Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Pillar[6]MaxQ Functions as an *In Vivo* Sequestrant for Rocuronium and Vecuronium

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General Experimental Details. All starting materials and solvents were purchased from commercial sources and were used without further purification unless otherwise stated. NMR spectra were measured on a Bruker 600 MHz spectrometer at 25 ˚C. ITC data were collected using a Malvern Microcal PEAQ-ITC instrument and analyzed using the software provided by the vendor. (EtO)12P6 was synthesized according to Hof's procedure (Wilson, C. R.; Chen, E. F. W.; Puckett, A. O.; Hof, F. *Org. Synth.* **2022,** *99,* 125-138). $(HO)_{12}$P6 and **P6MQ** were prepared according to modifications of the literature procedures (Xue, W.; Zavalij, P. Y.; Isaacs, L. *Angew. Chem. Int. Ed.* **2020**, *59*, 13312-13319 and Brockett, A. T.; Xue, W.; Deng, C.-L.; Zhai, C.; Shuster, M.; King, D.; Rastogi, S.; Briken, V.; Roesch, M. R.; Isaacs, L. *Chem* **2023**, *9*, 881-900).

^{1}H NMR stack plots recorded for **P6MQ** with selected guests

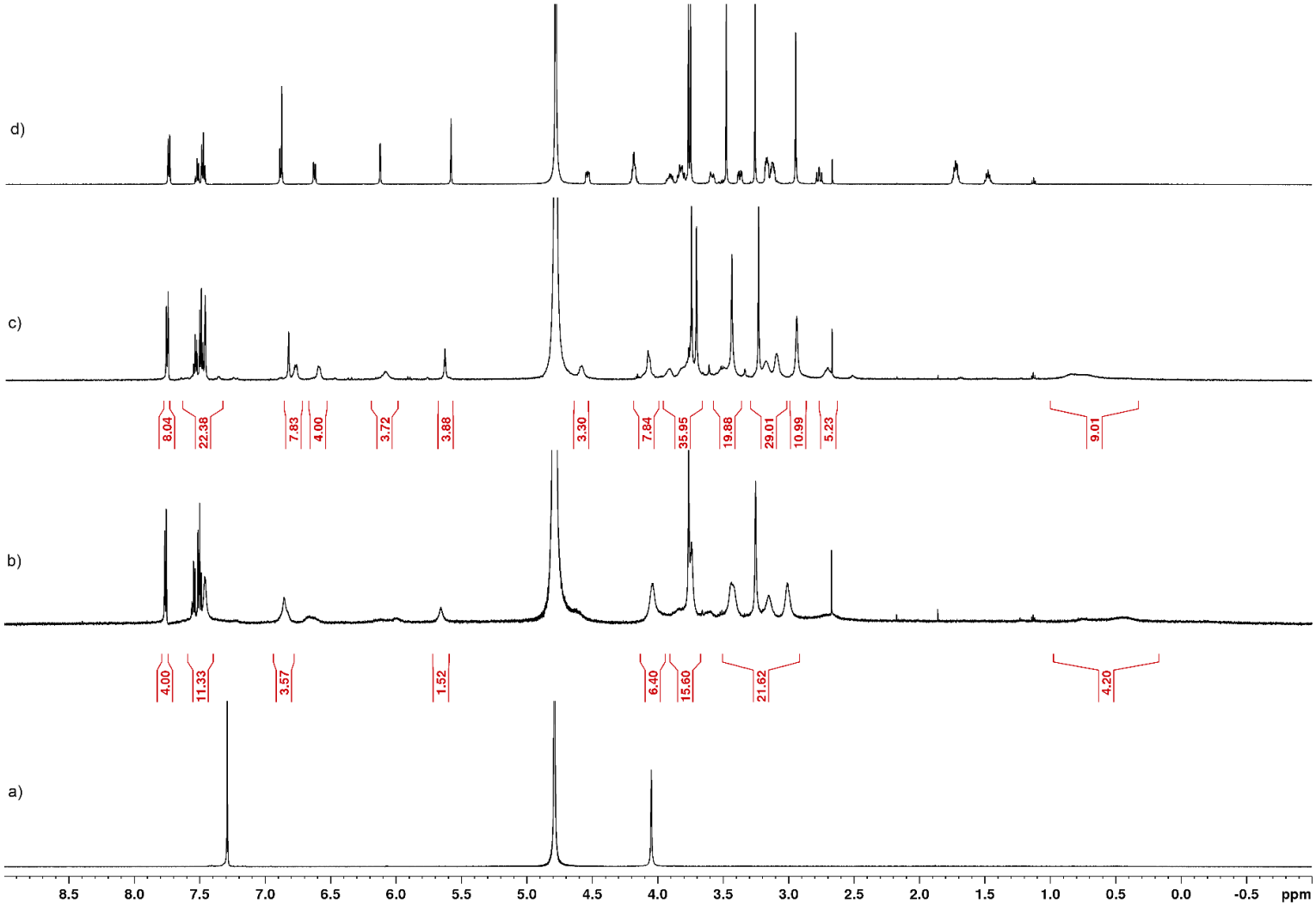

Figure S7. ^{1}H NMR spectra recorded (600 MHz, RT, D_2O) for: a) **P6MQ**, b) an equimolar mixture of **P6MQ** and **Cisatracurium** (0.5 mM), c) a 2:1 mixture of **Cisatracurium** (1 mM) and **P6MQ** (0.5 mM), and d) **Cisatracurium**.

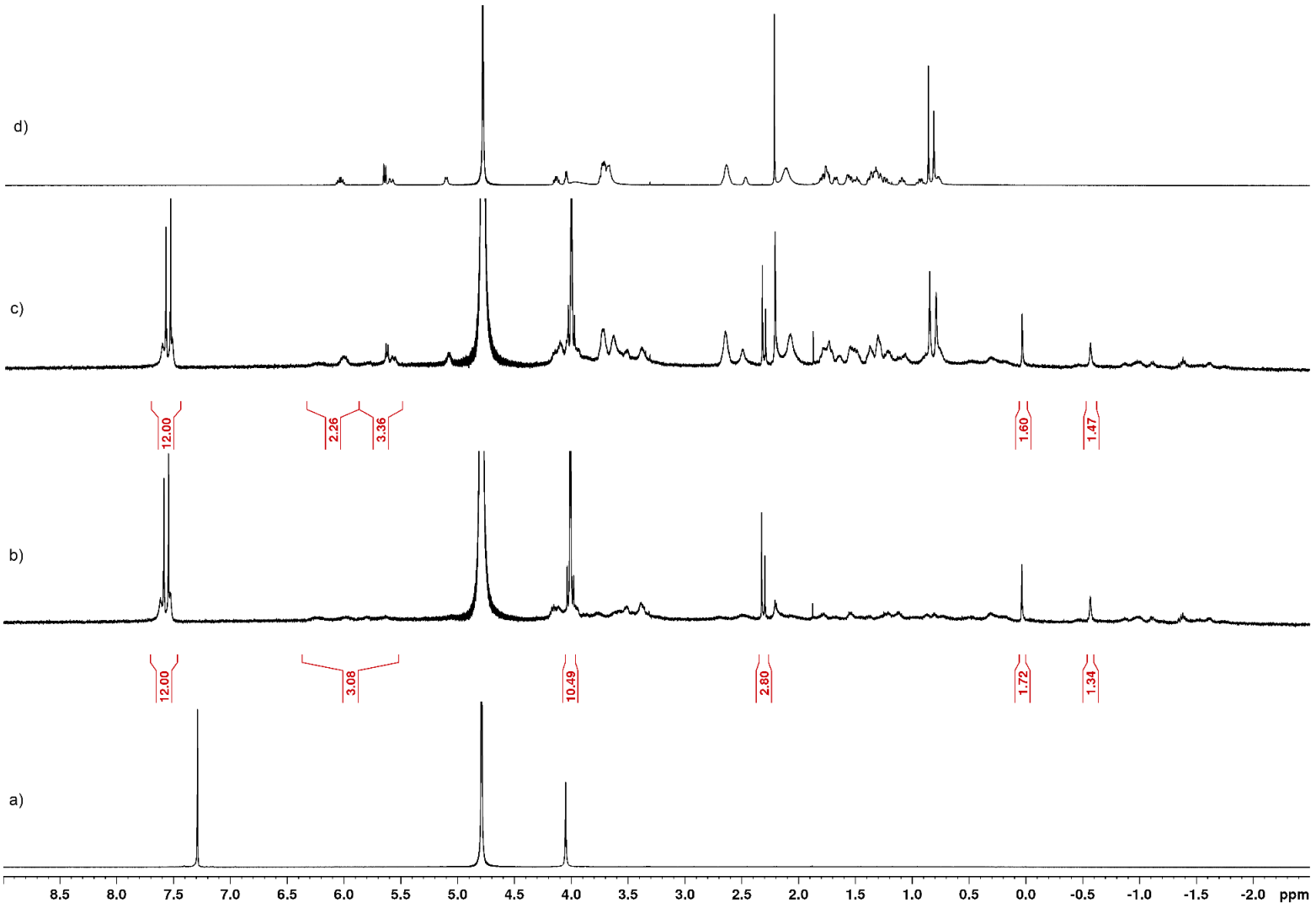

Figure S8. ^{1}H NMR spectra recorded (600 MHz, RT, D_2O) for: a) **P6MQ**, b) an equimolar mixture of **P6MQ** and **Rocuronium** (0.5 mM), c) a 2:1 mixture of **Rocuronium** (1 mM) and **P6MQ** (0.5 mM), and d) **Rocuronium**.

