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A convenient route to keto-glycosyl phosphates

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Abstract

The reagent combination, ruthenium dioxide/sodium periodate/benzyltriethyl ammonium chloride in dichloromethane/aqueous bicarbonate buffer simultaneously oxidises alcohol functions in the sugar ring and glycosyl H-phosphonates to yield keto-glycosyl phosphates. These can be coupled to the respective nucleoside diphosphates to render biosynthetically relevant sugar metabolites and derivatives thereof, useful for further investigation of the polysaccharide biosynthesis in bacteria and plants.

Keywords: carbohydrates; oxidation; phosphates; ruthenium.

The pathogenicity of the *O*-antigen of Gram-negative bacteria is largely influenced by the presence of deoxy sugars at the non-reducing terminus.[1] Furthermore, regulation of hydrophilicity of the aglycone in commercially applied drugs, and targetting of the glyco-drug towards the endogenous receptor is attributed to the presence of specifically deoxygenated hexoses.[2–4]

Cloning techniques made the gene clusters available, which account for the biosynthesis of bacterial and plant deoxysaccharides[5] and much insight has been gained on the respective mechanisms.[6,7] However, the ultimate goal of suppressing or modulating the biosynthesis of such a deoxy sugar-containing structure in order to gain control over the antigeneity of the respective organism, will only be met if the biosynthetic intermediates and their analogues are at hand for further investigation.

In addition to the reported enzyme-based procedures,[8] suitable chemical approaches would enrich the product spectrum by artificial analogues of the target metabolites for biochemical research.

However, straightforward routes for the chemical generation of keto-glycosyl phosphates are scarce, if not unavailable and the only method published hitherto has been reported by Schmidt et al.[9] An *exo*-methylene group masked the keto-function which was then generated upon ozonolysis.

The aim of this contribution is to report on a facile method for the oxidation of glycosyl phosphites and H-phosphonates, allowing for simultaneous oxidation of the C-3 or C-4 positions of the hexosyl moiety. Therefore, a series of experiments was set up to investigate: (i) the oxidation of glycosyl H-phosphonates; (ii) the mild oxidation of hydroxy-groups in the hexose and hence, suppressing imminent

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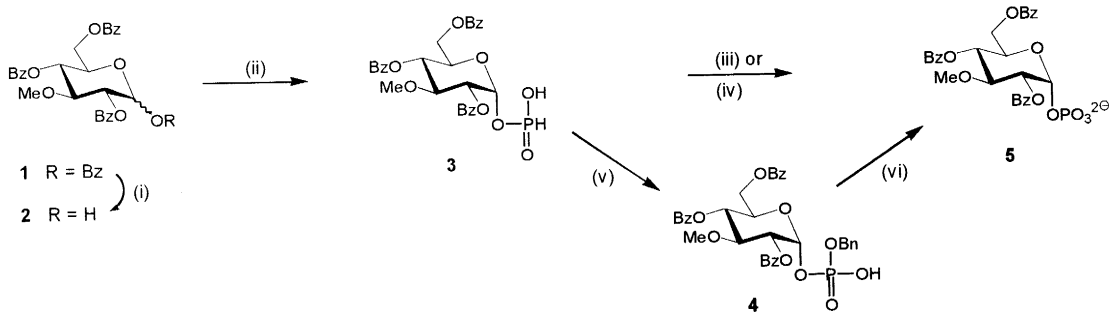
rearrangements and eliminations; and (iii) the simultaneous generation of both the keto-group and the glycosyl phosphate.

