Synthesis of a Biotinated Lipofullerene as a New Type of Transmembrane Anchor

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As a prototype for a new class of lipid membrane components, the lipophilic fullerene derivative (so-called lipofullerene) **1** was synthesized and characterized. This mixed [1:5]-hexakisadduct consists of ten long alkyl chains within five didodecyl malonate addends and a linker malonate carrying a (+)-biotin unit as part of an amphiphilic spacer. The malonates are attached to the fullerene core in an octahedral addition pattern, which was achieved by two successive cyclopropanation sequences with the functional precursor malonate **8** and dodecyl malonate **10**. The final step of the synthesis of **1** was the attachment of activated biotin **5** with the deprotected precursor **11**. Binding experiments followed by reflectometric interference spectroscopy [RIfS] proved the capability of **1** to bind specifically the protein streptavidin (SA) through the biotin unit. The amphiphilic behavior of **1** was demonstrated by Langmuir Blodgett (LB) film investigations.

Introduction

We have shown recently that T_h-symmetrical hexakisadducts of C_{60} containing six pairs of long alkyl chains form entirely new membrane composites with synthetic lecithin bilayers.[1] These lipofullerenes are easily accessible in large quantities via template mediated cyclopropanation of [6,6] double bonds with the corresponding dialkyl malonates in octahedral sites.[2,3] In membrane bilayers unprecedentedly high degrees of loading of up to 25 mol-% of lipofullerenes can be achieved. Such intercalated lipofullerenes form new supramolecular arrangements within the lipid bilayer consisting of rod-shaped nanostructures. At lower temperatures, in the gel phase of the bilayer, the rods exhibit long-range order, whereas at higher temperatures, in the fluid phase, this spatial correlation is drastically reduced. The bilayer itself is stabilized by the lipofullerene rods against deformation, e.g. in a magnetic field. This appears to be reminiscent of the stabilization of membranes by cytoskeletons. However, unlike the location of the cytoskeleton inside the cell membrane, the lipofullerene rods are located in between the bilayer. One implication of these discoveries is the possibility of a membrane-assisted formation of perfectly spherical lipofullerene polymers which we have demonstrated recently.[2f] Another challenging task is to take advantage of the pronounced noncovalent binding of the lipofullerenes within the lipid bilayer and to use them as transmembrane anchors for biologically active molecules located outside the membrane. As a biological function the recognition of proteins is considered. This requires a careful design of the linker connecting the lipofullerene and the biofunctional group. The linker must be able to span the lipid layer and must therefore consist of a lipophilic chain and a subsequent hydrophilic part. It has to be compatible with the dimensions of the corresponding lecithin DPPC (dipalmitoyl-*sn*-glycero-3-phosphatidylcholine) used as the membrane building block. The lipophilic part should match with the lipophilic fatty acid chains of the glycerides and the hydrophilic part with the phosphatidyl choline moiety. Moreover, the spacer chain must be long and flexible enough to allow for unrestricted molecular recognition events outside the membrane. As a suitable biofunctional group we have chosen (+)-biotin because of its strong and well-established binding interactions with the protein streptavidin. Streptavidin (SA) is a fungal protein with a MW of 60 000 Da. An advantage of this approach is that the lipofullerene anchor inside the bilayer is expected to prevent the bound protein from extracting the biotinated component out of the membrane.

We now report on the synthesis and complete characterization of the biotinated lipofullerene **1** as the first prototype of such a new class of transmembrane anchors. The dimensions and the design of the biotin linker of **1** was based on computer-generated models of DPPC-lipofullerene bilayer composites as depicted in Figure 1. We further report on the first coupling studies of **1** with streptavidin (SA) and on the assembly of transmembrane systems using a series of RIfS[4] and LB-film experiments.

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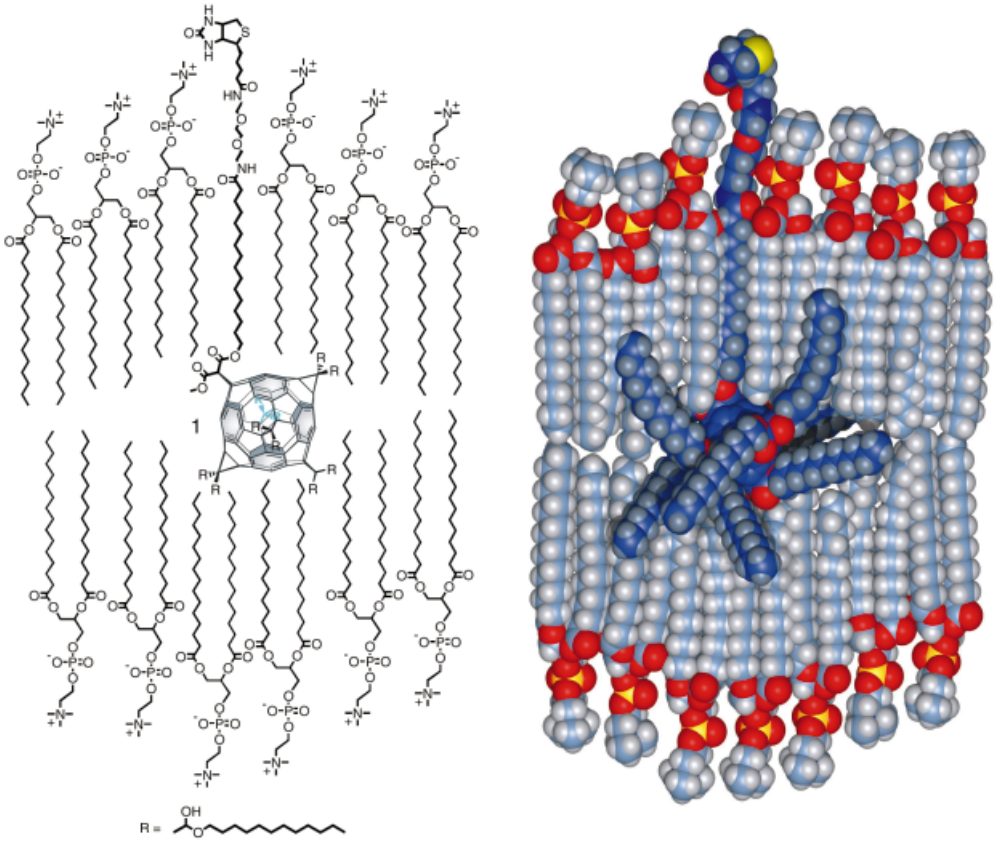
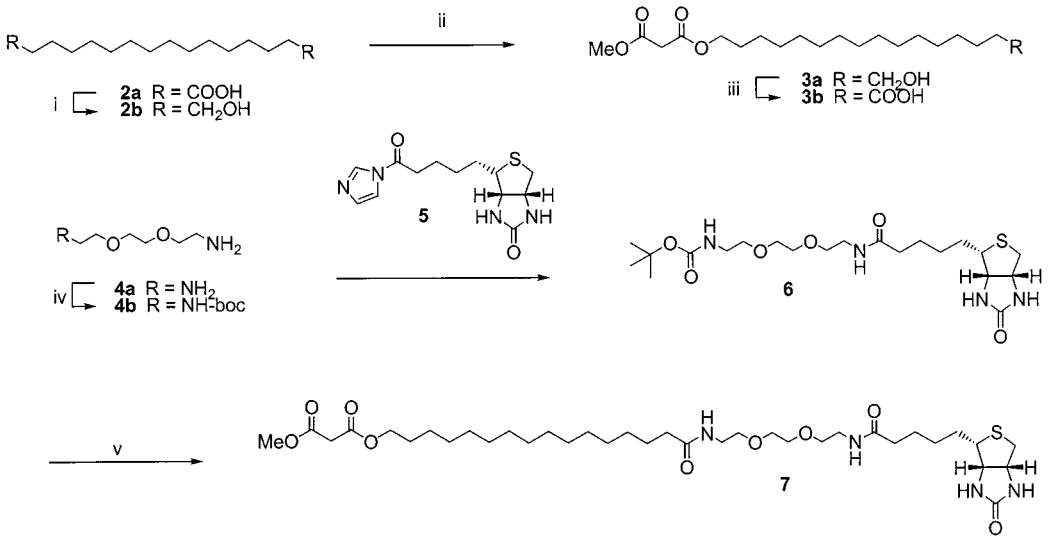


