A bis-boronic acid modified electrode for the sensitive and selective determination of glucose concentrations

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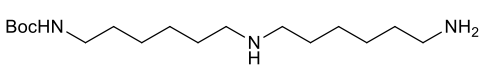
General

Commercially available solvents and reagents were used without further purification. ^{1}H NMR spectra were recorded at 300 MHz or 250 MHz on Bruker AVANCE 300 or Brucker AVANCE 250 NMR spectrometers respectively, ^{13}C NMR spectra at 75 MHz or 63 MHz on Bruker AVANCE 300 or Bruker AVANCE 250 NMR spectrometers respectively and are proton decoupled and ^{11}B NMR spectra were recorded at 96 MHz on a Bruker AVANCE 300 NMR spectrometer and are proton decoupled all spectra were recorded at room temperature unless otherwise stated, data was processed with Mestrec version 5.2.5-4731 and Topspin 2.0 (Version of: Nov 9th 2006). Chemical shifts (δ) are reported in ppm relative to TMS (δ 0.00) for the ^{1}H NMR and to chloroform (δ 77.0) for the ^{13}C NMR measurements, coupling constant *J* are expressed in Hertz. Mass spectra were recorded Bruker Daltonics MicroTOF Mass Spectrometer.

Experimental Procedures

Synthesis of tert-*butyl (6-((6-aminohexyl)amino)hexyl)carbamate (3)*



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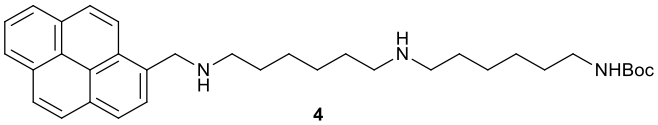
Bis(hexamethylene)triamine **2** (2.15 g, 0.01 mol) was dissolved in MeOH then cooled in an ice bath. $(Boc)_2O$ (0.436 g, 0.002 mol) in MeOH was added slowly into the solution at 0 °C then the mixture was

stirred continuously whilst warming from 0 °C to room temperature over 2 hours (monitoring by TLC). When the reaction finished, methanol was evaporated and water was added into the mixture. The pH of the mixture was adjusted to pH 7 and extracted with dichloromethane to remove the unwanted double addition adduct. Then the pH was adjusted (pH 10) and extracted with dichloromethane to give the title compound (**3**) as a white solid (0.543 g, 86%); mp 146-148 ^{o}C; IR (cm^{-1}) 1169, 1524, 1686, 2789, 2859, 2925, 3361; Acc Mass ESI [M+H] $C_{17}N_{38}N_3O_2$ calc 316.2964 found 316.2958; ^{1}H NMR (300 MHz, methanol-d_4, ppm) δ 1.37-1.50 (m, 21H), 1.64-1.65 (m, 4H), 2.85 (t, *J* = 6, 6H, -CH_2-N), 3.03 (t, *J* = 6, 2H, -CH_2-N-Boc); ^{13}C NMR (75 MHz, methanol-d_4, ppm) δ 27.58, 27.81, 27.85, 27.95, 28.45, 28.61, 29.26(3C), 30.13, 31.18, 41.47, 41.56, 49.88(2C), 80.18, 158.95.

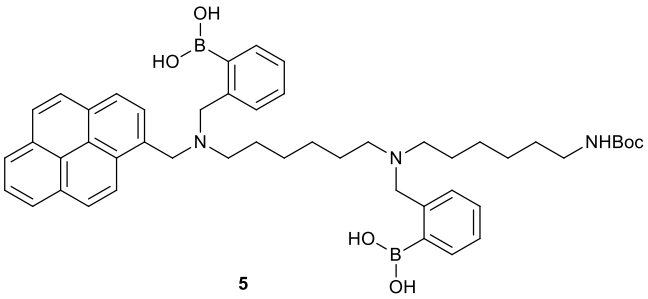
Synthesis of tert-*butyl (6-((6-((pyren-1-ylmethyl)amino)hexyl) amino)-hexyl)carbamate (4)*



