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Carbohydrate Research 343 (2008) 1840–1848
Carbohydrate RESEARCH

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# Fructose-fused γ-butyrolactones and lactams, synthesis and biological evaluation as GABA receptor ligands

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Received 5 February 2008; received in revised form 7 March 2008; accepted 9 March 2008
Available online 14 March 2008

**Abstract**—We describe the synthesis of sugar-fused β-disubstituted γ-butyrolactones, γ-butyrolactams and a lipophilic β-disubstituted GABA analogue as potential GABA receptor ligands, where the pharmacophore is engineered into the carbohydrate scaffold in the form of a C-fructoside. The products were characterized for receptor binding studies of $GABA_A$ receptors.


*Keywords:* C-Glycosides; Fructose; γ-Butyrolactones; γ-Butyrolactams; GABA receptors

Gamma-amino butyric acid (GABA, **1**, Fig. 1) is the primary inhibitory neurotransmitter in the mammalian central nervous system (CNS).[1] GABA operates through multiple receptors subdivided into the ionotropic $GABA_A$ and $GABA_C$ receptors and the metabotropic $GABA_B$ receptor.[2] These receptors are the target for many endogenous and exogenous modulators that regulate normal and pathological brain mechanisms, such as sleep, memory, epilepsy and emotions,[3] and for a number of drugs[4] including benzodiazepines, barbiturates and neurosteroids. Consequently, compounds that act at GABA receptors have considerable therapeutic interest for use in a variety of neurological disorders, such as epilepsy,[5] anxiety,[5b,6] schizophrenia,[5b,7] stiff-person syndrome[5b,8] and Huntington's chorea.[9]

While GABA itself has not been shown to be pharmacologically useful,[10] many attempts have been made to produce GABAergic drugs and prodrugs. Several GABA receptor ligands derived from GABA (Fig. 1), such as the selective agonists muscimol (**7**) and 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]-pyridine-3-ol (THIP, gaboxadol, **8**) and the antagonist gabazine (**10**), have been developed over the years.[4] In addition, the anticonvulsants Progabide (**2**), Vigabatrin (**3**) and Gabapentin (**4**), containing a GABA substructure are marketed as antiepileptic drugs.[11]

Also, γ-butyrolactones and γ-butyrolactams (Fig. 2) are of biological relevance as GABA receptor ligands.[12] γ-Butyrolactones are common structural motifs encountered in a number of naturally occurring compounds possessing therapeutic properties,[13] and in particular γ-butyrolactones and their derivatives are $GABA_A$ modulator agents of great interest[14] because some of them possess potent anticonvulsant activity in vivo.[15] The γ-lactams, in turn (Fig. 2), have been shown to possess anticonvulsant and antioxidant activity,[16] gabapentin-lactam (GBP-L) is neuroprotective in retinal ischaemia,[17] and in addition, γ-lactams can be useful as key intermediates in the synthesis of pyrrolidines, the biosynthetic precursors of GABA analogues.[18]

Here we describe the synthesis of sugar-fused β-disubstituted γ-butyrolactones **21**–**22**, (Scheme 1) γ-butyrolactams **24**–**27** and a lipophilic β-disubstituted GABA analogue **23** as potential GABA receptor ligands, where the pharmacophore is engineered into the carbohydrate scaffold in the form of a C-fructoside. A few examples of sugar-fused GABA analogues on a galactose or glucose

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doi:10.1016/j.carres.2008.03.013

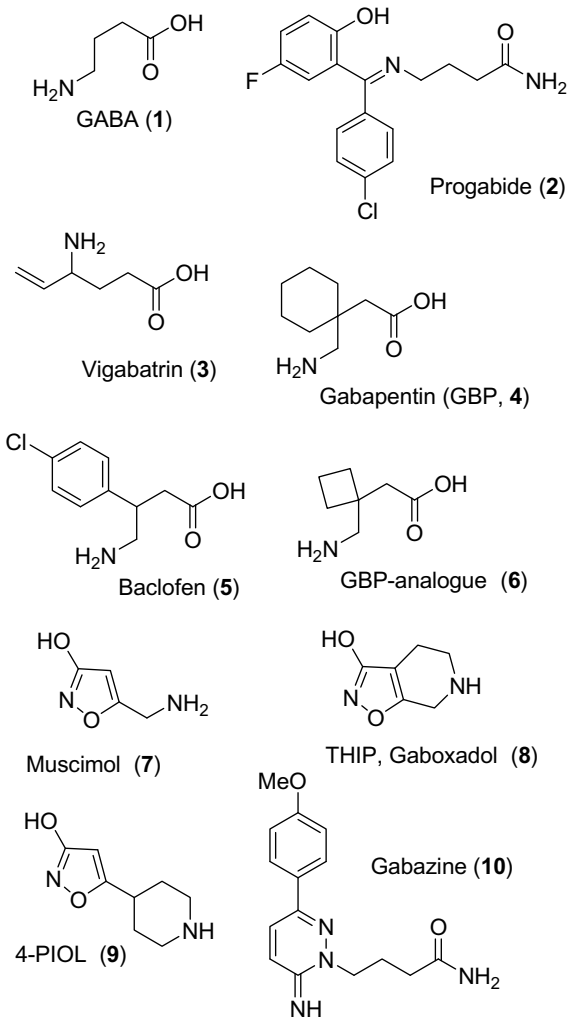



**Figure 1.** Chemical structure of some GABAergic ligands.

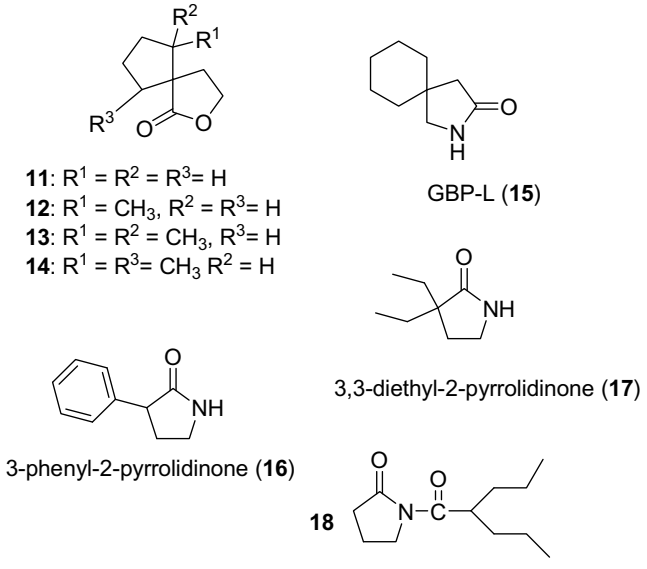
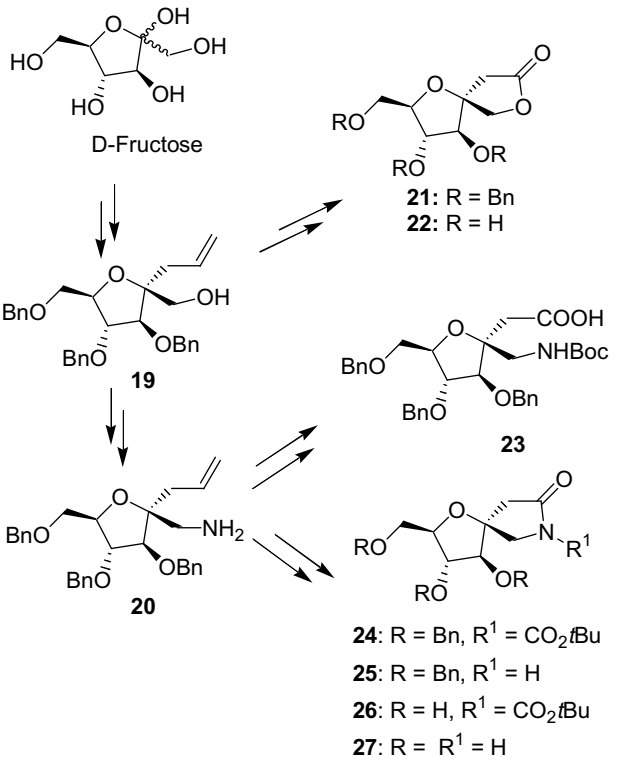



