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Note

Synthesis and enzyme-catalyzed hydrolysis of a radical-masked glycosylated spin-label reagent

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Abstract—N^1-Acetoxy-2,2,6,6-tetramethylpiperidin-4-yl 2,3,4,6-tetra-*O*-benzyl-α- and -β-D-glucopyranosides (**3**-α, β) and N^1-acetoxy-2,2,5,5-tetramethylpyrrolin-3-oyl 2,3,4,6-tetra-*O*-benzyl-α- and -β-D-glucopyranosylamines (**9**-α, β) were synthesized in good yield by Schmidt's glycosylation method. Their subsequent O-debenzylation was proceeded successfully to give the desired products **1**-α, and **1**-β in good yield, and **2**-α in a low yield, without **2**-β by only short-timed hydrogenolysis in the presence of palladium-on-carbon (Pd–C) in a $CHCl_3$–MeOH solvent system that included concentrated HCl. Upon enzyme-catalyzed hydrolysis, only **2**-α was hydrolyzed by the esterase, while both of **1**-α and **1**-β were not hydrolyzed by any other enzyme such as lipase. These **2**-α can likely be used as a new water-soluble radical-masked glycosylated spin-label reagent.

Keywords: Hydroxylamine, acyl-protected; Spin-label reagent, acetyl-protected; Glycosylation; Imidate; Hydrogenolysis; Enzyme-catalyzed hydrolysis; Esterase

Although a variety of spin-label reagents have been reported to date, only limited information is available concerning a protected spin-label reagent.[1] Recently, Yokoyama and co-workers developed acyl-protected hydroxylamines for use as spin-label reagents for ESR measurements of intracellular oxidative stress.[2] These compounds were stable, nonradical compounds and were readily hydrolyzed by esterase to yield a hydroxylamine, which was then oxidized to form an ESR-detectable nitroxide radical. When an acyl-protected hydroxylamine was used, a highly sensitive ESR determination procedure was successfully developed for analyzing oxidative stress in human leukocytes.

In the meantime, we have reported on the synthesis of 1-*N*-α- and -β-D-*gluco*- and galactopyranosyloxy-4-hydroxyl-2,2,6,6-tetramethylpiperidines and 3-carbamoyl-1-*N*-α- and -β-D-*gluco*- and galactopyranosyloxy-2,2,5,5-tetramethylpyrrolines, which would be expected to be more stable than the above acyl-protected hydroxylamines toward an enzyme as new, stable spin-label reagents.[3] However, these glycosyl-protected hydroxylamines were hydrolyzed by α- or β-D-glycosidases with difficulty.

Alternatively, based on the above results, we developed a new synthesis of N^1-acetoxy-2,2,6,6-tetramethylpiperidin-4-yl α- and β-D-glucopyranosides (**1**) and N^1-acetoxy-2,2,5,5-tetramethylpyrrolin-3-oyl α- and β-D-glucopyranosylamines (**2**). It is expected these compounds will be readily hydrolyzed by esterase giving glycosylated hydroxylamines, which are unstable and rapidly oxidized to form the corresponding glycosylated radicals. Glycosylated nitroxyl radicals are more water-soluble and stable under intracellular conditions than the above acyl-protected hydroxylamines.[4] For the synthesis of **1**, the protecting group for the hydroxyl group

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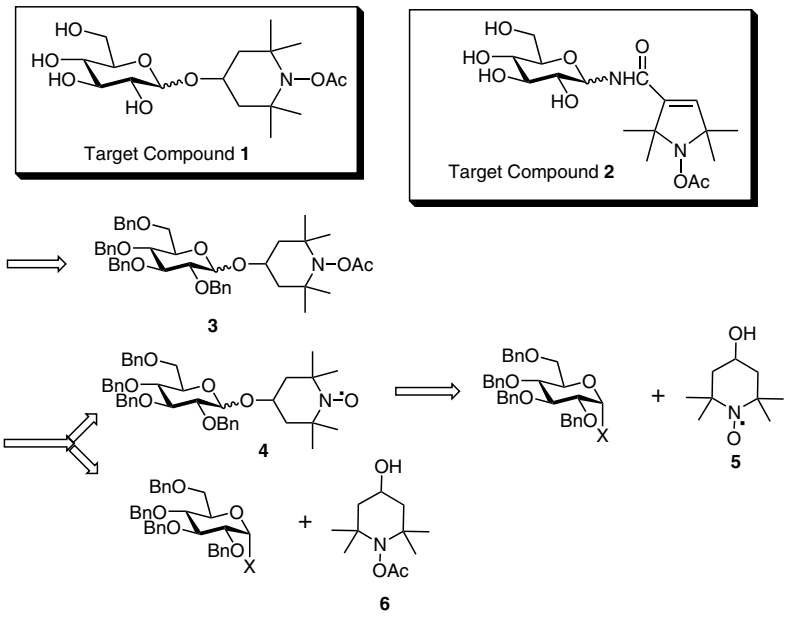
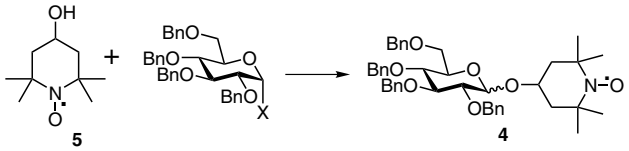


Scheme 1. Target compounds **1** and **2**, and retrosynthetic analysis.

of the glucose must be different from an acetyl group. We therefore employed the benzyl-protecting group for glucose in the glycosylation reaction as shown in Scheme 1, and examined two synthetic methods: (1) After the glycosylation of 4-hydroxyl-2,2,6,6-tetramethylpiperidine-1-oxyl (4-hydroxyl TEMPO, **5**), the nitroxyl radical moiety is reduced and then acetylated, and, finally, O-debenzylation is accomplished by hydrogenolysis. (2) After the glycosylation of 1-*N*-acetoxy-4-hydroxyl-2,2,6,6-tetramethylpiperidine (**6**), hydrogenolysis is performed. We initially explored the first plan. The O-glycosylation of **5** was examined by using 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl fluoride as a glycosyl donor (see Table 1). When boron trifluoride diethyl etherate ($BF_3{\cdot}OEt_2$) was used as a promoter, a yield of 66% (α/β = 70:30) was obtained. However, the reproducibility of this method was poor. We next attempted Schmidt's imidate method using 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl trichloroacetimidate as a glycosyl donor and trimethylsilyl trifluoromethanesulfonate (TMSOTf) as a promoter.[5] The use of 0.25 equiv of TMSOTf resulted in the highest yield, 70% (α/β = 38:62). This imidate method was found to be reliable and reproducible. Each anomer of the glycosyl radical thus obtained was reduced by hydrazine and acetylated by acetic anhydride to give the corresponding glycosylated acetyl-protected hydroxylamine **3** in the yield of 50% for the α- and 65% for the β-anomer, respectively.

