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Synthesis of pyrrolidine-based analogues of 2-acetamidosugars as *N*-acetyl glucosaminidase inhibitors

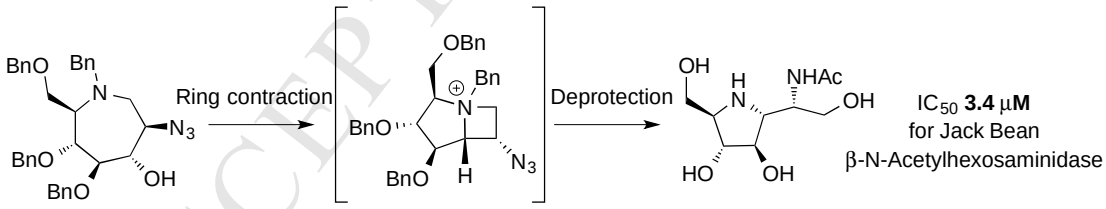
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Abstract. A ring-contraction strategy applied to β-azido,γ-hydroxyazepanes yielded after functional group manipulation new tetrahydroxylated pyrrolidines displaying an acetamido moiety, one of these iminosugars demonstrating low micromolar inhibition on *N*-acetylglucosaminidases.



Keywords. Iminosugars, Ring-contraction, Glycosidase inhibitors, Pyrrolidines

Highlights.

-A new family of amino-functionalised pyrolidines was synthesized.

-Aring-contraction of β-azido,γ-hydroxyazepanes was used.

-A low micromolar inhibitor of β-N-acetylehexosaminidase identified.

Polyhydroxylated pyrrolidines[1,2] are well-established powerful glycosidase inhibitors, even though their analogy with hexopyranoses, and therefore the structural basis of their inhibition, are less straightforward than for the corresponding piperidines.[3] Hexosaminidases are a very important class of glycosidases that cleave the pyranosidic *N*-acetyl-D-glucosamine unit from glycoconjugates. Several pyrrolidines bearing an acetamide group have been reported as potent hexosaminidases inhibitors. Interestingly, only one naturally occuring acetamido-containing pyrolidine was isolated so far: Pochonicine.[4-6] The main classes of synthetic nitrogen functionalized polyhydroxylated pyrrolidines are represented in Figure 1, the most studied one being the 2,5-dideoxy-2,5-imino-hexitols **A**[7-21] but other scaffolds such as **B**,[22-24] **C**,[25-26] **D**,[27] **E**[28] and **F**[8] have also been prepared. It is rather stricking that structure **G**, which can be seen as a combination of **A** and **E** possessing as many hydroxyl groups as the hexosaminidase substrate and product, has never been synthesized and assessed as a hexosaminidase inhibitor. The present study reports the synthesis and hexosaminidase inhibitory evaluation of molecules derived from scaffold **G**. (Figure 1)

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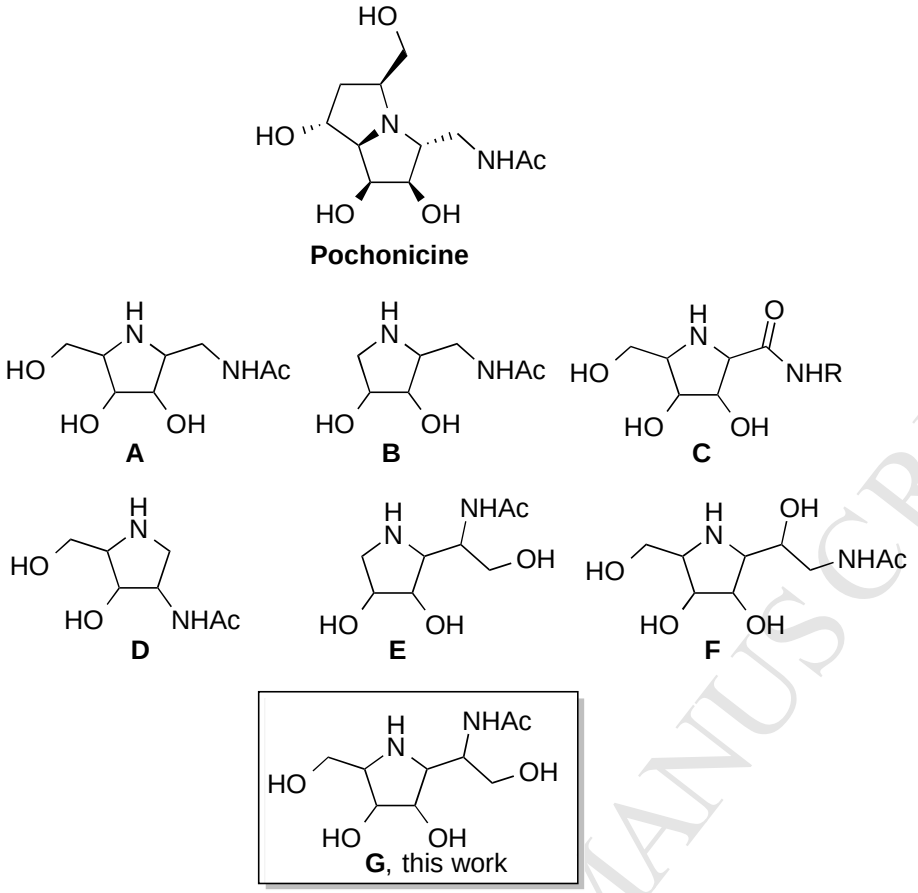
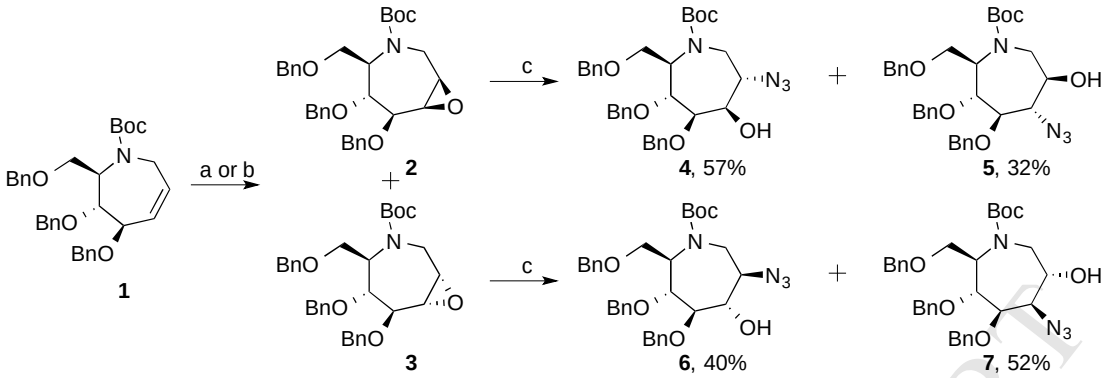


Figure 1: Structures of the existing classes of acetamido-pyrrolidines **A-F** and of the target scaffold **G**.

In the course of our studies aimed at the synthesis of GlcNAc-like piperidine homoiminosugars exploiting a ring-contraction methodology,[29-32] a 2,3-trans-2-hydroxy,3-azido-azepane was required and obtained from the unsaturated 7-membered ring **1**.[33] The obvious synthetic route transits via the formation of an epoxide, followed by its azidolysis. We observed that it was possible to operate the epoxydation with some degree of stereocontrol to afford either epoxides **2** or **3** as the main products.[33] These latter could then be opened using sodium azide to give, in both cases, a significant amount of the 2-azido derivatives **4** and **6** together with the desired 3-azido compounds **5** and **7**.[33] (Scheme 1)

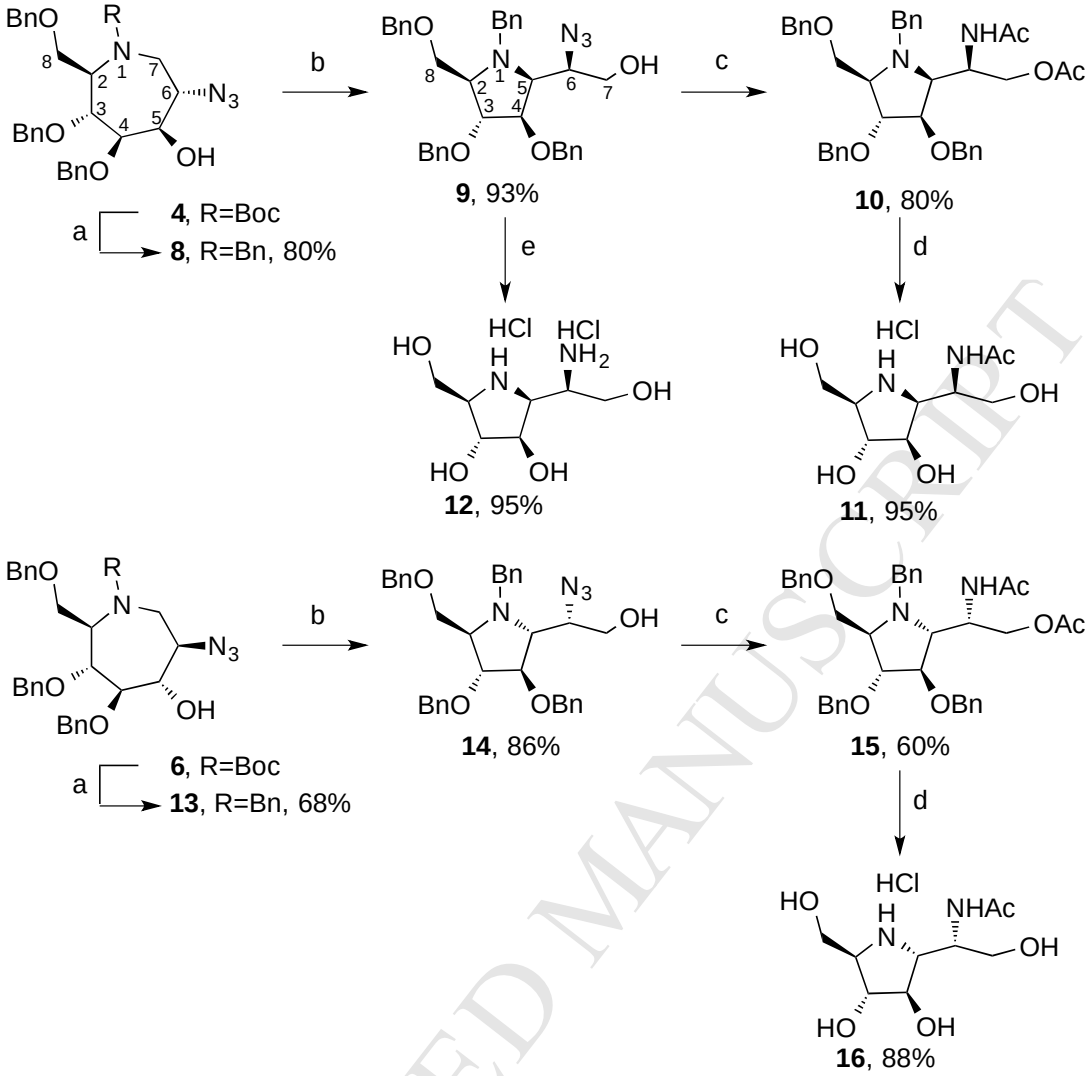
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Scheme 1 : Synthesis of azidoazepanes **4-7**. Reagents and conditions: a) Oxone, D-epoxone, $NaHCO_3$, **2** : 54%**,** b) Oxone, CF_3COCH_3, $NaHCO_3$, **2** : 29%, **3** : 51%; c) NaN_3, NH_4Cl, DMF/H_2O, 90°C

Compounds **4** and **6**[33] are also suitable candidates for a ring contraction reaction[34] to give pyrrolidine derivatives[35] through a γ-aminoacohol rearrangement. Hence, we decided to apply the TFAA-mediated ring contraction conditions developped by Cossy[36] to β-azidoazepanes **4** and **6** that were first converted into the *N*-benzyl derivatives **8** (80%) and **13** (68%) respectively, using TFA followed by *N*-benzylation (BnBr, K_2CO_3). Their ring contraction with TFAA furnished the azidopyrrolidines **9** (93%) and **14** (86%) respectively in good yield. Reduction of the azide moiety (PPh_3, THF/H_2O) followed by *N*-acetylation was achieved to provide the acetamide **10** (80%) and **15** (60%) respectively. Final *O*-deacetylation followed by hydrogenolysis yielded the target pyrrolidines **11** (95%) and **16** (88%). Compound **9** was also directly submitted to the action of hydrogen in the presence of Pd/C to give the diamine **12** in 95% yield as its hydrochloride salt (Scheme 2).