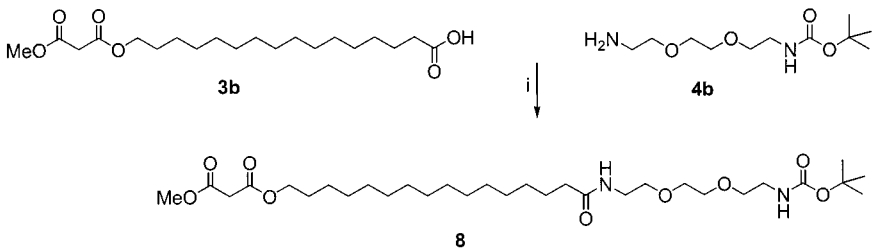
Figure 1. Schematic representation (left) and computer generated space-filling model (right) of the biotinated lipofullerene **1** embedded in a DPPC bilayer; the space-filling model is based on MM+ force field calculations using the program package HyperChem[5]

Results and Discussion

The synthesis of **1** consists of two main parts. The first part (Scheme 1 and Scheme 2) comprises the synthesis of amphiphilic malonate spacers. In the second part (Scheme 3), the malonate spacer is attached to C_{60} and the resulting monoadduct is transferred by fivefold addition of lipophilic malonates to a mixed [1:5]-hexakisadduct with an octahedral addition pattern. The final step is the coupling reaction with biotin.



Scheme 1. Stepwise synthesis of the amphiphilic spacer malonate **7** carrying a biotin anchor unit (i: lithium aluminium hydride, THF; ii: monomethyl malonyl chloride, pyridine, THF; iii: pyridinium dichromate, DMF; iv: boc anhydride, dioxane; v: TFA/CH_2Cl_2, CDI)



Scheme 2. Synthesis of the functionalized malonate **8** without a biotin moiety (i: CDI/THF; boc = *tert*-butyloxycarbonyl)

Molecular modelling investigations (Figure 1) showed, that the malonate spacer should have a C_{16} chain, elongated by a bis(2-aminoethyl)ethylene glycol ether moiety in order to have the right length to penetrate the thickness of a DPPC monolayer. The CH_2 chains of the spacer fit well with the length of the glyceride fatty acid chains, whereas the amino ether region of the spacer has the dimension of the phosphatidyl choline moiety. The biotin terminus protrudes from the outer bilayer surface with the right position to be recognised by the protein.

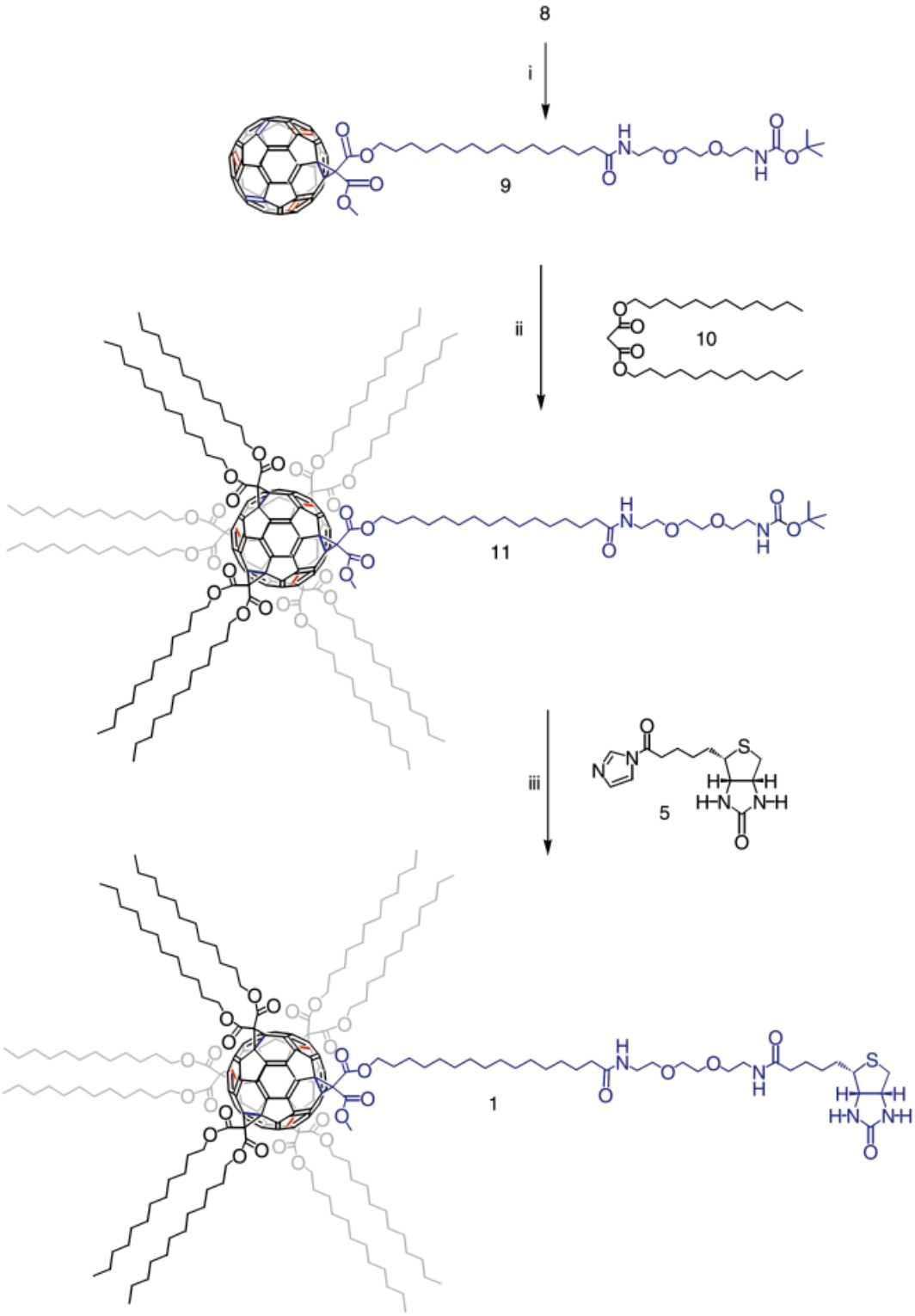
As starting material hexadecanedioic acid **2a** was taken and converted into the corresponding 1,16-hexadecanediol **2b** by reduction with lithium aluminium hydride, and then coupled with monomethyl malonyl chloride (Scheme 1). Malonate **3a** was subsequently converted into the carboxylic acid **3b** by treatment with pyridinium dichromate. For the introduction of the hydrophilic part of the spacer, the diamino ether **4a** was converted into the monoprotected compound **4b** by treatment with boc anhydride. Subsequent Staab coupling[6,7] of **4b** with the activated biotin derivative **5** obtained by treatment of (+)-biotin with a slight excess of carbonyldiimidazole (CDI), afforded the boc protected amine **6** in good yields. To complete the synthesis of **7**, the biotinated precursor **6** was deprotected by the action of trifluoroacetic acid (TFA) and subsequently coupled with **3b** by activation in situ with CDI (Scheme 1). However, an unsatisfactory yield of only 18% was obtained in the last deprotection and coupling step, indicating that the biotin might be too labile under the conditions of the deprotection/coupling procedure.

In order to improve the overall yield, we decided to use a spacer malonate carrying an amphiphilic spacer without the terminal biotin unit for the subsequent cyclopropanation of C_{60}. The coupling of the spacer with biotin was performed as the very last step. Indeed, Staab coupling of **3b** with **4b** (Scheme 2) afforded the functional malonate **8** in much better yield (64%) than was achieved with the reaction sequence leading to **7**. Another important reason to prefer this route was that we noticed that DBU, which is used in the following cyclopropanation reactions, could promote side reactions with the biotin moiety.

