

## **Electronic Supplementary Information**

### **Repairing faulty genes by aminoglycosides: Identification of new pharmacophore with enhanced suppression of diseases-causing nonsense mutations**

Jeyakumar Kandasamy<sup>†</sup>, Dana Atia-Glikin<sup>†</sup>, Valery Belakhov and Timor Baasov<sup>\*</sup>

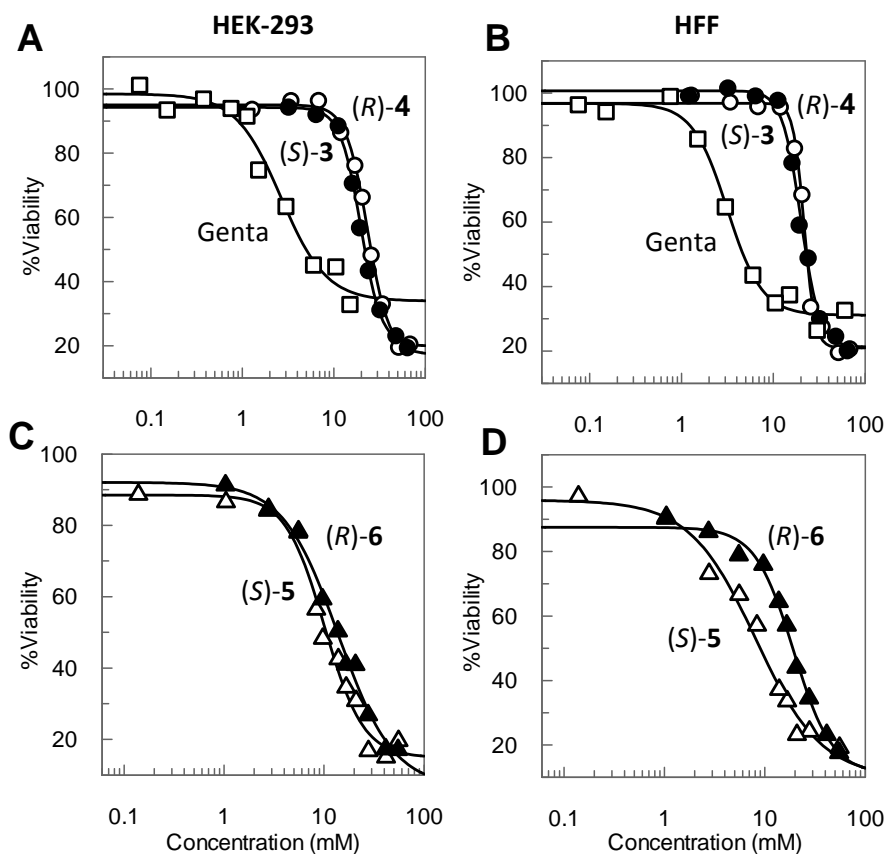
The Edith and Joseph Fischer Enzyme Inhibitors Laboratory, Schulich Faculty of Chemistry,  
Technion - Israel Institute of Technology, Haifa 32000, Israel

<sup>†</sup> Equal Contribution.

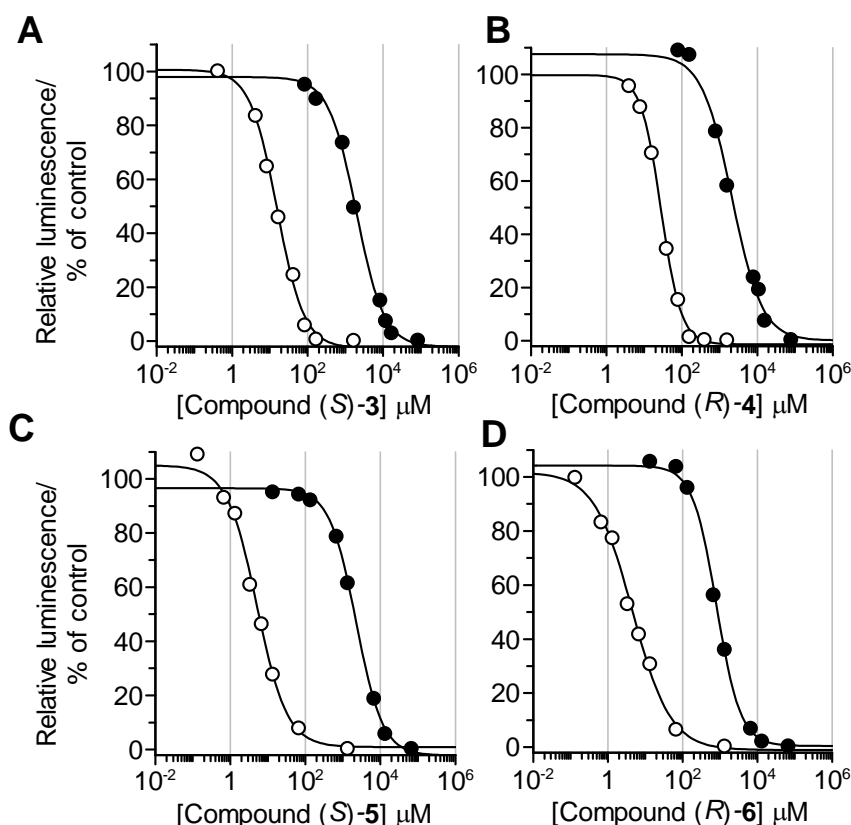
<sup>\*</sup> Corresponding author:

Timor Baasov: Tel: +972-4-829-2590; Fax: +972-4-829-5703;

Email: [chtimor@tx.technion.ac.il](mailto:chtimor@tx.technion.ac.il)



**Figure 1S.** Semilogarithmic plots of the percentages of cell viability versus concentration of the tested compound in HEK-293 (A and C) and in human foreskin fibroblasts (HFF) (B and D) cells, for gentamicin (□), compound (S)-3 (○), compound (R)-4 (●), compound (S)-5 (Δ), and compound (R)-6 (▲). The percentages of cell viability were calculated as the ratio between the numbers of living cells in cultures grown in the presence of the tested compounds, versus cultures grown under the identical protocol but without the tested compound. For the assay details see Experimental Section. The results are averages of at least three independent experiments.



**Figure 2S.** Semilogarithmic plots of in vitro translation inhibition in prokaryotic (●) and eukaryotic (○) systems measured for compound (S)-3 (A), compound (R)-4 (B) compound (S)-5 (C) and compound (R)-6 (D). Prokaryotic and eukaryotic translation inhibition was quantified in coupled transcription/translation assays by using active luciferase detection as described in the Experimental Section.

### Dual luciferase readthrough assays

DNA fragments derived from *PCDH15*, *CFTR*, *Dystrophin* and *IDUA* cDNAs, including the tested nonsense mutation or the corresponding wt codon, and four to six upstream and downstream flanking codons were created by annealing following pairs of complementary oligonucleotides:

#### Usher Syndrome:

*p.R3Xmut/wt:*

5'-GATCCCAGAAGATGTTTT/CGACAGTTTTATCTCTGGACAGAGCT-3'

and 5'-CTGTCAGAGATAAACTGTCA/GAAACATCTTCTG-3';

*p.R245Xmut/wt:*

5'-GATCCAAAATCTGAATGAGAGGT/CGAACCACCACCACCCTCGAGCT-3'

and 5'-CGAGGGTGGTGGTGGTTGTTTCG/ACCTCTCATTTCAGATTTTG-3';

Cystic Fibrosis:

*p.G542Xmut/wt:*

5'-TCGACCAATATAGTTCTTT/GGAGAAGGTGGAATCGAGCT-3' and  
and 5'- CGATTCCACCTTCTCA/GAAGAACTATATTGG-3';

*p.W1282Xmut/wt:*

5'- TCGACAACCTTTGCAACAGTGA/GAGGAAAGCCTTTGAGCT-3' and  
5'- CAAAGGCTTTCCTT/CCACTGTTGCAAAGTTG-3';

Duchene Muscular Dystrophy (DMD):

*p.R3381Xmut/wt:*

5'-TCGACAAAAACAAATTTTGA/CACCAAAAGGTATGAGCT-3' and  
5'- CATACTTTTGGTT/GCAAAATTTGTTTTTTG-3';

Hurler Syndrome:

*p.Q70Xmut/wt:*

5'-TCGACCCTCAGCTGGGACT/CAGCAGCTCAACCTCGAGCT-3' and  
5'-CGAGGTTGAGCTGCTA/GGTCCCAGCTGAGG-3'.

Fragments were inserted in frame into the polylinker of the p2Luc plasmid between either *Bam*HI and *Sac*I (*p.R3X* and *p.R245X*) or *Sal*I and *Sac*I (all the rest) restriction sites. For the *in vitro* readthrough assays, the obtained plasmids, with addition of the tested aminoglycosides were transcribed and translated using the TNT Reticulocyte Lysate Quick Coupled Transcription/Translation System. Luciferase activity was determined after 90 min of incubation at 30°C, using the Dual Luciferase Reporter Assay System (Promega<sup>TM</sup>). For the *ex vivo* readthrough assays, the constructs harboring the R3X, R245X, Q70X and W1282X mutations were transfected to HEK-293 cells with Lipofectamine 2000 (Invitrogen) and addition of the tested compounds was performed 6 h post transfection. The cells were harvested following 16 h incubation with the aminoglycosides. Stop codon readthrough was calculated as previously described (see G. Grentzmann, J. A. Ingram, P. J. Kelly, R. F. Gesteland, and J. F. Atkins, *RNA*, 1998, **4**, 479.)

**General methods.** 1D and 2D NMR spectras were routinely recorded on a Bruker Avance<sup>TM</sup> 500 spectrometer. Mass spectra analysis were obtained either on a Bruker Daltonix Apex 3 mass spectrometer under electron spray ionization (ESI), or by a TSQ-70B mass spectrometer (Finnigan Mat). Reactions were monitored by TLC on Silica Gel 60 F<sub>254</sub> (0.25 mm, Merck), and spots were visualized by charring with a yellow solution containing (NH<sub>4</sub>)Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O (120 g) and (NH<sub>4</sub>)<sub>2</sub>Ce(NO<sub>3</sub>)<sub>6</sub> (5 g) in 10% H<sub>2</sub>SO<sub>4</sub> (800 mL). Column chromatography was performed on a Silica Gel 60 (70-230 mesh). All reactions were carried out under an argon atmosphere with anhydrous solvents, unless otherwise noted. All chemicals unless otherwise stated, were obtained from commercial sources.

#### 4-Methylphenyl 2,3-*O*-1-methylethylidene-1-thio- $\beta$ -D-ribose-1,4-furanoside (**8**).

The mixture of 4-methylphenyl 1-thio- $\beta$ -D-ribofuranoside **7** (25 g, 0.097 mol) and 1,1-dimethoxypropane (22.3 ml, 0.39 mol) in acetone (500 ml) was stirred at room temperature for about five minutes and then catalytic amount of CSA (1.0 g) and MgSO<sub>4</sub> (5.0 g) were added. The reaction progress was monitored by TLC, which indicated completion after 5 h. The reaction mixture was diluted with ethyl acetate and washed with saturated NaHCO<sub>3</sub> and brine. The combined organic layer was dried over MgSO<sub>4</sub>, evaporated and subjected to column chromatography (EtOAc/Hexane) to afford the desired 2,3-isopropylidene derivative in 82% yield (23.5 g). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$ <sub>H</sub> 3.73-3.85 (m, 2H, H-5), 4.37 (m, 1H, H-4), 4.74 (dd, 1H,  $J_1 = 2.5$ ,  $J_2 = 6.0$  Hz, H-2), 4.80 (dd, 1H,  $J_1 = 1.7$ ,  $J_2 = 6.0$  Hz, H-3), 5.52 (d, 1H,  $J = 2.5$  Hz, H-1). The additional peaks in the spectrum were identified as follows:  $\delta$ <sub>H</sub> 1.37 (s, 3H, isopropylidene-CH<sub>3</sub>), 1.53 (s, 3H, isopropylidene-CH<sub>3</sub>), 2.35 (s, 3H, aryl-CH<sub>3</sub>), 7.16 (d, 2H,  $J = 8.0$  Hz), 7.42 (d, 2H,  $J = 8.0$  Hz). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>):  $\delta$ <sub>C</sub> 21.0 (CH<sub>3</sub>), 25.2 (CH<sub>3</sub>), 26.8 (CH<sub>3</sub>), 63.2 (C-5), 81.8 (C-3), 85.7 (C-2), 87.7 (C-4), 93.0 (C-1), 113.3 (quaternary-C), 129.2 (Ar), 129.9 (Ar), 132.3 (Ar), 138.0 (Ar). MALDI TOFMS calculated for C<sub>15</sub>H<sub>20</sub>O<sub>4</sub>Sn ([M+Na]<sup>+</sup>)  $m/e$  319.1; measured  $m/e$  319.09.

The product from the above step (22g, 0.074 mol) was stirred in dichloromethane (500 ml) at room temperature to which Dess-Martin periodinane (DMP, 34.6 g, 0.082 mol) and MgSO<sub>4</sub> (5.0 g) were added. The reaction progress was monitored by TLC, which indicated completion after 8 h. The reaction mixture was diluted with ether and washed with saturated NaHCO<sub>3</sub>, Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, and brine. The combined organic layer was dried over MgSO<sub>4</sub>, evaporated and subjected to column chromatography (EtOAc/Hexane) to yield the title compound **8** (18.0 g, 85% yield). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$ <sub>H</sub> 4.49 (s, 1H, H-4), 4.69 (d, 1H,  $J = 6.5$  Hz, H-2), 5.21 (d, 1H,  $J = 6.0$  Hz, H-3), 5.86 (s, 1H, H-1), 9.80 (s, 1H, H-5, CHO). The additional peaks

in the spectrum were identified as follows:  $\delta_{\text{H}}$  1.37 (s, 3H, isopropylidene-CH<sub>3</sub>), 1.52 (s, 3H, isopropylidene-CH<sub>3</sub>), 2.36 (s, 3H, Ar-CH<sub>3</sub>), 7.19 (d, 2H,  $J = 8.0$  Hz, Ar), 7.41 (d, 2H,  $J = 8.0$  Hz, Ar).  $^{13}\text{C}$  NMR (125 MHz, CDCl<sub>3</sub>):  $\delta_{\text{C}}$  21.0 (CH<sub>3</sub>), 25.1 (CH<sub>3</sub>), 26.2 (CH<sub>3</sub>), 87.1 (C-3), 84.5 (C-2), 89.9 (C-4), 92.6 (C-1), 113.3 (quaternary-C), 128.9 (Ar), 130.0 (Ar), 131.0 (Ar), 137.8 (Ar), 200.3 (CHO). MALDI TOFMS calculated for C<sub>15</sub>H<sub>19</sub>O<sub>4</sub>S ([M+H]<sup>+</sup>)  $m/e$  295.1; measured  $m/e$  295.1.

