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Influence of cyclic and acyclic cucurbiturils on the degradation pathways of VX

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CONTENT

Degradation of VX: Experimental Procedures

CAUTION

Chemical warfare nerve agents are regulated by the chemical weapons convention and related national regulation. These compounds are highly toxic and have to be handled with extreme care and appropriate safety precautions. All experiments that involved the use of nerve agents were performed in approved fume hoods at the Bundeswehr Institute of Pharmacology and Toxicology in Munich. Remaining nerve agents and contaminated material were decontaminated with 30% sodium hypochlorite prior to disposal.

General details. The organophosphate VX was made available by the German Ministry of Defence (Bonn, Germany). The purity of VX was checked by ^{1}H and ^{31}P NMR spectroscopy. The major other component, amounting to a content of 13%, was EMPA. This intrinsic EMPA content was considered in the kinetic measurements (see below).

The acyclic cucurbituril **1**[1] was prepared by using the reported procedures. CB[7] **2** was purchased from Sigma-Aldrich and D_2O from Deutero. The commercial **2** contains H_2O and HCl and the exact composition was therefore determined prior to preparing the stock solutions by elemental analysis.

For the NMR spectroscopic analyses, a Bruker Nanobay Ascend 400 spectrometer equipped with a BBO probe head was used. The NMR data were recorded at 298 K throughout and were analysed by using the Topspin software 3.5pl4. All ^{31}P NMR spectra are proton-decoupled (PCPD) spectra.

Mass scans of the solutions were recorded with an Agilent 6420 Triple Quadrupole mass spectrometer with dynamic multiple reaction monitoring (Agilent Technologies Inc., Santa Clara, CA, USA). Aliquots (5 µL) were removed from the NMR tube and diluted with distilled water in a ratio of 1:1000. The resulting solutions were injected by using a Harvard Apparatus model 11 Plus syringe (diameter: 4.61 mm) pump (Harvard Bioscience Inc., Holliston, MA, USA) at a flow rate of 20 µL/min. Full scan spectra (*m/z* 50-1700) were acquired in +ESI and –ESI mode in separate runs. The following parameters were used: positive ion mode, ion source temperature 250 °C, nitrogen drying gas flow, 7.0 L/min, nitrogen drying gas pressure, 30 psi, capillary voltage, 5500 V; negative ion mode: ion source temperature 300 °C, nitrogen drying gas flow, 3.0 L/min, nitrogen drying gas

pressure, 15 psi, capillary voltage, -4000 V. Acquisition and analysis of mass scans was performed by using the Agilent MassHunter Workstation Software Version B.04.01.

Qualitative Binding Studies. Stock solutions of VX (10 mM) and the receptor (4 mM) were prepared in D_2O or in phosphate buffer in D_2O (0.1 M, pD 7.81). The samples were set up by mixing the VX stock solution (60 μL) and the receptor stock solution (300 μL) and adding D_2O or phosphate buffer (240 μL). The samples were stored at room temperature and their ^{1}H and ^{31}P NMR spectra were recorded immediately after mixing and then every day for a period of up to 7 d. For recording the spectra of the pure components, the VX stock solution (60 μL) or the receptor stock solution (300 μL) was diluted with D_2O or phosphate buffer (0.1 M, pD 7.81) to obtain a total volume of 600 μL.

NMR Titrations. Stock solutions were prepared of VX (6.0 mM) and of **1** (3.6 mM) in D_2O buffered to pD 7.81 with phosphate buffer (0.1 M). Eleven NMR tubes were prepared by adding increasing amounts of the receptor stock solution to each tube (0-500 μL in steps of 50 μL). Phosphate buffer was added to obtain a total volume of 500 μL in each tube. The measurements were performed by adding the VX stock solution (100 μL) immediately before the measurement, thoroughly shaking the respective tube, and recording the ^{1}H NMR spectrum. In this way, the VX concentration was maintained at 1.0 mM throughout the titration, whereas the concentration of **1** varied between 0 and 3.0 molar equiv. The changes in the chemical shifts of the P-CH_3, ethyl-CH_3, and *iso*-propyl-CH_3 signals were monitored and used to generate binding isotherms that were globally fitted to a 1:1 complex equilibrium by using HypNMR (Protonic Software). An analogous titration was performed by using D_2O instead of phosphate buffer.

Time-Dependent Measurements. Stock solutions of VX (10 mM) and the receptor (6.25 mM) were prepared in D_2O. The samples were prepared by mixing the VX stock solution (120 μL) and the receptor stock solution (360 μL) and adding D_2O (120 μL). The samples were stored at room temperature and their ^{1}H and ^{31}P NMR spectra were recorded immediately after mixing and then every day for a period of up to 7 d. VX degradation in the absence of the receptor was followed by diluting the VX stock solution (120 μL) with D_2O (480 μL). The measurements in the presence of NaOH were performed by adding a stock solution of NaOH (120 μL, 10 mM for 1 molar equiv and

20 mM for 2 molar equiv) to the mixture of the VX and receptor stock solution instead of D_2O. The relative amounts of EMPT, VX, EA-2192, and EMPA were determined by integrating the corresponding signals in the ^{31}P NMR spectra at 75.7, 61.8, 42.2, and 26.4 ppm, respectively. The initial EMPA content of the VX used in the measurements was considered during the data treatment so that the curves depicted in Fig. 3 of the article and Figures S3 and S9 only illustrate EMPA formation beyond the amount originally present.

Degradation of VX: Figures

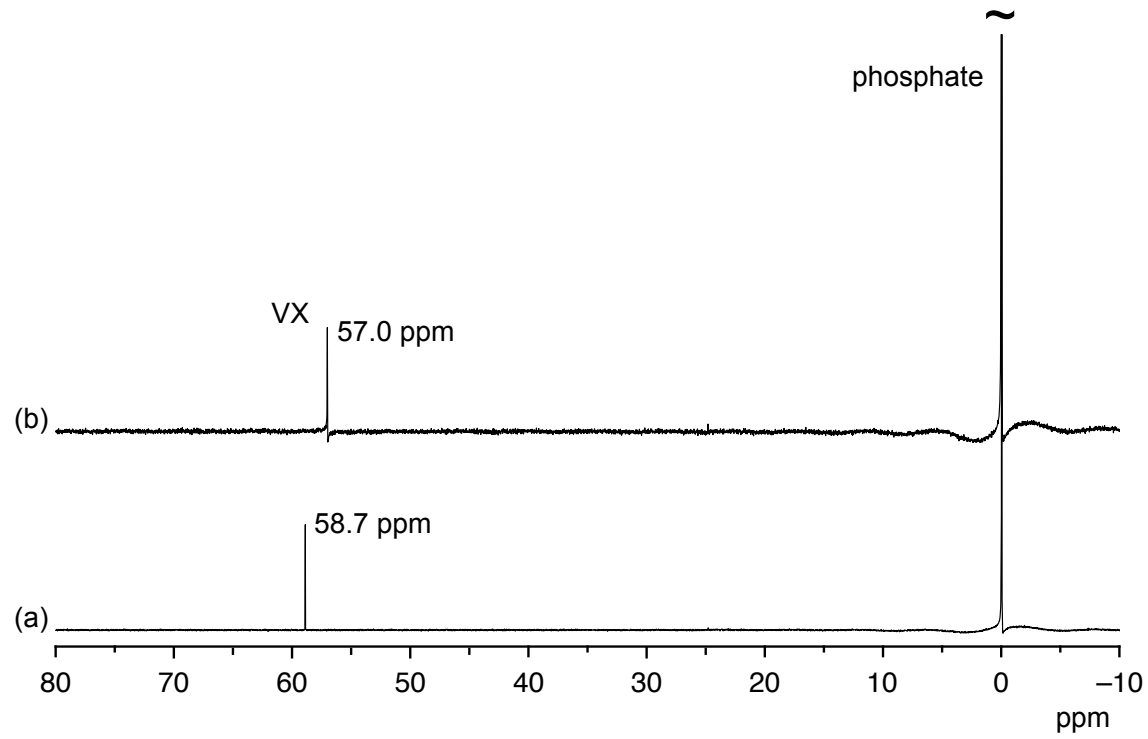
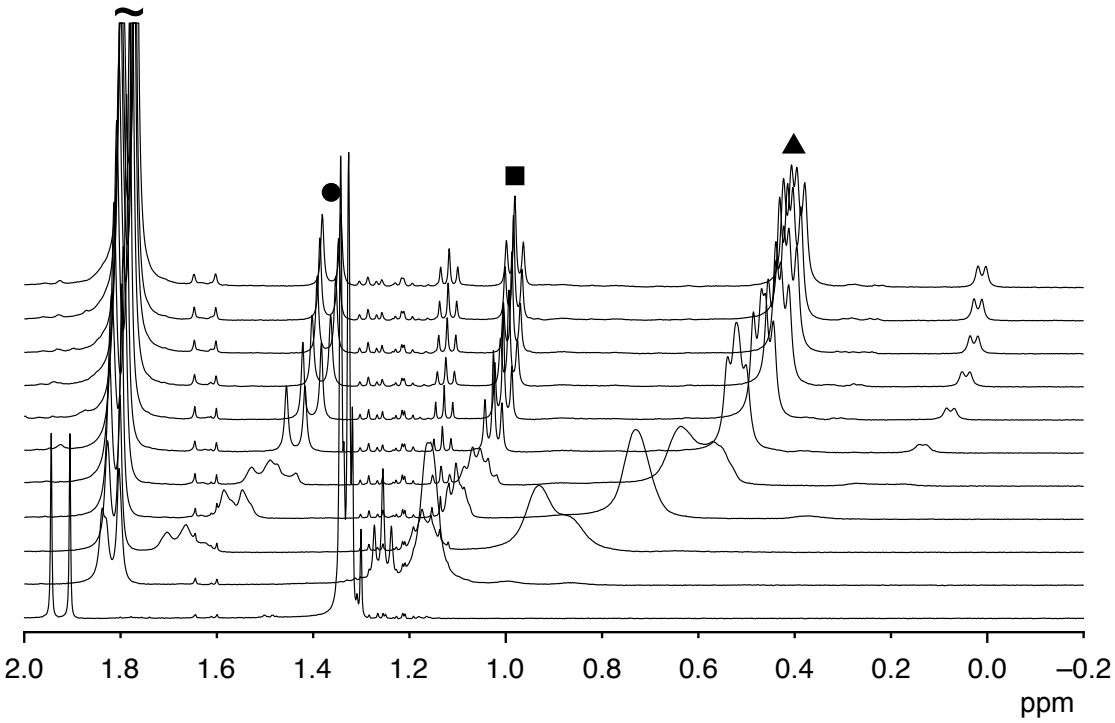


Fig. S1: ^{31}P NMR spectra of VX (1 mM) in the absence (a) and in the presence (b) of 2 molar equiv of **1** in 0.1 M phosphate buffer in D_2O at pD 7.81. The presence of **1** causes an upfield shift of the VX signal.

(a)



(b)

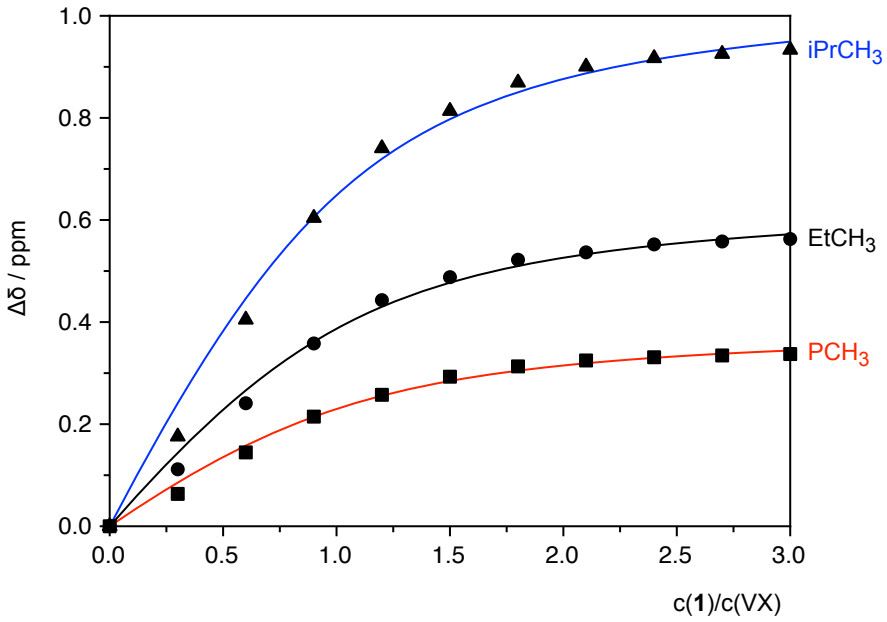


Figure S2: ^{1}H NMR spectra of VX in phosphate-buffered D_2O (0.1 M, pD 7.81) containing between 0 (bottom) and 3 molar equiv (top) of **1** in steps of 0.3 molar equiv (a). The shifts of the P-CH_3 (circles), ethyl-CH_3 (squares), and *iso*-propyl-CH_3 signals (triangles) were used to construct the binding isotherms in (b) that afforded the stability constant of the complex by fitting them to a 1:1 equilibrium. The dots in the graphs represent the experimental results and the lines the fitted isotherms. The stability constant log K_a amounts to 3.7 with an estimated error of 15%.