4

Compound **3** (0.67g, 1.81 mmol) and 1-pyrenecarbocaldehyde (0.416 g, 1.81 mmol) were dissolved in THF and methanol (50 mL of a 1:1 mixture) and heated at reflux for 2 hours. After inspection of the crude NMR spectrum confirmed that the corresponding imine had formed. Sodium borohydride (0.068 g, 1.81 mmol) was added, at room temperature, and the reaction stirred for further 2 hours. After the reaction was completed, unreacted $NaBH_4$ was quenched by addition of water. Volatile organic solvents were removed *in vacuo*, the aqueous residue was exacted with dichloromethane, dried over Na_2SO_4 and purified by flash column chromatography (DCM, DCM / MeOH 20:1, 10:1, 8:1) to afford the title compound (**4**) as a yellow powder (0.527 g, 55%). Mp 229 °C (dec); IR (cm^{-1}) 848, 1171, 1527, 1678, 2794, 2862, 2928, 3350; Acc Mass ESI [M+H] $C_{34}H_{48}N_3O_2$ calculated 530.3747 found 530.3763; ^{1}H NMR (250 MHz, $CDCl_3$, ppm) δ 1.21-1.46 (m, 25H), 2.44-2.47 (m, 4H), 2.69 (t, *J* = 7, 2H), 3.02-3.05 (m, 2H), 4.35 (s, 2H), 7.89-8.11 (m, 8H), 8.23 (d, *J* = 9.25, 1H); ^{13}C NMR (62 MHz, $CDCl_3$, ppm) δ 26.72, 27.05, 27.36 (2C), 28.54 (3C), 30.00, 30.03, 30.08, 30.12, 40.52, 49.91, 49.95, 49.98, 51.80, 78.81, 123.14, 124.64, 124.86, 124.93, 124.95, 125.00, 125.81, 126.82, 126.94, 127.44, 127.49, 128.94, 130.51, 130.80, 131.27, 134.09, 156.12

Synthesis of (2-(9-(2-(benzylboronic acid)-19,19-dimethyl-17-oxo-2-(pyren-1-ylmethyl)-18-oxa-2,9,16-triazaicosyl)phenyl)boronic acid (5)

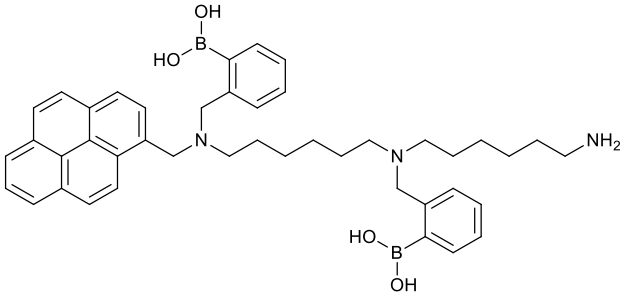


5

Compound **4** (0.109 g, 0.161 mmol) was dissolved in dry THF (20 mL). Sodium hydride (0.025 g, 0.62 mmol, 60% in mineral oil) was slowly added into the solution and stirred for 20 min, then pinacol protected 2-(bromomethyl)phenylboronic acid (2-(2-(bromomethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane) (0.135 g, 0.45 mmol) was added and the reaction was stirred for 2 hours. The reaction mixture was then quenched with water and poured into saturated $NaHCO_3$ solution and a precipitate formed. The precipitate was then filtered, washed with water, hexane and then dried to give the title compound (**5**) as a light yellow powder (0.134g, 83%). Mp 124 °C (dec). IR (cm^{-1}) 752, 844, 1380, 1694, 2924, 2981, 3659; AccMass ESI [M-H_2O+H] $C_{48}H_{65}B_2N_3O_5$ calculated

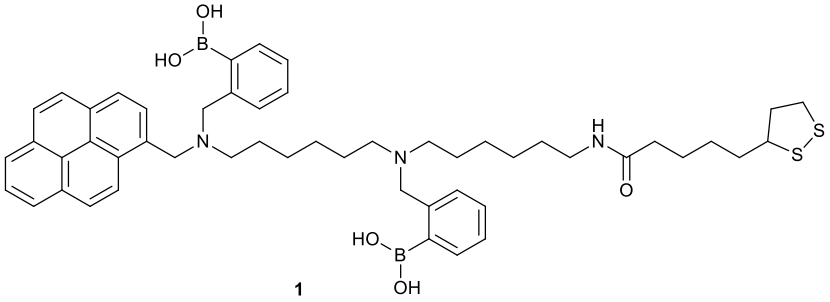
780.4719 found 780.4685; ^{11}B NMR (96 MHz, methanol-d_4, ppm) δ 8.15; ^{1}H NMR (300 MHz, methanol-d_4, ppm) δ 0.96-1.16 (m, 25H), 2.23-2.44 (m, 6H), 2.74 (t, *J* = 7.5, 2H), 3.07 (s, 4H), 3.60 (s, 2H), 6.66 (dd, *J* = 6 & 9, 1H), 6.80 (dd, *J* = 6 & 9, 1H), 6.94 (dd, *J* = 6 & 9, 1H), 7.06-7.16 (m, 3H), 7.34 (d, *J* = 9, 1H), 7.52 (d, *J* = 6, 1H), 7.74-7.95 (m, 9H); ^{13}C NMR (75 MHz, methanol-d_4, ppm) δ 23.90, 24.44, 27.17, 27.53, 27.62, 27.70, 29.21, 31.08, 31.31, 35.81, 41.43, 51.14, 51.72, 60.98, 62.13, 80.18, 123.86, 125.88, 126.23, 126.38, 126.54, 127.20, 127.43, 127.57, 128.03, 128.78, 129.23, 129.38, 129.82, 129.96, 130.65, 131.64, 131.88, 132.22, 132.76, 132.96, 136.43, 139.61, 158.94.