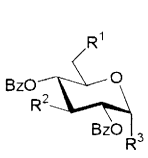
Scheme 1 depicts the experiments conducted on the oxidation of glycosyl phosphites to give the corresponding phosphates. Oxidation of glycosyl phosphates is known to be very sluggish due to the reported stability of the glycosyl H-phosphonates. Consequently, starting material **3** was converted according to the reports by van Boom et al.[10] to the respective glycosyl diester, either by in situ esterification to yield the disilyl phosphite or via the glycosyl monobenzyl-H-phosphonate **4**. Upon oxidation with iodine in pyridine of the former, or deprotection by hydrogenation of the latter, both derivatives were converted in moderate yields to furnish the target phosphate **5**. We were prompted to investigate alternatives to these methods, mainly due to two factors: (i) the facile cleavage under the conditions of oxidation which accounted for the instability of the disilylester and, therefore, low yields; and (ii) the extremely inconvenient two-step oxidation/hydrogenolysis sequence along the monobenzyl phosphonate/phosphate **4** route. Finally, screening led to the ruthenium tetraoxide reagent in a two-phase bicarbonate buffered system.[11] All hexosyl phosphonates **6**, **8** and **10** were oxidised in nearly quantitative yields. Reactions were complete after addition of 2 equivalents of $NaIO_4$ within approximately 1 h. Traces of ruthenium ions were removed by either adding EDTA to the aqueous eluents in SiO_2-chromatography or by filtration through cation exchange resins.



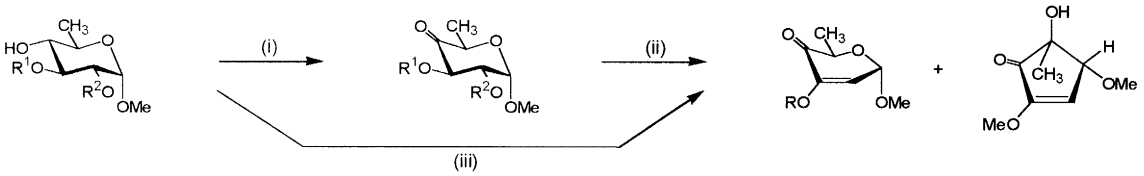
Scheme 1. Reagents and conditions: (i) $N_2H_5^+OH^-$ (71%), anhyd. DMF; (ii) $P(Imi)_3$, anhydr. CH_3CN, then pyridine/H_2O (93%); (iii) HMDSA, then I_2/pyridine (20–46%); (iv) RuO_2, $NaIO_4$, $BnEt_3N^+Cl^-$, $KHCO_3$, CH_2Cl_2, H_2O (87%); (v) BnOH, anhyd. pyr., then I_2, pyr./H_2O; (vi) H_2/Pd–C (75%)

To arrive at the targeted keto-hexosyl phosphates, the efficiency of the RuO_4 method was checked against selectively blocked methyl 6-deoxy-α-D-glucosides **12** and **13** (Scheme 2). Whereas, under Swern-conditions, a subsequent β-elimination was unavoidable and furnished the methyl 4-keto-hex-2-enopyranosides **16** and **17**, the RuO_4-oxidation under the above conditions gave the wanted 4-keto glycosides **14** and **15** in very good yields. Both compounds could be isolated, purified to produce excellent NMR and elementary analyses, and stored over longer periods without decomposition. Upon treatment with either tetrabutylammonium acetate or DBU, the β-elimination to render **16** and **17** was initiated for dibenzoate **14** in a very clean reaction. The dimethyl ether **15** yielded the ring-contracted furan **18** via an intramolecular aldoladdition as the main product, the stereochemistry of which was confirmed by NOE experiments.

Finally, the combined, one-step oxidation was checked for the preparation of dTDP-3-keto- (**25**) and dTDP-4-keto-6-deoxy-α-D-glucose (**26**, Scheme 3). The respective selectively blocked 6-deoxy-glucosyl phosphonates **19** and **21**, which were phosphitylated according to Shibaev et al.[12], were deblocked at O-3 (**20**) or O-4 (**22**) by hydrazinolysis. Application of the described oxidation procedure rendered, after the addition of 3 equivalents of $NaIO_4$, the wanted 6-deoxy-3-keto- and 6-deoxy-4-ketoglucosyl



