Pergamon

SCIENCE DIRECT®

Tetrahedron Letters 44 (2003) 9047–9049

TETRAHEDRON LETTERS

New facile synthesis of phosphoglycolohydroxamic acid and other phosphoglycolic acid derivatives

Philippe Weber, Matthieu Fonvielle and Michel Therisod*

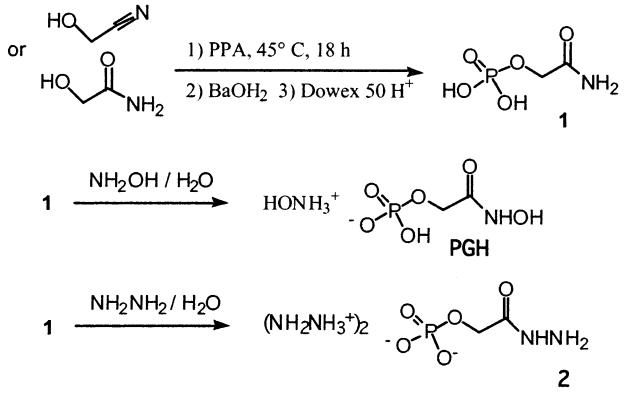
Lab. Chimie Bioorganique et Bioinorganique, ICMMO, Bat. 420, Université Paris-Sud, F-91405 Orsay, France

Received 31 July 2003; revised 19 September 2003; accepted 26 September 2003

Abstract—We report a new facile and efficient preparation of phosphoglycolohydroxamic acid, a known potent inhibitor of several enzymes acting on dihydroxyacetone phosphate. Phosphoglycolamide and phosphoglycolohydrazide can be obtained by the same method.

Glycolysis in animals (mammals) and plants utilises a Class I fructose-bis-phosphate aldolase. A very large number of inhibitors have been prepared and tested on this class of enzymes,[1] giving crucial structural and mechanistic information. These inhibitors are active on human aldolase and consequently have a limited therapeutic interest. It has been shown by Rutter[2] that some yeasts, fungi and bacteria have a zinc-containing Class II aldolase catalysing the same reaction with a very different mechanism. This microbial metalo-aldolase is an interesting target for the development of new antibiotics, and specific inhibitors would have a strong therapeutic potential, since class II aldolase is present in many pathogenic organisms, like *Candida albicans*, *Helicobacter pilori*, *Mycobacterium tuberculosis*. Surprisingly, only a limited number of Class II aldolases inhibitors have been synthesised. The best one, phosphoglycolohydroxamic acid (PGH), first introduced by Collins, Lewis and Lowe in two concomitant papers,[3,4] has a K_i in the nanomolar range. Phosphoglycolohydroxamic acid (or one of its deprotonated species) is isostructural with the enediol(ate) derived from dihydroxyacetone-phosphate. Consequently, it acts as an 'high energy intermediate-analogue' inhibitor (improperly called 'transition state-analogue' inhibitor) of several enzymes acting on this substrate. Thus, the crystal structure of the triosphosphate isomerase-PGH complex was first elucidated.[5] Crystals of several mutants of this enzyme complexed with PGH were then studied, giving useful information on its mechanism.[6–8] The compound was equally useful for the elucidation of the structure and catalytic mechanism of two class II aldolases: fructose-bisphosphate aldolase[9] and fuculose-1-phosphate aldolase,[10,11] and more recently for methylglyoxal synthase.[12] Only two independent syntheses of this important compound have been reported,[3,4] using expensive materials or tedious work-ups, via ethyl phosphoglycolate. We wish to report here an easier and more efficient preparation of PGH. The method is based on phosphorylation of glycolamide (or hydrolysis-phosphorylation of glycolonitrile) by polyphosphoric acid (PPA) and is summarised below in Scheme 1.

The synthesis involves well-known properties of nitriles (conversion to primary amides with polyphosphoric acid[13,14]) and primary amides (conversion to hydroxamic acids and hydrazides[15]). The first step of the synthesis, gives the barium salt of phosphoglycolamide **1** in 60% yield. We used the conditions described by



Scheme 1.

