First use of supramolecular recognition to extract and stabilize an enzymatic inhibitor of coagulation process

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Electronic Supplementary information (ESI)

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^{31}P NMR spectrometry of **1**:**4a** complex

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General experimental section

Unless otherwise stated, ^{1}H NMR, ^{13}C NMR, ^{31}P NMR spectra were recorded in deuterium solvent on a spectrometer Bruker AC 300, operating at 300, 75 and 121 MHz respectively. Chemical shifts are expressed in parts per million (ppm) and coupling constant in Hertz (Hz). The proton spectra are reported are reported as follows δ (multiplicity, coupling constant *J*, number of protons). Multiplicities are indicated by s (singlet), d (doublet), t (triplet), m (multiplet). IR Spectra were recorded on a Perkin-Elmer 16 PC FT-IR and expressed in cm^{-1}. Chemicals were used without further purification. All reactions were monitored by analytical thin-layer chromatography using pre-coated silica gel plates. Visualization was accomplished by UV-light (254 nm). Flash chromatography was performed using silica gel (mesh 230-400). For the complexation and filtration experiments, the light radiations were avoided.

^{31}P NMR spectrometry BABCH.2HCl 1:4a complex in D_2O

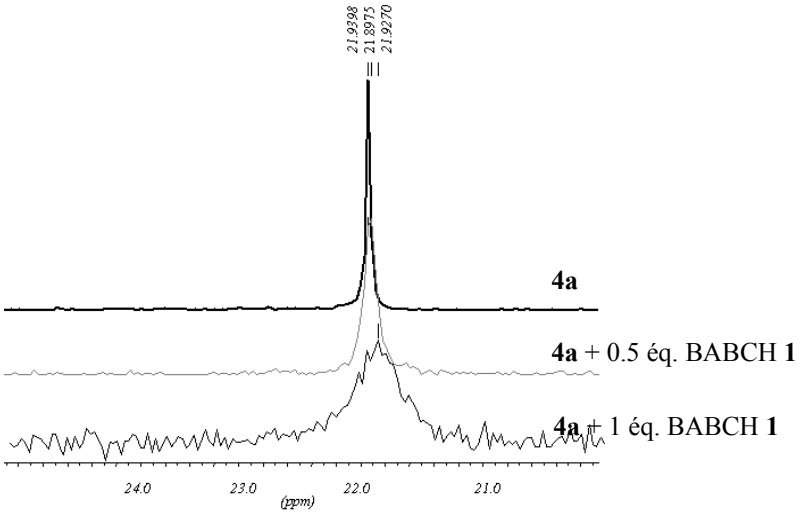


Figure 2 Phosphorus signal evolution after addition of the receptor **4a** on mixture of isomers BABCH.2HCl **1**

Isothermal Titration Calorimetry

Calorimetric titrations were performed using a MicroCal MCS-ITC instrument (MicroCal Inc., Northampton MA, U. S. A.), at 30 °C. Each BABCH.2HCl isomer **1a**, **1b** and **1c** (at 0.1–1 mM concentration) was titrated by a solution of bis-monoesterphosphonate of lithium **4a** at 10-20 fold larger concentration. Solution concentrations were precisely determined by the UV absorbance in water (ε_{45}, $\lambda_{299\ nm}$= 274.34 $L.cm^{-1}.mol^{-1}$; ε_{88b}, $\lambda_{246\ nm}$=61.574 $L.cm^{-1}.mol^{-1}$) in the linearity range of the Lambert-Bear law. The injection volume was adjusted in order to obtain a high sensitivity in

signal detection and a reliable number of titration points. The first injection of 2 υl was ignored in the final data analysis. Integration of the peaks corresponding to each injection and correction for the baseline were done using Origin-based software provided by the manufacturer. The dilution enthalpy of **4b**, determined by injecting **4b** into the buffer (Figure 3a), was deduced from the binding isotherms as a base line. Fitting of the data to an interaction model results in the stoichiometry (n), equilibrium binding constant (K_a) and enthalpy of complex formation (ΔH). The experimental data allow calculation of the free energy change (ΔG) and of the entropy term ($T\Delta S$) according to the classical thermodynamic formula:

$\Delta G=-RT\times \ln K_a$

$\Delta G=\Delta H-T\Delta S$

where R is the universal gas constant and T is the absolute temperature.