Synthesis of (2-(((6-((6-aminohexyl)(2-(benzylboronic acid)amino)-hexyl)(pyren-1-ylmethyl)amino)methyl)phenyl)boronic acid



Compound **5** was dissolved in dichloromethane and then trifluoroacetic acid (1 equiv.) was added into the solution. The reaction was monitored by TLC and mass spectrometry, more trifluoroacetic could be added if required. After the Boc protecting group was removed, all solvents were evaporated and residue was taken up and extracted in dichloromethane and NaOH solution (1 M) to give the title compound (quantitative yield) which was used without further purification in the next step. Acc Mass ESI [M-H_2O+H] $C_{43}H_{52}B_2N_3O_3$ calculated 680.4195 found 680.4295.

Synthesis of (2-(((6-((6-(5-(1,2-dithiolan-3-yl)pentanamido)hexyl)(2-benzylboronic acid)amino)hexyl)(pyren-1-ylmethyl)amino)methyl)-phenyl)boronic acid (1)



1

Aforementioned (2-(((6-((6-aminohexyl)(2-(benzylboronic acid)amino)-hexyl)(pyren-1-ylmethyl)amino)-methyl)phenyl)boronic acid (0. 503 g, 0.72 mmol) and the NHS ester of lipoic acid (0.438 g, 1.4 mmol) were dissolved in dichloromethane and stirred at room temperature overnight. The solvent was then removed *in vacuo* and the residue purified by flash column chromatography (DCM, DCM / MeOH 10:1, DCM / MeOH / 5 % propylamine) to give the title compound (**1**) as a light yellow powder (0.43 g, 67 %). Mp 100 °C (dec); IR (cm^{-1}) 733, 953, 1382, 1444, 1598, 2890, 2972, 2981, 3658; Acc Mass ESI [M-H_2O+H] $C_{51}H_{64}B_2N_3O_4S_2$ calculated 868.4524 found 868.4350; ^{11}B NMR (96 MHz, methanol-d_4, ppm) δ 7.81; ^{1}H NMR (250 MHz, $CDCl_3$, ppm) δ 0.99-1.59 (m, 26H), 1.84-2.49 (m, 10H), 3.11-3.14 (s*2, 4H), 3.53-3.54 (m, 2H), 3.88 (m, 1H), 4.21 (s, 2H), 7.29-7.39 (m, 6H), 7.87-8.14 (m, 11H); ^{13}C NMR (62 MHz, $CDCl_3$, ppm) δ 24.53, 25.02, 25.20, 25.45, 26.54, 26.89, 27.12, 28.51, 28.90, 29.27, 34.62, 36.36, 38.46, 39.34, 40.23, 51.75, 52.96, 53.53, 54.50, 56.48, 83.63, 123.08, 124.72, 124.77, 124.82, 125.16, 125.28, 125.97, 127.25, 127.45, 127.50, 127.75, 128.80, 129.85, 130.27, 130.48, 130.71, 130.92, 131.27, 131.29, 136.52, 141.82, 146.33, 172.82.

Details of the mathematical model

Consider the bis-boronic acid film covering the electrode that has continuous pores or channels from the solution to the electrode. Electrolysis of the redox probe in solution at such an electrode differs from that at the bare (unfilmed) electrode, and depends upon the extent of coverage of the electrode by the film, the size and distribution of the pores. Assuming the pores have uniform dimensions, degrees of tortuosity, and distribution within the film, theoretical idealised models could be applied to investigate such films. The theory for the models is closely related to that for ultramicroelectrode arrays.

In this case, the theoretical treatment for the film is according to the linear relationship between peak current and pinhole area. Assuming that the peak current on the electrode is at a maximum when it is totally unfilmed and placed in a solution containing electroreactant which undergos reversible electrode reaction. When the electrode surface is blocked by a non-conductive film with a fraction of θ, the fraction of the electrode surface unblocked will be (1-θ). Meanwhile, the peak current will be (1-θ) times that of the bare electrode. In our case, it can be assumed that the footprint of bis-boronic acid (receptor) is smaller than that binding with monosaccharide molecule (complex species). If the peak current is I_{max} with a surface blocked fraction, θ1 for receptor, the surface blocked fraction will be increased to θ2 when the receptor is fully replaced by complex species; meanwhile, the peak will be decreased to I_{min}.

