Electronic Supporting Information

Cyclooctyne [60]Fullerene Hexakis Adducts: a Globular Scaffold for Copper-Free Click Chemistry

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General. Reagents and solvents were purchased as reagent grade and used without further purification. 8,8-Dibromobicyclo[5.1.0]octane,¹ 2-[2'-(2''-azidoethoxy)ethoxy]ethanol² and 1-azidotetradecane³ were prepared according to previously reported procedures. For column chromatography silica gel 60 (230-400 mesh, 0.040-0.063 mm) was purchased from E. Merck. Thin Layer Chromatography (TLC) was performed on aluminium sheets coated with silica gel 60 F254 purchased from E. Merck, visualization by UV light. IR spectra (cm⁻¹) were measured on an ATI Mattson Genesis Series FTIR instrument. NMR spectra were recorded on a Bruker AC 400 or AC 500 with solvent peaks as reference. ¹H and ¹³C NMR spectra were obtained for solutions in CD₃OD, CDCl₃ and DMSO-*d*₆. All the assignments were confirmed by two-dimensional NMR experiments. MALDI-TOF-mass spectra were carried out on a Bruker BIFLEXTM matrix-assisted laser desorption time-of-flight mass spectrometer using dithranol or 2-[(*E*)-3-(4-*tert*-butylphenyl)-2methylprop-2-enylidene]propanedinitrile (DCTB) as matrix. ESI-mass spectra were recorded with an Esquire 6000 ESI-Ion Trap from Bruker Daltonics using CH₂Cl₂/MeOH as solvent system.

Synthesis and Characterization

Bis (4-(benzyloxy)butyl) malonate (1).



Malonyl chloride (100 µL, 0.10 mmol) was added dropwise to a solution of 4-benzyloxy-1butanol (434 µL, 2.40 mmol), Et₃N (334 µL, 2.40 mmol) and DMAP (5 mg, 0.04 mmol) in dry CH₂Cl₂ (6 mL) at 0 °C under Ar atmosphere. After 30 min, the mixture was allowed to slowly warm to room temperature, then stirred overnight. After the reaction, the solution was diluted with CH₂Cl₂ and washed with 1M HCl and brine. The organic layer was dried with anh. MgSO₄, filtered and concentrated. The resulting crude material was purified by silica gel chromatography column (EtOAc/*n*-hexane, 1:4), to give **1** (420 mg, 98%) as a colourless oil. ¹H-NMR (400 MHz, CDCl₃) & 7.40-7.22 (m, 10H, H-Ar), 4.49 (s, 4H, CH_{2Bn}), 4.17 (t, 4H, ³J_{H,H} = 6, OCH_2CH_2), 3.49 (t, 4H, ³J_{H,H} = 6, CH₂CH₂OBn), 3.36 (s, 2H, H_{malonate}), 1.76 (m, 4H, OCH₂CH₂), 1.68 (m, 4H, CH₂CH₂OBn); ¹³C-NMR (100 MHz, CDCl₃) & 166.6 (CO), 138.5 (C_{ipso}-Ar), 128.3, 127.6,

¹ Lallana, E.; Fernandez-Megia, E.; Riguera, R. J. Am. Chem. Soc. **2009**, 131, 5748-5750.

² Li, J.; Zacharek, S.; Chen, X.; Wang, J.; Zhang, W.; Janczuk, A.; Wang, P. G. *Bioorg. Med. Chem.*, **1999**, *7*, 1549-1558.

³ Pajk, S.; Garvas, M.; Strancar, J.; Pecar, S. Org. Biomol. Chem. **2011**, *9*, 4150-4159.

127.5 (C-Ar), 72.9 (CH_{2Bn}), 69.6 (CH₂CH₂OBn), 65.3 (OCH₂CH₂), 41.6 (CH_{2malonate}), 26.1 (CH₂CH₂OBn), 25.4 (OCH₂CH₂); ESI-MS: m/z calcd for C₂₅H₃₂O₆: 428.2; found: 451.3 [M+Na]⁺; ESI-HRMS: m/z calcd for C₂₅H₃₂O₆Na [M+Na]⁺: 451.2091; found: 451.2082.

Compound 2.



DBU (831 µL, 5.56 mmol) was added to a solution of fullerene C₆₀ (200 mg, 0.28 mmol), malonate **1** (1.43 g, 3.3 mmol) and CBr₄ (9.40 g, 27.8 mmol) in dry ODCB (50 mL) under Ar atmosphere. The mixture was stirred for 72 h at room temperature and evaporated. The resulting crude material was purified by silica gel chromatography column (toluene/acetone, $30:1 \rightarrow 20:1$), yielding hexakis-adduct **2** (455 mg, 50%) as a red glassy solid. IR (neat): 2946, 2856, 1743, 1214; ¹H-NMR (500 MHz, CDCl₃) & 7.27-7.14 (m, 60H, H-Ar), 4.38 (s, 24H, CH_{2Bn}), 4.16 (t, 24H, ³J_{H,H} = 7, OCH₂CH₂), 3.37 (t, 24H, ³J_{H,H} = 6, CH₂CH₂OBn), 1.69 (m, 24H, OCH₂CH₂), 1.57 (m, 24H, CH₂CH₂OBn); ¹³C-NMR (125.8 MHz, CDCl₃) & 163.9 (CO), 145.9, 141.2 (C_{sp2,fullerene}), 138.6 (C_{ipso}-Ar), 128.4, 127.7, 127.6 (C-Ar), 73.0 (CH_{2Bn}), 69.6 (C_{sp3,fullerene}), 69.2 (CH₂CH₂OBn), 66.8 (OCH₂CH₂), 45.5 (C_{q,brigde}), 26.2 (CH₂CH₂OBn), 25.4 (OCH₂CH₂); MALDI-ToF (dithranol): *m/z* calcd for C₂₁₀H₁₈₀O₃₆: 3279.7; found: 3279.1 [M]⁺.

Compound 3.



