Electronic Supplementary Material (ESI) for Chemical Communications
This journal is © The Royal Society of Chemistry 2012

Electronic Supplementary Information

Tunable, Dynamic and Electrically Stimulated Lectin-Carbohydrate Recognition on a Glycan-Grafted Conjugated Polymer

Shyh-Chyang Luo,[a] Eric Assen B. Kantchev,[*,b] Bo Zhu,[a] Yuen Wee Siang,[b] and Hsiao-hua Yu[*,a]

[a]Yu Initiative Research Unit, RIKEN Advanced Science Institute, 2-1 Hirosawa, Wako, Saitama, 351-0198, Japan, [b] Institute of Bioengineering and Nanotechnology, 138669, Singapore

Experimental Section

Materials and Reagents. Lithium perchlorate ($LiClO_4$) was purchased from Fluka, sodium dodecyl sulfate (SDS) from Alfa Aesar, bovine serum albumin (BSA) from Wako Pure Chemical Industries, BES buffer, Concanavalin A (Con A) and α-methyl mannoside from Sigma-Aldrich. All remaining reagents were also commercial unless specified otherwise. Hydroxymethyl-functionalized EDOT (EDOT-OH) and triethylene glycol-functionalized EDOT were synthesized according to the literature procedure.[1] Anhydrous solvents were purchased from Sigma-Aldrich in a sure-seal bottle, and introduced in the reaction flask under Ar using standard vacuum/inert gas manifold techniques. All other solvents were purchased from J. T. Baker (Phillipsburg, NJ). Deuterated solvents were purchased from Sigma-Aldrich or Cambridge Isotope Laboratories, Inc. All reagents and solvents were used without further purification, unless otherwise indicated. ^{1}H and ^{13}C NMR data were acquired at 25°C with a Bruker AV 400 spectrometer. Flash chromatography was performed on CombiFlash Companion or Rx16 on normal phase Silicagel cartridges. MS was carried out on a Finnigan/MAT LCQ Mass Spectrometer (ThermoFinnigan, San Jose, CA) fitted with an FAB probe. Indium tin oxide (ITO)-coated glass (Delta-Technologies, Ltd.) was cleaned by standard procedure prior to use. Au-coated crystal was cleaned by piranha solution before use.

Electropolymerization and Film Syntheses. The details of PEDOT thin film preparation is described previously.[2] PEDOT films from different functional EDOT monomers were electropolymerized on Au-coated crystal and ITO-coated glass from 10 mM of EDOT aqueous solutions containing 0.1 M of $LiClO_4$ as supporting electrolyte in the presence of 1 mM of HCl and 0.05 M of SDS by applying cy-

clic potentials (0 to 1.1 V *vs.* Ag/AgCl at a scan rate of 100 mV/s). A thin poly(**EDOT-OH**) film was deposited on ITO-coated first to enhance the adhesion of poly(**EDOT-Man**) and poly(**EDOT-EG3**) film for all experiments.

Quartz Microbalance Crystal Measurement. QCM experiment was conducted by a Q-Sense E4 (Q-Sense) system. The details of this technique are described elsewhere.[3] PEDOT was electropolymerized directly on Au-coated crystals with 14 mm diameter. Measurement was conducted at a fundamental frequency of 4.95 MHz. For test of Con A binding at high concentration (100 mg/mL), Con A was dissolved in 20 mM BES buffer containing 1 M NaCl and 1 mM $MnCl_2$ and $CaCl_2$. For test of Con A binding at low concentration (1 mg/mL), Con A was dissolved 1 mg/mL in 0.1 M Tris-buffer containing 1 M NaCl and 1 mM $MnCl_2$ and $CaCl_2$. 100 mM α-methyl mannoside and 100 mg/mL bovine serum albumin solutions were prepared by using the same buffered solutions. Solutions were conducted by using a microprocessor controlled dispensing pump (IPC-4, Ismatec) at a flow rate of 50 μL/min. Electrochemical experiment was conducted by using a electrochemical module of QCM-D system (QEC 401) and a potentiostat (PGSTAT128N, Autolab) with a Ag/AgCl electrode as reference electrode.

Surface Analysis. The surface morphology of polymer films was examined by using with atomic force microscopy (AFM). AFM was performed in the tapping mode at room temperature in air with BioScope Digital Instruments. Root-Mean-Square roughness (R_{rms}) was obtained by Nanoscope software under scan range at 10 × 10 μm.

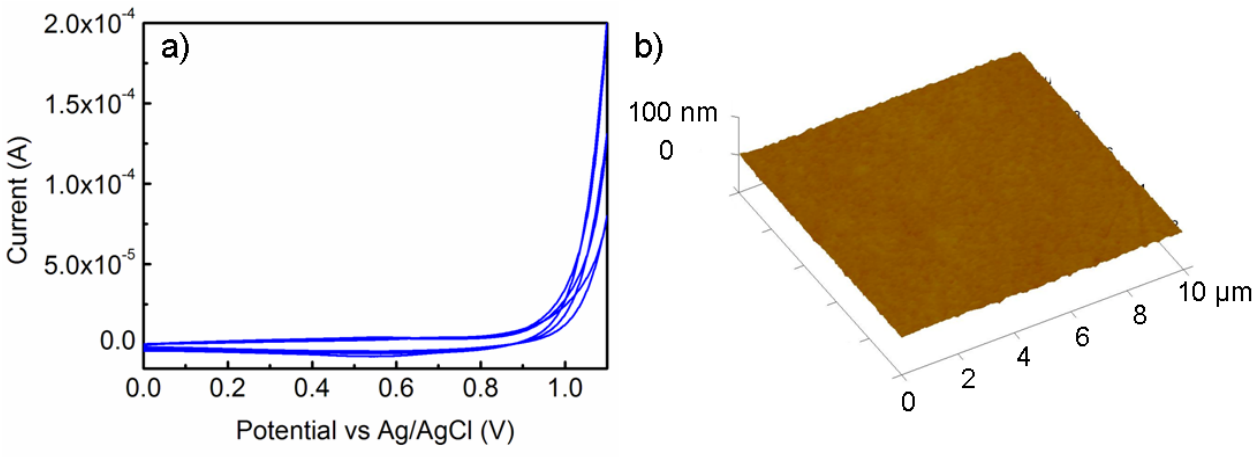


Figure S1. a) Electropolymerization curve and b) surface morphology from AFM for poly(**EDOT-Man**).

