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Iminosugar Analogues of Phosphatidyl Inositol as Potential Inhibitors of Protein Kinase B (Akt)

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A small virtual library of iminosugar derivatives was evaluated by docking experiments carried out by sampling a protein region corresponding to the phosphoinositide binding site of the PH domain of Akt. Four compounds were selected and efficiently synthesised from a common precursor. All compounds were subjected to preliminary biological evaluation on purified enzyme, and – among them – compound **9** exhibited the best inhibitory activity.

Introduction

Protein Kinase B (Akt) is a protooncogenic serine/threonine kinase involved in the PI3K/Akt transduction pathway. Its activation promotes cellular proliferation, growth and survival. Its hyperactivation and overexpression is a common event in many human cancers,[1,2] and this activation results in tumor cell survival, resistance to apoptosis, and promotion of tumor formation.[3] Selective inhibitors of such enzymes represent, therefore, interesting lead compounds for the development of new anticancer drugs. Akt activation[4] occurs by the binding of phosphatidylinositol diphosphate [PI(3,4)P2] (**1**) with a specific protein domain, the so-called pleckstrin homology domain (PH). Recently,[2,5] phosphatidylinositol ether lipid analogues with 3–30 μM IC_{50} inhibitory activities have been synthesized, and some of them presented good selectivity towards Akt. Among them, the carbonate derivative **2**[6] (Figure 1) (5.0 ± 1.9 μM), although less potent than the corresponding phosphate, is much more selective, and therefore is used as a lead compound. According to modeling studies,[6] the axial hydroxymethyl group in position 3 of the inositol ring of compound **2** has a strong hydrogen-bonding interaction with the Arg25 residue of the Akt PH domain that seems to be important for the selectivity. The corresponding compound with an equatorial hydroxymethyl group showed a sixfold reduction in Akt inhibition activity.

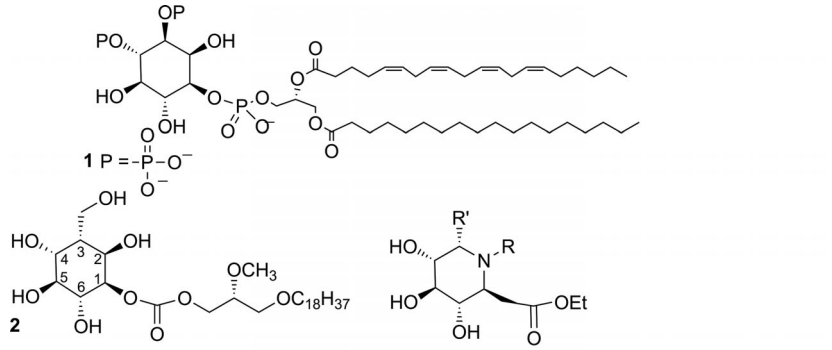

Figure 1. PH domain substrate (**1**), inhibitor (**2**) and members of the iminosugar-based virtual library (**3**–**13**).

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Previous X-ray crystallographic investigations showed that inositol-1,3,4,5-tetrakis(phosphate) (IP4) in the phosphoinositide binding site of the PH domain of Akt is positioned with the 2-hydroxy group oriented inside the binding cleft, and that this is also the orientation of [PI(3,4)P2] and [PI(3,4,5)P3] in the binding pocket.[2]

Moreover, a network of hydrogen bonds in the binding pocket involves the three phosphate groups linked to the equatorial 1-, 3- and 4-hydroxy groups of the inositol ring, as well as the axial hydroxy group in position 2.[7] These molecular features of the phosphoinositide binding site of the PH domain of Akt have been already exploited in previous studies to design and test Akt inhibitors.[3,6,7] Our analysis of the X-ray structure of the PH domain of Akt reveals also that, close to the polar pocket discussed above, a hydrophobic cleft including residues Ile74, Val83, Ile84 is present, suggesting that this hydrophobic cleft might be potentially exploited to design novel Akt inhibitors.

Results and Discussion

In light of previous considerations, and with the aim of generating a new class of inhibitors targeting the phosphoinositide binding site of the Akt PH domain, we have initially built and tested, using docking calculations, a small virtual library of iminosugar-based phosphatidyl inositol analogues. The library (Figure 1) was designed to include molecules **3**–**8**, **10**, which feature the axial hydroxymethyl group at C3 of inhibitor **2**, which seems crucial for the selectivity towards Akt. Moreover, we also designed compounds **9**, **11**–**13** with an axial carboxy group, which should better interact with Arg25, which in previous studies was shown to be one of the key protein residues involved in the interaction with phosphatidylinositol phosphate ligands. In order to possibly exploit the hydrophobic characteristics of the cleft close to the natural ligand binding site (including residues Ile74, Val83, Ile84, see above), the C2 carbon atom of inhibitor **2** was substituted with a ring nitrogen atom in order to allow the chemoselective introduction of hydrophobic substituents in this position. Thus, derivatives **4**–**13**, bearing a substituted ring nitrogen atom with different alkyl or carbamate groups, were considered. Finally, we planned to substitute the labile phosphate of the natural substrate by the more stable carboxymethyl group. Since the phosphatidyl moiety is not involved in the interaction with the PH domain, but is required in vivo to anchor the substrate to the cell membrane, we substituted the lipophilic moiety with a simple ethyl ester so to avoid water solubility problems in the in vitro biological test. In light of the above considerations, we carried out initial docking calculations on the series of compound members of the library.

In the first series of docking experiments carried out sampling a protein region corresponding to the phosphoinositide binding site (hereafter referred to as BOX1), it turned out that not all compounds were predicted to bind to the same protein region (see Figure 2). In particular, molecules **3**, **5**, **6** and **7** (blue in Figure 1) were found close to the box boundary. In order to avoid possible artifacts due to boundary effects, we carried out also a second set of docking experiments, in which the docking box was shifted and centered on the protein region where molecules **3**, **5**, **6** and **7** were docked (hereafter referred to as BOX2; see Experimental Section).

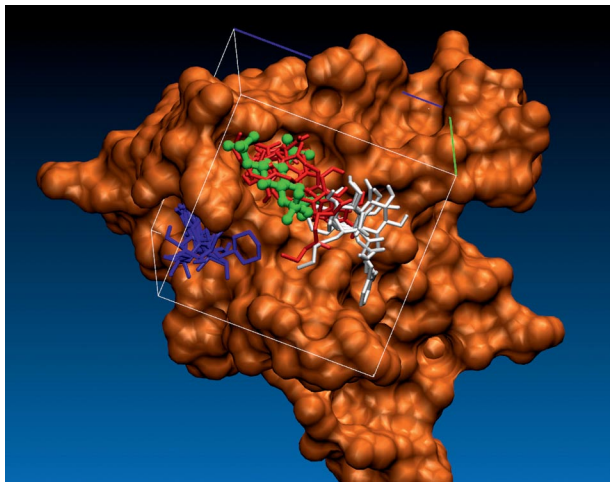


Figure 2. Docking experiments sampling a protein region corresponding to the phosphoinositide binding site (BOX1); inositol-1,3,4,5-tetrakis(phosphate) (green); ligands **3**, **5**, **6**, **7** (blue); ligands **4**, **8**, **10** (white); ligands **9**, **11**, **12**, **13** (red).

Results from docking calculations carried out on BOX1 and BOX2 are collected in Table 1.

Table 1. Docking energies [kcal/mol] computed for compounds **3**–**13** sampling the protein regions labeled as BOX1 (left) and BOX2 (right).

