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Nine of 16 Stereoisomeric Polyhydroxylated Proline Amides Are Potent β-*N*-Acetylhexosaminidase Inhibitors

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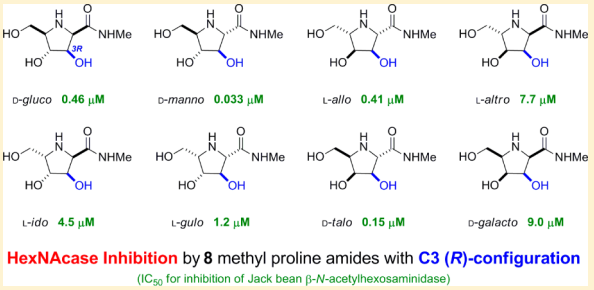
Supporting Information

ABSTRACT: All 16 stereoisomeric *N*-methyl 5-(hydroxymethyl)-3,4-dihydroxyproline amides have been synthesized from lactones accessible from the enantiomers of glucuronolactone. Nine stereoisomers, including all eight with a (3*R*)-hydroxyl configuration, are low to submicromolar inhibitors of β-*N*-acetylhexosaminidases. A structural correlation between the proline amides is found with the ADMDP-acetamide analogues bearing an acetamidomethylpyrrolidine motif. The proline amides are generally more potent than their ADMDP-acetamide equivalents. β-*N*-Acetylhexosaminidase inhibition by an azetidine ADMDP-acetamide analogue is compared to an azetidine carboxylic acid amide. None of the amides are good α-*N*-acetylgalactosaminidase inhibitors.



INTRODUCTION

In humans, deficiencies in the levels of β-*N*-acetylhexosaminidases (HexNAcases [EC 3.2.1.52]) can lead to lysosomal storage disorders (LSDs) such as Tay–Sachs and Sandhoff[1−3] and to Alzheimer's disease.[4] In particular, control of *O*-GlcNAc levels provides opportunities for intervention in many pathogenic conditions,[5,6] including diabetes,[7,8] cancer,[9,10] Parkinson's disease,[11] and osteoarthritis.[12] In addition, specific inhibition of α-*N*-acetylgalactosaminidases (α-GalNAcases [EC 3.2.1.49]) by chemotherapeutic agents has potential in the treatment of the Schindler–Kanzaki LSD[13] and of cancer by prevention of the degradation of the macrophage-activating factor.[14,15]

Although about 250 naturally occurring iminosugars have been isolated from plants, none of them are good HexNAcase inhibitors. Three microbial natural products, pochonicine **1**,[16−18] siastatin B **2**,[19−21] and nagstatin **3**,[22,23] are nanomolar inhibitors of HexNAcases (Figure 1). There are many synthetic examples of micromolar HexNAcase inhibitors that possess an acetamide (NHAc) group which include the pyrrolidines ADMDP-acetamide [(*N*-(((2*R*,3*R*,4*R*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidin-2-yl)methyl)acetamide] **4**[12,24] and LABNAc **5**;[2] the piperidines PUGNAc **6**,[25,26] NGT (*N*-acetyl-D-glucosamine-thiazoline) **7**,[27,28] DNJNAc **8**,[29] and DGJNAc **9**;[29] and azepanes including **10**.[30,31]

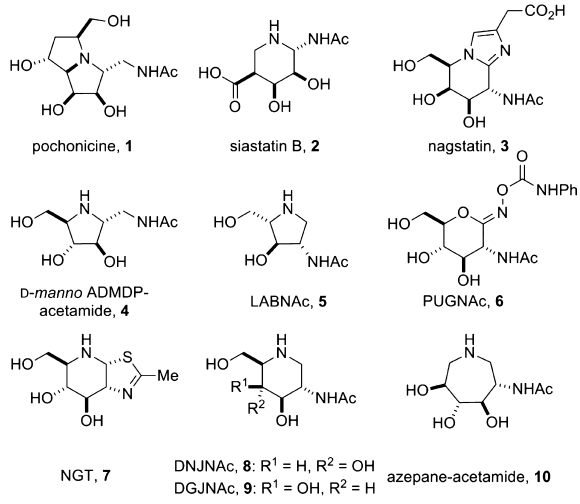


Figure 1. Examples of potent HexNAcase inhibitors containing an acetamide (NHAc) group.

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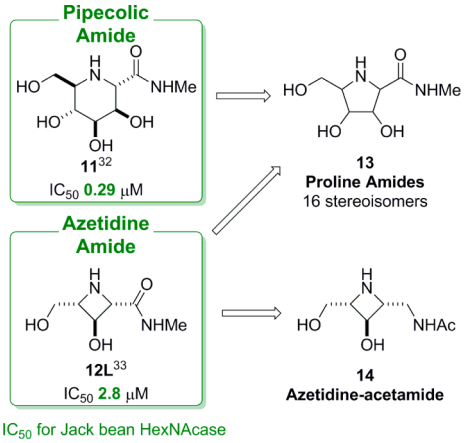
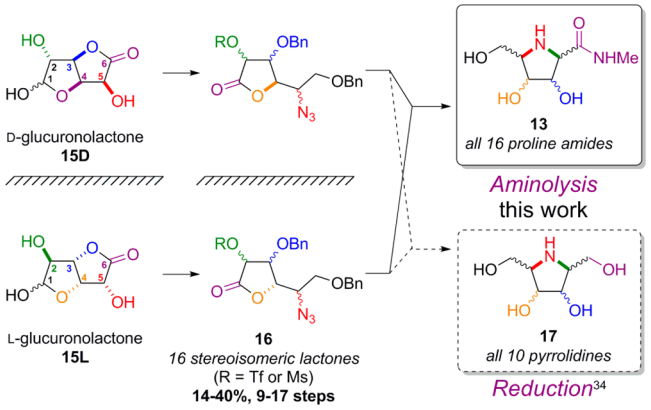


Figure 2. *N*-Methylamides of piperidine, pyrrolidine, and azetidine carboxylic acids and the azetidine-acetamide (-**D** and -**L** numbering suffixes are used where both enantiomers of a compound are discussed).

Scheme 1. Divergent Access to 10 Pyrrolidines from the Enantiomers of Glucuronolactone[34] and Proposed Access to Proline Amide Targets



Our group has described the synthesis and inhibitory activity of the *N*-methylamides of the hydroxylated pipecolic **11**[32] and azetidine **12L**[33] carboxylic acids as potent inhibitors of HexNAcases, which do not contain an NHAc group (Figure 2). An SAR investigation into the inhibitory activity of these amides toward HexNAcases has not been possible as the syntheses of amides **11** and **12L** do not readily permit preparation of their stereoisomers. In contrast, we have recently published a divergent pathway to the synthesis of all the stereoisomers of pyrrolidine-containing DMDP[34] [(2*R*,3*R*,4*R*,-5*R*)-2,5-bis(hydroxymethyl)pyrrolidine-3,4-diol] from the enantiomers of glucuronolactone[35] via lactone intermediates. We report herein the extension of this divergent methodology, and the utilization of these lactone intermediates, to provide access to all 16 stereoisomeric *N*-methyl proline amides **13**, which are five-membered analogues of *N*-methyl pipecolic **11** and azetidine amides **12L**. This permitted an SAR investigation of their activity toward HexNAcases which is also described. In addition, an SAR comparison is made between the *N*-methyl proline amides and the corresponding isosteric ADMDP-acetamides.

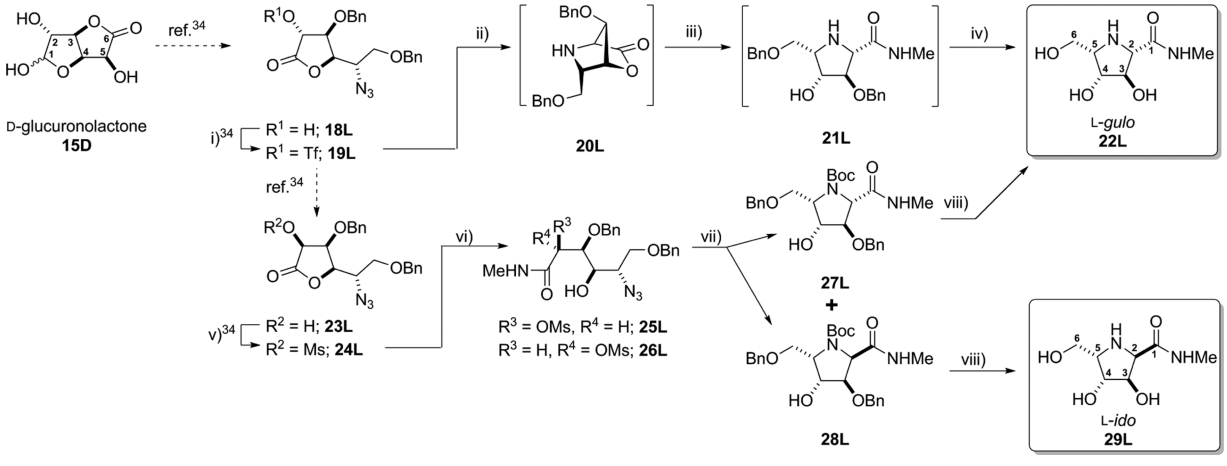
The *N*-methyl amide moiety in these targets is isosteric to the NHAc of inhibitors shown in Figure 1; with this is mind, we propose that the azetidine-acetamide **14**, the novel isosteric analogue of azetidine amide **12L**, would be an inhibitor of HexNAcases. The synthesis and biological evaluation of **14** is also reported.

RESULTS AND DISCUSSION

Synthesis. *1. N-Methyl 5-(Hydroxymethyl)-3,4-dihydroxyproline Amides.* D-Glucuronolactone **15D**, and its readily available enantiomer **15L**,[35] are well-established chirons.[36−45] We have previously reported the synthesis of all 10 stereoisomeric pyrrolidines **17** divergently from the enantiomers of glucuronolactone via the 16 lactones **16** (Scheme 1).[34] The proline amides **13** can be accessed from the lactones **16** by reaction with methylamine.

The lactones **16** are the key intermediates in the synthesis of the target proline amides **13**. They were accessed in excellent yield, divergently from the enantiomers of glucuronolactone **15D** and **15L**.[34] In the synthesis of L-*gulo* proline amide **22L** (Scheme 2), the lactone **18L** was utilized. Activation of the C2-hydroxyl by

Scheme 2. Synthesis of *Gulo*- and *Ido*-Configured Proline Amides[a]



[a]Reagents and conditions: (i) Tf_2O, pyr, DCM −40 °C, 1 h, 95%;[34] (ii) 10% Pd−C, H_2, 1,4-dioxane, rt, 1.5 h; (iii) $MeNH_2$ (33% in EtOH), 16 h, rt; (iv) 10% Pd−C, H_2, 1,4-dioxane−2 M HCl, rt, 16 h, 66% over three steps; (v) MsCl, pyr, 0 °C−rt, 2 h, 93%;[34] (vi) $MeNH_2$ (33% EtOH), 1,4-dioxane, rt, 16 h, 99%; (vii) 10% Pd−C, H_2, NaOAc, 1,4-dioxane, rt, 3 h, then Boc_2O, rt, 16 h, 86%; (viii) 10% Pd−C, H_2, 1,4-dioxane−2 M HCl, rt, 16 h, from **27L**, 77%, from **28L**, 75%.

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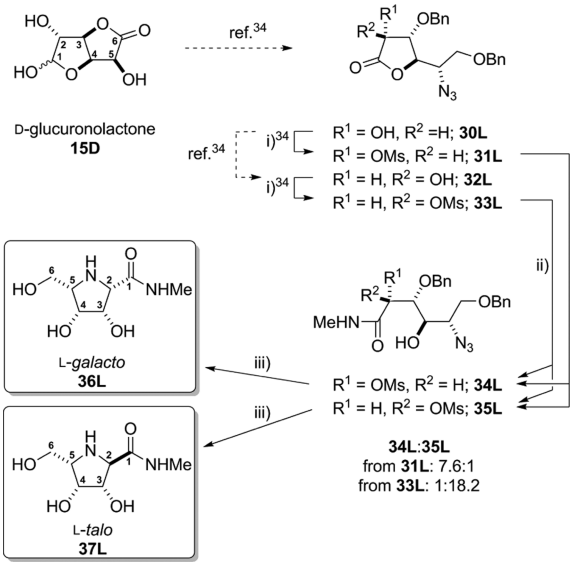
esterification with trifluoromethanesulfonic (triflic) anhydride in pyridine afforded the triflate **19L** (95%).[34] Hydrogenation of the azide moiety with palladium (10% on carbon) led initially to the strained bicycle **20L**, which upon addition of methylamine underwent aminolytic ring opening to form the dibenzyl intermediate **21L**. Further reduction under acidic conditions afforded the proline amide **22L** in 66% yield over three steps. The enantiomeric D-*gulo* proline amide **22D** was similarly formed from the lactone **18D** in a comparable yield. In order to access the C2-epimeric L-*ido* proline amide **29L**, the C2-epimeric lactone **23L** was employed. The C2-hydroxyl of the lactone **23L** was activated by esterification with methanesulfonyl (mesyl) chloride in pyridine (93%) to form the mesylate **24L**.[34] Here, aminolytic ring-opening was performed prior to reductive iminocyclization as a bicyclic lactone could not be formed. Treatment of the mesylate **24L** with methylamine afforded an inseparable mixture of the epimeric open-chain amides **25L** and **26L** (99%). Azide hydrogenation and subsequent Boc-protection of the iminocyclized prolines gave the separable, protected L-*gulo* **27L** (42%) and L-*ido* (44%) amides **28L**. Acidic hydrogenation of these amides afforded the L-*gulo* **22L** (77%) and L-*ido* **29L** (75%) proline amides, respectively. The D-*ido* target **29D** was accessed with the enantiomeric route in a comparable yield.

