Electronic Supporting Information for the Communication Ref.: B817059B

Title: Non-Covalent Functionalization of Carbon Nanotubes with Glycolipids: Glyconanomaterials with Specific Lectin-Affinity.

Mohyeddin Assali,[a] Manuel Pernía Leal,[a] Inmaculada Fernández,[b] and Rachid Baati,[c] Charles Mioskowski, [c] and Noureddine Khiar.*[a]

Contribution from:

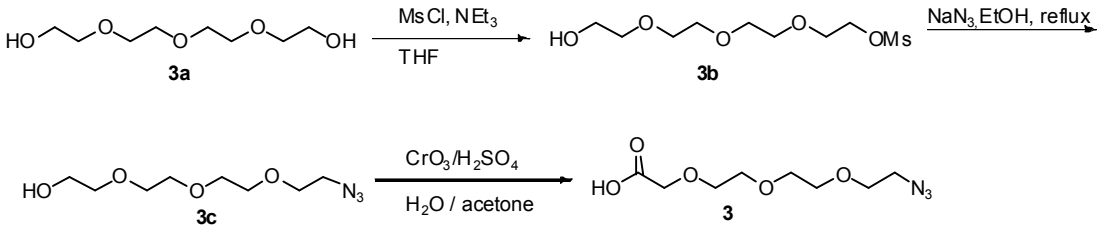
[a] Instituto de Investigaciones Químicas, C.S.I.C-Universidad de Sevilla, c/. Américo Vespucio, 4., Isla de la Cartuja, 41092 Sevilla, Spain. [b] Departamento de Química Orgánica y Farmacéutica, Facultad de Farmacia, Universidad de Sevilla, 41012 Sevilla, Spain. [c]Laboratoire de Synthèse Bio-Organique, Faculté de Pharmacie UMR 7175-LC1, Université Louis Pasteur de Strasbourg, 74 route du Rhin, 67 401 Illkirch-Graffenstaden, France.

Table of Contents

General Methods.

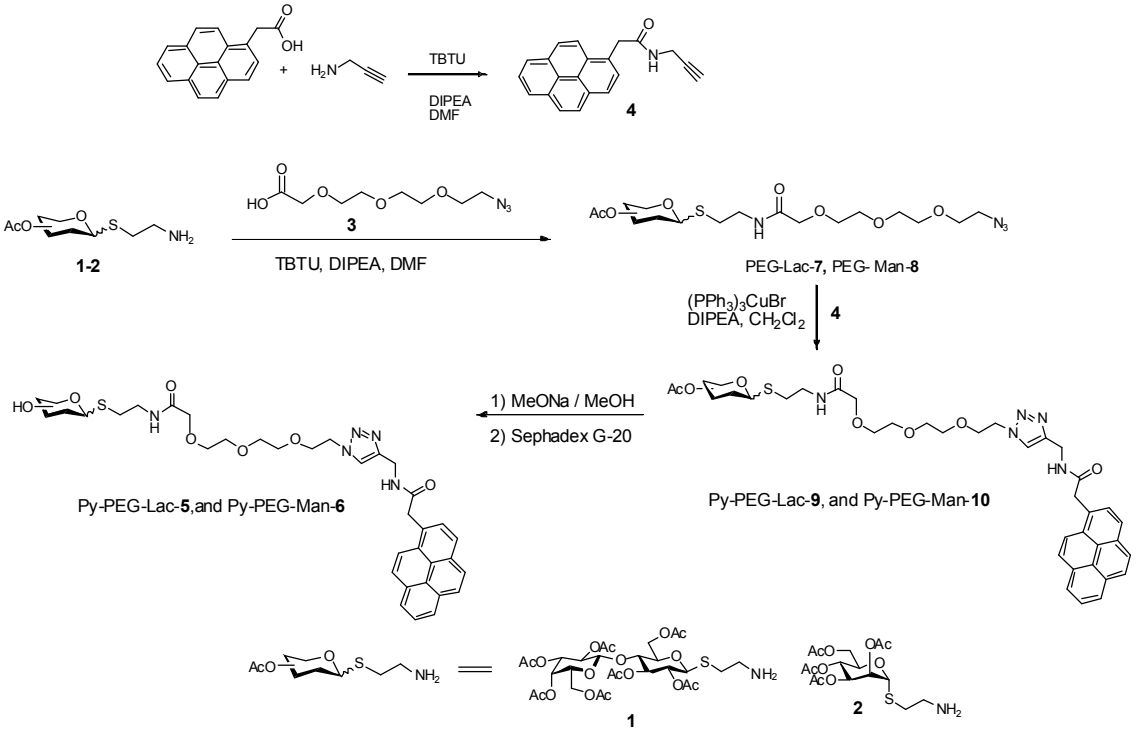
All reactions were run under an atmosphere of dry argon using oven-dried glassware and freshly distilled and dried solvents. THF and diethyl ether were distilled from sodium benzophenone ketyl. Dichloromethane was distilled from calcium hydride. TLC was performed on Silica Gel GF_{254} (Merck) with detection by charring with phosphomolybdic acid/EtOH and sulphuric acid/EtOH. Reagents were obtained from commercial suppliers and used without further purification. For flash chromatography, silica Gel (Merck 230-400 mesh) was used. Columns were eluted with positive air pressure. Chromatographic eluents are given as volume to volume ratios (v/v). NMR spectra were recorded with a Bruker AMX_{500} (^1H, 500 MHz) and Bruker Avance DRX_{500} (^1H, 500 MHz) spectrometers. Chemical shifts are reported in ppm, and coupling constants are reported in Hz. Routine spectra were referenced to the residual proton or carbon signals of the solvent. High-resolution mass spectra were recorded on a Kratos MS-80RFA 241-MC apparatus. Optical rotations were determined with a Perkin-Elmer 341 polarimeter. Elemental analyses were recorded on a leco CHNS-932 apparatus. The organic extracts were dried over anhydrous sodium sulfate and concentrated under vacuum.

