# Synthesis of new bifunctional chelating agents: (1*R**,2*R**,4*S**)-4-isothiocyanatocyclohexane-1,2-diamine-*N*,*N*,*N*′,*N*′-tetrakis-methanephosphonic acid (4-ICMP) and (1*R**,2*R**,4*S**)-4-isothiocyanatocyclohexane-1,2-diamine-*N*,*N*,*N*′,*N*′-tetrakis-ethanephosphonic acid (4-ICEP)

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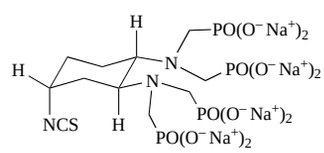
**Immunotherapy with radiolabelled antibodies should allow fairly specific targeting of certain cancers. Although $^{153}$Sm possesses favourable radiophysical characteristics for such therapy, a lack of radiolabelling stability *in vivo* limits its application. Yet the use of phosphonic semi-rigid bifunctional chelating agents (BCA) can improve its stability. Syntheses of (1*R**,2*R**,4*S**)-4-isothiocyanato-cyclohexane-1,2-diamine-*N*,*N*,*N*′,*N*′-tetrakismethanephosphonic acid (4-ICMP) and (1*R**,2*R**,4*S**)-4-isothiocyanatocyclohexane-1,2-diamine-*N*,*N*,*N*′,*N*′-tetrakisethanephosphonic acid (4-ICEP) are described in this paper. The complexing properties of these two compounds has been confirmed by labelling with $^{153}$Sm. A novel synthetic approach involving the introduction of methanephosphonic functions has also been developed.**

## Introduction

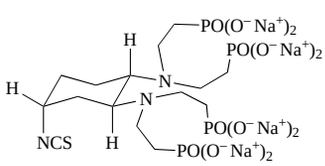
Although $^{153}$Sm has a half-life of 46.27 h, a mean $\beta^-$ energy [$E_{max}$ = 810 (20%), 710 (50%) and 640 (30%) keV] suitable for radioimmunotherapy[1] of microscopic tumours and a $\gamma$ emission with an energy of 103 keV (28%), facilitating gamma camera detection, its application is limited. Results obtained with antibodies radiolabelled with this isotope, using diethylenetriaminepentaacetic acid (DTPA, the ligand of reference),[2] 2-(*p*-isothiocyanatobenzyl)-6-methyldiethylenetriaminepentaacetic acid (MX-DTPA)[3] or 6-(*p*-isothiocyanatobenzyl)diethylenetriaminepentaacetic acid (CITCDTPA),[4] have shown that labelling stability, though improved with the last two bifunctional chelating agents (BCAs), is not satisfactory in biological environments, causing nonspecific uptake by liver and bone tissue. The incapacity of these BCAs to complex $^{153}$Sm in a stable manner led us to develop new ligands which provide more stable complexes *in vivo*.

This paper reports the synthesis of two new BCAs, (1*R**,2*R**,4*S**)-4-isothiocyanatocyclohexane-1,2-diamine-*N*,*N*,*N*′,*N*′-tetrakismethanephosphonic acid (4-ICMP) **1**, an analogue of cyclohexanediamine-*N*,*N*,*N*′,*N*′-tetrakismethanephosphonic acid (CDTMP) and (1*R**,2*R**,4*S**)-4-isothiocyanatocyclohexane-1,2-diamine-*N*,*N*,*N*′,*N*′-tetrakisethanephosphonic acid (4-ICEP) **2**. Studies confirmed the possibility of complexing a lanthanide such as $^{153}$Sm.

Our investigations were guided by two reports in the literature. A first study[5] performed on polyaminocarboxylic ligands incorporating the skeleton of ethylenediaminetetraacetic acid (EDTA) in a cyclohexane structure showed the influence of this semi-rigid structure on the stability of the resulting complexes. Through reduction of free rotation around the EDTA ethylene bridge, the effect of the cyclohexane design is to preorient the four acetate functions in a skew position.[6] A second study,[7] on the stability of polyaminophosphonic complexes of $^{153}$Sm, showed that ethylenediaminetetrakismethanephosphonic acid (EDTMP) derivatives allowed stable, quantitative $^{153}$Sm chelation to be obtained during several days. These results are compatible with certain stability constants[8] according to which poly(aminomethanephosphonic) ligands form stabler complexes with $^{153}$Sm than do their polyaminocarboxylic analogues.



