ELSEVIER

European Journal of Medicinal Chemistry 37 (2002) 773–781

EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY

www.elsevier.com/locate/ejmech

Preliminary communication

Synthesis of glycosylated β-amino acids as new class of antitubercular agents☆

R.P. Tripathi [a,*], R. Tripathi [a], V.K. Tiwari [a], Laxmi Bala [b], S Sinha [b], A. Srivastava [c], R. Srivastava [c], B.S. Srivastava [c]

[a] *Division of Medicinal Chemistry, Central Drug Research Institute, Lucknow 226 001, India*
[b] *Division of Biochemistry, Central Drug Research Institute, Lucknow 226 001, India*
[c] *Division of Microbiology, Central Drug Research Institute, Lucknow 226 001, India*

Received 3 January 2002; accepted 13 June 2002

Abstract

A series of glycosylated β-amino acids was prepared and evaluated against *Mycobacterium tuberculosis*, *M. avium*, *M. fortuitum* and *M. smegmatis*. The compounds were designed to mimic the enzyme D-alanine racemase and glycosyl transferase involved in the biosynthesis of essential cell wall peptidoglycan and arabinogalactan. Though most of the compounds exhibited little activity, however, one showed significant activity against all the strains in cell culture and activity was confirmed by BACTEC method.

Keywords: Tuberculosis; Glycosyl amino acids; Conjugate addition

1. Introduction

Tuberculosis (T.B.) is the most prevalent infectious disease worldwide and a leading killer caused by a single infectious agent i.e. *Mycobacterium tuberculosis* [1,2]. As a consequence of HIV epidemic and the lack of potency of multi-drug regimens, drug resistance has become more evident and the development of novel mechanism based antitubercular agents has become a high priority area globally [3,4]. The mycobacterial cell wall is an effective barrier that contributes to drug resistance [5]. Inhibition of cell wall biosynthesis is not only a potential antimycobacterial strategy but it also increases the susceptibility of the bacteria to other antimycobacterial agents [6]. The cell wall is dominated by covalently linked mycolic acids (complimented by glycolipids), D-arabinogalactan and peptidoglycan. D-Amino acids are important constituents of mycobacterial cell wall [7]. A cytoplasmic enzyme D-alanine racemase is required in the initial step of peptidoglycan biosynthesis to convert natural L- to D-alanine and has been identified as novel target for antitubercular drug development [8,9]. Inhibitors of this enzyme such as, D-cycloserine (a cyclic analog of D-alanine) and fludalanine are found to be active against *M. tuberculosis* both in vitro and in vivo [10], but because of toxicity none of them found their way to clinics [11]. Very recently, certain sugar derivatives and glycofuranose analogs have been reported to show significant anti T.B. and other biological activities [12–17]. Since last 40 years none of the new chemical entity has been appeared against this disease. Keeping in mind the above and the ability of sugars to offer better stability [18], better pharmacokinetics [19], better transport [20] and above all the less toxicity we were prompted to develop novel, selective and more potent glycosylated amino acid derivatives as antitubercular agents.

We envisaged that such compounds might be effective inhibitors of D-alanine racemase and D-alanylalanine synthetase, and being glycofuranosylated at the β-position these amino acid derivatives have chances to interfere the glycosyltransferase activity of *M. tuberculosis*. It was further contemplated that introducing the hydrophobic component would help in ligand binding

☆ CDRI communication No. 6067.

* Correspondence and reprints
E-mail address: rpt_56@yahoo.com (R.P. Tripathi).

0223-5234/02/$ - see front matter
PII: S0223-5234(02)01398-3

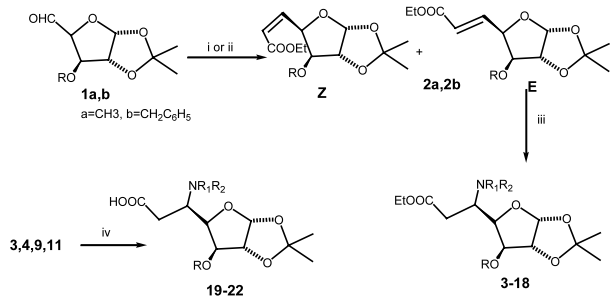

Fig. 1. Synthesis of glycosylated amino esters and acids. Reagents and conditions: (i) carbethoxymethylenetriphenylphosphorane, dry benzene, reflux, 12 h; (ii) triethylphosphonoacetate, LiOH, dry THF, r.t., 10 h; (iii) amines, ethanol, r.t., 3–10 h; (iv) triethyl amine–water–ethanol (2:2:1), r.t., 3 days or LiOH, THF–water (1:1), 0.5 h, r.t.

Compounds	R	NR_1R_2	Ratio of major and minor isomers
3	CH_3	NH_2	65:35
4	CH_2Ph	NH_2	75:25
5	CH_3	$PhCH_2NH$	65:35
6	CH_2Ph	$PhCH_2NH$	80:20
7	CH_3	*n*-butyl–NH	65:35
8	CH_2Ph	*n*-butyl–NH	75:25
9	CH_3	*n*-dodecyl–NH	64:36
10	CH_2Ph	*n*-dodecyl–NH	76:24
11	CH_3	*n*-hexadecyl–NH	64:36
12	CH_2Ph	*n*-hexadecyl–NH	76:24
13	CH_3	1,5-dimethylhexyl–NH	65:35
14	CH_2Ph	1,5-dimethylhexyl–NH	75:25
15	CH_3	2-carbethoxyethyl–NH	60:40
16	CH_2Ph	2-carbethoxyethyl–NH	70:30
17	CH_3	3-carbethoxypropyl–NH	60:40
18	CH_2Ph	3-carbethoxypropyl–NH	80:20
19	CH_3	NH_2	
20	CH_2Ph	NH_2	
21	CH_3	*n*-dodecyl–NH	
22	CH_3	*n*-hexadecyl–NH	

and the chelating moieties (–COOEt and –NH) might be involved in co-ordination with metal ions often found in the enzyme's active site.

2. Chemistry

The approach towards the synthesis of the desired glycosylated β-amino acid derivatives is represented in Fig. 1. Ethyl-3-[3-*O*-benzyl(methyl)-1,2-*O*-isopropylidene-5-deoxy-α-D-xylofuranos-5-yl]-carbethoxyprop-2-enoates (**2**) were derived from 1,2-*O*-isopropylidene-3-*O*-benzyl(methyl)-xylofuranos-5-ulose (**1**) either by Wittig olefination with carbethoxymethylene triphenylphophorane or with triethylphosphonoacetate [20,21]. In the former a mixture of (*E*) and (*Z*) in (90:10) ratio was obtained, however in the latter only (*E*) form was obtained in stereo-specific manner. Subsequently, 1,4-conjugate addition of different primary amines [viz. NH_3, benzyl-, *n*-butyl-, *n*-dodecyl-, *n*-hexadecyl-, 1,5-dimethyl hexyl-, 2-(carbethoxy)-ethyl- and 3-(carbethoxy)-propylamines] to the above esters **2** led to the corresponding glycosylated β-amino esters (**3**–**18**) in excellent yield. LiOH mediated hydrolysis led to the Li salt of the corresponding acids. However, the free acids (**19**–**22**) could be obtained by treatment of amino esters (**3**, **4**, **9** and **11**) with aqueous ethanolic triethylamine. Since compounds **9** and **11** (both as diastereoisomeric mixture) were the most active compounds, the individual isomers of compounds **9** and **11** were separated using column chromatography as the major and minor isomers.

3. Results and discussion

The conjugate addition of amines to ester **2** was diastereoselective as two diastereoisomers were obtained in different ratios as evident by ^{1}H-NMR spectral data of glycosylated amino esters (**3**–**18**). Explanation can be rationalised in terms of Felkin-Anh like transition states. Four transition states for the olefinic ester can be considered as shown in Fig. 2. In conformations **I** and **II**, the more electronegative C–O group of the furanose ring, whereas in **III** and **IV** the C-3 substituent (bezyloxy or methyl) were placed at right angles to the double bond. In such a model it is known that nucleophile should attack from the side opposite to the olefinic double bond. In case of benzyloxy substituent '*re*' face attack of nucleophile to the double bond in conformer **I** (to give the D-*gluco* isomer) and '*si*' face attack in conformer **II** (to give the L-*ido* isomer) is hindered by C-3 substituent. Therefore, conformers **III** and **IV** should be considered. It is presumed that conformer **III** has preference to the **IV** due to favourable alkene–arene interactions as per Houck's rule [22]. Whereas '*si*' face attack of amines by chelation with furanose ring oxygen explains the formation of L-*ido* isomer. However, in C-3 hydroxy or with non-bulky substituent arene π-stacking effect is absent, therefore, it is presumed that the furanose C–O bond will adopt the perpendicular position favoring the conformers **I** and **II** would then lead to product formation. Thus major isomer has '*S*' configuration at C-5 while minor isomer has '*R*' configuration.