**4-Methylphenyl 6-deoxy-2,3-*O*-1-methylethylidene-1-thio- $\beta$ -D-allofuranoside [(*R*)-9] and 4-methylphenyl 6-deoxy-2,3-*O*-1-methylethylidene-1-thio- $\alpha$ -L-talofuranoside [(*S*)-10].**

Aldehyde **8** (17 g, 0.057 mol) was stirred in THF (200 ml) at -30 °C for 30 minutes to which the solution of MeMgBr (1.4 M in THF/Toluene, 235 ml, 0.171 mol) was added drop wise with syringe. The reaction mixture was stirred for 2h at the same temperature and progress was monitored by TLC. After completion, the reaction mixture was quenched with saturated NH<sub>4</sub>Cl and extracted with ethyl acetate. The combined organic layer was dried over MgSO<sub>4</sub> and evaporated. The crude product was purified by column chromatography (EtOAc/Hexane) to afford 4:1 ratio of two C5-diastereomers in 88% yield: the major product (*5R*)-**9** (13 g,  $R_f = 0.38$  in EtOAc/Hexane 1:4) and the minor product (*5S*)-**10** (3 g,  $R_f = 0.48$  in EtOAc/Hexane 1:4). The absolute configuration at the C5-position was determined by using  $^1\text{H}$  NMR anisotropy method as described below.

Data for (*5R*)-**9**:  $[\alpha]_{\text{D}}^{20} = -191.4$  ( $c = 1.02$ , CHCl<sub>3</sub>).  $^1\text{H}$  NMR (500 MHz, CDCl<sub>3</sub>):  $\delta_{\text{H}}$  1.25 (d, 3H,  $J = 6.3$  Hz, CH<sub>3</sub>), 4.06 (m, 2H, H-4 and H-5), 4.68 (dd, 1H,  $J_1 = 2.8$ ,  $J_2 = 6.3$  Hz, H-2), 4.87 (t, 1H,  $J = 5.0$  Hz, H-3), 5.46 (d, 1H,  $J = 2.8$  Hz, H-1). The additional peaks in the spectrum were identified as follows:  $\delta_{\text{H}}$  1.37 (s, 3H, isopropylidene-CH<sub>3</sub>), 1.53 (s, 3H, isopropylidene-CH<sub>3</sub>), 2.34 (s, 3H, Ar-CH<sub>3</sub>), 7.15 (d, 2H,  $J = 8.0$  Hz, Ar), 7.42 (d, 2H,  $J = 8.0$  Hz, Ar).  $^{13}\text{C}$  NMR (125 MHz, CDCl<sub>3</sub>):  $\delta_{\text{C}}$  18.5 (C-6), 21.0 (CH<sub>3</sub>), 25.2 (CH<sub>3</sub>), 26.9 (CH<sub>3</sub>), 67.3 (C-5), 80.2 (C-3), 85.4 (C-2), 91.4 (C-4), 92.5 (C-1), 113.4 (quaternary-C), 129.2 (Ar), 129.8 (Ar), 132.3 (Ar), 137.9 (Ar). MALDI TOFMS calculated for C<sub>16</sub>H<sub>22</sub>O<sub>4</sub>SNa ([M+Na]<sup>+</sup>)  $m/e$  333.1; measured  $m/e$  333.1. Data for (*5S*)-**10**:  $[\alpha]_{\text{D}}^{20} = -199.7$  ( $c = 1.04$ , CHCl<sub>3</sub>).  $^1\text{H}$  NMR (500 MHz, CDCl<sub>3</sub>):  $\delta_{\text{H}}$  1.27 (d, 3H,  $J = 6.3$  Hz, CH<sub>3</sub>), 3.90 (m, 1H, H-5), 4.08 (dd, 1H,  $J_1 = 1.3$ ,  $J_2 = 5.6$  Hz, H-4), 4.71 (dd, 1H,  $J_1 = 1.3$ ,  $J_2 = 6.0$  Hz, H-3), 4.76 (dd, 1H,  $J_1 = 2.1$ ,  $J_2 = 6.0$  Hz, H-2), 5.57 (d, 1H,  $J = 2.0$  Hz, H-1). The additional peaks in the spectrum were identified as follows:  $\delta_{\text{H}}$  1.36 (s, 3H, isopropylidene-CH<sub>3</sub>), 1.54 (s, 3H, isopropylidene-CH<sub>3</sub>), 2.35 (s, 3H, Ar-CH<sub>3</sub>),

7.17 (d, 2H,  $J = 8.0$  Hz, Ar), 7.43 (d, 2H,  $J = 8.0$  Hz, Ar).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  19.2 (C-6), 21.0 ( $\text{CH}_3$ ), 25.2 ( $\text{CH}_3$ ), 26.8 ( $\text{CH}_3$ ), 67.9 (C-5), 82.4 (C-3), 85.7 (C-2), 91.6 (C-4), 93.0 (C-1), 113.3 (quaternary-C), 129.4 (Ar), 129.9 (Ar), 131.9 (Ar), 137.9 (Ar). MALDI TOFMS calculated for  $\text{C}_{16}\text{H}_{22}\text{O}_4\text{SNa}$  ( $[\text{M}+\text{Na}]^+$ )  $m/e$  333.1; measured  $m/e$  333.1.

**Preparation of esters (*R,X*)-27 and (*S,X*)-28 for the assignment of absolute configuration at C5.**

A mixture of (*R*)-2-methoxy-2(1-naphthyl)propanoic acid [(*R*)-M $\alpha$ NP] or (*S*)-M $\alpha$ NP (0.07 g, 0.0003 mol), 4-dimethylaminopyridine (DMAP, 0.05 g, 0.0004 mol), 10-camphorsulfonic acid (CSA, 0.025 g), and 1,3-dicyclohexylcarbodiimide (DCC, 0.240 g, 0.0016 mol) was stirred in  $\text{CH}_2\text{Cl}_2$  (30 mL) at  $0^\circ\text{C}$ . The major alcohol **9** from the above (0.1 g, 0.0003 mol), was dissolved in  $\text{CH}_2\text{Cl}_2$  (5 mL), slowly added to the above stirred mixture, and the reaction was left at room temperature for overnight. The mixture was diluted with EtOAc and washed with 1% HCl solution, saturated  $\text{NaHCO}_3$  and brine. The combined organic layer was dried over  $\text{MgSO}_4$ , evaporated and subjected to a column chromatography (EtOAc/Hexane) to yield the desired esters (*R,X*)-**27** (0.135 g, 80%) or (*S,X*)-**28** (0.138 g, 80%).

Data for (*R,X*)-**27**:  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  1.23 (d, 3H,  $J = 6.3$  Hz,  $\text{CH}_3$ ), 3.54 (d, 1H,  $J = 6.1$  Hz, H-3), 3.72 (d, 1H,  $J = 9.0$  Hz, H-4), 4.18 (dd, 1H,  $J_1 = 2.3$ ,  $J_2 = 6.1$  Hz, H-2), 5.08 (m, 1H, H-5), 5.32 (d, 1H,  $J = 2.4$  Hz, H-1). The additional peaks in the spectrum were identified as follows:  $\delta_{\text{H}}$  1.00 (s, 3H, isopropylidene- $\text{CH}_3$ ), 1.32 (s, 3H, isopropylidene- $\text{CH}_3$ ), 2.04 (s, 3H,  $\text{CH}_3$ ), 2.32 (s, 3H, Ar- $\text{CH}_3$ ), 3.14 (s, 3H,  $\text{OCH}_3$ ), 7.11 (d, 2H,  $J = 8.0$  Hz, Ar), 7.28-7.31 (m, 2H, Ar), 7.48-7.56 (m, 3H, Ar), 7.65 (d, 1H,  $J = 8.0$  Hz, Ar), 7.85 (dd, 2H,  $J_1 = 4.7$ ,  $J_2 = 8.0$  Hz, Ar), 8.47 (d, 1H,  $J = 8.0$  Hz, Ar).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  17.1 (C-6), 21.0 ( $\text{CH}_3$ ), 21.5 ( $\text{CH}_3$ ), 24.8 ( $\text{CH}_3$ ), 26.6 ( $\text{CH}_3$ ), 50.9 ( $\text{OCH}_3$ ), 70.5 (C-5), 81.2 (C-3), 81.3 (quaternary-C), 84.8 (C-2), 88.0 (C-4), 92.5 (C-1), 112.9 (quaternary-C), 124.7 (Ar), 125.0 (Ar), 125.7 (Ar), 125.8 (Ar), 126.6 (Ar), 128.8 (Ar), 129.5 (Ar), 129.7 (Ar), 130.3 (Ar), 131.2 (Ar), 131.3 (Ar), 134.0 (Ar), 134.6 (Ar), 137.2 (Ar), 173.1 (C=O). MALDI TOFMS calculated for  $\text{C}_{30}\text{H}_{34}\text{O}_6\text{SNa}$  ( $[\text{M}+\text{Na}]^+$ )  $m/e$  545.2; measured  $m/e$  545.2. Data for (*S,X*)-**28**:  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  0.95 (d, 3H,  $J = 6.3$  Hz,  $\text{CH}_3$ ), 3.84 (dd, 1H,  $J_1 = 1.5$ ,  $J_2 = 6.2$  Hz, H-3), 3.87 (dd, 1H,  $J_1 = 1.5$  and  $J_2 = 6.2$  Hz, H-4), 4.08 (dd, 1H,  $J_1 = 3.4$ ,  $J_2 = 6.1$  Hz, H-2), 5.06 (m, 1H, H-5), 5.27 (d, 1H,  $J = 3.4$  Hz, H-1). The additional peaks in the spectrum were identified as follows:  $\delta_{\text{H}}$  1.14 (s, 3H, isopropylidene- $\text{CH}_3$ ), 1.41 (s, 3H, isopropylidene- $\text{CH}_3$ ), 2.09 (s, 3H,  $\text{CH}_3$ ), 2.33 (s, 3H, Ar- $\text{CH}_3$ ), 3.14 (s, 3H,  $\text{OCH}_3$ ), 7.12 (d, 2H,  $J = 8.0$  Hz, Ar), 7.35 (d, 2H,  $J = 8.0$  Hz, Ar), 7.49-7.67 (m, 3H, Ar), 7.69 (d, 1H,  $J = 8.0$  Hz, Ar), 7.88 (d, 2H,  $J = 8.0$  Hz, Ar),

8.41 (d, 1H,  $J = 8.0$  Hz, Ar).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  16.0 (C-6), 21.0 ( $\text{CH}_3$ ), 21.5 ( $\text{CH}_3$ ), 24.9 ( $\text{CH}_3$ ), 26.8 ( $\text{CH}_3$ ), 50.8 ( $\text{OCH}_3$ ), 71.4 (C-5), 81.0 (C-3), 81.5 (quaternary-C), 84.7 (C-2), 87.5 (C-4), 92.6 (C-1), 113.3 (quaternary-C), 124.7 (Ar), 125.2 (Ar), 125.7 (Ar), 126.0 (Ar), 126.4 (Ar), 128.6 (Ar), 129.4 (Ar), 129.7 (Ar), 130.3 (Ar), 131.3 (Ar), 131.5 (Ar), 133.8 (Ar), 134.8 (Ar), 137.4 (Ar), 173.4 (C=O). MALDI TOFMS calculated for  $\text{C}_{30}\text{H}_{34}\text{O}_6\text{SNa}$  ( $[\text{M}+\text{Na}]^+$ )  $m/e$  545.2; measured  $m/e$  545.2.

**4-Methylphenyl 6-deoxy-5-*O*-tosyl-2,3-*O*-1-methylethylidene-1-thio- $\beta$ -D-allofuranoside [(*R*)-11].**

To a stirred solution of compound (*R*)-9 (13 g, 0.041 mol) in pyridine (200 ml) at 0 °C, were added tosyl chloride (15.6 g, 0.082 mol) and 4-DMAP (1 g). The reaction temperature was raised to room temperature and progress was monitored by TLC. After completion (36 h), the reaction mixture was diluted with ethyl acetate and sequentially washed with 1% aqueous HCl solution, saturated  $\text{NaHCO}_3$ , and brine. The combined organic layer was dried over  $\text{MgSO}_4$ , evaporated and subjected to column chromatography (EtOAc/Hexane) to obtain the title compound (*R*)-11 (16.0 g) in 82% yield.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  1.28 (d, 3H,  $J = 6.2$  Hz,  $\text{CH}_3$ ), 3.99 (d, 1H,  $J = 8.6$  Hz, H-4), 4.60 (dd, 1H,  $J_1 = 2.0$ ,  $J_2 = 6.2$  Hz, H-2), 4.67 (d, 1H,  $J = 6.2$  Hz, H-3), 4.92 (m, 1H, H-5), 5.48 (d, 1H,  $J = 1.8$  Hz, H-1). The additional peaks in the spectrum were identified as follows:  $\delta_{\text{H}}$  1.30 (s, 3H, isopropylidene- $\text{CH}_3$ ), 1.48 (s, 3H, isopropylidene- $\text{CH}_3$ ), 2.34 (s, 3H, Ar- $\text{CH}_3$ ), 2.45 (s, 3H, Ar- $\text{CH}_3$ ), 7.13 (d, 2H,  $J = 8.0$  Hz, Ar), 7.30-7.38 (m, 4H, Ar), 7.87 (d, 2H,  $J = 8.0$  Hz, Ar).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  18.0 (C-6), 21.0 ( $\text{CH}_3$ ), 21.6 ( $\text{CH}_3$ ), 25.0 ( $\text{CH}_3$ ), 26.6 ( $\text{CH}_3$ ), 77.1 (C-5), 81.2 (C-3), 85.0 (C-2), 87.9 (C-4), 92.3 (C-1), 113.6 (quaternary-C), 127.9 (Ar), 129.8 (2C, Ar), 129.9 (Ar), 131.0 (Ar), 133.8 (Ar), 137.4 (Ar), 144.8 (Ar). MALDI TOFMS calculated for  $\text{C}_{23}\text{H}_{29}\text{O}_6\text{S}_2$  ( $[\text{M}+\text{H}]^+$ )  $m/e$  465.1; measured  $m/e$  465.1.

