The first use of supramolecular recognition to extract and stabilize an enzymatic inhibitor of a coagulation process†

Arnaud Grandeury,‡[ab] Claudette Martin,[a] Samuel Petit,[b] Constantin T. Craescu§[c] and Géraldine Gouhier*[a]

Received (in Montpellier, France) 23rd February 2010, Accepted 12th April 2010
First published as an Advance Article on the web 6th May 2010
DOI: 10.1039/c0nj00147c

A supramolecular complex based on salt bridges between an artificial receptor and (*Z*,*Z*)-(BABCH), a synthetic antagonist of tissue-plasminogen activator (t-PA), leads to a notable increase of its stability in solution and a new purification route for this bioactive isomer.

Tissue-plasminogen activator (t-PA) is a serine protease located in the brain that is widely studied for its presumed critical role in the homeostasis of the central nervous system (CNS), regulating blood viscosity in case of vascular lesion. Investigations on thrombolysis processes have shown that the coagulation mechanism is divided into cascade enzymatic reactions and involving many factors subject to activation and inhibition processes. Among them, the X activated factor (FXa) plays a major part in fibrin synthesis. A deficiency of FXa leads to the risk of haemorrhage and a surplus of thromboses, pulmonary embolisms and myocardium infarction risks. In the light of the progression of vascular problems in recent decades, it is necessary to be able to stimulate or inhibit these mechanisms. In addition to the description of human FXa structure, Shaw *et al.* have shown high affinities between FXa and three synthetic isomers, (*Z*,*Z*), (*E*,*Z*) and (*E*,*E*)-, of 2,7-bis-(4-amidinobenzylidene)cycloheptan-1-one (BABCH) (**1**: **1a**, **1b** and **1c**, respectively; Fig. 1).[1]

Due to its high affinity with FXa (0.66 nM) and t-PA catalytic sites (16 nM), (*Z*,*Z*)-BABCH (**1a**) ($K_{i(E,E)\mathbf{1c}}/K_{i(Z,Z)\mathbf{1a}}$ = 25 000) has been exploited to understand the specific interactions in CNS biological processes. According to the literature, **1a** was isolated with a 4% global yield in three steps.[1] The photoisomerization of **1c**, which is thermodynamically more stable, gave a mixture of **1a**, **1b** and **1c** isomers with the ratio 55 : 40 : 5. The most active isomer, **1a**, was then isolated by preparative inverse phase HPLC.

Since the initial reports of (*Z*,*Z*)-BABCH activity,[1] the design of new FXa inhibitors has been described, and non-amidine structures such as rivaroxaban and apixaban are under clinical study as orally active FXa inhibitors for the prevention and treatment of thromboembolic diseases.[2] However (*Z*,*Z*)-BABCH is still one of the strongest inhibitors (K_i = 0.66 nM) ever reported and constitutes a reference model for comparative purposes. The incorporation of new substituents into the amidine inhibitor did not enhance the interactions with the enzymatic site.[3] In 2009, Fujimoto's group synthesized a new chiral inhibitor with K_i = 0.5 nM,[4] and only a few examples have been recently described having nanomolar activity.[2,5] Despite the low oral bioavailability of inhibitors containing an amidino group,[5b] a patent describing new amidine compounds as inhibitors of FXa for pharmaceutical applications was deposited in 2010.[6] The low synthesis yields, difficult purification steps and instability of (*Z*,*Z*)-BABCH could explain why further studies have not been pursued with this inhibitor. Therefore, it is relevant to develop new strategies to obtain a synthetically viable inhibitor template. Other methodologies have also been developed to improve the synthesis of the precursor of this inhibitor.[7]

To reduce the high photochemical instability of **1a**, we have focused our research on the development of complexation agents that are able to bind the desired isomer selectively through relevant molecular recognition mechanisms. Various synthetic hosts have been developed to recognize specific substrates and to simplify the mechanisms of enzymatic recognition.[8] Acceptors such as carboxylate, phosphonate and sulfonate functions have been used for their ability to form hydrogen bonds with the cationic amidine functions.

