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4-HYDROXYBUTYRIC ACID (AND ANALOGUES) DERIVATIVES OF D-GLUCOSAMINE

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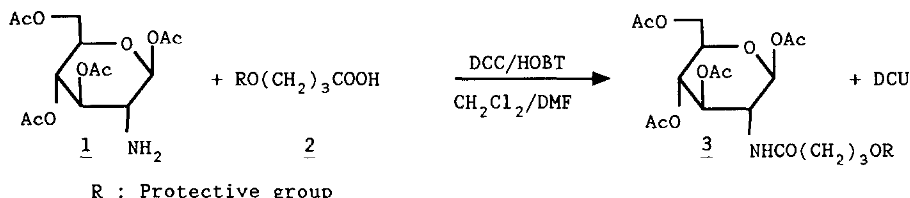
Abstract. The syntheses of 2-carboxamido 2-deoxy 1,3,4,6-tetra O-acetyl β-D-glucose and 2-carboxamido 2-deoxy D-glucose derivatives are described. These compounds are proposed as 4-hydroxybutyric acid (GHB) derivatives in attempt to test whether modifications in GHB structure could lead to more active biological substances.

INTRODUCTION

Gamma-hydroxybutyric acid (GHB) was first studied in 1960 by Laborit and coworkers as an isostere of γ-aminobutyric acid (GABA) able to cross the blood-brain barrier and was proposed as a hypnotic and general anaesthetic[1]. As GHB modulates dopaminergic activity[2] and plays a part in sleep regulation, it might be a neuromodulator or neurotransmitter in the central nervous system[3]. Moreover, an increasing number of results are pointing out the influence of the sugar moieties of glucopeptides in their biological activity[4] and several studies have detected a dopamine antagonist action of glucose[5]. In attempt to specify if modification in GHB structure with incorporation of a glucose moiety (via 2-amino 2-deoxy D-glucose) could lead to more active biological substances, we describe in the present paper the synthesis of new glycosyl GHB derivatives.

RESULTS AND DISCUSSION

4-hydroxybutyric acid (GHB) spontaneously undergoes cyclization in butyrolactone and thus, an O-protective group was required prior to condensation of GHB with 2-amino 1,3,4,6-tetra O-acetyl 2-deoxy β-D-glucose 1 according to the coupling method (DCC-HOBT) used for peptide synthesis[6,7] (*Scheme 1*).



- *Scheme 1* -

Protection of the GHB hydroxyl group must be carried out under non-acidic conditions to avoid preferential cyclization in butyrolactone.

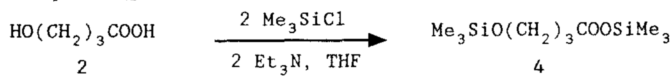
Three protective groups have been attempted for the protection of GHB-hydroxyl function as $RO(CH_2)_3COOH$:

-trimethylsilyl ether ($-SiMe_3$)

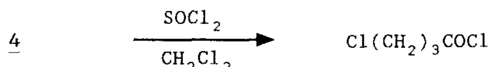
-methoxyethoxymethyl ether ($-CH_2OCH_2CH_2OCH_3$)

-acetate ($-COCH_3$)

a) The trimethylsilyl group has been widely used as a protective group for alcohols[8]. We prepared easily compound 4 as follows:

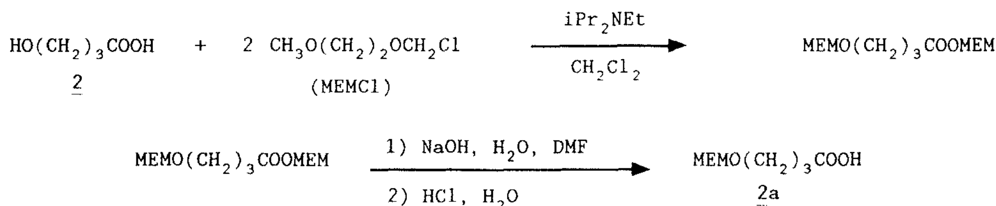


Compound 4 could not be selectively deprotected as $Me_3SiO(CH_2)_3COOH$ despite numerous trials. We tried to convert 4 to $Me_3SiO(CH_2)_3COCl$ by means of thionylchloride[9] for further coupling with 1. This attempt was not successful and instead, 4-chlorobutanoylchloride was obtained:



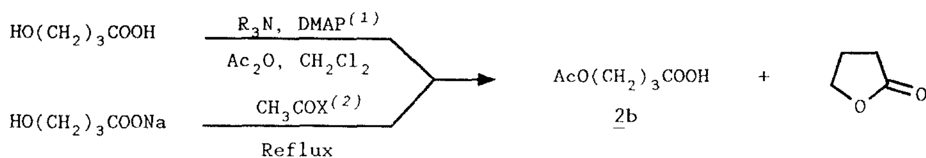
b) Since the trimethylsilyl group failed as GHB-hydroxyl protective group we used methoxyethoxymethyl (MEM) group as protective group. MEM-ethers are formed under aprotic-basic or aprotic-neutral conditions[10]. We prepared 4-(2-methoxyethoxymethoxy) butyric acid in a satisfactory yield (*scheme 2*).

