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

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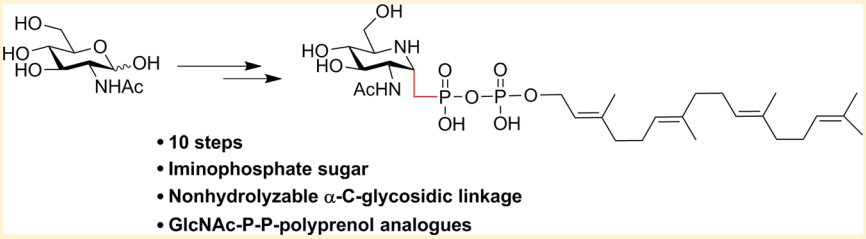
Supporting Information



ABSTRACT: We herein describe the first synthesis of iminosugar C-glycosides of α-D-GlcNAc-1-phosphate in 10 steps starting from unprotected 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.[1] 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.[2] 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.[1,3]

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.[4] Peptidoglycan transglycosylase (TGase) is the enzyme responsible for catalyzing the polymerization of β1,4-linked *N*-acetylglucosamine-*N*-acetylmuramic acid $(GlcNAc\text{-}MurNAc)_n$ disaccharide of lipid II to form the bacterial cell wall.[5] 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.[6] The only well-characterized TGase inhibitor, moenomycin (Moe), however, is not effective in humans because of its poor pharmacokinetic properties.[7] From recent structural studies,[8] the transglycosylation mechanism employed by TGase is that of a S_N2-like reaction, which is similar to other inverting glycosyltransferases (Figure 1).[9] Therefore, we expect iminosugar-based lipid II mimetics to be potent substrate-based inhibitors that mimic the transition state of TGase transglycosylation (Scheme 1).[10] However, the idea has been tested only with pyrrolidine mimics that were discovered by a combinatorial approach.[11] 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 phosphonate analogue **2** has to be realized. Only a few examples have been found for the synthesis of iminosugar analogues of GlcNAc.[12] 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.[13] Key steps are a

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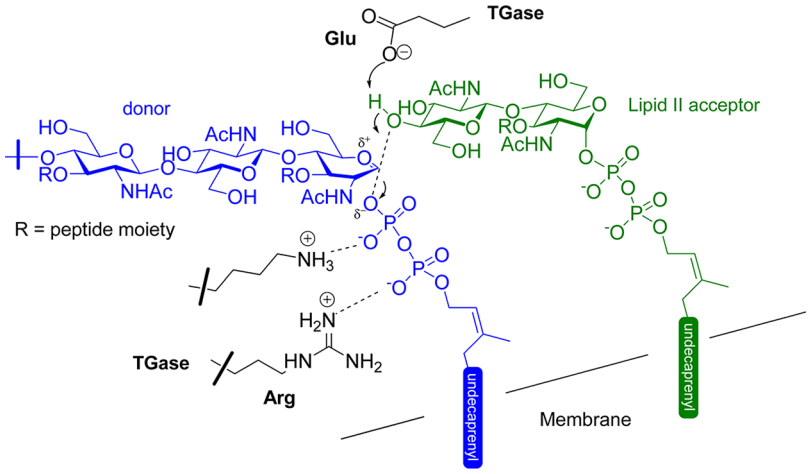
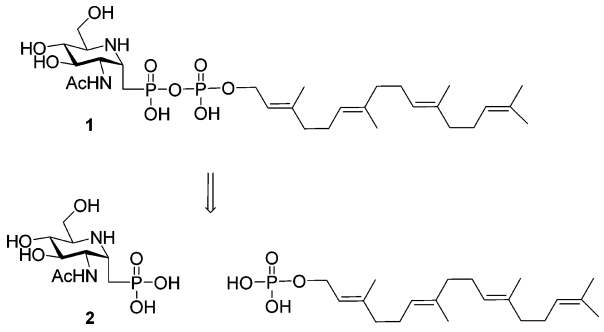


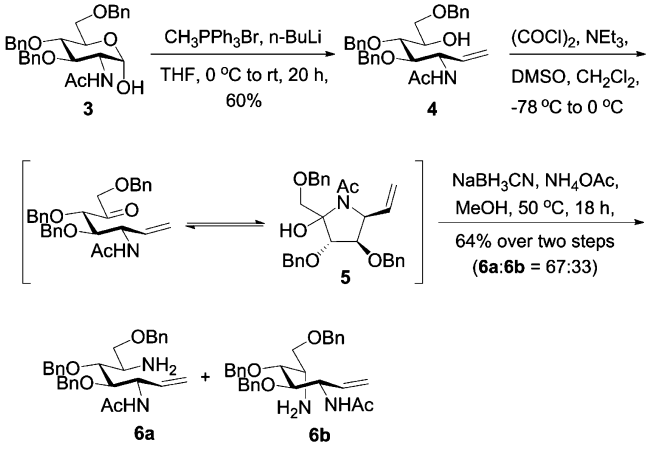
Figure 1. Transglycosylation by TGase to synthesize peptidoglycans.

Scheme 1. Iminosugar C-Glycosidic Lipid II Analogue



Wittig reaction followed by oxidation and reductive amination leading to a diaminohepenitol, followed by an NIS-mediated cyclization. Similar synthetic strategies were known before,[13] but have never been applied successfully to GlcNAc. The synthesis of diaminoheptenitols is depicted in Scheme 2.

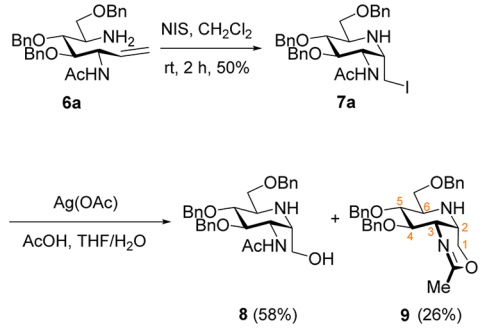
Scheme 2. Synthesis of Diaminoheptenitols 6a and 6b



Starting from GlcNAc, compound **3** was prepared[14] and subjected to optimized Wittig conditions. The obtained aminoheptenitol **4**[15] had to be transformed into the corresponding amine to construct the piperidine ring. Epimerization at C-6 of **4** followed by reinversion 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.[16] The ketone obtained by Swern oxidation was found to be in equilibrium with *N*-acyl hemiaminal **5** (typical ^{13}C NMR appearance of the C-2 quaternary carbon at δ = 91 ppm),[17] which was stable and could be subjected to reductive amination directly without purification. Optimization of the reaction conditions led to the use of excess $NaBH_3CN$/NH_4OAc (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 (6*R*) diastereomer being the main product in a ratio **6a**/**6b** (6*R*/6*S*) = 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 (6*R*) 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).[13b] The configuration of the stable compound **7a** was

Scheme 3. Oxazine 9 for Structural Determination



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

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configuration (see Figure S1). 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/H_2O (Scheme 3). The bicyclic system locks the sugar ring in the chair conformation ($^4C_1$),[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 D-gluco conformation.[19]