**4-Methylphenyl 6-deoxy-5-*O*-tosyl-2,3-*O*-1-methylethylidene-1-thio- $\alpha$ -L-allofuranoside[(*S*)-12]**

To a stirred solution of compound (*S*)-10 (10 g, 0.032 mol) in pyridine (200 ml) at 0 °C, were added tosyl chloride (15.6 g, 0.082 mol) and 4-DMAP (1 g). The reaction temperature was raised to room temperature and progress was monitored by TLC. After completion (36 h), the reaction mixture was diluted with ethyl acetate and sequentially washed with 1% aqueous HCl solution, saturated  $\text{NaHCO}_3$ , and brine. The combined organic layer was dried over  $\text{MgSO}_4$ , evaporated and subjected to column chromatography (EtOAc/Hexane) to obtain the title compound (*S*)-12 (14.0 g) in 88% yield.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  1.37 (d, 3H,  $J =$



6.4 Hz, CH<sub>3</sub>), 4.09 (dd, 1H,  $J_1 = 2.8$ ,  $J_2 = 4.3$  Hz, H-4), 4.48 (dd, 1H,  $J_1 = 2.8$ ,  $J_2 = 6.2$  Hz, H-3), 4.55 (dd, 1H,  $J_1 = 4.0$ ,  $J_2 = 6.2$  Hz, H-2), 4.82 (m, 1H, H-5), 5.25 (d, 1H,  $J = 4.0$  Hz, H-1). The additional peaks in the spectrum were identified as follows:  $\delta_{\text{H}}$  1.30 (s, 3H, isopropylidene-CH<sub>3</sub>), 1.50 (s, 3H, isopropylidene-CH<sub>3</sub>), 2.35 (s, 3H, Ar-CH<sub>3</sub>), 2.43 (s, 3H, Ar-CH<sub>3</sub>) 7.12 (d, 2H,  $J = 8.0$  Hz, Ar), 7.32 (d, 2H,  $J = 8.0$  Hz, Ar), 7.38 (d, 2H,  $J = 8.0$  Hz, Ar), 7.87 (d, 2H,  $J = 8.0$  Hz, Ar). <sup>13</sup>CNMR (125 MHz, CDCl<sub>3</sub>):  $\delta_{\text{C}}$  17.0 (C-6), 21.0 (Ar-CH<sub>3</sub>), 21.6 (Ar-CH<sub>3</sub>), 25.3 (isopropylidene -CH<sub>3</sub>), 27.2 (isopropylidene -CH<sub>3</sub>), 78.3 (C-5), 81.2 (C-3), 84.6 (C-2), 86.7 (C-4), 92.2 (C-1), 114.1 (quaternary-C), 127.7 (Ar), 129.6 (Ar), 129.7 (Ar), 129.8 (Ar), 132.3 (Ar), 134.1 (Ar), 137.6 (Ar), 144.7 (Ar). MALDI TOFMS calculated for C<sub>23</sub>H<sub>28</sub>O<sub>6</sub>S<sub>2</sub>Na ([M+Na]<sup>+</sup>)  $m/e$ : 487.1; measured  $m/e$ : 487.1

#### **4-Methylphenyl 5-azido-5,6-dideoxy-2,3-O-1-methylethylidene-1-thio- $\alpha$ -L-talofuranoside [(S)-13]**

To a stirred solution of compound (*R*)-**11** (15 g, 0.032 mol) in DMF (250 ml) were added NaN<sub>3</sub> (10 g, 0.15 mol) and HMPA (15 ml) at room temperature. The reaction temperature was raised to 70 °C and progress was monitored by TLC. After completion (10 h), the reaction mixture was diluted with ethyl acetate and sequentially washed with 1% aqueous HCl solution, saturated NaHCO<sub>3</sub>, and brine. The combined organic layer was dried over MgSO<sub>4</sub>, evaporated and subjected to column chromatography (EtOAc/Hexane) to obtain the title compound (*S*)-**13** (6 g) in 55% yield. <sup>1</sup>HNMR (500 MHz, CDCl<sub>3</sub>):  $\delta_{\text{H}}$  1.35 (d, 3H,  $J = 6.2$  Hz, CH<sub>3</sub>), 3.73 (m, 1H, H-5), 3.99 (dd, 1H,  $J_1 = 3.0$ ,  $J_2 = 6.7$  Hz, H-4), 4.56 (dd, 1H,  $J_1 = 3.0$ ,  $J_2 = 6.5$  Hz, H-3), 4.70 (dd, 1H,  $J_1 = 2.0$ ,  $J_2 = 6.2$  Hz, H-2), 5.39 (d, 1H,  $J = 3.2$  Hz, H-1). The additional peaks in the spectrum were identified as follows:  $\delta_{\text{H}}$  1.36 (s, 3H, isopropylidene-CH<sub>3</sub>), 1.53 (s, 3H, isopropylidene-CH<sub>3</sub>), 2.36 (s, 3H, Ar-CH<sub>3</sub>), 7.15 (d, 2H,  $J = 8.0$  Hz, Ar), 7.46 (d, 2H,  $J = 8.0$  Hz, Ar). <sup>13</sup>CNMR (125 MHz, CDCl<sub>3</sub>):  $\delta_{\text{C}}$  15.5 (C-6), 21.1 (Ar-CH<sub>3</sub>), 25.4 (isopropylidene-CH<sub>3</sub>), 27.1 (isopropylidene-CH<sub>3</sub>), 58.2 (C-5), 81.9 (C-3), 85.1 (C-2), 88.9 (C-4), 91.9 (C-1), 114.2 (quaternary-C), 129.5 (Ar), 129.7 (Ar), 132.4 (Ar), 138.8 (Ar). MALDI TOFMS calculated for C<sub>16</sub>H<sub>20</sub>N<sub>3</sub>O<sub>3</sub>S ([M-H]<sup>-</sup>)  $m/e$ : 334.1; measured  $m/e$ : 334.1.

#### **4-Methylphenyl 5-azido-5,6-dideoxy-2,3-O-1-methylethylidene-1-thio- $\beta$ -D-allofuranoside [(R)-14]**

To a stirred solution of compound (*S*)-**12** (13 g, 0.028 mol) in DMF (250 ml) were added NaN<sub>3</sub> (10 g, 0.15 mol) and HMPA (13 ml) at room temperature. The reaction temperature was raised to 70 °C and progress was monitored by TLC. After completion (10 h), the reaction mixture was diluted with ethyl acetate and sequentially washed with 1% aqueous HCl solution,

saturated NaHCO<sub>3</sub>, and brine. The combined organic layer was dried over MgSO<sub>4</sub>, evaporated and subjected to column chromatography (EtOAc/Hexane) to obtain the title compound (*R*)-**14** (9 g) in 97% yield. <sup>1</sup>HNMR (500 MHz, CDCl<sub>3</sub>): δ<sub>H</sub> 1.32 (d, 3H, *J* = 6.2 Hz, CH<sub>3</sub>), 3.81 (m, 1H, H-5), 3.89 (dd, 1H, *J*<sub>1</sub> = 2.1, *J*<sub>2</sub> = 8.3 Hz, H-4), 4.72 (dd, 1H, *J*<sub>1</sub> = 2.5, *J*<sub>2</sub> = 6.3 Hz, H-2), 4.77 (dd, 1H, *J*<sub>1</sub> = 2.1, *J*<sub>2</sub> = 6.3 Hz, H-3), 5.49 (d, 1H, *J* = 2.5 Hz, H-1). The additional peaks in the spectrum were identified as follows: δ<sub>H</sub> 1.37 (s, 3H, isopropylidene-CH<sub>3</sub>), 1.53 (s, 3H, isopropylidene-CH<sub>3</sub>), 2.35 (s, 3H, Ar-CH<sub>3</sub>), 7.15 (d, 2H, *J* = 8.0 Hz, Ar), 7.74 (d, 2H, *J* = 8.0 Hz, Ar). <sup>13</sup>CNMR (125 MHz, CDCl<sub>3</sub>): δ<sub>C</sub> 16.2 (C-6), 21.0 (Ar-CH<sub>3</sub>), 25.2 (isopropylidene-CH<sub>3</sub>), 26.1 (isopropylidene-CH<sub>3</sub>), 58.1 (C-5), 81.9 (C-3), 85.1 (C-2), 89.1 (C-4), 92.2 (C-1), 113.8 (quaternary-C), 129.7 (Ar), 129.8 (Ar), 131.6 (Ar), 137.6 (Ar). MALDI TOFMS calculated for C<sub>16</sub>H<sub>20</sub>N<sub>3</sub>O<sub>3</sub>S ([M-H]<sup>+</sup>) *m/e*: 334.1; measured *m/e*: 334.1.

#### 4-Methylphenyl 5-azido-5,6-dideoxy-2,3-*O*-dibenzoyl-1-thio- $\alpha$ -L-talofuranoside [(*S*)-**15**]

Compound (*S*)-**13** (6 g, 0.018 mol) was stirred in a mixture of acetic acid-water (100 ml, 8:2) at 70 °C for over night. The reaction progress was monitored by TLC, after completion, the reaction mixture was diluted with ethyl acetate and washed with saturated NaHCO<sub>3</sub> and brine. The combined organic layer was dried over MgSO<sub>4</sub>, evaporated and subjected to column chromatography (EtOAc/Hexane) to obtain desired isopropylidene deprotected product (5 g) in 96% yield. <sup>1</sup>HNMR (500 MHz, CDCl<sub>3</sub>): δ<sub>H</sub> 1.36 (d, 3H, *J* = 6.2 Hz, CH<sub>3</sub>), 3.61 (m, 1H, H-5), 3.82 (t, 1H, *J* = 4.8 Hz, H-4), 4.13 (m, 2H, H-3 and H-2), 5.18 (d, 1H, *J* = 3.7 Hz, H-1). The additional peaks in the spectrum were identified as follows: δ<sub>H</sub> 2.35 (s, 3H, Ar-CH<sub>3</sub>), 7.15 (d, 2H, *J* = 8.0 Hz, Ar), 7.45 (d, 2H, *J* = 8.0 Hz, Ar). <sup>13</sup>CNMR (125 MHz, CDCl<sub>3</sub>): δ<sub>C</sub> 15.2 (C-6), 21.0 (Ar-CH<sub>3</sub>), 58.2 (C-5), 72.0 (C-3), 74.9 (C-2), 86.5 (C-4), 90.5 (C-1), 128.8 (Ar), 129.7 (Ar), 133.0 (Ar), 138.1 (Ar). MALDI TOFMS calculated for C<sub>13</sub>H<sub>16</sub>N<sub>3</sub>O<sub>3</sub>S ([M-H]<sup>+</sup>) *m/e*: 294.1; measured *m/e*: 294.08.

The product from the above step was stirred in pyridine (200 ml) at 0°C to which BzCl (7.14 g, 0.051) and 4-DMAP (1g) was added slowly. The reaction temperature was raised to room temperature and stirred for overnight. The reaction progress was monitored by TLC, after completion, reaction mixture was diluted with ethyl acetate and washed with 1% HCl solution, saturated NaHCO<sub>3</sub> and brine. The combined organic layer was dried over MgSO<sub>4</sub>, evaporated and subjected to column chromatography (EtOAc/Hexane) to obtain title compound (*S*)-**15** (8.0 g) in 94% yield. <sup>1</sup>HNMR (500 MHz, CDCl<sub>3</sub>): δ<sub>H</sub> 1.38 (d, 3H, *J* = 6.2 Hz, CH<sub>3</sub>), 3.81 (m, 1H, H-5), 4.22 (m, 1H, H-4), 5.55 (m, 1H, H-1), 5.56-5.58 (m, 2H, H-2 and H-3). The additional

peaks in the spectrum were identified as follows:  $\delta_{\text{H}}$  2.37 (s, 3H, Ar-CH<sub>3</sub>), 7.21 (d, 2H,  $J$  = 8.0 Hz, Ar), 7.34-7.42 (m, 4H, Ar), 7.53-7.59 (m, 4H, Ar), 7.90 (dd, 2H,  $J_1$  = 1.2,  $J_2$  = 8.0 Hz, Ar), 7.99 (dd, 2H,  $J_1$  = 1.2,  $J_2$  = 8.0 Hz, Ar).  $^{13}\text{C}$ NMR (125 MHz, CDCl<sub>3</sub>):  $\delta_{\text{C}}$  15.2 (C-6), 21.1 (Ar-CH<sub>3</sub>), 57.9 (C-5), 72.6 (C-3), 74.4 (C-2), 85.1 (C-4), 88.4 (C-1), 127.8 (Ar), 128.3 (2C, Ar), 128.9 (Ar), 129.0 (Ar), 129.6 (Ar), 129.7 (Ar), 129.8 (Ar), 133.4 (2C, Ar), 133.9 (Ar), 138.6 (Ar), 164.9 (C=O), 165.2 (C=O). MALDI TOFMS calculated for C<sub>27</sub>H<sub>25</sub>N<sub>3</sub>O<sub>5</sub>SSNa ([M+Na]<sup>+</sup>)  $m/e$ : 526.2; measured  $m/e$ : 526.1.

