N-Alkyl Isofagomine Lactams: Synthesis, Glycosidase Inhibition, and Antimicrobial Assessments

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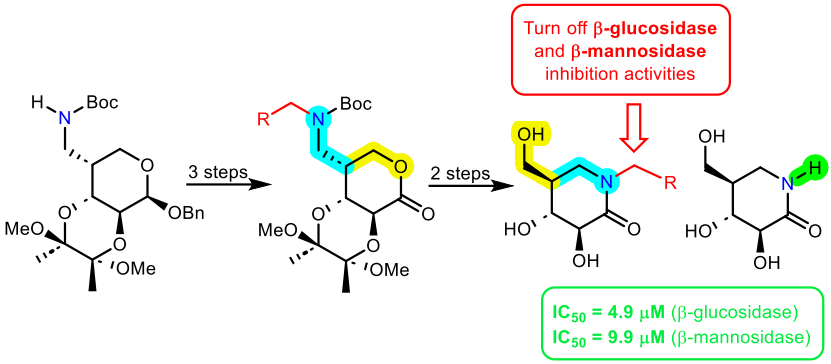

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A series of six *N*-alkyl isofagomine lactam derivatives were synthesized over ten steps from D-arabinose. The compounds were evaluated as glycosidase inhibitors. It was found that the *N*-alkyl groups have a detrimental effect on the glycosidase inhibition activities, as the *N*-alkyl isofagomine lactam derivatives are much less potent glycosidase inhibitors than native isofagomine lactam.

Key words Azasugar, Lactam, Glycosidase Inhibitor, Antimicrobial, and Synthesis

Iminosugars, exemplified by nojirimycin (**1**) (**Figure 1**), represent a group of carbohydrate mimetics where the inherent endocyclic oxygen atom of carbohydrates has been replaced by a nitrogen atom. Nojirimycin was one of the first iminosugars to be isolated from natural sources. Because this compound resembles glucose, it underwent testing as a β-glucosidase inhibitor with positive results.[1] Since then, iminosugars have been extensively investigated for their glycosidase inhibitory activities.[2] Such activity have made them attractive as lead compounds for the treatment of various diseases such as cancer,[3] diabetes,[4] lysosomal storage disorders,[5] and COVID-19.[6] Iminosugars are assumed to be potent glycosidase inhibitors, because in their *N*-protonated state, these compounds resemble the positive charge developed on the transition state of the glycosidase-mediated hydrolysis of glycosidic linkages; this allows them to interact with the carboxylate groups of the enzyme active site.[7] The biological activity of iminosugars has been extended to other targets, including for instance cholinesterase inhibition[8] as well as antimicrobial[1,9] and antiproliferative[10] activities.

Azasugars, exemplified by isofagomine (**2**) (**Figure 1**), is another group of carbohydrate mimetics closely related to iminosugars, containing a nitrogen atom instead of a carbon atom in the ring of a monosaccharide. Isofagomine was reported in 1994 as a mimic of glucose and mannose and was found to be a very potent β-glucosidase inhbitor.[11] The conjugated acid of this compound was expected to resemble the positive charge developed at the anomeric carbon in the transition state of enzymatic hydrolysis of glycosidic bonds,[11,12] in which the positively charged NH group was thought to establishe ionic interactions with the negatively charged active site carboxylate groups.[11] Even though X-ray crystallography has not confirmed the presence of any such interactions, it has unambiguously confirmed that the positively charged NH group in a cellobiose analogue of isofagomine interacts with the negatively charged carboxylate groups in the active site of cellulase Cel5a.[13] The importance of a basic NH group in the anomeric position of isofagomine was supported by the synthesis of isofagomine lactam (**3**) (**Figure 1**), which is roughly 260-fold less active β-glucosidase inhibitor than **2**.[14] A related compound to **3** is hydrazinolactam **4a** (**Figure 1**),[15] which exhibits a similar glycosidase inhibition profile as **3**. It was proposed that the NH lactam group of **4a** forms a hydrogen bond with an acceptor group in the active site of β-glucosidase and β-mannosidase. This was supported by the observation that the alkylation of the

NH group to provide compounds **4b**-**4d** essentially turned off the inhibition activity.[15]

Because the arming of isofagomine with alkyl groups can have either a detrimental[16] or positive impact[17] on glycosidase inhibitory activity compared to native isofagomine (**2**), we decided to prepare a series of six *N*-alkyl armed isofagomine lactam derivatives containing an *N*-methyl (**5a**), *N*-propyl (**5b**), *N*-butyl (**5c**), *N*-hexyl (**5d**), *N*-octyl (**5e**), or *N*-3-phenylpropyl group (**5f**) (**Figure 1**) and evaluate their performance as glycosidase inhibitors. The choice of *N*-alkyl substituents was inspired by earlier studies in which the attachment of an octyl group to isofagomine (**2**)[17] and a *N*-hexyl group or *N*-3-phenylpropyl group to 1-azafgomine[18] were found to have a positive impact on the glycosidase inhibition potency compared to their parent compounds. We also investigated the antimicrobial activity of the isofagomine lactam derivatives.

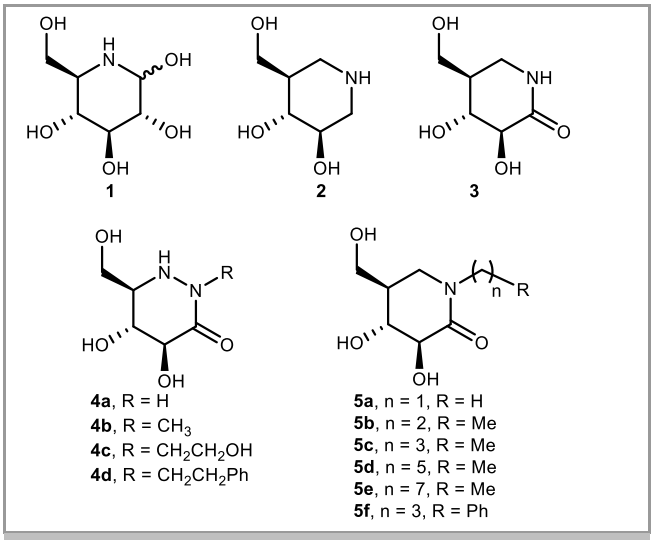
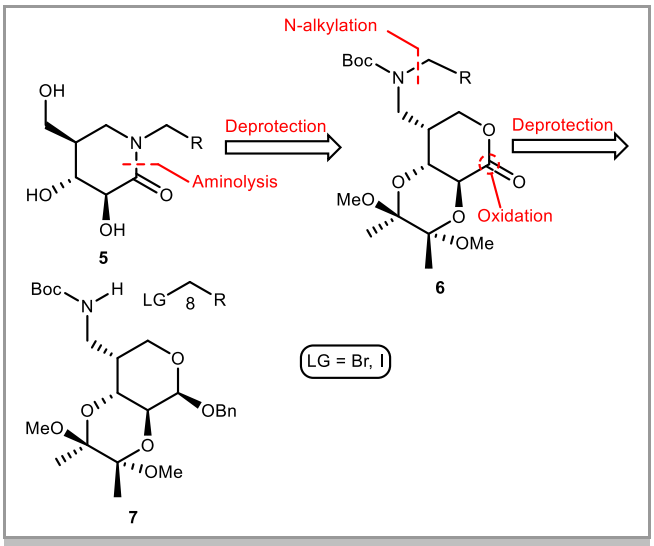


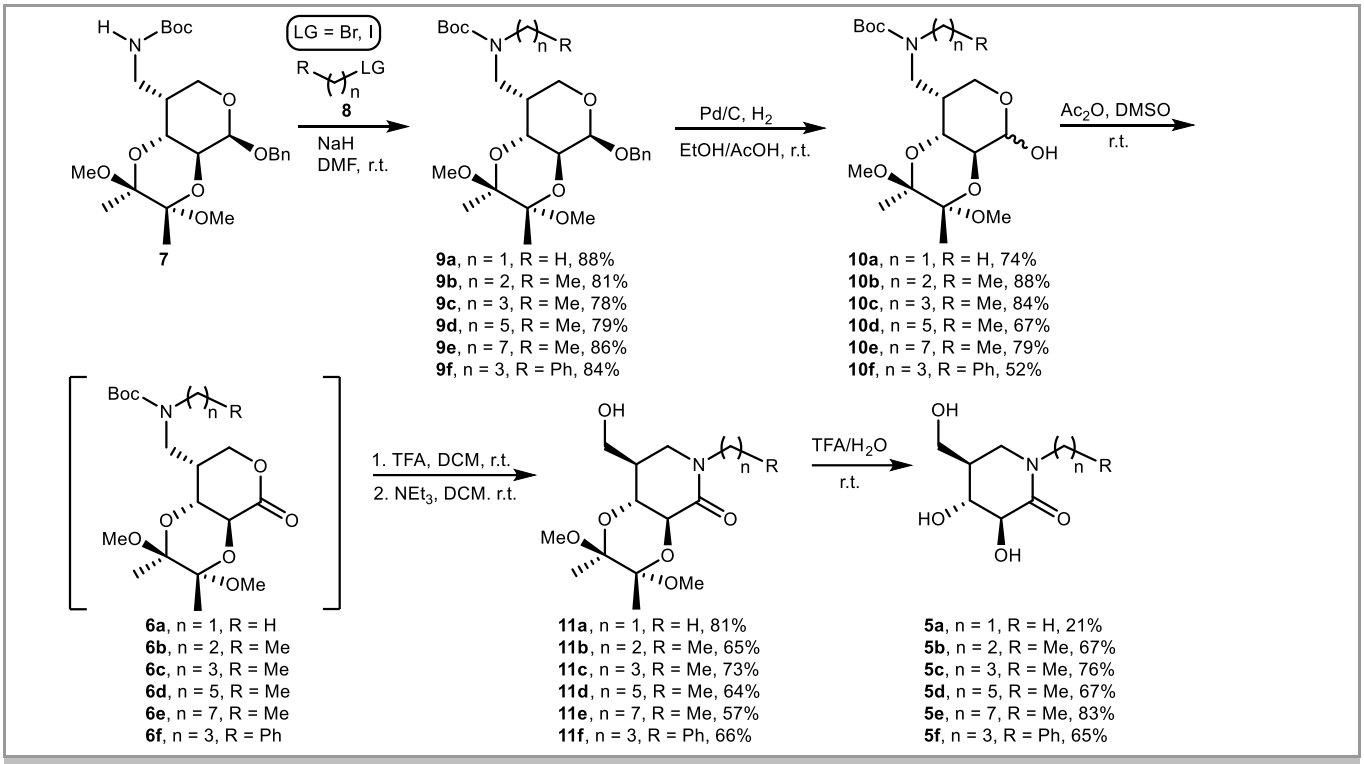
Figure 1 Compounds **1**-**4** are parts of earlier work and compounds of type **5** are related to this work.

The synthetic plan for *N*-alkylated isofagomine lactams of type **5** is presented in **Scheme 1**. We envisaged that the removal of the Boc protection group in carbamates of type **6** would trigger intramolecular aminolysis to establish the lactam moiety of **5**. The carbamates of type **6** would be achieved when reported compound **7**[19] is subjected to alkylation with a suitable alkyl halide of type **8**, followed by de-*O*-benzylation to afford a reducing sugar, which is oxidized to install the lactone group in **6**.



