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Calix[4]arene α-Aminophosphonic Acids: Asymmetric Synthesis and Enantioselective Inhibition of an Alkaline Phosphatase

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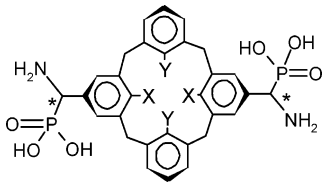
ABSTRACT



Chiral calix[4]arene α-aminophosphonic acids were obtained through diastereoselective Pudovik-type addition of sodium ethyl phosphites to the chiral calixarene imines, removal of chiral auxiliary groups, and mild dealkylation of phosphonate fragments. The diacids obtained show inhibitory activity toward porcine kidney alkaline phosphatase that depends considerably on the absolute configuration of the α-carbon atoms.

Inhibition of nonspecific alkaline phosphatases is of significant current medical interest[1] since these enzymes catalyze the hydrolysis of phosphate monoesters,[2] which are involved in a number of important biochemical pathways.

Recently, we have reported that calix[4]arenes bearing fragments of methylenebisphosphonic acid at the wide rim of the macrocycle are efficient inhibitors of calf intestine alkaline phosphatase.[3] Such an activity was explained by the coordination of bidentate bisphosphonic residues to metal ions in the enzyme active site. On the basis of these results, it was hypothesized that calix[4]arenes functionalized with chiral bidentate α-aminophosphonic acid residues[4] would possess a similarly enhanced inhibitory activity, which might depend on the configuration of the chiral carbon atoms.

Herein we report the asymmetric synthesis of calix[4]arene α-aminophosphonic acids and their efficient, enantioselective inhibitory activity toward porcine kidney alkaline phosphatase (PKAP).

The Pudovik reaction of (*S*)-imine **1**[5] (Scheme 1) with an

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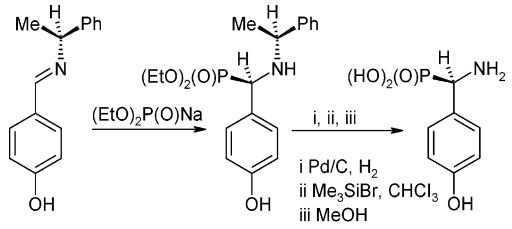
Scheme 1



excess of sodium diethyl phosphite in diethylphosphite solution at 10 °C afforded α-aminophosphonate **2** in 98% yield and 95% diastereomeric excess.[6] The subsequent removal of the phenylethyl group (Pd/C, H_2) and the dealkylation of the ester groups (Me_3SiBr, MeOH) transformed compound **2**[7] into acid **3**.[8] Single crystal X-ray analysis[9] showed that the α-carbon atom of aminophosphonate **2** has the (*R*) configuration, which is in agreement with the empirical rule for the Pudovik type addition to the α-phenylethylimines.[4]

Chiral iminocalix[4]arenes **4** and **5** were obtained in preparative yields through the condensation of mono- or 1,3-diformylcalix[4]arenes with (*R*)- or (*S*)-phenylethylamine.[10] The ensuing diastereoselective addition of sodium diethyl phosphite to the C═N bonds afforded aminophosphonates **6** and **7** in 60−80% yield and 75−85% diastereomeric excess (Scheme 2).[11]

The chiral auxiliary groups of compounds **6** and **7** were removed by catalytic hydrogenation (H_2, Pd/C) to give individual stereoisomers of calixarenes **8** and **9** in preparative yields.[12] The subsequent treatment of aminophosphonates **8** and **9** with Me_3SiBr and methanol gave mono- and di-α-aminophosphonic acids **10** and **11** in quantitative yields.[13]

The absolute configurations at the α-carbon atoms in calixarenes **8**−**11** were assigned on the basis of studies on the model compounds **2** and **3**. (*S*)-Imines give (*R*)-amino acids and vice versa (see above).

The ^{1}H NMR spectra of compounds **10** and **11** contain AB doublets for the methylene protons of the bridges that are characteristic of the C_{2V}-symmetrical pinched cone conformation.[14] There are two possible pinched cone conformers of calixarenes **10** and **11**: one with quasiparallel and the other with quasicoplanar distal unsubstituted aromatic rings. Unfortunately, the NMR spectra do not allow determination of which conformation is preferred in solution.

(6) Diastereomeric excesses were calculated through integration of ^{31}P NMR signals of diastereomers.

