PALACKY UNIVERSITY OLOMOUC

Faculty of Science

Department of Organic Chemistry

Study of New Systems for Non-catalyzed Conjugation Reaction with Azides

Ph.D. Thesis

Author: Mgr. Petra Jedináková

Supervisor: prof. RNDr. Jan Hlaváč, Ph.D.

Olomouc 2017

Bibliographic details

Title	Study of New Systems for Non-catalyzed Conjugation Reaction with Azides
Název	Studium nových systémů pro nekatalyzované konjugační reakce s azidy
Type	Ph.D. thesis
Author	Mgr. Petra Jedináková
Supervisor	prof. RNDr. Jan Hlaváč, Ph.D.
University	Palacký University Olomouc
Study programme	P1417 Chemistry
Field of study	Organic chemistry
Department	Organic chemistry
Language	English
Year	2017
Pages	
Avaliable at	http://portal.upol.cz

Declaration of originality

I declare that I have developed the presented dissertation independently under supervision of prof. RNDr. Jan Hlaváč, Ph.D. and that I have use only literature sources referred by citations. Neither the thesis nor any of its substantial parts were previously used for awarding of any other academic degree.

In Olomouc ……...2017

……
Petra Jedináková

Acknowledgement

At this place I would like to thank mainly to my supervisor prof. Jan Hlaváč for his professional leadership, patience, optimism, positive motivation and trust, valuable advice during working on presented dissertation. Further my thanks also belong to co-authors of my publications and also to all of my colleagues from Department of Organic Chemistry for their help, friendship and support.

Last but not least, I would like to thank to my family, especially to my husband, for their support, patience and help.

This research was financially supported by the Ministry of Education, Youth and Sport of the Czech Republic (IGA_PrF_2014030, IGA_PrF_2015_007 and IGA-PrF-2016-020), Technological Agency of the Czech Republic (projects TE01020028 and TE02000058) and by the European Social Fund (CZ.1.07/2.3.00/30.0060 and CZ.1.07/2.3.00/20.0009). The infrastructure of this project (Institute of Molecular and Translation Medicine) was supported by the National Program of sustainability (project LO1304).

Abstract

Non-catalyzed click reactions are widely used in range of biological applications. Although a number of derivatives of cyclic alkynes have been developed till now for these purposes, the most of these systems suffer from complicated and multi-step synthesis with low overall yields which limits their wider use. Often insufficient characterization of products formed by conjugation reaction of these alkynes with azides is another important problem in this field.

The presented thesis was focused on preparation of two types of compounds for non-catalyzed conjugation reaction with azides. Firstly, conjugation reactions of known aza-dibenzocyclooctyne with azides derived from 5-methyluridine were studied. Synthesized products were characterized by detail NMR study, which showed presence of conformational isomers at all prepared compounds. The computational study then helped to suggest the cause of occurrence and structure of these isomers. Then, the attention was paid to development of fast and simple procedure for fluorescently labelled derivatives of aza-dibenzocyclooctyne on solid phase. Unfortunately, the key formation of triple bond on cyclooctyne system has not been successful despite a number of optimizations. However, prepared precursors having double bond can be used for conjugation reactions using alkenes.

Derivatives of cycloalka-1,2,3-selenadiazoles were the second type of studied compounds. Cycloalka-1,2,3-selenadiazoles can decompose by high temperatures or UV irradiation to cycloalkynes undergoing the conjugation reactions with azides. The first part of work was focused on study of microwave assisted and photochemically initiated conjugation reactions of cycloocta[d][1,2,3]selenadiazole with structurally different azides. The both method resulted in target 1,2,3-triazoles. The potential application of photochemically initiated reaction was then successfully demonstrated on conjugation reaction of cycloocta[d][1,2,3]selenadiazole derivative with avidin-modified biotin complex. Then, derivatives of cycloocta[d][1,2,3]selenadiazole bearing substituent with different electronic properties were prepared to decrease the temperature necessary for conjugation reaction. Unfortunately, both type of synthesized derivatives exhibited much lower reactivity during reaction with azides than unsubstituted cycloocta[d][1,2,3]selenadiazole. In the last part, the attention was paid to preparation of dibenzothiepine- a dibenzoselenepine-1,2,3-selenadiazoles with the aim to enhance the reactivity at photochemically initiated conjugation reactions. All synthesized 1,2,3-selenadiazoles provided different products depending on 1,2,3-selenadiazole structure and used wavelength.

Souhrn

Nekatalyzované konjugační reakce alkynů s azidy jsou široce používané v řadě biologických aplikací. Ačkoliv byla vyvinuta řada derivátů cyklických alkynů pro tyto účely, jejich komplikovaná a multi-kroková příprava, často spojená s nízkými výtěžky, je limitací pro jejich rozsáhlejší využití. Dalším problémem je také často nedostatečná charakterizace produktů vznikajících samotnou konjugační reakcí těchto alkynů s azidy.

Disertační práce byla zaměřena na přípravu dvou typů sloučenin pro nekatalyzované konjugační reakce s azidy. Prvním typem byly deriváty již známého aza-dibenzocyclooctynu u kterého byly nejprve studovány konjugační reakce s azidy odvozenými od 5-methyluridinu. Vzniklé produkty byly charakterizovány pomocí detailní NMR studie, která vedla ke zjištění přítomnosti konformačních izomerů u všech připravených sloučenin. Příčina vzniku těchto izomerů a jejich struktury byly navrženy pomocí výpočetní studie. Dalším cílem bylo vyvinout rychlou a jednoduchou syntézu fluorescenčně značených derivátů aza-dibenzocyclooctynu na pevné fázi. Klíčová tvorba trojné vazby na cyclootynovém systému nebyla bohužel i přes řadu optimalizací úspěšná. Nicméně, připravené prekurzory nesoucí dvojnou vazbu mohou být použity pro konjugační reakce využívající alkeny.

Druhým typem studovaných sloučenin byly deriváty cycloalka-1,2,3-selenadiazolů, které se mohou působením vysokých teplot nebo UV záření rozkládat za vzniku cykloalkynů podléhajícím konjugačním reakcím s azidy. První část práce byla zaměřena na studium mikrovlnami a fotochemicky iniciovaných konjugačních reakcí cyklookta[d][1,2,3]selenadiazolu s různými azidy. Obě metody poskytují požadované 1,2,3-triazoly. Aplikace fotochemicky iniciované reakce byla demonstrována na konjugační reakci cyklookta[d][1,2,3]selenadiazolu s komplexem avidin-modifikovaný biotin. Dále byly připraveny deriváty cyklookta[d][1,2,3]selenadiazolu nesoucí substituenty s různými elektronovými vlastnostmi s cílem snížit teplotu potřebnou pro konjugační reakci. Bohužel, oba typy připravených derivátů vykazovaly při reakci s azidy mnohem nižší reaktivitu než nesubstituovaný cyklookta[d][1,2,3]selenadiazol. Poslední část pak byla zaměřena na přípravu dibenzothiepino- a dibenzoselenepino-1,2,3-selenadiazolů s cílem zvýšit reaktivitu u fotochemicky iniciovaných konjugačních reakcí. Všechny připravené 1,2,3-selenadiazoly poskytovaly různé produkty v závislosti na typu 1,2,3-selenadiazolu a na použité vlnové délce.

List of abbreviations

A549 cells	adenocarcinomic human alveolar basal epithelial cells
Ac4GalNAz	tetraacetylated *N*-Azidoacetylgalactosamine
Ac4ManNAz	tetraacetylated *N*-Azidoacetylmannosamine
AcOH	acetic acid
AgAAC	Ag(I) catalysed Azide-Alkyne Cycloaddition
AIBN	2,2′-Azobis(2-methylpropionitrile)
Alloc	allyloxycarbonyl
BALB/c mouse	albino, laboratory-bred strain of the house mouse
Boc	tert-butyloxycarbonyl
BOP	(Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate
BTPP	(tert-Butylimino)tris(pyrrolidino)phosphorane
CBzCl	benzyl chloroformate
CDI	*N*,*N*′-Carbonyldiimidazole
Cellosolve	2-ethoxyethanol
CuAAC	Cu(I) catalysed Azide-Alkyne Cycloaddition
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCE	1,2-dichlorethane
DCM	dichlormethane
DEA	diethylamine
DIBAL-H	diisobutylaluminium hydride
DIC	*N*,*N*′-Diisopropylcarbodiimide
DIEA	*N*,*N*-Diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMDO	dimethyldioxirane
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DOTA	1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid
EDC.HCl	*N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride
EGFR	epidermal growth factor receptor

EpCAM	epithelial cell adhesion molecule
EtOAc	ehylacetate
ETFA	ethyl trifluoroacetate
FAEEAA	{2-[2-(Fmoc-amino)ethoxy]ethoxy}acetic acid
Fmoc-Osu	9-Fluorenylmethyl *N*-succinimidyl carbonate
G5 PAMAM	polyamidoamine, generation 5
GC/MS	gas chromatography–mass spectrometry
GRPR	gastrin releasing peptide receptor
HATU	1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate
HEK293 cells	human embryonic kidney cells 293
HMDS	hexamethyldisilazane
HMPA	hexamethylphosphoramide
HOBt	1-Hydroxybenzotriazole
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
KHDMS	potassium bis(trimethylsilyl)amide
LDA	lithium diisopropylamide
LHDMS	lithium bis(trimethylsilyl)amide
Lys	lysin
MALDI	matrix-assisted laser desorption/ionization
m-CPBA	3-Chloroperbenzoic acid
MeCN	acetonitrile
MeOH	methanol
MW	microwave
NFSI	*N*-Fluorobenzenesulfonimide
NMP	*N*-Methyl-2-pyrrolidone
NMR	nuclear magnetic resonance
PCC	pyridinium chlorochromate
PCR	polymerase Chain Reaction
PEG	polyethylenglycol
PES	potential energy surface
PET	positron emission tomography
PivCl	pivaloyl chloride

PPA	polyphosphoric acid
PPTS	pyridinium *p*-toluenesulfonate
Proton sponge	1,8-Bis(dimethylamino)naphthalene
PyBOP	(Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
QD	quantum dots
RGD	arginylglycylaspartic acid
RhoB	rhodamine B
RNA	ribonucleic acid
rt	room temperature
RuAAC	Ru(I) catalysed Azide-Alkyne Cycloaddition
SiaNAz	*N*-azidoacetyl sialic acid
SPAAC	strain promoted Azide-Alkyne Cycloaddition
TBABr	tetrabutylammonium bromide
TBAF	tetrabutylammonium fluoride
TBAHS	tetrabutylammonium hydrogensulfate
TBSCl	*tert*-Butyldimethylsilyl chloride
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
TIPS	triisopropylsilyl group
TMP	trimethylolpropane
VACV	vaccinia virus

List of publications of the author related to the thesis

1. Smyslova, P.; Popa, I.; Lycka, A.; Tejral, G.; Hlavac, J. Non-catalyzed click reactions of ADIBO derivatives with 5-methyluridine azides and conformational study of the resulting triazoles *PLoS One* **2015,** *10* (12), e0144613-1-e0144613/33.

2. Jedinakova, P.; Sebej, P.; Slanina, T.; Klan, P.; Hlavac, J. Study and application of noncatalyzed photoinduced conjugation of azides and cycloocta-1,2,3-selenadiazoles *Chem. Commun. (Cambridge, U. K.)* **2016,** *52* (26), 4792-4795.

Contents

Acknowledgement **4**
Abstract **5**
Souhrn **6**
List of abbreviations **7**
List of publications of the author related to the thesis **10**
Contents **11**
1. Introduction **14**
2. State of the art **15**
2.1. Azide – alkyne cycloaddition 15
2.1.1. Types of azide – alkyne cycloadditions 15
2.1.1.1. Metal catalysed azide – alkyne cycloaddition 15
2.1.1.2. Strain promoted azide-alkyne cycloaddition 16
2.1.2. Preparation of cycloalkynes for SPAAC 20
2.1.2.1. Cyclooctyne 20
2.1.2.1.1. Synthesis of cyclooctyne from hydrazones and by elimination 20
2.1.2.1.2. Synthesis from cycloocta[d][1,2,3]selenadiazole 22
2.1.2.2. OCT 27
2.1.2.3. Fluorinated cyclooctynes 27
2.1.2.4. Dibenzocyclooctynes 30
2.1.2.5. Benzocyclooctynes 33
2.1.2.6. Thiacycloalkynes 35
2.1.2.7. Cycloalkynes with improved polarity 37
2.1.2.8. The others 38
2.1.3. Application of SPAAC 41
2.1.3.1. Chemical-biology applications 41
2.1.3.1.1. Cellular imaging 41
2.1.3.1.2. Nucleic acid modifications 44
2.1.3.1.3. Quantum dots conjugates 46
2.1.3.1.4. Preparation of probes for PET 47
2.1.3.2. Materials science applications 49
2.1.3.2.1. Dendrimers 49
2.1.3.2.2. Hydrogels 50

2.1.3.2.3. Polymers 50
2.1.3.2.4. Surface functionalization 51
3. Aims of the work 53
3.1. Summary of presented aims 53
3.1.1. **DIBAC** derivatives 53
3.1.2. Cycloalka-1,2,3-selenadiazoles 54
4. Results and discussion 55
4.1. **DIBAC** derivatives 55
4.1.1. SPAAC of **DIBAC** and characterization of resulting triazols 55
4.1.1.1. SPAAC with azides derived from 5-methyl uridine 55
4.1.1.1.1. Preparation of reagents 55
4.1.1.1.2. SPAAC 56
4.1.1.1.3. NMR study 57
4.1.1.1.4. Computational study 62
4.1.1.1.5. Effect of aliphatic chain on formation of conformers 67
4.1.2. Solid phase synthesis of fluorescently labelled **DIBAC** analogues 70
4.1.2.1. Immobilization of dihydrodibenzoazocine 70
4.1.2.2. Triple bond formation 73
4.1.2.3. Attachment of fluorescent label 76
4.2. 1,2,3-selenadiazoles 79
4.2.1. Reactions of cycloocta[d][1,2,3]selenadiazoles 79
4.2.1.1. Microwave assisted conjugation reactions of cycloocta[d][1,2,3]selenadiazole 80
4.2.1.2. Photochemically induced conjugation reactions of cycloocta[d][1,2,3]selenadiazole 81
4.2.2. Preparation of derivatives of cycloocta-1,2,3-selenadiazol 89
4.2.2.1. 4-nitro-cycloocta[d][1,2,3]selenadiazole 89
4.2.2.2. 4-amino-cycloocta[d][1,2,3]selenadiazole 90
4.2.2.3. 4,4-difluoro-cycloocta[d][1,2,3]selenadiazole 92
4.2.2.4. Thermally induced conjugation reaction with azides 93
4.2.3. Preparation and reactivity of dibenzo thia- or selena- cyclohepta-1,2,3-selenadiazoles 94
4.2.3.1. Synthesis of dibenzo thia- or selena- cyclohepta-1,2,3-selenadiazoles 94

4.2.3.2. Photoinduced conjugation reaction of dibenzo thia- or selena- cyclohepta-1,2,3-selenadiazoles 95
5. Conclusion 102
6. Experimental part 105
6.1. Materials and methods 105
6.1.1. Instrumentation 105
6.1.2. Description of methods for study of photochemically induced conjugation reaction of cycloocta[d][1,2,3]selenadiazole 106
6.1.2.1. Photochemical reaction of cyclooctaselenadiazole **65** and azide **5**, **6**, **68-73** 106
6.1.2.2. Photochemical transformation of cyclooctaselenadiazole **65** 107
6.1.2.3. Synthesis of triazoles **66**, **67** and **74-79** *via* photochemical reaction 107
6.1.2.4. Experimental procedures for conjugation reactions of derivative **80** and **81** 108
6.1.2.5. Kinetic study of a reaction between cyclooctyne and benzylazide **72** 108
6.1.2.6. Determination of quantum yield of triazole **78** appearance 109
6.1.3. Study of photochemically induced reactions of 1,2,3-selenadiazoles **105a-e** with benzylazide **72** 110
6.2. Synthesis of individual compounds 110
6.2.1. SPAAC of **DIBAC** and characterization of resulting triazoles 110
6.2.2. Solid phase synthesis of fluorescently labelled **DIBAC** analogues 124
6.2.3. Reactions of cycloocta[d][1,2,3]selenadiazole 136
6.2.3.1. Synthesis of triazoles **66**, **67**, **74-79** via thermal heating of cycloocta[d][1,2,3]selenadiazole **65** 136
6.2.3.2. Compounds from photochemically induced reactions of cycloocta[d][1,2,3]selenadiazole 139
6.2.4. Preparation of derivatives of cycloocta-1,2,3-selenadiazole 144
6.2.5. Preparation and reactivity of dibenzothiepine- and dibenzoselenepine-1,2,3-selenadiazoles 146
7. References 152

1. Introduction

Since discovery of Cu(I) catalysed cycloaddition of azides (CuAAC) with terminal alkynes by Sharpless[1] and Meldal[2] in 2002, this reaction, yielding selectively 1,4-triazoles, was widely studied and applied for range of bioconjugation reactions.[3] Bioconjugation techniques generally involve the covalent attachment of synthetic labels to a specific biomolecular target. Examples include the modification of proteins and nucleic acids by incorporation of fluorophores, ligands, chelates, radioisotopes and affinity tags.[3]

The applicability of CuAAC for bioconjugation was firstly reported by Meldal *et al.* in 2002.[2] They prepared first peptidotriazoles by utilization of solid phase synthesis techniques and the azide-alkyne cycloaddition.[2] Then CuAAC has been applied for functionalization of DNA, ligation and decoration of peptides and peptoids, for synthesis of peptidomimetics, surface modifications and many other applications.[3] Ideal bioconjugation reactions should be carried out without affecting living tissues via undesired interaction with biomolecules.[3] However Cu(I) ions are toxic for living organisms which limited the use of CuAAC for reactions in living systems.[4]

In 2004, Bertozzi *et al*. utilized the non-catalysed azide-alkyne conjugation reaction mediated by ring strain of cyclooctyne.[5] The biocompatibility of this approach was showed on labelling of Jurkat cells.[5] Their discovery started new era in field of bioconjugation reactions. Till now, many reactive cycloalkynes were developed and utilized for range of not only biological applications including modification of peptides and nucleic acids, tumor targeting, fluorescent labelling, radiolabelling or surface modifications and formation of polymer, hydrogels and dendrimers.[6]

The main drawback of non-catalyzed azide-alkyne cycloadditions is especially availability of reactive cycloalkynes. The most of the known systems require time wasting multi-step synthesis, often with very low overall yield.[6] Another problem is stability of some systems during storage.[6] Thus, development of new reactive cycloalkynes, which will be easy to prepare and modify, and will have satisfactory stability with good reactivity, is still important topic, which could be beneficial for extending of azide-alkyne cycloadditions applicability.

2. State of the art

2.1. Azide – alkyne cycloaddition

1,3-dipolar cycloaddition was firstly demonstrated by Michael in 1893[7] by reaction of azide **I** and alkyne **II** giving 1,2,3-triazole **III** (Figure I) The kinetic of this reaction was extensively studied by Huisgen in 1960s.[8] Despite the high potential, this reaction was not commonly used due to its high activation barrier, which could be overcome only by elevated temperature and pressure.[4,9] Moreover, 1,2,3-triazoles are formed as a mixture of regioisomers **V** and **VI** (Figure I) when an unsymmetrically substituted alkynes are used.[9]

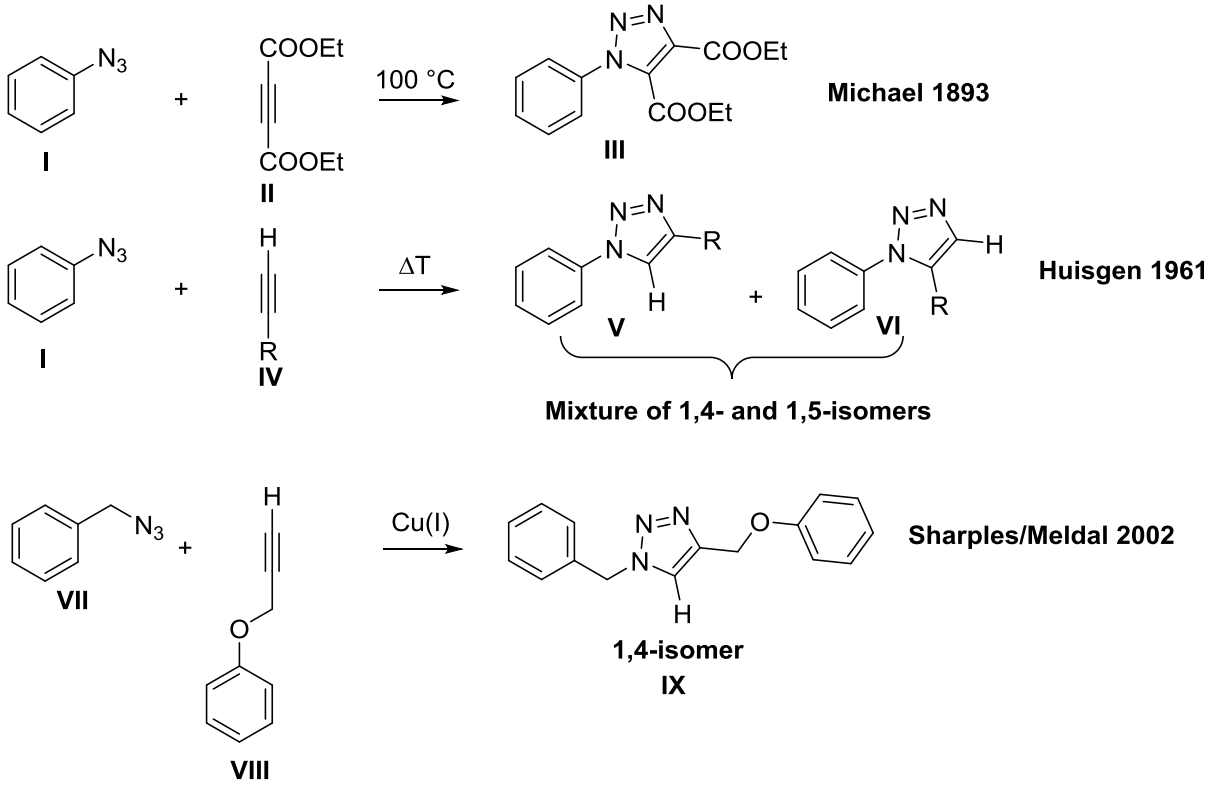


Figure I: Reactions of alkynes with azides.

2.1.1. Types of azide – alkyne cycloadditions

2.1.1.1. Metal catalysed azide – alkyne cycloaddition

Azide – terminal alkyne cycloaddition became more widely used after discovery of Sharpless[1] and Meldal[2] who independently found out, that Cu(I) catalysis dramatically increase the reaction rate. CuAAC (Cu-catalyzed azide-alkyne cycloaddition) proceeds very fast at ambient temperatures and only 1,4-isomers are formed (Figure I).[1,2,4] CuAAC is an example of "click chemistry" (click reaction must be modular, wide in scope, high yielding;

must generate only inoffensive product and be stereospecific[10]), which includes the range of biocompatible reactions designed primarily to join selected substrates with specific biomolecules. The term was coined by Sharpless and co-workers in 1998.[10] The scope of the reaction is limited only for terminal alkynes and in addition, the use of CuAAC in biological application is limited due to the toxicity of copper catalyst.[4]

The ruthenium variant of CuAAC (RuAAC), firstly described by group of V. Fokin in 2005, then resulted selectively in 1,5-disubstituted 1,2,3-triazole **XI** (Figure II).[11] In addition, both terminal and internal alkynes can participate in this reaction with azides.[9]

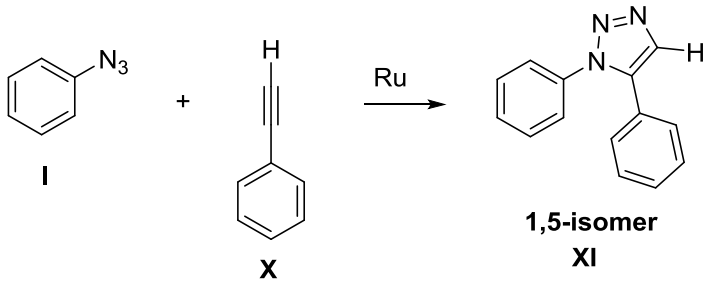


Figure II: Ruthenium catalysed alkyne azide cycloaddition.

In 2011 McNulty and co-workers developed silver(I) catalyst for cycloaddition of azides into terminal alkynes.[12] The AgAAC reaction occurs at room temperature or with heating to obtain selectively the corresponding 1,4-disubstituted-1,2,3-triazole.[12,13]

2.1.1.2. Strain promoted azide-alkyne cycloaddition

The high activation barrier of cycloaddition reaction can be also decreased by increase of ground state energy of reactants.[4] This may be achieved by introducing a strain into reactants as the alkyne in this case.[4] In 1961 Wittig and Krebs observed, that cyclooctyne reacted very fast with phenylazide yielding a single triazole product.[14] However, this reaction was considered as a chemical curiosity till 2004, when Bertozzi and co-workers prepared derivative of cyclooctyne **OCT** (Figure III) and used its biotinylated modification for labelling of Jurkat cells.[5] Discovery of strain promoted azide – alkyne cycloaddition (SPAAC) has led to development of new strained cycloalkynes and their use in biological applications.[4]

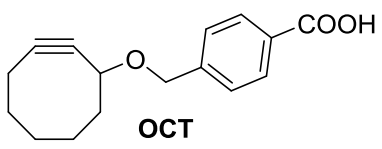


Figure III: Structure of **OCT**.

Cycloalkynes for SPAAC

Although biotinylated **OCT** was successfully used for cell surface labelling and exhibited no cellular toxicity,[5] slow reaction kinetic was the main limitation of this reaction.[4,6] According to this fact, following studies were focused on development of strained alkynes with improved kinetics in cycloaddition with azides. Theoretical studies show that reactivity of cycloalkyne depends on ring strain and LUMO energy. New cycloalkynes were then designed according to these two factors.[4,6]

In 2006 Bertozzi and co-workers introduced electron-withdrawing fluorine to the position adjacent to the triple bond of OCT to obtained monofluorinated cyclooctyne called **MOFO** (Figure IV) with reduced LUMO energy.[4-6] It resulted in rapid increase in reaction rate. Nonfluorinated analogue of **MOFO** termed **NOFO**[15] (Figure IV) exhibited slower reaction rate then **OCT**, which confirmed the positive effect of fluorine on cycloaddition reaction rate.[4,6] The further increase was achieved by synthesizing difluorinated cyclooctyne **DIFO** (Figure IV).[4,6,16] Due to low yielding multistep synthesis of **DIFO**, Bertozzi and co-workers developed more convenient **DIFO2** and **DIFO3** (Figure IV).[4,6,17]

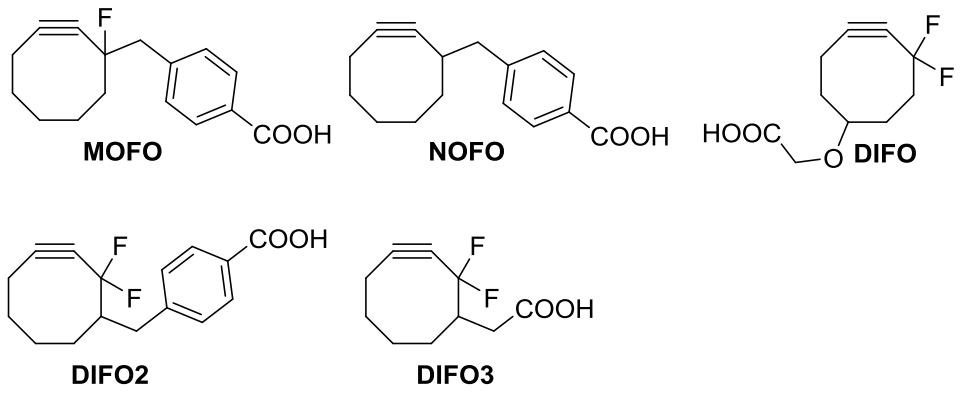


Figure IV: Structures of fluorinated cyclooctynes.

Other approaches for improving cyclooctyne kinetics were focused on increasing of the ring strain. Because of instability of seven-membered cycloalkynes, the best way was to change the hybridization of ring carbons.[4,6] As a result, dibenzocyclooctyne derivatives **DIBO1** and **DIBO2** (Figure V) were prepared with rate constant 25 times higher than **OCT**.[4,6,18] Aza analogues of **DIBO** termed **DIBAC**[19] and **BARAC**[20] (Figure V) then exhibited rate constant even more than 100 and 400 times higher respectively than **OCT**.[4,6] Another way how to increase the ring strain involved cyclopropyl ring fused to the cyclooctyne.[4,6] The resulting bicyclononyne **BCN** (Figure V), firstly described by van Delft and co-workers, then showed rate constant value between **DIFO** and **DIBAC**.[4,6,21]

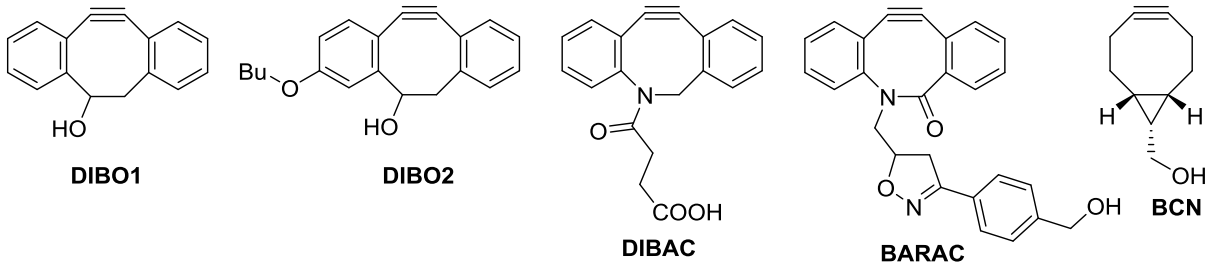


Figure V: Cyclooctynes with increased ring strain.

In 2010, Bertozzi and co-workers prepared difluorobenzocyclooctyne **DIFBO** (Figure VI) as a combination of both rate-amplifying effects.[4,6,22] The rate constant of **DIFBO** (~ 92 relative to **OCT**) was measured after its enclosing to β-cyclodextrin cavity to avoid of its spontaneous trimerization.[4,6,22] To study the individual effects of aromatic ring and fluorine substituents Bertozzi group synthesized **MOBO** (Figure VI) as non-fluorinated analogue of **DIFBO**. The relatively low value of rate constant confirmed the beneficial effect of fluorine to reactivity similarly to **MOFO** and **NOFO** (see above).[4,6,22]

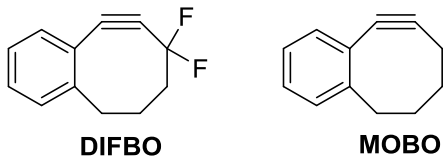


Figure VI: Monobenzocyclooctynes.

Two years later, Dudley and co-workers tested the effect of ring expansion on reaction rate of SPAAC. They synthesized benzocyclononyne **TMBN** (Figure VII). However, the rate constant was lower than in case of **OCT**.[4,6,23] A less significant expansion can be achieved by introducing endocyclic sulphur into cyclooctyne structure.[4,6] Thiacycloalkyne **thiaOCT** (Figure VII) prepared by Bertozzi group showed almost one order magnitude lower rate constant than **OCT**.[4,6,24] Similarly, cycloaddition of **thiaDIFBO** (Figure VII) was almost twenty times lower than cycloaddition on parent **DIFBO**.[4,6,24] On the other hand, unlike **DIFBO**, **thiaDIFBO** did not oligomerize during storage. Consequently, reactivity of more strain cycloalkyne containing endocyclic sulphur was also tested.[4,6,24] 3,3,6,6-tetramethylthiacycloheptyne **TMTH** (Figure VII), firstly prepared in 1970s by Krebs and Kimling,[25] is stable enough to be isolated and handle and displays four times higher rate constant than **BARAC** and three orders of magnitude greater than **OCT**. Till now **TMTH** is the most reactive SPAAC reagent.[6,24]

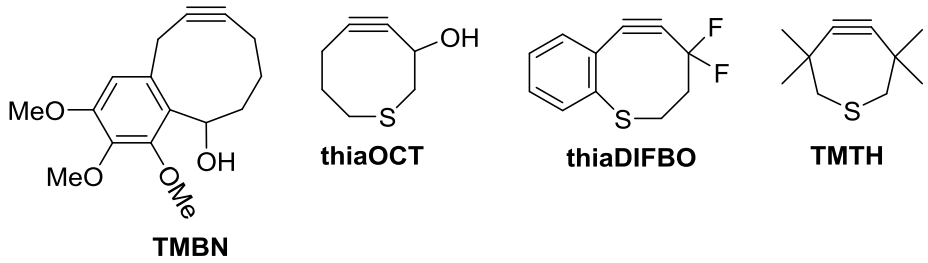


Figure VII: Cyclooalkynes with changed ring size.

Despite of improved reaction kinetics, the hydrophobicity of cyclooctynes could present a problem in terms of water solubility and nonspecific adhesion to membrane and other hydrophobic surfaces in biological systems.[4,6] To supress thess effects, modifications of known systems have been made.[4,6] Bertozzi and co-workers prepared aryl-less analogue of **OCT** termed **ALO**[4,15] (Figure VIII) and heterocyclic dimethylazacyclooctyne **DIMAC**[4,26] (Figure VIII). Boons and co-workers then described **S-DIBO** (Figure VIII) with two polar groups showing significantly improved water solubility.[6,27]

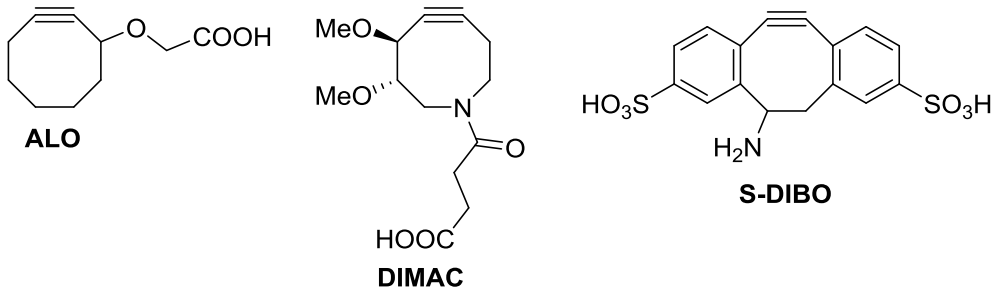
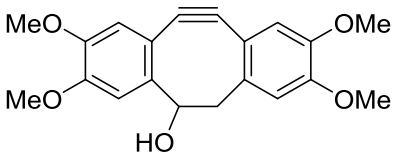
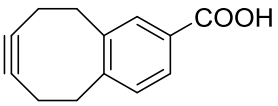


Figure VIII: Structures of more hydrophobic cyclooctynes.

Other problem, which can occur in some cases, is stability of reagents during storage or towards chemical conditions such as pH changes.[6] Design of new systems was focused on development of robust reagent with long shelf-life and easy handling.[6] Leeper and co-workers prepared **TMDIBO** (Figure IX) with reactivity similar to **DIFBO**.[6,28] **TMDIBO** can be stored for years at room temperature without any noticeable decomposition.[6,28] Kele and co-workers introduced **COMBO** (Figure IX) which has the same level reactivity with **DIFBO**.[6,29] **COMBO** is stable under acidic and strongly basic conditions. Moreover, potential side reaction with biologically relevant reagent was studied in presence of three-fold excess of glutathione. After 14 hours at 25 °C only 9% of **COMBO** reacted.[6,29]



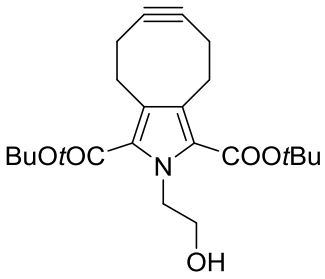
TMDIBO



COMBO

Figure IX: Structure of cyclooctynes with improved stability.

A major shortcoming of SPAAC is the formation of mixture of isomeric products with similar or equal ratios for most functionalized cyclooctynes.[30] In 2015 Berg and Gröst published preparation of the first functionalized cyclooctyne **PYRROC** (Figure X), which do not form isomers with azides due to its symmetry.[30] Kinetic study of its reaction with benzylazide provided value of rate constant located between **DIBO** and **DIFO**.[30]



PYRROC

Figure X: Structure of PYRROC.

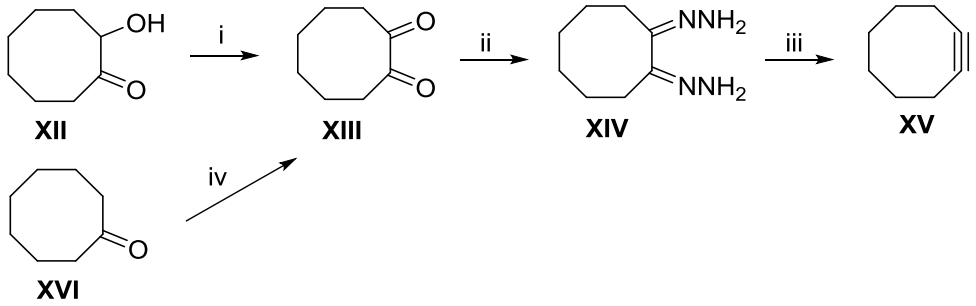
2.1.2. Preparation of cycloalkynes for SPAAC

2.1.2.1 Cyclooctyne

2.1.2.1.1. Synthesis of cyclooctyne from hydrazones and by elimination

The preparation of cyclooctyne as the smallest, stable and isolable cyclooalkyne was firstly studied by Domnin in 1938.[31] He reported its synthesis from 1-bromo-2-chlorocyclooctene,[31] but his claim was disputed by Blomquist and Liu.[32] They argued that his chemical evidence in support of cyclooctyne structure was insufficient and assumed that in fact the hydrocarbon was a mixture of cyclooctene and cyclooctane.[32] Blomquist and Liu then prepared cyclooctyne **XV** by alkali-catalysed oxidative decomposition of 1,2-cyclooctanedione dihydrazone **XIV** prepared from 2-hydroxycyclooctanone **XII** (Scheme I).[32]

Scheme I: Preparation of cyclooctyne according to Blomquist.[32,*a*]

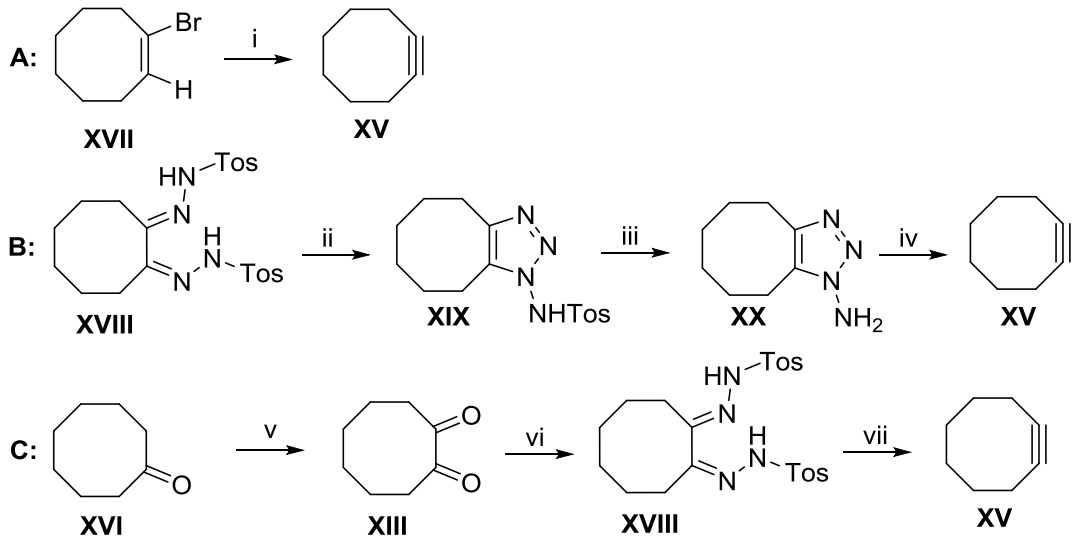


[*a*]Reagent: (i) $Cu(OAc)_2$, MeOH, AcOH, rt (ii) hydrazine monohydrate, rt (iii) HgO, KOH, benzene, ΔT (iv) SeO_2, dioxane, reflux.

In 1961 Wittig and Krebs published modification of this approach containing synthesis of cyclooctane dione **XIII** from cyclooctanone **XVI**, which was oxidized by selenium dioxide (Scheme I).[14] The following steps are analogical to procedure described previously.[32]

Seven years later Wittig and Dorsch prepared cyclooctyne by dehydrobromination of bromocyclooctene **XVII** in 30% yield or by oxidation of 1-aminocycloocteno-1,2,3-triazole **XX** in 74% yield (Scheme II, entry A or B).[33] In 1971 Freeman and Johnson described the formation of alkynes by photodecomposition of the dianions of the di-*p*-tosylhydrazones,[34] which was later used by Meier and Menzel for the synthesis of cycloalkynes. Cyclooctyne **XV** prepared by this method (Scheme II, entry C) was obtained in 36% yield.[35]

Scheme II: Other preparations of cyclooctyne.[33,35,*a*]



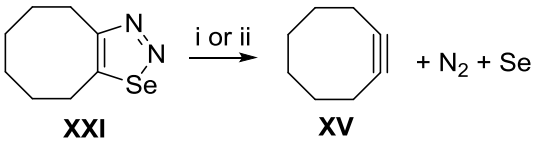
[*a*]Reagent: (i) $NaNH_2$, 200 – 210 °C (ii) AcOH (iii) 85% H_2SO_4, rt (iv) $Pb(OAc)_4$, DCM, rt (v) SeO_2, dioxane, H_2O, rt (vi) $TsNHNH_2$, AcOH, rt (vii) *hv*, NaOH, H_2O, MeOH, rt.

2.1.2.1.2. Synthesis from cycloocta[d][1,2,3]selenadiazole

Thermolysis of 1,2,3-selenadiazoles

In 1971 Meier and Menzel reported that cyclooctyne can be obtained by pyrolysis of cycloocta[d][1,2,3]selenadiazole **XXI** at 170 – 220 °C on glass powder in 49% yield with simultaneous N_2 and Se elimination (Scheme III).[36] In case of thermally labile substrates, the thermolysis can result in formation of side products. For this reason Meier and co-workers developed modification of mentioned pyrolysis.[37] They described, that cyclooctyne can be generated from cycloocta[d][1,2,3]selenadiazole or thiadiazole also by treatment with strong base such as *n*-butyllithium at low temperature (Scheme III). Cyclooctyne **XV** was obtained in 70% yield from 1,2,3-thiadiazole and in 80% yield from 1,2,3-selenadiazole.[37]

Scheme III: Preparations of cyclooctyne from cycloocta[d][1,2,3]selenadiazole.[36,37,*a*]

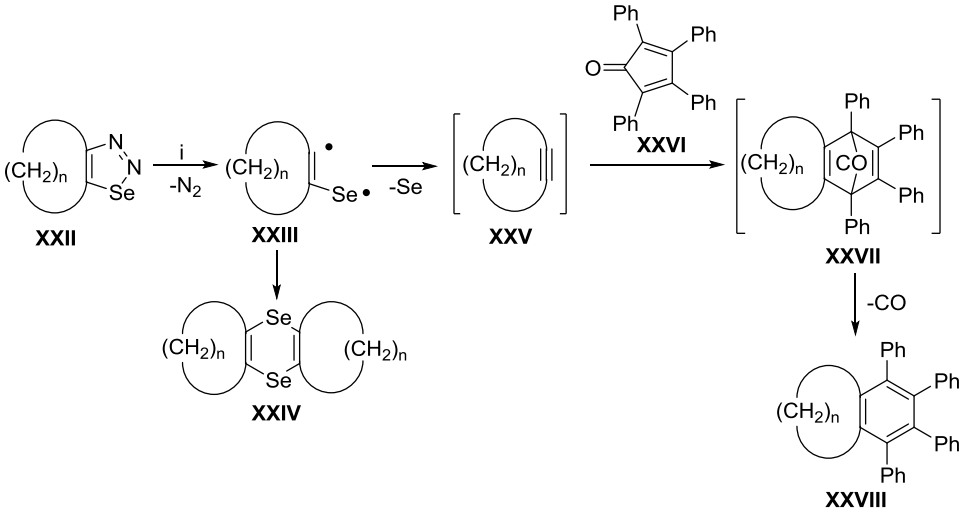


XXI **XV**

[*a*]Reagent: (i) 170 – 220°C, glass powder (ii) *n*BuLi, THF, hexane, -70°C.

Thermolysis of cycloalka-1,2,3-selenadiazoles was then studied in more detail. In 1972 Meier and Voight reported that cycloalka-1,2,3-selenadiazole heated under vacuum released nitrogen to form biradical **XXIII**, which dimerise to diselenine **XXIV** (Scheme IV). With increasing the temperature, selenium can be also eliminated from biradical. Resulted cycloalkynes **XXV** were trapped by tetraphenylcyclopentadione **XXVI** to form product **XXVIII** in 0-51% yield for n=3-6 (Scheme IV).[38]

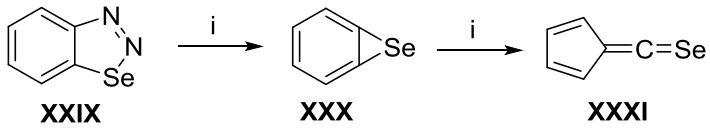
Scheme IV: Thermolysis of cycloalka-1,2,3-selenadiazoles.[38]



XXII **XXIII** **XXV** **XXVI** **XXVII** **XXIV** **XXVIII**

In 1972 Schweig and Schulz studied gas phase pyrolysis of 1,2,3-benzoselenadiazole **XXIX**.[39] They observed that thermal decomposition of **XXIX** lead to formation of 6-fulveneselenone **XXXI** (Scheme V) *via* formation of intermediate, which was characterised as benzoseleniren **XXX** (Scheme V) according to IR spectra identical to benzothiirene obtained previously by thermolysis of 1,2,3-benzothiadiazole.[39,40]

Scheme V: Gas phase pyrolysis of 1,2,3-benzoselenadiazole.[39,*a*]

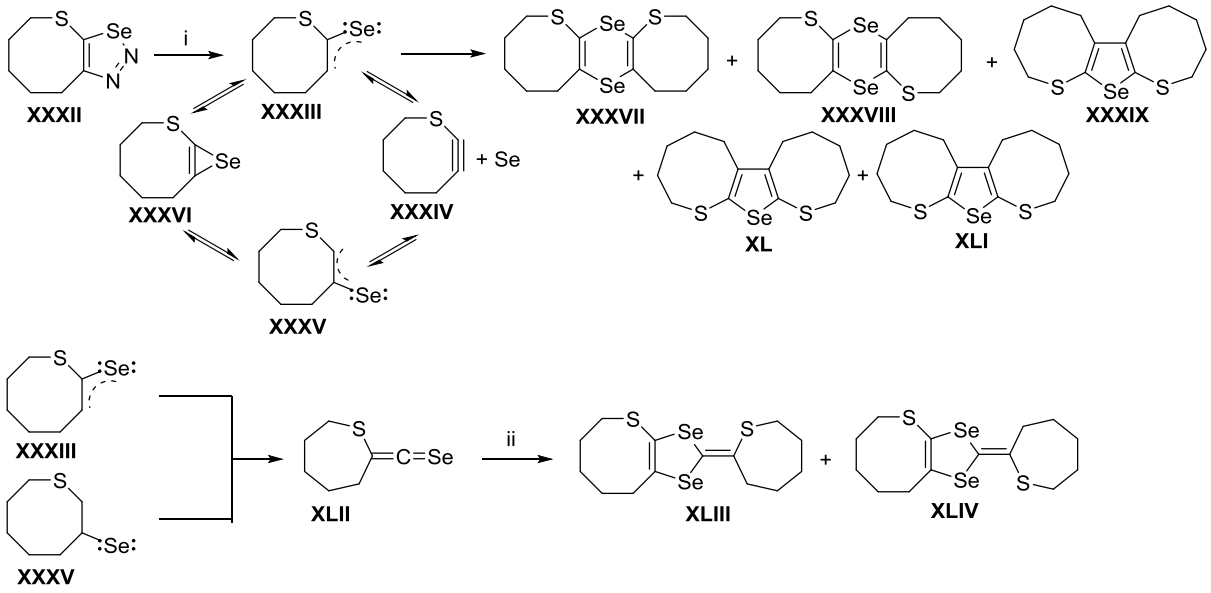


XXIX **XXX** **XXXI**

[*a*]Reagent: (i) 560 °C, 10^{-3} mbar.

Meier an co-workers described synthesis of 1-thia-2-cyclooctyne **XXXIV** (Scheme VI) by pyrolysis of corresponding 1,2,3-selenadiazole **XXXII** on copper powder.[41] Besides the cyclooctyne they reported also formation of other products such as diselenines **XXXVII** and **XXXVIII**. Moreover, they also as a first observed selenophenes **XXXIX** - **XLI** created probably by reaction of intermediates **XXXIII** and **XXXV** with cyclooctyne **XXXIV** (Scheme VI).[41] In addition, rearrangement of **XXXIII** and **XXXV** to selenoketen **XLII** was expected with regard of formation of diselenols **XLIII** and **XLIV**.[41]

Scheme VI: Thermolysis of 1,2,3-selenadiazole **XXXII**.[41,*a*]



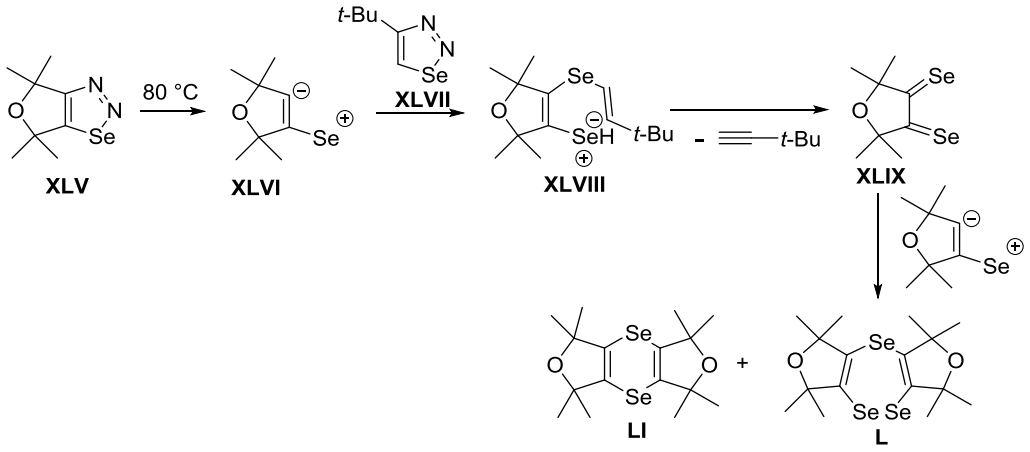
XXXII **XXXIII** **XXXVII** **XXXVIII** **XXXIX**
XXXVI **XXXIV** **XL** **XLI**
XXXV
XXXIII **XLII** **XLIII** **XLIV**
XXXV

[*a*]Reagent: (i) 160 °C, Cu powder (ii) **XXXIV**.

Thermolysis of 1,2,3-selenadiazoles in presence of other reagent can result in structurally different products besides those mentioned above. Ando and co-workers reported

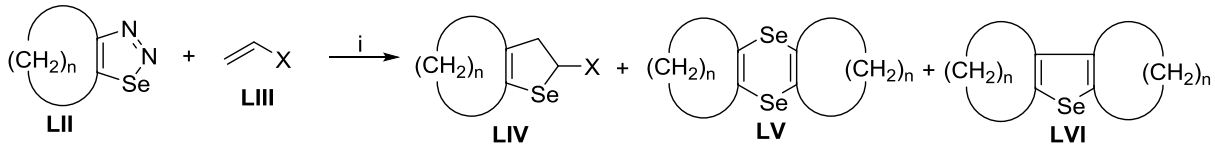
that if 1,2,3-selenadiazole **XLV** is heated with 4-tert-butyl-1,2,3-selenadiazole **XLVII**, the 1,2,5-triselepin **L** is formed together with diselenine **LI** (Scheme VII).[42] In the absence of **XLVII** in reaction mixture, only diselenine **LI** is obtained in high yield.[42]

Scheme VII: Thermolysis of 1,2,3-selenadiazole **XLV**.



When cycloalka-1,2,3-selenadiazoles **LII** were treated with excess of olefins **LIII** at 130 °C, the addition of radical formed *in situ* by the elimination of nitrogen from 1,2,3-selenadiazole to C-C double bond followed by intramolecular cyclization resulted in dihydroselenophenes **LIV** in moderate to good yields (Scheme VIII).[43] Corresponding diselenines **LV** and selenophenes **LVI** were formed as by-products.[43]

Scheme VIII: Thermolysis of 1,2,3-selenadiazoles in presence of olefins.[43]



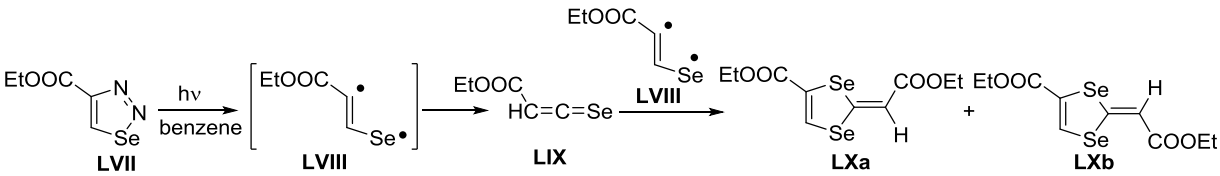
[a]Reagent: (i) 130 °C, 15 h.

The release of selenium after thermolysis of 1,2,3-selenadiazoles was utilized by Khanna and co-workers for preparation of various nanoparticles. They observed, that thermal reaction of cycloalkeno-1,2,3-selenadiazoles with metal salts can generate powders of metal selenides. During the last decade they use this methodology for preparation of CdSe,[43-47] Ag_2Se,[48,49] PbSe,[50] and HgSe[51] nanoparticles. They prepared also core-shell ZnSe-CdSe quantum dots.[52] In 2015 Khanna also reported formation of selenium nanoparticles by microwave irradiation of cycloocteno-1,2,3-selenadiazole.[53] Selenium nanoparticles were found to be more biological active and less toxic than bulk selenium.[54] Moreover selenium is proven to be good antimicrobial agent as well as antitumor agent against various cell lines.[53]

Photolysis of 1,2,3-selenadiazoles

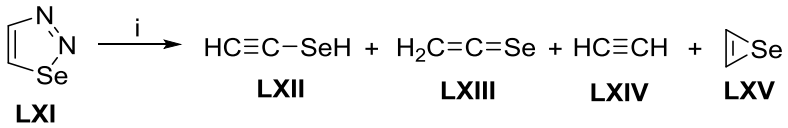
Photolysis of 1,2,3-selenadiazoles was firstly described in 1972 by Meier and Menzel.[55] They observed that photodecomposition of ethyl 1,2,3-selenadiazole-4-carboxylate **LVII** resulted in formation of fulvenoselenols **LXa** and **LXb** (Scheme IX). The proposed mechanism of fulvenoselenol formation included the creation of selenoketen **LIX** as primary photoproduct which reacted with biradical **LVIII** (Scheme IX).[55]

Scheme IX: Photolysis of 1,2,3-selenadiazole **LVII**.[55]



Four years later, Krantz and co-workers reported that irradiation with Pyrex-filtered mercury lamp of argon or nitrogen matrix-isolated 1,2,3-selenadiazole **LXI** at 8K resulted in ethynylselenol **LXII**, selenoketen **LXIII** and acetylene **LXIV** (Scheme X).[56] Few months later they described also formation of selenirene **LXV** (Scheme X) beside the products mentioned previously.[56,57]

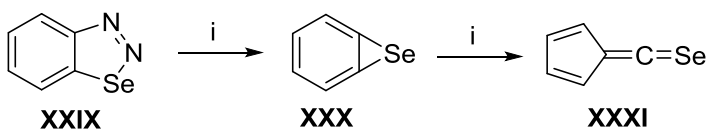
Scheme X: Photolysis of 1,2,3-selenadiazole **LXI**.[56,57]



[a]Reagent: (i) Ar, 8K, hν.

Irradiation (254 nm) of matrix-isolated 1,2,3-benzoselenadiazole **XXIX** produces reactive intermediate, which was characterized as benzoselenirene **XXX** (Scheme XI) according to bands in IR spectrum identical to those observed for benzothiirene.[39,58] Benzoselenirene by further irradiation rearranged to 6-fulvenoselenol **XXXI** (Scheme XI) as in case of gas phase pyrolysis mention above.[39]

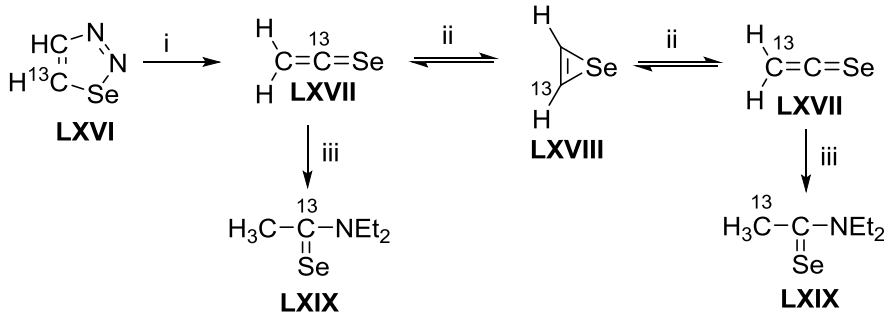
Scheme XI: Photolysis of 1,2,3-benzoselenadiazole.[39,a]



[a]Reagent: (i) Ar, hν.

In 1985 Holm and co-workers focused on formation of selenirene thus they compared photochemical fragmentation by a high pressure mercury arc (~ 300 nm) of 1,2,3-selenadiazole **LXI** (Scheme X) in fluid solution and in frozen EPA glass at liquid nitrogen temperature.[59] According to experiments with ^{13}C-labelled 1,2,3-selenadiazole **LXI** (**LXVI** in Scheme XII**)**, they confirmed that selenoketen **LXVII** is formed as the first photoproduct (Scheme XII). Seleniren **LXVIII** is then generated by secondary irradiation of selenoketen (Scheme XII).[59]

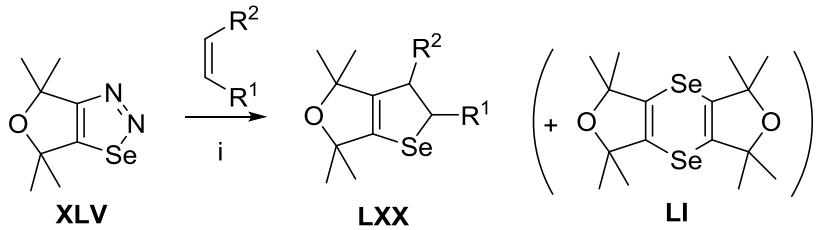
Scheme XII: Photolysis of ^{13}C-labelled 1,2,3-selenadiazole.[59,*a*]



[*a*]Reagent: (i) hν (ii) hν, 80K (iii) Et_2NH

Ando and co-workers were the first who studied photochemistry of bicyclic 1,2,3-selenadiazole in presence of olefin.[60] The 1,2,3-selenadiazole **XLV** was irradiated by a medium pressure mercury lamp together with olefin such as methyl acrylate, acrylonitrile or cyclopentadiene. Similarly to results obtained by pyrolysis of mixture of 1,2,3-selenadiazole and olefin described by Sonoda[43] (see above), the selenirenes **LXX** were obtained as a major products (Scheme XIII).[60] In case of cyclopentadiene, the diselenine **LI** was formed as a by-product.[60]

Scheme XIII: Photolysis of ^{13}C-labelled 1,2,3-selenadiazole.[60,*a*]

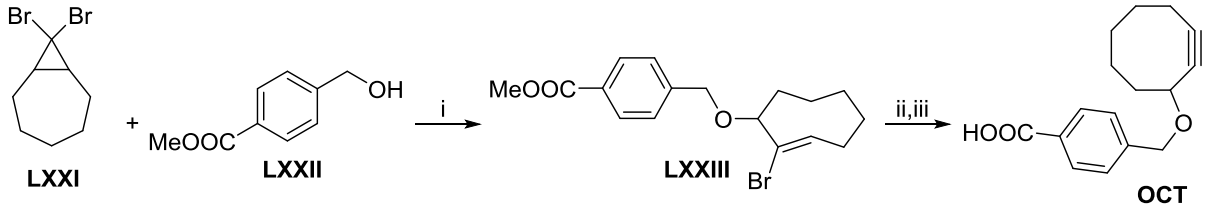


[*a*]Reagent: (i) hν, olefin.

2.1.2.2. OCT

Bertozzi and co-workers synthesized **OCT** according to methodology described by Reese and Show (Scheme XIV).[61] In this procedure, dibromobicyclononane **LXXI** was treated with silver perchlorate to open the ring to *trans*-allylic cation, which was reacted with methyl 4-(hydroxymethyl)-benzoate **LXXII** to afford bromo-trans-cyclooctene **LXXIII**.[5] Base mediated dehydrobromination followed by ester saponification yielded **OCT** in overall yield 73%.[5]

Scheme XIV: Preparation of OCT.[5,*a*]



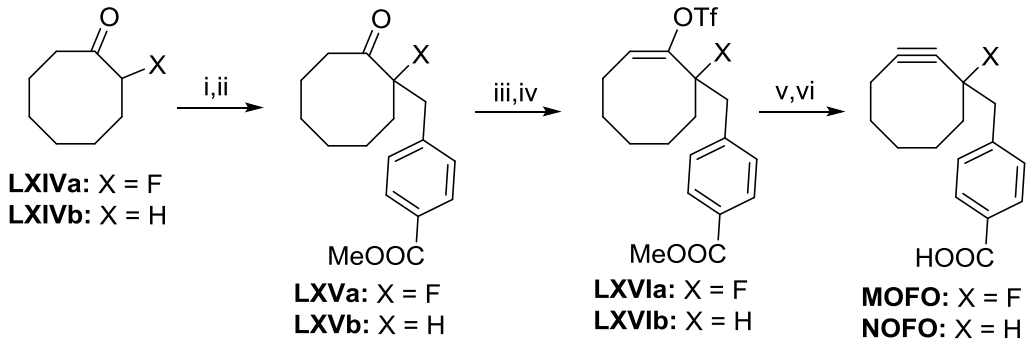
[*a*]Reagent: (i) $AgClO_4$, toluene, rt (ii) NaOMe, DMSO, rt (iii) LiOH, 20% H_2O/dioxane, rt.

2.1.2.3. Fluorinated cyclooctynes

MOFO

MOFO, the first member of group consists of fluorinated cyclooctynes was prepared in 2006 by Bertozzi group.[15] The synthesis of **MOFO** started by alkylation of 2-fluorocyclooctanone **LXIVa** followed by vinyl triflate **LXVIa** formation. Base mediated elimination and subsequent saponification resulted in **MOFO** in overall yield 18%.[15] Non-fluorinated analogue **NOFO** was synthetized via similar route (Scheme XV).[15]

Scheme XV: Preparation of **MOFO** and **NOFO**.[15,*a*]

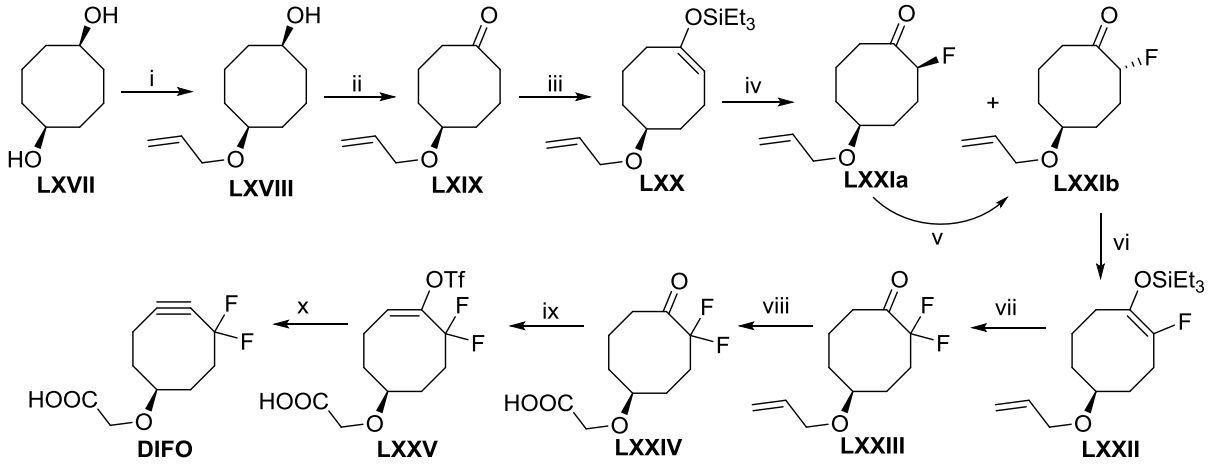


[*a*]Reagent: (i) LDA, THF, -78 °C (ii) Methyl 4-bromo-methylbenzoate, -78 °C to 25 °C (iii) KHDMS, THF, -78 °C (iv) Tf_2NPh, -78 °C to 25 °C (v) LDA, THF, 0 °C (vi) LiOH, dioxane/H_2O, 50 °C.

DIFO 1-3

In 2007 Bertozzi and co-workers introduced **DIFO**, which was prepared by multistep synthesis started from substitution of 1,5-cyclooctanediol **LXVII** with allylbromide (Scheme XVI).[16] Remaining alcohol was then oxidized to corresponding ketone **LXIX** followed by formation of silyl enol ether **LXX** and its subsequent fluorination by Selectfluor (Scheme 16).[16] Two diastereomeric monofluoroketones **LXXIa** and **LXXIb** were formed. Monofluorketone **LXXIb** was then converted to silyl enol-ether **LXXII**, which was fluorinated to difluoroketone **LXXIII** (Scheme XVI).[16] Oxidation of allyl group to carboxylic acid followed by formation and base mediated elimination of vinyl triflate yielded **DIFO** in overall yield 1.4%.[16]

Scheme XVI: Preparation of **DIFO**.[16,*a*]

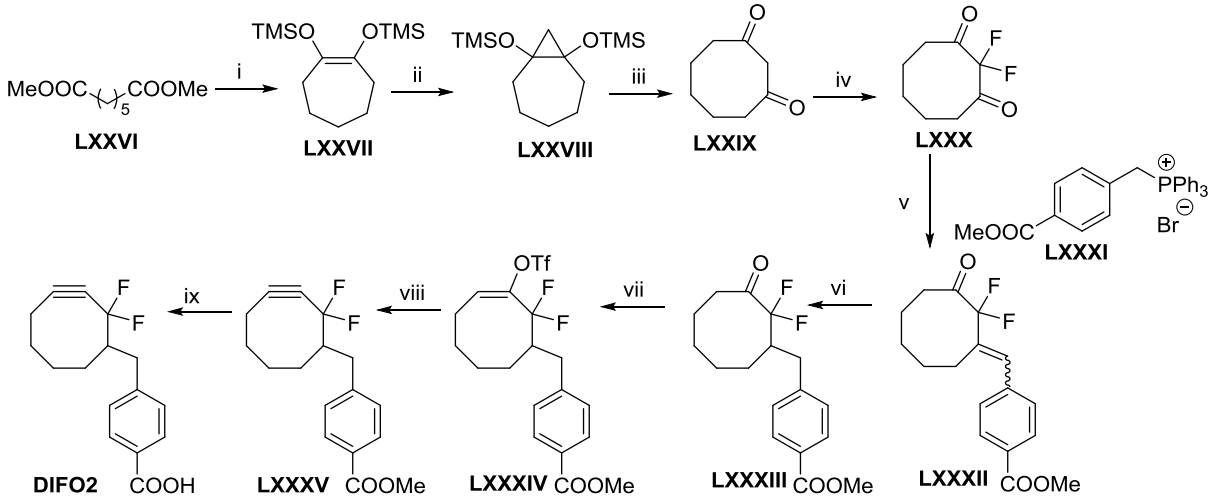


[*a*]Reagent: (i) NaH, allylbromide, rt (ii) PCC, rt (iii) LHDMS, -78 °C; Et_3SiCl (iv) Selectfluor, rt (v) cat. LHDMS, rt (vi) KHDMS, -78 °C; Et_3SiCl (vii) Selectfluor, rt (viii) $RuCl_3$, $NaIO_4$, rt (ix) KHDMS, -78 °C; Tf_2NPh (x) LDA, 0 °C.

A year later, Bertozzi group reported the second-generation **DIFO** reagents (**DIFO2** and **DIFO3**) with improved synthesis overcoming low overall yield of **DIFO** synthesis and problems with decomposition during the final steps.[17] Synthesis of **DIFO2** began with 1,3-cyclooctanedione **LXXIX**, which was prepared according to Webster and Pirrung (Scheme XVII).[62] In the first step, 1,3-cyclooctanedione **LXXIX** was difluorinated with Selectfluor and Cs_2CO_3.[17] Difluoro-1,3-cyclooctanedione **LXXX** underwent Wittig reaction using phosphonium salt **LXXXI** and DBU. Hydrogenation of the olefin to saturated compounds **LXXXIII** and subsequent transformation of ketone to vinyl triflate led to compound **LXXXIV**. **DIFO2** was finally formed by LDA mediated elimination followed by

methyl ester saponification. Overall yield of the synthesis starting from 1,3-cyclooctanedione was 36%.[17]

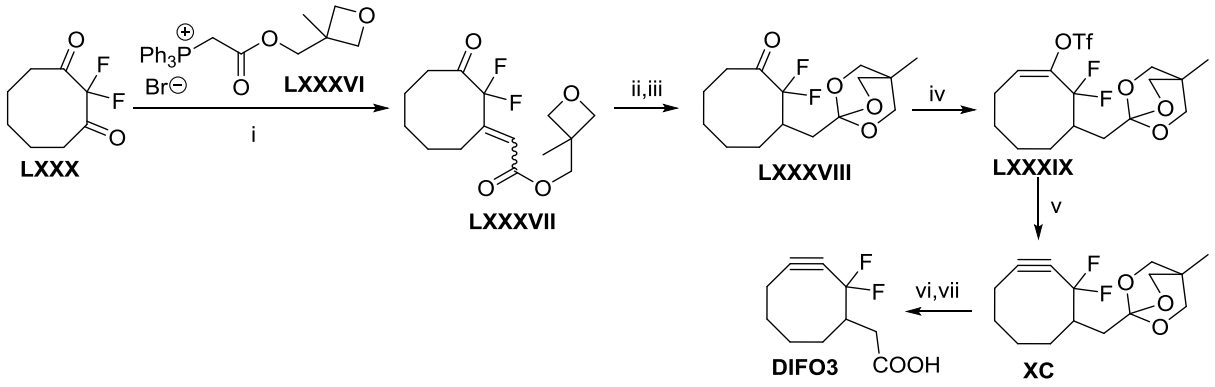
Scheme XVII: Preparation of **DIFO2**.[17,a]



[a]Reagent: (i) Na, TMSCl, reflux (ii) CH_2I_2, Et_2Zn, -10 °C to rt (iii) H_5IO_6, -10 °C (iv) Selectfluor, Cs_2CO_3, rt (v) DBU, THF, 0 °C to rt (vi) H_2, Pd/C, rt (vii) KHDMS, -78 °C; Tf_2NPh (viii) LDA, -20 °C (ix) LiOH, 55 °C.

In case of **DIFO3**, difluoro-1,3-cyclooctanedione **LXXX** reacted with phosphonium salt **LXXXVI** (Scheme XVIII).[17] Hydrogenation of olefin and transformation of oxetane ester to oxabicycloortho (OBO) ester using $BF_3.OEt_2$ resulted in ketone **LXXXVIII** (Scheme XVIII).[17] **LXXXVIII** underwent formation and elimination of vinyltriflate to form cyclooctyne **XC**. Two-step deprotection of the OBO ester yielded **DIFO3** with an overall yield of 28% from 1,3-cyclooctanedione (Scheme XVIII).[17]

Scheme XVIII: Preparation of **DIFO3**.[17,a]



[a]Reagent: (i) DBU, DCM, 0 °C to rt (ii) H_2, Pd/C, rt (iii) $BF_3.Et_2O$, 0 °C to rt (iv) KHDMS, -78 °C; Tf_2NPh (v) LDA, -20 °C (vi) PPTS, rt (vii) LiOH, rt.

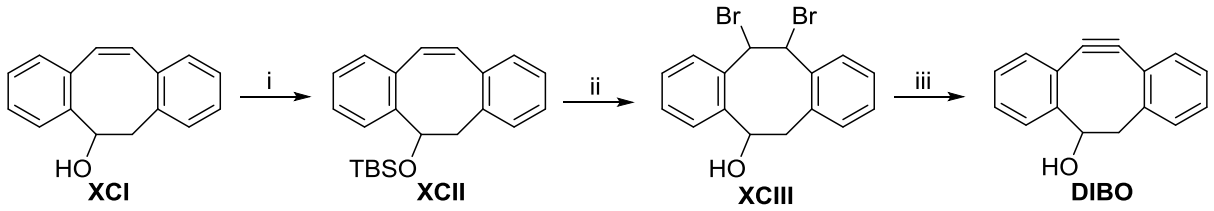
The main disadvantage of the synthesis of **DIFO2** and **DIFO3** is complicated producing of large amount of key synthetic intermediate 1,3-cyclooctanedione **LXXIX**.[63]

2.1.2.4. Dibenzocyclooctynes

DIBO

In 2008, Boons and co-workers introduced 4-dibenzocyclooctynol **DIBO**, which can be easily prepared from 3-hydroxy-1,2:5,6-dibenzocycloocta-1,5,7-triene **XCI** (Scheme XIX). After protection of hydroxyl group by TBS, bromination provided dibromide **XCIII**.[18] The TBS protecting group was removed during bromination, but the bromination of free alcohol provided low yields. Dehydrobromination of **XCIII** by LDA gave the **DIBO** in 27% overall yield.[18]

Scheme XIX: Preparation of **DIBO**.[18,*a*]



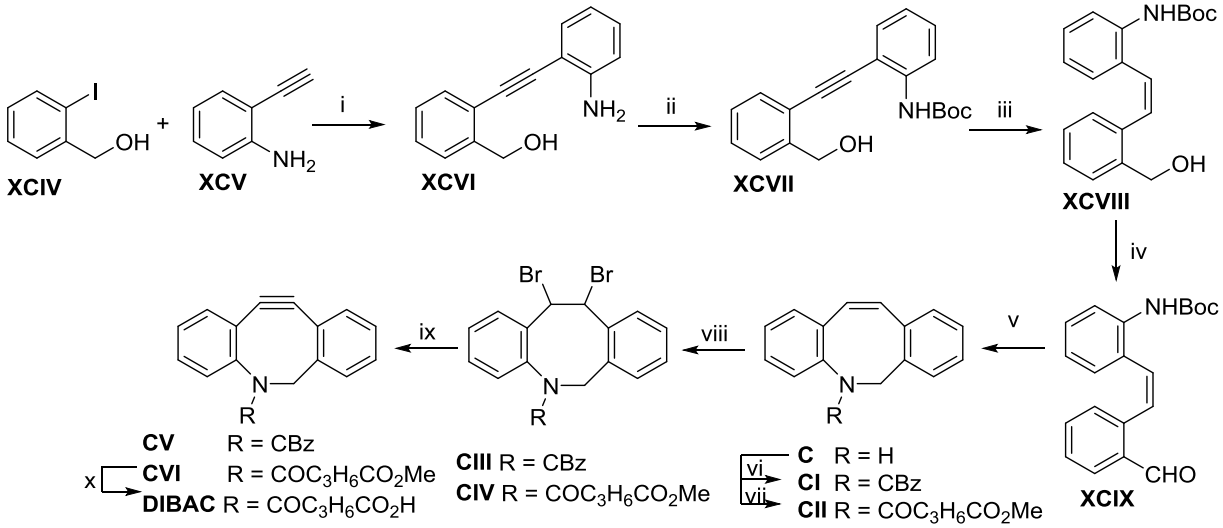
[*a*]Reagent: (i) TBSCl, pyridine, rt (ii) Br_2, $CHCl_3$, rt (iii) LDA, THF, rt.

DIBAC

In 2009, van Delft and co-workers designed aza-dibenzocyclooctyne **DIBAC** as a combination of favourable kinetics of **DIBO** and hydrophilicity of **DIMAC** (see above).[19] The synthesis of key intermediate dihydrodibenzoazocine **C** started by Sonogashira cross-coupling to product **XCVI** followed by Boc-protection (Scheme 20).[19] Partial hydrogenation resulted in compound **XCVIII**, which was then oxidized under Dess-Martin conditions to aldehyde **XCIX** (Scheme XX). Boc-deprotection under acidic condition led to formation of cyclic imine, which was reduced by $NaBH_4$ to secondary amine **C**. Unfortunately, direct bromination of **C** resulted in the intramolecular substitution of bromine, leading to indoline, thus CBz protecting group was introduced.[19] Consequent bromination and elimination with use of *t*BuOK resulted in compound **CV** (Scheme XX). Unfortunately, deprotection of CBz group using either acidic or basic conditions did not yield the free amine. Instead, 6*H*-isoindolo[2,1-*a*]indol (Figure XI) was formed.[19] Due to this fact, **C** was substituted by small linker leading to compound **CII** (Scheme XX). Subsequent bromination

and elimination resulted in alkyne **CVI** followed by hydrolysis of methylester to final **DIBAC** in overall yield of 41%.[19]

Scheme XX: Preparation of **DIBAC**.[19,*a*]



[*a*]Reagent: (i) $PdCl_2(PPh_3)_2$, CuI, Et_3N, THF, N_2/H_2 atmosphere, rt (ii) Boc_2O, THF, 70 °C (iii) 10% $Pd/BaSO_4$, quinolone, H_2, MeOH, rt (iv) Dess-Martin periodinane, $NaHCO_3$, DCM, rt (v) (1) 2M HCl in EtOAc, rt; (2) $NaBH_4$, H_2O, rt (vi) CBzCl, Na_2CO_3, H_2O, DCM, rt (vii) $ClCOC_3H_6COOMe$, Et_3N, DCM, rt (viii) Br_2, DCM, 0 °C (ix) *t*BuOK, THF, 0 °C to rt (CIX), -40 °C (CX) (x) LiOH, THF, H_2O, rt.

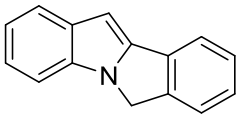
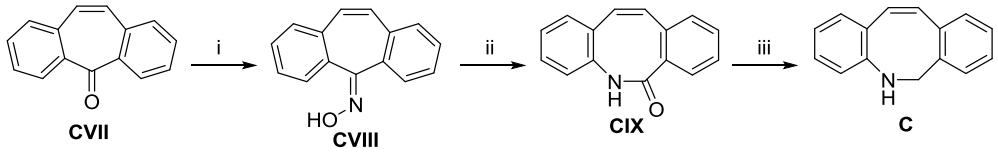


Figure XI: 6*H*-isoindolo[2,1-*a*]indol.