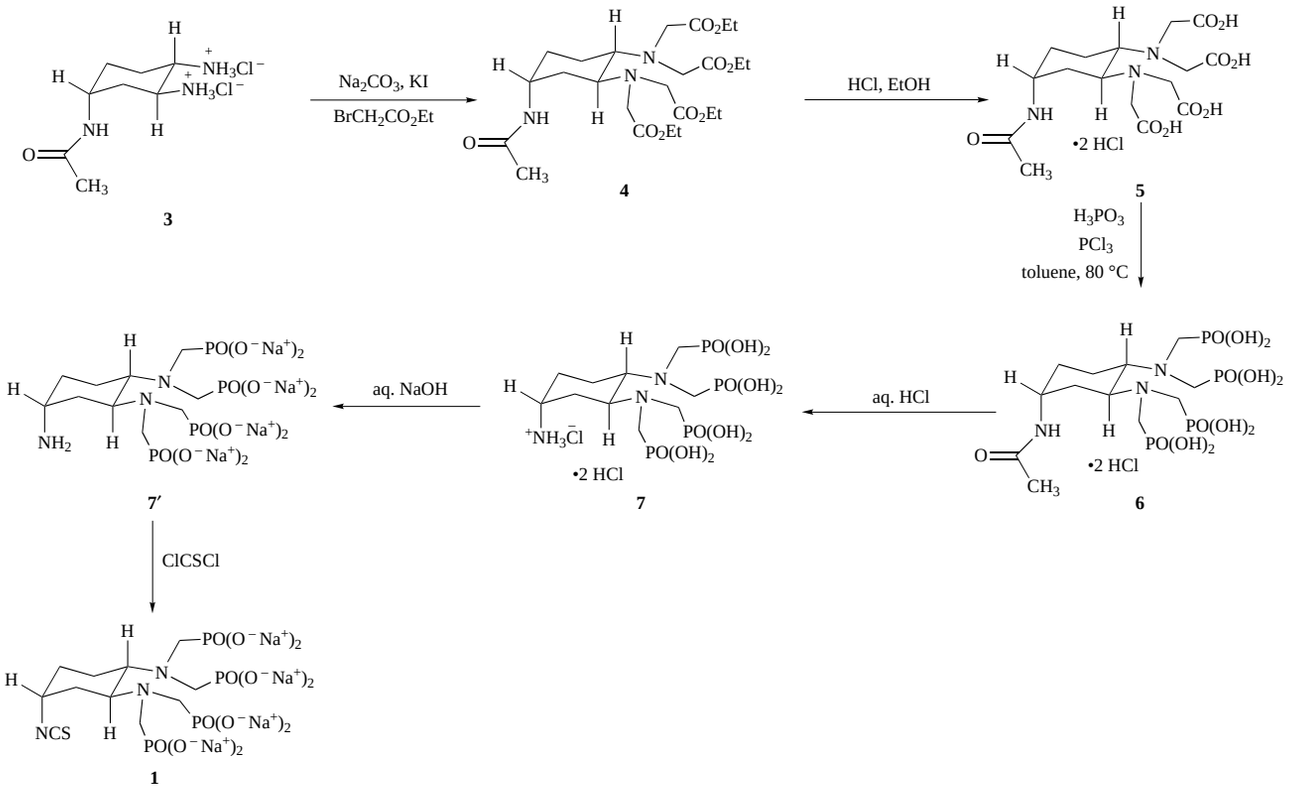

**1** 4-ICMP




**2** 4-ICEP

These observations led us to use these two properties in the synthesis of the two chelating agents. The stability of these new complexing cages was thus ensured by incorporation of the EDTA skeleton into a cyclohexanic semi-rigid structure, allowing the ligand cavity to be adapted to the size of the metallic element to be complexed, as well as the introduction of phosphonic acid functions more favourable to the chelation of $^{153}$Sm.

