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A CONVENIENT PREPARATIVE METHOD OF CARBOHYDRATE PHOSPHATES WITH BUTYLLITHIUM AND PHOSPHOROCHLORIDATE

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α-Glycosyl phosphates and 4-phosphates of N-acylglucosamine derivatives were prepared in high yields via the reaction of corresponding O-lithium salts with dibenzyl or diphenyl phosphorochloridate at low temperature.

Since phosphoric acid esters of carbohydrates are of many biological importances, convenient methods of their preparations are often required. We developed a quite efficient and simple procedure for this purpose by using butyllithium and dibenzyl or diphenyl phosphorochloridate at low temperature.

In the course of our synthetic study on the liposaccharide component of bacterial endotoxin, a convenient synthetic procedure was required for preparation of α-glycosyl phosphates of N-acylglucosamine derivatives.[1] Several new preparative methods were recently described for glucosyl phosphates, for example, those via 1-O-thallium salt[2] and glucosyl trichloroacetimidates.[3] On the contrary, few procedures have been described for 2-acylamino-2-deoxy sugars. Among them, the oxazoline method[4] seemed to be one of the choice. Indeed, α-glycosyl phosphates of N-tetradecanoylglucosamine and its disaccharides could be prepared via oxazoline intermediates,[1] but the procedure was rather tedious and yields of phosphates were not always satisfactorily high. Furthermore, it turned out that the oxazoline method could not be used for preparation of glycosyl phosphate of N-(3-hydroxyacyl)glucosamine derivatives.[5] Since this 3-hydroxyacyl-type structure was a typical feature of natural endotoxin and seemed to be important for endotoxic activities, a new versatile method for it had to be established.

In our new approach to glycosyl phosphate, we attempted an activation of glycosyl oxygen rather than its carbon, because the activation of glycosyl carbon in N-acylglucosamine derivatives by introduction of a good leaving group readily resulted in intramolecular cyclization to form oxazolines which were not always effective precursors for phosphorylation as described above. One successful example of 1-O-activation was phosphorylation of 1-O-thallium salt of glucose.[2] Therefore, this method was first applied to an N-acylgucosamine derivative. 3,4,6-Tri-O-acetyl-2-deoxy-2-tetradecanoylamino-α-D-glucopyranose (1)[6] was converted into its 1-O-thallium salt (addition of TlOEt to a solution of 1 in benzene-THF (1:1) and evaporation under reduced pressure), which was then treated with diphenyl

