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Short communication

A new protecting group: 9-fluorenylmethoxycarbonyl (FMOC) in the synthesis of 1,2-diacylglycerols

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Abstract

The synthesis of 1,2-L-dipalmitoyl-*sn*-glycerol, 1,2-L-distearoyl-*sn*-glycerol and 1,2-L-dioleoyl-*sn*-glycerol are described here using 9-fluorenylmethoxycarbonyl (FMOC) group for protection of the 3-position of glycerol which can be selectively removed by Et_3N treatment on the overall 60–70% yield based on 1,2-isopropylidene-*sn*-glycerol. Little or no acyl migration occured during deprotection and purification. © 1997 Elsevier Science Ireland Ltd.

1. Introduction

The glyceryl derivatives, especially the phospholipids, are important components of biological membranes (Jain, 1972; Han et al., 1994). Phospholipid synthesis requires a careful choice of protecting groups for the different functionalities and mild deprotection conditions, in order to avoid acyl group migration. The most frequently used glycerol protecting groups are: benzyl (Eibl and Wolley, 1986; Hong et al., 1988); allyl and trityl (Eibl and Wolley, 1986); and terahydropyranyl (Lok, 1978). The 2,2,2-trichloroethoxycarbonyl (Pfeiffer et al., 1970); trifluoroacetyl (Lok, 1978); levulinate (Fröling et al., 1984); methoxyethoxymethyl (Duralski et al., 1989); and *t*-butyldimetylsilyl (Sonnet, 1991), protecting groups have also been used in the synthesis of diacyl-*sn*-glycerols. Removal of these protecting groups from diacyl-*sn*-glycerols generally resulted in some acyl migration. We suggest using an 9-fluorenylmethoxycarbonyl (FMOC) protecting group which is removable by non-nucleophylic tertiary base with β-elimination.

The FMOC group was first used in peptide chemistry by Carpino and Han (1970). Later it was introduced to nucleosides by Heikkila and Chattopadhyaya (1983).

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2. Experimental procedures

^{1}H-NMR and ^{13}C-NMR spectra were measured with a Jeol Fx 90 Q spectrometer using tetramethylsilane as internal standard in δ scale. Merck Kieselgel G was used for short column chromatography.

Pyridine was dried by heating under reflux with calcium hydride for ca. 3 h then distilled at atmospheric preasure and stored over molecula sieves (4 A) in a dark bottle. 9-Fluorenyl chloroformate (FMOC-Cl), palmitic-, stearic- and oleic acid were purchased from Sigma.

Elemental analyses were performed in Uppsala. Results were within $\pm$ 0.4% of theoretical values.

*2.1. 1,2-Isopropylidene-3-FMOC-sn-glycerol (**2**)*

1,2-Isopropylidene-*sn*-glycerol (0.66 g, 5 mmol) was dissolved in 50 ml dry pyridine, 9-fluorenyl chloroformate (1.55 g, 6 mmol) was added and the reaction mixture was stirred for 1 h at RT. The reaction mixture was divided into 500 ml chloroform and 500 ml saturated ammonium bicarbonate. The organic phase was evaporated to dryness and coevaporated with toluene several times to remove all traces of pyridine. The oil residue was dissolved in dichloromethane, loaded on silica gel and eluted with dichloromethane and 5% methanol–dichloromethane. The proper fractions were evaporated and the resulting oil was 1.44 g (81.3%). ^{1}H-NMR ($CDCl_3$) δ: 7.23–8.0 (m, 8H) aromatic of FMOC; 3.6–4.5 (m, 8H) CH and CH_2 of FMOC, 2-HC, 3-H_2C and 1-H_2C; 1.45 (s, 3H) CH_3; 1.38 (s, 3H) CH_3.

*2.2. 3-FMOC-sn-glycerol (**3**)*

Compound **2** (0.46 g, 1.3 mmol) was dissolved in 10 ml dioxane and 10 ml of 80% formic acid was added. The reaction mixture was stirred at RT for 45 min then evaporated, loaded on silica gel column and eluted with 2–5% methanol–dichloromethane. The proper fractions were evaporated and the resulting oil was 0.37 g (89%). ^{1}H-NMR ($CDCl_3$) δ: 7.1–7.7 (m, 8H) aromatic of FMOC; 4.0–4.4 (m, 5H) CH_2, CH of FMOC and 3-H_2C; 3.8 (m, 1H) 2-HC; 3.6 (m, 2H) 1-H_2C; 2.73(bs, 1H) OH, 2.37 (bs, 1H) OH. ^{13}C-NMR ($CDCl_3$) δ: 155.13 (C=O); 142.99, 141.04, 127.71, 124.84, 119.86 (aromatic carbons of FMOC); 69.8, 68.45, 62.98 (glycerol carbons), 46.51 (CH_2 of FMOC).