The key intermediate dihydrodibenzoazocine **C** can be also prepared by alternative way as described by Tsourkas et. al. (Scheme XXI).[64] In this method, dibenzosuberone derivative **CVII** was converted into corresponding oxime **CVIII** followed by its rearrangement to amide **CIX** (Scheme XXI). The amide **CIX** was then reduced to secondary amine **C** which was obtained with 25% overall yield after three steps.[64]

Scheme XXI: Preparation of dihydrodibenzoazocine.[64,*a*]

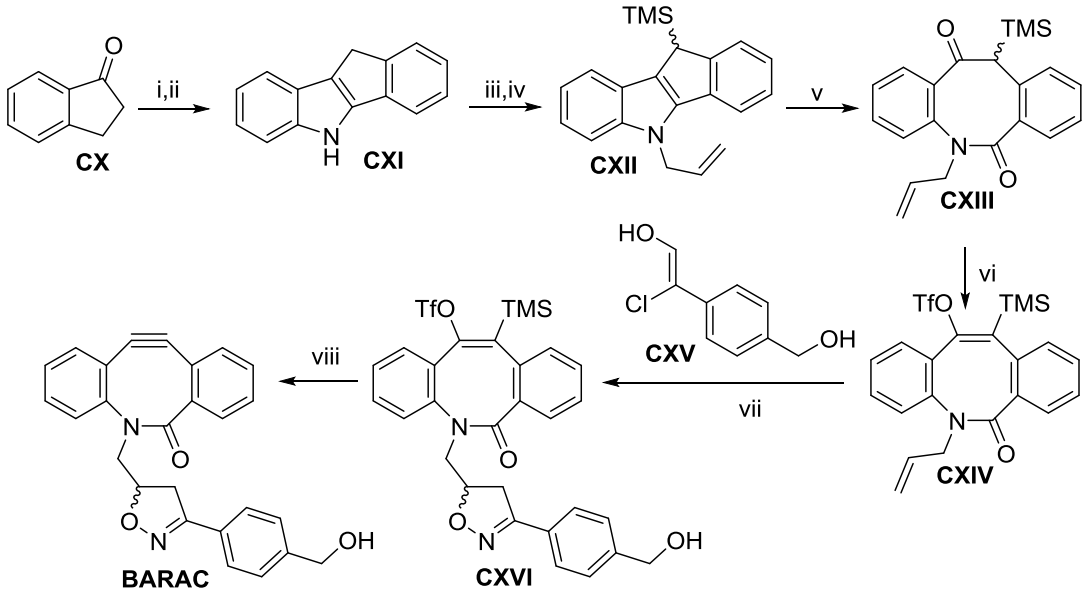


[*a*]Reagent: (i) $NH_2OH.HCl$, pyridine, rt (ii) PPA, 125 °C (iii) $LiAlH_4$, diethylether, reflux.

BARAC

Biarylazacyclooctynone (**BARAC**) was firstly prepared by Bertozzi group in 2010.[20] The preparation of **BARAC** started by Fischer indol synthesis from 1-indanone **CX** and phenyl hydrazine (Scheme XXII).[20] Resulting indole **CXI** was alkylated followed by installation of TMS group to obtain compound **CXII**. Oxidation of derivative **CXII** with *m*-CPBA led to opening of central rings to form cyclic keto-amide **CXIII**, which underwent formation of enol triflate **CXIV**.[20] In the next step, double bond is reacted with enol **CXIV** to obtain compound **CXVI**. Reaction of derivative **CXVI** with CsF resulted in final **BARAC** in 18% overall yield.[20]

Scheme XXII: Preparation of **BARAC**.[20,*a*]

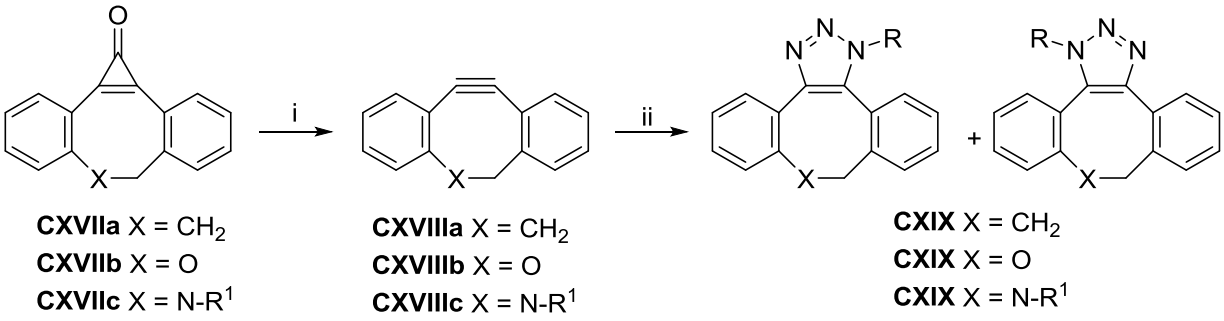


[*a*]Reagent: (i) $PhNHNH_2$, EtOH, AcOH, rt (ii) 12M HCl, reflux (iii) allyl bromide, KOH, TBABr, H_2O, toluene, rt (iv) *n*-BuLi, Et_2O, TMSCl, rt (v) *m*-CPBA, $NaHCO_3$, DCM, rt (vi) KHDMS, Tf_2O, THF, rt (vii) Et_3N, DCM (viii) CsF, MeCN, rt.

Photo-click

In 2003, Popik and co-workers demonstrated, that triple bond can be generated by photolysis of cyclopropenone.[65] They extended their study and introduced **DIBO**[66] and its oxa- and aza- analogues in which triple bond is replaced by cyclopropanone moiety (Scheme XXIII).[67] These compounds are thermally stable and do not react with organic azides. Irradiation of cyclopropenone **CXVIIa-c** at 350 nm results in the loss of carbon monooxide and formation of dibenzocyclooctynes **CXVIIIa-c**, which then undergo catalyst-free addition to azides (Scheme XXIII).[67]

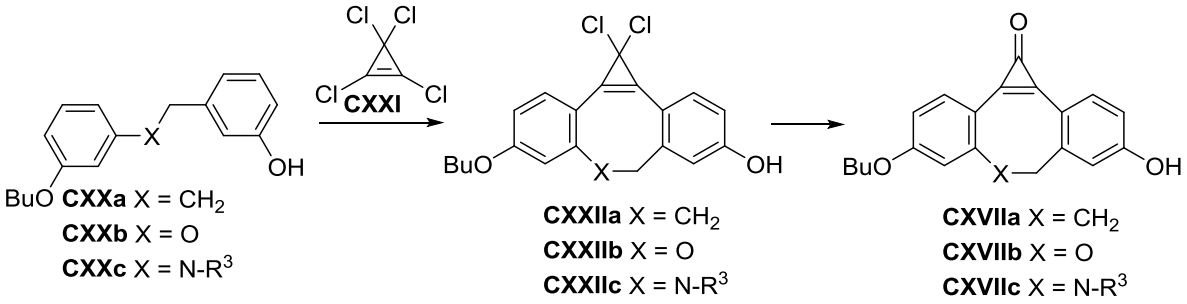
Scheme XXIII: Photoinduced formation of triple bond from cyclopropenone.[67,a]



[a]Reagent: (i) hν, 350 nm, rt (ii) R-N_3, rt.

Preparation of cyclooctynes required two step procedure started from appropriately substituted diphenylethane **CXXa**, *N*-phenyl-*N*-benzyl amide **CXXb** or phenyl benzyl ether **CXXc** (Scheme XXIV).[67] **CXXa-c** underwent double Friedel-Crafts alkylation with tetrachlorocyclopropene **CXXI** to obtained corresponding dichlorocyclopropene **CXXIIa-c**. Controlled hydrolysis of dichlorides resulted in target cyclopropanones **CXVIIa-c** in 20-67% overall yield (Scheme XXIV).[67]

Scheme XXIV: Formation of cyclopropenones.[67,a]



[a]Reagent: (i) $AlCl_3$, rt (ii) H_2O, rt.

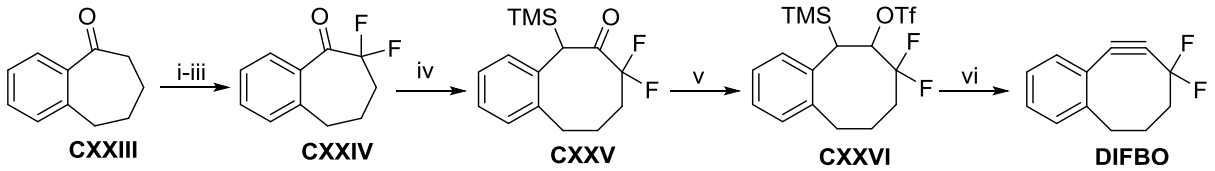
2.1.2.5. Benzocyclooctynes

DIFBO

Difluorobenzocyclooctyne **DIFBO** was designed by Bertozzi group as a combination of rate-enhancing factors of **DIFO** and **DIBO**.[22] The **DIFBO** was synthesized by four-step procedure started from reaction of benzosuberone **CXXIII** with hexylamine (Scheme XXV).[22] The resulting imine was treated with Selectfluor to obtain difluorobenzosuberone **CXXIV**. Difluorobenzosuberone then underwent reaction with trimethylsilyl diazomethane and trimethylaluminium to get compound **CXXV**, which was deprotonated by KHDMS followed by formation of enol triflate **CXXVI** (Scheme XXV).[22]

This intermediate was converted to **DIFBO** by reaction with caesium fluoride. However, the desired product could not be isolated in pure form because the spontaneous homotrimerization of **DIFBO**. For this reason, **DIFBO** was finally trapped by forming a stable inclusion complex with β-cyclodextrin.[22]

Scheme XXV: Formation of **DIFBO**.[22,*a*]

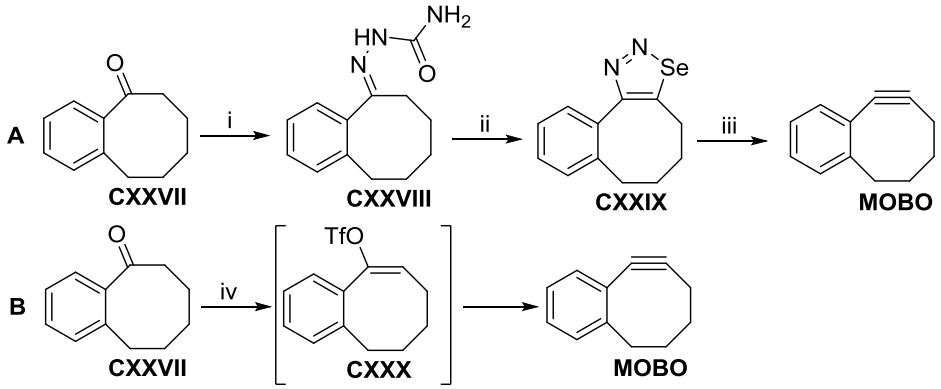


[*a*]Reagent: (i) Hexylamine, cyclohexane, TFA, reflux (ii) Selectfluor, MeCN, Na_2SO_4, reflux (iii) HCl, reflux (iv) $AlMe_3$, $TMSCHN_2$, DCM, -78 °C (v) KHDMS, Tf_2O, THF, -78 °C to -45 °C (vi) CsF, MeCN, rt.

MOBO

Non-fluorinated benzocyclooctyne **MOBO** was utilized by Bertozzi group to determine relative effect of fluorination to the reactivity of **DIFBO**.[22] **MOBO** was firstly prepared by thermolysis of corresponding 1,2,3-selenadiazole **CXXIX** (Scheme XXVI, eq. A) by Meier and co-workers in 1974.[68,69] However, the yield of reaction was low and by-products were formed.[68,69] Bertozzi group then prepared **MOBO** by syn elimination of an enol triflate **CXXX** in 33% yield (Scheme XXVI, eq. B).[22]

Scheme XXVI: Formation of **MOBO**.[22,68,69,*a*]



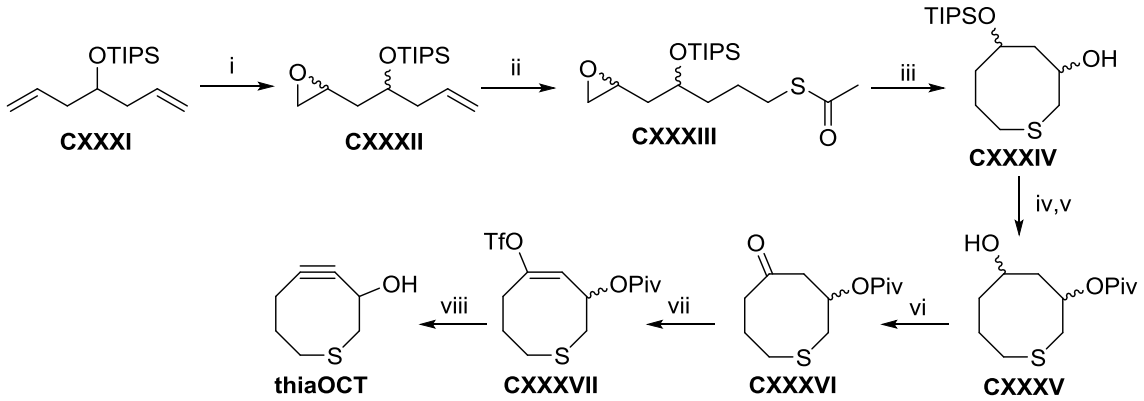
[*a*]Reagent: (i) $NH_2NHCONH_2$, rt (ii) SeO_2, dioxane, 40-50 °C (iii) 190 °C, glass powder (iv) KHDMS, *N*-phenyl bis(trifluormethanesulfonimide), THF, -78 °C to 0 °C.

2.1.2.6. Thiacycloalkynes

thiaOCT

ThiaOCT, the sulphur containing analogue of **OCT** was synthesized by Bertozzi and co-workers to assess the effect of endocyclic sulphur atom on cyclootyne reactivity.[24] The eight-step synthesis started by epoxidation of diene **CXXXI** to monoalkene **CXXXII** followed by thiol-ene reaction with thioacetic acid to give compound **CXXXIII** (Scheme XXVII).[24] Treatment of **CXXXIII** with sodium hydride led to the sulphur deprotection and subsequent cyclization to compound **CXXXIV**. Then free hydroxyl group was protected by pivaloyl and TIPS group was removed. Following oxidation afforded ketone **CXXXVI** (Scheme XXVII). **ThiaOCT** was then obtained by *syn* elimination of vinyl triflate **CXXXVII** in 5% overall yield.[24]

Scheme XXVII: Formation of **thiaOCT**.[24,*a*]

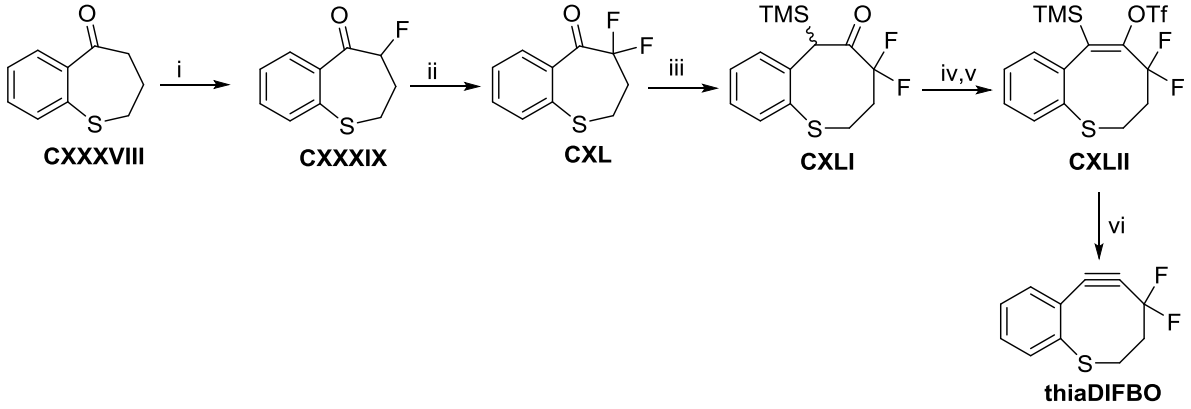


[*a*]Reagent: (i) DMDO, $NaHCO_3$, H_2O, 0 °C to rt (ii) thioacetic acid, AIBN, DCE, 70 °C (iii) NaH, EtOH, 80 °C (iv) PivCl, pyridine, 35 °C (v) TBAF, THF, 0 °C to rt (vi) Dess-Martin periodinane, DCM, H_2O, rt (vii) NaHMDS, Tf_2O, THF, -78 °C (viii) LDA, THF, 0 °C.

thiaDIFBO

After successful synthesis of **thiaOCT**, Bertozzi group also tested the effect of sulphur atom on stability of **DIFBO**.[24] **ThiaDIFBO** was prepared similarly to **DIFBO** (Scheme XXV) in 3% overall yield (Scheme XXVIII).[24] Its main advantage is that unlike **DIFBO**, **thiaDIFBO** did not oligomerize during storage.[24]

Scheme XXVIII: Formation of **thiaDIFBO**.[24,a]

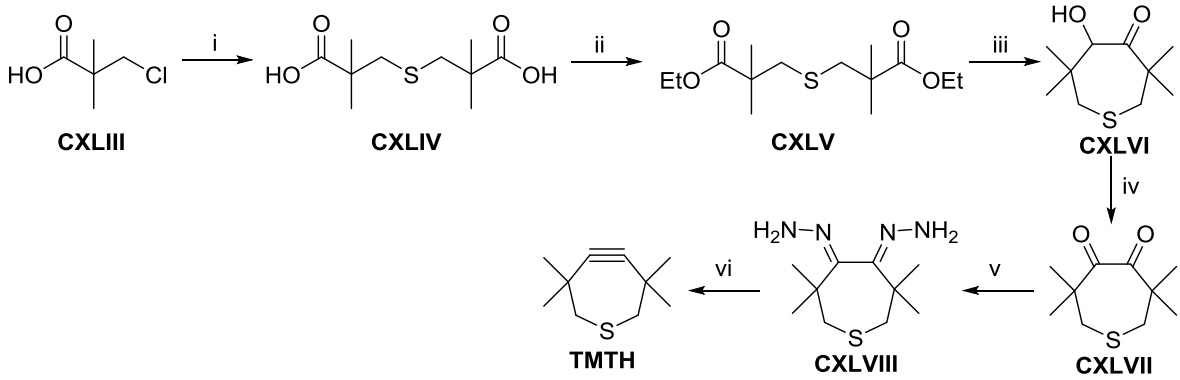


[a]Reagent: (i) a) LDA, - 78 °C to 0 °C; b) NFSI, - 78 °C (ii) a) LDA, - 78 °C to 0 °C; b) NFSI, - 78 °C (iii) $AlMe_3$, $TMSCHN_2$, 0 °C (iv) NaHDMS, - 78 °C (v) Tf_2O, - 78 °C to rt (vi) CsF, rt.

TMTH

3,3,6,6-tetramethylthiacycloheptyne **TMTH** was firstly reported by Krebs and Kimling in 1970 as a stable cyclooalkyne, which is able to react with phenylazide although the rate constant for this reaction was not determined.[70] Due to this fact, Bertozzi group prepared **TMTH** similarly to procedure described previously (Scheme XXIX) in overall yield below 1% and assed reaction of **TMTH** with phenylazide as the fastest cycloalkyne-azide cycloaddition reported to date.[24]

Scheme XXIX: Formation of **TMTH**.[24,a]



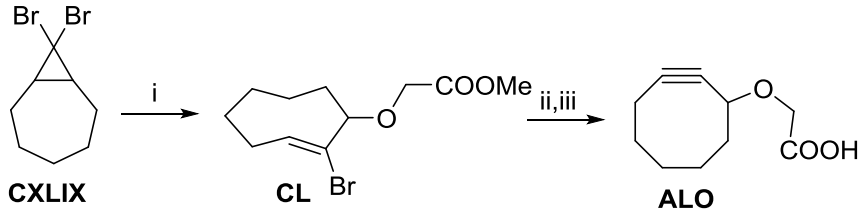
[a]Reagent: (i) Na_2S, Na_2CO_3, H_2O, rt (ii) EtOH, H_2SO_4, 90 °C (iii) Na, *m*-Xylene, reflux (iv) DMSO, oxalyl chloride, Et_3N, - 78 °C to 0 °C (v) anhydrous N_2H_4, $H_5N_2HSO_4$, ethylene glycol, 150 °C (vi) $Pb(OAc)_4$, DCM, 0 °C.

2.1.2.7. Cycloalkynes with improved polarity

ALO

Aryl-less cyclooctyne analogue of **OCT** termed **ALO** was introduced by Bertozzi and co-workers in 2006.[15] Cyclopropane ring in compound **CXLIX** was opened by treatment of silver perchlorate to obtain E-bromocyclooctene **CL**, which then underwent one-pot elimination and saponification to get desired **ALO** in 51% overall yield (Scheme XXX).[15]

Scheme XXX: Formation of **ALO**.[15,*a*]

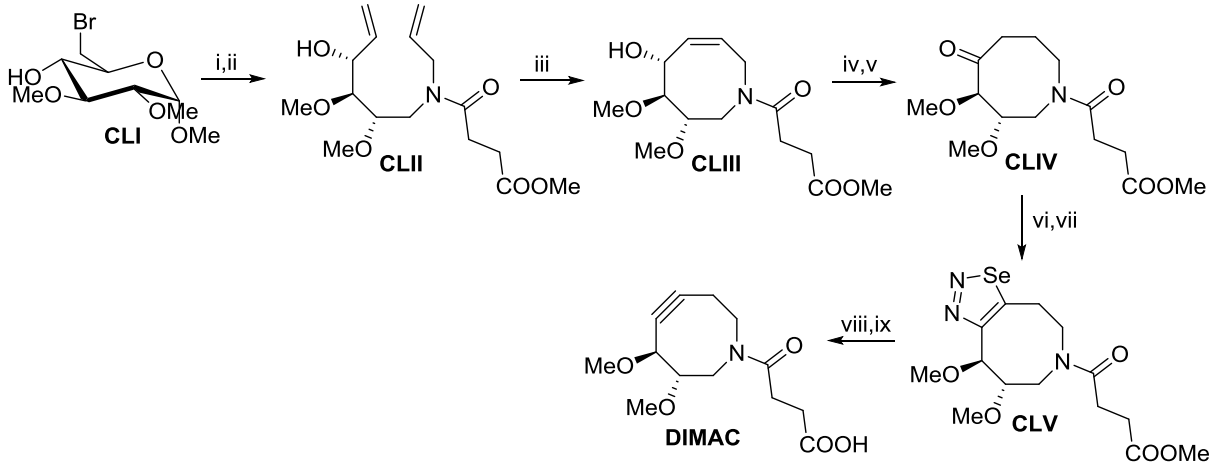


[*a*]Reagent: (i) $AgClO_4$, methyl glycolate, rt (ii) NaOMe, DMSO, 0 °C (iii) H_2O, rt.

DIMAC

Incorporation of nitrogen to cyclooctyne systems resulted in preparation of **DIMAC** by Bertozzi and co-workers in 2008.[26] Two methoxy groups then enhance polarity of the compound. The preparation of **DIMAC** consists from nine steps synthesis beginning from 6-bromglucopyranoside **CLI**, which was transformed to acyclic diene **CLII** (Scheme XXXI).[26]

Scheme XXXI: Formation of **DIMAC**.[26,*a*]



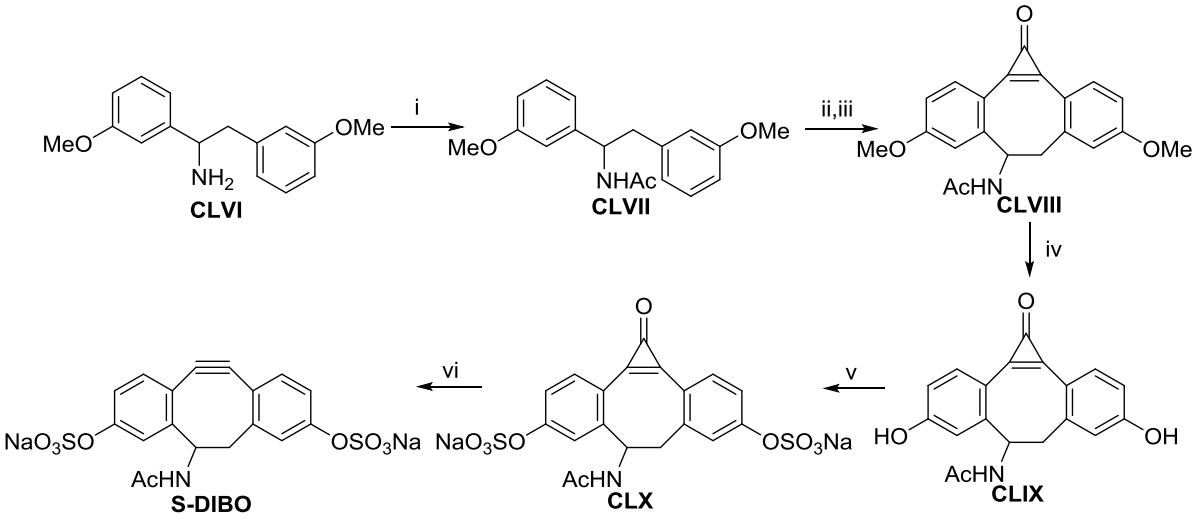
[*a*]Reagent: (i) Zn, $NaBH_3CN$, prop-2-en-1-amine, 1-propanole/H_2O 19:1, 90 °C (ii) methyl succinyl chloride, DIEA, rt (iii) Grubs II, DCM, reflux (iv) PCC, DCM, 40 °C (v) H_2, Pd/C, EtOH, rt (vi) semicarbazide hydrochloride, AcOH, aniline, EtOH/H_2O 1:1, rt (vii) SeO_2, dioxane/H_2O 1:1, rt (viii) *m*-Xylene, 115 °C (ix) LiOH, dioxane/H_2O 1:2, rt.

The eight membered ring was generated by a Grubs ring-closing metathesis. Alcohol **CLIII** was then converted to ketone **CLIV** followed by two-steps formation of 1,2,3-selenadiazole **CLV** (Scheme XXXI). Thermal decomposition of **CLV** followed by saponification resulted in **DIMAC** in 6% overall yield.[26]

S-DIBO

In 2012, Boons and co-workers developed the highly polar sulphated analogue of **DIBO** termed **S-DIBO** with excellent stability under moderately acidic and basic conditions.[27] The synthesis of **S-DIBO** started by Friedel-Crafts alkylation of **CLVII** with tetrachlorocyclopropene (Scheme XXXII). Cyclopropenone **CLVIII** then underwent methoxy groups deprotection followed by sulfatation to get cyclopropenone **CLX**. Photochemical decarbonylation resulted in formation of desired **S-DIBO** in 13% overall yield (Scheme XXXII).[27]

Scheme XXXII: Formation of **S-DIBO**.[27,*a*]



[*a*]Reagent: (i) Ac_2O, DIEA, 0 °C to rt (ii) perchlorocycloprop-1-en, $AlCl_3$, DCM, -20 °C to rt (iii) H_2O (iv) BBr_3, DCM, -78 °C to rt (v) SO_3.py, DMF, then Na^+ resin (vi) hν, 350 nm, H_2O.

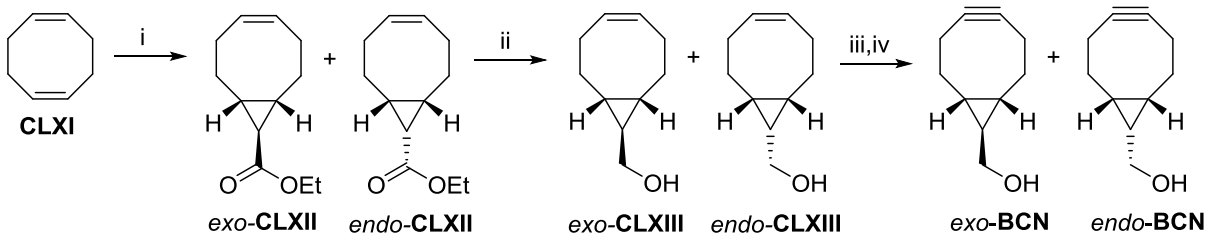
2.1.2.8. The others

BCN

Bicyclo[6.1.0]nonyne **BCN** was firstly described by van Delft and co-workers in 2010.[21] The synthesis of **BCN** started by reaction of ethyldiazoacetate with 1,5-cyclooctadiene in presence of rhodium acetate (Scheme XXXIII).[21] The resulting mixture of *exo*-**CLXII** and *endo*-**CLXII** was separated and both isomers were converted to

corresponding hydroxyalkynes **CLXIII** followed by bromination and elimination to get *endo*- and *exo*-**BCN** in overall yield 61% or 53% respectively (Scheme XXXIII).[21]

Scheme XXXIII: Formation of **BCN**.[21,*a*]

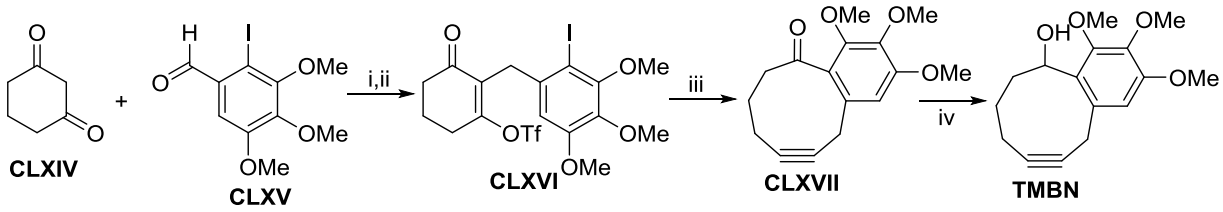


[*a*]Reagent: (i) ethyl diazoacetate, $Rh(OAc)_2$, DCM, 0 °C (ii) $LiAlH_4$, Et_2O, 0 °C (iii) Br_2, DCM, rt (iv) KO*t*Bu, THF, 0 °C to reflux.

TMBN

In 2012, Dudley and co-workers prepared benzocyclononyne **TMBN**, which synthesis started by preparation of acyl triflate **CLXVI** followed by tandem cyclization /ring expansion initiated by iodine-lithium exchange (Scheme XXXIV). **TMBN** was then obtained after reduction of ketone in 35% overall yield.[23]

Scheme XXXIV: Formation of **TMBN**.[23,*a*]

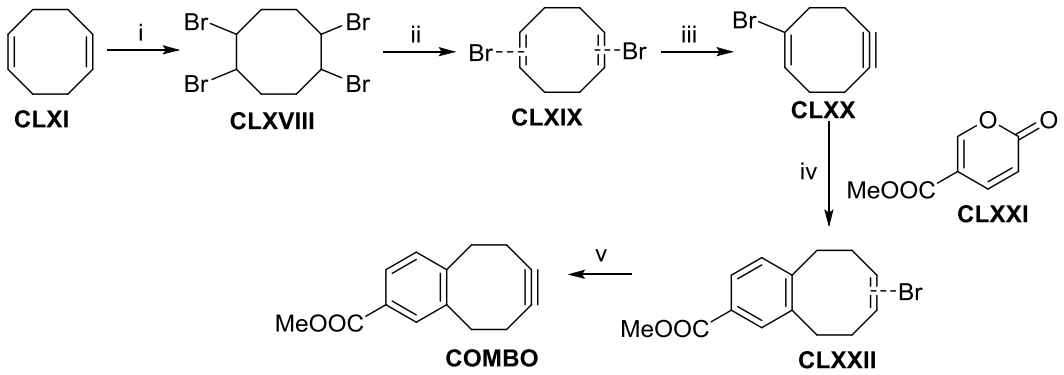


[*a*]Reagent: (i) L-proline, EtOH; then $NaBH_3CN$, 3A MS, 85 °C (ii) Tf_2O, DCM, -78 °C to rt (iii) *n*-BuLi, HMPA, -78 °C to rt (iv) DIBAL-H, DCM, rt.

COMBO

Carboxymethylmonobenzocyclooctyne **COMBO** was reported by group of Péter Kele in 2012 as non-fluorinated copper free click reagent with excellent kinetics.[29] **COMBO** was prepared by five step procedure (Scheme XXXV) started by tetrabromination of cyclooctadiene followed by partial elimination to dibromide **CLXIX**, which underwent further elimination resulting in eneyne **CLXX**.[29] **CLXX** was then subjected to inverse-electron-demand Diels-Alder reaction to afford compound **CLXXII**. Elimination of HBr from **CLXXII** resulted in **COMBO** in 12% overall yield.[29]

Scheme XXXV: Formation of **COMBO**.[29,*a*]

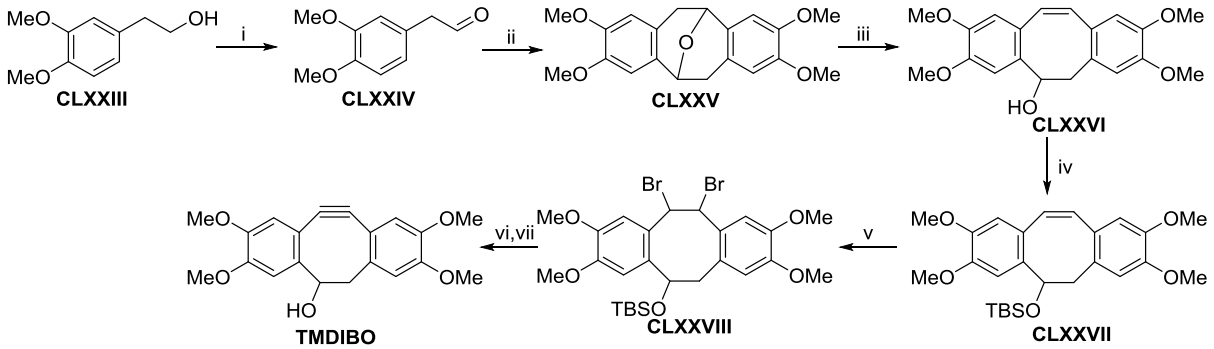


[*a*]Reagent: (i) Br_2, DCM, 0 °C (ii) *t*BuOK, Et_2O, rt (iii) *t*BuOK, 18-crown-6 ether, hexane, rt (iv) rt (v) *t*BuOK, 18-crown-6 ether, hexane, 58 °C.

TMDIBO

In 2011, Leeper and co-workers described the high yielding synthesis of tetramethoxydibenzocyclooctyne **TMDIBO**, which exhibited good stability to the set of conditions (air, acid, base, thiols).[28] Its synthesis started by oxidation of homoveratryl alcohol to aldehyde **CLXXIV**, which then underwent dimerization to compound **CLXXV** (Scheme XXXVI).[28] Ether **CLXXV** was treated with *n*-BuLi to get dibenzocyclooctanole **CLXXVI**. Free hydroxyl group was then protected to enable bromination of double bond. Desired **TMDIBO** was obtained after dehydrobromination and subsequent TBS deprotection in 57% overall yield (Scheme XXXVI).[28]

Scheme XXXVI: Formation of **TMDIBO**.[28,*a*]



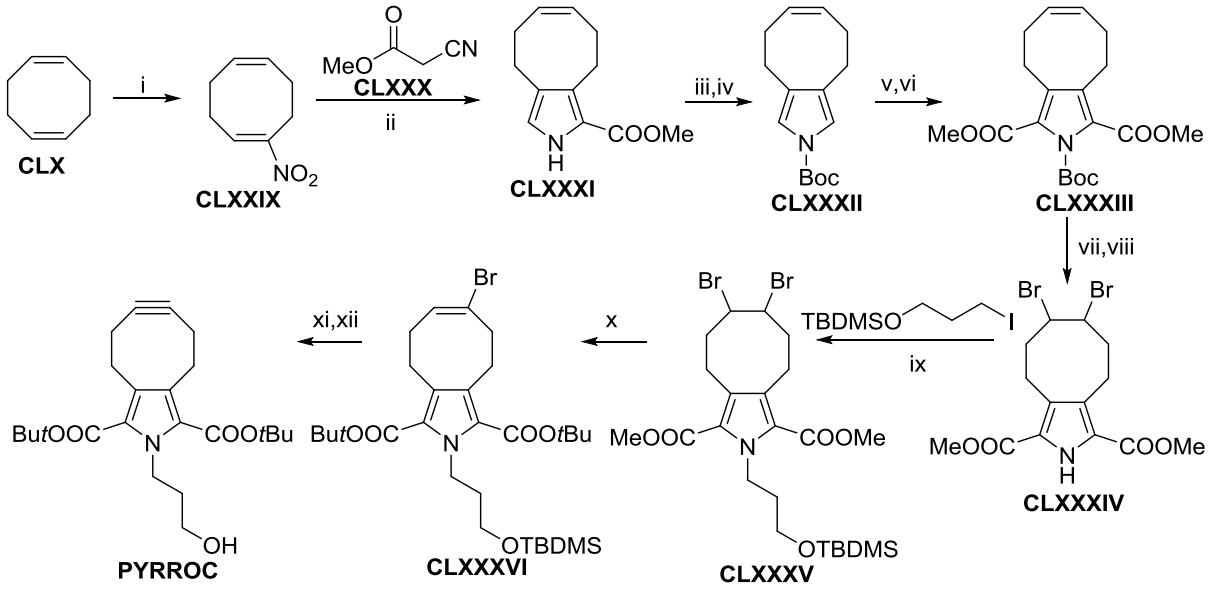
[*a*]Reagent: (i) Dess-Martin periodinane, DCM, rt (ii) TMSI, DCM, -78 °C (iii) n-BuLi, THF, rt (iv) TBSCl, pyridine, DCM, rt (v) Br_2, $CHCl_3$, -40 °C (vi) *t*BuOK, *N*-methylpiperazine, THF, rt (vii) TBAF, THF, 0 °C.

PYRROC

In 2015, Greg and Gröst reported the newest cyclooctyne **PYRROC**, main advantage of which lies in formation of only one isomer in the reaction with azides.[30] The synthesis of pyrrolocyclooctyne started from nitration of cyclooctadiene followed by Barton-Zang reaction

yielding pyrrolocyclooctene **CLXXXI** (Scheme XXXVII).[30] Saponification, decarboxylation and *N*-Boc protection get **CLXXXII**, which was then converted to diester **CLXXXIII**. Bromination and Boc deprotection resulted in compound **CLXXXIV**, which underwent alkylation to dibromide **CLXXXV**. **PYRROC** was received after two-step elimination and TBDSM deprotection in 3% overall yield (Scheme XXXVII).[30]

Scheme XXXVII: Formation of **PYRROC**.[30,*a*]



[*a*]Reagent: (i) $AgNO_2$, TEMPO, $CHCl_3$, rt (ii) DBU, THF, reflux (iii) NaOH, ethylene glycol, 195 °C (iv) Et_3N, DMAP, Boc_2O, THF, rt (v) TMP, *n*BuLi, -78 °C (vi) ClCOOMe, THF, -78 °C (vii) Br_2, DCM, -78 °C to rt (viii) TFA, DCM, rt (ix) K_2CO_3, 80 °C (x) 1M tBuOK in THF, Et_2O, -10 °C to 10 °C (xi) *t*BuOK, 18-crown-6, hexane, 55 °C (xii) 1M TBAF in THF, rt.

2.1.3. Application of SPAAC

2.1.3.1. Chemical-biology applications

Many functional groups have been already used in chemical biology including ketones, organic azides and alkynes.[71] Organic azides are the most suitable for biological applications because azido group is abiotic and nontoxic in animals and can be selectively derivatized under physiological conditions with cyclooctynes.[71]

2.1.3.1.1. Cellular imaging

The use of strain-promoted azide alkyne cycloaddition in chemical biology was firstly demonstrated by Bertozzi´s group in 2004.[5] They prepared biotinylated **OCT**, which was used for labelling of glycans with incorporated *N*-azidoacetyl sialic acid (SiaNAz).[5] Then, they also investigated the utility of SPAAC for live cell labelling. Jurkat cells were incubated with

SiaNAz, which was introduced to their cell-surface glycoproteins and reacted with biotinylated **OCT**. The cells were then labelled with avidin bearing fluorescein (FITC-avidin) and analysed by flow cytometry.[5]

Three years later, the same group reported *in-vivo* labelling of azido-glycans in mice.[16] Mice were injected with peracetylated *N*-azidoacetylmannosamine (Ac_4ManNAz) and then with **DIFO**-FLAG. Flow cytometry confirmed selective labelling inside a living mouse.[16] Later they extended their study also on other reagents including **OCT**, **ALO**, **DIBAC** and **MOFO**.[72] **DIFO** was finally identified as the most efficient reagent for labelling of serum or tissue-resident glycoproteins.[72]

Bertozzi and co-workers were focused on visualization of glycans as attractive targets because the cell-surface glycans form a rich source of information about cell physiological state.[73] They demonstrated imaging of glycans in live developing zebrafish with incorporated peracetylated *N*-azidoacetylgalactosamine (Ac_4GalNAz).[73] Labelling was performed with **DIFO**-AlexaFluor647 conjugate (Figure XII).[73]

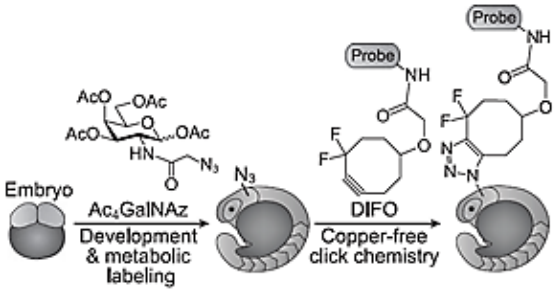


Figure XII: Schematic depicting the use of metabolic labelling with Ac_4GalNAz and SPAAC using **DIFO** probes for the non-invasive imaging of glycans during zebrafish development.[73]

The metabolic imaging approach was then extended to image sialic acids in this model organism.[74] Zebrafish embryos were incubated with Ac_4ManNAz, reacted with **DIFO**-AlexaFluor488 and visualised by confocal microscopy.[74]

In 2008, Boons and co-workers reported synthesis of **DIBO** and its application in labelling of Jurkat cells, which were cultured with Ac_4ManNAz to introduce SiaNAz into glycoproteins.[18] The cells were then labelled with biotinylated **DIBO** and stained with FITC-avidin.[18] The similar experiment was performed also with living cells of Chinese hamster ovary (CHO) labelled with avidin-AlexaFluor488.[18]

In 2012, Bests‘ group introduced the use of biotinylated **DIMAC** for labelling of membrane bilayers.[75] The strategy involved preparation of the azido-lipid conjugate presented on liposomes, which can be labelled via cyclooctyne reagent.[75]

In 2014, van Delft and co-workers reported new set of **BCN** derived structures with enhanced hydrophilicity, retained reactivity and high labelling specifity.[76] All derivatives were tested for cellular staining of metabolically labelled azidoglycans.[76] HEK293 cells were incubated with Ac_4ManNAz, then with biotinylated **BCN** probes and stained with streptavidin-AlexaFluor488.[76]

SPAAC was also used for visualization of plant cell. Ralph and co-workers in 2014 published the utilization of click chemistry for visualization of plant cell wall lignification, crucial biological process that terrestrial plants undertake throughout various tissues.[77] Cut stems from *Arabidopsis thaliana* were incubated with azidomodified coniferyl alcohol followed by reaction with **DIBO**-AlexaFluor488. Intense fluorescent signal was detected in areas, where lignins are typically produced.[77]

Recently, group of Cho and Kim reported the use of **DIBAC**-650 for labelling and tracking of chondrocytes *in vivo*.[78] Chondrocytes were incubated with Ac_4ManNAz to generate azido groups on the surface of the cells followed by treatment with **DIBAC**-650 (Figure XIII).[78] For the *in-vivo* cell tracking, DIBAC-650-labeled chondrocytes were implanted into mice and were effectively tracked using NIRF imaging technology.[78]

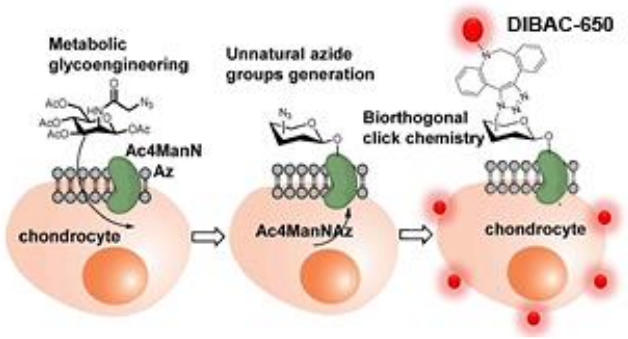


Figure XIII: Schematic illustration of metabolic glycoengineering and azide−alkyne click chemistry for labelling of chondrocytes.[78]

Tumor imaging/targeting

Early detection of cancer, which is the second major cause of death worldwide, is important factor of curability. Cancer diagnosis by molecular-imaging using aptamers, antibodies, peptides or nanocarrier is gaining increasing importance due to identification of cancer cell specific markers.[79] In 2014, Krishnakumar and co-workers reported the new system for cancer cells imaging based on SPAAC between fluorescent **DIBO**-AF594 and epithelial cell adhesion molecule (EpCAM) aptamer.[79] The conjugate showed target specific binding and imaging of the most tested cancer cell lines.[79]

In 2012 Kim and co-workers reported *in vivo* targeting strategy for nanoparticles with use of SPAAC.[80] They treated A549 human lung cancer cells with tetraacetyl *N*-azidoacetylmannosamine (Ac_4ManAz) followed by addition of nano-sized **DBCO** modified PEGylated liposomes bearing Cy5 fluorescent label to form the conjugate.[80] The similar approach was used also for *in vivo* labelling of A549 tumor cells in mice.[80] Later they extended this study to two steps tumor-targeting strategy consisting of intravenous injection of precursor-loaded glycol chitosan nanoparticles generated azido groups on tumor tissue by EPR effect (Figure XIV).[81] This resulted in enhance of the tumor-targeting ability of a second intravenous injection of **BCN** glycol-chitosan nanoparticles bearing photosensitizer chlorine e6 and increase in efficiency of photodynamic therapy *in vivo*.[81]

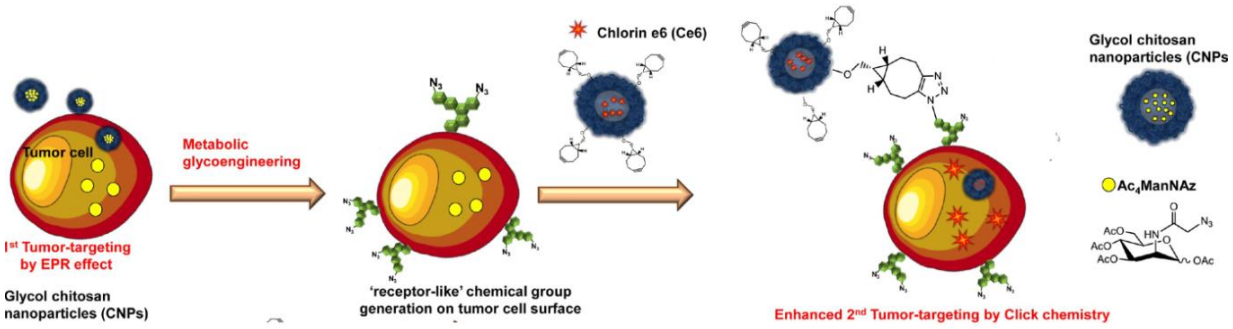


Figure XIV: Schematic illustration of the two-step *in vivo* tumor-targeting strategy for nanoparticles via metabolic glycoengineering and click chemistry.[81]

2.1.3.1.2 Nucleic acid modifications

DNA/RNA conjugates

Preparation of nucleic acid conjugates *via* SPAAC was firstly reported independently by groups of Manoharan[82] and Filipov[83] in 2010. Both authors were focused on solid phase preparation of **DIBO** modified RNA oligonucleotides.[82,83] The conjugation with azides through SPAAC was than performed in solution after oligonucleotide cleavage from solid support.[82,83] Conjugation reaction with nucleic acids directly on solid support was firstly described by Heaney and co-workers in 2011.[84] They prepared resin-supported DNA cyclootyne which was then successfully reacted with structurally different azides derived from biotin, coumarin, cholesterol and fluorescein with aim of potential application in cell biology and drug delievery.[84]

In 2012, Taton and co-workers utilized SPAAC for preparation of covalent protein-oligonucleotide conjugates which can be used in a variety of biological and biotechnological applications. The conjugates were prepared by the reaction between **DIBO** modified oligodeoxynucleotide and prenylazide-functionalized proteins.[85] Four years later, Schlierf and

Mukhortava reported two-step formation of site-specific proteins-DNA hybrids using SPAAC (Figure XV).[86] The first step included reaction of cysteine-engineered protein of interest (POI) with **DIBO**-maleimide (Figure XV). In the second step, **DIBO** was clicked with azido-modified oligonucleotides (Figure XV). This method enables to attach proteins to any positions on DNA oligonucleotides and could serve as valuable tool for single-molecule studies and DNA based nanotechnology involving functional protein-DNA hybrids.[86]

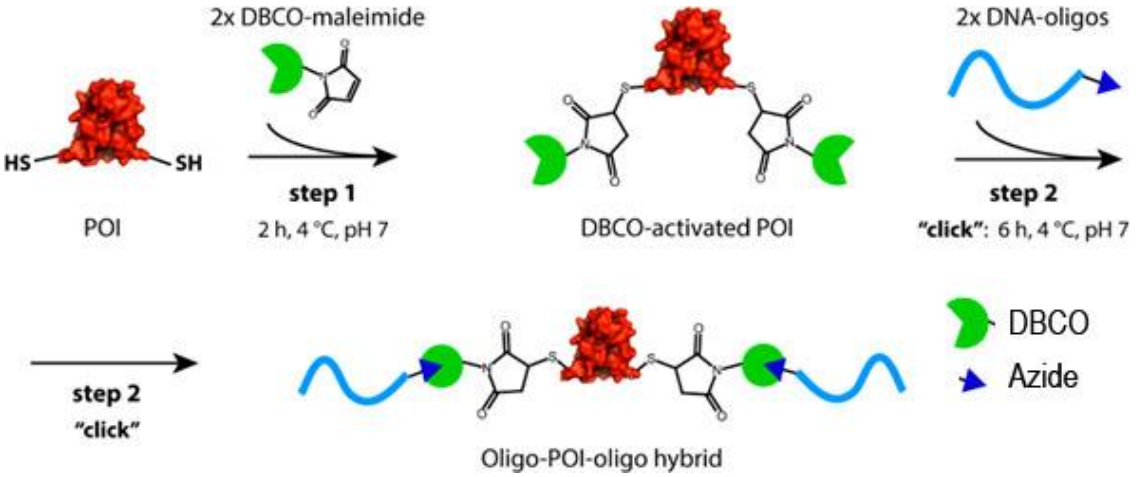


Figure XV: Preparation of protein-DNA hybrids.[86]

DNA labelling

In 2011, Taton and co-workers published article describing the solid phase synthesis of 5´-**DIBO**-modified oligonucleotides using **DIBO** phosphoroamidite.[87] These oligonucleotides reacted quantitatively with azides either on solid phase or in aqueous solution after oligonucleotide cleavage from solid support.[87] **DIBO**-modified oligonucleotides were used also as primers for PCR reactions followed by fluorescent labelling with Texas Red azide *via* SPAAC.[87]

One year later, El-Sagheer and Brown reported preparation of **DIBO**- or **BCN**-thymidine monomers, which were incorporated into oligonucleotide and cross-linked to azide-labelled complementary strands.[88] DIBO-monomer was also successfully used for site-specific labelling of genetic probes with multiple fluorophores.[88]

DNA ligation

In 2011, El-Sagheer and Brown introduced the synthesis of 5´-cyclooctyne and **DIBO** functionalised oligonucleotides.[89] These oligonucleotides were subjected to ligation with 3´-azido oligonucleotide with fluorescent dye at 5´-end (Figure XVI). Ligation proceeded very fast and efficiently. It was also observed, that presence of single mismatched base pair inhibited the ligation, which predestines this application in genetic analysis.[89]

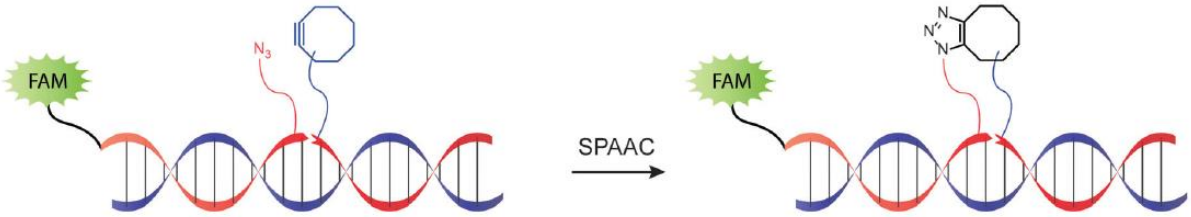


Figure XVI: SPAAC click DNA ligation between azide-labelled and cyclooctyne-labelled oligonucleotides - schematic of reaction (FAM = 6-carboxyfluorescein).[88,89]

Similarly, van Delft and co-workers were focused on incorporation of **BCN** into oligonucleotides *via* solid phase phosphoroamidite procedure.[90] **BCN** modified oligonucleotides were then coupled with azide-functionalized oligonucleotides to receive oligonucleotide dimer.[90] New methodology for ligation of single stranded DNA was reported by group of Kanaras in 2013.[91] They prepared gold nanoparticles functionalized with 3´-azide or 5´-**DIBO** oligonucleotides, which can be brought together *via* a splint strand and covalently clicked in one pot reaction which is beneficial in contrast with enzymatic ligation.[91]

2.1.3.1.3. Quantum dots conjugates

Inorganic semiconductor nanocrystals, known as quantum dots (QD), exhibit the significant spectral properties with tuneable, narrow and symmetric emission band, long luminescence lifetime and good photostability, which predestine their potential use for biological imaging purposes.[92] In 2010, Texier and co-workers described preparation of highly luminescent QD conjugates from **OCT** modified QD and Ac_4ManAz *via* SPAAC.[92] Moreover, they also use these **OCT**-QDs for *in vivo* imaging of azido-modified Chinese Hamster Ovary cells.[92] Two years later, Cais´ group reported imaging of living **DIBAC**-modified Baculovirus with azido-QDs.[93] Few months later, Xie and co-workers showed that enveloped viruses such as azido modified vaccinia virus (VACV) and avian influenza A virus (H9N2) can be specifically labelled with **DIBAC** modified QDs (Figure XVII).[94]

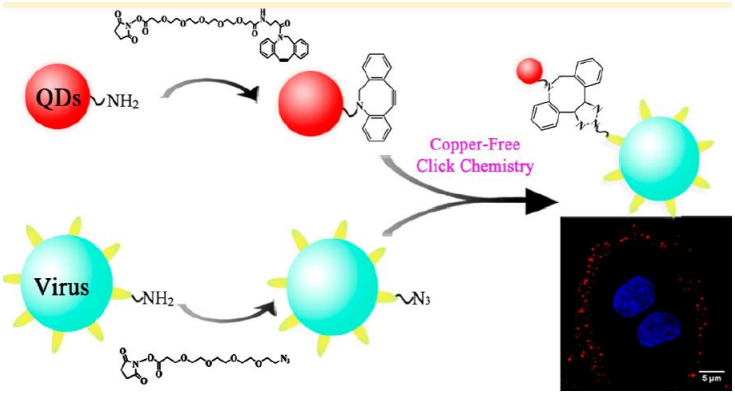


Figure XVII: Labelling of enveloped virus with **DIBAC** modified quantum dots.[94]

In 2014, Achilefus´group used SPAAC for development of QDs conjugates with anti-epidermal growth factor receptor (EGFR) antibodies for sensitive detection of cancer biomarkers through fluoroimmunoassays.[95]

2.1.3.1.4. Preparation of probes for PET

Positron emission tomography (PET) is imaging technique using positron emitted from suitable radionuclide such as ^{18}F, ^{64}Cu and the others. It provides important information about physiological, biochemical and pharmacological processes in living subjects. PET is often used in diagnostic medicine especially for diagnosis of cancer and Parkinson disease.

^{18}F Radiolabelling

Among the available PET radionuclides, the fluorine-18 is one of the most used due to its excellent chemical, physiological and nuclear properties, although its half-life is relatively short ($t_{1/2}$ = 109.8 min).[96] Due to this fact, labelling strategies for preparation of new PET probes must be rapid, simple and reliable.[96]

In 2011, Wuest and co-workers as a first reported radiolabelling reaction with ^{18}F with use of SPAAC.[96] They prepared ^{18}F-labeled derivative of **DIBAC** (referred to by authors as [^{18}F]FB-DIBAC), which was then tested towards reactivity with different azides.[96] [^{18}F]FB-DIBAC also showed promising radiopharmacological profile and fast blood clearance, which predestines its potential use in PET studies.[96] Approximately at the same time, the group of Elsinga and Feringa published ^{18}F radiolabelling of bombesin, 14 amino acid neuropeptide with high binding affinity to the gastrin-releasing peptide receptor (GRPR), which is massively overexpressed in variety of tumor cells.[97] They used **DIBAC** modified Lys[3]- bombesin and labelled it with ^{18}F containing azides. The prepared probes exhibited high affinity to GRPR in PC3 human prostate cancer cells.[97]

In the same year, Kostikov and co-workers utilized SPAAC for radiolabelling of peptides.[98] *N*-terminally azido-modified peptides were reacted with ^{18}F-DIBAC in high radiochemical yields.[98] In 2012, the group of Carroll reported the use of SPAAC as a potential tool for PET pretargeting.[99] They studied biodistribution of profile of [^{18}F]2-fluoroethylazide in BALB/c mice and its reactivity with different cyclooctynes.[99] The SPAAC proceeded efficiently in the most of cases with the best results for **TMDIBO**.[99] Two years later, groups of Kim and Lee introduced the use of SPAAC for preparation of **DIBAC** containing ^{18}F monomeric and dimeric RGD peptides and their application for *in vitro* and *in vivo* PET imaging (Figure XVIII).[100] All peptides exhibited high binding affinity to integrin $\alpha_v\beta_3$ expressing tumors both *in vitro* and *in vivo*.[100]

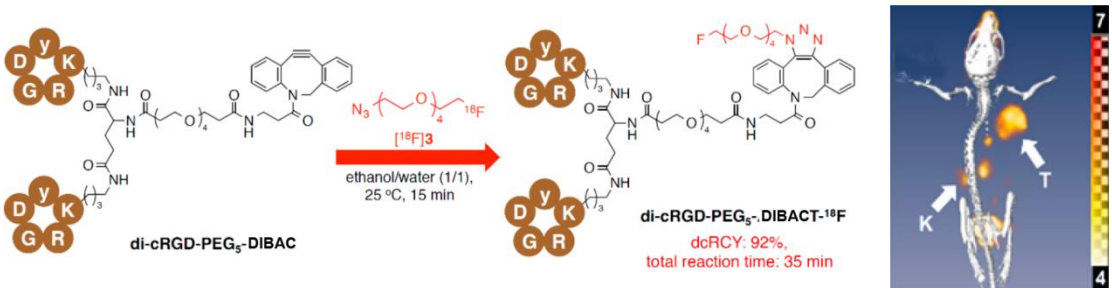


Figure XVIII: Schematic diagram of the procedure for the synthesis of di-cRGD-PEG5-**DIBAC**T-^{18}F and *in vivo* evaluation of di-cRGD-PEG$_5$-**DIBACT**-^{18}F in mouse; three-dimensional reconstruction. T = tumor, K = kidney.[100]

In 2016, Ross and Kettenbach published preparation of new ^{18}F labelled **DIBAC** which reacted with different azido-peptides in very high radiochemical yields and with fast kinnetics.[101]

^{64}Cu Radiolabelling

Due to short half live of fluorine, several metallic radionucleotides such as ^{64}Cu, ^{68}Ga, ^{86}Y and ^{89}Zr were utilized for PET imaging.[102] They are usually characterized with longer half-lives facilitating the monitoring of processes over several hours or even days. ^{64}Cu ($t_{1/2}$ = 12.7 h) is attractive especially due to its decay half-life, low positron energy and commercial availability.[102]

The first preparation of ^{64}Cu-labeled PET probes with use of SPAAC was reported in 2012 by Chen and Conti.[102] They synthesized **DIBAC** modified ^{64}Cu chelator, which was attached *via* click reaction to azido-modified cyclic RGD peptide (Figure XIX).[102] Resulting probes then exhibited both good binding affinity to the integrin $\alpha_v\beta_3$ receptor in mouse tumor model.[102]

Figure XIX: Schematic diagram of the preparation of ^{64}Cu labelled PET probe via SPAAC for tumor targeting *in vivo*.[102]

A year later, Kim and co-workers prepared ^{64}Cu glycol chitosan nanoparticles (CNPs) with the use of SPAAC.[103] **DIBAC**-PEG$_4$-Lys-DOTA was synthesized with use of solid phase synthesis, radiolabelled with ^{64}Cu and clicked with azido-modified CNPs.[103] ^{64}Cu-CNPs conjugates were then injected to tumor bearing mice exhibiting effective accumulation in the tumor region.[103] In 2014, Sutcliffe and colleagues reported preparation of [^{64}Cu]DOTA-**DIBAC**-Ala-PEG$_{28}$-A20FMDV2 radiotracer *via* SPAAC and its use for *in vitro* and *in vivo* imaging of integrin $\alpha_v\beta_6$ expressing tumors.[104]

2.1.3.2. Materials science applications

2.1.3.2.1. Dendrimers

Many types of drug delivery carriers including polymer microcapsules, liposomes and nanoparticles have been developed and utilized for biomedical applications.[105] Unlike the others, dendrimers have a numerous advantages such as controlled multivalence, low polydispersity and the globular shape of higher generation of dendrimers.[105] In 2010, Weck and co-workers presented SPAAC as effective strategy for functionalization of dendrons and dendrimers.[105] They prepared cyclooctyne derivatized by triethylenglycol ether, which was reacted with poly(amide)-based dendrons and dendrimers with azide terminus.[105] A year later, the group of Baker Jr. reported preparation of G5 polyamidoamine (PAMAM) dendrimer with 20 cyclooctyne molecules which were used for attachment of azido derivative of methotrexate.[106]

Then, they extended this work on synthesis of mono-, di- and tri-functional PAMAM dendrimer conjugates with folic acid, methotrexate and fluorescein (Figure XX).[107] Dendrimers containing folic acid were efficiently bound to KB human tumor cells.[107]

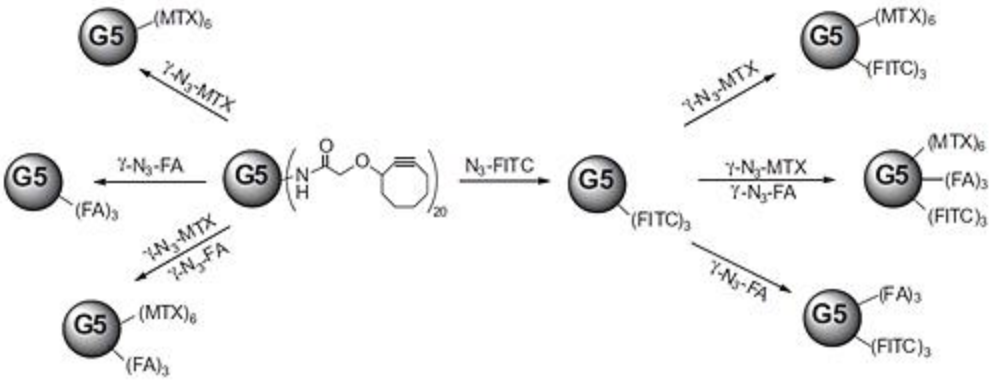


Figure XX: SPAAC of the dendrimer with azido modified functionalities.[107]

2.1.3.2.2. Hydrogels

Biocompatible hydrogels are widely used in biomedicinal research and pharmaceutical applications as protein microchips, drugs and genes delivery carriers, ophthalmic prostheses, and scaffolds for encapsulating cells.[108] Unlike naturally occurring hydrogels, synthetic ones have unique advantages due to the broader tunability of the properties.[108] In 2011, Song and co-workers described preparation of PEG-*co*-polycarbonate hydrogel through SPAAC of azido-functionalized PEG-*co*-polycarbonate macromers with **DIBAC**-functionalized PEG.[108] This gel allowed the encapsulation of Bone marrow stromal cells with high cellular viability.[108] A year later, Anseth and co-workers reported synthesis of hydrogels for photoreversible patterning of biomolecules.[109] These hydrogels were prepared by SPAAC between 4-arm PEG-DIFO and (alloc)-protected polypeptide.[109] In another study, they developed photo- and enzyme-labile hydrogels, which were prepared from four-arm PEG-**DIFO** and azido peptides (Figure XXI).[110]

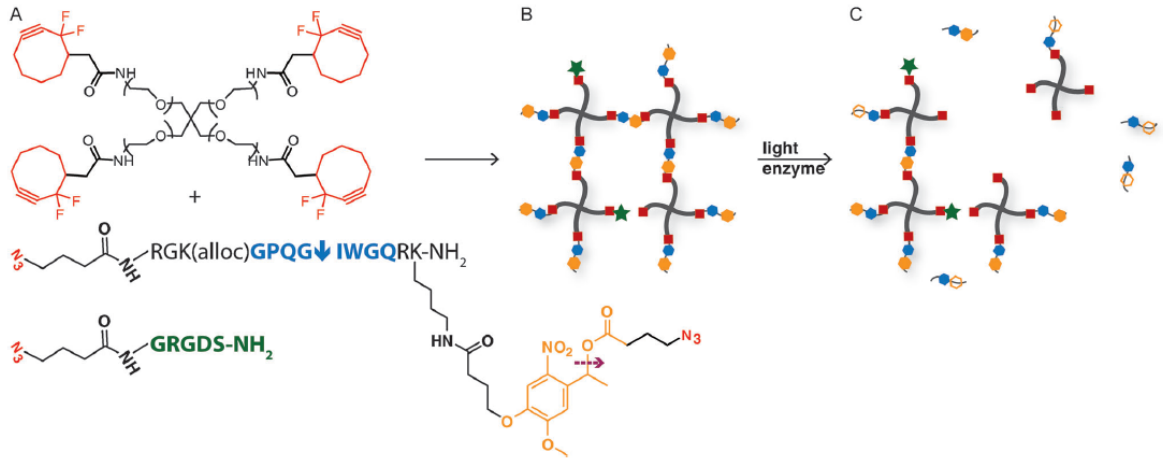


Figure XXI: Hydrogel synthesis and degradation.[110]

In 2012, Becker demonstrated, that human mesenchymal stem cells are able to be encapsulated into *in situ* forming hydrogels from glycerol exytholate triazide and **DIBO** functionalized PEG.[111] Santi et. al. then introduced hydrogels prepared through SPAAC for tunable drug release and degradation rates.[112] In 2013, Ito and co-workers as the first described preparation of *in situ* crosslinked hyaluronan hydrogels using SPAAC between azide- and **OCT**- modified hyaluronan.[113]

2.1.3.2.3. Polymers

Covalently crosslinked polymers, which swell but do not dissolve in a solvent given, played important role in a number of applications including tissue engineering, drug delivery or chemical sensing.[114] End-linked polymers gels are then promising for drug delivery

applications because of their easily controlled, homogenous pore sizes.[114] Turro *et al.* reported the use of bifunctional **MOFO** and **DIFO** for *in situ* crosslinking with tetraazido terminated poly(tert-butyl acrylate) star polymer yielding photodegradable polymeric model networks.[114]

Chitosan-g-PEG (CS-g-PEG) is widely studied as a stable polymeric carrier for drug delivery.[115] Functionalized CS-g-PEG in form of nanoparticles was utilized in brain delivery applicatons.[115] Fernandez-Megia and Riguera developed methodology for incorporation of molecule of interest on CS-g-PEG nanostructures via SPAAC between CS-g-PEG-N_3 and PEG-**OCT** bearing relevant ligands such as mannose, anti-BSA rabbit immunoglobulin G (IgG) and fluorescein (Figure XXII).[115]

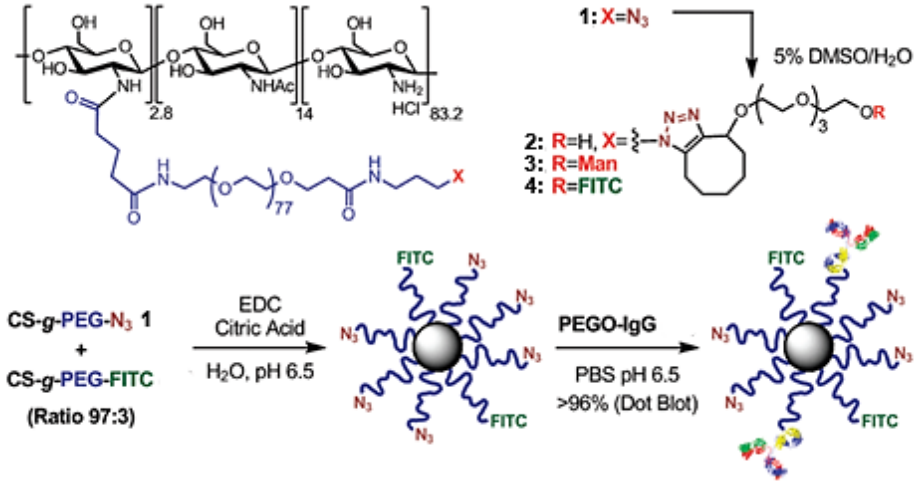


Figure XXII: SPAAC conjugation of CS-g-PEG and NPs.[115]

2.1.3.2.4. Surface functionalization

Surface immobilization of biomolecules is a key step in the formation of biosensors, microbeads, biochips and other devices.[116] In 2010, Popik and co-workers developed new methodology for efficient surface functionalization using SPAAC. They prepared **DIBAC** or azido functionalized glass slides, which were then patterned with **DIBAC**- or azido-fluorescent dyes (Figure XXIII).[116] In 2011, Andresen *et al.* reported a method for functionalization of liposomes via SPAAC.[117] They prepared **MOFO** functionalized PEGylated phospholipid which was incorporated into liposome. This liposome was then labelled with 3-azidocoumarin RGD peptides.[117]

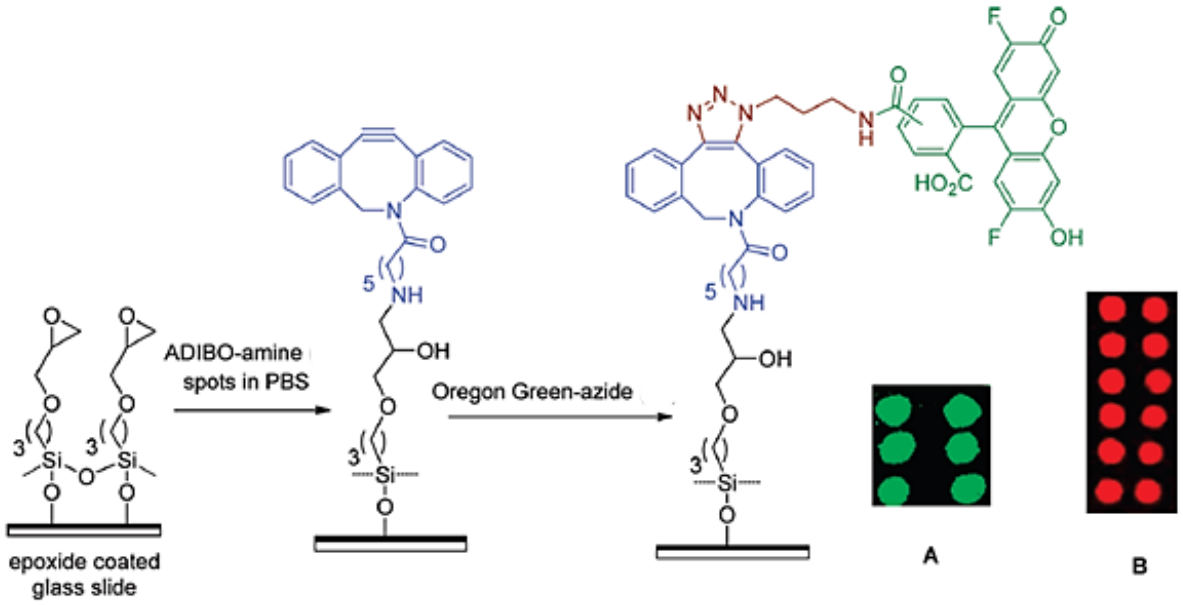


Figure XXIII: Schematic representation of **DIBAC** derivatization of an epoxy-coated slide followed by copper-free click immobilization of Oregon green dye. Inserts show fluorescent images **DIBAC** slides patterned with (A) Oregon green azide and (B) Lissamine rhodamine B azide.[116]

Silicon or silicon oxide combined with silicon nitride (Si_3N_4) is often used material for microcantilever-based biosensors, optical waveguides and for other applications.[118] Zuilhof and co-workers developed functionalization of Si_3N_4 with covalently attached organic monolayers.[118] **BCN** was bound on Si_3N_4 surface *via* four-step method followed by SPAAC reaction with a series of modified azides including a fluorescent dye, olligosacharides and a perfluorinated compound.[118] In 2014, Becker *et al.* reported efficient surface modification with conjugate of dopamine and **DIBO**.[119] Dopamine is known as an adhesive agents for surface functionalization.[119] **DIBO**-dopamine was attached to polydimethylsiloxane, which was then used for generation of patterns *via* SPAAC.[119]

In the above mentioned articles the site specificity of the labelling as well as regioselectivity of the SPAAC with respect to biomolecule/polymer conformation has not been studied.

3. Aims of the work

Based on the literature review mentioned above it is obvious, that SPAAC is useful method for (bio)conjugation. The crucial drawback of this reaction and following application lies in the complex multi-step and often also low-yielding synthesis of cyclooctyne systems. In addition, full characterization of click chemistry products, which can affect the conformation of biomolecules resulting in change of their function, is not always performed.

The first goal of presented dissertation was exploration of conjugation reaction between **DIBAC** derivatives and azidoderivatives of nucleic acid components. The attention was supposed to be paid to possible formation of conformation isomers possibly affecting the function of nucleic acids.

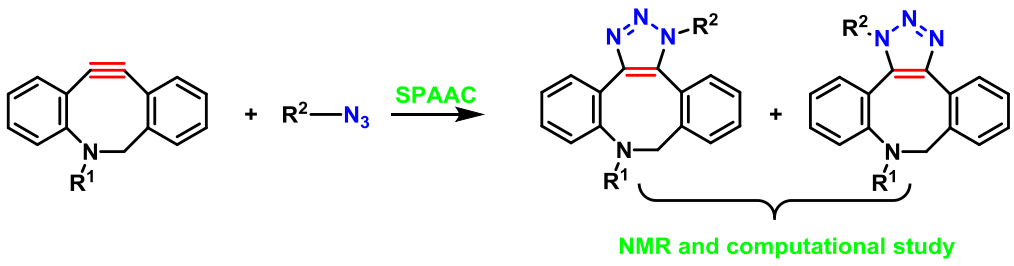
The second goal was focused on development of synthesis of new **DIBAC-Dye** systems suitable for non-catalyzed labelling of biomolecules.

The third goal of the work was aimed to possible use of cycloalka-1,2,3-selenadiazoles for conjugation reactions with azides under various initiation conditions including heating, microwave irradiation and photolysis. The synthesis of a system suitable for biorthogonal labelling was supposed to be developed and the system applicability was supposed to be verified in reaction with a biomolecule.

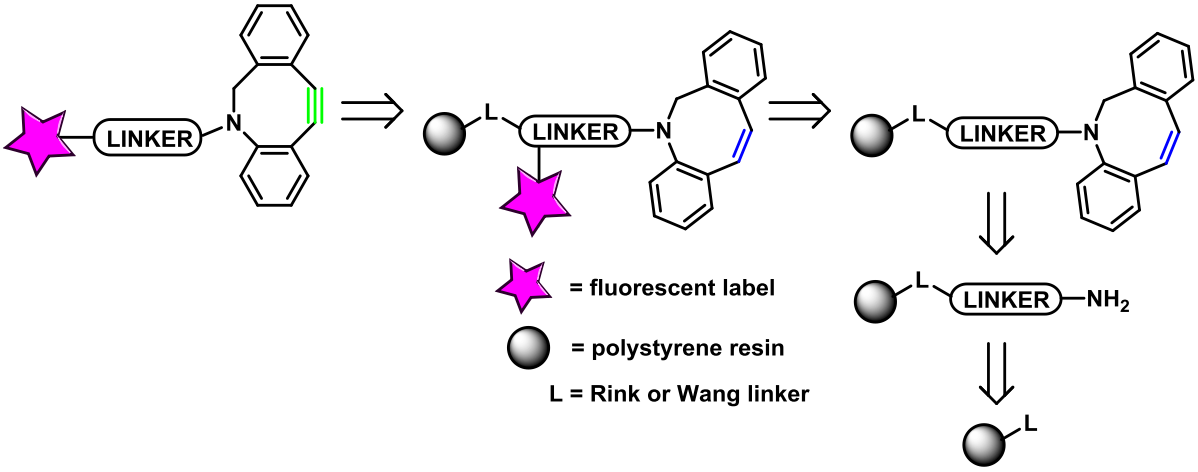
3.1. Summary of presented aims

3.1.1. DIBAC derivatives

- SPAAC of **DIBAC** analogues with different azides derived mainly from nucleosides and detail characterization of resulting triazoles

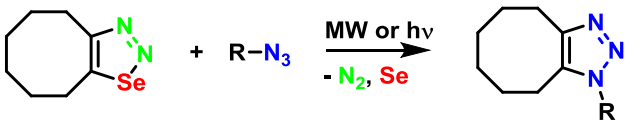


- development of solid phase synthesis of fluorescently labelled **DIBAC** analogues

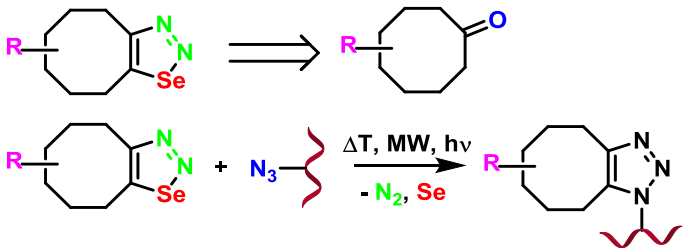


3.1.2. Cycloalka-1,2,3-selenadiazoles

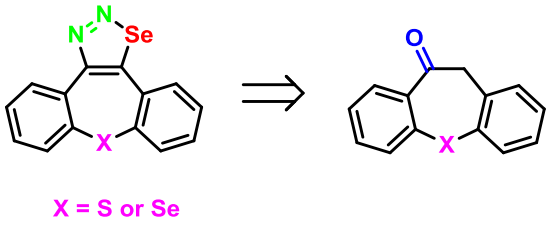
- study of reactions of cycloocta[d][1,2,3]selenadiazoles with structurally different azides



- development of synthesis of substituted cycloocta[d][1,2,3]selenadiazoles and study of conjugation reactions with azides (including derivatized biomolecules) under various conditions



- development of synthesis of dibenzothiepine- and dibenzoselenepine-1,2,3-selenadiazoles derivatives for photochemically induced conjugation reaction with azides.



4. Results and discussion

4.1. DIBAC derivatives

This part of work is dealing with strain promoted azide-alkyne cycloaddition reactions of aza-dibenzocyclooctyne derivatives (**DIBAC**) which was firstly described by van Delft in 2010.[19] Although **DIBAC** exhibits excellent kinetic towards reaction with azides, time-wasting multi-step synthesis of its derivatives is the main drawback of this system.[19] Also detail characterization of resulting conjugates published in literature is vague.

4.1.1. SPAAC of DIBAC and characterization of resulting triazoles

This part of work was focused on study of triazoles formed from **DIBAC** and various azides including mainly nucleic acid components. Copper-free click reactions with nucleobases using dibenzoazocine derivatives have been described in only two articles.[120,121] The structure of triazoles derived from **DIBAC** and oligonucleotides or nucleosides has not been studied to date, although the vicinity of the bulky aza-dibenzocyclooctyne group to the nucleic base can play a significant role in the conformation of the DNA duplex, RNA strain assembly or protein tertiary structure when used for biomolecule labeling. As a model substrate for this study we have chosen 5-methyl uridine bearing azido group in base as well as sugar part.