**Figure 2.** Chemical structure of some γ-butyrolactones and γ-butyrolactams as GABA receptor ligands.



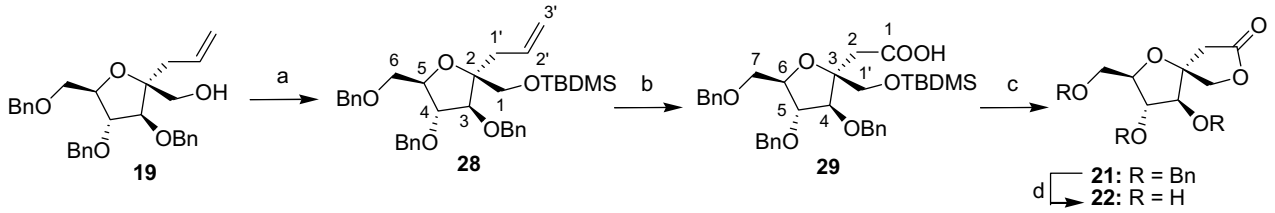

**Scheme 1.** Synthetic scheme towards GABA receptor ligands **21**–**27**.

scaffold have been recently proposed,[19] but no biological evaluation of these compounds was reported.

The fructose moiety acts as versatile scaffold,[20] being rich in stereochemistry and having a relatively rigid skeleton. For GABA receptor ligand action, penetration of the blood-brain barrier (BBB) is required, and lipophilicity is the most important parameter that crucially influences its penetration.[21] It is worthy of note that additional hydroxyl derivatization of the fructose scaffold may be used to increase lipophilicity, as well as to modulate the activity of pharmacophores or the receptor specificity.

The synthesis of title compounds **21**–**27** requires access to key intermediates **19** and **20**. Although the synthesis of compound **19** has been already described,[22] the synthesis of **20** was recently communicated without experimental details in a preliminary account,[23] and will be therefore given here in detail. The anomeric position and the hydroxyl group at C-1 of fructose were exploited for the construction of the butyrolactone or lactam rings with a spiro junction to the carbohydrate scaffold.

GABA lactones **21**–**22** were synthesized via key intermediate **19**, obtained from fully benzylated D-fructose (Scheme 2), after C-allylation of the anomeric position. *C*-Allyl fructofuranoside **19** was protected at the free hydroxyl group affording the corresponding *tert*-butyldimethylsilyl ether **28** with *tert*-butyldimethylchloro silane and imidazole in dry dimethylformamide at reflux (90% yield, Scheme 2).

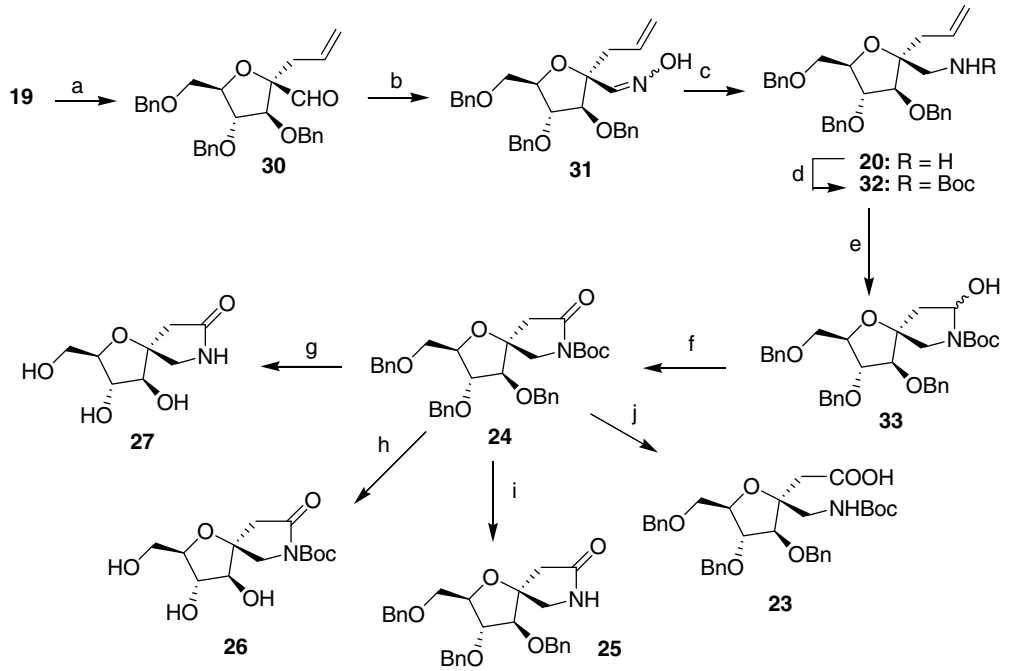



**Scheme 2.** Reagents and conditions: (a) TBDMSCl, imidazole, dry DMF, reflux, 90%; (b) (i) 0.016 M $OsO_4$ in *t*-BuOH, $NaIO_4$, $H_2O$–acetone–*t*-BuOH 1:1:1, rt; (ii) 1.25 M $NaH_2PO_4{\cdot}2H_2O$, $NaClO_2$, $CH_3CN$, rt, 71% over 2 steps; (c) TFA–$H_2O$ 9:1, $CH_2Cl_2$, rt, 94%; (d) $H_2$, $Pd(OH)_2$–C, HCl 37%, MeOH–EtOAc 4:1, rt, 95%.

Oxidative cleavage of the double bond in the *C*-propenyl appendage of compound **28** via a two-step degradative oxidation by reaction with osmium tetroxide–sodium periodate[24] followed by treatment with $NaH_2PO_4$–$NaClO_2$[25] (71% yield over two steps) afforded acid **29**, possessing the carboxylic function suitably positioned for the formation of the γ-butyrolactone ring. Attempts to oxidize the double bond of **19** without hydroxyl protection resulted in low yields in the desired product. Acidic hydrolysis of the silyl ether with aqueous trifluoroacetic acid in dichloromethane directly afforded benzylated γ-lactone **21**. Debenzylation by hydrogenolysis using palladium hydroxide as a catalyst afforded fully deprotected hydrophilic lactone **22** (95% yield).

The synthesis of γ-butyrolactams **24**–**27** and GABA analogue **23** takes advantage once more of the *C*-propenyl substituent for the introduction of the carboxylic group. In addition, the synthesis of these compounds required the introduction of the amino functionality in place of the hydroxyl group at C-1 of fructose. To this end, key intermediate **20** was synthesized from alcohol **19** and subsequently transformed into title compounds **23**–**27** (Scheme 3).

