Supplementary Material (ESI) for Chemical Communications

Supporting Information for:

Protein assembly along a supramolecular wire

Marion K. Müller,[a] Katja Petkau,[b] and Luc Brunsveld*[ab]

[a] *Chemical Genomics Centre of the Max Planck Society, Otto-Hahn Straße 15, 44227 Dortmund, Germany.*
[b] *Laboratory of Chemical Biology, Department of Biomedical Engineering, Technische Universiteit Eindhoven, Den Dolech 2, 5612AZ, Eindhoven, the Netherlands. Fax: (+) 31 402478367, Tel: (+) 31 402473737, E-mail: l.brunsveld@tue.nl*

Contents

1. Materials and Methods

Nuclear Magnetic Resonance Spectroscopy

^{1}H and ^{13}C NMR spectra were recorded on one of the following instruments:

Varian Mercury 400 (400 MHz, ^{1}H NMR; 100.6 MHz, ^{13}C NMR)

Bruker DRX 400 (400 MHz, ^{1}H NMR; 100.6 MHz, ^{13}C NMR)

Bruker DRX 500 (500 MHz, ^{1}H NMR; 125.8 MHz, ^{13}C NMR)

Bruker DRX 600 (600 MHz, ^{1}H NMR; 150.9 MHz, ^{13}C NMR)

with tetramethylsilane as the internal reference. The chemical shifts are provided in ppm and the coupling constants in Hz. The following abbreviations for multiplicities are used: s, singlet; d, doublet; dd, double doublet; t, triplet; q, quadruplet; m, multiplet; br, broad; ar, aromatic.

Mass Spectrometry (MS)

ESI-mass spectra were recorded on a Finnigan LCQ ESI spectrometer.

MALDI-TOF spectra were recorded on a Voyager- DE Pro MALDI-TOF with 2,5-dihydroxybenzoic acid (DHB) as matrix (20 mg DHB, 900 µL H2O, 90 µL ethanol, 10 µL TFA).

Reversed-Phase High-Pressure Liquid Chromatography (HPLC)

Analytical HPLC was recorded on an Agilent HPLC (1100 series machine) using a CC125/4 NUCLEODUR C18 Gravity, 3 µ (Macherey- Nagel) column with a flow of 0.1 ml/min. The standard gradient was raised from 5% to 100% acetonitrile over 11 min. TFA (0.1% v/v) was added to the HPLC solvents.

Preparative HPLC was run on an Agilent HPLC (1100 series) using a 125/21 NUCLEODUR C18 Gravity, 5 µ (Macherey- Nagel) column.

Flash Chromatography

Flash column chromatography was performed using flash silica gel (Merck, Darmstadt, 40-64 µM) or on Merck aluminum oxide 90 (70-230 mesh, activity II-III) with pressure ranging from 0.5 - 1.0 bar.

Preparative size exclusion chromatography

Preparative size exclusion chromatography was performed on BIO RAD Bio Beads S-X1 (200-400 mesh) swollen in CH_2Cl_2 or DMF.

Chemicals

Chemicals were obtained from the following suppliers and used without further purification: Acros, Aldrich, Fluka, Lancaster, Novabiochem. CH_2Cl_2 was distilled prior to use following standard protocols. Dry DMF and THF were purchased from Fluka.

NHS-PEO_4-Biotin, Pierce Biotechnology, 21330

Cy3-Streptavidin, Zymed, 43-4315

Cy3-Anti biotin Antibody, Jackson ImmunoResearch, 200-162-211

TexasRed-Streptavidin, Invitrogen, S872

Alexa633-Streptavidin, Invitrogen, S21375

Buffer

PBS stock solution (0.1 M, 10 fold):

1) 49.8 g Na_2HPO_4 x 2 H_2O in 700 mL H_2O

2) 19.3 g NaH_2PO_4 x 1 H_2O in 350 mL H_2O

3) 84.1 g NaCl

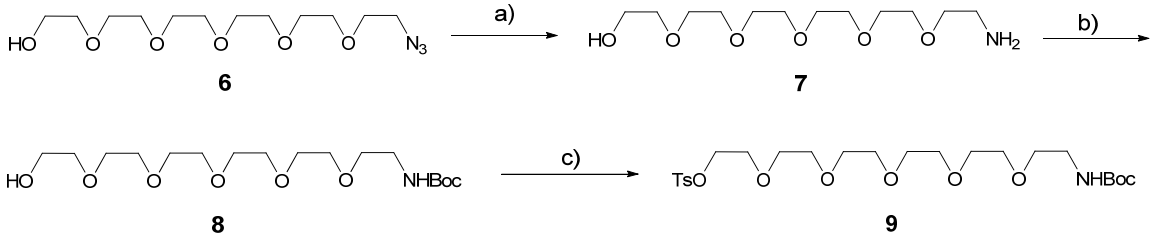
2) was added to 1) until the pH reached 7.3 and 3) was added subsequently.

Fluorescence Spectroscopy

Fluorescence data were recorded either on a JASCO FP-6500 fluorimeter or a Cary Eclipse fluorescence spectrophotometer.