Previous work[34] had shown that the *galacto*-system was not amenable to forming a bicyclic intermediate; the methodology utilized for accessing *ido*-configured targets was repeated here. The lactone **30L** was activated as the mesylate **31L** by esterification with mesyl chloride (95%, Scheme 3).[34] Treatment with methylamine led to the formation of two separable, epimeric open-chain amides **34L** and **35L** (95%, 7.6:1). Acidic hydrogenation of **34L** and **35L** led to the target proline amides L-*galacto* **36L** (94%) and L-*talo* **37L** (90%), respectively. In the *talo*-route, the lactone **32L** was activated as the mesylate **33L** in the usual manner (84%).[34] Methylamine-induced aminolysis gave an 18.2:1 mixture of the open-chain amides **35L** and **34L** (96%). These open-chain amides formed the target proline amides as described above. The enantiomeric proline amides D-*galacto* **36D** and D-*talo* **37D** were prepared via the same route.

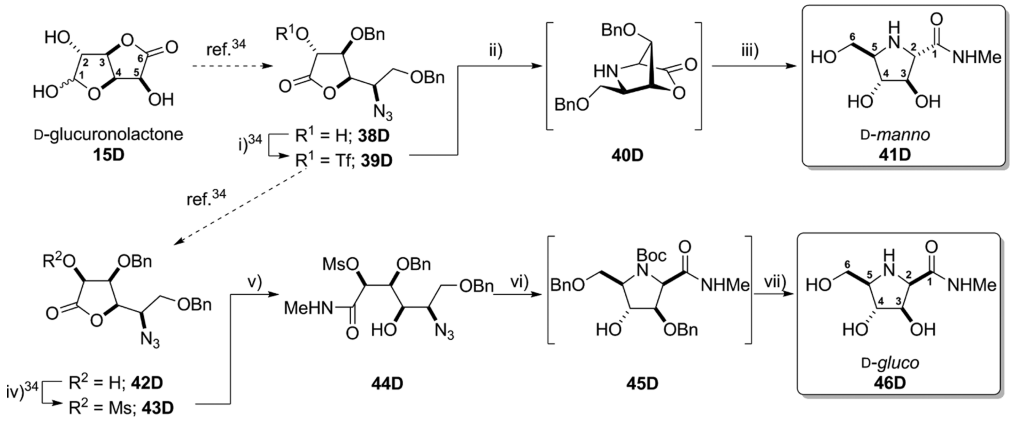
In contrast to the *galacto* route, the *manno* route has previously been shown to form a stable bicyclic intermediate.[45] The triflate **39D** was formed from the lactone **38D**, which with subsequent hydrogenation gave the intermediate bicycle **40D** (Scheme 4).[34,45] Aminolytic ring-opening and acidic hydrogenation led to the D-*manno* target **41D** (99% over three steps). For the synthesis of the D-*gluco* proline amide **46D**, the lactone **42D** was activated as the mesylate **43D**[34] (99%) which with methylamine formed the open-chain amide **44D** (92%). In contrast to previous *ido*, *galacto*, and *talo* routes that gave a mixture of epimers, retention of the C2 stereochemistry under the reaction conditions was observed. However, unlike these previous routes, azide hydrogenation gave a complex mixture. Boc-protection of the mixture allowed separation of the protected proline amide intermediate **45D** in an acceptable 52% yield. Further attempts at improving this step were not successful. Acidic hydrogenation of Boc-protected **45D** led to formation of the D-*gluco* proline amide **46D**

Scheme 3. Synthesis of *Galacto*- and *Talo*-Configured Proline Amides[a]



[a]Reagents and conditions: (i) MsCl, pyr, 0 °C rt, 1.5 h, from **30L** 95%, from **32L** 84%;[34] (ii) $MeNH_2$ (33% in EtOH), 1,4-dioxane, rt, 16 h, from **31L** 95%, from **33L** 96%; (iii) 10% Pd−C, H_2, NaOAc, 1,4-dioxane, rt, 3 h, then 2 M HCl, 19 h, from **34L**, 94%, from **35L**, 90%.

Scheme 4. Synthesis of *Manno*- and *Gluco*-Configured Proline Amides[a]



[a]Reagents and conditions: (i) Tf_2O, pyr, DCM, −40 °C, 1 h, 99%;[34] (ii) 10% Pd−C, H_2, 1,4-dioxane, rt, 1 h;[34] (iii) $MeNH_2$ (33% in EtOH), rt, 18 h; then 10% Pd−C, H_2, 1,4-dioxane−2 M HCl, rt, 18 h, 99% over three steps; (iv) MsCl, pyr, rt, 2 h, 99%;[34] (v) $MeNH_2$ (33% in EtOH), 16 h, rt, 92%; (vi) 10% Pd−C, H_2, NaOAc, 1,4-dioxane, rt, 18 h, then Boc_2O, rt, 24 h, 52%; (vii) 10% Pd−C, H_2, 1,4-dioxane−2 M HCl, rt, 20 h 97%.

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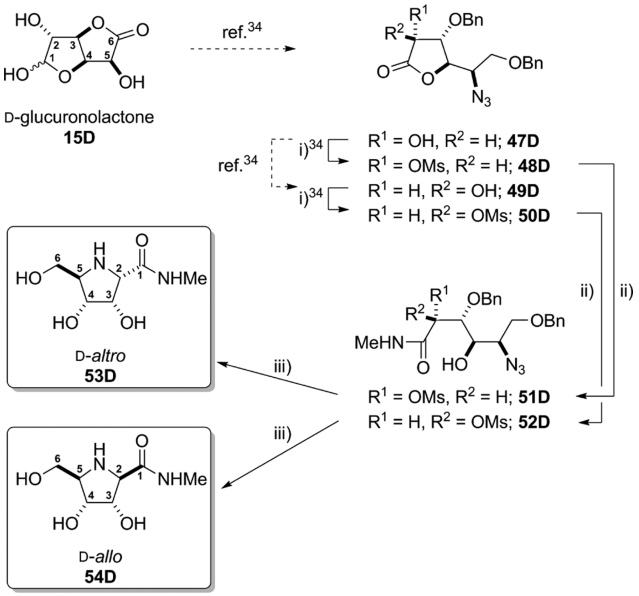
(97%). The enantiomeric L-*manno* **41L** and L-*gluco* **46L** amides were prepared in comparable yields via the enantiomeric route.

In the final two routes toward *altro*- and *allo*-configured targets, the two lactones **47D** and **49D** were treated identically (Scheme 5). Both lactones were activated as the mesylates **48D**

Scheme 5. Synthesis of *Altro*- and *Allo*-Configured Proline Amides[a]



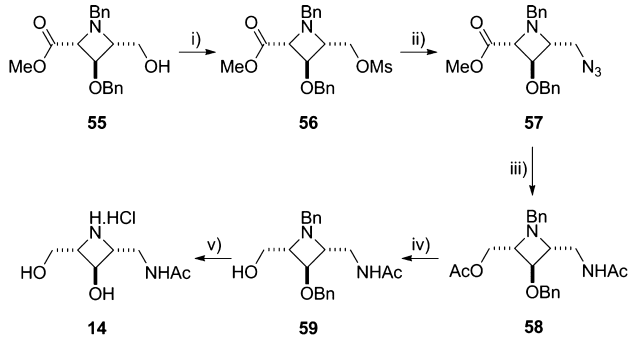
[a]Reagents and conditions: (i) MsCl, pyr, 0 °C–rt, 2 h, from **47D**, 70%, from **49D**, 84%;[34] (ii) $MeNH_2$ (33% in EtOH), 1,4-dioxane, rt, 17–18 h, from **48D**, 69%, from **50D**, 91%; (iii) 10% Pd–C, H_2, NaOAc, 1,4-dioxane, rt, 3–3.5 h, then 2 M HCl, 19–24 h, from **51D**, 94%, from **52D**, 91%.

(70%) and **50D** (84%), respectively.[34] Aminolysis with methylamine gave the open-chain amides **51D** (69%) and **52D** (91%), where in both cases no epimerization was observed under the reaction conditions. Finally, acidic hydrogenation afforded the target proline amides D-*altro* **53D** (94%) and D-*allo* **54D** (91%). The enantiomeric targets L-*altro* **53L** and L-*allo* **54L** were accessed by the same route in comparable yields.

The methodology developed to access the proline amide targets is simple, robust, and scalable. The D-*allo* isomer **54D** was accessed on a 1 g scale via the longest synthetic route from D-glucuronolactone **15D** (20 steps) in an overall yield of 16%.

2. Azetidine Acetamide ***14.*** The 5-hydroxymethylazetidine ester **55**[33] was activated as the mesylate **56** by esterification with MsCl in pyridine (88%, Scheme 6). Treatment of the mesylate

Scheme 6. Synthesis of Azetidine Acetamide[a]



[a]Reagents and conditions: (i) MsCl, pyr, rt, 1.5 h, 88%; (ii) NaN_3, DMF, 60 °C, 24 h, 78%; (iii) $LiAlH_4$ (1 M in THF), THF, −78 °C, 3.5 h, then Ac_2O, H_2O, rt, 15 h, 79% over two steps; (iv) NaOMe, MeOH, 60 °C, 25 h, 32%; (v) 10% Pd–C, H_2, 1 M HCl–1,4-dioxane–H_2O, rt, 5 d, 99%.

56 with sodium azide proceeded smoothly to afford the azetidine azide **57** (78%); this reaction occurred without neighboring group participation and subsequent ring expansion.[46,47] Reduction of both the ester and azide moieties was achieved with lithium aluminum hydride, with peracetylation affording the diacetate **58** (79% over two steps). Selective deprotection of the *O*-acetyl group was achieved with sodium methoxide in methanol and gave the protected acetamide **59** (32%). Final acidic hydrogenation afforded the hydrochloride salt of the azetidine acetamide **14** (99%).

Glycosidase Inhibition. The comparative inhibition of the 16 *N*-methyl proline amides against a panel of glycosidases showed that all eight enantiomers with a (3*R*)-hydroxyl configuration are low micromolar to nanomolar inhibitors of a range of HexNAcases (*β*-*N*-acetylhexosaminidases) (Table 1). The only amide with a (3*S*)-configuration that showed significant inhibition is L-*manno* **41L** (Jack bean HexNAcase: IC_{50} 7.3 μM), which is the enantiomer of the strongest inhibitor **41D** (IC_{50} 0.033 μM). For many glycosidases with potent iminosugar

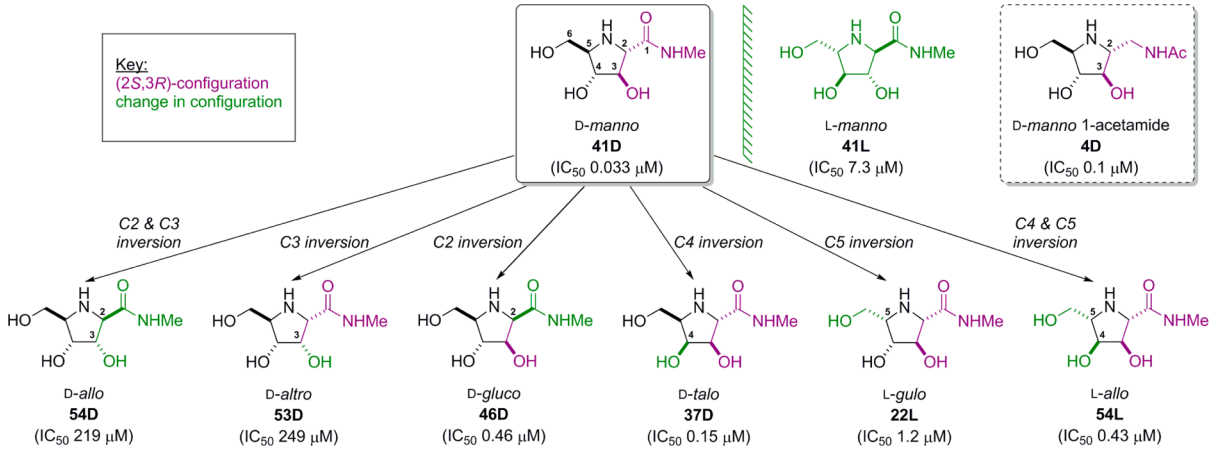


Figure 3. HexNAcase and proline amide SAR.