Ligand	BOX1		BOX2	
	LBE[a]	MBE[b]	LBE[a]	MBE[b]
3	–4.39	–3.86	–5.45	–4.57
4	–3.68	–2.43	–4.81	–3.75
5	–3.25	–3.25	–6.16	–5.00
6	–2.86	–2.30	–4.87	–3.97
7	–3.19	–2.16	–4.88	–3.83
8	–2.84	–2.39	–5.30	–4.46
9	–6.25	–5.56	–6.25	–6.08
10	–4.31	–3.24	–5.52	–4.13
11	–4.40	–3.78	–5.64	–4.75
12	–6.94	–5.67	–7.57	–6.24
13	–7.36	–6.67	–7.05	–6.21

[a] Lowest binding energy. [b] Mean binding energy; MBE is computed by using poses within 2 Å of the LBE pose.

According to the computational results, compounds **5**, **9**, **12** and **13** were predicted to have the highest affinity for the protein, followed by compounds **3**, **8**, **10** and **11**.

In docking experiments sampling the protein region labeled as BOX1 (Figure 2), molecules **9**, **11**, **12** and **13** were found to bind in the same pocket that hosts the IP4 ligand (referred to as pocket 1 hereafter). Compounds **4**, **8** and **10** were predicted to bind in a pocket partially overlapping the binding site of the natural ligand (referred to as pocket 2), whereas, as discussed above, molecules **3**, **5**, **6**, and **7** were predicted to bind in a novel pocket not superimposed on the phosphoinositide binding site (pocket 3). When docking

calculations were carried out sampling the protein portion labeled as BOX2, all ligands were found to bind to the same pocket, which essentially corresponded to pocket 3 of the BOX1 series of calculations (see Figure 3).

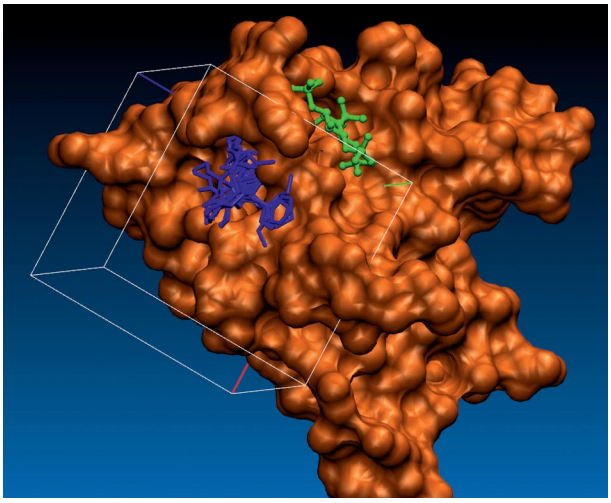
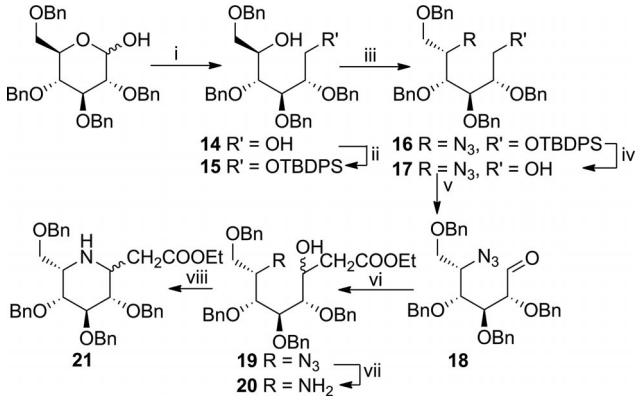


Figure 3. Docking experiments sampling a protein region corresponding to BOX2; ligands **3**, **4**, **5**, **6**, **7**, **8**, **9** (blue); inositol-1,3,4,5-tetrakis(phosphate) (green).

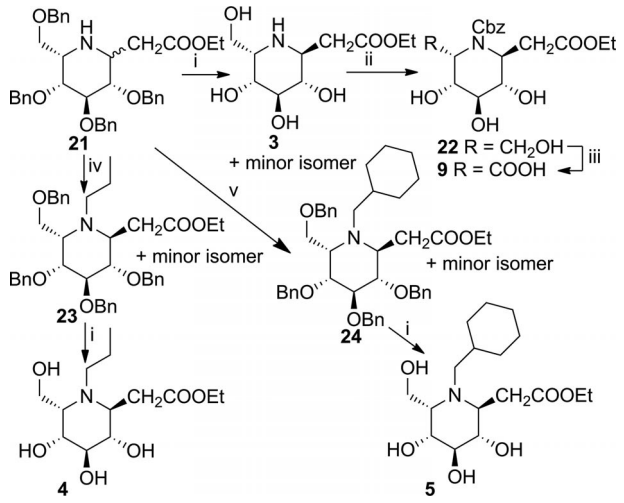
Notably, pocket 3 is sufficiently close to the natural ligand binding site to suggest that the interaction between the tested ligands and the protein might indirectly affect binding of the natural ligands. All molecules except molecule **13** show better docking scores for the BOX2 binding site. However, molecules **9**, **12** and **13** show very similar affinity for both BOX1 and BOX2 sites.

In the light of the docking results, four molecules (**3**, **4**, **5**, **9**), which are predicted to bind to the three different binding pockets, have been selected and synthesized. A preliminary in vitro biological inhibition assay has also been carried out.

Compounds **5** and **9** were selected from the group with the highest affinity for the protein and also as representatives of the group of compounds that bind pockets 3 and 1, respectively. Compound **3** was also selected to evaluate pocket 3, with the second highest affinity in the group of compounds binding to this pocket. Compound **4** was selected to evaluate the biological activity of the group of compounds that bound to pocket 2 in the computational experiments. The reason for this choice is the lowest binding affinity of **4** that resulted from the experiment in BOX 2, which corresponds to the binding to pocket 3. Therefore, compound **4** has the lowest probability of binding to pocket 3, which raises the probability of binding to pocket 2. Even though the highest affinities were obtained by compounds **12** and **13**, the difficulty of purchasing commercially available starting materials for their synthesis hampered their preparation. The synthesis was designed in order to obtain all the target compounds from a common precursor (Schemes 1 and 2), which could be differentially functionalized at the ring nitrogen atom and selectively oxidized at the primary OH group. Commercially available tetrabenzylglucopyranose was reduced to the diol **14**, which was protected at the primary hydroxy group. In order to obtain the correct stereochemistry at C6 of the target molecules, inversion of configuration was achieved through a Mitzunobu reaction with concomitant introduction of the azido function, precursor of the ring nitrogen atom (compound **16**). Removal of the TBDPS group and oxidation of the free OH group afforded aldehyde **18**. The introduction of the carboxymethyl moiety was done by exploiting a nucleophilic attack of AcOEt-derived enolate on aldehyde **18**. This reaction afforded azido alcohol **19** as an inseparable diastereoisomeric mixture in an approximate (*R*)/(*S*) ratio



Scheme 1. Reagents and conditions: (i) $NaBH_4$, CH_2Cl_2/EtOH (1:1), quantitative; (ii) imidazole, TBDPSiCl, CH_2Cl_2, 97%; (iii) PPh_3, DIAD, DPPA, THF, 75%; (iv) TBAF (1 M), THF, 90%; (v) Dess–Martin periodinane, CH_2Cl_2, 79%; (vi) DIPA, *t*BuLi (1 M), 0 °C, AcOEt, THF, –78 °C, 95%; (vii) Lindlar catalyst/C, H_2, AcOEt, 86%; (viii) PPh_3, DIAD, THF, 0 °C to room temp., 70%.



