Pergamon

Tetrahedron Letters, Vol. 36, No. 31, pp. 5627-5630, 1995
Elsevier Science Ltd
Printed in Great Britain
0040-4039/95 $9.50+0.00

0040-4039(95)01031-9

Single Step Stereoselective Synthesis of Unprotected 2,4-Dinitrophenyl Glycosides

Sunil K. Sharma, Guillermo Corrales and Soledad Penadés*

Grupo de Carbohidratos, Instituto de Química Orgánica, CSIC, Juan de la Cierva 3, 28006 Madrid (Spain)

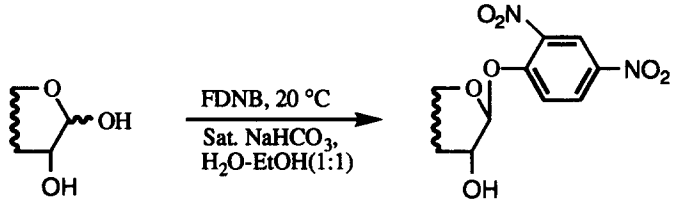
Abstract: Unprotected 2,4-dinitrophenyl glycosides have been synthesised in a single step by the reaction of mono- and disaccharides with 1-fluoro-2,4-dinitrobenzene in the presence of a solution of sodium bicarbonate.

As part of our studies on carbohydrate-carbohydrate interactions in water using model systems,[1] we are interested in a straightforward synthesis of 2,4-dinitrophenyl glycosides (DNP-gly). These compounds have proven to be useful substrates for the study of the mechanism of action of glycosidases and related enzymes.[2] The excellent UV properties of the 2,4-dinitrophenol (DNP) together with its good leaving ability[3] make the study of hydrolysis of any glycosyl-enzyme intermediate kinetically accessible.[4]

Unfortunately, up to now, the introduction of DNP group in sugar moieties has been rather unsatisfactory. The general method to obtain DNP-glycosides is the reaction of *O*-acetyl-α-glycosyl bromide or chloride with DNP in the presence of potassium carbonate to afford fully protected 1,2-*trans* glycoside.[5,6] This method is a slight modification of the method of Hengstenberg and Wallenfels,[7] who used trimethylsilylated bromohexose as glycosyl donor. The yields of DNP glycosides thus obtained varied between 7 and 50%. The yields were slightly improved by Koeners *et al.*,[8] for the gluco- and galacto- derivatives. They used 1-fluoro-2,4-dinitrobenzene (FDNB), in the presence of the tertiary base 1,4-diazabicyclo[2.2.2]-octane (DABCO), with DMF as solvent. But the deprotection of acetyl group is rather difficult as these glycosides are stable towards acids but are cleaved rapidly in basic conditions. In this way the synthesis of DNP-glycosides is still very tedious and overall yields range from 6 to 16% for the unprotected glycosides.

Direct anomeric *O*-arylation of unprotected sugars seems to be rather difficult because not only α- and ß-pyranosides but also α- and ß-furanosides can be expected; additionally, all the other hydroxyl groups are readily accessible to *O*-arylation. Recently, it has been reported that direct *O*-alkyl/aryl glycosylation does not require any protection of other hydroxyl groups.[9] Based on these findings we follow a new route for the introduction of DNP group at the anomeric centre of sugars. We studied the direct anomeric *O*-arylation of unprotected aldoses in a suitable system which is able to dissolve the glycosyl donor as well as partially the arylating agent.

We now report that FDNB can be used to introduce the DNP group directly at the anomeric centre of unprotected sugars in saturated sodium bicarbonate solution of water-ethanol (1:1).



In this procedure, a solution of the sugar (500 mg) and sodium bicarbonate (500 mg) in water (5 ml) was immediately treated with freshly prepared solution of FDNB (2 eq) in ethanol (5-10 ml). The reaction mixture was stirred under dark at 20 °C overnight and then acidified (pH 6.0-6.5) with amberlite IR-120(H). The ethanol was removed under reduced pressure followed by the removal of water by lyophilization. The crude reaction product so obtained was purified by flash chromatography on silica gel and eluted with CH_2Cl_2-MeOH (30:1-3:1). All these glycosides can be crystallized conveniently by acetone/acetone-diethyl ether.

