Electronic Supplementary Information (ESI)

Bis[zinc(II) dipicolylamino]-functionalised peptides as high affinity receptors for pyrophosphate ions in water

Karen K. Y. Yuen[a] and Katrina A. Jolliffe[a]*

[a] School of Chemistry, The University of Sydney, 2006, NSW, Australia.

Email: kate.jolliffe@sydney.edu.au; Fax: +61 2 9351 3329; Tel; +61 2 9351 2297

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Experimental procedures and characterisation data for compounds 1-10

General Remarks

Optical rotations were obtained using a Perkin Elmer model 341 polarimeter at 589 nm and 20 °C, using the indicated spectroscopic grade solvent.

^{1}H NMR (500 MHz) and ^{13}C{^{1}H} NMR (125 MHz) were determined on a Bruker Avance DPX 500 spectrometer. ^{1}H NMR (400 MHz) and ^{13}C{^{1}H} NMR (100 MHz) were determined on a Bruker Avance DPX 400 spectrometer. ^{1}H NMR (300 MHz) and ^{13}C{^{1}H} NMR (75 MHz) were determined on a Bruker Avance DPX 300 spectrometer. Chemical shifts for ^{1}H NMR are reported in parts per million (ppm) downfield shift from TMS using residual solvent peak of methanol-d_4 (δ_H 3.31 ppm) as internal reference. The data are reported as chemical shift (δ), multiplicity (br = broad, s = singlet, d = doublet, t = triplet, dd = doublet of doublets, dt = doublet of triplets, m = multiplet), coupling constant (*J* Hz), relative integral and assignment where possible. Chemical shifts for ^{13}C{^{1}H} NMR are reported in ppm, relative to the central line of a septet at δ = 49.00 ppm for methanol-d_4.

Infrared absorption spectra were recorded on a Bruker Alpha-E FT-IR spectrometer using attenuated total reflection (ATR) of a thin film. FT-IR are reported in wavenumbers (cm^{-1}).

Low resolution mass spectra were recorded on a Thermo Finnigan LCQ Deca Ion Trap mass spectrometer using electrospray ionisation (ESI, positive mode). HRMS (ESI) were obtained using a Bruker BioApex Fourier Transform Ion Cyclotron Resonance mass spectrometer (FTICR) with an analytical ESI source, operating at 4.7 T and reported as *m/z* (relative intensity).

Liquid chromatography mass spectrometry was performed on a Thermo Separation Products: Spectra System with a P400 Pump and a UV6000LP photodiode array detector. Separation was achieved using a Phenomenex Jupiter column (5 μm, 150 × 2.1 mm ID) coupled to a Thermoquest Finnigan LCQ Deca mass spectrometer (ESI, positive mode). Flow rate was maintained at 0.2 mL min^{-1} over a linear gradient from 0% to 100% solvent B (solvent A: 0.1% formic acid in Milli-Q water, solvent B: 0.1% formic acid in MeCN) over 30 min. Preparative reversed-phase high performance liquid chromatography (RP-HPLC) was performed on a Waters 600E Multisolvent Delivery System with a Waters U6K injector, Waters 490E programmable multiwavelength detector, Waters busSAT/IN module and Waters Empower 2 software. Separation was achieved on a Waters Sunfire™ PrepC_{18} OBD™ column (5 μm, 19 × 150 mm ID). Compounds were eluted at a flow rate of 7 mL min^{-1} over the stated linear gradient in a mobile phase comprising of 0.1% TFA in Milli-Q water (solvent A) and 0.1% TFA in MeCN (solvent B).

UV-Vis spectra were recorded using a Varian Cary 4000 UV-Vis spectrophotometer. Temperature control was provided by a Varian Cary PCB 150 Water Peltier System. pH values were determined using an Activon Model 209 pH/mV meter.

Commercial materials were used as received unless otherwise noted. Amino acids, coupling reagents and resins were obtained from Novabiochem or GL Biochem. Anhydrous CH_2Cl_2 was obtained by distillation over CaH_2 prior to use. Anhydrous DMF was purified by passage through neutral alumina using an Innovative Technology, Inc., PureSolv™ solvent purification system. HPLC grade DMF used for solid phase peptide synthesis was obtained from LabScan or Merck.

Synthesis

As the syntheses for all the compounds were similar, general outlines of the procedures used are given below.

Solid-Phase Peptide Synthesis

Solid-phase peptide synthesis was conducted in Torviq polypropylene fritted-syringes.

Iterative peptide assembly (Fmoc-SPPS)

Rink amide resin (0.62 mmol/g or 0.41 mmol/g as stated) was swollen in dry CH_2Cl_2 for 1 h. The resin was drained, then washed with DMF (× 5), CH_2Cl_2 (× 5) and DMF (× 5). The resin was treated with 10% piperidine/DMF (2 × 3 min) and subsequently washed with DMF (× 5), CH_2Cl_2 (× 5) and DMF (× 5). A solution of appropriate Fmoc-protected amino acid (2 eq. or 4 eq. relative to resin capacity for Lys or other amino acids respectively), HBTU (1.1 eq. relative to peptide) and $^{i}Pr_2NEt$ (2 eq. relative to peptide) in dry DMF (0.1 M) was added and the mixture was agitated at rt for 2 h. The resin was then washed with DMF (× 5), CH_2Cl_2 (× 5) and DMF (× 5).

Deprotection: The resin was treated with 10% piperidine/DMF (2 × 3 min) and washed with DMF (× 5), CH_2Cl_2 (× 5) and DMF (× 5).

Amino acid coupling: A preactivated solution of protected amino acid (2 eq. or 4 eq. relative to resin capacity for Lys or other amino acids respectively), HBTU (1.1 eq. relative to peptide) and $^{i}Pr_2NEt$ (2 eq. relative to peptide) in dry DMF (0.1 M) was added to the resin and agitated at rt for 2 h. The resin was then washed with DMF (× 5), CH_2Cl_2 (× 5) and DMF (× 5).

Acetylation: Upon removal of the Fmoc protecting group, the resin was treated with 20% acetic anhydride/pyridine (3 × 4 min), followed by washing with DMF (× 5), CH_2Cl_2 (× 5) and DMF (× 5).

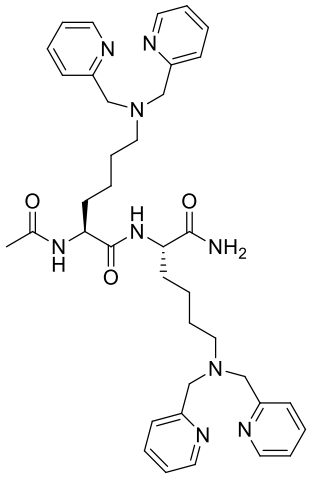
Allyloxycarbonyl (Alloc) deprotection: All Alloc-deprotected peptides were prepared following a modification of the method described by Kates *et al.*[1] The resin was swollen at rt or 15 min in $CHCl_3$/morpholine/acetic acid (90:5:5).
Tetrakis(triphenylphosphine)palladium (1.05 eq. relative to peptide) was added to the

suspension, and the syringe was shielded from light and agitated for 2 h. The resin was drained then washed with $CHCl_3$ (× 5) and a palladium chelating cocktail (DMF/diethyldithiocarbamic acid-3-water/triethylamine 25 mL:225 mg:250 µL). Traces of the chelating cocktail were removed *via* a basic wash (0.5% triethylamine in DMF, × 5). The resin was then washed with MeOH (× 5), DMF (× 5), CH_2Cl_2 (× 5) and DMF (× 5).

Reductive amination: All DPA functionalised peptides were prepared following a modification of the method described by Quinti *et al*.[2] The resin was swollen in dry DMF at rt for 30 min before being treated with 2-pyridinecarboxaldehyde (20 eq.) with 1% acetic acid in DMF and agitated at rt overnight. Sodium triacetoxyborohydride (25 eq.) was then added to the suspension and agitated at rt overnight. The resin was washed with DMF (× 5), CH_2Cl_2 (× 5) and DMF (× 5) and subjected to another cycle of reductive amination procedure to ensure completion of the reaction.

Cleavage: The resin was treated with a solution of trifluoroacetic acid/H_2O/tri*iso*propylsilane (95:2.5:2.5 v/v/v) for 1 h. The resin was drained and then washed with trifluoroacetic acid (× 4). The cleavage solution and acid washes were combined and concentrated *in vacuo*.

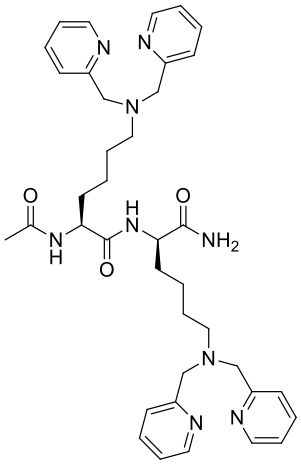
Ac-Lys(DPA)-Lys(DPA)-NH_2, 1



Dipeptide **1** was synthesised on Rink amide resin (0.422 g, 0.261 mmol, resin capacity 0.62 mmol g^{-1}), utilising the general methods for Fmoc-SPPS, Alloc deprotection and reductive amination. Cleavage from the resin was then achieved through treatment with a solution of TFA, tri*iso*propylsilane and water (95:2.5:2.5 v/v/v) for 1 h. The crude peptide was then purified by preparative RP-HPLC (0 to 50% B over 30 min). The appropriate fractions were lyophilised, affording the linear dipeptide **1** as a TFA salt, which was treated with basic anion exchange resin to give the desired dipeptide **1** (t_R = 17.3 min) as a yellow oil.

Yield: 28.2 mg, (16%); $[\alpha]_D$ -17 (*c* 0.6 in MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 8.42-8.43 (d, *J* 4.6 Hz, 4H, PyH), 7.78-7.81 (m, 4H, PyH), 7.61-7.63 (d, *J* 7.9 Hz, 4H, PyH), 7.26-7.28 (m, 4H, PyH), 4.24-4.29 (m, 2H, 2 × α-CH), 3.77 (s, 8H, DPA-CH_2), 2.49-2.52 (m, 4H, CH_2), 1.94 (s, 3H, CH_3), 1.51-1.77 (m, 8H, 2 × CH_2), 1.29-1.41 (m, 4H, CH_2), NH signals not observed; ^{13}C NMR (125 MHz, CD_3OD) δ 176.7, 174.4, 173.5, 160.9, 149.4, 138.8, 125.0, 123.8, 61.2, 55.5, 55.1, 54.3, 33.1, 32.9, 27.8, 27.8, 24.8, 24.7, 22.6; IR (thin film) ν_{max} 3303, 2941, 1652, 1591, 1569, 1434, 1201, 1127 cm^{-1}; MS (ESI) *m/z* = 680 ($[M + H]^+$, 15%), 702 ($[M + Na]^+$, 100); HRMS (ESI) *m/z* = 680.4041 $[M + H]^+$, 680.4031 calcd. for $C_{38}H_{50}N_9O_3^+$.

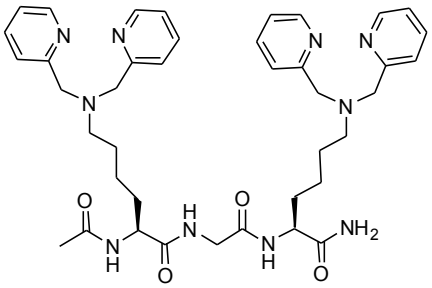
Ac-d-Lys(DPA)-Lys(DPA)-NH_2, 2



Dipeptide **2** was synthesised on Rink amide resin (0.618 g, 0.253 mmol, resin capacity 0.41 mmol g^{-1}), utilising the general methods for Fmoc-SPPS, Alloc deprotection and reductive amination. Cleavage from the resin was then achieved through treatment with a solution of TFA, tri*iso*propylsilane and water (95:2.5:2.5 v/v/v) for 1 h. The crude peptide was then purified by preparative RP-HPLC (0 to 50% B over 30 min). The appropriate fractions were lyophilised, affording the linear dipeptide **2** as a TFA salt, which was treated with basic anion exchange resin to give the desired dipeptide **2** (t_R = 17.4 min) as a yellow oil.

Yield: 31.0 mg, (18%); $[\alpha]_D$ -23 (*c* 0.7 in MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 8.42 (m, 4H, PyH), 7.76-7.78 (m, 4H, PyH), 7.58-7.62 (m, 4H, PyH), 7.24-7.28 (m, 4H, PyH), 4.21-4.25 (m, 1H, 1 × α-CH), 4.14-4.18 (m, 1H, 1 × α-CH), 3.75 (s, 8H, DPA-CH_2), 2.46-2.50 (m, 4H, CH_2), 1.95 (s, 3H, CH_3), 1.52-1.86 (m, 8H, 2 × CH_2), 1.29-1.39 (m, 4H, CH_2), NH signals not observed; ^{13}C NMR (100 MHz, CD_3OD) δ 177.0, 174.9, 173.6, 160.8, 149.4, 138.7, 124.8, 123.7, 61.1, 55.5, 55.4, 55.3, 54.4, 32.4, 32.4, 27.6, 24.9, 24.6, 22.3; IR (thin film) ν_{max} 3881, 3828, 3577, 3428, 3332, 1682, 1191, 1152 cm^{-1}; HRMS (ESI) *m/z* = 680.4041 $[M + H]^+$, 680.4031 calcd. for $C_{38}H_{50}N_9O_3^+$.

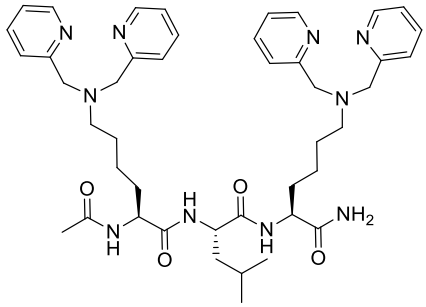
Ac-Lys(DPA)-Gly-Lys(DPA)-NH_2, 3



Tripeptide **3** was synthesised on Rink amide resin (0.401 g, 0.251 mmol, resin capacity 0.62 mmol g^{-1}), utilising the general methods for Fmoc-SPPS, Alloc deprotection and reductive amination. Cleavage from the resin was then achieved through treatment with a solution of TFA, tri*iso*propylsilane and water (95:2.5:2.5 v/v/v) for 1 h. The crude peptide was then purified by preparative RP-HPLC (0 to 50% B over 30 min). The appropriate fractions were lyophilised, affording the linear tripeptide **3** as a TFA salt, which was treated with basic anion exchange resin to give the desired tripeptide **3** (t_R = 17.0 min) as a yellow oil.

Yield: 26.4 mg, (14%); $[\alpha]_D$ -21 (*c* 0.7 in MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 8.41-8.43 (m, 4H, PyH), 7.77-7.83 (m, 4H, PyH), 7.61-7.65 (m, 4H, PyH), 7.25-7.29 (m, 4H, PyH), 4.25-4.29 (m, 1H, α-CH), 4.11-4.16 (m, 1H, α-CH), 3.77 (m, 10H, Gly-CH_2, DPA-CH_2), 2.49-2.52 (m, 4H, CH_2), 1.94 (s, 3H, CH_3), 1.51-1.78 (m, 8H, 2 × CH_2), 1.29-1.41 (m, 4H, CH_2), NH signals not observed; ^{13}C NMR (75 MHz, CD_3OD) δ 177.0, 175.3, 173.6, 171.5, 160.8, 149.3, 138.7, 124.9, 123.7, 61.1, 55.6, 55.4, 55.3, 54.6, 43.6, 32.7, 32.3, 27.7, 24.7, 22.5; IR (thin film) ν_{max} 3296, 1668, 1538, 1468, 1130 cm^{-1}; MS (ESI) *m/z* = 369 ([M + 2H]$^{2+}$, 100%), 737 ([M + H]$^+$, 40); HRMS (ESI), *m/z* = 737.4249 [M + H]$^+$, 737.4246 calcd. for $C_{40}H_{53}N_{10}O_4^+$.

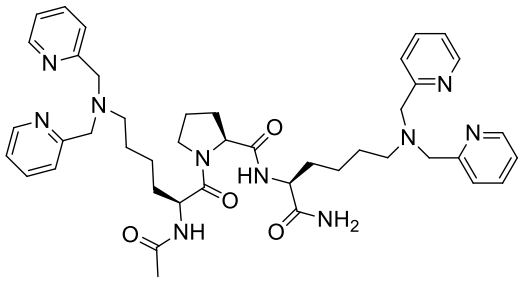
Ac-Lys(DPA)-Leu-Lys(DPA)-NH_2, 4



Tripeptide **4** was synthesised on Rink amide resin (0.505 g, 0.313 mmol, resin capacity 0.62 mmol g^{-1}), utilising the general methods for Fmoc-SPPS, Alloc deprotection and reductive amination. Cleavage from the resin was then achieved through treatment with a solution of TFA, tri*iso*propylsilane and water (95:2.5:2.5 v/v/v) for 1 h. The crude peptide was then purified by preparative RP-HPLC (0 to 70% B over 60 min). The appropriate fractions were lyophilised, affording the linear tripeptide **4** as a TFA salt, which was treated with basic anion exchange resin to give the desired tripeptide **4** (t_R = 23.8 min) as a yellow oil.

Yield: 40.6 mg, (16%); $[\alpha]_D$ -31 (*c* 0.8 in MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 8.63 (m, 4H, PyH), 7.86-7.89 (m, 4H, PyH), 7.48-7.50 (d, *J* 7.8 Hz , 4H, PyH), 7.41-7.44 (m, 4H, PyH), 4.59 (s, 8H, DPA-CH_2), 4.31 (m, 1H, α-CH), 4.22-4.28 (m, 2H, 2 × α-CH), 1.94 (s, 3H, CH_3), 1.75-1.89 (m, 8H, 2 × CH_2), 1.56-1.72 (m, 6H, CH_2, Leu-CH_2), 1.34-1.44 (m, 5H, CH_2, Leu-CH), 0.86-0.92 (dd, *J* 6.4, 6.4 Hz, 6H, 2 × Leu-CH_3), NH signals not observed; ^{13}C NMR (125 MHz, CD_3OD) δ 176.4, 174.7, 174.6, 173.7, 151.9, 151.8, 150.3, 150.3, 139.5, 125.5, 125.5, 58.5, 56.0, 55.0, 54.1, 53.5, 41.3, 32.3, 32.1, 25.9, 24.8, 24.8, 23.9, 23.8, 23.5, 22.5, 21.7; IR (thin film) ν_{max} 3369, 2956, 1666, 1594, 1542, 1440, 1200, 1178, 1130 cm^{-1}; HRMS (ESI), *m/z* = 397.2471 $[M + 2H]^{2+}$, 397.2472 calcd. for $C_{44}H_{62}N_{10}O_4^{2+}$.

