# Synthesis of Tetra(2-hydroxyethoxy)-Substituted Dibenzocyclooctyne Derivatives as Novel, Highly Hydrophilic Tool Compounds for Strain-Promoted Alkyne-Azide Cycloaddition Applications

Martin Golkowski,* Thomas Ziegler

Institut für Organische Chemie, Universität Tübingen, Auf der Morgenstelle 18, 72076 Tübingen, Germany
Fax +49(7071)295244; E-mail: Martin.Golkowski@uni-tuebingen.de

*Received: 17.02.2013; Accepted after revision: 05.03.2013*

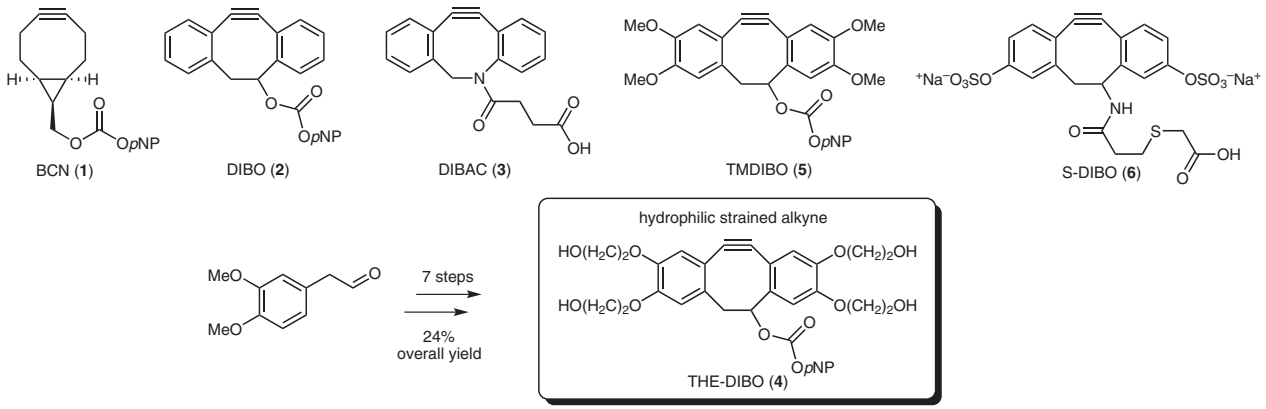
**Abstract:** The synthesis of a novel, hydrophilic dibenzocyclooctyne derivative for strain-promoted alkyne-azide cycloaddition (SPAAC) is described. Starting from 2-(3,4-dimethoxyphenyl)acetaldehyde, the corresponding activated carbonate of 2,3,8,9-tetrakis(2-hydroxyethoxy)-5,6-dihydro-11,12-didehydrodibenzo[*a*,*e*][8]annulene, termed THE-DIBO, was prepared in seven steps and 24% overall yield. A water-soluble THE-DIBO analogue showed a $k_2$ value of $(21.9 \pm 0.2) \times 10^{-2}$ $M^{-1}$ $s^{-1}$ for SPAAC with 2-azidoethanol in water. The high hydrophilicity and steric bulk of THE-DIBO derivatives should to suppress nonspecific thiol-yne addition of proteins to strained alkynes observed for SPAAC labeling in complex proteomes.

**Key words:** alkynes, azides, conjugation, proteins, SPAAC, click chemistry, thiol-yne addition, bioorthogonal chemistry

Over the last decade the copper-catalyzed Huisgen-cycloaddition reaction between an azide moiety and a terminal alkyne (CuAAC) has become a versatile chemical tool for the rapid assembly of complex organic structures.[1,2] Click reactions, such as the archetypical CuAAC, but also the Staudinger ligation reaction, oxime ligation, and natural chemical ligation, among others, are defined as processes that proceed chemoselectively, in high yield, and under mild reaction conditions.[3] The bioorthogonal character of CuAAC allowed efficient synthetic manipulation of biomolecules functionalized with the azide or alkyne moiety in vitro and in vivo.[4,5] More recently, the concept of strain-promoted azide-alkyne cycloaddition (SPAAC) has been introduced as a novel strategy for rapid and chemoselective conjugation of biomolecules and materials.[6] The methodology obviates the use of cytotoxic, environmentally harmful copper and can proceed at significantly higher reaction rates than classical CuAAC.

SPAAC has been shown to enable bioorthogonal conjugation of azide-functionalized biomolecules in various experimental settings, most prominently the labeling of azide-functionalized glycans on the surface of living cells. Nonetheless, in the more recent past reports on the incomplete chemoselectivity of SPAAC in a physiological environment have become more frequent.[6a,7,8] We and others have observed extensive nonspecific addition of biopolymers, i.e. proteins, to strained alkynes causing significant nonspecific background labeling alongside specific labeling of azide-bearing biomolecules.

Regarding this issue, cyclooctyne derivatives, such as compounds derived from bicyclo[6.1.0]nonyne (BCN) **1**,[9] dibenzocyclooctyne (DIBO) **2**,[6a] and azadibenzocyclooctyne (DIBAC) **3**[10] (Scheme 1), were shown to be susceptible to thiol-yne addition of proteins under physiological conditions. Whether the thiol-yne addition to strained alkynes follows a radical or ionic mechanism could not be elucidated unambiguously.[8,11] Thus, it was found that aliphatic thiols spontaneously add to cyclooctyne with exclusion of light. It was hypothesized that the conversion




**Scheme 1** Various cyclooctynes for SPAAC applications described in the literature and the novel, hydrophilic THE-DIBO **4**

**SYNTHESIS** 2013, 45, 1207–1214
Advanced online publication: 28.03.2013
DOI: 10.1055/s-0032-1316875; Art ID: SS-2013-T0888-OP

was triggered by reactive oxygen species and proceeds via free radicals, but the exact mechanism could not be clarified.[11]

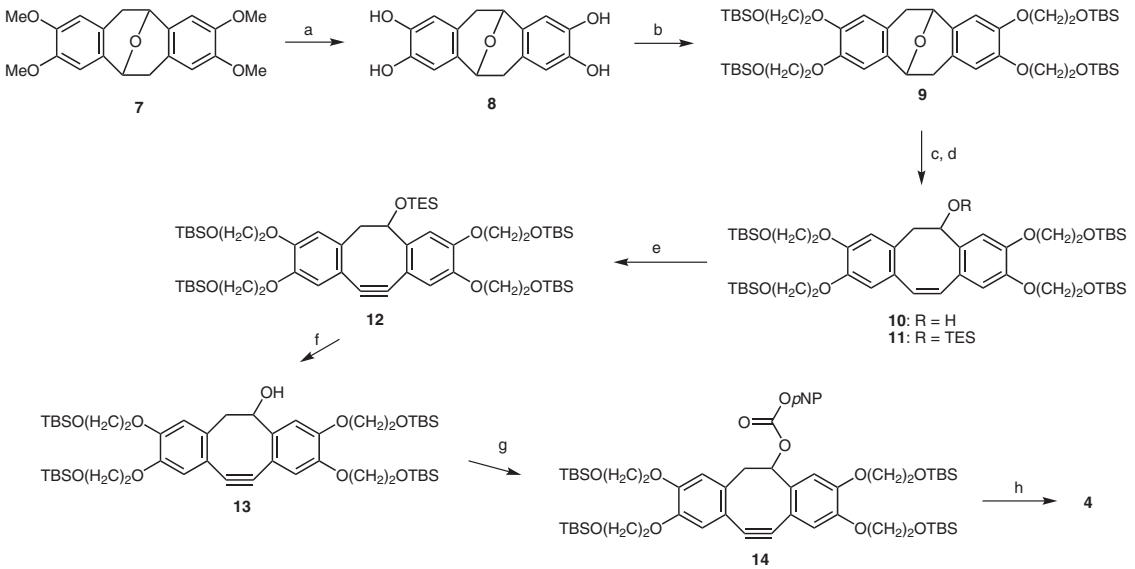
In a study published by van Geel et al.[8] it was found that thiol-yne addition to the strained alkynes **1**–**3** is not suppressed in the presence of sodium ascorbate. It was also found that small, soluble thiols [i.e., 2-mercaptoethanol (BME), dithiothreitol (DTT), and reduced glutathione (GSH)] as well as bovine serum albumin (BSA) compete for the thiol-yne addition by proteins only when present in large excess (>25 fold). Likewise, our findings and reports from other groups suggest that BME, DTT, and GSH do not readily add to strained alkynes derived from the DIBO scaffold, but that free thiols found in proteins (e.g., derived from whole-cell lysates) are capable of doing so (unpublished results from our group). Thus, it appeared feasible that reactive thiols in the hydrophobic environment of proteins preferentially engage in thiol-yne addition and said thiols are primarily responsible for nonspecific SPAAC labeling in complex proteomes.