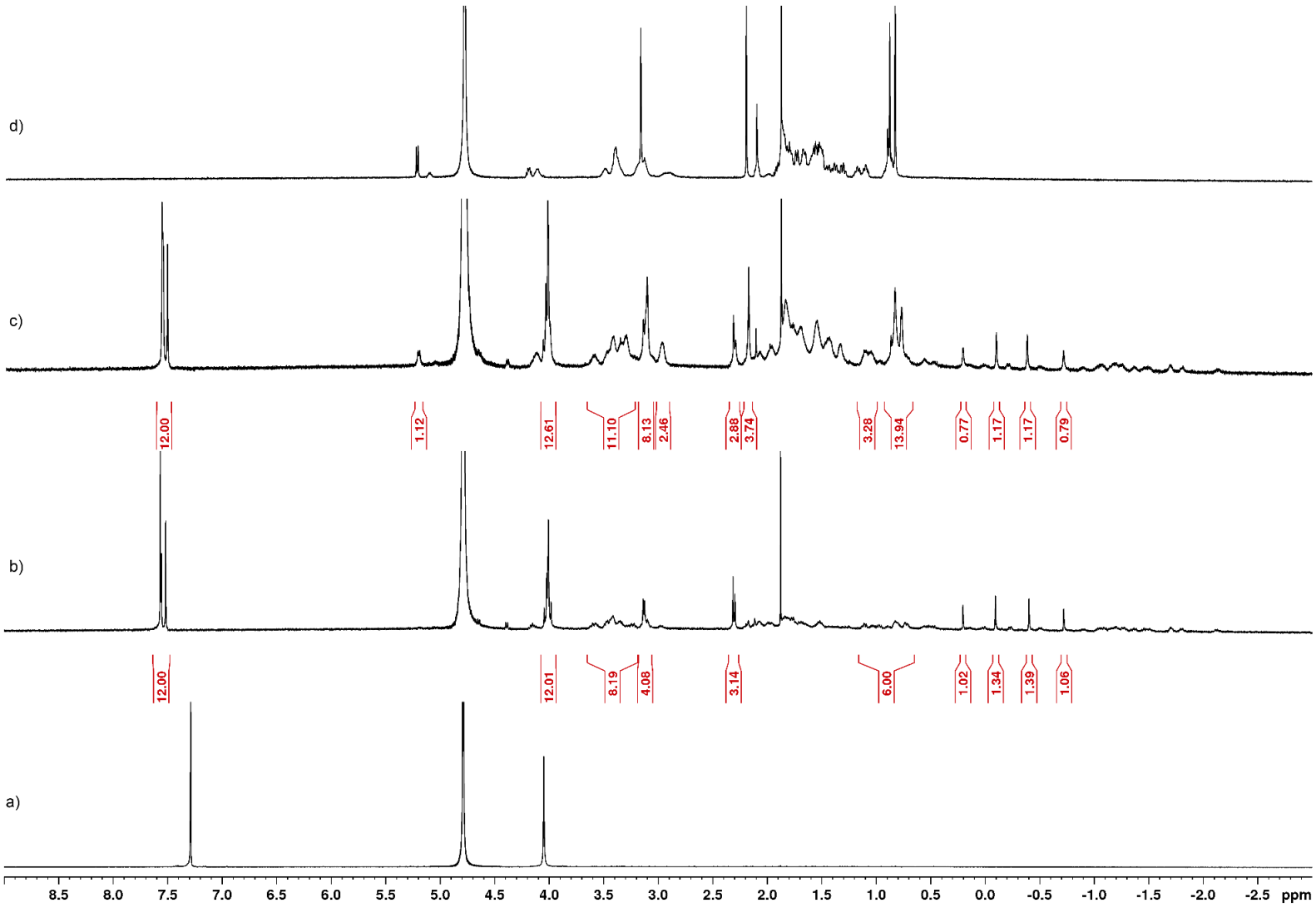

Figure S9. ^{1}H NMR spectra recorded (600 MHz, RT, D_2O) for: a) **P6MQ**, b) an equimolar mixture of **P6MQ** and **Vecuronium** (0.5 mM), c) a 2:1 mixture of **Vecuronium** (1 mM) and **P6MQ** (0.5 mM), and d) **Vecuronium**.

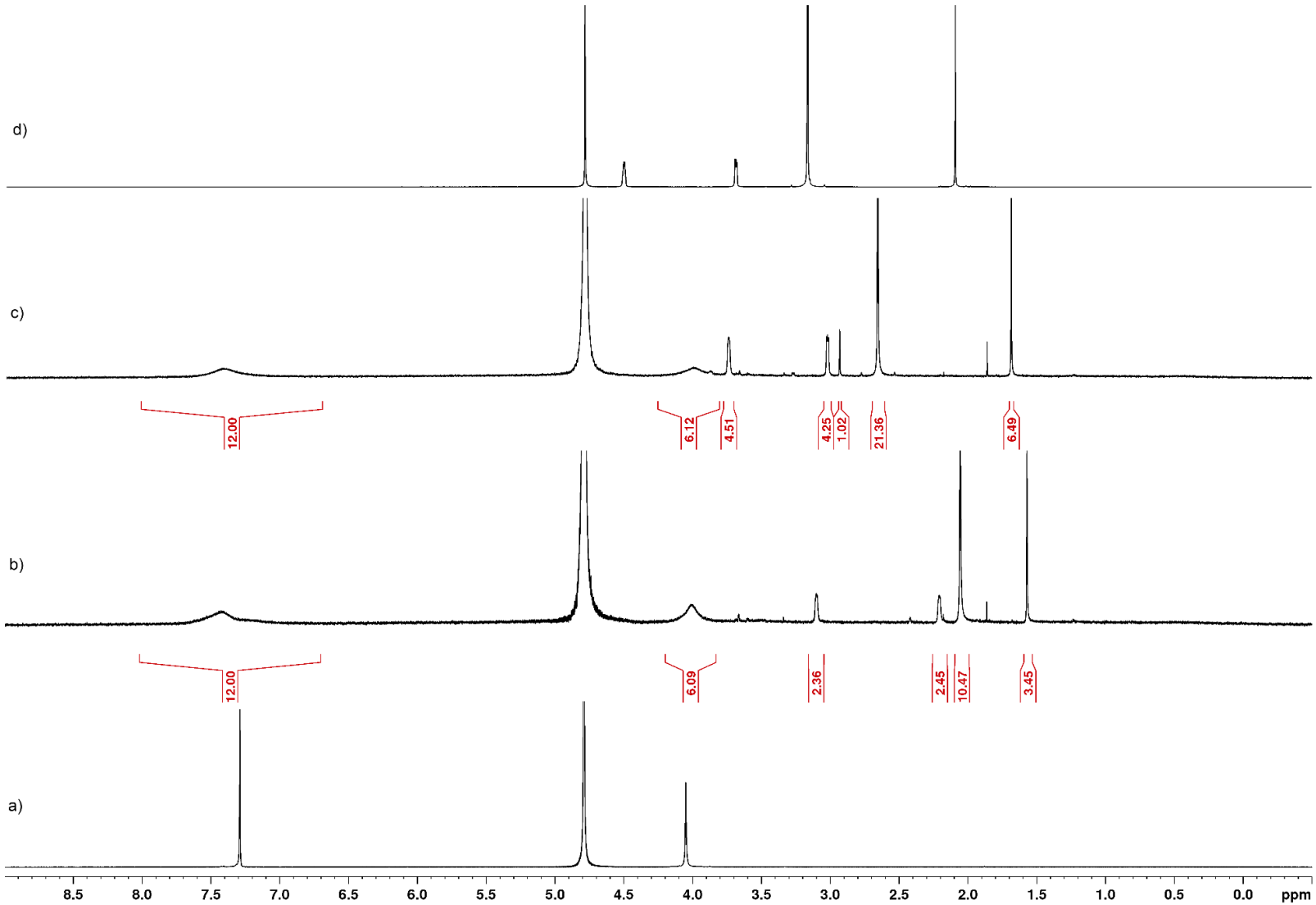

Figure S10. ^{1}H NMR spectra recorded (600 MHz, RT, D_2O) for: a) **P6MQ**, b) an equimolar mixture of **P6MQ** and **Acetylcholine** (0.5 mM), c) a 2:1 mixture of **Acetylcholine** (1 mM) and **P6MQ** (0.5 mM), and d) **Acetylcholine**.

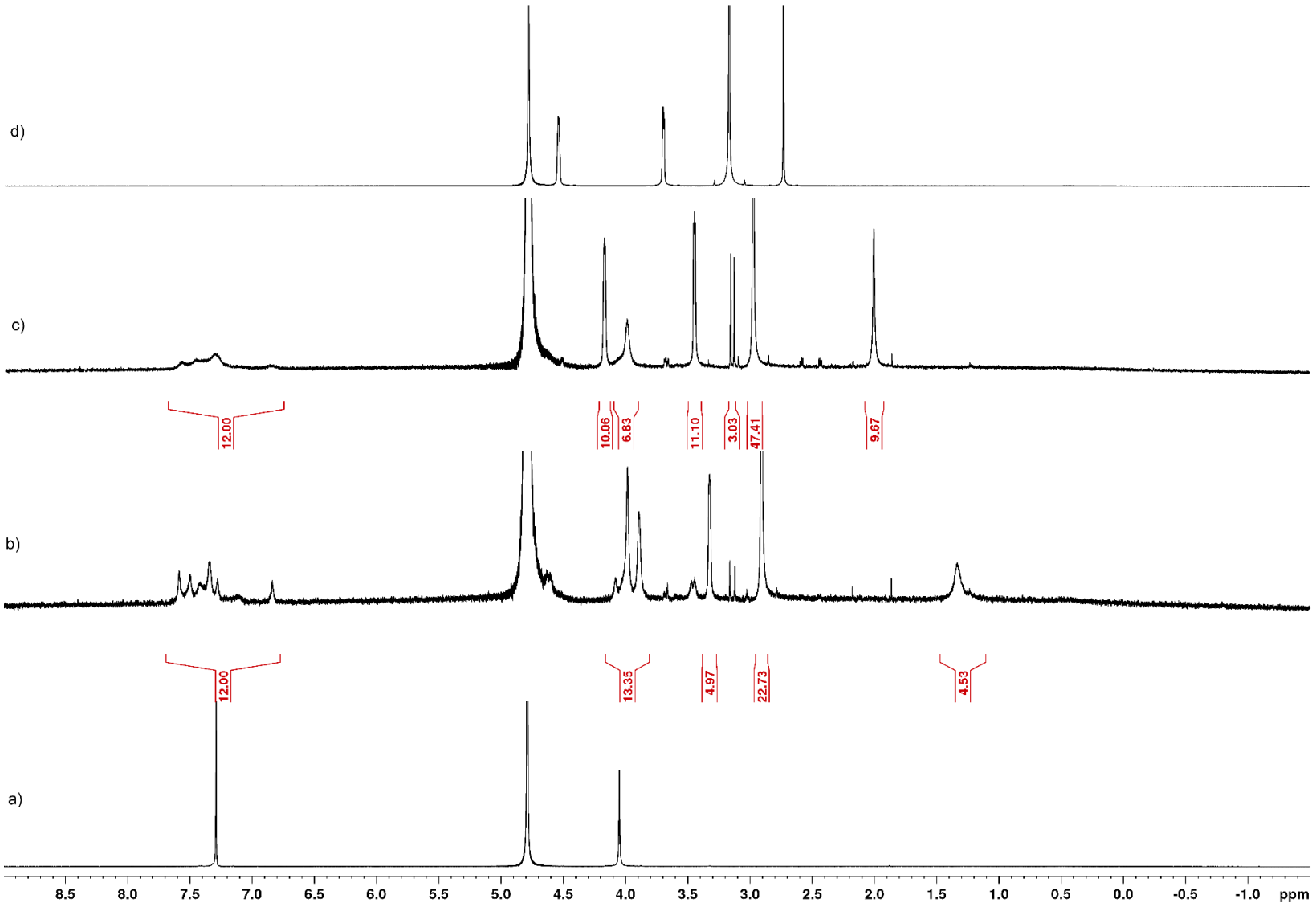

Figure S11. ^{1}H NMR spectra recorded (600 MHz, RT, D_2O) for: a) **P6MQ**, b) an equimolar mixture of **P6MQ** and **Succinylcholine** (0.5 mM), c) a 2:1 mixture of **Succinylcholine** (1 mM) and **P6MQ** (0.5 mM), and d) **Succinylcholine.**

