Optimized Synthesis, Structure, and Solution Dynamics of 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetrakis(methylenephosphonic acid) (H_8DOTP)

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There is an emerging interest in the application of polyazamacrocyclic polymethylenephosphonate ligands as effective metal ion chelators for divalent and trivalent metal ions. One recently reported ligand that is rapidly finding many applications in radiopharmaceuticals, medical imaging, and spectroscopy is DOTP (H_8DOTP = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis(methylene phosphonic acid)).[1-3] The acid–base and metal complexation properties of this ligand have been reported[1,2] and some of the lanthanide–DOTP complexes have found use as NMR shift and relaxation reagents for proteins[4,5] and for biological cations.[6-8] One complex, TmDOTP^{5-}, has emerged as perhaps the most favorable ^{23}Na shift reagent (SR) available for perfused tissues[9-11] and for in vivo animal spectroscopy and imaging studies.[12-14] The first preparation of H_8DOTP was reported[15] in 1984, and since then, at least four similar synthetic procedures for preparing H_8DOTP have appeared,[2,5,6,16] each leading to different yields or products with subtly differing chemical characteristics. Our goal has been to develop a simple, high-yield synthetic procedure for DOTP that may be easily followed by others interested in using this ligand. We report here the X-ray crystal structure of H_8DOTP, a temperature-dependent ^{31}P NMR study which shows that the solution structure of the ligand may be similar to that found in the solid-state, and a simple procedure for preparing this ligand it its purest form.

Results and Discussion

Synthesis. H_8DOTP was prepared by reaction of tetraazacyclododecane tetrahydrochloride (sold as tetraaza-12-crown-4·4HCl by Parish Chemical CO., Orem, UT), paraformaldehyde, and phosphorous acid in the ratios shown in Table I. This table also compares the mole ratios, reaction conditions, and yields for a number of previously published synthetic procedures for this ligand. In our earliest experiments, commercially available 37% aqueous formaldehyde solution was used, but this resulted in relatively low yields, likely due to the large volume of the reaction mixture. To avoid unnecessary dilution, further experiments were carried out using solid paraformaldehyde. The majority of the published procedures used virtually the same molar ratios of reactants. Workup of the reaction mixture usually included an evaporation or concentration step followed by precipitation with a large volume of ethanol,[2] 2-propanol,[5] or acetone.[16] The products were usually filtered off and dried, yielding rather impure solids that required further purification.

In our experiments, ^{31}P NMR was used to monitor the reaction during the optimization process. The following parameters were varied: molar ratios of the reactants, total volume of the reaction mixture, acidity, addition time of paraformaldehyde, and total reaction time. The volume and rate of addition of absolute ethanol during workup were varied as well. We have found that it is not necessary to use more than 50% excess paraformaldehyde and 100% excess phosphorous acid over the cyclic amine. Larger excesses of paraformaldehyde and phosphorous acid were found to inhibit crystal formation of the ligand.

The addition rate of paraformaldehyde and the additional reflux time both affected the yield. Very little product was isolated either when paraformaldehyde was added in one portion followed by 20 min of reflux or when paraformaldehyde was added in small portions over 14 h at reflux temperature. The optimal addition time for addition of paraformaldehyde was about 60 min, and the optimal reflux time following complete addition of paraformaldehyde was also about 60 min. The optimized synthesis described in Table I has been repeated several times on a 10-g scale over the past 18 months and has proven to be very reliable. Our yield is typically 78–82%, and the isolated product is pure as judged by ^{31}P NMR.

The major side reaction is partial N-methylation of free amino groups.[16] This reaction is suppressed in very acidic media but cannot be totally eliminated. Addition of hydrochloric acid to the reaction mixture is necessary, but a too large volume should be avoided. N-Methylation dominates above pH 4, and no DOTP is formed under basic conditions. Hydroxymethyl dihydrogen phosphite can also form in this reaction, but it is very soluble in water and its presence does not interfere with crystallization of H_8DOTP.

The workup procedure is the most crucial part of the synthesis. We found that pure H_8DOTP crystallized from the reaction mixture after an equal volume of absolute ethanol had been added slowly (using an infusion pump) over a period of 1–2 h. This method gave reproducibly high yields, and the isolated H_8DOTP was of satisfactory purity for most applications.

