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(54) Titre : COMPOSES POLYAZAMACROCYCLIQUES POUR LA COMPLEXATION D'IONS METALLIQUES

(54) Title: POLYAZAMACROCYCLIC COMPOUNDS FOR COMPLEXATION OF METAL IONS

(57) **Abrégé/Abstract:**

The present invention relates to a new polyazamacrocyclic compound or a salt thereof and its uses. The compound has formula (I), where x is 2, 3 or a combination of p 2(s) and q 3(s) where p+q=y; y is 3 or 4; R is $(CH_2)_zP(=O)(R^1)(OR^2)$; R¹ is R³ or OR³ where R³ is alkyl, cycloalkyl or aryl; R² is H, alkyl or a where R⁴ is alkyl, cycloalkyl or aryl; and z is 1 to 3. In one important embodiment, this compound may be complexed with a metal to be a polyazamacrocyclic compound-metal complex having formula (II), where r is 2 or 3; and Me is a metal ion. Such complexes are useful for enhancing a magnetic resonance image of a subject.





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<table style="width: 100%; border: none;"> <tr> <td style="width: 50%; vertical-align: top; border-right: 1px solid black; padding: 5px;"> (21) International Application Number: PCT/US91/08438 (22) International Filing Date: 12 November 1991 (12.11.91) (30) Priority data: 615,619 19 November 1990 (19.11.90) US (71) Applicant: BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM [US/US]; 201 West 7th Street, Austin, TX 78701 (US). (72) Inventors: SHERRY, A., Dean ; 6934 Spanky Branch, Dallas, TX 75248 (US). LAZAR, Istvan ; 601 W. Renner Rd., Apt. 124, Richardson, TX 75080 (US). RAMASAMY, Ravichandran ; 7220 McCullum Blvd., Apt. 905, Dallas, TX 74252 (US). BRUCHER, Erno ; Sestakert 5, H-4032 Debrecen (HU). </td> <td style="width: 50%; padding: 5px;"> (74) Agent: HODGINS, Daniel, S.; Arnold, White & Durkee, P.O. Box 4433, Houston, TX 77210 (US). (81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), GR (European patent), HU, IT (European patent), JP, KP, LU (European patent), NL (European patent), NO, SE (European patent). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> </td> </tr> </table>			(21) International Application Number: PCT/US91/08438 (22) International Filing Date: 12 November 1991 (12.11.91) (30) Priority data: 615,619 19 November 1990 (19.11.90) US (71) Applicant: BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM [US/US]; 201 West 7th Street, Austin, TX 78701 (US). (72) Inventors: SHERRY, A., Dean ; 6934 Spanky Branch, Dallas, TX 75248 (US). LAZAR, Istvan ; 601 W. Renner Rd., Apt. 124, Richardson, TX 75080 (US). RAMASAMY, Ravichandran ; 7220 McCullum Blvd., Apt. 905, Dallas, TX 74252 (US). BRUCHER, Erno ; Sestakert 5, H-4032 Debrecen (HU).	(74) Agent: HODGINS, Daniel, S.; Arnold, White & Durkee, P.O. Box 4433, Houston, TX 77210 (US). (81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), GR (European patent), HU, IT (European patent), JP, KP, LU (European patent), NL (European patent), NO, SE (European patent). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
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(54) Title: POLYAZAMACROCYCLIC COMPOUNDS FOR COMPLEXATION OF METAL IONS $\left[\{ (\text{CH}_2)_x \text{NR} \}_y \right] \quad (\text{I})$ $\left[\{ (\text{CH}_2)_x \text{NR} \}_y \right] \text{Me}^{+r}, \quad (\text{II})$ $\begin{array}{c} \text{O} \\ \parallel \\ \text{CR}^4 \end{array} \quad (\alpha)$				
(57) Abstract The present invention relates to a new polyazamacrocyclic compound or a salt thereof and its uses. The compound has formula (I), where x is 2, 3 or a combination of p 2(s) and q 3(s) where p+q=y; y is 3 or 4; R is (CH₂)₂P(=O)(R¹)(OR²); R¹ is R³ or OR³ where R³ is alkyl, cycloalkyl or aryl; R² is H, alkyl or α where R⁴ is alkyl, cycloalkyl or aryl; and z is 1 to 3. In one important embodiment, this compound may be complexed with a metal to be a polyazamacrocyclic compound-metal complex having formula (II), where r is 2 or 3; and Me is a metal ion. Such complexes are useful for enhancing a magnetic resonance image of a subject.				

**POLYAZAMACROCYCLIC COMPOUNDS FOR
COMPLEXATION OF METAL IONS**

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The present invention relates to a series of new
15 phosphorous containing macrocyclic chelates which bind
certain biological ions with relative specificity. The
 ^{31}P resonance of these chelators shift to a new position
in the NMR spectrum when a metal ion occupies the cavity
of the chelate and the NMR chemical shift of the bound
20 chelate differs for each metal ion. This property should
be useful for monitoring the intracellular concentrations
of certain biological cations including for example, Mg^{2+} ,
 Ca^{2+} , and Zn^{2+} , by ^{31}P NMR. There are several fluorescent
chelators commercially available for measuring
25 intracellular cation concentrations and 1 or 2 fluorine
containing chelators for NMR purposes. The fluorescent
chelators will likely not be applicable for human studies
and the ^{19}F chelators are not as likely to be applied
clinically as the ^{31}P chelators of the present invention.
30 Also, certain of the chelators when bound to Gd^{3+} has
properties which should make it a safe, effective
contrast agent for magnetic resonance imaging.

A great number of biological systems require the
35 diamagnetic cations, Ca^{2+} , Mg^{2+} , and Zn^{2+} to regulate
various reactions. The role of Ca^{2+} as an intracellular

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5 messenger ion in many types of cells is well established [1]. Mg^{2+} is a required cofactor for virtually all biological reactions involving ATP and may play an extensive role in the buffering of a greater variety of biological reactions [2]. Zn^{2+} is bound rather tightly in the active sites of several enzymes and its role appears to involve polarization of chemical bonds to aid bond hydrolysis, oxidation-reduction, or group transfer reactions. However, free Zn^{2+} may play a much wider role in some cells such as brain cells where it is known to be stored in neurons as storage granules and is mobilized during electrophysiologic activation [3].

15 An evaluation of the role of divalent cations in cell function has been limited by the availability of direct methods for measuring free cation concentration in cells and perfused organs. Presently available methods for measurement of divalent cations include indirect calculations based on equilibrium reactions [4], ion-selective micro-electrodes [5,6], and null point measurements using metallochromic dyes that are either microinjected into cells [7] or placed into the extracellular space with subsequent lysis of cells [8,9]. Virtually all of these methods are invasive in nature and require sample destruction prior to analysis. By contrast, NMR has made considerable advances as a non-invasive tool in measuring intracellular free divalent cation concentrations in perfused organs and intact cells.

30

Recently, fluorinated chelators have been effectively used to measure intracellular free Ca^{2+} [10,11] and Mg^{2+} [12,13] in cells and perfused organs by ^{19}F NMR. These chelators work reasonably well in isolated cell systems but suffer from unexpected line broadening when used in perfused organs [13,14]. Another

35

disadvantage with these systems is the synthetic routes to these compounds limits the possible chelate structures and hence metal-ion selectivity that may be designed into the chelate. Aspects of the present invention include
5 the synthesis and development of a series of triaza and tetraaza macrocyclic phosphonate monoesters and alkyl phosphinates as ^{31}P NMR indicators to detect intracellular free cations in biological systems.

10 The present invention also relates to NMR imaging of living subjects, sometimes referred to as MRI (magnetic resonance imaging). More specifically, it relates to agents which can be used to enhance NMR contrast in such subjects.

15 Nuclear magnetic resonance (NMR) has been used for many years as a means of chemical analysis. NMR is a type of radio frequency spectroscopy which is based upon small energy differences between electrically charged
20 atomic nuclei which are spinning parallel or antiparallel to an applied magnetic field. When radio frequency energy is applied to the sample, these spinning atomic nuclei change spin states and in doing so, absorb some of the radio frequency energy. Nuclei in slightly different
25 chemical environments within the same molecule change spin state at slightly different energies and this produces characteristic absorptions or resonances which help identify the molecular structure.

30 NMR has more recently been used in examinations of the human body. Other methods such as computerized axial tomography (CAT scanning) have been used in the past for this purpose, and still are. However, because NMR does not use ionizing radiation, it is believed to have some
35 safety advantages over CAT. Thus, NMR is an advantageous

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method of producing cross-sectional images of the human body.

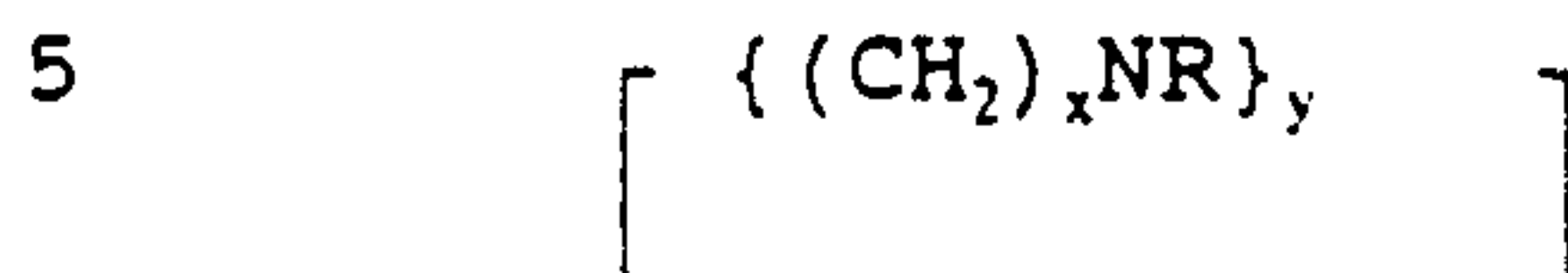
5 The quality of the images obtained from an NMR scan is based on two properties: the proton densities of the various tissues and differences in proton relaxation rates. The proton density of tissues cannot be readily altered. Proton relaxation rates can be adjusted by adding a paramagnetic relaxation agent, more commonly
10 known as a "contrast agent." Contrast agents enhance the contrast in NMR images between magnetically similar but histologically dissimilar tissues.

15 Gadolinium has been tested as a contrast agent in the past because it has a large magnetic moment, which efficiently relaxes magnetic nuclei. Gadolinium's strong paramagnetic properties are the result of its seven unpaired electrons.

20 One drawback of gadolinium as a contrast agent is its toxicity to animals. One possible remedy for this problem is to incorporate gadolinium in a compound that would pass through the body and be excreted without releasing toxic gadolinium ions. Unfortunately, the rare
25 earth elements, such as gadolinium, do not form stable covalent bonds with organic molecules, so such molecules can decompose in vivo and release the toxic ions.

30 There is a need for effective contrast agents which avoid the toxicity problems inherent in using gadolinium. Further, there is a need for new and better contrast agents, whether they include gadolinium or another paramagnetic metal.

The present invention relates to a new polyazamacrocyclic compound or a salt thereof and its uses. The compound has the formula



where

x is 2, 3 or a combination of p 2(s) and q 3(s)
 10 where $p + q = y$;
 y is 3 or 4;

R is $(\text{CH}_2)_z \text{P}(=\text{O}) \text{OR}^1 \text{R}^2$

R^1 is R^3 or OR^3

R^3 is alkyl, cycloalkyl or aryl

15

R^2 is H, alkyl or CR^4

R^4 is alkyl, cycloalkyl or aryl

20

(R^3 and R^4 may or may not be equal in the same molecule)

z is 1 to 3

25

A preferable alkyl is $\text{C}_n\text{H}_{1+2n}$ where n is 1-20; a preferable cycloalkyl is $\text{C}_m\text{H}_{2m-1}$ when m is 1-20; and a preferable aryl is phenyl.

30

In one important embodiment, this compound may be complexed with a metal to be a polyazamacrocyclic compound-metal complex having the formula



35

where

x is 2, 3 or a combination of p 2(s) and q 3(s)
 where $p + q = y$;
 y is 3 or 4;

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R is $(\text{CH}_2)_z\text{P}(=\text{O})\text{OR}^1\text{R}^2$;

R^1 is R^3 or OR^3 ;

R^3 is alkyl, cycloalkyl or aryl;

5

R^2 is H, alkyl or $\overset{\text{O}}{\parallel}\text{CR}^4$;

z is 1 to 3;

r is 2 or 3; and

Me is a metal ion.

10

The y designation characterizes the compound as triazamacrocyclic or tetraazamacrocyclic. The x is preferably 2, although 3 is feasible under many circumstances. Combinations of p 2(s) and q 3(s) for x are of course readily produced but the total of p + q must be y for the number of units in the polyaza macrocycle.

In a preferred embodiment of either the compound or its metal complex y is 3, p is 1 and q is 2 or p is 2 and q is 1.

In another preferred embodiment of the compound or its metal complex, y is 4, p is 1 and q is 3, p is 2 and q is 2 or p is 3 and q is 1 and z is most preferably 1. n is preferably 2.

In a more preferred embodiment x is 2, y is 4, z is 1, and R^1 is R^3 .

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In another preferred embodiment X is 2, y is 3, z is 1, R^1 is OR^2 and n is 2.

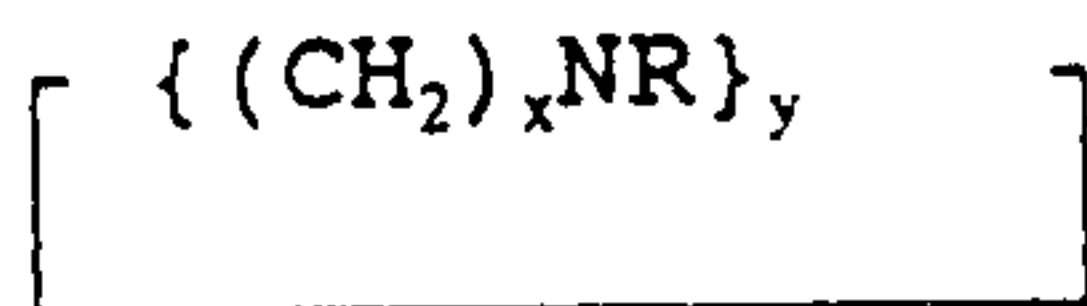
The Me^{+r} is preferably Ca^{+2} , Mg^{+2} , Zn^{+2} or Gd^{+3} . Other characteristic divalent or trivalent metal ions,

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particularly paramagnetic lanthanides may also be so complexed.

