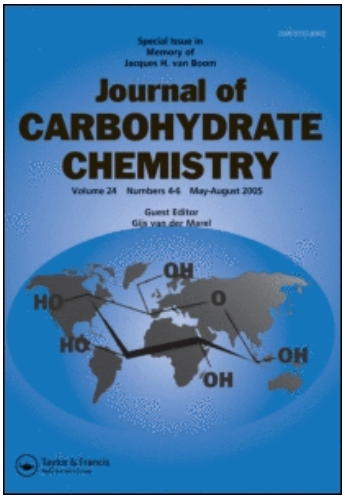

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Synthesis of (-)-Isofagomine

Irma Panfil[a]; Jolanta Solecka[b]; Marek Chmielewski[a]
[a] Institute of Organic Chemistry of the Polish Academy of Sciences, Warsaw, Poland [b] National Institute of Hygiene, Warsaw, Poland

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Synthesis of (−)-Isofagomine

Irma Panfil

Institute of Organic Chemistry of the Polish Academy of Sciences, Warsaw, Poland

Jolanta Solecka

National Institute of Hygiene, Warsaw, Poland

Marek Chmielewski

Institute of Organic Chemistry of the Polish Academy of Sciences, Warsaw, Poland

1,3-Dipolar cycloaddition of *N*-benzyl nitrone **2** to *D-threo* δ-lactone **15** proceeded with excellent stereoselectivity to provide only one adduct **16**. Cycloadduct **16** was subsequently subjected to a sequence of reactions involving rearrangement to γ-lactone, glycolic cleavage/reduction, protection of the terminal hydroxymethyl group, reduction of the lactone, desilylation/mesylation, and hydrogenolysis of the N-O bond providing (−)-isofagomine and its *N*-substituted derivatives. The biologic activity of *N*-substituted (−)-isofagomines toward commercially available α- and β-glucosidases, α-D-mannosidase, α-L-fucosidase, β-D-glucuronidase, and β-D-galactosidase was tested.

Keywords (−)-Isofagomine, Iminosugars, 1,3-Dipolar cycloaddition, Nitrones, Unsaturated aldonolactones

INTRODUCTION

Glycosidases and glycosyl transferases play an important role as carbohydrate-modifying enzymes. Carbohydrate mimics, such as iminosugars, carbasugars, or *C*-glycosides, display properties as specific and competitive inhibitors of these enzymes.[1] Consequently, they display many interesting biologic and therapeutic activities such as antiviral, anticancer, antidiabetic, and antihyperglycemic.[1–7] The discovery of isofagomine (**1**) by a Danish group[8] as one of the most potent β-glucosidase inhibitors has propelled, during the past years, a growing interest of many laboratories in the synthesis of iminosugars with nitrogen at the anomeric position.[9–21]

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Address correspondence to Marek Chmielewski, Institute of Organic Chemistry of the Polish Academy of Sciences, 01-224, Warsaw, Poland. E-mail: chmiel@icho.edu.pl

Recently we reported on 1,3-dipolar cycloaddition of *N*-benzyl nitrone **2** to *D-glycero* γ-lactone **3**, *L-erythro* δ-lactone **4**, and *D-threo* δ-lactone **5**.[22] The cycloaddition performed with **4** afforded two stereoisomers **7** and **8** in a ratio of about 2.5:1. Cycloaddition of the same nitrone **2** to lactones **3** and **5** yielded, in each case as a sole product, **6** and **9**, respectively (Chart 1).

Cycloadducts **6**–**9** were subsequently subjected to a sequence of reactions involving hydrogenolysis of the N-O bond and intramolecular alkylation of the nitrogen atom by the C-4 or C-5 carbon atom of the sugar backbone to afford iminosugars **10**–**14** with a hydroxymethyl group at the C-2 carbon atom.[23] This strategy proceeded with inversion of configuration at the secondary carbon atom (C-4 or C-5) of the starting sugar. In addition, the interchange of the C-1 carbon atom by the aminomethyl group caused inversion of configuration (according to the carbohydrate nomenclature) at the tertiary carbon atom (C-2).[16]

RESULTS AND DISCUSSION

In all cases, the high preference for the *anti* addition of the nitrone to the terminal methyl or substituted methyl groups in lactones was observed.[22–25] Low stereoselectivity of cycloadditions of simple nitrones to *erythro* lactones

