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Stereospecific Synthesis of Ether Phospholipids. Preparation of 1-O-(3'-Carboxypropyl)-Glycero-3-Phosphoserine From Glyceric Acid

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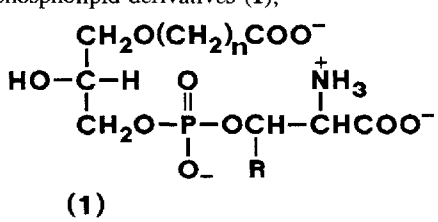
Abstract: *A new stereospecific synthesis of carboxyalkyl ether phospholipids is reported.*

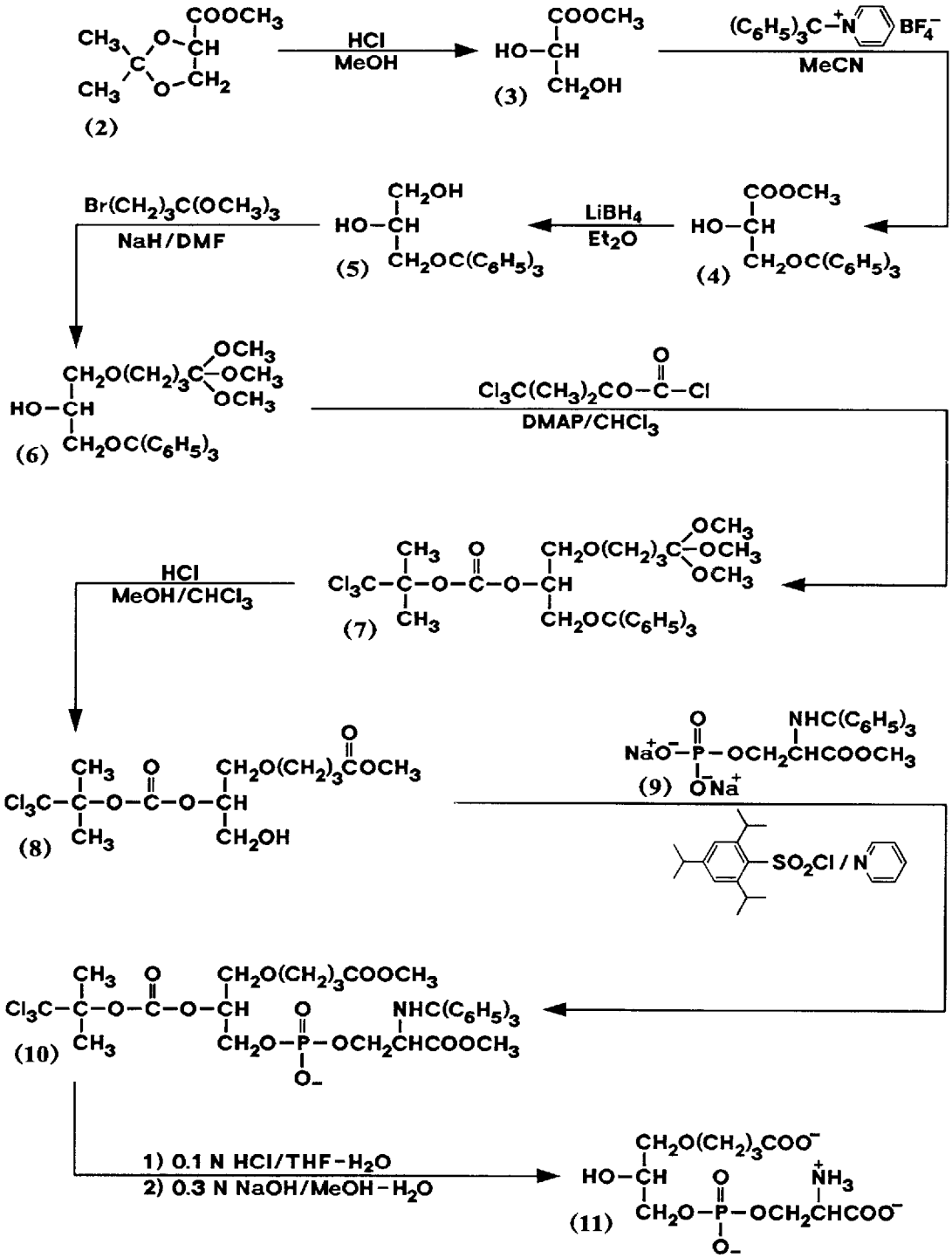
Structurally modified analogues of platelet-activating factor (1-0-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, PAF) represent a series of synthetic phospholipid compounds exhibiting high level of activity in a wide spectrum of biological systems.[1-4] Included among these are platelet-activating,[1] antihypertensive,[2] and antitumor active[3] alkyl ether phospholipids as well as immunological response modifiers.[4] PAF analogues have been shown to inhibit phosphatidylcholine biosynthesis,[5] protein kinase C activity[6] as well as phorbol ester stimulation of protein kinase C.[7] Despite ongoing vigorous investigation of the biochemistry and cell-biology of ether phospholipids their physiological function as well as their mechanism of action remains to be elucidated.