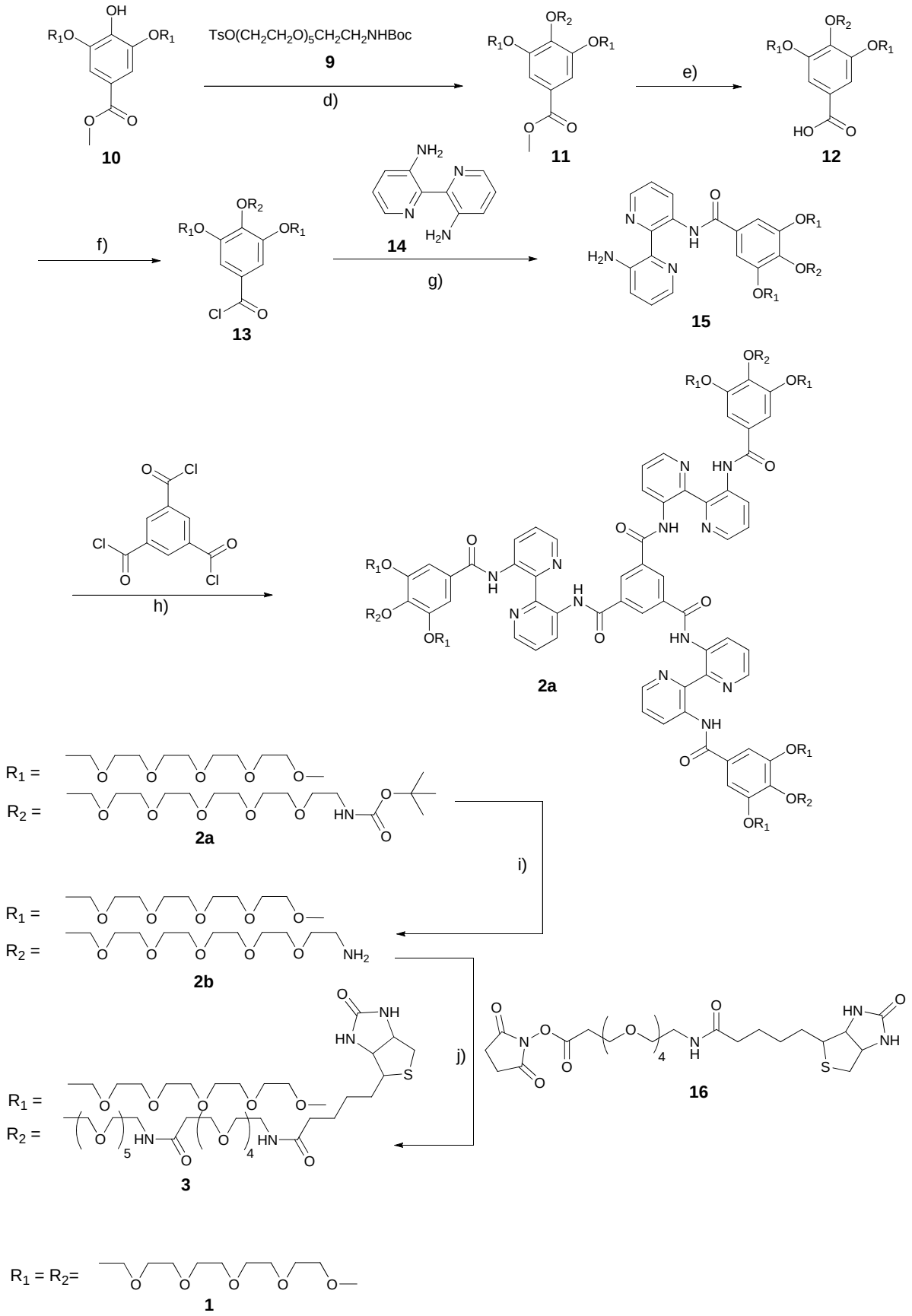
2. Synthesis Protocols

2,2'-Bipyridyl-3,3'-diamine **20**,[1] discotic **1,**[2] azido hexaethylene glycol **6,** and gallic acid derivative **10**[3] were synthesized as published.



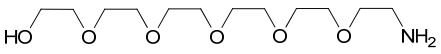
Scheme S1. Synthesis of the glycol side chain. a) H_2 (10 bar), Pd/C, ethanol/water; b) Boc_2O, TEA, dioxane, 0°C-rt; c) Tosyl-Cl, TEA, THF, rt.

[1] C. R. Rice, S. Onions, N. Vidal, J. D. Wallis, M.-C. Senna, Melanie Pilkington, H. Stoeckli-Evans, *Eur. J. Inorg. Chem.* **2002**, 1985-1997.
[2] L. Brunsveld, B. G. G. Lohmeijer, J. A. J. M. Vekemans, E. W. Meijer, *J. Incl. Phen. Macrocyclic Chem.* **2001**, *41*, 61-64.
[3] M. K. Müller, L. Brunsveld, *Angew. Chem. Int. Ed*. **2009**, *48*, 2921 –2924.



Scheme S2. Synthesis of the biotin discotic **3** via the amine discotic **2**. d) K_2CO_3, DMF; e) KOH, ethanol/water; f) $C_2O_2Cl_2$, DMF, CH_2Cl_2;g) TEA, CH_2Cl_2; h) TEA, CH_2Cl_2; i) TFA, CH_2Cl_2; j) TEA, CH_2Cl_2.

