Tetrahedron Letters Vol. 21, pp 2017 - 2020 0040-4039/80/0515-2017$02.00/0

A SIMPLE SYNTHESIS OF CHOLINE ALKYL PHOSPHATES

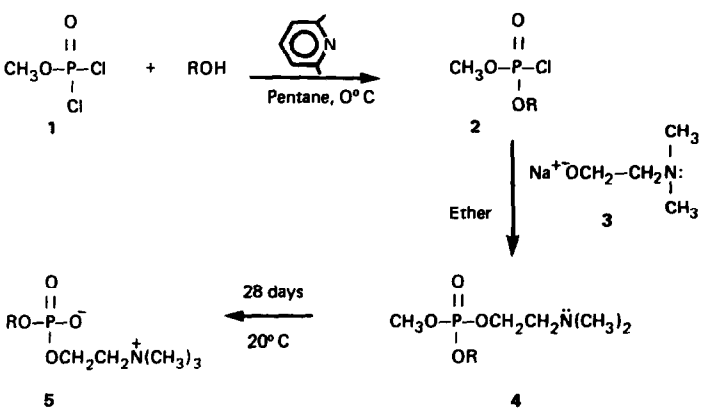
C. Jeffrey Lacey and Leslie M. Loew*
Department of Chemistry
State University of New York at Binghamton, Binghamton, New York 13901

ABSTRACT Alkyl (2-dimethylamino)ethyl methyl phosphates are prepared and isomerize smoothly to the corresponding alkyl choline phosphates.

The burgeoning field of membrane research has fostered the need for simple synthetic approaches to phospholipids and phospholipid analogues (e.g. membrane "probes"). As in nucleic acid chemistry, the key problem is the efficient preparation of a mixed diester of phosphoric acid. This paper presents a simple procedure using a methyl protecting group in a "phosphotriester approach",[1] which is especially suited for the synthesis of lecithins, the major class of phospholipid.

The chemistry is summarized in Scheme I. A smooth deprotection of methyl

SCHEME I



dialkyl phosphates has been demonstrated by Ramirez, et al[2] and Daub and Van Tamelen.[3] The latter authors used this as the basis of an oligonucleotide synthesis in which the starting material was methyl phosphorodichloridite and the resulting trialkylphosphite had to be oxidized to the phosphate prior to deprotection. The non-polar solvents and the use of 2,6-lutidine in Scheme I allow preservation of the methyl group[4] in the stepwise alkoxylation of methyl dichlorophosphate; attempts with various combinations of other bases (triethylamine, pyridine, or diisopropylethylamine) and other solvents (chloroform, methylene chloride, or acetonitrile) led to an intractable polymeric gum in this first alkoxylation step. Presumably, the second alkoxylation succeeds without appreciable demethylation because of the preference of the alkoxide for the hard acid center at the phosphorus. Thus, the procedure has the general potential for a simplified mixed dialkylphosphate synthesis.

We chose to concentrate on the special adaptation, illustrated in the last step of Scheme I, to alkyl choline phosphate synthesis. Here, a non-polar triester, which is easily separated from the polar side products in the steps leading to it, is converted by an isomerization to the desired polar diester; the separation of a methylated deprotection reagent is thus avoided. Attempts to speed this isomerization step by heating the crude triester led to a viscous gum which showed evidence for the desired product but could not be purified in any of the cases studied. The mechanism of the isomerization appears to be intermolecular, based on a recent kinetic study,[5] and competes with an intramolecular extrusion of aziridinium ion; therefore, the absence of solvent is also advantageous in the last step.

The procedure will now be detailed for R = propyl. Distilled methyl dichlorophosphate (1) (Aldrich Chemical) is stored and handled in a glove box under a dry nitrogen atmosphere and all reactions are carried out under nitrogen. A solution of 0.09 mol each of propanol and 2,6-lutidine in 35 ml pentane (ether is equally effective) is added dropwise over a period of ∿1/2 hr to an ice bath cooled and stirred solution of 0.11 mol of 1 in 50 ml of the same solvent. A white precipitate of 2,6-lutidine hydrochloride is immediately formed. After stirring at room temperature overnight the precipitate is filtered off with suction in the glove box, consistently affording a quantitative recovery of the base. The filtrate is evaporated and the residue distilled (0.2 torr, 47°-50°C) to yield 10.40 g (67%) of 2(R = propyl).[6] A solution of 0.02 mol of 3 in 50 ml of benzene is siphoned under a positive N_2 pressure from the excess sodium used in its preparation, the sodium washed with 25 ml fresh benzene, and the combined benzene solution added dropwise to an ice-bath cooled solution of 2(0.02 mol) in 50 ml ether. After stirring for 1 hour the precipitated sodium chloride is removed by centrifugation and washed with fresh solvent. Evaporation of the combined supernatant and washings provide the triester, 4(R = propyl).[7] The crude

triester isomerizes to propyl choline phosphate upon standing at room temperature for 28 days. The isomerization is easily followed by the separation of a glassy crystalline phase from the mobile liquid. The solid can be purified by recrystallization from isopropanol. The yield of 5(R = propyl) is 53% based on dimethyl ethanolamine.[8] The overall yield of choline propyl phosphate, based on propanol, is, thus, 36%.