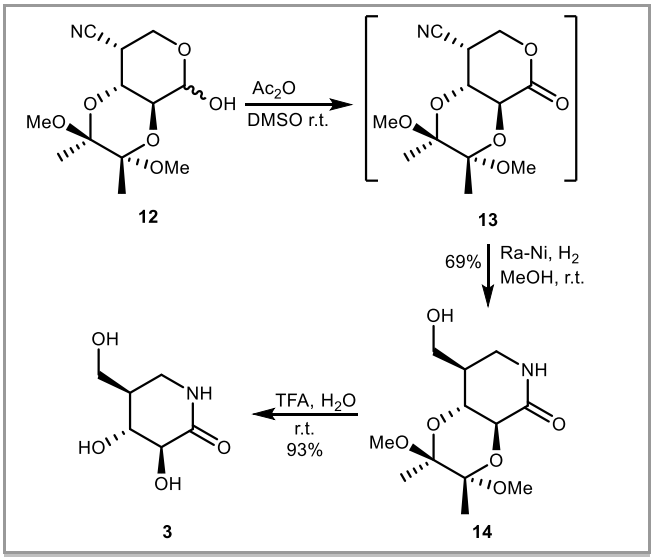
Scheme 1 Retrosynthetic analysis of lactams of type **5** (LG = leaving group).

The synthesis of *N*-alkyl lactams of type **5** is presented in **Scheme 2**. Thus, carbamate **7** underwent alkylation with alkyl halides of type **8** (LG = Br or I) to afford **9a**-**9f** in 78-88% yield. The benzyl group of glycosides **9a**-**9f** was removed by palladium-catalyzed hydrogenation to generate pyranoses **10a**-**10f** in 52-88% yield. These pyranoses were converted into lactones **6a**-**6f** through the Albright-Goldman oxidation.[20] As the lactones decomposed upon purification by silica gel flash column chromatography, after simple aqueous work-up, the crude lactones were used in the following step without further purification. The Boc-protection group of lactones **6a**-**6f** was selectively removed by treatment with TFA in dichloromethane (DCM) under anhydrous conditions. Subsequent treatment with base was required for the intramolecular aminolysis to take place, generating protected *N*-alkyl isofagomine lactams **11a**-**11f** in 57-81% yield over three steps from **10a**-**10f**. In the last step, the acetal ring was removed upon treatment with aqueous TFA to generate the target lactams **5a**-**5f** in 21-83% yield. The notable low yield of **5a** (21%) was attributed to tailing during flash column chromatography, which made it challenging to identify all collected fractions containing **5a**.



Scheme 2 Synthesis of *N*-alkyl lactams **5a**-**5f**.

The synthesis of lactam **3**[14] commenced from pyranose **12** (**Scheme 3**). The synthesis of **12** is presented in **Scheme S-1**. This compound underwent oxidation to form lactone **13**, which was used immediately in the following step where it underwent an intramolecular aminolysis after Raney nickel catalyzed hydrogenation of the nitrile group to form acetal protected lactam **14** in 69% yield. Compound **14** was liberated from the acetal ring when it was treated with an aqueous solution of TFA to form **3** in 93% yield.



Scheme 3 Synthesis of lactam **3**.

Compounds **3** and **5a**-**5f** were tested against a panel of six glycosidases including α-glucosidase (*Saccharomyces cerevisiae*), β-glucosidase (almonds), β-mannosidase (*Helix pomatia*), α-galactosidase (green coffee beans), and β-galactosidase (*Escherichia coli* and, *Aspergillus oryzae*) (**Table 1**). All *N*-alkyl isofagomine lactams **5a**-**5f** acted as poor inhibitors (IC_{50} > 50 μM) of all enzymes. Lactam **3** lacking an *N*-alkyl group exhibited a IC_{50} value of 4.9 ± 0.7 μM and 9.9 ± 2.5 μM for the inhibition of β-glucosidase and β-mannosidase, respectively. The poor binding of **5a**-**5f** to β-glucosidase and β-mannosidase may be a result of the *N*-alkyl groups being unable to establish interactions with the hydrophobic binding sites of the enzymes. However, as **5a** containing a small *N*-methyl group is a weak inhibitor of both β-glucosidase and β-mannosidase (IC_{50} > 50 μM for both enzymes) and **3** lacking a *N*-alkyl group is a micromolar inhibitor (4.9 ± 0.7 μM for β-glucosidase and and IC_{50} = 9.9 ± 2.5 μM for β-mannosidase) for the same enzymes, it is likely that the NH group of **3** is involved in hydrogen bonds with an acceptor (e.g. the catalytic nucleophile) in the active site of the enzymes. Indeed, Vasella and coworkers used the same argument to explain why **4a** but not **4b**-**4c** is a potent inhibitor of β-glucosidase and β-mannosidase.[15]

Previous work has shown that some iminosugars have antimicrobial activity.[9] Therefore, we decided to assess the antimicrobial activity of the newly synthesized compounds **5a**-**5e** against three genetically diverse bacteria, namely *E. coli*, and *P.* and *aeruginosa* (gram-negative), and *S. aureus* (gram-positive). The compounds did not inhibit the growth of these bacteria at the tested concentrations (50 and 100 μM), suggesting that these compounds do not have antimicrobial activity (see **Figures S3** and **S4**).

In the work presented herein, a series of six *N*-alkyl isofagomine lactam derivatives has been prepared. All of them appeared to

be weak inhibitors of a panel of six glycosidases. As native isofagomine lactam (**3**) is a potent inhibitor of β-glucosidase and β-mannosidase, we propose that the NH group of this compound is an important hydrogen bond donor for the binding to β-glucosidase. This assumption can be further investigated shifting the position of the alkyl groups from the 1-position to the 6-position, which is the position that belongs to the endocyclic oxygen atom in a monosaccharide.

Table 1 IC_{50} values in μM for the inhibition of a series of six enzymes.

Compound	IC_{50} (μM)					
	α-Glucosidase (*S. cerevisiae*)[a]	β-Glucosidase (almonds)[a]	β-Mannosidase (*Helix pomatia*)	α-Galactosidase (green coffee beans)[a]	β-Galactosidase (*E. coli*)[a]	β-Galactosidase (*A. oryzae*)[a]
3	> 50	4.9 ± 0.7	9.9 ± 2.5	> 50	> 50	> 50
5a	> 50	> 50	> 50	> 50	> 50	> 50
5b	> 50	> 50	> 50	> 50	> 50	> 50
5c	> 50	> 50	> 50	> 50	> 50	> 50
5d	> 50	> 50	> 50	> 50	> 50	> 50
5e	> 50	> 50	> 50	> 50	> 50	> 50
5f	> 50	> 50	> 50	> 50	> 50	> 50

[a]pH = 6.8. [b]pH = 5.6

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General information. TLC analyses were performed on Merck silica gel 60 F254 plates using UV light, $KMnO_4$, heat, or 5% of sulfuric acid in ethanol for detection. Silica gel NORMALSIL 60 40-63 μm was used as stationary phase for flash column chromatography. All chemicals were purchased from Aldrich/Merck or VWR. For petroleum ether (PE), the 40-65 °C fraction was used. Specific optical rotations ($[\alpha]_D$) were measured on an ADP440+ polarimeter. The concentration (*c*) for the measurement of $[\alpha]_D$ is in the unit of g/100 cm^3. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AscendTM 400 series, operating at 400.13 MHz for ^1H and 100.61 MHz for ^{13}C in $CDCl_3$, CD_3OD, or D_2O. Chemical shifts (δ) are reported in ppm relative to an internal standard of residual chloroform (δ = 7.26 ppm for ^1H NMR; δ = 77.16 ppm for ^{13}C NMR), residual methanol (δ = 3.31 ppm for ^1H NMR; δ = 49.00 ppm for ^{13}C NMR), or residual water (δ = 4.79 ppm for ^1H NMR).

General procedure for the preparation of 9a-9f

NaH (60% dispersion in mineral oil) (8 equiv.) was added in small portions to a 0.03 M solution of **7** in anhydrous DMF under an argon atmosphere at 0 °C. The suspension was stirred for 15 minutes. Then haloalkane (methyl iodide for **9a**, propyl iodide for **9b**, butyl iodide for **9c**, hexyl iodide for **9d**, octyl bromide for **9e**, and 1-bromo-3-phenylpropan for **9f**) (4 equiv.) was added dropwise. The mixture was kept stirring at room temperature until TLC analysis showed full conversion. The mixture was put on an ice bath and 10 mL of methanol was added. The volatiles were removed under reduced pressure. Water was added and the mixture was extracted with diethyl ether. The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude product was purified by silica gel flash column chromatography.

Benzyl 4-deoxy-4-*C*-[(*N*-*tert*-butyloxycarbonyl-*N*-methyl)]aminomethyl-2,3-*O*-((2'S,3'S)-2',3'-dimethoxybutan-2',3'-diyl)-β-D-arabinopyranoside (9a). The reaction was kept running for 3 hours. For the purification of crude product **9a** by silica gel flash column chromatography, eluent with gradient was applied (PE/EA 9:1 to 8:2). Compound **9a** was obtained as a colorless oil (88%, 0.136 g). R_f = 0.34 (PE/EA 7:3); $[\alpha]_D^{26\,°C}$ = - 25 (*c* = 0.24 in $CHCl_3$); **(NMR-signals are broadened due to rotamers)** ^1H NMR (400 MHz, $CDCl_3$, ppm): δ = 7.41-7.40 (m, 2H; 2 x ArH), 7.35-7.25 (m, 3H; 3 x ArH), 4.92-4.87 (m, 1H; H-1), 4.75 (d, *J* = 12.6 Hz, 1H; CHaPh), 4.67(d, *J* = 12.6 Hz, 1H; CHbPh), 4.28-4.24 (m, 1H; H-3), 3.82-3.74 (m, 3H, H-2, H-5a, H-4a'), 3.56-3.48 (m, 2H; H-5b, H-4b'), 3.24 (s, 3H; OCH_3), 3.20 (s, 3H; OCH_3), 2.85 (s, 3H; NCH_3), 2.07 (brs, 1H; H-4), 1.45 (s, 9H, $C(CH_3)_3$), 1.31 (s, 3H; CH_3), 1.24 (s, 3H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ = 156.3 (C=O), 137.9, 128.3 128.0, 127.5 (Ar), 100.1, 99.8 (C-2', C-3'), 96.8 (C-1), 79.4 ($C(CH_3)_3$), 69.0 (CH_2Ph), 66.4 (C-2), 64.8 (C-3), 62.1, 60.0 (C-5, rotamers), 48.0, 47.9 (OCH_3), 45.3 (C-4'), 39.2, 38.0 (C-4, rotamers), 35.6 (NCH_3), 28.5 ($C(CH_3)_3$), 18.0, 17.9 (CH_3); HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{25}H_{39}O_8NNa$: 504.2568; found: 504.2560.