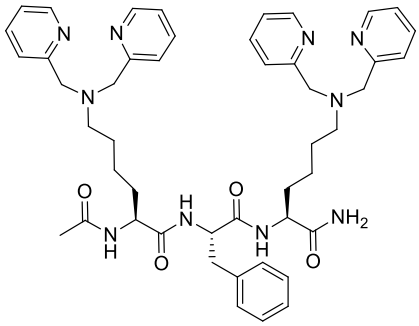
Ac-Lys(DPA)-Pro-Lys(DPA)-NH_2, 5



Tripeptide **5** was synthesised on Rink amide resin (0.502 g, 0.311 mmol, resin capacity 0.62 mmol g^{-1}), utilising the general methods for Fmoc-SPPS, Alloc deprotection and reductive amination. Cleavage from the resin was then achieved through treatment with a solution of TFA, tri*iso*propylsilane and water (95:2.5:2.5 v/v/v) for 1 h. The crude peptide was then purified by preparative RP-HPLC (0 to 70% B over 60 min). The appropriate fractions were lyophilised, affording the linear tripeptide **5** as a TFA salt, which was treated with basic anion exchange resin to give the desired tripeptide **5** (t_R = 24.7 min) as a colourless oil.

Yield: 25.9 mg, (11%); $[\alpha]_D$ = -27 (*c* 0.8 in MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 8.55 (m, 4H, PyH), 7.82-7.86 (dd, *J* 7.8, 7.8 Hz, 4H, PyH), 7.54-7.56 (d, *J* 7.7 Hz, 4H, PyH), 7.36 (m, 4H, PyH), 4.50-4.55 (m, 1H, α-CH), 4.39-4.42 (m, 1H, α-CH), 4.22-4.25 (m, 8H, DPA-CH_2), 3.78-3.84 (m, 1H, Pro-H), 3.55-3.61 (m, 1H, Pro-H), 2.92-3.00 (m, 4H, CH_2), 2.17-2.23 (m, 1H, Pro-H), 1.92-2.06 (m, 7H, 4 × Pro-H, CH_3), 1.55-1.81 (m, 8H, 2 × CH_2), 1.29-1.46 (m, 4H, CH_2), NH signals not observed; ^{13}C NMR (100 MHz, CD_3OD) δ 176.7, 174.3, 173.1, 172.9, 150.0, 150.0, 148.6, 139.0, 125.2, 124.7, 124.7, 61.6, 59.7, 55.9, 55.8, 54.4, 52.5, 32.5, 31.9, 30.6, 26.2, 26.1, 26.0, 24.2, 24.0, 22.3; IR (thin film) ν_{max} 3288, 2926, 1668, 1437, 1200, 1128 cm^{-1}; HRMS (ESI), *m/z* = 411.2134 $[M + 2Na]^{2+}$, 411.2135 calcd. for $C_{43}H_{56}N_{10}Na_2O_4^{2+}$.

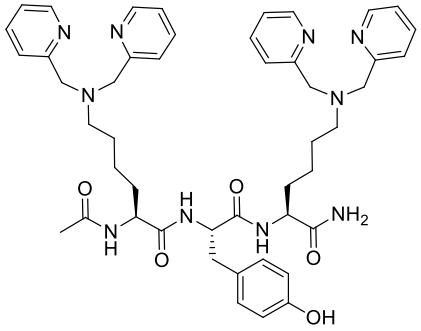
Ac-Lys(DPA)-Phe-Lys(DPA)-NH_2, 6



Tripeptide **6** was synthesised on Rink amide resin (0.478 g, 0.296 mmol, resin capacity 0.62 mmol g^{-1}), utilising the general methods for Fmoc-SPPS, Alloc deprotection and reductive amination. Cleavage from the resin was then achieved through treatment with a solution of TFA, tri*iso*propylsilane and water (95:2.5:2.5 v/v/v) for 1 h. The crude peptide was then purified by preparative RP-HPLC (0 to 70% B over 60 min). The appropriate fractions were lyophilised, affording the linear tripeptide **6** as a TFA salt, which was treated with basic anion exchange resin to give the desired tripeptide **6** (t_R = 24.6 min) as a colourless oil.

Yield: 29.0 mg, (12%); $[\alpha]_D$ -25 (*c* 0.8 in MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 8.42-8.44 (m, 4H, PyH), 7.78-7.81 (m, 4H, PyH), 7.60-7.63 (t, *J* 7.6 Hz, 4H, PyH), 7.20-7.28 (m, 8H, PyH, Phe-Ar-H), 7.12-7.16 (m, 1H, Phe-Ar-H), 4.61-4.64 (m, 1H, α-CH), 4.23-4.26 (m, 1H, α-CH), 4.15-4.18 (m, 1H, α-CH), 3.76-3.77 (d, *J* 8.0 Hz, 8H, DPA-CH_2), 3.13-3.17 (dd, *J* 5.7, 5.7 Hz, 1H, Phe-CH_2), 2.93-2.98 (dd, *J* 8.6, 8.6 Hz, 1H, Phe-CH_2), 2.44-2.51 (m, 4H, CH_2), 1.91 (s, 3H, CH_3), 1.48-1.71 (m, 8H, 2 × CH_2), 1.18-1.35 (m, 4H, CH_2), NH signals not observed; ^{13}C NMR (125 MHz, CD_3OD) δ 176.6, 174.4, 173.4, 173.1, 160.8, 149.4, 149.4, 138.7, 138.6, 138.2, 130.4, 129.5, 127.8, 124.8, 124.8, 123.7, 61.1, 61.1, 55.8, 55.4, 55.3, 55.2, 54.6, 38.4, 32.8, 32.5, 27.7, 27.7, 24.7, 24.5, 22.5; IR (thin film) ν_{max} 3291, 2938, 1644, 1593, 1541, 1434 cm^{-1}; HRMS (ESI), *m/z* = 849.4537 $[M + Na]^+$, 849.4535 calcd. for $C_{47}H_{58}N_{10}NaO_4^+$.

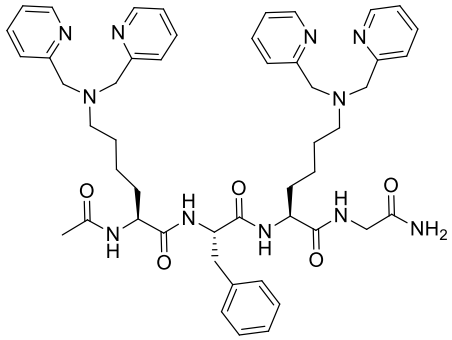
Ac-Lys(DPA)-Tyr-Lys(DPA)-NH_2, 7



Tripeptide **7** was synthesised on Rink amide resin (0.481 g, 0.298 mmol, resin capacity 0.62 mmol g^{-1}), utilising the general methods for Fmoc-SPPS, Alloc deprotection and reductive amination. Cleavage from the resin was then achieved through treatment with a solution of TFA, tri*iso*propylsilane and water (95:2.5:2.5 v/v/v) for 1 h. The crude peptide was then purified by preparative RP-HPLC (0 to 70% B over 60 min). The appropriate fractions were lyophilised, affording the linear tripeptide **7** as a TFA salt, which was treated with basic anion exchange resin to give the desired tripeptide **7** (t_R = 22.6 min) as a colourless oil.

Yield: 32.3 mg, (13%); $[\alpha]_D$ -20 (*c* 0.7 in MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 8.45 (m 4H, PyH), 7.80-7.83 (dd, *J* 7.4, 7.4 Hz, 4H, PyH), 7.62-7.65 (dd, *J* 7.8, 7.8 Hz, 4H, PyH), 7.27-7.31 (m, 4H, PyH), 7.03-7.04 (d, *J* 8.4 Hz, 2H, Tyr-Ar-H), 6.69-6.70 (d, *J* 8.4 Hz, 2H, Tyr-Ar-H), 4.52-4.55 (m, 1H, α-CH), 4.21-4.24 (m, 1H, α-CH), 4.13-4.16 (m, 1H, α-CH), 3.78-3.80 (d, *J* 7.5 Hz, 8H, DPA-CH_2), 3.04-3.08 (dd, *J* 5.7, 5.7 Hz, 1H, Tyr-CH_2), 2.88-2.91 (m, 1H, Tyr-CH_2), 2.48-2.55 (dt, *J* 2.4, 7.4 Hz, 4H, CH_2), 1.93 (s, 3H, CH_3), 1.50-1.74 (m, 8H, 2 × CH_2), 1.21-1.34 (m, 4H, CH_2), NH and OH signals not observed; ^{13}C NMR (125 MHz, CD_3OD) δ 176.7, 174.4, 173.6, 173.4, 160.8, 157.6, 151.3, 149.3, 138.7, 138.7, 131.4, 128.6, 124.9, 123.7, 116.4, 61.1, 61.1, 56.2, 56.1, 55.5, 55.4, 55.4, 54.7, 37.5, 32.7, 32.4, 30.7, 28.7, 27.7, 27.7, 24.7, 24.6, 22.5; IR (thin film) ν_{max} 3308, 2929, 1655, 1594, 1516, 1436, 1375 cm^{-1}; HRMS (ESI), *m/z* = 422.2368 $[M + 2H]^{2+}$, 422.2369 calcd. for $C_{47}H_{60}N_{10}O_5^{2+}$.

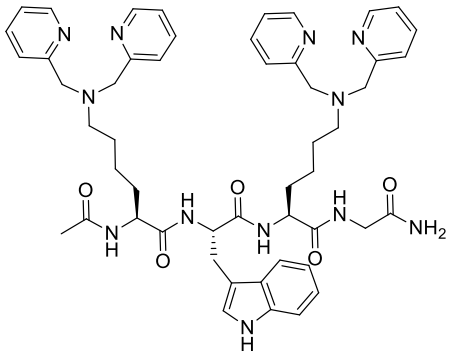
Ac-Lys(DPA)-Phe-Lys(DPA)-Gly-NH_2, 8



Tetrapeptide **8** was synthesised on Rink amide resin (0.617 g, 0.252 mmol, resin capacity 0.41 mmol g^{-1}), utilising the general methods for Fmoc-SPPS, Alloc deprotection and reductive amination. Cleavage from the resin was then achieved through treatment with a solution of TFA, tri*iso*propylsilane and water (95:2.5:2.5 v/v/v) for 1 h. The crude peptide was then purified by preparative RP-HPLC (0 to 70% B over 60 min). The appropriate fractions were lyophilised, affording the linear tetrapeptide **8** as a TFA salt, which was treated with basic anion exchange resin to give the desired tetrapeptide **8** (t_R = 26.8 min) as a colourless oil.

Yield: 34.0 mg, (15%); $[\alpha]_D$ -114 (*c* 0.2 in MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 8.50-8.51 (m, 4H, PyH), 7.80-7.85 (m, 4H, PyH), 7.55-7.58 (m, 4H, PyH), 7.31-7.35 (m, 4H, PyH), 7.11-7.21 (m, 5H, Phe-Ar-H), 4.55-4.60 (m, 1H, α-CH), 4.05-4.20 (m, 10H, α-CH, DPA-CH_2), 3.71-3.90 (diastereotopic d, *J* 17.1, 23.4 Hz, 2H, Gly-CH_2), 3.13-3.19 (m, 1H, Phe-CH_2), 2.89-2.98 (m, 1H, Phe-CH_2), 2.70-2.84 (m, 4H, 2 × CH_2), 1.91 (s, 3H, CH_3), 1.47-1.82 (m, 8H, 2 × CH_2), 1.17-1.31 (m, 4H, CH_2), NH signals not observed; ^{13}C NMR (75 MHz, CD_3OD) δ 174.5, 174.2, 174.2, 173.8, 173.7, 157.7, 157.2, 149.8, 149.8, 139.0, 138.9, 138.3, 130.4, 129.5, 127.8, 125.1, 125.1, 124.5, 124.4, 60.2, 60.1, 56.0, 55.7, 55.6, 55.3, 55.2, 43.2, 38.1, 32.2, 31.9, 26.6, 26.5, 24.3, 22.5, signals obscured or overlapping; IR (thin film) ν_{max} 3909, 3633, 3586, 3266, 3184, 3064, 2936, 1660, 1594, 1542, 1433 cm^{-1}; HRMS (ESI), *m/z* = 906.4732 $[M + Na]^+$, 906.4749 calcd. for $C_{49}H_{61}N_{11}NaO_5^+$.

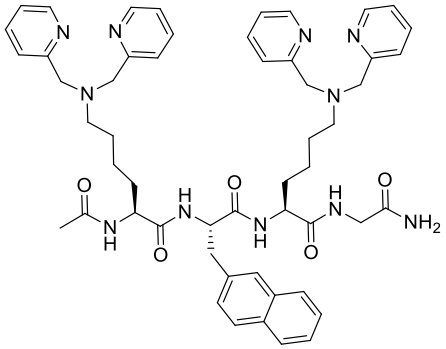
Ac-Lys(DPA)-Trp-Lys(DPA)-Gly-NH_2, 9



Tetrapeptide **9** was synthesised on Rink amide resin (0.609 g, 0.250 mmol, resin capacity 0.41 mmol g^{-1}), utilising the general methods for Fmoc-SPPS, Alloc deprotection and reductive amination. Cleavage from the resin was then achieved through treatment with a solution of TFA, tri*iso*propylsilane and water (95:2.5:2.5 v/v/v) for 1 h. The crude peptide was then purified by preparative RP-HPLC (0 to 70% B over 60 min). The appropriate fractions were lyophilised, affording the linear tetrapeptide **9** as a TFA salt, which was treated with basic anion exchange resin to give the desired tetrapeptide **9** (t_R = 27.7 min) as a colourless oil.

Yield: 52.0 mg, (23%); $[\alpha]_D$ -23 (*c* 0.9 in MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 8.49 (m, 4H, PyH), 7.78-7.83 (m, 4H, PyH), 7.53-7.56 (m, 4H, PyH), 7.27-7.33 (m, 4H, PyH), 7.14 (s, 1H, Trp-H), 6.95-7.06 (m, 4H, Trp-H), 4.57-4.61 (m, 1H, α-CH), 4.00-4.12 (m, 10H, DPA-CH_2, 2 × α-CH), 3.65-3.81 (diastereotopic d, *J* 12.6, 17.1 Hz, 2H, Gly-CH_2), 3.22-3.27 (m, 2H, Trp-CH_2), 2.67-2.74 (m, 4H, CH_2), 1.81 (s, 3H, CH_3), 1.50-1.70 (m, 8H, 2 × CH_2), 1.21-1.28 (m, 2H, CH_2), 1.05-1.13 (m, 2H, CH_2), NH signals not observed; ^{13}C NMR (75 MHz, CD_3OD) δ 174.7, 174.4, 174.3, 174.2, 173.9, 157.9, 157.5, 149.8, 149.7, 138.9, 138.9, 138.0, 128.7, 125.0, 125.0, 124.4, 124.3, 122.7, 120.1, 119.4, 112.5, 110.4, 60.3, 60.1, 55.8, 55.7, 55.6, 55.3, 43.2, 32.0, 31.7, 28.0, 26.7, 26.6, 24.3, 24.2, 22.4, signals obscured or overlapping ; IR (thin film) ν_{max} 3750, 3529, 3290, 2937, 2700, 1670, 1542, 1435, 1374, 1202, 1134 cm^{-1}; HRMS (ESI), *m/z* = 923.5039 $[M + H]^+$, 922.5039 calcd. for $C_{51}H_{63}N_{12}O_5^+$.

Ac-Lys(DPA)-Ala(1-naphthyl)-Lys(DPA)-Gly-NH_2, 10

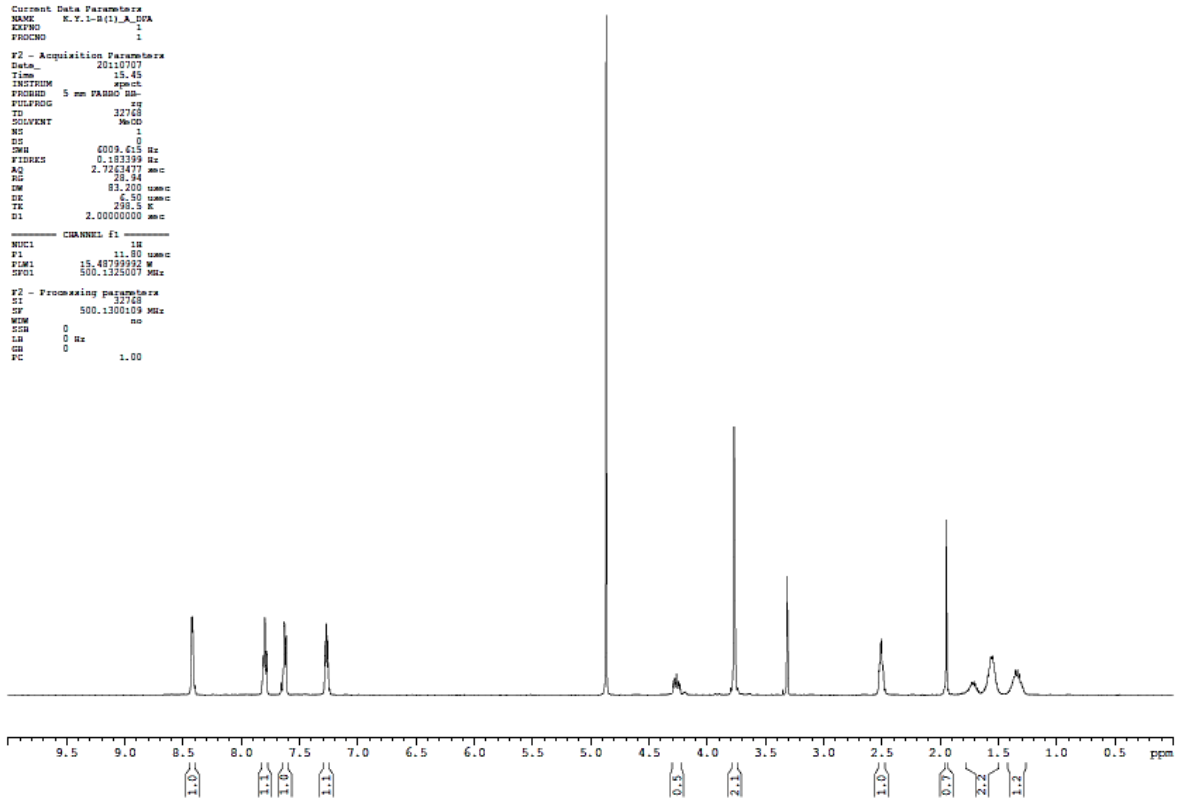


Tetrapeptide **10** was synthesised on Rink amide resin (0.609 g, 0.249 mmol, resin capacity 0.41 mmol g^{-1}), utilising the general methods for Fmoc-SPPS, Alloc deprotection and reductive amination. Cleavage from the resin was then achieved through treatment with a solution of TFA, tri*iso*propylsilane and water (95:2.5:2.5 v/v/v) for 1 h. The crude peptide was then purified by preparative RP-HPLC (0 to 70% B over 60 min). The appropriate fractions were lyophilised, affording the linear tetrapeptide **10** as a TFA salt, which was treated with basic anion exchange resin to give the desired tetrapeptide **10** (t_R = 27.2 min) as a colourless oil.