A solution of **2** (435 mg, 0.13 mmol) in a mixture of CH₂Cl₂/MeOH (16 mL, 3/1) was hydrogenated under atmospheric pressure at room temperature overnight using Pd-C (10%) as catalyst. Then, the solution was filtered through Celite, and the catalyst was washed with CH₂Cl₂/MeOH. The filtered solution was concentrated, furnishing pure **3** (291 mg, quant.) as an orange-red solid. IR (neat): 3327, 2926, 2856, 1740, 1222; ¹H-NMR (500 MHz, CD₃OD) δ : 4.36 (t, 24H, ³J_{H,H} = 6, OCH₂CH₂), 3.56 (t, 24H, ³J_{H,H} = 6, CH₂CH₂OH), 1.79 (m, 24H, OCH₂CH₂), 1.61 (m, 24H, CH₂CH₂OH); ¹³C-NMR (125.8 MHz, CD₃OD + CDCl₃) δ : 164.7 (CO), 146.6, 142.3 (C_{sp2,fullerene}), 70.3 (C_{sp3,fullerene}), 68.0 (OCH₂CH₂), 62.1 (CH₂CH₂OH), 47.1 (C_{q,brigde}), 29.8 (CH₂CH₂OH), 26.0 (OCH₂CH₂); MALDI-TOF (dithranol): *m/z* calcd for C₁₂₆H₁₀₈O₃₆: 2196.7; found: 2197.7 [M+H]⁺.

Synthesis of 4-{2'-[2"-(cyclooct-2"'-yn-1"'-yloxy)ethoxy]ethoxy}-4-oxobutanoic acid (4).



Compound 8.



To a solution of 8,8-dibromobicyclo[5.1.0]octane (5.70 g, 21.4 mmol) in a mixture of dry toluene/pyridine (1:1.25, 27 mL), a solution of diethyleneglycol (61 mL, 0.64 mol) and AgClO₄ (13.74 g, 64.3 mmol) in dry toluene (26 mL) was added. The mixture was refluxed in the dark for 4 h under Ar atmosphere. The solvent was evaporated, brine (100 mL) was added and the

insoluble silver salts were removed by filtration. The aqueous phase was extracted with Et₂O (7 x 150 mL) and the combined organic phases were washed with brine (200 mL) and water (200 mL), dried over MgSO₄, filtered and concentrated. The crude product was dissolved in a mixture of dry ⁱPrOH/pyridine (7:1, 184 mL), and KO^tBu (6.30 g, 53.6 mmol) was added. After 60 h of stirring at room temperature, the reaction was neutralized with 5% HCl and partitioned between CH₂Cl₂ and water. Then, the aqueous phase was extracted with CH₂Cl₂ (7 x 200 mL) and the combined organic phases were dried over MgSO₄, filtered and concentrated. The resulting crude material was purified by silica gel chromatography column (EtOAc/hexane, 1:3 \rightarrow 1:1), furnishing compound **8** (2.72 g, 60%) as a yellow oil. ¹H-NMR (400 MHz, CDCl₃) δ : 4.23 (m, 1H, H-1), 3.77-3.70 (m, 3H, CHHO, CH₂CH₂OH), 3.68 (m, 2H, CH₂O), 3.61 (m, 2H, CH₂CH₂OH), 3.52 (m, 1H, CHHO), 2.48 (br s, 1H, OH), 2.25 (m, 1H, H-6a), 2.21-2.09 (m, 2H, H-2a, H-6b), 2.03-1.88 (m, 2H, H-2b, H-5a), 1.88-1.76 (m, 2H, H-3a, H-5b), 1.65 (m, 2H, H-4), 1.44 (m, 1H, H-3b); ¹³C-NMR (100 MHz, CDCl₃) & 100.0 (C-7), 92.4 (C-8), 72.6 (C-1), 72.4 (CH₂CH₂OH), 70.0, 68.4 (each CH₂O), 61.4 (CH₂CH₂OH), 42.1 (C-2), 34.1 (C-5), 29.5 (C-4), 26.2 (C-3), 20.5 (C-6); ESI-MS: *m/z* calcd for C₁₂H₂₀O₃: 212.2; found: 235.1 [M+Na]⁺; ESI-HRMS: *m/z* calcd for C₁₂H₂₀O₃Na [M+Na]⁺: 235.1305; found: 235.1299.

Compound 4.



To a solution of compound **8** (2.44 g, 11.5 mmol) and succinic anhydride (1.42 g, 13.8 mmol) in dry CH₂Cl₂ (60 mL) under Ar atmosphere, DMAP (1.40 g, 11.5 mmol) was added. The reaction mixture was stirred at room temperature overnight. The solvent was removed under vacuum and Et₂O (30 mL) was added. The resulting solution was washed with 5% Na₂CO₃ solution (60 mL). The aqueous layer was brought to pH 4 by adding 1M HCl solution (60 mL). The resulting aqueous solution was extracted three times with Et₂O (400 mL). The organic layers were washed with brine, dried over MgSO₄, filtered and reduced under vacuum, to give compound **4** (3.23 g, 90%) as a yellow oil. ¹H-NMR (400 MHz, CDCl₃) & 4.30-4.22 (m, 3H, H-1, CH₂CH₂OCO), 3.75 (m, 1H, CHHO), 3.71-3.62 (m, 4H, CH₂O, CH₂CH₂OCO), 3.53 (m, 1H, CHHO), 2.71-2.62 (m, 4H, CH₂,_{succ.}), 2.25 (m, 1H, H-6a), 2.20-2.10 (m, 2H, H-2a, H-6b), 2.05-1.88 (m, 2H, H-2b, H-5a), 1.88-1.76 (m, 2H, H-3a, H-5b), 1.64 (m, 2H, H-4), 1.43 (m, 1H, H-3b); ¹³C-NMR (100 MHz, CDCl₃) & 177.3 (COOH), 172.2 (COO), 100.3 (C-7), 92.6 (C-8), 72.9 (C-1), 70.4 (CH₂O), 69.0

 (CH_2CH_2OCO) , 68.5 (CH₂O), 63.9 (CH₂CH₂OCO), 42.2 (C-2), 34.3 (C-5), 29.7 (C-4), 29.0 (CH_{2,succ.}), 26.4 (C-3), 20.7 (C-6); ESI-MS: *m/z* calcd for C₁₆H₂₄O₆: 312.2; found: 335.2 [M+Na]⁺, 351.2 [M+K]⁺; ESI-HRMS: *m/z* calcd for C₁₆H₂₄O₆Na [M+Na]⁺: 335.1465; found: 335.1456.