Scheme 3 depicts the two step attachment of the spacer malonate **8** followed by the fivefold cyclopropanation with the malonate **10** and the final coupling with the activated (+)-biotin **5** to give the target molecule **1**. The cyclopropanation of C_{60} with the spacer malonate **9** was promoted by CBr_4 and DBU.[2b] The nonoptimized yield of 29% of monoadduct **9** is acceptable for this type of reaction and indicates that side reactions of DBU with the amide bonds of **8** are not significant. The product **9** was purified by flash chromatography and fully characterized by IR, UV/Vis, ^{1}H- and ^{13}C-NMR and mass spectroscopy. The UV/Vis and NMR spectra are typical for [6,6]-bridged monoadducts of C_{60}.[8] In the UV/Vis spectrum the typical electronic absorption for [6,6] monoadducts appears at 426 nm.[8] The ^{13}C-NMR spectrum shows 16 signals between δ = 145.3 and 138.9 corresponding to 16 different types of sp^2-C atoms and one signal at δ = 71.5 corresponding to the sp^3-C atoms of **9** which have a local C_{2v} symmetry. SIMS mass spectroscopy (Cs^+) shows a molecular ion signal with m/z = 1321 as well as fragment ion peaks at m/z = 1222 and 720 corresponding to **9** with loss of the boc group and the C_{60} fragment, respectively.

The transformation of the monoadduct **9** to the mixed hexakisadduct **11** was carried out by template-mediated reaction with didodecyl malonate **10** in the presence of CBr_4 and DBU/DMA at room temperature (Scheme 3).[2] After purification by flash chromatography and preparative HPLC the hexakisadduct **11** was isolated in 44% yield. The UV/Vis spectrum shows the absorptions at 317 and 335 nm that are typical for hexakisadducts of C_{60} with an octahedral addition pattern.[2] The ^{13}C-NMR spectrum of **11** is very characteristic for such mixed hexakisadducts of C_{60}.[2] The sp^2 resonances of the fullerene core are separated into two groups of closely overlapping signals located at about δ = 141 and 145. This is about the peak position of the two magnetically different sorts of sp^2-C atoms within T_h-symmetrical hexakisadducts containing just one type of addend.[2] The FAB mass spectrum shows the molecular ion peak and a quasimolecular ion peak at m/z = 3513 and 3647 corresponding to M^+ and $[M + Cs]^+$.

For the synthesis of the biotinated lipofullerene **1** the boc protecting group was cleaved with a mixture of TFA and CH_2Cl_2 (1:1), and the resulting amine coupled with the activated (+)-biotin **5** (Scheme 3). This final step proceeds comparatively slowly in a yield of only 55% when 1.5 equivalents of biotin and CDI are used. With an excess of three equivalents of activated biotin the yield of **1** can be increased to 92% after purification by FC and HPLC. The UV/Vis spectrum of **1** is almost identical to that of **11** and shows the two characteristic absorptions of hexakisadducts with an octahedral addition pattern at 316 and 333 nm. The fullerene part of the ^{13}C-NMR spectrum of **1** is very similar to that of **11** with two sets of signals for the fullerene sp^2-C atoms at δ = 141 and 145. The resonances for the fullerene sp^3 C-atoms appear at δ = 69. The FAB mass spectrum



Scheme 3. Successive cyclopropanation of C_{60} leading to the hexakisadduct **11** and final coupling with biotin. (black: front C_{60} hemisphere, grey: rear hemisphere; i: C_{60}, CBr_4, DBU; ii: DMA, CBr_4, DBU; iii: TFA/CH_2Cl_2, (+)-biotin/CDI)

shows the molecular ion and quasimolecular ion peaks at m/z = 3641 and 3773 corresponding to M^+ and $[M + Cs]^+$.

Reflectometric Interference Spectroscopy (RIfS) Investigations

The capability of the fullerene-attached biotin unit in **1** to couple with streptavidin (SA) was proven by Reflectometric Interference spectroscopy (RIfS).[4] With RIfS, an immobilized layer of a biologically active haptophoric component is treated with a potential interaction partner. When the partner is recognised and adapted, a corresponding change in optical thickness of the layer is observed. Two different experiments have been carried out, one with immobilized biotin, the other with poly-streptavidin (poly-SA). These ex-

periments provide evidence for intense and selective coupling of poly-SA and **1**.

In the first experiment poly-SA was preincubated with **1** by mixing it with an excess of **1** in DMF and PBS buffer, in order to occupy the binding sites of poly-SA. The resulting solution was rinsed over an already biotinated transducer surface layer and, after 10 min, the layer was washed with buffer. No change in optical thickness was observed, indicating a complete blocking of the original poly-SA by **1**. As a control experiment, the transducer layer was washed with methanol and, subsequently, a solution of poly-SA in buffer and DMF, but without **1**, was rinsed over the same surface. As a result, an increase in optical thickness of about 3.7 nm

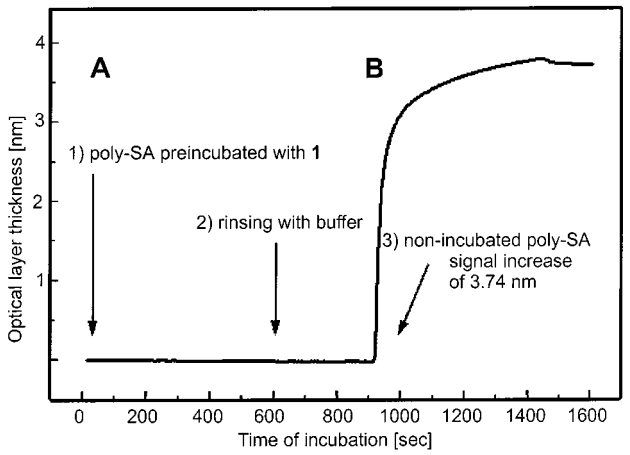


Figure 2. Reflectometric Interference spectroscopy [RIfS] experiment 1: **A**) poly-Streptavidin [poly-SA], preincubated with **1** to occupy its biotin adaptor sites, is exposed to a transducer surface covered with biotin; no binding interactions occur and hence no increase of thickness is observed; **B**) control: nonincubated poly-SA with free active sites in contact with biotin surface results in coupling interactions and in an increase of optical layer thickness

(Figure 2) was observed, caused by additional occupation of the biotin adaptor sites.

In the second experiment, poly-SA was immobilised on the transducer surface and a solution of **1** in PBS buffer and DMF was piped over the surface. Because of the relatively small size of **1**, no increase should be observed. The slight decrease in thickness shown in Figure 3 is possibly due to different refraction indices of poly-SA and the buffer/DMF solution. Subsequently, the surface was rinsed with buffer solution and incubated with biotinated BSA (bovine serum albumin). No increase in thickness was observed, the almost constant signal proved that **1** had effectively blocked the biotin binding pockets of the poly-SA at the transducer layer. The control experiment with buffer solution and DMF without **1** resulted in a signal increase of about 1.6 nm on account of the addition of biotinated BSA.

