UNIVERSAL STANDARD REAGENTS. THE USE OF 3-(2,4-DINITRO-ANILINO)PROPANOL (DNAP) AND 1-(2,4-DINITROPHENYL)-4-HYDROXYPIPERIDINE (DNPP) IN GLYCOSYLATION AND SELECTIVE HYDROLYSIS OF ESTER GLUCOSIDES[1]

Abraham Patchornik,[*] Alfred Hassner,[*] Maria Kramer, Hugo E. Gottlieb, and Miriam Cojocaru

Department of Chemistry, Bar-Ilan University, Ramat Gan 52900, Israel and the Weizmann Institute of Science, Rehovoth, Israel

Abstract We demonstrate the utility of the new principle of Universal Standard Reagents (USR) in glycosylation of protected sugars and selective hydrolysis of ester glycosides by means of 3-(2,4-dinitroanilinopropanol) (DNAP) and 1-(2,4-dinitrophenyl)-4-hydroxypiperidine (DNPP). Quantitative determination of each individual sugar derivative was carried out using extinction coefficients at λ_{max} (~350 nm) on the micromole scale, eliminating the need for specific standards. The selective hydrolysis of β-DNAP-tetra-*O*-acetyl- and *O*- aroyl-glycosides was examined by different O- and N-bases using quantitative assessment by TLC and HPLC. DCI/NH_3 MS of anomers and NMR-spectra of partially acylated glucosides are discussed.

INTRODUCTION

Visualization of molecules by means of a colored derivative can greatly aid in the detection and separation of closely related compounds. Usually the various modified compounds carrying the chromophore do not have the same molar extinction coefficients (ε). For example, dinitrophenylamino acids have been used extensively but different amino acids often give slightly different UV absorptions, so that quantitative comparison without standards becomes difficult.

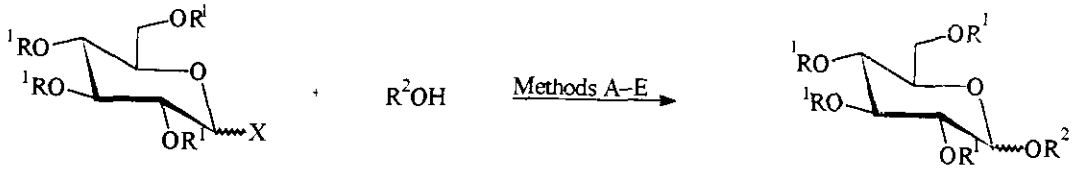
Universal standards which contain a chromophore and may give quantitative information about related molecules have been suggested as an analytical tool for determination of functional groups.[2] Thus, attachment of a chromophore *via* a tether to two or more related molecules should provide derivatives that have the same ε and give visually detectable spots by TLC, HPLC or column chromatography. This is possible if the tether does not transmit electronic interactions between the chromophore and the rest of the molecule. The ratio of these molecules can be assayed quantitatively by UV measurements of extinction coefficients without the benefit of added standards, since the extinction coefficient per mole should be essentially independent of the non-chromophore portion of the molecule.

RESULTS AND DISCUSSION

We decided to demonstrate the utility of the new principle of Universal Standard Reagents (USR) in sugar chemistry by attachment of the 2,4-dinitroanilinopropyl (DNAP) and the 2,4-dinitrophenylpiperidyl (DNPP) chain to *O*-1 of 2,3,4,6-tetra-*O*-protected D-glucopyranose and to study quantitatively the β/α anomer ratio and selective hydrolysis of such ester glycopyranosides. We also report here conditions for TLC as well as HPLC separation of α- and β-anomers of glycosides (**3-8**) and for quantitative assessment of the products of selective deesterification of β-DNAP-tetra-*O*-acetyl-, tetra-*O*-benzoyl- and tetra-*O*-veratroyl-D-glucopyranosides (**3, 7** and **8**). An advantage of the DNAP and DNPP over benzyl or related groups is the easy visual detection of colored derivatives.

Detection of Anomers.

The substrates (**3-8**) were readily prepared by glycosylation of the corresponding 2,3,4,6-tetra-*O*-protected D-glucose derivatives (**1a-1e**) with two models of aglycones, the primary alcohol 3-(2,4-dinitroanilino)propanol (DNAP) (**2a**) and the secondary alcohol 1-(2,4-dinitrophenyl)-4-hydroxypiperidine (DNPP) (**2b**), under a variety of conditions (Scheme 1).