Reaction of compound 2a with 1,3,4,6-tetra O-acetyl β-D-glucosamine 1 according to *scheme 1* affords 3a (R = MEM) in a 73% yield.



- *Scheme 2* -

c) In our structure-activity relationship study we also prepared 4-acetoxybutyric acid 2b. Classical procedures[11,12] applied to acetylation of GHB-hydroxyl group afforded 2b in very poor yield (15%) on account of preferential formation of butyrolactone (*Scheme 3*).

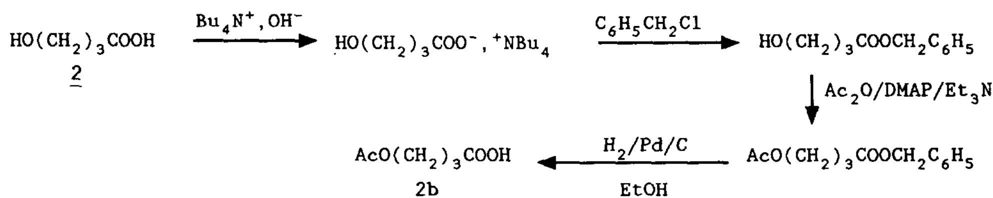


(1) R = Et or iPr_2, Et ; DMAP : dimethylaminopyridine

(2) X = Cl or $OCOCH_3$

- *Scheme 3* -

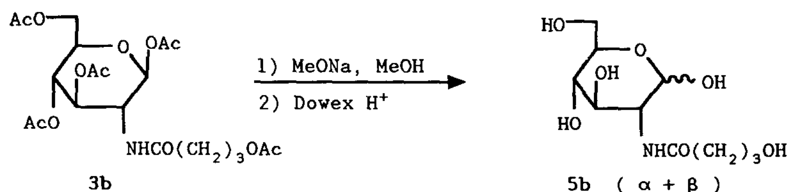
We improved significantly yield of 2b (81%) using the following reactional pathway (*Scheme 4*).



- *Scheme 4* -

Coupling of compound 2b with 1 affords 3b in 72% yield.

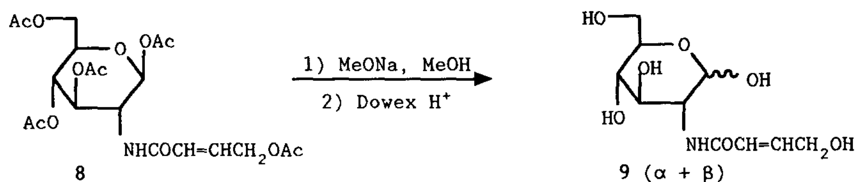
Within the context of lipophilic-activity comparison using *in vivo* tests, 3b was O-deacetylated with a catalytic amount of sodium methoxide to give 5b (anomeric mixture - *Scheme 5*).



- *Scheme 5* -

GHB exhibits good flexibility of the carbon chain. In an attempt to specify which GHB-conformation acts with different biological sites we synthesized two trans 4-hydroxycrotonic acid derivatives, each containing glucose moiety. Trans hydroxycrotonic acid has been recently identified as a naturally occurring substance in the central nervous system and it shows a better biological affinity than GHB[13].

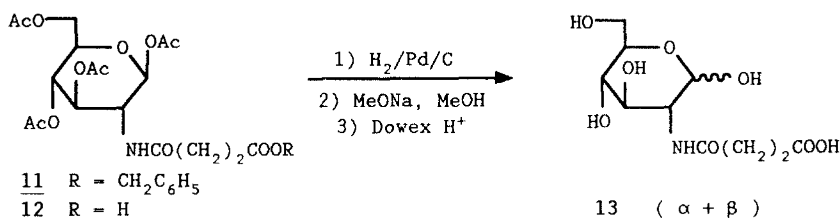
4-hydroxycrotonic acid 6 was prepared in good yield by alkaline hydrolysis of 4-bromocrotonic acid and protected as 4-acetoxycrotonic acid 7, $AcOCH_2CH{=}CHCOOH$, before the coupling reaction with 1 according to the procedure in *Scheme 1*. The resulting compound 8 was O-deacetylated in 9 (anomeric mixture).



- *Scheme 6* -

With the aim to establish if free hydroxyl or carboxylic groups of GHB are necessary for recognition by binding sites, an analogue of 3b and 8 was prepared using a succinic acid derivative.

Benzyl hydrogen succinate 10 $HOOCCH_2CH_2COOCH_2C_6H_5$ was prepared by heating a mixture of succinic anhydride and benzyl alcohol. Reaction of 10 with 1 (DCC, HOBT) affords 11. The benzyl group was removed by catalytic hydrogenation to give compound 12 which was O-deacetylated in 13 (*Scheme 7*).



- *Scheme 7* -

N.M.R. STUDY OF D-GLUCOSAMINE DERIVATIVES

A) 2-carboxamido 2-deoxy 1,3,4,6-tetra O-acetyl β-D-glucose derivatives.

1) **^{1}H-NMR SPECTRA**. Shift data[a)] and coupling constants[b)] are given in table I.