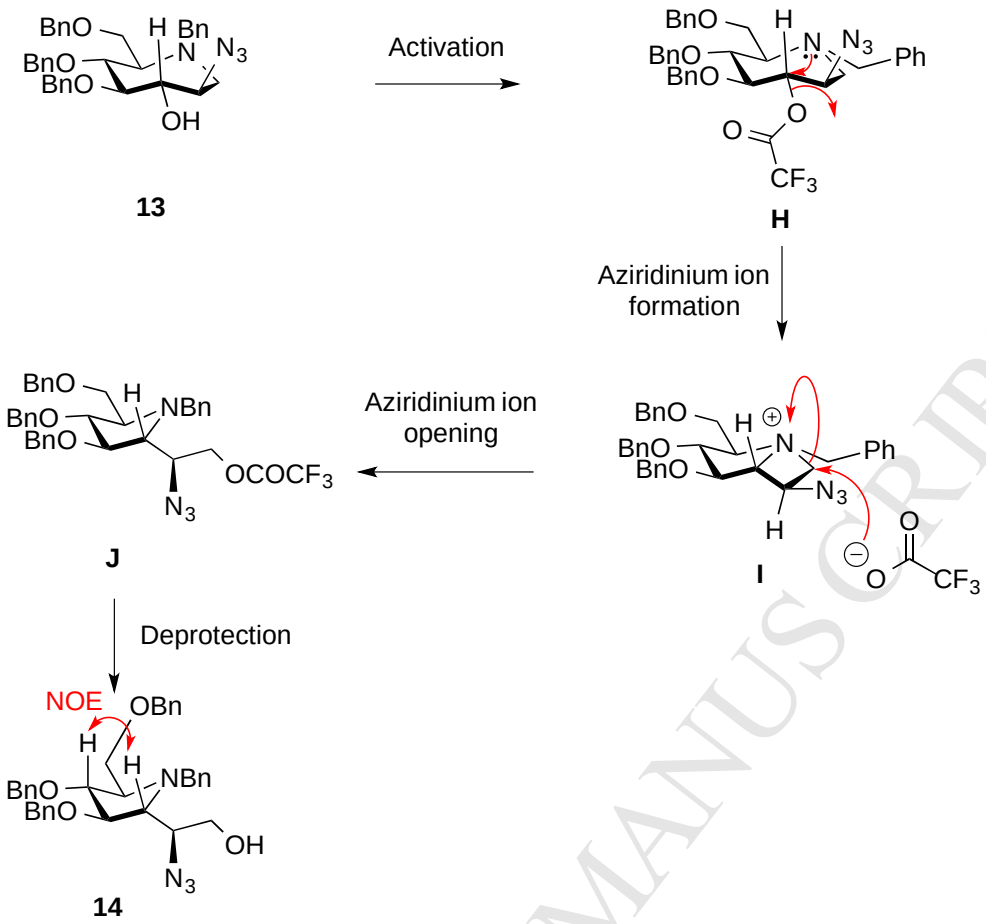
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Scheme 2 : Synthesis of NHAc derived pyrrolidines **11** and **16**. Reagents and conditions: a) i)TFA, DCM; ii) BnBr, K_2CO_3, EtOAc/H_2O; b) i) Trifluoroacetic anhydride (TFAA), Et_3N, toluene, reflux, ii) 10% aq. NaOH; c) i) PPh_3, THF/H_2O, 80°C, ii) Pyridine, Ac_2O; d) i) Et_3N, MeOH, H_2O, ii) H_2, Pd/C, MeOH, HCl; e) H_2, Pd/C, MeOH, HCl

The ring contraction reaction is initiated by the esterification of the free hydroxyl group in azepane **13** to give intermediate **H**, in which the amine displaces this leaving group to produce the fuded pyrrolidine-azetidinium ion **I**. Nucleophilic ring opening at the less hindered carbon affords pyrrolidine **J** which leads to the five-membered iminosugar **14** upon saponification. The stereochemistry of the ring-contracted product is the one expected by this mechanism as attested by the NOE cross-correlation between H-3 and H-5 on **14**. (Scheme 3)

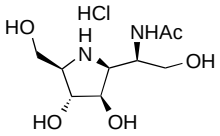
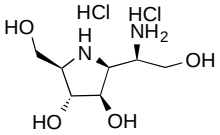
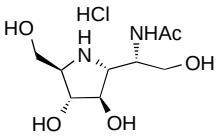
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Scheme 3 : Proposed mechanism for the ring contraction step.

The three pyrrolidines **11**, **12** and **16** were assayed as inhibitors of a panel of hexosaminidases and β-glucuronidases. Iminosugar **11** is a moderate inhibitor of β-*N*-acetylglucosaminidases with IC_{50} in the high micromolar range. The present work revealed that inversion of C-1 side chain in **11** to give **16** significantly enhanced its inhibition potency against these enzymes, pyrrolidine **16** demonstrating potent Jack bean β-*N*-acetylglucosaminidase inhibition, with a IC_{50} value of 3.4 μM. In contrast, replacement of the acetamide group by an amine as in **12** is detrimental to hexosaminidase inhibition (Table 1). It is noteworthy that pyrrolidine **11** showed low micromolar inhibition against bovine liver and *E.coli* β-glucuronidase, with IC_{50} values of 26 and 15 μM, respectively. Previous study suggested that β-glucuronidase recognized uronic acid and carboxylic acid part is required for tight binding.[37,38] Thus, pyrrolidine **11** is an interesting case for β-glucuronidase inhibition.

Table 1 . Concentration of iminosugars giving 50 % inhibition of various glycosidases

	IC_{50} (μM)		
	 **11**	 **12**	 **16**
Enzyme :			
β-*N*-Acetylglucosaminidase			
Aspergillus oryzae	241	NI (0%)	323
Bovine kidney	181	NI (0%)	31
HL60	538	NI (6.6%)	18
Human placenta	597	NI (0%)	15
Jack bean	61	NI (7.3%)	3.4
α-*N*-Acetylgalactosaminidase			
Chicken liver	[a]NI [b](26.2%)	NI (3.3%)	NI (7.2%)
β-glucuronidase			
Bovine liver	26	NI (0%)	NI (48.5%)
E.coli	15	NI (17.8%)	145

[a] NI : No inhibition (less than 50% inhibition at 1000 μM).

[b] ( ) : inhibition % at 1000 μM

In conclusion, a ring-contraction methodology applied to seven-membered iminosugars bearing an azido group in β position furnished a low micromolar hexosaminidase inhibitor after conversion of the azide function into an acetamide and final deprotection. This work complements previous work on the conversion of polyhydroxylated azepanes into six-membered NHAc-homoiminosugars.

1. Experimental

1.1 Material and methods

All commercial reagents were used as supplied. Solvents (DMF, THF) were distilled under anhydrous conditions. TLC plates (Macherey-Nagel, ALUGRAM® SIL G/UV_{254}, 0.2 mm silica gel 60 Å) were visualized under 254 nm UV light and/or by dipping the TLC plate into a solution of 3 g of phosphomolybdic acid in 100 mL of ethanol followed by heating with a heat gun. Flash column

chromatography was performed using Macherey-Nagel silica gel 60 (15-40 μm). NMR experiments were recorded with a Bruker AM- 400 spectrometer at 400 MHz for ^{1}H nuclei and at 100 MHz for ^{13}C nuclei. The chemical shifts are expressed in part per million (ppm) using residual $CHCl_3$ signal as internal reference ($\delta(^{1}H)$ =7.26 ppm and $\delta(^{13}C)$ =77.16 ppm) and the coupling constant *J* in hertz (Hz). NMR multiplicities are reported using the following abbreviations: b = broad, s = singulet, d = doublet, t = triplet, q = quadruplet, m = multiplet. HRMS were recorded on a Bruker microTOF spectrometer, using Tuning-Mix as reference. Optical rotations were measured on a Perkin–Elmer 341 digital polarimeter or a Jasco P-2000 polarimeter with a path length of 1 dm.

1.2 tert-butyl (2R,3R,4R,5R,6S)-6-azido-3,4-bis(benzyloxy)-2-((benzylo-xy)methyl)-5-hydroxyazepane-1-carboxylate (4)

Known epoxide **2**[33] (465 mg, 0.853 mmol) was dissolved in a DMF/H_2O mixture (9.0/1.0 mL), then NaN_3 (277 mg, 4.26 mmol) and NH_4Cl (226 mg, 4.26 mmol) were added. The resulting mixture was stirred at 90 °C for 3 days. After being cooled to room temperature, EtOAc and H_2O were added and the layers were separated. The aqueous layer was extracted twice with EtOAc and the combined organic layers were dried over $MgSO_4$, filtered and evaporated. The residue was purified by flash chromatography (Cy/EtOAc: 9/1) to give **4** (285 mg, 57%) as colorless oil and **5**[6] (160 mg, 32%) $[\alpha]_D$ +18.6 (*c* = 1.0, $CHCl_3$) ^{1}H NMR (400 MHz, $CDCl_3$, 2 rotamers): 7.39-7.26 (m, 26H, H_{ar}), 7.23-7.22 (m, 4H, H_{ar}), 4.81 (d, 1H, ^{2}J = 11.5 Hz , CH_2Ph), 4.75 (d, 1H, ^{2}J = 12.0 Hz, CH_2Ph), 4.69-4.59 (m, 4H, CH_2Ph), .4.49-4.40 (m, 6H, CH_2Ph), 4.02-3.91 (m, 6H, H_{8a}, H_2, H_3, H_3', H_5, H_5), 3.87-3.53 (m, 12H, H_{8b}, $H_{8a'}$, $H_{8b'}$, $H_{2'}$, H_4, $H_{4'}$, H_6, $H_{6'}$, H_{7a}, H_{7b}, $H_{7a'}$, $H_{7b'}$), 2.57 (bs, 0.8H, OH), 2.52 (bs, 0.8H, OH'),1.49 (s, 9H, CH_3, *Boc*), 1.41 (s, 9H, CH_3, *Boc*); ^{13}C NMR (100 MHz, $CDCl_3$, 2 rotamers): δ 155.4, 155.1 (CO, *Boc*), 138.3, 138.1, 138.0, 138.0, 137.9, 137.8 (C_{ipso}) 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.5 (CH_{ar}), 81.8, 81.4 (C_4, C_4'), 80.3, 80.2 ($C(CH_3)_3$, *Boc*) 74.4, 74.3 (C_3, $C_{3'}$), 74.0, 74.0, 73.4, 72.9 (2C), 72.8 (CH_2Ph), 72.5, 72.4 (C_5, $C_{5'}$), 69.5, 69.0 (C_8, $C_{8'}$), 63.1, 62.4 (C_6, $C_{6'}$), 58.9 (2C, C_2, $C_{2'}$), 45.1, 43.7 (C_7, $C_{7'}$), 28.3, 28.2 (CH_3, *Boc*); ESI-HRMS calcd. for $C_{33}H_{40}N_4NaO_6$ $[M+Na]^+$: 611.2846, found 611.2840.