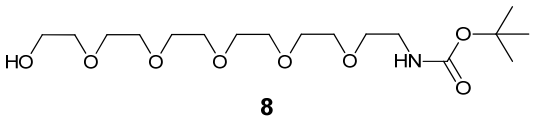
2[-2-(2-{2-[2-(2-Hydroxyethoxy)-ethoxy]-ethoxy}-ethoxy)-ethoxy]- ethyl amine (7)



7

6 (7.2 g, 23 mmol) was dissolved in ethanol (20 mL) and water (5 mL) and Pd/C (10%, 0.3 g) was added. The suspension was stirred for 48 h under an H_2-atmosphere (10 bar) in an autoclave and subsequently filtered through celite®. The solvents were evaporated *in vacuo* to yield **7** (6.2 g, 22 mmol, 96%). HPLC: R_t = 1.91 min; ^{1}H NMR (400 MHz, *CDCl$_3$)* δ = 3.74-3.50 (m, 22H, OC*H*$_2$C*H*$_2$O), 2.93 (s, 2H, N*H*$_2$), 2.87 (t, *J*=5.1, 2H, C*H*$_2$N*H*$_2$); ^{13}C NMR (101 MHz, *CDCl$_3$*) δ = 73.1, 72.8, 70.6, 70.5, 70.20, 61.4, 41.5; MS (ESI): calcd. for $[C_{12}H_{27}NO_6 + H]^+$ *m/z* = 282.18 found *m/z* = 282.13.

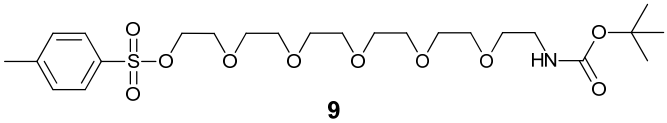
2[-2-(2-{2-[2-(2-Hydroxyethoxy)-ethoxy]-ethoxy}-ethoxy)-ethoxy]- ethyl- tert-butyl carbamate (8)



8

To a solution of **7** (6.2 g, 22 mmol) in dioxane (30 mL) di-*tert*-butyl dicarbonate (6.0 g, 27 mmol) was added at 0 °C and stirred for 3 h. Subsequently triethylamine (4 mL, 30 mmol) was given to the mixture and stirred over night at rt. The solvent was evaporated *in vacuo* and the crude product was purified by column chromatography (silica, 3% methanol in CH_2Cl_2) to yield **8** (5.1 g, 13 mmol, 59%). HPLC: R_t = 6.38 min; ^{1}H NMR (400 MHz, *CDCl$_3$*) δ = 5.01 (br, 1H, N*H*), 3.70-3.51 (m, 20H, OC*H*$_2$C*H*$_2$O), 3.47 (t, *J* = 5.2, 2H, OC*H*$_2$CH$_2$NHboc), 3.24 (dd, *J* = 10.1 and 5.2, 2H, OCH$_2$C*H*$_2$NHboc), 1.37 (s, 9H, OC(C*H*$_3$)$_3$); ^{13}C NMR (101 MHz, *CDCl3*) δ = 155.6, 79.07, 72.67-70.4, 61.8, 40.5, 28.6. MS (ESI): calcd. for $[C_{17}H_{35}NO_8 + H]^+$ *m/z* = 382.16 found *m/z* = 382.02.

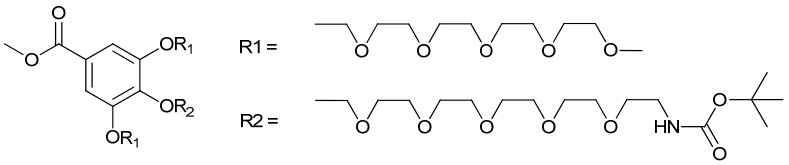
2[-2-(2-{2-[2-(2-tert-butoxycarbonylamino)-ethoxy)-ethoxy]-ethoxy}-ethoxy)-ethoxy]-ethyl p-tosylate (9)



9

To a solution of **8** (5.06 g, 13.3 mmol) and triethylamine (5.0 mL, 38.0 mmol) in THF (50 mL) *p*-toluenesulfonyl chloride (3.04 g, 16.1 mmol) in THF (50 mL) was added dropwise. The reaction was stirred over night and then washed with 1N HCl (3 x 50 mL) and brine (50 mL). Drying over $MgSO_4$ and evaporating the solvent *in vacuo* gave the crude product. Purification by column chromatography (silica, 3% methanol in CH_2Cl_2) yielded the pure compound **9** as a colorless oil (4.41 g, 8.2 mmol, 62%). HPLC: R_t = 8.97 min; ^{1}H NMR (400 MHz, *CDCl*$_3$) δ = 7.79 (d, *J* = 8.1, 2H, *H*-benzyl), 7.33 (d, *J* = 8.1, 2H, *H*-benzyl), 5.01 (br, 1H, N*H*), 4.15 (t, 2H, C*H*$_2$OSO$_2$-Ar), 3.71-3.49 (m, 20H, OC*H*$_2$C*H*$_2$O), 3.29 (dd, *J* = 4.4, 2H, C*H*$_2$NHboc), 2.44 (s, 3H, C*H*$_3$Ar), 1.43 (s, 9H, OC(C*H*$_3$)$_3$); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 155.98, 144.76, 133.01, 129.79, 127.96, 79.13, 70.73, 70.60, 70.57, 70.55, 70.54, 70.50, 70.21, 69.21, 68.67, 40.35, 28.41, 21.62. MS (ESI): calcd. for $[C_{24}H_{41}NO_{10}S + Na]^+$ *m/z* = 558.23 found *m/z* = 558.06.