In order to obtain a strained alkyne warhead exhibiting minimal interaction with proteins and thus having a lower tendency for nonspecific thiol-yne addition, the hydrophilic SPAAC reagent **4** was envisaged. Activated THE-DIBO **4** was synthesized in seven steps and 24% overall yield from commercially available starting materials. Furthermore, the reaction rate constants of the click reaction between a 1-methylpiperazine carbamate derivative of THE-DIBO **4** and 2-azidoethanol in water and methanol were determined by UV spectrophotometry[12] and compared with the corresponding DIBO **2** analogue. Finally, the stability of THE-DIBO **4** toward thiol-yne addition was studied by competitive treatment of our model compounds with 2-azidoethanol and GSH or BME in aqueous buffer solution using LC-MS.

The molecular architecture of **4** was inspired by the tetramethoxy-functionalized alkyne TMDIBO **5** (Scheme 1).[13] TMDIBO **5** was reported to be significantly more stable during storage and application in biological experimental settings than for example DIBO **2**, while retaining very fast reaction kinetics in the SPAAC reaction.[13] Due to these facts and the straightforwardness of the synthetic route leading to **5**, we deemed the tetraalkoxy-DIBO scaffold superior for our purposes as compared to the previously reported ionic S-DIBO **6** (Scheme 1).[12]

To increase the hydrophilicity as well as the steric bulk around the strained alkyne moiety, the methoxy groups found in TMDIBO **5** were replaced by 2-hydroxyethoxy moieties (Scheme 1). The synthetic route to 4-nitrophenyl (*p*NP) activated carbonate building block **4** starts from known tetrahydroxy aromatic **8**.[14] The latter could be obtained in 98% yield over two steps by treatment of 2-(3,4-dimethoxyphenyl)acetaldehyde with iodotrimethylsilane in dichloromethane[15] followed by demethylation of dimerization product **7** by boron tribromide in dichloromethane (Scheme 2).

Contrary to the literature report,[14] the direct route via condensation of 2-(3,4-dimethoxyphenyl)acetaldehyde with excess boron tribromide led to low yields of **8** and complex product mixtures. In order to establish the tetrakis(2-hydroxyethoxy) substitution pattern, **8** was peralkylated with TBS-protected 2-iodoethanol[16] using cesium carbonate as the base in refluxing acetonitrile. Hereby, tetraethoxy derivative **9** was obtained in 67% yield (Scheme 2). Attempts to peralkylate **8** with TBS-protected 2-bromo- or iodoethanol in *N*,*N*-dimethylformamide or dimethyl sulfoxide with bases such as potassium carbonate or sodium hydride were unsuccessful. This was most likely due to precipitation of the corresponding sodium or potassium phenolate salts formed in situ.




**Scheme 2** Synthesis of the activated THE-DIBO carbonate **4**. *Reagents and conditions*: (a) $BBr_3$, $CH_2Cl_2$, –78 °C to 25 °C, 2 h, 98%; (b) $I(CH_2)_2OTBS$, $Cs_2CO_3$, MeCN, reflux, 72 h, 67%; (c) BuLi, THF, –50 °C, 4 h; (d) TESCl, imidazole, DMF, 0 °C to 25 °C, 14 h, 96%; (e) 1. dioxane–$Br_2$, THF, 0 °C, 30 min, 2. KHDMS, THF, –78 °C to 25 °C, 1 h, 74%; (f) HF·Py, THF, –50 °C, 10 h, 61%; (g) 4-$O_2NC_6H_4OCOCl$, Py, $CH_2Cl_2$, 0 °C to 25 °C, 14 h, 94%; (h) HF·Py, THF, 0 °C, 2 h, 91%.