Langmuir-Blodgett Film Experiments

First compression experiments with the target molecule **1** spread as a monolayer at the air/water interface of a Langmuir trough at 20 °C suggest a (reversible) phase behavior which is qualitatively similar to that of a DPPC monolayer. The first increase of pressure upon compression

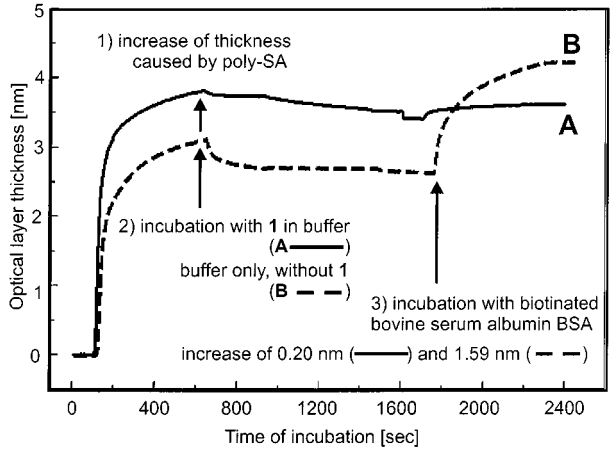


Figure 3. Reflectometric Interference spectroscopy [RIfS] experiment 2: **A**) an immobilised poly-SA transducer layer is exposed to **1** in buffer solution; because of the small molecular size, almost no increase in optical thickness is observed; after 3 min the washed surface is brought into contact with biotinated bovine serum albumin [BSA]; again, no binding interactions occur and hence no increase of thickness (solid line); **B**) control: immobilised poly-SA surface is exposed to buffer only; an exposure to biotinated BSA after 3 min results in coupling interactions and in an increase of optical layer thickness (dashed line)

is observed at a molecular area of 305 Å^2 and an LE–LC (liquid expanded to liquid condensed) transition is observed at 250 Å^2. Further compression causes a transition into an S-like (solid) phase at 140 Å^2 and the maximum compression was reached at 130 Å^2 (lateral pressure 34 mN/m). The latter molecular area is a factor of 2.7 higher than that of DPPC at identical lateral pressure.

Conclusions

We present here for the first time the synthesis of a lipophilic fullerene derivative **1** with a biofunctional group, which can be used as a transmembrane anchor. Biotin was chosen as the biofunctional group of the lipofullerene, since it exhibits high affinity towards the protein streptavidin. As a consequence, complex supramolecular assemblies reminiscent of those of biological membranes can be envisaged. The biotin carrying linker of **1** is thoroughly designed for lecithin membranes such as DPPC. Due to its ability to penetrate through a monolayer it does not only provide a suitable molecular recognition unit on the outer membrane surface but also guarantees a more homogeneous distribution of lipofullerenes within the lipid bilayer. This could lead to membrane morphologies of lecithin/lipofullerene vesicle composites different from the rod-like aggregation of plain lipofullerenes observed previously.[1] Detailed investigations of such membrane composites consisting of DPPC and target molecule **1** show that the biotinated lipofullerene indeed penetrates through the membrane of the liposome, as depicted in Figure 1, and binds streptavidin at the outer surface. These results will be reported in detail in an upcoming publication.

Experimental Section

General Remarks: ^{1}H and ^{13}C NMR: JEOL JNM EX 400 and JEOL JNM GX 400; MS: Micromass Zab Spec (FAB, EI), Varian MAT 311 A (EI), Micromass Tofspec (MALDI); IR: Bruker FT-IR IFS88 and FT-IR Vector 22; UV/Vis: Shimadzu UV 3102 PC; HPLC preparative: Shimadzu Class-LC10, SIL 10A, SPD 10A, CBM 10A, LC 8A, FRC 10A (Nucleosil, 5 μm, 200 × 4); HPLC analytical: Shimadzu Class-LC10, SIL 10A, SPD-M10A, CBM 10A, LC 10AT (Nucleosil, 5 μm, 250 × 21); flash chromatography: Merck, silica gel 60, 230–400 mesh; TLC: Riedel-de Haën, silica gel 60 F 254 (0.2 mm), visualization by UV or staining with molybdophosphoric acid and cerium sulfate in H_2SO_4 (aq) or with $KMnO_4$ (aq). – Materials and solvents were obtained from commercial suppliers and were used without further purification. Film balance experiments were performed at 25 °C on a subphase of ultrapure water using a 15 × 45 × 0.5 cm^3 Teflon trough equipped with a computer controlled movable Teflon barrier and a Wilhelmy pressure detection system (accuracy 0.1 mN/m). The barrier speed was 100 μm/s.

1,16-Hexadecanediol (2b): A solution of the commercially available hexadecanedioic acid **2a** (2.1 g, 7.34 mmol) in 75 mL of dry of THF was added dropwise to a suspension of lithium aluminium hydride (4.3 g, 113 mmol) in 40 mL of dry THF at room temperature under nitrogen.[9] The mixture was refluxed for 1 day and stirred at ambient temperature for 4 days. TLC control showed complete conversion. To destroy the excess of LAH, a mixture of methanol and diethyl ether (1:5) was added slowly. Subsequently dilute H_2SO_4 was added until two phases formed. The phases were separated and the aqueous phase was extracted three times with 50 mL of diethyl ether. The combined organic phase was washed with 5% aqueous $NaHCO_3$ solution and dried with $MgSO_4$. After evaporation of the solvent pure product **2b** was obtained as a white solid (1.75 g, 92%). TLC: (Et_2O/hexane 2:1, R_f = 0.4). – ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25 °C): δ = 4.31 (br t, J = 4.6 Hz, 2 H, O*H*), 3.34 (dt, J_1 = 5.9 Hz, J_2 = 8.8 Hz, 4 H, C*H*$_2$OH), 1.38 (tt, J_1 = J_2 = 6.6 Hz, 4 H, *C*H$_2$CH$_2$OH), 1.23 (m, 24 H, CH$_2$). – ^{13}C NMR (100.5 MHz, [D$_6$]DMSO, 25 °C): δ = 60.69 (2 C, *C*H$_2$OH), 32.52 (2 C, β-*C*H$_2$), 29.09, 29.03, 28.96 (10C, *C*H$_2$), 25.45 (2 C, γ-*C*H$_2$). – IR (KBr): $\tilde{\nu}$ = 3419, 3360, 2923, 2849, 1481, 1462, 1363, 1348, 1316, 1057, 1036, 1012, 995, 968, 728, 613 cm^{-1}. – MS (EI, 80 °C, $C_{16}H_{34}O_2$, calcd. 258.44): m/z = 259 [M$^+$], 222 ($C_{16}H_{30}$), 194, 166, 138, 124, 109, 96, 82, 69, 55 (100%). – $C_{16}H_{34}O_2$ (258.4): calcd. C 74.36, H 13.26; found C 73.92, H 13.20.

16-Hydroxyhexadecyl Methyl Malonate (3a): To a mixture of hexadecanediol **2b** (1.22 g, 4.72 mmol) and pyridine (0.382 mL, 4.72 mmol) in 60 mL of dry THF at room temperature under a protective atmosphere was added malonyl chloride monomethyl ester (0.50 mL, 4.72 mmol) in 20 mL of THF from a dropping funnel. After stirring for 2 days the solution was concentrated and separated by flash chromatography (SiO_2, hexane/ethyl acetate 3:2, R_f = 0.3) to give a white solid (820 mg, 48%). – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.10 (t, J = 6.7 Hz, 2 H, OC*H*$_2$), 3.71 (s, 3 H, OC*H*$_3$), 3.59 (t, J = 6.6 Hz, 2 H, C*H*$_2$OH), 3.35 (s, 2 H, OCC*H*$_2$CO), 1.60 (tt, 2 H, C*H*$_2$CH$_2$O), 1.52 (tt, 2 H, C*H*$_2$CH$_2$OH), 1.45 (br, 1 H, O*H*), 1.22 (m, 24 H, C*H*$_2$). – ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 167.03, 166.60 (2 C, *C*O), 65.73 (1 C, O*C*H$_2$), 63.01 (1 C, *C*H$_2$OH), 52.48 (1 C, *C*H$_3$O), 41.36 (1 C, OC*C*H$_2$CO), 32.77 (1 C, *C*H$_2$CH$_2$OH), 29.92, 29.83, 29.57, 29.50, 29.44, 29.39, 29.13, 28.40, 25.86, 25.72 (13 C, *C*H$_2$). – IR (KBr): $\tilde{\nu}$ = 3453, 3317, 3225, 2966, 2917, 2849, 1736, 1476, 1463, 1439, 1409, 1356, 1279, 1218, 1168, 1154, 1065, 1030, 995, 969, 927, 851, 731, 720, 689, 611, 581 cm^{-1}. – MS (EI, 80 °C, $C_{20}H_{38}O_5$, calcd. 358.51): m/z = 359 [M$^+$] (1%), 327 [M$^+$ – CO], 222 ($C_{16}H_{30}$, 4%), 119 [M$^+$ – C_{16}OH] (100%). – $C_{20}H_{38}O_5$ (358.5): calcd. C 67.00, H 10.68; found C 66.84, H 10.34.