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1.3 (3S,4R,5R,6R,7R)-3-azido-1-benzyl-5,6-bis(benzyloxy)-7-((benzyloxy)methyl)azepan-4-ol (8)

To a solution of **4** (46 mg, 0.078 mmol) in CH_2Cl_2 (2.0 mL) was added trifluoroacetic acid (2.0 mL) and the solution was stirred at room temperature for 1 hour. The solvents were evaporated and co-evaporated with toluene to remove completely the TFA. The obtained residue was dissolved in a mixture of EtOAc/H_2O (5.0/0.5 mL) and BnBr (13 µL, 0.101 mmol), K_2CO_3 (32 mg, 0.234 mmol) were added respectively. The mixture was refluxed for 18h. After being cooled to room temperature, H_2O and EtOAc were added and the layers were separated. The aqueous layer was extracted twice with EtOAc. Then the organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (Cy/EtOAc: 8.5/1.5) to give **8** as colorless oil (36 mg, 80%). $[\alpha]_D$ +19.0 (*c* = 1.0, $CHCl_3$); ^{1}H (400 MHz, $CDCl_3$): 7.37-7.19 (m, 20H, H_{ar}), 4.74 (d, 1H, ^2J = 11.5 Hz, CH_2Ph), 4.69 (d, 1H, ^2J = 11.5 Hz, CH_2Ph), 4.54 d, 1H, ^2J = 11.5 Hz, CH_2Ph), 4.41 (s, 2H, 2xCH_2Ph), 4.36 (d, 1H, ^2J = 11.5 Hz, CH_2Ph), 4.10 (ddd, 1H, $J_{H5\text{-}H4}$ = 1.5 Hz, $J_{H5\text{-}OH}$ = 4.0 Hz, $J_{H5\text{-}H6}$ = 6.0 Hz, H_5), 4.05 (dd, 1H, $J_{H4\text{-}H5}$ = 1.5 Hz, $J_{H4\text{-}H3}$ = 5.5 Hz, H_4), 4.01 (d, 1H, ^2J = 14.5 Hz, NCH_2Ph), 3.92 (d, 1H, ^2J = 14.5 Hz, NCH_2Ph), 3.82-3.75 (m, 2H, H_3, H_6), 3.68 (dd, 1H, $J_{H8a\text{-}H2}$ = 5.0 Hz, $J_{H8a\text{-}H8b}$ = 9.5 Hz, H_{8a}), 4.64 (dd, 1H, $J_{H1b\text{-}H2}$ = 5.0 Hz, $J_{H8b\text{-}H8a}$ = 9.5 Hz, H_{8b}), 3.33 (dd, 1H, $J_{H7a\text{-}H6}$ = 4.5 Hz, $J_{H7a\text{-}H7b}$ = 14.5 Hz, H_{7a}), 3.03 (dt, 1H, $J_{H2\text{-}H1}$ = 5.0 Hz, $J_{H2\text{-}H3}$ = 6.0 Hz, H_2), 2.69 (dd, 1H, $J_{H7b\text{-}H6}$ = 6.5 Hz, $J_{H7b\text{-}H7a}$ = 14.5 Hz, H_{7b}), 2.55 (d, 1H, $J_{OH\text{-}H5}$ = 4.0 Hz, OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 140.1, 138.3, 138.2, 138.1 (C_{ipso}), 128.5, 128.4, 128.4, 128.3, 128.0, 127.9, 127.7, 127.7, 127.6, 127.0 (CH_{ar}), 82.1 (C_4), 76.6 (C_3), 73.8 (CH_2Ph), 73.2 (2C, C_5, CH_2Ph), 72.7 (CH_2Ph), 68.9 (C_8), 63.9 (C_6), 63.5 (C_2), 57.2 (NCH_2Ph), 51.6 (C_7); ESI-HRMS calcd. for $C_{35}H_{39}N_4O_4$ $[M+H]^+$: 579.2971, found 579.2975

1.4 (R)-2-azido-2-((2S,3R,4R,5R)-1-benzyl-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)pyrrolidin-2-yl)ethan-1-ol (9)

To a solution of **8** (60 mg, 0.104 mmol) in toluene (1.0 mL) were added trifluoroacetic anhydride (28 µL, 0.194 mmol) and Et_3N (26 µL, 0.194 mmol). The obtained solution was refluxed for 3h and cooled to room temperature. A solution of

NaOH (10%, 5 mL) was added and the mixture was stirred for 30 minutes. EtOAc and H_2O were added and the layers were separated. The aqueous layer was extracted twice with EtOAc and the combined organic layers were dried on Na_2SO_4, filtered and evaporated. The obtained crude was purified by flash chromatography (Cy/EtOAc: 9/1) to give compound **9** (55 mg, 92%). $[\alpha]_D$ +1.3 (c = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38-7.27 (m, 18H, H_{ar}), 7.18-71.7 (m, 2H, H_{ar}), 4.60 (d, 1H, ^2J = 12.0 Hz, CH_2Ph), 4.56 (d, 1H, ^2J = 12.0 Hz, CH_2Ph), 4.53 (s, 2H, CH_2Ph), 4.27 (s, 2H, CH_2Ph), 4.15-4.09 (m, 2H, H_3, H_4), 4.07 (d, 1H, ^2J = 14.0 Hz, NCH_2Ph), 4.97-3.81 (m, 4H, H_6, H_{7a}, H_{7b}, NCH_2Ph), 3.45 (t, 1H, J_{H5-H4} = J_{H5-H6} = 6.5 Hz, H_5), 3.36-3.31 (m, 1H, H_{8a}), 3.18-3.12 (m, 2H, H_{8b}, H_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.2, 138.1, 138.1, 137.4 (C_{ipso}), 129.4, 128.4, 128.2, 127.7, 127.7, 127.5, 127.5, 127.4 (CH_{ar}), 83.5(C_4), 81.5 (C_3), 72.8, 72.4, 71.7 (CH_2Ph), 69.8 (C_8), 67.3 (C_5), 66.4 (C_2), 63.6 (C_7), 62.6 (C_6), 61.5 (NCH_2Ph); ESI-HRMS calcd. for $C_{35}H_{39}N_4O_4$ $[M+H]^+$: 579.2971, found 579.2947.

1.5 (R)-2-acetamido-2-((2R,3R,4R,5R)-1-benzyl-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)pyrrolidin-2-yl)ethyl acetate (10)

To a solution of azide **9** (28 mg, 0.044 mmol) in THF/H_2O (2.0 mL/1.0mL) was added Ph_3P (35 mg, 0.132 mmol) and the resulting solution was stirred at 65 °C for 2h. The solution was cooled to room temperature, solvents were evaporated and the crude was dried for 2h under reduced pressure. The residue was dissolved in pyridine (2.0 mL) and Ac_2O (1.0 mL) was added at 0°C. The resulting solution was then stirred for 12 h at room temperature. Pyridine and Ac_2O were removed by evaporation and co-evaporation with toluene (5 x 3 mL). The residue was purified by flash chromatography (cyclohexane/AcOEt: 6/4) to give **10** (22 mg, 76%). $[\alpha]_D$ -22.9 (c = 1.05, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): 7.36-7.22 (m, 16H, H_{ar}), 7.19-71.5 (m, 4H, H_{ar}), 4.73-4.67 (m, 1H, H_6), 4.62 (d, 1H, ^2J = 12.0 Hz, CH_2Ph), 4.51 (d, 1H, ^2J = 12.0 Hz, CH_2Ph), 4.41 (d, 1H, ^2J = 10.5 Hz, CH_2Ph), 4.34 (d, 1H, ^2J = 12.0 Hz, CH_2Ph), 4.31(d, 1H, ^2J = 10.5 Hz, CH_2Ph), 4.19 (d, 1H, ^2J = 12.0 Hz, CH_2Ph), 4.15 (dd, 1H, J_{H7a-H6} = 6.5 Hz, $J_{H7a-H7b}$ = 10.5 Hz, H_{7a}), 4.11 (dd, 1H, J_{H7b-H6} = 6.5 Hz, $J_{H7b-H7a}$ = 10.5 Hz, H_{7b}), 4.02-3.98 (m, 3H, H_3, H_4, NCH_2Ph), 3.55 (d, 1H, ^2J = 13.0 Hz, NCH_2Ph), 3.27 (t, 1H, J_{H5-H4} = J_{H5-H6} = 4.5 Hz, H_5), 3.20-3.14 (m, 2H, H_{8a}, H_2), 2.91 (dd, $J_{8b,2}$ = 10.0 Hz, $J_{8b,8a}$ = 16.0 Hz, H_{8b}), 2.05 (s, 3H, CH_3, *Ac*), 1.77 (s, 3H, CH_3,

Ac); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.9, 170.0, (CO), 138.5, 138.5, 138.1, 137.1 (C_{ipso}), 129.5, 128.7, 128.5, 128.3, 128.3, 127.9, 127.8, 127.8, 127.5, 127.5, 127.2 (CH_{ar}), 84.4 (C_4), 80.9 (C_3), 72.9, 71.7 (CH_2Ph), 71.5 (C_8), 70.8 (CH_2Ph), 68.0 (C_2), 64.8 (C_5), 64.6 (C_7), 57.4 (NCH_2Ph), 46.7 (C_6), 23.3, 21.0 (CH_3, *Ac*); ESI-HRMS calcd. for $C_{39}H_{45}N_2O_6$: $[M+H]^+$: 637.3278, found 637.3299.

1.6 N-((R)-1-((2R,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidin-2-yl)-2-hydroxyethyl)acetamide (11)

A solution of **10** (20 mg, 0.314 mmol) in MeOH/H_2O/Et_3N (4/0.5/0.5 mL) was stirred for 18h at room temperature. The solvents were evaporated and co-evaporated three times with toluene. The obtained residue was dissolved in MeOH (2 mL) and aqueous HCl (1M, 0.2 mL) was added under argon. After addition of Pd/C (10%, 20 mg), the argon was removed. The H_2 was introduced and the mixture was bubbled for 5 minutes. After stirring the solution for 24 under H_2 atmosphere, the mixture was filtered on micro-filter (0.3 µm). The solvent was evaporated to give compound **11** (6 mg, 82%) as a white solid. $[\alpha]^{18}_D$ +22.7 (*c* = 0.5, MeOH); ^{1}H NMR (400 MHz, D_2O): 4.46 (dt, 1H, $J_{H6\text{-}H7a} = J_{H6\text{-}H7b}$ = 5.5 Hz, $J_{H6\text{-}H5}$ = 10.5 Hz, H_6), 4.23 (d, 1H, $J_{H4\text{-}H5}$ = 2.5 Hz, H_4), 4.13 (dd, 1H, $J_{H3\text{-}H4}$ = 1.0 Hz, $J_{H3\text{-}H2}$ = 2.0 Hz, H_3), 4.01 (dd, 1H, $J_{H8a\text{-}H2}$ = 5.0 Hz, $J_{H8a\text{-}H8b}$ = 12.0 Hz, H_{8a}), 3.95-3.87 (m, 2H, H_{8b}, H_5), 3.84 (dd, 1H, $J_{H7a\text{-}H6}$ = 5.5 Hz, $J_{H7a\text{-}H7b}$ = 12.0 Hz, H_{7a}), 3.77 (dd, 1H, $J_{H7b\text{-}H6}$ = 5.5 Hz, $J_{H7b\text{-}H7a}$ = 12.0 Hz, H_{7b}), 3.71 (ddd, 1H, $J_{H2\text{-}H3}$ = 2.0 Hz, $J_{H2\text{-}H8a}$ = 5.0 Hz, $J_{H2\text{-}H8b}$ = 8.0 Hz, H_2), 2.08 (s, 3H, CH_3, *Ac*); ^{13}C NMR (100 MHz, D_2O): δ 174.6 (CO), 75.0 (C_4), 74.9 (C_3), 69.0 (C_2), 61.9 (C_5), 61.1 (C_7), 59.6 (C_8), 47.4 (C_6), 21.9 (CH_3, *Ac*); ESI-HRMS calcd. for $C_9H_{19}N_2O_5$ $[M+H]^+$: 235.1294, found 235.1297.

1.7 N-((R)-1-((2R,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidin-2-yl)-2-hydroxyethyl)amonium (12)

9 (10 mg, 0.017 mmol) was dissolved in MeOH (2 mL) and aqueous HCl (1M, 0.2 mL) was added under argon. After adding Pd/C (10%, 10 mg), the argon was removed. The H_2 was introduced and the mixture was bubbled for 5 minutes. After stirring the solution for 24 under H_2 atmosphere, the mixture was filtered on micro-filter 0.3 µm). The solvent was evaporated to give the desired product (4.5 mg, 95%).

$[\alpha]^{18}_{D}$ +63.7 (*c* = 0.2, MeOH); ^{1}H RMN (400 MHz, D_2O) δ 4.41 (dd, 1H, J_{H4-H3} = 1.0 Hz, J_{H4-H5} = 3.0 Hz, H_4), 4.22 (dd, 1H, J_{H3-H4} = 1.0 Hz, J_{H3-H2} = 2.5 Hz, H_3), 4.16 (dd, J_{H5-H4} = 3.0 Hz, J_{H5-H6} = 9.0 Hz, H_5), 4.07-4.00 (m, 3H, H_{8a}, H_6, H_{7a}), 3.94-3.89 (m, 2H, H_{8b}, H_{7b}), (ddd, 1H, J_{H2-H3} = 2.5 Hz, J_{H2-H8a} = 4.5 Hz, J_{H2-H8b} = 8.5 Hz, H_2); ^{13}C RMN (100 MHz, D_2O) δ 75.6 (C_3), 74.1 (C_4), 68.8 (C_2), 59.5 (2C, C_5, C_7), 59.0 (C_8), 48.6 (C_6), HRMS calcd for $C_7H_{17}N_2O_4$: $[MH]^+$: 193.1188 found 193.1191.