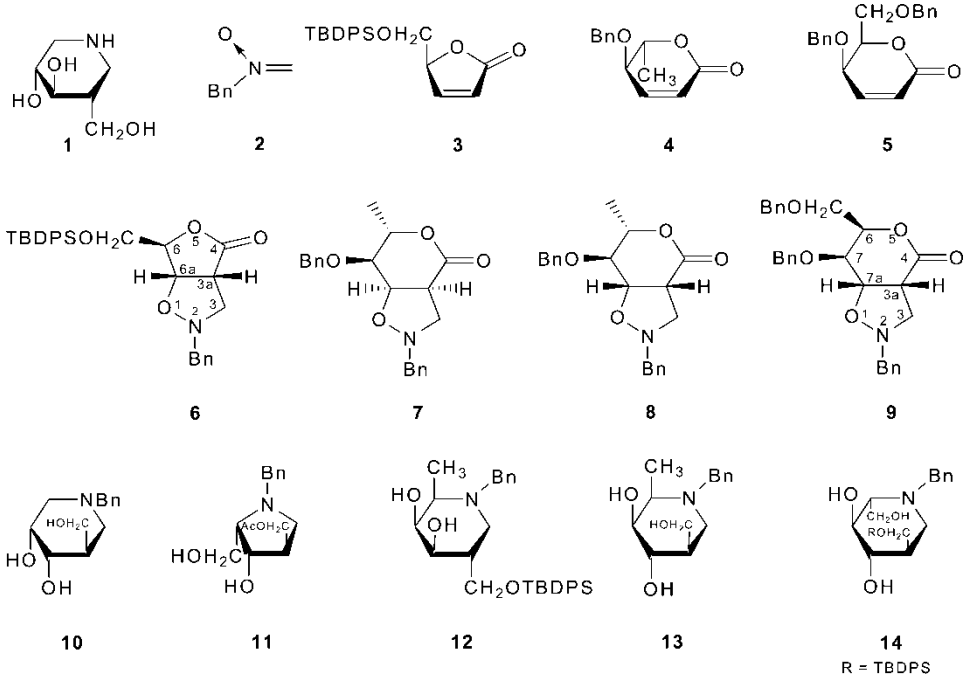
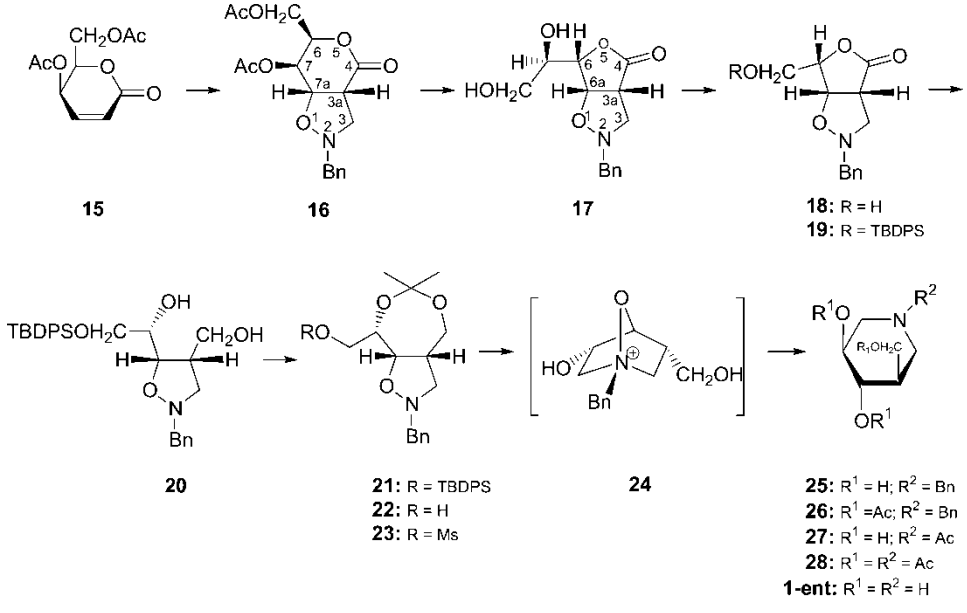


Chart 1

compared to *threo* congeners caused by the 4-*O*-benzyloxy group diminished the attractiveness of the former in the target-oriented synthesis. On the other hand, cycloadducts involving *threo* lactones provide a wide range of synthetic possibilities, particularly as the δ-lactone fragment can be rearranged into the γ one and the terminal carbon atom of the adduct can be easily removed (Scheme 1).

By a such strategy, (−)-isofagomine **1-ent**, the enantiomer of the natural compound **1**, can be synthesized. Adduct **16**, obtained by the cycloaddition of the acetylated lactone **15** and the nitrone **2**, was rearranged to the γ-lactone by deacetylation to afford diol **17**. Subsequent cleavage of the terminal diol in **17** followed by the reduction of the aldehyde group gave compound **18**, which was silylated yielding **19**.

