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Original article

Synthesis and glycosidase inhibition of C-7 modified casuarine derivatives

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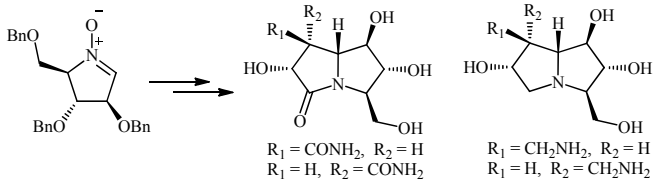
Graphical Abstract



A series of C-7 modified analogues of casuarine have been synthesized from sugar-derived nitrone and assayed against various glycosidases. Introduction of C-7 aminomethyl or amide group led to sharp decrease of the inhibitory activities.

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ABSTRACT

A series of C-7 modified analogues of casuarine have been synthesized from sugar-derived nitrone and assayed against various glycosidases. Introduction of C-7 aminomethyl or amide group led to sharp decrease of the inhibitory activities.

1. Introduction

Casuarine (**1**) was first isolated from the bark of *Casuarina equisetifolia* L. (Casuarinaceae) in 1994 [1] and then from the leaves and bark of *Eugenia uniflora* L. (Myrtaceae) [2], which are traditionally used for treatment of cancer and diabetes, respectively. Casuarine (**1**) and its related analogues constitute an important class of the polyhydroxylated pyrrolizidines for their six continuous stereogenic centres and also the most-oxygenated bicylic framework. This class of alkaloids has been shown to exhibit attractive biological activities. For example, casuarine (**1**) was a potent inhibitor of processing glycosidase I [3], rat intestinal maltase and rat intestinal isomaltase [4], and also showed potent inhibition of amyloglucosidase in a competitive manner [4,5]. Importantly, casuarine (**1**) was

able to inhibit human NtMGAM more strongly than acarbose [6]. The naturally occurring glucoside (**2**) not only retained the potent inhibition of amyloglucosidase of casuarine (**1**) [4,5], but also was a more powerful trehalase inhibitor [4,7] and notably one of the most potent inhibitors of Tre37 till now [6]. These noteworthy activities have endowed casuarine (**1**) and its derivatives promise for development of novel drugs against diabetes, cancer and HIV infection [6,8], and therefore promoted the syntheses of a series of natural and non-natural structural analogues and subsequent structure-activity relationship (SAR) studies.

According to the report of Bonaccini *et al.* [5] for casuarine (**1**) and its analogues (**2**-**9**) (Fig. 1), the C-6 and C-7 positions have no significant influence on their inhibitory activity as amyloglucosidase and glucoamylase inhibitors, but the replenishment of a lactam structure at the C-5 positions (compounds **5** and **8**) dramatically decreased their activities due to the block of ammonium cation formation and the limited conformational flexibility. Computational studies [5] showed that casuarine (**1**) interacted with the active site of *A. niger* amyloglucosidase through a hydrogen bond involving C-6 hydroxyl, whereas the C-7 hydroxyl did not present the correct geometry for hydrogen-bond formation. Therefore, the C-6 hydroxyl should be retained in potential glycosidase inhibitors, while the C-7 position could be modified by other electron donor, *i.e.*, an amino group. In combination of our recent SAR study of polyhydroxylated pyrrolidines and pyrrolizidines [9] and the previous report of 3-*epi*-casuarine (**10**) for lost of most inhibitory activity [10], the relative configuration of A ring of casuarine (**1**) was thereof believed to be vital for efficient docking of the molecule with corresponding enzymes. Herein, we report the synthesis and glycosidase inhibition of casuarine derivatives with C-7 hydroxyl replaced by an aminomethyl or amide group.