Table I

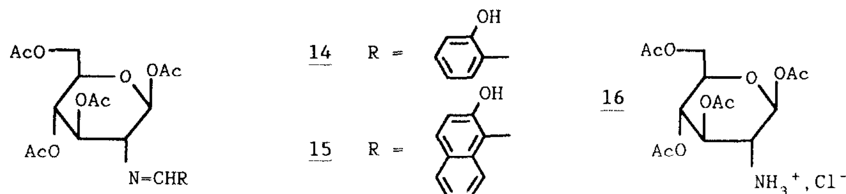
		3a	3b	8	11	12	14	15	16
H_1	δ	5.72,d	5.71,d	5.76,d	5.71,d	5.69,d	6.17,d	6.25,d	5.94,d
	J	^3J=8.9	^3J=8.8	^3J=8.8	^3J=8.8	^3J=8.8	^3J=8.2	^3J=8.3	^3J=8.6
H_2	δ	~ 4,m	~ 4,m*	~ 4,m	~ 4,m	~ 4,m	3.58,t	3.91,t	3.54,t
	J						^3J=9.2	^3J=9.3	^3J=9.5
H_3	δ	5.18,t	5.18,t	5.22,t	5.18,t	5.16,t	5.60,t	5.72,t	5.38,t
	J	^3J=9.5	^3J=9.6	^3J=9.7	^3J=9.7	^3J=9.7	^3J=9.7	^3J=9.5	^3J=9.6
H_4	δ	4.89,t	4.90,t	4.91,t	4.89,t	4.88,t	5.00,t	5.03,t	4.93,t
	J	^3J=9.5	^3J=9.6	^3J=9.6	^3J=9.5	^3J=9.5	^3J=9.7	^3J=9.5	^3J=9.6
H_5	δ	~ 4,m	~ 4,m*	~ 4,m	~ 4,m	~ 4,m	4.21,m	~ 4.3,m	~ 4,m
$H_{6,6'}$	δ	~ 4 4.2,m	~ 4 4.2,m*	~ 4 4.2,m	~ 4 4.2,m	~ 4 4.2,m	~ 4.3 4.1,m	4.25 4.07,m	~ 4 4.2,m

* With heteronuclear COSY experiment: 3.96 (H_2); 3.99 (H_5); 3.98, 4.19 ($H_{6,6'}$).

a) Solvent for all compounds: DMSO D_6; δ_{ppm} (from TMS) of glycosidic protons only. For the other ^{1}H-chemical shifts see experimental.

b) Only typical coupling constants (J_{Hz}).

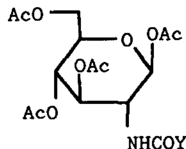
Upon examination of this table one can notice that H_2 and H_5 chemical shifts were virtually identical. A double irradiation experiment did not allow H_3 and H_4 identification. Compounds 14 and 15 were also synthesized for other purposes.



In the ^{1}H-NMR spectra of **14**, **15** and 2-amino 2-deoxy 1,3,4,6-tetra O-acetyl β-D-glucose hydrochloride **16**, resonance signals of H_2, H_5, $H_{6,6'}$ are clearly differentiated. Selective irradiation of H_2, H_5 signals permits in these cases the assignment of H_3 and H_4 chemical shifts. For these compounds the signal of H_4 is found at higher field than H_3. The chemical shift of H_4 remains almost constant throughout the series of compounds described here. The nature of the nitrogen substituant thus induces very little change on H_4 chemical shift, consequently the signal at higher field is assigned to H_4 for the other compounds of table I.

2) ^{13}C-NMR SPECTRA. Shift data are reported in table II.

Table II: Chemical shifts[a)] of



Y	$CH_2CH_2CH_2OMEM$[b)e)] (2' 3' 4') **3a**	$CH_2CH_2CH_2OAc$[c)] (2' 3' 4') **3b**	$CH{=}CHCH_2OAc$[c)] (2' 3' 4') **8**	CH_2CH_2COOH[c)] (2' 3' 4') **12**
C_1	92.60	91.80	91.62	91.85
C_2	52.84	52.05	52.06	52.04
C_3	72.85[d)]	72.21	72.11	72.17
C_4	68.28	68.31	68.16	68.40
C_5	72.72[d)]	71.62	71.47	71.67
C_6	61.87	61.57	61.40	61.61
$C_{2'}$	33.34	31.73	124.16	30.26
$C_{3'}$	25.77	24.27	136.67	29.11
$C_{4'}$	71.96	63.00	62.25	
CO	172.92, 170.94 170.65, 169.38	171.64, 170.19 169.88, 169.45 169.14, 168.68	169.67, 169.56 169.22, 168.95 168.46, 164.22	173.41, 171,35 169.88, 169.49 169.14, 167.73
$COOCH_3$	20.83, 20.64	20.54, 20.36 20.29, 20.19	20.22, 20.17 20.09, 20.02	20.37, 20.28 20.20

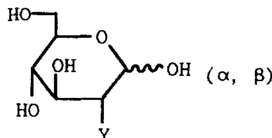
a) δ_{ppm} from TMS b) Solvent: $CDCl_3$ c) Solvent: DMSO D_6 d) C_3 or C_5
e) MEM: $CH_2OCH_2CH_2OCH_3$; δ $C_{5'}$: 95.68, $C_{6'}$,$C_{7'}$: 67.14, $C_{8'}$: 58.94

Assignments of C_1 and C_2 signals agree with ^{13}C-NMR data previously reported for tetra O-acetyl β-D-glucosamine[14]. For C_6 and C_9 signals on one hand and C_3, C_4 and C_5 on the other hand, assignments were not unambiguous. But once the chemical shifts of those carbon protons were known (*vide supra*) it was a simple matter to assign all carbon signals of **3b** using a heteronuclear correlation experiment. Chemical shifts of carbons of **3a**, **8**, **12** remained very

similar to **3b** signals and assignment of different carbons of those compounds was then established by analogy to **3b**.

