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Note

Synthesis and hypolipidemic activity of *N*-phthalimidomethyl tetra-*O*-acyl-α-D-mannopyranosides

Rajendra M. Srivastava,[a,*] Fernando J.S. Oliveira,[a] Ladjane P. da Silva,[a] João R. de Freitas Filho,[a] Shalom P. Oliveira,[b] Vera L.M. Lima[b]

[a]*Departamento de Química Fundamental, Universidade Federal de Pernambuco, Cidade Universitária, 50.740-540 Recife, PE, Brazil*

[b]*Departamento de Bioquímica, Universidade Federal de Pernambuco, Cidade Universitária, 50.670-420 Recife, PE, Brazil*

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Abstract

A facile synthesis of anomerically pure phthalimidomethyl 2,3,4,6-tetra-*O*-acetyl- and phthalimidomethyl 2,3-di-*O*-acetyl-4,6-di-*O*-benzoyl-α-D-mannopyranosides (**6** and **9b**) starting from *N*-hydroxymethylphthalimide and tri-*O*-acetyl-D-glucal is described. Compounds **3**, **6**, **8**, **9a** and **9b** have been tested for their hypolipidemic activity in mice. All these compound showed significant reduction of plasma cholesterol and triglyceride levels. Compound **9b** has been found to possess the highest activity.

Keywords: α-D-Mannopyranoside; *N*-Hydroxymethylphthalimide; Tri-*O*-acetyl-D-glucal

Phthalimide derivatives are known to display antitumor,[1] anticonvulsant,[2] and hypolipidemic[3,4] activities. Our own group has been interested in N-substituted phthalimides and found that *N*-[3-phenyl-1,2,4-oxadiazol-5-yl]methylphthalimide possesses enhanced analgesic activity.[5] A recent report describes the synthesis of methyl 2,6-anhydro-3-deoxy-3-phthalimido-α-D-mannopyranoside and its ^{15}N-labeled analog, for which the spectroscopic and stereochemical studies were carried out,[6] but no biological activity tests have been reported. The literature does not record a phthalimidoylalkyl moiety as an aglycone in mannopyranoside derivative. If such glycoside is prepared, the carbohydrate moiety might play an important role as a carrier of this group to an appropriate site suited for the pharmacological activity. With this objective in mind, the synthesis of phthalimidoylmethyl mannopyranoside was planned and, indeed, compounds **3**, **6**, **8**, **9a** and **9b** exhibited significant hypolipidemic activity. The results are described below.

The synthesis of *N*-phthalimidomethyl 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranoside (**3**) was achieved in high yield by the reaction of *N*-hydroxymethylphthal-

* Corresponding author. Tel.: +55-81-27184443; fax: +55-81-2718442.

E-mail address: rms@npd.ufpe.br (R.M. Srivastava).