Alcohol **19** was converted into aldehyde **30**; the carbonyl group of **30** was transformed into the corresponding amine required for the construction of the lactam ring by oximation to compound **31** ($NH_2OH{\cdot}HCl$, pH 4.5, 92% yield as a mixture of *E*/*Z* isomers), followed by reduction with $LiAlH_4$ to amine **20** (quantitative yield). The allyl-containing amine derivative **20** could provide γ-butyrolactam **24** by oxidation and γ-lactam formation.[26] Hence, protection of amine **20** in the presence of Boc anhydride and triethylamine afforded compound **32**. Oxidative cyclization of **32** to the corresponding lactam **24** proceeded in a two-step fashion




**Scheme 3.** Reagents and conditions: (a) PCC, $CH_2Cl_2$, 4 Å m.s., 85% (Ref. 20); (b) $NH_2OH{\cdot}HCl$ pH 4.5, MeOH–THF 1:1, 94%; (c) $LiAlH_4$, THF, quant. yield (d) $(Boc)_2$, TEA, $CH_2Cl_2$, rt, 87%; (e) (i) 0.016M $OsO_4$ in *t*-BuOH, NMO, THF–$H_2O$ 1:1; (ii) $NaIO_4$, THF–$H_2O$ 1:1, rt, 85%; (f) PCC, 4 Å m.s., $CH_2Cl_2$, rt, 78%; (g) $H_2$, $Pd(OH)_2$–C, HCl 37%, MeOH–EtOAc 4:1, rt, 74%; (h) $H_2$, $Pd(OH)_2$–C, acetic acid, MeOH–EtOAc 4:1, rt, 70%; (i) TFA–$H_2O$ 8:2, $CH_2Cl_2$, rt, 76%; (j) LiOH, THF, rt, 61%.

by initial osmylation–$NaIO_4$ oxidation to lactol **33**, followed by further oxidation using pyridinium chlorochromate (PCC). Hydrolysis of lactam **24** using 1 N lithium hydroxide provided the Boc-protected GABA analogue **23** in 61% yield. Any attempt to obtain a fully deprotected GABA analogue failed. Hydrogenolysis of lactam **24** using palladium hydroxide as the catalysts in the presence of hydrochloric acid afforded fully deprotected and hydrophilic γ-lactam **27** (74% yield); on the contrary, hydrogenolysis with the same catalyst but with acetic acid as the acid afforded *N*-Boc-protected lactam **26** (70% yield). Finally, treatment of lactam **25** with aqueous trifluoroacetic acid afforded benzylated lactam **25** (76% yield).

Compounds **21**–**27** were characterized in receptor binding studies at $GABA_A$ receptors using rat brain membrane preparations. The ability of the new compounds to bind to $GABA_A$ receptor was determined using [$^3$H]muscimol[27] and the results are reported in Table 1. Data show that compounds **21**, **22**, **24** and **26** possess affinity for $GABA_A$ receptor significantly inhibiting [$^3$H]muscimol binding in the μM range. In particular, the most potent compound in the displacement binding study is the *N*-Boc-protected lactam **26** with a 40% reduction of [$^3$H]muscimol specific binding.

These biological data demonstrate that both butyrolactones **21**–**22**, and N-substituted lactams **24** and **26**, are able to displace tritiated muscimol from the GABA binding site. By this preliminary evaluation we can argue that the sugar moiety does not seem to hinder the binding, as both benzylated and deprotected lactones and lactams had comparable activity. Hence, the carbohydrate moiety can effectively be used to modulate drug pharmacokinetic properties and lipophilicity. It is worth noting that the hydrophobic nature of benzyl groups can facilitate BBB crossing, which is one of the main issues to be addressed for CNS directed drugs.

In summary, the synthesis of diverse butyrolactones and lactams has been accomplished, and preliminary biological evaluation of title compounds as GABA receptor ligands has been performed.

**Table 1.** Binding competition study of the synthesized products with [$^3$H] muscimol at $GABA_A$ receptor performed on rat cerebral membranes

| Compound | % [$^3$H]Muscimol specific binding[a] | Significance versus control[b] |
|---|---|---|
| **Control** | 100.00 ± 3.053 | |
| **21** | 75.55 ± 1.013 | $P < 0.01$ |
| **22** | 77.84 ± 0.446 | $P < 0.05$ |
| **23** | 99.33 ± 2.073 | n.s. |
| **24** | 70.77 ± 4.391 | $P < 0.05$ |
| **25** | 76.75 ± 0.029 | n.s. |
| **26** | 60.42 ± 6.668 | $P < 0.05$ |
| **27** | 96.80 ± 1.976 | n.s. |

[a] Values are means ± SEM determined from at least three independent experiments.

[b] Statistical analysis is performed with Kruskal–Wallis ANOVA for nonparametric values followed by Dunns test.

## 1. Experimental

### 1.1. Synthesis, general procedures

All solvents were dried over molecular sieves (Fluka), for at least 24 h prior to use. When dry conditions were required, the reactions were performed under an argon atmosphere. Thin-layer chromatography (TLC) was performed on Silica Gel 60 $F_{254}$ plates (Merck) with detection with UV light when possible, or charring with a solution containing conc. $H_2SO_4$–EtOH–$H_2O$ in a ratio of 5:45:45 followed by heating at 180 °C. Flash column chromatography was performed on silica gel 230–400 mesh (Merck). The boiling range of petroleum ether used as eluent in column chromatography is 40–60 °C. NMR spectra were recorded at 400 MHz ($^1$H) and at 100.57 MHz ($^{13}$C) on a Varian MERCURY instrument at 300 K. Chemical shift values ($\delta$) are reported in ppm downfield from TMS as an internal standard; *J* values are given in Hertz. Mass spectra were recorded on a MALDI2 Kompact Kratos instrument, with gentisic acid (DHB) as the matrix. Compounds are numbered and named systematically as reported.[19b]

### 1.2. 1-Amino-2,5-Anhydro-3,4,6-tri-*O*-benzyl-1-deoxy-2-*C*-(prop-2-enyl)-D-glucitol (20)

Oxime **31** (0.500 g, 1.02 mmol) was dissolved in dry THF (5 mL), and 1.6 mL of 1 M $LiAlH_4$ solution in THF was added. The reaction mixture was stirred overnight, then quenched by the addition of EtOAc; the precipitate was filtered off, and the solvent evaporated under reduced pressure. Purification by flash chromatography (6:4 EtOAc–EtOH), afforded 0.480 mg of **20**, as a colourless oil. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.43–7.18 (m, 15H, Ph-*H*), 5.85–5.77 (m, 1H, H-2′), 5.13 (d, 1H, $J_{3'a,2} = 10.0$ Hz, H-3′a), 5.07 (d, 1H, $J_{3'b,2} = 17.8$ Hz, H-3′b), 4.68–4.51 (m, 8H, PhC$H_2$O, $NH_2$), 4.21 (dd, 1H, $J_{4,5} = 7.0$ Hz, $J_{3,4} = 5.5$ Hz, H-4), 4.09 (d, 1H, $J_{3,4} = 5.5$ Hz, H-3), 4.00 (dt, 1H, $J_{4,5} = 7.0$ Hz, $J_{5,6} = 4.0$ Hz, H-5), 3.68 (dd, 1H, $J_{6a,6b} = 10.6$ Hz, $J_{5,6a} = 4.0$ Hz, H-6a), 3.58 (dd, 1H, $J_{6a,6b} = 10.6$ Hz, $J_{5,6b} = 4.0$ Hz, H-6b), 2.90 (d, 1H, $J_{1a,1b} = 14.1$ Hz, H-1a), 2.70 (d, 1H, $J_{1a,1b} = 14.1$ Hz, H-1b), 2.39 (dd, 1H, $J_{1'a,1'b} = 13.9$ Hz, $J_{1'a,2'}$ 7.1 Hz, H-1′a), 2.30 (dd, 1H, $J_{1'a,1'b} = 13.9$ Hz, 13.9 Hz, $J_{1'b,2'}$ 7.5 Hz, H-1′b); $^{13}$C NMR (100.57 MHz, $CDCl_3$): $\delta$ 138.20, 138.14, 138.12 (3s, Cq arom.), 133.49 (d, C-2′), 119.03 (t, C-3′), 85.27 (s, C-2) 87.97, 84.66, 79.39 (3d, C-3, C-4, C-5), 73.74, 73.06, 72.94, 70.24 (4t, Ph*C*$H_2$O, C-6), 47.50 (t, C-1), 41.99 (t, C-1′). MALDI-MS *m*/*z* calcd for $C_{30}H_{35}NO_4$: 473.60. Found:

475 $[M+H]^+$, 497 $[M+Na]^+$. Anal. Calcd for $C_{30}H_{35}NO_4$: C, 76.08; H, 7.45; N, 2.96. Found: C, 76.10; H, 7.48; N, 2.93.