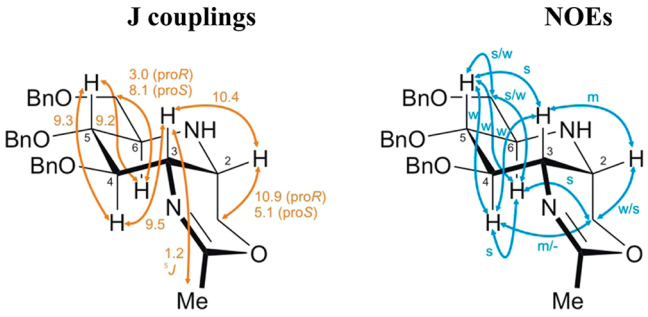
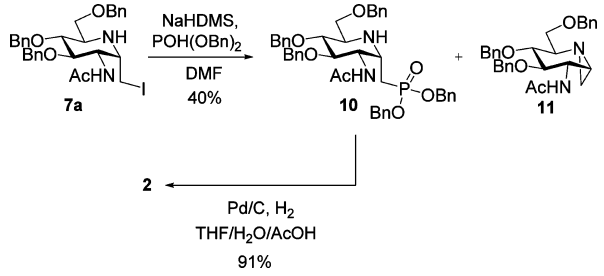


Figure 2. Coupling constants and NOEs of bicyclic iminosugar **9**. Abbreviations of the NOE intensities are as follows: s, strong; m, medium; and w, weak.

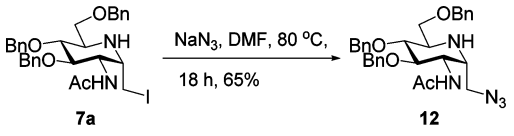
The synthesis of the first GlcNAc-derived iminosugar phosphonate was achieved for the D-gluco series of compounds, but initial attempts were not successful. The intramolecular formation of aziridine intermediate **11** dominated in reactions employing bases such as Cs_2CO_3 (Scheme 4). The problem

Scheme 4. Synthesis of Phosphonate Analogue 2



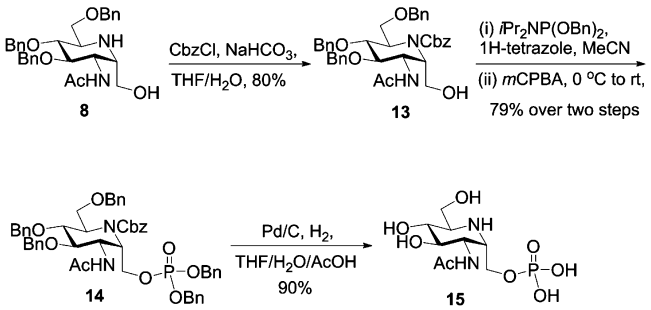
was solved by using a bulkier base such as NaHDMS. Substitution of the iodine of **7a** by deprotonated dibenzylphosphite using NaHDMS led to the isolation of protected phosphonate **10** in moderate yield (Scheme 4). Debenzylation of **10** was achieved to afford **2** in high yield with Pd/C under a H_2 atmosphere in a solvent mixture of THF/H_2O/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).

Scheme 5. Synthesis of Azido Derivative 12



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

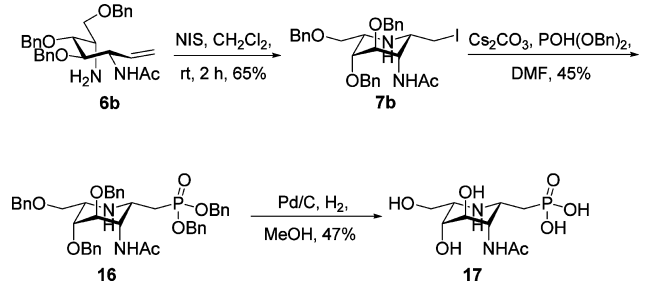
Scheme 6. Synthesis of Phosphate Analogue 15



alkyl or aryl protecting groups gave significant amounts of *N*-oxide after treatment with *m*CPBA. Deprotection of **14** by Pd/H_2 in THF/H_2O/AcOH afforded the desired glycosyl phosphate mimetic **15** in 90%.

Because L-iminosugars are being increasingly appreciated for pharmaceutical purposes,[21] the procedure also offers access to L-ido imino-C-glycosides of GlcNAc-1-phosphate **17** (Scheme 7), which allows biological studies to be extended to the

Scheme 7. Synthesis of L-ido Analogue 17



unnatural (L-ido) derivatives. The structure of **17** was determined as the L-ido-isomer in a $^1C_4$-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

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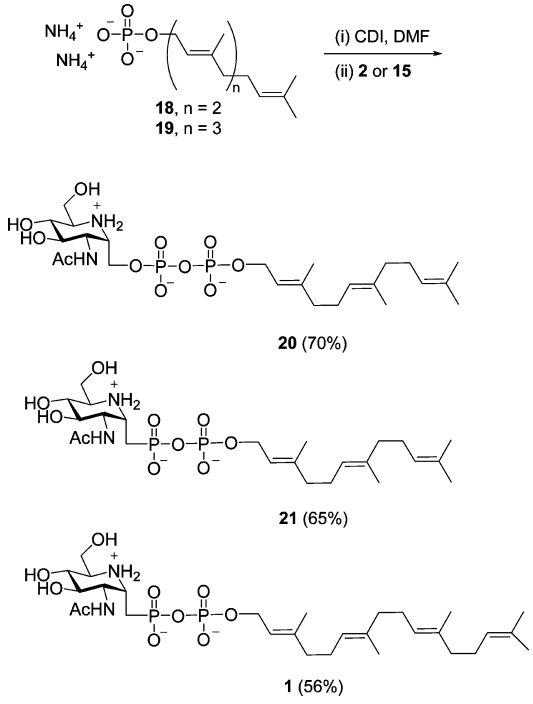
Scheme 8. Synthesis of Lipid-Linked Pyrophosphate Analogues



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 K_i value in the low micromolar range (Table 1), although the inhibition is still weak compared with that of moenomycin. The K_i value is determined by using our recent reported FRET-based TGase assay.[27]

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

compd	inhibition (%)[a] (500 μM)	inhibition (%) (100 μM)	K_i (μM)
2	—[b]	—	
15	—	—	
17	—	—	
20	50	0	
21	100	30	
1	100	100	6.3 ± 1.4[c]
Moe	100	100	0.0015 ± 0.0001[d]

[a]Inhibition percent calculated by comparing the reaction rate in the presence of the compound to the rate in the absence of any inhibitory compound. [b]— refers to no inhibition. [c]Shown are the mean values of the data generated from three independent experiments. [d]The data was adopted from ref 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 diaminoheptenitol **6a** and **6b**, versatile iodo intermediates **7a** and **7b**, and aminoheptenitol **4** which has been reported via a less direct route[15] (eight steps starting from D-arabinofuranose). Our result demonstrated that the connection of iminosugar 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*-acetylglucosamine 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 F_{254} (0.25 mm, E. Merck); detection was executed by spraying with a solution of Ce-$(NH_4)_2(NO_3)_6$ and $(NH_4)_6Mo_7O_{24}$ and subsequent heating on a hot plate. ^{1}H and ^{13}C NMR spectra were recorded on 500 and 600 MHz spectrometers. In ^{1}H NMR spectra, chemical shifts were referenced to internal tetramethylsilane (δ 0.00 ppm in $CDCl_3$), C_6D_5H (δ 7.16 ppm in C_6D_6), or HOD (δ 4.79 ppm in D_2O). 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 (^{1}H−^{1}H 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 NH_4OAc (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 $CaCl_2$, 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 H_2O (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 K_i Values of Transglycosylase Inhibitors. The K_i values of **1** 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 **1**. Initial velocity for each progression curve was calculated and used for K_i determination. The K_i value for **1** was determined with the competitive inhibition