Benzyl 4-deoxy-4-*C*-[(*N*-*tert*-butyloxycarbonyl-*N*-propyl)]aminomethyl-2,3-*O*-((2'S,3'S)-2',3'-dimethoxybutan-2',3'-diyl)-β-D-arabinopyranoside (9b). The reaction was kept running for 4 hours. For the purification of crude product **9b** by silica gel flash column chromatography, eluent with gradient was applied PE/EA (9:1 to 17:3). Compound **9b** was obtained as a colorless oil (81%, 0.133 g); R_f = 0.40 (PE/EA 7:3); $[\alpha]_D^{26\,°C}$ = - 31 (*c* = 0.26, $CHCl_3$); **(NMR-signals are broadened due to rotamers)** ^1H NMR (400 MHz, $CDCl_3$, ppm): δ = 7.42-7.40 (m, 2H; 2 x ArH), 7.35-7.26 (m, 3H; 3 x ArH), 4.92-4.88 (m, 1H; H-1), 4.75 (d, *J* = 12.6 Hz, 1H; CHaPh), 4.65 (d, *J* = 12.6 Hz, 1H; CHbPh), 4.28-4.24 (m, 1H; H-3), 3.92-3.19 (m, 11H; H-2, H-5a, H-5b, H-4a', H-4b', 2 x OCH_3), 3.11 (brs, 2H; NCH_2), 2.09 (brs, 1H; H-4), 1.61-1.54 (m, 2H; CH_2), 1.44 (s, 9H; $C(CH_3)_3$), 1.32 (s, 3H; CH_3), 1.25 (s, 3H; CH_3), 0.87 (t, *J* = 7.6 Hz, 3H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ = 156.2 (C=O), 137.9, 128.3, 128.0, 127.5 (Ar), 100.1, 99.7 (C-2', C-3'), 96.8 (C-1), 79.3 ($C(CH_3)_3$), 69.0 (CH_2Ph), 66.6, 66.3, 62.2 (C-2, C-3, double signal of C-2 or C-3 due to rotamers), 62.2, 60.5 (C-5, rotamers), 49.9 (NCH_2), 48.0 47.9 (2 x OCH_3), 43.0 (C-4'), 40.3, 39.6, 38.5 (C-4, rotamers), 28.5, 28.4 ($C(CH_3)_3$, rotamers), 18.1, 17.9 (2 x CH_3), 11.3 (CH_3); HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{27}H_{43}O_8NNa$: 532.2881; found: 532.2872.

Benzyl 4-deoxy-4-*C*-[(*N*-*tert*-butyloxycarbonyl-*N*-butyl)]aminomethyl-2,3-*O*-((2'S,3'S)-2',3'-dimethoxybutan-2',3'-diyl)-β-D-arabinopyranoside (9c). The reaction was kept running for 4 hours. For the purification of crude product **18c** by silica gel flash column chromatography, eluent with gradient was applied (PE/EA 19:1 to 9:1). Compound **9c** was obtained as a colorless oil (78%, 0.131 g). R_f = 0.68; (PE/EA 7:3). $[\alpha]_D^{26\,°C}$ = - 24 (*c* = 0.25, $CHCl_3$); **(NMR-signals are broadened due to rotamers)** ^1H NMR (400 MHz, $CDCl_3$, ppm) δ = 7.41-

7.40 (m, 2H; 2 x ArH), 7.32 (d, *J* = 7.3 Hz, 2H; 2 x ArH), 7.27-7.26 (m, 1H; ArH), 4.91-4.88 (m, 1H; H-1), 4.75 (d, *J* = 12.1 Hz, 1H; CHaPh), 4.65 (d, *J* = 12.1 Hz, 1H; CHbPh), 4.27-4.25 (m, 1H; H-3), 3.85-3.06 (m, 13H; H-2, NCH_2, H-5a, H-5b, H-4a', H-4b', 2 x OCH_3), 2.09 (brs, 1H; H-4), 1.50-1.42 (m, 11H; $C(CH_3)_3$, CH_2), 1.33-1.24 (m, 8H; 2 x CH_3, CH_2), 0.91 (t, *J* = 7.2 Hz, 3H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ = 156.2, 155.9 (C=O, rotamers) 137.9, 128.3 128.0 127.5 (Ar), 100.1, 99.7 (C-2', C-3'), 96.8 (C-1), 66.6, 66.3, 64.9 (C-2, C-3, double signal of C-2 or C-3 due to rotamers), 48.0, 47.9, 46.8 (NCH_2, 2 x OCH_3), 43.0 (C-4'), 40.3, 39.6, 38.5 (C-4, rotamers), 30.8, 30.3 (CH_2, rotamers), 28.5, 28.4 ($C(CH_3)_3$, rotamers), 20.1 (CH_2), 18.0, 17.9, 17.8 (2 x CH_3, rotamers), 14.0 (CH_3); (ESI): *m/z* $[M+Na]^+$ calcd for $C_{28}H_{45}O_8NNa$: 546.3037; found: 546.3027.

Benzyl 4-deoxy-4-*C*-[(*N*-*tert*-butyloxycarbonyl-*N*-hexyl)]aminomethyl-2,3-*O*-((2'S,3'S)-2',3'-dimethoxybutan-2',3'-diyl)-β-D-arabinopyranoside (9d). The reaction was kept running for 5 hours. For the purification of crude product **9d** by silica flash column chromatography, eluent with gradient was applied (PE/EA 49:1 to 9:1). Compound **9d** was obtained as a colorless oil (79%, 0.139 g). R_f = 0.55 (PE/EA 7:3); $[\alpha]_D^{26\,°C}$ = - 5 (*c* = 0.41, $CHCl_3$); **(NMR-signals are broadened due to rotamers)** ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ = 7.41-7.40 (m, 2H; 2 x ArH), 7.33 (d, *J* = 7.3 Hz, 2H; 2 x ArH), 7.28-7.25 (m, 1H; ArH), 4.91-4.87 (m, 1H; H-1), 4.75 (d, *J* = 13.0 Hz, 1H; CHaPh), 4.65 (d, *J* = 13.0 Hz, 1H; CHbPh), 4.27-4.23 (m, 1H; H-3), 3.81-3.13 (m, 13H; H-2, H-5a, H-5b, H-4a', H-4b', 2 x OCH_3), 2.09 (brs, 1H; H-4), 1.51-1.49 (m, 2H; CH_2), 1.44 (s, 9H; $C(CH_3)_3$), 1.34-1.25 (m, 12H; 2 x CH_3, 3 x CH_2), 0.88 (brs, 3H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ = 156.2, 155.9 (C=O, rotamers), 137.9, 128.3, 128.0, 127.5 (Ar), 100.1, 99.7 (C-2', C-3'), 96.8 (C-1), 79.2 ($C(CH_3)_3$), 69.0 (CH_2Ph), 66.6, 66.3, 64.9 (C-2, C-3, double signal of C-2 or C-3 due to rotamers), 62.2, 60.0 (C-5, rotamers), 48.3, 47.9 (2 x OCH_3, NCH_2), 43.0 (C-4'), 40.3, 39.6, 38.5 (C-4, rotamers), 31.7 (CH_2), 28.6 ($C(CH_3)_3$), 28.1, 26.6, 22.7 (CH_2), 18.1, 18.0, 17.9 (2 x CH_3, rotamers), 14.1 (CH_3); (ESI): *m/z* $[M+Na]^+$ calcd for $C_{30}H_{49}O_8NNa$: 574.3341; found: 574.3350.

Benzyl 4-deoxy-4-*C*-[(*N*-*tert*-butyloxycarbonyl-*N*-octyl)]aminomethyl-2,3-*O*-((2'S,3'S)-2',3'-dimethoxybutan-2',3'-diyl)-β-D-arabinopyranoside (9e). The reaction was kept running for 3.5 hours. For the purification of the crude product **9e** by silica flash column chromatography, eluent with gradient was applied PE/EA 49:1 to 9:1. Compound **9e** was obtained as a colorless oil (86%, 0.160 g). R_f = 0.61 (PE/EA 7:3); $[\alpha]_D^{26\,°C}$ = - 10 (*c* = 0.41, $CHCl_3$; **(NMR-signals are broadened due to rotamers)** ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ = 7.38-7.33 (m, 2H; 2 x ArH), 7.26 (d, *J* = 7.3 Hz, 2H; 2 x ArH), 7.21-7.19 (m, 1H; ArH), 4.85-4.81 (m, 1H; H-1), 4.68 (d, *J* = 12.5 Hz, 1H; CHaPh), 4.58 (d, *J* = 12.5 Hz, 1H; CHbPh), 4.20-4.17 (m, 1H; H-3), 3.77-3.05 (m, 13H; 2 x OCH_3, H-2, H-5a, H-5b, H-4a', H-4b', CH_2N), 2.02 (brs, 1H; H-4), 1.44-1.42 (m, 2H; CH2), 1.38 (m, 9H; $C(CH_3)_3$), 1.26-1.18 (m, 16H; 5 x CH_2, 2 x CH_3), 0.81 (t, *J* = 6.0 Hz, 3H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ = 156.2, 155.9 (C=O, rotamers), 137.9, 128.3, 128.0 127.5 (Ar), 100.1, 99.7 (C-2', C-3'), 79.2 ($C(CH_3)_3$), 69.0 (CH_2Ph), 66.6, 66.3 64.9 (C-2, C-3, double signal of C-2 or C-3 due to rotamers), 62.3, 60.1 (C-5, rotamers), 48.3, 48.1, 48.0, 47.9 (2 x OCH_3, NCH_2, rotamers), 43.0 (C-4'), 40.3, 39.6, 38.5 (C-4, rotamers), 31.9 (CH_2), 29.4 (2 x CH_2), 28.6 ($C(CH_3)_3$), 28.2, 27.0, 22.8 (CH_2), 18.1, 18.0, 17.9 (2 x CH_3, rotamers), 14.2 (CH_3); (ESI): *m/z* $[M+Na]^+$ calcd for $C_{32}H_{53}O_8NNa$: 602.3663; found: 602.3653.

Benzyl 4-deoxy-4-*C*-[(*N*-*tert*-butyloxycarbonyl-*N*-(3-phenyl)propyl)]aminomethyl-2,3-*O*-((2'S,3'S)-2',3'-dimethoxybutan-2',3'-diyl)-β-D-arabinopyranoside (9f). The reaction was kept running for 5.5 hours. For the purification of crude product **9f** by silica flash column chromatography, eluent with gradient was applied (PE/EA 19:1 to 9:1). Compound **9f** was obtained as a colorless oil (84%, 0.153 g). R_f = 0.69 (PE/EA 7:3); $[\alpha]_D^{27\,°C}$ = - 14 (*c* 0.43, $CHCl_3$); **(NMR-signals are broadened due to rotamers)** ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ = 7.41 (d, *J* = 7.3 Hz, 2H; 2 x ArH), 7.34 (t, *J* = 7.4 Hz, 2H; 2 x ArH), 7.29-7.26 (m, 3H; 3 x ArH), 7.20-7.18 (m, 3H; 3 x ArH), 4.91-4.88 (m, 1H, H-1), 4.75 (d, *J* = 12.6 Hz, 1H; CHaPh), 4.66 (d, *J* = 12.6 Hz, 1H; CHbPh), 4.25 (dd, *J* = 10.1 Hz, *J* = 5.0 Hz, 1H; H-3), 3.82-3.21 (m, 13H; H-2. H-5a, H-5b, H-4a', H-4b', NCH_2, 2 x OCH_3), 2.60 (t, *J* = 8.6 Hz, 2H; CH_2), 2.08 (brs, 1H; H-4), 1.94-1.82 (m, 2H; CH_2), 1.44 (brs, 9H; $C(CH_3)_3$), 1.32 (s, 3H; CH_3), 1.25 (s, 3H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ = 156.0 (C=O), 141.9 (Ar), 138.0 (Ar), 128.4 (2 x Ar), 128.3 (Ar), 128.0 (Ar), 127.5 (Ar), 125.9 (Ar), 100.1, 99.7 (C-2', C-3'), 96.7 (C-1), 79.4 ($C(CH_3)_3$), 69.0 (CH_2Ph), 66.6, 66.3, 64.9 (C-2, C-3, double signal of C-2 or C-3 due to rotamers), 62.2, 60.0 (C-5, rotamers), 47.9, 46.9 (2 x OCH_3, NCH_2), 43.1 (C-4'), 40.4, 39.6, 38.5 (C-4, rotamers), 30.1. 29.8, 28.5 (CH_2, $C(CH_3)_3$, double signal of CH_2 or $C(CH_3)_3$ due to rotamers), 18.0, 17.9 (2 x CH_3); HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{33}H_{47}O_8NNa$: 608.3194; found: 608.3187.