### 1.3. 3,6-Anhydro-4,5,7-tri-*O*-benzyl-2-deoxy-3-*C*-(hydroxymethyl)-D-*manno*-heptonic acid lactone (21)

To a solution of **29** (0.250 g, 0.412 mmol) in $CH_2Cl_2$ (8 mL), a mixture of 9:1 TFA–$H_2O$ (0.3 mL) was added at 0 °C. The reaction mixture was allowed to warm at room temperature and stirred for 1 h. The reaction was quenched by slowly adding $NaHCO_3$ until neutral pH. The product was extracted with $CH_2Cl_2$ and the organic layer was dried on sodium sulfate, filtered and evaporated. Flash column chromatography of the residue (8:2 petroleum ether–EtOAc) afforded **21** (0.184 g, 94%) as a dark yellow oil. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.40–7.22 (m, 15H, Ph-*H*), 4.63–4.51 (m, 6H, PhC$H_2$O), 4.39 (d, 1H, $J_{1'a,1'b} = 9$ Hz, H-1′a), 4.37 (d, 1H, $J_{1'a,1'b} = 9$ Hz, H-1′b), 4.23 (dt, 1H, $J_{6,7} = 6.1$ Hz, $J_{5,6} = 3$ Hz, H-6), 4.04 (dd, 1H, $J_{5,6} = 3$ Hz, $J_{4,5} = 1.7$ Hz, H-5), 3.94 (d, 1H, $J_{4,5} = 1.7$ Hz, H-4), 3.60 (dd, 1H, $J_{7a,7b} = 10$ Hz, $J_{6,7a} = 5.5$ Hz, H-7a), 3.52 (dd, 1H, $J_{7a,7b} = 10$ Hz, $J_{6,7b} = 6.4$ Hz, H-7b), 2.77 (d, 1H, $J_{2a,2b} = 18$ Hz, H-2a), 2.63 (d, 1H, $J_{2a,2b} = 18$ Hz, H-2b); $^{13}$C NMR (100.57 MHz, $CDCl_3$): $\delta$ 175.12 (s, *C*O), 138.02, 137.53, 137.09 (3s, Cq arom.), 87.36 (s, C-3), 85.81, 82.89, 82.77 (3d, C-4, C-5, C-6), 73.74 (t, C-1′), 72.38, 72.18, 72.02, 70.42 (4t, Ph*C*$H_2$O, C-7), 39.60 (t, C-2). MALDI-MS *m*/*z* calcd for $C_{29}H_{30}O_6$: 474.54. Found: 498 $[M+Na]^+$, 514 $[M+K]^+$. Anal. Calcd for $C_{29}H_{30}O_6$: C, 73.40; H, 6.37. Found: C, 73.38; H, 6.39.

### 1.4. 3,6-Anhydro-2-deoxy-3-*C*-(hydroxymethyl)-D-*manno*-heptonic acid lactone (22)

Compound **21** (0.250 g, 0.527 mmol) was dissolved in 4:1 MeOH–EtOAc mixture (15 mL) and a few drops of HCl 37% and catalytic $Pd(OH)_2$–C (10% w/w) were added. The flask was purged 3 times with Ar and then filled with $H_2$. After 12 h, the catalyst was removed by filtration, and the filtrate concentrated under reduced pressure. The crude residue was purified by silica gel flash chromatography (EtOAc) affording compound **22** as a yellow oil (0.102 g, 95%). $^1$H NMR (400 MHz, $D_2O$): $\delta$ 4.50 (d, 1H, $J_{1'a,1'b} = 10.6$ Hz, H-1′a), 4.23 (d, 1H, $J_{1'a,1'b} = 10.6$ Hz, H-1′b), 4.07 (d, 1H, $J_{4,5} = 3.8$ Hz, H-4), 3.89 (dd, 1H, $J_{5,6} = 5.1$ Hz, $J_{4,5} = 3.8$ Hz, H-5), 3.80 (dt, 1H, $J_{5,6} = 5.1$ Hz, $J_{6,7} = 3.5$ Hz, H-6), 3.60 (dd, 1H, $J_{7a,7b} = 12.3$ Hz, $J_{6,7a} = 3.5$ Hz, H-7a), 3.52 (dd, 1H, $J_{7a,7b} = 12.3$ Hz, $J_{6,7b} = 5.3$ Hz, H-7b), 2.81 (d, 1H, $J_{2a,2b} = 18$ Hz, H-2a), 2.68 (d, 1H, $J_{2a,2b} = 18$ Hz, H-2b); $^{13}$C NMR (100.57 MHz, $D_2O$): $\delta$ 178.83 (s, *C*O), 87.47 (s, C-3), 83.69, 79.48, 76.64 (3d, C-4, C-5, C-6), 74.88 (t, C-1′), 61.42 (t, C-7), 38.70 (t, C-2). MALDI-MS *m*/*z* calcd for $C_8H_{12}O_6$: 204.18. Found: 227 $[M+Na]^+$, 243 $[M+K]^+$. Anal. Calcd for $C_8H_{12}O_6$: C, 47.06; H, 5.92. Found: C, 47.04; H, 5.97.

### 1.5. 3,6-Anhydro-4,5,7-tri-*O*-benzyl-2-deoxy-3-*C*-(aminomethyl(*N*-*tert*-butoxycarbonyl))-D-*manno*-heptonic acid (23)

To a solution of **24** (0.250 g, 0.436 mmol) in dry THF (7 mL) 1 M $LiOH·H_2O$ (1.74 mL, 1.74 mmol) was added, and the resulting solution was stirred at room temperature for 2 h. THF was evaporated, and the resulting residue was dissolved in $Et_2O$ and filtered. The resulting filtrate was washed with $Et_2O$ and the aqueous layer was acidified with 1 M HCl and extracted with $Et_2O$. The combined organic layers were dried ($Na_2SO_4$), filtered and concentrated to dryness providing **23** as a yellow oil (0.157 g, 61%). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.25–7.16 (m, 15H, Ph-*H*), 5.05 (t, 1H, $J = 6.3$ Hz, N*H*), 4.53–4.33 (m, 6H, PhC$H_2$O), 4.23 (d, 1H, $J_{4,5} = 3.9$ Hz, H-4), 4.11–4.07 (m, 1H, H-6), 3.97 (dd, 1H, $J_{5,6} = 6.8$ Hz, $J_{4,5} = 3.9$ Hz, H-5), 3.47 (dd, 1H, $J_{7a,7b} = 10.3$ Hz, $J_{6,7a} = 4.6$ Hz, H-7a), 3.45–3.42 (m, 2H, H-1′), 3.40 (dd, 1H, $J_{7a,7b} = 10.3$ Hz, $J_{6,7b} = 4.6$ Hz, H-7b), 2.64 (d, 1H, $J_{2a,2b} = 14$ Hz, H-2a), 2.57 (d, 1H, $J_{2a,2b} = 14$ Hz, H-2b), 1.48 (s, 9H, C(C$H_3$)$_3$); $^{13}$C NMR (100.57 MHz, $CDCl_3$): $\delta$ 178.24 (s, *C*O-1), 156.76 (s, *C*O), 138.04, 137.83, 137.63 (3s, Cq arom.), 86.63, 85.51, 83.89 (3d, C-4, C-5, C-6), 85.48, 80.48 (2s, C-3,*C*($CH_3$)$_3$), 77.62, 77.30, 76.98, 69.99 (4t, Ph*C*$H_2$O, C-7), 44.20 (t, C-1′), 40.42 (t, C-2), 28.65, 28.58, 27.98 (3q, C(*C*$H_3$)$_3$). MALDI-MS *m*/*z* calcd for $C_{34}H_{41}NO_8$: 591.69. Found: 615 $[M+Na]^+$, 631 $[M+K]^+$. Anal. Calcd for $C_{34}H_{41}NO_8$: C, 69.02; H, 6.98; N, 2.37. Found: C, 69.08; H, 6.95; N, 2.40.