15-Carboxypentadecyl Methyl Malonate (3b): To a solution of 16-hydroxyhexadecyl methyl malonate **3a** (820 mg, 2.29 mmol) in 6 mL of dry DMF was added pyridinium dichromate (3.01 g, 8.0 mmol, 3.5 equiv.) in 6 mL of dry DMF. The mixture was stirred for 8 h and filtered by means of a short silica gel column.[10] The column was rinsed with ethanol and the combined solutions were concentrated in vacuo. Purification by flash chromatography (SiO_2, diethyl ether/hexane 2:1) gave 420 mg of product **3b** (49%) as a white solid. – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 11.06 (br, 1 H, COO*H*), 4.11 (t, J = 6.6 Hz, 2 H, OC*H*$_2$), 3.72 (s, 3 H, OC*H*$_3$), 3.36 (s, 2 H, OCC*H*$_2$CO), 2.32 (t, J = 7.4 Hz, 2 H, C*H*$_2$COOH), 1.61 (m, 4 H, C*H*$_2$CH$_2$O, C*H*$_2$CH$_2$COOH), 1.23 (m, 24 H, C*H*$_2$). – ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 179.75, 167.07, 166.60 (3 C, *C*O), 65.75 (1 C, O*C*H$_2$), 52.46 (1 C, *C*H$_3$O), 41.38 (1 C, OC*C*H$_2$CO), 33.98 (1 C, *C*H$_2$COOH), 29.92, 29.83, 29.57, 29.50, 29.44, 29.39, 29.13, 28.40, 25.86, 25.72 (13 C, *C*H$_2$). – IR (KBr): $\tilde{\nu}$ = 3443, 2964, 2920, 2850, 1749, 1737, 1722, 1699, 1466, 1441, 1418, 1336, 1300, 1285, 1245, 1200, 1154, 1039, 1022, 984, 954, 933, 862, 808, 724, 684, 613, 588, 545, 511 cm^{-1}. – MS (EI, $C_{20}H_{36}O_6$, calcd. 372.50): m/z = 373 [M$^+$] (27%), 354 [M$^+$ – H_2O], 341 [M$^+$ – CO], 255 [M$^+$ – C_{16}OH], 237, 119 [M$^+$ – C_{16}OH], 98 (80%), 29 (100%). – $C_{20}H_{36}O_6$ (372.5): calcd. C 64.49, H 9.74; found C 64.44, H 9.69.

2-{2-[Amino-2-(*N*-*tert*-butyloxycarbonyl)ethyloxy]ethyloxy}-ethylamine (4b): Boc anhydride (4.37 g, 0.02 mol) in 30 mL of dioxane was added dropwise to a solution of aminoethyloxyethyloxy ethylamine **4a** (17.8 mL, 0.122 mol) in 85 mL of dioxane within 5 h. After additional stirring for 5 h the solution was concentrated in vacuo. The resulting yellow oil was dissolved in 40 mL of water and washed four times with 50 mL of CH_2Cl_2. The combined organic phases were washed four times with 30 mL of saturated saline solution and dried with Na_2SO_4. TLC control (DCM/ethanol 9:1) showed almost no bis-protected diamine. The yellow oil (4.33 g, 87% with respect to boc_2O) was used without further purification. – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.18 (br, 1 H, N*H*), 3.51 (s, 4 H, OC*H*$_2$C*H*$_2$O), 3.44, 3.41 (2t, J = 5.4, 4 H, C*H*$_2$O), 3.21 (dt, 2 H, C*H*$_2$NH), 2.77 (t, J = 5.1, 2 H, C*H*$_2$NH$_2$), 1.42 (br, 2 H, N*H*$_2$), 1.34 (s, 9 H, C*H*$_3$). – ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 155.84 (1 C, *C*O), 78.88 [1 C, C(CH$_3$)$_3$-CO], 73.27 (1 C, *C*H$_2$O), 70.02 (2 C, *C*H$_2$O), 66.88 (1 C, *C*H$_2$O), 41.56 (1 C, *C*H$_2$NH$_2$), 40.15 (1 C, *C*H$_2$NH), 28.24 (3 C, *C*H$_3$). – IR (KBr): $\tilde{\nu}$ = 3369, 2975, 2931, 2868, 1713, 1521, 1456, 1391, 1366, 1274, 1251, 1174, 1123, 1042, 916, 872, 781, 509, 495, 482 cm^{-1}. – MS (EI, 60 °C, $C_{11}H_{24}N_2O_4$, calcd. 248.32): m/z = 248 [M$^+$], 219 [M$^+$ – CH_2NH_2] (2%), 206 [M$^+$ – $CH_2CH_2NH_2$] (2%), 175 [M$^+$ – *t*BuO] (27%), 163 (28%), 150 [M$^+$ – boc] (17%), 119, 106, 88. – $C_{11}H_{24}N_2O_4$ (248.3): calcd. C 53.20, H 9.74; found C 52.73, H 9.40.

19-[(3a*S*,4*S*,6a*R*)-2-Oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]-2,2-dimethyl-4,15-dioxo-3,8,11-trioxa-5,14-diazanonadecane (6): To a solution of monoprotected diamine **4b** (750 mg, 3.02 mmol, 1.5 equiv.) in 4 mL of DMF was added the imidazolide activated biotin **5** (2.05 mmol). The mixture was stirred for 6 h. Separation by flash chromatography (SiO_2; Ethanol/CH_2Cl_2 2:1, R_f = 0.44) gave 651 mg of an oil (67% with respect to biotin). – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.32, 6.88 (br, 1 H, *cis*/*trans*-CON*H*), 6.71 (br, 1 H, biotin–N*H*), 6.04 (br, 1 H, biotin–N*H*), 5.51, 5.20 (br, 1 H, *cis*/*trans*-CON*H*), 4.40, 4.21, (2m, 2 H, biotin–C*H*), 3.52 (s, 4 H, OC*H*$_2$C*H*$_2$O), 3.47, 3.45 (2t, J = 5.3 Hz, 4 H, C*H*$_2$O), 3.33 (dt,

$J_1 = J_2 = 5.4$ Hz, 2 H, CH_2NH), 3.20 (dt, 2 H, CH_2NHboc), 3.04 (m, 1 H, biotin–C*H*), 2.80 (dd, $J_1 = 4.9$ Hz, $J_2 = 12.7$ Hz, 1 H, CHH_{exo}S), 2.66 (d, $J = 12.7$ Hz, 1 H, CHH_{endo}S), 2.13 (t, $J = 7.6$, 2 H, CH_2CO), 1.51–1.67 (m, 4 H, biotin–CH_2), 1.35 (s, 9 H, CH_3), 1.32 (m, 2 H, biotin–CH_2). – ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 173.41, 164.20 (2 C, CO), 155.94 (1 C, *C*O boc), 79.06 (1 C, *C*CH_3), 69.95, 69.88 (4 C, *C*H_2O), 61.65, 60.06 (2 C, *C*H), 55.59, (1 C, *C*HS), 40.31, 40.15 (2 C, *C*H_2NH), 38.96 (1 C, *C*H_2S), 35.78 (1 C, *C*H_2CO), 28.26 (3 C, *C*H_3), 28.15, 27.93, 25.48 (3 C, *C*H_2). – IR (KBr): $\tilde{\nu}$ = 3298, 3088, 2974, 2930, 2869, 1706, 1646, 1552, 1530, 1461, 1392, 1366, 1280, 1249, 1170, 1142, 1122, 859, 653, 603 cm^{-1}. – MS (EI, 200 °C, $C_{21}H_{38}N_4O_6S$, calcd. 474.62): *m*/*z* = 474 [M^+], 401 [M^+ – *t*BuO] (4%), 374 [M^+ – boc] (17%), 332 [M^+ – CH_2CH_2NHboc] (10%), 227 [biotinyl] (14%), 59 [C_3H_7O] (90%), 41 (100%). – $C_{21}H_{38}N_4O_6S$ (474.3): calcd. C 53.14, H 8.07, N 11.80, S 6.76; found C 52.83, H 7.94, N 12.20, S 5.76.

