Supporting Information

Thermodynamics of Pillararene•Guest Complexation: Blinded Dataset for the SAMPL9 Challenge

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General experimental. Starting materials were purchased from commercial suppliers and used without further purification or were prepared by literature procedures. Melting points were measured on a Meltemp apparatus in open capillary tubes and are uncorrected. IR spectra were recorded on a JASCO FT/IR 4100 spectrometer and are reported in cm^{-1}. NMR spectra were measured on Bruker spectrometers operating at 400 or 600 MHz for ^{1}H and 100 or 125 MHz for ^{13}C using D_2O, or DMSO-d_6 as solvents. Chemical shifts (δ) are referenced relative to the residual resonances for HOD (4.80 ppm) and DMSO-d_6 (2.50 ppm for ^{1}H, 39.51 ppm for ^{13}C). Mass spectrometry was performed using a JEOL AccuTOF electrospray instrument (ESI). ITC data was collected on a Malvern Microcal PEAQ-ITC instrument and analyzed using the software provided by the vendor.

Table S1. Binding constants (K_a, M^{-1}), thermodynamic parameter (ΔH, kcal mol^{-1}) and C values for **WP6** and guests. Conditions: 1× PBS buffer, 298 K.

Guest	K_a with **WP6** (M^{-1}) ΔH and C *or app. C*	*Duplicate* K_a with **WP6** (M^{-1}) ΔH and C *or app. C*
G1[a]	$(5.29 \pm 0.07) \times 10^4$; −8.08 ± 0.02; *26*	$(5.15 \pm 0.16) \times 10^4$; −7.87 ± 0.03; *27.1*
G2[b]	$(4.59 \pm 0.35) \times 10^7$; −6.10 ± 0.02; *172*	$(4.48 \pm 0.21) \times 10^7$; −6.15 ± 0.02; *167*
(±)-**G3**[a]	$(6.45 \pm 0.18) \times 10^5$; −4.75 ± 0.02; *64.5*	$(6.25 \pm 0.30) \times 10^5$; −4.68 ± 0.02; *62.7*
G4[e]	$(5.08 \pm 0.11) \times 10^4$; −4.15 ± 0.02; *25.4*	$(4.97 \pm 0.16) \times 10^4$; −3.98 ± 0.02; *26.3*
G5[f]	$(9.01 \pm 0.23) \times 10^3$; −3.95 ± 0.03; *9*	$(9.31 \pm 0.22) \times 10^3$; −4.15 ± 0.03; *4.2*
G6[c]	$(7.09 \pm 0.44) \times 10^5$; −6.90 ± 0.07; *35.5*	$(7.26 \pm 0.44) \times 10^5$; −7.10 ± 0.04; *36.1*
G7[d]	$(1.31 \pm 0.05) \times 10^5$; −3.18 ± 0.02; *26.2*	$(1.29 \pm 0.05) \times 10^5$; −3.17 ± 0.02; *25.7*
G8[e]	$(2.35 \pm 0.04) \times 10^4$; −9.55 ± 0.05; *11.7*	$(2.30 \pm 0.06) \times 10^4$; −9.51 ± 0.05; *11.5*
G9[f]	$(3.75 \pm 0.31) \times 10^4$; −5.31 ± 0.08; *37.4*	$(3.68 \pm 0.37) \times 10^4$; −5.21 ± 0.08; *36.8*
G10[b]	$(1.61 \pm 0.08) \times 10^7$; −6.23 ± 0.02; *59.1*	$(1.60 \pm 0.12) \times 10^7$; −6.11 ± 0.02; *63*
G11[f]	$(3.37 \pm 0.05) \times 10^4$; −5.61 ± 0.02; *33.7*	$(3.31 \pm 0.05) \times 10^4$; −5.45 ± 0.03; *33.1*
(±)-**G12**[g]	$(9.43 \pm 0.31) \times 10^7$; −7.45 ± 0.02; *142*	$(8.40 \pm 0.51) \times 10^7$; −7.41 ± 0.02; *309*
G13[c]	$(1.63 \pm 0.11) \times 10^6$; −4.98 ± 0.04; *81.3*	$(1.61 \pm 0.11) \times 10^6$; −4.96 ± 0.03; *82*
G14[h]	$(4.69 \pm 0.09) \times 10^9$; −16.4 ± 0.02; 59.2	$(4.67 \pm 0.11) \times 10^9$; −16.1 ± 0.03; 500
G15[i]	$(1.76 \pm 0.06) \times 10^7$; −7.03 ± 0.03; 15.4	$(1.45 \pm 0.07) \times 10^7$; −7.13 ± 0.02; 135
G16[e]	$(1.32 \pm 0.03) \times 10^4$; −7.49 ± 0.06; 6.6	$(1.24 \pm 0.03) \times 10^4$; −7.00 ± 0.06; 6.27
G17[a]	$(2.29 \pm 0.06) \times 10^5$; −4.15 ± 0.02; 22.5	$(3.45 \pm 0.27) \times 10^5$; −3.52 ± 0.05; 34.5

[a] Measured directly by ITC during the titration of **WP6** (0.1 mM) in the cell with guest (1 mM) in the syringe. [b] Measured by the ITC competition by titrating a solution of **G7** (0.2 mM) and **WP6** (0.1 mM) in the cell with guest (1 mM) from the syringe. [c] Measured directly by the ITC during the titration of **WP6** (0.05 mM) in the cell with guest (0.5 mM) in the syringe. [d] Measured directly by the ITC during the titration of **WP6** (0.2 mM) in the cell with guest (2 mM) in the syringe. [e] Measured directly by the ITC during the titration of **WP6** (0.5 mM) in the cell with guest (5 mM) in the syringe. [f] Measured directly by the ITC during the titration of **WP6** (1 mM) in the cell with guest (10 mM) in the syringe. [g] Measured by the ITC competition by titrating a solution of **G7** (0.5 mM) and **WP6** (0.1 mM) in the cell with guest (1 mM) from the syringe. [h] Measured by the ITC competition by titrating a solution of **G15** (0.5 mM) and **WP6** (0.1 mM) in the cell with **G14** (1 mM) from the syringe. [i] Measured by the ITC competition by titrating a solution of **G7** (1.0 mM) and **WP6** (0.1 mM) in the cell with **G15** (1 mM) from the syringe.

^{1}H NMR spectra of WP6 with Guests.

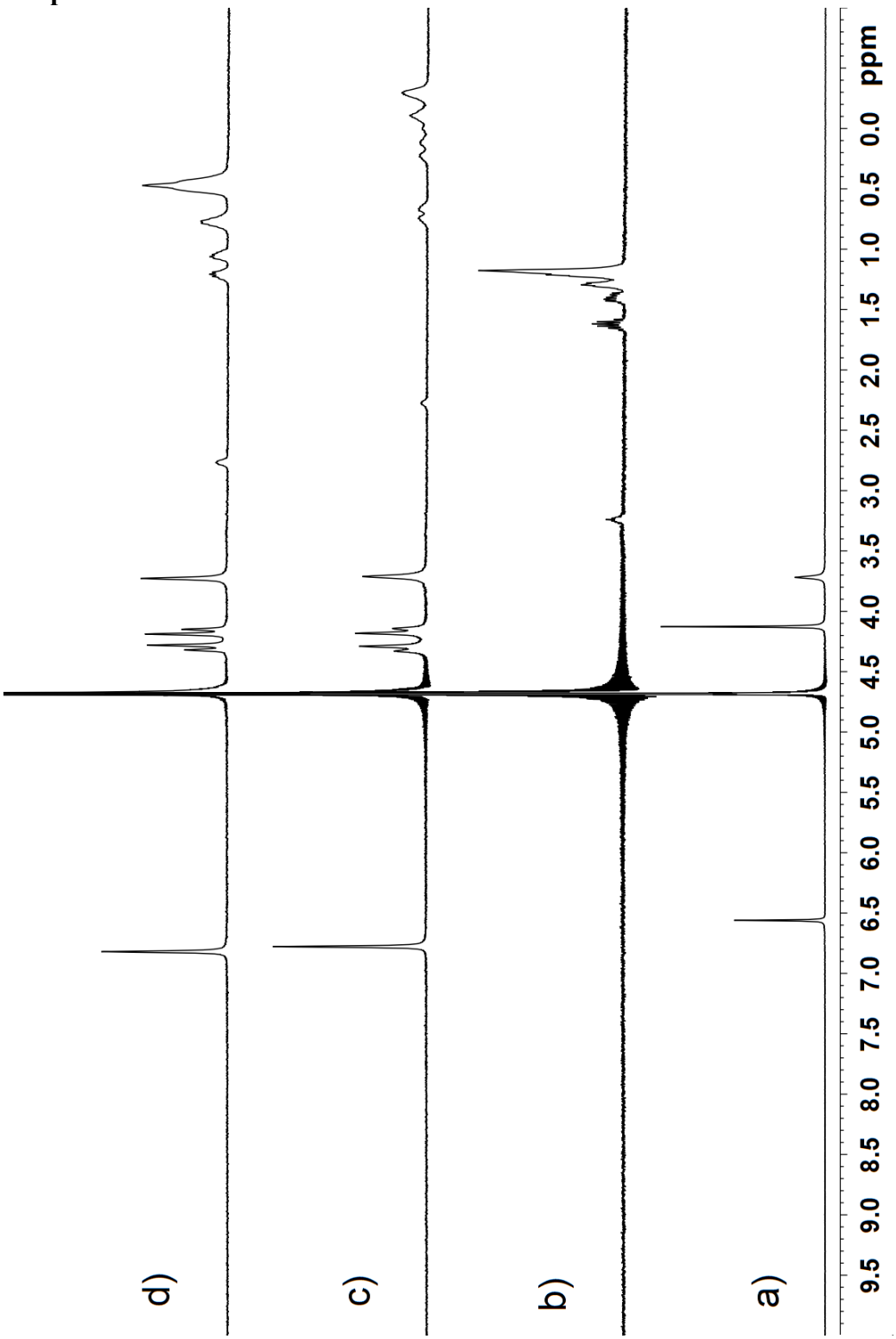

Figure S1. ^{1}H NMR spectra recorded (400 MHz, RT, D_2O) for: a) **WP6**, b) Guest **G1**, c) an equimolar mixture of **WP6** and Guest **G1** (1 mM), and d) a 2:1 mixture of Guest **G1** (2 mM) and **WP6** (1 mM).

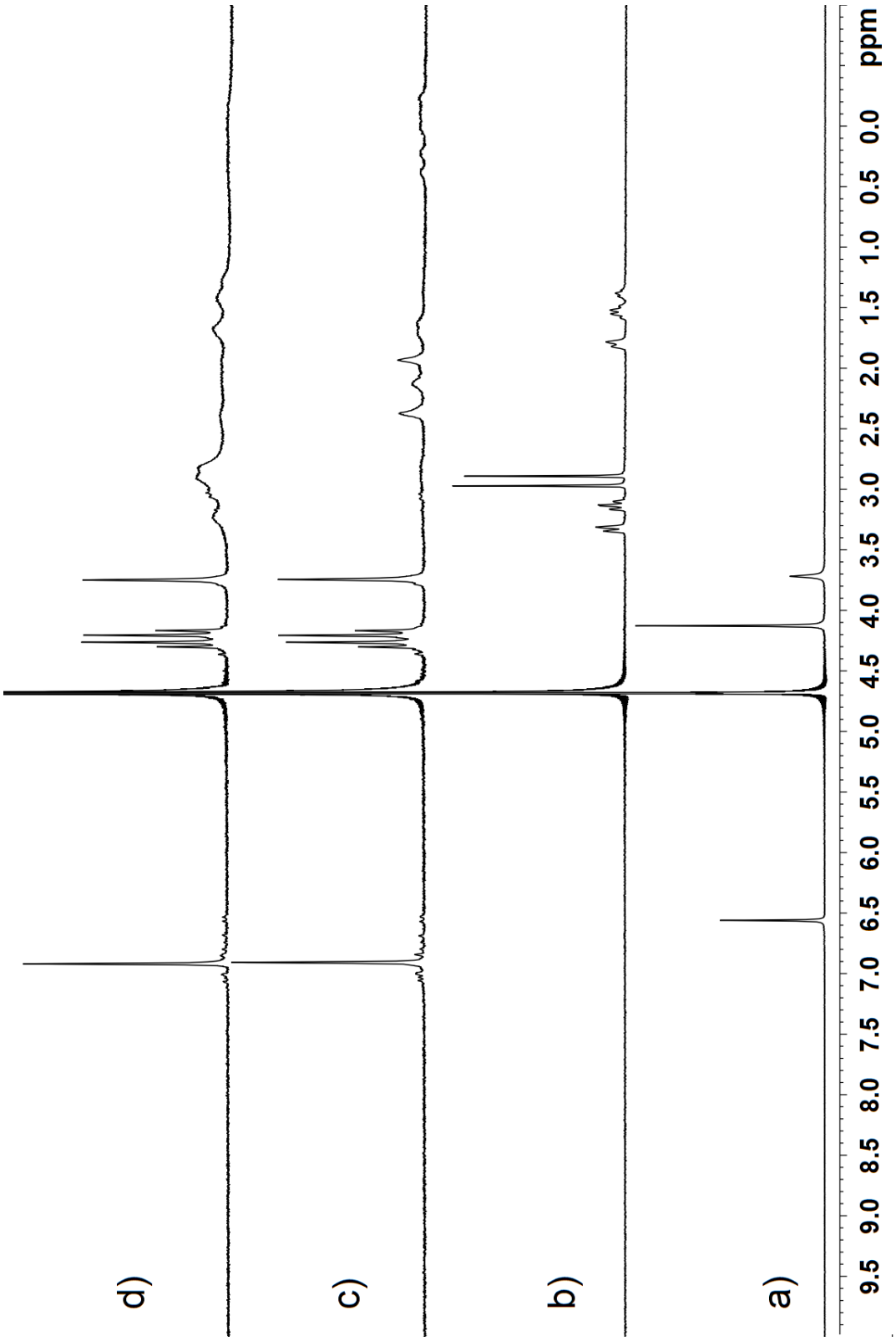

Figure S2. ^{1}H NMR spectra recorded (400 MHz, RT, D_2O) for: a) **WP6**, b) Guest **G2**, c) an equimolar mixture of **WP6** and Guest **G2** (1 mM), and d) a 2:1 mixture of Guest **G2** (2 mM) and **WP6** (1 mM).

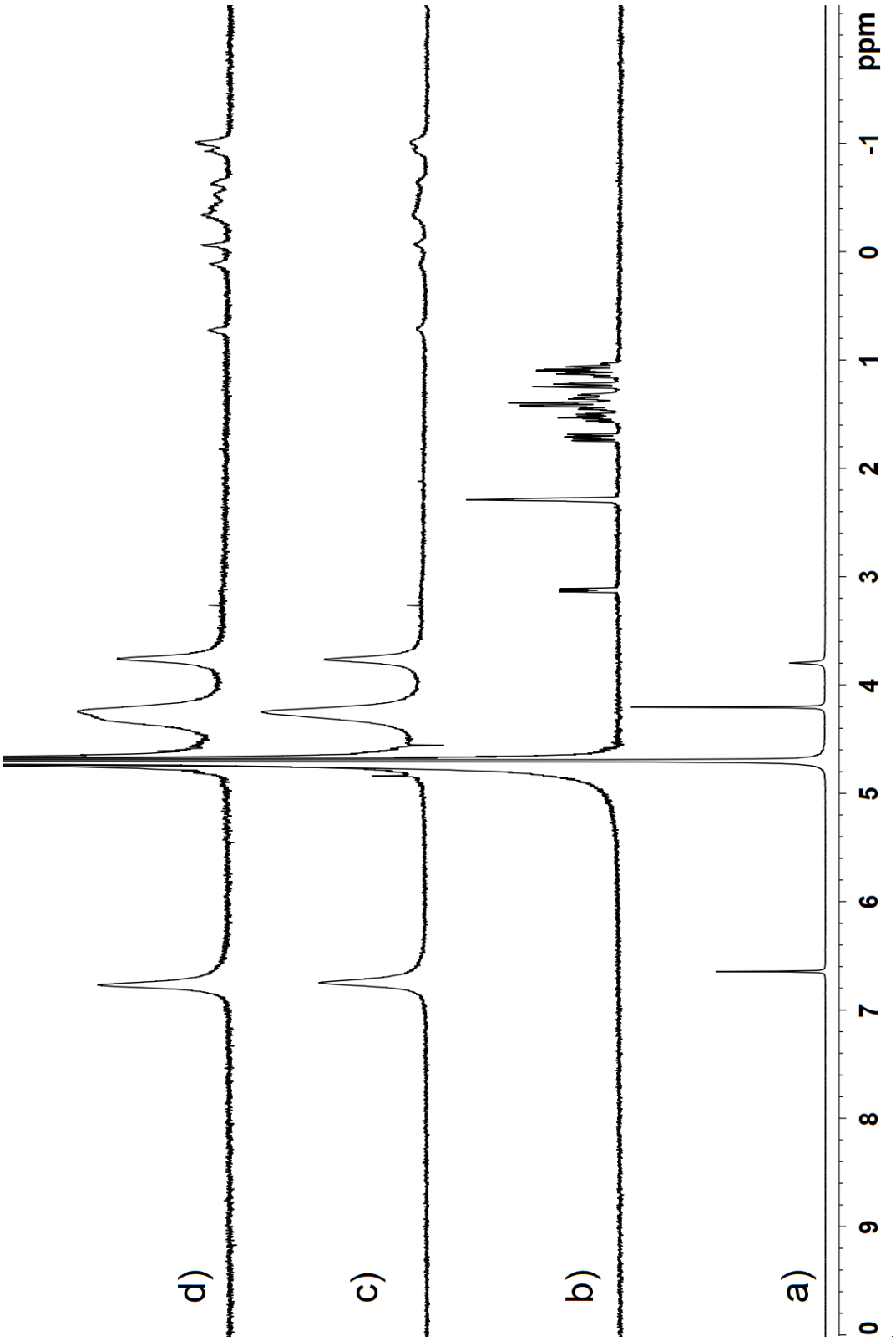

Figure S3. ^{1}H NMR spectra recorded (400 MHz, RT, D_2O) for: a) **WP6**, b) Guest **G3**, c) an equimolar mixture of **WP6** and Guest **G3** (1 mM), and d) a 2:1 mixture of Guest **G3** (2 mM) and **WP6** (1 mM).

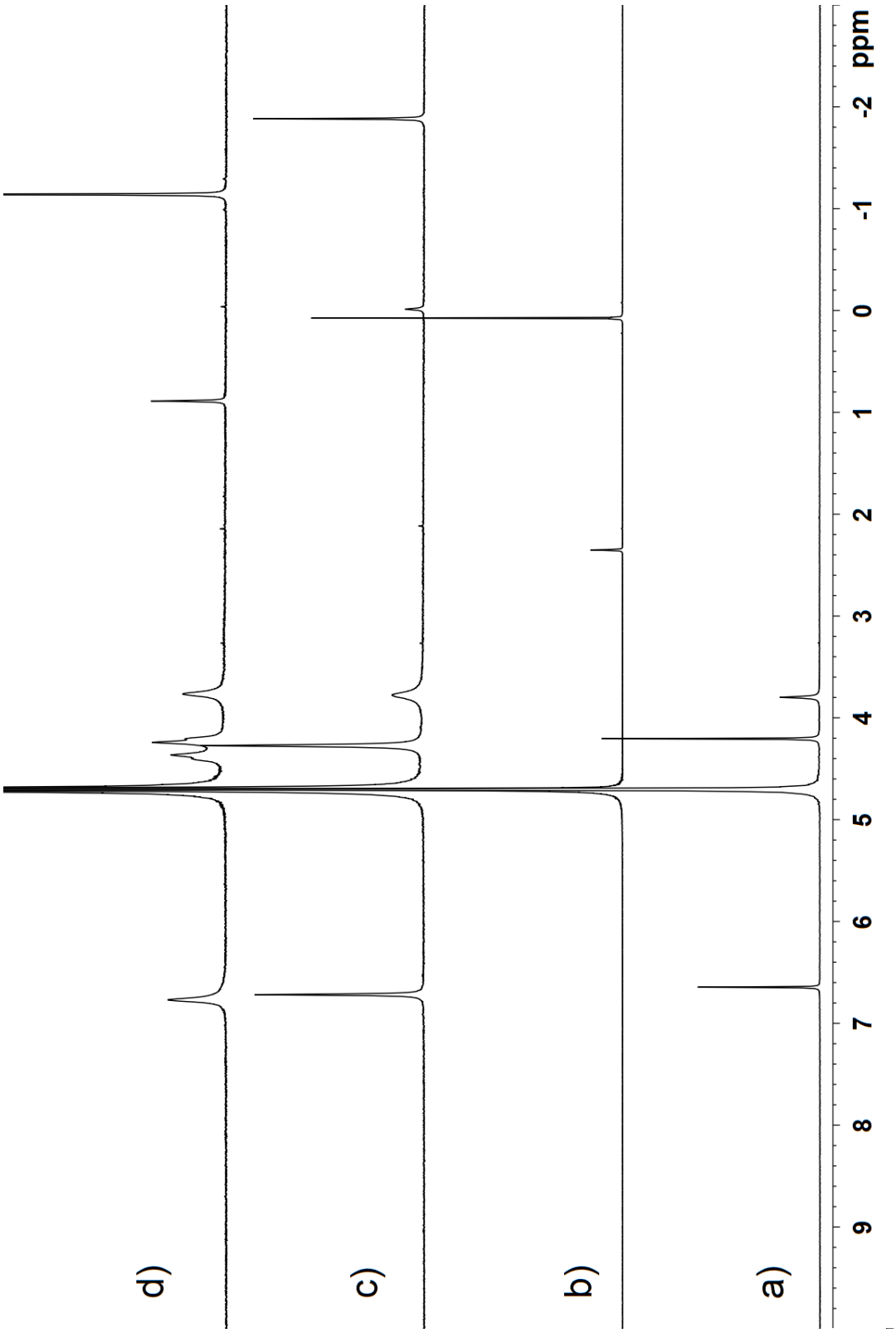

Figure S4. ^{1}H NMR spectra recorded (600 MHz, RT, D_2O) for: a) **WP6**, b) Guest **G4**, c) an equimolar mixture of **WP6** and Guest **G4** (1 mM), and d) a 2:1 mixture of Guest **G4** (2 mM) and **WP6** (1 mM).

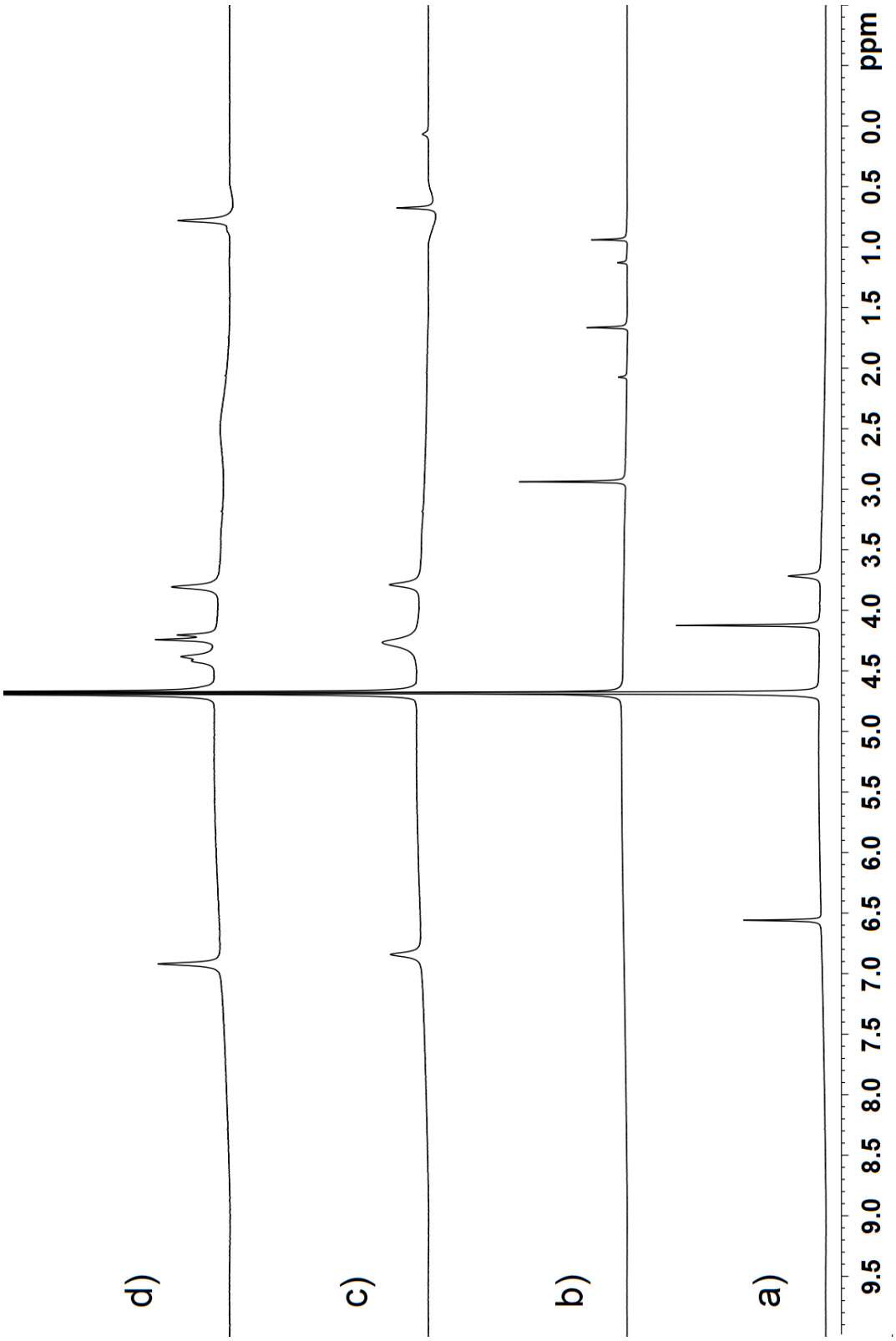

Figure S5. ^{1}H NMR spectra recorded (600 MHz, RT, D_2O) for: a) **WP6**, b) Guest **G5**, c) an equimolar mixture of **WP6** and Guest **G5** (1 mM), and d) a 2:1 mixture of Guest **G5** (2 mM) and **WP6** (1 mM).