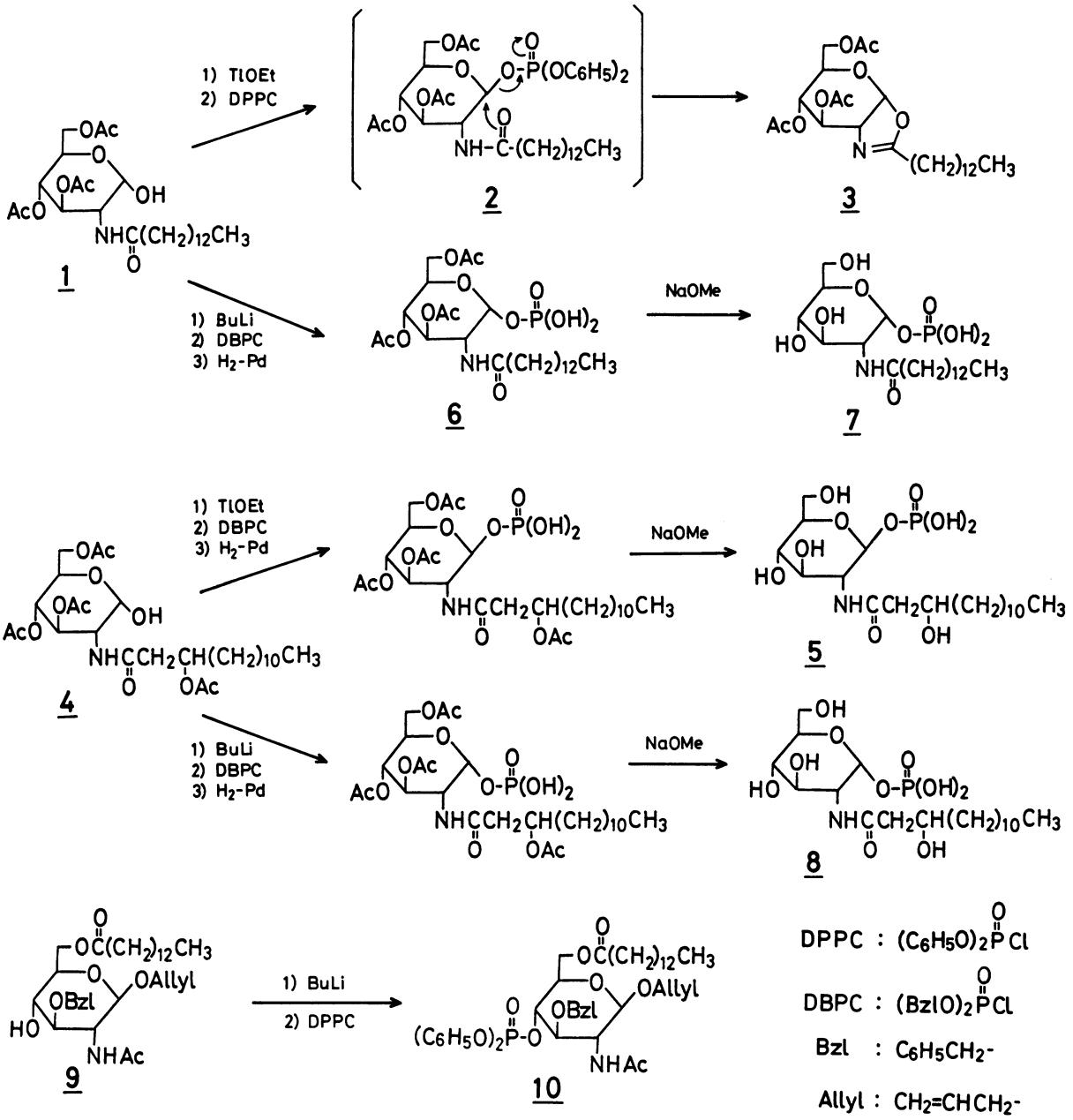
phosphorochloridate (in dry CH_3CN or benzene under ice-cooling). Unexpectedly, the product was not a 1-phosphate but an oxazoline 3, which was identified with an authentic specimen. This result can be explained as follows : the initial product was 1-β-phosphate diphenyl ester (2), from which diphenyl phosphate splitted off spontaneously with participation of the 2-acylamino function to form 3. In accordance with this explanation, application of the same method to a 2-[(R)-3-acetoxytetradecanoylamino]-2-deoxy-α-D-glucopyranose derivative (4)[6] followed by deprotection afforded a β-glycosyl phosphate (5).[7,8] The amide carbonyl group of N-(3-acetoxyacyl) moiety had low reactivity towards the anomeric center and therefore did not participate to form the corresponding oxazoline.

Because the 1-hydroxyl groups in the both starting compounds (1 and 4) possess α-configuration exclusively, almost complete inversion occurred during thallium salt formation and/or phosphorylation steps. As the result, no α-glycosyl phosphate could be obtained. Hence, we next attempted to avoid anomerization by means of metalation of α-glycosyl oxygen at low temperature followed by prompt phosphorylation of the metal salt. This approach could be realized by use of butyllithium as exemplified in 1-α-O-phosphorylation of 1 below.

One equivalent of butyllithium in hexane was added to a solution of 1 in dry THF at -70°C. After 2 min, dibenzyl phosphorochloridate (1.3 equivalent) in THF was added at the same temperature and the mixture was stirred for further 10 min at -60°C. The product was directly hydrogenolyzed by addition of Pd-black to the reaction mixture and stirring under atmosphere of hydrogen.[9] Purification with a column of DEAE-cellulose (5% triethylamine in methanol) afforded 6 as syrup which was identified with an authentic sample prepared by the oxazoline procedure. The anomeric configuration was further confirmed by ^{1}H-NMR after conversion with sodium methoxide (at 0°C for 2 h) to 2-deoxy-2-tetradecanoylamino-α-D-glucopyranose 1-phosphate (7) disodium salt (85% from 1; mp 168-174°C dec; $[\alpha]_D^{13}$ + 73.7° (c 0.50, H_2O); NMR δ 5.45ppm, 1Hq, $J_{1,2}$=3Hz, $J_{1,p}$=7Hz, H-1).

1-α-Phosphates of N-(3-hydroxyacyl)glucosamine derivatives could be also prepared in good yields. Thus, 4 was treated in a similar manner as described above to give disodium salt of 8 (83%; mp 176-181°C dec; $[\alpha]_D^{13}$ +51.5° (c 0.53, H_2O); NMR δ 5.49ppm, 1Hq, $J_{1,2}$=3Hz, $J_{1,p}$=7Hz, H-1).

