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Graphical Abstract

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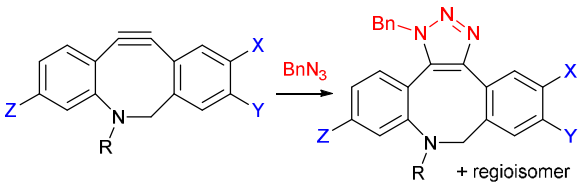

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ARTICLE

Synthesis of DIBAC analogues with excellent SPAAC rate constants

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In search for increased reactivity in strain-promoted azide alkyne cycloadditions (SPAAC), the synthesis of new and more reactive cyclooctynes is of pivotal importance. To identify cyclooctynes with enhanced reactivity, without loss of stability, the synthesis and kinetic analysis of new dibenzoazacyclooctyne (DIBAC) analogues were conducted. Starting from iodobenzyl alcohol analogues and *ortho*-ethynylaniline various substituted dihydrodibenzo[*b*,*f*]azocines were produced. Subsequent bromination and elimination proved to be difficult depending on the aromatic substitution pattern, yielding chloro-, bromo-, and methoxy-substituted DIBACs in moderate yield. In the elimination reaction towards nitro- and Br,Cl-DIBAC, the corresponding cyclooctene was obtained instead of the cyclooctyne. Additionally, a dimethoxy-substituted DIBAC analogue was prepared following an alternative route involving light-induced deprotection of a cyclopropenone derivative. In total, four DIBAC analogues were successfully prepared showing excellent rate constants in the SPAAC reaction ranging from 0.45 to 0.9 $M^{-1}s^{-1}$, which makes them comparable to the fastest cyclooctynes currently known.

Introduction

Selective bioorthogonal ligation strategies for the investigation of biological processes and biomolecule modification have become increasingly important in the last decade. Currently, the azide function is the most commonly applied reactive group in bioorthogonal chemistry being utilised in the Staudinger ligation,[1] the Cu(I)-catalysed azide–alkyne cycloaddition (CuAAC),[2,3] and the strain-promoted azide–alkyne cycloaddition (SPAAC).[4,5] The advantages of SPAAC over the Staudinger ligation include, depending on the cyclooctyne used, an increased reactivity and, as opposed to phosphines, the stability under ambient conditions. Unlike CuAAC, SPAAC avoids the use of a toxic Cu(I)-catalyst.

Since the first application of SPAAC in a biological system,[6] a wide range of cyclooctynes and dibenzocyclooctynes has been developed.[5,7,8] Each cyclooctyne displays specific advantageous characteristics, *e.g.* excellent rate constant,[9-11] good water solubility,[12,13] synthetic ease,[14] and/or fluorogenic properties, but nevertheless requires improvement.[15,16] In particular hydrophilicity, reactivity, stability, and selectivity are key aspects for a wider applicability of the strained alkynes in a biological context.

The fastest dibenzocyclooctyne currently known is BARAC (**2**) with a rate constant of 0.9 $M^{-1}s^{-1}$. It was recently shown that the reactivity of BARAC could be tuned by the introduction of substituents on the aromatic rings.[17] BARAC, however, has the disadvantage that it is susceptible to Michael addition by thiols.[10] Another interesting cyclooctyne is DIBAC (**1**)[9] (also referred to as ADIBO[18] or Aza-DBCO),[19] designed in analogy to DIBO,[20] displaying a rate constant of 0.3 ± 0.1 $M^{-1}s^{-1}$. Unlike BARAC, DIBAC shows no Michael addition product when reacted with glutathione, even at elevated temperatures. In addition, DIBAC showed complete shelf-stability when stored in neat form at –20 ºC, and was stored in aqueous solution for over a year at 4 ºC without noticeable loss of reactivity. Presumably, it is this optimal combination of reactivity and stability which has made DIBAC the most commonly used cyclooctyne for SPAAC applications.

We envisioned that the addition of substituents on the aromatic rings of DIBAC would lead to an increase in reactivity while retaining stability. Based on previous studies,[17,21] we anticipated that electron-withdrawing groups could have a substantial positive effect on the reactivity. In addition, we expected a change in reactivity based on the positioning of substituents on the aromatic rings. To investigate these hypotheses we aimed to prepare a series of DIBAC analogues (**3a-f**, Figure 1) following a previously reported synthetic route[9] and investigate the rate constants in SPAAC reactions with benzyl azide.

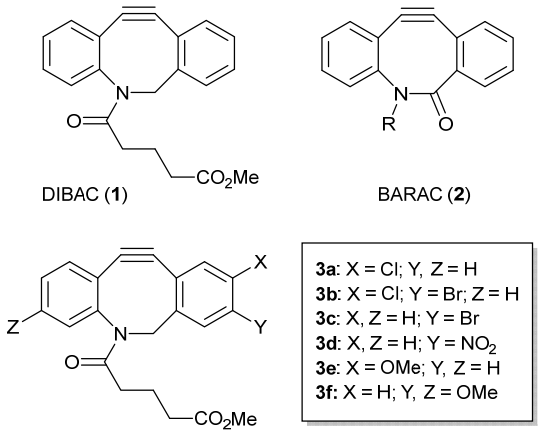
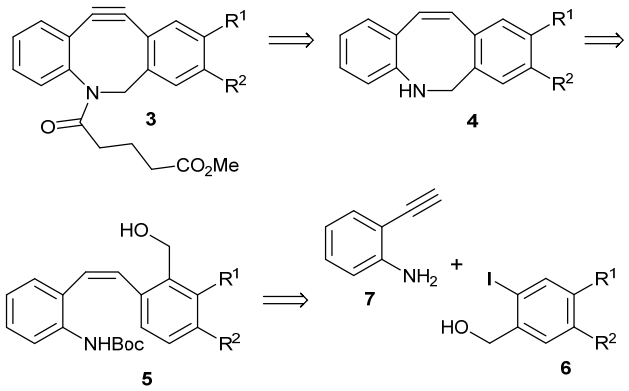