#### 4-Methylphenyl 5-azido-5,6-dideoxy-2,3-*O*-dibenzoyl-1-thio- $\beta$ -D-allofuranoside [(*R*)-16]

Compound (*R*)-14 (8 g, 0.023 mol) was stirred in a mixture of acetic acid-water (100 ml, 8:2) at 70 °C for over night. The reaction progress was monitored by TLC, after completion, the reaction mixture was diluted with ethyl acetate and washed with saturated NaHCO<sub>3</sub> and brine. The combined organic layer was dried over MgSO<sub>4</sub>, evaporated and subjected to column chromatography (EtOAc/Hexane) to obtain desired isopropylidene deprotected product (6.5 g) in 92% yield.  $^1\text{H}$ NMR (500 MHz, CDCl<sub>3</sub>):  $\delta_{\text{H}}$  1.36 (d, 3H,  $J$  = 6.2 Hz, CH<sub>3</sub>), 3.65 (m, 1H, H-5), 3.78 (dd, 1H,  $J_1$  = 2.5,  $J_2$  = 7.5 Hz, H-4), 4.09 (t, 1H,  $J$  = 5.0 Hz, H-2), 4.15 (t, 1H,  $J$  = 4.5 Hz, H-3), 5.18 (d, 1H,  $J$  = 5.0 Hz, H-1). The additional peaks in the spectrum were identified as follows:  $\delta_{\text{H}}$  2.36 (s, 3H, Ar-CH<sub>3</sub>), 7.15 (d, 2H,  $J$  = 8.0 Hz, Ar), 7.44 (d, 2H,  $J$  = 8.0 Hz, Ar).  $^{13}\text{C}$ NMR (125 MHz, CDCl<sub>3</sub>):  $\delta_{\text{C}}$  16.0 (C-6), 21.0 (Ar-CH<sub>3</sub>), 59.0 (C-5), 71.9 (C-3), 74.9 (C-2), 86.4 (C-4), 90.3 (C-1), 128.9 (Ar), 129.7 (Ar), 132.8 (Ar), 138.1 (Ar). MALDI TOFMS calculated for C<sub>13</sub>H<sub>16</sub>N<sub>3</sub>O<sub>3</sub>S ([M-H]<sup>-</sup>)  $m/e$ : 294.1; measured  $m/e$ : 294.08.

The product from the above step was stirred in pyridine (200 ml) at 0°C to which BzCl (7.14 g, 0.051) and 4-DMAP (1g) was added slowly. The reaction temperature was raised to room temperature and stirred for overnight. The reaction progress was monitored by TLC, after completion, reaction mixture was diluted with ethyl acetate and washed with 1% HCl solution, saturated NaHCO<sub>3</sub> and brine. The combined organic layer was dried over MgSO<sub>4</sub>, evaporated and subjected to column chromatography (EtOAc/Hexane) to obtain title compound (*R*)-16 (9.5 g) in 93% yield.  $^1\text{H}$ NMR (500 MHz, CDCl<sub>3</sub>):  $\delta_{\text{H}}$  1.42 (d, 3H,  $J$  = 6.7 Hz, CH<sub>3</sub>), 3.74 (m, 1H, H-5), 4.24 (t, 1H,  $J$  = 4.7 Hz, H-4), 5.53 (d, 1H,  $J$  = 5.6 Hz, H-1), 5.50 (t, 1H,  $J$  = 5.5 Hz, H-2), 5.65 (t, 1H,  $J$  = 5.5 Hz, H-3). The additional peaks in the spectrum were identified as follows:  $\delta_{\text{H}}$  2.38 (s, 3H, Ar-CH<sub>3</sub>), 7.20 (d, 2H,  $J$  = 8.0 Hz, Ar), 7.38 (t, 4H,  $J$  = 7.6 Hz, Ar), 7.51 (d, 2H,  $J$  = 8.0 Hz, Ar), 7.55 (t, 2H,  $J$  = 8.0 Hz, Ar), 7.93-7.96 (m, 4H, Ar).  $^{13}\text{C}$ NMR (125 MHz, CDCl<sub>3</sub>):  $\delta_{\text{C}}$  15.5 (C-6), 21.1 (Ar-CH<sub>3</sub>), 58.5 (C-5), 71.8 (C-3), 74.2 (C-2), 84.9 (C-4), 88.2 (C-

1), 127.8 (Ar), 128.3 (2C, Ar), 128.9 (2C, Ar), 129.6 (Ar), 129.7 (Ar), 129.8 (Ar), 133.3 (2C, Ar), 133.8 (Ar), 138.6 (Ar), 164.9 (C=O), 165.0 (C=O). MALDI TOFMS calculated for  $C_{27}H_{25}N_3O_5SNa$  ( $[M+Na]^+$ )  $m/e$ : 526.2; measured  $m/e$ : 526.2

**L-Talofuranose, 5-azido-5,6-dideoxy- 2,3-dibenzoate 1-(2,2,2-trichloroethanimidate) [(S)-17]**

Compound (S)-**15** (8 g, 0.016 mol) was stirred in a mixture of acetone-water (100 ml, 9:1) mixture at  $-30^\circ\text{C}$  for 10 minutes to which *N*-bromosuccinimide (9.16 g, 0.051 mol) was added slowly. The reaction mixture was stirred at same temperature and the progress was monitored by TLC. After completion (3h), reaction mixture was diluted with ethyl acetate and washed saturated  $\text{NaHCO}_3$ , saturated  $\text{Na}_2\text{S}_2\text{O}_3$  and brine. The combined organic layer was dried over  $\text{MgSO}_4$ , evaporated to obtain 6.3 g of corresponding hemiacetal. The hemiacetal was stirred in a mixture of dichloromethane (40 ml) and trichloroacetonitrile (5 ml) at  $0^\circ\text{C}$  for 10 minutes to which catalytic amount of DBU (0.3 ml) was added. The reaction mixture was stirred in same temperature and the progress was monitored by TLC. After completion (3h), the reaction mixture was diluted with DCM and washed with saturated  $\text{NH}_4\text{Cl}$ . The combined organic layer was dried over  $\text{MgSO}_4$  and concentrated to obtain the title compound (S)-**17** in 9 g. The crude product was directly used for the glycosylation reaction without purification.

**D-Allofuranose, 5-azido-5,6-dideoxy- 2,3-dibenzoate 1-(2,2,2-trichloroethanimidate) [(R)-18]**

Compound (R)-**16** (9 g, 0.018 mol) was stirred in a mixture of acetone-water (100 ml, 9:1) mixture at  $-30^\circ\text{C}$  for 10 minutes to which *N*-bromosuccinimide (9.0g, 0.050 mol) was added slowly. The reaction mixture was stirred at same temperature and the progress was monitored by TLC. After completion (3h), reaction mixture was diluted with ethyl acetate and washed saturated  $\text{NaHCO}_3$ , saturated  $\text{Na}_2\text{S}_2\text{O}_3$  and brine. The combined organic layer was dried over  $\text{MgSO}_4$ , evaporated to obtain 6.5 g of corresponding hemiacetal. The hemiacetal was stirred in a mixture of dichloromethane (50 ml) and trichloroacetonitrile (6 ml) at  $0^\circ\text{C}$  for 10 minutes to which catalytic amount of DBU (0.3 ml) was added. The reaction mixture was stirred in same temperature and the progress was monitored by TLC. After completion (3h), the reaction mixture was diluted with DCM and washed with saturated  $\text{NH}_4\text{Cl}$ . The combined organic layer was dried over  $\text{MgSO}_4$  and concentrated to obtain the title compound (R)-**18** in 9 g. The crude product was directly used for the glycosylation reaction without purification.

**5-*O*-(5-Azido-5,6-dideoxy-2,3-*O*-dibenzoyl- $\alpha$ -L-talofuranosyl)-3',4',6',6-tetra-*O*-acetyl-2',1,3-triazido paromamine [(*S*)-21]**

Anhydrous CH<sub>2</sub>Cl<sub>2</sub> (15mL) was added to a powdered, flame-dried 4 Å molecular sieves (2.0 g), followed by the addition acceptor **19** (0.75 g, 0.0013 mol) and donor (*S*)-**17** (2.1 g, 0.0039 mol). The reaction mixture was stirred for 10 min at room temperature and was then cooled to -20°C. A catalytic amount of BF<sub>3</sub>·Et<sub>2</sub>O (0.1 ml) was added and the mixture was stirred at -15 °C and the reaction progress was monitored by TLC, which indicated the completion after 60 min. The reaction mixture was diluted with ethyl acetate and washed with saturated NaHCO<sub>3</sub> and brine. The combined organic layer was dried over MgSO<sub>4</sub>, evaporated and subjected to column chromatography (EtOAc/Hexane) to obtain the titled compound (*S*)-**21** (1.0 g) in 80% yield. <sup>1</sup>HNMR (500 MHz, CDCl<sub>3</sub>): "**Ring I**"  $\delta_{\text{H}}$  3.65 (dd, 1H,  $J_1 = 4.2$ ,  $J_2 = 9.7$  Hz, H-2'), 4.20 (d, 1H,  $J = 11.1$  Hz, H-6'), 4.26 (dd, 1H,  $J_1 = 3.1$ ,  $J_2 = 12.6$  Hz, H-6'), 4.54 (m, 1H, H-5'), 5.08 (dd, 1H,  $J_1 = 9.3$ ,  $J_2 = 10.7$  Hz, H-4'), 5.41 (t, 1H,  $J = 9.9$  Hz, H-3'), 5.85 (d, 1H,  $J = 3.7$  Hz, H-1'); "**Ring II**"  $\delta_{\text{H}}$  1.64 (ddd, 1H,  $J_1 = J_2 = J_3 = 12.5$  Hz, H-2<sub>ax</sub>), 2.42 (td, 1H,  $J_1 = 4.5$ ,  $J_2 = 12.5$  Hz, H-2<sub>eq</sub>), 3.49-3.56 (m, 2H, H-1 and H-3), 3.74 (t, 1H,  $J = 9.5$  Hz, H-4), 3.87 (t, 1H,  $J = 8.7$  Hz, H-5), 5.02 (d, 1H,  $J = 10.1$  Hz, H-6); "**Ring III**"  $\delta_{\text{H}}$  1.27 (d, 3H,  $J = 6.9$  Hz, CH<sub>3</sub>), 3.72 (m, 1H, H-5''), 4.35 (t, 1H,  $J = 6.6$  Hz, H-4''), 5.43 (dd, 1H,  $J_1 = 5.1$ ,  $J_2 = 7.4$  Hz, H-3''), 5.62 (d, 1H,  $J = 3.8$  Hz, H-2''), 5.66 (s, 1H, H-1''). The additional peaks in the spectrum were identified as follows:  $\delta_{\text{H}}$  2.04 (s, 3H, OAc), 2.10 (s, 3H, OAc), 2.11 (s, 3H, OAc), 2.23 (s, 3H, OAc), 7.35-7.43 (m, 4H, Ar), 7.53-7.60 (m, 2H, Ar), 7.89-7.95 (m, 4H, Ar). <sup>13</sup>CNMR (125 MHz, CDCl<sub>3</sub>):  $\delta_{\text{C}}$  15.3 (C-6''), 20.5 (OAc), 20.6 (2C, OAc), 20.9 (OAc), 31.6 (C-1), 58.3, 58.5, 59.3, 61.7, 61.8, 68.0, 68.2, 70.9, 71.8, 73.6, 74.6, 78.1, 79.5, 84.4, 96.6 (C-1'), 107.6 (C-1''), 128.4 (Ar), 128.5 (2C, Ar), 128.7 (Ar), 129.6 (2C, Ar), 133.5 (Ar), 133.6 (Ar), 164.8 (C=O), 165.3 (C=O), 169.7 (C=O), 169.9 (C=O), 170.1 (C=O), 170.6 (C=O). MALDI TOFMS calculated for C<sub>40</sub>H<sub>43</sub>N<sub>12</sub>O<sub>16</sub> ([M-H]<sup>+</sup>)  $m/e$ : 947.3; measured  $m/e$ : 947.28.

**5-*O*-(5-Azido-5,6-dideoxy-2,3-*O*-dibenzoyl- $\beta$ -D-allofuranosyl)-3',4',6',6-tetra-*O*-acetyl-2',1,3-triazido paromamine ((*R*)-22)**

Anhydrous CH<sub>2</sub>Cl<sub>2</sub> (15mL) was added to a powdered, flame-dried 4 Å molecular sieves (2.0 g), followed by the addition acceptor **19** (0.75 g, 0.0013 mol) and donor (*R*)-**18** (2.1 g, 0.0039 mol). The reaction mixture was stirred for 10 min at room temperature and was then cooled to -20°C. A catalytic amount of BF<sub>3</sub>·Et<sub>2</sub>O (0.1 ml) was added and the mixture was stirred at -15 °C and the reaction progress was monitored by TLC, which indicated the

completion after 60 min. The reaction mixture was diluted with ethyl acetate and washed with saturated NaHCO<sub>3</sub> and brine. The combined organic layer was dried over MgSO<sub>4</sub>, evaporated and subjected to column chromatography (EtOAc/Hexane) to obtain the titled compound (*R*)-**22** (1.02 g) in 82% yield. <sup>1</sup>HNMR (500 MHz, CDCl<sub>3</sub>): "**Ring I**" δ<sub>H</sub> 3.55 (dd, 1H, *J*<sub>1</sub> = 4.5 and *J*<sub>2</sub> = 10.7 Hz, H-2'), 4.17 (d, 1H, *J* = 13.1 Hz, H-6'), 4.30 (dd, 1H, *J*<sub>1</sub> = 4.2 and *J*<sub>2</sub> = 12.4 Hz, H-6'), 4.56 (m, 1H, H-5'), 5.08 (t, 1H, *J* = 9.7 Hz, H-4'), 5.43 (t, 1H, *J* = 9.9 Hz, H-3'), 5.83 (d, 1H, *J* = 3.9 Hz, H-1'); "**Ring II**" δ<sub>H</sub> 1.64 (ddd, 1H, *J*<sub>1</sub>=*J*<sub>2</sub>=*J*<sub>3</sub>=12.5 Hz, H-2<sub>ax</sub>), 2.42 (td, 1H, *J*<sub>1</sub>=4.5 and *J*<sub>2</sub>=12.5 Hz, H-2<sub>eq</sub>), 3.49-3.56 (m, 2H, H-1 and H-3), 3.74 (t, 1H, *J* = 10.0 Hz, H-4), 3.92 (t, 1H, *J* = 9.1 Hz, H-5), 5.03 (d, 1H, *J* = 9.9 Hz, H-6); "**Ring III**" δ<sub>H</sub> 1.41 (d, 3H, *J* = 6.9 Hz, CH<sub>3</sub>), 3.76 (m, 1H, H-5"), 4.39 (t, 1H, *J* = 4.9 Hz, H-4"), 5.50 (dd, 1H, *J*<sub>1</sub> = 5.1 and *J*<sub>2</sub> = 7.0 Hz, H-3"), 5.60 (d, 1H, *J* = 4.9 Hz, H-2"), 5.68 (s, 1H, H-1"). The additional peaks in the spectrum were identified as follows: δ<sub>H</sub> 2.06 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.11 (s, 3H, OAc), 2.34 (s, 3H, OAc), 7.37-7.41 (m, 4H, Ar), 7.57 (m, 2H, Ar), 7.92 (d, 4H, *J* = 8.0 Hz Ar). <sup>13</sup>CNMR (125 MHz, CDCl<sub>3</sub>): δ<sub>H</sub> 15.1 (C-6"), 20.5 (OAc), 20.6 (OAc), 20.7 (OAc), 20.8 (OAc), 31.7 (C-1), 58.2 (2C), 58.6, 61.7 (2C), 68.0, 68.1, 70.7, 71.4, 73.7, 74.6, 77.8, 79.2, 83.9, 96.6 (C-1'), 107.1 (C-1"), 128.4 (2C, Ar), 128.7 (Ar), 128.8 (Ar), 129.6 (2C, Ar), 133.4 (Ar), 133.5 (Ar), 164.9 (C=O), 165.4 (C=O), 169.7 (2C, C=O), 169.9 (C=O), 170.6 (C=O). MALDI TOFMS calculated for C<sub>40</sub>H<sub>44</sub>N<sub>12</sub>O<sub>16</sub>Na ([M+Na]<sup>+</sup>) *m/e*: 971.3; measured *m/e*: 971.4.

