A novel dibenzoazacyclooctyne precursor in regioselective copper-free click chemistry. An innovative 3-step synthesis

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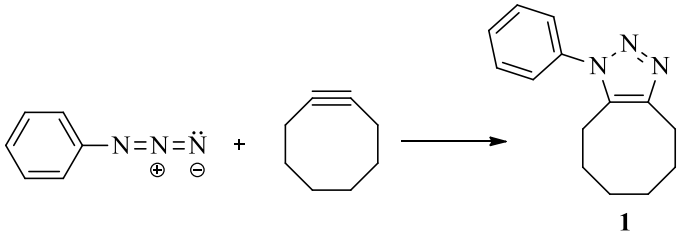
Abstract
A novel carboxylic acid substituted dibenzoazacyclooctyne precursor has been synthesized using a fast and innovative three-step synthesis. It can be easily converted into the corresponding alkyne through UV-irradiation. Due to its fast and regioselective reaction with azides, the alkyne is a promising agent for copper-free "click chemistry". The second order reaction rate constant was determined by ^{1}H-NMR.

Keywords: Bioorthogonal, cyclooctyne, azides, click chemistry

Introduction

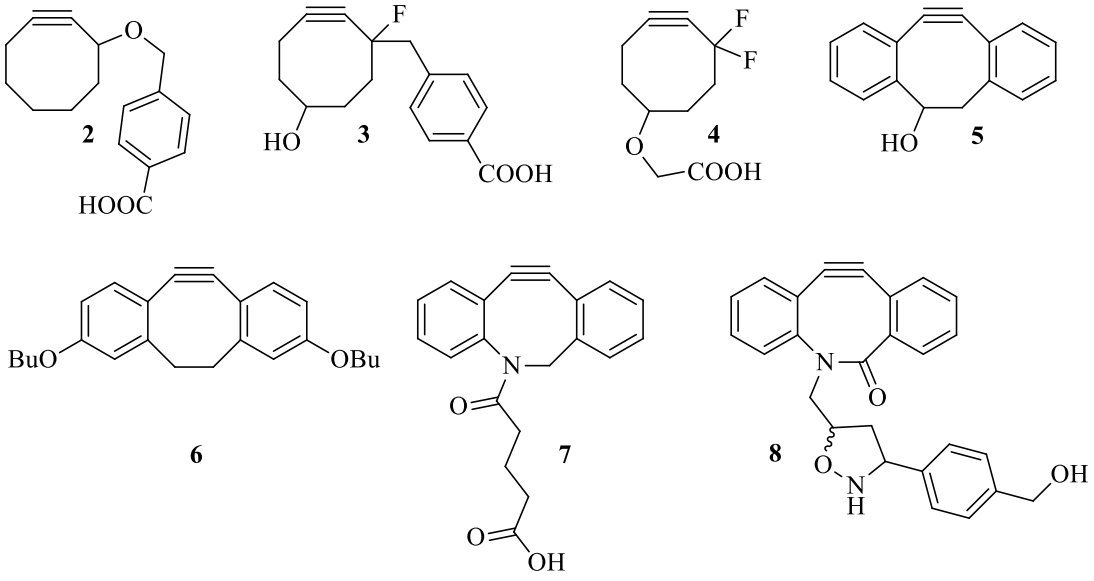
Studying the tremendous number of biochemical processes occurring in a living organism has always been a demanding challenge for scientists. To undergo the problem of monitoring a certain biomolecule in the complex and abundantly functionalized environment of a biological system, it was necessary to develop a new labeling method: The bioorthogonal chemical reporter strategy, which includes the introduction of a defined chemical reporter into the desired biomolecule and the subsequent reaction of the reporter group with a complementary molecule attached to any user-defined probe such as fluorophores or radioactive compounds.[1,2] Besides a fast reaction under physiological conditions, these so called bioorthogonal reactions also have to proceed highly selective in the presence of various functional groups and multiple metabolic reactions taking place simultaneously. Therefore, reagents should not be sensitive to water, nucleophilic attack from thiols or amines, or redox chemistry. Furthermore, they need to withstand metabolization by enzymes and should provide no toxic effects within the range of the required concentrations.[3] Only very few reactions fulfill all these criteria.

The most commonly used chemical reporter group is the azide group. Besides its stability against occurring biological functionalities, it provides interesting reactions with phosphines (Staudinger ligation) and alkynes. However, the cycloaddition of the azide with alkynes requires activation of the corresponding alkyne compound. This can be achieved either by catalyzation with copper(I), usually termed 'click chemistry', or through ring-strain. Due to the cytotoxic effects of the heavy metal, the application of the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) in living systems is very limited, making the strain-promoted variant the more preferable choice.[4] It was discovered by Wittig and Krebs, who observed the rapid reaction of cyclooctyne with phenyl azide providing a single product, the triazole **1** (Scheme 1).[5]



Scheme 1. Reaction of cyclooctyne with phenyl azide to triazole **1.**

In 2004 Bertozzi and co-workers rediscovered this observation and since the reaction of the first substituted cyclooctyne **2** with azides was published, this class of reactions was entitled as the strain-promoted azide-alkyne cycloaddition (SPAAC).[6] While the reaction rate of **2** was only moderately increased compared to linear alkynes, many new cyclooctyne compounds with strongly improved kinetics have been established since then (Scheme 2).



