ELSEVIER

Chemistry and Physics of Lipids
105 (2000) 189–191

CPL
CHEMISTRY AND PHYSICS OF LIPIDS

www.elsevier.com/locate/chemphyslip

A simple approach to DOTAP and its analogs bearing different fatty acids

Ulrich Massing *, Jörg T. Kley, Laura Gürtesch, Stefan Fankhaenel

Department of Medicinal Oncology, Division of Medicinal Research, Tumor Biology Center, Breisacher Str. 117, D-79106 Freiburg, Germany

Received 19 November 1999; received in revised form 11 January 2000; accepted 11 January 2000

Abstract

A simple synthesis of *N*-[1-(2,3-dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium methyl sulfate (DOTAP) and its analogs differing in the fatty acids is presented. The synthesis is designed as quasi-one-pot reaction and the precipitating products are purified by simple recrystallization.

Keywords: Synthesis; DOTAP; Analogs; Cationic lipids; Transfection; Imidazolides

1. Introduction

During the last decade, lipofection, the transfection of cells by means of cationic lipids has become one of the most promising strategies for safe gene transfer into a variety of cells (Behr, 1994; Cotten and Wagner, 1993). One of the cationic lipids most frequently used for both studies on lipofection and applied lipofection is *N*-[1-(2,3-dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium methyl sulfate (DOTAP[1]). Until now, two strategies for the preparation of DOTAP are described using only iodide as counter ion, but not methyl sulfate (counter ion in the commercially available DOTAP) (Leventis and Silvius, 1990; Bennet et al., 1997). These strategies include at least two reaction steps, column purifications for each step and also several extraction steps. Here we present a simple synthesis of DOTAP and its analogs (Fig. 1) differing in the fatty acids. The counter ion of the nicely crystalline products is methyl sulfate.

2. Material and methods

2.1. N-[1-(2,3-diacyloxy)propyl]-N,N,N-trimethylammonium methyl sulfate (DAcylTAP) (general description)

Oleic-, stearic-, palmitic-, myristic-, or lauric acid (15.8 mmol), was dissolved in 25 ml of THF. CDI, 2.82 g (17.4 mmol), was added and the mixture was stirred for 30 min at rt. 3-Dimethyl-

* Corresponding author. Tel.: +49-761-206-2177; fax: +49-761-206-2174.

E-mail address: umas@tumorbio.uni-freiburg.de (U. Massing)

[1] Originally referred to as 1,2-dioleoyl-3-(trimethylammonium)propane.

0009-3084/00/$ - see front matter
PII: S0009-3084(00)00121-3

amino-1,2-propanediol, 750 mg (6.30 mmol) and n-BuLi (15% in hexane) 200 µl (0.315 mmol) were added and the reaction was refluxed for two days. The solvent was removed using a rotatory evaporator. 100 ml of acetone and dimethyl sulfate, 2.0 ml (21.1 mmol) were added and the solution was stirred for further two days at 4°C. The precipitated product was isolated by filtration and recrystallized from acetone. Yield: 30–40%.

All compounds gave satisfactory spectroscopic and analytical data. Representative data for selected compounds: ^{1}H-NMR-spectra (Bruker AC250, 250 MHz, $CDCl_3/CD_3OD$ 3:1/TMS). **DOTAP**: δ (ppm) = 0.89 (t, J = 7.0 Hz, 6H; C$\underline{H}_3$), 1.23 (m_c, 40H; 4-7- und 12-17-C$\underline{H}_2$), 1.65 (m_c, 4H; 3-C$\underline{H}_2$), 2.02 (m_c, 8H; 8 und 11-C$\underline{H}_2$), 2.36 (m_c, 4H; 2-C$\underline{H}_2$), 3.20 (s, 9H; $N^+(CH_3)_3$), 3.62–3.80 (m, 5H; N-C$\underline{H}$H, N-CH$\underline{H}$, C$\underline{H}_3$OS), 4.05 (m_c, 1H; O-CH$\underline{H}$), 4.47 (m_c, 1H; O-C$\underline{H}$H), 5.34 (m_c, 4H, 9 and 10 = C$\underline{H}$), 5.60 (m_c, 1H; C$\underline{H}$-O). **DMTAP** (as a general example for the very similar 1H-NMR-spectra of the DOTAP-analogs bearing saturated fatty acid esters): δ (ppm) = 0.88 (t, J = 7.0 Hz, 6H; C$\underline{H}_3$), 1.15–1.35 (m, 40H; 4-13-C$\underline{H}_2$), 1.57–1.70 (m, 4H; 3-C$\underline{H}_2$), 2.36 (m_c, 4H; 2-C$\underline{H}_2$), 3.20 (s, 9H; $N^+(CH_3)_3$), 3.62-3.83 (m, 5H; N-C$\underline{H}$H, N-CH$\underline{H}$, C$\underline{H}_3$OS), 4.04 (dd, J = 5.5 Hz, 1H; O-CH$\underline{H}$), 4.42 (dd, J = 5.5 Hz, 1H; O-C$\underline{H}$H), 5.60 (m_c, 1H; C$\underline{H}$-O). Maldi ms-spectra (PerSeptive Biosystems; Voyager DE-RP (20 kV; matrix: 2.5DHB in acetonitrile/water 1:1 (v/v)). **DOTAP** (m/z: 662.129 (M^+-CH_3O_4S); $C_{43}H_{83}O_8NS$); **DSTAP** (m/z: 665.359 (M^+-CH_3O_4S); $C_{43}H_{87}O_8NS$); **DPTAP** (m/z: 610.384 (M^+-CH_3O_4S); $C_{39}H_{79}O_8NS$); **DMTAP** (m/z: 553.617 (M^+-CH_3O_4S); $C_{35}H_{71}O_8NS$); **DLTAP** (m/z: 498.397 (M^+-CH_3O_4S); $C_{31}H_{63}O_8NS$).

