Supporting Information

Polycationic calix[8]arenes able to recognize and neutralize heparin

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Spectral data of compounds **1a** and **2a**

Octa-lys-octa-*O*-propoxycalix[8]arene trifluoroacetic salt (**1a)**. ^{1}H NMR δ_H (400 MHz, DMF-d_6, 297 K) 0.78 (t, J = 7.2 Hz, 24 H, CH_3), 1.59 (m, 32 H, 2xCH_2), 1.79 (m, 16 H, CH_2), 2.02 (m, 16 H, CH_2), 3.03 (t, J = 6.5 Hz, 16 H, OCH_2), 3.53 (m, 16 H, $CH_2NH_3^+$), 3.98 (bs, 16 H, $ArCH_2Ar$), 4.23 (bt, J = 6.2 Hz, 8 H, CH), 7.45 (s, 16 H, ArH), 8.42 (bs, 24H, NH_3^+), 8.78 (bs, 24H, NH_3^+), 10.61 (s, 8H, NH). ES-MS calcd for $C_{128}H_{201}O_{16}N_{24}$ 2332.1672 ($M+H^+$), found 2331.2.

Octa-6-amino-hexanoic-octa-*O*-propoxycalix[8]arene trifluoroacetic salt (**2a)**. ^{1}H NMR δ_H (400 MHz, MeOH-d_4, 297 K) 0.80 (t, J = 7.2 Hz, 24 H, CH_3), 1.40 (m, 16 H, CH_2), 1.45 (m, 16 H, CH_2), 1.55 (m, 32 H, 2xCH_2), 2,31 (t, J = 7.1 Hz, 16 H, CH_2CO), 2,92 (t, J = 7.5 Hz, 16 H, OCH_2), 3.51 (bt, 16 H, $CH_2NH_3^+$), 3.96 (bs, 16 H, $ArCH_2Ar$), 7.22 (s, 16 H, ArH). ES-MS calcd for $C_{128}H_{193}O_{16}N_{16}$ 2211.0416 ($M+H^+$), found 2210.3.

Kinetic Model for Heparin–Polycations complexation

The multicharge equilibrium between heparin and polycations **1**, **2**, or protamine is too complex for an exact treatment of the kinetic equations. Practically, due to the non-specificity of the interactions, and the presence of cross-interactions between the species, each single ion pair formed in the overall mixture is characterized by different kinetic parameters. In order to simplify the problem, we can consider each ion pair formed independently from all other pairs, thus the overall recognition phenomenon can be represented as follows:

$$\sum_{\substack{m \\ m \le n}} A_m \; + \; \sum_{n} B_n \longrightarrow \sum_{m} P_m$$

The first term represents the sum of each single acid site, the second term represents the sum of each single basic site and the last term represents the sum of each single ion pair formed during the complexation. The kinetic expression for the single basic specie B_n can be represented by the equation (1):

$$-\frac{d[B_n]}{dt} = \sum_m k_{nm}[B_n][A_m] = [B_n]\left(k_{n1}[A_1] + k_{n2}[A_2] + ... + k_{nm}[A_m]\right) \qquad \text{[Eq. (1)]}$$

in which k_{nm} represents the rate constant for the interaction of B_n with A_m.

If we consider that the concentration of each single acid site in the medium has the same value, it can be represented by a value A' such as the Equation (2) is valid:

$$[A_1] = [A_2] = ... = [A_m] = [A'] \Rightarrow \sum_m [A_m] = m \cdot [A'] = [A]_{tot} \qquad \text{[Eq. (2)]}$$

where $[A]_{tot}$ represents the total concentration of acid groups.

The Equation (1) can be rearranged as Equation (3):

$$-\frac{d[B_n]}{dt} = \left(k_{n1} + k_{n2} + ... + k_{nm}\right)[B_n][A'] \qquad \text{[Eq. (3)]}$$

If we consider the medium value of the sum of the rate constants for the single basic specie B_n defined as in Equation (4):

$$\overline{k_n} = \frac{\sum_m k_{nm}}{m} \qquad \text{[Eq. (4)]}$$

then the kinetic expression for the single basic specie B_n [Equation (3)] can be rearranged as follows [Equation (5)]:

$$-\frac{d[B_n]}{dt} = \overline{k_n}[B_n][A]_{tot} \qquad \text{[Eq. (5)]}$$

The overall change in concentration over time of all the basic species will be equal to the sum of each single variation [Equation (6)]:

$$\sum_n -\frac{d[B_n]}{dt} = \sum_n \overline{k_n}[B_n][A]_{ot} = \left(\overline{k_1}[B_1] + \overline{k_2}[B_2] + ... + \overline{k_n}[B_n]\right)[A]_{tot} \qquad \text{[Eq. (6)]}$$

If we consider that the concentration of each single basic site in the medium has the same value, it can be represented by a value B' such as the Equation (7) is valid:

$$[B_1] = [B_2] = ... = [B_n] = [B'] \Rightarrow \sum_n [B_n] = n \cdot [B'] = [B]_{tot} \qquad \text{[Eq. (7)]}$$

where $[B]_{tot}$ represents the total concentration of basic groups.