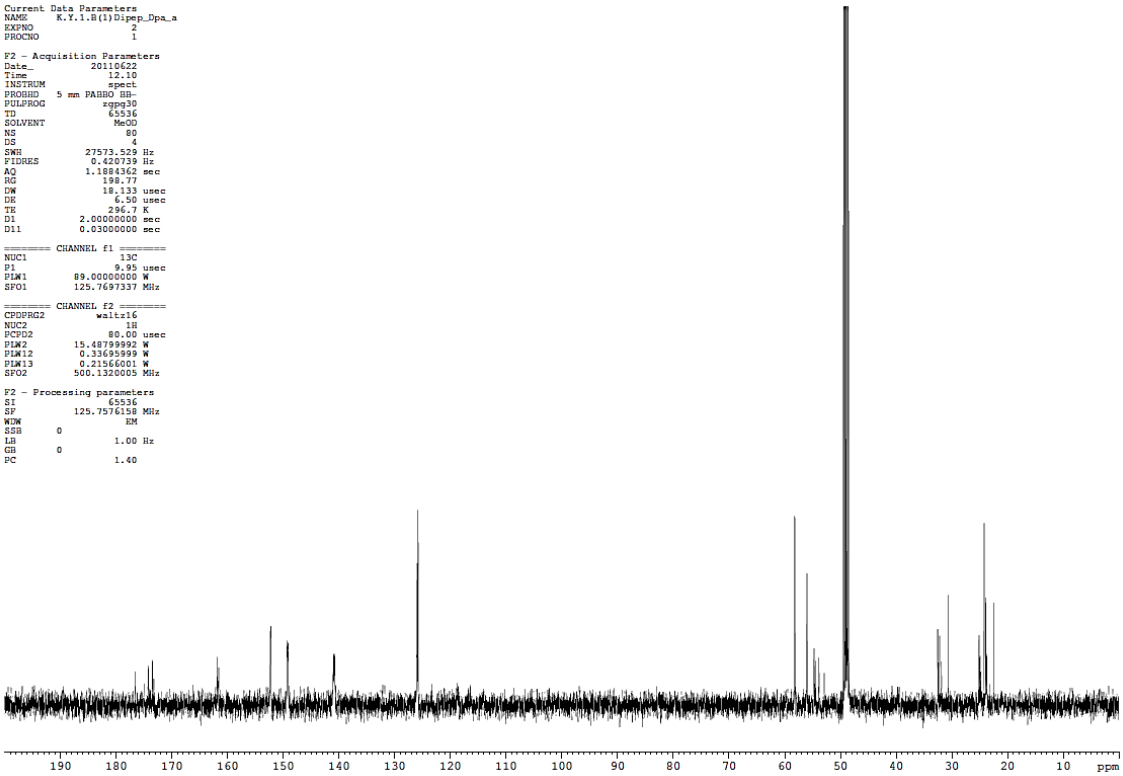
Yield: 39.5 mg, (17%); $[\alpha]_D$ -53 (*c* 0.3 in MeOH); ^{1}H NMR (300 MHz, CD_3OD) δ 8.41-8.42 (m, 4H, PyH), 7.55-7.81 (m, 12H, 3 × PyH), 7.34-7.41 (m, 3H, Ala(1-naphthyl)-Ar-H), 7.24-7.28 (m, 4H, Ala(1-naphthyl)-Ar-H), 4.68-4.73 (m, 1H, α-CH), 4.06-4.16 (m, 2H, 2 × α-CH), 3.59-3.83 (m, 10H, DPA-CH_2, Gly-CH_2), 3.08-3.16 (m, 2H, Ala(1-naphthyl)-CH_2), 2.32-2.47 (m, 4H, 2 × CH_2), 1.81 (s, 3H, CH_3), 1.11-1.72 (m, 12H, 3 × CH_2), NH signals not observed; ^{13}C NMR (75 MHz, CD_3OD) δ 174.6, 174.4, 174.3, 173.8, 173.6, 160.9, 160.8, 149.4, 138.7, 138.7, 135.8, 134.9, 133.9, 129.1, 129.1, 128.7, 128.7, 128.5, 127.2, 126.8, 124.8, 123.7, 61.1, 61.0, 55.8, 55.5, 55.5, 55.4, 55.2, 43.2, 38.4, 32.4, 32.1, 27.7, 27.6, 24.6, 24.5, 22.4, signals obscured or overlapping; IR (thin film) ν_{max} 3852, 3737, 3654, 3293, 2352, 1651, 1594, 1539, 1435, 1368, 1299 cm^{-1}; HRMS (ESI), *m/z* = 956.4909 $[M + Na]^+$, 956.4906 calcd. for $C_{53}H_{63}N_{11}NaO_5^+$.

1H and 13C NMR spectra of compounds 1-10

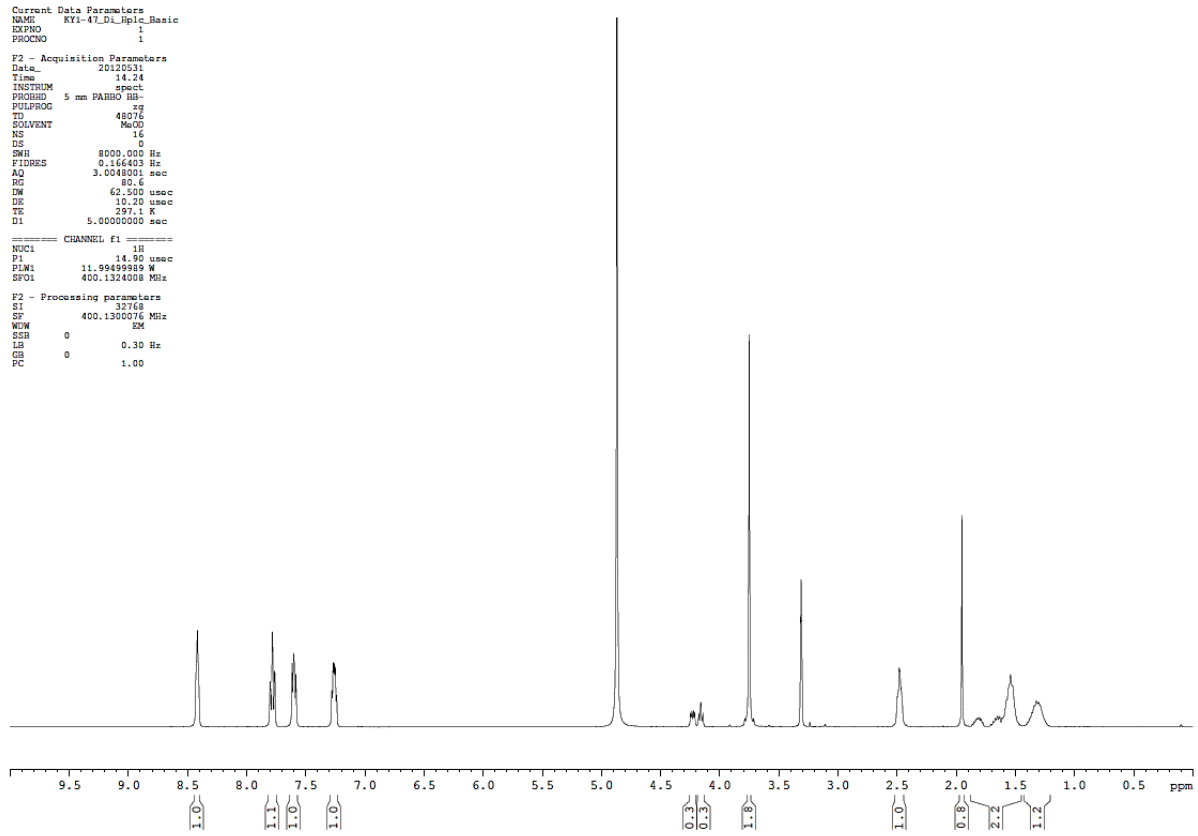
Compound 1, ^{1}H NMR (CD_3OD, 500 MHz)



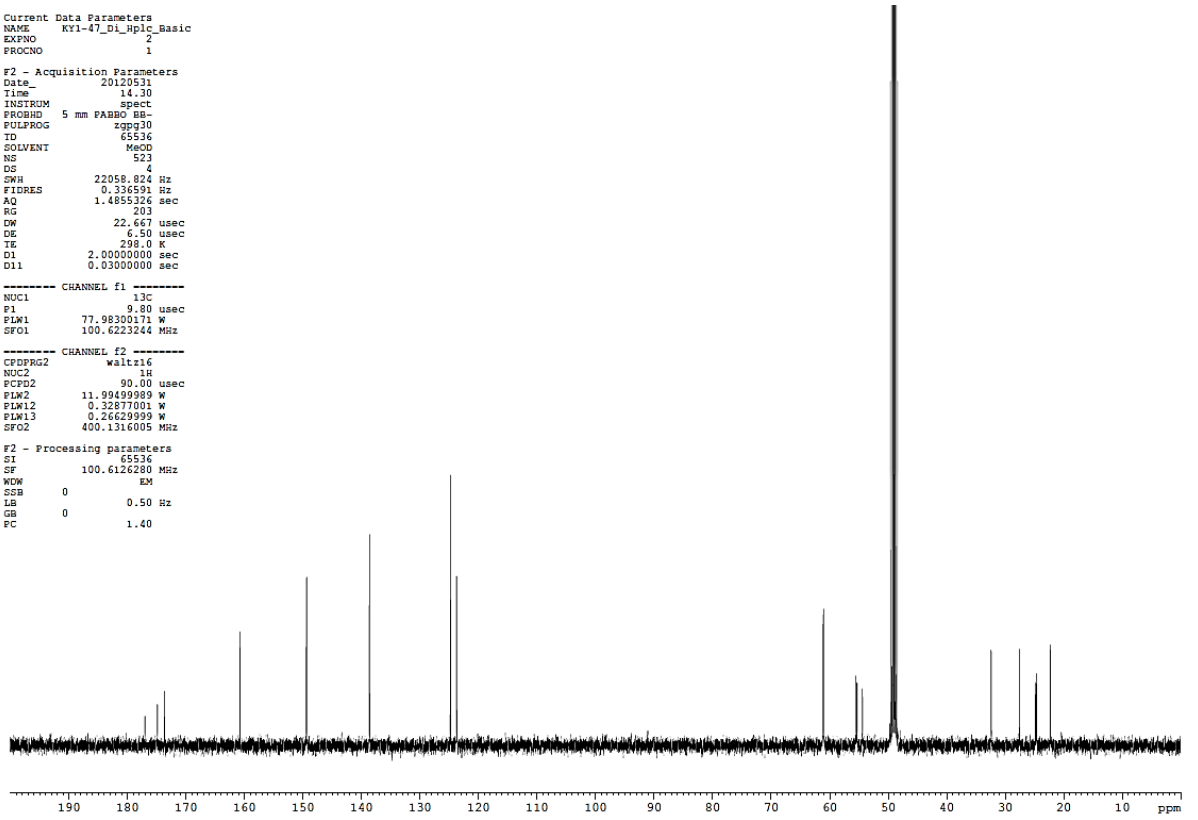
Compound 1, $^{13}C\{^{1}H\}$ NMR (CD_3OD, 125 MHz)



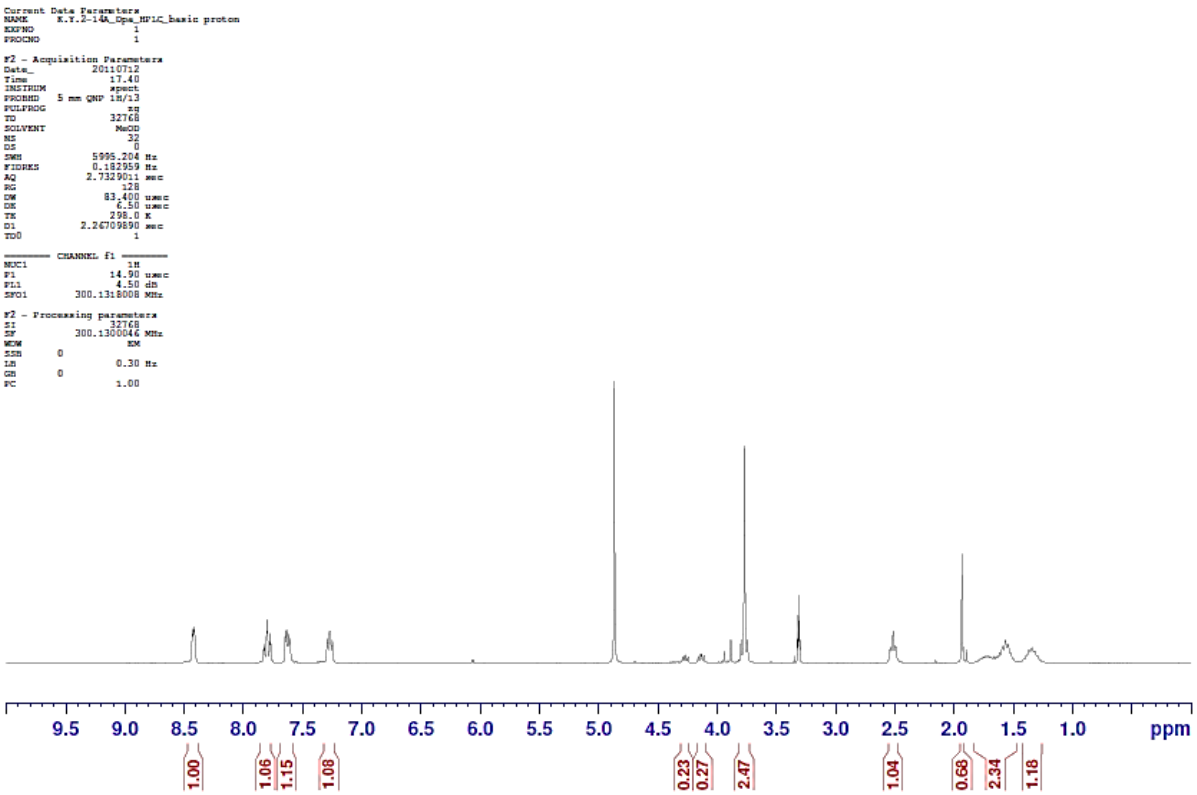
Compound 2, ^{1}H NMR (CD_3OD, 400 MHz)



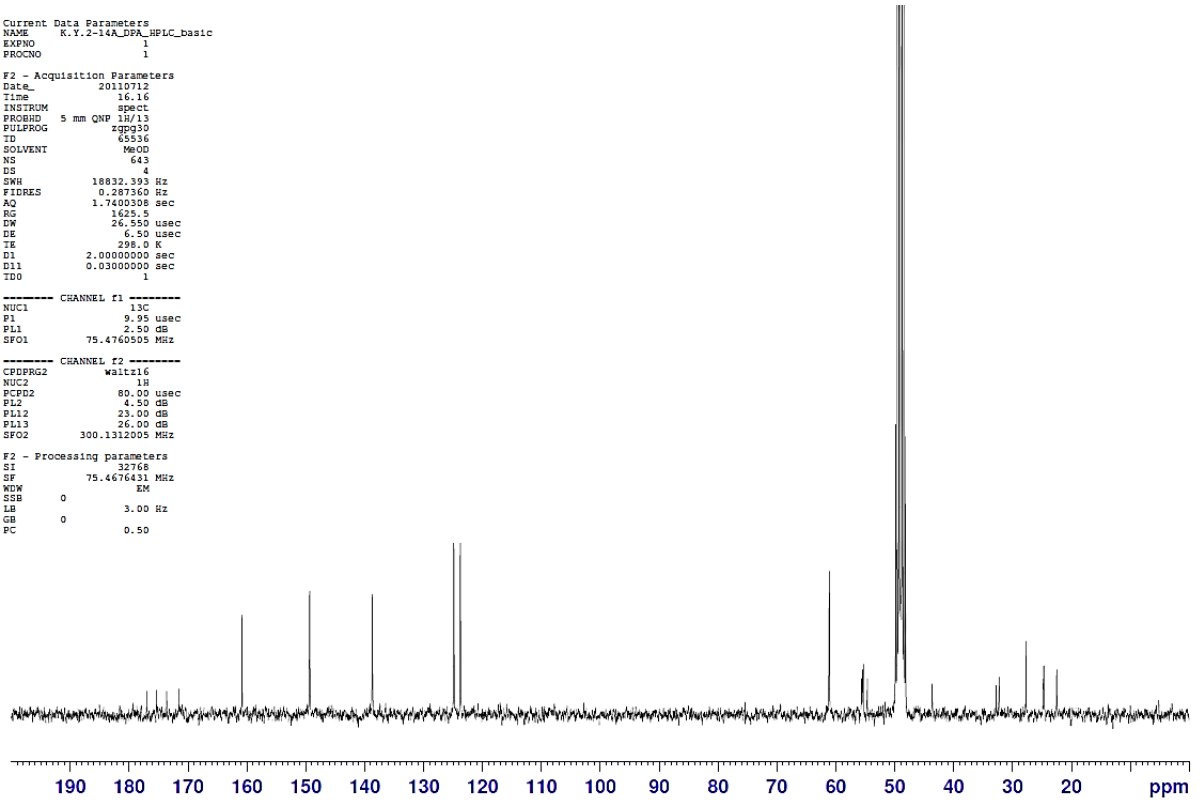
Compound 2, $^{13}C\{^{1}H\}$ NMR (CD_3OD, 100 MHz)



