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Novel glycosylation of the nitroxyl radicals with peracetylated glycosyl fluorides using a combination of $BF_3{\cdot}OEt_2$ and an amine base as promoters

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Abstract

Glycosylation of the nitroxyl radicals, 4-acetoxy-2,2,6,6-tetramethylpiperidin-1-oxyl (4-acetoxy-TEMPO) and 3-carbamoyl-2,2,5,5-tetramethylpyrollin-1-oxyl (3-carbamoyl-PROXYL) with peracetylglycosyl fluoride as the glycosyl donor, in the presence of boron trifluoride diethyl etherate ($BF_3{\cdot}OEt_2$) and an amine base afforded the corresponding hydroxylamine-*O*-glycosides in 25–100% yields.

Keywords: Nitroxyl radical; Peracetylglycosyl fluoride; $BF_3{\cdot}OEt_2$; Amine base; Glycosylation; Hydroxylamine-*O*-glycoside

1. Introduction

A number of nitroxyl radicals have been developed for use as spin-label reagents, but a radical-masked spin-label reagent has not yet been reported.[1] Quite recently, Yokoyama et al. reported on some non-radical acyl-protected hydroxylamine derivatives as spin-probe reagents for ESR measurements of intracellular oxidative stress.[2] These compounds were hydrolyzed by an esterase in vitro to form hydroxylamines and were rapidly transformed by oxidative stress to the corresponding nitroxyl radicals. If a sugar were to be employed in place of an acyl group, the resulting sugar-protected hydroxylamines would be stable toward hydrolysis by esterase enzymes. These compounds could be useful as new spin-probe reagents because of their high intracellular hydrophilicity and stability and their susceptibility to selective hydrolysis by glycosidase enzymes.

The synthesis of a sugar-protected hydroxylamine has only recently been reported by Yamago et al.[3] In this study, a glycosyl radical, produced by the thermolysis of a telluroglycoside, was trapped with 4-oxo-2,2,6,6-tetramethylpiperidin-1-oxyl to yield 1-*N*-hydroxyl-4-oxo-2,2,6,6,-tetramethylpiperidin-*O*-glucoside in 95% yield, with an α:β stereoselectivity of 33:67. However direct, the glycosylation of nitroxyl radicals using a general glycosylation method has not been reported to date. We have examined a

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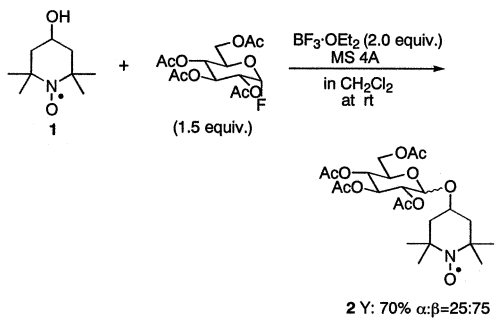
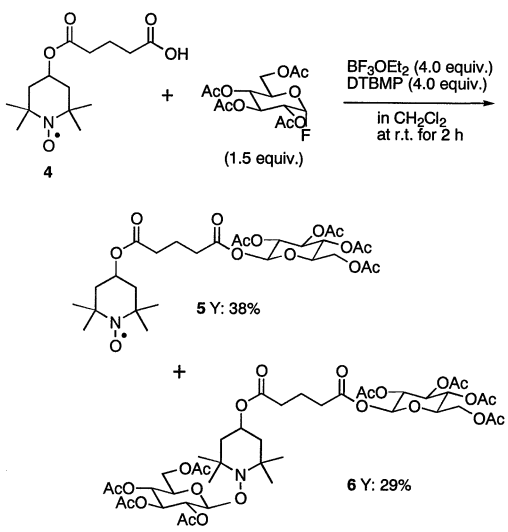
simple and practical synthesis of the *O*-glycoside of the nitroxyl radical (hydroxylamine-*O*-glycoside) as a new spin-probe reagent by employing the general glycosylation conditions developed to date. The results are presented herein.

2. Results and discussion

The nitroxyl radicals, 4-hydroxyl-TEMPO (**1**) and 3-carbamoyl-PROXYL (**9**), were employed as glycosyl acceptors. These compounds are well-known spin-label reagents,[1] and 2,3,4,6-tetra-*O*-acetyl-α- and -β-D-glucopyranosyl fluorides (Glc α- and β-F) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl fluoride (Gal α-F), the glycosyl donors, are readily prepared from glucose and galactose, because they are readily available and stable, have been used extensively.[4] A general glycosylation reaction of **1**, without the protection



Scheme 1.