To reach our objective, we have developed a proton acceptor system with a rigid structure that is able to act as a selective and non-covalent molecular clamp with **1a**. Taking into account the specific lateral chain orientation in **1c**, this strategy was applied for the first time to separate **1a** from the two other

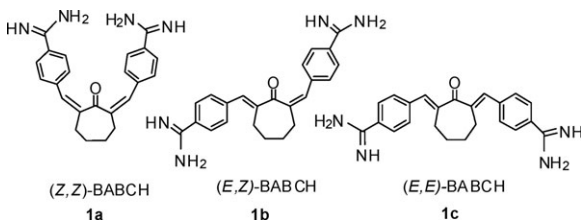
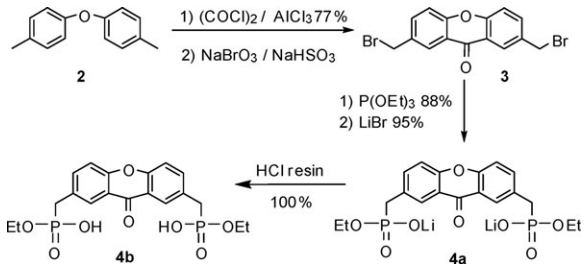
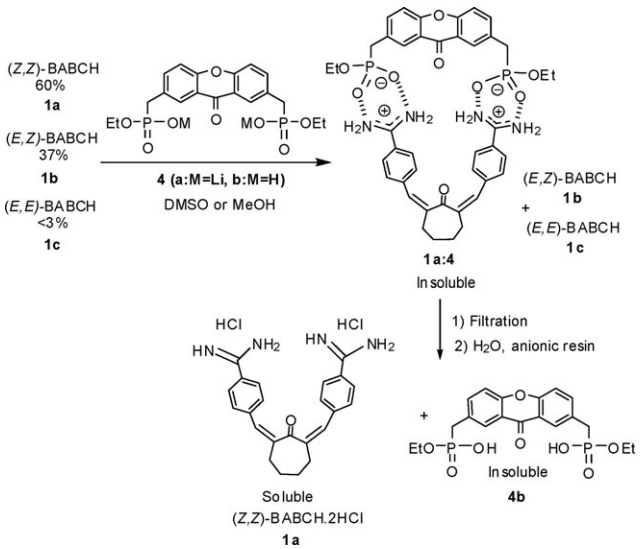


Fig. 1 The three synthetic isomers of BABCH.

[a] *UMR CNRS 6014, Université de Rouen, IRCOF, 1 Rue Tesnière, 76821 Mont Saint Aignan cedex, France. E-mail: geraldine.gouhier@univ-rouen.fr; Fax: +33 235522959; Tel: +33 235522909*
[b] *UPRES EA 3233, SMS, IRCOF, Université de Rouen, 1 Rue Tesnière, F-76821 Mont Saint Aignan cedex, France*
[c] *INSERM U759/Institut Curie-Centre de Recherche, Centre Universitaire Paris-Sud, Bâtiment 112, 91405 Orsay Cedex, France*
† Electronic supplementary information (ESI) available: Synthesis and analytical data of **4a**, **4b** and their precursors. NMR and ITC spectra of the complexes. See DOI: 10.1039/c0nj00147c
‡ Present address: Novartis Pharma AG, Klybeckstrasse 141, CH-4057 Basel, Switzerland.
§ Deceased September 2009.



Scheme 1 Synthesis route of bis-*O*-ethylphosphonic acid **4b** and its lithium salt **4a**.



Scheme 2 The extraction procedure of **1a** by molecular recognition.

isomers. Indeed, the formation of similar saline bridges has already been reported in the literature, but has not been applied as an efficient preparative purification tool. We report here the synthesis of a new specific receptor of this active antagonist and its capacity to exert molecular recognition.