1a. R^1 = Ac ; X = Br
1b. R^1 = Bn ; X = OH
1c. R^1 = Bn ; X = Cl
1d. R^1 = Bz ; X = Br
1e. R^1 = $COCH_2C_6H_3(OMe)_2$; X = Br

2a. R^2 = $-(CH_2)_3NH-C_6H_3(NO_2)_2$ (DNAP)

2b. R^2 = $-N(CH_2)_4CH-C_6H_3(NO_2)_2$ (DNPP)

3. R^1 = Ac ; R^2 = DNAP
4. R^1 = Ac ; R^2 = DNPP
5. R^1 = Bn ; R^2 = DNAP
6. R^1 = Bn ; R^2 = DNPP
7. R^1 = Bz ; R^2 = DNAP
8. R^1 = Ver ; R^2 = DNAP

Scheme 1

The total yields of glycosides and ratios of α- and β-anomers resulting from various methods are shown in Table 1.

Coupling of tetra-*O*-acetyl-α-D-glucopyranosylbromide (**1a**) with DNAP (**2a**) or with DNPP (**2b**) and also of tetra-*O*-benzoyl- and tetra-*O*-veratroylglycosyl bromides (**1d** and **1e**) with **2a** by the most

commonly employed Koenigs-Knorr method[3] (A) provided β-glycosides (**3,4,7** and **8**) preferentially (85-98%).

Table 1: Yields and ratio of α- to β- anomers of glycosides (3-8).

Entry	Method[a]	Glycosyl donor	Aglycone	Product	Yield (%)	Ratio of Anomers (%)		Retention time RT(min)	
						α	β	α	β
1	A	1a	2a	3	68.5	0.5	99.5		8.22
2	B	1a	2a	3	78.4	23	75	7.04	8.22
3	A	1a	2b	4	56.5	2	98	7.96	8.72
4	B	1a	2b	4	60.8	51	42		
5	C	1c	2a	5	68.5	53	47	27.21	28.41
6	D	1b	2a	5	80.0	23	69		
7	E	1b	2a	5	56.8	33	13		
8	D	1b	2b	6	75.0	26	60		
9	A	1d	2a	7	78.5	15	84	14.60	13.63
10	A	1e	2a	8	58.0	3	97	9.80	9.22

[a]See experimental

The glycosylation reaction under Helferich conditions[4] (B) [$Hg(CN)_2$, polar media] proceeded with low stereospecificity and afforded *ca.* 1:3 ratio of glycosides **(3α):(3β)** and a 1:1 ratio of anomers **(4α):(4β)**. α/β Selectivity remained the same, when the glycosylation catalyst $AgClO_4$ in MeCN (method C)[5] was used in the coupling 2,3,4,6-tetrabenzyl-D-glycosyl chloride (**1e**) with **2a** at low temperature; *ca.* 1:1 ratio of glycosides **(5α):(5β)** was formed. Use of 2,3,4,6-tetrabenzyl-D-glucose (**1b**) activated with trifluoromethanesulfonate (Me_3SiOTf) (method D)[6] gave 1:3 ratio of anomers **(5α):(5β)**.

Preferential α-glycosylation, *ca.* 3:1 ratio of **5α** to **5β** was achieved by the reaction sequence consisting of esterifying **1b** with triflic anhydride followed by coupling of the resulting triflate glycoside *in situ* with DNAP (method E).[7]

The advantage of the presence of the USR was immediately obvious in the ready detection of resulting yellow colored α- and β-glycosides. It was possible not only to separate anomers on a silica column by following colored bands, but also to assess the relative amounts by comparison of the UV maxima. This is not possible for benzyl derivatives of sugars. That various α- and β-DNAP-glycosides have nearly identical λ_{max} and extinction coefficients within *ca.* ±1% was ascertained (see Table 2). The values obtained after column chromatography fit well with those analyzed by HPLC.

Accurate HPLC analysis is sometimes difficult because of low absorbance of some sugars or due to background noise of trace impurities.[8] Therefore, the preparation of sugar derivatives containing chromophoric groups is useful for HPLC analysis of carbohydrates, by improving detection

Table 2: UV spectral data of α- and β-glycosides.

Product	M.W.	Molar Concentration C	Absorbance OD	λ_{max}EtOH (nm)	Molar absorbance ε
2a	241	$4.5 \cdot 10^{-5}$	0.734	348	16310
3o	571	$3.42 \cdot 10^{-5}$	0.5603	349	16300
3β	571	$9.28 \cdot 10^{-5}$	1.500	350	16160
5o	763	$4.58 \cdot 10^{-5}$	0.737	349	16100
5β	763	$5.28 \cdot 10^{-5}$	0.858	350	16250
2b	267	$1.18 \cdot 10^{-5}$	1.921	360	16270
4β	597	$6.42 \cdot 10^{-5}$	1.0477	360	16319

The sensitivity of our method, due to the high value of molar absorbance ε and long wavelength of absorption of the chromophore, allows one to carry out chemical reactions even on a 1 mg scale. On TLC one can detect selectively a few nanomoles of products, while by HPLC even 0.01-1.0 nanomole could be determined. For HPLC separation of the chemically very similar anomers a complex isocratic and gradient programming of the mobile phase was used successfully.

Mass Spectra.