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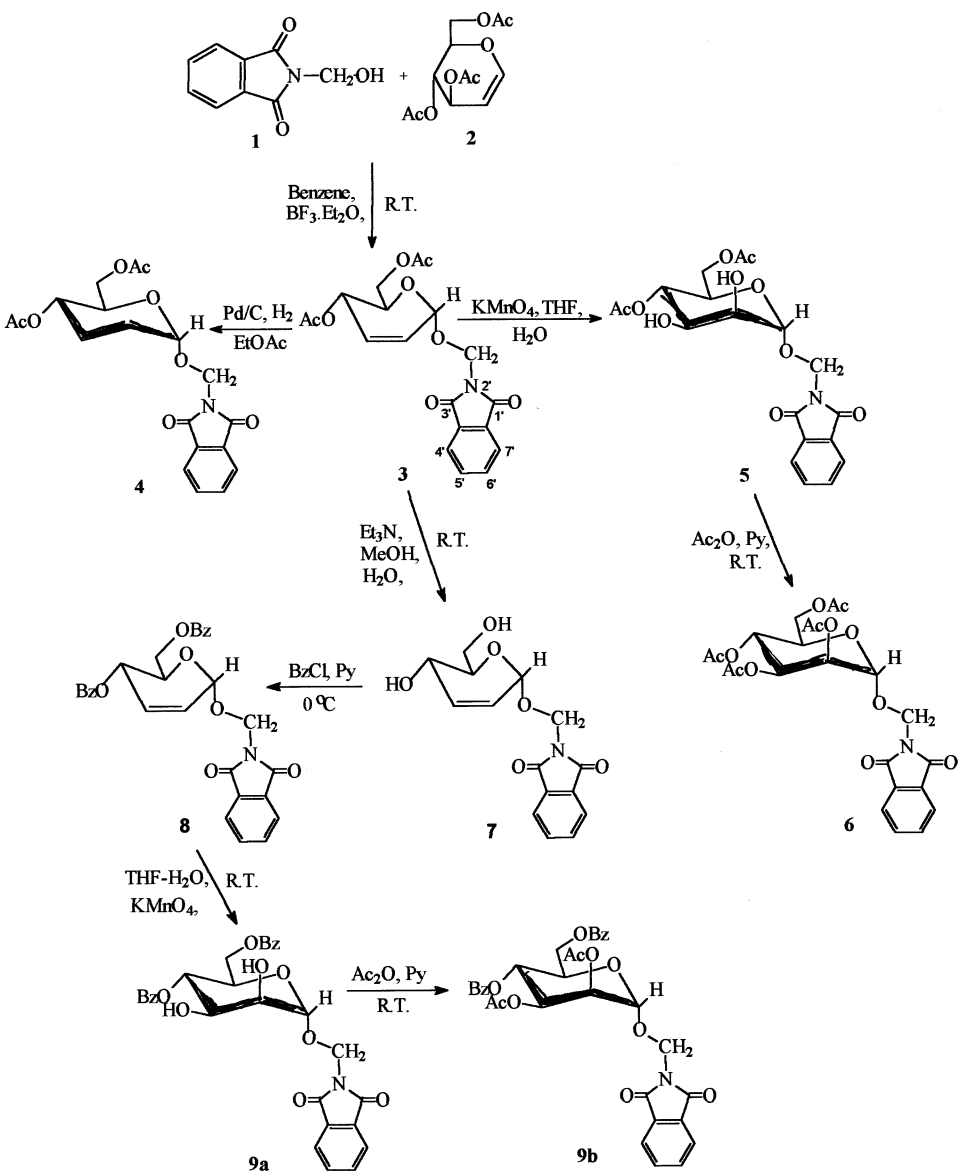
imide (**1**) with 3,4,6-tri-*O*-acetyl-1,2-dideoxy-D-*arabino*-hex-1-enopyranose (tri-*O*-acetyl-D-glucal) (**2**) using Ferrier's method[7] (Scheme 1). Although, it is known[7] that the unsaturated sugars formed by Ferrier's method contain more than 90% of α anomer, in the present work, only one product was isolated. In order to confirm the anomeric configuration of **3**, we hydrogenated it. The product **4** gave an anomeric proton at δ 5.07 ppm as a broad singlet ($W/2 \approx 4.08$ Hz) indicating the axial configuration of the phthalimidomethyloxy group. Hence, it is concluded that compound **3** is exclusively the α anomer. Once the anomeric configuration of **3** has been established, we proceeded to treat it with potassium permanganate. The reaction furnished presumably a diol **5**, but it was difficult to establish the configuration of the hydroxyl groups at C-2 and C-3 by ^{1}H NMR. We acetylated **5** and got the tetra-*O*-acetyl derivative **6**. The ^{1}H NMR spectrum of this compound showed four signals between δ 2.07 and 2.13 ppm due to acetyl groups. However, the ^{1}H NMR spectrum turned out to be more complicated because H-2, H-3, H-4 and –N–CH_2–O– protons appeared between δ 5.20 and 5.40 ppm. All attempts to determine the configurations at C-2 and C-3 by NMR spectroscopy failed. Even the shift reagent did not help to



Scheme 1.