1.8 tert-butyl (2R,3R,4R,5S,6R)-6-azido-3,4-bis(benzyloxy)-2-((benzyloxy)methyl)-5-hydroxyazepane-1-carboxylate (6)

To a solution of known epoxide **3**[33] (160 mg, 0.294 mmol) in a mixture of DMF/H_2O (2.9/0.3 mL) was added NaN_3 (88 mg, 1.358 mmol) followed by NH_4Cl (53 mg, 1.358 mmol). The mixture was stirred for 28 h at 90 °C. EtOAc (50 mL) and H_2O (50 mL) were added. The layers were separated and the aqueous layer was extracted with EtOAc (50 mL). The combined organic layers were dried on $MgSO_4$, filtered and evaporated. The residue was purified by flash chromatography (Cy/ EtOAc: 96/4 - 95/5) to give compound **7**[6] (90 mg, 52%) and **6** (70 mg, 40%) as a pale yellow oil. $[\alpha]_D$ -38.5 (*c* = 1,0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 2 rotamers): δ 7.37-7.26 (m, 26H, H_{ar}), 7.214-7.18 (m, 4H, H_{ar}), 4.78 (d, 2H, ^{2}J = 11.5 Hz , CH_2Ph), 4.72, 4.71 (2s, 4H, CH_2Ph), 4.51 (d, 2H, ^{2}J = 11.5 Hz , CH_2Ph), 4.47 (d, 2H, ^{2}J = 12.0 Hz , CH_2Ph), 4.38 (d, 2H, ^{2}J = 12.0 Hz , CH_2Ph), 4.27, 4.26 (2s, 2H, OH, OH'), 4.10-4.08 (m, 2H, H_2, $H_{2'}$), 4.02-3.84 (m, 10H, H_3, $H_{3'}$, H_4, $H_{4'}$, H_5, $H_{5'}$, H_6, $H_{6'}$, H_{7a}, $H_{7a'}$), 3.67 (dd, 2H, J_{H8a-H2} = 4.5 Hz, $J_{H8a-H8b}$ = 9.5 Hz, H_{8a}, $H_{8a'}$), 3.55 (dd, 2H, J_{H8b-H2} = 4.5 Hz, $J_{H8b-H8a}$ = 9.5 Hz, H_{8b}, $H_{8b'}$), 3.31 (dd, J_{H7b-H6} = 1.5 Hz, $J_{H7b-H7a}$ = 15.5 Hz, H_{7b}, $H7_{b'}$), 1.42 (s, 18H, CH_3, *Boc*); ^{13}C NMR (100 MHz, $CDCl_3$, 2 rotamers): δ 158.9 (CO, *Boc*), 138.0, 137.9, 137.7 (C_{ipso}) 128.4 128.4, 127.8, 127.7, 127.6, 127.5, 127.4 (CH_{ar}) 81.5 (2C, ($C(CH_3)_3$, *Boc*), 80.5 (2C, C_4, $C_{4'}$), 74.4 (2C, C_3, $C_{3'}$), 73.9, 72.9 (CH_2Ph, 72.0 (2C, C_5, $C_{5'}$) 68.9 (2C, C_8, $C_{8'}$), 66.1 (2C, C_6, $C_{6'}$), 57.6 (2C, C_2, $C_{2'}$), 44.7 (2C, C_7, $C_{7'}$), 28.2 (CH_3, *Boc*); ESI-HRMS calcd. for $C_{33}H_{40}N_4NaO_6$ $[M+Na]^+$: 611.2846, found 611.2858.

1.9 (3R,4S,5R,6R,7R)-3-azido-1-benzyl-5,6-bis(benzyloxy)-7-((benzyloxy)methyl)azepan-4-ol (13)

To a solution of **6** (42 mg, 0.071 mmol) in CH_2Cl_2 (4 mL) was added trifluoroacetic acid (2 mL) and the obtained solution was stirred at room temperature for 1h. The solution was evaporated and co-evaporated with toluene (3x5 mL). The residue was dissolved in EtOAc/H_2O (4/0.4 mL) and K_2CO_3 (49 mg, 0.355 mmol), BnBr (13 µL, 0.107 mmol) were added respectively. The resulting mixture was stirred at 80 °C for 16 h and cooled to room temperature. EtOAc and H_2O were added and the layers were separated. The aqueous layer was extracted twice with EtOAc and the combined organic layers were dried on Na_2SO_4, filtered and evaporated. The obtained crude was purified by flash chromatography to give **13** (28 mg, 68%). $[\alpha]_D$ +43.7 (*c* = 1,0, $CHCl_3$); ^{1}H NMR(400 MHz, $CDCl_3$): 7.40-7.26 (m, 18H, H_{ar}), 7.20-7.18 (m, 2H, H_{ar}), 5.06 (d, 1H, ^2J = 11.0 Hz, CH_2Ph), 4.93 (d, 1H, ^2J = 11.0 Hz, CH_2Ph), 4.63 (d, 1H, ^2J = 11.0 Hz, CH_2Ph), 4.47-4.40 (m, 3H, CH_2Ph), 3.95-3.88 (m, 2H, H_4, NCH_2Ph), 3.77 (d, 1H, ^2J = 13.0 Hz, NCH_2Ph), 3.10-3.63 (m, 2H, H_{8a}, H_3), 3.61 (dd, 1H, J_{H8b-H2} = 3.5 Hz, $J_{H8b-H8a}$ = 10.0 Hz, H_{8b}), 3.53 (t, 1H, J_{H5-H4} = J_{H5-H6} = 8.0 Hz, H_5), 3.31 (s, 1H, OH), 3.21 (ddd, 1H, J_{H6-H7b} = 4.0 Hz, J_{H6-H5} = 8.0 Hz, J_{H6-7a} = 11.5 Hz, H_6), 3.12 (dd, 1H, J_{H7a-H6} = 11.5 Hz, J_{H7a-7b} = 14.0 Hz, H_{7a}), 2.90 (dt 1H, J_{H2-H1a} = J_{H2-H1b} = 3.5 Hz, J_{H2-H3} = 9.0 Hz, H_2), 2.63 (dd, 1H, J_{H7b-H6} = 4.0, $J_{H7b-H7a}$ = 14.0 Hz, H_{7b}); ^{13}C NMR (100 MHz, $CDCl_3$): δ 139.1, 138.3, 138.1, 138.0 (C_{ipso}), 128.8, 128.6, 128.4, 128.4, 128.4, 128.1, 128.0, 127.7, 127.7, 127.4 (CH_{ar}), 83.2 (C_4), 79.2 (C_3), 78.1 (C_2), 76.1, 75.3, 73.2 (CH_2Ph), 67.8 (C_8), 64.4 (C_6), 63.7 (C_2), 59.6 (NCH_2Ph), 48.9 (C_7); ESI-HRMS calcd. for $C_{35}H_{39}N_4O_4$ $[M+H]^+$: 579.2971, found 579.2952.

1.10 (S)-2-azido-2-((2R,3R,4R,5R)-1-benzyl-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)pyrrolidin-2-yl)ethan-1-ol (14)

To a solution of **13** (28 mg, 0.048 mmol) in toluene (0.5 mL) were added trifluoroacetic anhydride (14 µL, 0.1 mmol) and Et_3N (13 µL, 0.097 mmol). The obtained solution was refluxed for 3 h and cooled to room temperature. A solution of NaOH (10%, 2 mL) was added and the mixture was stirred for 30 minutes. AcOEt and H_2O were added and the layers were separated. The aqueous layer was extracted twice with AcOEt and the combined organic layers were dried on Na_2SO_4, filtered and evaporated. The obtained crude was purified by flash chromatography (Cyclohexane/AcOEt: 95/5) to give compound **14** (24 mg, 86%). $[\alpha]^{19}_D$ +16.8 (*c* = 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38-7.22 (m, 20H, H_{ar}), 4.56 (d, 1H, ^2J

= 12.0 Hz, CH_2Ph), 4.50-4.43 (m, 5H, CH_2Ph), 4.16 (dd, 1H, J_{H4-H3} = 2.0 Hz, J_{H4-H5} = 4.0 Hz, H_4), 4.10 (t, 1H, J_{H3-H2} = J_{H3-H4} = 2.0 Hz, H_3), 4.05 (d, 1H, ^2J = 14.0 Hz, NCH_2Ph), 3.86 (td, 1H, J_{H6-H5} = 4.0 Hz, J_{H6-H7a} = J_{H6-H7b} = 6.5 Hz, H_6), 3.76 (d, 1H, ^2J = 14.0 Hz, NCH_2Ph), 3.68-3.56 (m, 4H, H_{8a}, H_{8b}, H_{7a}, H_{7b}), 3.46 (td, 1H, J_{H2-H3} = 2.0 Hz, J_{H2-H8a} = J_{H2-H8b} = 6.0 Hz, H_2), 3.27 (t, J_{H5-H4} = J_{H5-H6} = 4.0 Hz, H_5), 3.07 (s, 0.9 H, OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.2, 138.0, 137.9, 137.6 (C_{ipso}), 128.5, 128.4, 128.3, 128.3, 127.9, 127.9, 127.7, 127.6, 127.6, 127.1 (CH_{ar}), 85.7 (C_4), 83.6 (C_3), 73.2, 71.7, 71.4 (CH_2Ph), 68.9 (C_5), 66.6 (C_8), 63.2 (C_2), 62.9 (C_7), 62.0 (C_6), 51.9 (NCH_2Ph); ESI-HRMS calcd. for $C_{35}H_{39}N_4O_4$ $[M+H]^+$: 579.2971, found 579.2977.

1.11 (S)-2-acetamido-2-((2S,3R,4R,5R)-1-benzyl-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)pyrrolidin-2-yl)ethyl acetate (15)

To a solution of azide **14** (24 mg, 0.042 mmol) in THF/H_2O (2.0 mL/1.0mL) was added Ph_3P (32 mg, 0.125 mmol) and the resulting solution was stirred at 65 °C for 2 h. The solution was cooled to room temperature, solvents were evaporated and the reaction crude was dried for 2 h under reduced pressure. The residue was dissolved in pyridine (2.0 mL) and Ac_2O (1.0 mL) was added at 0°C. The resulting solution was then stirred for 12 h at room temperature. Pyridine and Ac_2O were removed by evaporation and co-evaporation with toluene (5 x 3 mL). The residue was purified by flash chromatography (Cy/EtOAc: 6.5/3.5) to give **15** as a white solid (16 mg, 60%). $[\alpha]_D$ -12.3 (*c* = 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): 7.36-7.26 (m, 20H, H_{ar}), 6.67 (d, 1H, J_{NH-H6} = 6.5 Hz, NHAc), 4.56 (d, 1H, ^2J = 11.0 Hz, CH_2Ph), 4.51-4.44 (m, 5H, CH_2Ph), 4.32-4.27 (m, 1H, H6), 4.14 (dd, 1H, J_{H7a-H6} = 5.0 Hz, $J_{H7a-H7b}$ = 11.0 Hz, H_{7a}), 4.08 (bs, 1H, H_3), 3.99 (dd, 1H, J_{H7b-H6} = 7.5 Hz, $J_{H7b-H7a}$ = 11.0 Hz, H_{7b}), 3.94 (bs, 1H, H_4), 3.90 (d, 1H, ^2J = 14.5 Hz, NCH_2Ph), 3.81 (d, 1H, ^2J = 14.5 Hz, NCH_2Ph), 3.71 (dd, 1H, J_{H8a-H2} = 4.5 Hz, $J_{H8a-H8b}$ = 9.0 Hz, H_{8a}), 3.54 (t, 1H, J_{H8b-H2} = $J_{H8b-H8a}$ = 9.0 Hz, H_{8b}), 3.50 (dd, 1H, J_{H2-H8a} = 4.5 Hz, J_{H2-H8b} = 9.0 Hz, H_2), 3.40-3.39 (m, 1H, H_5), 1.97 (s, 3H, CH_3, *Ac*), 1.56 (s, 3H, CH_3, *Ac*); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.7, 170.5 (CO), 139.0, 138.2, 137.6, 137.1 (C_{ipso}), 128.7, 128.6, 128.5, 128.4, 128.4, 128.4, 128.3, 128.2, 128.1, 127.9, 127.7, 127.6, 127.6, 127.0 (CH_{ar}), 83.9 (C_4), 82.6 (C_3), 73.3, 71.6, 71.4 (CH_2Ph), 68.0 (C_8) 66.7 (C_5), 64.2 (C_2), 63.7

(C_7), 50.7 ((NCH_2Ph), 47.0 (C_6), 22.8, 20.7 (CH_3, *Ac*); ESI-HRMS calcd. for $C_{39}H_{45}N_2O_6$ $[M+H]^+$: 637.3278 found 637.3281.

