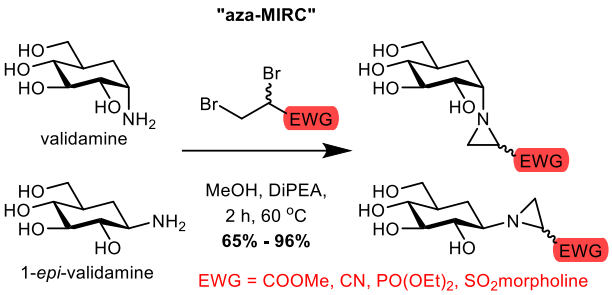
Design and synthesis of next generation carbohydrate-mimetic cyclitols: towards deactivators of inverting glycosidases and glycosyl transferases

Ofman, T.P.

Chapter 4

Design and synthesis of exocyclic cyclitol aziridines as potential mechanism-based glycosidase inactivators



ABSTRACT Eight exocyclic aziridine cyclitols were synthesized, envisioned as putative covalent inhibitors of inverting glucosidases. The constructs, bearing a range of electron withdrawing moieties, were obtained efficiently *via* an *aza*-Michael initiated ring closure reaction (*aza*-MIRC) on validamine or 1-*epi*-validamine. The synthetic methodologies and inhibitor design presented here can fuel the future discovery of covalent inhibitors of inverting glycosidases.

PUBLISHED Ofman, T. P.; van der Marel, G. A.; Codée, J. D. C.; Overkleeft, H. S. Design and synthesis of exocyclic cyclitol aziridines as potential mechanism-based glycosidase inactivators. *Eur. J. Org. Chem.* **2023**, *26*, e202300186.

Introduction

In 1990, cyclophellitol (**1**, Figure 1B) was isolated from samples of the *Phellinus sp.* mushroom and shown to be a potent, irreversible inactivator of retaining β-glucosidases.[1,2] Structural elucidation revealed cyclophellitol to have a carba-glucose core, functionalized with a β-oriented epoxide spanning the C-1 and C-7 position.[3,4] This epoxide forces the carba-glucose backbone to adopt a half-chair conformation, mimicking the conformation of the oxocarbenium ion transition state of β-D-glucopyranosides during hydrolysis by retaining β-glucosidase enzymes.[5]

Retaining α- and β-glucosidases generally employ a Koshland double displacement mechanism (Figure 1A).[6–12] The acid/base residues of these enzymes are in close proximity, with a relative distance of roughly 5.5 Å.[9,13] Upon binding of a substrate molecule in the enzyme pocket, in a first nucleophilic substitution reaction the carboxylate residue acts as a nucleophile and displaces the substrate aglycon, which is activated through protonation by the acid-base residue leading to a covalent intermediate. Subsequently, the aglycon leaves the enzyme active site allowing water to enter and upon deprotonation it then engages in a second displacement reaction to deliver the glucopyranose product with net retention of stereochemistry at the anomeric center. The covalently bound intermediate formed during hydrolysis has inspired the design of mechanism-based inhibitors that react to form stable, covalent adducts, effectively incapacitating the enzyme.[14,15] This in turn has formed the basis for the design and synthesis of activity based probes (ABPs) as tools to study enzyme activities.[15–18]

In previous studies, it has been shown that equipping cyclophellitol **1** and its nitrogen congener cyclophellitol aziridine **2** with a tag (for instance, a fluorophore or biotin) allows for selective and sensitive profiling of β-glucosidases.[19] Subsequently, inhibitors and probes of the 1,7-epimers (Figure 1B, **3** and **4**) were constructed to selectively inhibit and probe retaining α-glucosidases, revealing **3** and **4** to be irreversible inactivators with micromolar to nanomolar potencies.[20]

In an alternative design, the incorporation of an *N*-2-bromoacetyl warhead on a β-glucose scaffold results in efficient, covalent inactivators of retaining β-glucosidases (Figure 1B, **5**).[21–23] In this case the electrophilic site is transpositioned from the anomeric center to the more distal α-bromo amide, which traps the catalytic acid/base residue through a nucleophilic substitution reaction of the bromide to form a stable ester linkage.[23–27]

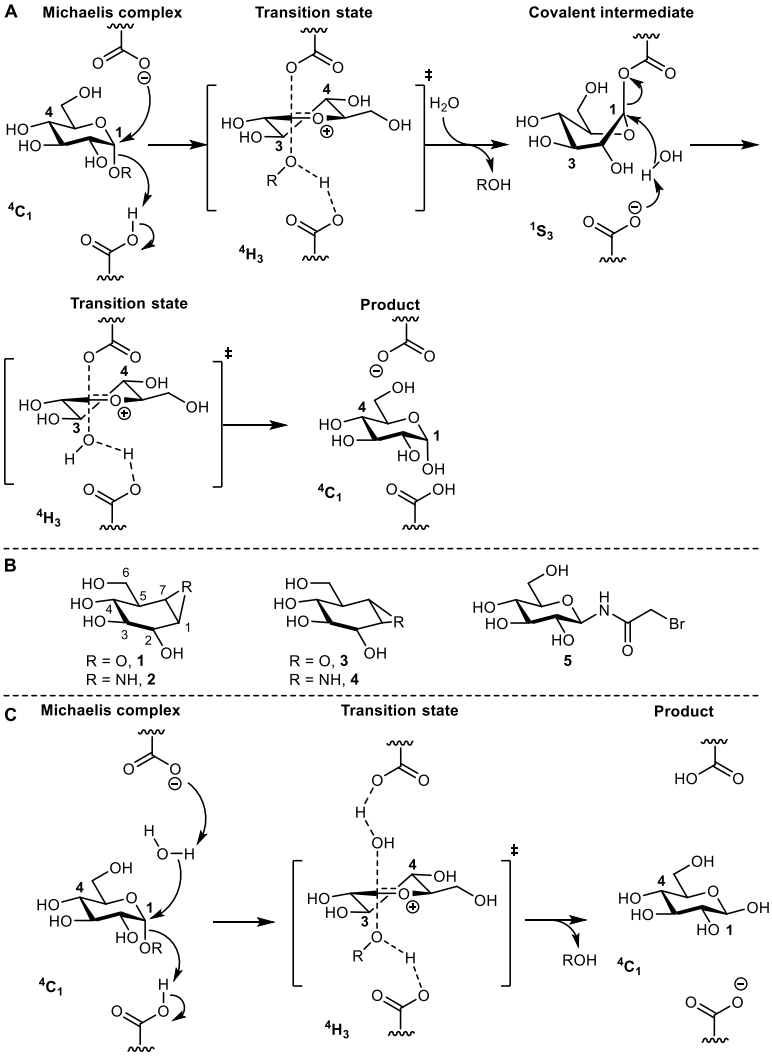


Figure 1. Conformational itinerary of inverting and retaining α-glucosidases *via* classic Koshland mechanisms, and potent, irreversible inhibitors **1** – **5**.[19,20,28,33] (A) Reaction itinerary of retaining α-glucosidases following a Koshland double displacement mechanism. (B) Potent, irreversible α- and β-glucosidase inhibitors; cyclophellitol **1**, cyclophellitol aziridine **2**, 1,7-*epi*-cyclophellitol **3**, 1,7-*epi*-cyclophellitol aziridine **4** and the structure of glucosyl-1-amine *N*-2-bromoacetyl **5**. (C) Reaction itinerary of inverting α-glucosidases following a Koshland single displacement mechanism.

Inverting glycosidases represent another large group of glycoside hydrolase (GH) and these hydrolases employ a different reaction mechanism than retaining glycosidases.[6,9,11,12] Inverting glycosidases employ a Koshland single displacement mechanism (Figure 1C).[9–11,28,29] The relatively large distance (6–12 Å) between the two catalytic side residues, which usually are two carboxylic acids, enables binding of the

substrate and a water molecule.[6,30–32] The active site carboxylate deprotonates the water molecule which concomitantly performs a nucleophilic substitution on the anomeric center expelling the aglycon, which is simultaneously protonated by the enzyme active site carboxylic acid. This results in net inversion of stereochemistry at the anomeric center of the thus produced glucopyranose.

Due to lack of a covalently bound intermediate during hydrolysis, the design of covalent inhibitors and probes for inverting glycosidases, in analogy to the *modus operandi* of cyclophellitol, is complicated. To date, this has led to an absence of covalent inhibitors and activity-based probes for selectively targeting inverting glycosidases.

In an attempt to identify such inhibitors, here a series of inhibitors is proposed based on 1-*epi*-validamine **6** and validamine **7**,[34–37] which are modified at the amine forming an exocyclic aziridine (Figure 2). This aziridine may act as a distal electrophile, for which it was reasoned there is enough space in the relatively large inverting glycosidase active site. It is hypothesized that the electrophile, further away from the anomeric position can bridge the relatively large distance between the carboxylic acid/carboxylate residues, allowing reaction with one of these – specifically, the one responsible for deprotonating the water molecule, which is replaced by the inhibitor in the enzyme pocket.

Here the synthesis of a panel of inhibitors **8** – **15** using an *aza*-Michael initiated ring closure reaction (*aza*-MIRC) as the key step is described.[38,39] Literature precedent has shown the *aza*-MIRC aziridine formation on primary amines to be high yielding and taking place under mild conditions.[38,39] To this end, validamine and 1-*epi*-validamine were considered suitable substrates for this transformation. A small series of dibromide coupling partners was composed, equipped with a diverse selection of electron withdrawing groups, all envisioned to be suitable for coupling under *aza*-MIRC conditions.

In turn, the inhibitor design and synthetic procedures presented here can fuel future design and synthesis of constructs to act on inverting glycosidases.

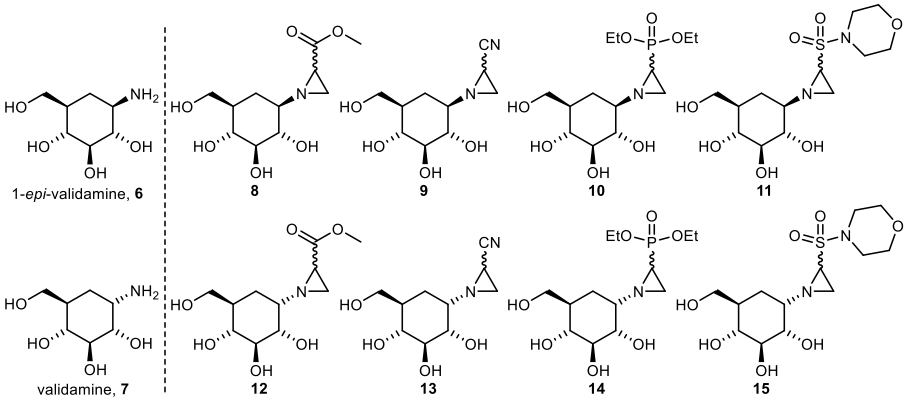


Figure 2. 1-*Epi*-validamine **6** and validamine **7** and eight 1-*N*-aziridine analogues **8** – **15** subject of the here-described studies.