The synthesis of (2' ethylamino) *per-O*-acetylated-1-thio-glycosides **1** and **2** was done in a one step manner from the corresponding peracetylated sugars and will be reported in due course. The hydrophilic spacer **3** was obtained from tetraethylene glycol in three steps using the following Scheme 1.



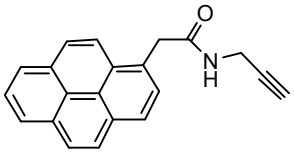
Scheme 1: Synthesis of the hydrophilic spacer 3.

The synthesis of neoglycoconjugates Py-PEG-Lac-**5**, and Py-PEG-Man-**6** has been done following Scheme 2.



Scheme 2: General synthetic method followed for the synthesis of neoglycoconjugates Py-PEG-Lac-5, and Py-PEG-Man-6

Synthesis of alkyne **4**



1-Pyreneacetic acid **(**200 mg, 0.77 mmol) was dissolved in DMF (3.8 mL). O-Benzotriazol-1-yl-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate (271 mg, 0.85 mmol) and *N,N*-diisopropylethylamine (DIPEA) (134 µL, 0.77 mmol) were added sequentially at room temperature. The solution was stirred for 5 min before a solution of propargylamine **(**53 µL, 0.77 mmol) and DIPEA (267 µL, 1.54 mmol) in DMF (7.7 mL) were added slowly. The solution was stirred overnight before the solvent was removed under vacuum. The residue was dissolved in CH_2Cl_2 (100 mL) and washed with 1M HCl (20 mL), saturated aqueous $NaHCO_3$ (40 mL) and brine (20 mL). After drying over Na_2SO_4 and removal of solvent, the crude product was purified by silica gel chromatography eluting with hexane/ethylacetate (3:1) to give 210 mg of an amorphous yellow solid **4** (99% yield): R_f= 0.43 (Hex/EtOAc 1:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ : 8.35-7.90 (m, 9H), 5.48 (s, 1H), 4.36 (s, 2H), 3.95 (s, 2H), 2.07 (s, 1H). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ : 170.5, 131.1, 130.7, 129.5, 127.2, 126.2, 125.5, 125.4, 125.0, 122.7, 79.0, 71.3, 41.7, 29.2. HRMS Calcd. for $C_{21}H_{15}NONa$ $[M+Na]^+$: m/z 297.1154. Found: 297.1148