Table 1
Effect of compounds **3**, **6**, **8**, **9a** and **9b** on mice plasma cholesterol and triglyceride levels of mice [a]

Compound	Cholesterol (mmol/L)		Triglycerides (mmol/L)	
	Before	After	Before	After
3 [b]	3.43 ± 0.29	2.71 ± 0.37 [e]	1.94 ± 0.23	1.44 ± 0.24 [d]
6 [b]	2.98 ± 0.26	2.42 ± 0.22 [f]	1.65 ± 0.08	1.35 ± 0.08 [e]
8 [c]	3.07 ± 0.34	2.54 ± 0.26 [f]	1.63 ± 0.15	1.28 ± 0.12 [f]
9a [c]	3.40 ± 0.46	2.91 ± 0.51 [e]	1.92 ± 0.18	1.48 ± 0.13 [d]
9b [c]	3.43 ± 0.26	2.93 ± 0.28 [d]	1.83 ± 0.25	1.33 ± 0.20 [e]
1% CMC	2.68 ± 0.16	2.66 ± 0.17	1.09 ± 0.04	1.09 ± 0.04

[a] Number of mice for each test group = 6. Results are shown as the mean ± S.D.
[b] Results for treatment with 20 mg/kg per day.
[c] Results for treatment with 10 mg/kg per day.
[d] $P<0.01$.
[e] $P<0.001$.
[f] $P<0.0001$.

separate the peaks sufficiently for configurational analysis. Frustrated with the tetra-*O*-acetyl compound **6**, we turned our attention to the 4,6-di-*O*-benzoyl derivative with the hope to get separation of the H-2, H-3 and H-4 protons for determining their correct configurations. To achieve this goal, compound **3** was hydrolyzed to **7** using a mixture of triethylamine, methanol and water. Benzoylation of **7** and usual work-up afforded a crystalline compound **8** which showed a single spot on TLC (R_f 0.70). The 300 MHz ^{1}H NMR spectrum of this derivative showed a broad signal for H-1 at δ 5.38 ppm with a narrow splitting at the top of the peak. It was therefore not possible to determine the anomeric configuration with certainty. However, the COSY (^{1}H–^{1}H) spectrum clearly showed that H-1 is coupled with H-2 at δ 5.88 and H-4 at δ 5.72. The latter coupling between H-1 and H-4 can only take place if H-1 and H-4 are on the same side, i.e., the anomeric proton at C-1 is oriented equatorially and H-4 is oriented axially. cis-Hydroylation of **8** gave **9a**, which was acetylated. The structure of the resulting di-*O*-acetyl derivative **9b** was deduced from its ^{1}H NMR spectrum. The anomeric proton appeared as a doublet (J 2.1 Hz), H-2 as a doublet of doublets at 5.28 (J 1.8 and 3.3 Hz), H-4 as a triplet (J 10.1 Hz) and H-3 as a doublet of doublets (J 10.2 and 3.3 Hz). With this information, it is obvious that **3** is a mannopyranoside with the aglycone portion oriented axially at C-1.

Compounds **3**, **6**, **8**, **9a**,**b** have been tested for their hypolipidemic activity. Phthalimidomethyl 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranoside (**3**) reduced the total plasma cholesterol and triglycerides levels by 21 and 25% in normolipidemic Swiss white male mice (Table 1) after 16 days of treatment with 20 mg/kg per day. A smaller reduction was found when the animals were treated with phthalimidomethyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (**6**), where the cholesterol and triglycerides levels decreased by 17–18%. An improvement in hypotriglyceridemic activity in vivo, but not in the hypocholesterolemic activity, was observed in compounds **8**, **9a** and **9b**, respectively. As shown in Table 1, the animals treated with phthalimidoylmethyl 4,6-di-*O*-benzoyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranoside (**8**), phthalimidoylmethyl 4,6-di-*O*-benzoyl-α-D-mannopyranoside (**9a**) and phthalimidoylmethyl 2,3-di-*O*-acetyl-4,6-di-*O*-benzoyl-α-D-mannopyranoside (**9b**), at 10/mg/kg per day, exhibited significant reduction of the plasma triglyceride concentrations (21, 23 and 27%, respectively). Therefore, although all compounds showed strong hypolipidemic activity for reducing triglycerides, compound **9b** has been found to possess the highest activity. Finally, it was found that administration of 1% carboxymethyl cellulose (CMC) to the animals had no significant changes in mice plasma cholesterol and triglycerides (2%).