4.1.1.1. SPAAC with azides derived from 5-methyl uridine

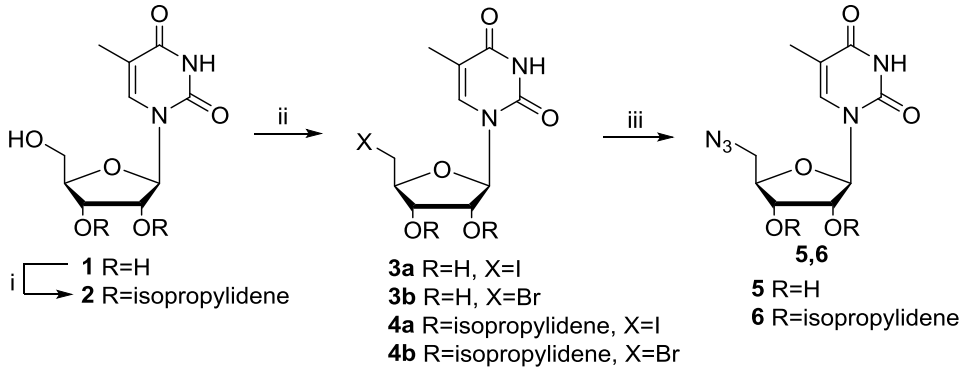
Presented work is focused on SPAAC of **DIBAC** derivative **13** (Scheme 2) with azides derived from 5-methyluridine bearing azidogroup in position 5 and 5´.

4.1.1.1.1. Preparation of reagents

Preparation of 5-methyluridine-5´-azides

The attention was paid to 5´-azidoderivative **5** and its protected analogue **6** previously developed within authors´ Diploma thesis using commercially available 5-methyluridine as a starting material (Scheme 1).[122] In case of 5´-azidoderivative **5**, the total yield of reaction reached approximately 60%, which is more efficient than the previously described synthesis[123] beginning with protection of 5-methyluridine by acetone. The developed synthetic protocol was also successfully tested in the synthesis of protected 5´-azidoderivative **6** starting from protected 5-methyluridine **2**, prepared in a very good yield using the described procedure.[124]

Scheme 1: Preparation of 5´-azide-5-methyluridines **5** and **6**.[122,*a*]

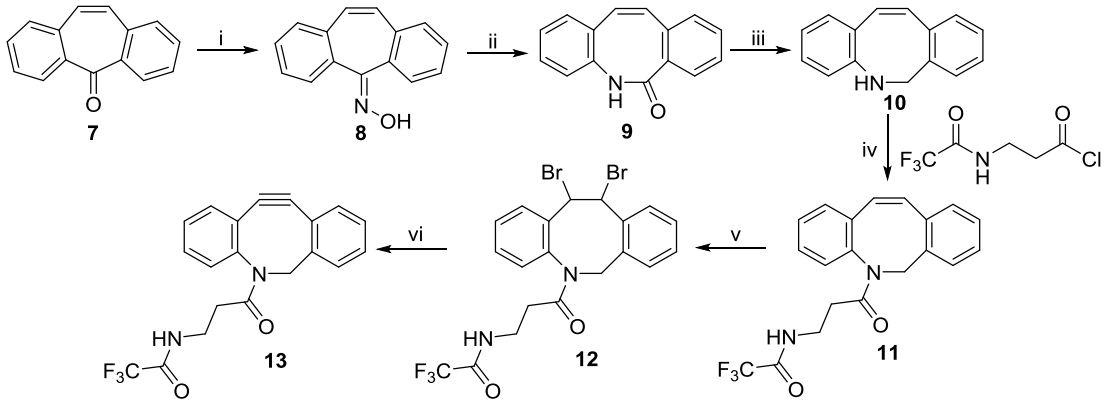


[*a*]Reagent: (i) aceton, H_2SO_4 (ii) I_2 or Br_2, PPh_3, imidazole, THF (iii) NaN_3, DMF, 90 °C.

*Preparation of **DIBAC** derivative*

Aza-dibenzocyclooctyne derivative **13** was prepared according to literature by six step procedure starting from dibenzosuberone **7** in 7% overall yield (Scheme 2).[64]

Scheme 2: Preparation of **DIBAC** derivative **13**.[64,*a*]

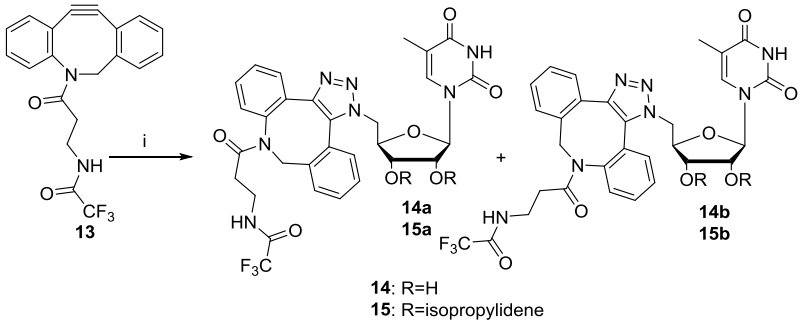


[*a*]Reagent: (i) $NH_2OH.HCl$, pyridine (ii) PPA, 125 °C (iii) $LiAlH_4$, diethylether, reflux (iv) pyridine, DCM (v) pyridinium tribromide, DCM (vi) *t*-BuOK, anhydrous THF.

4.1.1.1.2. SPAAC

Strain promoted click reaction of azides **5** and **6** with **DIBAC** derivative **13** (Scheme 3) was studied.[125] As expected, this compound was highly reactive, and all reactions in methanol were nearly instantaneous. Both products were produced as a mixture of two regioisomers in a 1:1 ratio.[125] The conversion of this reaction depended on the amount of compound **13**. Complete conversion of the azides required at least 1.4 equivalents of **13**.[125]

Scheme 3: SPAAC with azadibenzocyclooctyne **13** and azides **5** and **6**.[125,a]



[a]Reagent: (i) Azide **5** or **6**, MeOH.

Isomers **14a,b** and **15a,b** were successfully separated by semi-preparative HPLC, yielding products in >99% purity. All isomers were subjected to detailed NMR study.[125]

4.1.1.1.3 NMR study*

Although the purity of triazoles **14a,b** and **15a,b** was confirmed by HPLC before and after the NMR experiments and verified under several HPLC conditions, the ^{1}H NMR spectra revealed the presence of two isomers of the **14a** and **15a** derivatives and even more for the **14b** as well as **15b** derivatives.[125] The ^{13}C NMR spectra of $CDCl_3$ solutions also revealed more than the expected number of signals.

The structures of compounds **14a** and **15a** were clearly confirmed by the ^{1}H, ^{13}C and ^{15}N signals assigned via 2D experiments, particularly ^{1}H – ^{1}H gROESY and ^{1}H – ^{15}N gHMBC.[125] The correlation was based on the interaction of the **DIBAC** nitrogen with **DIBAC** methylene protons and with the aromatic proton next to the **DIBAC** nitrogen in ^{1}H – ^{15}N gHMBC. An interaction of the aromatic protons with the ribose methylene group and the **DIBAC** methylene group was observed in ^{1}H – ^{1}H gROESY as well (Figure 1).[125]

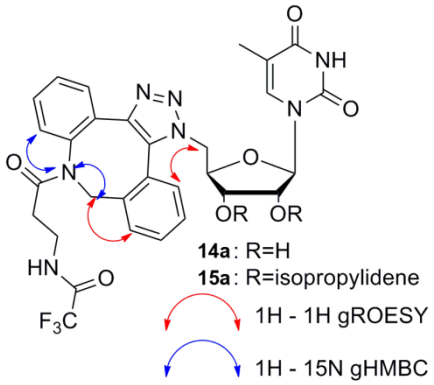


Figure 1: Homo and heteronuclear interactions used to determine the structures of derivatives **14a** and **15a**.[125]

* ^{19}F-^{19}F EXSY, ^{1}H-^{15}N gHMQC and ^{1}H-^{15}N gHMBC were measured by Antonín Lyčka from Faculty of Science, University of Hradec Králové.

Assigning the ^{1}H, ^{13}C and ^{15}N signals (measured in $CDCl_3$) of regioisomers **14b** and **15b** was problematic due to the presence of more forms of each pure compound (see above). Because the reaction course predetermines the formation of two regioisomers and the HRMS of couples **14a**/**14b** or **15a**/**15b** respectively afforded the same elemental composition, we conclude that the structures really correspond to the opposite regioisomers. The NMR spectra for **14b** and **15b** are described as the set of signals of all their present forms.[125]

For couple **15a**/**15b**, we performed standard ^{19}F NMR and ^{19}F-^{19}F EXSY. In both cases we observed two resonances for **15a** and four resonances for **15b** ($CDCl_3$) (Figure 2). ^{19}F-^{19}F EXSY of both compounds revealed a mutual slow exchange between all existing forms, as evidenced by positive cross-peaks in the spectra (Figure 2). These positive cross-peaks confirmed exchange between all four existing forms of compound **15b** for a relatively wide range of mixing times from 0.05 to 2 s, indicating that the relative rates of exchange must be very similar.[125]

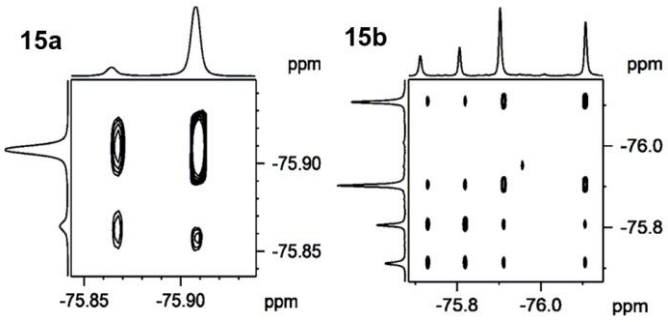


Figure 2: ^{19}F-^{19}F EXSY of triazoles **15a** and **15b** (mixing time 1 s).[125]

^{1}H-^{15}N gHMQC and ^{1}H-^{15}N gHMBC experiments in $CDCl_3$ suggested that the isomery was of conformational origin. Doubled signals of the pyrimidine NH group for compound **15a** at 150.75 ppm (Figure 3) likely hindered the rotation of the pyrimidine ring relative to the other parts of the molecule. Identical results were obtained in d_6-DMSO.[125]

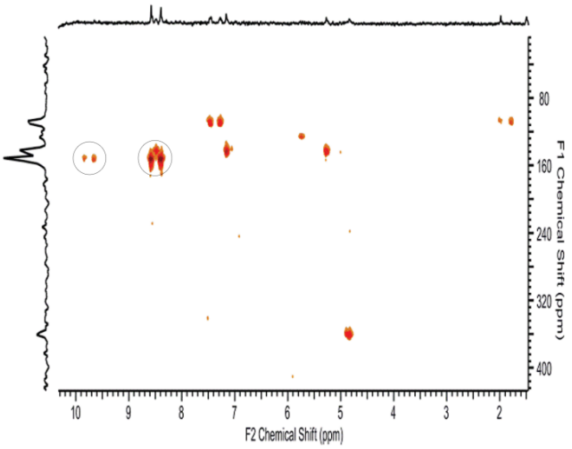


Figure 3: ^{1}H-^{15}N HMBC of **15a** in $CDCl_3$.[125]

The ^{1}H spectra of compounds **15a** and **15b** in d_6-DMSO were measured at various temperatures (25 °C, 50 °C, 100 °C and cooling back) to determine whether the number of isomers was affected by temperature. The spectral pattern was essentially unaffected until +50 °C. Signal coalescence was finally observed at +100 °C (Figure 4).[125] Identical results were obtained for standard ^{19}F spectra of **15a** and **15b** measured in DMSO, with signal coalescence at 100 °C (Figure 5).[125]

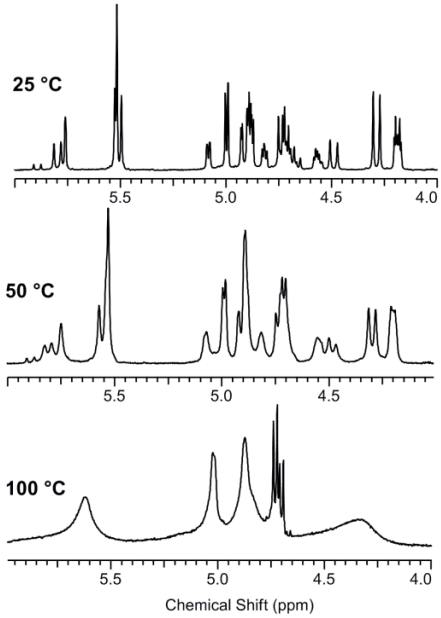


Figure 4: ^{1}H NMR spectra of **15a** at different temperatures.[125]

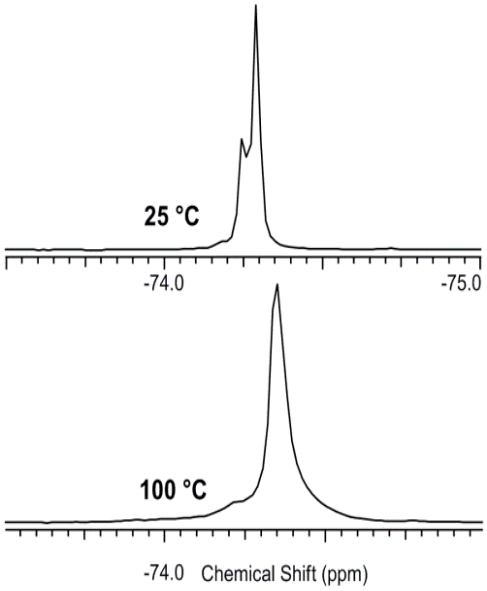


Figure 5: ^{19}F spectra of **15a** at different temperatures.[125]

To characterize the relationship between the number of isomers and the type of solvent, we extended the number of tested solvents to acetone, D_2O, MeOD, DMSO-d_6 and DMF-d_7 for derivatives **14a** and **15a**, which were selected as representative model compounds. The ratio of isomers was determined from the signals at around 5.75 and 6.0 (see red arrows in Figure 6). The only exception is spectrum in DMSO-d_6, in which the signals are not clear and therefore the couple of signals at 4.25 and 4.5 were selected. Changing the solvent not only shifted the signals but also affected their ratio (Figure 6).[125] On the other hand the number of isomers of compounds **14a** and **15a** remained constant. The dependence of the isomeric ratio on solvent is presented in Table 1.

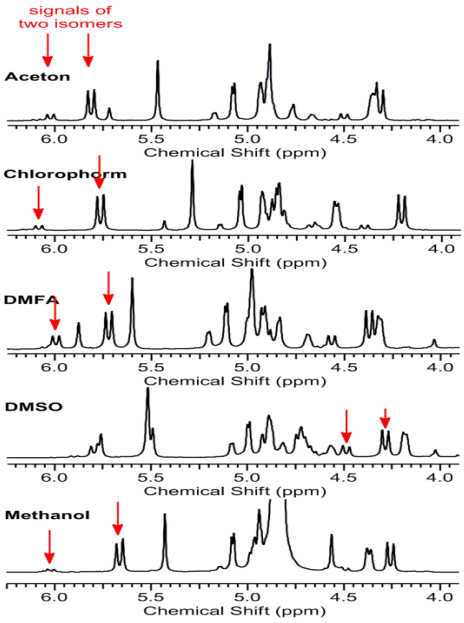


Figure 6: ^{1}H NMR spectra of **15a** in various solvents.[125]

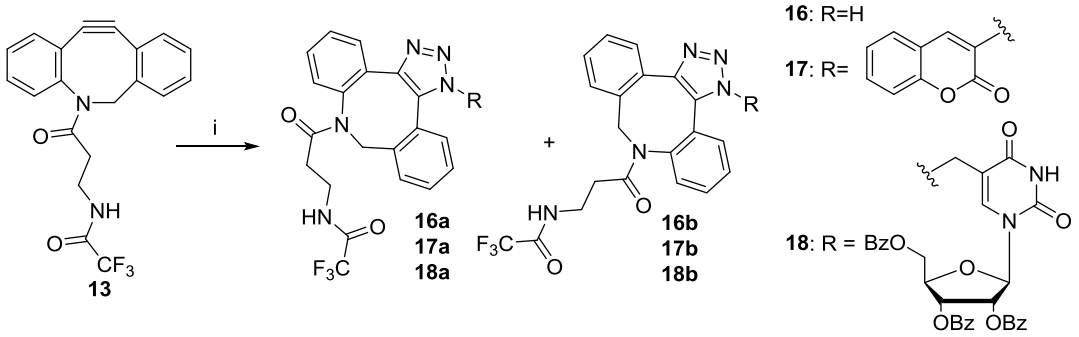
Table 1. The ratio of isomers of compounds **14a** and **15a**.

14a		**15a**	
Solvent	**Ratio***	**Solvent**	**Ratio***
CD_3OD	3.2:1	CD_3OD	9:1
$CDCl_3$	2.6:1	$CDCl_3$	6.6:1
DMF-d_7	1.7:1	DMF-d_7	2.6:1
D_2O	7.9:1	d_6-Acetone	4:1
DMSO-d_6	1.4:1	DMSO-d_6	2.2:1

*The ratio was determined from the peak integrals of the **DIBAC** methylene group protons at approximately 5.75 ppm and 6.00 ppm or at 4.25 ppm and 4.50 ppm.[125]

To determine whether the presence of isomers was caused by the nucleoside part of molecule or by s-cis, s-trans isomery of the amide groups on the **DIBAC** moiety, we prepared triazoles **16-18** substituted on nitrogen by only hydrogen, the sterically bulkier coumarin, and by 2´,3´,5´tribenzoyl-5-methyluridine (Scheme 4).[125]

Scheme 4: Preparation of triazoles **16, 17** and **18**.[125,a]



[a]Reagent: (i) Azide, MeOH.

The azidocoumarin as the starting material was synthesized as described previously.[126] 2´,3´,5´-Tribenzoyl-5-azidomethyluridine was synthesized using a simple procedure starting from 5-hydroxymethyleneuridine.[122]

Using a simple triazole with hydrogen, we obtained only a single regioisomer **16a**, whereas 3-azidocoumarine and protected 5-methyluridine formed two regioisomers, **17a/17b** and **18a/18b**, respectively.[125]

For triazoles **16a** and **17a**, we observed only one set of signals, with no isomery. Surprisingly, the ^{1}H spectra of triazole **17b** contained at least three sets of signals (Figure 7), similar to the ^{13}C NMR spectra, in which more than one set of signals was detected. These results confirm that the type of substituent on the triazole strongly influences the number of isomers in NMR spectra.[125] Moreover, the presence of isomery in **17b** and the lack of isomery in derivative **17a** indicate that the position of the aliphatic part of **DIBAC** relative to the triazole substituent is crucial for the number of isomers formed. These results also demonstrate that the presence of amide bonds in the **DIBAC** part of the molecule does not affect the isomery observed in the NMR spectra.[125]

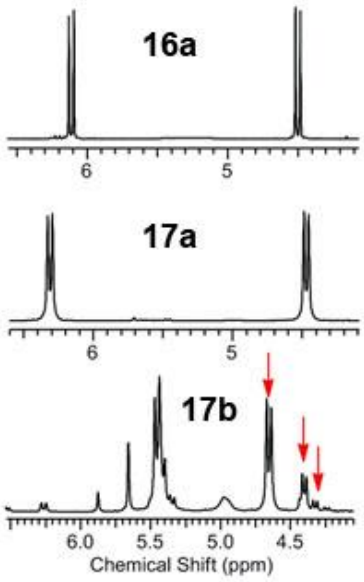


Figure 7: Detail of the ^{1}H spectra of compounds **16a**, **17a** and **17b**.[125]

Regioisomers **18a** and **18b** were inseparable under several HPLC conditions; the retention times of the isomers were nearly identical on semi-preparative C_{18} columns. The ^{1}H NMR spectrum of the mixture of **18a,b** in $CDCl_3$ revealed the presence of additional isomers, similar to compounds **14a,b** and **15a,b**. Thus, all possible isomers can also be expected when labeling oligonucleotides *via* nucleobase derivatization.[125]

To clearly identify the origin of the isomery, triazoles **14a,b** and **15a,b** were subjected to computational study.[125]

4.1.1.1.4. Computational study[2]

According to the NMR study described above, we assumed that the observed conformations of compounds **14a,b** and **15a,b** resulted from a combination of rotation about two bonds between the triazole and ribose rings. The rotations of these bonds were studied as the changes of two dihedral angles involving backbone atoms $N^{15}N^{14}C^{13}C^{12}$ and $N^{14}C^{13}C^{12}C^{11}$ (for numbering see Figure 8).[125]

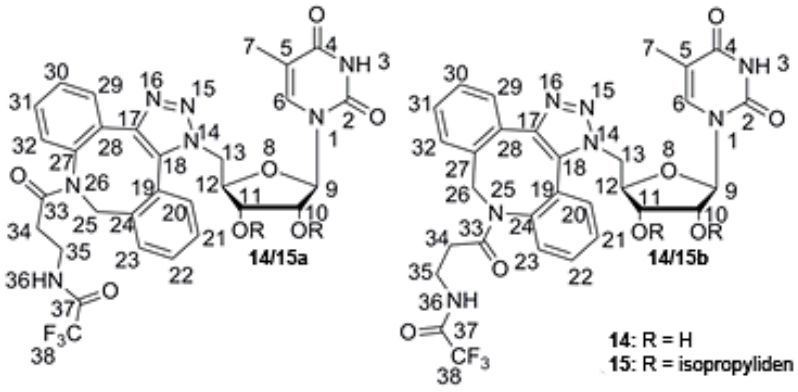


Figure 8: Numbering of atoms in **14a,b** and **15a,b** in the conformational study.[125]

Although the conformational changes are dependent on the solvent, the quantum calculation was simplified to a vacuum to assess the ability of the compounds to form stable conformers whose distributions further depend on the solvents. The theoretical model used in this study was the B3LYP method with a 6-31G(d,p) basis set in Gaussian 09.[125,127]

To determine the configurations with energies at the local minima, the dependencies of the energies on both dihedral angles were examined. The conformation with the lowest energy was selected as the zero point on the energy scale for each structure. The potential local energy minima were subsequently determined, and the fractional populations from the Maxwell-Boltzmann distribution were estimated. It was revealed that the most frequent conformers have potential energies lower than 30 kJ/mol.[125]

Compound **14a** formed 20 local minima with energy lower than 30 kJ/mol, and one conformer significantly predominated with a population close to 14%. Compound **14b** formed 16 local minima with energy < 30 kJ/mol, and 12 and 15 local minima satisfied these criteria for derivatives **15a** and **15b**, respectively. The local minima representing conformers with populations greater than 0.5% are summarized in Table 2.[125]

[2]Calculation of energies at local minima and conformer structures were provided by Gracián Tejral from Laboratory of Tissue Engineering, Institute of Experimental Medicine, Academy of Science of the Czech Republic.

Table 2. Local minima of derivatives **14a**, **14b**, **15a** and **15b** with conformer populations greater than 0.5%.[125]

Derivative	Local minima	Energy (kj/mol)	Population (%)
14a	1	0.00	13.93
	2	3.64	3.24
	3	5.71	1.41
	4	6.78	0.92
	5	7.84	0.60
14b	1	0.00	17.84
	2	5.93	1.66
	3	6.86	1.14
	4	7.08	1.04
	5	7.45	0.90
	6	8.03	0.71
15a	1	0.00	13.93
	2	5.47	3.24
	3	6.00	1.41
15b	1	0.00	16.40
	2	0.71	12.34

Analyses of compounds **14a,b** and **15a,b** with respect to changes in both dihedral angles revealed that the position of the aliphatic chain in structures (a) (intended **14a**/**15a**) and (b) (intended **14b**/**15b**) differed. For the (a) structures, both the left and right positions of the aliphatic chain (with respect to the triazole ring – see Figure 9) were observed (Figure 9A and 9B), but the positions on the right site were characteristic for only three local minima, with higher energies (26.03 – 26.79 kJ/mol) and a low conformers population (below 0.01%) for derivative **14a**; two local minima with populations less than 0.01% were observed for derivative **15a**. However, the (b) structures were characterized by the right position only (Figure 9C).

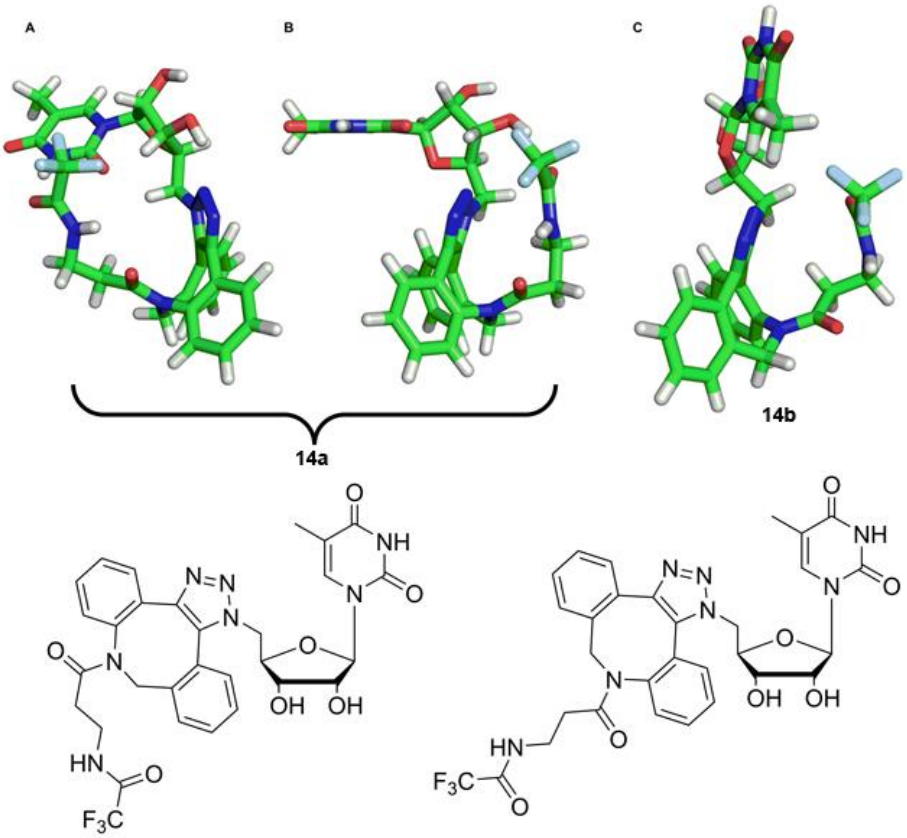


Figure 9: Possible orientation of the aliphatic chain exemplified by structures **14a** and **14b**. The orientation of the triazole ring plane perpendicular to the plane of the page with the orientation of the bond to 5-methyluridine behind the plane of the page was established as the reference plane in the description of the position of the aliphatic chain in structures **14a** ("left" for A and "right" for B) and **14b** ("right" for C).[125]

In addition, the interactions between the 5-methyluridine and the aliphatic chain as well as the distinct intermolecular hydrogen bonds were observed. The most frequent conformer at the first local minima of derivative **14a** (see Table 2) maintained the aliphatic chain in direct interaction with the pyrimidine ring. This interaction was enabled by a hydrogen bond between the carbonyl of trifluoroacetyl group 37 and imide hydrogen 3 of the pyrimidine ring (Figure 10 - 1). In the conformer representing the second most populated local minimum, the trifluoroacetyl carbonyl group of the aliphatic chain interacted with the ribose OH hydrogen (Figure 10 - 2).[125] Similar to conformation at the second most populated local minimum, the other local minima of derivative **14a** summarized in Table 2 exhibited interactions of the trifluoroacetyl group with the ribose OH hydrogens.[125]

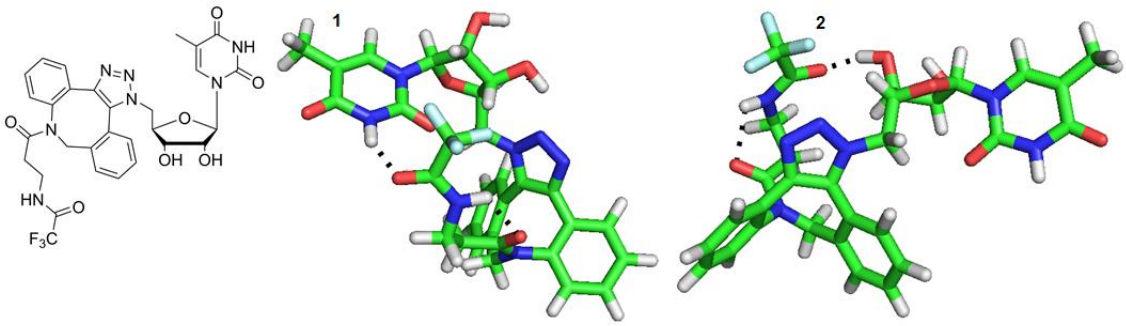


Figure 10: Two conformers 1 and 2 of derivative **14a** with different positions of the aliphatic chain.

Local minimum 1, in which the uracil carbonyl group directly interacts with the ribose hydroxyl group, predominated for derivative **14b** (Figure 11). In local minimum 4, interaction of both ribose OH hydrogens with the trifluoroacetyl carbonyl group was observed. Structures at other local minima (2, 3, 5 and 6) were again fixed by hydrogen bonds between the ribose OH hydrogen and uracil carbonyl group.[125]

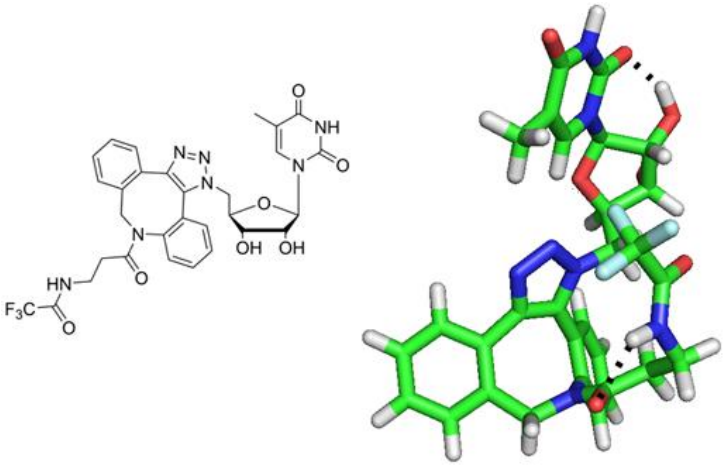


Figure 11: The most abundant conformation of derivative **14b**.

In derivative **15a**, in the conformer of the first local minimum, the aliphatic chain was located close to the pyrimidine ring; however, no hydrogen bond was observed between them (Figure 12-1). The conformer with local minimum 2 (Table 2) was characterized by the location of the pyrimidine part of the molecule distant from the aliphatic chain (Figure 12-2). Moreover, in the conformer with local minimum 3 (Table 2), an interaction between the pyrimidine imide group 3 and trifluoroacetyl carbonyl group 37 was detected (Figure 12-3).[125]

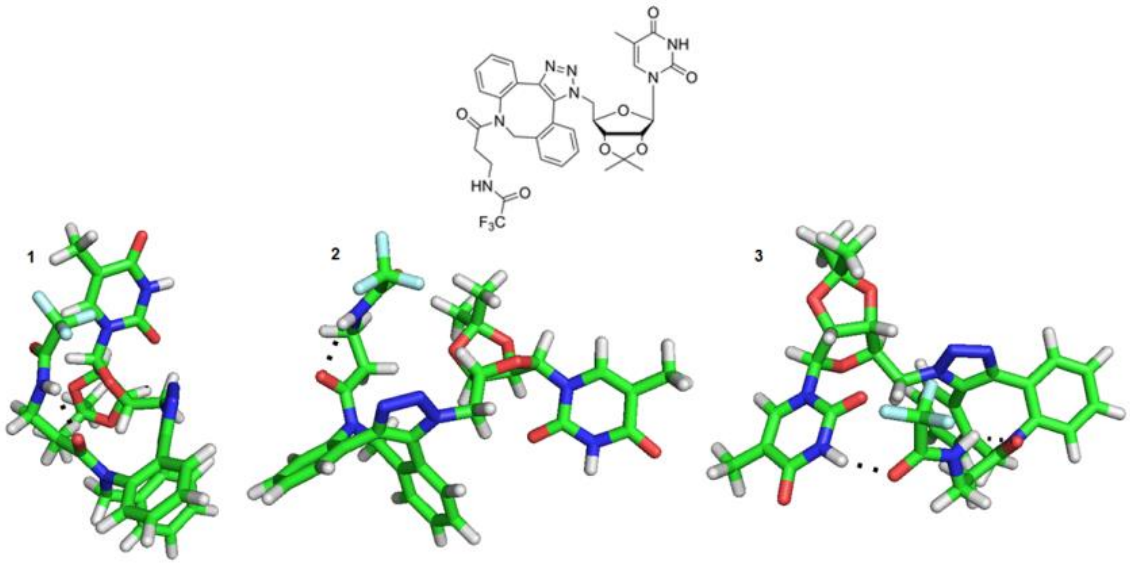


Figure 12: The most abundant conformers of derivative **15a**.[125]

The derivative **15b** was characterized by two predominant local minima with a total population of approximately 28% (Table 2). These two local minima, which differed only slightly in their combination of dihedral angles, exhibited a close position of the aliphatic chain and pyrimidine ring, although no hydrogen bond was observed between them (Figure 13-1 and 2).[125]

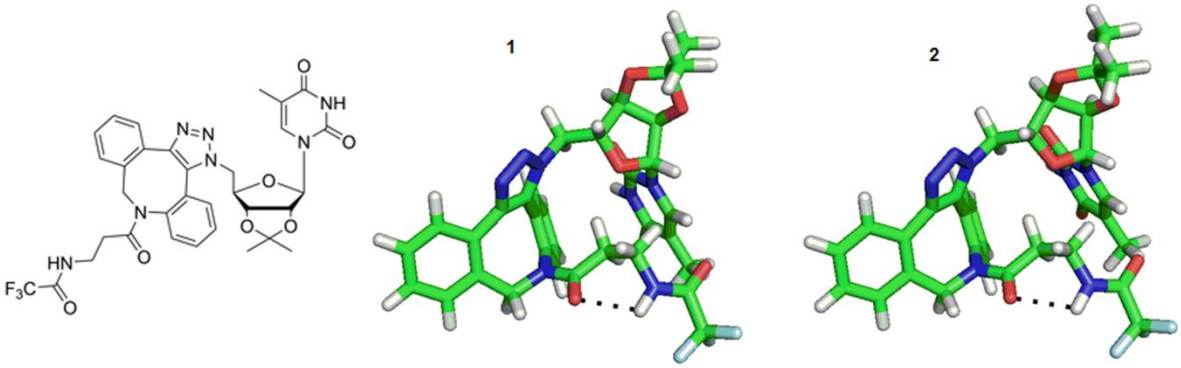


Figure 13: Conformation 1 and 2 of derivative **15b**.[125]

To elucidate the conformational changes and relationship among individual local minima, the pathways of the conformational changes with appropriate energy were determined. The pathways included all local minima of the appropriate derivatives with intrinsic energy lower than or equivalent to 30 kJ/mol (and a few intermediate states with higher energies).[125]

In derivative **14a,** the transition from local minimum 1 to 2 was connected with energy of 13.76 kJ/mol, whereas other changes required relatively high energy. Although the

transition between minima 2 and 3 required low energy, any transition to another local minimum involved overcoming a relatively high energy barrier. Thus, the transition to local minimum 4 and 5 was relatively complicated (Figure 14).[125]

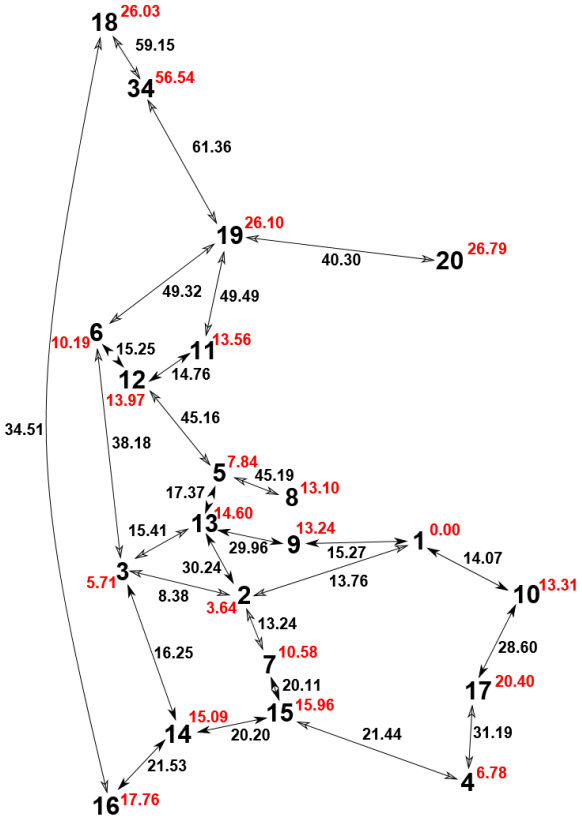


Figure 14: The transitions among the individual local minima of derivative **14a** characterized by adequate energies. The red numbers depict the intrinsic energies of individual local minima; black numbers describe the energy barriers for the conversion of local minima.

Identical diagrams were determined for structures **14b**, **15a** and **15b**. Similar to **14a**, the transitions among individual local minima were energetically demanding.[125]

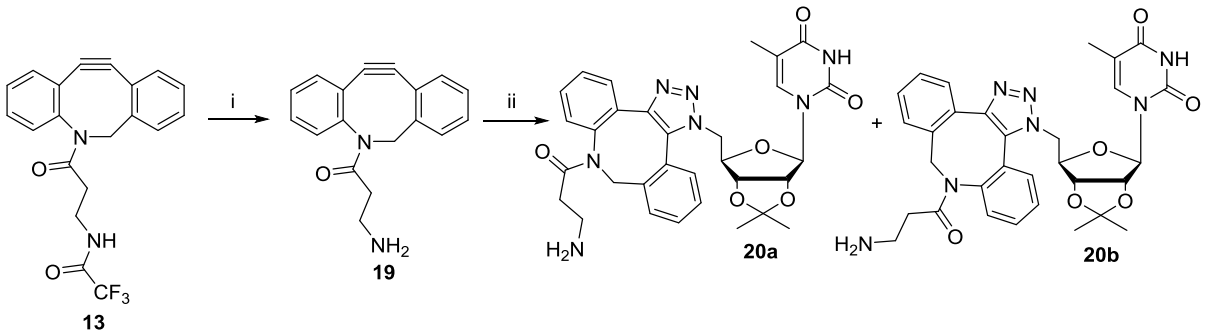
This conformational analysis using quantum mechanical investigations revealed that derivatives **14a/b** and **15a/b** can form more local minima on the PES, in which different interactions between the aliphatic chain (bearing a fluorine atom), uracil and ribose were observed, consistent with the differentiation of signals in the ^{1}H and ^{19}F NMR spectra.[125]

4.1.1.1.5. Effect of aliphatic chain on formation of conformers

To determine whether the presence of conformers depends on the length of aliphatic chain attached to aza-dibenzocyclooctyne, triazoles of aza-dibenzocyclooctyne bearing only hydrogen, trifluoroacetyl or *β*-alanine on nitrogen were prepared.

Triazoles with unprotected *β*-alanine on aza-dibenzocyclooctyne nitrogen **20a,b** were prepared by reaction of azide **6** with deprotected aza-dibenzocyclooctyne **19** (Scheme 5). Triazoles **20a,b** were separated with use of semipreparative HPLC in 20% yield.

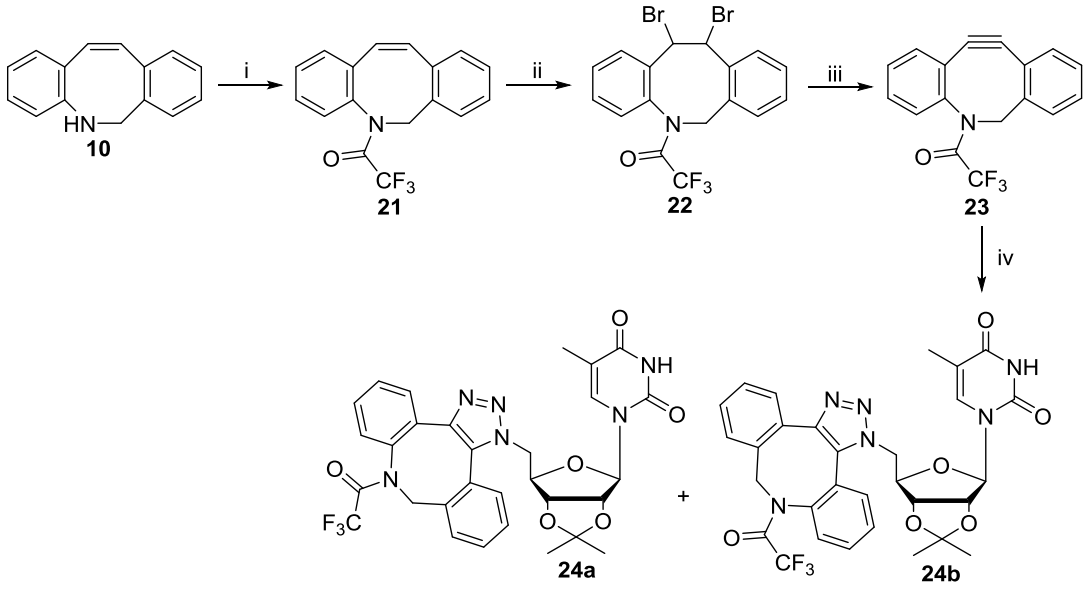
Scheme 5.: Preparation of triazoles **20a** and **20b**.[a]



[a]Reagent: (i) K_2CO_3, MeOH (ii) azide **6**, MeOH.

N-trifluoroacetyldibenzoazocine **21** was prepared by our developed procedure starting by trifluoroacetylation of dihydrodibenzoazocine **10** (Scheme 6) followed by dibromination with pyridinium tribromide. Further double dehydrobromination resulted in desired alkyne **23** together with product of trifluoracetyl deprotection, which was later identified as 6*H*-isoindolo[2,1-*a*]indole **25** (Scheme 7). This mixture was used for synthesis of triazoles **24a,b** which were successfully separated with the use of semipreparative HPLC.

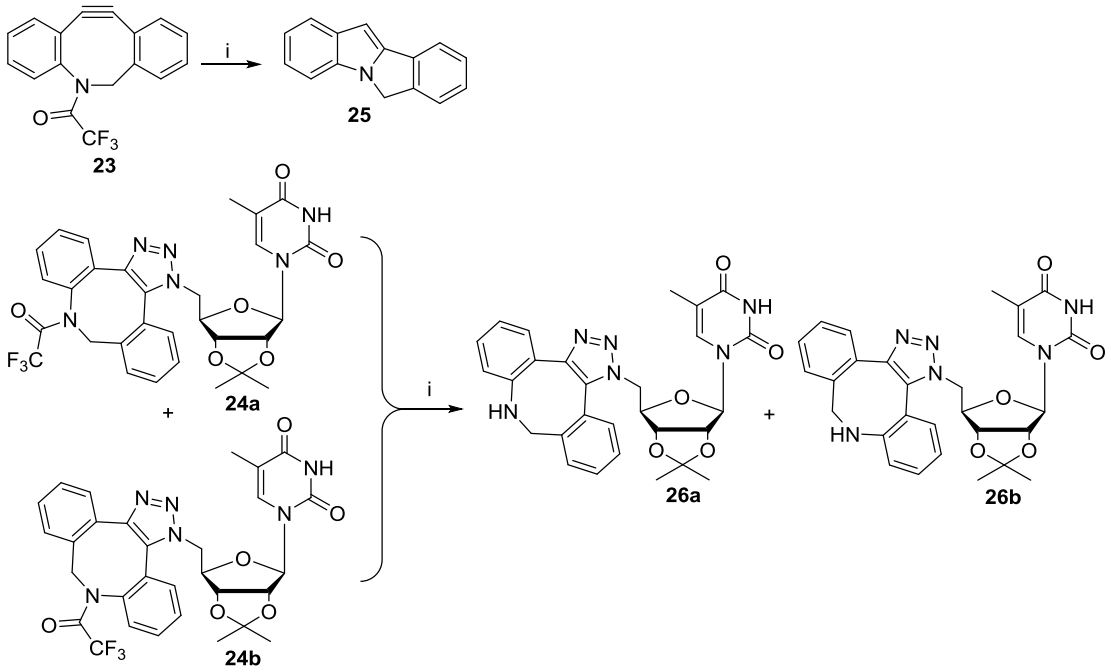
Scheme 6: Preparation of aza-dibenzocyclooctyne **23** and formation of triazoles **24a,b**.[a]



[a]Reagent: (i) TFAA, DCM (ii) pyridinium tribromide, DCM (iii) *t*BuOK, THF, 0 °C (iv) azide **6**, MeOH.

Next experiments were focused on preparation of triazoles with hydrogen on aza-dibenzocyclooctyne nitrogen. Direct deprotection of derivative **23** resulted in formation of 6*H*-isoindolo[2,1-*a*]indole **25** (Scheme 7) what corresponds to results described in the literature.[19] Due to this fact, desired triazoles **26a,b** were obtained by removal of trifluoroacetyl group from triazoles **24a,b** (Scheme 7).

Scheme 7: Preparation of triazoles **26a,b**.[a]



[a]Reagent: (i) K_2CO_3, MeOH.

^{1}H and ^{13}C NMR spectra of triazoles **20a,b**, **24a,b** and **26a,b**, showed, that number of isomers was not changed with reducing the aliphatic part of DIBAC. Similarly to compounds **15a**, triazoles **20a**, **24a** and **26a** exhibited presence of two isomers, triazoles **20b**, **24b** then exhibited presence of three isomers, in case of triazole **26b**, two isomers were formed. (Figure 15).

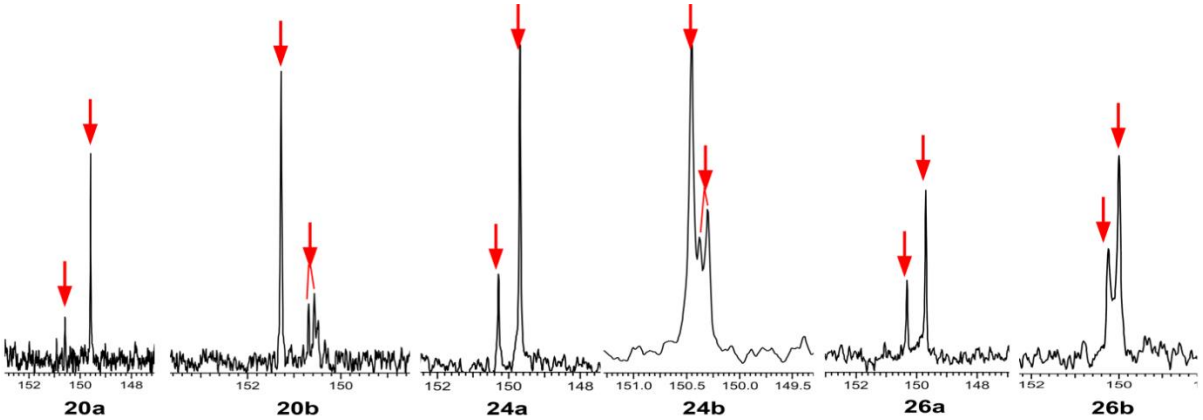


Figure 15: Isomery in ^{13}C Spectra.

These results indicated that the formation of conformers is not dependent on the length of aliphatic chain attached to aza-dibenzocyclooctyne nitrogen and that not only trifluoracetamido group is responsible for observed phenomenon. Isomery observed in case of triazoles **26a,b** thus shows, that probably also other type of interactions between aza-dibenzocyclooctyne and nucleoside parts of molecule will be involved in formation of individual conformers.

4.1.2. Solid phase synthesis of fluorescently labelled DIBAC analogues

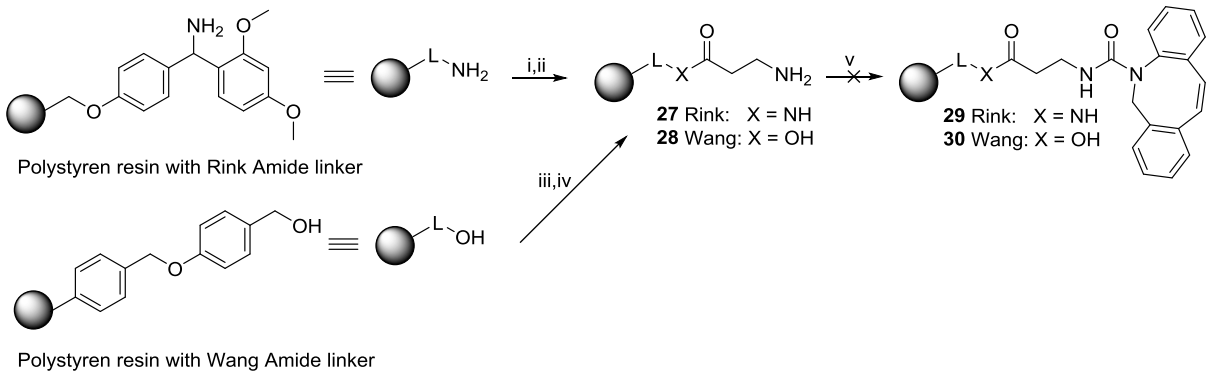
For the synthesis of **DIBAC** system bearing the fluorescent dye the solid-phase synthesis was suggested. The advantage of this approach lies especially in possible application of combinatorial chemistry for synthesis of systems with various type and length of the linker as well as various types of dyes. For the successful preparation of desired compound, it was firstly necessary to develop suitable method for immobilization of dihydrodibenzoazocine **10** to solid support and find out the conditions for formation of triple bond. This crucial step was supposed to be followed by incorporation of fluorescent label.

4.1.2.1. Immobilization of dihydrodibenzoazocine

Immobilization via urea linkage

The first approach for immobilization of dihydrodibenzoazocine was based on formation of urea linkage between solid supported *β*-alanine amino group and dihydrodibenzoazocine *via* CDI activation. For this purpose, model system with amino group from *β*-alanine **27** or **28** attached to the Rink Amide resin or Wang resin (Scheme 8) was used. Unfortunately, further reaction with CDI and azocine **10** to desired compound **29** or **30** did not proceed.

Scheme 8: Preparation of triazoles **26a,b**.[a]



[a]Reagent: (i) Fmoc-*β*-Ala-OH, HOBt, DIC, DMF, DCM (ii) = (iv) 50% piperidine in DMF (iii) Fmoc-*β*-Ala-OH, HOBt, DIC, DMAP, DMF, DCM (v) CDI, compound **10**, DCM.

Due to unsuccessful immobilization of **10** on solid support *via* urea linkage, reactivity of **10** with CDI was studied in detail in solution. Although the set of conditions with different bases, at elevated or at high temperatures including the use of microwave irradiation was tested, the desired product has never been obtained.

Immobilization via amide linkage

In the next approach, the attention was paid to immobilization of dehydrodibenzoazocine **10** as an amide of suitable solid supported carboxylic acid. For this intention, 4-fluoro-3-nitrobenzoic acid was chosen as a building block. Reactive fluorine atom allowed immobilization of acid to the solid support; nitro group can serve as a source of amino group for later incorporation of fluorescent label and carboxylic group can be used for attachment of azocine **10** (Figure 16).

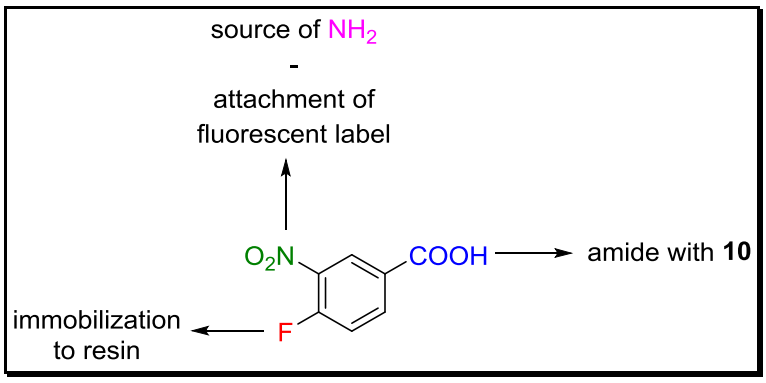
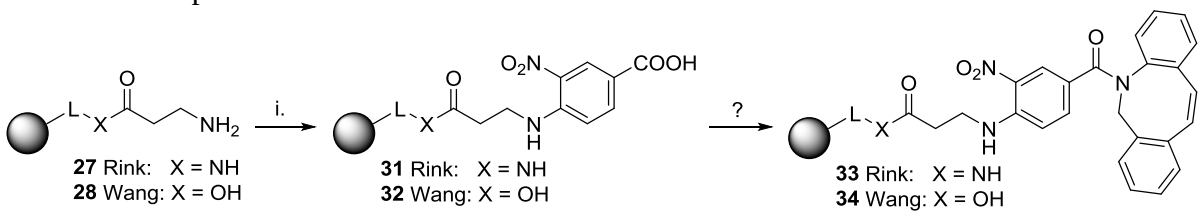


Figure 16: 4-fluoro-3-nitrobenzoic acid as a building block.

4-fluoro-3-nitrobenzoic acid was attached quantitatively *via* nucleophilic substitution of fluorine atom to amine of *β*-alanine **27** or **28** immobilized to the Rink Amide resin or Wang resin (Scheme 9). In the next step, conditions for formation of amide **33** and **34** were investigated.

Scheme 9: Preparation of amides **33** and **34**.[a]



[a]Reagent: (i) 4-fluoro-3-nitrobenzoic acid, pyridine, 75 °C.

Firstly, amide bond formation with use of coupling reagents was tested. At the beginning, combination of HOBt and DIC was chosen. Desired product was not formed and only starting material was presented. Addition of catalytic amount of DMAP led to formation

of 5% of product, however different temperatures and solvents did not significantly help with product yield (Table 3). Moreover, when DMF was used as a solvent or co-solvent, dimethylamide of carboxylic acid **31** or **32** respectively as the side product was formed at higher temperatures.

In the next experiments, DIC was changed to EDC reagent. Nevertheless, the yields of product were again very low and dimethylamide was formed at higher temperatures as well (Table 3). Then HOBt was changed to BOP reagent, but desired amine was not formed in any case (Table 3). The same results were obtained for modification of BOP called pyBOP, in addition, unknown side product was formed in high yield at elevated temperature (Table 3). Finally, the combination of reagent HATU with DIEA was tested, however only 5% of product was formed as a maximum (Table 3).

As the reactivity of carboxylic acids **31** and **32** was nearly identical, the results for both substrates are summarized in one table (Table 3).

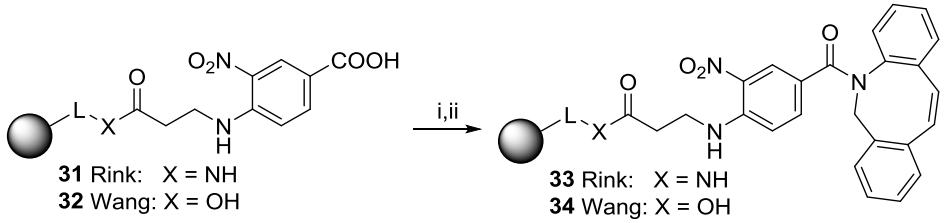
Table 3: Conditions for formation of amides **33** or **34**.

Reagents		**Base**	**Solvent**	**Temperature**	**[%][a] 31 or 32**	**[%][a] 33 or 34**	**[%][a] dimethylamide**
HOBt	DIC	-	DCM/DMF	rt	> 99	< 1	< 1
HOBt	DIC	DMAP	DCM/DMF	rt	~85	~5	< 1
HOBt	DIC	DMAP	DMF	40 °C	~75	~5	~10
HOBt	DIC	DMAP	DMF	80 °C	~15	~10	~65
HOBt	DIC	DMAP	DMSO	80 °C	~60	~25	< 1
HOBt	DIC	DMAP	DMSO	120 °C	~65	~10	< 1
HOBt	EDC	TEA	DMF	rt	~80	~5	< 1
HOBt	EDC	TEA	DMF	40 °C	~65	~5	~15
HOBt	EDC	TEA	DMF	80 °C	~55	< 1	~30
HOBt	EDC	DIEA	DMF	rt	~70	< 1	< 1
HOBt	EDC	DIEA	DMF	40 °C	~60	~10	~25
HOBt	EDC	DIEA	DMF	80 °C	~30	~5	~30
BOP	DIC	DMAP	DCM/DMF	rt	> 99	< 1	< 1
BOP	DIC	DMAP	DMSO	80 °C	> 99	< 1	< 1
BOP	DIC	-	DMF	rt	> 99	< 1	< 1
BOP	DIC	-	DMF	40 °C	50	< 1	~25
BOP	DIC	-	DMF	80 °C	< 1	< 1	~90
BOP	-	DIEA	DMF	rt	> 99	< 1	< 1
BOP	-	DIEA	DMF	40 °C	~55	< 1	~20
BOP	-	DIEA	DMF	80 °C	~10	< 1	~85
PyBOP	-	DIEA	DMF	rt	~75	< 1	< 1
PyBOP	-	DIEA	DMF	40 °C	~30	< 1	< 1
HATU	-	DIEA	DMF	rt	~55	< 1	< 1
HATU	-	DIEA	DMF	40 °C	~40	~5	~40

[a]Calculated form LC traces at 200 – 600 nm.

The use of coupling reagents has been proven to be inefficient, thus solid supported nitrobenzoic acid was converted to chloride and reacted with amine **10** (Scheme 10). With use of this method the desired product was obtained quantitatively in both cases. An excess of a base, such as DIEA, has to be used in chlorination step to avoid of undesired cleavage of material from resin by HCl forming during reaction.

Scheme 10: Preparation of amides **33** and **34**.[a]



[a]Reagent: (i) $SOCl_2$, DIEA, anhydrous DCM (ii) amine **10**, pyridine, anhydrous DCM.

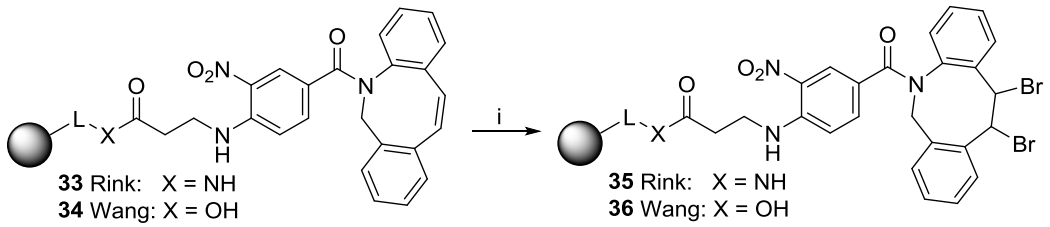
4.1.2.2. Triple bond formation

Formation of triple bond was suggested as two step synthesis based on bromination of azocine double bond followed by base mediated double dehydrobromination. This method was inspired by literature, where this sequence was performed in solution.[64]

Bromination of double bond

In the first experiments, conditions for solution-phase synthesis using pyridinium tribromide as a bromination agent were tested. However analysis of resin after reaction showed neither starting material nor product. Analysis of reaction solution then revealed cleavage of substrate from the resin during reaction. Addition of DIEA to the reaction mixture resulted in quantitative formation of dibromoderivatives **35** and **36** (Scheme 11). To achieve full conversion in preparative scale, the bromination had to be sometimes repeated.

Scheme 11: Preparation of dibromoderivatives **35** and **36**.[a]



[a]Reagent: (i) pyridinium tribromide, DIEA, DCM.

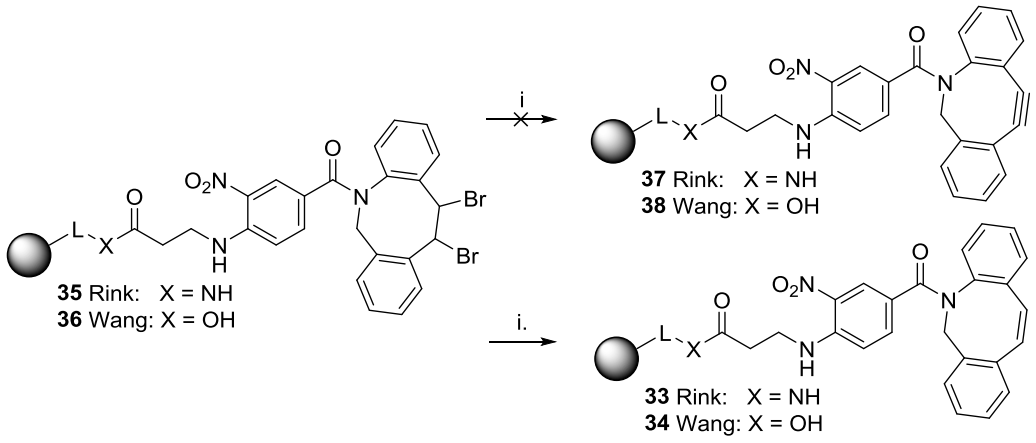
Formation of triple bond

Formation of triple bond in solution is frequently performed with use of strong base such as KOH[128] or *t*BuOK[87,129]. In the first experiments, *t*BuOK in different concentrations was tested. On Wang resin, partial dehydrobromination was observed after one hour and monobromalkene was observed as a main product. Unfortunately increase of reaction time led to progressive decomposition of material on resin. The same results were obtained also at low temperatures (- 30 °C). The use of these conditions on Rink resin resulted always in formation of mixture of compounds indicating decomposition of material on resin.

After that, set of organic bases (DIEA, DBU, proton sponge and BTTP) at different temperatures and solvents (anhydrous THF, DMF, DMSO, celosolve) were used. In case of material attached to Wang resin, partial debromination was observed in presence of DBU at room zemperature; reaction did not work for other base. At elevated temperature (60 °C), the best results were obtained for proton sponge in DMSO, where the reaction proceeded with 98% conversion with purity of the product 89% (Scheme 12).

The similar results were obtained also for Rink resin. In this case, the best result with 94% purity of product was obtained after treatment of **35** with BTPP in DMSO at 60 °C (Scheme 12). After optimization of the last step, the final products were prepared in preparative scale, cleaved from resin, purified with use of semipreparative HPLC and characterized. Surprisingly, ^{1}H and ^{13}C NMR spectra of both products did not confirm the alkyne structure. Instead of alkynes **37** and **38**, alkenes **33** and **34** were obtained after elimination step (Scheme 12), what indicates, that the dehalogenation instead of double dehydrohalogenation proceeds.

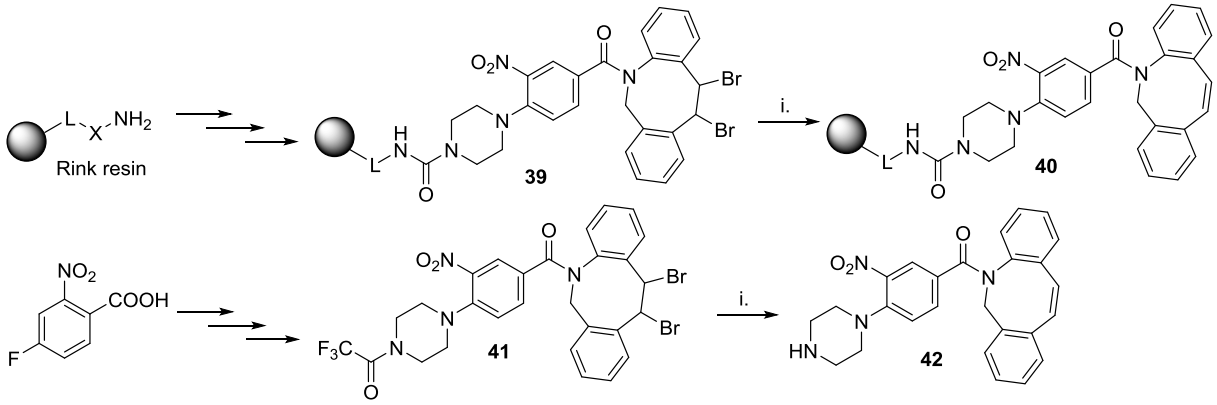
Scheme 12: Elimination of dibromoderivatives **35** and **36**.[a]



[a]Reagent: (i) Wang: proton sponge, DMSO, 60 °C; Rink: BTPP, DMSO, 60 °C.

To determine, if this course of elimination takes place only on solid phase or also in solution, the same dibromoderivatives were prepared by multistep synthesis (Scheme 13, for more detail see experimental part) and elimination in solution was tested. Alkene **40** was obtained after treatment of resin supported derivative **39** by BTTP or proton sponge, which corresponded to results received previously. In solution, both bases firstly induced removal of trifluoroacetyl group from piperazine followed by formation of deprotected alkene **42** (Scheme 13). These results confirmed preference of bases induced dehalogenation instead of dehydrohalogenation.

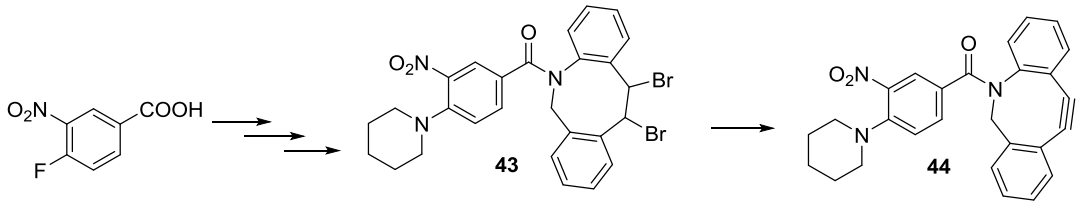
Scheme 13: Model dibromoderivatives **39** and **41** and products of elimination.[a]



[a]Reagent: (i) proton sponge, DMSO, 60 °C or BTPP, DMSO, 60 °C.

Because it was necessary to find other conditions, elimination of piperidine analogue of compound **41** (to avoid of side reactions, such as deprotection) was studied with use of different bases (Scheme 14). Desired alkyne **44** was obtained only after treatment of **43** by *t*BuOK at room temperature. The bases as NaOH and $Si(OMe)_3K$ were efficient only at 80 °C, but other compounds and impurities were formed during this reaction.

Scheme 14: Preparation of alkyne **44**.



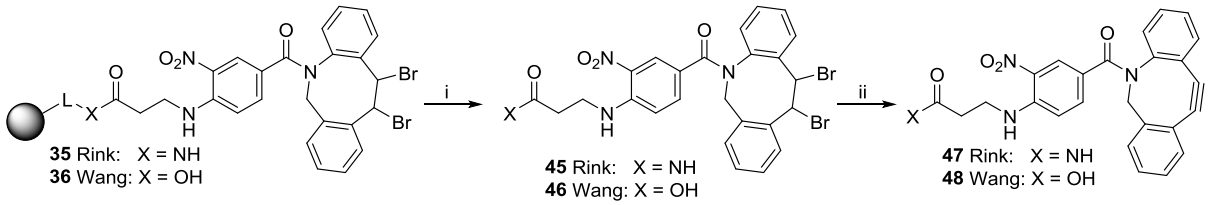
As a result, *t*BuOK was found to be the best base for formation of desired triple bond. However, on solid phase, decomposition of material on resin was observed (see above) after cleavage. We tested stability of **DIBAC** derivative **13** in cleavage cocktail used for releasing of compounds from solid support (50% TFA in DCM) and observed the decomposition of

substrate. Therefore we deduced that desired alkyne **37** or **38** respectively was formed by reaction with *t*BuOK, but it was destroyed during cleavage from resin.

To solve this problem, the use of different linkers allowing cleavage under **DIBAC** friendly conditions was tested. However problems with immobilization of dihydrodibenzoazocine **10** occurred. For example, in case of silyl linker, the desired compound was cleaved from resin during chlorination step; in case of hydrazone linker, it was not possible to immobilized dihydrodibenzoazocine **10** to solid support. Then, TentaGel resin cleavable by hydroxide solution was used. In this case, it was possible to prepare desired product, but in complicated mixture of compounds.

Alkynes **47** and **48** were finally prepared by *t*BuOK in solution after cleavage of dibromoderivative **35** or **36** respectively from resin (Scheme 15). In case of carboxylic acid derivative **48**, the product was obtained in 70 % purity; however the amide **47** was formed only in 25% purity. For this reason, synthesis of more complex compounds was done only on Wang resin.

Scheme 15: Preparation of azadibenzocyclooctynes **47** and **48**.[a]



[a]Reagent: (i) 50% TFA in DCM (ii) *t*BuOK, anhydrous THF.

4.1.2.3. Attachment of fluorescent label

After successful optimization of triple bond formation, the structure could be extended to fluorescent label. For this purpose, Rhodamine B (RhoB) was chosen as a label. Amino group obtained by reduction of nitro group of derivative **48** should have served for attachment of RhoB via aliphatic linker or RhoB itself (Figure 17).

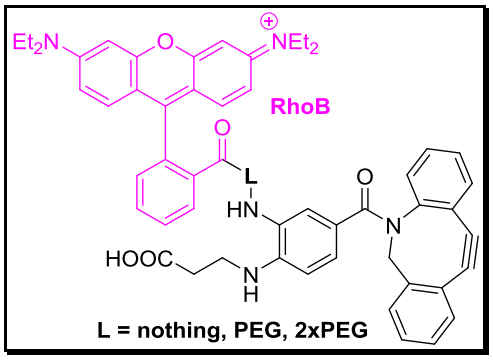
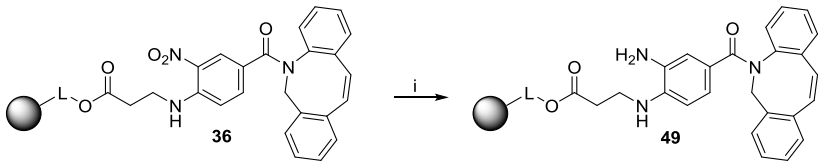


Figure 17: Structures of desired aza dibenzocyclooctynes with fluorescent label.

Reduction of nitro group

A methodology using sodium dithionite and phase transfer catalyst was chosen for reduction of nitro group. If tetrabutylammonium hydrogensulfate (TBAHS) was used as a phase transfer catalyst, formation of amine was observed but in mixture with one major impurity. Then TBAHS was changed to ethyl viologen diiodide and desired product was obtained nearly quantitatively (Scheme 16).

Scheme 16: Preparation of aminoderivative **49**.[a]

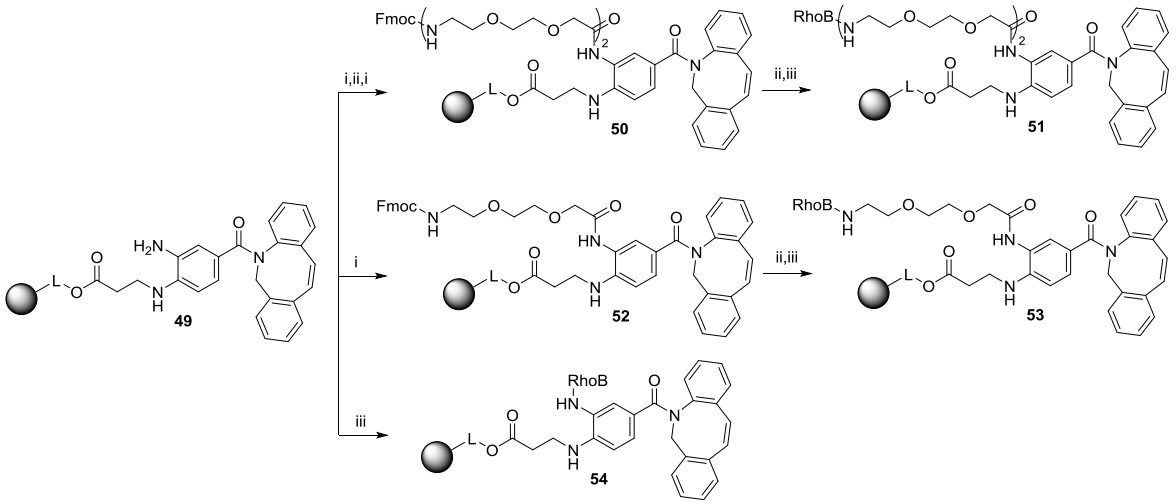


[a]Reagent: (i) Sodium dithionite, K_2CO_3, ethyl viologen diiodide, DCM/H_2O 1:1.

Pegylation of aminogroup and attachment of RhoB

For attachment of RhoB through aliphatic linker, amino group was acylated by Fmoc-*N*-PEG-COOH *via* HOBt/DIC method (Scheme 17). Desired products **50** and **52** were formed, however a side product with mass 18 units less were also occurred in significant amount. This product was probably a result of some kind of cyclization in obtained structure. After removal of Fmoc group from PEG linkers, RhoB was attached also *via* HOBt/DIC method using DMAP as a catalyst (Scheme 17). Compound **54** was obtained nearly quantitatively. Products **51** and **53** were formed as a mixture with cyclized side products analogically to previous step.

Scheme 17: Preparation of RhoB derivatives **51**, **53** and **54**.[a]

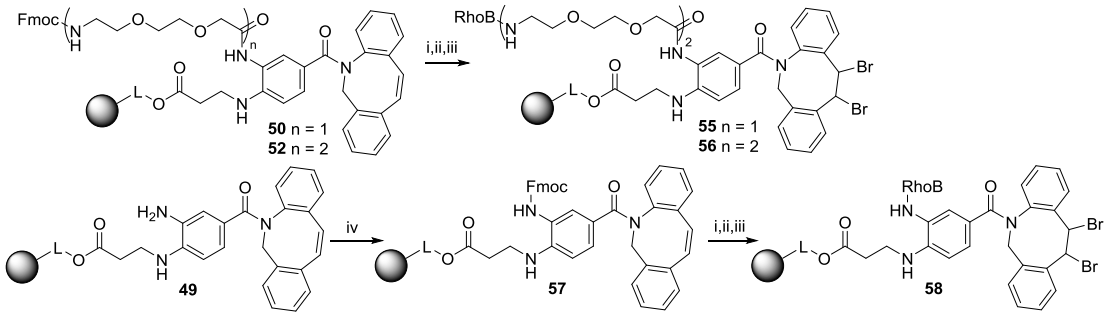


[a]Reagent: (i) FmocNH-PEG-COOH, HOBt, DIC, DIEA, DMF/DCM 1:1 (ii) 50% piperidine in DMF (iii) RhoB, HOBt, DIC, DMAP, DMF/DCM 1:1.

Bromination and formation of triple bond

Bromination of resin **51**, **53** and **54** was performed under standard conditions with pyridinium tribromide. Although desired product was obtained, its purity was decreased by presence of number of side products. The reason could lie in side reaction of pyridinium tribromide with RhoB part of molecule. For this reason, the reaction sequence was slightly modified. Quantitative bromination was carried out before rhodamine binding, it means on substrates **49**, **50** or **52** respectively. Acylation with RhoB was performed after that approximately in 70% purity (Scheme 18).

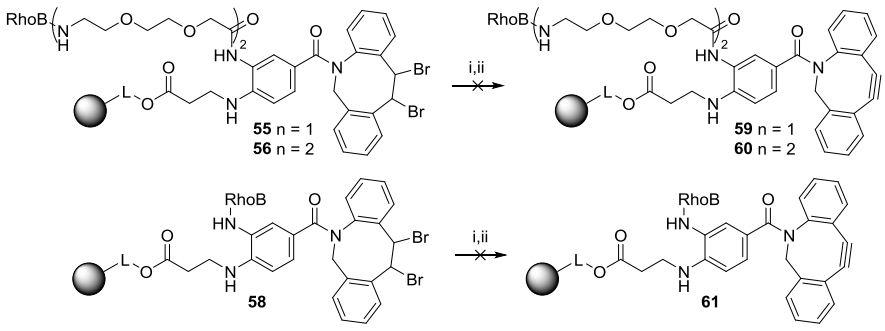
Scheme 18: Preparation of RhoB derivatives **55, 56** and **58**.[a]



[a]Reagent: (i) pyridinium tribromide, DIEA, DCM (ii) 50% piperidine in DMF (iii) RhoB, HOBt, DIC, DMAP, DMF/DCM 1:1 (iv) Fmoc-Cl, DIEA, DCM.

After successful preparation of dibromoderivatives **55**, **56** and **58**, formation of triple bond from these substrates could be performed. The dibromoderivatives were cleaved from resin, all solvents were evaporated and *t*BuOK in anhydrous THF was added. Unfortunately, cleavage of RhoB from all molecules occurred during reaction followed by total decomposition (Scheme 19). Changing of RhoB to other molecules such as coumarin-3-carboxylic acid or simple benzoic acid to compare the stability resulted in the same results. Thus it was not possible to prepare desired alkynes **59 - 61** or the analogues with another dye by this methodology.

Scheme 19: Preparation of RhoB derivatives **59 - 61**.



[a]Reagent: (i) 50% TFA in DCM (ii) *t*BuOK, anhydrous THF.

Isolation of alkenes

Although the synthesis of aza-cycloalkynes for SPAAC was unsuccessful, the alkene intermediates can be utilized for different type of biorthogonal reaction such as alkene ligation. For this reason, resin bound alkenes **51**, **53** and **54** were prepared in preparative scale, cleaved from resin, purified with use of semipreparative HPLC and characterized. According to LC/MS analysis, cyclized forms **62** and **63** of desired products from **51** and **53** (Figure 18) predominated. The final structure of obtained compounds was determined according to NMR spectra. It was found out, that cyclization takes place between carbonyl group of *o*-phenylendiamine amide and the adjacent amino group to form benzimidazole structures **62** and **63** (Figure 18). In addition, NMR spectra also showed that RhoB is attached in closed form in all cases (Figure 18).

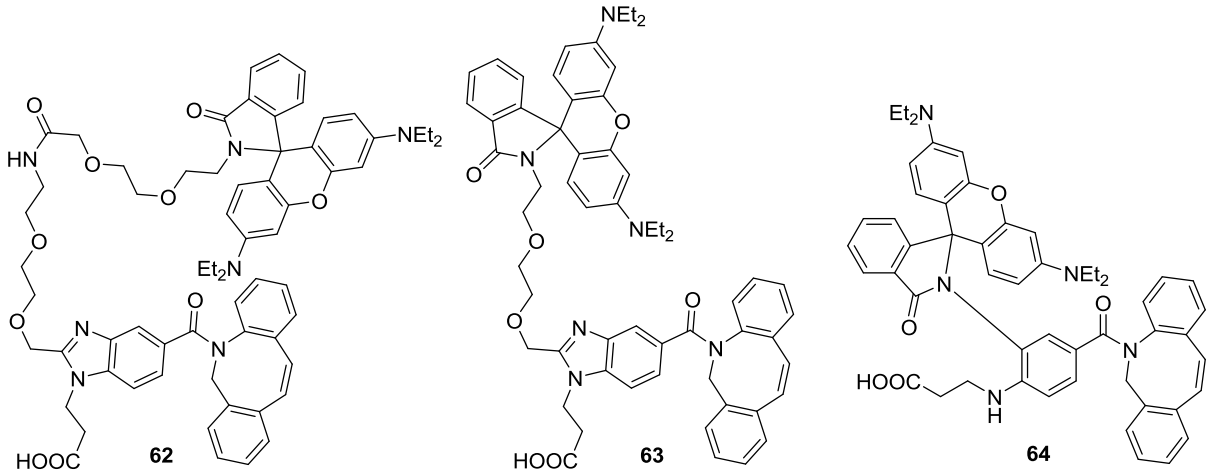


Figure 18: Structures of **62**, **63** and **64** determined by NMR spectra.