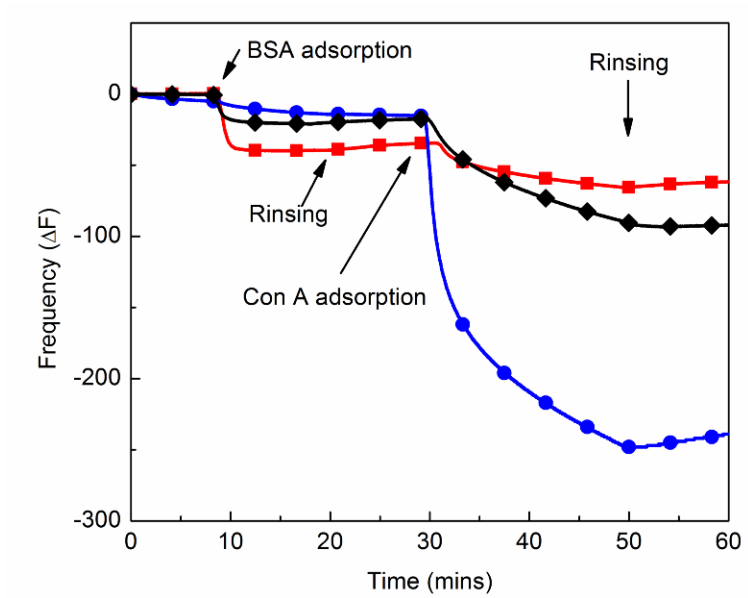


Figure S2. Real-time monitoring of BSA (1 mg/ml) and Con A (100 mg/ml) binding on Au (black/diamond), poly(**EDOT-OH**) (red/square), and poly(**EDOT-Man**) (blue/circle) by QCM-D.

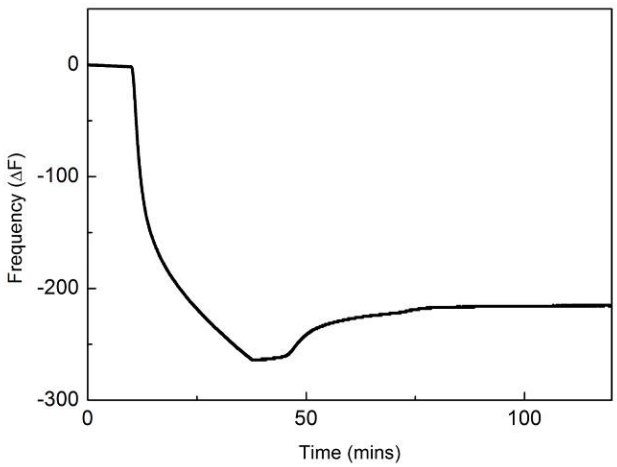


Figure S3. Real-time monitor of Con A binding on poly(**EDOT-OH**)-*co*-poly(**EDOT-Man**) films by using a QCM-D. Adding of Con A at 10 min, rinsing at 70 min, and 100 mM methyl-α-D-mannoside at 90 mins.

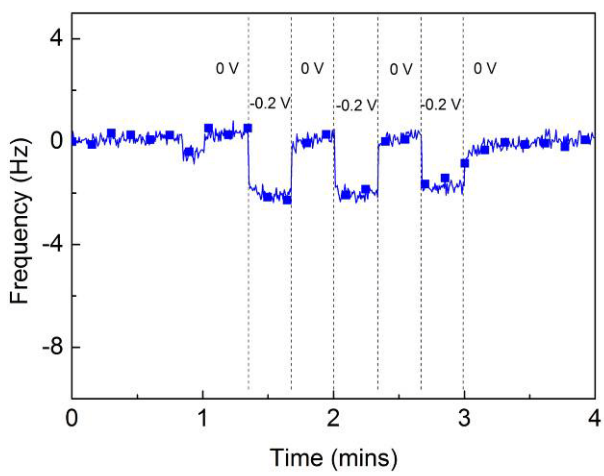


Figure S4. Real-time monitor of electric pulse effect on poly(**EDOT-EG3**)-*co*-(**EDOT-Man**) films by using a QCM-D with electrochemical modulus.

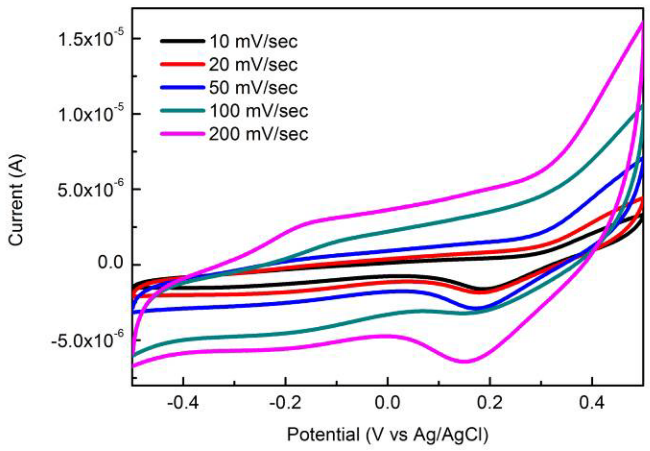


Figure S5. Cyclic voltammetry scan of poly(**EDOT-EG3**)-*co*-(**EDOT-Man**) films in 0.1 M Tris-buffer containing 1 M NaCl and 1 mM $MnCl_2$ and $CaCl_2$.

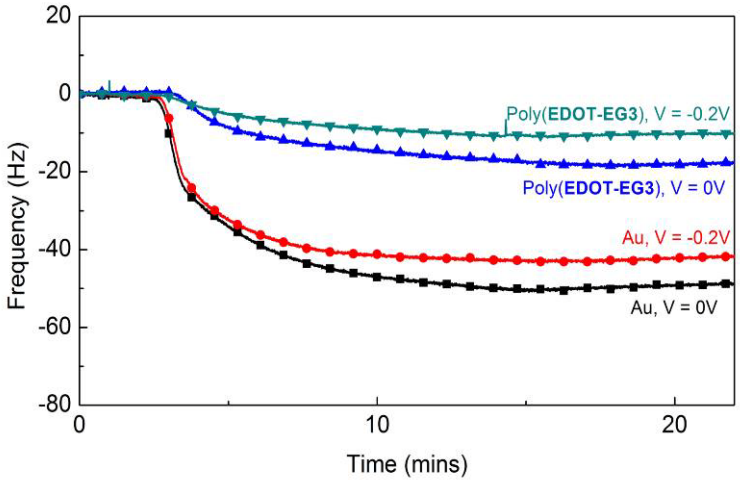


Figure S6. Real-time monitor of Con A binding (1 mg/ml) on bare Au surfaces and poly(**EDOT-EG3**) films by using a QCM-D when 0 V and -0.2 V *vs.* Ag/AgCl applied.