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Table 1. Concentration of Proline Amides Giving 50% Inhibition (IC_{50}) of Various Glycosidases

	IC_{50} (μM)							
Stereochemistry:	D-*gulo* **22D**	L-*gulo* **22L**	D-*ido* **29D**	L-*ido* **29L**	D-*galacto* **36D**	L-*galacto* **36L**	D-*talo* **37D**	L-*talo* **37L**
β-*N*-Acetylhexos-aminidase								
Human placenta	584	**5.3**	351	**38**	**54**	NI (37.4%)	**0.92**	NI (34.6%)
Bovine kidney	566	**4.8**	250	**34**	**60**	NI (34.0%)	**0.69**	NI (33.5%)
Jack bean	119	**1.2**	38	**4.5**	**9.0**	588	**0.15**	409
HL60	849	**6.1**	329	**88**	**24**	NI (31.5%)	**0.96**	NI (32.6%)
Aspergillus oryzae	NI[a] (9.3%)[b]	**6.6**	NI (1.3%)	**87**	NI (37.4%)	NI (11.7%)	**32**	NI (11.0%)
α-*N*-Acetylgalactos-aminidase								
Chicken liver	NI (10.0%)	NI (0%)	NI (6.7%)	NI (31.1%)	**99**	NI (23.2%)	312	NI (2.9%)
β-*N*-Acetylgalactos-aminidase								
HL60	NI (30.2%)	**26**	NI (41.8%)	269	83	NI (15.5%)	**3.6**	NI (12.7%)
Aspergillus oryzae	NI (9.6%)	**7.3**	NI (2.8%)	83	NI (37.4%)	NI (17.5%)	**25**	NI (11.0%)
β-Glucuronidase								
E. coli	NI (1..9%)	NI (0%)	NI (2.6%)	NI (3.5%)	NI (0%)	NI (10.0%)	NI (0%)	NI (16.1%)
Bovine liver	NI (2.6%)	NI (8.5%)	NI (6.6%)	NI (16.5%)	NI (0%)	NI (9.8%)	NI (2.6%)	NI (3.2%)

	IC_{50} (μM)							
Stereochemistry:	D-*manno* **41D**	L-*manno* **41L**	D-*gluco* **46D**	L-*gluco* **46L**	D-*altro* **53D**	L-*altro* **53L**	D-*allo* **54D**	L-*allo* **54L**
β-*N*-Acetylhexos-aminidase								
Human placenta	**0.20**	**41**	**2.6**	NI (13.0%)	NI (43.6%)	**11**	914	**0.72**
Bovine kidney	**0.22**	**32**	**3.1**	NI (18.1%)	NI (44.1%)	**4.0**	NI (44.6%)	**0.32**
Jack bean	**0.033**	**7.3**	**0.46**	NI (39.9%)	249	**7.7**	219	**0.41**
HL60	**0.20**	**50**	**2.8**	NI (18.7%)	NI (34.9%)	**14**	NI (42.9%)	**0.93**
Aspergillus oryzae	**0.30**	**53**	**3.6**	NI (3.4%)	NI (24.4%)	115	NI (29.8%)	**12**
α-*N*-Acetylgalactos-aminidase								
Chicken liver	NI (0%)	NI (9.6%)	NI (5.5%)	NI (0%)	NI (0.6%)	NI (0%)	NI (1.3%)	NI (16.0%)
β-*N*-Acetylgalactos-aminidase								
HL60	**1.0**	206	**11**	NI (1.8%)	NI (18.8%)	133	NI (22.4%)	**2.8**
Aspergillus oryzae	**0.35**	**84**	**5.4**	NI (0%)	NI (21.0%)	103	NI (28.0%)	**9.5**
β-Glucuronidase								
E. coli	NI (25.1%)	NI (26.9%)	NI (0.8%)	NI (0%)	NI (0%)	NI (1.5%)	NI (1.2%)	NI (10.0%)
Bovine liver	NI (0%)	NI (3.6%)	NI (12.5%)	NI (10.3%)	NI (0.1%)	NI (0%)	NI (1.4%)	NI (0%)

Red: (2*S*). Blue: (3*R*). [a]NI: no inhibition (less than 50% inhibition at 1000 μM). [b]Value in parentheses: inhibition % at 1000 μM.

inhibition there is a 100- to 1000-fold difference for the *same* enzymes between enantiomeric inhibitors.[48,49]

N-Methyl proline amide stereoisomers bearing a (2*S*,3*R*)-*trans* configuration, such as D-*talo* **37D** (IC_{50} 0.15 μM), are significantly more potent inhibitors than those with a (2*R*,3*R*)-*cis* motif, such as the corresponding C2-epimer D-*galacto* **36D** (IC_{50} 9.0 μM). Comparison of the strongest inhibitor D-*manno* **41D** with its (3*R*)-epimer D-*altro* **53D** (IC_{50} 249 μM) shows a 10000-fold decrease in inhibition (Figure 3). If the stereochemistry in D-*manno* **41D** is inverted (2*S*,3*R* → 2*R*,3*S*) the corresponding configuration is the weak inhibitor D-*allo* **54D** (IC_{50} 219 μM). Change of configuration of C2 (2*S*→2*R*) yields D-*gluco* **46D** (IC_{50} 0.46 μM) gives a better tolerated epimerization (a 10-fold decrease). Alteration of the disposition of other hydroxyls leads to similar decreases in activity: C4 leads to D-*talo* **37D** (5-fold decrease), C5 affords L-*gulo* **22L** (40-fold decrease), and changing both C4 and C5 gives L-*allo* **54L** (10-fold decrease).

None of the proline amides were found to inhibit β-glucuronidases (*E. coli* and bovine liver), and only two were found to show modest inhibition of α-GalNAcase (chicken liver): D-*galacto* **36D** and D-*talo* **37D** (IC_{50} 99 and 312 μM, respectively). Their inhibition may be due to an equivalent orientation between the C3, C4, and C5 hydroxyls of the iminosugars and of the GalNAc substrate TS (Figure 4). DGJNAc remains the only potent inhibitor of α-GalNAcase reported,[29] although DGJNAc is also a powerful inhibitor of HexNAcase and a modest inhibitor of α-galactosidases.[13]

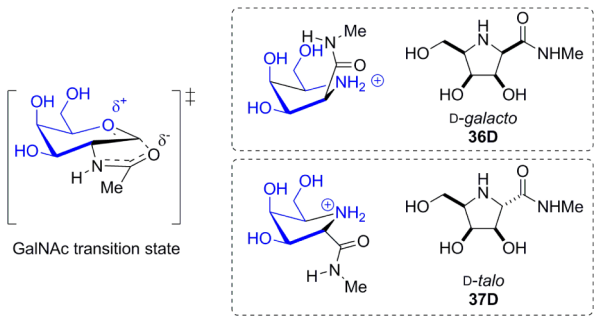

Figure 4. Comparison of GalNAc TS and the structures of the D-*galacto* and D-*talo* proline amide inhibitors.

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Table 2. Comparison of Known AMDP-acetamides and Proline Amides

	β-*N*-Acetylhexosaminidase (Jack bean) IC_{50} (μM)						
Stereochemistry:	D-*manno* **4D**	L-*manno* **4L**	D-*gluco* **60D**	D-*talo* **61D**	L-*talo* **61L**	D-*altro* **62D**	L-*galacto* **63L**
R = CH_2NHAc	**0.1** [50]	55 [50]	(K_i **0.24**) [12]	**0.2** [50]	500[a] [53]	NI[b] [54]	500[a] [53]
R = CONHMe	**0.033**	**7.3**	**0.46**	**0.15**	409	249	588

Red: (2*S*). Blue: (3*R*). [a]*Streptomyces cerevisiae*. [b]NI: no inhibition observed up to 1000 pM (*Streptomyces plicatus*). No biological data for R = CH_2NHAc L-*ido*,[55] L-*gulo*,[56] and L-*gluco*.[57] No known synthesis of R = CH_2NHAc D-*allo*, L-*allo*, L-*altro*, D-*galacto*, D-*gulo*, D-*ido*.

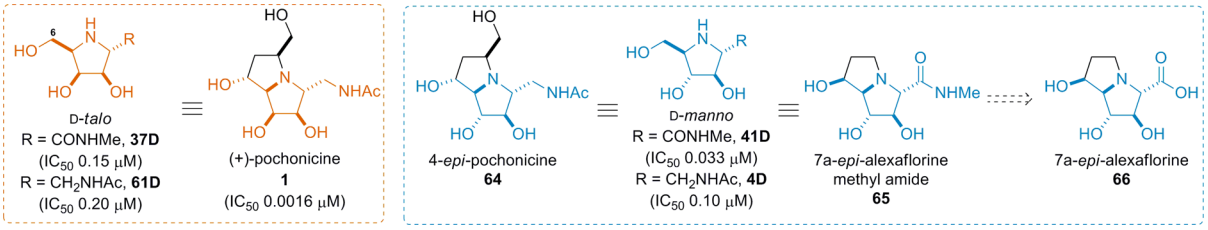

Figure 5. Pochonicine and D-*talo* proline amide comparison and prediction of pyrrolizidine inhibitors.

The structure–activity relationship (SAR) of the *N*-methyl proline amides parallels that of the known ADMDP-acetamides **4D**, **4L**, **60D**, **61D**, **61L**, **62D**, and **63L**[12,50−55] designed by Wong as pyrrolidine-based transition-state inhibitors (Table 2). The ADMDP-acetamides possess inhibition similar to that of the proline amides but are less specific in their glycosidase inhibition profiles. Pochonicine **1** (IC_{50} 0.0016 μM, Figure 5) is a conformationally restricted pyrrolizidine analogue of D-*talo* **37D** (IC_{50} 0.15 μM).[17] This constitutes a 100-fold improvement over the proline amide equivalent. On this basis, it may be predicted that (i) 4-*epi*-pochonicine **64**, bearing a D-*manno* configuration, should be an extremely potent inhibitor and (ii) *N*-methylamide **65** of naturally occurring,[56] but as yet not synthesized, 7a-*epi*-alexaflorine **66** also bearing a D-*manno* configuration, should also be an extremely potent inhibitor.

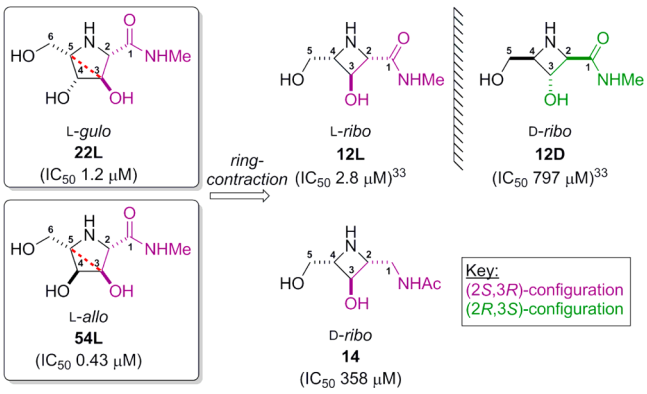

Figure 6. Azetidine SAR comparison.

The *N*-methyl azetidine amides[33] **12D** and **12L** correlate with the SAR established for the proline amides and ADMDP-acetamides (Figure 6). The azetidine amide **12L** may be considered as a ring-contracted analogue of the L-*gulo* **22L** and L-*allo* **54L** amides while maintaining the crucial (2*S*,3*R*)-configuration (Figure 3). Azetidine amide **12L** is a comparable inhibitor (Jack bean HexNAcase: IC_{50} 2.8 μM) (Table 3);[33] the ring-contraction leads to only a 2- to 7-fold drop in potency.

The enantiomeric amide **12D**, lacks the (2*S*,3*R*)-motif and possesses little activity (IC_{50} 797 μM),[33] analogous to the contracted proline amides **22D** (IC_{50} 119 μM) and **54D** (IC_{50} 219 μM). The inhibitory profile of the azetidine acetamide **14** showed only modest inhibition of HexNAcases (IC_{50} 358 μM).

Table 3. Concentration of Azetidine Acetamide Giving 50% Inhibition (IC_{50}) of Various Glycosidases

	IC_{50} (μM)		
	12L[33]	**12D**[33]	**14**
β-*N*-Acetylhexosaminidase			
Human placenta	**3.3**	NI (42.8%)	NI (5.6%)
Bovine kidney	**1.4**	NI (46.8%)	217
Jack bean	**2.8**	797	358
HL60	**4.2**	NI (34.7%)	378
Aspergillus oryzae	48	NI (21.4%)	376
α-*N*-Acetylgalactosaminidase			
Chicken liver	NI[a] (0%)[b]	NI (12.5%)	NI (13.6%)

Red: (2*S*). Blue:(3*R*). [a]NI: no inhibition (less than 50% inhibition at 1000 μM). [b]Values in parentheses: inhibition % at 1000 μM.