Most of the compounds showed marginal antimycobacterial activity in MABA and micro-dilution techni-

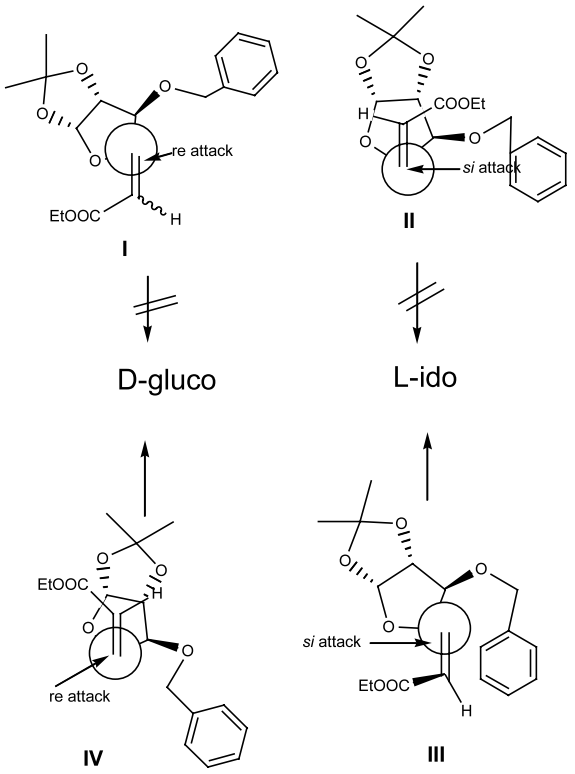

Fig. 2. Felkin-Anh transition state model for reaction mechanism.

Table 1
Antitubercular activities of glycosylated β-amino acid derivatives **3**–**23**

Compounds	MABA (μg mL^{-1})	Agar microdilution	BACTEC method
3	> 25	> 100	ND
4	> 25	> 100	ND
5	ND	> 100	ND
6	ND	50	ND
7	> 25	> 50	ND
8	ND	50	ND
9	> 25	50	ND
10	> 25	50	ND
11	12.5	6.25	6.25
12	> 25	50	> 25
13	> 25	ND	ND
14	> 25	25	ND
15	> 25	50	ND
16	> 25	50	ND
17	> 25	100	ND
18	> 25	50	50
19	> 100	50	ND
20	> 100	ND	ND
21	> 25	50	> 50
22	> 50	100	ND
21	> 50	100	ND
22	> 100	> 100	ND
Major isomer of **11**	3.12	6.25	6.25
Minor isomer of **11**	> 100	> 100	> 100

MIC, minimum inhibitory concentration; ND, not determined; MIC of the compounds used as control: ethambutol 1.5–5 μg mL^{-1}, rifampicin 0.75 μg mL^{-1}, INH 0.65 μg mL^{-1}, ethionamide 10 μg mL^{-1}.

ques (Table 1). Two compounds with long alkyl chain (**9** and **11**) exhibited significant activities in all the three-test system. Compound **11** caused complete inhibition of *M. tuberculosis* strain at 6.25 μg mL^{-1}. This compound showed very good activity in all the three strains of mycobacteria (*M. avium*, *M. smegmatis* and *M. tuberculosis*) at one or the other concentrations. The activity in compound **11** was confirmed using the BACTEC technique also, where the MIC was found to be 6.25 μg mL^{-1}. However, the activity in compound **11** is due to major diastereoisomer (β-L-ido isomer) only with MIC of 6.25 μg mL^{-1}, while the minor isomer (α-D-gluco isomer) was found to be inactive in BACTEC method.

On careful examination of the biological results, it is clear that glycosyl amino esters having long chain alkyl groups as amine substituent are more active than the short chain alkyl group. Conversion of glycosylated amino esters (**3**, **4**, **9** and **11**) into corresponding acids (**19**–**22**) results in drastic loss in antimycobacterial activity. Replacement of 3-*O*-methyl (**9**) with 3-*O*-benzyl (**10**) reduces the activity. Further replacement of amino function either with short or branched chain alkyl group or with β-amino ester (**15**–**18**) increases antitubercular activity profile in comparison to the corresponding amino esters.

4. Conclusion

In conclusion, we have shown glycosylated β-amino esters as novel class of antimycobacterial agents. The mechanism for the activity of these compounds against the mycobacteria is unclear. It is speculated that these compounds might be acting either by interfering in the cell wall biosynthesis by inhibiting the crucial enzymes (glycosyl transferase or D-alanine racemase) or through some immune modulation mechanism. The exact mode of action of these compounds is under investigation and will be reported in future communication.

5. Experimental

5.1. Biological activity

MICs of the test compounds were determined using three different techniques i.e. MABA assay, agar microdilution method and BACTEC 460-TB system.

For MABA assay *M. tuberculosis*, H37Ra was used as a suitable surrogate for the virulent H37 Rv strain. Young (7–8 days) cultures were diluted in liquid

medium to provide, an optimal density of 0.02 at 550 nm spectrophotometrically which gave colour change of Alamar blue 'oxidation reduction' dye (blue to pink) in 7–8 days. The standard antitubercular agents Rifamycin, isoniazid, *p*-aminosalicylic acid, ethambutol and ethionamide were taken as positive controls. The screening of the compounds was carried out as reported earlier [23].

In agar microdilution method serial twofold dilutions of each test compound was added into 7H10 agar and *M. tuberculosis* H37 Rv was used as test organism. The test was performed as reported by Saita et al. [24]. Stock solution of the test compounds prepared in DMSO at 1 mg mL^{-1} was sterilised by passage through 0.22 μm filters. Fifty microliters were added to 4 mL radiometric 7H12 Broth (BACTEC 12B; Becton Dickinson Diagnostic Instrument System, US) to achieve final concentrations. Controls received 50 μL DMSO. Ofloxacin, streptomycin and rifampicin (Sigma Chemical Co., St. Louis, MO) were included as positive drug control.

In BACTEC method, *M. tuberculosis* H37 Rv was scraped from fresh Lowenstein–Jensen slants resuspended in 3 mL diluting fluid and homogenised with glass beads (2 mm). Homogenous supernatant was taken, turbidity was adjusted to mcFarland 1 with diluting fluid and 0.1 mL injected into a BACTEC 12B vial which was used as a primary inoculum after growth index (GI) of 0 reached to 500–700. This suspension (0.1 mL) was used to inoculate 4 mL fresh BACTEC 12B broth containing the test compounds. An additional control vial was included which received a further 1:100 inoculum. Cultures were incubated at 37 °C and the GI determined daily. When the GI of 1:100 control vials reached 30, the test was read for an additional day and then terminated. If the drug difference in the GI values from the previous day (called ΔGI) in case of drug containing vials was less than ΔGI of the 1:100 control, then the bacteria was defined as 1−(GI of the test sample/GI of control) × 100. Assays were completed in 5–8 days and were carried out according to literature procedure [25].

5.2. Chemistry

M.P.s were determined on a Buchi 510 apparatus and are uncorrected. Elemental analysis for all new compounds were performed on a Carlo Erba Model 1108 elemental analyser and data of C, H, and N is within ± 0.4% of calculated values. Thin layer chromatography was used to monitor the reactions. IR spectra were recorded using Perkin–Elmer 881 spectrophotometer and the values are expressed as ν_{max} cm^{-1}. Mass spectral data were run on JEOL-300 spectrophotometer and PMR spectra were recorded on Bruker 200 and 300 MHz spectrophotometer.

5.2.1. General procedure for the preparation of compounds **3** and **4**

*5.2.1.1. Ethyl-[5-amino-3-O-methyl-5,6-dideoxy-1,2-O-isopropylidene]-α-D-gluco- and β-L-ido-heptofuranurnate (**3**).* Ethyl-[3-*O*-methyl-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-gluco]-heptfuran-5-en-uronate (3 g, 11.494 mmol) and ethanolic ammonia (25 mL) was magnetically stirred in a closed vessel for 24 h. Solvent and excess of NH_3 were evaporated under reduced pressure. The crude product, thus obtained, was chromatographed over SiO_2 column using $CHCl_3$–MeOH (98:2) as eluant to give the above compound as colourless oil (diastereoisomeric mixture ratio 65:35).

Colourless oil (90% yield); IR (KBr, cm^{-1}): ν_{max} 3382 (NH_2), 1720 (>C=O); MS (FAB): *m/z* 290 $[M+H]^+$; ^{1}H-NMR ($CDCl_3$): δ 5.91 and 5.90 (each d, $J = 3.7$ Hz, each 1H, diastereoisomeric H-1), 4.60 (d, $J = 3.7$ Hz, 1H, H-2), 4.10 (q, $J = 7$ Hz, 2H, OCH_2CH_3), 3.98 (d, $J = 2.8$ Hz, 1H, H-4), 3.82 (d, $J = 2.8$ Hz, 1H, H-3), 3.52 (m, 1H, H-5), 3.39 (s, 3H, $-OCH_3$), 2.40 (m, 2H, H-6), 1.80 (bs, 2H, NH_2), 1.48, 1.32 [each s, each 3H, $>C(CH_3)_2$], 1.26 (t, $J = 7$ Hz, 3H, $-OCH_2CH_3$); ^{13}C-NMR ($CDCl_3$): δ 172.06 (>C=O), 111.85 [$>C(CH_3)$],105.09 and 105.03 (C-1), 84.57 (C-2), 82.22 (C-4), 81.56 (C-3), 60.73 ($-OCH_2CH_3$), 57.84 ($-OCH_3$), 47.42 (C-5), 38.69 (C-6), 27.04 and 26.58 [$>C(CH_3)$, 14.49 ($-OCH_2CH_3$). Anal. $C_{13}H_{23}NO_6$ (C, H, N).