The present invention comprises a method for
 5 assessing intracellular concentration of one or more
 divalent metal ions. The method comprising treating
 cells, under conditions facilitating intracellular
 localization of a polyazamacrocyclic compound or a salt
 thereof, the compound having the formula

10



where

15

x is 2, 3 or a combination of p 2(s) and q 3(s)

where $p + q = y$;

y is 3 or 4;

R is $(\text{CH}_2)_z \text{P}(=\text{O})\text{OR}^1\text{R}^2$;

R^1 is R^3 or OR^3 ;

20

R^3 is alkyl, cycloalkyl or aryl

R^2 is H, alkyl or CR^4 ;
 R^4 is alkyl, cycloalkyl or aryl

25

z is 1 to 3

and measuring shifts in the ^{31}P NMR spectrum, said shifts
 being proportional to intracellular divalent metal
 concentration. In one embodiment cells are treated with
 30 the acid anhydride form of the compound of the present
 invention

(i.e. where R^2 is CR^4)

35

which passes into the cells, is there hydrolyzed to the
 acid form (i.e. where R^2 is H) and complexes with
 intracellular metal ions. The metals currently most
 amenable to such measurement are calcium, magnesium or
 zinc, magnesium being preferred.

In one important application, the present invention involves a method for enhancing a magnetic resonance image of a subject. This method comprises administering to the subject a polyazamacrocyclic compound-metal
 5 complex having the formula



10 where

x is 2, 3 or a combination of p 2(s) and q 3(s)
 where p
 + q = y;

15

y is 3 or 4;

R is $(\text{CH}_2)_z \text{P}(=\text{O})\text{OR}^1\text{R}^2$;

20

R^1 is R^3 or OR^3 ;

R^3 is alkyl, cycloalkyl or aryl

25

R^2 is H, alkyl or $\overset{\text{O}}{\parallel} \text{CR}^4$;

R^4 is alkyl, cycloalkyl or aryl

30

z is 1 to 3;

r is 2 or 3; and

Me is a paramagnetic metal (preferably gadolinium);

35

obtaining a magnetic resonance image of said subject.

Figure 1 schematically illustrates the structure of NOTPME (where R is CH_2CH_3 and R^1 is H).

40

Figure 2 describes potentiometric titration curves of 2 mM NOTPME (a) and those obtained in the presence of equivalent amounts of Ca^{2+} (b), Mg^{2+} (c), and Zn^{2+} (d).

Figure 3 shows ^{31}P NMR spectra of 5 mM NOTPME as a function of added (total) magnesium. Mg^{2+} concentrations from bottom to top are 0, 0.5, 1.5, 3 mM respectively. Sample constituents and spectral parameters are described in methods section.

Figure 4 shows ^{31}P NMR spectra of 5mM NOTPME in the presence of (A) 2.05 mM ZnCl_2 , and (B) 38.5 mM CaCl_2 . Sample composition and spectral parameters are given in methods section.

Figure 5 describes pH dependence of the ^{31}P NMR chemical shifts of 5 mM NOTPME (a), $\text{Ca}(\text{NOTPME})^-$ (b), $\text{Zn}(\text{NOTPME})^-$ (c), and $\text{Mg}(\text{NOTPME})^-$ (d).

Figure 6 shows ^{31}P NMR spectra of (a) normal RBCs and (b) NOTPME loaded RBCs.

Figure 7 schematically illustrates the structure of DOTE P .

Figure 8 shows the ^{31}P NMR spectrum of DOTE P and a DOTE P -calcium complex.

Figure 9 describes the decomposition rate of gadolinium DOTA and gadolinium-DOTE P complexes in 0.1N HCl.

Figure 10 schematically shows the compound 1,5,9-triazacyclododecane.

Figure 11 schematically shows the compound 1,4,8,12-tetracyclopentadecane.

Figure 12 schematically shows the compound 1,4,8,11-tetraazacyclotetradecane.

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EXAMPLE 1**Triazamacrocyclic Compounds****1,4,7-Triazacyclononane-N,N',N''-**

5 tris(methylenephosphonate monoethylester) (NOTPME) has been synthesized, characterized and analyzed for use as a ^{31}P NMR indicator of intracellular Mg^{2+} and Zn^{2+} ions. The ^{31}P NMR spectrum of this chelate in the presence of metal ions shows characteristic resonances for the free
10 chelate, $\text{Mg}(\text{NOTPME})^-$, and $\text{Zn}(\text{NOTPME})^-$ and $\text{Ca}(\text{NOTPME})^-$. Stability constants for NOTPME complexes with Ca^{2+} and Mg^{2+} have been obtained by potentiometry and NMR and its complex with Zn^{2+} by potentiometry. This chelate has a 10 fold higher affinity for Mg^{2+} than for Ca^{2+} at
15 physiological pH values. Its affinity for Zn^{2+} is so high that accurate binding constants could not be determined by NMR. In the presence of Mg^{2+} , NOTPME is readily loaded into red blood cells. ^{31}P chemical shifts of the free chelate and its metal complexes are far downfield from
20 the typical phosphorous containing metabolites observed in biological systems, thus making it possible to monitor intracellular cation concentration and cell energetics simultaneously.

25 Materials

1,4,7-triazacyclononane, paraformaldehyde, diethylphosphite, and activated carbon Darco G-60™ were purchased from Aldrich Chemical Company. MgSO_4 was from
30 Mallickrodt, sodium hydroxide, and benzene from J. T. Baker, and diethylether from Fisher Scientific. All chemicals were of highest purity and were used without further purification. Solutions of ZnCl_2 , CdCl_2 , MgCl_2 and Ca Cl_2 were standardized complexometrically.

35

Synthesis of NOTPME

1,4,7-Triazacyclononane (1.91 g, 14.71 mmol) and diethylphosphite (7.018 g, 16.94 mmol, 15% excess) were dissolved in 125 ml of benzene and heated to reflux. Anhydrous paraformaldehyde (1.727 g, 30% excess) was added in small portions to the above refluxing mixture while the benzene-water azeotropic mixture was removed by distillation. After the addition of paraformaldehyde was complete, the entire solution was boiled for 30 minutes and then evaporated to obtain a yellow viscous oil. The oil was dissolved in 150 ml anhydrous diethylether and dried with anhydrous MgSO_4 overnight. MgSO_4 , along with a white precipitate which formed, were filtered off and discarded. The filtrate was decolorized with activated carbon and filtered. The filtrate was evaporated in vacuum to obtain a viscous oil of 1,4,7-triazacyclononane- N,N',N'' -tris(methylenephosphonate diethylester) (NOTPDE). Pure NOTPDE was obtained in 96% yield (9.21 g, 14.17 mmol) and was used for the synthesis of NOTPME (structure shown in Figure 1) without further purification. ^1H NMR data of NOTPDE in CDCl_3 (TMS at zero) are as follows: δ (ppm) : 1.33 (t, 18H, $-\text{CH}_3$), 2.97 (s, 12H, $\text{N}-\text{CH}_2$), 3.00 (d, 6H, $\text{P}-\text{CH}_2$), 4.13 (p, 12H, $\text{O}-\text{CH}_2$).

25

9.20 g of NOTPDE (14.15 mmol) was mixed with 2.50 g of NaOH in 9 ml H_2O) and after 2 hours the entire reaction mixture was boiled until a clear solution was obtained (approximately 5 minutes). The solution was cooled to room temperature and was allowed to stand overnight. The crystals formed were filtered off from the viscous mother liquor using a pressure filter funnel with a coarse porosity grade filter disc. The crystals were washed once with cold absolute ethanol, three times with absolute ethanol-diethylether (1:1) mixture and finally with diethyl ether. The crystals of Na_3NOTPME were dried

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in dry nitrogen stream at 25°C for 2 hours. Traces of H₂O and ethanol were removed upon vacuum drying (10 mm Hg) NOTPME for 5 hours at 50°C. Pure NOTPME thus obtained were white crystals, very hygroscopic, readily soluble in H₂O, and fairly soluble in chloroform. The yield of pure NOTPME was 40.8% (3.24 g, 5.77 mmol).

¹H NMR (D₂O, HDO peak set as reference at 4.90 ppm), δ (ppm): 1.23 (t, 9H, -CH₃), 2.54 (s, broad, 6H, P-CH₂), 2.79 (s, broad, 12 H, N-CH₂), 3.91 (p, 6H, O-CH₂).

NMR Measurements

¹H NMR data was obtained on JEOL FX-200™ NMR spectrometer using a 10 mm probe operating at room temperature. Studies on solutions of NOTPME and its complexes were performed on a General Electric GN-500 NMR spectrometer. A 10 mm broad band probe was tuned to 202.4 MHz for ³¹P detection. Shifts for NOTPME and its complexes were measured using 85% H₃PO₄ as an external standard. Probe temperatures were accurate to $\pm 0.5^\circ\text{C}$.

Samples of NOTPME used for K_D determinations by NMR consisted of 115 mM KCl, 20 mM NaCl and 10 mM HEPES buffered with Tris base at pH 7.4. Varying concentrations (typically 0.5 to 10 mM) of Mg²⁺, Ca²⁺ or Zn²⁺ were added to the sample and the resulting ³¹P NMR spectrum obtained. Resonance areas were determined by integration of peaks using the GE software.

Potentiometric Measurements

Potentiometric titrations were conducted at 25°C using a Corning Ion Analyzer (model 250) and a Metrohm Dorsimat™ automatic burette (Brinkman Instruments). The hydrogen ion concentration was obtained from the measured

pH values by the method suggested by Irving et al. [15]. Na₃NOTPME was dissolved in 0.1 M tetramethylammonium chloride, the pH adjusted to low pH value with HCl and titrated with 0.098 M KOH. KOH was standardized by
5 potentiometric titration against potassium hydrogen phthalate and stored under N₂ atmosphere. The hydrogen ion activity coefficient and K_a were determined separately in these same salt solutions. Stability constants of Zn(NOTPME)⁻, Mg(NOTPME)⁻, and Ca(NOTPME)⁻ were
10 determined by potentiometric titration of solutions containing 1:1 ratio of metal and ligand. In all of the titrations, samples were covered by a layer of cyclohexane to exclude CO₂.

15 Protonation constants (K_{H_iL}) and stability constants (K_{ML}, and K_{MHL}) are defined by the following equations:

$$K_{H_iL} = [H_iL] / \{[H_{i-1}L] [H^+]\} \quad (1)$$

$$K_{ML} = [ML] / \{[M] [L]\} \quad (2)$$

20 $K_{MHL} = [MHL] / \{[ML] [H^+]\} \quad (3)$

Protonation and stability constants were obtained from the potentiometric data using a simplex non-linear algorithm [16] run on an IBM PC.

25 Red Blood Cell Loading of NOTPME

Fresh whole blood was obtained placed in heparinized tubes and centrifuged at 3000 g for 6 min. to remove the buffy coat. RBCs were then washed three times in 5 mM
30 phosphate buffered saline at pH 7.4. RBCs at 50% hematocrit were suspended in the loading medium containing 120 mM NaCl, 5 mM MgCl₂, 10 mM HEPES pH 7.4, 10 mM glucose, and 10 mM NOTPME and incubated at 37°C for 12 hours. No lysis of RBCs were observed during the loading
35 procedure. The RBCs were centrifuged and the supernatant discarded. The RBCs were washed twice with phosphate

buffered saline at pH 7.4 before suspension in an isotonic medium.

Protonation Studies

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A representative potentiometric titration curve for NOTPME in 0.1 M tetramethylammonium chloride and 25°C is illustrated in Figure 2. Three protonation constants ($pK_1 = 11.8$, $pK_2 = 3.65$, and $pK_3 = 1.4$) were obtained from these data. The ^{31}P NMR spectrum of NOTPME was also measured as a function of pH (data not shown) and the protonation constants obtained from these data were in general agreement with those obtained by potentiometry. The phosphorous shifts are very sensitive to protonation of the nitrogens in the polyaza ring, similar to that observed for the parent phosphonate NOTP [17]. The first two protonations ($\log K = 11.8, 3.65$) result in ^{31}P shifts to low frequency which is consistent with protonation at two ring nitrogens [17]. This indicates that the first nitrogen protonations in NOTP versus NOTPME are quite similar ($\log K_1 = 12.1$ versus 11.8, respectively) while the second nitrogen protonations are dramatically different ($\log K_2 = 9.4$ versus 3.65). These differences in pK_2 may reflect differences in the ability of the phosphonate versus the phosphonate monoester side chains to form internal hydrogen bonds with the protonated nitrogens. This would be consistent with the greater basicity of the phosphonate oxygens in NOTP (protonation constants between 6.0 and 7.5) over the phosphonate ester oxygens in NOTPME (protonations below 1.4). ^{31}P NMR spectra of NOTPME exhibited a single resonance over the entire pH range, indicating rapid exchange between all protonated species.

Complexation Studies

Figure 2 also shows potentiometric titration data for NOTPME in the presence of one equivalent of Mg^{2+} , Ca^{2+} , or Zn^{2+} . The stability constants for $Mg(NOTPME)^-$, $Ca(NOTPME)^-$, and $Zn(NOTPME)^-$ derived from these data are summarized in Table I.

TABLE I

10

Stability constants of the NOTPME complexes with Ca^{2+} , Mg^{2+} , and Zn^{2+} .

15

Metal ion	K_D	K_D	$\log K_{ML}$
	(at 25°C)	(at 37°C)	
Ca^{2+}	50 mM	47.62 mM	5.1
Mg^{2+}	5.77 mM	4.66 mM	6.3
Zn^{2+}	$(10^{-11} M)^a$	_____	15.4

20

30

35

K_D values were obtained from NMR data while $\log K$ values were determined by potentiometry at 25°C. aK_D estimated from the thermodynamic value ($\log K_{ML} = 15.4$) by considering the ligand pK values and the proton concentration at pH 7.4.