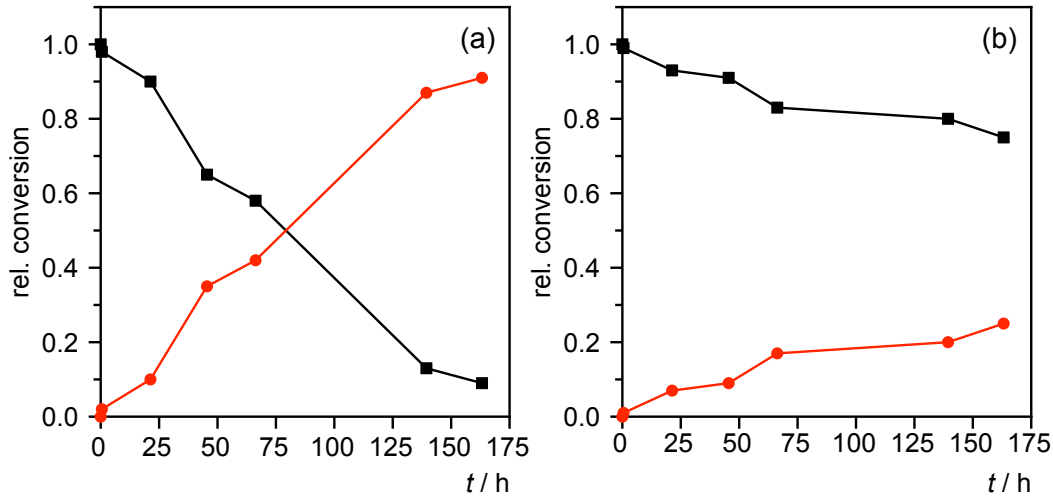


Fig. S3: Time-dependence of the formation of EMPA (red circles) when VX (black squares) (1 mM) was incubated in 0.1 M phosphate buffer in D_2O (a) and in the same solvent but in the presence of 2 molar equiv of **1** (b). The relative amounts of the different compounds were estimated by integrating their signals in the ^{31}P NMR spectra of the samples recorded every day over a period of a week. EMPT and EA-2192 were not formed under these conditions.

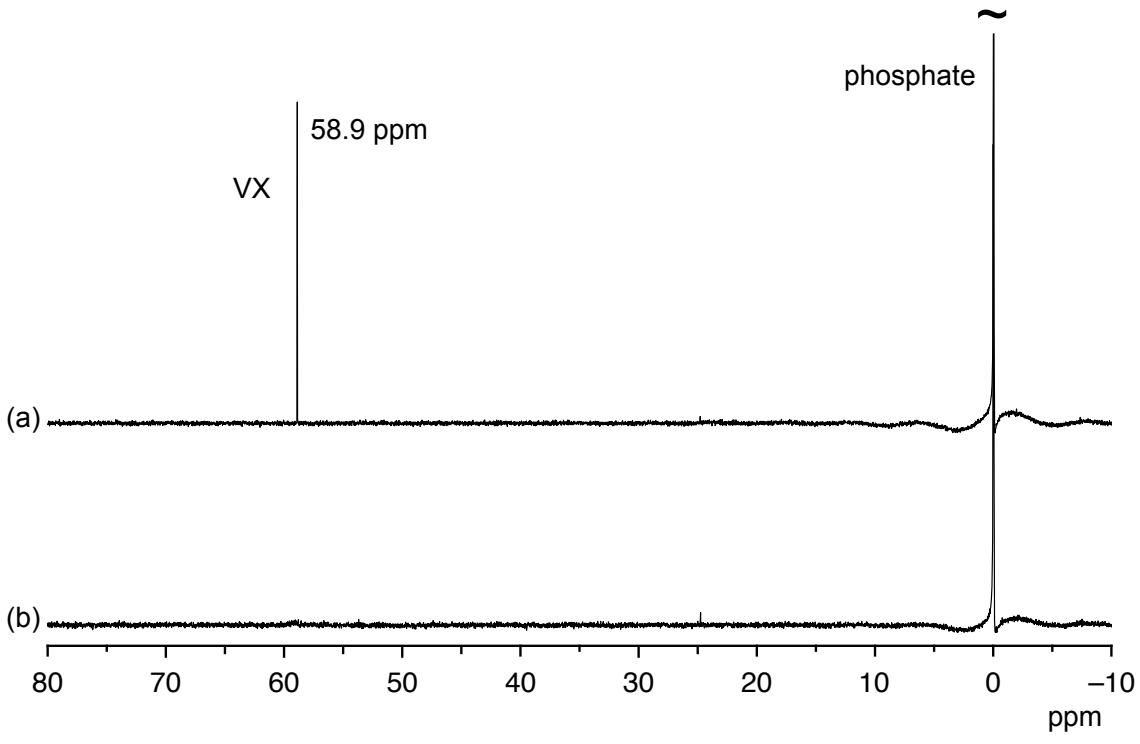


Fig. S4a: ^{31}P NMR spectra of VX (1 mM) in the absence (a) and in the presence (b) of 2 molar equiv of **2** in 0.1 M phosphate buffer in D_2O at pD 7.81. The presence of **2** causes the VX signal to broaden to such an extent that it disappears within the baseline.

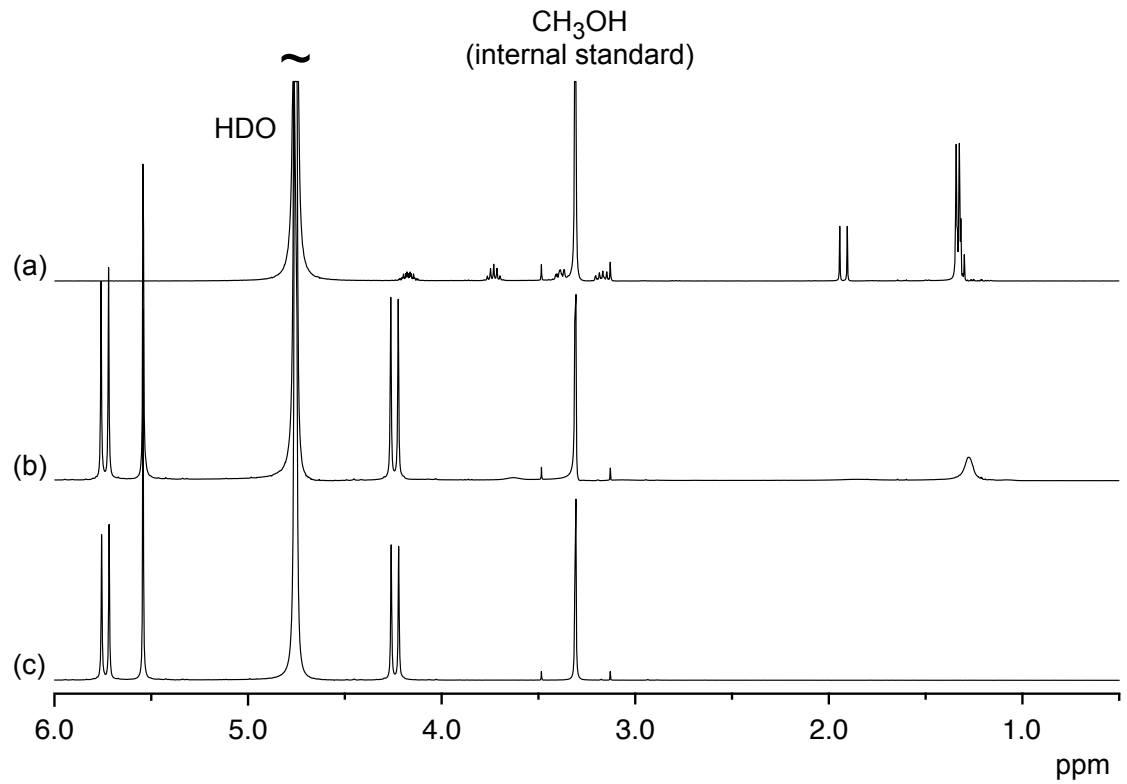


Fig. S4b: ^{1}H NMR spectra of VX (1 mM) in the absence (a) and in the presence (b) of 2 molar equiv of **2** in 0.1 M phosphate buffer in D_2O at pD 7.81. The trace in (c) shows the spectrum of **2**. The presence of **2** causes a pronounced broadening of the VX signals.

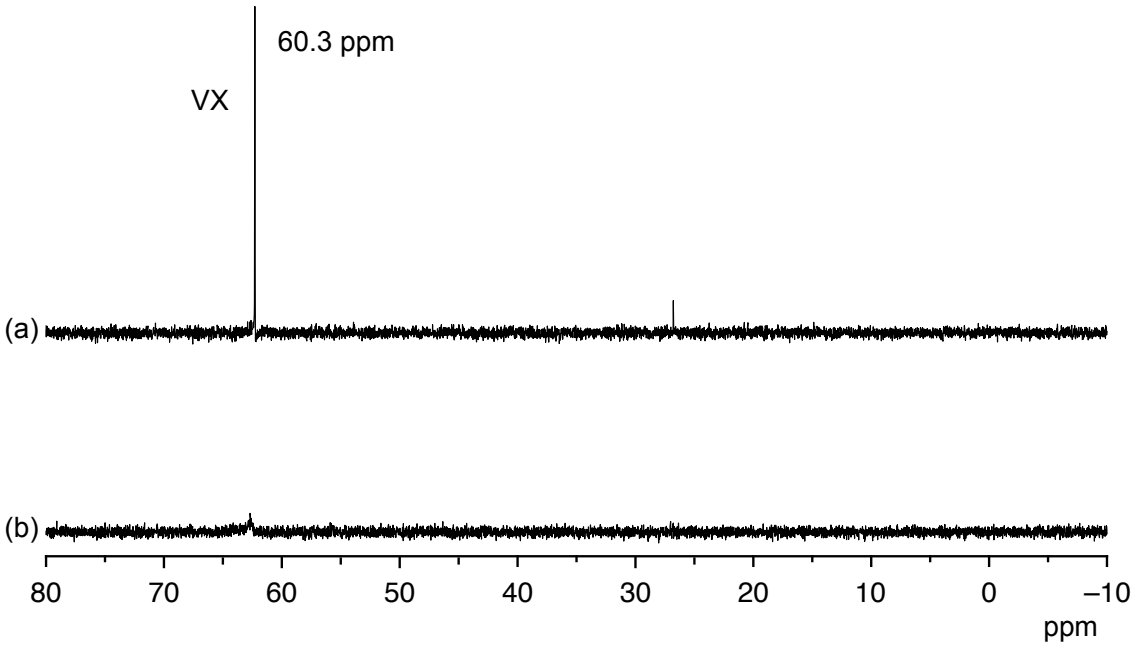


Fig. S4c: ^{31}P NMR spectra of VX (1 mM) in the absence (a) and in the presence (b) of 2 molar equiv of **2** in D_2O. The presence of **2** causes the VX signal to broaden to such an extent that it disappears within the baseline.