Scheme 2. Cyclooctyne derivatives for bioorthogonal reactions.

The attachment of one or two electron-withdrawing fluorine substituents to the cyclooctyne ring resulted in a significant increase of the reaction rates of the compounds **3** and **4** compared to **2**.[7,8] Boons and co-workers have reported the synthesis of the even faster dibenzocyclooctynes **5** and **6**, whose reactivities are enhanced by the additional ring-strain provided by the two phenyl rings.[9,10] The introduction of a nitrogen atom into the 8-ring of a dibenzocyclooctyne further elevates the ring-strain due to the shorter carbon-nitrogen bond and therefore additionally improves the reaction rates of dibenzoazacyclooctyne **7** and dibenzoazacyclooctynone **8**.[11,12] According to IUPAC nomenclature these compounds are named as derivatives from azocine, which is present in a good number of valuable natural substances like manzamine A or nakadomarin A.[13] Aside from this, azacyclooctynes are more hydrophilic, lowering the risk of unspecific binding when used *in vivo*.

Table 1. Second order reaction rate constants of cyclooctynes **2-8** with benzyl azide[4,6-12]

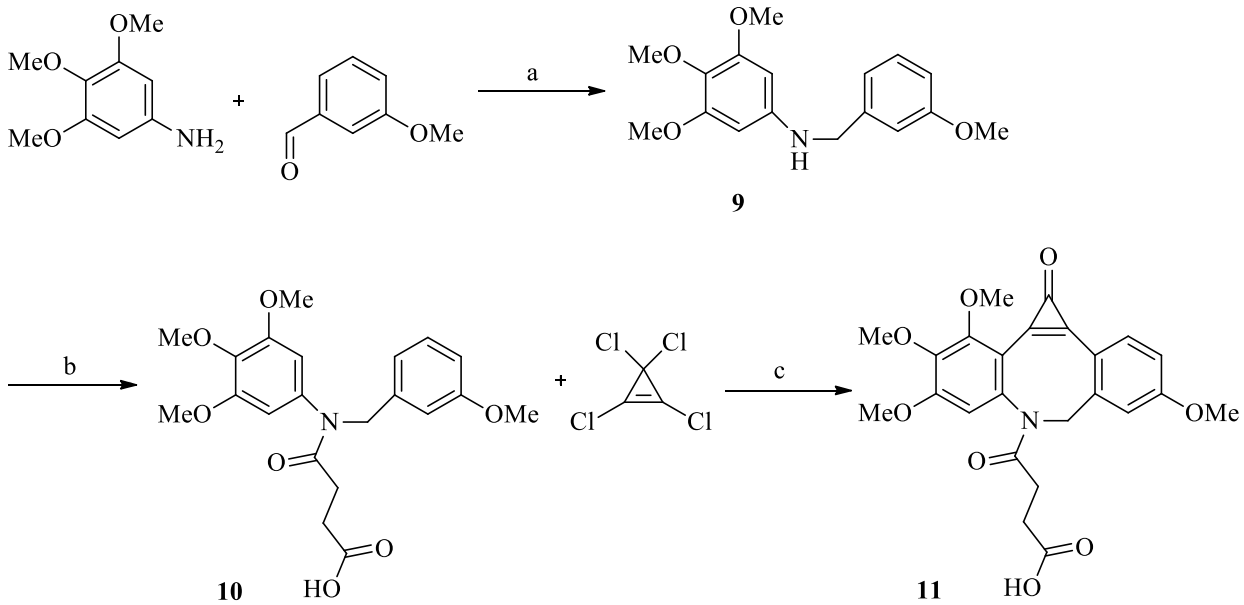
Entry	k [x 10^{-3} $M^{-1}s^{-1}$]	solvent
2	1.3	CD_3CN
3	4.3	CD_3CN
4	76	CH_3CN
5	120	CD_3CN
6	76	CH_3OH
7	310	CD_3OD
8	960	CH_3CN

Although lots of improvements have been made, the reaction rates of known cyclooctynes with azides are still perfectible. Especially for applications in living animals or humans, it is absolutely necessary to design a further improved, highly hydrophilic cyclooctyne with superior kinetics. One major problem, which delays the development of new cyclooctynes, is the complex and time-consuming production of the most promising compounds, especially for the dibenzoazacyclooctynes. For example, the synthesis of the fastest known cyclooctynes **7** and **8** requires up to nine steps. In this paper we describe a fast, easy and innovative three-step synthesis of a novel dibenzoazacyclooctyne precursor that carries a carboxylic acid group for easy coupling, its rapid conversion into the corresponding alkyne and the fast and regioselective reaction with several azides. The synthesis uses inexpensive starting materials and the three steps proceeded in 23% overall yield, making it an interesting method for the prospective production of further substituted dibenzoazacyclooctynes with suitable properties.