Amino acid conjugates of ether phospholipids are recent additions to the family of highly potent PAF analogues.[8-13] Among these compounds, particular attention has been directed toward a series of polar short-chain carboxyalkyl ether phospholipid derivatives (**1**),



(1)

that carry amino acid substituents as a part of the *sn*-3-phosphodiester function. Specifically, compounds with serine, threonine and other related amino acid derivatives have been suggested to function as the "endogenous low-molecular weight steroid-receptor complex activation inhibitor" called *"modulator."*[9,11,13] Two such compounds were isolated and purified recently from rat liver cytosol[10,12,13] and the two *"isoforms"* suggest that the modulator is an amino acid derivative of an alkyl ether glycerophospholipid, with a terminal carboxyl


HCl
MeOH
(2)
(3)
MeCN
LiBH4
Et2O
(4)
(5)
Br(CH2)3C(OCH3)3
NaH/DMF
(6)
DMAP/CHCl3
(7)
HCl
MeOH/CHCl3
(8)
(9)
SO2Cl / N
(10)
1) 0.1 N HCl/THF-H2O
2) 0.3 N NaOH/MeOH-H2O
(11)

Scheme 1

group in the *sn*-1-alkyl moiety, and an unsubstituted *sn*-2-hydroxy group in the glycerol portion of the molecule.[11]

Development of synthetic methods for the preparation of compound (**1**) is important not only to confirm the proposed structure of the modulator, but also to provide synthetic analogues to establish structure-activity relationships with regard to regulation (inhibition/activation) of modulator activity in the glucocorticoid receptor complex.[13]

We now describe a stereospecific synthesis of modulator phospholipid (**1**) providing a general route that should be applicable to preparation of a wide range of amino acid conjugated ether phospholipids. Our approach is based on the following elements: 1) L-glyceric acid is used as the stereocenter for construction of the optically active phospholipid molecule, 2) the carboxylalkyl ether function is introduced in the ω-orthoester form, 3) the *sn*-2-hydroxyl group is protected using the base-sensitive 2,2,2-trichloro-t-butoxycarbonyl group, and 4) coupling between the functionalized glycerol and the phosphorylated amino acid is achieved *via* the triisopropylbenzenesulfonyl chloride/pyridine route.