The early studies of carbohydrates using low-resolution electron-impact MS were not encouraging. The spectra exhibit low intensity molecular ions and very intense and complicated fragment ions. Desorption chemical ionization (DCI) was found to be the most suitable technique. In contrast to conventional solid probe electron impact or chemical ionization, the spectra obtained by direct sample insertion into the reagent gas plasma contains ions indicative of the molecular weight as well as structurally significant fragment ions. This method permits one to obtain the quasimolecular ion not only of the 2,3,4,6-tetra-derivatized sugars but also of the partially hydrolyzed products.

The DCI/NH_3 MS of the β-DNAP-2,3,4,6-tetra-*O*-acetyl-D-glucopyranoside is different from that of the α-anomer. In the β-anomer the base peaks is the ammonia cluster of the molecule MNH_4^+ (589, 100%), but in the α-anomer this ion is a very small one (<1%). The pseudo molecular ion MH^+=572 is a strong one in the β-anomer (34%) and not found in the α. Elimination of an AcOH group yields the m/z = 512 (8% in the β, not found in α). Loss of the DNAP-OH gives the common m/z = 331 (8% in β and 100% in α) of the well-known oxonium ion.

The energetically less favorable axial position[9] of the C-1 DNAP-O group in the α-anomer would be ex-

pected to promote its elimination from MH^+ and as a consequence, the ion at m/z = 331 is a very intense one. The same MS-behavior was found for the 2,3,4,6-tetra-*O*-acetyl-D-glucopyranoside α- and β-anomers where the chromophore bonded at C-1 was 2,4-dinitrophenyl-4-hydroxypiperidine. The single difference between the spectrum of the α- and β-anomers is the intensity of the m/z = 331 ion ($\alpha/\beta = 100/6 = 16.6$.).

The DCI/NH_3 MS of the β-DNAP-2,3,4,6-tetra-*O*-benzyl-D-glucopyranoside (**5**β) is similar to that of the α-anomer.

Table 3 shows the representative peaks which appear in the spectra of the β-*DNAP* and α-*DNAP* anomers of the tetra-*O*-benzyl-D-glucopyranoside (**5**).

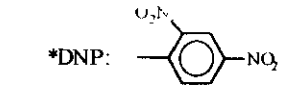
Selective Deacetylation.

The selective base catalyzed deacetylation of β-DNAP-tetra-*O*-acetylglucoside (**3**) was studied to obtain easily accessible information about the relative ease of cleavage of ester bonds in carbohydrate molecules.

Table 3: DCI/NH_3 MS of the β- and α-anomers of DNAP-tetra-*O*-benzylglycoside (5).

IONS/ %RIC	781 MNH_4^+	764 MH^+	734 MNH_4^+-HNO_2	614 MNH_4^+-DNP*	572 MNH_4^+-DNAE**	558 MNH_4^+-DNAP***
β	95	22	100	14	49	31
α	100	22	63	.3	0.17	.8

%RIC - relative abundance of the ions



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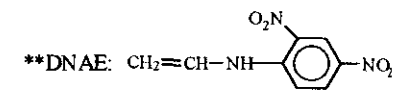

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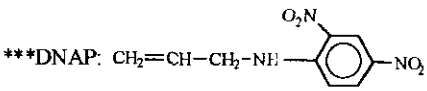

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Here, the presence of the visible DNAP chromophore permitted easy detection, which would have been much more difficult with, for instance, methyl tetra-*O*-acetylglycoside.

A comparative study of the deacetylation of tetraacetate (**3**β) by different *O*- and *N*-bases such as NaOMe, $Ba(OMe)_2$, NaOn-Bu, 25% aq. NH_4OH, n-$PrNH_2$, piperidine, TEA was carried out in methanolic solution at room temperature. Progress of hydrolysis was monitored by TLC or by HAPLY and the results are illustrated in Table 4. In all cases a mixture of products was formed including starting

tetraacetate (3β), completely deacetylated glucoside (**12**), monoacetate (**11**) and a very small amount of diacetate (**10**). The yields of these products varied depending on the reaction time and the hydrolyzing agent.

When we used sodium or barium methoxide, propylamine or piperidine in MeOH, the reaction was completed during 1 h and free sugar (**12**) was formed as the major product. With 25% aq. ammonia in MeOH the reaction was slower (*ca.* 15 h) and with TEA in MeOH the reaction was slowest (*ca.* 24 h).

A different behavior was observed in the deacetylation of tetraacetate (3β) in excess n-propylamine in the absence of MeOH. In addition to **11** and **12**, triacetate and diacetate glucosides (**9** and **10**) were obtained. Thus, after 15 min reaction in propylamine five components were detected: starting tetraacetate (3β) (45%), triacetate (**9**) (7%), diacetate (**10**) (25%), monoacetate (**11**) (20%) and a very small amount of free sugar (**12**) (1%). After 1 h a large amount of monoacetate (**11**) (62.5%) and free sugar (**12**) (26%) had formed. Similar results were obtained with n-butylamine, piperidine, morpholine and dimethylamine but the reaction was slower than with n-propylamine. In isopropylamine deacetylation was much slower (*ca.* 48 h) and *t*-butylamine was ineffective. As expected, this indicates strong steric

Table 4: HPLC analysis (%) of selective deacetylation of β-DNAP-tetraacetate -glycoside (3).