Scheme 2. Reagents and conditions: (i) $Pd(OH)_2$/C, CH_3COOH, H_2, MeOH, 67% for **3**, quantitative for **4** and **5**; (ii) CbzCl, $NaHCO_3$, MeOH, 25%; (iii) TEMPO, KBr, NaOCl 5%, H_2O, 84%; (iv) propionaldehyde, CH_3COOH, Na_2SO_4, $Na(AcO)_3BH$, 1,2-dichloroethane, 52%; (v) cyclohexanecarbaldehyde, CH_3COOH, Na_2SO_4, $Na(AcO)_3BH$, 1,2-dichloroethane, 79%.

of 2:1, determined by NMR spectroscopy on the final cyclized products. Reduction of the azido function and cyclization through an intramolecular Mitzunobu reaction led to the desired iminosugar scaffold **21**.

The first member of the library was obtained by deprotection of **21** through a catalytic hydrogenation [$Pd(OH)_2$/C, H_2]. Target compound **3** was efficiently separated by flash chromatography from the minor isomer. Compound **9** was obtained directly from pure compound **3**. First the ring nitrogen atom was functionalized as a carboxybenzylcarbamate, and then the primary hydroxy group was selectively oxidized to the corresponding carboxylic acid, affording compound **9** in 21% overall yield. The synthesis of derivatives **4** and **5** was carried out by reductive amination on precursor **21** with propionaldehyde and cyclohexanecarbaldehyde, respectively, followed by catalytic hydrogenation. This time the diastereoisomeric mixtures were efficiently separated before the deprotection step. The stereochemistry at C2 was determined on the deprotected compounds. As an illustrative example, we report the NMR values of the coupling constants of compound **3** (major isomer) (Figure 4). The values ($J_{3,2}$ = 8.7, $J_{4,3}$ = 8.7, Hz) were indicative of a *trans*-diaxial disposition of the protons, which indicated a $^4C_1$ conformation; moreover, the diaxial disposition of C(2)–H/C(3)–H allowed us to determine the absolute (*S*) configuration of the C2 center.

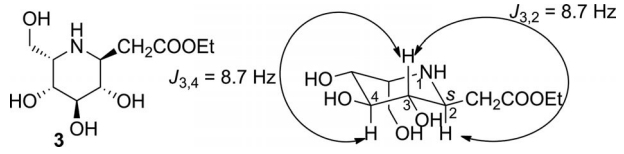


Figure 4. NMR values of the coupling constants of compound **3**.

Preliminary biological evaluations were performed through a safe, rapid and reliable method for screening of inhibitors of Akt: a solid-phase enzyme-linked immunosorbent assay (ELISA) that utilizes a synthetic peptide as a substrate for Akt and a polyclonal antibody that recognizes the phosphorylated form of the substrate. All the tested compounds (**3**, **4**, **5** and **9**) exhibited a significant inhibitory effect on the activity of the purified enzyme when assayed at 700 μM (Table 2). Compound **9** showed the best inhibitory activity, in accordance to the calculated binding energy obtained by docking experiments carried out in BOX1, which contains the phosphoinositide binding site. This activity may be ascribed to the presence of the carboxy group, probably involved in favourable interactions with basic residues present in the binding pocket. The hydrophobic group present on the ring nitrogen atom may also play a role in the interaction. For instance, compound **3**, which lacks this group, showed the lowest activity. Compounds **4** and **5** both present a hydrophobic group; in this case the slightly higher activity observed for compound **4** may be due to the more flexible propyl group, which may better fit the hydrophobic cleft. Moreover, from docking calculations, compounds **3** and **5** are predicted to bind the protein region named pocket 3, close to the phosphoinositide binding site; thus, their activity may be a consequence of the modification of the phosphoinositide binding pocket following their binding.

Table 2. Inhibition of Akt activity.

Ligand	Inhibition [%]
3	14.87 ± 0.133
4	25.67 ± 0.882
5	20.00 ± 0.577
9	31.70 ± 1.670
2[a]	75.46 ± 1.059

[a] Known inhibitor.[6]

Conclusions

In our search for Akt-selective inhibitors targeting the PH domain, we developed a unique iminosugar scaffold from which a variety of inhibitors have been synthesised by chemoselective derivatisation of the ring nitrogen atom and oxidation of the primary hydroxy group. Further members of the library can be chemoselectively generated by modification of the ester group and derivatization of the primary hydroxy group. The introduction of a lipophilic appendage at the ring nitrogen atom allowed us to decipher the role of the hydrophobic cleft close to the natural ligand binding site revealed by our analysis of the X-ray structure of the PH domain of Akt. Preliminary biological evaluation showed inhibitory activities that justify the choice of compound **9** as a lead compound for further development of iminosugar-based Akt inhibitors.

Experimental Section

Docking Calculations: The X-ray structure of the human Akt complex was obtained from the Protein Data Bank (code 1H10). Three-dimensional structures of ligands were generated by molecular mechanics optimization using the MMFF94 force field.[8] Docking calculations were carried out by using AutoDock 4.2.[9] The torsional degrees of freedom of the ligands were explicitly considered, whereas the protein structure was kept frozen to X-ray atomic coordinates. Two boxes were used: BOX1, whose center is fixed on coordinates X = 16.677, Y = 25.705, Z = 13.116 (centered in proximity of the natural ligand binding site); BOX2, was centered on coordinates X = 27.627, Y = 19.412, Z = 19.899 (centered in a contiguous site of the natural ligand binding site), and its dimension was set to $20.25 \times 21.75 \times 21$ Å^3. Atomic charges were calculated within AutoDock 4.2 by using the Gasteiger-Marsili algorithm.[10] The search for best poses was carried out with a Lamarckian genetic algorithm by using the following parameters: run, 100; population size, 300; maximum number of evaluations, 11000000. Two-dimensional representations of the best protein–ligand adducts were obtained by using MOE Ligand Interactions Structure-Based Design Tool.[11] In all in silico experiments, we used the PDB code 1H10 structure that contains the Akt PH domain fragment alone. The site identified with BOX2 sampling might be not accessible to ligands in vivo, since in the whole protein structure it could not be excluded that the binding site located by docking experiments is buried. However, we identified another protein with a PH domain showing a high sequence similarity to the Akt PH domain (data

not shown), in which the region corresponding to BOX2 is accessible. While our analysis was underway, a new X-ray structure of human Akt1 complexed with an allosteric inhibitor was uploaded in the Protein Data Bank (code 3O96). The structure of the newly reported PH domain is essentially superimposable with the structure used in our calculations.

Synthesis

General Remarks: All solvents were dried with molecular sieves for at least 24 h prior to use. Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} plates (Merck) with UV light detection when possible, or by charring with a solution of concd. H_2SO_4/EtOH/H_2O (10:45:45) or a solution of $(NH_4)_6Mo_7O_{24}$ (21 g), $Ce(SO_4)_2$ (1 g), concd. H_2SO_4 (31 mL) in water (500 mL). Flash column chromatography was performed on silica gel 230–400 mesh (Merck). ^1H and ^{13}C NMR spectra were recorded at 25 °C with a Varian Mercury 400 MHz instrument by using $CDCl_3$ as the solvent unless otherwise stated. Chemical shift assignments, reported in ppm, were referenced to the corresponding solvent peaks. HR mass spectra were recorded with a QSTAR elite LC/MS/MS system with a nanospray ion source. Optical rotations were measured at room temperature by using an Atago Polax-2 L polarimeter and are reported in units of 10^{-1} deg cm^2 g^{-1}.

