

Synthesis and Reaction of 1,5,3,7-Diazadiphosphocine-1,5-Dicarboxylic Acids

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In order to synthesize new bioactive compounds and contrasting agents, reactions of glycine and glutamic acid as an amino acid with paraformaldehyde and hypophosphorous acid were executed. Products are 3,7-dihydroxy-3,7-dioxoperhydro-1,5,3,7-diazadiphosphocine-1,5-diacetic acid **1** and 3,7-dihydroxy-3,7-dioxoperhydro-1,5,3,7-diazadiphosphocine-1,5-di-(2-glutaric acid) **3**. 2-[5-(1,2-Dicarboxyethyl)-3,7-dihydroxy-3,7-dioxo-315.715-[1,5,3,7] diazadiphosphocan-1-yl]-succinic acid **2** by using aspartic acid was not obtained. Esterification of 3,7-dihydroxy-3,7-dioxoperhydro-1,5,3,7-diaza-diphosphocine-1,5-diacetic acid **1** by treatment of methanol, ethanol, and propanol were executed. 3,7-Dihydroxy-3,7-dioxoperhydro-1,5,3,7-diazadiphosphocine-1,5-diacetic acid methyl ester **4**, 3,7-dihydroxy-3,7-dioxoperhydro-1,5,3,7-diazadiphosphocine-1,5-diacetic acid ethyl ester **5**, and 3,7-dihydroxy-3,7-dioxoperhydro-1,5,3,7-diazadiphosphocine-1,5-diacetic acid propyl ester **6** were respectively synthesized in good yields. Continuously, we will try synthesis of novel compounds and evaluation of biological activity.

Key words – Contrasting agent, esterification, amino acid, 1,5,3,7-diazadiphosphocine-1,5-dicarboxylic acid

Introduction

In the last few years, great efforts have been devoted to the development of efficient ligands for transition metal ions, in order to obtain complexes whose stability, physical properties and biodistribution could make them suitable for application as contrast agents for magnetic resonance imaging (MRI), diagnostic therapeutic radiopharmaceuticals or fluorescent bioassays [2,4,5,10,12].

Most of these ligands belong to the huge class of polyaminopolycarboxylic acids as diethylenetriaminopentaacetic acid (DTPA), 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) and the great array of their substituted or modified derivatives. Nevertheless an increasing interest is attracted by polyaminopolyphosphonic and -phosphinic acids, as witnessed in a recent review on their coordination properties. Despite the scarce literature on α -aminoalkyl phosphinic acid, they represent a very useful class of organic compounds. The close similarity with α -aminocarboxylic acids suggests them as potential isoteric substitutes of this ubiquitous moiety. Furthermore, alkylamino and bis

(alkylamino)phosphinic acids represent optimal structural scaffolds for the preparation of novel ligands with improved properties. In sharp contrast with carboxylic and phosphonic moieties, the bidentate phosphinic may be introduced as bridging group in linear or cyclic molecules, allowing the formation of a larger number of five-membered chelate rings, well known to provide high stability to the corresponding complexes [7,9,13-15].

In addition, the lower ionic charge relating to phosphonates helps to obtain easily neutral or almost neutral metal complexes, better tolerated in vivo applications in view of the lower osmolarity of their solutions [1,3,6,8].

To the best of our knowledge, there are no reports involving the reaction of hypophosphorous acid with formaldehyde and primary amines.