*5.2.1.2. Ethyl-[5-amino-3-O-benzyl-5,6-dideoxy-1,2-O-isopropylidene]-α-D-gluco- and β-L-ido-heptofuranurnate (**4**).* It was obtained by reaction of ethyl-[3-*O*-benzyl-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-gluco]-heptfuran-5-en-uronate and ethanolic ammonia as described above to give diastereoisomeric mixture ratio (75:25) of **4** as colourless oil (90% yield); IR (KBr, cm^{-1}): ν_{max} 3382 (NH_2), 1720 (COOEt); MS (FAB): *m/z* 366 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$): δ 7.32 (bs, 5H, Ar*H*), 5.94 and 5.92 (each d, $J = 3.7$ Hz, each 1H, diastereoisomeric H-1), 4.69 (d, $J = 11.9$ Hz, 1H, $-OCH_AAr$), 4.65 (d, $J = 3.7$ Hz, 1H, H-2), 4.44 (d, $J = 11.9$ Hz, 1H, $-OCH_BAr$), 4.09 (q, $J = 7$ Hz, 2H, OCH_2CH_3), 3.99 (d, $J = 2.9$ Hz, 1H, H-4), 3.87 (d, $J = 2.9$ Hz, 1H, H-3), 3.59 (m, 1H, H-5), 2.26 (m, 2H, H-6), 1.70 (bs, 2H, NH_2), 1.48, 1.32 [each s, each 3H, $C(>CH_3)_2$], 1.25 (t, $J = 7$ Hz, 3H, $-OCH_2CH_3$); ^{13}C-NMR ($CDCl_3$): δ 172.12 (>C=O), 137.37, 129.09, 128.94 and 128.32 (Ar–C), 112.09 [$>C(CH_3)$, 105.47 and 105.20 (C-1), 83.77 (C-2), 82.58 (C-4), 81.96 (C-3), 72.03 ($-OCH_2Ar$), 60.82 ($-OCH_2CH_3$), 47.82 (C-5), 38.65 (C-6), 27.14 and 26.69 [$>C(CH_3)$, 14.59 ($-OCH_2CH_3$). Anal. $C_{19}H_{27}NO_6$ (C, H, N).

*5.2.2. General procedure for the preparation of compounds **5**–**18***

*5.2.2.1. Ethyl-[3-O-methyl-5,6-dideoxy-5-benzylamino-1,2-O-isopropylidene]-α-D-gluco and β-L-ido-heptofurannuronates (**5**).* A solution of (1*R*, 2*R*, 3*S*, 4*R*)-ethyl-(3-*O*-methyl-1,2-isopropylidene-1,4-pentofuranose-4-yl)-hept-5-enoate (2.72 g, 10.0 mmol) dissolved in EtOH (15 mL), was magnetically stirred with benzylamine (1.16 mL, 10.8 mmol) at room temperature (25 °C) for 40 h. Solvent was evaporated under reduced pressure to get crude product. Column chromatography (SiO_2) of the crude product using C_6H_{14}–EtOAc (80:20) as eluant afforded the diastereomeric compounds in 65:35 ratios. Over all, yield was 96%.

Minor isomer: Colourless oil (15% yield); IR (KBr, cm^{-1}): ν_{max} 3351 (–NH), 3051, 2985, 2922 and 2848 (CH_3 and CH_2 stretching), 1721 (>C=O); MS (FAB): *m/z* 380 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$): δ 7.35 (m, 5H, Ar–H), 5.90 (d, $J = 3.7$ Hz 1H, H-1), 4.60 (d, $J = 3.7$ Hz, 1H, H-2), 4.28 and 4.23 (dd, $J = 7.0$ and 3.2 Hz, 1H, H-4), 4.16 (q, $J = 7.14$ Hz, 2H, –OCH_2CH$_3$), 3.89 (d, $J =$ 12 Hz, 1H, –NHCH_APh), 3.80 (d, $J = 12$ Hz, 1H, –NHCH_BPh), 3.74 (d, $J = 3.2$ Hz, 1H, H-3), 3.40 (m, 1H, H-5), 3.35 (s, 3H, –OCH$_3$), 2.56 and 2.50 (dd, $J =$ 15.6 and 4.7 Hz, 1H, H-6$_A$), 2.47 and 2.40 (dd, $J = 15.6$ and 4.7 Hz, 1H, H-6$_B$), 1.88 (s, 1H, –N*H*CH$_2$Ph), 1.49 and 1.30 [each s, each 3H, >C(CH_3)$_2$], 1.24 (t, $J = 7.14$ Hz, 3H, –OCH$_2$CH_3); ^{13}C-NMR ($CDCl_3$): δ 172.76 (>C=O), 141.15, 128.67, 128.51 and 127.28 (each Ar–C), 111.89 [>*C*(CH$_3$)$_2$], 105.13 (C-1), 84.17 (C-2), 82.36 (C-4), 82.26 (C-3), 60.67 (–O*C*H$_2$CH$_3$), 57.98 (–OCH$_3$), 52.75 (C-5), 51.94 (–NH*C*H$_2$Ph), 36.57 (C-6), 27.18 and 26.73 [2 × >C(*C*H$_3$)$_2$], 14.63 (–OCH$_2$*C*H$_3$).

Major isomer: Colourless oil (75% yield); IR (KBr, cm^{-1}): ν_{max} 3355 (–NH), 3048, 2978, 2923 and 2845 (CH_3 and CH_2 stretching), 1721 (>C=O); MS (FAB): *m/z* 380 $[M+1]^+$; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.32 (m, 5H, Ar–H), 5.90 (d, $J = 3.7$ Hz, 1H, H-1), 4.57 (d, $J = 3.7$ Hz, 1H, H-2), 4.23 and 4.18 (dd, $J = 8.6$ and 3.1 Hz, 1H, H-4), 4.18 (q, $J = 7.14$ Hz, 2H, –OCH_2CH$_3$), 3.90 (d, $J = 12$ Hz, 1H, –NHCH_APh), 3.85 (d, $J = 12$ Hz, 1H, –NHCH_BPh), 3.74 (d, $J = 3.2$ Hz, 1H, H-3), 3.39 (m, 1H, H-5), 3.36 (s, 3H, –OCH$_3$), 2.58 and 2.52 (dd, $J = 15.6$ and 4.7 Hz, 1H, H-6$_A$), 2.46 and 2.39 (dd, $J = 15.6$ and 6.6 Hz, 1H, H-6$_B$), 2.02 (s, 1H, –N*H*CH$_2$Ph), 1.47 and 1.30 [each s, each 3H, >C(CH_3)$_2$], 1.25 (t, $J = 7.14$ Hz, 3H, –OCH$_2$CH_3); ^{13}C-NMR ($CDCl_3$): δ 172.12 (>C=O), 140.96, 128.65, 127.18 (each Ar–C), 111.90 [>*C*(CH$_3$)$_2$], 105.18 (C-1), 84.55 (C-2), 82.83 (C-4), 81.59 (C-3), 60.77 (–O*C*H$_2$CH$_3$), 57.62 (–OCH$_3$), 54.33 (C-5), 51.96 (–NH*C*H$_2$Ph), 36.89 (C-6), 27.14 and 26.94 [2 × >C(*C*H$_3$)$_2$], 14.62 (–OCH$_2$*C*H$_3$). Anal. $C_{20}H_{29}NO_6$ (C, H, N).

*5.2.2.2. Ethyl-[3-O-benzyl-5,6-dideoxy-5-benzylamino-1,2-O-isopropylidene]-α-D-gluco and β-L-ido-heptofurannuronate (**6**). Minor isomer:* Colourless oil (25% yield); IR (KBr, cm^{-1}): ν_{max} 3350 (–NH), 3020, 2980, 2920 (CH_3 and CH_2 stretching), 1710 (>C=O); MS (FAB): *m/z* 456 $[M+1]^+$; ^{1}H-NMR (200 MHz, $CDCl_3$): δ 7.26 (m, 10H, Ar–H), 5.89 (d, $J = 3.77$ Hz, 1H, H-1), 4.67 (d, $J = 11.72$ Hz, 1H, –OCH$_A$Ph), 4.59 (d, $J = 3.7$ Hz, 1H, H-2), 4.53 (d, $J = 11.69$ Hz, 1H, –OCH$_B$Ph), 4.23 and 4.17 (dd, $J = 7.0$ and 2.7 Hz, 1H, H-4), 4.12 (q, $J = 7.2$ Hz, 2H, –OCH_2CH$_3$), 4.08 (d, $J =$ 3.2 Hz, 1H, H-3), 3.85 (d, $J = 12.8$ Hz, 1H, –NHCH$_A$Ph), 3.72 (d, $J = 12.8$ Hz, 1H, –NHCH$_B$Ph), 3.52 (m, 1H, H-5), 2.85 and 2.78 (dd, $J = 15.6$ and 4.4 Hz, 1H, H-6$_A$), 2.62 and 2.54 (dd, $J = 15.6$ and 6.7 Hz, 1H, H-6$_B$), 1.68 (s, 1H, –N*H*CH$_2$Ph), 1.47 and 1.31 [each s, each 3H, >C(CH$_3$)$_2$], 1.24 (t, $J = 7.14$ Hz, 3H, –OCH$_2$CH_3); ^{13}C-NMR ($CDCl_3$): δ 172.15 (>C=O), 141.07, 137.52, 128.92, 128.73, 128.66, 128.47, 128.32 and 127.19 (each Ar–C), 112.01 [>*C*(CH$_3$)$_2$], 105.29 (C-1), 82.76 (C-2), 82.26 (C-4), 82.19 (C-3), 71.91 (–O*C*H$_2$Ph), 60.80 (–O*C*H$_2$CH$_3$), 54.23 (C-5), 52.02 (–NH*C*H$_2$Ph), 36.91 (C-6), 27.19 and 26.75 [2 × >C(*C*H$_3$)$_2$], 14.62 (–OCH$_2$*C*H$_3$).