In conclusion, the results show that these drugs are able to reduce the plasma lipid levels in normolipidemic mice and that they offer excellent promise as new hypolipidemic agents.

1. Experimental

General methods.—Melting points were determined on an Electrothermal digital melting point apparatus (model 9100) and are uncorrected. Infrared spectra were measured with a

Bruker model IFS66 FT-IR spectrophotomer using potassium bromide discs. NMR spectra were recorded with a Varian Unity Plus 300 MHz instrument using $CDCl_3$ as solvent, unless otherwise stated, and Me_4Si as internal standard. The specific rotation of compound **8** was obtained on a Perkin–Elmer, model 241 polarimeter and the sp rotations of other compounds were measured on JASCO, model DIP-370 polarimeter. Silica gel coated plates with fluorescent indicator (PF_{254}) were used for thin-layer chromatography (TLC) and the spots were revealed under ultraviolet light. The solvent system for running the TLC plates was a mixture of 1:9 EtOAc–CH_2Cl_2.

*Phthalimidomethyl 4,6-di-*O*-acetyl-2,3-dideoxy-α-*D*-erythro-hex-2-enopyranoside* (**3**).—*N*-(Hydroxymethyl)phthalimide (**1**) (0.23 g, 1.30 mmol), and tri-*O*-acetyl-D-glucal (**2**) (0.33 g, 1.21 mmol) in dry benzene (40 mL), were stirred in a 150 mL round-bottom flask under nitrogen atmosphere. Borontrifluoride etherate (0.5 mL) was added to it and the stirring continued for 75 min at rt. Thin-layer chromatography showed a fast moving spot of R_f 0.62 (alcohol **1** had R_f 0.34). Neutralization of this solution with solid $NaHCO_3$, drying with anhyd Na_2SO_4 followed by solvent evaporation left a crystalline solid which, after chromatography over silica gel, provided crystals (0.36 g, 76.3% based on **2**), which after recrystallization from EtOH melted at 120–120.8 °C; $[\alpha]_D^{25}$ +46° ± 2 (*c* 0.9, $CHCl_3$); R_f 0.62; IR (KBr): 3055 (C–H, ar), 2938 (C–H, aliph.), 1760 (ν_{as} CO, of phthalimide part) and 1726 (ν_s CO of phthalimide group and CO of acetyl groups), 1609 cm^{-1} (C=C, ar). ^1H NMR ($CDCl_3$): δ 2.08 (s, 3 H, CH_3CO–), 2.09 (s, 3 H, CH_3CO–), 4.10–4.26 (m, 3 H, H-5, H-6, H-6′), 5.27 and 5.35 (2d, 2 H, *J* 10.20 Hz, –N–CH_2–O–), 5.38 (m, 2 H, H-1 and H-4), 5.80 (ddd, $J_{2,3}$ 10.20, $J_{1,2}$ 2.70, $J_{2,4}$ 1.5 Hz, H-2), 5.90 (d, $J_{3,2}$ 10.2 Hz, H-3), 7.78 (dd, 2 H, *J* 5.40, *J* 3.0 Hz, AA′ BB′ system, H-5′ and H-6′), 7.92 (dd, 2 H, *J* 5.40, *J* 3.00 Hz, AA′BB′ system, H-4′ and H-7′). Anal. Calcd for $C_{19}H_{19}NO_8$ (389.34): C, 58.61; H, 4.92; N, 3.60. Found. C, 58.34; H, 4.68; N, 3.58.