Results and Discussion

As outlined in Scheme 3, the initial step of our approach for the synthesis of the new substituted dibenzoazacyclooctyne precursor **11** was an indirect reductive amination using commercially

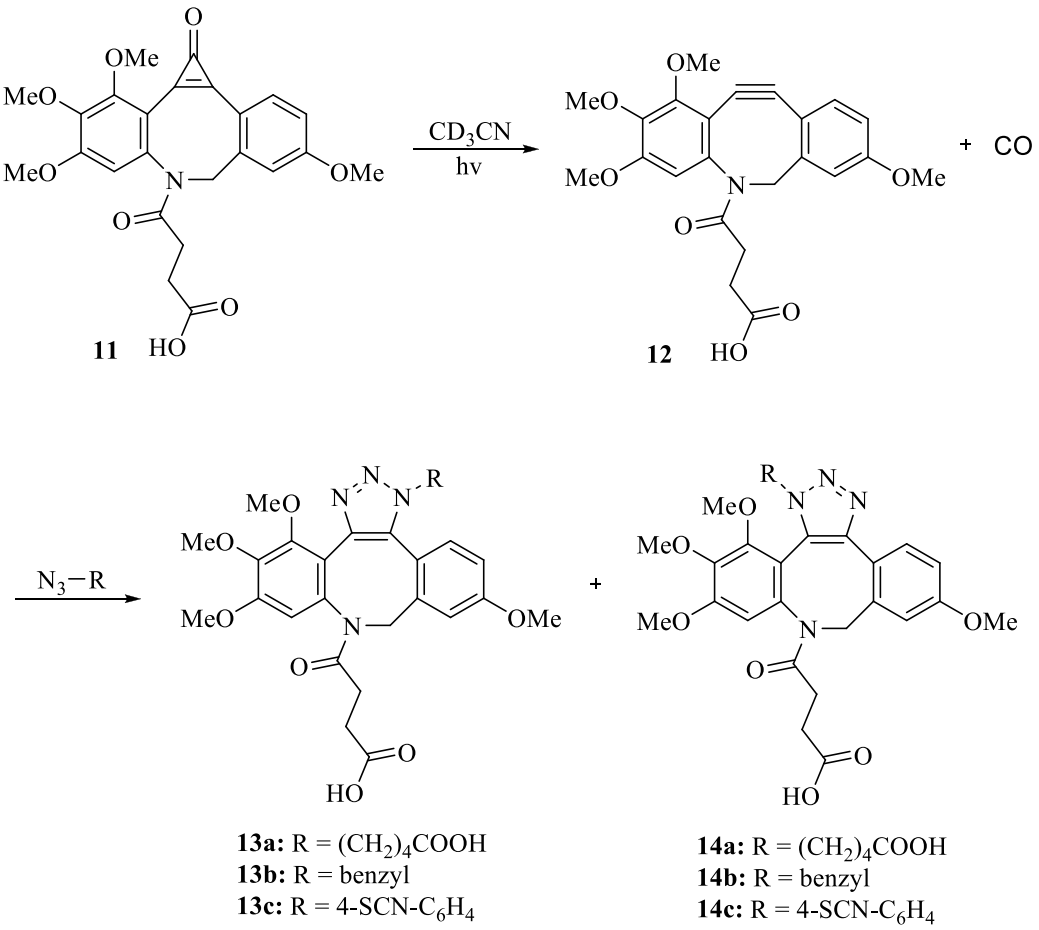
available 3,4,5-trimethoxyaniline and 3-methoxybenzaldehyde, followed by addition of sodium borohydride to obtain amine **9**. The selection of these two starting compounds was motivated by two intentions: On the one hand the number and position of methoxy substituents were selected for maximum support of the ring closing reaction in *ortho*-position, on the other hand steric hindrance at only one site of the dibenzoazacyclooctyne should induce regioselectivity of the 'click reaction'. The reaction of **9** with succinic anhydride, 4-dimethylaminopyridine and triethylamine in dichloromethane gave acid **10** in high yield. A double Friedel-Crafts alkylation of **10** with tetrachlorocyclopropene (TCCP) in the presence of aluminium chloride eventually yielded the desired cyclopropenone **11**.



Scheme 3. Synthesis route for the production of **11**. *Reagents and conditions:* a: MeOH, 3 h, $NaBH_4$. b: $CHCl_3$, NEt_3, DMAP, succinic anhydride, 48 h, NaOH. c: CH_2Cl_2, $AlCl_3$, -78 °C to RT, 19 h, 1 M HCl.