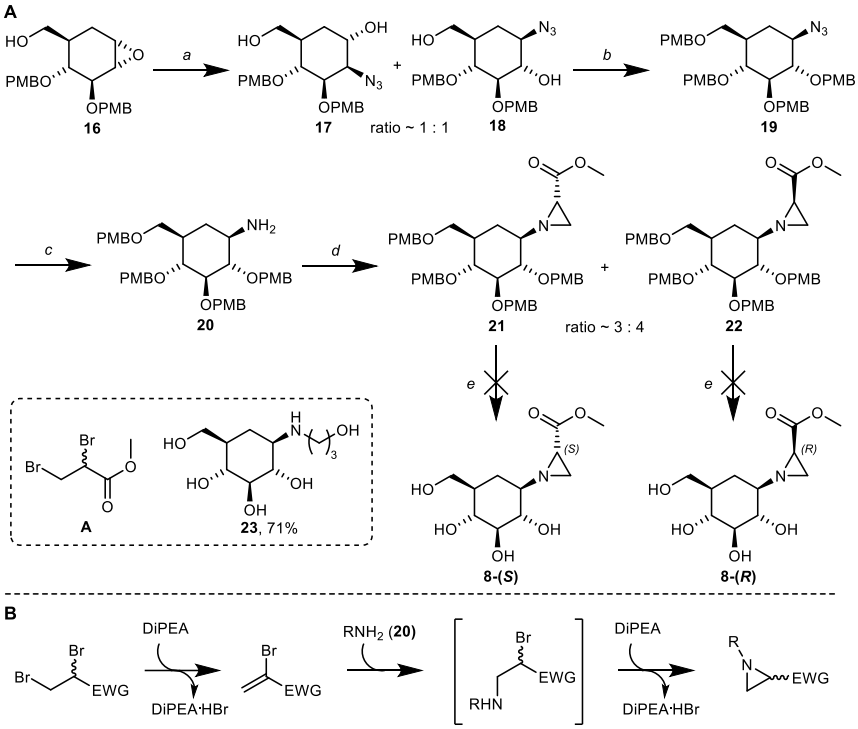
Results and discussion

The synthesis of the panel of target compounds as depicted in Figure 2 started with the preparation of 4-methoxybenzyl protected 1-*epi*-validamine **20**, which were envisioned to be suitably protected constructs to investigate the *aza*-MIRC reaction. To this end, epoxide **16**, the synthesis of which is part of the research described in chapter 3,[40] was treated with NaN_3 in DMF at elevated temperatures to yield a separable mixture of regioisomers **17** and **18** in a 1:1 ratio and an overall yield of 81% (Scheme 1A). Subsequent protection of the 2- and 6-OH in **18** under Williamson etherification conditions (NaH, PMBCl) yielded fully protected compound **19** in 77% yield. Reduction of the azide under Staudinger conditions (PMe_3, aq. NaOH, THF) transformed the azide into the corresponding primary amine **20** (74%).

With protected 1-*epi*-validamine **20** in hand, attention was then turned to the installation of the exocyclic aziridine. The protected 1-*epi*-validamine **20** was reacted with commercially available methyl 2,3-dibromopropanoate (**A**) in a polar, protic solvent (MeOH) using a non-nucleophilic base (DiPEA) to yield a separable mixture of diastereomers **21** and **22** in a 3:4 ratio, and an overall yield of 70%. Observed NOE interactions allowed for identification of both epimers.

The general mechanism of the efficient aziridine formation is shown in scheme 1B.[38,39] First, elimination of the primary bromide results in the *in situ* formation of the 2-bromovinyl intermediate which bears a Michael acceptor motive ready for a 1,4-addition of the primary amine of the protected 1-*epi*-validamine **20**. Subsequently, in an *aza*-Darzen reaction, the α-bromide is substituted by the resulting secondary amine to deliver the desired aziridine functionality.

Scheme 1. Attempted synthesis of two exocyclic aziridine epimers *via* an *aza*-MIRC reaction (A) and the mechanism of the *aza*-MIRC aziridine formation (B).

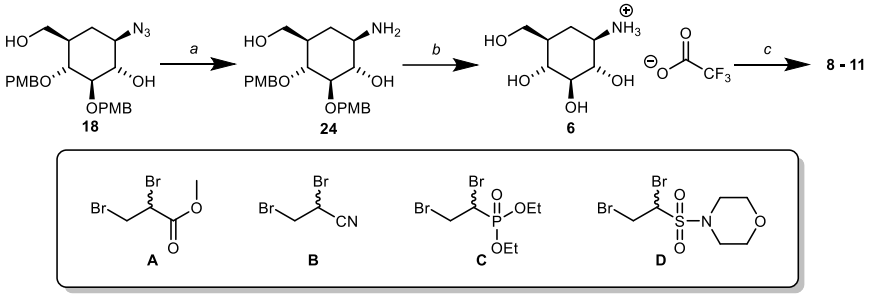


Reagents and conditions: *a)* NaN_3, DMF, 16 h, 130 °C, 39% (**17**), 42% (**18**); *b)* PMBCl, NaH, DMF, 16 h, rt (77%); *c)* PMe_3, NaOH, THF, H_2O, rt, 16 h (74%); *d)* methyl 2,3-dibromopropanoate, DiPEA, MeOH, 2 h, 60 °C, 30% (**21**), 40% (**22**); *e)* Na, NH_3, *t*-BuOH, 1 h, -60 °C (isolated **23** in 71%).

Unfortunately, all attempts to deprotect the exocyclic aziridines **21** and **22** resulted in degradation of the starting material, or led to undesired side reactions. Both reductive conditions (Pd/C, H_2) and acidic conditions (TFA, TES) resulted in complete degradation of material, while removal of the PMB ethers under Birch conditions led to the clean formation of compound **23**,[41] in which the reductive cleavage of the PMB groups was accompanied by reduction of the aziridine and methyl ester to from the *N*-propan-3-ol adduct.

Prompted by the robustness of the *aza*-MIRC reaction, it was hypothesized that the problematic PMB deprotection could be circumvented by the use of unprotected substrates.[39] Therefore, the use of unprotected 1-*epi*-validamine **6**, which was prepared from azide **18**, was explored (Scheme 2). Reduction of the azide under Staudinger conditions (PMe_3, NaOH, H_2O, THF) transformed the azide into the corresponding amine **24** (78%), of which the PMB protecting groups were removed under acidic conditions (TFA, TES, DCM) to yield 1-*epi*-validamine **6** as its TFA salt.

Scheme 2. Construction of target compounds **8** – **11** *via* an *aza*-MIRC reaction with 1-*epi*-validamine **6**.



Reagents and conditions: *a)* PMe_3, NaOH, THF, H_2O, rt, 16 h (78%); *b)* TFA, DCM, 1 h, 0 °C, (91%); *c)* DiPEA, dibromide (**A** – **D**), MeOH, 1 h, 80 °C, 96% (**8**), 82% (**9**), 73% (**10**), 79% (**11**).

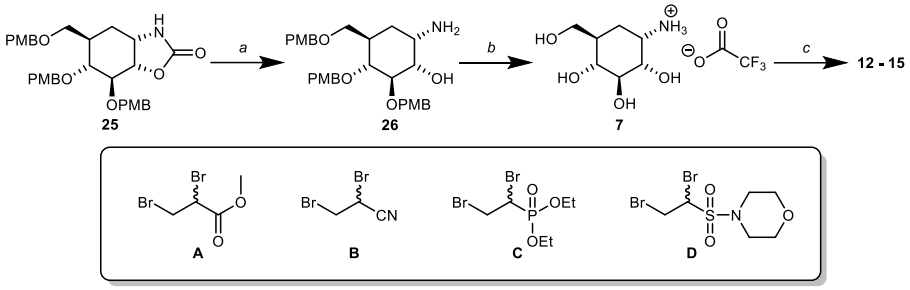
Next, 1-*epi*-validamine **6** was reacted, under the agency of DiPEA, with dibromides **A** – **D** (either commercially available (**A**) or easily accessible *via* known literature procedures (**B** – **D**),[42–44] see Scheme S1, Appendix), in MeOH at elevated temperatures. Gratifyingly, clean conversion towards the desired target structures was observed, yielding target compounds **8** – **11** in moderate to excellent yields after purification (73% – 96%).

With effective conditions in hand to generate the exocyclic aziridines, the assembly of the diasteroisomeric set of target compounds from validamine **7** was undertaken (Scheme 3).

To this end, compound **25**, previously described and synthesized in chapter 3, was considered as a suitable starting point.[40] The cyclic carbamate in **25** was hydrolyzed under alkaline conditions using NaOH in EtOH under elevated temperatures to afford the deprotected amino alcohol **26** (98%). Global deprotection using TFA and TES resulted in validamine **7** which was obtained as its TFA salt in quantitative yield.

Following the procedures applied to the 1-*epi*-validamine substrate **6**, validamine **7** was transformed into the set of target compounds **12** – **15** using dibromides **A** – **D**. Also these reactions proceeded uneventfully to cleanly provide **12** – **15** which were isolated in 65% to 87% yield.

Scheme 3. Construction of target compounds **12** – **15** *via* an *aza*-MIRC reaction with validamine **7**.



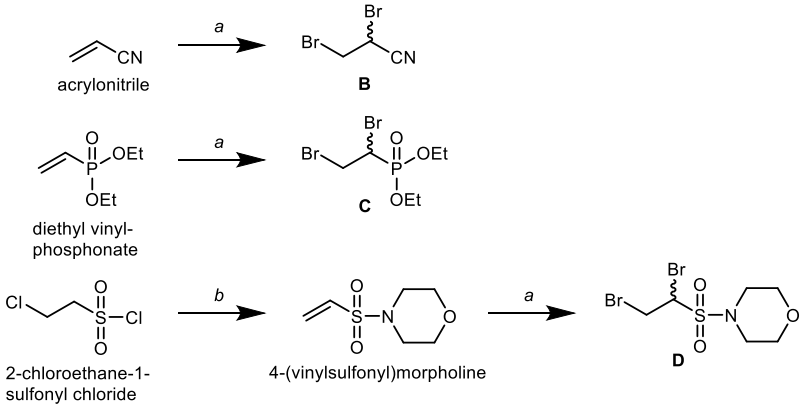
Reagents and conditions: *a)* NaOH, EtOH, 16 h, 80 °C (98%); *b)* TFA, DCM, 1 h, 0 °C, (quant.); *c)* DiPEA, dibromide (**A** – **D**), MeOH, 1 h, 80 °C, 85% (**12**), 82% (**13**), 65% (**14**), 87% (**15**).

Conclusion

In conclusion**,** this report describes the design and synthesis of functionalized validamines **8** – **15**, bearing an exocyclic aziridine motif as putative inhibitors of inverting glucosidases. These compounds were designed and synthesized on the premise that the exocyclic aziridine functionality can bridge the distance between the carboxylic acid/carboxylate residues in the enzyme pocket, potentially allowing for the formation of a covalent bond with the enzyme active site nucleophile, effectively incapacitating the enzyme. Key in the synthesis schemes has been an *aza*-Michael initiated ring closure (*aza*-MIRC) reaction, which in a single step converts unprotected 1-*epi*-validamine **6** and validamine **7** into target compounds **8** – **15**, proving the sturdiness and robustness of these aziridine forming reactions on complex, unprotected substrates. Suitable inhibition assays are currently being developed to probe whether this novel class of carbomimetics is capable of inhibiting inverting glucosidases. If so, the inhibitor design and synthetic procedures presented here, can fuel the future design and synthesis of constructs to effectively act on inverting glycosidases.

