Tetrahedron Letters,Vol.28,No.16,pp 1729-1732,1987 0040-4039/87 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

STEREOSPECIFIC SYNTHESIS OF PAF ANALOGUES. PREPARATION OF 1-HEXADECYL 2-THIOACETYL-2-DEOXYGLYCEROPHOSPHOCHOLINE (2-ThioPAF)

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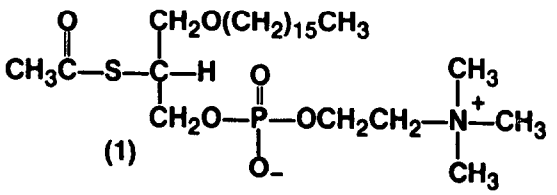
SUMMARY: A new stereospecific synthesis of ether-phospholipids leading to 2-ThioPAF is reported.

Structurally modified derivatives of platelet activating factor (PAF)[1] represent an important class of exceptionally potent biologically active compounds.[2-5] Specifically, a number of alkylglycerophosphocholines have been shown to exhibit platelet activating[1], antihypertensive[2] and immunomodulating[3] activities, as well as selective tumor cytotoxicity against a series of human cancer cells[4]. The high potency and wide spectrum of biological activity of PAF itself are generating a great deal of current interest[5], yet elucidation of its mechanism of action in biochemical as well as in physiological-regulatory processes remains to be accomplished. Consequently, development of efficient synthetic methods for the preparation of biologically active structural analogues of platelet activating factor has become one of the most timely problems in lipid chemistry and biochemistry today.

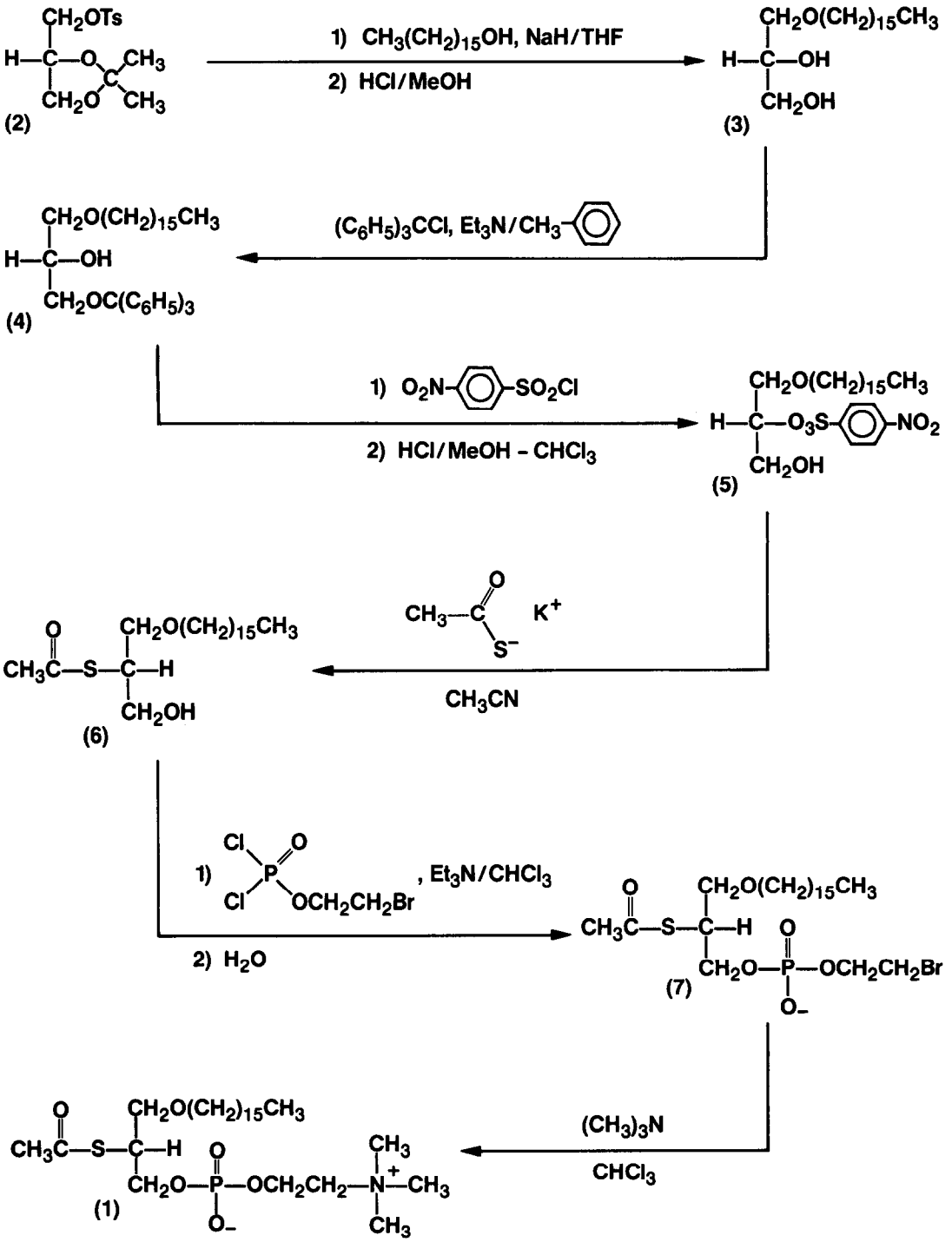
In the present communication we describe a new stereospecific phospholipid synthesis focusing on the preparation of 1-hexadecyl-2-thioacetyl-2-deoxy-sn-glycero-3-phosphocholine (2-thioPAF, (1)) a thiol-producing probe for the study of PAF-metabolizing enzymes [5a](Scheme 1). The sequence should be readily applicable to the synthesis of a series of related ether-phospholipid derivatives for structural, chemical and cell-biological studies.



