

Supporting Information for:

Tuning the Binding Properties of a New Heteroditopic Salt Receptor Through Embedding in a Polymeric System*

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GENERAL INFORMATIONS

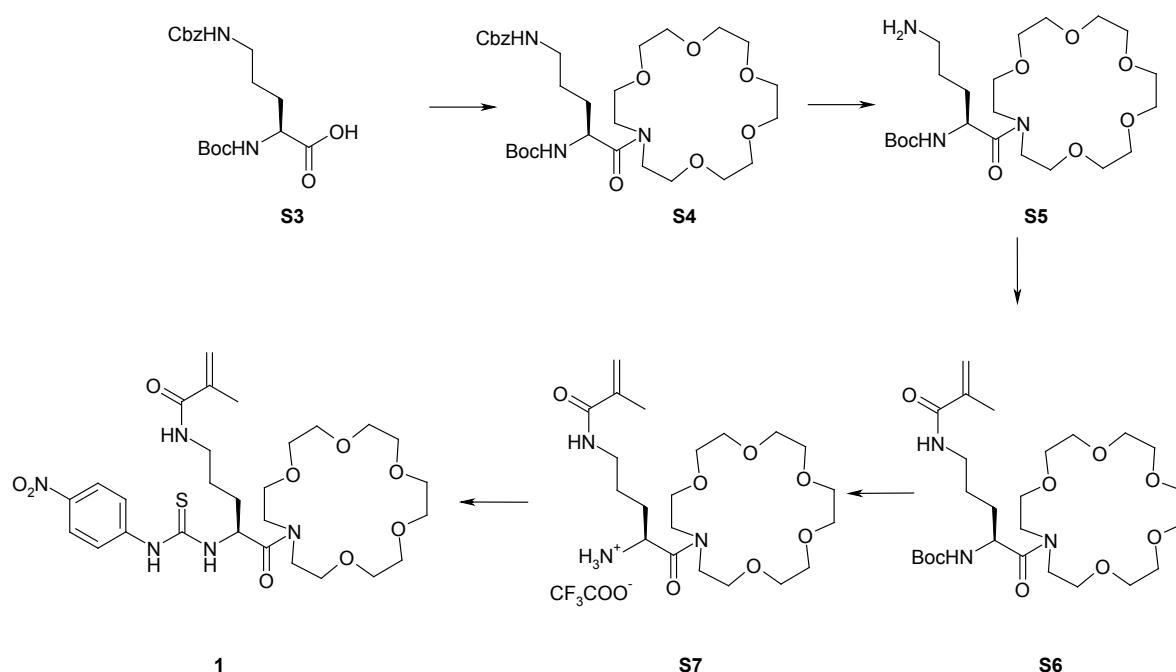
Unless specifically indicated, all other chemicals and reagents used in this study were purchased from commercial sources and used as received. 18-aza-crown-6 was prepared according to literature procedure.¹ Purification of products was performed using column chromatography on silica gel (Merck Kieselgel 60, 230-400 mesh) with mixtures of chloroform/methanol. Thin-layer chromatography (TLC) was performed on silica gel plates (Merck Kieselgel 60 F254).

¹H and ¹³C NMR spectra used in the characterization of products were recorded on Varian Unity 200 spectrometer using a TMS ($\delta=0.00$) or residual protonated solvent as internal standard. The following abbreviations are used to indicate the multiplicity: s - singlet; d - doublet; t - triplet; q - quartet; m - multiplet, b – broad signal.

High resolution mass spectra (HRMS) were measured on a Quattro LC Micromass unit using ESI technique.

UV-vis analyses were performed using Thermo Spectronic Unicam UV500 Spectrophotometer. Atomic absorption measurements were performed using Perkin Elmer AAnalyst 300 spectrometer.

SYNTHESIS



Scheme S1. Synthesis of receptor 1. *Reagents and conditions:* a) DCC, 1-aza-18-crown-6, CH₂Cl₂, 0°C to r.t., 92%; b) H₂, Pd/C, MeOH-THF, r.t., quantitative; c) methacryloyl chloride, Et₃N, CH₂Cl₂, 0°C to r.t., 50%; d) TFA-CH₂Cl₂ (1:1), r.t., quantitative; e) 4-nitrophenyl isothiocyanate, Et₃N, THF, reflux, 72%.

¹ H. Maeda, S. Furuyoshi, Y. Nakatsuji, M. Okahara, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 212-218

Compound S4:

To a solution of N_{α} -Boc- N_{δ} -Cbz-L-ornithine (**S3**) (3.51 g, 9.58 mmol) and 1,3-dicyclohexylocarbodiimide (DCC) (2.13 g, 10.3 mmol) in 30 ml of dry dichloromethane 2.52 g, 9.58 mmol of 1-aza-18-crown-6 in 30 ml of dry dichloromethane at 0 °C (ice bath) was added. The reaction mixture was stirred for 30 min and then left at room temperature overnight. The precipitate was filtered off, washed with dichloromethane and solvent was evaporated. The residue was purified by silica gel column chromatography (2% methanol in chloroform) to give the title product as a white oil (5.39 g, 92% yield)

HRMS (ESI): calcd for $C_{30}H_{49}N_3O_{10}Na$ $[M+Na]^+$: 634.3315, found: 634.3328.

1H NMR (200 MHz, $CDCl_3$) δ : 1.42 (s, 9H, $C(CH_3)_3$); 1.48-1.85 (m, 4H, CH_2-CH_2); 3.12-3.30 (m, 2H, $CH_2-NHCbz$); 3.56- 3.74 (m, 24H, crown ether CH_2); 4.66 (bs, 1H, $\alpha-CH$); 5.08 (s, 2H, CH_2-Ph), 5.22 (bs, 1H, $NH-Cbz$); 5.35 (bd, $J=7,8$ Hz , 1H, $NH-Boc$); 7.25- 7.40 (m, 5H, Ph).