Compound 5.



To a solution of **3** (100 mg, 45.5 μ mol), cyclooctyne derivative **4** (256 mg, 0.82 mmol) and DPTS (80 mg, 0.27 mmol) in a dry mixture of CH₂Cl₂/DMF (3.4 mL, 7.5:1) under Ar atmosphere, DCC (171 mg, 0.82 mmol) in dry CH₂Cl₂ (2 mL) was added. The reaction mixture was stirred at room temperature overnight. Once the reaction was complete, the dicyclohexylurea was filtered off in a fritted glass filter and washed with CH₂Cl₂. The crude product was purified by size-exclusion chromatography (Sephadex LH-20, MeOH/CH₂Cl₂ 1:1), to give **5** (259 mg, 99%) as a

red oil. IR (neat): 2928, 2858, 1736, 1214; ¹H-NMR (500 MHz, CDCl₃) δ : 4.29 (m, 24H, OCH₂CH₂), 4.26-4.17 (m, 36H, H-1, CH₂O), 4.11 (m, 24H, CH₂CH₂OCO), 3.75-3.66 (m, 36H, CHHO, CH₂O), 3.64 (m, 24H, CH₂O), 3.50 (m, 12H, CHHO), 2.69-2.58 (m, 48H, CH_{2,succ.}), 2.24 (m, 12H, H-6a), 2.20-2.06 (m, 24H, H-2a, H-6b), 2.01-1.88 (m, 24H, H-2b, H-5a), 1.87-1.64 (m, 84H, H-3a, H-4a, H-5b, OCH₂CH₂, CH₂CH₂OCO), 1.60 (m, 12H, H-4b), 1.43 (m, 12H, H-3b); ¹³C-NMR (125.8 MHz, CDCl₃) δ : 172.3, 172.2 (CO_{succ}), 163.6 (CO), 145.8, 141.1 (C_{sp2,fullerene}), 100.1 (C-7), 92.8 (C-8), 72.8 (C-1), 70.4 (CH₂O), 69.1 (C_{sp3,fullerene}), 69.0 (CH₂O), 68.5 (CH₂O), 66.4 (OCH₂CH₂), 63.9 (CH₂CH₂OCO), 63.8 (CH₂O), 45.3 (C_{q,brigde}), 42.3 (C-2), 34.3 (C-5), 29.7 (C-4), 29.0 (CH_{2,succ.}), 26.4 (C-3), 25.1 (OCH₂CH₂CH₂CH₂OCO), 20.7 (C-6); MALDI-TOF (DCTB): *m/z* calcd for C₃₁₈H₃₇₂O₉₆: 5730.4; found: 5753.4 [M+Na]⁺.

Compound 6c.



To a solution of 2-[2'-(2"-azidoethoxy)ethoxy]ethanol (143 mg, 0.82 mmol), biotin (300 mg, 1.23 mmol) and DPTS (120 mg, 0.41 mmol) in dry CH₂Cl₂ (3 mL) under Ar atmosphere, DCC (426 mg, 2.05 mmol) in dry CH₂Cl₂ (1.5 mL) was added. The reaction mixture was stirred at room temperature overnight. Once the reaction was complete, the dicyclohexylurea was filtered off in a fritted glass filter and washed with a small volume of CH₂Cl₂. The crude product was purified by silica gel chromatography column (CH₂Cl₂/MeOH, 30:1) to give 6c (323 mg, 98%) as a white amorphous solid. IR (neat): 3212, 2923, 2865, 2101, 1697, 1119; ¹H-NMR (400 MHz, CDCl₃) & 5.95 (s, 1H, NH), 5.37 (s, 1H, NH), 4.49 (m, 1H, H-7_{biotin}), 4.30 (ddd, 1H, J_{6.7} = 8, $J_{6,5} = 5$, $J_{6,NH} = 1$, H-6_{biotin}), 4.22 (m, 2H, CH₂O), 3.70 (t, 2H, ${}^{3}J_{H,H} = 5$, CH₂O), 3.68-3.63 (m, 6H, $CH_2CH_2N_3$, CH_2O), 3.38 (t, 2H, ${}^{3}J_{H,H}$ = 5, CH_2N_3), 3.14 (ddd, 1H, $J_{5,4a}$ = 11, $J_{5,4b}$ = 6, H-5_{biotin}), 2.90 (dd, 1H, J_{8a.8b} = 13, J_{8a.7} = 5, H-8a_{biotin}), 2.73 (d, 1H, H-8b_{biotin}), 2.36 (t, 2H, J_{1.2} = 8, H-1_{biotin}), 1.79-1.59 (m, 4H, H-2_{biotin}, H-4_{biotin}), 1.44 (m, 2H, H-3_{biotin}); ¹³C-NMR (100 MHz, CDCl₃) & 173.7 (CO_{ester}), 164.1 (CO_{urea}), 70.6, 70.5 (CH₂O), 70.0 (CH₂CH₂N₃), 69.2 (CH₂O), 63.4 (CH₂O), 62.0 (C-6_{biotin}), 60.1 (C-7_{biotin}), 55.6 (C-5_{biotin}), 50.7 (CH₂N₃), 40.5 (C-8_{biotin}), 33.8 (C-1_{biotin}), 29.3 (C-3_{biotin}), 28.2 (C-4_{biotin}), 24.7 (C-2_{biotin}); ESI-MS: *m*/*z* calcd for C₁₆H₂₇N₅O₅S: 401.2; found: 402.2 [M+H]⁺, 424.2 $[M+Na]^+$; ESI-HRMS: m/z calcd for $C_{16}H_{27}N_5O_5SNa$ $[M+Na]^+$: 424.1625; found: 424.1616.