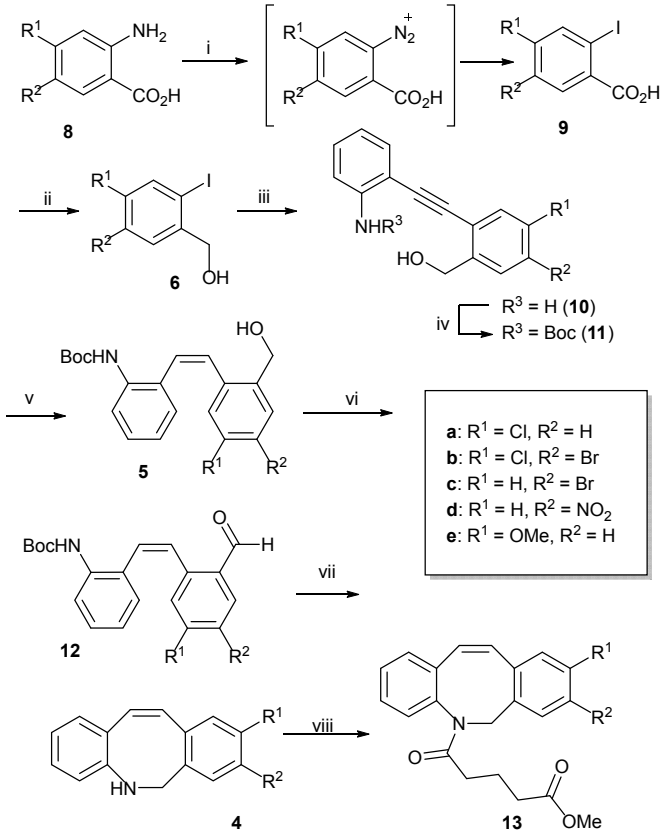
Figure 1. DIBAC (**1**), BARAC (**2**) and target DIBAC-analogues **3a-f**.

Retrosynthesis of the proposed DIBAC analogues **3a-e** involved acylation of dihydrodibenzoazocine **4**, bromination and subsequent elimination (Scheme 1). The key intermediate **4** was envisaged to be prepared from Z-olefin **5** in two steps, which in turn was prepared from 2-ethynylaniline (**7**) and substituted iodobenzyl alcohol derivatives **6**. A different strategy was envisaged for DIBAC analogue **3f**.



Scheme 1. Retrosynthetic analysis of the synthesis of DIBAC analogues **3a-e**.

Synthesis of cyclooctene intermediates. The synthesis commenced with the readily available anthranilic acids **8**, which were transformed into the aryl iodides **9** via the corresponding diazonium salts (Scheme 2). Subsequently, the carboxylic acids were reduced to the corresponding alcohols **6**, which underwent Sonogashira coupling with *ortho*-ethynylaniline (**7**) to produce the chloro-, bromo-, nitro- and methoxy-substituted iodobenzyl alcohols **10a**, **c-e** in excellent yields (89-100%, Table 1).[9] The *para*-bromo derivative **10b** was obtained via a similar pathway after bromination of 4-chloroanthranilic acid **8a**.



Scheme 2. Synthesis of dihydroazocines **13a-e**. *Reagents:* (i) 1) 30% H_2SO_4, $NaNO_2$, DMSO; 2) KI, H_2O; (ii) 1) $ClCO_2Et$, NEt_3, CH_2Cl_2; 2) $NaBH_4$, H_2O; (iii) 2-ethynylaniline (**7**), $PdCl_2(PPh_3)_2$, CuI, Et_3N, THF, N_2/H_2-atmosphere; (iv) Boc_2O, THF, 70 ºC; (v) 10% $Pd/BaSO_4$, quinoline, H_2-gas, MeOH; (vi) Dess-Martin periodinane, $NaHCO_3$, CH_2Cl_2; (vii) 1) 2 M HCl in EtOAc; 2) $NaBH_4$, H_2O; (viii) $ClCO_2C_3H_6CO_2Me$, NEt_3, CH_2Cl_2.

Acetylenes **10a-e** were Boc-protected yielding compounds **11** (Scheme 2), which proceeded smoothly except for analogues containing strongly electron-withdrawing substituents (*i.e.* **10b** and **d**, Table 1). Next, partial Z-selective hydrogenation of the triple bond was in order. For compounds **11a-c** the hydrogenation gave excellent yields, while for compounds **11d** and **e** full reduction to the alkane was observed indicating a strong effect of the electron-withdrawing and electron-donating groups on the hydrogenation rate. Fortunately, compounds **5d** and **e** could be obtained in high yields by increasing the amount of quinoline (0.5 and 5 equivalents for the conversion of **11d** and **e**, respectively). Subsequent steps towards dihydrodibenzoazocines **4** included Dess-Martin oxidation, yielding **12**, Boc-deprotection, and ring-closing reductive amination. For almost all substrates, these reactions proceeded in good to excellent yields (Table 1). Only in case of compound **4b**, reductive amination was troublesome, since the reduction of the imine went sluggishly and resulted in side product formation.