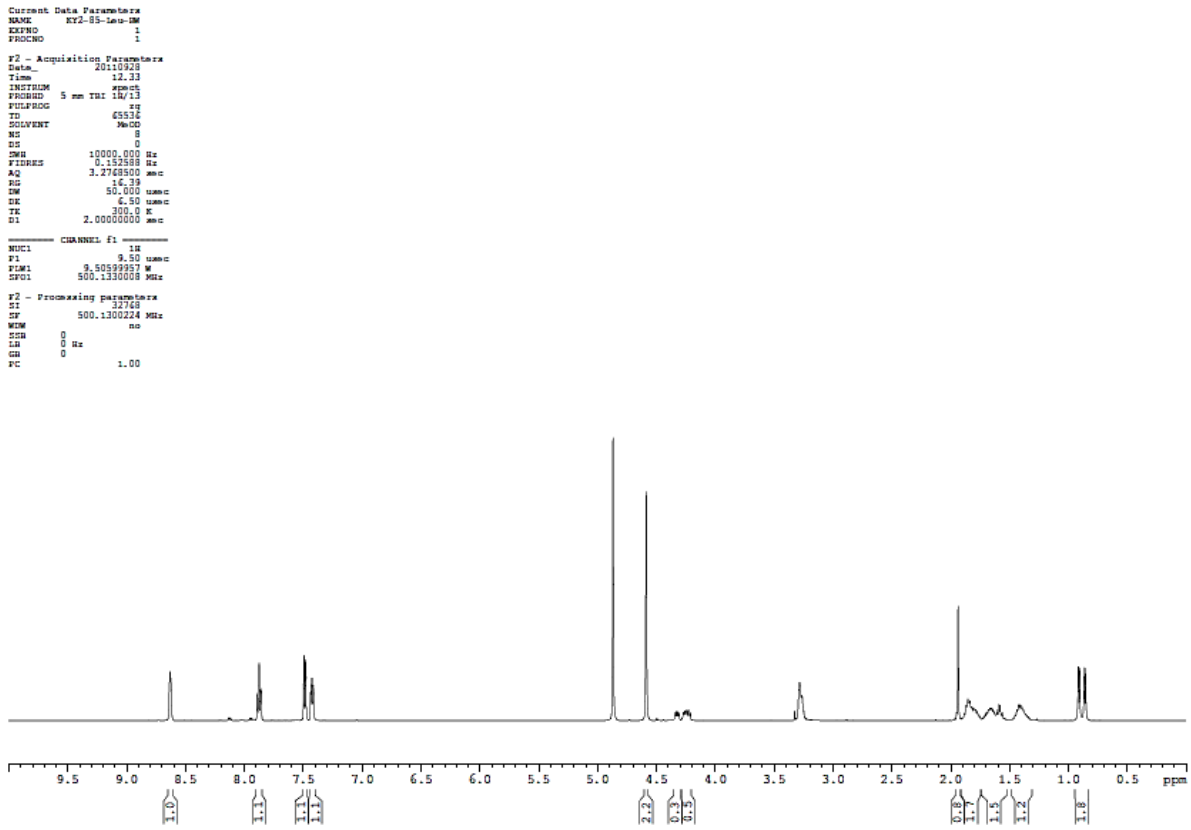
Compound 3, ^{1}H NMR (CD_3OD, 300 MHz)



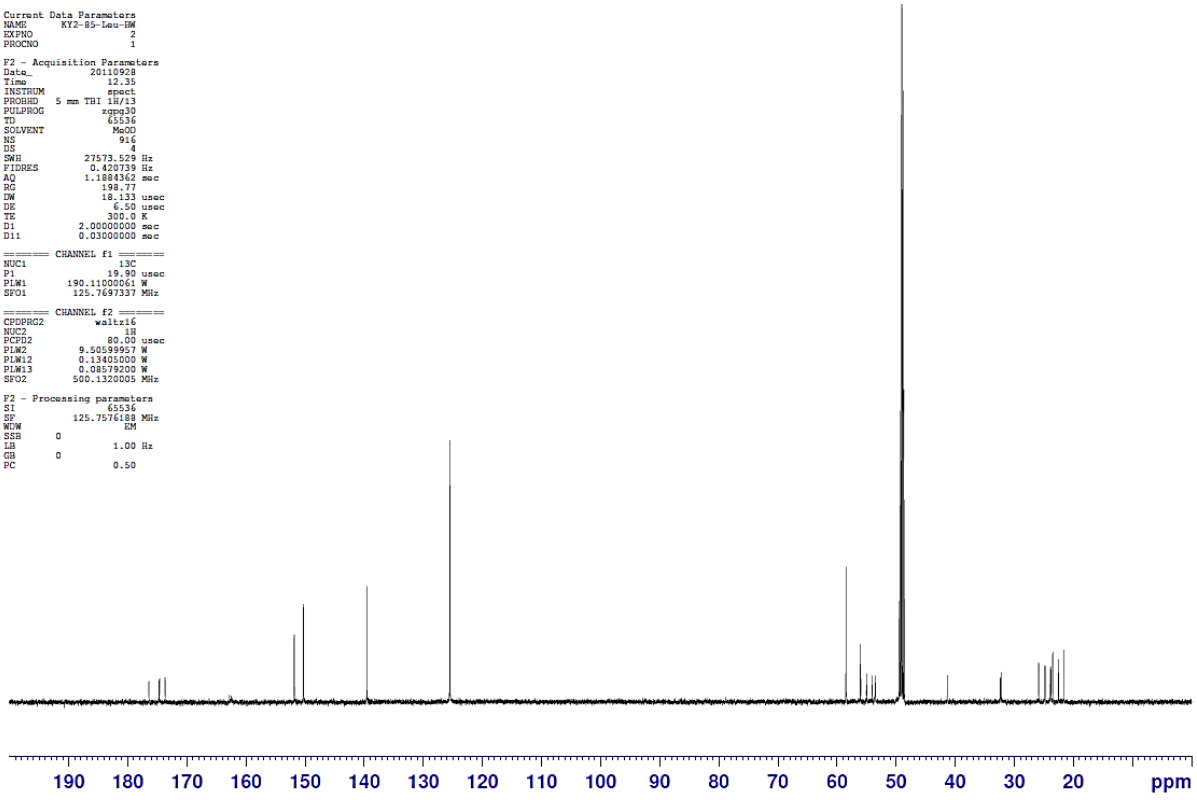
Compound 3, $^{13}C\{^{1}H\}$ NMR (CD_3OD, 75 MHz)



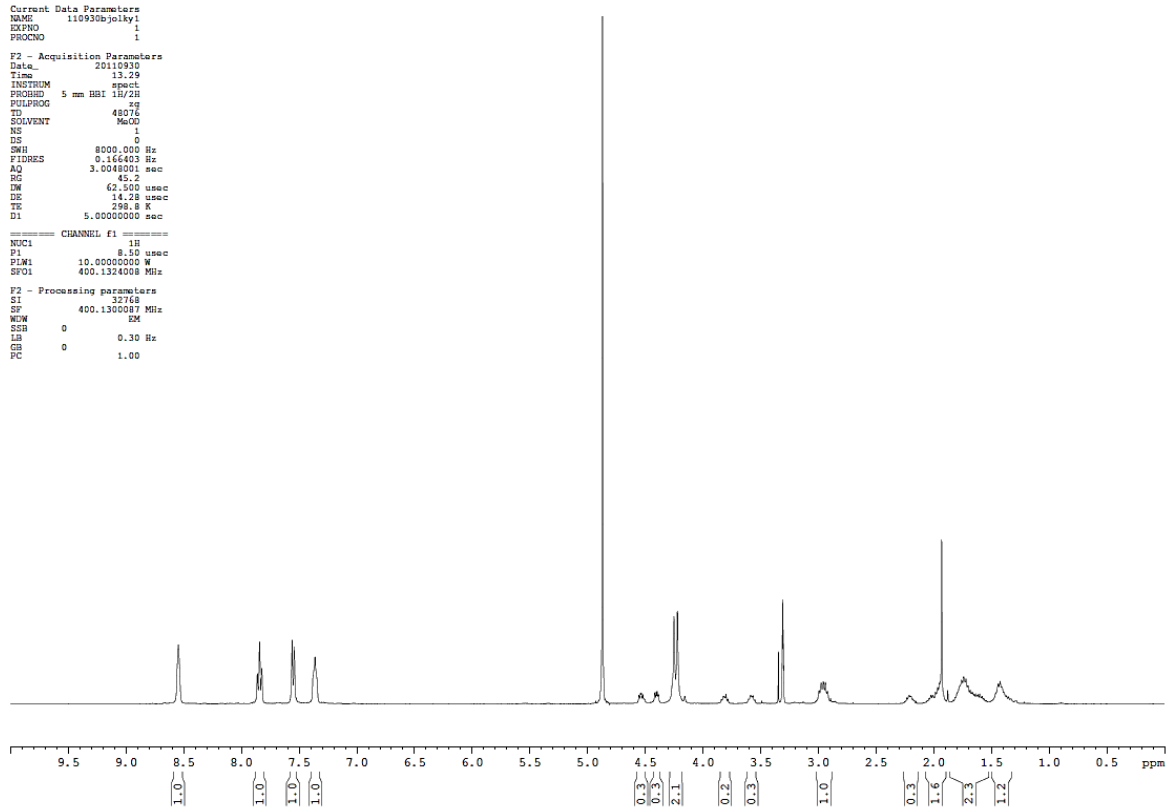
Compound 4, ^{1}H NMR (CD_3OD, 500 MHz)



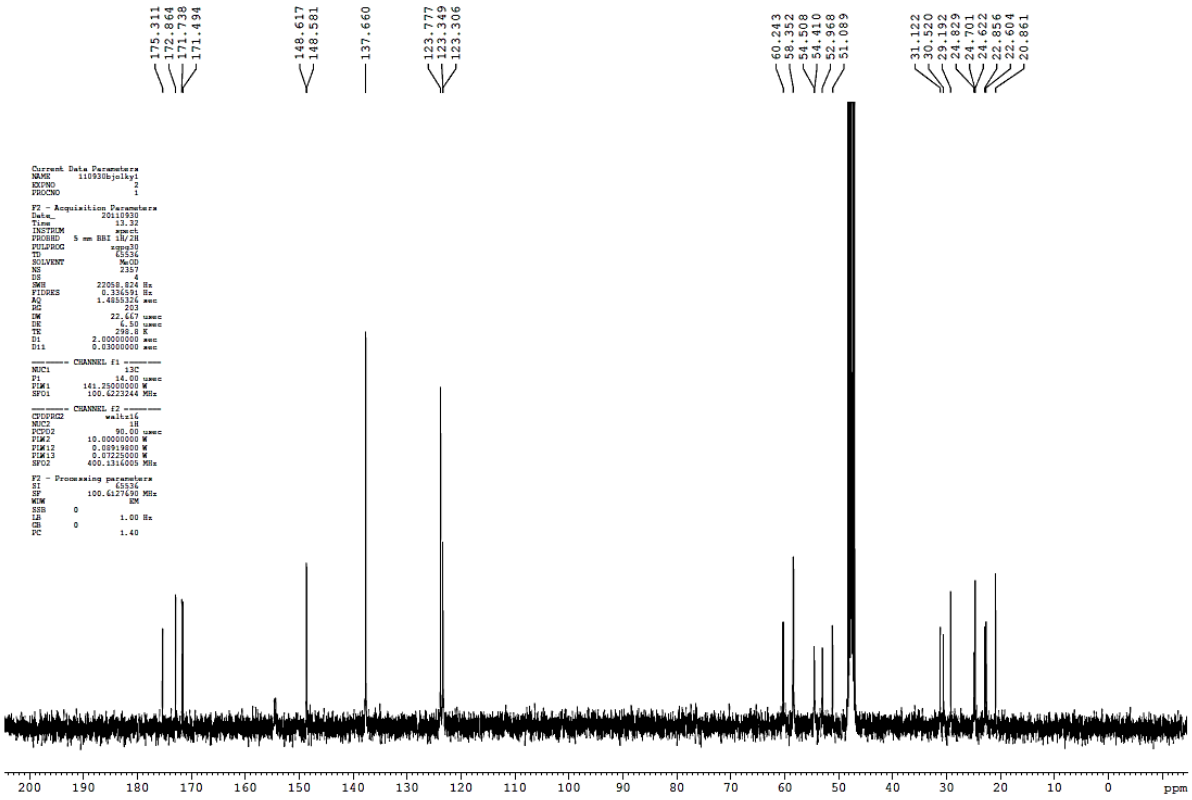
Compound 4, ^{13}C{^{1}H} NMR (CD_3OD, 125 MHz)



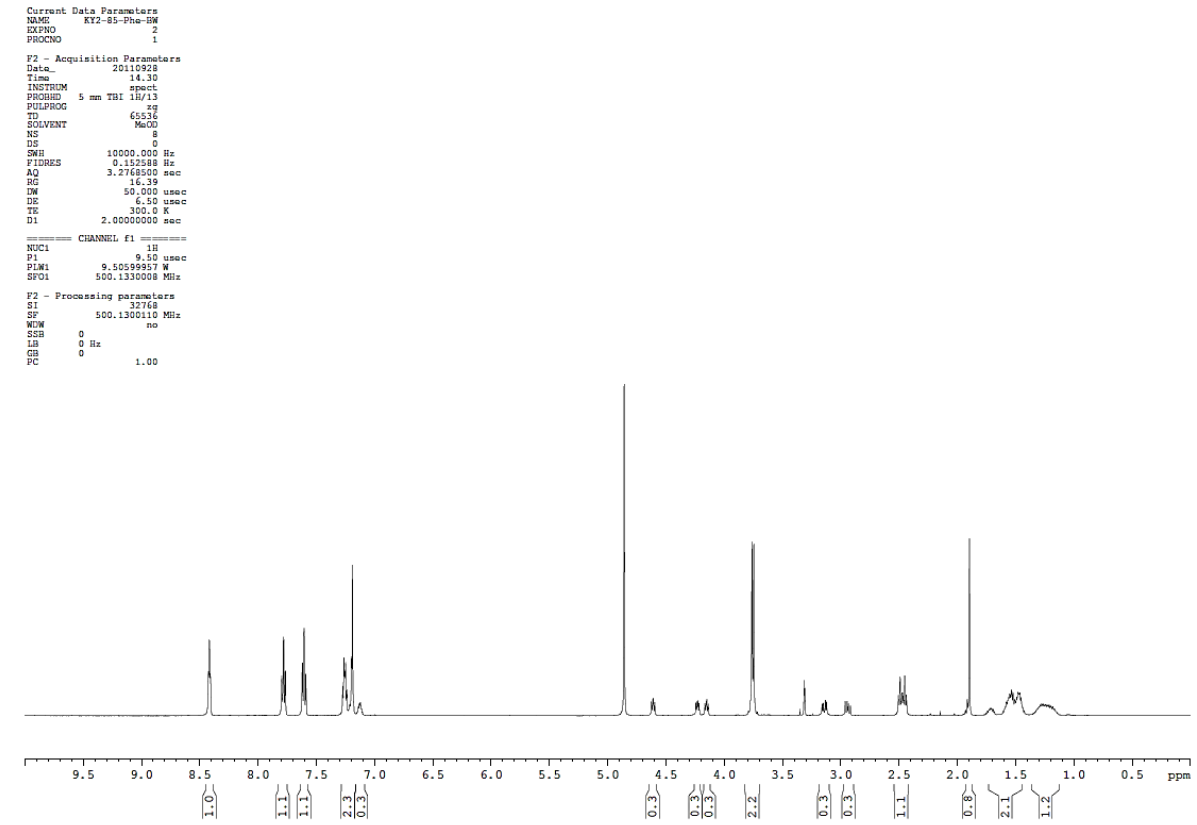
Compound 5, ^{1}H NMR (CD_3OD, 400 MHz)