General procedure for the preparation of compounds 10a-10f

A stirred solution of **9a-f** (0.025 M) in EtOH/AcOH (10:1, v/v) under an argon atmosphere was added Pd/C (10 wt%, 2 x mass of **9a-f**). Thereafter, the mixture was degassed, and hydrogen atmosphere was introduced (1 atm). The mixture was kept stirring at room temperature for 24 hours. The mixture was then filtered through celite, and the filtrate was concentrated under reduced pressure. The crude product was purified by silica gel flash column chromatography.

4-Deoxy-4-*C*-[(*N*-*tert*-butyloxycarbonyl-*N*-methyl)]aminomethyl-2,3-*O*-((2'S,3'S)-2',3'-dimethoxybutan-2',3'-diyl)-β-D-arabinopyranose (10a). For the purification of crude product **10a** by silica gel flash column chromatography, eluent with gradient was applied (DCM/MeOH 99:1 to 97:3). Compound **10a** was obtained as a colorless viscous oil (74%, 81 mg) as 54:46 mixture of anomers. R_f = 0.27 (DCM/MeOH 47:3); $[\alpha]_D^{26\,°C}$ = + 71 (*c* = 0.14, $CHCl_3$); **(NMR-signals are broadened due to rotamers)** ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ = 5.21 (brs, 0.46H; H-1 minor), 4.63 (d, *J* = 7.2 Hz, 0.54H; H-1 major), 4.21 (brs, 0.46H; H-3 minor), 4.06 (d, *J* = 12.4 Hz, 0.46H; H-5a minor), 3.92-3.43 (m, 5.08H; H-2 major, H-2 minor, H-3 major, H-4a' major, H-4a' minor, H-4b' major, H-4b' minor, H-5a major, H-5b major, H-5b minor), 3.29-3.23 (m, 6H; 2 x OCH_3 major, 2 x OCH_3 minor), 2.89 (brs, 3H; NCH_3 major, NCH_3 minor), 2.04 (brs, 1H; H-4 major, H-4 minor), 1.44 (brs, 9H; $C(CH_3)_3$ major, $C(CH_3)_3$ minor), 1.28-1.23 (m, 6H; 2 x CH_3 major, 2 x CH_3 minor); ^{13}C NMR (100 MHz, $CDCl_3$. ppm) δ = 156.2, 156.1 (C=O major, C=O minor), 100.3, 99.9 99.8 (C-2' major, C-2' minor, C-3'major, C-3' minor), 96.1 (C-1 major) 92.0 (C-1 minor), 79.6 ($C(CH_3)_3$ major, $C(CH_3)_3$ minor), 69.5 (C-2 major), 68.7 (C-2 minor), 66.5 (C-3 major), 64.7 (C-5 major), 64.4 (C-3 minor), 60.0 (C-5 minor), 48.0-47.9 (2 x OCH_3 major, 2 x OCH_3 minor), 45.9, 45.6, 45.0 (C-4' major, C-4' minor, double signal of C-4' major or C-4' minor due to rotamers) 39.3, 39.1, 38.0 (C-4 major, C-4 minor, double signal of C-4 major or C-4 minor due to rotamers), 35.8, 35.4 (NCH_3 major, NCH_3 minor), 28.6, 28.5 ($C(CH_3)_3$ major, $C(CH_3)_3$ minor), 17.9, 17.8, 17.6 (2 x CH_3 major, 2 x CH_3 minor); HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{18}H_{33}O_8NNa$: 414.2098; found: 414.2098.

4-Deoxy-4-*C*-[(*N*-*tert*-butyloxycarbonyl-*N*-propyl)]aminomethyl-2,3-*O*-((2'S,3'S)-2',3'-dimethoxybutan-2',3'-diyl)-β-D-arabinopyranose (10b). For the purification of crude product **10b** by silica flash column chromatography, eluent with gradient was applied DCM/MeOH 99:1 to 49:1. Compound **10b** was obtained as a colorless viscous oil (88%, 107 mg) as 60:40 mixture of isomers. R_f = 0.47 (DCM/MeOH 9:1); $[\alpha]_D^{27\,°C}$ = + 71 (*c* = 0.14, $CHCl_3$); **(NMR-signals are broadened due to rotamers)** ^{1}H NMR (400 MHz, $CDCl_3$. ppm): δ = 5.19 (brs, 0.4H; H-1 minor), 4.59 (brs, 0.6H; H-1 major), 4.19-4.18 (m, 1H; OH major, H-3 minor), 4.02 (d, *J* = 11.7 Hz, 0.4H; H-5a minor), 3.91-3.00 (m, 13.2H; H-2 major, H-2 minor, H-3 major, H-4a' major, H-4a' minor, H-4b' major, H-4b' minor, H-5a major, H-5b major, H-5b minor, NCH_2 major, NCH_2 minor, 2 x OCH_3 major, 2 x OCH_3 minor), 2.36 (brs, 0.4H; OH minor), 2.18-2.04 (m, 1H; H-4 major, H-4 minor), 1.61-1.50 (m, 2H; CH_2 major, CH_2 minor), 1.41 (brs, 9H; $C(CH_3)_3$ major, $C(CH_3)_3$ minor), 1.28-1.21 (m, 6H; 2 x CH_3 major, 2 x CH_3 minor), 0.85-0.81 (m, 3H; CH_3 major CH_3 minor); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ = 155.9 (C=O major, C=O minor),100.2, 100.1, 99.8, 99.7 (C-2' major, C-2' minor, C-3'major, C-3' minor), 96.1, 95.5 (C-1 major, double signal due to rotamers), 91.9 (C-1 minor), 79.4 ($C(CH_3)_3$ major, $C(CH_3)_3$ minor), 72.5, 70.1, 69.4, 68.8 (C-2 major, C-2 minor, double signals of C-2 major and C-2 minor due to rotamers), 66.4, 66.2, 64.6, 64.4, 62.1, 60.0 (C-3 major, C-3 minor, C-5 major, C-5 minor, six signals due to rotamers), 50.4, 50.1, 50.0 (NCH_2 major, NCH_2 minor, double signal of NCH_2 major or NCH_2 minor due to rotamers), 48.0 (2 x OCH_3 major, 2 x OCH_3 minor), 44.0, 43.3, 43.0, 42.7 (C-4' major, C-4' minor, double signals of C-4' major and C-4' minor due to rotamers), 39.6, 38.3, 38.0 (C-4 major, C-4 minor, double signal of C-4 major or C-4 minor due to

rotamers), 28.5 (C(CH_3)$_3$ major, C(CH_3)$_3$ minor), 21.8, 21.3 (CH_2 major, CH_2 minor), 17.9-17.7 (2 x CH_3 major, 2 x CH_3 minor), 11.3 (CH_3 major, CH_3 minor). HRMS (ESI): m/z [M+Na]$^+$ calcd for $C_{20}H_{37}O_8NNa$: 442.2411; found: 442.2404.

4-Deoxy-4-*C*-[(*N*-*tert*-butyloxycarbonyl-*N*-butyl)]aminomethyl-2,3-*O*-((2'S,3'S)-2',3'-dimethoxybutan-2',3'-diyl)-β-D-arabinopyranose (10c). For the purification of crude product **10c** by silica flash column chromatography, eluent with gradient was applied (DCM/MeOH 99:1 to 49:1). Compound **10c** was obtained as a colorless viscous oil (84%, 91 mg) as 56:44 mixture of isomers. R_f = 0.52 (DCM/MeOH 9:1); $[\alpha]_D^{27°C}$ = + 93 (c = 0.15, $CHCl_3$); **(NMR-signals are broadened due to rotamers)** ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ = 5.20 (brs, 0.44H; H-1 minor), 4.60 (brs, 0.56H; H-1 major), 4.19 (brs, 0.44H; H-3 minor), 4.03 (d, J = 11.4 Hz, 0.44H: H-5a minor), 3.94-3.04 (m, 13.68H; H-2 major, H-2 minor, H-3 major, H-5a major, H-5b major, H-5b minor, H-4a' major, H-4a' minor, H-4b' major, H-4b' minor, NCH_2 major, NCH_2 minor, OH major, 2 x OCH_3 major, 2 x OCH_3 minor), 2.25-2.05 (m, 1.44H; H-4 major. H-4 minor, OH minor), 1.48-1.42 (m, 11H; C(CH_3)$_3$ major, C(CH_3)$_3$ minor, CH_2 major, CH_2 minor), 1.29-1.22 (m, 8H; 2 x CH_3 major, 2 x CH_3 minor, CH_2 major, CH_2 minor), 0.91-0.87 (m, 3H; CH_3 major, CH_3 minor); ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ = 155.9 (C=O major, C=O minor),100.2, 99.9, 99.8, 99.7 (C-2' major, C-2' minor, C-3'major, C-3' minor), 96.1, 95.5 (C-1 major, double signal due to rotamers), 92.0 (C-1 minor), 79.3 (C(CH_3)$_3$ major, C(CH_3)$_3$ minor), 72.6, 70.1, 69.5, 68.8 (C-2 major, C-2 minor, double signals of C-2 major and C-2 minor due to rotamers), 66.4, 64.8, 64.5, 60.1 (C-3 major, C-3 minor, C-5 major, C-5 minor), 48.3, 48.0, 47.9, 46.9, 46.8 (2 x OCH_3 major, 2 x OCH_3 minor, NCH_2 major, NCH_2 minor), 44.1, 43.3, 43.0, 42.8 (C-4' major, C-4' minor, double signals of C-4' major and C-4' minor due to rotamers), 39.7, 38.4 38.1 (C-4 major, C-4 minor, double signal of C-4 major or C-4 minor due to rotamers), 30.7, 30.3 (CH_2 major, CH_2 minor), 28.5 (C(CH_3)$_3$ major, C(CH_3)$_3$ minor), 20.1 (CH_2 major, CH_2 minor), 17.9-17.7 (2 x CH_3 major, 2 x CH_3 minor), 14.0 (CH_3 major, CH_3 minor); HRMS (ESI): m/z [M+Na]$^+$ calcd for $C_{21}H_{39}O_8NNa$: 456.2568; found: 456.2561.