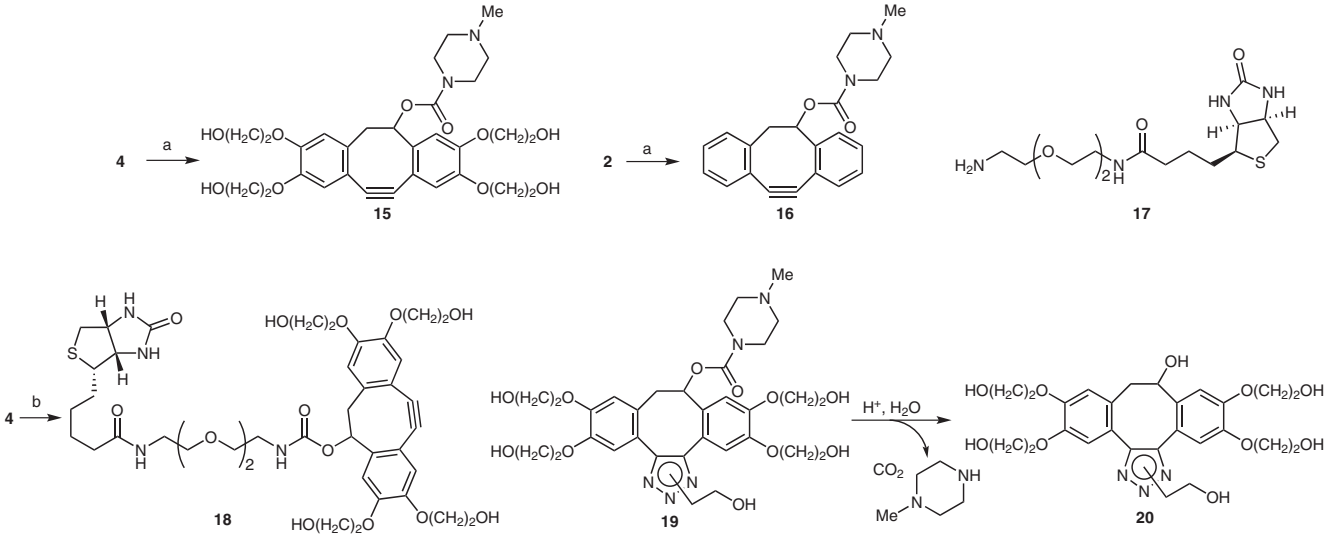
 

In the next stage, the epoxy scaffold in **9** was cleaved utilizing excess butyllithium in tetrahydrofuran–hexane. Unexpectedly, the elimination reaction proceeded smoothly at temperatures as low as –50 °C leading to alcohol **10** (compare[13]). Due to the highly acid labile nature of **10**, the free alcohol was directly converted into the corresponding triethylsilyl ether with chlorotriethylsilane and imidazole in *N*,*N*-dimethylformamide to yield **11** in 96% yield over two steps (Scheme 2). Like **10**, **11** was acid labile and column chromatography was performed on pre-neutralized silica gel. Introduction of the alkyne moiety by a sequence of bromination and elimination was found to be more difficult than expected. Treatment with bromine in dichloromethane at low temperature followed by brief workup and elimination with excess potassium *tert*-butoxide/1-methylpiperazine in tetrahydrofuran, as utilized in the synthesis of TMDIBO **5**, led to very low yields of alkyne **12** (<10%).[13] Likewise, treatment of **11** with bromine in dichloromethane at different temperatures and variations of the workup procedure followed by elimination with potassium *tert*-butoxide in tetrahydrofuran alone were unsuccessful. Gratifyingly, efficient bromination–elimination could be achieved by treatment of **11** with dioxane–bromine complex in tetrahydrofuran at 0 °C immediately followed by treatment with excess potassium hexamethyldisilazanide at –78 °C and warming to 25 °C. Hereby, the alkyne **12** was obtained in 74% yield (Scheme 2). Next, the transiently installed secondary TES ether group was selectively removed in presence of the primary TBS ether moieties. Here, treatment of **12** with 2 vol% hydrogen fluoride–pyridine in tetrahydrofuran at –50 °C led to the best result and free alkynol **13** was obtained in 61% yield. Reacting free alcohol **13** with 4-nitrophenyl chloroformate under standard conditions[6a] led to carbonate **14** in 94% yield. Finally, deprotection of the TBS groups in **14** was achieved with excess hydrogen fluoride–pyridine in tetrahydrofuran at 0 °C giving the activated THE-DIBO building block **4** in 91% yield.

In order to obtain model alkynes for further tests in aqueous systems and complex proteomes, the water soluble piperazine conjugates **15** and **16** and THE-DIBO-biotin **18** were prepared. Thus, **4** was reacted with excess 1-methylpiperazine in *N*,*N*-dimethylformamide to give **15** in 94% yield (Scheme 3). Likewise, *p*NP-DIBO **2** (Scheme 1) was converted into the corresponding piperazine carbamate **16** in virtually quantitative yield; **16** should serve as a water-soluble reference compound for the measurement of SPAAC rate constants and the stability of **15** toward thiol reagents. As expected, the THE-DIBO warhead **15** was found to be highly soluble in aqueous buffer solutions, e.g. 50 mM sodium acetate, 200 mM ammonium acetate, or 50 mM sodium phosphate buffer. To obtain THE-DIBO-biotin **18**, activated THE-DIBO **4** was reacted with biotin derivative **17**[6a] (Scheme 3) giving **18** in 89% yield. Biotin conjugate **18** possesses a remarkable water solubility of approx. 6 mM and exhibits the same stability characteristics as the piperazine conjugate **15** (see below).

Second-order rate constants of the SPAAC reaction with THE-DIBO analogue **15** were determined by UV-spectrophotometric measurement of the disappearance of the alkyne absorption band at around 310 nm as reported previously.[12] Plotting of the pseudo-first-order rate constants obtained by treatment of **15** and **16** with excess alkyl azide against azide concentration yielded the $k_2$ values. To get an impression of the impact of the aryl substituents in **15**, the nature of the azide and the solvent used on the SPAAC rates, DIBO analogue **16** was included in the experiments as a control. The alkynes were reacted with either 2-azidoethanol or benzyl azide in methanol and/or water, respectively (Table1).

Markedly, the second-order rate constants for THE-DIBO conjugate **15** increased slightly (approx. 1.5-fold) compared to DIBO analogue **16** for the azides and solvents tested (Table 1). Further, the rates for reaction between 2-azidoethanol and **15**/**16** decreased slightly compared to




**Scheme 3** Synthesis of the water-soluble model alkynes **15**, **16**, THE-DIBO-biotin **18** and acid-catalyzed fragmentation of carbamate **19**. *Reagents and conditions*: (a) 1-methylpiperazine, DMF, r.t., 12 h, 94% (**15**), 99% (**16**); (b) **17**·TFA, DIPEA, DMF, r.t., 24 h, 89%.

**Table 1** Measurement of the SPAAC Rate Constants[a]

| Alkyne | Solvent | 2-Azidoethanol $k_2$ ($M^{-1}$ $s^{-1}$) | Solvent | Benzyl azide $k_2$ ($M^{-1}$ $s^{-1}$) |
|---|---|---|---|---|
| **15** | $H_2O$ | $(21.8 \pm 0.2) \times 10^{-2}$ | MeOH | $(16.7 \pm 0.3) \times 10^{-2}$ |
| | MeOH | $(11.4 \pm 0.6) \times 10^{-2}$ | | |
| **16** | $H_2O$ | $(14.6 \pm 0.2) \times 10^{-2}$ | MeOH | $(9.3 \pm 0.4) \times 10^{-2}$ |
| | MeOH | $(6.9 \pm 0.3) \times 10^{-2}$ | | |

[a] T = 295 K.

the reaction with benzyl azide. It should also be mentioned that the $k_2$ value for SPAAC between benzyl azide and THE-DIBO analogue **15** was found to be 1.5-fold higher than the $k_2$ value for a TMDIBO **5** analogue in the same reagent system that was reported in the literature (evaluated by $^1$H NMR at 300 K[13]). Also, a twofold rate enhancement for SPAAC between 2-azidoethanol and **15**/**16** could be observed when switching solvent from methanol to more polar water. This is in good accordance to the observation that polar solvents can accelerate cycloaddition reactions, e.g. water in Diels–Alder-type reactions.[17] This effect was previously described for SPAAC reactions.[6a,9]

To further highlight the utility of our THE-DIBO-based SPAAC reagents, the storability of **4**, **15**, and **18** as well as the susceptibility of **15** toward thiol-yne addition was evaluated. Activated carbonate **4** could be stored at 7 °C under ambient atmosphere for nine months without noticeable loss of activity in the synthesis of follow-up compounds. The corresponding TFA salt of **15** and THE-DIBO **18** did not show any sign of decomposition upon storage under ambient conditions for at least six months as judged by LC-MS analyses. Further, the aqueous stock solution of **15** used in the UV experiments was found to be stable for at least four weeks at 7 °C (LC-MS). Thiol-yne addition to the water-soluble strained alkynes was investigated by LC-MS. Thus, 1.4mM solutions of **15** and **16** in 200 mM aqueous ammonium acetate were treated either with two equivalents of 2-azidoethanol (2.8 mM azide, control), two equivalents of 2-azidoethanol, and 10 equivalents of GSH or BME (14 mM thiol, competition) or blank buffer (control), respectively. After stirring for 24 hours under ambient conditions, the mixtures were analyzed by LC-MS. With the exception of the sample containing DIBO-conjugate **16**, 2-azidoethanol and GSH, no addition of thiol to the alkynes and only formation of the corresponding triazoles was observed. In the given sample, the **16**-GSH adduct ($t_R$ = 15.4 min, $m/z$ = 654.4 $[M + H]^+$) was present only in trace amounts (approx. 1–2%). In a further control experiment **15** and **16** were treated with GSH and BME alone under the aforementioned conditions. LC-MS analyses of the sample mixtures after 24 hours indicated that no addition of thiol to **15** had occurred. The adducts **16**-BME ($t_R$ = 17.0 min, $m/z$ = 448.0 $[M + Na]^+$) and **16**-GSH were traceable in the total ion chromatogram (TIC), but the bands (220 nm) were of too low intensity to be integrated (<1%). These findings support the assumption that small, hydrophilic thiols do not readily add to DIBO-derived SPAAC reagents.