In our previously reported DIBAC synthesis, we observed that acyl protection of the ring-nitrogen prior to formation of the alkyne was required, either using a protecting group or a suitable linker. Attempts to form DIBAC with an unprotected nitrogen resulted in indole formation.[9] Linker attachment to compound **4** proceeded swiftly, producing **13b-c**, and **e** in good yields (Table 1). Linker attachment to compounds **4a** and **4d**

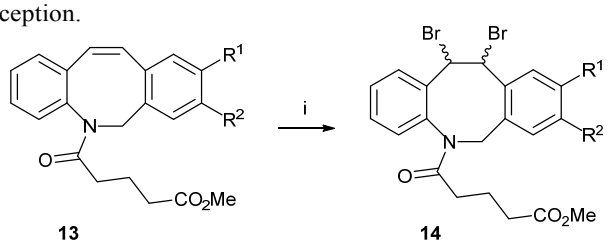
led to the formation of side products, resulting in relatively low yields (46 and 36%, respectively).

Table 1. Results for the synthesis of dihydroazocines **13a-e**.

Entry	R^1	R^2	**6** (%)	**10** (%)	**11** (%)	**5** (%)	**12** (%)	**4** (%)	**13** (%)
1	Cl	H	**a** (75)	**a** (95)	**a** (81)	**a** (97)	**a** (94)	**a** (95)	**a** (46)
2[a]	Cl	Br	**b** (46)	**b** (94)	**b** (57)[b]	**b** (100)	**b** (88)	**b** (50)	**b** (82)
3	H	Br	**c** (54)	**c** (100)	**c** (88)	**c** (90)	**c** (86)	**c** (91)	**c** (90)
4	H	NO_2	**d** (55)	**d** (89)	**d** (51)[b]	**d** (78)	**d** (86)	**d** (100)	**d** (37)
5	OMe	H	**e** (54)	**e** (91)	**e** (80)	**e** (90)	**e** (78)	**e** (87)	**e** (76)

[a] Substrate **6b** was prepared from **6a** using Br_2 in AcOH, resulting in a mixture of two products, which could be separated at the iodobenzyl alcohol stage. The crude yield was 81%. [b] For **9b**, 87% based on recovered starting material (b.r.s.m.), for **9d**, 91% b.r.s.m. [c] Yield starting from compound **6a**.

Bromination yields *cis*- and *trans*-isomers. Crucial steps in the formation of the substituted cyclooctynes are bromination and subsequent double elimination. Bromination of **13** to form dibromides **14** generally proceeded rapidly and in good yields with the bromination of compound **13e** being the only exception.



Scheme 3. Bromination of dihydroazocines **13** yielding dibromides **14**. *Reagents:* (i) Br_2, CH_2Cl_2.

Surprisingly, bromination led to two diastereoisomers (**X** and **Y**), being the corresponding *cis*- and *trans*-isomers. The ratio between the two isomers changed depending on the substituents on the aromatic ring and was determined either by separation by column chromatography or by comparing the signals originating from one of the two benzylic protons in the ^{1}H-NMR spectra of the crude reaction mixtures (assigned with either * or º in Figure 2). This phenomenon was already observed in the synthesis of DIBAC (**1**) showing a ratio of 1:0.18 (**X**:**Y**). While synthesising the DIBAC analogues, we noted that electron-withdrawing groups on the aromatic ring significantly changed the ratio between the two isomers. For example, in case of Br,Cl-compound **14b** and nitro-compound **14d** isomer **Y** was obtained as the major isomer (Table 2, entries 2 and 4, and Figure 2).

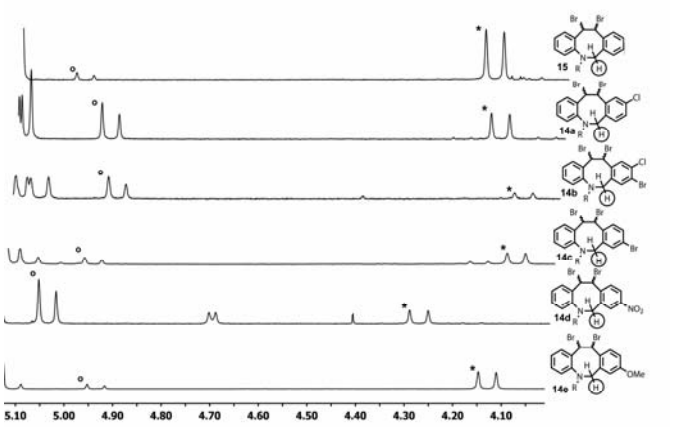


Figure 2. Diastereoisomers **X** (marked with *) and **Y** (marked with º) of dibromides **14a-e** and **15**. The ratio as observed in the displayed spectra is not always representative for the ratio of the isolated isomers.

Table 2. Overview of synthesis of dibromides **14**.