*Phthalimidomethyl 4,6-di-*O*-acetyl-2,3-dideoxy-α-*D*-erythro-hexopyranoside* (**4**).—Compound **3** (0.1g, 0.26 mmol) in EtOAc (5.0 mL) was hydrogenated at rt in the presence of 5% palladium on charcoal (15 mg) for 4 h at 101 KPa. Removal of the catalyst by filtration left a clear solution which showed one spot on TLC with R_f 0.50; the R_f of the starting material in this system was 0.39. Solvent evaporation left a solid which weighed 75 mg (74.6%). Crystallization and recrystallization of **4** from EtOH gave crystals with mp 97.2–97.7 °C; $[\alpha]_D^{25}$ +79.9° ± 0.4 (*c* 2.0, $CHCl_3$); R_f 0.50; IR (KBr): 3052 (C–H, Ar), 2984 (νCH_3), 2925 (ν_{as}-CH_2–), 2993 (ν_s-CH_2–), 1778 (ν_{as}, CO of phthalimide function), 1733 (ν_s, CO of phthalimide and acetyl groups), 1615 (νC=C, Ar). ^1H NMR ($CDCl_3$): δ 1.68–1.88 (m, 4 H, $2CH_2$), 1.96 (s, 3 H, CH_3CO–), 2.00 (s, 3 H, CH_3CO–), 3.92–4.1 (m, 2 H, H-6, H-6′), 4.20 (m, 1 H, H-5), 4.60 (m, 1 H, H-4), 5.07 (bs, 1 H, H-1), 5.12 and 5.22 (2d, 2 H, *J* 10.20 Hz, N–CH_2–O–), 7.72 (dd, 2 H, *J* 5.7, *J* 3.3 Hz, H-5′ and H-6′), 7.85 (dd, 2 H, *J* 5.7, *J* 3.3 Hz, H-4′ and H-7′). Anal. Calcd For $C_{19}H_{21}NO_8$ (391.36): C, 58.31; H, 5.37; N, 3.58. Found: C, 58.01; H, 5.25; N, 3.45.

*Phthalimidomethyl 4,6-di-*O*-acetyl-α-*D*-mannopyranoside* (**5**).—Compound **3** (0.30 g, 0.77 mmol) in THF (18 mL) was treated with a solution of $KMnO_4$ (0.14 g, 0.89 mmol in 10 mL of water) and the contents were stirred at rt for 5 h. TLC showed the disappearance of the starting material and appearance of a new product with R_f 0.30. Filtration over celite followed by solvent removal under reduced pressure gave a crude product which was chromatographed over a column containing silica gel. Pure **5** was eluted from 4:1 $CHCl_3$–EtOAc. Fractions containing **5** were combined, and the solvent evaporated to give 0.235 g of chromatographically pure product which, after crystallization from EtOH, yielded 0.21 g (64.4%) of pure **5**, mp 115.2–115.5 °C; $[\alpha]_D^{25}$ +26° ± 2 (*c* 0.7, $CHCl_3$); R_f 0.30; IR (KBr): 3100–3661 (broad, OH), 1781 (ν_{as}-CON–), 1725 (ν_sCO of phthalimide group and νCO of acetyl groups), 1611 cm^{-1}(ν-C=C–, ar). ^1H NMR ($CDCl_3$): δ 2.07–2.13 (2s, 6 H, $2CH_3CO$–), 2.9–3.5 (b, 2 H, OH), 3.80–4.10 (m, 4 H, H-2, H-3, H-6, H-6′), 4.20–4.36 (m, 1 H, H-5), 5.01–5.18 (dd, 1 H, *J* 10.2, *J* 9.6 Hz, H-4,), 5.2 (bs, 1 H, H-1), 5.26 and 5.29 (2d, 2 H, *J* 10.20 Hz, –N–CH_2–O–) 7.80 (m, 2 H, *J* 5.40, *J* 3.0 Hz, H-5′ and H-6′), 7.94 (m, 2 H, *J* 5.40, *J* 3.0 Hz, H-4′ and H-7′).