As a preliminary step, molecular modelling tools were used to design a suitable complexing agent based on molecular modelling of the three isomers.[9] The distances between the two amidinium functions were established as $d_{C-C} \sim 11$ Å and $d_{NH-NH} \sim 13$ Å for **1a**, $d_{C-C} \sim 13$ Å and $d_{NH-NH} \sim 17$ Å for **1b**, and $d_{C-C} \sim 16$ Å and $d_{NH-NH} \sim 19$ Å for **1c**. The rigid skeleton of xanthen-9-one and a bis-monoesterphosphonic acid were chosen.[10] A Friedel–Crafts reaction between *p*-tolylether **2** and oxalyl chloride led to 2,7-dimethylxanthen-9-one in 77% yield (Scheme 1). Bromination reactions using NBS under light irradiation or in the presence of a radical initiator afforded a mixture of inseparable side-bromination products in low yields. Sodium bromate combined with a reducing reagent such as sodium bisulfite at room temperature led to **3** in excellent conversion after a simple filtration step.[11]

The direct Arbusov reaction of **3** led to a white crystalline solid, bis-*O*,*O*-diethylphosphonate, that was easily isolated by simple filtration on silica in 88% yield in two steps. A mono-deprotection afforded lithium bis-*O*-ethylphosphonate **4a** in 95% yield, which was protonated to form **4b** by treatment with an ion exchange resin. Both receptors **4a** and **4b** are insoluble in apolar solvents and were solubilized in DMSO or methanol; **4a** is also soluble in water.

After screening solvents, irradiation times and concentrations, the displacement of the photoisomerization equilibrium was optimized. Thus, in dry methanol, a one hour irradiation of **1c** at a 10^{-3} mol L^{-1} concentration generated a new ratio of 60, 37 and <3% for **1a**, **1b** and **1c**, respectively. The same experiment was then attempted in the presence of one equivalent of **4a** in order to form exclusively **1a**. However, degradation products were observed that were produced by the instability of the xanthone structure under these conditions.

The strategy designed to produce **1a** consisted of (i) selectively forming supramolecular insoluble salt bridges **1a**:**4**, (ii) isolating the ionic complex and (iii) separating the isomer involved in the complexation (Scheme 2). It was assumed that, depending on the strength of the non-covalent associations, a dynamic equilibrium between the solvated species would displace the system towards the most thermodynamically stable salt. Assays were therefore attempted by mixing **4a** or **4b** with a mixture of **1** (in their ionic or molecular forms) in DMSO or MeOH. The recognition of bis-functional hydrogen bonding receptors displays divergent thermodynamics in different solvent systems.[12] Water is competitive due to the hydrogen bond formed between the host and the guest, induced as this solvent is a more dissociating medium. Therefore, the study was carried out in DMSO and methanol to reinforce the ion pair binding.

The concentration and temperature ranges were varied from 10^{-3} to 5×10^{-5} M and from 25 to −78 °C, respectively. New non-crystalline solids appeared after 12 h, suggesting the formation of salt pairs in solution. The precipitate was separated by centrifugation or filtration on a polytetrafluoroethylene (PTFE) membrane, dissociated in an acidic medium and the selectivity evaluated by HPLC. Insoluble receptor **4b** was then regenerated.

Only lithium bis-*O*-ethylphosphonate **4a** brought about enrichment in **1a**·2HCl. The data collected indicated that very poor molecular discrimination was observed in DMSO (entries 1 and 2, Table 1). Solvent, temperature and solution concentration have an influence on the isomeric enrichment. At room temperature, the selectivity increased with dilution (entries 1–5, Table 1) and a maximum enrichment was obtained at 5×10^{-5} M (entry 5, Table 1). At this concentration, the effect of temperature was low since no increase of selectivity was observed between −20 and −78 °C in methanol (entries 6 and 7, Table 1).

In methanol at 25 °C and at a concentration of 5×10^{-5} M, the complex **1a**:**4a** was isolated and separated through an ion exchange resin. The mixture enriched in **1a** was introduced into successive extraction cycles (Table 2).