By this method the glycosides of monosaccharides as well as disaccharides can be obtained directly in a single step. Table 1 shows the yields and physical data of the DNP glycosides. Attempts to improve the yield either by increasing the reaction time or by using other solvent systems (water-DMF, water-acetone, water-acetonitrile and water-pyridine) were unsuccessful. The introduction of the DNP ether function at the anomeric centre is highly stereoselective and leads to the formation of chiefly 1,2-*trans* glycosides as it could be ascertained by ^1H NMR spectroscopy.[10] This stereoselectivity of the reaction cannot be attributed to the anchimeric assistance from the neighbouring group. It seems, therefore, that the formation of 1,2-*trans* glycosides is under kinetic control. This method represents an improvement of the methods described in the literature as protection and deprotection steps are not necessary. Besides, the yields of the crystallized products obtained in this work are higher than those described using other methods.[6,7] Furthermore, the high stereoselectivity, the broad scope of the reaction and the easy isolation of the products, make it an efficient method to obtain these valuable compounds for the study of the mechanism of action of ß-glycosidases and related enzymes.[11-13]

Table 1. Physical Data of the 2,4-Dinitrophenyl Glycopyranosides

Sugar residue	Yield (%)	mp (°C)	Lit. mp (°C)	$[\alpha]^{25}_D$ (c, solv.)	Lit.$[\alpha]^{25}_D$ value (c, solv.)
ß-D-Glucose (**1**)	20	100-02	98-100[6] 100-01[7]	-110(0.24, MeOH)	-102(1,MeOH)[6] -93(1.1, MeOH)[7]
ß-D-Galactose (**2**)	15	147-49	161-63[6] 150-51[7]	-106(0.2, MeOH)	-97(1, MeOH)[6] -105(1, MeOH)[7]
α-D-Mannose (**3**)	25	116-18	149[7]	+169(0.24, MeOH)	+161(1, MeOH)[7]
α-D-Arabinose (**4**)	15	163-65	-	+85(0.2, MeOH)	-
α-L-Arabinose (**5**)	15	162-63	158-60[6] 167[7]	-82(0.2, MeOH)	-94(1, Me_2NCHO)[6] -103(1, Me_2NCHO)[7]
ß-D-Xylose (**6**)	20	146-48	148-50[6] 158-59[7]	-134(0.24, MeOH)	-121(1, MeOH)[6] -105(1.1, MeOH)[7]
ß-D-Fucose (**7**)	15	129-30	135-36[6]	-52(0.25, MeOH)	-87(1, MeOH)[6]
ß-L-Fucose (**8**)	15	112-14	-	+50(0.25, MeOH)	-
ß-D-Maltose (**9**)	28	125-28	-	-29(0.32, MeOH)	-
ß-D-Lactose (**10**)	30	179-80	-	-109(0.33, MeOH)	-

Acknowledgement

We thank Dr. A. Fernández-Mayoralas for helpful discussions and Prof. M. Martin-Lomas for his continuous support. This work was supported by DGCYT (Dirección General de Ciencia y Tecnologia). One of us (SKS) thanks the Spanish Ministerio de Educación y Ciencia for a postdoctoral fellowship.

References and Notes

1. Coterón, J. M.; Vicent, C.; Bosso, C.; Penadés, S. *J. Am. Chem. Soc.* **1993**, 115, 10066-10076.
2. Withers, S. G.; Rupitz, K.; Trimbur, D.; Warren, R. A. J. *Biochemistry* **1992**, 31, 9979-9985; Wang, Q.; Tull, D.; Meinke, A.; Gilkes, N. R.; Warren, R. A. J.; Aebersold, R.; Withers, S. G. *J. Biol. Chem.* **1993**, 268, 14096-14103; Miao, S.; Ziser, L.; Aebersold, R.; Withers, S. G. *Biochemistry* **1994**, 33, 7027-7032; Gebler, J. C.; Aebersold, R.; Withers,

S.G. *J. Biol. Chem.* **1992**, 267, 11126-11130; Withers, S. G.; Warren, R. A. J.; Street, I. P.; Rupitz, K.; Kempton, J. B.; Aebersold, R. *J. Am. Chem. Soc.* **1990**, 112, 5887- 5889; MacLeod, A. M.; Lindhorst, T.; Withers, S. G.; Warren, R. A. J. *Biochemistry* **1994**, 33, 6371-6376; Kempton, J. B.; Withers, S. G. *Biochemistry* **1992**, 31, 9961-9969; Tull, D.; Withers, S. G. *Biochemistry* **1994**, 33, 6363-6370; Withers, S. G.; Street, I. P.; Bird, P.; Dolphin, D. H. *J. Am. Chem. Soc.* **1987**, 109, 7530-7531.