Synthesis of 6d.



ÑHFmoc

To a solution of 2-[2'-(2"-azidoethoxy)ethoxy]ethanol (150 mg, 0.86 mmol), Fmoc-Phe(OH) (508 mg, 1.29 mmol) and DPTS (126 mg, 0.43 mmol) in dry CH₂Cl₂ (4 mL) under Ar atmosphere, DCC (446 mg, 2.14 mmol) in dry CH₂Cl₂ (2 mL) was added. The reaction mixture was stirred at room temperature overnight. Once the reaction was complete, the dicyclohexylurea was filtered off in a fritted glass filter and washed with a small volume of CH₂Cl₂. The crude product was purified by silica gel chromatography column (EtOAc/n-hexane, 1:3 \rightarrow 1:1) to give 9 (460 mg, 99%) as a colourless oil. ¹H-NMR (400 MHz, CDCl₃) δ : 7.78 (d, 2H, J = 8, H-Ar), 7.58 (t, 2H, J = 6, H-Ar), 7.41 (t, 2H, J = 7, H-Ar), 7.36-7.23 (m, 5H, H-Ar), 7.15 (d, 2H, J = 7, H-Ar), 5.37 (br d, 1H, J_{NH,CH} = 8, NH), 4.73 (m, 1H, CH), 4.46 (dd, 1H, ²J_{H,H} = 11, ³J_{H,H} = 7, CHH_{Fmoc}), 4.38-4.26 (m, 3H, CHH_{Fmoc}, CH₂O), 4.22 (br t, 2H, ³J_{H,H} = 7, CH_{Fmoc}), 3.74-3.57 (m, 8H, CH₂CH₂N₃, CH₂O), 3.35 (t, 2H, ${}^{3}J_{H,H} = 5$, CH₂N₃), 3.19 (dd, 1H, ${}^{2}J_{H,H} = 14$, ${}^{3}J_{H,CH} = 6$, CHH_{Bn}), 3.13 (dd, 1H, ${}^{3}J_{H,CH} = 6$, CHH_{Bn}); 13 C-NMR (100 MHz, CDCl₃) & 171.5 (CO_{ester}), 155.6 (CO_{Fmoc}), 143.9, 143.8, 141.4, 135.8 (C_{ipso}-Ar), 129.5, 128.6, 127.8, 127.2, 127.1, 125.2, 125.1, 120.0 (C-Ar), 70.7 (CH₂O), 70.2 (CH₂CH₂N₃), 68.9 (CH₂O), 67.0 (CH_{2Fmoc}), 64.6 (CH₂O), 54.8 (CH), 50.7 (CH₂N₃), 47.2 (CH_{Fmoc}), 38.2 (CH_{2Bn}); ESI-MS: *m*/*z* calcd for C₃₀H₃₂N₄O₆: 544.2; found: 545.2 [M+H]⁺, 567.2 [M+Na]⁺; ESI-HRMS: *m*/*z* calcd for C₃₀H₃₂N₄O₆Na [M+Na]⁺: 567.2214; found: 567.2205.

Compound 6d.



Compound **9** (460 mg, 0.85 mmol) was dissolved in piperidine/DMF (20%) (5 mL), and the mixture was stirred at r.t. for 15 min. Then, the solvent was removed and the residue was

purified by column chromatography (EtOAc/MeOH, 100:0 \rightarrow 9:1) affording the unprotected amine **6d** (270 mg, 99%) as a colourless oil. IR (neat): 3379, 2924, 2867, 2099, 1733, 1118; ¹H-NMR (400 MHz, CDCl₃) & 7.42-7.22 (m, 5H, H-Ar), 4.33 (t, 2H, ³J_{H,H} = 5, CH₂O), 3.83 (m, 1H, CH), 3.79-3.65 (m, 8H, CH₂CH₂N₃, CH₂O), 3.43 (t, 2H, ³J_{H,H} = 5, CH₂N₃), 3.16 (dd, 1H, ²J_{H,H} = 13, ³J_{H,H} = 5, CHH_{Bn}), 2.95 (dd, 1H, ³J_{H,H} = 8, CHH_{Bn}), 1.62 (br s, 2H, NH₂); ¹³C-NMR (100 MHz, CDCl₃) & 174.7 (CO_{ester}), 137.0 (C_{ipso}-Ar), 129.0, 128.2, 126.4 (C-Ar), 70.3, 70.2 (CH₂O), 69.7 (CH₂CH₂N₃), 68.7 (CH₂O), 63.6 (CH₂O), 55.4 (CH), 50.3 (CH₂N₃), 40.6 (CH_{2Bn}); ESI-MS: *m/z* calcd for C₁₅H₂₂N₄O₄: 322.2; found: 323.2 [M+H]⁺, 345.2 [M+Na]⁺; ESI-HRMS: *m/z* calcd for C₁₅H₂₃N₄O₄ [M+H]⁺: 323.1714; found: 323.1706.

Compound 6e.