At this point it should be mentioned that the triazole **19** (Scheme 3) formed by SPAAC reaction between **15** and 2-azidoethanol seems to posses only a limited half-life in neutral to slightly acidic buffer media (pH 7 to 6.3). Thus, reexamination of these sample mixtures after 48 hours under ambient conditions by LC-MS analyses showed clear signs of fragmentation of the triazole carbamate **19** ($t_R$ = 6.2 min, $m/z$ = 674.5 $[M + H]^+$) to the corresponding benzyl alcohol **20** ($t_R$ = 9.5 min, $m/z$ = 547.8 $[M + H]^+$; approx. 5%; Scheme 3). This effect was neither observed for the parent alkyne **15** nor for the biotin conjugate **18** (see above). Also, the corresponding DIBO-derived **2** triazole carbamates did not show any fragmentation under the same conditions. Apparently the 4-alkoxybenzyl position in **19** experiences significant activation upon transformation of **15** to the triazole in regard to acid-catalyzed hydrolysis via an $S_N1$ mechanism. When shifting to more basic buffer media (TRIS pH 8.1) carbamate fragmentation in **19** was suppressed and only trace amounts of the corresponding benzyl alcohol could be detected by LC-MS after 48 hours under ambient conditions. Based on these findings we strongly recommend that the triazoles formed from THE-DIBO derivatives are kept in basic buffers when stored for longer periods of time.

In conclusion, we described a practical synthetic route to the novel, highly hydrophilic and stable tetrakis(2-hydroxyethoxy)-substituted dibenzocyclooctyne THE-DIBO **4**, which is a versatile building block for the assembly of strained alkyne probes for SPAAC applications. Activated THE-DIBO **4** was obtained in seven steps from commercially available 2-(3,4-dimethoxyphenyl)acetaldehyde in an overall yield of 24%; **4** was further coupled to 1-methylpiperazine and biotin conjugate **17** to yield the water-soluble model alkyne **15** and THE-DIBO-biotin **18**. The second-order reaction rate constant of the click reaction between model compound **15** and 2-azidoethanol was found to be $(21.8 \pm 0.2) \times 10^{-2}$ $M^{-1}$ $s^{-1}$ as measured by UV spectrophotometry in water. Further, alkyne **15** proved to be highly resistant toward thiol-yne addition by small hydrophilic thiols GSH and BME. A possible weakness of the synthetic route presented here is the low yield in the alkylation step (step b in Scheme 2) and the TES-deprotection step (step f in Scheme 2) with 67% and 61% yields, respectively. Furthermore, the potential liability to acid-catalyzed hydrolysis of the triazoles obtained from THE-



DIBO conjugates might diminish their usefulness in applications where long term stability of the triazole conjugates is important. A possible solution would be to switch from the apparently labile carbamate linkage to more stable ether bonds. Derivatives of THE-DIBO are currently tested for their performance in our previously reported capture-release protocol for azide tagged biomolecules.[18] We hope that probes derived from THE-DIBO **4** are less prone to nonspecific thiol-yne addition, which is a major obstacle for successful application of SPAAC in complex proteomes like cell lysates, the interior of living cells or in animals.

All commercially available chemicals were used as received unless noted otherwise. Dry solvents were prepared according to standard methods, distilled, and stored over molecular sieves 3 Å under an atmosphere of $N_2$ until used. NMR spectra were recorded on a Bruker Avance 400 spectrometer and calibrated for the solvent-signal ($^1$H-MeOH-$d_4$: δ = 3.31; $^{13}$C-MeOH-$d_4$: δ = 49.00, $^1$H-DMSO-$d_6$: δ = 2.50; $^{13}$C-DMSO-$d_6$: δ = 39.52; $^1$H-acetone-$d_6$: δ = 2.05; $^{13}$C-acetone-$d_6$: δ = 28.84). SPAAC reaction kinetics were measured by UV spectrophotometry following the published protocol[12] on a Perkin-Elmer Lambda 25 UV-Vis spectrophotometer using a 1-cm quartz cuvette and were measured in duplicate. Absorbance was measured at 316 nm (**15**) and 305 nm (**16**), respectively. FT-ICR-MS spectra were recorded on a Bruker Apex II FT-ICR-MS (FAB) spectrometer and FAB-spectra on a Finnigan model TSQ 70. Melting points were determined with a Büchi Melting Point M-560 and are uncorrected. Elemental analysis was performed on a HEKAtech Euro EA Analyzer. IR spectra were recorded on a Bruker Tensor 27. TLC-analysis was performed with Macherey-Nagel Polygram SIL G/UV pre-coated polyester sheets. Column chromatography was performed with silica gel bead size 0.04–0.063 mm. Pre-neutralized silica gel was prepared by adding 5 vol% 7 M $NH_3$ in MeOH to a suspension of silica gel in $CHCl_3$ followed by brief shaking and removal of the solvent under reduced pressure. All reactions were performed in flame-dried glassware under an atmosphere of $N_2$ using dry solvents unless stated otherwise. 2-(3,4-Dimethoxyphenyl)acetaldehyde and 2,3,8,9-tetramethoxy-5,6,11,12-tetrahydro-5,11-epoxydibenzo[*a*,*e*][8]annulene (**7**)[15] and *tert*-butyl(2-iodoethoxy)dimethylsilane[16] were prepared according to the published procedures. The SPAAC/thiol-yne addition competition experiments with **15** an **16** were conducted by treatment of a 1.4 mM soln of the water-soluble strained alkyne component in 200 mM $NH_4OAc$ buffer with 2 mol equiv 2-azidoethanol (2.8 mM) alone (control), with additional 10 mol equiv (14 mM) of GSH or BME (competition) or blank buffer (control), respectively. After stirring for 24 h at r.t. under ambient atmosphere the mixtures were analyzed by LC-MS (ESI; Bruker Daltonics Esquire 3000 plus) on a Macherey-Nagel EC-Nucleodur 100-5 C18 ec column eluting with a MeOH–$H_2O$ + 1 vol% $HCO_2H$ gradient of 20 vol% MeOH to 100 vol% MeOH in 30 min.