Methyl {31-[(3a*S*,4*S*,6a*R*)-2-Oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]-16,27-dioxo-20,23-dioxa-17,26-diazahentriacont-1-yl} Malonate (7): Complete deprotection of **6** (160 mg, 0.337 mmol) was achieved with 1.5 mL of TFA in 1.5 mL of CH_2Cl_2 after 2 min. After evaporation to remove the excess acid, CH_2Cl_2 was added. The solution was neutralised with 120 μL of triethylamine and successively washed with saturated $NaHCO_3$ and a little cold water. The dried organic phase was added to a mixture of malonate **3b** (150 mg, 0.4 mmol) and CDI (195 mg, 1.2 mmol, 3 equiv.) after stirring for 2 h. After stirring for 2 days at room temperature TLC control (ethanol) remained unchanged and separation by flash chromatography (SiO_2; ethanol/diethyl ether 1:1) gave **7** (45 mg, 18%) as a white solid. – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.67, 6.38, 6.37, 5.98 (4br, 4 H, N*H*), 4.47, 4.28 (2m, 2 H, C*H*NH), 4.11 (t, $J = 6.8$ Hz, 2 H, OCH_2), 3.72 (s, 3 H, OCH_3), 3.58 (s, 4 H, OCH_2CH_2O), 3.53, (m, 4 H, OCH_2), 3.41 (m, 4 H, CH_2NH), 3.35 (s, 2 H, OCCH_2CO), 3.13 (m, 1 H, C*H*S), 2.86 (dd, $J_1 = 8.8$ Hz, $J_2 = 4.2$ Hz, 1 H, CHH_{exo}S), 2.71 (d, $J = 12.2$ Hz, 1 H, CHH_{endo}S), 2.16 (2t, $J = 7.8$ Hz, 2 H, CH_2CO), 1.59 (m, 8 H, CH_2), 1.40 (m, 2 H, biotin–CH_2), 1.22 (m, 24 H, CH_2). – ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 173.57, 167.06, 166.59, 156.39 (5 C, *C*O), 70.06, 70.02, 69.87 (4 C, *C*H_2O), 65.73 (1 C, *C*H_2OCO), 61.93, 60.35 (2 C, *C*HNH), 55.48 (1 C, *C*HS), 52.42 (1 C, OCH_3), 41.37 (1 C, OC*C*H_2CO), 40.35 (1 C, *C*H_2S), 39.09 (2 C, *C*H_2NH), 36.62, 35.76 (2 C, *C*H_2CO), 29.62, 29.51, 29.46, 29.38, 29.31, 29.15, 28.40, 28.12, 27.96, 25.73, 25.40 (16 C, *C*H_2); IR (KBr): $\tilde{\nu}$ = 3386, 3287, 2956, 2921, 2851, 1737, 1702, 1649, 1545, 1516, 1463, 1377, 1366, 1331, 1265, 1147, 1028, 881, 815, 750, 720, 699, 587 cm^{-1}. – MS (MALDI-Tof/CCA, $C_{36}H_{64}N_4O_9S$, calcd. 728.98): *m*/*z* = 768 [M^+ + K] (100%), 751 [M^+ + Na] (24%); MS (FAB/NBA): *m*/*z* = 861 [M^+ + Cs] (4%), 751 [M^+ + Na] (70%), 729 [M^+] (76%).

Methyl (29,29′-Dimethyl-16,27-dioxo-20,23,28-trioxa-17,26-diazatriacontyl) Malonate (8): 4-Carbonyldiimidazole (50 mg, 2.8 mmol) was added gradually to a solution of malonate **3b** (416 mg, 1.12 mmol) in dry THF under a nitrogen atmosphere until TLC control (ethyl acetate/hexane 2:1) showed no more starting material. To the imidazolide activated acid monoprotected diamine **4b** (334 g, 1.34 mmol, 1.2 equiv.) was added. The mixture was stirred for 3 days and, after evaporation of the solvent in vacuo, the product **8** was separated by flash chromatography (SiO_2, diethyl ether/ethanol 10:1) to give 435 mg (64%) of a white solid. – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.03, 5.00 (2br, 2 H, N*H*), 4.08 (t, $J = 6.6$ Hz, 2 H, OCH_2), 3.69 (s, 3 H, CH_3O), 3.55 (m, 4 H, OCH_2CH_2O), 3.50 (t, $J = 5.2$ Hz, 4 H, CH_2O), 3.40 (dt, $J_1 = J_2 = 5$ Hz, 2 H, CH_2NH), 3.33 (s, 2 H, OCCH_2CO), 3.27 (dt, 2 H, CH_2NH), 2.12 (t, $J = 7.4$ Hz, 2 H, CH_2CO), 1.59 (m, 4 H, CH_2CH$_2$O), 1.39 (s, 9 H, CCH_3), 1.19 (m, 22 H, CH_2). – ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 173.19 (1 C, *C*ONH), 166.97, 166.50 (2 C, *C*OO), 155.89 (1 C, *C*ONHboc), 79.23 [1 C, *C*$(CH_3)_3$], 70.09, 69.91, 65.65 (5 C, O*C*H$_2$), 52.36 (1 C, O*C*H$_3$), 41.30 (1 C, OC*C*H_2CO), 40.22, 39.02 (2 C, *C*H_2NH), 36.62 (1 C, *C*H_2CO), 29.54, 29.44, 29.40, 29.29, 29.23, 29.08 (12 C, *C*H_2), 28.31 [3 C, C(*C*H_3)$_3$], 25.66 (1 C, *C*H_2). – IR (KBr): $\tilde{\nu}$ = 3346, 3319, 3090, 2918, 2850, 1754, 1735, 1686, 1642, 1542, 1472, 1441, 1392, 1366, 1279, 1251, 1165, 1027, 971, 933, 869, 782, 720, 689, 606, 461, 405 cm^{-1}. – MS (FAB/NBA, $C_{31}H_{58}N_2O_9$, calcd. 602.80): *m*/*z* = 603 [M^+] (21%), 503 [M^+ – boc] (100%). – $C_{31}H_{58}N_2O_9$ (602.8): calcd. C 61.77, H 9.70, N 4.65; found C 61.22, H 9.67, N 5.14.

[Methoxycarbonyl(29,29′-dimethyl-16,27-dioxo-20,23,28-trioxa-17,26-diazatriacontyloxycarbonyl)]methano-1,2-dihydro[60]fullerene (9): To a mixture of malonate **8** (290 mg, 0.48 mmol), C_{60} (446 mg, 0.62 mmol, 1.25 equiv.) and CBr_4 (160 mg, 0.48 mmol, 1 equiv.) in 250 mL of toluene was added DBU (82 μL, 0.53 mmol, 1.1 equiv.) and the resulting mixture stirred for 12 h. After evaporation of the solvent, separation by flash chromatography (SiO_2; toluene/ethanol 9:1, $R_f = 0.30$) gave 185 mg of monoadduct **9** as a reddish brown solid (29%). – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.11, 5.98 (br, 1 H, *cis*-/*trans*-N*H*), 5.32, 4.97 (br, 1 H, *cis*-/*trans*-N*H*), 4.48 (t, $J = 6.4$ Hz, 2 H, OCH_2), 4.07 (s, 3 H, OCH_3), 3.58 (m, 4 H, OCH_2-CH_2O), 3.53 (t, $J = 5.0$ Hz, 4 H, CH_2O), 3.43 (dt, $J_1 = J_2 = 5$ Hz, 2 H, CH_2NH), 3.30 (dt, 2 H, CH_2NHboc), 2.15 (t, $J = 7.5$ Hz, 2 H, CH_2CO), 1.81 (m, 2 H, OCH$_2$CH_2), 1.59 (m, 2 H, CH_2CH$_2$CO), 1.42 [m, 2 H, CH_2; s, 9 H, C(CH_3)$_3$], 1.33 (m, 2 H, CH_2), 1.25 (m, 18 H, CH_2). – ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 173.28, 164.11, 163.60, 155.94 (4 C, *C*O), 145.33, 145.26, 145.17, 145.11, 144.87, 144.67, 144.60, 143.87, 143.06, 142.99, 142.39, 142.19, 141.90, 140.95, 139.10, 138.90 (58 C, C_{60} sp^2-*C*), 79.38 [1 C, *C*$(CH_3)_3$], 71.54 (2 C, C_{60} sp^3-*C*), 70.20, 70.02, 67.54 (5 C, O*C*H$_2$), 53.98 (1 C, O*C*H$_3$), 52.17 (1 C, methano–C), 40.31, 39.11 (2 C, *C*H$_2$NH), 36.75 (1 C, *C*H$_2$CO), 29.68, 29.61, 29.52, 29.41, 29.33, 29.21, 28.55 (11 C, *C*H$_2$), 28.40 [3 C, C(*C*H_3)$_3$], 25.97, 25.75 (2 C, *C*H$_2$). – IR (KBr): $\tilde{\nu}$ = 3442, 3425, 2923, 2852, 1746, 1709, 1644, 1547, 1532, 1460, 1429, 1389, 1365, 1262, 1238, 1174, 1102, 1022, 800, 704, 666, 579, 525 cm^{-1}. – UV/Vis ($CHCl_3$): λ_{max} (ε) = 257 (120000), 326 (37000), 426 (2900), 481 (1900), 683 nm (260). – MS (FAB/NBA, $C_{91}H_{56}N_2O_9$, calcd. 1321.43): *m*/*z* = 1321 [M^+] (4%), 1222 [M^+ – boc] (27%), 720 [C_{60}] (100%). – $C_{91}H_{56}N_2O_9$ (1321.4): calcd. C 82.71, H 4.27, N 2.12; found C 80.96, H 4.56, N 1.78.