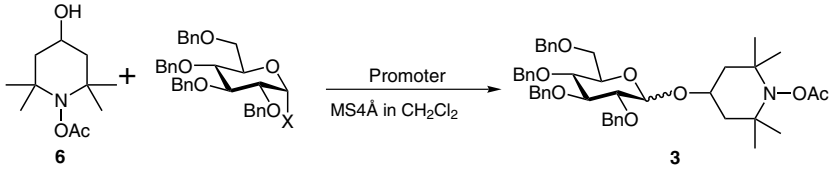
We next explored the second plan, the glycosylation of **6** under the same reaction conditions as the former method (see Table 2). When glycosyl fluoride was employed as a donor and $BF_3{\cdot}OEt_2$ as a promoter, the yield was similar to that obtained above (entries 1–4). However, the use of the imidate and TMSOTf lead to a nearly quantitative yield (α/β = 40:60) (entry 5), and the reaction proceeded essentially to completion within 1 h, and was reproducible. Glycosylation of the another nitroxyl radical, 1-*N*-acetoxy-3-carbamoyl-2,2,5,5-tetramethylpyrroline (**8**) was carried out in a same manner to give the corresponding N^1-acyl glycosyl amine (**9**) in 87% yield (α/β = 72:28) (see Scheme 2).

Table 1. Glycosylation of 4-hydroxyl TEMPO (**5**)

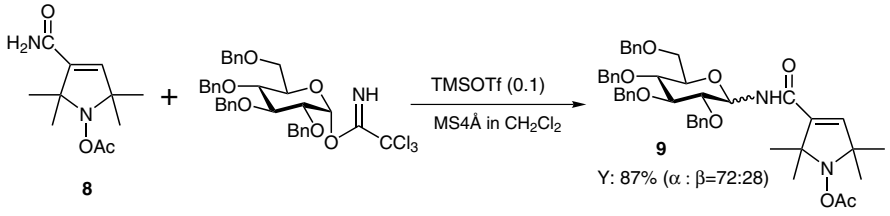


Entry	Acceptor (equiv)	Donor X (equiv)	Promoter (equiv)	Solvent	Temperature (°C)	Time (h)	Yield of **4** (%) (α/β)
1	**5** (2.0)	F (1.0)	$BF_3{\cdot}OEt_2$ (3.5) MS4 Å	CH_2Cl_2	0	2	66 (70:30)
2	**5** (2.0)	F (1.0)	Cp_2ZnCl_2 (1.0), $AlClO_4$ (2.0) MS4 Å	CH_2Cl_2	−78 to rt	24	46 (70:30)
3	TMS ether of **5** (1.0)	F (1.0)	TMSOTf (1.5)	CH_3CN	0	24	0
4	**5** (1.5)	C(=NH)CCl_3 (1.0)	TMSOTf (0.25) MS4 Å	CH_2CI_2	0	2	70 (38:62)

Table 2. Glycosylation of 1-*N*-acetoxy-4-hydroxy-2,2,6,6-tetramethylpiperidine (**6**)



Entry	Acceptor **6** (equiv)	Donor X (equiv)	Promoter (equiv)	Temperature (°C)	Time (h)	Yield of **3** (%) (α/β)
1	1.0	F (2.0)	$BF_3{\cdot}OEt_2$ (2.0)	0	1	49 (70:30)
2	1.0	F (1.1)	$BF_3{\cdot}OEt_2$ (3.0), *i*-Pr_2NEt (1.5)	0	2	63 (70:30)
3	1.0	F (1.1)	$BF_3{\cdot}OEt_2$ (2.5), *i*-Pr_2NEt (1.5)	−78	5	10 (70:30)
4	1.0	F (1.1)	$BF_3{\cdot}OEt_2$ (2.5), *i*-Pr_2NEt (1.5)	−20	2.5	57 (70:30)
5	1.0	C(=NH)CCl_3 (1.2)	TMSOTf (O.1) MS4 Å	0	2	96 (40:60)



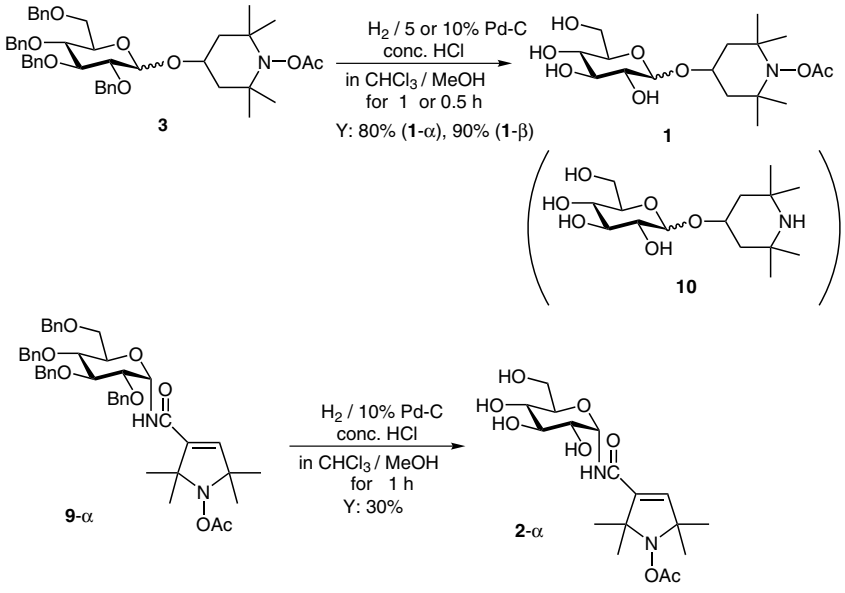
Scheme 2. Glycosylation of 1-*N*-acetoxy-3-carbamoyl-2,2,5,5-tetramethylpyrroline (**8**).