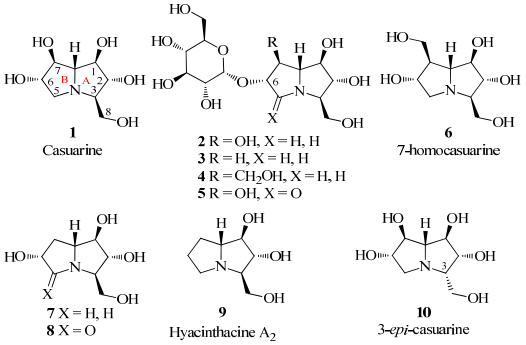
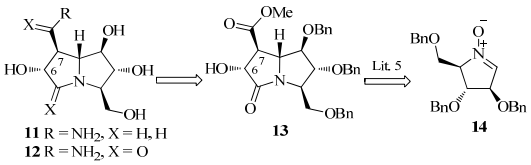


Fig. 1. Casuarine and related analogues.

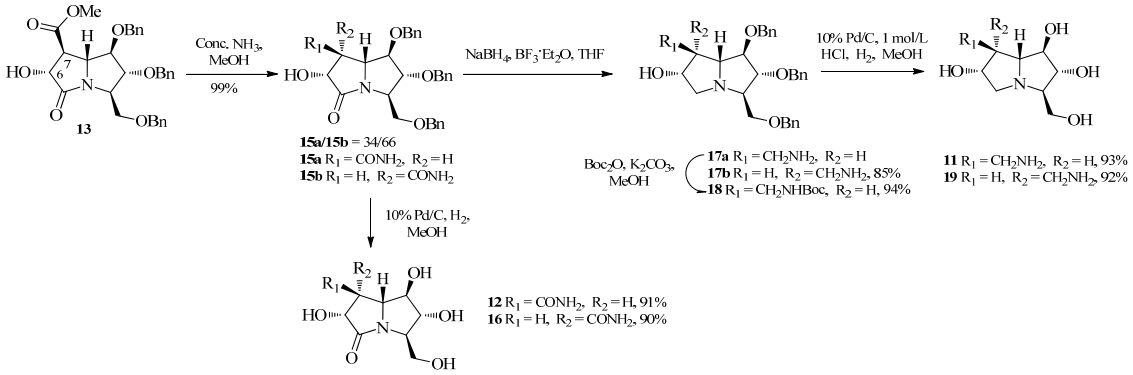
2. Results and discussions

1,3-Dipolar cycloaddition of polyhydroxylated cyclic nitrones with alkenes is amongst the most efficient methods of constructing the polyhydroxylated pyrrolizidine frameworks. Based on the previous efforts of Goti *et al.* [6,11] and their cooperators [5], the target casuarine derivatives **11** and **12** could be achieved from the 1,3-dipolar cycloaddition product **13** of nitrone **14** [12] and dimethyl maleate (Scheme 1).



Scheme 1 Retrosynthesis of casuarine derivatives from nitrone.

According to literature method [5], lactam **13** was obtained by the stereocontrolled cycloaddition of D-arabinose derived nitrone **14** followed by a subsequent reductive ring-opening/cyclization in 89% total yield. Lactam **13** was then put under ammonolysis procedure to give the expected amide **15a** together with its C-7 epimer **15b** in quantitative total yield (Scheme 2). The emergence of epimer **15b** can be explained by the acidity of H-7 and the subsequent participation of an enol intermediate. The C-7 configuration of compound **15a** was determined as *S*-configuration through NOESY experiments since a strong interaction of H-1 and H-7 was observed (See supplementary information), and the C-7 configuration of compound **15b** was thereof determined as *R*-configuration. Since hydrogenolysis of **15a** and **15b** in existence of hydrochloric acid would lead to partial hydrolysis of the C-7 amides to the corresponding acids, the reaction was performed without addition of any acid, affording casuarine analougues **12** and **16** in excellent yield, respectively.



Scheme 2. Synthesis of C-7 modified casuarine derivatives.