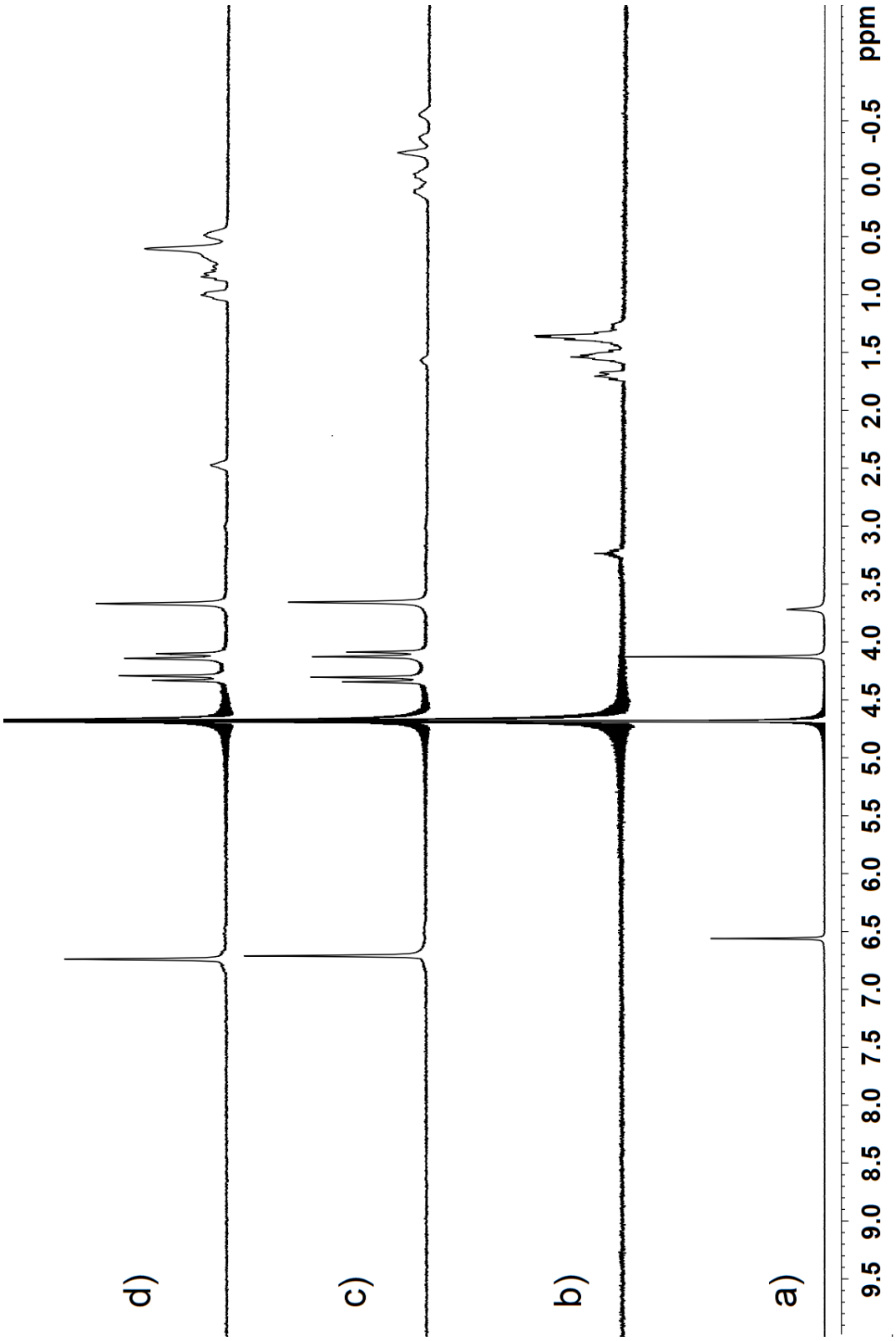

Figure S6. ^{1}H NMR spectra recorded (400 MHz, RT, D_2O) for: a) **WP6**, b) Guest **G6**, c) an equimolar mixture of **WP6** and Guest **G6** (1 mM), and d) a 2:1 mixture of Guest **G6** (2 mM) and **WP6** (1 mM).

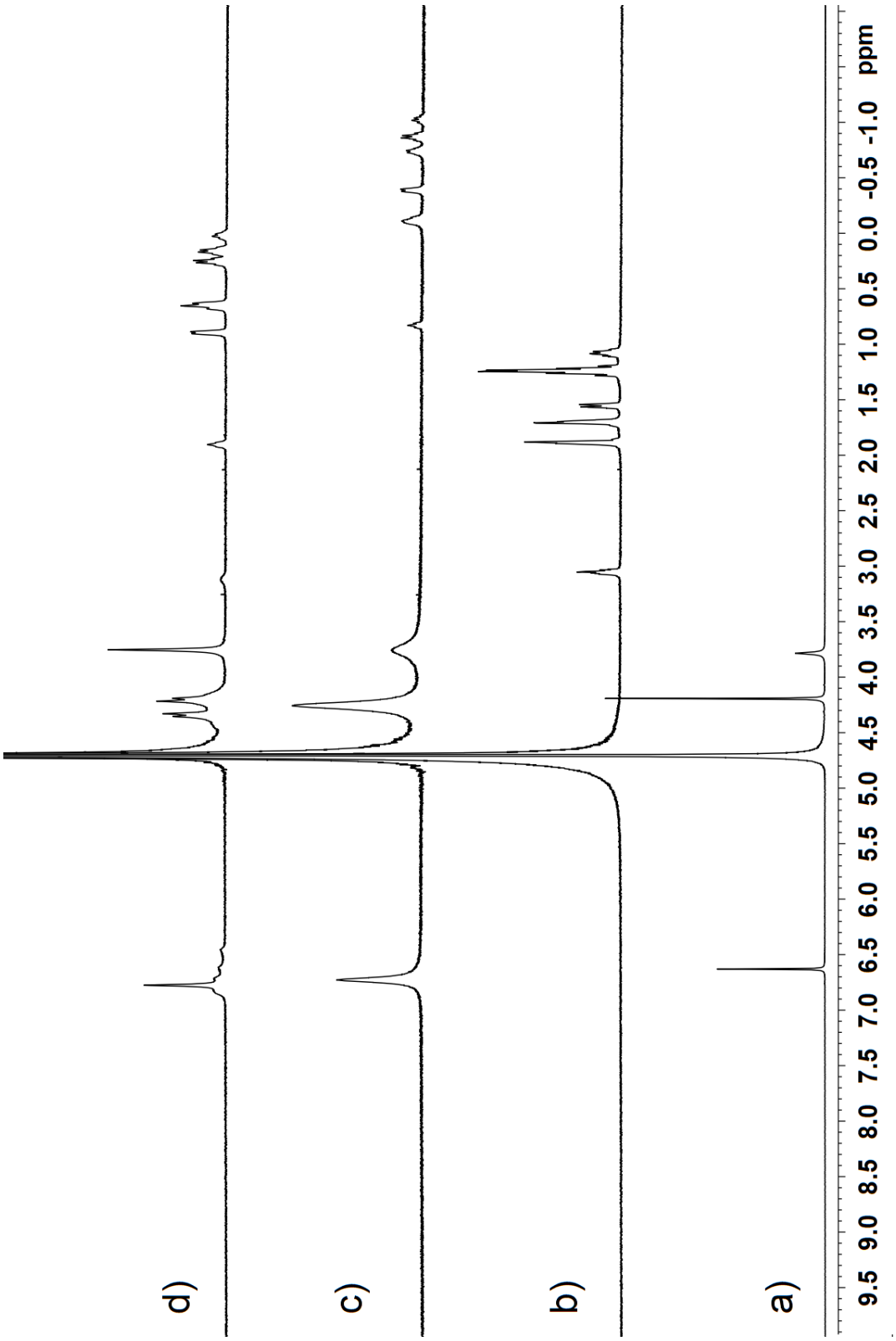

Figure S7. ^{1}H NMR spectra recorded (400 MHz, RT, D_2O) for: a) **WP6**, b) Guest **G7**, c) an equimolar mixture of **WP6** and Guest **G7** (1 mM), and d) a 2:1 mixture of Guest **G7** (2 mM) and **WP6** (1 mM).

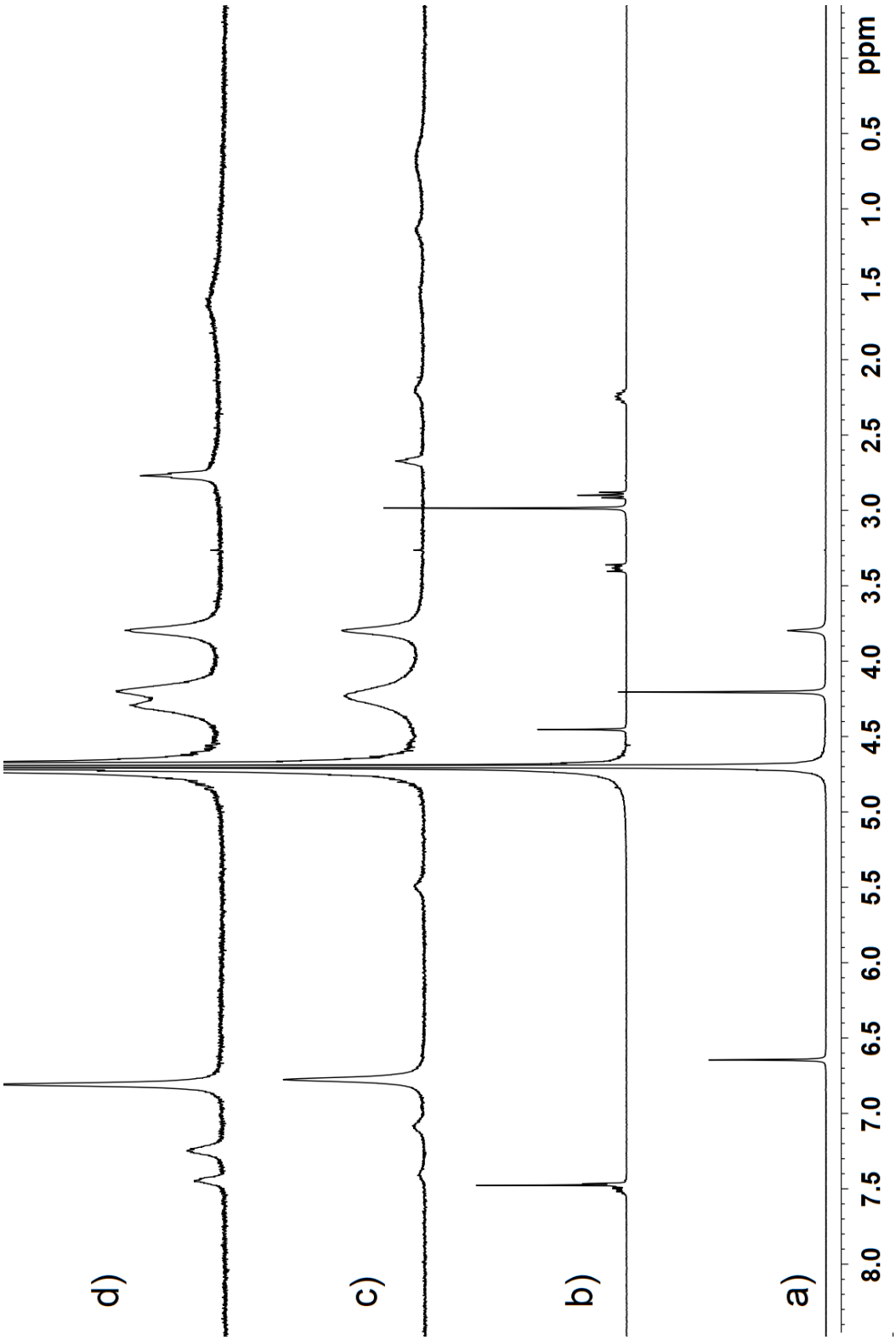

Figure S8. ^{1}H NMR spectra recorded (600 MHz, RT, D_2O) for: a) **WP6**, b) Guest **G8**, c) an equimolar mixture of **WP6** and Guest **G8** (1 mM), and d) a 2:1 mixture of Guest **G8** (2 mM) and **WP6** (1 mM).

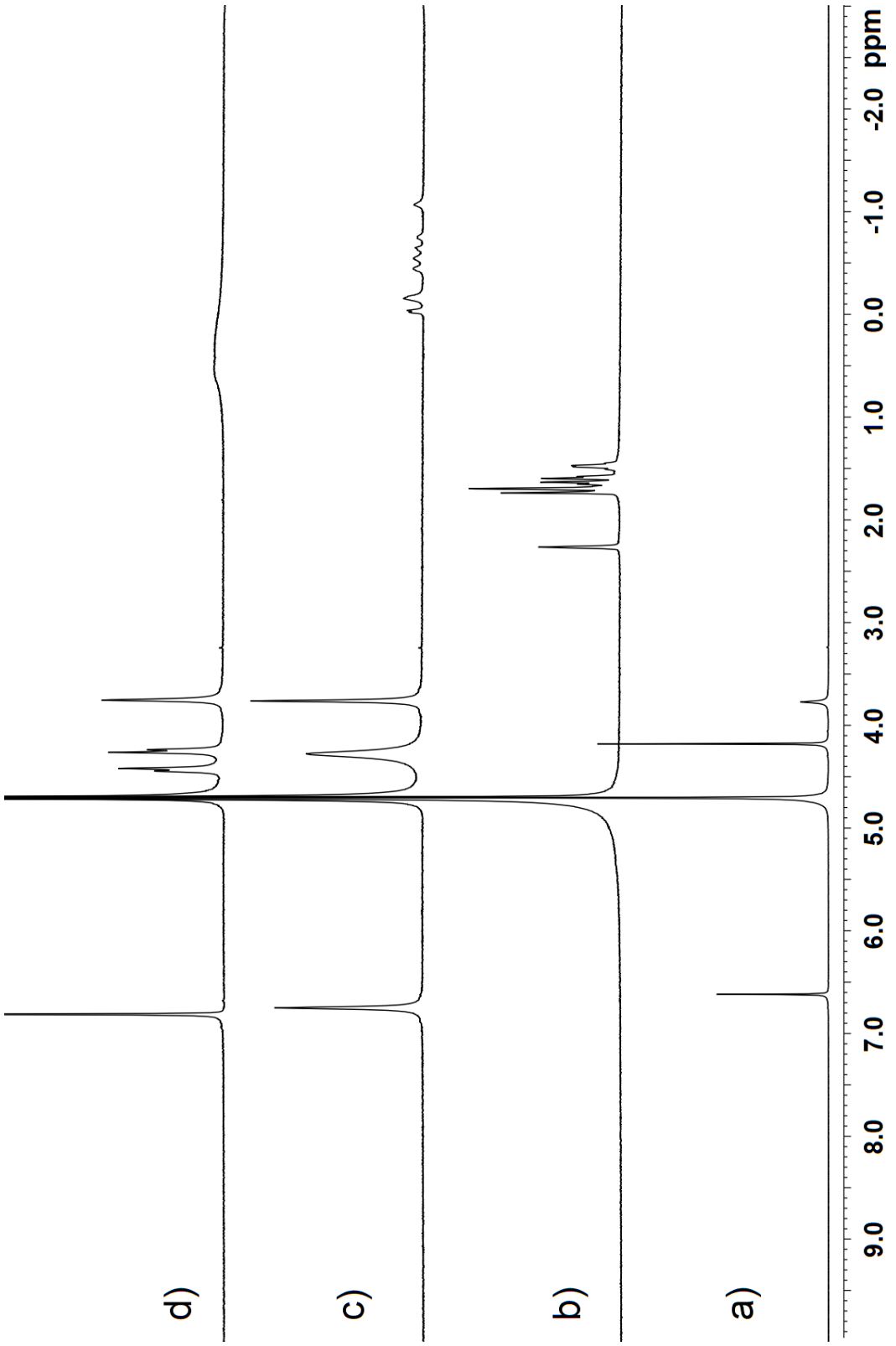

Figure S9. ^{1}H NMR spectra recorded (600 MHz, RT, D_2O) for: a) **WP6**, b) Guest **G9**, c) an equimolar mixture of **WP6** and Guest **G9** (1 mM), and d) a 2:1 mixture of Guest **G9** (2 mM) and **WP6** (1 mM).

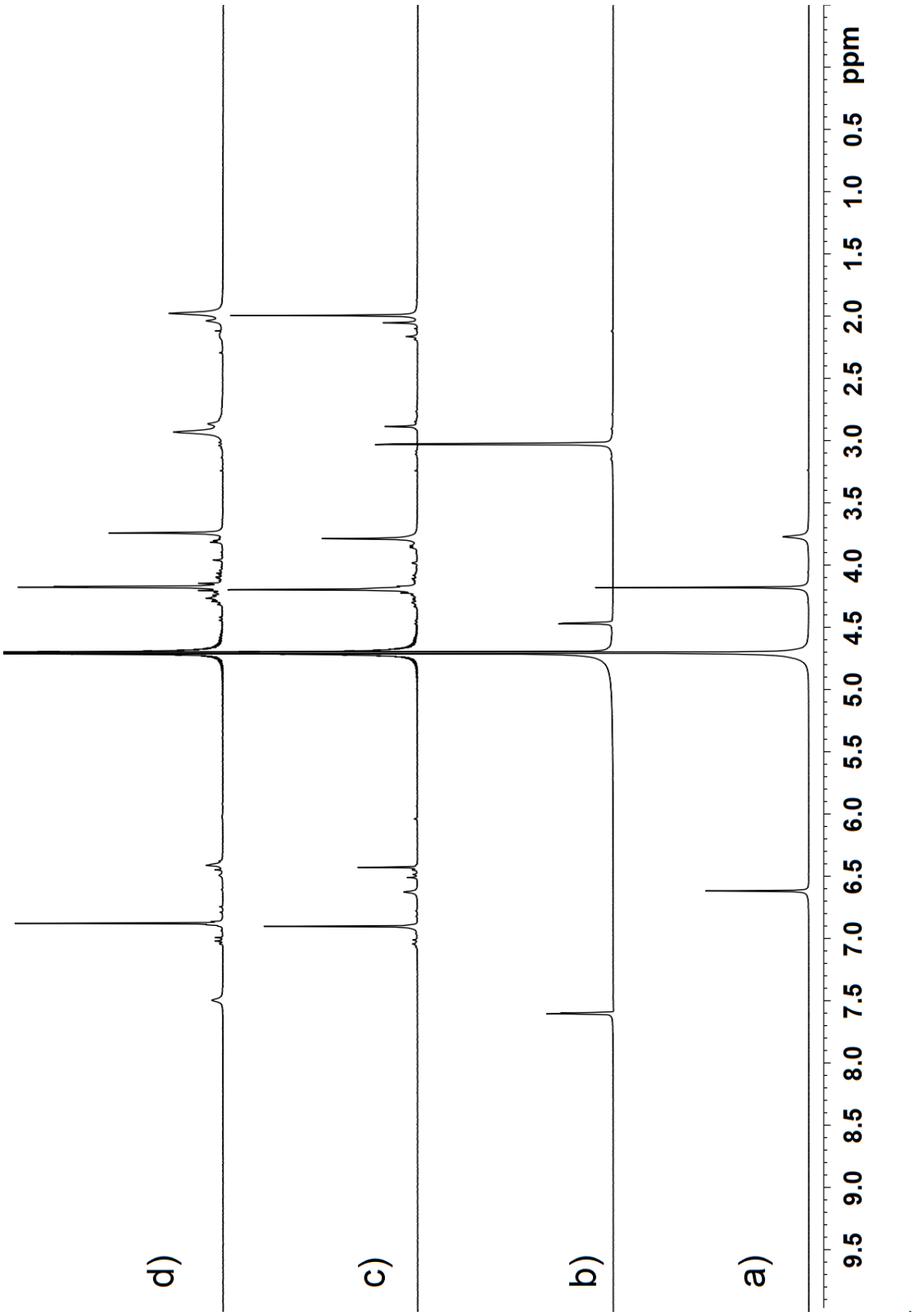

Figure S10. ^{1}H NMR spectra recorded (600 MHz, RT, D_2O) for: a) **WP6**, b) Guest **G10**, c) an equimolar mixture of **WP6** and Guest **G10** (1 mM), and d) a 2:1 mixture of Guest **G10** (2 mM) and **WP6** (1 mM).

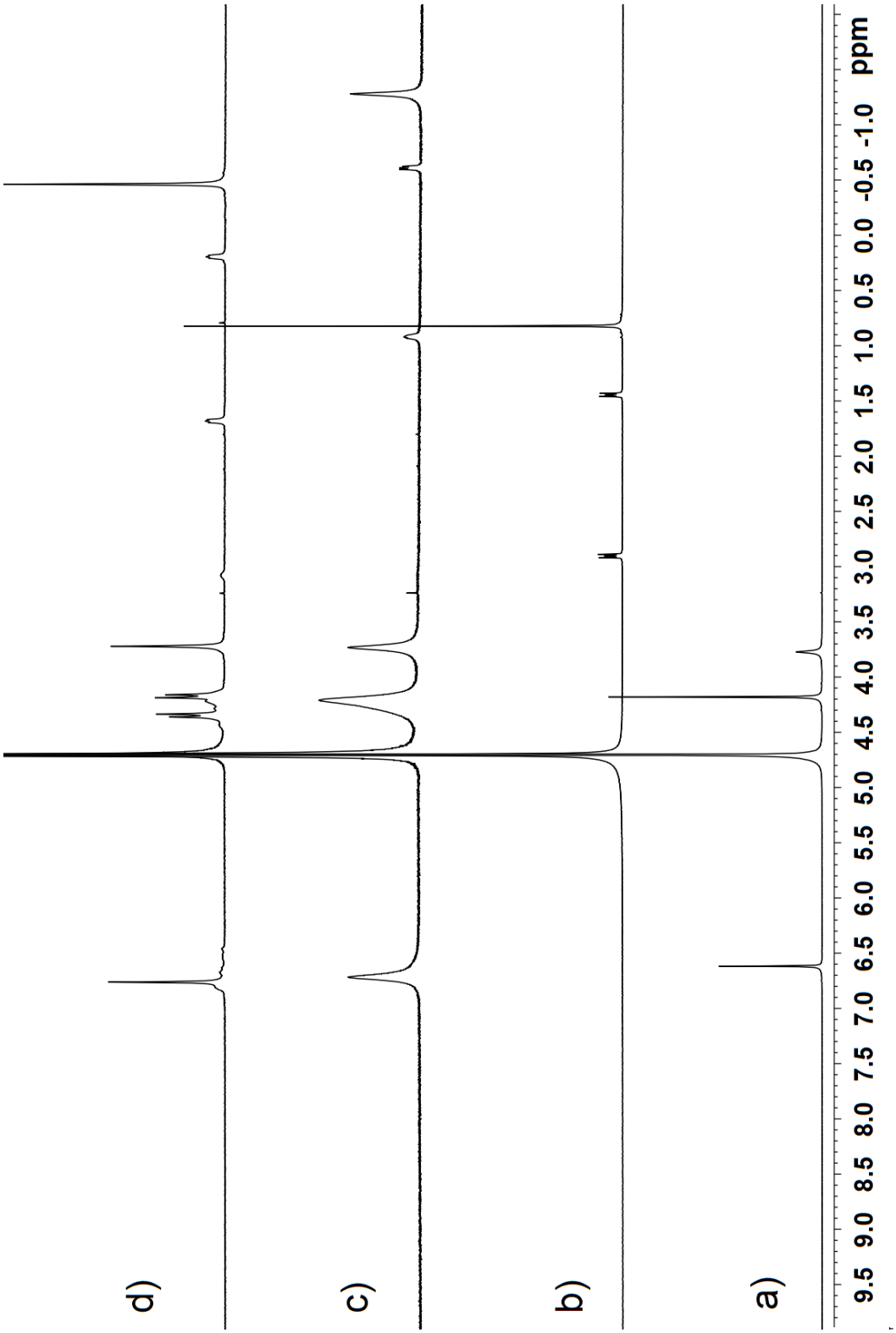

Figure S11. ^{1}H NMR spectra recorded (600 MHz, RT, D_2O) for: a) **WP6**, b) Guest **G11**, c) an equimolar mixture of **WP6** and Guest **G11** (1 mM), and d) a 2:1 mixture of Guest **G11** (2 mM) and **WP6** (1 mM).