Since a satisfactory result was obtained on the glycosylation reaction, we next examined the O-debenzylation of N^1-acetoxy-2,2,6,6-tetramethylpiperidin-4-yl 2,3,4,6-tetra-*O*-benzyl-α- and -β-D-glucopyranosides (**3**) by hydrogenolysis using Pd–C. However, hydrogenolysis using Pd–C surprisingly gave mainly 2,2,6,6-tetramethylpiperidin-4-yl glucopyranoside (**10**). It was found that the acetyl-protected hydroxylamine was readily reduced by hydrogenolysis to give piperidine. The β-anomer was apparently more susceptible to hydrogenolysis than the α-anomer, and no **1** was obtained. By using a mixture of $CHCl_3$, MeOH, and a small amount of AcOH as the solvent system, the hydrogenolysis of the α-anomer yielded **1** in the highest (20%) yield with the major product, 2,2,6,6-tetramethylpiperidin-4-yl α-D-glucopyranoside (**10**-α). This difference might be due to a greater degree of steric hindrance by the glucopyranosyl moiety of the α-anomer than that of the β-anomer.

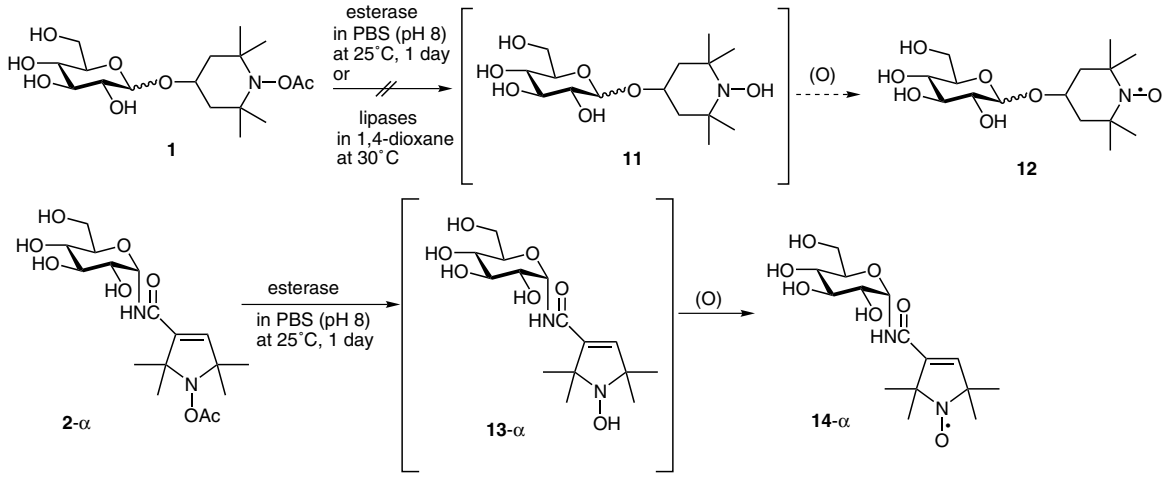
We examined the hydrogenolysis reaction conditions by using a series of mixed solvent systems; $CHCl_3$, EtOAc, MeOH, EtOH, a small amount of AcOH, or 2 M HCl. Using these systems, the *N*-acetoxy group was more susceptible to hydrogenolysis than the benzyl group, giving a mixture of partially benzyl-protected 2,2,6,6-tetramethylpiperidin-4-yl α-D-glucopyranosides. Another O-debenzylation reaction using excess amounts of iron(III) trichloride was also unsuccessful.[6] However, hydrogenolysis by Pd–C in strongly acidic solution in which concentrated HCl (0.5 mL) was added to a solution of $CHCl_3$ (2 mL) and MeOH (2 mL) surprisingly proceeded rapidly to afford the desired acetyl-protected hydroxylamine in good yield; the β-glycoside underwent hydrogenolysis within 1 hour using 5% Pd–C to give **1**-β in 80% yield. Hydrogenolysis of the α-glycoside was completed within 0.5 h by means of a small amount of 10% Pd–C to give **1**-α in 90% yield. On the O-debenzylation of **9** in the same manner, the *N*-acetoxy group of **9** was found to be more sensitive than that of **3** under the hydrogenolysis conditions, and the α-anomer yielded the desired product **2**-α in a low 30% yield, but gave no β-anomer (see Scheme 3).

We finally examined the enzyme-catalyzed hydrolysis of the synthesized **1**-α, **1**-β, and **2**-α (Scheme 4). On the hydrolysis of **1**-α, **1**-β, and **2**-α by esterase in 0.1 M PBS buffer (pH 8) at 25 °C, only **2**-α was hydrolyzed and then rapidly oxidized to afford the corresponding nitroxyl radical (**14**-α) with a few amount of hydroxylamine (**13**-α) on TLC. In a similar way, 1-*N*-acetoxy-3-carbamoyl-2,2,5,5-tetramethylpyrroline (**8**) was also easily hydrolyzed by esterase and rapidly oxidized to give 3-carbamoyl-2,2,5,5-tetramethylpyrroline-1-oxyl (**7**), but no 1-*N*-acetoxy-4-hydroxy-2,2,6,6-tetramethylpiperidine (**6**). On screening by 13 lipases, compounds **1**-α, **1**-β, and **6** were not hydrolyzed by any lipases.[7] Compounds **6** and its *O*-glycoside **1** are more inert than **8** and its *N*-glucoside **2**-α for enzymes as well as hydrogenolysis.

In conclusion, we were able to efficiently synthesize the target compound **1**-α and **1**-β in good yield and



Scheme 3. Hydrogenolysis of glucosides.