The key step of this approach is the closure of the azocine ring with tetrachlorocyclopropene (TCCP). This reagent has already been used to obtain a substituted dibenzocyclooctyne[10], but not for the production of dibenzoazacyclooctynes. During the reaction, tetrachlorocyclopropene forms a trichlorocyclopropenium cation with anhydrous aluminium chloride. This cation is highly reactive and can perform the first electrophilic aromatic substitution on compound **10**. The resulting aryltrichlorocyclopropene can undergo an intramolecular Friedel-Crafts reaction

giving a diaryldichlorocyclopropene.[14] This intermediate was then hydrolyzed rapidly to cyclopropenone **11** by the addition of hydrochloric acid.



Scheme 4. Production of dibenzoazacyclooctyne **12** and its reaction with azides.

Cyclopropenone **11** was finally converted into dibenzoazacyclooctyne **12** by UV-irradiation whereat the release of carbon monoxide bubbles was observed. The completion of this reaction can be monitored in ^{13}C-NMR and IR-spectroscopy. The ^{13}C-NMR signals of the cyclopropenone (DMSO-d_6 δ = 135.7, 137.2 and 162.4 ppm) disappeared, while two new carbon signals of the alkyne function of **12** (DMSO-d_6, δ = 105.0 and 108.0 ppm) came out. According to this, the characteristic intensive carbonyl IR absorption band of cyclopropenone **11** at 1855 cm^{-1} disappeared during the formation of the alkyne **12**. Compound **12** reacts quickly with several azides to produce the triazoles **13a-c** and their corresponding regioisomers **14a-c**. The cycloaddition proceeded in high regioselectivity. Presumably due to the greater steric demand of the trimethoxy substituted phenyl ring - especially that of the methoxy group in *ortho*-position to the alkyne - the formation of **14** is less preferred. The use of aryl substituted azides like 4-azidophenyl isothiocyanate did not elevate the isomeric excess of **13c**.

Table 2. Ratio of regioisomers of triazoles **13a-c**/**14a-c** (for structures see Scheme 4)

Resulting triazoles	Ratio of regioisomers	
	triazole **13**	triazole **14**
a	4,0	1
b	3,8	1
c	1,2	1

The reaction of alkyne **12** with azides can be monitored by ^{1}H-NMR spectroscopy. To determine the reaction kinetics, the decrease of the signal of the proton located at the trimethoxy substituted phenyl ring was observed, as well as the increase of the two corresponding signals of the two newly formed triazole regioisomers. The second order reaction rate constants for the reaction of **12** with three different azides were ascertained.