While refluxing the lactams with $LiAlH_4$ led to complex mixtures, reduction of **15a** and **15b** with $NaBH_4$ in the presence of $BF_3 \cdot Et_2O$ gave the corresponding amines **17a** and **17b**, respectively. **17b** was obtained smoothly after purification by silica gel column chromatography, but purification of **17a** was somewhat difficult due to the big polarity. **17a** was thereof transformed to the *N*-Boc derivative **18**, which can then be separated easily. Final hydrogenolysis of **18** and **17b** according to routine procedures afforded the target casuarine analogues **11** and **19** respectively.

The synthesized C-7 derivatives of casuarines **11**, **12**, **16**, **19** were assayed against a series of glycosidases (for details, see Supporting information). In contrast to casuarine (**1**) and 7-homocasuarine (**6**) [4,5], lactam **12** and **16** are completely inactive to all the evaluated enzymes. Amine **11** was a weak inhibitor of yeast *α*-glucosidase (IC_{50} = 1000 μmol/L) and *A. niger* amyloglucosidase (IC_{50} = 151 μmol/L), and no significant inhibitory activities were observed for its C-7 epimer **19**. The above assay results further confirmed the importance of reserving the free tertiary amine unit in effective interaction with enzymes. In the case of **11** and **19**, though the replacement of C-7 hydroxymethyl with an aminomethyl group might not affect the capability of forming hydrogen bond with amino acid residues, addition of the primary amine group would obviously change pK_a value of compounds **11** and **19**, and finally affect their inhibitory activities.

3. Conclusion

In conclusion, casuarine analogues with C-7 aminomethyl or amide groups have been designed and synthesized from D-arabinose-derived nitrones with 1,3-dipolar cycloaddition as key step. Evaluation of the analogues against a series of glycosidases showed sharply decreased inhibitory activities, which may be attributed to obvious pK_a change or limitation of configurations.

4. Experimental

4.1 General methods

All reagents were used as received from commercial sources or prepared according to the literature. Analytical TLC were performed with 0.20 mm silica gel 60F plates and visualized by ultraviolet light or by treatment with a spray of Pancaldi reagent $(NH_4)_6MoO_4$, $Ce(SO_4)_2$, H_2SO_4, H_2O. Column chromatographic purification of products was carried out on silica gel (200–300 mesh). Melting points were determined using an electrothermal melting point apparatus and were uncorrected. Infrared spectra were recorded on an FT-IR spectrometer. ^1H NMR spectra were measured in $CDCl_3$ (with TMS as an internal standard) or D_2O on a magnetic resonance spectrometer (^1H at 300 or 500 MHz, ^{13}C at 75 MHz). High resolution mass spectra (HRMS) were recorded on an LTQ/FT linear ion trap mass spectrometer. Polarimetry was carried out using an Optical Activity AA-10R or Rudolph AutopolVI polarimeter and the measurements were made at the sodium D-line with a 0.5 dm or 1 dm path length cell. Concentrations (*c*) are given in gram per 100 mL.

*4.2 1,2,8-Tribenzyl-5-oxo-7-deoxy-7-methylcarboxylate casuarine (**13**)*

To a solution of nitrone **14** (7.0 g, 16.8 mmol) in dichloromethane (50 mL, distilled from CaH_2) was added dimethyl maleate (4.2 mL, 33.6 mmol) and stirred overnight. After TLC showed completion of the reaction, the mixture was concentrated in vacuo, and the crude product was directly purified by flash column chromatography (silica gel, petroleum ether/EtOAc = 4:1) to give the cycloaddition product as a light yellow solid (9.2 g). The intermediate was then dissolved in acetic acid/water (55 mL, 10:1), followed by activated zinc powder (10.6 g, 0.16 mol), the suspension was stirred at 50 °C for 4 h until TLC showed completion of the reaction. The mixture was filtered, washed with EtOAc (3 × 50 mL), and then concentrated in vacuo. The residue was dissolved in dichloromethane/water (100 mL, 1:1), neutralized by aqueous $NaHCO_3$ solution, and then extracted with dichloromethane (2 × 20 mL). The organic layers were combined, dried over $MgSO_4$, and concentrated in vacuo. The crude product was recrystallized in petroleum ether/EtOAc (3:1) to give the target product **13** as a light yellow solid (7.9 g, 89% yield). Mp: 106–108 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 7.33–7.24 (m, 15H, *Ph*CH_2O), 4.77 (d, 1H, J = 9.6 Hz, H6), 4.56–4.43 (m, 6H, PhCH_2O), 4.28–4.21 (m, 2H, H2 and H3), 3.98–3.89 (m, 2H, H1 and H7a), 3.77 (s, 3H, OMe), 3.58–3.48 (m, 2H, H8), 3.04 (t, 1H, J = 9.0 Hz, H7); ^{13}C NMR (75 MHz, $CDCl_3$):