Zn^{2+} forms a considerably more stable complex with NOTPME than either Mg^{2+} or Ca^{2+} . This largely reflects the propensity of Zn^{2+} to form stronger M^{2+} -N bonds than more ionic species, Mg^{2+} and Ca^{2+} . Both $Mg(NOTPME)^-$ and $Ca(NOTPME)^-$ are less stable than their respective NOTP complexes [18], which again reflects the more acidic nature of phosphonate ester side-chain ligands. However, NOTPME, like NOTP forms more stable complexes with the

smaller Mg^{2+} ion than with Ca^{2+} . This reflects the size selectivity of the triazacyclononane macrocycle in both cases.

5 Figure 3 displays the ^{31}P NMR spectrum of a solution containing 5 mM NOTPME as a function of added Mg^{2+} . The spectra show that Mg^{2+} bound NOTPME is in slow exchange with "free" NOTPME. Figure 4 shows ^{31}P NMR spectra of 5
10 mM NOTPME in the presence of 2.05 mM Zn^{2+} and 38.5 mM Ca^{2+} . As expected based upon the potentiometrically determined stability constants, there is more $\text{Zn}(\text{NOTPME})$ present in this solution than in an equivalent solution containing Mg^{2+} . Interestingly, the chemical shifts of Mg^{2+} and Zn^{2+} bound NOTPME are quite different and,
15 indeed, it is possible to obtain characteristic resonances for $\text{Zn}(\text{NOTPME})$ and $\text{Mg}(\text{NOTPME})$ in a mixture containing both metal ions. Ca^{2+} , as expected, binds much more weakly and a resonance characteristic of $\text{Ca}(\text{NOTPME})$ only becomes visible after 32 mM Ca^{2+} has been added. The
20 chemical shift of $\text{Ca}(\text{NOTPME})$ (22.1 ppm) was also different from those of $\text{Mg}(\text{NOTPME})$ (23.5 ppm) and $\text{Zn}(\text{NOTPME})$ (23.1 ppm). This probably reflects the larger size of Ca^{2+} versus Mg^{2+} or Zn^{2+} and its inability to fit into the cyclononane cavity.

25

 The area of resonances corresponding to free and metal bound NOTPME were obtained by integration and their concentrations estimated. The K_D values obtained at 25°C by NMR for Mg^{2+} and Ca^{2+} complexes are 5.77 mM and 50 mM,
30 respectively. In comparison, the fluorinated chelator, MF-APTRA, reported by others [12] has a K_D of 1 mM for Mg^{2+} and 12 μM for Ca^{2+} .

 Table I also shows the K_D for $\text{Mg}(\text{NOTPME})$, and
35 $\text{Ca}(\text{NOTPME})$ obtained by NMR at 37°C. Clearly, the

magnitude of differences in stabilities between $\text{Mg}(\text{NOTPME})^-$, and $\text{Ca}(\text{NOTPME})^-$ at 37°C remain the same. This reconfirms the higher affinity of NOTPME for Mg^{2+} over Ca^{2+} at physiological temperature. It was not possible to
5 obtain K_D values for $\text{Zn}(\text{NOTPME})^-$ from the NMR data because the titrated Zn^{2+} was completely bound to NOTPME under virtually all measurable conditions.

Figure 5 shows the ^{31}P NMR chemical shifts of the
10 NOTPME complexes of Ca^{2+} , Mg^{2+} , and Zn^{2+} as a function of pH between 6 and 10. In solutions containing 1:1 metal ion and ligand, the resonance characteristic of $\text{Zn}(\text{NOTPME})^-$ is visible over this entire pH range, that characteristic of $\text{Mg}(\text{NOTPME})^-$ is detectable only above pH
15 6.4, while that characteristic of $\text{Ca}(\text{NOTPME})^-$ is only visible above pH 7.8. These differences clearly demonstrate the greater selectivity of NOTPME for Mg^{2+} over Ca^{2+} at physiological pH (ca. 7.4). As shown, chemical shifts of the metal bound species are all
20 virtually independent of pH over this range.

Determination of Intracellular Free Mg^{2+} Concentration in Human Erythrocytes

25 Figure 6 illustrates one application of NOTPME to detect intracellular free Mg^{2+} in RBCs. NOTPME loaded RBCs (Figure 6.b) were prepared by incubating RBCs at 37°C in a loading medium containing NOTPME in the presence of 5mM MgCl_2 for 12 hours. NOTPME loading into
30 RBCs was not observed in the absence of MgCl_2 in the loading medium. Control RBCs are shown in Figure 6a.

As in the in vitro studies, two resonances were observed corresponding to free and Mg^{2+} bound NOTPME in
35 RBCs. The intracellular free Mg^{2+} concentration in RBCs was estimated using the following equation:

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$$[\text{Mg}^{2+}] = K_D [\text{MgNOTPME}] / [\text{NOTPME}]$$

An intracellular free Mg^{2+} concentration of 1.71 mM was obtained in this particular example. Since intracellular
5 free Mg^{2+} levels in RBCs normally range between 0.2 - 0.3 mM [12,19,20], the high concentration of intracellular free Mg^{2+} observed in this experiment must have resulted from transport of Mg^{2+} as a complex with NOTPME. Upon
10 addition of the divalent cation ionophore A23187 to the suspension medium, an increase in intracellular free Mg^{2+} concentration (to 1.90 mM) was observed (data not shown). These results show that NOTPME can easily detect small changes in free Mg^{2+} levels in intact cells.

15 The complex forming characteristics of NOTPME are strongly influenced by the very high value of the first protonation constant as well as by the conformational and size requirements of the triaza ring. The fact that Zn^{2+} , Ca^{2+} , and Mg^{2+} complexes of NOTPME could be studied by
20 usual potentiometric method indicates that the complexes are formed relatively quickly in solution. As seen in Table I, Zn^{2+} forms a more stable complex with NOTPME than does either Mg^{2+} or Ca^{2+} . This same trend was observed for the triazacyclononane triacetate chelate, NOTA, with
25 these same ions; reported log K values for $\text{Zn}(\text{NOTA})$, $\text{Mg}(\text{NOTA})$, and $\text{Ca}(\text{NOTA})$ are 18.3, 9.69, and 8.92, respectively [21]. Thus, the carboxylate chelate forms more stable complexes with all three metal ions perhaps due to the greater basicity of the carboxylate oxygen
30 ligands over the phosphonate oxygen ligands. The parent phosphonate, NOTP, shows the same trend in its metal complexing ability with Zn^{2+} , Mg^{2+} , and Ca^{2+} but in this case the stability constants of the resulting complexes are all several orders of magnitude higher than the
35 corresponding values with NOTPME or NOTA. The reported log K values for $\text{Zn}(\text{NOTP})^+$, $\text{Mg}(\text{NOTP})^+$, and $\text{Ca}(\text{NOTP})^+$ are

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24.9, 11.0, 6.4 respectively [18]. Since the nitrogens and the phosphonate oxygens in NOTP are considerably more basic than those in NOTPME, the resulting stability constants with these metal ions are all larger. The
5 trend $\text{Zn}^{2+} \gg \text{Mg}^{2+} > \text{Ca}^{2+}$, however, is preserved.

NOTPME has several advantages over the fluorinated chelators such as BAPTA and APTRA for measurement of intracellular Mg^{2+} in biological systems. These include
10 (a) ease of synthesis, (b) higher affinity for Mg^{2+} than for Ca^{2+} , (c) no significant exchange contribution to the bound and free ligand resonances, and (d) the three magnetically equivalent ^{31}P nuclei in this chelate provides an attractive NMR nucleus for biological tissue
15 since the cell energetics may be measured simultaneously. The significant selectivity for Mg^{2+} over Ca^{2+} exhibited by NOTPME at physiological pH is of great advantage for monitoring Mg^{2+} in biological systems without concern about interference from Ca^{2+} . It is important to
20 emphasize that the resonances of NOTPME and its magnesium complex do not overlap with the phosphorous containing metabolites in tissue. Thus NOTPME provides versatile approach of measuring changes in intracellular free magnesium as well as changes in metabolites during
25 various physiological events by ^{31}P NMR. Although it is fortuitous that the K_D $\text{Mg}(\text{NOTPME})^-$ at physiological pH is in the range required for measurement of intracellular Mg^{2+} in many biological systems, it may prove possible to fine tune the affinity of NOTPME for Mg^{2+} by changing the
30 alkyl substituents on the phosphonate oxygens or by adding an alkyl chain to the carbon which links the phosphonate groups to the triaza ring.

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EXAMPLE 2**Tetraazamacrocyclic compounds****DOTEP Synthesis**

5 DOTEP, shown in Figure 7, was prepared as follows.
2 ml of dichloroethylphosphine was slowly mixed with ice
to form the corresponding ethylphosphinic acid. After
warming to room temperature, 390 mg of 1,4,7,10-
tetraazacyclododecane tetrahydrochloride (cyclen.4HCl)
10 (Parrish Chem. Co., Ogden, Utah) was added and the
mixture heated to boiling under a nitrogen atmosphere. A
solution containing 157 mg of paraformaldehyde dissolved
in 10 ml of 6M HCl was added at a rate of 0.5 ml/hr,
while the mixture continued to reflux. The final mixture
15 was refluxed an additional 4 hours then cooled to room
temperature. This solution was concentrated under vacuum
to a viscous oil, redissolved into 6 ml of water and
loaded onto a DOWEX™ 50Wx4 (hydrogen form) cation exchange
column (7.5 ml bed volume). The column was washed to
20 neutrality with water and the product eluted with 60 ml
of 0.66 M HCl. The fractions containing DOTEP were
combined, evaporated, redissolved in absolute ethanol and
evaporated to a white solid. This solid was dispersed
into anhydrous ether, filtered off, pre-dried under
25 nitrogen and dried under vacuum at 60-70°C to yield a
white, very hygroscopic solid (360 mg, 44% yield). This
solid was stored in sealed ampoules. Elemental analysis
and potentiometry shows the solid to be DOTEP.2HCl

30

DOTEP APPLICATIONS

A. Ca(DOTEP)⁻ as a ³¹P NMR indicator of [Ca²⁺]_{free}

35 The ³¹P spectrum shown in Figure 9 was obtained on a
solution containing approximately 1 mM Ca²⁺ and 3 mM DOTEP
in an aqueous solution at physiological pH. Clearly, the

³¹P resonances of the free and bound forms of DOTE^P have distinct chemical shifts and neither overlaps with the ³¹P resonances of metabolites normally observed in tissue. This indicates that when DOTE^P is loaded into cells by making an appropriate hydrolysable ester, the DOTE^P trapped in cells would give a direct measure of [Ca²⁺]_{free} using the simple relationship,

$$[Ca^{2+}]_{free} = K_D \cdot \frac{[Ca(DOTE^{P})]}{[DOTE^{P}]_{free}}$$

where $K_D = 11 \mu M$ at pH 7.4

The value of K_D at pH 7.4 reported above was determined by ³¹P NMR (measuring the free and bound integrals as a function of pH in solutions containing known amounts of [Ca²⁺]_{total} and [DOTE^P]_{total}) and by pH potentiometry. This value of K_D indicates that DOTE^P will be useful for measuring [Ca²⁺]_{free} over the approximate range, 1-50 μM .

B. Gd(DOTE^P)⁻ as a MRI contrast agent.

DOTE^P also forms stable complexes with Gd³⁺ (log K approximately equal to 16). The resulting complex has a favorable water proton relaxivity ($R = 5.1 s^{-1}mM^{-1}$) and the complex has many of the same kinetic advantages as Gd(DOTA)⁻, as shown by the data of Figure 10. In this experiment, Gd(DOTE^P)⁻ and Gd(DOTA)⁻ were dissolved in 0.1M HCl and their decomposition to free Gd³⁺ and free ligand were followed by water relaxation measurements. As shown, the half-lives for decomposition of the two complexes in strong acid were approximately 110 hrs. for Gd(DOTA)⁻ versus 30 hrs. for Gd(DOTE^P)⁻. This indicates that the decomposition rates for both complexes at pH 7.4 will be several orders of magnitude longer than this (or

about 10^6 hrs.) and therefore should be quite safe for use in humans.

EXAMPLE 3

5

Polyazamacrocyclic N-alkyl phosphonate or phosphine acid anhydrides

Synthesis of NOTPME-AC

10

[NOTPME-AC = 1,4,7-triazacyclononane-N,N',N''-tris(methylenephosphonate monoethylester acetic acid anhydride)]

NOTPME trisodium salt (178.63 mg, 0.318 mmol) was dissolved in 4.0 ml anhydrous chloroform (freshly distilled from over P_4O_{10}) and 120 microliter acetyl chloride was added. The solution was stirred at room temperature for 24 hours in a tightly closed vessel. The solution was then evaporated under dry nitrogen atmosphere to a yellowish, very viscous oil. To the oil anhydrous ether was added and NOTPME-AC (precipitated as a sodium chloride adduct) was filtered off and dried at 35-40°C under dry nitrogen. NOTPME-AC (234.24 mg, 92.3% calculated on the basis of NOTPME-AC.3NaCl formula weight) is an extremely hygroscopic, water sensitive powder. It is readily soluble in water, chloroform and DMSO.

1H NMR ($CDCl_3/TMS$): 1.31(t, 9H, $-CH_3$), 2.24 & 2.21 (two sg(s), 9H, $C(O)-CH_3$), 3.29 (sg, v.br., 18H, $N-CH_2-C$ and $N-CH_2-P$), 4.12 & 4.30 (two m(s), 6H, $O-CH_2$).

Synthesis of NOTPME-B

[NOTPME-B = 1,4,7-triazacyclononane-N,N',N''-tris(methylenephosphonate monoethylester benzoic acid anhydride)]

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NOTPME trisodium salt (164.82 mg, 0.294 mmol) and benzoyl chloride (105 microliter, 0.883 mmol) were reacted in 4.0 ml anhydrous chloroform at room temperature for 24 hours. The reaction mixture was worked up with identical method than was used in the synthesis of NOTPME-AC. The product NOTPME-B was get as a sodium chloride adduct (204.32 mg, 70.7% calculated on the basis of NOTPME-B.3NaCl formula weight). NOTPME-B is a very hygroscopic, water sensitive powder. It is soluble in water and chloroform and DMSO.