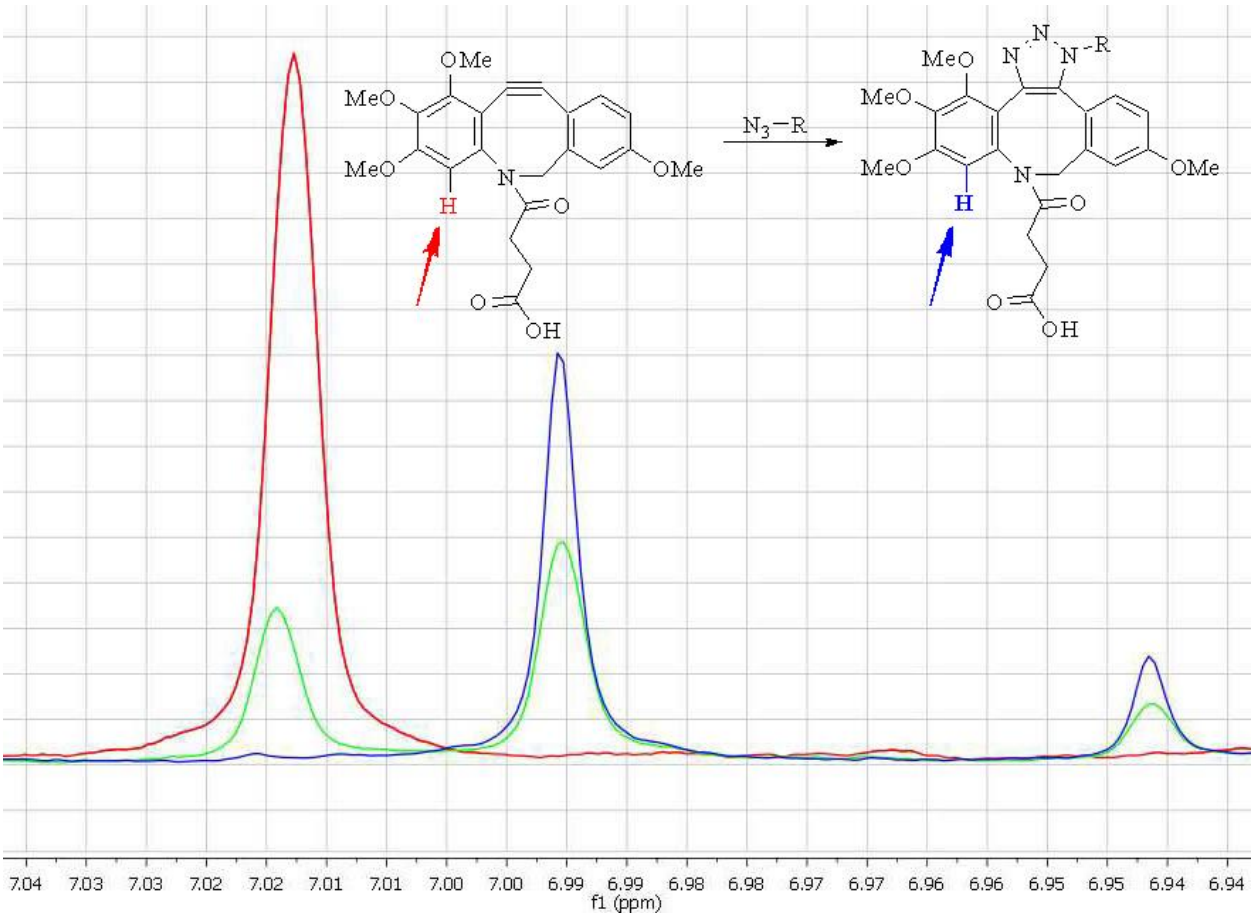


Figure 1. ^{1}H-NMR spectra of the reaction of **12** with 5-azidopentanoic acid forming triazole **13a** and regioisomer **14a** after 0 min (red), 70 min (green) and 20 h (blue) in CD_3CN.

Table 3. Second order reaction rate constants of **12** with azides

Azide	Resulting Triazoles	Temperature (°C)	k [x 10^{-3} M^{-1} s^{-1}]
5-azidopentanoic acid	**13a**, **14a**	25	6.3[a]
benzyl azide	**13b**, **14b**	25	8.0[b]
benzyl azide	**13b**, **14b**	37	8.8[b]
4-azidophenyl isothiocyanate	**13c**, **14c**	25	2.3[a]
4-azidophenyl isothiocyanate	**13c**, **14c**	37	3.7[a]

[a]in CD_3CN. [b]in DMSO-d_6.

As depicted in table 3, the reaction of dibenzoazacyclooctyne **12** with azides reached second order reaction rate constants up to 8.8 x 10^{-3} M^{-1} s^{-1}, which is, as we expected, throughout below those of the known dibenzoazacyclooctynes **7** and **8**. It is known, that a higher electron density at the alkyne function lowers the rate constant of the reaction with azides. The reaction is therefore decelerated because of the mesomeric effect of the four electron releasing methoxy groups. Future work will focus on the introduction of electron withdrawing groups into **11**, to significantly improve the kinetics of the cycloaddition. Also, the use of anilines and benzaldehydes with varying substituents as starting materials should be taken into consideration.

Conclusions

In summary, a fast and easy synthetic route for the production of the novel substituted dibenzoazacyclooctyne **12** has been accomplished in high yields, starting from commercial available and inexpensive 3,4,5-trimethoxyaniline and 3-methoxybenzaldehyde. Its fast and regioselective reaction with several azides makes **12** an interesting agent for copper free click reactions, e.g. for the attachment of an appropriate compound to an azide labeled bioactive molecule under mild conditions at room temperature. According to our expectations, the rate constant of the reaction of **12** with azides was lower than that of known dibenzoazacyclooctynes, preventing its application for *in vivo* usage. However, due to the fact that plenty of substituted anilines and benzaldehydes are commercially available, this strategy seems to be a convenient method for the synthesis of further substituted dibenzoazacyclooctynes with suitable properties for bioorthogonal reactions.

Experimental Section

General. Chemical reagents and solvents were purchased from commercial sources and used without further purification. UV-irradiation was carried out using an UV-lamp by Benda (2x6 Watt, wavelength 254 and 366 nm, respectively), whereat always both wavelengths were used.

^{1}H- and ^{13}C-NMR spectra were recorded on a 400 MHz Varian Inova spectrometer. The chemical shifts (δ, ppm) are relative to TMS. Elemental analyses were performed on a Leco Elemental Analyzer CHNS-932. Electrospray ionization mass spectrometry (ESI-MS) was carried out using a micromass tandem quadropole mass spectrometer (Quattro LC). Melting points were determined on a Mikroheiztisch Boetius apparatus by VEB Carl Zeiss Jena.