Subsequently, compound **19** was reduced to the diol **20**, and both hydroxyl groups were protected by the isopropylidene residue and provided bicyclic compound **21**, which was subsequently desilylated to afford the alcohol **22**. The primary hydroxy group in **22** was mesylated to give compound **23**. Subsequent deprotection of hydroxy groups caused immediate intramolecular alkylation of the nitrogen atom. The crude ammonium salt **24** was subjected to hydrogenolysis of the N-O bond to provide iminosugar **25**, which was characterized as its triacetate **26**. The relative configuration of **26** was proven by trans diaxial coupling constants between H-2, H-3, H-4, H-5, and H-6 protons equal to 9.7, 8.9, 10.1, and 11.0 Hz. The hydrogenolysis of **26** followed by acetylation/deacetylation sequence provided (−)-*N*-acetyl-isofagomine (**28**). Deacetylation



Scheme 1

of **26** followed by hydrogenolysis of the *N*-benzyl substituent afforded (−)-isofagomine (**1-ent**). Compound **1-ent** and its *N*-benzyl derivative **25** have been synthesized before from 1-tert-butoxycarbonyl-1,2,3,6-tetrahydropyridyl-3-methanol[13] and (+)-L-tartaric acid (Scheme 1).[15b]

The biologic activity of compounds **1-ent, 25** and **28**, toward commercially available α- and β-glucosidases, α-D-mannosidase, α-L-fucosidase, β-D-glucuronidase, and β-D-galactosidase were tested. (−)-*N*-Acetyl-isofagomine (**28**) was found to be a better inhibitor of tested enzymes than (−)-isofagomine (**1-ent**). It showed moderate/weak activity against bovine kidney α-L-fucosidase (50% inhibition required 3.5 mM), bovine liver β-D-galactosidase (50% inhibition required at 6.6 mM), and bovine liver β-D-glucuronidase (50% inhibition required at 9.1 mM), and little or no activity against rice α-, almond β-D-glucosidases, and jack bean α-D-mannosidase. (−)-Isofagomine (**1-ent**) showed moderate/weak activity against α-L-fucosidase, β-D-galactosidase, and β-D-glucuronidase, and a little or no activity against α-D-glucosidases and α-D-mannosidase. (−)-*N*-Benzyl-isofagomine (**25**) showed only inhibition of α-L-fucosidase (50% inhibition at 5.1 mM).

CONCLUSION

It was demonstrated that 1,3-dipolar cycloaddition of the simple *N*-benzyl nitrone to unsaturated *threo* 1,5-lactone **15** proceeded exclusively *anti* to the substituents in the sugar. The adduct **16** after rearrangement to the γ-lactone 17 and removal of the terminal hydroxymethyl group could be the attractive substrate for the synthesis of enantiomer of natural isofagomine (**1-ent**). Examination of the glycosidases' inhibitory activity showed that (−)-*N*-acetyl-isofagomine (**28**) was a better inhibitor of tested enzymes than (−)-isofagomine (**1-ent**).

EXPERIMENTAL

Melting points were determined on a Koefler hot-stage apparatus. ^{1}H NMR spectra were recorded using Bruker Avance 500 and Varian Mercury 400 instruments. Absorptions of aromatic protons were not reported. IR spectra were recorded on a Perkin-Elmer FTIR Spectrum 200 spectrophotometer. Mass spectra were recorded using AMD-604 Inectra GmbH and HPLC-MS with Mariner and API 356 detectors. Optical rotations were measured using a JASCO P 3010 polarimeter at 22 ± 3°C. Column chromatography was performed using E. Merck Kiesel Gel (230–400 mesh).

(3a*R*, 6*R*, 7*R*, 7a*S*)-7-Acetoxy-6-acetoxymethyl-2-benzyl-4-oxo-tetrahydropyrano[3,4-d]isoxazolidine (16). Compound **16** was obtained according to the procedure described earlier.[22] Syrup; $[\alpha]_D + 44.2$ (*c* 1.5, CH_2Cl_2); IR

(film): 1748 cm^{-1}; ^{1}NMR (500 MHz, toluene-d_8, 100°C) δ: 5.09 (ddt, 1H, *J* 1.6, 3.3 Hz, H-7), 4.88 (dt, 1H, *J* 1.6, 6.3 Hz, H-6), 4.11 (dd, 1H, *J* 3.3, 8.0 Hz, H-7a), 4.11 (dd, 1H, *J* 6.3, 11.4 Hz, C*H*HOAc), 4.09 (dd, 1H, *J* 6.3, 11.4 Hz, CH*H*OAc), 3.69, 3.63 (2d, 2H, *J* 13.3 Hz, *N*Bn), 3.22 (dt, 1H, *J* 5.4, 8.0 Hz, H-3a), 2.89 (bs, 1H, H-2), 2.83 (bt, 1H, H-2′); HRMS (ESI) *m/z* $[M + Na]^+$, Calcd. for $C_{18}H_{21}NO_7Na$: 386.1210. Found: 386.1230.