Appendix

Scheme S3. preparation of dibromide **B** – **D** *via* literature procedures.[42–44]



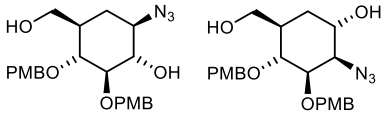
Reagents and conditions: *a)* Br_2, quant. (**B**), 68% (**C**); *b)* morpholine, Et_3N, DCM, 2 h, rt (78% over 2 steps).

Synthetic procedures.

General procedure A: *aza*-MIRC reaction of (1-*epi*)- validammonium trifluoroacetates (6 and 7) with corresponding dibromides.

Validammonium trifluoroacetate **6** or 1-*Epi*-validammonium trifluoroacetate **7** (29 mg, 0.1 mmol) was dissolved in MeOH (0.1 M). Subsequently, DiPEA (8.0 eq.) and the corresponding dibromide **A** – **D** (4.0 eq.) were added. The reaction mixture was stirred for 2 h at 60 °C after which full conversion was observed (MeOH:DCM, 2:8, v:v). The reaction mixture was concentrated under reduced pressure. Flash column chromatography (MeOH:DCM), and when mentioned followed by a second flash column (acetone:DCM), yielded the title compound as a mixture of diastereomers in roughly 1:1 ratios.

1-Deoxy-1-azido-3,4-di-*O*-(4-methoxybenzyl)-7-carba-β-D-glucose (18) and 2-Deoxy-2-azido-3,4-di-*O*-(4-methoxybenzyl)-7-carba-α-D-mannose (17).



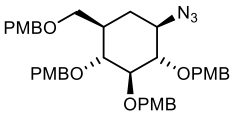
1,2-Anhydro-3,4-di-*O*-(4-methoxybenzyl)-7-carba-α-D-glucose (12.4 g, 31 mmol) was dissolved in DMF (310 mL, 0.1 M) followed by the addition of NaN_3 (20.1 g, 0.31 mol, 10 eq.). The reaction mixture was heated to 130 °C and stirring continued for 16 hours. Upon full conversion (R_f 0.8 and 0.4 for compound **18** and **17** respectively (EtOAc:pentane, 7:3, v:v)), the mixture was concentrated under reduced pressure to a quarter of its original volume and diluted with water. The aqueous layer was extracted with ethyl acetate (3x) followed by washing the combined organic layers with sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (50:50 EtOAc:pentane → 80:20 EtOAc:pentane) yielded title compounds **18** as a white solid (5.82 g, 13.1 mmol, 42%) and **17** as a colorless oil (5.40 g, 12.2 mmol, 39%).

Analytical data for **18**: ^{1}H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.38 – 7.16 (m, 4H, CH_{arom}), 6.98 – 6.82 (m, 4H, CH_{arom}), 4.91 (d, *J* = 11.0 Hz, 1H, CH*H* PMB), 4.87 (d, *J* = 10.8 Hz, 1H, C*H*H PMB), 4.71 (d, *J* = 11.0 Hz, 1H, CH*H* PMB), 4.60 (d, *J* = 10.9 Hz, 1H, C*H*H PMB), 3.80 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.66 – 3.52 (m, 2H, H-6), 3.44 – 3.28 (m, 4H, H-1, H-2, H-3, H-4), 2.67 (s, 1H, 2-OH), 1.88 (ddd, *J* = 13.1, 3.8, 3.8 Hz, 1H, H-7), 1.79 (s, 1H, 6-OH), 1.65 (dddd, *J* = 17.5, 10.1, 4.0, 4.0 Hz, 1H, H-5), 1.30 – 1.21 (m, 1H, H-7); ^{13}C NMR (101 MHz, $CDCl_3$, HSQC): δ 159.6, 159.5, 130.5, 130.1, 130.0 ($C_{q\text{-}arom}$), 129.7, 114.2, 114.2 (CH_{arom}), 86.2, 80.6, 76.6 (C-2, C-3, C-4), 75.4, 74.7 (CH_2 PMB), 63.6 (C-6), 62.5 (C-1), 55.4 (OMe), 41.3 (C-5), 29.6 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{23}H_{29}N_3NaO_6$ 466.2256; Found 466.1947.

Analytical data for **17**: ^{1}H NMR (300 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.40 – 7.14 (m, 4H, CH_{arom}), 6.96 – 6.79 (m, 4H, CH_{arom}), 4.78 (d, *J* = 10.9 Hz, 1H, CH*H* PMB), 4.69 – 4.59 (m, 2H, CH_2 PMB), 4.55 (d, *J* = 10.9 Hz, 1H, C*H*H PMB), 3.96 (m, 2H, H-1, H-3), 3.80 (m, 7H, OMe, OMe, H-2), 3.67 – 3.48 (m, 3H, H-4, H-6), 2.41 (bs, 1H, 6-OH), 2.36 (bs, 1H, 1-OH), 2.01 (dq, *J* = 9.3, 4.7 Hz, 1H, H-5), 1.62 – 1.53 (m, 2H, H-7); ^{13}C NMR (75 MHz, $CDCl_3$, HSQC): δ 159.5, 130.4, 130.1, 130.0 ($C_{q\text{-}arom}$), 129.7, 129.7, 129.7, 114.1, 114.0, 114.0 (CH_{arom}), 80.9 (C-1/C-3), 78.9 (C-4), 74.2, 72.8 (CH_2 PMB), 67.8

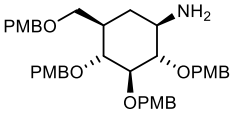
(C-1/C-3), 65.2 (C-6), 64.1 (C-2), 55.4, 55.4 (OMe), 38.9 (C-5), 30.1 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{23}H_{29}N_3NaO_6$ 466.2256; Found 466.1950.

1-Deoxy-1-azido-2,3,4,6-tetra-*O*-(4-methoxybenzyl)-7-carba-β-D-glucose (19).



Compound **18** (133 mg, 0.3 mmol) was dissolved in anhydrous DMF (3.0 mL, 0.1 M) and cooled on ice. PMBCl (0.10 mL, 0.75 mmol, 2.5 eq.) and NaH (60% in mineral oil, 60 mg, 1.5 mmol, 5.0 eq.) was subsequently added. The reaction mixture was allowed to attain to room temperature and stirring continued overnight. Upon full conversion (R_f 0.7 (EtOAc:pentane, 1:1, v:v)), the mixture was diluted with water. The aqueous layer was extracted with Et_2O (3x) followed by washing the combined organic layers with sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (10:90 EtOAc:pentane → 30:70 EtOAc:pentane) yielded the title compound **19** (159 mg, 0.23 mmol, 77%). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.33 – 7.04 (m, 8H, CH_{arom}), 6.94 – 6.76 (m, 8H, CH_{arom}), 4.87 – 4.72 (m, 5H, CH*H* PMB, CH*H* PMB, CH*H* PMB, C*H*H PMB, C*H*H PMB), 4.45 – 4.32 (m, 3H, CH*H* PMB, C*H*H PMB, C*H*H PMB), 3.80 (s, 3H, OMe), 3.79 (s, 6H, OMe, OMe), 3.78 (s, 3H, OMe), 3.54 – 3.28 (m, 6H, H-1, H-2, H-3, H-4, H-6), 1.99 (ddd, *J* = 13.5, 4.1, 4.1 Hz, 1H, H-7), 1.75 – 1.64 (m, 1H, H-5), 1.40 (q, *J* = 12.9 Hz, 1H, H-7); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.4, 159.3, 159.3, 159.2, 131.0, 130.7, 130.4, 130.3, 130.0 ($C_{q\text{-}arom}$), 129.7, 129.7, 129.4, 129.3, 129.3, 114.0, 114.0, 114.0, 113.9, 113.9, 113.8 (CH_{arom}), 86.6, 84.7, 80.3 (C-2, C-3, C-4), 75.5, 75.1, 72.9 (CH_2 PMB), 69.3 (C-6), 63.1 (C-1), 55.4, 55.4 (OMe), 40.0 (C-5), 30.7 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{39}H_{45}N_3NaO_8$ 706.3104; Found 706.3099.

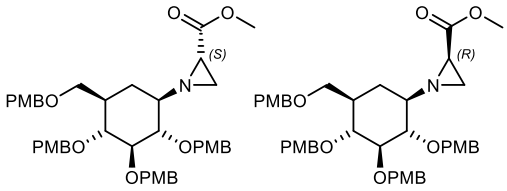
1-*Epi*-2,3,4,6-tetra-*O*-(4-methoxybenzyl)-validamine (20).



Compound **19** (159 mg, 0.23 mmol) was dissolved in THF (4.6 mL, 0.05 M) followed by the addition of aq. NaOH (1.0 M solution in water, 0.92 mL, 0.92 mmol, 4.0 eq.) and PMe_3 (0.92 mL, 0.92 mmol, 4.0 eq.). Stirring continued overnight upon which full conversion was observed (R_f 0.1 (EtOAc:pentane, 1:1, v:v)). The mixture was diluted with water and subsequently the aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (30:70 EtOAc:pentane → 100:0 EtOAc:pentane) yielded the title compound **20** (112 mg, 0.17 mmol, 74%).^{1}H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.34 – 7.08 (m, 8H, CH_{arom}), 6.90 – 6.79 (m, 8H, CH_{arom}), 4.93 (d, *J* = 10.8 Hz, 1H, CH*H* PMB), 4.86 – 4.76 (m, 3H, CH*H* PMB, CH*H* PMB, C*H*H PMB), 4.61 (d, *J* = 10.9 Hz, 1H, C*H*H PMB), 4.47 – 4.35 (m, 3H, CH*H* PMB, C*H*H PMB, C*H*H PMB), 3.79 (s, 3H, OMe), 3.79 (s, 6H, OMe, OMe), 3.78 (s, 3H, OMe), 3.56 – 3.40 (m, 4H, H-3, H-4, H-6), 3.10 (t, *J* = 9.3 Hz, 1H, H-2), 2.73 (ddd, *J* = 11.9, 9.4, 4.2 Hz, 1H, H-1), 1.87 (ddd, *J* = 13.3, 4.0, 4.0 Hz, 1H, H-7), 1.74 (ddd, *J* = 13.9, 13.0, 7.1 Hz, 1H, H-5), 1.29 (q, *J* = 12.7 Hz, 1H, H-7); ^{13}C NMR (101 MHz, $CDCl_3$, HSQC): δ 159.4, 159.3, 159.2, 159.2, 131.1, 131.0, 130.9, 130.6 ($C_{q\text{-}arom}$), 129.7, 129.6, 129.4, 129.3 (CH_{arom}), 87.3 (C-3), 87.2 (C-2), 81.3 (C-4), 75.3, 75.3, 75.0, 72.8

(CH_2 PMB), 69.8 (C-6), 55.4, 55.3, 52.9 (OMe), 40.6 (C-5), 33.3 (C-7); HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{39}H_{48}NO_8$ 658.3380; Found 658.3369.