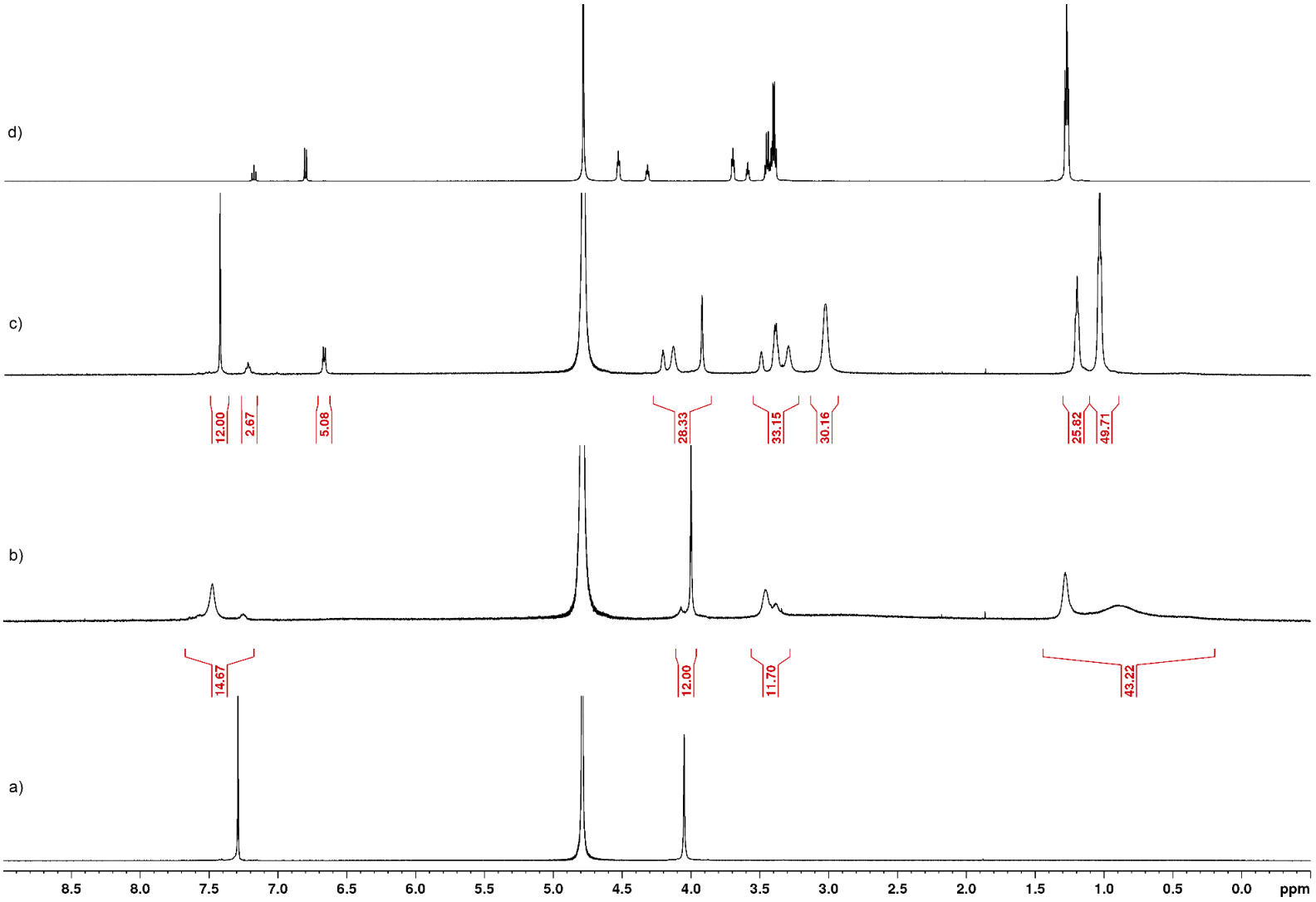

Figure S12. ^{1}H NMR spectra recorded (600 MHz, RT, D_2O) for: a) **P6MQ**, b) an equimolar mixture of **P6MQ** and **Gallamine triethiodide** (0.5 mM), c) a 2:1 mixture of **Gallamine triethiodide** (1 mM) and **P6MQ** (0.5 mM), and d) **Gallamine triethiodide**.

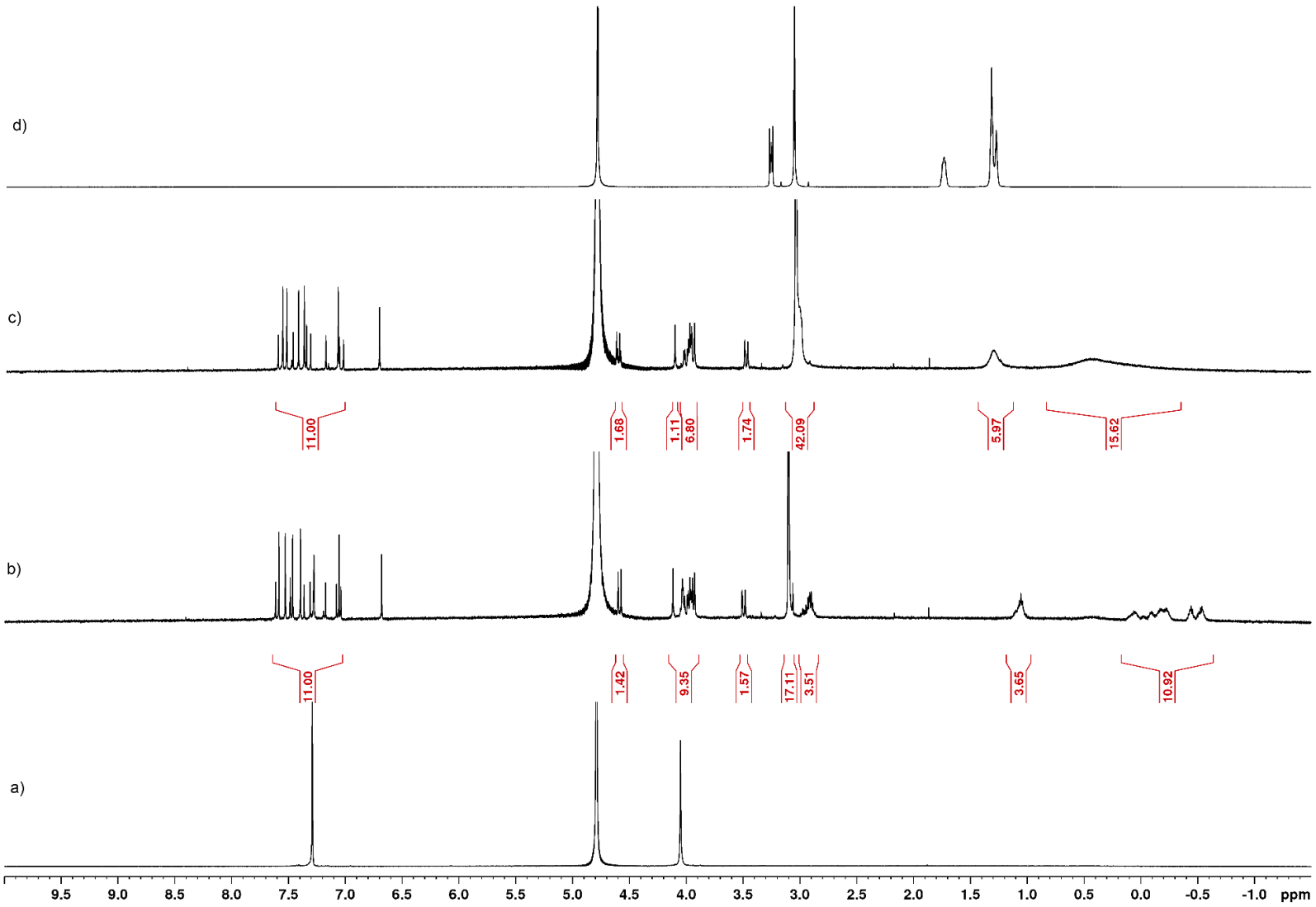

Figure S13. ^{1}H NMR spectra recorded (600 MHz, RT, D_2O) for: a) **P6MQ**, b) an equimolar mixture of **P6MQ** and Decamethonium bromide (0.5 mM), c) a 2:1 mixture of Decamethonium bromide (1 mM) and **P6MQ** (0.5 mM), and d) Decamethonium bromide.

Isothermal titration calorimetry experiments used to determine the thermodynamic parameters for P6MQ•guest complexes. 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 50 μL capacity. In each case, the host and guest solutions were prepared in PBS buffer (pH 7.4) prepared using Tablets (Catalog number 2810305) from MP Biomedicals. The sample cell was rinsed (using the PEAQ ITC integrated washing protocol), washed with 100 μL of HPLC grade H_2O (manually injected using the syringe) 3 times to ensure that any remnants of cleaning solvent from the machine was removed, and then coated with 50 μL of host solution, which was then discarded, prior to each usage. The sample cell was then filled to capacity (200 μL) with the host solution using the following process to minimize the potential for air bubbles to form: insert the syringe into sample cell, with about 1-2 mm of space between the syringe tip and the bottom of the cell, and slowly inject the host solution into the cavity until the solution is just barely visible in the bowl above the sample cell. Proceed to vigorously pull up and re-inject the host solution (about 20-50 μL worth) several times in order to agitate the solution and push out any air pockets present in the sample cell. If any air is present within the cell then small bubbles should come to the surface of the bowl during each agitation. After the agitation process is complete (no bubble should rise), pull up about 50 μL of host solution and re-inject it as the syringe is slowly pulled out of the cavity. Pull out any remaining host solution present in the bowl and discard. The guest solution was then titrated in by the instrument in equal increments of time (first injection = 0.4 μL with subsequent 18 injections = 2 μL). The binding data was fitted using the 1:1 binding model or the competitive binding model in MicroCal PEAQ-ITC analysis software.