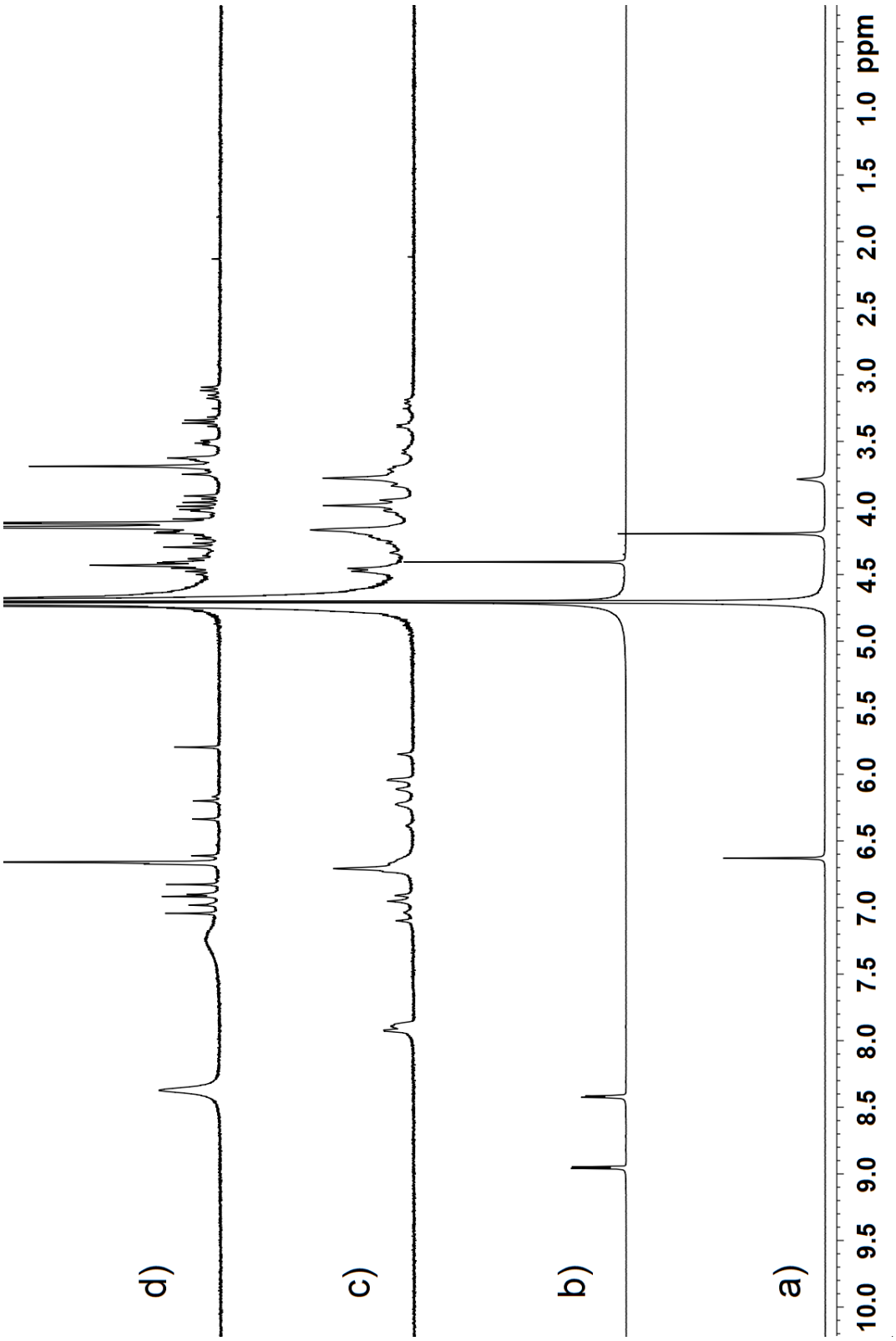

Figure S12. ^{1}H NMR spectra recorded (600 MHz, RT, D_2O) for: a) **WP6**, b) Guest **G13**, c) an equimolar mixture of **WP6** and Guest **G13** (1 mM), and d) a 2:1 mixture of Guest **G13** (2 mM) and **WP6** (1 mM).

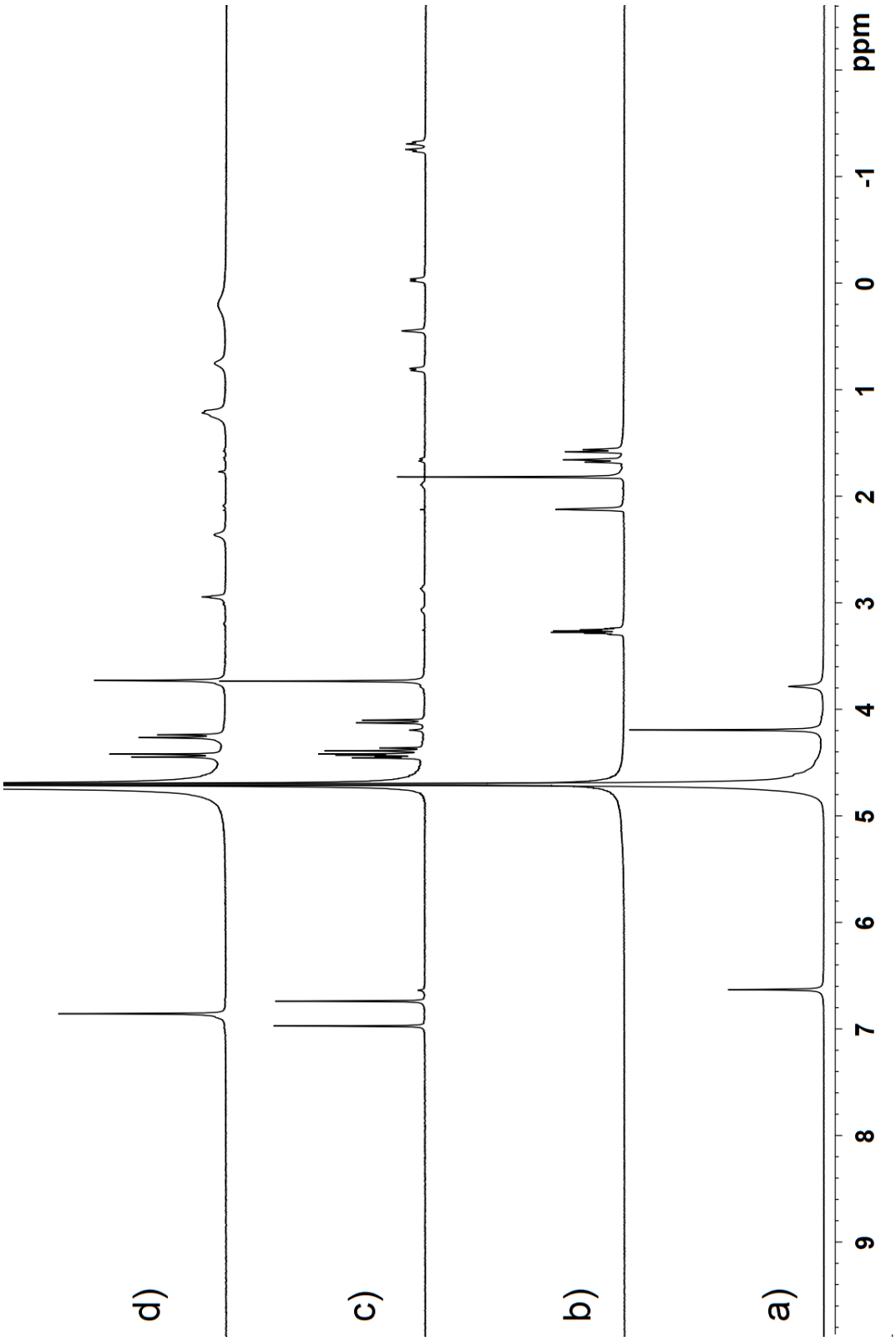

Figure S13. ^{1}H NMR spectra recorded (600 MHz, RT, D_2O) for: a) **WP6**, b) Guest **G14**, c) an equimolar mixture of **WP6** and Guest **G14** (1 mM), and d) a 2:1 mixture of Guest **G14** (2 mM) and **WP6** (1 mM).

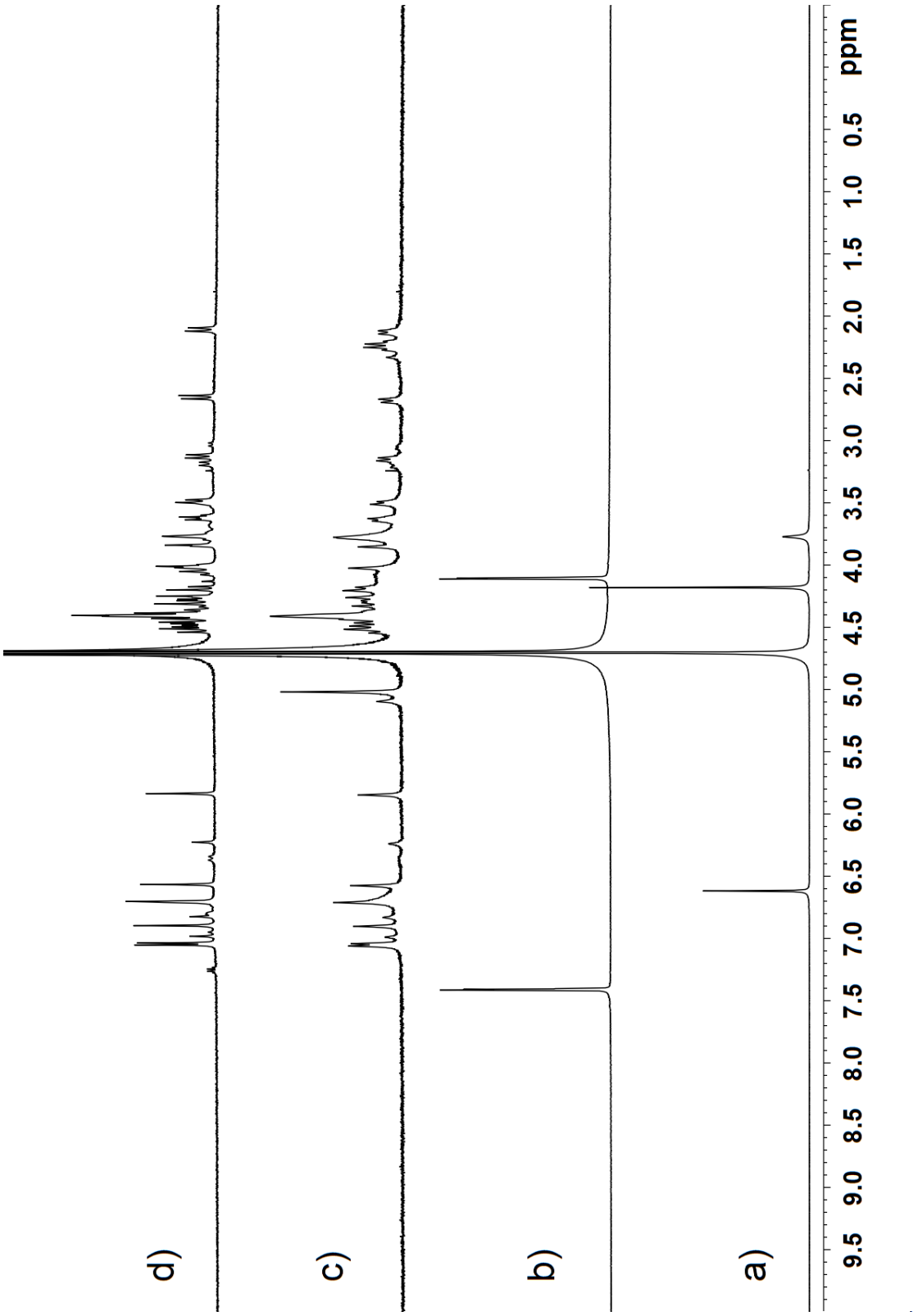

Figure S14. ^{1}H NMR spectra recorded (600 MHz, RT, D_2O) for: a) **WP6**, b) Guest **G15**, c) an equimolar mixture of **WP6** and Guest **G15** (1 mM), and d) a 2:1 mixture of Guest **G15** (2 mM) and **WP6** (1 mM).

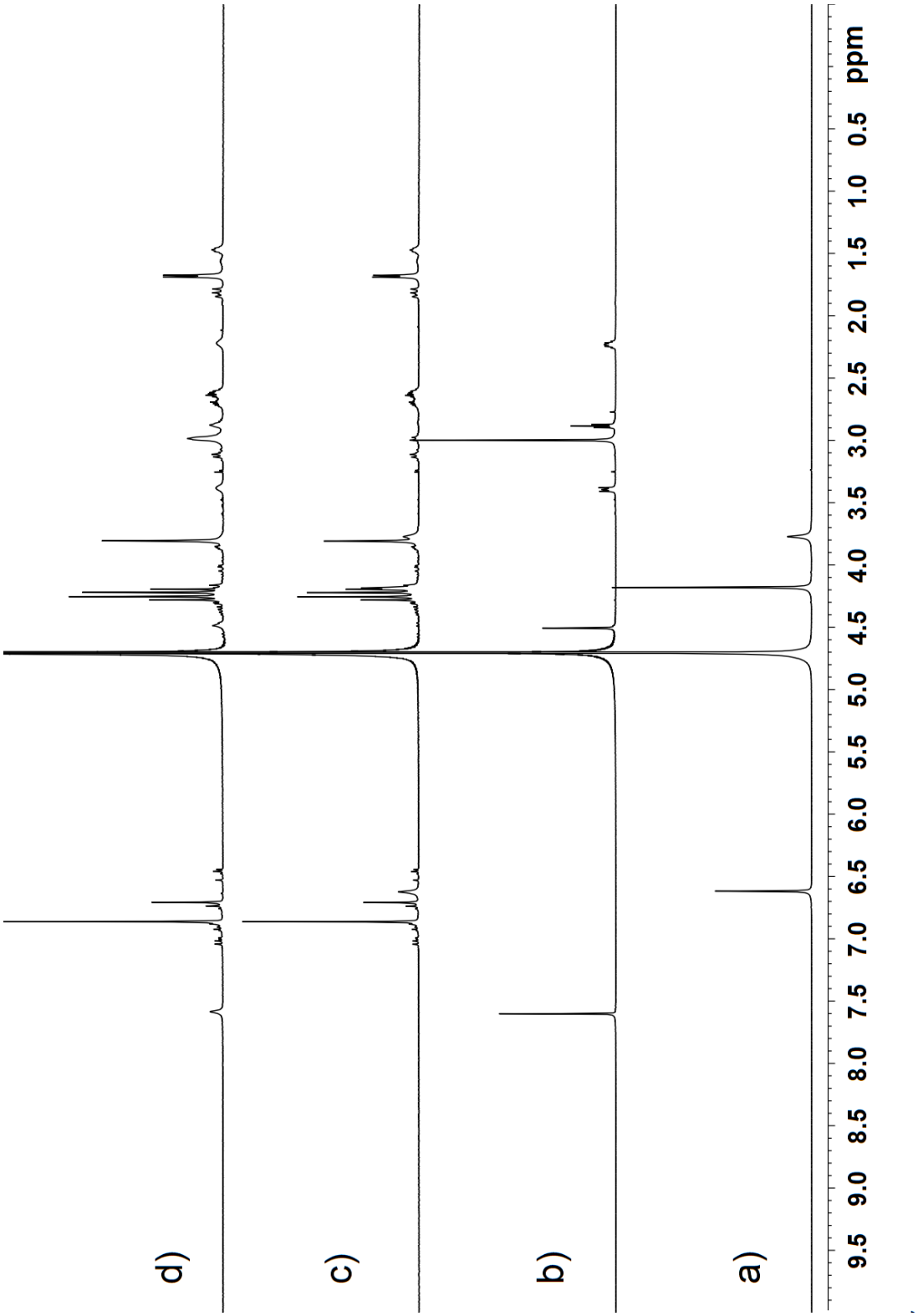

Figure S15. ^{1}H NMR spectra recorded (600 MHz, RT, D_2O) for: a) **WP6**, b) Guest **G16**, c) an equimolar mixture of **WP6** and Guest **G16** (1 mM), and d) a 2:1 mixture of Guest **G16** (2 mM) and **WP6** (1 mM).

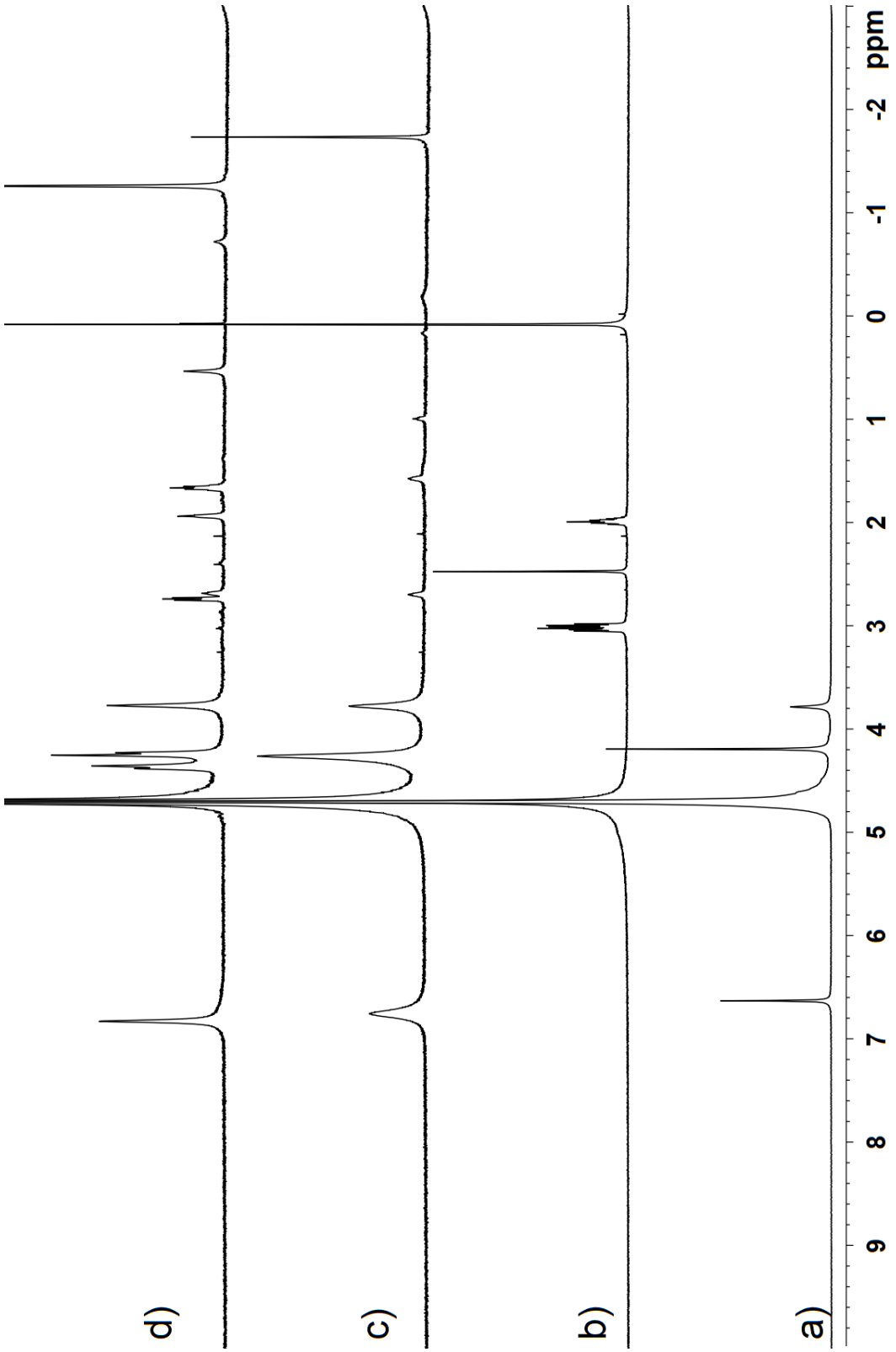

Figure S16. ^{1}H NMR spectra recorded (600 MHz, RT, D_2O) for: a) **WP6**, b) Guest **G17**, c) an equimolar mixture of **WP6** and Guest **G17** (1 mM), and d) a 2:1 mixture of Guest **G17** (2 mM) and **WP6** (1 mM).

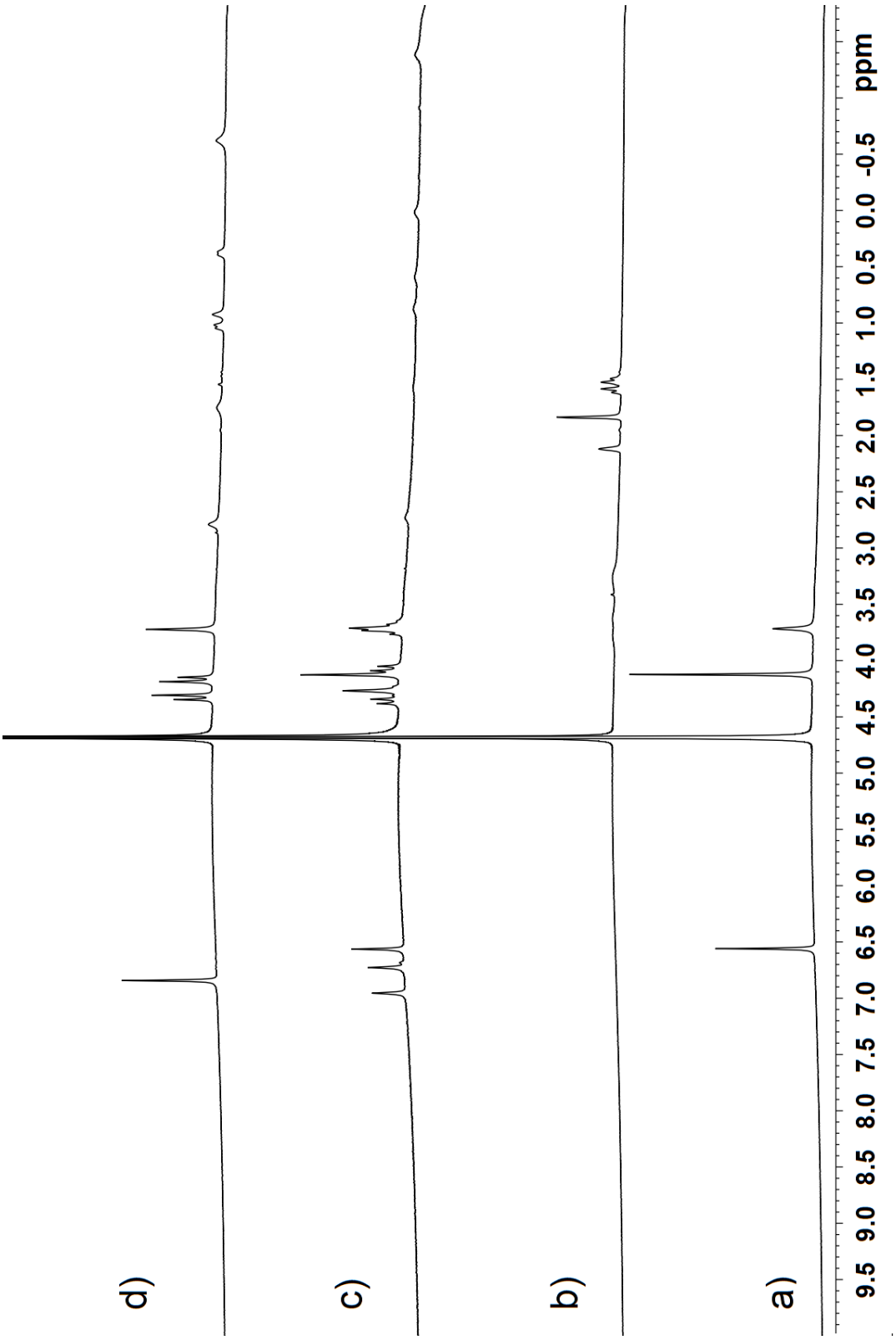

Figure S17. ^{1}H NMR spectra recorded (600 MHz, RT, D_2O) for: a) **WP6**, b) Guest **G18**, c) an equimolar mixture of **WP6** and Guest **G18** (1 mM), and d) a 2:1 mixture of Guest **G18** (2 mM) and **WP6** (1 mM).