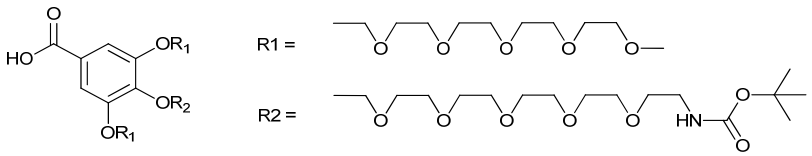
Methyl-4-{2[-2-(2-{2-[2-(2-tert-butoxycarbonylamino-ethoxy)-ethoxy]-ethoxy}-ethoxy)-ethoxy]-ethyl}-3,5-bis[2-(2-{2-[2-(2-methoxyethoxy)-ethoxy]-ethoxy}-ethoxy)-ethoxy]-benzoat (11)



11

A mixture of **9** (5.60 g, 10.5 mmol), **10** (6.52 g, 10.0 mmol) and K_2CO_3 (17.11 g, 124 mmol) was stirred 4 h at 100 °C in dry DMF (100 mL). The reaction mixture was poured into water (150 mL) and extracted with CH_2Cl_2 (3 x 150 mL). The combined organic layers were washed with brine (100 mL), dried over $MgSO_4$ and evaporated *in vacuo*. The crude product was purified by column chromatography (alumina, 1% ethanol in chloroform) affording pure compound **11** (9.57 g, 9.4 mmol, 90%). HPLC: R_t = 8.16 min; ^{1}H NMR (400 MHz, *CDCl*$_3$) δ = 7.26 (s, 2H, *H*-benzoyl), 5.01 (br, 1H, N*H*), 4.18 (t, 4H, *m*-OC*H*$_2$CH$_2$O), 4.16 (t, 2H, *p*-OC*H*$_2$CH$_2$O), 4.07 (t, 2H, *m*-OCH$_2$C*H*$_2$O), 3.85 (s, 3H, COOC*H*$_3$), 3.83 (t, 4H, *p*-OCH$_2$C*H*$_2$O), 3.79-3.45 (m, 46H, OC*H*$_2$C*H*$_2$O), 3.34 (s, 6H, OC*H*$_3$), 3.27 (d, 2H, OCH$_2$C*H*$_2$NHboc), 1.41 (s, 9H, OC(C*H*$_3$)$_3$); ^{13}C NMR (101 MHz, *CDCl*$_3$) δ = 171.3, 168.3, 152.3, 144.6, 127.0, 109.1, 79.1, 71.9, 70.8-70.2, 69.6, 68.9, 59.0, 40.3, 28.4; MS (ESI): calcd. for $[C_{47}H_{85}NO_{22} + NH_4]^+$ *m/z* = 1033.59 found *m/z* = 1033.33.

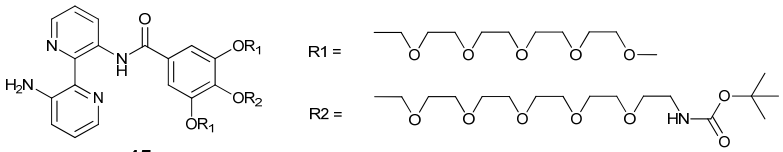
4-{2[-2-(2-{2-[2-(2-tert-butoxycarbonylamino-ethoxy)-ethoxy]-ethoxy}-ethoxy)-ethoxy]-ethyl}-3,5-bis[2-(2-{2-[2-(2-methoxyethoxy)-ethoxy]-ethoxy}-ethoxy)-ethoxy]-benzoic acid (12)



12

A solution of **11** (9.57 g, 9.4 mmol) and KOH (5.4 g, 28.1 mmol) in ethanol (40 mL) and water (40 mL) was heated under reflux overnight. Subsequently, the solution was acidified to pH = 2, cooled and extracted with CH_2Cl_2 (4 x 100 mL). The CH_2Cl_2 layer was washed with brine (70 mL). Drying over $MgSO_4$, evaporating *in vacuo* and drying over P_2O_5 afforded the pure compound **12** (8.81 g, 8.8 mmol, 94%). HPLC: R_t = 7.41 min; ^{1}H NMR (400 MHz, *CDCl*$_3$) δ = 7.35 (s, 2H, *H*-benzoyl), 5.01 (br, 1H, N*H*), 4.23 (t, 4H, *m*-OC*H*$_2$CH$_2$O), 4.19 (t, 2H, *p*-OC*H*$_2$CH$_2$O), 3.85 (t, 4H, *m*-OCH$_2$C*H*$_2$O), 3.82 (t, 2H, *p*-OCH$_2$C*H*$_2$O), 3.79-3.49 (m, 90H, OC*H*$_2$C*H*$_2$O), 3.36 (s, 6H, OC*H*$_3$), 3.30 (d, 2H, OCH$_2$C*H*$_2$NHboc), 1.43 (s, 9H, OC(C*H*$_3$)$_3$); ^{13}C NMR (101 MHz, *CDCl*$_3$) δ = 168.3, 156.1, 152.2, 142.9, 124.6, 109.8, 79.1, 72.0, 70.9-69.8, 59.0, 40.3, 28.4; MS (ESI): calcd. for $[C_{46}H_{83}NO_{22} + NH_4]^+$ *m/z* = 1019.57 found *m/z* = 1019.31.