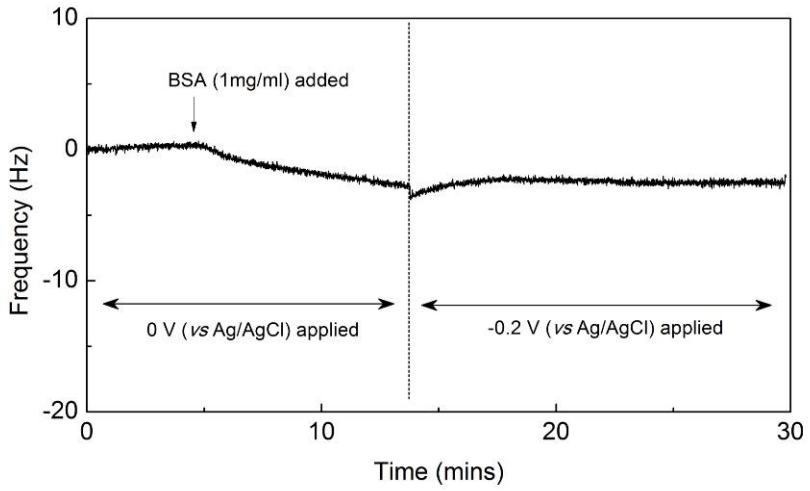


Figure S7. Real-time monitor of BSA binding (1 mg/ml) on poly(**EDOT-EG3**)-*co*-(**EDOT-Man**) films by using a QCM-D when 0 V and -0.2 V *vs.* Ag/AgCl applied.

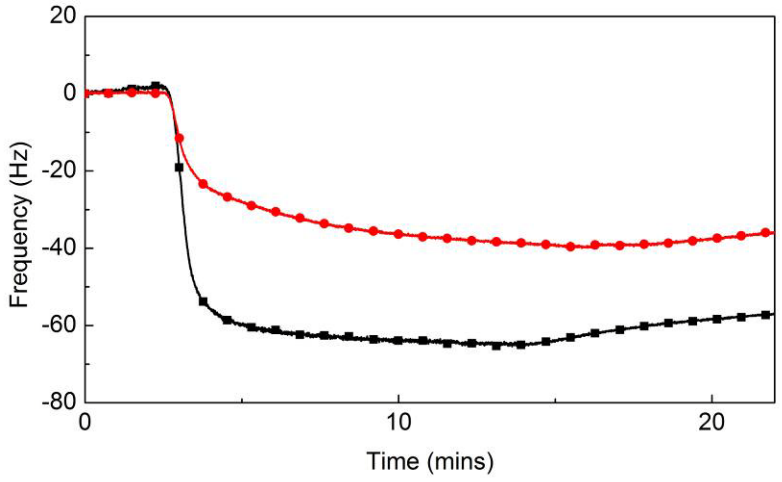


Figure S8. Real-time monitor of Con A binding (1 mg/ml) on poly(**EDOT-EG3**)-*co*-(**EDOT-Man**) films by using a QCM-D without adding 1 mM glucose (Black) and with adding 1 mM glucose (Red).

Synthetic procedures and characterization data

Scheme S1. Conditions: a) TrCl, pyridine, neat, RT, 18 h. b) MsCl, Et_3N, CH_2Cl_2, 0 °C → RT, 18 h. c) EDOT-OH (**2**), cat. NaI, NaH, DMF, RT, 18 h. d) Amberlite IR-120 (H^+ form), methanol, 60 °C, 6 h. e) **4** (1.2 equiv), $BF_3{\cdot}OEt_2$ (30 mol%), CH_2Cl_2, RT, 3 h. f) NH_3 in MeOH (2M), RT, 18 h.

2-(2-(2-(trityloxy)ethoxy)ethoxy)ethyl methanesulfonate (1). In a 100-mL round bottomed flask, triyl chloride (5.58 g, 20 mmol) were dissolved in a mixture of triethyleneglycol (13.3 mL, 15.02 g, 100 mmol) and CH_2Cl_2 (25 mL). Pyridine (1.6 mL, 1.58 g, 20 mmol) were added and the mixture was stirred overnight, then transferred into a separatory funnel with CH_2Cl_2 (50 mL) and washed with water (4 × 100 mL). The organic phase was dried ($MgSO_4$) and the volume was reduced to ~ 50 mL under reduced pressure. Et_3N (5.0 mL, 3.64 g, 36 mmol) were added and the mixture was cooled to 0 °C (ice bath). Next, methylsulfonyl chloride (MsCl) (2.3 mL, 3.44 g, 30 mmol) were added dropwise with vigorous stirring, the mixture was allowed to warm up to room temperature and stirred overnight. The excess MsCl was quenched by addition of water (50 mL) and stirring over 30 min, transferred into a separatory funnel with CH_2Cl_2 (30 mL) and washed successively with water and satd. $NaHCO_3$ solution (50 mL each). The organic phase was dried ($MgSO_4$) and the solvent removed under reduced pressure. Column chromatography on Silicagel (120 g Combiflash cartridge, ethyl acetate in hexane gradient: 10 to 40% over 20 min) afforded **1** (7.68 g, 82%) as colorless viscous liquid. The ^{1}H NMR spectrum is consistent two slow-interconverting conformational isomers: ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.47 (m, 3H),

