Pergamon

Tetrahedron Letters 39 (1998) 1607–1610

TETRAHEDRON LETTERS

Synthesis of Isotopically Labelled Cardiolipins

Andrzej A. Duralski, Paul J.R. Spooner, Saffron E. Rankin and Anthony Watts.

Department of Biochemistry, University of Oxford,

South Parks Road, Oxford, OX1 3QU, UK.

Received 19 November 1997; accepted 12 December 1997

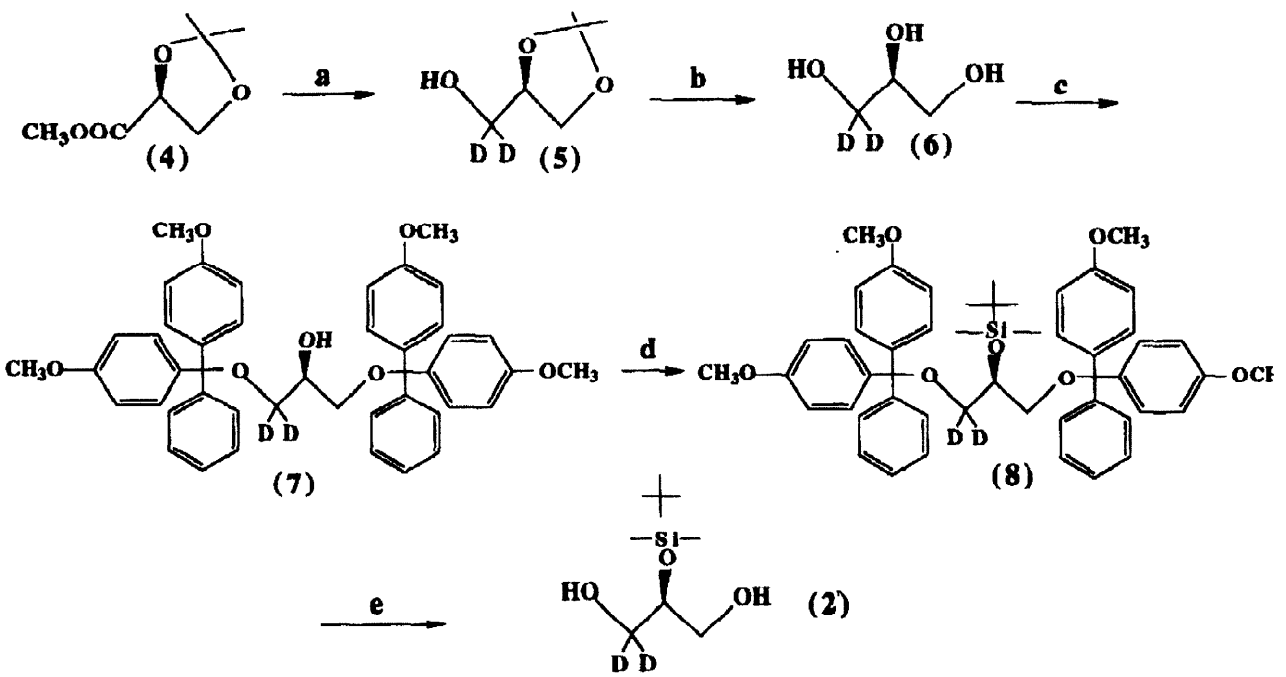
Abstract; A phosphotriester approach is used to access the complex phospholipid, cardiolipin (**1**) with polyunsaturated fatty acid chains. The synthetic method allows the specific incorporation of isotopic labels within the molecule and sets up the desired configuration at all three chiral centres which corresponds to the configuration of the natural phospholipid. Using such a methodology a range of pure optically active cardiolipin molecular species have been synthesized.