3,4,5-Trimethoxy-*N*-(3-methoxybenzyl)aniline (9). To a solution of 3,4,5-trimethoxyaniline (4 g, 21.83 mmol) in 60 ml anhydrous methanol was added 3-methoxybenzaldehyde (2.973 g, 21.83 mmol). After stirring for 3 h at room temperature, the yellow solution was treated with $NaBH_4$ (1.4 g, 37 mmol) and stirred for another 20 min. The reaction mixture was then quenched with 130 ml NaOH solution (c = 1 mol/l) and extracted with diethyl ether (3 x 60 ml). The combined organic phase was washed with brine, dried over Na_2SO_4 and concentrated in vacuum to provide **9** as a dark oil (6.572 g, 99.3% yield). **9** was used without further purification. ^{1}H NMR (399.91 MHz, $CDCl_3$), δ 3.70 – 3.80 (m, 12H), 4.24 (s, 2H), 5.85 (s, 2H), 6.78 – 6.80 (m, 1H), 6.90 – 6.94 (m, 2H), 7.21 – 7.25 (t, 1H, *J* = 7.80 Hz). ^{13}C-NMR ($CDCl_3$), δ 49.1, 55.4, 56.1, 61.3, 90.7, 112.9, 113.4, 120.0, 129.9, 130.4, 141.2, 145.2, 154.1, 160.1. ESI-MS, *m/z* = 304.56 $[M + H]^+$, 326.57 $[M + Na]^+$, 607.94 $[2M + H]^+$, 629.95 $[2M + Na]^+$, 302.44 $[M - H]^-$.

4-((3-Methoxybenzyl)(3,4,5-trimethoxyphenyl)amino)-4-oxobutanoic acid (10). To a solution of amine **9** (6.07 g, 20.01 mmol), triethylamine (3.04 g, 30.04 mmol) and DMAP (0.25 g, 2.05 mmol) in 50 ml anhydrous $CHCl_3$ was added succinic anhydride (4.1 g, 40.97 mmol) and the resulting mixture was stirred for 48 h at room temperature. The reaction was quenched by the addition of 80 ml NaOH solution (c = 1 mol/l), stirred for another 30 min, acidified by the addition of 110 ml hydrochloric acid (c = 1 mol/l) and extracted with $CHCl_3$ (3 x 50 ml). The combined organic phase was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. Purification by column chromatography using CH_2Cl_2: ethanol (7:1) gave **10** (6.7 g, 83% yield) as a slowly solidifying oil. ^{1}H NMR (399.91 MHz, $CDCl_3$), δ 2.41 (t, 2H, *J* = 7.2 Hz), 2.65 (t, 2H, *J* = 7.2 Hz), 3.63 – 3.78 (m, 12H), 4.76 (s, 2H), 6.16 (s, 2H), 6.71 – 6.74 (m, 3H), 7.12 (t, 1H, *J* = 8.20 Hz). ^{13}C-NMR ($CDCl_3$), δ 29.2, 29.8, 53.3, 55.4, 56.3, 61.1, 105.9, 113.4, 114.6, 121.5, 129.5, 137.4, 138.0, 139.1, 153.7, 159.8, 172.9, 177.1. ESI-MS, *m/z* = 404.52 $[M + H]^+$, 426.54 $[M + Na]^+$, 442.52 $[M + K]^+$. Anal. Calcd for $C_{21}H_{25}NO_7$: C, 62.52; H, 6.25; N, 3.47%. Found: C, 62.53; H, 6.25; N, 3.45 %.

4-Oxo-4-(2,3,4,9-tetramethoxy-1-oxo-1*H*-dibenzo[*b,f*]cyclopropa[*d*]azocin-6(7*H*)-yl)-butanoic acid (11). $AlCl_3$ (1.05 g, 7.87 mmol) and acid **10** (2.1 g, 5.20 mmol) were suspended in 120 ml anhydrous CH_2Cl_2 and cooled to -78 °C. A suspension of tetrachlorocyclopropene (0.934 g, 5.25 mmol) and $AlCl_3$ (1.6 g, 12 mmol) in 30 ml dry CH_2Cl_2 was added dropwise to the reaction mixture. After stirring for 3 h at -78°C, it was warmed to room temperature and stirred for another 16 h. The resulting mixture was quenched with 150 ml hydrochloric acid (c = 1 mol/l) and extracted with $CHCl_3$ (3 x 40 ml). The combined organic phase was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. Purification by column chromatography using CH_2Cl_2: ethanol (7:1) gave **11** (0,65 g, 28% yield) as a white solid. Mp