^{13}C NMR (50 MHz, $CDCl_3$) δ : 25.6 ($\gamma-CH_2$), 28.5 ($C(CH_3)_3$), 31.1 ($\beta-CH_2$), 40.9 ($\delta-CH_2$), 47.0, 49.1 (crown ether CH_2-N rotamers), 49.9 ($\alpha-CH$), 66.7 (CH_2-Ph), 69.7 (crown ether CH_2-CH_2-N), 70.5 (crown ether), 70.7 (crown ether), 70.8 (crown ether), 71.1 (crown ether), 79.6 ($C(CH_3)_3$), 128.2 (Ph), 128.3 (Ph), 128.7 (Ph), 136.8 (Ph), 155.5 ($COC(CH_3)_3$), 156.6 ($COBn$), 172.7 (CO -crown).

Compound S5:

To a degassed solution of **S4** (4.88 g, 7.99 mmol) in 100 ml of THF/MeOH (1:4) catalytic amounts of 10% Pd/C was added. The reaction mixture was kept under H_2 atmosphere (balloon pressure) at room temperature overnight. The catalyst was removed by filtration through a pad of Celite and washed with MeOH. The filtrate was concentrated under reduced pressure to give the crude product in quantitative yield (3.81 g). The amine was used in next step without further purification.

HRMS (ESI): calcd for $C_{22}H_{44}N_3O_8$ $[M+H]^+$: 478.3128, found: 478.3108.

1H NMR (200 MHz, $CDCl_3$) δ : 1.42 (s, 9H, $C(CH_3)_3$); 1.60-2.05 (m, 4H, CH_2-CH_2); 2.74 (bs, 2H, NH_2), 2.95-3.20 (m, 2H, CH_2-NH_2); 3.40-4.05 (m, 24H, crown ether CH_2); 4.64 (bs, 1H, $\alpha-CH$); 5.61 (bs, 1H, $NH-Boc$).

^{13}C NMR (50 MHz, $CDCl_3$) δ : 23.7 ($\gamma-CH_2$), 28.6 ($C(CH_3)_3$), 30.2 ($\beta-CH_2$), 39.4 ($\delta-CH_2$), 47.8, 48.5 (crown ether CH_2-N rotamers), 50.1 ($\alpha-CH$), 69.4 (crown ether CH_2-CH_2-N), 69.8 (crown ether), 70.4 (crown ether), 70.5 (crown ether), 70.7 (crown ether), 78.1 ($C(CH_3)_3$), 155.6 ($COC(CH_3)_3$), 173.2 (CO -crown).

Compound S6:

To a stirred solution of **S5** (3.52 g, 7.38 mmol) and triethylamine (1.49 g, 14.8 mmol) in 100 ml of dry dichloromethane at 0 °C a methacryloyl chloride (0.926 g, 8.86 mmol) was added dropwise. The reaction mixture was stirred at that temperature for 1h and then at room

temperature overnight. The reaction mixture was washed two times with 0.5M HCl, then with water, dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (2,5% methanol in chloroform) to give the title product as a white oil (2 g, 50% yield)

HRMS (ESI): calcd for C₂₆H₄₇N₃O₉Na [M+Na]⁺: 568.3210, found: 568.3203.

¹HNMR (200 MHz, CDCl₃) δ: 1,43 (s, 9H, C(CH₃)₃); 1,55- 1,80 (m, 4H, CH₂-CH₂); 1,97 (s, 3H, methacryl CH₃); 3,34 (m, 2H, δ-CH₂); 3,55-3,85 (m, 24H, crown ether CH₂); 4,69 (bs, 1H, α-CH); 5,31 (m, 1H, C=CH₂); 5,35-5,45 (bs, 1H, NH-Boc); 5,70 (m, 1H, C=CH₂); 6,32 (bs, 1H, CH₂-NH-methacryl).

¹³CNMR (50 MHz, CDCl₃) δ: 18.9 (methacryl CH₃), 25.1 (γ-CH₂), 28.5 (C(CH₃)₃), 31.8 (β-CH₂), 39.8 (δ-CH₂), 47.2, 49.1 (crown ether CH₂-N rotamers), 50.1 (α-CH), 69.7 (crown ether CH₂-CH₂-N), 70.6 (crown ether), 70.8 (crown ether), 71.0 (crown ether), 71.1 (crown ether), 79.8 (C(CH₃)₃), 119.5 (C=CH₂), 140.3 (C=CH₂), 155.3 (COC(CH₃)₃), 168.5 (CO-C=CH₂), 172.9 (CO-crown).

Compound S7:

The compound **S6** (2g, 3.67 mmol) was dissolved in 20 ml of dichloromethane and 5 ml of trifluoroacetic acid was added. The reaction mixture was stirred at room temperature until starting material was consumed (TLC monitoring). The mixture was evaporated *in vacuo* to yield the crude product as trifluoroacetate salt in quantitative yield (2 g). The ammonium salt was used in next step without further purification.

HRMS (ESI): calcd for C₂₁H₃₉N₃O₇Na [M+Na]⁺: 468.2686, found: 468.2685.

¹HNMR (200 MHz, CDCl₃) δ: 1,50-1,80 (m, 4H, CH₂-CH₂); 1,93 (s, 3H, methacryl CH₃), 3,20-3,40 (m, 2H, δ-CH₂); 3,40-3,91 (m, 24H, crown ether CH₂); 4,51 (bs, 1H, α-CH); 5,30 (s, 1H, C=CH₂); 5,76 (s, 1H, C=CH₂); 7,22 (bs, 1H, CH₂-NH-methacryl); 8,52 (s, 3H, NH₃⁺).

¹³CNMR (50 MHz, CDCl₃) δ: 18.7 (methacryl CH₃), 24.3 (γ-CH₂), 30.9 (β-CH₂), 38.9 (δ-CH₂), 48.9, 49.2 (crown ether CH₂-N rotamers), 50.5 (α-CH), 69.3 (crown ether CH₂-CH₂-N), 69.6 (crown ether), 70.0 (crown ether), 120.2 (C=CH₂), 139.8 (C=CH₂), 169.3 (CO-C=CH₂), 172.5 (CO-crown).