Major isomer: Yellow solid (m.p. 74 °C); IR (KBr, cm^{-1}): ν_{max} 3350 (–NH), 3020, 2980, 2920 (CH_3 and CH_2 stretching), 1710 (>C=O); MS (FAB): *m/z* 456 $[M+H]^+$; ^{1}H-NMR (200 MHz, $CDCl_3$): δ 7.25 (m, 10H, Ar–H), 5.94 (d, $J = 3.89$ Hz, 1H, H-1), 4.69 (d, $J = 11.82$ Hz, 1H, –OCH$_A$Ph), 4.64 (d, $J = 3.93$ Hz, 1H, H-2), 4.44 (d, $J = 11.8$ Hz, 1H, –OCH$_B$Ph), 4.25 and 4.19 (dd, $J = 8.8$ and 3.2 Hz, 1H, H-4), 4.10 (q, $J = 7.14$ Hz, 2H, –OCH_2CH$_3$), 3.94 (d, $J = 3.19$ Hz, 1H, H-3), 3.84 (s, 2H, –NHCH$_2$Ph), 3.52 (m, 1H, H-5), 2.46 and 2.40 (dd, $J = 15.6$ and 4.7 Hz, 1H, H-6$_A$), 2.34 and 2.28 (dd, $J = 15.6$ and 6.6 Hz, 1H, H-6$_B$), 1.68 (bs, 1H, –N*H*CH$_2$Ph), 1.47 and, 1.31 [each s, each 3H, >C(CH$_3$)$_2$], 1.24 (t, $J = 7.14$ Hz, 3H, –OCH$_2$CH_3); ^{13}C-NMR ($CDCl_3$): δ 172.80 (>C=O), 141.07, 137.97, 128.86, 128.69, 128.42, 128.26, 128.12 and 127.27 (each Ar–C), 111.98 [>*C*(CH$_3$)$_2$], 105.20 (C-1), 82.60 (C-2), 82.40 (C-4), 82.22 (C-3), 72.49 (–O*C*H$_2$Ph), 60.66 (–O*C*H$_2$CH$_3$), 53.07 (C-5), 51.67 (–NH*C*H$_2$Ph), 36.26 (C-6), 27.22 and 26.78 [2 × >C(*C*H$_3$)$_2$], 14.57 (–OCH$_2$*C*H$_3$). Anal. $C_{26}H_{33}NO_6$ (C, H, N).

*5.2.2.3. Ethyl-[3-O-methyl-5,6-dideoxy-5-butylamino-1,2-O-isopropylidene]-α-D-gluco and β-L-ido-heptofurannuronate (**7**).* Colourless oil (80% yield); IR (KBr, cm^{-1}): ν_{max} 2980, 2940, 2860, 2808 (CH_3 and CH_2 stretching), 1720 (>C=O); MS (FAB): *m/z* 346 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$): δ 5.91 (d, $J = 3.7$ Hz 1H, H-1, major isomer), 5.87 (d, $J = 3.7$ Hz 1H, H-1, minor isomer), 4.60 (d, $J = 3.7$ Hz, 1H, H-2, major isomer), 4.57 (d, $J = 3.7$ Hz, 1H, H-2, minor isomer), 4.12 (q, $J =$ 7.14 Hz, 2H, –OCH_2CH$_3$), 4.10 and 4.05 (dd, $J = 12$

and 8 Hz, 1H, H-4), 3.80 and 3.71 (d, $J = 3.1$ Hz, 1H, H-3), 3.38 (s, 3H, $-OCH_3$), 3.32 (m, 1H, H-5), 2.67 (m, 2H, $-NCH_2$), 2.50 and 2.45 (two dd, $J = 12$ and 8 Hz 1H, H-6_A), 2.40 and 2.35 (two dd, $J = 12$ and 8 Hz 1H, H-6_B), 1.77 [bs, 1H, $-NH(CH_2)_3CH_3$], 1.49 [s, 3H, $>C(CH_3)_2$], 1.42 [m, 2H, $-NH(CH_2CH_2)$], 1.32 [m, 2H, $-NH(CH_2)_2CH_2)$], 1.30 [s, 3H, $>C(CH_3)_2$], 1.27 (t, $J = 7.14$ Hz, 3H, $-OCH_2CH_3$), 0.90 [t, 3H, $J = 7.15$ Hz, $-NH(CH_2)_3CH_3$]; ^{13}C-NMR ($CDCl_3$): δ 172.20 ($>C=O$), 112.16 [$>C(CH_3)_2$], 105.39 (C-1), 83.99 (C-2), 82.30 (C-4), 81.79 (C-3), 60.74 ($-OCH_2CH_3$), 54.76 (C-5), 46.60 ($-NHCH_2$), 33.24 (C-6), 31.18 ($-NHCH_2CH_2$), 27.12 and 26.51 [$2 \times >C(CH_3)_2$], 20.22 ($-NHCH_2CH_2CH_2CH_3$), 14.52 ($-OCH_2CH_3$), 13.91 [$-NH(CH_2)_3CH_3$]. Anal. $C_{17}H_{31}NO_6$ (C, H, N).

*5.2.2.4. Ethyl-[3-O-benzyl-5,6-dideoxy-5-butylamino-1,2-O-isopropylidene]-α-D-gluco and β-L-ido-heptofurannuronate (**8**).* Colourless oil (80% yield); IR (KBr, cm^{-1}): ν_{max} 3350 (–NH), 3040, 2990, 2880 (CH_3 and CH_2 stretching), 1730 ($>C=O$); MS (FAB): *m/z* 422 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$): δ 7.35 (m, 5H, Ar–H), 5.95 (d, $J = 3.7$ Hz, 1H, H-1, major isomer), 5.87 (d, $J = 3.7$ Hz 1H, H-1, minor isomer), 4.72 (d, $J = 12$ Hz, 1H, $-OCH_APh$), 4.65 (d, $J = 3.7$ Hz, 1H, H-2, major isomer), 4.60 (d, $J = 3.7$ Hz, 1H, H-2, minor isomer), 4.45 (d, $J = 12$ Hz, 1H, $-OCH_BPh$), 4.14 (dd, $J = 12$ and 8 Hz, 1H, H-4), 4.10 (q, $J = 7.14$ Hz, 2H, $-OCH_2CH_3$), 3.90 (d, $J = 3.2$ Hz, 1H, H-3), 3.40 (m, 1H, H-5), 2.65 (m, 2H, $-NHCH_2$), 2.38 (dd, 1H, $J = 12$ and 8 Hz, 1H, H-6_A), 2.25 (dd, $J = 12$ and 7 Hz, 1H, H-6_B), 1.72 [bs, 1H, $-NH(CH_2)_3CH_3$], 1.50 [s, 3H, $>C(CH_3)_2$], 1.42 (m, 2H, $-NHCH_2CH_2$), 1.32 [s, 3H, $>C(CH_3)_2$], 1.30 [(m, 2H, $-NHCH_2CH_2CH_2$), 1.28 (t, $J = 7.1$ Hz, 3H, $-OCH_2CH_3$), 0.88 (t, $J = 7.2$ Hz, 3H, $-NH(CH_2)_3CH_3$); *^{13}C-NMR ($CDCl_3$):* δ 172.20 ($>C=O$), 137.51, 129.10, 128.42 and 128.14 (each Ar–C), 111.90 [$>C(CH_3)_2$], 105.21 (C-1), 82.63 (C-2), 82.26 (C-4), 82.12 (C-3), 71.88 ($-OCH_2Ph$), 60.68 ($-OCH_2CH_3$), 54.44 (C-5), 47.34 ($-NHCH_2$), 36.74 (C-6), 32.90 ($-NHCH_2CH_2$), 27.14 and 26.73 [$2 \times >C(CH_3)_2$], 20.81 [$-NH(CH_2)_2$-CH_2CH_3], 14.57 ($-OCH_2CH_3$), 14.36 [$-NH(CH_2)_3$-CH_3]. Anal. $C_{23}H_{35}NO_6$ (C, H, N).