Bis(dodecyl) Malonate 10: A solution of dodecanol (1.86 mg, 10 mmol) and 0.74 mL of pyridine in 50 mL of dry CH_2Cl_2 was cooled to 0° C. Malonyl dichloride (0.44 mL, 4.54 mmol) in 5 mL of dry CH_2Cl_2 was added dropwise. The mixture was then allowed to warm to room temperature and stirred for another 6 h. After washing with water and drying with $MgSO_4$ separation by flash chromatography (SiO_2; CH_2Cl_2/hexane 1:1, $R_f = 0.37$) gave a colorless oil, which crystallised after a few days as a white solid (yield: 1.73 g, 86%). – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.14 (t, $J = 6.7$ Hz, 4 H, CH_2O), 3.37 (s, 2 H, OCCH_2CO), 1.64 (tt, 4 H, CH_2CH$_2$O), 1.26 (m, 36 H, CH_2), 0.88 (t, $J = 6.6$ Hz, 6 H, CH_3). – ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 166.71 (2 C, *C*O), 65.70 (*C*H$_2$O), 41.73 (1 C, OC*C*H$_2$CO), 31.94, 29.67, 29.60, 29.54, 29.38, 29.25, 28.50, 25.82, 22.71 (20C, *C*H$_2$), 14.12 (2 C, *C*H$_3$). – IR (KBr): $\tilde{\nu}$ = 3464, 2956, 2926, 2855, 1756, 1739, 1467, 1412, 1380, 1330, 1270, 1149, 1010, 893, 722, 685, 583 cm^{-1}. – MS (EI, 80 °C, $C_{27}H_{52}O_4$, calcd 440.70): *m*/*z* = 441 [M + H$^+$], 273 [M^+ – C_{12}] (45%), 105 [M^+ – C_{12} – C_{12}] (100%). – $C_{27}H_{52}O_4$ (440.7): calcd. C 73.59, H 11.89; found C 73.582, H 11.94.

{[Methoxycarbonyl(29,29′-dimethyl-16,27-dioxo-20,23,28-trioxa-17,26-diazatriacontyloxycarbonyl)]methano}-18,36:22,23:27,45:31,32:55,60-pentakis[di(dodecyloxycarbonyl)methano]-1,2:18,36:22,23:27,45:31,32:55,60-dodecahydro[60]fullerene (11): A solution of monoadduct **9** (205 mg, 0.15 mmol) and DMA (320 mg, 1.55 mmol, 10 equiv.) in 40 mL of dry toluene was stirred for 6 h at room temperature. After adding 10 equiv. of malonate **10** (683 mg), 10 equiv. of CBr_4 (514 mg) and 20 equiv. of DBU (465 μL) in this order, the mixture was stirred for 2 days at room temperature. Separation by flash chromatography (SiO_2, ethyl acetate/hexane 1:1, $R_f = 0.35$; pentakisadducts $R_f < 0.3$) and purification by preparative HPLC (SiO_2, ethyl acetate/hexane 6:4) gave 240 mg (44%) of an amber solid. – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.94, 4.97 (br, 2 H, N*H*), 4.21 (t, *J* = 6.9 Hz, 22 H, OCH_2), 3.83 (s, 3 H, OCH_3), 3.59 (m, 4 H, OCH$_2$CH_2O), 3.54 (t, *J* = 5.2 Hz, 4 H, CH_2O), 3.44 (dt, J_1 = 5.5 Hz, J_2 = 5.0 Hz, 2 H, CH_2NH), 3.31 (dt, 2 H, CH_2NH), 2.15 (t, *J* = 7.7 Hz, 2 H, CH_2CO), 1.66 (m, 24 H, CH_2), 1.43 [s, 9 H, C(CH_3)$_3$], 1.24 (m, 202 H, CH_2), 0.86 (t, *J* = 6.6 Hz, 30 H, CH_3). – ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 164.30, 163.87 (14 C, *C*O), 146.01, 145.77, 145.74, 145.49, 141.35, 141.20, 141.17, 141.14, 140, 83 (48 C, C_{60} sp^2-*C*), 79.38 [1 C, *C*(CH$_3$)$_3$], 70.26, 70.14 (4 C, *C*H$_2$O), 69.11, 68.99 (12 C, C_{60} sp^3-*C*), 66.98, 66.86 (11 C, *C*H$_2$OCO), 53.55 (1 C, O*C*H$_3$), 45.41 (6 C, methano-*C*), 39.11 (2 C, *C*H$_2$NH), 37.00 (1 C, *C*H$_2$CO) 31.93 (11 C, *C*H$_2$CH$_2$O), 29.80, 29.68, 29.65, 29.58, 29.42, 29.38, 29.27, 28.50, 28.44 (81 C, *C*H$_2$), 28.39 [3 C, C(*C*H$_3$)$_3$], 25.84, 25.78 (11 C, *C*H$_2$), 22.68 (10C, *C*H$_2$), 14.11 (10C, *C*H$_3$). – IR (KBr): $\tilde{\nu}$ = 3415, 3343, 2925, 2854, 1747, 1679, 1514, 1466, 1365, 1264, 1217, 1125, 1080, 997, 828, 760, 716, 670, 529 cm^{-1}. – UV/Vis (CH_2Cl_2): λ_{max} (ε) = 245 (114000), 271 (88000), 280 (92000), 317 (56000, sh), 335 nm (45000, sh). – MS (FAB/NBA, $C_{226}H_{306}N_2O_{29}$, calcd. 3514.84): *m*/*z* = 3647 [M$^+$ + Cs] (1%), 3513 [M$^+$] (5%), 3414 [M$^+$ – boc] (11%), 720 [C_{60}] (100%). – $C_{226}H_{306}N_2O_{29}$ (3194.7): calcd. C 84.96, H 9.65, N 0.87; found C 84.12, H 9.21, N 0.52.

