Stereoselective Syntheses of the Methylene- and α-Fluoromethylene-phosphonate Analogues of 2-Phospho-D-Glyceric Acid

G. Michael Blackburn* and Abdul Rashid

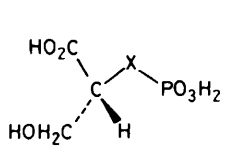
Department of Chemistry, The University, Sheffield S3 7HF, U.K.

An enantioselective synthesis is described for the preparation of (2*R*)-2-carboxy-3-hydroxypropane-1-phosphonic acid (**1**), an isosteric analogue of 2-phospho-D-glyceric acid (**2**), from L-threose; the route is extended to provide diastereoselective syntheses of both (1*R*,2*R*)- and (1*S*,2*R*)-2-carboxy-1-fluoro-3-hydroxypropane-1-phosphonic acids (**3**) and (**4**) respectively which provide the first examples of the synthesis of α-fluoroalkanephosphonates of defined chirality at C-1.

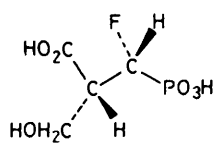
The use of phosphonate analogues[1] of biological phosphates for the study of enzyme-catalysed reactions in glycolysis achieved major significance with the synthesis[2,3] and enzymatic evaluation[2,4] of the racemic modification of the isosteric analogue (**4**) of 3-phospho-D-glyceric acid, 3-PGA. Knowles[4] showed that (**1**) is a substrate for phosphoglycerate kinase, PGK, with similar values of k_{cat} to the natural substrate although with binding to the enzyme 60-fold weaker than 3-PGA at pH 6—9. That difference can be attributed in part to the weaker acidity of the phosphonic acid relative to the parent phosphate.[4,5]

We have argued[5] that α-fluoroalkanephosphonic acids should exhibit improved performance as analogues of the corresponding phosphate esters with regard to both static and dynamic aspects of binding to enzymes and there are examples which support this hypothesis.[6—8] Hitherto, among the analogues of glycolytic intermediates, an enantiospecific synthesis has been described only in the case of the two isomers of 3,4-dihydroxybutanephosphonic acid which are analogues of D- and L-glycerol-3-phosphate.[9] We now report stereoselective syntheses for the methylene- and monofluoromethylene-analogues of 2-phospho-D-glyceric acid (**2**).

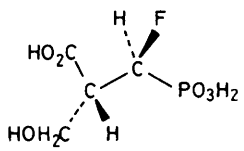
1,2-*O*-Isopropylidene-L-threose (**5**) was obtained from 2,4-*O*-benzylidene-L-threose[10] by treatment with toluene-*p*-sulphonic acid in dry acetone, an improvement on the previously reported 2-step procedure.[11] Oxidation of (**5**) using pyridinium chlorochromate (PCC) in dry dichloromethane[12] was superior to the preparation of (**6**) by oxidation with ruthenium tetroxide,[13] and gave the oily ketone (**6**) in 76% yield. This



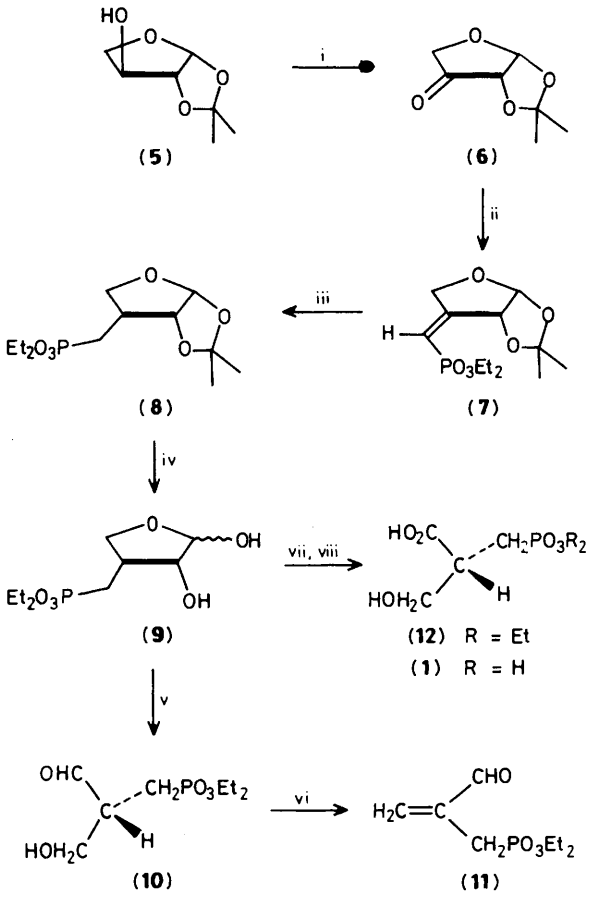
(**1**) X = CH_2
(**2**) X = O



(**3**)