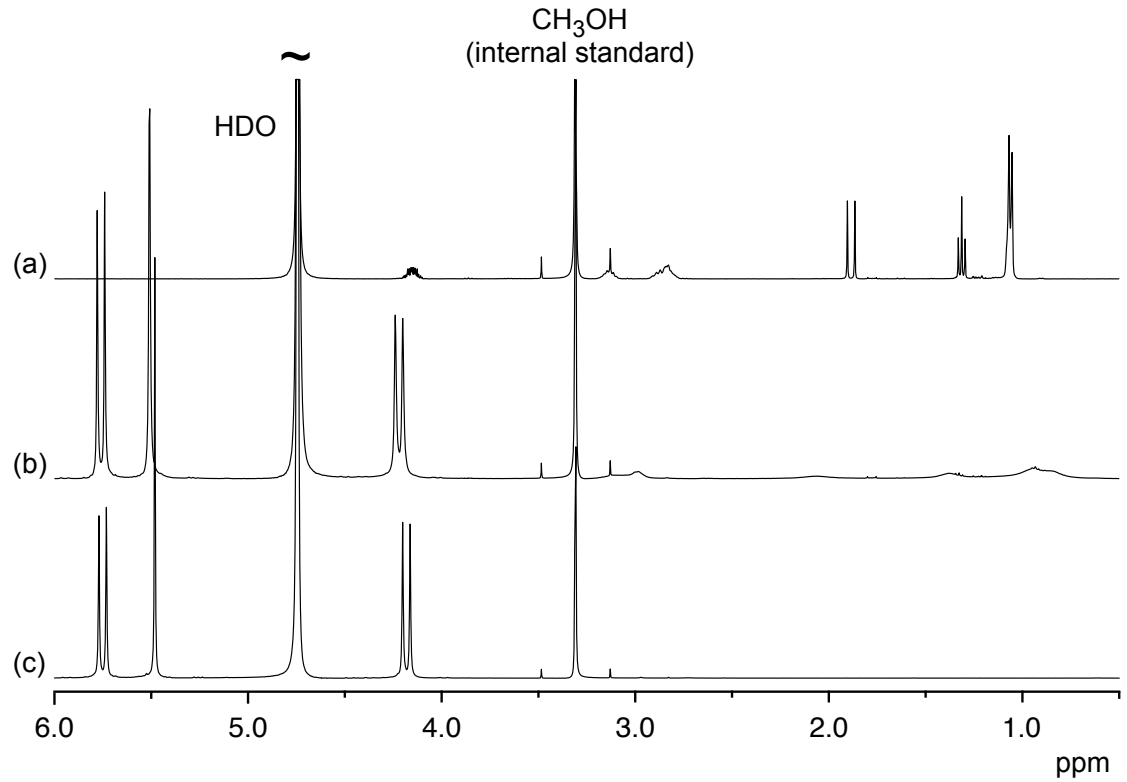


Fig. S4d: ^{1}H NMR spectra of VX (1 mM) in the absence (a) and in the presence (b) of 2 molar equiv of **2** in D_2O. The trace in (c) shows the spectrum of **2**. The presence of **2** causes a pronounced broadening of the VX signals.

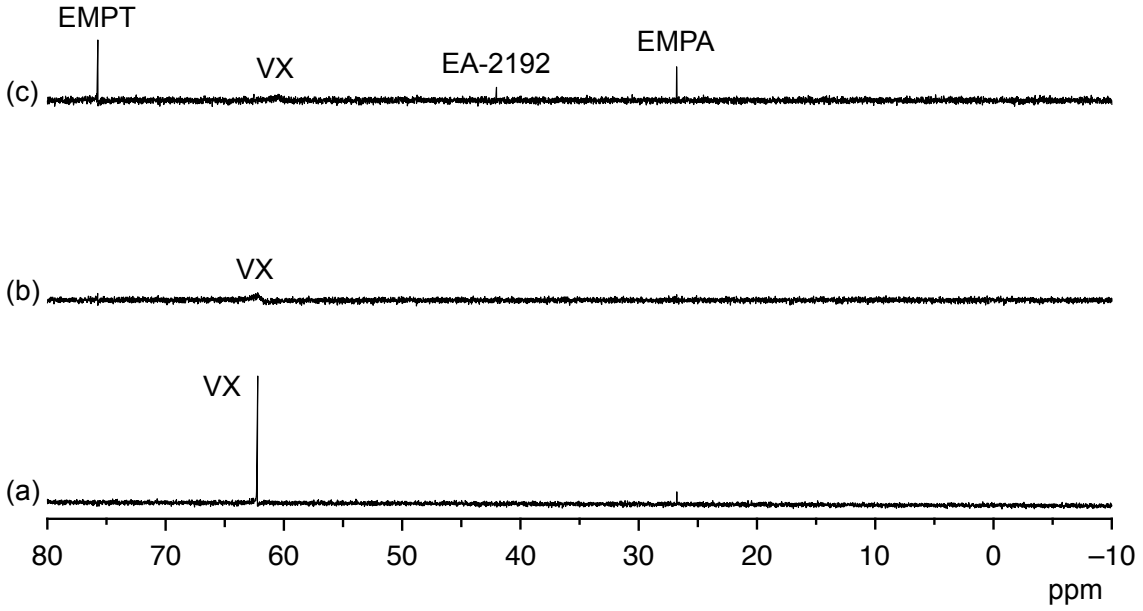
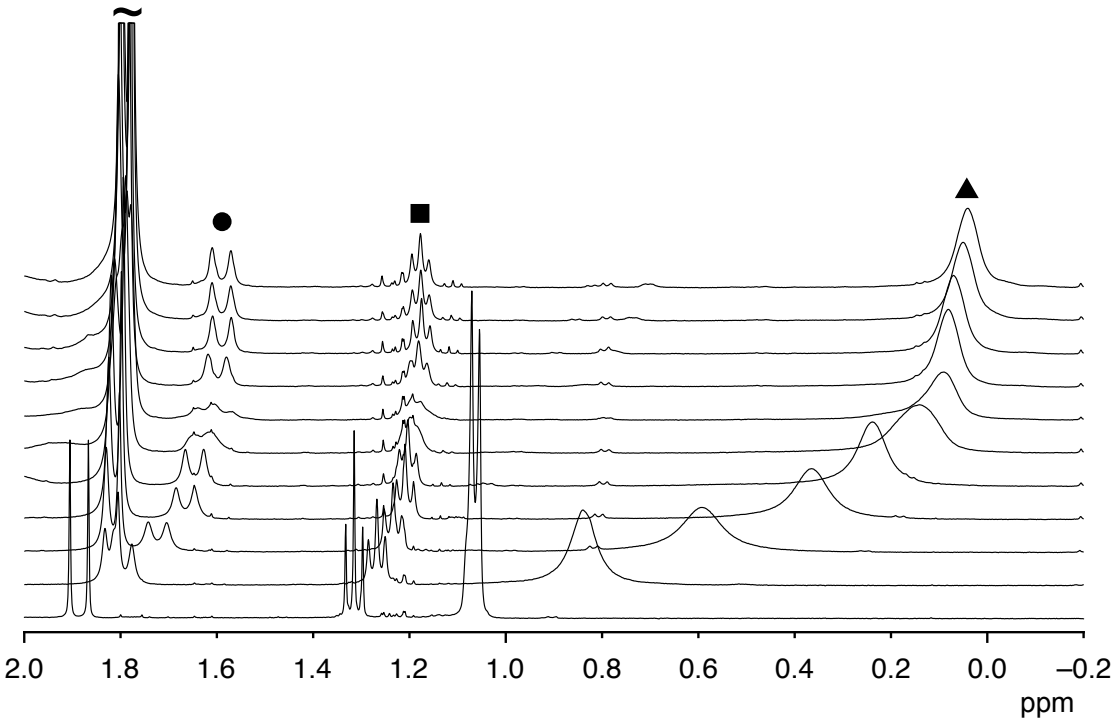


Fig. S5: ^{31}P NMR spectra of VX (1 mM) in the absence (a) and in the presence of 2 molar equiv of **1** in unbuffered D_2O 30 min after mixing (b). The trace in (c) shows the spectrum resulting of the mixture of VX (1 mM) and **1** (2 mM) in D_2O after 48 h.

(a)



(b)

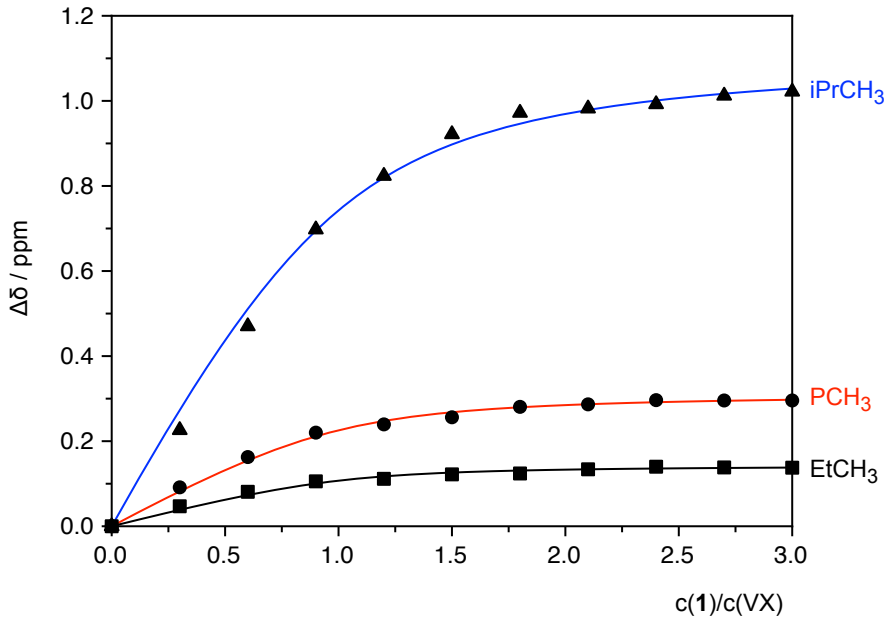


Figure S6: ^{1}H NMR spectra of VX in D_2O containing between 0 (bottom) and 3 molar equiv (top) of **1** in steps of 0.3 molar equiv (a). The shifts of the P-CH_3 (circles), ethyl-CH_3 (squares), and *iso*-propyl-CH_3 signals (triangles) were used to construct the binding isotherms in (b) that afforded the stability constant of the complex by fitting them to a 1:1 equilibrium. The dots in the graphs represent the experimental results and the lines the fitted isotherms. The stability constant log K_a amounts to 3.9 with an estimated error of 15%.