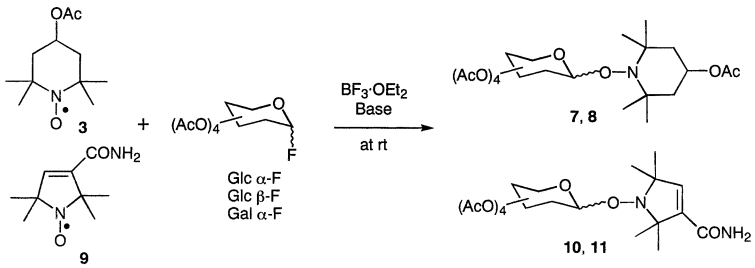


Scheme 2.

of the nitroxyl radical, using Glc α-F as a donor and $BF_3{\cdot}OEt_2$ as a promoter in the presence of powdered molecular sieves 4Å in CH_2Cl_2 gave rise to 4-*O*-(2,3,4,6-tetra-*O*-acetylglucopyranosyl)-TEMPO (**2**) in 70% yield (see Scheme 1). Since the glycosylation of 4-*O*-acetyl-protected **1** (**3**) under the same conditions did not proceed, we conclude that, under these glycosylation conditions, the nitroxyl radical was stable. A glycosylation reaction of the hydroxylamine (4-*O*-acetyl-1-*N*,4-dihydroxyl-2,2,6,6-tetramethylpiperidine) prepared by the hydrogenation of **3** under the same conditions also did not give the hydroxylamine-*O*-glycoside. Further, the Williamson ether synthesis type glycosylation reaction in which the hydroxylamine was treated with a base such as Cs_2CO_3, NaH, or BuLi, to give an alkoxide ion, followed by the addition of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (Glc α-Br), also did not give a reaction product. When only BuLi was used, the corresponding *O*-glycoside was produced in only trace amounts. Thus, the glycosylation methods developed thus far are not suitable for the synthesis of hydroxylamine-*O*-glycosides.

Recently, the glycosylation of acidic hydroxyl groups, such as those on phenols and carboxylic acids, by Glc α-F using a combination of $BF_3{\cdot}OEt_2$ and an amine base, 1,1,3,3-tetramethylguanidine (TMG) or 2,6-di-*tert*-butyl-4-methylpiridine (DTBMP) has been established by Yamaguchi et al.[5] and Kondo et al.[6] In these procedures, the *O*-glycoside was produced in good yield with β-selectivity. When these glycosyl conditions were employed for the glycosylation of glutaric acid TEMPO-4-yl monoester (**4**), surprisingly, it was found that the glutaric acid 4-(1-β-*O*-glucosyl-1-*N*,4-dihydroxylpiperidinyl) 1-β-*O*-glucosyl diester (**5**) was produced with glutaric acid 1-β-*O*-glucosyl TEMPO-4-yl diester (**6**) in 29 and 38% yield, respectively, as shown in Scheme 2. We then applied these glycosylation conditions to the glycosylation of **3**. The results are summarized in Table 1. Initially, employing Kondo's glycosylation conditions, $BF_3{\cdot}OEt_2$ (4 equiv) and DTBMP (4 equiv) as a promoter and CH_2Cl_2 as a solvent, hydroxylamine-β-*O*-glycoside (**7**) was afforded in 25%

Table 1
Glycosylation of the nitroxyl radicals



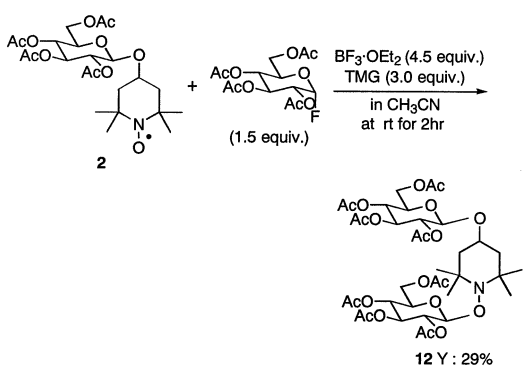
Entry	Radical	Glycosyl F (equiv)	$BF_3{\cdot}OEt_2$ (equiv)	Base (equiv)	Solvent	Reaction time (h)	Yield (%) [a]	Selectivity [b] (α:β)
1	**3**	Glc α-F (1.5)	4	none	CH_2Cl_2	4	0	
2	**3**	Glc α-F (1.5)	4	DTBMP (4)	CH_2Cl_2	2	25	**7** (β)
3	**3**	Glc α-F (1.5)	4	DTBP (4)	CH_2Cl_2	2	47	**7** (β)
4	**3**	Glc α-F (1.2)	1.5	DTBMP (3)	CH_3CN	0.7	50	**7** (16:84)
5	**3**	Glc α-F (1.2)	1.9	DTBP (4)	CH_3CN	1	52	**7** (13:87)
6	**3**	Glc α-F (1.5)	4	TMG (4)	CH_2Cl_2	2	0	
7	**3**	Glc α-F (1.5)	6	TMG (4)	CH_2Cl_2	2	26	**7** (29:71)
8	**3**	Glc α-F (1.5)	4.5	TMG (3)	CH_2Cl_2	2	29	**7** (29:71)
9	**3**	Glc α-F (1.2)	4.5	TMG (3)	CH_3CN	1	53	**7** (19:81)
10	**3**	Glc β-F (1.2)	4.5	TMG (3)	CH_3CN	0.5	50	**7** (14:86)
11	**3**	Gal α-F (1.2)	4.5	TMG(3)	CH_3CN	2.5	63	**8** (29:71)
12	**9**	Glc α-F (1.2)	4.5	TMG (3)	CH_3CN	0.7	73	**10** (37:63)
13	**9**	Gal α-F (1.2)	4.5	TMG (3)	CH_3CN	2	55	**11** (41:59)