Receptor 1:

To the solution of **S7** (2 g, 3.58 mmol) and triethylamine (0.725 g, 7.16 mmol) in 40 ml of dry THF the 4-nitrophenylthiocyanate (0.645 g, 3.58 mmol) was added. After stirring overnight at room temperature, the reaction mixture was concentrated and residue was partitioned between dichloromethane and water. The organic layer was dried over anhydrous MgSO₄. After filtration the solvent was evaporated, and the crude material was purified by silica gel column chromatography (2,5% methanol in chloroform) to give receptor **1** as pale-yellow solid (1.56 g, 70% yield).

Mp= 58-62 °C

HRMS (ESI): calcd for C₂₁H₃₉N₃O₇Na [M+Na]⁺: 648.2679, found: 648.2678.

¹HNMR (200 MHz, CDCl₃) δ: 1,60- 1,89 (m, 4H, CH₂-CH₂); 1,94 (s, 3H, methacryl CH₃); 3,36 (m, 2H, δ-CH₂); 3,45- 4,10 (m, 24H, crown ether CH₂), 5,32 (s, 1H, C=CH₂); 5,41 (bs,

1H, α -CH); 5.67 (s, 1H, C=CH₂); 6.58 (bt, J=5.6, 1H, CH₂-NH-methacryl); 7.70 (d, J=9, 2H, ArH), 8.05 (d, J=9, 2H, ArH), 8.54 (bd, J=6.6, 1H, NHCSN₂Ar); 9.16 (s, 1H, NHCSN₂Ar).

¹³CNMR (50 MHz, CDCl₃) δ : 18.9 (methacryl CH₃), 26.3 (γ -CH₂), 30.4 (β -CH₂), 39.5 (δ -CH₂), 47.7, 49.6 (crown ether CH₂-N rotamers), 54.6 (α -CH), 69.1, 69.5, 70.6, 70.8, 70.9, 71.1 (all crown ether rotamers), 119.9 (C=CH₂), 122.2 (ArH), 124.4 (ArH), 140.1 (C=CH₂), 143.5 (ArNO₂), 145.1 (ArNH), 169.0 (CO-C=CH₂), 174.3 (CO-crown), 180.8 (C=S).

Polymer 2:

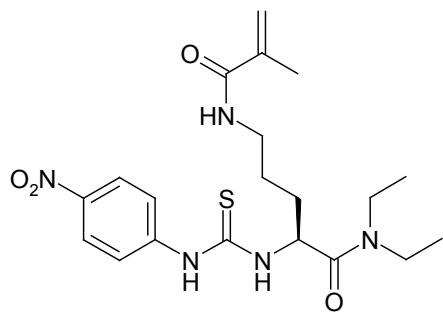
Receptor **1** (500 mg, 0.8 mmol), butyl methacrylate (1.02 g, 7.2 mmol) and azobisisobutyronitrile (AIBN) (26.27 mg, 0.16 mmol) were dissolved in 1.5 ml of dry THF. The solution was degassed and then placed at 70 °C for 16 h under atmosphere of nitrogen. The resulting viscous solution was added dropwise to excess methanol. The precipitate was isolated, dissolved in small amount of THF and again added dropwise into excess methanol. The polymer **2** was subsequently isolated by decantation and dried *in vacuo* to give a yellow solid in 72% yield.

¹HNMR (200 MHz, CDCl₃) δ : 0.60-1.20 (bm, 57.53H, 2xCH₃), 1.20-1.50 (bm, 25.12H, CH₂), 1.50-2.20 (bm, 46.44H, CH₂), 3.67 (bs, 16.97H, CH₂ crown ether), 3.95 (bs, 20H, OCH₂), 5.51 (bs, 8.89H, CH), 7.76 and 8.15 (bs, 1.62 and 1.99H, ArH).

GPC: M_n: 96.1 kDa, PDI: 2.8

Reference compound:

Reference compound was synthesized in analogues manner as receptor **1**. Instead of 1-aza-18-crown-6 at first step the diethylamine was used.



HRMS (ESI): calcd for C₂₀H₂₉N₅O₄SNa [M+Na]⁺: 458.1838, found: 458.1826.

¹HNMR (200 MHz, CDCl₃) δ : 1.11 (t, J=7.1, 3H, CH₂CH₃ rotamer), 1.38 (t, J=7.2, 3H, CH₂CH₃ rotamer), 1.65-1.90 (m, 4H, CH₂-CH₂), 1.94 (s, 3H, methacryl CH₃), 3.30-3.60 (m, 4H, δ -CH₂+CH₂CH₃ rotamer), 3.68 (q, J=7.8, CH₂CH₃ rotamer), 5.33 (s, 1H, C=CH₂), 5.39 (bs, 1H, α -CH), 5.69 (s, 1H, C=CH₂), 6.53 (t, J=6, 1H, CH₂-NH-methacryl), 7.69 (d, J=9.2, ArH), 8.04 (d, J=9.2, ArH), 8.69 (bd, J=7.4, 1H, NHCSN₂Ar), 9.22 (s, 1H, NHCSN₂Ar).

¹³CNMR (50 MHz, CDCl₃) δ : 13.0, 14.5 (CH₂CH₃ rotamers), 18.9 (methacryl CH₃), 26.7 (γ -CH₂), 30.8 (β -CH₂), 39.5 (δ -CH₂), 41.6, 43.0 (CH₂CH₃ rotamers), 54.5 (α -CH), 119.9

(C=CH₂), 122.2 (ArH), 124.4 (ArH), 140.1 (C=CH₂), 143.5 (ArNO₂), 145.1 (ArNH), 169.0 (CO-C=CH₂), 173.4 (CO-NEt₂), 181.0 (C=S).