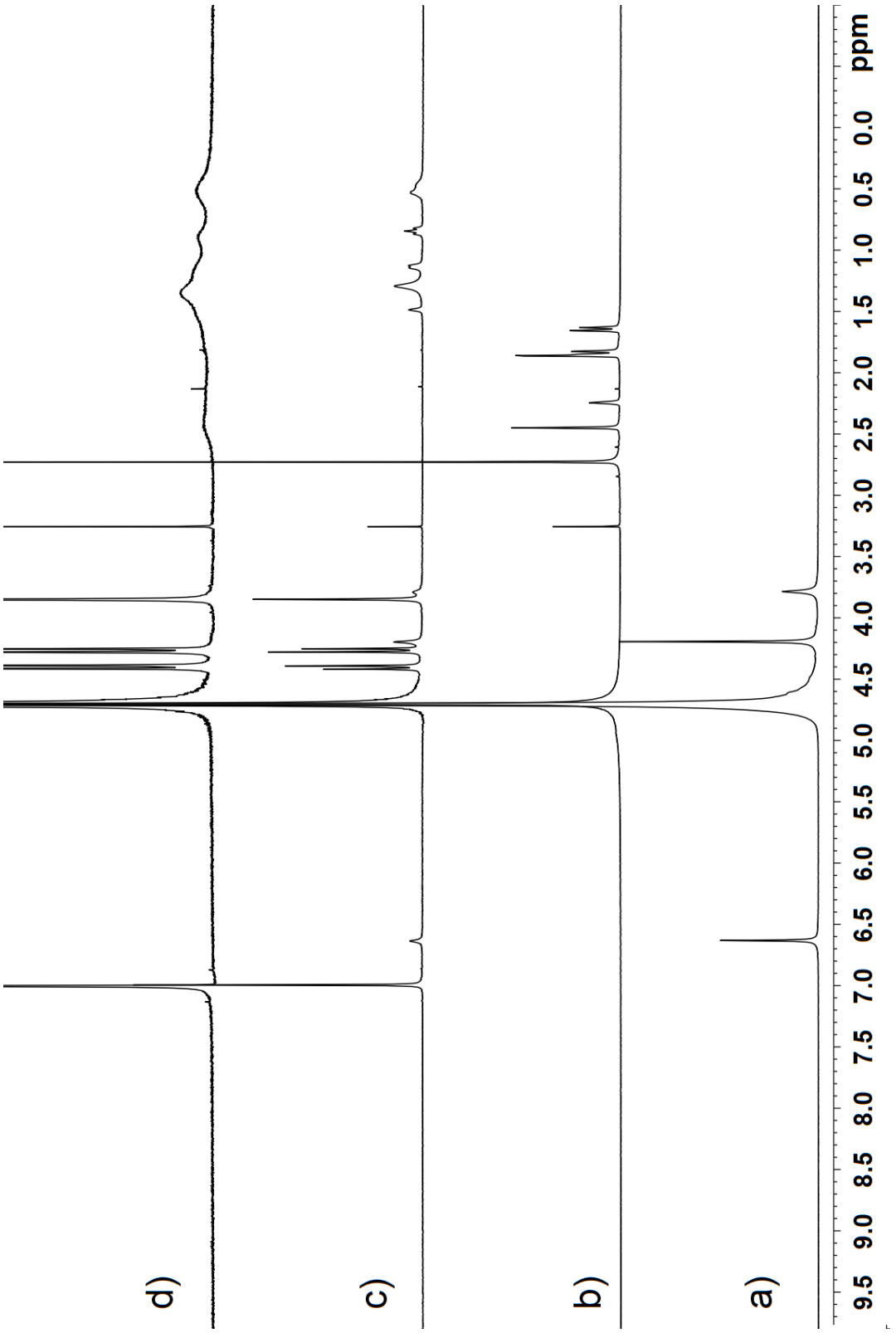

Figure S18. ^{1}H NMR spectra recorded (600 MHz, RT, D_2O) for: a) **WP6**, b) Guest **G19**, c) an equimolar mixture of **WP6** and Guest **G19** (1 mM), and d) a 2:1 mixture of Guest **G19** (2 mM) and **WP6** (1 mM).

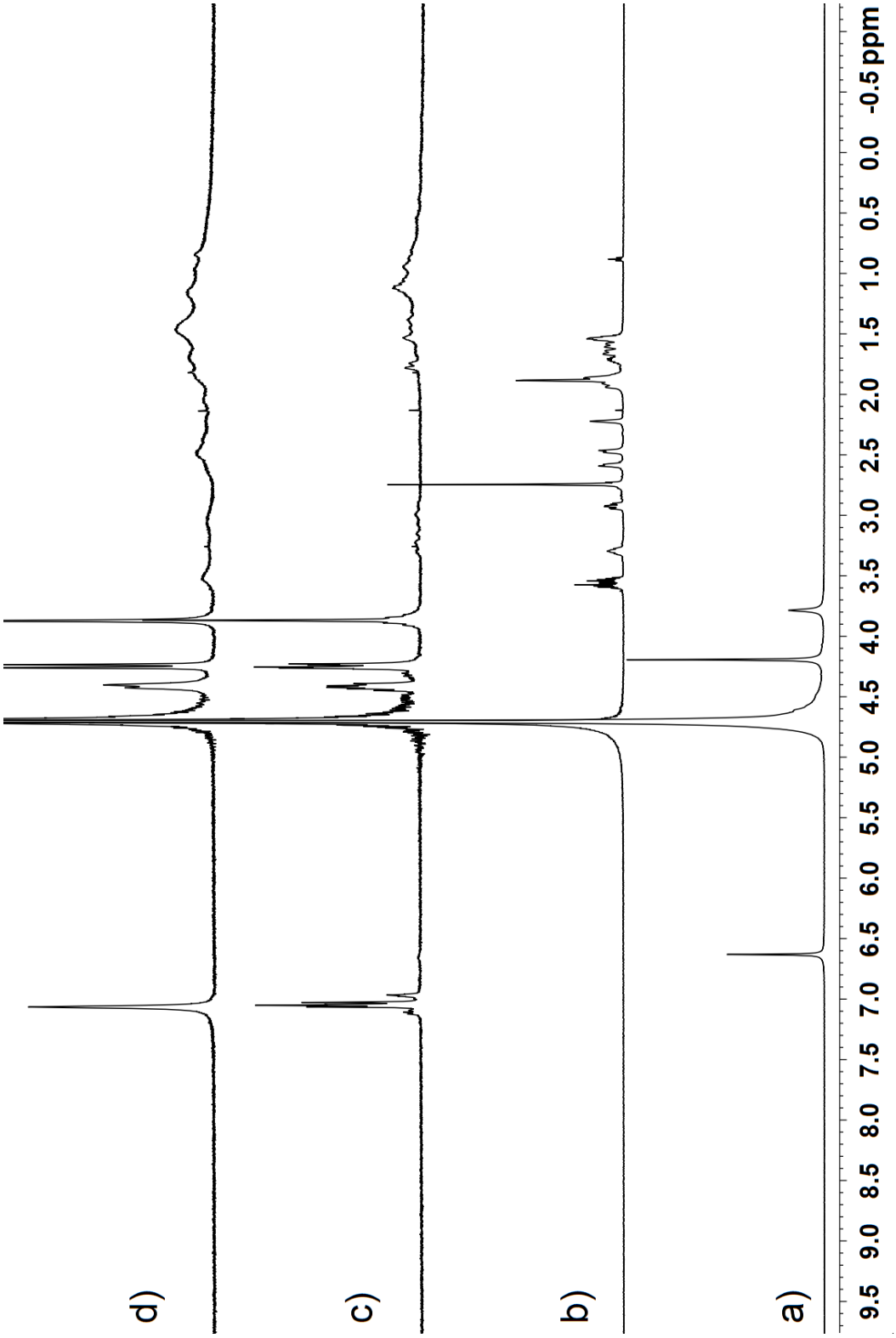

Figure S19. ^{1}H NMR spectra recorded (600 MHz, RT, D_2O) for: a) **WP6**, b) Guest **G20**, c) an equimolar mixture of **WP6** and Guest **G20** (1 mM), and d) a 2:1 mixture of Guest **G20** (2 mM) and **WP6** (1 mM).

Determination of K_a between various hosts and drugs of abuse using Isothermal Titration Calorimetry (ITC). Normal Guests

All ITC experiments were conducted in the 200 μL working volume of the sample cell of the PEAQ ITC instrument. We used an injection syringe of 40 μL capacity. In each case, the host and guest solutions were prepared in phosphate buffered saline (PBS) at pH 7.4. The sample cell was filled to capacity (200 μL) with the host solution and the guest solution was titrated in (first injection = 0.4 μL, subsequent 18 injections = 2 μL). In select cases, competitive titrations were required where host and an excess of weaker binding guest were included in the cell and the tighter binding guest was titrated into the cell. For direct titrations, the binding data was fitted using the 1:1 binding model in MicroCal PEAQ-ITC analysis software whereas for competitive titrations the competition binding model was used.

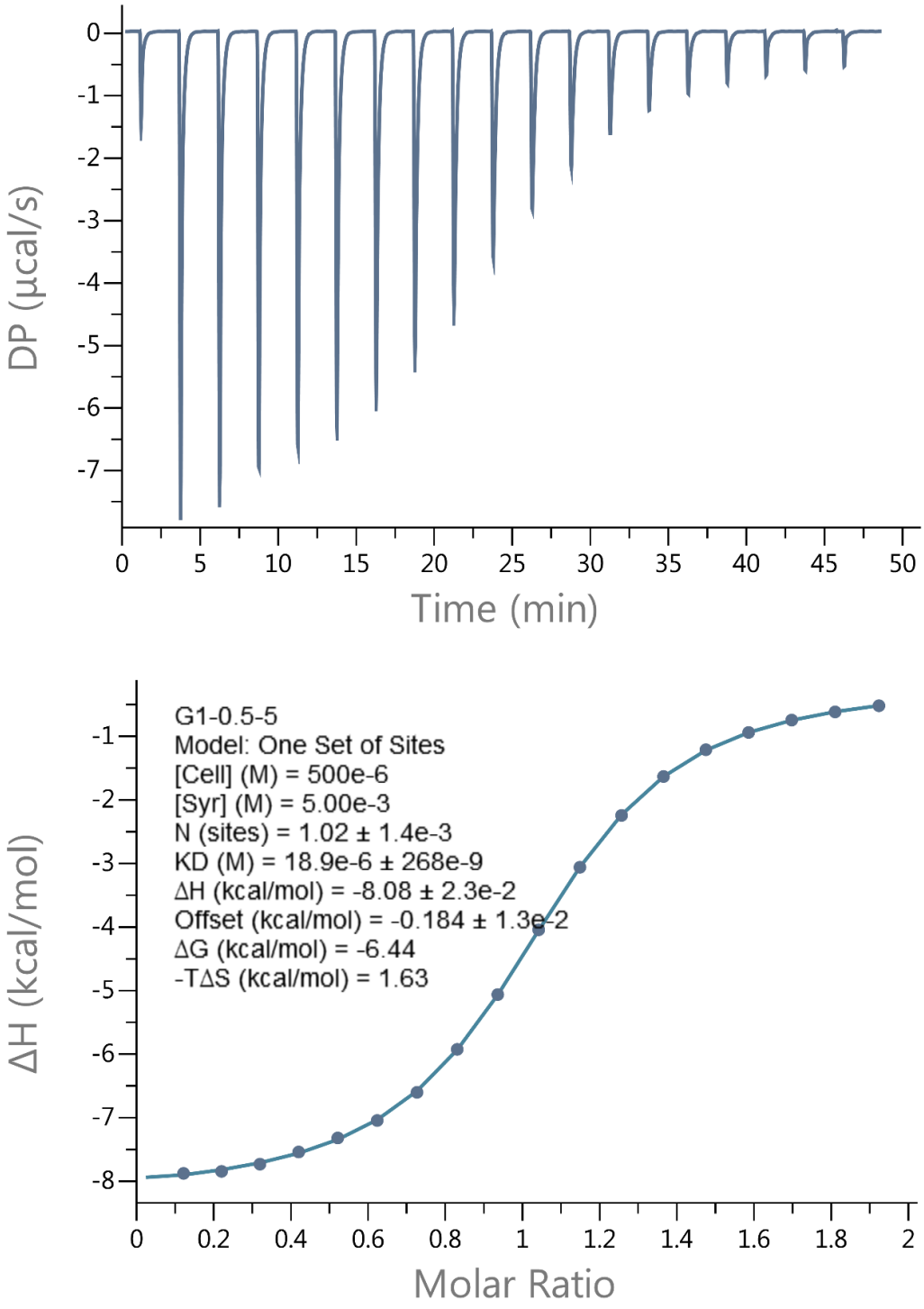

Figure S20. Top) Plot of DP vs time from the titration of molecular container **WP6** (500 µM) in the cell with guest **G1** (5.00 mM) in 1× PBS buffer solution; Bottom) plot of the ΔH as a function of molar ratio. The solid line represents the best non-linear fit of the data to a 1:1 binding model (K_a = (5.29 ± 0.07) × 10^4 M^{-1}, ΔH = -8.08 ± 0.02 kcal/mol, -TΔS = 1.63 kcal/mol).

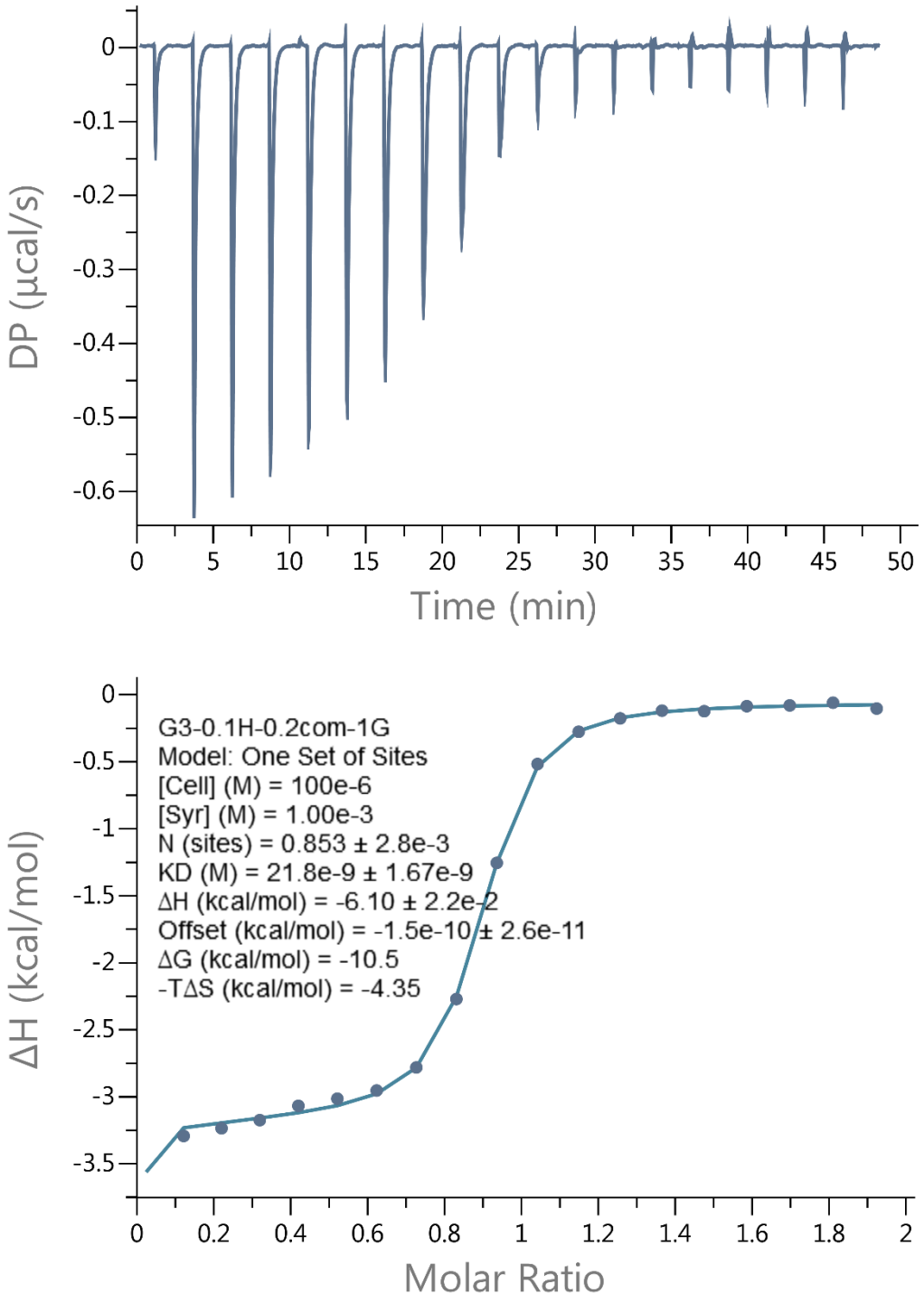

Figure S21. Top) Plot of DP vs time from the titration of molecular container **WP6** (100 µM) and competitive guest **G7** (200 µM) in the cell with guest **G2** (1.00 mM) in 1× PBS buffer solution; Bottom) Plot of the ΔH as a function of molar ratio. The solid line represents the best non-linear fit of the data to a 1:1 binding model (K_a = (4.59 ± 0.35) × 10^7 M^{-1}, ΔH = –6.10 ± 0.02 kcal/mol, −TΔS = −4.35 kcal/mol).

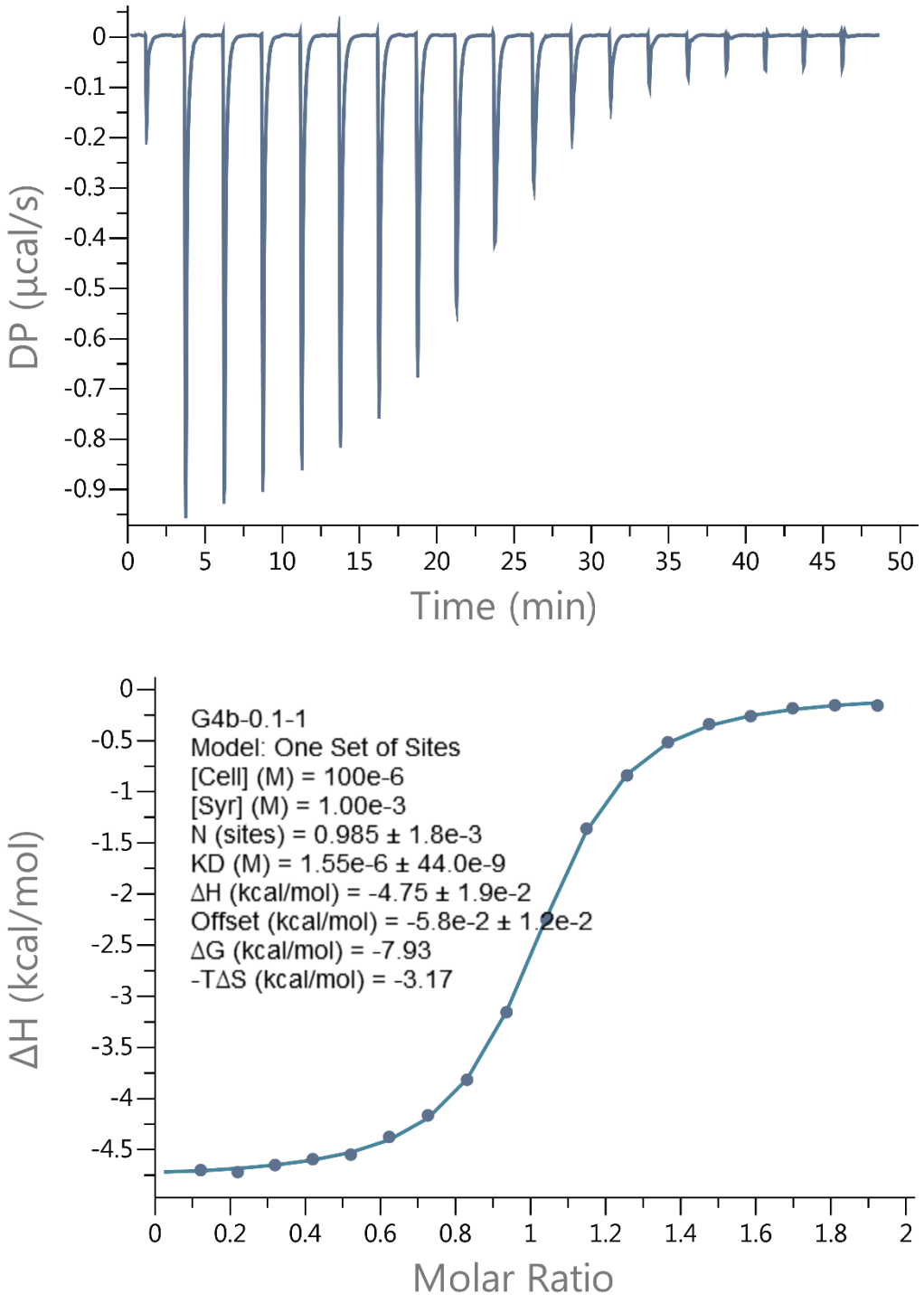

Figure S22. Top) Plot of DP vs time from the titration of molecular container **WP6** (100 µM) in the cell with guest **G3** (1.00 mM) in 1× PBS buffer solution; Bottom) plot of the ΔH as a function of molar ratio. The solid line represents the best non-linear fit of the data to a 1:1 binding model (K_a = (6.45 ± 0.18) × 10^4 M^{-1}, ΔH = -4.75 ± 0.02 kcal/mol, -TΔS = -3.17 kcal/mol).

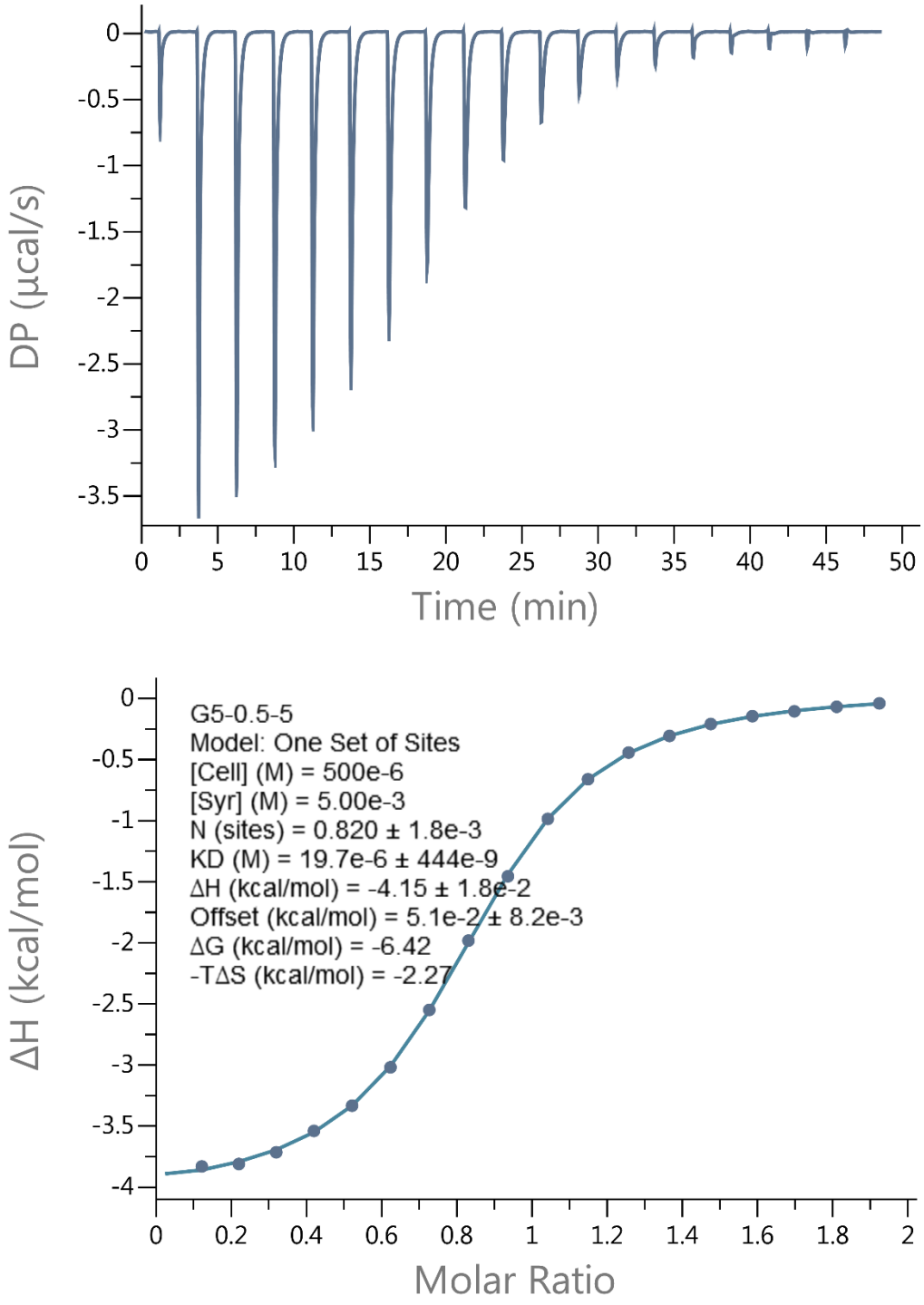

Figure S23. Top) Plot of DP vs time from the titration of molecular container **WP6** (500 µM) in the cell with guest **G4** (5.00 mM) in 1× PBS buffer solution; Bottom) plot of the ΔH as a function of molar ratio. The solid line represents the best non-linear fit of the data to a 1:1 binding model (K_a = (5.08 ± 0.11) × 10^4 M^{-1}, ΔH = -4.15 ± 0.02 kcal/mol, -TΔS = -2.27 kcal/mol).