R^1	R^2	R^3	
OMe	Obz	OP(O)HOH	**6**
OMe	Obz	OPO_3^{2-}	**7**
OBz	H	OP(O)HOH	**8**
OBz	H	OPO_3^{2-}	**9**
H	Obz	OP(O)HOH	**10**
H	Obz	OPO_3^{2-}	**11**

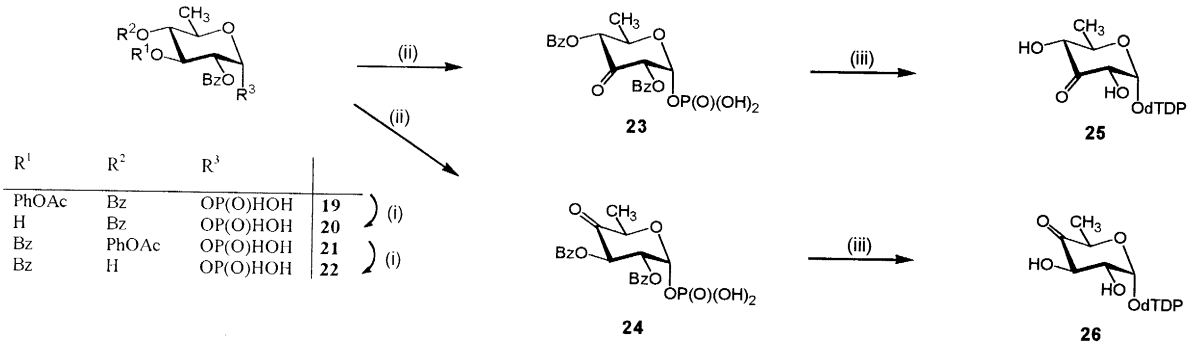


12 $R^1 = R^2 = Bz$ **14** $R^1 = R^2 = Bz$ (83%) **16** R = Bz **18**

13 $R^1 = R^2 = Me$ **15** $R^1 = R^2 = Me$ (61%) **17** R = Me

Scheme 2. Reagents and conditions: (i) RuO_2, $NaIO_4$, $BnEt_3N^+Cl^-$, $KHCO_3$, CH_2Cl_2, H_2O (83% for **14**; 61% for **15**); (ii) TBA^+OAc^-, DBU (89% for **16**; 3% for **17** and 21% for **18**), (iii) DMSO, ClOCCOCl (70% for **17**)

phosphates **23** and **24**, respectively, as the mixed Na^+/K^+-salts.[13] Coupling, according to previously described procedures,[14] gave the wanted biosynthetic intermediates **25** and **26** in good overall yields.



R^1	R^2	R^3	
PhOAc	Bz	OP(O)HOH	**19**
H	Bz	OP(O)HOH	**20**
Bz	PhOAc	OP(O)HOH	**21**
Bz	H	OP(O)HOH	**22**

Scheme 3. Reagents and conditions: (i) $N_2H_5^+OH^-$, anhyd. DMF, rt, 5 h (95% for **20**; 91% for **22**); (ii) RuO_2, $NaIO_4$, $BnEt_3N^+Cl^-$, $KHCO_3$, CH_2Cl_2, H_2O (87% for **23**; 71% for **24**); (iii) dTMP-morpholidate in CH_3CN, 3 d then aqueous LiOH, pH=11, 3 h, then ion exchange chromatography, size exclusion chromatography (47% for **25**; 38% for **26**)

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References

1. Lindberg, B. *Adv. Carbohydr. Chem. Biochem.* **1990**, *48*, 279–318. Raetz, C. R. H. In *Escherichia coli and Salmonella: Cellular and Molecular Biology*, 2nd ed.; Neidhardt, F. C.; Reznikoff, W. S.; Riley, M.; Schaechter, M.; Umbarger, H. E., Eds.; ASM Press: Washington, D.C., 1966; Vol 1, pp. 1035–1063.