Moreover, the functionalization of the cyclohexanic structure by an isothiocyanate termination[9] allowed coupling to an antibody or any other biological substance such as a hapten. Certain methods[10,11] use a coordination site for functionalization, but Mease[12] has generated this function opposite the complexation site, at position 4 of the cycle, leaving the coordination centres free.



**Scheme 1** Synthesis of 4-ICMP, a BCA ready to be used in a coupling reaction

## Results and discussion

Several access routes to α-aminomethanephosphonic acids from a primary amine have been described in the literature. The two methods which appear to be most commonly used (the Moedritzer–Irani[13] and Schwarzenbach reactions[14]) were considered for the synthesis of phosphonic tetraacid **6**. Despite the apparent simplicity of the purification step described by the authors, compound **6** has never been obtained in this way with satisfactory purity. In fact, the application of these two procedures to our diamine **3** produced an acid mixture which could not be correctly separated on a Dowex-type ion-exchange column.

More recently, the method of Krüger and Bauer,[15] rediscovered by Baily and Burgada[16] in their work on α-aminophosphonic acids, allowed CDTMP to be obtained in a single step from cyclohexane-1,2-diamine-*N*,*N*,*N*′,*N*′-tetraacetic acid (CDTA), with a yield of 82%. Given the drawbacks of the first two methods cited, our strategy was to integrate the synthesis of carboxylic tetraacid **5**, the precursor of phosphonic tetraacid **6**.

In a first step (Scheme 1), 4-acetamido-*trans*-1,2-diaminocyclohexane **3** was alkylated by ethyl bromoacetate under the conditions recommended by Studer[17] (KI and $Na_2CO_3$), to give tetraester **4**. Hydrolysis of the ester functions was performed in 3 M hydrochloric acid under conditions such that the amide function was not affected. In a second step, the carboxylic functions were converted into phosphonic functions by the $H_3PO_3$/$PCl_3$ in toluene at 80 °C. Twenty (20) h were required to ensure the total conversion of the four carboxylic functions. The primary amine function of compound **6** was released in the form of the amine salt by cleavage of the acetamide function in acid medium (3 M HCl) for 7 days at 100 °C, to give compound **7**. When treated by 3 M NaOH, acid **7** yielded its sodium salt **7**′, thus releasing its primary free amine function. In a final step,[18] thiophosgene reacted with salt **7**′ to give the bifunctional chelating agent **1** 4-ICMP.

The synthesis of chelating agent **2** (Scheme 2) was simpler, requiring fewer steps. To gain access to such a structure with two carbon atoms between the nitrogen and phosphorus atoms, the Michael-type addition[19] of a nucleophile derivative on an activated double bond (as described in the literature) was performed successfully.