### 1.6. 3,6-Anhydro-4,5,7-tri-*O*-benzyl-2-deoxy-3-*C*-(aminomethyl(*N*-*tert*-butoxycarbonyl))-D-*manno*-heptonic acid lactam (24)

To a solution of **33** (0.250 g, 0.434 mmol) and molecular sieves (4 Å, 300 mg) in dry $CH_2Cl_2$ (8 mL) pyridinium chlorochromate (PCC, 0.187 g, 0.868 mmol) was added and the mixture was stirred for 2 h. The resulting mixture was filtered through a Celite pad eluting with EtOAc. The filtrate was concentrated and purified by silica gel flash chromatography (7:3 petroleum ether–EtOAc) to give lactam **24** as a white solid (0.194 g, 78% yield). Mp = 82–85 °C; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.37–7.22 (m, 15H, Ph-*H*), 4.60–4.37 (m, 6H, PhC$H_2$O), 4.2–4.15 (m, 1H, H-6), 4.02 (d, 1H, $J_{1'a,1'b} = 12.4$ Hz, H-1′a), 3.99 (dd, 1H, $J_{5,6} = 3.2$ Hz, $J_{4,5} = 1.8$ Hz, H-5), 3.85 (d, 1H, $J_{1'a,1'b} = 12.4$ Hz, H-1′b), 3.82 (d, 1H, $J_{4,5} = 1.8$ Hz, H-4), 3.57 (dd, 1H, $J_{7a,7b} = 10$ Hz, $J_{6,7a} = 5.6$ Hz, H-7a), 3.49 (dd, 1H, $J_{7a,7b} = 10$ Hz, $J_{6,7b} = 6.1$ Hz, H-7b), 2.77 (d, 1H,

$J_{2a,2b} = 18$ Hz, H-2a), 2.64 (d, 1H, $J_{2a,2b} = 18$ Hz, H-2b), 1.5 (s, 9H, C($CH_3$)$_3$); $^{13}$C NMR (100.57 MHz, $CDCl_3$): $\delta$ 171.70 (s, *C*O-1), 149.97 (s, *C*O), 138.12, 137.66, 137.34 (3s, Cq arom.), 85.9, 83.21, 82.23 (3d, C-4, C-5, C-6), 83.2, 83.0 (2s, C-3,*C*($CH_3$)$_3$), 73.60, 72.11, 71.95, 70.44 (4t, Ph*C*$H_2$O, C-7), 53.06 (t, C-2), 43.92 (t, C-1′), 28.96, 28.27, 27.75 (3q, C(*C*$H_3$)$_3$). MALDI-MS *m*/*z* calcd for $C_{34}H_{39}NO_7$: 573.68. Found: 597 $[M+Na]^+$, 613 $[M+K]^+$. Anal. Calcd for $C_{34}H_{39}NO_7$: C, 71.18; H, 6.85; N, 2.44. Found: C, 71.20; H, 6.90; N, 2.40.

### 1.7. 3,6-Anhydro-4,5,7-tri-*O*-benzyl-2-deoxy-3-*C*-(aminomethyl)-D-*manno*-heptonic acid lactam (25)

A solution of **24** (0.250 g, 0.436 mmol) in dry $CH_2Cl_2$ (7 mL) and 80% aqueous TFA (13 mL) was stirred for 2 h at room temperature. Then, the mixture was neutralized with an satd aq $NaHCO_3$, and extracted with $CH_2Cl_2$. The organic layer was dried ($Na_2SO_4$), filtered and concentrated to dryness and then purified by silica gel flash chromatography (5:5 petroleum ether–EtOAc) to give **25** as a yellow oil (0.157 g, 76% yield). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.3–7.14 (m, 15H, Ph-*H*), 5.5 (s, 1H, N*H*), 4.52–4.31 (m, 6H, PhC$H_2$O), 4.12–4.10 (m, 1H, H-6), 3.91 (dd, 1H, $J_{5,6} = 3.5$ Hz, $J_{4,5} = 1.9$ Hz, H-5), 3.8 (d, 1H, $J_{4,5} = 1.9$ Hz, H-4), 3.7 (d, 1H, $J_{1'a,1'b} = 12$ Hz, H-1′a), 3.5 (dd, 1H, $J_{7a,7b} = 10$ Hz, $J_{6,7a} = 5.6$ Hz, H-7a), 3.42 (dd, 1H, $J_{7a,7b} = 10$ Hz, $J_{6,7b} = 6.1$ Hz, H-7b), 3.4 (d, 1H, $J_{1'a,1'b} = 12$ Hz, H-1′b), 2.52 (d, 1H, $J_{2a,2b} = 17.5$ Hz, H-2a), 2.44 (d, 1H, $J_{2a,2b} = 17.5$ Hz, H-2b); $^{13}$C NMR (100.57 MHz, $CDCl_3$): $\delta$ 175.4 (s, *C*O), 138.16, 137.74, 137.44 (3s, Cq arom.), 87.45 (s, C-3), 86.14, 83.41, 82.10 (3d, C-4, C-5, C-6), 73.59, 72.06, 71.89, 70.52 (4t, Ph*C*$H_2$O, C-7), 48.74 (t, C-2), 41.45 (t, C-1′). MALDI-MS *m*/*z* calcd for $C_{29}H_{31}NO_5$: 473.56 Found: 497 $[M+Na]^+$, 513 $[M+K]^+$. Anal. Calcd for $C_{29}H_{31}NO_5$: C, 73.55; H, 6.60; N, 2.96. Found: C, 73.50; H, 6.64; N, 2.99.