To a solution of 2-[2'-(2''-azidoethoxy)ethoxy]ethanol (72 mg, 0.41 mmol), Boc-PNA-T-OH (250 mg, 0.62 mmol) and DPTS (61 mg, 0.21 mmol) in dry CH₂Cl₂ (2 mL) under Ar atmosphere, DCC (215 mg, 1.03 mmol) in dry CH₂Cl₂ (1 mL) was added. The reaction mixture was stirred at room temperature overnight. Once the reaction was complete, the dicyclohexylurea was filtered off in a fritted glass filter and washed with a small volume of CH₂Cl₂. The crude product was purified by silica gel chromatography column (CH₂Cl₂/MeOH, 30:1) to give **6e** (210 mg, 94%) as a white amorphous solid. IR (neat): 3340, 3201, 2931, 2105, 1666, 1142; Due to the limited rotation around the secondary amide, several of the signals were doubled (indicated in the list by mj. for major and mi. for minor conformers): ¹H-NMR (400 MHz, CD₃OD) δ : 7.29 (s, '1H', mj., H-6_{thymine}), 7.27 (s, '1H', mi., H-6_{thymine}), 4.74 (s, '2H', mj., CH₂CON), 4.58 (s, '2H', mi., CH₂CON), 4.37 (m, '2H', mi., CH₂O), 4.35 (s, '2H', mi., CH₂COO), 4.28 (m, '2H', mj., CH₂O), 4.16 (s, '2H', mj., CH₂COO), 3.78 (m, '2H', mi., CH₂O), 3.72 (m, '2H', mj., CH₂O), 3.70-3.62 (m, 6H, CH₂CH₂N₃, CH2O), 3.54 (m, '2H', mj., NCH2), 3.49 (m, '2H', mi., NCH2), 3.37 (m, 2H, CH2N3), 3.31 (under CD₃OD, '2H', mj., NCH₂), 3.20 (m, '2H', mi., NCH₂), 1.88 (s, 3H, CH_{3,thymine}), 1.45 (s, 9H, mj., CH_{3,Boc}), 1.44 (s, 9H, mi., CH_{3,Boc}); ¹³C-NMR (100 MHz, CD₃OD) & 170.8, 170.7 (CO_{ester}), 169.9, 169.6 (C-4_{thymine}), 166.7 (CO_{amide}), 158.2 (CO_{Boc}), 152.9, 152.8 (C-2_{thymine}), 143.5 (C-6_{thymine}), 110.9, 110.8 (C-5_{thymine}), 80.5, 80.1 (C(CH₃)₃), 71.5 (CH₂O), 71.4 (CH₂CH₂N₃), 71.1, 71.0 (CH₂O), 69.9,

69.8 (CH₂O), 65.8, 65.5 (CH₂O), 51.7 (CH₂N₃), 50.8, 49.7 (CH₂COO), under CD₃OD (NCH₂, CH₂CON), 39.5, 39.1 (NCH₂), 28.7 (CH_{3,Boc}), 12.3 (CH_{3,thymine}); ESI-MS: m/z calcd for C₂₂H₃₅N₇O₉: 541.2; found: 564.2 [M+Na]⁺; ESI-HRMS: m/z calcd for C₂₂H₃₅N₇O₉Na [M+Na]⁺: 564.2388; found: 564.2384.

General procedure for SPAAC.



Cyclooctyne derivative **5** (50 mg, 8.7 μ mol) and compound **6a-e** (0.16 mmol), were dissolved in DMSO (1 mL) in a sealed microwave vial. The solution was heated at 50 °C in a microwave oven for 30 minutes. After that time, the solution was purified by size-exclusion chromatography (Sephadex LH-20, CH₂Cl₂/MeOH 1/1), furnishing compounds **7a-e**.

Compound 7a.

$$\begin{array}{c} \begin{array}{c} & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ \end{array} \end{array}$$

Following the general procedure and using compound **6a** as starting material, compound **7a** (70 mg, 93%) was obtained as a orange-red oil. IR (neat): 2924, 2854, 1733, 1160; Due to the formation of two expected regioisomers, several of the signals were doubled (indicated in the list by mj. for major and mi. for minor): ¹H-NMR (500 MHz, CDCl₃) δ : 4.82 (dd, '12H', J_{1.2a} = 5, $J_{1,2b}$ = 4, mj., H-1), 4.69 (dd, '12H', $J_{1,2a}$ = 9, $J_{1,2b}$ = 3, mi., H-1), 4.32 (t, '24H', ³ $J_{H,H}$ = 7, mi., CH₂CH₂N), 4.26 (m, 24H, OCH₂CH₂), 4.19 (m, 24H, CH₂O), 4.15 (m, '24H', mj., CH₂CH₂N), 4.07 (t, 24H, ³J_{H,H} = 6, CH₂CH₂OCO), 3.73-3.41 (m, 72H, CH₂O), 3.06, 2.78 (2m, '24H', both isomers, H-6), 2.67-2.52 (m, 48H, CH_{2.succ.} + '24H', both isomers, H-6), 2.14 (m, '24H', both isomers, H-2a), 1.95 (m, '24H', mi., H-2b), 1.89-1.39 (m, 120H, CH₂CH₂N, OCH₂CH₂, CH₂CH₂OCO, H-3, H-5 + '24H', mj., H-2b + '24H', both isomers, H-4), 1.34-1.12 (m, 262H, CH_{2.aliohatic chain}), 1.00 (m, '24H', both isomers, H-4), 0.84 (t, 36H, ${}^{3}J_{H,H}$ = 7, CH₃); 13 C-NMR (125.8 MHz, CDCl₃) δ : 172.3, 172.2 (CO_{succ}), 163.7 (CO), 145.8 (C_{sp2,fullerene}), 144.9 (C-7, mj.), 144.8 (C-8, mi.), 141.1 (C_{sp2,fullerene}), 133.5 (C-8, mj.), 132.4 (C-7, mi.), 74.7 (C-1, mj.), 72.2 (C-1, mi.), 70.6, 70.4 (CH₂O, both isomers), 69.2, 69.0 (CH₂O, both isomers), 69.1 (C_{sp3,fullerene}), 67.8, 67.7 (CH₂O, both isomers), 66.5 (OCH₂CH₂), 63.9 (CH₂CH₂OCO), 63.9, 63.8 (CH₂O, both isomers), 48.8 (CH₂CH₂N, mi.), 47.7 (CH₂CH₂N, mj.), 45.3 (C_{q,brigde}), 35.6 (C-2, mj.), 31.9 (CH_{2,aliphatic chain} + C-2, mi.), 30.5, 30.4 (CH₂CH₂N, both isomers), 29.7-29.4 (CH_{2,aliphatic chain}), 29.3 (CH_{2,succ.}), 29.2, 29.1, 29.0, 28.9 (CH_{2,aliphatic chain}), 28.5 (C-5, mi.), 27.3 (C-5, mj.), 26.8, 26.7 (CH_{2,aliphatic chain}), 25.6 (C-4, mj.), 25.2 (OCH₂CH₂CH₂CH₂OCO), 24.7 (C-4, mi.), 24.5 (C-6, mi.), 22.9 (C-3, mi.), 22.7 (CH₂CH₃), 20.9 (C-3, mj.), 20.1 (C-6, mj.), 14.2 (CH₃); MALDI-TOF (DCTB): *m/z* calcd for C₄₈₆H₇₂₀N₃₆O₉₆: 8601; found: 8624 [M+Na]⁺.