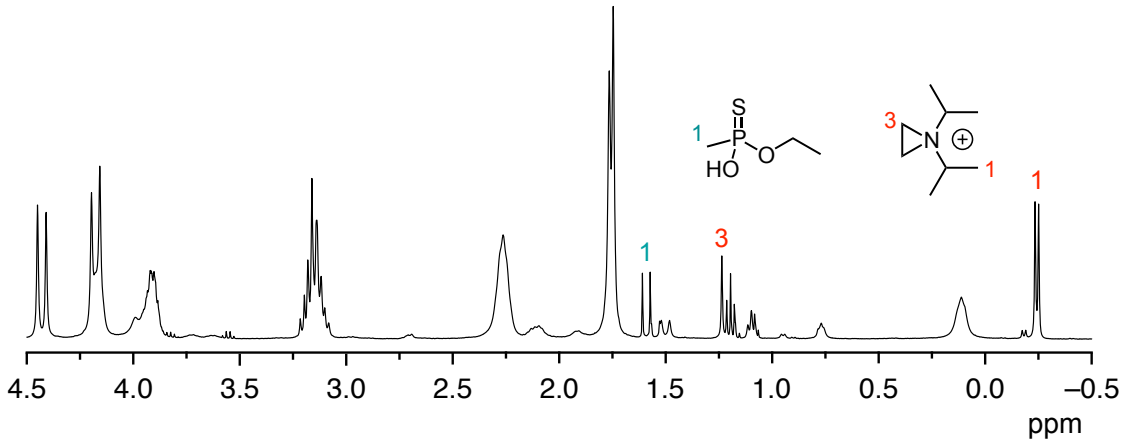
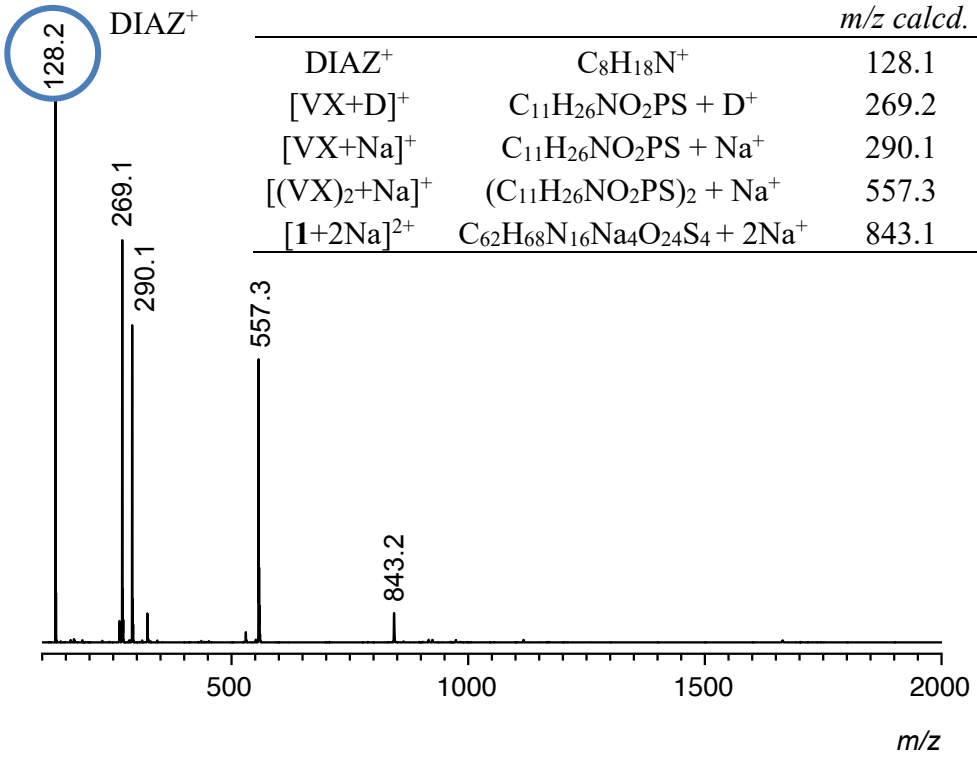
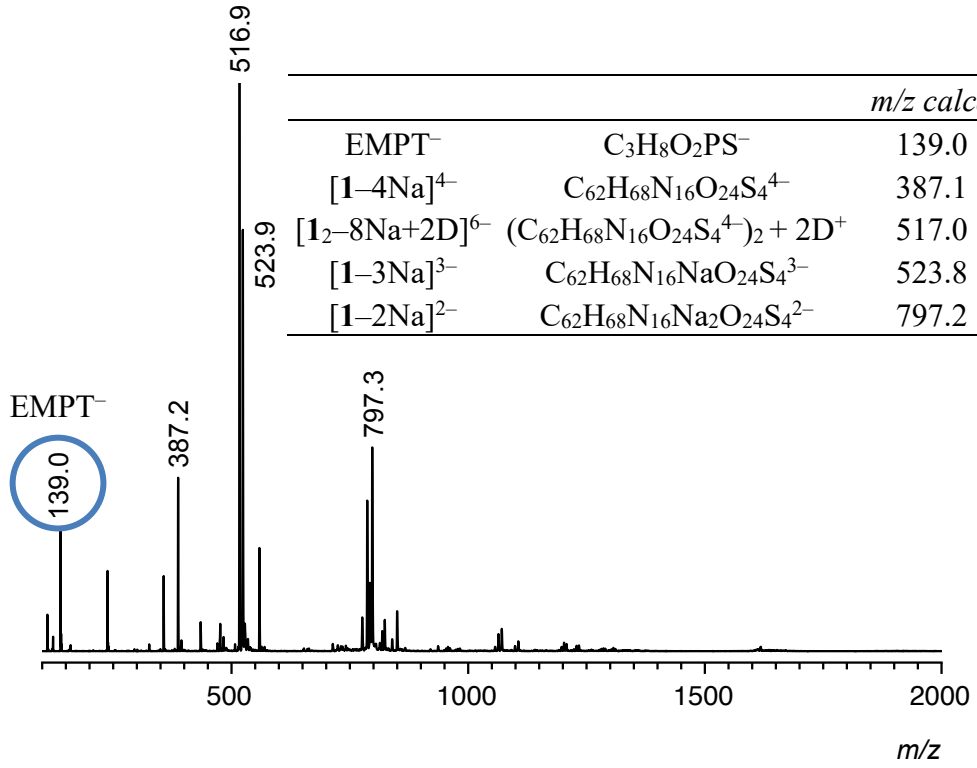


Fig. S7: ^{1}H NMR spectra of VX (1 mM) in the region 4.5 to –0.5 ppm in the presence of 2 molar equiv of **1** in unbuffered D_2O after 48 h. Prominent signals assigned to DIAZ and EMPT are marked.



		m/z calcd.	*m/z exp.*
$DIAZ^+$	$C_8H_{18}N^+$	128.1	128.2
$[VX+D]^+$	$C_{11}H_{26}NO_2PS + D^+$	269.2	269.1
$[VX+Na]^+$	$C_{11}H_{26}NO_2PS + Na^+$	290.1	290.1
$[(VX)_2+Na]^+$	$(C_{11}H_{26}NO_2PS)_2 + Na^+$	557.3	557.3
$[\mathbf{1}+2Na]^{2+}$	$C_{62}H_{68}N_{16}Na_4O_{24}S_4 + 2Na^+$	843.1	843.2



		m/z calcd.	*m/z exp.*
$EMPT^-$	$C_3H_8O_2PS^-$	139.0	139.0
$[\mathbf{1}–4Na]^{4-}$	$C_{62}H_{68}N_{16}O_{24}S_4^{4-}$	387.1	387.2
$[\mathbf{1}_2–8Na+2D]^{6-}$	$(C_{62}H_{68}N_{16}O_{24}S_4^{4-})_2 + 2D^+$	517.0	516.9
$[\mathbf{1}–3Na]^{3-}$	$C_{62}H_{68}N_{16}NaO_{24}S_4^{3-}$	523.8	523.9
$[\mathbf{1}–2Na]^{2-}$	$C_{62}H_{68}N_{16}Na_2O_{24}S_4^{2-}$	797.2	797.3

Fig. S8: ESI-mass spectra of a solution of VX (2 mM) containing 1.5 molar equiv of **1** in unbuffered D_2O recorded after an incubation period of 16 h at room temperature in the positive (top) and negative (bottom) mode. The signals observed in these spectra are assigned in the tables to ions with the corresponding *m/z* ratios.

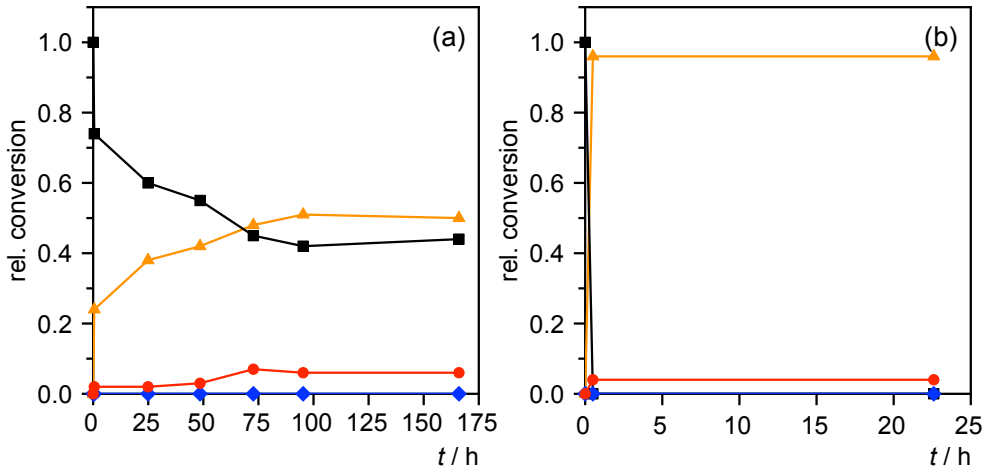


Fig. S9: Time-dependence of the formation of EMPT (orange triangles), EA-2192 (blue diamonds), and EMPA (red circles) when VX (black squares) (2 mM) was incubated with **2** (3.75 mM) in D_2O in the presence of 1 molar equiv (a) and 2 molar equiv of NaOH (b). The graphs illustrating the product distribution when VX is incubated under the same conditions in the absence of **2** are shown in Fig. 3d and Fig. 3f of the main article. Note the change in the scale in (b).

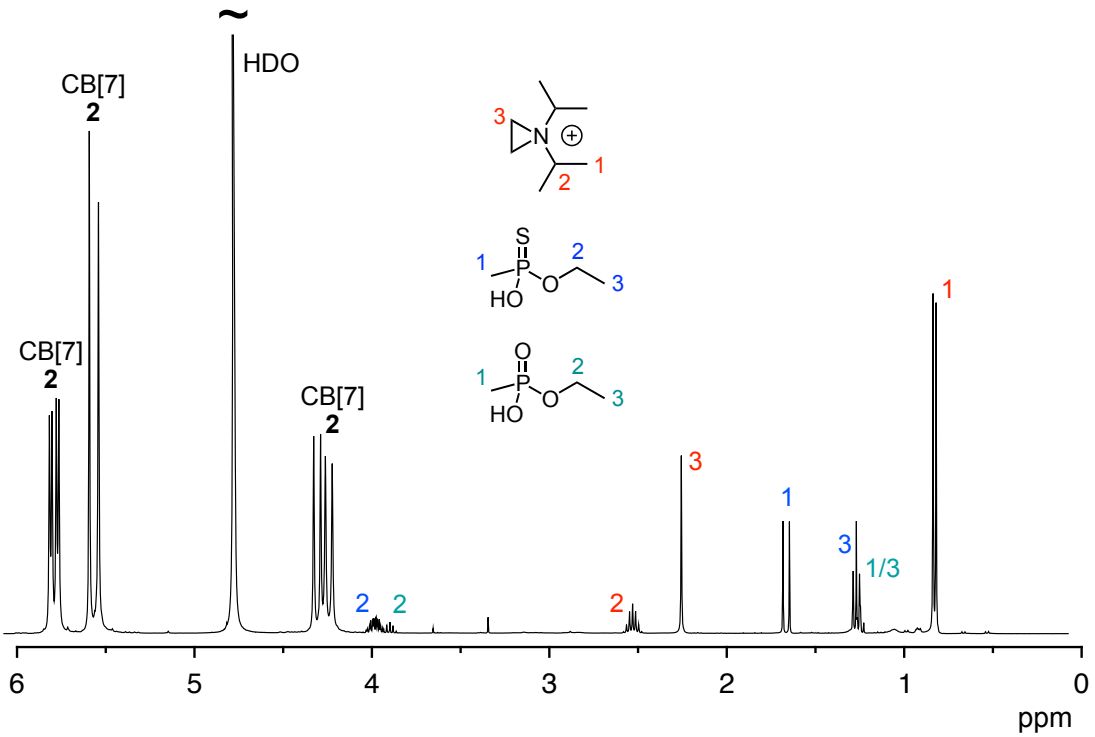


Fig. S10: ^{1}H NMR spectrum of the product mixture resulting after incubating a solution of VX (1 mM) in the presence of 2 molar equiv of **2** and 2 molar equiv of NaOH in unbuffered D_2O for 24 h.

The peaks in this spectrum that can be assigned to the protons of the reaction products DIAZ, EMPT and EMPA are marked. Additional signals of 2-(diisopropylamino)ethanethiol or the corresponding disulfide are small and partly hidden under the signals of **2**. Compound **2** shows twice the number of signals than normally likely because the complexation of DIAZ is slow on the NMR time-scale. One signal set thus represents the molar equivalent of **2** that is involved in complex formation whereas the other signal set represents uncomplexed **2**. The integrals of the signals of **2** in relation to those of DIAZ support this interpretation.

Degradation of VX Mimics: Experimental Procedures

General details. The substrates 2-chloroethyldiisopropylamine DIACl and 2-chloroethyldiethyl-amine DEACl were purchased from Sigma-Aldrich as their respective hydrochloride salts. The hydrochloride of *S*-(2-(diisopropylamino)ethyl) ethanethioate DIASAc was prepared by synthesising the corresponding amine as described,[2] dissolving it in diethyl ether and treating the solution with HCl. The precipitate was recrystallized from dichloromethane/diethylether, washed with diethyl ether, and dried. The acyclic cucurbituril **1**[1] and tetrasulfonatocalix[4]arene **3**[3] were prepared by using the reported procedures. CB[7] **2** was purchased from Sigma-Aldrich and Strem Chemicals, and D_2O from Deutero. The exact composition of the commercial **2** was determined prior to preparing the stock solutions by elemental analysis.

For the NMR spectroscopic analyses, a Bruker AVANCE 600 spectrometer was used. Data analysis was performed by using Topspin. The kinetic measurements were started with the command xau multizg to perform 50 experiments with 256 scans per experiment. These measurements were performed by using the zg30 pulse program with the specifications: size of fid 65536, dummy scans 0, spectral width 20.6225, acquisition time 2.6476543.

Determination of pD values. For the pH/pD measurements, stock solutions of DIASAc (20 mM), NaOH (20 mM), **1** (7.50 mM) and **2** (6.25 mM) were prepared in D_2O. These solutions were mixed in the ratios specified in Table S1, stirred over a period of 20 min, and their pD* values were then determined by using a calibrated pH electrode. The obtained pD* values were converted into pD values as described in the literature.[4] Since DIASAc was used as the hydrochloride, the solutions containing 1 molar equiv of NAOH correlate with VX solutions prepared in the absence of NaOH, while those containing 2 and 3 molar equiv of NaOH relate to the VX solutions with, respectively, 1 and 2 molar equiv of NaOH.

Table S1: Compositions of the solutions used of the pD measurements, concentrations of the individual components, measured pD* values and calculated pD values.