Phthalimidomethyl 2,3,4,6-tetra-O-*acetyl*-α-D-*mannopyranoside* (**6**).—Compound **5** (0.14 g, 0.33 mmol) was dissolved in dry pyridine (2.0 mL), cooled to 0 °C under N_2 atmosphere followed by the addition of Ac_2O (1.39 g, 1.5 mL, 13.6 mmol). The contents were left under stirring overnight at rt. Solvent evaporation left a viscous mass with R_f 0.5. Liquid chromatography over silica gel using 1:9 hexane–$CHCl_3$ provided 0.135 g (80.4%) of pure **6**; $[\alpha]_D^{25}$ $+31° \pm 1$ (*c* 1.2, $CHCl_3$); R_f 0.5; IR (KBr): 2961 (νC–H), 1778 (ν_{as}CO of phthalimide group), 1752 (ν_sCO of phthalimide groups), 1726 (νCO of acetyl groups), 1610 cm^{-1} (ν-C=C–, ar). ^1H MNR ($CDCl_3$): δ 1.97 (s, 3 H, CH_3CO–), 2.03 (s, 3 H, CH_3CO–), 2.06 (s, 3 H, CH_3CO–), 2.16 (s, 3 H, CH_3CO–), 4.00 (dd, 1 H, *J* 12.30, *J* 2.10, H-6 or H-6′), 4.12 (m, 1 H, H-5), 4.27 (dd, 1 H, *J* 12.30, *J* 4.50 Hz, H-6 or H-6′), 5.15 (d, 1 H, *J* 2.10 Hz, H-1), 5.22–5.40 (m, 5 H, H-2, H-3, H-4 and –N–CH_2–O–), 7.81 (dd, 2 H, *J* 5.70, *J* 3.30 Hz AA′BB′, H-5′ and H-6′), 7.94 (dd, 2 H, *J* 5.7, *J* 3.20 Hz, AA′BB′, H-4′ and H-7′). Anal. Calcd. for $C_{23}H_{25}NO_{12}$ (507.43): C, 54.43; H, 4.93; N, 2.76. Found: C, 54.32; H,5.16; N, 2.59.

Phthalimidomethyl 2,3-dideoxy-α-D-erythro-*hex*-2-*enopyranoside* (**7**).—Compound **3** (2.75 g, 7.06 mmol) was dissolved in a 9:6:1 mixture of MeOH (27.0 mL), water (18.0 mL) and Et_3N (3.0 mL) and stirred at rt for 5 h. TLC plate revealed the disappearance of the starting material and appearance of a new spot (R_f 0.05). Solvent evaporation left 2.42 g of crude **7** which, after column chromatography over silica gel provided 1.84 g (86%) of pure **7** as a semi-solid mass; IR (KBr): 3311 (b, νOH), 2944 (νCH_2), 2914 (νCH), 1779 (ν_{as}CO of phthalimide group), 1734 (ν_sCO of phthalimide group), 1610 cm^{-1} (νC=C, ar). ^1H NMR ($CDCl_3$): δ 1.4–2.4 (b, 2 H, OH), 3.70–3.81 (m, 3 H, H-5, H-6, H-6′), 4.20 (dd, *J* 9.0 Hz, 1.8 Hz, H-4), 5.27 (narrow multiplet, 1 H, *J* 2.1 Hz, H-1), 5.31 (s, 2 H, N–CH_2–O), 5.72 (ddd, 1 H, *J* 10.5, *J* 2.7, *J* 2.1 Hz, H-2), 5.98 (dt, 1 H, *J* 10.5, *J* 1.2 Hz, H-3), 7.78 (dd, 2 H, *J* 5.4, *J* 3.0 Hz, H-5′ and H-6′), 7.92 (dd, 2 H, *J* 5.7, *J* 3.0 Hz, H-4′, and H-7′). Anal. Calcd for $C_{15}H_{15}NO_6$ (305.28): C, 59.01; H, 4.95; N, 4.58. Found: C, 59.48; H, 5.69, N, 4.26.