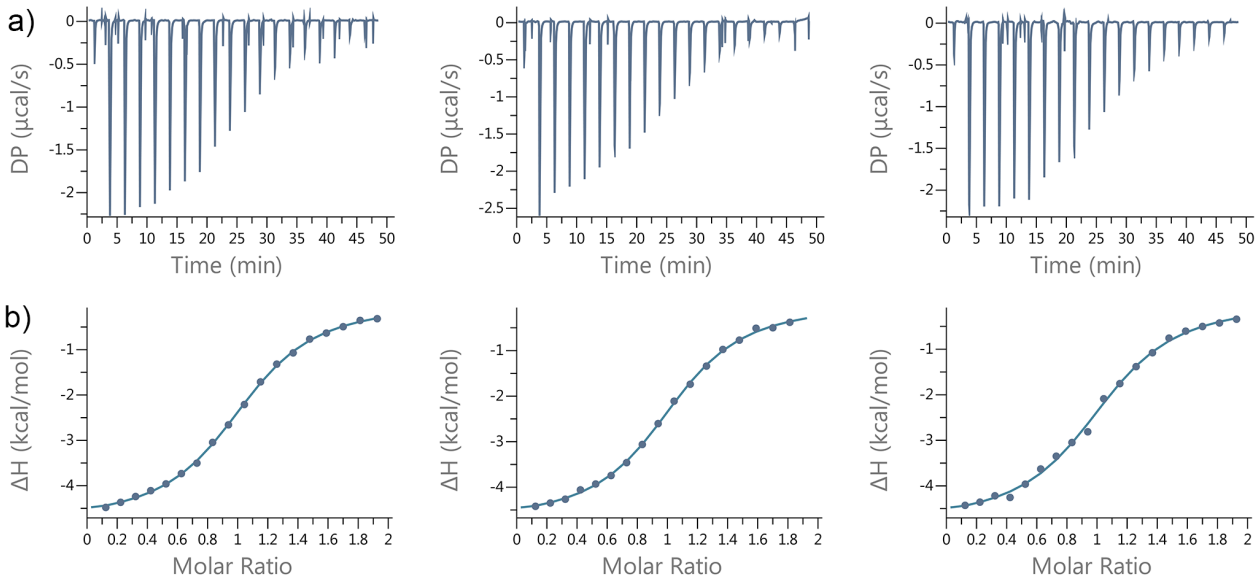

Figure S14. a) Plots of DP vs time from the direct titration of **P6MQ** (250 µM) with **C3DA** (2.50 mM) in PBS buffer (pH 7.4); b) plots of ΔH as a function of guest:host molar ratio. The solid lines represent the best non-linear fit of the data to a 1:1 binding model (triplicate averages: K_a = (5.95 ± 0.32) × 10^4 M^{-1}, ΔH = -4.79 ± 0.06 kcal/mol, -TΔS = -1.73 ± 0.06 kcal/mol).

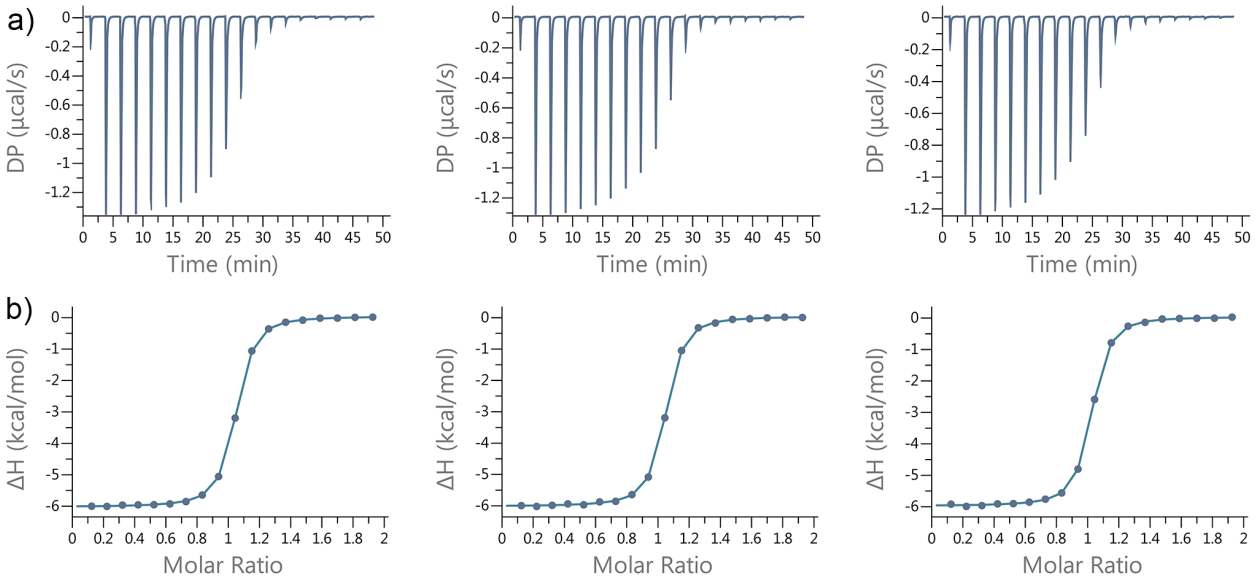

Figure S15. a) Plots of DP vs time from the direct titration of **P6MQ** (100 µM) with **C4DA** (1.00 mM) in PBS buffer (pH 7.4); b) plots of ΔH as a function of guest:host molar ratio. The solid lines represent the best non-linear fit of the data to a 1:1 binding model (triplicate averages: K_a = (3.53 ± 0.05) × 10^6 M^{-1}, ΔH = -6.04 ± 0.01 kcal/mol, -TΔS = -2.90 ± 0.01 kcal/mol).

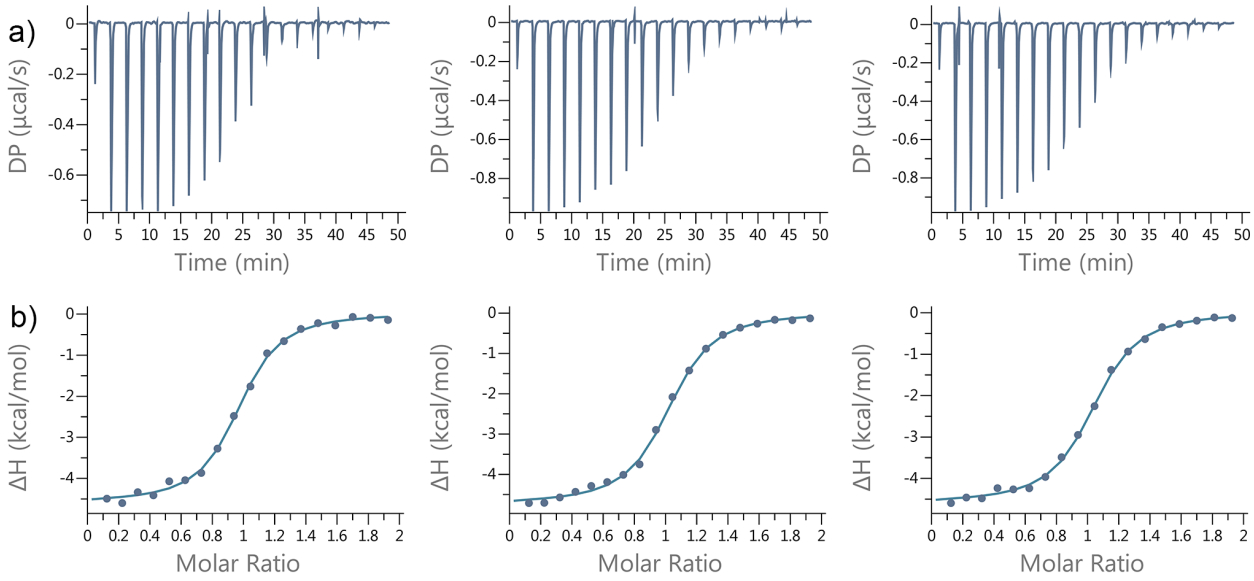

Figure S16. a) Plots of DP vs time from the competitive titration of **P6MQ** (100 µM) and **C3DA** (10.00 mM) in the cell with **C6DA** (1.00 mM) in PBS buffer (pH 7.4) from the syringe; b) plots of ΔH as a function of guest:host molar ratio. The solid lines represent the best non-linear fit of the data to a competition binding model (triplicate averages: $K_a = (2.73 \pm 0.03) \times 10^8$ M^{-1}, ΔH = -9.48 ± 0.03 kcal/mol, -TΔS = -2.03 ± 0.03 kcal/mol).

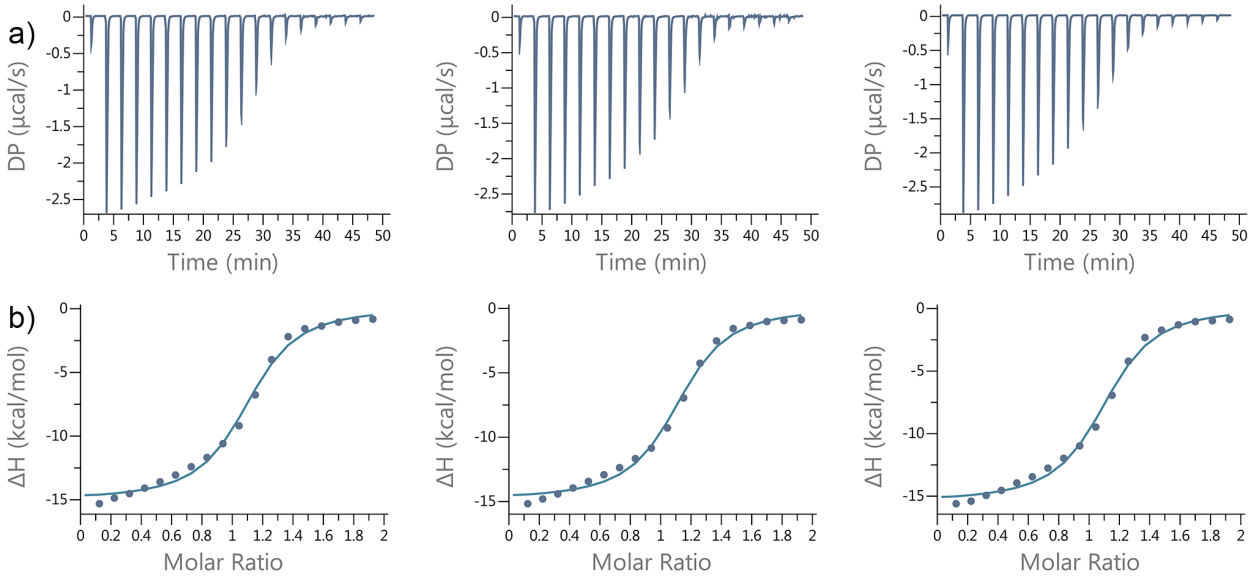

Figure S17. a) Plots of DP vs time from the direct titration of **P6MQ** (100 µM) with cisatracurium (1.00 mM) in PBS buffer (pH 7.4); b) plots of ΔH as a function of guest:host molar ratio. The solid lines represent the best non-linear fit of the data to a 1:1 binding model (triplicate averages: $K_a = (3.89 \pm 0.39) \times 10^5$ M^{-1}, ΔH = -15.17 ± 0.26 kcal/mol, -TΔS = 7.55 ± 0.26 kcal/mol).