4-Deoxy-4-*C*-[(*N*-*tert*-butyloxycarbonyl-*N*-hexyl)]aminomethyl-2,3-*O*-((2'S,3'S)-2',3'-dimethoxybutan-2',3'-diyl)-β-D-arabinopyranose (10d). For the purification of crude product **10d** by silica flash column chromatography, eluent with gradient was applied (DCM/MeOH 99.5:0.5 to 49:1). Compound **10d** was obtained as a colorless viscous oil (67%, 75 mg) as 56/44 mixture of anomers. R_f = 0.53 (DCM/MeOH 9:1); $[\alpha]_D^{27°C}$ = + 67 (c = 0.15, $CHCl_3$); **(NMR-signals are broadened due to rotamers)** ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ = 5.20 (brs, 0.44H; H-1 minor), 4.61 (brs, 0.56H; H-1 major), 4.20 (brs, 0.44H; H-3 minor), 4.03 (d, J = 11.0 Hz, 0.44H: H-5a minor), 3.93-3.04 (m, 13.68H; H-2 major, H-2 minor, H-3 major, H-5a major, H-5b major, H-5b minor, H-4a' major, H-4a' minor, H-4b' major, H-4b' minor, NCH_2 major, NCH_2 minor, OH major, 2 x OCH_3 major, 2 x OCH_3 minor), 2.19-2.05 (m, 1.44H; H-4 major. H-4 minor, OH minor), 1.49-1.42 (m, 11H; C(CH_3)$_3$ major, C(CH_3)$_3$ minor, CH_2 major, CH_2 minor), 1.28-1.22 (m, 12H; 2 x CH_3 major, 2 x CH_3 minor, 3 x CH_2 major, 3 x CH_2 minor), 0.86 (m, 3H; CH_3 major, CH_3 minor); ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ = 155.9 (C=O major, C=O minor),100.2, 99.9, 99.8, 99.7 (C-2' major, C-2' minor, C-3'major, C-3' minor), 96.1, 95.5 (C-1 major, double signal due to rotamers), 92.0 (C-1 minor), 79.3 (C(CH_3)$_3$ major, C(CH_3)$_3$ minor), 72.6, 70.1, 69.5, 68.8 (C-2 major, C-2 minor, double signals of C-2 major and C-2 minor due to rotamers), 66.4 (C-3 major), 64.8 (C-5 major), 64.5 (C-3 minor), 60.1 (C-5 minor), 48.8, 48.5, 48.1, 48.0 47.1 (2 x OCH_3 major, 2 x OCH_3 minor, NCH_2 major, NCH_2 minor), 44.0, 43.3, 43.0, 42.7 (C-4' major, C-4' minor, doubles signals of C-4' major and C-4' minor due to rotamers), 39.7, 38.4, 38.1 (C-4 major, C-4 minor, double signal of C-4 major or C-4 minor due to rotamers), 31.7 (CH_2 major, CH_2 minor), 28.5 (C(CH_3)$_3$ major, C(CH_3)$_3$ minor), 28.1 (CH_2 major, CH_2 minor), 26.5 (CH_2 major, CH_2 minor), 22.7 (CH_2 major, CH_2 minor), 17.9-17.7 (2 x CH_3 major, 2 x CH_3 minor), 14.1 (CH_3 major, CH_3 minor); HRMS (ESI): m/z [M+Na]$^+$ calcd for $C_{23}H_{43}O_8NNa$: 484.2881; found: 484.2872.

4-Deoxy-4-*C*-[(*N*-*tert*-butyloxycarbonyl-*N*-octyl)]aminomethyl-2,3-*O*-((2'S,3'S)-2',3'-dimethoxybutan-2',3'-diyl)-β-D-arabinopyranose (10e). For the purification of crude product **10e** by silica flash column chromatography, eluent with gradient was applied DCM/MeOH (99.5:0.5 to 49:1). Compound **10e** was obtained as a colorless viscous oil (79%, 104 mg) as 56/44 mixture of anomers. R_f = 0.55 (DCM/MeOH 9:1); $[\alpha]_D^{27°C}$ = +67 (c = 0.18, $CHCl_3$); **(NMR-signals are broadened due to rotamers)** ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ = 5.19 (brs, 0.44H; H-1 minor), 4.59 (brs, 0.56H; H-1 major), 4.18 (brs, 0.44H; H-3 minor), 3.93-3.04 (m, 14.12H; H-2 major, H-2 minor, H-3 major, H-5a major, H-5a minor, H-5b major, H-5b minor, H-4a' major, H-4a' minor, H-4b' major, H-4b' minor, NCH_2 major, NCH_2 minor, OH major, 2 x OCH_3 major, 2 x OCH_3 minor), 2.37-2.04 (m, 1.44H; H-4 major. H-4 minor, OH minor), 1.48-1.21 (m, 27H; C(CH_3)$_3$ major, C(CH_3)$_3$ minor, 2 x CH_3 major, 2 x CH_3 minor, 6 x CH_2 major, 2 x CH_2 minor), 0.85-0.82 (m, 3H; CH_3 major, CH_3 minor); ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ =155.8 (C=O major, C=O minor), 100.2, 100.1, 99.8, 99.7 (C-2' major, C-2' minor, C-3'major, C-3' minor), 96.1, 95.5 (C-1 major, double signal due to rotamers), 92.0 (C-1 minor), 79.3 (C(CH_3)$_3$ major, C(CH_3)$_3$ minor), 72.5, 70.1, 69.4, 68.8 (C-2 major, C-2 minor, double signals of C-2 major and C-2 minor due to rotamers), 66.5, 64.7, 64.5. 60.0 (C-3 major, C-3 minor, C-5 major, C-5 minor), 48.8, 48.5, 48.2, 48.1, 48.0, 47.1 (2 x OCH_3 major, 2 x OCH_3 minor, NCH_2 major, NCH_2 minor), 44.5, 44.0, 43.3, 43.0, 42.7 (C-4' major, C-4' minor, multiple signals of C-4' major and C-4' minor due to rotamers), 39.7, 38.4, 38.1(C-4 major, C-4 minor, double signal of C-4 major or C-4 minor due to rotamers), 31.8, 29.4, 28.5 28.2, 26.9, 22.7 (C(CH_3)$_3$ major, C(CH_3)$_3$ minor, CH_2 major, CH_2 minor), 17.8-17.7 (2 x CH_3 major, 2 x CH_3 minor), 14.2 (CH_3 major, CH_3 minor); HRMS (ESI): m/z [M+Na]$^+$ calcd for $C_{25}H_{47}O_8NNa$: 512.3194; found: 512.3184.

4-Deoxy-4-*C*-[(*N*-*tert*-butyloxycarbonyl-*N*-(3-phenyl)propyl)]aminomethyl-2,3-*O*-((2'S,3'S)-2',3'-dimethoxybutan-2',3'-diyl)-β-D-arabinopyranose (10f). For the purification of the crude product **10f** by silica flash column chromatography, eluent gradient was applied (DCM/MeOH 99.5:0.5 to 99:1). Compound **10f** was obtained as a white foam (52%, 65 mg) 54:46 mixture of isomers. R_f = 0.49 (DCM/MeOH 9:1); $[\alpha]_D^{27°C}$ = + 62 (c = 0.13, $CHCl_3$); **(NMR-signals are broadened due to rotamers)** ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ = 7.22-7.11 (m, 5H; ArH), 5.15 (brs, 0.46H; H-1 minor), 4.55 (brs, 0.54H; H-1 major), 4.14 (brs, 0.46H; H-3 minor), 3.98 (d, J = 11.1 Hz, 0.46H; H-5a minor), 3.87-3.15 (m, 13.62H; H-2 major, H-2 minor, H-3 major, H-5a major, H-5b major, H-5b minor, H-4a' major, H-4a' minor, H-4b' major, H-4b' minor, NCH_2 major, NCH_2 minor, OH major, 2 x OCH_3 major, 2 x OCH_3 minor), 2.55-2.51 (m, 2H; CH_2 major, CH_2 minor), 2.13-1.99 (m, 1.46H; H-4 major. H-4 minor, OH minor), 1.81-1.78 (m, 2H; CH_2 major, CH_2 minor), 1.37 (brs, 9H; C(CH_3)$_3$ major, C(CH_3)$_3$ minor), 1.25-1.18 (m, 6H; CH_3 major, CH_3 minor); ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ =155.9 (C=O major, C=O minor), 142.0, 128.5, 128,4, 125.9 (Ar), 100.3, 99.9, 99.8 (C-2' major, C-2' minor, C-3'major, C-3' minor), 79.5 (C(CH_3)$_3$ major, C(CH_3)$_3$ minor), 72.6, 70.1, 69.5, 68.8 (C-2 major, C-2 minor, double signals of C-2 major and C-2 minor due to rotamers), 66.4 (C-3 major), 64.7 (C-5 major), 64.5 (C-3 minor), 60.1 (C-5 minor), 48.3-46.9 (2 x OCH_3 major, 2 x OCH_3 minor, NCH_2 major, NCH_2 minor), 44.3, 43.4, 42.8 (C-4' major, C-4' minor, double signal of C-4' major or C-4' minor due to rotamers), 39.5, 38.4, 38.1 (C-4' major, C-4' minor, double signal of C-4' major or C-4' minor due to rotamers), 33.3 (CH_2 major, CH_2 minor), 30.2, 29.8 (CH_2 major, CH_2 minor) 28.5 (C(CH_3)$_3$ major, C(CH_3)$_3$ minor, 17.9-17.7 (2 x CH_3 major, 2 x CH_3 minor); HRMS (ESI): m/z [M+Na]$^+$ calcd for $C_{26}H_{41}O_8NNa$: 442.2411; found: 442.2404. HRMS (ESI): m/z [M+Na]$^+$ calcd for $C_{20}H_{37}O_8NNa$: 518.2724; found: 518.2716.

General procedure for the preparation of compounds 11a-11f

Step 1: A 0.06 M solution of **10a-10f** in DMSO was diluted to 0.04 M upon addition of acetic anhydride. The mixture was kept stirring at room temperature overnight. The mixture was then diluted with diethyl ether. The organic layer was washed two times with water, tree times with 10 mass% of an aqueous sodium bicarbonate solution, and two times with brine. However, for the reaction with substrate **10a**, the workup was slightly modified as the organic layer was washed two times with aqueous sodium bicarbonate solution, and one time with brine. The organic layer was dried ($MgSO_4$), filtered, and concentrated. The resulting concentrate was dissolved three times in toluene and concentrated under reduced pressure. **Step 2:** The concentrate was dissolved in DCM under an argon atmosphere to obtain a 0.048 M solution, which was diluted to 0.040 M upon addition of TFA. The mixture was kept stirring at room temperature and the progress of the reaction was followed by ^{1}H NMR spectroscopy. When it showed full conversion, the volatiles were removed under

reduced pressure. **Step 3**: The concentrate was dissolved in DCM (0.08 M) and triethylamine was added (2 equiv.). The mixture was kept stirring at room temperature overnight. Then, the volatiles were removed under reduced pressure and the crude product was purified by silica gel flash column chromatography.

(3*S*, 4*R*, 5*R*)-5-(Hydroxymethyl)-3,4-*O*-((3'S,4'S)-3',4'-dimethoxybutan-2',3'-dioxyl)-1-methylpiperidin-2-one (11a). Reaction time **step 1**: 18 hours, reaction time **step** 2: 2 hours, and reaction time **step 3**: 19 hours. For the purification of crude product **11a** by silica gel flash column chromatography, eluent with gradient was applied (DCM/MeOH 99:1 to 97:3). Compound **11a** was obtained as a white solid (81%, 65 mg). R_f = 0.31 (DCM/MeOH 9:1); m.p. 148.3-150.2 °C; $[\alpha]_D^{26°C}$ = + 133 (*c* = 0.81, CH_2Cl_2). ^{1}H NMR (400 MHz, CD_3OD, ppm) δ = 4.10 (d, *J* = 10.3 Hz, 1H; H-3), 3.80-3.85 (m, 2H; H-4, CHaOH), 3.67 (dd, *J* = 11.0 Hz, *J* = 6.5 Hz, 1H; CHbOH), 3.45 (dd, *J* = 12.6 Hz, *J* = 7.0 Hz, 1H; H-6a), 3.29-3.28 (m, 4H; H-6b, OCH_3), 3.27 (s, 3H; OCH_3), 2.91 (s, 3H; NCH_3), 2.26-2.16 (m, 1H; H-5), 1.34 (s, 3H; CH_3), 1.27 (s, 3H; CH_3); ^{13}C NMR (100 MHz, CD_3OD, ppm) δ = 169.2 (C-2), 100.6, 100.3 (C-3', C-4'), 70.1 (C-3), 67.0 (C-4), 60.5 (CH_2OH), 50.7 (C-6), 48.5, 48.2 (2 x OCH_3, overlap with the solvent signal) 39.6 (C-5), 34.3 (NCH_3), 17.9, 17.7 (2 x CH_3); HRMS (ESI): *m/z* [M+Na]$^+$ calcd for $C_{13}H_{23}O_6NNa$: 312.1418; found: 312.1416.