Assuming the peak current on a totally unfilmed electrode is I0, it can be easily stated that:

$$\theta_1/\theta_2 = (I_0 - I_{max})/(I_0 - I_{min}) \quad (1)$$

It's equal to the equation 2

$$[\![\ 1-\theta\ ]\!]_1/(1-\theta_2) = I_{max}/I_{min} \quad (2)$$

When the working electrode modified by bis-boronic acid film is incubated in monosaccharide solution, the following reaction takes place:

$$R + S \leftrightarrow RS \quad (3)$$

Where R, S and RS represent the free bis-boronic acid, monosaccharide molecule and complex species, respectively. Generally, the total mole number for bis-boronic acid on the electrode remains constant, and therefore the total surface coverage will be unchanged. The complexation equilibrium equations for the complexation: decomplexation equilibria can be described as below:

$$K = \Gamma_{RS}/(\Gamma_R \times [S]) = (\Gamma_0 - \Gamma_R)/(\Gamma_R \times [S]) \quad (4)$$

Γ represent the surface coverage of different species on electrode surface; Γ_0 is the total surface coverage of bis-boronic acid; [S] is the concentration of saccharide in solution. When the surface coverage at a specific concentration of saccharide is Γ_R, the surface block θ and peak current intensity I for a particular sugar concentration can also be evaluated:

$$\theta = \theta_1\ \Gamma_R/\Gamma_0 + \theta_2\ (\Gamma_0 - \Gamma_R)/\Gamma_0 \quad (5)$$

hence

$$I = I_0\ (1-\theta) = I_{max}\ \Gamma_R/\Gamma_0 + I_{min}\ (\Gamma_0 - \Gamma_R)/\Gamma_0 \quad (6)$$

By combining Eq. (4) and Eq. (6), Eq. (7) can be obtained:

$$I = I_{max}\ 1/(K[S]+1) + I_{min}\ (K[S])/(K[S]+1) \quad (7)$$

Using the equation (7) and the experimentally determined peak current values at various concentration of saccharide gives the solid line shown as Figure 3 presented in the main body

of the manuscript, which is titled “An electrochemical method for the sensitive and selective determination of glucose concentration using bis-boronic acid modified electrode”. Curve fitting the peak current intensity (I) versus the concentration of saccharide (both I_{min} and K were varied) also gives the calculated stability constant (K) and final peak current intensity when Γ_R infinitely approaches zero.

When the rate constant is slow enough, the peak current doesn’t show obvious variation towards the changing rate constant. Therefore, an experiment at higher concentrations could be suggested where the peak current is not the affected by irreversibility behaviour of the heterogeneous electron transfer.

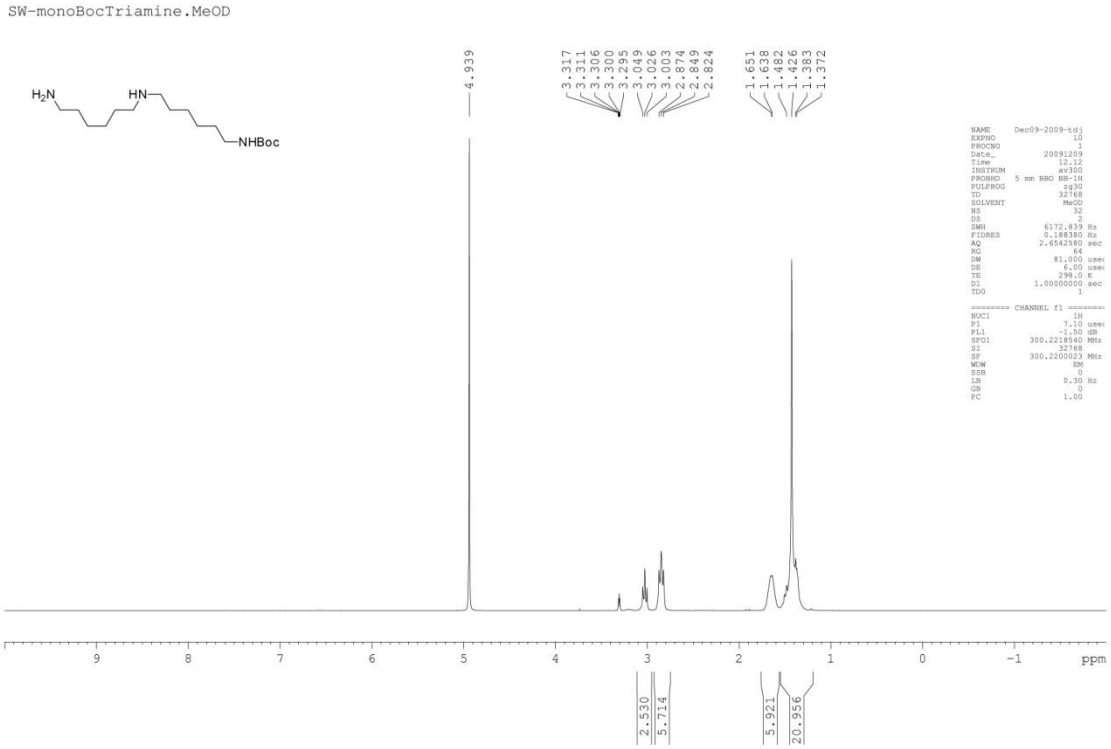
Possible explanation for high electrochemical sensitivity