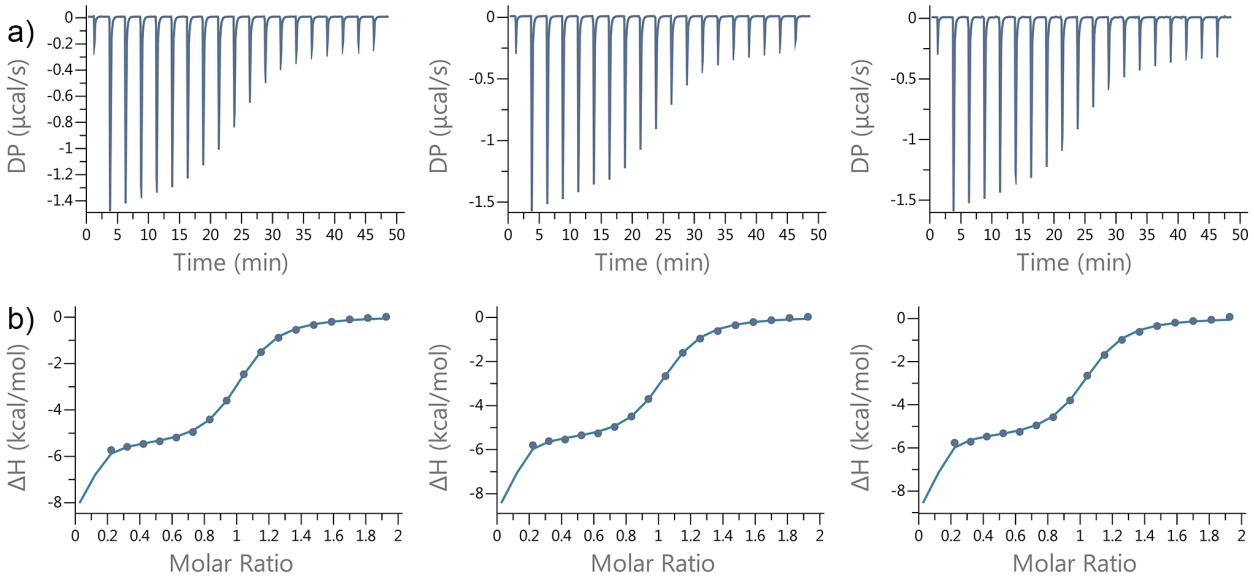

Figure S18. a) Plots of DP vs time from the competitive titration of **P6MQ** (100 µM) and **C4DA** (100 µM) in the cell with pancuronium (1.00 mM) in PBS buffer (pH 7.4) from the syringe; b) plots of ΔH as a function of guest:host molar ratio. The solid lines represent the best non-linear fit of the data to a competition binding model (triplicate averages: $K_a = (2.18 \pm 0.11) \times 10^8$ M^{-1}, $\Delta H = -11.60 \pm 0.04$ kcal/mol, $-T\Delta S = 0.21 \pm 0.04$ kcal/mol).

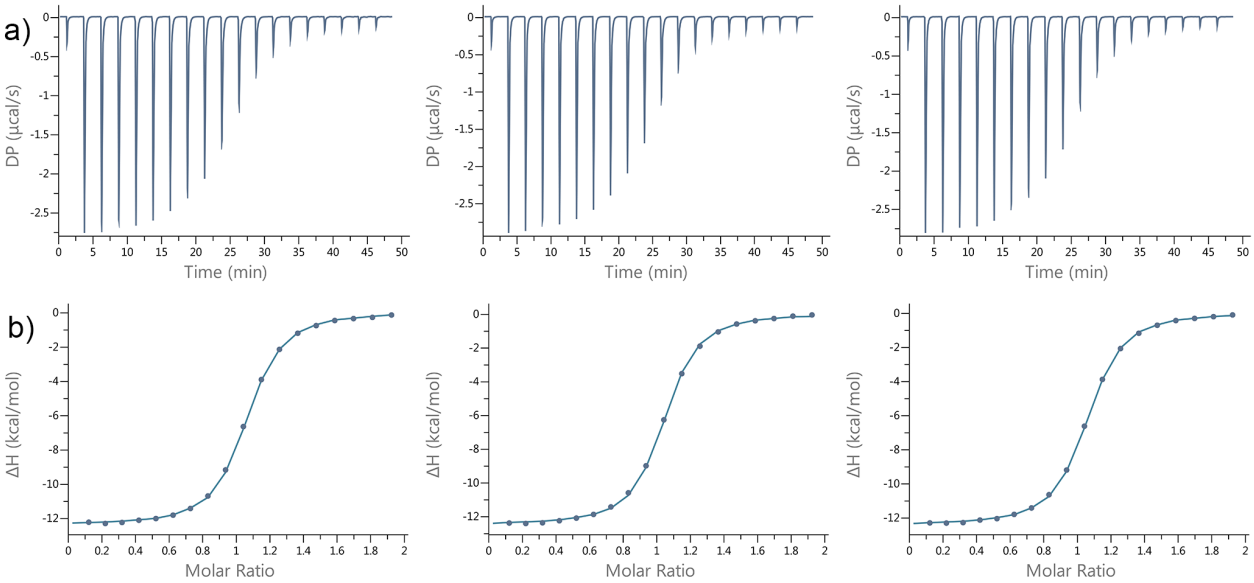

Figure S19. a) Plots of DP vs time from the competitive titration of **P6MQ** (100 µM) and **C6DA** (1.00 mM) in the cell with rocuronium (1.00 mM) in PBS buffer (pH 7.4) from the syringe; b) plots of ΔH as a function of guest:host molar ratio. The solid lines represent the best non-linear fit of the data to a competition binding model (triplicate averages: $K_a = (2.536 \pm 0.004) \times 10^{11}$ M^{-1}, $\Delta H = -22.03 \pm 0.02$ kcal/mol, $-T\Delta S = 6.44 \pm 0.02$ kcal/mol).

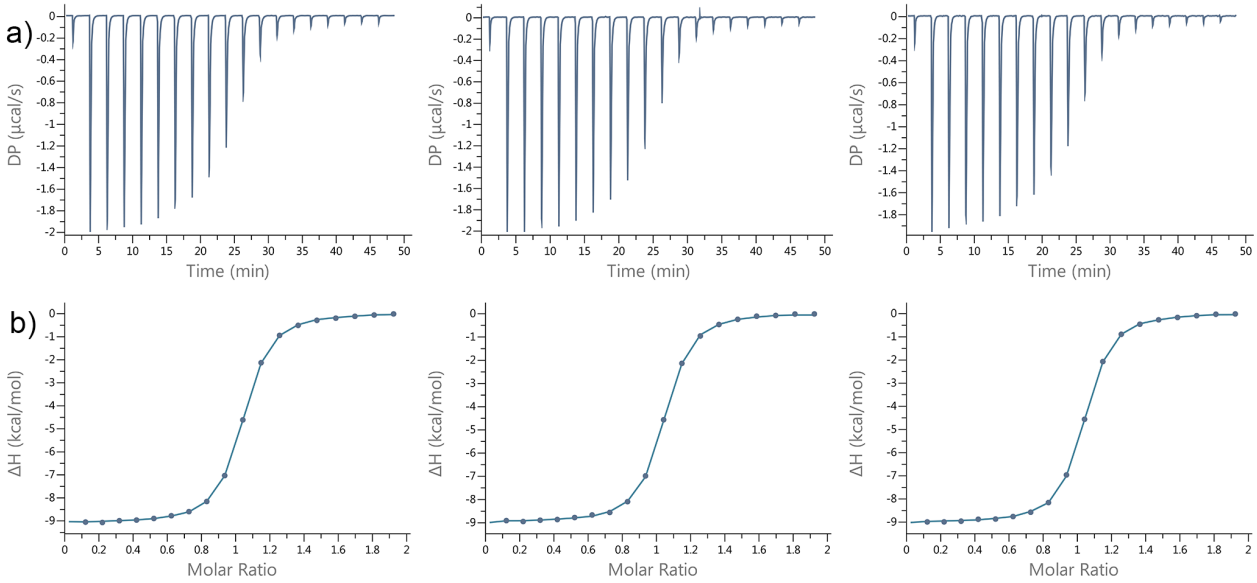

Figure S20. a) Plots of DP vs time from the competitive titration of **P6MQ** (100 µM) and **C6DA** (1.00 mM) in the cell with vecuronium (1.00 mM) in PBS buffer (pH 7.4) from the syringe; b) plots of ΔH as a function of guest:host molar ratio. The solid lines represent the best non-linear fit of the data to a competition binding model (triplicate averages: $K_a = (4.41 \pm 0.01) \times 10^{11}$ M^{-1}, ΔH = -18.60 ± 0.01 kcal/mol, -TΔS = 2.71 ± 0.01 kcal/mol).

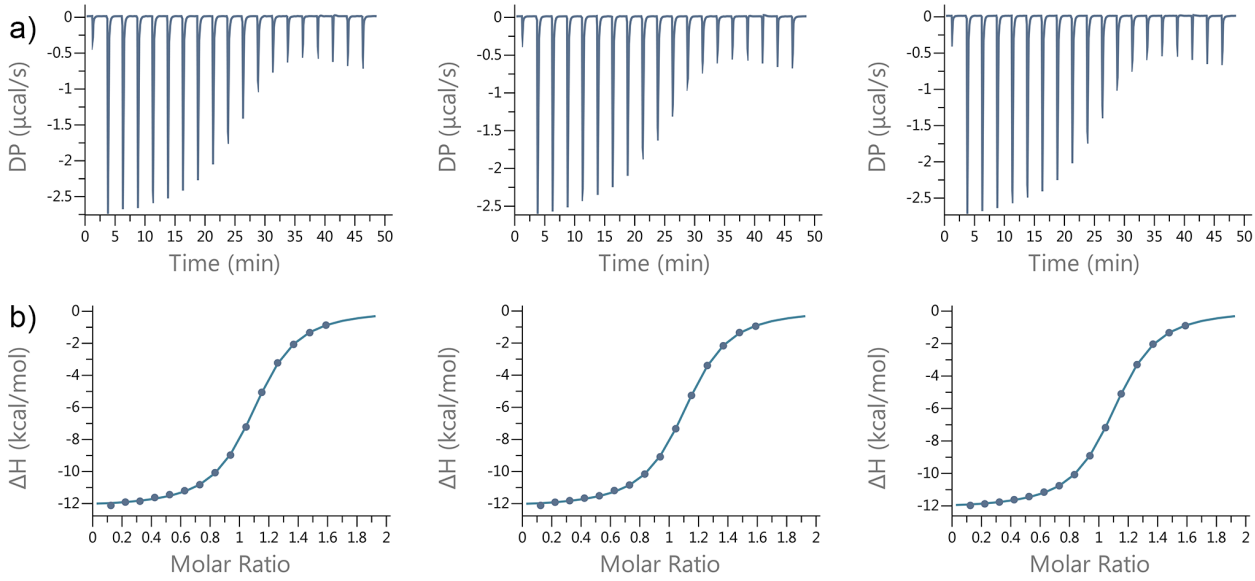

Figure S21. a) Plots of DP vs time from the direct titration of **P6MQ** (100 µM) with tubocurarine (1.00 mM) in PBS buffer (pH 7.4); b) plots of ΔH as a function of guest:host molar ratio. The solid lines represent the best non-linear fit of the data to a 1:1 binding model (triplicate averages: $K_a = (5.03 \pm 0.10) \times 10^5$ M^{-1}, ΔH = -12.27 ± 0.05 kcal/mol, -TΔS = 4.49 ± 0.05 kcal/mol).

