Iminosugar C-Glycoside Analogues of α-D-GlcNac-1-Phosphate: Synthesis and Bacterial Transglycosylase Inhibition

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ABSTRACT: We herein describe the first synthesis of iminosugar C-glycosides of α-D-GlcNac-1-phosphate in 10 steps starting from unprotected α-D-GlcNAc. A diastereoselective intramolecular iodoamination–cyclization as the key step was employed to construct the central piperidine ring of the iminosugar and the C-glycosidic structure of α-D-GlcNac. Finally, the iminosugar phosphonate and its elongated phosphate analogue were accessed. These phosphorus-containing iminosugars were coupled efficiently with lipophilic monophosphates to give lipid-linked pyrophosphate derivatives, which are lipid II mimetics endowed with potent inhibitory properties toward bacterial transglycosylases (TGase).

INTRODUCTION

Iminosugars are central structural motifs of small molecules that act by inhibition of glycosidases or glycosyltransferases. Their strong inhibitory activity is attributed to electrostatic binding interactions of the protonated nitrogen under physiological conditions and to their “oxocarbenium-ion-like” structure that mimics the transition state of the formation/cleavage of the glycosidic linkage. Iminosugars have shown potential as therapeutics toward cancer as well as viral and bacterial infections and are used for the treatment of type 2 diabetes.

Because of the rise of infections from antibiotic-resistant bacteria such as methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Enterococci (VRE), it is of great interest to develop new drugs targeting the conserved region of the bacterial cell wall. Peptidoglycan transglycosylase (TGase) is the enzyme responsible for catalyzing the polymerization of β1,4-linked N-acetylglucosamine-N-acetylmuramic acid (GlcNAc-MurNAc), disaccharide of lipid II to form the bacterial cell wall. TGases are attractive targets for antibacterial agents because they are essential enzymes located on the external cell surface and because no eukaryotic counterpart is known. The only well-characterized TGase inhibitor, moenomycin (Moe), is, however, not effective in humans because of its poor pharmacokinetic properties. From recent structural studies, the transglycosylation mechanism employed by TGase is that of a SN2-like reaction, which is similar to other inverting glycosyltransferases (Figure 1). Therefore, we expect iminosugar-based lipid II mimetics to be potent substrate-based inhibitors that mimic the transition state of TGase transglycosylation (Scheme 1). However, the idea has been tested only with pyrrolidine mimics that were discovered by a combinatorial approach. Six-membered iminosugars would better represent the natural N-acetylglucosamine hydroxyl configurations, but they have yet to be studied.

Herein, we report structural iminosugar C-glycosidic lipid II analogues such as compound 1 (Scheme 1), which contains a simplified structure (e.g., shorter lipid chain and no peptide moiety) when compared to that of lipid II but still possesses a core structure with the potential to inhibit TGase. Also, a molecule with a C-glycosidic linkage is expected to be stable toward enzymatic hydrolysis. The synthesis of compound 1 is challenging because the iminosugar α-C-glycoside of phosphate analogue 2 has to be realized. Only a few examples have been found for the synthesis of iminosugar analogues of GlcNAc. Herein, we present the synthesis of phosphonate 2 and its phosphate analogue. These compounds can be used to efficiently construct lipid-linked pyrophosphate analogues such as 1 that have the potential to display potent bacterial TGase inhibition.

RESULTS AND DISCUSSION

To construct the iminosugar C-glycoside core of GlcNAc, we utilized a ring-opening/cyclization strategy. Key steps are a
Wittig reaction followed by oxidation and reductive amination leading to a diaminoheptenitol, followed by an NIS-mediated cyclization. Similar synthetic strategies were known before, but have never been applied successfully to GlcNAc. The synthesis of diaminoheptenitols is depicted in Scheme 2.

Starting from GlcNAc, compound 3 was prepared and subjected to optimized Wittig conditions. The obtained aminohexenitol had to be transformed into the corresponding amine to construct the piperidine ring. Epimerization at C-6 of 4 followed by reversion with a nitrogen nucleophile was attempted but was found not to be applicable to GlcNAc derivatives because the nitrogen substituent in the 3-position leads to the formation of a pyrrolidine when the hydroxyl group of 4 is transformed into a good leaving group. Converting the hydroxyl group to an amine was best completed by an oxidation/reductive amination sequence. The ketone obtained by Swern oxidation was found to be in equilibrium with N-acyl hemiaminal 5 (typical 13C NMR appearance of the C-2 quaternary carbon at δ = 91 ppm), which was stable and could be subjected to reductive amination directly without purification. Optimization of the reaction conditions led to the use of excess NaBH₃CN/NH₄OAc (10 equiv) at 50 °C in methanol. Under the optimal reaction conditions, diaminoheptenitols 6a and 6b were synthesized in 64% over two steps, with the (6R) diastereomer being the main product in a ratio 6a/6b (6R/6S) = 67:33. The ratio was determined by NMR analysis of the NH protons of 6a and 6b acetamides in the crude product. It is noteworthy that the use of other amine sources such as benzyl amine gave higher yield and selectivity of the (6R) diastereomer, but the subsequent cyclization reaction was not successful.