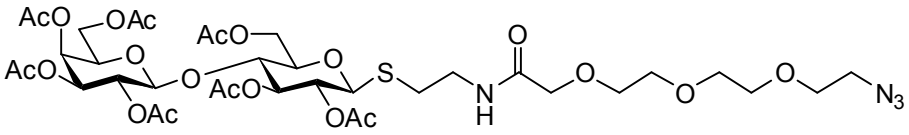
Synthesis of spacer **3** (See scheme A),

A solution of tetraethylene glycol **3a** (22.7 mL, 140 mmol), Et_3N (15 mL) and THF (100 mL) was cooled to 0ºC. To this was added dropwise methanesulfonyl chloride solution (10.8 mL, 140 mmol). The reaction mixture was then allowed to warm to room temperature and stirred vigorously overnight. The solution was diluted with CH_2Cl_2 (250 mL) and washed with saturated NH_4Cl (50 mL) and brine (20 mL). The organic solution was dried over Na_2SO_4 and concentrated in vacuum to give the crude product. The oily residue was purified by flash column chromatography, eluting with dichlorometane/methanol (20:1) to give 12.9 g of the mesylated compound **3b** as an oil (37% yield): R_f= 0.5 in dichlorometane/methanol (9:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.41- 4.35 (m, 2H), 3.81-3.60 (m, 14H), 3.09 (s, 3H), 2.97 (s, 1H). ^{13}C NMR (125.6 MHz, $CDCl_3$) δ: 72.5, 70.4, 70.3, 70.1, 69.4, 68.9, 61.4, 37.4. HRMS Calcd. for $C_9H_{20}O_7SNa$ $[M+Na]^+$: m/z 273.1008. Found: 273.0997.

A mixture of mesylated compound **3b** (9.1 g, 33.4 mmol) and sodium azide **(**2.4 g., 36.8 mmol) in ethanol (50 mL) was heated at reflux overnight, cooled to room temperature and concentrated in vacuo. The residue was diluted with ether (250 mL), washed with brine (50 mL), and dried over Na_2SO_4. Solvent was removed under vacuum to yield the crude product, which was purified by flash column chromatography, eluting with a gradient of hexane/ethyl acetate (1:1) to give 6.4 g of the azide **5c** as an oil (97% yield): R_f = 0.18 in hexane/ethyl acetate (1:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.58 (t, 2H, *J* = 4.8 Hz), 3.57-3.51 (m, 10H), 3.46 (t, 2H, *J* = 4.8 Hz), 3.26 (t, 2H, *J* = 4.8 Hz), 3.10 (brs, 1H). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ: 72.5, 70.6, 70.5, 70.4, 70.2, 69.9, 61.5, 50.5. HRMS calcd for $C_8H_{17}N_3O_4Na$ $[M+Na]^+$: m/z 220.1297. Found: 220.1288.

A solution of azido alcohol **3c** (2.4 g, 10 mmol) in acetone (100 mL) was cooled to 0ºC. To this was added freshly prepared Jones reagent (44 mL). The reaction mixture was then allowed to warm to room temperature and stirred vigorously overnight. To the orange suspension was added dropwise propan-2-ol until the green colour was observed, then the reaction mixture was filtered over Celite® to remove chromium (IV) oxide and concentrated in vacuum to give the crude product. The oil residue was purified by flash column chromatography, eluting with dichloromethane/methanol (9:1) to give 1.8 g of **3** as an oil (75% yield): R_f = 0.4 in dichloromethane/methanol (9:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.30 (brs, 1H), 4.20-4.10 (m, 2H), 3.80-3.60 (m, 10H), 3.40-3.20 (m, 2H). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ: 173.5, 70.9, 70.4, 70.3, 70.2, 69.9, 68.3, 50.5. HRMS calcd for $C_8H_{15}N_3O_5Na$ $[M+Na]^+$: m/z 234.1089. Found: 234.1085.