Reagent/ Solvent	Reaction time (min)	Tetraacetate (3) RT =6.88 min	Triacetate (9) RT=4.45 min	Diacetate (10) RT=3.09 min	Monoacetate (11) RT=2.43 min	Free sugar (12) RT=2.12 min
MeONa/MeOH	30	32.8	-	1.6	3.7	61.5
sec – BuONa/sec-BuOH	30	58.7	-	11.4	7.0	21.3
$C_5H_{10}NH$/MeOH	30	38.1	<1	3.9	26.2	31.7
25% aq. NH_4OH/MeOH	30	58.7	3.0	2.7	15.1	18.5
n-$PrNH_2$/MeOH	30	28.5	-	<1	20.5	51.0
n-$PrNH_2$ excess	30	23.2	3.6	21.6	43.1	8.1
Et_3N/MeOH	30	97.0	-	2.5	<1	<1
MeONa/MeOH	60	0.8	-	<1	<1	99.0
sec-BuONa/sec-BuOH	60	46.2	-	14.6	3.0	36.1
$C_5H_{10}NH$/MeOH	60	2.4	-	<1	7.6	89.9
25% aq. NH_4OH/MeOH	60	50.7	2.41	2.0	15.9	28.2
n-$PrNH_2$/MeOH	60	3.5	-	<1	13.2	82.9
n-$PrNH_2$ excess	60	6.7	<1	4.1	62.5	26.8
Et_3N/MeOH	60	78.5	-	2.5	10.4	8.6

(RT = retention time).

effects during deacetylation. For n-propylamine different solvents show a shift in the reaction rate as follows: $CHCl_3$<n-$PrNH_2$<MeOH.

In a similar manner, selective hydrolysis of the tetra-*O*-benzoate (**7**) or the tetra-*O*-veratroate (**8**) β-DNAP D-glucosides in n-propylamine produced as much as 66% of the respective 6-monoaroylesters (**13**, **14**) (see Table 5).

The products (**9-12**) were separated either by column chromatography or by HPLC. By lowering the polarity of the mobile phase in a linear gradient mode, the solute was separated according to the shift of polarity of its components from high to lower values (Figure 1).

The structure of the hydrolysis products was determined by MS, ^{1}H-NMR and COSY experiments. Only one monoacetate, namely β-DNAP 6-*O*-acetyl-D-glucopyranoside (**11**) was found. The diacetate (**10**),

Table 5: HPLC analysis (%) of selective hydrolysis of β-DNAP-tetra-*O*-aroylglycosides (7,8).

Compound	Hydrolysing Agent	Reaction time (h)	Tetraaroyl	Triaroyl	Diaroyl	Monoaroyl	Free Sugar
7	n-$PrNH_2$ (excess)	1	52.2	3.0	18.7	21.1	2.2
		4	35.7	<1	24.2	25.4	5.9
		24	-	-	-	66.1	33.9
8	n-$PrNH_2$	1	99.5	<1	-	-	-
		12	73.1	-	20.4	6.4	-
		24	27.8	-	28.2	37.1	6.9
		48	3.0	-	5.5	65.8	25.6
7	MeONa/MeOH	0.5	-	-	-	14.2	84.7
		1	-	-	-	>1	98.7
8	MeONa/MeOH	0.5	42.1	-	6.5	27.0	24.4
		1.5	21.9	-	5.3	20.8	52.0
		3.4	<1	-	-	2.5	95.5

which showed only one peak on HPLC, was indicated by NMR to be an inseparable mixture of 2,6-(65%), 4,6-(24%), and 3,6-(11%) β-DNAP-diacetyl-D-glucosides and the triacetate (also one HPLC peak) was a mixture of 2,3,6-(55%), 3,4,6-(30%), and 2,4,6-(15%) DNAP-triacetyl-D-glucosides.