[a] Isolated yields after purification by silica-gel column chromatography.
[b] Stereoselectivities were determined by isolation of pure isomers or HPLC analysis or ^{1}H NMR spectroscopy.

yield (entry 2). When 2,6-di-*tert*-butylpyridine (DTBP) was used in the place of DTBMP, the yield of the β-glycoside increased to 47% (entry 3). Both reactions gave 4-acetoxy-1-*N*-hydroxyl-2,2,6,6-tetramethylpiperidine (hydroxylamine) as a byproduct in ca. 50% yield. The disappearance of the nitroxyl radical was observed by the appearance of a colorless **3** (λ_{max} 420 nm). When the order of the addition of the amine and Lewis acid was changed, both the yield and selectivity remained unchanged.[5] When $BF_3{\cdot}OEt_2$ was added to a solution of the nitroxyl radical and the amine base, the nitroxyl radical was not completely consumed in the absence of Glc α-F. When Glc α-F was finally added to the resulting mixture, the radical completely disappeared, and the corresponding hydroxylamine-*O*-glycoside was produced. On changing the solvent from CH_2Cl_2 to CH_3CN, the yields increased to 50 and 52%, but the β-selectivity decreased to α:β = 16:84 and 13:87, respectively (entries

4 and 5). Yamaguchi's conditions using a combination of $BF_3{\cdot}OEt_2$ and TMG as a promoter and CH_2Cl_2 as a solvent were next employed. The use of $BF_3{\cdot}OEt_2$ (4 equiv) and TMG (4 equiv) did not afford a glycoside (entry 6), but $BF_3{\cdot}OEt_2$ (6 equiv) and TMG (4 equiv) resulted in a 26% yield with a selectivity of $\alpha{:}\beta = 29{:}71$ (entry 7). When the solvent was changed from CH_2Cl_2 to CH_3CN, the yield increased to 53% with a selectivity of $\alpha{:}\beta = 19{:}81$ (entry 9). Since the change in the glycosyl donor from Glc α-F to Glc β-F had no effect on the yield and stereoselectivity (entry 10), it was assumed that the glycosylation reaction under these reaction conditions might proceed, not by a S_N2-type process, but, rather by the neighboring group participation of the carbonyl oxygen at the C-2.[5,6] Glycosylation by Gal α-F also gave the corresponding galactoside **8** in 63% yield with an α:β selectivity of 27:71 (entry 11). When 3-carbamoyl-PROXYL (**9**) was employed as a glycosyl acceptor with CH_3CN as the solvent, the yield of the glycosylation by Glc α-F and Gal α-F reached 73 and 55% with an α:β selectivity of 37:63 and 41:59, respectively (entries 12 and 13). Finally, when Yamaguchi's conditions were used in the glycosylation of the 4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl) TEMPO (**2**), 1-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-1-*N*,4-dihydroxyl-2,2,6,6-tetramethylpiperadine (**12**) was produced in 29% yield (Scheme 3).

Thus, the use of TMG in CH_3CN gave the hydroxylamine-*O*-glycoside (**10**) in a maximum of 73% yield (radical **9** is not soluble in CH_2Cl_2). The use of DTBMP or DTBP as the amine base in CH_2Cl_2 gave only the β-glycoside in low to moderate yield. In this series of glycosylation reactions of nitroxyl radicals **3** or **9**, the corresponding hydroxylamine and an unknown compound were obtained as byproducts. However, since the direct glycosylation of hydroxylamine under the same conditions gave no the corresponding glycoside, it would appear that, under these reaction conditions, the glycosylation of the nitroxyl radical does not proceed via the hydroxylamine. (Although the glycosylation reaction of the hydroxylamine, in the presence of a combination of $BF_3{\cdot}OEt_2$ and TMG, in fact, gave the hydroxylamine-*O*-glycoside in ca. 3% yield, it seems reasonable to assume that these products were afforded by the glycosylation of the nitroxyl radical that was partially formed by the oxidation of the hydroxylamine in situ.) It is known that the glycosylation conditions employed here also do not proceed via radicals. In fact, hydroxylamine and a small amount of the unknown non-radical product were produced as byproducts. In the absence of an amine base, the glycosylation of the nitroxyl radicals produced no glycoside (Table 1, entry 1), though the glycosylation of an acidic hydroxyl group proceeded without an amine base in high yield and with α-selectivity.[5] A combination of $BF_3{\cdot}OEt_2$ and the less hindered amine, 2,6-lutidine yielded no glycoside. We then used 2 mols of 4-acetoxy-TEMPO (**3**) per mol of glycosyl fluoride. The results are summarized in Table 2. As expected, the yield increased to 98% (entry 3). When a threefold excess of **3** was used, the glycoside was produced in quantitative yield (entry 8). In addition to the data in Table 1, variations in the order of addition of reagents had no influence on yield and selectivity. Using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as a strongly basic amine, the same result was obtained as for TMG (entry 9). Since Kondo et al. reported that these reaction conditions are not applicable to the glycosylation of simple alcohols such as cyclohexanol and α,β-phenethyl alcohol,[6] it is assumed that the hydroxylamine prepared by hydrogenation could not react under these reaction condi-



Scheme 3.