A stereocontroled synthesis of glucosyl fatty acid esters in the same type of reaction was described by Pfeffer et al.[10] On reaction of 1-O-lithium salt of 2,3,4,6-tetra-O-benzyl-D-glucose with an acyl chloride, predominant formation of 1-α- or 1-β-ester was observed by using THF or benzene as solvent respectively. Since the starting tetrabenzylglucose and its lithium salt were both anomeric mixtures, the selective formation of one of the anomers was interpreted by means of potential energy levels of intermediates. In case of our phosphorylation reaction of glucosamine, it was difficult to evaluate whether anomerization of lithium salt occured in the reaction mixture.[11] A possibility could not be excluded that a similar kinetic control proposed by Pfeffer et al. also operated for the anomeric selectivity. Nevertheless, we rather assume at present time that the configuration of the starting material is in the most part reflected in that of the product. This assumption was supported by a parallel experiment where acetyl chloride was used in place of phosphorochloridate.[12]



As expected from the type of the reaction, this phosphorylation procedure was not restricted to glycosyl positions but could be successfully applied to the other hydroxyl groups. For example, compound 9 was treated in dry THF first with butyllithium (1 equivalent, at -70°C) and then with diphenyl phosphorochloridate (at -50°C for 10 min) as above. The structure of the syrupy product 10 (67%; $[\alpha]_D^{19}$ +13.1° (c 3.28, $CHCl_3$)) isolated by silica gel column chromatography was confirmed by means of 360MHz ^{1}H-NMR spectrum, in which all signals of sugar protons could be assigned. A quartet signal of H-4 at δ 4.70ppm ($J_{3,4}=J_{4,5}=J_{4,P}=9$Hz) clearly indicates that the phosphate moiety is on the hydroxyl group on C-4 as expected.

In conclusion, the present method has an obvious advantage that free hydroxyl groups can be readily phosphorylated by a simple procedure without use of strong

dehydrating agents. High yields can be usually obtained only by careful elimination of moisture from the reaction system. The method is, as described, especially useful for the selective formation of α-glycosyl phosphates of N-acylglucosamine derivatives as well as for phosphorylation of such rather hindered hydroxyl groups as that on C-4 of glucopyranoside. Since the reaction proceeds so quickly (usually within a few minutes at -70°C), use of butyllithium does not cause appreciable cleavage or transposition of ester-type protecting groups.

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References

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4. C. D. Warren, A. Herscovics, and R. W. Jeanloz, Carbohydr. Res., 61, 181 (1978) and references cited therein.
5. On reaction with dibenzyl phosphate, oxazolines derived from N-(3-benzyloxyacyl)- and N-(3-acetoxyacyl)glucosamines did not give corresponding glycosyl phosphates but undergo elimination of benzyl alcohol and acetic acid, respectively, to give α,β-unsaturated oxazolines.
6. Compounds 1 and 4 were obtained from the corresponding β-allyl glycosides (isomerization with $Rh(PPh_3)_3Cl$ followed by cleavage with $HgO\text{-}HgCl_2$). The initial products, which sometimes contained appreciable amounts of β-anomers, were converted to pure α-anomers by dissolving in THF-acetic acid (1:1) and evaporating in vacuo after 24 hr. Anomeric purity was checked by ^{13}C-NMR.
7. After reaction of thallium salt of 4 with dibenzyl phosphorochloridate (in a mixture of benzene-CH_3CN (1:2) at -40°C for 10 min), the product was immediately subjected to hydrogenolysis (Pd-black in benzene-CH_3CN-THF) and then treated with 0.1N sodium methoxide (at 0°C for 3 h). Neutralization with Amberlite IRC-CG50 (H^+ form) afforded disodium salt of 5 (55% from 4; mp 193-194°C dec; $[\alpha]_D^{13}$ -3.13° (c 0.51, H_2O); NMR δ 5.05ppm, 1Ht, $J_{1,2}=J_{1,p}=8$Hz, H-1).
8. The selective formation of β-phosphate by the thallium method may be useful in the future synthesis of structural analogs.
9. Immediate hydrogenolytic removal of the benzyl residue was essential to obtain a high yield of the free 1-phosphate, although dibenzyl esters of α-glycosyl phosphates were more stable than the β-anomers.
10. P. E. Pfeffer, E. S. Rothman, and G. G. Moore, J. Org. Chem., 41, 2925 (1976).
11. Determination of α/β ratio of 1-O-lithium salt by NMR[10] was not possible. Decomposition of the substrate occured during ^{13}C-NMR measurement of the lithium salt even at low temperatures.
12. When 1 was treated with butyllithium and then with acetyl chloride, only α- but no β-acetate was obtained. On the contrary, a mixture of α- and β-acetate was formed in one experiment where a mixture of 1 and its β-anomer was used as starting material. Since the 1-O-acetyl group has no tendency to split off. the 1-β-acetate was detected as it formed.

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