Our interests in α -aminoalkylphosphinic and bis(α -aminoalkyl)phosphinic acids lies in their coordination ability towards metal ions, thereby providing useful structural motifs for the preparation of multi-sited ligands. We were particularly interested (i) in assessing the behavior of primary amino acids in the condition described above, and (ii) in searching a route to obtain mixed carboxylic phosphinic ligands. The ditopic nature of hypophosphorous acid (a formal P(O)(OH)⁻ dinucleophile) and of the primary amino group (a formal RN(CH₂)₂ dielectrophile), could give rise either

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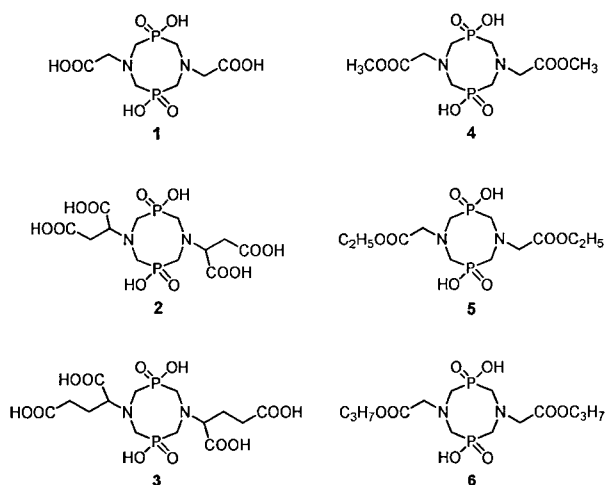


Fig 1. Structural diagram of 3,7-dihydroxy-3,7-dioxoperhydro-1,5,3,7-diazadiphosphocine-1,5-dicarboxylic acids 1, 2, 3 and its derivatives 4, 5, 6.

to linear polymeric or to cyclic oligomeric products [1].

As a part of a research program related to the synthetic study of pharmacologically interesting compounds and good chelating agents for transition metal ions, we report here the synthesis of an unusual medium-sized ring heterocyclic ligand with mixed carboxylic-amino-phosphonic donating groups (Fig. 1).

Materials and Methods

Experimental Section

Melting points were determined on an electrothermal capillary melting point apparatus and uncorrected. TLC was performed on glass plates coated with silicon oxide (silicagel 60F₂₅₄) and compounds were visualized using a uv lamp. ¹H and ¹³C NMR spectra were obtained with Bruker AC 2000 (200 MHz) and Varian Gemini (200 or 300MHz) spectrometers. Mass spectra were measured with HP 5890 GC/Mass (70 eV, EI). The organic solvents and chemicals were obtained from commercial products and purified by the appropriate methods before use.

Materials

Except where noted, all starting materials were purchased from Aldrich, Fluka, Fisher, Lancaster, or TCI chemical companies and used as received. The following known compounds were prepared by literature procedures [11]: ethanol, DMSO, hexane, chloroform, water, butanol, propanol, and methanol. Known compounds prepared by modified procedures have been included in the supplemental information:

Synthesis

3,7-Dihydroxy-3,7-dioxoperhydro-1,5,3,7-diazadiphosphocine-1,5-diacetic acid 1.

A mixture of glycine (0.75 g, 0.01 mol), hypophosphorous acid (0.55 mL, 0.01 mol), paraformaldehyde (1.8 g, 0.02 mol), and 6 M HCl (10 mL) was stirred for 30 min. And then the clear solution was left standing 3 days. A white solid product (0.26 g, yield 8%), was then collected by filtration, washed with a small amount of cold water, ethanol and dried in vacuo. Unreacted starting materials remained in solution.: mp 273-275°C; IR (*v*, KBr, cm⁻¹) 3445(OH), 2999, 1718(C=O), 1652; ¹H NMR (D₂O, pH 10, 200 MHz) δ 3.87 (s, 4H), 3.50(d, 8H, *J*=9.3 Hz); ¹³C NMR (D₂O, pH 10, 50 MHz) δ 178.5, 59.2, 55.6; MS (MALDI-TOF), *m/z* 331 (Anal. calcd for C 26.24; H, 5.50; N, 7.65; P, 16.92 Found; C, 26.50; H, 5.33; N, 7.36; P, 16.71).

3,7-Dihydroxy-3,7-dioxoperhydro-1,5,3,7-diazadiphosphocine-1,5-di-(2-glutaric acid) 3.