3. Page, I. D.; Pritt, J. R.; Whiting, M. C. *J. Chem. Soc., Perkin Trans. 2* **1972**, 906-911.
4. Wang, Q.; Graham, R. W.; Trimbur, D.; Warren, R. A. J.; Withers, S. G. *J. Am. Chem. Soc.* **1994**, 116, 11594-11595; Sinnott, M. L.; Viratelle, O. M. *Biochem. J.* **1973**, 133, 81-87; Sinnott, M. L.; Souchard, I. J. L. *ibid* **1973**, 133, 89-98.
5 Latham, H. G.; May, E. L.; Mosettig, E. *J. Org. Chem.* **1950**, 15, 884-889.
6. Ballardie, F.; Capon, B.; Sutherland, J. D. G.; Cocker, D.; Sinnott, M. L. *J. Chem. Soc., Perkin Trans. 1*, **1973**, 2418-2419.
7. Hengstenberg, W.; Wallenfels, K. *Carbohydr. Res.* **1969**, 11, 85-91.
8. Koeners, H. J.; de Kok, A. J.; Romers, C.; van Boom, J. H. *Recl. Trav. Chim. Pays-Bas* **1980**, 99, 355-362.
9. Schmidt, R. R.; Klotz, W. *Synlett.* **1991**, 168-170; Klotz, W.; Schmidt, R. R. *Liebigs Ann. Chem.* **1993**, 683-690; El Arabi Aouad, M.; El Meslouti, A.; Uzan, R.; Beaupere, D. *Tetrahedron Lett.* **1994**, 35, 6279-6282.
10. Due to their thermal lability, all the glycosides were dried at room temperature, particularly the arabino- and fuco- derivatives, which are highly unstable as they decompose even in an aqueous solution. All the glycosides are characterized by elemental analysis and NMR spectroscopy. ^{1}H NMR (200 MHz) δ (ppm) : **1** (D_2O) : 5.31 (d, H_1, $J_{1,2}$ = 7.46 Hz); **2** (CD_3OD) : 5.10 (d, H_1, $J_{1,2}$ = 7.42 Hz); **3** (acetone-d_6) : 5.92 (d, H_1, $J_{1,2}$ = 1.76 Hz); **4** (CD_3OD) : 5.20 (d, H_1, $J_{1,2}$ = 6.34 Hz); **5** (CD_3OD) : 5.41 (d, H_1, $J_{1,2}$ = 6.36 Hz); **6** (CD_3OD) : 5.14 (d, H_1, $J_{1,2}$ = 6.78 Hz); **7** (CD_3OD) : 5.32 (d, H_1, $J_{1,2}$ = 7.60 Hz); **8** (CD_3OD) : 5.11 (d, H_1, $J_{1,2}$ = 7.60 Hz); **9** (D_2O) : 5.24 (d, H_1, $J_{1,2}$ = 7.50 Hz), 5.27 (d, $H_{1'}$, $J_{1',2'}$ = 3.50 Hz); **10** (CD_3OD) : 4.42 (d, $H_{1'}$, $J_{1',2'}$ = 6.69 Hz), 5.35 (d, H_1, $J_{1,2}$ = 7.01 Hz).
11. Legler, G. *Adv. Carbohydr. Chem. Biochem.* **1990**, 48, 319-384.
12. Sinnott, M. L. *Chem. Rev.* **1990**, 90, 1171-1202.
13. McCarter, J. D.; Withers, S. G. *Curr. Opin. Struct. Biol.* **1994**, 4, 885-892.

(*Received in UK* 5 *May* 1995; *accepted* 9 *June* 1995)