CONCLUSION

The 16 stereoisomeric *N*-methyl proline amides prepared from the enantiomers of glucuronolactone constitute a remarkable new family of specific HexNAcase inhibitors; that 9 of the 16 amides are low to submicromolar inhibitors is noteworthy (Figure 7). The SAR of the *N*-methyl proline amides correlates with HexNAcase data for the isosteric Wong ADMDP-acetamide series. Two amides demonstrate moderate activity against α-GalNAcase. In addition, a 4-membered azetidine-acetamide was also synthesized and shown to be an inhibitor of HexNAcases; it

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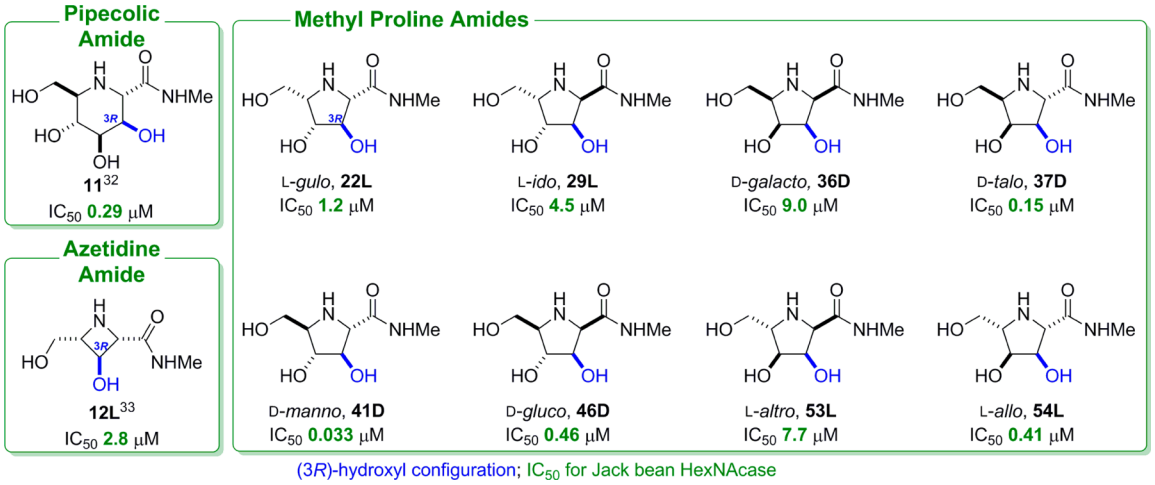

Figure 7. Comparison of the *N*-methyl amides of piperidine, pyrrolidine, and azetidine carboxylic acids.

is likely that other azetidine and pipecolic acid derivatives will also provide a new range of hexosaminidase inhibitors. The inhibitory profile of the proline amides makes them important scaffolds in the exploitation of their therapeutic potential toward a range of pathologies. The D-*manno* *N*-methylproline amide **41D** is among the most potent inhibitors of HexNAcases yet described and is easy to make in gram-quantities; further study of its derivatives is in progress to evaluate its future as a chemotherapeutic agent. The *N*-methyl amide **65** of 7a-*epi*-alexaflorine **66** would provide a conformationally locked equivalent of **41D** and may prove to be an even more potent inhibitor.

EXPERIMENTAL SECTION

***In Vitro* Enzyme Inhibition: IC_{50} Determination.** The enzymes *β*-*N*-acetylhexosaminidases (from human placenta, bovine kidney, jack bean, and *Aspergillus oryzae*), *α*-*N*-acetylgalactosaminidase (from chicken liver), *β*-*N*-acetylgalactosaminidase (from *Aspergillus oryzae*), *β*-glucuronidases (*Escherichia coli* and bovine liver), and *p*-nitrophenyl glycosides were purchased from Sigma-Aldrich Co. The cell lysate of human acute amyloid leukemia cell line HL-60 (RBRC) was cultured in RPMI 1640 medium containing 10% fetal calf serum, 100 units·mL^{-1} of penicillin, and 100 *μ*g·mL^{-1} streptomycin (Invitrogen) at 37 °C under 5% CO_2 and used as the source of *β*-*N*-acetylhexosaminidase and *β*-*N*-acetylgalactosaminidase. The glycosidase activities were determined using an appropriate *p*-nitrophenyl glycoside as substrate at the optimum pH of each enzyme (the HL60 lysate assay was performed at pH 4.5). The reaction mixture (1 mL) contained 2 mM of the substrate and the appropriate amount of enzyme. The reaction was stopped by adding 2 mL of 400 mM Na_2CO_3. The released *p*-nitrophenol was measured spectrometrically at 400 nm.

General Experimental Methods. All commercial reagents were used as supplied. Pyridine and DMF were purchased dry. All other solvents were used as supplied, without prior purification. All reactions were performed under an inert nitrogen or argon atmosphere and were maintained by an inflated balloon. All solutions are saturated unless otherwise stated. Thin-layer chromatography (TLC analysis) was performed on aluminum sheets coated with 60 F_{254} silica. Sheets were visualized using a dip of 0.2% w/v cerium(IV) sulfate and 5% ammonium molybdate in 2 M sulfuric acid solution. Flash chromatography was performed on Sorbsil C60 40/60 silica. Melting points were recorded on a Kofler hot block and are uncorrected. Optical rotations were recorded on a polarimeter with a path length of 1 dm. Concentrations are quoted in g·100 mL^{-1}. Infrared spectra were recorded using thin films on either NaCl or Ge plates. Only the characteristic peaks are quoted. Nuclear magnetic resonance (NMR) spectra were recorded in the deuterated solvent stated where residual signals where used as internal references; however, where D_2O was used as the solvent acetonitrile was used as internal reference. All chemical shifts (δ) are quoted in ppm and coupling constants (J) in Hz and were assigned used 2D COSY and HSQC spectra. Low-resolution mass spectra (m/z) were recorded on ESI ToF spectrometer. High-resolution mass spectra (HRMS m/z) were carried out on a ToF spectrometer (resolution = 10000 fwhm).

Synthesis of *Gulo*- And *Ido*-Configured Proline Amides (Scheme 2). *Methyl 2,5-Dideoxy-2,5-imino-*L*-gulonamide* ***22L.*** From triflate **19L**: Palladium (10% on carbon, 12 mg) was added to a solution of the triflate **19L**[34] (64 mg, 0.21 mmol) in 1,4-dioxane (0.6 mL). The reaction vessel was evacuated and flushed with hydrogen and stirred for 1.5 h, after which TLC analysis (2:1, cyclohexane−ethyl acetate) indicated the complete consumption of starting material (R_f 0.59) and the formation of a major product (R_f 0.19). The reaction mixture was filtered through glass microfiber (GF/B). Methylamine (33% in ethanol, 0.01 mL, 0.23 mmol) was added to the filtrate and the reaction mixture stirred at rt for 16 h, after which TLC analysis (ethyl acetate) indicated the complete consumption of the intermediate bicyclic lactone **20L** (R_f 0.29) and the formation of a single product (R_f 0.02). The reaction mixture was concentrated in vacuo to afford the crude dibenzyl amide **21L**, which was reacted on without further purification. Partial data for **21L**. δ_C (100 MHz, CD_3OD): 26.2 (*N*-Me), 62.0 (C-5), 66.6 (C-6), 70.5 (C-4), 74.3 (C-2), 75.2 (C-3), 128.7, 128.8, 128.9, 128.9, 129.3, 129.4, 129.5, 129.5 (ArCH), 139.3, 139.5 (ArCC), 174.6 (C-1). m/z (ESI +ve): 371 (M + H$^+$, 80), 393 (M + Na$^+$, 85), 741 (2M + H$^+$, 100), 763 (2M + Na$^+$, 96). HRMS m/z (ESI +ve): found 371.1962 [M + H$^+$], $C_{21}H_{27}N_2O_4$ requires 371.1965. Palladium (10% on carbon, 4.5 mg) was added to a solution of the crude dibenzyl amide **21L** in a 5:1 1,4-dioxane−2 M aqueous hydrochloric acid mixture (2.5 mL). The flask was evacuated and flushed with hydrogen and stirred for 16 h, after which the reaction mixture was filtered through glass microfiber (GF/B) and concentrated in vacuo. The crude residue was dissolved in 2 M aqueous hydrochloric acid and loaded onto a short column of Dowex (50W-X8, H$^+$) and the resin washed with water. The L-*gulo* amide **22L** was liberated with 2 M aqueous ammonia, and the ammoniacal fractions were concentrated in vacuo to afford the L-*gulo* amide **22L** (15.5 mg, 66% over three steps) as a yellow oil. From Boc-protected **27L**: Palladium (10% on carbon, 24 mg) was added to a solution of the dibenzyl ether **27L** (61 mg, 0.13 mmol) in a 1:1 1,4-dioxane−2 M aqueous hydrochloric acid mixture (2.5 mL). The reaction vessel was evacuated and flushed with hydrogen and stirred for 16 h. The reaction mixture was filtered and the filtrate stirred at 50 °C for 2 h and concentrated in vacuo. The crude residue was loaded onto a short column of Dowex (50W-X8, H$^+$) and the resin washed with water. The L-*gulo* amide **22L** was liberated with 2 M aqueous ammonia and the ammoniacal fractions concentrated in vacuo to afford the L-*gulo* amide **22L** (19 mg, 77%) as a pale-yellow oil: $[\alpha]^{25}_D$ −9.0 (*c* 0.32 in H_2O) [lit.[57] $[\alpha]^{25}_D$ −4.3 (*c* 0.675 in H_2O)]. ν_{max} (thin film): 3325 (br s, NH,

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OH), 1648 (s, CO), 1562 (s, CO). δ_H (400 MHz, D_2O): 2.63 (3 H, s, *N*-Me), 3.40 (1 H, a-q, H-5, $J_{5,4} = J_{5,6} = J_{5,6'}$ 5.6), 3.50 (1 H, d, H-2, $J_{2,3}$ 2.5), 3.56 (1 H, dd, H-6, $J_{6,5}$ 6.8, $J_{6,6'}$ 11.2), 3.68 (1 H, dd, H-6′, $J_{6',5}$ 6.1, $J_{6',6}$ 11.2), 3.98 (1 H, dd, H-4, $J_{4,3}$ 2.6, $J_{4,5}$ 4.8), 4.06 (1 H, a-t, H-3, $J_{3,2} = J_{3,4}$ 2.6). δ_C (100 MHz, D_2O): 26.1 (*N*-Me), 61.1 (C-5), 61.4 (C-6), 66.4 (C-2), 76.6 (C-4), 80.8 (C-3), 175.6 (C-1). HRMS *m*/*z* (ESI +ve): found 213.0850 [M + Na^+], $C_7H_{14}N_2NaO_4$ requires 213.0846. *m*/*z* (ESI +ve): 191 (M + H^+, 100). For the enantiomer **22D**; $[\alpha]^{25}_D$ +7.9 (*c* 0.30 in H_2O).

Methyl 5-Azido-3,6-di-O-benzyl-5-deoxy-2-O-methanesulfonyl-L-idonamide ***25L****/Methyl 5-Azido-3,6-di-O-benzyl-5-deoxy-2-O-methanesulfonyl-L-gulonamide* ***26L***. Methylamine (33% in ethanol, 0.11 mL, 0.86 mmol) was added to a solution of the mesylate **24L**[34] (132 mg, 0.29 mmol) in 1,4-dioxane (6 mL) at rt. The reaction mixture was stirred for 16 h, after which TLC analysis (3:7, cyclohexane−ethyl acetate) indicated the complete consumption of the starting material (R_f 0.72) and the formation of two major, cospotting products (R_f 0.60). The reaction mixture was concentrated in vacuo and coevaporated with dichloromethane (3 × 10 mL) and the residue purified by flash chromatography (9:1 to 1:1 cyclohexane−ethyl acetate) to afford an inseparable 1.3:1 (A:B) epimeric mixture of the L-*gulo* amide **26L** and the L-*ido* amide **25L** (140 mg, 99%) as a yellow oil. ν_{max} (thin film): 3405 (br s, OH), 2100 (s, N_3), 1668 (s, CO), 1558 (s, CO), 1358 (s, SO_2), 1176 (s, SO_2). δ_H (400 MHz, CD_3OD): 2.75 (3 H, s, *N*-Me^B), 2.76 (3 H, s, *N*-Me^A), 3.14 (3 H, s, SO_2-Me^B), 3.14 (3 H, s, SO_2-Me^A), 3.52−3.56 (2 H, m, H-6^A, H-6^B), 3.61−3.67 (2 H, m, H-$6'^A$, H-$6'^B$), 3.75 (1 H, ddd, H-5^A or H-5^B, *J* 3.7, *J* 5.6, *J* 6.9), 3.77−3.80 (1 H, m, H-5^A or H-5^B), 3.82 (1 H, dd, H-4^B, $J_{4,5}$ 4.5, $J_{4,3}$ 5.4), 3.90 (1 H, dd, H-4^A, $J_{4,3}$ 4.3, $J_{4,5}$ 5.6), 3.96 (1 H, dd, H-3^A, $J_{3,4}$ 4.3, $J_{3,2}$ 5.1), 4.05 (1 H, dd, H-3^B, $J_{3,2}$ 4.3, $J_{3,4}$ 5.4), 4.49 (1 H, d, OCH_2Ph, J_{gem} 11.9), 4.50 (1 H, d, OCH_2Ph, J_{gem} 12.1), 4.53 (1 H, d, OCH_2Ph, J_{gem} 11.3), 4.54 (1 H, d, OCH_2Ph, J_{gem} 11.9), 4.56 (1 H, d, OCH_2Ph, J_{gem} 12.1), 4.62 (1 H, d, OCH_2Ph, J_{gem} 10.9), 4.64 (1 H, d, OCH_2Ph, J_{gem} 11.3), 4.70 (1 H, d, OCH_2Ph, J_{gem} 10.9), 5.13 (1 H, d, H-2^B, $J_{2,3}$ 4.3), 5.18 (1 H, d, H-2^A, $J_{2,3}$ 5.1), 7.26−7.35 (20 H, m, Ar-H^A, Ar-H^B). δ_C (100 MHz, CD_3OD): 26.5, 26.5 (*N*-Me^A, *N*-Me^B), 38.4, 38.6 (SO_2-Me^A, SO_2-Me^B), 63.1, 64.4 (C-5^A, C-5^B), 71.0, 71.1 (C-6^A, C-6^B), 71.6 (C-4^B), 71.8 (C-4^A), 74.2, 74.3, 75.2, 76.7 (OCH_2Ph^A, OCH_2Ph^B), 79.0 (C-2^A), 79.8 (C-2^B), 80.6, 80.6 (C-3^A, C-3^B), 128.8, 128.9, 129.0, 129.4, 129.4, 129.5 (ArCH^A, ArCH^B), 138.9, 139.2, 139.3 (ArCC^A, ArCC^B), 169.6 (C-1^A, C-1^B). *m*/*z* (ESI +ve): 515 (M + Na^+, 18), 1007 (2M + Na^+, 100). (ESI −ve): 491 ([M − H]$^-$, 65), 983 ([2M − H]$^-$, 100). HRMS *m*/*z* (ESI +ve): found 515.1567 [M + Na^+], $C_{22}H_{28}N_4NaO_7S$ requires 515.1571. For the enantiomeric mixture **25D**/**26D**: −.