Crystal Structure. The structure of H_8DOTP was determined via single-crystal X-ray diffractometry (see Figure 1). Tables II and III list the pertinent crystallographic data and the atomic positional parameters. The four nitrogens are on the same side of the ring, resulting in a conformation common to tetraoxo- and tetraazacyclododecanes.[17-19] The overall geometry of the ring is very similar to that reported for 1,4,7,10-tetraazacyclododecane-

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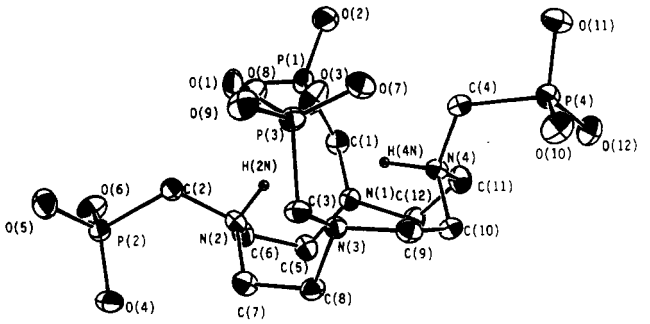

Figure 1. View of the H_8DOTP molecule showing the atom-labeling scheme.

Table I. Comparison of Molar Ratios and Reaction Conditions of H_8DOTP Syntheses, Calculated for 1 mmol of Secondary Amino Group of 1,4,7,10-Tetraazacyclododecane

mmol		μL			
H_3PO_3	HCHO	tot. H_2O	conc HCl	yield, %	ref
2.02	3.99	620	206	42	6
2.01	3.66	510	390	97[a]	16
2.03	3.67	530	320	76[b]	15
2.00	4.05	470	210	48–54	5
1.00	2.43	570	450	37[c]	2
1.0–4.1	1.1–23	0–1800	0–270	0–87	*d*
2.00	1.50	175	64	78–82	*e*

[a] Purity 78%. [b] Purity not specified; yield after purification 36%. [c] Additional crop was collected from the mother liquor by adding ethanol; no yield was given. [d] Present work; optimization range. [e] Present work; optimized conditions; purity 95%+.

Table II. Crystallographic Data

compd	$C_{12}H_{32}N_4O_{12}P_4$
mol wt	560
space group	$P2_1/n$
a, Å	9.834 (2)
b, Å	16.452 (3)
c, Å	13.493 (9)
β, deg	103.82 (9)
cell vol, Å^3	2119.9
molecules/unit cell	4
d(calcd), g/cm^3	1.75
radiation	Mo Kα
max cryst dimens, mm	0.15 × 0.1 × 0.22
std reflns	$\bar{3}30$, $\bar{2}50$, $0\bar{6}1$
decay of stds, %	2.6
no. of reflns measd	2883
2θ range, deg	$4 \leq 2\theta \geq 50$
no. of obsd reflns	2086
no. of params varied	297
struc refinement	*a*
R^b	0.036
R_w	0.041

[a] The structure was solved by direct methods. Refinement was performed by full-matrix least-squares techniques, first isotropically and then anisotropically for all non-hydrogen atoms. All hydrogen atoms were either located in difference maps or put in idealized calculated positions. They were included in the calculation but not refined. [b] $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$. Unit weights were used.

N,N',N'',N'''-tetraacetic acid (H_4DOTA).[20] The arrangement of the phosphonate groups appears to be quite unique however. Unlike in DOTA where all four carboxylates are positioned above the plane of the ring nitrogens, two of the the phosphonate groups (P1 and P3 in the figure) are positioned above the plane of the ring while the other pair (P2 and P4) are oriented away from the ring. Examination of the P–O bond lengths reveals that two of the phosphonates are fully protonated (P1–O1 = 1.487 (4) Å, P1–O2 = 1.550 (4) Å, P1–O3 = 1.574 (4) Å, P4–O10 = 1.492 (4) Å, P4–O11 = 1.537 (4) Å, P4–O12 = 1.533 (4) Å) while the other two are monoprotonated –1 anions (P2–O4 = 1.506 (4) Å, P2–O5 = 1.506 (4) Å, P2–O6 = 1.554 (4) Å, P3–O7 = 1.568 (4) Å, P3–O8 = 1.504 (4) Å, P3–O9 = 1.503 (4) Å). This was confirmed by locating the hydrogens on the six oxygen atoms by difference Fourier electron density maps. Two of the nitrogens, N2 and N4, are also protonated, resulting in an overall neutral molecule.