We wish to report herein a simple, efficient, stereospecific procedure for the synthesis of cardiolipin (**1**) labelled specifically with deuterium at the C-1 carbon of the glycerol head group. Cardiolipin (**1**) is a complex phospholipid composed of three glycerol units linked via the primary hydroxyl groups through phosphodiester linkages. The two terminal glycerol units are acylated with long chain fatty acids. Cardiolipin (**1**), which can be considered as a family of compounds rather than a single compound (depending on type and distribution of fatty acid chains), is found in the electron transporting membranes of many eukaryotic cells, including mitochondria, where it constitutes up to 25 mole % of the total phospholipid content. It has been suggested that cardiolipin (**1**) plays an important role in many mitochondrial processes,[1-5] however currently no specific molecular details of this functional involvement of cardiolipin (**1**) is available. In order to study the biophysical behavior of cardiolipin (**1**) using deuterium NMR spectroscopy, it is necessary to label specific sites in this lipid with deuterons. Hitherto, the labelled lipid has only been obtained by a low yielding biosynthetic route[6] which does not provide for control of the fatty acyl chain composition. Previous synthetic approaches[7,8] have not been adapted to provide such specific labelling. In addition our procedure can be carried out on a gramme scale yielding quantities previously unrealized.

The synthetic methodology which we have developed involves the application of a phosphotriester approach commonly used in oligonucleotide synthesis.[9] Such an approach has not been used for cardiolipin synthesis. The synthesis involves the condensation of a deuterium labelled glycerol fragment (**2**) with a diacylglycerol (**3**) which ultimately results with the formation of symmetrically substituted cardiolipin (**1**). The key stereochemical building block for the labelled moiety (**2**) is derived from methyl 2,3-O-isopropylidene-L-glycerate (**4**) which was reduced with $LiAlD_4$ to yield 1-[$^2H_2$]-2,3-isopropylidene-*sn*-glycerol (**5**) in 75% yield. The isopropylidine group was removed by treating (**5**) with 80% aq. acetic acid at 60lC for 1 hour to yield glycerol (**6**) labelled with deuterium at the C-1 carbon. This labelled glycerol (**6**) was not purified but allowed to react in a crude state. Conveniently the C-1 and C-3 hydroxyl groups of (**6**) could then be selectively protected as 4,4'-dimethoxytrityl ethers to yield the bis protected glycerol (**7**) by treating (**6**), in dry pyridine at room temperature under nitrogen with 4-