The procedure has also been applied to R = allyl and R = the acetonide of glycerol. In both of these cases, 5 can be used as a starting material in a phospholipid synthesis.[9,10] The distillation of 2, however, is accompanied by considerable decomposition in these cases. For R = allyl, a 55% yield of distilled 2 (50°C, 0.7 torr) was obtained which, in turn, provided a 53% yield of 5. For R = glycerol acetone, crude 2 was used directly, affording a 28% overall yield of 5. This success with crude 2 encourages us to reoptimize the general procedure to completely avoid this costly intermediate purification step. Even these yields, however, are quite competitive with the still popular, but complex, glycerylphosphorylcholine synthesis of Baer and Kates,[11] which is based on stepwise phosphorylation with phenyldichlorophosphate followed by hydrogenolysis.

It should be emphasized that, although this procedure is especially promising as a route to lecithins, the simplicity of the triester synthesis shown in the first two steps of Scheme I, coupled with the mild procedures available for demethylation,[2,3] make this a route which should be explored in general for mixed dialkyl phosphate synthesis. Also, the yields reported above are all based on the first alcohol to be phosphorylated. Some of the best previously reported procedures require an excess of unrecoverable alcohol in the first step (if yields were calculated on the basis of the first nucleoside in references 3 and 4, for example, figures in the range of 25% and 5%, respectively are obtained). A number of mixed phosphate diesters have been prepared in this way in our laboratory[12] and will be the subjects of an expanded report.

ACKNOWLEDGEMENT This investigation was supported by Grant Number CA-23838 awarded by the National Cancer Institute, USPHS. The support of the Research Corporation is also gratefully acknowledged.

NOTES AND REFERENCES

1) R. L. Letsinger and K. K. Ogilvie, J. Am. Chem. Soc., 91, 3350 (1969).
2) F. Ramirez, S. Raghupathy, Y. F. Chaw, T. McCaffrey, J. Marecek, B. McKeever and D. Nierman, ibid., 99, 5385 (1977).
3) G. W. Daub and E. E. Van Tamelen, ibid., 99, 3526 (1977).
4) M. Rubinstein and A. Patchornik, Tetrahedron, 31, 2107 (1965), used the

demethylation of 1 by pyridine as the basis of a direct oligonucleotide synthesis.

5) P. A. Manninen, Acta Chemica Scandinavica, B32, 269 (1978).

6) ^{1}HNMR ($CDCl_3$/TMS): δ1.00 (t, 3H, $-CH_3$); 1.75 (m, 2H, $-CH_2-$); 3.92 (d. 3H, J=11 Hz, $-O-CH_3$); and 4.15 (m, 2H, $-O-CH_2-$) ppm.

7) ^{1}HNMR ($CDCl_3$/TMS): δ0.98 (t, 3H, $-CH_3$); 1.70 (m, 2H, $-CH_2-$); 2.25 (S, 6H, $-N-(CH_3)_2$); 2.55 (t, 2H, $-CH_2-NMe_2$); 3.73 (d, 3H, J=11 Hz, $-O-CH_3$) and 4.10 (m, 4H, $-O-CH_2-CH_2NMe_2$ $-O-CH_2-Et$) ppm.

8) ^{1}HNMR (D_2O/DSS): δ1.10 (t, 3H, $-CH_3$); 1.80 (m, 2H, $-O-CH_2-CH_2-CH_3$); 3.40 (S, 9H, $-\overset{+}{N}(CH_3)_3$); 3.75 (t, 2H, $-O-CH_2-CH_2-NMe_3$); 4.00 (m, 2H, $-O-CH_2-Et$); and 4.35 (m, 2H, $O-CH_2-CH_2\overset{+}{N}ME_3$)ppm. Calculated for $C_8H_{20}NO_4P\cdot H_2O$: C, 39.50%; H, 9.11%; N, 5.75%; P, 12.75%. Found: C, 39.50%; H, 9.25%; N, 5.80%; P, 12.65%. (Analysis performed by Galbraith Laboratories, Knoxville, Tennessee.)

9) C. M. Lok, Chemistry and Physics of Lipids, 22, (1978), pp. 323-337.

10) C. M. Gupta, R. Radhakrishnan, and H. G. Khorana, Proc. Natl. Acad. Sci. U.S.A., 74, 4315 (1977).

11) E. Baer and M. Kates, J. Am. Chem. Soc., 70, 1394, (1948).

12) M. Jove, C. J. Lacey, and L. M. Loew, unpublished results.

(Received in USA 15 January 1980)