(7) Sodium (0.3 g, 13 mmol) was added to diethyl phosphite (5 mL) (caution!) at room temperature followed by compound **1** (4 mmol). The reaction mixture was stirred at 10 °C for 12 h, quenched with water (100 mL), and extracted with chloroform (3 × 50 mL). The chloroform layer was evaporated and the residue was washed with hexane and dried in vacuo. **(*R*,*S*)-α-Phenylethylaminodiethoxyphosphonylmethyl-4-hydroxybenzene 2.** $[\alpha]^{20}_D = +20°$ ($CHCl_3$). Colorless solid, yield 96%. Mp 119−120 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.16, 1.36 (two t, J = 7.5 Hz, each 3H), 1.30 (d, J = 7.5 Hz, 3H), 3.80−4.20 (m, 6H), 6.64 (d, J = 7.5 Hz, 2H), 7.08 (dd, J = 7.5 Hz, J = 2 Hz, 2H), 7.21−7.28 (m, 5H). ^{31}P NMR (121 MHz, $CDCl_3$) δ: 25.01.

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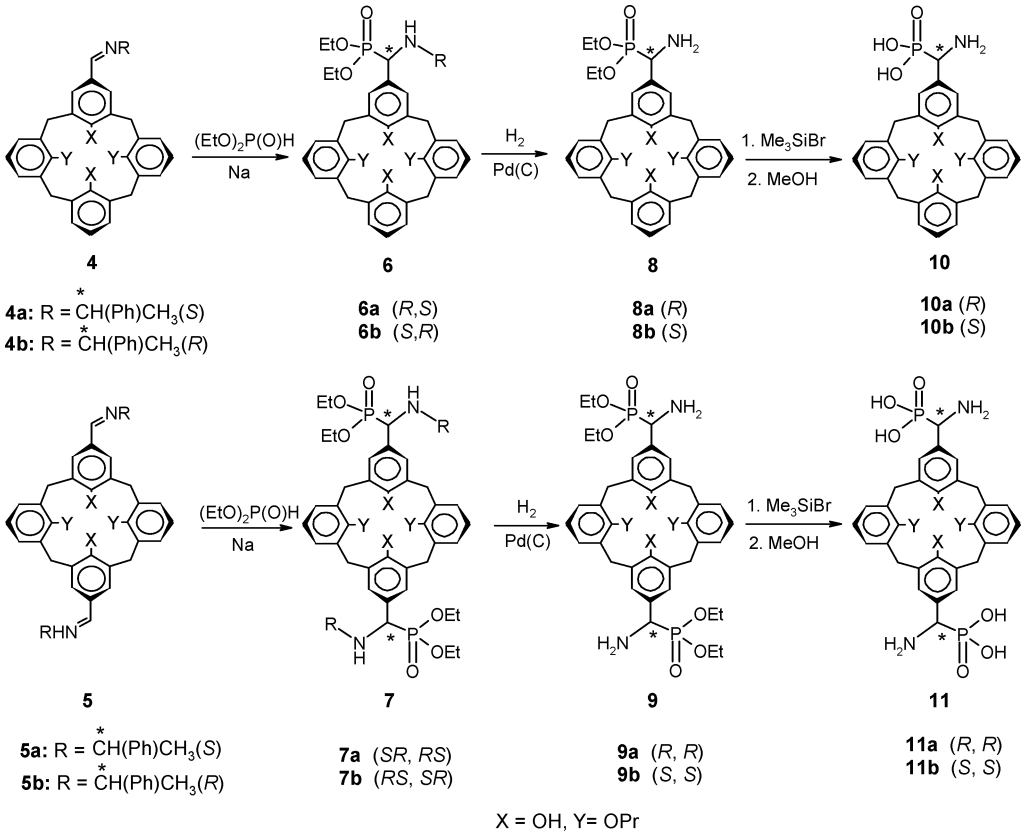
(11) **Synthesis of Aminophosphonates 6 and 7.** Sodium (0.3 g, 13 mmol) was added to diethyl phosphite (5 mL) by portions (with caution!) at room temperature. Calixarene monoimine **4** (4 mmol) or diimine **5** (2 mmol) was added to the resulting solution. The reaction mixture was stirred at 10 °C for 12 h, quenched by water (100 mL), and extracted with chloroform (3 × 50 mL). The chloroform extract was evaporated, and the residue was washed with hexane and dried in vacuo. **5-(α-Phenylethylaminodiethoxyphosphonylmethyl)-25,27-dipropoxycalix[4]arenes 6a,b.** Purified by flash column chromatography (silica, $CHCl_3$/acetone 4:1) R_f = 0.5. $[\alpha]^{28}_D = +12°$ ($CHCl_3$) for **6a**, $[\alpha]^{28}_D = -12°$ ($CHCl_3$) for **6b**. Colorless solids, yields 75−80%. Mp 119−120 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.85, 1.32 (two t, 3H+3H, J = 7.5 Hz), 1.30, 1.31 (two t, J = 7.5 Hz, each 3H), 1.33 (d, J = 7.5 Hz, 3H), 2.01 (m, 4H), 3.30 (m, 1H), 3.32, 3.34 (two d, J = 13.2 Hz, each 2H), 3.75, 4.15 (two m, each 2H), 3.91 (d, J = 11 Hz, 1H), 3.96, 3.98 (two t, J = 7.5 Hz, each 2H), 4.24, 4.26, 4.28, 4.31 (four d, J = 13.2 Hz, each 1H), 6.80−7.40 (m, 11H), 8.40, 8.55 (two s, each 1H). ^{31}P NMR (121 MHz, $CDCl_3$) δ: 26.4; **5,17-Bis(α-phenylethylaminodiethoxyphosphonylmethyl)-25,27-dipropoxycalix[4]arenes 7a,b.** Purified by column chromatography (silica, $CHCl_3$/acetone 4:1) R_f = 0.7. $[\alpha]^{28}_D = +7°$ ($CHCl_3$) for **7a**, $[\alpha]^{28}_D = -7°$ ($CHCl_3$) for **7b**. White solids, yields 60−65%. Mp 82−83 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.66, 1.32 (two t, J = 7.5 Hz, each 6H), 1.33 (m, 6H), 1.41 (d, J = 7.5 Hz, 6H), 2.01 (m, 4H), 3.36 (m, 6H), 3.66, 3.81 (two m, each 2H), 3.92 (d, J = 11 Hz, 2H), 3.96 (t, J = 7.5 Hz, 4H), 4.09 (m, 4H), 4.26, 4.30 (two d, J = 13.0 Hz, each 2H), 6.67 (t, J = 7.5 Hz, 2H), 6.81, 6.90 (two d, J = 7.5 Hz, each 2H), 6.98, 7.11 (two s, each 2H), 7.25 (m, 10H), 8.26 (s, 2H). ^{31}P NMR (121 MHz, $CDCl_3$) δ: 25.7.