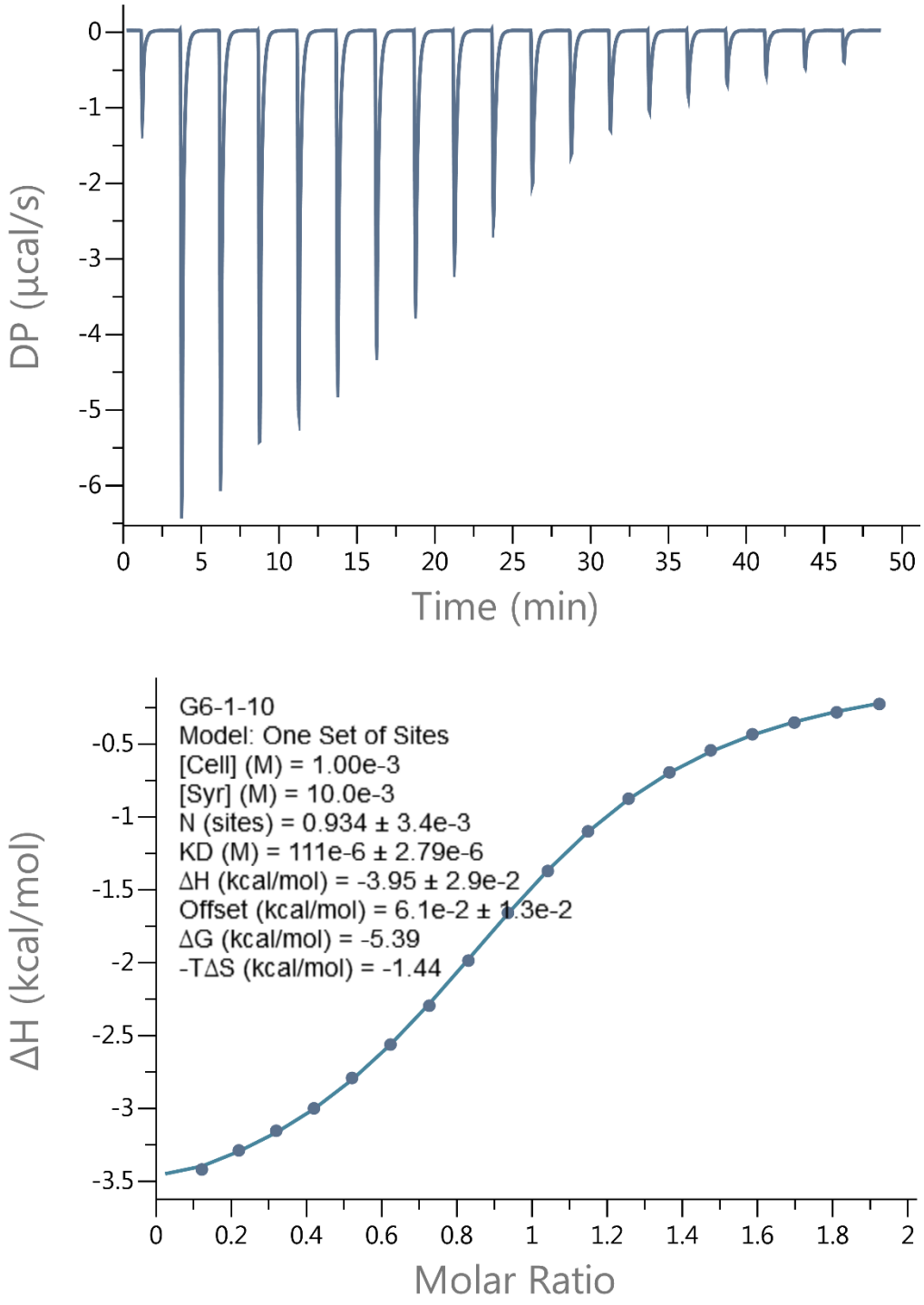

Figure S24. Top) Plot of DP vs time from the titration of molecular container **WP6** (1.00 mM) in the cell with guest **G5** (10.00 mM) in 1× PBS buffer solution; Bottom) plot of the ΔH as a function of molar ratio. The solid line represents the best non-linear fit of the data to a 1:1 binding model (K_a = (9.01 ± 0.23) × 10^3 M^{-1}, ΔH = -3.95 ± 0.03 kcal/mol, -TΔS = -1.44 kcal/mol).

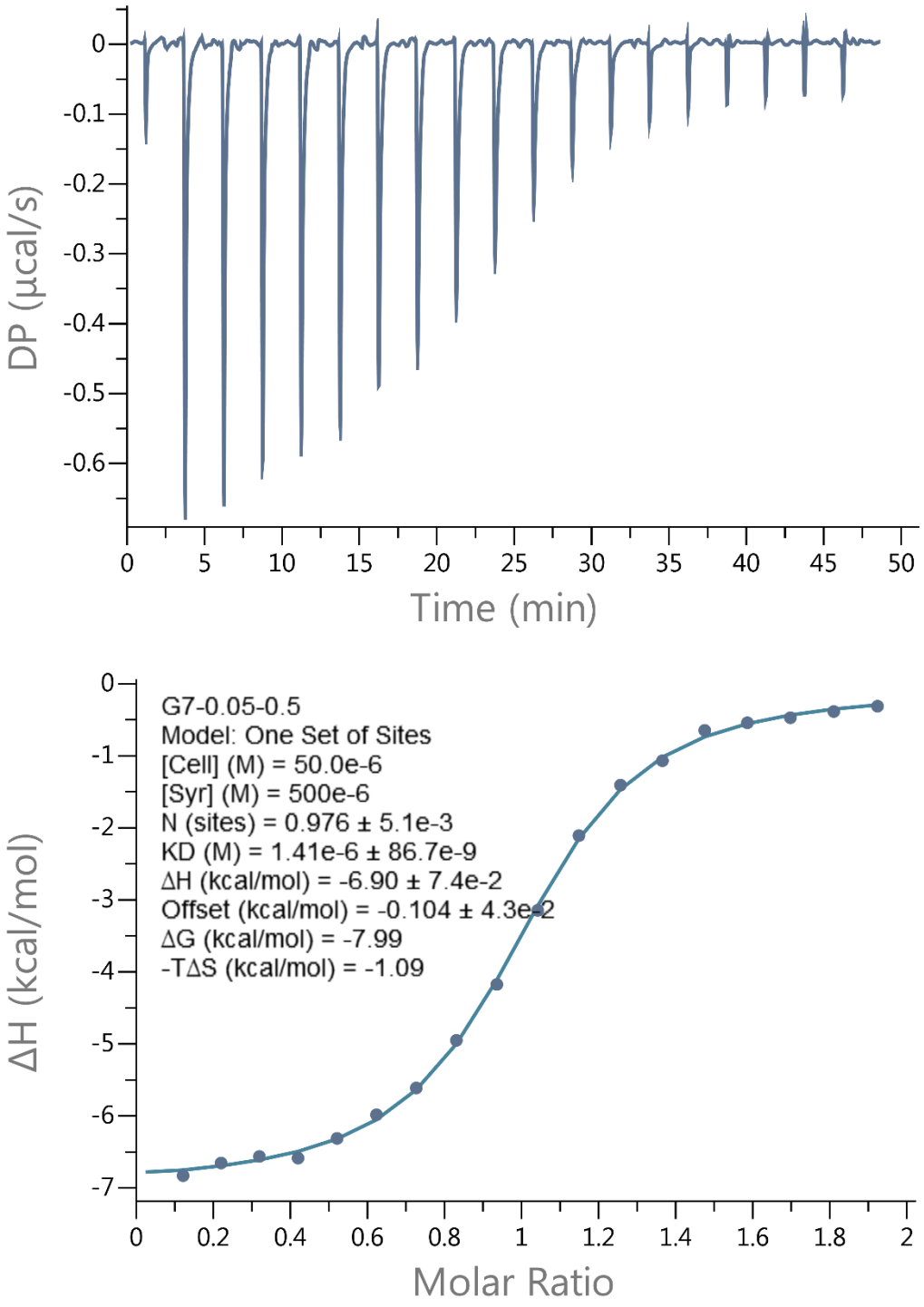

Figure S25. Top) Plot of DP vs time from the titration of molecular container **WP6** (50 µM) in the cell with guest **G6** (0.5 mM) in 1× PBS buffer solution; Bottom) plot of the ΔH as a function of molar ratio. The solid line represents the best non-linear fit of the data to a 1:1 binding model (K_a = (7.09 ± 0.44) × 10^5 M^{-1}, ΔH = –6.90 ± 0.07 kcal/mol, $-T\Delta S$ = –1.09 kcal/mol).

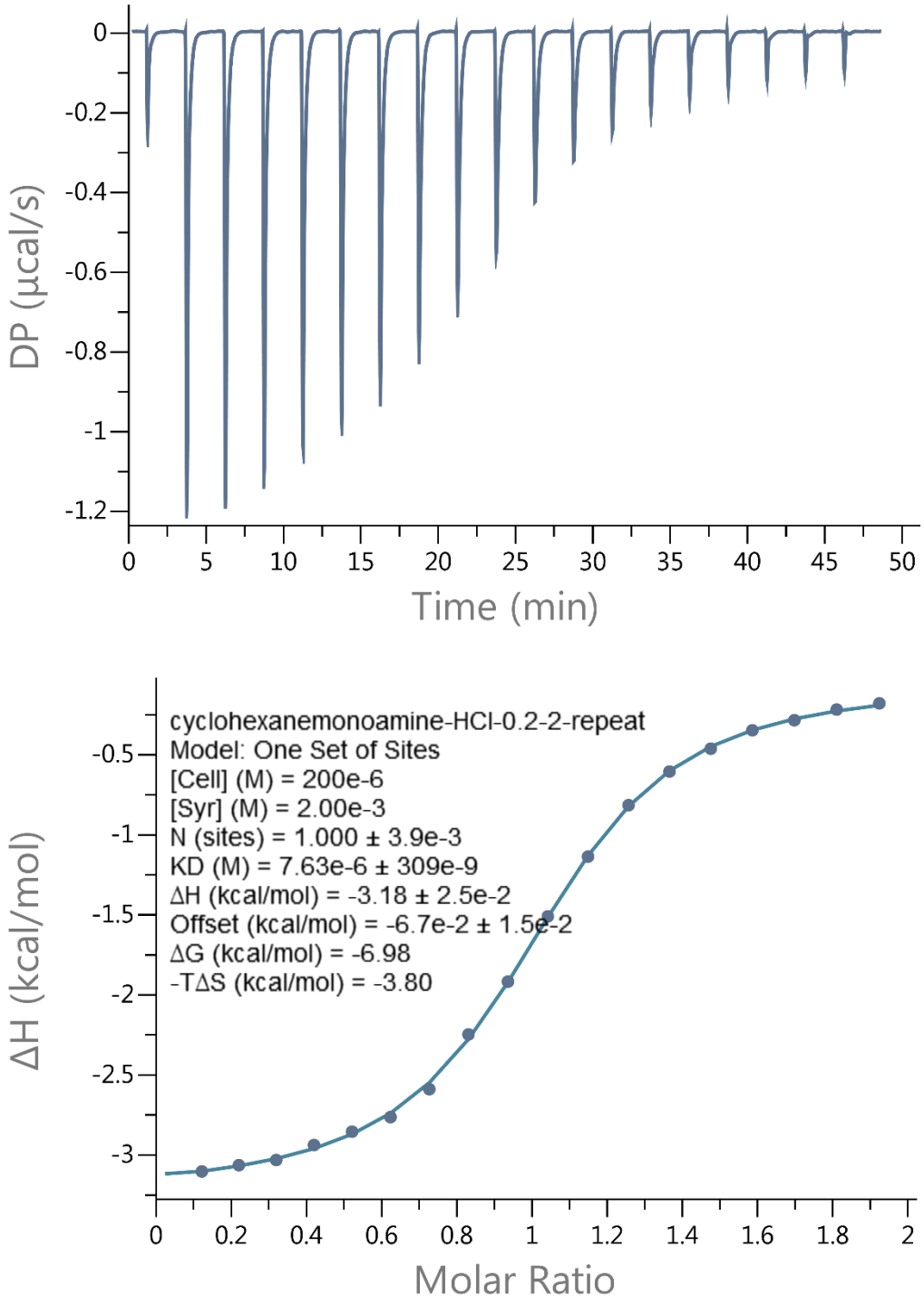

Figure S26. Top) Plot of DP vs time from the titration of molecular container **WP6** (200 µM) in the cell with guest **G7** (2.0 mM) in 1× PBS buffer solution; Bottom) plot of the ΔH as a function of molar ratio. The solid line represents the best non-linear fit of the data to a 1:1 binding model (K_a = (1.31 ± 0.05) × 10^5 M^{-1}, ΔH = –3.18 ± 0.02 kcal/mol, $-T\Delta S$ = –3.80 kcal/mol).

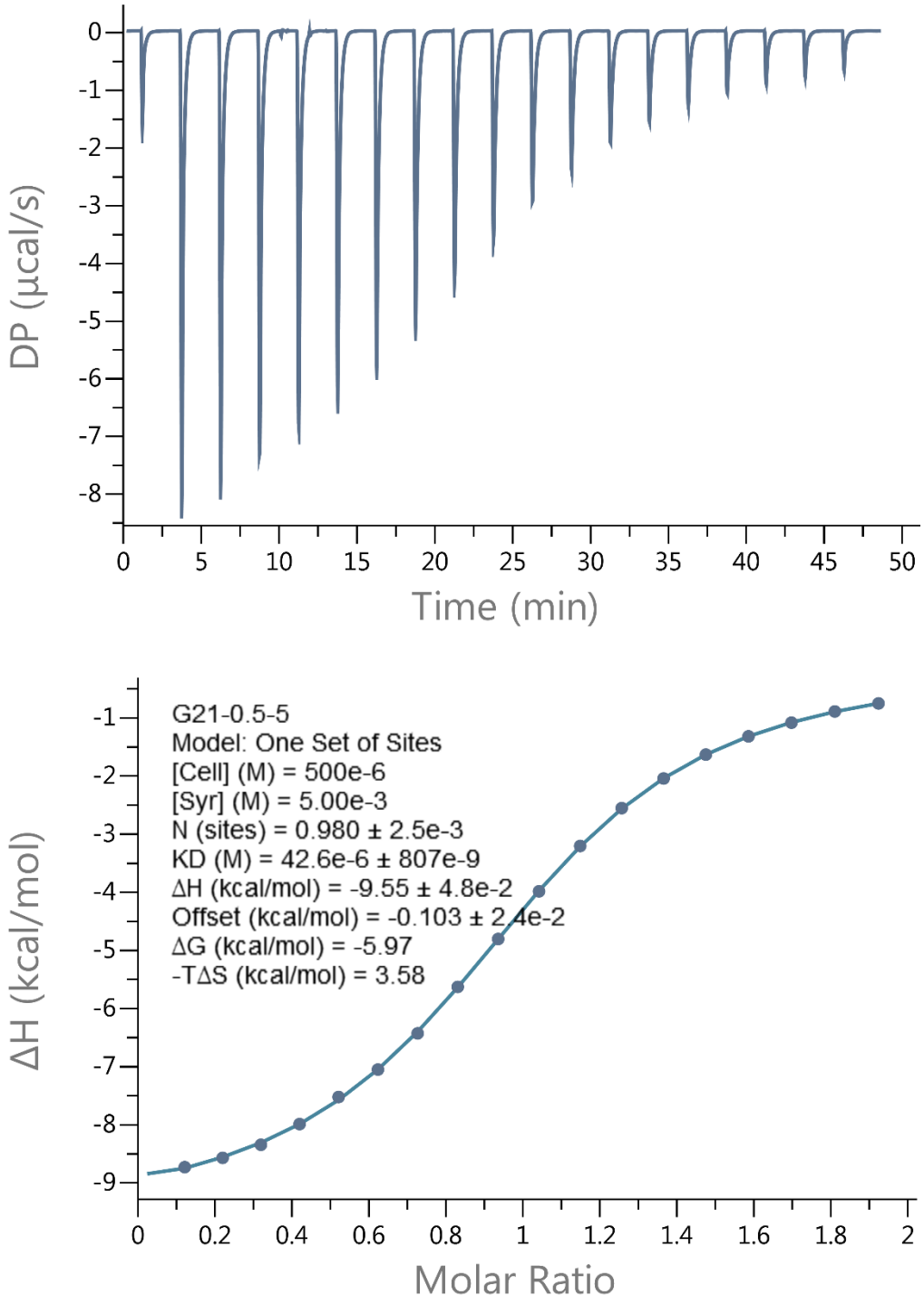

Figure S27. Top) Plot of DP vs time from the titration of molecular container **WP6** (0.50 mM) in the cell with guest **G8** (5.00 mM) in 1× PBS buffer solution; Bottom) plot of the ΔH as a function of molar ratio. The solid line represents the best non-linear fit of the data to a 1:1 binding model (K_a = (2.35 ± 0.04) × 10^5 M^{-1}, ΔH = −9.55 ± 0.05 kcal/mol, $-T\Delta S$ = 3.58 kcal/mol).

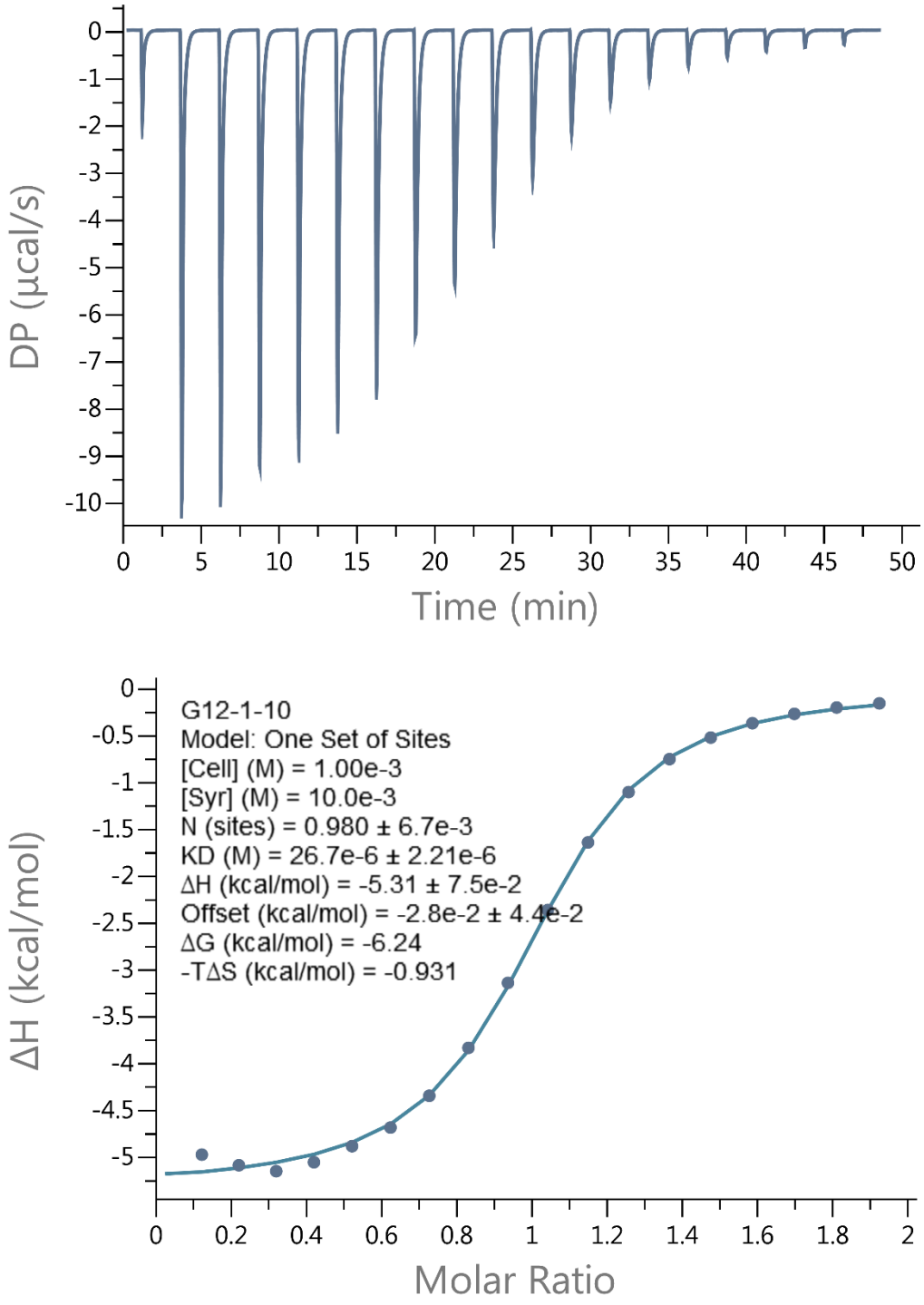

Figure S28. Top) Plot of DP vs time from the titration of molecular container **WP6** (1.00 mM) in the cell with guest **G9** (10.00 mM) in 1× PBS buffer solution; Bottom) plot of the ΔH as a function of molar ratio. The solid line represents the best non-linear fit of the data to a 1:1 binding model (K_a = (3.75 ± 0.31) × 10^4 M^{-1}, ΔH = −5.31 ± 0.08 kcal/mol, $-T\Delta S$ = −0.93 kcal/mol).

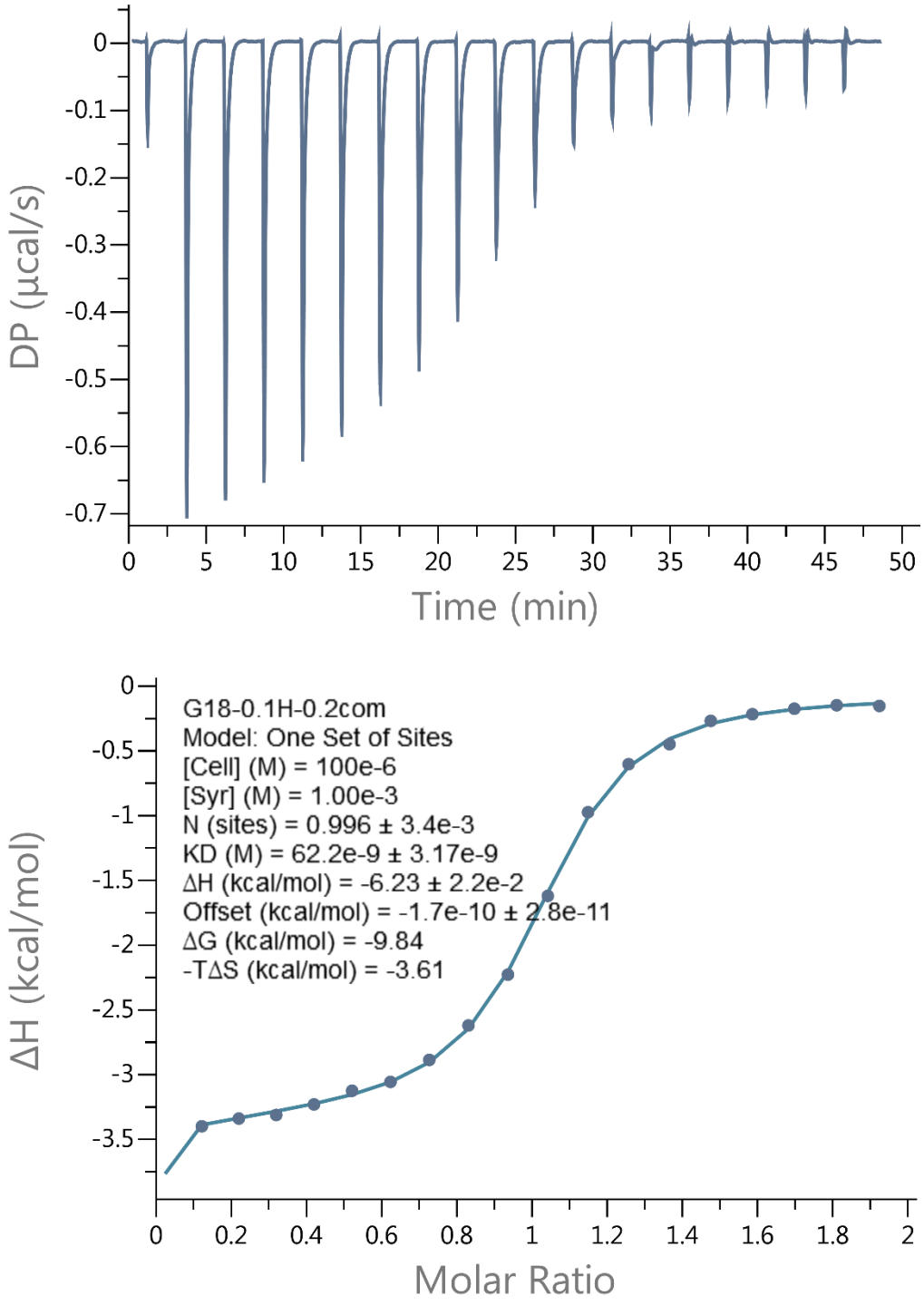

Figure S29. Top) Plot of DP vs time from the titration of molecular container **WP6** (100 µM) and competitive guest **G7** (200 µM) in the cell with guest **G10** (1.00 mM) in 1× PBS buffer solution; Bottom) plot of the ΔH as a function of molar ratio. The solid line represents the best non-linear fit of the data to a 1:1 binding model (K_a = (4.59 ± 0.35) × 10^7 M^{-1}, ΔH = –6.10 ± 0.02 kcal/mol, −TΔS = −4.35 kcal/mol).