Compound 7b.

Following the general procedure and using compound **6b** as starting material, compound **7b** (68 mg, 99%) was obtained as an orange-red solid. IR (neat): 3436, 2927, 2866, 1733, 1216; Due to the formation of two expected regioisomers, several of the signals were doubled (indicated in the list by mj. for major and mi. for minor): ¹H-NMR (500 MHz, CD₃OD + CDCl₃) δ : 4.88 (dd, '12H', J_{1,2a} = 8, J_{1,2b} = 3, mi., H-1), 4.79 (dd, '12H', J_{1,2a} = 6, J_{1,2b} = 4, mj., H-1), 4.66, under water (2m, '24H', mi., OCH₂CH₂N), 4.41 (t, '24H', ³J_{H,H} = 5, mj., OCH₂CH₂N), 4.32 (m, 24H, OCH_2CH_2), 4.21 (m, 24H, CH_2O), 4.10 (t, 24H, ${}^{3}J_{H,H}$ = 6, CH_2CH_2OCO), 3.90 (m, 24H, OCH_2CH_2N), 3.74-3.52 (m, 144H, CH₂O, OCH₂CH₂OH), 3.49 (m, 24H, OCH₂CH₂OH), 3.08, 2.78 (2m, 24H, both isomers, H-6), 2.63 (m, 48H, CH_{2.succ.}), 2.22 (m, '24H', mi., H-2a), 2.12 (m, '24H', mj., H-2a), 2.02 (m, '24H', mi., H-2b), 1.89-1.39 (m, 120H, OCH₂CH₂, CH₂CH₂OCO, H-3, H-4, H-5 + '24H', mj., H-2b); ¹³C-NMR (125.8 MHz, CD₃OD + CDCl₃) δ: 173.2 (CO_{succ}), 164.3 (CO), 146.4 (C_{sp2.fullerene}), 145.1 (C-7, mj.), 144.7 (C-8, mi.), 141.8 (Csp2,fullerene), 136.1 (C-8, mj.), 135.1 (C-7, mi.), 75.1 (C-1, mj.), 73.1 (OCH2CH2OH), 72.4 (C-1, mi.), 71.2, 70.1, 70.0, 70.8 (CH2O), 70.5, 70.2 (OCH2CH2N, both isomers), 69.7 (C_{sp3,fullerene}), 69.6, 69.5 (CH₂O, both isomers), 68.5, 68.2 (CH₂O, both isomers), 67.2 (OCH₂CH₂), 64.6 (CH₂CH₂OCO), 64.5, 64.4 (CH₂O, both isomers), 61.7 (OCH₂CH₂OH), under CD₃OD (OCH₂CH₂N, mi.), 48.2 (OCH₂CH₂N, mj.), 46.2 (C_{q,brigde}), 36.1 (C-2, mj.), 31.3 (C-2, mi.), 29.4 (CH_{2,succ.}), 28.6 (C-5, mi.), 27.5 (C-5, mj.), 26.1 (C-4, mj.), 25.7 (OCH₂CH₂CH₂CH₂OCO), 24.8 (C-4, mi.), 24.6 (C-6, mi.), 23.3 (C-3, mi.), 21.5 (C-3, mj.), 20.6 (C-6, mj.); MALDI-TOF (DCTB): *m/z* calcd for C₃₉₀H₅₂₈N₃₆O₁₃₂: 7831; found: 7854 [M+Na]⁺.

Compound 7c.



Following the general procedure and using compound 6c as starting material, compound 7c (91 mg, 99%) was obtained as a orange-red solid. IR (neat): 3368, 2928, 2865, 1731, 1696, 1211; Due to the formation of two expected regioisomers, several of the signals were doubled (indicated in the list by mj. for major and mi. for minor): ¹H-NMR (500 MHz, CD₃OD + CDCl₃) δ : 4.90 (m, '12H', mi., H-1), 4.80 (m, '12H', mj., H-1), 4.69, 4.57 (2m, '24H', mi., OCH₂CH₂N), 4.49 (dd, 12H, $J_{7.6} = 7$, $J_{7.8a} = 5$, H-7_{biotin}), 4.42 (t, '24H', ³ $J_{H,H} = 5$, mj., OCH₂CH₂N), 4.38-4.26 (m, 36H, OCH2CH2, H-6biotin), 4.26-4.14 (m, 48H, CH2O), 4.11 (m, 24H, CH2CH2OCO), 3.90 (m, 24H, OCH2CH2N), 3.76-3.43 (m, 144H, CH2O), 3.17 (m, 12H, H-5biotin), 3.08 (m, '24H', both isomers, H-6), 2.90 (dd, 12H, J_{8a,8b} = 13, H-8a_{biotin}), 2.80 (m, '24H', both isomers, H-6), 2.71 (d, 12H, H-8b_{biotin}), 2.63 (m, 48H, CH_{2,succ.}), 2.35 (m, 24H, H-1_{biotin}), 2.23 (m, '24H', mi., H-2a), 2.13 (m, '24H', mj., H-2a), 2.03 (m, '24H', mi., H-2b), 1.90-1.49 (m, 144H, OCH₂CH₂, CH₂CH₂OCO, H-3, H-5, H-2_{biotin}, H-4_{biotin} + '24H', mj., H-2b + '24H', both isomers, H-4), 1.44 (m, 24H, H-3_{biotin}), 1.24, 1.07 (2m, '24H', both isomers, H-4); ¹³C-NMR (125.8 MHz, CD₃OD + CDCl₃) & 174.7 (CO_{biotin}), 173.4 (CO_{succ}), 165.2 (CO_{urea}), 164.4 (CO), 146.5 (C_{sp2,fullerene}), 145.3 (C-7, mj.), 144.8 (C-8, mi.), 141.9 (C_{sp2.fullerene}), 136.3 (C-8, mj.), 135.3 (C-7, mi.), 75.3 (C-1, mj.), 72.5 (C-1, mi.), 71.3, 71.2, 71.1, 71.0 (CH₂O), 70.7, 70.5 (OCH₂CH₂N, both isomers), 69.9 (C_{sp3,fullerene}), 69.9, 69.7, 69.6 (CH₂O), 68.7, 68.4 (CH₂O, both isomers), 67.4 (OCH₂CH₂), 64.7 (CH₂CH₂OCO), 64.6, 64.5 (CH₂O, both isomers), 64.1 (CH₂O), 62.8 (C-6_{biotin}), 61.0 (C-7_{biotin}), 56.4 (C-5_{biotin}), under CD₃OD (OCH₂CH₂N, mi.), 48.4 (OCH₂CH₂N, mj.), 46.5 (C_{q,brigde}), 40.8 (C-8_{biotin}), 36.2 (C-2, mj.), 34.4 (C-1_{biotin}), 31.5 (C-2, mi.), 29.6 (CH_{2,succ.}), 29.2 (C-3_{biotin}), 29.0 (C-4_{biotin}), 28.8 (C-5, mi.), 27.7 (C-5, mj.), 26.3 (C-4, mj.), 25.9 (OCH₂CH₂CH₂CH₂OCO), 25.4 (C-2_{biotin}), 25.0 (C-4, mi.), 24.7 (C-6, mi.), 23.5 (C-3, mi.), 21.7 (C-3, mj.), 20.7 (C-6, mj.).