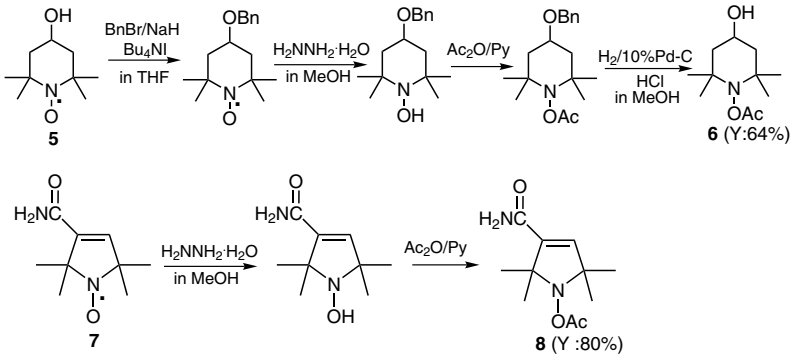


Scheme 4. Enzyme-catalyzed hydrolysis of **1**-α, **1**-β, and **2**-α.

2-α in a low yield by O- or N-glycosylation of acetyl-protected hydroxylamines using the imidate method and subsequent O-debenzylation by hydrogenolysis using a solvent system that included concentrated HCl. 1-*N*-Acetoxy-3-carbamoyl-2,2,5,5-tetramethylpyrroline (**8**) and its 3-*N*-α-glucoside (**2**) were readily hydrolyzed by esterase, but neither 1-*N*-acetyoxy-4-hydroxy-2,2,6,6-tetramethylpiperidine (**6**) nor its 4-*O*-α- and -β-glucosides (**1**) were hydrolyzed by any enzymes.

1. Experimental

Anhydrous CH_2Cl_2 used in this reaction was prepared in situ by distillation from CaH_2 under N_2. For separation and purification, flash column chromatography was performed on silica-gel (230–400 mesh, Fuji-Silysia Co., Ltd, BW-300). HPLC was performed using an Inertsil ODS-3 column (GL Science: 5 μm, 4.6 × 250 mm for analytical use, 20 × 250 mm for preparative use; mobile phase: MeOH–water). Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Mass spectral data were obtained by fast-atom bombardment (FABMS) method using 3-nitrobenzyl alcohol (NBA) or glycerol as the matrix on a JEOL JMS-AX505HA instrument. Optical rotations were recorded on a JASCO DIP-370 polarimeter. Elemental analyses were performed on a Perkin–Elmer PE 2400 II. NMR spectra were recorded on a Varian Inova 500 spectrometer using Me_4Si as the internal reference.



Scheme 5. Syntheses of 1-*N*-acetoxy-4-hydroxy-2,2,6,6-tetramethylpiperidine (**6**) and 1-*N*-acetoxy-3-carbamoyl-2,2,6,6-tetramethylpyrroline (**8**).

1.1. 1-*N*-Acetoxy-4-hydroxy-2,2,6,6-tetramethylpiperidine (6)

Compound **6** was synthesized from 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (**5**)[1b] in four steps in a total yield of 64%, as shown in Scheme 5.

Colorless prisms; mp 82 °C (from diisopropyl ether); FABMS (NBA, *m*/*z*): 216 $(M+H)^+$; ^{1}H NMR (DMSO-d_6) δ: 0.95 and 1.12 (each 6H, s, $CH_3 \times 2$), 1.38 (2H, t, *J* 12.0 Hz, CH_2), 1.76 (2H, dd, *J* 3.0 and 12.0 Hz, CH_2), 2.04 (3H, s, NO*Ac*), 3.81 (1H, m, >CH–), 4.61 (1H, d, *J* 4.9 Hz, OH); IR (KBr) *v*: 3465, 3282, 3004, 2968, 2945, 1763, 1738, 1365, 1248, 1203, 1182, and 1045 cm^{-1}. Anal. Calcd for $C_{11}H_{21}NO_3$: C, 61.14; H, 9.83; N, 6.51. Found: C, 61.39; H, 9.91; N, 6.46.

1.2. 1-*N*-Acetoxy-3-carbamoyl-2,2,5,5-tetramethylpyrroline (8)

Compound **8** was synthesized from 3-carbamoyl-2,2,5,5-tetramethylpyrroline-1-oxyl (**7**)[1b] in two steps in a total yield of 80%, as shown in Scheme 5.

Colorless prisms; mp 87.5 °C (from EtOAc); FABMS (NBA, *m*/*z*); 227 $(M+H)^+$, ^{1}H NMR ($CDCl_3$) δ: 1.29 (6H, s, $CH_3 \times 2$), 1.40 and 1.45 (each 3H, s, $CH_3 \times 2$), 2.15 (3H, s, OAc), 6.06 (2H, br s, NH_2), 6.14 (1H, s, olefinic H); IR (KBr) *v*: 3435, 3354, 3228, 3068, 2989, 2945, 1774, 1662, 1604, 1414, 1365, and 1203 cm^{-1}. Anal. Calcd for $C_{11}H_{18}N_2O_3$: C, 58.39; H, 8.02; N, 12.38. Found: C, 58.61; H, 8.09; N, 12.31.

1.3. 4-(2,3,4,6-Tetra-*O*-benzyl-α- and -β-D-glucopyranosyloxy)-2,2,6,6-tetramethylpiperidine-1-oxyl (4)

To a stirred mixture of **5** (7.755 g, 4.39 mmol), benzyl-protected α-imidate (2.00 g, 2.928 mmol), and 2 g of powdered molecular sieves 4 Å in dry CH_2Cl_2 (5 mL) at 0 °C under Ar, TMSOTf (106 μL, 0.293 mmol) was added and the resulting mixture was stirred at 0 °C. After 2 h, satd aq $NaHCO_3$ solution was added to the stirred reaction mixture, which was then extracted twice with EtOAc. The combined extract was washed with water, brine, and dried over anhyd Na_2SO_4. After removing the EtOAc by evaporation, the resulting residue was purified by flash column chromatography on silica-gel (hexane–EtOAc) to give **4** (1.43 g, 70%) as a pale-red viscous oil. Separation of α- and β-anomers was performed by HPLC (ODS-3 column: 5 μm, 20 × 250 mm; mobile phase: (98:2 MeOH–H_2O) to give the α-anomer (0.543 g) as a red viscous oil and the β-anomer (0.886 g) as pale-red prisms.

