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Synthesis via a Cyclic Dioxatrichlorophosphorane of 1,3-Dibenzyl-2-Phosphonooxy Citrate

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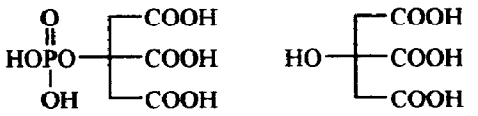
Abstract: Phosphorylation of tribenzyl citrate with PCl_5 proceeds via a cyclic dioxatrichlorophosphorane and results in the formation of 1,3-dibenzyl-2-phosphonooxy citrate. A mechanism for the phosphorylation and ester hydrolysis is proposed and the usefulness of the diester for producing triesters is discussed.

Continuing research into the chemistry[1] and biological applications[2] of the phosphorylated tricarboxylic acid 2-phosphonooxy-1,2,3-propane tricarboxylic acid (phosphocitrate, PC; fig. 1a) has focussed on the use of prodrugs of PC as tools to possibly increase its membrane permeability[3,4] or impart tissue selectivity onto the compound.[5] Accordingly, selective esterification and/or protection of the carboxylic or phosphate moieties has been an integral facet of the synthetic procedures investigated.



(a) (b)

Figure 1. Structures of (a) phosphocitric acid and (b) citric acid.

Previous research routinely required the production of esterified derivatives of citric acid (fig. 1b) suitable for susequent phosphorylation and deprotection. Phosphorylation of the α-carboxy tertiary alcohol functional group in citric acid is difficult and powerful phosphorylating reagents are necessary to gain respectable yields. Previously published phosphorylations of citrate using diphenyl chlorophosphate[6] or *o*-phenylene phosphorochloridate[7] required hydrogenation over Adam's catalyst resulting in modest yields and necessitating the use of suitable protecting groups for the carboxylic acid moieties. Although oxidation (i.e. Br_2, $Pb(Ac)_4$) also has been examined for the removal of the *o*-phenylene group,[8] such a procedure is cumbersome and not appropriate for citrate derivatives. Another agent, 2-cyanoethyl phosphate has been used to prepare ^{32}P-labelled and unlabelled PC albeit with low yields.[9]

Whilst our laboratory has had particular success producing PC, the use of halo-phosphorus compounds as direct phosphorylating agents for citrate esters requires further investigation. It is known that PCl_5

phosphorylates α-hydroxy-carboxylic acids such as D,L-malic when the latter is either the free acid or esterified.[10] However, investigations with citric acid derivatives has been limited. The use of PCl_5 for the formulation of a suitable phosphorylation method for citric acid esters was therefore, an interesting possibility The present study has focussed on the use of benzyl (Bnz) esters of citrate as these are known to be useful protecting groups for the phosphate and carboxyl moieties, requiring only mild hydrogenolysis deprotection.[11]

The reaction of two mole portions of tribenzyl citrate[12] (1) with one mole of PCl_5 (figure 2) and controlled aqueous hydrolysis of the products unexpectedly resulted in the formation of 1,3-dibenzyl-2-phosphonooxy citrate **3** in 61% yield and unreacted **1** From previous investigations this reaction was expected to produce 1,2,3-tribenzyl-2-phosphonooxy citrate (**5**, see experimental) and 2-chloro-tribenzyl tricarballylate[10] The absence of any symmetrical dibenzyl citrate in LSIMS (liquid secondary ion mass spectroscopy) of the products suggested that non-specific acid catalysed hydrolysis of the central ester was not responsible for the formation of **3**.

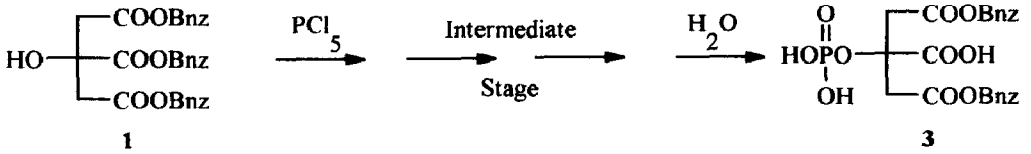


Figure 2. General scheme for the phosphorylation of tribenzyl citrate

Hence, we propose that hydrolysis of the central ester occurred during phosphorylation with PCl_5 according to the scheme outlined in fig. 3.

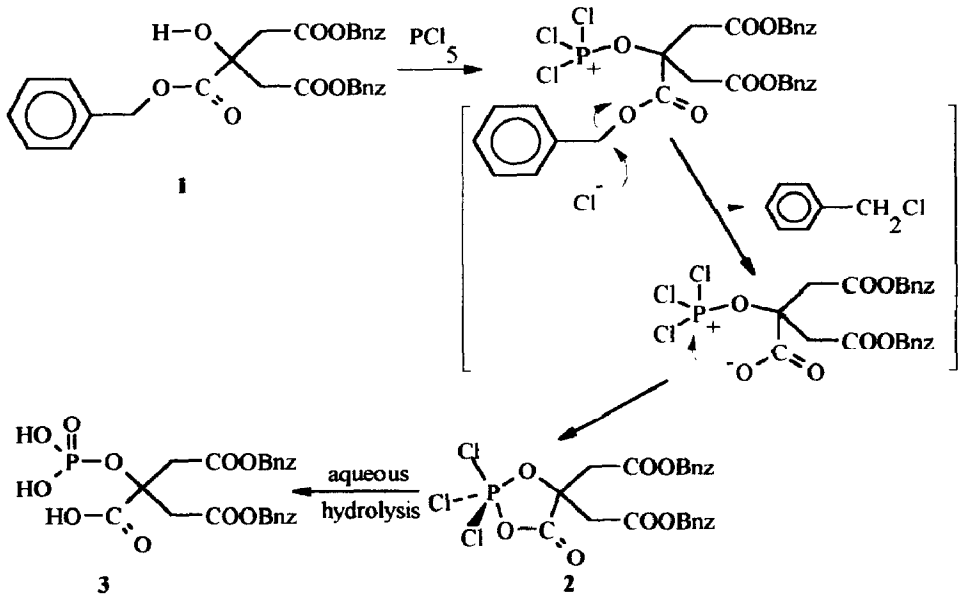


Figure 3. Mechanism for intramolecular hydrolysis occurring during phosphorylation