In the next step toward the synthesis of 7a, the cyclization of 6a by internal iodoamination proceeds with a very high degree of stereoselectivity to form exclusively the α-epimer (Scheme 3). The configuration of the stable compound 7a was determined by NMR analysis. Early attempts to determine the two newly formed stereocenters at C-2 and C-6 on the basis of NMR parameters of 7a failed. However, small coupling constants and the absence of 1,3-diaxial NOE contacts indicated that the conformation of 7a was not a chair, but it was difficult to obtain unambiguous proof of its exact...
The structure of 7a was finally determined by analyzing bicyclic oxazine 9, a side product in the synthesis of 8 (a C2-acetamido analogue of homonojirimycin, HNJ), that was obtained by treating iodo derivative 7a with silver acetate in THF/H2O (Scheme 3). The bicyclic system locks the sugar ring in the chair conformation (4C1),18 in which the C-2 substituent is in an axial position. NOEs indicate spatial proximity of H3 and H5 on one side of the ring and of H4 and H6 on the other side, respectively (Figure 2). In addition, larger coupling constants throughout the molecule are observed, which indicates larger vicinal diaxial coupling constants associated with the β-glucos conformation.19

The synthesis of the first GlcNAc-derived iminosugar phosphonate was achieved for the β-glucos series of compounds, but initial attempts were not successful. The intramolecular formation of aziridine intermediate 11 dominated in reactions employing bases such as Cs2CO3 (Scheme 4). The problem was solved by using a bulkier base such as NaHDMS. Substitution of the iodine of 7a by deprotonated dibenzylphosphite using NaHDMS led to the isolation of protected phosphonate 10 in moderate yield (Scheme 4). Debenzylation of 10 was achieved to afford 2 in high yield with Pd/C under a H2 atmosphere in a solvent mixture of THF/H2O/AcOH. In addition to the phosphonate group, the versatile iodo intermediate 7a enabled the introduction of structural diversity, such as the introduction of the azido group (Scheme 5).

We are also interested in the synthesis of an analogue with an extended phosphate group that has been found in several glycosyltransferase inhibitors.20 The endocyclic secondary amine function of 8 was initially required to be protected because the amine group would engage in the phosphorylation reaction, causing low yields. As a result, carboxybenzyl (Cbz)-protected iminoheptitol 13 was prepared and transformed into phosphate 14 in good yield by using the phosphoramidite method (Scheme 6). The nitrogen was Cbz-protected because alkyl or aryl protecting groups gave significant amounts of N-oxide after treatment with mCPBA. Deprotection of 14 by Pd/H2 in THF/H2O/AcOH afforded the desired glycosyl phosphate mimic 15 in 90%.

Because β-iminosugars are being increasingly appreciated for pharmaceutical purposes,21 the procedure also offers access to β-ido imino-C-glycosides of GlcNAc-1-phosphate 17 (Scheme 7), which allows biological studies to be extended to the unnatural (β-ido) derivatives. The structure of 17 was determined as the β-ido-isomer in a 1C4-configuration by NMR analysis (see Figure S2).

To synthesize lipid II analogues, phosphonate 2 and phosphate 15 were transformed into the corresponding pyrophosphates (Scheme 8). C15- (18, farnesyl) and C20-phosphates (19, geranylgeranyl) were attached using the carbonyldiimidazole (CDI) method.22 Accordingly, we treated the ammonium salt of lipid monophosphates 18 and 19 with CDI to generate a highly reactive monophosphate imidazolide in situ, which was subsequently reacted with iminosugars 2 and 15. Surprisingly, pyrophosphate products 20, 21, and 1 were obtained in good yields (56−70%) with only a small amount of self-coupled product of lipid monophosphate.

Clostridium difficile is a Gram-positive spore-forming bacterium that can cause severe infectious diarrhea. The frequency C. difficile infection has significantly increased in North America and worldwide in recent years and is becoming

Figure 2. Coupling constants and NOEs of bicyclic iminosugar 9. Abbreviations of the NOE intensities are as follows: s, strong; m, medium; and w, weak.

Scheme 4. Synthesis of Phosphonate Analogue 2

Scheme 5. Synthesis of Azido Derivative 12

Scheme 6. Synthesis of Phosphate Analogue 15

Scheme 7. Synthesis of β-ido Analogue 17
one of the major causes of morbidity in healthcare facilities.23 Because targeting C. difficile cell wall synthesis has proved to be successful,24 we are interested in developing new C. difficile TGase inhibitors by our newly synthesized iminosugars. The inhibitory activity of compounds 2, 15, and 17 against C. difficile TGase was then evaluated by the HPLC-based TGase assay.25 Unfortunately, none of these derivatives (lacking a lipophilic moiety) displayed a measurable inhibition at 500 and 100 μM. It is noted that the TGase inhibition results of the three lipid-linked compounds, 20, 21, and 1, showed increased inhibition against TGase (Table 1). It is clear that the presence of a lipid chain provides increased potency, possibly due to increased lipophilicity and increased binding to TGases.26 By comparison of 20 and 21, the elongation of the bond length between the lipid and the sugar was found to decrease the inhibition activity from 100 to 50% (500 μM) and 30 to 0% (100 μM), suggesting that the phosphonate moiety is a better precursor for this design. The C20 derivative 1 exhibited the best inhibitory potency against C. difficile TGase, with a Kᵢ value in the low micromolar range (Table 1), although the inhibition is still weak compared with that of moenomycin. The Kᵢ value is determined by using our recent reported FRET-based TGase assay.27