0040-4039/98/$19.00
PII: S0040-4039(97)10829-2

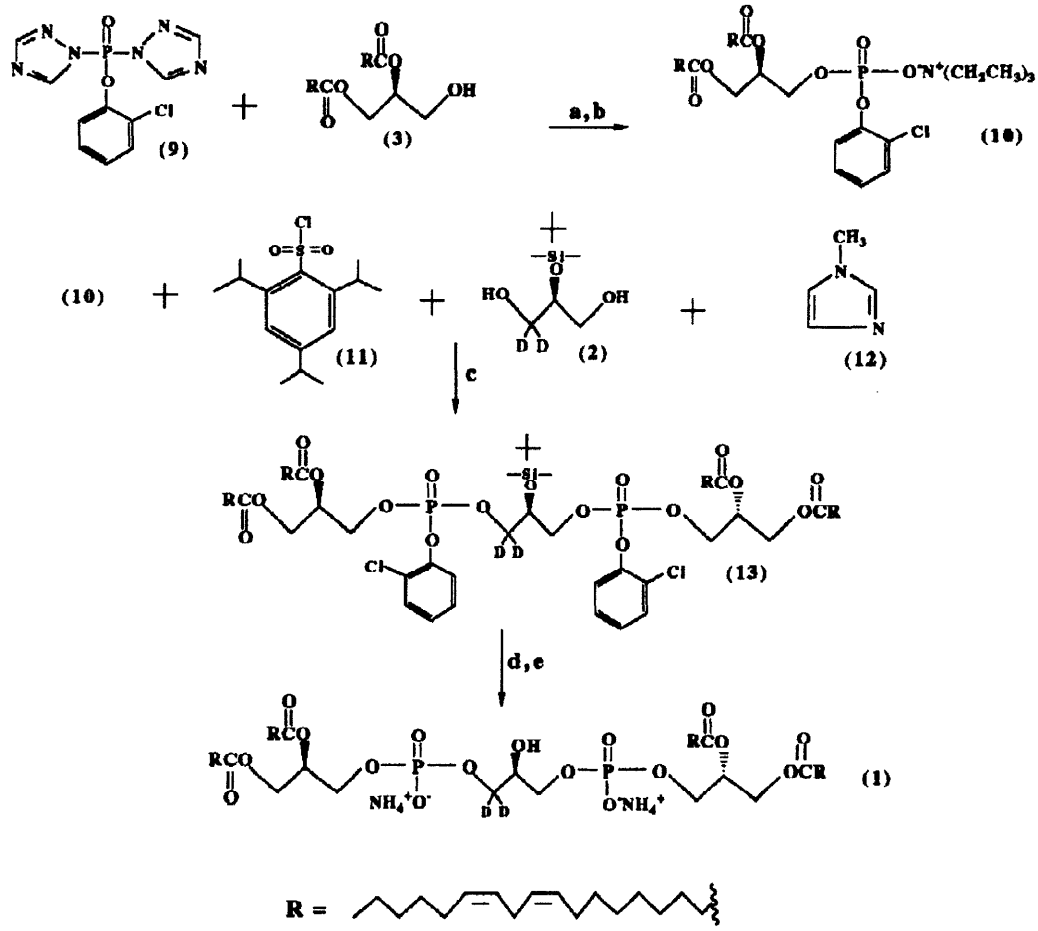
dimethylaminopyridine (0.1 molar equivalents), triethylamine (2.8 molar equivalents) and 4,4'-dimethoxytritylchloride (2.2 molar equivalents) for 1 hour. After an aqueous work up the product (**7**) was purified by column chromatography [hexane/ethyl acetate 80/20;v/v] in 66% yield. The remaining secondary hydroxyl group of (**7**) was then protected as a *tert*-butyldimethylsilyl (TBDMS) ether by stirring overnight under nitrogen in dry DMF at room temperature with imidazole (3.1 molar equivalents) and TBDMSCl (1.6 molar equivalents). Following an aqueous work up the product was purified by column chromatography [hexane/ethyl acetate 90/10;v/v] to yield the fully protected glycerol fragment (**8**) in 95% yield. The 4,4'-dimethoxytrityl ethers of (**8**) could then be selectively removed in the presence of the TBDMS ether by stirring in the presence of 4% *p*-toluenesulphonic acid in a solvent mixture of $CHCl_3$/MeOH (2:1 v/v) at 0IC for 30 minutes, to yield the desired fragment, 1-[$^2H_2$]-2-*tert*-butyldimethylsilyl-*sn*-glycerol (**2**). Following an aqueous work up the product was purified, in 63% yield, by column chromatography using CH_2Cl_2/MeOH (100/0 I 92/8;v/v) as the eluting solvent.



(a) $LiAlD_4$, 45IC, 1 hour. (b) 80% aq. CH_3COOH, 60IC, 1 hour. (c) 4-Dimethylaminopyridine, triethylamine, 4,4'-dimethoxytrityl chloride, 1 hour, room temperature. (d) Imidazole, TBDMSCl, room temperature, overnight. (e) 4% p-Toluenesulphonic acid, $CHCl_3$/MeOH, 0IC, 30 minutes.

Diacylglycerols such as 1,2-dilinoleoyl-sn-glycerol (**3**) where synthesized according to the procedure previously reported.[10] The diacylglycerol (**3**) was coupled simultaneously at both the C-1 and C-3 carbons of the deuterium labelled moiety (**2**) via a phosphotriester linkage using the bifunctional phosphorylating agent 2-chlorophenyl phosphorodi-(1,2,4-triazolide) (**9**). This bifunctional phosphorylating agent (**9**) was generated *in situ* by the action of 1,2,4-triazole (2.05 molar equivalents) on 2-chlorophenyl phosphodichloridate (1 molar equivalent) in the presence of triethylamine (2.09 molar equivalents) in dry acetonitrile at room temperature under nitrogen and was formed within 15 minutes. Initial reaction of the bifunctional phosphorylating reagent (**9**) (2.4 molar equivalents) with the diacylglycerol (**3**) (1 molar equivalent) at room temperature under nitrogen is followed after 20 minutes by treatment with a solution of triethylamine (6.3 molar equivalents) and water (6.7 molar

equivalents) in pyridine which results in the formation of the triethylamine salt of the mono 2-chlorophenyl ester of phosphatidic acid (**10**).