Methyl 2-N-(tert-Butoxycarbonyl)-3,6-di-O-benzyl-2,5-dideoxy-2,5-imino-L-gulonamide ***27L****/Methyl 2-N-(tert-Butoxycarbonyl)-3,6-di-O-benzyl-2,5-dideoxy-2,5-imino-L-idonamide* ***28L***. Palladium (10% on carbon, 54 mg) and sodium acetate (45 mg, 0.46 mmol) were added to a solution of the inseparable L-*gulo* and L-*ido* amides **25L**/**26L** (1:1.3 mixture, 134 mg, 0.27 mmol) in 1,4-dioxane (5 mL). The reaction vessel was evacuated and flushed with hydrogen and stirred for 3 h, after which TLC analysis (2:1, toluene−acetone) indicated the complete consumption of the starting materials (R_f 0.54) and the presence of a major product (R_f 0.07). The reaction was filtered through glass microfiber (GF/A), and di-*tert*-butyl dicarbonate (45 mg, 0.46 mmol) was added portionwise to the filtrate. This reaction mixture was stirred for 16 h, after which TLC analysis (2:1, toluene−acetone) indicated the complete consumption of the intermediate product (R_f 0.07) and the formation of two products (R_f 0.54, 0.36). The reaction mixture was concentrated in vacuo and the crude residue purified by flash chromatography (1:0 to 3:2, toluene−acetone) to afford the cyclized Boc-protected amides, L-*gulo* **27L** (R_f 0.54, 54 mg, 42%) and L-*ido* **28L** (R_f 0.36, 56 mg, 44%) as clear oils. Partial data for L-*gulo* amide **27L** (R_f 0.54): $[\alpha]^{25}_D$ −10.0 (*c* 0.58 in $CHCl_3$). ν_{max} (thin film): 3321 (br s, OH), 1666 (s, CO), 1552 (s, CO). *m*/*z* (ESI +ve): 493 (M + Na^+, 30), 963 (2M + Na^+, 100). HRMS *m*/*z* (ESI +ve): found 493.2306 [M + Na^+], $C_{26}H_{34}N_2NaO_6$ requires 493.2309. For the enantiomer **27D** (R_f 0.54): $[\alpha]^{25}_D$ +2.9 (*c* 1.0 in $CHCl_3$). Partial data for L-*ido* amide **28L** (R_f 0.36): $[\alpha]^{25}_D$ −21.7 (*c* 1.17 in $CHCl_3$). ν_{max} (thin film): 3326 (br s, OH), 1657 (s, CO), 1550 (s, CO). *m*/*z* (ESI +ve): 493 (M + Na^+, 25), 963 (2M + Na^+, 100). HRMS *m*/*z* (ESI +ve): found 493.2310 [M + Na^+], $C_{26}H_{34}N_2NaO_6$ requires 493.2309. For the enantiomer **28D** (R_f 0.36): $[\alpha]^{25}_D$ +15.8 (*c* 0.96 in $CHCl_3$).

Methyl 2,5-Dideoxy-2,5-imino-L-idonamide ***29L***. Palladium (10% on carbon, 20 mg) was added to a solution of the Boc-protected amide **28L** (48 mg, 0.102 mmol) in a 1:1 1,4-dioxane−2 M aqueous hydrochloric acid mixture (4 mL). The reaction vessel was evacuated and flushed with hydrogen and stirred for 16 h. The reaction mixture was filtered and the filtrate stirred at 45 °C for 2 h and concentrated in vacuo. The crude residue was loaded onto a short column of Dowex (50W-X8, H^+) and the resin washed with water. The product was liberated with 2 M aqueous ammonia, and the ammoniacal fractions were combined and concentrated in vacuo to afford the L-*ido* amide **29L** as a pale yellow oil (14.5 mg, 75%): $[\alpha]^{25}_D$ +44.3 (*c* 0.92 in $CHCl_3$). ν_{max} (thin film): 3450 (br s, NH, OH), 1646 (s, CO), 1560 (s, CO). δ_H (500 MHz, D_2O): 2.68 (3 H, s, *N*-Me), 3.69 (1 H, m, H-5), 3.70 (1 H, dd, H-6, $J_{6,5}$ 6.8, $J_{6,6'}$ 11.4), 3.79 (1 H, dd, H-6′, $J_{6',5}$ 6.1, $J_{6',6}$ 11.4), 4.18 (1 H, d, H-2, $J_{2,3}$ 4.5), 4.25 (1 H, dd, H-4, $J_{4,3}$ 1.0, $J_{4,5}$ 3.3), 4.32 (1 H, dd, H-3, $J_{3,4}$ 1.0, $J_{3,2}$ 4.5). δ_C (125 MHz, D_2O): 26.2 (*N*-Me), 58.9 (C-6), 62.7 (C-5), 63.8 (C-2), 76.4 (C-4), 77.2 (C-3), 170.1 (C-1). *m*/*z* (ESI +ve): 191 (M + H^+, 100). HRMS *m*/*z* (ESI +ve): found 213.0843 [M + Na^+], $C_7H_{14}N_2NaO_4$ requires 213.0846. For the enantiomer **29D**: $[\alpha]^{25}_D$ −46.7 (*c* 0.95 in H_2O).

Synthesis of *Galacto*- and *Talo*-Configured Proline Amides (Scheme 3). *Methyl 5-Azido-3,6-di-O-benzyl-5-deoxy-2-O-methanesulfonyl-L-talonamide* ***34L****/Methyl 5-Azido-3,6-di-O-benzyl-5-deoxy-2-O-methanesulfonyl-L-galactonamide* ***35L***. From lactone **31L**: Methylamine (33% in ethanol, 90 μL, 2.13 mmol) was added to a solution of the lactone **31L**[34] (197 mg, 0.426 mmol) in 1,4-dioxane (10 mL). The reaction was stirred for 16 h, after which TLC analysis (3:7, cyclohexane−ethyl acetate) indicated the almost complete consumption of the starting material (R_f 0.96) and the formation of a major (R_f 0.35) and a minor product (R_f 0.64). The reaction mixture was concentrated in vacuo and the crude residue purified by flash chromatography (7:3 to 1:4, cyclohexane−ethyl acetate) to afford the L-*talo* amide **34L** (R_f 0.35, 165 mg, 84%) as a clear, viscous oil and the minor L-*galacto* amide **35L** (R_f 0.64, 22 mg, 11%) as a clear oil which crystallized on standing. From lactone **33L**: Methylamine (33% in EtOH, 0.11 mL, 0.89 mmol) was added to a solution of the lactone **33L** (137 mg, 0.297 mmol) in 1,4-dioxane (2.5 mL) under argon. The reaction mixture was stirred for 16 h, after which TLC analysis (3:7, cyclohexane−ethyl acetate) indicated the complete consumption of the starting material (R_f 0.96) and the formation of a major (R_f 0.64) and a minor product (R_f 0.35). The reaction was concentrated in vacuo and the crude residue was purified by flash chromatography (7:3 to 11:9, cyclohexane−ethyl acetate) to afford the L-*talo* amide **34L** (R_f 0.35, 7 mg, 5%) as a clear oil and the L-*galacto* amide **35L** (R_f 0.64, 133 mg, 91%) as a white crystalline solid. Data for L-*talo* amide **34L**: $[\alpha]^{25}_D$ +66.6 (*c* 2.01 in $CHCl_3$). ν_{max} (thin film): 3418 (br s, OH), 2105 (s, N_3), 1666 (s, CO), 1560 (s, CO), 1455 (s, SO_2), 1179 (s, SO_2). δ_H (400 MHz, $CDCl_3$): 2.65 (1 H, d, 4-OH, $J_{OH,4}$ 8.8), 2.83 (3 H, d, *N*-Me, $J_{H,NH}$ 5.1), 3.05 (3 H, s, SO_2-Me), 3.76 (1 H, dd, H-6, $J_{6,5}$ 5.1, $J_{6,6'}$ 9.9), 3.78 (1 H, dd, H-6′, $J_{6',5}$ 6.6, $J_{6',6}$ 9.9), 3.82 (1 H, ddd, H-4, $J_{4,5}$ 1.3, $J_{4,OH}$ 8.8, $J_{4,3}$ 9.3), 3.85 (1 H, ddd, H-5, $J_{5,4}$ 1.3, $J_{5,6}$ 5.1, $J_{5,6'}$ 6.6), 4.24 (1 H, dd, H-3, $J_{3,2}$ 1.4, $J_{3,4}$ 9.3), 4.55 (1 H, d, OCH_2Ph, J_{gem} 11.9), 4.57 (1 H, d, OCH_2Ph, J_{gem} 10.9), 4.59 (1 H, d, OCH_2Ph, J_{gem} 11.9), 4.77 (1 H, d, OCH_2Ph, J_{gem} 10.9), 5.46 (1 H, d, H-2, $J_{2,3}$ 1.4), 6.46 (1 H, q, *N*-H, $J_{NH,H}$ 5.1), 7.30−7.40 (10 H, m, Ar-H). δ_C (100 MHz, $CDCl_3$): 26.4 (*N*-Me), 39.1 (SO_2-Me), 60.7 (C-5), 70.3 (C-4), 71.3 (C-6), 73.4, 73.8 (OCH_2Ph), 79.0 (C-3), 79.4 (C-2), 127.9, 128.1, 128.6, 128.8 (ArCH), 136.5, 137.4 (ArCC), 167.4 (C-1). *m*/*z* (ESI +ve): 515 (M + Na^+, 100). (ESI −ve): 491 ([M − H]$^-$, 64), 527 (M + $^{35}Cl^-$, 52), 529 (M + $^{37}Cl^-$, 18), 983 ([2M − H]$^-$, 100). HRMS *m*/*z* (ESI +ve): found 515.1568 [M + Na^+], $C_{22}H_{28}N_4NaO_7S$ requires 515.1571. For the enantiomer **34D**: $[\alpha]^{25}_D$ −71.1 (*c* 2.05 in $CHCl_3$). Data for L-*galacto* amide **35L**: mp 114−116 °C. $[\alpha]^{25}_D$ +33.9 (*c* 1.08 in $CHCl_3$). ν_{max} (thin film): 3407 (br s, OH), 2105 (s, N_3), 1668 (s, CO), 1555 (s, CO), 1455 (s, SO_2), 1178 (s, SO_2). δ_H (400 MHz, $CDCl_3$): 2.86 (3 H, d, *N*-Me, $J_{H,NH}$ 4.5), 3.11 (3 H, s, SO_2-Me), 3.15 (1 H, d, 4-OH, $J_{OH,4}$ 8.2), 3.70 (1 H, ddd, H-4, $J_{4,5}$ 1.3, $J_{4,OH}$ 8.2, $J_{4,3}$ 9.3), 3.75 (1 H, ddd, H-5, $J_{5,4}$ 1.3, $J_{5,6}$ 4.3, $J_{5,6'}$ 6.6), 3.84 (1 H, dd, H-6, $J_{6,5}$ 4.3, $J_{6,6'}$ 10.4), 3.87 (1 H, dd, H-6′, $J_{6',5}$ 6.6, $J_{6',6}$ 10.4), 4.30 (1 H, dd, H-3, $J_{3,2}$ 1.7, $J_{3,4}$ 9.3), 4.52 (1 H, d,

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OCH_2Ph, J_{gem} 11.1), 4.58 (3 H, a-d, 3 × OCH_2Ph, J 11.4), 5.32 (1 H, d, H-2, $J_{2,3}$ 1.7), 6.63 (1 H, q, *N*-H, $J_{NH,H}$ 4.5), 7.24−7.37 (10 H, m, Ar-H). δ_C (100 MHz, $CDCl_3$): 26.6 (*N*-Me), 38.3 (SO_2-Me), 59.9 (C-5), 70.4 (C-4), 71.5 (C-6), 73.8, 75.2 (OCH_2Ph), 78.7 (C-3), 79.2 (C-2), 127.9, 128.1, 128.2, 128.4, 128.7, 128.7 (ArCH), 137.1, 137.3 (ArCC), 168.4 (C-1). *m*/*z* (ESI +ve): 515 (M + Na^+, 100). HRMS *m*/*z* (ESI +ve): found 515.1575 [M + Na^+], $C_{22}H_{28}N_4NaO_7S$ requires 515.1571. For the enantiomer **35D**: mp 114−116 °C. $[\alpha]^{25}_D$ −36.1 (*c* 1.10 in $CHCl_3$).