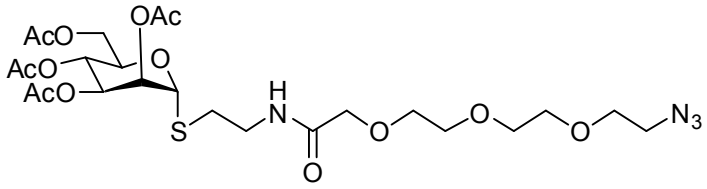
Synthesis of Compound PEG-Lac-**7**



The azido acid **3** **(**68 mg, 0.29 mmol) was dissolved in DMF (0.5 mL). O-Benzotriazol-1-yl-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate (93 mg., 0.29 mmol) and DIPEA (70 μL, 0.43 mmol) were added sequentially at room temperature. The solution was stirred for 5 min before a solution of lactose derived amine **1** **(**223 mg., 0.29 mmol) and DIPEA (70 μL, 0.43 mmol) in DMF (1 mL) was added slowly. The solution was stirred

for 2 h before the solvent was removed under vacuum. The residue was dissolved in CH_2Cl_2 (100 mL) and washed with 1M HCl (20 mL), saturated aqueous $NaHCO_3$ (40 mL) and brine (20 mL). After drying over Na_2SO_4 and removal of solvent, the crude product was purified by silica gel chromatography eluting with dichloromethane/methanol 20:1 to give 225 mg of a yellow oil PEG-Lac-**7** (85% yield): R_f = 0.21 (CH_2Cl_2/MeOH 20:1); $[\alpha]_D$ = -7.66 (c 0.85, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.20 (t, 1H, *J* = 1.1 Hz), 5.33 (d, 1H, *J* = 2.4 Hz), 5.18 (t, 1H, *J* = 9.2 Hz), 5.08 (t, 1H, *J* = 10.0 Hz), 4.97-4.89 (m, 2H), 4.55-4.45 (m, 3H), 4.15-4.05 (m, 3H), 3.98 (s, 2H), 3.85 (t, 1H, *J* = 6.8 Hz), 3.77 (t, 1H, *J* = 9.5 Hz), 3.70-3.60 (s, 11H), 3.60-3.40 (m, 2H), 3.38 (t, 2H, *J* = 4.9 Hz), 2.80-2.83 (m, 1H), 2.80 (s, 1H), 2.78-2.70 (m, 1H), 2.13 (m, 3H), 2.09 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.02 (s, 6H), 1.94 (s, 3H). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ: 170.3, 170.1, 170.0, 169.9, 169.6 (2C), 169.0, 101.1, 83.7, 76.1, 73.7, 71.0, 70.8, 70.7, 70.6, 70.5, 70.4, 70.3, 70.2, 70.0, 69.1, 66.6, 62.1, 60.8, 50.6, 38.9, 30.4, 20.8, 20.7, 20.6. HRMS calcd for $C_{36}H_{54}N_4O_{21}SNa$ $[M+Na]^+$: m/z 933.2899. Found: 933.2854.

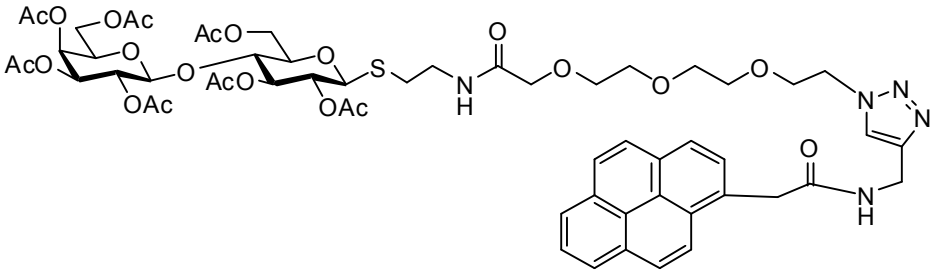
Synthesis of Compound PEG-Man-**8**



The azido acid **3** (190.5 mg, 0.82 mmol) was dissolved in DMF (2 mL). O-Benzotriazol-1-yl-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate (262 mg, 0.82 mmol) and DIPEA (194 µL, 1.1 mmol) were added sequentially at room temperature. The solution was stirred for 5 min before a solution of 2' ethyl amino-(2,3,4-tetra-*O*-acetyl-1-thio)-α-manopyranoside **2** (300 mg, 0.74 mmol) and DIPEA (194 µL, 1.1 mmol) in DMF (2 mL) was added slowly. The solution was stirred for 2 h before the solvent was removed under vacuum. The residue was dissolved in CH_2Cl_2 (100 mL) and washed with 1M HCl (20 mL), saturated $NaHCO_3$ aqueous solution (40 mL) and brine (20 mL). After drying over Na_2SO_4 and removal of solvent, the crude product was purified by silica gel chromatography eluting with ethyl acetate to give 414 mg of a yellow oil Py-PEG-Man-**8** (90% yield): R_f = 0.25 in ethyl acetate. $[\alpha]_D$ = + 43.6 (c 1.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.27 (brs, 1H), 5.35-5.20 (m, 4H), 4.40-4.30