¹H NMR (CDCl₃/TMS): 1.29 (t, -CH₃), 3.32 (m, V.br., N-CH₂-C and N-CH₂-P), 4.10 & 4.35 (two m(s), O-CH₂), 7.49 (m, Ar-H), 8.02(d, Ar-H).

It is understood to those of skill in the art that a variety of aryl halides may be used to prepare analogous phosphonate or phosphine acid anhydrides with only a minimum of efforts to define optimal synthetic procedures. Additionally, it is clear that facile leaving groups such as carbonyl-containing alkyls may be preferred for greater stability in an aqueous medium and yet still pass into cells for partial decomposition and use in NMR metal analyses.

25

Red Blood Cell Loading of NOTPME Derivatives

NOTPME derivatives used are the benzoyl and acetyl derivatives. Fresh whole blood was obtained in heparinized tubes and centrifuged at 3000g for 3 minutes to remove the buffy coat. Packed red blood cells are then washed three times in 5 mM phosphate buffered saline at pH 7.4. Red blood cells at 50% hematocrit were suspended in the NOTPME derivative containing loading medium. The loading medium contained 130 mM NaCl, 5 mM NOTPME derivative (benzoyl or acetyl derivative), 1 mM

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adenine, 10 mM glucose, and 10 mM HEPES at pH 7.4. Red cells in the above loading medium were incubated at 25°C for 1 hour. No lysis of red blood cells were observed during the loading procedure. After loading with NOTPME derivatives for one hour, the red cell suspension was centrifuged and the supernatant discarded. The red cells are washed twice with 5 mM phosphate buffered saline at pH 7.4 before suspension in an isotonic saline for NMR measurements. The ³¹P NMR measurements indicated that these NOTPME derivatives get loaded inside red cells and get hydrolysed to NOTPME. After NMR measurements the sample was centrifuged and the supernatant analyzed for any leakage of NOTPME. No leak of NOTPME was observed during the time course of NMR measurements. thus, it is clear that these NOTPME derivatives enter the red cells, get hydrolyzed to NOTPME and remain inside red cells.

EXAMPLE 4

Prophetic Synthesis of Generic Polyazamacrocyclic N-Alkylphosphonates, Polyazamacrocyclic N-Alkylphosphines or Acid Anhydrides thereof

The procedures in Examples 1, 2 and 3 may be readily adapted by those of skill in the art of synthetic organic chemistry to any suitably stable polyazamacrocyclic compound. Suitable polyazamacrocyclic compounds include the following triaza and tetraaza macrocyclic compounds available from the Aldrich Chemical Co, Milwaukee, Wisconsin:

- 1,5,9-triazacyclododecane (see Figure 10);
- 1,4,8,12-tetraazacyclopentadecane (see Figure 11);
- and
- 1,4,8,11-tetraazacyclotetradecane (see Figure 12).

-25-

5

REFERENCES

1. E. Carafoli and J.T. Penniston, *Sci. Amer.* 253:70 (1985).
- 10 2. R.D. Grubbs and M.E. Maguire, *Magnesium* 6:113 (1983).
3. G. Charton, G. Rovira, Y. Ben-Ari and V. Leviel, *Exp Brain Res.* 58:202 (1985).
- 15 4. D. Veloso, R.W. Gwynn, M. Oskarsson, and R.L. Veech, *J. Biol Chem* 248:4811 (1973).
- 20 5. J.R. Lopez, L. Alamo, C. Caputo, J. Vergara, and R. Di Polo, *Biochim. Biophys. Acta* 804:1 (1984).
6. C. H. Fry, *Magnesium* 5:306 (1986).
7. F.J. Brinkley and A. Scarpa, *FEBS Lett* 50:82 (1975).
- 25 8. T.J. Rink, R.Y. Tsien, and T. Pozzan, *J. Cell. Biol.* 95:189 (1982).
9. B.E. Corkey, J. Duszynski, T.L. Rich, B. Matschinsky, and J.R. Williamson, *J. Biol. Chem.* 258:14294 (1986).
- 30 10. G. A. Smith, T. R. Hesketh, J.C. Metcalfe, J. Feeney, and P.G. Morris, *Proc. Natl. Acad. Sci. USA* 80:7178 (1983).
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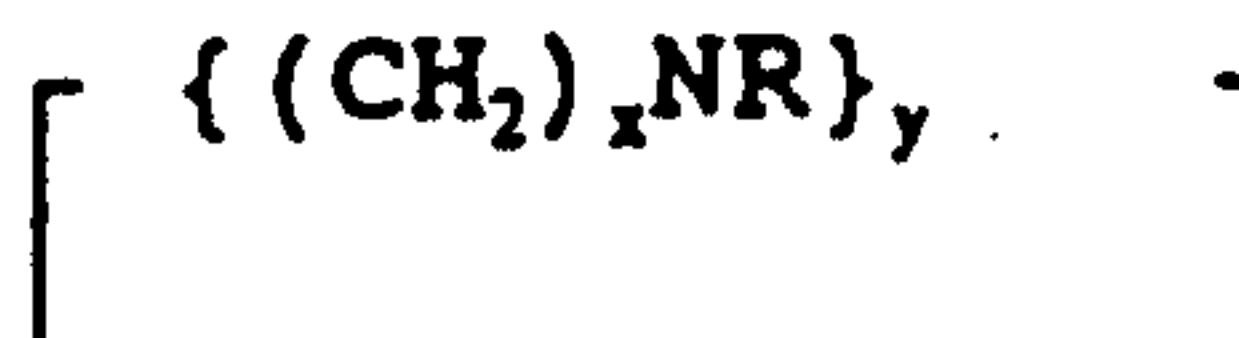
-26-

11. J.C. Metcalfe, T.R. Hesketh, and G.A. Smith, *Cell Calcium* 6:183 (1985).
12. L.A. Levy, E. Murphy, B. Raju, and R.E. London, *Biochemistry* 27:4041 (1988).
13. E. Murphy, C. Steenbergen, L.A. Levy, B. Raju, and R.E. London, *J. Biol. Chem.* 264:5622 (1989).
14. E. Marban, M. Kitakaze, H. Kusuoka, J.K. Poterfield, D.T. Yue, and V.P. Chacko, *Proc. Natl. Acad. Sci. USA* 84:6005 (1987).
15. H.M. Irving, M.C. Miles, and L.D. Petit, *Anal. Chim. Acta* 38 475 (1967).
16. M.S. Cacei and W.P. Cacheris, *Byte* 5:340 (1984).
17. (a) C.F.G.C. Geraldles, A.D. Sherry, and W.P. Cacheris, *Inorg. Chem.* 28:336 (1989). (b) S. Cortes, E. Brucher, C.F.G.C. Geraldles, and A.D. Sherry *Inorg. Chem.* 29:5 (1990)/
18. M.I. Kabachnik, T.Ya. Medved, Yu.M. Polikarpov, B.K. Scherbakov, F.I. Bel'skii, E.I. Matrosov, and M.P. Pasechnik, *Izv. Akad. Nauk. SSSR Ser Khim* 769 (1984).
19. R.K. Gupta, J.L. Benovic, and Z.B. Rose, *J. Biol. Chem.* 253:6472 (1978).
20. P.W. Flatman and V.L. Lew, *Nature (London)* 267:360 (1977).
21. A. Bevilacqua, R.T. Gelb, W.B. Hobard, and L.F. Zompa, *Inorg. Chem.* 26:2699 (1987).

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CLAIMS:

1. A polyazamacrocyclic compound or a salt thereof, the compound having the formula:



where

x is 2, 3 or a combination of p 2(s) and q 3(s) where $p + q = y$;

y is 3 or 4;

R is $\text{CH}_2)_z \text{P}(=\text{O})\text{OR}^1\text{R}^2$;

when y is 3, R^1 is 0-alkyl and R^2 is attached to an oxygen atom and is alkyl or

$\begin{array}{c} \text{O} \\ || \\ \text{CR}^4 \end{array}$, where R^4 is alkyl, cycloalkyl or aryl; and

z is 1 to 3;

where y is 4, R^1 is R^3 or OR^3 where

R^3 is alkyl with 2 or more carbon atoms and R^2 is attached to an oxygen atom and is hydrogen, alkyl or

$\begin{array}{c} \text{O} \\ || \\ \text{CR}^4 \end{array}$, where R^4 is alkyl, cycloalkyl or aryl; and
z is 1 to 3.

2. A polyazamacrocyclic compound-metal complex having the formula:



where

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where

x is 2, 3 or a combination of p 2(s) and q 3(s) where $p + q = y$;

y is 3 or 4;

R is $\text{CH}_2)_z\text{P}(=\text{O})\text{OR}^1\text{R}^2$;

when y is 3, R^1 is 0-alkyl and R^2 is attached to an oxygen atom and is alkyl or

$\begin{array}{c} \text{O} \\ || \\ \text{CR}^4 \end{array}$ where R^4 is alkyl, cycloalkyl or aryl; and

z is 1 to 3; and

where y is 4, R^1 is R^3 or OR^3 where

R^3 is alkyl with 2 or more carbon atoms

and R^2 is attached to an oxygen atom and is hydrogen, alkyl or

$\begin{array}{c} \text{O} \\ || \\ \text{CR}^4 \end{array}$ where R^4 is alkyl, cycloalkyl or aryl; and

z is 1 to 3;

r is 2 or 3; and

Me is a metal ion.

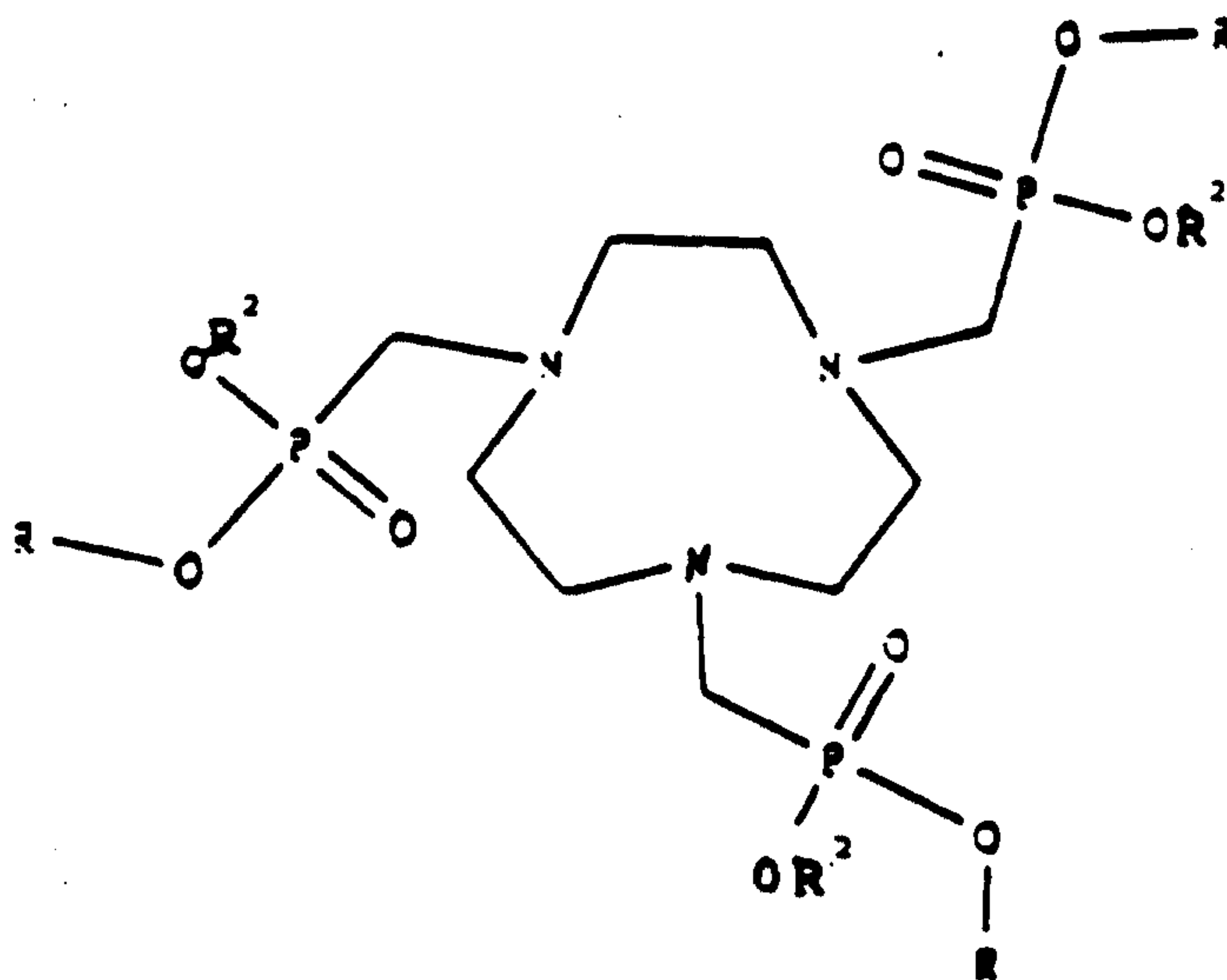
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3. The compound of claim 1 or 2 where y is 3.
4. The compound of claim 1 or 2 where y is 4.
5. The compound of claim 1 or 2 where y is 3 and x is 2.
6. The compound of claim 1 or 2 where y is 4 and x is 2.
7. The compound of claim 1 or 2 where y is 3, and either p is 1 and q is 2, or p is 2 and q is 1.
8. The compound of claim 1 or 2 where y is 4, and p is 1 and q is 3, or p is 2 and q is 2, or p is 3 and q is 1.
9. The compound of claim 1 or 2 where z is 1.
10. The compound of claim 1 or 2 where alkyl is ethyl.