A mixture of L-glutamic acid (1.47 g, 0.01 mol), hypophosphorous acid (0.55 mL, 0.01 mol), paraformaldehyde (1.8 g, 0.02 mol), and 6 M HCl (20 mL) was stirred for 30 min. And then the clear solution was left standing 3 days. In order to precipitate solid, the clear solution in refrigerator was kept for 24 hr. After filtering precipitated solid, it was washed by hexane and chloroform. A white solid product (0.32 g, yield 6.8%) was then collected: mp 304-306°C; IR (*v*, KBr, cm⁻¹) 3448(OH), 2956, 1731(C=O), 1655; ¹H NMR (D₂O, pH 10, 200 MHz) δ 4.27(s, 2H), 3.87(s, 4H), 3.50(d, 8H, *J*=9.3 Hz), 2.42 (t, 4H, *J*=13.8 Hz), 2.08(m, 4H); ¹³C NMR (D₂O, pH 10, 50 MHz) δ 173.0, 172.6, 170.4, 169.7, 52.3, 51.5, 48.9, 25.8.

3,7-Dihydroxy-3,7-dioxoperhydro-1,5,3,7-diazadiphosphocine-1,5-diacetic acid methyl ester 4.

A mixture of synthesized 1,5-diacetic acid 1 (0.99 g, 3 × 10⁻³ mol) with methanol (15 mL) as solvent and reagent was stirred for 30 min. After the reaction mixture was refluxed under N₂ for 24 hr and dried. The organic layer was filtered and concentrated. A light green solid product (0.80 g, yield 74.5%) then collected: mp 273-275°C; IR (*v*, KBr, cm⁻¹) 3383, 1749 (C=O); ¹H NMR (D₂O, pH 10, 200 MHz) δ 4.12 (s, 4H), 3.57(s, 6H), 3.42(d, 8H, *J*=9.34 Hz); ¹³C NMR (D₂O, pH 10, 50 MHz) δ 179.7, 68.2, 66.0, 64.8.

3,7-Dihydroxy-3,7-dioxoperhydro-1,5,3,7-diazadiphosphocine-1,5-diacetic acid ethyl ester 5.

A mixture of synthesized 1,5-diacetic acid 1 (0.99 g, 3 ×

10^{-3} mol) with ethanol(15 mL) as solvent and reagent was stirred for 30 min. After the reaction mixture was refluxed under N_2 for 5 hr, the organic layer was filtered, concentrated, and dried. A light yellow solid product (0.82 g, yield 71%) was then collected: mp 312-314°C; IR(v, KBr, cm^{-1}) 3385, 2930, 1750(C=O); 1H NMR (D_2O , pH 10, 200 MHz) δ 4.10 (s, 4H), 4.01(d, 6H, $J=7.1$ Hz), 3.46(d, 8H, $J=7.4$ Hz), 1.00(t, 6H, $J=14.2$ Hz); ^{13}C NMR (D_2O , pH 10, 50 MHz) δ 179.1, 74.8, 67.9, 68.4, 66.1, 24.6.

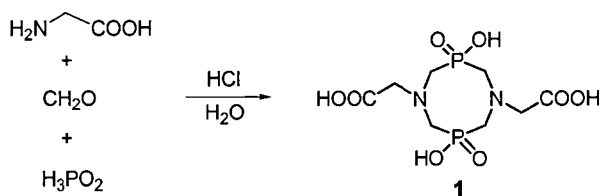
3,7-Dihydroxy-3,7-dioxoperhydro-1,5,3,7-diazadiphosphocine-1,5-diacetic acid propyl ester 6.