The concentrations of the solutions were:

Heat of the dilution de **4b**: [**4b**]=19.6 mM

Titration of (*Z,Z*)-BABCH.2HCl **1a**: [**1a**]=0.12 mM, [**4b**]=11.9 mM

Titration of (*E,Z*)-BABCH.2HCl **1b**: [**1b**]=1.02 mM, [**4b**]=19.9 mM

Titration of (*E,E*)-BABCH.2HCl **1c**: [**1c**]= 1.07 mM, [**4b**]=20.4 mM

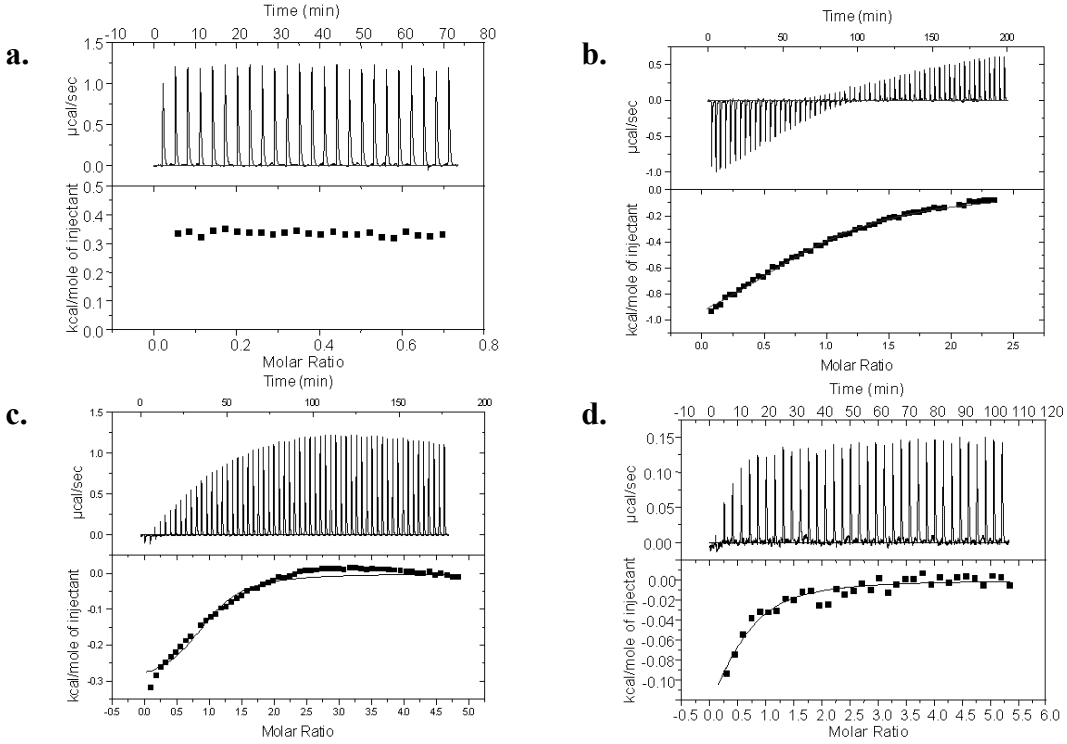
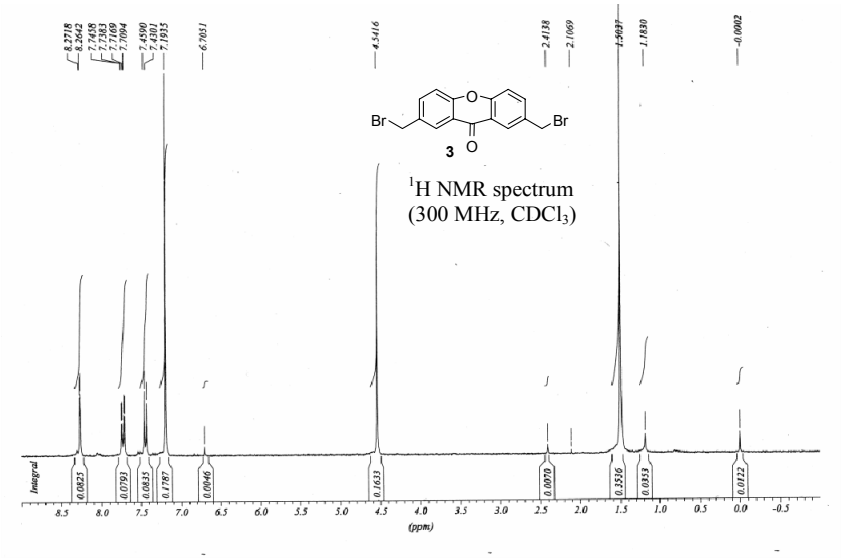
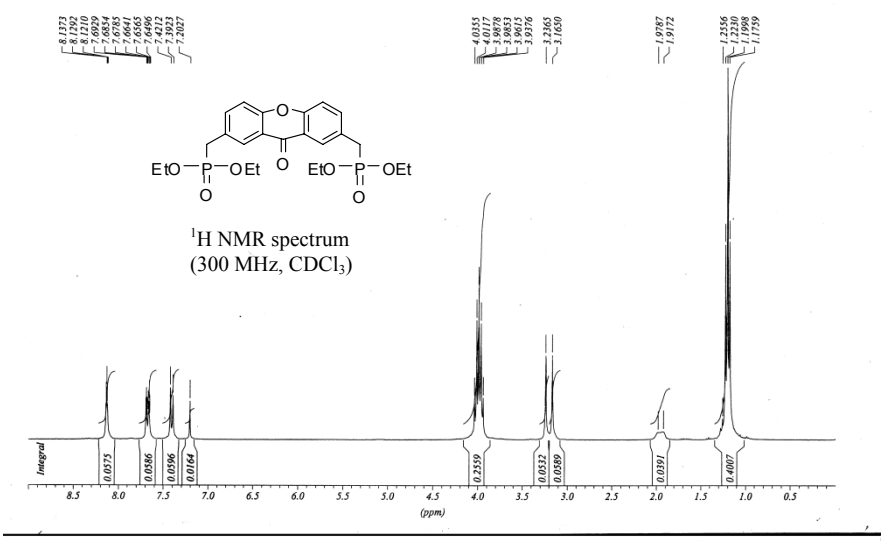