The chemical shifts of the side chain (spacer group and aryl protons) show a clear bimodal distribution depending only on the presence or absence of a 2-acetate function. The main effect is felt on the NH, which has a δ of 8.68-8.72 ppm when O-2 is acetylated and 8.87-8.95 when it isn't. This is apparently due to H-bonding to the NH group. The chemical shifts of the sugar portion are very consistent and permit construction of Table 6 showing the effect of acetylation at various positions. A detailed analysis of the shifts in Table 6 gives consistent deshielding effects on the sugar ring methines on acetylation: 1.43 ± 0.06 ppm for the hydrogen on the acylated position and 0.13 ± 0.03, 0.06 ● 0.02 and 0.02 ± 0.01 ppm for the β-, γ- and δ-hydrogens, respectively. The formation of 6-monoacetate (**11**), a primary alcohol acetate, in 62% yield from deacetylation of tetraacetate (**3β**) raises the question of whether this monoacetate is the

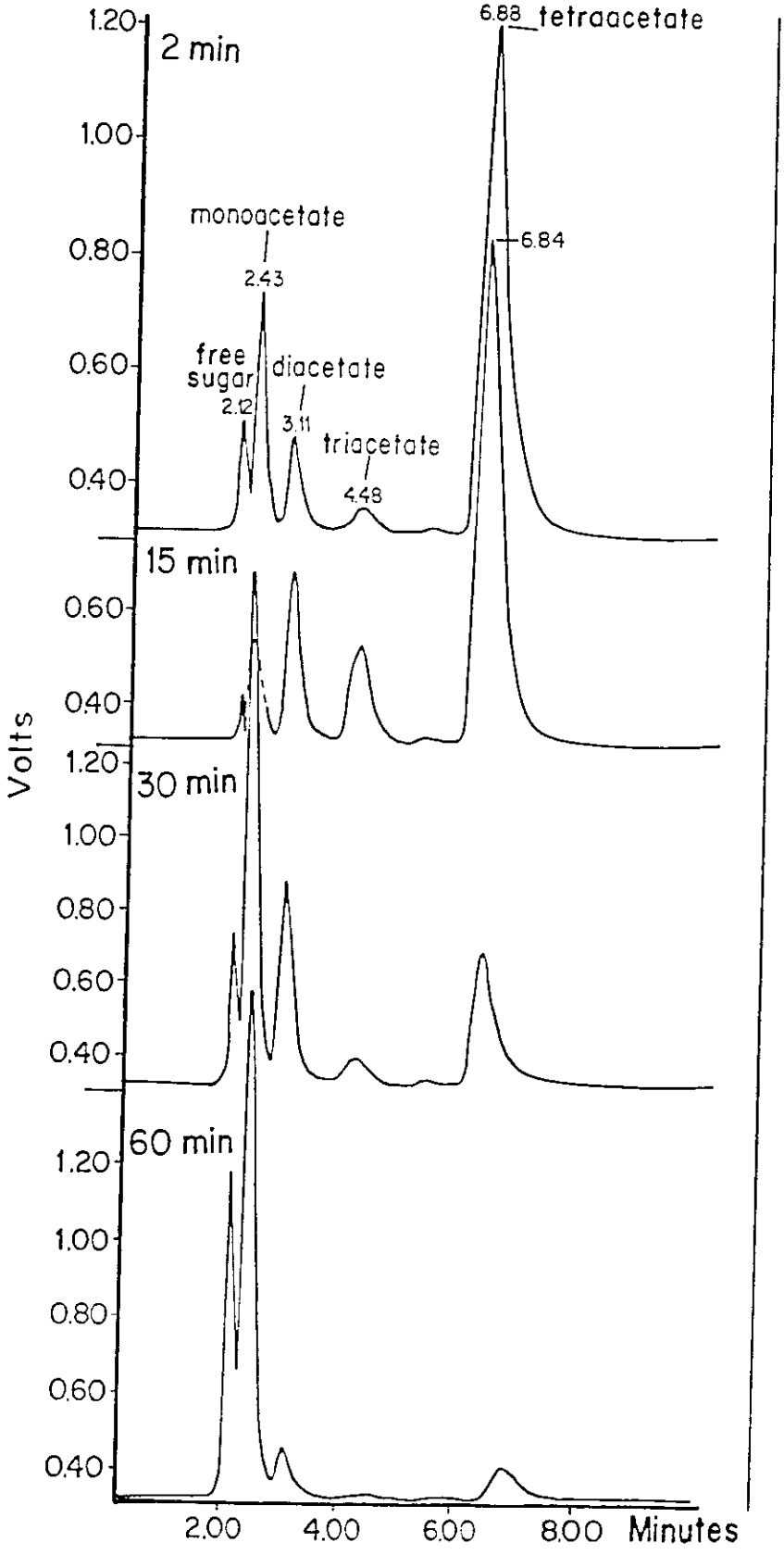

Figure 1. HPLC chromatogram of selective hydrolysis of β-DNAP-2,3,4,6-tetra-*O*-acetyl-D-glucopyranosides (3) by propylamine

Table 6. ^{1}H NMR chemical shifts of tetraacetate 3β and products of selective deacetylation.