Entry	Starting Material	R^1	R^2	Product (%)	Ratio **X**:**Y**[a]
1	**13a**	Cl	H	**14a** (71)	1:0.8
2	**13b**	Cl	Br	**14b** (96)	0.32:1
3	**13c**	H	Br	**14c** (80)	1:0.47
4	**13d**	H	NO_2	**14d** (84)	0.42:1
5	**13e**	OMe	H	**14e** (39)	1:0.2

[a] For the non-substituted dibromide (**15**), the ratio between **X**:**Y** was 1:0.18.

Remarkably, while applying the bromination/elimination methodology this observation has largely been ignored by various groups constructing DIBAC.[18,19] Recently, Schubert and co-workers described in their synthesis of DIBAC, utilising a Hiyama-Heck coupling to form the dibenzo[*b,f*]azocine core structure, the formation of two conformers upon bromination.[22] This effect was ascribed to the cisoid/transoid interconversion of the exocyclic amide bond. Our results, however, suggest that the observed peaks in ^{1}H-NMR are the result of different diastereoisomers, namely the *cis*- and *trans*-isomer, being formed. Consequently we set out to identify the different, isolated isomers of the bromination reaction. Focussing on the differences in NMR, the most notable dissimilarity is the difference in chemical shift of one of the benzylic protons (H^2, * vs. º, Figure 2), possibly caused by the shielding of H^2 by one of the bromides. In addition, the coupling constants for H^3 and H^4 are different for the *cis*- and *trans*-isomer for all analogues (J = 10 Hz (for **X**) vs. J = 9.4 Hz (for **Y**)), indicative of a difference in dihedral angle between the two protons.

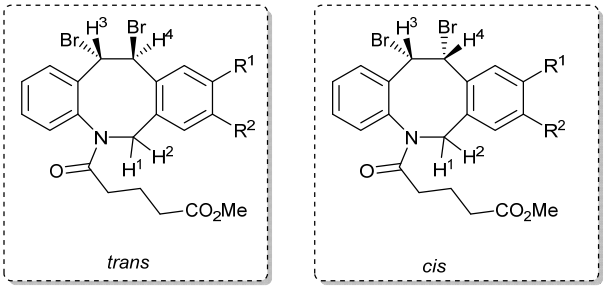


Figure 3. The *trans*- and *cis*-isomers obtained by cyclooctene bromination.

To determine the distance between the bromides and H^2 and the dihedral angle between H^3 and H^4, the *trans*- and *cis*-

compounds (Figure 3) were modelled using ChemBio3D with MOPAC interface. The results of these structure minimisations are depicted in Figure 4. For the *trans*-conformer (–146.8 kcal/mol), one bromide atom is in close proximity of H^2 (2.8 Å), while for the *cis*-conformers two energy minima were obtained (–89.3 kcal/mol and –90.6 kcal/mol) which showed a distance between H^2 and the closest bromide atom equals either 4.4 Å or 2.8 Å (*cis*-**1** and *cis*-**2**, respectively). The average distance between a bromide atom and H^2 is smaller for the *trans*-isomer compared to the *cis*-isomer, suggesting that for this isomer H^2 is more shielded, and hence shows a smaller chemical shift, thus suggesting that conformer **X** corresponds to the *trans*-isomer.

An additional support for this assignment was obtained by comparing the dihedral angle between H^3 and H^4 (highlighted in yellow in Figure 4) for the *cis*- and *trans*-isomers (113.2º, and 19º, respectively). According to the Karplus curve coupling constants become larger with increasing deviation from the 90º dihedral angle. Since the *trans*-isomer shows a larger coupling constant, this is yet another strong indication for **X** being the *trans*-isomer.

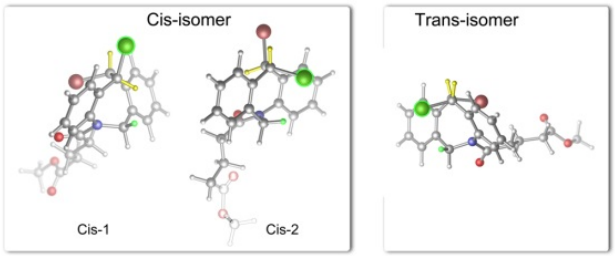
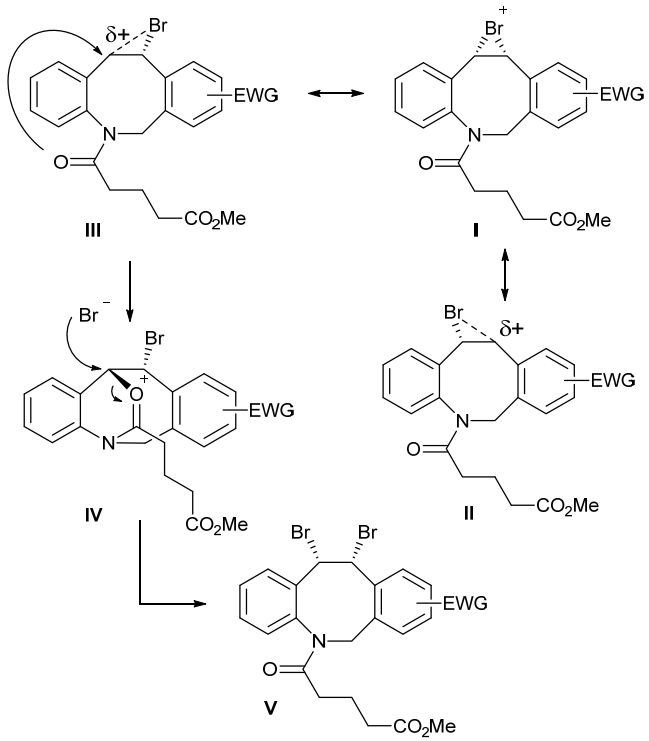


Figure 4. 3D models of energy-minimised structures of the *cis*- and *trans*-isomers of compound **15**. For the *cis*-isomer, two energy minima were observed. To clarify the atoms used for distance measurements in all models, H^2 and one bromide are highlighted in green, H^3 and H^4 are highlighted in yellow.