1.3.1. Data for the α-anomer. Reddish viscous oil; R_f 0.33 (3:1 hexane–EtOAc); HPLC: t_R 6.77 min (mobile phase: 98:2 MeOH–H_2O); $[\alpha]_D^{25}$ + 50.2 (*c* 1.54, $CHCl_3$); ^{1}H NMR ($CDCl_3$+hydrazobenzene) δ: 1.07, 1.10, 1.15, and 1.20 (each 3H, s, $CH_3 \times 4$), 1.49 (1H, t, *J* 12.0 Hz, CH_2), 1.61 (1H, t, *J* 12.0 Hz, CH_2), 1.82 (2H, m, CH_2), 3.54 (1H, dd, *J* 9.5 and 3.6 Hz, H-2′), 3.60 (1H, t, *J* 9.5 Hz, H-3′), 3.63 (1H, dd, *J* 1.5 and 12.5 Hz, H-6′a), 3.71 (1H, dd, *J* 4.0 and 12.5 Hz, H-6′b), 3.83 (1H, m, H-4), 3.96 (1H, t, *J* 9.5 Hz, H-4′), 4.45 (2H, d, *J* 11.5 Hz, benzylic CH_2), 4.61 (2H, d, *J* 13.5 Hz, benzylic CH_2), 4.79–4.86 (2H, m, benzylic CH_2), 4.82 (1H, d, *J* 3.6 Hz, H-1′), 4.83 (1H, d, *J* 11.5 Hz, benzylic CH_2), 4.99 (1H, d, *J* 11.0 Hz, benzylic CH_2), 6.80–7.20 (20H, m, *Ph*$CH_2 \times 4$). FABMS (NBA, *m*/*z*) 695 $(M+H)^+$, 415, 181; IR (KBr) *v*: 3087, 3062, 3029, 2973, 2931, 2865, 1496, 1454, 1361, 1072, 736, and 698 cm^{-1}. Anal. Calcd for $C_{43}H_{52}NO_7$: C, 74.32; H, 7.54; N, 2.02. Found: C, 73.92; H, 7.56; N, 1.83.

1.3.2. Data for the β-anomer. Pale-red needles; mp 123–124 °C (from diethyl ether) R_f 0.37 (3:1 hexane–EtOAc); HPLC: t_R 7.37 min (mobile phase: 98:2 MeOH–H_2O); $[\alpha]_D^{25}$ + 4.77 (*c* 0.965, $CHCl_3$); ^{1}H NMR ($CDCl_3$+hydrazobenzene) δ: 1.12, 1.13, 1.18, and 1.19 (each 3H, s, $CH_3 \times 4$), 1.53 (1H, t, *J* 12.0 Hz, CH_2), 1.62 (1H, t, *J* 12.0 Hz, CH_2), 1.95 (1H, dt, *J* 3.5 and 12.0 Hz, CH_2), 2.07 (1H, dt, *J* 3.5 and 12.0 Hz, CH_2), 3.48 (1H, m, H-5′), 3.53 (1H, t, *J* 8.9 Hz, H-3′), 3.65 (1H, dd, *J* 5.3 and 10.7 Hz, H-6′a), 3.73 (1H, dd, *J* 1.9 and 10.7 Hz, H-6′b), 3.99 (1H, m, H-4), 4.48 (1H, d, *J* 7.9 Hz, H-1′), 4.55 (1H, d, *J* 11.0 Hz, benzylic CH_2),

4.56 (1H, d, J 12.0 Hz, benzylic CH_2), 4.60 (1H, d, J 12.0 Hz, benzylic CH_2), 4.71 (1H, d, J 11.0 Hz, benzylic CH_2), 4.78 (1H, d, J 11.0 Hz, benzylic CH_2), 4.82 (1H, d, J 11.0 Hz, benzylic CH_2), 4.92 (1H, d, J 11.0 Hz, benzylic CH_2), 4.93 (1H, d, J 11.0 Hz, benzylic CH_2), 6.80–7.20 (20H, m, $PhCH_2 \times 4$); FABMS (NBA, m/z): 695 $(M+H)^+$, 415, 181; IR (KBr) ν: 3087, 3062, 3027, 2973, 2935, 2871, 1496, 1454, 1363, 1066, 754, and 698 cm^{-1}. Anal. Calcd for $C_{43}H_{52}NO_7$: C, 74.32; H, 7.54; N, 2.02. Found: C, 74.49; H, 7.37; N, 1.94.

1.4. N^1-Acetoxy-2,2,6,6-tetramethylpiperidin-4-yl 2,3,4,6-tetra-*O*-benzyl-α- and -β-D-glucopyranoside (3)

Experimental procedure for the glycosylation reaction was carried out in the same way as that described for **4**. Separation of α- and β-anomers was performed by flash column chromatography on silica-gel (hexane–EtOAc).

1.4.1. Data for the α-anomer. Colorless oil; R_f 0.38 (3:1 hexane–EtOAc); $[\alpha]_D^{21}$ +51.7 (c 0.995, $CHCl_3$); ^1H NMR ($CDCl_3$) δ: 1.04, 1.10, 1.11, and 1.14 (each 3H, s, $CH_3 \times 4$), 1.71 (1H, t, J 12.5 Hz, CH_2), 1.80 (1H, t, J 12.5 Hz, CH_2), 1.83 (1H, dt, J 3.5 and 12.5 Hz, CH_2), 1.88 (1H, dt, J 3.5 and 12.5 Hz, CH_2), 2.08 (3H, s, OAc), 3.54 (1H, dd, J 3.5 and 9.5 Hz, H-2′), 3.58 (1H, t, J 9.5 Hz, H-4′), 3.62 (1H, d, J 3.5 and 10.0 Hz, H-6′a), 3.69 (1H, dd, J 4.5 and 10.0 Hz, H-6′b), 3.83 (1H, ddd, J 3.5, 4.5, and 9.5 Hz, H-5′), 3.95 (1H, t, J 9.5 Hz, H-3′), 4.44 and 4.59 (each 1H, d, J 14.0 Hz, benzylic CH_2), 4.45 and 4.83 (each 1H, d, J 12.5 Hz, benzylic CH_2), 4.59 and 4.78 (each 1H, d, J 14.0 Hz, benzylic CH_2), 4.80 and 4.98 (each 1H, d, J 12.5 Hz, benzylic CH_2), 4.82 (1H, d, J 3.5 Hz, H-1′), 6.8–7.2 (20H, m, $PhCH_2 \times 4$); FABMS (Glycerol, m/z): 738 $(M+H)^+$; IR (KBr) ν: 3087, 3062, 3029, 2975, 2925, 2865, 1766, 1496, 1365, 1247, 736, and 698 cm^{-1}. Anal. Calcd for $C_{45}H_{55}NO_8$: C, 73.24; H, 7.51; N, 1.90. Found: C, 73.26; H, 7.51; N, 1.87.