3'-(4-{2[-2-(2-{2-[2-(2-tert-butoxycarbonylamino-ethoxy)-ethoxy]-ethoxy}-ethoxy)-ethoxy]-ethyl}-3,5-bis[2-(2-{2-[2-(2-methoxyethoxy)-ethoxy]-ethoxy}-ethoxy)-ethoxy]-benzoylamino)-2,2'-bipyridine-3-amine (15)

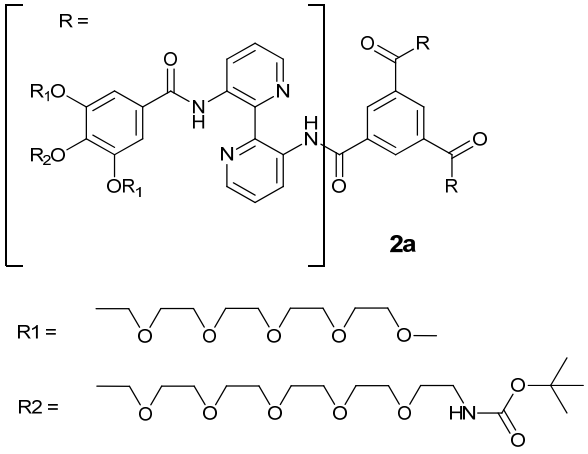


15

A solution of oxalyl chloride (330 mg, 2.6 mmol, 0.22 mL) in dry CH_2Cl_2 (5 mL) containing a catalytic amount of DMF (3 drops) was added dropwise to a solution of **12** (2.17 g, 2.17 mmol) in dry CH_2Cl_2 (5 mL). The reaction mixture was stirred overnight at rt in the absence of light. Evaporating the solvent *in vacuo* afforded the crude product **13** (2.38 g, 2.17 mmol, quant.). This product was dissolved in dry CH_2Cl_2 (25 mL) and added dropwise to a stirred solution of 2,2'-bipyridyl-3,3'-diamine **14** (504 mg, 2.7 mmol) and triethylamine (0.17 mL, 1.22 mmol) in dry CH_2Cl_2 (25 mL) within 1.5 h, while the temperature was kept below 5 °C. After stirring for 2 h the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography (silica, ethylacetate followed by 5% methanol in chloroform) and size exclusion chromatography to yield **15** (1.34 g, 1.14 mmol, 52%) HPLC: R_t = 7.92 min; ^{1}H NMR (400 MHz, *CDCl*$_3$) δ = 14.39 (s, 1H, N*H*CO), 9.19 (dd, *J* = 8.4 and 1.4, 1H, *H*-4'), 8.31 (dd, *J* = 4.5 and 1.4, 1H, *H*-6'), 8.02 (t, *J* = 2.9, 1H, *H*-6), 7.30 (s, 2H, *H*-benzoyl), 7.28 (m, 1H, *H*-5'),

7.13 (d, *J* = 2.9, 2H, *H*-4 and *H*-5), 6.57 (s, 2H, NH_2), 5.05 (s, 1H, N*H*), 4.24 (t, 4H, *m*-OCH_2CH_2O), 4.23 (t, 2H, *p*-OCH_2CH_2O), 3.86 (t, 4H, *m*-OCH_2CH_2O), 3.80 (t, 2H, *p*-OCH_2CH_2O), 3.72-3.50 (m, 46H, OCH_2CH_2O), 3.34 (s, 6H, *m*-OCH_3), 3.28 (d, 2H, OCH_2CH_2NHboc), 1.42 (s, 9H, $OC(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 165.7, 156.0, 152.6, 145.1, 143.6, 142.0, 140.8, 138.5, 136.1, 135.0, 130.8, 128.6, 125.3, 124.3, 122.7, 107.9, 79.1, 72.4, 71.9, 70.8, 70.6, 70.6, 70.6, 70.5, 70.5, 70.2, 69.7, 69.2, 59.0, 40.3, 28.4; MS (ESI): calcd. for $[C_{56}H_{91}NO_{21} + H]^+$ *m/z* = 1170.63 found *m/z* = 1170.37.

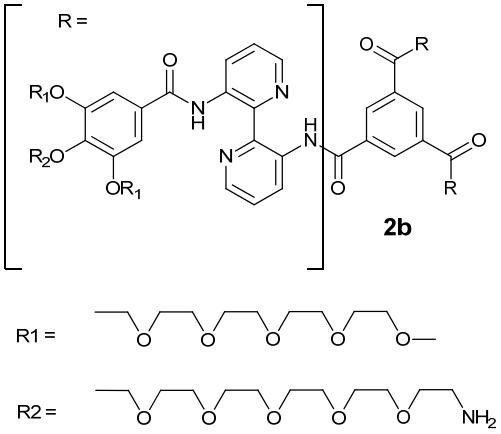
N,N',N''-Tris{3[3'-(4-{2[-2-(2-{2-[2-(2-tert-butoxycarbonylamino-ethoxy)-ethoxy]-ethoxy}-ethoxy)-ethoxy]-ethyl}-3,5-bis[2-(2-{2-[2-(2-methoxyethoxy)-ethoxy]-ethoxy}-ethoxy)-ethoxy]-benzoylamino]-2,2'-bipyridyl}benzene-1,3,5-tricarboxamide (2a)