		Monoacetate (11)	Diacetates (10)		Triacetates (9)			Tetracetates (3)	
H	**Free sugar 12[a]**	**6-Ac**	**2,6-Ac$_2$**	**4,6-Ac$_2$**	**2,3,6-Ac$_3$**	**3,4,6-Ac$_3$**	**2,4,6-Ac$_3$**	**2,3,4,6-Ac$_4$**	**Multiplicity[b]**
1	4.34	4.34	4.47	4.35	4.52	4.41	4.49	4.56	d, 8
2	3.36	3.50	4.86	3.58	4.98	3.69	4.92	5.05	dd, 9.5, 8
3	*ca.*3.45	3.58	3.64	3.68	5.06	5.12	3.75	5.23	t, 9.5
4	*ca.*3.45	3.44	3.48	4.92	3.61	5.04	4.95	5.10	t, 9.5
5	3.22	3.48	3.48	3.64	3.53	3.70	3.65	3.73	ddd, 9.5, 4.5, 2.5
6	3.78	4.49	4.46	4.26	4.48	4.26	4.26	4.26	dd, 12.5, 4.5
6a	3.86	4.29	4.32	4.13	4.33	4.13	4.18	4.16	dd, 12.5, 2.5
OCH_2	3.74	3.78	3.70	3.80	3.70	3.80	3.70	3.70	ddd, 11, 6.5, 4.5
OCH_2	4.09	4.13	3.99	4.12	3.99	4.14	4.01	4.00	ddd, 11, 6.5, 4.5
CH_2	2.08	2.12	2.04	2.12	2.01	2.13	2.04	2.03	m
CH_2	3.62	3.59	3.55	3.60	3.53	3.58	3.53	3.54	td, 7, 5.5
NH	8.87	8.95	8.72	8.92	8.69	8.91	8.69	8.68	br t, 5.5
3'	9.12	9.12	9.13	9.11	9.14	9.13	9.14	9.14	d, 2.5
5'	8.27	8.29	8.28	8.27	8.29	8.27	8.29	8.29	ddd, 9.5, 2.5, 0.5[c]
6'	7.03	6.94	6.96	6.96	6.95	6.94	6.96	6.96	d, 9.5
CH_3CO	-	2.10	2.09	2.06	2.02	2.04	2.07	2.02	s
CH_3CO	-	-	2.10	2.13	2.10	2.06	2.10	2.02	s
CH_3CO	-	-	-	-	2.11	2.10	2.12	2.04	s
CH_3CO	-	-	-	-	-	-	-	2.08	s

a. ~10% CD_3OD in $CDCl_3$; all others in $CDCl_3$; b. Measured from the spectrum of the tetraacetate; for others, it's very similar (±0.5 Hz).

c. Coupling to NH (:); d. 3,6-AC_2: H-1 at δ 4.40, methyl: 2.09 and 2.02.

result of selective hydrolysis of the secondary over the primary acetates or whether acetyl migration is involved. Facile migration of acyl groups in acetylated sugars are well documented[10] and has been held responsible for isolation of 6-*O*-acetylglucose from hydrolysis of glucose pentaacetate with KOH (22% yield).[11] The same compound has also been prepared by partial acetylation of glucose.[12] Similarly, hydrolysis of methyl 2,3,6-tri-*O*-benzoyl-D-galactoside with KOH in MeOH-water[13] furnishes the 6-monobenzoyl derivative. Nevertheless, Haines and coworkers showed by deuterium labeling that formation of 2,3,4,6,1',6' hexa-*O*-acetylsucrose by deacetylation of the octaacetate with propylamine did not proceed by acetyl migration.[14] Similar results for partial hydrolysis of 1,2,6-tri-*O*-acetyl-3,5-dimethyl-D-glucofuranose with NaOMe may also indicate a preference for hydrolysis to the 6-*O*-acetylsugar without acyl migration.[15] Hence, it is possible that in propylamine preferential hydrolysis of the secondary acetates has occurred. We did observe some selectivity between the 2- and 3- acetate in the hydrolysis of β-DNAP-4,6-benzylidene-2,3-di-*O*-acetylglycoside, which was synthesized in 85% yield by condensation of β-DNAP-D-glucopyranose with α,α-dimethoxytoluene in MeCN in the presence of *p*-TsOH as a catalyst, followed by acylation with excess acetic anhydride in pyridine.[16,17] Use of n-propylamine as hydrolyzing agent for 14 min led to a mixture of four components. As shown by TLC and HPLC the faster moving component was the starting 2,3-di-*O*-acetate, the second and the third ones were identified as 2-monoacetate and 3-monoacetate derivatives of β-DNAP-4,6-benzylideneglysoside. The compound of lowest mobility was the 2,3-dihydroxy derivative. Though the β-DNAP-4,6-benzylidenemonoacetates were obtained in low yield, there was more 2-acetate than 3-acetate remaining unhydrolyzed.