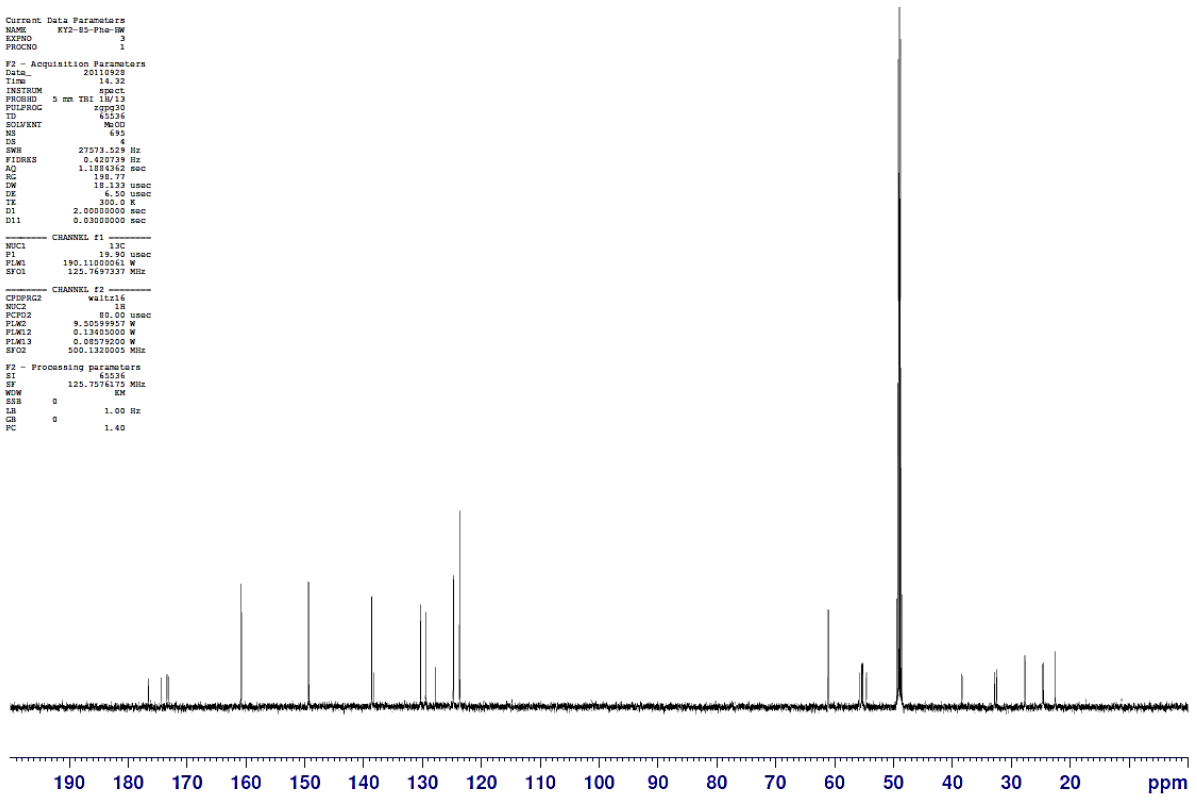
Compound 5, $^{13}C\{^{1}H\}$ NMR (CD_3OD, 100 MHz)



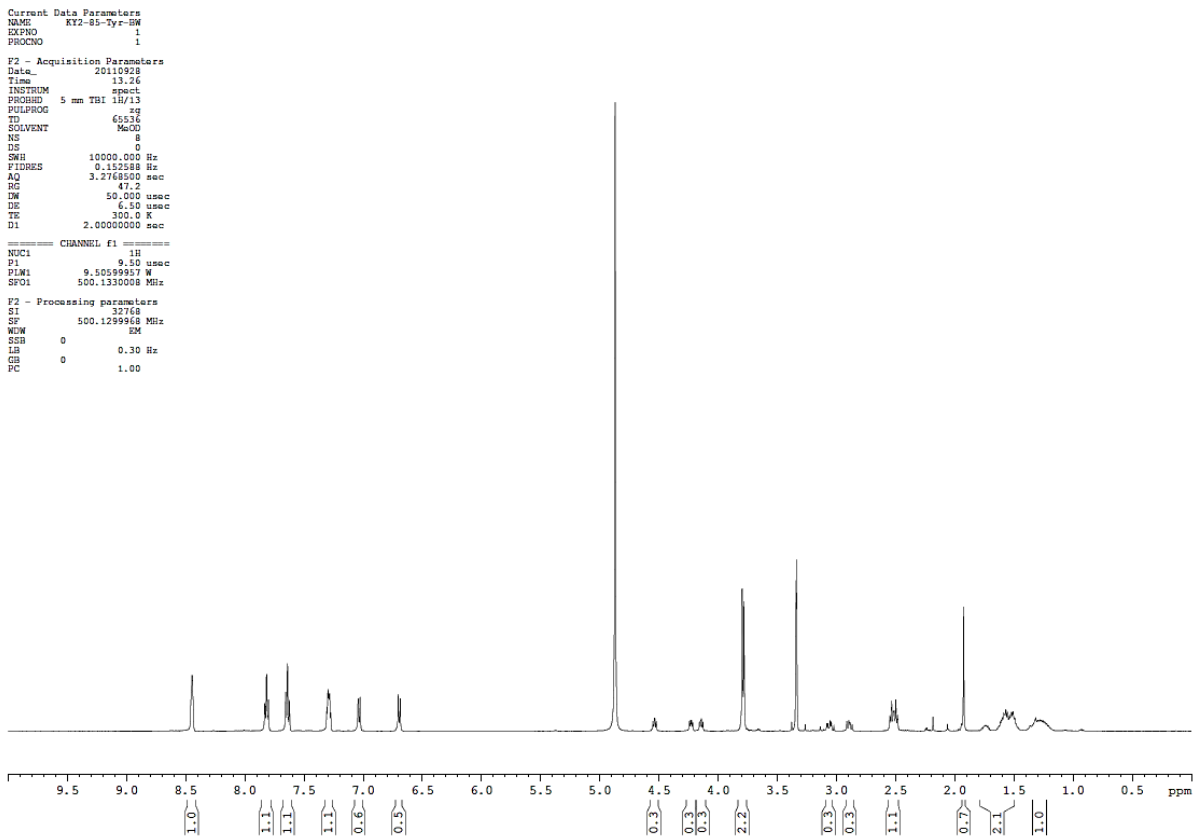
Compound 6, ^{1}H NMR (CD_3OD, 500 MHz)



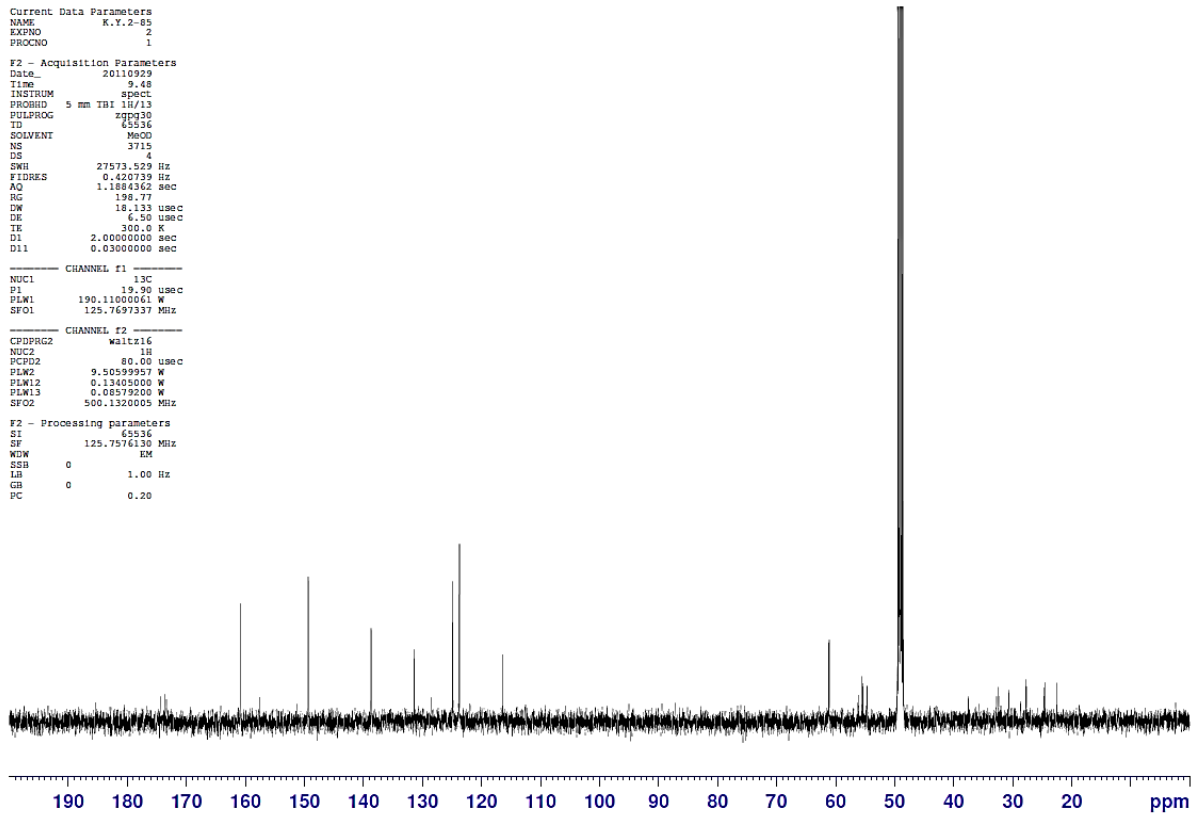
Compound 6, $^{13}C\{^{1}H\}$ NMR (CD_3OD, 125 MHz)



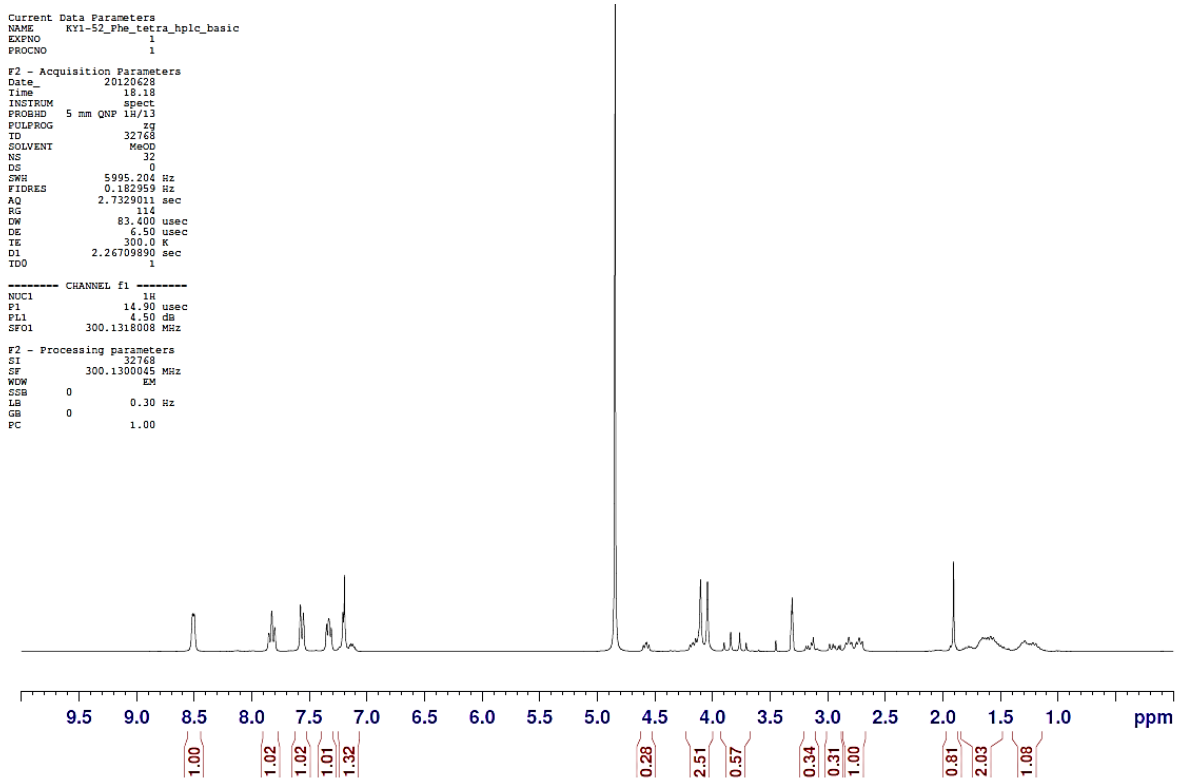
Compound 7, ^{1}H NMR (CD_3OD, 500 MHz)



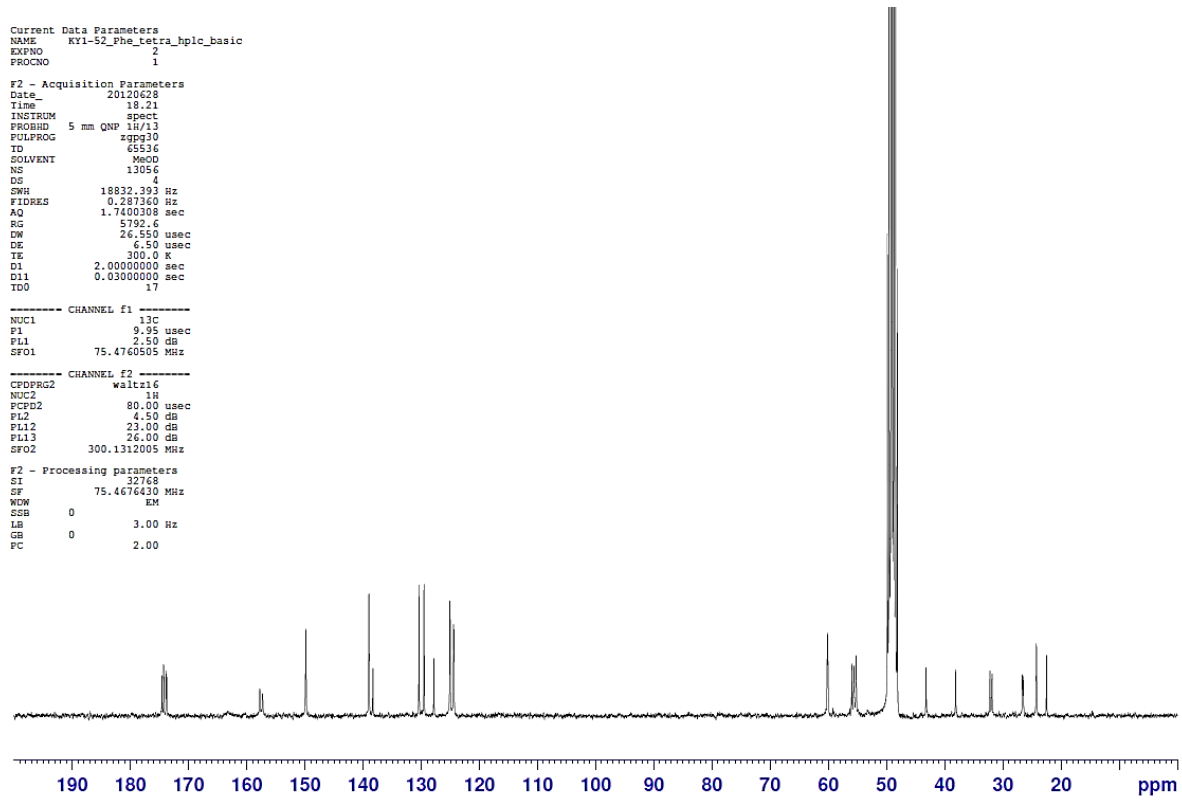
Compound 7, $^{13}C\{^{1}H\}$ NMR (CD_3OD, 125 MHz)



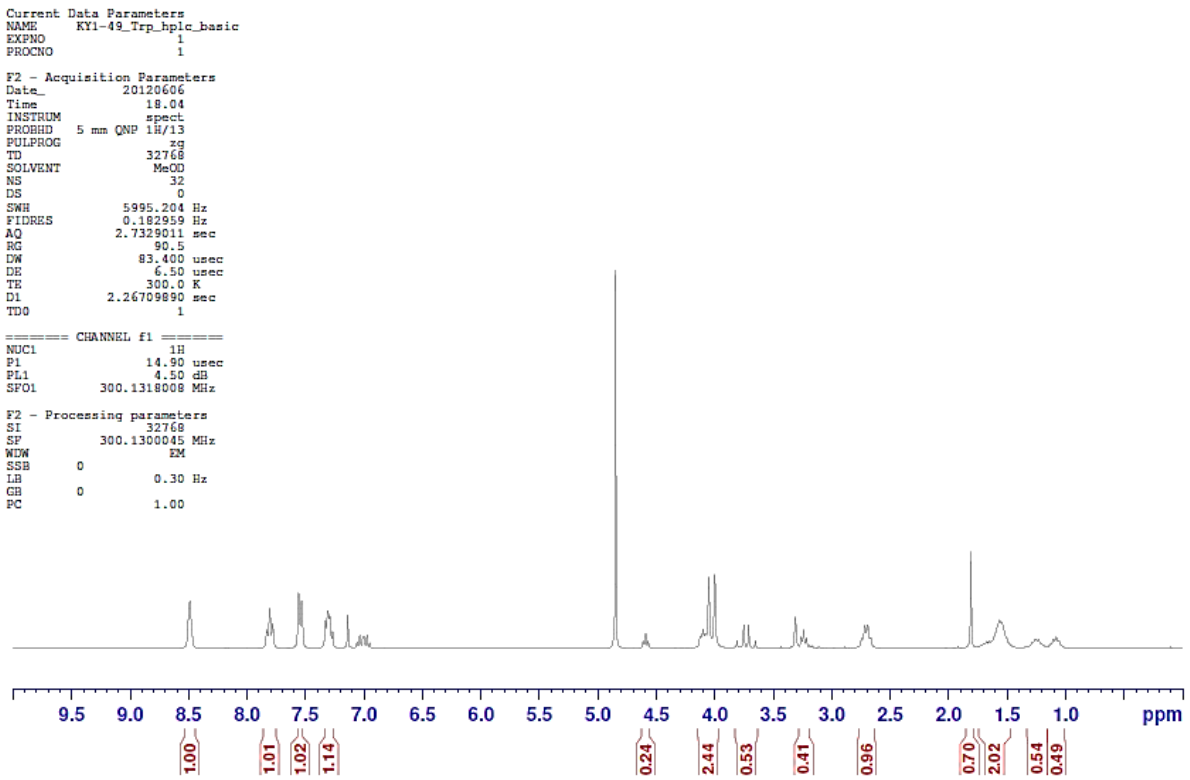
Compound 8, ^{1}H NMR (CD_3OD, 300 MHz)