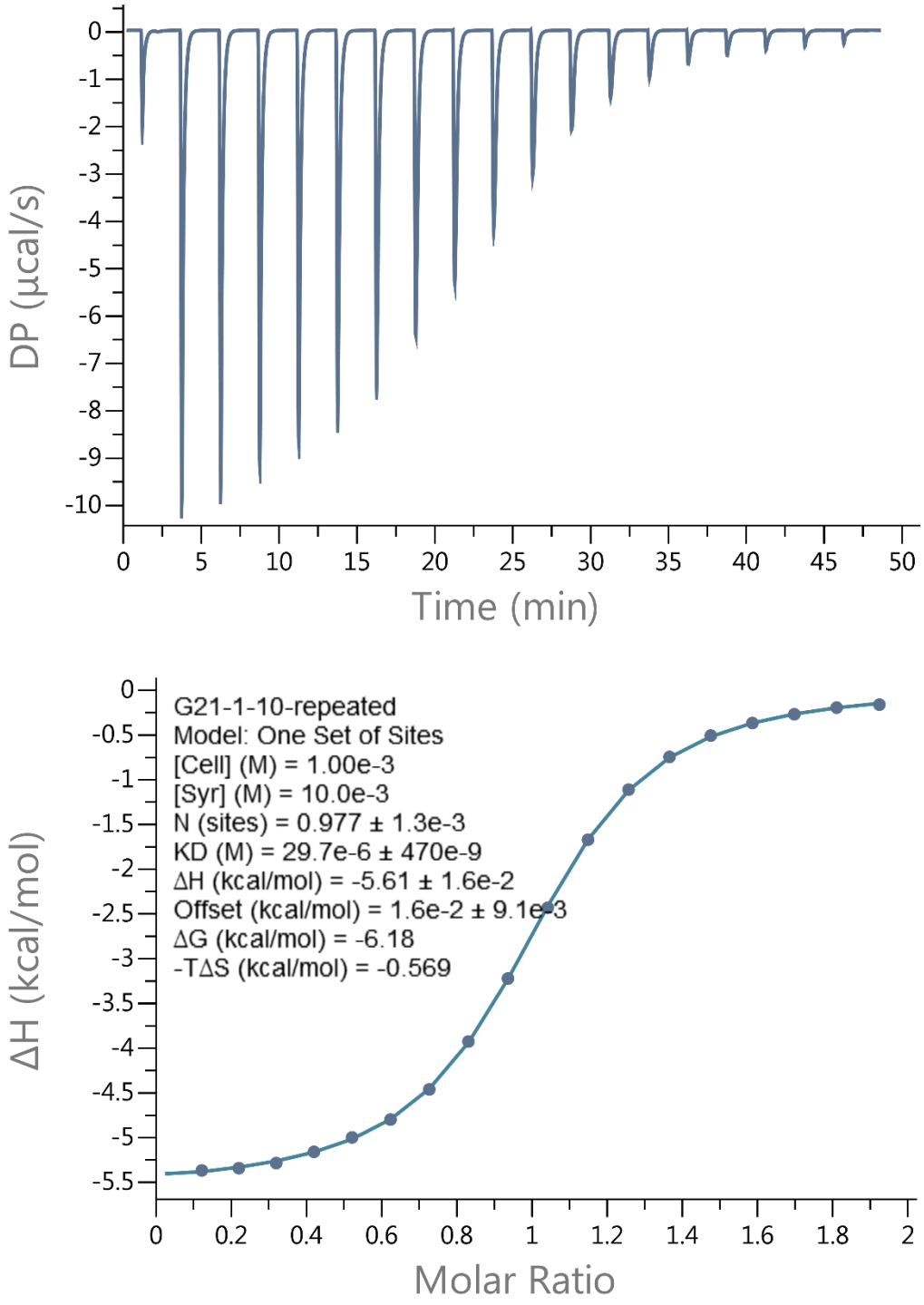

Figure S30. Top) Plot of DP vs time from the titration of molecular container **WP6** (1.00 mM) in the cell with guest **G11** (10.00 mM) in 1× PBS buffer solution; Bottom) plot of the ΔH as a function of molar ratio. The solid line represents the best non-linear fit of the data to a 1:1 binding model (K_a = (3.37 ± 0.05) × 10^4 M^{-1}, ΔH = −5.61 ± 0.02 kcal/mol, $-T\Delta S$ = −0.57 kcal/mol).

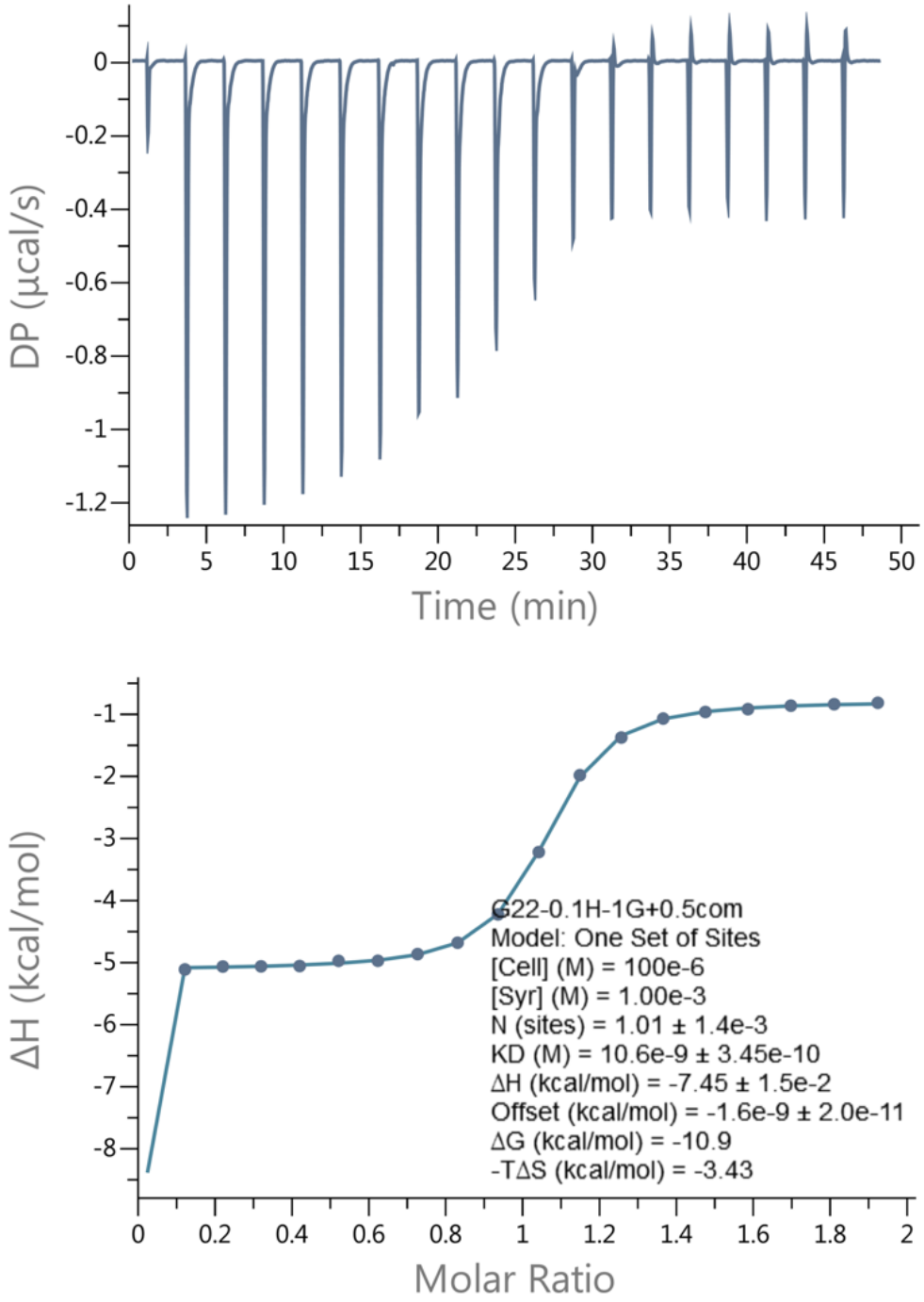

Figure S31. Top) Plot of DP vs time from the titration of molecular container **WP6** (100 µM) and competitive guest **G7** (500 µM) in the cell with guest **G12** (1.00 mM) in 1× PBS buffer solution; Bottom) plot of the ΔH as a function of molar ratio. The solid line represents the best non-linear fit of the data to a 1:1 binding model (K_a = (9.43 ± 0.31) × 10^7 M^{-1}, ΔH = −7.45 ± 0.02 kcal/mol, −TΔS = −3.43 kcal/mol).

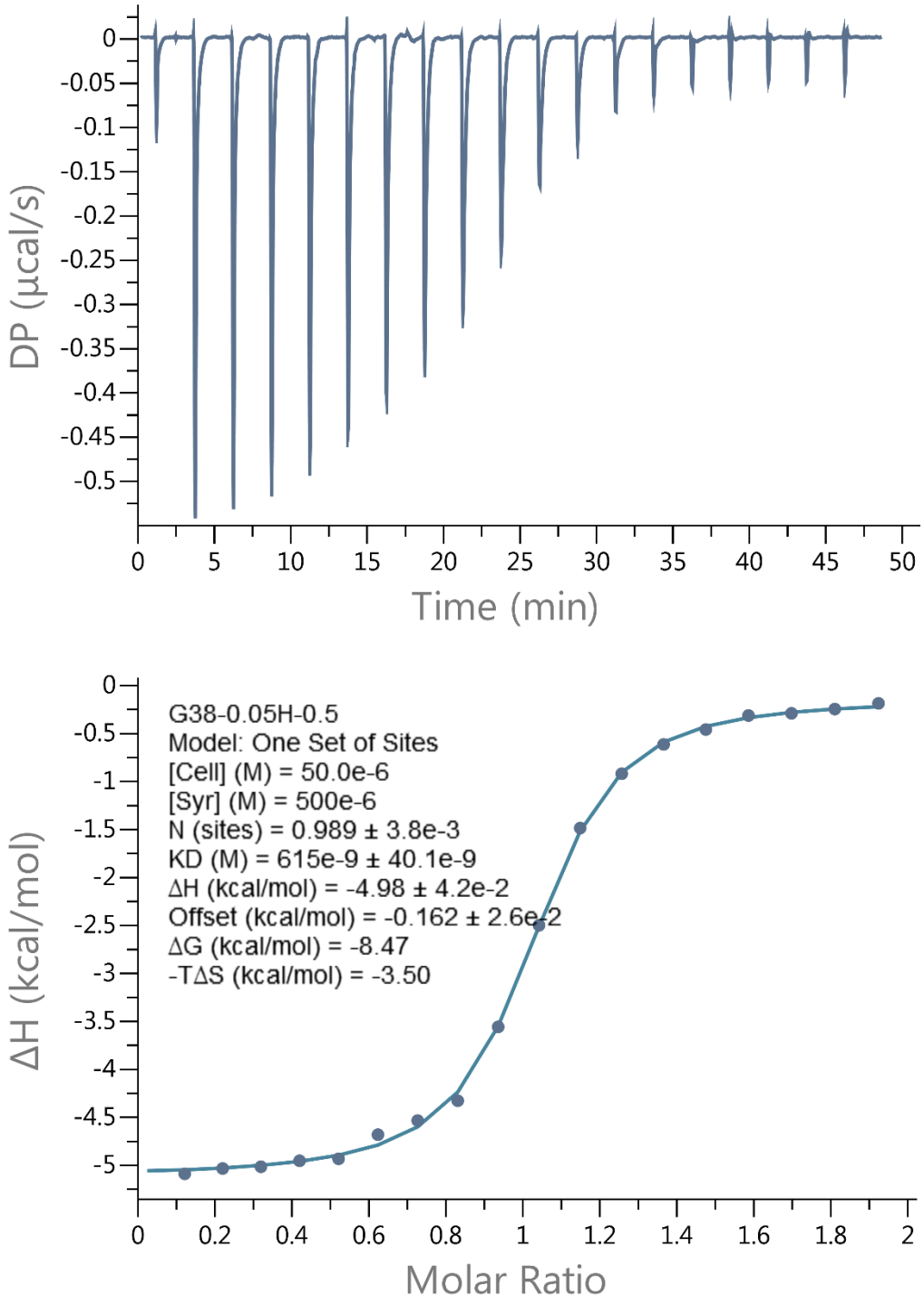

Figure S32. Top) Plot of DP vs time from the titration of molecular container **WP6** (50 µM) in the cell with guest **G13** (0.50 mM) in 1× PBS buffer solution; Bottom) plot of the ΔH as a function of molar ratio. The solid line represents the best non-linear fit of the data to a 1:1 binding model (K_a = (1.63 ± 0.11) × 10^6 M^{-1}, ΔH = –4.98 ± 0.04 kcal/mol, −TΔS = −3.50 kcal/mol).

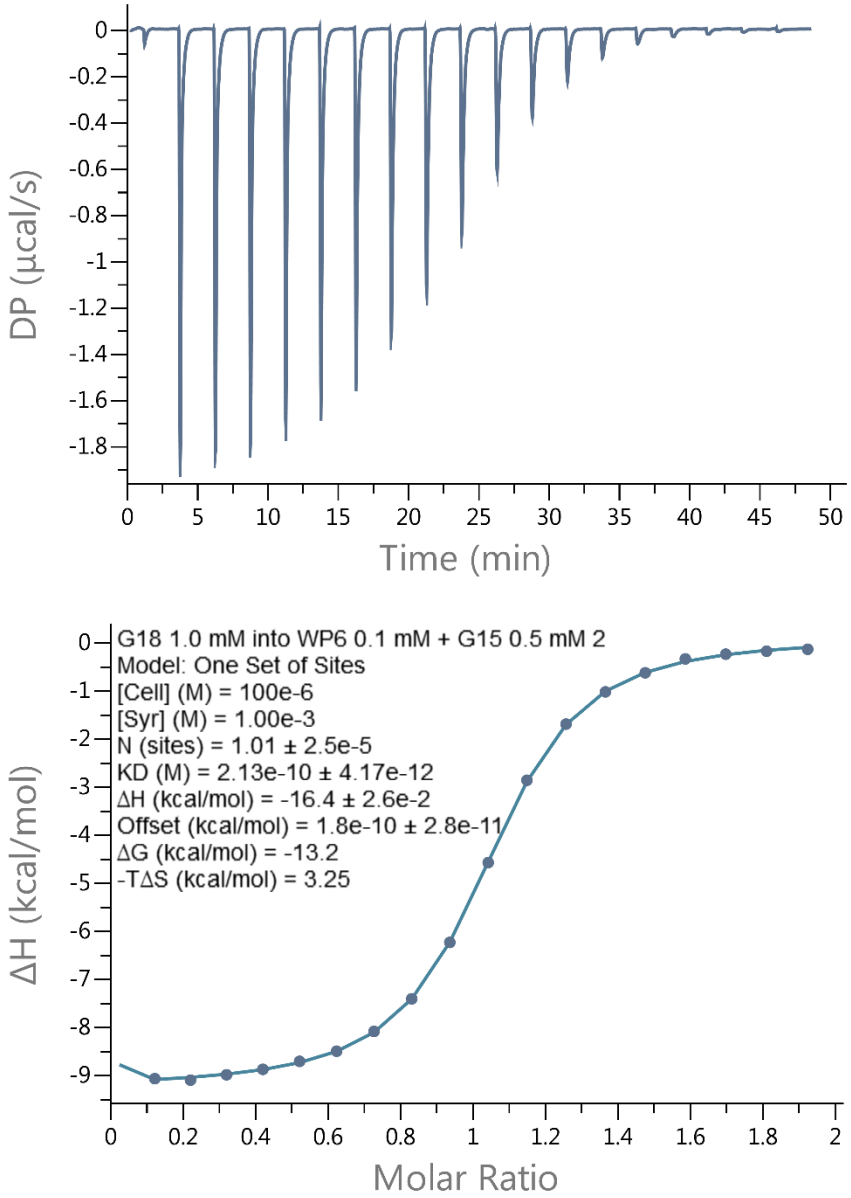

Figure S33. Top) Plot of DP vs time from the titration of molecular container **WP6** (100 µM) and competitive guest **G15** (500 µM) in the cell with guest **G14** (1.00 mM) in 1× PBS buffer solution; Bottom) plot of the ΔH as a function of molar ratio. The solid line represents the best non-linear fit of the data to a competition binding model (K_a = (4.69 ± 0.09) × 10^9 M^{-1}, ΔH = −16.4 ± 0.02 kcal kcal/mol, −TΔS = 3.25 kcal/mol).

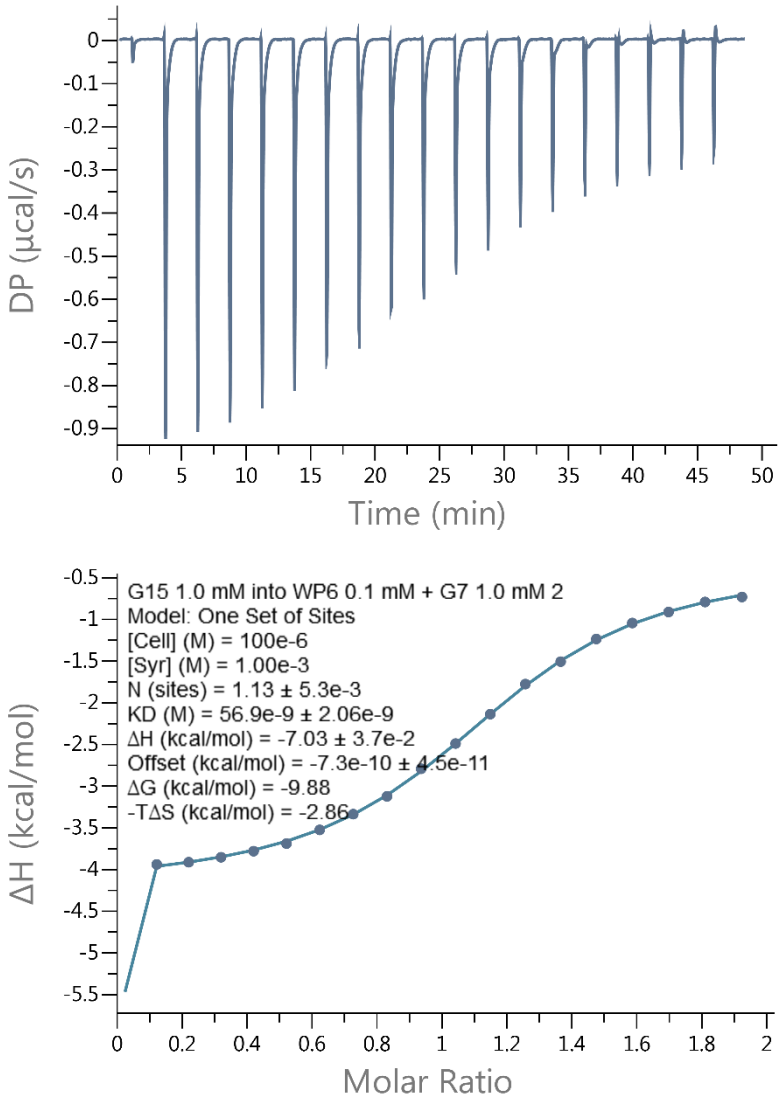

Figure S34. Top) Plot of DP vs time from the titration of molecular container **WP6** (100 µM) and competitive guest **G7** (1.00 mM) in the cell with guest **G15** (1.00 mM) in 1× PBS buffer solution; Bottom) plot of the ΔH as a function of molar ratio. The solid line represents the best non-linear fit of the data to a competition binding model (K_a = (1.76 ± 0.06) × 10^7 M^{-1}, ΔH = −7.03 ± 0.03 kcal kcal/mol, −TΔS = -2.86 kcal/mol).

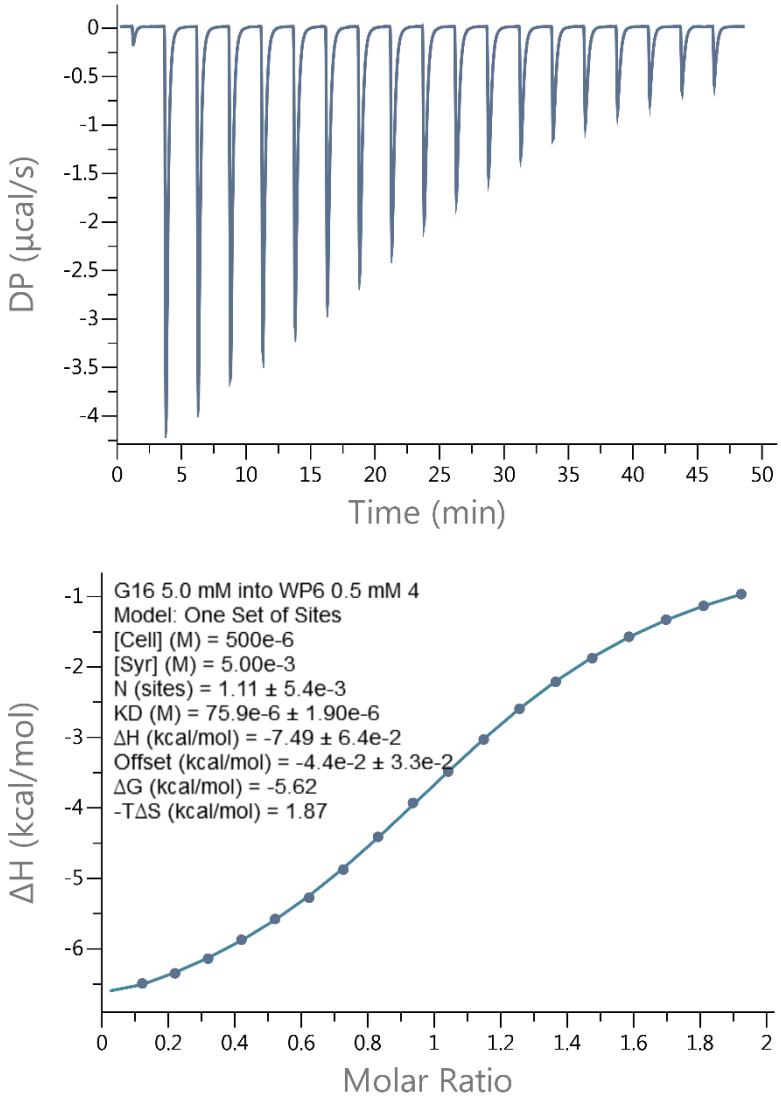

Figure S35. Top) Plot of DP vs time from the titration of molecular container **WP6** (500 µM) in the cell with guest **G16** (5.00 mM) in 1× PBS buffer solution; Bottom) plot of the ΔH as a function of molar ratio. The solid line represents the best non-linear fit of the data to a 1:1 binding model (K_a = (1.32 ± 0.03) × 10^4 M^{-1}, ΔH = −7.49 ± 0.06 kcal/mol, −TΔS = 1.87 kcal/mol).