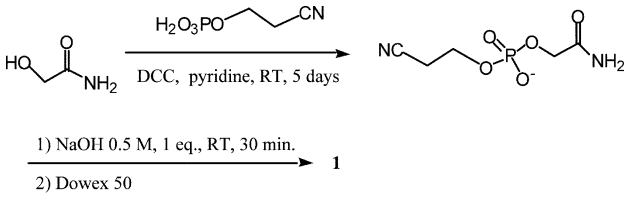
* Corresponding author. Tel.: 33 (0)1 69 15 63 11; fax: 33 (0)1 69 15 72 81; e-mail: therisod@icmo.u-psud.fr

0040-4039/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2003.09.210

Cherbuliez and Rabinowitz[16] The authors describe the conversion of glycolonitrile and glycolamide to their respective phosphoesters. However, in our hands, both starting materials gave the same unique product (phosphoglycolamide). Obviously, the fate of glycolonitrile in this reaction strongly depends on the concentration of water present in PPA. Moreover, by using milder reaction conditions, the yield was significantly increased as compared to the original method (25→60%). Phosphoglycolamide was also described and tested as an (inactive) inhibitor by Lewis and Lowe.[17] Treatment of this intermediate (either in acidic form or as its cyclohexylammonium salt) with a large excess of aqueous hydroxylamine affords **PGH** quantitatively. The new phosphoglycolohydrazide **2** is similarly prepared by reaction of 4 molar equivalents of hydrazine hydrate on **1**. One further advantage of using phosphoglycolamide instead of phosphoglycolate esters is that no hydrolysis can take place in the basic aqueous medium used (for instance, ethyl phosphoglycolate, when treated with aqueous hydroxylamine under our conditions gives a mixture of 80% hydroxamic acid and 20% phosphoglycolic acid, to be separated). The three compounds are easily converted to their free acid forms and to the corresponding bis-cyclohexylammonium salts for characterisation and purification through recrystallisation. Although **1** is obtained in only a moderate yield, the very simple procedure makes the whole method satisfactory as compared to those described previously. Alternatively, we have developed another method for phosphorylation of glycolamide through treatment with β-cyanoethylphosphate in pyridine in the presence of DCC, as summarised in Scheme 2 which gave compound **1** in an 80% yield.

Typical procedure and selected analytical data

Anhydrous glycolonitrile (Fluka, 10 mmol; water was removed by co-evaporation with pyridine) or (preferably) glycolamide was dissolved in an excess of commercial polyphosphoric acid (Acros, 4 ml). The mixture was warmed at 45°C under vacuum on a rotary evaporator for 1 h, and then left at the same temperature in a stoppered flask. After cooling, the mixture was diluted with cold water and neutralised (pH 8) with solid barium carbonate and barium hydroxide. The white precipitate was filtered off and the aqueous residue concentrated to a small volume until the barium salt of phosphoglycolamide **1** precipitated.



Scheme 2.

The barium salt (1 mmol) was dissolved in water by shaking in the presence of an excess of Dowex 50 (H^+). After removal of the resin, 0.7 ml (10 mmol) of commercial (Aldrich) aqueous hydroxylamine was added and the resulting solution kept overnight at room temperature. The conversion to hydroxamic acid was quantitative (as checked by NMR). Water and excess hydroxylamine were exhaustively evaporated under vacuum, and the CHA salt formed by addition of an excess of cyclohexylamine in water, followed by evaporation. The CHA salt of hydrazide **2** was prepared similarly by reaction of 4 molar equivalents of hydrazine hydrate on **1**, followed by ion exchange of the reaction medium on a Dowex 50 (CHA^+) column and evaporation.

1: (CHA salt, EtOH): ^{1}H NMR (D_2O) δ 4.05 (2H, d, 8 Hz) (2.95: 2H, m; 0.8–1.3: 10H, m; 1.3–1.9, 10H, m: CHA). ^{13}C NMR (D_2O) δ 177.6, 177.5, 63.7, 63.6 (51.1, 31.2, 25.2, 24.7: CHA).