Methyl (*S*)-1-(1-*epi*-validamine)aziridine-2-carboxylate (21) and methyl (*R*)-1-(1-*epi*-validamine)aziridine-2-carboxylate (22).

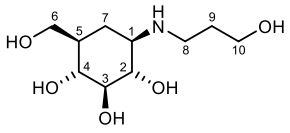


Compounds **21** and **22** were prepare according to **general procedure A** using **20** (133 mg, 0.2 mmol), DiPEA (0.28 mL, 1.6 mmol, 8.0 eq.) and methyl 2,3-dibromopropanoate (100 µL, 0.8 mmol, 4.0 eq.) in MeOH (2.0 mL, 0.1 M). Flash column chromatography (25:75 EtOAc:pentane → 40:60 EtOAc:pentane) yielded the title compounds **21** as a white solid (45 mg, 60 µmol, 30%) and **22** as a white solid (59 mg, 80 µmol, 40%). R_f 0.4 and 0.3 for compound **21** and **22** respectively (EtOAc:pentane, 7:3, v:v).

Analytical data for **21**: ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HH-NOESY, HSQC): δ 7.33 – 7.02 (m, 8H, CH_{arom}), 6.91 – 6.75 (m, 8H, CH_{arom}), 4.90 – 4.71 (m, 5H, C*H*H PMB, C*H*H PMB, C*H*H PMB, CH*H* PMB, CH*H* PMB), 4.43 – 4.36 (m, 3H, C*H*H PMB, CH*H* PMB, CH*H* PMB), 3.79 (s, 3H, OMe), 3.79 (s, 6H, OMe, OMe), 3.78 (s, 3H, OMe), 3.74 (s, 3H, OMe), 3.66 (dddd, *J* = 10.2, 6.6, 3.3, 3.3 Hz, 1H, H-2), 3.53 – 3.47 (m, 2H, H-6), 3.43 – 3.38 (m, 2H, H-3, H-4), 2.31 (d, *J* = 3.2 Hz, 1H, H-8), 2.10 (d, *J* = 6.9 Hz, 1H, H-8), 2.03 (dd, *J* = 6.5, 3.3 Hz, 1H, H-9), 1.96 – 1.85 (m, 1H, H-7), 1.64 (m, 2H, H-5, H-7), 1.47 – 1.38 (m, 1H, H-1); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 171.8 (C=O), 159.2, 159.2, 159.2, 159.1, 131.1, 130.9, 130.8, 130.5 ($C_{q\text{-}arom}$), 129.6, 129.5, 129.3, 129.2, 113.9, 113.9, 113.8, 113.8 (CH_{arom}), 87.0 (C-3/C-4), 85.4 (C-2), 80.7 (C-3/C-4), 75.5, 75.3, 75.0, 72.8 (CH_2 PMB), 70.0 (C-1), 69.8 (C-6), 55.3, 55.3, 55.3, 52.4 (OMe), 40.2 (C-5), 36.1 (C-8), 34.6 (C-9), 31.2 (C-7); HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{43}H_{52}NO_{10}$ 742.3591; Found 742.3582.

Analytical data for **22**: ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HH-NOESY, HSQC): δ 7.32 – 7.06 (m, 8H, CH_{arom}), 6.91 – 6.74 (m, 8H, CH_{arom}), 4.83 (d, *J* = 10.6 Hz, 1H, C*H*H PMB), 4.79 – 4.75 (m, 3H, C*H*H PMB, C*H*H PMB, CH*H* PMB), 4.70 (d, *J* = 10.5 Hz, 1H, CH*H* PMB), 4.43 – 4.37 (m, 3H, C*H*H PMB, CH*H* PMB, CH*H* PMB), 3.80 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.78 (s, 6H, OMe, OMe), 3.63 (t, *J* = 9.2 Hz, 1H, H-2), 3.56 (s, 3H, OMe), 3.51 (dd, *J* = 8.8, 2.3 Hz, 1H, H-6), 3.47 – 3.37 (m, 3H, H-3, H-4, H-6), 2.56 (dd, *J* = 6.7, 3.2 Hz, 1H, H-9), 2.04 (d, *J* = 3.2 Hz, 1H, H-8), 1.91 – 1.85 (m, 1H, H-7), 1.72 – 1.62 (m, 2H, H-5, H-7), 1.59 (d, *J* = 6.8 Hz, 1H, H-8), 1.49 (ddd, *J* = 11.2, 9.2, 4.2 Hz, 1H, H-1); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 171.4 (C=O), 159.3, 159.2, 159.1, 159.0, 131.0 ($C_{q\text{-}arom}$), 130.9, 130.8, 130.5, 129.7, 129.2, 129.2, 128.9 (CH_{arom}), 86.9 (C-3/C-4), 85.6 (C-2), 80.9 (C-3/C-4), 75.5, 75.0, 72.8 (CH_2 PMB), 69.9 (C-6), 69.3 (C-1), 55.3, 52.1 (OMe), 40.3 (C-5), 39.0 (C-9), 31.1 (C-7), 30.8 (C-8); HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{43}H_{52}NO_{10}$ 742.3591; Found 742.3575.

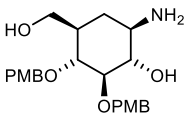
3-*N*-Propan-1-ol-(1-*epi*-2,3,4,6-tetra-*O*-(4-methoxybenzyl)-validamine) (23).



To liquid ammonia (3 mL) at -60 °C, sodium metal (74 mg, 3.2 mmol, 40 eq.) was added. This mixture was stirred for 30 minutes while maintaining a temperature of -60 °C. Subsequently, Compound **22** (60 mg, 81 µmol) was dissolved in THF (1.0 mL) followed by the addition *t*-BuOH (74 µL, 0.81 mmol, 10 eq.). This solution was added dropwise to

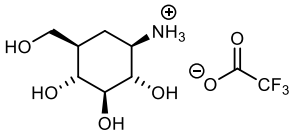
the flask containing ammonia. The solution was stirred for 1 hour while maintaining a temperature of -60 ºC. The reaction was quenched by addition of water (500 µL), let to attain to room temperature and concentrated under reduced pressure. The residue was purified by size exclusion chromatography over HW-40 eluted with water to obtain the title compound **23** as a colorless oil (5.0 mg, 10 µmol, 91%). Flash column chromatography (10:90 MeOH:DCM → 40:60 MeOH:DCM) yielded the title compound (14 mg, 58 µmol, 71%). (R_f 0.3 (MeOH:DCM, 2:8, v:v)); ^{1}H NMR (600 MHz, D_2O, HH-COSY, HSQC): δ 3.97 (dd, *J* = 11.4, 3.5 Hz, 1H, H-6), 3.96 – 3.90 (m, 2H, H-10), 3.87 (dd, *J* = 11.4, 5.9 Hz, 1H, H-6), 3.72 (dd, *J* = 10.4, 8.7 Hz, 1H, H-2), 3.61 – 3.45 (m, 4H, H-1, H-3, H-4, H-8), 3.39 (ddd, *J* = 12.5, 8.6, 6.3 Hz, 1H, H-8), 2.43 (ddd, *J* = 12.9, 4.0, 4.0 Hz, 1H, H-7), 2.25 – 2.08 (m, 2H, H-9), 1.92 (ddddd, *J* = 13.3, 9.6, 6.8, 3.6, 3.6 Hz, 1H, H-5), 1.65 (q, *J* = 12.7 Hz, 1H, H-7); ^{13}C NMR (151 MHz, D_2O, HSQC): δ 78.3 (C-3), 73.6 (C-2), 73.3 (C-4), 63.2 (C-6), 60.8 (C-10), 60.0 (C-1), 44.6 (C-8), 42.1 (C-5), 29.2 (C-9), 26.6 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{10}H_{22}NNaO_5$ 258.1317; Found 258.1504.

1-*Epi*-3,4-di-*O*-(4-methoxybenzyl)-validamine (24).



Compound **18** (0.22 g, 0.5 mmol) was dissolved in THF (10 mL, 0.05 M) followed by the addition of aq. NaOH (1.0 M solution, 2.0 mL, 2.0 mmol, 4.0 eq.) and PMe_3 (1.0 M solution in THF, 2.0 mL, 2.0 mmol, 4.0 eq.). The reaction mixture was stirred for 16 hours at room temperature. Upon full conversion (R_f 0.2 (MeOH:DCM, 1:9, v:v)), the mixture was diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (5:95 MeOH:DCM → 10:90 MeOH:DCM) yielded the title compound **24** (162 mg, 0.39 mmol, 78%). ^{1}H NMR (500 MHz, MeOD, HH-COSY, HSQC): δ 7.35 – 7.17 (m, 4H, CH_{arom}), 6.95 – 6.78 (m, 4H, CH_{arom}), 4.88 – 4.83 (m, 1H, CH*H* PMB), 4.74 (m, 2H, CH*H* PMB, C*H*H PMB), 4.52 (d, *J* = 10.5 Hz, 1H, CH*H* PMB), 3.78 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.70 (dd, *J* = 10.7, 3.1 Hz, 1H, H-6), 3.58 (dd, *J* = 10.7, 5.9 Hz, 1H, H-6), 3.37 – 3.27 (m, 2H, H-3, H-4), 3.14 (ddd, *J* = 9.3, 6.8, 2.4 Hz, 1H, H-2), 2.61 (ddd, *J* = 11.9, 9.6, 4.3 Hz, 1H, H-1), 1.91 (ddd, *J* = 13.3, 4.0, 4.0 Hz, 1H, H-7), 1.62 (dddd, *J* = 12.7, 9.6, 3.3, 3.3 Hz, 1H, H-5), 1.17 (q, *J* = 12.9 Hz, 1H, H-7); ^{13}C NMR (126 MHz, MeOD, HSQC): δ 160.8, 160.7, 132.5, 132.1 ($C_{q\text{-}arom}$), 130.6, 130.6, 114.7, 114.6 (CH_{arom}), 88.1, 82.1 (C-3, C-4), 79.9 (C-2), 76.2, 75.6 (CH_2 PMB), 63.2 (C-2), 55.7 (OMe), 54.3 (C-1), 43.5 (C-5), 33.1 (C-7); HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{23}H_{32}NO_6$ 418.2230; Found 418.2225.

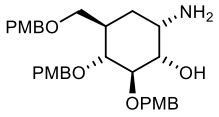
1-*Epi*-validammonium trifluoroacetate (6).