*5.2.2.5. Ethyl-[3-O-methyl-5,6-dideoxy-5-(dodec-1-yl)-1,2-O-isopropylidene]-α-D-gluco and β-L-ido-heptofuranuronate (**9**). Minor isomer:* Colourless oil (15% yield); IR (KBr, cm^{-1}): ν_{max} 3600 (NH), 2928, 2856, 2402, 2348 (CH_3 and CH_2 stretching), 1728 (COOEt); MS (FAB): *m/z* 458 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$): δ 5.86 (d, $J = 3.9$ Hz, 1H, H-1), 4.56 (d, $J = 3.9$ Hz, 1H, H-2), 4.12 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3), 4.04 (d, $J = 2.7$ Hz, 1H, H-4), 3.72 (d, $J = 2.7$ Hz, 1H, H-3), 3.41 (s, 3H, OCH_3), 3.30 (m, 1H, H-5), 2.65 (m, 3H, H-6_A and $NHCH_2$), 2.44 (dd, $J = 13.6$ and 5.5 Hz, H-6_B), 1.69 (bs, 1H, NH), 1.47 and 1.31 [each s, each 3H, $>C(CH_3)_2$], 1.28 (m, 31H, methylene protons of alkyl chain and $-OCH_2CH_3$), 0.90 (t, $J = 6.6$ Hz, 3H, alkyl CH_2CH_3); ^{13}C-NMR ($CDCl_3$): δ 172.5 ($>C=O$), 111.9 [$>C(CH_3)_2$], 104.6 (C-1), 84.8 (C-2), 81.9 (C-4), 81.4 (C-3), 60.2 (OCH_2), 57.6 (OCH_3), 52.5 (C-5), 47.7 ($NHCH_2$), 36.7 (C-6), 32.3, 30.8, 30.0, 29.7, 26.7, 26.9 (alkyl CH_2's), 26.7 and 26.3 [$>C(CH_3)_2$], 14.2 and 14.1 (OCH_2CH_3 and alkyl CH_2CH_3).

Major isomer: Colourless oil (75% yield); IR (KBr, cm^{-1}): ν_{max} 3690 (–NH), 2928, 2856, 2404 (CH_3 and CH_2 stretching), 1724 (ester); MS (FAB): *m/z* 458 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$): δ 5.83 (d, $J = 3.9$ Hz, 1H, H-1), 4.51 (d, $J = 3.9$ Hz, 1H, H-2), 4.08 (q, $J = 7.1$ Hz, 2H, OCH_2), 4.02 (d, $J = 3.15$ Hz, 1H, H-4), 3.65 (d, $J = 3.16$ Hz, 1H, H-3), 3.31 (s, 3H, OCH_3), 3.22 (m, 1H, H-5), 2.58 (dd, $J = 19.7$ and 4.2 Hz, 1H, H-6_A), 2.65 (m, 3H, H-6_B and $NHCH_2$), 1.57 (bs, 1H, NH), 1.41 and 1.28 [each s, each 3H, $>C(CH_3)_2$], 1.24 (m, 20H, alkyl protons), 1.18 (t, $J = 7.1$ Hz, OCH_2CH_3), 0.80 (t, $J = 6.67$ Hz, alkyl CH_3); ^{13}C-NMR ($CDCl_3$): δ 172.05 ($>C=O$), 111.76 [$>C(CH_3)_2$], 105.06 (C-1), 84.97 (C-2), 82.58 (C-4), 81.53 (C-3), 60.55 (OCH_2), 58.46 (OCH_3), 54.56 (C-5), 47.58 ($NHCH_2$), 36.71 (C-6), 32.2, 31.0, 30.01, 29.95, 29.71 and 27.68 (alkyl carbons), 26.70 and 26.14 [$C(CH_3)_2$], 14.50 and 14.37 (OCH_2CH_3 and CH_2CH_3). Anal. $C_{25}H_{47}NO_6$ (C, H, N).

*5.2.2.6. Ethyl-[3-O-benzyl-5,6-dideoxy-5-(dodec-1-yl)-1,2-O-isopropylidene]-α-D-gluco- and β-L-ido-heptofuranuronate (**10**).* Colourless oil (91% yield); IR (KBr, cm^{-1}): ν_{max} 3332 (–NH), 2921 and 2852 (CH_3 and CH_2 stretching), 1730 (ester); MS (FAB): *m/z* 535 $[M+2]^+$; ^{1}H-NMR ($CDCl_3$): δ 7.33 (m, 5H, Ar–H), 5.91 and 5.88 (d, $J = 3.7$ Hz, 1H, H-1, distereomeric proton), 4.67 (d, $J = 11.8$ Hz, 1H, $-OCH_APh$), 4.62 (d, $J = 3.7$ Hz, 1H, H-2), 4.47 (d, $J = 11.8$ Hz, 1H, $-OCH_BPh$), 4.13 (d, $J = 2.8$ Hz, 1H, H-4), 4.09 (q, $J = 7.1$ Hz, 2H, OCH_2), 3.90 (d, $J = 2.8$ Hz, 1H, H-3), 3.26 (m, 1H, H-5), 2.60 and 2.41 (m, 4H, H-6 and $NHCH_2$), 1.69 (bs, 1H, NH), 1.47 and 1.31 [each s, each 3H, $>C(CH_3)_2$], 1.25 (m, 23H, methylene protons of alkyl chain and $-OCH_2CH_3$), 0.84 (t, $J = 6.7$ Hz, 3H, CH_2CH_3); ^{13}C-NMR ($CDCl_3$): δ 172.18 ($>C=O$), 137.58, 129.32, 128.36 and 128.31 (Ar–C), 112.07 [$>C(CH_3)_2$], 105.31 (C-1), 83.32 (C-2), 82.41 (C-4), 82.22 (C-3), 71.82 ($-OCH_2Ph$), 60.64 (OCH_2CH_3), 54.46 (C-5), 47.68 ($NHCH_2$), 36.71 (C-6), 32.32, 30.85, 27.76 and 23.07 (alkyl carbons), 27.19 and 26.79 [$C(>CH_3)_2$], 14.57 and 14.43 (OCH_2CH_3 and CH_2CH_3). Anal. $C_{31}H_{51}NO_6$ (C, H, N).

*5.2.2.7. Ethyl-[3-O-methyl-5,6-dideoxy-5-(hexadec-1-yl)-1,2-O-isopropylidene]-α-D-gluco and β-L-ido-heptofuranuronate (**11**). Minor isomer:* Colourless oil (35% yield); IR (KBr, cm^{-1}): ν_{max} 3570 (–NH), 2931, 2843, 2504 (CH_3 and CH_2 stretching), 1718 (ester); MS

(FAB): *m/z* 514 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$): δ 5.86 (d, $J=3.8$ Hz, 1H, H-1), 4.56 (d, $J=3.8$ Hz, 1H, H-2), 4.17–4.04 (m, 3H, H-4 and OCH_2), 3.79 (d, $J=3.0$ Hz, 1H, H-3), 3.41 (s, 3H, $-OCH_3$), 3.31 (m, 1H, H-5), 2.65 (dd, $J=18.3$ and 4.5 Hz, 1H, H-6_A), 2.46 (m, 3H, H-6_B and $NHCH_2$), 1.62 (bs, 1H, NH), 1.48 and 1.33 [each s, each 3H, $>C(CH_3)_2$], 1.25 (m, 31H, methylene protons of alkyl chain and $-OCH_2CH_3$), 0.88 (t, $J=6.6$ Hz, 3H, CH_2CH_3).

Major isomer: Colourless oil (65% yield); IR (KBr, cm^{-1}): ν_{max} 3650 (–NH), 2913, 2843, 2504 (CH_3 and CH_2 stretching), 1720 (ester); MS (FAB): *m/z* 514 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$): δ 5.93 (d, $J=3.8$ Hz, 1H, H-1), 4.61 (d, $J=3.8$ Hz, 1H, H-2), 4.21 (d, $J=3.07$ Hz, 1H, H-4), 4.15 (q, $J=7.1$ Hz, 2H, OCH_2), 3.65 (d, $J=3.07$ Hz, 1H, H-3), 3.39 (s, 3H, OCH_3), 3.31 (m, 1H, H-5), 2.66 (dd, $J=19.7$ and 4.2 Hz, 1H, H-6_A), 2.47 (m, 3H, H-6_B and $NHCH_2$), 1.76 (bs, 1H, NH), 1.45 and 1.32 [each s, each 3H, $>C(CH_3)_2$], 1.28 (m, 31H, methylene protons of alkyl chain and $-OCH_2CH_3$), 0.87 (t, $J=6.6$ Hz, 3H, CH_2CH_3); ^{13}C-NMR ($CDCl_3$): δ 172.25 (>C=O), 111.93 [$>C(CH_3)_2$], 105.14 (C-1), 84.47 (C-2), 82.69 (C-4), 81.59 (C-3), 60.75 (OCH_2), 57.61 (OCH_3), 54.56 (C-5), 47.58 ($NHCH_2$), 36.71 (C-6), 32.2, 31.0, 30.01, 29.95, 29.71, 27.75, 27.68 and 23.07 (alkyl carbons), 26.70 and 26.14 [$C(>CH_3)_2$], 14.61 and 14.49 (OCH_2CH_3 and CH_2CH_3). Anal. $C_{29}H_{55}NO_6$ (C, H, N).