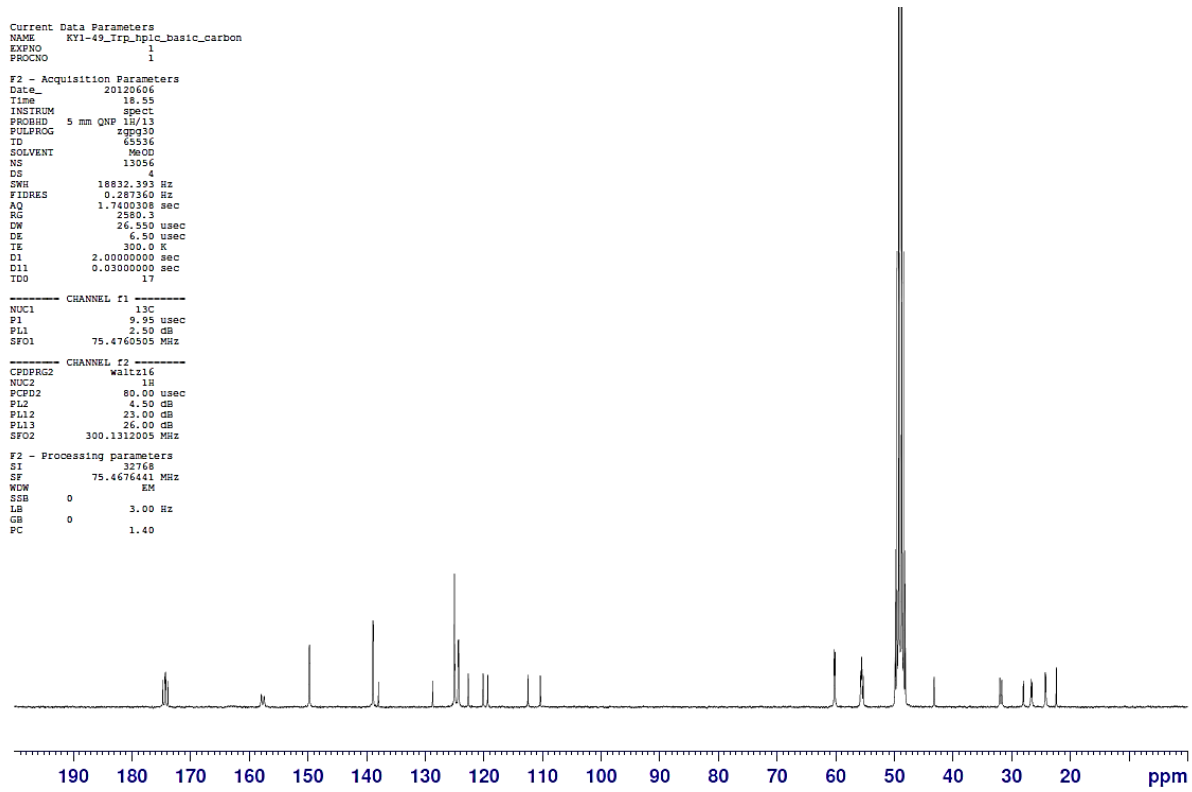
Compound 8, $^{13}C\{^{1}H\}$ NMR (CD_3OD, 75 MHz)



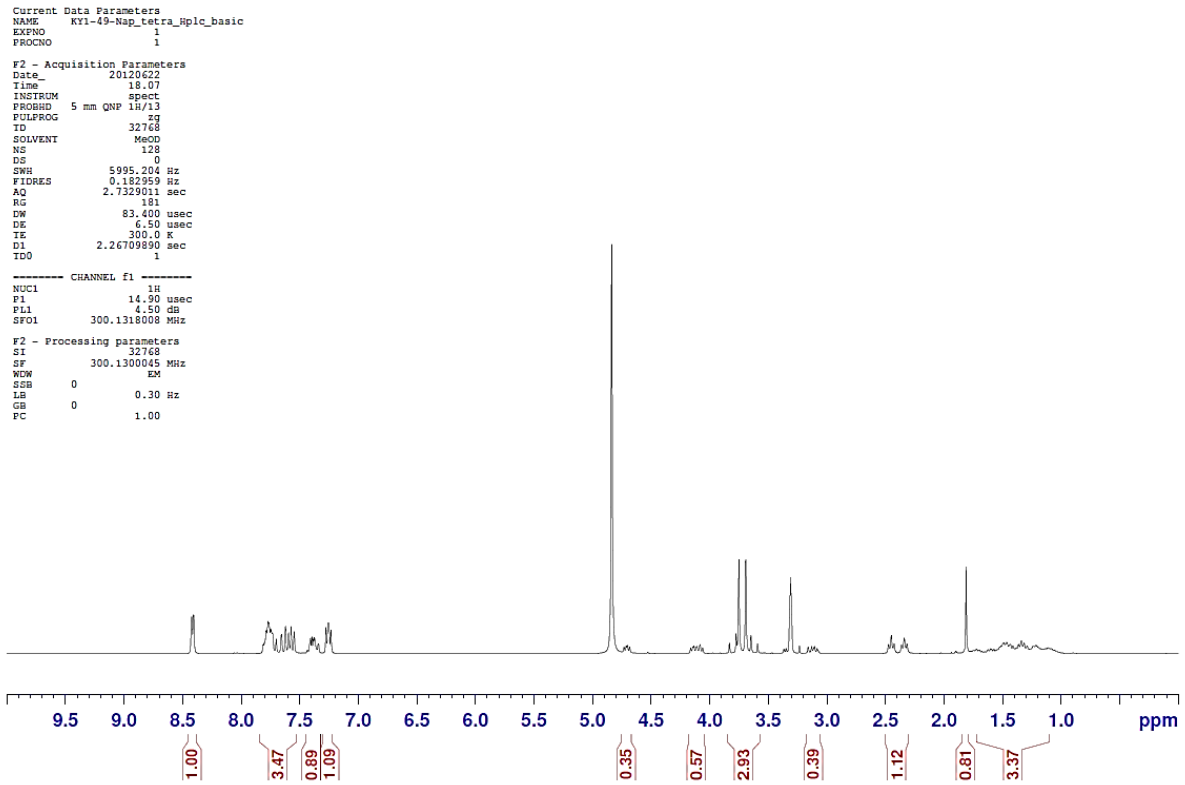
Compound 9, ^{1}H NMR (CD_3OD, 300 MHz)



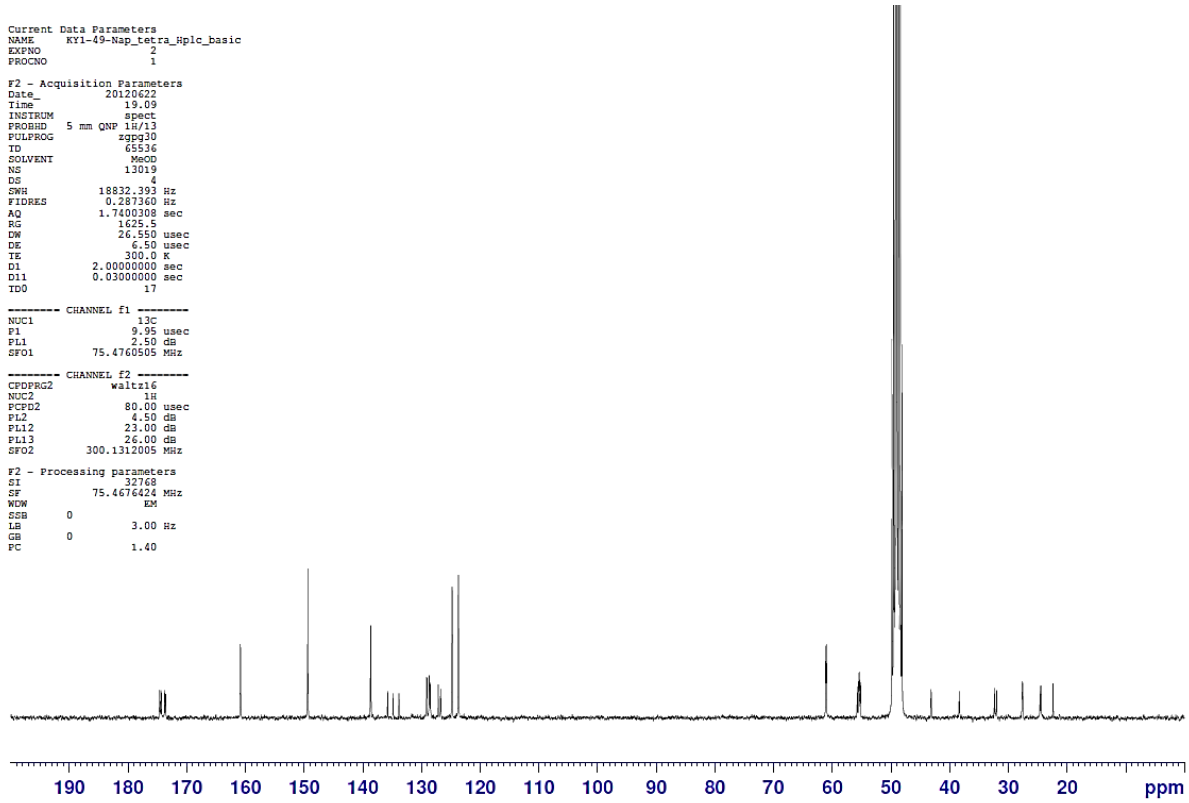
Compound 9, $^{13}C\{^{1}H\}$ NMR (CD_3OD, 75 MHz)



Compound 10, ^{1}H NMR (CD_3OD, 300 MHz)



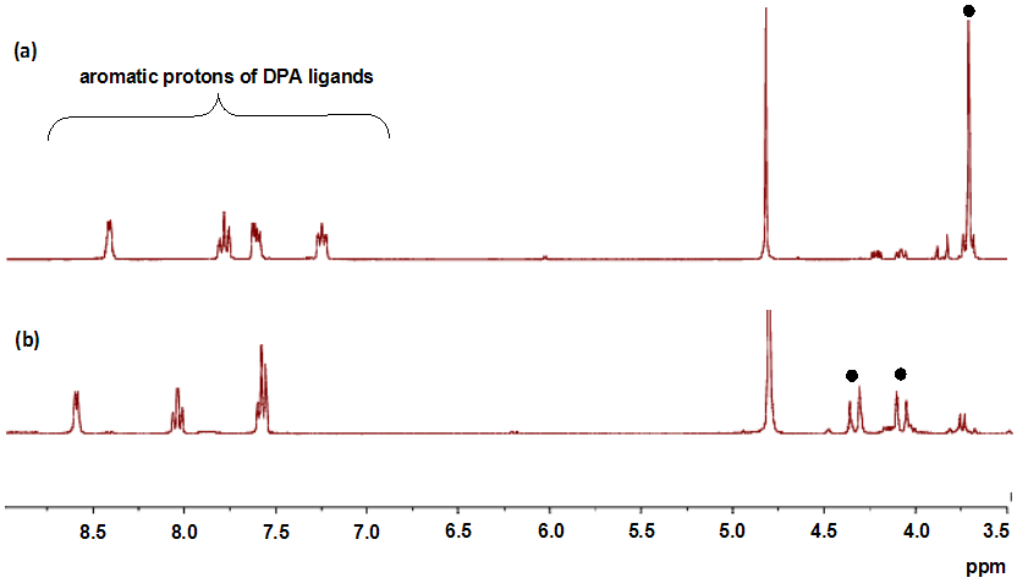
Compound 10, $^{13}C\{^{1}H\}$ NMR (CD_3OD, 75 MHz)



Formation of bis[zinc(II)] complexes

A solution of the appropriate peptide in MeOH (1 mL) was added to an aqueous solution of $Zn(NO_3)_2$ (2 eq. relative to peptide) and the mixture was stirred at rt for 30 min. The mixture was then concentrated under reduced pressure and the residue lyophilised, affording the bis[Zn(II)] complex as a nitrate salt.

Zinc Complexation - Representative Spectra of Compound 1



Partial ^{1}H NMR spectra in CD_3OD of compound **1 (a)** before and **(b)** after addition of two equiv. of $Zn(NO_3)_2$ to yield the bis[Zn(II)] complex. Benzylic protons denoted by black filled circle.

Anion Binding Studies under Mimicked Physiological Conditions

All titrations were performed in aqueous solutions buffered at pH 7.4 HEPES (5 mM) in the presence of 145 mM sodium chloride at 25 °C.

UV-Vis Titrations of Indicator with Receptor

Stock solutions of indicator **11** (20 μM) and of each **1-10•Zn$_2$** receptor was prepared (1000 μM). 2.5 mL of the HEPES buffer solution was transferred to one of a matched pair of 1 cm quartz glass cuvettes as a reference. The other cuvette was filled with 2.5 mL of indicator **11** solution and the UV absorbance (λ_{abs} = 250-750 nm) was recorded. Aliquots of the receptor solution were then added to both cuvettes. After each addition, the resulting solution was stirred with a magnetic stirrer and the UV absorbance was recorded. Typically, up to 10 equivalents of the receptor were added.

To determine the association constants for the indicator-receptor complexes, the UV absorbance (λ_{abs} = 250-750 nm) was plotted as a function of receptor concentration and the raw titration data was analysed using nonlinear least squares curve procedure based on the equilibria for 1:1 binding,[4] utilising the commercially available software programme HypSpec® (Hyperquad® package).[3]

UV-Vis Titrations of Indicator-Receptor Ensembles with Anions

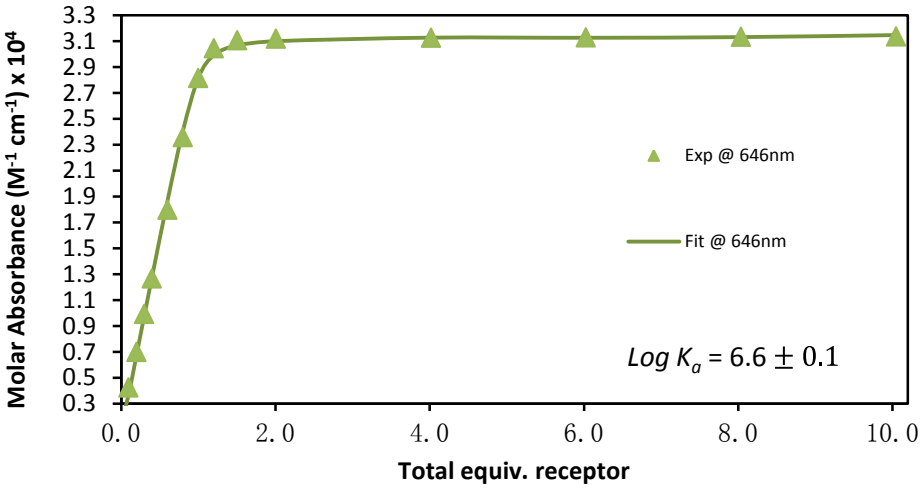
A stock solution of receptor (20 μM) was prepared. A stock solution of 1:1 indicator **11**-receptor ensemble solution (20 μM) was also prepared. 2.5 mL of the receptor solution was transferred to one of a matched pair of 1 cm quartz glass cuvettes as a reference. The other cuvette was filled with 2.5 mL of the indicator **11**-receptor solution and the UV absorbance was recorded for 250-750 nm. Aliquots of the anion solution (2000 μM) were then added to both cuvettes. After each addition, the resulting solution was stirred with a magnetic stirrer and the UV absorbance was recorded. Typically, up to 10 equivalents of the anion were added.

To determine the association constants for the anion-receptor complexes, the UV absorbance (λ_{abs} = 250-750 nm) was plotted as a function of anion concentration and the raw titration data was analysed using nonlinear least squares curve fitting procedure based on the equilibria for indicator displacement,[4] utilising the commercially available software programme HypSpec® (Hyperquad® package).[3]

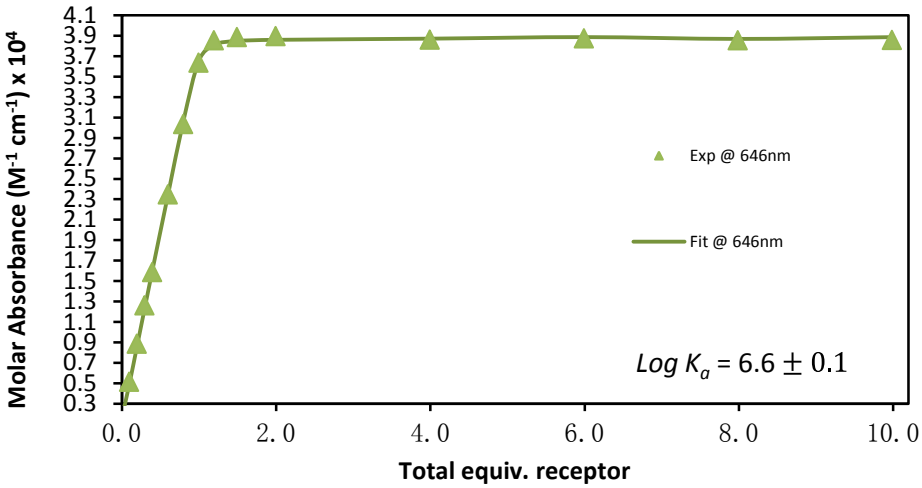
Titrating indicator 11 with complexes 1-10·Zn_2 in aqueous solution

Measurement conditions: aqueous solution of HEPES buffer (5 mM, pH 7.4), λ_{abs} = 250-750 nm, 25 °C.