B) 2-carboxamido 2-deoxy D-glucose derivatives.

1) ^{1}H-NMR SPECTRA (Table III)

Table III. Chemical shifts[a] of  (α, β)

Y	NHCOCH$_2$CH$_2$CH$_2$OH **5b** α	β	NHCOCH=CHCH$_2$OH **9** α	β	NHCOCH$_2$CH$_2$COOH **13** α	β
H_1	5.22 (^{3}J=3.4)	4.73 (^{3}J=8.3)	5.24 (^{3}J=3.5)	4.76 (^{3}J=8.3)	5.21 (^{3}J=3.4)	4.75 (^{3}J=8.2)
H_2	3.90	3.71	4.00	3.79	3.92	3.69
H_3	3.80	3.55	3.81	3.54	3.81	3.59
H_4	3.50	3.48	3.53	3.53	3.52	3.50
H_5	3.87	3.47	3.85	3.47	3.86	3.49
$H_{6,6'}$	3.84	3.84	{3.88, 3.77	{3.88, 3.77	3.84	3.84
$H_{2'}$	2.40	2.38	6.23 (^{3}J=15.5) (^{4}J=1.9)	6.18 (^{3}J=15.5) (^{4}J=1.8)	2.57[b]	2.57
$H_{3'}$	1.87	1.87	6.89 (^{3}J=15.5) (^{4}J=4.2)	6.88 (^{3}J=15.5)	2.58[b]	2.58
$H_{4'}$	3.64	3.64	4.31	4.31		

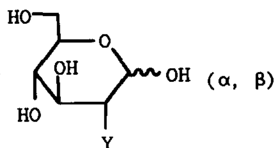
a) δ_{ppm} in D_2O; determined with 2D-heteronuclear correlation experiment.

b) H_2 or H_3.

The α and β anomeric H_1 protons of the different derivatives can be clearly differentiated and assigned on the basis of their chemical shifts and spin-spin coupling constants. The physical constants were in agreement with the literature data for β and α D-aldohexoses[15] ($JH_1\alpha H_2$ = 3.5 Hz; $JH_1\beta H_2$ = 8.3 Hz).

Assignment of H_2, H_3, H_4, H_5 chemical shifts of the α-anomer (major species) and of H_2 β-anomer was performed with a homonuclear correlation experiment (COSY). The unambiguous assignment of H_3, H_4, H_5 β-anomer and $H_{6,6'}$ α and β anomer is not straightforward due to strong resonance overlap. But the heteronuclear correlation experiment (^{13}C-^{1}H) enabled assignment of all signals.

2) ^{13}C-NMR SPECTRA (Table IV)

Table IV. Chemical shifts[a] of  (α, β)

Y	NH_3^+ (14)		$NHCOCH_2CH_2CH_2OH$		$NHCOCH=CHCH_2OH$		$NHCOCH_2CH_2COOH$	
	17		5b		9		13	
	α	β	α	β	α	β	α	β
C_1	90.0	93.6	91.9	95.9	91.8	95.8	91.9	95.9
C_2	55.3	57.8	55.1	57.7	55.0	57.8	55.1	58.0
C_3	70.5	72.9	71.8	74.8	71.7	74.8	71.9	74.9
C_4	70.5	70.5	71.2	71.0	71.1	70.9	71.3	71.1
C_5	72.4	76.9	72.6	76.9	72.5	76.8	72.7	77.0
C_6	61.3	61.3	61.7	61.7	61.6[b]	61.6	61.8	61.9
$C_{2'}$			33.3	33.6	122.3	122.6	32.8[c]	32.8
$C_{3'}$			28.7	28.7	144.8	144.6	33.1[c]	33.1
$C_{4'}$			62.0	62.0	61.7[b]	61.7	–	–
CO			177.7	177.9	169.4	169.8	177.0	177.4
							180.9	180.9

a) δ_{ppm} in D_2O. Reference: dioxan (δ: 67.4).

b) C_6 or $C_{4'}$.

c) $C_{2'}$ or $C_{3'}$.

^{13}C resonances corresponding to both α and β anomers are clearly differentiated. The more intense resonances of the anomeric mixture are assigned to the α-form on the basis of α/β ratio obtained from ^1H-NMR spectra.

Carbon resonances for glucopyranosyl ring are assigned by comparison with their parent compound 2-amino 2-deoxy α (and β)-D-glucopyranose 17[14]. For the three derivatives, chemical shifts are almost constant for all carbons. The heteronuclear correlation experiment (^{13}C-^1H) then provides all ^1H signals assignments.