Using this methodology, **1a** was obtained in high purity and in a 31% yield after four cycles. Compared to semi-preparative inverse phase HPLC, our strategy appears to be a small scale alternative, and could be attractive for larger scale production by increasing the initial concentration and the number of cycles.

The ion pair binding strategy was also applied to improve the stabilization of the (*Z*,*Z*)-BACBH inhibitor. Indeed, in aqueous solution, **1a** is highly sensitive to the intensity of light

Table 1 Effect of various conditions on the selectivity (initial mixture **1** ((*Z*,*Z*), (*E*,*Z*), (*E*,*E*))-BABCH·2HCl 60/37/3) in the presence of **4a**

Entry	Solvent	*T*/°C	Concentration in **1**/M	**1a** : **1b** : **1c** ratio[a]	Enrichment of **1a** (%)
1	DMSO	25	10^{-3} M	60 : 37 : 3	0
2	DMSO	25	10^{-4} M	65 : 32 : 3	8
3	MeOH	25	10^{-3} M	64 : 33 : 3	7
4	MeOH	25	10^{-4} M	67 : 28 : 3	11
5	MeOH	25	5×10^{-5} M	70 : 27 : 3	15
6	MeOH	−20	5×10^{-5} M	74 : 20 : ε[b]	19
7	MeOH	−78	5×10^{-5} M	75 : 20 : ε[b]	20

[a] Impurities excluded. [b] Non-quantifiable < 3%.

Table 2 Evolution of the purity of (*Z*,*Z*)-BABCH·2HCl (**1a**) during the successive cycles of salt formation from BABCH·2HCl **1** ((*Z*,*Z*)/(*E*,*Z*)/(*E*,*E*), 60 : 37 : 3)

Number of cycles	0	1	2	3	4
Purity of **1a** (%)	60	75	91	98	99
Global yield of **1** after dissociation (%)	100	75	56	42	31

radiation. Concentrated solutions ($>10^{-3}$ M) have a good stability (several weeks) but more dilute solutions are unstable. For example, HPLC used for isomer separation involving high dilution (10^{-5} M) generates fast photoisomerization of pure **1a**, probably due to the low conformational energy between **1a** and **1c**; estimated to be 2.1 kcal mol^{-1}.[13] Therefore, a stability study was carried out using a 10^{-4} M aqueous solution of **1** in the presence or absence of **4a** under indirect natural sun light exposure. The two test solutions were analyzed by HPLC. Table 3 shows a slower degradation of the initial ratio in presence of receptor **4a**.

This stabilization may be attributed to stronger molecular constraints or to a change of the optical density of the solution. However, total control was not reached.

In order to follow the association phenomena *via* salt bridges, ^{31}P NMR spectroscopy of **4a** at 10^{-4} M was performed in D_2O with the **1a–c** mixture and with the pure isomers **1a**, **1b** or **1c**. In all cases, a broadening of the phosphorus signal with a complex multiplicity occurred, probably associated with the formation of complex species in solution.† To evaluate the possible formation of oligomers, the influence of the concentration was studied with **1** and **4a** (1 equivalent with respect to **1a**) at 10^{-2} to 10^{-4} M using dialysis tubes in water. For the 10^{-4} M diluted solution, all the species passed through the membrane. For the 10^{-2} M concentrated solution, species were detected in the tube with a ratio **4a** : **1** of 1 : 1. This retention of matter is probably associated with stable aggregate formation having dimensions larger than the membrane pores (cut-off at 25 000 Da).[14] The degree of oligomerization was not evaluated because of the low sensitivity of the physicochemical indicators of the complexation. Assays of titration experiments by NMR or fluorescence spectroscopy did not allow the determination of binding association constants (K_a) in solution.[15] To obtain more information about these solvated associations, isothermal titration calorimetry (ITC) was then considered.[12] Three titrations in water with **4a** at 10^{-3} M afforded association constants in solution for each isolated isomer, **1a**, **1b** or **1c**, and revealed a real selectivity between the three inhibitors and **4a**. The results of these titration experiments are summarized in Table 4.†

The increase of the K_a value from the **1c** to the **1a** isomers explains the molecular discrimination between isomer **1a** and receptor **4a**.