**5,6,11,12-Tetrahydro-5,11-epoxydibenzo[*a*,*e*][8]annulene-2,3,8,9-tetraol (8)**

2,3,8,9-Tetramethoxy-5,6,11,12-tetrahydro-5,11-epoxydibenzo[*a*,*e*][8]annulene[15] (**7**, 1 g, 2.92 mmol) was dissolved in $CH_2Cl_2$ (50 mL) and the soln cooled to –78 °C. Then, $BBr_3$ (1.13 mL, 11.7 mmol, 4 equiv) was added dropwise via a syringe and the soln allowed to warm to 25 °C over a period of 1 h. The mixture was stirred for an additional 1 h at 25 °C and then poured onto ice-water (100 mL), stirred for 1 h, transferred to a separatory funnel, and extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine (3 × 20 mL) and dried ($MgSO_4$); the solvent was removed under reduced pressure to yield pure tetraol **8**. An analytical sample of **8** was obtained by recrystallization (benzene–acetone); light beige solid/colorless crystals; yield: 819 mg (98%); mp 285 °C (dec); $R_f$ = 0.45 (toluene–acetone, 1:1).

IR (KBr): 3346, 2917, 2886, 2830, 1610, 1528, 1459, 1438, 1348, 1287, 1264, 1190, 1160, 1106, 1051, 1018, 933, 866, 827, 800, 515 cm$^{-1}$.

$^1$H NMR (400 MHz, DMSO-$d_6$): δ = 2.32 (d, $J$ = –15.8 Hz, 1 H, $ArCH_2$), 3.14 (dd, $J$ = –15.8, 5.9 Hz, 1 H, $ArCH_2$), 4.96 (d, $J$ = 5.9 Hz, 1 H, ArCHO), 6.33 (s, 2 H, ArH), 6.45 (s, 2 H, ArH), 8.63 (s, 4 H, OH).

$^{13}$C NMR (100 MHz, DMSO-$d_6$): δ = 35.2, 68.2, 112.0, 115.2, 122.0, 128.5, 143.4, 144.2.

MS (FAB): $m/z$ (%) = 152.6 (100), 287.1 (40) $[M + H]^+$.

Anal. Calcd for $C_{16}H_{14}O_5$: C, 67.13; H, 4.93. Found: C, 67.36; H, 4.92.

**2,3,8,9-Tetrakis[2-(*tert*-butyldimethylsiloxy)ethoxy]-5,6,11,12-tetrahydro-5,11-epoxydibenzo[*a*,*e*][8]annulene (9)**

Tetraol **8** (5.70 g, 19.9 mmol) was dissolved in MeCN (300 mL). $Cs_2CO_3$ (38.9 g, 119 mmol, 6 equiv) and *tert*-butyl(2-iodoethoxy)dimethylsilane[16] (34.1 g, 119 mmol, 6 equiv) were added and the mixture was heated at reflux temperature for 72 h. After cooling to 25 °C, the mixture was concentrated under reduced pressure, the residue taken up in EtOAc (400 mL), and the slurry was transferred to a separatory funnel. The organic phase was washed with $H_2O$ ( 3 × 200 mL) and brine (100 mL) and dried ($MgSO_4$); the solvent was removed under reduced pressure. The residue was subjected to column chromatography (silica gel, toluene–EtOAc, 39:1) to afford pure TBS-protected ether **9** as a colorless viscous oil; yield: 12.2 g (67%); $R_f$ = 0.31 (toluene–EtOAc, 39:1).

IR (neat/KBr): 2929, 2857, 2738, 1612, 1516, 1472, 1428, 1361, 1343, 1254, 1197, 1110, 960, 835, 778 cm$^{-1}$.

$^1$H NMR (400 MHz, acetone-$d_6$): δ = 0.08 (s, 6 H, TBS-$CH_3$), 0.09 (s, 6 H, TBS-$CH_3$), 0.12 (s, 12 H, TBS-$CH_3$), 0.89 (s, 18 H, TBS-*t*-Bu), 0.92 (s, 18 H, TBS-*t*-Bu), 2.69 (d, $J$ = –16.0 Hz, 2 H, $ArCH_2$), 3.33 (dd, $J$ = –16.0, 6.0 Hz, 2 H, $ArCH_2$), 3.90–4.06 (m, 16 H, $OCH_2$), 5.13 (d, $J$ = 5.7 Hz, 2 H, ArCHO), 6.58 (s, 2 H, ArH), 6.78 (s, 2 H, ArH).

$^{13}$C NMR (100 MHz, acetone-$d_6$): δ = –5.0, 18.8, 18.9, 26.3, 26.3, 36.2, 62.9, 62.9, 69.7, 71.1, 71.3, 112.0, 115.1, 125.1, 131.4, 148.3, 148.9.

HRMS (FAB): $m/z$ $[M + Na]^+$ calcd for $C_{48}H_{86}O_9Si_4Na$: 941.5241; found: 941.5243.

**2,3,8,9-Tetrakis[2-(*tert*-butyldimethylsiloxy)ethoxy]-5-(triethylsiloxy)-5,6-dihydrodibenzo[*a*,*e*][8]annulene (11)**

TBS-protected ether **9** (196 mg, 213 μmol) was dissolved in THF (5 mL) and the soln cooled to –78 °C followed by dropwise addition of 2.5 M BuLi in hexanes (596 μL, 7 equiv) via a syringe. Then, the mixture was allowed to warm to –50 °C and stirring was continued for 4 h. Excess BuLi was quenched by the addition of $H_2O$ (1 mL), the mixture was diluted with $Et_2O$ (25 mL) and transferred to a separatory funnel. The aqueous layer was separated and the organic layer washed with $H_2O$ (2 × 10 mL) and brine (10 mL), and dried ($MgSO_4$); the solvent removed under reduced pressure. The crude alcohol **10** was obtained as an amber-colored viscous oil and used for the next step without further purification. Crude alcohol **10** was dissolved in DMF (1 mL) and the soln cooled to 0 °C. Imidazole (31.9 mg, 469 μmol, 2.2 equiv) and TESCl (39.3 μL, 234 μmol, 1.1 equiv) were added and the mixture stirred for 2 h at 0 °C and an additional 12 h at 25 °C. Subsequently, the mixture was diluted with $Et_2O$ (25 mL), transferred to a separatory funnel, and washed with $H_2O$ (3 × 10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$); the solvent was removed under reduced pressure. Column chromatography of the residue (pre-neutralized silica gel, light petroleum ether–EtOAc, 22:1) yielded pure TES ether **11** as a col-

orless oil; yield: 211 mg (96%); $R_f$ = 0.33 (light petroleum ether–EtOAc, 22:1).

IR (neat/KBr): 2930, 2737, 1604, 1512, 1462, 1361, 1254, 1188, 1105, 1107, 960, 835, 778, 743 cm$^{-1}$.

$^1$H NMR (400 MHz, acetone-$d_6$): δ = 0.08–0.16 (m, 24 H, TBS-$CH_3$), 0.55–0.63 (m, 6 H, TES-$CH_2$), 0.87–0.99 (m, 45 H, TBS-*t*-Bu + TES-$CH_3$), 3.13 (dd, *J* = –15.1, 10.1 Hz, 1 H, Ar$CH_2$), 3.36 (dd, *J* = –15.1, 5.6 Hz, 1 H, Ar$CH_2$), 3.90–4.14 (m, 16 H, $OCH_2$), 5.41 (dd, *J* = 10.1, 5.6 Hz, 1 H, ArCHO), 6.65 (d, *J* = 12.3 Hz, 1 H, ArCH), 6.67 (s, 2 H, ArH), 6.72 (s, 1 H, ArH), 6.74 (d, *J* = 12.3 Hz, 1 H, ArCH), 7.12 (s, 1 H, ArH).