Figure 3 Representative ITC titrations of the bis-monoesterphosphonate of lithium **4b** into BABCH.2HCl **1** isomers, obtained at 30 °C. Upper panel: the raw data; lower panel: the binding (dilution) isotherm. (a) dilution of **4b** (19.6 mM) into buffer; (b) **4b** (20.4 mM) into (*E,E*)-BABCH.2HCl **1c** (1.07 mM); (c) **4b** (19.9 mM) into *(E,Z)*-BABCH.2HCl **1b** (1.02 mM); (d) **4b** (11.9 mM) into *(Z,Z)*-BABCH.2HCl **1a** (0.12 mM).

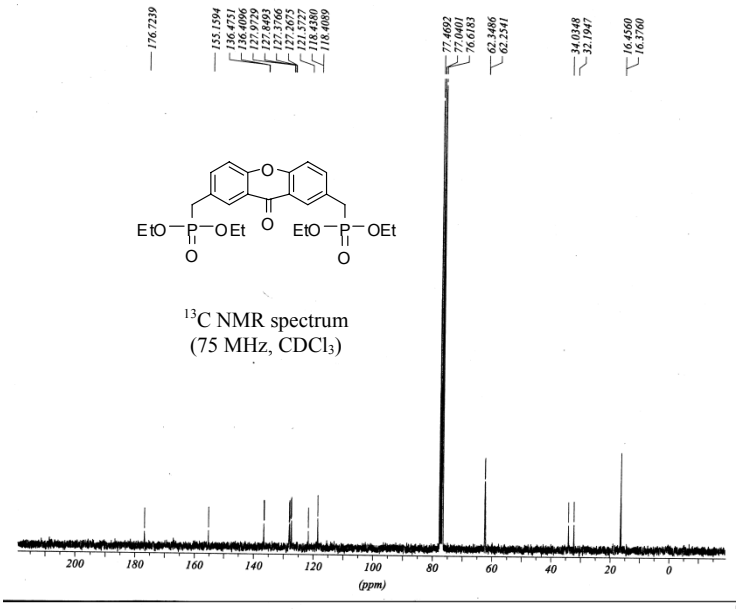
Supplementary Material (ESI)