Regarding the free energy of complexation, the association is driven by the entropy term, whatever isomer is being considered. Their values vary by a factor of two from **1c** to **1a**. This large variation reinforces the hypothesis of oligomer formation with **1b** and **1c**, which may be associated with a larger (unfavourable) entropic term due to the decrease of translational and rotational degrees of freedom. However, it appears that the entropically favourable term related to the release of water molecules and the increase of solvent entropy during complexation overcomes the associated molecular motions.[12]

To sum up, the functionalized receptor of xanthone **4a** discriminated the mixture of BABCH isomers. This positive effect was quantified by ITC using the isolated isomers. Although the difference of stability between the salt bridges was significant, no highly efficient separation occurred. This selectivity is solvent, concentration and temperature dependent, probably in connection with the contribution of the entropy term, which influences the variation of the association constant. This system turned out to be more complex than the one initially proposed, probably due to the competitive formation of oligomers, instead of the dimers initially expected. However, a new procedure was successfully developed to isolate the desired isomer and avoid the use of preparative inverse phase HPLC. We have also observed a notable increase in the stability of (*Z*,*Z*)-isomer **1a** in solution in the presence of receptor **4a**. This original work constitutes the first use of molecular recognition by the formation of salt bridges

Table 3 The evolution of the isomer ratio of BABCH·2HCl **1** ((*Z*,*Z*) : (*E*,*Z*) : (*E*,*E*)) in a 10^{-4} M aqueous solution under indirect natural light, with or without **4a** (1 equivalent)

Exposition time	0 day	5 days	10 days	15 days	20 days
1 without **4a**	60 : 37 : 3	55 : 33 : 12	41 : 30 : 29	30 : 27 : 33	29 : 29 : 41
1 with **4a**	60 : 37 : 3	58 : 37 : 5	55 : 40 : 5	51 : 43 : 7	46 : 42 : 12

Table 4 Thermodynamic data representing the association between BABCH·2HCl isomers **1a–c** and lithium bis-monoesterphosphonate **4a** obtained by ITC at 30 °C

	K_a/L mol^{-1}	ΔG/kcal mol^{-1}	ΔH/kcal mol^{-1}	$T\Delta S$/kcal mol^{-1}
1a	$3.1 \times 10^4 \pm 0.9 \times 10^4$	-6.2 ± 0.20	-0.10 ± 0.01	6.1 ± 0.4
1b	$8.2 \times 10^3 \pm 2.0 \times 10^3$	-5.4 ± 0.05	-0.31 ± 0.02	5.1 ± 0.3
1c	$2.7 \times 10^3 \pm 0.2 \times 10^3$	-4.8 ± 0.02	-1.31 ± 0.05	3.5 ± 0.1

for an extraction procedure and an isomeric stabilization. The synthesis of (*Z*,*Z*)-BACBH using additional molecular constraints, such as π-stacking, is in progress.

We are grateful to Region Haute-Normandie *via* the PunchOrga network for the A. G.'s PhD fellowship.

Experimental

p-Tolylether **2** (2 g, 10.1 mmol) and fresh aluminium trichloride (2 g, 15 mmol) were mixed under nitrogen in dry dichloromethane (50 mL). At 0 °C, oxalyl chloride (1 mL, 11.8 mmol) was added dropwise to the stirred solution. The mixture was heated under reflux for 2 h. After cooling to 0 °C, the solution was hydrolyzed by 0.1 M HCl (50 mL). After three extractions with ether, the organic phases were washed with a saturated NaCl solution. The dried ($MgSO_4$) organic layer was evaporated under reduced pressure. Recrystallization in a mixture of ethyl acetate/cyclohexane (9 : 1) gave 2,7-dimethylxanthen-9-one (1.7 g, 7.8 mmol, 77%) as a white solid. m.p. 143 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.06 (s, 2H), 7.46 (d, $^3J_{H,H}$ = 8.4 Hz, 2H), 7.32 (d, $^3J_{H-H}$ = 8.4 Hz, 2H), 2.40 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 177.8, 154.8, 136.3, 121.7 (2C), 133.8, 126.3 (2C), 118.1, 21.2; IR (CH_2Cl_2): ν = 1658 (C=O), 1383 (C–O); mass IE (70 eV) calc. 224.0837 (100.00%); found 224.0833 (100.00%); elemental analysis calc. (%) for $C_{15}H_{12}O_2$: C 80.35, H 5.36; found C 80.37, H 5.31.