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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(benzyloxy)-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. *n*BuLi (2.2 M in hexanes, 103 mmol, 46 mL) was added dropwise over 15 min using the droping funnel, forming a red−orange solution. This was stirred for 10 min at 0 °C and 40 min at rt, forming a dark red solution. Then a suspension of **3** (20.3 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 [CH_2Cl_2/MeOH 20:1, R_f(**3**) = 0.27, R_f(**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 × 250 mL), separating large amounts of unreacted phosphonium salts. The combined organic extracts were dried over Na_2SO_4, and the solvent was removed in vacuo. Separation from side products SP1 and SP2 [(R_f(**3**) = 0.20, R_f(SP1) = 0.15, cerium molybdate, R_f(SP2) = 0.15, UV, toluene/MeOH 9:1] by column chromatography on silica gel (separation from SP2: 50 cm column, 1.500 g silica, toluene/MeOH 40:1; separation from SP1: 50 cm column, 500 g silica, hexanes/EtOAc 1:1) led to the isolation of **4** (5.97 g, 60%) as a colorless oil. Analytical data was in full accordance with the reported data. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.89 (s, 3H, CH_3), 2.96 (d, J = 5.6 Hz, 1 H), 3.60−3.57 (m, 3H), 3.82 (dd, J = 6.0, 2.0 Hz, 1H), 3.94−3.99 (m, 1H), 4.5−4.7 (m, 6 H, 3CH_2Ph), 4.78−4.82 (m, 1H), 5.12 (d, J = 10.3 Hz, 1H, 1-H_Z), 5.16 (d, J = 17.2 Hz, 1H, 1-H_E), 5.82 (ddd, J = 17.2, 10.4, 5.2 Hz, 1 H, 2-H), 6.15 (d, J = 8.6 Hz, 1H, NH), 7.12−7.34 (m, 15 H, Ar-H). ^{13}C NMR ($CDCl_3$, 125 MHz), δ 23.3 (CH_3), 52.7 (C-3), 70.9 (t, C-7), 71.0, 73.4, 74.5, 75.1 (3 t, OCH_2Ph), 80.4, 81.0, 115.5 (C-1), 125.3, 127.7, 128.8, 127.9, 128.0, 128.3, 128.4, 128.4, 128.5 (9 d, Ar), 129.1, 136.9 (C-2), 138.0, 138.3 (3 s, Ar), 169.7 (C═O). HRMS (ESI-TOF) calcd for $C_{30}H_{36}NO_5$ (M + H)$^+$, 490.2593; found, 490.2588.

*N-((3R,4R,5R,6S)-6-Amino-4,5,7-tris(benzyloxy)hept-1-en-3-yl)-acetamide (**6a**) and N-((3R,4S,5R,6R)-6-Amino-4,5,7-tris-(benzyloxy)hept-1-en-3-yl)acetamide (**6b**).* Oxalyl chloride (12 mmol, 1.02 mL) was added at −78 °C to a solution of DMSO (16 mmol, 1.13 mL) in dry CH_2Cl_2 (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 CH_2Cl_2 (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, NEt_3 (32 mmol, 4.44 mL) was added followed by 40 min of stirring at −78 °C. The solution was warmed to 0 °C within 10 min with an ice bath, and CH_2Cl_2 (20 mL) and a saturated aqueous solution of $NaHCO_3$ (50 mL) were added followed by extraction of the aqueous phase with CH_2Cl_2 (3 × 40 mL). The combined organic extracts were dried over Na_2SO_4, and the solvent was removed in vacuo. A crude, instable intermediate was obtained as a yellow oil [CH_2Cl_2/MeOH 20:1, R_f(**4**) = 0.39, R_f(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 $NaBH_3CN$ (20 mmol, 1.30 g). After the resulting solution was stirred for 18 h at 50 °C, TLC monitoring [2 × CH_2Cl_2/MeOH 20:1, R_f(ketone) = 0.66, R_f(alcohol) = 0.58, R_f(**6b**) = 0.41, R_f(**6a**) = 0.34, cerium molybdate], saturated $NaHCO_3$ (40 mL) was added to the reaction mixture followed by the removal of MeOH. The residual aqueous solution was extracted by CH_2Cl_2 (120 mL) twice. The collected organic phase was dried over $MgSO_4$ 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, CH_2Cl_2/MeOH 25:1) led to the isolation of **6a** (836 mg, 42%) and **6b** (411 mg, 22%) as colorless oils.

For **6a**, $[\alpha]_D^{20} \cong -36.3$ (c 1.0, CH_2Cl_2). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39−7.26 (m, 15H), 6.31 (d, J = 8.7 Hz, 1H, NHAc), 5.82 (ddd, J = 17.2, 10.4, 5.1 Hz, 1H, 2-H), 5.19−5.13 (m, 2H, 1-H), 4.81−4.76 (m, 2H, CH_2Ph, 3-H), 4.72 (d, J = 10.8 Hz, 1H, CH_2Ph), 4.60 (dd, J = 10.6 Hz, 2H, CH_2Ph), 4.54 (s, 2H, CH_2Ph), 3.88 (dd, J = 7.1, 1.7 Hz, 1H, 4-H), 3.66 (dd, J = 9.2, 5.2 Hz, 1H, 7-H_a), 3.60 (dd, J = 7.0, 5.1 Hz, 1H, 5-H), 3.50 (dd, J = 9.2, 6.1 Hz, 1H, 7-H_b), 3.24 (ddd, J = 5.6, 5.6, 5.3 Hz, 1H, 6-H), 1.99 (s, 3H, NHAc). ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.5 (C═O), 138.5, 138.0, 137.9, 136.8 (C-2), 128.4, 128.3, 128.2, 127.9, 127.8, 127.7, 127.6, 127.4, 115.2 (C-1), 82.7 (C-5), 81.4 (C-4), 74.9, 73.2, 72.1 (C-7), 52.2 (C-6), 52.1 (C-3), 23.3 (CH_3). HRMS (ESI-TOF) calcd for $C_{30}H_{37}N_2O_4$ (M + H)$^+$, 489.2753; found, 489.2748.

For **6b**, $[\alpha]_D^{20} \cong -43.8$ (c 1.0, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.07 (s, 3H, CH_3), 3.23 (dd, J = 6.8, 6.8 Hz, 1H), 3.43 (dd, J = 7.6, 7.6 Hz, 1H), 3.54 (dd, J = 8.4, 6.5 Hz, 1H), 3.71 (d, J = 8.4 Hz, 1H), 4.06 (d, J = 8.4 Hz, 1H), 4.5−4.6 (m, 3H), 4.74 (d, J = 10.6 Hz, 1H, CH_2Ph), 4.78 (d, J = 10.6 Hz, 1 H, CH_2Ph), 4.86−4.91 (m, 2H), 5.27−5.37 (m, 2H, 1-H), 6.04 (ddd, J = 15.1, 10.5, 3.8 Hz, 1H, 2-H), 7.05 (d, J = 8.3 Hz, 1 H, NH), 7.30−7.43 (m, 15 H, Ar-H). ^{13}C NMR ($CDCl_3$, 125 MHz), δ 23.6 (CH_3), 51.2, 51.8 (2 *C*NHR), 73.1, 73.5, 74.9, 75.4 (4 t, OCH_2Ph, C-7), 80.4, 83.1, 115.6 (C-1), 127.7, 127.7, 127.8, 127.8, 128.0, 128.3, 128.4, 128.5, 128.54, 128.56 (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 $C_{30}H_{37}N_2O_4$ (M + H)$^+$, 489.2753; found, 489.2758.