Table III. Atomic Positional Parameters

atom	*x*	*y*	*z*	*B*,[a] Å^2
P1	0.1201 (1)	0.11597 (7)	0.29839 (9)	1.70 (2)
P2	0.5687 (1)	0.07338 (7)	0.12865 (9)	1.69 (2)
P3	0.3067 (1)	0.37277 (8)	0.19760 (9)	1.75 (2)
P4	–0.2586 (1)	0.39227 (8)	0.14915 (9)	1.71 (2)
O1	0.2616 (3)	0.0814 (2)	0.3047 (2)	2.10 (7)
O2	0.0440 (3)	0.0821 (2)	0.3774 (2)	2.38 (7)
O3	0.1262 (3)	0.2104 (2)	0.3150 (2)	2.33 (7)
O4	0.6107 (3)	0.0861 (2)	0.0296 (2)	2.30 (7)
O5	0.6854 (3)	0.0818 (2)	0.2232 (2)	2.35 (7)
O6	0.4914 (4)	–0.0083 (2)	0.1318 (2)	2.53 (7)
O7	0.1967 (3)	0.4302 (2)	0.2286 (2)	2.48 (7)
O8	0.2629 (4)	0.2869 (2)	0.2114 (2)	2.77 (8)
O9	0.4495 (3)	0.3986 (2)	0.2565 (2)	2.93 (8)
O10	–0.3518 (3)	0.3609 (2)	0.0530 (2)	2.17 (7)
O11	–0.3119 (3)	0.3774 (2)	0.2453 (2)	2.76 (7)
O12	–0.2191 (4)	0.4813 (2)	0.1388 (2)	2.46 (7)
N1	0.0593 (4)	0.1350 (2)	0.0949 (3)	1.46 (8)
N2	0.3357 (4)	0.1806 (2)	0.0544 (3)	1.46 (8)
N3	0.2035 (4)	0.3246 (2)	–0.0031 (3)	1.66 (8)
N4	–0.0443 (4)	0.3022 (2)	0.0900 (3)	1.55 (8)
C1	0.0017 (5)	0.0971 (3)	0.1767 (3)	1.8 (1)
C2	0.4471 (5)	0.1536 (3)	0.1450 (3)	1.7 (1)
C3	0.2926 (5)	0.3870 (3)	0.0612 (3)	1.9 (1)
C4	–0.0966 (5)	0.3332 (3)	0.1799 (3)	1.8 (1)
C5	0.1529 (5)	0.0761 (3)	0.0618 (3)	1.8 (1)
C6	0.2448 (5)	0.1133 (3)	–0.0009 (3)	1.7 (1)
C7	0.3913 (5)	0.2320 (3)	–0.0197 (4)	2.1 (1)
C8	0.2749 (5)	0.2874 (3)	–0.0760 (3)	2.0 (1)
C9	0.0623 (5)	0.3534 (3)	–0.0526 (3)	2.0 (1)
C10	–0.0252 (5)	0.3731 (3)	0.0219 (3)	2.0 (1)
C11	–0.1358 (5)	0.2339 (3)	0.0347 (4)	2.0 (1)
C12	–0.0523 (5)	0.1635 (3)	0.0086 (3)	1.8 (1)

[a] Anisotropically refined atoms are given in the form of the isotropic equivalent displacement parameter defined as $(4/3)[a^2B(1,1) + b^2B(2,2) + c^2B(3,3) + ab(\cos\gamma)B(1,2) + ac(\cos\beta)B(1,3) + bc(\cos\alpha)B(2,3)]$.

NMR Studies. The ^{31}P NMR spectrum of H_8DOTP is a singlet at all pH values[1] at room temperature and above. However, when an aqueous solution containing the ligand at pH 7 was cooled to slightly below room temperature, the single resonance broadened and split into two resonances of equal area separated by about 13 ppm (see Figure 2). A line shape analysis of these data gives an activation energy of 56 kJ mol^{-1} for this process. A very similar ^{31}P temperature dependence was observed at lower pH values, but at pH 10.6 the ^{31}P resonance of DOTP broadened upon lowering the temperature to 0 °C but did not separate into two resonances. The temperature-dependent behavior of the ^{31}P spectrum likely reflects the same nonequivalency of the two phosphonates, as found in the solid-state. We and others[2] have noted that H_8DOTP reacts more rapidly with lanthanide ions than does H_4DOTA under similar solution conditions and that, unlike the $LnDOTA^-$ complexes, the $LnDOTP^{5-}$ complexes tend to aggregate to a small extent in aqueous solution.[21] Both observations may be related to the conformational preferences of the side-chain phosphonates in DOTP versus the carboxylates in DOTA.