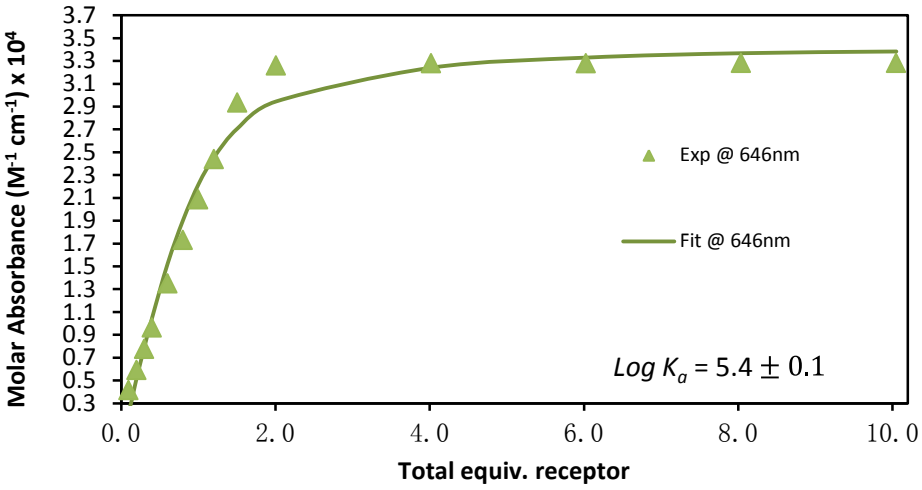
Receptor 1·Zn_2



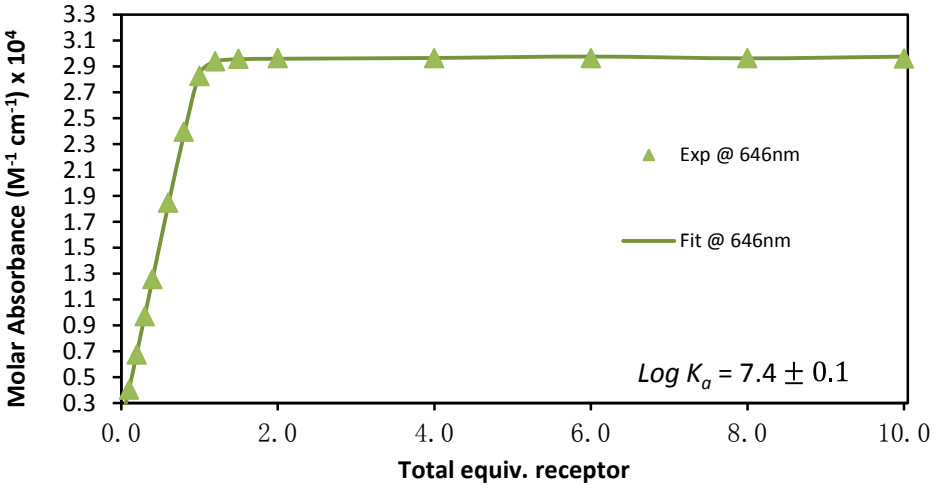
Receptor 2·Zn_2



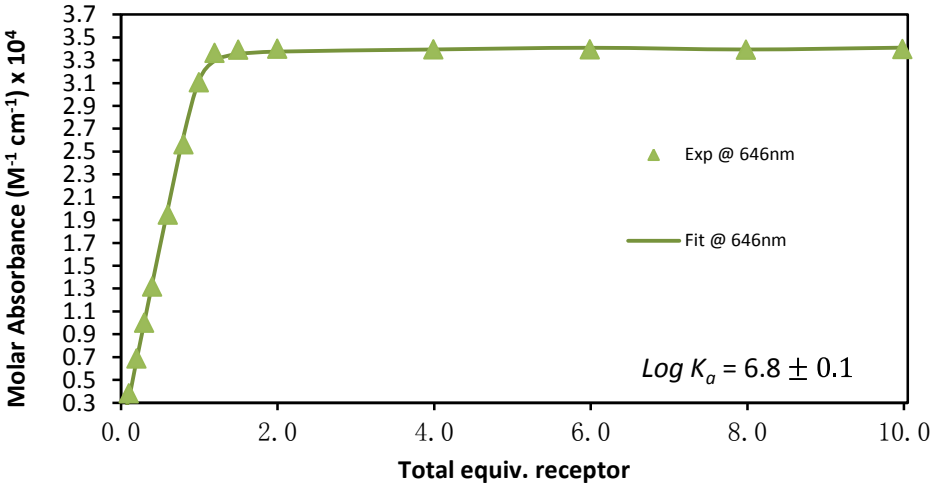
Receptor 3·Zn$_2$



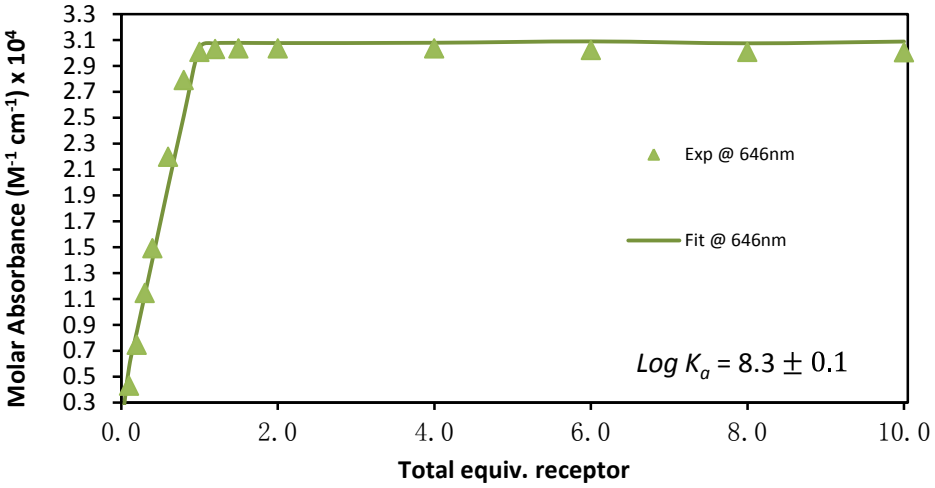
Receptor 4·Zn$_2$



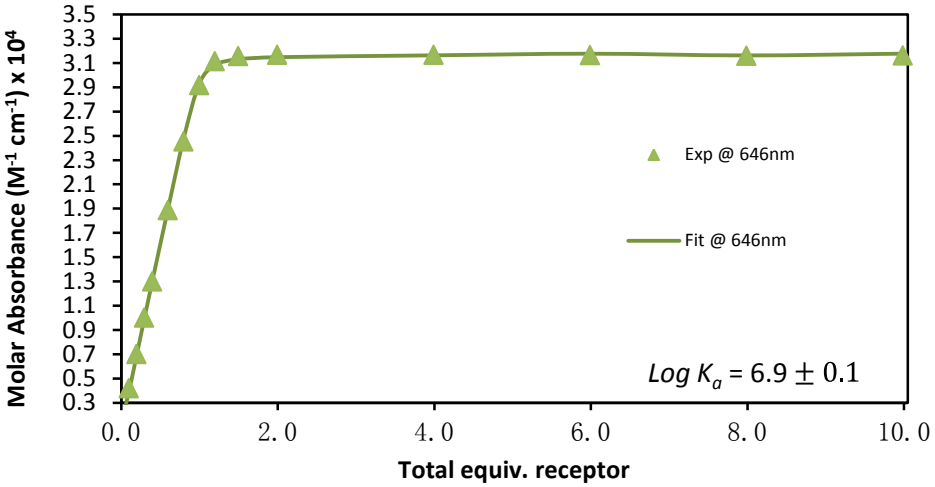
Receptor 5·Zn₂



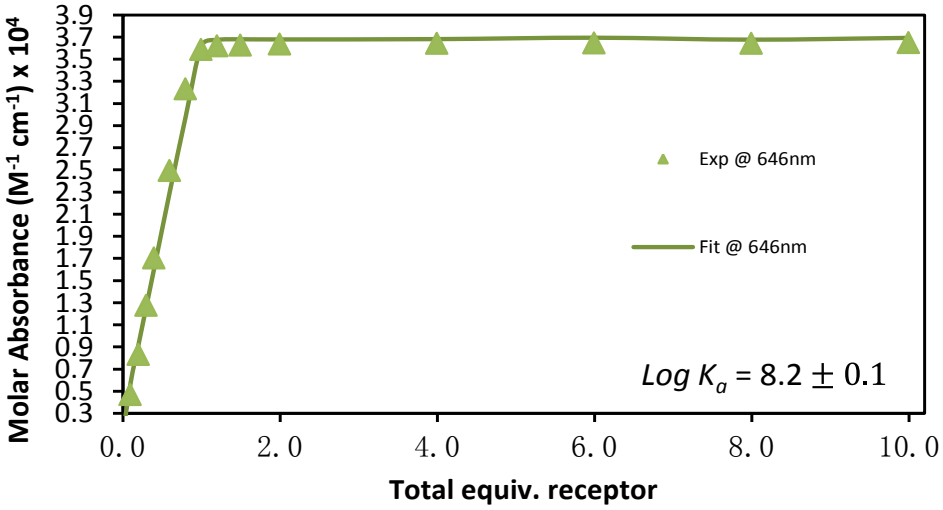
Receptor 6·Zn₂



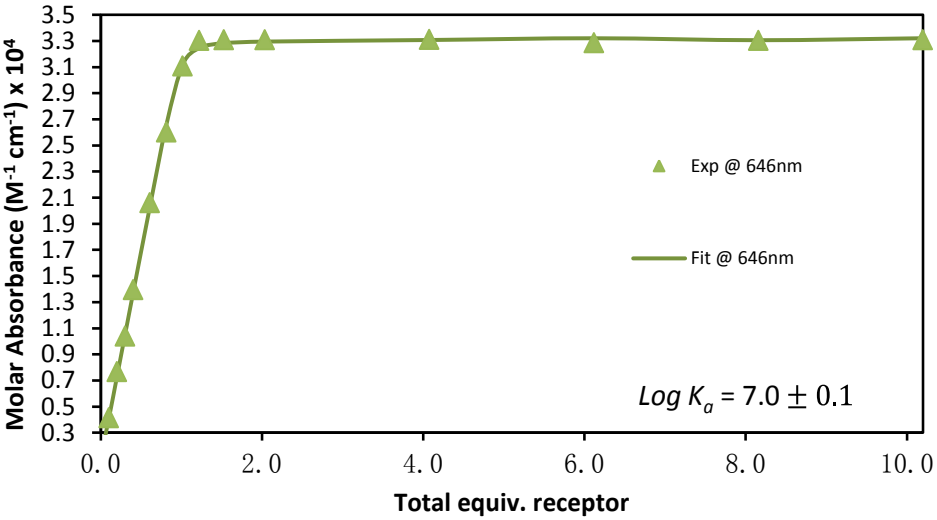
Receptor 7·Zn₂



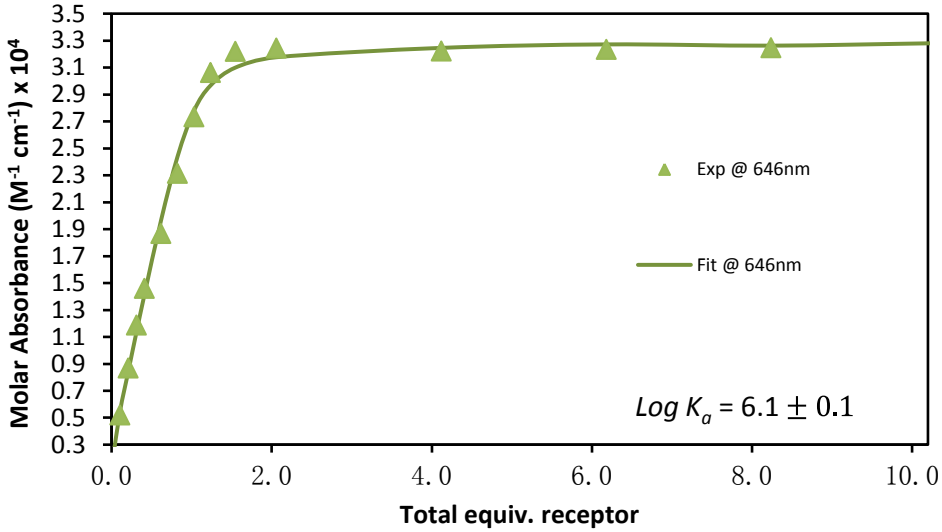
Receptor 8·Zn₂



Receptor 9·Zn$_2$



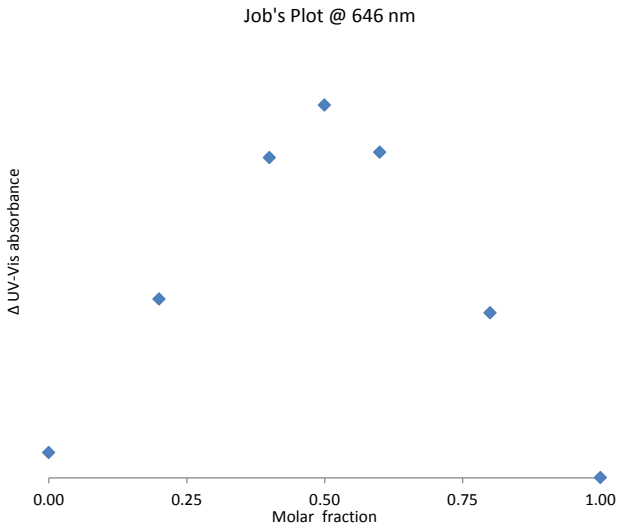
Receptor 10·Zn$_2$



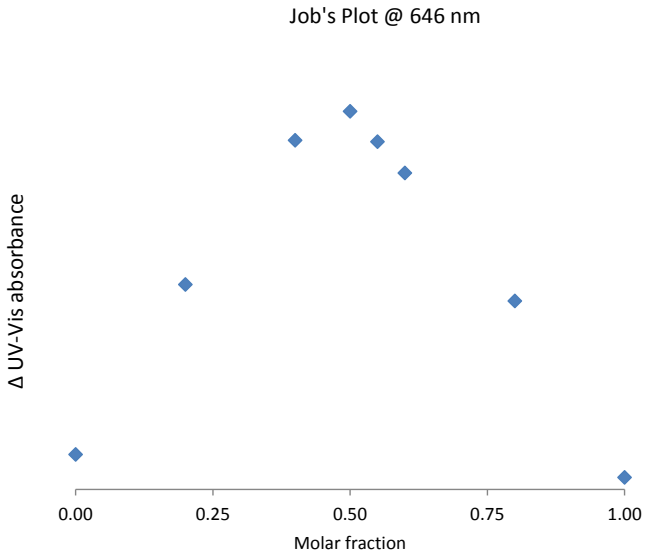
Job Plot for the indicator-receptor binding of 11 and 1, 8-10·Zn_2

Measurement conditions: aqueous solution of HEPES buffer (5 mM, pH 7.4), λ_{abs} = 250-750 nm, 25 °C.

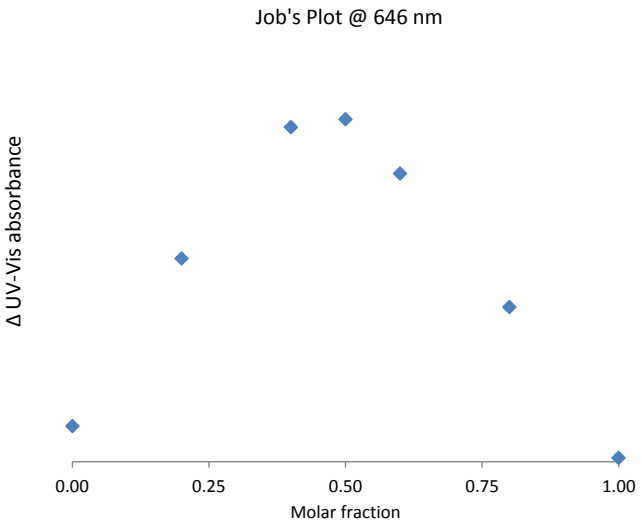
Receptor 1·Zn_2



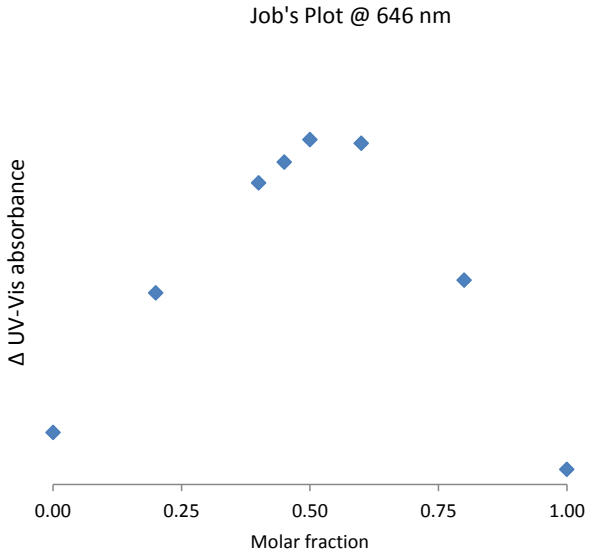
Receptor 8·Zn_2



Receptor 9·Zn$_2$



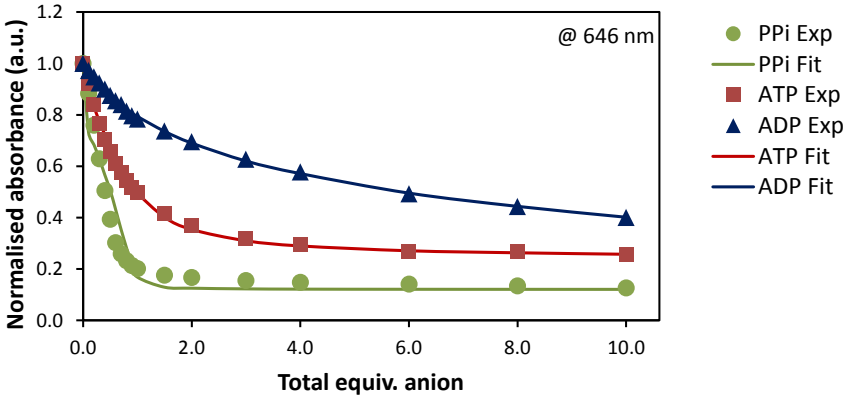
Receptor 10·Zn$_2$



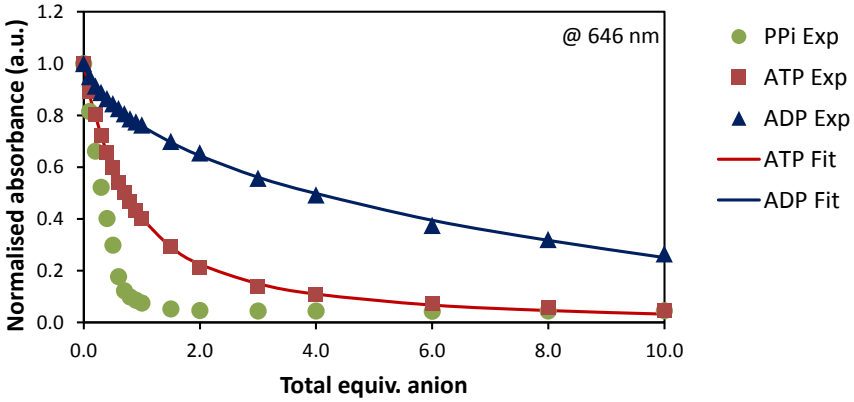
Titrating indicator-receptor ensembles with various anions in aqueous solution

Measurement conditions: aqueous solution of HEPES buffer (5 mM, pH 7.4), λ_{abs} = 250-750 nm, 25 °C.