δ 173.5, 171.4, 137.8, 137.43, 137.36, 128.50, 128.49, 128.4, 128.0, 127.9, 127.8, 127.7 (Ph), 87.4 (C1), 84.7 (C2), 74.3 (C6), 73.2, 72.2, 71.9 (Ph*C*H_2O), 68.2 (C8), 62.7 (C7a), 59.3 (C3), 54.8 (C7), 52.6 (Me) (Lit.[5]).

*4.3 1,2,8-Tribenzyl-5-oxo-7-deoxy-7-carboxamide casuarine (**15a**) and 1,2,8-tribenzyl-5-oxo-7-deoxy-7-epi-carboxamide casuarine (**15b**)*

To a suspension of compound **13** (3.61 g, 6.80 mmol) in methanol (50 mL) was added aqueous NH_3 (15 mol/L, 50 mL), and stirred at room temperature for three days. After TLC showed completion of the reaction, the mixture was concentrated in vacuo, and purified by flash column chromatography (silica gel, MeOH/EtOAc = 1:5) to afford the target product **15a** (1.17 g, 33% yield) together with its C-7 epimer **15b** (2.30 g, 66% yield), both as yellow solids.

Data for **15a**: Mp: 111–113 °C; $[\alpha]_D^{20}$ –21.1 (*c* 0.38 in MeOH); IR (KBr, cm^{-1}): 3343 m, 2923 m, 2866 m, 1686 s, 1454 m, 1364 m, 1266 m, 1100 s, 1028 m, 736 s, 699 s; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.37–7.25 (m, 15H, *Ph*CH_2O), 6.30 (s, 1H, $CONH_2$), 5.95 (s, 1H, $CONH_2$), 4.95 (s, 1H, br, OH), 4.69 (dd, 2H, *J* = 26.5, 12.0 Hz), 4.59–4.46 (m, 4H), 4.33–4.30 (m, 1H), 4.23 (t, 1H, *J* = 2.8 Hz), 4.14 (dd, 1H, *J* = 9.0, 5.0 Hz), 3.91 (t, 1H, *J* = 4.0 Hz), 3.56 (qd, 2H, *J* = 21.8, 6.5, 5.5 Hz), 2.90 (t, 1H, *J* = 10.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.0, 172.8, 137.9, 137.7, 137.6, 128.52, 128.48, 128.0, 127.9, 127.8 (Ph), 87.1, 85.1, 74.4, 73.2, 72.0, 71.9, 68.2, 61.6, 59.2, 54.9; HRMS (ESI) calcd. for $C_{30}H_{33}N_2O_6^+$ 517.2333 $[M+H]^+$, found 517.2337.