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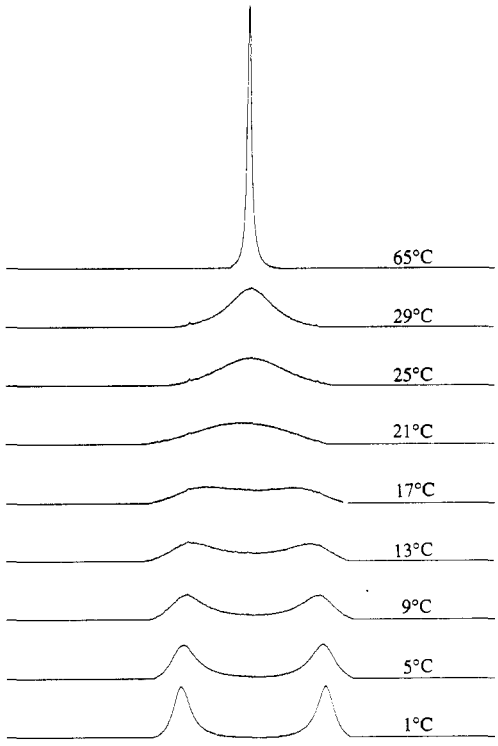

Figure 2. Temperature dependence of the ^{31}P NMR spectrum of aqueous DOTP, pH 7.

Experimental Section

Chemicals and Equipment. The following chemicals were purchased as the highest grade available and used without purification: 1, 4, 7, 10-tetraazacyclododecane tetrahydrochloride (Parish Chemical Co.), anhydrous ether (Mallinckrodt), hydrochloric acid (Fisher Scientific), paraformaldehyde and anhydrous phosphorous acid (Aldrich Chemical Co.), absolute ethanol (Aaper Alcohol and Chemical Co.). The elemental analyses were carried out by Oneida Research Services Inc., Whitesboro, NY. $^1H/^{13}C$ and ^{31}P NMR spectra were recorded on 200- and 250-MHz instruments, respectively.

Optimized Synthesis of H_8DOTP. 1,4,7,10-Tetraazacyclododecane tetrahydrochloride (10.00 g, 31.43 mmol) and anhydrous phosphorous acid (20.62 g, 251.5 mmol, 100% excess for each nitrogen) were dissolved in 22 mL of water and 8.0 mL of concentrated hydrochloric acid. The mixtures was refluxed, and dry, solid paraformaldehyde (5.66 g, calculated as 188.5 mmol of formaldehyde, 50% excess for each nitrogen) was added in small portions over 60 min. This mixture was further refluxed for 60 min. The solution was then cooled to room temperature or below, and absolute ethanol (30 mL) was added dropwise under vigorous stirring at a rate of 1 drop/3–4 s. The final mixture was stirred for an additional 1 h, and H_8DOTP was filtered through a medium-porosity-filter funnel. The solid was thoroughly washed with water (3 × 20 mL), absolute ethanol (3 × 30 mL), and anhydrous ether and dried to a constant weight under reduced pressure at 90 °C (13.80 g, 25.2 mmol, 80%). This product may be used directly for biological applications. Anal. Calcd for $C_{12}H_{32}N_4O_{12}P_4$ (M_r = 548.30): C, 26.29; H, 5.88; N, 10.22; P, 22.60. Found: C, 26.22; H, 5.94; N, 10.03; P, 22.41. ^1H NMR (15% DCl/D_2O, reference TSP): 3.54 (d, 8 H, NCH_2P, J_{PH} = 14 Hz), 3.50 ppm (s, 16 H, NCH_2).

Preparation of Single Crystals for X-ray Study. H_8DOTP was dissolved in 0.1 M hydrochloric acid, and the solution was loaded onto a Dowex 1X8-200 anion-exchange resin (chloride form). The resin was washed to neutrality with deionized water, and H_8DOTP was eluted with 0.5 M hydrochloric acid. The effluent was allowed to stand and the liquid allowed to slowly evaporated in a Petri dish covered with filter paper at room temperature. After several days, the crystals were isolated, washed with a drop of water, and dried.

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Supplementary Material Available: Textual details of the structure solution and tables of bond lengths and angles, refined atomic coordinates, and anisotropic thermal parameters (10 pages). Ordering information is given on any current masthead page.