*5.2.2.8. Ethyl-[3-O-benzyl-5,6-dideoxy-5-(hexadec-1-yl)-1,2-O-isopropylidene]-α-D-gluco and β-L-ido-heptofuranuronate (**12**).* Colourless solid (92% yield); IR (KBr, cm^{-1}): ν_{max} 3332 (–NH), 2921 and 2852 (CH_3 and CH_2 stretching), 1730 (ester); MS (FAB): *m/z* 591 $[M+2]^+$; ^{1}H-NMR ($CDCl_3$): δ 7.32 (m, 5H, Ar–H), 5.94 and 5.91 (d, $J=3.7$ Hz, 1H, H-1, distereomeric proton), 4.68 (d, $J=11.8$ Hz, 1H, $-OCH_APh$), 4.64 (d, $J=3.7$ Hz, 1H, H-2), 4.47 (d, $J=11.8$ Hz, 1H, $-OCH_BPh$), 4.14 (d, $J=2.7$ Hz, 1H, H-4), 4.08 (q, $J=7.1$ Hz, 2H, OCH_2), 3.92 (d, $J=2.7$ Hz, 1H, H-3), 3.43 (m, 1H, H-5), 2.61 and 2.34 (m, 4H, H-6 and $NHCH_2$), 1.68 (bs, 1H, NH), 1.47 and 1.31 [each s, each 3H, $>C(CH_3)_2$], 1.24 (m, 31H, methylene protons of alkyl chain and $-OCH_2CH_3$), 0.87 (t, $J=6.6$ Hz, 3H, CH_2CH_3); ^{13}C-NMR ($CDCl_3$): δ 172.22 (>C=O), 137.53, 128.87, 128.42 and 128.26 (Ar–C), 111.97 [$>C(CH_3)_2$], 105.22 (C-1), 82.52 (C-2), 82.22 (C-4), 82.11 (C-3), 71.89 ($-OCH_2Ph$), 60.69 (OCH_2CH_3), 54.46 (C-5), 47.75 ($NHCH_2$), 36.79 (C-6), 32.32, 30.83, 30.09, 27.75, and 23.08 (alkyl carbons), 27.15 and 26.72 [$C(>CH_3)_2$], 14.58 and 14.49 (OCH_2CH_3 and CH_2CH_3). Anal. $C_{35}H_{59}NO_6$ (C, H, N).

*5.2.2.9. Ethyl-[1,5-(dimethyl hex-1-yl-amino)-5,6-dideoxy-1,2-O-isopropylidene-3-O-methyl]-α-D-gluco- and β-L-ido-heptofuranuronate (**13**).* Colourless oil (90% yield); IR (KBr, cm^{-1}): ν_{max} 3332, 2908, 2884, 1731; MS (FAB): *m/z* 402 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$): δ 5.89 (d, $J=3.6$ Hz, 1H, H-1), 4.57 (d, $J=3.6$ Hz, 1H, H-2), 4.16 (d, $J=2.9$ Hz, 1H, H-3), 4.13 (q, $J=7.12$ Hz, 2H, $-OCH_2CH_3$), 3.71 (dd, $J=4.1$ and 3.4 Hz, 1H, H-4), 3.42 (m, 1H, H-5), 3.40 (s, 3H, $-OCH_3$), 2.50 (m, 1H, NHC*H*), 2.39 (m, 2H, H-6), 1.58 (bs, 1H, NH), 1.36, 1.31 [each s, each 3H, $>C(CH_3)_2$], 1.30 (m, 9H, $3\times CH_2$ and OCH_2CH_3), 1.02 (d, $J=6.5$ Hz, 3H, $CHCH_3$), 0.85 [d, $J=6.5$ Hz, 6H, $CH(CH_3)_2$]. Anal. $C_{21}H_{39}NO_6$ (C, H, N).

*5.2.2.10. Ethyl-[3-O-benzyl-1,5-(dimethyl hex-1-yl-amino)-5,6-dideoxy-1,2-O-isopropylidene]-α-D-gluco- and β-L-ido-heptofuranuronate (**14**).* Colourless oil (90% yield); MS (FAB): *m/z* 478 $[M+1]^+$; IR (KBr, cm^{-1}): ν_{max} 3334, 2949, 2869, 1733; ^{1}H-NMR ($CDCl_3$): δ 7.33 (m, 5H, ArH), 5.91 (d, $J=3.6$ Hz, 1H, H-1), 4.68, 4.55 (two dd, each $J=11.9$ Hz, 2H, benzylic CH_2), 4.60 (d, $J=3.6$ Hz, 1H, H-2), 4.16 (d, $J=2.9$ Hz, 1H, H-3), 4.13 (q, $J=7.12$ Hz, 2H, $-OCH_2CH_3$), 3.71 (dd, $J=4.1$ Hz, 3.4 Hz, 1H, H-4), 3.42 (m, 1H, H-5), 3.40 (s, 3H, OCH_3), 2.50 (m, 1H, NHC*H*), 2.39 (m, 2H, H-6), 1.58 (bs, 1H, NH), 1.36 and 1.31 [each s, each 3H, $C(CH_3)_2$], 1.30 (m, 9H, $3\times CH_2$ and OCH_2CH_3), 1.03 (d, $J=6.5$ Hz, 3H, $CHCH_3$), 0.87 [d, $J=6.5$ Hz, 6H, $CH(CH_3)_2$]; ^{13}C-NMR ($CDCl_3$): δ 172.45 (C=O), 137.5, 128.8, 128.4, 128.28 (Ar–C), 111.87 [$C(CH_3)_2$], 105.21 (C-1), 83.18 (C-2), 82.87 (C-4), 82.33 (C-3), 71.83 (OCH_2Ph), 60.65 ($-OCH_2CH_3$), 51.74, 51.57, 51.04, 50.78 (diastereoisomeric $NHCHCH_3$ and C-5), 39.53, 38.81, 37.88, 37.49 (CH_2's), 27.13 and 26.71 [$C(CH_3)_2$], 24.40, 23.90 (CH_2), 22.92, 21.7 (CH_3), 20.70 [$CH(CH_3)_2$], 14.57 (CH_3). Anal. $C_{27}H_{43}NO_6$ (C, H, N).

*5.2.2.11. Ethyl-[5-(carbethoxy ethyl amino)-5,6-dideoxy-1,2-O-isopropylidene-3-O-methyl]-α-D-gluco and β-L-ido-heptofuranuronate (**15**). Minor isomer:* Colourless oil (20% yield), IR (KBr, cm^{-1}): ν_{max} 3648, 2982, 1730; MS (FAB): *m/z* 389 $[M]^+$; ^{1}H-NMR ($CDCl_3$): δ 5.85 (d, $J=3.69$ Hz, 1H, H-1), 4.57 (d, $J=3.8$ Hz, 1H, H-2), 4.20 (m, 5H, H-4, $2\times OCH_2CH_3$), 3.70 (d, $J=2.85$ Hz, 1H, H-3), 3.42 (s, 3H, $-OCH_3$), 3.38 (m, 1H, H-5), 2.95 and 2.50 (each m, each 3H, H-6 and $NHCH_2$), 1.75 (bs, 1H, NH), 1.47 and 1.31 [each s, each 3H, $>C(CH_3)_2$], 1.25 (t, $J=7.13$ Hz, 6H, $-OCH_2CH_3$'s); ^{13}C-NMR ($CDCl_3$): δ 173 (C=O), 112.0 [$>C(CH_3)_2$], 105.2 (C-1), 84.03 (C-2), 82.23 (C-4), 81.79 (C-3), 60.67 ($-OCH_2CH_3$), 57.92 ($-OCH_3$), 52.78 (C-5), 42.9, 36.88, 35.81 ($-CH_2$'s), 27.14, 26.6 and 14.6 (CH_3's).

Major isomer: Colourless oil (70% yield); IR (KBr, cm^{-1}): ν_{max} 3340, 2930, 1738; MS (FAB): *m/z* 389 $[M]^+$; ^{1}H-NMR ($CDCl_3$): δ 5.89 (d, $J=3.8$ Hz, 1H, H-1), 4.55 (d, $J=3.69$ Hz, 1H, H-2), 4.20 (m, 5H, H-4, $2\times OCH_2CH_3$), 3.70 (d, $J=2.96$ Hz, 1H, H-3), 3.37 (s, 3H,

–OCH_3), 3.34 (m, 1H, H-5), 2.96 (t, $J = 6.6$ Hz, 2H, $NHCH_2$), 2.49 (m, each 4H, H-6 and $COCH_2$), 1.99 (bs, 1H, NH), 1.48 and 1.31 [each s, each 3H, $C(CH_3)_2$], 1.23 (t, $J = 7.13$ Hz, 6H, $-OCH_2CH_3$'s); ^{13}C-NMR ($CDCl_3$): δ 172.15 (C=O), 112.0 [$C(CH_3)_2$], 105.16 (C-1), 84.49 (C-2), 82.58 (C-4), 81.49 (C-3), 60.86 and 60.73 ($-OCH_2CH_3$), 57.66 ($-OCH_3$), 54.27 (C-5), 43.00, 36.78 and 35.52 (CH_2's), 27.12, 26.67 and 14.60 (CH_3's). Anal. $C_{18}H_{31}NO_8$ (C, H, N).