An aqueous solution of sodium bromate (1.5 mmol mL^{-1}, 14 mL, 20.5 mmol) was mixed with dimethylxanthen-9-one (1.1 g, 5 mmol) diluted in 10 mL of ethyl acetate. An aqueous solution of sodium bisulfite (1.4 mmol mL^{-1}, 14 mL, 20.2 mmol) was added dropwise to the stirred solution. After 24 h of vigorous stirring at room temperature, 20 mL of dichloromethane was added. After extraction, the organic phases were washed twice with 30 mL of a sodium thiosulfate solution. After drying over $MgSO_4$, the organic solvents were evaporated under reduced pressure. The mixture was filtered over silica with cyclohexane/ethyl acetate (90 : 1) as the eluent. 2,7-Bis(bromomethyl)-9*H*-xanthen-9-one (**3**) was isolated and directly used in the Arbusov reaction.

2,7-Bis-(bromomethyl)-9*H*-xanthen-9-one (**3**) (350 mg, 0.9 mmol) and triethylphosphite (600 mg, 3.6 mmol) were heated under reflux for 12 h. The excess triethylphosphite was evaporated under reduced pressure. The residual 2,7-dimethylxanthone was eliminated by filtration over silica with ethyl acetate as the eluent. The white solid 2,9-(9-oxo-9*H*-xanthen-7-yl)bismethyldiethylphosphite was isolated after filtration with ethanol as the eluent (448 mg, 0.9 mmol, 88% over two steps). m.p. 110–112 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.13 (s, 2H), 7.67 (d, $^3J_{H-H}$ = 8.6 Hz, 2H), 7.40 (d, $^3J_{H-H}$ = 8.6 Hz, 2H), 3.98 (q, $^3J_{H-H}$ = 7.1 Hz, 8H), 3.20 (d, $^3J_{H-P}$ = 21.4 Hz, 4H), 1.21 (t, $^3J_{H-H}$ = 7.1 Hz, 12H); ^{31}P NMR (121 MHz, $CDCl_3$): δ = 25.44; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 176.7, 155.1, 136.4, 127.9, 127.3, 121.5, 118.4, 62.3, 33.1 (d, $^3J_{C-P}$ = 138.0 Hz), 16.4; IR (CH_2Cl_2): ν = 3053, 2985, 1660 (C=O), 1265 (C–O–C), 1053 (P–O); mass ESI$^+$ calc. 497.1494 (100.00%); found 497.1500 (100.00%).

2,9-(9-Oxo-9*H*-xanthen-7-yl)-bis-methyldiethylphosphite (495 mg, 1 mmol), lithium bromide (191 mg, 2.2 mmol) and dried pentan-2-one (6 mL) were heated under nitrogen for 3 h. After evaporation under reduced pressure, the residue was washed with ether and recrystallized with a mixture of ethanol/acetone (7 : 3) as the eluent. A white solid of **4a** (428 mg, 0.9 mmol, 95%) was obtained. m.p. > 250 °C; ^{1}H NMR (300 MHz, MeOD-d_4): δ = 8.07 (s, 2H), 7.72 (d, $^3J_{H-H}$ = 8.7 Hz, 2H), 7.39 (d, $^3J_{H-H}$ = 8.7 Hz, 2H), 3.76 (q, $^3J_{H-H}$ = 7.2 Hz, 4H), 3.00 (d, $^3J_{H-P}$ = 20.3 Hz, 4H), 1.09 (t, $^3J_{H-H}$ = 6.9 Hz, 6H); ^{31}P NMR (121 MHz, MeOD-d_4): δ = 19.34; ^{13}C NMR (75 MHz, MeOD-d_4): δ = 178.8, 156.1, 138.3, 133.7, 127.5, 122.2, 118.7, 61.3, 35.5 (d, $^3J_{C-P}$ = 129.8 Hz), 17.09; mass FAB (matrix glycerol, negative): 439 (MH^-), 411 (MH^- – Et), 330 (MH^- – P(O)OEt); IR (KBr): ν = 1658 (C=O), 1098 (P–O).