A mixture of synthesized 1,5-diacetic acid 1 (0.99 g, 3×10^{-3} mol) with propanol(15 mL) as solvent and reagent was stirred for 30 min. After the reaction mixture was refluxed under N_2 for 5 hr, the organic layer was filtered, concentrated, and dried. A light yellow solid product (0.86 g, yield 69.4%) was then collected: mp 335-337°C; IR (v, KBr, cm^{-1}) 3384, 2932, 1748(C=O); 1H NMR (D_2O , pH 10, 200 MHz) δ 4.05 (s, 4H), 3.86(d, 4H, $J=6.7$ Hz), 3.36(d, 8H, $J=9.3$ Hz), 1.35(dd, 4H, $J=7.1$ Hz), 0.59(t, 6H, $J=14.8$ Hz); ^{13}C NMR (D_2O , pH 10, 50 MHz) δ 179.7, 80.0, 68.1, 66.0, 32.5, 20.8.

Results and Discussion

As a part of research program related to the synthetic study of pharmacologically interesting compounds and good chelating agents for transition metal ions, we report here the synthesis and esterification of an unusual medium sized ring heterocyclic ligand with mixed carboxylicamino-phosphonic donating groups. In order to synthesize 3,7-dihydroxy-3,7-dioxoperhydro-1,5,3,7-diazadiphosphocine-1,5-diacetic acid 1, the reaction was then performed, adopting glycine as a model amino acid in aq. HCl (Scheme 1).

The strongly acidic medium is required to promote the second reaction of H_3PO_2 and to avoid the side reactions of the iminium ion such as the reduction by means of formaldehyde to *N*-methyl derivatives.

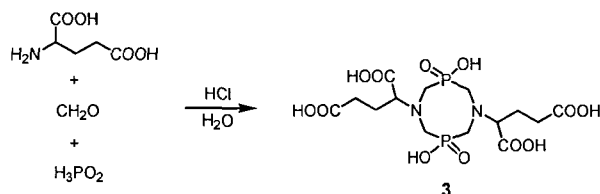


Scheme 1

The reaction was found to be highly dependant on the experimental conditions employed. High concentrations of the reactant, heat and very long reaction times led to extensive formation of polymeric products; conversely, low acidity (pH >1) and low reactant concentrations gave rise to complex mixtures. A clean reaction was effected dissolving glycine and H_3PO_2 in 6 M HCl to obtain a 1.0 M solution in both reagents and adding paraformaldehyde in slight excess (3 equiv.) in one portion. Complete dissolution was achieved by stirring for 30 min and then the clear solution was left standing for 30 min and then the clear solution was left standing for 3 days. A white solid product was then collected by filtration, washed with a small amount of cold water, ethanol and dried in vacuo.

NMR analysis of the product showed a highly symmetrical molecule, (two signals in 1H NMR and three signals in the ^{13}C NMR) with a molecular weight of 330 a.m.u., characterized as 3,7-dihydroxy-3,7-dioxoperhydro-1,5,3,7-diazadiphosphocine-1,5-diacetic acid 1. This heterocyclic ligand results from the assembly of two molecules of glycine, two molecules of H_3PO_2 and four molecules of formaldehyde; its striking feature is that each atom of this eight-membered ring is originated from eight single different molecules, representing a formal '1+1+1+1+1+1+1+1' cyclocondensation. The yield is satisfactory despite the number of elemental steps involved in the overall transformation and of the ring size, usually unfavorable for entropic reasons. And also reactions were performed by treatment of aspartic acid or glutamic acid in behalf of glycine in aq HCl(Scheme 2).

The reaction of glutamic acid with paraformaldehyde and H_3PO_2 gave 3,7-dihydroxy-3,7-dioxo-perhydro-1,5,3,7-diazadiphosphocine-1,5-di-(2-glutaric acid) 3. MS showed a protonated molecular ion at m/z 475 corresponding to the molecular formula $C_8H_{16}N_2O_8P_2$. 1H -NMR showed a singlet at δ 4.27 for two protons, a singlet at δ 3.87 for four protons, a doublet at δ 3.50 for eight protons, a triplet at δ 2.42 for four protons, and a multiplet at δ 2.08 for four protons. From these observation this product was proposed to have the structure of carboxylic acid 3.