*N-((2S,3S,4R,5R,6R)-4,5-Bis(benzyloxy)-6-(benzyloxymethyl)-2-(iodomethyl)piperidin-3-yl)acetamide (**7a**).* Under an atmosphere of argon in the dark, *N*-iodosuccinimide (0.65 mmol, 147 mg) was added in one portion at rt to a solution of **6a** (0.65 mmol, 320 mg) in dry CH_2Cl_2 (20 mL). The resulting solution was stirred at rt for 2 h (TLC monitoring, CH_2Cl_2/MeOH 20:1, R_f(**6a**) = 0.25, R_f(7**a**) = 0.60, cerium molybdate]. Then, a saturated aqueous solution of sodium thiosulfate (10 mL) was added followed by extraction with CH_2Cl_2 (3 × 20 mL). The combined organic extracts were dried over $MgSO_4$, and the solvent was removed in vacuo. Purification by silica gel chromatography (CH_2Cl_2/MeOH 100:1) gave **7a** (168 mg, 50%) as a white solid. $[\alpha]_D^{20} \cong +20.5$ (c 0.4, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.82 (s, 3H, CH_3), 2.99 (t, J = 10.2 Hz, 1H, 1-H_a), 3.20 (dd, J = 10.2, 3.3 Hz, 1H, 1-H_b), 3.36−3.40 (m, 2H, 2-H and 7-Ha), 3.43 (m, 1H, 5-H), 3.47 (m, 1H, 6-H), 3.56 (m, 1H, 4-H), 3.82 (dd, J = 9.4, 4.9 Hz, 1H, 7-H_b), 4.33 (m, 1H, 3-H), 4.41−4.45 (t, 2H, CH_2Ph), 4.60−4.49 (m, 4H, CH_2Ph), 6.61 (d, J = 9.1 Hz, 1 H, NH), 7.19−7.33 (m, 15 H, Ar-H). ^{13}C NMR ($CDCl_3$, 150 MHz) 9.8 (CH_2I), 23.3 (CH_3), 48.9 (C-3), 49.8 (C-2), 56.0 (C-6), 68.0 (C-7), 72.0, 72.1, 73.4 (OCH_2Ph), 74.5 (C-5), 75.4 (C-4), 138.0, 137.5, 137.3, 128.5, 128.5, 128.4, 128.0, 127.9, 127.8, 127.7, 127.7, 127.5 (Ar), 137.3, 137.5, 138.0 (Ar), 170.0 (C═O). HRMS (ESI-TOF) calcd for $C_{30}H_{36}IN_2O_4$ (M + H)$^+$, 615.1714; found, 615.1686.

*N-((2S,3S,4R,5R,6R)-4,5-Bis(benzyloxy)-6-((benzyloxy)methyl)-2-(hydroxymethyl)piperidin-3-yl)acetamide(**8**) and (4aS,6R,7R,8-R,8aS)-7,8-Bis(benzyloxy)-6-((benzyloxy)methyl)-2-methyl-4a,5,6,7,8,8a-hexahydro-4H-pyrido[3,2-d][1,3]oxazine (**9**).* A solution of **7a** (0.33 mmol, 200 mg) in THF/H_2O 1:1 (44 mL) was treated with Ag(OAc) (0.9 mmol, 150 mg) and stirred at rt for 30 min followed by the addition of AcOH (200 μL). After 3 h (TLC monitoring, CH_2Cl_2/MeOH 20:1, R_f(**8**) = 0.38, R_f(**9**) = 0.5, cerium molybdate], the mixture was filtered, and the solvent was removed in vacuo. Dichloromethane (100 mL) was added to the residue, which was washed with saturated aqueous $NaHCO_3$. The collected organic phase was dried by $MgSO_4$ and concentrated in vacuo. Purification by column chromatography on silica gel (CH_2Cl_2/MeOH/Et_3N 30:1:0.3) led to the isolation of **8** (96.5 mg, 58%) and **9** (41.7 mg, 26%) as white solid and colorless oil, respectively.

*Compound **8**.* ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.38−7.14 (m, 15H), 6.98 (d, J = 7.3 Hz, 1H, N*H*Ac), 4.60 (m, 2H, CH_2Ph), 4.51−4.40 (m, 4H, CH_2Ph), 4.21 (m, 1H, 3-H), 3.95 (d, J = 6.2 Hz, 1H, OH), 3.81 (t, J = 8.9 Hz, 1H, 7-H_a), 3.63 (m, 1H, 4-H), 3.58 (m, 1H, 5-H), 3.48 (dd, J = 9.4, 5.9 Hz, 1H, 7-H_b), 3.42−3.35 (m, 2H, 6-H, 1-H_b), 3.28−3.24 (m, 1H, 2-H), 3.23−3.18 (m, 1H, 1-H_a), 1.86 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.4, 138.1, 137.5, 137.2, 128.7,

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128.5, 128.4, 128.2, 127.9, 127.9, 127.7, 127.4, 74.6 (C-4), 74.5 (C-5), 73.3, 72.2, 71.9 (OCH_2Ph), 68.4 (C-7), 62.2 (C-1), 55.0 (C-6), 48.9 (C-2), 46.2 (C-3), 23.2 (CH_3). HRMS (ESI-TOF) calcd for $C_{30}H_{37}N_2O_5$ $(M + H)^+$, 505.2697; found, 505.2698.

*Corrected ^{1}H NMR Values for Compound **9** (Lorentz-to-Gauss Transformed Spectra). Compound **9**.* ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41−7.07 (m, 15H, Ar-H), 4.87 (dd, J = 10.7, 6.4 Hz, 2H, CH_2Ph), 4.73 (d, J = 10.7 Hz, 1H, CH_2Ph), 4.53 (ddd, J = 10.9, 10.4, 5.1 Hz, 1H, 2-H), 4.48−4.41 (m, 2H, CH_2Ph), 4.36 (d, J = 10.8 Hz, 1H), 4.29 (ddd, J = 10.4, 9.5, 1.2 Hz, 1H, 3-H), 3.79 (dd, J = 9.0, 3.0 Hz, 1H, 7-H_a), 3.61 (dd, J = 9.5, 9.3 Hz, 1H, 4-H), 3.38 (dd, J = 9.0, 8.1 Hz, 1H, 7-H_b), 3.25 (dd, J = 13.6, 5.1 Hz, 1H, 1-H_a), 3.19 (dd, J = 9.3, 9.2 Hz, 1H, 5-H), 2.94 (ddd, J = 9.2, 8.1, 3.0 Hz, 1H, 6-H), 2.58 (dd, J = 13.6, 10.9 Hz, 1H, 1-H_b), 1.94 (d, J = 1.2 Hz, 3H). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 164.31(C═O), 138.98, 138.25, 137.86, 128.46, 128.29, 128.13, 127.97, 127.96, 127.83, 127.58, 127.28 (Ar), 84.53 (C-4), 82.52 (C-5), 79.84 (C-2), 75.88, 75.50, 73.38 (OCH_2Ph), 72.18 (C-3), 71.50 (C-7), 63.30 (C-6), 47.90 (CH_2O), 14.02 (CH_3). HRMS (ESI-TOF) calcd for $C_{30}H_{35}N_2O_4$ $(M + H)^+$, 487.2591; found, 487.2616.