2,3,4,6-Tetra-*O*-benzyl-D-glucitol (14): $NaBH_4$ (420 mg, 11.09 mmol) was added to a stirred solution of 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose (2.00 g, 3.70 mmol) in CH_2Cl_2/EtOH (1:1) (20 mL). After 48 h, the solvent was evaporated in vacuo, and the solid was treated with Na_2CO_3 (10 mL of satd. solution), with stirring, for 10 min. The reaction mixture was diluted with CH_2Cl_2, washed with distilled water, dried with Na_2SO_4 and filtered. The solvent was evaporated in vacuo to afford compound **14** (2.01 g, quantitative) as a colorless syrup. $[\alpha]_D^{20}$ = +3.1 (*c* = 1.2, $CHCl_3$). ^1H NMR (400 MHz): δ = 3.60 (dd, *J* = 11.9, 4.8 Hz, 1 H, 1a-H), 3.64–3.72 (m, 2 H, 6a,b-H), 3.77 (dd, *J* = 11.9, 4.3 Hz, 1 H, 1b-H), 3.80–3.86 (m, 2 H, 2-H, 4-H), 3.94 (dd, *J* = 6.2, 3.6 Hz, 1 H, 3-H), 4.06–4.10 (m, 1 H, 5-H), 4.54 (d, *J* = 11.9 Hz, 1 H, C*H*Ph), 4.57–4.59 (m, 2 H, C*H*Ph), 4.63 (d, *J* = 11.4 Hz, 1 H, C*H*Ph), 4.66 (d, *J* = 11.5 Hz, 1 H, C*H*Ph), 4.69 (d, *J* = 11.2 Hz, 1 H, C*H*Ph), 4.70 (d, *J* = 11.5 Hz, 1 H, C*H*Ph), 4.75 (d, *J* = 11.2 Hz, 1 H, C*H*Ph), 7.20–7.40 (m, 20 H, Ar-H) ppm. ^{13}C NMR (100 MHz): δ = 62.2 [C(1)], 71.1 [C(5)], 71.5 [C(6)], 73.4, 73.6, 73.8, 74.9 (4 *C*H_2Ph), 77.7, 79.4, 79.8 [C(2), C(3), C(4)], 128.0–128.7 (*C*HAr), 138.0, 138.1, 138.2, 138.4 (*C*qAr) ppm. HRMS: calcd. for $C_{34}H_{39}O_6$ $[M + H]^+$ 543.2741; found 543.2957.

2,3,4,6-Tetra-*O*-benzyl-1-*O*-*tert*-butyldiphenylsilyl-D-glucitol (15): Compound **14** (2.00 g, 3.68 mmol) was dissolved in dry CH_2Cl_2 (12 mL) under argon. Imidazole (550 mg, 8.10 mmol) and TBDPSiCl (1.04 mL, 4.05 mmol) were added. After 1 h, the reaction mixture was quenched with methanol (0.5 mL), diluted with H_2O, and extracted with CH_2Cl_2. The organic layer was dried with Na_2SO_4, filtered, and the solvent was evaporated in vacuo. Purification by flash chromatography (petroleum ether/ethyl acetate, 8:2) afforded pure compound **15** (2.79 g, 97% yield). $[\alpha]_D^{25}$ = +12.8 (*c* = 1.0, $CHCl_3$). ^1H NMR (400 MHz): δ = 1.08 [s, 9 H, $C(CH_3)_3$], 2.97 (d, *J* = 4.9 Hz, 1 H, OH), 3.62–3.64 (m, 2 H, 6a,b-H), 3.76–4.03 (m, 6 H, 1a,b-H, 2-H, 3-H, 4-H, 5-H), 4.48–4.59 (m, 5 H, 5 C*H*Ph), 4.64–4.70 (m, 3 H, 3 C*H*Ph), 7.20–7.80 (m, 30 H, Ar-H) ppm. ^{13}C NMR (100 MHz): δ = 19.7 [$C(CH_3)_3$], 27.3 [$C(CH_3)_3$], 63.5, 71.6 [C(1), C(6)], 71.4 [C(5)], 73.4, 73.6, 73.7, 74.6 (4 *C*H_2Ph), 77.8, 78.3, 79.9 [C(2), C(3), C(4)], 127.8–129.9 (*C*HAr), 133.5, 133.6 (*C*qAr), 135.8 (*C*HAr), 138.3, 138.3, 138.4, 138.6 (4 *C*qAr) ppm. HRMS: calcd. for $C_{50}H_{57}O_6Si$ $[M + H]^+$ 781.3919; found 781.3900.

5-Azido-2,3,4,6-tetra-*O*-benzyl-1-*O*-*tert*-butyldiphenylsilyl-5-deoxy-L-iditol (16): Compound **15** (5.28 g, 6.76 mmol) was dissolved in dry THF (20 mL) under argon. Triphenylphosphane (5.32 g, 20.30 mmol) was added. The reaction mixture was cooled to 0 °C, and DIAD (3.93 mL, 20.30 mmol) was added dropwise, resulting in a yellow precipitate. Diphenylphosphoryl azide (4.68 mL, 21.64 mmol) was added. After 2 h, the solvent was evaporated in vacuo. Purification by flash chromatography (petroleum ether/ethyl acetate, 9:1) afforded pure compound **16** (4.10 g, 75.2% yield). $[\alpha]_D^{25}$ = –1.7 (*c* = 1.2, $CHCl_3$). ^1H NMR (400 MHz): δ = 1.07 [s, 9 H, $C(CH_3)_3$], 3.23–3.26 (m, 1 H, 5-H), 3.34 (dd, *J* = 9.5, 4.8 Hz, 1 H, 6a-H), 3.54–3.58 (m, 2 H, 2-H, 6b-H), 3.82 (dd, *J* = 7.8, 3.0 Hz, 1 H, 4-H), 3.86–3.91 (m, 2 H, 1a,b-H), 4.04 (dd, *J* = 7.8, 2.8 Hz, 1 H, 3-H), 4.31 (d, *J* = 11.8 Hz, 1 H, C*H*Ph), 4.38 (d, *J* = 11.9 Hz, 1 H, C*H*Ph), 4.39 (d, *J* = 11.9 Hz, 1 H, C*H*Ph), 4.54 (d, *J* = 11.5 Hz, 1 H, C*H*Ph), 4.62 (d, *J* = 11.8 Hz, 1 H, C*H*Ph), 4.66 (d, *J* = 11.2 Hz, 1 H, C*H*Ph), 4.76 (d, *J* = 11.2 Hz, 1 H, C*H*Ph), 4.78 (d, *J* = 11.5 Hz, 1 H, C*H*Ph), 7.20–7.80 (m, 30 H, Ar-H) ppm. ^{13}C NMR (100 MHz): δ = 19.6 [$C(CH_3)_3$], 27.3 [$C(CH_3)_3$], 61.6 [C(5)], 62.6, 70.2 [C(1)], 72.7, 73.5, 75.2, 75.6 (4 *C*H_2Ph), 78.1 [C(2)], 78.6 [C(4)], 79.3 [C(3)], 127.8–130.1 (*C*HAr), 133.40 (*C*qAr), 135.8–135.9 (*C*HAr), 138.0, 138.1, 138.3, 138.4 (4 *C*qAr) ppm. HRMS: calcd. for $C_{50}H_{56}N_3O_5Si$ $[M + H]^+$ 806.3984; found 806.3798.