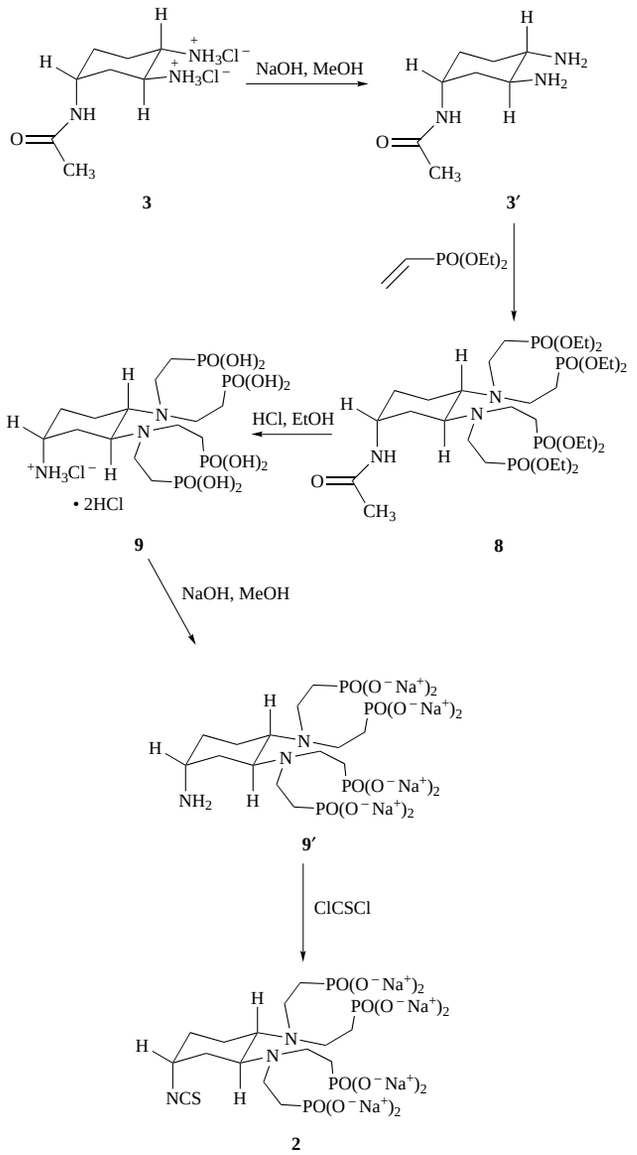
The free primary functions of diamine **3**′, obtained after treatment of compound salt **3** with 3 M NaOH, reacted with diethyl ethylenephosphonate to give tetraphosphonate **8**. Hydrolysis of the ester functions as well as cleavage of the acetamide group was performed in 3 M HCl for 7 days at 100 °C. Finally, released amine **9**′ reacted with thiophosgene, giving compound **2**: 4-ICEP. Owing to its relative instability, compound **2** is prepared just prior to reaction with the suitable amino-group-carrying vector. Presence of the SCN function is confirmed by its 2300 $cm^{-1}$ IR band.

### Complexation tests

Tests performed with the two synthesized BCA revealed the complexing properties for a $^{153}Sm$ : BCA ratio ranging from 1 : 1 to 1 : 10. No significant differences were observed as a result of the increased size of the cycle. Studies of complexation kinetics and stability in serum medium should provide further information on this point.

Our intermediate compound **3** [(1*R**,2*R**,4*S**)-4-acetamido-1,2-diaminocyclohexane dihydrochloride], which we consider quite promising in this field of investigation, allowed access to two new semi-rigid phosphonic ligands with different cage sizes. The structure of these BCAs resulted from a combination of different properties. They possess methane- or ethanephosphonic acid functions propitious to the chelation of $^{153}Sm$ which, thanks to the incorporation of the EDTA skeleton into a cyclohexane structure, are preoriented in a skew orientation and thus ensure the stability of the complexing cage. Moreover, the isothiocyanate function, grafted to the rear of the cycle, does not hinder access to the complexation site and allows coupling to the antibody.

Thus, after the failure of classical methods, compound 4-ICMP was synthesized by a method which has until now been rarely used. This approach, despite the disadvantage of adding



**Scheme 2** Synthesis of 4-ICEP, a BCA ready to be used in a coupling reaction

two steps to the synthesis procedure, allows compound 4-ICMP to be obtained with very satisfactory purity.