3. Results and discussion

In analogy with the procedures described by Leventis and Silvius for the synthesis of DOTAP (Leventis and Silvius, 1990) with iodide as counter ion and of its ether analog DOTMA (Felgner et al., 1987), the synthesis is based on the commercially available 3-dimethyl-1,2-propanediol (1). In contrast to the method published by Leventis, who acylated 1 using oleic acid chlorides in pyridine, our reaction sequence applies fatty acid imidazolides as acylating agents. They are prepared in situ from the respective fatty acids and *N*,*N*'-carbonyldiimidazole (CDI) (Staab and Mannschreck, 1962). As both the imidazolide formation and the following acylation of 1 (in refluxing THF with a catalytic amount of n-BuLi) occur without formation of insoluble by-products like pyridine hydrochlorides, neither extraction nor filtration is necessary.

The products, the 1,2-diacyl-3-dimethylaminopropanes (2a–e) need not to be isolated, only the solvent has to be replaced by acetone. For subsequent methylation, dimethyl sulfate is added. Due to the solvent characteristics of acetone, the methylated (quaternized) products (3a–e) precipitate and can easily be isolated by filtration (Massing and Eibl, 1994). This precipitation from acetone is not possible with iodide as counter ion. For final purification, one additional crystalliza-

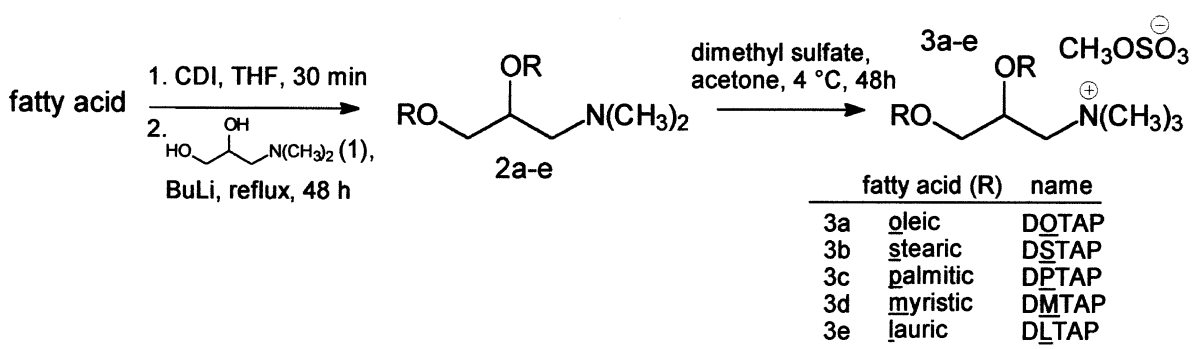

Fig. 1. Reaction sequence for the preparation of DOTAP and its analogs.

tion step from acetone is sufficient. The overall yields range from 30 to 40%, which is only slightly lower than described for the much more complicated original DOTAP-synthesis (49% for DOTAP).

Acknowledgements

This work was supported by the Bundesministerium für Bildung und Forschung (01 GE 9605/2).

References

Behr, J.-P., 1994. Gene transfer with synthetic cationic amphiphiles: prospects for gene therapy. Bioconjugate Chem. 5, 382–389.

Bennet, M.J., Aberle, A.M., Balasubramaniam, R.P., Malone, J.G., Malone, R.W., Nantz, M.H., 1997. Cationic lipid-mediated gene delivery to murine lung: correlation of lipid hydration with in vivo transfection activity. J. Med. Chem. 40, 4069–4078.

Cotten, M., Wagner, E., 1993. Non-viral approaches to gene therapy. Curr. Opin. Biotechnol. 4, 705–710.

Felgner, P.L., Gadek, T.R., Holm, M., Roman, R., Chan, H.W., Wenz, M., 1987. Lipofection, a highly efficient, lipid-mediated DNA-transfection procedure. PNAS 84, 7413–7417.

Leventis, R., Silvius, J.R., 1990. Interactions of mammalian cells with lipid dispersions containing novel metabolizable cationic amphiphiles. BBA 1023, 124–132.

Massing, U., Eibl, H., 1994. Synthesis of enantiomerically pure 1-*O*-phosphocholine-2-*O*-acyl-octadecane and 1-*O*-phosphocholine-2-*N*-acyl-octadecane. Chem. Phys. Lipids 69, 105–120.

Staab, H.A., Mannschreck, A., 1962. Synthese von Carbonsäureestern nach der Imidazolidmethode. Chem. Ber. 95, 1284–1297.