1.4.2. Data for the β-anomer. Colorless prisms; mp 152–153 °C (from EtOAc); R_f 0.42 (3:1 hexane–EtOAc); $[\alpha]_D^{21}$ +3.64 (c 0.990, $CHCl_3$); ^1H NMR ($CDCl_3$) δ: 1.09, 1.10, 1.17, and 1.18 (each 3H, s, $CH_3 \times 4$), 1.78 (1H, t, J 12.5 Hz, CH_2), 1.88 (1H, dd, J 3.5 and 12.5 Hz, CH_2), 1.96 (1H, dd, J 3.5 and 12.5 Hz, CH_2), 2.06 (1H, t, J 12.5 Hz, CH_2), 2.09 (3H, s, OAc), 3.42 (1H, dd, J 8.0 and 9.0 Hz, H-2′), 3.47 (1H, ddd, J 1.5, 5.0, and 9.0 Hz, H-5′), 3.54 (1H, t, J 9.0 Hz, H-4′), 3.63 (1H, t, J 9.0 Hz, H-3′), 3.65 (1H, dd, J 1.5 and 10.5 Hz, H-6′a), 3.74 (1H, dd, J 5.0 and 10.5 Hz, H-6′b), 4.44 and 4.59 (each 1H, d, J 14.0 Hz, benzylic CH_2), 4.45 and 4.83 (each 1H, d, J 12.5 Hz, benzylic CH_2), 4.59 and 4.78 (each 1H, d, J 14.0 Hz, benzylic CH_2), 4.80 and 4.98 (each 1H, d, J 12.5 Hz, benzylic CH_2), 4.82 (1H, d, J 3.5 Hz, H-1′), 6.8–7.2 (20H, m, $PhCH_2 \times 4$); FABMS (glycerol, m/z) 738 $(M+H)^+$; IR (KBr) ν: 3089, 3064, 3033, 2979, 2912, 2869, 1766, 1496, 1452, 1365, 1070, 752, and 702 cm^{-1}. Anal. Calcd for $C_{45}H_{55}NO_8$: C, 73.24; H, 7.51; N, 1.90. Found: C, 73.36; H, 7.64; N, 1.87.

1.5. 2,2,6,6-Tetramethylpiperidin-4-yl α- and β-D-glucopyranoside (10)

1.5.1. Data for the α-anomer. Colorless powder; $[\alpha]_D^{23}$ +97.9 (c 0.515, MeOH); ^1H NMR (DMSO-d_6) δ: 1.38 and 1.44 (each 6H, s, $CH_3 \times 4$), 1.51 and 1.56 (each 1H, d, J 10.5 and 13.0 Hz, CH_2), 1.98 and 2.05 (each 1H, d, J 3.5 and 13.0 Hz, CH_2), 3.03 (1H, m, H-5′), 3.17 (1H, ddd, 2.0, 5.5, and 10.0 Hz, H-6′a), 3.20 (ddd, J 3.5, 5.0, and 9.5 Hz, H-2′), 3.36 (1H, ddd, J 6.5, 9.5, and 9.5 Hz, H-3′), 3.64 (ddd, J 5.5, 6.5, and 10.0 Hz, H-6′b), 4.07 (1H, m, H-4), 4.52 (1H, t, J 5.5 Hz, 6′-OH), 4.71 (1H, d, J 6.5 Hz, 3′-OH), 4.80 (1H, d, J 5.0 Hz, 2′-OH), 4.94 (1H, d, J 5.5 Hz, 4′-OH); FABMS (NBA, m/z) 320 $(M+H)^+$; IR (KBr) ν: 3370, 2931, 1439, 1390, 1226, 1147, and 1022 cm^{-1}. Anal. Calcd for $C_{15}H_{29}NO_6 \cdot 2.5H_2O$: C, 49.43; H, 9.40; N, 3.84. Found: C, 49.52; H, 9.23; N, 3.78.

1.5.2. Data for the β-anomer. Colorless prisms; mp 145–147 °C (from MeOH); $[\alpha]_D^{24}$ −18.9 (c 0.55, MeOH); ^1H NMR (DMSO-d_6) δ: 1.40 (6H, s, $CH_3 \times 2$), 1.42 and 1.43 (each 3H, s, $CH_3 \times 2$), 1.47 (1H, t, J 13.2 Hz, CH_2), 1.54 (1H, t, J 12.9 Hz, CH_2), 2.02 (1H, dd, J 2.9 and 13.2 Hz, CH_2), 2.07 (1H, dd, J 2.6 and 12.9 Hz, CH_2), 3.12 (1H, t, J 9.0 Hz, H-4′), 3.27 (1H, dd, J 9.0 and 3.9 Hz, H-2′), 3.46 (1H, t, J 9.0 Hz, H-3′), 3.52 (2H, m, H-5′ and 6′a), 3.71 (1H, dd, J 5.0 and 14 Hz, H-6′b), 4.11 (1H, m, >CH–), 4.92 (1H, d, J 3.9 Hz, H-1′); FAB-MS (NBA m/z): 320 $(M+H)^+$; IR (KBr) ν: 3370, 2927, 1570, 1406, 1228, 1081, and 1037 cm^{-1}. Anal. Calcd for $C_{15}H_{29}NO_6 \cdot 2.5H_2O$: C, 49.43; H, 9.40; N, 3.84. Found: C, 49.32; H, 9.53; N, 3.80.