Compound **24** (162 mg, 0.39 mmol) was dissolved in anhydrous DCM (7.8 mL, 0.05M) and cooled on ice. Subsequently, TFA (0.30 mL, 3.9 mmol, 10 eq.) was added and the reaction was stirred for 1 hour while keeping on ice. Upon full conversion was observed (R_f 0.2 (MeOH:DCM, 1:1, v:v + 2% Et_3N)), the mixture was concentrated under reduced pressure and co-evaporated twice with water. The solid precipitate was filtered off using a cotton plug and rinsed with water. The filtrate was concentrated under reduced pressure to give the title compound **6** (103 mg, 0.35 mmol, 91%). ^{1}H NMR (500 MHz, D_2O, HH-COSY, HSQC): δ 3.71 (dd, *J* = 11.3, 3.4 Hz, 1H, H-6), 3.60 (dd, *J* = 11.3, 6.0 Hz, 1H, H-6), 3.35 (dd, *J* = 10.2, 9.3 Hz, 1H, H-2), 3.32

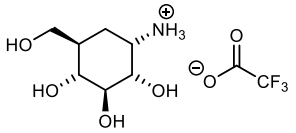
– 3.23 (m, 2H, H-3, H-4), 3.15 (ddd, *J* = 12.4, 10.1, 4.3 Hz, 1H, H-1), 2.06 (ddd, *J* = 13.0, 4.0, 4.0 Hz, 1H, H-7), 1.70 – 1.59 (m, 1H, H-5), 1.38 (q, *J* = 12.7 Hz, 1H, H-7); ^{13}C NMR (126 MHz, D_2O, HSQC): δ 76.9 (C-3/C-4), 73.1 (C-2), 71.9 (C-3/C-4), 61.5 (C-6), 52.4 (C-1), 40.8 (C-5), 27.6 (C-7); ^{19}F NMR (376 MHz, MeOD): δ -76.81; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_7H_{16}NO_4$ 178.1074; Found 178.1074.

3,4,6-Tri-*O*-(4-methoxybenzyl)-validamine (30).



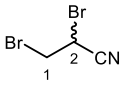
Compound **29** (1.0 g, 1.8 mmol) was dissolved in EtOH (18 mL, 0.1 M) after which NaOH (1.5 g, 37 mmol, 20 eq.) was added. The reaction was heated to 80 °C and stirred for 16 hours. Upon full conversion was observed (R_f 0.4 (MeOH:DCM, 1:9, v:v)), the mixture was diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (0:100 MeOH:DCM → 10:90 MeOH:DCM) yielded the title compound **30** (0.96 g, 1.8 mmol, 98%). ^{1}H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.29 – 7.12 (m, 6H, CH_{arom}), 6.89 – 6.80 (m, 6H, CH_{arom}), 4.82 (d, *J* = 11.0 Hz, 1H, C*H*H PMB), 4.71 (d, *J* = 10.6 Hz, 1H, C*H*H PMB), 4.64 (d, *J* = 11.1 Hz, 1H, CH*H* PMB), 4.45 (d, *J* = 10.6 Hz, 1H, CH*H* PMB), 4.40 (d, *J* = 11.7 Hz, 1H, C*H*H PMB), 4.36 (d, *J* = 11.6 Hz, 1H, CH*H* PMB), 3.79 (d, *J* = 1.0 Hz, 6H, OMe), 3.78 (s, 3H, OMe), 3.73 – 3.65 (m, 1H, H-3), 3.64 – 3.58 (m, 1H, H-6), 3.53 (bs, 1H, H-2), 3.43 (m, 2H, H-4, H-6), 3.33 (bs, 1H, H-1), 2.72 (bs, 3H, 1-NH_2, 2-OH), 2.15 (m, 1H, H-5), 1.78 (d, *J* = 14.1 Hz, 1H, H-7), 1.65 (bs, 1H, H-7); ^{13}C NMR (101 MHz, $CDCl_3$, HSQC): δ 159.4, 159.3, 159.2, 131.0, 130.8, 130.6 ($C_{q\text{-}arom}$), 129.6, 129.4, 114.1, 113.9, 113.9 (CH_{arom}), 82.6 (C-3), 80.3 (C-4), 74.7, 74.2 (CH_2 PMB), 73.8 (C-2), 72.8 (CH_2 PMB), 69.9 (C-6), 55.4 (OMe), 55.4 (OMe), 49.2 (C-1), 37.4 (C-5), 29.5 (C-7); HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{31}H_{40}NO_7$ 538.2805; Found 538.2800.

Validammonium trifluoroacetate (7).



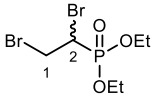
Compound **30** (0.32 g, 0.60 mmol) was dissolved in anhydrous DCM (12 mL, 0.05M) and cooled on ice. Subsequently, TFA (0.46 mL, 6.0 mmol, 10 eq.) was added and the reaction was stirred for 1 hour while keeping on ice. Upon full conversion was observed (R_f 0.2 (MeOH:DCM, 1:1, v:v + 2% Et_3N)), the mixture was concentrated under reduced pressure and co-evaporated twice with water. The solid precipitate was filtered off using a cotton plug and rinsed with water. The filtrate was concentrated under reduced pressure to give the title compound **7** (0.18 g, 0.60 mmol, quant.). Analytical data in full agreement with literature data.[34–37] ^{1}H NMR (500 MHz, D_2O, HH-COSY, HSQC): δ 3.78 – 3.68 (m, 3H, H-1, H-2, H-6), 3.65 (dd, *J* = 11.3, 5.2 Hz, 1H, H-6), 3.47 (t, *J* = 9.5 Hz, 1H, H-3), 3.31 (dd, *J* = 10.2, 9.2 Hz, 1H, H-4), 2.01 (dd, *J* = 11.7, 2.5 Hz, 1H, H-7), 1.77 – 1.66 (m, 2H, H-5, H-7); ^{13}C NMR (126 MHz, D_2O, HSQC): δ 73.8 (C-3), 72.1 (C-4), 70.0 (C-2), 61.5 (C-6), 51.3 (C-1), 38.0 (C-5), 26.0 (C-7); ^{19}F NMR (376 MHz, D_2O): δ -75.75; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_7H_{16}NO_4$ 178.1074; Found 178.1074.

2,3-Dibromopropanenitrile (B).



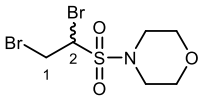
Prepared according to literature procedure.[44] Acrylonitrile (1.3 mL, 20 mmol) was dissolved in acetonitrile (10 mL, 2.0 M) and cooled on ice. Bromine (1.0 mL, 20 mmol, 1.0 eq.) was added dropwise and the reaction was allowed to attain to room temperature over a period of 2 hours after which the reaction was quenched by addition of sat. aq. $Na_2S_2O_3$ solution and diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with H_2O, sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the title compound **B** as an inseparable mixture of stereoisomers (4.4 g, 20 mmol, quant.). Analytical data in full agreement with literature data.[44] ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.54 (ddd, *J* = 9.1, 6.4, 1.4 Hz, 1H, H-1), 3.79 (d, *J* = 3.4 Hz, 1H, H-2), 3.77 (d, *J* = 0.8 Hz, 1H, H-2).

Diethyl (1,2-dibromoethyl)phosphonate (C).



Prepared according to literature procedure.[42] diethyl vinylphosphonate (1.2 mL, 10 mmol) was dissolved in DCM (50 mL, 0.2 M) and cooled on ice. Bromine (0.77 mL, 15 mmol, 1.5 eq.) was added dropwise and the reaction was allowed to attain to room temperature over a period of 30 minutes after which the reaction was quenched by addition of sat. aq. $Na_2S_2O_3$ solution and diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with H_2O, sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (40:60 EtOAc:pentane → 60:40 EtOAc:pentane) yielded the title compound **C** as an inseparable mixture of stereoisomers (2.0 g, 6.8 mmol, 68%). Analytical data in full agreement with literature data.[42] (R_f 0.1 (EtOAc:pentane 1:1 v:v)); ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 4.28 – 4.18 (m, 4H, CH_2 OEt, CH_2 OEt), 4.09 – 3.97 (m, 2H, H-1, H-2), 3.62 (tdd, *J* = 9.2, 7.8, 4.4 Hz, 1H, H-1), 1.40 – 1.31 (m, 6H, CH_3 OEt, CH_3 OEt); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 64.5, 64.5, 64.3, 64.2 (CH_2 OEt), 42.8, 41.6 (C-1), 32.1, 32.1 (C-2), 16.6, 16.5 (CH_3 OEt); ^{31}P NMR (202 MHz, $CDCl_3$) δ 17.26.

4-((1,2-Dibromoethyl)sulfonyl)morpholine (D).

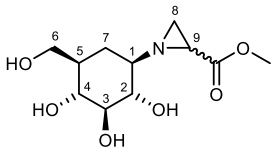


Prepared according to literature procedure.[43] 2-chloroethane-1-sulfonyl chloride (0.52 mL, 5.0 mmol) was dissolved in anhydrous DCM (10 mL, 0.5 M) and cooled on ice, Et_3N (2.1 mL, 15 mmol, 3.0 eq.) and morpholine (0.48 mL, 5.5 mmol, 1.1 eq.) were added subsequently and the reaction was allowed to attain to room temperature over a period of 2 hours upon which the reaction was quenched by addition of sat. aq. $NaHCO_3$ solution and diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with H_2O, sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo.*

The crude intermediate was dissolved in acetonitrile (5.0 mL, 1.0 M) and cooled on ice. Bromine (0.26 mL, 5.0 mmol, 1.0 eq.) was added dropwise and the reaction was allowed to attain to room temperature over a period of 2 hours after which the reaction was quenched by addition of sat. aq. $Na_2S_2O_3$ solution and diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with H_2O, sat. aq. $NaHCO_3$ and brine

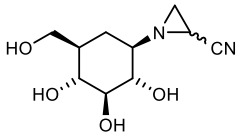
respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (10:90 EtOAc:pentane → 30:70 EtOAc:pentane) yielded the title compound **D** as an inseparable mixture of stereoisomers (1.3 g, 3.9 mmol, 78%). Analytical data in full agreement with literature data.[43] (R_f 0.3 (EtOAc:pentane 2:8 v:v)); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.94 (dd, *J* = 10.0, 3.1 Hz, 1H, H-2), 4.20 (dd, *J* = 11.5, 3.2 Hz, 1H, H-1), 3.77 – 3.66 (m, 5H, H-1, CH_2 morpholine, CH_2 morpholine), 3.51 – 3.41 (m, 4H, CH_2 morpholine, CH_2 morpholine).

Methyl 1-(1-*epi*-validamine)aziridine-2-carboxylate (8).