**5-*O*-(5-Azido-5,6-dideoxy-2,3-*O*-dibenzoyl-α-*L*-talofuranosyl)-3',4',6',6-tetra-*O*-acetyl-2',3-diazido-1-*N*-[(*S*)-4-azido-2-*O*-acetyl-butanoyl]paromamine [(*S*)-**23**]**

Anhydrous CH<sub>2</sub>Cl<sub>2</sub> (15mL) was added to a powdered, flame-dried 4 Å molecular sieves (2.0 g), followed by the addition acceptor **20** (1.0 g, 0.0014 mol) and donor (*S*)-**17** (2.2 g, 0.0042 mol). The reaction mixture was stirred for 10 min at room temperature and was then cooled to -20°C. A catalytic amount of BF<sub>3</sub>-Et<sub>2</sub>O (0.1 ml) was added and the mixture was stirred at -15 °C and the reaction progress was monitored by TLC, which indicated the completion after 60 min. The reaction mixture was diluted with ethyl acetate and washed with saturated NaHCO<sub>3</sub> and brine. The combined organic layer was dried over MgSO<sub>4</sub>, evaporated and subjected to column chromatography (EtOAc/Hexane) to obtain the titled compound (*S*)-**23** (1.19 g) in 79% yield. <sup>1</sup>HNMR (500 MHz, CDCl<sub>3</sub>): "**Ring I**" δ<sub>H</sub> 3.63 (dd, 1H, *J*<sub>1</sub> = 4.2, *J*<sub>2</sub> = 10.4 Hz, H-2'), 4.18 (d, 1H, *J* = 10.8 Hz, H-6'), 4.29 (dd, 1H, *J*<sub>1</sub> = 2.9, *J*<sub>2</sub> = 12.4 Hz, H-6'), 4.54 (m, 1H, H-5'), 5.09 (t, 1H, *J* = 10.2 Hz, H-4'), 5.42 (t, 1H, *J* = 10.2 Hz, H-3'), 5.84 (d, 1H, *J* = 3.9 Hz, H-1'); "**Ring II**" δ<sub>H</sub> 1.50 (ddd, 1H, *J*<sub>1</sub>=*J*<sub>2</sub>=*J*<sub>3</sub>=12.5 Hz, H-2<sub>ax</sub>), 2.53 (td, 1H, *J*<sub>1</sub> = 4.5, *J*<sub>2</sub> = 12.5 Hz, H-2<sub>eq</sub>), 3.60 (m, 1H, H-3), 3.74 (t, 1H, *J* = 9.5 Hz, H-4), 3.96 (t, 1H, *J* = 10.0 Hz, H-

5), 4.06 (m, 1H, H-1), 4.93 (d, 1H,  $J = 9.9$  Hz, H-6); "**Ring III**"  $\delta_{\text{H}}$  1.33 (d, 3H,  $J = 6.9$  Hz, CH<sub>3</sub>), 3.70 (m, 1H, H-5"), 4.33 (t, 1H,  $J = 6.0$  Hz, H-4"), 5.55 (dd, 1H,  $J_1 = 4.9$ ,  $J_2 = 7.7$  Hz, H-3"), 5.57 (m, 2H, H-2" and H-1"). The additional peaks in the spectrum were identified as follows:  $\delta_{\text{H}}$  2.04-2.10 (m, 2H, H-8 and H-8), 2.06 (s, 3H, OAc), 2.09 (s, 6H, OAc), 2.26 (s, 3H, OAc), 2.35 (s, 3H, OAc), 3.37 (dd, 2H,  $J_1 = 6.0$ ,  $J_2 = 7.5$  Hz, H-9 and H-9), 5.20 (dd, 1H,  $J_1 = 1.5$ ,  $J_2 = 8.5$  Hz, H-7), 6.69 (d, 1H,  $J = 7.5$  Hz, NH), 7.35 (t, 2H,  $J = 8.0$  Hz, Ar), 7.43 (t, 2H,  $J = 8.0$  Hz, Ar), 7.53 (t, 1H,  $J = 8.0$  Hz, Ar), 7.55 (t, 1H,  $J = 8.0$  Hz, Ar), 7.87 (dd, 2H,  $J_1 = 1.1$ ,  $J_2 = 8.2$  Hz, Ar), 7.95 (dd, 2H,  $J_1 = 1.2$ ,  $J_2 = 8.2$  Hz, Ar).  $^{13}\text{C}$ NMR (125 MHz, CDCl<sub>3</sub>):  $\delta_{\text{C}}$  15.4 (C-6"), 20.6 (4C, OAc), 20.9 (OAc), 31.9 (C-1), 47.0, 48.5, 58.4, 58.7, 61.7, 61.8, 68.0, 68.2, 70.8, 70.9, 71.4, 73.1, 74.7, 78.3, 79.7, 83.7, 96.7 (C-1'), 107.5 (C-1"), 128.4 (Ar), 128.5 (2C, Ar), 128.7 (Ar), 129.6 (Ar), 129.7 (Ar), 133.5 (Ar), 133.6 (Ar), 165.0 (C=O), 165.2 (C=O), 168.8 (C=O), 169.7 (2C, C=O), 169.8 (C=O), 170.6 (C=O), 172.5 (C=O). MALDI TOFMS calculated for C<sub>46</sub>H<sub>54</sub>N<sub>13</sub>O<sub>19</sub> ([M+H]<sup>+</sup>)  $m/e$ : 1092.3; measured  $m/e$ : 1092.3.

**5-*O*-(5-Azido-5,6-dideoxy-2,3-*O*-dibenzoyl- $\beta$ -D-allofuranosyl)-3',4',6',6-tetra-*O*-acetyl-2',3-diazido-1-N-[(*S*)-4-azido-2-*O*-acetyl-butanoyl]paromamine [(*R*)-**24**]**

Anhydrous CH<sub>2</sub>Cl<sub>2</sub> (15mL) was added to a powdered, flame-dried 4 Å molecular sieves (2.0 g), followed by the addition acceptor **20** (1.0 g, 0.0014 mol) and donor (*R*)-**18** (2.2 g, 0.0042 mol). The reaction mixture was stirred for 10 min at room temperature and was then cooled to -20°C. A catalytic amount of BF<sub>3</sub>-Et<sub>2</sub>O (0.1 ml) was added and the mixture was stirred at -15 °C and the reaction progress was monitored by TLC, which indicated the completion after 60 min. The reaction mixture was diluted with ethyl acetate and washed with saturated NaHCO<sub>3</sub> and brine. The combined organic layer was dried over MgSO<sub>4</sub>, evaporated and subjected to column chromatography (EtOAc/Hexane) to obtain the titled compound (*R*)-**24** (1.27 g) in 89% yield.  $^1\text{H}$ NMR (500 MHz, CDCl<sub>3</sub>): "**Ring I**"  $\delta_{\text{H}}$  3.53 (dd, 1H,  $J_1 = 4.7$ ,  $J_2 = 10.7$  Hz, H-2'), 4.18 (d, 1H,  $J = 10.1$  Hz, H-6'), 4.30 (dd, 1H,  $J_1 = 3.9$ ,  $J_2 = 12.3$  Hz, H-6'), 4.56 (m, 1H, H-5'), 5.09 (t, 1H,  $J = 10.2$  Hz, H-4'), 5.44 (t, 1H,  $J = 9.7$  Hz, H-3'), 5.84 (d, 1H,  $J = 3.9$  Hz, H-1'); "**Ring II**"  $\delta_{\text{H}}$  1.48 (ddd, 1H,  $J_1 = J_2 = J_3 = 12.5$  Hz, H-2<sub>ax</sub>), 2.52 (td, 1H,  $J_1 = 4.5$ ,  $J_2 = 12.5$  Hz, H-2<sub>eq</sub>), 3.60 (m, 1H, H-3), 3.74 (t, 1H,  $J = 9.5$  Hz, H-4), 4.00-4.08 (m, 2H, H-5 and H-1), 4.93 (t, 1H,  $J = 9.9$  Hz, H-6); "**Ring III**"  $\delta_{\text{H}}$  1.41 (d, 3H,  $J = 6.9$  Hz, CH<sub>3</sub>), 3.83 (m, 1H, H-5"), 4.37 (dd, 1H,  $J_1 = 4.1$ ,  $J_2 = 5.7$  Hz, H-4"), 5.60 (t, 1H,  $J = 6.5$  Hz, H-3"), 5.64 (d, 1H,  $J = 6.5$  Hz, H-2"), 5.70 (s, 1H, H-1"). The additional peaks in the spectrum were identified as follows:  $\delta_{\text{H}}$  2.04-2.10 (m, 2H, H-8 and H-8), 2.06 (s, 3H, OAc), 2.10 (s, 3H, OAc), 2.11 (s, 3H, OAc), 2.22 (s, 3H, OAc), 2.27 (s, 3H, OAc), 3.37 (dd, 2H,  $J_1 = 6.0$ ,  $J_2 = 7.5$  Hz, H-9 and H-9),

5.19 (dd, 1H,  $J_1 = 1.5$ ,  $J_2 = 8.5$  Hz, H-7), 6.69 (d, 1H,  $J = 7.5$  Hz, NH), 7.35-7.43 (m, 4H, Ar), 7.53-7.59 (m, 2H, Ar), 7.87-7.92 (m, 4H, Ar).  $^{13}\text{C}$ NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{C}}$  15.3 (C-6"), 20.5 (OAc), 20.5 (OAc), 20.6 (OAc), 20.7 (OAc), 20.8 (OAc), 30.4, 32.1, 47.0, 48.4, 58.2, 58.5, 61.6, 61.7, 68.0, 68.1, 70.7, 70.8, 70.9, 73.4, 74.7, 78.0, 79.5, 83.3, 96.8 (C-1'), 106.9 (C-1"), 128.4 (2C, Ar), 128.7 (2C, Ar), 129.5 (Ar), 129.6 (Ar), 133.5 (2C, Ar), 164.9 (C=O), 165.2 (C=O), 168.9 (C=O), 169.6 (C=O), 169.7 (C=O), 169.8 (C=O), 170.6 (C=O), 172.3 (C=O). MALDI TOFMS calculated for  $\text{C}_{46}\text{H}_{54}\text{N}_{13}\text{O}_{19}$  ( $[\text{M}+\text{H}]^+$ )  $m/e$ : 1092.3; measured  $m/e$ : 1092.3.