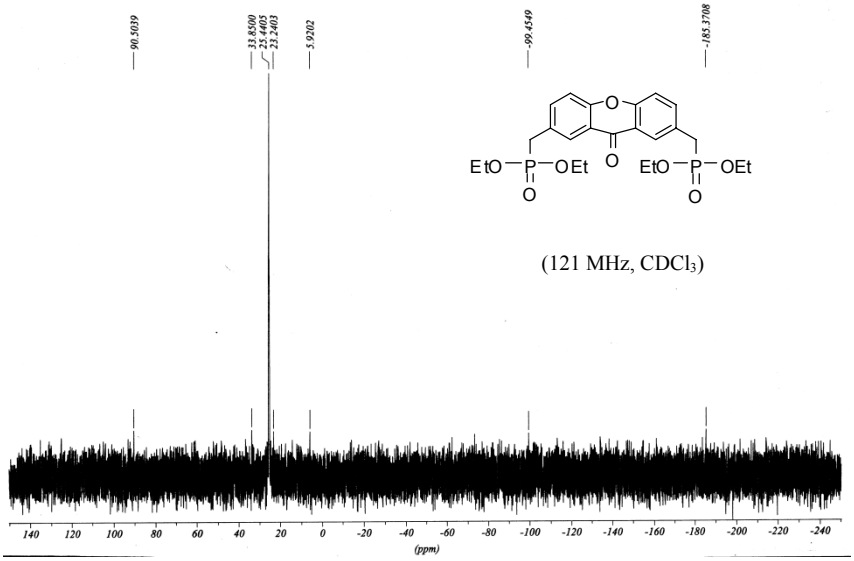
^{1}H NMR spectrum
(300 MHz, $CDCl_3$)



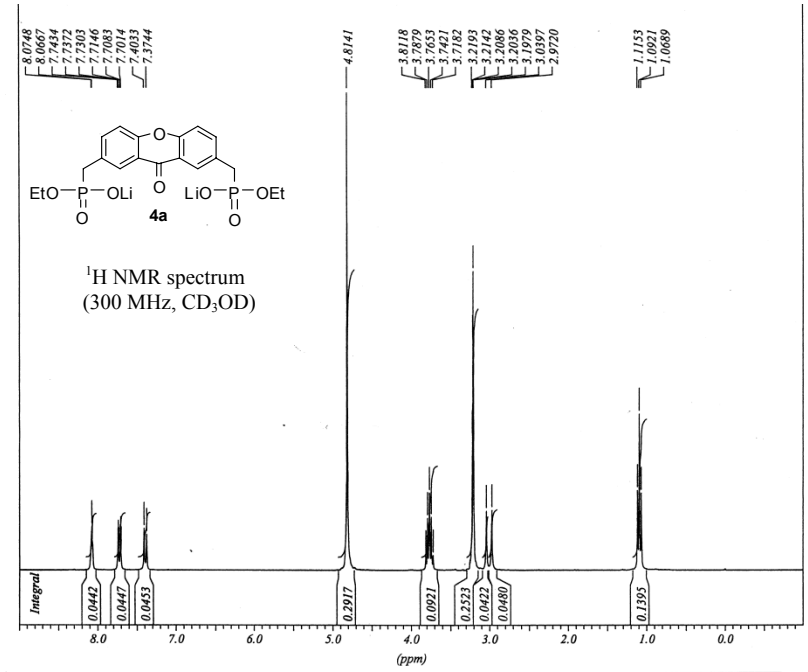
^{1}H NMR spectrum
(300 MHz, $CDCl_3$)