PGH: (CHA salt, EtOH): ^{1}H NMR (D_2O) δ 4.15 (2H, d, 8 Hz) (2.95: 2H, m; 0.8–1.3: 10H, m; 1.3–1.9, 10H, m: CHA). ^{13}C NMR (D_2O) δ 169.4, 169.5, 62.1, 62 (51.15, 31.4, 25.4, 24.9: CHA). IR (KBr) 1653 cm^{-1} (br), 1558 cm^{-1}. Anal. calcd for C_{14} $H_{32}N_3O_6P$: C,45.52; H, 8.73; N, 11.38; P, 8.39. Found: C, 45.01; H, 8.64; N, 11.11; P, 8.64.

2: (CHA salt, EtOH)): ^{1}H NMR (D_2O) δ 4.1 (2H, d, 7 Hz); (2.95: 2H, m; 0.8–1.3: 10H, m; 1.3–1.9, 10H, m: CHA). ^{13}C NMR (D_2O) δ 172.5,172.6, 63.7, 63.6 (51.15, 31.4, 25.4, 24.9: CHA). IR (KBr) 1670 cm^{-1}, 1558 cm^{-1}. Anal. calcd for $C_{14}H_{33}N_4O_5P$: C, 45.64; H, 9.03; N, 15.21; P, 8.41 Found: C, 44.74; H, 8.91; N, 15.24; P, 8.4.

References

1. Gefflaut, T.; Blonski, C.; Perié, J.; Willson, M. *Prog. Biophys. Mol. Biol.* **1995**, *63*, 301–340.
2. Rutter, W. J. *Federation Proc.* **1964**, *23*, 1248–1257.
3. Collins, K. D. *J. Biol. Chem.* **1974**, *249*, 136–142.
4. Lewis, D. J.; Lowe, G. *J. Chem. Soc., Chem. Commun.* **1973**, 713–715.
5. Davenport, R. C.; Bash, P. A.; Seaton, B. A.; Karplus, M.; Petsko, G. A.; Ringe, D. *Biochemistry* **1991**, *30*, 5821–5826.
6. Zhang, Z.; Sugio, S.; Komives, E. A.; Liu, K. D.; Knowles, J. R.; Petsko, G. A.; Ringe, D. *Biochemistry* **1994**, *33*, 2830–3837.
7. Komives, E. A.; Lougheed, J. C.; Zhang, Z.; Sugio, S.; Narayana, N.; Xuong, N. H.; Petsko, G. A.; Ringe, D. *Biochemistry* **1996**, *35*, 14474–14484.
8. Zhang, Z.; Komives, E. A.; Sugio, S.; Blacklow, S. C.; Narayana, N.; Xuong, N. H.; Stock, A. M.; Petsko, G. A.; Ringe, D. *Biochemistry* **1999**, *38*, 4389–4397.
9. Hall, D. R.; Leonard, G. A.; Reed, C. D.; Watt, C. I.; Berry, A.; Hunter, W. N. *J. Mol. Biol.* **1999**, *287*, 383–394.
10. Dreyer, M. K.; Schulz, G. E. *J. Mol. Biol.* **1996**, *259*, 458–466.

11. Fessner, W.-D.; Schneider, A.; Held, H.; Sinerius, G.; Walter, C.; Hixon, M.; Scloss, J. V. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2219–2221.
12. Marks, G. T.; Harris, T. K.; Massiah, M. A.; Mildvan, A. S.; Harrison, D. H. T. *Biochemistry* **2001**, *40*, 6805–6818.
13. Snyder, H. R.; Elston, C. T. *J. Am. Chem. Soc.* **1954**, *76*, 3039–3040.
14. Hauser, C. R.; Erby, C. J. *J. Am. Chem. Soc.* **1957**, *79*, 725–727.
15. Hoffmann, C. *Chem. Ber.* **1889**, *22*, 2854–2856.
16. Cherbuliez, E.; Rabinowitz, J. *Helv. Chim. Acta* **1956**, *174*, 1455–1461.
17. Lewis, D. J.; Lowe, G. *Eur. J. Biochem.* **1977**, *80*, 119–133.