In the system of boronic acid and saccharide, two types of binding models should be considered. One is shown as Scheme 2(b) in the main body of the manuscript, in which saccharide is bonded with two boronic acid functional groups. Another is considering that in the dense packing self-assembled monolayer only one of them is bonded with a saccharide molecule, or two boronic acid functional groups in two individual molecules are bonded with a saccharide molecule.

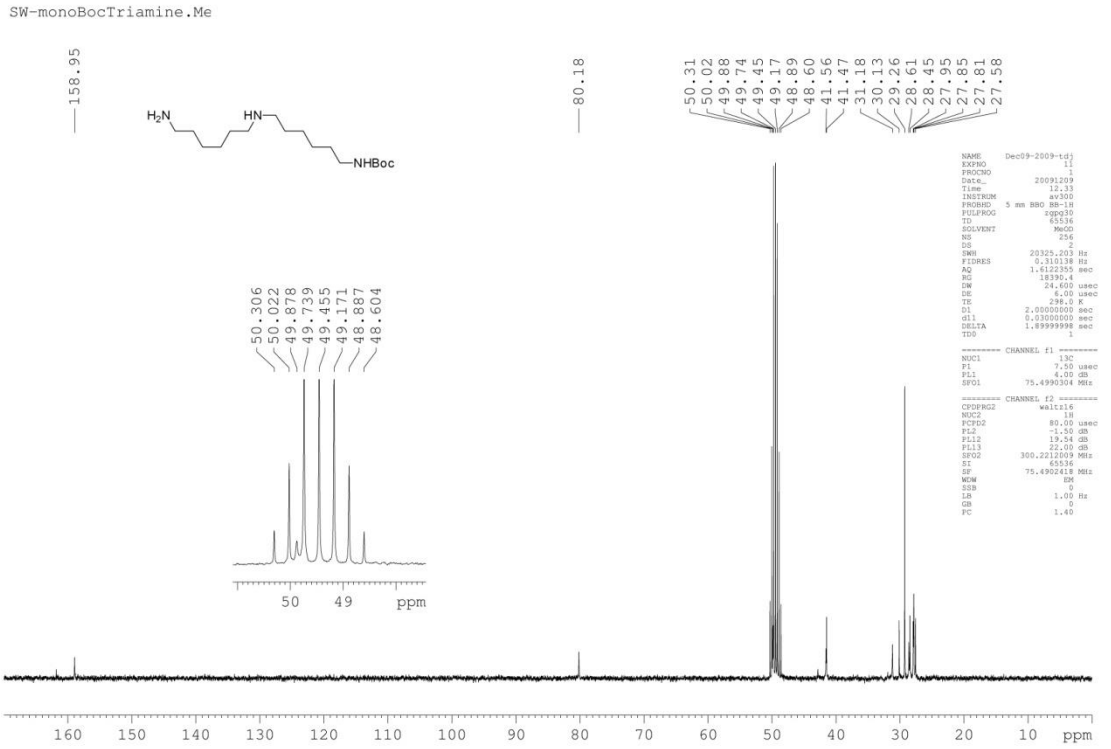
In the first binding type, the footprint of complex species is similar to that of a free bis-boronic acid. On the contrary, the footprint of complex species will be significantly enlarged, and then the well-packed self monolayer of bis-boronic acid will be also influenced. The free bis-boronic acid molecules surrounding the complex species would be pushed away and deeply block the pores or channels, through which the redox probe in solution diffuse to the electrode surface, therefore the electron transfer resistance will be increased by a large extent. In this way, at low concentrations of saccharide, the irreversibility of the voltammetric behaviours and charge transfer resistance in EIS curves both increased significantly.

