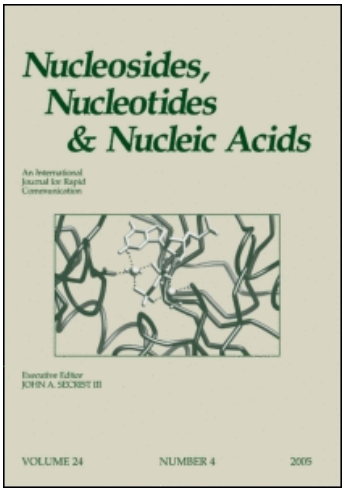

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An Improved Synthesis of 1-β-D-Arabinofuranosylcytosine 5'-Phosphate-L-1,2-diacylglycerols

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AN IMPROVED SYNTHESIS OF 1-β-D-ARABINOFURANOSYLCYTOSINE 5′-PHOSPHATE-L-1,2-DIACYLGLYCEROLS

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ABSTRACT: 5′-O-MMTr-cytosine arabinoside was prepared on a large scale from 5′-O-MMTr-cytidine with diphenyl carbonate via 5′-protected cytidine - 2′,3′-carbonate -- ara-cytidine-2′,2-anhydro derivative at a 67 % yield. The synthesis of 1,2-L-dipalmitoyl-sn-glycerol, 1,2-L-distearoyl-sn-glycerol and 1,2-L-dioleoyl-sn-glycerol described here using 9-fluorenylmethoxycarbonyl (FMOC) group for protection of 3-position of glycerol which can be selectively removed by Et_3N treatment on the overall 60-70 % yield based on 1,2,-isopropilidene-sn-glycerol. These glycerols were phosphorylated first with 2-chlorophenyl-phosphoro-bis-triazolide quantitatively[1] in order to avoid acyl migration, then the glycerophosphate intermediates were condensed with 2′,3′,N^4-trileulinyl-1-β-D-arabinofuranosylcytosine in the presence of 2-mesytilenesulphonyl chloride (MsCl) and 1-methylimidazole (MeIm)- which was used in the coupling of nucleotides[2]- in an 85-95 % yield compared with the low yielding diester method of Ryu[3]. Deblocking was carried out in two steps with tetrabutylammonium fluoride (TBAF) and hydrazine hydrate, producing target compouns (**14a**, **14b**, **14c**) at a 50 % yield.

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INTRODUCTION

Cytosine arabinoside (ara-C) is an important drug used against leukemia[4].

A survey of literature shows, that ara-C has been prepared via 2,2'-anhydro derivative, namely by the reaction of cytidine with phosphoryl chloride[5] or with thionyl chloride[6]. Ogilvie[7] has introduced a one step process from cytidine using diphenyl carbonate. Beranek et al.[8] have improved the reproducibility with several additions of diphenyl carbonate and purification of the reaction mixture with Zerolite FF and Dowex 50WX2 (H^+). However the purification was tedious and time consuming and gave ara-C in only a 32 % yield overall. These papers did not give NMR data of the product.

The clinical effectivness of ara-C may be improved with granulocite colony-stimulating factor (G-CSF)[9] and interleukin-3 (IL-3)[10]. Furthermore the use of hydrocortison[11] will protect cells from the toxic effect of ara-C. Another way to improve the effectivness of ara-C and overcome the problems associated with its use as chemotherapeutic agent is to synthesize ara-C-phospholipid. Such problems include rapid catabolism to the biologically ineffective ara-U, the eventual development by cells of resistance to the drug, and the extensive toxicity of ara-C. Ryu[3] and Hong[12] have prepared several conjugates. Their in vivo and in vitro studies concentrated mostly on ara-C diphosphate analogue. Ryu at al.[3] have synthesized one ara-C monophosphate analogue by diester method, thus the yield of condensation of protected ara-C and L-α-diacylglycero-phosphoric acid did not exceed 22%. It should be worth preparing monophosphate analogue with different lenghts of fatty acids also, because the 1-β-D-arabinofuranosylcytosine 5'-phosphate-1,2-dipalmitoyl-sn-glycerol showed antiproliferative activity[3].

The synthesis of phospholipid requires a careful choice of protecting groups for the different functionalities and mild deprotection condition in order to avoid acyl migration and racemism. Since the activation method of coupling could cause isomerisation, the glycerol part should be phosphorylated first[1].

The most frequently used glycerol protecting groups and removing conditions are shown in TABLE 1.

We suggest using an FMOC protecting group which is removable by mild condition, non-nucleophylic tertiary base with β-elimination. The FMOC group was first

TABLE 1. Protecting groups in glycerol chemistry.