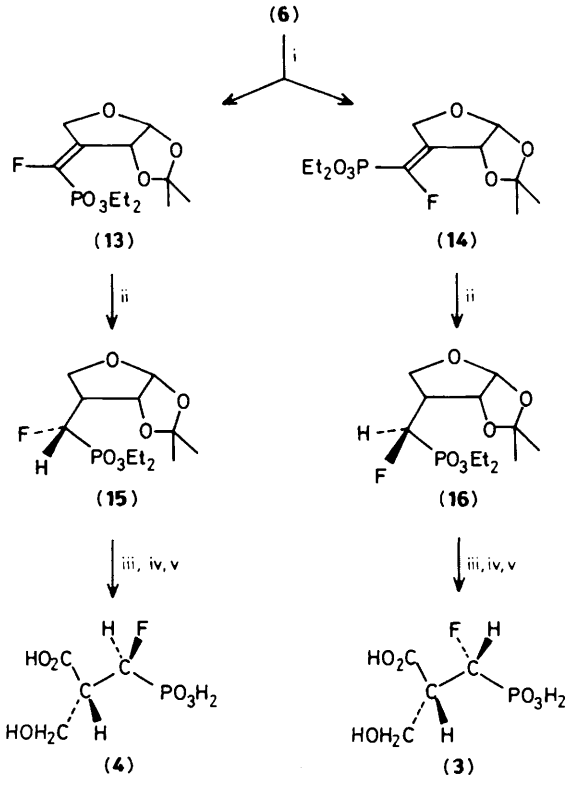
(**4**)



Scheme 1. *Reagents and conditions*: i, PCC, CH_2Cl_2, NaOAc; ii, $\{(EtO)_2P(O)\}_2CHLi$, tetrahydrofuran; iii, H_2, Pd–C; iv, 90% trifluoroacetic acid; v, $NaIO_4$, aq. $NaHCO_3$, 20 °C, 1 h; vi, SiO_2, EtOAc; vii, excess $NaIO_4$, aq. $NaHCO_3$, 20 °C, 3 h; viii, Me_3SiI, MeCN then MeOH.

product condensed smoothly with tetraethyl-, but not tetraisopropyl-, methylenebisphosphonate as its lithium derivative to give a single diastereoisomeric form of the α-vinylphosphonate (**7**). Analysis of the ^{13}C n.m.r. spectrum of (**7**) revealed a large coupling from phosphorus to C-4 ($^3J_{CP}$ 24 Hz) and a small coupling to C-2 ($^3J_{CP}$ 5 Hz) supporting the assignment of the *Z*-configuration shown (Scheme 1). Catalytic hydrogenation of (**7**) using 10% Pd–C in ethanol proceeded stereospecifically as expected to give the D-*erythro*-phosphonate (**8**) as the sole product, identified by ^1H n.m.r. analysis. The isopropylidene group of (**8**) was smoothly removed by treatment with trifluoroacetic acid/water (9/1) at room temperature and the lactol (**9**) oxidised without further purification to the aldehyde (**10**) with sodium metaperiodate in water. The attempted purification of this protected analogue of D-glyceraldehyde-2-phosphate by chromatography on silica resulted in its dehydration to diethyl 2-methylene-3-oxopropanephosphonate (**11**). When, however, the lactol (**9**) was oxidised for a longer time with a small excess of sodium metaperiodate, the desired acid ester (**12**) was obtained as an oil and directly treated with iodotrimethylsilane[14] in acetonitrile followed by methanolysis to give the required product (**1**), which was fully characterised as its *p*-anisidinium and trilithium salts.†

† All new compounds were fully characterised by spectroscopic and composition analysis and were homogeneous by h.p.l.c. or t.l.c. analysis.



Scheme 2. *Reagents and conditions*: i, $[(EtO)_2PO]_2CFLi$, tetrahydrofuran; ii, H_2, Pd–C; iii, 90% trifluoroacetic acid; iv, $NaIO_4$, aq., $NaHCO_3$, 20 °C, 4 h; v, Me_3SiI, MeCN, then MeOH.