(3a*R*, 6*R*, 6*aS*, 1′*R*)-2-Benzyl-6-(1′,2′-dihydroxyethyl)-4-oxo-isoxazolidino[4,5-c]tetrahydrofurane (17). Adduct **16** (0.36 g, 1 mmol) was dissolved in methanol (10 mL) containing 1.5% of ammonia and left at rt for 24 h. Subsequently, solvent was evaporated and the crude product was purified by chromatography using AcOEt as an eluent to afford **17** (0.26, 95%). m.p. 95–97°C; $[\alpha]_D + 25.2$ (*c* 0.5, CH_2Cl_2); IR (film): 3588, 1777 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.99 (bt, 1H, H-6a), 4.65 (dd, 1H, *J* 3.7, 6.5 Hz, H-6), 4.10, 3.89 (2d, 2H, *J* 12.4 Hz, *N*Bn), 4.06 (dd, 1H, *J* 5.1, 9.6 Hz, C*H*HOH), 3.74–3.56 (m, 4H, C*H*OH, CH*H*OH, H-3, H-3a), 2.82 (bm, 1H, H-3′); HRMS (LSIMS) *m/z* $[M + H]^+$, Calcd. for $C_{14}H_{18}NO_5$: 280.1185. Found: 280.1197.

(3a*R*, 6*R*, 6a*S*, 1′*R*)-6-(1′,2′-Diacetoxyethyl)-2-benzyl-4-oxo-isoxazolidino [4,5-c]tetrahydrofurane (17Ac). Syrup, $[\alpha]_D + 1.15$ (*c* 0.8, CH_2Cl_2); IR (film): 1779, 1744 cm^{-1}; ^{1}H NMR (500 MHz, toluene-d_8) δ: 5.50 (ddd, 1H, *J* 3.1, 4.2, 8.9 Hz, CHOAc), 4.22 (dd, 1H, *J* 3.1, 12.4 Hz, C*H*HOAc), 4.20 (dd, 1H, *J* 5.4, 7.1 Hz, H-6a), 4.16 (dd, 1H, *J* 5.4, 8.9 Hz, H-6), 4.10 (dd, 1H, *J* 4.2, 12.4 Hz, CH*H*OAc), 3.63, 3.58 (2d, 2H, *J* 13.2 Hz, *N*Bn), 3.17 (bd, 1H, *J* 9.3 Hz, H-3), 2.74 (t, 1H, *J* 7.1 Hz, H-3a), 2.33 (bt, 1H, H-3′) 1.76, 1.70 (2s, 6H, 2Ac); HRMS (ESI) *m/z* $[M + Na]^+$, Calcd. for $C_{18}H_{21}NO_7Na$: 386.1210. Found: 386.1232.

(3a*R*, 6*R*, 6a*R*)-2-Benzyl-6-hydroxymethyl-4-oxo-isoxazolidino[4,5-c] tetrahydrofurane (18). Compound **17** (0.36 g, 1.0 mmol) was dissolved in methanol/water 2:1 v/v (15 mL) and treated with sodium metaperiodate at rt (0.24 g, 1.1 mmol). After 1.5 h the reaction was completed. After evaporation of the solvent, water was added into the flask and the resulting solution was extracted with AcOEt. Subsequently, the extract was evaporated and the residue was dissolved in dry CH_2Cl_2(15 mL) and reduced with 1.5 molar equiv. of $NaBH(OAc)_3$(0.29 g). Standard workup provided the product, which was purified on a silica gel column using AcOEt as an eluent to afford **18** (0.16 g, 60%). Syrup; $[\alpha]_D + 31.0$ (*c* 0.84, CH_2Cl_2); IR (film): 3564, 1778 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.94 (bt, 1H, H-6a), 4.22 (dt, 1H, *J* 5.1, 5.8, 5.8 Hz, H-6), 3.99, 3.95 (2bd, 2H, *J* 12.9 Hz, Bn), 3.89 (dd, 1H *J* 4.7, 12.3 Hz, C*H*HOH), 3.83 (dd, 1H *J* 5.6, 12.3 Hz, CH*H*OH), 3.66–3.54 (m, 2H, H-3, H-3′), 2.80 (bs, 1H, H-3a); HRMS (EI) *m/z* M^+, Calcd. for $C_{13}H_{15}NO_4$: 249.1001. Found: 249.1007.