(12) **General Procedure for Synthesis of Aminophosphonates 8 and 9.** Compound **6** (1.38 mmol) or **7** (1.07 mmol) was added to a suspension of Pd/C (0.2 g) in methanol (25 mL). The reaction mixture was stirred under hydrogen atmosphere at 40 °C for 72 h. The catalyst was filtered off, and the solution was evaporated in vacuo. The residue was washed by diethyl ether. **5-(Aminodiethoxyphosphonylmethy)-25,27-dipropoxycalix-[4]arenes 8a,b.** $[\alpha]^{28}_D = +30°$ ($CHCl_3$) for **8a**, $[\alpha]^{28}_D = -30°$ ($CHCl_3$) for **8b**. White solids, yields 60−65%. Mp 95−97 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.51, 0.62 (two t, J = 7.5 Hz, each 2H), 1.31 (t, J = 7.5 Hz, 6H), 2.01 (m, 4H), 3.55 (m, 4H), 3.96−3.98 (m, 4H), 4.28, 4.31 (two d, J = 13.2 Hz, each 2H), 4.60 (d, J = 11 Hz, 1H), 6.60 (t, J = 7.5 Hz, 2H) 6.71 (t, J = 7.5 Hz, 1H), 6.90−7.15 (m, 6H), 7.28, 7.30 (two s, each 1H), 8.41, 8.62 (two s, each 2H). ^{31}P NMR (121 MHz, $CDCl_3$) δ: 26.4. Calcd for $C_{39}H_{48}NO_7P$: C 69.52, H 7.18, N 2.08 P 4.60, found C 69.31, H 7.26, N 2.15 P 4.46. *m*/*z* (FAB) 536.7 ([M − P(O)OEt_2]$^+$, 80%) 657.7 ([M − NH_2]$^+$, 70%); **5,17-Bis(aminodiethoxyphosphonylmethyl)-25,27-dipropoxycalix[4]arenes 9a,b.** $[\alpha]^{28}_D = -21°$ ($CHCl_3$) for **9a**, $[\alpha]^{28}_D = +21°$ ($CHCl_3$) for **9b**. White solids, yields 85−90%. Mp 65−67 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.88, 0.95 (two t, J = 7.5 Hz, each 6H), 1.23 (t, J = 7.5 Hz, 6H), 2.01 (m, 4H), 3.32 (d, J = 13.0 Hz, 4H), 3.54, 3.75 (two m, each 2H), 3.90 (m, 8H), 4.04 (d, J = 15.0 Hz, 2H), 4.22 (d, J = 13.0 Hz, 4H), 6.61 (t, J = 7.2 Hz, 2H), 6.82, 6.84(two d, J = 7.2 Hz, each 2H), 7.09, 7.12 (two s, each 2H) 8.18 (s, 2H). ^{31}P NMR ($CDCl_3$) δ: 24.9. Calcd for $C_{44}H_{60}N_2O_{10}P_2$: C 63.00, H 7.21, N 3.34 P 4.60, found C 62.89, H 7.15, N 3.42 P 4.68 (**9a**); found C 62.91, H 7.13, N 3.38 P 4.52 (**9b**).