- 30 -

11. The complex of claim 2 where Me^{+} is Ca^{+2} , Mg^{+2} , Zn^{+2} or Gd^{+3} .

12. A compound or salt thereof, the compound having the formula:

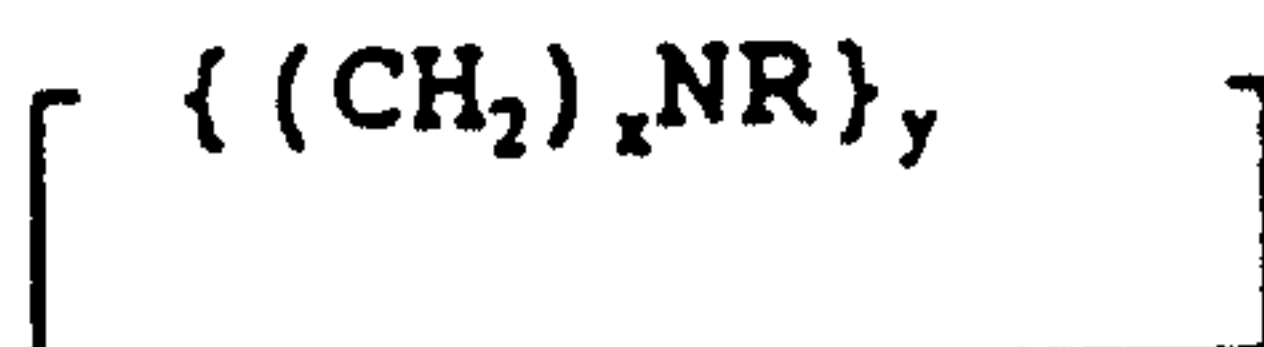


where R is C_8H_{17} ; and

- 31 -

R^2 is alkyl or $\overset{\text{O}}{\parallel}\text{CR}^4$ where R^4 is alkyl, cycloalkyl or aryl; and n is 1 to 20.

13. A method for assessing intracellular concentration of a divalent metal ion, the method comprising treating cells in vitro, under conditions facilitating intracellular localization, of a polyazamacrocyclic compound or a salt thereof, the compound having the formula



where

x is 2, 3 or a combination of p 2(s) and q 3(s)

where $p + q = y$;

y is 3 or 4;

R is $\text{CH}_2)_2\text{P}(=\text{O})\text{OR}^1\text{R}^2$;

when y is 3, R^1 is O-alkyl and R^2 is attached to an oxygen atom and is alkyl or

$\overset{\text{O}}{\parallel}\text{CR}^4$ where R^4 is alkyl, cycloalkyl or aryl; and

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z is 1 to 3 and
 where y is 4, R^1 is R^3 or OR^3 where R^3 is
 alkyl with 2 or more carbon atoms and R^2 is
 attached to the oxygen atom and is hydrogen, alkyl
 or

$$\begin{array}{c} \text{O} \\ || \\ \text{CR}^4 \end{array}$$
 where R^4 is alkyl, cycloalkyl or aryl; and
 z is 1 to 3;

and measuring changes in the ^{31}P NMR spectrum, said
 changes being proportional to intracellular divalent
 metal concentration.

14. The method of claim 13 wherein the metal is
 calcium, magnesium or zinc.

15. The method of claim 13 wherein the metal is
 magnesium.

16. The method of claim 13 wherein x is 2, y is 3 and z
 is 1 and alkyl is ethyl.

17. The method claim 13 wherein x is 2, y is 4 and z is
 1 and alkyl is ethyl.

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18. The use of a polyazamacrocyclic compound metal complex as defined in claim 2, where Me is a paramagnetic metal; as a magnetic resonance image enhancement agent.

19. The use of claim 18 wherein the metal is a lanthanide.

20. The use of claim 18 wherein the metal is gadolinium and r is 3.

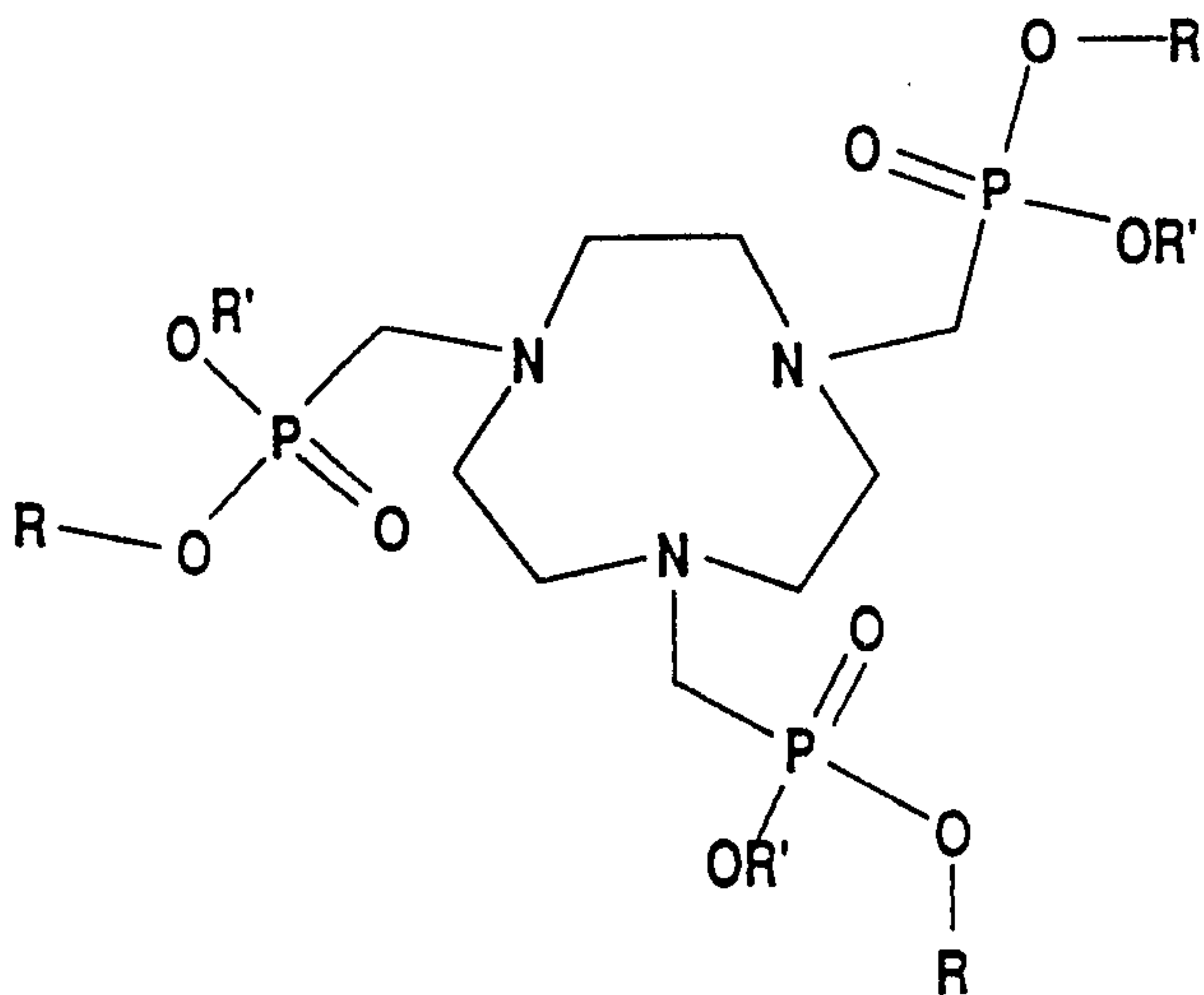


Fig. 1

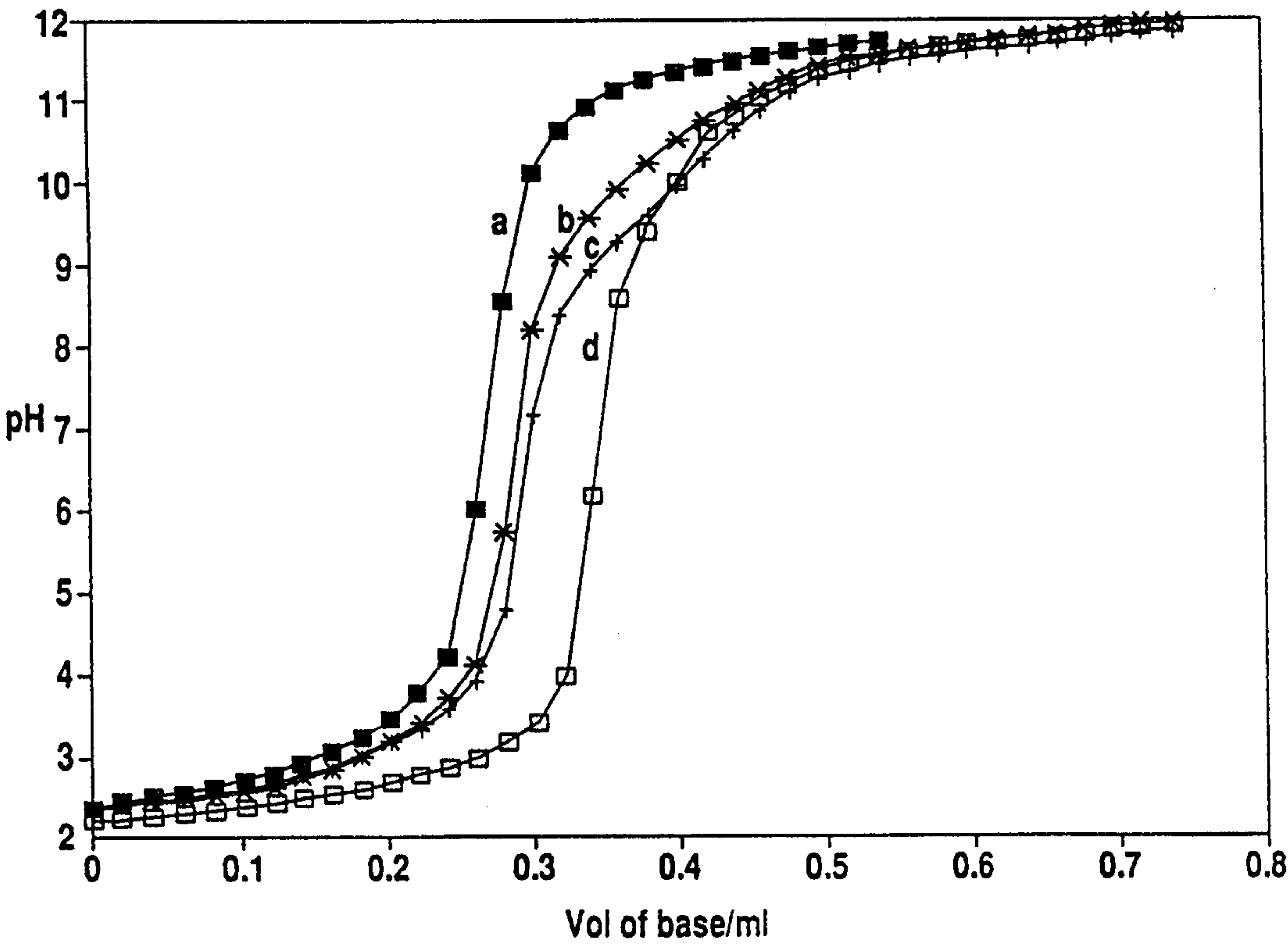


Fig. 2

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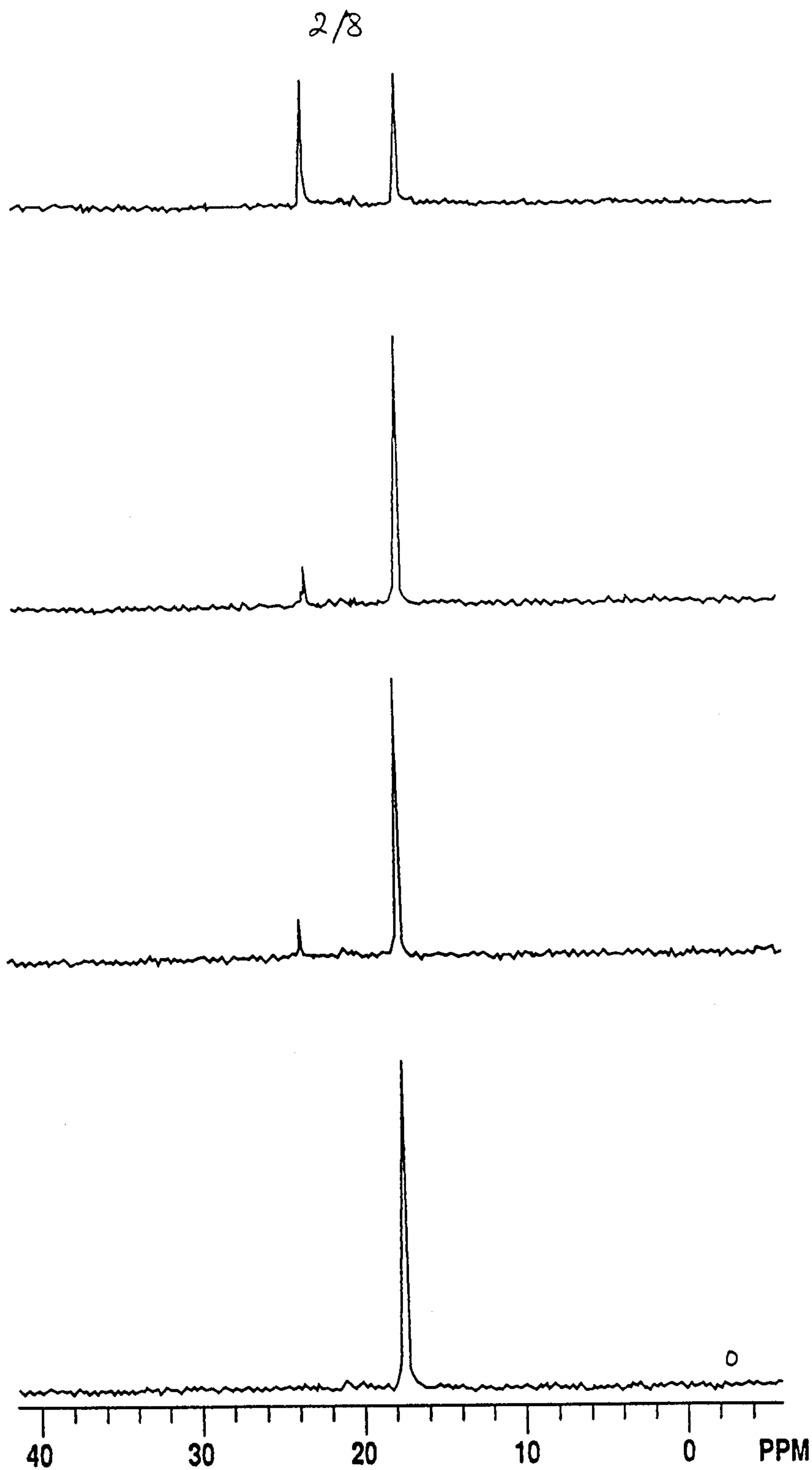