### 1.8. 3,6-Anhydro-2-deoxy-3-*C*-(aminomethyl(*N*-*tert*-butoxycarbonyl))-D-*manno*-heptonic acid lactam (26)

Compound **24** (0.250 g, 0.436 mmol) was dissolved in 4:1 MeOH–EtOAc mixture (15 mL) and a few drops of acetic acid and catalytic $Pd(OH)_2$–C (10% w/w) were added. The flask was purged 3 times with Ar and then filled with $H_2$. After 12 h, the catalyst was removed by filtration, and the filtrate concentrated under reduced pressure. The crude residue was purified by silica gel flash chromatography (9:1 EtOAc–MeOH) affording compound **26** as a yellow oil (0.093 g, 70%). $^1$H NMR (400 MHz, $D_2O$): $\delta$ 3.97 (d, 1H, $J_{4,5} = 4.4$ Hz, H-4), 3.93 (d, 1H, $J_{1'a,1'b} = 12.4$ Hz, H-1′a), 3.88 (m, 1H, H-5), 3.8–3.76 (m, 1H, H-6), 3.66 (d, 1H, $J_{1'a,1'b} = 12.4$ Hz, H-1′b), 3.56 (dd, 1H, $J_{7a,7b} = 12.4$ Hz, $J_{6,7a} = 5.3$ Hz, H-7a), 3.5 (dd, 1H, $J_{7a,7b} = 12.4$ Hz, $J_{6,7b} = 5.4$ Hz, H-7b), 2.77 (d, 1H, $J_{2a,2b} = 18$ Hz, H-2a), 2.62 (d, 1H, $J_{2a,2b} = 18$ Hz, H-2b), 1.34 (s, 9H, C($CH_3$)$_3$); $^{13}$C NMR (100.57 MHz, $D_2O$): $\delta$ 178.19 (s, *C*O-1), 153.52 (s, *C*O), 87.94, 85.3 (2s, C-3, *C*($CH_3$)$_3$), 85.55, 82.38, 81.99 (3d, C-4, C-5, C-6), 63.90 (t, C-7), 56.27 (t, C-2), 45.68 (t, C-1′), 28.94, 28.54, 27.85 (3q, C(*C*$H_3$)$_3$). MALDI-MS *m*/*z* calcd for $C_{13}H_{21}NO_7$: 303.31. Found: 326 $[M+Na]^+$, 342 $[M+K]^+$. Anal. Calcd for $C_{13}H_{21}NO_7$: C, 51.48; H, 6.98; N, 4.62. Found: C, 51.53; H, 6.92; N, 4.65.

### 1.9. 3,6-Anhydro-2-deoxy-3-*C*-(aminomethyl)-D-*manno*-heptonic acid lactam (27)

Compound **24** (0.250 g, 0.436 mmol) was dissolved in 4:1 MeOH–EtOAc mixture (15 mL) and a few drops of HCl 37% and catalytic $Pd(OH)_2$–C (10% w/w) were added. The flask was purged 3 times with Ar and then filled with $H_2$. After 12 h, the catalyst was removed by filtration, and the filtrate concentrated under reduced pressure. The crude residue was purified by silica gel flash chromatography (7.5:2:0.5 EtOAc–MeOH–$H_2O$) affording compound **27** as a yellow oil (0.088 g, 74%). $^1$H NMR (400 MHz, $D_2O$): $\delta$ 4.24 (s, 1H, N*H*), 3.99 (d, 1H, $J_{4,5} = 4$ Hz, H-4), 3.88 (dd, 1H, $J_{5,6} = 5.3$ Hz, $J_{4,5} = 4$ Hz, H-5), 3.8–3.76 (m, 1H, H-6), 3.66 (d, 1H, $J_{1'a,1'b} = 12$ Hz, H-1′a), 3.6 (dd, 1H, $J_{7a,7b} = 12.3$ Hz, $J_{6,7a} = 3.6$ Hz, H-7a), 3.51 (dd, 1H, $J_{7a,7b} = 12.3$ Hz, $J_{6,7b} = 5.4$ Hz, H-7b), 3.24 (d, 1H, $J_{1'a,1'b} = 12$ Hz, H-1′b), 2.58 (d, 1H, $J_{2a,2b} = 18$ Hz, H-2a), 2.42 (d, 1H, $J_{2a,2b} = 18$ Hz, H-2b); $^{13}$C NMR (100.57 MHz, $D_2O$): $\delta$ 180.72 (s, *C*O), 89.85 (s, C-3), 85.58, 82.38, 79.20 (3d, C-4, C-5, C-6), 63.99 (t, C-7), 52.15 (t, C-2), 43.41 (t, C-1′). MALDI-MS *m*/*z* calcd for $C_8H_{13}NO_5$: 203.19. Found: 226 $[M+Na]^+$, 242 $[M+K]^+$. Anal. Calcd for $C_8H_{13}NO_5$: C, 47.29; H, 6.45; N, 6.89. Found: C, 47.20; H, 6.40; N, 6.91.

### 1.10. 2,5-Anhydro-3,4,6-tri-*O*-benzyl-2-*C*-(prop-2-enyl)-1-*O*-*tert*-butyldimethylsilyl-D-glucitol (28)

To a solution of **19** (0.250 g, 0.527 mmol) in dry DMF (10 mL), *tert*-butyldimethylsilyl chloride (TBDMSCl, 0.198 g, 1.32 mmol) and imidazole (0.106 g, 1.58 mmol) were added under argon atmosphere, and the resulting mixture was stirred under reflux for 12 h. The solvent was then removed under reduced pressure and the residue dissolved in $CH_2Cl_2$ and washed with brine. The organic layer was dried ($Na_2SO_4$), filtered and evaporated. The crude residue was purified by silica gel flash chromatography (9:1 petroleum ether–EtOAc), affording **28** as a yellow oil (0.279 g, 90% yield). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.38–7.25 (m, 15H, Ph-*H*), 5.81–5.79 (m, 1H, H-2′), 5.10 (d, 1H,

$J_{3'a,2}$ = 10.2 Hz, H-3′a), 4.98 (d, 1H, $J_{3'b,2}$ = 17 Hz, H-3′b), 4.75–4.46 (m, 6H, PhC$H_2$O), 4.10 (dq, 1H, $J_{4,5}$ = 5.3 Hz, $J_{5,6}$ = 1.3 Hz, H-5), 4.04 (dd, 1H, $J_{4,5}$ = 5.3 Hz, $J_{3,4}$ = 3.6 Hz, H-4), 3.96 (d, 1H, $J_{3,4}$ = 3.6 Hz, H-3), 3.69 (dd, 1H, $J_{6a,6b}$ = 10.0 Hz, $J_{5,6a}$ = 1.4 Hz, H-6a), 3.63 (dd, 1H, $J_{6a,6b}$ = 10.0 Hz, $J_{5,6b}$ = 1.2 Hz, H-6b), 3.60–3.52 (m, 2H, H-1), 2.52–2.41 (m, 2H, H-1′), 0.88 (s, 9H, C(C$H_3$)$_3$), 0.04 (s, 3H, SiC$H_3$), −0.08 (s, 3H, SiC$H_3$); $^{13}$C NMR (100.57 MHz, $CDCl_3$): δ 138.27, 138.16, 137.69 (3s, Cq arom.), 133.05 (d, C-2′), 119.18 (t, C-3′), 84.40 (s, C-2), 86.69, 83.58, 79.46 (3d, C-3, C-4, C-5), 73.74, 73.25, 70.96, 69.72 (4t, PhC$H_2$O, C-6), 66.51 (t, C-1), 40.45 (t, C-1′), 26.94, 25.17, 24.53 (3q, C(C$H_3$)$_3$), 20.15 (s, C(CH$_3$)$_3$), −4.6, −4.8 (2q, SiC$H_3$). MALDI-MS *m*/*z* calcd for $C_{36}H_{48}O_5Si$: 588.33. Found: 611 $[M+Na]^+$, 627 $[M+K]^+$. Anal. Calcd for $C_{36}H_{48}O_5Si$: C, 73.43; H, 8.22; Si, 4.77. Found: C, 73.46; H, 8.20; Si, 4.73.