(13) **Synthesis of Aminophosphonic Acids 10 and 11.** Bromotrimethylsilane (8-fold molar excess per phosphonate group) was added to a solution of aminophosphonates **8** or **9** (1 mmol) in dry chloroform (5 mL). The reaction mixture was stirred at room temperature for 30 h. Then the solvent was evaporated under reduced pressure, and the residue was dissolved in absolute methanol (15 mL). The reaction mixture was stirred at 50 °C for 2 h and then was evaporated in vacuo. The residue was dried in vacuo (0.05 mm) for 10 h. **5-(Aminodihydroxyphosphonylmethyl)-25,27-dipropoxycalix[4]arenes 10a,b.** $[\alpha]^{28}_D = +18°$ ($CHCl_3$) for **10a**, $[\alpha]^{28}_D = -18°$ ($CHCl_3$) for **10b**. White solids, yields 90−95%. Mp 89 °C (decomp). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.35 (t, 6H, J = 7.5 Hz, 6H), 2.05 (m, 4H), 3.33 (br m, 4H), 3.96−3.98 (m, 4H), 4.22 (d, J = 11 Hz, 1H), 4.30 (br m, 4H), 6.62−6.71 (m, 3H), 6.92−7.17 (m, 6H), 7.28−7.30 (br s, 2H), 8.39, 8.55 (two s, each 2H). ^{31}P NMR (121 MHz, DMSO-d_6) δ: 15.75. Calcd for $C_{35}H_{40}NO_7P$: C 68.06, H 6.53, N 2.27 P 5.01, found C 68.31, H 6.64, N 2.16 P 4.91. *m*/*z* (FAB MASS) 536.7 ([M − P(O)$(OH)_2$]$^+$, 80%) 601.6 ([M − NH_2]$^+$, 75%). **5,17-Bis(aminodihydroxyphosphonylmethyl)-25,27-dipropoxycalix[4]arenes 11a,b.** $[\alpha]^{28}_D = -12°$ ($CHCl_3$) for **11a**, $[\alpha]^{28}_D = +12°$ ($CHCl_3$) for **11b**. Colorless solids, yields 90−95%. Mp 95 °C (decomp). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.24 (m, 6H), 2.01 (m, 4H), 3.32 (br m, 4H), 3.95 (br m, 4H), 4.07 (br d, J = 15 Hz, 2H), 4.19 (br m, 4H), 6.81 (br. m, 4H), 7.01(br m, 4H), 7.21 (br m, 4H), 8.61 (br s, 2H). ^{31}P NMR (121 MHz, DMSO-d_6) δ: 13.78. Calcd for $C_{36}H_{44}N_2O_{10}P_2$: C 59.50, H 6.10, N 3.85 P 8.52, found C 59.63, H 6.01, N 3.76, P 8.65 (**11a**); found C 59.41, H 6.15, N 3.79, P 8.61 (**11b**).

Scheme 2



When assayed against the hydrolysis of *p*-nitrophenylphosphate by the porcine kidney alkaline phosphatase, calixarenes **10** and **11** were found to be reversible inhibitors.