To a solution of **15** (375 mg, 0.32 mmol) and triethylamine (55 µL, 0.4 mmol) in dry CH_2Cl_2 (6 mL), a solution of trimesic chloride (26.5 mg, 0.10 mmol) in dry CH_2Cl_2 (4 mL) was added dropwise at room temperature. Stirring was continued overnight, after which the solution was diluted with CH_2Cl_2 (10 mL) and washed with water (2 x 10 mL). The combined water layers were extracted with CH_2Cl_2 (4 x 15 mL). The combined dichloromethane layers were washed with brine (10 mL), dried over $MgSO_4$ and evaporated *in vacuo*. The crude product was purified by size exclusion chromatography (in CH_2Cl_2), column chromatography (silica, 10% methanol with 1% triethylamine in chloroform) and size exclusion chromatography (in CH_2Cl_2). Drying over P_2O_5 afforded **2a** (210 mg, 57 µmol, 57%). ^1H NMR (400 MHz, *CDCl*$_3$) δ = 15.52 (s, 3H, N*H*CO), 14.35 (s, 3H, N*H'*CO), 9.60 (dd, *J* = 8.5 and 1.4, 3H, *H*-4), 9.40 (dd, *J* = 8.6 and 1.5, 3H, *H*-4'), 9.29 (s, 3H, *o*-*H*), 9.06 (dd, *J* = 4.6 and 1.4, 3H, *H*-6'), 8.52 (dd, *J* = 4.6 and 1.5, 3H, *H*-6), 7.57 (dd, *J* = 8.6 and 4.6, 6H, *H*-5 and *H*-5'), 7.36 (s, 6H, *H*-benzoyl), 5.05 (s, 1H, N*H*), 4.29 (t, 12H, *m*-OCH_2CH_2O), 4.27 (t, 6H, *p*-OCH_2CH_2O), 3.91 (t, *J* = 4.9, 12H, *m*-OCH_2CH_2O), 3.91 (m, 6H, *p*-OCH_2CH_2O), 3.74-3.48 (m, 150H, OCH_2CH_2O), 3.35 (s, 18H, *m*-OCH_3), 3.31 (d, 6H, *p*-

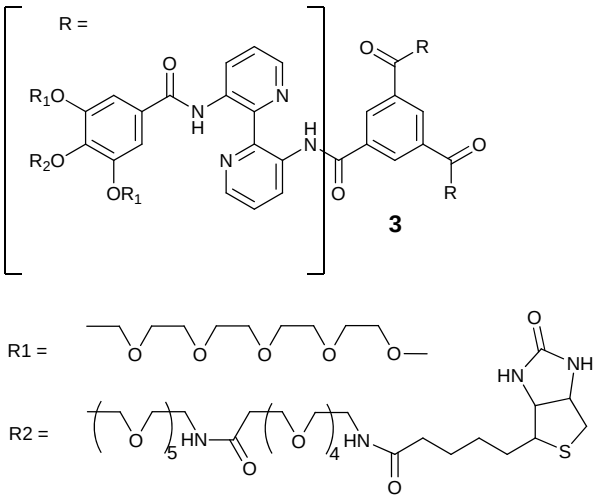
OCH_2CH_2NHboc), 1.44 (s, 27H, $OC(CH_3)_3$). ^{13}C NMR (151 MHz, *CDCl*$_3$) δ = 169.1, 166.0, 164.9, 153.0, 142.6, 142.0, 141.8, 141.7, 137.8, 135.2, 132.0, 131.8, 130.2, 128.8, 128.7, 124.9, 124.6, 108.3, 79.1, 72.7-69.6, 59.2, 40.4, 28.4. MS (MALDI-TOF): calcd. for $[C_{177}H_{273}N_{15}O_{66} + Na]^+$ *m/z* = 3688.9, found *m/z* = 3689.0.

N,N',N''-Tris{3[3'-(4-{2[-2-(2-{2-[2-(2-amino-ethoxy)-ethoxy]-ethoxy}-ethoxy)-ethoxy]-ethyl}-3,5-bis[2-(2-{2-[2-(2-methoxyethoxy)-ethoxy]-ethoxy}-ethoxy)-ethoxy]-benzoylamino]-2,2'-bipyridyl}benzene-1,3,5-tricarboxamide (2b)



2a (200 mg, 54 μmol) and trifluoracetic acid (1 mL) in CH_2Cl_2 (3 mL) were stirred for 3 h. Toluene (3 mL) was added and the solvents were evaporated *in vacuo*. The crude product was purified by size exclusion chromatography (in CH_2Cl_2) affording **2b** (186 mg, 54 μmol, quant.). ^1H NMR (400 MHz, *CDCl*$_3$) δ = 15.43 (s, 3H, N*H*CO), 14.45 (s, 3H, N*H'*CO), 9.57 (d, *J* = 8.4, 3H, *H*-4), 9.32 (d, *J* = 8.4, 3H, *H*-4'), 9.19 (s, 3H, *o*-*H*), 9.03 (d, *J* = 4.1, 3H, *H*-6'), 8.45 (s, 3H, *H*-6), 7.55 (br, 6H, *H*-5 and *H*-5'), 7.30 (s, 6H, *H*-benzoyl), 4.27 (br, 12H, *m*-OCH_2CH_2O), 4.26 (br, 6H, *p*-OCH_2CH_2O), 3.91 (br, 12H, *m*-OCH_2CH_2O), 3.84 (br, 6H, *p*-OCH_2CH_2O), 3.77-3.49 (m, 150H, OCH_2CH_2O), 3.33 (s, 18H, *m*-OCH_3), 3.21 (br, 6H, *p*- $OCH_2CH_2NH_2$). ^{13}C NMR (151 MHz, *CDCl*$_3$) δ = 165.7, 164.1, 152.8, 141.8, 141.7, 141.1, 140.7, 139.5, 137.7, 135.2, 131.4, 131.2, 130.2, 129.7, 127.5, 124.9, 124.7, 107.5, 72.7-69.3, 67.2, 59.2, 40.4. MS (MALDI-TOF): calcd. for $[C_{162}H_{249}N_{15}O_{60} + H]^+$ *m/z* = 3387.63, found *m/z* = 3388.71.