BIOLOGICAL TESTS

The followed compounds were tested for biological activities: **GHB**, **2b**, **3b**, **5b***, **9***, **12**, **13***(*= mixture α + β).

Male mice (30-35 g) were obtained from IFFA CREDO (Saint-Germain sur l'Arbresle, France). Compounds were intraperitoneally administred. Control mice received vehicle alone.

Compounds were first evaluated on general behaviour. At 0.63 mmol/Kg the compounds didn't produce any remarkable change on gross behaviour. At 2.10 mmol/Kg GHB and 2b induced a narcotic state, mice laid on flank, locomotor activity was highly decreased from ten minutes to one hour. Other compounds produced no change.

The biochemical compounds action were investigated by mesuring dopamine (DA) and its

main catabolites: homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) in mice striatum. Mice were killed by decapitation one hour after treatment. Striata were rapidly dissected[16] and kept at -80°C until analysis. The simultaneous assay of DA, DOPAC and HVA was performed by HPLC with electrochemical detection[17]. For each compound a 0.63 mmol/Kg dosage administration did not affect the DA and DA catabolites striatal levels. For the 2.10 mmol/Kg dosage, GHB increased the level of mice striatal DA (+ 19%, $n = 6$, $p < 0.05$), DOPAC (+ 96.5%, $n = 6$, $p < 0.01$) and HVA (+ 16%, $n = 6$, $p < 0.05$); compound 2b was as effective as GHB. Other compounds did not have any activity.

The compound 2b effectiveness certainly resulted of an enzymic hydrolysis[18]. So 2b might be used as a prodrug of GHB. The other compounds showed no signs of GHB like biological activities. These results are in agreement with the observation that free carboxylic group is essential for GHB like activities[19].

EXPERIMENTAL

Melting points were determined with a Kofler hot-stage and are uncorrected. N.M.R. spectra were recorded with a Bruker WH-250 spectrometer (250 MHz for ^1H and 62.89 MHz for ^{13}C). I.R. spectra were recorded with a Pye-Unicam Philips SP3-200 spectrophotometer (KBr). Optical rotations were measured at 25°C with a Polartronic D Schmidt-Haensch polarimeter. T.L.C. was performed on silica gel F_{254} (Merck) with detection using iodine vapor or U.V. light. Preparative liquid chromatography was performed using Chromatospac (Jobin-Yvon): *columns* ϕ 40 mm; *packing pressure* 1.5 MPa; *elution pressure* 0.7 MPa; *silica gel* 15 µm; *detector* differential refractometer Knauer.

1-hydroxybenzotriazole was recrystallized from methanol. All solvents were distilled before use. The following abbreviations are used: THF, tetrahydrofuran; DMF, N,N-dimethyl formamide; HOBT, 1-hydroxybenzotriazole; DCC, N,N'-dicyclohexylcarbodiimide.

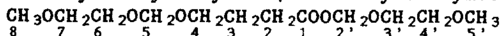
2-amino 2-deoxy 1,3,4,6-tetra O-acetyl β-D-glucose 1.

1 was prepared by the method of M. Bergmann and L. Zervas[20].

4-hydroxybutyric acid (GHB) 2.

Aqueous solution of sodium 4-hydroxybutyrate was acidified with hydrochloric acid (pH = 3.6) and then concentrated under vacuum. The residue was extracted with ethyl acetate, dried ($MgSO_4$) and then concentrated. Yield: 86%. ^1H NMR (DMSO D_6); δ: 1.7 (m, 2H, H_3); 2.3 (t, 2H, H_2); 3.45 (t, 2H, H_4).

Methoxyethoxymethyl 4-(2-methoxyethoxymethoxy) butyrate.



To a solution of GHB (38.5 mmol) in THF (20 ml) at 0°C was added dropwise a solution of di-isopropylamine (77 mmol in 10 ml THF). The mixture was stirred at room temperature for 1/2 hr and then cooled at 0°C. MEM-chloride (77 mmol) was added and the mixture was stirred for 18 hr at room temperature. Water (20 ml) was added and aqueous solution extracted with chloroform (3 x 20 ml). The combined extracts were dried, concentrated to give an oil purified by preparative liquid chromatography (eluent: CH_2Cl_2, MeOH: 9/1). Yield: 82%. ^1H NMR ($CDCl_3$); δ: 2.0 (m, 2H, H_3); 2.5 (t, 2H, H_2); 3.4 (s, 6H, $H_{8,5'}$); 3.65 (m, 10H, $H_{6,3',7,4',4}$); 4.77 (m, 2H, H_5); 5.4 (s, 2H, $H_{2'}$).

Anal: Calc for $C_{12}H_{24}O_7$: C, 51.41; H, 8.63. Found: C, 51.46; H, 8.60.

4-(2-methoxyethoxymethoxy) butyric acid 2a.

A solution of MEM (4-OMEM) butyrate (20 mmol) in DMF, NaOH 1M (20 ml/ 20 ml) was refluxed for 3hr. The concentrated mixture was diluted with H_2O and extracted with chloroform (2 x 20 ml). The aqueous solution was then acidified to pH = 3.6 with

hydrochloric acid and extracted with chloroform (3 x 20 ml). The chloroformic solution was dried ($MgSO_4$) and concentrated. The product (oil - 71%) was sufficiently pure for use in the next step. ^1H NMR ($CDCl_3$); δ: 2.0 (m, 2H, H_3); 2.5 (t, 2H, H_2); 3.48 (s, 3H, H_8); 3.7 (m, 6H, $H_{4,6,7}$); 4.77 (m, 2H, H_5).

