Supplementary Material (ESI) for Chemical Communications

Synthetically Accessible, High-Affinity Phosphate Anon Receptors

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1. Potentiometric Titrations

Potentiometric measurements were conducted using a Metrohm 792SM Titrino automated burette fitted with a combined glass electrode and an in-house designed jacketed glass cell thermostated at 25 °C suitable for small sample volumes (5 mL). The cell was sealed under an inert atmosphere. All solutions were made up using ultrapure CO_2-free water from a Millipore Simplicity 185 system. The sample solutions contained 0.1 M KCl as a background electrolyte. The titrant base was made up from commercial (Riedel-de Haën) CO_2-free concentrates of KOH, diluted to the required concentration (0.020 M) with a 0.080 M KCl solution to avoid problems of different ionic strengths. The glass electrode was calibrated by titrating well-known amounts of HCl (made up from Riedel-de Haën concentrates) with the base and determining the equivalence point using Gran's method[1] yielding the ionic product of water (pKw = 13.78), which was used as a constant in the subsequent analyses. Sample concentrations were typically $1.0 - 5.0 \times 10^{-3}$ M. A known excess of HCl was added to ensure full protonation of the receptor. The degree of protonation of our receptors was calculated from the onset of titration curve. For the phosphate binding studies an equimolar mixture of KH_2PO_4 and receptor was used. A minimum of 250 points in the pH-range of 2.5-11.0 was taken for every titration, with at least 30 seconds between each addition to ensure equilibration. The computer program HYPERQUAD[2] was used to determine the protonation and stability constants. For each system a minimum of three titrations were first treated as individual sets and then merged and fitted simultaneously to give the final constants.

1 G. Gran, *Analyst*, **1952**, *77*, 661-671.

2 (*a*) P. Gans, A. Sabatini and A. Vacca, *Talanta* **1996**, *43*, 1739-1753. (*b*) A. Sabatini, A. Vacca and P. Gans, *Coord. Chem. Rev.* **1992**, *120*, 389-405.

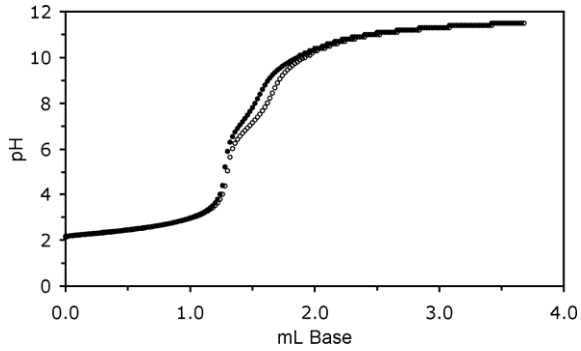

Figure 4: Experimental titration curves of receptor **2** in the presence (●) and absence (○) of phosphate.

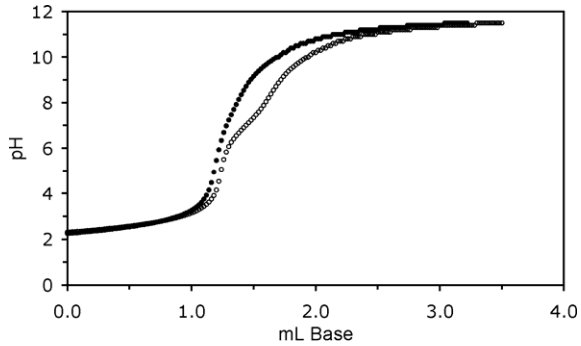

Figure 5: Experimental titration curves of receptor **3** in the presence (●) and absence (○) of phosphate.

2. Fluorimetric Titrations

Titrations were performed on a Spex Fluoromax-II instrument at 25 °C in a 0.1 M TRIS buffer of pH 7.0, using an excitation wavelength of 324 nm. The fluorophore 4-methylumbelliferyl phosphate was purchased from Sigma and its concentration was kept constant at 5.0×10^{-6} M. The data was fitted to a 1:1 binding model with a non-linear least-squares algorithm.