NMR Spectrums

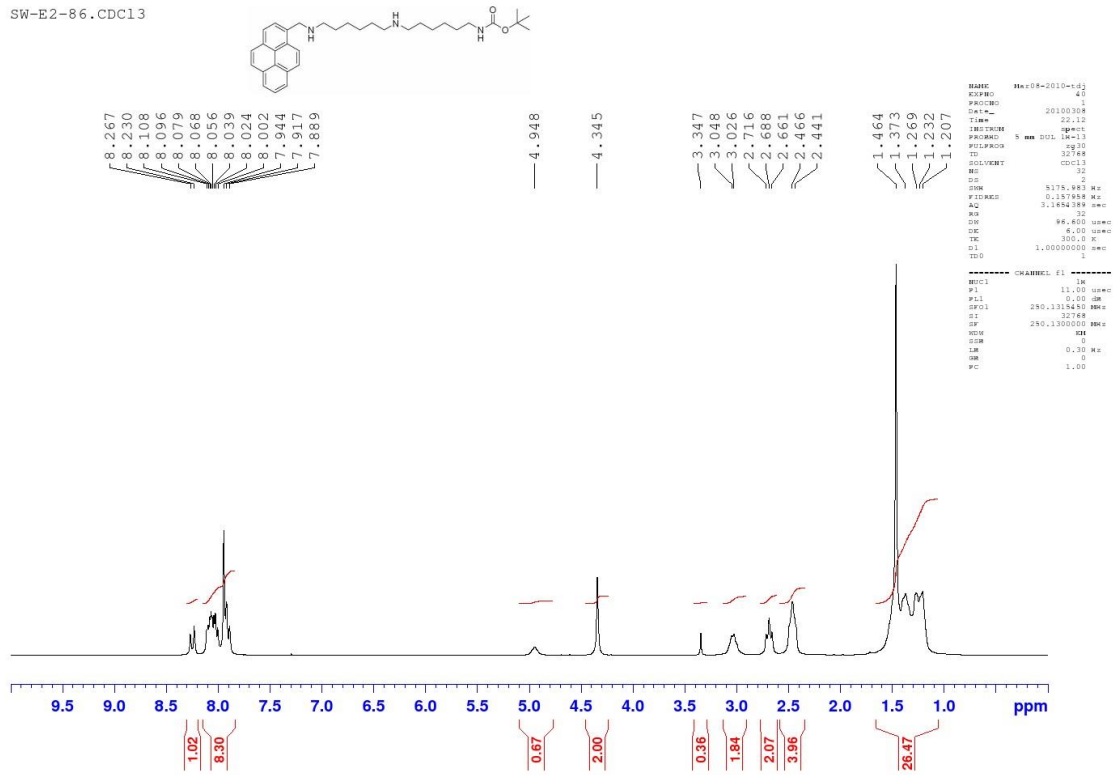
Proton NMR Spectrum of Compound 3



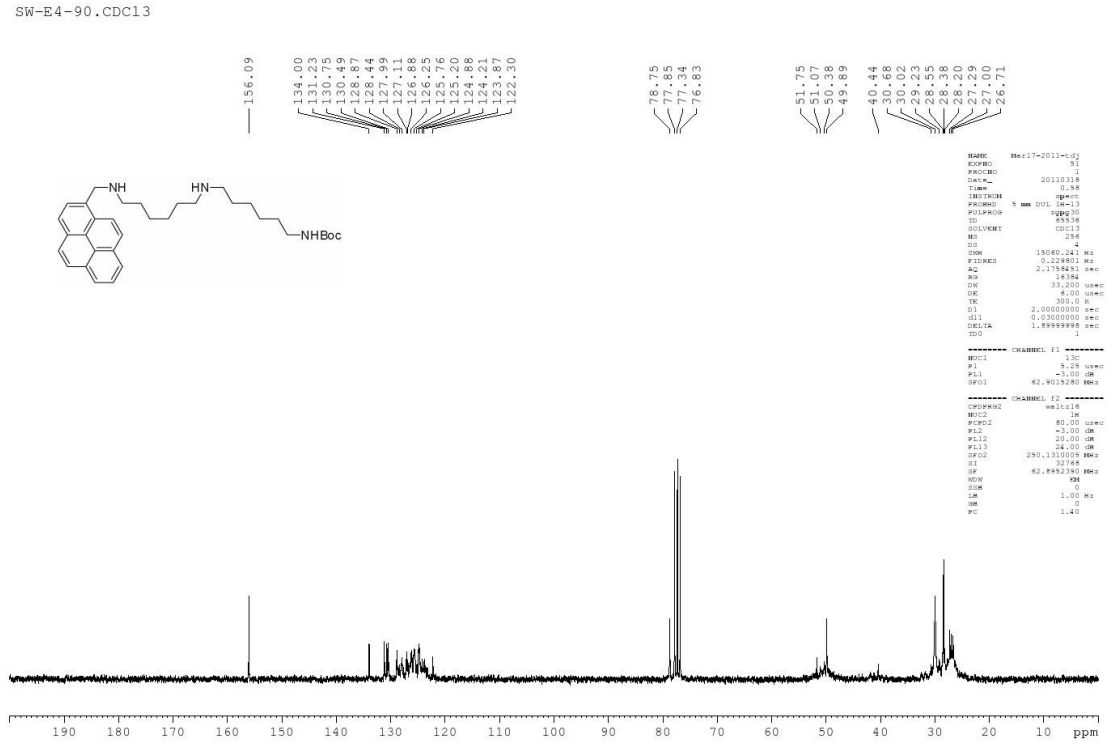
Carbon NMR Spectrum of Compound 3