1.12 N-((S)-1-((2S,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidin-2-yl)-2-hydroxyethyl)acetamide (16)

A solution of **15** (8 mg, 0.013 mmol) in MeOH/H_2O/Et_3N (2/0.25/0.25 mL) was stirred for 18 h at room temperature. The solvents were evaporated and co-evaporated three times with toluene. The obtained residue was dissolved in MeOH (1 mL) and aqueous HCl (1M, 0.1 mL) was added under argon. After adding Pd/C (10%, 10 mg), the argon was removed. The H_2 was introduced and the mixture was bubbled for 5 minutes. After stirring the solution for 24 under H_2 atmosphere, the mixture was filtered on micro-filter (0.3 µm). The solvent was evaporated to give compound **16** (3 mg, 88%). $[\alpha]^{22}_D$ = +18.3 (*c* = 0.16, MeOH); ^{1}H NMR (400 MHz, D_2O): 4.44 (q, 1H, $J_{H6\text{-}H7a} = J_{H6\text{-}H7b} = J_{H6\text{-}H5}$ = 5.5 Hz, H_6), 4.20 (dd, 1H, $J_{H4\text{-}H3}$ = 6.5 Hz, $J_{H4\text{-}H5}$ = 8.0 Hz, H_4), 4.14 (dd, 1H, $J_{H3\text{-}H4}$ = 6.5 Hz, $J_{H3\text{-}H2}$ = 8.0 Hz, H_3), 3.98 (dd, 1H, $J_{H8a\text{-}H2}$ = 3.5 Hz, $J_{H1a\text{-}H1b}$ = 12.5 Hz, H_{8a}), 3.91 (dd, 1H, $J_{H8b\text{-}H2}$ = 5.5 Hz, $J_{H8b\text{-}H8a}$ = 12.5 Hz, H_{8b}) 3.84 (2d, 2H, *J* = 5.5 Hz, H_{7a}, H_{7b}), 3.78 (dd, 1H, $J_{H5\text{-}H6}$ = 5.5 Hz, $J_{H5\text{-}H4}$ = 8.0 Hz, H_5), 3.63 (ddd, 1H, $J_{H2\text{-}H8a}$ = 3.5 Hz, $J_{H2\text{-}H8b}$ = 5.5 Hz, $J_{H2\text{-}H3}$ = 8.0 Hz, H_2), 2.14 (s, 3H, CH_3, *Ac*); ^{13}C NMR (100 MHz, D_2O): δ 175.9 (CO), 74.8 (C_4), 74.3 (C_3), 62.7 (C_2), 61.7 (C_5), 60.5 (C_7), 57.5 (C_8), 51.0 (C_6), 21.8 (CH_3, *Ac*); ESI-HRMS calcd. for $C_9H_{18}N_2NaO_4$ $[M+Na]^+$: 257.1113, found 257.1108.

Acknowledgements

Support for this research was provided by Sanfilippo foundation Switzerland, and Dorphan.

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ACCEPTED MANUSCRIPT

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Highlights.

-A new family of amino-functionalised pyrolidines was synthesized.

-Aring-contraction of β-azido,γ-hydroxyazepanes was used.

-A low micromolar inhibitor of β-N-acetylehexosaminidase identified.

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SUPPLEMENTARY INFORMATION

Synthesis of pyrrolidine-based analogues of 2-acetamidosugars as *N*-acetyl glucosaminidase inhibitors

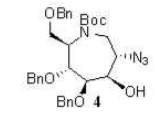
Anh Tuan Tran,[a] Bo Luo,[a] Jagadeesh Yerri,[b] Nicolas Auberger,[b] Jérôme Désiré,[b] Shinpei Nakagawa,[c] Atsushi Kato,[c] Yongmin Zhang,[a] Yves Blériot,[b] * Matthieu Sollogoub[a] *

[a] *Sorbonne Universités, UPMC Univ Paris 06, Institut Universitaire de France, UMR-CNRS 8232, IPCM, F-75005 Paris, France. E-mail : matthieu.sollogoub@upmc.fr*

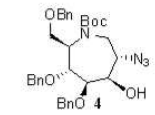
[b] *Glycochemistry Group of "Organic Synthesis" Team, Université de Poitiers, UMR-CNRS 7285 IC2MP, 4 rue Michel Brunet, 86073 Poitiers Cedex 9, France. E-mail: yves.bleriot@univ-poitiers.fr*

[c] *Department of Hospital Pharmacy, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan*

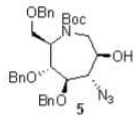
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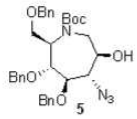
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Nucleus	1H



Parameter	Value
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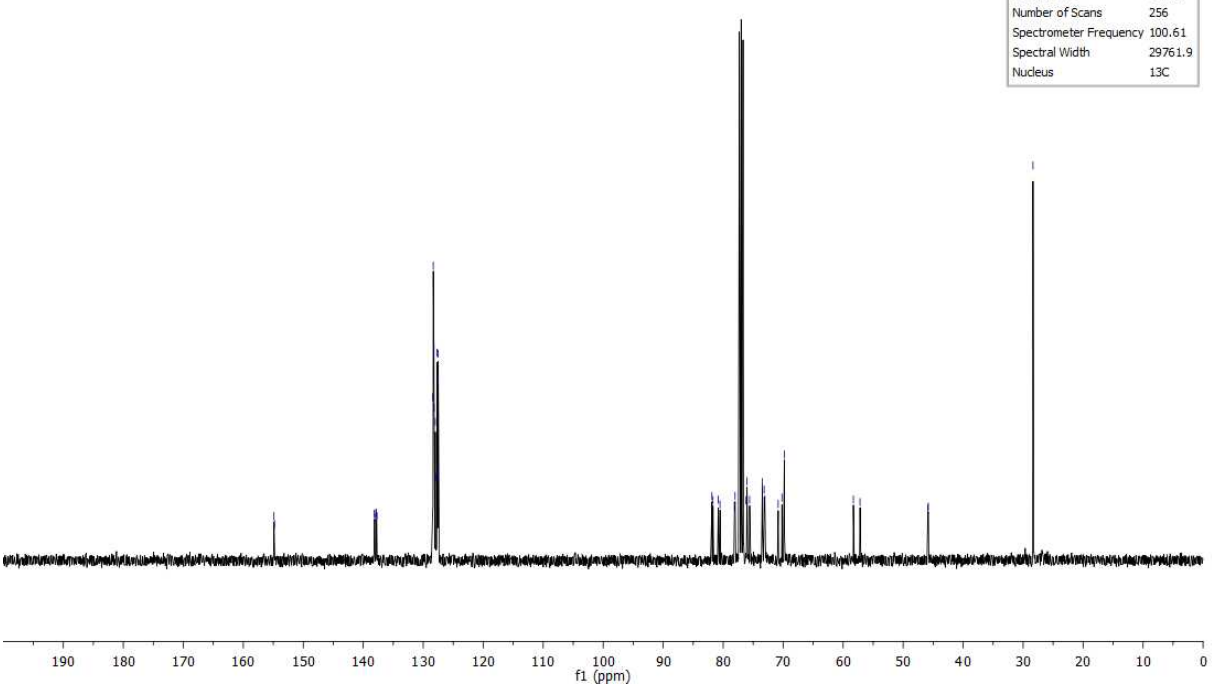


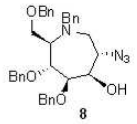
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Nucleus	1H





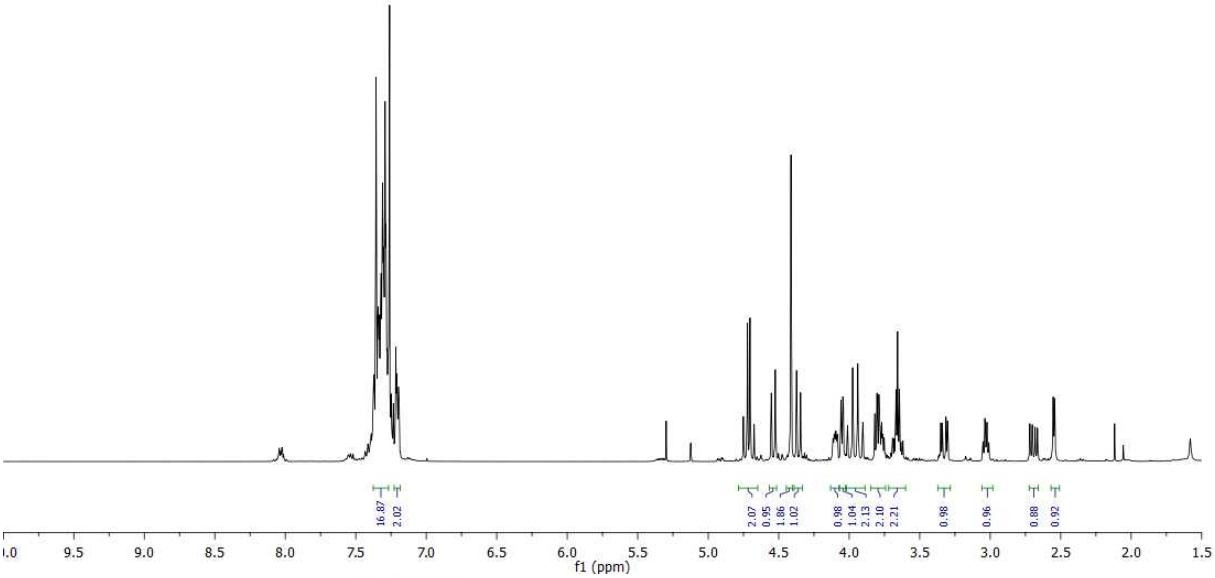
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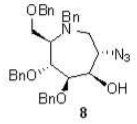