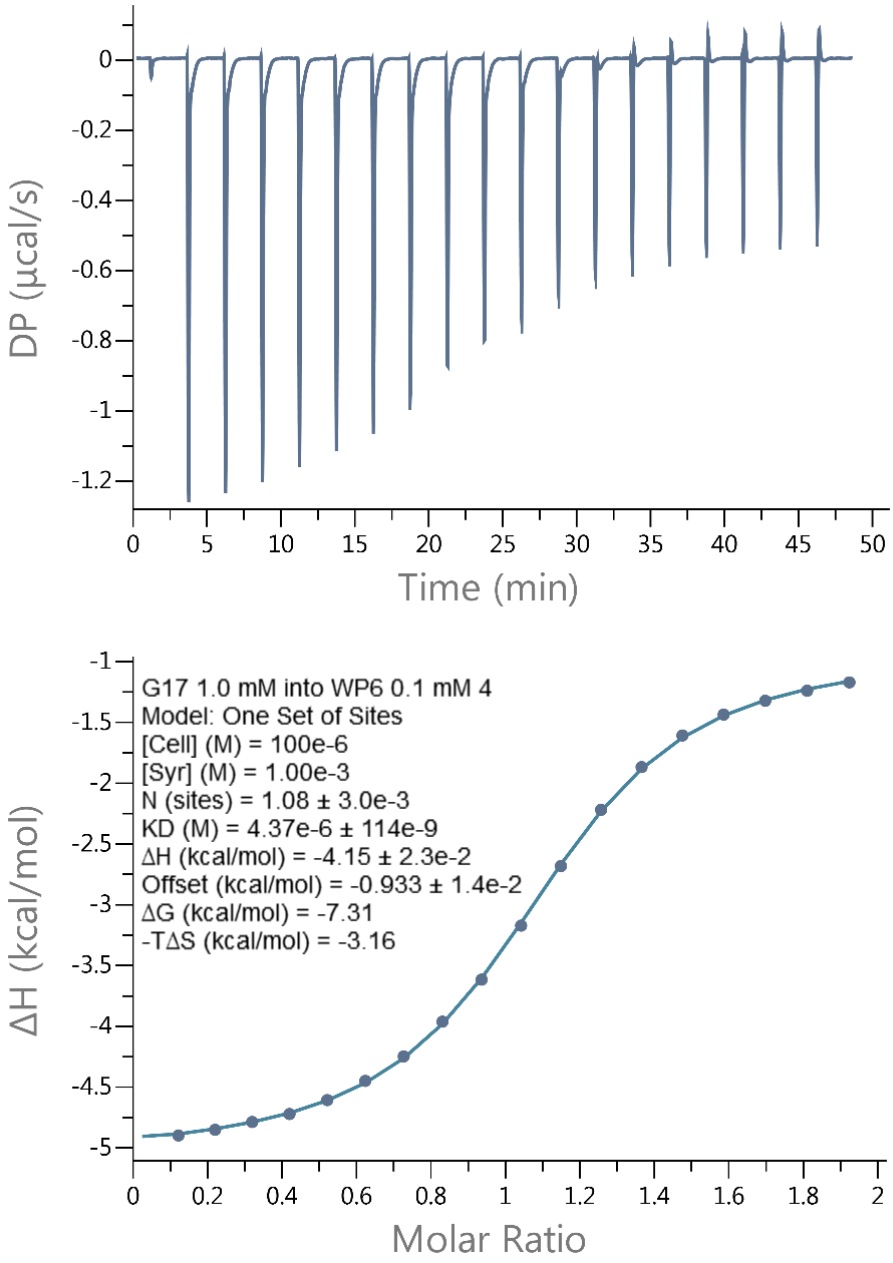

Figure S36. Top) Plot of DP vs time from the titration of molecular container **WP6** (100 µM) in the cell with guest **G17** (1.00 mM) in 1× PBS buffer solution; Bottom) plot of the ΔH as a function of molar ratio. The solid line represents the best non-linear fit of the data to a 1:1 binding model (K_a = (2.29 ± 0.06) × 10^5 M^{-1}, ΔH = −4.15 ± 0.02 kcal/mol, −TΔS = -3.16 kcal/mol).

X-ray crystal structure of Guest G2

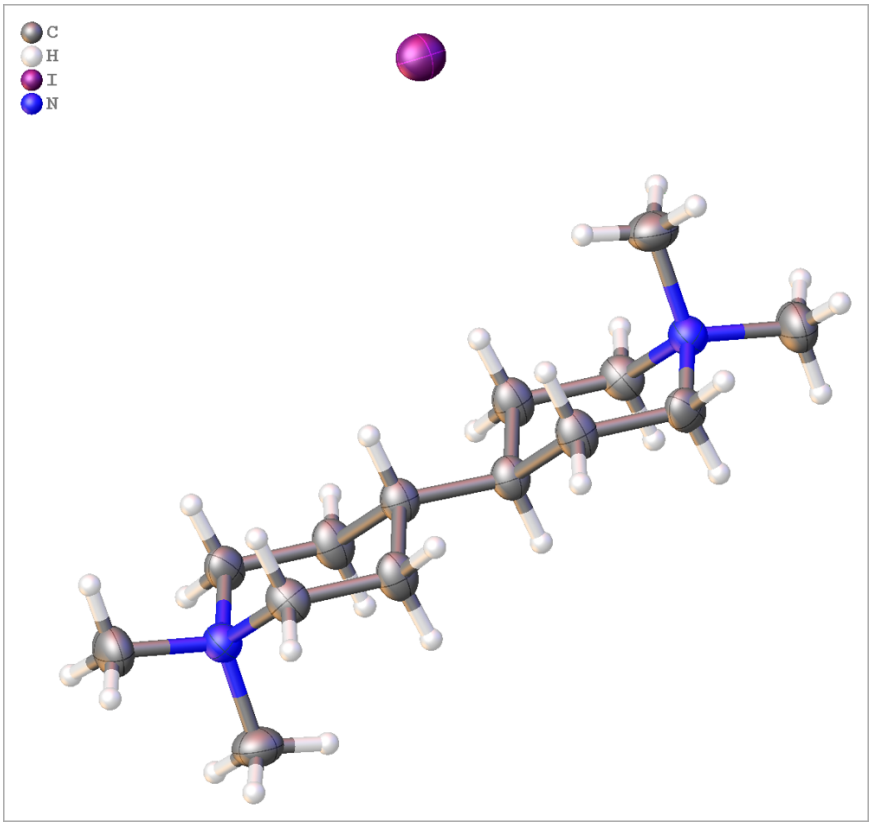

Table 2 Crystal data and structure refinement for UM3483b.

Identification code	UM3483b
Empirical formula	$C_{14}H_{30}N_2I_2$
Formula weight	480.20
Temperature/K	296(2)
Crystal system	monoclinic
Space group	$P2_1/c$
a/Å	6.5208(2)
b/Å	14.5116(5)
c/Å	10.1283(3)
α/°	90
β/°	104.2182(5)
γ/°	90
Volume/Å^3	929.05(5)
Z	2
ρ_{calc}g/cm^3	1.717
μ/mm^{-1}	3.375

F(000)	468.0
Crystal size/mm^3	0.5 × 0.42 × 0.25
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection/°	5.01 to 62.496
Index ranges	-9 ≤ h ≤ 9, -21 ≤ k ≤ 21, -14 ≤ l ≤ 14
Reflections collected	16743
Independent reflections	3037 [R_{int} = 0.0262, R_{sigma} = 0.0147]
Data/restraints/parameters	3037/0/85
Goodness-of-fit on F^2	1.056
Final R indexes [I>=2σ (I)]	R_1 = 0.0240, wR_2 = 0.0539
Final R indexes [all data]	R_1 = 0.0268, wR_2 = 0.0553
Largest diff. peak/hole / e Å^{-3}	1.21/-1.10

Table 3 Fractional Atomic Coordinates and Equivalent Isotropic Displacement Parameters (Å²×10³) for UM3483b. U_{eq} is defined as 1/3 of of the trace of the orthogonalised U_{IJ} tensor.

Atom	*x*	*y*	*z*	U(eq)
I1	0.89162(2)	0.84473(2)	0.65652(2)	41.48(7)
N1	0.3572(3)	0.62374(12)	0.78449(17)	31.3(3)
C5	0.2873(3)	0.58592(16)	0.5339(2)	36.8(4)
C7	0.2493(4)	0.62752(19)	0.8995(2)	45.5(5)
C4	0.4501(3)	0.50783(14)	0.56055(19)	30.8(4)
C6	0.1928(3)	0.60279(16)	0.6546(2)	35.1(4)
C3	0.6173(3)	0.52955(16)	0.6923(2)	35.9(4)
C2	0.5197(3)	0.54706(15)	0.8118(2)	34.7(4)
C8	0.4610(4)	0.71506(16)	0.7770(3)	43.7(5)

Table 4 Anisotropic Displacement Parameters (Å²×10³) for UM3483b. The Anisotropic displacement factor exponent takes the form: -2π²[h²a*²U_{11}+2hka*b*U_{12}+…].

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
I1	35.53(9)	38.62(9)	47.78(10)	3.20(6)	5.46(6)	4.13(5)
N1	32.0(8)	31.1(8)	32.2(8)	−1.2(6)	10.8(6)	0.0(6)
C5	31.6(9)	45.3(11)	31.5(9)	0.6(8)	4.0(7)	11.7(8)
C7	51.8(13)	50.6(13)	40.3(11)	−4.8(10)	23.4(10)	2.3(11)

Table 4 Anisotropic Displacement Parameters ($Å^2 \times 10^3$) for UM3483b. The Anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U_{11}+2hka^*b^*U_{12}+...]$.

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
C4	27.6(8)	35.6(9)	28.4(8)	1.1(7)	5.5(6)	6.0(7)
C6	25.9(8)	40.6(11)	37.6(10)	−4.4(8)	5.9(7)	4.0(7)
C3	29.5(8)	45.2(11)	30.6(9)	0.0(8)	3.2(7)	9.8(8)
C2	36.8(9)	36.3(10)	29.5(8)	2.3(7)	5.4(7)	5.9(8)
C8	44.6(11)	31.9(10)	54.3(13)	−2.6(9)	11.5(10)	−6.5(9)

Table 5 Bond Lengths for UM3483b.

Atom	Atom	Length/Å	Atom	Atom	Length/Å
N1	C7	1.502(3)	C5	C6	1.518(3)
N1	C6	1.510(3)	C4	C4[1]	1.540(4)
N1	C2	1.514(3)	C4	C3	1.534(3)
N1	C8	1.498(3)	C3	C2	1.520(3)
C5	C4	1.531(3)			

[1]1-X,1-Y,1-Z

Table 6 Bond Angles for UM3483b.

Atom	Atom	Atom	Angle/°	Atom	Atom	Atom	Angle/°
C7	N1	C6	108.35(17)	C5	C4	C4[1]	112.3(2)
C7	N1	C2	108.99(17)	C5	C4	C3	108.36(17)
C6	N1	C2	109.04(15)	C3	C4	C4[1]	112.01(19)
C8	N1	C7	108.08(18)	N1	C6	C5	113.08(16)
C8	N1	C6	111.52(17)	C2	C3	C4	112.33(16)
C8	N1	C2	110.78(17)	N1	C2	C3	112.87(16)
C6	C5	C4	112.24(17)				

[1]1-X,1-Y,1-Z

Table 7 Torsion Angles for UM3483b.

A	B	C	D	Angle/°	A	B	C	D	Angle/°
C5	C4	C3	C2	54.4(2)	C6	N1	C2	C3	54.4(2)
C7	N1	C6	C5	−173.10(19)	C6	C5	C4	C4[1]	−178.7(2)
C7	N1	C2	C3	172.50(19)	C6	C5	C4	C3	−54.4(2)
C4	C5	C6	N1	56.8(3)	C2	N1	C6	C5	−54.6(2)
C4[1]	C4	C3	C2	178.8(2)	C8	N1	C6	C5	68.1(2)
C4	C3	C2	N1	−56.5(2)	C8	N1	C2	C3	−68.7(2)

[1]1-X,1-Y,1-Z

Table 8 Hydrogen Atom Coordinates and Isotropic Displacement Parameters (Å²×10³) for UM3483b.

Atom	*x*	*y*	*z*	U(eq)
H5A	0.354643	0.642013	0.513777	44
H5B	0.174816	0.570692	0.454736	44
H7A	0.351673	0.641049	0.982935	68
H7B	0.184176	0.569127	0.907349	68
H7C	0.143122	0.674812	0.88157	68
H4	0.377433	0.451004	0.575005	37
H6A	0.094589	0.65402	0.633755	42
H6B	0.113437	0.548681	0.668738	42
H3A	0.71519	0.478275	0.714159	43
H3B	0.696889	0.583517	0.678045	43
H2A	0.452852	0.490879	0.832057	42
H2B	0.631034	0.562805	0.891279	42
H8A	0.533161	0.713791	0.705011	66
H8B	0.560896	0.727283	0.861987	66
H8C	0.355417	0.762601	0.759129	66

Experimental:

A suitable single crystals of $C_{14}H_{30}N_2I_2$ **(UM3483b)** was selected and measured on a Bruker Smart Apex II CCD diffractometer [1]. The crystal was kept at 296(2) K during data collection. The integral intensity were correct for absorption using SADABS software [2] using multi-scan method. Resulting minimum and maximum transmission are 0.303 and 0.430 respectively. The structure was solved with the ShelXT (Sheldrick, 2015a) [3] program

and refined with the ShelXL (Sheldrick, 2015c) [4] program and least-square minimisation using ShelX software package [4]. Number of restraints used = 0.

Crystal structure determination:

Crystal Data for $C_{14}H_{30}N_2I_2$ (*M* =480.20 g/mol): monoclinic, space group $P2_1/c$ (no. 14), *a* = 6.5208(2) Å, *b* = 14.5116(5) Å, *c* = 10.1283(3) Å, *β* = 104.2182(5)°, *V* = 929.05(5) $Å^3$, *Z* = 2, *T* = 296(2) K, μ(MoKα) = 3.375 mm^{-1}, *Dcalc* = 1.717 g/cm^3, 16743 reflections measured (5.01° ≤ 2Θ ≤ 62.496°), 3037 unique (R_{int} = 0.0262, R_{sig} = 0.0147) which were used in all calculations. The final R_1 was 0.0240 (I > 2σ(I)) and wR_2 was 0.0553 (all data).

Refinement details: H atoms were positioned from the geometric considerations and refined as riding on the attached atoms with Uiso constrained to be 20% (50% for methyl group) larger than Ueqv of the attached group. Orientation of methyl groups was optimized.

References:

1. Bruker (2010). Apex2. Bruker AXS Inc., Madison, Wisconsin, USA.
2. Krause, L., Herbst-Irmer, R., Sheldrick, G.M., Stalke, D. (2015). J. Appl. Cryst. 48, 3-10.
3. Sheldrick, G. M. (2015a). Acta Cryst. A17, 3-8.
4. Sheldrick, G. M. (2015c). Acta Cryst. C17, 3-8.
5. Dolomanov, O.V., Bourhis, L.J., Gildea, R.J, Howard, J.A.K. & Puschmann, H. (2009), J. Appl. Cryst. 42, 339-341.

This report has been created with Olex2 [5], compiled on 2020.11.12 svn.r5f609507 for OlexSys.

XYZ coordinates of the MMFF minimized geometry for WP6•G5 and WP6•G14

For **WP6•G5**:

C	4.613741	-1.486469	1.517660
C	5.356010	1.211475	1.394940
C	4.508175	-0.715523	2.682884
C	4.894041	-0.874472	0.278088
C	5.268293	0.483377	0.194200
C	4.887626	0.641054	2.598415
C	4.173890	-1.326546	4.036116
C	5.608623	-3.566422	1.053533
C	6.747572	2.785115	2.509990
C	6.126795	-4.696860	1.976251
C	7.854326	3.819422	2.183141
C	0.944891	-3.343133	4.645195
C	1.834870	-0.694782	4.891196
C	0.023842	-2.358915	5.037545
C	2.245457	-2.969172	4.237733
C	2.717275	-1.639052	4.352894
C	0.501933	-1.043693	5.177801
C	-1.415415	-2.672753	5.424830
C	1.541042	-5.579669	5.194493
C	2.466766	0.743559	6.592805
C	0.907101	-6.737752	6.008798
C	2.711528	2.216340	7.003557
C	-3.950922	-2.659615	2.531931
C	-3.204736	-0.990966	4.653958
C	-4.521356	-1.380425	2.610845
C	-2.884816	-3.012954	3.384874
C	-2.496183	-2.188101	4.462861
C	-4.135490	-0.558061	3.688445
C	-5.622622	-0.912562	1.670853
C	-4.699875	-4.876588	2.198875
C	-4.186535	0.182520	6.452935
C	-6.020989	-5.522296	1.714671
C	-3.905485	0.694674	7.884582
C	-5.033595	-0.286642	-2.078713
C	-4.898378	1.153334	0.327056
C	-4.582806	1.046543	-2.112013
C	-5.252453	-0.922639	-0.837346
C	-5.202863	-0.214644	0.382868

C	-4.524044	1.753620	-0.892046
C	-4.283498	1.776308	-3.415300
C	-6.410744	-1.819157	-3.277043
C	-5.920219	3.009697	1.313279
C	-7.082562	-1.917831	-4.671195
C	-6.488454	3.477026	2.671994
C	-0.723950	1.348322	-4.814933
C	-2.224498	3.309237	-3.483426
C	-0.078333	2.528429	-4.414601
C	-2.044683	1.083544	-4.391650
C	-2.817776	2.059080	-3.724868
C	-0.853210	3.504445	-3.751072
C	1.366694	2.839444	-4.783643
C	-0.860467	0.003465	-6.755641
C	-2.826310	5.586499	-3.685499
C	-0.042650	-0.174974	-8.058851
C	-4.085819	6.486676	-3.611743
C	4.177709	0.728001	-3.209453
C	2.948131	3.223876	-2.793059
C	4.558504	1.489774	-2.090999
C	3.083074	1.139196	-4.000396
C	2.453357	2.391126	-3.810624
C	3.934125	2.742959	-1.906121
C	5.684207	1.066215	-1.153251
C	5.157609	-0.600229	-4.917145
C	3.471137	5.504415	-2.428748
C	6.518341	-1.296209	-5.177604
C	3.061073	6.903296	-2.945996
C	1.016173	4.776400	0.914197
C	1.599092	2.356361	1.265056
C	-0.503873	2.981671	0.028425
C	0.131156	1.933665	0.983460
C	-0.450890	4.400793	0.643999
C	1.664379	3.771599	1.882221
C	0.875593	3.776067	3.204967
C	-1.230230	4.391106	1.977300
C	-0.646208	1.964031	2.334506
C	-0.607693	3.383687	2.977763
C	3.116962	4.178060	2.148015
C	-1.076740	5.423462	-0.309363
C	0.997177	0.359604	-0.905580

C	-1.330769	0.082363	-0.137723
C	0.542223	-0.627232	1.311234
C	-2.931821	3.373562	4.158601
C	-1.053549	2.225897	5.244241
C	-1.093971	4.650914	5.194135
H	4.899517	-1.480926	-0.626901
H	4.871516	1.239559	3.506525
H	4.560418	-0.671621	4.828885
H	4.771116	-2.239833	4.159790
H	6.469502	-2.910451	0.865856
H	5.274226	-3.986832	0.098392
H	6.095494	3.208386	3.283607
H	7.241750	1.889029	2.906030
H	2.932104	-3.731254	3.868438
H	-0.164066	-0.295451	5.591820
H	-1.596998	-2.261276	6.426783
H	-1.548258	-3.752916	5.565515
H	2.262812	-5.078405	5.854478
H	2.074725	-5.991530	4.331041
H	1.590744	0.406241	7.160451
H	3.334787	0.137745	6.883279
H	-2.392021	-3.974671	3.256520
H	-4.618011	0.410933	3.794720
H	-6.300884	-0.244904	2.220463
H	-6.266975	-1.767142	1.424281
H	-4.759139	-4.851804	3.295760
H	-3.849456	-5.500824	1.903985
H	-4.615688	1.022236	5.894878
H	-4.913967	-0.638485	6.499397
H	-5.527077	-1.976289	-0.812014
H	-4.241910	2.806158	-0.909675
H	-4.854061	2.714702	-3.415567
H	-4.706929	1.228113	-4.266114
H	-7.183037	-1.485888	-2.571596
H	-6.054885	-2.811436	-2.978888
H	-5.384130	3.855494	0.868258
H	-2.509648	0.132422	-4.643902
H	-0.383556	4.450957	-3.489779
H	1.463838	3.919937	-4.955417
H	1.584668	2.415856	-5.774147
H	-1.678034	0.696975	-6.996586

H	-1.288378	-0.959689	-6.453679
H	-1.999882	6.122606	-3.204702
H	-2.581653	5.423864	-4.743670
H	2.743043	0.506907	-4.819007
H	4.268201	3.365569	-1.078297
H	6.342722	1.932585	-1.003682
H	6.338253	0.337167	-1.649166
H	5.171633	0.352619	-5.462082
H	4.350508	-1.226850	-5.315874
H	3.614359	5.562541	-1.343002
H	4.424474	5.238914	-2.903188
H	2.185212	2.360406	0.342097
H	0.020846	2.997968	-0.932204
H	1.575163	4.799421	-0.029808
H	2.081161	1.650699	1.949760
H	-1.550607	2.735534	-0.178055
H	0.952674	4.780561	3.639707
H	1.355995	3.085484	3.905415
H	-1.237358	5.403296	2.398805
H	-2.272548	4.128993	1.759275
H	-0.184435	1.248323	3.019634
H	-1.683836	1.654637	2.182841
H	3.175512	5.186933	2.570818
H	3.706000	4.172030	1.225041
H	3.591658	3.494740	2.860657
H	-1.059117	6.430595	0.122718
H	-2.121367	5.173581	-0.524930
H	-0.531023	5.462994	-1.256866
H	0.720356	1.131599	-1.621423
H	0.847278	-0.627440	-1.355592
H	2.036068	0.450830	-0.584907
H	-1.618023	0.735856	-0.962790
H	-2.017371	0.162899	0.704074
H	-1.305026	-0.953820	-0.491529
H	1.534386	-0.378484	1.684498
H	0.580157	-1.578308	0.769984
H	-0.187893	-0.704471	2.119461
H	-3.235097	4.326283	3.720644
H	-3.400477	3.251472	5.140166
H	-3.175738	2.529623	3.512349
H	0.025574	2.144806	5.317907

H	-1.472932	1.337626	4.778644
H	-1.488040	2.378825	6.233879
H	-1.264304	5.545796	4.590816
H	-0.061680	4.606870	5.545466
H	-1.765549	4.664791	6.059951
H	-6.756699	2.724356	0.661430
H	1.071726	5.790768	1.330850
N	0.082940	0.471207	0.333357
N	-1.402667	3.420434	4.351705
O	4.484333	-2.852874	1.634449
O	5.942346	2.462534	1.346787
O	6.434560	-4.378811	3.165083
O	9.016020	3.348146	1.965759
O	6.285037	-5.830067	1.420182
O	7.533519	5.044022	2.275081
O	0.531971	-4.657658	4.711508
O	2.250013	0.592486	5.162658
O	0.634807	-7.800858	5.370004
O	1.927042	3.089524	6.525744
O	0.805254	-6.554304	7.263441
O	3.636485	2.390411	7.859696
O	-4.500853	-3.553187	1.635828
O	-2.968385	-0.266902	5.804865
O	-5.901051	-6.602812	1.054920
O	-3.201130	1.746333	7.974919
O	-7.099924	-4.983444	2.109967
O	-4.471980	0.058507	8.827239
O	-5.281377	-0.907917	-3.286848
O	-4.993935	1.902082	1.480745
O	-6.704564	-2.874652	-5.416073
O	-5.639909	3.819821	3.550401
O	-8.029159	-1.100501	-4.899309
O	-7.754147	3.533828	2.757912
O	-0.042541	0.498082	-5.662809
O	-3.029478	4.321577	-3.003650
O	0.131484	-1.373215	-8.447287
O	-4.369638	6.984991	-2.480955
O	0.282364	0.884720	-8.677400
O	-4.670111	6.717170	-4.719708
O	4.922332	-0.395706	-3.500153
O	2.453799	4.509918	-2.715830

O	6.499885	-2.562681	-5.264265
O	2.404203	7.630568	-2.139277
O	7.512181	-0.531028	-5.385173
O	3.507769	7.232190	-4.089973

For **WP6•G14**:

C	5.235928	0.575886	-1.727748
C	5.236612	-1.683653	-0.061105
C	4.923802	-0.671349	-2.292540
C	5.304969	0.703000	-0.324183
C	5.289519	-0.417832	0.537452
C	4.950658	-1.797711	-1.439325
H	5.415782	1.690411	0.116057
H	4.786747	-2.782924	-1.870072
C	4.641953	-0.858624	-3.783181
H	5.091177	-1.814812	-4.079479
H	5.194787	-0.109413	-4.364733
O	5.501867	1.627081	-2.586795
O	5.522954	-2.781478	0.726141
C	6.514086	2.547497	-2.101429
H	7.258700	2.030206	-1.481982
H	6.017065	3.318577	-1.501589
C	6.382056	-3.747765	0.066553
H	5.743506	-4.508262	-0.397065
H	7.001396	-3.278701	-0.709707
C	7.311906	3.219948	-3.247981
C	7.372584	-4.422916	1.047838
O	8.068037	2.474088	-3.942855
O	8.246735	-3.678642	1.589342
O	7.236539	4.489302	-3.307618
O	7.292313	-5.690825	1.129067
C	1.099868	0.498012	-4.432609
C	2.570658	-1.818067	-4.999086
C	0.434239	-0.555403	-5.071024
C	2.421506	0.353871	-3.967505
C	3.178038	-0.806024	-4.224866
C	1.197750	-1.714754	-5.341648
H	2.879721	1.178754	-3.424643
H	0.709881	-2.531908	-5.867937
C	-0.988450	-0.430125	-5.619108
H	-1.141089	-1.209161	-6.377558

H	-1.038922	0.498586	-6.205879
O	0.453964	1.696421	-4.251738
O	3.376614	-2.861788	-5.418953
C	1.047491	2.729529	-5.074434
H	1.174019	2.368062	-6.102936
H	2.018484	3.025734	-4.661531
C	2.954284	-3.537600	-6.628141
H	2.174713	-4.264988	-6.373681
H	2.559323	-2.818557	-7.357267
C	0.152567	3.983855	-5.093167
C	4.127584	-4.290031	-7.308879
O	-0.533038	4.208880	-4.045234
O	4.355134	-5.474513	-6.911485
O	0.205656	4.687395	-6.147780
O	4.686214	-3.694189	-8.285034
C	-3.671281	0.802534	-3.154788
C	-2.979889	-1.572715	-4.445056
C	-4.368757	-0.354984	-2.796883
C	-2.557478	0.748907	-4.013985
C	-2.166550	-0.443489	-4.651410
C	-4.006814	-1.543886	-3.467429
H	-2.014023	1.669464	-4.215602
H	-4.563270	-2.453571	-3.247556
C	-5.542484	-0.337772	-1.823136
H	-6.202742	-1.184773	-2.052566
H	-6.172231	0.532021	-2.056383
O	-4.029787	2.017334	-2.625736
O	-2.765926	-2.667206	-5.261103
C	-4.746981	2.810011	-3.601438
H	-5.753378	2.394159	-3.726961
H	-4.230180	2.819614	-4.568783
C	-3.983293	-3.326734	-5.694062
H	-4.204366	-4.136429	-4.989459
H	-4.833385	-2.632109	-5.718906
C	-4.856795	4.275400	-3.130817
C	-3.848670	-3.904263	-7.126086
O	-3.972790	4.667456	-2.302942
O	-3.762106	-5.169321	-7.217329
O	-5.798400	4.948629	-3.651567
O	-3.951220	-3.076380	-8.085034
C	-4.943486	0.954866	1.765895