TITRATION EXPERIMENTS

The ¹H NMR titrations were performed on a Varian UnityPlus 200MHz spectrometer, at 298K in CD₃CN. In each case, a 500 μL of freshly prepared 2.7 mM solution of receptor **1** was added to a 5mm NMR tube. Where applicable the solution also contained 1 molar equivalent of sodium hexafluorophosphate. Small aliquots of 10-20 mM solution of tetrabutylammonium anion salts, containing **1** at 2.7 mM concentration, were added and a spectrum was acquired after each addition. Titration isotherms for NH protons were fitted to a 1:1 binding model using the HypNMR 2000 program. The 1:1 binding stoichiometries were verified by a Job plot analysis.

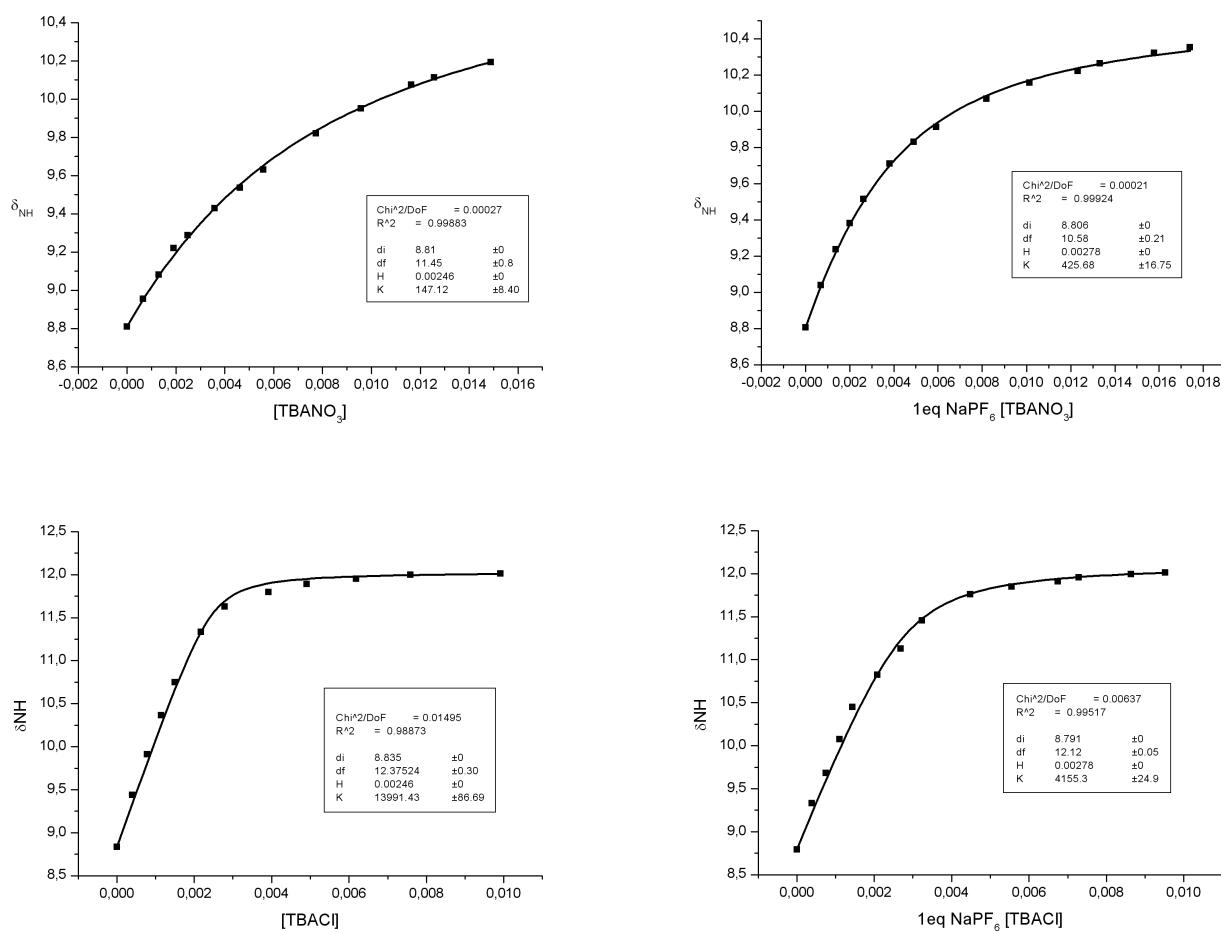


Figure S1. Selected binding isotherms. Experimental (squares) and calculated (line) chemical shifts.

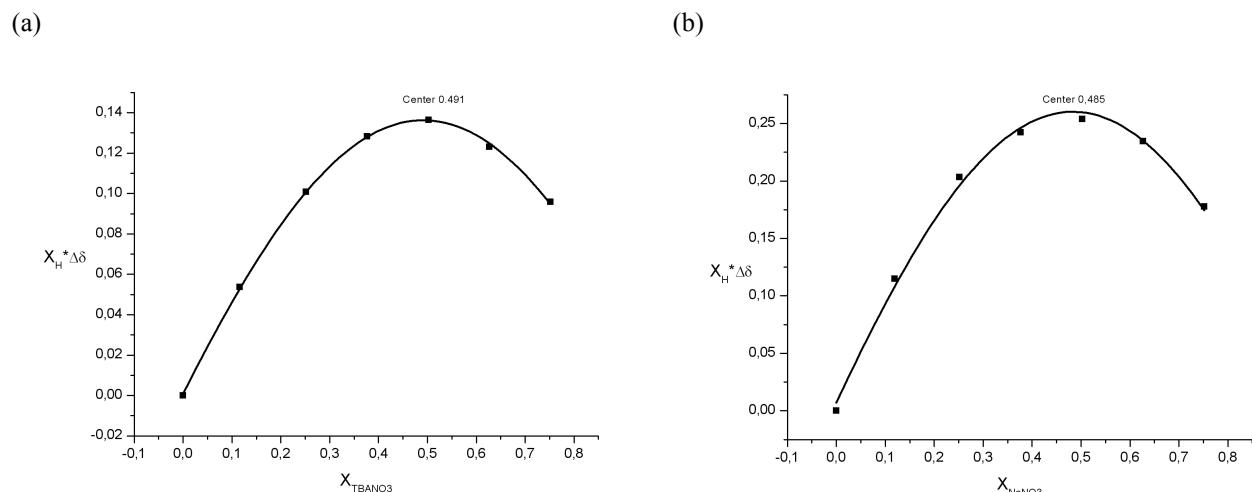


Figure S2. Selected Job plots (a) $TBANO_3$ (b) 1eq $NaPF_6 + TBANO_3$. Experimental (squares) and calculated (line) chemical shifts.