Fig. 3

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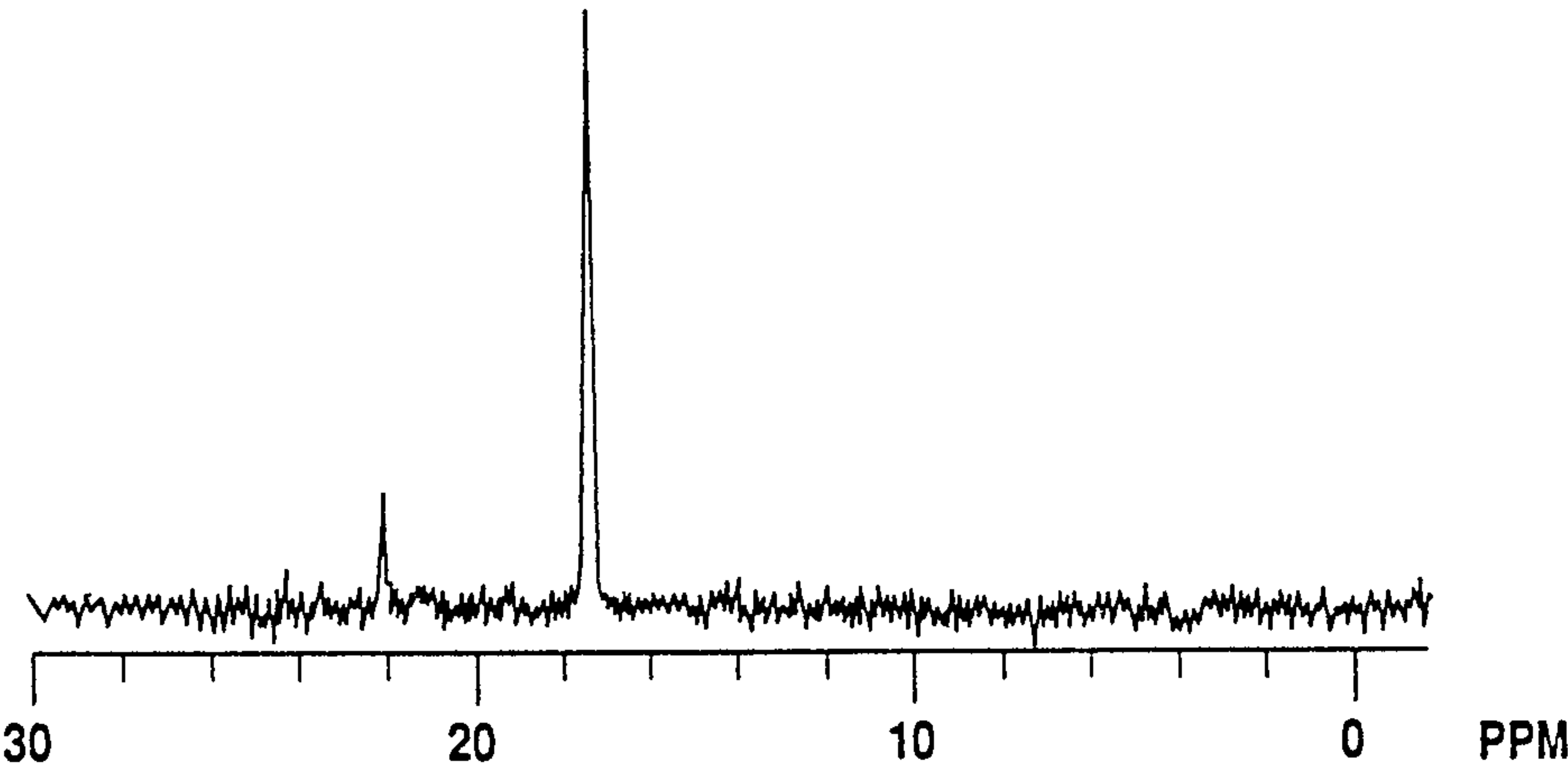


Fig. 4B

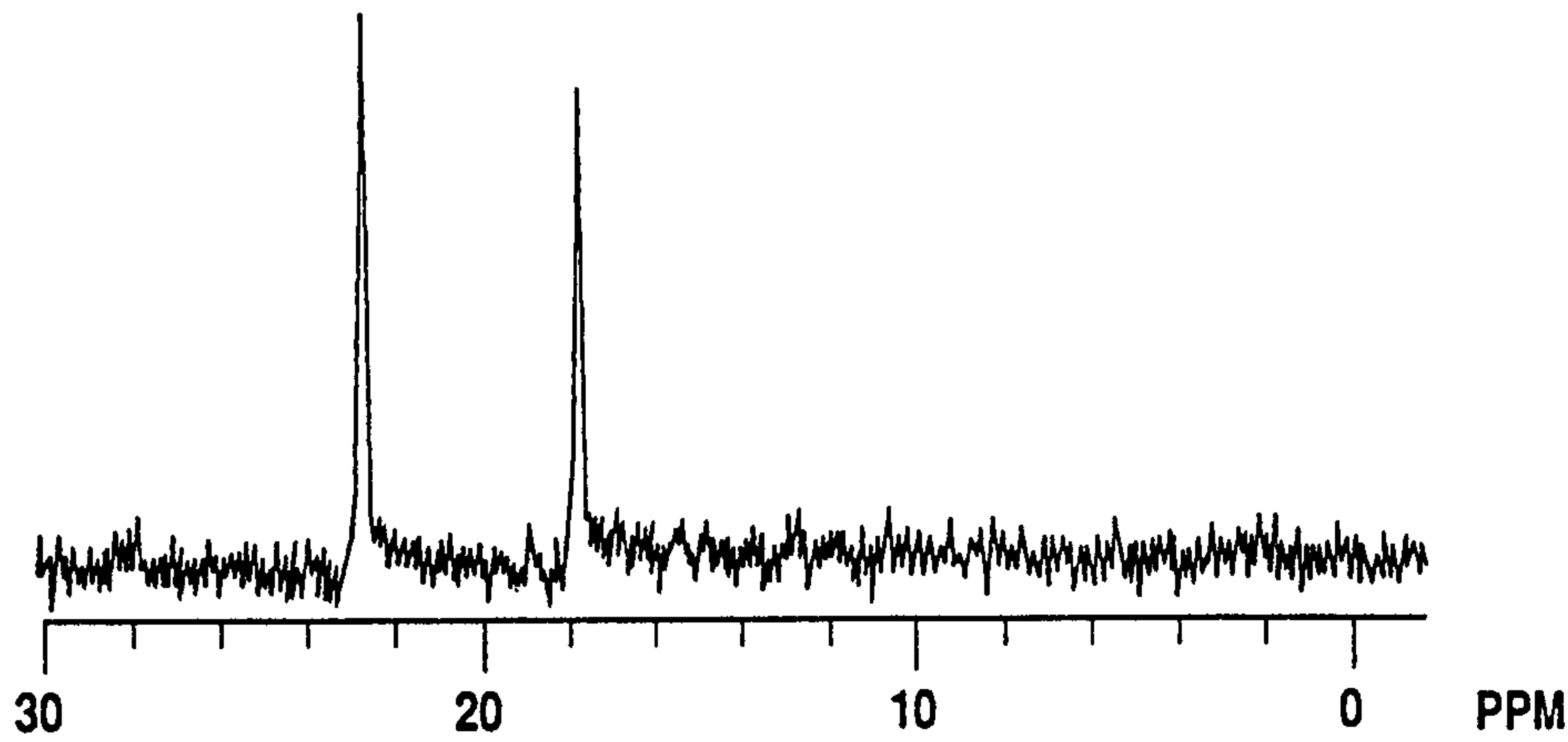


Fig. 4A

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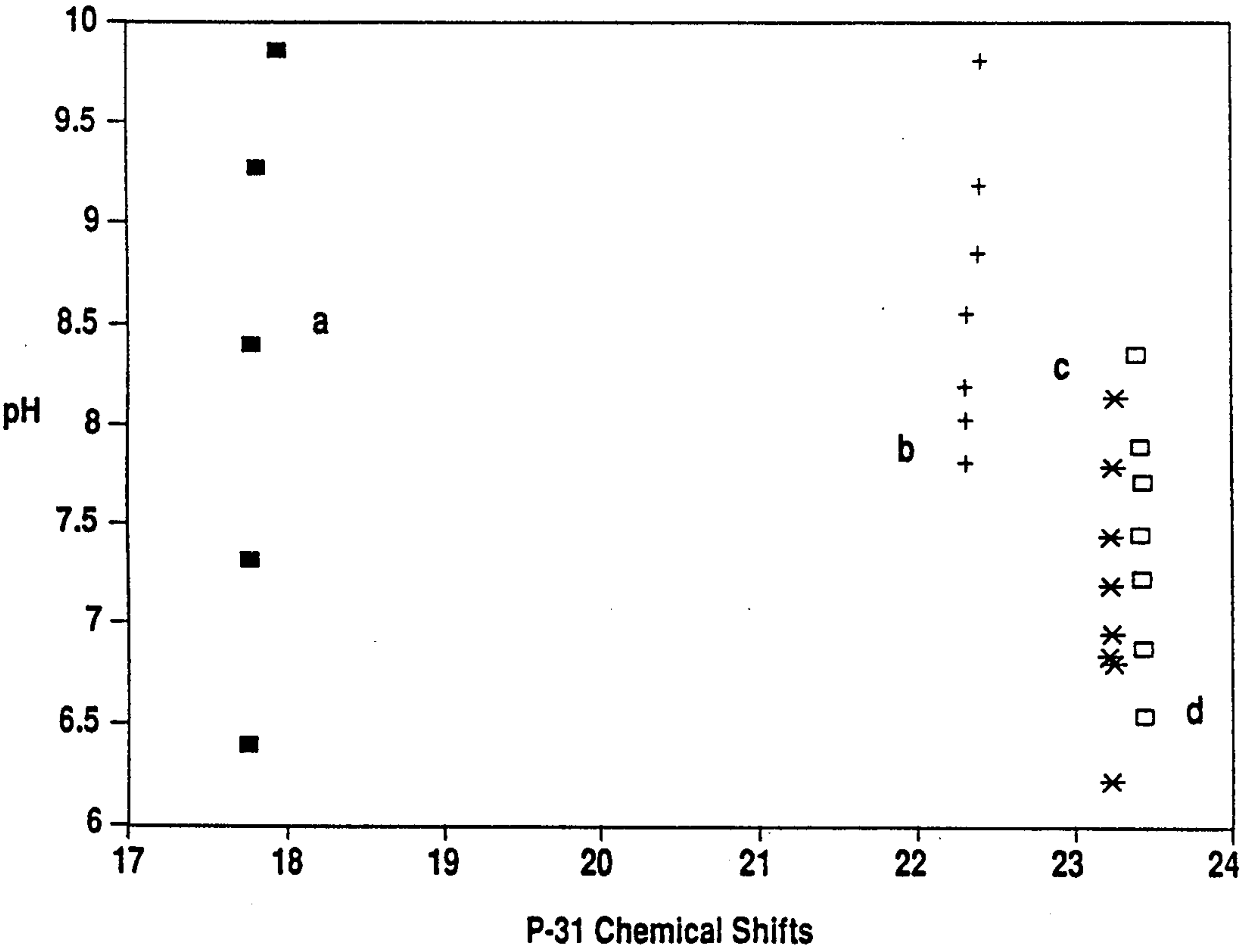