EXPERIMENTAL

General Methods:- NMR spectra were recorded on a Bruker AM-300 spectrometer in $CDCl_3/Me_4Si$ and UV spectra on a UV VisSpectrometer, Cary 1E/Cary 3E. MS were recorded on a Finnigan 4021 quadrupole low resolution instrument; the spectra were obtained in the DCI mode using as reagent gas ammonia or methane. In this technique, the sample was deposited as a solution on the filament of the DCI probe; after the solvent was evaporated, the sample was introduced into the ion source *via* the vacuum lock. Two experimental factors had to be optimized initially: the position of the wire with respect to the ion beam[17] and the heating current, to obtain the best emitter temperature.[18] At this temperature the compound was instantly volatilized. The scanning rate was about 1 a.m.u./msec, the source temperature 230° and the electron energy 70 eV.

TLC on Kieselgel F254 (Merck) was used to monitor the reaction and to ascertain the purity of the products. Column chromatography was performed on Kieselgel S (Merck, 230-400 mesh). HPLC analyses were performed on a Waters HPLC instrument equipped with Waters 501 solvent delivery pumps and Waters 484 UV-detector using a reverse phase Econosil-C_{18} column (250 mm x 4.6 mm). Optical density measurements were obtained at λ=350 nm. The mobile phase was HPLC grade H_2O/MeCN operated in the isocratic and gradient mode at a flow rate of 1.2 mL/min and roomtemperature The anomers separation was made in a combined isocratic-gradient program as follows: mobile phase A: H_2O, B: MeCN - 5 min isocratic 30/70; linear concentration change from 70% B to 90% B in two steps from 70% to 85% in 5 min and from 85% to 90% B in another 5 min. The ratio H_2O: MeCN 10:90 was maintained constant for 5 min followed by a new linear concentration change from 90% B to 95% B in 5 min. This concentration was maintained constant for 5 min.

The hydrolyzed products were separated on the same column using the same mobile phase A:H_2O and B: MeCN (40% A and 60% B) for 5 min followed by a linear increase of the % B from 60% to 70% for 5 min.

Methods of glycosylation. The products resulting from glycosylation according to methods A-E were analyzed and separated by HPLC and the ratio of α- and β-glucosides are given in Table 1. Nearly identical ratios were obtained by column chromatography. Structural assignments of the anomers are based on ^{1}H-NMR and COSY experiments.

Method A - 2,3,4,6-Tetraacetyl-α-bromo-D-glucose (**1a**)[19] or 2,3,4,6-tetra-*O*-benzoyl-α-D-glucose[20] (1.1 mmol) was added to a stirred mixture of aglycone (**3a**)[21] or (**3b**) (1.13 mmol), Ag_2CO_3 (0.2 g, 0.7 mmol) and Drierite (0.2 g) in anhydrous C_6H_6 (10 mL). The mixture was stirred for 20-24 h at rt with exclusion of moisture and light. The course of the reaction was monitored by TLC. The solid was removed by filtration and the solution was washed with saturated $NaHCO_3$, NaCl solution, H_2O and dried ($MgSO_4$). The filtrate was evaporated and the residue was purified on a silica gel column by using chloroform-ethyl acetate 6:1 and then ether-carbon tetrachloride 4:1 and/or subjected to HPLC.

Method B - A solution of 0.39 g (0.7 mmol) of **1a** in anhydrous MeCN (3 mL) was added portionwise to a stirred mixture of aglycone (0.5 mmol), Hg $(CN)_2$ (0.25 g, 1 mmol) and molecular sieves 4A (1 g) in MeCN (5 mL). The reaction and work-up were performed as above (Method A).

Method C - To 2,3,4,6-tetrabenzyl-D-glycosyl chloride (**1c**)[22] (0.56 g, 1 mmol) in dry MeCN (20 mL) was added anhydrous silver perchlorate (0.2 g, 1.2 mmol) at -15°C. After 40 min aglycone (2 mmol) was

added and the mixture was stirred for 0.5 h at this temperature and 24 h at rt with exclusion of moisture and light. The reaction mixture was neutralized with anhydrous Na_2CO_3, the organic material was separated with water/chloroform, the chloroform extract dried with $MgSO_4$ and evaporated. Column chromatography using ethyl acetate-chloroform 1:6 gave a mixture of α- and β-anomers and unreacted alcohol. The anomers were separated by using ether-CCl_4 (4:1) and also analyzed by HPLC.

Method D - To a strictly anhydrous solution of tetra-*O*-benzyl-D-glucose (**1b**)[23] (0.54 g, 1 mmol) and aglycone (1 mmol) in 1,2-DCE (10 mL) at -10 to -15°C was added TMS-Tfl (0.2 mL, 1 mmol) in DCE (5 mL). The reaction was monitored by TLC and stirring was continued for 3-4 h. To this reaction mixture was added pyridine (0.2 mL, 1 mmol) in DCE (5 mL), the mixture was stirred for 15-20 min, washed with H_2O and the organic layer was dried ($MgSO_4$). After evaporation of solvent the residue was purified by column chromatography.