Protecting group	Removing condition	Reference
Isopropilidene	acid	14
benzyl	Pd/C, bromodimethylborane	15, 25
allyl	to propenyl by Pd/C, bromination in water or acid	15
trityl	acid (HCl or acetic acid) or bromodimethylborane or converting to trifluoroacetyl	15, 25
tetrahydropyranyl	acid or converting to trifluor-acetyl	16
2,2,2/trichlorethoxycarbonyl	Zn in acetic acid	17
trifluoroacetyl	methanol, pyridine	16
levulinate	hydrazine hydrate	18
β-methoxy ethoxymethyl	1 M $TiCl_4$ in CH_2Cl_2	19
silyl	NBS	20

used in peptide chemistry by Carpino[21]. Later it was introduced to nucleosides by Heikkila[22].

EXPERIMENTAL PROCEDURES

^1H NMR, ^{13}C NMR and ^{31}P NMR spectra were recorded with a Jeol FX90 spectrometer using tetramethylsilane or 80 % phosphoric acid as internal standard in δ scale.

UV absorption spectra were recorded with a Varian-Cary 2200 spectrometer in ethanol.

Short column chromatographic separations were carried out using Merck G 60 silica gel eluted with a linear gradient of mixtures of n-hexane-CH_2Cl_2 and MeOH-CH_2Cl_2.

Diphenyl carbonate, TBAF, 9-fluorenyl chloroformate, palmitic-, stearic- and oleic acid were purchased from Sigma.

Pyridine was dried by heating under reflux with calcium hydride for ca. 3 h then distilled at atmospheric preasure and stored over molecula sieves in a dark bottle.

Elemental analyses were performed in Uppsala. Results within ±0.4 % of the theoretical values.

5′-O-MMTr-cytidine (2)

The 5′-OH function of cytidine (2.43 g, 10 mmol) was protected with monomethoxytrityl chloride (MMTrCl) (3.71 g, 12 mmol) in dry pyridine overnight in the dark. The reaction mixture was partitioned between chloroform (500 ml) and saturated sodium bicarbonate (500 ml), followed by evaporation of the organic phase and coevaporation with toluene in vacuo. One aliquot (about 20 mg) was purified on silica gel column with dichloromethane followed by 10 % methanol-dichloromethane in order to record ^{1}H NMR spectrum of 5′-O-MMTr-cytidine **2**. ^{1}H NMR ($CDCl_3$, CD_3OD) δ: 8.1 (d, 1H, $J_{H\text{-}5,\ H\text{-}6}$ = 9.0 Hz) H-6, 7.3 (m, 12H) aromatic, 6.9 (d, 2H) aromatic, 5.8 (d, 1H, $J_{H\text{-}1',H\text{-}2'}$ =2.0 Hz) H-1′, 5.4 (d, 1H) H-5, 4.2 (m, 3H) H-2′, H-3′ and H-4′, 3.8 (s, 3H) OCH_3, 3.5 (m, 2H) H-5′ and H-5″. UV (pH2): λ_{max} 285 nm; (pH7) 276 nm; (pH12) 274 nm.

5′-O-MMTr-ara-cytidine (3)

The major part of the reaction mixture was treated with diphenyl carbonate (9.0 g, 42 mmol) and sodium bicarbonate (6.7 g, 80 mmol) in dimethylformamide (24 ml) for 3 h at 80 °C. After evaporation in vacuo the residue was dissolved in chloroform (about 20 ml) and purified by silica gel chromatography with chloroform then 10 % methanol-dichloromethane as eluents. Evaporation of the appropriate fractions gave compound **3** (3.3 g, 67 % for two reaction steps) ^{1}H NMR ($CDCl_3$, CD_3OD) δ: 7.9 (d, 1H, $J_{5,6}$ = 9.0 Hz) H-6, 7.3 (m, 12 H) aromatic, 6.8 (d, 2H) aromatic, 6.2 (d, 1H, $J_{1',2'}$ = 5.1 Hz) H-1′, 5.5(d, 1H) H-5, 4.1 (m, 3H) H-2′, H-3′ and H-4′, 3.8 (s, 3H) OCH_3, 3.4 (m, 2H) H-5′, H-5″. UV (pH2): λ_{max} 285 nm; (pH7) 276 nm; (pH12) 274 nm.