*5.2.2.12. Ethyl-[3-O-benzyl-5-(carbethoxy ethyl amino)-5,6-dideoxy-1,2-O-isopropylidene]-α-D-gluco- and β-L-ido-heptofuranuronate (**16**).* Colourless oil (90% yield); IR (KBr, cm^{-1}): ν_{max} 3444, 2982, 1732; MS (FAB): *m/z* 465 $[M]^+$; ^{1}H-NMR ($CDCl_3$): δ 7.42 (m, 5H, ArH), 5.92 (d, $J = 3.74$ Hz, 1H, H-1), 4.66 (d, $J = 3.74$ Hz, 1H, H-2), 4.62 and 4.15 (two d, $J = 12$ Hz, each 1H, benzylic CH_2), 4.07 (m, 5H, H-4, 2 × –OCH_2), 3.91 (d, $J = 3.06$ Hz, 1H, H-3), 3.35 (m, 1H, H-5), 2.93 (t, $J = 6.78$ Hz, 2H, $NHCH_2$), 2.44 (t, $J = 6.7$ Hz, 1H, $COCH_2$), 2.32 and 2.28 (each d, $J = 4.6$ and 6.7 Hz, each 1H, H-6), 1.27 and 1.31 [each s, each 3H, $C(CH_3)_2$], 1.23 (t, $J = 7.0$ Hz, 6H, OCH_2CH_3); ^{13}C-NMR ($CDCl_3$): δ 172.92, 172.04 (2 × *C*OOEt), 137.42 (ArC), 128.85, 128.42, 128.28 (ArC), 111.95 [$C(CH_3)_2$], 105.20 (C-1), 82.54 and 82.13 (C-2, C-4), 71.86 (C-3), 60.76 and 60.14 (2 × –OCH_2), 54.02 (Ar*C*H_2), 43.01, 36.65 and 35.53 (CH_2's), 27.09, 26.66 and 14.53 (CH_3's). Anal. $C_{24}H_{35}NO_8$ (C, H, N).

*5.2.2.13. Ethyl-[3-O-methyl-5-(carbethoxypropyl amino)-5,6-dideoxy-1,2-O-isopropylidene]-α-D-gluco- and β-L-ido-heptofuranuronate (**17**).* Colourless oil (90% yield); IR (KBr, cm^{-1}): ν_{max} 2980, 2930, 2880, 1730 (ester); MS (FAB): 405 $[M+2]^+$; ^{1}H-NMR ($CDCl_3$): δ 5.89 (d, $J = 4.02$ Hz, 1H, H-1), 4.58 (d, $J = 4.02$ Hz, 1H, H-2), 4.13 (m, 5H, H-4, 2 × –OCH_2), 3.70 (d, $J = 2.84$ Hz, 1H, H-3), 3.37 (s, 3H, OCH_3), 3.36 (m, 1H, H-5), 2.78 (t, $J = 6.7$ Hz, 2H, $NHCH_2$), 2.31 (m, 4H, H-6 and $COCH_2$), 1.78 (m, 2H, $NHCH_2CH_2CH_2$), 1.32 and 1.28 [each s, each 3H, $C(CH_3)_2$], 1.24 (t, $J = 7.1$ Hz, 6H, 2 × –OCH_2 CH_3). Anal. $C_{19}H_{33}NO_8$ (C, H, N).

*5.2.2.14. Ethyl-[3-O-benzyl-5-(carbethoxypropyl amino)-5,6-dideoxy-1,2-O-isopropylidene]-α-D-gluco and β-L-ido-heptofuranuronate (**18**). Minor isomer:* Colourless oil (20% yield); IR (KBr, cm^{-1}): ν_{max} 3681, 3022, 2987, 2837, 1728; MS (FAB): *m/z* 480 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$): δ 7.33 (m, 5H, ArH), 5.87 (d, $J = 3.7$ Hz, 1H, H-1), 4.58 (d, $J = 3.7$ Hz, 1H, H-2), 4.66 and 4.54 (two d, $J = 12$ Hz, each 1H, benzylic CH_2), 4.10 (m, 6H, H-4, H-3, 2 × –OCH_2), 3.35 (m, 1H, H-5), 2.52 (m, 4H, H-6, $NHCH_2$), 2.28 (t, $J = 7.1$ Hz, 2H, $COCH_2$), 1.66 (t, $J = 7.1$ Hz, 2H, $NHCH_2CH_2CH_2$), 1.52 (bs, 1H, NH), 1.47 and 1.31 [each s, each 3H, $>C(CH_3)_2$], 1.25, 1.22 (each t, each $J = 7.1$ Hz, each 3H, 2 × OCH_2CH_3); ^{13}C-NMR ($CDCl_3$): δ 175.0, 174.19 (2 × *C*OOEt), 139.36, 130.24, 129.65, 129.51 (ArC), 113.3 (qC), 106.5 (C-1), 83.92, 83.48, 82.10 (C-2, C-4, C-3), 72.39 (benzylic CH_2), 61.52 (OCH_2), 54.34 (C-5), 47.86 ($NHCH_2$), 37.57 (C-6), 32.44 ($COCH_2$), 26.22 ($NHCH_2CH_2CH_2$), 27.17, 26.72 and 14.59 (CH_3's).

Major isomer: Colourless oil (70% yield); IR (KBr, cm^{-1}): ν_{max} 3400, 2928, 1730; MS (FAB): *m/z* 480 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$): δ 7.33 (m, 5H, ArH), 5.87 (d, $J = 3.7$ Hz, 1H, H-1), 4.57 (d, $J = 3.7$ Hz, 1H, H-2), 4.60 and 4.53 (two d, $J = 13.3$ Hz, each 1H, benzylic CH_2), 4.07 (m, 6H, H-4, H-3, 2 × –OCH_2), 3.40 (m, 1H, H-5), 2.66 (m, 4H, $NHCH_2$, H-6), 2.28 (t, $J = 7.1$ Hz, 2H, $COCH_2$), 1.79 (bs, 1H, NH), 1.66 (t, $J = 7.1$ Hz, 2H, $NHCH_2CH_2CH_2$), 1.30, 1.28 [each s, each 3H, $C(CH_3)_2$], 1.25 and 1.22 (each t, each $J = 7.1$ Hz, each 3H, 2 × –OCH_2 CH_3); ^{13}C-NMR ($CDCl_3$): δ 172.2, 170.5 (2 × *C*OOEt), 137.5, 128.88, 128.45, 128.30 (ArC), 111.5 [$C(CH_3)_2$], 105.20 (C-1), 82.55, 82.21, 82.08 (C-2, C-4, C-3), 71.90 (benzylic CH_2), 60.71, 60.52 (2 × –OCH_2), 54.19 (C-5), 46.73 ($NHCH_2$), 36.76 (C-6), 32.52 ($COCH_2$), 26.08 ($NHCH_2CH_2CH_3$), 27.12, 26.68 and 14.58 (CH_3's). Anal. $C_{25}H_{37}NO_8$ (C, H, N).

*5.2.3. General procedure for the preparation of compounds **19**–**22***

*5.2.3.1. 5-Amino-5,6-dideoxy-1,2-O-isopropylidene-3-O-methyl-α-D-gluco- and β-L-ido-heptofuranuronic acid (**19**).* To the magnetically stirred solution of the ethyl-[5-amino-5,6-dideoxy-3-*O*-methyl-1,2-*O*-isopropylidene]-α-D-gluco- and β-L-ido-heptofuranurnate (2.82 g, 9.757 mmol) in aq. EtOH (50%, 57 mL), Et_3N (14.5 mL) was added and the contents were stirred for 50 h. Solvent evaporated under reduced pressure with an azeotrop of EtOH and $C_6H_5CH_3$ to give a residual mass which was subjected to column chromatography over SiO_2 using $CHCl_3$–MeOH (9:1) as eluant to **19** as colourless solid, m.p. 200 °C (91% yield); IR (KBr, cm^{-1}): ν_{max} 2900, 2800 (CH_3CH_2 stretching); MS (FAB): *m/z* 262 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$): δ 5.89 (d, $J = 3.2$ Hz, 1H, H-1), 4.52 (d, $J = 3.2$ Hz, 1H, H-2), 4.33 (d, $J = 6.3$ Hz, H-4), 4.08 (d, $J = 3.2$ Hz, 1H, H-3), 3.98 (m, 1H, H-5), 3.40 (–O–CH_3), 2.50 (m, 2H, H-6), 1.43 and 1.33 [each s, each 3H, $>C(CH_3)$]; ^{13}C-NMR ($CDCl_3$): δ 168.5 ($>C=O$), 111.5 [$>C(CH_3)_2$], 105.19 (C-1), 83.77 (C-2), 82.57 (C-4), 81.93 (C-3), 58.21 (–OCH_3), 47.81 (C-5), 38.62 (C-6), 27.13 and 26.69 [$>C(CH_3)_2$]. Anal. $C_{11}H_{19}NO_6$ (C, H, N).