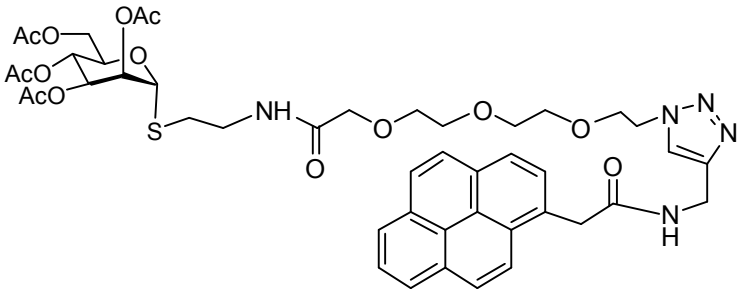
(m, 1H), 4.28 (dd, 1H, J = 12.2 Hz, J =5.5 Hz), 4.09 (dd, 1H, J = 12.2 Hz, J = 1.7 Hz), 3.99 (s, 2H), 3.70-3.60 (m, 10H), 3.54-3.51 (m, 2H), 3.38 (t, 2H, J = 4.9 Hz), 2.85-2.60 (m, 2H), 2.15 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 1.97 (s, 3H). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ: 170.6, 170.1, 169.9, 169.8, 82.5, 71.0, 70.9, 70.7, 70.6, 70.5, 70.4, 70.3, 70.0, 69.4, 69.1, 66.3, 62.4, 50.7, 38.1, 31.1, 31.0, 20.8, 20.7, 20.6. HRMS calcd for $C_{24}H_{38}N_4O_{13}SNa$ $[M+Na]^+$: m/z 645.2054. Found: 645.2046.

Synthesis of compound Py-PEG-Lac-**9**



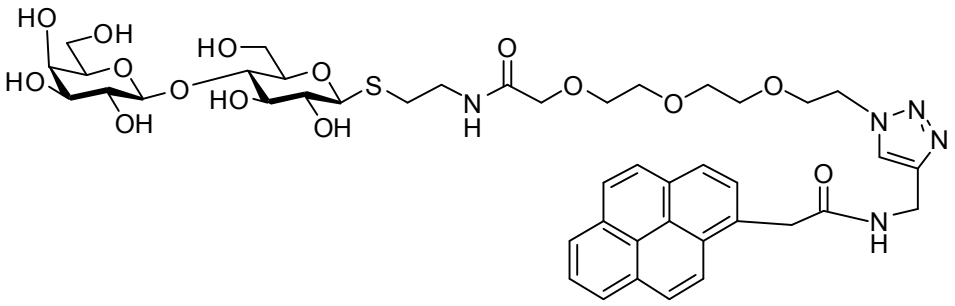
To a solution of the azide PEG-Lac-**7** (81.5 mg., 0.09 mmol) and the alkyne **4** (29 mg, 0.10 mmol) in anhydrous CH_2Cl_2 (2 mL), were added DIPEA (47 μL, 0.27 mmol) and Bromotris(triphenylphosphine)copper(I) (8.3 mg., 0.01 mmol). The resulting solution was stirred under argon overnight at room temperature. After dilution with dichloromethane (100 mL), washing with 1M HCl (20 mL), saturated aqueous $NaHCO_3$ (40 mL) and brine (20 mL), the organic phase was dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by silica gel chromatography eluting with ethylacetate/methanol (9:1) to give 46 mg of a yellow oil Py-PEG-Lac-**9** (56% yield): R_f = 0.6 (EtOAc/MeOH 9:1). $[\alpha]_D$ = -5.35 (c 0.6, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ : 8.25-7.95 (m, 9H), 7.55 (s, 1H), 7.21 (t, 1H, J= 5.7 Hz), 6.35 (t, 1H, J = 5.7 Hz), 5.40-5.20 (m, 5H), 4.50-4.30 (m, 9H), 4.15-4.05 (m, 4H), 3.80-3.70 (m, 2H), 3.59-3.54 (m, 2H), 3.50-3.45(m, 9H), 2.85-2.65 (m, 2H), 2.13, 2.09, 2.04, 2.02 (4s, 21H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ : 170.9, 170.2, 170.0, 169.8, 169.5, 169.0, 131.2, 130.9, 130.6, 129.4, 128.4, 128.2, 127.4, 127.2, 126.1, 125.4, 125.2, 125.0, 124.5, 123.0, 101.0, 83.5, 76.0, 73.5, 70.9, 70.6, 70.5, 70.4, 70.1, 69.1, 69.0, 66.5, 62.0, 60.6, 50.0, 41.7, 38.7, 35.0, 30.3, 20.6, 20.5, 20.4, 20.3. HRMS calcd for $C_{57}H_{69}N_5O_{22}SNa$ $[M+Na]^+$: m/z 1230.4053. Found: 1230.4053.