Data for **15b**: Mp: 92–94 °C; $[\alpha]_D^{20}$ +15.0 (*c* 1.47 in MeOH); IR (KBr, cm^{-1}): 3346 m, 2924 m, 1699 s, 1453 m, 1365 m, 1204 m, 1098 s, 1028 s, 737 m, 698 m; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.25 (s, br, 1H,), 7.22–7.18 (m, 15H, *Ph*CH_2O), 4.85 (d, 1H, *J* = 9.4 Hz), 4.60–4.35 (m, 6H, PhC*H*$_2$O), 4.21–4.15 (m, 2H), 3.98–3.92 (m, 2H), 3.46 (s, 2H), 3.10 (t, 1H, *J* = 8.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.4, 174.1, 137.8, 137.60, 137.56, 128.5, 127.96, 127.92, 127.88, 127.82 (Ph), 87.4, 84.9, 74.6, 73.2, 72.3, 71.8 (Ph*C*H_2O), 68.1, 62.9, 59.3, 55.3; HRMS (ESI) calcd. for $C_{30}H_{33}N_2O_6^+$ 517.2333 $[M+H]^+$, found 517.2337.

*4.4 5-Oxo-7-deoxy-7-carboxamide casuarine (**12**) and 5-oxo-7-deoxy-7-epi-carboxamide casuarine (**16**)*

Compound **15a** (0.30 g, 0.58 mmol) or **15b** (0.21 g, 0.41 mmol) was dissolved in methanol (20 mL), followed by 10% Pd/C (30 mg). The suspension was stirred under hydrogen atmosphere for 48 h when TLC showed completion of the reaction. Hydrogen was replaced by nitrogen, catalyst was removed from the reaction mixture by filtration, and then washed with MeOH/H_2O (1:1, 3 × 30 mL). The filtrate was concentrated in vacuo to give the target debenzylated product **12** (0.13 g, 91% yield) or **16** (0.09 g, 90% yield), both as light yellow syrup.

Data for **12**: $[\alpha]_D^{20}$ –13.5 (*c* 0.74 in H_2O); IR (KBr, cm^{-1}): 3340 s, 2926 m, 1675 s, 1436 m, 1331 m, 1055 m; ^{1}H NMR (300 MHz, D_2O): δ 4.70 (s, 1H), 4.06 (dt, 1H, *J* = 6.6, 3.3 Hz), 3.81–3.77 (m, 1H), 3.76–3.71 (m, 1H), 3.65 (t, 1H, *J* = 8.1 Hz), 3.59–3.54 (m, 2H), 2.96 (dd, 1H, *J* = 9.9, 8.1 Hz); ^{13}C NMR (75 MHz, D_2O): δ 174.2, 173.7, 79.6, 77.5, 74.4, 61.3, 61.1, 59.5, 55.3; HRMS (ESI) calcd. for $C_9H_{14}N_2O_6Na^+$ 269.0744 $[M+Na]^+$, found 269.0742.

Data for **16**: $[\alpha]_D^{20}$ –17.8 (*c* 0.67 in H_2O); IR (KBr, cm^{-1}): 3340 s, 2919 m, 1694 s, 1422 m, 1275 m, 1193 m, 1032 m; ^{1}H NMR (300 MHz, D_2O): δ 4.74 (d, 1H, *J* = 10.2 Hz), 4.07 (t, 1H, *J* = 6.2 Hz), 3.83 (t, 1H, *J* = 7.2 Hz), 3.76–3.68 (m, 2H), 3.66–3.55 (m, 2H), 3.13–3.02 (m, 1H); ^{13}C NMR (75 MHz, D_2O): δ 173.7, 173.6, 79.7, 77.6, 73.9, 61.1, 59.7, 54.0; HRMS (ESI) calcd. for $C_9H_{14}N_2O_6Na^+$ 269.0744 $[M+Na]^+$, found 269.0742.