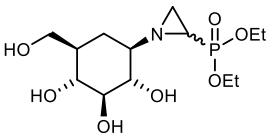
Compound **8** was prepared according to **general procedure A** using 1-*epi*-validamine **6** (29 mg, 0.1 mmol), DiPEA (0.14 mL, 0.8 mmol, 8.0 eq.) and methyl 2,3-dibromopropanoate **A** (50 µL, 0.4 mmol, 4.0 eq.) in MeOH (1.0 mL, 0.1 M). Flash column chromatography (0:100 MeOH:DCM → 20:80 MeOH:DCM) yielded the title compound **8** (25 mg, 96 µmol, 96%). R_f 0.3 (MeOH:DCM, 2:8, v:v); ^{1}H NMR (500 MHz, MeOD, HH-COSY, HSQC): δ 3.79 – 3.70 (m, 8H, H-6, H-6', OMe, OMe'), 3.61 – 3.56 (m, 2H, H-6, H-6'), 3.46 – 3.36 (m, 2H, H-2, H-2'), 3.26 – 3.21 (m, 2H, H-4, H-4'), 3.18 – 3.13 (m, 2H, H-3, H-3'), 2.64 (dd, *J* = 6.7, 3.5 Hz, 1H, H-9), 2.30 – 2.25 (m, 2H, H-8', H-9'), 2.16 (dd, *J* = 6.0, 1.1 Hz, 1H, H-8'), 1.98 (dd, *J* = 3.5, 0.8 Hz, 1H, H-8), 1.91 (t, *J* = 3.9 Hz, 1H, H-7/H-7'), 1.88 (t, *J* = 3.9, Hz, 1H, H-7/H-7'), 1.75 (dd, *J* = 6.7, 0.8 Hz, 1H, H-8), 1.58 – 1.47 (m, 4H, H-1, H-1', H-5, H-5'), 1.38 – 1.24 (m, 2H, H-7, H-7'); ^{13}C NMR (126 MHz, MeOD, HSQC): δ 173.2, 172.9 (C=O, C=O'), 79.4, 79.3 (C-3, C-3'), 78.0, 77.9 (C-2, C-2'), 74.7, 74.6 (C-4, C-4'), 70.6, 70.1 (C-1, C-1'), 64.2, 64.1 (C-6, C-6'), 52.8, 52.7 (OMe, OMe'), 42.9, 42.8 (C-5, C-5'), 39.6 (C-9), 36.6 (C-8'), 34.5 (C-9'), 31.3, 31.0 (C-7, C-7'), 30.8 (C-8); HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{11}H_{19}NO_6$ 262.1291; Found 262.1285.

1-(1-*Epi*-validamine)aziridine-2-carbonitrile (9).



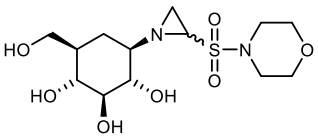
Compound **9** was prepared according to **general procedure A** using 1-*epi*-validamine **6** (29 mg, 0.1 mmol), DiPEA (0.14 mL, 0.8 mmol, 8.0 eq.) and 2,3-dibromopropanenitrile **B** (85 mg, 0.4 mmol, 4.0 eq.) in MeOH (1.0 mL, 0.1 M). Flash column chromatography (0:100 MeOH:DCM → 20:80 MeOH:DCM) and consecutively (50:50 acetone:DCM → 70:30 acetone:DCM) yielded the title compound **9** (19 mg, 82 µmol, 82%). R_f 0.3 (MeOH:DCM, 2:8, v:v); ^{1}H NMR (500 MHz, MeOD, HH-COSY, HSQC): δ 3.80 – 3.71 (m, 2H, H-6, H-6'), 3.62 – 3.53 (m, 2H, H-6, H-6'), 3.46 – 3.37 (m, 2H, H-2, H-2'), 3.27 – 3.17 (m, 2H, H-4, H-4'), 3.14 – 3.06 (m, 2H, H-3, H-3'), 2.61 (dd, *J* = 6.6, 3.5 Hz, 1H, H-9), 2.39 – 2.32 (m, 2H, H-8', H-9'), 2.20 (d, *J* = 6.4 Hz, 1H, H-8'), 2.11 (d, *J* = 3.5 Hz, 1H, H-8), 1.97 (ddd, *J* = 12.6, 3.4, 3.4 Hz, 1H, H-7/H-7'), 1.87 (ddd, *J* = 13.3, 3.9, 3.9 Hz, 1H, H-7/H-7'), 1.81 (d, *J* = 6.6 Hz, 1H, H-8), 1.58 – 1.22 (m, 6H, H-1, H-1', H-5, H-5', H-7, H-7'); ^{13}C NMR (126 MHz, MeOD, HSQC): δ 120.3, 120.1 (CN, CN'), 79.7, 79.4 (C-3, C-3'), 78.0, 77.9 (C-2, C-2'), 74.5 (C-4, C-4'), 70.8, 70.5 (C-1, C-1'), 64.1, 64.0 (C-6, C-6'), 43.0, 42.9 (C-5, C-5'), 36.5 (C-8'), 31.2, 31.0 (C-7, C-7'), 30.5 (C-8), 25.7 (C-9), 20.3 (C-9'); HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{10}H_{17}N_2O_4$ 229.1188; Found 229.1183.

Diethyl 1-(1-*epi*-validamine)aziridine-2-phosphonate (10).



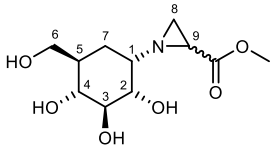
Compound **10** was prepared according to **general procedure A** using 1-*epi*-validamine **6** (29 mg, 0.1 mmol), DiPEA (0.14 mL, 0.8 mmol, 8.0 eq.) and diethyl (1,2-dibromoethyl)phosphonate **D** (130 mg, 0.4 mmol, 4.0 eq.) in MeOH (1.0 mL, 0.1 M). Flash column chromatography (0:100 MeOH:DCM → 20:80 MeOH:DCM) and consecutively (70:30 acetone:DCM → 95:5 acetone:DCM) yielded the title compound **10** (25 mg, 73 µmol, 73%). R_f 0.4 (MeOH:DCM, 2:8, v:v); ^{1}H NMR (500 MHz, MeOD, HH-COSY, HSQC): δ 4.24 – 4.11 (m, 8H, CH_2 OEt, CH_2 OEt, CH_2 OEt', CH_2 OEt'), 3.76 (dd, *J* = 10.8, 4.0 Hz, 2H, H-6, H-6'), 3.56 (dd, *J* = 10.8, 6.3 Hz, 2H, H-6, H-6'), 3.42 (dd, *J* = 9.1, 9.0 Hz, 2H, H-2, H-2'), 3.22 (dd, *J* = 10.4, 9.0 Hz, 2H, H-4, H-4'), 3.13 (dd, *J* = 9.1, 9.0 Hz, 2H, H-3, H-3'), 2.13 (dd, *J* = 7.0, 3.9 Hz, 1H, H-9), 2.09 (dd, *J* = 7.0, 3.9 Hz, 1H, H-9'), 1.98 (ddd, *J* = 9.6, 3.9, 0.7 Hz, 2H, H-8, H-8'), 1.90 (ddd, *J* = 13.0, 3.7, 3.6 Hz, 2H, H-7, H-7'), 1.70 (ddd, *J* = 7.8, 6.9, 0.7 Hz, 2H, H-8, H-8'), 1.52 – 1.44 (m, 2H, H-5, H-5'), 1.43 – 1.26 (m, 16H, H-1, H-1', H-7, H-7', CH_3 OEt, CH_3 OEt, CH_3 OEt', CH_3 OEt'); ^{13}C NMR (126 MHz, MeOD, HSQC): δ 79.5 (C-3, C-3'), 78.4 (C-2, C-2'), 74.7 (C-4, C-4'), 71.8, 71.8 (C-1, C-1'), 64.3, 64.2, 64.2 (C-6, C-6', CH_2 OEt, CH_2 OEt', CH_2 OEt, CH_2 OEt'), 43.0 (C-5, C-5'), 34.4 (C-9), 32.6 (C-9'), 31.4 (C-7, C-7'), 28.6, 28.6 (C-8, C-8'), 16.7, 16.7, 16.7, 16.7 (CH_3 OEt, CH_3 OEt', CH_3 OEt, CH_3 OEt'); ^{31}P NMR (202 MHz, MeOD): δ 23.82; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{13}H_{27}NO_7P$ 340.1525; Found 340.1519.

Morpholino 1-(1-*epi*-validamine)aziridine-2-sulfonamide (11).



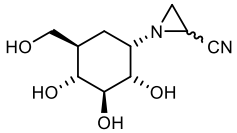
Compound **11** was prepared according to **general procedure A** using 1-*epi*-validamine **6** (29 mg, 0.1 mmol), DiPEA (0.14 mL, 0.8 mmol, 8.0 eq.) and 4-((1,2-dibromoethyl)sulfonyl)morpholine **D** (135 mg, 0.4 mmol, 4.0 eq.) in MeOH (1.0 mL, 0.1 M). Flash column chromatography (0:100 MeOH:DCM → 20:80 MeOH:DCM) and consecutively (70:30 acetone:DCM → 95:5 acetone:DCM) yielded the title compound **11** (28 mg, 79 µmol, 79%). R_f 0.5 (MeOH:DCM, 2:8, v:v); ^{1}H NMR (500 MHz, D_2O, HH-COSY, HSQC): δ 5.48 (dd, *J* = 8.9, 4.0 Hz, 1H, H-9), 5.42 (dd, *J* = 7.7, 4.6 Hz, 1H, H-9'), 3.83 – 3.76 (m, 10H, H-6, H-6', H-10, H-10'), 3.67 – 3.61 (m, 2H, H-6, H-6'), 3.59 – 3.46 (m, 10H, H-8, H-8', H-10, H-10'), 3.36 – 3.17 (m, 8H, H-2, H-2', H-3, H-3', H-4, H-4', H-8, H-8'), 2.75 – 2.56 (m, 2H, H-1, H-1'), 2.08 – 2.02 (m, 2H, H-7, H-7'), 1.68 – 1.58 (m, 2H, H-5, H-5'), 1.14 – 1.01 (m, 2H, H-7, H-7'); ^{13}C NMR (126 MHz, D_2O, HSQC): δ 77.6, 77.6 (C-3/C-4, C-3'/C-4'), 75.6, 75.5 (C-2, C-2'), 72.7, 72.7 (C-3/C-4, C-3'/C-4'), 66.6 (C-11, C-11'), 62.3 (C-6, C-6'), 61.1, 60.9 (C-9, C-9'), 57.8, 56.5 (C-1, C-1'), 48.1, 48.0, 47.1, 47.0 (C-8, C-8'), 46.8 (C-10, C-10'), 41.1, 41.0 (C-5, C-5'), 28.7, 28.2 (C-7, C-7'); HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{13}H_{25}N_2O_7S$ 353.1383; Found 353.1376.

Methyl 1-(validamine)aziridine-2-carboxylate (12).