C	-5.223246	-1.498747	0.439928
C	-4.725552	-0.225890	2.498186
C	-5.102066	0.900176	0.361351
C	-5.244117	-0.324238	-0.327997
C	-4.876018	-1.451784	1.809628
H	-5.185019	1.827107	-0.202215
H	-4.779821	-2.382475	2.366998
C	-4.489096	-0.228587	4.008425
H	-5.005680	-1.098089	4.437605
H	-5.026966	0.618006	4.456967
O	-5.045954	2.139196	2.470459
O	-5.581421	-2.677352	-0.181932
C	-6.078303	3.031441	1.971497
H	-6.873654	2.480830	1.451737
H	-5.613797	3.731790	1.267859
C	-6.580497	-3.422257	0.562247
H	-6.062185	-4.187387	1.151063
H	-7.150209	-2.775646	1.243102
C	-6.764705	3.825888	3.111277
C	-7.618103	-4.092066	-0.374961
O	-6.349023	5.009982	3.304531
O	-7.686928	-5.361265	-0.314001
O	-7.760770	3.266291	3.669147
O	-8.375421	-3.320424	-1.040523
C	-1.129223	1.110319	5.359280
C	-2.340550	-1.374258	4.841839
C	-0.352812	-0.054935	5.490280
C	-2.420138	1.035538	4.787542
C	-3.047525	-0.203439	4.513170
C	-0.983675	-1.292191	5.230400
H	-2.978452	1.951653	4.602318
H	-0.411649	-2.204501	5.383912
C	1.090693	-0.033331	6.003066
H	1.174838	-0.853768	6.727257
H	1.267752	0.872717	6.596369
O	-0.585972	2.289592	5.830058
O	-3.028085	-2.573048	4.803686
C	-1.535170	3.175986	6.476079
H	-1.933370	3.864934	5.722566
C	-2.668340	-3.487314	5.871934
H	-1.823660	-4.098091	5.532948

H	-2.379298	-2.943941	6.781401
C	-0.871243	3.999914	7.612321
C	-3.843863	-4.413583	6.273665
O	-0.276312	5.070155	7.276476
O	-3.689345	-5.650614	6.021052
O	-1.072560	3.584584	8.798923
O	-4.797744	-3.882053	6.921787
C	3.294472	0.777762	2.941628
C	3.300550	-1.020619	5.110008
C	4.295589	-0.201910	2.986680
C	2.251575	0.777644	3.891281
C	2.213651	-0.132143	4.969554
C	4.277040	-1.092084	4.087904
H	1.448833	1.507806	3.796906
H	5.071436	-1.831951	4.150751
C	5.493288	-0.222737	2.034705
H	6.210365	-0.983086	2.373455
H	6.035312	0.721123	2.192817
O	3.351545	1.699933	1.930294
O	3.371395	-1.759137	6.279155
C	3.099440	3.068373	2.312523
H	3.992149	3.636128	2.025327
H	2.964761	3.209744	3.390904
C	4.680495	-2.269146	6.632289
H	4.881794	-3.170123	6.041176
H	5.454474	-1.517472	6.431127
C	1.892974	3.639221	1.553288
C	4.777788	-2.639547	8.135674
O	0.942754	2.838379	1.285000
O	4.322130	-3.773243	8.480495
O	1.948101	4.869400	1.254160
O	5.414451	-1.819619	8.873613
C	-1.064379	-2.932358	0.849732
C	-0.261082	-0.696557	1.627029
C	-1.529816	-0.872848	-0.509474
C	-0.396584	-0.121620	0.205142
C	-1.221348	-2.377854	-0.575332
C	0.073842	-2.196334	1.574081
H	-1.193156	-0.547799	2.182285
H	-2.472720	-0.710785	0.015910
H	-2.001879	-2.811598	1.405613

H	0.520592	-0.165886	2.170871
H	-1.650967	-0.498550	-1.527983
H	-2.035320	-2.901568	-1.088674
H	0.183203	-2.590609	2.590057
C	1.385087	-2.398086	0.796332
H	1.638550	-3.464562	0.758086
H	2.208825	-1.894498	1.314609
C	1.240901	-1.840701	-0.629144
H	2.168297	-1.996076	-1.185191
C	0.926440	-0.334293	-0.550525
H	1.749820	0.191297	-0.051658
H	0.860121	0.071678	-1.562752
C	0.094187	-2.575916	-1.344998
H	0.325655	-3.645274	-1.422444
H	-0.011676	-2.198982	-2.368705
N	-0.712430	1.334492	0.324105
H	0.142266	1.831602	0.714939
H	-1.376827	1.481906	1.094018
C	-1.168651	2.096500	-0.880953
H	-0.643938	1.707373	-1.755174
H	-2.235379	1.892804	-0.961195
C	-0.921329	3.610916	-0.751399
H	-1.324094	4.002233	0.187402
H	0.143938	3.840516	-0.824799
N	-1.560737	4.373464	-1.830717
H	-1.189874	4.143144	-2.792558
H	-1.385943	5.372500	-1.768547
H	-2.608223	4.308478	-1.922584
H	-2.366718	2.614713	6.921988
H	-0.852474	-4.007864	0.813023

Platinum Standard pK_a Report

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Project No.:	P210127
Laboratory Notebook:	RR003-061

Contents

Prepared by: Rebeca Ruiz (Global Analytical Services Manager) **Date:** 24th September 2021

Reviewed by: Imogen Anastasiou (Biopharmaceutical Product Specialist) **Date:** 27th September 2021

Summary

Table 1 – Platinum Standard pK_a Results

Pion No.	Compound ID	Batch ID	pK_a Result	Method
210464	**WP6**	CLD001	**3.52 ± 0.03** (Acid*) **3.62 ± 0.01** (Acid*) **4.16 ± 0.01** (Acid*) **4.41 ± 0.03** (Acid*) **4.80 ± 0.07** (Acid*) **5.66 ± 0.01** (Acid*)	**pH-metric**

** The type of ionisable groups are based on our observations.*

Introduction

The experiments were designed to determine the pK_a values of the submitted compounds.

Experimental Procedure

Table 2 – Experimental Conditions

Compound ID	Method	Assay ID	pH Range	Media	Temperature	Ionic Strength Background
WP6	pH-metric	21H-27007	2.0 – 12.0	MeCN/ water	25.0 ± 0.2 °C	0.15M KCl
	pH-metric	21I-15005	2.0 – 12.0	Dioxane/ water	25.0 ± 0.2 °C	0.15M KCl
	pH-metric	21I-09004 21I-17004	2.0 – 12.0	THF/water	25.0 ± 0.2 °C	0.15M KCl
	pH-metric	21I-24004 21I-24007 21I-24014 21I-24017	2.0 – 12.0	THF/water	25.0 ± 0.2 °C	0 M

Further experimental information can be found under "General Platinum Standard pK_a Procedure".

Platinum Standard pK_a: Results and Discussions

The Platinum Standard pK_a assays for the submitted compound(s) were performed using the UV-metric (spectrometric) and pH-metric (potentiometric) techniques.

Study Compound: WP6

Sample Preparation:

pH-metric:
The sample was prepared for the assay by weighing sample powder into a 5 mL glass assay vial. For this assay 1.11 – 2.53 mg of the sample was used.

Measurement:

pH-metric:
The sample was titrated potentiometrically between pH 2.0 and 12.0 in a triple titration under several co-solvent experimental conditions, due to the low solubility of the sample (especially at low pH). The pK_as were finally determined under tetrahydrofuran-water co-solvent conditions at zero ionic strength.

Acetonitrile/water (ionic strength 0.15M KCl):
A triple titration was carried out under acetonitrile-water co-solvent conditions from pH 12.0 – 2.0 at concentrations of 1.0 – 0.8 mM (the acetonitrile mixing ratio varied from 42.4 to 35.2 % w/w). Precipitation of the sample from solution was observed in all the titrations preventing the accurate determination of any sample pK_as using the potentiometric technique.

Dioxane/water (ionic strength 0.15M KCl):
A triple titration was carried out under dioxane-water co-solvent conditions from pH 12.0 – 2.0 at concentrations of 0.9 – 0.6 mM (the dioxane mixing ratio varied from 60.2 to 41.2 % w/w). Precipitation of the sample from solution was observed in all the titrations preventing the accurate determination of any sample pK_as using the potentiometric technique.

Tetrahydrofuran /water (ionic strength 0.15M KCl):
Two triple titrations were carried out under tetrahydrofuran-water co-solvent conditions from pH 12.0 – 2.0 at concentrations of 1.8 – 0.5 mM (the tetrahydrofuran mixing ratio varied from 56.4 to 26.1 % w/w). Precipitation of the sample from solution was observed bellow pH 4.8 at the highest sample concertation (1.8 mM). No precipitation was observed below concentrations of 0.8 mM, however data points were also observed to be inconsistent. Therefore, four further triple titrations were performed with zero ionic strength, due the lower solubility of the sample in a high background electrolyte.

Tetrahydrofuran-water (ionic strength 0 M):
A triple titration was carried out under tetrahydrofuran-water co-solvent conditions from pH 2.0 – 12.0 at concentrations of 1.1 – 0.7 mM (the tetrahydrofuran mixing ratio varied from 51.3 to 32.0 % w/w). No precipitation of the sample from solution was observed and six pK_as, with aqueous values of 3.52 ± 0.03, 3.62 ± 0.01, 4.16 ± 0.01, 4.41 ± 0.03, 4.80 ± 0.07 and 5.66 ± 0.01 were determined from the potentiometric data collected, by Yasuda-Shedlovsky extrapolation of the individual results obtained.

Please note that the pK_a values obtained should be treated with caution. This is due to the close proximity of the pK_as as well as the poor solubility observed with the compound.

Table 3 – Assay Audit Trail

Compound ID	Date	Instrument ID (Electrode ID)	Assay Files	Audit Trail
WP6	27th August 2021	SiriusT311054 (PBM0412)	21H-27007	..\ AS_Customer Samples\ University of Maryland\September\P210127 \Data
	09th – 24th September 2021	SiriusT310026 (PBM0272)	21I-09004 21I-15005 21I-17004 21I-24004 21I-24007 21I-24014 21I-24017	

Instrument Calibration

Details of the instrument calibration files are included in the individual results printouts.

Quality Control Checks

Table 4 – Instrument Quality Control Checks Audit Trail

Assay	QC compound	Date	Instrument ID	Assay Files	Audit Trail
Fast-UV p_sK_a	Propranolol	3rd August 2021	SiriusT311054 (T3E01145)	21H-03008	..\Data 2021 \T311054\ August
pH-metric logP		2nd August 2021		21H-02014	
Fast-UV p_sK_a	Propranolol	1st September	SiriusT310026 (PBM0272)	21I-01008	..\Data 2021 \T310026\ September
pH-metric logP				21I-01009	

Results Printouts

Individual results printouts are displayed at the end of the report.

General pK_a Platinum Standard Procedure

Apparatus

An automated titrator system with an incorporated UV-Vis spectrometer[1] (SiriusT3™, Pion Inc.) was used to acquire the spectrometric and/or potentiometric data. The optical system consisted of a photodiode array detector with a deuterium lamp and a fibre optic dip probe.

The titrator module consisted of a temperature controller (by Peltier device with in-situ thermocouple), pH electrode, an overhead stirrer, and motorised dispensers for the automatic delivery of assay titrants and reagents via capillaries. The instrumentation was operated using SiriusT3Control software (V2.0). Data processing and generation of the reported pK_a values was carried out using SiriusT3Refine software (V2.0).

Reagents

The titrants used were 0.5 M hydrochloric acid (product code J/4330/17, Fisher Scientific) and 0.5 M potassium hydroxide (product code J/6630C/90, Fisher Scientific), standardised using Potassium Hydrogen Phthalate (Sigma Aldrich).

ACS or HPLC grade methanol, dioxane, acetonitrile, DMSO or MDM[2] cosolvent may be used for pK_a determination. Spectrophotometric experiments are carried out in the presence of a mid-range buffer solution (15 mM Di-potassium hydrogen orthophosphate, AR grade) or Neutral Linear Buffer™ (reference number T1404007 Pion Inc.) to prevent uncontrolled pH-drift at mid-range pH.

Procedure – Platinum standard pK_a

All experiments are carried out at a controlled temperature 25.0 ± 0.2 °C. The pH range of titration assays is set between pH 2.0 to pH 12.0, the pH range is adjusted accordingly to suit sample stability and/or extremely high or low pK_as. Prior to use, 0.5 M KOH base titrant is standardised by the titration of approximately 15 mg of potassium hydrogen phthalate, in triplicate. 0.5 M HCl titrant is subsequently standardised against the base titrant. The assay media for pK_a determination is kept at a constant ionic strength of 0.15 M KCl and under argon atmosphere. The pH electrode is calibrated daily using the Avdeef–Bucher four–parameter equation[3].

For the determination of pK_a values, both pH-metric and UV-metric methods are employed. pK_a results will be reported for compounds from data acquired using the UV-metric technique where pK_as are shown to exhibit UV activity and pH-metric titrations are performed to supplement UV data. pK_a values determined by the pH-metric technique will be provided where pK_as cannot be identified using the UV-metric assay. The pK_a values obtained from spectrophotometric experiments agree excellently with those derived from potentiometric titrations[4, 5].

When cosolvent is incorporated into the assay design, the aqueous pK_a values are extrapolated from the apparent pK_a values measured in the presence of cosolvents (p_sK_a) using Yasuda-Shedlovsky extrapolation equation[6]; where p_sK_a versus reciprocal function of the dielectric constant (ε) at different cosolvent mixtures is represented. A minimum of three ratios of water/cosolvent are titrated to determine the aqueous pK_a values. The co-solvent of choice will be altered accordingly to suit sample solubility.

The spectrometric (UV-metric) method[7] uses a fibre optic dip probe, a UV light source (deuterium lamp), and a photodiode array detector to capture the absorption spectra of a sample solution over the course of a titration. The compound to be analysed must contain a chromophore(s) in close proximity to the ionisation centre(s) such that the optical properties of the sample solution vary as a function of pH.

A 10 mM DMSO stock solution is prepared and 5 µL (unless otherwise stated) of the stock solution is pipetted into the vial for titration. Neutral linear buffer (25 µL), or phosphate buffer 15 mM (25 µL) and 0.15 M KCl water (1.5 mL) are dispensed into the assay vial. Acid or base is added to bring the pH to the desired starting pH. Up to three titrations can be performed in one vial, reported values are determined from a minimum of three titrations.

Over the chosen pH range, any spectral changes due to ionisation are captured by the photodiode array detector for subsequent analysis. Dedicated software applies principle component analysis to the data to determine the pK_a values of the sample and resolve the molar absorptivity spectra of each sample species.

The spectrophotometric method is ideal for sparingly soluble samples because it requires a lower sample concentration than the potentiometric method. However, the pK_a can also be determined in a mixture of cosolvent and water if necessary. In cases such as these, the aqueous pK_a is determined using the Yasuda-Shedlovsky technique[6].

The potentiometric (pH metric) method is carried out by weighing approximately 0.5 – 1.0 mg of pure drug (i.e. FW 400 g/mol) substance into an assay vial. 0.15M KCl water is added with optional cosolvent to dissolve the compound, followed by an acid or base titrant addition to decrease or increase the pH to the desired starting pH. The solution is then titrated with acid or base to the end pH. The pK_a values are determined by analysis of the obtained titrant volume difference curves by dedicated software. Up to three titrations can be performed in one vial, reported values are determined from a minimum of three titrations. For drug substances that are sparingly soluble in water, the aqueous pK_a is determined by Yasuda-Shedlovsky extrapolation[5] from mixtures of water and cosolvent.

Errors

The reported error arises from the non-linear, least squares regression analysis utilised in the dedicated refinement software. Matrix algebra is used to generate the uncertainty of each parameter of the least squares fit. The regression analysis also contains a weighting scheme that allows greater significance to be accorded to the regions of the titration curve that contain information about the ionisation of the sample. For a pK_a value obtained from extrapolation using cosolvent, additional information about the quality of the extrapolation is also contained in the reported error.

References

[1]*Comer et al., Comp. Med. Chem. II., 2006, 5, 357.*
[2]*Gergely Volgyi et al., Anal. Chim. Acta, 2007, 583, 418.*
[3]*Avdeef and Bucher, Anal. Chem., 1978, 50, 2137.*
[4]*Mitchell et al., J. Pharma. Biomed. Anal., 1999, 20, 289.*
[5]*Tam et al., Talanta, 1999, 49, 539*
[6]*Avdeef et al., Anal. Chem., 1993, 65, 42.*
[7]*Allen et al., J. Pharm. Biomed. Anal., 1998, 17, 699*

Yasuda-Shedlovsky Result

Sample name: **WP6 THF_ I zero**
Assay name: **pH-metric psKa**
Assay ID: **21I-24004**
Quality: **Good**
Filename: **I:\Analytical Services Lab\Data 2021\AS_Customer Samples\University of Maryland\September\P210127\Data\21I-24004_WP6_I zero_pH-metric psKa_THF_6pKas.t3r**

Experiment start time: **24/09/2021 12:01:40**
Analyst: **RR**
Instrument ID: **T310026**

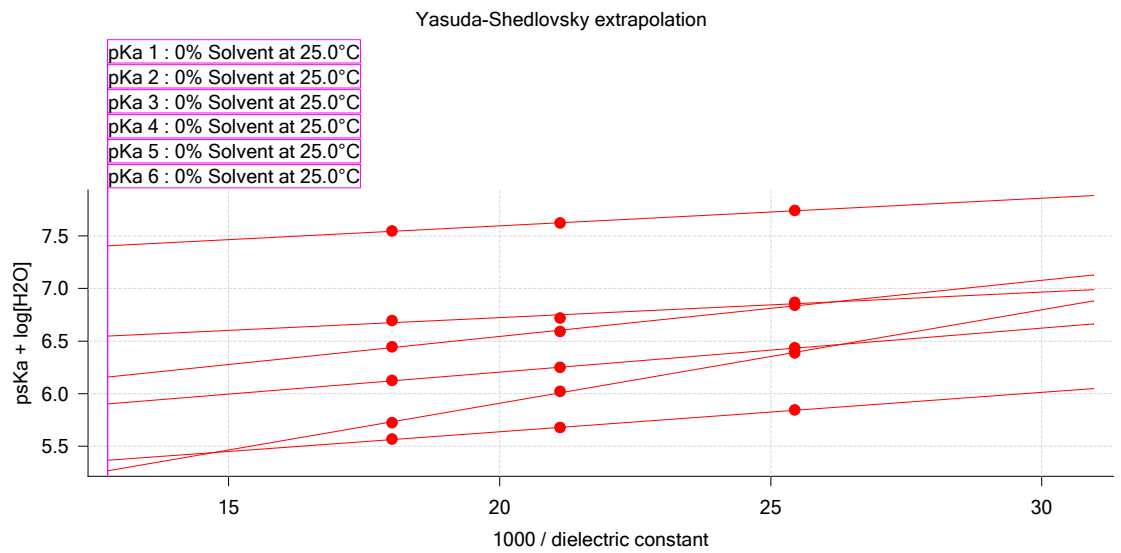
Yasuda-Shedlovsky result

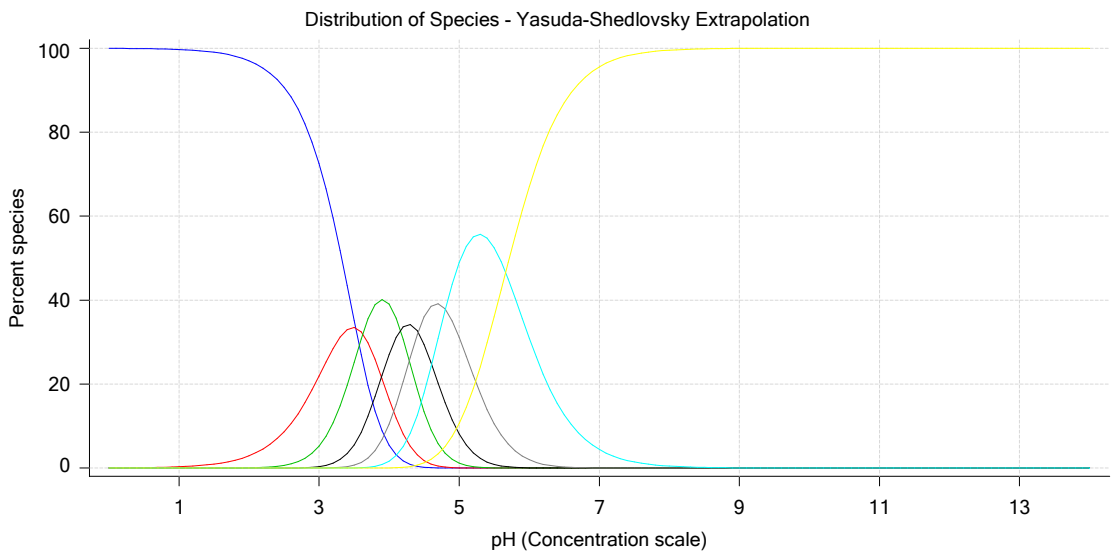
Extrapolation type	pKa 0%	SD	Intercept	Slope	R²	Ionic strength	Temperature
Yasuda-Shedlovsky	3.62	±0.01	4.89	37.4791	0.9992	0.178 M	25.0°C
Yasuda-Shedlovsky	3.52	±0.03	4.13	89.0581	0.9986	0.178 M	25.0°C
Yasuda-Shedlovsky	4.16	±0.00	5.37	41.8154	0.9999	0.178 M	25.0°C
Yasuda-Shedlovsky	4.41	±0.03	5.47	53.4146	0.9970	0.178 M	25.0°C
Yasuda-Shedlovsky	4.80	±0.07	6.24	24.3499	0.9159	0.178 M	25.0°C
Yasuda-Shedlovsky	5.66	±0.01	7.07	26.3571	0.9986	0.178 M	25.0°C

Component assay results

Titration	THF weight%	Direction	Result type	Dielectric constant	[H2O]	Ionic strength	Temperature	psKa 1	psKa 2	psKa 3	psKa 4	psKa 5	psKa 6
21I-24004 Points 89 to 133	31.96 %	Up	pH-metric	55.5	36.1 M	0.182 M	25.0°C	4.01	4.17	4.57	4.89	5.14	5.99
21I-24004 Points 46 to 88	41.69 %	Up	pH-metric	47.4	30.5 M	0.179 M	25.0°C	4.19	4.54	4.77	5.11	5.23	6.14
21I-24004 Points 3 to 45	51.32 %	Up	pH-metric	39.3	25.2 M	0.172 M	25.0°C	4.44	4.99	5.03	5.44	5.47	6.34

Graphs





Sample name: **WP6 THF_ I zero**
Assay name: **pH-metric psKa**
Assay ID: **21I-24004**
Quality: **Good**
Filename: **I:\Analytical Services Lab\Data 2021\AS_Customer Samples\University of Maryland\September\P210127\Data\21I-24004_WP6_I zero_pH-metric psKa_THF_6pKas.t3r**

Experiment start time: **24/09/2021 12:01:40**
Analyst: **RR**
Instrument ID: **T310026**

Calibration Settings

Setting	Value	Date/Time changed	Imported from
Four-Plus alpha	0.369	25/09/2021 14:51:03	I:\Analytical Services Lab\Data 2021\AS_Customer Samples\University of Maryland\September\P210127\Data\THF-37\21I-24021_Blank standardisation- THF.t3r
Four-Plus S	0.9969	25/09/2021 14:51:03	I:\Analytical Services Lab\Data 2021\AS_Customer Samples\University of Maryland\September\P210127\Data\THF-37\21I-24021_Blank standardisation- THF.t3r
Four-Plus jH	-0.0	25/09/2021 14:51:04	I:\Analytical Services Lab\Data 2021\AS_Customer Samples\University of Maryland\September\P210127\Data\THF-37\21I-24021_Blank standardisation- THF.t3r
Four-Plus jOH	-78.0	25/09/2021 14:51:04	I:\Analytical Services Lab\Data 2021\AS_Customer Samples\University of Maryland\September\P210127\Data\THF-37\21I-24021_Blank standardisation- THF.t3r
Base concentration factor	1.017	24/09/2021 12:01:40	I:\Analytical Services Lab\Data 2021\T310026\September\September_KHP Multiset Pass.t3r
Acid concentration factor	1.006	24/09/2021 12:01:40	I:\Analytical Services Lab\Data 2021\T310026\September\21I-24003_Blank standardisation.t3r