*Dibenzyl(((2S,3S,4R,5R,6R)-3-acetamido-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)piperidin-2-yl)methyl)phosphonate (**10**).* To a stirring solution of dibenzyl phosphate (111 μL, 0.48 mmol) in dry DMF (2.5 mL) was added molecular sieve MS 4Å (150 mg), and the mixture was stirred for 30 min at rt. NaHMDS (2 M solution in THF, 230 μL, 0.46 mmol) was then added. After 1 h, compound **7a** (51 mg, 0.08 mmol) in dry DMF (1.5 mL) was added to the reaction mixture at rt, and the mixture was stirred for another 24 h (R_f (**10**) = 0.38, UV, EtOAc/Hex 4:1). The reaction mixture was concentrated to remove DMF and purified by silica gel chromatography (CH_2Cl_2/MeOH 100:1 to 10:2, 5.0 g silica) to afford **10** (24.8 mg, 40%) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.33−7.18 (m, 25H), 6.60 (d, J = 8.2 Hz, 1H, N*H*Ac), 4.97 (m, CH_2Ph, 2H), 4.92−4.87 (m, CH_2Ph, 2H), 4.56 (d, J = 11.8 Hz, CH_2Ph, 1H), 4.52 (d, J = 11.5 Hz, CH_2Ph, 1H), 4.45−4.40 (m, CH_2Ph, 2H), 4.34 (d, J = 11.9 Hz, CH_2Ph 1H), 4.10 (brd, J = 9.4 Hz, 1H, 3-H), 3.71−3.63 (m, 2H, 7-Ha and 2-H), 3.57 (m, 1H, 4-H), 3.53−3.47 (m, 2H, 7-Hb and 5-H), 3.32 (m, 6-H), 1.94−1.87 (m, 1H, 1-H_a), 1.84−1.75 (m, 4H, $COCH_3$ and 1-H_b). ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.95 (C═O), 138.3, 137.7, 137.5, 136.2, 136.2, 136.2, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 127.9, 127.8, 127.8, 127.6, 127.5, 75.4 (C-4), 74.1 (C-5), 73.2, 72.1, 68.8 (C-7), 67.2, 67.2, 67.2, 67.2 (dd, $J_{c,P}$ = 4.2 Hz, 2C, $POCH_2Ph$), 55.4 (C-6), 49.8, 49.7 (d, $J_{c,P}$ = 16.2 Hz, C-3), 43.6 (C-2), 29.3, 28.4 (d, $J_{c,P}$ = 140.4 Hz, C-1), 23.3 (CH_3). ^{31}P NMR ($CDCl_3$, 202 MHz) δ 31.9. HRMS (ESI-TOF) calcd for $C_{44}H_{49}N_2O_7P$ $(M + H)^+$, 749.3350; found, 749.3360.

*(((2S,3S,4R,5R,6R)-3-Acetamido-4,5-dihydroxy-6-(hydroxymethyl)piperidin-2yl)methyl) phosphonic acid (**2**).* To a round-bottomed flask contained starting material **10** (20 mg, 0.027 mmol) were added THF (2.5 mL), H_2O (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, 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. ^{1}H NMR (600 MHz, D_2O) δ 4.10 (brd, J = 6.8 Hz, 1H, 3-H), 3.91−3.75 (m, 3H, 7-Ha, 7-Hb, 2-H), 3.68 (t, J = 9.5 Hz, 1H, 4-H), 3.56 (t, J = 9.0 Hz, 1H, 5-H), 3.22 (d, J = 8.2 Hz, 1H, 6-H), 2.01 (s, 3H), 1.79 (m, 1H, 1-Ha), 1.66 (m, 1H, 1-Hb). ^{13}C NMR (151 MHz, D_2O) δ 176.9 (C═O), 71.9 (C-4), 71.6 (C-5), 60.4 (C-7), 56.3 (C-6), 53.9 (C-2), 53.7−53.6 (C-3, Jc_P = 28.9 Hz), 24.3. ^{31}P NMR (D_2O, 202 MHz) δ 19.7 (brs). HRMS (ESI-TOF) calcd for $C_9H_{19}N_2O_7P$ $(M - H)^-$, 297.0846; found, 297.0860.

*N-((2R,3S,4R,5R,6R)-2-(Azidomethyl)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)piperidin-3-yl)acetamide (**12**).* To a stirred solution of iodo compound **7a** (58 mg, 0.094 mmol) in dry DMF (6 mL) was added NaN_3 (12.2 mg, 0.188 mmol). The reaction mixture was heated at 80 °C and reacted for 8 h. After the disappearance of **7a** (toluene/EtOAc 3:2, R_f = 0.25, cerium molybdate) on TLC, the reaction mixture was cooled to rt. CH_2Cl_2 (10 mL) was added, and the mixture was washed twice with water (5 mL), dried with $MgSO_4$, and concentrated. The crude residue was purified by flash silica gel column chromatography (toluene/EtOAc 7:3) to afford compound **12** (32.3 mg, 65%) as colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.40−7.31 (m, 11H), 7.29 (m, 2H), 7.25−7.22 (m, 2H), 6.68 (d, J = 9.1 Hz, 1H), 4.61 (t, J = 12.0 Hz, 2H), 4.55 (q, J = 12.0 Hz, 2H), 4.48 (t, J = 11.3 Hz, 2H), 4.22 (m, 1H, 3-H), 3.88 (t, J = 8.6 Hz, 1H), 3.63 (t, J = 3.0 Hz, 1H), 3.54 (td, J = 8.9, 3.3 Hz, 1H), 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). ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.9, 138.1, 137.5, 137.3, 128.5, 128.5, 128.4, 128.0, 127.8, 127.8, 127.7, 127.5, 75.0, 74.4, 73.3, 72.0, 67.9, 55.4, 53.6, 47.2, 23.3. HRMS (ESI-TOF) calcd for $C_{30}H_{35}N_5O_4$ $(M + H)^+$, 530.2762; found, 530.2776.