$^{13}$C NMR (100 MHz, acetone-$d_6$): δ = –5.0, –5.0, –5.0, 5.6, 7.3, 18.9, 26.3, 47.2, 62.7, 62.8, 62.9, 71.0, 71.0, 71.2, 71.2, 72.9, 112.8, 114.9, 116.7, 116.7, 128.3, 129.6, 129.7, 130.4, 132.8, 137.2, 147.4, 148.0, 148.7, 148.7, 149.0.

HRMS (FAB): *m*/*z* [M + Na]$^+$ calcd for $C_{54}H_{100}O_9Si_5Na$: 1055.6106; found: 1055.6102.

**2,3,8,9-Tetrakis[2-(*tert*-butyldimethylsiloxy)ethoxy]-5-(triethylsiloxy)-11,12-didehydro-5,6-dihydrodibenzo[*a*,*e*][8]annulene (12)**

Alkene **11** (870 mg, 842 μmol) was dissolved in THF (20 mL) and the soln cooled to 0 °C. Dioxane–bromine complex (292 mg, 1.18 mmol, 1.4 equiv) was added in one portion and the mixture stirred for 30 min at 0 °C in the dark. Subsequently, the mixture was cooled to –78 °C and 1 M KHDMS in THF (8.42 mL, 8.42 mmol, 10 equiv) was added via a syringe. The mixture was allowed to warm to 25 °C over a period of 30 min and stirred for additional 30 min at 25 °C. The mixture was quenched by addition of sat. $NH_4Cl$ soln (5 mL), transferred to a separatory funnel, and diluted with $H_2O$ (10 mL) and $Et_2O$ (25 mL). The organic layer was separated and the aqueous phase extracted with $Et_2O$ (2 × 10 mL). The combined organic layers were washed with $H_2O$ (2 × 10 mL) and brine (10 mL), and dried ($MgSO_4$); the solvent was removed under reduced pressure. The residue was subjected to column chromatography (silica gel, light petroleum ether–EtOAc, 24:1) to give pure, protected alkynol **12** as a colorless viscous oil; yield: 643 mg (74%); $R_f$ = 0.33 (light petroleum ether–EtOAc, 24:1).

IR (neat/KBr): 2929, 2856, 1601, 1562, 1503, 1471, 1407, 1329, 1252, 1224, 1186, 1134, 1107, 1082, 1006, 969, 835, 776 cm$^{-1}$.

$^1$H NMR (400 MHz, acetone-$d_6$): δ = 0.11–0.14 (m, 24 H, TBS-$CH_3$), 0.55–0.63 (m, 6 H, TES-$CH_2$), 0.89–0.972 (m, 45 H, TBS-*t*-Bu + TES-$CH_3$), 2.79 (dd, *J* = –14.4, 3.5 Hz, 1 H, Ar$CH_2$), 3.06 (dd, *J* = –14.4, 1.8 Hz, 1 H, Ar$CH_2$), 3.98–4.21 (m, 16 H, $OCH_2$), 4.50–4.54 (m, 1 H, ArCHO), 6.91 (s, 1 H, ArH), 6.93 (s, 1 H, ArH), 7.09 (s, 1 H, ArH), 7.40 (s, 1 H, ArH).

$^{13}$C NMR (100 MHz, acetone-$d_6$): δ = –5.0, –5.0, 5.4, 7.3, 18.9, 26.3, 26.3, 51.0, 62.8, 62.8, 63.0, 63.0, 71.1, 71.4, 71.5, 71.5, 76.9, 110.6, 112.0, 112.3, 113.0, 113.6, 116.8, 117.2, 146.1, 148.3, 148.6, 149.5, 149.9, 151.0.

HRMS (FAB): *m*/*z* [M + Na]$^+$ calcd for $C_{54}H_{98}O_9Si_5Na$: 1053.5949; found: 1053.5945.

**2,3,8,9-Tetrakis[2-(*tert*-butyldimethylsiloxy)ethoxy]-5-hydroxy-11,12-didehydro-5,6-dihydrodibenzo[*a*,*e*][8]annulene (13)**

In a 15-mL polypropylene tube equipped with a stirring bar and a rubber septum, TES ether **12** (593 mg, 575 μmol) was dissolved in THF (9 mL) under an atmosphere of $N_2$. The soln was cooled to –50 °C and HF·Py (180 μmol, 6.3 mmol, 11 equiv) was added at a final concentration of 2 vol%. The mixture was stirred for 10 h at –50 °C, poured into ice-cold sat. $NaHCO_3$ soln, and transferred to a separatory funnel. The mixture was extracted with $Et_2O$ (3 × 15 mL) and the combined organic layers were washed with $H_2O$ (2 × 10 mL) and brine (10 mL), and dried ($MgSO_4$); the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel, light petroleum ether–EtOAc, 17:3) to yield pure alkynol **13**. An analytical sample of **13** was obtained by recrystallization (*n*-hexane); white solid/colorless needles; yield: 322 mg (61%); mp 135 °C; $R_f$ = 0.33 (light petroleum ether–EtOAc, 17:3).

IR (KBr): 3511, 2953, 2930, 2884, 2857, 2857, 1602, 1561, 1502, 1471, 1328, 1255, 1222, 1136, 1109, 1078, 964, 836, 779 cm$^{-1}$.

$^1$H NMR (400 MHz, acetone-$d_6$): δ = 0.13, 0.13 (2 s, 24 H, TBS-$CH_3$), 0.92 (s, 36 H, TBS-*t*-Bu), 2.74 (dd, *J* = –14.3, 3.5 Hz, 1 H, Ar$CH_2$), 3.07 (dd, *J* = –14.3, 1.9 Hz, 1 H, Ar$CH_2$), 3.98–4.05 (m, 8 H, $OCH_2$), 4.08–4.18 (m, 8 H, $OCH_2$), 4.42–4.47 (m, 1 H, ArCHO), 4.80 (d, *J* = 4.64 Hz, 1 H, OH), 6.91, 6.92 (2 s, 2 H, ArH), 7.10 (s, 1 H, ArH), 7.45 (s, 1 H, ArH).

$^{13}$C NMR (100 MHz, acetone-$d_6$): δ = –5.0, 18.9, 26.3, 50.2, 62.9, 62.9, 63.0, 63.0, 71.2, 71.4, 71.6, 75.6, 110.9, 112.1, 112.4, 112.5, 112.8, 113.8, 116.9, 117.2, 146.8, 148.2, 148.5, 149.7, 150.1, 151.7.

HRMS (FAB): *m*/*z* [M + Na]$^+$ calcd for $C_{48}H_{84}O_9Si_4Na$: 939.5085; found: 939.5084.

Anal. Calcd for $C_{48}H_{84}O_9Si_4$: C, 62.83; H, 9.23. Found: C, 62.78; H, 9.28.

**4-Nitrophenyl {2,3,8,9-Tetrakis[2-(*tert*-butyldimethylsiloxy)ethoxy]-5,6-dihydro-11,12-didehydrodibenzo[*a*,*e*][8]annulen-5-yl} Carbonate (14)**

Alkynol **13** (260 mg, 282 μmol) was dissolved in $CH_2Cl_2$ (2 mL) and the soln cooled to 0 °C. Then, pyridine (115 μL, 1.42 mmol, 5 equiv) was added followed by 4-nitrophenyl chloroformate (115 mg, 574 μmol, 2 equiv), the mixture stirred for 2 h at 0 °C and for a further 12 h at 25 °C. Subsequently a few drops of butane-1,4-diol were added and the mixture stirred for 30 min at 25 °C in order quench excess 4-nitrophenyl chloroformate. The mixture was diluted with EtOAc (25 mL) and transferred to a separatory funnel, the organic layer washed with $H_2O$ (3 × 10 mL) and brine (10 mL), and dried ($MgSO_4$); the solvent was removed under reduced pressure. The residue was subjected to column chromatography (light petroleum ether–EtOAc, 7:1) to give pure activated carbonate **14** as a light yellow resin; yield: 289 mg (94%); $R_f$ = 0.37 (light petroleum ether–EtOAc, 7:1).