*4.5 1,2,8-Tribenzyl-7-deoxy-7-(tert-butoxycarbonyl)aminomethyl casuarine (**18**) and 1,2,8-tribenzyl-5-oxo-7-deoxy-7-epi-aminomethyl casuarine (**17b**)*

To the solution of compound **15a** or **15b** (0.15 g, 0.29 mmol) in THF (20 mL, dried over 4 Å molecular sieve) was added $NaBH_4$ (65.0 mg, 1.76 mmol) at 5 °C. The mixture was stirred for 5 min, and $BF_3{\cdot}Et_2O$ (0.26 mL, 2.07 mmol) was added dropwise. The system was stirred at room temperature for 0.5 h, then heated at 50 °C for 3 h, TLC showed disappearance of the raw material. The suspension was cooled to room temperature, and poured into iced 1 mol/L HCl carefully, solvent and water was then removed in vacuo. The resulting mixture was basified by aqueous $NaHCO_3$ solution, and extracted with EtOAc (3 × 20 mL). The organic layers were combined, dried over $MgSO_4$, and concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel, MeOH/EtOAc = 1:10) to give the reductive product **17a** (0.17 g, with impurities) or **17b** (0.12 g, 85% yield), both as light yellow syrup. Compound **17a** was then dissolved in methanol (20 mL), followed by K_2CO_3 (0.12 g, 0.87 mmol) and Boc_2O (0.10 g, 0.55 mmol), and then reacted at room temperature for 1 h. After TLC showed completion of the reaction, solvent was removed in vacuo, and water (20 mL) as added. The mixture was extracted with EtOAc (3 × 20 mL), the organic phases were combined and dried over $MgSO_4$, then concentrated in vacuo. The crude product were purified by flash column chromatography (silica gel, MeOH/EtOAc = 1:10) to give the target product **18** (0.16 g, 94% yield for 2 steps) as a light yellow syrup.

Data for **18**: $[\alpha]_D^{20}$ +5.9 (*c* 3.07 in CH_2Cl_2); IR (KBr, cm^{-1}): 3344 m, 2930 m, 1699 m, 1497 m, 1454 m, 1367 m, 1252 m, 1166 s, 1073 s, 1028 s, 738 m, 699 m; ^{1}H NMR (300 MHz; $CDCl_3$): δ 7.22–7.12 (m, 15H, *Ph*CH_2O), 5.36 (s, 1H, NH), 4.51–4.39 (m, 8H, PhC*H*$_2$O and OH), 4.05-3.97 (m, 3H), 3.57–3.31 (m, 5H), 3.14–3.09 (m, 1H), 3.02–2.94 (m, 1H), 2.88 (dd, 1H, *J* = 10.5, 5.4 Hz), 2.19 (t, 1H, *J* = 6.3 Hz), 1.50 (s, 2H, br, H_2O), 1.30 (s, 9H, Boc); ^{13}C NMR (75 MHz; $CDCl_3$): δ 156.9, 137.8, 137.7, 137.6, 128.5, 128.4, 127.94, 127.89, 127.79 (Ph), 86.7, 84.6, 79.7, 74.7, 73.4, 72.6, 72.1, 70.8, 70.3, 69.3, 61.0, 51.8, 40.8, 28.4; HRMS (ESI) calcd. for $C_{35}H_{45}N_2O_6^+$ 589.3272 $[M+H]^+$, found 589.3272.

Data for **17b**: $[\alpha]_D^{20}$ +9.9 (*c* 1.62 in MeOH); IR (KBr, cm^{-1}): 3347 m, 2870 m, 1454 m, 1366 m, 1074 s, 1028 s, 738 m, 698 m; ^{1}H NMR (300 MHz; $CDCl_3$): δ 7.32–7.19 (m, 15H, *Ph*CH_2O), 5.45 (s, br, 3H), 4.61–4.43 (m, 6H, PhC*H*$_2$O), 4.32–4.28 (m, 1H), 4.16 (t,

1H, *J* = 4.5 Hz), 4.04 (t, 1H, *J* = 4.5 Hz), 3.73–3.49 (m, 7H), 3.08 (dd, 1H, *J* = 11.4, 4.8 Hz), 2.30 (t, 1H, *J* = 5.7 Hz); ^{13}C NMR (75 MHz; $CDCl_3$): δ 137.8, 137.6, 137.4, 128.5, 128.4, 128.0, 127.93, 127.89, 128.82, 127.77 (Ph), 86.6, 84.3, 74.1, 73.3, 72.6, 72.1, 70.4, 70.1, 69.0, 61.4, 61.3, 53.4; HRMS (ESI) calcd. for $C_{30}H_{37}N_2O_4^+$ 489.2748 $[M+H]^+$, found 489.2744.