Table 2
Glycosylation of the nitroxyl radical with per-*O*-acetylglucopyraosyl fluoride using a combination of $BF_3{\cdot}OEt_2$ and amine base

Entry	Nitroxyl radical (equiv)	Glc α-F (equiv)	$BF_3{\cdot}OEt_2$ (equiv)	Base (equiv)	Solvent	Time (h)	Product yield (%)	Selectivity (α:β)
1	**3** (2.0)	1.0	6.0	TMG (4.0)	CH_3CN	2	**7** (91)	22:78
2	**3** (2.0)	1.0	9.0	TMG (6.0)	CH_3CN	1.5	**7** (95)	20:80
3	**3** (2.0)	1.0	9.0	TMG (6.0)	CH_3CN	16	**7** (98)	28:72
4	**3** (2.0)	1.0	9.0	TMG (6.0)	CH_3CN	1.5	**7** (95)	20:80
5	**3** (2.0)	1.0	3.0	DTBMP (2.0)	CH_2Cl_2	16	**7** (56)	13:87
6	**3** (2.0)	1.0	3.0	DTBMP (6.0)	CH_3CN	48	**7** (87)	29:71
7	**9** (2.0)	1.0	9.0	TMG (6.0)	CH_3CN	1	**10** (78)	15:85 [a]
8	**3** (3.0)	1.0	13.5	TMG (9.0)	CH_3CN	2	**7** (quant)	20:80
9	**3** (3.0)	1.0	13.5	DBU (9.0)	CH_3CN	5	**7** (quant)	24:76

[a] Determined by ^{1}H NMR spectrum.

tions. Although a highly hindered amine, DTBMP, has used to improve the yield and β-selectivity of the reaction,[6] and the strong basic amine, TMG, for the β-selectivity,[5] it seems that, in this reaction, these amines promote the formation of the hydroxylamine anion. Therefore, strongly basic amines, such as TMG and DBU, resulted in a better yield than the highly hindered amine, DTBMP (entries 5 and 6). This is the first description of a one-pot glycosylation of nitroxyl radicals by a per-*O*-acetylglycosyl fluoride, though Cinget et al. reported that the hydroxylamine-*O*-glucoside, in the triflate glycosylation of 1-*O*-acetyl-1-*N*,4-dihydroxyl-2,2,6,6-tetramethylpiperid ine, was afforded as a byproduct in ca. 7% yield.[7] Further considerations of the mechanism is under study.

3. Experimental

Dry CH_2Cl_2, CH_3CN, and MeOH used in this reaction were prepared by distillation from CaH_2 or Mg. For separation and purification (1) flash-column chromatography was performed on silica gel (230–400 mesh, Fuji-Silysia Co., Ltd., BW-300); (2) HPLC was performed using an Inertsil ODS-3 column (GL Science; 5 μm, 20 × 250 mm, mobile phase; MeOH–water). Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Mass spectra data were obtained by the electron-ionization (EI) method and fast-atom bombardment (FAB) method using 3-nitrobenzyl alcohol as the matrix on a JEOL JMS-AX505HA mass spectrometer. Optical rotations were recorded on a JASCO DIP-370 polarimeter. Elemental analyses were performed on a Perkin–Elmer PE 2400 II. The ^{1}H NMR spectra were recorded on a Varian Inova 500 spectrometer using Me_4Si as the internal reference.

Per-*O*-acetyl-α-D-gluco- and galactopyranosyl fluorides were, respectively, prepared from per-*O*-acetyl-D-glucose and D-galactose with the HF–pyridine complex.[8] Per-*O*-acetyl-β-D-glucopyranosyl fluoride was prepared from per-*O*-acetyl-α-D-glucopyranosyl bromide with silver fluoride in dry CH_3CN.[9] Per-*O*-acetyl-α-D-glucopyranosyl bromide was prepared from per-*O*-acetyl-D-glucose with 30% HBr in AcOH. 4-Hydroxyl-TEMPO (**1**)

was prepared by oxidation of 4-hydroxyl-2,2,6,6,-tetramethylpiperidine using sodium tungstate, hydrogen peroxide and disodium salts of ethylenediaminetetraacetic acid.[1b] 4-Acetoxy-TEMPO (**3**) was prepared by acetylation of **1** with Ac_2O and pyridine. 3-Carbamoyl-PROXYL (**9**) was prepared from 2,2,6,6-tetramethyl-4-piperidone via a three-reaction process.[1b]

4-(2,3,4,6-Tetra-O*-acetyl-α- and -β-*D*-glucopyranosyloxy)-2,2,6,6,-tetramethylpiperidine* N*-oxide* (**2**).—To a stirred mixture of 4-hydroxy-TEMPO (**3**, 400 mg, 2.32 mmol), Glc α-F (813 mg, 2.32 mmol), and powdered molecular sieves 4 Å (200 mg) in dry CH_2Cl_2 (4 mL), $BF_3{\cdot}OEt_2$ (584 μL, 4.64 mmol) was added at 0 °C, and the mixture was stirred at rt for 6 h under an Ar atmosphere. The reaction mixture was quenched with cold water and filtered through a Celite® pad. The filtrate was extracted with EtOAc three times. The extract was washed with brine and dried over anhyd Na_2SO_4. The organic solvent was removed under reduced pressure. The residual syrup was separated and purified by silica-gel column chromatography (2:1 hexane–EtOAc, then 20:1 $CHCl_3$–MeOH) to give the α anomer (204 mg, 17.5%) as a reddish viscous oil and the β anomer (512 mg, 52.5%) as pale-red needles.