1.6. N^1-Acetoxy-2,2,6,6-tetramethylpiperidin-4-yl α- and β-D-glucopyranoside (1)

To a solution of **4**-α-anomer (200 mg, 0.271 mmol) in $CHCl_3$ (2 mL) and MeOH (2 mL), 2 mL of concd HCl and 40 mg of 10% Pd–C were added. The atmosphere was replaced with H_2 and the mixture was stirred vigorously at room temperature for 0.5 h. After monitoring the progress of reaction by TLC, the reaction mixture was neutralized with 2 M aq Na_2CO_3 solution and filtered through a Celite pad. The filtrate was evaporated in vacuo. The residual solid was purified by flash column chromatography on silica-gel (20:1 and 10:1

$CHCl_3$–MeOH) to give **1**-α (93 mg, 90%) as a colorless solid.

The preparation of the β-anomer was carried out in the same manner as that used for the α-anomer, using 5% Pd–C.

1.6.1. Data for the α-anomer. Colorless amorphous powder; R_f 0.56 (5:1 $CHCl_3$–MeOH); $[\alpha]_D^{19}$ +98.8 (*c* 1.00, MeOH); ^{1}H NMR (DMSO-d_6) δ: 0.97 and 0.98 (each 3H, s, $CH_3 \times 2$), 1.13 (6H, s, $CH_3 \times 2$), 1.40 and 1.50 (each 1H, t, *J* 12.2 Hz, CH_2), 1.95 (2H, m, CH_2), 2.04 (3H, s, OAc), 3.03 (1H, dt, *J* 5.6 and 9.0 Hz, H-4′), 3.16 (1H, m, *J* 3.9, 6.8, and 9.0 Hz, H-2′), 3.37 (1H, dt, *J* 4.6 and 9.0 Hz, H-3′), 3.43 (2H, m, H-5′ and 6′a), 3.62 (1H, dd, *J* 5.6 and 9.5 Hz, H-6b′), 3.91 (1H, m, H-4), 4.51 (1H, t, *J* 5.6 Hz, 6′-OH),. 4.55 (1H, t, *J* 6.8 Hz, 2′-OH), 4.75 (1H, t, *J* 4.6 Hz, 3′-OH), 4.79 (1H, t, *J* 3.9 Hz, H-1′), 4.87 (1H, t, *J* 5.6 Hz, 4′-OH); FABMS (glycerol, *m*/*z*): 578 $(M+H)^+$; IR (KBr) *v*: 3400, 2975, 2931, 1766, 1367, 1251, 1203, 1188, 1049, and 1028 cm^{-1}. Anal. Calcd for $C_{17}H_{31}NO_8 \cdot 0.25H_2O$: C, 53.46; H, 8.31; N, 3.67. Found: C, 53.56; H, 8.44; N, 3.63.

1.6.2. Data for the β-anomer. Colorless viscous oil; R_f 0.56 (5:1 $CHCl_3$–MeOH); $[\alpha]_D^{19}$ −33.0 (*c* 0.995, MeOH); ^{1}H NMR (DMSO-d_6) δ: 0.97 and 0.98 (each 3H, s, $CH_3 \times 2$), 1.13 (6H, s, $CH_3 \times 2$), 1.40 and 1.47 (each 1H, t, *J* 12.2 Hz, CH_2), 1.96 (2H, m, CH_2), 2.04 (3H, s, OAc), 2.90 (1H, br t, *J* 7.5 and 9.0 Hz, H-2′), 3.02 (1H, t, *J* 9.0 Hz, H-4′), 3.11 (1H, t, *J* 9.0 Hz, H-3′), 3.13 (1H, m, H-5′), 3.45 (1H, dd, *J* 6.5 and 11.5 Hz, H-6a′), 3.65 (1H, d, *J* 11.5 Hz, H-6b′), 3.97 (1H, m, H-4), 4.23 (1H, d, *J* 7.5 Hz, H-1′), 4.47 (1H, t, *J* 5.7 Hz, 6′-OH),. 4.90 (1H, t, *J* 4.6 Hz, 4′-OH), 4.92 (1H, d, *J* 4.8 Hz, 3′-OH), 4.94 (1H, t, *J* 4.4 Hz, 2′-OH); FABMS (glycerol, *m*/*z*): 578 $(M+H)^+$; IR (KBr) *v*: 3400, 2974, 2931, 1766, 1367, 1253, 1205, 1188, 1076, 1049, and 1028 cm^{-1}. Anal. Calcd for $C_{17}H_{31}NO_8 \cdot 0.25H_2O$: C, 53.46; H, 8.31; N, 3.67. Found: C, 53.68; H, 8.62; N, 3.63.

1.7. N^1-Acetoxy-2,2,5,5-tetramethylpyrrolin-3-oyl 2,3,4,6-tetra-*O*-benzyl-α- and -β-D-glucopyranosylamine (9)

1.7.1. Data for the α-anomer. Colorless amorphous powder; R_f 0.47 (2:1 hexane–EtOAc); $[\alpha]_D^{26}$ +50.9 (*c* 1.08, $CHCl_3$); ^{1}H NMR ($CDCl_3$, at 40 °C) δ: 1.28 (6H, s, $CH_3 \times 2$), 1.39 (6H, br s, $CH_3 \times 2$), 2.13 (3H, s, OAc), 3.63–3.69 (3H, m, H-3′, 4′, and 5′), 3.73 (1H, br d, *J* 9.5 Hz, H-6′a), 3.77 (1H, dd, *J* 2.7 and 9.5 Hz, H-6′b), 3.85 (1H, dd, *J* 5.2 and 9.1 Hz, H-2′), 4.58 (2H, s, benzylic CH_2), 4.49 and 4.61 (each 1H, d, *J* 11 Hz, benzylic CH_2), 4.51 and 4.77 (each 1H, d, *J* 11 Hz, benzylic CH_2), 4.80 and 4.92 (each 1H, d, *J* 11 Hz, benzylic CH_2), 5.80 (1H, t, *J* 5.2 Hz, H-1′), 5.58 and 6.05 (each 0.5H, br s, olefinic H), 6.4 (0.5H, br d, *J* 5.2 Hz, > NH–), 6.5 (0.5H, br s, enol OH); FABMS (NBA, *m*/*z*): 749 $(M+H)^+$; IR (KBr) *v*: 3340, 2931, 2869, 1774, 1668, 1514, 1363, 1203, and 1072 cm^{-1}. Anal. Calcd for $C_{45}H_{52}N_2O_8$: C, 72.17; H, 7.00; N, 3.74. Found: C, 72.19; H, 7.01; N, 3.66.

