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SYNTHESIS OF OPTICALLY ACTIVE POLYUNSATURATED DIACYLGLYCEROLS

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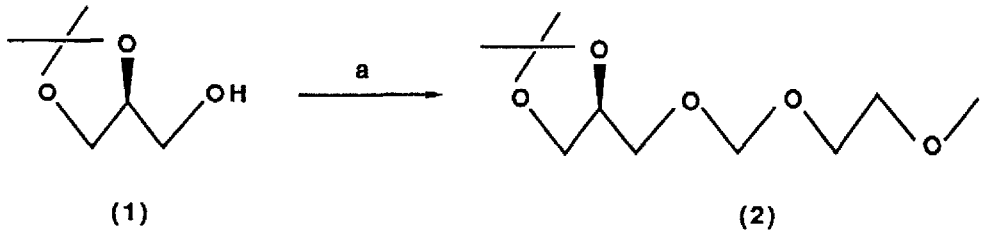
Abstract; 1,2-Isopropylidene-3-methoxyethoxymethyl-*sn*-glycerol (**2**) is used to access complex polyunsaturated diacylglycerols of high optical purity such as 1,2-dilinoleoyl-*sn*-glycerol (**5**). Using a similar methodology its enantiomer, 2,3-dilinoleoyl-*sn*-glycerol, can be obtained from 1-methoxyethoxymethyl-2,3-isopropylidene-*sn*-glycerol.

We wish to report herein a simple, efficient, stereospecific procedure for the synthesis of optically active polyunsaturated 1,2- and 2,3-diacyl-*sn*-glycerols. This procedure is the first example of the application of the versatile β-methoxyethoxymethyl (MEM)[1] protecting group to the synthesis of diacylglycerols, with potential for subsequent phospholipid synthesis.

Many authors have described the synthesis of optically active 1,2-diacyl-*sn*-glycerols. Sowden and Fischer[2] were the first to achieve the synthesis of enantiomerically pure diacyl-*sn*-glycerols. However, these contained only saturated fatty acid substituents and it was some years before Baer and Buchnea[3] reported a complete synthesis of monounsaturated diacylglycerols. This was achieved by converting the fatty acid substituents of 1,2-dioleoyl-3-benzyl-*sn*-glycerol[4] into 9,10-dibromostearic acids, removing the benzyl protecting group by catalytic hydrogenolysis and then regenerating the double bonds with activated zinc. This method is unsuitable for polyunsaturated diacylglycerols and always results with the formation of small amounts of the more thermodynamically stable 1,3- isomer. Pfeiffer *et. al*[5] were the first to synthesize polyunsaturated diacylglycerols *via* glycerol carbonates employing 2,2,2-trichloroethoxycarbonyl as a protecting group, however, this group is removed under conditions liable to induce migration of the fatty acid substituents. Buchnea[6] was able to obtain saturated and unsaturated 1,2- and 2,3-diacyl-*sn*-glycerols by using a trityl protecting group. Detritylation can be accomplished without inducing facile chain migration or adversely affecting the double bonds by chromatography on a silicic acid/boric acid column[7]. In our hands this lengthy method provided unsatisfactory yields.

The key stereochemical building block used for the polyunsaturated 1,2-diacyl-*sn*-glycerols is 1,2-



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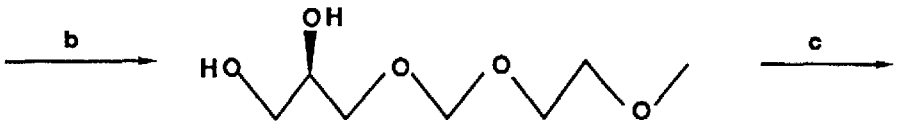

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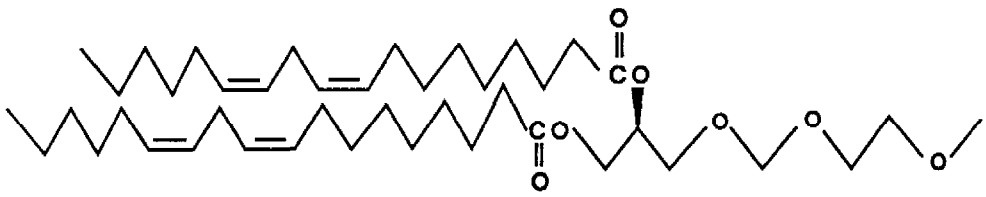

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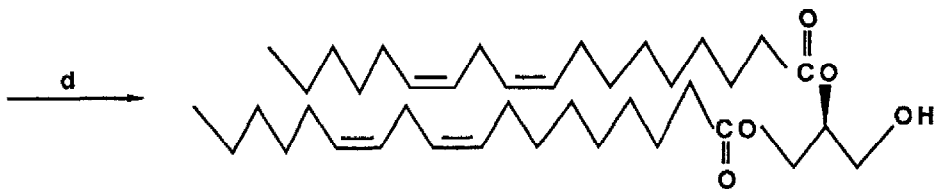

(5)

(a) MEMCl, N,N-Diisopropylethylamine, CH_2Cl_2. (b) 10% aq. Acetic acid.