### 1.11. 3,6-Anhydro-4,5,7-tri-*O*-benzyl-2-deoxy-3-*C*-(hydroxymethyl(*O*-*tert*-butyldimethylsilyl))-D-*manno*-heptonic acid (29)

To a solution of **28** (0.250 g, 0.424 mmol) in 1:1:1 $H_2O$–acetone-*t*-BuOH (10 mL), 0.016M $OsO_4$ in *t*-BuOH (1.3 mL, 0.021 mmol) was added. After 30 min the mixture was treated with sodium periodate (0.181 g, 0.848 mmol) and 2 h later the reaction solution was filtered and the solvents evaporated in vacuo without heating, affording the desired aldehyde that was used without further purification. To a solution of the aldehyde (0.250 g, 0.423 mmol) in dry $CH_3CN$ (8 mL) a solution of $NaH_2PO_4{\cdot}2H_2O$ 1.25 M (3.3 mL, 4.23 mmol) and sodium chlorite (0.382 g, 4.23 mmol) was added at rt. After 3 h, the solvents were evaporated in vacuo and the resulting residue was dissolved in $CH_2Cl_2$, filtered and evaporated. The residue was purified by flash column chromatography (8:2 petroleum ether–EtOAc) affording **29** as a yellow oil (0.182 g, 71% yield). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.30–7.19 (m, 15H, Ph-*H*), 4.54–4.45 (m, 6H, PhC$H_2$O), 4.28–4.26 (m, 1H, H-6), 3.98 (dd, 1H, $J_{5,6}$ = 5.6 Hz, $J_{4,5}$ = 2.2 Hz, H-5), 3.96 (d, 1H, $J_{4,5}$ = 2.2 Hz, H-4), 3.84 (d, 1H, $J_{1'a,1'b}$ = 10.3 Hz, H-1′a), 3.73 (d, 1H, $J_{1'a,1'b}$ = 10.3 Hz, H-1′b), 3.56 (dd, 1H, $J_{7a,7b}$ = 9.8 Hz, $J_{6,7a}$ = 6.0 Hz, H-7a), 3.46 (dd, 1H, $J_{7a,7b}$ = 9.8 Hz, $J_{6,7b}$ = 6.1 Hz, H-7b), 2.92 (d, 1H, $J_{2a,2b}$ = 15 Hz, H-2a), 2.74 (d, 1H, $J_{2a,2b}$ = 15 Hz, H-2b), 0.86 (s, 9H, C(C$H_3$)$_3$), 0.03 (s, 3H, SiC$H_3$), −0.02 (s, 3H, SiC$H_3$); $^{13}$C NMR (100.57 MHz, $CDCl_3$): δ 173.5 (s, *C*O), 138.77, 138.68, 137.91 (3s, Cq arom.), 83.95 (s, C-3), 86.19, 82.45, 78.93 (3d, C-4, C-5, C-6), 73.17, 72.95, 72.07, 70.25 (4t, PhC$H_2$O, C-7), 63.43 (t, C-1′), 36.50 (t, C-2), 26.21, 25.95, 23.43 (3q, C(C$H_3$)$_3$), 21.25 (s, C(CH$_3$)$_3$), −3.9, −4.2 (2q, SiC$H_3$). MALDI-MS *m*/*z* calcd for $C_{35}H_{46}O_7Si$: 606.82. Found: 630 $[M+Na]^+$, 646 $[M+K]^+$. Anal. Calcd for $C_{35}H_{46}O_7Si$: C, 69.27; H, 7.64; Si, 4.63. Found: C, 69.29; H, 7.60; Si, 4.60.

### 1.12. 2,5-Anhydro-3,4,6-tri-*O*-benzyl-2-*C*-(prop-2-enyl)-D-glucose oxime (31)

Aldehyde **30** (0.570 g, 1.21 mmol) was dissolved in a 1:1 THF–EtOH mixture (5 mL), and 0.252 g of hydroxylamine hydrochloride (3.63 mmol) dissolved in a pH 4.5 acetate buffer (2.5 mL) was added. After 4 h, the reaction mixture was extracted with dichloromethane, the organic layer dried over $Na_2SO_4$, filtered and evaporated to dryness. The crude was purified by flash chromatography (8.5:1.5 petroleum ether–EtOAc), affording 0.545 g of **31**, as a colourless oil (92% yield). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.51 (s, 1H, OH), 7.38–7.22 (m, 16H, Ph-*H*, H-1), 5.95–5.82 (m, 1H, H-2′), 5.13 (d, 1H, $J_{3'a,2'}$ = 10.2 Hz, H-3′a), 5.09 (d, 1H, $J_{3'b,2}$ = 17.0 Hz, H-3′b), 4.59–4.49 (m, 6H, PhC$H_2$O), 4.16 (q, 1H, $J_{4,5}$ = $J_{5,6a}$ = $J_{5,6b}$ 4.8 Hz, H-5), 4.14–4.09 (m, 2H, H-3, H-4), 3.69–3.52 (m, 2H, H-6), 2.66 (bd, 2H, H-1′); $^{13}$C NMR (100.57 MHz, $CDCl_3$): δ 152.14 (d, C-1), 138.25, 137.95, 137.71 (3s, Cq arom.), 133.00 (d, C-2′), 119.31 (t, C-3′), 84.20 (s, C-2) 87.48, 84.72, 81.25 (3d, C-3, C-4, C-5), 73.77, 72.71, 72.55, 70.67 (4t, Ph*C*H2O, C-6), 40.11 (t, C-1′). MALDI-MS *m*/*z* calcd for $C_{30}H_{33}NO_5$: 487.59. Found: 489 $[M+H]^+$, 511 $[M+Na]^+$, 527 $[M+K]^+$. Anal. Calcd for $C_{30}H_{33}NO_5$: C 73.90; H 6.82; N 2.87. Found: C, 73.88; H, 6.80; N, 2.89.

### 1.13. 1-Amino-2,5-Anhydro-3,4,6-tri-*O*-benzyl-1-deoxy-2-*C*-(prop-2-enyl)-*N*-*tert*-butoxycarbonyl-D-glucitol (32)

To a solution of **20** (0.250 g, 0.528 mmol) and $Et_3N$ (0.223 mL, 1.58 mmol) in dry $CH_2Cl_2$ (7 mL) Boc anhydride (0.138 g, 0.634 mmol) was added at 0 °C. Then the mixture was warmed to room temperature and stirred at rt overnight. Volatiles were removed, and the residue was dissolved in EtOAc and washed with $H_2O$. The organic layer was dried ($Na_2SO_4$), filtered, concentrated to dryness and then purified by silica gel flash chromatography (9:1 petroleum ether–EtOAc) to give the desired product **32** as a yellow oil (0.264 g, 87% yield). $^1$H NMR (400 MHz, $CDCl_3$): δ 7.38–7.24 (m, 15H, Ph-*H*), 5.89–5.79 (m, 1H, H-2′), 5.37 (t, 1H, $J_{NH,1a}$ = $J_{NH,1b}$ = 5.6 Hz, N*H*), 5.13 (dd, 1H, $J_{3'a,2}$ = 10.2 Hz, $J_{3'a,2}$ = 2.1 Hz, H-3′a), 5.06 (dd, 1H, $J_{3'b,2}$ = 17.1 Hz, $J_{3'a,2}$ = 2.1 Hz, H-3′b), 4.63–4.47 (m, 6H, PhC$H_2$O), 4.20 (dd, 1H, $J_{4,5}$ = 6.7 Hz, $J_{3,4}$ = 5.4 Hz, H-4), 4.09 (d, 1H, $J_{3,4}$ = 5.4 Hz, H-3), 3.99 (m, 1H, H-5), 3.61 (dd, 1H, $J_{6a,6b}$ = 10.5 Hz, $J_{5,6a}$ = 4.0 Hz, H-6a), 3.49 (dd, 1H, $J_{6a,6b}$ = 10.5 Hz, $J_{5,6b}$ = 4.0 Hz, H-6b), 3.38 (m, 2H, H-1), 2.43 (dd, 1H, $J_{1'a,1'b}$ = 14.2 Hz, $J_{1'a,2'}$ = 7.0 Hz, H-1′a), 2.34 (dd, 1H, $J_{1'a,1'b}$ = 14.2 Hz, $J_{1'b,2'}$ = 7.6 Hz, H-1′b), 1.45 (s, 9H,