4.2. 1,2,3-selenadiazoles

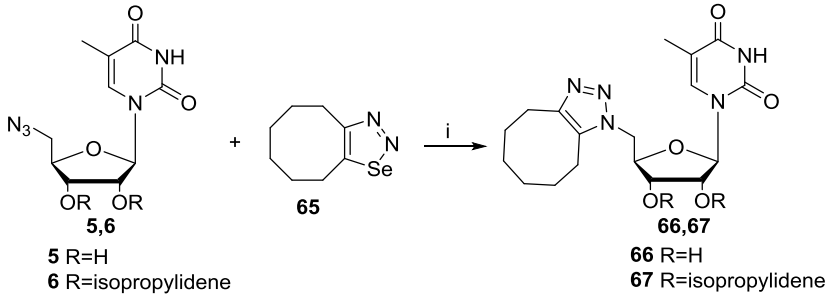
This part of work is focused on thermally, microwave assisted and photochemically induced conjugation reaction of azides and cycloalka-1,2,3-selenadiazoles as a precursors of cycloalkynes. First part deals with reactions of cycloocta[d][1,2,3]selenadiazole. Other parts are focused on preparation of new cycloalka-1,2,3-selenadiazoles and their application in reactions with azides.

4.2.1. Reactions of cycloocta[d][1,2,3]selenadiazoles

Within author´s diploma theses was found out, that cycloocta[d][1,2,3]selenadiazole **65**, prepared according to described procedure,[130] heated in mixture with azide **5** and **6** can form corresponding triazole **66** and **67** (Scheme 20).[122] However, to perform the reaction, the

high temperature has to be used. The decreasing of temperature led to significant increase of reaction time (Table 4).[122]

Scheme 20: Reactions of azides **5** and **6** with 1,2,3-selenadiazole **65**.[122,a]



[a]Reagent: (i) NMP, ΔT.

Table 4: Reaction times for full conversion of azides at different temperatures.[122]

	FULL CONVERSION OF AZIDE				
T (°C)	180	150	130	110	90
TIME	10 min	1 h	20 h	20 h	48 h

4.2.1.1. Microwave assisted conjugation reactions of cycloocta[d][1,2,3]selenadiazole

Due to long reaction time required for decomposition of cycloocta[d][1,2,3]selenadiazole **65** at temperatures below 110 °C, the use of microwave irradiation was tested to accelerate the reaction. In the first experiment, the mixture of azide **5** or **6** and 1,2,3-selenadiazole **65** (1.5 equiv.) in NMP was used. The temperature was set to 110 °C and conversion of azide was monitored using HPLC/MS each ten minutes. The effect of MW power and also effect of reaction mixture concentration was compared (Table 5).

Table 5: Reaction times for full conversion of azides **5** and **6** into triazoles **66** and **67** under microwave irradiation.

	FULL CONVERSION OF AZIDES 5 and 6								
T (°C)	110								
c_{azide} (mg/ml) [a]	10			2			0.4		
POWER (W)	50	100	150	50	100	150	50	100	150
TIME (min)	110 - 130			110 - 120			120		

[a] cycloocta[d][1,2,3]selenadiazole was used in 1,5 equivalent.

In comparison to standard heating discussed above, the significant decrease of reaction time to approximately 2 hours at 110 °C was observed if microwave was used. Surprisingly no difference among used microwave power and concentration of reaction mixture occurred.

On the other hand effect of excess of cycloocta[d][1,2,3]selenadiazole **65** (Table 6) was observed. Reactions with concentration of azides 2 mg/ml were tested at 110 °C with different excess of cycloocta[d][1,2,3]selenadiazole (Table 6).

Table 6: Effect of amount of cycloocta[d][1,2,3]selenadiazole on reaction time at 110 °C.

	FULL CONVERSION OF AZIDE 5 and 6			
c_{azide} (mg/ml)	2			
POWER (W)	150			
SELENADIAZOLE	1.5 eq	2 eq	3 eq	4 eq
TIME (min)	120	120	70	70

The results showed, that there is no difference between the 1.5 and 2 equivalent of cycloocta[d][1,2,3]selenadiazole **65**. However, increase in amount of 1,2,3-selenadiazole **65** to three or four equivalent led to decrease in time nearly to half (70 min).

Finally, the optimal conditions were chosen (2 mg/ml of azide **5** or **6**, three equivalents of cycloocta[d][1,2,3]selenadiazole, 150 W) according to results discussed above and used for study of this reaction at lower temperatures. The reaction conversion was monitored after 70 minutes. Obtained values were compared with cycloocta[d][1,2,3]selenadiazole **65** full conversion at 110 °C (Table 7). Similarly to results described above, the temperatures below 110 °C dramatically reduced the conversion of azide, thus the reaction time required for its full transformation to desired triazole would be non-acceptably long

Table 7: Conversion of azides at different temperatures.

T (°C)	110	90	70	50
Conversion after 70 min (%)	100	25	15	8

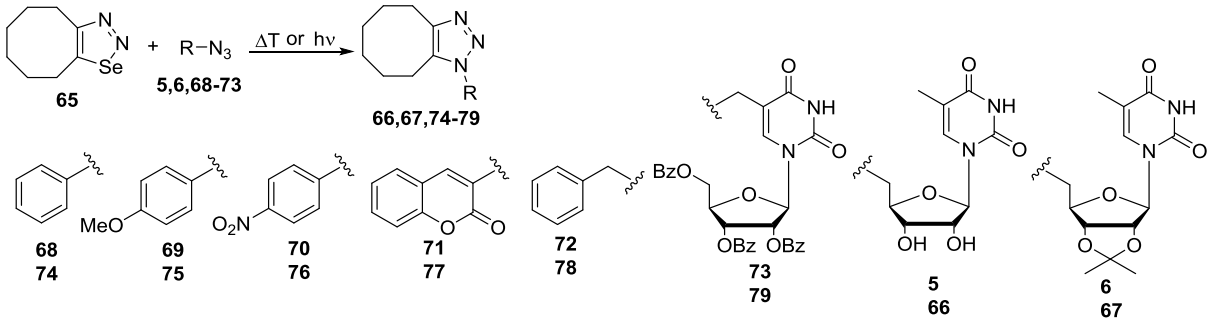
4.2.1.2. Photochemically induced conjugation reactions of cycloocta[d][1,2,3]selenadiazole

Photochemically-induced non-catalyzed conjugation reaction of cyclooctyne generated in situ from cycloocta[d][1,2,3]selenadiazole **65** with structurally different azides **5**, **6**, **68-73** (Scheme 21) was studied. [131] The azides represent substrates possessing different electronic and steric properties.

Azides **68-70**[132] and **71**[126] were synthesized from the corresponding amines. Benzyl azide **72**[133] was prepared from benzylchloride. The analytical standards, triazoles **66**, **67** and **74-79**, were prepared by heating a mixture of

cycloocta[d][1,2,3]selenadiazole **65** and the corresponding azide **5**, **6** and **68-73** (Scheme 21).[131]

Scheme 21: Non-catalyzed conjugation reactions of cycloocta[d][1,2,3]selenadiazole **65**.[131]



The irradiation wavelength had to be carefully chosen for each pair of reactants. As the absorption spectrum of the principal chromophore cycloocta[d][1,2,3]selenadiazole **65** partially overlaps with those of the azides **5**, **6**, **68-73** as well as the photoproducts **66**, **67**, **74-79**, the undesired light absorption by the azides should have been avoided or at least largely suppressed. The absorption tail of the starting material **65** allowed us to use irradiation wavelengths in range approximately 300-325 nm (Figure 19).[131]

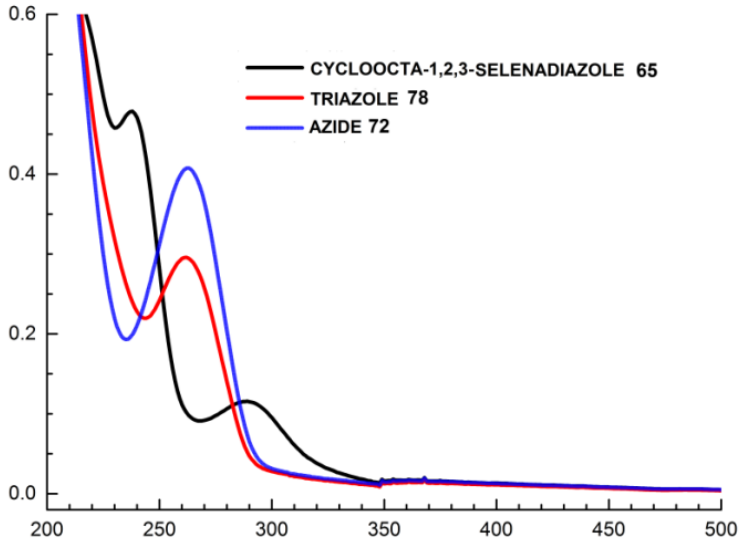


Figure 19: Absorption spectra of 1,2,3-selenadiazole **65**, azide **72** and triazole **78** in methanol.[131]

The benzylazide **72** has been selected as a reagent for an initial photochemical reaction study because it shows no absorption above 300 nm. During the irradiation of equimolar amounts of **65** and **72** in methanol ($c = 3 \times 10^{-3}$ mol dm^{-3}) at 313 nm, a red

solid precipitated. According to UV/VIS spectrum having absorption maximum at λ_{abs} of approximately 500 nm, the solid was identified as selenium nanoparticles with a diameter of approximately 180 nm[134] (Figure 20), which may become an important internal optical filter.[131]

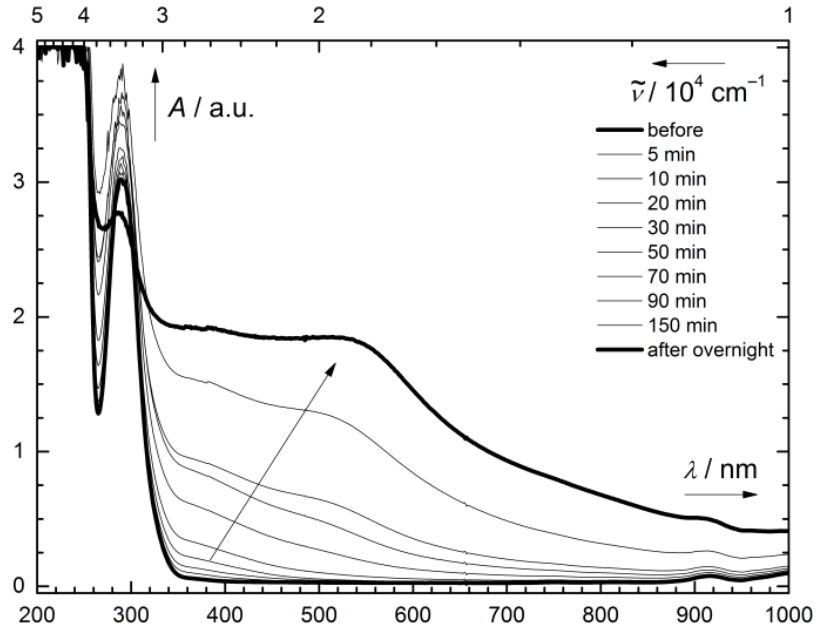


Figure 20: Change of absorption spectra of 1,2,3-selenadiazole **65** and benzylazide **72** in methanol upon irradiation with medium-pressure Hg arc filtered through a 313 nm filter.[131]

Upon exhaustive irradiation, triazole **78** was found to be the major product formed in a 28% yield, whereas 62% of azide **72** remained unreacted in the solution according to GC/MS analysis. Due to the thermal instability of the 1,2,3-selenadiazoles,[38] GC/MS could not be used to determine the concentration of the unreacted derivative **65**. Therefore, NMR was used to monitor reaction progress.[131]

Upon irradiation of a solution of **65** (c ~ 0.03 mol dm^{-3}) and **72** (c ~ 0.03 mol dm^{-3}) in methanol-d_4 in a NMR tube (Pyrex glass; λ > 280 nm) by a medium-pressure Hg lamp for 2 h, the conversion reached 38%, and the triazole **78** was a major product.[131] The product formation was evident from appearance of the new triplet signals at 2.81 and 2.94 ppm assigned to the methylene group of the triazole derivative and a decreased signal intensity for the methylene groups of 1,2,3-selenadiazole **65** at 3.32 ppm (Figure 21). The ^{1}H NMR spectrum of the product obtained by irradiation corresponds to that of the isolated triazole **78** prepared by thermolysis.[131]

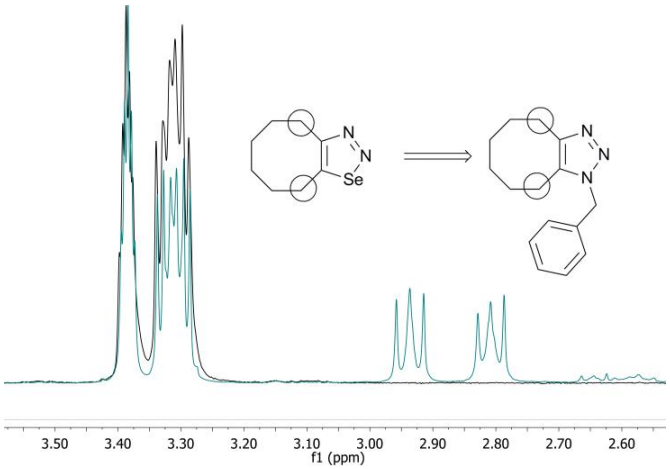


Figure 21: A detail of the ^{1}H NMR spectra of a mixture of 1,2,3-selenadiazole **65** and triazole product **78** before irradiation (black line) and after 2 h of irradiation (blue line).

To detect cyclooctyne as a possible primary photoproduct, irradiation of cycloocta[d][1,2,3]selenadiazole **65** in methanol-d_4 (c ~ 0.04 mol dm^{-3}) was performed for 1 h.[131] The ^{1}H NMR showed the signals at 1.64 and 2.10 ppm, what corresponds to the spectrum of cyclooctyne reported in the literature.[135] A new signal in the ^{13}C NMR spectrum at 93.8 ppm and three signals in the range of 20–35 ppm (Figure 22) were also attributed to cyclooctyne in accordance with the published data.[136]

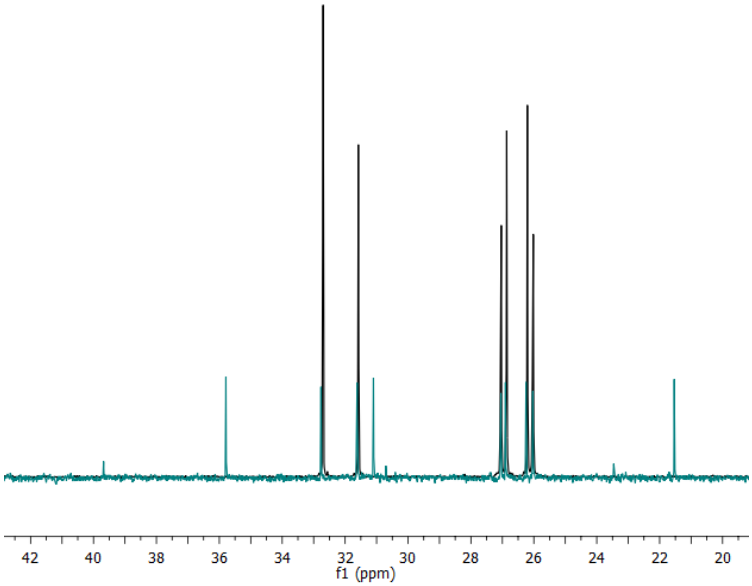


Figure 22: A detail of ^{13}C NMR spectra of cycloocta-1,2,3-selenadiazole **65** before (black line) and after irradiation (blue line) with a medium-pressure Hg arc filtered through a Pyrex glass of the NMR tube.[131]

The cyclooctyne half-life in the mixture with azide **72** was estimated to be approximately 40 min).[131] The quantum yield[3] of triazole **78** formation upon irradiation of a methanolic solution of the 1,2,3-selenadiazole **65** (c = 1.9 × 10^{-3} mol dm^{-3}) in the presence of **72** (c = 6.1 × 10^{-3} mol dm^{-3}) at λ_{irr} = 313 nm was determined to be Φ (**78**) = 0.10 ± 0.05 using valerophenone[137,138] as an actinometer.[131]

To determine the multiplicity of the excited state involved in the cyclooctyne formation, the influence of oxygen as a triplet quencher on the reaction rate was studied.[131] Degassed, aerated and oxygenated solutions of **65** and **72** in methanol-d_4 were irradiated using a medium-pressure Hg lamp in an NMR tube. The changes of the concentration of **65** at different reaction conversions were followed by ^{1}H NMR. The presence of oxygen evidently, but not significantly, slowed down the reaction, thus the productive triplet-excited state lifetime must be relatively short and its reaction competes with a diffusion-limited quenching, or the singlet state is also involved in the reaction.[131]

In addition, sensitization of **65** by triplet-excited benzophenone was performed to find whether the same process occurs in the triplet manifold. Irradiation of a degassed solution of **65**, **72** and benzophenone in methanol resulted in the formation of **78** (Table 8) in substantially higher yields compared to those observed when the same reaction mixture was purged with oxygen.[131] The irradiation wavelength was 366 ± 1 nm to ensure that benzophenone is the only absorbing species. Table 8 also demonstrates that oxygen quenched the sensitized production of **78**. Such a result supports our assumption that **78** is produced at least partially from triplet-excited **65**.[131]

Table 8. Sensitization of **65**[a]

Irradiation time (h)	Yield of[a] **78**/%	
	O_2[b]	N_2[c]
2	4	7
16	24	41

[a] **65** (c ~ 0.03 mol dm^{-3}), **72** (c ~ 0.03 mol dm^{-3}) and benzophenone (c ~ 0.3 mol dm^{-3}) in methanol-d_4 irradiated at 366 nm; the yields were determined by ^{1}H NMR. [b] Sample was purged with oxygen for 10 min. [c] Sample was purged with nitrogen for 10 min.[131]

[3] Estimation of cyclooctyne half-life and quantum yield was performed by Peter Šebej from Department of Chemistry and RECETOX, Faculty of Science, Masaryk University, Brno, Czech Republic; Present address: Department of Chemistry, Columbia University, New York, USA.

The photolysis of an equimolar mixture of **65** and the corresponding azide **5**, **6**, **68-71**, **73** (c ~ 0.03 mol dm^{-3}) was carried out afterwards, and the reaction conversion was monitored by NMR (see Table 9, Method I).[131] The lowest triazole yield (6-8%) was observed for 3-azidocoumarine **71**, which has been reported to be photolabile.[139] For the other azides **68-70** and **73**, triazole yields were 11−39%. Further irradiation did not increase the conversion, which may indicate a role of selenium as an internal filter and potential side photoreactions of the azides known from literature.[140-142]

Table 9: Conversions of cycloocta-1,2,3-selenadiazole **65** after irradiation with azides **5**, **6**, **68-71** and **73**.[131]

	CONVERSION (%)					
	METHOD I			METHOD II		
Azide	**A**[*]	**B**[*]	**C**[*]	**D**[*]	**E**[*]	**F**[*]
5	32	35	37	12	22	24
6	23	28	39	-	-	-
68	11	11	11	15	17	17
69	22	24	24	17	28	28
70	30	31	32	12	14	14
71	6	8	8	18	23	23
73	6	22	32	7	12	17

[*]A Conversion was measured directly after 1 h irradiation of a mixture of **65**and azide
[*]B Conversion was measured after 1 h of irradiation of a mixture of **65** and azide and subsequent 2 h keeping in dark
[*]C Conversion was measured after 1 h of irradiation of **65** and azide and subsequent 16 h keeping in dark
[*]D Conversion was measured directly after 1 h of irradiation of **65** and subsequent addition of azide
[*]E Conversion was measured after 1 h of irradiation of **65**, subsequent addition of azide and keeping the mixture in dark for 2 h
[*]F Conversion was measured after 1 h of irradiation of **65**, subsequent addition of azide and keeping the mixture in dark for 16h

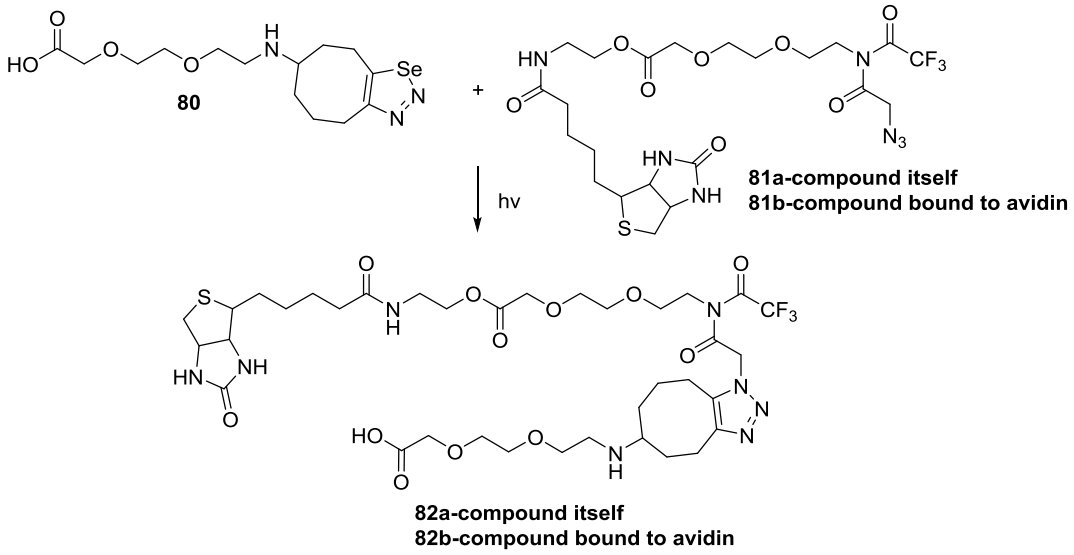
To enhance the reactivity of cycloocta-1,2,3-selenadiazole **65** by avoiding inadvertent photolysis of the azides, a solution of **65** in methanol-d_4 was initially irradiated for 1 hour to form cyclooctyne, and then an equimolar amount of the corresponding azide **5**, **6**, **68-71** and **73** was added.[131] Subsequently, the mixture was kept in the dark, and the reaction conversions were monitored by NMR (Table 9, Method II). Surprisingly, the triazole yields were similar or lower compared to those previously determined. The only exception was the reaction of azide **71**, where the post-irradiation addition of this azide prevented its photodecomposition and the total yield of triazole increased. In all cases, the starting cycloocta-1,2,3-selenadiazole **65** always remained in the reaction mixture. Because of selenium internal filter formation

we were not able to further increase the yield of the triazoles under the conditions used.[131]

To demonstrate the applicability of cycloocta-1,2,3-selenadiazole photoconjugation reaction with azides, labeling of biomolecules was studied. Selenium nanoparticles, which are formed during the reaction, have already been proven to be nontoxic,[143] what meets the basic requirements for conjugation reactions applied in molecular biology.[131]

First we prepared a modified derivative of cycloocta[d][1,2,3]selenadiazole **80** having a PEG moiety for its better water solubility and the azidobiotine **81a** (Scheme 22, the synthesis is described in experimental part) as model reaction partners and performed the photoinduced conjugation.[131]

Scheme 22: Conjugation reaction of modified cycloocta-1,2,3-selenadiazole **80** and appropriate azido derivatives **81a** and **81b** .[131]



The reaction mixture was irradiated with a medium-pressure Hg arc filtered through the Pyrex glass ($\lambda > 280$ nm) of an NMR tube for 1 h. The ^{1}H NMR analysis showed the formation of new signals at approximately 5.80 ppm (Figure 23) and a decrease in the signal intensity of azidomethylene group in the starting material **81a** at approximately 3.42 ppm. The MS spectrum of the reaction product provided evidence for the formation of triazole **82a**.[131]

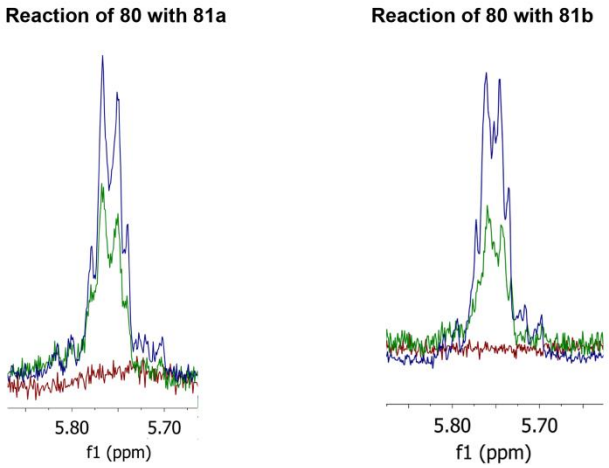


Figure 23: A details of the ^{1}H NMR spectra of the mixture of modified biotin **81a/81b** with cycloocta-1,2,3-selenadiazole **80** before reaction (red line), after 1h irradiation (green line) and subsequent 1h irradiation (blue line).

The reactivity was then tested on modified protein **81b** as a biomolecule representative. Complex **81b** was prepared by mixing of avidin with modified biotin **81a** (c ~ 0.013 mol dm^{-3}).[131] The concentration of **81a** was higher than corresponds to the binding capacity of avidin to allow monitoring of the reaction course by an NMR analysis of the non-bound biotinylated azide **81a** present in reaction mixture in sufficient concentration. After the addition of 1,2,3-selenadiazole **80**, the reaction mixture was irradiated with a medium-pressure mercury arc filtered through the Pyrex glass of an NMR tube for 1 h. In ^{1}H NMR spectrum we observed the same changes as in reaction without avidin (Figure 23).[131]

Further evidence for the reaction on avidin-bound azide **81b**, was brought by MALDI[4] analyses of the modified protein, where the corresponding appearance of higher-mass compound was observed. As the binding constant of avidin-biotin is very high at room temperature (K_D = 10^{-15} mol dm^{-3}),[144] we assume that the reaction of azide immobilized by avidin proceeded on a bound substrate and not on a substrate temporarily released from the protein.[131]

Interestingly the conversion of 1,2,3-selenadiazole **80** for both reactions with substrates **81a** and **81b** (bound and non-bound to avidine) was possible to enhance by the irradiation for additional one hour.[131] The intensity of the newly formed signals described above significantly increased (about 2-fold), whereas the signals at

[4] MALDI analysis was performed by Tomáš Oždian from Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University Olomouc, Olomouc, Czech Republic.

approximately 3.17, 3.44 and 4.35 ppm nearly disappeared. We assume that the conversion in this case was higher, because the selenium as internal filter was formed in low amounts because of lower concentration of selenadiazole.[131]

According to these observations, the conjugation reaction between the cyclooctyne analogue obtained from the corresponding 1,2,3-selenadiazole derivative and the biotinylated azide immobilized by avidin proceeds successfully under photoirradiation at room temperature.[131]

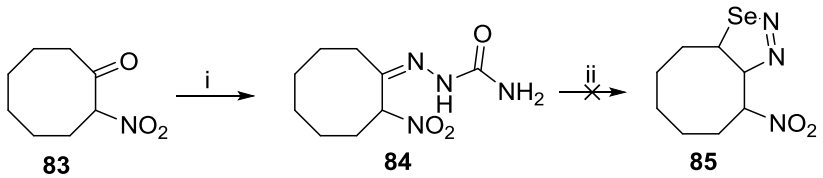
4.2.2. Preparation of derivatives of cycloocta-1,2,3-selenadiazole

This part of work was focused on preparation of substituted cycloocta[d][1,2,3]selenadiazoles bearing substituents possessing different electronic properties at position 4. Thermally induced conjugation reactions of these derivatives were then studied and influence of substituents should have been evaluated in comparison to unsubstituted cycloocta[d][1,2,3]selenadiazole **65**.

4.2.2.1. 4-nitro-cycloocta[d][1,2,3]selenadiazole

The strategy for synthesis of substituted cycloocta[d][1,2,3]selenadiazoles was same as synthesis of other known 1,2,3-selenadiazoles – transformation of corresponding ketone to semicarbazone and subsequent cyclization with selenium dioxide. Thus, 2-nitrocyclooctanone **83** was prepared by two-step synthesis described in literature.[145] Following reaction with semicarbazide hydrochloride under basic conditions in refluxing mixture of EtOH/H_2O 3:5 (v/v) totally failed. For this reason, acidic conditions were used, and 2-(2-nitrocyclooctylidene)hydrazine-1-carboxamide **84** was obtained quantitatively (Scheme 23).

Scheme 23: Preparation of 4-nitro-cycloocta[d][1,2,3]selenadiazole **85**.[a]



83 **84** **85**

[a]Reagent: (i) semicarbazide hydrochloride, 50% AcOH in H_2O (ii) SeO_2, dioxane, H_2O or SeO_2, AcOH.

Surprisingly, final cyclization to 1,2,3-selenadiazole **85** was proved to be problematic. Firstly, cyclization in aqueous dioxane was tested. At room temperature, only starting material **84** together with small amount of impurities was presented in reaction mixture. Heating of

reaction solution to 50 °C resulted in mixture of starting material **84** and unknown compound in ratio 1:1. Many impurities were also formed. Additional heating to 80 °C then led to formation of complicated mixture of compounds. Desired product **85** was not formed in any case.

Then, cyclization was also tested in acetic acid. Similarly to reaction in dioxane, 1,2,3-selenadiazole **85** was not obtained. Reaction mixture consisted from starting material and unknown compound in ratio 1:1; other impurities were also presented. The same unknown compound as above was isolated from reaction mixture by column chromatography and characterized. Surprisingly ^{13}C NMR showed presence of keto-group instead of nitro group suggesting, that nitro group was oxidize by selenium dioxide to corresponding ketone **86** (Figure 24). The proposed structure of compound **86** was then confirmed also by HRMS analysis.

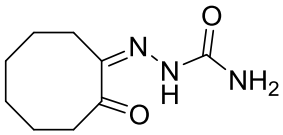
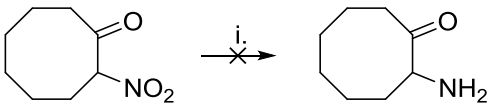


Figure 24: Structure of compound **86**.

4.2.2.2. 4-amino-cycloocta[d][1,2,3]selenadiazole

After unsuccessful preparation of 4-nitro-cycloocta[d][1,2,3]selenadiazole, the effort was focused on the synthesis of its amino analogue 4-amino-cycloocta-1,2,3-selenadiazole. 2-nitrocyclooctanone **83** was used as a starting material again. It was necessary to find out, which phase of synthesis is the best for crucial reduction of nitro group to amino group. Firstly, reduction as a first step of synthesis was studied (Scheme 24).

Scheme 24: Preparation of 2-aminocyclooctanone **87**.[a]



83 **87**

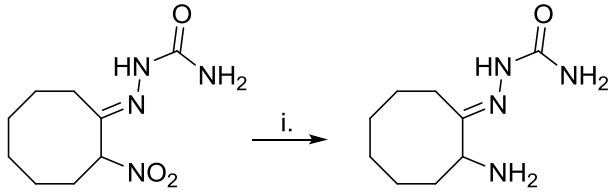
[a]Reagent: (i) H_2, Pd/C, MeOH or EtOAc.

The reduction was performed by hydrogen under Pd/C catalysis in MeOH or EtOAc (Scheme 24). However, mixture of unknown compounds was obtained in all cases and desired product **87** has never been observed. This result might be caused by side reactions of carbonyl and amino groups.

Thus, in next approach, protection of carbonyl group was suggested to prevent the side reactions. Firstly, ethylenglycol was chosen for ketal formation. Although different methods were tested, desired product was not obtained. Then, MeOH with sulphuric acid was used for preparation of corresponding dimethylketal. Reactivity was observed, but desired product was not detected either on LC/MS or on NMR. The reason could be in acidic cleavage of α-nitroketones described by Feuer and Pivawer in 1969.[146] Due to mentioned facts we decided to perform reduction after formation of semicarbazone **84**.

The reduction of semicarbazone **84** was tested with use of hydrogen on Pd/C (Scheme 25). In MeOH, the starting material was fully consumed after 3 hours; however product **88** was obtained only in 15% purity in complex mixture of compounds. Similar result was observed also in EtOAc with one difference; approximately 50% of starting material remained unreacted after 20 hours. Complex mixture was formed again with 13% purity of product **88**.

Scheme 25: Preparation of 2-(2-aminocyclooctylidene)hydrazine-1-carboxamide **88**.[a]

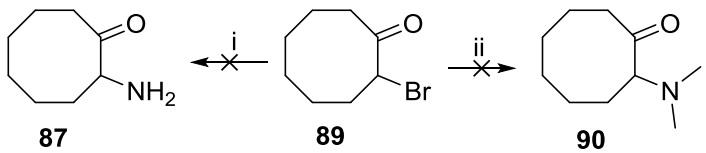


84 **88**

[a]Reagent: (i) H_2, Pd/C, MeOH or EtOAc.

In the next approach, attention was paid to substitution of 2-bromocyclooctanone **89** to desired 2-aminocycloctanone. 2-bromocyclooctanone **89** was prepared according to described procedures[63,147]. Substitution was done according to Utsukihara[148] in Microwave reactor (Scheme 26). When 7% aqueous ammonia was used, unknown compounds were obtained similarly to reduction of 2-nitrocyclooctanone (see above). Then, 7% diethylamine in water was tested. Nevertheless, formation of compounds containing bromine in their structure (according to Br isotope profile in MS) was observed.

Scheme 26: Preparation of 2-aminocyclooctanone **87** and **90**.[a]

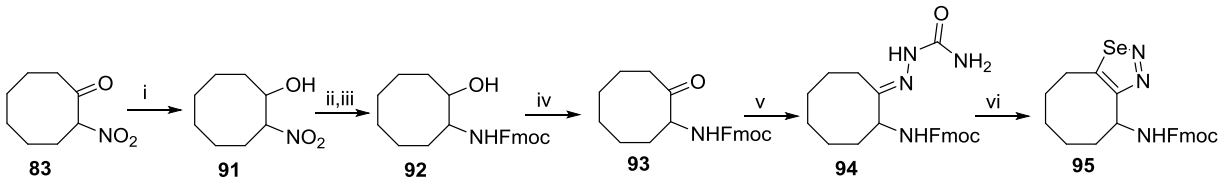


87 **89** **90**

[a]Reagent: (i) 7% NH_3/H_2O, 105 °C, 150 W (ii) 7% DEA/H_2O, 105 °C, 150 W.

Finally, 2-nitrocyclooctanone **83** was used as a starting material, but reduction of nitro group was performed after reduction of carbonyl group (Scheme 27). 2-nitrocyclooctanol **91** was prepared according to literature.[149] Nitro group was then reduced by hydrogen under Pd/C catalysis and resulting amine was protected by Fmoc group. Protection is important for following steps. Moreover, Fmoc group also allows visualization of 2-aminocyclooctanole on LC/MS and thus enables easier analysis. In the next step, hydroxyl group was oxidized to ketone **93** by Dess-Martin periodinane followed by reaction with semicarbazide hydrochloride. Compound **94** was then treated with selenium dioxide. 1,2,3-selenadiazole **95** was obtained in 43% purity together with unknown compound formed in 34% purity. 1,2,3-selenadiazole **95** was isolated and purified with use of semipreparative HPLC with 5% overall yield. The compound **95** was then used for study of conjugation reaction with azides (see later).

Scheme 27: Preparation Fmoc-4-amino-cycloocta[d][1,2,3]selenadiazole **95**.[a]

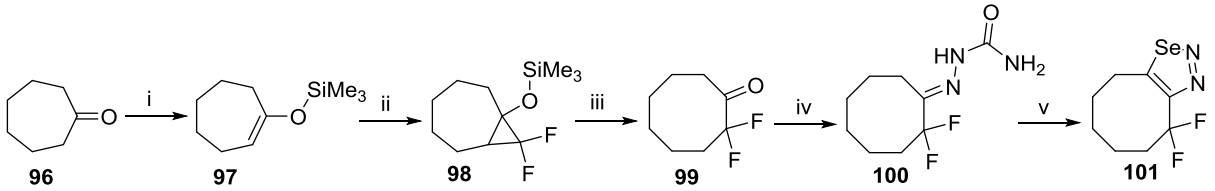


[a]Reagent: (i) $NaBH_4$, anhydrous EtOH, 0 °C (ii) H_2, Pd/C, MeOH (iii) Fmoc-OSu, DCM (iv) Dess-Martin periodinane, DCM (v) semicarbazide hydrochloride, 50% AcOH in H_2O (vi) SeO_2, AcOH.

4.2.2.3. 4,4-difluoro-cycloocta[d][1,2,3]selenadiazole

Inspired by reactivity of **DIFO** and its derivatives, the optimizations for the synthesis of difluorocycloocta[d][1,2,3]selenadiazole were carried out. 2,2-difluorocyclooctanone **99** as a crucial reaction intermediate was prepared by described three-step synthesis. At the beginning, cycloheptanone **96** is converted to trimethylsilylenol ether **97** (Scheme 28) as published in literature,[150] which is then reacted with sodium bromodifluoroacetate to form compound **98** (Scheme 28) according to described procedure.[151] Treatment of compound **98** by Na_2CO_3 then resulted in desired 2,2-difluorocyclooctanone **99**.[151]

Scheme 28: Preparation 4,4-difluoro-cycloocta[d][1,2,3]selenadiazole **101**.[a]



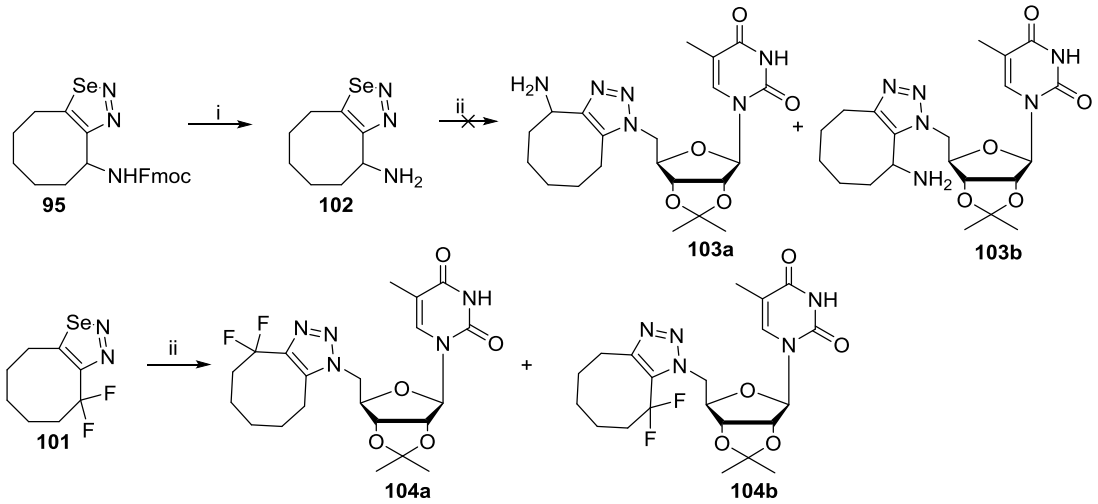
[a]Reagent: (i) NaBr, Me_3SiCl, DIEA, DMF (ii) sodium bromodifluoroactetate, anhydrous diglyme, 150 °C (iii) Na_2CO_3, anhydrous MeOH (iv) semicarbazide hydrochloride, 50% AcOH in H_2O (v) SeO_2, AcOH, 50 °C.

2,2-difluorocyclooctanone **99** was then converted to compound **100** by reaction with semicarbazide hydrochloride (Scheme 28). Treatment of **100** by selenium dioxide in acetic acid at room temperature did not result in product, no reactivity was observed. After heating to 50 °C, 1,2,3-selenadiazole **101** was formed in 50% purity in mixture with unknown compound and starting material, which was hydrolysed back to ketone **99**. The same results were obtained in aqueous dioxane. Desired 1,2,3-selenadiazole **101** was purified with use of column chromatography in 15 % overall yield and used for the following study of conjugation with azides.

4.2.2.4. Thermally induced conjugation reaction with azides

Thermally induced conjugation reactions of prepared 1,2,3-selenadiazoles **95** and **101** with azide **6** were studied. Firstly, Fmoc-4-amino-cycloocta[d][1,2,3]selenadiazole **95** was deprotected by piperidine to form amine **102**, which was then reacted with azide **6** at 90 °C. In contrast to non-substituted cycloocta-1,2,3-selenadiazole **65** fully convertable to triazole **67** after 48 h (see Table 4), only starting materials were recovered in case of 4-amino-cycloocta[d][1,2,3]selenadiazole **102** (Scheme 29).

Scheme 29: Conjugation reaction of 1,2,3-selenadiazoles **101** and **102** with azide **6**.[a]

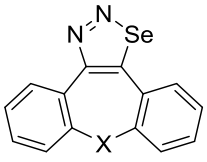


[a]Reagent: (i) pyridine, NMP (ii) azide **6**, NMP, 90 °C.

Heating of mixture of 4,4-difluoro-cycloocta[d][1,2,3]selenadiazole **101** with azide **6** to 90 °C resulted after 48 hours in formation of products **104a,b** (Scheme 29), but only in very low yield (below 10 %). Starting materials were still presented in reaction mixture. Surprisingly, the reaction did not proceed at all if electron donating group had been introduced. These preliminary results showed that presence of substituent on cyclooctane ring did not help with reaction rate in thermally induced conjugation. The reaction with 1,2,3-selenadiazole bearing electron withdrawing group occurred, but significantly slower than in case of cycloocta-1,2,3-selenadiazole itself.

4.2.3. Preparation and reactivity of dibenzo thia- or selena- cyclohepta-1,2,3-selenadiazoles

The last part of work was focused on preparation of thia- or selena-dibenzo-1,2,3-selenadiazoles **105a-e** (Figure 25) and on study of their photochemically induced conjugation reactions with azides. Benzylazide **72** was chosen as a reaction partner due to its good stability to photolysis and easy preparation. As dibenzocyclooheptyne undergoes spontaneous trimerization at temperature around 80K,[152] introduction of more bulky sulphur and selenium heteroatom instead of CH_2 group to cycloheptyne ring should increase the ring strain. Resulted thia- or selena- dibenzocycloheptynes should be more stable than dibenzocycloheptyne itself, but still more reactive then dibenzocyclooctynes. Oxides of sulphur and selenium then can affect electronic properties of each 1,2,3-selenadiazoles resulted in differences in their reactivity. In addition, two benzene rings condensed to seven-membered ring could also enable irradiation at higher wavelengths more compatible with biological applications.



105a-e

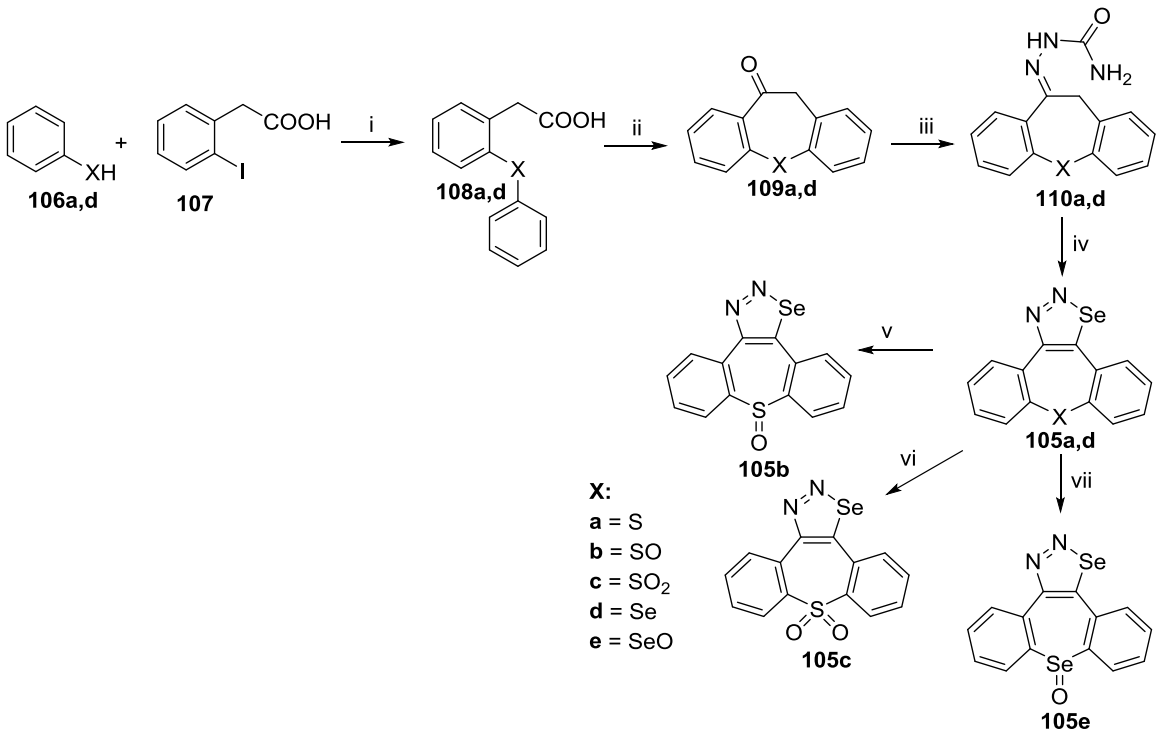
X:
a = S
b = SO
c = SO_2
d = Se
e = SeO

Figure 25: Thia- and selena- dibenzo-1,2,3-selenadiazoles **105a-e**.

4.2.3.1. Synthesis of dibenzo thia- or selena- cyclohepta-1,2,3-selenadiazoles

Dibenzothiepino-1,2,3-selenadiazole **105a** and dibenzoselenepino-1,2,3-selenadiazole **105d** were prepared *via* dibenzothiepin-10- and dibenzoselenepin-10-hydrazine carboxamides **84a** and **84d** which were generated from corresponding ketones **109a** and **109d** (Scheme 30).

Scheme 30: Preparation of 1,2,3-selenadiazoles **105a-e**.[a]



[a]Reagent: (i) KOH, Cu, H_2O, reflux (ii) PPA, toluene, 110 °C (iii) semicarbazide hydrochloride, AcOH/H_2O 1:1, DMF (iv) selenium dioxide, AcOH, DMF (v) 30% H_2O_2, Me_3SiCl, MeCN (vi) 30% H_2O_2, AcOH, DCM (vii) 30% H_2O_2, $CHCl_3$/MeOH 4:1.

Dibenzo-10-thiepinone **109a** was prepared by two step procedure described in literature.[153,154] In the first step, alkylation of thiophenol by 2-iodophenylacetic acid was carried out followed by cyclization in polyphosphoric acid (Scheme 30). The analogical conditions were used also for preparation of dibenzoselenepin-10-one **109d** (Scheme 30). 1,2,3-selenadiazoles **105b,c,e** were then prepared by oxidation of 1,2,3-selenadiazoles **105a** and **105d** by hydrogen peroxide (Scheme 30).

4.2.3.2. Photoinduced conjugation reaction of dibenzo thia- or selena- cyclohepta-1,2,3-selenadiazoles

Photoinduced conjugation reactions of 1,2,3-selenadiazoles **105a-e** were studied with benzylazide **72**. Irradiation wavelengths were carefully chosen according to UV/VIS spectra of individual compounds **105a-e** (Figure 26).

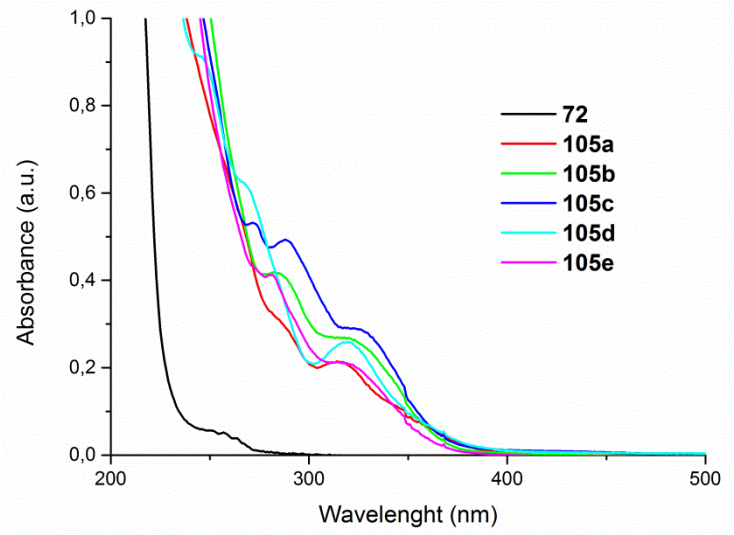
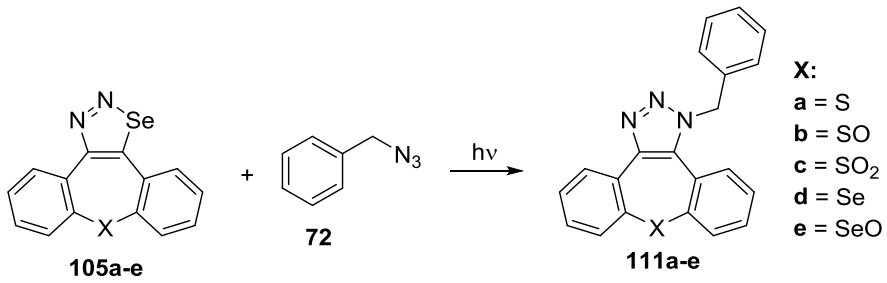


Figure 26: UV/VIS spectra of 1,2,3-selenadiazoles **105a-e**.

As the absorption spectra of the 1,2,3-selenadiazoles **105a-e** partially overlaps with absorption spectrum of benzylazide **72**, the undesired light absorption of benzylazide should be suppressed. The absorption maxima of starting materials **105a-e** allowed us to use irradiation wavelengths higher than 280 nm.

Benzylazide **72** (c ~ 0.03 mol dm^{-3}) and 1,2,3-selenadiazole **105a-e** (c ~ 0.03 mol dm^{-3}) in MeOH (Scheme 31) were irradiated by light emitted from medium-pressure Hg lamp filtered through Pyrex glass (λ > 280 nm) or through 300, 316 and 366 nm filter. The reaction was monitored with use of LC-UV/MS after two, four and six hours of irradiation.

Scheme 31: Photochemically induced conjugation reactions of 1,2,3-selenadiazoles **105a-e** and benzylazide **72**.



Dibenzothiepino-1,2,3-selenadiazole

Two hours of irradiation of dibenzothiepino-1,2,3-selenadiazole **105a** with benzylazide **72** at λ > 280 nm resulted in its quantitative conversion and triazole **111a** was formed as a major product in 72 % yield. The yield remained nearly constant also after subsequent irradiation (Table 10). Surprisingly, beside triazole **111a** one significant side

product was formed during irradiation of the reaction mixture at 300, 316 and 366 nm. The highest yield of this compound was obtained at 366 nm (Table 10). According to 1D and 2D NMR spectra and detail mass spectroscopy analysis, the structure of this compound was identified as diselenine derivative **112a** depicted in Figure 27. In addition, small amount of unreacted 1,2,3-selenadiazole **105a** (below 5%) remained unreacted in reaction mixture even after six hours of irradiation (Table 10).

Table 10: Percentages of photoproducts after irradiation of 1,2,3-selenadiazole **105a**.

λ [nm]	**Time [h]**	**[%][a] 105a**	**[%][a] 111a**	**[%][a] 112a**
> 280	2	0	72	-
	4	0	68	-
	6	0	74	-
300	2	4	41	35
	4	4	41	31
	6	2	31	19
316	2	13	26	24
	4	3	39	35
	6	2	39	33
366	2	4	31	49
	4	4	31	48
	6	4	29	55

[a]Calculated form LC traces at 200 – 600 nm.

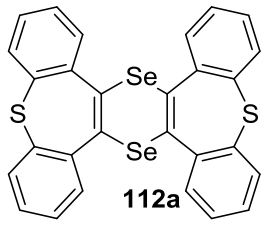


Figure 27: Structure of compound **112a**.

The results also showed that the use of higher wavelengths in this case brought no benefit, because of more than half decrease in yield due to formation of side product **112a**. Nevertheless, full conversion of **105a** after two hours at λ > 280 nm with 72% yield of **111a** confirmed the enhanced reactivity of these systems in comparison to cycloocta[d][1,2,3]selenadiazole with ~ 40% yield at the same condition.[131]

Dibenzothiepino-1,2,3-selenadiazole-8-oxide

In contrast to 1,2,3-selenadiazole **105a**, six hours irradiation of the analog **105b** at λ > 280 nm resulted in its total decomposition (Table 11). When reaction time was shortened to four hours, the product **111b** was represented by 15% in LC spectrum and significant amount of selenophene **114b** (67%) (Figure 28) was observed as well (Table 11). The third significant product of this reaction was compound with unknown structure represented by 6%. Two hours irradiation afforded only this unknown compound (14%) and selenophene **114b** (44%) as products and more than 40% of starting material remained unreacted. From these facts we can deduce that the reaction proceeds independently by two main pathways – the faster one affords derivative **114b** and the slower one expected product **111b**.

Table 11: Percentages of photoproducts after irradiation of 1,2,3-selenadiazole **105b**.

λ [nm]	Time [h]	[%][a] 105b	[%][a] 111b	[%][a] 112b	[%][a] 114b
> 280	2	41	0	-	44
	4	0	15	-	67
	6	DECOMPOSITION			
300	2	0	20	-	67
	4	0	19	-	67
	6	0	23	1	67
316	2	0	16	-	55
	4	0	15	-	53
	6	0	16	2	54
366	2	0	19	-	43
	4	0	26	8	43
	6	0	19	24	32

[a]Calculated form LC traces at 200 – 600 nm.

When optical filters 300, 316 or 366 nm were used, the desired product **111b** was represented by 15-26% in LC spectra. The dominant product was again derivative **114b**, which amount was decreasing with increasing irradiation wavelength. In addition irradiation at 366 nm resulted also in formation of diselenine **112b** (Figure 27) represented by 24% after six hours of irradiation.

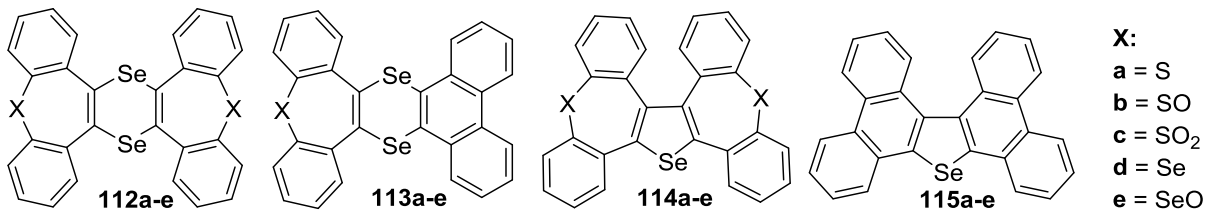


Figure 28: Structures of compounds **112-115**.

Dibenzothiepino-1,2,3-selenadiazole-8,8-dioxide

Irradiation of dibenzothiepino-1,2,3-selenadiazole-*S,S*-dioxide **105c** resulted in all cases in complex mixture of photoproducts (Table 12), which were not isolated due to their low abundance. Composition of reaction mixture was almost unchanged within the time and there were no significant differences among used wavelengths (Table 12). Conversion of starting 1,2,3-selenadiazole **105c** ranged from 81 to 95% after six hours of irradiation (Table 12).

Table 12: Percentages of photoproducts after irradiation of 1,2,3-selenadiazole **105c**.

λ [nm]	**Time [h]**	**[%][a] 105c**	**[%][a] 111c**	**[%][a] 112c**	**[%][a] 113c**
> 280	2	25	8	11	10
	4	21	11	13	13
	6	19	10	9	15
300	2	11	19	18	27
	4	11	23	23	26
	6	9	21	24	22
316	2	16	10	5	18
	4	16	11	12	25
	6	17	14	13	22
366	2	10	13	10	23
	4	8	16	13	18
	6	5	26	15	20

[a]Calculated form LC traces at 200 – 600 nm.

According to mass spectra and information from literature[42,155] we suggested structures of two main side products, which correspond to compounds **112c** and **113c** (Figure 28, Table 12). Expected triazole **111c** was detected in all cases in yield 10-26% after six hours of irradiation (Table 12). The observed photochemical extrusion of SO_2 from cyclic sulfones which took place during formation of product **113c** is well known process resulting in alkyl or aryl radicals which can undergo various reactions including formation of C-C bond.[155]

Dibenzoselenepino-1,2,3-selenadiazole

Irradiation of equimolar mixture of dibenzoselenepino-1,2,3-selenadiazole **105d** and benzylazide **72** (c ~ 0.03 mol dm^{-3}) afforded in all cases mixture of desired **111d** and diselenine **112d** as a major side product (Table 13, Figure 27). The best ratio of these products was observed when 316 nm filter was used and reaction proceeded for 6 hours (Table 13).

Table 13: Percentages of photoproducts after irradiation of 1,2,3-selenadiazole **105d**.

λ [nm]	Time [h]	[%][a] 105d	[%][a] 111d	[%][a] 112d
> 280	2	0	35	13
	4	0	38	11
	6	0	34	9
300	2	0	49	27
	4	0	50	21
	6	0	54	14
316	2	0	43	31
	4	0	50	26
	6	0	56	17
366	2	0	29	48
	4	0	31	54
	6	0	38	46

[a]Calculated form LC traces at 200 – 600 nm.

Dibenzoselenepino-1,2,3-selenadiazole-8-oxide

In contrast to reaction of sulphur analogue **105b** no formation of desired product **111e** was observed under the same conditions for derivative **105e**. From LC/MS spectra we can deduce formation of derivatives **114e** and **115e** (Figure 27, Table 14), which were not possible to isolate because of complexity of reaction mixture. According to typical Se pattern in mass spectra, the majority of the side products contained Se in their structure. The cleavage of bond between phenyl and SeO moiety by UV light is in accordance with the observations described in the literature.[156] In some cases, compound with structure **112e** was detected as minor product as well (Table 14).

Table 14: Percentages of photoproducts after irradiation of 1,2,3-selenadiazole **105e**.

λ [nm]	Time [h]	[%][a] 105e	[%][a] 111e	[%][a] 112e	[%][a] 114e	[%][a] 115e
> 280	2	5	0	-	22	-
	4	5	0	15	23	3
	6	1	0	17	17	6
300	2	4	0	-	29	14
	4	3	0	-	43	17
	6	7	0	-	28	-
316	2	3	0	-	28	13
	4	3	0	-	25	10
	6	7	0	-	51	11
366	2	0	0	2	41	25
	4	1	0	2	39	19
	6	5	0	5	25	2

[a]Calculated form LC traces at 200 – 600 nm.

The best results in formation of triazoles were finally obtained by dibenzothiepino-1,2,3-selenadiazole **105a** at $\lambda > 280$ nm and by dibenzoselenepino-1,2,3-selenadiazole **105d** at $\lambda = 315$ nm. Triazoles **111a** and **111d** were isolated, purified with use of semipreparative HPLC and characterized. Unfortunately, the presence of side products observed in all cases showed that it is not possible to selectively convert the 1,2,3-selenadiazoles **105a-e** to corresponding triazoles by this method.

5. Conclusion

The main goal of presented dissertation was to prepare and study new systems suitable for non-catalyzed conjugation reactions (SPAAC) with azides. The whole work was focused on two types of system. The first part of dissertation deals with aza-dibenzocyclooctyne (**DIBAC**) derivatives; the second part is focused on preparation and reactivity of cycloalka-1,2,3-selenadiazoles.

In the first part of work, the attention was firstly paid to preparation and especially characterization of 1,2,3-triazoles formed by SPAAC between known **DIBAC** derivative **13** and 5-methyl uridine-5´-azides **5** and **6**. ^{1}H-^{15}N HMQC and ^{1}H-^{15}N HMBC NMR spectra predicted the formation of conformational isomers due to hindered rotation of the pyrimidine and azocine parts of the molecule and ^{19}F-^{19}F EXSY experiments proved exchange between individual conformers. The computational study of the studied compounds revealed that observed isomery is caused either by different positions of the aliphatic chain relative to the nucleoside part of the molecule or by the formation of intramolecular hydrogen bonds. The similar isomery was studied subsequently also for derivatives, in which the nucleoside was replaced by hydrogen (**16**) or coumarine scaffold (**17a**/**17b**). For the substrates **16** and **17a** no isomery was observed, while for derivative **17b** at least three conformers were detected.

Secondly, 1,2,3-triazoles, in which the previous aliphatic chain was replaced by *β*-alanine (**20a**/**20b**), trifluoracetyl group (**24a**/**24b**) and hydrogen (**26a**/**26b**), were prepared and studied. Similarly to triazoles described above, isomery was observed in NMR spectra of all prepared compounds. These results indicate, that observed isomery is independent on the length of aliphatic chain.

As a result, the formation of triazoles on a nucleic acid sequence can caused deformation of the nucleic acid structure, which can have a negative effect on imaging processes.

The last aim of this part was to developed solid-phase synthesis for fluorescently labelled **DIBAC** derivatives. At the beginning, it was necessary to find a way, how to immobilized dihydrodibenzoazocine **10** on solid support. Although the reaction of **10** with solid supported carboxylic acid with use of coupling reagents completely failed, it was possible to prepared desired amide **33** and **34** quantitatively *via* corresponding acyl chloride. Following bromination proceeded without any problems, however subsequent formation of triple bond proved to be challenging. Although the different bases were tested, elimination proceeded satisfactory only when *t*BuOK was used. Due to instability of aza-

dibenzocyclooctyne towards acidic conditions used for cleavage from resin, it was firstly necessary to cleave dibromoderivatives **35** or **36** from resin and then perform the key elimination in solution. Unfortunately, this methodology could not be used for RhoB labelled dibromoderivatives **55**, **56** and **58** as RhoB was cleaved from the substrate during elimination step. Although the preparation of fluorescently labelled aza-dibenzocyclooctynes was unsuccessful, RhoB labelled dihydrodibenzoazocines **62**, **63** and **64**, which can be later used for alkene ligation, were isolated and characterized.

In the second part of work, the attention was firstly paid to study of microwave assisted and photochemically induced click reactions of cycloocta[d][1,2,3]selenadiazole **65** with azides. In comparison to thermally induced click reaction of **65**, where the quantitative conversion to triazole **67** was achieved after 20 hours at 110 °C, the use of microwave (110 °C, 150 W, 3 eq. of **65**) helped to shorten the reaction time to approximately 70 minutes. Unfortunately, the conversion of **65** at temperatures below 100 °C significantly decreased, thus the reaction time required for full transformation of **65** to desired triazoles **66** and **67** would be non-acceptably long.

On the other hand, conjugation reactions of cyclooctyne, which was photochemically generated in situ from cycloocta[d][1,2,3]selenadiazole **65**, with various azides at room temperature, provided satisfactory yields of the corresponding 1,2,3-triazoles. The reaction was successfully tested for the labelling of an avidin–biotin complex, which predestines cyclooctaselenadiazole derivatives for applications in molecular biology. Although the conversion of 1,2,3-selenadiazole **65** is not quantitative, it can be sufficient for application in various conjugation reactions, such as labelling of biomolecules.

Second aim of this part was to prepare derivatized cycloocta[d][1,2,3]selenadiazoles and study, if substituent adjacent to 1,2,3-selenadiazole ring can influence the rate of thermally induced click reaction. Synthesis of 4-nitro[d][1,2,3]selenadiazole **85** was unsuccessful due to oxidation of nitro group to 2-oxocyclooctylidene)hydrazine-1-carboxamide **86** by selenium dioxide. After optimization, *N*-Fmoc-4-amino[d][1,2,3]selenadiazole **95** was prepared by seven step synthesis started from 2-nitrocylooctanone in 5% overall yield. The target 4-amino[d][1,2,3]selenadiazole **102** was then prepared by quantitative Fmoc deprotection before click reaction itself. Then 4,4-difluoro-1,2,3-selenadiazole **101** was prepared from 2,2-difluorocyclooctanone in 15% overall yield. 1,2,3-selenadiazoles **101** and **102** were reacted with azide **6** at 90 °C for 48 h. In case of 4-amino derivative **102**, only starting material was recover; in case of difluoro

derivative **101**, the reaction proceeded but in very low yield (below 10 %). These results showed that both chosen electron withdrawing and electron donating substituents have negative effect on conjugation reaction rate in comparison to unsubstituted cycloocta[d][1,2,3]selenadiazole **65**, where the conversion was quantitative after 48 h.

Lastly, conjugation reactions of dibenzocycloheptyne derivatives, which were photochemically generated *in situ* from dibenzothiepino- or dibenzoselenepino-1,2,3-selenadiazoles **105a-e**, with benzylazide **72** were studied. Expected triazoles were formed in all cases with exception of dibenzoselenepino-1,2,3-selenadiazole-8-oxide **105e**. The yields and triazole purities differed relative to structure of starting 1,2,3-selenadiazole and used wavelengths. The best results were obtained for dibenzothiepino-1,2,3-selenadiazole **105a** at $\lambda > 280$ nm, where triazole **111a** was formed in 74% yield after 6 hours of irradiation.

6. Experimental part

6.1. Materials and methods

6.1.1. Instrumentation

LC/MS analyses were performed using UHPLC/MS with an UHPLC chromatograph Acquity with a PDA detector and a single quadrupole mass spectrometer (Waters) with an X-Select C18 column at 30 °C and a flow rate of 600 μL/min. The mobile phase consisted of 0.01 mol dm^{-3} ammonium acetate in water and acetonitrile; a linearly programmed gradient was used over the course of 2.5 min. and then maintained the corresponding concentrations for 1.5 min. The APCI ionization operated at a discharge current of 5 μA, vaporizer temperature of 350 °C and capillary temperature of 200 °C.

Purity of the compounds was determined as a ratio of the given peak area to of the total area of all peaks of the mixture.

Purification was performed using semipreparative HPLC with a Waters 1500 series HPLC instrument equipped with an Autosampler 2707, a Binary HPLC pump 1525, a Waters Photodiode Array Detector 2998 and a Waters Fraction Collector III with a YMC C18 reverse phase column, 20 × 100 mm, with 5 μm particles. The mobile phase consisted of acetonitrile and 10 mmol dm^{-3} aqueous ammonium acetate gradient over six min.

NMR spectra were measured in $CDCl_3$, DMSO-d_6, methanol-d_4 and D_2O using a Jeol (400 and 500 MHz) spectrometer, Bruker Avance 300 MHz instrument and a Bruker Avance 500 MHz instrument. The chemical shifts (δ) are reported in parts per million (ppm), and coupling constants (*J*) are reported in Hertz (Hz).

HRMS analysis was performed using either an Orbitrap Elite high-resolution mass spectrometer (Thermo Fischer Scientific, MA, USA) or LC-MS an Orbitrap Elite high-resolution mass spectrometer (Dionex Ultimate 3000, Thermo Exactive plus, MA, USA). The MALDI/TOF spectra were recorded on Bruker Ultraflextreme MALDI-TOF instrument using sinapinic acid matrix prepared according to published procedure.[157] The mass spectra were exported to text files, normalized using Microsoft Excel and visualized with mMass 5.5.0.[158]

The UV-vis spectra were obtained with matched 1.0 cm quartz cells on an Agilent 8453 diode-array spectrophotometer against air as a background reference.

Microwave reactions were carried out in a CEM-Discover microwave reactor operating at 2.45 GHz with continuous irradiation of 300W maximum power. CEM-Discover microwave reactor uses external IR temperature measurement. The microwave irradiation of

required watts was used and the temperature was continuously raised for 2 minutes. Once the desired temperature was reached, the mixture was held at this temperature for given time.

The solid-phase syntheses were performed in plastic reaction vessels (syringes equipped with one porous disk each) using a manually operated synthesizer.[159] The volume of the wash solvent was 10 mL per gram of the resin. For washing, the resin slurry was shaken with fresh solvent for at least 1 min before the solvent was replaced. The yields of the crude products were calculated with respect to the loading of the first building block.

1-(-6-(Hydroxymethyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione **6**,[124] 5-hydroxymethylene-uracil,[160] 3-azidocoumarine **71**,[126] phenylazide **68**,[132] 1-azido-4-methoxybenzene **69**,[132] 1-azido-4-nitrobenzene **70**,[132] benzylazide **72**,[133] 2-nitrocyclooctanone **83**,[145] 2-nitrocyclooctanole **91**,[149] 2,2-difluorocyclooctanone **99**,[151] 2-(2-(phenylthio)phenyl)acetic acid **108a**[154] and dibenzo[b,f]thiepin-10(11H)-one **109a**[153] were prepared as described in the literature.

6.1.2. Description of methods for study of photochemically induced conjugation reaction of cycloocta[d][1,2,3]selenadiazole

6.1.2.1. Photochemical reaction of cyclooctaselenadiazole 65 and azide 5, 6, 68-73

Reaction with UV/VIS and GC/MS monitoring

A solution of selenadiazole **65** (0.38 mg, 1.8×10^{-3} mmol) and benzyl azide **72** (0.24 mg, 1.8×10^{-3} mmol) in methanol (3 mL) was irradiated by light emmited from a medium-pressure mercury arc filtered through a 313 nm filter ($\lambda_{irr} = 313.5 \pm 1.5$ nm). The reaction was monitored with time (before the irradiation started and then after 5, 10, 20, 30, 50, 70, 90, 150 min and after 16 h) using UV/VIS spectrophotometer. GC/MS analysis of the reaction mixture was performed after 16 hours of irradiation.

Reaction with NMR monitoring

^{1}H NMR of a solution of selenadiazole **65** (3.8 mg, 0.018 mmol) and benzyl azide **72** (2.4 mg, 0.018 mmol) in methanol-d_4 (0.6 mL) was measured and the reaction mixture was then irradiated using light from a medium-pressure mercury arc effectively filtered through the Pyrex glass of the NMR tube (transparent at $\lambda > 280$ nm) for 2 hours. After irradiation, ^{1}H NMR of the reaction mixture was measured again.

Determination of singlet or triplet state

Oxygenated, aerated and degassed solutions of selenadiazole **65** (0.67 mg, 3.1×10^{-3} mmol), benzyl azide **72** (0.4 mg, 3.1×10^{-3} mmol) and TMS as internal standard in

methanol-d_4 (0.7 mL) were irradiated by light emmited from a medium-pressure mercury arc filtered through the Pyrex glass of the NMR tube. The reaction was monitored with time using ^{1}H NMR (before the irradiation started and then after 5, 10, 20, 30, 50, 70 min and after 16 h keeping in dark).

Reactions with sensitizer

^{1}H NMR of a solution of selenadiazole **65** (4 mg, 0.018 mmol), benzyl azide **72** (2.4 mg, 0.018 mmol) and benzophenone (1, 10 or 50 equivalent) in methanol-d_4 (0.6 mL) was measured and the reaction mixture was then irradiated using light from a medium-pressure mercury arc effectively filtered through a 365 nm filter. The reaction was monitored after 2 hours and 16 hours of irradiation.

Reaction with sensitizer – effect of oxygen

Oxygenated and degassed solutions of selenadiazole **65** (4 mg, 0.018 mmol), benzyl azide **72** (2.4 mg, 0.018 mmol) and benzophenone (10 equivalent) in methanol-d_4 (0.7 mL) were irradiated using light from a medium-pressure mercury arc filtered through 365 nm filter. The reaction was monitored using ^{1}H NMR after 2 hours and 16 hours of irradiation.

6.1.2.2. Photochemical transformation of cyclooctaselenadiazole 65

^{1}H NMR of a solution of selenadiazole **65** (5.2 mg, 0.024 mmol) in methanol-d_4 (0.6 mL) was measured and the solution was irradiated using light from a medium-pressure mercury arc effectively filtered through the Pyrex glass of the NMR tube (transparent at $\lambda > 280$ nm) for 1 hour. After irradiation, ^{1}H and ^{13}C NMR spectra of the reaction mixture were measured again.

6.1.2.3. Synthesis of triazoles 66, 67 and 74-79 *via* photochemical reaction

Irradiation of mixture of cyclooctaselenadiazole 65 and azide (Method I)

General procedure: ^{1}H NMR spectrum of a solution of equimolar amounts of selenadiazole **65** and azide (**5**,**6** and **68-73**) in methanol-d_4 (c = 0.03 mol dm^{-3}) was measured and the was then irradiated using light from a medium-pressure mercury arc effectively filtered through a Pyrex glass of the NMR tube (transparent at $\lambda > 280$ nm) for 1 hour. After irradiation, ^{1}H NMR of reaction mixture was measured again. The reaction mixture was then kept in dark and ^{1}H NMR spectra were measured after 2 and 16 hours of standing.

Irradiation of cyclooctaselenadiazole 65 followed by addition of azide (Method II)

General procedure: A solution of selenadiazole **65** in methanol-d_4 (c = 0.03 mol dm^{-3}) in NMR tube was irradiated using light from a medium-pressure mercury arc effectively filtered

through a Pyrex glass of the NMR tube (transparent at λ > 280 nm) for 1 hour. After irradiation, equimolar amount of azide (**5**,**6** and **68-73**) was added to reaction mixture and ^{1}H NMR of the reaction mixture was measured. The reaction mixture was then kept in dark and ^{1}H NMR spectra were measured again after 2 and 16 hours of standing.

6.1.2.4. Experimental procedures for conjugation reactions of derivative 80 and 81

Synthesis of 2-(2-(2-((1-(2,11,16-trioxo-20-(2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-3-(2,2,2-trifluoroacetyl)-6,9,12-trioxa-3,15-diazaicosyl)-4,5,6,7,8,9-hexahydro-1H-cycloocta[d][1,2,3]triazol-6-yl)amino)ethoxy)ethoxy)acetic acid (82):

Reaction without presence of avidin

^{1}H NMR spectrum of a solution of PEG-selenadiazole **80** (3 mg, 0.008 mmol) and azide **81** (5 mg, 0.008 mmol) in D_2O (0.6 mL) was measured and the reaction mixture was then irradiated using light from a medium-pressure mercury arc effectively filtered through a Pyrex glass of the NMR tube (transparent at λ > 280 nm) for 1 hour. After irradiation, ^{1}H NMR spectrum of the reaction mixture was measured. The reaction mixture was then irradiated for another 1 hour and the ^{1}H NMR spectrum of the reaction mixture was measured again.

6.1.4.2. Reaction in presence of avidin

To a solution of **81** (8 mg, 0.013 mmol) in D_2O (0.6 mL) in a NMR tube was added commercially available avidin (20 mg; 65 kDa) followed by addition of PEG-selenadiazole **80** (5 mg, 0.013 mmol). The reaction mixture was then irradiated with a medium-pressure mercury arc filtered through a Pyrex glass of the NMR tube (transparent at λ > 280 nm) for 1 hour. After irradiation, ^{1}H NMR spectrum of reaction mixture was measured. The reaction mixture was then irradiated for another 1 hour and the ^{1}H NMR spectrum of the reaction mixture was measured again.

6.1.2.5. Kinetic study of a reaction between cyclooctyne and benzylazide 72

A solution of **65** (c = 23 mmol dm^{-3}) in methanol-d_4 (0.6 mL) was irradiated with a medium-pressure mercury arc filtered through a Pyrex glass of the NMR tube (transparent at λ > 280 nm) until no starting material was observable by ^{1}H NMR (ca. 2 h). Benzylazide **72** (5 μL) was then added to the reaction mixture, agitated and the following reaction was monitored by ^{1}H NMR spectroscopy. The change of the relative concentration of cyclooctyne as reactant and triazole **78** as product (calculated from the ratio of the integrals of the

characteristic peaks of cyclooctyne and **78** against the integral of the peak of non-deuterated methanol as an impurity) was plot and the data were fit with pseudo-first order kinetic equation which allowed to calculate observed rate constant of the cyclooctyne disappearance to be $k_{obs} = 4.4 \times 10^{-3}$ s^{-1} and estimate the half-life to be $\tau_{1/2} \sim 38$ min under the experimental conditions.

6.1.2.6. Determination of quantum yield of triazole 78 appearance

Samples of methanolic solution (A ~ 1.1 at λ_{irr} = 313 nm) of cycloocta[d][1,2,3]selenadiazole **65** (c = 1.9×10^{-3} mol.dm^{-3}) and benzylazide **72** (c = 6.1×10^{-3} mol.dm^{-3}) were prepared by dilution of stock solutions and irradiated using a 40 W medium pressure mercury arc equipped with a 313 nm band-pass filter (λ_{em} = 313.5 ± 1.5 nm) in matched 1.000 cm quartz cells. Appearance of acetophenone (Φ_{app} = 0.30) upon irradiation of valerophenone solution (c = 1.9×10^{-3} mol.dm^{-3}; A ~ 0.3 at λ_{irr} = 313 nm) was used as an actinometer.[138] All samples (both **65** and benzylazide **72**, and valerophenone, respectively) were bubbled with dry nitrogen 10 minutes prior to irradiation and the concentration of both triazole **78** and acetophenone was followed by gas chromatography.

Both valerophenone and cycloocta[d][1,2,3]selenadiazole **65** and benzylazide **72** samples, respectively, were irradiated as at least three independently prepared samples and each sample was analyzed at least five times. Hexadecane (c = 1.1×10^{-3} mol.dm^{-3}) was used as an internal standard in all samples and concentrations of analytes (acetophenone and triazole **78**) were calculated from calibration curves (all of them consists of at least five concentration points and were linear in the range c = $0.1–5.0 \times 10^{-3}$ mol.dm^{-3} with $R^2 > 0.99$ for linear fit to data). The samples of triazole **78** were kept at least six hours in dark after irradiation and prior to determination of concentration of triazole **78** to allow high conversion of cyclooctyne to the triazole **78** (six hours equals to about 10 half-lives – for more details see text above).

The conversion was kept below 25% for **72** and below 10% for valerophenone to avoid the interference of photoproducts. The relative standard deviation of the mean for the acetophenone concentrations was found to be below 10%. Higher standard deviations of the mean of concentration of triazole **78** in the samples were mainly caused by non-uniform adsorption of elementary selenium on the cuvette surfaces acting as an effective light filter in all UV and visible range of light.

All stock solutions (with 10-fold higher concentration than used in the experiments) were prepared by direct weighting of the respective compounds into a volumetric flasks and filling with a solvent. The stock solutions were kept in fridge and used within a week from preparation.

6.1.3. Study of photochemically induced reactions of 1,2,3-selenadiazoles 105a-e with benzylazide 102

General procedure: LC/MS analysis of a solution of equimolar amounts of selenadiazole **105a-e** and benzylazide **72** in methanol (c = 0.03 mol dm^{-3}) was measured and the reaction was then irradiated using light from a medium-pressure mercury arc effectively filtered through a Pyrex glass of the NMR tube (transparent at λ > 280 nm) or through 300, 316 and 366 nm filters for two hours. After irradiation, LC/MS of reaction mixture was measured again. The reaction mixture was irradiated again and composition of reaction mixture was compared after two and four hours.

6.2. Synthesis of individual compounds

6.2.1. SPAAC of DIBAC and characterization of resulting triazoles

1-(3,4-Dihydroxy-5-iodomethyl-tetrahydrofuran-2-yl)-5-methyl-1*H*-pyrimidin-2,4-dione (3a)

5-Methyluridine **1** (1.18 g, 4.57 mmol), PPh_3 (1.85 g, 7.05 mmol) and imidazole (479 mg, 7.04 mmol) were dissolved in anhydrous THF (30 ml). Then, a solution of iodine (1.28 g, 5.04 mmol) in anhydrous THF (15 ml) was added. The reaction mixture was stirred at room temperature for two hours. The white precipitate was removed by filtration, and the THF was evaporated under reduced pressure. The resulting brown solid was purified on a silica gel column with a mobile phase of $CHCl_3$/MeOH (6:1 v/v). Yellow solid, 1.19 g (70%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.80 (s, 3 H), 3.40 (dd, *J*=10.52, 6.58 Hz, 1 H), 3.57 (dd, *J*=10.52, 6.14 Hz, 1 H), 3.83 (td, *J*=6.25, 3.73 Hz, 1 H), 3.90 (dd, *J*=5.48, 3.73 Hz, 1 H), 4.20 (t, *J*=5.92 Hz, 1 H), 5.81 (d, *J*=6.14 Hz, 1 H), 7.52 (d, *J*=0.88 Hz, 1 H), 11.37 (s, 1 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 7.98, 12.29, 72.17, 73.01, 83.21, 88.09, 110.14, 136.75, 151.01, 163.86. HRMS m/z calculated for $C_{10}H_{14}IN_2O_5$ $[M+H]^+$ 368.9942, found 368.9941.