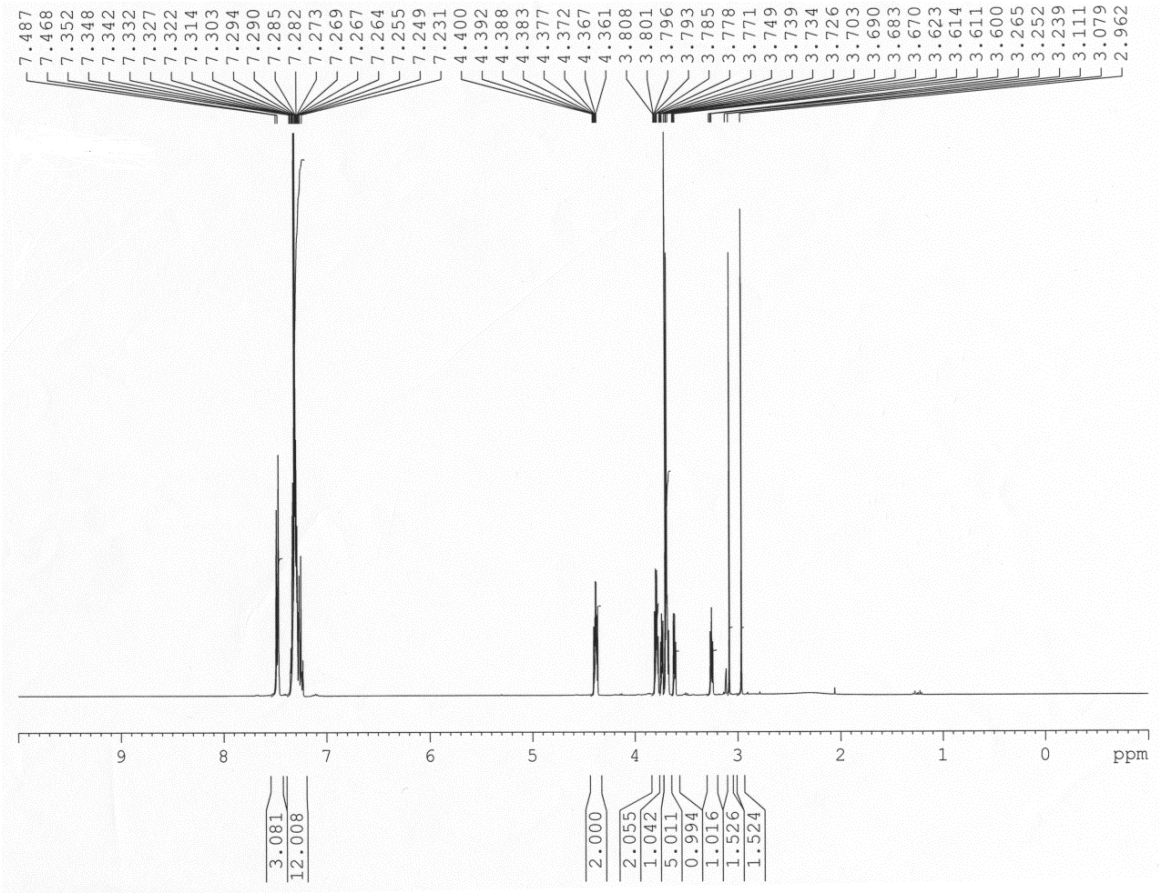
7.35-7.23 (m, 2H), 4.38 (m, 2H), 3.78 (m, 2H), 3.73 (m, 2H), 3.72-3.68 (m, 5H), 3.61 (m, 1H), 3.25 (t, *J* = 5.2 Hz, 1H), 3.08 (s, 1.5H), 3.00 (s, 1.5H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 146.9, 144.0, 128.7, 127.9, 127.8, 127.3, 127.0, 86.6, 82.0, 72.5, 70.8, 70.7 (3 carbons), 70.3, 69.3, 69.1, 69.0 (2 carbons), 63.3, 61.7, 37.6, 31.5. HR-MS (FAB): calcd. for $C_{26}H_{31}O_6{}^{32}S$ ([M+H] $^+$): 471.1841; found 471.1846.

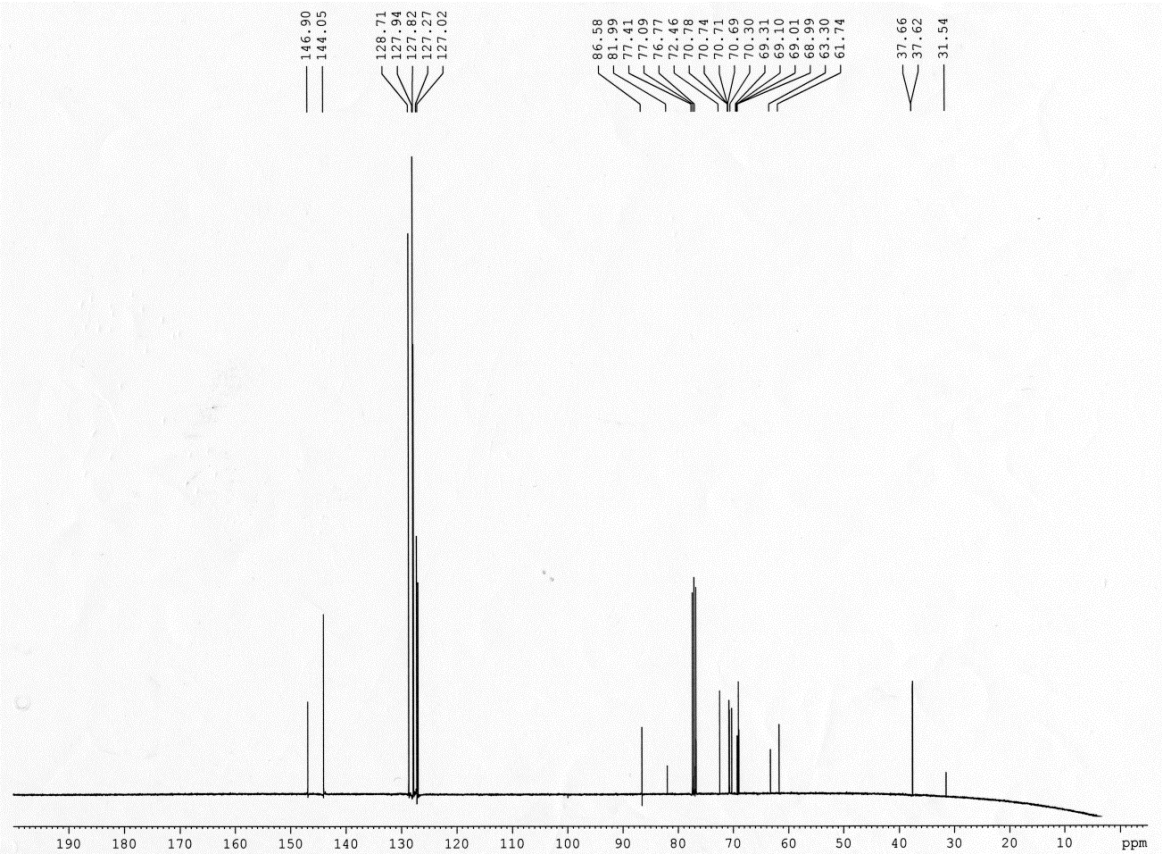
2-(2-(2-((2,3-dihydrothieno[3,4-*b*][1,4]dioxin-2-yl)methoxy)ethoxy)ethoxy)ethanol (3). A 100-mL round-bottomed flask charged with a magnetic stirrer bar, **2** (2.76 g, 16 mmol) and NaI (0.24 g, 1.6 mmol) was capped with a rubber septum and backfilled with Ar (3 ×). Next, dry DMF (20 mL) were added via syringe followed by careful addition of NaH (60% in mineral oil; 0.96 g, 24 mmol) against a weak back stream of Ar (CAUTION! Vigorous evolution of a highly flammable gas, H_2). The reaction mixture was stirred under Ar for 15 min, followed by addition of a solution of **1** (7.55 g, 16 mmol) in dry DMF (15 mL). The reaction mixture was stirred under Ar over 16 h, transferred into a separatory funnel with CH_2Cl_2 (100 mL) and washed with H_2O (4 × 250 mL). The organic layer was dried ($MgSO_4$) and the solvents removed under reduced pressure followed by a brief drying in high vacuum. The residue was mixed with MeOH (200 mL) and Amberlite IR-120 (H^+ form, washed with copious volumes of MeOH and Et_2O and thoroughly dried before use; 20 g) were added. The solution was stirred at 60 C over 6 h, the resin was filtered and the solvents removed under reduced pressure followed by thorough drying in high vacuum. Column chromatography (80 g Combiflash cartridge, ethyl acetate gradient in hexane: 50 to 100% over 20 min) afforded **5** (4.16 g, 85%) as a viscous colorless liquid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 6.33 (dd, *J* = 5.6, 4.0 Hz, 2H), 4.33 (m, 1H), 4.26 (dd, *J* = 11.6, 2.4 Hz, 1H), 4.07 (dd, *J* = 11.6, 4.4 Hz, 1H), 3.79-3.65 (m, 12H), 3.61 (m, 2H), 2.82 (broad s, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 141.5, 141.4, 99.7, 99.6, 72.6 (2 carbons), 71.1, 70.7, 70.5 70.3, 69.6, 66.1, 61.7, 60.4. HR-MS (FAB): calcd. for $C_{13}H_{21}O_6{}^{32}S$ ([M+H] $^+$): 305.1053; found 305.1047.