**CONCLUSIONS**

A variety of imino-C-glycosides of α-D-GlcNAc-1-phosphate, 2, and 15, their L-ido analogue 17, and lipid-linked analogues 20, 21, and 1 were synthesized and evaluated for C. difficile TGase inhibition. The synthetic procedure provides easy access to dianimoheptenitol 6a and 6b, versatile iodo intermediates 7a and 7b, and aminooheptenitol 4 which has been reported via a less direct route15 (eight steps starting from D-arabinofuranose). Our result demonstrated that the connection of iminosugars phosphonate 2 with a lipid chain via a pyrophosphate group results in a new design for generating active TGase inhibitors. Because the lipid part greatly contributes to TGase inhibition, optimization may offer even better TGase inhibitors. In addition, such a glycolipid could be further elaborated by enzymes such as N-acetylgalactosamine transferase to increase the glycan length to better fit the donor site of TGase, which can accommodate a large substrate.8 Further investigations based on these results are in progress. The efficient pyrophosphate coupling could also be used for the synthesis of analogues of UDP-GlcNAc, which could result in potential inhibitors of GlcNAc transferases such as OGT.28

**EXPERIMENTAL SECTION**

**General.** All chemicals were obtained from commercial sources and used without further purification. Anhydrous solvents were purchased and used without additional purification. TLC was performed on glass plates precoated with Silica Gel 60 F254 (0.25 mm, E. Merck); detection was executed by spraying with a solution of Ce-(NH₄)₂(NO₃)₆ and (NH₄)₆Mo₇O₂₄ and subsequent heating on a hot plate. 1H and 13C NMR spectra were recorded on 500 and 600 MHz spectrometers. In 1H NMR spectra, chemical shifts are referenced to internal tetramethylsilane (δ 0.00 ppm in CDCl₃), CD₃H (δ 7.16 ppm in CD₃), or HOD (δ 4.79 ppm in D₂O). Chemical shifts are in ppm and are calibrated using the resonances of the carbon and the residual proton of the deuterated solvent. Proton peaks were assigned with the aid of 2D NMR techniques (1H COSY, HSQC, DEPT135, DEPT90, and HMBC). Mass spectra (MS) were recorded on an ESI-TOF mass spectrometer.

**Measurement of the Percent Inhibition of TGase Inhibitors.** HPLC analysis was performed on an anion-exchange column (4.6 mm × 25 mm, 5 μm, LC-SAZ1, Supelco Co.). A linear gradient of NH₄OAc (from 20 mM to 1 M in MeOH) was eluted at a flow rate of 1.0 mL/min over 30 min. Inhibition assays were composed of buffer (1 μL, 0.85% decyl-PEG, 500 mM HEBES, 100 mM CaCl₂, pH 8.0), DMSO/MeOH (1:1, 2.5 μL), NBD-lipid II (200 μM)/internal standard (4 μM) (2:3, 1 μL), inhibitors (1 μL, different concentrations ranging from 10 to 100 mM), TGase (CD) (1 μL, 25 μg/mL), and H₂O (4.5 μL). After incubation at 37 °C for 2 h, reactions were stopped by addition of moenomycin A (1 μL, 100 μM). The reaction mixture was analyzed by HPLC analysis. The percent inhibition was calculated by the relative peak area of the remaining NDB-lipid II and NBD internal standard via integration.

**Determination of Kᵢ Values of Transglycosylase Inhibitors.** The Kᵢ values of inhibitors were determined using our recently reported FRET-based assays.27 The reaction was performed and monitored as described above with various concentrations of FRET-based lipid II analogue (0–15 μM) in the presence of 0–40 μM I. Initial velocity for each progression curve was calculated and used for Kᵢ determination. The Kᵢ value for I was determined with the competitive inhibition

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**Table 1. Inhibition of Clostridium difficile TGase by Synthesized Iminosugars**

| compd | inhibition (%)a (500 μM) | inhibition (%) (100 μM) | Kᵢ (μM) |
|-------|--------------------------|-------------------------|--------|
| 2     | —                        | —                       | —      |
| 15    | —                        | —                       | —      |
| 17    | —                        | —                       | —      |
| 20    | 50                       | 0                       | —      |
| 21    | 100                      | 30                      | —      |
| Moe   | 100                      | 100                     | 6.3 ± 1.4b |

aInhibition percent calculated by comparing the reaction rate in the presence of the compound to the rate in the absence of any inhibitory compound. bRefers to no inhibition. cShown are the mean values of the data generated from three independent experiments. dThe data was adopted from ref 27.
model using GraphPad Prism 5 (GraphPad Software Inc.). The data shown are the mean value from three independent experiments.