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Nucleus	1H

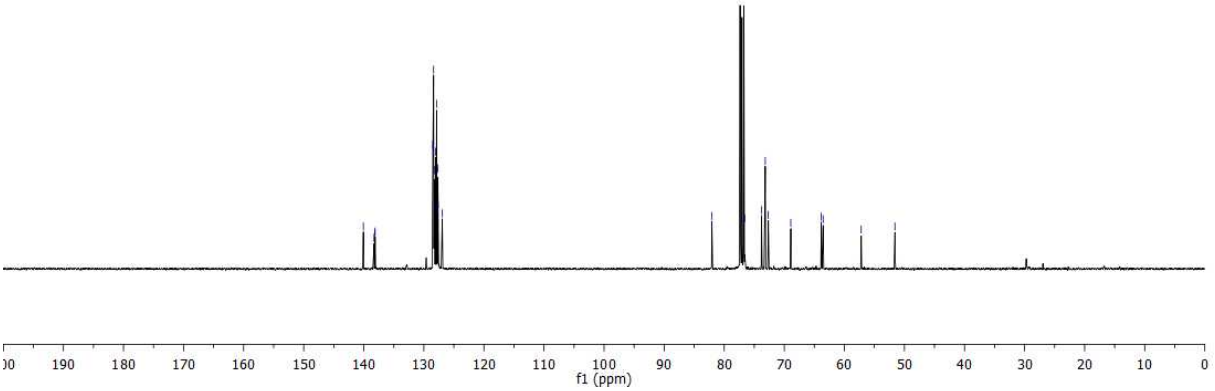


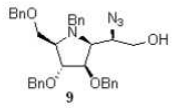


8

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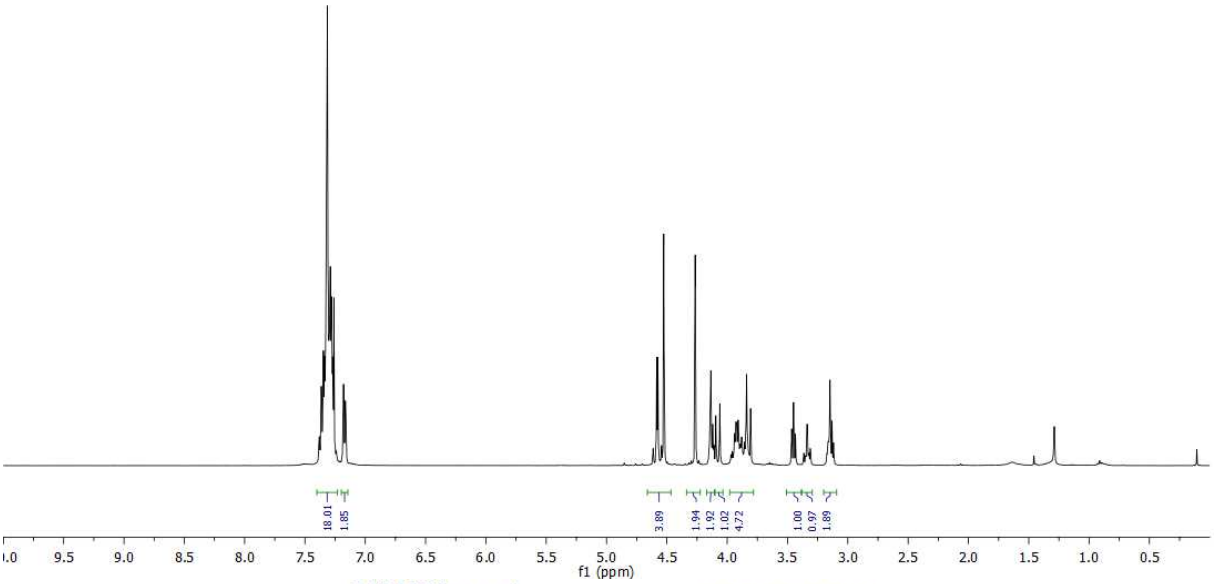
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Nucleus	13C

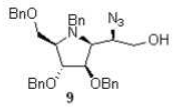




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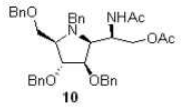
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Number of Scans	8
Spectrometer Frequency	400.13
Spectral Width	7211.5
Nucleus	1H





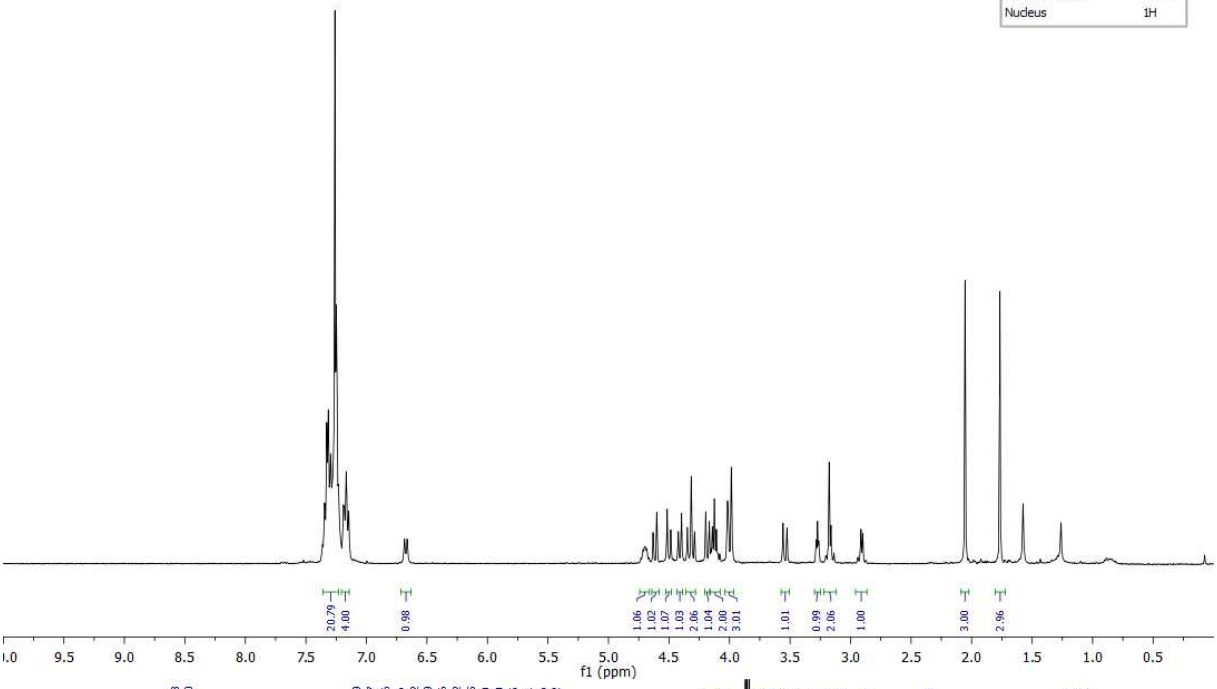
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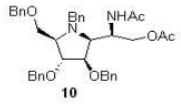
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Number of Scans	2048
Spectrometer Frequency	100.61
Spectral Width	29761.9
Nucleus	13C



10

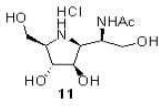
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Spectrometer Frequency	400.13
Spectral Width	7211.5
Nucleus	1H





10

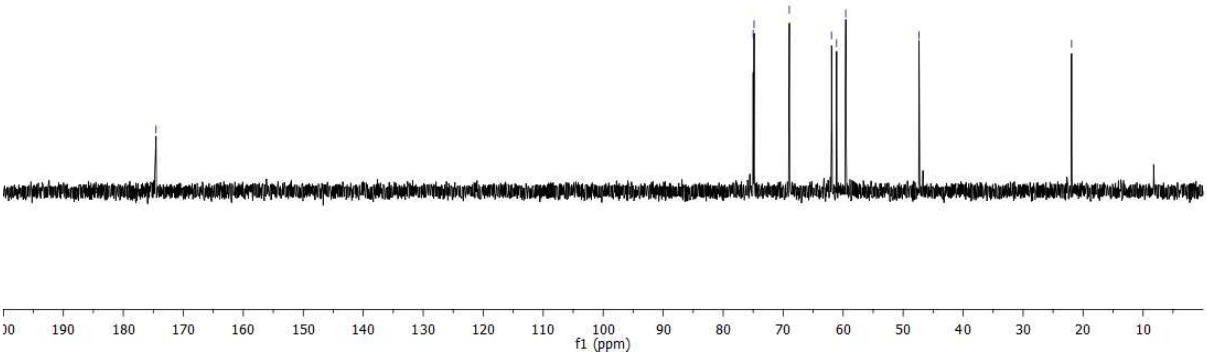
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Spectrometer Frequency	100.61
Spectral Width	29761.9
Nucleus	13C

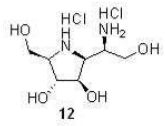


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Spectrometer Frequency	400.13
Spectral Width	7211.5
Nucleus	1H



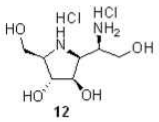
Parameter	Value
Spectrometer	spect
Solvent	D2O
Temperature	300.0
Number of Scans	2048
Spectrometer Frequency	100.61
Spectral Width	29761.9
Nucleus	13C





12

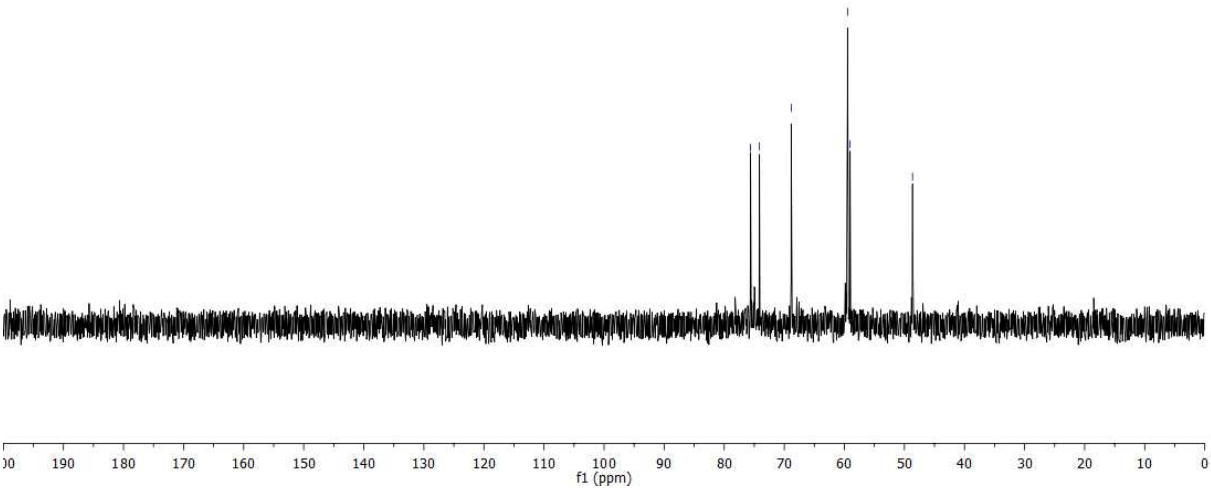
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Spectrometer	spect
Solvent	D2O
Temperature	300.0
Number of Scans	32
Spectrometer Frequency	400.13
Spectral Width	7211.5
Nudeus	1H