Data for the α anomer. Reddish viscous oil; R_f 0.39 (2:1 hexane–EtOAc); $[\alpha]_D^{25}$ +99.7° (*c* 1.10, $CHCl_3$); ^{1}H NMR ($CDCl_3$ + hydrazobenzene): δ 1.19, 1.22, 1.25, 1.27 (each 3 H, s, $CH_3 \times 4$), 1.51–1.80 (2 H, m, CH_2), 1.82–2.02 (2 H, m, CH_2), 2.02, 2.04, 2.07, 2.09 (each 3 H, s, OAc × 4), 4.05–4.13 (2 H, m, *J* 2.5, 12.7 Hz, H-6′b, 5′), 4.09 (1 H, dd, *J* 2.5, 12.7 Hz, H-6′a), 4.13 (1 H, dd, *J* 3.6, 10.2 Hz, H-2′), 5.06 (1 H, t, *J* 9.9 Hz, H-3′), 5.22 (1 H, d, *J* 3.6 Hz, H-1′), 5.45 (1 H, t, *J* 9.9 Hz, H-4′); EIMS (*m*/*z*): 502 $[M]^+$; Anal. Calcd for $C_{23}H_{36}NO_{11}{\cdot}CH_3OH$: C, 53.92; H, 7.54; N, 2.62. Found: C, 53.70; H, 7.30; N, 2.25.

Data for the β anomer. Pale-red needles (from EtOAc); R_f 0.34 (2:1 hexane–EtOAc); mp 150–152 °C; $[\alpha]_D^{25}$ −13.4° (*c* 1.08, $CHCl_3$); ^{1}H NMR ($CDCl_3$ + hydrazobenzene): δ 1.16 (6 H, s, $CH_3 \times 2$), 1.19, 1.20 (each 3 H, s, $CH_3 \times 2$), 1.40–1.65 (2 H, m, CH_2), 1.79–2.00 (2 H, m, CH_2), 2.01, 2.03, 2.04, 2.08 (each 3 H, s, OAc × 4), 3.71 (1 H, m, H-5′), 3.91 (1 H, m, H-4), 4.00 (1 H, dd, *J* 2.0, 12.0 Hz, H-6′a), 4.25 (1 H, dd, *J* 4.8, 12.0 Hz, H-6′b), 4.60 (1 H, d, *J* 7.9 Hz, H-1′), 4.95 (1 H, dd, *J* 7.9, 9.2 Hz, H-2′), 5.06 (1 H, dd, *J* 9.6, 9.9 Hz, H-4′), 5.21 (1 H, dd, *J* 9.2, 9.6 Hz, H-3′); EIMS (*m*/*z*): 502 $[M]^+$; Anal. Calcd for $C_{23}H_{36}NO_{11}$: C, 54.97; H, 7.22; N, 2.79. Found: C, 54.76; H, 7.18; N, 2.72.

2,2,6,6-tetramethylpiperidin-4-yl N*-oxide) (2,3,4,6-tetra*-O*-acetyl-β-*D*-glucopyranosyl)-glutarate* (**5**).—Pale-red prisms (from ether); mp 127–128 °C; $[\alpha]_D^{17}$ +5.40° (*c* 1.00, $CHCl_3$); ^{1}H NMR (+hydrazobenzene, in $CDCl_3$):?δ 1.197, 1.205 (each 6 H, s, $CH_3 \times 6$), 1.55 (2 H, t, *J* 11.7 Hz), 1.89 (2 H, dd, *J* 4.0, 12.5 Hz), 1.92 (2 H, t, *J* 7.3 Hz), 2.01, 2.03, 2.04, 2.09 (each 3 H, s, OAc × 4), 2.32 (2 H, t, *J* 7.3 Hz, CH_2), 2.44 (2 H, dt, *J* 2.9, 7.5 Hz, CH_2), 3.84 (1 H, ddd, *J* 2.2, 4.5, 9.6 Hz, H-5′), 4.11 (1 H, dd, *J* 2.2, 12.4 Hz, H-6a), 4.29 (1 H, dd, *J* 4.5, 12.4 Hz, H-6b), 5.05 (1 H, m, >CH–), 5.13 (1 H, t, *J* 9.6 Hz, H-4′), 5.14 (1 H, dt, *J* 8.3, 9.7 Hz, H-2′), 5.25 (1 H, t, *J* 9.4 Hz, H-3′), 5.73 (1 H, d, *J* 8.3 Hz, H-1′). FABMS (NBA, *m*/*z*) 617 [M + H], 301, 331, 169; Anal. Calcd for $C_{28}H_{42}NO_{14}$: C, 54.54; H, 6.87; N, 2.27. Found: C, 54.76; H, 6.87; N, 2.21.