(3a*R*, 6*R*, 6a*R*)-6-Acetoxymethyl-2-benzyl-4-oxo-isoxazolidino[4,5-c] tetrahydrofurane (18Ac). m.p. 108–110°C; $[\alpha]_D - 5.14$ (*c* 1.0, CH_2Cl_2); IR

(film): 1778, 1741 cm^{-1}; ^1H NMR (500 MHz, toluene-d_8, 100°C) δ: 4.32 (dd, 1H, *J* 4.1, 12.2 Hz, CH*H*OAc), 4.24 (dd, 1H, *J* 7.6, 12.2 Hz, CH*H*OAc), 4.15 (ddd, 1H, *J* 4.1, 5.5, 7.6 Hz, H-6), 4.11 (dd, 1H, *J* 5.5, 7.2 Hz, H-6a), 3.66, 3.58 (2d, 2H, *J* 13.2 Hz, *N*Bn), 3.14 (bd, 1H, *J* 9.3 Hz, H-3), 2.71 (dt, 1H, *J* 1.6 7.2 Hz, H-3a), 2.31 (bt, 1H, H-3′), 1.69 (s, 3H, Ac); HRMS (LSIMS) *m/z* $[M+H]^+$, Calcd. for $C_{15}H_{18}NO_5$: 292.1185. Found: 292.1192.

(3a*R*, 6*R*, 6a*R*)-2-Benzyl-6-t-butyldiphenylsiloxymethyl-4-oxo-isoxazolidino[4,5-c]tetrahydrofurane (19). Compound **18** (0.25 g, 1 mmol) dissolved in CH_2Cl_2(15 mL) was treated with *t*-butyldiphenylchlorosilane (0.302, 1.1 mmol) and DMAP (1.1 mmol). The mixture was left overnight. Subsequently, the solvent was evaporated and the residue was purified by chromatography using hexane/AcOEt 4:1 v/v as an eluent to give **19** (0.35 g, 73%). Syrup, $[\alpha]_D + 26.3$ (*c* 0.9, CH_2Cl_2); IR (film): 1772 cm^{-1}; ^1H NMR (500 MHz, toluene-d_8, 100°C) δ: 4.22–4.16 (m, 2H, H-6, H-6a), 4.03 (dd, 1H, *J* 6.4, 11.1 Hz, C*H*HOTBDPS), 3.98 (dd, 1H, *J* 5.1, 11.1 Hz, CH*H*OTBDPS), 3.57, 3.54 (2d, 2H, *J* 13.3 Hz, *N*Bn), 3.13 (bd, 1H, *J* 9.3 Hz, H-3), 2.73 (dt, 1H, *J* 1.7, 7.0 Hz, H-3a), 2.33 (bt, 1H, H-3′), 1.14 (s, 9H, *t*-Bu); HRMS (ESI) *m/z* $[M+H]^+$, Calcd. for $C_{29}H_{33}NO_4Si$: 488.2252. Found: 488.2251.

(3a*R*, 8*R*, 8a*R*)-2-Benzyl-8-tert-butyldiphenylsiloxymethyl-6,6-dimethyl-5,7-dioxa-isoxazolidino[4,5]cycloheptane (21). Compound **18** (0.49 g, 1 mmol) was dissolved in dry THF and treated with $LiAlH_4$(0.04 g, 1 mmol). The mixture was stirred for 0.5 h and then, consecutively, AcOEt (1 mL) and water (1 mL) were added. Subsequently, the mixture was filtered through Celite and solvents were evaporated. The residue was dissolved in brine (5 mL) and extracted with AcOEt. The extract was dried and evaporated. The residue (crude **20**) was dissolved in dimethoxypropane (10 mL), treated with catalytic amount of *p*-TsOH, and refluxed for 2 h. Subsequently, the mixture was neutralized with Na_2CO_3 and evaporated. The crude product was purified by chromatography using hexane/AcOEt 7:3 v/v as an eluent to afford **21** (0.38 g, 71%). m.p. 84–86°C; $[\alpha]_D + 24.5$ (*c* 1.0, CH_2Cl_2); ^1H NMR (500 MHz, toluene-d_8, 100°C) δ: 4.17–4.12 (m, 2H, H-8, H-8a), 3.95 (d, 2H, CH_2OTBDPS), 3.87 (t, 1H, *J* 10.4, 11.9 Hz, H-4), 3.84, 3.82 (2d, 2H, *J* 13.1 Hz, *N*Bn), 3.20 (dd, 1H, *J* 5.2, 11.9 Hz, H-4′), 2.76 (bt, 1H, *J* 7.8, 9.1 Hz, H-3), 2.38 (m, 1H, H-3a), 2.19 (bdd, 1H, H-3′), 1.31, 1.29 (2s, 6H, $2CH_3$), 1.14 (s, 9H, *t*-Bu); HRMS (ESI) *m/z* $[M+H]^+$, Calcd. for $C_{32}H_{42}NO_4Si$: 532.2878. Found: 532.2860.