Methyl 2,5-Dideoxy-2,5-imino-L-galactonamide ***36L***. Palladium (10% on carbon, 75 mg) was added to a solution of the L-*talo* amide **34L** (165 mg, 0.34 mmol) and sodium acetate (72 mg, 0.88 mmol) in 1,4-dioxane (8 mL). The flask was evacuated and flushed with hydrogen and stirred for 6 h, after which TLC analysis (99:1, ethyl acetate−triethylamine) indicated the complete consumption of the starting material (R_f 0.72) and the formation of a major product (R_f 0.08). Aqueous hydrochloric acid (2 M, 1.4 mL) was added, the reaction vessel evacuated and flushed with hydrogen, and the reaction stirred for 16 h, after which the reaction mixture was filtered through glass microfiber (GF/A) and concentrated in vacuo. The crude residue was loaded onto a short column of Dowex (50W-X8, H^+) and the resin washed with water. The product was liberated with 2 M aqueous ammonia, and the ammoniacal fractions were combined and concentrated in vacuo to afford the L-*galacto* amide **36L** (60 mg, 94%) as a yellow oil: $[\alpha]^{25}_D$ −41.1 (*c* 0.24 in H_2O). ν_{max} (thin film): 3332 (br s, OH, NH), 1645 (s, CO), 1554 (s, CO). δ_H (400 MHz, D_2O): 2.66 (3 H, a-s, *N*-Me), 3.34 (1 H, a-q, H-5, $J_{5,4}$ = $J_{5,6}$ = $J_{5,6'}$ 5.8), 3.53 (1 H, dd, H-6, $J_{6,5}$ 6.3, $J_{6,6'}$ 11.5), 3.61 (1 H, dd, H-6′, $J_{6',5}$ 4.8, $J_{6',6}$ 11.5), 3.81 (1 H, d, H-2, $J_{2,3}$ 6.1), 4.23 (1 H, dd, H-4, $J_{4,3}$ 5.5, $J_{4,5}$ 6.2), 4.27 (1 H, a-t, H-3, $J_{3,2}$ = $J_{3,4}$ 5.8). δ_C (100 MHz, D_2O): 25.9 (*N*-Me), 59.9 (C-5), 61.6 (C-6), 62.5 (C-2), 72.5 (C-4), 72.8 (C-3), 174.6 (C-1). *m*/*z* (ESI +ve): 191 (M + H^+, 100), 213 (M + Na^+, 68). HRMS *m*/*z* (ESI +ve): found 191.1025 [M + H^+], $C_7H_{15}N_2O_4$ requires 191.1026. For the enantiomer **36D**: mp 85−87 °C (from 2-propanol). $[\alpha]^{25}_D$ +46.6 (*c* 0.26 in H_2O).

Methyl 2,5-Dideoxy-2,5-imino-L-talonamide ***37L***. Palladium (10% on carbon, 10 mg) was added to a solution of the mesylate **35L**[34] (22 mg, 0.04 mmol) and sodium acetate (16 mg, 0.20 mmol) in 1,4-dioxane (2 mL). The flask was evacuated, flushed with hydrogen, and stirred for 6 h, after which TLC analysis (99:1, ethyl acetate−triethylamine) indicated the complete consumption of the starting material (R_f 0.82) and the formation of a major product (R_f 0.18). Aqueous hydrochloric acid (2 M, 0.4 mL) was added, the reaction vessel evacuated and flushed with hydrogen, and the reaction stirred for 16 h. The reaction mixture was filtered through glass microfiber (GF/A) and concentrated in vacuo. The crude residue loaded onto a short column of Dowex (50W-X8, H^+) and the resin washed with water. The product was liberated with 2 M aqueous ammonia, and the ammoniacal fractions were combined and concentrated in vacuo to afford the L-*talo* amide **37L** (5.5 mg, 90%) as a yellow oil: $[\alpha]^{25}_D$ −5.0 (*c* 0.28 in H_2O). ν_{max} (thin film): 3335 (br s, OH, NH), 1648 (s, CO), 1558 (s, CO). δ_H (400 MHz, D_2O): 2.66 (3 H, s, *N*-Me), 3.30 (1 H, a-td, H-5, $J_{5,4}$ 4.1, $J_{5,6}$ = $J_{5,6'}$ 6.6), 3.44 (1 H, d. H-2, $J_{2,3}$ 8.2), 3.52 (1 H, dd, H-6, $J_{6,5}$ 6.6, $J_{6,6'}$ 11.0), 3.67 (1 H, dd, H-6′, $J_{6',5}$ 6.6, $J_{6',6}$ 11.0), 4.05 (1 H, dd, H-3, $J_{3,4}$ 4.1, $J_{3,2}$ 8.2), 4.08 (1 H, t, H-4, $J_{4,3}$ = $J_{4,5}$ 4.1). δ_C (100 MHz, D_2O): 26.3 (*N*-Me), 60.9 (C-5), 61.0 (C-6), 63.7 (C-2), 72.7 (C-4), 76.9 (C-3), 175.5 (C-1). *m*/*z* (ESI +ve): 191 (M + H^+, 100), 213 (M + Na^+, 55). HRMS *m*/*z* (ESI +ve): found 191.1028 [M + H^+], $C_7H_{15}N_2O_4$ requires 191.1026. For the enantiomer **37D**: $[\alpha]^{25}_D$ +6.9 (*c* 0.30 in H_2O).

Synthesis of *Manno*- and *Gluco*-Configured Proline Amides (Scheme 4). *Methyl 2,5-Dideoxy-2,5-imino-D-mannonamide* ***41D***. Palladium (10% on carbon, 32 mg) was added to a solution of triflate **39D**[34] (150 mg, 0.3 mmol) in 1,4-dioxane (2 mL). The reaction was purged with argon and then hydrogen and allowed to stir for 1 h, after which TLC analysis (2:1, cyclohexane−ethyl acetate) indicated the complete consumption of the starting material (R_f 0.80) and the formation of a major product (R_f 0.20). The reaction was filtered through glass microfiber (GF/B) and methylamine (33% in EtOH, 0.07 mL, 0.58 mmol) was added. The reaction was stirred at rt for 18 h after which TLC analysis indicated only baseline material. Palladium (10% on carbon, 64 mg) and 2 M aqueous HCl (0.6 mL) were added, and the reaction was purged with argon and then hydrogen and stirred for 18 h. The reaction was filtered through glass microfiber (GF/B) and concentrated in vacuo. The crude residue was dissolved in 2 M aqueous HCl and loaded onto a short column of Dowex (50W-X8, H^+), the column washed with water, and the product liberated with 2 M aqueous ammonia to afford the proline amide **41D** (58 mg, 99%) as a yellow oil: $[\alpha]^{25}_D$ −3.8 (*c* 1.01 in MeOH). ν_{max} (thin film): 3331 (br s, OH, NH), 1651 (s, CO), 1547 (s, CO). δ_H (400 MHz, D_2O): 2.65 (3 H, s, *N*-Me), 3.00 (1 H, ddd, H-5, $J_{5,6'}$ 4.2, $J_{5,6}$ 6.2, $J_{5,4}$ 7.2), 3.41 (1 H, d, H-2, $J_{2,3}$ 6.5), 3.50 (1 H, dd, H-6, $J_{6,5}$ 6.2, $J_{6,6'}$ 11.6), 3.61 (1 H, dd, H-6′, $J_{6',5}$ 4.2, $J_{6',6}$ 11.6), 3.72 (1 H, dd, H-4, $J_{4,3}$ 6.5, $J_{4,5}$ 7.2), 3.94 (1 H, t, H-3, $J_{3,2}$ = $J_{3,4}$ 6.5). δ_C (100 MHz, D_2O): 26.2 (*N*-Me), 62.3 (C-6), 63.0 (C-5), 64.2 (C-2), 78.1 (C-4), 80.5 (C-3), 175.1 (C-1). *m*/*z* (ESI +ve): 191 (M + H^+, 100), 213 (M + Na^+, 68), 403 (2M + Na^+, 32). (ESI −ve): 189 ([M − H]$^-$, 95), 225 (M + Cl^-, 100), 379 ([2M − H]$^-$, 52). HRMS *m*/*z* (ESI +ve): found 191.1026 [M + H^+], $C_7H_{15}N_2O_4$ requires 191.1026. For the enantiomer **41L**: $[\alpha]^{25}_D$ +1.9 (*c* 1.01 in MeOH).

Methyl 5-Azido-3,6-di-O-benzyl-5-deoxy-2-O-methanesulfonyl-D-mannonamide ***44D***. Methylamine solution (33% in absolute ethanol, 0.24 mL, 1.90 mmol) was added to a solution of the mesylate **43D**[34] (0.18 g, 0.38 mmol) in 1,4-dioxane (3 mL) at rt. The reaction was stirred for 16 h, after which TLC analysis (1:1, ethyl acetate−cyclohexane) indicated the complete consumption of the starting material (R_f 0.83) and the formation of a major product (R_f 0.29). The reaction was concentrated in vacuo, and the remaining residue was purified by flash chromatography (1:4, ethyl acetate−cyclohexane) to afford the amide **44D** (170 mg, 92%) as a colorless oil: $[\alpha]^{20}_D$ −52.8 (*c* 0.71 in $CHCl_3$). ν_{max} (thin film): 3394 (br s, OH, NH), 2101 (s, N_3), 1668 (s, CO). δ_H (400 MHz, $CDCl_3$): 2.85 (3 H, d, *N*-Me, $J_{H,NH}$ 4.8), 3.06 (3 H, s, SO_2-Me), 3.51 (1 H, br s 4-OH), 3.58 (1 H, ddd, H-5, $J_{5,6'}$ 3.4, $J_{5,6}$ 6.4, $J_{5,4}$ 9.6), 3.71 (1 H, m, H-4), 3.72 (1 H, dd, H-6, $J_{6,5}$ 6.4, $J_{6,6'}$ 10.0), 3.89 (1 H, dd, H-6′, $J_{6',5}$ 3.4, $J_{6',6}$ 10.0), 4.25 (1 H, dd, H-3, $J_{3,4}$ 1.4, $J_{3,2}$ 4.0), 4.57 (2 H, s, OCH_2Ph), 4.63 (1 H, d, OCH_2Ph, J_{gem} 10.8), 4.75 (1 H, d, OCH_2Ph, J_{gem} 10.8), 5.25 (1 H, d, H-2, $J_{2,3}$ 4.0), 6.55 (1 H, q, *N*-H, $J_{NH,H}$ 4.8), 7.28−7.40 (10 H, m, Ar-H). δ_C (100 MHz, $CDCl_3$): 26.6 (*N*-Me), 39.1 (SO_2-Me), 61.9 (C-5), 70.3 (C-6), 70.4 (C-4), 73.7, 74.3 (OCH_2Ph), 77.1 (C-3), 79.7 (C-2), 127.8, 128.0, 128.6, 128.7, 128.7, 128.7, 128.8 (ArCH), 136.8, 137.7 (ArCC), 167.1 (C-1). *m*/*z* (ESI +ve): 493 (M + H^+, 9), 515 (M + Na^+, 30), 1007 (2M + Na^+, 100). HRMS *m*/*z* (ESI +ve): found 515.1567 [M + Na^+], $C_{22}H_{28}N_4NaO_7S$ requires 515.1571. For the enantiomer **44L**: $[\alpha]^{20}_D$ +59.2 (*c* 0.74 in $CHCl_3$).

Methyl 2,5-Dideoxy-2,5-imino-D-gluconamide ***46D***. Palladium (10% on carbon, 56 mg) and sodium acetate (47 mg, 0.57 mmol) were added to a solution of the amide **44D** (140 mg, 0.28 mmol) in 1,4-dioxane (10 mL). The flask was evacuated and flushed three times with argon and three times with hydrogen. The reaction was stirred at rt for 18 h, after which TLC analysis (1:1, ethyl acetate−cyclohexane) indicated the complete consumption of starting material (R_f 0.29) and the formation of a baseline product. The reaction mixture was filtered through glass microfiber (GF/B) and was concentrated in vacuo to afford a yellow oil. The crude residue was dissolved in 1,4-dioxane (10 mL), and di-*tert*-butyl dicarbonate (0.13 mL, 0.57 mmol) was added. After 24 h, TLC analysis (2:1, toluene−acetone) indicated the consumption of starting material and the formation of a major product (R_f 0.42). The solvents were removed in vacuo, and the remaining residue was purified by flash chromatography (9:1, toluene−acetone) to yield the *N*-Boc-protected intermediate **45D** as a yellow oil (69 mg, 52%). This intermediate was dissolved in a 1:1 1,4-dioxane−2 M HCl mixture (2.8 mL), and palladium (10% on carbon, 27 mg) was added. The flask was evacuated and flushed three times with argon and three times with hydrogen. The reaction was stirred at rt for 20 h, after which TLC analysis (2:1, toluene−acetone) indicated the complete consumption of starting material (R_f 0.42) and the formation of a baseline product. The reaction mixture was filtered through glass microfiber (GF/B) and concentrated in vacuo, and the residue was loaded onto a short column of Dowex (50W-X8, H^+) and washed with water. The product was liberated with 2 M aqueous ammonia, and the ammoniacal fractions were combined and concentrated in vacuo to afford the proline amide **46D** (27.0 mg, 97%) as a colorless oil: $[\alpha]^{25}_D$ +49.7 (*c* 0.86 in H_2O) [lit.[57] $[\alpha]^{23}_D$ +65.06 (*c* 0.8 in H_2O)]. ν_{max} (thin film): 3304 (br s, OH, NH), 1636 (s, CO), 1544 (s, CO). δ_H (400 MHz,

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D_2O): 2.65 (3 H, s, *N*-Me), 3.04 (1 H, m, H-5), 3.52 (1H, dd, H-6, $J_{6,5}$ 6.4, $J_{6,6'}$ 11.6), 3.59 (1 H, dd, H-6′, $J_{6',5}$ 4.8, $J_{6',6}$ 11.6), 3.80 (1 H, dd, H-4, $J_{4,5}$ 4.0, $J_{4,3}$ 3.2), 3.86 (1 H, d, H-2, $J_{2,3}$ 5.8), 4.13 (1 H, dd, H-3, $J_{3,4}$ 3.2, $J_{3,2}$ 5.8). δ_C (100 MHz, D_2O): 25.9 (*N*-Me), 62.8 (C-6), 63.7 (C-2), 64.7 (C-5), 77.9 (C-3), 78.2 (C-4), 173.9 (C-1). *m*/*z* (ESI +ve): 191 (M + H^+, 56), 213 (M + Na^+, 100). HRMS *m*/*z* (ESI +ve): found 213.0849 [M + Na^+], $C_7H_{14}N_2NaO_4$ requires 213.0846. For the enantiomer **46L**: $[\alpha]^{20}_D$ −54.0 (*c* 0.44 in H_2O).