An ion exchange resin of sulfonic acid was activated with 6N HCl and then washed with pure water (18 MΩ). An aqueous solution of lithium salt **4a** (400 mg, 0.9 mmol) was deposited on the resin and eluted with a mixture of water/methanol (4 : 6). A white solid (389 mg, 0.9 mmol, 100%) was obtained. m.p. > 250 °C; ^{1}H NMR (300 MHz, D_2O): δ = 7.88 (s, 2H), 7.62 (d, $^3J_{H-H}$ = 8.5 Hz, 2H), 7.41 (d, $^3J_{H-H}$ = 8.3 Hz, 2H), 3.75 (q, $^3J_{H-H}$ = 7.0 Hz, 4H), 3.02 (d, $^3J_{H-P}$ = 20.3 Hz, 4H), 1.06 (t, $^3J_{H-H}$ = 7.1 Hz, 6H); ^{31}P NMR (121 MHz, D_2O): δ = 21.87; ^{13}C NMR (75 MHz, D_2O): δ = 154.89, 137.33, 136.13, 131.06, 125.7, 118.2, 61.2, 32.7, 15.18; IR (KBr): ν = 1658 (C=O), 1098 (P–O); mass ESI$^+$ calc. 439.0706 (100.00%); found 439.0712 (100.00%).

Representative procedure

At 25 °C, a solution of **4a** and a mixture of isomers **1a**/**1b**/**1c** (60 : 37 : 3) in anhydrous MeOH was prepared at 10^{-5} M so that a 1 : 1 stoichiometry between **1a**/**4a** was in place. After 12 h of stirring, the precipitate was centrifuged. The absence of **4a** in the filtrate was confirmed by ^{31}P NMR and by disappearance of fluorescence. The complex was passed through an ion exchange resin (dowex). The enriched mixture was introduced in a new cycle.

Notes and references

1 (*a*) K. J. Shaw, W. J. Guilford, J. L. Dallas, S. K. Koovakkaat, M. A. McCarrick, A. Liang, D. R. Light and M. M. Morrissey, *J. Med. Chem.*, 1998, **41**, 3551–3556; (*b*) W. J. Guilford, K. J. Shaw, J. L. Dallas, S. K. Koovakkaat, W. Lee, A. Liang,

