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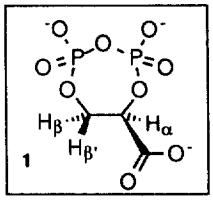
A Synthesis of cyclo-2,3-Diphospho-D-glycerate from D-Mannitol

Albrecht Berkessel*, Urs Geisel and David A. Hérault

Organisch-Chemisches Institut der Ruprecht-Karls-Universität Heidelberg, Im Neuenheimer Feld 270, D-69120 HEIDELBERG, Germany

Abstract: cyclo-2,3-Diphospho-D-glycerate (c-DPG) was synthesized from D-mannitol in seven steps on a gram-scale. Key feature of the synthetic route is the cyclization of methyl 2,3-diphospho-D-glycerate using dicyclohexylcarbodiimide. The preparation described makes the natural product c-DPG available on a larger scale for the first time.

In 1983, a novel diphosphate was discovered in methanogenic archaea. On the basis of spectroscopic investigations, the material was identified as cyclo-2,3-diphosphoglycerate[1,2]. Later on, the absolute configuration of this new natural product was established by stereospecific degradation: The novel diphosphate was found to be cyclo-2,3-diphospho-D-glycerate **1** ("cyclic 2,3-diphosphoglycerate", "c-DPG")[3]. The physiological function of c-DPG is still a matter of ongoing debate. So far, the limited availability of c-DPG from natural sources precluded a systematic investigation of its biochemical role. Therefore, a synthetic source of this novel



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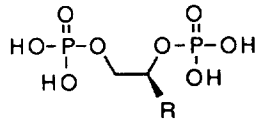

2a: R = CO_2H; **2b**: R = CO_2CH_3

natural product appeared highly desirable. In principle, c-DPG **1** can be synthesized from well known 2,3-diphospho-D-glycerate **2a** ("DPG") by carbodiimide condensation. In fact, this type of cyclization was already used by Kanodia and Roberts as a part of their structural assignment[1]. However, as could be expected, carbodiimide condensation of the trifunctional substrate DPG **2a** does not selectively afford c-DPG **1**, but gives rise to a variety of side-products. We therefore reasoned that carbodiimide cyclization of a carboxyl-protected DPG derivative should afford c-DPG in better yield (after deprotection). Herein we describe the synthesis of c-DPG **1** on a gram-scale using the methyl ester of DPG (**2b**) as the starting material for the diimide condensation.

We prepared the starting material **2b** necessary for our cyclization procedure on a gram-scale from D-mannitol by a six-step sequence of established transformations: (1) formation of the 1,2;5,6-diacetonide[4,5] (53 %); (2) ruthenium-catalyzed diol cleavage, affording sodium 2,3-isopropylidene-D-glycerate[4,6] (93 %); (3) esterification using diazomethane[4,7] (50 %); (4) cleavage of the acetonide affording methyl D-glycerate[4,8] (68 %); (5) twofold phosphorylation using diphenoxyphosphoryl chloride[4,9] (51 %); and finally (6) deprotection of the phosphoryl groups by catalytic hydrogenation[4,9], affording **2b** in quantitative yield. Thus, the methyl ester **2b** was obtained in an overall yield of 9 % from readily available D-mannitol. None of the synthetic steps required chromatographic separation of reaction products.

The transformation of **2b** into **1** was achieved as follows: To a solution of 5.82 g (20.8 mmol) of the methyl ester **2b** in 350 ml acetonitrile was added pyridine (25 ml) and dicyclohexylcarbodiimide (6.43 g, 31.2 mmol). The mixture was kept at ca. 20 °C for 20 h and rota-evaporated. The residue was taken up in water (70 ml). After removal of N,N'-dicyclohexylurea by filtration, the solution was lyophylized. The residue was taken up in 10 ml of water, and a trace amount of phenolphthalein was added. Aqueous sodium hydroxide (1N) was added until the indicator showed alkaline pH, plus an additional one-third of the amount of base

added so far. The reaction mixture was kept at 35 °C for 20 h and lyophylized. The crude product was chromatographed on Sephadex QAE (40 g, pre-equilibrated with 50 mM aqueous ammonium acetate). The products were eluted with aqueous ammonium acetate (gradient, 0.1 - 1.5 M). Fractions were assayed for organic phosphorus[10]. The desired cyclic diphosphate c-DPG **1** eluted second, preceded by a peak which contained mostly DPG **2a**. After lyophylization, 1.64 g (4.08 mmol, 20 %) of 80 % pure c-DPG **1** was obtained as the triammonium salt, the remainder being ammonium acetate[11]. The product is obtained as a colorless and extremely hygroscopic powder.