*Benzyl(2S,3S,4R,5R,6R)-3-acetamido-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-2-(hydroxymethyl)piperidine-1-carboxylate (**13**).* A solution of compound **8** (220 mg, 0.44 mmol) in dioxane/H_2O (3:2, 25 mL) was treated with $NaHCO_3$ (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 CH_2Cl_2 (30 mL) and washed with water (15 mL) and brine (15 mL) sequentially. The organic layer was dried with $MgSO_4$ and concentrated, and the residue was purified by flash silica gel column chromatography (EtOAc/hexane 55:45 to 70:30) to afford **13** (347 mg, 92%) as a viscous oil. ^{1}H NMR (600 MHz, C_6D_6, 45 °C) δ 7.38−7.07 (m, 20H, Ar-H), 6.61 (d, J = 9.1 Hz, 1H, NH), 5.26 (m, 1H, 6-H), 5.21 (d, J = 11.9 Hz, 1H, 7-H_a), 5.05 (d, J = 9.8 Hz, 1H, 7-H_b), 4.92 (ddd, J = 9.2, 2.3, 2.3 Hz, 1H, 3-H), 4.57 (m, 1H, 1-H_a), 4.5−4.49 (m, 2H, 1-H_b, CH_2Ph), 4.46 (d, J = 11.9 Hz, 1H, CH_2Ph), 4.43−4.37 (m, 2H, CH_2Ph), 4.31 (d, J = 11.9 Hz, 1H, CH_2Ph), 4.17 (d, J = 11.3 Hz, 1H, CH_2Ph), 4.14−4.11 (ddd, J = 8.0, 6.0, 2.3 Hz, 1H, 1-H), 4.04 (t, J = 7.1 Hz, 1H, OH), 3.84 (p, J = 9.5 Hz, 3H, 5-H, CH_2Ph), 3.74 (m, 1H, 4-H), 1.62 (s, 3H, CH_3). ^{13}C NMR (151 MHz, C_6D_6, 45 °C) δ 169.9 (C═O), 156.6 (C═O), 138.6, 137.8, 137.7, 137.0, 128.4, 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 (OCH_2Ph), 67.0 (C-7), 61.4 (C-1), 55.7 (C-6), 55.0 (C-2), 46.8 (C-3), 22.3 (CH_3). HRMS (ESI-TOF) calcd for $C_{38}H_{42}N_2O_7$ $(M + H)^+$, 639.3065; found, 639.3084.

*Benzyl(2S,3S,4R,5R,6R)-3-acetamido-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-2-(((bis(benzyloxy)phosphoryl)oxy)methyl)-piperidine-1-carboxylate (**14**).* To a stirred solution of **13** (16 mg, 0.03 mmol) and tetrazole (9.7 mg, 0.14 mmol) in MeCN (2 mL) at 0 °C was slowly added bis(benzyloxy)(diisopropylamino)phosphane (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 0 °C followed by the addition of *m*CPBA (75%, 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 $NaHCO_3$ (1.5 mL). The organic phase was collected. The aqueous phase was extracted with CH_2Cl_2 (10 mL) three times. The combined organic phase was dried over $MgSO_4$, filtered, and concentrated. The reside was purified by silica gel chromatography (EtOAc/hexanes 55:45) to yield compound **14** (21.3 mg, 79% over two steps) as a colorless liquid. ^{1}H NMR (600 MHz, C_6D_6, 45 °C) δ 7.37−7.06 (m, 35H), 6.91 (brs, 1H, NHAc), 5.08−5.04 (m, 10H), 4.55 (brs, 2H), 4.43 (m, 4H), 4.22 (d, J = 11.5 Hz, 1H), 4.02−3.85 (m, 4H), 1.79 (brs, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.0 ($NHCOCH_3$), 156.4 (NHCOOBn), 138.0, 137.5, 137.3, 136.2, 135.8, 135.7, 135.7, 128.5, 128.4, 128.3, 128.1, 127.9, 127.9, 127.9, 127.8, 127.6, 127.5, 74.3, 73.0, 71.8, 71.2, 69.3, 67.9, 67.5, 52.3, 47.7, 23.0 ($NHCOCH_3$). ^{31}P NMR ($CDCl_3$, 202 MHz) δ 0.32. HRMS (ESI-TOF) calcd for $C_{52}H_{55}N_2O_{10}P$ $(M + H)^+$, 899.3667; found, 899.3692.

*((2S,3S,4R,5R,6R)-3-Acetamido-4,5-dihydroxy-6-(hydroxymethyl)-piperidin-2-yl)methyl Dihydrogen Phosphate (**15**).* To a round-bottomed flask containing starting material **14** (20 mg) were added THF (2.5 mL), H_2O (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 P2 led to the isolation of **15** (6.5 mg, 91%) as a colorless solid. ^{1}H NMR (600 MHz, D_2O) δ 4.07 (m, 2H, 3-H and 1-H_a), 3.97 (m, 1H,

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1-H_b), 3.88 (m, 2H, 4-H and 7-H_a), 3.76 (d, J = 12.3 Hz, 1H, 7-H_b), 3.61 (m, 1H, 2-H), 3.42 (m, 2H, 5-H and 6-H), 1.98 (s, 3H, CH_3). ^{13}C NMR (151 MHz, D_2O) δ 174.6 (C═O), 71.0 (C-4), 70.1 (C-5), 60.1 (C-1), 58.8 (C-7), 55.7 (C-6), 53.9 (C-2), 50.9 (C-3), 21.8 (CH_3). ^{31}P NMR (D_2O, 202 MHz) δ 3.3. HRMS (ESI-TOF) calcd for $C_9H_{19}N_2O_8P$ $(M - H)^-$, 313.0795; found, 313.0808.

*N-((2S,3S,4R,5R,6S)-4,5-Bis(benzyloxy)-6-(benzyloxymethyl)-2-(iodomethyl)piperidinyl) Acetamide (**7b**).* Under an atmosphere of argon in the dark, *N*-iodosuccinimide (0.38 mmol, 85.5 mg) was added in one portion at 0 °C to a solution of **6b** (0.3 mmol, 147 mg) in dry CH_2Cl_2 (10 mL). The resulting solution was stirred at 0 °C for 2 h until TLC monitoring [CH_2Cl_2/MeOH 20:1, R_f(**6b**) = 0.38, R_f(**7b**) = 0.67, cerium molybdate] showed full consumption of the starting material. Then, a saturated aqueous solution of sodium thiosulfate (15 mL) and a saturated aqueous solution of Na_2CO_3 (15 mL) were added followed by extraction with CH_2Cl_2 (3 × 20 mL). The combined organic extracts were dried over Na_2SO_4, and the solvent was removed in vacuo. Purification by column chromatography on silica gel (20 g silica, CH_2Cl_2/MeOH 99:1) led to the isolation of **7b** (132 mg, 72%) as a colorless oil. $[\alpha]_D^{20} \cong +25.9$ (c 1.0, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.79 (s, 3H, CH_3), 3.04 (dd, J = 10.9 Hz, J = 10.9 Hz, 1H, 1-H_a), 3.31 (dd, J = 10.7 Hz, J = 3.5 Hz, 1H, 1-H_b), 3.29−3.33 (m, 1H, 2-H), 3.37 (ddd, J = 8.5 Hz, J = 4.7 Hz, 2.1 Hz, 1H, 6-H), 3.43 (dd, J = 3.2 Hz, J = 2.1 Hz, 1H, 5-H), 3.53 (dd, J = 9.0 Hz, J = 4.7 Hz, 1H, 7-H_a), 3.60 (dd, J = 3.1 Hz, J = 3.1 Hz, 1H, H-4), 3.63 (dd, J = 9.0 Hz, J = 9.0 Hz, 1H, 7-H_b), 4.37 (d, J = 11.2 Hz, 1H, CH_2Ph), 4.42 (d, J = 11.2 Hz, 1H, CH_2Ph), 4.46 (ddd, J = 9.7 Hz, J = 3.2 Hz, 2.7 Hz, 1H, 3-H), 4.49 (d, J = 11.9 Hz, 2H, CH_2Ph), 4.59 (d, J = 11.8 Hz, 1H, CH_2Ph), 4.68 (d, J = 11.8 Hz, 1H, CH_2Ph), 6.68 (d, J = 9.7 Hz, 1H, NH), 7.17−7.40 (m, 15H, Ar-H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 9.6 (t, CH_2I), 23.4 (CH_3), 48.8 (C-3), 55.5 (C-6), 55.9 (C-2), 71.0 (C-7), 71.8, 73.06, 73.4 (3 t, OCH_2Ph), 73.5 (C-5), 75.1 (C-4), 127.7, 127.8, 127.8, 128.0, 128.4, 128.4, 128.5, 128.6, 128.7 (9 d, Ar), 137.2, 137.7, 138.1 (3 s, Ar), 170.0 (C═O). HRMS (ESI-TOF) calcd for $C_{30}H_{36}IN_2O_4$ $(M + H)^+$, 615.1714; found, 615.1716.