(3*S*, 4*R*, 5*R*)-5-(Hydroxymethyl)-3,4-*O*-((3'S,4'S)-3',4'-dimethoxybutan-2',3'-dioxyl)-1-propylpiperidin-2-one (11b). Reaction time **step 1**: 18 hours, reaction time **step 2**: 3.5 hours, and reaction time **step 3**: 20 hours. For the purification of crude product **11b** by silica gel flash column chromatography, eluent with gradient was applied (DCM/MeOH 99:1 to 49:1). Compound **11b** was obtained as a colorless viscous oil (65%, 53 mg, the ^{1}H NMR spectrum indicated that the substance is contaminated with silicone grease). R_f = 0.44 (DCM/MeOH 9:1); $[\alpha]_D^{27°C}$ = + 65 (*c* = 0.95, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ = 4.10 (d, *J* = 10.4 Hz, 1H; H-3), 3.86-3.81 (m, 2H; H-4, CHaOH), 3.69 (dd, *J* = 10.4 Hz, *J* = 5.7 Hz, 1H; CHbOH), 3.37-3.29 (m, 5H; OCH_3, H-6a, NCHa), 3.24-3.19 (m, 4H; OCH_3, NCHb), 3.13 (dd, *J* = 12.1 Hz, *J* = 10.3 Hz, 1H; H-6b), 2.50 (brs, 1H; OH), 2.32-2.24 (m, 1H; H-5), 1.58-1.49 (m, 2H; CH_2), 1.37 (s, 3H, CH_3), 1.29 (s, 3H; CH_3), 0.87 (t, *J* = 7.4 Hz, 3H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ = 166.3 (C-2), 100.4, 99.3 (C-3', C-4'), 68.9 (C-3), 68.0 (C-4), 62.0 (CH_2OH), 48.5 (OCH_3, NCH_2), 48.0 (OCH_3), 47.1 (C-6), 38.4 (C-5), 20.2 (CH_2), 17.8 (CH_3), 17.7 (CH_3), 11.4 (CH_3); HRMS (ESI): *m/z* [M+Na]$^+$ calcd for $C_{15}H_{27}O_6NNa$: 340.1731; found: 340.1724.

(3*S*, 4*R*, 5*R*)-1-Butyl-5-(hydroxymethyl)-3,4-*O*-((3'S,4'S)-3',4'-dimethoxybutan-2',3'-dioxyl)piperidin-2-one (11c). Reaction time **step 1**: 17 hours, reaction time **step 2**: 3 hours, and reaction time **step 3**: 21 hours. For the purification of crude product **11c** by silica gel flash column chromatography, eluent with gradient was applied (DCM/MeOH 99.5:0.5 to 49:1). Compound **11c** was obtained as a colorless viscous oil (73%, 49 mg). R_f = 0.29 (DCM/MeOH 9:1); $[\alpha]_D^{27°C}$ = + 133 (*c* = 0.21, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ = 4.11 (d, *J* = 10.3 Hz, 1H; H-3), 3.88-3.82 (m, 2H; H-4, CHaOH), 3.71 (dd, *J* = 10.7 Hz, *J* = 5.6 Hz, 1H; CHbOH), 3.42-3.21 (m, 9H; 2 x OCH_3, H-6a, NCH_2), 3.15-3.10 (m, 1H; H-6a), 2.32-2.24 (m, 2H; H-5, OH), 1.53-1.46 (m, 2H; CH_2), 1.38 (s, 3H; CH_3), 1.33-1.24 (s, 5H; CH_3, CH_2), 0.90 (t, *J* = 7.4 Hz, 3H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ = 166.2 (C-2), 100.5, 99.3 (C-3', C-4'), 68.9 (C-3), 68.2 (C-4), 62.3 (CH_2OH), 48.5 (OCH_3), 48.0 (OCH_3), 47.0 (C-6), 46.7 (NCH_2), 38.5 (C-5), 29.1 (CH_2), 20.2 (CH_2), 17.8 (2 x CH_3), 13.9 (CH_3); HRMS (ESI): *m/z* [M+Na]$^+$ calcd for $C_{16}H_{29}O_6NNa$: 354.1887; found: 354.1882.

(3*S*, 4*R*, 5*R*)-1-Hexyl-5-(hydroxymethyl)-3,4-*O*-((3'S,4'S)-3',4'-dimethoxybutan-2',3'-dioxyl)piperidin-2-one (11d). Reaction time **step 1**: 17 hours, reaction time **step 2**: 3 hours, and reaction time **step 3**: 20 hours. For the purification of crude product **11d** by silica gel flash column chromatography, eluent with gradient was applied (DCM/MeOH 99.5:0.5 to 49:1). Compound **11d** was obtained as a colorless viscous oil (64%, 37 mg). R_f = 0.40 (DCM/MeOH 9:1); $[\alpha]_D^{27°C}$ + 100 (*c* = 0.16, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ = 4.11 (d, *J* = 10.9 Hz, 1H; H-3), 3.89-3.82 (m, 2H; H-4, CHaOH), 3.71 (dd, *J* = 8.9 Hz, *J* = 4.9 Hz, 1H; CHbOH), 3.40-3.20 (m, 9H; 2 x CH_3, H-6a, NCH_2), 3.15-3.05 (m, 1H, H-6b), 2.34-2.25 (m, 2H; OH, H-5), 1.54-1.49 (m, 2H; CH_2), 1.38 (s, 3H, CH_3), 1.29-1.24 (m, 9H; CH_3, 3 x CH_2), 0.86 (t, *J* = 7.1 Hz, 3H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ = 166.1 (C-2), 100.5, 99.3 (C-3', C-4'), 68.9 (C-3), 68.2 (C-4), 62.3 (CH_2OH), 48.5 (OCH_3), 48.0 (OCH_3), 47.0 (NCH_2, C-6), 38.5 (C-5), 31.6 (CH_2), 26.9 (CH_2), 26.6 (CH_2), 22.6 (CH_2), 17.8 (2 x CH_3), 14.1 (CH_3); HRMS (ESI): *m/z* [M+Na]$^+$ calcd for $C_{18}H_{33}O_6NNa$: 382.2200; found: 382.2194.

(3*S*, 4*R*, 5*R*)-5-(Hydroxymethyl)-3,4-*O*-((3'S,4'S)-3',4'-dimethoxybutan-2',3'-dioxyl)-1-octylpiperidin-2-one (11e). Reaction time **step 1**: 22 hours, reaction time **step 2**: 3 hours, and reaction time **step 3**: 22 hours. For the purification of crude product **11e** by silica gel flash column chromatography, eluent with gradient was applied (DCM/MeOH 99.5:0.5 to 49:1). Compound **11e** was obtained as a colorless viscous oil (57%, 45 mg); R_f = 0.35 (DCM/MeOH 9:1); $[\alpha]_D^{27°C}$ = +200 (*c* = 0.16, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ = 4.09 (d, *J* = 10.1 Hz, 1H; H-3), 3.86-3.81 (m, 2H; H-4, CHaOH), 3.69 (dd, *J* = 10.5 Hz, *J* = 5.6 Hz, 1H; CHbOH), 3.37-3.19 (m, 9H; 2 x CH_3, H-6a, NCH_2), 3.15-3.09 (m, 1H, H-6b), 2.47 (brs, 1H, OH), 2.32-2.22 (m, 1H; H-5), 1.51-1.47 (m, 2H; CH_2), 1.29-1.25 (m, 16H; 2 x CH_3, 5 x CH_2), 0.85 (t, *J* = 7.0 Hz, 3H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ = 166.2 (C-2), 100.4, 99.3 (C-3', C-4'), 68.9 (C-3), 68.0 (C-4), 62.1 (CH_2OH), 48.4, (OCH_3), 48.0 (OCH_3), 47.1 (C-6), 47.0 (NCH_2), 38.4 (C-5), 31.9 (CH_2), 29.4 (CH_2), 29.2 (CH_2), 27.0 (CH_2), 26.9 (CH_2), 22.7 (CH_2), 17.8 (CH_3), 17.7 (CH_3), 14.2 (CH_3); HRMS (ESI): *m/z* [M+Na]$^+$ calcd for $C_{20}H_{37}O_6NNa$: 410.2513; found: 410.2506.

(3*S*, 4*R*, 5*R*)-5-(Hydroxymethyl)-3,4-*O*-((3'*S*,4'S)-2',3'-dimethoxybutan-2',3'-dioxyl)-1-(3-phenylpropyl)piperidin-2-one (11f). Reaction time **step 1**: 22 hours, reaction time **step 2**: 2.5 hours, and reaction time **step 3**: 22 hours. For the purification of the crude product **11f** by silica gel flash column chromatography, eluent with gradient was applied (DCM/MeOH 99.5:0.5 to 49:1). Compound **11f** was obtained as a colorless viscous oil (66%, 23 mg); R_f = 0.42 (DCM/MeOH 9:1); $[\alpha]_D^{27°C}$ = +71 (*c* = 0.48, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ =7.22-7.16 (m, 2H; ArH), 7.12-7.09 (m, 3H; ArH), 4.01 (d, *J* =10.2 Hz, 1H; H-3), 3.78-3.72 (m, 2H; H-4, CHaOH), 3.62 (dd, *J* = 10.7 Hz, *J* = 5.1 Hz, 1H; CHbOH), 3.41-3.34 (m, 1H; NCHa), 3.28-3.17 (m, 8H, NCHb, H-6a, 2 x OCH_3), 3.02 (dd, *J* = 12.1 Hz, *J* = 10.7 Hz, 1H; H-6b), 2.55 (t, *J* = 7.7 Hz,; 2H, CH_2Ph), 2.22-2.13 (m, 2H; H-5, OH), 1.86-1.76 (m, 2H, CH_2), 1.32 (s, 3H; CH_3), 1.24 (s, 3H; CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ =166.4 (C-2), 141.5, 128.5, 128.4, 126.0 (Ar), 100.5, 99.3 (C-3', C-4'), 68.9 (C-3), 68.0 (C-4), 62.2 (CH_2OH), 48.5, 48.0 (2 x OCH_3); 47.0, 46.7 (NCH_2, C-6), 33.3 (CH_2Ph), 28.3 (CH_2), 17.8, 17.7 (2 x CH_3); HRMS (ESI): *m/z* [M+Na]$^+$ calcd for $C_{21}H_{31}O_6NNa$: 416.2036; found: 416.2036.

General procedure for the preparation of compounds 5a-f

A solution of **5a**, **5b**, **5c**, **5d**, **5e**, or **5f** (0.04 M) in TFA/H_2O (95:5, v/v) was kept stirring at room temperature overnight. The progress of the reaction was monitored by TLC (DCM/MeOH 9:1). When the TLC analysis showed complete conversion, the volatiles were removed under reduced pressure and the crude product was purified by silica gel flash column chromatography.