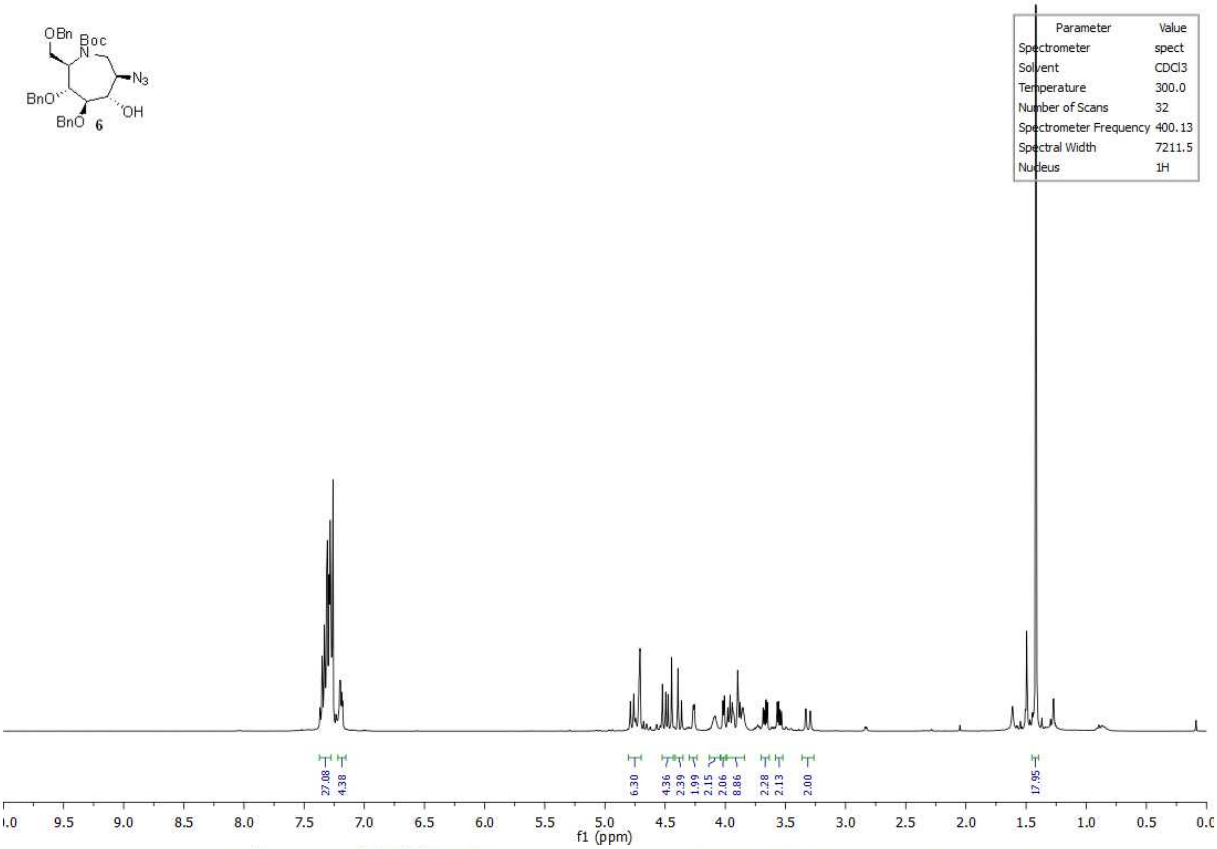


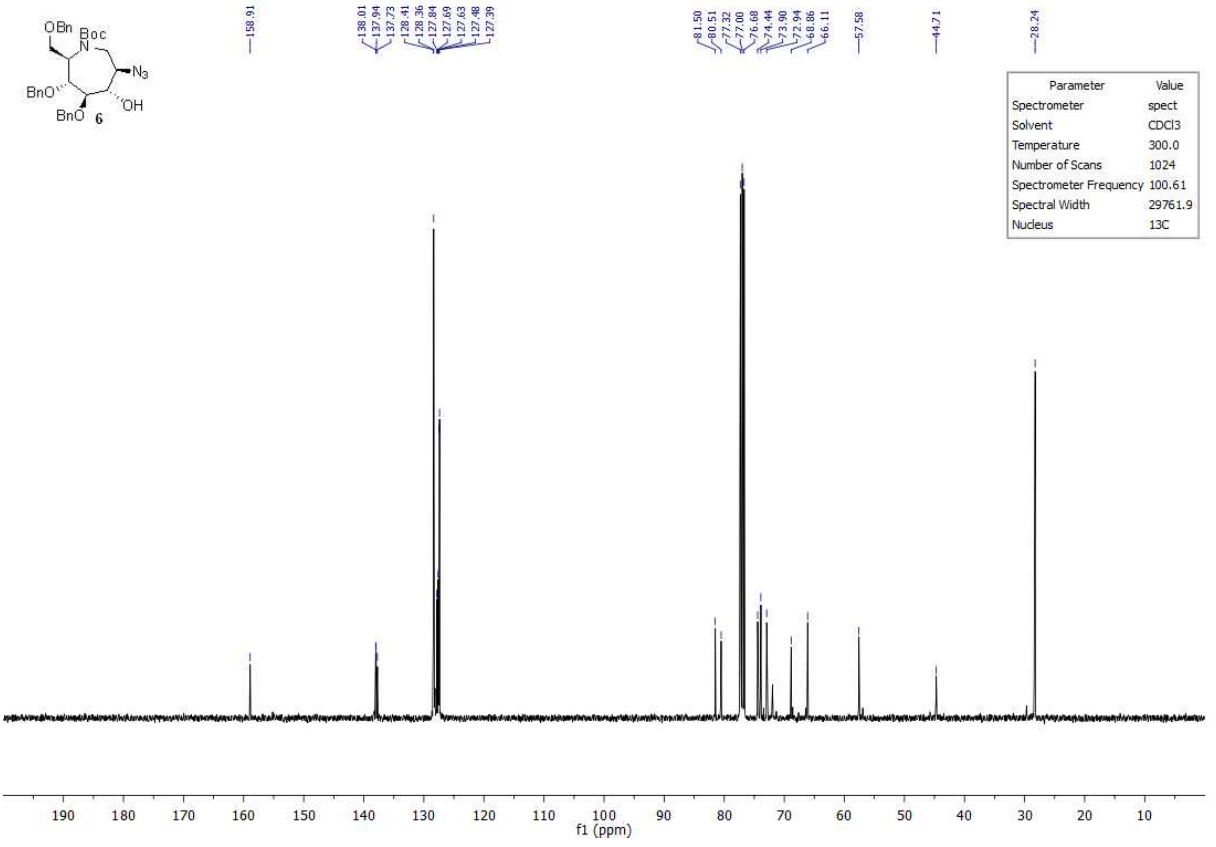
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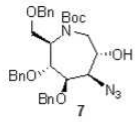


Parameter	Value
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Nucleus	13C

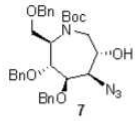



OBn
Boc
N
N3
BnO
OH
BnO
6
Parameter Value
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2.06
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2.00
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f1 (ppm)

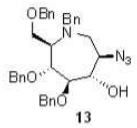

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N3
BnO
OH
BnO
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80.51
77.32
77.00
76.68
74.44
73.90
72.94
68.86
66.11
57.58
44.71
28.24
Parameter Value
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Solvent CDCl3
Temperature 300.0
Number of Scans 1024
Spectrometer Frequency 100.61
Spectral Width 29761.9
Nucleus 13C
f1 (ppm)


OBn
Boc
N
OH
BnO
N3
BnO
7

Parameter Value
Spectrometer spect
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Temperature 300.0
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Spectral Width 7211.5
Nucleus 1H
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2.59
2.71
5.33
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9.12
f1 (ppm)

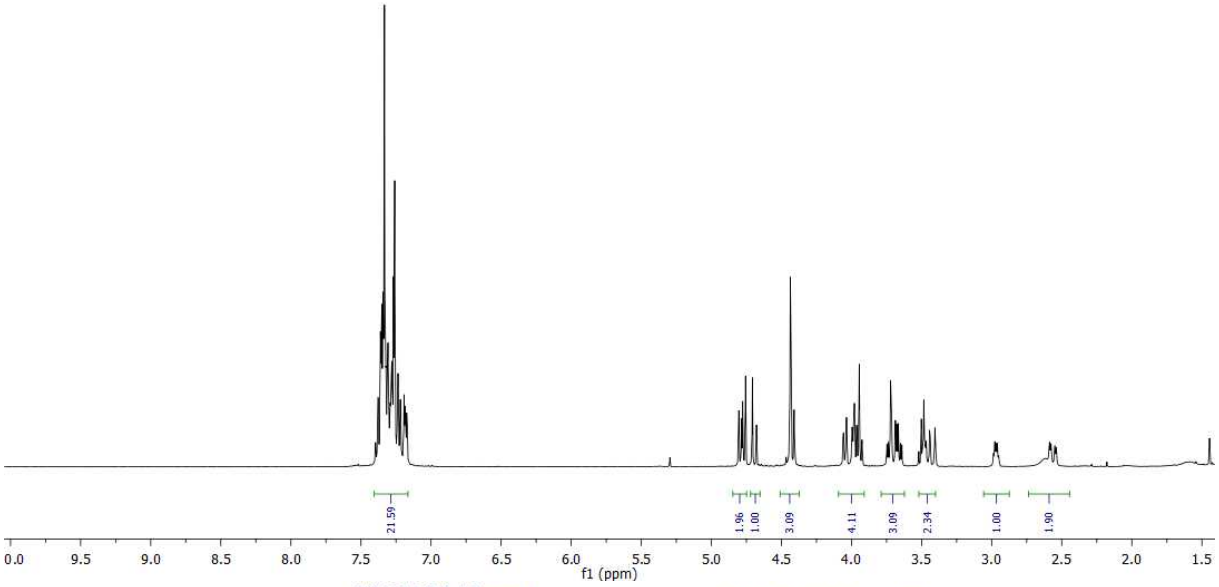

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N3
BnO
7

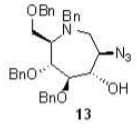
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Nucleus 13C
f1 (ppm)



13

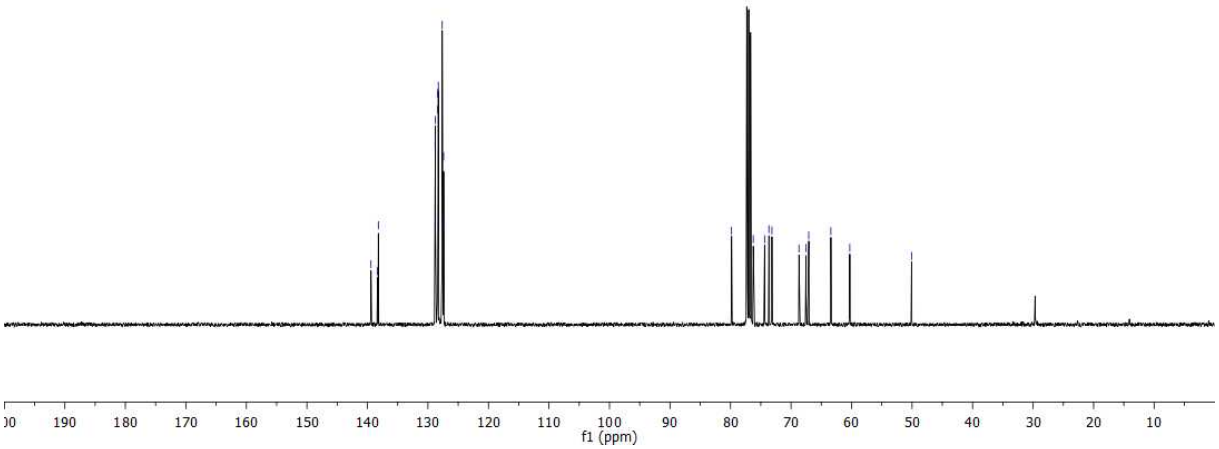
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Nucleus	1H

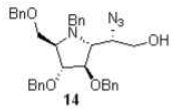




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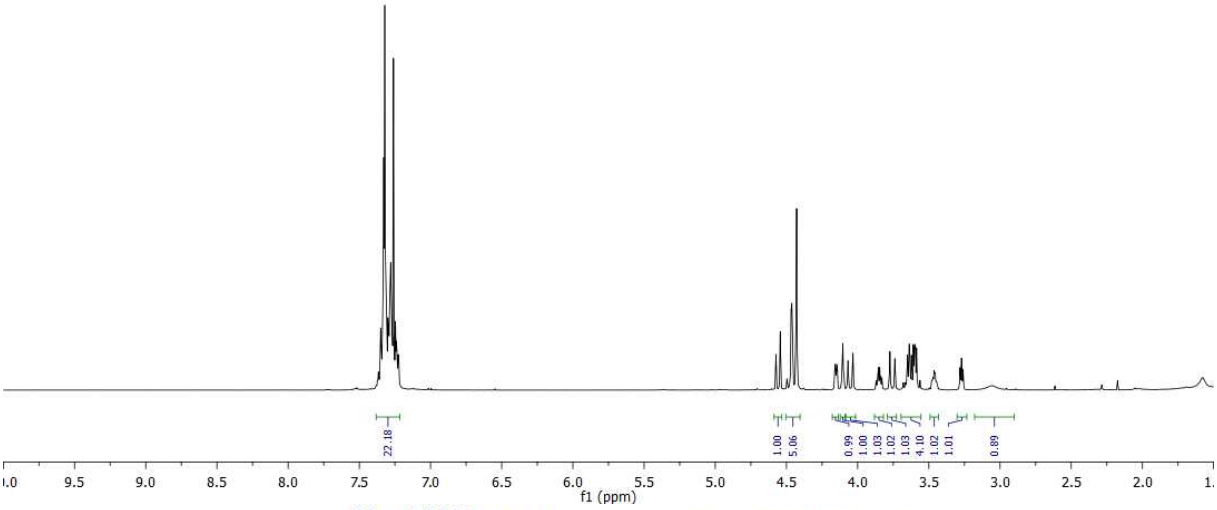
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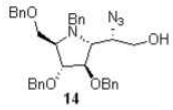




14

Parameter	Value
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Spectral Width	7211.5
Nucleus	1H