When this route was applied to the synthesis of (1*S*,2*R*)-2-carboxy-1-fluoro-3-hydroxypropanephosphonic acid (**4**), the condensation of tetraethyl fluoromethylenebisphosphonate as its lithium salt[15] with (**5**) showed little stereoselectivity and gave a mixture of (*Z*)- and (*E*)-isomers of the desired condensation product in a 2:3 ratio. These were separated with some difficulty by h.p.l.c. while their stereochemistries were confidently assigned on the basis of ^1H n.m.r. and nuclear Overhauser enhancement (n.O.e.) data. In particular, the (*Z*)-isomer (**13**) shows an n.O.e. relating the CH_2 groups in the phosphonate ester to H-2 which is not seen in the spectrum of the *E*-isomer (**10**). Neither isomer shows any n.O.e. between the ester hydrogens and either H-4′ or H-4″. These stereoisomers were separately reduced (10% Pd–C in ethanol) without loss of fluorine[11] in a stereospecific fashion to give the two diastereoisomers of the α-fluoro-derivatives of (**8**). This reduction not only introduces the required D-stereochemistry at C-3 of the tetrose but also controls the absolute stereochemistry at the carbon adjacent to phosphorus. It follows that the (*Z*)-isomer (**13**) is converted into the (1*S*,2*R*) product (**15**) while the (*E*)-diastereoisomer (**14**) gives the (1*R*,2*R*) product (**16**). In practice, the reduction could be performed more conveniently on the mixture of (**13**) and (**14**) since the epimers (**15**) and (**16**) are readily separable by medium pressure liquid chromatography and independently identified by n.m.r. analysis (Scheme 2).

The syntheses of the target fluoromethylene analogues (**4**) and (**3**) respectively, of 2-phospho-D-glyceric acid (**2**) was concluded by operations on (**15**) and (**16**) as for the conversion of (**8**) into (**1**).

The availability of the discrete epimers of the monofluorophosphonate analogue of 2-phospho-D-glyceric acid should

permit assessment of whether the presence of a fluorine atom adjacent to the phosphoryl group gives a scalar or a vectorial advantage to such analogues of biological phosphate esters. Results on the evaluation of the activity of analogues (**1**), (**3**), and (**4**) towards appropriate glycolytic enzymes will be reported in due course.

We thank the S.E.R.C. for financial support.

Received, 30th September 1987; Com. 1417

References

1 R. Engel, *Chem. Rev.*, 1977, **77**, 349.
2 H. B. F. Dixon and M. J. Sparkes, *Biochem. J.*, 1974, **141**, 715.
3 F. R. Pfeiffer, J. Mier, and J. A. Weisbach, *J. Med. Chem.*, 1974, **17**, 112.
4 G. A. Orr and J. R. Knowles, *Biochem. J.*, 1974, **141**, 721.
5 G. M. Blackburn, *Chem. Ind. (London)*, 1981, 134.
6 G. M. Blackburn, F. Eckstein, D. E. Kent, and T. D. Perrée, *Nucleosides, Nucleotides*, 1985, **4**, 145.
7 *cf.* K. E. Stremler and C. D. Poulter, *J. Am. Chem. Soc.*, 1987, **109**, 5542.
8 C. Bisbal, M. Silhol, M. Lemaître, B. Bayard, T. Salehzada, B. Lebleu, T. D. Perrée, and G. M. Blackburn, *Biochemistry*, 1987, **26**, 5172.
9 K.-C. Tang, B. E. Tropp, and R. Engel, *Tetrahedron*, 1978, **34**, 2873.
10 A. B. Foster, A. H. Haines, J. Homer, J. Lehmann, and L. F. Thomas, *J. Chem. Soc.*, 1961, 5005.
11 M. Steiger and T. Reichstein, *Helv. Chim. Acta*, 1936, **19**, 1016.
12 *cf.* D. H. Hollenberg, R. S. Klein, and J. J. Fox, *Carbohydr. Res.*, 1978, **67**, 491.
13 V. M. Parikh and J. K. N. Jones, *Can. J. Chem.*, 1965, **43**, 3452.
14 G. M. Blackburn and D. Ingleson, *J. Chem. Soc., Perkin Trans. 1*, 1980, 1150.
15 G. M. Blackburn and M. J. Parratt, *J. Chem. Soc., Perkin Trans. 1*, 1986, 1417.