### 5-O-(5-Amino-5,6-dideoxy- $\alpha$ -L-talofuranosyl)-paromamine [(S)-3]

The glycosylation product (S)-**21** (1.0 g, 0.001 mol) was treated with a solution of  $\text{MeNH}_2$  (33% solution in EtOH, 50 mL) and the reaction progress was monitored by TLC (EtOAc/MeOH 85:15), which indicated completion after 8 hours. The reaction mixture was evaporated to dryness and the residue was dissolved in a mixture of THF (5 mL) and aqueous NaOH (1 mM, 5.0 mL). The mixture was stirred at room temperature for 10 minutes, after which  $\text{PMe}_3$  (1 M solution in THF, 5.0 mL, 5.0 mmol) was added. The reaction progress was monitored by TLC [ $\text{CH}_2\text{Cl}_2$ /MeOH/ $\text{H}_2\text{O}$ / $\text{MeNH}_2$  (33% solution in EtOH) 10:15:6:15], which indicated completion after 1 hour. The product was purified by column chromatography on a short column of silica gel. The column was washed with the following solvents: THF (800 mL),  $\text{CH}_2\text{Cl}_2$  (800 mL), EtOH (200 mL), and MeOH (400 mL). The product was then eluted with a mixture of 20%  $\text{MeNH}_2$  (33% solution in EtOH) in 80% MeOH. Fractions containing the product were combined and evaporated to dryness. The residue was re-dissolved in a small volume of water and evaporated again (2-3 repeats) to afford the free amine form of **3**. The analytically pure product was obtained by passing the above product through a short column of Amberlite CG50 ( $\text{NH}_4^+$  form). The column was first washed with a mixture of MeOH/ $\text{H}_2\text{O}$  (3:2), then the product was eluted with a mixture of MeOH/ $\text{H}_2\text{O}$ / $\text{NH}_4\text{OH}$  (80:10:10) to afford compound **3** (0.405 g, 82% yield). For the storage and biological tests, compound was converted to its sulfate salt form: the free base was dissolved in water, the pH was adjusted around 7.0 with  $\text{H}_2\text{SO}_4$  (0.1 N) and lyophilized.  $[\alpha]_{\text{D}}^{20} = +38.4$  ( $c = 0.2$ , MeOH).  $^1\text{H}$ NMR (500 MHz,  $\text{CD}_3\text{OD}$ ): "Ring I"  $\delta_{\text{H}}$  2.64 (dd, 1H,  $J_1 = 3.7$ ,  $J_2 = 10.4$  Hz, H-2'), 3.27 (t, 1H,  $J = 9.7$  Hz, H-4') 3.52 (t, 1H,  $J = 10.8$  Hz, H-3'), 3.67 (dd, 1H,  $J_1 = 6.0$ ,  $J_2 = 11.8$  Hz, H-6'), 3.79 (m, 1H, H-5'), 3.87 (dd, 1H,  $J_1 = 2.0$ ,  $J_2 = 11.9$  Hz, H-6') 5.20 (d, 1H,  $J = 3.4$  Hz, H-1'); "Ring II"  $\delta_{\text{H}}$  1.20 (ddd, 1H,  $J_1=J_2=J_3= 12.5$  Hz, H-2<sub>ax</sub>), 1.97 (td, 1H,  $J_1 = 4.5$ ,  $J_2 = 12.5$  Hz, H-2<sub>eq</sub>), 2.64 (m, 1H, H-1), 2.78 (m, 1H, H-3), 3.21 (t, 1H,  $J = 9.3$  Hz, H-6), 3.38 (t, 1H,  $J = 9.5$  Hz, H-4), 3.50 (t, 1H,  $J = 9.2$ , H-5); "Ring III"  $\delta_{\text{H}}$  1.18 (d, 3H,  $J = 6.2$  Hz,  $\text{CH}_3$ ), 2.96 (m, 1H, H-5"), 3.57 (t,



1H,  $J = 6.9$  Hz, H-4"), 4.02 (t, 1H,  $J = 5.5$  Hz, H-3"), 4.06 (dd, 1H,  $J_1 = 2.9$ ,  $J_2 = 5.4$  Hz, H-2"), 5.25 (d, 1H,  $J = 2.7$  Hz, H-1").  $^{13}\text{C}$ NMR (125 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta_{\text{C}}$  19.3 (C-6"), 37.5 (C-1), 50.6, 52.3, 52.6, 57.8, 62.7 (C-6'), 72.1, 72.2, 75.3, 75.4, 76.2, 78.6, 84.6, 87.4, 88.6, 102.0 (C-1'), 109.5 (C-1"). MALDI TOFMS calculated for  $\text{C}_{18}\text{H}_{37}\text{N}_4\text{O}_{10}$  ( $[\text{M}+\text{H}]^+$ )  $m/e$ : 469.2; measured  $m/e$ : 469.2.

#### 5-O-(5-Amino-5,6-dideoxy- $\beta$ -D-allofuranosyl)-paromamine [(*R*)-4]

The glycosylation product (*R*)-**22** (1.0 g, 0.001 mol) was treated with a solution of  $\text{MeNH}_2$  (33% solution in EtOH, 50 mL) and the reaction progress was monitored by TLC (EtOAc/MeOH 85:15), which indicated completion after 8 hours. The reaction mixture was evaporated to dryness and the residue was dissolved in a mixture of THF (5 mL) and aqueous NaOH (1 mM, 5.0 mL). The mixture was stirred at room temperature for 10 minutes, after which  $\text{PMe}_3$  (1 M solution in THF, 5.0 mL, 5.0 mmol) was added. The reaction progress was monitored by TLC [ $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{H}_2\text{O}/\text{MeNH}_2$  (33% solution in EtOH) 10:15:6:15], which indicated completion after 1 hour. The product was purified by column chromatography on a short column of silica gel. The column was washed with the following solvents: THF (800 mL),  $\text{CH}_2\text{Cl}_2$  (800 mL), EtOH (200 mL), and MeOH (400 mL). The product was then eluted with a mixture of 20%  $\text{MeNH}_2$  (33% solution in EtOH) in 80% MeOH. Fractions containing the product were combined and evaporated to dryness. The residue was re-dissolved in a small volume of water and evaporated again (2-3 repeats) to afford the free amine form of **4**. The analytically pure product was obtained by passing the above product through a short column of Amberlite CG50 ( $\text{NH}_4^+$  form). The column was first washed with a mixture of MeOH/ $\text{H}_2\text{O}$  (3:2), then the product was eluted with a mixture of MeOH/ $\text{H}_2\text{O}/\text{NH}_4\text{OH}$  (80:10:10) to afford compound **4** (0.398 g, 80% yield). For the storage and biological tests, compound was converted to its sulfate salt form: the free base was dissolved in water, the pH was adjusted around 7.0 with  $\text{H}_2\text{SO}_4$  (0.1 N) and lyophilized.  $[\alpha]_{\text{D}}^{20} = +37.0$  ( $c = 0.2$ , MeOH).  $^1\text{H}$ NMR (500 MHz,  $\text{CD}_3\text{OD}$ ): "**Ring I**"  $\delta_{\text{H}}$  2.64 (dd, 1H,  $J_1 = 3.4$ ,  $J_2 = 10.2$  Hz, H-2'), 3.27 (t, 1H,  $J = 9.1$  Hz, H-4'), 3.52 (t, 1H,  $J = 8.9$  Hz, H-3'), 3.68 (t, 1H,  $J = 6.1$  Hz, H-6'), 3.79 (m, 1H, H-5'), 3.87 (dd, 1H,  $J_1 = 2.5$ ,  $J_2 = 12.2$  Hz, H-6'), 5.20 (d, 1H,  $J = 3.6$  Hz, H-1'); "**Ring II**"  $\delta_{\text{H}}$  1.21 (ddd, 1H,  $J_1 = J_2 = J_3 = 12.5$  Hz, H-2<sub>ax</sub>), 1.97 (td, 1H,  $J_1 = 4.5$ ,  $J_2 = 12.5$  Hz, H-2<sub>eq</sub>), 2.64 (m, 1H, H-1), 2.78 (m, 1H, H-3), 3.18 (t, 1H,  $J = 9.1$  Hz, H-6), 3.37 (t, 1H,  $J = 9.5$  Hz, H-4), 3.46 (t, 1H,  $J = 9.2$  Hz, H-5); "**Ring III**"  $\delta_{\text{H}}$  1.16 (d, 3H,  $J = 6.2$  Hz,  $\text{CH}_3$ ), 3.09 (m, 1H, H-5"), 3.70 (t, 1H,  $J = 5.3$  Hz, H-4"), 4.04 (dd, 1H,  $J_1 = 3.3$ ,  $J_2 = 5.3$  Hz, H-2"), 4.15 (t, 1H,  $J = 5.5$  Hz, H-3"), 5.21 (d, 1H,  $J = 2.7$  Hz, H-1").  $^{13}\text{C}$ NMR (125 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta_{\text{C}}$  18.8 (C-6"), 37.6 (C-1), 49.4, 52.1, 52.6,

57.8, 62.8 (C-6'), 70.8, 72.1, 75.2, 75.4, 76.1, 78.4, 84.7, 87.8, 88.2, 102.0 (C-1'), 109.5 (C-1").  
MALDI TOFMS calculated for C<sub>18</sub>H<sub>37</sub>N<sub>4</sub>O<sub>10</sub> ([M+H]<sup>+</sup>) *m/e*: 469.2; measured *m/e*: 469.2

**5-O-(5-Amino-5,6-dideoxy- $\alpha$ -L-talofuranosyl)-1-N-[(S)-4-amino-2-hydroxy-butanoyl]  
paromamine [(S)-5]**

The glycosylation product (S)-**23** (1.1 g, 0.001 mol) was treated with a solution of MeNH<sub>2</sub> (33% solution in EtOH, 50 mL) and the reaction progress was monitored by TLC (EtOAc/MeOH 85:15), which indicated completion after 8 hours. The reaction mixture was evaporated to dryness and the residue was dissolved in a mixture of THF (5 mL) and aqueous NaOH (1 mM, 5.0 mL). The mixture was stirred at room temperature for 10 minutes, after which PMe<sub>3</sub> (1 M solution in THF, 5.0 mL, 5.0 mmol) was added. The reaction progress was monitored by TLC [CH<sub>2</sub>Cl<sub>2</sub>/MeOH/H<sub>2</sub>O/MeNH<sub>2</sub> (33% solution in EtOH) 10:15:6:15], which indicated completion after 1 hour. The product was purified by column chromatography on a short column of silica gel. The column was washed with the following solvents: THF (800 mL), CH<sub>2</sub>Cl<sub>2</sub> (800 mL), EtOH (200 mL), and MeOH (400 mL). The product was then eluted with a mixture of 20% MeNH<sub>2</sub> (33% solution in EtOH) in 80% MeOH. Fractions containing the product were combined and evaporated to dryness. The residue was re-dissolved in a small volume of water and evaporated again (2-3 repeats) to afford the free amine form of **5**. The analytically pure product was obtained by passing the above product through a short column of Amberlite CG50 (NH<sub>4</sub><sup>+</sup> form). The column was first washed with a mixture of MeOH/H<sub>2</sub>O (3:2), then the product was eluted with a mixture of MeOH/H<sub>2</sub>O/NH<sub>4</sub>OH (80:10:10) to afford compound **5** (0.450 g, 79% yield). For the storage and biological tests, compound was converted to its sulfate salt form: the free base was dissolved in water, the pH was adjusted around 7.0 with H<sub>2</sub>SO<sub>4</sub> (0.1 N) and lyophilized.  $[\alpha]_D^{20} = +35.4$  (*c* = 0.2, H<sub>2</sub>O). <sup>1</sup>HNMR (500 MHz, CD<sub>3</sub>OD) "**Ring I**"  $\delta_H$  2.65 (dd, 1H, *J*<sub>1</sub> = 3.7 and *J*<sub>2</sub> = 10.3 Hz, H-2'), 3.26 (t, 1H, *J* = 8.9 Hz, H-4'), 3.54 (t, 1H, *J* = 9.2 Hz, H-3'), 3.68 (dd, 1H, *J*<sub>1</sub> = 5.9 and *J*<sub>2</sub> = 11.8 Hz, H-6'), 3.80 (m, 1H, H-5'), 3.87 (dd, 1H, *J*<sub>1</sub> = 1.7 and *J*<sub>2</sub> = 11.7 Hz, H-6'), 5.21 (d, 1H, *J* = 3.3 Hz, H-1'); "**Ring II**"  $\delta_H$  1.34 (ddd, 1H, *J*<sub>1</sub>=*J*<sub>2</sub>=*J*<sub>3</sub>= 12.5 Hz, H-2<sub>ax</sub>), 1.99 (td, 1H, *J*<sub>1</sub> = 4.5 and *J*<sub>2</sub> = 12.5 Hz, H-2<sub>eq</sub>), 2.84 (m, 1H, H-3), 3.40 (t, 1H, *J* = 9.0 Hz, H-4), 3.50-3.59 (m, 2H, H-5 and H-6), 3.81 (m, 1H, H-1); "**Ring III**"  $\delta_H$  1.17 (d, 3H, *J* = 6.7 Hz, CH<sub>3</sub>), 2.95 (m, 1H, H-5"), 3.57 (t, 1H, *J* = 6.5 Hz, H-4"), 4.01 (t, 1H, *J* = 5.7 Hz, H-3"), 4.08 (dd, 1H, *J*<sub>1</sub> = 2.7 and *J*<sub>2</sub> = 5.4 Hz, H-2"), 5.26 (d, 1H, *J* = 2.5 Hz, H-1"). The additional peaks in the spectrum were identified as follows:  $\delta_H$  1.82 (m, 1H, H-8), 1.94 (m, 1H, H-8), 2.83 (t, 2H, *J* = 6.4 Hz, H-9 and H-9), 4.14 (dd, 1H, *J*<sub>1</sub> = 4.1 and *J*<sub>2</sub> = 7.6 Hz, H-7). <sup>13</sup>CNMR (125 MHz, CD<sub>3</sub>OD):  $\delta_C$  19.2 (C-6"), 35.9, 37.8, 38.9, 50.8,