Synthesis of compound Py-PEG-Man-**10**



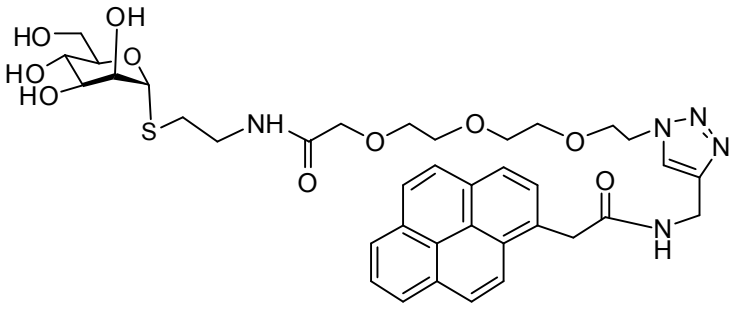
To a solution of the azide PEG-Man-**8** (111 mg, 0.18 mmol) and the alkyne **4** (55 mg, 0.20 mmol) in anhydrous CH_2Cl_2 (2 mL), were added DIPEA (93µL, 0.54mmol) and Bromotris(triphenylphosphine)-cooper(I) (17 mg, 0.02 mmol). The resulting solution was stirred under argon overnight at room temperature. After dilution with dichloromethane (100 mL), washing with 1M HCl (20 mL), saturated aqueous $NaHCO_3$ (40 mL) and brine (20 mL), the organic phase was dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by silica gel chromatography eluting with ethylacetate/methanol (9:1) to give 90 mg of the triazol Py-PEG-Man-**10** as a yellow oil (53% yield): R_f = 0.29 (EtOAc/MeOH 9:1). $[\alpha]_D$ = 28.4 (c 0.5, MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ : 8.30-7.90 (m, 9H), 7.55 (s, 1H), 7.20 (t, 1H, *J* = 6.3 Hz), 6.30 (t, 1H, *J* = 5.9 Hz), 5.40-5.20 (m, 4H), 4.50-4.25 (m, 7H), 4.10 (d, 1H, *J* = 11.8 Hz), 3.94 (s, 2H), 3.76 (t, 2H, *J* = 5.0 Hz), 3.6 (s, 2H), 3.60-3.40 (m, 9H), 2.85-2.65 (m, 2H), 2.15 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 1.97 (s, 3H). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ : 171.1, 170.6, 170.0, 169.9, 169.8, 169.6, 144.5, 131.3, 131.0, 130.8, 129.5, 129.0, 128.5, 128.4, 128.3, 128.2, 127.5, 127.4, 126.2, 125.5, 125.3, 125.1, 124.6, 123.1, 123.0, 82.4, 70.9, 70.8, 70.5, 70.4, 70.3, 70.1, 69.4, 69.2, 69.1, 66.2, 62.4, 50.1, 41.8, 38.0, 35.1, 31.1, 20.9, 20.7, 20.6, 20.4. HRMS calcd for $C_{45}H_{53}N_5O_{14}SNa$ $[M+Na]^+$: m/z 942.3207. Found: 942.3220.