1-(2,3,4,6-tetra-O*-acetyl-β-*D*-glucopyranosyloxy-2,2,6,6-tetramethylpiperidin-4-yl) (2,3,4,6-tetra*-O*-acetyl-β-*D*-glucopyranosyl)glutarate* (**6**).—White prisms (from ether); mp 112–113 °C; $[\alpha]_D^{17}$ +1.57° (*c* 1.02, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.15 (6 H, s, $CH_3 \times 2$), 1.21 (3 H, s, CH_3), 1.27 (3 H, s, CH_3), 1.53 (2 H, m, CH_2), 1.84 (2 H, m, CH_2), 1.91 (2 H, t, *J* 7.3 Hz, CH_2), 2.31 (2 H, t, *J* 7.2 Hz, CH_2), 2.43 (2 H, dt, *J* 3.2, 7.3 Hz, CH_2), 3.69 (1 H, ddd, *J* 2.5, 4.9, 7.5 Hz, H-5″), 3.84 (1 H, ddd, *J* 2.4, 4.4, 6.7 Hz, H-5′), 4.11 (1 H, dd, *J* 2.0, 12.5 Hz, H-6′a), 4.14 (1 H, dd, *J* 2.0, 12.5 Hz, H-6″a), 4.22 (1 H, dd, *J* 5.0, 12.5 Hz, H-6″b), 4.29 (1 H, dd, *J* 4.5, 12.5 Hz, H-6′b), 4.83 (1 H, d, *J* 7.5 Hz, H-1″), 5.00 (1 H, m, >CH–), 5.05 (1 H, t, *J* 9.5 Hz, H-3″), 5.07 (1 H, t, *J* 9.5 Hz, H-2″), 5.13 (1 H, t, *J* 9.5 Hz, H-3′), 5.135 (1 H, t, *J* 9.0 Hz, H-2′), 5.20 (1 H, t, *J* 9.5 Hz, H-4′), 5.25 (1 H, t, *J* 9.7 Hz, H-4″); FABMS (NBA, *m*/*z*) 948 [M + H], 601, 331, 169; Anal. Calcd for $C_{42}H_{61}NO_{23}$: C, 53.21; H,

6.49; N, 1.48. Found: C, 52.88; H, 6.38; N, 1.31.

1-(2,3,4,6-Tetra-O-*acetyl-α- and -β-*D-*glucopyranosyloxy)-4-acetoxy-2,2,6,6-tetramethylpiperidine* (**7**).—To a mixture of nitroxyl radical (**3**, 1.00 g, 4.67 mmol), Glc α-F (1.96 g, 5.61 mmol), and TMG (1.76 mL, 14.0 mmol) in dry CH_3CN (5 mL), $BF_3{\cdot}OEt_2$ (2.59 mL, 21.0 mmol) was added at rt under an Ar atmosphere. After stirring for 2 h, the mixture was quenched with satd aq $NaHCO_3$ and extracted twice with EtOAc. The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4. The organic solvent was removed under reduced pressure. The residual syrup was purified by chromatography on silica gel (hexane–EtOAc) to give the α anomer (0.260 g) and β anomer (1.107 g) in 53.7% total yield. The β anomer was recrystallized from ether or EtOAc to give colorless prisms.

Data for the α anomer. Colorless; R_f 0.34 (2:1 hexane–EtOAc); $[\alpha]_D^{25}$ +83.4° (*c* 1.14, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.20, 1.26, 1.29, 1.33 (each 3 H, s, $CH_3 \times 4$), 1.50–1.62 (2 H, m, CH_2), 1.85, 1.90 (each 1 H, m, CH_2), 2.00, 2.01, 2.02, 2.04, 2.05, 2.07 (each 3 H, s, OAc × 6), 4.08 (1 H, d, *J* 2.2, 12.2 Hz, H-6′a), 4.16 (1 H, ddd, *J* 2.2, 4.1, 9.3 Hz, H-5′), 4.26 (1 H, dd, *J* 4.1, 12.2 Hz, H-6′b), 4.99 (1 H, m, H-4), 5.11 (1 H, t, *J* 9.3 Hz, H-4′), 5.18 (1 H, dd, *J* 4.4, 10.7 Hz, H-2′), 5.34 (1 H, d, *J* 4.4 Hz, H-1′), 5.39 (1 H, dd, *J* 9.3, 10.7 Hz, H-3′); FABMS (*m*/*z*): 546 [M + H], 331, 214; Anal. Calcd for $C_{25}H_{39}NO_{12}$: C, 55.03; H, 7.21; N, 2.57. Found: C, 55.29; H, 7.57; N, 2.70.