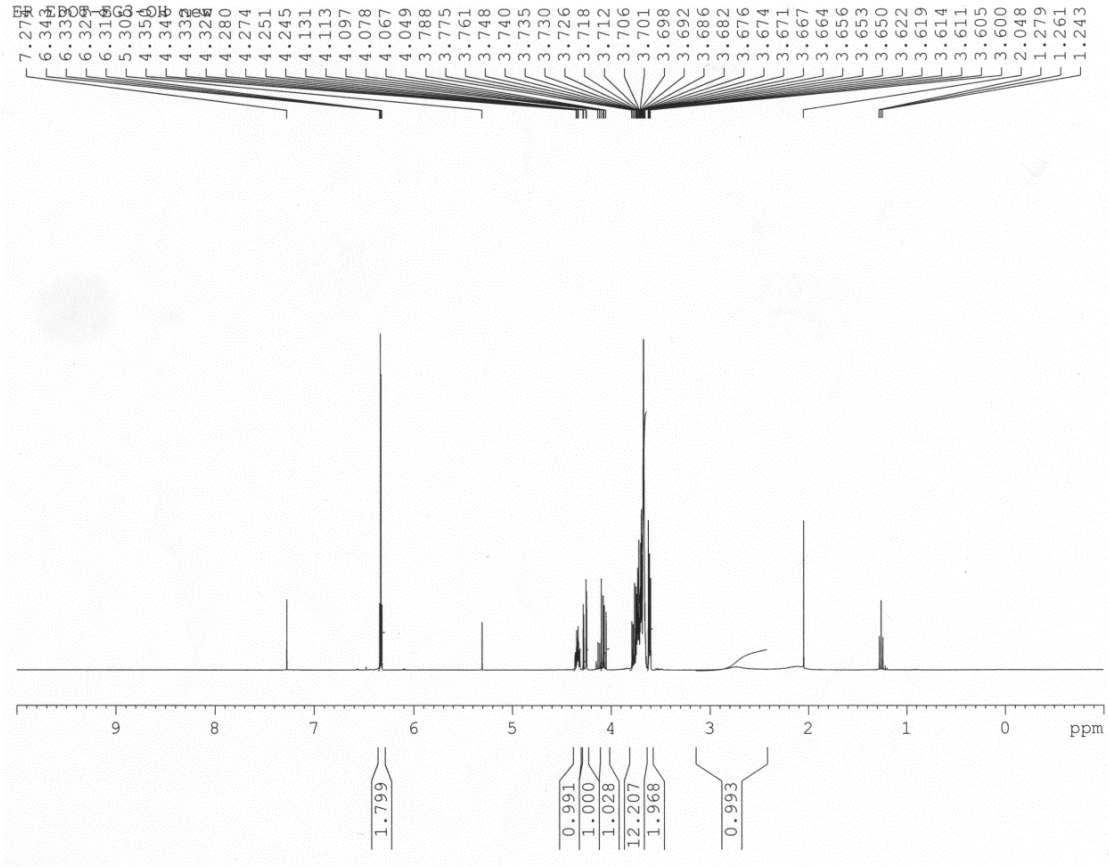
1-*O*-(2-(2-(2-((2,3-dihydrothieno[3,4-*b*][1,4]dioxin-2-yl)methoxy)ethoxy)ethoxy)ethoxy))-2,3,4,6-*O*,*O*,*O*,*O*-tetraacetyl-α-*D*-mannoside (5). In a 25-mL round-bottomed flask charged with a magnetic