Compound 7d.



Following the general procedure and using compound 6d as starting material, compound 7d (82 mg, 98%) was obtained as a orange-red solid. IR (neat): 3386, 2926, 2864, 1732, 1214; Due to the formation of two expected regioisomers, several of the signals were doubled (indicated in the list by mj. for major and mi. for minor): ¹H-NMR (500 MHz, CDCl₃) δ : 7.32-7.08 (m, 60H, H-Ar), 4.82 (m, 12H, both isomers, H-1), 4.56 (m, '24H', mi., OCH₂CH₂N), 4.34 (t, '24H', ³J_{H,H} = 5, mj., OCH₂CH₂N), 4.27 (m, 24H, OCH₂CH₂), 4.24-4.15 (m, 48H, CH₂O), 4.08 (m, 24H, CH₂CH₂OCO), 3.89 (m, 24H, OCH2CH2N), 3.74 (m, 12H, CHNH2), 3.71-3.42 (m, 144H, CH2O), 3.13-3.00 (m, 12H, CHH_{Bn} + '24H', both isomers, H-6), 2.92-2.77 (m, 12H, CHH_{Bn} + '24H', both isomers, H-6), 2.67 (m, '24H', both isomers, H-6), 2.61 (m, 48H, CH_{2,succ.}), 2.16 (m, '24H', both isomers, H-2a), 1.97 (m, '24H', mi., H-2b), 1.90-1.49 (m, 144H, OCH2CH2, CH2CH2OCO, H-3, H-4, H-5, NH2 + '24H'. mj., H-2b); ¹³C-NMR (125.8 MHz, CDCl₃) δ : 175.1 (CO_{Phe}), 172.4, 172.3 (CO_{succ}), 164.7 (CO), 145.9 (C_{sp2,fullerene}), 144.8 (C-7, mj.), 144.5 (C-8, mi.), 141.1 (C_{sp2,fullerene}), 137.2 (C_{ipso}-Ar), 134.9 (C-8, mj.), 133.7 (C-7, mi.), 129.4, 128.6, 126.9 (C-Ar), 74.7 (C-1, mj.), 72.1 (C-1, mi.), 70.7-71.4 (CH₂O), 70.3, 70.0 (OCH₂CH₂N, both isomers), 69.2 (C_{sp3,fullerene}), 69.1, 69.0 (CH₂O), 67.9, 67.5 (CH₂O, both isomers), 66.5 (OCH₂CH₂), 64.0, 63.9, 63.8 (CH₂CH₂OCO, CH₂O both isomers), 55.8 (CHNH₂), 48.5 (OCH₂CH₂N, mi.), 47.5 (OCH₂CH₂N, mj.), 45.4 (C_{q,brigde}), 41.1 (CH_{2Bn}), 35.6 (C-2, mj.), 31.2 (C-2, mi.), 29.0, 28.9 (CH_{2,succ.}), 28.3 (C-5, mi.), 27.1 (C-5, mj.), 25.6 (C-4, mj.), 25.2 (OCH₂CH₂CH₂CH₂OCO), 24.5 (C-4, mi. + C-6, mi.), 23.0 (C-3, mi.), 21.0 (C-3, mj.), 20.1 (C-6, mj.).

Compound 7e.