227 – 228 °C, IR (ν_{max}, cm^{-1}): 1854 (s, C=O). ^{1}H NMR (399.91 MHz, DMSO-d_6), δ 1.79 – 2.58 (m, 4H), 3.80 (s, 3H), 3.84 (s, 3H), 3.88 (s, 3H), 3.98 (s, 3H), 4.20 (d, 1H, *J* = 14.40 Hz), 4.97 (d, 1H, *J* = 14.80 Hz), 7.00 (dd, 1H, J^2 = 8.60 Hz, J^3 = 2.50 Hz), 7.15 (s, 1H), 7.20 (s, 1H), 7.70 (d, 1H, *J* = 8.80 Hz), 11.94 (s, 1H); ^{13}C-NMR (DMSO-d_6): δ 29.6, 56.2, 56.3, 57.3, 61.5, 63.2, 110.5, 110.9, 113.7, 116.0, 118.8, 135.7, 137.2, 141.4, 141.7, 143.1, 143.5, 151.3, 154.8, 157.3, 162.4, 171.9, 174.3. ESI-MS, *m/z* = 454.64 $[M + H]^+$, 476.60 $[M + Na]^+$, 492.60 $[M + K]^+$, 945.99 $[2M + K]^+$, 163.27 $[M + H + 2\ NH_4]^{3+}$. Anal. Calcd for $C_{24}H_{23}NO_8$-CO: C, 64.93; H, 5.45; N, 3.29 %. Found: C, 65.31; H, 5.28; N, 3.08%.

Oxo(1,2,3,8-tetramethoxy-11,12-didehydrodibenzo[*b,f*]azocin-5(6*H*)-yl)butanoic acid (12). Cyclopropenone **11** (20 mg, 47 µmol) was suspended in 1 ml acetonitrile in a quartz glass tube and irradiated for 1 h to give a yellow solution. The solvent was removed under reduced pressure to give **12** (18 mg, 96% yield) as a brown solid. Mp 227 – 228 °C. ^{1}H NMR (399.91 MHz, CD_3CN), δ 2.25 – 2.43 (m, 2H), 2.68 – 2.75 (m, 2H), 3.65 (d, 1H, *J* = 14.00 Hz), 3.81 (s, 3H), 3.82 (s, 3H), 3.84 (s, 3H), 4.04 (s, 3H), 4.97 (d, 1H, *J* = 14.00 Hz), 6.86 (dd, 1H, J^2 = 8.40, J^3 = 2.60 Hz), 7.02 (s, 1H), 7.16 (d, 1H, *J* = 8.40 Hz), 7.19 (d, 1H, *J* = 2.40 Hz). ^{13}C NMR (399.91 MHz, CD_3CN): δ 29.1, 29.5, 55.5, 55.9, 56.4, 60.8, 60.9, 104.7, 108.5, 110.3, 112.9, 115.0, 115.5, 119.0, 126.5, 141.3, 147.4, 150.3, 150.5, 153.9, 159.6, 172.2, 173.5; ESI-MS: *m/z* = 426.60 $[M + H]^+$, 448.65 $[M + Na]^+$, 868.97 $[2M + NH4]^+$. Anal. Calcd for $C_{23}H_{23}NO_7$: C, 64.93; H, 5.45; N, 3.29 %. Found: C, 65.17; H, 5.31; N, 3.10%.

General procedure for the determination of the second order rates of the reaction of (4) with azides

Cyclopropenone **11** (20.0 mg, 47 µmol) was suspended in 1 ml CD_3CN or 1 ml DMSO-d_6 respectively in a quartz glass tube and irradiated for 1 h, releasing alkyne **12**. To the resulting solution, azide (47 µmol) was added and the reaction was monitored by ^{1}H-NMR spectroscopy. The conversion was calculated by the disappearance of **12** relative to the formation of the triazoles as determined by integration (see figure 1). The second order reaction rate constant was determined by plotting the reciprocal of the concentration of alkyne **12** versus time. The plot was fitted to a linear regression and the slope equates to the second order reaction rate constant.

No products other than the two triazole regioisomers were apparent by ^{1}H-NMR. Since these experiments were focused on the examination of the reaction rate constants only, no attempts for isolation or separation of the resulting compounds have been made. NMR spectroscopic data is given for the main products **13a-c** only.

5-(8-(3-Carboxypropanoyl)-4,5,6,11-tetramethoxy-8,9-dihydro-1*H*-dibenzo[*b,f*][1,2,3]-triazolo[4,5-*d*]azocin-1-yl)pentanoic acid (**13a**) and **5-(8-(3-carboxypropanoyl)-4,5,6,11-tetramethoxy-8,9-dihydro-3*H*-dibenzo[*b,f*][1,2,3]triazolo[4,5-d]azocin-3-yl)pentanoic acid (14a).** Reaction in CD_3CN. ^{1}H NMR (399.91 MHz, CD_3CN), δ 1.59 – 1.61 (m, 2H), 1.68 – 1.76 (m, 2H), 1.94 (quintet, 2H, *J* = 2.5 Hz), 2.05 – 2.27 (m, 2H), 2.30 (t, 2H, *J* = 7.0 Hz), 3.30 (t, 2H, *J* = 6.3 Hz), 3.50 (s, 3H), 3.76 (s, 3H), 3.84 (s, 3H), 3.87 (s, 3H), 4.37 (d, 1H, *J* = 16.8 Hz), 5.80

(d, 1H, *J* = 16.8 Hz), 6.78 (d, 1H, *J* = 2.5 Hz), 6.81 – 6.87 (m, 1H), 7.00 (s, 1H), 7.36 (d, 1H, *J* = 8.6 Hz). ESI-MS, *m/z* = 569.63 $[M + H]^+$, 591.65 $[M + Na]^+$.