Biotin Discotic (3)



To a solution of **2b** (5 mg, 1.5 µmol) in CH_2Cl_2 (0.3 mL) BiotinPEO_4-NHS **16** (8 mg, 13.5 µmol) was slowly added. After stirring for 1 h, triethylamine (8 µL) was given dropwise to the mixture and stirred for another 4 h. The reaction was monitored by MALDI-TOF MS. After 4 h only traces of mono- and double-acylated side products were left. After adding more BiotinPEO_4-NHS (3 mg, 5.0 µmol) and stirring for another 24 h no change could be observed. The solvent was evaporated *in vacuo* and the crude product was purified by size exclusion chromatography (in DMF) affording **3** (4.6 mg, 1 µmol, 65%). ^{1}H NMR (500 MHz, *CDCl*$_3$) δ = 15.32 (s, 3H, N*H*CO), 14.28 (s, 3H, N*H'*CO), 9.60 (d, *J* = 8.0, 3H, *H*-4), 9.39 (d, *J* = 8.8, 3H, *H*-4'), 9.29 (s, 3H, *o-H*), 9.06 (d, *J* = 3.7, 3H, *H*-6'), 8.53 (d, *J* = 4.8, 3H, *H*-6), 7.57 (dd, *J* = 2.7, 7.2, 6H, *H*-5 and *H*-5'), 7.36 (s, 6H, *H*-benzoyl), 4.50 (m, 3H, biotin-NHC*H*), 4.30 (t, 12H, *m*-OC*H*$_2$CH$_2$O), 4.29 (m, 3H, biotin-NHC*H*), 4.28 (t, 6H, *p*-OC*H*$_2$CH$_2$O), 3.91 (t, 12H, *m*-OCH$_2$C*H*$_2$O), 3.83 (t, 6H, *p*-OCH$_2$C*H*$_2$O), 3.79-3.50 (m, 216H, OC*H*$_2$C*H*$_2$O), 3.46-3.39 (m, 12H, biotin-SC*H*$_2$), 3.36 (s, 18H, *m*-OC*H*$_3$), 3.19-3.09 (m, 3H, biotin-SC*H*$_2$), 2.55-2.41 (m, 6H, *H*-Alkyl), 2.27-2.14 (m, 6H, *H*-Alkyl), 1.51-1.38 (m, 6H, *H*-Alkyl), 1.27-1.22 (m, 6H, *H*-Alkyl). MS (MALDI-TOF): calcd. for $[C_{225}H_{354}N_{24}O_{81}S_3 + Na]^+$ *m/z* = 4810.53 found *m/z* = 4810.33.

3. FRET measurements

All samples for spectrophotometric measurements were prepared under ambient conditions in quartz cuvettes of 1 cm path length and 2 mL volume in phosphate buffer (pH 7.3). All data were acquired at 20°C.

3.1. Assembly of Cy3 labeled streptavidin

A series of concentrations of Cy3® labeled streptavidin (SA-Cy3, 10^{-6} M in phosphate buffer) ranging from 10^{-9} to 2.5 x 10^{-7} M was added to either **3** or **1** (each at 10^{-6} M in phosphate buffer) at 20 °C. The mixtures were stirred for 5 min before measurement. Of each sample fluorescence spectra (λex = 340 nm, λem = 450 - 650 nm) were measured. As reference the same range of concentration of Cy3® labeled streptavidin was added to pure phosphate buffer and spectra were recorded under the same conditions. These spectra were used for correction of Cy3® background fluorescence.

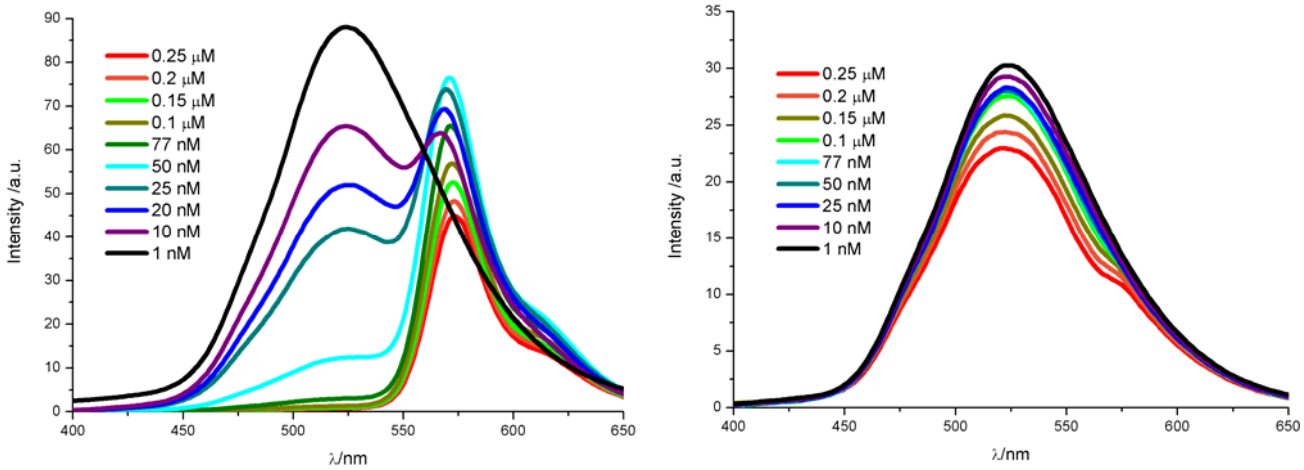


Figure S1: Left: titration of SA-Cy3 to **3**; right: titration of SA-Cy3 to **1**; all the data are corrected for the background fluorescence of SA-Cy3 in phosphate buffer at the excitation wavelength of 340 nm.