Synthesis of Compound Py-PEG-Lac-**5**



To a solution of Py-PEG-Lac-**9** (50 mg, 0.04 mmol) in dry methanol (1 mL) was added NaOMe (1.7 mg, 0.03 mmol). The reaction was allowed to proceed at rt for 1 h at which time the reaction was judged complete by TLC analysis. A sufficient quantity of Amberlyst Ir-120 (plus) (H^+. form) resin was added to the mixture to render the pH of the solution neutral. The resin was removed by filtration and the solvent removed under vacuum. The crude product was purified by size-exclusion chromatography (sephadex® 20) eluting with methanol. Lyophylization of the solvent gave 40 mg of a white solid Py-PEG-Lac-**5** (80% yield): R_f= 0.62 ($MeCN/H_2O/NH_4OH$ 6:1:1); $[\alpha]_D$ = -4.87 (c 0.9, MeOH). ^1H NMR (500 MHz, MeOD) δ : 8.53 (brs, 1H), 8.29-7.95 (m, 9H), 7.72 (s, 1 H), 4.50-4.30 (m, 9H), 3.90-3.20 (m, 26 H), 2.90-2.60 (m, 2 H). ^{13}C NMR (125.7 MHz, MeOD): δ : 173.9, 172.8, 132.7, 132.1, 132.0, 130.7, 129.7, 128.8, 128.5, 128.2, 127.2, 126.3, 126.2, 1126.1, 126.0, 125.8, 124.6, 105.1, 86.9, 80.5, 80.5, 77.8, 77.1, 74.8, 74.1, 72.5, 71.8, 71.5, 71.3, 71.2, 70.3, 70.2, 62.5, 62.1, 51.4, 50.0, 41.6, 40.4, 35.9, 30.5. HRMS calcd for $C_{43}H_{55}N_5O_{15}SNa$ $[M+Na]^+$: m/z 936.3313. Found: 936.3313.

Synthesis of Compound Py-PEG-Man-**6**



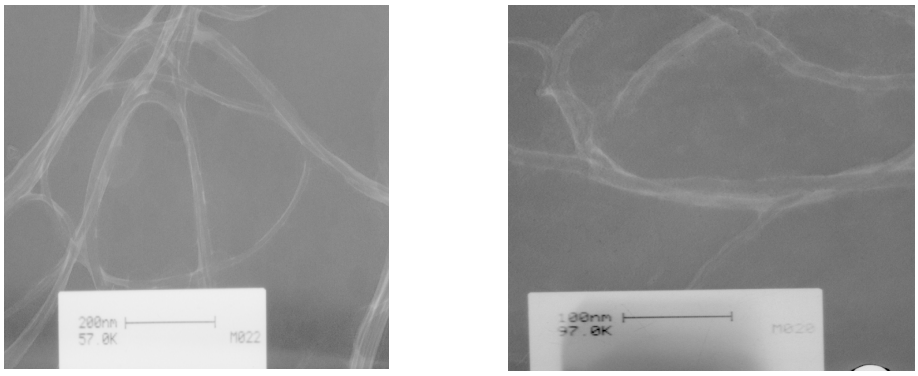
To a solution of Py-PEG-Man-**10** (90 mg, 0.10 mmol) in dry methanol (1 mL) was added NaOMe (2.1 mg, 0.04 mmol). The reaction was allowed to proceed at RT for 1 h at which time the reaction was judged complete by TLC analysis. A sufficient quantity of Amberlyst Ir-120 (plus) (H^+. form) resin was added to the mixture to render the pH of the solution neutral. The resin was removed by filtration and the solvent removed under vacuum. The crude product was purified by size-exclusion chromatography (sephadex® G20) eluting with methanol. Lyophylization of the solvent gave 50 mg of a yellow solid Py-PEG-Man-**6** (56% yield) R_f = 0.33 (CH_2Cl_2/MeOH 9:1); $[\alpha]_D$ = + 43.8 (c 0.6, MeOH). ^{1}H NMR (500 MHz, MeOD) δ : 8.23-7.92 (m, 9H), 7.69 (s, 1H), 5.27 (s, 1H), 4.45 (s, 2H) ,4.41 (t, 2H, *J* = 5.2 Hz), 4.26 (s, 2H), 3.94-3.80 (m, 6H), 3.75-3.60 (m, 6H), 3.45-3.25 (m, 14H), 2.80-2.60 (m, 2H). ^{13}C NMR (125.7 MHz, MeOD) δ : 173.9, 172.8, 146.2, 132.7, 132.1, 131.9, 130.6, 129.4, 128.4, 128.2, 127.4, 127.2, 126.1, 125.4, 125.2, 125.0, 124.5, 86.5, 75.2, 73.6, 73.1, 71.7, 71.1, 71.0, 70.1, 68.9, 62.8, 51.3, 41.5, 39.6, 35.9, 31.4. HRMS calcd for $C_{37}H_{45}N_5O_{10}SNa$ $[M+Na]^+$: m/z 774.2785. Found: 774.2774.