2-(4-OMEM) butyramido 2-deoxy 1,3,4,6-tetra O-acetyl β-D-glucose 3a.

To a solution of 2a (10 mmol) in CH_2Cl_2 (20 ml) at 0°C were added HOBT (12 mmol) in DMF (10 ml) and DCC (10 mmol) in CH_2Cl_2 (10 ml). The mixture was stirred 1/2 hr at room temperature under argon. A solution of 1 in CH_2Cl_2 (10 mmol in 10 ml) was added dropwise and the mixture was stirred for 15 hr at room temperature. Dicyclohexylurea was removed by filtration and washed with a small amount of CH_2Cl_2-DMF. The solvents were evaporated. The remaining syrup was dissolved in chloroform (or ethyl acetate), washed with potassium bicarbonate solution, water, dried ($MgSO_4$) and evaporated to a residue which was chromatographed (eluent: ethyl acetate, dichloromethane 50/50). Yield: 73%. m.p: 93°C. IR: ν_{max} 3300 (NH), 1740 (C=O ester), 1650, 1530 (C=O amide). $[\alpha]_D$: +1.0° (c 0.997, $CHCl_3$). For ^{13}C NMR data see table II. ^1H NMR; δ: glycosidic protons see table I; 1.68 (m, 2H, $H_{3'}$); 1.92, 1.98, 2.01, 2.04 (s, 4 x 3H, CH_3COO); 2.08 (t, 2H, $H_{2'}$); 3.25 (s, 3H, $H_{8'}$); 3.38 (t, 2H, $H_{4'}$); 3.45 and 3.55 (m, 4H, $H_{6',7'}$); 4.58 (s, 2H, $H_{5'}$); 7.99 (d, 1H, NH, J = 9.2 Hz).

Anal: Calc for $C_{22}H_{35}NO_{13}$: C, 50.66; H, 6.76; N, 2.69. Found: C, 50.73; H, 6.81; N, 2.65.

4-acetoxybutyric acid 2b (according to scheme 4).

To a solution of 4-hydroxybutyric acid (50 mmol) in water (30 ml) was added tetrabutylammonium hydroxyde (50 mmol) (pH = 7). The mixture was evaporated to dryness. The resulting syrup was dissolved in DMF and benzylchloride (50 mmol) was added. After 15 hr stirring, water (30 ml) was added and benzyl 4-hydroxybutyrate was extracted with diethyl ether. After drying and evaporation of solvent the resulting ester was distilled ($E_{0.05}$ = 115°C, yield: 91%) and dissolved (40 mmol) in CH_2Cl_2 (30 ml). To the cooled solution (0°C) were successively added dimethylaminopyridine (600 mg), triethylamine (40 mmol) and acetic anhydride (40 mmol). The mixture was stirred 3 hr at room temperature, then water (30 ml) was added and benzyl 4-acetoxybutyrate was extracted with chloroform (3 x 30 ml). Yield: 98%.

The resulting ester (20 mmol) was hydrogenated in ethanol (50 ml) in the presence of 10% palladium on charcoal for 1/2 hr at room temperature and atmospheric pressure. After filtration the solvent was evaporated and crude 4-acetoxybutyric acid was distilled[12] ($E_{0.01}$ = 74°C) or chromatographed (eluent: CH_2Cl_2, MeOH, AcOH: 30/2/0.8). Yield: 90%. ^1H NMR:

a) $HO\underset{4}{CH_2}\underset{3}{CH_2}\underset{2}{CH_2}\underset{1}{COO}\underset{2'}{CH_2}C_6H_5$ ($CDCl_3$)

δ: 1.95 (m, 2H, H_3); 2.5 (t, 2H, H_2); 3.7 (t, 2H, H_4); 5.2 (s, 2H, $H_{2'}$); 7.6 (s, 5H, C_6H_5); 2.9 (broad, 1H, OH).

b) $\underset{6}{CH_3}\underset{5}{COO}\underset{4}{CH_2}\underset{3}{CH_2}\underset{2}{CH_2}\underset{1}{COO}\underset{2'}{CH_2}C_6H_5$ ($CDCl_3$)

δ: 2.0 (s + m, 5H, H_3, H_6); 2.5 (t, 2H, H_2); 4.17 (t, 2H, H_4); 5.2 (s, 2H, $H_{2'}$); 7.6 (s, 5H, C_6H_5).

c) $\underset{6}{CH_3}\underset{5}{COO}\underset{4}{CH_2}\underset{3}{CH_2}\underset{2}{CH_2}\underset{1}{COOH}$ ($CDCl_3$)

δ: 2.0 (m, 2H, H_3); 2.1 (s, 3H, H_6); 2.5 (t, 2H, H_2); 4.20 (t, 2H, H_4).

Anal: Calc for $C_6H_{10}O_4$: C, 49.31; H, 6.90. Found: C, 49.26; H, 6.85.