5-Azido-2,3,4,6-tetra-*O*-benzyl-5-deoxy-L-iditol (17): Compound **16** (4.10 g, 5.09 mmol) was dissolved in dry THF (15 mL) under argon. A solution of TBAF (1 M, 15.26 mL, 15.26 mmol) was added slowly. After 3 h, the solvent was evaporated in vacuo. Purification by flash chromatography (petroleum ether/ethyl acetate, 8:2) afforded pure compound **17** (2.61 g, 90.4% yield). $[\alpha]_D^{25}$ = +11.0 (*c* = 1.1, $CHCl_3$). ^1H NMR (400 MHz): δ = 3.40 (dd, *J* = 9.4, 4.8 Hz, 1 H, 6a-H), 3.47–3.55 (m, 3 H, 2-H, 5-H, 6b-H), 3.56–3.61 (m, 1 H, 1a-H), 3.67–3.73 (m, 1 H, 1b-H), 3.77–3.83 (m, 2 H, 3-H, 4-H), 4.32 (d, *J* = 11.8 Hz, 1 H, C*H*Ph), 4.36 (d, *J* = 11.8 Hz, 1 H, C*H*Ph), 4.49 (d, *J* = 11.5 Hz, 2 H, 2 C*H*Ph), 4.54 (d, *J* = 11.5 Hz, 1 H, C*H*Ph), 4.56 (d, *J* = 11.2 Hz, 1 H, C*H*Ph), 4.62 (d, *J* = 11.2 Hz, 1 H, C*H*Ph), 4.69 (d, *J* = 11.5 Hz, 1 H, C*H*Ph), 7.15–7.30 (m, 20 H, Ar-H) ppm. ^{13}C NMR (100 MHz): δ = 61.4 [C(5)], 61.7 [C(1)], 69.8 [C(6)], 72.7, 73.6, 75.0, 75.1 (4 *C*H_2Ph), 78.2, 78.3, 79.2 [C(2), C(3), C(4)], 127.9–128.7 (*C*HAr), 137.8, 137.9, 137.9, 138.0 (4 *C*qAr) ppm. HRMS: calcd. for $C_{34}H_{38}N_3O_5$ $[M + H]^+$ 568.2806; found 568.2789.

5-Azido-2,3,4,6-tetra-*O*-benzyl-5-deoxy-L-idose (18): Compound **17** (2.61 g, 4.60 mmol) was dissolved in CH_2Cl_2 (20 mL). Dess–Martin periodinane (2.93 g, 6.90 mmol) was added slowly. After 40 min, a 1:1 solution of satd. $NaHCO_3$/10% $Na_2S_2O_3$ (100 mL) was added. After stirring for 30 min, the reaction mixture was diluted with CH_2Cl_2 and then washed with water. The organic layer was dried with Na_2SO_4, filtered, and the solvent was evaporated in vacuo. Purification by flash chromatography (petroleum ether/ethyl acetate, 8:2) afforded pure aldehyde **18** (1.98 g, 79.1% yield), which was immediately used in the next reaction without further characterization, due to its low chemical stability.

Ethyl (3*R*/*S*,4*S*,5*S*,6*R*,7*R*)-7-Azido-4,5,6,8-tetrakis(benzyloxy)-3-hydroxyoctanoate (19): Diisopropylamine (4.94 mL, 34.99 mmol) was dissolved in dry THF (10 mL) under argon. At 0 °C, a solution of *tert*-butyllithium (1 M, 21.87 mL, 34.99 mmol) was added. After stirring for 0.5 h, the temperature was reduced to –78 °C, and dry ethyl acetate (3.42 mL, 34.99 mmol) was added. After stirring for 0.5 h, a solution of compound **18** (1.98 g, 3.50 mmol) in dry THF (5 mL) was added. After 1 h, the reaction mixture was treated with a satd. solution of NH_4Cl (30 mL), taken to room temperature, and

neutralized with 5% HCl. The reaction mixture was dissolved in CH_2Cl_2 and washed with water. The organic layer was dried with Na_2SO_4, filtered, and the solvent was evaporated in vacuo. Purification by flash chromatography (petroleum ether/ethyl acetate, 8:2) afforded compound **19** as a mixture of stereoisomers [2.18 g, 95.1% yield, (3*R*)/(3*S*) = 2:1]. **Major Isomer:** ^{1}H NMR (400 MHz): δ = 1.23 (m, 3 H, OCH_2CH_3), 2.49–2.56 (m, 1 H, 2a-H), 2.72 (dd, *J* = 16.0, 3.6 Hz, 1 H, 2b-H), 3.50–3.68 (m, 4 H, 4-H, 5-H, 6-H, 7-H), 3.96 (dd, *J* = 7.0, 3.0 Hz, 1 H, 8a-H), 4.00 (dd, *J* = 7.0, 3.6 Hz, 1 H, 8b-H), 4.09–4.18 (m, 2 H, CH_2CO_2Et), 4.26–4.32 (m, 1 H, 3-H), 4.40–4.49 (m, 2 H, CH_2Ph), 4.59–4.72 (m, 5 H, CHPh), 4.80 (d, *J* = 11.6 Hz, 1 H, CHPh), 7.23–7.40 (m, 20 H, Ar-H) ppm. ^{13}C NMR (100 MHz): δ = 14.4 (OCH_2CH_3), 38.7, 39.1 [C(2)], 60.8 (OCH_2CH_3), 61.3, 61.4 [C(7)], 67.5, 69.0 [C(3)], 69.6, 69.9, 73.2, 73.3, 73.4, 74.1, 74.5, 74.7, 74.8, 75.0 [CH_2Ph, C(8)], 77.8, 78.1, 78.1, 78.9, 79.0, 79.7 [C(4), C(5), C(6)], 127.9–128.7 (CHAr), 137.6, 137.7, 137.8, 137.9, 138.0, 138.1, 138.2 (CqAr), 172.2, 172.6 [C(1)] ppm. HRMS: calcd. for $C_{38}H_{44}N_3O_7$ [M + H]$^+$ 654.3174; found 654.3748.

Ethyl (3*R*/*S*,4*S*,5*S*,6*R*,7*R*)-7-Amino-4,5,6,8-tetrakis(benzyloxy)-3-hydroxyoctanoate (20): Compound **19** (240 mg, 0.36 mmol) was dissolved in ethyl acetate (3 mL). A catalytic amount of Lindlar catalyst was added, and the reaction mixture was stirred under H_2 overnight. The catalyst was filtered through a Celite pad (eluent ethyl acetate), and the solvent was evaporated in vacuo. Purification by flash chromatography (petroleum ether/ethyl acetate, 3:7) afforded compound **20** as a mixture of stereoisomers [190 mg, 85.6% yield, (3*R*)/(3*S*) = 2:1). **Major Isomer:** ^{1}H NMR (400 MHz): δ = 1.20–1.30 (m, 3 H, OCH_2CH_3), 2.49–2.60 (m, 1 H, 2a-H), 2.75 (dd, *J* = 16.0, 3.7 Hz, 1 H, 2b-H), 3.12 (dt, *J* = 6.4, 2.4 Hz, 1 H, 7-H), 3.30–3.45 (m, 2 H, 8a,b-H), 3.61 (dd, *J* = 6.6, 3.6 Hz, 1 H, 4-H), 3.89 (dd, *J* = 7.6, 2.5 Hz, 1 H, 6-H), 4.04–4.15 (m, 3 H, OCH_2CH_3, 5-H), 4.28–4.36 (m, 1 H, 3-H), 4.03 (d, *J* = 11.9 Hz, 1 H, CHPh), 4.44 (d, *J* = 11.9 Hz, 1 H, CHPh), 4.51 (d, *J* = 11.2 Hz, 1 H, CHPh), 4.58 (d, *J* = 11.3 Hz, 1 H, CHPh), 4.64–4.84 (m, 4 H, CHPh), 7.20–7.40 (m, 20 H, Ar-H) ppm. ^{13}C NMR (100 MHz): δ = 14.1 (OCH_2CH_3), 38.5, 38.7 [C(2)], 51.4, 51.5 [C(7)], 60.4 (OCH_2CH_3), 67.7, 68.8 [C(3)], 72.9, 73.4, 74.1, 74.3, 74.4, 74.5, 74.6 [CHPh, C(8)], 78.6, 78.9, 79.1, 79.4, 79.8, 80.1 [C(4), C(5), C(6)], 127.7, 128.6 (CHAr), 138.1, 138.2, 138.3, 138.5, 138.6 (CqAr), 172.2 [C(1)] ppm. HRMS: calcd. for $C_{38}H_{46}NO_7$ [M + H]$^+$ 628.3269; found 628.3371.