(3*S*, 4*R*, 5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)-1-methylpiperidin-2-one (5a). Reaction time: 24 h. For the purification of crude product **5a** by silica gel flash column chromatography, eluent with gradient was applied (DCM/MeOH/NH_4OH) to 95:5:0.1 to 90:10:1.2. Compound **5a** was obtained as a light-yellow oil (21%, 8 mg); R_f = 0.39 (DCM/MeOH 4:1); $[\alpha]_D^{26°C}$ = +21 (*c* = 0.28, MeOH); ^{1}H NMR (400 MHz, CD_3OD, ppm) δ = 3.85 (d, *J* = 9.3 Hz, 1H; H-3), 3.81 (dd, *J* = 10.9 Hz, *J* = 3.5 Hz, 1H; CHaOH), 3.66 (dd, *J* = 10.9 Hz, *J* = 6.9 Hz, 1H; CHbOH), 3.58-3.53 (m, 1H, H-4), 3.42 (dd, *J* = 12.6 Hz, *J* = 5.9 Hz, 1H; H-6a), 3.23 (dd, *J* = 12.6 Hz, *J* = 10.4 Hz, 1H; H-6b), 2.94 (s, 3H; NCH_3), 2.10-2.01 (m, 1H; H-5); ^{13}C NMR (100 MHz, CD_3OD, ppm) δ = 172.3 (C-2), 75.1 (C-3), 72.0 (C-4), 62.0 (CH_2OH), 50.4 (C-6), 42.3 (C-5), 34.6 (NCH_3); HRMS (ESI): *m/z* [M+Na]$^+$ calcd for $C_7H_{13}O_4NNa$: 198.0737; found: 198.0736.

(3*S*, 4*R*, 5*R*)- 3,4-Dihydroxy-5-(hydroxymethyl)-1-propylpiperidin-2-one (5b). Reaction time: 23 h. For the purification of the product **5b** by silica gel flash column chromatography, eluent with gradient was applied (DCM/MeOH/NH_4OH to 95:5:0.1 to 90:10:0.6). Compound **5b** was obtained as a colorless viscous oil (67%, 22 mg). R_f = 0.22 (DCM/MeOH 9:1); $[\alpha]_D^{27°C}$ = + 16 (*c* = 0.90, MeOH); ^{1}H NMR (400 MHz, CD_3OD, ppm) δ = 3.76 (d, *J* = 9.6 Hz, 1H; H-3), 3.71 (dd, *J* = 11.0 Hz, *J* = 3.9 Hz, 1H; CHaOH), 3.53 (dd, *J* = 11.0 Hz, *J* = 7.4 Hz, 1H; CHbOH), 3.42 (t, *J* = 9.6 Hz, 1H; H-4), 3.33 (dd, *J* = 12.9 Hz, *J* = 5.9 Hz, 1H; H-6a), 3.26-3.20 (m, 2H; NCH_2), 3.15 (dd, *J* = 12.9 Hz, *J* = 9.6 Hz, 1H; H-6b), 1.95-1.88 (m, 1H; H-5), 1.48 (sextet,

J = 7.4 Hz, 2H; CH_2), 0.81 (t, *J* = 7.4 Hz, 3H; CH_3); ^{13}C NMR (100 MHz, CD_3OD, ppm): δ = 172.1 (C-2), 75.1 (C-3), 72.2 (C-4), 61.8 (CH_2OH), 49.8 (NCH_2), 48.1 (C-6), 42.6 (C-5), 21.2 (CH_2), 11.5 (CH_3); HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_9H_{17}O_4NNa$: 226.1050; found: 226.1049.

(3*S*, 4*R*, 5*R*)-1-Butyl-3,4-dihydroxy 5-(hydroxymethyl)-1-piperidin-2-one (5c). Reaction time: 23 h. For the purification of crude product **5c** by silica gel flash column chromatography, eluent with gradient was applied (DCM/MeOH/NH_4OH to 95:5:0.1 to 90:10:0.4). Compound **5c** was obtained as a colorless oil (76%, 25 mg). R_f = 0.22 (DCM/MeOH 9:1); $[\alpha]_D^{26°C}$ = + 16 (*c* = 0.98, MeOH); ^1H NMR (400 MHz, CD_3OD, ppm) δ = 3.85 (d, *J* = 9.1 Hz, 1H; H-3), 3.81 (dd, *J* = 11.1 Hz, *J* = 4.1 Hz, 1H; CHaOH), 3.62 (dd, *J* = 11.1 Hz, *J* = 7.4 Hz, 1H; CHbOH), 3.51 (t, *J* = 9.1 Hz, 1H; H-4), 3.45-3.34 (m, 3H; H-6a, NCH_2), 3.24 (dd, *J* = 12.7 Hz, *J* = 9.6 Hz, 1H; H-6b), 2.06-1.97 (m, 1H; H-5), 1.54 (q, *J* = 7.4 Hz, 2H; CH_2), 1.33 (sextet, *J* = 7.4 Hz, 2H; CH_2), 0.95 (t, *J* = 7.4 Hz, 3H; CH_3); ^{13}C NMR (100 MHz, CD_3OD, ppm) δ = 172.0, (C-2), 75.1 (C-3), 72.2 (C-4), 61.8 (CH_2OH), 48.1 (C-6), 47.9 (NCH_2), 42.6 (C-5), 30.1 (CH_2), 21.0 (CH_2), 14.1 (CH_3); HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{10}H_{19}O_4NNa$: 240.1206; found: 240.1207.

(3*S*, 4*R*, 5*R*)-1-Hexyl-3,4-dihydroxy 5-(hydroxymethyl)-piperidin-2-one (5d). Reaction time: 23 h. For the purification of crude product **5d** by silica gel flash column chromatography, eluent with gradient was applied (DCM/MeOH/NH_4OH 95:5:0.1 to 90:10:0.25). Compound **5d** was obtained as a colorless oil (67%, 16 mg). R_f = 0.14 (DCM/MeOH 9:1); $[\alpha]_D^{26°C}$ = + 18 (*c* = 0.11, MeOH); ^1H NMR (400 MHz, CD_3OD, ppm) δ = 3.90 (d, *J* = 9.3 Hz, 1H; H-3), 3.81 (dd, *J* = 11.0 Hz, *J* = 3.8 Hz, 1H; CHaOH), 3.62 (dd, *J* = 11.0 Hz, *J* = 7.2 Hz, 1H; CHbOH), 3.52 (t, *J* = 9.3 Hz, 1H; H-4), 3.46-3.33 (m, 3H; H-6a, NCH_2), 3.26 (dd, *J* = 12.7 Hz, *J* = 9.4 Hz, 1H; H-6b), 2.06-1.97 (m, 1H; H-5), 1.59-1.54 (m, 2H; CH_2); 1.32-1.29 (m, 6H; 3 x CH_2), 0.91 (t, *J* = 7.0 Hz, 3H; CH_3); ^{13}C NMR (100 MHz, CD_3OD, ppm) δ = 172.1 (C-2), 75.2 (C-3), 72.1 (C-4), 61.8 (CH_2OH), 48.2 (NCH_2), 48.1 (C-6), 42.6 (C-5), 32.7 (CH_2), 27.9 (CH_2), 27.6 (CH_2), 23.6 (CH_2), 14.3 (CH_3); HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{12}H_{23}O_4NNa$: 268.1519; found: 268.1521.

(3*S*, 4*R*, 5*R*)-3,4-Dihydroxy 5-(hydroxymethyl)-1-octylpiperidin-2-one (5e). Reaction time: 24 h. For the purification of crude product **5e** by silica gel flash column chromatography, eluent with gradient was applied (DCM/MeOH/NH_4OH 95:5:0.1 to 90:10:0.1). Compound **5e** was obtained as a white solid (83%, 23 mg). R_f = 0.18 (DCM/MeOH 9:1); m.p. 72.9-73.5 °C; $[\alpha]_D^{26°C}$ = + 14 (*c* = 0.14, MeOH); ^1H NMR (400 MHz, CD_3OD, ppm) δ = 3.87 (d, *J* = 9.4 Hz, 1H; H-3), 3.83 (dd, *J* = 10.9 Hz, *J* = 3.6 Hz, 1H; CHaOH), 3.64 (dd, *J* = 10.9 Hz, *J* = 7.3 Hz, 1H; CHbOH), 3.53 (t, *J* = 9.4 Hz, 1H; H-4), 3.45 (dd, *J* = 12.7 Hz, *J* = 5.7 Hz, 1H; H-6a), 3.40-3.31 (m, 2H; NCH_2), 3.26 (dd, *J* = 12.7 Hz, *J* = 9.7 Hz, 1H; H-6b), 2.08-2.01 (m, 1H; H-5), 1.61-1.56 (m, 2H; CH_2), 1.34 (brs, 10H; 5 x CH_2), 0.92 (t, *J* = 7.1 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, CD_3OD, ppm) 172.0 (C-2), 75.1 (C-3), 72.2 (C-4), 61.8 (CH_2OH), 48.1 (NCH_2, C-6), 42.6 (C-5), 33.0 (CH_2), 30.4 (CH_2), 30.3 (CH_2), 28.0 (CH_2), 27.9 (CH_2), 23.7 (CH_2), 14.4 (CH_3); HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{14}H_{27}O_4NNa$: 296.1832; found: 296.1832.

(3*S*, 4*R*, 5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)-1-(3-phenylpropyl)piperidin-2-one (5f). Reaction time: 22 h. Crude product **5f** was purified two time by silica gel flash column chromatography. Eluent with gradient was applied both times (column 1: DCM/MeOH 98:2 to 9:1 and column 2: DCM/MeOH 97:3 to DCM/MeOH/NH_4OH 95:4.5:0.5. Compound **5f** was obtained as a light beige solid (65%, 13 mg). R_f = 0.16 (DCM/MeOH 9:1); m.p. = 107.4-108.4 °C; $[\alpha]_D^{27°C}$ = + 24 (*c* 0.34, MeOH); ^1H NMR (400 MHz, CD_3OD, ppm) δ = 7.27-7.13 (m, 5H; ArH), 3.81-3.77 (m, 2H; H-3, CHaOH), 3.61 (dd, *J* = 11.2 Hz, *J* = 7.2 Hz, 1H; CHbOH), 3.51-3.34 (m, 4H; NCH_2, H-4, H-6a), 3.21 (dd, *J* = 12.5 Hz, *J* = 9.5 Hz, 1H; H-6b), 2.62 (t, *J* = 7.8 Hz, 2H; CH_2), 2.00-1.85 (m, 3H; H-5, CH_2); ^{13}C NMR (100 MHz, CD_3OD, ppm) δ = 172.2 (C-2), 142.9 (Ar), 129.4 (Ar), 129.3 (Ar), 126.9 (Ar), 75.1 (C-3), 72.2 (C-4), 61.8 (CH_2OH), 48.1, 48.0 (NCH_2, C-6), 42.5 (C-5), 34.2 (CH_2), 29.6 (CH_2); HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{15}H_{21}O_4NNa$: 302.1363; found: 302.1362.