2-(4-acetoxy) butyramido 2-deoxy 1,3,4,6-tetra O-acetyl β-D-glucose 3b.

The procedure was identical to 3a. The purification was performed by preparative liquid chromatography (eluent: AcOEt, CH_2Cl_2: 55/45). Yield: 72%. mp: 113°C. IR: ν_{max} 3360 (NH), 1740 (C=O ester), 1665, 1520 (C=O amide). $[\alpha]_D$: +3.0° (c 1.0, $CHCl_3$). For ^{13}C NMR data see table II. ^1H NMR; δ: glycosidic protons see table I; 1.74 (m, 2H, $H_{3'}$); 1.91, 1.98, 1.99, 2.01, 2.04 (s, 5 x 3H, CH_3COO); 2.09 (t, 2H, $H_{2'}$); 3.92 (t, 2H, $H_{4'}$); 8.04 (d, 1H, NH, J = 9.1 Hz).

Anal: Calc for $C_{20}H_{29}NO_{12}$: C, 50.52; H, 6.15; N, 2.95. Found: C, 50.47; H, 6.12; N, 2.91.

2-(4-hydroxy) butyramido 2-deoxy D-glucose 5b.

To a solution of <u>3b</u> (2 mmol) in dry methanol (30 ml) was added (under argon) a solution of sodium methoxide (0.1 ml; 1% in methanol). Deacetylation was monitored by TLC ($CHCl_3$, MeOH: 10/1 then 10/5) and was performed for 5 hr. The solution was then neutralized with DOWEX 5X8-400 (H^+) resin. After filtration, methanol was evaporated and <u>5b</u> was crystallized and washed with diethyl ether (yield: 88%). IR: ν_{max} 3600-3100 (OH, NH), 1660, 1550 (C=O amide). For NMR data see tables III and IV. Ratio α/β = 65/35.

Anal: Calc for $C_{10}H_{19}NO_7$: C, 45.28; H, 7.22; N, 5.28. Found: C, 45.32; H, 7.24; N, 5.17.

4-hydroxybutene 2-(E) oic acid <u>6</u>.

Acid <u>6</u> was obtained from trimethylsilyl 4-bromocrotonate which was prepared according to the experimental procedure described in reference[21]. E_{12} = 95°C. Yield: 80%. ^1H NMR ($CDCl_3$): $BrCH_2CH{=}CHCOOSi(CH_3)_3$; δ: 0.4 (s, 9H, $Si(CH_3)_3$); 4.0 (d, 2H, H_4); 6.0 (d, 1H, H_2, JH_2H_3 = 17 Hz); 7.0 (d x t, 1H, H_3).

To a solution of trimethylsilyl 4-bromocrotonate (70 mmol) in water (120 ml) was added dropwise a 2M KOH solution (240 ml) at 0°C. After the addition the solution was heated for 5 mn (100°C), cooled in an ice bath and acidified (pH = 3.6). The mixture was concentrated and extracted with ethyl acetate and chromatographed on silica gel column (eluent: AcOEt, MeOH: 97/3). Yield: 80%. mp: 109°C. ^1H NMR (DMSO D_6); δ: 4.2 (m, 2H, H_4); 6.0 (d, 1H, H_2); 7.1 (d x t, 1H, H_3, JH_2H_3 = 16 Hz).

Anal: Calc for $C_4H_6O_3$: C, 47.06; H, 5.92. Found: C, 47.00; H, 5.83.

4-acetoxybutene 2-(E) oic acid <u>7</u>.

To a solution of acid <u>6</u> (50 mmol) in dichloromethane (20 ml) was added at 5°C, triethylamine (100 mmol). The medium was stirred for 1/2 hr at 20°C and then cooled to -10°C. Dimethylaminopyridine (2.5 mmol) and acetic anhydride were added. After 2 hr at 20°C the mixture was diluted with acidified water (pH = 4) and extracted with ethyl acetate. The solvent was evaporated and the compound <u>7</u> recrystallized from diethyl ether, petroleum ether (60/40). Yield 58%. ^1H NMR (DMSO D_6); δ: 2.1 (s, 3H, H_6); 4.8 (d, 2H, H_4); 5.9 (d, 1H, H_2, JH_2H_3 = 15.5 Hz); 6.9 (d x t, 1H, H_3).

Anal: Calc for $C_6H_8O_4$: C, 50.00; H, 5.60. Found: C, 49.96; H, 5.56.

2-(4-acetoxybutene 2) amido 2-deoxy 1,3,4,6-tetra O-acetyl β-D-glucose <u>8</u>.

Compound <u>8</u> was prepared as previously described for <u>3a</u> and <u>3b</u>. Crude product was purified by preparative liquid chromatography (eluent: AcOEt, CH_2Cl_2: 1/1) to yield <u>8</u> (45%). mp: 133°C. IR: ν_{max} 3360 (NH), 1750 (C=O ester), 1675, 1530 (C=O amide), 1640 (C=C). $[\alpha]_D$: +12.5° (c 0.88, $CHCl_3$). For ^{13}C NMR data see table II. ^1H NMR; δ: glycosidic protons see table I; 1.89, 1.98, 2.01, 2.03, 2.08 (s, 5 x 3H, CH_3COO); 4.69 (d x d, 2H, $H_{4'}$, ^3J = 4.4 Hz, ^4J = 1.5 Hz); 6.0 (d x t, 1H, $H_{2'}$, ^3J = 15.5 Hz, ^4J = 1.5 Hz); 6.64 (d x t, 1H, $H_{3'}$, ^3J = 15.5 Hz, ^4J = 4.4 Hz); 8.27 (d, 1H, NH, J = 9.1 Hz).