**N-(3R,4R,5R)-4,5,7-Tris(benzoyl)-6-hydroxyhept-1-en-3-yl-acetamide (4)***. Under an atmosphere of argon in a three-necked flask equipped with a dropping funnel, dry THF (100 mL) was added to methyltriphenylphosphonium bromide (dried by heating at 150 °C at 0.01 mbar), forming a suspension that was cooled to 0 °C. BuLi (2.2 M in hexanes, 103 mmol, 46 mL) was added dropwise over 15 min using the dropping funnel, forming a red–orange solution. This solution was stirred for 10 min at 0 °C and 40 min at rt, forming a dark red solution. Then a suspension of 3 (203 mmol, 10.0 g) in dry THF (150 mL) was added over 10 min via dropping funnel at 0 °C. The mixture was stirred for 1 h at 0 °C, 12 h at rt, and 8 h under reflux when TLC monitoring [CH2Cl2/MeOH 20:1, Rf(3) = 0.27, Rf(4) = 0.39, cerium molybdate] showed full consumption of the starting material. Then, saturated aqueous solutions of ammonium chloride (250 mL) and diethyl ether (250 mL) were added followed by separation of the phases and extraction of the aqueous phase with diethyl ether (3×50 mL), separating large amounts of unreacted phosphonium salts. The combined organic extracts were dried over Na2SO4 and the solvent was removed in vacuo. Separation from side products SP1 and SP2 (separation from SP2: 50 cm column, 1.500 g silica, toluene/MeOH 20:1, UV, toluene/MeOH 9:1) by column chromatography on silica gel (CDCl3, 500 MHz) [α]D20 = 7.6, 6.6, 6.5 Hz, 1H], 3.54 (dd, 8.4, 6.5 Hz, 1H), 3.71 (d, 8.4 Hz, 1H), 4.06 (d, 8.4 Hz, 1H), 4.45–4.6 (3H, 3H), 4.74 (d, 10.6 Hz, 1H, CH2Ph), 4.78 (d, 10.6 Hz, 1H, CH2Ph), 4.86–4.91 (2H, 9.2, 7.6, 7.6 Hz, 1H), 5.07 (d, 8.3 Hz, 1H, NH), 7.30–7.43 (4.0 H, 15 H, Ar-H).13C NMR (CDCl3, 125 MHz) δ 23.6 (CH2), 51.2, 51.8 (2 CNHR2), 73.1, 73.5, 74.9, 75.4 (4 t, OCH2Ph, C7), 80.4, 83.1, 115.6 (C-1), 127.7, 127.8, 128.0, 128.3, 128.4, 128.5, 128.6 (9 d, Ar), 135.8 (C-2), 138.2, 138.3, 138.7 (3 s, Ar), 169.8 (C=O). HRMS (ESI-TOF) calcd for C20H22N2O4 (M+H)+, 458.2753; found, 458.2785.

**N-(25,35,45,5R,6R)-4,5-Bis(benzoxyl)-6-(benzoxylmethyl)-2-isoxazolidinylipiperidinyl-3-ylacetamide (6a)**. Under an atmosphere of argon in the dark, N-iodoucinnimide (0.65 mmol, 147 mg) was added in one portion at rt to a solution of 6a (0.65 mmol, 320 mg) in dry CH2Cl2 (20 mL). The resulting solution was stirred at rt for 2 h (TLC monitoring, CH2Cl2/MeOH 20:1, Rf(6a) = 0.25, Rf(7a) = 0.60, cerium molybdate). Then, a saturated aqueous solution of sodium thiosulfate (10 mL) was added followed by extraction with CH2Cl2 (3×20 mL). The combined organic extracts were dried over MgSO4, and the solvent was removed in vacuo. Purification by silica gel chromatography (CH2Cl2/MeOH 100:1) gave 7a (168 mg, 50%) as a white solid. [α]D20 = +20.5 (c 0.4, CH3Cl2).1H NMR (CDCl3, 600 MHz) δ 1.82 (3H, CH3), 2.99 (s, 1H, 10.2 Hz, 1H, 1-Ha), 3.21 (d, 10.2, 3,3 Hz, 1H, 1-Hb), 3.35–3.40 (m, 2H, 2-H and 7-Ha), 3.43 (m, 1H, 5-H), 3.47 (m, 1H, 5-H), 3.56 (m, 1H, 4-H), 3.82 (d, 9.4, 4.9 Hz, 1H, 7-Ha), 4.33 (m, 1H, 3-H), 4.41–4.45 (2H, 2H, CH2Ph), 4.60–4.49 (4H, 4H, CH2Ph), 6.61 (d, 9.1 Hz, 1H, 1H), 7.19–7.33 (20 H, m, 15 H, Ar-H).13C NMR (CDCl3, 150 MHz) δ 23.3 (CH2), 23.6 (CH3), 48.9 (C-3), 49.8 (C-2), 56.0 (C-6), 68.0 (C-7), 72.0, 72.1, 73.4 (OCH2Ph), 74.5 (C-5), 75.4 (C-4), 130.0, 135.7, 137.5, 138.3, 138.5, 138.6 (9 d, Ar), 137.3, 137.5, 138.0 (17O, Ar). HRMS (ESI-TOF) calcd for C20H22N2O4 (M+H)+, 490.2593; found, 490.2588.