Supporting evidence for the mechanism was gained from ^{31}P-NMR examination of the reaction between equimolar amounts of PCl_5 and **1**. The spectrum displayed one major peak with chemical shift of -38 ppm indicative of the formation of the cyclic dioxatrichlorophosphorane **2**. Also, ^{13}C-NMR analysis revealed the total disappearence of the central ester carbonyl carbon signal at 173.4 ppm indicating that hydrolysis had occurred. The production of stoichiometric amounts of benzyl chloride during reaction (isolated through extraction of the reaction mixture with petroleum ether (b.p. 40-60°C)) also supports the proposed mechanism. Formation of similar dioxatrichlorophosphoranes has been previously observed for the reaction between some α-hydroxy carboxylic acids and PCl_5.[10,13] Of particular interest here is that its formation involves the hydrolysis of the 2-benzyl ester.

We have examined the usefulness of **3** for the production of symmetrical triesters of PC The general procedure involved the reaction of **3** (1 mol) in acetonitrile with 1.3-1.5 mol of either ethyl bromide (for compound **4**) or benzyl bromide (for compound **5**) in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; 2 mol) at room temperature for 24 h. After solvent evaporation the residue was dissolved in ethyl acetate and washed with 1% HCl followed by water before drying over Na_2SO_4. Removal of the solvent afforded the crude triesters with yields (determined from ^{1}H-NMR) typically 67% for **4** and 84% for **5**. Esterification of the phosphate hydroxyls is restricted if 2 molar equivalents of DBU are used due to the reduced nucleophilicity of the phosphate monoanion compared with the carboxylate anion.

The preparation of 1,3-dibenzyl-2-phosphonooxy citrate **3** now permits easy access to a range of additional compounds with the phosphorylated tricarboxylate backbone

EXPERIMENTAL

^{31}P-NMR was performed at 121.496 MHz in toluene-d_8 using 85% H_3PO_4 as an external standard. ^{13}C-NMR was performed in toluene-d_8 at 75.469 MHz. ^{1}H-NMR was performed at 300.13 MHz. All NMR spectroscopy was run on a Bruker Aspect 3000.

1,3-dibenzyl-2-phosphonooxy citrate (3) Tribenzyl citrate (120 g) was dissolved in benzene (500 ml) and cooled to 4°C using an ice-salt bath. The flask was fitted with a thermometer and connected to a vacuum line (water pump). PCl_5 (54 g) was ground to a fine powder and then added slowly through a solid dropping funnel into the reaction mix under vacuum. The temperature was maintained between 4-7°C with complete addition requiring 1.5 h. Following addition, the mix was stirred for a further 0.5 h at 5°C. Water (19 ml) was added slowly via a dropping funnel ensuring no rise in temperature. A white precipitate appeared after most of the water has been added. The slurry was stirred for a further 30 mins, filtered through a glass sinter and the solid washed with 100 ml benzene. The solid was dried over KOH in a vacuum dessicator to give 70g (61% yield) of white solid. ^{1}H-NMR in acetone-d_6 δ (ppm): 3.58 (s, 4H, C$\underline{H_2}$-C-C$\underline{H_2}$); 5.26 (s, 4H, -C$\underline{H_2}$-Ar); 7.52 (m, 10H, Ar-$\underline{H}$); 10.74 (s, 2H, P-O$\underline{H}$). Hydrogenation of **3** over Pd/C yielded quantitative amounts of PC. ^{1}H-NMR in D_2O δ (ppm)· 3.08-3 26 (2xd, 4H, C$\underline{H_2}$-C-C$\underline{H_2}$, J_{ab}=16.47 Hz).

1,3-dibenzyl-2-ethyl-2-phosphonooxy citrate (4). ^{1}H-NMR in acetone-d_6 δ (ppm): 1.28 (t, 3H, -CH_2-C$\underline{H}_3$); 3.6 (s, 4H, C$\underline{H}_2$-C-C$\underline{H}_2$); 4.26 (q, 2H, -C$\underline{H}$2-CH_3); 5.25 (s, 4H, -C$\underline{H}_2$-Ar); 7.47 (m, 10H, Ar-$\underline{H}$); 9 75 (s, 2H, P-O$\underline{H}$). Yield from ^{1}H-NMR = 67%

1,2,3-tribenzyl-2-phosphonooxy citrate (5). ^{1}H-NMR in acetone-d_6 δ (ppm): 3.59 (s, 4H, C$\underline{H}_2$-C-C$\underline{H}_2$); 5.21 (s, 4H, -C$\underline{H}_2$-Ar [1,3]); 5.27 (s, 2H, -C$\underline{H}_2$-Ar [2]); 7.49 (m, 15H, Ar-$\underline{H}$), 9.36 (s, 2H, P-O$\underline{H}$). Yield from ^{1}H-NMR = 84 %.

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