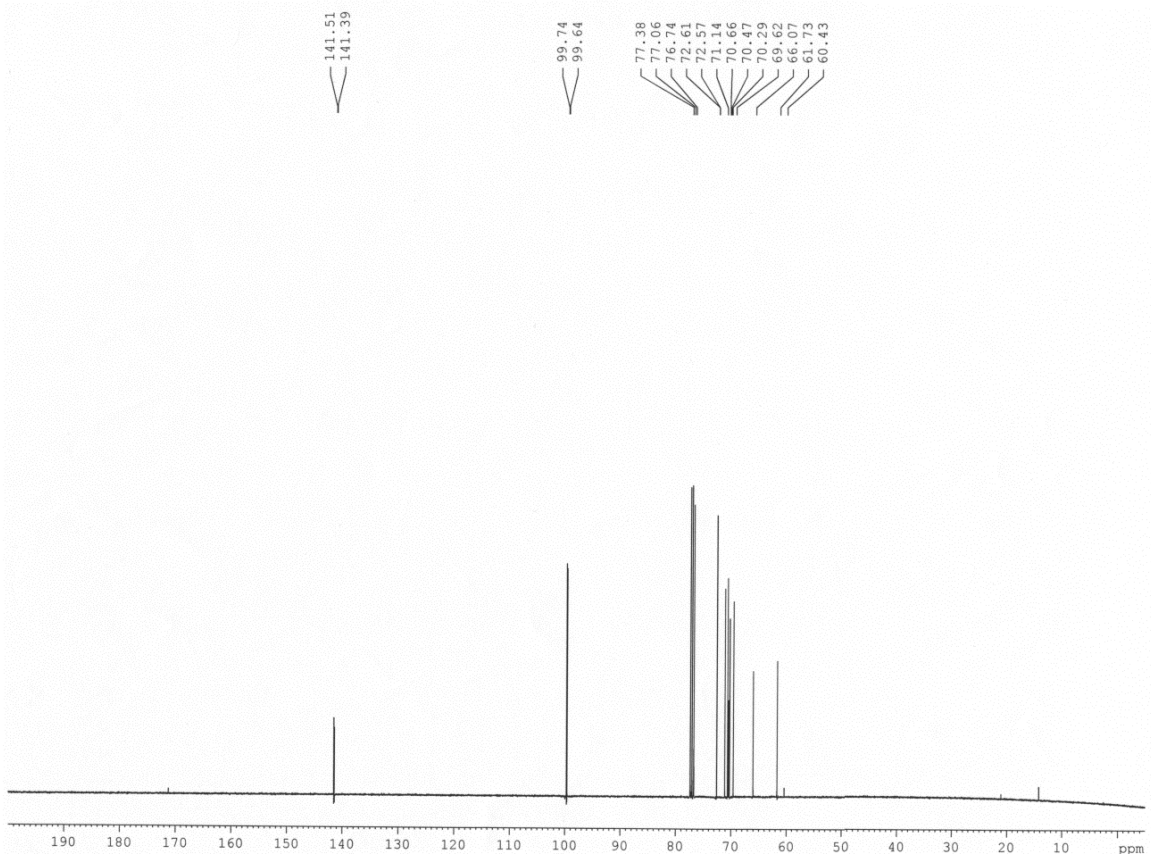
stirrer bar, **3** (609 mg, 2.0 mmol) and **4**[4] (1.18 g, 2.4 mmol) were dried in high vacuum over P_2O_5 for 2 h. The flask vas backfilled with Ar and dry CH_2Cl_2 (4.0 mL) were introduced via syringe. Next, $BF_3{\cdot}OEt_2$ (75 μL, 86 mg, 0.60 mmol) were added and the mixture was stirred at room temperature over 3 h. Next, the mixture was partitioned between CH_2Cl_2 (50 mL) and water (50 mL), the organic layer washed with sat. $NaHCO_3$ solution (2 × 30 mL), brine (30 mL), dried ($MgSO_4$) and the solvent removed under reduced pressure. Column chromatography (40 g Combiflash cartridge, ethyl acetate gradient in hexane: 30 to 100% over 28 min) afforded **5** (856 mg, 67 %) a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 6.31 (dd, *J* = 4.4, 4.0 Hz, 2H), 5.37-5.26 (m, 4H), 4.87 (d, *J* = 1.2 Hz, 1H), 4.35-4.23 (m, 3H), 4.10-4.03 (m, 2H), 3.83-3.74 (m, 2H), 3.71-3.63 (m, 12H), 2.14 (s, 3H), 2.10 (s, 3H), 2.03 (s, 3H), 1.99 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 170.7, 170.1, 169.9, 169.7, 141.6, 141.5, 99.7, 99.6, 97.7, 77.4, 77.1, 76.8, 72.6, 71.2, 70.7 (2 carbons), 70.5 70.0, 69.6, 69.5, 69.1, 68.3, 67.4, 66.1, 62.4, 60.4, 20.9, 20.8, 20.7 (2 carbons). HR-MS (FAB): calcd. for $C_{27}H_{39}O_{15}{}^{32}S$ ([M+H] $^+$): 635.2004; found 635.2006.

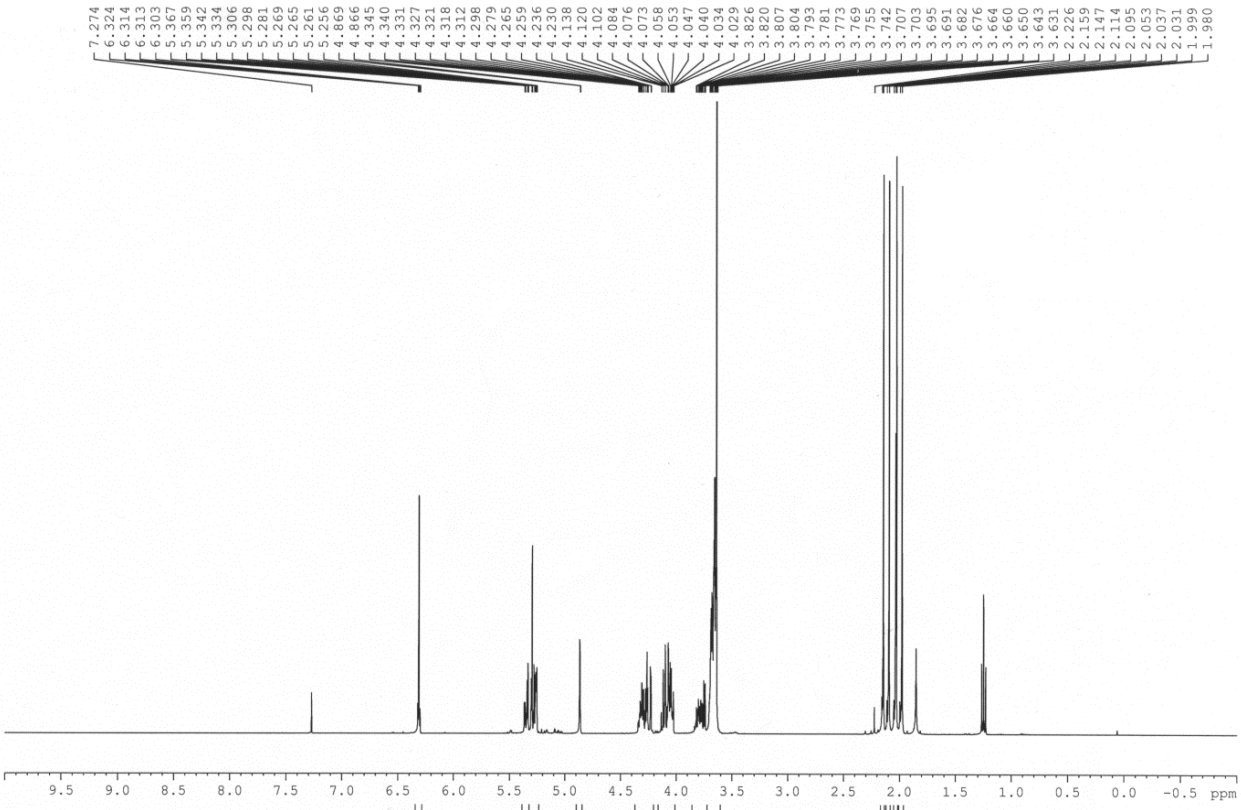
1-*O*-(2-(2-(2-((2,3-dihydrothieno[3,4-*b*][1,4]dioxin-2-yl)methoxy)ethoxy)ethoxy)ethoxy))-2,3,4,6-*O,O,O,O*-tetraacetyl-α-*D*-mannoside (6). Into a 50-mL round-bottomed flask charged with a magnetic stirrer bar, **5** (761 mg, 1.2 mmol) and a solution of NH_3 in anhydrous MeOH (2M; 6 mL, 12 mmol) were added in succession and the mixture stirred overnight. The solvent was evaporated to dryness and the residue was dried in high vacuum affording **6** (565 mg, 100%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 6.58 (m, 2H), 4.72 (t, *J* = 6.0 Hz, 2H), 4.62 (d, *J* = 1.2 Hz, 1H), 4.56 (d, *J* = 6.0 Hz, 1H), 4.45 (t, *J* = 6.0 Hz, 1H), 4.30 (m, 1H), 4.24 (dd, *J* = 12.0, 2.4 Hz, 1H), 3.97 (dd, *J* = 12.0, 4.4 Hz, 1H), 3.71-3.27 (m, 17H), 3.35 (broad s, 4H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ (ppm) 141.8 (2 carbons), 100.4, 100.2, 100.1, 74.4, 72.9, 71.4, 70.8, 70.7, 70.3, 70.2 (2 carbons), 69.9, 69.3, 67.3 66.1, 65.9, 61.7. HR-MS (FAB): calcd. for $C_{19}H_{31}O_{11}{}^{32}S$ ([M+H] $^+$): 467.1587; found 467.1581.