Method E - To **1b**, (0.27 g, 0.55 mmol) and 1,2,2,4,4-pentamethylpiperidine (0.12 mL, 0.66 mmol) in dry DCM (10 mL) precooled at -40 to -50°C was dropped slowly triflic anhydride (0.11 mL, 0.66 mmol) and mixture was stirred for 0.5 h at the same temperature. Then additional pentamethylpiperidine (0.12 mL, 0.66 mmol) and aglycone (0.45 mmol) in DCM (5 mL) were added. The temperature was raised to rt and stirring was continued for 3-4 h. To the mixture was added dry ether, the white crystals of salt were filtrated and the filtrate was evaporated. The residue was purified by column chromatography.

β-DNAP-2,3,4,6-tetra-*O*-acetyl-D-glucopyranoside (3β) was obtained by method A in 68% yield, mp 131-132°C, $[\alpha]_D^{27}$ -19.77° (c 3,5, $CHCl_3$). ^{1}H-NMR - see Table 6. Anal. Calcd for $C_{23}H_{29}N_3O_{14}$: C, 48.34; H, 5.11; N, 7.35. Found C, 48.52; H, 5.41; N, 7.05.

β-DNAP-2,3,4,6-tetra-*O*-benzoyl-D-glucopyranoside (7β) was obtained by method A in 78% yield, mp 75-76°C, $[\alpha]_D^{27}$ + 16.90° (c 4.1, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): δ (H-1), 5.58 (H-2). 5.93 (H-3), 5.71 (H-4), 4.19 (H-5), 4.48 (H-6), 4.69 (H-6a), 4.10 and 3.79 (OCH_2), 1.98-2.03 (CH_2), 3.41 (NCH_2), 8.59 (NH), 8.96 (H-3'), 8.07 (H-5'), 6.67 (H-6'); for multiplicities and coupling constants, see Table 6. Benzoate groups: 8.00, 7.91, 7.87, 7.82 (ortho); 7.40, 7.35, 7.30, 7.28 (meta); 7.55, 7.50, 7.47, 7.42 (para). Anal. Calcd for $C_{43}H_{37}N_3O_{14}$: C, 63.00; H, 4.55; N, 5.13. Found C, 63.01; H, 4.83; N, 4.86.

β-DNAP-2,3,4,6-tetra-*O*-veratroyl-D-glucopyranoside (8β) was obtained by method A with 56% yield, mp 119-120°C, $[\alpha]_D^{27}$ + 4.62° (c 0.5, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): δ 4.94 (H-1), 5.54 (H-2), 5.86 (H-3), 5.65 (H-4), 4.20 (H-5), 4.44 (H-6), 4.72 (H-6a) 4.10 and *ca.* 3.8 (OCH_2), 1.94-2.12 (CH_2), 3.42 (NCH_2),

8.65 (NH), 8.95 (H-3'), 8.10 (H-5'), 6.70 (H-6'); for multiplicities and coupling constants, see Table 6. Veratroate groups: 7.28, 7.32, 7.37, 7.51 (each, d, J=2, H=2"); 7.46, 7.50, 7.55, 7.63 (each, dd, J=8, 2, H=5"); 6.71, 6.72, 6.78, 6.81 (each, d, J=8, H=6"); 3.83, 3.84, 3.86, 3.87, 3.89, 3.89, 3.92, 3.93 (each, s, 3H, OMe).

Selective hydrolysis of tetra-*O*-acetyl-β-glycoside (3β) and tetra-*O*-aroyl-β-glycosides (7β, 8β).

(a) In propylamine - A solution of **3β** (0.11 g, 0.2 mmol) in 1 mL of propylamine was stirred at rt for 30 with $CHCl_3$ to give tetraacetate (**3β**) as the first fraction, followed by a second fraction which afforded triacetate (**9**). The third, fourth and fifth fractions, using $CHCl_3$-EtOH (7:3), afforded diacetate (**10**), monoacetate(**11**) and completely deacetylated glycoside (**12**), respectively. Column chromatography [$CHCl_3$-EtOH (7:3)] of the residue obtained on concentration of the reaction mixture after 1 h gave 6-monoacetyl-D-glucopyranoside (**11**) as the major component. See Table 4.

The hydrolysis of **7β** or **8β** was performed in the same manner as above. The 6-monobenzoyl- and 6-monoveratroylglycosides (**13, 14**) were isolated as major components from reaction mixture after 24 h and 48 h, respectively. See Table 5.

(b) In methanolic solution - To a solution of **3β**, **7β** or **8β** (0.2 mmol) in dry MeOH (1 mL) was added 0.01 mL of 0.01 M MeONa at rt. The reaction was monitored by TLC. After neutralization with Amberlite IR-120(H^+), filtration and evaporation of the solvent, the residue was fractionated on a column as above. Similar procedures were carried out with ammonia and amines in MeOH solution (see Tables 4,5).

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