Functionalization of MWNTs and SWNTs with the neoglycolipids Py-PEG-Lac-5 and Py-PEG-Man-6.

In a typical preparation experiment, 0.5 mg of CNTs (Nanoledge, and mer) was suspended in 1 ml of aqueous glycolipid solution. The mixture was sonicated using a water-bath sonicator for 1 h, then was incubated all the night, after that the mixture was sonicated another 30 mins. The insoluble material was removed by centrifugation.

TEM characterization of the aggregates.

TEM images of glycolipid-coated CNTs were obtained on a Philips CM-200 operating at electron energy of 200 keV and magnification of 10-300 thousand times. Samples were prepared by depositing 15 µl of the suspension onto grids, allowing the grids to absorb for 2 mins.

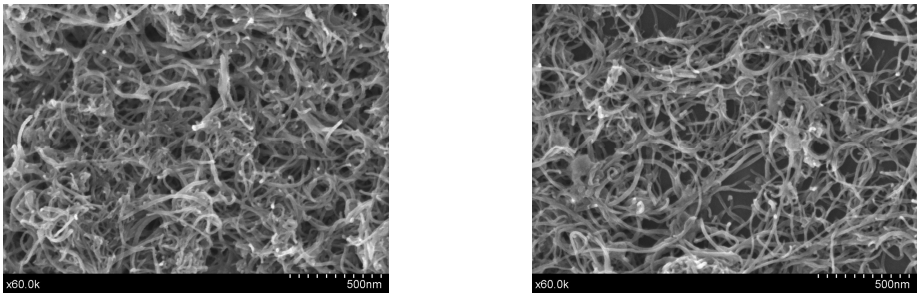


a. **b.**

Figure S1: a. TEM image MWCNTs-Py-PEG-Man-**6**, **b.** TEM image SWCNTs-Py-PEG-Man-**6**.

SEM Characterization.

SEM images of glycolipid-coated CNTs were obtained on a JEOL JSM-5400 magnification of 15 thousand times. Samples were prepared by depositing 15 µl of the suspension onto grids, allowing the grids to absorb for 2 mins.



a. **b.**

Figure S2: a. SEM image of MWCNTs-Py-PEG-Lac-**5**, **b.** SEM image of MWCNTs-Py-PEG-Man-**6**

Lectin binding assay.

A 1 mL solution of PNA-FITC (100 μg/mL) in buffer (0.10 M Tris, and 0.15 M NaCl, pH 8.0) was added to the suspensions of glycolipid-coated nanotubes in H_2O (1 mL). An additional 0.5 mL buffer was added and the reactions were incubated all the night at rt in the dark. Lactose inhibition of PNA binding to glycolipid-CNTs was tested by pre-incubating 1 mL of PNA-FITC solution in buffer (100 μg/mL) with 0.5 ml of lactose in buffer (500 mg/mL) for 1h at rt in the dark. This pre-incubated solution was added to the suspension of glycolipid-CNTs in H_2O (1 mL), and the resulting solution was incubated all the night at rt in the dark, as described above. After incubation, the solutions were centrifuged three times for removing the unreacted lectins. The centrifuged solutions were analyzed at 510-550 nm using a fluorescence microplate reader (excitation wavelength 492 nm).