Anal: Calc for $C_{20}H_{27}NO_{12}$: C, 50.74; H, 5.75; N, 2.96. Found: C, 50.58; H, 5.86; N, 3.05.

2-(4-hydroxybutene 2) amido 2-deoxy D-glucose <u>9</u>.

Deacetylation of <u>8</u> was performed as for <u>5b</u>. Compound <u>9</u> was recrystallized from ethanol (yield: 85%). IR: ν_{max} 3600-3100 (OH, NH), 1660, 1545 (C=O amide), 1610 (C=C). For ^1H NMR data see tables III and IV. Ratio α/β = 70/30.

Anal: Calc for $C_{10}H_{17}NO_7$: C, 45.62; H, 6.51; N, 5.32. Found: C, 45.70; H, 6.54; N, 5.37.

Benzyl hydrogen succinate <u>10</u> : $HOOCCH_2CH_2COOCH_2C_6H_5$.

Compound <u>10</u> was performed by a general procedure[22]. A mixture of succinic anhydride (1 mol) and benzyl alcohol (1.2 mol) was stirred at 100-110°C for 2 hr. After cooling, compound <u>10</u> crystallized and was filtered and washed with diethyl ether, petroleum ether (30/70). Crude <u>10</u> was dissolved in water (50 ml) and a sodium hydroxyde solution (2M) was added until pH = 10. The solution was washed with ethyl acetate (2 x 20 ml). The aqueous solution was then acidified (pH = 2), extracted with ethyl acetate (3 x 20 ml), washed with brine, and

dried on magnesium sulfate. The solvent was evaporated and the solid washed with diethyl ether, petroleum ether (30/70). Yield 67%. mp: 60°C. ^{1}H NMR ($CDCl_3$); δ: 2.70 (s, 4H, H_2, H_3); 5.25 (s, 2H, $H_{2'}$); 7.52 (s, 5H, C_6H_5).

Anal: Calc for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81. Found: C, 63.47; H, 5.90.

2-benzylsuccinamido 2-deoxy 1,3,4,6-tetra O-acetyl β-D-glucose 11.

Compound 11 was synthetized according to the experimental procedure described for 3a. After evaporation of the solvent, the solid was washed with diethyl ether. Yield: 83%. IR: ν_{max} 3350 (NH), 1740 (C=O ester), 1680, 1530 (C=O amide). ^{1}H NMR (DMSO D_6); δ: glycosidic protons see table I; 1.91, 1.97, 2.01, 2.02 (s, 4 x 3H, CH_3COO); 2.32 (t, 2H, $H_{2'}$); 2.55 (t, 2H, $H_{3'}$); 5.06 (s, 2H, $H_{5'}$); 7.35 (s, 5H, C_6H_5); 8.05 (d, 1H, NH, J = 9.2 Hz).

Anal: Calc for $C_{25}H_{31}NO_{12}$: C, 55.86; H, 5.81; N, 2.61. Found: C, 55.91; H, 5.78; N, 2.56.

2-succinamido 2-deoxy 1,3,4,6-tetra O-acetyl β-D-glucose 12.

Compound 11 (3.7 mmol) was hydrogenated in methanol (50 ml) in the presence of 10% palladium on charcoal for 1/2 hr at room temperature and atmospheric pressure. After filtration and evaporation of the solvent, crude 12 was purified by preparative liquid chromatography (eluent: CH_2Cl_2, MeOH, AcOH: 45.95/4/0.05). Yield: 63%. mp: 170°C. IR: ν_{max} 3380, 3300-2800 (NH, OH), 1740, 1700 (C=O ester, acide), 1660, 1510 (C=O amide). $[\alpha]_D$: +6.8° (c 1.02, $CHCl_3$). ^{1}H NMR (DMSO D_6); δ: glycosidic protons see table I; 1.92, 1.97, 2.01, 2.04 (s, 4 x 3H, CH_3COO); 2.23 (t, 2H, $H_{2'}$); 2.29 (t, 2H, $H_{3'}$); 7.99 (d, 1H, NH, J = 9.3 Hz).

Anal: Calc for $C_{18}H_{25}NO_{12}$: C, 48.32; H, 5.63; N, 3.13. Found: C, 48.26; H, 5.55; N, 2.87.

2-succinamido 2-deoxy D-glucose 13.

Deacetylation of 12 was performed as for 5b. After evaporation of methanol the solid was washed with diethyl ether. Yield: 86%. For ^{1}H NMR data (D_2O) see tables III and IV. Ratio α/β = 60/40. IR: ν_{max} 3600-2800 (OH, NH), 1700 (C=O acide), 1650, 1530 (C=O amide).

Anal: Calc for $C_{10}H_{17}NO_8$: C, 43.01; H, 6.14; N, 5.02. Found: C, 42.92; H, 6.22; N, 5.18.

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