D. R. Light, M. A. McCarrick, M. Whitlow, B. Ye and M. M. Morrissey, *J. Med. Chem*., 1999, **42**, 5415–5425.
2 Y. Shi, C. Li, S. P. O'Connor, J. Zhang, M. Shi, S. N. Bisaha, Y. Wang, D. Sitkoff, A. T. Pudzianowski, C. Huang, H. E. Klei, K. Kish, J. Yanchunas, E. C.-K. Liu, K. S. Hartl, S. M. Seiler, T. E. Steinbacher, W. A. Schumacher, K. S. Atwal and P. D. Stein, *Bioorg. Med. Chem. Lett*., 2009, **19**, 6882–6889.
3 (*a*) G. B. Phillips, B. O. Buckman, D. D. Davey, K. A. Eagen, W. J. Guilford, J. Hinchman, E. L. Ho, S. Koovakkat, A. Liang, D. R. Light, M. H. P. Ng, J. M. Post, K. J. Shaw, D. Smith, B. Subramanyam, M. E. Sullivan, L. Trinh, R. Vergona, J. Walters, K. White, M. Whilow, S. Wu, W. Xu and M. M. Morrissey, *J. Med. Chem*., 1998, **41**, 3557–3562; (*b*) G. B. Phillips, D. D. Davey, K. A. Eagen, S. Koovakkat, A. Liang, M. H. P. Ng, M. Pinkerton, L. Trinh, M. Whitlow, A. M. Beatty and M. M. Morrissey, *J. Med. Chem*., 1999, **42**, 1749–1756.
4 T. Fujimoto, M. Tobisu, N. Konishi, M. Kawamura, N. Tada, T. Takagi and K. Kubo, *Bioorg. Med. Chem*., 2009, **17**, 7993–8002.
5 (*a*) Y. Imaeda, T. Kawamoto, M. Tobisu, N. Konishi, K. Hiroe, M. Kawamura, T. Tanka and K. Kubo, *Bioorg. Med. Chem*., 2008, **16**, 3125–3140; (*b*) T. Nagata, T. Yoshino, N. Haginoya, K. Yoshikawa, M. Nagamochi, S. Kobayashi, S. Komoriya, A. Yokomizo, R. Muto, M. Yamaguchi, K. Osanai, M. Suzuki and H. Kanno, *Bioorg. Med. Chem*., 2009, **17**, 1193–1206; (*c*) Y. Shi, J. Zhang, M. Shi, S. P. O'Connor, S. N. Bisaha, C. Li, D. Sitkoff, A. T. Pudzianowski, S. Chong, H. E. Klei, K. Kish, J. Yanchunas, E. C.-K. Liu, K. S. Hartl, S. M. Seiler, T. E. Steinbacher, W. A. Schumacher, K. S. Atwal and P. D. Stein, *Bioorg. Med. Chem. Lett*., 2009, **19**, 4034–4041.
6 Y. Matsumoto, M. Sugiki, M. Takayanagi, Y. Nogi, S. Taniguchi, S. Ueno and Y. Shirai, Amidine Derivatives, *WO Pat*. WO2010005087, 2010.
7 (*a*) A. Grandeury, P. Petit, S. Coste, G. Coquerel, C. Perrio and G. Gouhier, *Chem. Commun*., 2005, 4007–4009; (*b*) F. Crestey, C. Lebargy, M.-C. Lasne and C. Perrio, *Synthesis*, 2007, **21**, 3406–3410.
8 (*a*) M. W. Göbel, J. W. Bats and G. Durner, *Angew. Chem., Int. Ed. Engl*., 1992, **31**, 207–209; (*b*) S. Rensing, M. Arendt, A. Springer, T. Grawe and T. Schrader, *J. Org. Chem*., 2001, **66**, 5814–5821; (*c*) S. Rensing and T. Schrader, *Org. Lett*., 2002, **4**, 2161–2164; (*d*) K. Sada, T. Tani and S. Shinkai, *Synlett*, 2006, 2364–2374.
9 Molecular modelling calculations used Gasteiger–Marsilli charges and the force field Dreiding II compiled in *Cerius*^{2} software (v. 4.9, Accelrys Inc., 2003).
10 No complexation occurred in the presence of bis-methylsulfonic or bis-methylcarboxylic acid functions.
11 D. Kikuchi, S. Sakaguchi and Y. Ishii, *J. Org. Chem*., 1998, **63**, 6023–6026.
12 B. R. Linton, M. S. Goodman, E. Fan, S. A. van Arman and A. D. Hamilton, *J. Org. Chem*., 2001, **66**, 7313–7319.
13 J. Cui, D. Crich, D. Wink, M. Lam, A. L. Rheingold, D. A. Case, W. Fu, Y. Zhou, M. Rao, A. J. Olson and M. E. Johnson, *Bioorg. Med. Chem*., 2003, **11**, 3379–3392.
14 A. Zafar, S. J. Geib, Y. Hamuro and A. D. Hamilton, *New J. Chem*., 1998, **22**, 137–141.
15 T. Grawe, T. Schrader, R. Zadmard and A. Kraft, *J. Org. Chem*., 2002, **67**, 3755–3763.