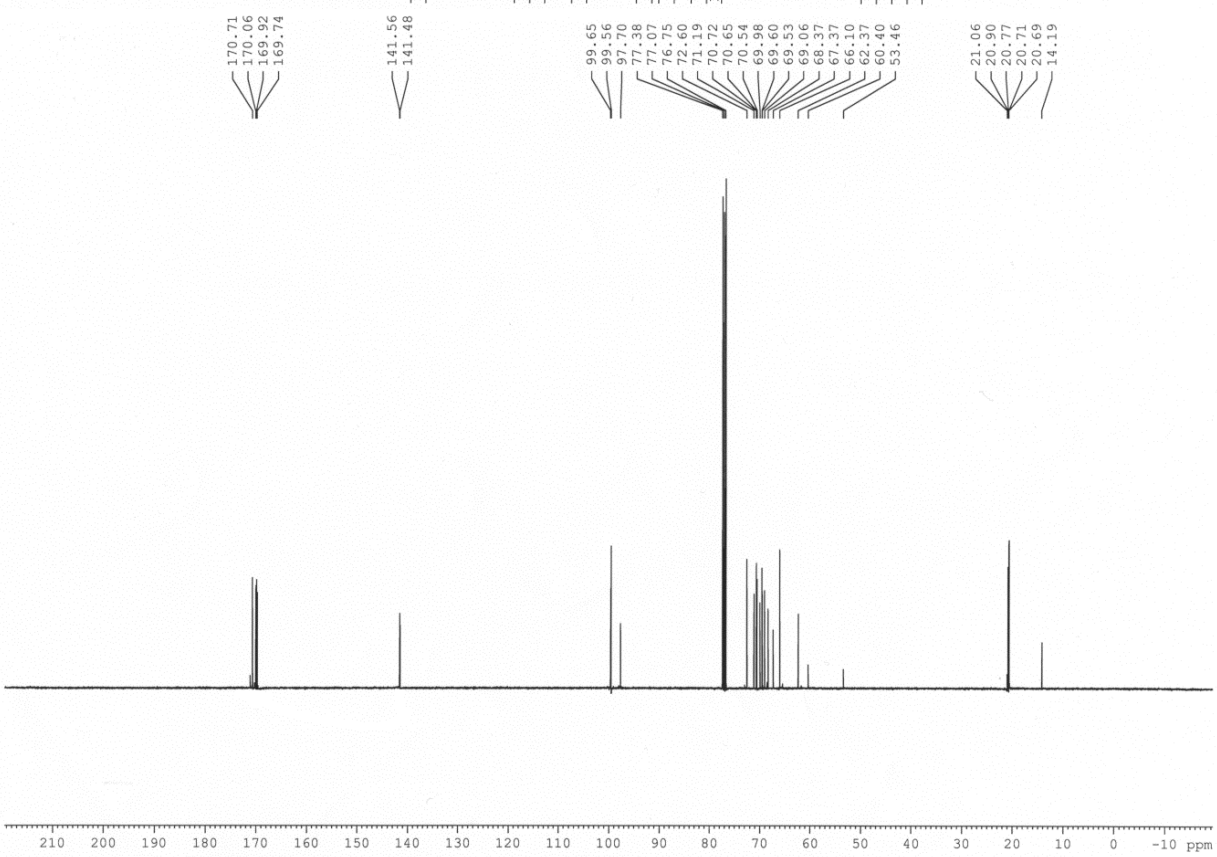

7.487
7.468
7.352
7.348
7.342
7.332
7.327
7.322
7.314
7.303
7.294
7.290
7.285
7.282
7.273
7.269
7.267
7.264
7.255
7.249
7.231
4.400
4.392
4.388
4.383
4.377
4.372
4.367
4.361
3.808
3.801
3.796
3.793
3.785
3.778
3.771
3.749
3.739
3.734
3.726
3.703
3.690
3.683
3.670
3.623
3.614
3.611
3.600
3.265
3.252
3.239
3.111
3.079
2.962
9
8
7
6
5
4
3
2
1
0
ppm
3.081
12.008
2.000
2.055
1.042
5.011
0.994
1.016
1.526
1.524