Ethyl {(2*S*/*R*,3*S*,4*R*,5*R*,6*S*)-3,4,5-Tris(benzyloxy)-6-[(benzyloxy)-methyl]piperidin-2-yl}acetate (21): Compound **20** (170 mg, 0.27 mmol) was dissolved in dry THF (1 mL) under argon. The reaction mixture was chilled to 0 °C, and triphenylphosphane (140 mg, 0.54 mmol) was added. DIAD (0.10 mL, 0.54 mmol) was added dropwise, and the reaction mixture was stirred overnight. The solvent was removed in vacuo, and purification by flash chromatography (petroleum ether/ethyl acetate, 7:3) afforded compound **21** as a mixture of stereoisomers [0.12 g, 70.4% yield, (2*S*)/(2*R*) = 2:1]. **Major Isomer:** ^{1}H NMR (400 MHz): δ = 1.21–1.24 (m, 3 H, OCH_2CH_3), 2.32 (dd, *J* = 15.5, 8.1 Hz, 1 H, $CHCO_2Et$), 2.82 (dd, *J* = 15.5, 4.3 Hz, 1 H, $CHCO_2Et$), 3.20–3.28 (m, 2 H, 2-H, 3-H), 3.49–3.58 (m, 1 H, CHOBn), 3.62–3.68 (m, 2 H, 4-H, CHOBn), 3.77 (bt, *J* = 9.9 Hz, 1 H, 5-H), 3.79 (dd, *J* = 9.4, 5.9 Hz, 1 H, 6-H), 4.01–4.15 (m, 2 H, OCH_2CH_3), 4.52–4.69 (m, 5 H, CHPh), 4.74 (d, *J* = 10.8 Hz, 1 H, CHPh), 4.91 (d, *J* = 11.4 Hz, 1 H, CHPh), 4.94 (d, *J* = 11.1 Hz, 1 H, CHPh), 7.20–7.40 (m, 20 H, Ar-H) ppm. ^{13}C NMR (100 MHz): δ = 14.4 (OCH_2CH_3), 29.9 (CH_2CO_2Et), 50.7, 54.3 [C(2), C(6)], 60.8, 65.6 (OCH_2CH_3, CH_2OBn), 73.0, 73.8, 75.5, 75.7 (CH_2Ph), 80.4, 82.2, 83.5 [C(3), C(4), C(5)], 127.7–128.8 (CHAr), 138.3, 138.4, 138.4, 138.8 (CqAr), 172.2 (C=O) ppm. HRMS: *m*/*z* = calcd. for $C_{38}H_{44}NO_6$ [M + H]$^+$ 610.3163; found 610.4024.

Ethyl [(2*S*,3*S*,4*R*,5*R*,6*S*)-3,4,5-Trihydroxy-6-(hydroxymethyl)piperidin-2-yl]acetate (3): The diastereoisomeric mixture **21** (26.4 mg, 0.043 mmol) was dissolved in methanol (2 mL). Catalytic amounts of $Pd(OH)_2$ and acetic acid (0.1 mL) were added, and the reaction mixture was stirred under H_2 overnight. The catalyst was filtered through a Celite pad (eluent methanol), and the solvent was evaporated in vacuo. Purification by flash chromatography (ethyl acetate/methanol, 8:2) afforded pure compound **3** as the major isomer (7.2 mg, 66.7% yield). $[\alpha]_D^{25}$ = –30.1 (*c* = 0.7, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ = 1.26 (t, *J* = 7.1 Hz, 3 H, OCH_2CH_3), 2.36 (dd, *J* = 16.3, 8.8 Hz, 1 H, $CHCO_2Et$), 2.91 (dd, *J* = 16.3, 3.3 Hz, 1 H, $CHCO_2Et$), 3.10 (t, *J* = 8.7 Hz, 1 H, 3-H), 3.17 (ddd, *J* = 9.0, 8.9, 3.4 Hz, 1 H, 2-H), 3.20–3.27 (m, 1 H, 6-H), 3.42 (t, *J* = 8.7 Hz, 1 H, 4-H), 3.70 (dd, *J* = 9.2, 5.4 Hz, 1 H, 5-H), 3–75–3.80 (m, 2 H, 7a,b-H), 4.12–4.19 (m, 2 H, OCH_2CH_3) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 14.5 (OCH_2CH_3), 38.2 (CH_2CO_2Et), 52.1, 59.0, 73.3, 76.0, 76.3 [C(2), C(3), C(4), C(5), C(6)], 58.1, 61.8, (OCH_2CH_3, CH_2OH), 174.0 (C=O) ppm. HRMS: calcd. for $C_{10}H_{20}NO_6$ [M + H]$^+$ 250.1285; found 250.1246.

Ethyl {(2*S*,3*S*,4*R*,5*R*,6*S*)-3,4,5-Tris(benzyloxy)-6-[(benzyloxy)methyl]-1-propylpiperidin-2-yl}acetate (23): The mixture of isomers **21** (115.4 mg, 0.19 mmol) was dissolved in dry 1,2-dichloroethane (5 mL), then propionaldehyde (41 μL, 0.57 mmol) and acetic acid (0.108 mL, 1.89 mmol) were added. The reaction mixture was dried with Na_2SO_4 and stirred for 0.5 h. $Na(AcO)_3BH$ (160.4 mg, 0.76 mmol) was added, and the mixture was stirred overnight. The reaction mixture was neutralized with a satd. solution of $NaHCO_3$, dissolved in CH_2Cl_2, and washed with water. The organic layer was dried with Na_2SO_4, filtered, and the solvent was evaporated in vacuo. Purification by flash chromatography (petroleum ether/ethyl acetate, 9:1) afforded pure compound **23** (64.0 mg, 51.9% yield) as the major isomer. $[\alpha]_D^{25}$ = –38.6 (*c* = 1.4, $CHCl_3$). ^{1}H NMR (400 MHz): δ = 0.83 [t, *J* = 7.3 Hz, 3 H, $N(CH_2)_2CH_3$], 1.17 (t, *J* = 7.1 Hz, 3 H, OCH_2CH_3), 1.37–1.49 (m, 2 H, $NCH_2CH_2CH_3$), 2.40–2.53 (m, 2 H, $CHCO_2Et$, $NCHCH_2CH_3$), 2.62 (ddd, *J* = 12.6, 8.6, 6.2 Hz, 1 H, $NCHCH_2CH_3$), 2.73 (dd, *J* = 15.1, 3.9 Hz, 1 H, $CHCO_2Et$), 3.32–3.44 (m, 3 H, 2-H, 3-H, 6-H), 3.66–3.74 (m, 1 H, CHOBn), 3.74–3.81 (m, 2 H, CHOBn, 4-H), 3.84 (dd, *J* = 10.1, 3.4 Hz, 1 H, 5-H), 3.97–4.10 (m, 2 H, OCH_2CH_3), 4.50–4.78 (m, 6 H, 6 CHPh), 4.89–4.98 (m, 2 H, 2 CHPh), 7.20–7–43 (m, 20 H, Ar-H) ppm. ^{13}C NMR (100 MHz): δ = 11.90 [$N(CH_2)_2CH_3$], 14.42 (OCH_2CH_3), 22.52 ($NCH_2CH_2CH_3$), 36.15 (CH_2CO_2Et), 49.99 ($NCH_2CH_2CH_3$), 56.75, 57.93 [C(2), C(6)], 60.76, 67.84 (OCH_2CH_3, CH_2OBn), 72.98, 73.65, 75.00, 75.50 (4 CH_2Ph), 79.08, 80.94, 84.37 [C(3), C(4), C(5)], 127.6–129.6 (CHAr),138.45, 138.45, 139.05, 139.05 (CqAr), 172.25 (C=O) ppm. HRMS: calcd. for $C_{41}H_{50}NO_6$ [M + H]$^+$ 652.3416; found 652.3633.