Data for the β anomer. Colorless prisms (from EtOAc); R_f 0.29 (2:1 hexane–EtOAc); mp 177.5–178.5 °C; $[\alpha]_D^{25}$ −33.0° (*c* 1.05, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.15 (6 H, s, $CH_3 \times 2$), 1.21, 1.27 (each 3 H, s, $CH_3 \times 2$), 1.55 (2 H, m, CH_2), 1.85 (2 H, m, CH_2), 3.69 (1 H, ddd, *J* 2.5, 5.0, 9.5 Hz, H-5′), 4.14 (1 H, dd, *J* 2.5, 12.0 Hz, H-6′a), 4.22 (1 H, dd, *J* 5.0, 12.0 Hz, H-6′b), 4.84 (1 H, d, *J* 8.5 Hz, H-1′), 4.99 (1 H, m, H-4), 5.05 (1 H, dd, *J* 8.5, 9.5 Hz, H-3′), 5.22 (1 H, t, *J* 9.5 Hz, H-4′); FABMS (*m*/*z*): 546 [M + H], 331, 214; Anal. Calcd for $C_{25}H_{39}NO_{12}$: C, 55.03; H, 7.21; N, 2.57. Found: C, 55.30; H, 7.34; N, 2.53.

1-(2,3,4,6-Tetra-O-*acetyl-α- and -β-*D-*galactopyranosyloxy)-4-acetoxy-2,2,6,6-tetramethylpiperidine* (**8**).—The reaction, work-up, and isolation were carried out in the same manner mentioned above.

Data for the α anomer. Colorless; $[\alpha]_D^{25}$ +86.6° (*c* 1.10, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 1.19, 1.27, 1.32 (12 H, s, $CH_3 \times 4$), 1.52–1.62 (2 H, m, CH_2), 1.84–1.92 (2 H, m, CH_2), 1.98, 2.02, 2.07, 2.15 (12 H, s, OAc × 4), 4.07 (1 H, dd, *J* 6.5, 16.0 Hz, H-6′a), 4.15 (1 H, dd, *J* 6.5, 16.0 Hz, H-6′b), 4.38 (1 H, m, H-5′), 4.99 (1 H, m, H-4), 5.26 (1 H, dd, *J* 3.3, 11.5 Hz, H-3′), 5.37 (1 H, d, *J* 4.3 Hz, H-1′), 5.47 (1 H, dd, *J* 4.3, 11.5 Hz, H-2′), 5.51 (1 H, d, *J* 3.3 Hz, H-4′); FABMS (*m*/*z*): 546 [M + H]; Anal. Calcd for $C_{25}H_{39}NO_{12}$: C, 55.03; H, 7.21; N, 2.57. Found: C, 55.16; H, 7.69; N, 2.28.

Data for the β anomer. White prisms (from ether); mp 77–78 °C; $[\alpha]_D^{25}$ −23.8° (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.16, 1.17, 1.22, 1.27 (each 3 H, s, $CH_3 \times 4$), 1.55, 1.85 (each 2 H, m, $CH_2 \times 2$), 1.98, 2.02, 2.04, 2.08, 2.17 (each 3 H, s, OAc × 5), 3.90 (1 H, ddd, *J* 1.1, 6.5, 7.1 Hz, H-5′), 4.08 (1 H, dd, *J* 7.1, 11.0 Hz, H-6′a), 4.18 (1 H, dd, *J* 6.5, 11.0 Hz, H-6′b), 4.81 (1 H, d, *J* 8.4 Hz, H-1′), 5.00 (1 H, m, H-4), 5.04 (1 H, dd, *J* 3.4, 10.5 Hz, H-3′), 5.23 (1 H, dd, *J* 8.4, 10.5 Hz, H-2′), 5.36 (1 H, dd, *J* 1.1, 3.4 Hz, H-4′); FABMS (*m*/*z*): 546 [M + H]; Anal. Calcd for $C_{25}H_{39}NO_{12}$: C, 55.03; H, 7.21; N, 2.57. Found: C, 54.90; H, 7.40; N, 2.42.

1-(2,3,4,6-Tetra-O-*acetyl-α- and -β-*D-*glucopyranosyloxy)-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrole-3-carboxamide* (**10**).—Since the α,β-mixture was inseparable by silica-gel TLC or HPLC, separation and purification were carried out as follows: the β anomer from an EtOAc extract was isolated by direct crystallization with ether. Removal of the β anomer by precipitation from the mother liquor with Et_2O–isopropyl ether several times isolated the α anomer.

Data for the α anomer. Colorless; $[\alpha]_D^{24}$ +72.7° (*c* 1.03, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.22–1.55 (12 H, m, $CH_3 \times 4$), 2.02 2.05, 2.09, 2.11 (each 3 H, s, OAc × 4), 4.16 (1 H, m, H-6′a), 4.25 (2 H, m, H-6′b, 5′), 5.07 (2 H, m, *J* 4.3, 10.6 Hz, H-2′, 4′), 5.30 (1 H, brs, H-1′), 5.44 (1 H, t, *J* 10.0 Hz, H-3′), 5.70 (2 H, brs, $CONH_2$), 6.04 (1 H, s, olefinic H); FABMS

(m/z): 515 [M + H]; Anal. Calcd for $C_{23}H_{34}N_2O_{11}$: C, 53.69; H, 6.66; N, 5.44. Found: C, 53.59; H, 6.93; N, 5.31.