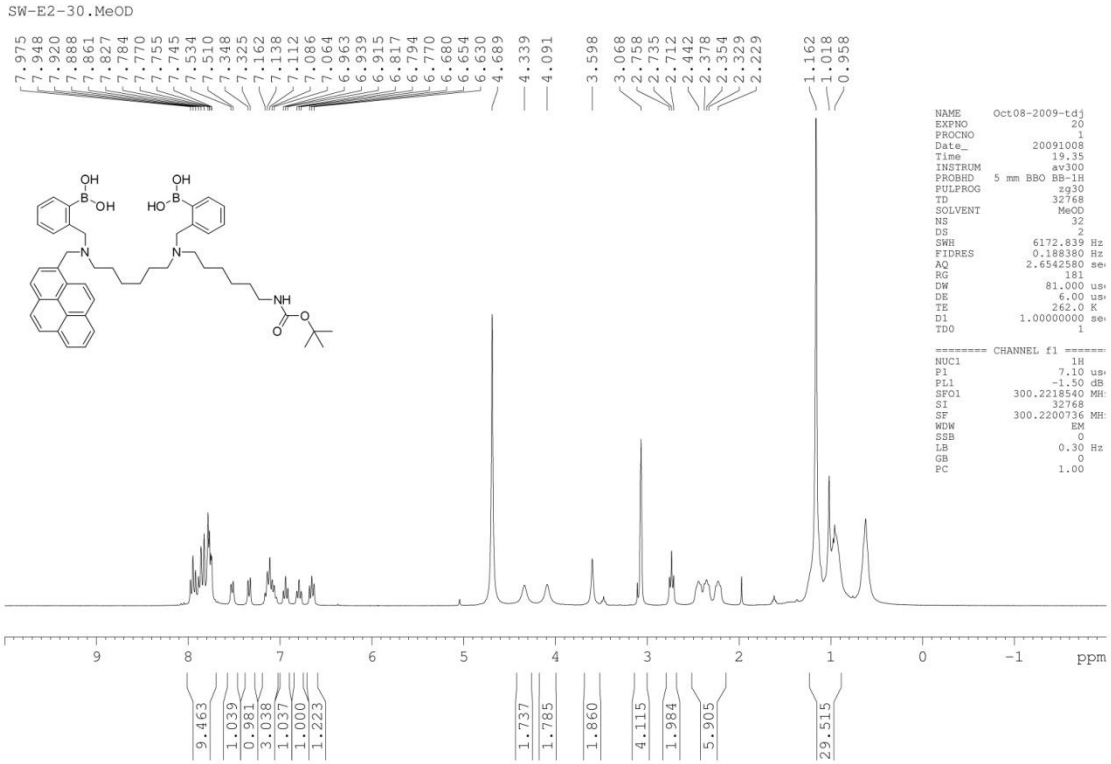
Proton NMR Spectrum of Compound 4



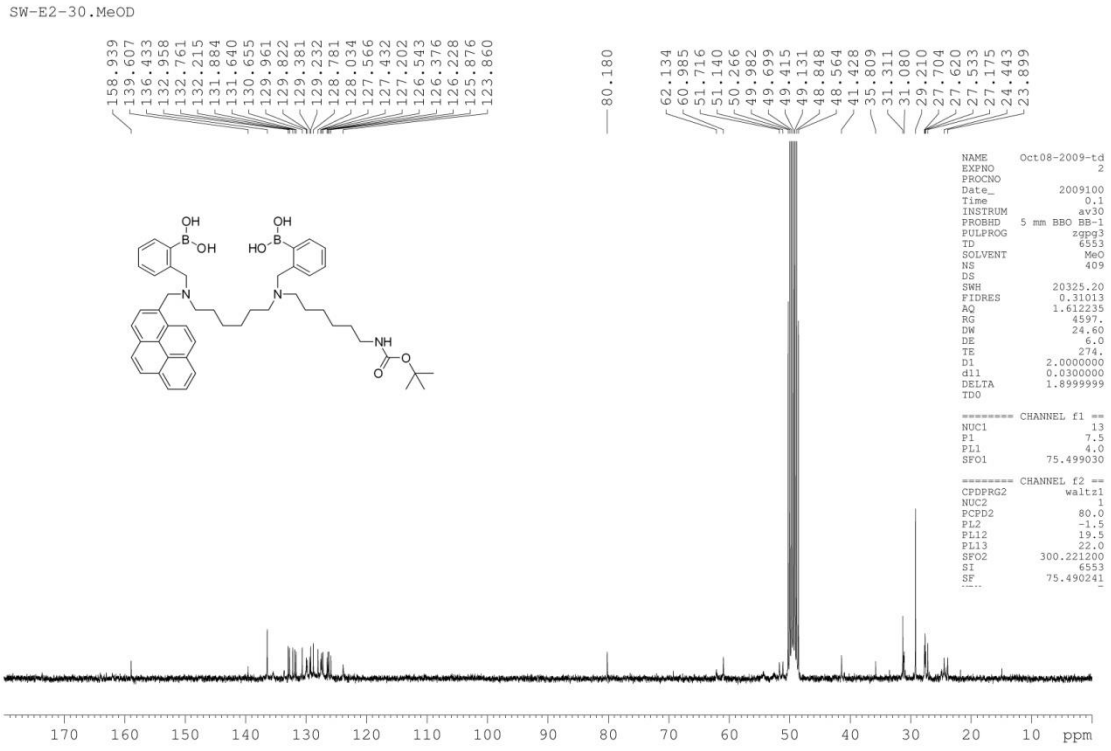
Carbon NMR Spectrum of Compound 4