EXTRACTION EXPERIMENTS

The 1.5 mM aqueous solution of $NaNO_3$ or $NaCl$ was extracted with 12.5 mM (effective concentration) of $CHCl_3$ solution of **1** or **2**, respectively. After phase separation a sample of organic phase was diluted with ethyl acetate and the content of extracted sodium cation was determined *via* atomic absorption spectroscopy. A calibration curve was generated using a standard solution of sodium hexafluorophosphate in ethyl acetate/chloroform (9/1 v/v).

The organic phase was then back-extracted into H_2O . A sample of aqueous phase was diluted with water and content of NO_3^- was determined by NO_2^-/NO_3^- colorimetric test (Roche Applied Science). A calibration curve was generated by plotting an absorbance at 540 nm in a function of sodium nitrate concentration (25 to 0.05 mg/L of NO_3^-).

The results are summarized in Table S1.

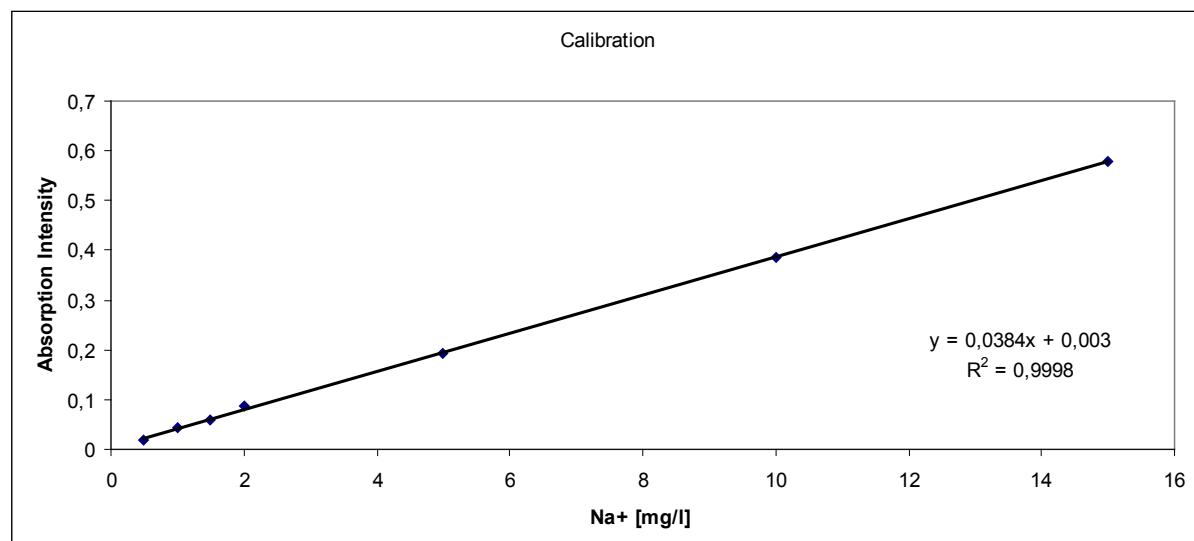


Figure S3. Calibration curve generated by measuring the peak intensities produced by $NaPF_6$ standard solutions.

Table S1. Summary of extraction data.

	Polymer 2			Receptor 1			Chloroform (blank)		
	AI ^a	c [mg/l]	Extraction efficiency [%]	AI	c [mg/l]	Extraction efficiency [%]	AI	c [mg/l]	Extraction efficiency [%]
NO ₃ ⁻	0.487	12.63	44 32 ^b	0.032	0.832	2.9 1.3 ^b	0.001	0.031	0.1 n.d ^c
Cl ⁻	0.219	5.60	19	0.025	0.637	2.2	0.002	0.060	0.2