**N-(25,35,45,5R,6R,6S)-6-Amino-4,5,7-tris(benzoyl)-hept-1-en-3-yl-acetamide (6a) and N-(25,35,45,5R,6R)-6-Amino-4,5,7-tris(benzoyl)-hept-1-en-3-yl-acetamide (6b)***. Oxalyl chloride (20 mmol) was added to a solution of 3 (120 mmol, 1.02 mL) at −78 °C to a solution of DMSO (16 mmol, 1.13 mL) in dry CH2Cl2 (20 mL) under an atmosphere of argon in a Schlenk flask. After 30 min of stirring, a solution of 4 (4.0 mmol, 1.96 g) in dry CH2Cl2 (8 mL) was added carefully via syringe over 10 min (note that precooling the cooled wall of the flask is necessary!). After 1 h of stirring at −78 °C, NEt3 (32 mmol, 4.44 mL) was added by 40 min of stirring at −78 °C. The solution was warmed to 0 °C within 10 min with an ice bath, and CH2Cl2 (20 mL) and a saturated aqueous solution of NaHCO3 (50 mL) were added followed by extraction of the aqueous phase with CH2Cl2 (3×40 mL). The combined organic extracts were dried over Na2SO4 and the solvent was removed in vacuo. A crude, instable intermediate was obtained as a yellow oil [CH2Cl2/MeOH 20:1, Rf(4) = 0.39, Rf(ketone) = 0.49, cerium molybdate]. Without purification, dry methanol (40 mL) was added to the reaction crude under an atmosphere of argon, and the resulting solution was treated with ammonium acetate (100%, 40 mmol, 3.08 g) and NaBH4CN (20 mmol, 1.30 g). After the resulting solution was stirred for 18 h at 50 °C, TLC monitoring [2×CH2Cl2/MeOH 20:1, Rf(ketone) = 0.66, Rf(alkalol) = 0.58, Rf(benzyl) = 0.41, Rf(6a) = 0.34, cerium molybdate], saturated NaHCO3 (40 mL) was added to the reaction mixture followed by the removal of MeOH. The residual aqueous solution was extracted with CH2Cl2 (120 mL) twice. The collected organic phase was dried over MgSO4 and concentrated. NMR of the crude product showed a ratio of 6a/6b = 67:33. Purification by column chromatography on silica gel (196 g silica, CH2Cl2/MeOH 25:1) led to the isolation of 6a (836 mg, 42%) and 6b (411 mg, 22%) as colorless oils.
7-Hb), 3.25 (dd, J = 10.7 Hz, 1H, 3-H), 3.79 (d, J = 9.0, 3.0 Hz, 1H, H-1), 2.48 (s, J = 8.8 Hz, 3H), 2.34 (m, J = 9.3 Hz, 1H, H-1), 3.50 (dd, J = 12.2, 3.8 Hz, 1H), 3.48–3.42 (m, 2H), 3.34–3.31 (m, 1H), 3.17 (dd, J = 12.1, 9.5 Hz, 1H), 1.86 (s, 3H). 13C NMR (151 MHz, CDCl3) δ 169.9, 138.1, 137.5, 137.3, 128.5, 128.5, 128.4, 128.0, 127.8, 127.7, 127.5, 75.0, 74.4, 73.3, 72.0, 67.9, 55.4, 53.6, 47.2, 23.3 (HRESI-TOF) calc for C27H37N2O2 (M + H)+, 390.2762; found, 350.2776.

**Benzyl(2S,3S,4R,5R,6R)-3-acetamido-4,5-bis(benzoxyl)-6-((benzoxyl)imethyl)-2-(hydroxymethyl)pyrrolidine-1-carboxylic acid (14).** A solution of compound 8 (220 mg, 0.14 mmol) in dioxane/H2O (3:2, 3.2 mL) was treated with NaHCO3 (361 mg, 4.40 mmol) and CbzCl (503 μL, 3.52 mmol) at 0°C. After stirring at rt for 4 h, the reaction mixture was diluted with CHCl3 (30 mL) and washed with water (15 mL) and brine (15 mL) sequentially. The organic layer was dried with MgSO4 and concentrated, and the residue was purified by flash silica gel column chromatography (EtOAc/hexane 55:45 to 70:30) to afford 13 (16 mg, 92%) as a viscous oil. 1H NMR (600 MHz, CDCl3) δ 7.38–7.07 (m, 20H, Ar-H), 6.61 (d, J = 9.1 Hz, 1H, NH), 5.26 (m, 1H, -H), 5.21 (d, J = 11.9 Hz, 1H, -H), 5.05 (d, J = 9.8 Hz, 1H, -H), 4.32 (m, J = 9.2, 2.3 Hz, 1H, -H), 3.3, 2.5, 2.2, 2.3, 1.8, 1.7, 1.6, 1.5, 1.4, 1.3, 1.2, 1.1 Hz. 13C NMR (151 MHz, CDCl3) δ 169.9 (C-1), 156.4 (N-H), 138.6, 137.8, 137.7, 137.0, 128.4, 128.4, 128.3, 128.1, 128.0, 127.9, 127.7, 127.6 (Ar), 76.4 (C-4), 74.6 (C-5), 73.0, 71.6, 71.3, 67.9 (OCH2Ph), 67.0 (C-7), 61.4 (C-1), 55.7 (C-6), 55.0 (C-2), 46.8 (C-3), 22.3 (CH3). HRESI-TOF calc for C39H40N4O4 (M + H)+, 639.3085; found, 639.3086.