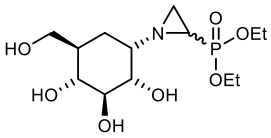
Compound **12** was prepared according to **general procedure A** using validamine **7** (29 mg, 0.1 mmol), DiPEA (0.14 mL, 0.8 mmol, 8.0 eq.) and commercially available methyl 2,3-dibromopropanoate **A** (40 µL, 0.4 mmol, 4.0 eq.) in MeOH (1.0 mL, 0.1 M). Flash column chromatography (0:100 MeOH:DCM → 20:80 MeOH:DCM) and consecutively (70:30 acetone:DCM → 95:5 acetone:DCM) yielded the title compound **12** (22 mg, 85 µmol, 85%). R_f 0.3 (MeOH:DCM, 2:8, v:v); ^{1}H NMR (500 MHz, MeOD, HH-COSY, HSQC): δ 3.93 (t, *J* = 9.3 Hz, 1H, H-3/H-3'), 3.86 (t, *J* = 9.3 Hz, 1H, H-3/H-3'), 3.74 – 3.65 (m, 8H, H-6, H-6', OMe, OMe'), 3.63 – 3.55 (m, 2H, H-6, H-6'), 3.46 – 3.39 (m, 2H, H-2, H-2'), 3.24 – 3.16 (m, 2H, H-4, H-4'), 2.47 (dd, *J* = 6.4, 3.2 Hz, 1H, H-9), 2.26 (dd, *J* = 3.1, 1.1 Hz, 1H, H-8'), 2.23 – 2.14 (m, 1H, H-5/H-5'), 2.10 – 1.97 (m, 3H, H-5/H-5', H-8', H-9'), 1.94 – 1.87 (m, 3H, H-1, H-1', H-8), 1.86 – 1.78 (m, 2H, H-7, H-7'), 1.50 (dd, *J* = 6.5, 1.3 Hz, 1H, H-8), 1.38 – 1.28 (m, 2H, H-7, H-7'); ^{13}C NMR (126 MHz, MeOD, HSQC): δ 173.8, 173.7 (C=O, C=O'), 76.5 (C-3, C-3'), 76.4, 76.2 (C-2, C-2'), 75.9, 75.8 (C-4, C-4'), 69.4, 69.0 (C-1, C-1'), 64.5, 64.5 (C-6, C-6'), 52.6, 52.5 (OMe, OMe'), 40.6, 40.5 (C-5, C-5'), 40.0 (C-9), 37.4 (C-8'), 34.2 (C-9'), 30.9, 30.6 (C-7, C-7'), 30.5 (C-8); HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{11}H_{20}NO_6$ 262.1291; Found 262.1285.

1-(Validamine)aziridine-2-carbonitrile (13).



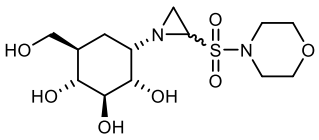
Compound **13** was prepared according to **general procedure A** using validamine **7** (29 mg, 0.1 mmol), DiPEA (0.14 mL, 0.8 mmol, 8.0 eq.) and 2,3-dibromopropanenitrile **B** (85 mg, 0.4 mmol, 4.0 eq.) in MeOH (1.0 mL, 0.1 M). Flash column chromatography (0:100 MeOH:DCM → 20:80 MeOH:DCM) and consecutively (50:50 acetone:DCM → 70:30 acetone:DCM) yielded the title compound **13** (18 mg, 82 µmol, 82%) as a 6:1 mixture of diastereoisomers. R_f 0.4 (MeOH:DCM, 2:8, v:v); data for the major stereoisomer: ^{1}H NMR (500 MHz, MeOD, HH-COSY, HSQC): δ 3.89 (t, *J* = 9.2 Hz, 1H, H-3), 3.73 (dd, *J* = 10.7, 4.0 Hz, 1H, H-6), 3.60 (dd, *J* = 10.7, 6.1 Hz, 1H, H-6), 3.45 (dd, *J* = 9.6, 3.4 Hz, 1H, H-2), 3.22 (dd, *J* = 10.7, 8.9 Hz, 1H, H-4), 2.49 (dd, *J* = 6.4, 3.3 Hz, 1H, H-9), 2.15 – 2.06 (m, 1H, H-5), 2.05 (d, *J* = 3.2 Hz, 1H, H-8), 1.87 (td, *J* = 3.2, 3.1 Hz, 1H, H-1), 1.81 (ddd, *J* = 14.3, 3.6, 3.6 Hz, 1H, H-7), 1.62 (d, *J* = 6.2 Hz, 1H, H-8), 1.34 (ddd, *J* = 14.3, 12.8, 2.9 Hz, 1H, H-7); ^{13}C NMR (126 MHz, MeOD, HSQC): δ 120.5 (CN), 76.8 (C-3), 76.0 (C-2), 75.5 (C-4), 69.1 (C-1), 64.4 (C-6), 40.7 (C-5), 30.5 (C-7), 30.2 (C-8), 26.0 (C-9); data for the minor stereoisomer: ^{1}H NMR (500 MHz, MeOD, HH-COSY, HSQC): δ 3.82 – 3.75 (m, 1H, H-3), 3.53 (dd, *J* = 9.7, 3.6 Hz, 1H, H-2), 2.57 (dd, *J* = 5.3, 3.4 Hz, 1H, H-9), 2.40 (d, *J* = 5.3 Hz, 1H, H-8), 2.37 (d, *J* = 3.3 Hz, 1H, H-1), 2.02 (dd, *J* = 3.5, 3.4 Hz, 1H, H-7), 1.47 – 1.39 (m, 1H, H-7); ^{13}C NMR (126 MHz, MeOD, HSQC): δ 118.9 (CN), 76.7 (C-3), 76.0 (C-2), 75.7 (C-4), 66.1 (C-1), 40.6 (C-5), 36.9 (C-8), 30.5 (C-7), 18.4 (C-9); HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{10}H_{17}N_2O_4$ 229.1188; Found 229.1183.

Diethyl 1-(validamine)aziridine-2-phosphonate (14).



Compound **14** was prepared according to **general procedure A** using validamine **7** (29 mg, 0.1 mmol), DiPEA (0.14 mL, 0.8 mmol, 8.0 eq.) and diethyl (1,2-dibromoethyl)phosphonate **C** (130 mg, 0.4 mmol, 4.0 eq.) in MeOH (1.0 mL, 0.1 M). Flash column chromatography (0:100 MeOH:DCM → 15:85 MeOH:DCM) and consecutively (70:30 acetone:DCM → 90:10 acetone:DCM) yielded the title compound **14** (22 mg, 65 µmol, 65%). R_f 0.5 (MeOH:DCM, 2:8, v:v); ^{1}H NMR (500 MHz, MeOD, HH-COSY, HSQC): δ 4.21 – 4.12 (m, 8H, CH_2 OEt, CH_2 OEt, CH_2 OEt', CH_2 OEt'), 3.87 (dd, *J* = 9.6, 8.9 Hz, 2H, H-3, H-3'), 3.73 (dd, *J* = 10.7, 4.0 Hz, 2H, H-6, H-6'), 3.59 (dd, *J* = 10.7, 6.1 Hz, 2H, H-6, H-6'), 3.40 (dd, *J* = 9.7, 3.3 Hz, 2H, H-2, H-2'), 3.22 (dd, *J* = 10.6, 8.8 Hz, 2H, H-4, H-4'), 2.15 – 2.07 (m, 2H, H-5, H-5'), 2.05 (dd, *J* = 6.7, 3.6 Hz, 1H, H-9), 2.01 (dd, *J* = 6.7, 3.6 Hz, 1H, H-9'), 1.97 – 1.93 (m, 2H, H-8, H-8'), 1.88 – 1.83 (m, 4H, H-1, H-1', H-7, H-7'), 1.57 (ddd, *J* = 7.7, 6.7, 1.0 Hz, 2H, H-8, H-8'), 1.42 – 1.27 (m, 14H, H-7, H-7', CH_3 OEt, CH_3 OEt, CH_3 OEt', CH_3 OEt'); ^{13}C NMR (126 MHz, MeOD, HSQC): δ 77.0 (C-3, C-3'), 76.8 (C-2, C-2'), 75.8 (C-4, C-4'), 70.1, 70.1 (C-1, C-1'), 64.6, 64.6, 64.5, 64.2, 64.2 (C-6, C-6', CH_2 OEt, CH_2 OEt', CH_2 OEt, CH_2 OEt'), 40.7 (C-5, C-5'), 34.9 (C-9), 33.2 (C-9'), 31.0 (C-7, C-7'), 28.4, 28.4 (C-8, C-8'), 16.8, 16.8, 16.7, 16.7 (CH_3 OEt, CH_3 OEt', CH_3 OEt, CH_3 OEt'); ^{31}P NMR (202 MHz, MeOD): δ 25.71; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{13}H_{27}NO_7P$ 340.1525; Found 340.1519.

Morpholino 1-(validamine)aziridine-2-sulfonamide (15).



Compound **15** was prepared according to **general procedure A** using validamine **7** (29 mg, 0.1 mmol), DiPEA (0.14 mL, 0.8 mmol, 8.0 eq.) and 4-((1,2-dibromoethyl)sulfonyl)morpholine **D** (135 mg, 0.4 mmol, 4.0 eq.) in MeOH (1.0 mL, 0.1 M). Flash column chromatography (0:100 MeOH:DCM → 20:80 MeOH:DCM) and consecutively (50:50 acetone:DCM → 75:25 acetone:DCM) yielded the title compound **15** (31 mg, 87 µmol, 87%). R_f 0.5 (MeOH:DCM, 2:8, v:v); ^{1}H NMR (500 MHz, MeOD, HH-COSY, HSQC): δ 5.38 (dd, *J* = 8.3, 4.0 Hz, 1H, H-9), 5.32 (dd, *J* = 8.0, 4.4 Hz, 1H, H-9), 3.78 – 3.67 (m, 6H, H-6, H-6', H-11, H-11'), 3.65 – 3.55 (m, 4H, H-3, H-3', H-6, H-6'), 3.53 – 3.38 (m, 12H, H-2, H-2', H-8, H-8', H-10, H-10'), 3.24 – 3.15 (m, 3H, H-4, H-4', H-8/H-8'), 3.13 – 3.03 (m, 3H, H-1, H-1', H-8/H-8'), 2.03 – 1.85 (m, 4H, , H-5, H-5', H-7, H-7'), 1.32 – 1.19 (m, 2H, H-7, H-7'); ^{13}C NMR (126 MHz, MeOD, HSQC): δ 76.4, 76.4 (C-3, C-3'), 75.6, 75.6 (C-4, C-4'), 75.5, 75.4 (C-2, C-2'), 68.0, 68.0 (C-11, C-11'), 64.5 (C-6, C-6'), 63.7, 63.4 (C-9, C-9'), 58.0, 56.6 (C-1, C-1'), 51.0, 50.3 (C-8, C-8'), 48.3, 48.2 (C-10, C-10'), 39.7, 39.7 (C-5, C-5'), 28.9, 28.3 (C-7, C-7'); HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{13}H_{25}N_2O_7S$ 353.1383; Found 353.1371.

References

(1) Atsumi, S.; Iinuma, H.; Naganawa, H.; Nakamura, H.; Takeuchi, T.; Umezawa, K.; Iitaka, Y. Production, isolation and structure determination of a novel β-glucosidase inhibitor, cyclophellitol, from Phellinus Sp. *J. Antibiot.* **1990**, *43*, 49–53.

(2) Atsumi, S.; Iinuma, H.; Nosaka, C.; Umezewa, K. Biological activities of cyclophellitol. *J. Antibiot.* **1990**, *43*, 1579–1585.

(3) Shing, T. K. M.; Cui, Y.; Tang, Y. Facile syntheses of pseudo-α-D-glucopyranose and pseudo-α-D-mannopyranose. *J. Chem. Soc., Chem. Commun.* **1991**, *11*, 756–757.

(4) Tatsuta, K.; Niwata, Y.; Umezawa, K.; Toshima, K.; Nakata, M. Syntheses and enzyme inhibiting activities of cyclophellitol analogs. *J. Antibiot.* **1991**, *44*, 912–914.

(5) Gloster, T. M.; Madsen, R.; Davies, G. J. Structural basis for cyclophellitol inhibition of a β-glucosidase. *Org. Biomol. Chem.* **2007**, *5*, 444–446.