*2.3. 3-FMOC-1,2-dipalmitoyl-sn-glycerol (**4a**)*

Compound **3** (0.3 g, 0.94 mmol) and palmitic acid (0.48 g, 1.88 mmol) were dissolved in 13 ml dichloromethane and cooled to 0°C. DCC (1.32 g, 6.4 mmol) and DMAP (24.4 mg, 0.2 mmol) were dissolved in 10 ml dichloromethane and added to the reaction mixture. After 2–3 h stirring the reaction mixture was evaporated and dissolved in 50 ml dichloromethane and the dicyclohexylurea was then filtered. The product **4a** was purified on silica gel column and eluted with 50% hexane–dichloromethane. The proper fractions were evaporated giving the compound **4a** (0.8 g, 100%). ^{1}H-NMR ($CDCl_3$) δ: 7.23–7.8 (m, 8H) aromatic of FMOC; 5.3 (m, 1H) 2-HC; 4.38 (m, 3H) CH and CH_2 of FMOC; 3.2 (m, 3H) 3-H_2C and 1-H_2C; 2.33 (m, 4H) OCH_2; 1.2–2.1 (m, 26H) CH_2; 0.87 (bs, 6H) CH_3.

*2.4. 3-FMOC-1,2-Distearoyl-sn-glycerol (**4b**)*

Compound **4b** was prepared in the same way as compound **4a** (0.57 g, 87%). ^{1}H-NMR ($CDCl_3$) δ:7.25–8.0 (m, 8H) aromatic of FMOC; 5.3 (m, 1H) 2-HC; 4.38 (m, 3H) CH and CH_2 of FMOC; 3.2 (m, 4H) 3-H_2C and 1-H_2C; 2.33 (m, 4H) 2 OCH_2, 1.1–2.2 (m, 30H) CH_2; 0.877 (bs, 6H) CH_3.

*2.5. 3-FMOC-1,2-Dioleoyl-sn-glycerol (**4c**)*

Compound **4c** was prepared in the same way as compound **4a** (0.5 g, 95%). ^{1}H-NMR ($CDCl_3$) δ:7.19–7.91 (m, 8H) aromatic of FMOC; 5.33 (m, 5H) 2-HC and 2CH=CH; 4.36 (m, 3H) CH and CH_2 of FMOC; 3.23 (m, 3H) 3-H_2C and 1-H_2C; 2.33 (m, 4H) 2 OCH_2, 1.1–2.19 (m, 26H) CH_2; 0.88 (m, 6H) CH_3.

*2.6. 1,2-Dipalmitoyl-sn-glycerol (**5a**)*

Compound **4a** (0.63 g, 0.79 mmol) was dissolved in 8 ml dry pyridine and dry Et_3N (0.8 ml, 7.95 mmol) was then added. The reaction mixture was stirred at RT for 2 h, evaporated and loaded on silica gel column. The column was eluted with 50% hexane–dichloromethane and the product (**5a**) was eluted with dichloromethane. The proper fractions were poured together and evaporated giving the compound **5a** 0.38 g (84%). ^{1}H-NMR ($CDCl_3 + CD_3OD$) δ: 4.99 (m, 1H) 2-HC; 4.19 (m, 2H) 1-H_2C; 3.6 (m, 2H) 3-H_2C; 2.27 (m, 4H) 2 CH_2CO; 1.22–1.73 (m, 26H) 2 $(CH_2)_{13}$; 0.81 (t, 6H) 2 CH_3. ^{13}C-NMR ($CDCl_3$ + CD_3OD) δ: 173.65 (C=O); 71.86, 62.16, 60.64 (glycerol carbons); 48.51 and 33.88 (CH_2–C=O); 31,66 (CH_2–CH_2–CH_3); 29.44 ($(CH_2)_{10}$ both acyl chain); 13.84 (CH_3 both acyl chain).

*2.7. 1,2-Distearoyl-sn-glycerol (**5b**)*

Compound **5b** was produced similar to **5a** (0.37 g, 80%). ^{1}H-NMR ($CDCl_3$) δ: 5.0 (m, 1H) 2-HC; 4.2 (m, 2H) 1-H_2C; 3.65 (m, 2H) 3-H_2C, 2.3 (m, 4H) 2 CH_2CO; 1.5 (m, 30H) CH_2; 0.88 (t, 6H) 2 CH_3.

*2.8. 1,2-Dioleoyl-sn-glycerol (**5c**)*

Compound **5c** was produced similar to compound **5a** (0.25 g, 80%). ^{1}H-NMR ($CDCl_3$) δ: 5.4 (m, 4H) 2 CH=CH; 4.26 (m, 2H) 1-H_2C; 3.72 (m, 2H) 3-H_2C; 2.34 (m, 4H) 2 CH_2CO; 1.27 (m, 26H) CH_2; 0.88 (t, 6H) 2 CH_3.