1-(3,4-Dihydroxy-5-bromomethyl-tetrahydrofuran-2-yl)-5-methyl-1*H*-pyrimidin-2,4-dione (3b)

5-Methyluridine **1** (0.5 g, 1.94 mmol), PPh_3 (0.78 g, 2.98 mmol) and imidazole (200 mg, 2.98 mmol) were dissolved in anhydrous THF (10 ml), and bromine was added (0.11 ml, 2.13 mmol). The reaction mixture was stirred at room temperature for two hours. The white precipitate was removed by filtration, and the THF was evaporated under reduced pressure. The resulting brown solid was purified on a silica gel column with a mobile phase of $CHCl_3$/MeOH (6:1 v/v). White solid 0.42 g (68%).

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.76–1.83 (m, 3 H), 3.67 (dd, *J*=10.74, 5.92 Hz, 1 H), 3.76–3.84 (m, 1 H), 3.96 (dq, *J*=8.50, 4.48 Hz, 2 H), 4.18 (t, *J*=5.70 Hz, 1 H), 5.81 (d, *J*=6.14 Hz, 1 H), 7.51 (d, *J*=0.88 Hz, 1 H), 11.37 (s, 1 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 12.11, 33.82, 71.63, 71.92, 82.75, 87.78, 109.94, 136.39, 150.81, 163.66. HRMS m/z calculated for $C_{10}H_{14}BrN_2O_5$ $[M+H]^+$ 321.0081, found 321.0080.

5´-Deoxy-5´-iodo-5-methyl-2´,3´-*O*-isopropyliden-uridine (4a)

Compound **2** (1.27 g, 4.26 mmol), PPh_3 (1.71 g, 6.52 mmol) and imidazole (448 mg, 6.58 mmol) were dissolved in anhydrous THF (25 ml). Then, a solution of iodine (1.19 g, 4.69 mmol) in anhydrous THF (15 ml) was added. The reaction mixture was stirred at room temperature for two hours. The white precipitate was removed by filtration, and the THF was evaporated under reduced pressure. The resulting brown solid was purified on a silica gel column with a mobile phase of Tol/AcN (5:2 v/v). Yellow solid 1.31 g (75%).

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.29 (s, 3 H), 1.48 (s, 3 H), 1.77 (s, 3 H), 3.36–3.40 (m, 1 H), 3.48 (dd, *J*=10.10, 8.00 Hz, 1 H), 4.12 (ddd, *J*=7.56, 6.03, 3.95 Hz, 1 H), 4.73 (dd, *J*=6.58, 3.95 Hz, 1 H), 5.08 (dd, *J*=6.58, 2.19 Hz, 1 H), 5.80 (d, *J*=2.19 Hz, 1 H), 7.60 (d, *J*=1.32 Hz, 1 H), 11.45 (s, 1 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 6.68, 12.17, 25.28, 27.06, 83.66, 83.90, 86.29, 92.61, 109.91, 113.61, 138.90, 150.49, 164.04. HRMS m/z calculated for $C_{13}H_{18}IN_2O_5$ $[M+H]^+$ 409.0255, found 409.0253.

5´-Bromo-5´-deoxy-5-methyl-2´,3´-*O*-isopropyliden-uridine (4b)

Compound **2** (1.23 g, 4.12 mmol), PPh_3 (1.66 g, 6.33 mmol) and imidazole (432 mg, 6.35 mmol) were dissolved in anhydrous THF (40 ml), and bromine (0.24 ml, 4.56 mmol) was added. The reaction mixture was stirred at room temperature for two hours. The white precipitate was removed by filtration, and the THF was evaporated under reduced pressure. The resulting solid was purified on a silica gel column with a mobile phase of Tol/AcN (5:2 v/v). White solid 0.94 g (63%).

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.29 (s, 3 H), 1.48 (s, 3 H), 1.77 (d, *J*=1.32 Hz, 3 H), 3.63 (dd, *J*=10.50, 6.10 Hz, 1 H), 3.73 (dd, *J*=10.00, 7.00 Hz, 1 H), 4.19 (td, *J*=6.47, 3.73 Hz, 1 H), 4.79 (dd, *J*=6.58, 3.95 Hz, 1 H), 5.08 (dd, *J*=6.58, 2.19 Hz, 1 H), 5.79 (d, *J*=2.63 Hz, 1 H), 7.59 (s, 1 H), 11.45 (s, 1 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 11.98, 25.06, 26.86, 32.87, 82.56, 83.51, 85.74, 92.47, 109.71, 113.44, 138.67, 150.32, 163.85. HRMS m/z calculated for $C_{13}H_{18}BrN_2O_5$ $[M+H]^+$ 361.0394, found 361.0394.

1-(5-(Azidomethyl)-3,4-dihydroxytetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (5)

Compound **3a** (1 g, 2.72 mmol) or **3b** (850 mg, 2.72 mmol) was dissolved in anhydrous DMF (15 ml), and sodium azide (555 mg, 8.5 mmol) was added. The suspension was stirred at 90 °C for 2 hours. The remaining sodium azide was removed by filtration. Water (15 ml) was added, and the mixture was extracted with ethyl acetate (4x25 ml). The organic layer was dried over sodium sulphate, and the solvent was evaporated. The product was dried with use of freeze dryer. Yield 620 mg (81%).

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.79 (s, 3 H), 3.58 (d, *J*=4.82 Hz, 1 H), 3.88–3.97 (m, 2 H), 4.11–4.19 (m, 1 H), 5.28 (br. s., 1 H), 5.45 (d, *J*=4.39 Hz, 1 H), 5.78 (d, *J*=5.70 Hz, 1 H), 7.51 (s, 1 H), 11.37 (s, 1 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 12.05, 51.68, 70.39, 72.03, 82.17, 88.30, 109.85, 136.53, 150.78, 163.70. HRMS m/z calculated for $C_{10}H_{14}N_5O_5$ $[M+H]^+$ 284.0989, found 284.0987.

5´-Azido-5´-deoxy-5-methyl-2´,3´-*O*-isopropyliden-uridine (6)

Compound **4a** (1.3 g, 3.18 mmol) or **4b** (1.14 g, 3.18 mmol) was dissolved in anhydrous DMF (20 ml), and sodium azide (0.65 g, 10 mmol) was added. The suspension was stirred at 90 °C for 2 hours. The remaining sodium azide was removed by filtration, and DMF was removed by lyophilization. The solid was then suspended in water (20 ml), and the product was obtained as a white precipitate by filtration. Yield 0.83 g (82%).

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.29 (s, 3 H), 1.48 (s, 3 H), 1.77 (s, 3 H), 3.58 (d, *J*=5.70 Hz, 2 H), 4.12 (q, *J*=4.80 Hz, 1 H), 4.76 (dd, *J*=6.58, 4.39 Hz, 1 H), 5.06 (dd, *J*=6.58, 2.19 Hz, 1 H), 5.80 (d, *J*=2.63 Hz, 1 H), 7.57 (s, 1 H), 11.43 (br. s., 1 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 11.96, 25.14, 26.92, 51.53, 80.97, 83.13, 84.49, 91.79, 109.71, 113.55, 138.41, 150.31, 163.82. HRMS m/z calculated for $C_{13}H_{18}N_5O_5$ $[M+H]^+$ 324.1302, found 324.1303.

***N*-(3-(1-((-3,4-Dihydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)methyl)-1*H*-dibenzo[b,f][1,2,3]triazolo[4,5-d]azocin-8(9*H*)-yl)-3-oxopropyl)-2,2,2-trifluoroacetamide (14a)**

***N*-(3-(3-((-3,4-Dihydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)methyl)-3*H*-dibenzo[b,f][1,2,3]triazolo[4,5-d]azocin-8(9*H*)-yl)-3-oxopropyl)-2,2,2-trifluoroacetamide (14b)**

Azido derivative **5** (50 mg, 0.18 mmol) was dissolved in MeOH (2 ml), and aza-dibenzocyclooctyne **13** (93 mg, 0.25 mmol) was added. The solution was stirred at room temperature for 5 minutes, diluted with MeOH to 8 ml and then purified by semi-preparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to change from 20% to 50% over the course of 6 min. The derivatives **14a** and **14b** were collected separately. Derivative **14a** was the first compound and was isolated as a white solid, 44 mg (35%). Derivative **14b** was the second compound and was isolated as a white solid, 38 mg (30%).

14a:

^{1}H NMR (500 MHz, $CDCl_3$-*d*) δ ppm 1.77 (dt, *J*=17.0, 5.60 Hz 1 H), 1.83 (s, 3 H), 2.08 (dt, *J*=17.0, 5.60 Hz 1 H), 3.24 (m, 2 H), 4.02 (t, *J*=5.50 Hz 1 H), 4.08 (q, *J*=5.50 1 H), 4.18 (t, *J*=4.60 Hz 1 H), 4.32 (d, *J*=16.60 Hz 1 H), 4.71 (dd, *J*=13.80, 5.20 Hz 1 H), 4.95 (dd, *J*=13.80, 4.50 Hz 1 H), 5.61 (d, *J*=3.70 Hz 1 H), 6.03 (d, *J*=16.60 Hz 1 H), 7.05 (d, *J*=7.0 Hz 1 H), 7.14 (s, 1 H), 7.18 (m, 1 H), 7.26 (m, 1 H), 7.28 (m, 1 H), 7.35 (tt, *J*=6.70, 2.0 Hz 1 H), 7.46 (m, 1 H), 7.47 (m, 1 H), 7.65 (dd, *J*=6.70, 2.0 Hz 1 H), 7.82 (t, *J*=5.50 Hz 1 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ ppm 12.31, 33.31, 35.39, 50.22, 51.32, 70.72, 73.37, 82.12, 92.05, 111.05, 115.79 (q, *J*=287.9), 124.32, 127.33, 127.44, 127.77, 129.82, 129.86, 129.92, 131.05, 131.10, 131.13, 131.83, 135.11, 135.77, 137.36, 139.27, 142.97, 150.78, 157.17 (q, *J*=36.5), 164.32, 171.86. HRMS m/z calculated for $C_{30}H_{29}F_3N_7O_7$ $[M+H]^+$ 656.2075, found 656.2072.

14b:

Because it was not possible to identify the major ^{1}H and ^{13}C NMR signals, characterization was carried out for mixture of all conformers.

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.60 - 1.63 (m, 2 H) 1.65 - 1.69 (m, 1 H) 1.81 (d, *J*=0.86 Hz, 3 H) 1.84 (s, 1 H) 1.87 (s, 1 H) 2.03 - 2.11 (m, 2 H) 2.15 - 2.25 (m, 1 H) 2.35 - 2.37 (m, 1 H) 2.47 (quin, *J*=1.86 Hz, 1 H) 2.51 - 2.53 (m, 1 H) 2.62 - 2.65 (m, 1 H) 2.70 - 2.80 (m, 1 H) 2.87 - 2.94 (m, 1 H) 2.98 - 3.05 (m, 1 H) 3.08 (dt, *J*=13.53, 6.55 Hz, 1 H) 3.13 - 3.18 (m, 1 H) 3.19 - 3.27 (m, 2 H) 4.03 (t, *J*=4.44 Hz, 1 H) 4.26

(br. s., 2 H) 4.28 - 4.32 (m, 1 H) 4.35 - 4.40 (m, 2 H) 4.41 - 4.45 (m, 1 H) 4.46 - 4.51 (m, 2 H) 4.52 - 4.57 (m, 1 H) 4.65 (d, *J*=4.87 Hz, 1 H) 4.78 - 4.83 (m, 1 H) 4.94 - 5.00 (m, 1 H) 5.36 - 5.49 (m, 2 H) 5.51 - 5.59 (m, 2 H) 5.74 (d, *J*=3.44 Hz, 2 H) 5.78 (d, *J*=4.30 Hz, 1 H) 5.81 - 5.86 (m, 1 H) 5.88 (s, 1 H) 7.07 - 7.10 (m, 1 H) 7.25 - 7.32 (m, 5 H) 7.37 (ddd, *J*=7.73, 6.73, 2.15 Hz, 1 H) 7.40 - 7.43 (m, 2 H) 7.52 - 7.54 (m, 2 H) 7.55 - 7.59 (m, 1 H) 7.59 - 7.63 (m, 1 H) 7.63 - 7.68 (m, 3 H) 7.73 - 7.76 (m, 1 H) 9.21 - 9.27 (m, 1 H) 9.28 - 9.37 (m, 1 H) 10.96 - 11.21 (m, 1 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 11.95, 12.05, 31.35, 31.85, 32.08, 35.46, 35.51, 49.44, 49.78, 51.74, 51.87, 54.29, 70.77, 70.86, 71.87, 72.16, 80.85, 81.87, 89.07, 90.20, 109.86, 110.05, 114.59, 116.88, 126.46, 126.52, 126.83, 126.95, 127.61, 127.99, 128.05, 128.08, 128.22, 128.41, 128.66, 128.81, 129.00, 129.07, 129.13, 129.36, 129.43, 130.15, 130.42, 131.52, 131.63, 132.01, 132.23, 132.30, 132.73, 133.72, 133.80, 136.09, 137.05, 140.81, 140.86, 143.85, 143.91, 150.50, 150.58, 150.65, 155.94, 156.02, 156.21, 156.31, 163.49, 163.73, 163.77, 168.70, 168.84. HRMS m/z calculated for $C_{30}H_{29}F_3N_7O_7$ $[M+H]^+$ 656.2075, found 656.2072.

***N*-(3-(1-((2,2-Dimethyl-6-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)-1*H*-dibenzo[b,f][1,2,3]triazolo[4,5-d]azocin-8(9*H*)-yl)-3-oxopropyl)-2,2,2-trifluoroacetamide (15a)**

***N*-(3-(3-((2,2-Dimethyl-6-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)-3*H*-dibenzo[b,f][1,2,3]triazolo[4,5-d]azocin-8(9H)-yl)-3-oxopropyl)-2,2,2-trifluoroacetamide (15b)**

Azido derivative **6** (50 mg, 0.15 mmol) was dissolved in MeOH (2 ml), and aza-dibenzocyclooctyne **13** (78 mg, 0.21 mmol) was added. The solution was stirred at room temperature for 5 minutes, diluted with MeOH to 8 ml and then purified by semi-preparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to change from 30% to 60% over the course of 6 min. Derivatives **15a** and **15b** were collected separately. Derivative **15a** was the first compound and was isolated as a white solid, 31 mg (30%). Derivative **15b** was the second compound and was isolated as a white solid, 24 mg (23%).

15a:

^{1}H NMR (500 MHz, $CDCl_3$-*d*) δ ppm 1.30 (s, 3 H), 1.48 (s, 3 H), 1.80 (m, 1 H), 1.96 (d, *J*=1.40 Hz 3 H), 2.00 (m, 1 H), 3.22 (m, 1 H), 3.32 (m, 1 H), 4.19 (d, *J*=16.5 Hz 1 H), 4.53 (dt, *J*=9.90, 3.30 Hz 1 H), 4.79 (dd, *J*=13.80, 2.60 Hz 1 H), 4.85 (dd, *J*= 13.80, 2.60 Hz 1 H), 4.91 (dd, *J*=6.50, 3.40 Hz 1 H), 5.02 (d, *J*=6.30 1 H), 5.27 (m, 1 H), 5.75 (d, *J*=16.5 Hz 1 H), 6.95 (m, 1 H), 7.03 (m, 1 H), 7.16 (s, 1 H), 7.25 (m, 1 H), 7.26 (m, 2 H), 7.38 (t, *J*=5.70 Hz 1

H), 7.44 (m, 2 H), 7.65 (dd, *J*=5.90, 3.40 Hz 1 H), 8.34 (br. s., 1 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ ppm 12.31, 33.31, 35.39, 50.22, 51.32, 70.72, 73.37, 82.12, 92.05, 111.05, 115.79 (q, *J*=287.9), 124.32, 127.33, 127.44, 127.77, 129.82, 129.86, 129.92, 131.05, 131.10, 131.13, 131.83, 135.11, 135.77, 137.36, 139.27, 142.97, 150.78, 157.17 (q, *J*=36.5), 164.32, 171.86. HRMS m/z calculated for $C_{33}H_{33}F_3N_7O_7$ 696.2388, found 696.2385.

15b

Because it was not possible to identify the major ^{1}H and ^{13}C NMR signals, characterization was carried out for mixture of all conformers.

^{1}H NMR (500 MHz, $CDCl_3$-*d*) δ ppm 1.33 - 1.39 (m, 9 H), 1.51 (s, 1 H), 1.53 - 1.57 (m, 7 H), 1.71 (ddd, *J*=17.18, 6.87, 4.01 Hz, 1 H), 1.81 - 1.86 (m, 8 H), 1.87 (dd, *J*=4.58, 2.86 Hz, 1 H), 1.91 (s, 1 H), 1.98 (dd, *J*=7.45, 3.44 Hz, 1 H), 2.00 (s, 1 H), 2.01 - 2.03 (m, 1 H), 2.09 (s, 1 H), 2.16 (ddd, *J*=17.18, 7.45, 4.58 Hz, 2 H), 2.28 - 2.36 (m, 1 H), 2.65 - 2.75 (m, 1 H), 3.18 - 3.25 (m, 1 H), 3.26 - 3.49 (m, 6 H), 4.40 (dd, *J*=16.90, 4.30 Hz, 2 H), 4.57 - 4.67 (m, 2 H), 4.68 - 4.73 (m, 4 H), 4.79 - 4.83 (m, 1 H), 4.85 - 4.95 (m, 2 H), 4.99 - 5.05 (m, 1 H), 5.09 (dd, *J*=6.59, 2.00 Hz, 1 H), 5.11 - 5.15 (m, 1 H), 5.16 (dd, *J*=6.30, 3.44 Hz, 1 H), 5.23 (dd, *J*=6.30, 3.44 Hz, 1 H), 5.35 (br. s., 1 H), 5.37 - 5.43 (m, 1 H), 5.53 (d, *J*=1.15 Hz, 1 H), 5.57 (d, *J*=1.72 Hz, 1 H), 5.65 (d, *J*=1.72 Hz, 1 H), 5.81 (d, *J*=16.61 Hz, 1 H), 6.03 (d, *J*=17.18 Hz, 1 H), 6.88 - 6.92 (m, 2 H), 7.06 - 7.08 (m, 1 H), 7.09 - 7.13 (m, 1 H), 7.13 - 7.18 (m, 2 H), 7.22 - 7.27 (m, 4 H), 7.29 - 7.34 (m, 3 H), 7.35 (s, 1 H), 7.36 - 7.41 (m, 5 H), 7.41 - 7.46 (m, 3 H), 7.46 - 7.51 (m, 3 H), 7.51 - 7.56 (m, 2 H), 7.58 - 7.68 (m, 3 H), 9.12 (s, 1 H), 9.32 (s, 1 H), 9.41 (s, 1 H), 9.66 (s, 1 H); ^{13}C NMR (126 MHz, $CDCl_3$-*d*) δ ppm 11.85, 12.02, 12.18, 25.14, 25.25, 25.45, 26.86, 26.91, 27.01, 27.09, 31.95, 32.06, 33.19, 34.82, 35.03, 35.19, 35.34, 49.57, 49.93, 50.40, 50.69, 52.81, 53.34, 55.08, 55.13, 82.27, 82.71, 82.84, 84.00, 84.10, 84.38, 85.43, 85.72, 85.90, 87.30, 95.05, 95.94, 96.32, 96.50, 111.02, 111.18, 111.88, 112.28, 114.36, 114.58, 114.67, 114.92, 116.87, 126.81, 127.09, 127.22, 127.35, 127.39, 127.43, 127.50, 127.59, 128.03, 128.09, 128.19, 128.34, 128.45, 128.61, 128.71, 128.86, 129.02, 129.07, 129.20, 129.33, 129.53, 130.02, 130.33, 131.09, 131.79, 131.84, 131.92, 132.26, 132.42, 132.82, 132.91, 133.54, 138.51, 139.01, 139.41, 139.52, 140.67, 140.94, 141.87, 142.00, 143.12, 145.02, 145.37, 150.04, 150.18, 150.58, 157.08, 157.20, 157.42, 157.48, 163.82, 163.90, 163.96, 164.00, 170.30, 170.49, 171.16, 171.64. HRMS m/z calculated for $C_{33}H_{33}F_3N_7O_7$ 696.2388, found 696.2385.

***N*-(3-(1*H*-dibenzo[b,f][1,2,3]triazolo[4,5-d]azocin-8(9*H*)-yl)-3-oxopropyl)-2,2,2-trifluoroacetamide (16a)**

Aza-dibenzocyclooctyne **13** (50 mg, 0.13 mmol) was dissolved in MeOH (5 ml), and sodium azide (13 mg, 0.19 mmol) was added. The solution was stirred at room temperature for 5 minutes, diluted with MeOH to 8 ml and then purified by semi-preparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to change from 30% to 60% over the course of 6 min. White solid, 38 mg (70%).

^{1}H NMR (500 MHz, $CDCl_3$-*d*) δ ppm 1.91 (dt, *J*=17.18, 6.00 Hz, 1 H), 2.14 (dt, *J*=17.26, 6.00 Hz, 1 H), 3.28 (q, *J*=6.00 Hz, 2 H), 4.50 (d, *J*=17.30 Hz, 1 H), 6.11 (d, *J*=17.30 Hz, 1 H), 7.21 (d, *J*=7.50 Hz, 1 H), 7.23 - 7.27 (m, 1 H), 7.29 - 7.33 (m, 1 H), 7.42 (dd, *J*=8.02, 0.86 Hz, 1 H), 7.46 - 7.50 (m, 1 H), 7.52 (dd, *J*=7.73, 1.15 Hz, 1 H), 7.55 - 7.59 (m, 1 H), 7.62 - 7.67 (m, 1 H), 7.72 (br. s, 1 H), 7.73 (d, *J*=1.15 Hz, 1 H); ^{13}C NMR (126 MHz, $CDCl_3$-*d*) δ ppm 32.95, 35.36, 52.60, 115.94 (q, *J*=287.9 Hz), 124.46, 127.49, 128.09, 128.72, 128.93, 129.65, 129.97, 130.18, 131.95, 132.78, 134.14, 139.14, 139.97, 140.94, 157.19 (q, *J*=36.5 Hz), 171.46. HRMS m/z calculated for $C_{20}H_{17}F_3N_5O_2$ 416.1329, found 416.1328.

2,2,2-trifluoro-*N*-(3-oxo-3-(1-(2-oxo-2*H*-chromen-3-yl)-1*H*-dibenzo[b,f][1,2,3]triazolo[4,5-d]azocin-8(9*H*)-yl)propyl)acetamide (17a)

2,2,2-trifluoro-*N*-(3-oxo-3-(3-(2-oxo-2*H*-chromen-3-yl)-3*H*-dibenzo[b,f][1,2,3]triazolo[4,5-d]azocin-8(9*H*)-yl)propyl)acetamide (17b)

Aza-dibenzocyclooctyne **13** (149 mg, 0.4 mmol) was dissolved in MeOH (4 ml) and 3-azidocoumarine (50 mg, 0.26 mmol) was added. The solution was stirred at room temperature for 15 minutes, diluted with MeOH to 8 ml and then purified by semi-preparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to change from 50% to 80% over the course of 6 min. Derivatives **17a** and **17b** were collected separately. Derivative **17a** was the first compound and was isolated as a white solid, 27 mg (19%). Derivative **17b** was the second compound and was isolated as a white solid, 31 mg (21%).

17a:

^{1}H NMR (500 MHz, $CDCl_3$-*d*) δ ppm 1.83 - 1.90 (m, 1 H), 2.07 (ddd, *J*=17.47, 8.59, 4.01 Hz, 1 H), 3.25 (ddt, *J*=13.50, 9.06, 4.47, 4.47 Hz, 1 H), 3.39 - 3.47 (m, 1 H), 4.45 (d, *J*=16.61 Hz, 1 H), 6.29 (d, *J*=16.60 Hz, 1 H), 6.91 (d, *J*=7.73 Hz, 1 H), 7.06 - 7.10 (m, 1 H), 7.21 - 7.24 (m, 1 H), 7.26 - 7.28 (m, 1 H), 7.30 - 7.34 (m, 1 H), 7.36 (d, *J*=0.86 Hz, 1 H), 7.36 - 7.40 (m,

2 H), 7.50 - 7.53 (m, 2 H), 7.61 - 7.66 (m, 2 H), 7.74 - 7.78 (m, 1 H), 8.20 (s, 1 H); ^{13}C NMR (126 MHz, $CDCl_3$-*d*) δ ppm 33.31, 35.16, 51.67, 115.86 (q, *J*=287.9 Hz), 117.01, 117.73, 123.60, 124.72, 125.35, 127.33, 127.73, 129.13, 129.69, 129.82, 130.04, 130.08, 130.33, 130.90, 131.10, 133.46, 135.09, 136.24, 139.75, 140.12, 142.46, 153.68, 155.93, 156.61 (q, *J*=36.5 Hz), 171.43. HRMS m/z calculated for $C_{29}H_{21}F_3N_5O_4$ 560.1540, found 560.1540.

17b:

^{1}H NMR (500 MHz, $CDCl_3$-*d*) δ ppm 2.34 (dd, *J*=7.16, 4.58 Hz, 1 H), 2.37 (dd, *J*=6.44, 4.44 Hz, 1 H), 3.42 - 3.49 (m, 1 H), 3.60 - 3.68 (m, 1 H), 4.68 (d, *J*=15.50 Hz, 1 H), 5.49 (d, *J*=15.47 Hz, 1 H), 7.10 - 7.12 (m, 2 H), 7.21 - 7.24 (m, 1 H), 7.29 - 7.31 (m, 2 H), 7.31 - 7.35 (m, 2 H), 7.40 (d, *J*=8.31 Hz, 2 H), 7.60 - 7.63 (m, 2 H), 7.67 - 7.71 (m, 1 H), 7.74 (dd, *J*=8.02, 1.43 Hz, 1 H), 8.39 (s, 1 H); ^{13}C NMR (126 MHz, $CDCl_3$-*d*) δ ppm 33.36, 35.76, 53.76, 115.85 (q, *J*=289.0 Hz), 117.03, 117.84, 123.04, 125.23, 125.71, 127.43, 127.99, 128.80, 128.92, 129.29, 129.75, 130.14, 131.04, 131.30, 131.97, 133.20, 133.89, 137.41, 138.82, 140.05, 141.38, 153.67, 156.22, 157.19 (q, *J*=36.6 Hz), 171.85. HRMS m/z calculated for $C_{29}H_{21}F_3N_5O_4$ 560.1540, found 560.1539.

2-((benzoyloxy)methyl)-5-(2,4-dioxo-5-((8-(3-(2,2,2-trifluoroacetamido)propanoyl)-8,9-dihydro-1*H*-dibenzo[b,f][1,2,3]triazolo[4,5-d]azocin-1-yl)methyl)-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-3,4-diyl dibenzoate 18a

2-((benzoyloxy)methyl)-5-(2,4-dioxo-5-((8-(3-(2,2,2-trifluoroacetamido)propanoyl)-8,9-dihydro-3*H*-dibenzo[b,f][1,2,3]triazolo[4,5-d]azocin-3-yl)methyl)-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-3,4-diyl dibenzoate 18b

Aza-dibenzocyclooctyne **13** (46 mg, 0.12 mmol) was dissolved in MeOH (4 ml) and 2′,3′,5′tribenzoyl-5-azidomethyluridine (50 mg, 0.08 mmol) was added. The solution was stirred at room temperature for 15 minutes, diluted with MeOH to 8 ml and then purified using semipreparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to change from 50% to 80% over the course of 6 min. The compound **18** was separated as a mixture of two inseparable regioisomers. Because it was not possible to identify the ^{1}H and ^{13}C NMR signals for individual regisomers, characterization was performed for both regioisomers together. White solid 35 mg (44%).

^{1}H NMR (500 MHz, $CDCl_3$-*d*) δ ppm 1.69 - 1.80 (m, 6 H), 1.81 - 1.90 (m, 1 H), 1.92 - 2.01 (m, 1 H), 2.07 - 2.17 (m, 1 H), 2.22 - 2.28 (m, 1 H), 2.62 - 2.72 (m, 1 H), 3.09 - 3.18 (m, 1 H), 3.19 - 3.34 (m, 2 H), 3.36 - 3.47 (m, 1 H), 4.23 - 4.31 (m, 1 H), 4.39 - 4.45 (m, 1 H), 4.59 -

4.73 (m, 4 H), 4.74 - 4.82 (m, 2 H), 4.84 - 4.90 (m, 1 H), 4.98 - 5.02 (m, 1 H), 5.25 - 5.31 (m, 1 H), 5.62 - 5.67 (m, 1 H), 5.82 (dt, *J*=15.90, 5.80 Hz, 1 H), 5.88 - 5.93 (m, 1 H), 5.94 - 5.99 (m, 1 H), 6.01 - 6.11 (m, 1 H), 6.21 (dd, *J*=12.60, 4.87 Hz, 1 H), 6.26 - 6.29 (m, 1 H), 6.34 (d, *J*=5.44 Hz, 1 H), 6.92 - 6.96 (m, 1 H), 7.04 - 7.08 (m, 1 H), 7.23 - 7.29 (m, 7 H), 7.31 - 7.42 (m, 12 H), 7.43 - 7.48 (m, 4 H), 7.51 - 7.60 (m, 8 H), 7.90 - 7.94 (m, 4 H), 7.95 - 8.01 (m, 3 H), 8.07 - 8.12 (m, 3 H), 8.76 - 9.07 (m, 2 H); ^{13}C NMR (126 MHz, $CDCl_3$-*d*) 33.06, 33.10, 33.36, 33.39, 35.16, 35.27, 35.37, 44.13, 44.99, 45.60, 51.31, 52.86, 63.57, 64.00, 70.82, 71.26, 71.35, 74.01, 74.19, 80.32, 80.49, 80.91, 89.15, 89.29, 89.68, 89.78, 107.94, 108.62, 109.13, 127.09, 127.29, 127.45, 127.49, 128.37, 128.53, 128.63, 128.89, 129.61, 129.78, 129.83, 129.93, 130.87, 131.21, 131.41, 131.89, 132.68, 133.50, 133.73, 133.79, 133.83, 134.57, 135.09, 135.39, 139.60, 139.66, 139.96, 140.37, 140.42, 142.24, 142.62, 142.71, 144.60, 145.40, 149.31, 149.38, 149.40, 149.48, 156.84, 157.13, 157.42, 161.34, 161.45, 161.85, 161.91, 165.23, 165.28, 165.31, 165.35, 166.01, 170.12, 170.27, 171.45, 171.50.

2-(5-(Azidomethyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-5-((benzoyloxy)methyl)tetrahydrofuran-3,4-diyl dibenzoate

5-hydroxymethylenuracil (3.25 g, 22.8 mmol) was suspended in HMDS (120 ml), and a catalytic amount of ammonium sulphate was added. The mixture was stirred under reflux for 2 hours and evaporated. The resulting solid was dissolved in 1,2-dichloroethane (85 ml). 1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose (11.2 g, 22.2 mmol) and trimethylsilyl trifluormethansulfonate (4.69 ml, 25.9 mmol) were added. The reaction was stirred at room temperature for 18 hours. Then, extraction with saturated sodium hydrogen carbonate solution was performed. The organic layer was dried over anhydrous sodium sulphate, and the solvent was evaporated. The crude product 5-hydroxymethylenuridin (15 g) was dissolved without purification in acetonitrile (300 ml), and thionyl chloride (2.3 ml) was added. The reaction mixture was stirred at room temperature for 30 minutes, and triethylamine (4.5 ml) was added to neutralize the reaction. The solvent was evaporated, the resulting solid was dissolved in anhydrous DMF (187.5 ml), and sodium azide (9.5 g, 146.7 mmol) was added. The reaction was stirred at 90 °C overnight and then diluted with water (1.5 L). The white precipitate was obtained by filtration and washed with water. The crude product was purified first on a silica gel column with a mobile phase of Tol:EtOAc:HCOOH (7:2:0.2 v/v/v). White solid 2.75 g (33%). For characterization the product was purified again using semipreparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to shift from 50% to 80% over the course of 6 min.

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.01 (dd, *J*=13.60, 7.00 Hz, 1 H), 4.64 (dd, *J*=11.90, 5.20 Hz, 1 H), 4.71 (d, *J*=3.73 Hz, 1 H), 4.73 - 4.79 (m, 1 H), 5.91 - 5.97 (m, 1 H), 6.19 - 6.23 (m, 1 H), 7.41 - 7.48 (m, 1 H), 7.48 - 7.53 (m, 1 H), 7.61 - 7.68 (m, 1 H), 7.89 (ddd, *J*=8.22, 4.17, 1.21 Hz, 1 H), 7.99 - 8.01 (m, 1 H), 8.02 (d, *J*=0.88 Hz, 1 H), 11.76 (br. s., 1 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 46.66, 63.62, 70.42, 73.26, 78.79, 89.08, 109.09, 128.42, 128.72-128.75 (3C), 129.19-129.32 (3C), 129.36, 133.57, 133.85, 133.96, 141.02, 150.07, 162.84, 164.59, 164.61, 165.48. HRMS m/z calculated for $C_{31}H_{26}N_5O_9$ $[M+H]^+$ 612.1725, found 612.1723.

1-(6-((8-(3-aminopropanoyl)-8,9-dihydro-1*H*-dibenzo[b,f][1,2,3]triazolo[4,5-d]azocin-1-yl)methyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (20a)

1-(6-((8-(3-aminopropanoyl)-8,9-dihydro-3*H*-dibenzo[b,f][1,2,3]triazolo[4,5-d]azocin-3-yl)methyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (20b)

Solution of K_2CO_3 (105 mg, 0.76 mmol) in water (1 ml) was added to solution of aza-dibenzocyclooctyne **13** (150 mg, 0.40 mmol) in MeOH (1.5 ml). Reaction mixture was stirred at room temperature for 18 hours. Then azide **6** (105 mg, 0.32 mmol) was added to reaction mixture. The solution was stirred at room temperature for 20 minutes, diluted with MeOH to 8 ml and then purified by semi-preparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to change from 20% to 50% over the course of 6 min. Derivatives **20a** and **20b** were collected separately. Derivative **20a** was the first compound and was isolated as a white solid, 27 mg (14%). Derivative **20b** was the second compound and was isolated as a white solid, 20 mg (10%).

20a:

^{1}H NMR (500 MHz, $CDCl_3$-*d*) δ ppm 1.30 - 1.33 (m, 3 H) 1.49 (s, 3 H) 1.74 - 1.81 (m, 1 H) 1.95 (s, 3 H) 1.99 - 2.06 (m, 1 H) 2.65 - 2.73 (m, 1 H) 2.80 - 2.87 (m, 1 H) 4.21 (d, *J*=16.60 Hz, 1 H) 4.55 (dt, *J*=10.17, 2.94 Hz, 1 H) 4.80 (dd, *J*=13.60, 2.70 Hz, 1 H) 4.84 - 4.88 (m, 1 H) 4.90 (dd, *J*=5.87, 3.87 Hz, 1 H) 5.02 (d, *J*=6.30 Hz, 1 H) 5.45 (s, 1 H) 5.81 (d, *J*=16.60 Hz, 4 H) 6.93 - 6.98 (m, 1 H) 7.03 - 7.07 (m, 1 H) 7.26 – 7.27 (m, 1 H) 7.27 - 7.29 (m, 1 H) 7.31 (dd, *J*=5.87, 3.58 Hz, 1 H) 7.33 (br. s., 1 H) 7.43 - 7.47 (m, 2 H) 7.68 (dd, *J*=5.58, 3.29 Hz, 1 H); ^{13}C NMR (126 MHz, $CDCl_3$-*d*) δ ppm 12.03, 22.71, 25.21, 26.84, 33.95, 36.14, 51.02, 82.14, 85.07, 89.13, 96.96, 110.07, 114.13, 124.65, 127.48, 127.78, 129.22, 129.32, 129.55,

129.87, 130.75, 131.50, 131.70, 135.40, 136.20, 139.56, 141.51, 142.50, 149.59, 164.00, 170.80. MS m/z calculated for $C_{31}H_{34}N_7O_6$ $[M+H]^+$ 600.26, found 600.32.

20b:

^{1}H NMR (500 MHz, $CDCl_3$-*d*) δ ppm 1.39 (s, 3 H) 1.58 (s, 3 H) 1.71 - 1.76 (m, 1 H) 1.78 (s, 3 H) 2.25 - 2.35 (m, 1 H) 2.81 - 2.87 (m, 1 H) 2.88 – 2.94 (m, 1 H) 4.39 (d, *J*=16.04 Hz, 1 H) 4.62 - 4.70 (m, 1 H) 4.72 (d, *J*=4.58 Hz, 1 H) 4.73 - 4.77 (m, 1 H) 5.04 - 5.08 (m, 1 H) 5.40 - 5.44 (m, 1 H) 5.50 (d, *J*=16.04 Hz, 1 H) 5.75 (s, 1 H) 6.69 (s, 1 H) 7.10 - 7.14 (m, 1 H) 7.14 - 7.18 (m, 1 H) 7.20 (d, *J*=8.02 Hz, 1 H) 7.24 (d, *J*=9.17 Hz, 1 H) 7.26 (br. s., 1 H) 7.39 (d, *J*=8.02 Hz, 1 H) 7.45 - 7.48 (m, 1 H) 7.48 - 7.53 (m, 2 H); ^{13}C NMR (126 MHz, $CDCl_3$-*d*) δ ppm 12.04, 25.26, 27.01, 32.64, 35.57, 49.79, 52.61, 82.61, 83.72, 85.68, 94.07, 112.33, 114.76, 127.00, 127.62, 128.66, 128.89, 129.49, 129.69, 129.87, 130.29, 131.57, 131.72, 133.18, 134.05, 137.20, 140.51, 145.61, 151.28, 164.46, 170.01. MS m/z calculated for $C_{31}H_{34}N_7O_6$ $[M+H]^+$ 600.26, found 600.52.

(Z)-1-(dibenzo[b,f]azocin-5(6*H*)-yl)-2,2,2-trifluoroethan-1-one (21)

(Z)-5,6-dihydrodibenzo[b,f]azocine **6** (1.6 g, 7.73 mmol) was dissolved in DCM (50 ml) and trifluoracetic anhydride (4.2 ml, 30 mmol) was added. Reaction mixture was stirred at room temperature for 1 h. Then saturated Na_2CO_3 solution was added (30 ml) and mixture was stirred vigorously for 30 min. Organic layer was separated, washed with water (30 ml) and brine (30 ml) and dried over $MgSO_4$. Solvent was removed under reduced pressure and crude product was purified on column chromatography with mobile phase consists of hexane:DCM (3:2 v/v). Brown solid, 1.68 g (72%).

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 4.41 (d, *J*=15.11 Hz, 1 H) 5.33 (d, *J*=15.11 Hz, 1 H) 6.72 (d, *J*=13.28 Hz, 1 H) 6.87 (d, *J*=13.28 Hz, 1 H) 7.20 - 7.24 (m, 1 H) 7.24 - 7.27 (m, 2 H) 7.34 - 7.38 (m, 2 H) 7.39 - 7.41 (m, 2 H) 7.46 – 7.49 (m, 1 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 56.28, 115.86 (q, *J*=288.99 Hz), 127.37, 127.60, 127.62, 128.25, 128.27, 129.13, 130.67, 130.90, 131.68, 132.37, 132.67, 135.54, 135.90, 137.22, 154.93 (q, *J*=35.15 Hz). MS m/z calculated for $C_{17}H_{13}F_3NO$ $[M+H]^+$ 304.09, found 304.23.

1-(11,12-dibromo-11,12-dihydrodibenzo[b,f]azocin-5(6*H*)-yl)-2,2,2-trifluoroethan-1-one (22)

Compound **21** (1.68 g, 5.5 mmol) was dissolved in DCM (12 ml) and pyridinium tribromide (1.90 g, 5.94 mmol) was added. Reaction mixture was stirred at room temperature for 18 hours. After that time, reaction mixture was diluted with DCM (50 ml), washed with 5% HCl (50 ml) and with brine (50 ml) and dried over $MgSO_4$. Solvent was removed under

reduced pressure and crude product was purified on column chromatography with mobile phase consists of hexane:DCM (2:3 v/v). Compound **22** was isolate as a mixture of inseparable regiosiomers; characterization was performed for all isomers together. Brown solid, 2.14 g (84%).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 4.59 (d, *J*=14.60 Hz, 2 H) 4.94 (d, *J*=14.00 Hz, 1 H) 5.23 (d, *J*=14.00 Hz, 1 H) 5.73 - 5.77 (m, 3 H) 5.77 - 5.81 (m, 2 H) 5.90 (d, *J*=9.74 Hz, 2 H) 6.28 (d, *J*=9.45 Hz, 1 H) 6.99 - 7.06 (m, 3 H) 7.10 - 7.17 (m, 5 H) 7.21 (d, *J*=8.02 Hz, 2 H) 7.23 - 7.28 (m, 7 H) 7.28 - 7.33 (m, 4 H) 7.59 (d, *J*=7.73 Hz, 1 H) 7.66 (d, *J*=7.73 Hz, 2 H); ^{13}C NMR (126 MHz, $CDCl_3$-*d*) δ ppm 52.72, 53.72, 54.59, 56.28, 58.67, 60.56, 115.61 (q, *J*=287.91 Hz), 128.22, 128.26, 128.68, 128.93, 129.22, 129.32, 129.37, 129.64, 129.96, 129.99, 130.12, 130.24, 130.30, 130.92, 130.99, 133.83, 133.88, 134.18, 136.24, 137.19, 140.29, 155.11 (q, *J*=35.99 Hz), 156.32 (q, *J*=35.99 Hz). MS m/z calculated for $C_{17}H_{13}F_3NO$ $[M+H]^+$ 461.93, found 461.95.

1-(1,2-didehydrodibenzo[b,f]azocin-5(6*H*)-yl)-2,2,2-trifluoroethan-1-one (23)

Solution of *t*BuOK (990 mg, 8.82 mmol) in anhydrous THF (30 ml) was added to solution of dibromoderivative **22** (1.5 g, 3.25 mmol) in anhydrous THF (15 ml) at 0 °C. Solution was stirred at 0 °C for 30 min, then diluted with EtOAc (60 ml) and washed with 5% HCl (50 ml), brine (50 ml) and dried over $MgSO_4$. Crude product was purified by semi-preparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to change from 60% to 80% over the course of 6 min. Yellowish solid, 520 mg (53%).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 3.90 (d, *J*=14.32 Hz, 1 H) 5.23 (d, *J*=14.32 Hz, 1 H) 7.32 - 7.38 (m, 1 H) 7.38 - 7.42 (m, 1 H) 7.45 (dd, *J*=6.87, 2.29 Hz, 1 H) 7.50 - 7.54 (m, 1 H) 7.56 (d, *J*=4.58 Hz, 2 H) 7.67 - 7.71 (m, 2 H); ^{13}C NMR (126 MHz, $CDCl_3$-*d*) δ ppm 56.96, 115.79 (q, *J*=289.11 Hz), 121.61, 122.38, 125.66, 126.73, 128.23, 128.37, 128.51, 128.83, 128.96, 129.08, 129.64, 132.62, 146.19, 148.04, 155.76 (q, *J*=35.99 Hz). MS m/z calculated for $C_{17}H_{11}F_3NO$ $[M+H]^+$ 302.08, found 302.19.

1-(2,2-dimethyl-6-((8-(2,2,2-trifluoroacetyl)-8,9-dihydro-1*H*-dibenzo[b,f][1,2,3]triazolo[4,5-d]azocin-1-yl)methyl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (24a)

1-(2,2-dimethyl-6-((8-(2,2,2-trifluoroacetyl)-8,9-dihydro-3*H*-dibenzo[b,f][1,2,3]triazolo[4,5-d]azocin-3-yl)methyl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (24b)

Aza-dibenzocyclooctyne **23** (520 mg, 1.73 mmol) and azide **6** (404 mg, 1.25 mmol) were dissolved in MeOH (10 ml). The solution was then purified by semi-preparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to change from 40% to 70% over the course of 6 min. Derivatives **24a** and **24b** were collected separately. Derivative **24a** was the first compound and was isolated as a white solid, 223 mg (29%). Derivative **24b** was the second compound and was isolated as a white solid, 197 mg (25%).

24a:

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.26 (s, 3 H) 1.45 (s, 3 H) 1.72 (s, 3 H) 4.19 - 4.25 (m, 1 H) 4.53 (d, *J*=15.50 Hz, 1 H) 4.72 - 4.84 (m, 2 H) 4.88 (dd, *J*=6.16, 3.58 Hz, 1 H) 4.95 (dd, *J*=14.32, 2.86 Hz, 1 H) 5.01 (d, *J*=6.30 Hz, 1 H) 5.16 (d, *J*=15.50 Hz, 1 H) 5.54 (s, 1 H) 7.13 - 7.18 (m, 3 H) 7.25 - 7.30 (m, 1 H) 7.41 - 7.46 (m, 3 H) 7.57 - 7.61 (m, 1 H) 10.91 (br. s, 1 H); ^{13}C NMR (126 MHz, $CDCl_3$-*d*) δ ppm 11.91, 24.88, 26.65, 50.59, 53.14, 81.47, 84.24, 88.56, 94.40, 109.22, 112.90, 115.61 (q, *J*=289.11 Hz), 125.54, 126.41, 128.50, 129.18, 129.26, 129.45, 129.94, 130.00, 130.34, 131.89, 132.44, 135.50, 136.00, 139.52, 149.69, 155.95 (q, *J*=34.76 Hz), 163.73. MS m/z calculated for $C_{30}H_{28}F_3N_6O_6$ $[M+H]^+$ 625.20, found 625.21.

24b:

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.33 (s, 3 H) 1.54 (s, 3 H) 1.79 (d, *J*=1.15 Hz, 3 H) 4.37 - 4.44 (m, 1 H) 4.68 - 4.73 (m, 2 H) 4.75 (d, *J*=16.90 Hz, 1 H) 5.05 - 5.09 (m, 2 H) 5.16 (dd, *J*=6.59, 2.00 Hz, 1 H) 5.68 (d, *J*=16.90 Hz, 1 H) 5.82 (d, *J*=1.72 Hz, 1 H) 7.31 - 7.34 (m, 3 H) 7.40 - 7.44 (m, 2 H) 7.68 - 7.71 (m, 2 H) 7.84 (d, *J*=8.02 Hz, 1 H) 11.48 (br. s, 1 H); ^{13}C NMR (126 MHz, $CDCl_3$-*d*) δ ppm 11.91, 25.17, 26.98, 50.01, 54.42, 81.40, 83.65, 86.42, 93.23, 109.66, 113.63, 115.63 q, *J*=289.00 Hz), 125.58, 126.45, 127.53, 128.25, 128.60, 129.21, 129.50, 129.65, 129.79, 131.55, 132.07, 132.78, 137.57, 139.36, 143.57, 150.45, 154.88 (q, *J*=34.68 Hz), 163.94 MS m/z calculated for $C_{30}H_{28}F_3N_6O_6$ $[M+H]^+$ 625.20, found 625.24.

1-(6-((8,9-dihydro-1*H*-dibenzo[b,f][1,2,3]triazolo[4,5-d]azocin-1-yl)methyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (26a)

Solution of K_2CO_3 (76 mg, 0.55 mmol) in water (4 ml) was added to solution of triazole **24a** (170 mg, 0.27 mmol) in MeOH (8 ml). Reaction mixture was stirred at room temperature for 18 hours. The solution was then purified by semi-preparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to change from 30% to 60% over the course of 6 min. White solid, 35 mg (24%).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.25 (s, 3 H) 1.40 (s, 3 H) 1.73 (s, 3 H) 3.60 - 3.68 (m, 1 H) 4.10 - 4.16 (m, 1 H) 4.31 (dd, *J*=14.61, 7.45 Hz, 1 H) 4.58 (dd, *J*=14.18, 9.31 Hz, 1 H) 4.84 - 4.89 (m, 1 H) 4.97 (d, *J*=6.30 Hz, 1 H) 5.44 (s, 1 H) 6.24 (t, *J*=7.59 Hz, 1 H) 6.46 - 6.52 (m, 2 H) 6.88 - 6.93 (m, 1 H) 7.06 (d, *J*=8.31 Hz, 1 H) 7.17 - 7.21 (m, 1 H) 7.26 - 7.31 (m, 2 H) 7.35 - 7.38 (m, 2 H) 7.46 - 7.52 (m, 1 H) 11.10 (br. s, 1 H); ^{13}C NMR (126 MHz, $CDCl_3$-*d*) δ ppm 11.87, 25.02, 26.79, 46.14, 50.00, 81.26, 84.13, 87.76, 93.47, 109.28, 112.95, 113.32, 115.87, 117.57, 127.88, 127.99, 128.73, 129.02, 129.51, 130.06, 133.11, 133.19, 139.30, 140.05, 145.94, 146.26, 149.70, 163.87. MS m/z calculated for $C_{28}H_{29}N_6O_5$ $[M+H]^+$ 529.22, found 529.39.

1-(6-((8,9-dihydro-3*H*-dibenzo[b,f][1,2,3]triazolo[4,5-d]azocin-3-yl)methyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (26b)

Solution of K_2CO_3 (66 mg, 0.48 mmol) in water (3 ml) was added to solution of triazole **24a** (147 mg, 0.24 mmol) in MeOH (6 ml). Reaction mixture was stirred at room temperature for 18 hours. The solution was then purified by semi-preparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to change from 30% to 60% over the course of 6 min. White solid, 59 mg (46%).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.26 (br. s., 3 H) 1.44 (br. s., 3 H) 1.75 (br. s., 3 H) 3.80 - 3.93 (m, 1 H) 4.17 - 4.32 (m, 1 H) 4.78 - 4.86 (m, 2 H) 4.89 - 5.00 (m, 1 H) 5.01 - 5.10 (m, 1 H) 5.60 - 5.67 (m, 1 H) 6.31 - 6.52 (m, 1 H) 6.71 - 6.92 (m, 2 H) 7.39 (d, *J*=1.15 Hz, 5 H) 7.46 - 7.54 (m, 3 H) 11.19 (br. s, 1 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 11.95, 25.08, 26.79, 46.12, 50.43, 81.69, 84.09, 87.13, 93.41, 107.73, 109.33, 113.04, 116.04, 117.79, 127.82, 127.97, 128.86, 129.46, 129.75, 131.94, 132.55, 132.71, 137.25, 139.19,

143.34, 146.88, 150.00, 163.93. MS m/z calculated for $C_{28}H_{29}N_6O_5$ $[M+H]^+$ 529.22, found 529.44.

6.2.2. Solid phase synthesis of fluorescently labelled DIBAC analogues

Resin 27

Rink Resin (1 g) was washed three times with DCM, three times with DMF and a solution of 50% piperidine in DMF (10 ml) was added. The resin was shaken for 15 min, washed five times with DMF and five times with DCM. Then, solution of Fmoc-*β*-Ala-OH (622 mg, 2 mmol), HOBt (306 mg, 2 mmol) and DIC (312 μL, 2 mmol) in 10 mL DCM/DMF (1:1, v/v) was added. The resin was shaken for 18 hours, washed five times with DMF and five times with DCM. LC/MS analysis of cleaved product: MS (ESI) exact mass calculated for $C_{18}H_{19}N_2O_3$ $[M+H]^+$ 311.14; found 311.13, t_R = 2.87 min, purity: 97%.

Resin 28

Wang Resin (1 g) was washed three times with DCM and solution of Fmoc-*β*-Ala-OH (622 mg, 2 mmol), HOBt (306 mg, 2 mmol), DMAP (61 mg, 0.5 mmol) and DIC (312 μL, 2 mmol) in 10 mL DCM/DMF (1:1, v/v) was added. The resin was shaken for 18 hours, washed five times with DMF and five times with DCM. LC/MS analysis of cleaved product: MS (ESI) exact mass calculated for $C_{18}H_{18}NO_4$ $[M+H]^+$ 312.12; found 312.39, t_R = 1.76 min, purity: 93%.

Resin 31 and 32

Resin **27** (500 mg) or **28** (500 mg) was washed three times with DCM, three times with DMF and a solution of 50% piperidine in DMF (5 ml) was added. The resin was shaken for 15 min, washed five times with DMF and five times with DCM. Then, solution of 4-fluoro-3-nitrobenzoic acid (555 mg, 2.5 mmol) in pyridine (5 ml) was added. The resin was shaken for 18 hours at 80 °C, washed three times with H_2O/DMF 1:1, three times with 20% AcOH in DMF, five times with DMF and five times with DCM. LC/MS analysis of cleaved products:

31: MS (ESI) exact mass calculated for $C_{10}H_{12}N_3O_5$ $[M+H]^+$ 254.08; found 254.66, t_R = 0.55 min, purity: 93%.

32: MS (ESI) exact mass calculated for $C_{10}H_{11}N_2O_6$ $[M+H]^+$ 255.06; found 255.50, t_R = 0.98 min, purity: 88%.

Resin 33 and 34

Resin **31** (500 mg) or **32** (500 mg) was washed three times with anhydrous DCM and a solution of $SOCl_2$ (75 μL, 1 mmol) and DIEA (165 μL, 2 mmol) in anhydrous DCM (5 ml)

was added. The resin was shaken for 18 hours and washed five times with anhydrous DCM. Then, solution of (Z)-5,6-dihydrodibenzo[b,f]azocine **6** (208 mg , 1 mmol), pyridine (80 μL, 1 mmol) in anhydrous DCM (5 ml) was added. The resin was shaken for two hours and washed five times with DCM. LC/MS analysis of cleaved products:

33: MS (ESI) exact mass calculated for $C_{25}H_{23}N_4O_4$ $[M+H]^+$ 443.17; found 443.53, t_R = 2.67 min, purity: 97%.

34: MS (ESI) exact mass calculated for $C_{25}H_{22}N_3O_5$ $[M+H]^+$ 444.16; found 444.31, t_R = 2.24 min, purity: 94%

Resin 35 and 36

Resin **33** (500 mg) or **34** (500 mg) was washed three times with DCM and a solution of pyridinium tribromide (320 mg, 1 mmol) and DIEA (165 μL, 1 mmol) in DCM (5 ml) was added. The resin was shaken for 18 hours and washed five times with DCM. LC/MS analysis of cleaved products:

35: MS (ESI) exact mass calculated for $C_{25}H_{23}Br_2N_4O_4$ $[M+H]^+$ 601.01; found 601.31, t_R = 2.59 min, purity: 95%.

36: MS (ESI) exact mass calculated for $C_{25}H_{22}Br_2N_3O_5$ $[M+H]^+$ 601.99; found 602.20, t_R = 2.39 min, purity: 95%.

Resin 37

Resin **35** (250 mg) was washed three times with DMSO and a solution of BTPP (320 mg, 1 mmol) in DMSO (3 ml) was added. The resin was shaken at 70 °C for 18 hours and washed three times with DMSO and five times with DCM. LC/MS analysis of cleaved products: MS (ESI) exact mass calculated for $C_{25}H_{23}N_4O_4$ $[M+H]^+$ 443.17; found 443.53, t_R = 2.68 min, purity: 94%.

(Z)-3-((4-(5,6-dihydrodibenzo[b,f]azocine-5-carbonyl)-2-nitrophenyl)amino)propanamide 47

Resin **37** (250 mg) was treated by TFA in DCM (1:1) at room temperature for 1 hour. The cleavage cocktail was evaporated by a stream of nitrogen. Solution containing crude product was purified using semipreparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to shift from 30% to 60% over the course of 6 min. Yellowish oil, 21 mg (32%).

^{1}H NMR (500 MHz, $CDCl_3$-*d*) δ ppm 2.50 - 2.55 (m, 2 H) 3.56 - 3.60 (m, 2 H) 4.21 - 4.27 (m, 1 H) 5.36 - 5.56 (m, 2 H) 6.59 - 6.63 (m, 1 H) 6.64 - 6.68 (m, 1 H) 6.82 - 6.86 (m, 2 H) 7.02 - 7.08 (m, 2 H) 7.17 - 7.21 (m, 1 H) 7.24 - 7.26 (m, 2 H) 7.29 - 7.33 (m, 2 H) 7.41 - 7.43 (m, 1 H) 7.56 - 7.59 (m, 1 H) 8.12 (d, *J*=2.29 Hz, 1 H) 8.20 - 8.24 (m, 1 H); ^{13}C NMR (126 MHz,

$CDCl_3$-*d*) δ ppm 34.53, 38.70, 56.35, 112.55, 122.63, 127.08, 127.11, 127.43, 127.64, 128.13, 128.57, 128.93, 129.89, 131.11, 131.46, 131.76, 133.81, 134.27, 135.23, 136.36, 136.40, 143.82, 145.58, 167.81, 171.91. MS (ESI) exact mass calculated for $C_{25}H_{23}N_4O_4$ $[M+H]^+$ 443.17; found 443.53.

Resin 38

Resin **36** (250 mg) was washed three times with DMSO and a solution of proton sponge (267 mg, 1.5 mmol) in DMSO (3 ml) was added. The resin was shaken at 70 °C for 18 hours and washed three times with DMSO and five times with DCM. LC/MS analysis of cleaved products: MS (ESI) exact mass calculated $C_{25}H_{22}N_3O_5$ $[M+H]^+$ 444.16; found 444.43, t_R = 2.02 min, purity: 91%.

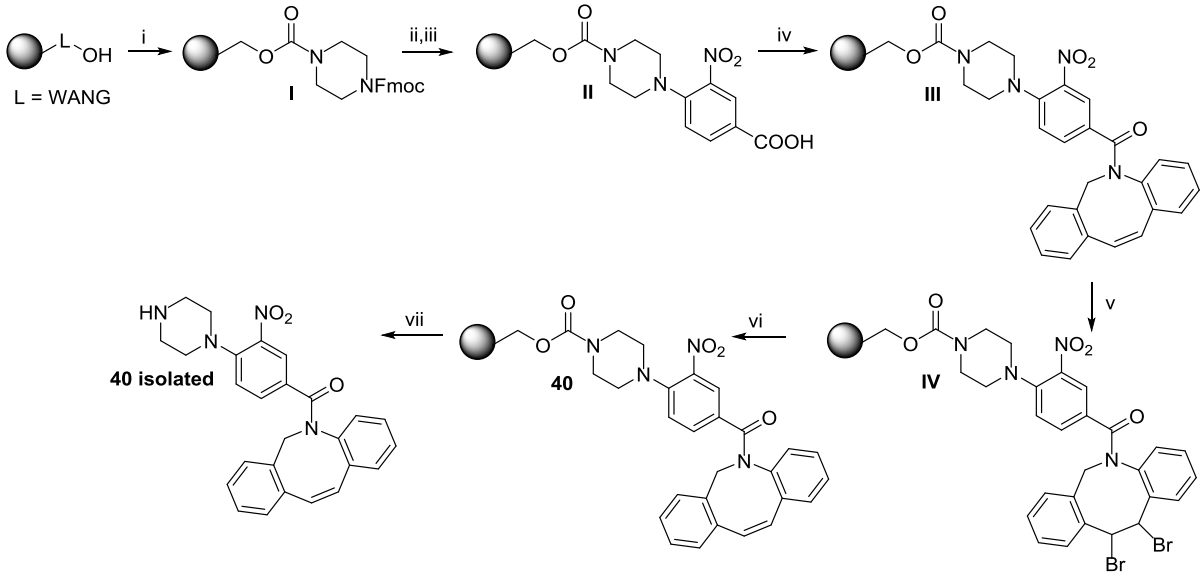
(Z)-3-((4-(5,6-dihydrodibenzo[b,f]azocine-5-carbonyl)-2-nitrophenyl)amino)propanoic acid 48

Resin **38** (250 mg) was treated by TFA in DCM (1:1) at room temperature for 1 hour. The cleavage cocktail was evaporated by a stream of nitrogen. Solution containing crude product was purified using semipreparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to shift from 20% to 50% over the course of 6 min. Yellowish oil, 44 mg (40%).

^{1}H NMR (500 MHz, $CDCl_3$-*d*) δ ppm 2.47 - 2.58 (m, 2 H) 3.36 - 3.46 (m, 2 H) 4.15 - 4.29 (m, 1 H) 5.38 - 5.51 (m, 1 H) 6.07 - 6.33 (m, 1 H) 6.49 (d, *J*=9.16 Hz, 1 H) 6.64 (d, *J*=14.32 Hz, 1 H) 6.79 - 6.84 (m, 1 H) 6.86 (d, *J*=7.73 Hz, 1 H) 7.02 (td, *J*=7.59, 1.15 Hz, 1 H) 7.16 (td, *J*=7.52, 1.00 Hz, 1 H) 7.20 - 7.25 (m, 3 H) 7.28 (d, *J*=2.86 Hz, 1 H) 7.39 (d, *J*=7.16 Hz, 1 H) 7.50 - 7.59 (m, 1 H) 8.07 (d, *J*=2.00 Hz, 1 H) 8.19 - 8.32 (m, 1 H); ^{13}C NMR (126 MHz, $CDCl_3$-*d*) δ ppm 33.64, 38.40, 56.34, 112.54, 122.22, 127.06, 127.18, 127.38, 127.62, 128.19, 128.54, 128.88, 130.87, 131.42, 131.73, 133.74, 134.12, 134.23, 135.07, 136.26, 136.33, 143.60, 145.58, 168.05, 175.70. MS (ESI) exact mass calculated $C_{25}H_{22}N_3O_5$ $[M+H]^+$ 444.16; found 444.43.

(Z)-dibenzo[b,f]azocin-5(6*H*)-yl(3-nitro-4-(piperazin-1-yl)phenyl)methanone (40 isolated)

Derivative **40** was prepared according to the following reaction sequence:



(i) CDI, Fmoc-piperazine, pyridine, DCM, rt (ii) 50% piperidine/DMF, rt (iii) 4-fluoro-3-nitrobenzoic acid, pyridine, 75 °C (iv) $SOCl_2$, DIEA, anhydrous DCM, rt; then azocine **6**, pyridine, angydrous DCM, rt (v) pyridinium tribromide, DIEA, DCM, rt (vi) BTPP, DMSO, 65 °C (vii) 50% TFA/DCM, rt.

Resin I

Wang Resin (1 g) was washed three times with DCM and a solution of CDI (810 mg, 5 mmol) and pyridine (400 μL, 5 mmol) in 10 mL DCM was added. The resin was shaken for two hours and washed three times with DCM. Then, solution of piperazine (431 mg, 5 mmol) in DCM (10 ml) was added. The resin was shaken for 18 h and washed five times with DCM. LC/MS analysis of cleaved product: MS (ESI) exact mass calculated for $C_{19}H_{21}N_2O_2$ $[M+H]^+$ 309.16; found 309.29, t_R = 2.64 min, purity: 94%.

Resin II

Resin **I** (1 g) was washed three times with DCM, three times with DMF and a solution of 50% piperidine in DMF (10 ml) was added. The resin was shaken for 15 min, washed five times with DMF and five times with DCM. Then, solution of 4-fluoro-3-nitrobenzoic acid (1.11 g, 5 mmol) in pyridine (10 ml) was added. The resin was shaken for 18 hours at 80 °C, washed three times with H_2O/DMF 1:1, three times with 20% AcOH in DMF, five times with DMF and five times with DCM. LC/MS analysis of cleaved product: MS (ESI) exact mass calculated for $C_{11}H_{14}N_3O_4$ $[M+H]^+$ 252.09; found 252.46, t_R = 0.52 min, purity: 92%.

Resin III

Resin **II** (1 g) was washed three times with anhydrous DCM and a solution of $SOCl_2$ (150 μL, 2 mmol) and DIEA (330 μL, 4 mmol) in anhydrous DCM (10 ml) was added. The resin was shaken for 18 hours and washed five times with anhydrous DCM. Then, solution of (Z)-5,6-dihydrodibenzo[b,f]azocine **6** (416 mg, 2 mmol), pyridine (160 μL, 2 mmol) was added. The resin was shaken for two hours and washed five times with DCM. LC/MS analysis of cleaved product: MS (ESI) exact mass calculated for $C_{26}H_{25}N_4O_3$ $[M+H]^+$ 441.19; found 441.27, t_R = 2.69 min, purity: 84%.

Resin IV

Resin **III** (750 mg) was washed three times with DCM and a solution of pyridinium tribromide (1.1 g, 3.5 mmol) and DIEA (580 μL, 3.5 mmol) in DCM (7 ml) was added. The resin was shaken for 18 hours and washed five times with DCM. LC/MS analysis of cleaved product: MS (ESI) exact mass calculated for $C_{26}H_{25}Br_2N_4O_3$ $[M+H]^+$ 599.03; found 599.42, t_R = 2.92 min, purity: 87%.

Resin 40

Resin **IV** (250 mg) was washed three times with DMSO and a solution of BTPP (320 mg, 1 mmol) in DMSO (3 ml) was added. The resin was shaken at 70 °C for 18 hours and washed three times with DMSO and five times with DCM. LC/MS analysis of cleaved products: MS (ESI) exact mass calculated for $C_{26}H_{25}N_4O_3$ $[M+H]^+$ 441.19; found 441.27, t_R = 2.71 min, purity: 82%.

(Z)-dibenzo[b,f]azocin-5(6H)-yl(3-nitro-4-(piperazin-1-yl)phenyl)methanone (40 isolated)

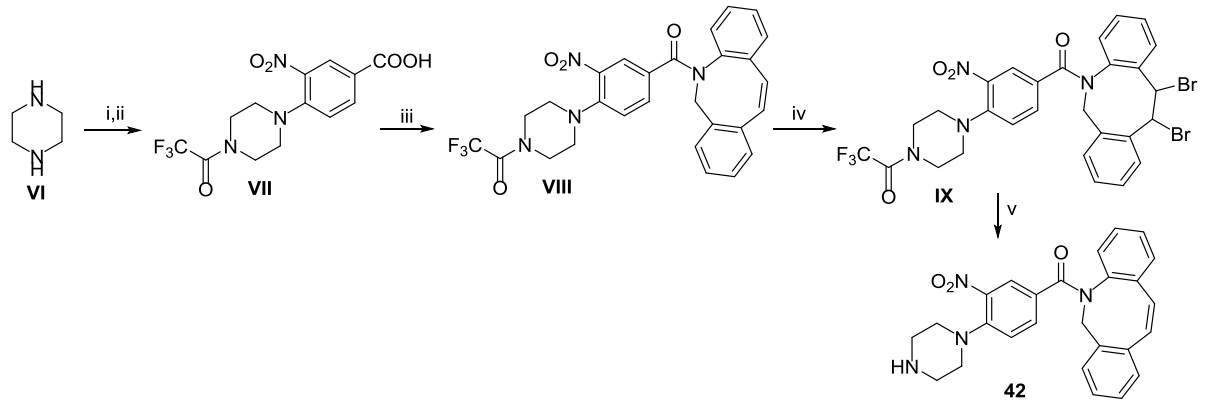
Resin **40** (250 mg) was treated by TFA in DCM (1:1) at room temperature for 1 hour. The cleavage cocktail was evaporated by a stream of nitrogen. Solution containing crude product was purified using semipreparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to shift from 30% to 60% over the course of 6 min. Yellowish oil, 42 mg (39%).

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.68 - 2.76 (m, 4 H) 2.85 - 2.91 (m, 4 H) 4.20 (d, *J*=14.20 Hz, 1 H) 5.36 (d, *J*=14.20 Hz, 1 H) 6.69 (d, *J*=14.50 Hz, 1 H) 6.86 (d, *J*=14.50 Hz, 1 H) 6.99 (d, *J*=8.82 Hz, 1 H) 7.05 (d, *J*=7.79 Hz, 1 H) 7.11 - 7.18 (m, 2 H) 7.21 - 7.27 (m, 3 H) 7.27 - 7.30 (m, 1 H) 7.36 - 7.40 (m, 1 H) 7.49 - 7.53 (m, 1 H) 7.56 (d, *J*=2.08 Hz, 1 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 45.43, 51.68, 56.20, 120.08, 126.69, 127.55, 127.58, 127.62, 127.83, 128.06, 128.99, 129.20, 131.53, 131.79, 133.99, 134.29, 134.39, 134.49,

135.75, 136.50, 139.35, 143.91, 146.94, 167.07. MS (ESI) exact mass calculated for $C_{26}H_{25}N_4O_3$ $[M+H]^+$ 441.19; found 441.35.

(Z)-dibenzo[b,f]azocin-5(6H)-yl(3-nitro-4-(piperazin-1-yl)phenyl)methanone (42)

Derivative **42** was prepared according to the following reaction sequence:



(i) ETFA, piperazine **VI**, anhydrous THF, rt (ii) 4-fluoro-3-nitrobenzoic acid, pyridine, 75 °C (iii) $SOCl_2$, DIEA, anhydrous DCM, rt; then azocine **6**, pyridine, anhydrous DCM, rt (iv) pyridinium tribromide, DIEA, DCM, rt (v) BTPP, DMSO, 65 °C.

Compound VIII

Ethyl trifluoroacetate (3.4 ml, 29 mmol) was added to a suspension of piperazine (2.5 g, 29 mmol) in anhydrous THF under N_2 atmosphere. The solution was stirred at room temperature for 30 minutes. Solvent was removed under reduced pressure. The oily residue was mixed with ether and filtered. Filtrate was evaporated to give the crude *N*-trifluoroacetylpiperazine. *N*-trifluoroacetylpiperazine (1 g, 5.4 mmol) was dissolved in pyridine (20 ml) and 4-fluro-3-nitrobenzoic acid (1 g, 5.4 mmol) was added. Solution was stirred at room temperature for 18 hours. Solvent was removed under reduced pressure. Crude product **VII** (1 g, 2.9 mmol) was dissolved in anhydrous DCM (20 ml) and $SOCl_2$ (310 µL, 4 mmol) was added. Solution was stirred at room temperature for 18 hours. Solvent was removed under reduced pressure, oily residue was dissolved in anhydrous DCM (20 ml) and (Z)-5,6-dihydrodibenzo[b,f]azocine **6** (720 mg, 3.5 mmol) with pyridine (600 µL, 7 mmol) were added. Reaction mixture was stirred at room temperature for 1 hour, washed with 5% HCl (20 ml), brine (20 ml) and dried over $MgSO_4$. Solvent was removed under reduced pressure and crude product was purified on column chromatography with mobile phase consists of 2% MeOH in DCM. Orange solid, 1.02 g (66%).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 3.05 - 3.13 (m, 4 H) 3.58 - 3.66 (m, 4 H) 4.22 (d, *J*=14.03 Hz, 1 H) 5.34 (d, *J*=14.03 Hz, 1 H) 6.70 (d, *J*=14.61 Hz, 1 H) 6.87 (d, *J*=14.61 Hz, 1 H) 7.02 - 7.10 (m, 2 H) 7.14 (t, *J*=7.30 Hz, 1 H) 7.19 - 7.25 (m, 2 H) 7.25 - 7.30 (m, 2 H) 7.37

(d, *J*=7.45 Hz, 1 H) 7.50 (d, *J*=7.45 Hz, 1 H) 7.63 (d, *J*=1.72 Hz, 1 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 42.60, 44.81, 49.30, 49.86, 54.87, 55.59, 116.18 (q, *J*=287.91 Hz), 120.08, 126.83, 126.99, 127.07, 127.35, 127.48, 127.58, 128.45, 128.67, 131.06, 131.16, 133.47, 133.65, 133.96, 135.10, 135.90, 139.35, 143.12, 145.49, 154.33 (q, *J*=34.79 Hz), 166.40. MS (ESI) exact mass calculated for $C_{28}H_{24}F_3N_4O_4$ $[M+H]^+$ 537.17; found 537.19.

Compound IX

Alkene **VIII** (420 mg, 0.78 mmol) was dissolved in DCM (10 ml) and pyridinium tribromide (360 mg, 1.13 mmol) was added. Solution was stirred at room temperature for 18 hours, diluted with DCM (10 ml) washed with 5% HCl (20 ml), brine (20 ml) and dried over $MgSO_4$. Solvent was removed under reduced pressure and crude product was purified on column chromatography with mobile phase consists of 2% MeOH in DCM. Compound **IX** was isolate as a mixture of inseparable regiosiomers; characterization was performed for all isomers together. Brown oil, 417 mg (77%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.08 - 3.13 (m, 4 H) 3.16 (d, *J*=5.97 Hz, 6 H) 3.65 (br. s., 11 H) 4.56 (d, *J*=14.53 Hz, 2 H) 4.96 (d, *J*=13.75 Hz, 1 H) 5.60 (d, *J*=13.49 Hz, 1 H) 5.81 (d, *J*=16.87 Hz, 2 H) 5.88 (d, *J*=9.60 Hz, 1 H) 6.10 (d, *J*=10.12 Hz, 2 H) 6.57 (d, *J*=9.60 Hz, 1 H) 6.81 - 6.88 (m, 1 H) 7.02 - 7.08 (m, 2 H) 7.08 - 7.17 (m, 10 H) 7.17 - 7.21 (m, 2 H) 7.23 - 7.28 (m, 3 H) 7.29 - 7.35 (m, 3 H) 7.58 (dd, *J*=7.92, 1.43 Hz, 1 H) 7.63 - 7.70 (m, 3 H) 7.88 (br. s., 1 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 42.65, 44.83, 49.13, 49.44, 49.68, 50.01, 51.21, 53.87, 54.91, 55.02, 56.42, 59.88, 60.71, 117.65, 119.29, 120.02, 125.94, 126.96, 127.81, 128.25, 128.57, 128.83, 128.95, 129.02, 129.25, 129.45, 129.70, 130.03, 130.42, 131.38, 131.52, 131.96, 132.80, 133.43, 133.59, 135.08, 137.05, 137.82, 137.98, 138.65, 139.58, 140.47, 145.08, 145.53, 154.20, 166.23, 166.33. MS (ESI) exact mass calculated for $C_{28}H_{24}Br_2F_3N_4O_4$ $[M+H]^+$ 695.01; found 695.18.

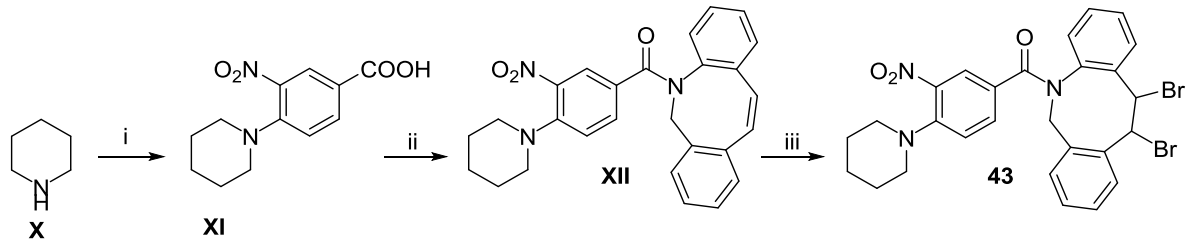
(Z)-dibenzo[b,f]azocin-5(6*H*)-yl(3-nitro-4-(piperazin-1-yl)phenyl)methanone (42)

Dibromoderivative **IX** (250 mg, 0.36 mmol) was dissolved in DMSO (5 ml) and BTPP (265 μL, 0.9 mmol) was added. Reaction mixture was stirred at 65 °C for 1 hour. Solution containing crude product was purified using semipreparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to shift from 30% to 60% over the course of 6 min. Yellow solid, 85 mg (54%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.73 - 2.80 (m, 4 H) 2.89 - 2.95 (m, 4 H) 4.17 (d, *J*=14.01 Hz, 1 H) 5.34 (d, *J*=14.01 Hz, 1 H) 6.69 (d, *J*=14.47 Hz, 1 H) 6.86 (d, *J*=14.47 Hz, 1 H) 7.00 (d, *J*=8.82 Hz, 1 H) 7.05 (d, *J*=7.79 Hz, 1 H) 7.11 - 7.18 (m, 2 H) 7.21 - 7.27 (m, 3 H)

7.27 - 7.31 (m, 1 H) 7.35 - 7.40 (m, 1 H) 7.51 (d, *J*=7.79 Hz, 1 H) 7.57 (d, *J*=1.82 Hz, 1 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 45.19, 51.22, 56.20, 120.21, 126.92, 127.55, 127.63, 127.85, 128.07, 129.00, 129.21, 131.54, 131.77, 131.79, 134.01, 134.29, 134.40, 134.50, 135.74, 136.50, 139.45, 143.88, 146.83, 167.05. MS (ESI) exact mass calculated for $C_{26}H_{25}N_4O_3$ $[M+H]^+$ 441.19; found 441.27.

11,12-dibromodibenzo[b,f]azocin-5(6*H*)-yl(3-nitro-4-(piperidin-1-yl)phenyl)methanone (43)

Derivative **43** was prepared according to the following reaction sequence:



(i) 4-fluoro-3-nitrobenzoic acid, pyridine, 75 °C (ii) $(CO)_2Cl_2$, DMF, DCM, rt; then azocine **6**, pyridine, anhydrous DCM, rt (iii) pyridinium tribromide, DCM, rt.

Compound XI

4-fluro-3-nitrobenzoic acid (1 g, 5.4 mmol) was dissolved in pyridine (20 ml) and piperidine (535 μL, 5.4 mmol) was added. Solution was stirred at 75 °C for 3 hours. Solvent was removed under reduced pressure. Crude product was recrystallized from MeOH. Orange needles, 1.04 g (77%).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.53 - 1.66 (m, 6 H) 3.09 – 3.13 (m, 4 H) 7.28 (d, *J*=8.86 Hz, 1 H) 7.96 (dd, *J*=8.86, 2.19 Hz, 1 H) 8.24 (d, *J*=2.19 Hz, 1 H) 13.01 (br. s, 1 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 23.30, 25.23, 51.35, 120.08, 120.65, 127.86, 134.12, 138.83, 148.55, 165.77. MS (ESI) exact mass calculated for $C_{12}H_{15}N2O_4$ $[M+H]^+$ 251.10; found 251.18.

Compound XII

Compound **XI** (1.04 g, 4.2 mmol) was dissolved in DCM (11 ml) and 2M oxalyl chloride in DCM (11 ml, 22 mmol) and DMF (35 μL, 0.45 mmol) was added. Solution was stirred at room temperature for 3 hours. Solvent was removed under reduced pressure, oily residue was dissolved in DCM (20 ml) and (Z)-5,6-dihydrodibenzo[b,f]azocine **6** (1 g, 4.8 mmol) with pyridine (384 μL, 4.8 mmol) were added. Reaction mixture was stirred at room temperature for 18 hours, washed with 5% HCl (20 ml), brine (20 ml) and dried over $MgSO_4$.

Solvent was removed under reduced pressure and crude product was purified on column chromatography with mobile phase consists of 100% DCM. Orange solid, 700 mg (39%).

^{1}H NMR (500 MHz, $CDCl_3$-*d*) δ ppm 1.60 - 1.66 (m, 6 H) 2.93 - 2.98 (m, 4 H) 4.23 (d, *J*=14.03 Hz, 1 H) 5.46 (d, *J*=14.03 Hz, 1 H) 6.64 (d, *J*=14.61 Hz, 1 H) 6.75 (d, *J*=8.88 Hz, 1 H) 6.83 (d, *J*=14.61 Hz, 2 H) 7.06 (td, *J*=7.59, 1.43 Hz, 1 H) 7.18 - 7.21 (m, 1 H) 7.24 (dd, *J*=2.15, 1.29 Hz, 1 H) 7.25 - 7.27 (m, 1 H) 7.30 - 7.32 (m, 1 H) 7.41 (dd, *J*=7.88, 1.29 Hz, 1 H) 7.56 - 7.60 (m, 1 H) 7.71 (d, *J*=2.01 Hz, 1 H); ^{13}C NMR (126 MHz, $CDCl_3$-*d*) δ ppm 23.91, 25.68, 52.15, 56.46, 119.03, 126.13, 127.15, 127.21, 127.54, 127.77, 128.26, 128.78, 131.63, 131.83, 133.90, 133.92, 134.21, 134.40, 134.42, 135.27, 136.49, 139.75, 143.83, 147.65, 167.77. MS (ESI) exact mass calculated for $C_{27}H_{26}N_3O_3$ $[M+H]^+$ 440.20; found 440.37.