Fig. 5

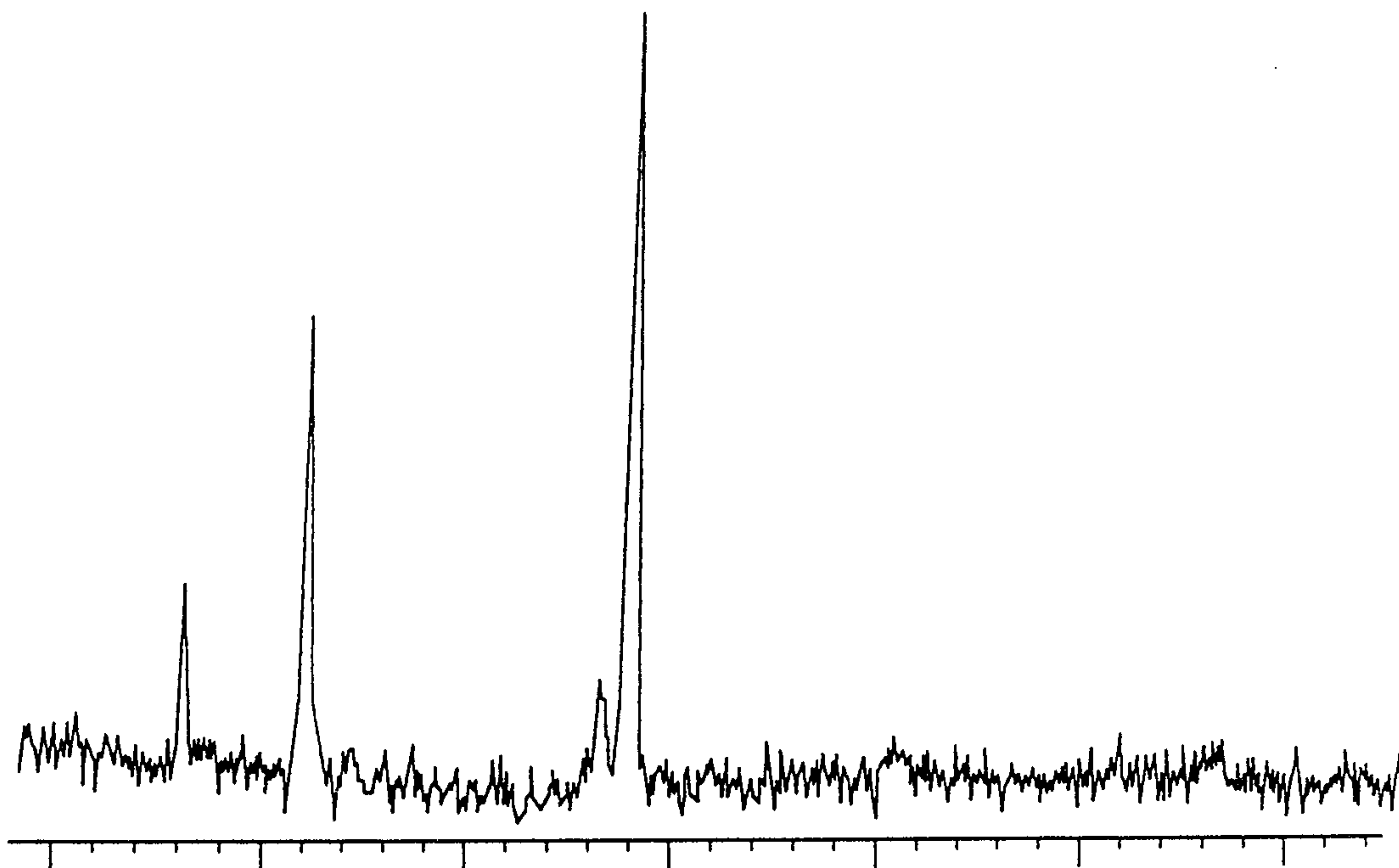


Fig. 6B

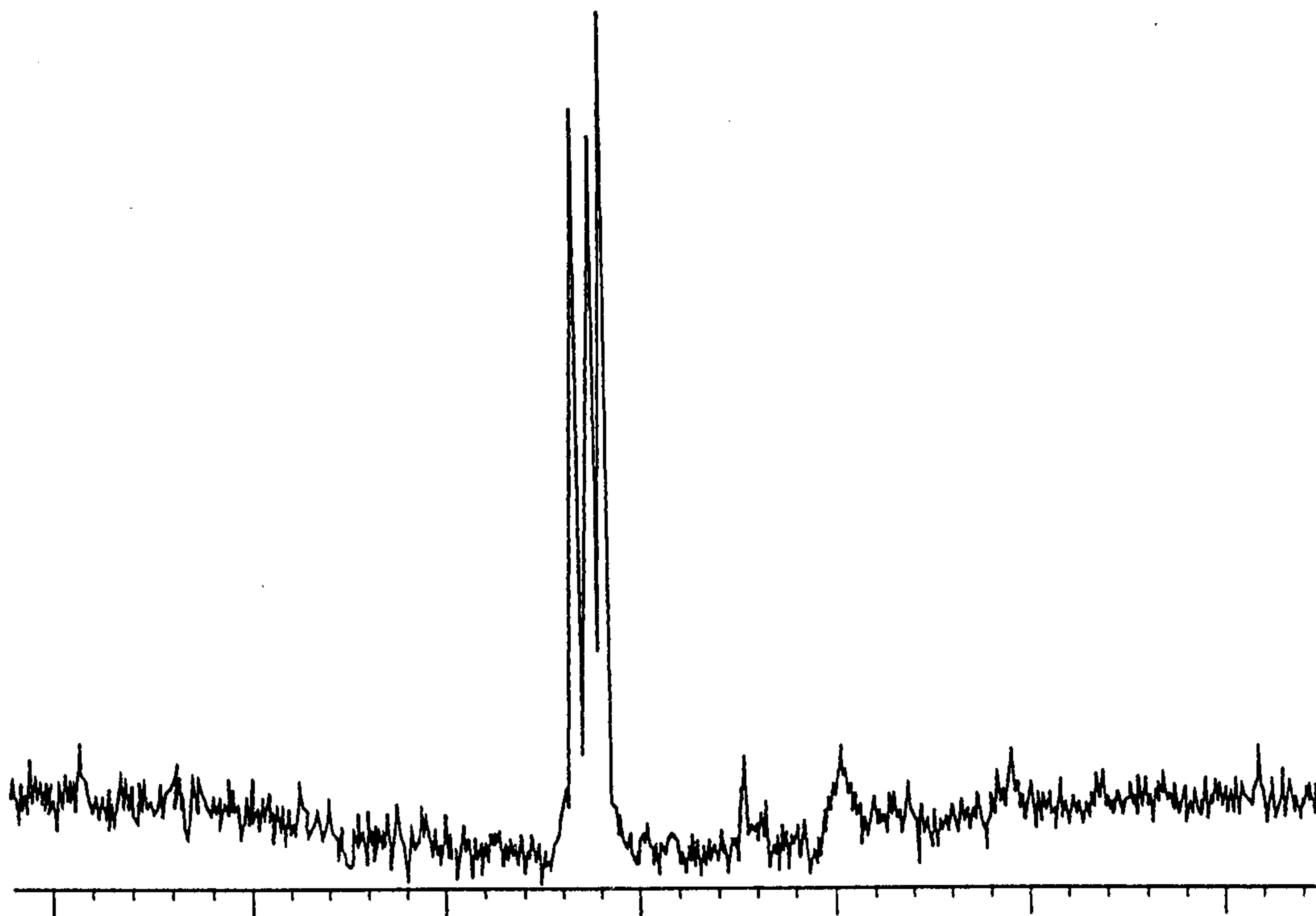


Fig. 6A

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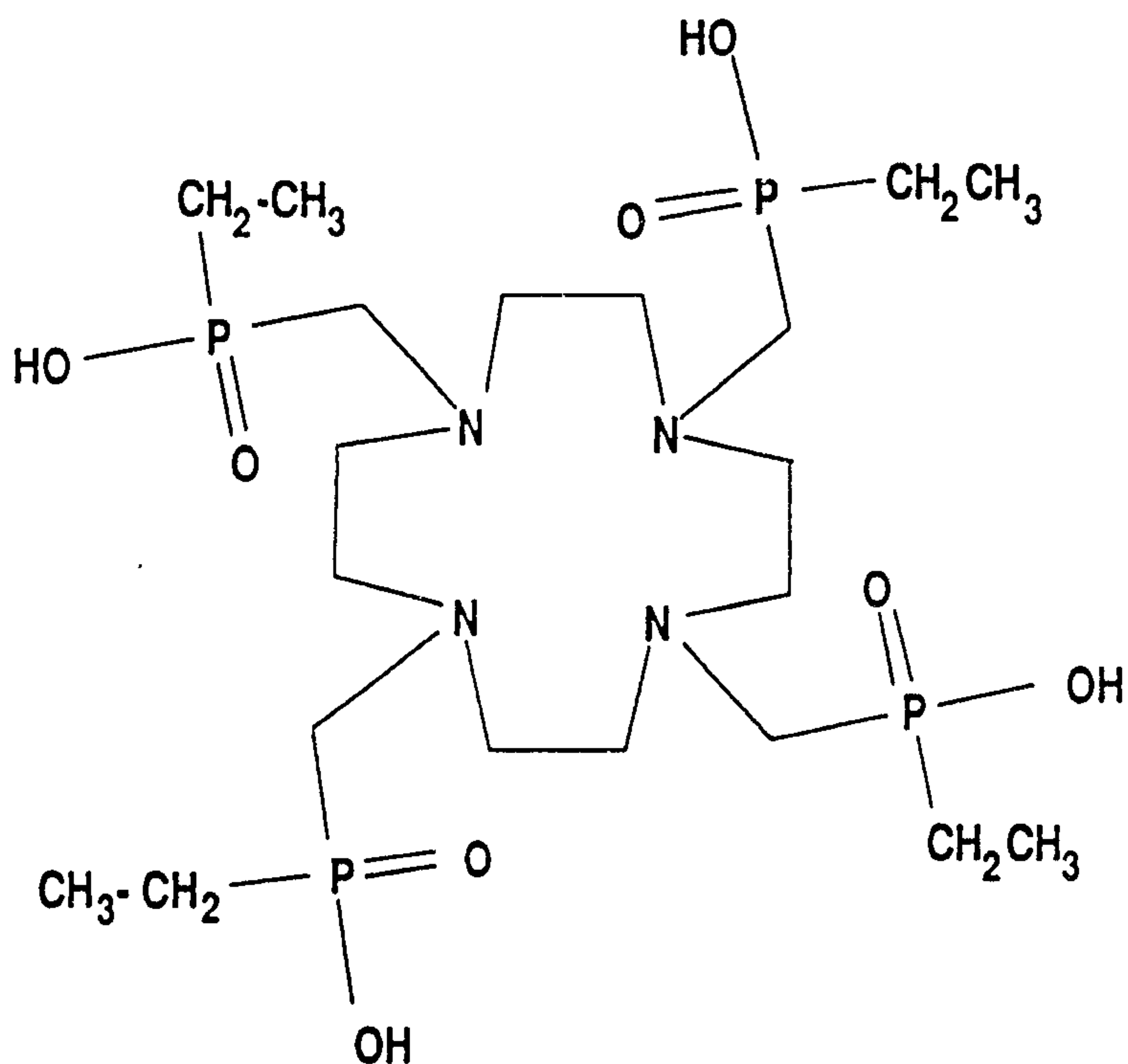