5′-O-MMTr-2′,3′,N^4-trilevulinyl-1-β-D-arabinofuranosylcytosine (4)

5′-O-MMTr-ara-C was levulinated as in the literature of Ryu[3] with a 96 % yield. ^{1}H NMR ($CDCl_3$, CD_3OD) δ: 7.0 (d, 1H, $J_{5,6}$ = 7.6 Hz) H-6, 7.3 (m, 14 H) aromatic of MMTr, 6.8 (d, 1H, $J_{5,6}$ = 7.6 Hz) H-5, 6.2 (d, 1H, $J_{1',2'}$ = 4.2 Hz) H-1′, 5.2 (m, 1H) H-2′,

5.1 (m, 1H) H-3', 4.2 (m, 1H) H-4', 3.7 (s, 3H) OCH_3, 3.4 (m, 2H) H-5', H-5'', 2.7-2.4 (m, 12H) CH_2 of Lev, 2.1-2.0 (m, 9H) CH_3 of Lev.

2',3',N^4-trilevulinyl-1-β-D-arabinofuranosylcytosine (5)

Compound **4** (0.802, 1.07 mmol) was dissolved in 10 ml 80 % aqueous acetic acid. The reaction mixture was stirred at room temperature for 3 h then evaporated and coevaporated with water several times. The resulting foam was dissolved in CH_2Cl_2 and loaded onto silica gel. The target compound was eluted with CH_2Cl_2. The yield was 0.34 g, 67 %. ^{1}H NMR ($CDCl_3$, CD_3OD) δ: 8.2 (d, 1H, $J_{5,6}$= 7.8 Hz) H-6, 7.4 (d, 1H, $J_{5,6}$= 7.8 Hz) H-5, 6.2 (d, 1H, $J_{1,2}$ = 4.2 Hz) H-1'. 5.5 (m, 1H) H-2', 5.2 (m, 1H) H-3', 4.1 (m, 1H) H-4', 3.9 (m, 2H) H-5', H-5'', 2.7-2.4 (m, 12H) CH_2 of Lev, 2.1-2.0 (m, 9H) CH_3 of Lev.

1,2-isopropylidene-3-FMOC-sn-glycerol (7)

1,2-isopropylidene-sn-glycerol **6** (0.66 g, 5 mmol) was dissolved in 50 ml dry pyridine and 9-fluorenyl chloroformate (FMOC-Cl) (1.55 g, 6 mmol) was added and the reaction mixture was stirred for 1 h at room temperature. 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 (CDCl3, CD_3OD) δ: 7.2-8.0 (8H, m) aromatic of FMOC, 3.6-4.5 (m, 8H) CH and CH_2 of FMOC, 2-HC, 3-H_2C and 1-H_2C, 1.5 (s, 3H) CH_3, 1.4 (s, 3H) CH_3.

3-FMOC-sn-glycerol (8)

Compound **7** (0.46 g, 1.3 mmol) was dissolved in 10 ml dioxane and 10 ml 80 % formic acid was added. The reaction mixture was stirred at room temperature 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 ($CDCl_3$, CD_3OD) 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, 2.7(bs, 1H) OH, 2.4 (bs, 1H) OH. ^{13}C NMR ($CDCl_3$) δ: 155.1 (C=O); 143.0, 141.0, 127.7, 124.8, 119.9 (aromatic carbons of FMOC); 69.8, 68.5, 63.0 (glycerol carbons), 46.5 (CH_2 of FMOC).

3-FMOC-1,2-dipalmitoyl-sn-glycerol (9a)

Compound **8** (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 **9a** was purified on silica gel column and eluted with 50 % hexane-dichloromethane. The proper fractions were evaporated giving compound **9a** (0.8 g, 100 %). ^{1}H NMR ($CDCl_3$) δ: 7.2-7.8 (m, 8H) aromatic of FMOC, 5.3 (m, 1H) 2-HC, 4.4 (m, 3H) CH and CH_2 of FMOC, 3.2 (m, 3H) 3-H_2C and 1-H_2C, 2.3 (m, 4H) OCH_2, 1.2-2.1 (m, 26H) CH_2, 0.9 (bs, 6H) CH_3.

3-FMOC-1,2-distearoyl-sn-glycerol (9b)

Compound **9b** was prepared in the same way as compound **9a** (0.57 g, 87 %). ^{1}H NMR ($CDCl_3$) δ: 7.3-8.0 (m, 8H) aromatic of FMOC, 5.3 (m, 1H) 2-HC, 4.4 (m, 3H) CH and CH_2 of FMOC, 3.2 (m, 4H) 3-H_2C and 1-H_2C, 2.3 (m, 4H) 2 OCH_2, 1.1-2.2 (m, 30H) CH_2, 0.9 (bs, 6H) CH_3.