1.7.2. Data for the β-anomer. Colorless prisms; R_f 0.34 (2:1 hexane–EtOAc); mp 122–125 °C (from diethyl ether–diisopropyl ether); $[\alpha]_D^{24}$ −27.9 (*c* 1.005, $CHCl_3$); ^{1}H NMR ($CDCl_3$, at 40 °C) δ: 1.28 (6H, s, $CH_3 \times 2$), 1.39 (6H, s, $CH_3 \times 2$), 2.13 (3H, s, OAc), 3.39 (1H, t, *J* 9.0 Hz, H-2′), 3.52 (1H, d, *J* 9.0 Hz), 3.69–3.79 (4H, m), 4.46 and 4.59 (each 1H, d, *J* 11 Hz, benzylic CH_2), 4.69 and 4.78 (each 1H, d, *J* 11 Hz, benzylic CH_2), 4.54 and 4.81 (each 1H, d, *J* 11 Hz, benzylic CH_2), 4.89 (2H, s, benzylic CH_2), 5.15 (1H, t, *J* 9.0 Hz, H-1′), 5.66 and 5.76 (total 1H, br s, olefinic H), 5.88 (1H, br s, NH), 7.25–7.33 (20H, m, $CH_2Ph \times 4$); FABMS (NBA, *m*/*z*): 749 $(M+H)^+$; IR (KBr) *v*: 3346, 2931, 2868, 1774, 1676, 1531, 1363, 1205, and 1070 cm^{-1}. Anal. Calcd for $C_{45}H_{52}N_2O_8$: C, 72.17; H, 7.00; N, 3.74. Found: C, 72.00; H, 6.84; N, 3.76.

1.8. N^1-Acetoxy-2,2,5,5-tetramethylpyrrolin-3-oyl α-D-glucopyranosylamine (2-α)

The preparation was carried out in the same manner as that used for **1**, using 10% Pd–C. Colorless amorphous powder; R_f 0.42 (3:1 $CHCl_3$–MeOH); $[\alpha]_D^{23}$ +68.1 (*c* 1.045, MeOH); ^{1}H NMR (DMSO-d_6) δ: (pyrroline moiety) 1.16 (6H, s, $CH_3 \times 2$), 1.21 and 1.25 (each 3H, s, $CH_3 \times 2$), 2.09 (3H, s, OAc), 6.53 (1H, s, olefinic H), 7.80 (1H, br d, *J* 8.5 Hz, NH); (sugar moiety) 3.1, 3.2, 3.4, 3.5, 3.6, and 3.7 (each 1H, m), 4.5 (1H, br m, 6′-OH), 4.81 (1H, d, *J* 4.2 Hz, OH), 4.92 (1H, d, *J* 4.6 Hz, OH), 5.06 (1H, d, *J* 4.6 Hz, OH), 5.47 (1H, m, H-1′); FABMS (NBA, *m*/*z*): 389 $(M+H)^+$; IR (KBr) *v*: 3417, 2983, 2933, 1770, 1754, 1668, 1621, 1519, 1211, and 1061 cm^{-1}. Anal. Calcd for $C_{17}H_{28}N_2O_8 \cdot 1.25H_2O$: C, 49.69; H, 7.48; N, 6.82. Found: C, 49.89; H, 7.12; N, 6.48.

1.9. Enzyme-catalyzed hydrolysis of 1-α, 1-β, and 2-α

Porcine liver esterase (40 μL) was added to a PBS buffer solution (pH 8.0, 1 mL) of **1** (2 mg), and the mixture was shaken (120 rpm) at 25 °C for 1 day. The progress of the reaction was monitored by silica-gel TLC (0.25 mm silica-gel F_{254} plates (E. Merck), solvent system: 5:1 $CHCl_3$–MeOH), which was observed by the emission using UV 254 nm light-irradiation and the coloring using a 5% ethanolic solution of phosphomolybdic acid with heat. The enzymes used included the following: Porcine liver esterase (Sigma Chemical Co.); CAL (Novozyme 435 and 525L®, Novo Nordisk Bio Industry Co., and Chirazyme L-2®, Boehringer Mannheim Co.); Lipase AH, PS, AY, and AK (Amano Pharmaceu-

tical Co.); CCL and PPL (Sigma Chemical Co.); Lipase MY and OF (Meito Sangyou Co.).

1.10. 1-Oxyl-2,2,5,5-tetramethylpyrrolin-3-oyl α-D-glucopyranosylamine (14-α)

Pale-yellow solid; R_f 0.39 (3:1 $CHCl_3$–MeOH); $[\alpha]_D^{23}$ +70.9 (*c* 1.30, MeOH); ^{1}H NMR (DMSO-d_6+hydrazobenzene) δ: (PROXYL moiety) 1.16 (6H, s, $CH_3 \times 2$), 1.21 and 1.25 (each 3H, s, $CH_3 \times 2$), 6.54 (1H, s, olefinic H), 7.30 (1H, br d, *J* 8.5 Hz, NH); (sugar moiety) 3.13 (2H, m), 3.34, 3.45, 3.55, and 3.69 (each 1H, m), 4.42 (1H, br m, 6′-OH), 4.80 (1H, m, OH), 4.92 (1H, m, OH), 5.05 (1H, m, OH), 5.47 (1H, m, H-1′); FABMS (NBA, *m*/*z*): 346 $(M+H)^+$; IR (KBr) *v*: 3359, 2977, 2933, 1670, 1622, 1521, 1159, 1103, and 1060 cm^{-1}.

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