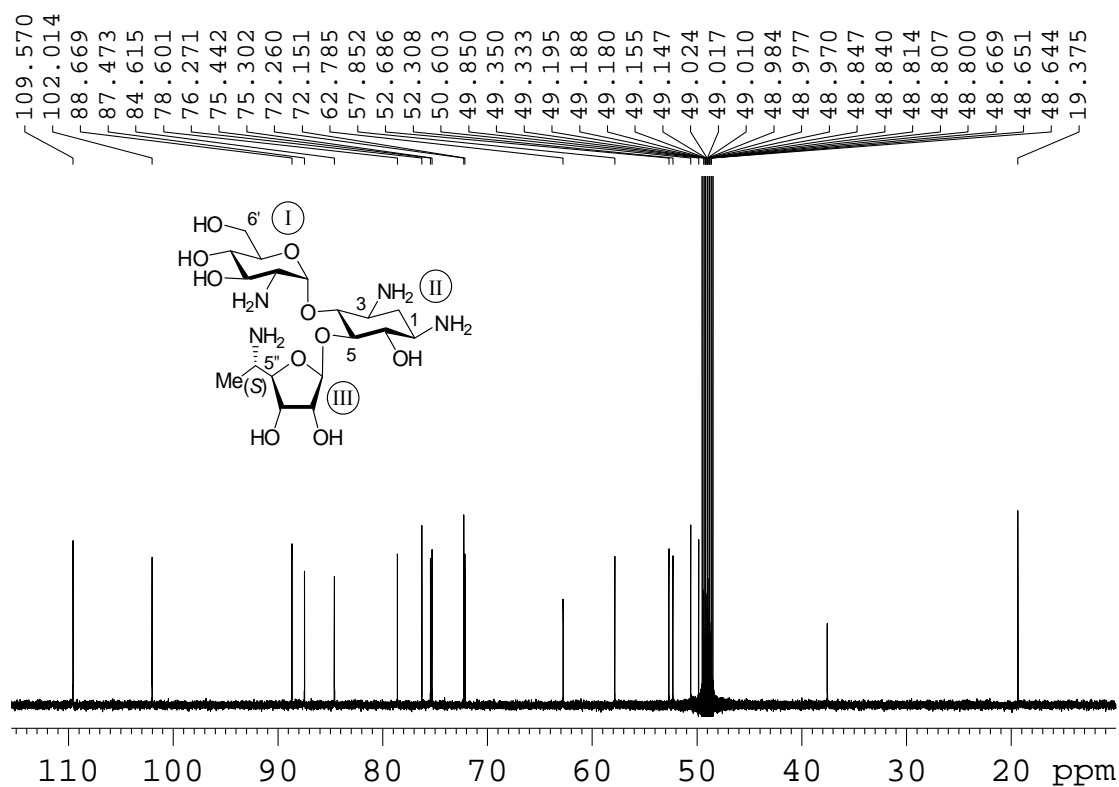
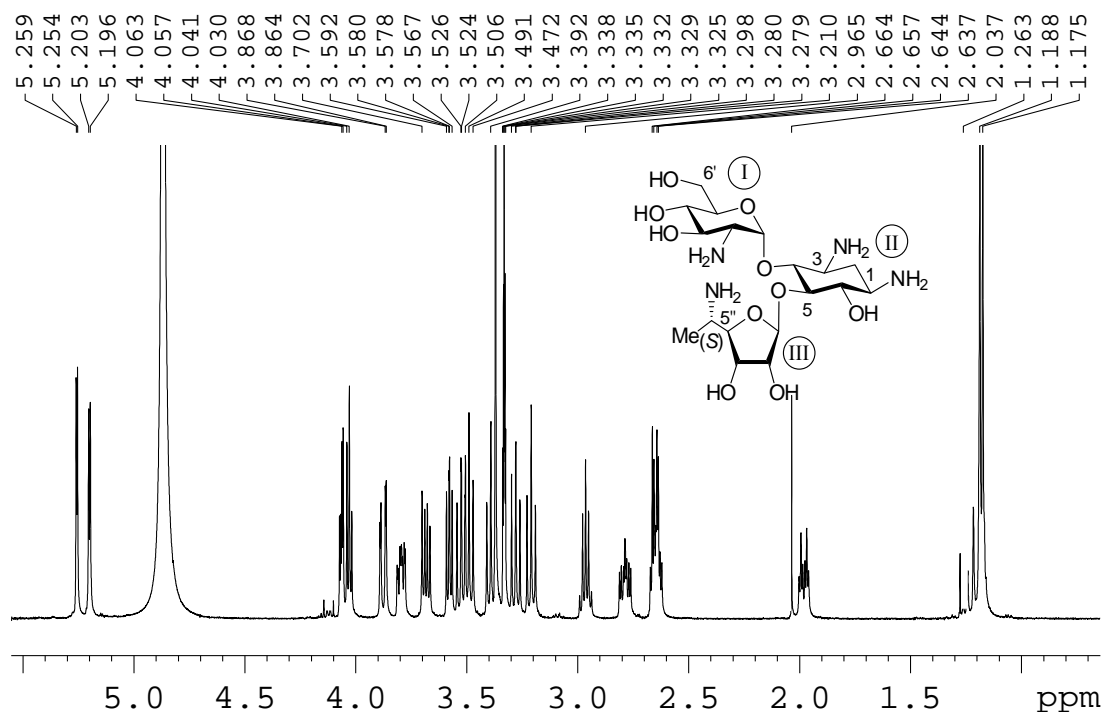
50.9, 52.4, 57.8, 62.8, 71.7, 72.1, 72.3, 75.3, 75.4, 75.6, 76.3, 84.7, 86.9, 88.6, 101.9 (C-1'), 109.9 (C-1''), 177.1 (C=O). MALDI TOFMS calculated for C<sub>22</sub>H<sub>44</sub>N<sub>5</sub>O<sub>12</sub> ([M+H]<sup>+</sup>) *m/e*: 570.3; measured *m/e*: 570.27.

**5-*O*-(5-Amino-5,6-dideoxy- $\beta$ -D-allofuranosyl)-1-*N*-[(*S*)-4-amino-2-hydroxy-butanoyl] paromamine [(*R*)-6]**

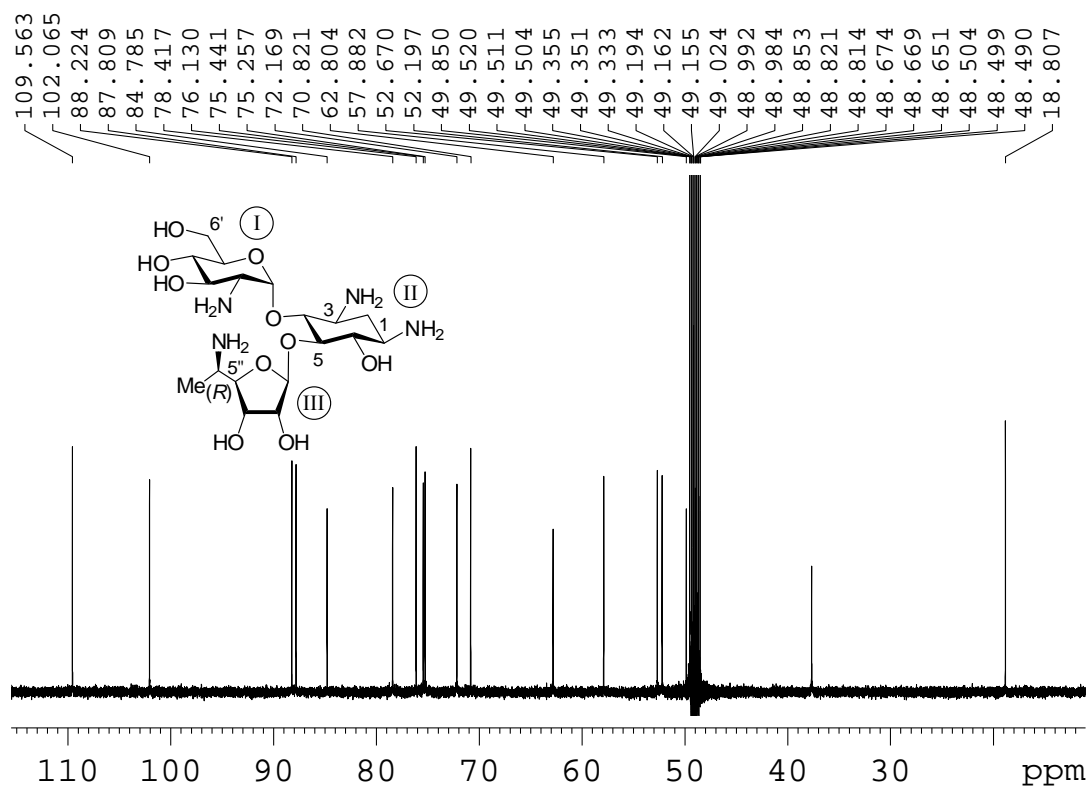
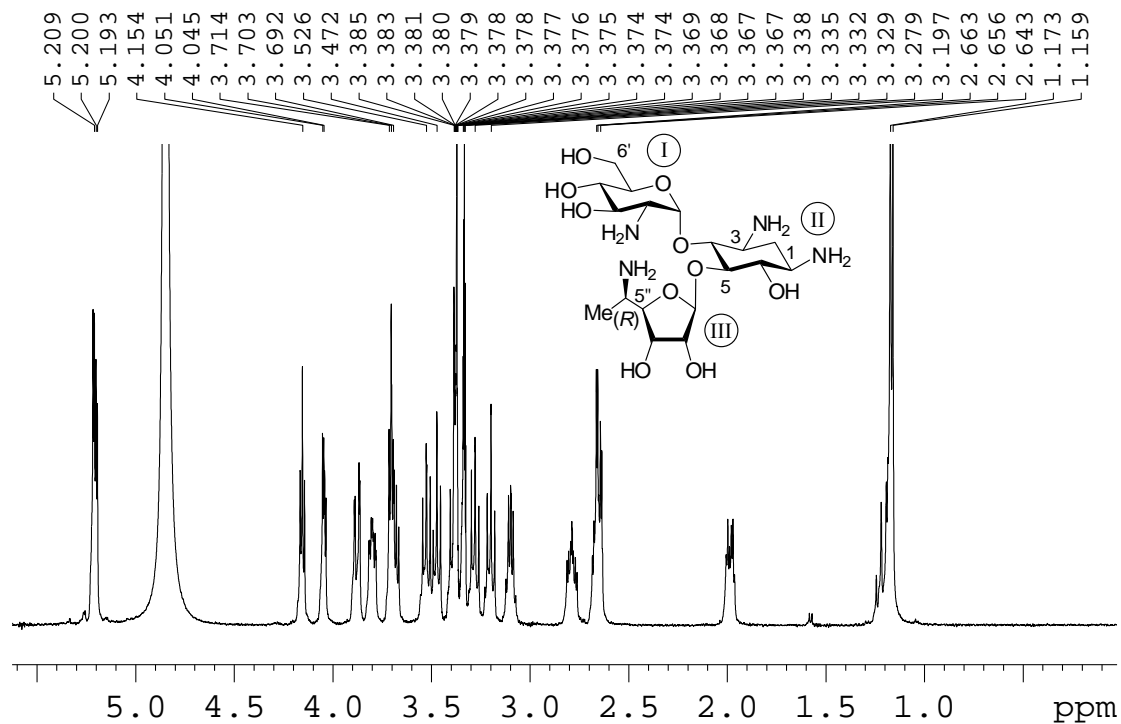
The glycosylation product (*R*)-**24** (1.2 g, 0.0011 mol) was treated with a solution of MeNH<sub>2</sub> (33% solution in EtOH, 50 mL) and the reaction progress was monitored by TLC (EtOAc/MeOH 85:15), which indicated completion after 8 hours. The reaction mixture was evaporated to dryness and the residue was dissolved in a mixture of THF (5 mL) and aqueous NaOH (1 mM, 5.0 mL). The mixture was stirred at room temperature for 10 minutes, after which PMe<sub>3</sub> (1 M solution in THF, 5.0 mL, 5.0 mmol) was added. The reaction progress was monitored by TLC [CH<sub>2</sub>Cl<sub>2</sub>/MeOH/H<sub>2</sub>O/MeNH<sub>2</sub> (33% solution in EtOH) 10:15:6:15], which indicated completion after 1 hour. The product was purified by column chromatography on a short column of silica gel. The column was washed with the following solvents: THF (800 mL), CH<sub>2</sub>Cl<sub>2</sub> (800 mL), EtOH (200 mL), and MeOH (400 mL). The product was then eluted with a mixture of 20% MeNH<sub>2</sub> (33% solution in EtOH) in 80% MeOH. Fractions containing the product were combined and evaporated to dryness. The residue was re-dissolved in a small volume of water and evaporated again (2-3 repeats) to afford the free amine form of **6**. The analytically pure product was obtained by passing the above product through a short column of Amberlite CG50 (NH<sub>4</sub><sup>+</sup> form). The column was first washed with a mixture of MeOH/H<sub>2</sub>O (3:2), then the product was eluted with a mixture of MeOH/H<sub>2</sub>O/NH<sub>4</sub>OH (80:10:10) to afford compound **6** (0.510 g, 82% yield). For the storage and biological tests, compound was converted to its sulfate salt form: the free base was dissolved in water, the pH was adjusted around 7.0 with H<sub>2</sub>SO<sub>4</sub> (0.1 N) and lyophilized.  $[\alpha]_D^{20} = +32.2$  (*c* = 0.2, H<sub>2</sub>O). <sup>1</sup>HNMR (500 MHz, CD<sub>3</sub>OD) "**Ring I**"  $\delta_H$  2.65 (dd, 1H, *J*<sub>1</sub> = 3.4, *J*<sub>2</sub> = 10.0 Hz, H-2'), 3.27 (t, 1H, *J* = 9.0 Hz, H-4'), 3.54 (t, 1H, *J* = 9.1 Hz, H-3'), 3.66 (dd, 1H, *J*<sub>1</sub> = 6.0, *J*<sub>2</sub> = 12.0 Hz, H-6'), 3.81 (m, 1H, H-5'), 3.88 (dd, 1H, *J*<sub>1</sub> = 2.0, *J*<sub>2</sub> = 12.0 Hz, H-6'), 5.21 (d, 1H, *J* = 3.5 Hz, H-1'); "**Ring II**"  $\delta_H$  1.33 (ddd, 1H, *J*<sub>1</sub>=*J*<sub>2</sub>=*J*<sub>3</sub>= 12.5 Hz, H-2<sub>ax</sub>), 1.99 (td, 1H, *J*<sub>1</sub>=4.5, *J*<sub>2</sub>= 12.5 Hz, H-2<sub>eq</sub>), 2.85 (m, 1H, H-3), 3.39 (t, 1H, *J* = 9.0 Hz, H-4), 3.49-3.57 (m, 2H, H-5 and H-6), 3.82 (m, 1H, H-1); "**Ring III**"  $\delta_H$  1.16 (d, 3H, *J* = 6.7 Hz, CH<sub>3</sub>), 3.09 (m, 1H, H-5''), 3.70 (t, 1H, *J* = 5.4 Hz, H-4''), 4.08 (dd, 1H, *J*<sub>1</sub> = 2.6, *J*<sub>2</sub> = 5.1 Hz, H-2''), 4.14 (t, 1H, *J* = 5.7 Hz, H-3''), 5.22 (d, 1H, *J* = 2.7 Hz, H-1''). The additional peaks in the spectrum were identified as follows:  $\delta_H$  1.82 (m, 1H, H-8), 1.94 (m, 1H, H-8), 2.84 (t, 2H, *J* = 7.2 Hz, H-9 and H-9), 4.15 (dd, 1H, *J*<sub>1</sub> = 4.0, *J*<sub>2</sub> = 7.5 Hz, H-7).

$^{13}\text{C}$ NMR (125 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta_{\text{H}}$  18.8 (C-6"), 35.9, 37.6, 38.9, 49.6, 40.8, 52.3, 57.8, 62.8, 71.0, 71.6, 72.1, 75.2, 75.3, 75.4, 76.2, 85.0, 87.1, 87.9, 101.9 (C-1'), 110.0 (C-1"), 177.0 (C=O). MALDI TOFMS calculated for  $\text{C}_{22}\text{H}_{44}\text{N}_5\text{O}_{12}$  ( $[\text{M}+\text{H}]^+$ )  $m/e$ : 570.3; measured  $m/e$ : 570.27.