3-FMOC-1,2-dioleoyl-sn-glycerol (9c)

Compound **9c** was prepared in the same way as compound **9a** (0.5 g, 95 %). ^{1}H NMR ($CDCl_3$) δ: 7.2-7.9 (m, 8H) aromatic of FMOC; 5.3 (m, 5H) 2-HC and 2CH=CH, 4.4 (m, 3H) CH and CH_2 of FMOC, 3.2 (m, 3H) 3-H_2C and 1-H_2C, 2.3 (m, 4H) 2 OCH_2, 1.1-2.2 (m, 26H) CH_2, 0.9 (m, 6H) CH_3.

1,2-Dipalmitoyl-sn-glycerol (10a)

Compound **9a** (0.63 g, 0.79 mmol) was dissolved in 8 ml dry pyridine and dry Et_3N (0.8 ml, 7.95 mmol). The reaction mixture was stirred at room temperature for 2 h, evaporated and loaded on silica gel column. The column was eluted with 50 % hexane-dichloromethane and the product (**10a**) was eluted with dichloromethane. The proper fractions were poured together and evaporated giving compound **10a** 0.38 g (84 %). ^{1}H NMR ($CDCl_3$ + CD_3OD) δ: 5.0 (m, 1H) 2-HC, 4.2 (m, 2H) 1-H_2C, 3.6 (m, 2H) 3-H_2C, 2.3 (m, 4H) 2 CH_2CO, 1.2-1.7 (m, 26H) 2 $(CH_2)_{13}$, 0.8 (t, 6H) 2 CH_3. ^{13}C NMR ($CDCl_3$ + CD_3OD) δ: 173.6 (C=O), 71.9, 62.2, 60.6 (glycerol carbons), 48.5 and 33.9 (CH_2-C=O), 31,7 (CH_2-CH_2-CH_3), 29.4 ($(CH_2)_{10}$ of both acyl chains), 13.8 (CH_3 of both acyl chains).

1,2-Distearoyl-sn-glycerol (10b)

Compound **10b** was produced similar to compound **10a** (0.37 g, 80 %). ^{1}H NMR ($CDCl_3$) δ: 5.0 (m, 1H) 2-HC; 4.2 (m, 2H) 1-H_2C, 3.7 (m, 2H) 3-H_2C, 2.3 (m, 4H) 2 CH_2CO, 1.5 (m, 30H) CH_2, 0.9 (t, 6H) 2 CH_3.

1,2-Dioleoyl-sn-glycerol (10c)

Compound **10c** was produced similar to compound **10a** (0.25 g, 80 %). ^{1}H NMR ($CDCl_3$) δ: 5.4 (m, 4H) 2 CH=CH; 4.3 (m, 2H) 1-H_2C, 3.7 (m, 2H) 3-H_2C, 2.3 (m, 4H) 2 CH_2CO, 1.3 (m, 26H) CH_2, 0.9 (t, 6H) 2 CH_3.

1,2-Dipalmitoyl-sn-glycero-3-(2-chlorophenyl)-phosphate triethylammonium salt (11a)

1,2-dipalmitoyl-sn-glycerol (0.219, 0.384 mmol) was dissolved in 2 ml dry CH_2Cl_2 and 2 ml dry pyridine. 3 ml 2-chlorophenyl-phosphoro-bis-triazolide solution (0.768 mmol, in acetonitrile) was added. The reaction mixture was stirred at room temperature for 20 min, then quenched with 0.5 M triethylammonium bicarbonate solution (10 ml) and partitioned between 50 ml CH_2Cl_2 and 50 ml saturated sodium bicarbonate. The organic layer was extracted with water (2 x 100 ml) then evaporated and coevaporated several times to remove any traces of pyridine. The resulting gum was 0.324 g, 100 %. ^{31}P NMR ($CDCl_3$, CD_3OD) δ: - 7.4.

1,2-Distearoyl-sn-glycero-3-(2-chlorophenyl)-phosphate triethylammonium salt (11b)

Distearoyl-sn-glycerol was phosphorylated analogously to the preparation of **11a** to give **11b** (0.32 g, 100 %). ^{31}P NMR ($CDCl_3$, CD_3OD) δ:- 7.1

1,2-Dioleoyl-sn-glycero-3-(2-chlorophenyl)-phosphate triethylammonium salt (11c)

Dioleoyl-sn-glycerol was phosphorylated analogously to the preparation of **11a**. The yield was 0.41 g, 93.5 %. ^{31}P NMR ($CDCl_3$, CD_3OD) δ:- 6.5.

2′,3′,N^4-trilevulinyl-1-β-arabinofuranosylcytosine 5′-(2-chlorophenyl)-phosphate-1,2-dipalmitoyl-sn-glycerol (12a)

Compound **11a** (0.1 g, 0.12 mmol) and compound **5** (0.047 g, 0.098 mmol) were dissolved in 1 ml dry pyridine. MsCl (0.077 g, 0.35 mmol) and MeIm (0.058g, 0.71 mmol) were added to the reaction mixture. After 20 min stirring the reaction mixture was partitioned between 10 ml CH_2Cl_2 and 10 ml citric acid solution. The organic phase was evaporated and dissolved in 2 ml CH_2Cl_2 and loaded on silica gel. 50 % CH_2Cl_2-n-hexane and CH_2Cl_2 eluted the target compound. The proper fractions were evaporated and gave a foam, 0.123 g (86 %). ^{31}P NMR ($CDCl_3$, CD_3OD) δ: - 6.6, - 6.8.

2′,3′,N^4-trilevulinyl-1-β-arabinofuranosylcytosine 5′-(2-chlorophenyl)-phosphate-1,2-distearoyl-sn-glycerol (12b)

Compound **11b** and **5** were coupled analogously to **11a**. The yield was 0.335 g, 95 %. ^{31}P NMR ($CDCl_3$, CD_3OD) δ: - 6.6, - 6.8.

2',3',N^4-trilevulinyl-1-β-arabinofuranosylcytosine 5'-(2-chlorophenyl)-phosphate-1,2-dioleoyl-sn-glycerol (12c)

Compound **11c** and **5** were coupled analogously to **11a**. The yield was 0.38 g, 85 %. ^{31}P-NMR ($CDCl_3$, CD_3OD) δ: - 6.7, -6.9.

2',3',N^4-trilevulinyl-1-β-arabinofuranosylcytosine 5'-monophosphate-1,2dipalmitoyl-sn-glycerol tributylammonium salt (13a)

Compound **12a** (0.129 g, 0.115 mmol) was dissolved in 31 ml pyridine/H_2O/THF(tetrahydrofuran) = 1:1:8 and 1 M TBAF solution in THF (1.55 ml) was added. After 4.5 h stirring at room temperature the reaction mixture was evaporated and the residue was dissolved in 2 ml CH_2Cl_2 and loaded on silica gel. Compound **13a** was eluted with 10 % MeOH-CH_2Cl_2. The proper fractions were combined and evaporated. The yield was 0.185 g, 88 %. ^{31}P NMR ($CDCl_3$, CD_3OD) δ: - 2.3.

2',3',N^4-trilevulinyl-1-β-arabinofuranosylcytosine 5'-monophosphate-1,2-distearoyl-sn-glycerol tributylammonium salt (13b)

Compound **12b** was treated with TBAF and worked up analogously to compound **12a**. The yield was 0.188 g, 80 %.^{31}P NMR ($CDCl_3$, CD_3OD) δ: -1.9.

2',3',N^4-trilevulinyl-1-β-arabinofuranosylcytosine 5'-monophosphate-1,2-dioleoyl-sn-glycerol tributylammonium salt (13c)

Compound **12c** was partially deblocked analogously to compound **12a**. The yield was 0.352 g, 100 %. ^{31}P NMR ($CDCl_3$, CD_3OD) δ: - 1.0.

1-β-arabinofuranosylcytosine 5'-monophosphate-1,2-dipalmitoyl-sn-glycerol tributylammonium salt (ara-CMP-dipalmitin) (14a)

Compound **13a** was delevulinated with hydrazine hydrate as described in the literature of Ryu[3] The yield was 0.076 g, 50 %. ^{1}H NMR ($CDCl_3$-CD_3OD-D_2O, 2:3:1) δ: 7.8 (d, 1H, $J_{5,6}$ = 7.8 Hz) H-6, 6.1 (d, $J_{1',2'}$ = 4.4 Hz) H-1', 6.0 (d, 1H, $J_{5,6}$ = 7.8 Hz) H-5,

5.2 (m, 1H)2-CH of glycerol, 4.3-3.9 (m, 15H) H-2′, H-3′, H-4′, H-5′, H-5″ + glycerol CH_2′s + tributylamine CH_2′s, 2.3 (m, 2H) CH_2CO, 1.6-1.3 (m, 26H) CH_2 of palmitoyl, 0.9 (t, 9H) CH_3 of palmitoyl and tributylamine. ^{31}P NMR ($CDCl_3$, CD_3OD) δ: - 2.3. UV (pH2) λ_{max} 285 nm; (pH7) 276 nm; (pH12) 274 nm.

1-β-arabinofuranosylcytosine 5′-monophosphate-1,2-distearoyl-sn-glycerol tributylammonium salt (ara-CMP-dipalmitin) (14b)

Compound **13b** was treated with hydrazine hydrate analogously to compound **13a**. The yield was 0.073 g, 52 %. ^1H NMR ($CDCl_3$-CD_3OD-D_2O, 2:3:1) δ: 7.8 (d, 1H, $J_{5,6}$ = 7.6 Hz) H-6, 6.1 (d, 1H, $J_{1',2'}$ = 4.4 Hz) H-1′, 5.9 (d, 1H, $J_{5,6}$ = 7.6 Hz) H-5, 5.2 (m, 1H) CH of glycerol. 4.7-3.8 (m, 15H) H-2′, H-3′, H-4′, H-5′, H-5″ + glycerol CH_2′s + tributylamine CH_2′s, 2.3 (m, 4H) CH_2CO, 1.6-1.0 (m, 30H) CH_2 of stearoyl, 0.9 (t, 9H) CH_3 of stearoyl and tributylamine. ^{31}P NMR ($CDCl_3$, CD_3OD) δ: - 0.9. UV (pH2) λ_{max} 285 nm; (pH7) 276 nm; (pH12) 274 nm.

1-β-arabinofuranosylcytosine 5′-monophosphate-1,2-dioleoyl-sn-glycerol tributylammonium salt (ara-CMP-dipalmitin) (14c)

Compound **13c** was deblocked analogously to compound **13a**. The yield was 0.067 g, 53 %. ^1H NMR ($CDCl_3$-CD_3OD-D_2O, 2:3:1) δ: 7.9 (d, 1H, $J_{5,6}$ = 7.6 Hz) H-6, 6.1 (d, 1H, $J_{1',2'}$ = 4.4 Hz) H-1′, 5.9 (d, 1H, $J_{5,6}$ = 7.6 Hz) H-5, 5.3 (m, 5H) CH of glycerol + $(CH{=}CH)_2$, 4.7-3.7 (m, 15H) H-2′, H-3′, H-4′, H-5′, H-5″ + glycerol CH_2′s + tributylamine CH_2′s, 2.3 (m, 4H) CH_2CO, 1.6-1.3 (m, 26H) CH_2 of oleoyl, 0.9 (m, 9H) CH_3 of oleoyl and tributylamine. ^{31}P NMR ($CDCl_3$, CD_3OD) δ: - 0.6. UV (pH2) λ_{max} 285 nm; (pH7) 276 nm; (pH12) 274 nm.

RESULTS AND DISCUSSIONS

In the synthesis of 5′-O-MMTr-ara-C (FIG. 1) we showed that increasing the liphophility of the starting material (cytidine) by protecting the 5′-OH of cytidin with monomethoxytrityl chloride we could prepare 5′-O-MMTr-ara-C **3** with the Ogilvie procedure[7] then purify from the remaining few percent of starting material with fast short

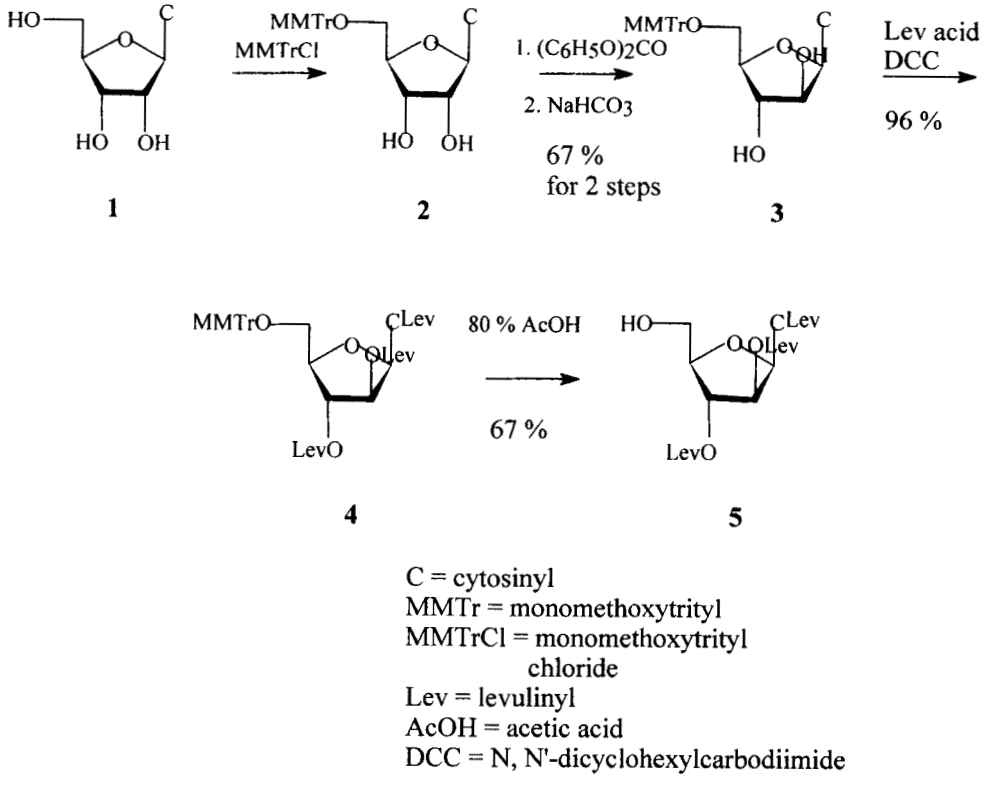


FIG. 1. Synthesis of the protected ara-C.

silica gel chromatography instead of with Zerolite FF and Dowex 50WX2 (H^+) by Beranek[8]. Compound **3** could be used directly with the temporary 5'-O-MMTr protection of ara-C for the synthesis of ara-C phospholipid. The UV spectra of 5'-O-MMTr-cytidine **2** and 5'-O-MMTr-ara-cytidine **3** are the same and the ^1H NMR spectra corroborate the structure of compound **3**. In the 5'-O-MMTr-ara-C **3** the anomeric proton becomes more shielded comparing to compound **2**. This relative shielding of H-1' may be due to a lone pair effect of the C-2' substituent[13]. The H-1' chemical shift of compound **3** (6.2 ppm) is 0.4 ppm more shielded than that of compound **2** (5.8 ppm). $J_{H,H}$ of 2' or 3' increases as the configuration of the substituent - which is directly attached to it - chanches[13]. We have noticed the same tendency because in the 5'-O-MMTr-cytidin **2** $J_{H\text{-}1',H\text{-}2'}$ = 2.0 Hz and in the 5'-O-MMTr-ara-cytidine **3** it has a higher value (5.1 Hz).

The synthesis of phospholipid was carried out employing Eibl method[14] for the preparation of isopropilidene glycerol. The 3 position of glycerol was protected by FMOC in an 80 % yield and the isopropilidene group was removed with formic acid treatment. The fatty acids were introduced quantitative with fatty acids and DCC in the presence of DMAP[23] then the FMOC group was removed by dry triethylamine.

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

1. It is easy to introduce with FMOC-Cl after 1 h at RT.

2. The products **7, 8, 9, 10** 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 **8** can be easily seen.

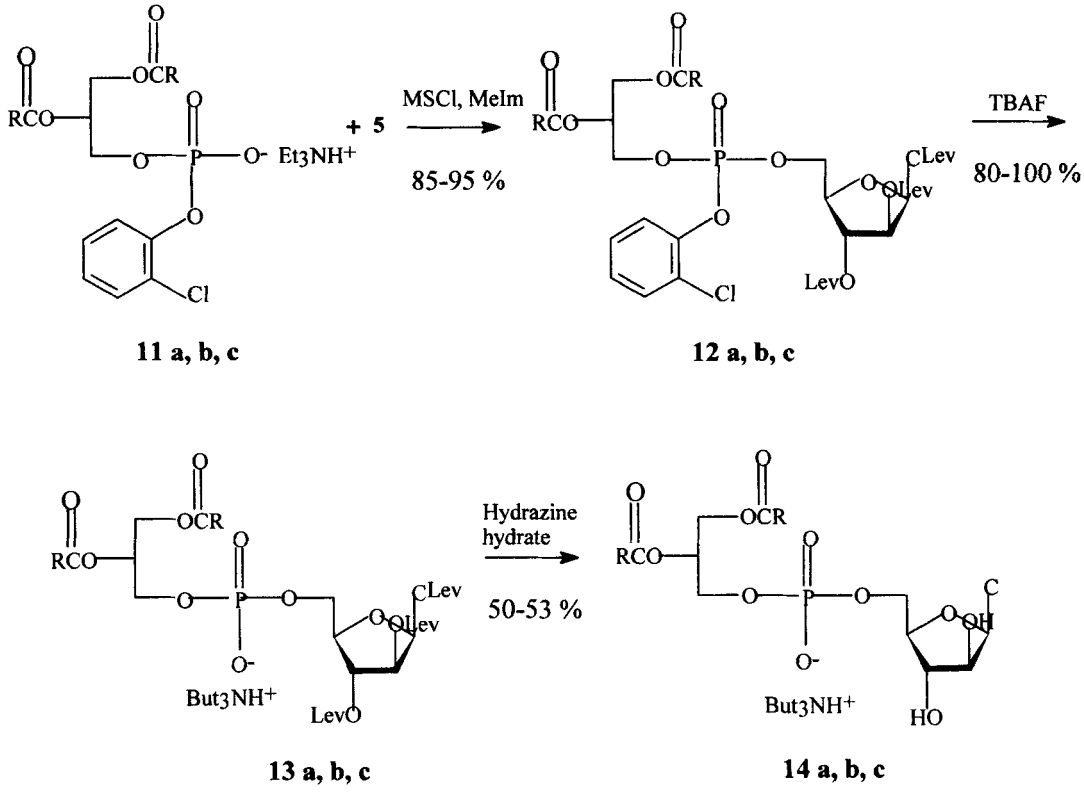
4. The fatty acids can be introduced almost quantitatively despite of the bulkyness of the protecting group.

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 the removal of benzyl with Pd/C can not be done in the case of unsaturated fatty acids, but debenzylation and detrtitylation are possible with bromodimethylborane showed by Kodali[25].

7. ^{1}H NMR and ^{13}C NMR spectra of compound **10a** are similar to that of in the literature[25].

The levulination of 5′-O-MMTr-ara-C was carried out as in the literature of Ryu[3], and the MMTr group was removed with aqueous acetic acid treatment at room temperature for 3 h, giving compound **5** (67 %) (FIG. 1). The phosphorylation of diacyl-sn-glycerols was performed with 2-chlorophenyl-phosphoro-bis-triazolide[1] giving the phosphoglycerols (**10a, 10b, 10c**) quantitative. Condensation of phosphoglycerols with 2′,3′,N^4-trilevulinyl-ara-C **5** (FIG. 2) was performed also with a high yield in the presence of MsCl and MeIm[2]. The TBAF treatment and delevulination with hydrazine hydrate gave the target compounds (**14a, 14b, 14c**) at a 50 % yield, which were characterized by UV, ^{1}H-NMR and ^{31}P-NMR spectroscopy. The λ_{max} at different pH was the same as that of cytidine. ^{1}H-NMR spectrum of compound **14a** is similar to the literature of Ryu[3].



a : R = palmitoyl
b : R = stearoyl
c : R = oleoyl

TBAF = tetrabutylammonium fluorid
MSCl = mesitylenesulphonyl chloride
Lev = levulinyl
C = cytosinyl

FIG. 2. Synthesis of ara-C-phospholipid conjugates.

Chemical shifts of ^{31}P NMR spectra of triesters (**12a**, **12b**, **12c**) and diesters (**13a, b, c, 14a, b, c**) were similar to the dinucleotides[2].

SUMMARY

We have improved the yield of the synthesis of ara-C from 32 to 67 % with MMTr protection of 5'-OH of cytidine and shortened the time of purification with silica gel of the reaction mixture.

We have characterized the 5′-O-MMTr-ara-C by ^{1}H NMR spectroscopy comparing to the spectrum of 5′-O-MMTr-C.

We showed here that the 1,2-diacylglycerols synthesized by FMOC protection on sn-3 position could be phosphorylated with 2-chlorophenyl-phosphoro-bis-triazolide and could be used for the preparation of ara-C-phospholipid conjugates without acyl migration, within the limit of the sensitivity of ^{1}H NMR.

Employing phosphotriester methodology[1] instead of the diester method of Ryu[3] the yield of condensation of phosphoglycerols and 2′,3′,N^4-trilevulinyl-ara-C could be improved from 22 to 85-95 %.

The 1-β-D-arabinofuranosylcytosine 5'-phosphate-L-1,2-diacylglycerols are not substrate of the phospholipase C, which is 3-5 fold higher in both human and rat tumor cells[24]. The biological activity of 1-β-D-arabinofuranosylcytosine 5'-phosphate-L-1,2-diacylglycerols most probably bases on the selective decomposition of the conjugate to the prodrug by the higher pH (8) of the tumor cells[24].

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