Synthesis of *Altro*- and *Allo*-Configured Proline Amides (Scheme 5). *Methyl 5-Azido-3,6-di-O-benzyl-5-deoxy-2-O-methanesulfonyl-D-allonamide* ***51D***. Methylamine (33% in ethanol, 0.11 mL, 0.85 mmol) was added to a solution of mesylate **48D**[34] (75 mg, 0.17 mmol) in 1,4-dioxane (3.8 mL). The reaction was stirred at rt for 17 h under argon, after which TLC analysis (3:7, cyclohexane−ethyl acetate) indicated the complete consumption of starting material (R_f 0.91) and the formation of a major product (R_f 0.44). The reaction mixture was concentrated in vacuo and purification by flash chromatography (4:1 to 1:1, cyclohexane−ethyl acetate) to afford the amide **51D** (55 mg, 69%) as a colorless oil: $[\alpha]^{25}_D$ +18.4 (*c* 0.89 in $CHCl_3$). ν_{max} (thin film): 3419 (br s, NH, OH), 2101 (s, N_3), 1665 (s, CO). δ_H (400 MHz, $CDCl_3$): 2.84 (3 H, d, *N*-Me, $J_{H,NH}$ 4.8), 3.04 (3 H, s, SO_2-Me), 3.53 (1 H, dd, H-6, $J_{6,5}$ 6.8, $J_{6,6'}$ 11.4), 3.63−3.68 (2 H, m, H-5, H-6′), 3.95 (1 H, dd, H-4, $J_{4,5}$ 2.5, $J_{4,3}$ 8.4), 4.20 (1 H, dd, H-3, $J_{3,2}$ 1.6, $J_{3,4}$ 8.4), 4.42 (1 H, d, OCH_2Ph, J_{gem} 11.1), 4.46 (1 H, d, OCH_2Ph, J_{gem} 11.7), 4.49 (1 H, d, OCH_2Ph, J_{gem} 11.7), 4.67 (1 H, d, OCH_2Ph, J_{gem} 11.1), 5.44 (1 H, d, H-2, $J_{2,3}$ 1.6), 6.50 (1 H, d, *N*-H, $J_{NH,H}$ 4.8), 7.26−7.38 (10 H, m, Ar-H). δ_C (100 MHz, $CDCl_3$): 26.4 (*N*-Me), 39.1 (SO_2-Me), 61.7 (C-5), 69.8 (C-6), 71.2 (C-4), 72.3, 73.9 (OCH_2Ph), 78.8 (C-3), 78.9 (C-2), 128.1, 128.2, 128.6, 128.7, 128.8, 128.8, 128.9 (ArCH), 136.5, 137.1 (ArCC), 167.6 (C-1). *m*/*z* (ESI +ve): 493 (M + H^+, 51), 515 (M + Na^+, 100). (ESI −ve): 491 ([M − H]$^-$, 34), 527 (M + $^{35}Cl^-$, 100), 529 (M + $^{37}Cl^-$, 60). HRMS *m*/*z* (ESI +ve): found 515.1567 [M + Na^+], $C_{22}H_{28}N_4NaO_7S$ requires 515.1571. For the enantiomer **51L**: $[\alpha]^{25}_D$ −17.7 (*c* 0.98 in $CHCl_3$).

Methyl 2,5-Dideoxy-2,5-imino-D-altronamide ***53D***. Palladium (10% on carbon, 11 mg) and sodium acetate (20 mg, 0.24 mmol) were added to a solution of amide **51D** (55 mg, 0.12 mmol) in 1,4-dioxane (2.2 mL). The flask was evacuated and flushed three times with argon and three times with hydrogen. The reaction was stirred at rt for 3 h under hydrogen, after which TLC analysis (ethyl acetate) indicated the complete consumption of starting material (R_f 0.71) and the formation of a single product (R_f 0.06). Aqueous hydrochloric acid (2 M, 0.44 mL) was added to the reaction mixture and the flask evacuated and flushed three times with argon and three times with hydrogen. The reaction was stirred for a further 19 h. The reaction was filtered through glass microfiber (GF/B) and the filtrate concentrated in vacuo. The crude residue was dissolved in water and loaded onto a column of Dowex (50W-X8, H^+). The column was washed with water and the amide **53D** liberated with 2 M aqueous ammonia. The ammoniacal fractions were concentrated in vacuo to afford the amide **53D** (20 mg, 94%) as a yellow solid: mp 154−156 °C. $[\alpha]^{25}_D$ −29.8 (*c* 0.83 in H_2O). ν_{max} (thin film): 3297 (br s, NH, OH), 1637 (s, CO). δ_H (400 MHz, D_2O): 2.67 (3 H, s, *N*-Me), 3.11 (1 H, ddd, H-5, $J_{5,6'}$ 3.8, $J_{5,6}$ 5.4, $J_{5,4}$ 9.1), 3.54 (1 H, dd, H-6, $J_{6,5}$ 5.4, $J_{6,6'}$ 11.9), 3.72 (1 H, dd, H-6′, $J_{6',5}$ 3.8, $J_{6',6}$ 11.9), 3.84−3.88 (2 H, m, H-2, H-4), 4.25 (1 H, a-t, H-3, $J_{3,2} = J_{3,4}$ 4.3). δ_C (100 MHz, D_2O): 26.0 (*N*-Me), 61.8 (C-6), 62.0 (C-5), 62.8 (C-2 or C-4), 73.3 (C-3), 73.8 (C-2 or C-4), 173.5 (C-1). *m*/*z* (ESI +ve): 191 (M + Na^+, 90), 213 (M + Na^+, 100). HRMS *m*/*z* (ESI +ve): found 213.0849 [M + Na^+], $C_7H_{14}N_2NaO_4$ requires 213.0846. For the enantiomer **53L**: mp 156−158 °C. $[\alpha]^{25}_D$ +39.9 (*c* 0.88 in H_2O).

Methyl 5-Azido-3,6-di-O-benzyl-5-deoxy-2-O-methanesulfonyl-D-altronamide ***52D***. Methylamine (33% in ethanol, 2.75 mL, 22.3 mmol) was added to a solution of the mesylate **50D**[34] (3.43 g, 7.43 mmol) in 1,4-dioxane (75 mL) at rt. The reaction was stirred for 18 h, after which TLC analysis (3:1, toluene−acetone) indicated complete consumption of the starting material (R_f 0.80) and the formation of a major product (R_f 0.37). The reaction mixture was concentrated in vacuo and the residue purified by flash chromatography (7:3 to 13:7, cyclohexane−ethyl acetate) to afford the amide **52D** (3.31 g, 91%) as a white crystalline solid: mp 86−88 °C. $[\alpha]^{25}_D$ −1.50 (*c* 1.72 in $CHCl_3$). ν_{max} (thin film): 3402 (br s, OH), 2101 (s, N_3), 1666 (s, CO), 1544 (s, CO), 1360 (s, SO_2) 1176 (s, SO_2). δ_H (400 MHz, CD_3CN): 2.76 (3 H, d, *N*-Me, $J_{H,NH}$ 4.8), 3.16 (3 H, s, SO_2-Me), 3.63 (1 H, dd, H-6, $J_{6,5}$ 7.7, $J_{6,6'}$ 10.4), 3.69 (1 H, dd, H-6′, $J_{6',5}$ 3.8, $J_{6',6}$ 10.4), 3.71 (1 H, d, 4-OH, $J_{OH,4}$ 6.6), 3.80 (1 H, ddd, H-4, $J_{4,5}$ 3.0, $J_{4,OH}$ 6.6, $J_{4,3}$ 9.3), 3.87 (1 H, a-dt, H-5, $J_{5,4} = J_{5,6'}$ 3.5, $J_{5,6}$ 7.7), 4.05 (1 H, dd, H-3, $J_{3,2}$ 1.8, $J_{3,4}$ 9.3), 4.43 (1 H, d, OCH_2Ph, J_{gem} 11.1), 4.46 (1 H, d, OCH_2Ph, J_{gem} 11.1), 4.48 (1 H, d, OCH_2Ph, J_{gem} 12.0), 4.51 (1 H, d, OCH_2Ph, J_{gem} 12.0), 5.12 (1 H, d, H-2, $J_{2,3}$ 1.8), 7.05 (1 H, br q, *N*-H, $J_{NH,H}$ 4.8), 7.25−7.38 (10 H, m, Ar-H). δ_C (100 MHz, CD_3CN): 26.5 (*N*-Me), 38.7 (SO_2-Me), 64.5 (C-5), 70.3 (C-6), 71.0 (C-4), 73.8, 74.8 (OCH_2Ph), 79.1 (C-2), 79.9 (C-3), 128.7, 128.8, 129.3, 129.4 (ArCH), 138.4, 139.1 (ArCC), 168.9 (C-1). *m*/*z* (ESI +ve): 493 (M + H^+, 16), 515 (M + Na^+, 100). (ESI −ve): 491 ([M − H]$^-$, 100), 427 (M + $^{35}Cl^-$, 65), 429 (M + $^{37}Cl^-$, 25), 983 ([2M − H]$^-$, 45). HRMS *m*/*z* (ESI +ve): found 515.1570 [M + Na^+], $C_{22}H_{28}N_4NaO_7S$ requires 515.1571. For the enantiomer **52L**: mp 85−87 °C. $[\alpha]^{25}_D$ +2.4 (*c* 1.60 in $CHCl_3$).

Methyl 2,5-Dideoxy-2,5-imino-D-allonamide ***54D***. Palladium (10% on carbon, 650 mg) and sodium acetate (1.08 g, 13.21 mmol) were added to a solution of the amide **52D** (3.25 g, 6.61 mmol) in 1,4-dioxane (65 mL) at rt. The flask was evacuated and flushed with hydrogen and stirred for 3.5 h, after which TLC analysis (1:1, toluene−ethyl acetate) indicated the complete consumption of starting material (R_f 0.70) and the formation of a major product (R_f 0.28). Aqueous hydrochloric acid (2 M, 13 mL) was added to the reaction mixture and the flask evacuated and flushed with hydrogen. The reaction mixture was stirred for 24 h. The reaction was filtered glass microfiber (GF/B) and the filtrate concentrated in vacuo. The crude residue was loaded onto a short column of Dowex (50W-X8, H^+) and the resin washed with water. The product was liberated with 2 M aqueous ammonia and the ammoniacal fractions were combined and concentrated in vacuo to afford the D-*allo* amide **54D** (1.15 g, 91%) as a yellow oil: $[\alpha]^{25}_D$ +14.6 (*c* 0.27 in H_2O). ν_{max} (thin film): 3272 (br s, OH, NH), 1643 (s, CO), 1543 (s, CO). δ_H (400 MHz, D_2O): 2.66 (3 H, s, *N*-Me), 3.31 (1 H, a-td, H-5, $J_{5,6'}$ 4.0, $J_{5,4} = J_{5,6}$ 6.1), 3.58 (1 H, dd, H-6, $J_{6,5}$ 5.6, $J_{6,6'}$ 12.1), 3.69 (1 H, dd, H-6′, $J_{6',5}$ 4.0, $J_{6',6}$ 12.1), 3.74 (1 H, d, H-2, $J_{2,3}$ 4.3), 3.84 (1 H, dd, H-4, $J_{4,3}$ 4.7, $J_{4,5}$ 6.7), 4.07 (1 H, a-t, H-3, $J_{3,2} = J_{3,4}$ 4.5). δ_C (100 MHz, D_2O): 26.3 (*N*-Me), 61.1 (C-6), 63.2 (C-5), 64.8 (C-2), 71.9 (C-4), 75.0 (C-3), 172.8 (C-1). *m*/*z* (ESI +ve): 191 (M + H^+, 100). HRMS *m*/*z* (ESI +ve): found 213.0842 [M + Na^+], $C_7H_{14}N_2NaO_4$ requires 213.0846. For the enantiomer **54L**. $[\alpha]^{25}_D$ −17.3 (*c* 0.33 in H_2O).