	DIASAc		NaOH		receptor		D_2O	V_{tot} /	pD*	pD
	V / mL	c / mM	V / mL	c / mM	V / mL	c / mM	V / mL	mL		
no receptor	0.3	2	0	0	0	0	2.7	3	7.01	7.45
	0.3	2	0.3	2	0	0	2.4	3	9.41	9.85
	0.3	2	0.6	4	0	0	2.1	3	10.29	10.73
	0.3	2	0.9	6	0	0	1.8	3	11.47	11.91
1	0.3	2	0	0	1.5	3.75	1.2	3	7.70	8.14
	0.3	2	0.3	2	1.5	3.75	0.9	3	11.25	11.69
	0.3	2	0.6	4	1.5	3.75	0.6	3	11.61	12.05
	0.3	2	0.9	6	1.5	3.75	0.3	3	11.80	12.24
2	0.3	2	0	0	1.8	3.75	0.9	3	2.24	2.68
	0.3	2	0.3	2	1.8	3.75	0.6	3	2.83	3.27
	0.3	2	0.6	4	1.8	3.75	0.3	3	9.57	10.01
	0.3	2	0.9	6	1.8	3.75	0	3	10.91	11.35

Qualitative Binding Studies. For the qualitative binding studies involving DIACl and DIAZ, the same ^{1}H NMR spectra were used that also served in the kinetic measurements. The influence of the receptors on the chemical shifts of the DIACl protons was evaluated by comparing the spectrum of DIACl in the absence of a receptor with the spectra obtained in the presence of the receptors right at the beginning of the kinetic measurements. In the case of **2**, even the first spectrum contained substantial amounts of DIAZ, while minor amounts of this product were initially observed in the presence of the other receptors. The complexation-induced shifts the receptors induced on the DIAZ signals were determined by using the respective final spectra of the kinetic measurements.

For the binding study involving DIASAc, stock solutions in phosphate buffer (pD 6.10, 67.2 mM) of the receptors (4 mM) and DIASAc (10 mM) were prepared. The spectra of the mixtures were recorded by diluting a receptor stock solution (300 µL) with buffer (288 µL), adding the stock solution of DIASAc (12 µL), shaking, and recording the ^{1}H NMR spectrum immediately afterwards. For the

spectrum of pure DIASAc, the DIASAc stock solution (12 µL) was mixed with phosphate buffer (588 µL). All NMR spectra were referenced to the signal methanol (δ = 3.34 ppm) that was present in the buffer as an internal standard.

ITC Titrations. The ITC titrations were carried out in the same phosphate buffer that was used in the kinetic measurements. DIACl and DIASAc were used as hydrochloride salts. These salts and receptors **1**, **2** and **3** were weighed by using an analytical precision balance and dissolved in exact volumes of the phosphate buffer to obtain the desired concentrations. A stock solution of DIACl (10 mM) was prepared and diluted with phosphate buffer to give a final concentration of 0.3 mM. This solution was stored for 17 h at room temperature under stirring. Afterwards, an ^{1}H NMR spectrum of the solution was recorded to confirm that the conversion of DIACl into DIAZ was complete. For the ITC titrations with DIASAc, a stock solution with a concentration of 10 mM was initially prepared and subsequently diluted to obtain a final concentration of 0.3 mM.

The solutions of DIAZ or DIASAc were filled in the cell of the calorimeter and titrated with 5 mM solutions of the receptors in the same phosphate buffer that was also used to prepare the guest solutions. The measurements were carried out at 25 °C by using a reference power of 20 µCal/s, a stirring speed of 307 rpm, a filter period of 2 s, an injection delay of 8 s, and a spacing time of 180 s. For the first injection, an injection volume of 2 µl was used and for injections 2 to 38 an injection volume of 6 µl. Automated baseline assignment and peak integration of raw thermograms were accomplished by singular value decomposition and peak-shape analysis using NITPIC.[5]

The titrations with the receptor in the cell and DIASAc in the syringe were performed analogously. The individual receptor concentrations were: 0.5 mM for **1**, 4 mM for **2**, and 1 mM for **3**. The concentration of the DIASAc solution was twenty times higher than the respective receptor solution. Because of large heats developed during these titrations, the reference power had to be set to 34.8 µCal/s. All other parameters and data treatment were the same as in the other measurements.

Kinetic Measurements. The NMR spectroscopic kinetic measurement were performed in phosphate buffer, which was prepared as follows: a stock solution of KH_2PO_4 (0.91 g, 6.67 mmol) in H_2O (100 mL) and another stock solution of Na_2HPO_4 (1.19 g, 8.36 mmol) in H_2O (100 mL) were prepared.

The KH_2PO_4 solution (97 mL) and the Na_2HPO_4 (3 mL) were mixed and the pH adjusted to 5.65 by adding one or the other stock solution if necessary. The solvent was evaporated to a volume of 10 mL and the remaining solution freeze-dried. The residue was dissolved in D_2O and methanol (1 mL) was added as internal standard. The determination of the pD according to the described method yielded a value of 6.10.[4]

For the measurements, stock solutions of the receptor (4 mM, ca. 1 mL) and of the substrate (10 mM, ca. 2 mL) were prepared in phosphate buffer. The stock solution of the substrate was prepared immediately before the measurement. The stock solution of the receptor (300 µL) was diluted with buffer (288 µL). The stock solution of the substrate (12 µL) was added, the resulting mixture thoroughly shaken. The final concentrations thus amounted to 0.2 mM of the substrate and 2 mM of the receptor. The first ^{1}H NMR spectrum was recorded immediately after mixing and further NMR spectra were measured in time intervals of 15.6 min for an overall duration of 13 h.

Product formation was followed by integrating the growing signal of the diisopropyl methyl signal of DIAZ and relating this integral to the sum of the integrals of the diisopropyl methyl signals of DIAZ and the substrate. The results were plotted *vs.* the time, and the rate constant of the reaction determined by fitting the resulting curve to the rate equation of a first-order reaction by using Origin 2019b (OriginLab).

Computations. All computations were performed by using Spartan 18 (Wavefunction, Inc.). The structures of DIACl, DIAZ, and the protonated and unprotonated forms of VX were obtained by performing a Monte Carlo conformational search (equilibrium conformer search) by using the MMFF force field implemented in Spartan 18 followed by a DFT (B3LYP/6-31G*) optimisation of the thus obtained structures in the gas phase.

These structures were then included into the cavity of a cucurbit[7]uril ring, pre-optimized at the semi-empirical PM6 level, and the respective complexes again optimised in the same way as described above but this time by using a continuum water model for the DFT calculation. The xyz files of all calculated structures are available for download as separate files.

Degradation of VX Mimics: Figures

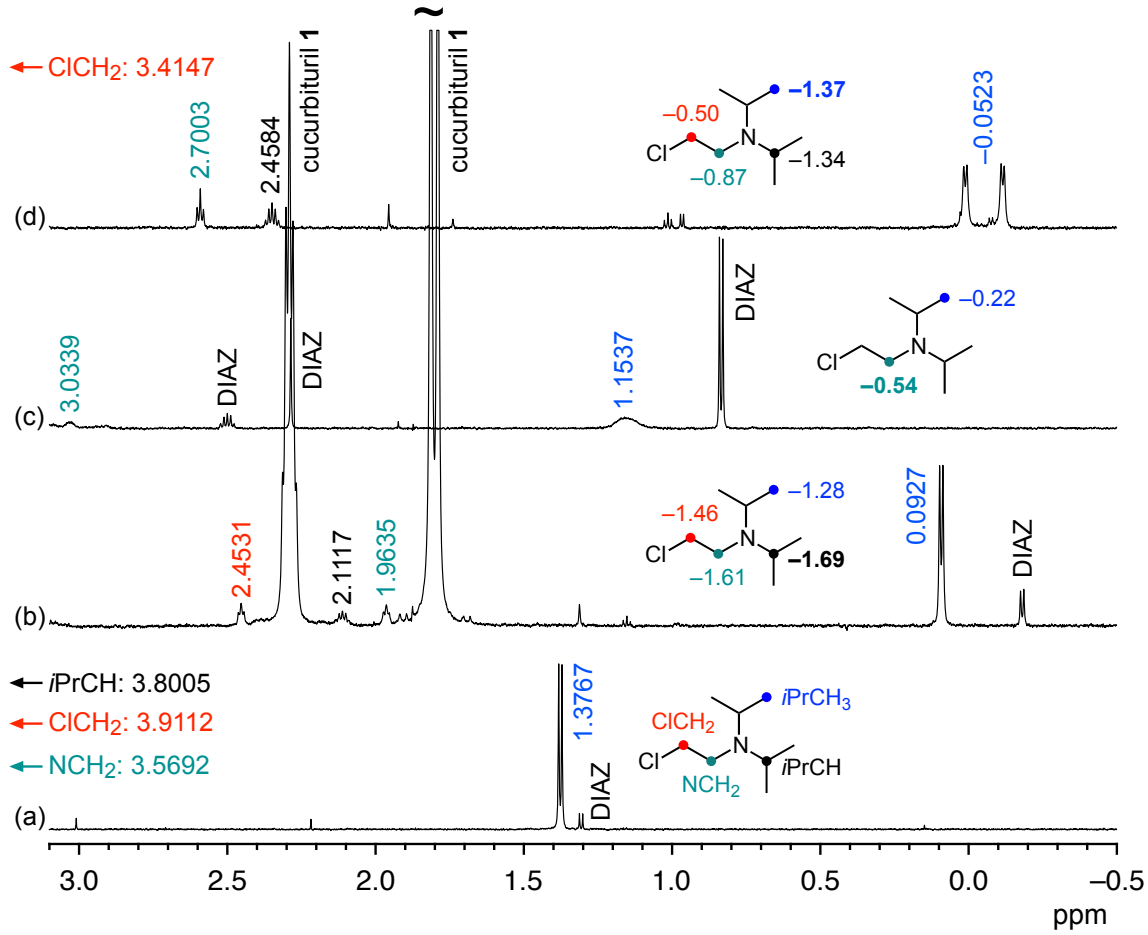


Fig. S11: ^{1}H NMR spectrum of DIACl (0.2 mM) in phosphate buffer (67.2 mM, pD 6.10) (a) and spectra in the same solvent of DIACl (0.2 mM) in the presence of 10 molar equiv of receptors **1** (b), **2** (c), and **3** (d). The signals of the substrate are marked and the structures in the spectra (b), (c), and (d) show the extents in terms of Δδ to which the different signals shift in the presence of the receptor. Upfield shifts are denoted with a negative sign. In the presence of **2**, major amounts of DIAZ are already present even in the first spectrum of the measurement.