L-glyceric acid methyl ester (**3**) was prepared by acid catalyzed deprotection of isopropylidene (**2**) in saturated methanolic HCl, and converted to the *sn*-3-triphenylmethyl ether (**4**) using tritylpyridinium tetrafluoroborate in acetonitrile at r.t. overnight (70%).[14] The carbomethoxy group of compound (**4**) was reduced to the corresponding carbinol (**5**) with $LiBH_4$ in ether (83%, mp 92°),[15] and the resulting product (**5**) was alkylated with trimethyl bromoorthobutyrate in anhydrous dimethyl formamide, in the presence of 1 equiv. NaH at r.t. to give compound (**6**), which was isolated and purified by flash chromatography on neutral alumina ($CHCl_3$-hexane 1:1) as a white gum (83%, $[\alpha]_D^{23}$ +5.3 (c. 1.0, $CHCl_3$) ^{1}H-NMR(80 MHz, $CDCl_3$): δ = 1.62 - 1.70[m, 4H,$(CH_2)_2$], 2.50(m, 1H, OH), 3.20 [s, 9H, $(OCH_3)_3$], 3.45 - 3.70 [m, 6H $(OCH_2)_3$], 3.9 - 3.98 (m, 1H, CH), 7.25 - 7.37 [m, 15H, $(C_6H_5)_3$]. Treatment of the product (**6**) with 2,2,2-trichloro-t-butyl chloroformate/4-(dimethylamino)pyridine in chloroform yielded the corresponding carbonate (**7**, 79%), which was converted to alcohol (**8**) with stoichiometric amounts of 12M HCl in a $CHCl_3$-MeOH (1:1) solution of the trityl ether for 2h. The resulting *sn*-3-carbinol could be isolated and purified (89%) without migration of the *sn*-2-trichloro-t-butoxycarbonyl group[16] (mp 41°, $[\alpha]_D^{23}$ +1.65° (c 1.0, CH_3OH) calcd. for $C_{13}H_{21}Cl_3O_7$: C, 39.46; H, 5.35; Cl, 26.88; found C, 39.48; H, 5.38; Cl, 26.93. Coupling of this functionalized glycerol (**8**) with N-trityl-O-phosphoserine methyl ester disodium salt (**9**) using triisopropylbenzenesulfonyl chloride in pyridine gave the phosphodiester (**10**) in 52% yield.[17] Deprotection of the amino group with 0.1 N HCl (THF - H_2O 1:1) followed by sequential base hydrolyses of 1) the serine methyl ester, and 2) the trichloro-t-butyl carbonate and the ω-carboxylic ester together, led to the final product (**11**) in 64, 42, and 72% yields successively.[17] The target amino phosphodiester (**11**) was purified by silica-gel chromatography ($CHCl_3$ - MeOH - H_2O 3:6:1, R_f = 0.36) as the disodium salt, yielding a hygroscopic white solid; ^{1}H-NMR(80 MHz, D_2O)δ=1.59-1.90(m, 2H, $CH_2C\underline{H}_2CH_2$), 2.06-2.25(t, 2H, $C\underline{H}_2COO^-$), 3.39-3.53[m, 4H, $(CH_2)_2O$], 3.77-3.92[m, 4H, $^-O_2P(OCH_2)_2$]; 4.03-4.15(m, 2H, $C\underline{H}\overset{+}{N}H_3$ and $C\underline{H}OH$); $[\alpha]_D^{23}$ -5.50 (c 1.0, H_2O) calcd. for $C_{10}H_{18}NO_{10}PNa_2 \cdot 0.5H_2O$) : C, 30.16; H, 4.81; N, 3.51; P, 7.77; Na, 11.55; found C, 30.29, H, 5.01; N, 3.23; P, 7.83; Na, 11.54.

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16. Employing the 2,2,2-trichloro-t-butoxycarbonyl rather than the 2,2,2-trichloroethoxycarbonyl group (Hasegawa, E.; Eshima, K.; Matsushita, Y.; Nishide, H.; Tsuchida, E. *Synthesis* **1987,** 60-62) for protection of the *sn*-2-glycerol function provides a readily isolable, stable, white crystalline derivative, that is much less susceptible to undergo either cyclization or acyl migration to the neighboring primary hydroxyl group.
17. Regarding the problem of recovery of phospholipids from silica-gel columns cf. Chandrakumar, N.S.; Hajdu, J. *J. Org. Chem.* **1983,** *48,* 1197-1202.

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