(3*S*,4*S*,5*S*) *N*-Benzyl-3,4-diacetoxy-5-(acetoxymethyl)piperidine [(−)-*N*-Benzyl-tri-*O*-acetyl-isofagomine] (26). Compound **21** (0.266 g, 0.5 mmol) was dissolved in THF (10 mL), treated with TBAF (0.16 g, 0.5 mmol), and stirred at rt for 3 h. The reaction mixture was evaporated and crude product was passed through a silica gel column using AcOEt as an

eluent. Subsequently, the solvent was evaporated and the product (**22**, 0.13 g, 90%) was dissolved in CH_2Cl_2(10 mL), treated with Et_3N (0.06 g) and mesyl chloride (0.06 g, 0.5 mmol), and left for 1 h at rt. The mixture was then washed with water and evaporated. The product was dissolved in methanol (10 mL) and acetic acid (2 mL) was added, the resulting solution was refluxed for 10 min and evaporated, and the residue acetylated with acetic anhydride/pyridine 1:1 mixture (2 mL). After standard workup and evaporation, the crude mixture was dissolved in methanol (15 mL), treated with K_2CO_3(20 mg), and hydrogenated using Degussa 10% Pd/C at rt for 24 h. The mixture was filtered through Celite, evaporated, and acetylated using 1:1 acetic anhydride/pyridine mixture. After standard workup, the crude product was purified by chromatography using hexane/AcOEt 1:1 v/v mixture to afford **26** (0.07 g, 50%). Syrup, $[\alpha]_D$ − 10.5 (*c* 0.9, CH_2Cl_2); IR ($CHCl_3$): 1738 cm^{-1}; ^1H NMR (500 MHz, toluene-d_8, 100°C) δ: 5.07 (ddd, 1H, *J* 5.0, 8.9, 9.7 Hz, H-3), 4.91 (dd, 1H, *J* 8.9, 10.1 Hz, H-4) 3.98 (dd, 1H, *J* 6.2, 11.4 Hz, C*H*HOAc), 3.83 (dd, 1H, *J* 3.8, 11.4 Hz, CH*H*OAc), 2.96 (ddd, 1H, *J* 2.1, 5.0, 10.9 Hz, H-2), 2.68 (ddd, 1H, *J* 2.1, 4.1, 11.5 Hz, H-6), 2.02 (m, 1H, H-5), 1.97 (dd, 1H, *J* 9.7, 10.9 Hz, H-2′), 1.87 (t, 1H, *J* 11.1 Hz, H-6′), 1.74, 1.68, 1.67 (3s, 12H, 3Ac); HRMS (ESI) m/z $[M+H]^+$, Calcd. for $C_{19}H_{26}NO_6$: 364.1755. Found: 364.1748.

(−)-*N*-Benzyl-isofagomine (25). Deacetylation of **26** with 1% of ammonia in methanol gave **25** (90%). Colorless crystals; m.p. 147–150°C; $[\alpha]_D$ − 16.5 (*c* 0.4, CH_2Cl_2), lit.[15b] $[\alpha]_D$ − 13.2 (*c* 1.1, ethanol); ^1H NMR (400 MHz, methanol-d_4) δ: 3.78 (dd, 1H, *J* 3.7, 11.0 Hz, C*H*HOH), 3.58, 3.53 (2d, 2H, *J* 12.8 Hz, Bn), 3.51 (m, 1H, H-3), 3.49 (dd, 1H, *J* 6.9 11.0 Hz, CH*H*OH), 3.07 (dd, 1H, *J* 8.8, 10.3 Hz, H-4), 3.01 (ddd, 1H, *J* 2.3, 3.8, 11.4 Hz, H-2), 2.96 (ddd, 1H, *J* 2.3, 4.8, 10.8 Hz, H-3), 1.89 (t, 1H, J 11.4 Hz, H-2′), 1.86 (t, 1H, *J* 10.8 Hz, H-3′), 1.76 (m, 1H, H-5); HRMS (ESI) m/z $[M+H]^+$, Calcd. for $C_{13}H_{20}NO_3$: 238.1438. Found: 238.1428.