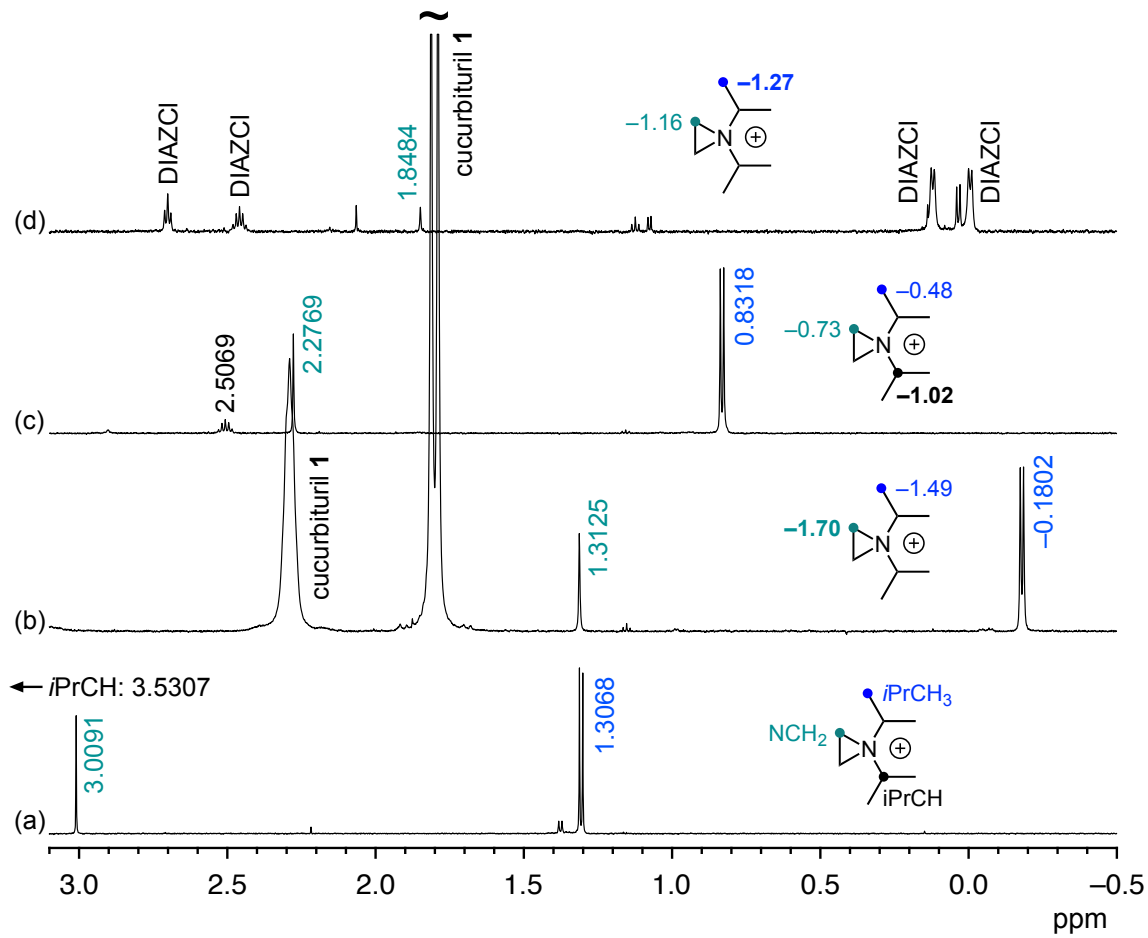


Fig. S12: ^{1}H NMR spectrum of DIAZ (0.2 mM) in phosphate buffer (67.2 mM, pD 6.10) (a) and spectra in the same solvent of DIAZ (0.2 mM) in the presence of 10 molar equiv of receptors **1** (b), **2** (c), and **3** (d). The signals of the substrate are marked and the structures in the spectra (b), (c), and (d) show the extents in terms of Δδ to which the different signals shift in the presence of the receptor. Upfield shifts are denoted with a negative sign. Only minor amounts of DIAZ were formed in the presence of receptor **3**.

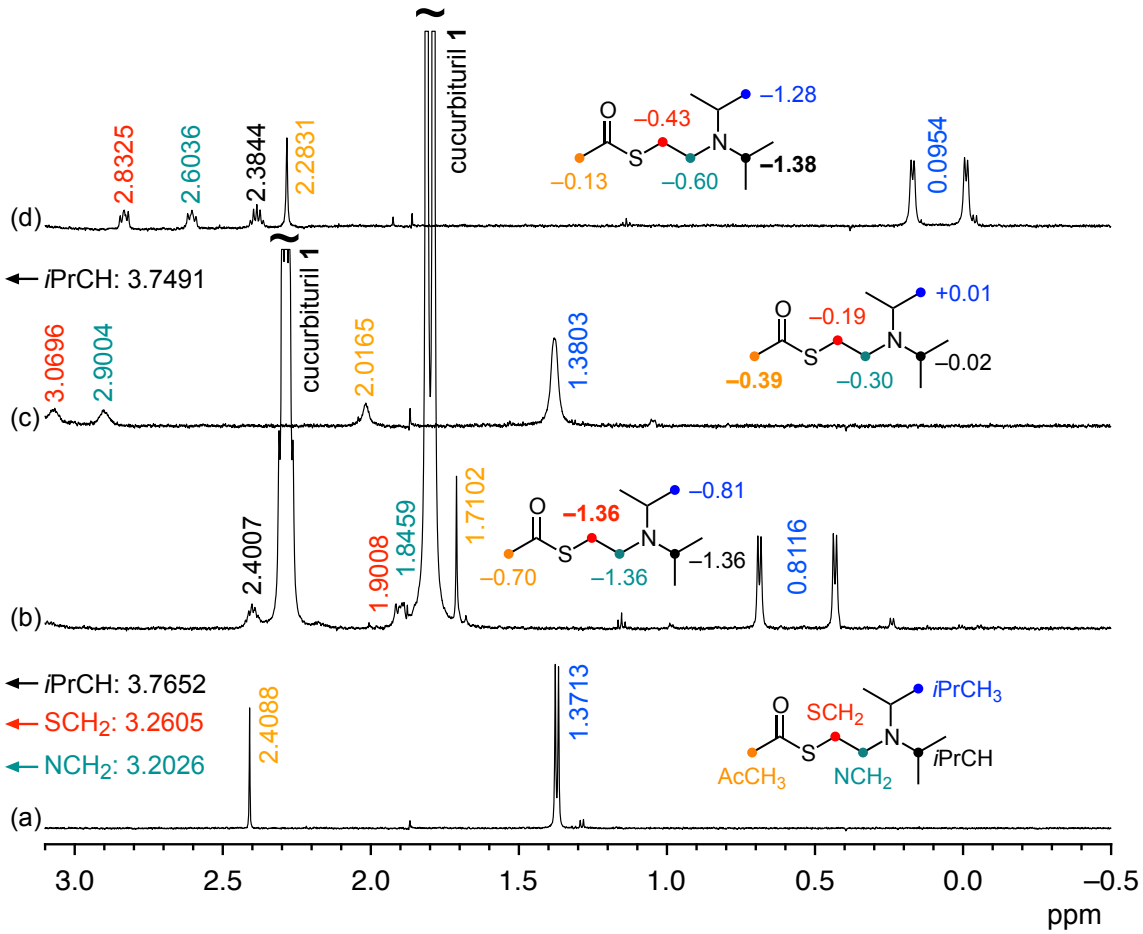


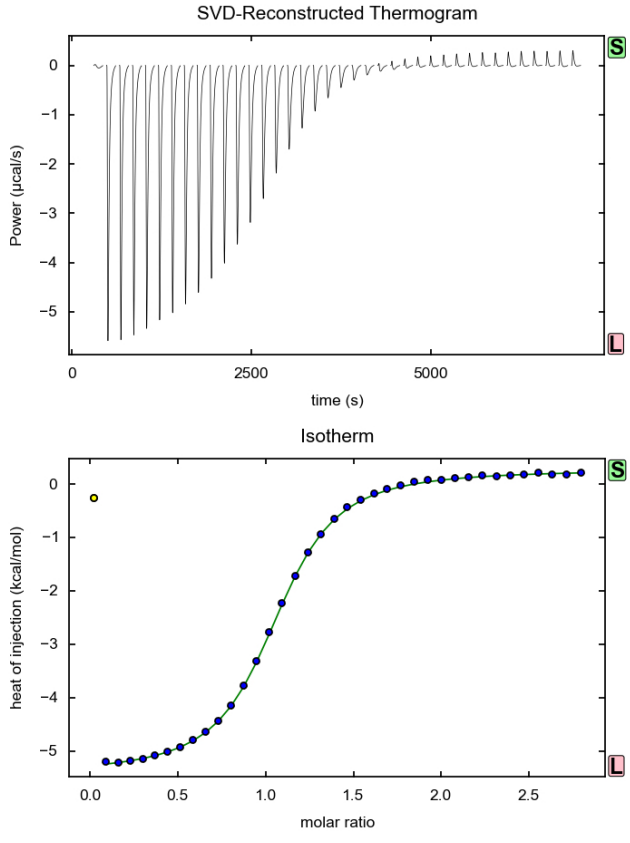
Fig. S13: ^{1}H NMR spectrum of DIASAc (0.2 mM) in phosphate buffer (67.2 mM, pD 6.10) (a) and spectra in the same solvent of DIASAc (0.2 mM) in the presence of 10 molar equiv of receptors **1** (b), **2** (c), and **3** (d). The signals of the substrate are marked and the structures in the spectra (b), (c), and (d) show the extents in terms of Δδ to which the different signals shift in the presence of the receptor. Upfield shifts are denoted with a negative sign.

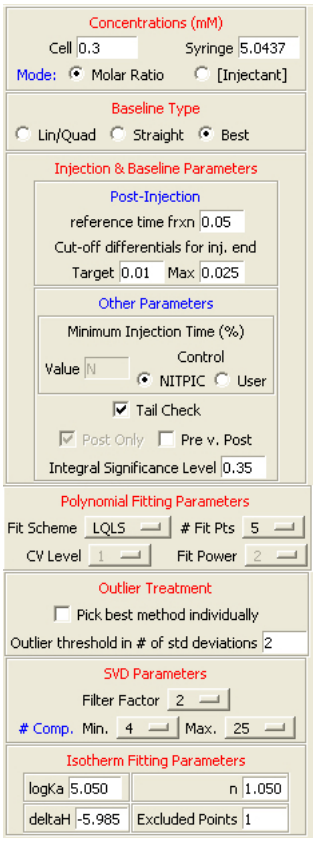
Table S2: Thermodynamic parameters $\Delta G°$, $\Delta H°$, and $T\Delta S°$ obtained in the ITC titrations for the complexes of receptors **1**, **2**, and **3** with DIASAc and DIAZ. All measurements were performed in triplicate at 298 K with the error specified. The stoichiometry factors n indicates that only 1:1 complexes were formed.

substrate	receptor	log K_a	$\Delta G°$ / kJ mol^{-1}	$\Delta H°$ / kJ mol^{-1}	$T\Delta S°$ / kJ mol^{-1}	n
DIASAc[a]	**1**	5.1 ± 0.1	–28.8 ± 0.2	–24.5 ± 0.6	4.3 ± 0.7	1.01 ± 0.03
	2	heat released during titration too small to allow evaluation				
	3	4.1 ± 0.1	–23.2 ± 0.2	–23.2 ± 0.1	0.0 ± 0.2	1.03 ± 0.04
DIASAc[b]	**1**	5.0 ± 0.1	–28.6 ± 0.1	–27.3 ± 0.2	1.3 ± 0.1	0.89 ± 0.02
	2	2.8 ± 0.1	–16.0 ± 0.5	–8.6 ± 1.0	7.4 ± 1.5	0.80 ± 0.06
	3	4.0 ± 0.1	–22.9 ± 0.1	–23.7 ± 1.1	–0.8 ± 1.1	0.94 ± 0.07
DIAZ	**1**	5.1 ± 0.2	–29.3 ± 1.0	–15.6 ± 0.6	13.7 ± 0.5	1.04 ± 0.01
	2	6.1 ± 0.1	–34.6 ± 0.8	–26.4 ± 1.9	8.2 ± 1.3	0.99 ± 0.10
	3	4.5 ± 0.1	–25.8 ± 0.2	–27.9 ± 0.3	–2.1 ± 0.5	0.98 ± 0.01

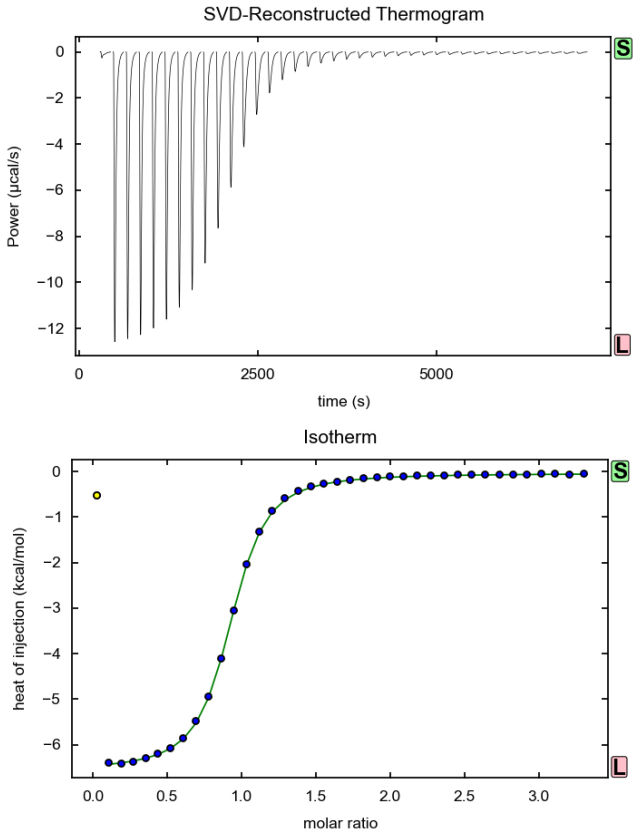
[a] titration performed with the substrate in the cell and the receptor in the syringe; [b] titration performed with the substrate in the syringe and the receptor in the cell

Titration of DIASAc with **1** (substrate in cell)