*Dibenzyl((2S,3S,4R,5R,6S)-3-acetamido-4,5-bis(benzyloxy)-6-(benzyloxymethyl) piperidin-2-yl)methylphosphonate (**16**).* Under an atmosphere of argon and strictly dry conditions, Cs_2CO_3 (0.6 mmol, 195 mg) was added to a solution of freshly distilled dibenzyl phosphite (0.9 mmol, 237 mg) in dry DMF (1.5 mL). After 1 h of stirring at rt, **7b** (0.147 mmol, 90 mg) was added, and the resulting solution was stirred for 12 h in the dark until TLC monitoring [CH_2Cl_2/MeOH 20:1, R_f(**7b**) = 0.64, R_f(**16**) = 0.39, cerium molybdate] showed full consumption of the starting material. Then, the solvent was removed in vacuo. Purification by column chromatography on silica gel (20 g silica, CH_2Cl_2/MeOH 98:2) led to the isolation of **16** (49 mg, 45%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.77 (s, 3H, CH_3), 3.29−3.34 (m, 1H), 3.39−3.53 (m, 4H), 3.60 (dd, J = 4.9, 2.7 Hz, 1H), 4.19 (d, J = 9.7 Hz, 1H), 4.34 (d, J = 11.2 Hz, 1H), 4.38 (d, J = 12.8 Hz, 1H), 4.42 (d, J = 12.4 Hz, 1H), 4.51 (d, J = 11.9 Hz, 1H), 4.62 (d, J = 11.9 Hz, 1H), 4.88−5.02 (m, 4 H), 6.71 (d, J = 10.0 Hz, 1H, NH), 7.15−7.35 (m, 25H, Ar-H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 23.4 (CH_3), 49.2, 49.4, 49.6 (3 CNHR), 55.0, 67.2, 67.3, 67.43, 67.49, 70.5, 71.6, 73.0, 73.2, 73.4, 74.4, 127.6, 127.7, 127.8, 127.9, 128.0, 128.0, 128.3, 128.3, 128.4, 128.4, 128.4, 128.5, 128.62, 128.65, 128.65 (15 d, Ar), 136.2, 136.3, 137.4, 137.8, 138.1 (5 s, Ar), 169.9 (C═O). ^{31}P NMR ($CDCl_3$, 400 MHz) δ 32.4. HRMS (ESI-TOF) calcd for $C_{44}H_{50}N_2O_7P$ $(M + H)^+$, 749.3350; found, 749.3337.

*((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 (mini column, 2 g RP silica, eluent: H_2O) led to the isolation of L-ido-**17** (6.8 mg, 47%) as a colorless solid. ^{1}H NMR (600 MHz, D_2O) δ 4.31 (m, 1H, 3-H), 4.04−4.02 (m, 2H, 4-H and 5-H), 3.96−3.90 (m, 1H, 2-H), 3.88 (dd, J = 12.1, 4.5 Hz, 1H, 7-H_a), 3.85−3.79 (m, 1H, 7-H_b), 3.69 (dd, J = 9.8, 3.3 Hz, 1H, 6-H), 2.06 (s, 3H, NHAc), 1.91−1.83 (m, 2H, 1H), 1.35 (d, J = 13.7 Hz, 1H). ^{13}C NMR (151 MHz, D_2O) δ 173.9 (C═O), 67.2(C4), 65.9 (C5), 59.0 (C7), 57.0 (C6), 51.6 (C2, $J_{c,p}$ = 2.6 Hz), 51.0, 50.9 (C-3, $J_{c,p}$ = 13.6 Hz), 27.4, 26.5 (C-1, Jc,p = 129.1 Hz), 24.6, 22.2 (CH_3). ^{31}P NMR (202 MHz, D_2O) δ −19.01. HRMS (ESI-TOF) calcd for $C_9H_{20}N_2O_7P$ $(M + H)^+$, 299.1003; found, 299.1006.

*(((2S,3S,4R,5R,6R)-3-Acetamido-4,5-dihydroxy-6-(hydroxymethyl)piperidin-2-yl)methyl)phosphoric ((2Z,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl phosphoric) Anhydride (**20**).* A round-bottomed flask containing lipid phosphate **18** (17 mg, 0.051 mmol) was dried in vacuo for 1 h. Anhydrous DMF (1 mL) was then added to the flask followed by the addition of CDI (32.8 mg, 0.203 mmol). The resulting solution was kept stirring for 3 h. MeOH (30 μL) was added to quench the extra CDI for 1.5 h. The reaction residue was then transferred to a vial containing compound **15** (5.3 mg, 0.016 mmol) followed by the addition of tetrazole solution in dry DMF (10 mg/mL, 0.041 mmol). The reaction was kept stirring for 48 h. The mixture was transferred to a round-bottomed flask, and the vial was washed with MeOH (1 mL) three times. The solvent was removed in vacuo and purified by silica gel chromatography ($CHCl_3$/MeOH/NH_4OH 7:3:0.5 to MeOH/H_2O 1:1). The collected fractions containing product with 90% purity were concentrated and further purified by HPLC (20 mM NH_4HCO_3/MeCN) to afford compound **20** (6.7 mg, 70%) as a white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.47 (td, J = 7.1, 1.0 Hz, lipid-C═CHCH$_2$O, 1H), 5.25−5.15 (m, 2 × C═CH, 2H), 4.50 (m, lipid-C═CHCH_2O, 2H), 4.25−4.20 (m, 1H, 1-Ha), 4.08 (dt, J = 10.9, 3.8 Hz, 1H, 1-Hb), 4.01 (dd, J = 10.9, 6.1 Hz, 1H, 3-H), 3.90 (dd, J = 11.6, 2.9 Hz, 1H, 7-Ha), 3.85 (dd, J = 10.8, 9.0 Hz, 1H, 4-H), 3.60 (dd, J = 11.7, 6.6 Hz, 1H, 7-Hb), 3.38 (m, 1H, 2-H), 3.27 (t, J = 9.5 Hz, 1H, 5-H), 3.12−3.07 (m, 1H, 6-H), 2.20−2.09 (m, 3 × lipid-CH_2, 6H), 2.07 (s, NHAc, 3H), 2.04 (m, lipid-CH_2, 2H), 1.73 (s, lipid-CH_3, 3H), 1.70 (s, lipid-CH_3, 3H), 1.64 (s, 2 × lipid-CH_3, 6H). ^{13}C NMR (151 MHz, D_2O) δ 174.5 (C═O), 160.2 (NH_4HCO_3), 143.0, 136.6, 133.4 (lipid CH_3CH_2C═CH), 124.4, 124.2 (2 X C═CH), 119.4, 119.3 (lipid-C═CHCH$_2$O), 72.6 (C-5), 72.0 (C-4), 64.0, 63.9 (C-1), 63.0, 63.03 (lipid-C═CHCH$_2$O), 61.8 (C-7), 55.1 (C-6), 53.2, 53.1 (C-2), 52.8 (C-3), 38.7, 38.7, 25.7, 25.5 (4C, lipid-CH_2), 24.8 (lipid-CH_3), 21.9 (NHCOCH_3), 16.9 (lipid-CH_3), 15.6 (lipid-CH_3), 15.2 (lipid-CH_3). ^{31}P NMR (202 MHz, D_2O) δ −10.91 (d, J = 21.7 Hz), −11.51 (d, J = 21.8 Hz). HRMS (ESI-TOF) calcd for $C_{24}H_{44}N_2O_{11}P_2$ $(M - H)^-$, 597.2337; found, 597.2337.