**Benzyl(2S,3S,4R,5R,6R)-3-acetamido-4,5-bis(benzoyl)-6-((benzoxyl)imethyl)-2-(hydroxymethyl)pyrrolidine-1-carboxylic acid (12).** To a stirred solution of 3 (16 mg, 0.03 mmol) and tetrazole (97 mg, 0.14 mmol) in MeCN (2 mL) at 0°C slowly was added bis(benzylidene)disopropylphosphine 40 μL, 0.125 mmol) followed by removal of the ice bath. When the TLC analysis showed consumption of the starting material, the reaction mixture was cooled to 4°C followed by the addition of acetonitrile (75%), H2O (10%) and NaCl (9.3 mg, 0.032 mmol). The ice bath was removed, and the reaction mixture was heated to rt. After 1 h, the reaction was quenched by saturated NaHCO3 (1.5 mL). The organic phase was collected. The aqueous phase was extracted with CHCl3 (10 mL) three times. The combined organic phase was dried over MgSO4, and concentrated. The residue was purified by silica gel chromatography (EtOAc/hexane 55:45 to yield compound 14 (21.3 mg, 79% over two steps) as a colorless liquid. 1H NMR (600 MHz, CDCl3, 45 °C) δ 7.37–7.06 (m, 2SH), 6.91 (bs, 1H, NHAc), 5.08–5.04 (m, 10H), 4.55 (bs, 2H), 4.43 (m, 4H), 4.22 (d, J = 11.5 Hz, 1H), 4.02–3.85 (m, 4H), 1.79 (bs, 4H, CH2), 0.89 (bs, 3H, CH3). 13C NMR (151 MHz, CDCl3) δ 170.0 (NHCOCH3), 156.4 (NHCOOBn), 138.0, 137.5, 137.3, 136.2, 135.8, 135.7, 135.7, 135.7, 128.5, 128.4, 128.3, 128.1, 127.9, 127.9, 127.8, 127.6, 127.5, 74.3, 73.0, 71.8, 69.3, 67.9, 67.5, 52.3, 47.7, 23.0 (NHCOCH3). 13C NMR (CDCl3, 202 MHz) δ 0.32. HRESI-TOF calc for C41H39N3O4P (M + H)+, 899.3667; found, 899.3692.

**Benzyl(2S,3S,4R,5R,6R)-3-acetamido-4,5-bis(benzoyl)-6-((benzoxyl)imethyl)-2-(hydroxymethyl)pyrrolidine-2-yl)methyl Dihydrogen Phosphate (15).** To a round-bottomed flask containing starting material 14 (20 mg) were added THF (2.5 mL), H2O (2.5 mL), and AcOH (50 μL). The solution was treated with Pd/C (10%; Degussa type, 26.0 mg) under a hydrogen atmosphere and stirred at rt for 18 h. The mixture was filtered through a pad of Celite, and the solvent was removed in vacuo. Purification by biogel P2 chromatography (eluted by water) led to the isolation of 2 (7.2 mg, 90%) as a white solid after lyophilization.

1H NMR (600 MHz, CDCl3, 45 °C) δ 7.40–7.32 (m, 14H, Ar-H), 7.17–7.11 (m, 4H, Ar-H), 7.07–7.02 (m, 2H, Ar-H), 6.82 (d, J = 9.0 Hz, 1H, 5-H), 6.78 (dd, J = 10.4, 9.5 Hz, 1H), 4.55 (q, J = 7.2 Hz, 1H, C-4), 4.48 (d, J = 11.3 Hz, 2H), 4.22 (m, 1H, 4-H), 3.88 (t, J = 8.6 Hz, 3H), 3.52 (d, J = 3.0 Hz, 1H), 3.49 (dd, J = 8.8, 3.3 Hz, 1H), 3.1 (dd, J = 12.2, 3.8 Hz, 1H), 3.48–3.42 (m, 2H), 3.34–3.31 (m, 1H), 3.17 (dd, J = 12.1, 9.5 Hz, 1H), 1.86 (s, 3H). 13C NMR (151 MHz, CDCl3) δ 169.9, 138.1, 137.5, 137.3, 128.5, 128.4, 128.0, 127.8, 127.7, 127.5, 75.0, 74.4, 73.3, 72.0, 67.9, 55.4, 53.6, 47.2, 23.3 (HRESI-TOF) calc for C41H39N3O4P (M + H)+, 899.3667; found, 899.3692.