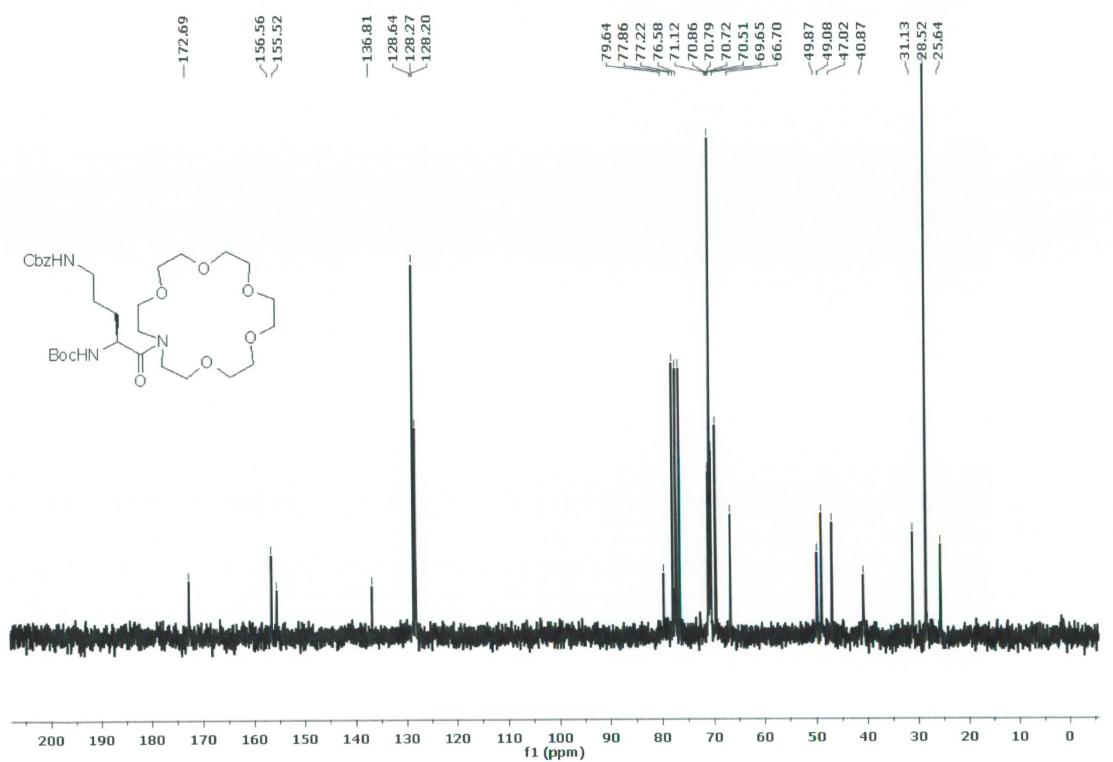
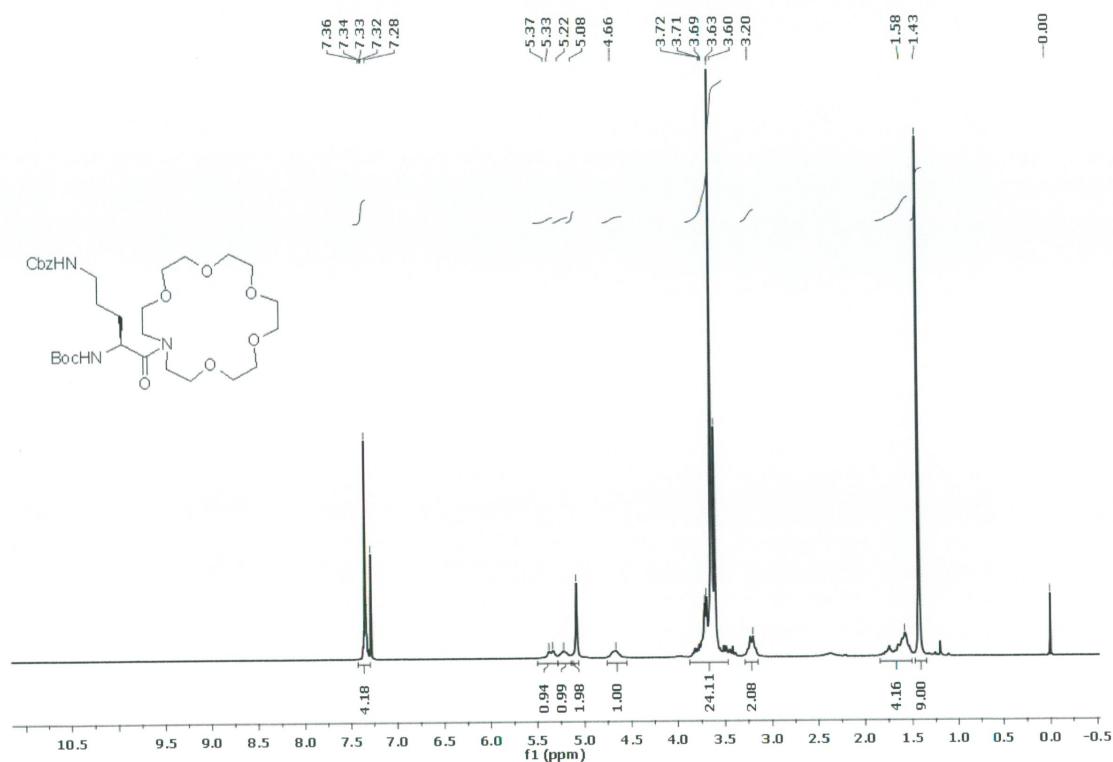
^a Absorption Intensity

^b determined by NO₂⁻/NO₃⁻ colorimetric test (Roche Applied Science) after back-extraction into H₂O