4-(1-Benzyl-4,5,6,11-tetramethoxy-1*H*-dibenzo[*b,f*][1,2,3]triazolo[4,5-*d*]azocin-8(9*H*)-yl)-4-oxobutanoic acid (13b) and **4-(3-benzyl-4,5,6,11-tetramethoxy-3*H*-dibenzo[*b,f*][1,2,3]triazolo-[4,5-*d*]azocin-8(9*H*)-yl)-4-oxobutanoic acid (14b).** Reaction in DMSO-d_6. ^{1}H NMR (399.91 MHz, DMSO-d_6), δ 1.65 – 2.38 (m, 4H), 3.39 (s, 3H), 3.70 (s, 3H), 3.80 (s, 3H), 3.83 (s, 3H), 4.35 (d, 1H, *J* = 17.0 Hz), 5.36 (d, 1H, *J* = 15.3 Hz), 5.49 (d, 1H, *J* = 15.2 Hz), 5.67 (d, 1H, *J* = 17.0 Hz), 6.77 (s, 1H), 6.82 – 6.87 (m, 2H), 7.04 (s, 1H), 7.13 – 7.31 (m, 5H). ESI-MS, *m/z* = 559.75 $[M + H]^+$, 581.76 $[M + Na]^+$.

4-(1-(4-Isothiocyanatophenyl)-4,5,6,11-tetramethoxy-1*H*-dibenzo[*b,f*][1,2,3]triazolo[4,5-*d*]-azocin-8(9*H*)-yl)-4-oxobutanoic acid (13c) and 4-(3-(4-isothiocyanatophenyl)-4,5,6,11-tetramethoxy-3*H*-dibenzo[*b,f*][1,2,3]triazolo[4,5-*d*]azocin-8(9*H*)-yl)-4-oxobutanoic acid (14c). Reaction in CD_3CN. ^{1}H NMR (399.91 MHz, CD_3CN), δ 2.45 – 2.54 (m, 2H), 2.74 – 2.82 (m, 2H), 3.13 (s, 3H), 3.59 (s, 3H), 3.79 (s, 3H), 3.87 (s, 3H), 4.94 (d, 1H, *J* = 18.4 Hz), 5.53 (d, 1H, *J* = 18.4 Hz), 6.88 (m, 1H), 6.90 (m, 1H), 7.01 (s, 1H), 7.36 - 7.39 (m, 2H), 7.46 (d, 1H, *J* = 8.6 Hz), 7.54 – 7.58 (m, 2H). ESI-MS, *m/z* = 602.55 $[M + H]^+$, 624.58 $[M + Na]^+$.

References

1. Sletten, E. M.; Bertozzi, C. R. *Angew. Chem. Int. Ed.* **2009**, *48*, 6974.
2. Ning, X.; Temming, R. P.; Dommerholt, J.; Guo, J.; Ania, D. B.; Debets, M. F.; Wolfert, M. A.; Boons, G.-J.; van Delft, F. L. *Angew. Chem. Int. Ed.* **2010**, *49*, 3065.
3. Jewett, J. C.; Bertozzi, C. R. *Chem. Soc. Rev.* **2010**, *39*, 1272.
4. Debets, M. F.; van der Doelen, C. W. J.; Rutjes, F. P. J. T.; van Delft, F. L. *ChemBioChem* **2010**, *11*, 1168.
5. Wittig, G.; Krebs, A. *Chem. Ber.* **1961**, *94*, 3260.
6. Agard, N. J.; Prescher, J. A.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2004**, *126*, 15046.
7. Agard, N. J.; Baskin, J. M.; Prescher, J. A.; Lo, A.; Bertozzi, C. R. *ACS Chem. Biol.* **2006**, *1*, 644.
8. 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. USA*, **2007**, *104*, 16793.
9. Ning, X.; Guo, J.; Wolfert, M. A.; Boons, G.-J. *Angew. Chem.* **2008**, *120*, 2285.
10. Poloukhtine, A. A.; Mbua, N. E.; Wolfert, M. A.; Boons, G.-J.; Popik, V. V. *J. Am. Chem. Soc.* **2009**, *131*, 15769.
11. 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*, 97.
12. Jewett, J. C.; Sletten, E. M.; Bertozzi, C. R. *J. Am. Chem. Soc*. **2010**, *132*, 3688.
13. Majumdar, K. C.; Nandi, R. K.; Samanta, S.; Chattopadhyay, B. *Synthesis* **2010**, *6*, 985.
14. Poloukhtine, A.; Popik, V. V. *J. Org. Chem.* **2003**, *68*, 7833.