C($CH_3$)$_3$); $^{13}$C NMR (100.57 MHz, $CDCl_3$): $\delta$ 161.74 (s, *C*O), 138.45, 138.34, 138.22 (3s, Cq arom.), 132.89 (d, C-2′), 119.94 (t, C-3′), 88.17, 85.18, 79.74 (3d, C-3, C-4, C-5), 85.67, 85.11 (2s, C-2,*C*($CH_3$)$_3$), 73.54, 73.09, 72.59, 69.64 (4t, Ph*C*$H_2$O, C-6), 40.85 (t, C-1), 38.46 (t, C-1′), 28.20, 26.7, 25.6 (3q, C(*C*$H_3$)$_3$). MALDI-MS *m/z* calcd for $C_{35}H_{43}NO_6$: 573.72. Found: 597 $[M+Na]^+$, 613 $[M+K]^+$. Anal. Calcd for $C_{35}H_{43}NO_6$: C, 73.27; H, 7.55; N, 2.44. Found: C, 73.31; H, 7.50; N, 2.54.

### 1.14. 3,6-Anhydro-4,5,7-tri-*O*-benzyl-2-deoxy-3-*C*-(aminomethyl(*N*-*tert*-butoxycarbonyl))-D-*manno*-heptonic acid lactol (33)

To a solution of **32** (0.250 g, 0.436 mmol) in dry THF (6 mL) 4-methylmorpholine *N*-oxide (NMO, 0.147 g, 1.09 mmol) and 0.016 M $OsO_4$ in *t*-BuOH (1.38 mL, 0.022 mmol) were added at 0 °C, and the resulting mixture was stirred at room temperature for 5 h. Additional NMO (0.071 g) was added, and stirring was continued overnight. The mixture was poured into 1 M $Na_2S_2O_3$ and extracted with EtOAc. The organic layer was dried ($Na_2SO_4$), filtered, concentrated to dryness to give the diol intermediate that was used without further purification. To a solution of the diol in THF (5 mL) aqueous solution of $NaIO_4$ (158 mg, 0.741 mmol in 1 mL) was added at 0 °C, and the mixture was stirred for 3 h, poured into ice water and extracted with EtOAc. The organic layer was dried ($Na_2SO_4$), filtered, concentrated to dryness and then purified by silica gel flash chromatography (7:3 petroleum ether–EtOAc) to give lactol **33** as a yellow oil (0.213 g, 85% yield). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.28–7.12 (m, 15H, Ph-*H*), 5.37 (t, 1H, $J_{1,2} = 6$ Hz, H-1), 4.51–4.30 (m, 6H, PhC$H_2$O), 4.10–4.02 (m, 1H, H-6), 4.0 (d, 1H, $J_{4,5} = 1.6$ Hz, H-4), 3.85 (dd, 1H, $J_{5,6} = 3.5$ Hz, $J_{4,5} = 1.6$ Hz, H-5), 3.81 (d, 1H, $J_{1'a,1'b} = 12$ Hz, H-1′a), 3.51 (dd, 1H, $J_{7a,7b} = 17$ Hz, $J_{6,7a} = 5.8$ Hz, H-7a), 3.40 (dd, 1H, $J_{7a,7b} = 17$ Hz, $J_{6,7b} = 7$ Hz, H-7b), 3.37 (d, 1H, $J_{1'a,1'b} = 12$ Hz, H-1′b), 2.2 (dd, 1H, $J_{2a,2b} = 14$ Hz, $J_{1,2a} = 5.2$ Hz, H-2a), 1.9 (dd, 1H, $J_{2a,2b} = 14$ Hz, $J_{1,2b} = 6.8$ Hz, H-2b), 1.15 (s, 9H, C(CC(*C*$H_3$)$_3$)); $^{13}$C NMR (100.57 MHz, $CDCl_3$): $\delta$ 151.15 (s, *C*O), 138.54, 138.78, 137.25 (3s, Cq arom.), 85.87, 83.54, 82.31 (3d, C-4, C-5, C-6), 83.60, 83.38 (2s, C-3,*C*($CH_3$)$_3$), 73.55, 72.63, 71.97, 70.50 (4t, Ph*C*$H_2$O, C-7), 54.43 (t, C-1′), 39.54 (t, C-2), 28.94, 28.35, 27.80 (3q, C(*C*$H_3$)$_3$). MALDI-MS *m/z* calcd for $C_{34}H_{41}NO_7$: 575.69. Found: 599 $[M+Na]^+$, 615 $[M+K]^+$. Anal. Calcd for $C_{34}H_{41}NO_7$: C, 70.93; H, 7.18; N, 2.43. Found: C, 71.00; H, 7.20; N, 2.40.

### 1.15. In vitro pharmacology: $GABA_A$ receptor binding assay

Rat brain membranes were prepared and assayed for $GABA_A$ receptor binding by using the method described by Frosini et al.[27] with slight modifications. The whole brain was homogenized in 10 vol of cold 0.32 M sucrose and the homogenate was centrifuged at 2500*g* for 10 min at 4 °C. The supernatant was then centrifuged at 48,000*g* for 30 min at 4 °C and the pellet (crude synaptic membranes) was resuspended in Tris–HCl 50 mM, pH 7.4 (1 mL/1 g wet weight) and frozen (−20 °C) for 24 h. Thawed membranes were then resuspended in Tris–HCl containing Triton X-100 (0.05% w/v) to obtain a final protein concentration of 1 mg/mL. The mixture was incubated for 30 min at 37 °C and then centrifuged at 48,000*g* for 20 min at 4 °C. The pellet was then washed three times with Tris–HCl and frozen at −20 °C before use. The pellet, resuspended in Tris–HCl to obtain a final protein concentration of 2 mg/mL, was used for displacement binding studies. [$^3$H]Muscimol (10 nM) served as radioligand and nonspecific binding was determined in the presence of nonlabelled muscimol (500 μM) and represented about 30–40% of the total binding. Compounds **22**, **26** and **27** were diluted in Tris–HCl 50 mM pH 7.4, to obtain the final concentration of 500 μM, whereas stock solutions of compounds **21**, **24** and **25** were prepared in ethanol and then diluted in Tris–HCl to obtain the final concentration of 500 μM. The binding reaction consisted in 0.1 mg of membranes incubated with the radioligand in the presence or absence of the test compounds and was stopped after 30 min at 4 °C by rapid filtration under vacuum through glass-fibre filter (GO/B, Whatman). The filters were washed twice with 2 mL portions of ice-cold Tris–HCl buffer and then dissolved in 8 mL of scintillation fluid (UltimaGold). Filter-bound radioactivity was determined by a scintillation counter. For all compounds three independent experiments were performed in triplicate. Results are expressed as percentage of control [$^3$H]Muscimol specific binding and analyzed by Graph Pad Prism, using the Kruskal–Wallis ANOVA for nonparametric data followed by Dunns test for specific comparisons.

## Acknowledgements

This work was supported by Ministero dell'Università e della Ricerca under Contract MIUR-PRIN 2005 n.2005037725 and Fundação para a Ciência e Tecnologia (FCT) SFRH/BD/17815/2004; we gratefully acknowledge Dr. Pietro Fumagalli for his technical support for $GABA_A$ receptor binding assays.

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