(c) Linoleic acid, 4-Dimethylaminopyridine, N,N-Dicyclohexylcarbodimide, CCl_4.

(d) CH_2Cl_2, 1M $TiCl_4$ in CH_2Cl_2

isopropylidene-*sn*-glycerol (**1**). This is obtained in high yields either from D-mannitol as described by Eibl[8] or from D-serine as described by Lok[9]. Similarly 2,3-isopropylidene-*sn*-glycerol is used to obtain polyunsaturated 2,3-diacyl-*sn*-glycerols.

The MEM protecting group is introduced into 1,2-isopropylidene-*sn*-glycerol (**1**) by stirring with 1.5 molar equivalents of MEMCl and 1.5 molar equivalents of N,N-diisopropylethylamine at room temperature for 30min in dry dichloromethane [10ml/g of (**1**)] under nitrogen. After an aqueous work up the product is purified in yields greater than 85% by flash column chromatography (hexane/ethyl acetate; 96/4) to give 1,2-isopropylidene-3-methoxyethoxymethyl-*sn*-glycerol (**2**) as a colourless oil. The isopropylidene group is removed by stirring (2) in 10% aq. acetic acid at 60°C for 1 hour. The diol (3) is purified by flash column chromatography (ethyl acetate/hexane; 95/5) and the pure diol (**3**) is formed as a colourless oil in nearly quantitative yield.

The diol (3) is condensed with linoleic acid in by adding a solution of the diol (**3**) in dry carbon tetrachloride to 2 molar equivalents of linoleic acid in dry carbon tetrachloride at 0°C. This is followed by the addition of 2 molar equivalents of 4-dimethylaminopyridine. When all these components have dissolved a solution of N,N-dicyclohexylcarbodimide (2 molar equivalents) in dry carbon tetrachloride is added to the mixture which is then allowed to stir at room temperature for 2 hours. The resultant mixture is filtered and the precipitate washed with carbon tetrachloride. Evaporation of the filtrate under reduced pressure gives the crude product which is purified in yields greater than 75% by flash column chromatography (hexane/ethyl acetate; 95/5).

The MEM protecting group from 1,2-dilinoleoyl-3-methoxyethoxymethyl-*sn*-glycerol (**4**) is easily removed under aprotic conditions in yields in excess of 70% by stirring in dry dichloromethane [25ml/g of (**4**)] with 1.8 molar equivalents of a 1M solution of $TiCl_4$ in CH_2Cl_2 under nitrogen at 0°C for one hour. Following this period ammonia solution was added to the mixture in a careful dropwise manner until a white precipitate was formed. This was then filtered through celite and the filtrate washed with water and then brine. Attempts to remove the MEM protecting group using $ZnBr_2$ provided unsatisfactory yields. Column chromatography using a silicic acid/boric acid [9/1 w/w] mixture is used to purify the diacylglycerol. The boric acid is added in aqueous solution to the dry silicic acid (100 mesh) to form a slurry, which is air dried before activation overnight at 110°C. The use of such a mixture prevents the facile migration of the fatty acid substituents in the 1,2-diacylglycerol to form the more thermodynamically stable 1,3-diacylglycerol. The eluting solvent is hexane/ethyl acetate, the ratio of ethyl acetate is gradually increased in a stepwise manner from 100/0 to 80/20. Thin layer chromatography on silica gel plates, impregnated with boric acid, using solvent systems known to separate 1,2-diacylglycerols from the isomeric 1,3-diacylglycerols[10] showed no detectable amount of the 1,3- isomer under loading conditions (100μg lipid) capable of detecting 1% of this impurity after charring with 25% aq. H_2SO_4.

Using the above procedure polyunsaturated diacylglycerols, such as 1,2-dilinoleoyl-*sn*-glycerol (**5**), $[\alpha]_D$ -1.82° (c 1.2, $CHCl_3$), [reported $[\alpha]_D$ -2.00° (c 1.1, $CHCl_3$)][11], have been synthesized in gram quantities. The enantiomer 2,3-dilinoleoyl-*sn*-glycerol has been synthesized using L-serine as the starting material. This efficient method is also convenient to produce all 1,2- and 2,3-diacyl-*sn*-glycerols and is particularly useful for

the synthesis of polyunsaturated diacylglycerols as the removal of the MEM group does not adversely effect the double bonds.

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