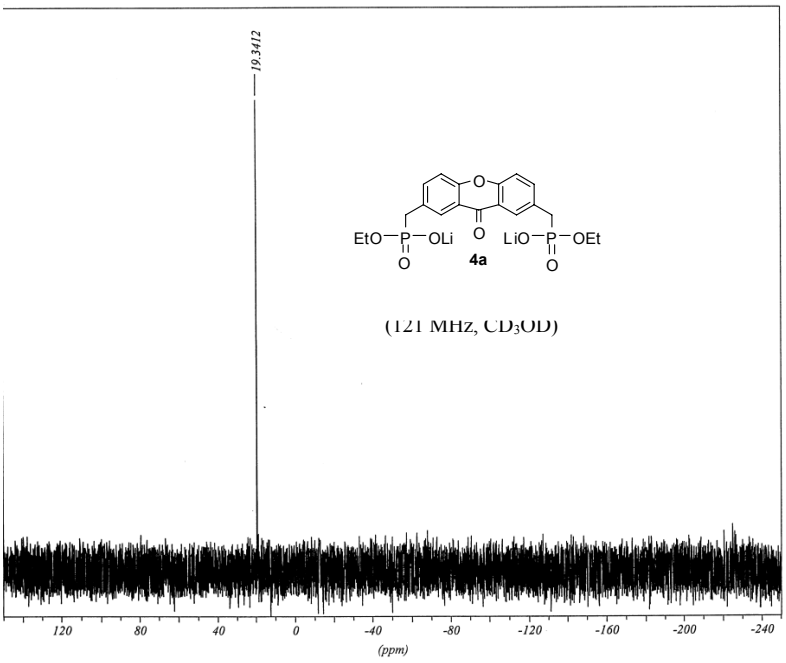
^{13}C NMR spectrum
(75 MHz, $CDCl_3$)



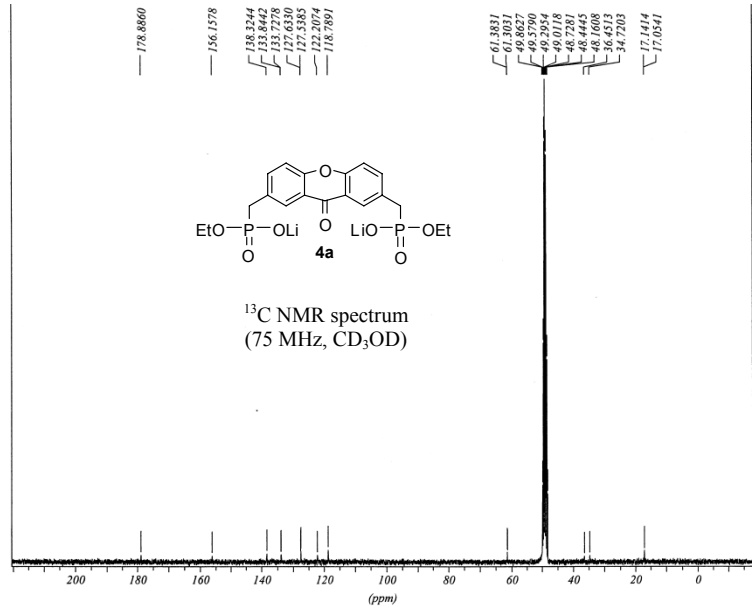
(121 MHz, $CDCl_3$)



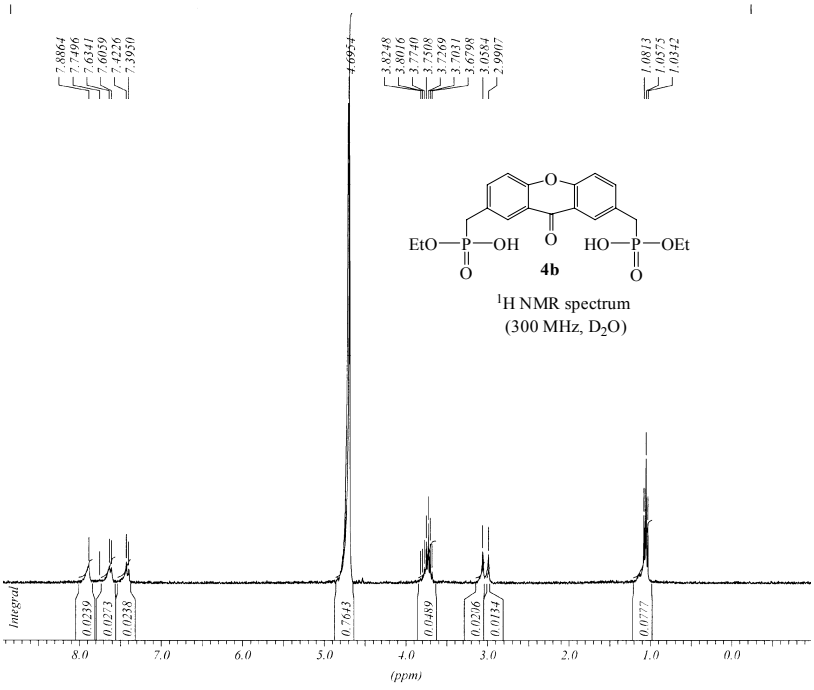
^{1}H NMR spectrum
(300 MHz, CD_3OD)