(1)

Our approach to the synthesis is based on the following elements: 1) 1-tosyl-2,3-isopropylidene-D-glycerol[6] provides a suitable chiral conduit to construct the target molecule around the optically active center, 2) the thioacetyl group is introduced by nucleophilic sulfur-displacement at the p-nitrobenzenesulfonyl-activated secondary glycerol function (through inversion of the configuration of the sn-2-carbon), and 3) the phosphocholine moiety is elaborated via a β-bromoethyl phosphodichloridate - trimethylamine sequence. Significantly, the synthesis requires only minimal use of protecting groups and it presents a general route to a wide spectrum of structurally well-defined PAF analogues.[7]

SCHEME 1


CH2OTs
H—C—O
CH3
C
CH2O
CH3
(2)
1) CH3(CH2)15OH, NaH/THF
2) HCl/MeOH
CH2O(CH2)15CH3
H—C—OH
CH2OH
(3)
(C6H5)3CCl, Et3N/CH3
CH2O(CH2)15CH3
H—C—OH
CH2OC(C6H5)3
(4)
1) O2N
SO2Cl
2) HCl/MeOH - CHCl3
CH2O(CH2)15CH3
H—C—O3S
NO2
CH2OH
(5)
CH3—C
O
S−
K+
CH3CN
O
CH3C—S—C—H
CH2O(CH2)15CH3
CH2OH
(6)
1)
Cl
Cl
P
O
OCH2CH2Br
, Et3N/CHCl3
2) H2O
O
CH3C—S—C—H
CH2O(CH2)15CH3
CH2O—P—OCH2CH2Br
O
O−
(7)
(CH3)3N
CHCl3
O
CH3C—S—C—H
CH2O(CH2)15CH3
CH2O—P—OCH2CH2—N+—CH3
O
O−
CH3
CH3
(1)

1-D-hexadecylglycerol (3) was obtained from a reaction of D-2,3-isopropylideneglycerol tosylate (2) with hexadecanol in the presence of 1 equiv. NaH in tetrahydrofuran, followed by acid-catalyzed deprotection of the diol function using methanolic HCl at r.t. for 1 hr (65% from (2)). Tritylation of the alkyl glycerol (3) with trityl chloride/triethylamine gave compound (4) in 75% yield, which then was allowed to react with excess p-nitrobenzenesulfonyl chloride/4-(dimethylamino)pyridine in anhydrous chloroform for 48 hrs. at r.t.. The resulting p-nitrobenzenesulfonate was detritylated with HCl gas in chloroform-methanol (1:1) at r.t. for 1 hr yielding compound (5) in 90% (m.p. 54°). Anal. calc. for $C_{25}H_{43}NO_7S$; C, 59.85; H, 8.64; N, 2.79; S, 6.39; found C, 60.16; H, 8.55; N, 2.87; S, 6.82. The alcohol (5) was treated with potassium thioacetate in dry acetonitrile at r.t. for 6 hrs. to give the thioester (6) which was purified by Sephadex LH-20 chromatography.[8] Compound (6) was dried in vacuo over P_2O_5, and phosphorylated with β-bromoethyl phosphodichloridate[9] in dry chloroform, in the presence of excess triethylamine at r.t. for 18 hrs.. The crude bromoethyl phospholipid was stirred with aq. 0.1 M KCl for 1 hr, extracted at pH 3.0 with chloroform, dried over P_2O_5 and then treated with anhydrous trimethylamine in $CHCl_3$ at 60° (in a pressure - bottle) for 14 hrs.. Passage of the product (1) through silica gel column ($CHCl_3$-MeOH-H_2O, 65:25:4, Rf = 0.31) gave chromatographically pure phospholipid (47% isolated yield[10] from alcohol (6)). ^{1}H-n.m.r. ($CDCl_3$), δ0.88 (br t, 3H, - CH_3), 1.26(s, 28H, - CH_2), 2.34 (s, 3H, - $COCH_3$), 3.45 (s, 9H, - $N(CH_3)_3$), 3.45 - 4.35 (m, 11 H). Anal. Calc. for $C_{26}H_{54}NO_6PS \cdot H_2O$, C, 55.99; H, 10.12; N, 2.51; P, 5.55; S, 5.75; found C, 55.82; H, 10.11; N, 2.69; P, 4.48; S, 5.45.[11] The stereochemistry of the product (1) was ascertained by enzymatic hydrolysis using bee-venom phospholipase A_2. Exhaustive hydrolysis of 2-thioPAF in mixed micelles with Triton X-100 (1:8) at 40°C gave 97.0 ± 5% chiral purity.[12]

Preliminary results indicate that compound (1) exhibits potent hypotensive activity at the picomolar level.[13]

Acknowledgements. We thank Ms. Caroline Balet of our research group for the enzymatic experiments. We are grateful to Professor Fred Snyder for testing the compound in his assay system. We thank the National Institutes of Health (AM 36215 and CA 41666) for financial support.

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