IR (KBr): 3442, 2930, 2857, 1772, 1604, 1561, 1529, 1506, 1471, 1345, 1256, 1220, 1108, 964, 835, 778 cm$^{-1}$.

$^1$H NMR (major isomer, 400 MHz, acetone-$d_6$): δ = 0.09–0.17 (m, 24 H, TBS-$CH_3$), 0.89–0.96 (m, 36 H, TBS-*t*-Bu), 2.88 (dd, *J* = –15.2, 3.8 Hz, 1 H, Ar$CH_2$), 3.39 (dd, *J* = –15.2, 1.8 Hz, 1 H, Ar$CH_2$), 3.97–4.07 (m, 8 H, $OCH_2$), 4.09–4.22 (m, 8 H, $OCH_2$), 5.45–5.49 (m, 1 H, ArCHO), 6.96 (s, 1 H, ArH), 7.02 (s, 1 H, ArH), 7.20 (s, 1 H, ArH), 7.22 (s, 1 H, ArH), 7.59–7.65 (m, 2 H, *p*NP), 8.32–8.38 (m, 2 H, *p*NP).

$^{13}$C NMR (mixture of isomers, 100 MHz, acetone-$d_6$): δ = –5.1, –5.0, 18.9, 26.3, 26.3, 39.9, 46.5, 62.8, 62.8, 62.9, 62.9, 71.3, 71.5, 71.5, 71.6, 71.6, 79.2, 82.6, 109.6, 110.1, 111.1, 111.4, 111.5, 112.1, 112.4, 112.6, 113.3, 114.1, 116.2, 116.5, 117.6, 119.3, 119.4, 119.5, 122.9, 123.2, 125.9, 126.1, 140.8, 141.8, 144.4, 145.1, 146.2, 146.5, 148.9, 149.0, 149.0, 149.0, 149.9, 150.0, 150.2, 152.5, 152.7, 156.4, 156.7.

HRMS (FAB): *m*/*z* [M + Na]$^+$ calcd for $C_{55}H_{87}NO_{13}Si_4Na$: 1104.5147; found: 1104.5147.

**4-Nitrophenyl [2,3,8,9-Tetrakis(2-hydroxyethoxy)-5,6-dihydro-11,12-didehydrodibenzo[*a*,*e*][8]annulen-5-yl] Carbonate (4)**

In a 10-mL polypropylene tube equipped with a stirring bar and a rubber septum activated carbonate **14** (52.6 mg, 49 μmol) was dissolved in THF (1 mL) under an atmosphere of $N_2$. The soln was cooled to 0 °C, HF·Py (100 μL, 3.48 mmol, 71 equiv) was added, and the mixture was stirred for 2 h at 0 °C. Then the soln was diluted with EtOAc (2 mL) and silica gel (1 g) was added in order to quench excess HF·Py. The soln was filtered, the solids were washed with MeOH (3 × 5 mL), and the combined organic layers were concen-



trated under reduced pressure. The residue was subjected to column chromatography ($CHCl_3$–MeOH, 12:1) to afford pure deprotected active THE-DIBO **4**. An analytical sample of **4** could be obtained by recrystallization (acetone); white solid/colorless crystals; yield: 27.9 mg (91%); mp 145 °C (dec); $R_f$ = 0.28 ($CHCl_3$–MeOH, 12:1).

IR (KBr): 3406, 2934, 1758, 1602, 1562, 1506, 1334, 1221, 1082, 982, 860 $cm^{-1}$.

$^1H$ NMR (major isomer, 400 MHz, DMSO-$d_6$): δ = 2.75 (dd, $J$ = –15.2, 3.9 Hz, 1 H, $ArCH_2$), 3.42 (dd, $J$ = –15.2, 1.5 Hz, $ArCH_2$), 3.66–3.79 (m, 8 H, $OCH_2$), 3.98–4.11 (m, 8 H, $OCH_2$), 4.82–4.95 (m, 4 H, OH), 5.29–5.34 (m, 1 H, ArCHO), 6.99 (s, 1 H, ArH), 7.07 (1s, 1 H, ArH), 7.09 (s, 1 H, ArH), 7.24 (s, 1 H, ArH), 7.64–7.69 (m, 2 H, *p*NP), 8.32–8.38 (m, 2 H, *p*NP).

$^{13}C$ NMR (mixture of isomers, 100 MHz, DMSO-$d_6$): δ = 45.1, 59.4, 59.5, 59.6, 70.6, 70.8, 77.8, 81.3, 108.7, 109.3, 110.3, 110.7, 111.1, 111.4, 111.7, 112.5, 112.5, 114.6, 114.8, 116.9, 117.7, 118.3, 118.6, 122.1, 122.8, 125.4, 125.5, 139.7, 140.7, 143.1, 144.1, 145.1, 145.3, 147.4, 147.5, 147.6, 147.8, 148.6, 148.8, 149.0, 151.1, 151.4, 154.9, 155.3.

HRMS (ESI): $m/z$ [M + Na]$^+$ calcd for $C_{31}H_{31}NO_{13}Na$: 648.1687; found: 648.1685.

**2,3,8,9-Tetrakis(2-hydroxyethoxy)-5,6-dihydro-11,12-didehydrodibenzo[*a*,*e*][8]annulen-5-yl 4-Methylpiperazine-1-carboxylate (15)**

To a soln of activated carbonate **4** (100 mg, 160 μmol) in DMF (2 mL) was added 1-methylpiperazine (80.1 mg, 800 μmol, 5 equiv) and the mixture was stirred for 12 h at r.t. Then, the solvent was removed under reduced pressure and the residue subjected to column chromatography ($CHCl_3$–MeOH–7 M $NH_3$ in MeOH, 20:4:1), which led to pure piperazine conjugate **15**. The basic alkyne could later be converted into its TFA salt by dissolving **15** in MeOH, adding an equimolar amount of TFA, and removal of the solvent under reduced pressure; colorless foam; yield: 88.2 mg (94%); $R_f$ = 0.55 ($CHCl_3$–MeOH–7 M $NH_3$ in MeOH, 20:4:1).

IR (KBr): 3419, 2929, 2875, 2742, 1683, 1602, 1561, 1506, 1460, 1432, 1408, 1321, 1285 $cm^{-1}$.

$^1H$ NMR (mixture of isomers, 400 MHz, MeOH-$d_4$): δ = 1.37–1.50, 1.82–1.96 (2 m, 1 H, pip-$CH_2$), 2.16–2.31, 2.44–3.15 (2 m, 6 H, pip-$CH_2$ + $ArCH_2$), 2.19, 2.40 (2 s, 3 H, pip-$CH_3$), 3.40–3.65 (m, 2 H, pip-$CH_2$ + $ArCH_2$), 3.66–3.95 (m, 9 H, pip-$CH_2$ + $OCH_2$), 3.98–4.19 (m, 8 H, $OCH_2$), 5.33–5.38, 6.07–6.13 (2 m, 1 H, ArCHO), 6.85, 6.88, 6.88, 6.93, 6.96, 7.00, 7.02, 7.15 (8 s, 4 H, ArH).