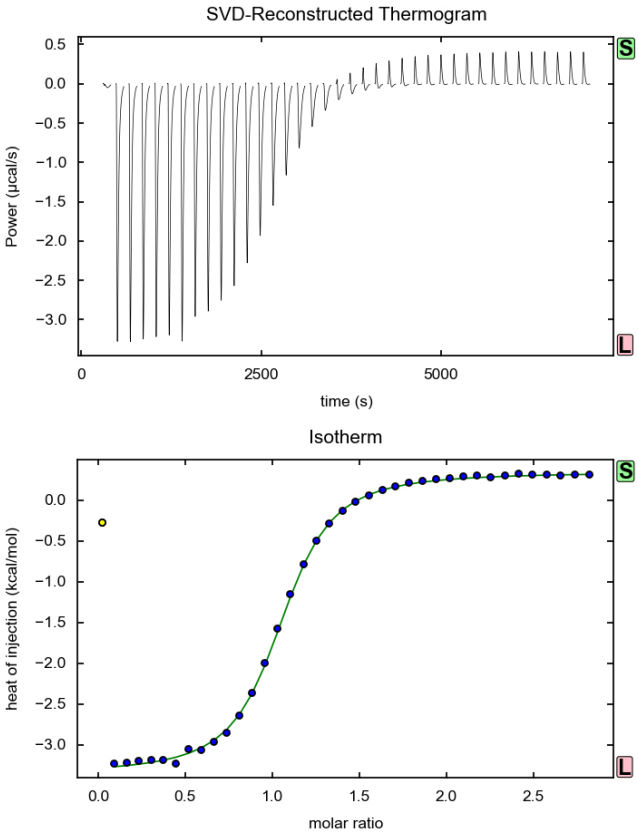


Titration of DIASAc with **1** (receptor in cell)



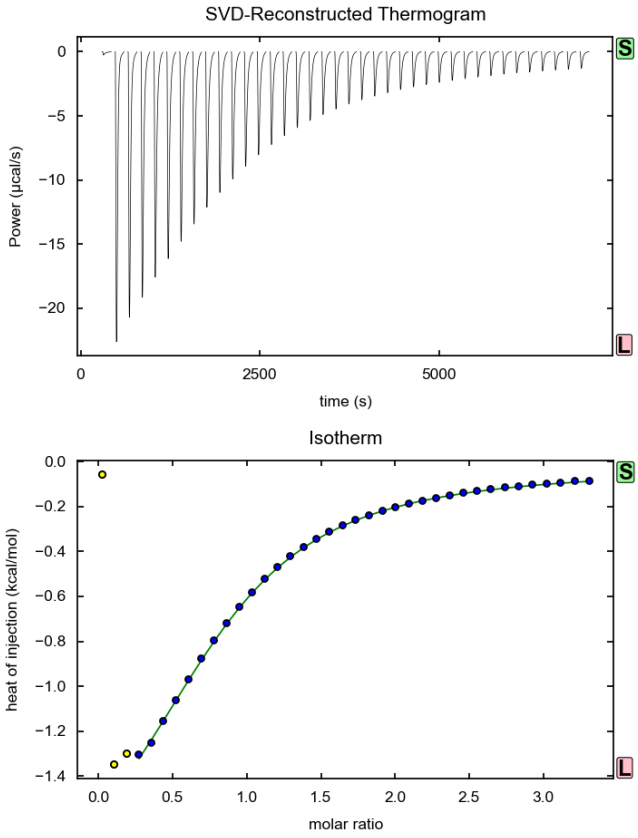
Concentrations (mM)
Cell 0.5069 Syringe 10.0705
Mode: Molar Ratio [Injectant]
Baseline Type
Lin/Quad Straight Best
Injection & Baseline Parameters
Post-Injection
reference time frxn 0.05
Cut-off differentials for inj. end
Target 0.01 Max 0.025
Other Parameters
Minimum Injection Time (%)
Value Control NITPIC User
Tail Check
Post Only Pre v. Post
Integral Significance Level 0.35
Polynomial Fitting Parameters
Fit Scheme LQLS # Fit Pts 5
CV Level 1 Fit Power 2
Outlier Treatment
Pick best method individually
Outlier threshold in # of std deviations 2
SVD Parameters
Filter Factor 2
Comp. Min. 4 Max. 25
Isotherm Fitting Parameters
logKa 5.029 n 0.903
deltaH -6.526 Excluded Points 1

Titration of DIAZ with **1** (substrate in cell)



Concentrations (mM)
Cell 0.3 Syringe 5.0886
Mode: Molar Ratio [Injectant]
Baseline Type
Lin/Quad Straight Best
Injection & Baseline Parameters
Post-Injection
reference time frxn 0.05
Cut-off differentials for inj. end
Target 0.01 Max 0.025
Other Parameters
Minimum Injection Time (%)
Value Control NITPIC User
Tail Check
Post Only Pre v. Post
Integral Significance Level 0.35
Polynomial Fitting Parameters
Fit Scheme LQLS # Fit Pts 5
CV Level 1 Fit Power 2
Outlier Treatment
Pick best method individually
Outlier threshold in # of std deviations 2
SVD Parameters
Filter Factor 2
Comp. Min. 4 Max. 25
Isotherm Fitting Parameters
logKa 5.194 n 1.037
deltaH -3.830 Excluded Points 1

Titration of DIASAc with **2** (receptor in cell)



Concentrations (mM)
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Mode: Molar Ratio [Injectant]

Baseline Type
Lin/Quad Straight Best

Injection & Baseline Parameters
Post-Injection
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Cut-off differentials for inj. end
Target 0.01 Max 0.025
Other Parameters
Minimum Injection Time (%)
Value Control
NITPIC User
Tail Check
Post Only Pre v. Post
Integral Significance Level 0.35

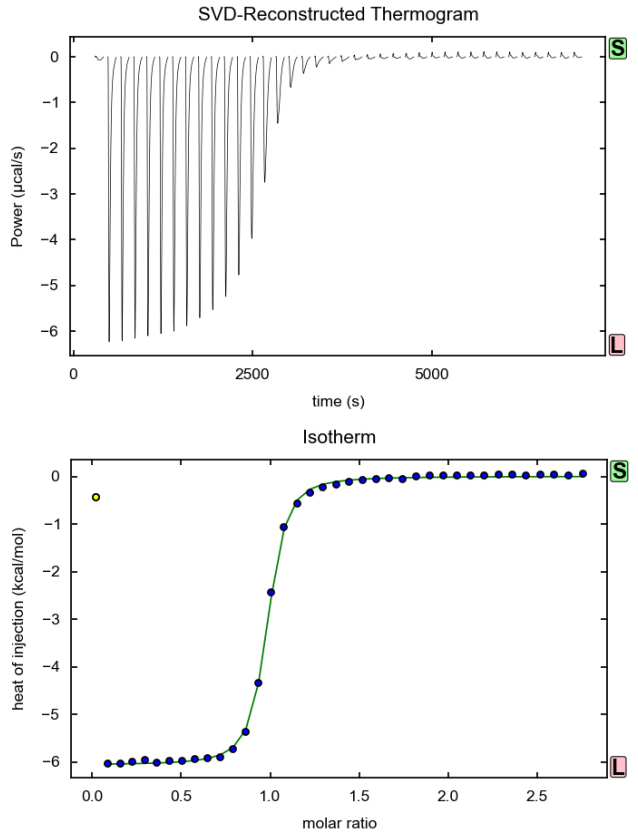
Polynomial Fitting Parameters
Fit Scheme LQLS # Fit Pts 5
CV Level 1 Fit Power 2

Outlier Treatment
Pick best method individually
Outlier threshold in # of std deviations 2

SVD Parameters
Filter Factor 2
Comp. Min. 4 Max. 25

Isotherm Fitting Parameters
logKa 2.809 n 0.799
deltaH -2.086 Excluded Points 3

Titration of DIAZ with **2** (substrate in cell)



Concentrations (mM)
Cell 0.3 Syringe 4.97
Mode: Molar Ratio [Injectant]

Baseline Type
Lin/Quad Straight Best

Injection & Baseline Parameters
Post-Injection
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Cut-off differentials for inj. end
Target 0.01 Max 0.025
Other Parameters
Minimum Injection Time (%)
Value Control
NITPIC User
Tail Check
Post Only Pre v. Post
Integral Significance Level 0.35

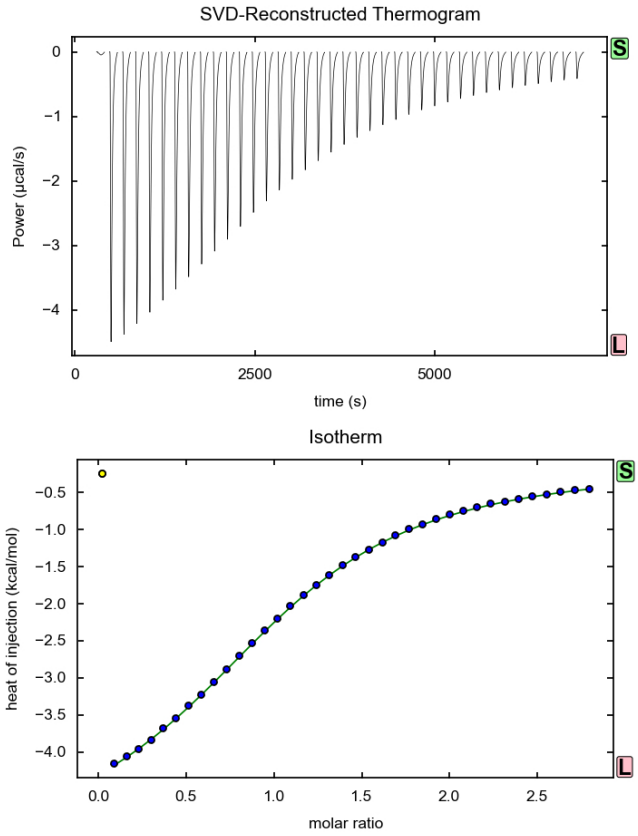
Polynomial Fitting Parameters
Fit Scheme LQLS # Fit Pts 5
CV Level 1 Fit Power 2

Outlier Treatment
Pick best method individually
Outlier threshold in # of std deviations 2

SVD Parameters
Filter Factor 2
Comp. Min. 4 Max. 25

Isotherm Fitting Parameters
logKa 6.060 n 0.954
deltaH -6.069 Excluded Points 1

Titration of DIASAc with **3** (substrate in cell)



Concentrations (mM)
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Mode: Molar Ratio [Injectant]

Baseline Type
Lin/Quad Straight Best

Injection & Baseline Parameters
Post-Injection
reference time frxn 0.05
Cut-off differentials for inj. end
Target 0.01 Max 0.025
Other Parameters
Minimum Injection Time (%)
Value Control NITPIC User
Tail Check
Post Only Pre v. Post
Integral Significance Level 0.35

Polynomial Fitting Parameters
Fit Scheme LQLS # Fit Pts 5
CV Level 1 Fit Power 2

Outlier Treatment
Pick best method individually
Outlier threshold in # of std deviations 2

SVD Parameters
Filter Factor 2
Comp. Min. 4 Max. 25

Isotherm Fitting Parameters
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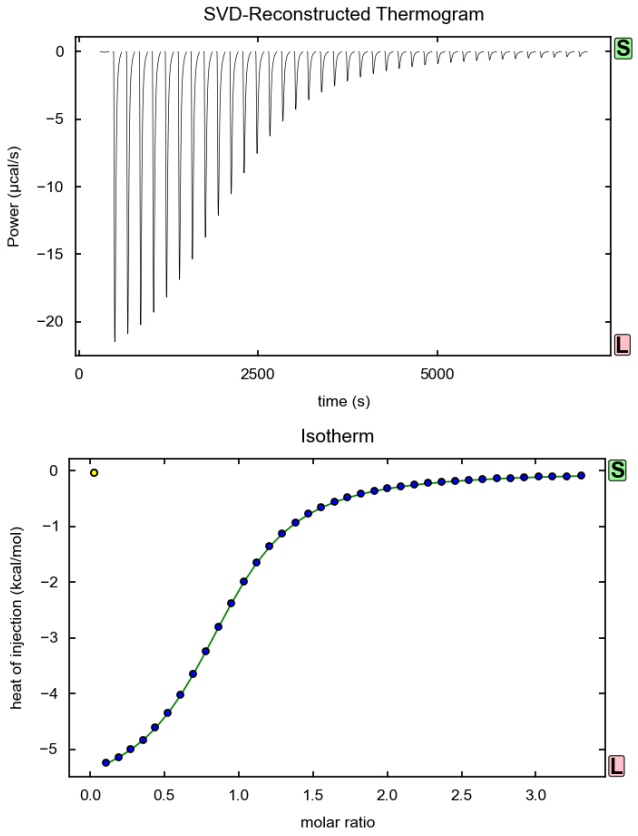
deltaH -5.561 Excluded Points 1

Titration of DIASAc with **3** (receptor in cell)



Concentrations (mM)
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Mode: Molar Ratio [Injectant]