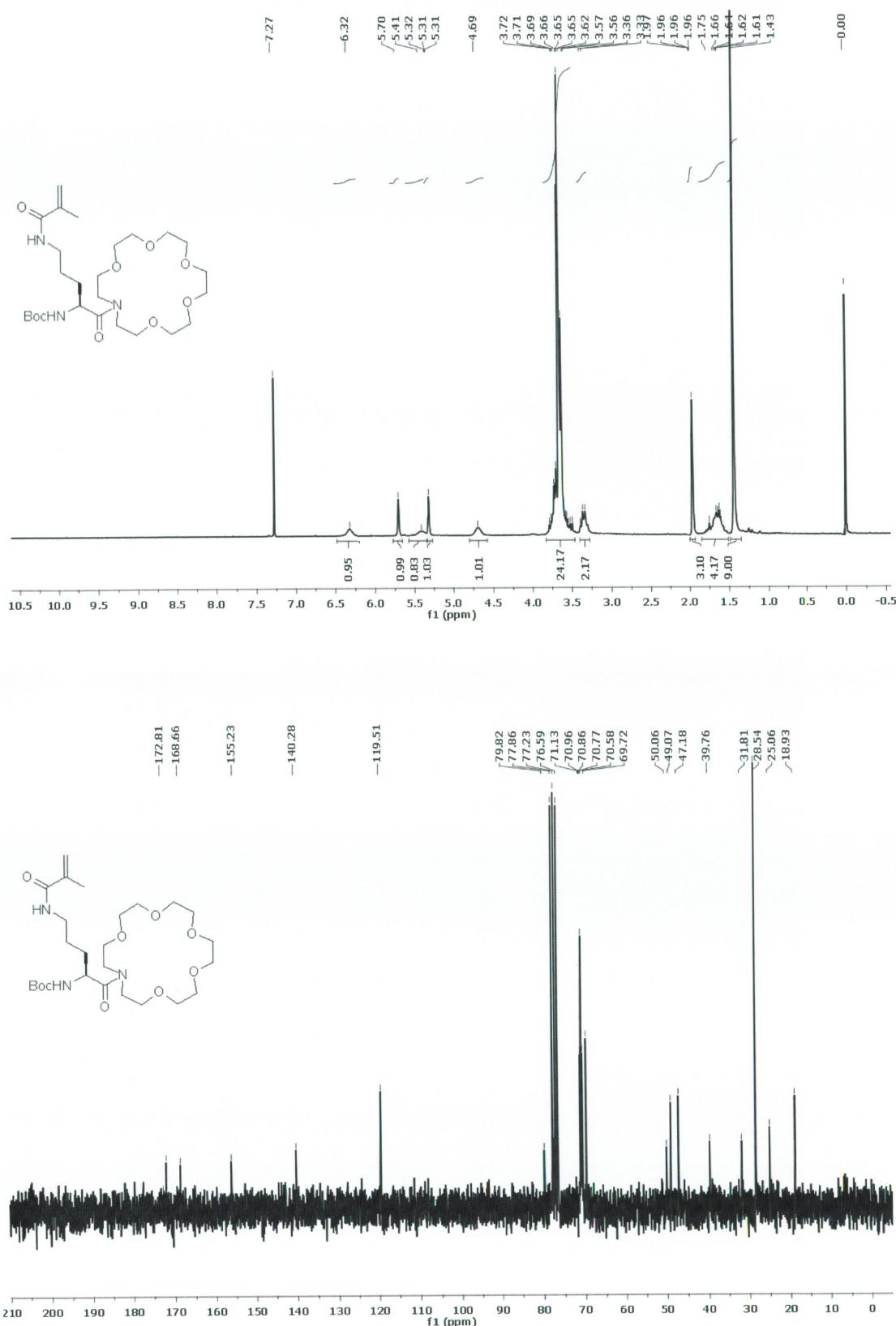
^c not detected

NMR SPECTRA

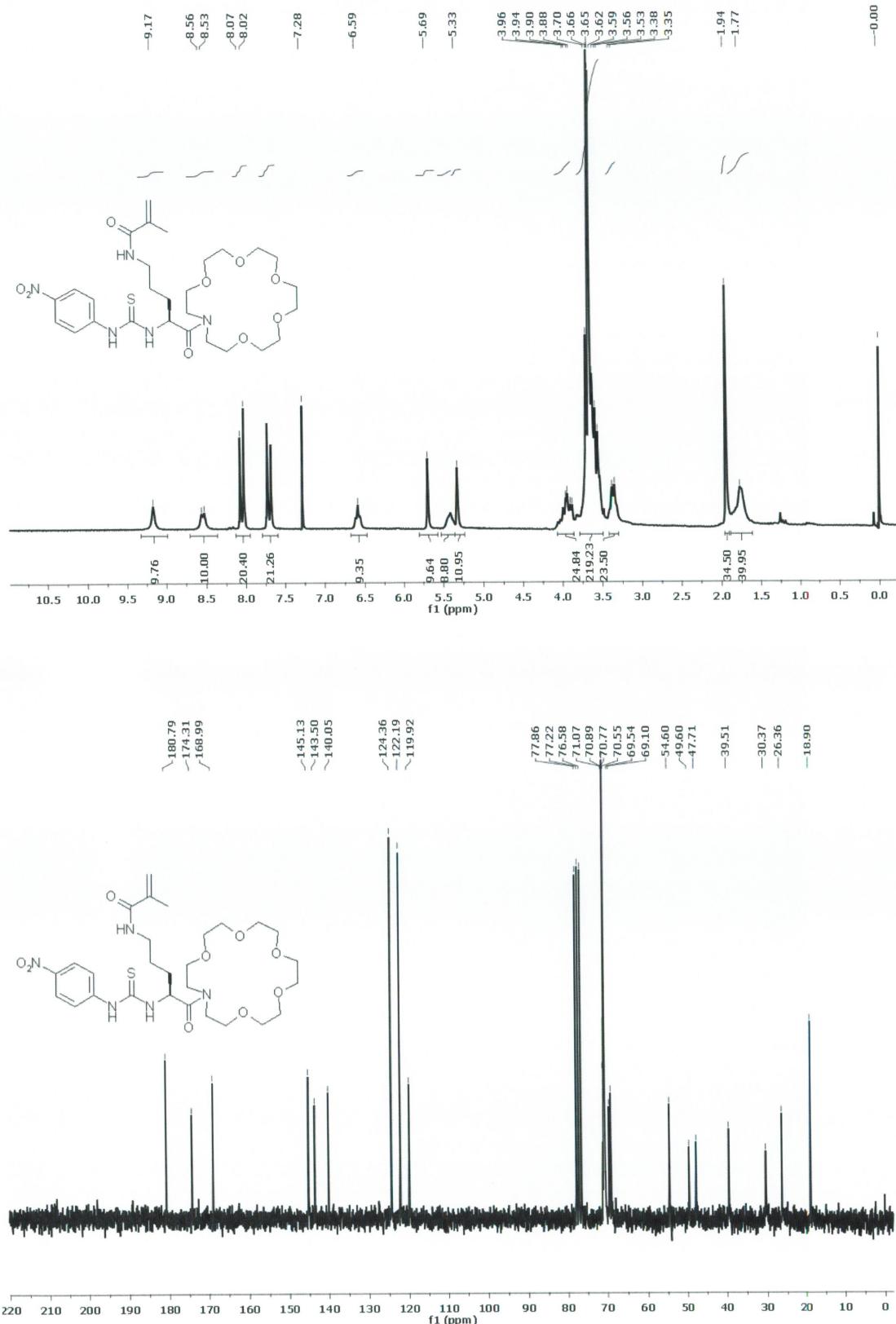
^1H and ^{13}C NMR of S4:



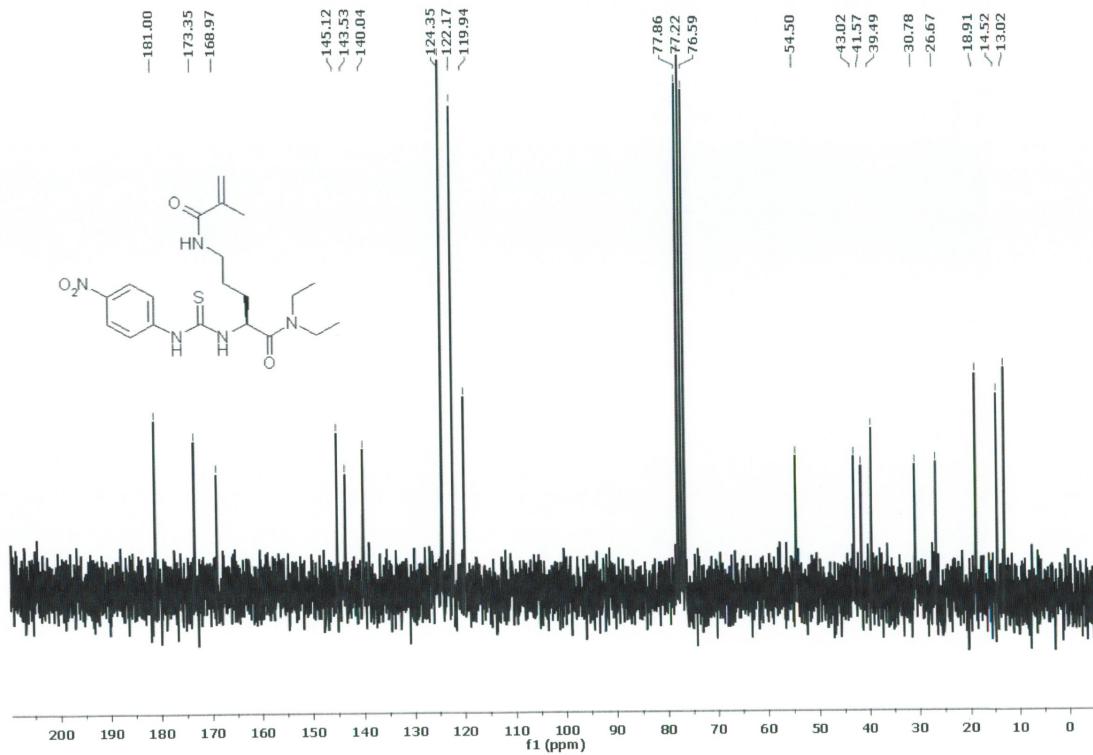
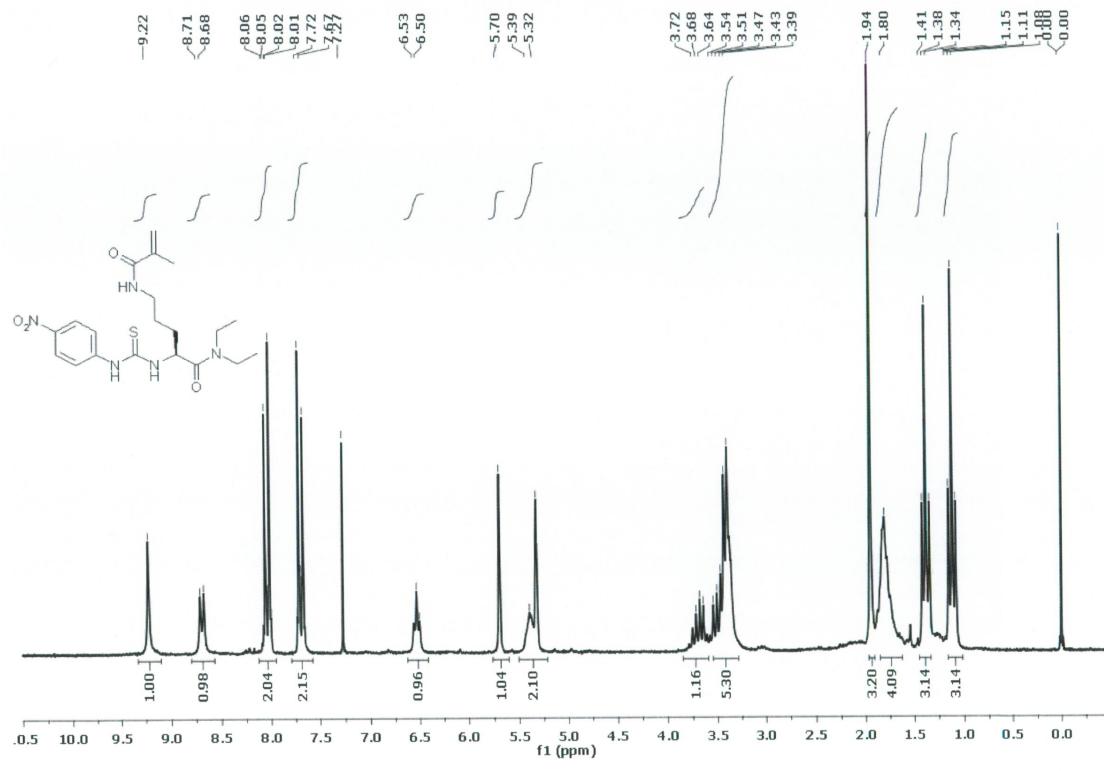
¹H and ¹³C NMR of S6:



¹H and ¹³C NMR of 1:



¹H and ¹³C NMR of reference compound:



¹H NMR of polymer 2:

