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Synthesis of Phosphoserine and Phosphothreonine Ether-Glycerolipids *via* 2,2,2-Trichloro-*t*-Butyl Phosphodichloridite Coupling

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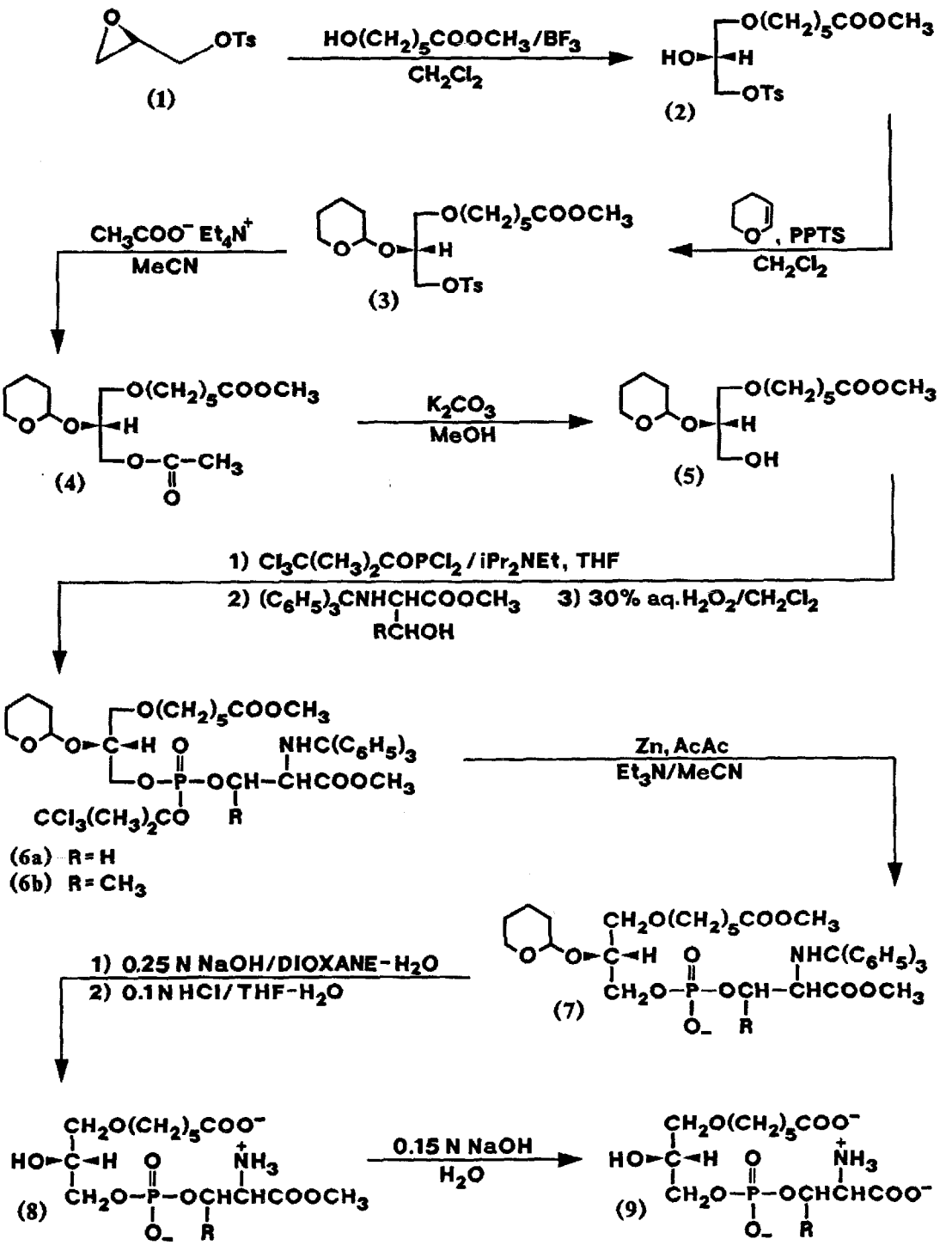
Abstract: *A facile and efficient phosphite coupling procedure for the synthesis of ether phospholipids with serine and threonine polar headgroup substituents is reported.*

Amino acid derivatives of ether phospholipids represent a series of highly potent biologically active synthetic analogues of platelet-activating factor (1-octadecyl-2-acetyl-*sn*-glycero-3-phosphocholine, PAF). Included among these are antitumor active ether phospholipids[1] as well as a series of recently discovered "modulator phospholipids"[2,3] both carrying amino acid components incorporated into the *sn*-3-polar headgroup of the molecule. Special attention has been focused recently on the latter series as two such amino acid derivatives of ether phospholipids, isolated from rat liver cytosol, have shown potent inhibition of glucocorticoid receptor-complex activation.[3] Although the proposed structures of the isolated modulator phospholipids remain to be confirmed, based on physicochemical studies aimed at characterization of the naturally occurring compounds it has been suggested that an *sn*-1-(carboxy)alkyl ether function, a free hydroxyl at the *sn*-2-glycerol position, and an *sn*-3 phosphorylated amino acid moiety (serine, threonine or related amino acid derivative) comprise the substituents of the phospholipid molecule.[3]

Development of new synthetic methods for preparation of these phospholipid derivatives is an important prerequisite to establishing the structures of the naturally occurring compounds, and to providing access to analogues for elucidation of structure-activity relationships in the series. In the present communication we describe the use of 2,2,2-trichloro-*t*-butyl phosphodichloridite for coupling a substituted glycerol component with a suitably protected amino acid alcohol function that provides a flexible and efficient method for the preparation of a wide range of related ether aminophosphoglycerides. Specifically, our approach is based on the following elements: 1) *(R)*-glycidyl tosylate is used as chiral substituted glycerol precursor, 2) BF_3-catalyzed epoxide ring opening with the corresponding alcohol is employed for introduction of the incipient *sn*-1-(carboxy)alkyl ether function,[4] and 3) coupling of the substituted glycerol with the N-protected hydroxy amino acid methyl ester is accomplished using 2,2,2-trichloro-*t*-butyl phosphodichloridite to provide the


OTs
(1)
HO(CH2)5COOCH3/BF3
CH2Cl2
O(CH2)5COOCH3
HO
H
OTs
(2)
CH3COO⁻ Et4N⁺
MeCN
(3)
PPTS
CH2Cl2
O(CH2)5COOCH3
H
O–C–CH3
(4)
K2CO3
MeOH
OH
(5)
1) Cl3C(CH3)2COPCl2 / iPr2NEt, THF
2) (C6H5)3CNHCHCOOCH3
RCHOH
3) 30% aq.H2O2/CH2Cl2
NHC(C6H5)3
O–P–OCH–CHCOOCH3
CCl3(CH3)2CO
R
(6a) R=H
(6b) R=CH3
Zn, AcAc
Et3N/MeCN
CH2O(CH2)5COOCH3
(7)
1) 0.25 N NaOH/DIOXANE-H2O
2) 0.1 N HCl/THF-H2O
CH2O(CH2)5COO⁻
NH3
(8)
0.15 N NaOH
H2O
(9)

Scheme 1

phosphite triester intermediate. Oxidation to the corresponding phosphotriester is achieved using hydrogen peroxide.[5] Finally, reductive cleavage of the tertiary phosphoester function followed by sequential hydrolysis of the protecting groups yields the desired target phospholipid (**9**).

Methyl-6-hydroxyhexanoate, prepared by esterification of the parent acid in anhydrous methanolic HCl (100%) was allowed to react with *(R)*-glycidyl tosylate in CH_2Cl_2 in the presence of 1 equiv. boron trifluoride etherate at r.t. for 4h. The product (**2**) was readily purified on silica-gel chromatography (hexane-EtOAc 2:3, 89%, >99% e.e. based on ^{1}H-NMR analysis (360 MHz) of the Mosher ester of (**2**)[6], $[\alpha]_D^{25}$ -7.5±0.2 (c 1.0, MeOH)), and then converted to the *sn*-2-tetrahydropyranyl ether derivative (**3**) in 69% yield. The analytically pure mixture of stereoisomers (**3**)[7] was used for the next step in reaction with anhydrous tetraethylammonium acetate in acetonitrile for displacement of the *sn*-3-toluenesulfonate[8] to afford the corresponding acetoxy compound (**4**, 90%, calcd. for $C_{17}H_{30}O_6$: C, 58.94; H, 8.73; found C, 58.66, H, 8.49). The product (**4**) readily underwent selective hydrolysis at the *sn*-3-position in methanolic K_2CO_3 at r.t. for 30 min. to give the alcohol (**5**, 75%, calcd. for $C_{15}H_{28}O_6$: C, 59.19; H, 9.27; found: C, 59.27; H, 9.24). Coupling of this alcohol (**5**) with N-trityl serine methyl ester[9] using stoichiometric amounts of 2,2,2-trichloro-*t*-butyl phosphodichloridite in the presence of 3 equiv. diisopropyl ethylamine in tetrahydrofuran at -78° gave the trialkyl phosphite (69%), which was purified on silica-gel chromatography (hexane-EtOAc 2:1); (FAB-MS calcd. for $C_{42}H_{56}O_{10}NPCl_3$ ($M+H^+$):870.2707, found 870.2715), and treated with 30% aq. hydrogen peroxide in a biphasic system with methylene chloride at r.t. for 2h to obtain the phosphotriester (**6a**, 95%, FAB-MS calcd. for $C_{42}H_{56}O_{11}NPCl_3$ ($M+H^+$): 886.2656, found: 886.2612). The corresponding threonine derivative (**6b**) was prepared in a similar way, except that in this case the secondary alcohol was phosphorylated first, followed by addition of the primary alcohol (**5**) to the reaction mixture. Compound (**6b**) was obtained in 45% from (**5**) (FAB-MS calcd. for $C_{43}H_{57}O_{11}NPCl_3$ (M^+): 899.2734, found 899.2693). Reductive cleavage to the phosphodiester (**7a**) was achieved using zinc powder in the presence of acetyl acetone/triethylamine in acetonitrile at r.t. for 3h.[10] The purified phosphodiester (**7a**, 61%) was subjected to sequential hydrolysis 1) 0.25 N NaOH in dioxane-water (5:1) to form the ω-carboxylate,[11] 2) 0.1 N HCl in THF-water (1:1) to remove the acid labile N-trityl and O-tetrahydropyranyl groups, and 3) 0.15 N aq.NaOH to obtain the free serine carboxylate in 76, 82, and 100% yields respectively. The final product (**9a**) was purified by silica-gel chromatography ($CHCl_3$-MeOH-H_2O 3:6:1, R_f = 0.42)[12] : ^{1}H-NMR(200 MHz, D_2O) δ = 1.20(m, 2H, $CH_2C\underline{H}_2CH_2$), 1.42(m, 4H, $OCH_2C\underline{H}_2CH_2C\underline{H}_2CH_2COOH$), 2.23 (t, 2H, $C\underline{H}_2COOH$), 3.38 (m, 4H, $C\underline{H}_2OC\underline{H}_2$), 3.70 (m, 2H, $OCHC\underline{H}_2OP$), 3.83(m, 1H, $OC\underline{H}CH_2OP$), 3.93(m, 1H, $H_3\overset{+}{N}C\underline{H}CH_2OP$), 4.10(m, 2H, $H_3\overset{+}{N}CHC\underline{H}_2OP$); $[\alpha]_D^{25}$-6.5° (c 1.0, H_2O), FAB-MS calcd. for $C_{12}H_{25}NO_{10}P$ ($M+H^+$) : 374.1216, found: 374.1194. The corresponding threonine derivative (**9b**) was obtained using the same procedure, sequential hydrolysis of phosphodiester (**7b**) proceeded in 78, 80 and 96% yields, respectively; (**9b**) : $[\alpha]_D^{25}$-10° (c, 1.0, H_2O), ^{1}H-NMR (200 MHz, D_2O) δ = 1.25 (m, 2H, $CH_2C\underline{H}_2CH_2$), 1.38 (d, 3H, OCH-C$\underline{H}_3$), 1.52 (m, 4H, $C\underline{H}_2CH_2C\underline{H}_2$), 2.15 (t, 2H, $C\underline{H}_2COOH$), 3.50 (m, 4H, $C\underline{H}_2OC\underline{H}_2$), 3.62 (m, 1H, $OC\underline{H}CH_2OP$), 3.77 (m, 2H, $OCHC\underline{H}_2OP$), 3.93 (m, 1H, $H_3\overset{+}{N}C\underline{H}CHOP$), 4.62 (m, 1H, $H_3\overset{+}{N}CHC\underline{H}OP$); R_f = 0.44 ($CHCl_3$ - MeOH - H_2O 3:6:1); calcd. for $C_{13}H_{24}NO_{10}PNa_2 \cdot 0.5\ H_2O$: C, 35.46; H, 5.72; N, 3.18; found C, 35.32; H, 6.11; N, 2.99.

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6. Mosher esters of compound (**2**) and its enantiomer (obtained by ring opening of the corresponding *(S)*-glycidyl tosylate in a similar fashion) were prepared according to Dale, J.A.; Dull, D.L.; Mosher, H.S. *J. Org. Chem.* **1969,** *34,* 2543-2549, and analyzed by high-field ^{1}H-NMR spectroscopy. The 360 MHz ^{1}H-NMR spectrum of (**2**) in $CDCl_3$ exhibited a multiplet for the proton at the chiral sn-2-glycerol carbon centered at δ = 5.3413, showing not even traces of absorption (but baseline, signal-to-noise ratio higher than 100:1) at δ = 5.3996 and 5.4135 which are part of the multiplet centered at 5.833 assigned to the proton at the *(S)*-glyceryl stereocenter of the Mosher ester prepared from the enantiomer of compound (**2**). These findings are clearly consistent with the well-documented stereospecificity of BF_3-catalyzed epoxy-ring opening by alcohol nucleophiles (see ref. 4 for details).
7. The diastereoisomers formed on introduction of the tetrahydropyranyl group appear as a single spot on silica-gel chromatography. This, however, does not interfere with the stereospecificity of the synthesis, since subsequent deprotection of the *sn*-2-hydroxyl function yields a single enantiomer.
8. We found this procedure to be a great deal more convenient than the alternative method using CsOAc/DMF-DMSO 4:1, cf. Dijkstra, G.; Kruizinga, W.H.; Kellogg, R.W. *J. Org. Chem.* **1987,** *52,* 4230-4234.
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10. This procedure employed for the removal of the 2,2,2-trichloro-*t*-butyl group gives better yields and higher purity of the product (**7**) than either zinc/acac/pyridine, or tributyl phosphine/triethylamine/ DMF/aq.NH_3 used in oligonucleotide syntheses, cf. Letsinger, R.L.; Groody, E.P.; Tanaka, T. *J. Am. Chem. Soc.* **1982,** *104,* 6806-6808.
11. There is a clear difference in reactivity between the two carboxylic esters, the neighboring trityl group prevents hydrolysis of the serine methyl ester under these reaction conditions.
12. The phospholipid product (**9**) obtained as a disodium salt was converted to the acid form *via* ion exchange chromatography using Dowex 50W-X8 (H^+ form).

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