(−)-*N*-Acetyl-tri-O-acetyl-isofagomine (27). Compound **26** was hydrogenated in methanol under standard condition. After evaporation of the solvent, the residue was acetylated with acetic anhydride pyridine mixture, which after evaporation was purified on a silica gel column using AcOEt/MeOH 4:1 v/v as an eluent to afford **27** (90%) as a mixture of two rotamers in a ratio of about 2:1. Colorless crystals; m.p. 89–90°C; $[\alpha]_D$ − 14.7 (*c* 0.9, CH_2Cl_2); IR (film): 1774, 1649 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ: major rotamer 5.03 (bm, 1H, H-3), 4.73 (bm, 1H, H-4), 4.49 (bd, 1H, C*H*HOAc), 4.09 (m, 1H, CH*H*Ac), 4.03 (bm, 1H, H-2), 4.01 (bm, 2H, H-2, H-6), 3.11 (m, 1H, H-2′), 2.81 (bt, 1H, H-6′), 2.05 (m, 1H, H-5), 2.13, 2.11, 2.06, 2.05 (4s, 12H, 4Ac); minor rotamer 5.02 (bs, 1H, H-3), 4.84 (bm, 1H, H-4), 4.38 (bm, 1H, C*H*HOAc), 4.11 (m, 1H, CH*H*OAc), 3.77 (bm, 1H, H-3), 3.21 (m, 1H, H-6), 3.08 (bm, 1H, H-3′), 3.05 (m, 1H, H-6′), 1.56 (bm, 1H, H-5), 2.10, 2.09, 2.08, 2.03 (4s, 12H,

4Ac); HRMS (ESI), for the mixture of rotamers, m/z $[M + Na]^+$, Calcd. for $C_{14}H_{21}NO_7Na$: 338.1210. Found: 338.1226.

(−)-*N*-Acetyl-isofagomine (28). Deacetylation of compound **27** with 1% of ammonia in methanol gave **28** (90%) as a mixture of two rotamers in a ratio of about 1:1. Colorless crystals; m.p. 60–62°C; $[\alpha]_D$ − 8.7 (*c* 0.7, methanol); IR (film): 1637 cm^{-1}; ^1H NMR (500 MHz, methanol-d_4) δ: 4.53, 4.50 (2 m, 1H, H-4a and b), 3.95, 3.89 (2ddd, 1H, *J* 2.4, 4.4, 13.8 Hz, H-2a and b), 3.86, 3.78 (2dd, 1H, *J* 3.7, 11.1 Hz, C*H*HOHa and b), 3.60 (2dd, 0.5H, J 6.7, 11.1 Hz, CH*H*OHa), 3.55 (2dd, 0.5H, *J* 7.5, 11.0 Hz, CH*H*OHb), 3.39 (ddd, 0.5H, *J* 4.8, 8.3, 10.1 Hz, H-6a), 3.35–3.24 (m, 1.5H, H-4a and b, H-6b), 2.96 (dd, 0.5H, *J* 10.3, 13.3 Hz, H-2′a), 2.93 (2dd, 0.5H, *J* 11.5, 13.1 Hz, H-2′b), 2.56 (dd, 0.5H, *J* 11.5, 13.1 Hz, H-6′a), 2.47 (dd, 0.5H, *J* 10.3, 12.7 Hz, H-6′b), 2.11, 2.10 (2s, 3H, Ac a and b), 1.64, 1.55 (2 m, 1H, H-5a and b); HRMS (ESI), for the mixture of rotamers, m/z $[M + Na]^+$, Calcd. for $C_8H_{15}NO_4Na$: 212.0893. Found: 212.0890.

(−)-Isofagomine (1-ent). Compound **1-ent** was obtained from **25** by hydrogenation in methanol in the presence of 10% Pd/C (Degussa). Standard workup and purification on a silica gel column using i-PrOH/NH_3aq 3:1 v/v as an eluent afforded **1-ent** in 90% yield. Syrup; $[\alpha]_D$ − 17.8 (*c* 0.5, methanol), lit.[15b] $[\alpha]_D$ − 15.7 (*c* 0.19, ethanol); IR (film): 3631, 3533, 3415 cm^{-1}; ^1H NMR (500 MHz, methanol-d_4) δ: 3.78 (dd, 1H, *J* 3.8, 11.0 Hz, C*H*HOH), 3.55 (dd, 1H, *J* 6.8, 11.0 Hz, CH*H*OH), 3.40 (ddd, 1H, *J* 5.0, 8.7, 10.6 Hz, H-3), 3.20 (dd, 1H, *J* 8.8, 10.2 Hz, H-4), 3.08 (m, 2H, H-2, H-6), 2.39 (dd, 1H, *J* 11.8, 12.8 Hz, H-2′), 2.37 (dd, 1H, *J* 10.7, 12.4 Hz, H-6′), 1.64 (m, 1H, H-5); HRMS (EI) m/z M^+, Calcd. for $C_6H_{13}NO_3$: 147.0895. Found: 147.0892.