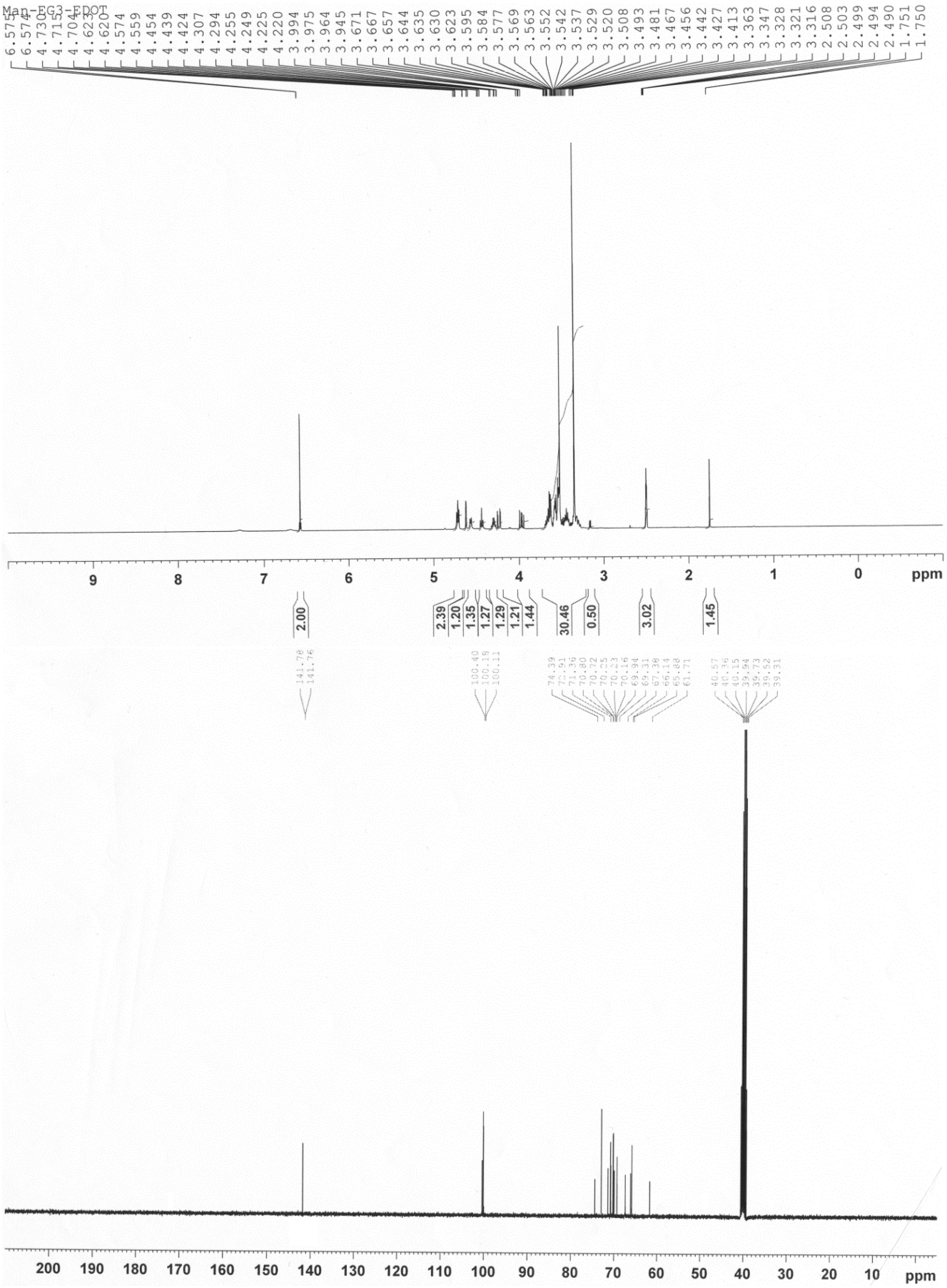

146.90
144.05
128.71
127.94
127.82
127.27
127.02
86.58
81.99
77.41
77.09
76.77
72.46
70.78
70.74
70.71
70.69
70.30
69.31
69.10
69.01
68.99
63.30
61.74
37.66
37.62
31.54
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


7.279
6.341
6.331
6.321
6.311
5.305
4.357
4.347
4.337
4.327
4.280
4.274
4.251
4.245
4.131
4.113
4.097
4.078
4.067
4.049
3.788
3.775
3.761
3.748
3.740
3.735
3.730
3.726
3.718
3.712
3.706
3.701
3.698
3.692
3.686
3.682
3.676
3.674
3.671
3.667
3.664
3.656
3.653
3.650
3.622
3.619
3.614
3.611
3.605
3.600
2.048
1.279
1.261
1.243
9
8
7
6
5
4
3
2
1
0
ppm
1.799
0.991
1.000
1.028
12.207
1.968
0.993


141.51
141.39
99.74
99.64
77.38
77.06
76.74
72.61
72.57
71.14
70.66
70.47
70.29
69.62
66.07
61.73
60.43
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


7.274 6.324 6.314 6.313 6.303 5.367 5.359 5.342 5.334 5.306 5.298 5.281 5.269 5.265 5.261 5.256 4.869 4.866 4.345 4.340 4.331 4.327 4.321 4.318 4.312 4.298 4.279 4.265 4.259 4.236 4.230 4.138 4.120 4.102 4.084 4.076 4.073 4.058 4.053 4.047 4.040 4.034 4.029 3.826 3.820 3.807 3.804 3.793 3.781 3.773 3.769 3.755 3.742 3.707 3.703 3.695 3.691 3.682 3.676 3.664 3.660 3.650 3.643 3.631 2.226 2.159 2.147 2.114 2.095 2.053 2.037 2.031 1.999 1.980
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 ppm
2.00
1.30
3.02
1.18
3.65
4.37
2.53
13.19
3.38
3.56
4.57
3.51


170.71 170.06 169.92 169.74
141.56 141.48
99.65 99.56 97.70
77.38 77.07 76.75 72.60 71.19 70.72 70.65 70.54 69.98 69.60 69.53 69.06 68.37 67.37 66.10 62.37 60.40 53.46
21.06 20.90 20.77 20.71 20.69 14.19
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 ppm


Man-EG3-EDOT
6.575
6.574
4.730
4.715
4.704
4.623
4.620
4.574
4.559
4.454
4.439
4.424
4.307
4.294
4.255
4.249
4.225
4.220
3.994
3.975
3.964
3.945
3.671
3.667
3.657
3.644
3.635
3.630
3.623
3.595
3.584
3.577
3.569
3.563
3.552
3.542
3.537
3.529
3.520
3.508
3.493
3.481
3.467
3.456
3.442
3.427
3.413
3.363
3.347
3.328
3.321
3.316
2.508
2.503
2.499
2.494
2.490
1.751
1.750
9 8 7 6 5 4 3 2 1 0 ppm
2.00
2.39
1.20
1.35
1.27
1.29
1.21
1.44
30.46
0.50
3.02
1.45
141.78
141.76
100.40
100.15
100.11
74.39
72.91
71.36
70.80
70.72
70.25
70.23
70.16
69.94
69.31
67.38
66.14
65.18
61.71
40.57
40.36
40.15
39.94
39.73
39.52
39.31
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

References:

1. Preparation of **2**: Lima, A.; Schottland, P.; Sadki, S.; Chevrot, C. *Synth. Met.* **1998**, *93*, 33.
2. Luo, S. -C.; Ali, E. M.; Tansil, N. C.; Yu, H. -h.; Gao, S.; Kantchev, E. A. B.; Ying, J. Y. *Langmuir* **2008**, *24*, 8071.
3. Rogahl, M.; Höök, F.; Krozer, A.; Brzezinski, P.; Kasemo, B. *Rev. Sci. Instrum.* **1995**, 66, 3924.
4. Preparation of **4**: Tosin, M; Murphy, P. V.; *J. Org. Chem.* **2005**, *70*, 4107.