The influence of compounds **3**, **10**, and **11** on the enzyme activity is in agreement with a mixed-type inhibition mechanism. The inhibition constants K_i and K_i'[15] (Table 1) of calixarene **10a** are considerably smaller than the values for the model compound **3**. This corresponds to an increase in the stabilization of the enzyme−inhibitor complex by 1.2 kcal/mol. Such a reinforcement of the enzyme−inhibitor interactions may be caused by multiple van der Waals attractions between the bulky calixarene fragment and the surface of the binding site.

Table 1. Inhibition Constants (μM) for the Inhibition of PKAP by Compounds **3**, **10**, and **11**[a] and Free Energies (kcal/mol) for the Formation of Enzyme−Inhibitor Complexes of PKAP and BIAP

inhibitor	K_i (PKAP)	K_i' (PKAP)	ΔG^b (PKAP)	ΔG^b (BIAP)
3	580 ± 110	6800 ± 840	−4.4	−4.5
10a *(R)*	73 ± 13	540 ± 90	−5.6	−6.5
10b *(S)*	32 ± 5	780 ± 100	−6.1	−6.6
11a *(R,R)*	1.7 ± 3	130 ± 30	−7.8	−7.2
11b *(S,S)*	86 ± 11	610 ± 210	−5.5	−6.4

[a] 0.1 M Tris-HCI buffer (pH 9), 296 K. [b] $\Delta G = -RT \ln(K_i^{-1})$.

The inhibition of PKAP is sensitive to the absolute configuration of the calixarene α-aminophosphonic acids. The K_i value for isomer **10b** is about two times smaller than for its counterpart **10a**. However in terms of free energy, this difference (0.5 kcal/mol) is practically negligible. The enantioselectivity of the inhibition is drastically increased in the case of bis-α-aminophosphonic acids **11**. Namely, the (*R*,*R*) isomer **11a** binds to PKAP about 50 times stronger than the (*S*,*S*) isomer **11b**, which corresponds to a difference in free energy of 2.3 kcal/mol. Although the exact cause of this enantioselectivity remains unrevealed, it seems plausible that, at least partly, it can be attributed to the asymmetric disposition of the amino acid residues around the binding site, which may result in different sterical demands to the binding of the enantiomeric inhibitors.

Even after statistical correction, the K_i value for acid **10a** is 20 times higher than that for diacid **11a**. This can be attributed to the cooperative binding of the two α-aminophosphonate fragments of molecule **11a** to the metal cations and the arginine residue of the alkaline phosphatase binding site.[2,16] It should be noted that the free energy for the formation of the enzyme−inhibitor complex with compound **10b** is lower by 1 kcal/mol than the statistically corrected

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(15) These are dissociation constants of enzyme−inhibitor and enzyme−substrate−inhibitor complexes, respectively: Dixon, M.; Webb, E. C. *Enzymes*; Mir: Moskow, 1982 (in Russian).

value of the free energy in the case of **11b**. This shows that the attachment of the second (*S*)-α-aminophosphonate residue to the calixarene scaffold contributes to the destabilization of the enzyme−inhibitor complex. Similar effects have been observed also for the binding of compounds **10** and **11** to the enzyme−substrate complex, which is characterized by inhibition constants K_i' (Table 1).

As is seen from Table 1, compounds **10** and **11** display almost identical affinities to bovine intestinal alkaline phosphatase (BIAP). This can be explained by the coordination of only one α-aminophosphonate residue at the binding site. The impact of the calixarene is more pronounced than in the case of PKAP inhibition most probably as a result of the better fit between the inhibitor and enzyme surfaces. Surprisingly the enantioselectivity of the BIAP inhibition by calixarenes **11a** and **11b** is not pronounced, with a difference in free energy of only 0.8 kcal/mol. These results may reflect subtle differences in the active sites of BIAP and PKAP.

In conclusion, the preorganization of two α-aminophosphonic acid fragments on the calix[4]arene platform results in a significant increase in the inhibition of alkaline phosphatase. The strength of the enzyme−inhibitor interactions is determined both by cooperative effects and the goodness of the fit of the chiral inhibitor to the chiral space around the binding site. To the best of our knowledge compound **11a** is the first alkaline phosphatase inhibitor of α-aminophosphonic acid type that has an affinity for the enzyme in the micromolar range.

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