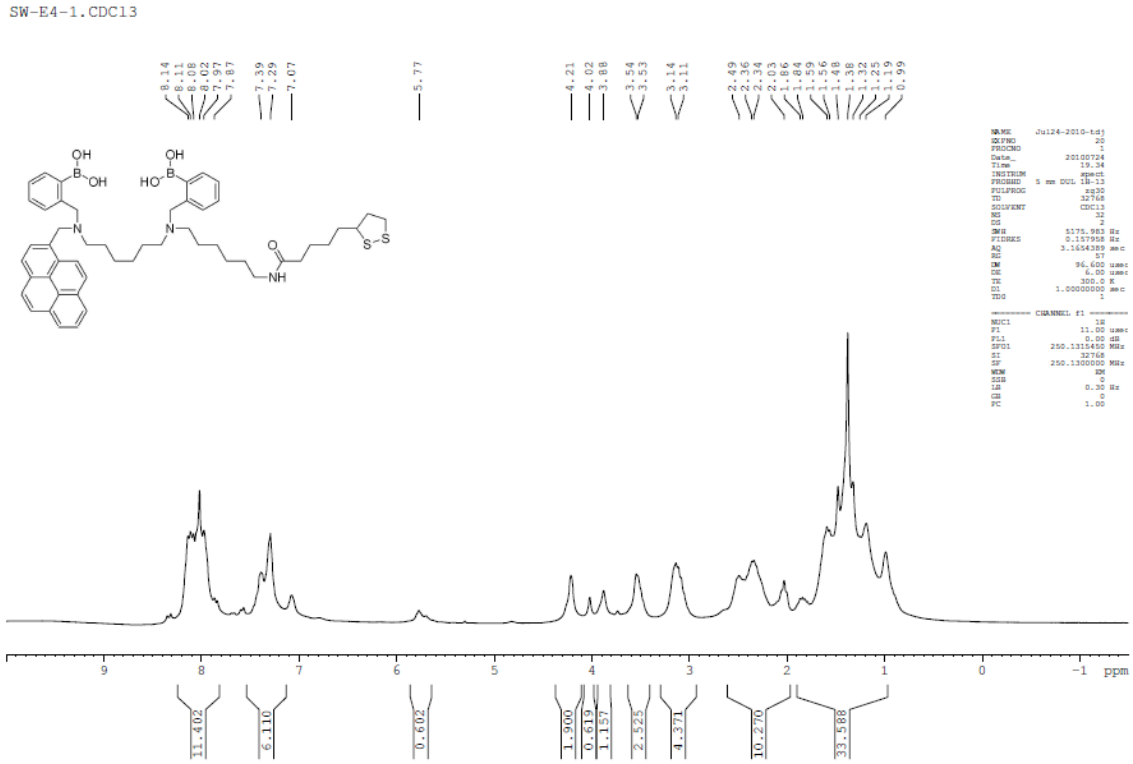
Proton NMR Spectrum of Compound 5



Carbon NMR Spectrum of Compound 5



Proton NMR Spectrum of Compound 1



Carbon NMR Spectrum of Compound 1