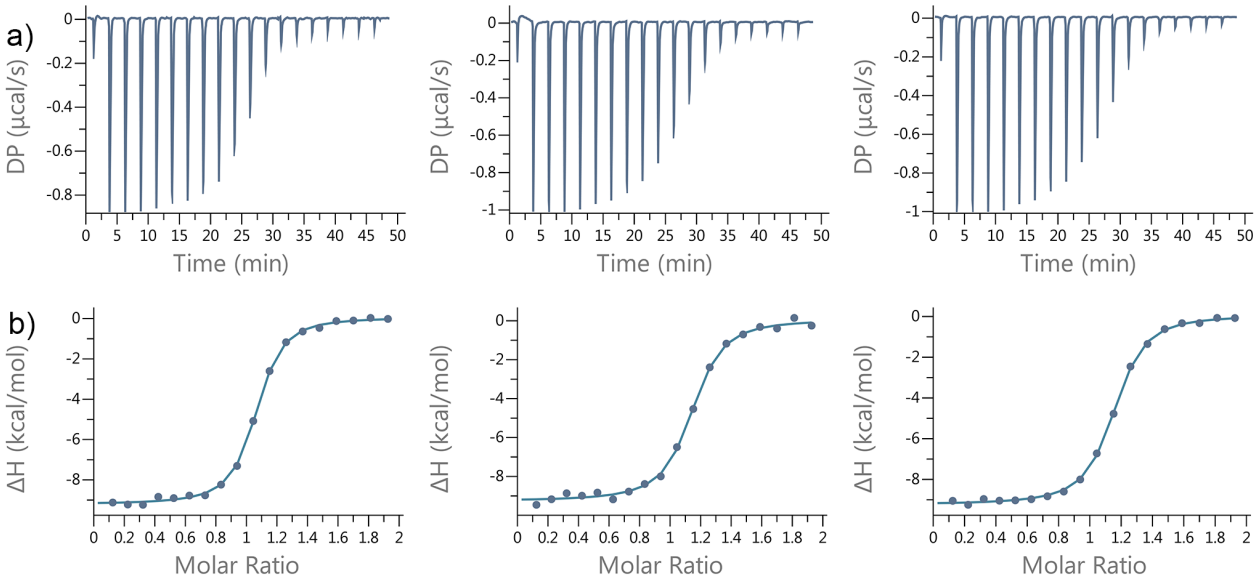

Figure S22. a) Plots of DP vs time from the direct titration of **P6MQ** (50 µM) with acetylcholine (500 µM) in PBS buffer (pH 7.4); b) plots of ΔH as a function of guest:host molar ratio. The solid lines represent the best non-linear fit of the data to a 1:1 binding model (triplicate averages: K_a = (2.55 ± 0.15) × 10^6 M^{-1}, ΔH = -9.29 ± 0.06 kcal/mol, -TΔS = 0.54 ± 0.06 kcal/mol).

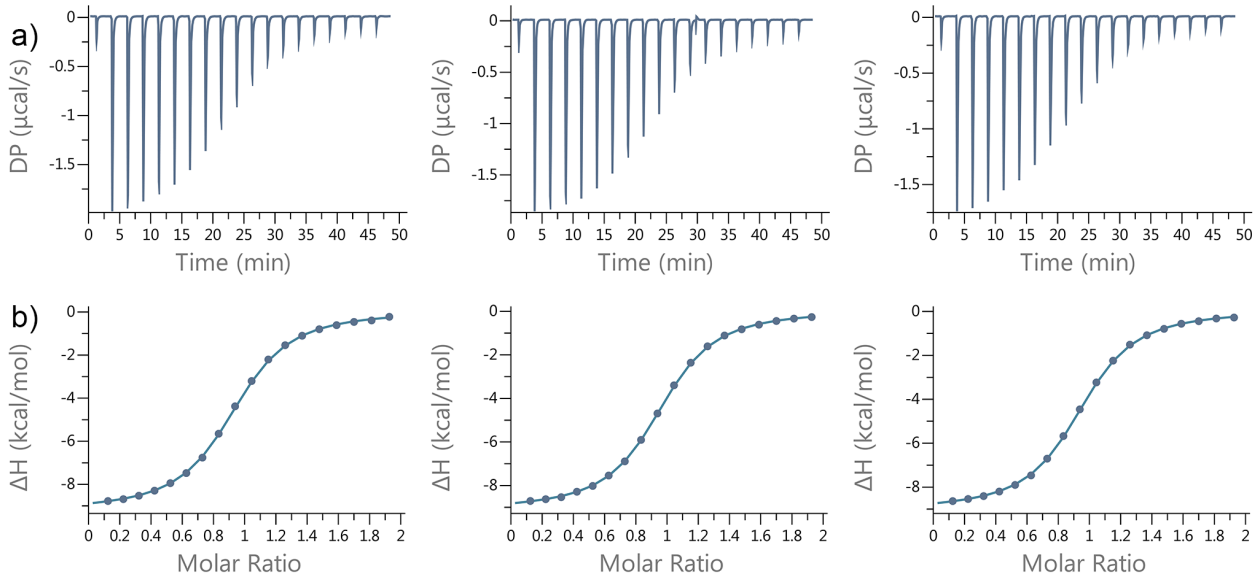

Figure S23. a) Plots of DP vs time from the competitive titration of **P6MQ** (100 µM) and **C4DA** (1.00 mM) in the cell with succinylcholine (1.00 mM) in PBS buffer (pH 7.4) from the syringe; b) plots of ΔH as a function of guest:host molar ratio. The solid lines represent the best non-linear fit of the data to a competition binding model (triplicate averages: K_a = (9.20 ± 0.13) × 10^8 M^{-1}, ΔH = -15.23 ± 0.02 kcal/mol, -TΔS = 3.01 ± 0.02 kcal/mol).

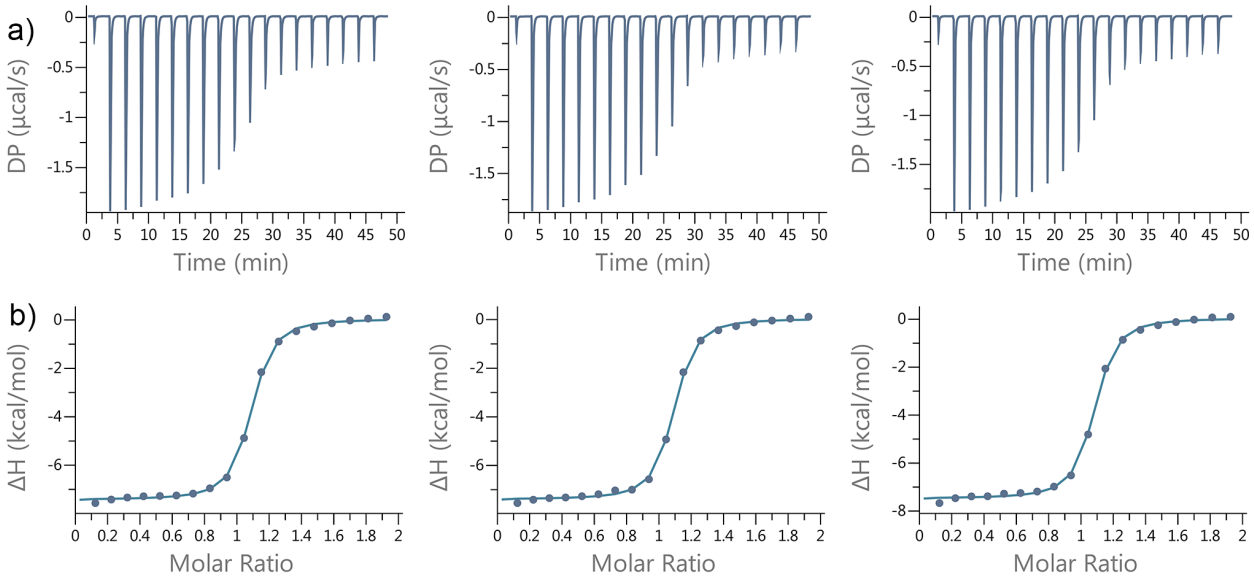

Figure S24. a) Plots of DP vs time from the competitive titration of **P6MQ** (100 µM) and **C4DA** (1.00 mM) in the cell with gallamine (1.00 mM) in PBS buffer (pH 7.4) from the syringe; b) plots of ΔH as a function of guest:host molar ratio. The solid lines represent the best non-linear fit of the data to a competition binding model (triplicate averages: $K_a = (8.11 \pm 0.44) \times 10^9$ M^{-1}, ΔH = -13.53 ± 0.04 kcal/mol, -TΔS = 0.04 ± 0.04 kcal/mol).