With this material in hand, we were for the first time able to conduct a thorough spectroscopic characterization of the novel cyclic diphosphate **1**[12].The NMR (^{1}H, ^{13}C, ^{31}P) data of our synthetic pyrophosphate are in agreement with those reported for the material isolated from natural sources[1,2]. Furthermore, MALDI-TOF[13] mass spectroscopy of our product shows only one strong signal at m/z = 247 [(c-DPG+2H)$^{+}$].

In summary, we have for the first time synthesized and isolated in pure form the cyclic diphosphate c-DPG **1**. Optimization of the cyclization procedure may further increase the yield of this crucial step. Nevertheless, c-DPG **1** is already at this stage available in gram-quantities. The synthetic approach to c-DPG **1** described in this note is hoped to pave the way for further studies on its biochemical function.

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11. Calculated from the ^{1}H-NMR spectrum of the mixture, using the integrals of the acetate CH_3-signal and of the CH/CH_2-signals of the pyrophosphate **1**.
12. ^{1}H-NMR (D_2O, 42 °C, 500 MHz): δ = 4.34 (dddd, $^{2}J_{H\beta,\beta'\text{-}H\beta',\beta}$ = 12.5 Hz, $^{3}J_{H\beta,\beta'\text{-}H\alpha}$ = 9.9 Hz, $^{3}J_{H\beta,\beta'\text{-}P\beta}$ = 8.2 Hz, $^{4}J_{H\beta,\beta'\text{-}P\alpha}$ = 0.4 Hz; 1H, $H_{\beta,\beta'}$), 4.42 (ddd, $^{2}J_{H\beta',\beta\text{-}H\beta,\beta'}$ = 12.5 Hz, $^{3}J_{H\beta',\beta\text{-}H\alpha}$ = 2.4 Hz, $^{3}J_{H\beta',\beta\text{-}P\beta}$ = 22.4 Hz; 1H, $H_{\beta',\beta}$), 4.91 (dddd, $^{3}J_{H\alpha\text{-}H\beta,\beta'}$ = 9.9 Hz, $^{3}J_{H\alpha\text{-}H\beta',\beta}$ = 2.4 Hz, $^{3}J_{H\alpha\text{-}P\alpha}$ = 6.6 Hz, $^{4}J_{H\alpha\text{-}P\beta}$ = 0.4 Hz; 1H, Hα); reported in ref. 1: complex 2H-multiplet at δ = 4.20 and 1H-multiplet at δ = 4.76, partially obscured by HOD. A tentative assignment of the diastereotopic protons Hβ (pro-*S*) and Hβ' (pro-*R*) can be done based on the Karplus-curve and the dihedral angles H_α-C-C-$H_{\beta,\beta'}$ obtained from computer modelling of **1**[3]: According to this analysis, the resonance at δ = 4.34 can be assigned to Hβ, and δ = 4.42 to Hβ'. ^{13}C-NMR (D_2O, 75 MHz): δ = 70.17 (td, $^{2}J_{COP\beta}$ = 7.2 Hz; CH_2), 78.69 (dd, $^{2}J_{COP\alpha}$ = 7.1 Hz; CH), 174.50 (sd, $^{3}J_{CCOP\alpha}$ = 10.6 Hz; C=O); reported in ref 1: δ = 69.39 (td, $^{2}J_{COP\beta}$ = 7 Hz; CH_2), 77.86 (dd, $^{2}J_{COP\alpha}$ = 9 Hz; CH), 173.74 (sd, $^{3}J_{CCOP\alpha}$ = 11 Hz; C=O); ^{31}P-NMR (D_2O, 81 MHz): δ = -10.51 (d, $^{2}J_{POP}$ = 17.42 Hz; P_α), -9.19 (d, $^{2}J_{POP}$ = 17.42 Hz; P_β), assignment based on H-coupled spectra; reported in ref. 2: AB quartet pattern with lines at δ = -11.08, -10.78, -9.78, -9.48; reported in ref. 1: AB quartet pattern centered at δ = 9.7. The purified mixture of the cyclic pyrophosphate **1** and ammonium acetate as described in the text had $[\alpha]_D^{20}$ = +34.1 ° (c = 1, H_2O).
13. Matrix: cyanohydroxycinnamic acid.

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