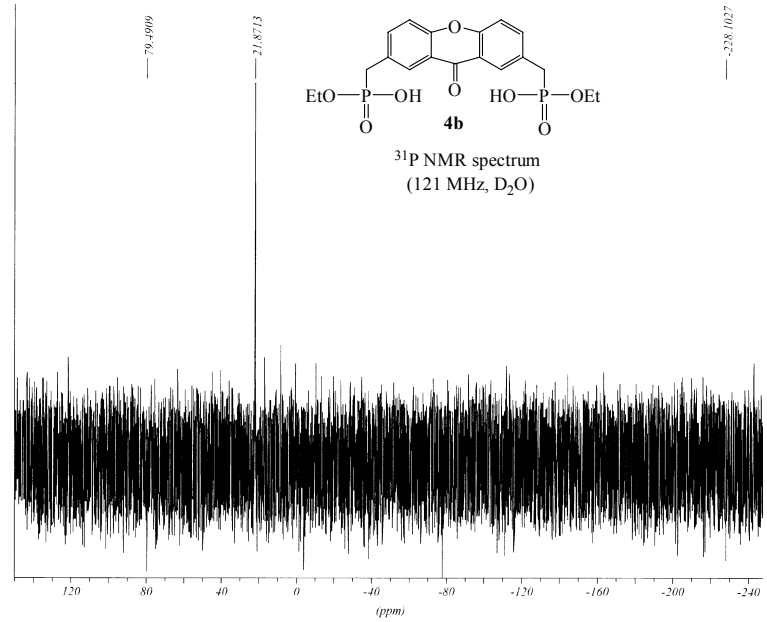
(121 MHz, CD_3OD)



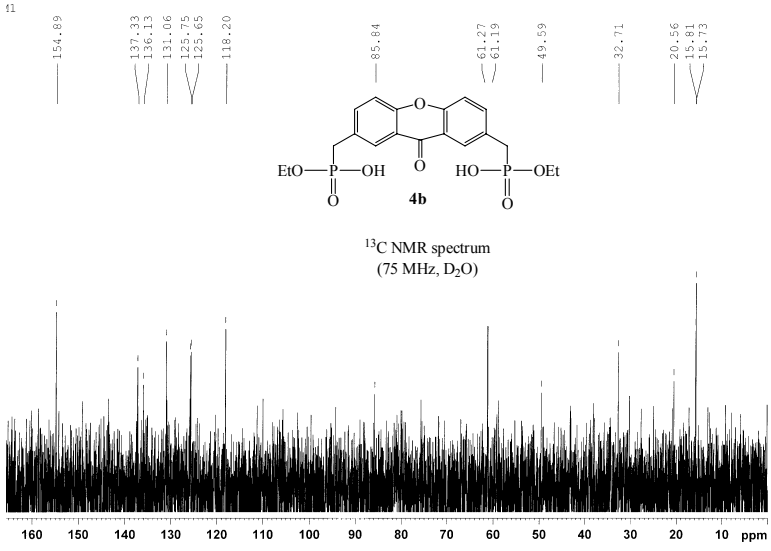
^{13}C NMR spectrum
(75 MHz, CD_3OD)



^{1}H NMR spectrum
(300 MHz, D_2O)



^{31}P NMR spectrum
(121 MHz, D_2O)



^{13}C NMR spectrum
(75 MHz, D_2O)