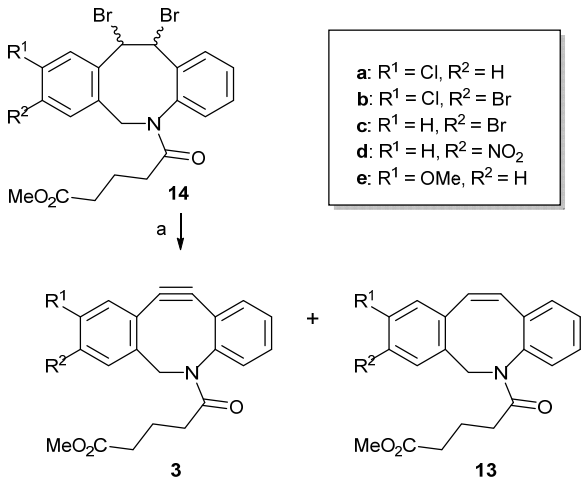
In addition, we observed that **Y** slowly isomerises to **X** in solution, which also indicates that **X** is the *trans*-isomer, as this is the thermodynamically more stable isomer. Summarising, we conclude that the *trans*-isomer is the main product for dibromides **15**, **14a**, **14c**, and **14e**, while the *cis*-isomer is the main product for dibromides **14b** and **14d**.

The rationale for the formation of the unexpected *cis*-isomer most likely involves neighbouring group participation.[23] Scheme 4 depicts the possible resonance structures **II** and **III** of bromonium ion **I**, which are both stabilised by the aromatic rings. However, with electron-withdrawing groups on one of the rings, a (partial) positive charge (**II**) is significantly destabilised, compared to **III**. Possibly, when structure **III** is favoured, neighbouring group attack is more likely compared to bromide attack, forming intermediate **IV** as shown in Scheme 4. Attack of the bromide anion on this intermediate structure would then lead to *cis*-isomer **V**.



Scheme 4. Tentative mechanism for formation of *cis*-isomer **V** from bromonium ion **I**.

Bromide elimination. The last step in the synthesis of the DIBAC analogues involved double elimination of the two bromides. Optimisation performed for DIBAC (**1**) had shown that elimination proceeds smoothly using slightly more than two equivalents of a 1 M KO^{t}Bu solution in THF. To investigate whether the elimination would proceed differently for the two stereoisomers of **14a**, elimination was performed with one of the isomers and compared to the outcome of the elimination on a 1:1 mixture (only the major isomer could be obtained in pure form). Upon reaction with KO^{t}Bu, the major isomer was successfully converted into alkyne **3a** (yield 35%) with only slight contamination of alkene **13a** (2%), whereas for the 1:1 mixture alkyne **3a** was obtained with a 20% contamination of alkene **13a**. The latter indicated that the minor diastereoisomer had yielded 24% of alkene and 76% of alkyne. Notably, the isomer giving the best conversion to the alkyne, was the major isomer for compound **14** (see also Table 2). Treatment of the *anti*-isomer of **14c** and **14e** with KO^{t}Bu successfully yielded cyclooctynes **3c** and **3e** in moderate yields (35 and 34%, respectively) with little to no alkene formation (2 and 0%, respectively). In contrast, double elimination of analogues **14b** and **14d** (isomer mixtures) mainly resulted in alkene formation, while no alkyne was observed (Table 3). In both cases, alkene product formation was confirmed by mass analysis and ^{1}H NMR spectroscopy (see: Supporting Information Figure S1).



Scheme 5. Elimination of dibromides **14a-e**, yielding alkynes **3** and/or alkenes **13**. *Reagents:* (a) 1 M KO^{t}Bu in THF.

Table 3. Products obtained after elimination of compounds **14**.

Entry	**14**	R^1	R^2	**3** (%)	**13** (%)
1	**a**	Cl	H	**a** (35)	**a** (2)
2	**b**	Cl	Br	**b** (0)	**b** (n.d.[a])
3	**c**	H	Br	**c** (35)	**c** (2)
4	**d**	H	NO_2	**d** (0)	**d** (n.d.[a])
5	**e**	OMe	H	**e** (34)	**e** (0)

[a] Yield was not determined (n.d.)