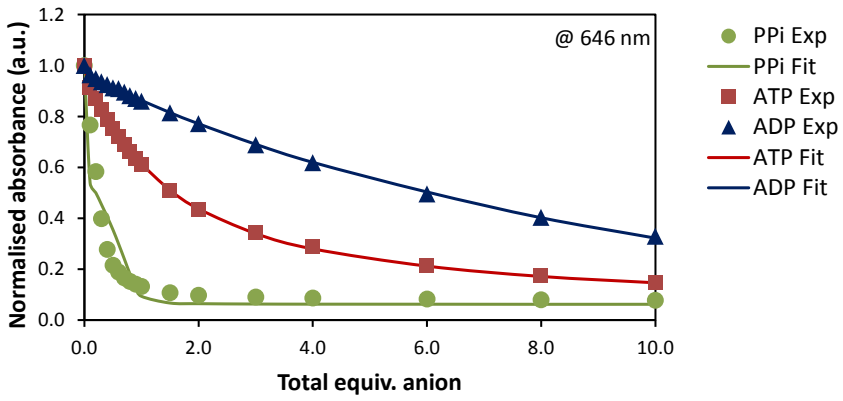
Receptor 1·Zn₂



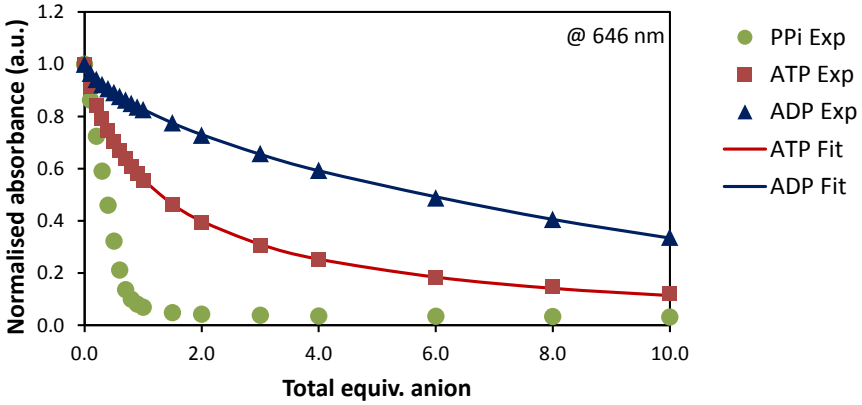
Receptor 2·Zn₂



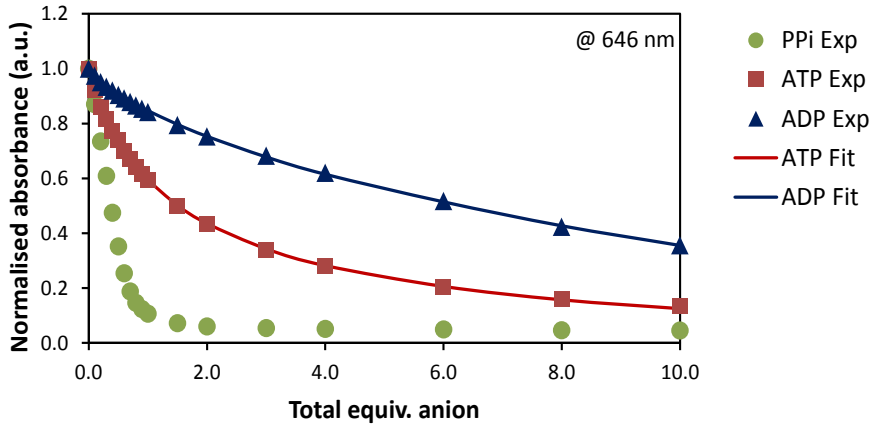
Receptor 3·Zn₂



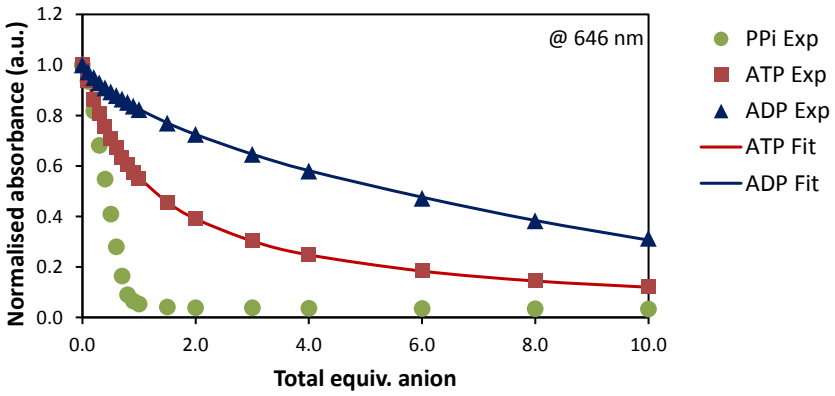
Receptor 4·Zn₂



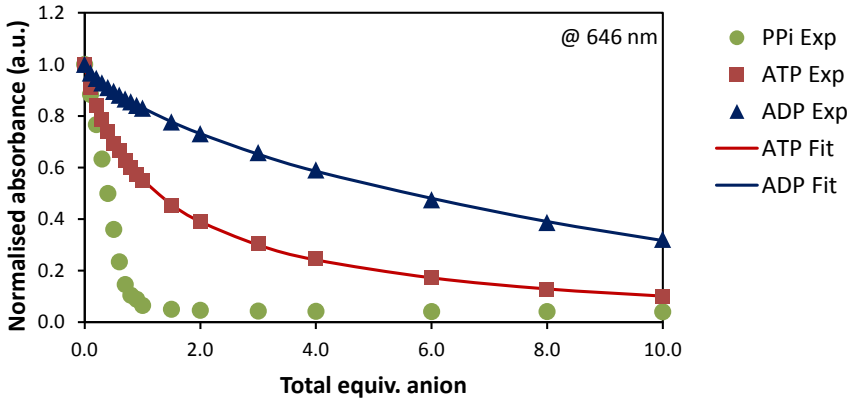
Receptor 5·Zn₂



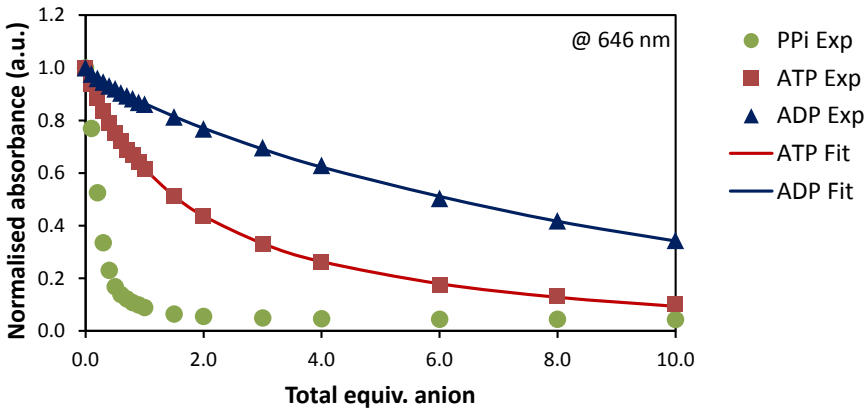
Receptor 6·Zn₂



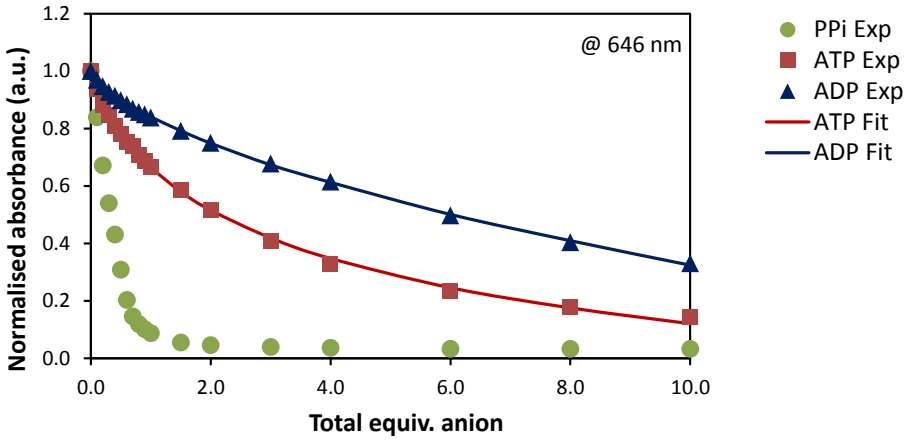
Receptor 7·Zn₂



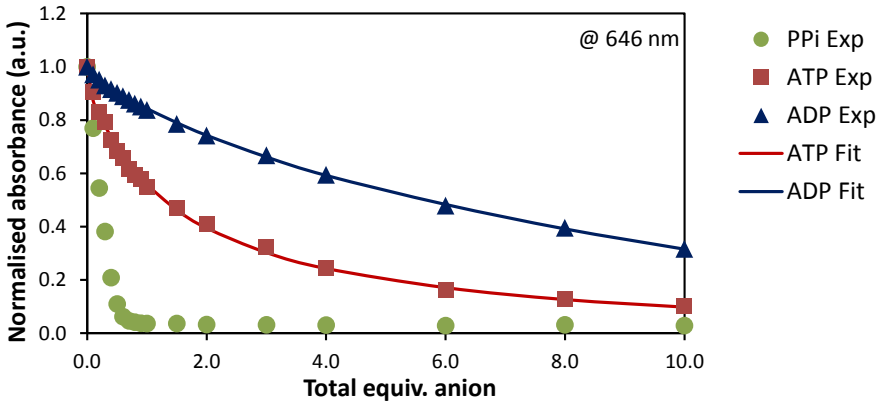
Receptor 8·Zn₂



Receptor 9·Zn$_2$



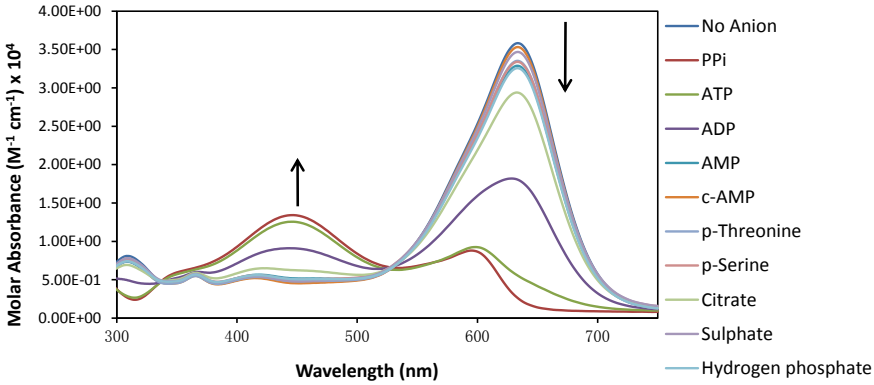
Receptor 10·Zn$_2$

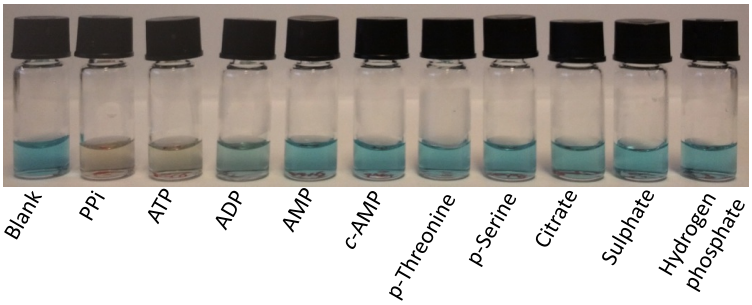


Qualitative screening of indicator-receptor ensembles with various anions in aqueous solution

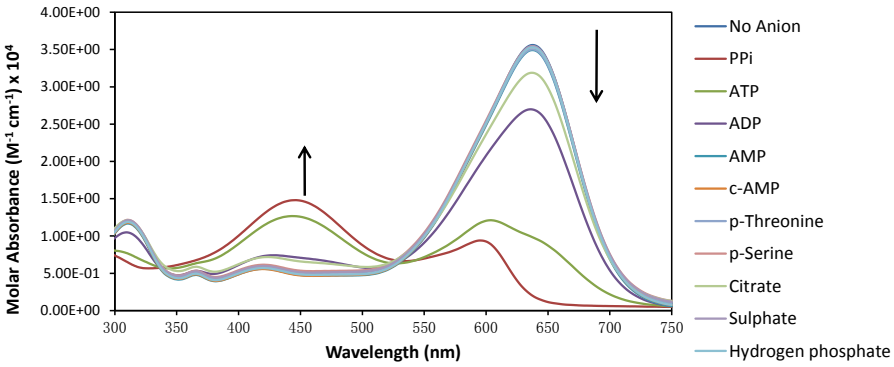
Measurement conditions: aqueous solution of HEPES buffer (5 mM, pH 7.4), λ_{abs} = 250-750 nm, 25 °C.

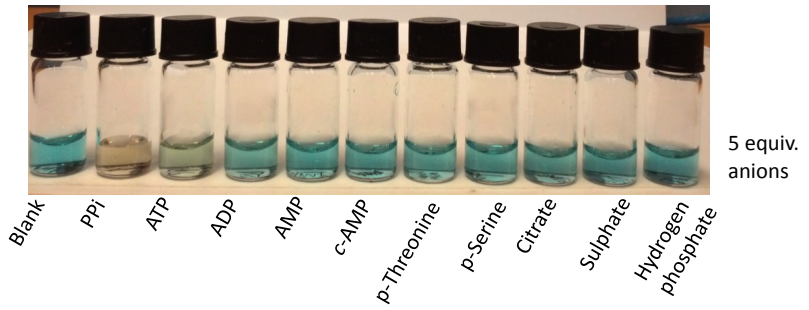
Receptor 2·Zn$_2$



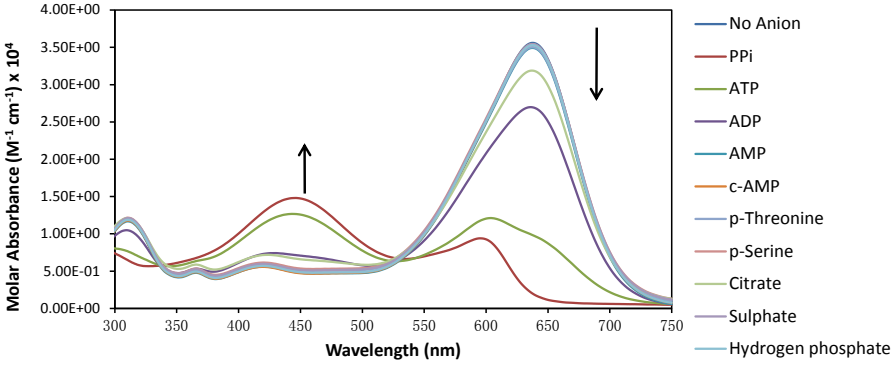


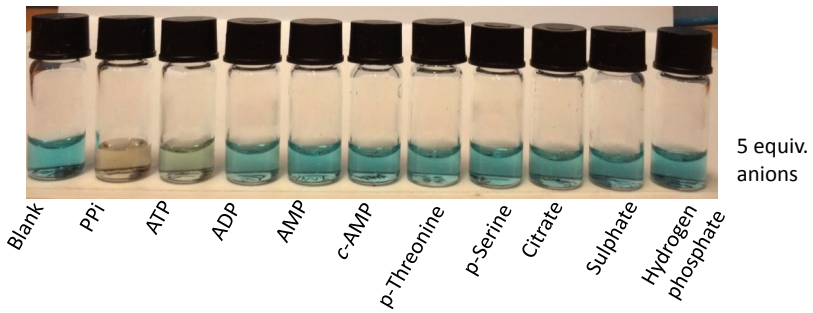
Receptor 6·Zn₂



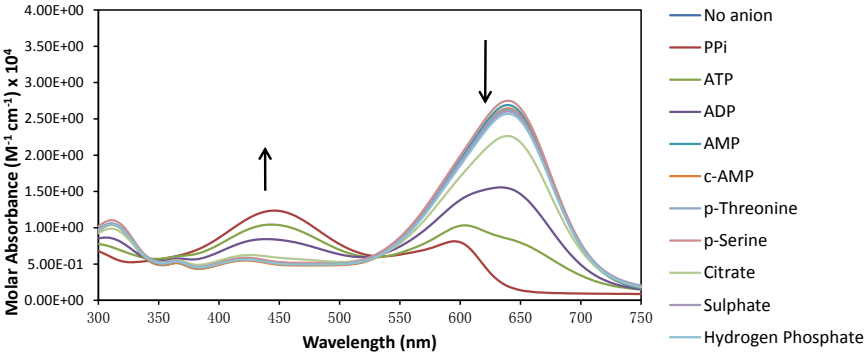


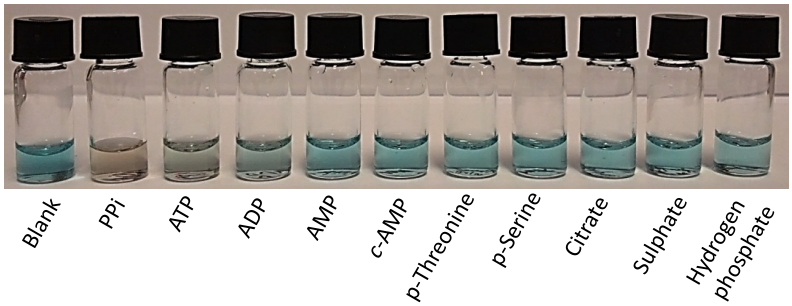
Receptor 8·Zn₂





Receptor 9·Zn$_2$





5 equiv. anions

References

1. S. A. Kates, S. B. Daniels and F. Albericio, *Anal. Biochem*., 1993, **212**, 303.
2. L. Quinti, R. Weissleder and C.-H. Tung, *Nano Lett*., 2006, **6**, 488.
3. P. Gans, A. Sabatini and A. Vacca, *Talanta*, 1996, **43**, 1739.
4. K. A. Connors, *Binding Constants: The Measurements of Molecular Complex Stability*, John Wiley and Sons, 1987.