*(((2S,3S,4R,5R,6R)-3-Acetamido-4,5-dihydroxy-6-(hydroxymethyl)piperidin-2-yl)methyl)phosphonic((2Z,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl phosphoric) Anhydride (**21**).* A round-bottomed flask containing lipid phosphate **18** (13.6 mg, 0.041 mmol) was dried in vacuo for 1 h. Anhydrous DMF (1 mL) was then added to the flask followed by the addition of CDI (26.2 mg, 0.163 mmol). The resulting solution was kept stirring for 3 h. MeOH (25 μL) was added to quench the extra CDI for 1.5 h. The reaction residue was then transferred to a vial containing compound **2** (4.1 mg, 0.014 mmol). The reaction was completed when the reaction mixture turned from cloudy to clear after 48 h of stirring. The mixture was transferred to a round-bottomed flask, and the vial was washed with MeOH (1 mL) three times. The solvent was removed in vacuo and purified by silica gel chromatography ($CHCl_3$/MeOH/NH_4OH 7:3:0.5 to MeOH/H_2O 1:1). The collected fractions containing product with 90% purity were concentrated and further purified by HPLC (20 mM NH_4HCO_3/MeCN) to afford compound **20** (5.0 mg, 62%) as a white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.47 (brt, J = 6.3 Hz, lipid-C═CHCH$_2$O,1H), 5.26−5.17 (m, 2 × C═CH, 2H), 4.48 (t, J = 6.8 Hz, lipid-C═CHCH_2O, 2H), 3.99 (brd, J = 7.1 Hz, 1H, 3-H), 3.93 (dd, J = 12.0, 2.8 Hz, 1H, 7-H_a), 3.63 (m, 3H, 7-H_b, 2-H, 4-H), 3.32 (t, J = 9.1 Hz, 1H, 5-H), 3.04 (brs, 1H, 6-H), 2.21−1.92 (m, lipid-CH_2, NHAc, 1-H, 13H), 1.74 (s, lipid-CH_3, 3H), 1.70 (s, lipid-CH_3, 3H), 1.64 (s, lipid-CH_3, 6H). ^{13}C NMR (151 MHz, D_2O) δ 174.3 (C═O), 160.2 (NH_4HCO_3), 143.1, 136.6, 133.5 (lipid-CH_3C═CH), 124.4, 124.2 (2 × C═CH), 119.4, 119.3 (lipid-C═CHCH$_2$O), 72.4 (C-5), 71.1 (C-4), 62.9, 62.8 (lipid-C═CHCH$_2$O), 61.7 (C-7), 54.1 (C-6), 53.3 (C-3), 50.3, 50.3 (C-2), 38.7, 38.7, 25.7, 25.5 (4C,

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lipid-CH_2), 24.8 (lipid-CH_3), 24.5, 23.6 (C-1), 22.0 ($NHCOCH_3$), 16.9 (lipid-CH_3), 15.6 (lipid-CH_3), 15.2 (lipid-CH_3). ^{31}P NMR (202 MHz, D_2O) δ 14.27 (d, J = 23.7 Hz), −10.71 (d, J = 26.3 Hz). HRMS (ESI-TOF) calcd for $C_{24}H_{44}N_2O_{10}P_2$ $(M - H)^-$, 581.2387; found, 581.2373.

(((2S,3S,4R,5R,6R)-3-Acetamido-4,5-dihydroxy-6-(hydroxymethyl)piperidin-2-yl)methyl)phosphonic((2Z,6E,10E)-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraen-1-yl phosphoric) Anhydride (1). A round-bottomed flask containing lipid phosphate **19** (13.3 mg, 0.036 mmol) was dried in vacuo for 1 h. Anhydrous DMF (1 mL) was then added to the flask followed by the addition of CDI (23.3 mg, 0.144 mmol). The resulting solution was kept stirring for 3 h. MeOH (25 μL) was added to quench the extra CDI for 1.5 h. The reaction residue was then transferred to a vial containing compound **2** (3.6 mg, 0.012 mmol). The reaction was completed when the reaction mixture turned from cloudy to clear after 48 h of stirring. The mixture was transferred to a round-bottomed flask, and the vial was washed with MeOH (1 mL) three times. The solvent was removed in vacuo and purified by silica gel chromatography ($CHCl_3$/MeOH/NH_4OH 7:3:0.5 to MeOH/H_2O 1:1). The collected fractions containing product with 90% purity were concentrated and further purified by HPLC (20 mM NH_4HCO_3/MeCN) to afford compound **1** (4.3 mg, 56%) as a white solid after lyophilization. ^1H NMR (600 MHz, D_2O) δ 5.38 (m, lipid-C=C*H*CH_2O, 1H), 5.13 (m, 2 × C=C*H*, 3H), 4.39 (t, J = 6.6 Hz, lipid-C=CHC*H*$_2$O, 2H), 3.88−3.83 (m, 2H, 3-H and 7-H_a), 3.52−3.47 (m, 3H, 7-H_b, 2-H, 4-H), 3.19 (t, J = 9.3 Hz, 1H, 5-H), 2.90 (m, 1H, 6-H), 2.12−1.80 (m, 17H, lipid-CH_2, NHAc, 1-H), 1.66 (s, 3H, lipid-CH_3), 1.62 (s, 3H, lipid-CH_3), 1.55 (m, 9H, lipid-CH_3). ^{13}C NMR (151 MHz, D_2O) δ 174.3 (C=O), 160.3 NH_4HCO_3), 143.1, 136.6, 136.4, 133.4 (lipid-CH_3C=CH), 124.5, 124.4, 124.3 (3 × C=CH), 119.4, 119.3 (lipid-C=*C*HCH$_2$O), 72.9 (C-5), 71.3 (C-4), 62.8, 62.8 (lipid-C=CH*C*H$_2$O), 62.2 (C-7), 54.0 (C-6), 53.8, 53.7 (C-3), 50.3 (C-2), 38.8, 38.7, 25.6, 25.6, 25.5 (6C, lipid-CH_2), 24.8 (lipid-CH_3), 24.7, 23.8 (C-1), 22.0 ($NHCOCH_3$), 16.9 (lipid-CH_3), 15.6 (lipid-CH_3), 15.2 (lipid-CH_3), 15.2 (lipid-CH_3). ^{31}P NMR (202 MHz, D_2O) δ 14.66 (brs), −10.74 (d, J = 26.5 Hz). HRMS (ESI-TOF) calcd for $C_{29}H_{52}N_2O_{10}P_2$ $(M - H)^-$, 649.3013; found, 649.3040.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^1H NMR, ^{13}C NMR, and ^{31}P 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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