## Experimental

### General procedures

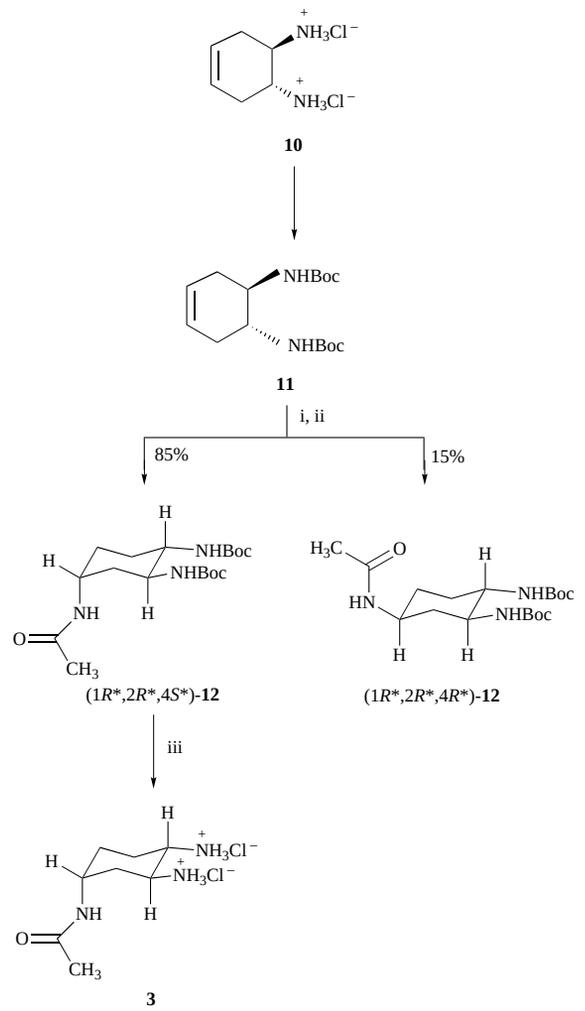
All experiments were performed under nitrogen. Solvents were distilled prior to reactions. The primary chemicals used were commercial products (Sigma-Aldrich Company). Product purity and reaction progress were monitored on TLC plates (60 $F_{254}$, Merck), and liquid chromatography was carried out on a silica gel column (Merck 60, 70–230 mesh). TLC revelation was performed under UV light (254 nm) or by iodine.

### Nuclear magnetic resonance (NMR) spectrometry

$^{1}H$ and $^{13}C$ NMR spectra were recorded on a Bruker AC 250 spectrometer (250 MHz) and $^{31}P$ NMR spectra on a Bruker AC 300 spectrometer. Chemical shifts are reported in ppm to phosphoric acid as reference (85% $H_3PO_4$ in 'heavy water'), positive values being downfield. Chemical shifts ($\delta$) are reported in ppm. Coupling constants $J$ are reported in Hertz (Hz).

### Mass spectrometry (MS)

MS spectra were recorded on a Mat Finnigan LCQ Ion Trap mass apparatus using the electrospray method in negative or positive mode.



**Scheme 3** Synthesis of (1*R**,2*R**,4*S**)-4-acetamido-1,2-diaminocyclohexane dihydrochloride. *Reagents:* i, $Hg(NO_3)_2$, $CH_3CN$; ii, $NaBH_4$, NaOH; iii, HCl, acetone.

### Starting material

Compound **3** was synthesized in our laboratory according to the procedure of Sweet *et al.*[20] with minor modifications. Briefly, in the acetamidomercuriation–demercuriation stage,[21] a mixture of two non-isolated diastereoisomers was obtained. The improvement over the procedure described in our previous work [22] (see Scheme 3) consisted in separating the two diastereoisomers (1*R**,2*R**,4*S**)-4-acetamido-1,2-bis(*tert*-butoxycarbonylamino)cyclohexane (1*R**,2*R**,4*S**)-**12** and (1*R**,2*R**,4*R**)-4-acetamido-1,2-bis(*tert*-butoxycarbonylamino)cyclohexane (1*R**,2*R**,4*R**)-**12**, enabling us to continue our syntheses on the deprotected major diastereoisomer **3** (1*R**,2*R**,4*S**)-4-acetamido-1,2-diaminocyclohexane dihydrochloride.

### Synthesis and spectroscopic data

**Tetraethyl (1*R**,2*R**,4*S**)-4-acetamidocyclohexane-1,2-diamine-*N*,*N*,*N*′,*N*′-tetraacetate 4.** To a suspension of the salt **3** (1 g, 4.10 mmol) in 50 ml of freshly distilled $CH_3CN$ were added $Na_2CO_3$ (2.22 g, 20.90 mmol) and KI (0.75 g, 4.51 mmol). After stirring of the mixture for 1 h at 60 °C, $BrCH_2CO_2Et$ (2.32 ml, 20.92 mmol) was added dropwise. The reaction mixture was kept at this temperature over a period of 48 h prior to cooling to room temp., filtration and concentration under reduced pressure. The residue was taken up in $CHCl_3$ (200 ml) and washed with water. The organic layer was dried ($Na_2SO_4$) and concentrated under reduced pressure to give a viscous brown oil. The crude product was purified by column