Fig. 7

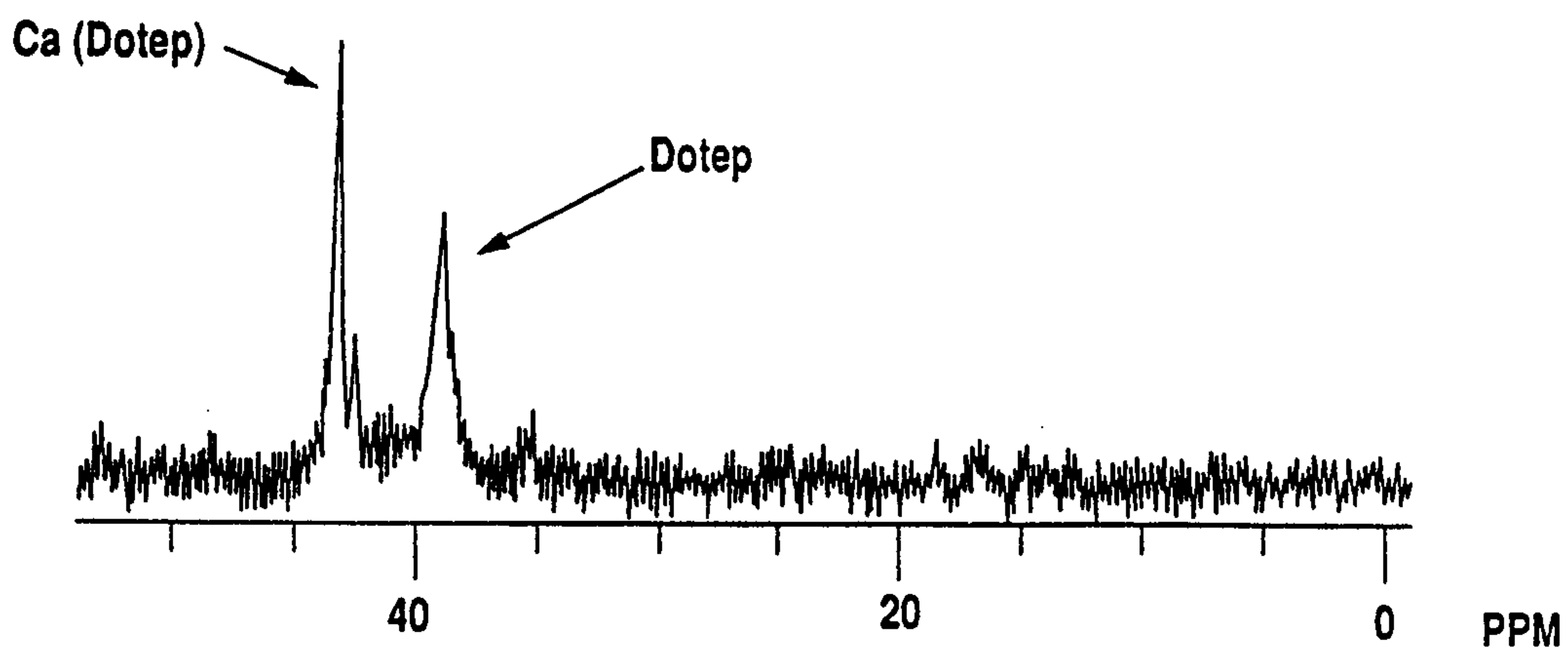


Fig. 8

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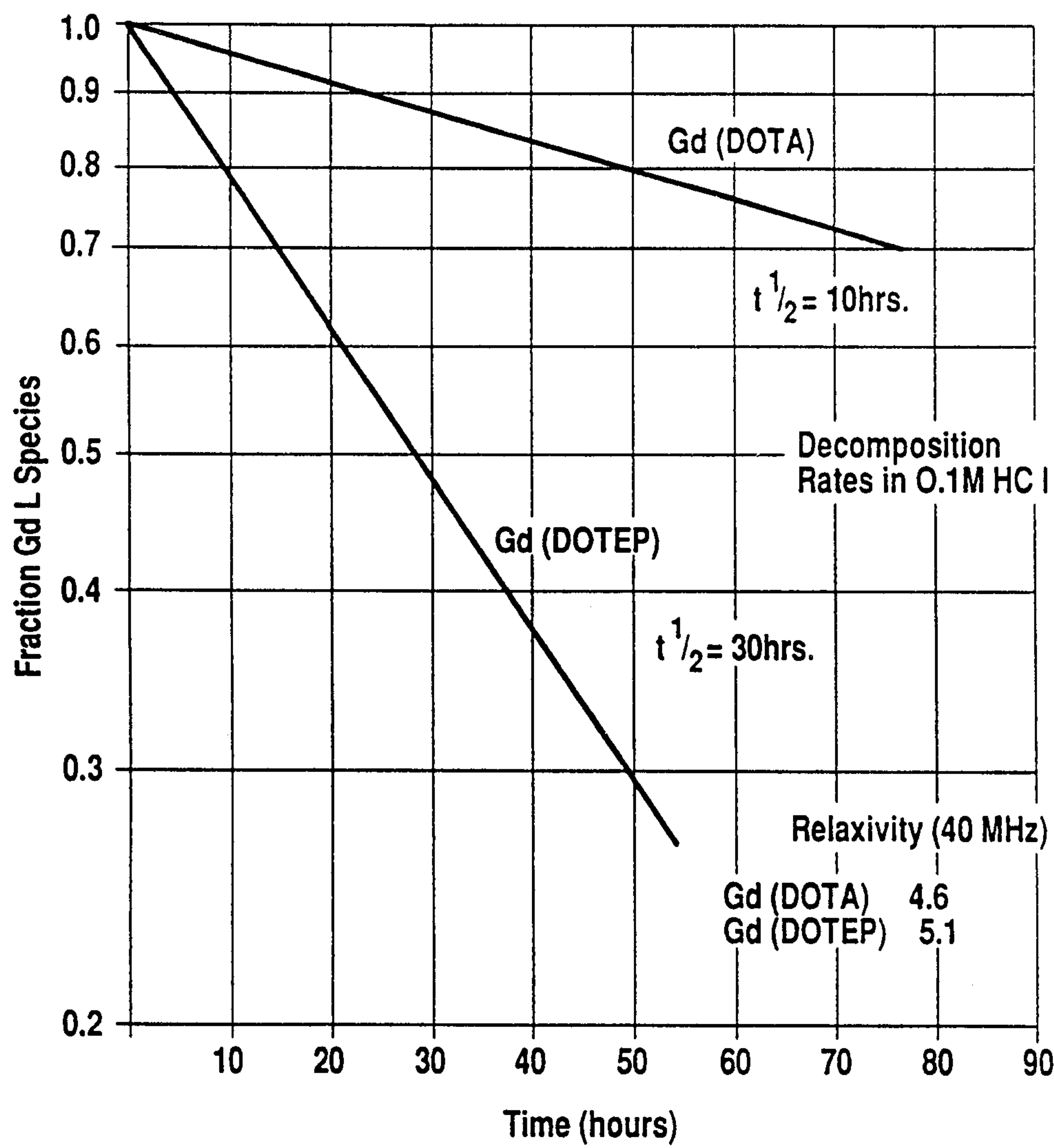


Fig. 9

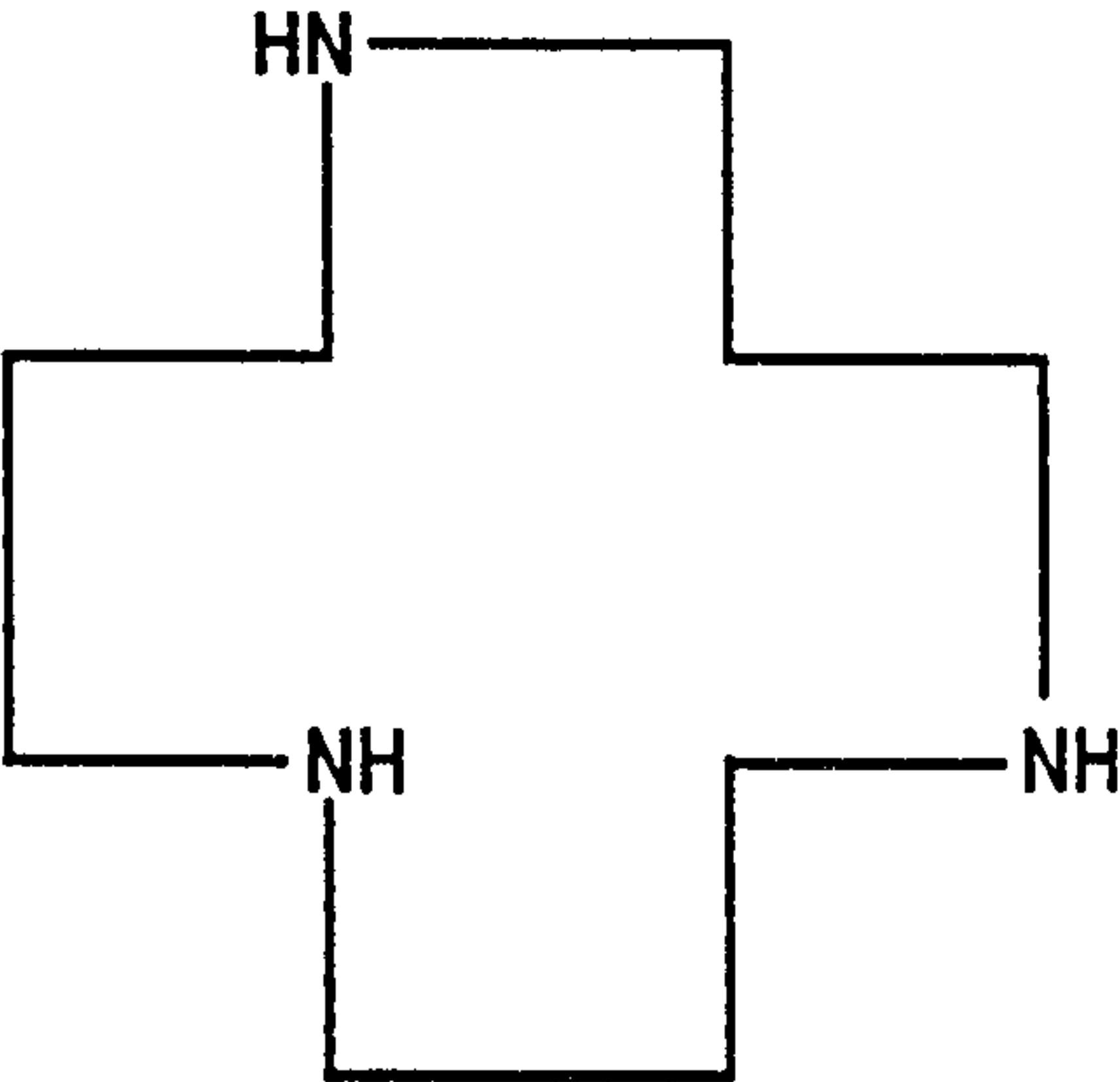


Fig. 10

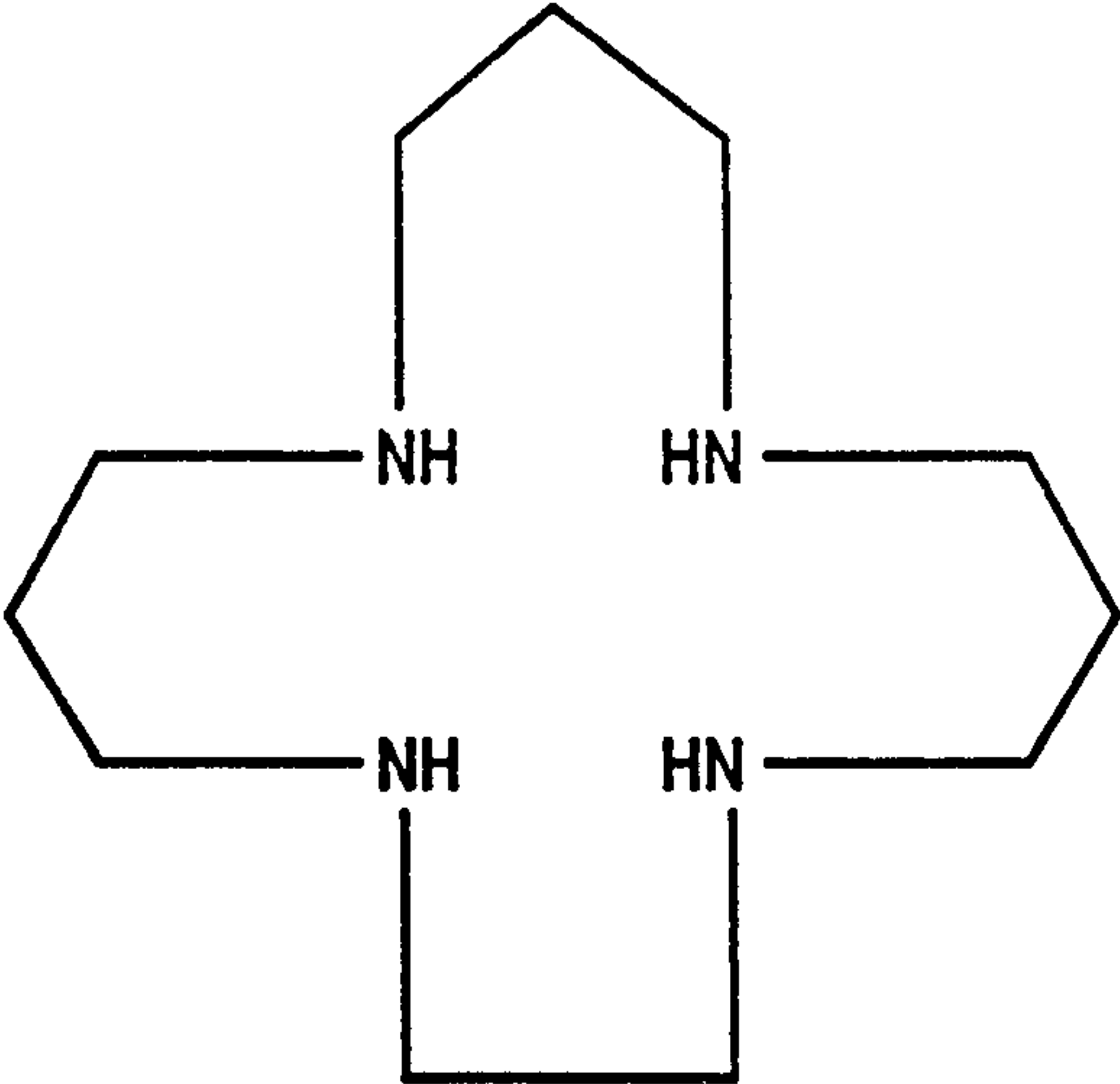


Fig. 11

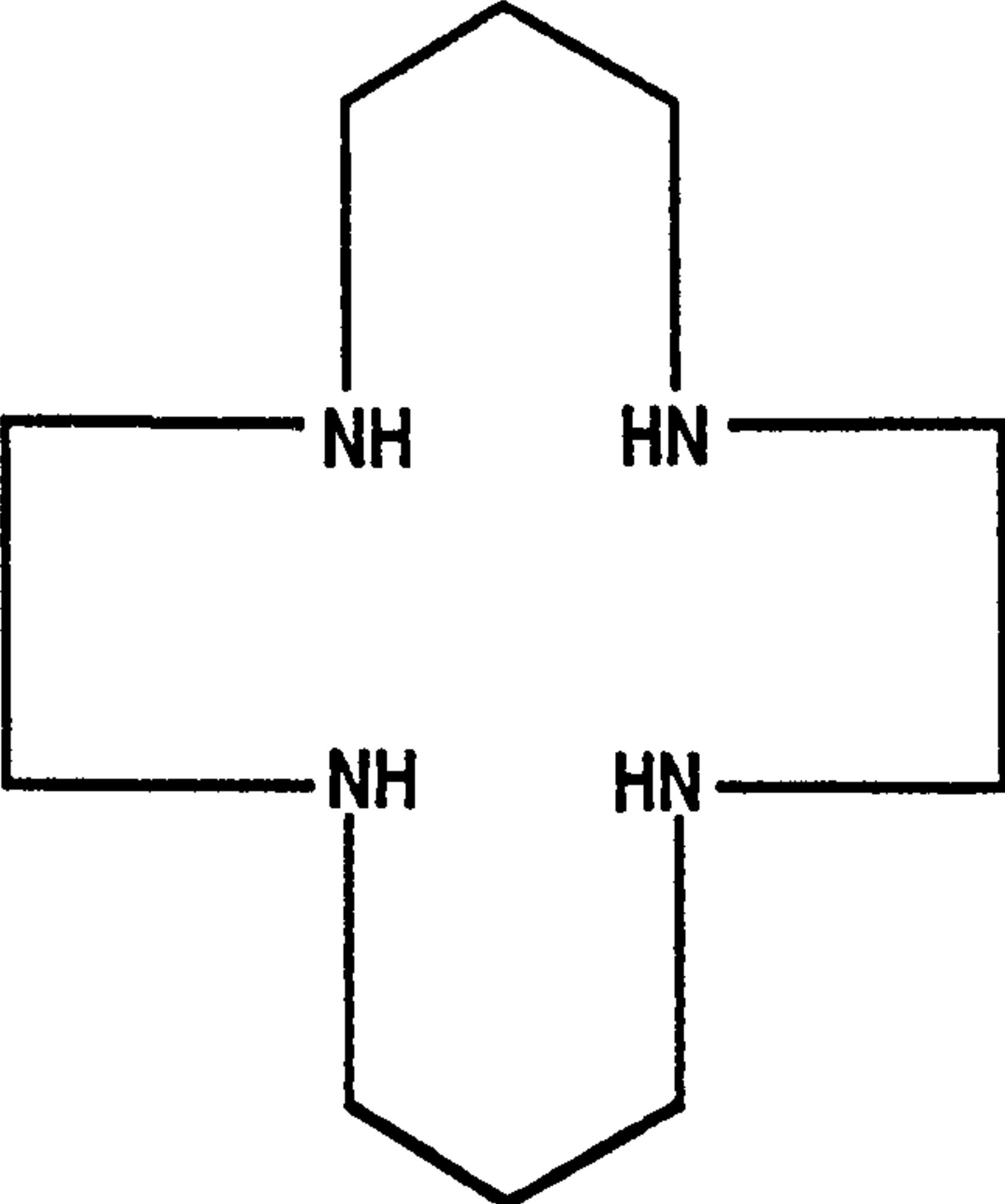


Fig. 12