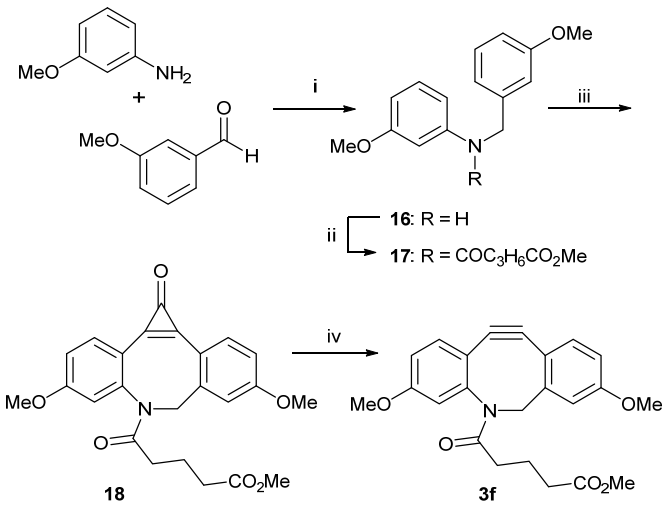
In literature, the elimination of vicinal halogen atoms to give the corresponding alkene has been described for dihalides of stilbenes.[24] In addition, when the acetylene is extremely hard to form via dibromide elimination, alkene formation has been previously observed.[25,26] Furthermore, a potential mechanism for alkene formation using alkoxides was proposed by Ramazan and Osman in 2004.[27] In an attempt to obtain Br,Cl-DIBAC (**3b**) and NO_2-DIBAC (**3d**) via bromination and subsequent elimination, screening of different bases was performed. Bases which had already proven unsuccessful in the synthesis of DIBAC were LDA, aqueous NaOH and ^{n}BuLi.[9] In addition, we tried NaH,[28,29] KH, DBU, Verkade's base and NaHMDS, however, none of them was successful (Supporting Information, Table S1). As an alternative, we envisioned that the alkyne could also be obtained via dihydroxylation of the alkene (**13b** or **d**), followed by triflation and elimination. Unfortunately, double hydroxylation was not successful in our hands. Mono-hydroxylation, followed by oxidation and formation of the enol triflate did not result in formation of the desired product either.

In conclusion, Cl-DIBAC (**3a**), Br-DIBAC (**3c**), and MeO-DIBAC (**3e**) were obtained in moderate yields over 10 steps (6, 7, and 2.5%, respectively). In addition, Cl-DIBAC (**3a**) was stored at –20 ºC for two years without detectable degradation or loss of activity.

DIBAC analogue via a cyclopropenone. The aforementioned results demonstrated that alkyne formation is the limiting step in the synthesis of DIBAC derivatives. To prepare additional DIBAC analogues, an alternative strategy was pursued based on the work described by Popik *et al*. involving a method to prepare dibenzocyclooctynes via the formation of a cyclopropenone ring, followed by UV irradiation.[13, 30]

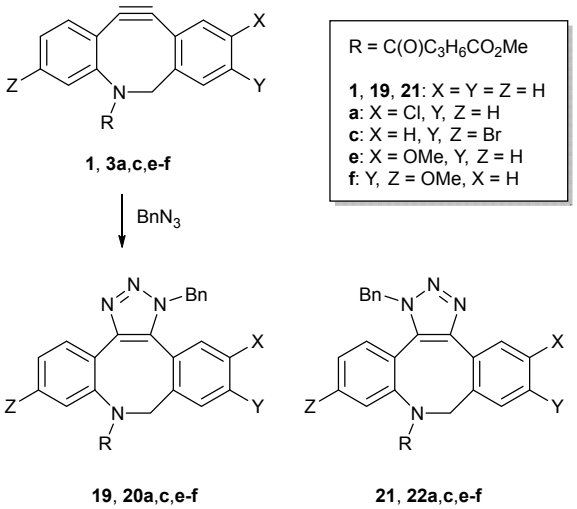
Pietzsch *et al.* utilised this method for the preparation of a DIBAC analogue containing four methoxy groups which, however, showed a drop in reaction rate (SPAAC with benzyl azide) of two orders of magnitude.[31] This decrease in reactivity was most likely caused by steric hindrance of the methoxy group positioned at the *ortho*-position relative to the alkyne. It has been shown that *ortho*-substituents cause a significant decrease in reactivity for BARAC, while *para*-substitution did not show this effect.[17] In order to increase reactivity, while retaining the beneficial hydrophilicity increase by the methoxy-groups we aimed for synthesis of compound **3f**.

The synthesis of **3f** started with a reductive amination of 3-methoxybenzaldehyde with 3-methoxyaniline, yielding amine **16** in good yield (98%). Next, a functionalisable linker was attached, giving rise to compound **17** in 54% yield. Compound **17** was subjected to a double Friedel-Crafts alkylation (Scheme 6), providing cyclopropenone **18** in a moderate yield (30%). This low yield was mainly caused by the formation of side products as a result of poor regioselectivity of the double Friedel-Crafts alkylation. In the final step, cyclopropenone **18** was subjected to UV light irradiation (320-500 nm, absorption spectrum shown in Supporting Information) to produce alkyne **3f**. While clean and full conversion was observed by ^{1}H-NMR, product isolation by column chromatography only yielded 35% of the desired product. This indicates poor silica stability of the product and hence explains the one-pot procedure described in literature, *i.e.* alkyne formation and subsequent cycloaddition.[30] Although this route involves significantly less steps, overall yields are comparable to the aforementioned route. Disadvantage of the cyclopropenone route is that electron-donating substituents are required to direct the Friedel-Craft reaction, thus limiting the scope of this route.