3. Selected Characterisation Data

Receptor 1. ^{1}H NMR (400 MHz, D_2O, 298K) δ: 1.56 (quintet, $^{3}J_{H,H}$ = 7.5 Hz, 4H), 1.66 (quintet, $^{3}J_{H,H}$ = 7.8 Hz, 4H), 2.12 (s, 6H), 2.35 (m, 8H), 2.59 (t, $^{3}J_{H,H}$ = 7.2 Hz, 4H), 3.23 (t, $^{3}J_{H,H}$ = 6.8 Hz, 4H), 6.77 (s, 2H). ^{13}C NMR (100 MHz, D_2O, 298K) δ: 25.44, 28.13, 38.15, 38.95, 41.05, 53.91, 54.08, 132.44, 166.55. ESI MS (MeOH) m/z

(%): calculated for $C_{18}H_{38}N_6O_2$ = 370, found 371 (100) [M^++H]. IR (KBr) ν (cm^{-1}): 1546 (amide II), 1635 (amide I), 2973 (CH).

Receptor 2. ^{1}H NMR (500 MHz, D_2O, 298K) δ: 1.74 (quintet, $^3J_{H,H}$ = 7.5 Hz, 4H), 1.78 (quintet, $^3J_{H,H}$ = 7.0 Hz, 4H), 2.28 (s, 6H), 2.51 (m, 8H), 2.72 (dq, $^3J_{H,H}$ = 5.5 Hz, $^3J_{H,H}$ = 7.0 Hz, 4H), 3.30 (br. t, $^3J_{H,H}$ = 7.0 Hz, 4H), 5.97 (d, $^3J_{H,H}$ = 11.0 Hz, 1H), 6.39 (d, $^3J_{H,H}$ = 12.0 Hz, 1H). ^{13}C NMR (125 MHz, D_2O, 298K) δ: 26.85, 29.60, 29.75, 39.10, 39.24, 40.38, 40.40, 42.43, 42.46, 55.32, 55.38, 55.52, 55.55, 126.07, 137.28, 169.57, 175.98. ESI MS (MeOH) m/z (%): calculated for $C_{18}H_{38}N_6O_2$ = 370, found 371 (100) [M^++H]. IR (KBr) ν (cm^{-1}): 1558 (amide II), 1627 (amide I), 1716 (amide I), 2960 (CH), 3452 (NH).

Receptor 3. More peaks than expected are observed in the NMR spectra due to the presence of exchanging conformations. ^{1}H NMR (500 MHz, D_2O/NaOD, 298K) δ: 1.54 (br. m, 8H), 2.12 (br. s, 6H), 2.24-2.55 (m, 12H), 3.17 (br. t, 4H), 3.67 (br. t, 4H), 5.87 (d, $^3J_{H,H}$ = 12.2 Hz, 2H), 5.95 (s, 2H), 6.28 (d, $^3J_{H,H}$ = 12.3 Hz, 2H), 7.23-7.59 (br. m, 4H). ^{13}C NMR (125 MHz, D_2O/NaOD, 298K) δ: 25.33, 25.58, 25.77, 25.82, 28.86, 37.59, 38.97, 40.85, 40.92, 40.96, 41.55, 45.45, 45.56, 46.04, 48.86, 52.19, 53.78, 53.85, 53.96, 54.02, 54.27, 54.32, 61.38, 124.21, 124.32, 127.28, 128.57, 128.76, 130.41, 135.30, 135.93, 139.54, 167.84, 175.37, 179.82, 181.60. ESI MS (MeOH) m/z (%): calculated for $C_{26}H_{44}N_6O_2$ = 472, found 472 (100) [M^++H]. IR (KBr) ν (cm^{-1}): 1575 (amide II), 1702 (amide I), 3029 (CH), 3467 (NH).