Data for the β anomer. Colorless (from ether); mp 205 °C; $[\alpha]_D^{24}$ +4.60° (*c* 1.00, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.16–1.48 (12 H, m, $CH_3 \times 4$), 2.01, 2.04, 2.06, 2.09 (each 3 H, s, OAc × 4), 3.75 (1 H, m, H-5′), 4.18 (2 H, m, H-6′a, b), 4.78 (1 H, d, *J* 8.5 Hz, H-1′), 5.04 (1 H, dd, *J* 8.5, 9.5 Hz, H-2′), 5.08 (1 H, t, *J* 9.5 Hz, H-4′), 5.21 (1 H, t, *J* 9.5 Hz, H-3′), 5.43 (2 H, brs, $CONH_2$), 6.03 (1 H, s, olefinic H); FABMS (m/z): 515 [M + H]; Anal. Calcd for $C_{15}H_{26}N_2O_7$: C, 52.01; H, 7.57; N, 8.09. Found: C, 52.20; H, 7.99; N, 7.73.

1-(2,3,4,6-Tetra-O-*acetyl-α- and -β-*D-*galactopyranosyloxy)-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrole-3-carboxamide* (**11**).—Separation and purification were carried out as follows: the β anomer from an EtOAc extract with ether from (colorless prisms, 100 mg, 23.2%). The residual mother liquor was separated by HPLC (ODS-column, mobile phase: 7:1 MeOH–water, t_R: 4.7 min (β anomer), 5.5 min (α anomer)) to give the β anomer (40 mg, 9.3%) and α anomer (97 mg, 22.5%) as a colorless amorphous powder.

Data for the α anomer. Colorless; $[\alpha]_D^{24}$ +109° (*c* 1.02, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.22–1.53 (12 H, m, $CH_3 \times 4$), 2.00, 2.04, 2.12, 2.16 (each 3 H, s, OAc × 4), 4.13 (2 H, m, H-6′a, b), 4.45 (1 H, m, H-5), 5.33 (3 H, m, H-1′, 2′, 3′), 5.51 (1 H, dd, *J* 1.5, 2.5 Hz, H-4′), 6.03 (1 H, s, olefinic H), 5.22–5.70 (2 H, brs, NH_2); FABMS (m/z): 515 [M + H]; Anal. Calcd for $C_{23}H_{34}N_2O_{11}$: C, 53.69; H, 6.66; N, 5.44. Found: C, 53.60; H, 6.99; N, 5.06.

Data for the β anomer. Colorless prisms (from ether); mp 125 °C; $[\alpha]_D^{24}$ +7.56° (*c* 1.00, $CHCl_3$); ^1H NMR ($CDCl_3$):?δ 1.27–1.59 (12 H, m, $CH_3 \times 4$), 1.99, 2.02, 2.10, 2.17 (each 3 H, s, OAc × 4), 3.95 (1 H, brt, *J* 6.6 Hz, H-5′), 4.12–4.20 (2 H, m, H-6′a, b), 4.75 (1 H, d, *J* 8.5 Hz, H-1′), 5.03 (1 H, dd, *J* 10.3, 3.7 Hz, H-3′), 5.27 (1 H, dd, *J* 10.3, 8.5 Hz, H-2′), 5.38 (1 H, m, H-4′), 5.20–5.66 (2 H, brs, NH_2), 6.03 (1 H, s, olefinic H); FABMS (m/z): 515 [M + H]; Anal. Calcd for $C_{23}H_{34}N_2O_{11}\cdot 0.3\ H_2O$: C, 53.13; H, 6.71; N, 5.39. Found: C, 53.16; H, 6.88; N, 5.35.

1,4-Bis-(2,3,4,6-tetra-O-*acetyl-β-*D-*gluco-pyranosyloxy) - 2,2,6,6 - tetramethylpiperidine* (**12**).—Colorless prisms (from MeOH–EtOH); mp 218–220 °C; 486, 331, 169; $[\alpha]_D^{17}$ −22.8° (*c* 0.92, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.09, 1.10, 1.19, 1.26 (each 3 H, s, $CH_3 \times 3$), 1.45 (1 H, t, *J* 12.2 Hz, CH_2), 1.53 (1 H, t, *J* 12.2 Hz, CH_2), 1.74 (1 H, m, CH_2), 1.91 (1 H, m, CH_2), 2.00 (6 H, s, OAc × 2), 2.02, 2.03, 2.04 (each 3 H, s, OAc × 3), 2.07 (9 H, s, OAc × 3), 3.69 (2 H, m, H-5′, 5″), 3.86 (1 H, m, >CH–), 4.12 (2 H, m, H-6′a, 6″a), 4.23 (2 H, dd, *J* 5.3, 12.5 Hz, H-6′b, 6″b), 4.56 (1 H, d, *J* 7.8 Hz, H-1″), 4.81 (1 H, d, *J* 8.3 Hz, H-1′), 4.93 (1 H, dd, *J* 8.3, 9.2 Hz, H-2″), 5.01–5.08 (3 H, m, H-2′, 4′, 4″), 5.17–5.23 (2 H, m, H-3′, 3″); FABMS (NBA, m/z) 834 [M + H], Anal. Calcd for $C_{37}H_{55}O_{20}N$: C, 53.29; H, 6.65; N, 1.68. Found: C, 53.48; H, 6.67; N, 1.56.

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