Scheme 6. Synthesis of DIBAC-analogue **3f**. *Reagents and conditions:* (i) 1) dry MeOH, r.t., 1.5 h; 2) $NaBH_4$, r.t., 30 min (98%); (ii) $ClCO_2C_3H_6CO_2Me$, Et_3N, CH_2Cl_2, 0 ºC, 1.5 h (54%); (iii) 1) $AlCl_3$, perchlorocycloprop-1-ene, CH_2Cl_2, -78 ºC to r.t., o.n.; 2) 1M HCl, r.t., 5 min; (30%); (iv) MeCN, UV-light (320-500 nm), r.t. 65 min (35%).

Kinetics of DIBAC analogues. With various DIBAC analogues in hand, we set out to determine the influence of the different substitutions on the reaction rate of cycloaddition. All DIBAC derivatives were reacted with benzyl azide in deuterated methanol, as depicted in Scheme 7.



Scheme 7. Cycloaddition of DIBAC (**1**) and DIBAC analogues (**3a**, **c**, **e**, and **f**)) with benzyl azide, yielding both regioisomers.

To increase the comparability of the kinetics for **1**, **3a**, **c**, **e**, and **f**, the same stock solution of benzyl azide in deuterated methanol was used for all experiments. The rate constants obtained are depicted in Figure 5. The results show that having substituents on the DIBAC core structure gave rise to an increase of the rate constant, corresponding to the observations made for BARAC analogues.[17] The electron-withdrawing effect of the halogens (*i.e.* **3a** and **3c**) proved to have the largest influence on the rate of the cycloaddition. Comparing the rate constants for MeO-DIBAC (**3e**) and $(MeO)_2$-DIBAC (**3f**) we found a rate constant for **3e** comparable to DIBAC (**1**), whereas **3f** reacted notably faster than DIBAC (**1**). The DIBAC analogue as reported by Pietzsch showed a rate constant which was two orders of magnitude smaller (0.8×10^{-2} $M^{-1}s^{-1}$).

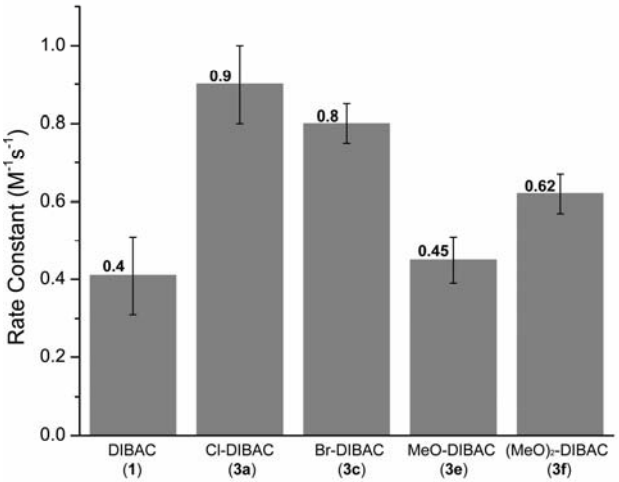


Figure 5. Rate constants for DIBAC and DIBAC-analogues. Standard errors are calculated based on the average of two to five measurements.

Examining the effect of the halogen substitution of DIBAC derivatives **3a** and **3c** on the rate constant, a two-fold increase in reaction rate compared to DIBAC was observed (0.9 and 0.8 $M^{-1}s^{-1}$, respectively). These rate constants are comparable to BARAC (**2**), the fastest non-substituted cyclooctyne currently known. It seems that the chloride substituent has a slightly larger positive effect on the rate constant than the bromide. This can possibly be explained by the larger electron-withdrawing effect of the chloride compared to the bromide. Interestingly, substituents on DIBAC appear to have a larger effect on the rate than observed for BARAC. For example, a fluoride substituent on the *meta*-position of BARAC relative to the alkyne, resulted in a 1.1 to 1.3-fold rate enhancement, while a *meta*-chloride substituent on DIBAC, being less electron-withdrawing than a fluoride, resulted in a 2.2 fold rate enhancement. A similar pattern was observed for substituents on the *para*-position.

Conclusions

In conclusion, substituted DIBAC analogues have been successfully prepared following our previously reported route for DIBAC (**1**). Until the synthesis of the intermediate cyclooctenes, the individual steps proceeded virtually identical to non-substituted DIBAC, proving the envisioned versatility of the route. Dibromination of these cyclooctenes proceeded in moderate to good yields, whereby it was noted that not only the expected *trans*-isomer was obtained, but also the *cis*-isomer. Depending on the substitution pattern on the aromatic rings, the *cis*/*trans* ratio varied; the amount of *cis*-isomer increased with an increasing number of electron-withdrawing groups on the aromatic ring. The identity of the *cis*- and *trans*-isomers was confirmed by NMR spectroscopy in combination with modelling studies.