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5.02 (m, 4 H), 6.71 (d, 2-H), 3.37 (ddd, 4-H), 4.49 (d, 5-H and 6-H), 3.42 (m, 2H, 3-H). 13C NMR (CDCl3, 100 MHz) δ 112.0, 143.0, 136.6, 133.4 (lipid CH3CH2O, 1H), 119.3, 119.4, 143.0 (lipid CH3CH2C=CH), 124.4, 124.2 (2 × CH=CH), 119.4, 119.3 (lipid-C=CH2CH2O, 2H), 72.6 (C-3), 72.5 (C-2), 64.0, 63.9 (C-1), 63.0, 63.0 (lipid-C=CH2O, 5H), 138.1 (3 × ar), 170.0 (C=O). HRMS (ESI-TOF) calc for C34H36N2O2P (M + H)+: 573.2295, found 573.2295.

((2S,3S,4R,5R,6S)-3-Acetamido-4,5-dihydroxy-6-(hydroxymethyl)piperidin-2-yl)methylphosphonic acid (17). A solution of 16 (0.059 mmol, 37 mg) in MeOH (2 mL) was treated with Pd/C (10%, Degussa type, 5.3 mg) and stirred at rt for 3 d under a hydrogen atmosphere. The mixture was filtered, and the solvent was removed in vacuo. Purification by column chromatography on RP silica gel (20 g silica, CH2Cl2/MeOH 98:2) led to the isolation of 16 (64 mg, 45%) as a colorless oil. H NMR (CDCl3, 500 MHz) δ 1.17 (s, 3H), 1.77–2.05 (m, 15H, Ar-H). 13C NMR (CDCl3, 125 MHz) δ 29.4 (CH2), 32.9 (C-3), 55.0 (C-6), 72.5 (CH2), 124.4, 124.2 (2 × CH=CH), 119.4, 119.3 (lipid-C=CH2CH2O, 2H), 170.0 (C=O). HRMS (ESI-TOF) calc for C34H36N2O2P (M + H)+: 573.2295, found 573.2295.

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5.02 (m, 4 H), 6.71 (d, 2-H), 3.37 (ddd, 4-H), 4.49 (d, 5-H and 6-H), 3.42 (m, 2H, 3-H). 13C NMR (CDCl3, 100 MHz) δ 112.0, 143.0, 136.6, 133.4 (lipid CH3CH2O, 1H), 119.3, 119.4, 143.0 (lipid CH3CH2C=CH), 124.4, 124.2 (2 × CH=CH), 119.4, 119.3 (lipid-C=CH2CH2O, 2H), 72.6 (C-3), 72.5 (C-2), 64.0, 63.9 (C-1), 63.0, 63.0 (lipid-C=CH2O, 5H), 138.1 (3 × ar), 170.0 (C=O). HRMS (ESI-TOF) calc for C34H36N2O2P (M + H)+: 573.2295, found 573.2295.
The vial was washed with MeOH (1 mL) three times. The solvent was removed in vacuo and purified by flash chromatography (CHCl₃/MeOH/NH₄OH 7:3:0.5 to MeOH/H2O 1:1). The collected fractions (4.3 mg, 56%) as a white solid after lyophilization. HRMS (ESI-TOF) calcld for C₂₅H₂₅N₃O₇P₂ (M – H)⁻: 649.3013; found, 649.3040.

**ASSOCIATED CONTENT**

1H NMR, 13C NMR, and 31P NMR data, NOEs of compound 7a and 17, and substrate–velocity curve of 1. 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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**REFERENCES**

