Tetrahedron Letters No. 37, pp 3561 - 3564. 0040-4039/79/0908-3561$02.00/0

SYNTHESIS OF GLUCOSYLPHOSPHATIDYLGLYCEROL *VIA* A PHOSPHOTRIESTER INTERMEDIATE.

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Complete deblocking of a fully-protected intermediate, which was prepared by using *inter alia* a selective protection of 1- or 3-O-allyl-sn-glycerol with one pivaloyl group and effective phosphorylation and coupling procedures, afforded glucosylphosphatidylglycerol.

It is well known now[1] that glucosylphosphatidylglycerol (i.e., 8d) is part of the phospholipid pool of moderately halophilic halotolerant Gram-negative bacteria.

In order to get more insight into the biosynthesis and function of naturally occurring glycophospholipids we embarked upon a programme to develop efficient synthetic methods for the preparation of this type of compounds.

In this paper we wish to present a convenient and attractive procedure for the synthesis of glucosylphosphatidylglycerol (8d).

The compound to be synthesized contains two ester bonds, one α-glucosidic linkage and one phosphodiester linkage. In order to introduce the latter two types of linkages, and keeping in mind the base lability of the two ester bonds, we adopted the following strategy. The α-linkage between D-glucose and sn-glycerol was introduced by condensing 2,3,4,6-tetra-O-benzyl-D-glucosyl bromide[2] (1, R^1=benzyl) with the secondary hydroxyl group of a suitably protected sn-glycerol derivative (i.e., 2) under the conditions as developed by Lemieux[3]. For the protection of the two primary hydroxyl groups of sn-glycerol we selected the allyl and the pivaloyl groups. The choise of the latter relatively base stable[4] group had several attractive features. For instance, under the conditions of Lemieux, which are necessary for the required α-glucosidic linkage, no migration of this group was observed, further, the removal of this group could be accomplished selectively to give a free primary hydroxyl function which was necessary for the formation of the required phosphoester bond with the phosphatidic derivative (7). Another noteworthy feature adherent to the use of the pivaloyl group was that it could be introduced selectively at a primary hydroxyl of a sn-glycerol derivative. Thus treatment of 1-O-allyl-sn-glycerol[5] (15 mmol) with pivaloyl chloride (15 mmol) in dry pyridine at -10°C gave, after purification by short column chromatography[6], pure 2[7a,b] (R^2= $(CH_3)_3CCO$; 13.8 mmol). In the same way, starting from 3-O-allyl-sn-glycerol[5], the enantiomeric form of 2 was obtained. The identity of both compounds was established by ^{1}H-NMR and ^{13}C-NMR spectroscopy.

Condensation of the glucopyranosyl bromide 1 (R^1=benzyl ; 3.5 mmol) with the sn-glycerol derivative 2 (2.5 mmol), under the conditions of Lemieux, afforded, after purification by silica gel chromatography, the required product 3 (1.2 mmol) as a homogeneous oil ($[\alpha]_D^{25}$ = +31.6°, c=1, $CHCl_3$). In the same way, starting from 1-O-pivaloyl-3-O-allyl-sn-glycerol, an other diastereomer of 3 ($[\alpha]_D^{25}$ = +38.8°, c=1, $CHCl_3$) was obtained. The two compounds