Compound 43

Alkene **XII** (700 mg, 1.59 mmol) was dissolved in DCM (3 ml) and pyridinium tribromide (600 mg, 1.8 mmol) was added. Solution was stirred at room temperature for 18 hours, diluted with DCM (10 ml) washed with 5% HCl (15 ml), brine (15 ml) and dried over $MgSO_4$. Solvent was removed under reduced pressure and crude product was purified on column chromatography with mobile phase consists of 100% DCM. Compound **43** was isolated as a mixture of inseparable regiosiomers; characterization was performed for all isomers together. Brown solid, 440 mg (46%).

^{1}H NMR (500 MHz, $CDCl_3$-*d*) δ ppm 1.58 (br. s., 5 H) 1.66 (br. s., 10 H) 3.03 (d, *J*=12.60 Hz, 9 H) 4.48 (d, *J*=14.89 Hz, 1 H) 5.09 (d, *J*=14.32 Hz, 1 H) 5.19 (d, *J*=9.74 Hz, 1 H) 5.34 (d, *J*=9.45 Hz, 1 H) 5.64 (d, *J*=14.32 Hz, 1 H) 5.80 (d, *J*=14.32 Hz, 1 H) 6.00 - 6.14 (m, 2 H) 6.40 (d, *J*=7.16 Hz, 1 H) 6.76 - 6.89 (m, 5 H) 6.90 - 7.00 (m, 3 H) 7.02 - 7.17 (m, 10 H) 7.19 - 7.31 (m, 3 H) 7.59 - 7.77 (m, 4 H) 7.78 - 7.97 (m, 3 H); ^{13}C NMR (126 MHz, $CDCl_3$-*d*) δ ppm 23.67, 25.45, 52.09, 52.17, 52.37, 53.40, 54.26, 54.89, 55.36, 60.03, 60.61, 118.66, 119.24, 124.62, 126.19, 128.17, 128.31, 128.53, 129.05, 129.14, 129.21, 129.40, 129.46, 129.62, 129.73, 129.95, 130.39, 131.27, 131.51, 131.61, 132.30, 133.22, 133.71, 135.26, 135.36, 137.22, 137.40, 138.05, 138.21, 139.11, 139.74, 140.09, 146.93, 147.03, 167.11, 167.18. MS (ESI) exact mass calculated for $C_{27}H_{26}Br_2N_3O_3$ $[M+H]^+$ 598.03; found 598.13.

Resin 49

Resin **46** (1 g) was washed three times with DCM and solution of sodium dithionite (1.7 g, 10 mmol), K_2CO_3 (1.7 g, 12 mmol) and ethyl viologen diiodide (200 mg, 0.4 mmol) in DCM/H_2O 1:1 (20 ml) was added. The resin was shaken at room temperature for 18 hours

and washed three times with DCM/H_2O 1:1, three times with DMF and five times with DCM. LC/MS analysis of cleaved products: MS (ESI) exact mass calculated for $C_{25}H_{24}N_3O_3$ $[M+H]^+$ 414.18; found 414.50, t_R = 2.09 min, purity: 76%.

Resin 50

Resin **49** (300 mg) was washed three times with DCM and solution of FAEEAA (231 mg, 0.6 mmol), HOBt (84 mg, 0.6 mmol), DIEA (102 μL, 0.6 mmol) and DIC (96 μL, 0.6 mmol) in DCM/DMF 1:1 (3 ml) was added. The resin was shaken at room temperature for 18 hours and washed three times with DMF and five times with DCM. Then 50% piperidine in DMF (3 ml) was added. The resin was shaken at room temperature for 15 minutes and washed five times with DMF and three times with DCM. Then, solution of FAEEAA (231 mg, 0.6 mmol), HOBt (84 mg, 0.6 mmol), DIEA (102 μL, 0.6 mmol) and DIC (96 μL, 0.6 mmol) in DCM/DMF 1:1 (3 ml) was added. The resin was shaken at room temperature for 18 hours and washed three times with DMF and five times with DCM. LC/MS analysis of cleaved products: MS (ESI) exact mass calculated for $C_{52}H_{56}N_5O_{11}$ $[M+H]^+$ 926.40; found 926.83, t_R = 2.59 min, purity: 64%.

Resin 51

Resin **50** (300 mg) was washed three times with DCM and three times with DMF. Then, 50% piperidine in DMF (3 ml) was added. The resin was shaken at room temperature for 15 minutes and washed five times with DMF and three times with DCM. Then, solution of RhoB (442 mg, 0.9 mmol), HOBt (122 mg, 0.9 mmol), DMAP (20 mg, 0.2 mmol) and DIC (144 μL, 0.9 mmol) in DCM/DMF 1:1 (3 ml) was added. The resin was shaken at room temperature for 18 hours and washed three times with DMF and five times with DCM. LC/MS analysis of cleaved products: MS (ESI) exact mass calculated for $C_{65}H_{74}N_7O_{11}$ $[M+H]^+$ 1128.54; found 1128.98, t_R = 3.73 min, purity: 17%.

Cyclized form: MS (ESI) exact mass calculated for $C_{65}H_{72}N_7O_{10}$ $[M+H]^+$ 1110.54; found 1110.99, t_R = 3.60 min, purity: 67%.

(Z)-3-(2-(16-(3',6'-bis(diethylamino)-3-oxospiro[isoindoline-1,9'-xanthen]-2-yl)-9-oxo-2,5,11,14-tetraoxa-8-azahexadecyl)-5-(5,6-dihydrodibenzo[b,f]azocine-5-carbonyl)-1*H*-benzo[d]imidazol-1-yl)propanoic acid (62)

Resin **51** (300 mg) was treated by TFA in DCM (1:1) at room temperature for 1 hour. The cleavage cocktail was evaporated by a stream of nitrogen. Solution containing crude product was purified using semipreparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to shift from 70% to 90% over the course of 6 min. Purple solid, 47 mg (14%).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.79 – 7.75 (m, 1H), 7.52 – 7.44 (m, 5H), 7.40 (t, *J*=7.5 Hz, 2H), 7.31 (s, 1H), 7.27 (td, *J*=7.4, 1.5 Hz, 1H), 7.23 (t, *J*=7.1 Hz, 1H), 7.12 (dd, *J*=11.5, 4.7 Hz, 2H), 7.03 – 7.00 (m, 1H), 6.98 (d, *J*=7.2 Hz, 1H), 6.90 (d, *J*=7.2 Hz, 1H), 6.87 (d, *J*=14.7 Hz, 1H), 6.71 (d, *J*=14.7 Hz, 1H), 6.37 (d, *J*=2.3 Hz, 2H), 6.34 (dd, *J*=8.9, 2.4 Hz, 2H), 6.31 (d, *J*=8.8 Hz, 2H), 5.38 (d, *J*=13.9 Hz, 1H), 4.70 (d, *J*=4.7 Hz, 2H), 4.37 (t, *J*=7.2 Hz, 2H), 4.17 (d, *J*=13.2 Hz, 1H), 3.75 – 3.72 (m, 2H), 3.54 – 3.49 (m, 2H), 3.45 (dt, *J*=6.4, 3.4 Hz, 2H), 3.34 (dd, *J*=7.8, 3.8 Hz, 4H), 3.29 (q, *J*=7.0 Hz, 9H), 3.19 (dd, *J*=10.5, 5.1 Hz, 4H), 3.14 (t, *J*=6.9 Hz, 2H), 3.01 (t, *J*=6.9 Hz, 2H), 2.67 (t, *J*=7.2 Hz, 2H), 2.54 (s, 1H), 1.06 (t, *J*=7.0 Hz, 12H); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 172.26, 169.33, 169.08, 166.97, 153.37, 152.59, 152.09, 148.37, 144.36, 140.74, 136.13, 136.08, 135.57, 133.99, 133.77, 132.68, 131.29, 130.76, 130.26, 129.59, 128.69, 128.31, 128.25, 128.14, 127.43, 127.25, 126.96, 126.65, 123.59, 123.13, 122.27, 119.79, 109.97, 108.13, 104.91, 97.25, 69.91, 69.83, 69.33, 69.21, 69.00, 68.82, 67.02, 64.87, 63.96, 60.73, 55.75, 43.63, 40.43, 38.88, 37.86, 33.93, 12.33. MS (ESI) exact mass calculated for $C_{65}H_{72}N_7O_{10}$ $[M+H]^+$ 1110.54; found 1110.68.

Resin 52

Resin **49** (300 mg) was washed three times with DCM and solution of FAEEAA (231 mg, 0.6 mmol), HOBt (84 mg, 0.6 mmol), DIEA (102 μL, 0.6 mmol) and DIC (96 μL, 0.6 mmol) in DCM/DMF 1:1 (3 ml) was added. The resin was shaken at room temperature for 18 hours and washed three times with DMF and five times with DCM. LC/MS analysis of cleaved products: MS (ESI) exact mass calculated for $C_{46}H_{45}N_4O_8$ $[M+H]^+$ 781.32; found 781.63, t_R = 2.76 min, purity: 65%.

Resin 53

Resin **42** (300 mg) was washed three times with DCM and three times with DMF. Then, 50% piperidine in DMF (3 ml) was added. The resin was shaken at room temperature for 15 minutes and washed five times with DMF and three times with DCM. Then, solution of RhoB (442 mg, 0.9 mmol), HOBt (122 mg, 0.9 mmol), DMAP (20 mg, 0.2 mmol) and DIC (144 μL, 0.9 mmol) in DCM/DMF 1:1 (3 ml) was added. The resin was shaken at room temperature for 18 hours and washed three times with DMF and five times with DCM. LC/MS analysis of cleaved products: MS (ESI) exact mass calculated for $C_{59}H_{63}N_6O_8$ $[M+H]^+$ 983.47; found 983.83, t_R = 4.02 min, purity: 8%.

Cyclized form: MS (ESI) exact mass calculated for $C_{59}H_{61}N_6O_7$ $[M+H]^+$ 965.46; found 965.82, t_R = 3.78 min, purity: 75%.

(Z)-3-(2-((2-(2-(3',6'-bis(diethylamino)-3-oxospiro[isoindoline-1,9'-xanthen]-2-yl)ethoxy)ethoxy)methyl)-5-(5,6-dihydrodibenzo[b,f]azocine-5-carbonyl)-1*H*-benzo[d]imidazol-1-yl)propanoic acid (63)

Resin **53** (300 mg) was treated by TFA in DCM (1:1) at room temperature for 1 hour. The cleavage cocktail was evaporated by a stream of nitrogen. Solution containing crude product was purified using semipreparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to shift from 70% to 90% over the course of 6 min. Purple solid, 44 mg (15%).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.79 – 7.74 (m, 1H), 7.52 – 7.48 (m, 3H), 7.48 – 7.44 (m, 2H), 7.40 (d, *J*=7.5 Hz, 1H), 7.35 – 7.29 (m, 2H), 7.28 – 7.21 (m, 2H), 7.14 – 7.08 (m, 2H), 7.03 – 6.95 (m, 2H), 6.88 (d, *J*=14.7 Hz, 2H), 6.71 (d, *J*=14.7 Hz, 1H), 6.38 – 6.24 (m, 6H), 5.38 (d, *J*=13.5 Hz, 1H), 4.66 – 4.57 (m, 2H), 4.30 (d, *J*=5.9 Hz, 2H), 4.20 – 4.12 (m, 1H), 3.41 – 3.37 (m, 1H), 3.34 (dd, *J*=5.5, 3.5 Hz, 2H), 3.27 (q, *J*=6.9 Hz, 8H), 3.14 (dd, *J*=10.3, 5.5 Hz, 2H), 3.08 (t, *J*=6.8 Hz, 2H), 2.94 (t, *J*=6.9 Hz, 2H), 2.60 (t, *J*=7.1 Hz, 2H), 1.07 – 1.01 (m, 12H); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 172.27, 169.34, 166.88, 153.33, 152.57, 152.06, 148.34, 144.40, 140.75, 136.14, 136.06, 135.58, 133.79, 132.66, 131.28, 130.75, 130.33, 129.48, 128.68, 128.33, 128.24, 128.19, 128.14, 127.44, 127.26, 126.97, 126.62, 123.60, 123.09, 122.25, 119.80, 109.88, 108.10, 104.93, 97.17, 69.02, 68.95, 66.96, 64.84, 63.92, 60.72, 55.76, 43.63, 38.85, 34.01, 29.19, 12.32. MS (ESI) exact mass calculated for $C_{59}H_{61}N_6O_7$ $[M+H]^+$ 965.46; found 965.48.

Resin 54

Resin **49** (300 mg) was washed three times with DCM and solution of RhoB (442 mg, 0.9 mmol), HOBt (122 mg, 0.9 mmol), DMAP (20 mg, 0.2 mmol) and DIC (144 μL, 0.9 mmol) in DCM/DMF 1:1 (3 ml) was added. The resin was shaken at room temperature for 18 hours and washed three times with DMF and five times with DCM. LC/MS analysis of cleaved products: MS (ESI) exact mass calculated for $C_{53}H_{52}N_5O_5$ $[M+H]^+$ 838.40; found 838.83, t_R = 4.25 min, purity: 87%.

(Z)-3-((2-(3',6'-bis(diethylamino)-3-oxospiro[isoindoline-1,9'-xanthen]-2-yl)-4-(5,6-dihydrodibenzo[b,f]azocine-5-carbonyl)phenyl)amino)propanoic acid (64)

Resin **54** (300 mg) was treated by TFA in DCM (1:1) at room temperature for 1 hour. The cleavage cocktail was evaporated by a stream of nitrogen. Solution containing crude product was purified using semipreparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to shift from 70% to 90% over the course of 6 min. Purple solid, 69 mg (28%).

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.11 (t, *J*=6.98 Hz, 12 H) 2.05 (t, *J*=7.21 Hz, 2 H) 2.79 - 2.87 (m, 2 H) 3.34 (q, *J*=6.87 Hz, 8 H) 4.25 - 4.33 (m, 1 H) 5.98 - 6.03 (m, 1 H) 6.22 - 6.29 (m, 3 H) 6.32 (d, *J*=8.47 Hz, 1 H) 6.43 (d, *J*=8.01 Hz, 2 H) 6.51 - 6.59 (m, 3 H) 6.68 - 6.77 (m, 2 H) 7.13 - 7.20 (m, 3 H) 7.20 - 7.25 (m, 2 H) 7.27 - 7.33 (m, 2 H) 7.36 (d, *J*=7.33 Hz, 1 H) 7.60 - 7.70 (m, 2 H) 7.86 - 7.91 (m, 1 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 12.05, 33.10, 38.02, 43.40, 54.88, 67.01, 97.35, 106.08, 108.16, 120.43, 122.05, 122.50, 123.90, 124.24, 125.86, 126.45, 126.87, 127.16, 127.56, 128.15, 128.32, 128.80, 130.17, 130.44, 130.82, 131.60, 132.54, 132.84, 133.03, 133.15, 133.43, 135.17, 135.97, 144.13, 146.57, 148.50, 151.17, 153.21, 165.20, 168.04, 172.37. MS (ESI) exact mass calculated for $C_{53}H_{52}N_5O_5$ $[M+H]^+$ 838.40; found 838.58.

6.2.3. Reactions of cycloocta[d][1,2,3]selenadiazole

6.2.3.1. Synthesis of triazoles 66, 67, 74-79 via thermal heating of cycloocta-1,2,3-selenadiazole 65

General procedure:

Corresponding azide **5**, **6** and **68-73** (50 mg) was dissolved in DMSO (4 mL) and cycloocta[d][1,2,3]selenadiazole **65** (1.5 equivalent) was added. The reaction mixture was stirred at 110 °C overnight. DMSO was removed using a freeze dryer. Resultant solid was suspended in methanol, insoluble selenium was filtered off and crude product as methanolic solution was purified using semipreparative HPLC. Solvents were removed using a freeze dryer and all products were obtained as white or yellowish amorphous solid.

1-((2R,3R,4S,5R)-5-((4,5,6,7,8,9-Hexahydro-1*H*-cycloocta[d][1,2,3]triazol-1-yl)methyl)-3,4-dihydroxytetrahydrofuran-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (66)

Crude product was purified using semipreparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to shift from 20% to 50% over the course of 6 min. White solid, 40 mg (57%).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.29–1.39 (m, 4 H), 1.61 (quint, *J*=5.70 Hz, 2 H), 1.64–1.70 (m, 2 H), 1.76 (d, *J*=1.15 Hz, 3 H), 2.72–2.79 (m, 4 H), 4.03–4.11 (m, 3 H), 4.49 (dd, *J*=14.70, 6.30 Hz, 1 H), 4.60 (dd, *J*=14.70, 4.20 Hz, 1 H), 5.74 (d, *J*=4.87 Hz, 1 H), 7.20 (d, *J*=1.15 Hz, 1 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 11.95, 20.75, 23.87, 24.31, 25.47, 25.85, 28.09, 48.33, 70.46, 71.81, 81.79, 88.42, 109.76, 134.20, 136.38, 143.27, 150.69, 163.61. HRMS m/z calculated for $C_{18}H_{26}N_5O_5$ $[M+H]^+$ 392.1928, found 392.1926.

1-((3aR,4R,6R,6aR)-6-((4,5,6,7,8,9-Hexahydro-1*H*-cycloocta[d][1,2,3]triazol-1-yl)methyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (67)

Crude product was purified using semipreparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to shift from 30% to 60% over the course of 6 min. White solid, 31 mg (48%).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.28–1.30 (m, 3 H), 1.31–1.36 (m, 4 H), 1.47 (s, 3 H), 1.56–1.64 (m, 4 H), 1.75 (d, *J*=1.15 Hz, 3 H), 2.66 (q, *J*=5.60 Hz, 2 H), 2.75 (td, *J*=6.50, 2.60 Hz, 2 H), 4.28–4.32 (m, 1 H), 4.50 (dd, *J*=14.30, 7.70 Hz, 1 H), 4.62 (dd, *J*=14.30, 4.30 Hz, 1 H), 4.93 (dd, *J*=6.59, 4.30 Hz, 1 H), 5.10 (dd, *J*=6.44, 1.86 Hz, 1 H), 5.74 (d, *J*=1.72 Hz, 1 H), 7.46 (d, *J*=1.15 Hz, 1 H), 11.49 (s, 1 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 11.85, 20.70, 23.77, 24.22, 25.12, 25.45, 25.85, 26.86, 28.09, 48.49, 81.20, 83.49, 85.55, 92.66, 109.61, 113.44, 133.96, 138.93, 143.21, 150.40, 163.81. HRMS m/z calculated for $C_{21}H_{30}N_5O_5$ $[M+H]^+$ 432.2241, found 432.2240.

1-Phenyl-4,5,6,7,8,9-hexahydro-1*H*-cycloocta[d][1,2,3]triazole (74)

Crude product was purified using semipreparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to shift from 40% to 70% over the course of 6 min. White solid, 24 mg (26%).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.42–1.51 (m, 4 H), 1.64–1.77 (m, 4 H), 2.68–2.76 (m, 2 H), 2.84–2.90 (m, 2 H), 7.49–7.53 (m, 2 H), 7.55–7.63 (m, 3 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 21.39, 23.74, 24.52, 25.27, 26.48, 27.95, 125.21, 129.36, 129.57, 133.96, 136.27, 143.92. HRMS m/z calculated for $C_{14}H_{18}N_3$ $[M+H]^+$ 228.1495, found 228.1496.

1-(4-Methoxyphenyl)-4,5,6,7,8,9-hexahydro-1*H*-cycloocta[d][1,2,3]triazole (75)

Crude product was purified using semipreparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to shift from 40% to 70% over the course of 6 min. White solid, 49 mg (48%).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.42–1.49 (m, 4 H), 1.65–1.73 (m, 4 H), 2.65–2.69 (m, 2 H), 2.83–2.88 (m, 2 H), 3.84 (s, 3 H), 7.10–7.15 (m, 2 H), 7.40–7.43 (m, 2 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 21.35, 23.77, 24.52, 25.27, 26.46, 27.96, 55.52, 114.58, 126.74, 129.12, 134.07, 143.58, 159.73. HRMS m/z calculated for $C_{15}H_{20}N_3O$ $[M+H]^+$ 258.1601, found 258.1602.

1-(4-Nitrophenyl)-4,5,6,7,8,9-hexahydro-1*H*-cycloocta[d][1,2,3]triazole (76)

Crude product was purified using semipreparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to shift from 40% to 70% over the course of 6 min. Yellow solid, 43 mg (52%).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.44–1.52 (m, 4 H), 1.68–1.79 (m, 4 H), 2.80–2.84 (m, 2 H), 2.87–2.92 (m, 2 H), 7.85–7.89 (m, 2 H), 8.41–8.46 (m, 2 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 21.49, 23.71, 24.37, 25.33, 26.32, 27.91, 125.06, 125.91, 134.36, 141.08, 144.76, 147.34. HRMS m/z calculated for $C_{14}H_{17}N_4O_2$ $[M+H]^+$ 273.1346, found 273.1347.

3-(4,5,6,7,8,9-Hexahydro-1*H*-cycloocta[d][1,2,3]triazol-1-yl)-2*H*-chromen-2-one (77)

Crude product was purified using semipreparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to shift from 40% to 70% over the course of 6 min. Yellow solid, 13 mg (17%).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.42–1.52 (m, 4 H), 1.72 (dq, *J*=12.06, 6.10 Hz, 4 H), 2.75 (t, *J*=6.30 Hz, 2 H), 2.88 (t, *J*=6.30 Hz, 2 H), 7.46–7.51 (m, 1 H), 7.56 (d, *J*=8.30 Hz, 1 H), 7.75–7.80 (m, 1 H), 7.87 (dd, *J*=7.70, 1.40 Hz, 1 H), 8.56 (s, 1 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 20.97, 23.55, 24.39, 25.35, 25.77, 27.64, 116.48, 118.08, 122.57, 125.26, 129.67, 133.60, 135.85, 142.37, 143.44, 153.35, 156.89. HRMS m/z calculated for $C_{17}H_{18}N_3O_2$ $[M+H]^+$ 296.1394, found 296.1393.

1-Benzyl-4,5,6,7,8,9-hexahydro-1*H*-cycloocta[d][1,2,3]triazole (78)

Crude product was purified using semipreparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to shift from 40% to 70% over the course of 6 min. White solid, 47 mg (49%).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.27–1.34 (m, 4 H), 1.41–1.47 (m, 2 H), 1.59–1.64 (m, 2 H), 2.67–2.70 (m, 2 H), 2.76–2.79 (m, 2 H), 5.52 (s, 2 H), 7.13–7.16 (m, 2 H), 7.27–7.31 (m, 1 H), 7.33–7.37 (m, 2 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 20.67, 23.84, 24.30, 25.38, 25.52, 28.11, 50.44, 126.98, 127.74, 128.66, 133.14, 136.34, 143.95. HRMS m/z calculated for $C_{15}H_{20}N_3$ $[M+H]^+$ 242.1652, found 242.1653.

(2R,3R,4R,5R)-2-((Benzoyloxy)methyl)-5-(5-((4,5,6,7,8,9-hexahydro-1*H*-cycloocta[d][1,2,3]triazol-1-yl)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-3,4-diyl dibenzoate (79)

Crude product was purified using semipreparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to shift from 50% to 80% over the course of 6 min. White solid, 25 mg (43%).

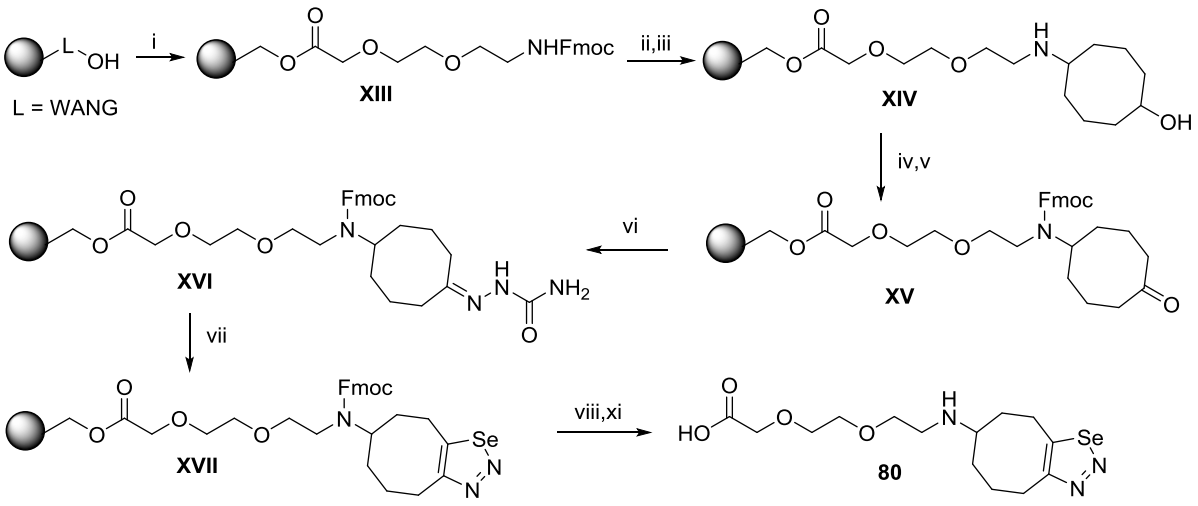
^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.29–1.42 (m, 4 H), 1.55–1.62 (m, 2 H), 1.67–1.75 (m, 2 H), 2.75 (t, *J*=6.40 Hz, 2 H), 2.82 (t, *J*=6.20 Hz, 2 H), 4.63 (dd, *J*=12.10, 5.70 Hz, 1H), 4.70 (dd, *J*=12.10, 3.70 Hz, 1 H), 4.75 (td, *J*=5.73, 4.12 Hz, 1 H), 4.94 (d, *J*=15.00 Hz, 1 H), 5.01 (d, *J*=15.00 Hz, 1 H), 5.93 (t, *J*=6.40 Hz, 1 H), 5.95–5.99 (m, 1 H), 6.20 (d, *J*=3.97 Hz, 1 H), 7.41–7.47 (m, 4 H), 7.49 (t, *J*=7.88 Hz, 2 H), 7.62–7.67 (m, 3 H), 7.86–7.91 (m, 4 H), 7.99–8.02 (m, 3 H), 11.74 (br s, 1 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 20.81, 23.91, 24.12, 25.44, 25.69, 28.16, 43.24, 63.63, 70.41, 73.20, 78.72, 89.91, 109.14, 128.43, 128.51, 128.68, 128.71, 128.72, 129.20, 129.30, 129.31, 129.33, 133.40, 133.47, 133.82, 133.93, 141.97, 143.22, 149.96, 162.28, 164.59, 164.61, 165.46.

6.2.3.2. Compounds from photochemically induced reactions of cycloocta[d][1,2,3]selenadiazole

Solid-phase synthesis of derivatives 80 and 81

2-(2-(2-((4,5,6,7,8,9-Hexahydrocycloocta[d][1,2,3]selenadiazol-7-yl)amino)ethoxy)ethoxy)acetic acid (80)

Derivative **80** was prepared according to the following reaction sequence:



(i) FAEEAA, HOBt, DIC, DIEA, DCM/DMF 1:1, rt (ii) 50% piperidine/DMF, rt (iii) 5-hydroxycyclooctanone, 20% AcOH/DMF, rt; $NaBH(OAc)_3$, rt (iv) Fmoc-OSu, DCM, rt (v) Dess-Martin periodinane, DCM, rt (vi) Semicarbazide hydrochloride, 20% AcOH/DMF, rt (vii) Selenium dioxide, 20% AcOH/DMF, rt (viii) 50%TFA/DCM, rt (xi) 20% piperidine/MeOH, rt.

Resin XIII

Wang Resin (1 g) was washed with DCM and a solution of FAEEAA (1.16 g, 3.0 mmol), HOBt (405 mg, 3.0 mmol), DIEA (68 μL, 0.4 mmol) and DIC (464 μL, 3.0 mmol) in 10 mL DCM/DMF (1:1, v/v) was added. The resin was shaken for 18 hours, washed five

times with DMF and five times with DCM. LC/MS analysis of cleaved product: MS (ESI) exact mass calculated for $C_{21}H_{24}NO_6$ $[M+H]^+$ 386.14; found 386.36, t_R = 1.85 min, purity: 93%.

Resin XIV

Resin **XIII** (1 g) was washed three times with DMF. A solution of 10 mL of 50% piperidine in DMF was added to the resin and the slurry was shaken for 15 minutes. The resin was washed three times with DMF, three times with DCM and three times with anhydrous DMF. A solution of 5-hydroxycylooctanone[161] (710 mg, 5 mmol) in 10 mL 20% AcOH/anhydrous DMF was added and the slurry was shaken overnight. The next day, $NaBH(OAc)_3$ (1.1 g, 5.0 mmol) was added in three portions and the syringe was punctured with a needle just below the plunger to enable hydrogen gas evolve. The slurry was shaken for two hours after each portion. The resin was washed three times with 5% AcOH/DMF (v/v), three times with DMF, neutralized with 20% (v/v) piperidine/DMF for five minutes, washed three times with DMF and three times with DCM. For identification, Fmoc-OSu (170 mg, 0.5 mmol) in DCM (1 mL) was added to resin **XIV** (20 mg). The slurry was shaken for 30 minutes. The resin was washed three times with DCM. LC/MS analysis of cleaved product: MS (ESI) exact mass calculated for $C_{29}H_{38}NO_7$ $[M+H]^+$ 512.26; found 512.42, tR = 1.92 min, purity: 42%.

Resin XV

Resin **XIV** (1 g) was washed three times with DCM. A solution of Fmoc-OSu (1.7 g, 5.0 mmol) in DCM (10 mL) was added to the resin and the slurry was shaken for 30 minutes. The resin was washed three times with DCM. A solution of 0.2 M Dess-Martin periodinane in DCM (10 mL) was added to the resin and the slurry was shaken for 30 minutes. The resin was washed five times with DCM. LC/MS analysis of cleaved product: MS (ESI) exact mass calculated for $C_{29}H_{36}NO_7$ $[M+H]^+$ 510.24; found 510.40, t_R = 2.04 min, purity: 40%.

Resin XVI

Resin **XV** (1 g) was washed three times with DMF. A solution of semicarbazide hydrochloride (558 mg, 5.0 mmol) in 20% (v/v) AcOH/DMF (10 mL) was added to the resin and the slurry was shaken for 18 hours. The resin was washed three times with 5% (v/v) AcOH/DMF, three times with DMF and three times with DCM. LC/MS analysis of cleaved product: MS (ESI) exact mass calculated for $C_{30}H_{39}N_4O_7$ $[M+H]^+$ 567.27; found 567.48, t_R = 1.95 min, purity: 40%.

Resin XVII

Resin **XVI** (1 g) was washed three times with DMF. A solution of selenium dioxide (550 mg, 5 mmol) in 20% (v/v) AcOH/DMF (10 mL) was added to the resin and the slurry was shaken for 18 hours. The resin was washed three times with 5% (v/v) AcOH/DMF, three times with DMF and three times with DCM.

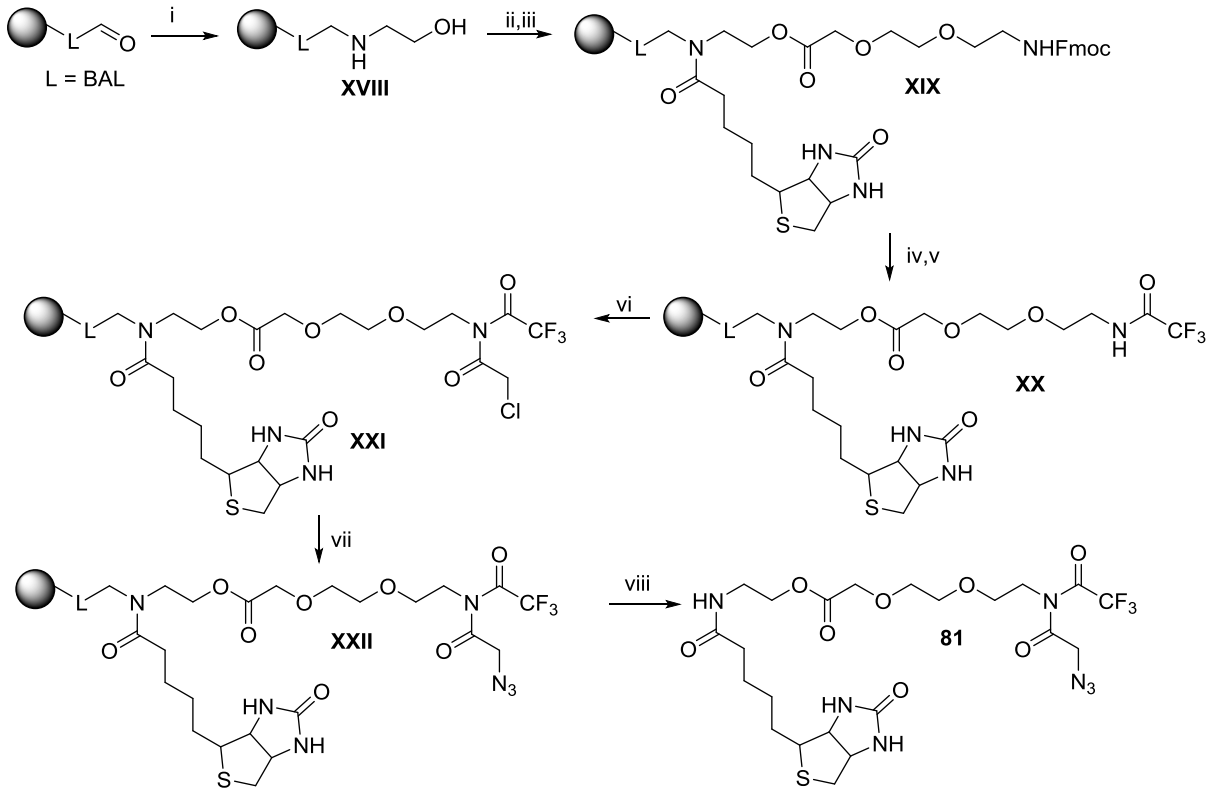
2-(2-(2-((4,5,6,7,8,9-Hexahydrocycloocta[d][1,2,3]selenadiazol-6-yl)amino)ethoxy)ethoxy)acetic acid (80)

Resin **XVII** (1 g) was treated by TFA in DCM (1:1) at room temperature for 1 hour. The cleavage cocktail was evaporated by a stream of nitrogen. 20% piperidine in methanol (5 mL) was added, solution was stirred for 30 minutes. Solution containing crude product was purified using semipreparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to shift from 10% to 40% over the course of 6 min. Colourless oil, 31 mg (25%).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.33–1.42 (m, 1 H), 1.59–1.71 (m, 3 H), 1.89–1.97 (m, 1 H), 1.99–2.07 (m, 1 H), 2.31–2.37 (m, 1 H), 2.62–2.69 (m, 1 H), 2.70–2.75 (m, 1 H), 3.04–3.11 (m, 1 H), 3.16–3.25 (m, 1 H), 3.28–3.38 (m, 2 H), 3.43–3.52 (m, 6 H), 3.62 (s, 2 H), 5.27 (br s, 1 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 23.18, 26.02, 27.63, 31.84, 35.35, 45.17, 56.88, 68.72, 69.13, 69.43, 70.38, 159.53, 160.78, 173.19. HRMS m/z calculated for $C_{14}H_{24}N_3O_4Se$ $[M+H]^+$ 378.0927, found 378.0926.

2-(5-(2-Oxohexahydro-1*H*-thieno[3,4-d]imidazol-4-yl)pentanamido)ethyl 2-(2-(2-(N-(2-azidoacetyl)-2,2,2-trifluoroacetamido)ethoxy)ethoxy)acetate (81)

Derivative **81** was prepared according to the following reaction sequence:



(i) aminoethanole, 20% AcOH/DMF, rt; $NaBH(OAc)_3$, rt (ii) Biotin, HOBt, DIC, DMF, rt (iii) FAEEAA, HOBt, DIC, DIEA, DCM/DMF 1:1, rt (iv) 50% piperidine/DMF, rt (v) Ethyl trifluoracetate, DBU, DMF, rt (vi) Chloroacetylchloride, pyridine, DCM, rt (vii) Sodium azide, DMF, 80 °C (viii) 50%TFA/DCM, rt.

Resin XVIII

Aminomethyl resin with BAL linker[162] (1 g) was washed three times with DCM and three times with dry DMF. A solution of 2-aminoethanol (302 µL, 5.0 mmol) in 10 mL 20% (v/v) AcOH/anhydrous DMF was added and the slurry was shaken for 18 hours. The next day, $NaBH(OAc)_3$ (1.1 g, 5.0 mmol) was added in three portions and the syringe was punctured with a needle just below the plunger to enable hydrogen gas evolve. The slurry was shaken for two hours after each portion. The resin was washed three times with DMF, neutralized with 20% (v/v) piperidine/DMF for ten minutes, washed three times with DMF and three times with DCM. For identification, Fmoc-OSu (170 mg, 0.5 mmol) in DCM (1 mL) was added to resin **VI** (20 mg). The slurry was shaken for 30 minutes. The resin was washed three times with DCM. LC/MS analysis of cleaved product: MS (ESI) exact mass calculated for $C_{17}H_{18}NO_3$ $[M+H]^+$ 284.12; found 284.47, t_R = 2.55 min, purity: 88%.

Resin XIX

Resin **XVIII** (1 g) was washed three times with DMF. Biotin (489 mg, 2.0 mmol) was dissolved in DMF (10 mL) at higher temperature (~80°C). The solution was cooled to room temperature, HOBt (270 mg, 2.0 mmol) and DIC (310 μL, 2.0 mmol) was added, the resulting solution was added to the resin and the slurry was shaken for 18 hours. The resin was washed five times with DMF and five times with DCM. Solution of FAEEAA (1.16 g, 3.0 mmol), HOBt (405 mg, 3.0 mmol), DIEA (68 μL, 0.4 mmol) and DIC (464 μL, 3.0 mmol) in 10 mL DCM/DMF (1:1, v/v) was added. The resin was shaken for 18 hours, washed five times with DMF and five times with DCM. LC/MS analysis of cleaved product: MS (ESI) exact mass calculated for $C_{33}H_{43}N_4O_8S$ $[M+H]^+$ 655.27; found 655.34, t_R = 2.35 min, purity: 86%.

Resin XX

Resin **XIX** (1 g) was washed three times with DMF. A solution of 10 mL of 50% piperidine in DMF was added to the resin and the slurry was shaken for 15 minutes. The resin was washed five times with DMF. A solution of ETFA (120 μL, 1.0 mmol), DBU (180 μL, 1.2 mmol), in DMF (10 mL) was added. The resin was shaken for 18 hours, washed five times with DMF and five times with DCM. LC/MS analysis of cleaved product: MS (ESI) exact mass calculated for $C_{20}H_{32}F_3N_4O_7S$ $[M+H]^+$ 529.18; found 529.32, t_R = 3.75 min, purity: 86%.

Resin XXI

Resin **XX** (1 g) was washed three times with anhydrous DCM. A solution of chloracetlychloride (170 μL, 2 mmol), pyridine (320 μL, 4 mmol), in anhydrous DCM (10 mL) was added. The resin was shaken for 2 hours and washed five times with DCM. MS (ESI) exact mass calculated for $C_{22}H_{33}ClF_3N_4O_8S$ $[M+H]^+$ 604.15; found 604.84, t_R = 4.52 min, purity: 79%.

Resin XXII

Resin **XIX** (1 g) was washed three times with DMF. A solution of sodium azide (300 mg, 5 mmol), in DMF (10 mL) was added. The resin was shaken at 80 °C for 2 hours and washed three times with DMF and three times with DCM. MS (ESI) exact mass calculated for $C_{22}H_{33}F_3N_7O_8S$ $[M+H]^+$ 612.20; found 611.98, t_R = 4.60 min, purity: 73%.

2-(5-(2-oxohexahydro-1*H*-thieno[3,4-d]imidazol-4-yl)pentanamido)ethyl 2-(2-(2-(N-(2-azidoacetyl)-2,2,2-trifluoroacetamido)ethoxy)ethoxy)acetate (81)

Resin **X** (1 g) was treated by TFA in DCM (1:1, v/v) at room temperature for 1 hour. The cleavage cocktail was evaporated by a stream of nitrogen. Oily product was dissolved in methanol (5 mL) and purified by semipreparative HPLC with light scattering detector. The

mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to shift from 35% to 50% over the course of 6 min. White solid, 57 mg (25%).

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.25–1.39 (m, 2 H), 1.43–1.56 (m, 3 H), 1.59–1.69 (m, 1 H), 2.07 (t, *J*=7.33 Hz, 2 H), 2.89 (d, *J*=13.30 Hz, 1 H), 3.00 (dd, *J*=13.20, 5.60 Hz, 1 H), 3.21 (ddd, *J*=8.64, 6.01, 4.35 Hz, 1 H), 3.25–3.32 (m, 3 H), 3.34 (t, *J*=5.72 Hz, 2 H), 3.51 (t, *J*=5.80 Hz, 2 H), 3.54–3.56 (m, 2 H), 3.58–3.60 (m, 1 H), 4.07 (t, *J*=5.72 Hz, 2 H), 4.11 (s, 2 H), 4.20 (dd, *J*=7.67, 4.24 Hz, 1 H), 4.44 (d, *J*=17.90 Hz, 1 H), 4.53 (d, *J*=17.90 Hz, 1 H), 4.78–4.83 (m, 1 H), 7.92 (t, *J*=5.61 Hz, 1 H), 8.02–8.08 (m, 1 H), 9.45 (br s, 1 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 25.04, 27.76, 28.13, 35.02, 37.19, 37.44, 51.81, 54.60, 58.17, 61.06, 62.78, 67.55, 67.78, 69.45, 69.91, 117.33, 155.87, 156.57, 167.71, 168.65, 170.10, 172.30. HRMS m/z calculated for $C_{22}H_{33}F_3N_7O_8S$ $[M+H]^+$ 612.2058, found 612.2068.

6.2.4. Preparation of derivatives of cycloocta[d][1,2,3]selenadiazole

2-(2-nitrocyclooctylidene)hydrazine-1-carboxamide (84)

2-nitrocyclooctanone **83** (3 g, 17.4 mmol) was suspended in 50% AcOH in H_2O (80 ml) and semicarbazide hydrochloride (2.4 g, 21.3 mmol) was added. Reaction mixture was stirred at room temperature for 18 hours. Solution was diluted with water (50 ml). Product in form of white precipitation was filtered off and washed with water. White solid, 2.23 g (56%).

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.97 - 1.11 (m, 1 H) 1.23 - 1.36 (m, 1 H) 1.48 - 1.66 (m, 6 H) 2.19 - 2.45 (m, 3 H) 2.74 - 2.83 (m, 1 H) 5.15 (dd, *J*=12.25, 3.55 Hz, 1 H) 6.41 (br. s., 2 H) 9.60 - 9.68 (m, 1 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 23.78, 24.12, 24.43, 24.69, 24.88, 27.51, 91.46, 144.63, 156.68. MS m/z calculated for $C_9H_{17}N_4O_3$ $[M+H]^+$ 229.13, found 229.34.

(2-oxocyclooctylidene)hydrazine-1-carboxamide (86)

Compound **84** (1 g, 4.4. mmol) was suspended in AcOH (15 ml) and SeO_2 (580 mg, 5.3 mmol) was added. Reaction mixture was stirred at room temperature for 18 hours followed by dilution with $CHCl_3$ (30 ml). Solution was washed with saturated Na_2CO_3, water, brine and dried over $MgSO_4$. Solvent was removed under reduced pressure and crude product was purified on column chromatography with mobile phase consists of 5% MeOH in DCM. Beige solid, 120 mg (14%).

^{1}H NMR (500 MHz,) δ ppm 1.47 – 1.54 (m, 2H), 1.54 – 1.61 (m, 2H), 1.63 – 1.72 (m, 2H), 1.79 (ddd, *J*=7.9, 7.3, 4.7 Hz, 2H), 2.67 – 2.71 (m, 2H), 2.72 – 2.77 (m, 2H), 5.32 (br. s., 1H), 6.33 (br. s., 1H), 9.41 (s, 1H); ^{13}C NMR (126 MHz,) δ ppm 25.30, 25.62, 26.31, 26.89, 27.84, 40.53, 148.96, 158.03, 203.06. HRMS m/z calculated for $C_9H_{16}N_3O_2$ $[M+H]^+$ 198.1237, found 198.1239.

(9*H*-fluoren-9-yl)methyl(4,5,6,7,8,9-hexahydrocycloocta[d][1,2,3]selenadiazol-4-yl)carbamate (95)

2-nitrocyclooctanole **91** (913 mg, 5.3 mmol) was dissolved in MeOH (10 ml) and Pd/C (100 mg) was added. Reaction mixture was equipped with balloon filled with H_2 and stirred at room temperature for 12 hours. Solvent was removed under reduced pressure and solution of FmocCl (1.36 g, 5.3 mmol) in DCM (10 ml) was added. Solution was stirred at room temperature for three hours, solvent was removed under reduced pressure and crude Fmoc-aminocyclooctanole was purified on column chromatography with mobile phase consists of 2% MeOH in DCM. Fmoc-aminocyclooctanole **92** (413 mg, 1,13 mmol) was dissolved in anhydrous DCM (45 ml) and Dess-Martin periodinane (1.4 g, 3.3 mmol) was added. Solution was stirred at room temperature for two hours, solvent was removed under reduced pressure and crude Fmoc-aminocyclooctanone was purified on column chromatography with mobile phase consists of 2% MeOH in DCM. Fmoc-aminocyclooctanone **93** (240 mg, 0.66 mmol) was suspended in 50 % AcOH/H_2O (2 ml) and semicarbazide hydrochloride (258 mg, 2.16 mmol) was added in three portions during three hours. Reaction mixture was stirred at room temperature for 18 hours. Crude product was filtered off from resulting suspension and washed with water. Crude semicarbazone **94** (32 mg, 0.076 mmol) was suspended in AcOH (3 ml) and SeO_2 (60 mg, 0.52 mmol) was added in three portions during three hours. Reaction mixture was stirred at room temperature for 18 hours followed by dilution with $CHCl_3$ (10 ml). Solution was washed with saturated Na_2CO_3, water, brine and dried over $MgSO_4$. Solvent was removed under reduced pressure and crude product was purified by semipreparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to shift from 60% to 80% over the course of 6 min. White solid, 13 mg (0.5%).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.34 - 1.68 (m, 8 H) 2.80 - 2.89 (m, 1 H) 3.52 - 3.62 (m, 2 H) 4.24 - 4.28 (m, 2 H) 4.95 - 5.02 (m, 1 H) 7.39 - 7.44 (m, 4 H) 7.88 (dt, *J*=7.45, 0.86 Hz, 4 H) 8.29 (d, *J*=4.40 Hz, 1 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 23.66, 25.22, 31.52, 36.05, 46.65, 48.32, 65.57, 109.72, 120.01, 121.35, 125.27, 127.03, 127.26, 128.90, 137.40, 155.65, 161.34. MS m/z calculated for $C_{23}H_{24}N_3O_2Se$ $[M+H]^+$ 454.10, found 454.28.

2-(2,2-difluorocyclooctylidene)hydrazine-1-carboxamide (100)

2,2-difluorocyclooctanone (500 mg, 3.09 mmol) was suspended in 50% AcOH in H_2O (10 ml) and semicarbazide hydrochloride (347 mg, 3.09 mmol) was added. Reaction mixture was stirred at room temperature for 1 hour, diluted with water (10 ml) and extracted three times to EtOAc. Combine organic layers were washed with saturated solution of $NaHCO_3$, brine and dried over $MgSO_4$. Solvent was removed under reduced pressure and crude product was purified by semipreparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to shift from 20% to 50% over the course of 6 min. White solid, 273 mg (40%).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.34 - 1.47 (m, 4 H) 1.50 - 1.58 (m, 2 H) 1.58 - 1.66 (m, 2 H) 2.18 - 2.29 (m, 2 H) 6.33 (br. s, 2 H) 9.58 (br. s, 1 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 20.86, 23.66, 24.12, 24.63, 24.73, 32.91 (t, *J*=25.79 Hz), 120.81 (t, *J*=241.73 Hz), 143.16 (t, *J*=22.19 Hz), 156.55. MS m/z calculated for $C_9H_{16}F_2N_3O$ $[M+H]^+$ 220.13, found 220.38.

4,4-difluoro-4,5,6,7,8,9-hexahydrocycloocta[d][1,2,3]selenadiazole (101)

Compound **74** (200 mg, 0.91 mmol) was dissolved in AcOH (3 ml) and SeO_2 (110 mg, 1 mmol) was added. Solution was stirred at 50 °C for 18 hours. Solution was diluted with H_2O (10 ml) and extracted three times with DCM. Combine organic layers were washed with saturated solution of Na_2CO_3, brine and dried over $MgSO_4$. Solution was evaporated under reduced pressure and crude product was purified on column chromatography with mobile phase consists of hexane:DCM 1:4 (v/v). Orange solid, 46 mg (20%).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.26 - 1.32 (m, 2 H) 1.65 - 1.77 (m, 6 H) 3.46 (t, *J*=6.80 Hz, 2 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 20.21, 21.91, 22.86, 28.71, 36.40 (t, *J*=24.59 Hz), 120.50 (t, *J*=240.53 Hz), 155.49 (t, *J*=26.39 Hz), 164.90. MS m/z calculated for $C_8H_{11}F_2N_2Se$ $[M+H]^+$ 253.01, found 253.22.

6.2.5. Preparation and reactivity of dibenzothiepine- and dibenzoselenepine-1,2,3-selenadiazoles

Dibenzo[2,3:6,7]thiepino[4,5-d][1,2,3]selenadiazole (105a)

Compound **110a** (385 mg, 1.36 mmol) was dissolved in AcOH (7 ml) and SeO_2 (177 mg, 1.61 mmol) was added. Reaction mixture was stirred at room temperature for 5 hours, diluted with DCM (20 ml) and washed with saturated solution of Na_2CO_3, brine and dried over $MgSO_4$. Solution was evaporated under reduced pressure and crude product was

purified on column chromatography with mobile phase consists of hexane:DCM 1:1 (v/v). Orange solid, 310 mg (72%).

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.42 - 7.47 (m, 1 H) 7.54 - 7.57 (m, 2 H) 7.57 - 7.59 (m, 2 H) 7.65 - 7.68 (m, 1 H) 7.72 - 7.75 (m, 2 H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 129.43, 129.73, 130.47, 130.57, 131.76, 132.94, 133.60, 133.64, 134.12, 134.74, 135.00, 135.16, 157.28, 160.63. HRMS m/z calculated for $C_{14}H_9N_2SSe$ $[M+H]^+$ 316.9646, found 316.9644.

Dibenzo[2,3:6,7]thiepino[4,5-d][1,2,3]selenadiazole 8-oxide (105b)

1,2,3-selenadiazole **105a** (527 mg, 1.67 mmol) was suspended in AcCN (20 ml) and 30% H_2O_2 (335 μL, 3.28 mmol) and Me_3SiCl (211 μL, 1.67 mmol) were added. Solution was stirred at room temperature for 30 minutes. Then, reaction was quenched by addition of water (25 ml) and product was extracted three times to EtOAc (15 ml). Combine organic extract were dried over $MgSO_4$, solvent was removed under reduced pressure and crude product was purified on column chromatography with mobile phase consists of 100% DCM - > DCM:MeOH 300:1 (v/v) . Orange solid, 314 mg (57%).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.58 (td, J=7.37, 1.58 Hz, 1 H) 7.69 - 7.74 (m, 2 H) 7.84 - 7.87 (m, 1 H) 7.87 - 7.93 (m, 3 H) 8.09 - 8.13 (m, 1 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 121.06, 121.35, 124.59, 125.80, 129.90, 130.56, 130.79, 130.96, 132.10, 134.35, 142.19, 142.75, 153.79, 156.62. HRMS m/z calculated for $C_{14}H_9N_2OSSe$ $[M+H]^+$ 332.9595, found 332.9596.

Dibenzo[2,3:6,7]thiepino[4,5-d][1,2,3]selenadiazole 8,8-dioxide (105c)

1,2,3-selenadiazole **105a** (110 mg, 0.35 mmol) was dissolved in DCM (11 ml) and 30% H_2O_2 (1.5 mL, 14.69 mmol) and AcOH (367 μL) were added. Solution was stirred at room temperature for 72 hours. Then, reaction was diluted by DCM (10 ml) and washed three times with water and brine. Organic layer was dried over $MgSO_4$, solvent was removed under reduced pressure and crude product was purified on column chromatography with mobile phase consists of 100% DCM - > DCM:MeOH 300:1 (v/v) . Orange solid, 84 mg (69%).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.81 - 7.86 (m, 1 H) 7.88 - 7.91 (m, 2 H) 7.96 - 7.99 (m, 2 H) 8.24 - 8.27 (m, 2 H) 8.42 - 8.45 (m, 1 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 125.89, 126.19, 127.68, 128.91, 130.54, 131.72, 131.78, 134.28, 134.45, 136.33, 138.51, 138.99, 155.17, 158.71. HRMS m/z calculated for $C_{14}H_9N_2O_2SSe$ $[M+H]^+$ 348.9544, found 348.9544.

Dibenzo[2,3:6,7]selenepino[4,5-d][1,2,3]selenadiazole (105d)

Compound **110d** (1.5 g, 4.5 mmol) was dissolved in AcOH (15 ml) and SeO_2 (545 mg, 4.95 mmol) was added. Reaction mixture was stirred at room temperature for five hours, diluted with DCM (20 ml) and washed with saturated solution of Na_2CO_3, brine and dried over $MgSO_4$. Solution was evaporated under reduced pressure and crude product was purified on column chromatography with mobile phase consists of hexane:DCM 3:7 (v/v). Orange solid, 750 mg (45%).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.41 - 7.45 (m, 1 H) 7.48 - 7.53 (m, 2 H) 7.55 - 7.59 (m, 1 H) 7.65 (dd, *J*=7.66, 1.22 Hz, 1 H) 7.84 (dt, *J*=7.73, 1.29 Hz, 2 H) 8.00 - 8.03 (m, 1 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 127.31, 129.12, 129.42, 130.28, 130.78, 131.43, 131.66, 133.76, 134.22, 134.33, 134.93, 135.63, 158.08, 161.54. HRMS m/z calculated for $C_{14}H_9N_2Se_2$ $[M+H]^+$ 364.9091, found 364.9090.

Dibenzo[2,3:6,7]selenepino[4,5-d][1,2,3]selenadiazole 8-oxide (105e)

1,2,3-selenadiazole **105d** (740 mg, 2.03 mmol) was dissolved in $CHCl_3$/MeOH 4:1 (20 ml) and 30% H_2O_2 (510 μL, 5 mmol) was added in two portions during two hours. Solution was stirred at room temperature for 1 hour. Then, solvents were removed under reduced pressure and crude product was purified on column chromatography with mobile phase consists of 5% MeOH in DCM. Orange solid, 578 mg (75%).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.53 - 7.57 (m, 1 H) 7.68 (d, *J*=8.02 Hz, 1 H) 7.78 - 7.84 (m, 2 H) 7.87 - 7.90 (m, 1 H) 7.90 - 7.94 (m, 2 H) 8.01 - 8.05 (m, 1 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 122.98, 123.27, 127.08, 128.46, 130.24, 130.54, 130.76, 131.78, 134.04, 135.23, 141.40, 142.53, 154.00, 157.22. HRMS m/z calculated for $C_{14}H_9N_2OSe_2$ $[M+H]^+$ 380.9040, found 380.9042.

2-(2-(phenylselanyl)phenyl)acetic acid (108d)

Benzeneselenol (1.57 g, 9.94 mmol) was added to solution of KOH (1.84 g, 32 mmol) in H_2O (45 ml). Reaction mixture was heated to 50 °C. Then, Cu (204 mg, 3.21 mmol) and 2-iodophenylacetic acid (2.46 g, 9.39 mmol) were added. Suspension was refluxed for 18 hours. After cooling to room temperature, reaction mixture was filtered. Filtrate was acidified with 1M HCl and extracted three times to EtOAc. Combine organic layers were washed with brine and dried over $MgSO_4$. Solvent was removed under reduced pressure and oily crude product was sonicated with water to get white suspension. Product was filtered off and washed with water. White solid, 1.74 g (71 %).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 3.78 (s, 2 H) 7.19 - 7.23 (m, 1 H) 7.28 - 7.31 (m, 3 H) 7.32 - 7.33 (m, 1 H) 7.34 - 7.35 (m, 1 H) 7.35 - 7.36 (m, 1 H) 7.37 - 7.40 (m, 1 H) 7.40 - 7.42 (m, 1 H) 12.33 (br. s, 1 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 41.20, 119.43, 127.22, 128.10, 128.33, 129.54, 131.17, 131.65, 131.85, 134.92, 137.69, 172.10. HRMS m/z calculated for $C_{14}H_{13}O_2Se$ $[M+H]^+$ 293.0075, found 293.0077.

Dibenzo[b,f]selenepin-10(11*H*)-one (109d)

Compound **108d** (1.74 g, 5.95 mmol) was dissolved in toluene (40 ml) and PPA (15 g) was added. Reaction mixture was refluxed for 18 hours and concentrated under reduced pressure. Oily residue was dissolved in EtOAc (100 ml), washed with water (100 ml), brine (100 ml) and dried over $MgSO_4$. Solution was evaporated under reduced pressure and crude product was purified on column chromatography with mobile phase consists of hexane:DCM 2:3 (v/v). Orange solid, 845 mg (53%).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 4.33 (s, 2 H) 7.24 (td, *J*=7.52, 1.29 Hz, 1 H) 7.40 - 7.43 (m, 1 H) 7.43 - 7.46 (m, 1 H) 7.49 (dd, *J*=7.88, 1.58 Hz, 1 H) 7.51 - 7.54 (m, 1 H) 7.77 - 7.82 (m, 2 H) 8.06 (dd, *J*=7.88, 1.58 Hz, 1 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 51.35, 120.01, 127.87, 127.91, 130.10, 130.65, 131.91, 132.75, 132.87, 133.27, 136.07, 138.06, 138.31, 191.95. HRMS m/z calculated for $C_{14}H_{11}OSe$ $[M+H]^+$ 274.9970, found 274.9968.

2-(dibenzo[b,f]thiepin-10(11*H*)-ylidene)hydrazine-1-carboxamide (110a)

Dibenzo[b,f]thiepin-10(11H)-one **109a** (414 mg, 1.83 mmol) was dissolved in 50% AcOH in H_2O (20 ml) and DMF (15 ml) and semicarbazide hydrochloride (750 mg, 6.6 mmol) was added in three portions during three hours. Solution was stirred at room temperature for 1 hour. Solid product was filtered off and washed with water. White solid, 404 mg (78%).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 4.25 (s, 2 H) 6.44 - 6.53 (m, 2 H) 7.22 - 7.26 (m, 2 H) 7.27 - 7.31 (m, 1 H) 7.38 (td, *J*=7.52, 1.15 Hz, 1 H) 7.45 (dd, *J*=7.80, 1.36 Hz, 1 H) 7.59 (dd, *J*=7.66, 1.07 Hz, 1 H) 7.72 (dd, *J*=7.52, 1.15 Hz, 1 H) 7.97 - 8.00 (m, 1 H) 9.96 (s, 1 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 33.58, 126.65, 127.34, 128.59, 129.16, 129.23, 129.48, 129.72, 130.91, 133.45, 134.04, 137.02, 139.38, 141.27, 157.11. HRMS m/z calculated for $C_{15}H_{14}N_3OS$ $[M+H]^+$ 284.0852, found 284.0851.

2-(dibenzo[b,f]selenepin-10(11*H*)-ylidene)hydrazine-1-carboxamide (110d)

Dibenzo[b,f]selenepin-10(11H)-one **109d** (845 mg, 3.08 mmol) was dissolved in 50% AcOH in H_2O (30 ml) and DMF (20 ml) and semicarbazide hydrochloride (1.26 mg, 11.1 mmol) was added in three portions during three hours. Solution was stirred at room

temperature for 1 hour. Solid product was filtered off and washed with water. White solid, 658 mg (65%).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 4.22 (s, 2 H) 6.45 (s, 2 H) 7.20 (td, *J*=7.52, 1.29 Hz, 1 H) 7.23 - 7.27 (m, 2 H) 7.37 (td, *J*=7.45, 1.15 Hz, 1 H) 7.54 - 7.57 (m, 1 H) 7.66 - 7.70 (m, 1 H) 7.77 (dd, *J*=7.45, 0.86 Hz, 1 H) 7.88 - 7.92 (m, 1 H) 9.87 (s, 1 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 34.60, 126.59, 127.25, 128.50, 128.83, 129.39, 129.50, 130.08, 130.30, 131.33, 131.71, 138.97, 139.50, 142.00, 157.02. HRMS m/z calculated for $C_{15}H_{14}N_3OSe$ $[M+H]^+$ 332.0297, found 322.0295.

1-benzyl-1*H*-dibenzo[2,3:6,7]thiepino[4,5-d][1,2,3]triazole (111a)

1,2,3-selenadiazole **105a** (76 mg, 0.24 mmol) was dissolved in MeOH (40 ml) and benzylazide **72** (30 mg, 0.22 mmol) was added. Reaction was irradiated by medium pressure mercury arc at λ > 280 nm for 20 hours. Then, solvent was removed under reduced pressure and crude product was purified by semipreparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to shift from 60% to 80% over the course of 6 min. White solid, 10 mg (12%).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 5.87 (d, *J*=15.90 Hz, 1 H) 5.98 (d, *J*=15.90 Hz, 1 H) 7.08 - 7.12 (m, 2 H) 7.27 - 7.33 (m, 3 H) 7.46 - 7.48 (m, 1 H) 7.49 - 7.51 (m, 2 H) 7.52 - 7.56 (m, 1 H) 7.65 - 7.68 (m, 1 H) 7.71 - 7.74 (m, 2 H) 7.90 - 7.93 (m, 1 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 52.06, 126.82, 127.17, 127.49, 127.90, 128.09, 128.71, 129.42, 129.85, 130.84, 132.59, 133.02, 133.64, 133.79, 134.12, 134.82, 134.97, 135.74, 145.70. HRMS m/z calculated for $C_{21}H_{16}N_3S$ $[M+H]^+$ 342.1059, found 342.1059.

1-benzyl-1*H*-dibenzo[2,3:6,7]selenepino[4,5-d][1,2,3]triazole (111d)

1,2,3-selenadiazole **105d** (40 mg, 0.11 mmol) was dissolved in MeOH (40 ml) and benzylazide **72** (25 mg, 0.18 mmol) was added. Reaction was irradiated by medium pressure mercury arc at λ = 300 nm for 20 hours. Then, solvent was removed under reduced pressure and crude product was purified by semipreparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to shift from 60% to 80% over the course of 6 min. White solid, 12 mg (28%).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 5.83 (d, *J*=16.04 Hz, 1 H) 5.91 (d, *J*=16.04 Hz, 1 H) 7.04 - 7.07 (m, 2 H) 7.26 - 7.32 (m, 3 H) 7.41 - 7.46 (m, 2 H) 7.46 - 7.50 (m, 1 H) 7.50 - 7.55 (m, 1 H) 7.70 (dd, *J*=7.59, 1.58 Hz, 1 H) 7.75 - 7.78 (m, 1 H) 7.81 - 7.84 (m, 1 H) 7.86 - 7.89 (m, 1 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 51.94, 126.77, 127.84, 128.50, 128.63, 129.01, 129.20, 129.24, 129.80, 129.91, 130.39, 130.76, 131.33, 133.83, 134.76, 134.97,

135.75, 135.84, 146.70. HRMS m/z calculated for $C_{21}H_{16}N_3Se$ $[M+H]^+$ 390.0504, found 390.0506.

Dibenzo[3,4:6,7]thiepine[1,2-b]dibenzo[3,4:6,7]thiepine[1,2-e][1,4]diselenine (112a)

1,2,3-selenadiazole **105a** (76 mg, 0.24 mmol) was dissolved in MeOH (40 ml) and benzylazide **72** (30 mg, 0.22 mmol) was added. Reaction was irradiated by medium pressure mercury arc at $\lambda > 280$ nm for 20 hours. Then, solvent was removed under reduced pressure and crude product was purified by semipreparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to shift from 60% to 80% over the course of 6 min. White solid, 8 mg (10%).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.06 - 7.11 (m, 4 H) 7.38 - 7.44 (m, 8 H) 7.48 - 7.53 (m, 4 H); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 126.08, 129.29, 130.33, 132.77, 133.02, 134.74, 139.97. HRMS m/z calculated for $C_{28}H_{17}S_2Se_2$ $[M+H]^+$ 576.9097, found 576.9095.

5,14-dithia[3,4:6,7]cyclohepta[1,2-b]dibenzo[3,4:6,7]cyclohepta[1,2-d]selenophene-*S*,*S*´-dioxide (114b)

1,2,3-selenadiazole **105b** (76 mg, 0.24 mmol) was dissolved in MeOH (40 ml) and benzylazide **72** (30 mg, 0.22 mmol) was added. Reaction was irradiated by medium pressure mercury arc at $\lambda > 300$ nm for 20 hours. Then, solvent was removed under reduced pressure and crude product was purified by semipreparative HPLC. The mobile phase consisted of (A) 0.01 M ammonium acetate in water and (B) acetonitrile, with B linearly programmed to shift from 60% to 80% over the course of 6 min. White solid, 10 mg (10%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.87 (dd, *J*=7.9, 1.1 Hz, 1H), 7.81 (ddd, *J*=8.7, 7.6, 1.0 Hz, 2H), 7.78 – 7.74 (m, 1H), 7.64 – 7.59 (m, 1H), 7.59 – 7.55 (m, 1H), 7.19 (td, *J*=7.6, 1.3 Hz, 1H), 6.86 (dd, *J*=7.8, 0.8 Hz, 1H). HRMS m/z calculated for $C_{28}H_{17}O_2S_2Se$ $[M+H]^+$ 528.9830, found 528.9834.

7. References

1. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem. , Int. Ed.* **2002,** *41* (14), 2596-2599.
2. Tornoe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002,** *67* (9), 3057-3064.
3. Moses, J. E.; Moorhouse, A. D. *Chem. Soc. Rev.* **2007,** *36* (8), 1249-1262.
4. Baskin, J. M.; Bertozzi, C. R. *Aldrichimica Acta* **2010,** *43* (1), 15-23.
5. Agard, N. J.; Prescher, J. A.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2004,** *126* (46), 15046-15047.
6. Ostrovskis, P.; Volla, C. M. R.; Turks, M.; Markovic, D. *Curr. Org. Chem.* **2013,** *17* (6), 610-640.
7. Michael, A. *J. Pr. Chem.* **1906,** *48* (2), 94-95.
8. Huisgen, R. *Angew. Chem.* **1963,** *75* (13), 604-637.
9. Hein, J. E.; Fokin, V. V. *Chem Soc Rev* **2010,** *39* (4), 1302-1315.
10. Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem. , Int. Ed.* **2001,** *40* (11), 2004-2021.
11. Zhang, L.; Chen, X.; Xue, P.; Sun, H. H. Y.; Williams, I. D.; Sharpless, K. B.; Fokin, V. V.; Jia, G. *J. Am. Chem. Soc.* **2005,** *127* (46), 15998-15999.
12. McNulty, J.; Keskar, K.; Vemula, R. *Chem. Eur. J.* **2011,** *17* (52), 14727-14730.
13. McNulty, J.; Keskar, K. *Eur. J. Org. Chem.* **2012,** *2012* (28), 5462-5470.
14. Wittig, G.; Krebs, A. *Chem. Ber.* **1961,** *94*, 3260-3275.
15. Agard, N. J.; Baskin, J. M.; Prescher, J. A.; Lo, A.; Bertozzi, C. R. *ACS Chem. Biol.* **2006,** *1* (10), 644-648.
16. Baskin, J. M.; Prescher, J. A.; Laughlin, S. T.; Agard, N. J.; Chang, P. V.; Miller, I. A.; Lo, A.; Codelli, J. A.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. U. S. A. , Early Ed.* **2007,** *104* (43), 16793-16797.
17. Codelli, J. A.; Baskin, J. M.; Agard, N. J.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2008,** *130* (34), 11486-11493.
18. Ning, X.; Guo, J.; Wolfert, M. A.; Boons, G. J. *Angew. Chem. , Int. Ed.* **2008,** *47* (12), 2253-2255.
19. Debets, M. F.; van Berkel, S. S.; Schoffelen, S.; Rutjes, F. P. J. T.; van Hest, J. C. M.; van Delft, F. L. *Chem. Commun. (Cambridge, U. K.)* **2010,** *46* (1), 97-99.
20. Jewett, J. C.; Sletten, E. M.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2010,** *132* (11), 3688-3690.

21. Dommerholt, J.; Schmidt, S.; Temming, R.; Hendriks, L. J. A.; Rutjes, F. P. J. T.; van Hest, J. C. M.; Lefeber, D. J.; Friedl, P.; van Delft, F. L. *Angew. Chem. , Int. Ed.* **2010,** *49* (49), 9422-9425.
22. Sletten, E. M.; Nakamura, H.; Jewett, J. C.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2010,** *132* (33), 11799-11805.
23. Tummatorn, J.; Batsomboon, P.; Clark, R. J.; Alabugin, I. V.; Dudley, G. B. *J. Org. Chem.* **2012,** *77* (5), 2093-2097.
24. de Almeida, G.; Sletten, E. M.; Nakamura, H.; Palaniappan, K. K.; Bertozzi, C. R. *Angew. Chem. , Int. Ed.* **2012,** *51* (10), 2443-2447.
25. Krebs, A.; Kimling, H. *Justus Liebigs Ann. Chem.* **1975,** (12), 2074-2084.
26. Sletten, E. M.; Bertozzi, C. R. *Org. Lett.* **2008,** *10* (14), 3097-3099.
27. Friscourt, F.; Ledin, P. A.; Mbua, N. E.; Flanagan-Steet, H. R.; Wolfert, M. A.; Steet, R.; Boons, G. J. *J. Am. Chem. Soc.* **2012,** *134* (11), 5381-5389.
28. Stoeckmann, H.; Neves, A. A.; Stairs, S.; Ireland-Zecchini, H.; Brindle, K. M.; Leeper, F. J. *Chem. Sci.* **2011,** *2* (5), 932-936.
29. Varga, B. R.; Kallay, M.; Hegyi, K.; Beni, S.; Kele, P. *Chem. Eur. J.* **2012,** *18* (3), 822-828.
30. Groest, C.; Berg, T. *Org. Biomol. Chem.* **2015,** *13* (13), 3866-3870.
31. Domnin, N. A. *Zh. Obshch. Khim.* **1938,** *8*, 851-868.
32. Blomquist, A. T.; Liu, L. H. *J. Am. Chem. Soc.* **1953,** *75*, 2153-2154.
33. Wittig, G.; Dorsch, H. L. *Justus Liebigs Ann. Chem.* **1968,** *711*, 46-54.
34. Freeman, P. K.; Johnson, R. C. *J. Org. Chem.* **1969,** *34* (6), 1746-1750.
35. Meier, H.; Menzel, I. *Synthesis* **1971,** (4), 215-216.
36. Meier, H.; Menzel, I. *J. Chem. Soc. D.* **1971,** (18), 1059.
37. Buehl, H.; Gugel, H.; Kolshorn, H.; Meier, H. *Synthesis* **1978,** (7), 536-537.
38. Meier, H.; Voigt, E. *Tetrahedron* **1972,** *28* (1), 187-198.
39. Schulz, R.; Schweig, A. *Tetrahedron Lett.* **1984,** *25* (22), 2337-2340.
40. Bock, H.; Aygen, S.; Solouki, B. *Z. Naturforsch. , B: Anorg. Chem. , Org. Chem.* **1983,** *38B* (5), 611-615.
41. Meier, H.; Stavridou, E.; Storek, C. *Angew. Chem.* **1986,** *98* (9), 838-839.
42. Ando, W.; Kumamoto, Y.; Tokitoh, N. *Tetrahedron Lett.* **1987,** *28* (46), 5699-5702.
43. Nishiyama, Y.; Hada, Y.; Iwase, K.; Sonoda, N. *J. Organomet. Chem.* **2000,** *611* (1-2), 488-493.
44. Khanna, P. K.; Gorte, R. M.; Morley, C. P. *Mater. Lett.* **2003,** *57* (8), 1464-1466.

45. Khanna, P. K.; Gorte, R. M.; Gokhale, R. *Mater. Lett.* **2004,** *58* (6), 966-969.
46. Khanna, P. K.; Singh, N.; Charan, S.; Lonkar, S. P.; Reddy, A. S.; Patil, Y.; Viswanath, A. K. *Mater. Chem. Phys.* **2006,** *97* (2-3), 288-294.
47. Khanna, P. K. *Synth. React. Inorg. , Met. -Org. , Nano-Met. Chem.* **2008,** *38* (5), 409-413.
48. Khanna, P. K.; Das, B. K. *Mater. Lett.* **2004,** *58* (6), 1030-1034.
49. Khanna, P. K.; Singh, N.; Charan, S.; Patil, K. R. *Mater. Lett.* **2006,** *60* (17-18), 2080-2085.
50. Khanna, P. K.; Singh, N.; Charan, S.; Viswanath, A. K.; Patil, K. R. *Mater. Res. Bull.* **2007,** *42* (8), 1414-1421.
51. Singh, N.; Patil, K. R.; Khanna, P. K. *Mater. Sci. Eng. B* **2007,** *142* (1), 31-36.
52. Bhanoth, S.; More, P. V.; Jadhav, A.; Khanna, P. K. *RSC Adv.* **2014,** *4* (34), 17526-17532.
53. Jadhav, A. A.; Khanna, P. K. *RSC Adv.* **2015,** *5* (56), 44756-44763.
54. Wang, H.; Zhang, J.; Yu, H. *Free Radical Biol. Med.* **2007,** *42* (10), 1524-1533.
55. Meier, H.; Menzel, I. *Tetrahedron Lett.* **1972,** (5), 445-448.
56. Laureni, J.; Krantz, A.; Hajdu, R. A. *J. Am. Chem. Soc.* **1976,** *98* (24), 7872-7874.
57. Krantz, A.; Laureni, J. *J. Am. Chem. Soc.* **1977,** *99* (14), 4842-4844.
58. Torres, M.; Clement, A.; Bertie, J. E.; Gunning, H. E.; Strausz, O. P. *J. Org. Chem.* **1978,** *43* (12), 2490-2493.
59. Harrit, N.; Rosenkilde, S.; Larsen, B. D.; Holm, A. *J. Chem. Soc., Perkin Trans. 1* **1985,** (5), 907-911.
60. Ando, W.; Kumamoto, Y.; Tokito, N. *Tetrahedron Lett.* **1986,** *27* (50), 6107-6110.
61. Reese, C. B.; Shaw, A. *J. Chem. Soc. , Chem. Commun.* **1972,** (13), 787-788.
62. Pirrung, M. C.; Webster, N. J. G. *J. Org. Chem.* **1987,** *52* (16), 3603-3613.
63. Sims, E. A.; DeForest, C. A.; Anseth, K. S. *Tetrahedron Lett.* **2011,** *52* (16), 1871-1873.
64. Cheng, Z.; Elias, D. R.; Kamat, N. P.; Johnston, E. D.; Poloukhtine, A.; Popik, V.; Hammer, D. A.; Tsourkas, A. *Bioconjugate Chem.* **2011,** *22* (10), 2021-2029.
65. Poloukhtine, A.; Popik, V. V. *J. Org. Chem.* **2003,** *68* (20), 7833-7840.
66. Poloukhtine, A. A.; Mbua, N. E.; Wolfert, M. A.; Boons, G. J.; Popik, V. V. *J. Am. Chem. Soc.* **2009,** *131* (43), 15769-15776.
67. Arumugam, S.; Orski, S. V.; Mbua, N. E.; McNitt, C.; Boons, G. J.; Locklin, J.; Popik, V. V. *Pure Appl. Chem.* **2013,** *85* (7), 1499-1513.
68. Meier, H.; Layer, M.; Zetzsche, A. *Chem. Ztg.* **1974,** *98* (9), 460.

69. Meier, H.; Layer, M.; Combrink, W.; Schniepp, S. *Chem. Ber.* **1976,** *109* (5), 1650-1656.
70. Krebs, A.; Kimling, H. *Tetrahedron Lett.* **1970,** (10), 761-764.
71. Fernandez-Suarez, M.; Baruah, H.; Martinez-Hernandez, L.; Xie, K. T.; Baskin, J. M.; Bertozzi, C. R.; Ting, A. Y. *Nat. Biotechnol.* **2007,** *25* (12), 1483-1487.
72. Chang, P. V.; Prescher, J. A.; Sletten, E. M.; Baskin, J. M.; Miller, I. A.; Agard, N. J.; Lo, A.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. U. S. A.* **2010,** *107* (5), 1821-1826.
73. Laughlin, S. T.; Baskin, J. M.; Amacher, S. L.; Bertozzi, C. R. *Science (Washington, DC, U. S.)* **2008,** *320* (5876), 664-667.
74. Dehnert, K. W.; Baskin, J. M.; Laughlin, S. T.; Beahm, B. J.; Naidu, N. N.; Amacher, S. L.; Bertozzi, C. R. *ChemBioChem* **2012,** *13* (3), 353-357.
75. Bostic, H. E.; Smith, M. D.; Poloukhtine, A. A.; Popik, V. V.; Best, M. D. *Chem. Commun. (Cambridge, U. K.)* **2012,** *48* (10), 1431-1433.
76. Leunissen, E. H. P.; Meuleners, M. H. L.; Verkade, J. M. M.; Dommerholt, J.; Hoenderop, J. G. J.; van Delft, F. L. *ChemBioChem* **2014,** *15* (10), 1446-1451.
77. Tobimatsu, Y.; Wouwer, D. V. d.; Allen, E.; Kumpf, R.; Vanholme, B.; Boerjan, W.; Ralph, J. *Chem. Commun. (Cambridge, U. K.)* **2014,** *50* (82), 12262-12265.
78. Yoon, H. I.; Yhee, J. Y.; Na, J. H.; Lee, S.; Lee, H.; Kang, S. W.; Chang, H.; Ryu, J. H.; Lee, S.; Kwon, I. C.; Cho, Y. W.; Kim, K. *Bioconjugate Chem.* **2016,** *27* (4), 927-936.
79. Subramanian, N.; Sreemanthula, J. B.; Balaji, B.; Kanwar, J. R.; Biswas, J.; Krishnakumar, S. *Chem. Commun. (Cambridge, U. K.)* **2014,** *50* (80), 11810-11813.
80. Koo, H.; Lee, S.; Na, J. H.; Kim, S. H.; Hahn, S. K.; Choi, K.; Kwon, I. C.; Jeong, S. Y.; Kim, K. *Angew. Chem. Int. Ed.* **2012,** *51* (47), 11836-11840.
81. Lee, S.; Koo, H.; Na, J. H.; Han, S. J.; Min, H. S.; Lee, S. J.; Kim, S. H.; Yun, S. H.; Jeong, S. Y.; Kwon, I. C.; Choi, K.; Kim, K. *ACS Nano* **2014,** *8* (3), 2048-2063.
82. Jayaprakash, K. N.; Peng, C. G.; Butler, D.; Varghese, J. P.; Maier, M. A.; Rajeev, K. G.; Manoharan, M. *Org. Lett.* **2010,** *12* (23), 5410-5413.
83. van Delft, P.; Meeuwenoord, N. J.; Hoogendoorn, S.; Dinkelaar, J.; Overkleeft, H. S.; van der Marel, G. A.; Filippov, D. V. *Org. Lett.* **2010,** *12* (23), 5486-5489.
84. Singh, I.; Freeman, C.; Heaney, F. *Eur. J. Org. Chem.* **2011,** *2011* (33), 6739-6746.
85. Khatwani, S. L.; Kang, J. S.; Mullen, D. G.; Hast, M. A.; Beese, L. S.; Distefano, M. D.; Taton, T. A. *Bioorg. Med. Chem.* **2012,** *20* (14), 4532-4539.
86. Mukhortava, A.; Schlierf, M. *Bioconjugate Chem.* **2016,** *27* (7), 1559-1563.