The alkyne formation appeared the most troublesome step for all analogues in the cyclooctyne synthesis. Cl-DIBAC, Br-DIBAC, and MeO-DIBAC were obtained in moderate yields with little or no cyclooctene contamination. Inversely, despite extensive elimination attempts to produce Cl,Br-DIBAC and NO_2-DIBAC, the corresponding cyclooctenes were formed instead. To avoid the troublesome bromide elimination, a different route towards the diMeO-DIBAC analogue was successfully pursued, forming the cyclooctyne by UV-irradiation of a cyclopropenone precursor, following previously published precedent.[30]

Finally, the rate constants of the 1,3-dipolar cycloaddition with benzyl azide were determined for all four new DIBAC derivatives showing increased rate constants (0.9 (Cl), 0.8 (Br), 0.45 (MeO), and 0.62 $(MeO)_2$ $M^{-1}s^{-1}$) compared to the rate constant of DIBAC (0.4 $M^{-1}s^{-1}$). Gratifyingly, the rate constant of Cl-DIBAC is competitive with the rate constant of BARAC, while the stability of Cl-DIBAC is significantly higher, thus making this an unparalleled cyclooctyne.

Notes and references

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1 E. Saxon and C. R. Bertozzi, *Science*, 2000, **287**, 2007-2010.
2 V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem. Int. Ed.*, 2002, **41**, 2596-2599.
3 C. W. Tornøe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057-3064.
4 E. M. Sletten and C. R. Bertozzi, *Acc. Chem. Res.*, 2011, **44**, 666-676.

5 M. F. Debets, S. S. van Berkel, J. Dommerholt, A. J. Dirks, F. P. J. T. Rutjes and F. L. van Delft, *Acc. Chem. Res.*, 2011, **44**, 805-815.
6 N. J. Agard, J. A. Prescher and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2004, **126**, 15046-15047.
7 M. F. Debets, C. W. J. van der Doelen, F. P. J. T. Rutjes and F. L. van Delft, *ChemBioChem*, 2010, **11**, 1168-1184.
8 J. C. Jewett and C. R. Bertozzi, *Chem. Soc. Rev.*, 2010, **39**, 1272.
9 M. F. Debets, S. S. van Berkel, S. Schoffelen, F. P. J. T. Rutjes, J. C. M. van Hest and F. L. van Delft, *Chem. Commun.*, 2010, **46**, 97-99.
10 J. C. Jewett, E. M. Sletten and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2010, **132**, 3688-3690.
11 B. R. Varga, M. Kállay, K. Hegyi, S. Béni and P. Kele, *Chem. Eur. J.*, 2012, **18**, 822-828.
12 E. M. Sletten and C. R. Bertozzi, *Org. Lett.*, 2008, **10**, 3097-3099.
13 F. Friscourt, P. A. Ledin, N. E. Mbua, H. R. Flanagan-Steet, M. A. Wolfert, R. Steet and G.-J. Boons, *J. Am. Chem. Soc.*, 2012, **134**, 5381-5389.
14 J. Dommerholt, S. Schmidt, R. P. Temming, L. J. A. Hendriks, F. P. J. T. Rutjes, J. C. M. van Hest, D. J. Lefeber, P. Friedl and F. L. van Delft, *Angew. Chem. Int. Ed.*, 2010, **49**, 9422-9425.
15 F. Friscourt, C. J. Fahrni and G.-J. Boons, *J. Am. Chem. Soc.*, 2012, **134**, 18809-18815.
16 J. C. Jewett and C. R. Bertozzi, *Org. Lett.*, 2011, **13**, 3097-3099.
17 C. G. Gordon, J. L. Mackey, J. C. Jewett, E. M. Sletten, K. N. Houk and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2012, **134**, 9199-9208.
18 A. Kuzmin, A. A. Poloukhtine, M. A. Wolfert and V. V. Popik, *Bioconjugate Chem.*, 2010, **21**, 2076-2085.
19 L. S. Campbell-Verduyn, L. Mirfeizi, A. K. Schoonen, R. A. Dierckx, P. H. Elsinga and B. L. Feringa, *Angew. Chem. Int. Ed.*, 2011, **50**, 11117-11120.
20 X. Ning, J. Guo, M. A. Wolfert and G.-J. Boons, *Angew. Chem. Int. Ed.*, 2008, **47**, 2253-2255.
21 J. M. Baskin, J. A. Prescher, S. T. Laughlin, N. J. Agard, P. V. Chang, I. A. Miller, A. Lo, J. A. Codelli and C. R. Bertozzi, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 16793-16797.
22 M. Jager, H. Gorls, W. Gunther and U. S. Schubert, *Chem. Eur. J.*, 2013, **19**, 2150-2157.
23 G. Bellucci, R. Bianchini and S. Vecchiani, *J. Org. Chem.*, 1987, **52**, 3355-3362.
24 T. L. Jacobs, *Organic Reactions*, John Wiley & Sons , Inc., New York, 4 edn., 1949.
25 C. Harries and H. von Splawa-Neyman, *Ber. Dtsch. Chem. Ges.*, 1909, **42**, 693-698.
26 C. Harries, *Ber. Dtsch. Chem. Ges.*, 1912, **45**, 809-816.
27 E. Ramazan and C. Osman, *J. Chem. Res.*, 2004, 566-569.
28 I. Singh and F. Heaney, *Chem. Commun.*, 2011, **47**, 2706-2708.
29 C. Joce, J. Caryl, P. G. Stockley, S. Warriner and A. Nelson, *Org. Biomol. Chem.*, 2009, **7**, 635-638.
30 A. A. Poloukhtine, N. E. Mbua, M. A. Wolfert, G.-J. Boons and V. V. Popik, *J. Am. Chem. Soc.*, 2009, **131**, 15769-15776.
31. F. Starke, M. Walther and H.-J. Pietzsch, *ARKIVOC*, 2010, 350-359 (xi).