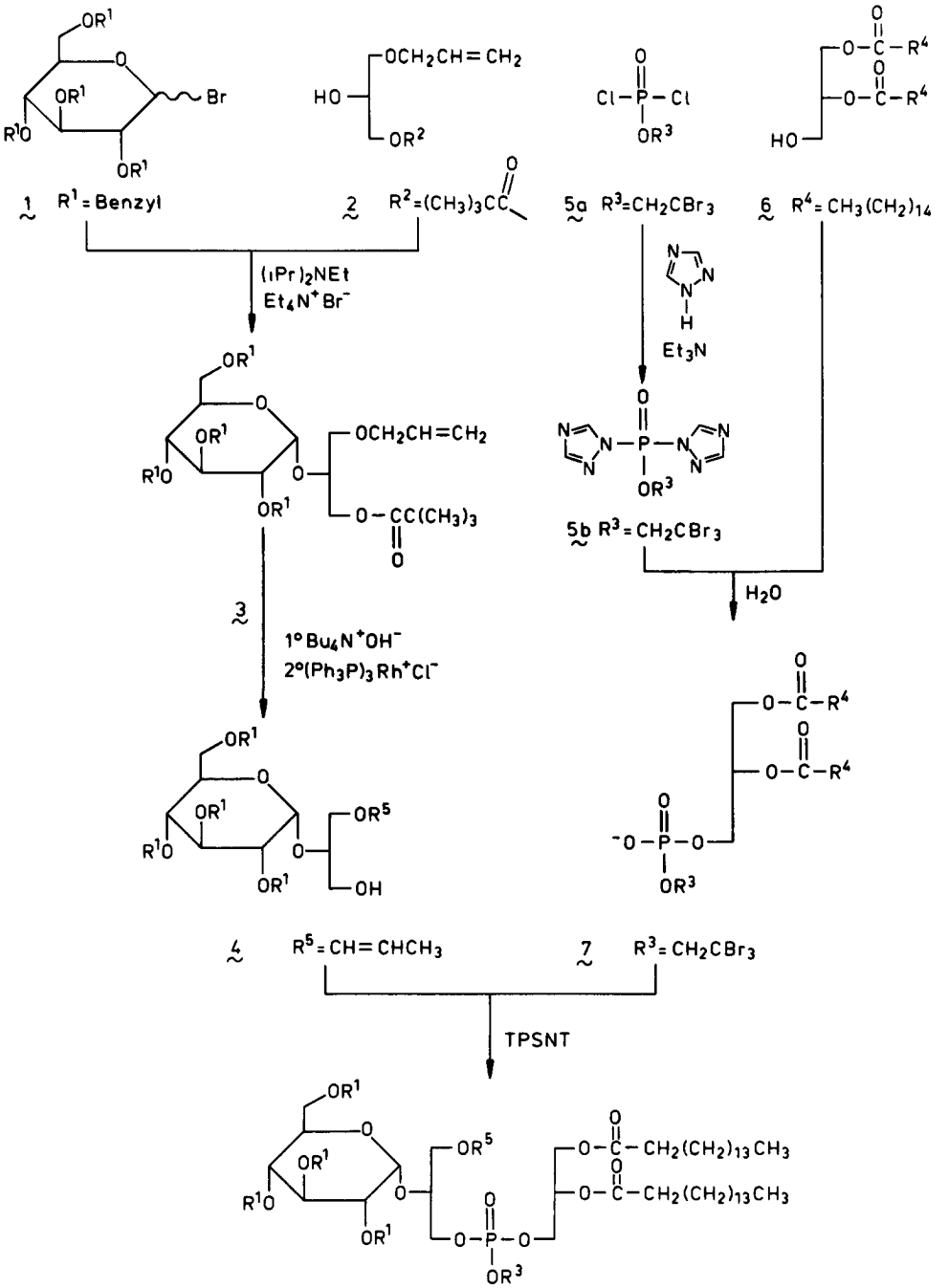
thus obtained could also be distinguished by ^{1}H-NMR spectroscopy. Thus the anomeric proton H1 of the glucose part of the 1-O-allyl derivative of 3 had a δ-value of 5.05 (d, J = 3.1 Hz) and the 3-O-allyl derivative of 5.18 (d, J = 3.1 Hz).

Treatment of a solution of 3 (0.73 mmol) in dioxane-water with tetrabutylammonium hydroxide for 4 h at 20°C gave, after work-up and purification by short column chromatography, 4[7a,b] (R^5= $CH_2CH=CH_2$; 0.64 mmol) as a homogeneous syrup.

The derivative 4 (R^5= $CH=CHCH_3$) necessary for the coupling with the phosphatidic derivative 7 was obtained by treating 4 (R^5= $CH_2CH=CH_2$; 0.5 mmol), in ethanol/water (9:1,v/v) containing 1,4-diazabicyclo[2,2,2]octane (20 mg), with $(Ph_3P)_3RhCl$[8] (45 mg) for 2.5 h at 85°C. Work-up of the reaction mixture followed by short column chromatography afforded 4 (R^5= $CH=CHCH_3$; 0.48 mmol) as an oil. Analysis of this oil by ^{1}H-NMR spectroscopy revealed the presence of 4 (R^5= $CH_2CH_2CH_3$; ca. 10%). The latter impurity which accompanies[9] the isomerization of the allyl group into the prop-1-enyl group could easily be removed in the last step of the synthesis of the crucial intermediate 8b.