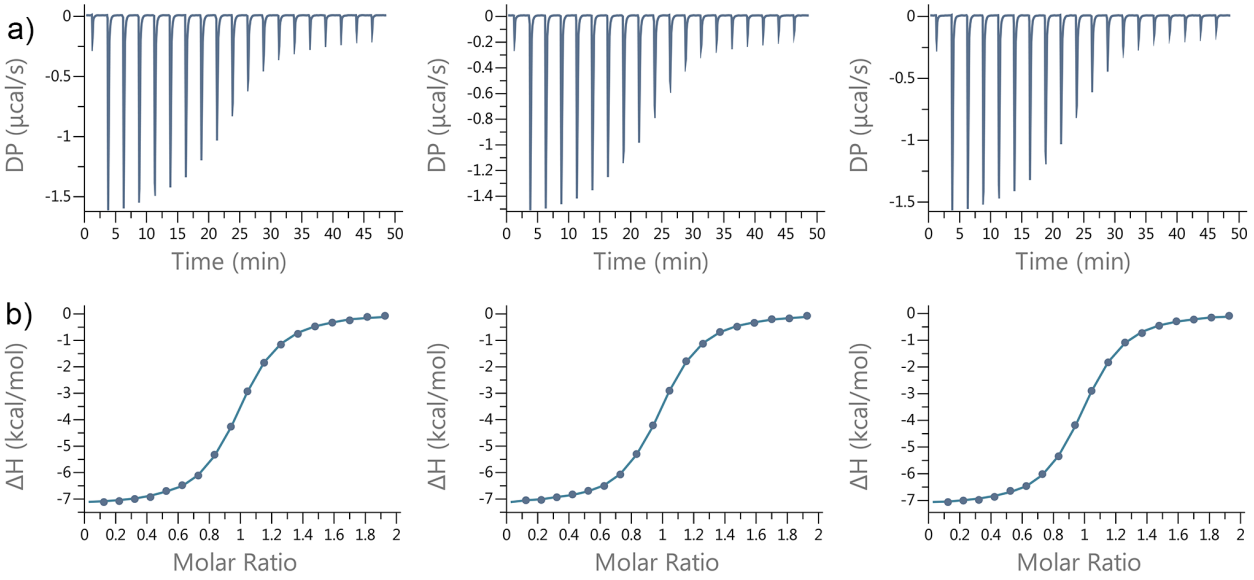

Figure S25. a) Plots of DP vs time from the titration of **P6MQ** (100 µM) and **C6DA**(1.00 mM) with decamethonium (1.00 mM) in PBS buffer (pH 7.4) from the syringe; b) plots of ΔH as a function of guest:host molar ratio. The solid lines represent the best non-linear fit of the data to a competition binding model (triplicate averages: $K_a = (1.327 \pm 0.001) \times 10^{11}$ M^{-1}, ΔH = -16.77 ± 0.01 kcal/mol, -TΔS = 1.60 ± 0.01 kcal/mol).

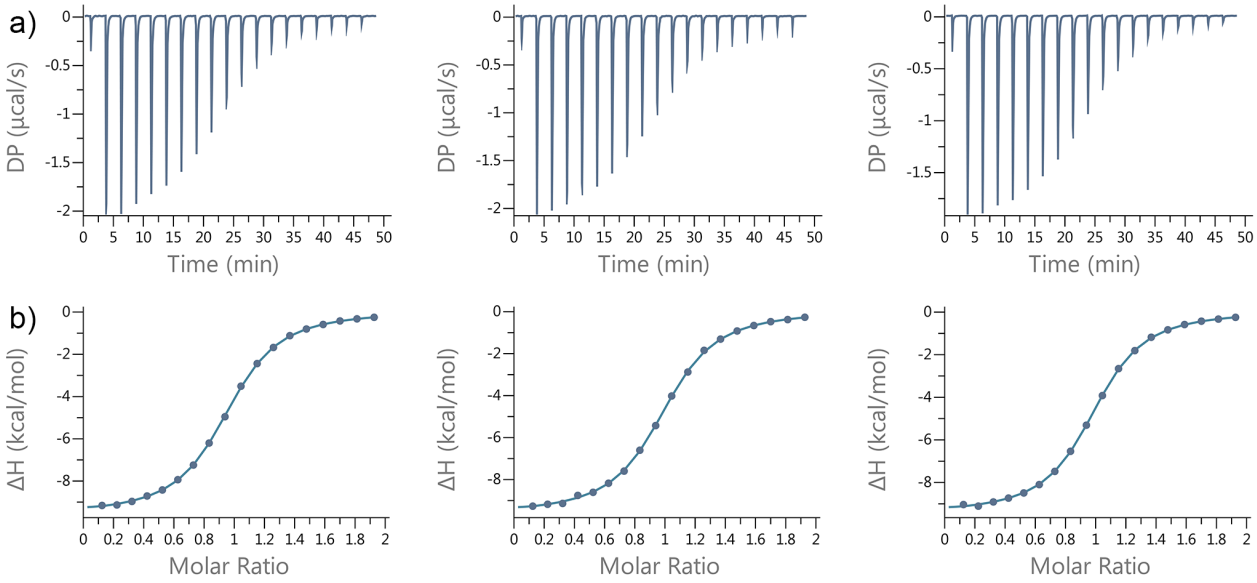

Figure S26. a) Plots of DP vs time from the direct titration of **P6MQ** (100 µM) with tyramine (1.00 mM) in PBS buffer (pH 7.4); b) plots of ΔH as a function of guest:host molar ratio. The solid lines represent the best non-linear fit of the data to a 1:1 binding model (triplicate averages: K_a = (3.19 ± 0.05) × 10^5 M^{-1}, ΔH = -9.60 ± 0.03 kcal/mol, -TΔS = 2.09 ± 0.03 kcal/mol).

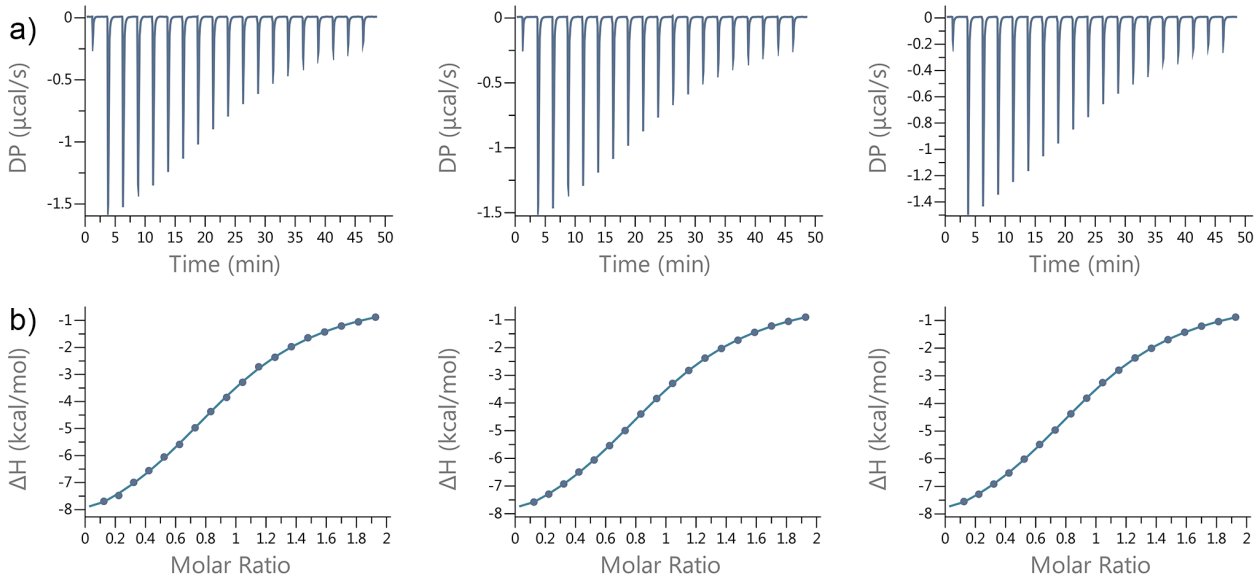

Figure S27. a) Plots of DP vs time from the direct titration of **P6MQ** (100 µM) with tryptamine (1.00 mM) in PBS buffer (pH 7.4); b) plots of ΔH as a function of guest:host molar ratio. The solid lines represent the best non-linear fit of the data to a 1:1 binding model (triplicate averages: K_a = (5.35 ± 0.11) × 10^4 M^{-1}, ΔH = -9.40 ± 0.07 kcal/mol, -TΔS = 2.94 ± 0.07 kcal/mol).

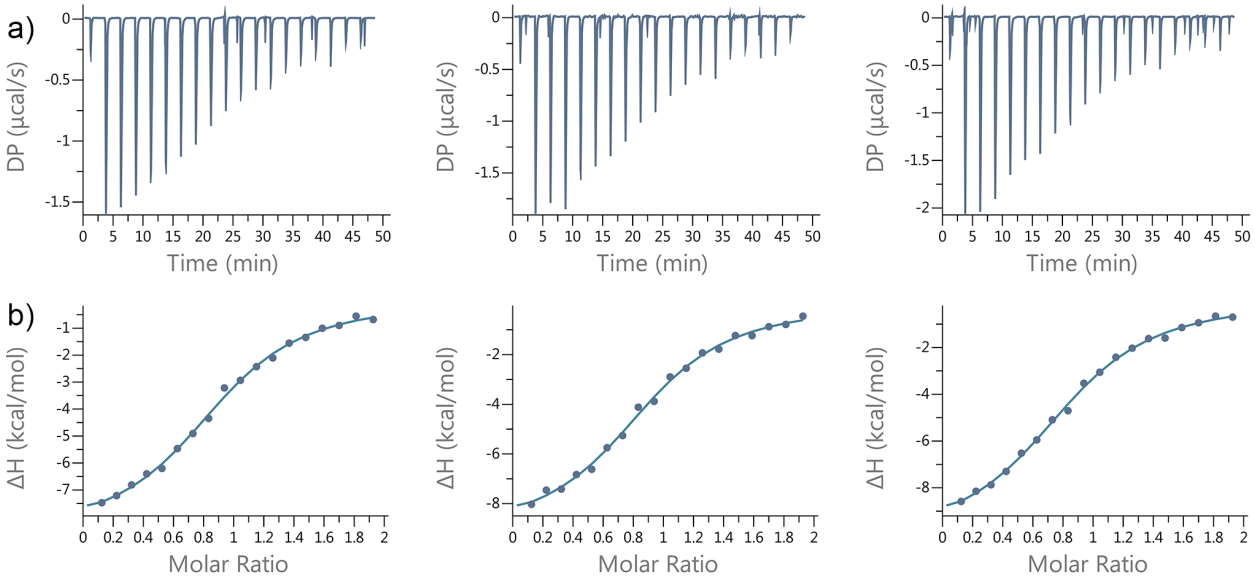

Figure S28. a) Plots of DP vs time from the direct titration of **P6MQ** (100 µM) with dopamine (1.00 mM) in PBS buffer (pH 7.4); b) plots of ΔH as a function of guest:host molar ratio. The solid lines represent the best non-linear fit of the data to a 1:1 binding model (triplicate averages: K_a = (8.02 ± 0.76) × 104 M^{-1}, ΔH = -9.40 ± 0.26 kcal/mol, -TΔS = 2.72 ± 0.26 kcal/mol).