3. Results and discussion

In our strategy we have employed the method used by Eibl (1981) for the synthesis of 1,2-isopropylidene-*sn*-glycerol. We have protected the 3-position with FMOC-Cl in 82% yield, the isopropilidene group was removed with formic acid, and the fatty acids, either saturated or unsaturated, were introduced using the method of Neises and Steiglich (1978) almost quantitatively. The FMOC group was removed from diacyl-*sn*-glycerols with Et_3N in dry pyridine, resulting in an 80% yield.

What are the similarities and advantages of FMOC group compared to the other protecting groups in glycerol chemistry?

1. It is easy to introduce FMOC-Cl after 1 h at RT.
2. The products **2**,**3**,**4**,**5** can be quickly purified on silica gel.
3. In the ^{1}H-NMR and ^{13}C-NMR spectra, the aromatic part and CH_2 of FMOC in compound **3** can be easily seen.
4. The fatty acids can be introduced almost quantitatively despite the bulkiness of the protecting group.
5. The deblocking condition, Et_3N in dry pyridine for 2 h, does not cause acyl migration, as no other product was observed on TLC.
6. The overall yield of diacyl-*sn*-glycerols is similar to using benzyl protection. However, benzyl can not be used in the case of unsaturated fatty acids, therefore removal with Pd/C, debenzylation and detrtitylation can be achieved using bromodimethylborane as shown by Kodali and Duclos (1992).
7. ^{1}H-NMR and ^{13}C-NMR spectra of compound **5a** are similar to that of Kodali and Duclos (1992).

We will show in our next paper, the use of the above prepared diacyl-*sn*-glycerols in the synthesis of 1-β-D-arabinofuranosylcytosine-phospholipid.

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References

Carpino, L.A., Han, G.Y., 1970. The 9-fluorenylmethoxycarbonyl function, a new base-sensitive aminoprotecting group. J. Am. Chem. Soc. 92, 5748–5750.

Duralski, A.A., Spooner, P.J.R., Watts, A., 1989. Synthesis of optically active polyunsaturated diacylglycerols. Tetrahedron Lett. 30, 3585–3588.

Eibl, H., Wolley, P., 1986. Synthesis of enantiomerically pure glycerol esters and ethers. 1. Methods employing the precursor 1,2-isopropilidene-s*n*-glycerol. Chem. Phys. Lipids 41, 53–63.

Eibl, H., 1981. An improved method for the preparation of 1,2-isopropylidene-s*n*-glycerol. Chem. Phys. Lipids 28, 1–6.

Froling, A., Pabon, H.J.J., Ward, J.P., 1984. Synthesis of long-chain 1-saturated-2-unsaturated diacylglycerols via levulinoyl protection. Chem. Phys. Lipids 36, 29–38.

Han, G.W., Ruble, J.R., Craven, B.M., 1994. The crystal structure of 1,2-dipalmitoyl-s*n*-glycerol at 123 K. Chem. Phys. Lipids 71, 219–225.

Heikkila, J., Chattopadhyaya, J., 1983. The 9-fluorenylmethoxy-carbonyl (FMOC) group for the protection of amino function of cytidine, adenosine, guanosine and their 2′-deoxysugar derivatives. Acta Chem. Scand. B37 (3), 263–265.

Hong, C.I., An, S.H., Schliselfield, L., Buchheit, D.J., Nechaev, A., Kirisits, A.J., West, C.R., 1988. Nucleoside conjugates. 10. Synthesis and antitumor activity of 1-β-D-arabinofuranosylcytosine 5′-diphosphate-1,2-dipalmitins. J. Med. Chem. 31, 1793–1798.

Jain, J.K., 1972. The Biomolecular Lipid Membrane: A System, von Nostrand-Reinhold, New York.

Kodali, D.R., Duclos, R.I., 1992. Debenzylation and detritylation by bromodimethylborane: Synthesis of mono-acid or mixed-acid 1,2 or 2,3-diacylglycerols. Chem. Phys. Lipids 61, 169–173.

Lok, C.M., 1978. Versatile methods for the synthesis of mixed-acid 1,2-diacylglycerols. Chem. Phys. Lipids 22, 323–337.

Neises, B., Steiglich, W., 1978. Simple method for the esterification of carboxylic acids. Angew. Chem. Int. Ed. 17 (7), 522–523.

Pfeiffer, F.R., Miao, C.K., Weisbach, J.A., 1970. Glycerolipids. III. Stereospecific synthesis of D-and L-1,2-diglycerides via glycerol carbonates. J. Org. Chem. 35, 221–224.

Sonnet, P.E., 1991. A short highly region-and stereoselective synthesis of triacylglycerols. Chem. Phys. Lipids 58, 35–39.