3.2. Assembly of Cy3 labeled anti-biotin antibody

A series of concentrations of Cy3® labeled anti-biotin antibody (AB-Cy3, 10^{-5} M in phosphate buffer) ranging from 10^{-9} to 1.1 x 10^{-6} M was added to either **3** or **1** (each at 10^{-6} M in phosphate buffer) at 20 °C. The mixtures were stirred for 5 min before measurement.. Of each sample fluorescence spectra (λex = 340 nm, λem = 450 - 650 nm) were measured. As reference the same range of concentration of Cy3® labeled anti biotin antibody was added to phosphate buffer and spectra were recorded under the same conditions. These spectra were used for correction of Cy3® background fluorescence.

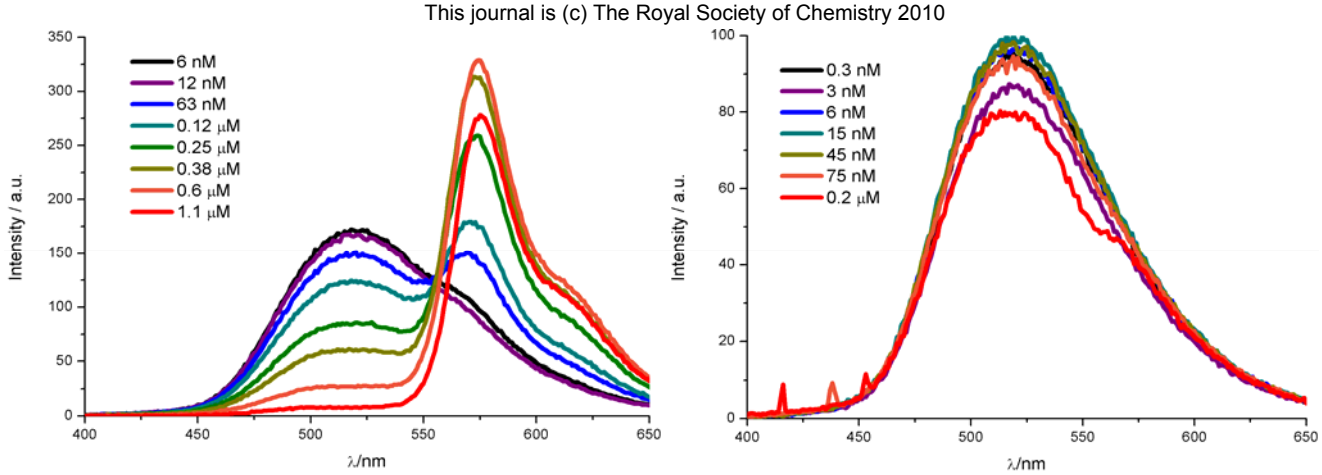


Figure S2: Left: titration of AB-Cy3 to **3**; right: titration of AB-Cy3 to **1**; all the data are corrected for the background fluorescence of AB-Cy3 in phosphate buffer at the excitation wavelength of 340 nm.

3.3. Assembly of Alexa Fluor 633 and Texas Red labeled streptavidin

Phosphate buffered solutions of Texas Red® labeled streptavidin (SA-TR) and Alexa Fluor 633® labeled streptavidin (SA-Alexa633) were mixed in a 1 to 1 ratio 2 h prior to the experiments. This protein mixture was added to **3** or **1** (10^{-6} M in phosphate buffer) or to phosphate buffer alone to reach a protein concentration of 10^{-7} M each. The mixture was stirred for 10 min before fluorescence spectra were measured (λ_{ex} = 584 nm, λ_{em} = 600 - 750 nm). Also, Alexa Fluor 633® labeled streptavidin alone was titrated to phosphate buffer and this data was used for correction of Alexa633® background fluorescence.

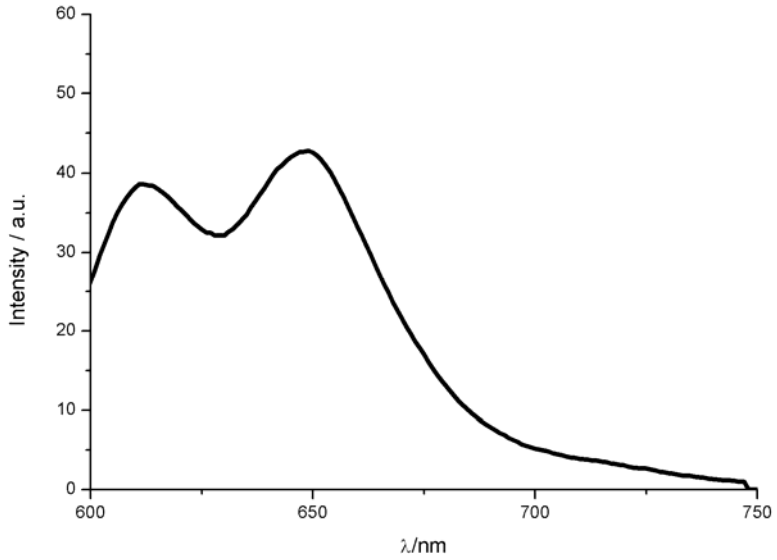


Figure S3: Fluorescence of a 1 to 1 mixture of SA-TR and SA-Alexa633 in phosphate buffer; data is corrected for the background fluorescence of SA-Alexa633 in phosphate buffer at the excitation wavelength of 584 nm.