*5.2.3.2. 5-Amino-5,6-dideoxy-1,2-O-isopropylidene-3-O-benzyl-α-D-gluco- and β-L-ido-heptofuranuronic acid (**20**).* Colourless solid, m.p. 164 °C (90% yield); IR (KBr, cm^{-1}): ν_{max} 2980, 2930, 2880, 1730; MS (FAB): *m/z* 338 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$): δ 7.35 (m, 5H, ArH), 5.89 (d, $J = 3.18$ Hz, 1H, H-1), 4.56 (d, $J = 3.18$

Hz, 1H, H-2), 4.52 (m, 3H, benzylic CH_2 and H-4), 4.08 (d, $J = 2$ Hz, 1H, H-3), 3.69 (m, 1H, H-5), 2.39 (bs, 2H, NH_2), 1.43 and 1.33 [each s, each 3H, $>C(CH_3)_2$]; ^{13}C-NMR ($CDCl_3$): δ 171.36 (COOH), 135.84, 126.73, 126.20, (ArC), 109.58 [$C(CH_3)_2$], 102.86 (C-1), 79.51, 78.55, 77.60 (C-2, C-4 and C-3), 69.24 (–OCH_2Ph), 34.68 (C-6), 46.98, 46.38 (diastereoisomeric C-5), 26.91 and 26.47 [$>C(CH_3)_2$]. Anal. $C_{17}H_{23}NO_6$ (C, H, N).

*5.2.3.3. 5,6-Dideoxy-5-(dodecyl amino)-1,2-O-isopropylidene-3-O-methyl-α-D-gluco- and β-L-ido-heptofuran-6-oic acids (**21**).* Colourless foam (84% yield); IR (KBr, cm^{-1}): ν_{max} 3986.6 (–OH), 2856, 2270 (CH_3 and CH_2 stretching), 1585 (>C=O); MS (FAB): *m/z* 430 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$): δ 5.84 and 5.79 (d, $J = 3.7$ Hz, 1H, H-1), 4.55 (d, $J = 3.5$ Hz, 1H, H-2), 4.22 (d, $J = 2.4$ Hz, 1H, H-4), 3.68 (d, $J = 2.4$ Hz, 1H, H-3), 3.37 (s, 3H, –OCH_3), 3.16 (m, 1H, H-5), 2.63 and 2.23 (each m, each 2H, H-6 and NHCH_2), 1.87 (bs, 1H, NH), 1.47 and 1.29 [each s, each 3H, $>C(CH_3)_2$], 1.24 (m, 20H, alkyl protons), 0.85 (t, $J =$ 7.1 Hz, alkyl CH_3); ^{13}C-NMR ($CDCl_3$): δ 182.48 (>C=O), 116.48 [$>C(CH_3)_2$], 109.78 (C-1), 88.79 (C-2), 86.31 (C-4), 83.96 (C-3), 62.46 (C-5), 59.28 (OCH_3), 51.74 (NHCH_2), 36.70 (C-6), 36.02, 35.50, 35.13, 33.31, 33.20 and 28.46 (alkyl carbons), 32.70 and 32.26 [$>C(CH_3)_2$], 20.02 (–CH_2CH_3). Anal. $C_{23}H_{43}NO_6$ (C, H, N).

*5.2.3.4. 5,6-Dideoxy-5-(hexadecyl amino)-1,2-O-isopropylidene-3-O-methyl-α-D-gluco- and β-L-ido-heptofuran-6-oic acids (**22**).* Colourless foam (75% yield); IR (KBr, cm^{-1}): ν_{max} 3876.5 (–OH), 2873, 2673 (CH_3 and CH_2 stretching), 1585 (>C=O); MS (FAB): *m/z* 488 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$): δ 5.92 and 5.89 (d, $J = 3.8$ Hz, 1H, H-1), 4.53 (d, $J = 3.8$ Hz, 1H, H-2), 4.21 (d, $J = 2.6$ Hz, 1H, H-4), 3.71 (d, $J = 2.6$ Hz, 1H, H-3), 3.39 (s, 3H, –OCH_3), 3.20 (m, 1H, H-5), 2.60 and 2.36 (each m, each 2H, H-6 and $NHCH_2$), 1.85 (bs, 1H, NH), 1.52 and 1.31 [each s, each 3H, $>C(CH_3)_2$], 1.24 (m, 28H, alkyl protons), 0.85 (t, $J =$ 7.0 Hz, alkyl CH_3); ^{13}C-NMR ($CDCl_3$): δ 178.78 (>C=O), 112.04 [$>C(CH_3)_2$], 105.01 (C-1), 84.47 (C-2), 82.09 (C-4), 81.07 (C-3), 57.89 (OCH_3), 54.45 (C-5), 32.29 (C-6), 36.97, 30.12, 29.74, 27.79 and 23.05 (CH_2's), 27.12 and 26.763 [$>C(CH_3)_2$], 14.46 (–CH_2CH_3). Anal. $C_{27}H_{51}NO_6$ (C, H, N).

Acknowledgements

Authors gratefully acknowledge the financial assistance from Department of Ocean Development, India and ICMR, New Delhi for financial Assistance. Authors are also thankful to Dr A.P.B. for his valuable comments during the study.

References

[1] P. Sudre, G. ten Dam, A. Kochi, Bull.World Health Organ. 70 (1992) 149.
[2] R.E. Huebner, K.G. Castro, Ann. Rev. Med. 46 (1995) 47.
[3] V. St. Georgiev, Int. J. Antimicrob. Agents 4 (1994) 157.
[4] R.F. Jacobs, Clin. Infect. Dis. 19 (1994) 1.
[5] J. Trias, R. Benz, Mol. Microbiol. 19 (1994) 283.
[6] N.K. Rastogi, S. Goh, H.L. David, Antimicrob. Agents Chemother. 34 (1990) 750.
[7] P.J. Brennan, H. Nikaido, Ann. Rev. Biochem. 64 (1995) 63.
[8] M. Julius, C.A. Free, G.T. Barry, Methods Enzymol. Sect. A 17 (1970) 171.
[9] N.E. Caceres, N.B. Harris, J.F. Wellehan, Z. Feng, V. Kapur, R.G. Baletta, J. Bacteriol. 179 (1997) 5046.
[10] (a) P.R.J. Gangadharam, M.D. Iseman (Eds.), Antimicrob. Agents Annals, vol. 2, Elsevier Science Publishers BV, Amsterdam, 1987, p. 14;
(b) R. Wise, J.M. Address, Antimicrob. Agents Chemother. 25 (1984) 612.
[11] F.C. Neuhas, D. Gotlich, P.L. Shaw (Eds.), Antibiotics, vol. 1, Springer-Verlag, Heidelberg, Germany, 1967, p. 40.
[12] F.C. Hammes, W.P. Hammes, Pharmacol. Ther. 14 (1981) 265.
[13] J.A. Maddry, N. Bansal, L.E. Bermudez, R.N. Comber, I.M. Orme, W.J. Suling, L.N. Wilson, R.C. Raynolds, Biorg. Med. Chem. Lett. 8 (1998) 237.
[14] T.B. John, D. Varalakshmi, S. Todd, K. Takayama, J.B. Patrick, G.S. Besra, Science 276 (1997) 1420.
[15] (a) G.A. Chung, Z. Aktar, S. Jackson, K. Duncan, Antimicrob. Agents Chemother. 39 (1995) 2235;
(b) J.M. Dickinson, D.A. Mitchison, Tubercle 66 (1985) 109.
[16] Identification of inhibitors of DNA Topoisomerase-II from a synthetic library of glycoconjugates, R.P. Tripathi, S.K. Rastogi, B. Kundu, J.K. Saxena, V.J.M. Reddy, S. Srivastava, S. Chandra, A.P. Bhaduri, Comb. Chem. High Throughput Screen. 4 (2001) 237–244.
[17] A. Namane, C. Goujette, M.P. Fillion, G. Fillion, T. Huynh Dinh, J. Med. Chem. 35 (1992) 3039.
[18] J.F. Fischer, A.W. Harrison, G.L. Bundy, K.F. Wilkinson, B.D. Rush, M.J. Ruwart, J. Med. Chem. 34 (1991) 4140.
[19] E. Negre, M.L. Chance, S.Y. Hanbuta, M. Monsigny, A.C. Roche, R.M. Mayer, Antimicrob. Agents Chemother. 35 (1992) 2228.
[20] M.L. Wolform, S. Hanessian, J. Org. Chem. 27 (1962) 1800.
[21] K. Freudenberg, W. Dun, H. von Hochstetter, Ber. Dtsch. Chem. Ges. 61 (1928) 1732.
[22] K.N. Houk, N.M. Paddon Row, N.G. Rondon, Y.D. Wu, F.K. Brown, C.D. Spellmeyer, J.T. Metz, Y.L. Li, R.J. Loncharich, Science 231 (1986) 1108.
[23] L.A. Collins, S.G. Franzblan, Antimicrob. Agents Chemother. 41 (1997) 1004.
[24] H. Saita, H. Tomioka, K. Sato, T. Yamne, K. Yamashita, K. Hosol, Antimicrob. Agents Chemother. 35 (1991) 542.
[25] C.N. Lee, L.B. Heifets, Am. Rev. Respir. Dis. 136 (1967) 349.