chromatography (silica gel; $CH_2Cl_2$–EtOH 100 : 5). The fractions containing pure product were collected and dried to give a *yellow oil* (0.92 g, 43%) (Found: C, 55.72; H, 8.12; N, 8.28. $C_{24}H_{41}N_3O_9$ requires C, 55.91; H, 8.01; N, 8.15%); $\delta_H(CDCl_3)$ 1.26 (m, 12 H), 1.29–1.70 (m, 4 H), 1.96 (s, 3 H), 1.91–2.16 (m, 2 H), 2.81 (m, 1 H), 3.10 (m, 1 H), 3.61 (d, 8 H), 4.13 (m, 9 H) and 5.64 (m, 1 H); $\delta_C(CDCl_3)$ 14.17 (4 C), 23.12, 23.44, 28.52, 31.58, 44.77, 52.74 (2 C), 52.91 (2 C), 58.08, 60.57 (4 C), 60.79, 169.68, 171.99 (2 C) and 172.19 (2 C); $(M + H^+)$, 516.

**(1*R**,2*R**,4*S**)-4-Acetamidocyclohexane-1,2-diamine-*N*,*N*,*N*′,*N*′-tetraacetic acid 5.** To a solution of the resulting oil **4** in 20 ml of ethanol was added 3 M hydrochloric acid (7.15 ml, 21.50 mmol). The reaction mixture was then stirred overnight under reflux. The refrigerant was removed, and the reaction mixture was kept at 70 °C to evaporate the ethanol. Additional 3 M hydrochloric acid (7.15 ml, 21.50 mmol) was then added, and the solution was heated to dryness. The crude residue was dissolved in a minimum volume of water prior to precipitation of acid **5** with acetonitrile. The resulting solid was filtrated off, and washed with warm acetonitrile.The purification step was repeated twice to give *title acid* **5** as a solid, which was dried under vacuum and kept under nitrogen (0.62 g, 73%) [Found: C, 40.42; H, 5.53; N, 8.93. $C_{16}H_{25}N_3O_9(HCl)_2$ requires C, 40.34; H, 5.71; N, 8.82%]; $\delta_H(D_2O)$ 1.55–2.45 (m, 6 H), 1.97 (s, 3 H), 3.35 (m, 1 H), 3.50 (m, 1 H), 3.55–4.10 (m, 9 H) and 4.14 (m, 1 H); $\delta_C(D_2O)$ 20.95, 24.72, 30.05, 30.25, 47.55, 55.16 (2 C), 55.56 (2 C), 60.29, 65.58, 173.26, 173.92, 175.61, 176.83 and 176.06; $(M - H^+)$, 402.

**(1*R**,2*R**,4*S**)-4-Acetamidocyclohexane-1,2-diamine-*N*,*N*,*N*′,*N*′-tetrakismethanephosphonic acid 6.** A mixture of tetraacid **5** (0.2 g, 0.42 mmol) and phosphorous acid (0.152 g, 1.84 mmol) in dry toluene was heated to 80 °C and stirred for 30 min. $PCl_3$ (0.16 ml, 1.84 mmol) was then added dropwise, and the reaction mixture was kept at this temperature for 20 h before being cooled to room temp. The solvent was discarded and the residual product was dissolved in a small volume of water. After filtration, the filtrate was evaporated to give a residue, which was purified by precipitation in warm acetone and collected by filtration. The purification step was repeated twice to give title *phosphonic tetraacid* **6** which was dried under vacuum and kept under nitrogen (0.18 g, 69%) [Found: C, 23.31; H, 4.89; N, 6.84. $C_{12}H_{29}N_3O_{13}P_4(HCl)_2$ requires C, 23.24; H, 5.04; N, 6.77%]; $\delta_H(D_2O)$ 1.55–3.00 (m, 6 H), 1.96 (s, 3 H) and 2.85–4.25 (m, 12 H); $\delta_C(D_2O)$ 20.74, 24.73, 28.86, 30.04, 47.73, 52.49 (2 C, d, $J_{CP}$ 143), 60.55 (2 C, d, $J_{CP}$ 120), 61.41, 66.36 and 176.90; $\delta_P(D_2O$; pH 14) 4 lines at 19.56, 19.24, 18.63 and 18.91; $(M - H^+)$, 546.