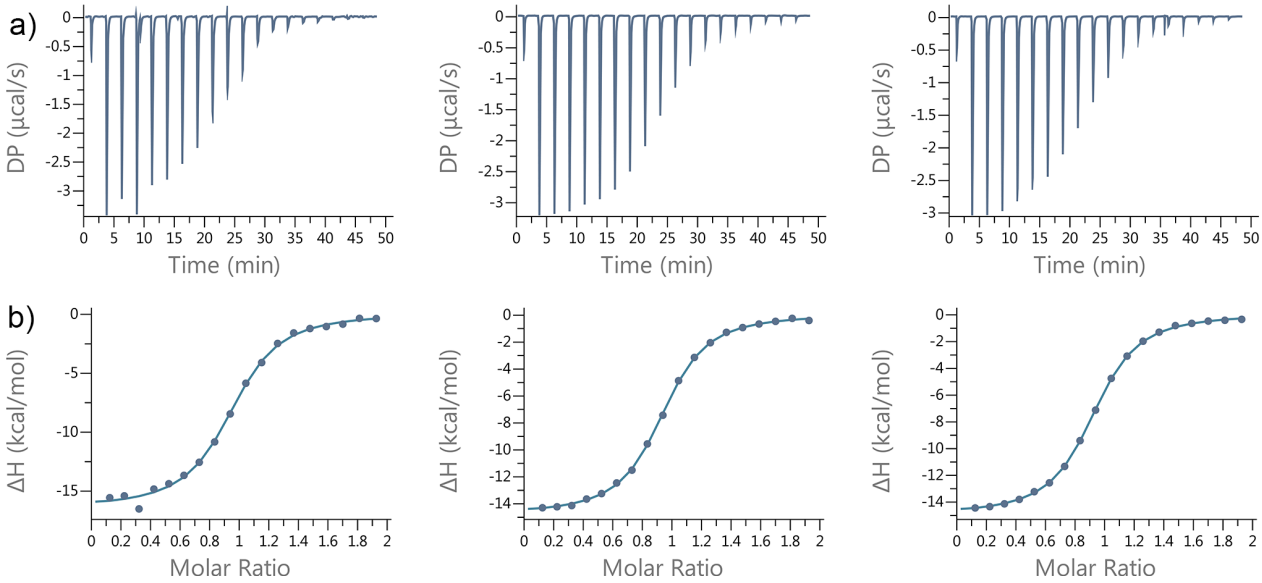

Figure S29. a) Plots of DP vs time from the direct titration of **P6MQ** (100 µM) with histamine (1.00 mM) in PBS buffer (pH 7.4); b) plots of ΔH as a function of guest:host molar ratio. The solid lines represent the best non-linear fit of the data to a 1:1 binding model (triplicate averages: K_a = (3.77 ± 0.20) × 10^5 M^{-1}, ΔH = -15.47 ± 0.14 kcal/mol, -TΔS = 7.85 ± 0.14 kcal/mol).

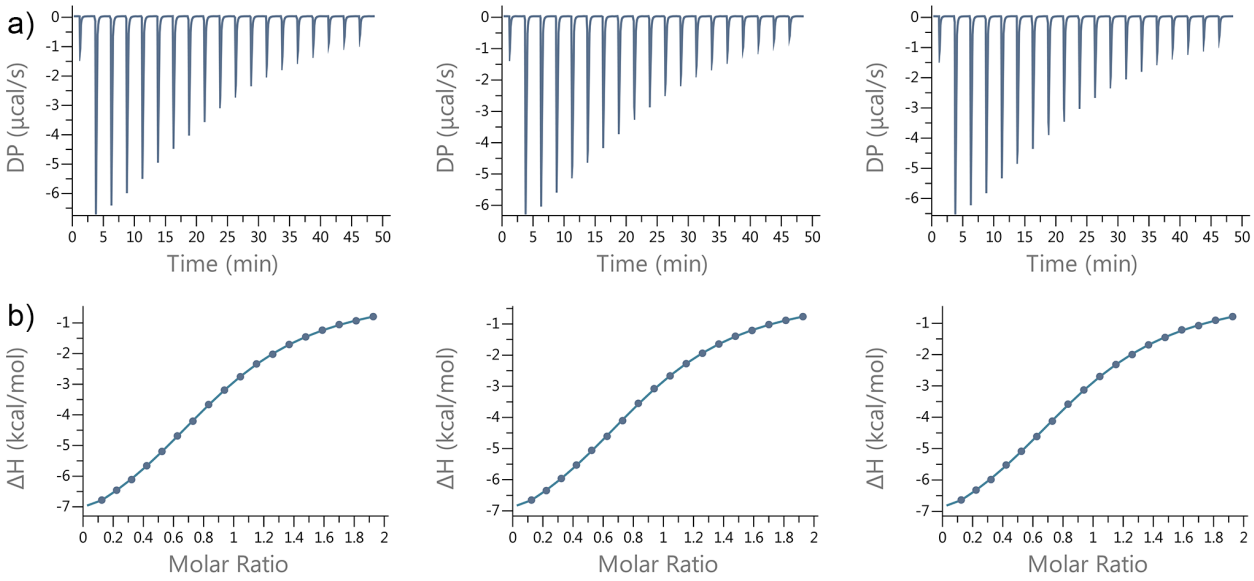

Figure S30. a) Plots of DP vs time from the direct titration of **P6MQ** (500 µM) with noradrenaline (5.00 mM) in PBS buffer (pH 7.4); b) plots of ΔH as a function of guest:host molar ratio. The solid lines represent the best non-linear fit of the data to a 1:1 binding model (triplicate averages: K_a = (9.06 ± 0.14) × 10^3 M^{-1}, ΔH = -8.64 ± 0.05 kcal/mol, -TΔS = 3.25 ± 0.05 kcal/mol).

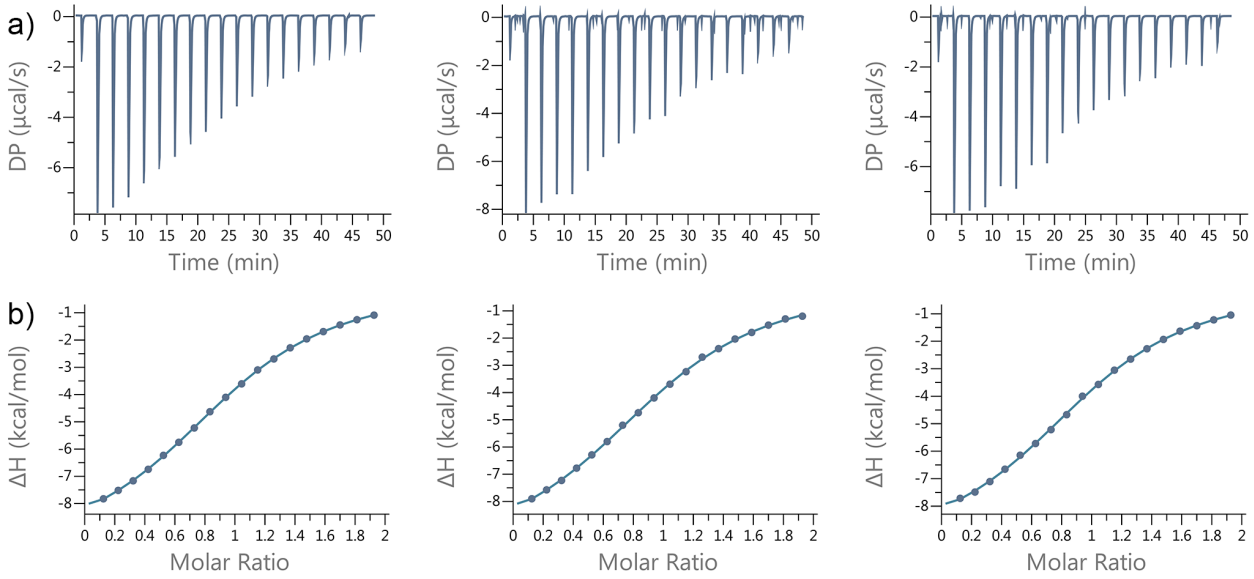

Figure S31. a) Plots of DP vs time from the direct titration of **P6MQ** (500 µM) with serotonin (5.00 mM) in PBS buffer (pH 7.4); b) plots of ΔH as a function of guest:host molar ratio. The solid lines represent the best non-linear fit of the data to a 1:1 binding model (trplicate averages: K_a = (9.04 ± 0.26) × 10^3 M^{-1}, ΔH = -9.88 ± 0.11 kcal/mol, -TΔS = 4.47 ± 0.011 kcal/mol).

Details of the *In Vivo* efficacy studies.

Animals. Sprague−Dawley rats were obtained from Chongqing Medical University. All animal-related procedures were approved by the Institutional Animal Care and Use Committees at Chongqing Medical University (Approval number: IACUC-CQMU-2022-0025). The experiments involving animals were conducted at Chongqing Medical University and strictly followed the Animal Management Rules of the Ministry of Health of the People's Republic of China (No. 55, 2001) and the guidelines for the Care and Use of Laboratory Animals of the Chongqing Medical University.

***In vivo* efficacy studies.** *In vivo* efficacy studies were conducted using the methods previously reported with slight modifications.[1] Male and female rats weighing between 220 g and 320 g were anesthetized with 5% isoflurane and maintained with 1% isoflurane before endotracheal intubation. To maintain the normal body temperature (37.0 ± 1 °C), a thermostatic heating plate was used. Drug administration was done through the left jugular vein, and the right femoral nerve was stimulated using two subcutaneous electrodes supramaximally. The stimulation was set and maintained at 1 Hz for a minimum of 10 minutes after the twitch height stabilized. Mechanical ventilation was initiated prior to administering the NMBAs, and the reversal agents or saline were administered 30 seconds later. The rats were observed for at least 20 minutes, and the time to TOF recovery (90%) was recorded.

Drugs. Roc (3.5 mg kg^{-1}, 5.74 μmol/kg), Vec (0.7 mg kg^{-1}, 1.1 μmol/kg), **P6MQ** (5.74 μmol/kg for roc; 1.1 μmol/kg for vec) and sugammadex (5.74 μmol/kg for roc; 1.1 μmol/kg for vec) were diluted in 0.5 mL 0.9% saline.

Statistical Analysis. Statistical analysis was conducted using unpaired two-tailed Student's t-test with SPSS Statistics 18.0 software. Error bars depict means and standard deviation. P values of ≤0.05 were considered significant, * $p < 0.05$; ** $p < 0.01$.

Reference (*In Vivo* Efficacy).

1) F. Haerter, J. C. Simons, U. Foerster, I. Moreno Duarte, D. Diaz-Gil, S. Ganapati, K. Eikermann-Haerter, C. Ayata, B. Zhang, M. Blobner, L. Isaacs, M. Eikermann, *Anesthesiology*, 2015, **123**, 1337-1349. DOI: 10.1097/ALN.0000000000000868.