Measurements of Enzymes Inhibition

The following hydrolases were used: α-L-glucosidase from rice (type V, 63.43 U/mg, 1.34 mg/mL, Sigma), β-D-glucosidase from almonds (25.8 U/mg, 95.4% protein, Sigma), α-D-mannosidase from jack bean (6.2 mg prot./mL, 22 U/mg, Sigma), α-L-fucosidase from bovine kidney (28.0 U/mg, 0.55 mg prot./mL, Sigma), β-D-galactosidase from bovine liver (0.148 U/mg, Sigma) solution (0.562 U/mL) and β-D-glucuronidase from bovin liver (2630 U/mg, Sigma) solution (4909 U/mL) (Table 1).

These enzymes were assayed with appropriate p-nitrophenyl glycoside substrates (phenolphthalein β-glucuronide for β-glucuronidase), which were purchased from Sigma. Hydrolase activities were measured by modification of the procedures described previously.[24–29] The reaction mixtures (for each enzyme) consisted of 25 μL of 16.5 mM p-nitrophenyl α-D-glucopyranoside, 403 μL of 0.1 M acetate buffer (pH 4.0), 50 μL of inhibitor solution (water or methanol), and 22 μL of α-L-glucosidase solution (10 × diluted) for

Table 1: Effect of (−)-isofagomine (**1-ent**) and (−)-*N*-acetyl-isofagomine (**28**) on various glycosidases.

	(−)-Isofagomine (1-ent)		(−)-*N*-Accetyl-isofagomine (28)	
Enzyme tested	**Concentration of inhibitor (mM)**	**Enzyme inhibition (%)**	**Concentration of inhibitor (mM)**	**Enzyme inhibition (%)**
α-L-Fucosidase	12.0	50	3.5	50
β-D-Galactosidase	8.5	50	6.6	50
β-D-Glucuronidase	15.0	50	9.1	50
α-D-Glucosidase	10.0	lack	10.0	Lack
β-D-Glucosidase	9.0	50	50.0	8.5
α-D-Mannosidase	11.0	lack	6.9	16

α-glucosidase activity; 100 μL of 14 mM p-nitrophenyl β-D-glucopyranoside, 250 μL of 0.2 M acetate buffer (pH 4.6), 50 μL of inhibitor solution, and 100 μL of β-D-glucosidase solution (30 μg/mL) for β-D-glucosidase activity; 5 μL of 20 mM p-nitrophenyl α-D-mannopyranoside, 200 μL of 0.1 M acetate buffer (pH 4.5), 25 μL of inhibitor solution, and 20 μL of α-D-mannosidase solution (100 × diluted) for α-D-mannosidase activity; 20 μL of α-L-fucosidase solution (100 × diluted), 25 μL of inhibitor solution, 155 μL of 0.1 M acetate buffer (pH 5.0), and 50 μL of 20 mM 4-nitrophenyl-α-L-fucopyranoside for α-L-fucosidase activity; 90 μL of 4 mM phenolphthalein β-D-glucuronide, 95 μL of 0.1 M acetate buffer (pH 5.0), 25 μL of inhibitor solution, and 40 μL of β-D-glucuronidase solution for β-D-glucuronidase activity; 25 μL of 20 mM 4-nitrophenyl β-D-galactopyranoside, 195 μL of 0.1 M acetate buffer (pH 5.0), 25 μL of inhibitor solution, and 5 μL of β-D-galactosidase solution for β-D-galactosidase activity. Generally, enzyme and inhibitor were mixed in buffer and reaction was started by the addition of the substrate. Each reaction mixture was incubated for 30 min (α- and β-glucosidases for 15 min) at 37°C and was terminated by adding 0.250 mL of 2% sodium carbonate. The absorbance of liberated p-nitrophenol was measured at 405 nm (liberated phenolphthalein at 540 nm).

(−)-*N*-Benzyl-isofagomine (**25**) showed 50% inhibition of α-L-fucosidase activity in concentration 5.1 mM, trace inhibition of β-D-glucosidase activity in concentration 18 mM, and lack of inhibition of α-D-glucosidase, β-D-galactosidase, β-D-glucuronidase, and α-D-mannosidase.

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