The Equation (6) can be transformed into Equation (8):

$$-n \cdot \frac{d[B']}{dt} = \left(\overline{k_1} + \overline{k_2} + ... + \overline{k_n}\right)[B'][A]_{tot} \qquad \text{[Eq. (8)]}$$

If we consider the overall medium value for the sum of all the medium rate constants coming from Equation (4) defined as in Equation (9):

$$\bar{k} = \frac{\sum \overline{k_n}}{n} \qquad \text{[Eq. (9)]}$$

that Equation (8) can be rearranged to give the final kinetic expression [Equation (10)]:

$$-\frac{d[\mathrm{B}]_{tot}}{dt} = \bar{k}[\mathrm{B}]_{tot}[\mathrm{A}]_{tot} \qquad \text{[Eq. (10)]}$$

At this point it is possible to use the competitive titration method to calculate the ratio of the medium rate constants for the substances under investigation.[1]

Let us consider two competitive reactions that occur in the same solution following the kinetic model just described:

$$\mathrm{A} + \mathrm{B}_1 \rightarrow \mathrm{P}_1$$
$$\mathrm{A} + \mathrm{B}_2 \rightarrow \mathrm{P}_2$$

The Equation (11) represent the quotient of the rates of disappearing of compounds B_1 and B_2:

$$\frac{-\frac{d[\mathrm{B}_1]}{dt}}{-\frac{d[\mathrm{B}_2]}{dt}} = \frac{d[\mathrm{B}_1]}{d[\mathrm{B}_2]} = \frac{\overline{k_1}[\mathrm{A}][\mathrm{B}_1]}{\overline{k_2}[\mathrm{A}][\mathrm{B}_2]} = \frac{\overline{k_1}[\mathrm{B}_1]}{\overline{k_2}[\mathrm{B}_2]} \qquad \text{[Eq. (11)]}$$

Rearranging the equation(11):

$$\frac{d[\mathrm{B}_1]}{[\mathrm{B}_1]} = \frac{\overline{k_1}}{\overline{k_2}} \cdot \frac{d[\mathrm{B}_2]}{[\mathrm{B}_2]} \qquad \text{[Eq. (12)]}$$

The solution of this differential equation is the following logarithmic expression [Equation (13)]:

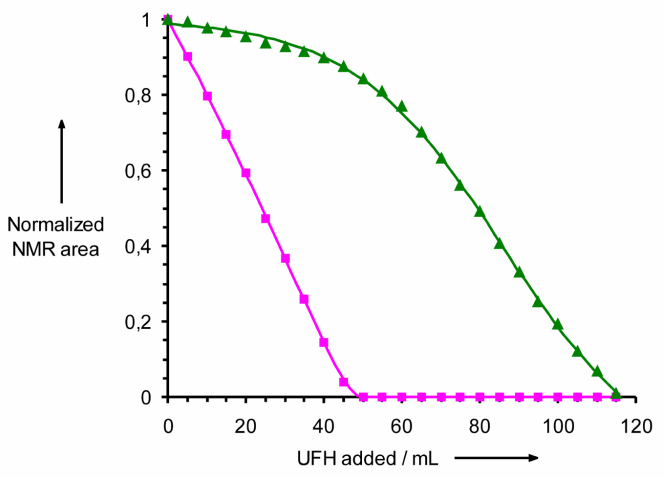
$$\ln\left(\frac{[\mathrm{B}_1]_n}{[\mathrm{B}_1]_0}\right) = \frac{\overline{k_1}}{\overline{k_2}} \cdot \ln\left(\frac{[\mathrm{B}_2]_n}{[\mathrm{B}_2]_0}\right) \qquad \text{[Eq. (13)]}$$

in which the terms $[B_1]_0$, $[B_1]_n$ and $[B_2]_0$, $[B_2]_n$ represent the concentrations of the species B_1 and B_2 after 0 (initial condition) and n additions of small amount of compound A.