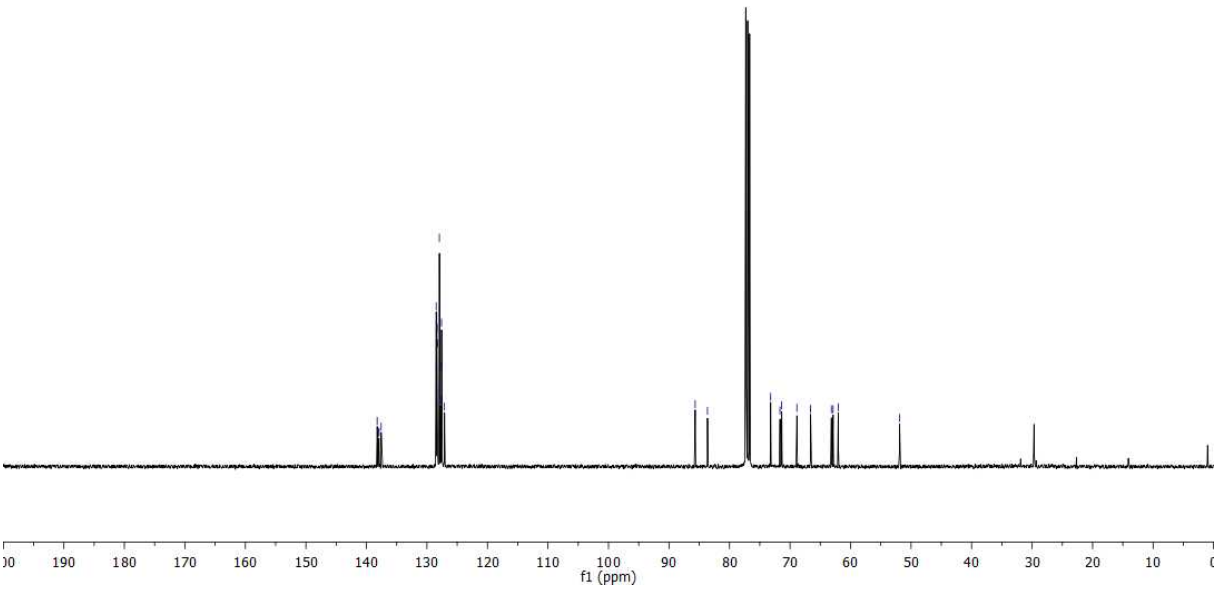




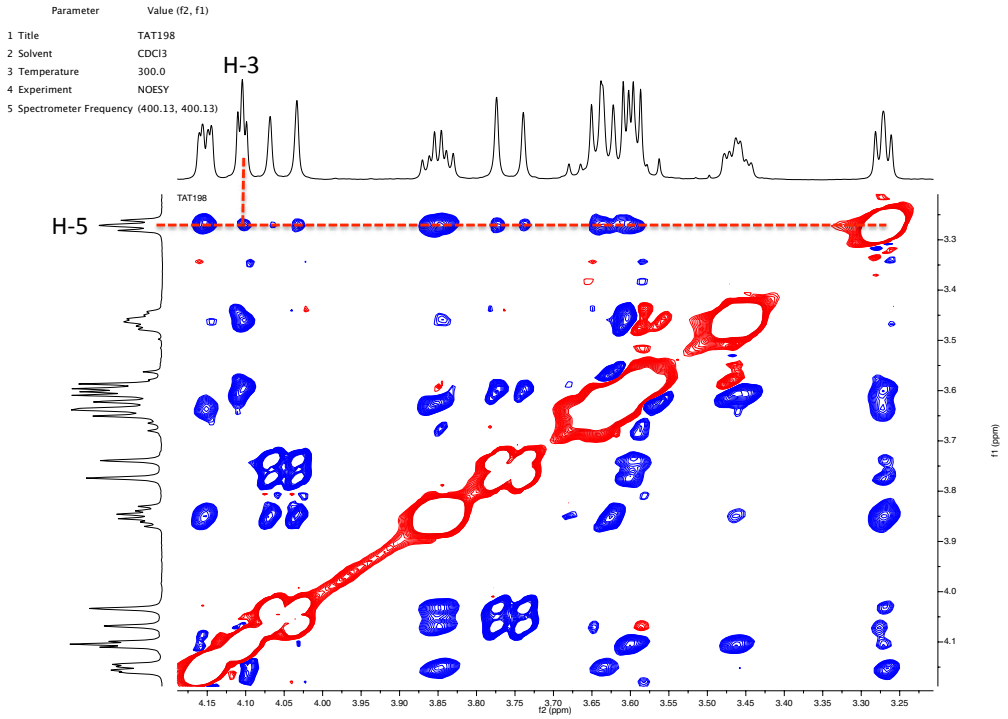
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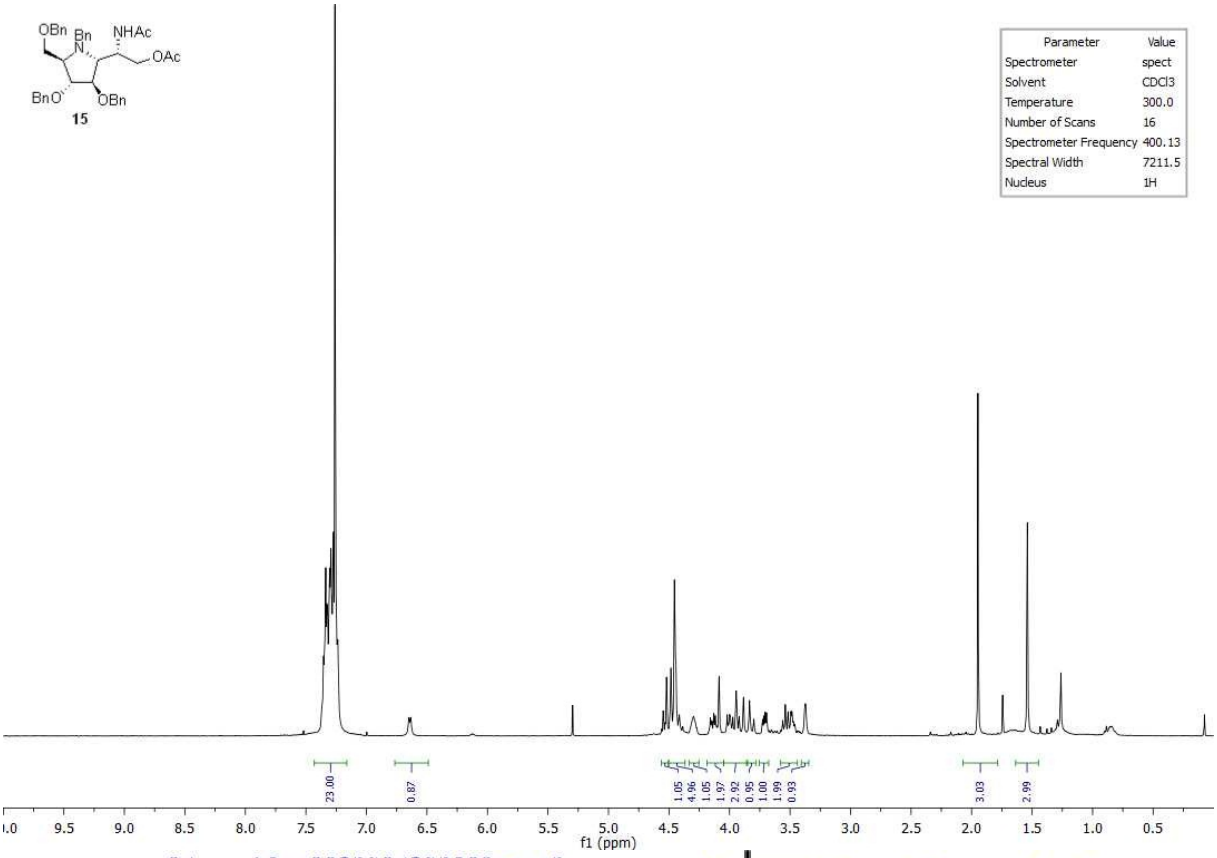
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62.04
51.90

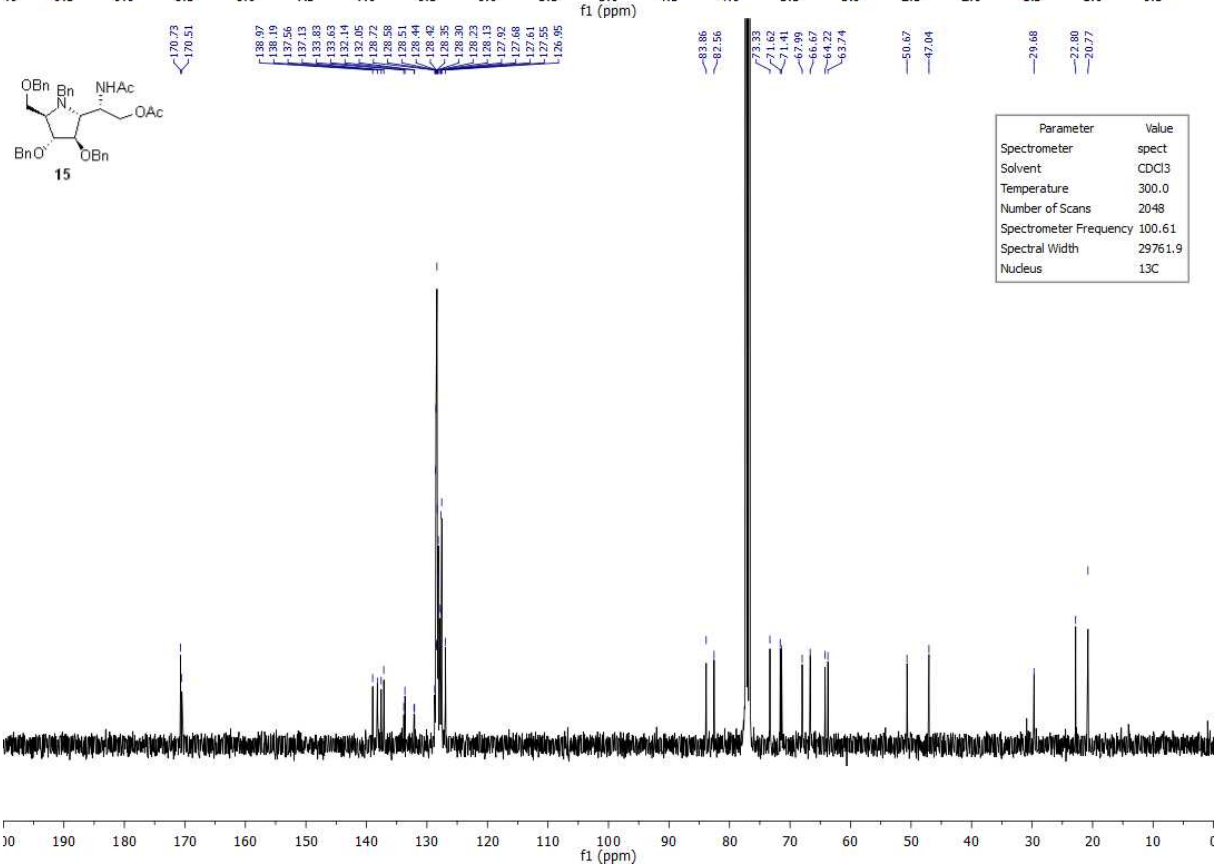
Parameter	Value
Spectrometer	spect
Solvent	CDCl3
Temperature	300.0
Number of Scans	2048
Spectrometer Frequency	100.61
Spectral Width	29761.9
Nucleus	13C

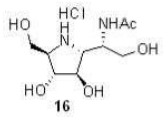


ACCEPTED MANUSCRIPT



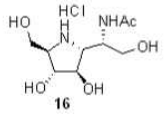

OBn
Bn
N
NHAc
OAc
BnO
OBn
15
Parameter Value
Spectrometer spect
Solvent CDCl3
Temperature 300.0
Number of Scans 16
Spectrometer Frequency 400.13
Spectral Width 7211.5
Nucleus 1H
23.00
0.87
1.05
4.96
1.05
1.97
2.92
0.95
1.00
1.99
0.93
3.03
2.99
f1 (ppm)


170.73
170.51
138.97
138.19
137.56
137.13
133.83
133.63
132.14
132.05
128.72
128.58
128.51
128.44
128.42
128.35
128.30
128.23
128.13
127.92
127.68
127.61
127.55
126.95
83.86
82.56
73.33
71.62
71.41
67.99
66.67
64.22
63.74
50.67
47.04
29.68
22.80
20.77
OBn
Bn
N
NHAc
OAc
BnO
OBn
15
Parameter Value
Spectrometer spect
Solvent CDCl3
Temperature 300.0
Number of Scans 2048
Spectrometer Frequency 100.61
Spectral Width 29761.9
Nucleus 13C
f1 (ppm)



16

Parameter	Value
Spectrometer	spect
Solvent	D2O
Temperature	300.0
Number of Scans	64
Spectrometer Frequency	400.13
Spectral Width	7211.5
Nucleus	1H



16

Parameter	Value
Spectrometer	spect
Solvent	D2O
Temperature	300.0
Number of Scans	2048
Spectrometer Frequency	100.61
Spectral Width	29761.9
Nucleus	13C