(a) Pyridine, 20 minutes, room temperature. (b) Triethylamine, water, pyridine, room temperature, 5 minutes. (c) CH_3CN/CH_2Cl_2 (4/1; v/v), 2 hours, room temperature. (d) THF, room temperature, 2-nitrobenzaldoxime, N,N,N,N-tetramethylguanidine, water, 2.5 hours. (e) $THF/H_2O/CH_3COOH$ (3/1.5/1; v/v/v), room temperature, 24 hours.

The phospholipid (**10**) is not purified but after work up was allowed to react at room temperature for 2 hours with 1-[$^2H_2$]-2-*tert*-butyldimethylsilyl-*sn*-glycerol (**2**) (0.48 molar equivalents) in the presence of 2,4,6-triisopropylsulphonyl chloride (**11**) (2.5 molar equivalents) as the condensing agent and N-methyl imidazole (**12**) (3 molar equivalents) as the catalyst in a solvent mixture of dry acetonitrile/dichloromethane (4/1; v/v) under nitrogen. The product was purified by column chromatography using hexane/ethyl acetate (80/20; v/v) as the eluting solvent to yield the fully protected cardiolipin molecule (**13**).

The phosphate protecting groups were removed by stirring (**13**) in dry THF with 9.9 molar equivalents of 2-nitrobenzaldoxime and 9.1 molar equivalents of the strong organic base N,N,N,N-tetramethylguanidine in the presence of a few drops of deionised water at room temperature for 2.5 hours. The mixture was then neutralised by the addition of acetic acid and partially purified over a small bed of silica gel ($CHCl_3$/MeOH 98/2 I 92/8; v/v) to remove any non-phosphorus containig species. Finally the TBDMS ether was cleaved using an acidic mixture of THF/acetic acid/water (3/1.5/1; v/v) stirring the mixture at room temperature for 24 hours. This deprotection procedure was found to proceed in a reaction yield of 55% (over 2 steps). Purification of the target compound

was achieved using column chromatography with a basic eluting solvent system ($CHCl_3$/MeOH/NH_3; 100/15/1 | 65/15/1; v/v/v) to give cardiolipin (**1**) as its ammonium salt. The synthetic cardiolipin was compared to the natural product (beef heart mitochondrial cardiolipin, Lipid Products, Surrey, UK) by NMR, IR Mass spec and optical rotation and found to be the same. A range of cardiolipins have been synthesized using the above method.

ACKNOWLEDGEMENTS

Funding was provided by the Science and Engineering Research Council (SERC) for a studentship (A.A.D.) and on grants GR/E/56683 and GR/F/54006 (P.J.R.S and A.W.). Dr A.A. Duralski wishes to thank Dr A. Mohtto for many useful discussions.

REFERENCES

1. Awasthi, Y.C.; Chuang, T.F.; Keenan, T.W.; Crane, F.C. *Biochim. Biophy. Acta.*, **1971**, *226*, 42.
2. Vik, S.B.; Georgevich, G.; Capaldi, R.A. Proc. *Natl. Acad. Sci., USA.*, **1981**, *78*, 1456.
3. Robinson, N.C. *Biochemistry*, **1982**, *21*, 184.
4. Cheneval, D.; Muller, M.; Carafoli, E. *FEBS Lett.*, **1983**, *159*, 123.
5. Rietveld, A.; Jardi, W.; de Kruijff, B. *J. Biol. Chem.*, **1986**, *261*, 3848.
6. Allegrini, P.R.; Pluschke, G.; Seelig, J. *Biochemistry*, **1984**, *23*, 6452.
7. Ramirez, F.; Ioannou, P.V.; Marecek, J.F.; Dodd, G.M.; Golding, B.T. *Tetrahedron*, **1977**, *33*, 599.
8. Keana, J.F.; Shimizu, M.; Jernstedt, K.K. *J. Org. Chem.*, **1986**, *51*, 2297.
9. Reese, C.B. *Tetrahedron*, **1978**, *34*, 3143.
10. Duralski, A.A.; Spooner, P.J.R.; Watts, A. *Tet. Letts*, **1989**, *30*, 3585.