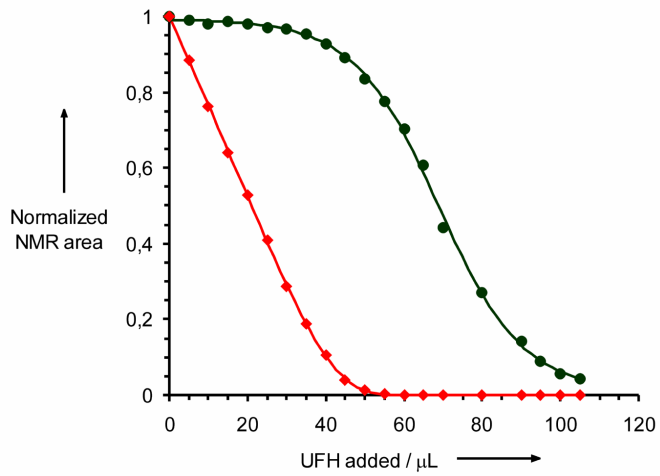
The ratio of the medium rate constants for the two competitive reactions is easily obtained [Equation (14)]:

$$\frac{\overline{k_1}}{\overline{k_2}} = \frac{\ln\left(\frac{[B_1]_n}{[B_1]_0}\right)}{\ln\left(\frac{[B_2]_n}{[B_2]_0}\right)} \qquad \text{[Eq. (14)]}$$

Competitive titrations

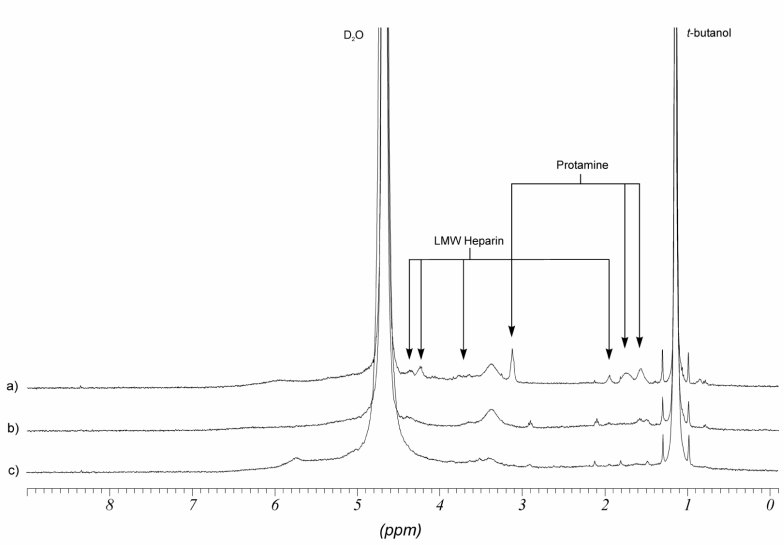


NMR competitive titration between **2a** (■) and protamine sulfate (▲). Small aliquots of UFH solution (20 mg mL^{-1}) were added to the NMR sample containing 500 µL PBS buffer (10 mM, pH 5.4 NaCl 150 mM), **2a** (2.25 mg), protamine sulfate (1.62 mg), and *t*-butanol (0.516 µL) as standard for NMR area calculation.



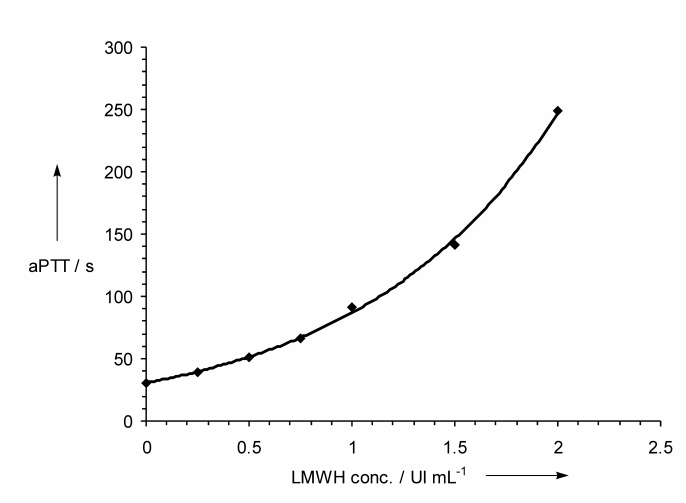
NMR competitive titration between **1a** (◆) and polylysine (●). Small aliquots of UFH solution (20 mg mL^{-1}) were added to the NMR sample containing 500 µL PBS buffer (10 mM, pH 7.2, NaCl 150 mM), **1a** (1.43 mg), polylysine (1.05 mg), and *t*-butanol (0.516 µL) as standard for NMR area calculation.

Low Molecular Weight Heparin (LMWH) NMR titration



NMR spectra (PBS buffer 10 mM pH 7.2, NaCl 150 mM, *t*-butanol 0.1 % v/v as standard for NMR area calculation) of the end point of LMW Heparin titration with a): Protamine, b): **1b,** c): **2b.** Only in the spectrum a) it is possible to see the contemporary presence of signals referred to LMWH and protamine, showing the inability of protamine to completely eliminate LMWH from the solution. The residual area relative to the heparin peak at δ=1.95 ppm is about 20% of the initial value.

Activated partial thromboplastin time (aPTT) calibration curve for LMWH.



(1) Espenson, J. H. Chemical Kinetics and Reaction Mechanisms–2nd ed., McGraw-Hill, Inc, 1995, p. 62.