**(1*R**,2*R**,4*S**)-4-Aminocyclohexane-1,2-diamine-*N*,*N*,*N*′,*N*′-tetrakismethanephosphonic acid 7.** Compound **6** (0.1 g, 0.16 mmol) was dissolved in 3 M hydrochloric acid (10 ml, 30 mmol) and the solution was stirred under reflux for 7 days. The reaction mixture was concentrated to small volume and added dropwise to acetone to give a precipitate, which was collected by filtration, washed with acetone, and dried under vacuum to obtain *title compound* **7** as a solid (0.080 g, 81%) [Found: C, 19.72; H, 4.77; N, 6.75. $C_{10}H_{27}N_3O_{12}P_4(HCl)_3$ requires C, 19.54; H, 4.92; N, 6.84%]; $\delta_H(D_2O)$ 1.50–2.50 (m, 8 H) and 2.80–4.15 (m, 11 H); $\delta_C(D_2O)$ 20.11, 27.78, 33.08, 49.44, 52.90 (2 C, d, $J_{CP}$ 140), 59.38, 60.82 (2 C, d, $J_{CP}$ 120) and 67.06; $(M - H^+)$, 504.

**(1*R**,2*R**,4*S**)-4-Isothiocyanatocyclohexane-1,2-diamine-*N*,*N*,*N*′,*N*′-tetrakismethanephosphonic acid octasodium salt 1.** Compound **7** (0.080 g, 0.13 mmol) was dissolved in water, the pH adjusted to 12 using aq. 3 M sodium hydroxide and the solution was stirred for 30 min. After removal of water under reduced pressure, the residue was dissolved in MeOH. Filtration and evaporation yielded octasodium salt **7′** which was dissolved without further purification in freshly distilled methanol (20 ml). A 0.2 M solution of thiophosgene (0.20 mmol, 1 ml) in dried methylene dichloride was added to the reaction mixture and the whole was stirred for 2 h at room temp. The yellow precipitate **1** was filtered off, washed with acetone, and dried under vacuum (0.082 g, 87%); $\nu_{max}$(KBr)/$cm^{-1}$ new band at 2100 (SCN).

**Tetraethyl (1*R**,2*R**,4*S**)-4-acetamidocyclohexane-1,2-diamine-*N*,*N*,*N*′,*N*′-tetrakisethanephosphonate 8.** Compound **3** (1 g, 4.10 mmol) was dissolved in water and the pH was adjusted to 12 with aq. 3 M sodium hydroxide. The solution was then stirred for 30 min. After removal of water under reduced pressure, the residue was taken up with MeOH. Salt residues were filtered out, and the resulting solution was evaporated to give free diamine **3′** without further purification. To a mixture of diamine **3′** in dry MeOH (50 ml) was added dropwise diethyl ethylenephosphonate (2.83 ml, 18.45 mmol). The solution was then stirred under reflux for 18 h before the reaction mixture was concentrated under reduced pressure. The residue was then taken up in $CH_2Cl_2$ and washed with water. The combined organic layers were dried ($Na_2SO_4$) and concentrated under reduced pressure to give a yellow oil. Chromatography of the residual oil over silica gel (MeOH) yielded the pure *tetraphosphonate* **8** (1.62 g, 48%) (Found: C, 46.25; H, 8.64; N, 4.92. $C_{32}H_{69}N_3O_{13}P_4$ requires C, 46.43; H, 8.40; N, 5.08%); $\delta_H(CD_3OD)$ 1.34 (m, 24 H), 1.37–1.56 (m, 4 H), 1.98 (s, 3 H), 1.75–2.41 (m, 8 H + 2 H), 2.50–3.10 (m, 8 H + 2 H) and 4.11 (m, 17 H); $\delta_P(CD_3OD)$ 33.31 (d, 4 P); $(M + H^+)$, 829.