$^{13}C$ NMR (mixture of isomers, 400 MHz, MeOH-$d_4$): δ = 40.5, 43.5, 44.5, 45.6, 45.9, 47.1, 54.9, 55.5, 61.5, 61.6, 61.6, 72.0, 72.0, 72.0, 72.1, 72.2, 76.0, 79.4, 79.5, 110.2, 110.9, 111.0, 111.3, 111.7, 112.4, 112.8, 112.8, 113.4, 115.0, 117.1, 117.2, 117.5 119.0, 119.1, 119.8, 142.5, 144.2, 146.2, 146.8, 148.9, 148.9, 149.1, 149.2, 150.0, 150.0, 150.1, 150.3, 155.7, 155.7.

HRMS (FAB): $m/z$ [M + H]$^+$ calcd for $C_{30}H_{39}N_2O_{10}$: 587.2599; found: 587.2597.

LC-MS: $t_R$ = 14.3 min; $m/z$ = 587.5 [M + H]$^+$.

**5,6-Dihydro-11,12-didehydrodibenzo[*a*,*e*][8]annulen-5-yl 4-Methylpiperazine-1-carboxylate (16)**

Analogous to the synthesis of **15** DIBO carbonate **2** (100 mg, 259 μmol) was treated with 1-methylpiperazine (130 mg, 1.30 mmol, 5 equiv). Column chromatography ($CHCl_3$–7 M $NH_3$ in MeOH, 49:1) yielded pure piperazine conjugate **16**. DIBO derivative **16** could later be converted into the corresponding TFA salt analogous to **15**; colorless foam; yield: 85.4 mg (99%); $R_f$ = 0.25 ($CHCl_3$–7 M $NH_3$ in MeOH, 49:1).

IR (KBr): 3436, 3021, 2955, 2472, 1706, 1465, 1429, 1259, 1200, 1133, 1026, 977, 760 $cm^{-1}$.

$^1H$ NMR (mixture of isomers, 400 MHz, $CDCl_3$): δ = 1.36–1.50, 1.77–2.60 (2 m, 4 H, pip-$CH_2$), 2.09, 2.34 (2 s, 3 H, pip-$CH_3$), 2.71–3.90 (m, 4 H, pip-$CH_2$), 2.84, 2.93 (2 dd, $J$ = –13.6, 2.0, –14.9, 3.9 Hz, 1 H, $ArCH_2$), 3.16, 3.71 (2 dd, $J$ = –14.9, 2.1, –13.6, 10.3 Hz, 1 H, $ArCH_2$), 5.52–5.57 + 6.28 (m + dd, $J$ = 10.3, 2.0 Hz, 1 H, ArCHO), 7.23–7.51 (m, 8 H, ArH).

$^{13}C$ NMR (mixture of isomers, 400 MHz, $CDCl_3$): δ = 39.8, 43.3, 44.1, 46.1, 46.3, 46.5, 54.1, 54.4, 54.9, 74.3, 77.5, 109.9, 110.3, 110.8, 113.1, 121.4, 123.2, 123.7, 123.8, 125.1, 126.0, 126.1, 126.4, 126.9, 127.1, 127.2, 127.8, 128.0, 128.1, 128.2, 129.0, 130.0, 131.4, 132.2, 148.2, 149.7, 151.1, 152.1, 154.2, 154.3.

HRMS (FAB): $m/z$ [M + H]$^+$ calcd for $C_{22}H_{23}N_2O_2$: 347.1754; found: 347.1756.

LC-MS: $t_R$ = 18.9 min; $m/z$ = 369.9 [M + Na]$^+$.

**2,3,8,9-Tetrakis(2-hydroxyethoxy)-5,6-dihydro-11,12-didehydrodibenzo[*a*,*e*][8]annulen-5-yl {2-[2-(2-{5-[(3a*S*,4*S*,6a*R*)-2-Oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanamido}ethoxy)ethoxy]ethyl}carbamate (18)**

To a soln of activated carbonate **4** (200 mg, 319 μmol) in DMF (2 mL) was added the TFA salt of biotin conjugate **17**[6a] (234 mg, 479 μmol, 1.5 equiv) and DIPEA (124 mg, 957 μmol, 3 equiv) and the mixture was stirred for 24 h at r.t. After removal of the volatiles under reduced pressure, the residue was subjected to column chromatography ($CHCl_3$–MeOH, 4:1) to furnish pure THE-DIBO-biotin **18** as a colorless foam; yield: 240 mg (89%); $R_f$ = 0.45 ($CHCl_3$–MeOH, 10:3).

IR (KBr): 3389, 2929, 2870, 2482, 2482, 2150, 1694, 1642, 1603, 1560, 1504, 1456, 1408, 1332, 1263, 1222, 1082, 1039, 934, 904 $cm^{-1}$.

$^1H$ NMR (major isomer, 400 MHz, MeOH-$d_4$): δ = 1.29–1.42 (m, 2 H, biotin-$CH_2$), 1.46–1.72 (m, 4 H, biotin-$CH_2$), 2.15 (t, $J$ = 7.0 Hz, 2 H, biotin-$CH_2$), 2.61–2.76 (m, 2 H, biotin-$SCH_2$ + $ArCH_2$), 2.86 (ddd, $J$ = –12.7, 4.9, 1.9 Hz, 1 H, biotin-$SCH_2$), 3.05–3.16 (m, 2 H, biotin-$SCH_2$ + $ArCH_2$), 3.24–3.38 (m, 4 H, $NCH_2$), 3.41–3.73 (m, 8 H, $OCH_2$), 3.82–3.95 (m, 8 H, $OCH_2$), 3.99–4.16 (m, 8 H, $OCH_2$), 4.20 (dd, $J$ = 7.7, 4.4 Hz, 1 H, biotin-NCH), 4.42 (dd, $J$ = 7.7, 4.9 Hz, 1 H, biotin-NCH), 5.27–5.36 (m, 1 H, ArCHO), 6.85 (s, 1 H, ArH), 6.87 (1s, 1 H, ArH), 7.01 (s, 1 H, ArH), 7.19 (s, 1 H, ArH).

$^{13}C$ NMR (major isomer, 400 MHz, MeOH-$d_4$): δ = 26.8, 29.4, 29.7, 36.7, 40.2, 41.0, 41.7, 47.2, 56.9, 58.3, 61.4, 61.5, 63.2, 70.5, 70.9, 71.2, 71.3, 71.8, 71.8, 71.9, 78.2, 110.3, 111.4, 112.1, 113.4, 114.8, 117.2, 146.4, 147.5, 148.6, 148.6, 148.9, 149.7, 149.9, 150.0, 157.9, 165.9, 176.0.

HRMS (FAB): $m/z$ [M + Na]$^+$ calcd for $C_{41}H_{56}N_4O_{14}SNa$: 883.3406; found: 883.3410.

LC-MS: $t_R$ = 20.2 min; $m/z$ = 883.1 [M + Na]$^+$.

## Acknowledgment

We wish to thank Dr. Markus Kramer, Dr. Dorothee Wistuba, and Paul Schuler for their analytical services.

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