Scheme 2

However, the reaction of aspartic acid with paraformaldehyde and H_3PO_2 did not give the expected product, 2-[5-(1,2-dicarboxyethyl)-3,7-dihydroxy-3,7-dioxo-315.715-[1,5,3,7]-diazadiphosphocan-1-yl]-succinic acid **2**. We will try synthesis of carboxylic acids by treatment of various amino acid.

Esterification of synthesized compound **1** by methanol, ethanol and propanol gave acid ester compound **4**, **5**, **6**. In the case of acid ester **4**, 1H NMR showed a singlet at δ 4.12 for four protons, a singlet δ 3.57 for six protons and a doublet at δ 3.42 for eight protons ($J=9.4$ Hz). And MS showed a protonated molecular ion at m/z 358 corresponding to the molecular formula $C_{10}H_{20}N_2O_8P_2$. From these observations, this product was proposed to have the structure of 3,7-dihydroxy-3,7-dioxoperhydro-1,5,3,7-diazadiphosphocane-1,5-diacetic acid methyl ester **4**. Structures of acid ester **5** and acid ester **6** was suggested by the similar manner as acid ester **4**.

The relative position of the functional groups is particularly interesting in view of the possible application of carboxylic acid **1** as ligand for metal ions. The $N-CH_2COOH$ and $N-CH_2-P(O)OH-CH_2-N$ moieties are known to chelate efficiently through formation of five-membered rings with the metal atom. Furthermore, the latter is embraced by the six donor atoms in a nearly ideal octahedral arrangement, highly advantageous for the complexation of the hexacoordinated transition metal ions. Hence we will start a preliminary investigation on the binding properties of carboxylic acid **1** towards Mn^{2+} and Gd^{3+} , two paramagnetic ions of choice in the design of contrast agents for MRI, with different chemical behaviors and whose magnetic features help in the investigation of the solution structures of the corresponding adducts.

Acknowledgment

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초록 : 1,5,3,7-Diazadiphosphocine-1,5-Dicarboxylic Acids의 합성과 반응정대일* · 조승환¹ · 송주현 · 이도훈 · 이용균 · 박유미 · 최순규[†] · 한정태²(동아대학교 화학과, ¹한미제약, ²영동대학교 뷰티케어과)

본 연구는 MRI 조영제나 새로운 생리활성 물질을 개발키 위해 paraformaldehyde와 hypophosphorous acid를 아미노산인 glycine 혹은 glutamic acid와 함께 반응시켜 3,7-dihydroxy-3,7-dioxoperhydro-1,5,3,7-diazadiphosphocine-1,5-diacetic acid **1**와 3,7-dihydroxy-3,7-dioxoperhydro-1,5,3,7-diazadiphosphocine-1,5-di-(2-glutaric acid) **3**을 합성하였다. 그러나 aspartic acid에 의한 2-[5-(1,2-dicarboxy-ethyl)-3,7-dihydroxy-3,7-dioxo-315.715-[1,5,3,7]diazadiphosphocan-1-yl]-succinic acid **2**는 얻지 못했다. 합성된 3,7-dihydroxy-3,7-dioxoperhydro-1,5,3,7-diazadiphosphocine-1,5-diacetic acid **1**을 산 촉매에 의한 에스테르화반응시켜 화합물 3,7-dihydroxy-3,7-dioxoperhydro-1,5,3,7-diazadiphosphocine-1,5-diacetic acid methyl ester **4**, 3,7-dihydroxy-3,7-dioxoperhydro-1,5,3,7-diazadiphosphocine-1,5-diacetic acid ethyl ester **5**, 그리고 3,7-dihydroxy-3,7-dioxoperhydro-1,5,3,7-diazadiphosphocine-1,5-diacetic acid propyl ester **6**을 합성하였다. 계속해서 다양한 반응을 통해 새로운 화합물을 합성할 것이며 생리활성검색도 실시할 예정이다.