Ethyl [(2*S*,3*S*,4*R*,5*R*,6*S*)-3,4,5-Trihydroxy-6-(hydroxymethyl)-1-propylpiperidin-2-yl]acetate (4): Compound **23** (64.0 mg, 0.098 mmol) was dissolved in ethyl acetate (2 mL) and methanol (4 mL). Catalytic amounts of $Pd(OH)_2$ and acetic acid (0.1 mL) were added, and the reaction mixture was stirred under H_2 overnight. The catalyst was filtered through a Celite pad (eluent methanol), and the solvent was evaporated in vacuo to afford pure compound **4** (29.2 mg, quant.). $[\alpha]_D^{25}$ = +2.1 (*c* = 0.3, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ = 0.84 [t, *J* = 7.4 Hz, 3 H, $N(CH_2)_2CH_3$], 1.25 (t, *J* = 7.1 Hz, 3 H, OCH_2CH_3), 1.30–1.47 (m, 2 H, $NCH_2CH_2CH_3$), 2.42–2.54 (m, 2 H, $CHCO_2Et$, $NCHCH_2CH_3$), 2.66–2.77 (m, 1 H, $NCHCH_2CH_3$), 2.86 (dd, *J* = 16.4, 4.2 Hz, 1 H, $CHCO_2Et$), 3.10–3.21 (m, 2 H, 2-H, 6-H), 3.24 (t, *J* = 9.6 Hz,

1 H, 3-H), 3.39 (t, J = 9.0 Hz, 1 H, 4-H), 3.73 (d, J = 7.4 Hz, 2 H, CH_2OH), 3.82 (dd, J = 9.5, 6.2 Hz, 1 H, 5-H), 4.13 (q, J = 7.2 Hz, 2 H, OCH_2CH$_3$) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 11.62, 14.50 [N(CH$_2$)$_2$CH_3, OCH$_2$CH_3], 24.06 (NCH$_2$CH_2CH$_3$), 34.98 (CH_2CO$_2$Et), 49.67, 57.58, 61.75 (NCH_2CH$_2$CH$_3$, OCH_2CH$_3$, CH_2OH), 55.76, 62.15, 70.44, 72.66, 77.57 [C(2), C(3), C(4), C(5), C(6)], 174.31 (C=O) ppm. HRMS: calcd. for $C_{13}H_{26}NO_6$ [M + H]$^+$ 292.1755; found 292.1720.

Ethyl {(2*S*,3*S*,4*R*,5*R*,6*S*)-3,4,5-Tris(benzyloxy)-6-[(benzyloxy)methyl]-1-(cyclohexylmethylene)piperidin-2-yl}acetate (24): Compound **21** (86.5 mg, 0.142 mmol) was dissolved in dry dichloroethane (4 mL), then cyclohexanecarbaldehyde (0.051 mL, 0.426 mmol) and acetic acid (0.081 mL, 1.42 mmol) were added. The reaction mixture was dried with Na_2SO_4 and stirred for 0.5 h. Na(OAc)$_3$BH (120.2 mg, 0.567 mmol) was added, and the mixture was stirred overnight. The reaction mixture was neutralized with a satd. solution of $NaHCO_3$, dissolved in CH_2Cl_2, and washed with water. The organic layer was dried with Na_2SO_4, filtered, and the solvent was evaporated in vacuo. Purification by flash chromatography (petroleum ether/ethyl acetate, 9:1) afforded pure compound **24** (72.3 mg, 79.4% yield). $[\alpha]_D^{25}$ = –18.3 (c = 1.0, $CHCl_3$). ^{1}H NMR (400 MHz): δ = 0.63–0.82 [m, 2 H, 2 CH(Cy)], 1.15 (t, J = 7.1 Hz, 3 H, OCH$_2$CH_3), 1.06–1.40 [m, 3 H, 3 CH(Cy)], 1.58–1.80 [m, 6 H, 6 CH(Cy)], 2.21–2.32 (m, 1 H, NCHCy), 2.35–2.51 (m, 2 H, CHCO$_2$Et, NCHCy), 2.69 (dd, J = 15.5, 4.7 Hz, 1 H, CHCO$_2$Et), 3.22–3.30 (m, 1 H, 6-H), 3.31–3.38 (m, 1 H, 3-H), 3.38–3.49 (m, 1 H, 2-H), 3.67–3.75 (m, 1 H, 4-H), 3.75–3.82 (m, 2 H, 5-H, 7a-H), 3.85 (dd, J = 10.1, 3.4 Hz, 1 H, 7b-H), 3.91–4.00 (m, 1 H, OCHCH$_3$), 4.00–4.09 (m, 1 H, OCHCH$_3$), 4.54–4.63 (m, 3 H, 3 CHPh), 4.64–4.71 (m, 2 H, 2 CHPh), 4.73 (d, J = 10.8 Hz, 1 H, CHPh), 4.93 (d, J = 10.9 Hz, 2 H, 2 CHPh), 7.24–7.37 (m, 20 H, Ar-H) ppm. ^{13}C NMR (100 MHz): δ = 14.27 (OCH$_2$CH_3), 26.19, 26.26, 27.00, 31.29, 31.39 [5 CH_2(Cy)], 37.00 [CH(Cy)], 36.03 (CH_2CO$_2$Et), 54.51, 60.47, 67.35 (OCH_2CH$_3$, NCH_2Cy, CH_2OBn), 56.26, 58.69 [C(2), C(6)], 78.41, 80.85, 84.38 [C(3), C(4), C(5)], 72.84, 73.35, 74.89, 75.45 (4 OCH_2Ph), 127.4–128.5 (CHAr), 138.5, 138.6, 138.9, 138.9 (4 CqAr), 172.2 (C=O) ppm. HRMS: calcd. for $C_{45}H_{56}NO_6$ [M + H]$^+$ 706.4102; found 706.4029.