1. Iminosugars: From Synthesis to Therapeutic Applications; Compain, P., Martin, O. R., Eds.; John Wiley and Sons: Chichester, West Sussex, England, 2007.
2. Bals, M. Acc. Chem. Res. 1998, 31, 1.
3. (a) Gostler, T. M.; Voovalo, D. J. Nat. Chem. Biol. 2012, 8, 683.
4. (b) Horne, G.; Wilson, F. Prog. Med. Chem. 2011, 50, 135.
5. Taubes, G. Science 2008, 321, 356.
6. (a) Gauert, A.; Vyas, R.; Tewari, R. Crit. Rev. Biotechnol. 2011, 31, 295.
7. (a) Halliday, J.; McKeveney, D.; Muldoon, C.; Rajaratnam, P.; Meutermans, W. Biochem. Pharmacol. 2006, 71, 957. (b) Ostabh, B.; Walker, S. Curr. Opin. Chem. Biol. 2005, 9, 459.
8. (a) Goldman, R. C.; Gange, D. Curr. Med. Chem. 2000, 7, 801.
9. (a) Huang, C. Y.; Shih, H. W.; Lin, L. Y.; Tien, Y. W.; Cheng, T. J. R.; Cheng, W. C.; Wong, C. H.; Ma, C. Proc. Natl. Acad. Sci. U.S.A. 2012, 109, 6496. (b) Lovering, A. L.; de Castro, L. H.; Lim, D.; Styrmadka, N. C. J. Chem. Soc. 2007, 315, 1402. (c) Yuan, Y. Q.; Barrett, D.; Zhang, Y.; Kahne, D.; Siln, P.; Walker, S. Proc. Natl. Acad. Sci. U.S.A. 2007, 104, 5348.
10. (a) Lairson, L. L.; Henriissat, B.; Davies, G. J.; Withers, S. G. Annu. Rev. Biochem. 2008, 77, 521.
11. (a) Whalen, L. J.; Greenberg, W. A.; Mitchell, M. L.; Wong, C.-H. In Iminosugars: From Synthesis to Therapeutic Applications; Compain, P., Martin, O. R., Eds.; John Wiley & Sons: Chichester, West Sussex, England, 2007, p 153.
12. (a) Shih, H. W.; Chen, K. T.; Chen, S. K.; Huang, C. Y.; Cheng, T. J. R.; Ma, C.; Wong, C. H.; Cheng, W. C. Org. Biomol. Chem. 2010, 8, 2586.
13. (a) Granier, T.; Vasella, A. Helv. Chim. Acta 1998, 81, 865.
14. (b) Sellogoub, M.; Bleriot, Y.; Pons, E.; Auberge, N. PCT Int. Patent CA284015A1, 2003.
15. (a) Goujon, J. Y.; Gueyrard, D.; Compain, P.; Martin, O. R.; Hoffmann, M.; Burkhart, F.; Hessler, G.; Kessler, H. Helv. Chim. Acta 1996, 79, 1519.
16. (a) Yoda, H.; Suzuki, Y.; Takabe, K. Tetrahedron Lett. 2004, 45, 1599.
17. (a) Wennerkes, T.; Lang, B.; Leeman, M.; van der Marel, G. A.; Smith, E.; Weber, M.; van Wiltenburg, J.; Wolberg, M.; Aerts, J. M. F. G.; Overkleeft, H. S. Org. Process Res. Dev. 2008, 12, 414.
18. (a) Kumar, V.; Ramesh, N. G. Tetrahedron 2006, 62, 1877.
19. (a) Schwarz, J. C. P. J. Am. Chem. Soc. 1963, 85, 2870.
20. (b) Alonzo, D.; Guaragna, A.; Palumbo, G. Curr. Med. Chem. 2006, 13, 4719.
21. (b) Kumar, V.; Ramesh, N. G. Tetrahedron 2006, 62, 1877.
22. (a) Martin, O. R.; Liu, L.; Yang, F. Crit. Rev. Biotechnol. 1996, 16, 351.
23. (a) Liu, P. S. J. Org. Chem. 1987, 52, 4717.
24. (b) Hoffmann, M.; Burkhart, F.; Hessler, G.; Kessler, H. Helv. Chim. Acta 1996, 79, 1519.
25. (b) Ye, X. Y.; Lo, M. C.; Alonzo, D.; Guaragna, A.; Palumbo, G. Curr. Med. Chem. 2006, 13, 4719.
26. (a) D’Alonzo, G.; Guaragna, A.; Palumbo, G. Curr. Med. Chem. 2006, 13, 4719.
27. (a) Wagner, G. K.; Pesnot, T.; Field, R. A. Curr. Med. Chem. 2006, 13, 4719.
28. (a) Kelly, C. P.; LaMont, J. T. J. Am. Chem. Soc. 2008, 130, 12608.
29. (b) Pearlstein, D. L.; Wang, T. S. A.; Doud, E. H.; Kahne, D.; Walker, S. J. Am. Chem. Soc. 2010, 132, 48. (b) Ye, X. Y.; Lo, M. C.; Brunner, L.; Walker, D.; Kahne, D.; Walker, S. J. Am. Chem. Soc. 2001, 123, 3155.
30. (a) Liu, C. Y.; Guo, C. W.; Chang, Y. F.; Wang, J. T.; Hsu, Y. F.; Chen, C. W.; Chen, S. K.; Wang, Y. C.; Cheng, T. J. R.; Ma, C.; Wong, C. H.; Fang, J. M.; Cheng, W. C. Org. Lett. 2010, 12, 1608.
31. (a) Perlinstein, D. L.; Wang, T. S. A.; Doud, E. H.; Kahne, D.; Walker, S. J. Am. Chem. Soc. 2010, 132, 48. (b) Ye, X. Y.; Lo, M. C.; Brunner, L.; Walker, D.; Kahne, D.; Walker, S. J. Am. Chem. Soc. 2001, 123, 3155.
32. (a) Huang, S. H.; Wu, W. S.; Huang, L. Y.; Huang, W. F.; Fu, W. C.; Chen, P. T.; Fang, J. M.; Cheng, W. C.; Cheng, T. J. R.; Wong, C. H. J. Am. Chem. Soc. 2013, 135, 17078.
(28) Gloster, T. M.; Zandberg, W. F.; Heinonen, J. E.; Shen, D. L.; Deng, L. H.; Vocadlo, D. J. Nat. Chem. Biol. 2011, 7, 174.