(3*S*, 4*R*, 5*R*)-5-(Hydroxymethyl)-3,4-*O*-((2'S,3'S)-2',3'-dimethoxybutan-2',3'-dioxyl)-1-methylpiperidin-2-one (14). Step 1. Acetic acid anhydride (0.55 mL) was added to a solution of **12** (20 mg, 0.07 mmol) in DMSO (1.3 mL). The mixture was kept stirring at room temperature overnight. After this time, it was diluted with ethyl acetate (15 mL) and washed with brine (2x8 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated. **Step 2.** The crude product from the previous step was dissolved in toluene and evaporated three times. The concentrate was dissolved in MeOH (2 mL) under argon and was then added Ra-Ni (slurry in water, 0.5 mL). Then, the mixture was degassed, and hydrogen atmosphere was introduced (1 atm). The mixture was kept stirring at room temperature overnight. After this time, it was filtered through celite, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography (eluent with gradient DCM/MeOH 49:1 to 93:7). Compound **14** was obtained as a colorless oil (69%, 14 mg). R_f = 0.34 (DCM/MeOH 9:1); $[\alpha]_D^{26°C}$ = + 160 (*c* 0.25, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ = 6.37 (s, 1H; NH), 4.20 (d, *J* = 10.4 Hz, 1H; H-3), 3.98-3.91 (m, 1H; H-4), 3.83-3.80 (m, 1H; CHaOH), 3.74-3.71 (m, 1H; CHbOH), 3.39-3.33 (m, 4H; OCH_3, H-6a), 3.27 (s, 3H; OCH_3), 3.16-3.10 (m, 1H; H-6b), 2.35-2.28 (m, 1H; H-5), 2.19 (brs, 1H; OH), 1.40 (s, 3H; OCH_3); 1.32 (s, 3H; OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ = 169.5 (C-2), 100.5, 99.6 (C-2', C-3'), 68.7 (C-3), 68.3 (C-4),62.2 (CH_2OH), 48.6, 48.1 (2 x OCH_3), 41.2 (C-6), 38.8 (C-5), 17.8, 17.7 (2 x OCH_3): HRMS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{12}H_{21}O_6NNa$: 298.1261; found: 298.1260.

(3*S*, 4*R*, 5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)-1-piperidin-2-one (3). A solution of **14** (57 mg, 0.21 mmol) in TFA/H_2O (95:5, v/v, 5.2 mL) was kept stirring at room temperature for 24 hours. The volatiles were removed under reduced pressure and the residue was purified by silica gel flash column chromatography. Eluent with gradient was applied DCM/MeOH 97:3 to DCM/MeOH/NH_4OH 90:10:1 (column 1.) after that MeCN/H_2O 99:1 to MeCN/H_2O 9:1 (column 2.). Compound **3** was obtained as a colorless oil (93%, 31 mg). R_f = 0.18 (DCM/MeOH 8:2); $[\alpha]_D^{27°C}$ = + 143 (*c* 0.28, MeOH). The observed NMR data are in full agreement with reported data:[14] ^1H NMR (400 MHz, D_2O, ppm): δ = 4.03 (d, *J* = 9.3 Hz, 1H; H-3), 3.82 (dd, *J* = 11.4 Hz, *J* = 3.6, 1H; CHaOH), 3.72-3.66 (m, 2H, H-4, CHbOH), 3.42 (dd, *J* = 12.6 Hz, *J* = 5.6 Hz, 1H; H-6a), 3.16 (dd, *J* = 12.6 Hz, *J* = 10.3 Hz, 1H; H-6b), 2.20-2.11 (m, 1H; H-5); ^{13}C NMR (100 MHz, D_2O, ppm): δ = 174.2 (C-2), 73.3 (C-3), 71.2 (C-4), 60.8 (CH_2OH), 41.2, 41.1 (C-5, C-6).

Glycosidase inhibition testing

Evaluation of the inhibitory profile of lactams prepared herein was accomplished according to the protocol previously used by our group.[21] Enzyme kinetics in the presence and in the absence of the inhibitor (50 μM concentration) was monitored using UV-Vis spectroscopy (400 nm) in a Thermo Scientific™ Varioskan™ LUX microplate reader. The following commercially-available enzymes (Sigma-Aldrich) were used: α-glucosidase (*Saccharomyces cerevisiae*), β-glucosidase (almonds), α-galactosidase (green coffee beans), β-galactosidases (*Escherichia coli* and, *Aspergillus orizae*) and β-mannosidase (*Helix pomatia*). As substrates, the corresponding p-nitrophenyl α/β-d-glycopyranosides were used, except for β-galactosidases (*o*-nitrophenyl derivatives). For β-mannosidase, a mixture of 100 mM phosphate-citrate buffer (50 μL, pH 5.6), 1.0 mM DMSO solution of inhibitor (or DMSO for control, 5 μL) or DMSO, substrate (10xK_M, 10 μL), appropriate diluted enzyme (velocity of reaction of 0.12-0.15 Abs/min at 4xK_M substrate concentration, 5 μL) and H_2O (30 μL) were incubated at 35 ºC during 4 minutes. Then, 1 M Na_2CO_3 (150 μL) was added to quench the reaction and the absorbance was measured at 400 nm.

For the rest of glycosidases, a mixture of 100 mM phosphate buffer (pH 6.8, 100 μL), substrate (10xK_M, 20 μL), 1.0 mM DMSO solution of inhibitor (or DMSO for control, 10 μL), and H_2O (60 μL) was used. Reaction was initiated by the addition of appropriate diluted enzyme (velocity of reaction of 0.12-0.15 Abs/min at 4xK_M substrate concentration, 10 μL), and the reaction was monitored without incubation at 25 ºC for 120 s at 400 nm.

Percentage of inhibition at [S] = K_M was obtained (4.0 mM for β-glucosidase, 0.57 mM for β-mannosidase, 50 μM inhibitor concentration). When strong inhibition was found, IC_{50} values were calculated (6-7 different inhibitor concentrations). For this purpose, GraphPad Prism 8.02 software was used, via a non-linear regression. Plots (%I vs. [I]) were obtained using the Quest Graph™ IC50 Calculator." AAT Bioquest, Inc., 17 Sep. 2024, (https://www.aatbio.com/tools/ic50-calculator). Data are expressed as mean ± SD (duplicate).

Antimicrobial testing

Testing of antimicrobial activity was performed as follows: Suspensions from three bacterial strains (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 29213) were prepared in Muller-Hinton-Broth (MHB) from fresh overnight LB agar cultures and adjusted to an optical density of 0.5 (McFarland scale). These precultures were used (1:100, corresponding to a final cell density of $5*10^5$ CFU/mL) to inoculate 96-well plates (flat-bottom, Cat.-No. 655180, Greiner) containing 100 microL MHB per well with varying compound concentrations (either 100 or 50 microM final concentration for all compounds except for gentamycin, which was used at 10 microM final concentration). Subsequently, 100 microL mineral oil (M3516, Sigma-Aldrich) was added to each well to prevent evaporation. Culture growth was monitored (as determined by OD600) every 10 minutes for at least 20h in an automated plate reader (Tecan Nano M+) using an incubation temperature of 37ºC and continuous shaking (linear shaking with 1 mm amplitude). Growth curves were analyzed using custom MATLAB scripts (Version R2021a) following procedures described previously.[22] Briefly, raw OD600 time courses from each well were blank-corrected and smoothed using a moving average window with size 3. Area-under-the-growth-curve (AUC) was calculated using the trapz function, and the maximal growth rate (m_{max}) of each curve during exponential growth was calculated using a slide window of six consecutive time points and above an OD600 threshold of > 0.02.

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Supporting Information

YES (this text will be updated with links prior to publication)

Primary Data

NO.

Conflict of Interest

The authors declare no conflict of interest.

References

(1) Niwa, T.; Inouye, S.; Tsuruoka, T.; Koaze, Y.; Niida, T. *Agric. Biol. Chem.* **1970,** 34, 966.
(2) Stütz, A. E.; Wrodnigg, T. M. *Adv. Carbohydr. Chem. Biochem.* **2011,** *66*, 187.
(3) Goss, P. E.; Reid, C. L.; Bailey, D.; Dennis, J. W. *Clin. Cancer Res.* **1997,** *3*, 1077.
(4) Ferhati, X.; Matassini, C.; Fabbrini, M. G.; Goti, A.; Morrone, A.; Cardona, F.; Moreno-Vargas, A. J.; Paoli, P. *Bioorg. Chem.* **2019,** *87*, 534.
(5) Sánchez-Fernández, E. M.; García Fernández, J. M.; Mellet, C. O. *Chem. Commun.* **2016,** *52*, 5497.
(6) Karade, S. S.; Franco, E. J.; Rojas, A. C.; Hanrahan, K. C.; Kolesnikov, A.; Yu, W.; MacKerell, A. D., Jr.; Hill, D. C.; Weber, D. J.; Brown, A. N.; Treston, A. M.; Mariuzza, R. A. *J. Med. Chem.* **2023,** *66*, 2744.
(7) Heightman, T. D.; Vasella, A. T. *Angew. Chem. Int. Ed. Engl.* **1999,** *38*, 750
(8) (a) Vaaland, I. C.; López, Ó.; Puerta, A.; Fernandes, M. X.; Padrón, J. M.; Fernández-Bolaños, J. G.; Sydnes, M. O.; Lindbäck, E. *J. Enzyme Inhib. Med. Chem.* **2023,** *38*, 349. (b) Evangelista, T. C. S.; López, Ó.; Puerta, A.; Fernandes, M. X.; Ferreira, S. B.; Padrón, J. M.; Fernández-Bolaños, J. G.; Sydnes, M. O.; Lindbäck, E. *J Enzyme Inhib. Med. Chem.* **2022,** *37*, 2395. (c) Oliveira de Santana, Q. L.; Santos Evangelista, T. C.; Imhof, P.; Ferreira, S. B.; Fernández-Bolaños, J. G.; Sydnes, M. O.; López, Ó.; Lindbäck, E. *Org. Biomol. Chem.* **2021,** *19*, 2322.
(9) De Gregorio, E.; Esposito, A.; Vollaro, A.; De Fenza, M.; D'Alonzo, D.; Migliaccio, A.; Iula, V. D.; Zarrilli, R.; Guaragna, A. *Antibiotics (Basel)* **2020,** *9*, 362.
(10) Vaaland Holmgard, I. C.; González-Bakker, A.; Poeta, E.; Puerta, A.; Fernandes, M. X.; Monti, B.; Fernández-Bolaños, J. G.; Padrón, J. M.; López, Ó.; Lindbäck, E. *Org. Biomol. Chem.* **2024,** *22*, 3425.
(11) Jespersen, T. M.; Dong, W.; Sierks, M. R.; Skrydstrup, T.; Lundt, I.; Bols, M. *Angew. Chem. Int. Ed. Engl.* **1994,** *33*, 1778.
(12) Bols, M. *Acc. Chem. Res.* **1998,** *31*, 1.
(13) Varrot, A.; Tarling, C. A.; Macdonald, J. M.; Stick, R. V.; Zechel, D. L.; Withers, S. G.; Davies, G. J. *Am. Chem. Soc.* **2003,** *125*, 7496.
(14) Lillelund, V. H.; Liu, H.; Liang, X.; Søhoel, H.; Bols, M. *Org. Biomol. Chem.* **2003,** *1*, 282.
(15) Venkata Ramana, C.; Vasella, A. *Helv. Chim. Acta* **2000,** *83*, 1599.
(16) Ichikawa, Y.; Igarashi, Y.; Ichikawa, M.; Suhara, Y. *J. Am. Chem. Soc.* **1998,** *120*, 3007.
(17) Zhu, X.; Sheth, K. A.; Li, S.; Chang, H.-H.; Fan, J.-Q. *Angew. Chem. Int. Ed. Engl.* **2005,** *44*, 7450.
(18) López López, Ó.; Bols, M. *ChemBioChem* **2007,** 8, (6), 657.
(19) Lindbäck, E.; López, Ó.; Fernández-Bolaños, J. G.; Sauer, S. P.; Bols, M. *Org. Lett.* **2011,** *13*, 2908.
(20) Albright, J. D.; Goldman, L. *J. Am. Chem. Soc.* **1965,** *87*, 4214.
(21) Haarr, M. B.; López, Ó.; Fernández-Bolaños, J. G.; Lindbäck, E.; Sydnes, M. O. *Synthesis* **2022,** *54*, 2916.
(22) Gerosa, L.; Kochanowski, K.; Heinemann, M.; Sauer, U. *Mol. Syst. Biol.* **2013,** *9*, 658.