Ethyl [(2*S*,3*S*,4*R*,5*R*,6*S*)-1-(Cyclohexylmethylene)-3,4,5-trihydroxy-6-(hydroxymethyl)piperidin-2-yl]acetate (5): Compound **24** (72.3 mg, 0.113 mmol) was submitted to catalytic hydrogenation according to the same procedure used for the synthesis of compound **3**, affording pure compound **5** (40.0 mg, 100% yield). $[\alpha]_D^{25}$ = –38.0 (c = 4.1, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ = 0.66–0.79 [m, 1 H, CH(Cy)], 0.80–0.92 [m, 1 H, CH(Cy)], 1.11–1.24 [m, 2 H, 2 CH(Cy)], 1.26 (t, J = 7.1 Hz, 3 H, OCH$_2$CH_3), 1.28–1.43 [m, 2 H, 2 CH(Cy)], 1.62–1.78 [m, 6 H, 6 CH(Cy)], 2.36 (dd, J = 13.5, 6.1 Hz, 1 H, NCHCy), 2.49 (dd, J = 17.1, 10.5 Hz, 1 H, CHCO$_2$Et), 2.57 (dd, J = 13.5, 8.1 Hz, 1 H, NCHCy), 2.87 (dd, J = 17.1, 3.7 Hz, 1 H, CHCO$_2$Et), 3.14–3.23 (m, 2 H, 2-H, 6-H), 3.27 (dd, J = 10.5, 8.5 Hz, 1 H, 3-H), 3.41 (t, J = 9.0 Hz, 1 H, 4-H), 3.74–3.80 (m, 2 H, CH_2OH), 3.85 (dd, J = 9.5, 6.1 Hz, 1 H, 5-H), 4.14 (q, J = 7.1 Hz, 2 H, OCH_2CH$_3$) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 17.31 (OCH$_2$CH_3), 29.84, 30.04, 30.58, 34.81, 35.24, 37.09 [5 CH_2(Cy), CH_2CO$_2$Et], 41.51 [CH(Cy)], 57.69, 60.62, 64.59 (CH_2OH, NCH_2Cy, OCH_2CH$_3$), 58.26, 65.78, 72.96, 74.88, 80.31 [C(2), C(3), C(4), C(5), C(6)], 177.12 (C=O) ppm. HRMS: calcd. for $C_{17}H_{32}NO_6$ [M + H]$^+$ 346.2224; found 346.2165.

Ethyl [(2*S*,3*S*,4*R*,5*R*,6*S*)-1-(Benzyloxycarbonyl)-3,4,5-trihydroxy-6-(hydroxymethyl)piperidin-2-yl]acetate (22): To a solution of compound **3** (27.3 mg, 0.109 mmol) in methanol (2 mL), benzyl chloroformate (0.047 mL, 0.329 mmol) and $NaHCO_3$ (18.4 mg, 0.219 mmol) were added. After stirring overnight, the solvent was evaporated in vacuo. Purification by flash chromatography (ethyl acetate) afforded pure compound **22** (10.5 mg, 25.1% yield). $[\alpha]_D^{25}$ = +83.3 (c = 0.1, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ = 1.19 (t, J = 7.1 Hz, 3 H, OCH$_2$CH_3), 2.88 (dd, J = 16.8, 5.9 Hz, 1 H, CHCO$_2$Et), 3.12 (dd, J = 16.4, 8.5 Hz, 1 H, CHCO$_2$Et), 3.39 (dd, J = 7.8, 6.7 Hz, 1 H, 3-H), 3.56 (dd, J = 8.2, 6.7 Hz, 1 H, 4-H), 3.67 (dd, J = 8.3, 6.1 Hz, 1 H, 5-H), 3.86–4.00 (m, 3 H, 2-H, CH_2OH), 4.01–4.11 (m, 2 H, OCH_2CH$_3$), 4.35–4.46 (m, 1 H, 6-H), 5.03–5.13 (m, 2 H, CH_2Ph), 7.26–7.45 (m, 5 H, Ar-H) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 14.43 (OCH$_2$CH_3), 36.15 (CH_2CO$_2$Et), 54.88, 59.46, 72.48, 74.17, 76.81 [C(2), C(3), C(4), C(5), C(6)], 58.94, 61.76, 68.31 (CH_2OH, OCH_2CH$_3$, OCH_2Ph), 128.96–129.53 (CHAr), 137.86 (CqAr), 157.88 (CO_2Bn), 174.09 (CO_2Et) ppm. HRMS: calcd. for $C_{18}H_{26}NO_8$ [M + H]$^+$ 384.1653; found 384.1774.

Ethyl [(2*S*,3*S*,4*R*,5*R*,6*S*)-1-(Benzyloxycarbonyl)-6-carboxy-3,4,5-trihydroxypiperidin-2-yl]acetate (9): To a solution of compound **22** (10.5 mg, 0.027 mmol) in water (1.5 mL), TEMPO (0.27 mg, 0.00175 mmol) and KBr (9.8 mg, 0.0822 mmol) were added. The reaction mixture was cooled to 0 °C, and 5% NaOCl (0.54 mL, 0.363 mmol) was added. After stirring for 4 h, methanol (1 mL) was added, the reaction mixture was acidified with HCl (5%), and the solvent was removed in vacuo. The white solid was suspended in ethanol, the suspension was filtered, and the filtrate was concentrated in vacuo. Purification by flash chromatography (ethyl acetate/methanol, 8:2) afforded pure compound **9** (9.7 mg, 84.4% yield). $[\alpha]_D^{25}$ = +83.3 (c = 0.1, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ = 1.20 (t, J = 7.1 Hz, 3 H, OCH$_2$CH_3), 2.66–2.87 (m, 2 H, CH_2CO$_2$Et), 3.52–3.65 (m, 2 H, 3-H, 4-H), 3.75–3.87 (m, 1 H, 5-H), 4.06 (dd, J = 13.6, 6.6 Hz, 2 H, OCH_2CH$_3$), 4.19–4.39 (m, 1 H, 2-H), 4.56 (d, J = 5.8 Hz, 1 H, 6-H), 5.10 (m, 2 H, OCH_2Ph), 7.23–7.41 (m, 5 H, HAr) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 14.45 (OCH$_2$CH_3), 38.33 (CH_2CO$_2$Et), 56.73, 61.13, 72.50, 75.49, 78.26 [C(2), C(3), C(4), C(5), C(6)], 61.69, 68.50 (OCH_2CH$_3$, OCH_2Ph), 128.8–129.5 (CHAr), 135.8 (CqAr), 149.8, 157.3, 173.8 (CO_2 H, CO_2Bn, CO_2Et) ppm. HRMS: calcd. for $C_{18}H_{23}NO_9Na$ [M + Na]$^+$ 420.1265; found 420.1181.

Akt Inhibition Assay: The Akt kinase activity assay was performed by employing a commercial ELISA kit designed specifically to screen inhibitors or activators of Akt/PKB, purchased from Assay Designs (Ann Arbor, MI 48108, USA; Cat. No. EKS-400A). Briefly, the substrate, which is readily phosphorylated by purified Akt, is precoated at 2.5 μg per well on the wells of the microtiter plate. The samples to be assayed are added. Specifically, 30 μL of each of the following was added to appropriate wells: blank (kinase assay dilution buffer), negative control [inhibitor diluent alone (distilled water)], samples (inhibitors at 700 μM), positive control at 50 μM. This was followed by the addition of ATP to initiate the reaction, which was terminated 90 min later. An antibody, which binds specifically to the phosphorylated substrate, was added to the wells and incubated for 60 min. After washing, this antibody was subsequently bound by a peroxidase-conjugated secondary antibody, which was added to the wells for 30 min. The assay was developed with tetramethylbenzidine (TMB) substrate, and a colour developed in proportion to the Akt phosphotransferase activity. The colour development was stopped with acid stop solution, and the intensity of the colour was measured in a microplate reader at 450 nm. As positive control of the test, a known Akt inhibitor [1L6-hydroxymethyl-chiro-inositol-2-(*R*)-2-*O*-methyl-3-*O*-octadecyl-*sn*-glycerocarbonate, compound **2** (Figure 1), purchased from Calbiochem (San Diego, CA, USA; Cat. No. 124005)] was used.

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