(6) McCarter, J. D.; Withers, G. S. Mechanisms of enzymatic glycoside hydrolysis. *Curr. Opin. Struct. Biol.* **1994**, *4*, 885–892.

(7) Brada, D.; Dubach, U. C. Isolation of a homogeneous glucosidase II from pig kidney microsomes. *Eur. J. Biochem.* **1984**, *141*, 149–156.

(8) O'Keefe, S.; Roebuck, Q. P.; Nakagome, I.; Hirono, S.; Kato, A.; Nash, R.; High, S. Characterizing the selectivity of ER α-glucosidase inhibitors. *Glycobiology* **2019**, *29*, 530–542.

(9) Rye, C. S.; Withers, S. G. Glycosidase mechanisms. *Curr. Opin. Chem. Biol.* **2000**, *4*, 573–580.

(10) Zechel, D. L.; Withers, S. G. Dissection of nucleophilic and acid–base catalysis in glycosidases. *Curr. Opin. Chem. Biol.* **2001**, *5*, 643–649.

(11) Vasella, A.; Davies, G. J.; Böhm, M. Glycosidase mechanisms. *Curr. Opin. Chem. Biol.* **2002**, *6*, 619–629.

(12) Kazlauskas, R. J.; Bornscheuer, U. T. 7.22 Enzyme catalytic promiscuity: expanding the catalytic action of enzymes to new reactions. *Compr. Chir.* **2012**, *7*, 465–480.

(13) Pang, P.; Cao, L.; Liu, Y.; Xie, W.; Wang, Z. Structures of a glucose-tolerant β-glucosidase provide insights into its mechanism. *J. Struct. Biol.* **2017**, *198*, 154–162.

(14) Artola, M.; Wu, L.; Ferraz, M. J.; Kuo, C.-L.; Raich, L.; Breen, I. Z.; Offen, W. A.; Codée, J. D. C.; van der Marel, G. A.; Rovira, C.; Aerts, J. M. F. G.; Davies, G. J.; Overkleeft, H. S. 1,6-Cyclophellitol cyclosulfates: a new class of irreversible glycosidase inhibitor. *ACS Cent. Sci.* **2017**, *3*, 784–793.

(15) McDevitt, R. E.; Fraser-Reid, B. A divergent route for a total synthesis of cyclophellitol and epicyclophellitol from a [2.2.2]oxabicyclic glycoside prepared from D-glucal. *J. Org. Chem.* **2002**, *59*, 3250–3252.

(16) de Boer, C.; McGregor, N. G. S.; Peterse, E.; Schröder, S. P.; Florea, B. I.; Jiang, J.; Reijngoud, J.; Ram, A. F. J.; van Wezel, G. P.; van der Marel, G. A.; Codée, J. D. C.; Overkleeft, H. S.; Davies, G. J. Glycosylated cyclophellitol-derived activity-based probes and inhibitors for cellulases. *RSC Chem. Biol.* **2020**, *1*, 148–155.

(17) Schröder, S. P.; van de Sande, J. W.; Kallemeijn, W. W.; Kuo, C.-L.; Artola, M.; van Rooden, E. J.; Jiang, J.; Beenakker, T. J. M.; Florea, B. I.; Offen, W. A.; Davies, G. J.; Minnaard, A.

J.; Aerts, J. M. F. G.; Codée, J. D. C.; van der Marel, G. A.; Overkleeft, H. S. Towards broad spectrum activity-based glycosidase probes: synthesis and evaluation of deoxygenated cyclophellitol aziridines. *Chem. Commun.* **2017**, *53*, 12528–12531.

(18) Artola, M.; Kuo, C.-L.; McMahon, S. A.; Oehler, V.; Hansen, T.; van der Lienden, M.; He, X.; van den Elst, H.; Florea, B. I.; Kermode, A. R.; van der Marel, G. A.; Gloster, T. M.; Codée, J. D. C.; Overkleeft, H. S.; Aerts, J. M. F. G. New irreversible α-L-iduronidase inhibitors and activity-based probes. *Chem. A Eur. J.* **2018**, *24*, 19081–19088.

(19) Li, K.-Y.; Jiang, J.; Witte, M. D.; Kallemeijn, W. W.; Donker-Koopman, W. E.; Boot, R. G.; Aerts, J. M. F. G.; Codée, J. D. C.; van der Marel, G. A.; Overkleeft, H. S. Exploring functional cyclophellitol analogues as human retaining beta-glucosidase inhibitors. *Org. Biomol. Chem.* **2014**, *12*, 7786–7791.

(20) Jiang, J.; Artola, M.; Beenakker, T. J. M.; Schröder, S. P.; Petracca, R.; de Boer, C.; Aerts, J. M. F. G.; van der Marel, G. A.; Codée, J. D. C.; Overkleeft, H. S. The synthesis of cyclophellitol-aziridine and its configurational and functional isomers. *Eur. J. Org. Chem.* **2016**, *2016*, 3671–3678.

(21) Legler, G. Glucosidases. *Methods Enzymol.* **1977**, *46*, 368–381.

(22) Jain, N.; Tamura, K.; Déjean, G.; van Petegem, F.; Brumer, H. Orthogonal active-site labels for mixed-linkage endo-β-glucanases. *ACS Chem. Biol.* **2021**, *16*, 1968–1984.

(23) Black, T. S.; Kiss, L.; Tull, D.; Withers, S. G. N-bromoacetyl-glycopyranosylamines as affinity labels for a β-glucosidase and a cellulase. *Carbohydr. Res.* **1993**, *250*, 195–202.

(24) Fenger, T. Synthesis and analysis of specific covalent inhibitors of endo-xyloglucanases. *Chem. Bio. Chem.* **2015**, *16*, 575–583.

(25) Yariv, J.; Wilson, K. J.; Hildesheim, J.; Blumberg, S. Labelling of the active site of β-galactosidase by N-bromoacetyl β-D-galactopyranosylamine. *FEBS Lett.* **1971**, *15*, 24–26.

(26) Tull, D.; Burgoyne, D. L.; Chow, D. T.; Withers, S. G.; Aebersold, R. A Mass spectrometry-based approach for probing enzyme active sites: identification of Glu 127 in cellulomonas fimi exoglycanase as the residue modified by N-bromoacetyl cellobiosylamine. *Anal. Biochem.* **1996**, *234*, 119–125.

(27) Black, T. S.; Kiss, L.; Tull, D.; Withers, S. G. N-bromoacetyl-glycopyranosylamines as affinity labels for a β-glucosidase and a cellulase. *Carbohydr. Res.* **1993**, *250*, 195–202.

(28) Koshland, D. E. Application of a theory of enzyme specificity to protein synthesis. *Proc. Natl. Acad. Sci.* **1958**, *44*, 98–104.

(29) Zhang, R.; Yip, V. L. Y.; Withers, S. G. Mechanisms of enzymatic glycosyl transfer. *Compr. Nat. Prod.* **2010**, *8*, 385–422.

(30) Lai, E. C. K.; Morris, S. A.; Street, I. P.; Withers, S. G. Substituted glycals as probes of glycosidase mechanisms. *Bioorg. Med. Chem.* **1996**, *4*, 1929–1937.

(31) Kallemeijn, W. W.; Witte, M. D.; Wennekes, T.; Aerts, J. M. F. G. Mechanism-based inhibitors of glycosidases: design and applications. *Adv. Carbohydr. Chem. Biochem.* **2014**, *71*, 297–338.

(32) Mhlongo, N. N.; Skelton, A. A.; Kruger, G.; Soliman, M. E. S.; Williams, I. H. A critical survey of average distances between catalytic carboxyl groups in glycoside hydrolases. *Proteins Struct. Funct. Bioinf.* **2014**, *82*, 1747–1755.

(33) Ofman, T. P.; Küllmer, F.; van der Marel, G. A.; Codée, J. D. C.; Overkleeft, H. S. An

orthogonally protected cyclitol for the construction of nigerose- and dextran-mimetic cyclophellitols. *Org. Lett.* **2021**, *23*, 9516–9519.

(34) Tatsuta, K.; Mukai, H.; Takahashi, M. Novel synthesis of natural pseudo-aminosugars, (+)-valienamine and (+)-validamine. *J. Antibiot.* **2000**, *53*, 430–435.

(35) Horii, S.; Fukase, H.; Kameda, Y. Stereoselective conversion of valienamine and validamine into valiolamine. *Carbohydr. Res.* **1985**, *140*, 185–200.

(36) Horii, S.; Iwasa, T.; Mizuta, E.; Kameda, Y. Studies on validamycins, new antibiotics. VI validamine, hydroxyvalidamine and validatol, new cyclitols. *J. Antibiot.* **1971**, *24*, 59–63.

(37) Yoshikawa, M.; Cha, B. C.; Okaichi, Y.; Takinami, Y.; Yokokawa, Y.; Kitagawa, I. Syntheses of validamine, epi-validamine, and valienamine, three optically active pseudo-amino-sugars, from D-glucose. *Chem. Pharm. Bull.* **1988**, *36*, 4236–4239.

(38) Fedotova, A. I.; Komarova, T. A.; Romanov, A. R.; Ushakov, I. A.; Legros, J.; Maddaluno, J.; Rulev, A. Y. Adamantyl aziridines via aza-michael initiated ring closure (Aza-MIRC) Reaction. *Tetrahedron* **2017**, *73*, 1120–1126.

(39) Sternativo, S.; Marini, F.; Del Verme, F.; Calandriello, A.; Testaferri, L.; Tiecco, M. One-pot synthesis of aziridines from vinyl selenones and variously functionalized primary amines. *Tetrahedron* **2010**, *66*, 6851–6857.

(40) Frau, I.; Di Bussolo, V.; Favero, L.; Pineschi, M.; Crotti, P. Stereodivergent synthesis of diastereoisomeric carba analogs of glycal-derived vinyl epoxides: a new access to carbasugars. *Chirality* **2011**, *23*, 820–826.

(41) Birch, A. J. The birch reduction in organic synthesis. *Pure Appl. Chem.* **1996**, *68*, 553–556.

(42) Zenina, A. A.; Rakhmanov, E. V.; Bobyleva, A. A.; Lukovskaya, E. V.; Kuzmina, L. G.; Abramov, A. A.; Anisimov, A. V. New functionalized dithia-12(13)-crown-4 ethers: synthesis, complex formation, and extraction capacity. *Chem. Heterocycl. Compd.* **2007**, *43*, 835–843.

(43) Aumaitre, G.; Chanet-Ray, J.; Durand, J.; Vessière, R.; Lonchambon, G. Synthesis of aziridinylsulfonic acid derivatives. *Synthesis.* **1983**, *10*, 816–821.

(44) Sainz-Díaz, C. I.; Gálvez-Ruano, E.; Hernández-Laguna, A.; Bellanato, J. Synthesis, molecular structure, and spectroscopical properties of alkenylphosphonic derivatives. 1. vinyl-, propenyl-, (bromoalkenyl)-, and (cyanoalkenyl)phosphonic compounds. *J. Org. Chem.* **1995**, *60*, 74–83.