5-[(3a*S*,4*S*,6a*R*)-4-[5-(1*H*-imidazol-1-yl)-5-oxopentyl]tetrahydro-1*H*-thieno-[3,4-*d*]imidazol-2(3*H*)-one (5): Biotin (500 mg, 2.05 mmol) in 20 mL of of dry DMF was gently heated to obtain a clear solution. Carbonyldiimidazole [CDI] (332 mg, 2.05 mmol) was then added. The mixture was stirred until no more CO_2 formation was observed and used without further purification in order to prevent solubility problems and decomposition. If necessary the imidazole can be easily removed by sublimation. NMR showed almost complete conversion. – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.41(s, 1 H, NC*H*N), 7.69 (d, *J* = 1.7 Hz, 1 H, NC*H*), 7.63 (s, 1 H, NC*H*N imidazole), 7.05 (d, *J* = 1.7 Hz, 1 H, NC*H*), 7.01, 7.00 (2s, 2 H, NC*H* imidazole), 6.47, 6.39 (2br, 2 H, biotin–N*H*), 4.30, 4.14 (2m, 2 H, C*H*), 3.11 (m, 1 H, C*H*), 3.01 (dt, J_1 = 4.7 Hz, J_2 = 2.6 Hz, 2 H, CH_2CO), 2.83 (dd, J_1 = 14 Hz, J_2 = 5 Hz, 1 H, CHH_{exo}S), 2.57 (d, *J* = 12.7 Hz, 1 H, CHH_{endo}S), 2.19 (t, *J* = 7 Hz, CH_2COOH corresponding to 7% nonactivated biotin or decomposed biotin imidazolide), 1.65, 1.51, 1.41 (3m, 6 H, CH_2). – ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 170.48, 162.75 (2 C, *C*O), 137.01 (1 C, N*C*HN), 135.14 (1 C, N*C*HN imidazole), 130.23 (1 C, *C*HN), 121.71 (2 C, *C*HN imidazole), 116.43 (1 C, *C*HN), 61.02, 59.20 (2 C, *C*H), 55.33 (1 C, *C*HS), 39.90 (1 C, *C*HS), 34.16 (1 C, *C*H$_2$), 27.99, 27.83, 23.62 (3 C, *C*H$_2$).

{[Methoxycarbonyl(31-[(3a*S*,4*S*,6a*R*)-2-oxohydro-1*H*-thieno[3,4-*d*]-imidazol-4-yl]-16,27-dioxo-20,23-dioxa-17,26-diazahentriacontyl-carbonyl)]methano}-18,36:22,23:27,45:31,32:55,60-pentakis-[bis(dodecyloxycarbonyl)methano]-1,2:18,36:22,23:27,45:31,32:55,60-dodecahydro[60]fullerene (1): To a solution of hexaadduct **11** (190 mg, 0.05 mmol) in 2.0 mL of cold CH_2Cl_2 was added TFA (2.0 mL, 26 mmol) to deprotect the boc-protected amino group. After 1 h stirring at room temperature the mixture was diluted with 5 mL of cold CH_2Cl_2, and triethylamine (3.62 mL, 26 mmol) was added slowly for neutralisation. After washing three times with saturated $NaHCO_3$ the phases were separated. The organic phase was washed a few times with a small amount of water and the combined aqueous phases were extracted with CH_2Cl_2. The combined organic phases were dried with $MgSO_4$. To this solution of deprotected amine in about 20 mL of DCM was added a mixture of biotin (39 mg, 0.16 mmol, 3 equiv.) and CDI (26 mg, 0.16 mmol) in 3 mL of dry DMF after 12 h of stirring. The coupling of the biotin was followed by TLC (toluene/ethanol 3:1, $R_f = 0.36$, with free amino group $R_f = 0.15$). After 2 days of stirring at room temperature TLC control showed complete reaction. Purification by flash chromatography (SiO_2; toluene/ethanol 4:1, increased to 3:1) followed by preparative HPLC (SiO_2, toluene/ethanol 9:1) gave hexakisadduct **1** (182 mg, 92%) as an amber solid. – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.66 (br t, *J* = 5.1 Hz, 1 H, N*H*), 6.48 (br, 1 H, biotin–NH), 6.35 (br t, *J* = 5.4 Hz, 1 H, N*H*), 5.53 (br, 1 H, biotin–N*H*), 4.46 (m, 1 H, biotin), 4.27 (m, 1 H, biotin), 4.20 (t, *J* = 6.8 Hz, 22 H, CH_2O), 3.81 (s, 3 H, OCH$_3$), 3.57 (s, 4 H, OCH_2CH_2O), 3.54 (2t, 4 H, CH_2O), 3.41 (dt, 4 H CH_2NH), 3.11 (m, 1 H, biotin–C*H*S), 2.86 (dd, J_1 = 7.8 Hz, J_2 = 4.9 Hz, 1 H, CHH_{exo}S), 2.70 (d, *J* = 13.0 Hz, 1 H, CHH_{endo}S), 2.20 (t, *J* = 7.6 Hz, 2 H, biotin–CH_2CO), 2.15 (t, *J* = 7.6 Hz, 2 H, CH_2CO), 1.65 (m, 28 H, CH_2), 1.40 (m, 2 H, biotin–CH_2), 1.26 (m, 202 H, CH_2), 0.84 (t, *J* = 6.8 Hz, 30 H, CH$_2$CH_3). – ^{13}C NMR (100.5 MHz, $CDCl_3$): δ = 173.48, 173.28, 164.22, 164.00, 163.80, 162.45 (15 C, *C*O), 145.93, 145.71, 145.42, 141.07, 140.76 (48 C, C_{60} sp^2-*C*), 70.00, 69.95, 69.80 (4 C, *C*H$_2$O), 69.05, 68.94 (12 C, C_{60} sp^3-*C*), 66.90 (11 C, *C*H$_2$OCO), 61.71 (1 C, biotin-CHNH), 60.17 (1 C, biotin-CHNH), 55.46 (1 C, *C*HS), 53.34 (1 C, O*C*H$_3$), 45.38, 45.16 (6 C, methano-C), 40.41 (1 C, *C*H$_2$S), 39.07 (2 C, *C*H$_2$NH), 36.59, 35.86 (2 C, *C*H$_2$CO), 31.86 (11 C, *C*H$_2$CH$_2$O), 29.59, 29.48, 29.30, 29.19, 28.38 (81 C, *C*H$_2$), 28.00 (2 C, biotin-CH$_2$CH$_2$), 25.72 (11 C, *C*H$_2$), 25.46 (1 C, biotin-CH$_2$), 22.61 (10C, *C*H$_2$), 14.02 (10C, *C*H$_3$). – IR (KBr): $\tilde{\nu}$ = 3287, 2924, 2853, 1748, 1703, 1652, 1543, 1466, 1379, 1353, 1264, 1215, 1124, 1079, 998, 826, 761, 716, 670, 529 cm^{-1}. – UV/Vis (CH_2Cl_2): λ_{max} (ε) = 245 (99000), 271 (72000), 281 (76000), 316 (47000, sh), 333 nm (38000, sh). – MS (FAB/NBA, $C_{231}H_{312}N_4O_{29}S$, calcd 3641.02): *m*/*z* = 3773 [M$^+$ + Cs], 3641 [M$^+$], 3309, 720 [C_{60}] (100%). – $C_{231}H_{312}N_4O_{29}S$ (3641.0): calcd. C 76.20, H 8.64, N 1.54, S 0.88; found C 75.55, H 8.16, N 1.13, S 0.50.

RIfS Experiments

Experiment 1: poly-SA (50 μg) in 997 μL of PBS buffer was mixed with a solution of 150 μg of **1** in 3μL of DMF. The mixture was allowed to stand for 10 minutes with occasional agitation. This solution was piped for 10 min over a transducer surface covalently linked to biotin. The transducer layer was rinsed thoroughly with methanol for 5 min, and the same surface was subsequently exposed to a control solution of 50 μg of poly-SA in 997 μL of PBS buffer and 3 μL of DMF.

Experiment 2: Compound **1** (150 μg) was dissolved in 997 μg of PBS buffer and 3μL of DMF. This solution was exposed on a transducer surface carrying immobilized poly-SA with an optical thickness between 2.71 nm and 3.75 nm. The signal showed a slight decrease of 0.21 nm. The thickness remained nearly the same during incubation with BSA. In the two analogous control experiments with 997 μL of PBS buffer, 3 μL of DMF (without **1**) and biotinated bovine serum albumin BSA an increase in layer thickness of 2.2 nm and 1.56 nm, respectively, was detected.

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