**(1*R**,2*R**,4*S**)-4-Aminocyclohexane-1,2-diamine-*N*,*N*,*N*′,*N*′-tetrakisethanephosphonic acid 9.** To a solution of oil **8** in 10 ml of ethanol was added 3 M hydrochloric acid (13.3 ml, 40 mmol). The refrigerant was removed and the reaction mixture was kept at 70 °C overnight. After the evaporation off of ethanol, additional 3 M hydrochloric acid (13.3 ml, 40 mmol) was added, and the solution was stirred under reflux for 7 days. The solution was then concentrated to small volume and added dropwise to acetone to give a precipitate, which was collected by filtration, washed with acetone, and dried under vacuum to obtain *title acid* **9** as a solid (1.1 g, 83%) [Found: C, 25.33; H, 5.56; N, 6.42. $C_{14}H_{35}N_3O_{12}P_4(HCl)_3$ requires C, 25.07; H, 5.71; N, 6.27%]; $\delta_H(D_2O)$ 1.70–2.50 (m, 6 H), 2.10 (m, 8 H) and 3.20–3.75 (m, 11 H); $\delta_C(D_2O)$ 23.31, 26.03, 27.01 (4 C, d, $J_{CP}$ 124), 29.11, 44.98 (2 C), 45.11 (2 C), 46.68, 55.63 and 56.06; $\delta_P(D_2O)$ 24 (d, 4 P); $(M - H^+)$, 560.

**(1*R**,2*R**,4*S**)-4-Isothiocyanatocyclohexane-1,2-diamine-*N*,*N*,*N*′,*N*′-tetrakisethanephosphonic acid octasodium salt 2.** Compound **9** (0.2 g, 0.298 mmol) was dissolved in water and the pH was adjusted to 12 using aq. 3 M sodium hydroxide. The solution was then stirred for 30 min. After removal of water under reduced pressure, the residue was redissolved in MeOH. Filtration and evaporation yielded octasodium salt **9′**, which was dissolved in freshly distilled methanol (20 ml) without further purification. A 0.2 M solution of thiophosgene (0.45 mmol, 2.25 ml) in dried methylene dichloride was then added and the reaction mixture was stirred for 2 h at room temp. The yellow precipitate was filtered off, washed with acetone, and dried under vacuum to give title acid **2** as a yellow solid (0.213 g, 92%); $\nu_{max}$(KBr)/$cm^{-1}$ new band at 2100 (SCN).

### Complexation tests

Complexation studies for 4-ICEP and 4-ICMP were performed at a $^{153}$Sm : BCA ratio ranging from 1 : 1 to 1 : 10 at pH 6 [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer 0.1 M]. Solutions of compounds **1** ($1.0 \times 10^{-6}$ g; $1.68 \times 10^{-9}$ mol) and ($1.0 \times 10^{-5}$ g; $1.68 \times 10^{-8}$ mol) in anhydrous dimethyl sulfoxide (DMSO) were added to a solution of $^{153}$Sm ($2.0 \times 10^{-3}$ ml; $1.68 \times 10^{-9}$) and HEPES buffer (20 μl). Likewise, solutions of compound **12** ($1.3 \times 10^{-6}$ g; $1.68 \times 10^{-9}$ mol) and ($1.3 \times 10^{-5}$ g; $1.68 \times 10^{-8}$ mol) in anhydrous DMSO were added to a solution of $^{153}$Sm ($2.0 \times 10^{-3}$; $1.68 \times 10^{-9}$ mol) and HEPES buffer (20 μl). The solutions were stirred and then left at room temp. for 1 h. Complexation was measured on a Phosphorimager 445SI apparatus after Gelman ITLC using as

eluate a citrate buffer mixture (0.1 M; pH 5 : MeOH; 1 : 4 for compound **1** and 1 : 16 for compound **2**).

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