2. Nicolaou, K. C.; Ajito, K.; Komatsu, H.; Smith, B. M.; Bertinato, P.; Gomez-Paloma, L. *Chem. Commun.* **1996**, 1495–1496; Nicolaou, K. C.; Ajito, K.; Komatsu, H.; Smith, B. M.; Bertinato, P.; Gomez-Paloma, L., Tor, Y. *J. Am. Chem. Soc.* **1996**, *118*, 2303–2304. Liu, C.; Smith, B. M.; Ajito, K.; Komatsu, H.; Gomez-Paloma, L.; Li, T.; Theodorakis, E. A.; Nicolaou, K. C.; Vogt, P. K. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 940–944.
3. Gao, X.; Patel, D. J. *Biochemistry* **1989**, *28*, 751–762.
4. Banville, D. L.; Keniry, M.; Shafer, R. H. *Biochemistry* **1990**, *29*, 9294–9304.
5. Lindqvist, L.; Schweda, K. H.; Reeves, P. R.; Lindberg, A. A. *Eur. J. Biochem.* **1994**, *225*, 863–872.
6. Liu, H.-W.; Thorson, J. S. *Annu. Rev. Microbiol.* **1994**, *48*, 223–256.
7. For recent mechanistic studies in the synthesis of CDP-activated sugars, see: Hallis, T. M.; Liu, H.-W. *J. Am. Chem. Soc.* **1999**, *121*, 6765–6766, and references cited therein.
8. A preparative bio-synthetic synthesis is presented in: Stein, A.; Kula, M.-R.; Elling, L.; Verseck, S.; Klaffke, W. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1748–1749.
9. Müller, T.; Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1328–1329.
10. Westerduin, P; Veeneman, G. H.; Marugg, J. E.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1986**, *27*, 1211–1214; van Boom, J. H., personal note, 1995.
11. Morris, P. E.; Kiely, D. E. *J. Org. Chem.* **1987**, *52*, 1149–1152.
12. Nikolaev, A. V.; Ivanova, I. A.; Shibaev, V. N. *Carbohydr. Res.* **1993**, *242*, 91–107.
13. Standard procedure for the oxidation of hexosyl H-phosphonates to ketohexosyl phosphates (for the oxidation of H-phosphonates to phosphates, the second addition of $NaIO_4$ is omitted): 0.5 mmol of the partially blocked hexosyl H-phosphonate, $KHCO_3$ (100 mg, 1.0 mmol), $RuO_2{*}\times H_2O$ (2.6 mg, 0.016 mmol, 3 mol%), $BnEt_3N^+Cl^-$ (2.8 mg, 0.010 mmol, 2 mol%) are dissolved in 10 ml CH_2Cl_2 and 10 ml dist. water. Upon addition of $NaIO_4$ (260 mg, 1.2 mmol, 2.4 equiv.) the phosphate is formed within 60 min, after which a further aliquot of $NaIO_4$ (130 mg, 0.6 mmol, 1.2 equiv.) and 50 mg $KHCO_3$ is added. Vigorous stirring is maintained for 20 h, and the reaction is quenched with 2 ml 2-propanol and 50 mg $KHCO_3$ for 10 min. The mixture is directly applied to a cellulose column (20×3 cm, EtOH) and filtered. Co-evaporation of the concentrated appropriate fractions renders the ketohexosyl phosphate, which can be used without further purification. Selected analytical data for **21**: ^{1}H NMR (CD_3OD): δ=8.14/8.06 (2d, 4H, *o*-Bz), 7.14–7.49 (m, 6H, *p*-Bz, *m*-Bz), 6.03 (dd, 1H, H-1), 5.75 (ddd, 1H, H-2), 5.33 (dd, 1H, H-4), 4.58 (dq, 1H, H-5), 1.17 (d, 3H, H-6); $J_{1,2}$=3.9, $J_{1,P}$=6.7, $J_{2,4}$=1.0, $J_{4,5}$=9.8, $J_{5,6}$=6.2; negative FAB-MS (2.3 eV): *m/z* (%)=449.0 (100) [M−Na/K].
14. Liemann, S.; Klaffke, W. *Liebigs Ann. Chem.* **1995**, 1779–1787.