*4.6 7-Deoxy-7-aminomethyl casuarine (**11**) and 5-oxo-7-deoxy-7-epi-aminomethyl casuarine (**19**)*

Compound **18** (0.06 g, 0.10 mmol) or **17b** (0.08 g, 0.16 mmol) was dissolved in methanol (10 mL), followed by 10% Pd/C (30 mg) and 1 mol/L HCl (5 mL). The suspension was stirred under hydrogen atmosphere for 24 h when TLC showed completion of the reaction. Hydrogen was replaced by nitrogen, catalyst was removed from the reaction mixture by filtration, and then washed with MeOH/H_2O for three times. The filtrate was concentrated in vacuo and neutralized with conc. NH_3 and concentrated again. The residue was then purified by an acidic ion exchange column (Dowex 5W×8-400, H^+ form, Aldrich, column size: 1.3 cm ×14 cm), eluting with distilled water (100 mL) and then 1 mol/L NH_4OH (50 mL), affording the target debenzylated product **11** (25.0 mg, 93% yield) or **19** (32.8 mg, 92% yield), both as light yellow syrup.

Data for **11**: $[\alpha]_D^{20}$ +32.5 (*c* 1.36 in MeOH); IR (KBr, cm^{-1}): 3347 s, 2921 m, 1366 m, 1074 m, 1028 m; ^{1}H NMR (300 MHz; D_2O) δ 4.54 (dd, 1H, *J* = 10.8, 5.4 Hz), 4.41 (t, 1H, *J* = 7.4 Hz), 4.09 (dd, 1H, *J* = 9.0, 7.5 Hz), 3.99 (dd, 1H, *J* = 12.9, 3.3 Hz), 3.89 (dd, 1H, *J* = 5.4, 2.4 Hz), 3.85 (dd, 1H, *J* = 5.4, 1.5 Hz), 3.81 (d, 1H, *J* = 6.9 Hz), 3.77–3.71 (m, 1H), 3.46 (dd, 1H, *J* = 12.6, 5.7 Hz), 3.22 (d, 2H, *J* = 7.8 Hz), 2.85 (td, 1H, *J* = 13.5, 7.2 Hz); ^{13}C NMR (75 MHz; D_2O): δ 78.0, 74.5, 73.6, 72.3, 70.5, 59.0, 56.9, 47.2, 39.9; HRMS (ESI) calcd. for $C_9H_{19}N_2O_4^+$ 219.1339 $[M+H]^+$, found 219.1338.

Data for **19**: $[\alpha]_D^{20}$ +30.5 (*c* 1.64 in MeOH); IR (KBr, cm^{-1}): 3344 s, 2925 m, 1368 m, 1095 m, 1032 m; ^{1}H NMR (300 MHz; D_2O): δ 4.15 (d, 1H, *J* = 4.8 Hz), 4.04 (t, 1H, *J* = 7.8 Hz), 3.74–3.66 (m, 2H), 3.55–3.42 (m, 3H), 3.19 (dd, 1H, *J* = 11.4, 4.2 Hz), 3.03–2.95 (m, 2H), 2.81 (d, 1H, *J* = 7.1 Hz), 2.21 (t, 1H, *J* = 5.7 Hz); ^{13}C NMR (75 MHz; D_2O): δ 79.9, 77.0, 74.2, 70.6, 68.6, 61.9, 61.0, 60.2, 52.6; HRMS (ESI) calcd. for $C_9H_{19}N_2O_4^+$ 219.1339 $[M+H]^+$, found 220.1340.

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