Baseline Type
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Injection & Baseline Parameters
Post-Injection
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Cut-off differentials for inj. end
Target 0.01 Max 0.025
Other Parameters
Minimum Injection Time (%)
Value Control NITPIC User
Tail Check
Post Only Pre v. Post
Integral Significance Level 0.35

Polynomial Fitting Parameters
Fit Scheme LQLS # Fit Pts 5
CV Level 1 Fit Power 2

Outlier Treatment
Pick best method individually
Outlier threshold in # of std deviations 2

SVD Parameters
Filter Factor 2
Comp. Min. 4 Max. 25

Isotherm Fitting Parameters
logKa 3.994 n 0.894
deltaH -5.851 Excluded Points 1

Titration of DIAZ with **3** (substrate in cell)

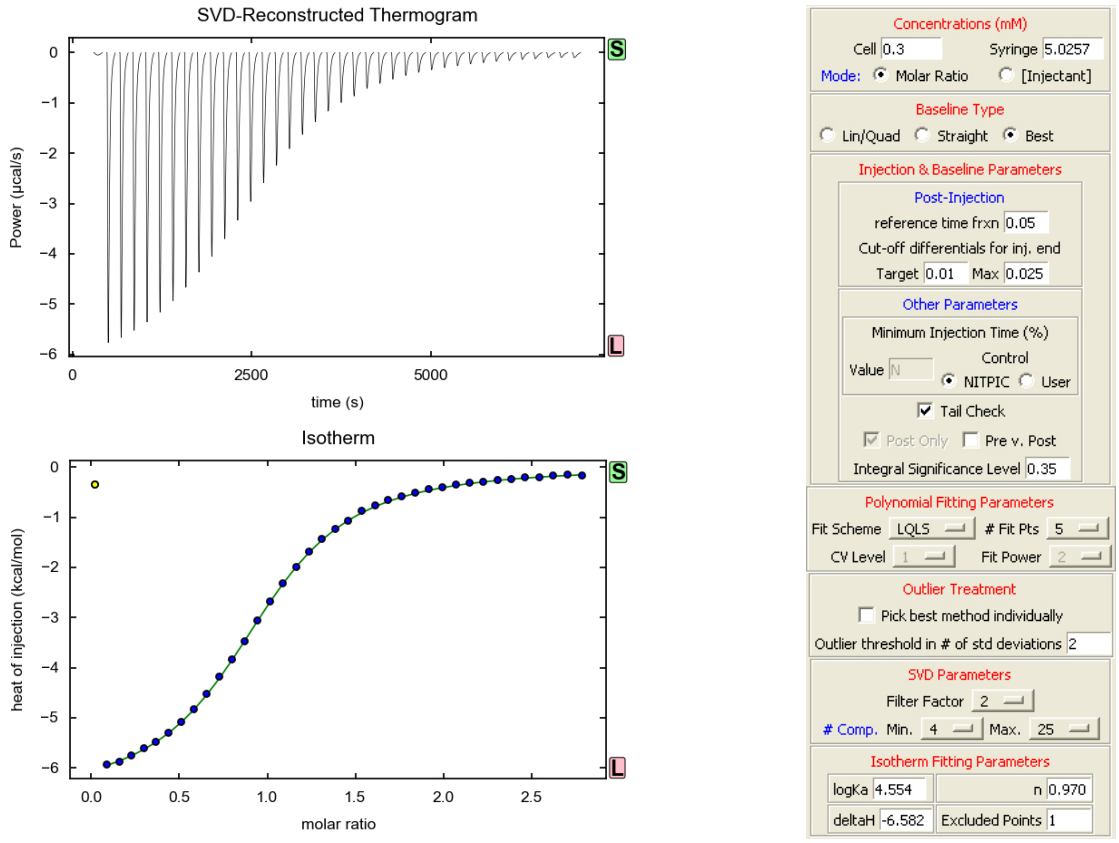
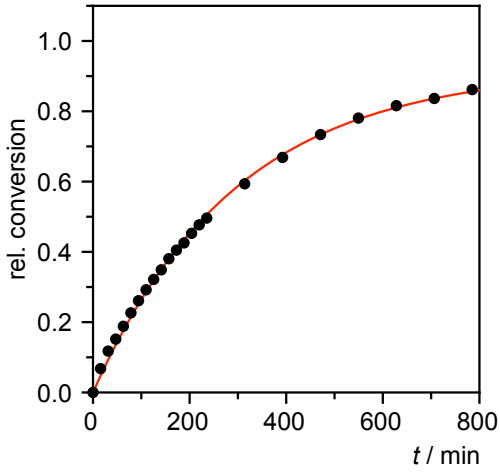
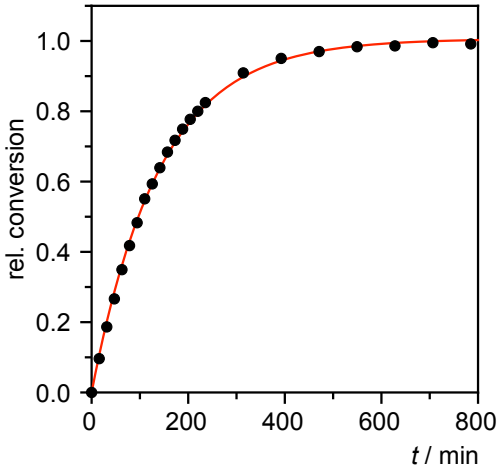


Fig. S14: Selected examples of thermograms, binding isotherms, and NITPIC outputs obtained in the ITC titrations.

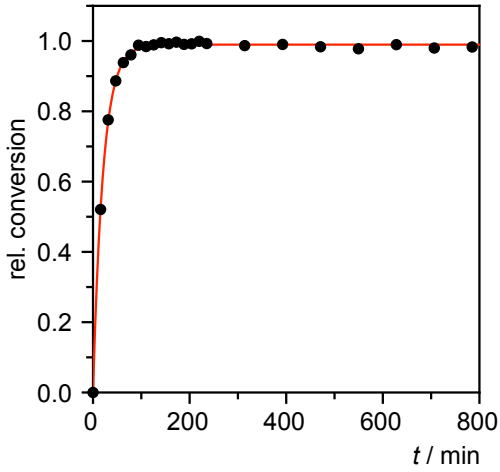
(a) DIACl (0.2 mM) without receptor



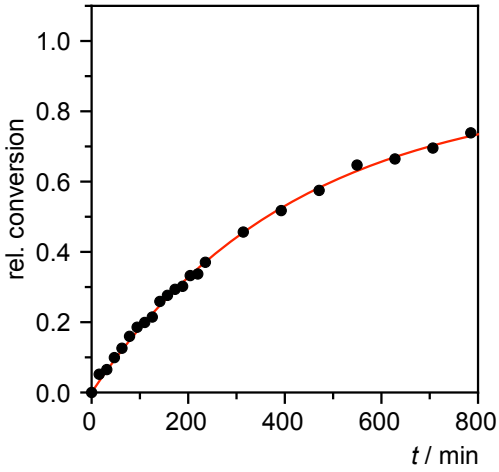
(b) DIACl (0.2 mM) + **1** (2 mM)



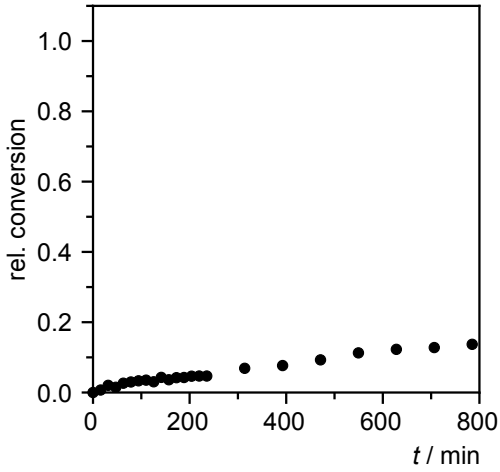
(c) DIACl (0.2 mM) + **2** (2 mM)



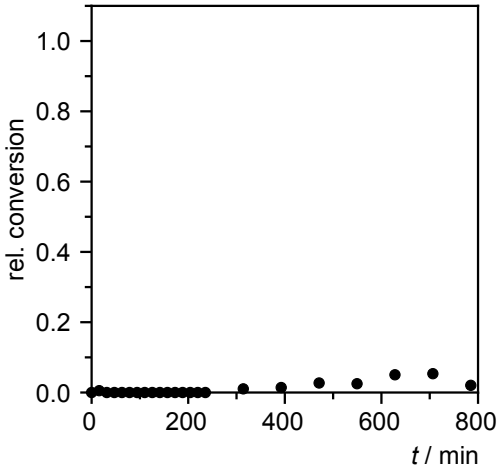
(d) DIACl (0.2 mM) + **2**/TAI (2 mM)



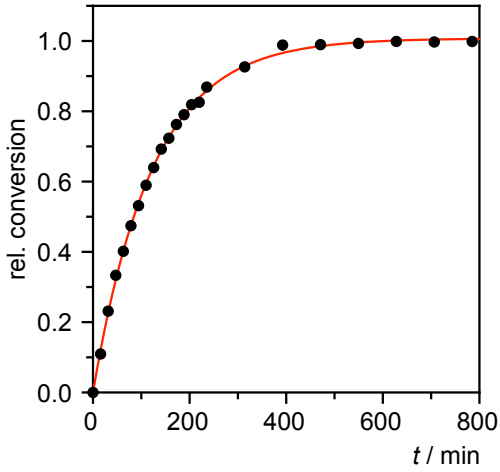
(e) DIACl (0.2 mM) + **3** (2 mM)



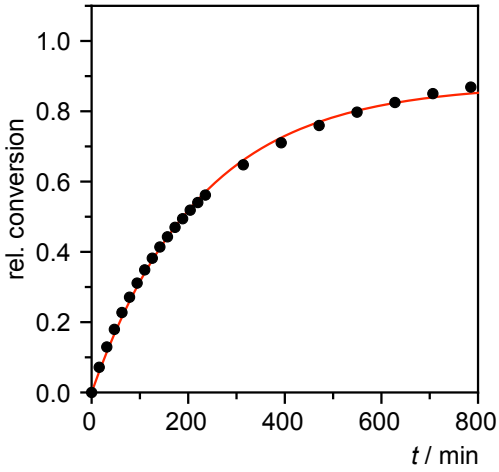
(f) DEACl (0.2 mM) without receptor



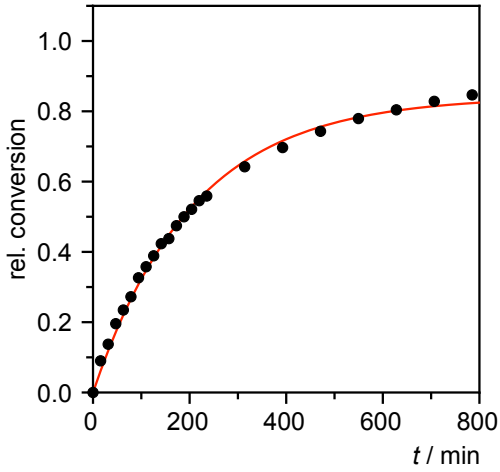
(g) DEACl (0.2 mM) + **2** (2 mM)



(h) DIACl (2 mM) without receptor



(i) DIACl (2 mM) + **1** (0.2 mM)



(j) DIACl (2 mM) + **2** (0.2 mM)

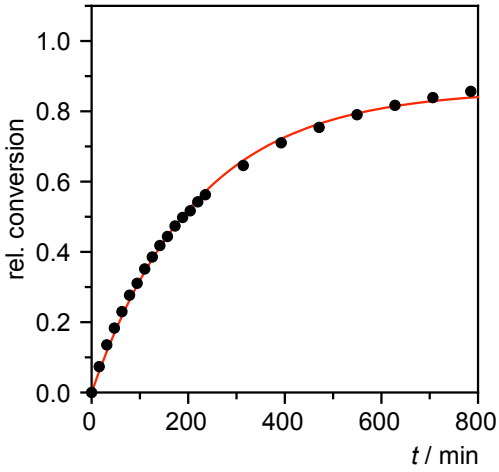


Fig. S15: Selected results of the kinetic measurements during which the rate of formation of DIAZ was followed in solutions of DIACl or DEACl (0.2 mM) in phosphate buffer (pD 6.10, 67.2 mM) and at 25 °C in the absence or the presence of 10 molar equiv of a receptor. Graphs (h), (i), and (j) depict the rate of product formation in solutions in which the concentrations of the substrate and the receptor amounted to 2 mM and 0.2 mM, respectively. Graph (d) describes the rate of the reaction when 1 molar equiv of *N*,*N*,*N*-trimethyladamantan-1-aminium iodide (TAI) with respect to **2** was additionally present.

References

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