87. Marks, I. S.; Kang, J. S.; Jones, B. T.; Landmark, K. J.; Cleland, A. J.; Taton, T. A. *Bioconjugate Chem.* **2011,** *22* (7), 1259-1263.
88. Shelbourne, M.; Brown, T.; El-Sagheer, A. H.; Brown, T. *Chem. Commun. (Cambridge, U. K.)* **2012,** *48* (91), 11184-11186.
89. Shelbourne, M.; Chen, X.; Brown, T.; El-Sagheer, A. H. *Chem. Commun. (Cambridge, U. K.)* **2011,** *47* (22), 6257-6259.
90. Jawalekar, A. M.; Malik, S.; Verkade, J. M. M.; Gibson, B.; Barta, N. S.; Hodges, J. C.; Rowan, A.; van Delft, F. L. *Molecules* **2013,** *18*, 7346-7363.
91. Heuer-Jungemann, A.; Kirkwood, R.; El-Sagheer, A. H.; Brown, T.; Kanaras, A. G. *Nanoscale* **2013,** *5* (16), 7209-7212.
92. Bernardin, A.; Cazet, A.; Guyon, L.; Delannoy, P.; Vinet, F.; Bonnaffe, D.; Texier, I. *Bioconjugate Chem.* **2010,** *21* (4), 583-588.
93. Zhang, P.; Liu, S.; Gao, D.; Hu, D.; Gong, P.; Sheng, Z.; Deng, J.; Ma, Y.; Cai, L. *J. Am. Chem. Soc.* **2012,** *134* (20), 8388-8391.
94. Hao, J.; Huang, L. L.; Zhang, R.; Wang, H. Z.; Xie, H. Y. *Anal. Chem. (Washington, DC, U. S.)* **2012,** *84* (19), 8364-8370.
95. Kotagiri, N.; Li, Z.; Xu, X.; Mondal, S.; Nehorai, A.; Achilefu, S. *Bioconjugate Chem.* **2014,** *25* (7), 1272-1281.
96. Bouvet, V.; Wuest, M.; Wuest, F. *Org. Biomol. Chem.* **2011,** *9* (21), 7393-7399.
97. Campbell-Verduyn, L. S.; Mirfeizi, L.; Schoonen, A. K.; Dierckx, R. A.; Elsinga, P. H.; Feringa, B. L. *Angew. Chem. , Int. Ed.* **2011,** *50* (47), 11117-11120.
98. Arumugam, S.; Chin, J.; Schirrmacher, R.; Popik, V. V.; Kostikov, A. P. *Bioorg. Med. Chem. Lett.* **2011,** *21* (23), 6987-6991.
99. Evans, H. L.; Slade, R. L.; Carroll, L.; Smith, G.; Nguyen, Q. D.; Iddon, L.; Kamaly, N.; Stoeckmann, H.; Leeper, F. J.; Aboagye, E. O.; Spivey, A. C. *Chem. Commun. (Cambridge, U. K.)* **2012,** *48* (7), 991-993.
100. Kim, H. L.; Sachin, K.; Jeong, H. J.; Choi, W.; Lee, H. S.; Kim, D. W. *ACS Med. Chem. Lett.* **2015,** *6* (4), 402-407.
101. Kettenbach, K.; Ross, T. L. *MedChemComm* **2016,** *7* (4), 654-657.
102. Chen, K.; Wang, X.; Lin, W. Y.; Shen, C. K. F.; Yap, L. P.; Hughes, L. D.; Conti, P. S. *ACS Med. Chem. Lett.* **2012,** *3* (12), 1019-1023.
103. Lee, D. E.; Na, J. H.; Lee, S.; Kang, C. M.; Kim, H. N.; Han, S. J.; Kim, H.; Choe, Y. S.; Jung, K. H.; Lee, K. C.; Choi, K.; Kwon, I. C.; Jeong, S. Y.; Lee, K. H.; Kim, K. *Mol. Pharmaceutics* **2013,** *10* (6), 2190-2198.

104. Satpati, D.; Bauer, N.; Hausner, S. H.; Sutcliffe, J. L. *J. Radioanal. Nucl. Chem.* **2014,** *302* (2), 765-771.
105. Ornelas, C.; Broichhagen, J.; Weck, M. *J. Am. Chem. Soc.* **2010,** *132* (11), 3923-3931.
106. Huang, B.; Desai, A.; Zong, H.; Tang, S.; Leroueil, P.; Baker, J. R., Jr. *Tetrahedron Lett.* **2011,** *52* (13), 1411-1414.
107. Huang, B.; Kukowska-Latallo, J. F.; Tang, S.; Zong, H.; Johnson, K. B.; Desai, A.; Gordon, C. L.; Leroueil, P. R.; Baker, J. R. *Bioorg. Med. Chem. Lett.* **2012,** *22* (9), 3152-3156.
108. Xu, J.; Filion, T. M.; Prifti, F.; Song, J. *Chem. Asian J.* **2011,** *6* (10), 2730-2737.
109. DeForest, C. A.; Anseth, K. S. *Angew. Chem. , Int. Ed.* **2012,** *51* (8), 1816-1819.
110. Kloxin, A. M.; Lewis, K. J. R.; DeForest, C. A.; Seedorf, G.; Tibbitt, M. W.; Balasubramaniam, V.; Anseth, K. S. *Integr. Biol.* **2012,** *4* (12), 1540-1549.
111. Zheng, J.; Smith Callahan, L. A.; Hao, J.; Guo, K.; Wesdemiotis, C.; Weiss, R. A.; Becker, M. L. *ACS Macro Lett.* **2012,** *1* (8), 1071-1073.
112. Ashley, G. W.; Henise, J.; Reid, R.; Santi, D. V. *Proc. Natl. Acad. Sci. U. S. A.* **2013,** *110* (6), 2318-2323.
113. Takahashi, A.; Suzuki, Y.; Suhara, T.; Omichi, K.; Shimizu, A.; Hasegawa, K.; Kokudo, N.; Ohta, S.; Ito, T. *Biomacromolecules* **2013,** *14* (10), 3581-3588.
114. Johnson, J. A.; Baskin, J. M.; Bertozzi, C. R.; Koberstein, J. T.; Turro, N. J. *Chem. Commun. (Cambridge, U. K.)* **2008,** (26), 3064-3066.
115. Lallana, E.; Fernandez-Megia, E.; Riguera, R. *J. Am. Chem. Soc.* **2009,** *131* (16), 5748-5750.
116. Kuzmin, A.; Poloukhtine, A.; Wolfert, M. A.; Popik, V. V. *Bioconjugate Chem.* **2010,** *21* (11), 2076-2085.
117. Jolck, R. I.; Sun, H.; Berg, R. H.; Andresen, T. L. *Chem. Eur. J.* **2011,** *17* (12), 3326-3331.
118. Manova, R. K.; Pujari, S. P.; Weijers, C. A. G. M.; Zuilhof, H.; van Beek, T. A. *Langmuir* **2012,** *28* (23), 8651-8663.
119. Zheng, J.; Chen, Y.; Karim, A.; Becker, M. L. *ACS Macro Lett.* **2014,** *3* (10), 1084-1087.
120. Zayas, J.; Annoual, M.; Das, J. K.; Felty, Q.; Gonzalez, W. G.; Miksovska, J.; Sharifai, N.; Chiba, A.; Wnuk, S. F. *Bioconjugate Chem.* **2015,** *26* (8), 1519-1532.
121. Zhou, Q.; Gui, J.; Pan, C. M.; Albone, E.; Cheng, X.; Suh, E. M.; Grasso, L.; Ishihara, Y.; Baran, P. S. *J. Am. Chem. Soc.* **2013,** *135* (35), 12994-12997.

122. Smyslova, P. Preparation and reactivity of selected 5 and 5′ substituted 5-methyluridines. Diploma thesis, Palacky University Olomouc, 2012.
123. Garland, W.; Frenzel, B.; Karg, T.; Wipke, T.; Konopelski, J. Methotrexate adjuvants to reduce toxicity and methods for using the same. WO 2009114325, 2009.
124. Wittmann, J. G.; Heinrich, D.; Gasow, K.; Frey, A.; Diederichsen, U.; Rudolph, M. G. *Structure (Cambridge, MA, U. S.)* **2008,** *16* (1), 82-92.
125. Smyslova, P.; Popa, I.; Lycka, A.; Tejral, G.; Hlavac, J. *PLoS One* **2015,** *10* (12), e0144613/1-e0144613/33.
126. Rajaganesh, R.; Ravinder, P.; Subramanian, V.; Mohan Das, T. *Carbohydr. Res.* **2011,** *346* (15), 2327-2336.
127. Gaussian 09, Revision D.01, Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. Gaussian, Inc., Wallingford CT, 2009.
128. Friscourt, F.; Fahrni, C. J.; Boons, G. J. *J. Am. Chem. Soc.* **2012,** *134* (45), 18809-18815.
129. Sachin, K.; Jadhav, V. H.; Kim, E. M.; Kim, H. L.; Lee, S. B.; Jeong, H. J.; Lim, S. T.; Sohn, M. H.; Kim, D. W. *Bioconjugate Chem.* **2012,** *23* (8), 1680-1686.
130. Lalezari, I.; Shafiee, A.; Yalpani, M. *Tetrahedron Lett.* **1969,** (58), 5105-5106.
131. Jedinakova, P.; Sebej, P.; Slanina, T.; Klan, P.; Hlavac, J. *Chem. Commun. (Cambridge, U. K.)* **2016,** *52* (26), 4792-4795.
132. Altimari, J. M.; Niranjan, B.; Risbridger, G. P.; Schweiker, S. S.; Lohning, A. E.; Henderson, L. C. *Bioorg. Med. Chem.* **2014,** *22* (9), 2692-2706.
133. Kamalraj, V. R.; Senthil, S.; Kannan, P. *J. Mol. Struct.* **2008,** *892* (1-3), 210-215.

134. Singh, S. C.; Mishra, S. K.; Srivastava, R. K.; Gopal, R. *J. Phys. Chem. C* **2010,** *114* (41), 17374-17384.
135. Fairbanks, B. D.; Sims, E. A.; Anseth, K. S.; Bowman, C. N. *Macromolecules (Washington, DC, U. S.)* **2010,** *43* (9), 4113-4119.
136. Chen, W.; Wang, D.; Dai, C.; Hamelberg, D.; Wang, B. *Chem. Commun. (Cambridge, U. K.)* **2012,** *48* (12), 1736-1738.
137. Klan, P.; Janosek, J.; Kriz, Z. *J. Photochem. Photobiol. , A* **2000,** *134* (1-2), 37-44.
138. Wagner, P. J.; Kochevar, I. E.; Kemppainen, A. E. *J. Am. Chem. Soc.* **1972,** *94* (21), 7489-7494.
139. Ito, K.; Higuchi, Y.; Tame, C.; Hariya, J. *Heterocycles* **1993,** *35* (2), 937-947.
140. Daehne, L.; Bendig, J.; Stoesser, R. *J. Prakt. Chem. /Chem. Ztg.* **1992,** *334* (8), 707-710.
141. Doering, W.; Odum, R. A. *Tetrahedron* **1966,** *22* (1), 81-93.
142. Leyva, E.; Platz, M. S.; Persy, G.; Wirz, J. *J. Am. Chem. Soc.* **1986,** *108* (13), 3783-3790.
143. He, Y.; Chen, S.; Liu, Z.; Cheng, C.; Li, H.; Wang, M. *Life Sci.* **2014,** *115* (1-2), 44-51.
144. Livnah, O.; Bayer, E. A.; Wilchek, M.; Sussman, J. L. *Proc. Natl. Acad. Sci. U. S. A.* **1993,** *90* (11), 5076-5080.
145. Giorgi, G.; Miranda, S.; Lopez-Alvarado, P.; Avendano, C.; Rodriguez, J.; Menendez, J. C. *Org. Lett.* **2005,** *7* (11), 2197-2200.
146. Feuer, H.; Pivawer, P. M. *J. Org. Chem.* **1969,** *34* (10), 2917-2919.
147. Piel, I.; Pawelczyk, M. D.; Hirano, K.; Froehlich, R.; Glorius, F. *Eur. J. Org. Chem.* **2011,** *2011* (28), 5475-5484, S5475-1.
148. Utsukihara, T.; Nakamura, H.; Watanabe, M.; Horiuchi, C. A. *Tetrahedron Lett.* **2006,** *47* (52), 9359-9364.
149. Milner, S. E.; Brossat, M.; Moody, T. S.; Elcoate, C. J.; Lawrence, S. E.; Maguire, A. R. *Tetrahedron: Asymmetry* **2010,** *21* (8), 1011-1016.
150. Mathys, M.; Kraft, P. *Chem. Biodiversity* **2014,** *11* (10), 1597-1607.
151. Kageshima, Y.; Suzuki, C.; Oshiro, K.; Amii, H. *Synlett* **2015,** *26* (1), 63-66.
152. Krebs, A.; Cholcha, W.; Mueller, M.; Eicher, T.; Pielartzik, H.; Schnoeckel, H. *Tetrahedron Lett.* **1984,** *25* (44), 5027-5030.
153. Botta, M.; Castiglioni, E.; Di Fabio, R.; Spinosa, R.; Togninelli, A. Spiro compounds useful as antagonists of the H1 receptor and their asymmetric preparation,

pharmaceutical compositions and use in the treatment of sleep disorders. WO2009016085A1, Feb 5, 2009.

154. Boudhar, A.; Ng, X. W.; Loh, C. Y.; Chia, W. N.; Tan, Z. M.; Nosten, F.; Dymock, B. W.; Tan, K. S. W. *Eur. J. Med. Chem.* **2016,** *119*, 231-249.
155. Still, I. W. J. Photochemistry of sulfoxides and sulfones. Wiley: 1988; pp 873-887.
156. Yamazaki, Y.; Tsuchiya, T.; Hasegawa, T. *Bull. Chem. Soc. Jpn.* **2003,** *76* (1), 201-202.
157. Song, F. *J. Am. Soc. Mass Spectrom.* **2007,** *18* (7), 1286-1290.
158. Strohalm, M.; Kavan, D.; Novak, P.; Volny, M.; Havlicek, V. *Anal. Chem. (Washington, DC, U. S.)* **2010,** *82* (11), 4648-4651.
159. Krchnak, V.; Padera, V. *Bioorg. Med. Chem. Lett.* **1998,** *8* (22), 3261-3264.
160. Chiacchio, U.; Corsaro, A.; Mates, J.; Merino, P.; Piperno, A.; Rescifina, A.; Romeo, G.; Romeo, R.; Tejero, T. *Tetrahedron* **2003,** *59* (26), 4733-4738.
161. Zheng, T.; Narayan, R. S.; Schomaker, J. M.; Borhan, B. *J. Am. Chem. Soc.* **2005,** *127* (19), 6946-6947.
162. Jensen, K. J.; Alsina, J.; Songster, M. F.; Vagner, J.; Albericio, F.; Barany, G. *J. Am. Chem. Soc.* **1998,** *120* (22), 5441-5452.

PALACKY UNIVERSITY OLOMOUC

Faculty of Science

Department of Organic Chemistry

Study of New Systems for Non-catalyzed Conjugation Reaction with Azides

Ph.D. Thesis

Author: Mgr. Petra Jedináková

Supervisor: prof. RNDr. Jan Hlaváč, Ph.D.

Olomouc 2017

This Ph.D. thesis was carried out within the framework of presence Ph.D. study of program P1417 Organic Chemistry guaranteed by the Department of Organic Chemistry, Faculty of Science, Palacky University Olomouc, over the years 2013-2017

Ph.D. candidate:	**Mgr. Petra Jedináková** Department of Organic Chemistry Faculty of Science, Palacky University Olomouc
Supervisor:	**prof. RNDr. Jan Hlaváč, Ph.D.** Department of Organic Chemistry Faculty of Science, Palacky University Olomouc
Opponents:	**prof. Ing. Radek Cibulka, Ph.D.** **doc. RNDr. Ctibor Mazal, CSc.**

The summary of the Ph.D. thesis was sent for distribution on.....

The oral defense will take place on…….11. 2017 before the Commission for Ph.D. thesis of the study program Chemistry at the Department of Organic Chemistry, Faculty of Science, Palacky University Olomouc, 17. listopadu 12, Olomouc

The Ph.D. thesis is available at the Department of Organic Chemistry, 17. Listopadu 12, Olomouc.

Souhrn

Nekatalyzované konjugační reakce alkynů s azidy jsou široce používané v řadě biologických aplikací. Ačkoliv byla vyvinuta řada derivátů cyklických alkynů pro tyto účely, jejich komplikovaná a multi-kroková příprava, často spojená s nízkými výtěžky, je limitací pro jejich rozsáhlejší využití. Dalším problémem je také často nedostatečná charakterizace produktů vznikajících samotnou konjugační reakcí těchto alkynů s azidy.

Disertační práce byla zaměřena na přípravu dvou typů sloučenin pro nekatalyzované konjugační reakce s azidy. Prvním typem byly deriváty již známého aza-dibenzocyclooctynu u kterého byly nejprve studovány konjugační reakce s azidy odvozenými od 5-methyluridinu. Vzniklé produkty byly charakterizovány pomocí detailní NMR studie, která vedla ke zjištění přítomnosti konformačních izomerů u všech připravených sloučenin. Příčina vzniku těchto izomerů a jejich struktury byly navrženy pomocí výpočetní studie. Dalším cílem bylo vyvinout rychlou a jednoduchou syntézu fluorescenčně značených derivátů aza-dibenzocyclooctynu na pevné fázi. Klíčová tvorba trojné vazby na cyclootynovém systému nebyla bohužel i přes řadu optimalizací úspěšná. Nicméně, připravené prekurzory nesoucí dvojnou vazbu mohou být použity pro konjugační reakce využívající alkeny.

Druhým typem studovaných sloučenin byly deriváty cycloalka-1,2,3-selenadiazolů, které se mohou působením vysokých teplot nebo UV záření rozkládat za vzniku cykloalkynů podléhajícím konjugačním reakcím s azidy. První část práce byla zaměřena na studium mikrovlnami a fotochemicky iniciovaných konjugačních reakcí cyklookta[d][1,2,3]selenadiazolu s různými azidy. Obě metody poskytují požadované 1,2,3-triazoly. Aplikace fotochemicky iniciované reakce byla demonstrována na konjugační reakci cyklookta[d][1,2,3]selenadiazolu s komplexem avidin-modifikovaný biotin. Dále byly připraveny deriváty cyklookta[d][1,2,3]selenadiazolu nesoucí substituenty s různými elektronovými vlastnostmi s cílem snížit teplotu potřebnou pro konjugační reakci. Bohužel, oba typy připravených derivátů vykazovaly při reakci s azidy mnohem nižší reaktivitu než nesubstituovaný cyklookta[d][1,2,3]selenadiazol. Poslední část pak byla zaměřena na přípravu dibenzothiepino- a dibenzoselenepino-1,2,3-selenadiazolů s cílem zvýšit reaktivitu u fotochemicky iniciovaných konjugačních reakcí. Všechny připravené 1,2,3-selenadiazoly poskytovaly různé produkty v závislosti na typu 1,2,3-selenadiazolu a na použité vlnové délce.

Abstract

Non-catalyzed click reactions are widely used in range of biological applications. Although a number of derivatives of cyclic alkynes have been developed till now for these purposes, the most of these systems suffer from complicated and multi-step synthesis with low overall yields which limits their wider use. Often insufficient characterization of products formed by conjugation reaction of these alkynes with azides is another important problem in this field.

The presented thesis was focused on preparation of two types of compounds for non-catalyzed conjugation reaction with azides. Firstly, conjugation reactions of known aza-dibenzocyclooctyne with azides derived from 5-methyluridine were studied. Synthesized products were characterized by detail NMR study, which showed presence of conformational isomers at all prepared compounds. The computational study then helped to suggest the cause of occurrence and structure of these isomers. Then, the attention was paid to development of fast and simple procedure for fluorescently labelled derivatives of aza-dibenzocyclooctyne on solid phase. Unfortunately, the key formation of triple bond on cyclooctyne system has not been successful despite a number of optimizations. However, prepared precursors having double bond can be used for conjugation reactions using alkenes.

Derivatives of cycloalka-1,2,3-selenadiazoles were the second type of studied compounds. Cycloalka-1,2,3-selenadiazoles can decompose by high temperatures or UV irradiation to cycloalkynes undergoing the conjugation reactions with azides. The first part of work was focused on study of microwave assisted and photochemically initiated conjugation reactions of cycloocta[d][1,2,3]selenadiazole with structurally different azides. The both method resulted in target 1,2,3-triazoles. The potential application of photochemically initiated reaction was then successfully demonstrated on conjugation reaction of cycloocta[d][1,2,3]selenadiazole derivative with avidin-modified biotin complex. Then, derivatives of cycloocta[d][1,2,3]selenadiazole bearing substituent with different electronic properties were prepared to decrease the temperature necessary for conjugation reaction. Unfortunately, both type of synthesized derivatives exhibited much lower reactivity during reaction with azides than unsubstituted cycloocta[d][1,2,3]selenadiazole. In the last part, the attention was paid to preparation of dibenzothiepine- a dibenzoselenepine-1,2,3-selenadiazoles with the aim to enhance the reactivity at photochemically initiated conjugation reactions. All synthesized 1,2,3-selenadiazoles provided different products depending on 1,2,3-selenadiazole structure and used wavelength.

Contents

Souhrn **3**

Abstract **4**

Contents **5**

8. Introduction **6**

9. Aims of the work **7**

9.1. Summary of presented aims 7

9.1.1. **DIBAC** derivatives 7

9.1.2. Cycloalka-1,2,3-selenadiazoles 8

10. Results and discussion **9**

10.1. **DIBAC** derivatives 9

10.1.1. SPAAC of **DIBAC** and characterization of resulting triazols 9

10.1.1.1. SPAAC with azides derived from 5-methyl uridine 9

10.1.1.1.1. Preparation of reagents 9

10.1.1.1.2. SPAAC 10

10.1.1.1.3. NMR study 11

10.1.1.1.4. Computational study 14

10.1.1.1.5. Effect of aliphatic chain on formation of conformers 18

10.1.2. Solid phase synthesis of fluorescently labelled **DIBAC** analogues 20

10.1.2.1. Immobilization of dihydrodibenzoazocine 20

10.1.2.2. Triple bond formation 22

10.1.2.3. Attachment of fluorescent label 24

10.2. 1,2,3-selenadiazoles 27

10.2.1. Reactions of cycloocta[d][1,2,3]selenadiazoles 27

10.2.1.1. Microwave assisted conjugation reactions of cycloocta[d][1,2,3]selenadiazole 28

10.2.1.2. Photochemically induced conjugation reactions of cycloocta[d][1,2,3]selenadiazole 29

10.2.2. Preparation of derivatives of cycloocta-1,2,3-selenadiazol 35

10.2.3. Preparation and reactivity of dibenzo thia- or selena- cyclohepta-1,2,3-selenadiazoles 39

11. Conclusion **44**

12. References **46**

1. Introduction

Since discovery of Cu(I) catalysed cycloaddition of azides (CuAAC) with terminal alkynes by Sharpless[1] and Meldal[2] in 2002, this reaction, yielding selectively 1,4-triazoles, was widely studied and applied for range of bioconjugation reactions.[3] Bioconjugation techniques generally involve the covalent attachment of synthetic labels to a specific biomolecular target. Examples include the modification of proteins and nucleic acids by incorporation of fluorophores, ligands, chelates, radioisotopes and affinity tags.[3]

The applicability of CuAAC for bioconjugation was firstly reported by Meldal *et al.* in 2002.[2] They prepared first peptidotriazoles by utilization of solid phase synthesis techniques and the azide-alkyne cycloaddition.[2] Then CuAAC has been applied for functionalization of DNA, ligation and decoration of peptides and peptoids, for synthesis of peptidomimetics, surface modifications and many other applications.[3] Ideal bioconjugation reactions should be carried out without affecting living tissues via undesired interaction with biomolecules.[3] However Cu(I) ions are toxic for living organisms which limited the use of CuAAC for reactions in living systems.[4]

In 2004, Bertozzi *et al*. utilized the non-catalysed azide-alkyne conjugation reaction mediated by ring strain of cyclooctyne.[5] The biocompatibility of this approach was showed on labelling of Jurkat cells.[5] Their discovery started new era in field of bioconjugation reactions. Till now, many reactive cycloalkynes were developed and utilized for range of not only biological applications including modification of peptides and nucleic acids, tumor targeting, fluorescent labelling, radiolabelling or surface modifications and formation of polymer, hydrogels and dendrimers.[6]

The main drawback of non-catalysed azide-alkyne cycloadditions is especially availability of reactive cycloalkynes. The most of the known systems require time wasting multi-step synthesis, often with very low overall yield.[6] Another problem is stability of some systems during storage.[6] Thus, development of new reactive cycloalkynes, which will be easy to prepare and modify, and will have satisfactory stability with good reactivity, is still important topic, which could be beneficial for extending of azide-alkyne cycloadditions applicability.

2. Aims of work

Based on the literature review mentioned above it is obvious, that SPAAC is useful method for (bio)conjugation. The crucial drawback of this reaction and following application lies in the complex multi-step and often also low-yielding synthesis of cyclooctyne systems. In addition, full characterization of click chemistry products, which can affect the conformation of biomolecules resulting in change of their function, is not always performed.

The first goal of presented dissertation was exploration of conjugation reaction between **DIBAC** derivatives and azidoderivatives of nucleic acid components. The attention was supposed to be paid to possible formation of conformation isomers possibly affecting the function of nucleic acids.

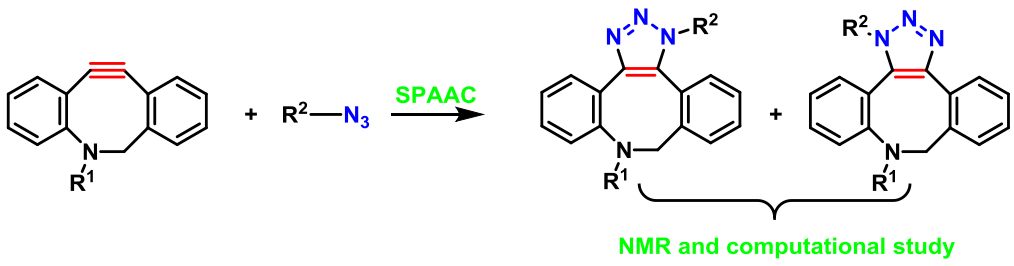
The second goal was focused on development of synthesis of new **DIBAC-Dye** systems suitable for non-catalyzed labelling of biomolecules.

The third goal of the work was aimed to possible use of cycloalka-1,2,3-selenadiazoles for conjugation reactions with azides under various initiation conditions including heating, microwave irradiation and photolysis. The synthesis of a system suitable for biorthogonal labelling was supposed to be developed and the system applicability was supposed to be verified in reaction with a biomolecule.

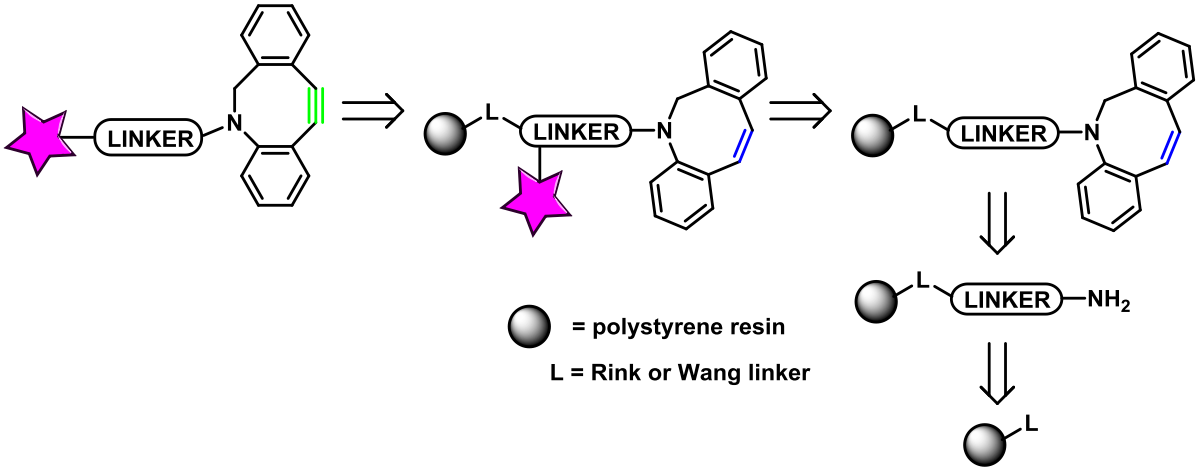
2.1. Summary of presented aims

2.1.1. DIBAC derivatives

- SPAAC of **DIBAC** analogues with different azides derived mainly from nucleosides and detail characterization of resulting triazoles

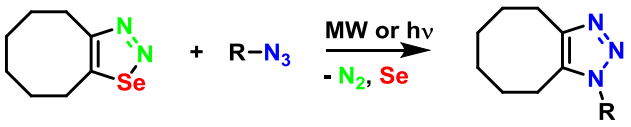


- development of solid phase synthesis of fluorescently labelled **DIBAC** analogues

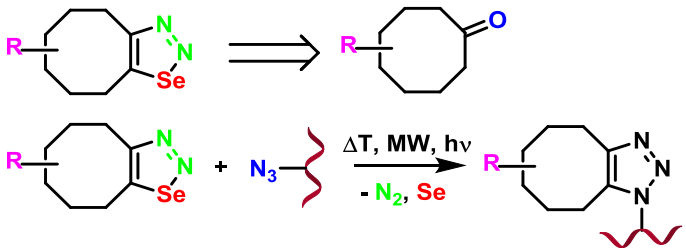


2.1.2. Cycloalka-1,2,3-selenadiazoles

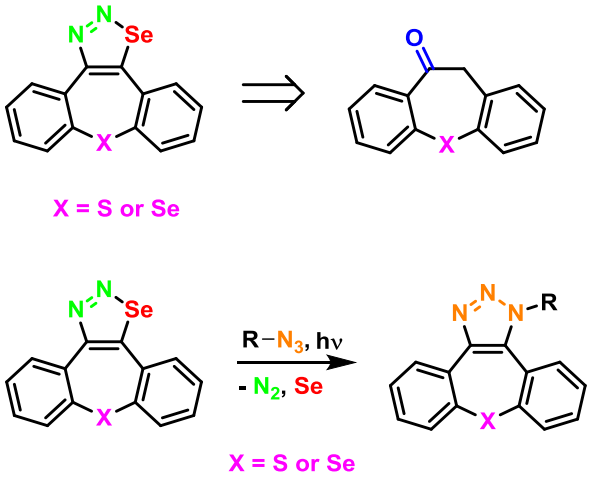
- study of reactions of cycloocta-1,2,3-selenadiazole with structurally different azides



- development of synthesis of substituted cycloocta-1,2,3-selenadiazoles and study of conjugation reactions with azides (including derivatized biomolecules) under various conditions



- development of synthesis of dibenzothiepine- and dibenzoselenepine-1,2,3-selenadiazoles derivatives for photochemically induced conjugation reaction with azides.



3. Results and discussion

3.1. DIBAC derivatives

This part of work is dealing with strain promoted azide-alkyne cycloaddition reactions of aza-dibenzocyclootyne derivatives (**DIBAC**) which was firstly described by van Delft in 2010.[7] Although **DIBAC** exhibits excellent kinetic towards reaction with azides, time-wasting multi-step synthesis of its derivatives is the main drawback of this system.[7] Also detail characterization of resulting conjugates published in literature is vague.

3.1.1. SPAAC of DIBAC and characterization of resulting triazols

This part of work was focused on study of triazoles formed from **DIBAC** and various azides including mainly nucleic acid components. Copper-free click reactions with nucleobases using **DIBAC** derivatives have been described in only two articles.[8,9] The structure of triazoles derived from **DIBAC** and oligonucleotides or nucleosides has not been studied to date.

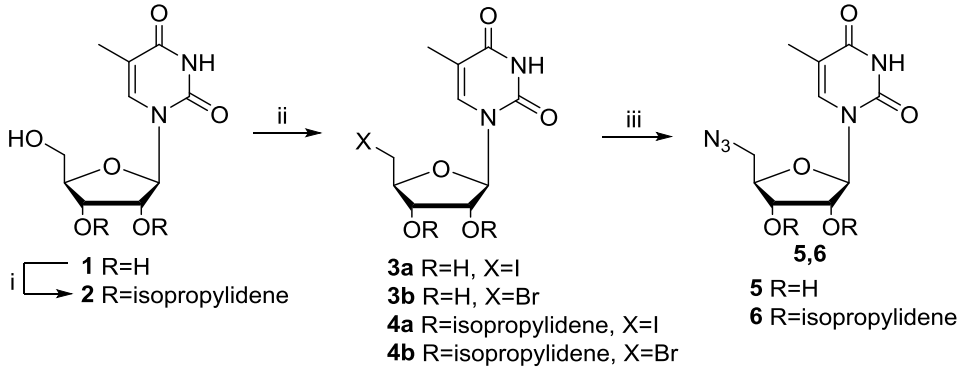
3.1.1.1. SPAAC with azides derived from 5-methyl uridine

3.1.1.1.1. Preparation of reagents

Preparation of 5-methyluridine-5´-azides

The attention was paid to 5´-azidoderivative **5** and its protected analogue **6** previously developed within authors´ Diploma thesis using commercially available 5-methyluridine as a starting material (Scheme 1).

Scheme 1: Preparation of 5´-azide-5-methyluridines **5** and **6**.[10,*a*]

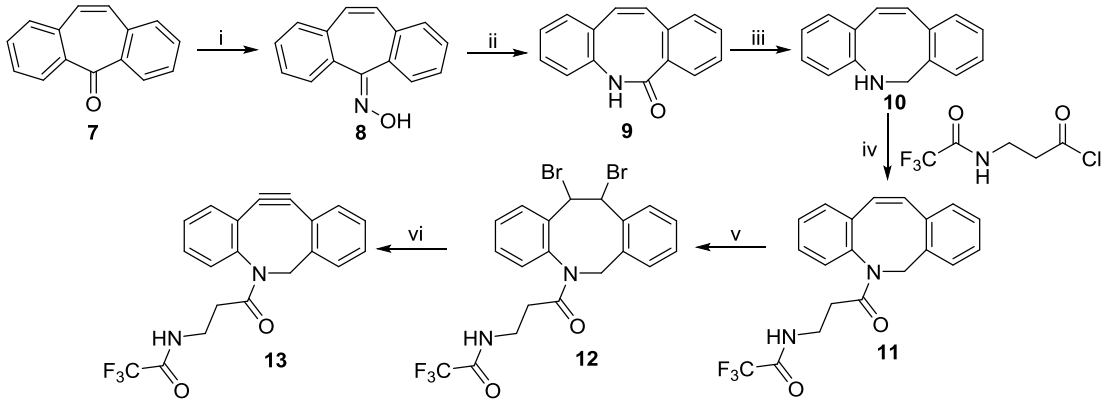


[*a*]Reagent: (i) aceton, H_2SO_4 (ii) I_2 or Br_2, PPh_3, imidazole, THF (iii) NaN_3, DMF, 90 °C.

*Preparation of **DIBAC** derivative*

Azadibenzocyclooctyne derivative **13** was prepared according to literature by six step procedure starting from dibenzosuberone **7** in 7% overall yield (Scheme 2).[11]

Scheme 2: Preparation of **DIBAC** derivative **13**.[11,a]

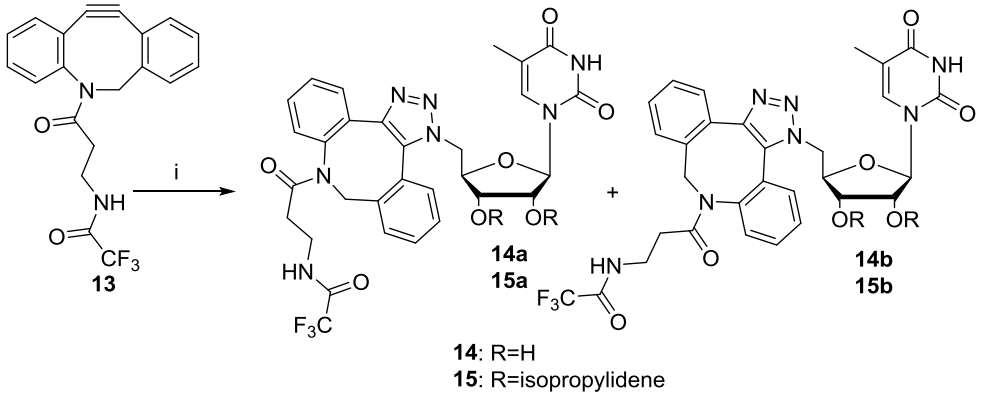


[a]Reagent: (i) $NH_2OH.HCl$, pyridine (ii) PPA, 125 °C (iii) $LiAlH_4$, diethylether, reflux (iv) pyridine, DCM (v) pyridinium tribromide, DCM (vi) *t*-BuOK, THF.

3.1.1.1.2. SPAAC

Strain promoted click reaction of azides **5** and **6** with **DIBAC** derivative **13** (Scheme 3) was studied.[12] As expected, this compound was highly reactive, and all reactions in methanol were nearly instantaneous. Both products were produced as a mixture of two regioisomers in a 1:1 ratio.[12]

Scheme 3: SPAAC with azadibenzocyclooctyne **13** and azides **5** and **6**.[12,a]



[a]Reagent: (i) Azide **5** or **6**, MeOH.

Isomers **14a,b** and **15a,b** were successfully separated by semi-preparative HPLC, yielding products in >99% purity. All isomers were subjected to detailed NMR study.[12]

3.1.1.1.3 NMR study[5]

Although the purity of triazoles **14a,b** and **15a,b** was confirmed by HPLC before and after the NMR experiments and verified under several HPLC conditions, the ^{1}H NMR spectra revealed the presence of two isomers of the **14a** and **15a** derivatives and even more for the **14b** as well as **15b** derivatives.[12] The ^{13}C NMR spectra of $CDCl_3$ solutions also revealed more than the expected number of signals.

The structures of compounds **14a** and **15a** were clearly confirmed by the ^{1}H, ^{13}C and ^{15}N signals assigned via 2D experiments.[12] Assigning the ^{1}H, ^{13}C and ^{15}N signals (measured in $CDCl_3$) of regioisomers **14b** and **15b** was problematic due to the presence of more forms of each pure compound (see above). Because the reaction course predetermines the formation of two regioisomers and the HRMS of couples **14a**/**14b** or **15a**/**15b** respectively afforded the same elemental composition, we conclude that the structures really correspond to the opposite regioisomers.[12]

For couple **15a**/**15b**, we performed standard ^{19}F NMR and ^{19}F-^{19}F EXSY. In both cases we observed two resonances for **15a** and four resonances for **15b** ($CDCl_3$) (Figure 1). ^{19}F-^{19}F EXSY of both compounds revealed a mutual slow exchange between all existing forms, as evidenced by positive cross-peaks in the spectra (Figure 1).[12]

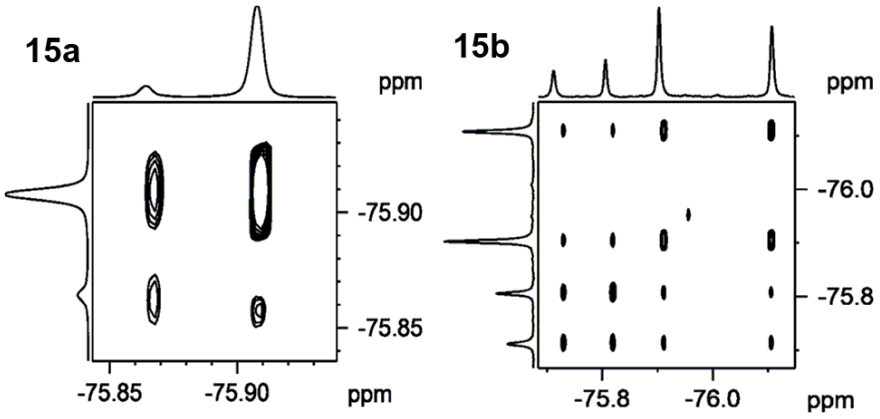


Figure 1: ^{19}F-^{19}F EXSY of triazoles **15a** and **15b** (mixing time 1 s).[12]

^{1}H-^{15}N gHMQC and ^{1}H-^{15}N gHMBC experiments in $CDCl_3$ suggested that the isomery was of conformational origin. Doubled signals of the pyrimidine NH group for compound **15a** at 150.75 ppm (Figure 2) likely hindered the rotation of the pyrimidine ring relative to the other parts of the molecule. Identical results were obtained in d_6-DMSO.[12]

[5] ^{19}F-^{19}F EXSY, ^{1}H-^{15}N gHMQC and ^{1}H-^{15}N gHMBC were measured by Antonín Lyčka from Faculty of Science, University of Hradec Králové.

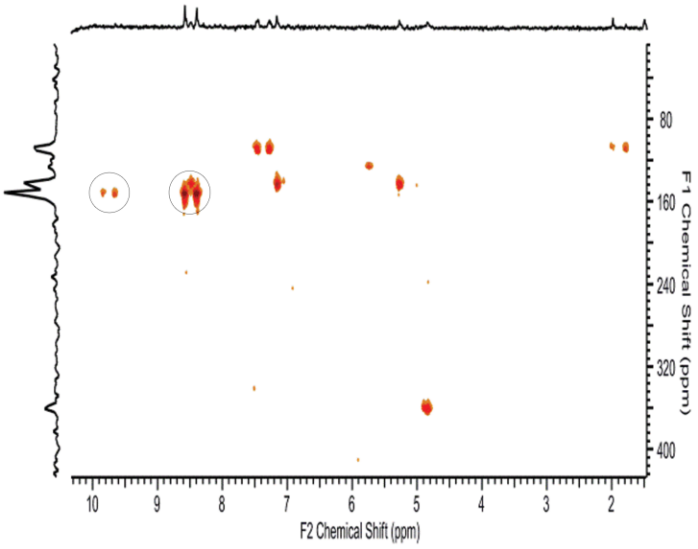


Figure 2: ^{1}H-^{15}N HMBC of **15a** in $CDCl_3$.[12]

The ^{1}H spectra of compounds **15a** and **15b** in d_6-DMSO were measured at various temperatures (25 °C, 50 °C, 100 °C and cooling back) to determine whether the number of isomers was affected by temperature. The spectral pattern was essentially unaffected until +50 °C. Signal coalescence was finally observed at +100 °C (Figure 3).[12] Identical results were obtained for standard ^{19}F spectra of **9a** and **9b** measured in DMSO, with signal coalescence at 100 °C (Figure 4).[12]

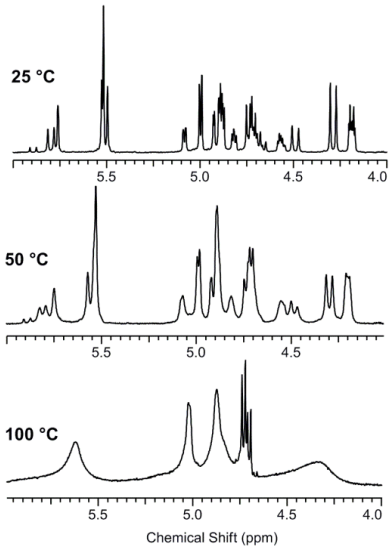


Figure 3: ^{1}H NMR spectra of **15a** at different temperatures.[12]

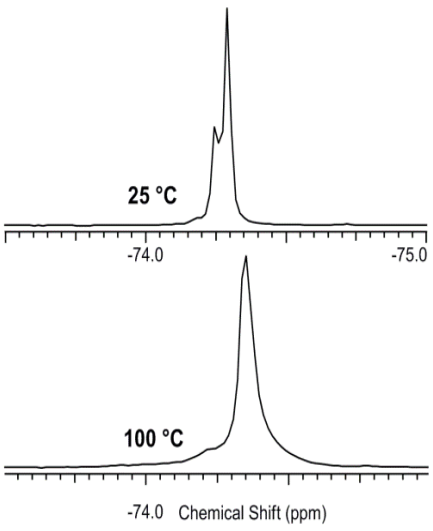


Figure 4: ^{19}F spectra of **15a** at different temperatures.[12]

To characterize the relationship between the number of isomers and the type of solvent, we extended the number of tested solvents to acetone, D_2O, MeOD, DMSO-d_6 and DMF-d_7 for derivatives **14a** and **15a**, which were selected as representative model compounds. Changing the solvent not only shifted the signals but also affected their ratio

(Figure 5).[12] On the other hand the number of isomers of compounds **14a** and **15a** remained constant. The dependence of the isomeric ratio on solvent is presented in Table 1.

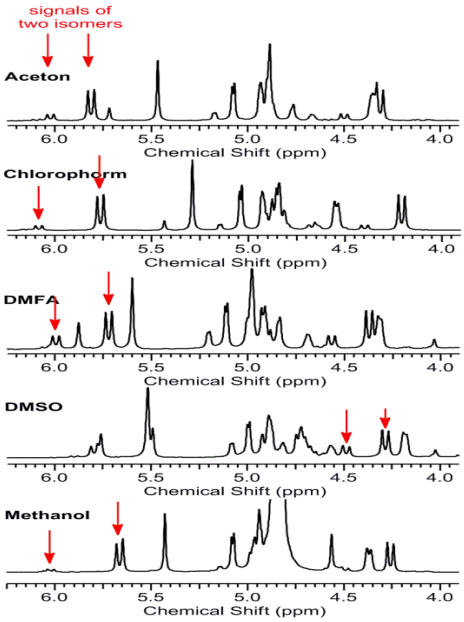


Figure 5: ^{1}H NMR spectra of **15a** in various solvents.[12]

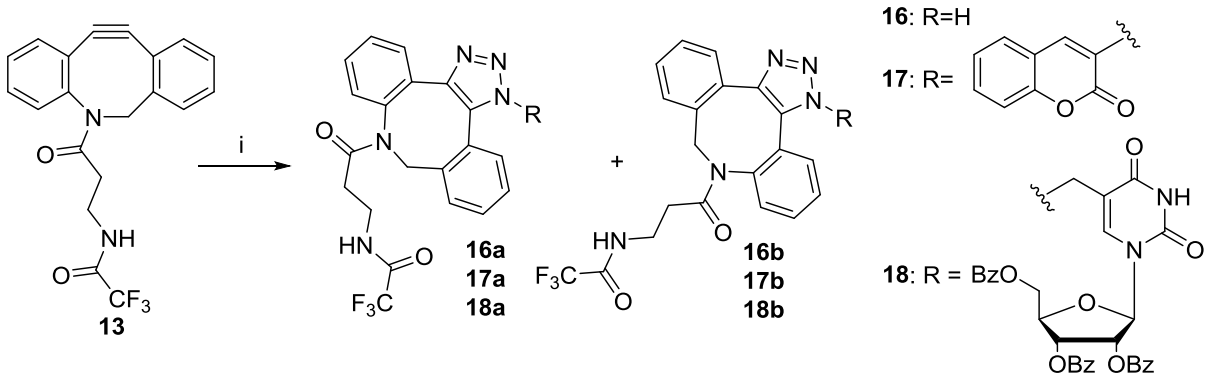
Table 1. The ratio of isomers of compounds **14a** and **15a**.

14a		**15a**	
Solvent	**Ratio***	**Solvent**	**Ratio***
CD_3OD	3.2:1	CD_3OD	9:1
$CDCl_3$	2.6:1	$CDCl_3$	6.6:1
DMF-d_7	1.7:1	DMF-d_7	2.6:1
D_2O	7.9:1	d_6-Acetone	4:1
DMSO-d_6	1.4:1	DMSO-d_6	2.2:1

*The ratio was determined from the peak integrals of the **DIBAC** methylene group protons at approximately 5.75 ppm and 6.00 ppm or at 4.25 ppm and 4.50 ppm.[12]

To determine whether the presence of isomers was caused by the nucleoside part of molecule or by s-cis, s-trans isomery of the amide groups on the azocine moiety, we prepared triazoles **16-18** substituted on nitrogen by only hydrogen, the sterically bulkier coumarin, and by 2´,3´,5´tribenzoyl-5-methyluridine (Scheme 4).[12]

Scheme 4: Preparation of triazoles **16, 17** and **18**.[12,a]



[a]Reagent: (i) Azide, MeOH.

For triazoles **16a** and **17a**, we observed only one set of signals, with no isomery. Surprisingly, the ^{1}H spectra of triazole **17b** contained at least three sets of signals, similar to the ^{13}C NMR spectra in which more than one set of signals was detected. These results confirm that the type of substituent on the triazole strongly influences the number of isomers

in NMR spectra.[12] Moreover, the presence of isomery in **17b** and the lack of isomery in derivative **17a** indicate that the position of the aliphatic part of azocine relative to the triazole substituent is crucial for the number of isomers formed. These results also demonstrate that the presence of amide bonds in the azocine part of the molecule does not affect the isomery observed in the NMR spectra.[12]

Regioisomers **18a** and **18b** were inseparable under several HPLC conditions; the retention times of the isomers were nearly identical on semi-preparative C_{18} columns. The ^{1}H NMR spectrum of the mixture of **18a,b** in $CDCl_3$ revealed the presence of additional isomers, similar to compounds **14a,b** and **15a,b**. Thus, all possible isomers can also be expected when labeling oligonucleotides *via* nucleobase derivatization.[12]

To clearly identify the origin of the isomery, triazoles **14a,b** and **15a,b** were subjected to computational study.[12]

3.1.1.1.4. Computational study[††]

According to the NMR study described above, we assumed that the observed conformations of compounds **14a,b** and **15a,b** resulted from a combination of rotation about two bonds between the triazole and ribose rings. The rotations of these bonds were studied as the changes of two dihedral angles involving backbone atoms $N^{15}N^{14}C^{13}C^{12}$ and $N^{14}C^{13}C^{12}C^{11}$ (for numbering see Figure 6).[12]

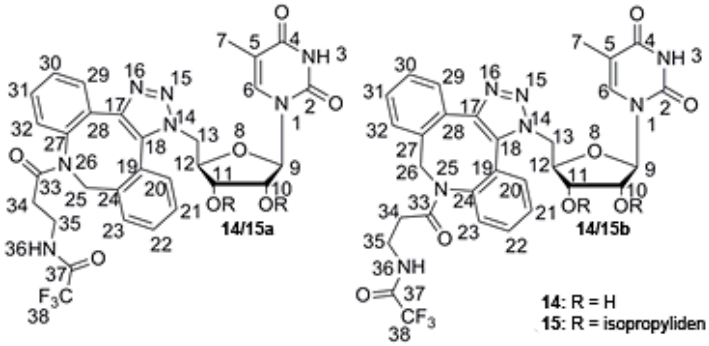


Figure 6: Numbering of atoms in **14a,b** and **15a,b** in the conformational study.[12]

The quantum calculation was simplified to a vacuum to assess the ability of the compounds to form stable conformers whose distributions further depend on the solvents. The theoretical model used in this study was the B3LYP method with a 6-31G(d,p) basis set in Gaussian 09.[12,13]

[††]Calculation of energies at local minima and conformer structures were provided by Gracián Tejral from Laboratory of Tissue Engineering, Institute of Experimental Medicine, Academy of Science of the Czech Republic.

To determine the configurations with energies at the local minima, the dependencies of the energies on both dihedral angles were examined. The conformation with the lowest energy was selected as the zero point on the energy scale for each structure. The potential local energy minima were subsequently determined, and the fractional populations from the Maxwell-Boltzmann distribution were estimated. It was revealed that the most frequent conformers have potential energies lower than 30 kJ/mol.[12]

Analyses of compounds **14a,b** and **15a,b** with respect to changes in both dihedral angles revealed that the position of the aliphatic chain in structures (a) (intended **14a**/**15a**) and (b) (intended **14b**/**15b**) differed. For the (a) structures, both the left and right positions of the aliphatic chain (with respect to the triazole ring – see Figure 7) were observed (Figure 7A and 7B). However, the (b) structures were characterized by the right position only (Figure 7C).

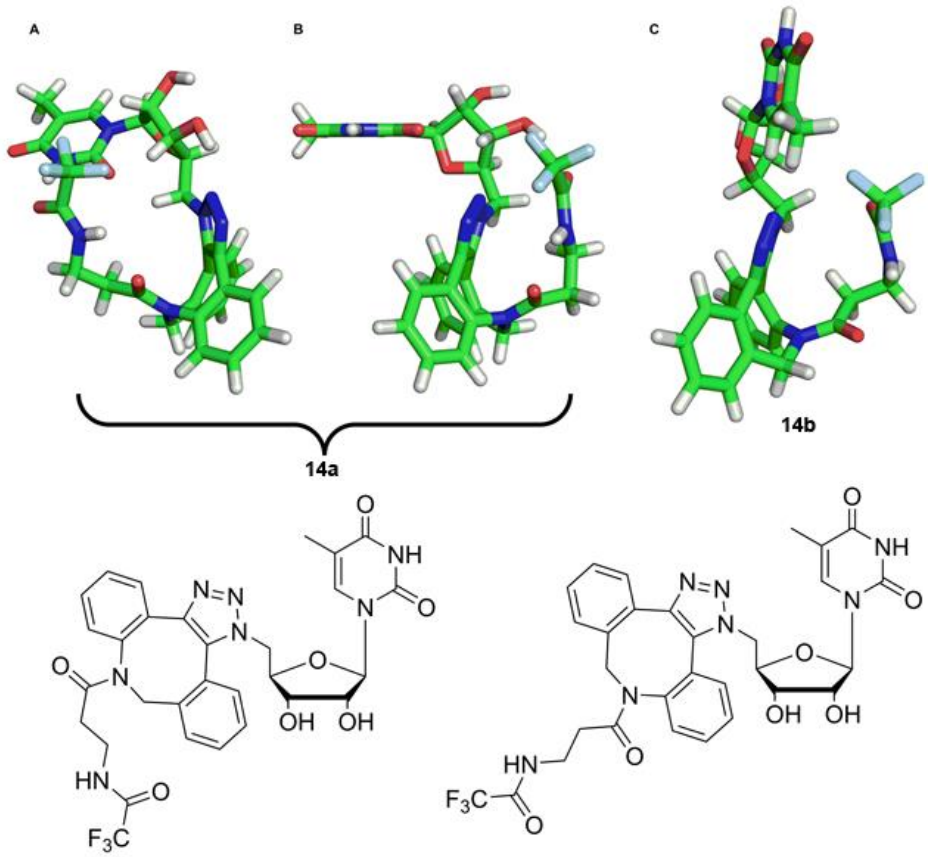


Figure 7: Possible orientation of the aliphatic chain exemplified by structures **14a** and **14b**.

In addition, the interactions between the 5-methyluridine and the aliphatic chain as well as the distinct intermolecular hydrogen bonds were observed. The most frequent conformer at the first local minima of derivative **14a** maintained the aliphatic chain in direct interaction with the pyrimidine ring. This interaction was enabled by a hydrogen bond between the carbonyl of trifluoroacetyl group and imide hydrogen of the pyrimidine ring (Figure 8 - 1). In the conformer representing the second most populated local minimum, the

trifluoroacetyl carbonyl group of the aliphatic chain interacted with the ribose OH hydrogen (Figure 8 - 2).[12]

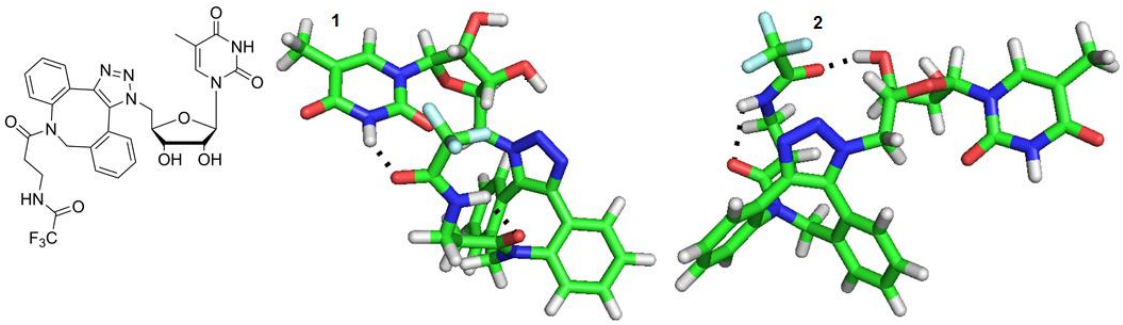


Figure 8: Two conformers 1 and 2 of derivative **14a** with different positions of the aliphatic chain.

Local minimum 1, in which the uracil carbonyl group directly interacts with the ribose hydroxyl group, predominated for derivative **14b** (Figure 9). In local minima 2-6, interaction of both ribose OH hydrogens with the trifluoroacetyl carbonyl group was observed.[12]

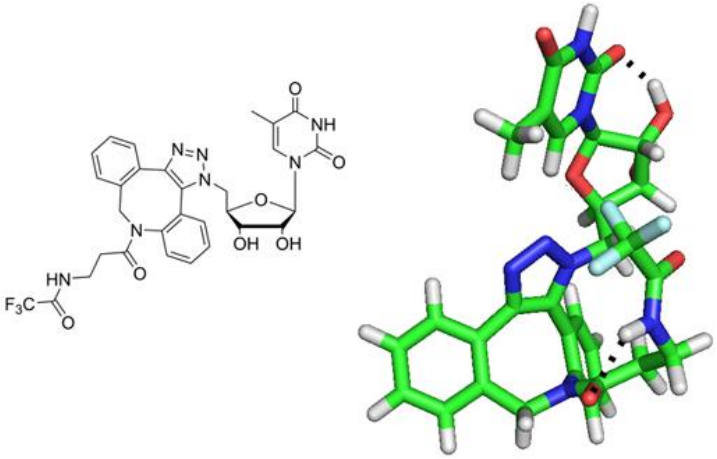


Figure 9: The most abundant conformation of derivative **14b**.

In derivative **15a**, in the conformer of the first local minimum, the aliphatic chain was located close to the pyrimidine ring; however, no hydrogen bond was observed between them (Figure 10-1). The conformer with local minimum 2 was characterized by the location of the pyrimidine part of the molecule distant from the aliphatic chain (Figure 10-2). Moreover, in the conformer with local minimum 3, an interaction between the pyrimidine imide group 3 and trifluoroacetyl carbonyl group 37 was detected (Figure 10-3).[12]

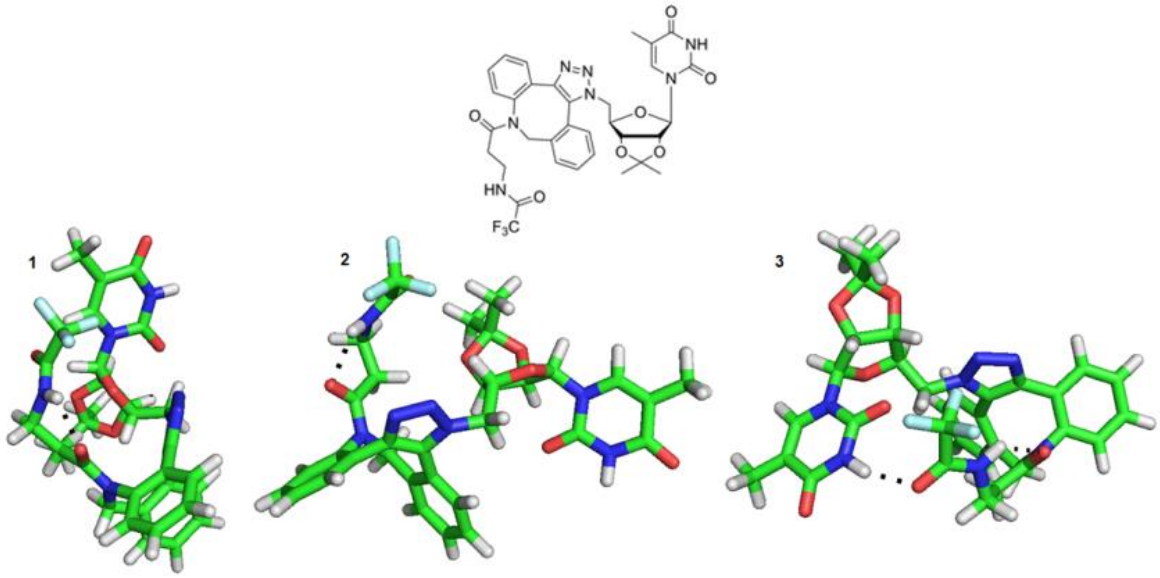


Figure 10: The most abundant conformers of derivative **15a**.[12]

The derivative **15b** was characterized by two predominant local minima with a total population of approximately 28%. These two local minima, which differed only slightly in their combination of dihedral angles, exhibited a close position of the aliphatic chain and pyrimidine ring, although no hydrogen bond was observed between them (Figure 11-1 and 2).[12]

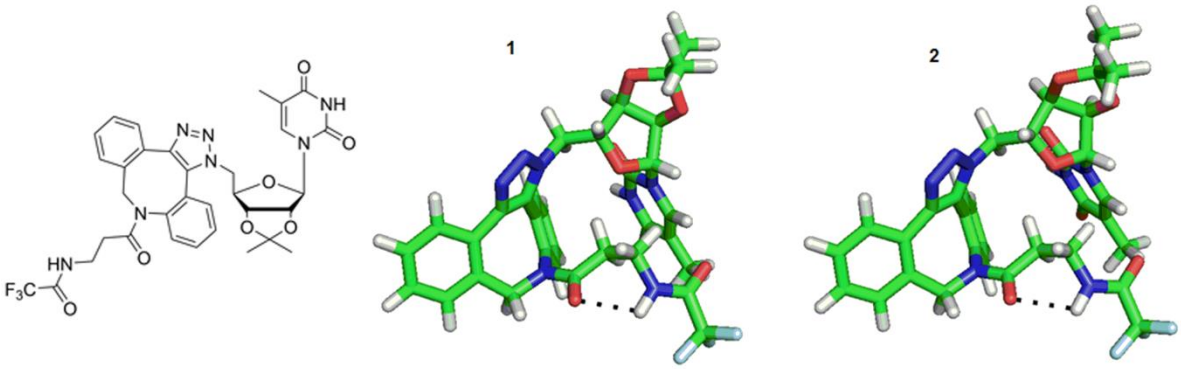


Figure 11: Conformation 1 and 2 of derivative **15b**.[12]

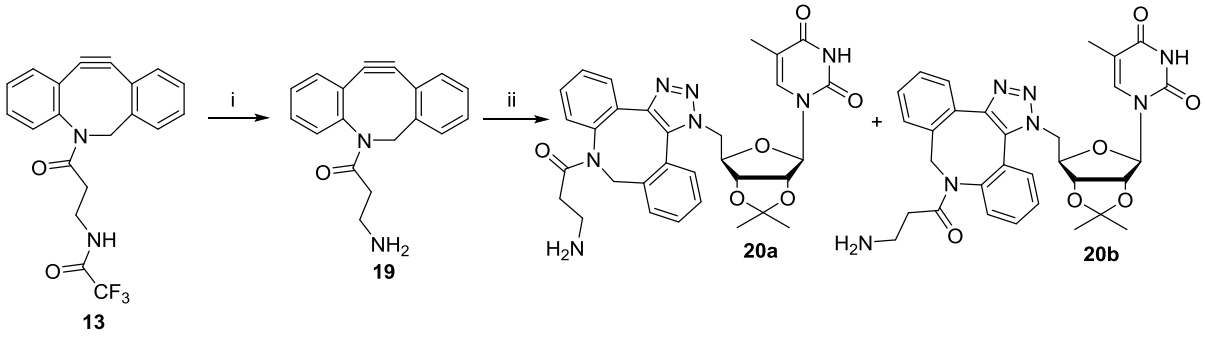
This conformational analysis using quantum mechanical investigations revealed that derivatives **14a/b** and **15a/b** can form more local minima on the PES in which different interactions between the aliphatic chain (bearing a fluorine atom), uracil and ribose were observed, consistent with the differentiation of signals in the ^{1}H and ^{19}F NMR spectra.[12]

3.1.1.1.5. Effect of aliphatic chain on formation of conformers

To determine whether the presence of conformers depends on the length of aliphatic chain attached to aza-dibenzocyclooctyne, triazoles of aza-dibenzocyclooctyne bearing only hydrogen, trifluoroacetyl or *β*-alanine on nitrogen were prepared.

Triazoles with unprotected *β*-alanine on aza-dibenzocyclooctyne nitrogen **20a,b** were prepared by reaction of azide **6** with deprotected aza-dibenzocyclooctyne **19** (Scheme 5). Triazoles **20a,b** were separated with use of semipreparative HPLC in 20% yield.

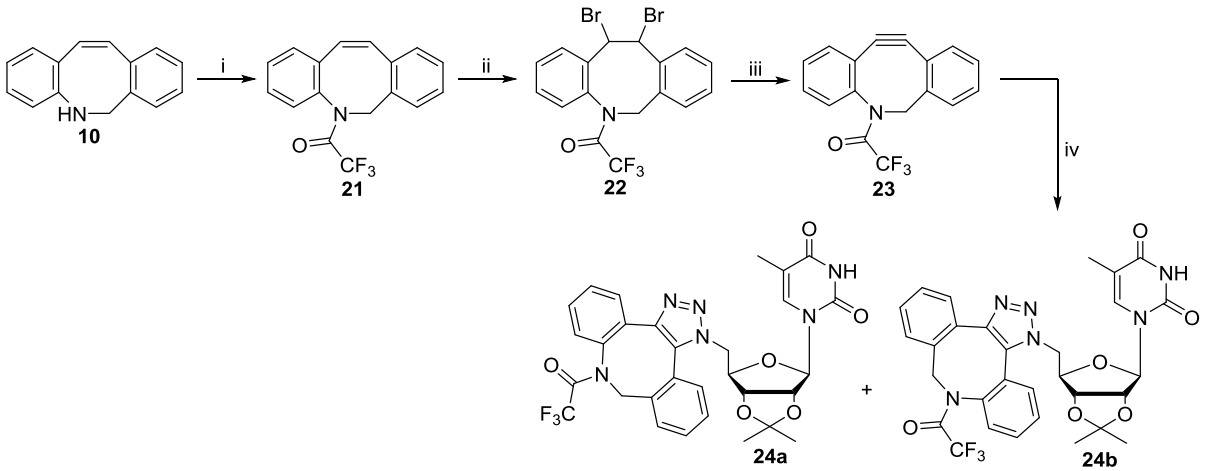
Scheme 5.: Preparation of triazoles **20a** and **20b**.[a]



[a]Reagent: (i) K_2CO_3, MeOH (ii) azide **6**, MeOH.

N-trifluoroacetyldibenzoazocine **21** was prepared by our developed procedure starting by trifluoroacetylation of dihydrodibenzoazocine **10** (Scheme 6). Double dehydrobromination resulted in desired alkyne **23** together with product of trifluoracetyl deprotection which was later identified as 6*H*-isoindolo[2,1-*a*]indole **25** (Scheme 7). This mixture was used for synthesis of traizoles **24a,b** which were successfully separated with the use of semipreparative HPLC.

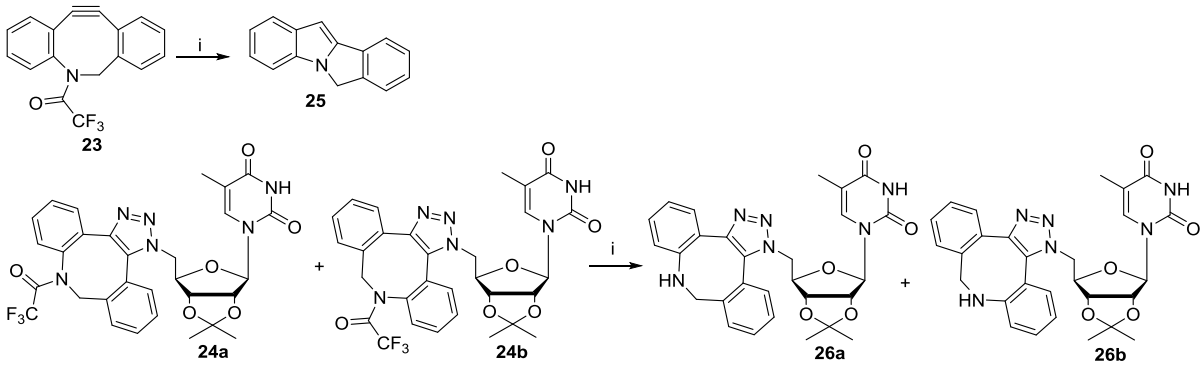
Scheme 6: Preparation of aza-dibenzocyclooctyne **23** and formation of triazoles **24a,b**.[a]



[a]Reagent: (i) TFAA, DCM (ii) pyridinium tribromide, DCM (iii) *t*BuOK, THF, 0 °C (iv) azide **6**, MeOH.

Next experiments were focused on preparation of triazoles with hydrogen on aza-dibenzocyclooctyne nitrogen. Direct deprotection of derivative **23** resulted in formation of 6*H*-isoindolo[2,1-*a*]indole **25** (Scheme 7). Due to this fact, desired triazoles **26a,b** were obtained by removal of trifluoroacetyl group from triazoles **24a,b** (Scheme 7).

Scheme 7: Preparation of triazoles **26a,b**.[a]



[a]Reagent: (i) K_2CO_3, MeOH.

^1H and ^{13}C NMR spectra of triazoles **20a,b**, **24a,b** and **26a,b**, showed, that number of isomers was not changed with reducing the aliphatic part of azocine. Similarly to compounds **15a**, triazoles **20a**, **24a** and **26a** exhibited presence of two isomers, triazoles **20b**, **24b** then exhibited presence of three isomers, in case of triazole **26b**, two isomers were formed. (Figure 12).

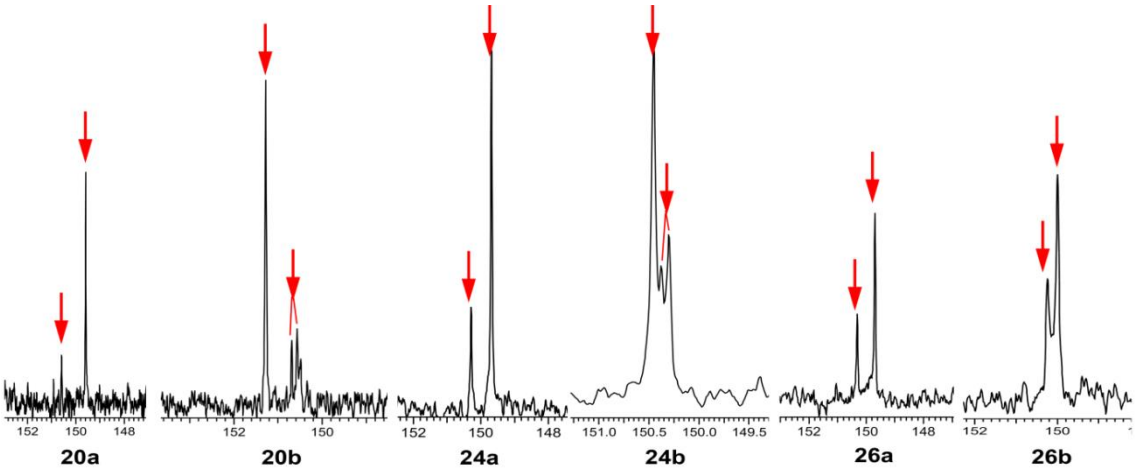


Figure 12: Isomery in ^{13}C Spectra.

These results indicated that the formation of conformers is not dependent on the length of aliphatic chain attached to aza-dibenzocyclooctyne nitrogen and that not only trifluoracetamido group is responsible for observed phenomenon.

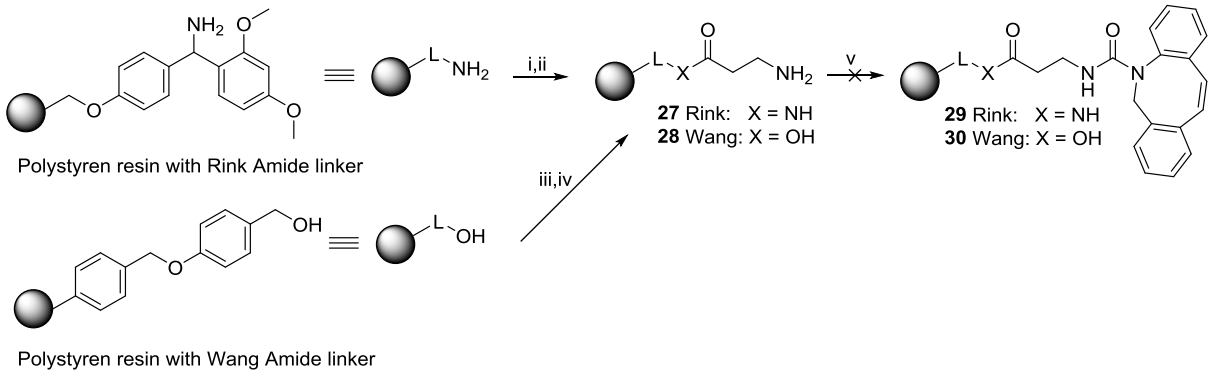
3.1.2. Solid phase synthesis of fluorescently labelled DIBAC analogues

For the synthesis of **DIBAC** system bearing the fluorescent dye the solid-phase synthesis was suggested. The advantage of this approach lies especially in possible application of combinatorial chemistry for synthesis of systems with various type and length of the linker as well as various types of dyes.

3.1.2.1. Immobilization of dihydrodibenzoazocine

The first approach for immobilization of dihydrodibenzoazocine was based on formation of urea linkage between solid supported *β*-alanine amino group and dihydrodibenzoazocine *via* CDI activation (Scheme 8). Unfortunately, reaction with CDI and azocine **10** to desired compound **29** or **30** completely failed.

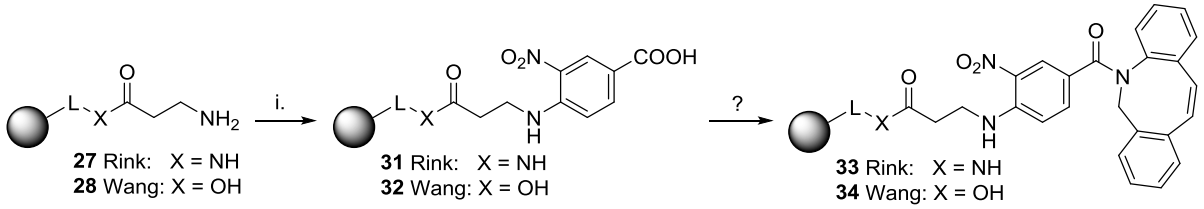
Scheme 8: Preparation of triazoles **26a,b**.[a]



[a]Reagent: (i) Fmoc-*β*-Ala-OH, HOBt, DIC, DMF, DCM (ii) = (iv) 50% piperidine in DMF (iii) Fmoc-*β*-Ala-OH, HOBt, DIC, DMAP, DMF, DCM (v) CDI, compound **10**, DCM.

In the next approach, the attention was paid to immobilization of dehydrodibenzoazocine **10** as an amide of suitable solid supported carboxylic acid. For this intention, 4-fluoro-3-nitrobenzoic acid was chosen as a building block. 4-fluoro-3-nitrobenzoic acid was attached quantitatively *via* nucleophilic substitution of fluorine atom to amine of *β*-alanine **27** or **28** immobilized to the Rink Amide resin or Wang resin (Scheme 9). In the next step, conditions for formation of amide **33** and **34** were investigated.

Scheme 9: Preparation of amides **33** and **34**.[a]



[a]Reagent: (i) 4-fluoro-3-nitrobenzoic acid, pyridine, 75 °C.

Firstly, amide bond formation with use of coupling reagents was tested. Although a number of conditions included different solvents, temperatures, bases and coupling reagents have been tested (Table 2), immobilization of azocine **10** by this way was unsuccessful.

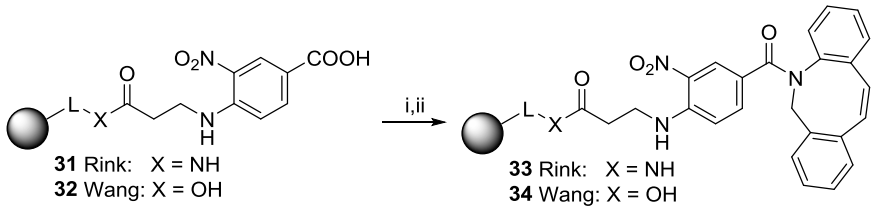
Table 2: Conditions for formation of amides **33** or **34**.

Reagents		Base	Solvent	Temperature	[%][a] 31 or 32	[%][a] 33 or 34	[%][a] dimethylamide
HOBt	DIC	-	DCM/DMF	rt	> 99	< 1	< 1
HOBt	DIC	DMAP	DCM/DMF	rt	~85	~5	< 1
HOBt	DIC	DMAP	DMF	40 °C	~75	~5	~10
HOBt	DIC	DMAP	DMF	80 °C	~15	~10	~65
HOBt	DIC	DMAP	DMSO	80 °C	~60	~25	< 1
HOBt	DIC	DMAP	DMSO	120 °C	~65	~10	< 1
HOBt	EDC	TEA	DMF	rt	~80	~5	< 1
HOBt	EDC	TEA	DMF	40 °C	~65	~5	~15
HOBt	EDC	TEA	DMF	80 °C	~55	< 1	~30
HOBt	EDC	DIEA	DMF	rt	~70	< 1	< 1
HOBt	EDC	DIEA	DMF	40 °C	~60	~10	~25
HOBt	EDC	DIEA	DMF	80 °C	~30	~5	~30
BOP	DIC	DMAP	DCM/DMF	rt	> 99	< 1	< 1
BOP	DIC	DMAP	DMSO	80 °C	> 99	< 1	< 1
BOP	DIC	-	DMF	rt	> 99	< 1	< 1
BOP	DIC	-	DMF	40 °C	50	< 1	~25
BOP	DIC	-	DMF	80 °C	< 1	< 1	~90
BOP	-	DIEA	DMF	rt	> 99	< 1	< 1
BOP	-	DIEA	DMF	40 °C	55	< 1	~20
BOP	-	DIEA	DMF	80 °C	10	< 1	~85
PyBOP	-	DIEA	DMF	rt	75	< 1	< 1
PyBOP	-	DIEA	DMF	40 °C	30	< 1	< 1
HATU	-	DIEA	DMF	rt	55	< 1	< 1
HATU	-	DIEA	DMF	40 °C	40	~5	~40

[a]Calculated form LC traces at 200 – 600 nm.

As the use of coupling reagents has been proven to be inefficient, the solid supported nitrobenzoic acid was converted to chloride and reacted with amine **10** (Scheme 10). With use of this method the desired product was obtained quantitatively in both cases. An excess of a base, such as DIEA, has to be used in chlorination step to avoid of undesired cleavage of material from resin by HCl forming during reaction.

Scheme 10: Preparation of amides **33** and **34**.[a]



[a]Reagent: (i) $SOCl_2$, DIEA, anhydrous DCM (ii) amine **10**, pyridine, anhydrous DCM.

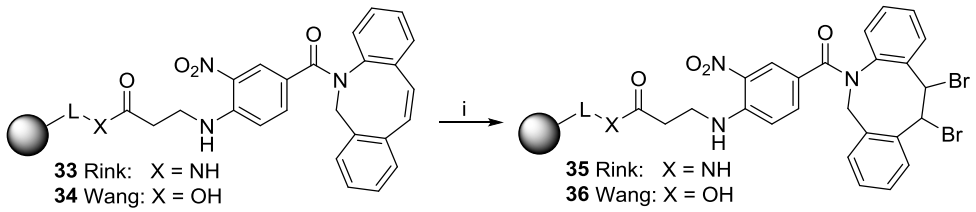
3.1.2.2. Triple bond formation

Formation of triple bond was suggested as two step synthesis based on bromination of azocine double bond followed by base mediated double dehydrobromination. This method was inspired by literature, where this sequence was performed in solution.[11]

Bromination of double bond

In the first experiments, conditions for solution-phase synthesis using pyridinium tribromide as a bromination agent were tested. Unfortunately quantitative cleavage of substrate from the resin during reaction was observed. Nevertheless, the addition of DIEA to reaction mixture resulted in quantitative formation of dibromoderivatives **35** and **36** (Scheme 11).