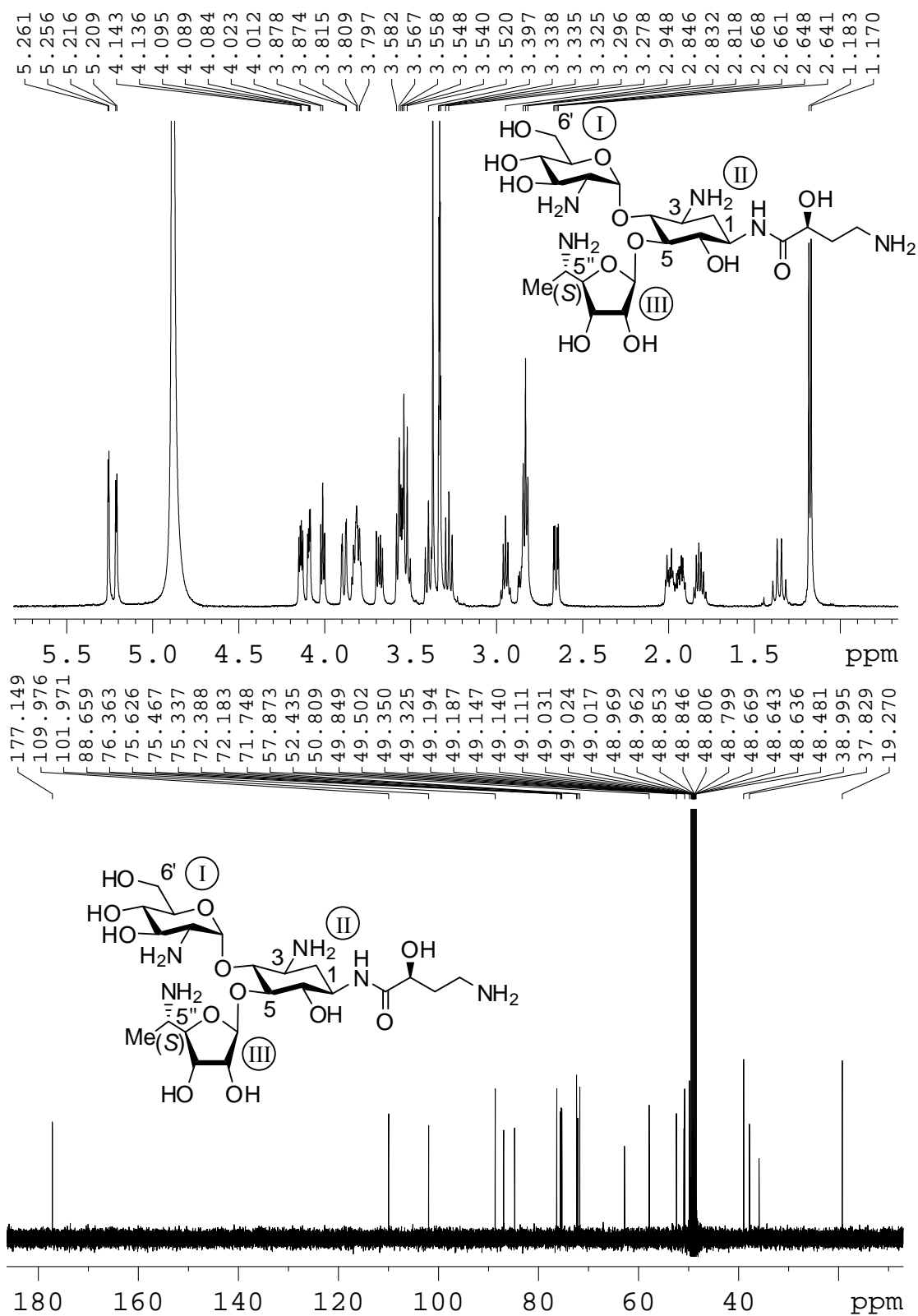
**$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of compound [(*S*)-3] in  $\text{CD}_3\text{OD}$**



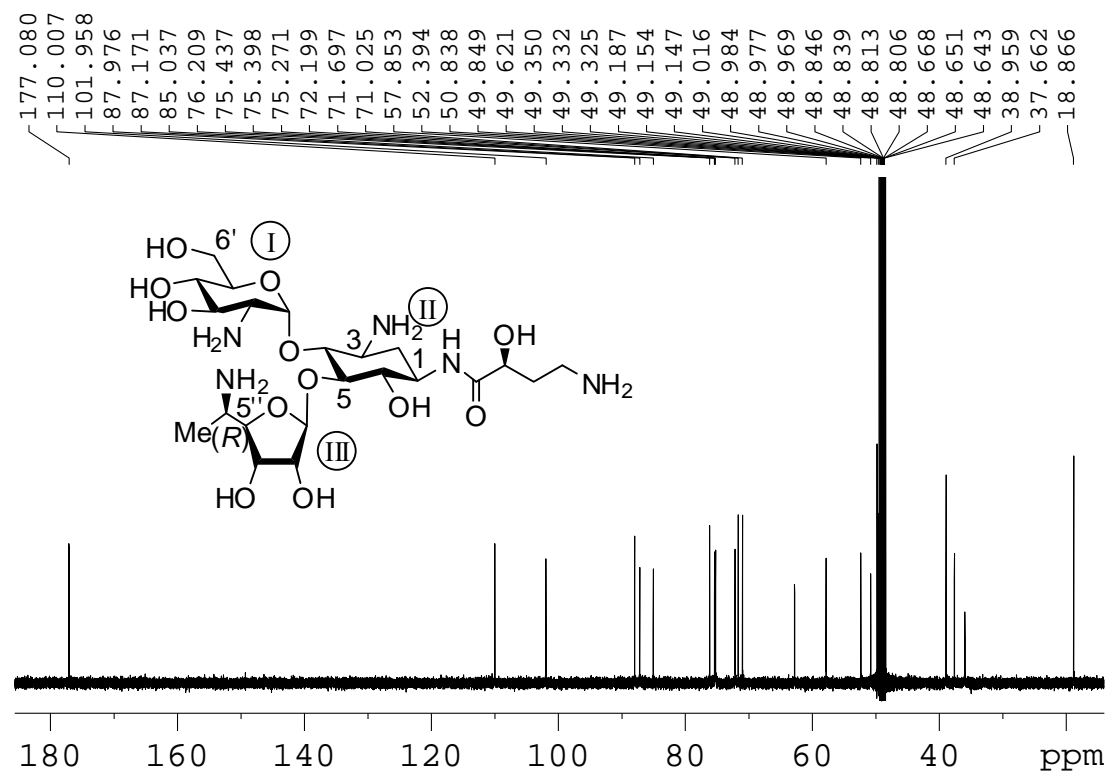
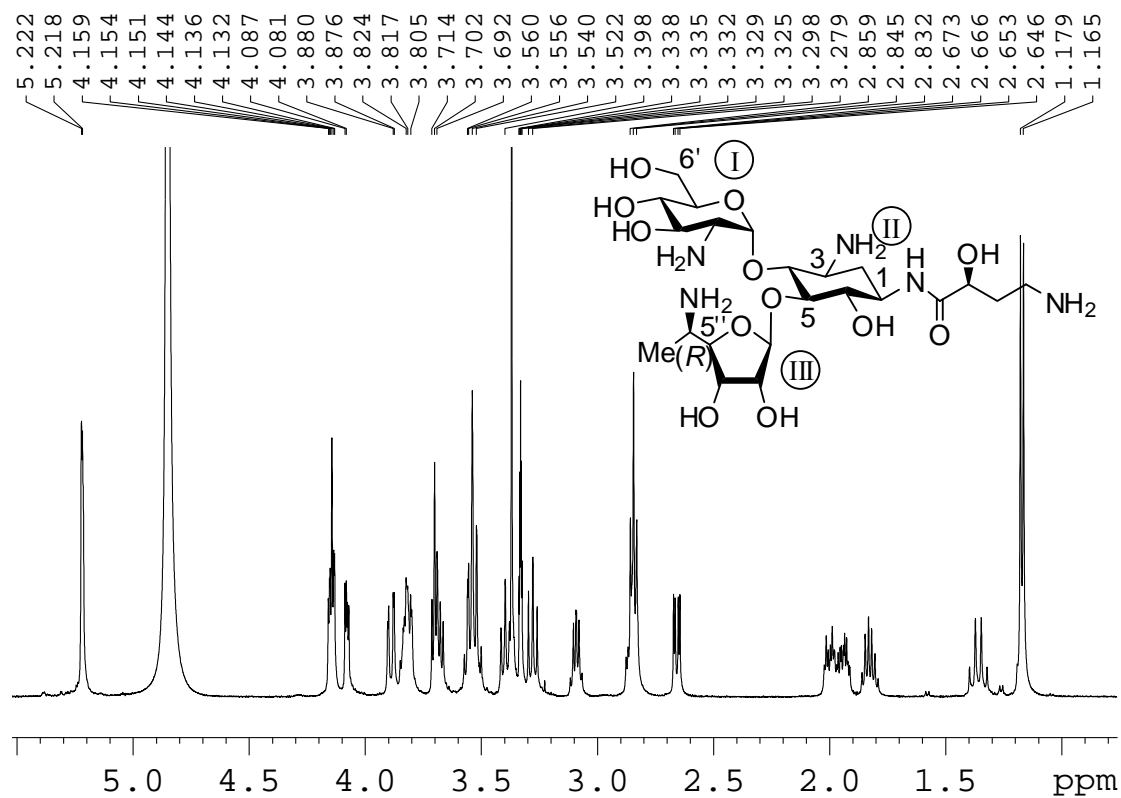
**$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of compound [(*R*)-4] in  $\text{CD}_3\text{OD}$**



**$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of compound [(*S*)-5] in  $\text{CD}_3\text{OD}$**

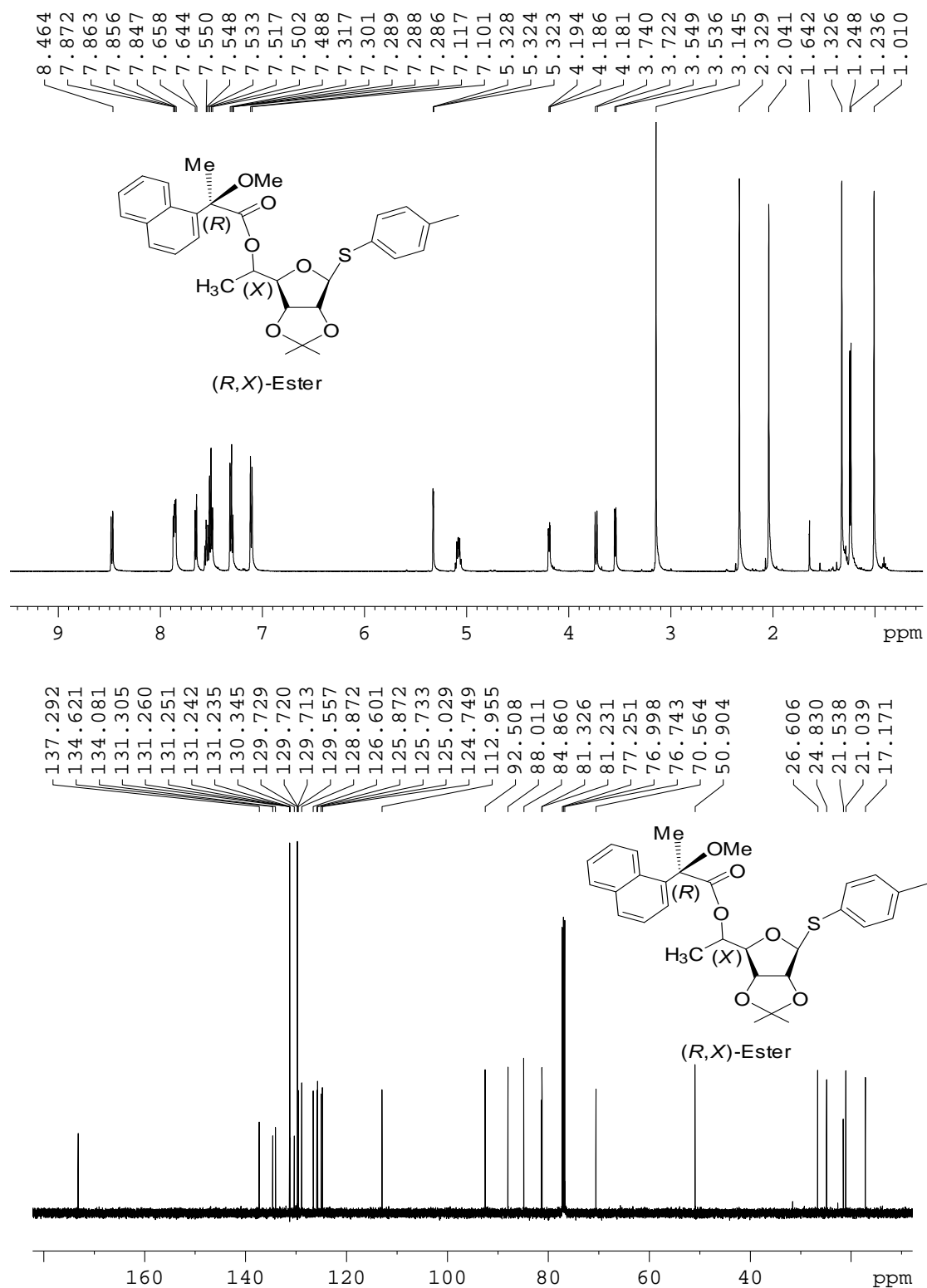


**$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of compound [(*R*)-6] in  $\text{CD}_3\text{OD}$**





**$^1\text{H}$  and  $^{13}\text{C}$  NMR spectrum of (*R, X*)-27 in  $\text{CDCl}_3$**



**$^1\text{H}$  and  $^{13}\text{C}$  NMR spectrum of (S, X)-28 in  $\text{CDCl}_3$**