Synthesis of the Azetidine-acetamide (Scheme 6). *Methyl 2-N,3-O-Dibenzyl-2,4-dideoxy-2,4-imino-5-O-methanesulfonyl-D-ribonate* ***56***. Methanesulfonyl chloride (0.08 mL, 2.45 mmol) was added to a solution of the methyl ester **5S**[33] (520 mg, 1.52 mmol) in pyridine (5.0 mL) at rt under argon. The reaction mixture was stirred for 90 min, after which TLC analysis (1:1, cyclohexane−ethyl acetate) indicated the complete consumption of the starting material (R_f 0.46) and the formation of a major product (R_f 0.64). The reaction mixture was diluted with ethyl acetate (10 mL), and the mixture was washed with 2 M aqueous hydrochloric acid (2 × 10 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo to afford the mesylate **56** (562 mg, 88%) as a yellow oil, which was reacted on without further purification: $[\alpha]^{20}_D$ +39.0 (*c* 0.41 in CH_3Cl). ν_{max} (thin film): 1740 (s, CO). δ_H (400 MHz, $CDCl_3$): 2.93 (3 H, s, SO_2-Me), 3.32 (1 H, a-q, H-4, $J_{4,3} = J_{4,5} = J_{4,5'}$ 5.0), 3.62 (1 H, d, H-2, $J_{2,3}$ 4.0), 3.62 (3 H, s, CO_2-Me), 3.77 (1 H, d, NCH_2Ph, J_{gem} 12.8), 3.84 (1 H, dd, H-5, $J_{5,4}$ 4.4, $J_{5,5'}$ 11.2), 3.88 (1 H, d, NCH_2Ph, J_{gem} 12.8), 4.00 (1 H, dd, H-5′, $J_{5',4}$ 4.4, $J_{5',5}$ 11.2), 4.10 (1 H, a-t, H-3, $J_{3,2} = J_{3,4}$ 5.2), 4.49 (1 H, d, OCH_2Ph, J_{gem} 11.6), 4.60 (1 H, d, OCH_2Ph, J_{gem} 11.6), 7.27−7.37 (10 H, m, Ar-H). δ_C (100 MHz, $CDCl_3$): 37.6 (SO_2-Me), 52.5 (CO_2-Me), 60.0 (NCH_2Ph), 67.5 (C-2), 68.3 (C-5), 68.7 (C-4), 72.4 (OCH_2Ph), 72.9 (C-3), 128.1, 128.4, 128.7, 128.7, 128.9, 130.1 (ArCH), 136,9 (ArCC), 169.7 (C-1). *m*/*z* (ESI +ve): 420 (M + H^+, 100). HRMS *m*/*z* (ESI +ve): found 442.1299 [M + Na^+], $C_{21}H_{25}NNaO_6S$ requires 442.1295.

Methyl 5-Azido-2-N,3-O-dibenzyl-2,4,5-trideoxy-2,4-imino-D-ribonate ***57***. Sodium azide (110 mg, 1.69 mmol) was added to a solution of the mesylate **56** (500 mg, 1.30 mmol) in DMF (5.00 mL) at rt. The reaction mixture was heated to 60 °C and stirred for 24 h, after which TLC analysis (1:1, cyclohexane−ethyl acetate) indicated the

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complete consumption of the starting material (R_f 0.64) and the formation of a major product (R_f 0.78). The reaction mixture was diluted with ethyl acetate (10 mL) and washed with a 1:1 brine−water mixture (2 × 10 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash chromatography (7:1 to 5:1, cyclohexane−ethyl acetate) to afford the azide **57** (372 mg, 78%) as a yellow oil: $[\alpha]^{20}_D$ +70.3 (*c* 0.92 in CH_3Cl). ν_{max} (thin film): 2099 (s, N_3), 1741 (s, CO). δ_H (400 MHz, $CDCl_3$): 2.89 (1 H, dd, H-5, $J_{5,4}$ 4.4, $J_{5,5'}$ 12.8), 3.02 (1 H, d, H-5′, $J_{5'4}$ 4.8, $J_{5',5}$ 12.8), 3.22 (1 H, a-q, H-4, $J_{4,3} = J_{4,5} = J_{4,5'}$ 4.8), 3.58 (1 H, d, H-2, $J_{2,3}$ 5.2), 3.66 (3 H, s, CO_2-Me), 3.74 (1 H, d, NCH_2Ph, J_{gem} 12.6), 3.93 (1 H, d, NCH_2Ph, J_{gem} 12.6), 4.10 (1 H, a-t, H-3, $J_{3,2} = J_{3,4}$ 5.2), 4.47 (1 H, d, OCH_2Ph, J_{gem} 11.6), 4.61 (1 H, d, OCH_2Ph, J_{gem} 11.6), 7.26−7.37 (10 H, m, Ar-H). δ_C (100 MHz, $CDCl_3$): 52.1 (CO_2-Me), 52.4 (C-5), 61.3 (NCH_2Ph), 68.5 (C-4), 69.7 (C-2), 71.9 (OCH_2Ph), 74.3 (C-3), 127.8, 128.0, 128.1, 128.5, 128.6, 129.8 (ArCH), 137.4 (ArCC), 171.5 (C-1). *m*/*z* (ESI +ve): 367 (M + H^+, 100). HRMS *m*/*z* (ESI +ve): found 389.1590 [M + Na^+], $C_{20}H_{22}N_4NaO_3$ requires 389.1584.

5-Acetamido-1-O-acetyl-2-N,3-O-dibenzyl-2,4,5-trideoxy-2,4-imino-D-ribitol ***58***. Lithium aluminum hydride (1 M in THF, 2.84 mL, 2.84 mmol) was added dropwise to a solution of the azide **57** (237 mg, 0.71 mmol) in THF (5 mL) at −78 °C under argon. The reaction mixture was stirred for 3.5 h, after which mass spectrometry indicated the absence of a peak corresponding to the starting material (*m*/*z* 389 [M + Na^+]) and the presence of a peak corresponding to the intermediate amine (*m*/*z* 335 [M + Na^+]). The reaction as quenched with saturated aqueous ammonium chloride and the reaction mixture concentrated in vacuo. A 1:1 acetic anhydride−water mixture (6 mL) was added to the crude residue. The reaction mixture was stirred at rt for 15 h, after which the mixture was concentrated in vacuo and the crude residue was purified by flash chromatography (1:1:0 to 0:100:1, cyclohexane−ethyl acetate−methanol) to afford the diacetate **58** (207 mg, 79%) as a yellow oil: $[\alpha]^{20}_D$ −20.5 (*c* 0.39 in MeOH). ν_{max} (thin film): 1737 (s, CO), 1651 (s, CO). δ_H (400 MHz, $CDCl_3$): 2.04 (6 H, a-s, NHCOCH_3, OCOCH_3), 2.84 (1 H, br s, H-5), 3.09−3.33 (3 H, m, H-2, H-4, H-5′), 3.68−3.74 (2 H, m, H-3, NCH_2Ph), 3.86−3.91 (1 H, m, NCH_2Ph), 3.93 (1 H, dd, H-1, $J_{1,2}$ 5.6, $J_{1,1'}$ 10.0), 3.98 (1 H, dd, H-1′, $J_{1',2}$ 5.6, $J_{1',1}$ 10.0), 4.44 (1 H, d, OCH_2Ph, J_{gem} 12.0), 4.49 (1 H, d, OCH_2Ph, J_{gem} 12.0), 7.28−7.36 (10 H, m, Ar-H). δ_C (100 MHz, $CDCl_3$): 21.1 (NHCOCH_3, OCOCH_3), 40.7 (C-5), 61.4 (NCH_2Ph), 64.2 (C-1), 68.5, 69.5 (C-2, C-4), 72.0 (OCH_2Ph), 73.0 (C-3), 128.0, 128.2, 128.2, 128.6, 128.8, 129.6 (ArCH), 137.4 (ArCC), 170.9, 171.0 (OCOCH_3, NHCOCH_3). *m*/*z* (ESI +ve): 397 (M + H^+, 100). HRMS *m*/*z* (ESI +ve): found 397.2125 [M + H^+], $C_{23}H_{29}N_2O_4$ requires 397.2122.

5-Acetamido-2-N,3-O-dibenzyl-2,4,5-trideoxy-2,4-imino-D-ribitol ***59***. Sodium methoxide (2.7 mg, 0.05 mmol) was added to a solution of the diacetate **58** (191 mg, 0.48 mmol) in methanol (5 mL) at rt under argon. The reaction mixture was heated to 60 °C for 25 h, after which mass spectrometry indicated the consumption of a peak corresponding to the starting material (*m*/*z* 397 [M + H^+]) and the presence of a peak corresponding to the monoacetylated product (*m*/*z* 335 [M + H^+]). The reaction mixture was concentrated in vacuo, and the residue redissolved in a 1:2 1,4-dioxane−water mixture (5 mL) and loaded onto a short column of Dowex (50W-X8, H^+). The column was washed with water and the product liberated with 2 M aqueous ammonia. The ammoniacal fractions were combined and concentrated in vacuo to afford the acetamido **59** (62 mg, 38%) as a light yellow oil: $[\alpha]^{20}_D$ −12.1 (*c* 0.77 in MeOH). ν_{max} 3301 (br s, OH, NH), 1650 (s, CO). δ_H (400 MHz, CD_3OD): 1.90 (3 H, s, NHCOCH_3), 2.95 (1 H, dd, H-5, $J_{5,4}$ 5.2, $J_{5,5'}$ 13.8), 3.06 (1 H, dt, H-2, $J_{2,1} = J_{2,1'}$ 4.3, $J_{2,3}$ 5.4), 3.14 (1 H, a-q, H-4, $J_{4,3} = J_{4,5} = J_{4,5'}$ 5.0), 3.21 (1 H, dd, H-5′, $J_{5'4}$ 4.3, $J_{5,5'}$ 13.8), 3.26 (1 H, dd, H-1, $J_{1,2}$ 4.5, $J_{1,1'}$ 11.8), 3.30 (1 H, dd, H-1′, $J_{1',2}$ 4.5, $J_{1',1}$ 11.8), 3.71−3.77 (3 H, m, H-3, NCH_2Ph), 4.48 (2 H, a-s, OCH_2Ph), 7.24−7.37 (10 H, m, Ar-H). δ_C (100 MHz, CD_3OD): 22.6 (NHCOCH_3), 42.4 (C-5), 62.7 (NCH_2Ph), 63.2 (C-1), 70.2 (C-4), 72.5 (OCH_2Ph), 72.8 (C-2), 75.4 (C-3), 128.5, 128.8, 128.9, 129.4, 130.6 (ArCH), 139.2, 139.5 (ArCC), 173.3 (NHCOCH_3). *m*/*z* (ESI +ve): 355 (M + H^+, 100). HRMS *m*/*z* (ESI +ve): found 355.2017 [M + H^+], $C_{21}H_{27}N_2O_3$ requires 355.2016.

5-Acetamido-2,4,5-trideoxy-2,4-imino-D-ribitol Hydrochloride ***14***. Palladium (10% on carbon, 5 mg) and 1 M aqueous hydrochloric acid (0.05 mL) were added to a solution of dibenzyl **59** (62.0 mg, 0.18 mmol) in 1:1 1,4-dioxane−water (2 mL). The reaction vessel was evacuated and flushed with nitrogen, argon, and hydrogen gas sequentially. The reaction mixture was stirred vigorously for 5 days at rt until mass spectrometry indicated the absence of the starting material (*m*/*z* 355 [M + H^+]). Completion of the reaction was confirmed by mass spectrometry. The reaction mixture was filtered through glass microfiber (GF/A) and concentrated in vacuo to afford the acetamide **14** (40.3 mg, 100%) as the hydrochloride salt and as a yellow gum: $[\alpha]^{20}_D$ +11.3 (*c* 0.42 in MeOH). ν_{max} 3336 (br s, OH, NH), 1634 (s, CO). δ_H (400 MHz, D_2O): 1.91 (3 H, s, NHCOCH_3), 3.49 (1 H, dd, H-5, $J_{5,4}$ 6.4, $J_{5,5'}$ 15.0), 3.56 (1 H, dd, H-5′, $J_{5'4}$ 4.4, $J_{5',5}$ 15.0), 3.74 (1 H, dd, H-1, $J_{1,2}$ 3.2, $J_{1,1'}$ 13.2), 3.77 (1 H, dd, H-1′, $J_{1',2}$ 3.2, $J_{1,1'}$ 13.2), 4.17−4.23 (2 H, m, H-2, H-4), 4.40 (1 H, t, H-3, $J_{3,2} = J_{3,4}$ 6.8). δ_C (100 MHz, D_2O): 22.1 (NHCOCH_3), 38.9 (C-5), 57.9 (C-1), 65.2, 65.4 (C-2, C-4), 67.1 (C-3), 176.1 (NHCOCH_3). *m*/*z* (ESI +ve): 175 (M + H^+, 100). HRMS *m*/*z* (ESI +ve): found 175.1075 [M + H^+], $C_7H_{15}N_2O_3$ requires 175.1077.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Copies of ^1H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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