Scheme 11: Preparation of dibromoderivatives **35** and **36**.[a]



[a]Reagent: (i) pyridinium tribromide, DIEA, DCM.

Formation of triple bond

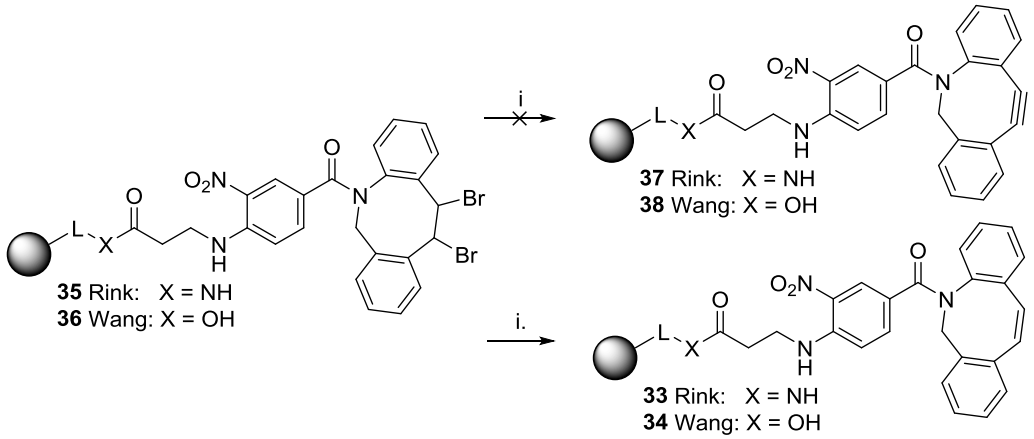
In the first experiments, *t*BuOK in different concentrations was tested. On Wang resin, partial dehydrobromination was observed after one hour and monobromalkene was observed as a main product. Unfortunately increase of reaction time led to progressive decomposition of material on resin. The same results were obtained also at low temperatures (- 30 °C). The use of these conditions on Rink resin resulted always in formation of mixture of compounds indicating decomposition of material on resin.

After that, set of organic bases (DIEA, DBU, proton sponge and BTTP) at different temperatures and solvents (anhydrous THF, DMF, DMSO, celosolve) were used. In case of material attached to Wang resin, the best results were obtained for proton sponge in DMSO, where the reaction proceeded with 98% conversion with purity of the product 89% (Scheme 12).

The similar results were obtained also for Rink resin. In this case, the best result with 94% purity of product was obtained after treatment of **35** with BTPP in DMSO at 60 °C (Scheme 12). After optimization of the last step, the final products were prepared in preparative scale, cleaved from resin, purified with use of semipreparative HPLC and

characterized. Surprisingly, ^{1}H and ^{13}C NMR spectra of both products did not confirm the alkyne structure. Instead of alkynes **37** and **38**, alkenes **33** and **34** were obtained after elimination step (Scheme 12), what indicates, that the dehalogenation instead of double dehydrohalogenation proceeds.

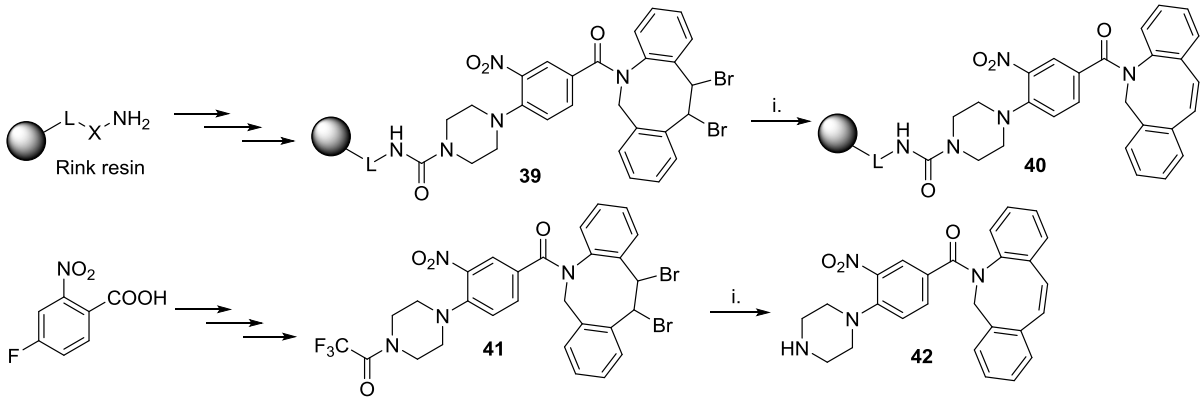
Scheme 12: Elimination of dibromoderivatives **35** and **36**.[a]



[a]Reagent: (i) Wang: proton sponge, DMSO, 60 °C; Rink: BTPP, DMSO, 60 °C.

To determine, if this course of elimination takes place only on solid phase or also in solution, the same dibromoderivatives were prepared by multistep synthesis (Scheme 13) and elimination in solution was tested. Alkene **40** was obtained after treatment of resin supported derivative **39** by BTTP or proton sponge, which corresponded to results received previously. In solution, both bases firstly induced removal of trifluoroacetyl group from piperazine followed by formation of deprotected alkene **42** (Scheme 13). These results confirmed preference of bases induced dehalogenation instead of dehydrohalogenation.

Scheme 13: Model dibromoderivatives **39** and **41** and products of elimination.[a]

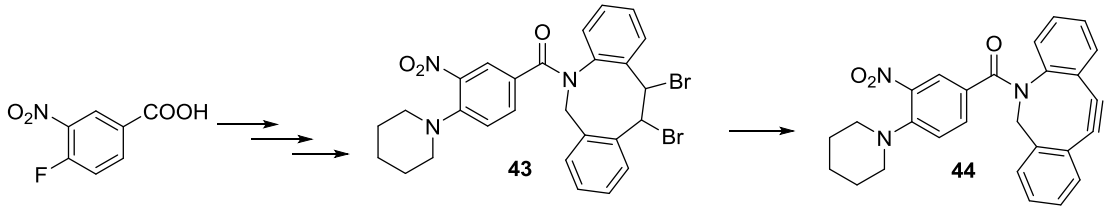


[a]Reagent: (i) proton sponge, DMSO, 60 °C or BTPP, DMSO, 60 °C.

Because it was necessary to find other conditions, elimination of piperidine analogue of compound **41** (to avoid of side reactions, such as deprotection) was studied with use of

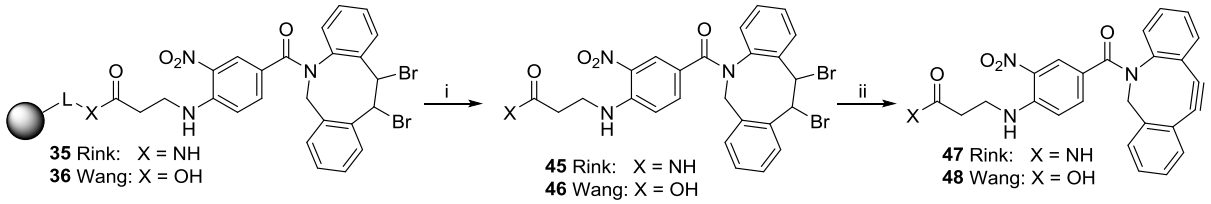
different bases (Scheme 14). Desired alkyne **44** was obtained only after treatment of **43** by *t*BuOK at room temperature. The bases as NaOH and $Si(OMe)_3K$ were efficient only at 80 °C, but other compounds and impurities were formed during this reaction.

Scheme 14: Preparation of alkyne **44**.



As a result, *t*BuOK was found to be the best base for formation of desired triple bond. However, on solid phase, decomposition of material on resin was observed (see above) after cleavage. Alkynes **47** and **48** were finally prepared by *t*BuOK in solution after cleavage of dibromoderivative **35** or **36** from resin (Scheme 15). In case of carboxylic acid derivative **48**, the product was obtained in 70 % purity; however the amide **47** was formed only in 25% purity. For this reason, synthesis of more complex compounds was done only on Wang resin.

Scheme 15: Preparation of azadibenzocyclooctynes **47** and **48**.[a]



[a]Reagent: (i) 50% TFA in DCM (ii) *t*BuOK, anhydrous THF.

3.1.2.3. Attachment of fluorescent label

After successful optimization of triple bond formation, the structure could be extended to fluorescent label. For this purpose, Rhodamine B (RhoB) was chosen as a label. Amino group obtained by reduction of nitro group of derivative **48** should have served for attachment of RhoB via aliphatic linker or RhoB itself (Figure 13).

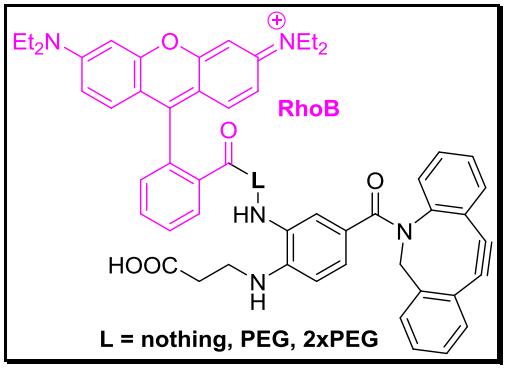
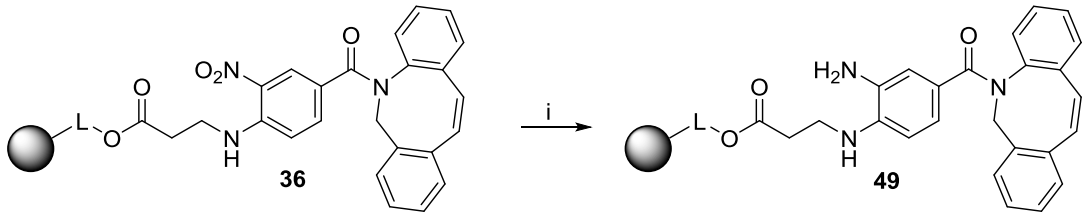


Figure 11: Structures of desired aza dibenzocyclooctynes with fluorescent label.

Reduction of nitro group

A methodology using sodium dithionite and phase transfer catalyst was chosen for reduction of nitro group. In case of ethyl viologen diiodide as phase transfer catalyst, the desired product was obtained nearly quantitatively (Scheme 16).

Scheme 16: Preparation of aminoderivative **49**.[a]

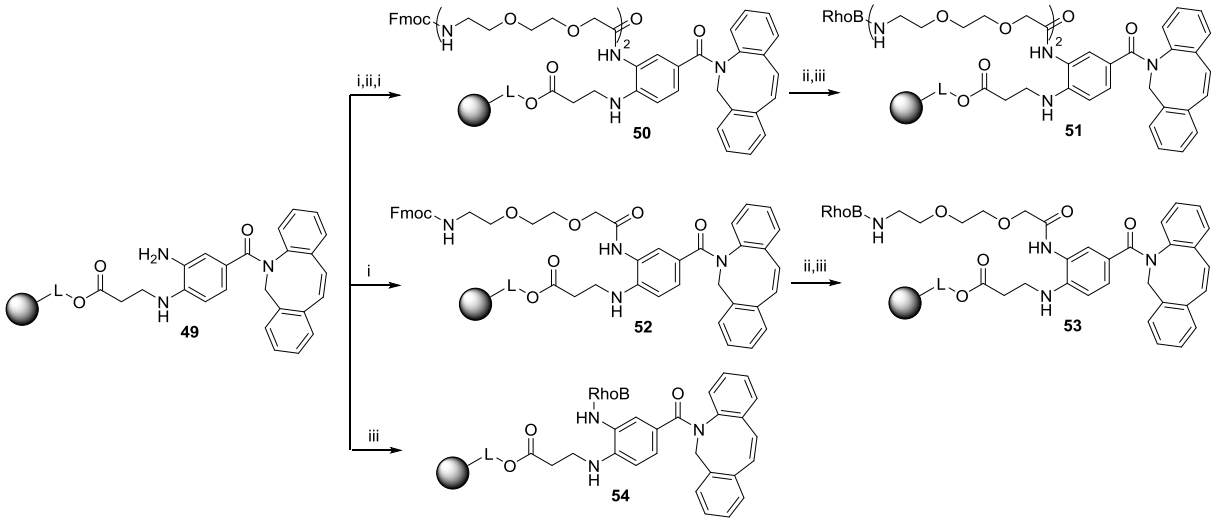


[a]Reagent: (i) Sodium dithionite, K_2CO_3, ethyl viologen diiodide, DCM/H_2O 1:1.

Pegylation of aminogroup and attachment of RhoB

For attachment of RhoB through aliphatic linker, amino group was acylated by Fmoc-*N*-PEG-COOH *via* HOBt/DIC method (Scheme 17). Desired products **50** and **52** were formed, however a side product with mass 18 units less were also occurred in significant amount. This product was probably a result of some kind of cyclization in obtained structure. After removal of Fmoc group from PEG linkers, RhoB was attached also *via* HOBt/DIC method using DMAP as a catalyst (Scheme 17). Compound **54** was obtained nearly quantitatively. Products **51** and **53** were formed as a mixture with cyclized side products analogically to previous step.

Scheme 17: Preparation of RhoB derivatives **51** and **53**, **54**.[a]

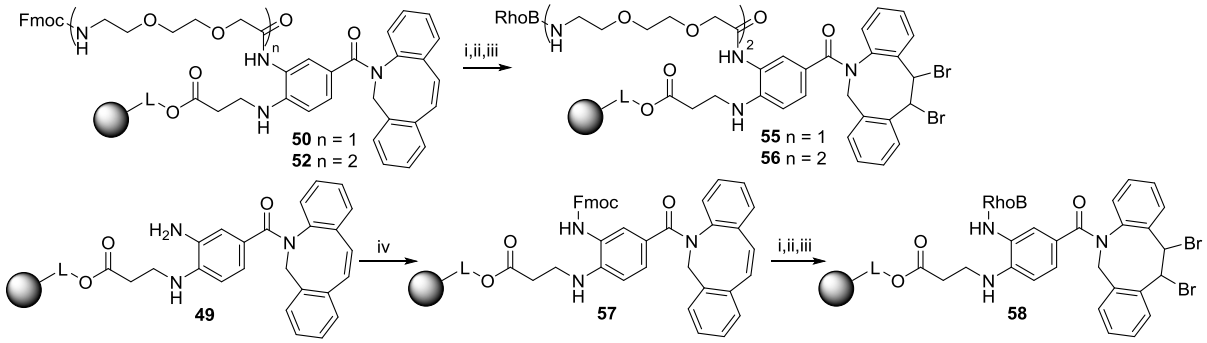


[a]Reagent: (i) FmocNH-PEG-COOH, HOBt, DIC, DIEA, DMF/DCM 1:1 (ii) 50% piperidine in DMF (iii) RhoB, HOBt, DIC, DMAP, DMF/DCM 1:1.

Bromination and formation of triple bond

Bromination of resin **51**, **53** and **54** was performed under standard conditions with pyridinium tribromide. Although desired product was obtained, its purity was decreased by presence of number of side products. For this reason, the reaction sequence was slightly modified. Quantitative bromination was carried out before rhodamine binding, it means on substrates **49**, **50** or **52** respectively. Acylation with RhoB was performed after that and desired products were obtained approximately in 70% purity (Scheme 18).

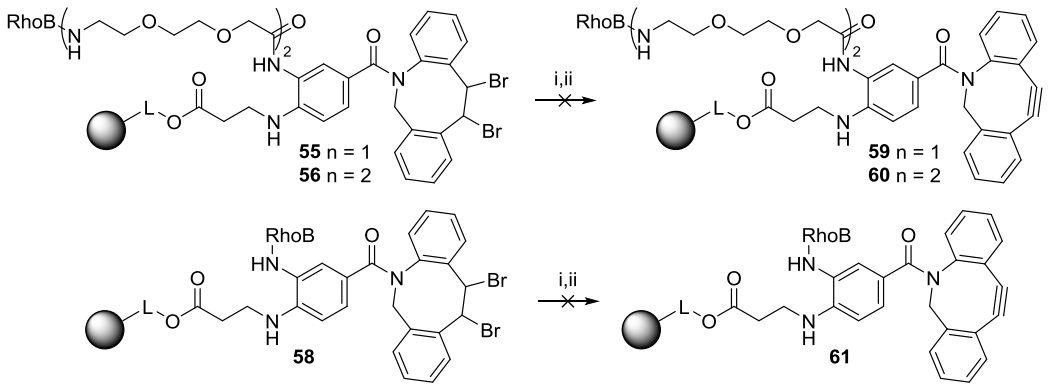
Scheme 18: Preparation of RhoB derivatives **55, 56** and **58**.[a]



[a]Reagent: (i) pyridinium tribromide, DIEA, DCM (ii) 50% piperidine in DMF (iii) RhoB, HOBt, DIC, DMAP, DMF/DCM 1:1 (iv) Fmoc-Cl, DIEA, DCM.

Then the dibromoderivatives were cleaved from resin, all solvents were evaporated and *t*BuOK in anhydrous THF was added to obtained final alkynes. Unfortunately, cleavage of RhoB from all molecules occurred during reaction followed by total decomposition (Scheme 19). Changing of RhoB to other molecules such as coumarin-3-carboxylic acid or simple benzoic acid to compare the stability resulted in the same results. Thus it was not possible to prepare desired alkynes **59 - 61** or the analogues with another dye by this methodology.

Scheme 19: Preparation of RhoB derivatives **59 - 61**.



[a]Reagent: (i) 50% TFA in DCM (ii) *t*BuOK, anhydrous THF.

Although the synthesis of aza-cycloalkynes for SPAAC was unsuccessful, the alkene intermediates can be utilized for different type of biorthogonal reaction such as alkene ligation. For this reason, resin bound alkenes **51**, **53** and **54** were prepared in preparative scale, cleaved from resin, purified with use of semipreparative HPLC and characterized. According to LC/MS analysis, cyclized form of desired products from **51** and **53** (Figure 14) predominated. The final structure of obtained compounds was determined according to NMR spectra, which also showed that RhoB is attached in closed form in all cases (Figure 14).

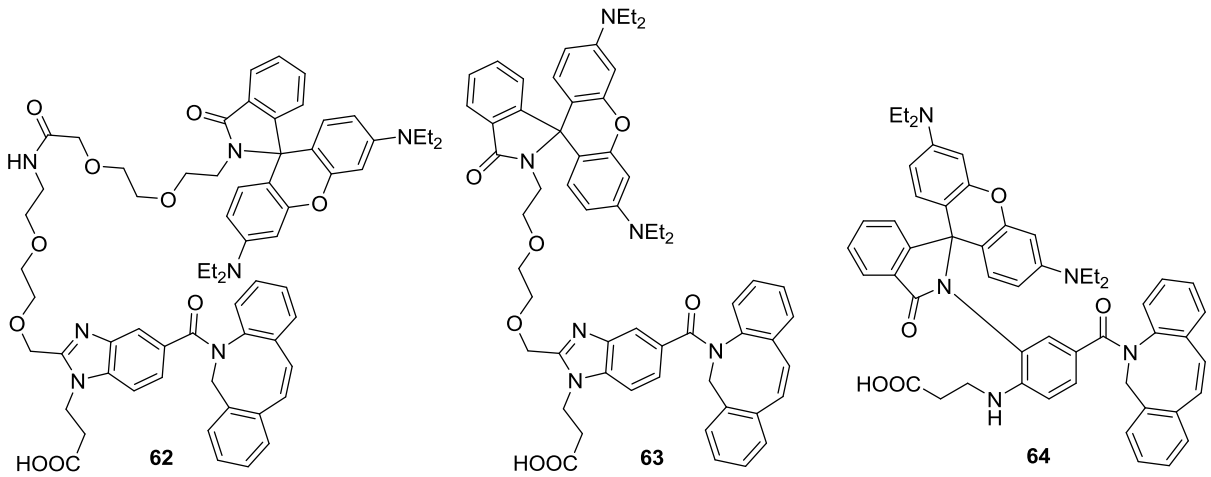


Figure 14: Structures of **62**, **63** and **64** determined by NMR spectra.

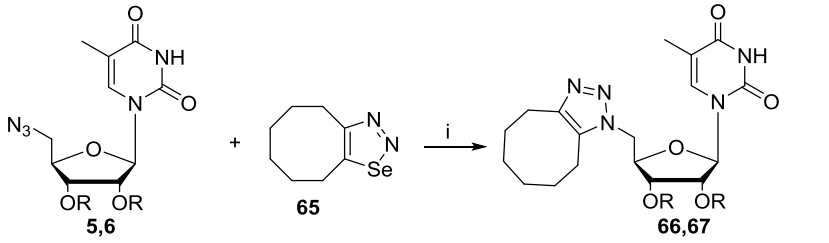
3.2. 1,2,3-selenadiazoles

This part of work is focused on thermally, microwave assisted and photochemically induced conjugation reaction of azides and cycloalka-1,2,3-selenadiazoles as a precursors of cycloalkynes. First part deals with reactions of cycloocta[d][1,2,3]selenadiazole. Other parts are focused on preparation of new cycloalka-1,2,3-selenadiazoles and their application in reactions with azides.

3.2.1. Reactions of cycloocta[d][1,2,3]selenadiazoles

Within author´s diploma theses was found out, that cycloocta[d][1,2,3]selenadiazole **65**, prepared according to described procedure,[14] heated in mixture with azide **5** and **6** can form corresponding triazole **66** and **67** (Scheme 20).[10] However, to perform the reaction, the high temperature has to be used. The decreasing of temperature led to significant increase of reaction time (Table 3).[10]

Scheme 20: Reactions of azides **5** and **6** with 1,2,3-selenadiazole **65**.[10,a]



[a]Reagent: (i) NMP, ΔT.

Table 3: Reaction times for full conversion of azides at different temperatures.[10]

	FULL CONVERSION OF AZIDE				
T (°C)	180	150	130	110	90
TIME	10 min	1 h	20 h	20 h	48 h

3.2.1.1. Microwave assisted conjugation reactions of cycloocta[d][1,2,3]selenadiazole

Due to long reaction time required for decomposition of cycloocta[d][1,2,3]selenadiazole **65** at temperatures below 110 °C, the use of microwave irradiation was tested to accelerate the reaction. In the first experiment, the mixture of azide **5** or **6** and 1,2,3-selenadiazole **65** (1.5 equiv.) in NMP was used. The temperature was set to 110 °C and conversion of azide was monitored using HPLC/MS each ten minutes. The effect of MW power and also effect of reaction mixture concentration was compared.

In comparison to standard heating discussed above, the significant decrease of reaction time to approximately 2 hours at 110 °C was observed if microwave was used. Surprisingly no difference among used microwave power and concentration of reaction mixture occurred. On the other hand effect of excess of cycloocta[d][1,2,3]selenadiazole **65** was observed. The results showed, that increase in amount of 1,2,3-selenadiazole **65** to 3 or 4 equivalent led to decrease in time nearly to half (70 min).

Finally, the optimal conditions were chosen (2 mg/ml of azide **5** or **6**, 3 equivalents of cycloocta[d][1,2,3]selenadiazole, 150 W) and used for study of this reaction at lower temperatures. The reaction conversion was monitored after 70 minutes. Obtained values were compared with full conversion at 110 °C (Table 4). Similarly to results described above, the temperatures below 110 °C dramatically reduced the conversion of azide, thus the reaction time required for its full transformation to desired triazole would be non-acceptably long.

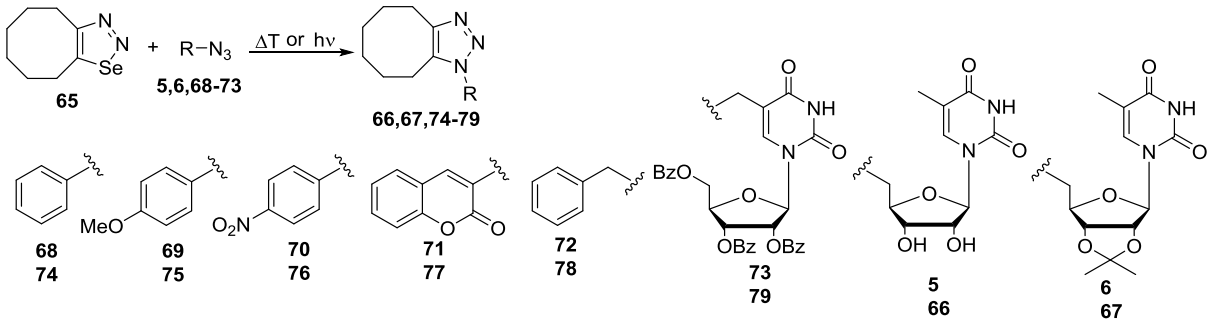
Table 4: Conversion of azides at different temperatures.

T (°C)	110	90	70	50
Conversion after 70 min (%)	100	25	15	8

3.2.1.2. Photochemically induced conjugation reactions of cycloocta[d][1,2,3]selenadiazole

Photochemically-induced noncatalyzed conjugation reaction of cyclooctyne generated in situ from cycloocta[d][1,2,3]selenadiazole **65** with structurally different azides **5**, **6**, **68-73** (Scheme 21) was studied.[15] The azides represent substrates possessing different electronic and steric properties.[15]

Scheme 21: Non-catalyzed conjugation reactions of cycloocta[d][1,2,3]selenadiazole **65**.[15]



The irradiation wavelength had to be carefully chosen for each pair of reactants. Althpugh the absorption spectrum of the cycloocta[d][1,2,3]selenadiazole **65** partially overlaps with those of the azides **5**, **6**, **68-73** as well as the photoproducts **66**, **67**, **74-79**, the absorption tail of the starting material **65** allowed us to use irradiation wavelengths in range approximately 300-325 nm (Figure 15).[15]

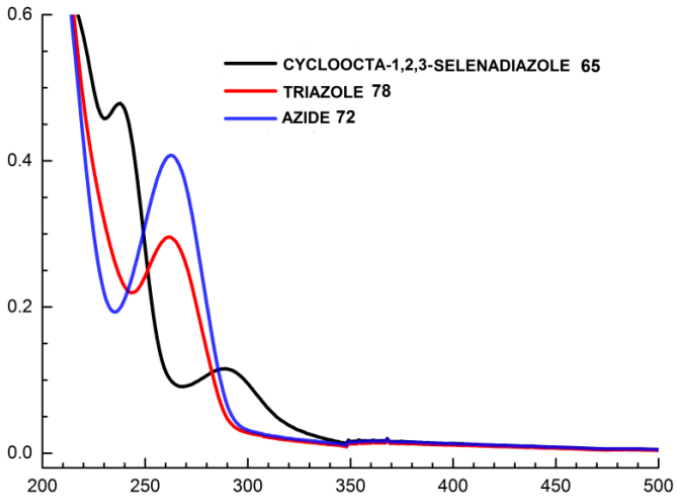


Figure 15: Absorption spectra of selenadiazole **65**, azide **72** and triazole **78** in methanol.[15]

The benzylazide **72** has been selected as a reagent for an initial photochemical reaction study because it shows no absorption above 300 nm. During the irradiation of equimolar amounts of **65** and **72** in methanol ($c = 3 \times 10^{-3}$ mol dm^{-3}) at 313 nm, a red

solid precipitated. According to UV/VIS spectrum having absorption maximum at λ_{abs} of approximately 500 nm, the solid was identified as selenium nanoparticles with a diameter of approximately 180 nm[16] (Figure 16), which may become an important internal optical filter.[15]

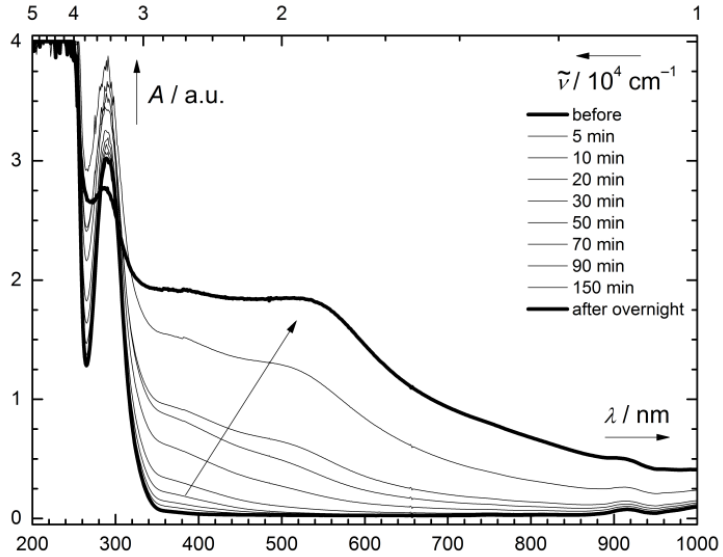


Figure 16: Change of absorption spectra of 1,2,3-selenadiazole **65** and benzylazide **72** in methanol upon irradiation with medium-pressure Hg arc filtered through a 313 nm filter.[15]

Upon exhaustive irradiation, triazole **78** was found to be the major product formed in a 28% yield, whereas 62% of azide **72** remained unreacted in the solution according to GC/MS analysis. Due to the thermal instability of the 1,2,3-selenadiazoles,[17] GC/MS could not be used to determine the concentration of the unreacted derivative **65**. Therefore, NMR was used to monitor reaction progress.[15]

Upon irradiation of a solution of **65** (c ~ 0.03 mol dm^{-3}) and **72** (c ~ 0.03 mol dm^{-3}) in methanol-d_4 in a NMR tube (Pyrex glass; λ > 280 nm) by a medium-pressure Hg lamp for 2 h, the conversion reached 38%, and the triazole **78** was a major product.[15] The product formation was evident from appearance of the new triplet signals at 2.81 and 2.94 ppm assigned to the methylene group of the triazole derivative and a decreased signal intensity for the methylene groups of 1,2,3-selenadiazole **65** at 3.32 ppm (Figure 17). The ^{1}H NMR spectrum of the product obtained by irradiation corresponds to that of the isolated triazole **78** prepared by thermolysis.[15]

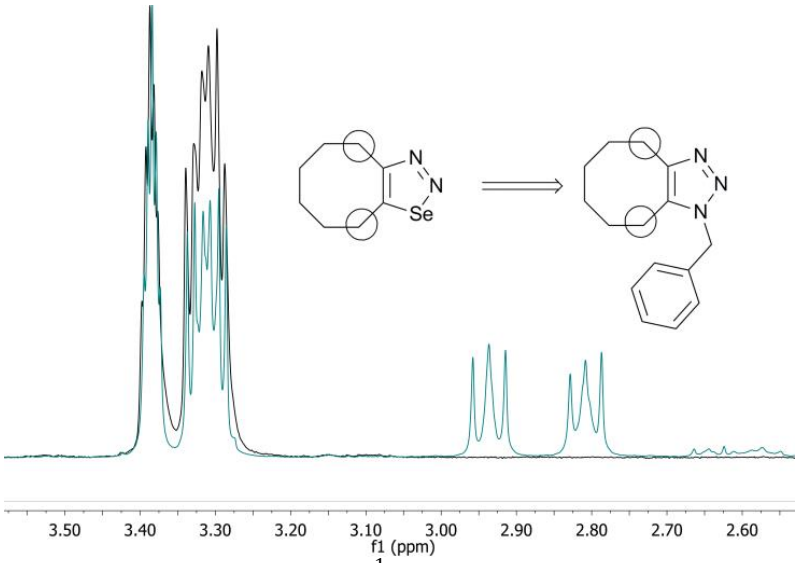


Figure 17: A detail of the ^{1}H NMR spectra of a mixture of 1,2,3-selenadiazole **65** and triazole product **78** before irradiation (black line) and after 2 h of irradiation (blue line).

To detect cyclooctyne as a possible primary photoproduct, irradiation of **65** in methanol-d_4 (c ~ 0.04 mol dm^{-3}) was performed for 1 h.[15] The ^{1}H NMR showed the signals at 1.64 and 2.10 ppm, what corresponds to the spectrum of cyclooctyne reported in the literature.[18] A new signal in the ^{13}C NMR spectrum at 93.8 ppm and three signals in the range of 20–35 ppm (Figure 18) were also attributed to cyclooctyne in accordance with the published data.[19]

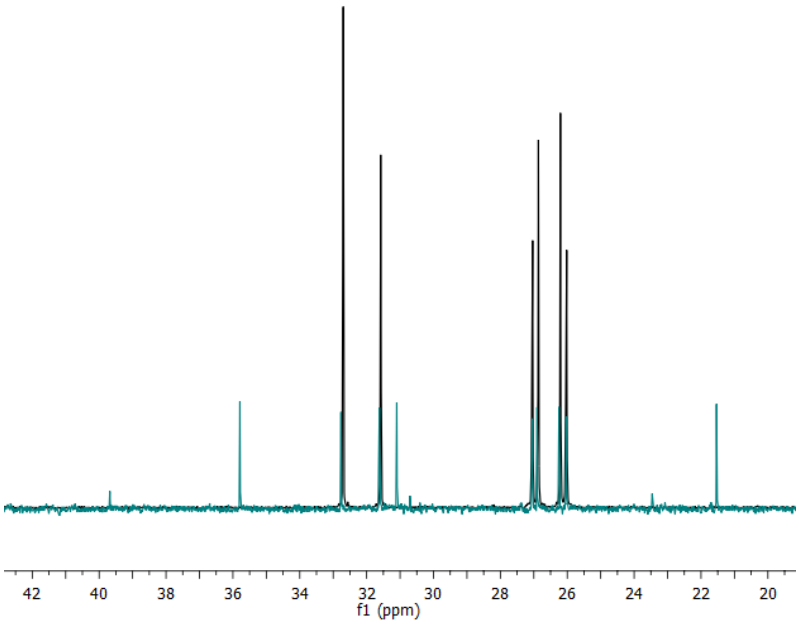


Figure 18: A detail of ^{13}C NMR spectra of cycloocta-1,2,3-selenadiazole **65** before (black line) and after irradiation (blue line) with a medium-pressure Hg arc filtered through a Pyrex glass of the NMR tube.[15]

The cyclooctyne half-life in the mixture with azide **72** was estimated to be approximately 40 min).[15] The quantum yield[‡‡] of triazole **78** formation upon irradiation of a methanolic solution of the 1,2,3-selenadiazole **65** (c = 1.9 × 10^{-3} mol dm^{-3}) in the presence of **72** (c = 6.1 × 10^{-3} mol dm^{-3}) at λ_{irr} = 313 nm was determined to be Φ (**78**) = 0.10 ± 0.05 using valerophenone[20,21] as an actinometer.[15]

To determine the multiplicity of the excited state involved in the cyclooctyne formation, the influence of oxygen as a triplet quencher on the reaction rate was studied.[15] Degassed, aerated and oxygenated solutions of **65** and **72** in methanol-d_4 were irradiated using a medium-pressure Hg lamp in an NMR tube. The changes of the concentration of **65** at different reaction conversions were followed by ^{1}H NMR. The presence of oxygen evidently, but not significantly, slowed down the reaction, thus the productive triplet-excited state lifetime must be relatively short and its reaction competes with a diffusion-limited quenching, or the singlet state is also involved in the reaction.[15]

In addition, sensitization of **65** by triplet-excited benzophenone was performed to find whether the same process occurs in the triplet manifold. Irradiation of a degassed solution of **65**, **72** and benzophenone in methanol resulted in the formation of **78** (Table 5) in substantially higher yields compared to those observed when the same reaction mixture was purged with oxygen.[15] Table 5 also demonstrates that oxygen quenched the sensitized production of **78**. Such a result supports our assumption that **78** is produced at least partially from triplet-excited **65**.[15]

Table 5. Sensitization of **65**[a]

Irradiation time (h)	Yield of[a] **78**/%	
	O_2[b]	N_2[c]
2	4	7
16	24	41

[a] **65** (c ~ 0.03 mol dm^{-3}), **72** (c ~ 0.03 mol dm^{-3}) and benzophenone (c ~ 0.3 mol dm^{-3}) in methanol-d_4 irradiated at 366 nm; the yields were determined by ^{1}H NMR. [b] Sample was purged with oxygen for 10 min. [c] Sample was purged with nitrogen for 10 min.[15]

[‡‡] Estimation of cyclooctyne half-life and quantum yield was performed by Peter Šebej from Department of Chemistry and RECETOX, Faculty of Science, Masaryk University, Brno, Czech Republic; Present address: Department of Chemistry, Columbia University, New York, USA.

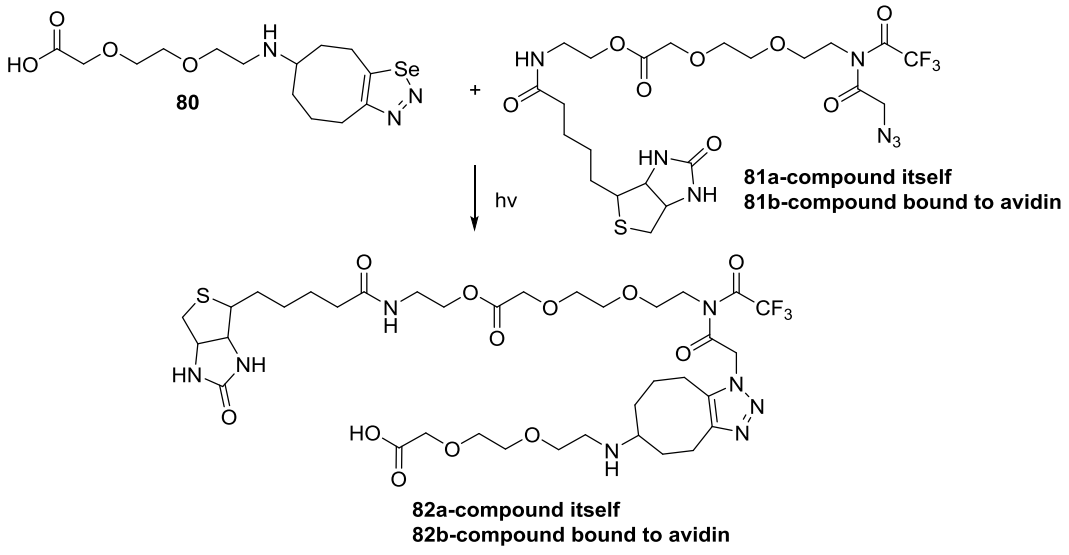
The photolysis of an equimolar mixture of **65** and the corresponding azide **5**, **6**, **68-71**, **73** (c ~ 0.03 mol dm^{-3}) was carried out afterwards, and the reaction conversion was monitored by NMR.[15] The lowest triazole yield (6-8%) was observed for 3-azidocoumarine **71**, which has been reported to be photolabile.[22] For the other azides **68-70** and **73**, triazole yields were 11−39%. Further irradiation did not increase the conversion, which may indicate a role of selenium as an internal filter and potential side photoreactions of the azides known from literature.[23-25]

To enhance the reactivity of cycloocta[d][1,2,3]selenadiazole **65** by avoiding inadvertent photolysis of the azides, a solution of **65** in methanol-d_4 was initially irradiated for 1 h to form cyclooctyne, and then an equimolar amount of the corresponding azide **5**, **6**, **68-71**, **73** was added.[15] Subsequently, the mixture was kept in the dark, and the reaction conversions were monitored by NMR. Surprisingly, the triazole yields were similar or lower compared to those previously determined. The only exception was the reaction of azide **71**, where the post-irradiation addition of this azide prevented its photodecomposition and the total yield of triazole increased.[15]

To demonstrate the applicability of cycloocta[d][1,2,3]selenadiazole photoconjugation reaction with azides, labeling of biomolecules was studied.[15]

First we prepared a modified derivative of cycloocta-1,2,3-selenadiazole **80** having a PEG moiety for its better water solubility and the azidobiotine **81a** (Scheme 22) as model reaction partners and performed the photoinduced conjugation.[15]

Scheme 22: Conjugation reaction of modified cycloocta-1,2,3-selenadiazole **80** and appropriate azido derivatives **81a** and **81b** .[15]



The reaction mixture was irradiated with a medium-pressure Hg arc filtered through the Pyrex glass ($\lambda > 280$ nm) of an NMR tube for 1 h. The ^{1}H NMR analysis showed the formation of new signals at approximately 5.80 ppm (Figure 19) and a decrease in the signal intensity of azidomethylene group in the starting material **81a** at approximately 3.42 ppm. The MS spectrum of the reaction product provided evidence for the formation of triazole **82a**.[15]

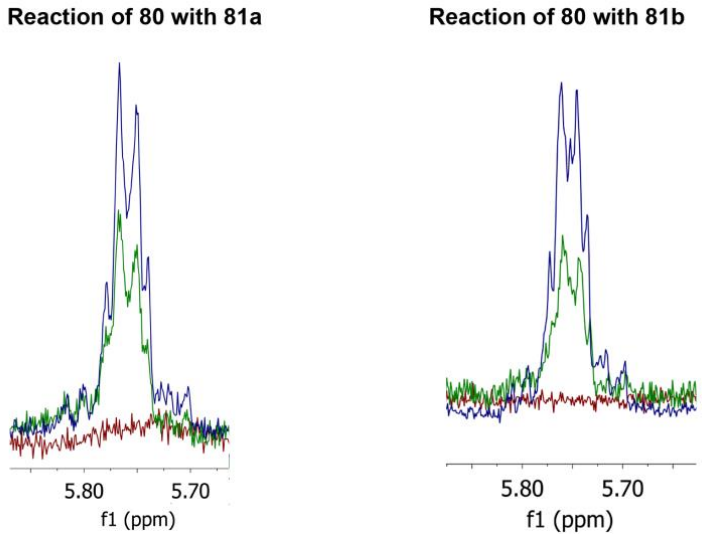


Figure 19: A details of the ^{1}H NMR spectra of the mixture of modified biotin **81a/81b** with cycloocta-1,2,3-selenadiazole **80** before reaction (red line), after 1h irradiation (green line) and subsequent 1h irradiation (blue line).

The reactivity was then tested on modified protein **81b** as a biomolecule representative. Complex **81b** was prepared by mixing of avidin with modified biotin **81a** ($c \sim 0.013$ mol dm^{-3}).[15] The concentration of **81a** was higher than corresponds to the binding capacity of avidin to allow monitoring of the reaction course by an NMR analysis of the non-bound biotinylated azide **81a** present in reaction mixture in sufficient concentration. After the addition of 1,2,3-selenadiazole **80**, the reaction mixture was irradiated with a medium-pressure mercury arc filtered through the Pyrex glass of an NMR tube for 1 h. In ^{1}H NMR spectrum we observed the same changes as in reaction without avidin (Figure 19).[15]

Further evidence for the reaction on avidin-bound azide **81b**, was brought by MALDI[§§] analyses of the modified protein, where the corresponding appearance of

[§§] MALDI analysis was performed by Tomáš Ožd ian from Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University Olomouc, Olomouc, Czech Republic.

higher-mass compound was observed. As the binding constant of avidin-biotin is very high at room temperature ($K_D = 10^{-15}$ mol dm^{-3}),[26] we assume that the reaction of azide immobilized by avidin proceeded on a bound substrate and not on a substrate temporarily released from the protein.[15]

According to these observations, the conjugation reaction between the cyclooctyne analogue obtained from the corresponding 1,2,3-selenadiazole derivative and the biotinylated azide immobilized by avidin proceeds successfully under photoirradiation at room temperature.[15]

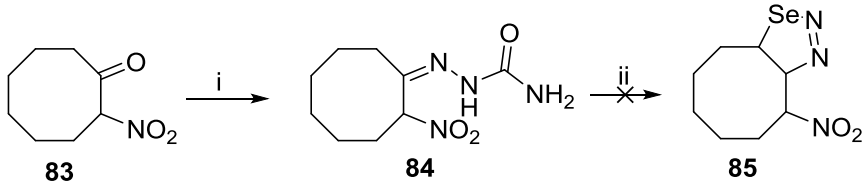
3.2.2. Preparation of derivatives of cycloocta-1,2,3-selenadiazole

This part of work was focused on preparation of substituted cycloocta[d][1,2,3]selenadiazoles bearing substituents possessing different electronic properties at position 4. Thermally induced conjugation reactions of these derivatives were then studied and influence of substituents should have been evaluated in comparison to unsubstituted cycloocta[d][1,2,3]selenadiazole **65**.

4-nitro-cycloocta[d][1,2,3]selenadiazole

The strategy for synthesis of substituted cycloocta[d][1,2,3]selenadiazoles was same as synthesis of other known 1,2,3-selenadiazoles – transformation of corresponding ketone to semicarbazone and subsequent cyclization with selenium dioxide. Thus, 2-nitrocyclooctanone **83** was prepared by two-step synthesis described in literature.[27] 2-(2-nitrocyclooctylidene)hydrazine-1-carboxamide **84** was obtained quantitatively in 50% AcOH (Scheme 23).

Scheme 23: Preparation of 4-nitro-cycloocta[d][1,2,3]selenadiazole **85**.[a]



83 **84** **85**

[a]Reagent: (i) semicarbazide hydrochloride, 50% AcOH in H_2O (ii) SeO_2, dioxane, H_2O or SeO_2, AcOH.

Surprisingly, final cyclization to 1,2,3-selenadiazole **85** was proved to be problematic. Firstly, cyclization in aqueous dioxane was tested. At room temperature, only starting material **84** together with small amount of impurities was presented in reaction mixture. Heating of reaction solution to 50 °C resulted in mixture of starting material **84** and unknown compound

in ratio 1:1. Many impurities were also formed. Additional heating to 80 °C then led to formation of complicated mixture of compounds. Desired product **85** was not formed in any case.

Then, cyclization was also tested in acetic acid. Similarly to reaction in dioxane, 1,2,3-selenadiazole **85** was not obtained. Reaction mixture consisted from starting material and unknown compound in ratio 1:1; other impurities were also presented. The same unknown compound as above was isolated from reaction mixture by column chromatography and characterized. Surprisingly ^{13}C NMR showed presence of keto-group instead of nitro group suggesting, that nitro group was oxidized by selenium dioxide to corresponding ketone **86** (Figure 20). The proposed structure of compound **86** was then confirmed also by HRMS analysis.

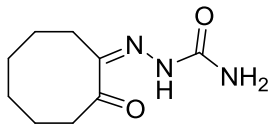
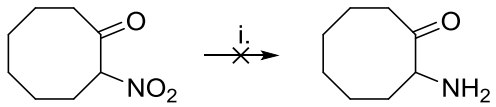


Figure 20: Structure of compound **86**.

4-amino-cycloocta[d][1,2,3]selenadiazole

After unsuccessful preparation of 4-nitro-cycloocta[d][1,2,3]selenadiazole, the effort was focused on the synthesis of its amino analogue 4-amino-cycloocta-1,2,3-selenadiazole. 2-nitrocyclooctanone **83** was used as a starting material again. It was necessary to find out, which phase of synthesis is the best for crucial reduction of nitro group to amino group. Firstly, reduction as a first step of synthesis was studied (Scheme 24). However, mixture of unknown compounds was obtained in all cases and desired product **87** has never been observed.

Scheme 24: Preparation of 2-aminocyclooctanone **87**.[a]

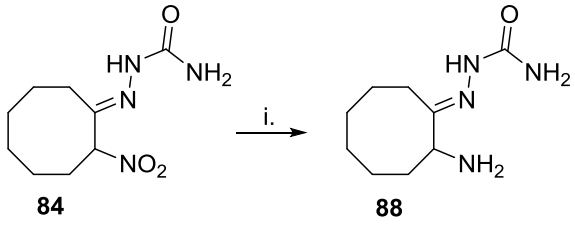


83 **87**

[a]Reagent: (i) H_2, Pd/C, MeOH or EtOAc.

Then, the reduction of semicarbazone **84** was tested with use of hydrogen on Pd/C (Scheme 25). Unfortunately, the mixture of compounds was obtained again in case of all tested reactions.

Scheme 25: Preparation of 2-(2-aminocyclooctylidene)hydrazine-1-carboxamide **88**.[a]

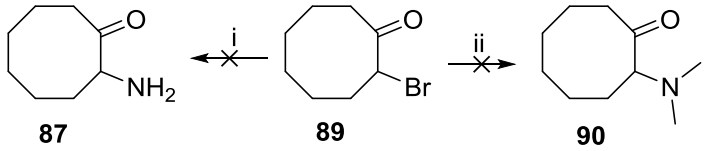


84 88

[a]Reagent: (i) H_2, Pd/C, MeOH or EtOAc.

In the next approach, attention was paid to substitution of 2-bromocyclooctanone **89** to desired 2-aminocycloctanone. 2-bromocyclooctanone **89** was prepared according to described procedures[28,29]. Substitution was done according to Utsukihara[30] in Microwave reactor (Scheme 26). Nevertheless, only formation of compounds containing bromine in their structure (according to Br isotope profile in MS) was observed.

Scheme 26: Preparation of 2-aminocyclooctanone **87** and **90**.[a]

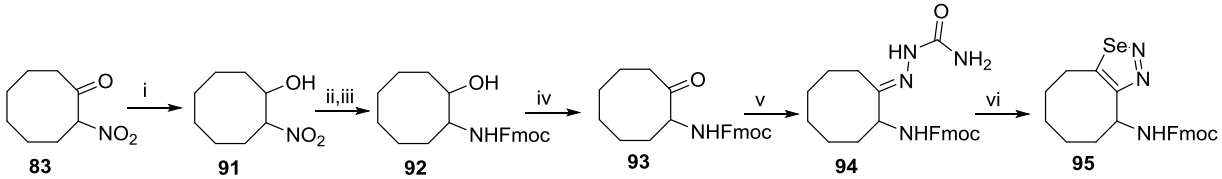


87 89 90

[a]Reagent: (i) 7% NH_3/H_2O, 105 °C, 150 W (ii) 7% DEA/H_2O, 105 °C, 150 W.

Finally, 2-nitrocyclooctanone **83** was used as a starting material, but reduction of nitro group was performed after reduction of carbonyl group (Scheme 27). 2-nitrocyclooctanol **91** was prepared according to literature.[31] Nitro group was then reduced by hydrogen under Pd/C catalysis and resulting amine was protected by Fmoc group. In the next step, hydroxyl group was oxidized to ketone **93** by Dess-Martin periodinane followed by reaction with semicarbazide hydrochloride. Compound **94** was then treated with selenium dioxide. 1,2,3-selenadiazole **95** was obtained in 43% purity together with unknown compound formed in 34% purity. 1,2,3-selenadiazole **95** was isolated and purified with use of semipreparative HPLC with 5% overall yield.

Scheme 27: Preparation Fmoc-4-amino-cycloocta[d][1,2,3]selenadiazole **95**.[a]



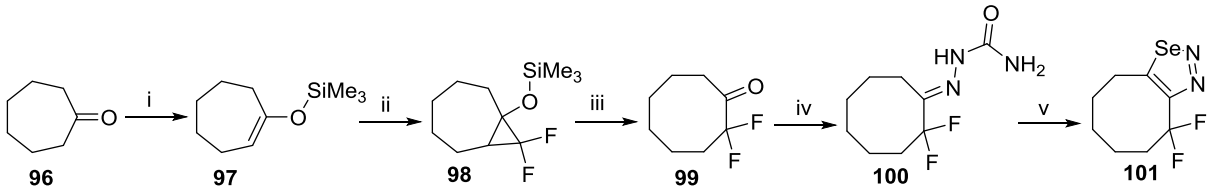
83 91 92 93 94 95

[a]Reagent: (i) $NaBH_4$, anhydrous EtOH, 0 °C (ii) H_2, Pd/C, MeOH (iii) Fmoc-OSu, DCM (iv) Dess-Martin periodinane, DCM (v) semicarbazide hydrochloride, 50% AcOH in H_2O (vi) SeO_2, AcOH.

4,4-difluoro-cycloocta[d][1,2,3]selenadiazole

Inspired by reactivity of **DIFO** and its derivatives, the optimizations for the synthesis of difluorocycloocta[d][1,2,3]selenadiazole were carried out. 2,2-difluorocyclooctanone **99** as a crucial reaction intermediate was prepared by described three-step synthesis. At the beginning, cycloheptanone **96** is converted to trimethylsilylenol ether **97** (Scheme 28) as published in literature,[32] which is then reacted with sodium bromodifluoroacetate to form compound **98** (Scheme 28) according to described procedure.[33] Treatment of compound **98** by Na_2CO_3 then resulted in desired 2,2-difluorocyclooctanone **99**.[33]

Scheme 28: Preparation 4,4-difluoro-cycloocta[d][1,2,3]selenadiazole **101**.[a]



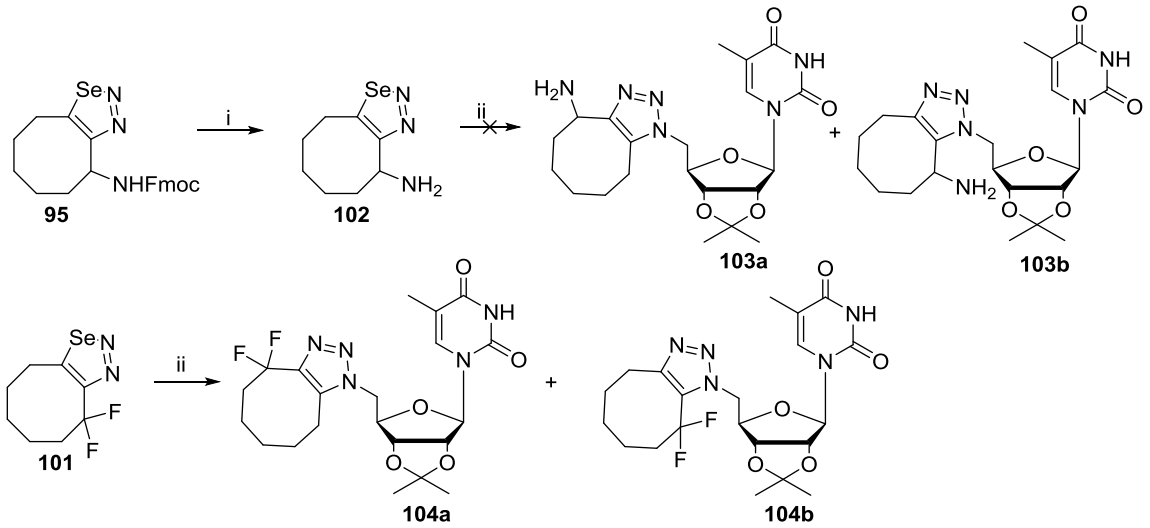
[a]Reagent: (i) NaBr, Me_3SiCl, DIEA, DMF (ii) sodium bromodifluoroactetate, anhydrous diglyme, 150 °C (iii) Na_2CO_3, anhydrous MeOH (iv) semicarbazide hydrochloride, 50% AcOH in H_2O (v) SeO_2, AcOH, 50 °C.

2,2-difluorocyclooctanone **99** was then converted to compound **100** by reaction with semicarbazide hydrochloride (Scheme 28). Treatment of **100** by selenium dioxide in acetic acid at room temperature did not result in product, no reactivity was observed. After heating to 50 °C, 1,2,3-selenadiazole **101** was formed in 50% purity in mixture with unknown compound and starting material, which was hydrolysed back to ketone **99**. The same results were obtained in aqueous dioxane. Desired 1,2,3-selenadiazole **101** was purified with use of column chromatography in 15 % overall yield and used for the following study of conjugation with azides.

Thermally induced conjugation reaction with azides

Thermally induced conjugation reactions of prepared 1,2,3-selenadiazoles **95** and **101** with azide **6** were studied. Firstly, Fmoc-4-amino-cycloocta[d][1,2,3]selenadiazole **95** was deprotected by piperidine to form amine **102**, which was then reacted with azide **6** at 90 °C. In contrast to non-substituted cycloocta[d][1,2,3]selenadiazole **65** fully convertable to triazole **67** after 48 h (see Table 4), only starting materials were recovered in case of 4-amino-cycloocta[d][1,2,3]selenadiazole **102** (Scheme 29).

Scheme 29: Conjugation reaction of 1,2,3-selenadiazoles **101** and **102** with azide **6**.[a]

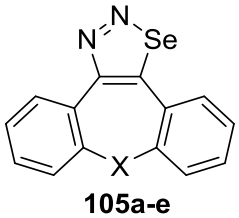


[a]Reagent: (i) pyridine, NMP (ii) azide **6**, NMP, 90 °C.

Heating of mixture of 4,4-difluoro-cycloocta[d][1,2,3]selenadiazole **101** with azide **6** to 90 °C resulted after 48 hours in formation of products **104a,b** (Scheme 29), but only in very low yield (below 10 %). Starting materials were still presented in reaction mixture. These preliminary results showed that presence of substituent on cyclooctane ring did not help with reaction rate in thermally induced conjugation. The reaction with 1,2,3-selenadiazole bearing electron withdrawing group occurred, but significantly slower than in case of cycloocta[d][1,2,3]selenadiazole itself.

3.2.3. Preparation and reactivity of dibenzo thia- or selena- cyclohepta-1,2,3-selenadiazoles

The last part of work was focused on preparation of thia- or selena-dibenzo-1,2,3-selenadiazoles **105a-e** (Figure 21) and on study of their photochemically induced conjugation reactions with azides. Benzylazide **72** was chosen as a reaction partner due to its good stability to photolysis and easy preparation.



105a-e

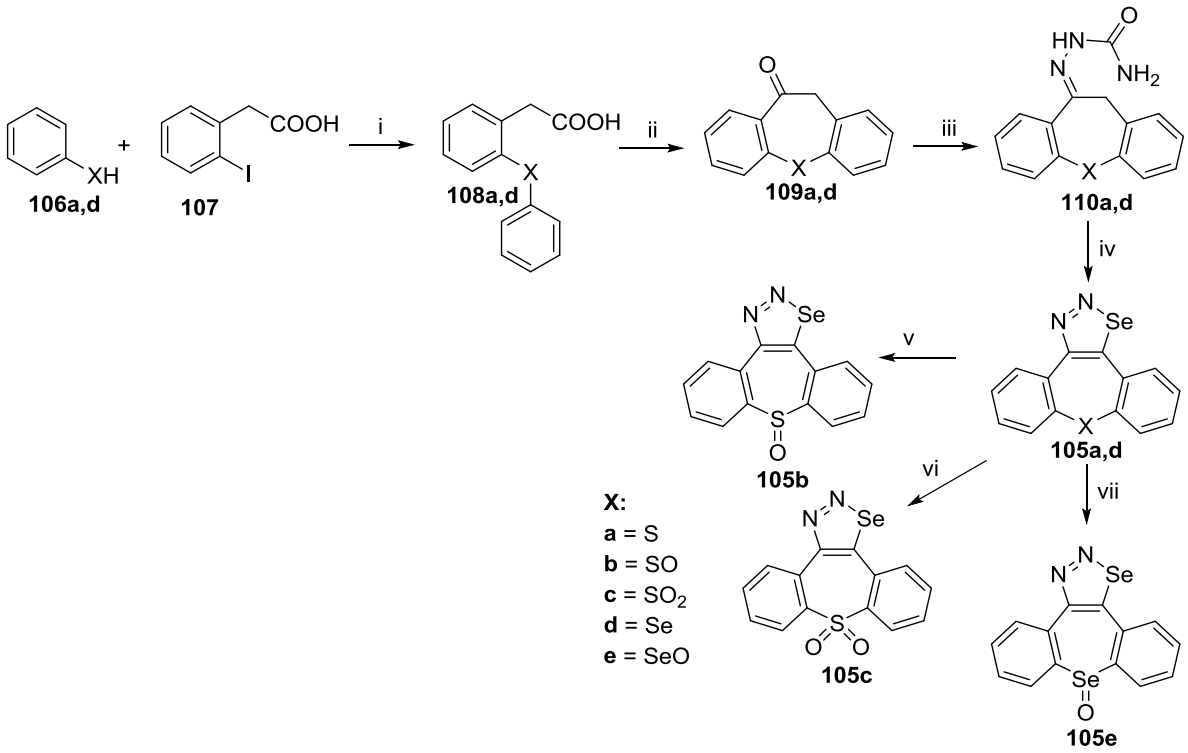
X:
a = S
b = SO
c = SO_2
d = Se
e = SeO

Figure 21: Thia- and selena- dibenzo-1,2,3-selenadiazoles **105a-e**.

Synthesis of dibenzo thia- or selena- cyclohepta-1,2,3-selenadiazoles

Dibenzothiepino-1,2,3-selenadiazole **105a** and dibenzoselenepino-1,2,3-selenadiazole **105d** were prepared *via* dibenzothiepin-10- and dibenzoselenepin-10-hydrazine carboxamides **84a** and **84d** which were generated from corresponding ketones **109a** and **109d** (Scheme 30).

Scheme 30: Preparation of 1,2,3-selenadiazoles **105a-e**.[a]



[a]Reagent: (i) KOH, Cu, H_2O, reflux (ii) PPA, toluene, 110 °C (iii) semicarbazide hydrochloride, AcOH/H_2O 1:1, DMF (iv) selenium dioxide, AcOH, DMF (v) 30% H_2O_2, Me_3SiCl, MeCN (vi) 30% H_2O_2, AcOH, DCM (vii) 30% H_2O_2, $CHCl_3$/MeOH 4:1.

Dibenzo-10-thiepinone **109a** was prepared by two step procedure described in literature.[34,35] The analogical conditions were used also for preparation of dibenzoselenepin-10-one **109d** (Scheme 30). 1,2,3-selenadiazoles **105b,c,e** were then prepared by oxidation of 1,2,3-selenadiazoles **105a** and **105d** by hydrogen peroxide (Scheme 30).

Photoinduced conjugation reaction of dibenzo thia- or selena- cyclohepta-1,2,3-selenadiazoles

Photoinduced conjugation reactions of 1,2,3-selenadiazoles **105a-e** were studied with benzylazide **72**. Irradiation wavelengths were carefully chosen according to UV/VIS spectra of individual compounds **105a-e** (Figure 22).

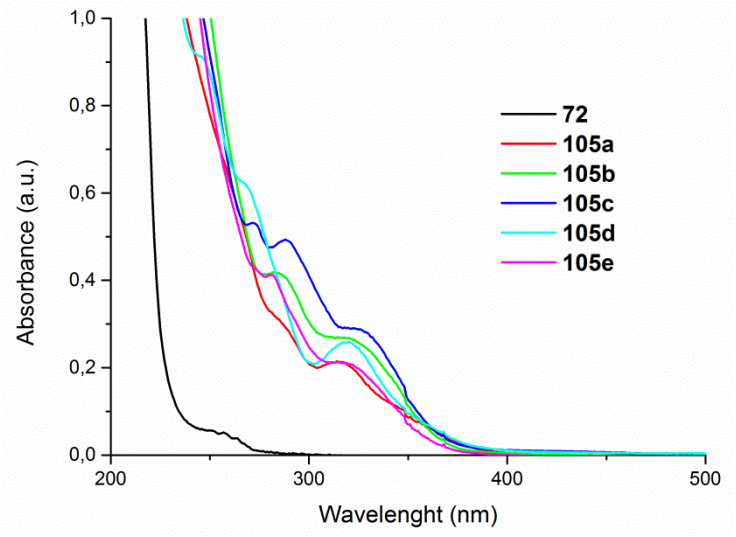
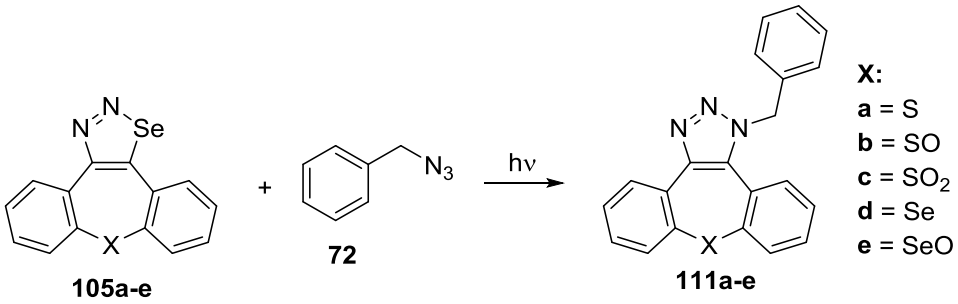


Figure 22: UV/VIS spectra of 1,2,3-selenadiazoles **105a-e**.

As the absorption spectra of the 1,2,3-selenadiazoles **105a-e** partially overlaps with absorption spectrum of benzylazide **72**, the undesired light absorption of benzylazide should be suppressed. The absorption maxima of starting materials **105a-e** allowed us to use irradiation wavelengths higher than 280 nm.

Benzylazide **72** (c ~ 0.03 mol dm^{-3}) and 1,2,3-selenadiazole **105a-e** (c ~ 0.03 mol dm^{-3}) in MeOH (Scheme 31) were irradiated by light emitted from medium-pressure Hg lamp filtered through Pyrex glass (λ > 280 nm) or through 300, 316 and 366 nm filter. The reaction was monitored with use of LC-UV/MS after two, four and six hours of irradiation.

Scheme 31: Photochemically induced conjugation reactions of 1,2,3-selenadiazoles **105a-e** and benzylazide **72**.



The desired triazole **111** was formed as a major product only in case of dibenzothiepino-1,2,3-selenadiazole **105a** and dibenzoselenepino-1,2,3-selenadiazole **105d**. Full conversion of **105a** after two hours at λ > 280 nm with 72% yield of **111a** (Table 6) confirmed the enhanced reactivity of these systems in comparison to cycloocta[d][1,2,3]selenadiazole with ~ 40% yield at the same condition.[15] When the optical filteres were used, the yield of triazoles **111a**

and **111d** was decreased by formation of side products (Table 6) which structures were determined according to mass spectrometry and NMR (Figure 23).

Table 6: Percentages of photoproducts after 6 hours of irradiation.

Selenadiazole		λ (nm)	105[%][a]	111 [%][a]	112[%][a]	113 [%][a]	114 [%][a]	115 [%][a]
a	**S**	> 280	0	74	-	-	-	-
		300	2	31	19	-	-	-
		316	2	39	33	-	-	-
		366	4	29	55	-	-	-
b	**SO**	> 280	DECOMPOSITION					
		300	0	23	-	-	67	-
		316	0	16	-	-	54	-
		366	0	19	24	-	32	-
c	**SO_2**	> 280	19	10	9	15	-	-
		316	9	21	24	22	-	-
		316	17	14	13	22	-	-
		366	5	26	15	20	-	-
d	**Se**	> 280	0	34	9	-	-	-
		300	0	54	14	-	-	-
		316	0	56	17	-	-	-
		366	0	38	46	-	-	-
e	**SeO**	> 280	1	0	-	17	17	6
		300	7	0	-	-	28	-
		316	7	0	-	-	51	11
		366	5	0	-	5	25	2

[a]Calculated form LC traces at 200 – 600 nm.

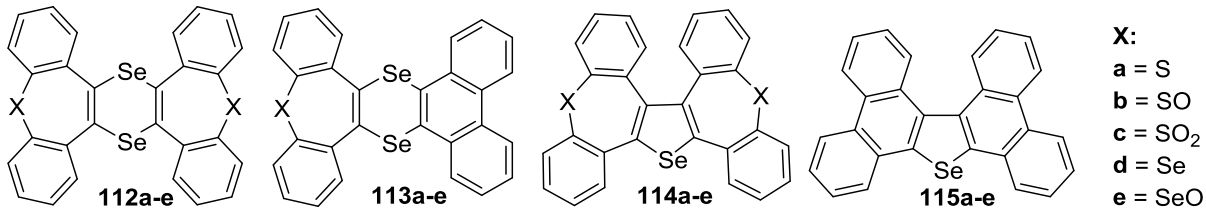


Figure 24: Structures of compounds **112-115**.

In case of selenadiazole **105b** the product **111b** was formed, but selenophene **114b** predominated (Table 6, Figure 23). Irradiation of dibenzothiepino-1,2,3-selenadiazole-*S*,*S*-dioxide **105c** resulted in all cases in complex mixture of photoproducts (Table 6) which were not isolated due to their low abundance.

Surprisingly, no formation of desired product **111e** was observed in case of derivative **105e**. From LC/MS spectra we can deduce formation of derivatives **114e** and **115e** (Figure 23), which were not possible to isolate because of complexity of reaction mixture.

The best results in formation of triazoles were finally obtained by dibenzothiepino-1,2,3-selenadiazole **105a** at $\lambda > 280$ nm and by dibenzoselenepino-1,2,3-selenadiazole **105d** at $\lambda = 315$ nm. Triazoles **111a** and **111d** were isolated, purified with use of semipreparative HPLC and characterized. Unfortunately, the presence of side products observed in all cases showed that it is not possible to selectively convert the 1,2,3-selenadiazoles **105a-e** to corresponding triazoles by this method.

4. Conclusion

The main goal of presented dissertation was to prepare and study new systems suitable for non-catalyzed conjugation reactions (SPAAC) with azides. The work was focused on two types of system - derivatives of known aza-dibenzocyclooctyne (**DIBAC**) and cycloalka-1,2,3-selenadiazoles.

In the first part of work, the attention was firstly paid to preparation and especially characterization of 1,2,3-triazoles formed by SPAAC between known **DIBAC** derivative **13** and 5-methyl uridine-5´-azides **5** and **6**. 1D and 2D NMR spectra predicted the formation of conformational isomers due to hindered rotation of the pyrimidine and azocine parts of the molecule and ^{19}F-^{19}F EXSY experiments proved exchange between individual conformers. The computational study of the studied compounds revealed that observed isomery is caused either by different positions of the aliphatic chain relative to the nucleoside part of the molecule or by the formation of intramolecular hydrogen bonds. The similar isomery was studied subsequently also for derivatives, in which the nucleoside was replaced by hydrogen (**16**) or coumarine scaffold (**17a**/**17b**). For the substrates **16** and **17a** no isomery was observed, while for derivative **17b** at least three conformers were detected.

Secondly, 1,2,3-triazoles, in which the previous aliphatic chain was replaced by *β*-alanine (**20a**/**20b**), trifluoracetyl group (**24a**/**24b**) and hydrogen (**26a**/**26b**), were prepared and studied. Similarly to triazoles described above, isomery was observed in NMR spectra of all prepared compounds. These results indicate, that observed isomery is independent on the length of aliphatic chain.

The last aim of this part was to developed solid-phase synthesis for fluorescently labelled **DIBAC** derivatives. At the beginning, it was necessary to find a way, how to immobilized dihydrodibenzoazocine **10** on solid support. Compound 10 was finally attached as an amide **33** or **34** *via* corresponding acyl chloride. Following bromination proceeded without any problems, however subsequent formation of triple bond proved to be challenging. Although the different bases were tested, elimination proceeded satisfactory only when *t*BuOK was used. Due to instability of aza-dibenzocyclooctyne towards acidic conditions used for cleavage from resin, it was firstly necessary to cleave dibromoderivatives **35** or **36** from resin and then perform the key elimination in solution. Unfortunately, this methodology could not be used for RhoB labelled dibromoderivatives **55**, **56** and **58** as RhoB was cleaved from the substrate during elimination step. Although the preparation of fluorescently labelled aza-

dibenzocyclooctynes was unsuccessful, RhoB labelled dihydrodibenzoazocines **62**, **63** and **64**, which can be later used for alkene ligation, were isolated and characterized.

In the second part of work, the attention was firstly paid to study of microwave assisted and photochemically induced click reactions of cycloocta[d][1,2,3]selenadiazole **65** with azides. In comparison to thermally induced click reaction of **65**, where the quantitative conversion to triazole **67** was achieved after 20 hours at 110 °C, the use of microwave (110 °C, 150 W, 3 eq. of **65**) helped to shorten the reaction time to approximately 70 minutes. Unfortunately, the conversion of **65** at temperatures below 100 °C significantly decreased.

On the other hand, conjugation reactions of cyclooctyne, which was photochemically generated in situ from cycloocta[d][1,2,3]selenadiazole **65**, with various azides at room temperature, provided satisfactory yields of the corresponding 1,2,3-triazoles. The reaction was successfully tested for the labelling of an avidin–biotin complex, which predestines cyclooctaselenadiazole derivatives for applications in molecular biology.

Second aim of this part was to prepare derivatized cycloocta[d][1,2,3]selenadiazoles and study, if substituent adjacent to 1,2,3-selenadiazole ring can influence the rate of thermally induced click reaction. Synthesis of 4-nitro[d][1,2,3]selenadiazole **85** was unsuccessful due to oxidation of nitro group to 2-oxocyclooctylidene)hydrazine-1-carboxamide **86** by selenium dioxide. After optimization, *N*-Fmoc-4-amino[d][1,2,3]selenadiazole **95** was prepared by seven step synthesis started from 2-nitrocylooctanone in 5% overall yield. The target 4-amino[d][1,2,3]selenadiazole **102** was then prepared by quantitative Fmoc deprotection before click reaction itself. Then 4,4-difluoro-1,2,3-selenadiazole **101** was prepared from 2,2-difluorocyclooctanone in 15% overall yield. 1,2,3-selenadiazoles **101** and **102** were reacted with azide **6** at 90 °C for 48 h. The results showed that both chosen electron withdrawing and electron donating substituents have negative effect on conjugation reaction rate in comparison to unsubstituted cycloocta[d][1,2,3]selenadiazole **65**, where the conversion was quantitative after 48 h.

Lastly, conjugation reactions of dibenzocycloheptyne derivatives, which were photochemically generated *in situ* from dibenzothiepino- or dibenzoselenepino-1,2,3-selenadiazoles **105a-e**, with benzylazide **72** were studied. Expected triazoles were formed in all cases with exception of dibenzoselenepino-1,2,3-selenadiazole-8-oxide **105e**. The yields and triazole purities differed relative to structure of starting 1,2,3-selenadiazole and used wavelengths. The best results were obtained for dibenzothiepino-1,2,3-selenadiazole **105a** at $\lambda > 280$ nm, where triazole **111a** was formed in 74% yield after 6 hours of irradiation.

5. References

1. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem. , Int. Ed.* **2002,** *41* (14), 2596-2599.
2. Tornoe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002,** *67* (9), 3057-3064.
3. Moses, J. E.; Moorhouse, A. D. *Chem. Soc. Rev.* **2007,** *36* (8), 1249-1262.
4. Baskin, J. M.; Bertozzi, C. R. *Aldrichimica Acta* **2010,** *43* (1), 15-23.
5. Agard, N. J.; Prescher, J. A.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2004,** *126* (46), 15046-15047.
6. Ostrovskis, P.; Volla, C. M. R.; Turks, M.; Markovic, D. *Curr. Org. Chem.* **2013,** *17* (6), 610-640.
7. Debets, M. F.; van Berkel, S. S.; Schoffelen, S.; Rutjes, F. P. J. T.; van Hest, J. C. M.; van Delft, F. L. *Chem. Commun.* **2010,** *46* (1), 97-99.
8. Zayas, J.; Annoual, M.; Das, J. K.; Felty, Q.; Gonzalez, W. G.; Miksovska, J.; Sharifai, N.; Chiba, A.; Wnuk, S. F. *Bioconjugate Chem.* **2015,** *26* (8), 1519-1532.
9. Zhou, Q.; Gui, J.; Pan, C. M.; Albone, E.; Cheng, X.; Suh, E. M.; Grasso, L.; Ishihara, Y.; Baran, P. S. *J. Am. Chem. Soc.* **2013,** *135* (35), 12994-12997.
10. Smyslova, P. Preparation and reactivity of selected 5 and 5´ substituted 5-methyluridines. Palacky University Olomouc, 2012.
11. Cheng, Z.; Elias, D. R.; Kamat, N. P.; Johnston, E. D.; Poloukhtine, A.; Popik, V.; Hammer, D. A.; Tsourkas, A. *Bioconjugate Chem.* **2011,** *22* (10), 2021-2029.
12. Smyslova, P.; Popa, I.; Lycka, A.; Tejral, G.; Hlavac, J. *PLoS One* **2015,** *10* (12), e0144613/1-e0144613/33.
13. Gaussian 09, Revision D.01, Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.;

Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. Gaussian, Inc., Wallingford CT, 2009.

14. Lalezari, I.; Shafiee, A.; Yalpani, M. *Tetrahedron Lett.* **1969,** (58), 5105-5106.
15. Jedinakova, P.; Sebej, P.; Slanina, T.; Klan, P.; Hlavac, J. *Chem. Commun.* **2016,** *52* (26), 4792-4795.
16. Singh, S. C.; Mishra, S. K.; Srivastava, R. K.; Gopal, R. *J. Phys. Chem. C* **2010,** *114* (41), 17374-17384.
17. Meier, H.; Voigt, E. *Tetrahedron* **1972,** *28* (1), 187-198.
18. Fairbanks, B. D.; Sims, E. A.; Anseth, K. S.; Bowman, C. N. *Macromolecules* **2010,** *43* (9), 4113-4119.
19. Chen, W.; Wang, D.; Dai, C.; Hamelberg, D.; Wang, B. *Chem. Commun* **2012,** *48* (12), 1736-1738.
20. Klan, P.; Janosek, J.; Kriz, Z. *J. Photochem. Photobiol. , A* **2000,** *134* (1-2), 37-44.
21. Wagner, P. J.; Kochevar, I. E.; Kemppainen, A. E. *J. Amer. Chem. Soc.* **1972,** *94* (21), 7489-7494.
22. Ito, K.; Higuchi, Y.; Tame, C.; Hariya, J. *Heterocycles* **1993,** *35* (2), 937-947.
23. Daehne, L.; Bendig, J.; Stoesser, R. *J. Prakt. Chem. /Chem. Ztg.* **1992,** *334* (8), 707-710.
24. Doering, W.; Odum, R. A. *Tetrahedron* **1966,** *22* (1), 81-93.
25. Leyva, E.; Platz, M. S.; Persy, G.; Wirz, J. *J. Am. Chem. Soc.* **1986,** *108* (13), 3783-3790.
26. Livnah, O.; Bayer, E. A.; Wilchek, M.; Sussman, J. L. *Proc. Natl. Acad. Sci. U. S. A.* **1993,** *90* (11), 5076-5080.
27. Giorgi, G.; Miranda, S.; Lopez-Alvarado, P.; Avendano, C.; Rodriguez, J.; Menendez, J. C. *Org. Lett.* **2005,** *7* (11), 2197-2200.
28. Piel, I.; Pawelczyk, M. D.; Hirano, K.; Froehlich, R.; Glorius, F. *Eur. J. Org. Chem.* **2011,** *2011* (28), 5475-5484.
29. Sims, E. A.; DeForest, C. A.; Anseth, K. S. *Tetrahedron Lett.* **2011,** *52* (16), 1871-1873.
30. Utsukihara, T.; Nakamura, H.; Watanabe, M.; Horiuchi, C. A. *Tetrahedron Lett.* **2006,** *47* (52), 9359-9364.
31. Milner, S. E.; Brossat, M.; Moody, T. S.; Elcoate, C. J.; Lawrence, S. E.; Maguire, A. R. *Tetrahedron: Asymmetry* **2010,** *21* (8), 1011-1016.
32. Mathys, M.; Kraft, P. *Chem. Biodiversity* **2014,** *11* (10), 1597-1607.
33. Kageshima, Y.; Suzuki, C.; Oshiro, K.; Amii, H. *Synlett* **2015,** *26* (1), 63-66.
34. Botta, M.; Castiglioni, E.; Di Fabio, R.; Spinosa, R.; Togninelli, A. Spiro compounds useful as antagonists of the H1 receptor and their asymmetric preparation, pharmaceutical

compositions and use in the treatment of sleep disorders. WO2009016085A1, Feb 5, 2009.

35. Boudhar, A.; Ng, X. W.; Loh, C. Y.; Chia, W. N.; Tan, Z. M.; Nosten, F.; Dymock, B. W.; Tan, K. S. W. *Eur. J. Med. Chem.* **2016,** *119*, 231-249.