The last steps involved in the synthesis of 8d consisted of the introduction of a phosphotriester linkage between the derivative 4 and the phosphatidic derivative 7 followed by a stepwise and selective removal of all protecting groups. The synthesis of 1,2-dipalmitoyl-sn-glycero-3-phospho-2,2,2-tribromoethanol (7) was accomplished by phosphorylation of 1,2-dipalmitoyl-sn-glycerol[10] (6) with the *in situ* prepared 2,2,2-tribromoethyl-phosphoroditriazolide (5b). The latter phosphorylating agent was prepared[11] by reacting together equimolar amounts of 5a[12] in THF with triazole in the presence of triethylamine. A solution of 6 (0.35 mmol) in dry pyridine was added dropwise to crude 5b (0.53 mmol) which was obtained after filtering off triethylamine hydrochloride. After two hours, when TLC analysis ($CHCl_3$/MeOH/H_2O, 80:20:1,v/v) showed the reaction to be complete. The reaction mixture was worked-up and product 7 was isolated by silica gel chromatography to afford, after conversion of compound 7 into the triethylammonium salt by extraction of a solution of the compound in chloroform with triethylammonium bicarbonate (2 M, pH 7.5), 7[7b] (0.31 mmol) as a waxy compound. It is noteworthy to mention that the above described phosphorylation procedure, despite the use of the bifunctional phosphorylating agent 5a, gives no rise to the formation of symmetrical products.

Coupling of 4 (0.4 mmol) with 7 (0.46 mmol) to give 8a was effected in dry pyridine under the influence of the coupling agent 2,4,6-tri-isopropylbenzenesulphonyl-3-nitro-1,2,4-triazolide[13] (TPSNT; 0.48 mmol). After 2 h at 20°C, the crude reaction mixture was worked-up and purified by short column chromatography, to give compound 8a (0.33 mmol) which was contaminated with ca. 10% of 8a (R^5= $CH_2CH_2CH_3$). Treatment of 8a (0.25 mmol) with $HgCl_2$/HgO[14] in acetone during 20 min. at 20°C gave, after work-up and purification by short column chromatography, 8b (0.19 mmol) as a viscous oil (α_D^{25}= +19.7°, c=1, $CHCl_3$) which was now devoid of the impurity introduced during the isomerization of the allyl group into the prop-1-enyl group (i.e., isomerization of 3 into 4). Removal of the 2,2,2-tribromoethyl group from the phosphotriester 8b to give 8c was easily accomplished by treating a solution of 8b in pyridine with zinc dust which was activated by the addition of a few drops of pentane-2,4-dione[15]. After stirring the reaction mixture for 10 min at 40°C, followed by work-up of the reaction mixture, 8c was obtained as a light yellow oil. ($[\alpha]_D^{25}$= +29.7°,


1 R¹ = Benzyl
2 R² = (CH₃)₃CC
5a R³ = CH₂CBr₃
6 R⁴ = CH₃(CH₂)₁₄
(iPr)₂NEt
Et₄N⁺Br⁻
Et₃N
5b R³ = CH₂CBr₃
H₂O
3
1° Bu₄N⁺OH⁻
2° (Ph₃P)₃Rh⁺Cl⁻
4 R⁵ = CH=CHCH₃
7 R³ = CH₂CBr₃
TPSNT
8a R¹ = Benzyl, R³ = CH₂CBr₃, R⁵ = CH=CHCH₃
8b R¹ = Benzyl, R³ = CH₂CBr₃, R⁵ = H
8c R¹ = Benzyl, R³ = H, R⁵ = H
8d R¹ = H, R³ = H, R⁵ = H

c=1, $CHCl_3$). The homogeneity and identity of 8c was established by TLC-analysis, ^{1}H-NMR, ^{13}C-NMR and ^{31}P-NMR spectroscopy.

Conversion of 8c into 8d was accomplished by hydrogenation of 8c (0.095 mmol) in isopropanol/ethylacetate/acetic acid (6:3:1,v/v) over palladium on charcoal for 2 days at 20°C. Work-up of the reaction mixture and fractionation by short column chromatography, followed by conversion of the compound into the triethylammonium salt, gave pure 8d (0.065 mmol) as a waxy solid. The identity and homogeneity of 8d was unambiguously confirmed by ^{1}H-NMR, ^{13}C-NMR and ^{31}P-NMR spectroscopy.

In conclusion, the methodology described in this paper presents an elegant way for the synthesis of naturally occurring glycophospholipids. For instance, derivative 8b is a very convenient intermediate for the synthesis of a recently[16] isolated glucosylated diphosphatidylglycerol from B Streptococci.

We wish to thank Dr. C.M. Lok, Unilever Research laboratory, Vlaardingen, for his stimulating discussions and advice.

FOOTNOTES AND REFERENCES

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(Received in UK 4 June 1979)