Following the general procedure and using compound 6e as starting material, compound 7e (100 mg, 94%) was obtained as a orange-red solid. IR (neat): 3352, 3199, 2921, 2863, 1730, 1216; Due to the formation of two expected regioisomers, several of the signals were doubled (indicated in the list by mj. for major and mi. for minor). Therefore, some of the signals of thymine were doubled owing to the limited rotation around the secondary amide: ¹H-NMR (500 MHz, DMSO-d₆) δ: 11.28 (br s, 12H, NH_{thymine}), 7.28, 7.24 (2s, 12H, H-6_{thymine}), 6.91, 6.72 (2s, 12H, NH_{Boc}), 4.84 (m, '12H', mi., H-1), 4.71 (m, '12H', mj., H-1), 4.65 (s, 24H, CH₂CON_{thymine}), 4.51 (m, '24H', mi., OCH₂CH₂N), 4.38 (m, '24H', mj., OCH₂CH₂N), 4.28 (m, 24H, OCH₂CH₂), 4.24-4.03 (m, 72H, CH₂O, CH₂COO_{thymine}), 4.00 (m, 24H, CH₂CH₂OCO), 3.77 (m, 24H, OCH₂CH₂N), 3.65-3.24 (m, 168H, CH₂O, NCH_{2,thymine}), 3.16, 3.01 (2m, 24H, NCH_{2,thymine}), 2.95, 2.73 (2m, 24H, both isomers, H-6), 2.54 (m, 48H, CH_{2,succ.}), 2.11 (m, '24H', mi., H-2a), 1.99 (m, '24H', mj., H-2a), 1.91 (m, '24H', mi., H-2b), 1.74 (s, 36H, CH_{3.thvmine}), 1.72-1.41 (m, 96H, OCH₂CH₂, CH₂CH₂OCO, H-3, H-5 + '24H', mj., H-2b + '24H', both isomers, H-4), 1.36 (br s, 108H, CH_{3.Boc}), 1.30-0.90 (m, '24H', both isomers, H-4); ¹³C-NMR (125.8 MHz, DMSO-d₆) δ: 171.9, 171.8 (CO_{succ}), 169.3, 169.0 (CO_{ester,thymine}), 167.6, 167.3 (C-4_{thymine}), 164.3 (CO_{amide,thymine}), 162.7 (CO), 155.7 (CO_{Boc}), 150.9 (C-2_{thymine}), 145.0 (C_{sp2,fullerene}), 143.8 (C-7, mj.), 143.0 (C-8, mi.), 141.9 (C-6_{thymine}), 140.7 (C_{sp2,fullerene}), 134.6 (C-8, mj.), 133.7 (C-7, mi.), 108.2, 108.1 (C-5_{thymine}), 78.0, 77.7 (C(CH₃)₃), 73.9 (C-1, mj.), 70.7 (C-1, mi.), 69.7-69.6 (CH₂O), 69.4, 69.2 (OCH₂CH₂N, both isomers), 68.7 (C_{sp3,fullerene}), 68.2, 68.1 (CH₂O, both isomers), 67.2, 67.0 (CH₂O, both isomers), 66.7 (OCH₂CH₂), 64.2, 63.8 (CH₂CH₂OCO), 63.4, 63.3 (CH₂O, both isomers), 49.0 (CH₂COO_{thymine}), 47.8 (OCH₂CH₂N, mi.), 47.6 (CH₂COO_{thymine}), 47.5 (CH₂CON_{thymine}), 46.9 (NCH_{2,thyime}, OCH₂CH₂N, mj.), 45.5 (C_{q,brigde}), 38.1, 37.6 (NCH_{2,thvmine}), 35.0 (C-2, mj.), 30.3 (C-2, mi.), 28.4 (CH_{2,succ.}), 28.1 (CH_{3,Boc}), 27.9 (C-5, mi.), 26.4 (C-5, mj.), 25.2 (C-4, mj.), 24.6 (OCH₂CH₂CH₂CH₂OCO), 23.9 (C-4, mi.), 23.6 (C-6, mi.), 22.1 (C-3, mi.), 20.7 (C-3, mj.), 19.4 (C-6, mj.), 11.9 (CH_{3.thymine}); MALDI-TOF (DCTB): *m/z* calcd for C₅₈₂H₇₉₂N₈₄O₂₀₄: 12231; found: 12254 [M+Na]⁺.

¹H NMR (top) and ¹³C NMR (bottom) spectra



¹H-NMR (400 MHz, CDCl₃): compound **1**



 $^{\rm 13}\text{C-NMR}$ (100 MHz, CDCl_3): compound 1



7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 f1 (ppm)

¹H-NMR (500 MHz, CDCl₃): compound **2**



 $^{\rm 13}\text{C-NMR}$ (125.8 MHz, CDCl₃): compound $\boldsymbol{2}$



¹H-NMR (500 MHz, CD₃OD): compound **3**



 $^{13}\text{C-NMR}$ (125.8 MHz, CD₃OD + $\epsilon\text{CDCl}_3\text{)}\text{:}$ compound **3**



7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 f1 (ppm)

¹H-NMR (400 MHz, CDCl₃): compound **8**





¹H-NMR (400 MHz, CDCl₃): compound **4**



 $^{\rm 13}\text{C-NMR}$ (100 MHz, $\text{CDCl}_{\rm 3}\text{):}$ compound 4







¹³C-NMR (125.8 MHz, CDCl₃): compound **5**



¹H-NMR (400 MHz, CDCl₃): compound **6c**







¹³C-NMR (100 MHz, CDCl₃): compound **9**



¹H-NMR (400 MHz, CDCl₃): compound **6d**



¹³C-NMR (100 MHz, CDCl₃): compound **6d**







 $^{\rm 13}\text{C-NMR}$ (100 MHz, CD₃OD): compound 6e



¹H-NMR (500 MHz, CDCl₃): compound **7a**





¹H-NMR (500 MHz, CD₃OD + CDCl₃): compound **7b**



¹³C-NMR (125.8 MHz, CD₃OD + CDCl₃): compound **7b**



7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 f1 (ppm)

¹H-NMR (500 MHz, CD₃OD + CDCl₃): compound **7c**



 $^{13}\text{C-NMR}$ (125.8 MHz, CD₃OD + CDCl₃): compound **7c**



7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8 f1 (ppm)

¹H-NMR (500 MHz, CDCl₃): compound **7d**



¹³C-NMR (125.8 MHz, CDCl₃): compound **7d**



¹³C-NMR (125.8 MHz, DMSO-*d*₆): compound **7e**

MS spectra







MALDI-ToF (matrix: dithranol): compound **3**



MALDI-ToF (matrix: DCTB): compound 5



MALDI-ToF (matrix: DCTB): compound 7a



MALDI-ToF (matrix: DCTB): compound 7b



MALDI-ToF (matrix: DCTB): compound 7c



MALDI-ToF (matrix: DCTB): compound 7d



MALDI-ToF (matrix: DCTB): compound 7e