Phthalimidomethyl 4,6-di-O-*benzoyl-2,3-dideoxy*-α-D-erythro-*hex*-2-*enopyranoside* (**8**).—To compound **7** (2.15 g, 7.04 mmol) in dry pyridine (3.0 mL) at 0 °C and under N_2 atmosphere was added BzCl (2.47 g, 2.04 mL, 17.6 mmol). The contents were maintained at this temperature for 3 h under stirring. At this point, completion of the reaction was checked by TCL (R_f 0.7; starting compound **7** had R_f 0.05). Neutralization of the contents with $NaHCO_3$ and extraction with CH_2Cl_2, drying ($NaSO_4$) and solvent evaporation afforded 2.53 g of the crude product. Liquid chromatography over silica gel using 4:1 hexane–EtOAc gave 2.42g (66.9%) of chromatographically pure **8**. Crystallization and recrystallization from EtOH furnished crystals, mp 125.7–126.4 °C; $[\alpha]_D^{20}$ $+91.65°$ (*c* 2, $CHCl_3$); R_f 0.7; IR (KBr): 3033 (νC=C, ar), 3070 (νC=C, ar), 1785 (ν_{as}CO of phthalimide group), 1723 (ν_s CO of phthalimide part and νCO of benzoyl groups), 1600 (νC=C, ar). ^1H NMR ($CDCl_3$): δ 3.8–4.58 (m, 3 H, H-5, H-6, and H-6′), 5.31 and 5.39 (2d, 2 H, *J* 10.2 Hz, O–CH_2–N), 5.44 (nm, 1 H, H-1), 5.72 (d, 1 H, *J* 7.8 Hz, H-4), 5.87 (dt, 1 H, *J* 10.5, *J* 2.7, *J* 2.1 Hz, H-2), 6.05 (d, 1 H, *J* 10.5 Hz, H-3), 7.39 (t, 2 H, *J* 7.5 Hz, meta protons of one benzoyl group), 7.42 (t, 2 H, *J* 7.5 Hz, meta protons of the other benzoyl group), 7.49–7.60 (m, 2 H, para protons of the benzoyl groups), 7.76 (dd, 2 H, *J* 5.55, *J* 3.30 Hz, H-5′, H-6′), 7.90 (dd, 2 H, *J* 5.40, *J* 3.00 Hz, H-4′ and H-7′), 8.0 (dd, 2 H, *J* 8.40, *J* 1.65 Hz, ortho protons of one benzoyl group), 8.04 (dd, 2 H, *J* 8.40, *J* 1.20 Hz, ortho protons of other benzoyl group). Anal. Calcd for $C_{29}H_{23}NO_8$ (513.47): C, 67.83; H, 4.51; N, 2.73. Found: C, 67.81; H, 4.54; N, 2.90.

Phthalimidomethyl 4,6-di-O-*benzoyl*-α-D-*mannopyranoside* (**9a**).—To a stirred solution of **8** (0.20 g, 0.039 mmol) in THF (6.0 mL) was added $KMnO_4$ (0.07 g, 0.44 mmol) dropwise in few minutes and the contents were left for 5 h at rt. TLC showed the formation of a new product with a R_f value of 0.17. Removal of MnO_2 by filtration followed by solvent evaporation furnished a semi-solid material, which after chromatography over a short column using 1:1.5 hexane–EtOAc gave 0.13 (61%) of pure **9a**; $[\alpha]_D^{23}$ $+52° \pm 3$ (*c* 0.7, $CHCl_3$); R_f 0.17; IR (KBr): 3494 (b, νOH),

2953 (CH, aliph.), 1779 (ν_{as}CO of phthalimide group), 1723 (ν_{s}CO of phthalimide and benzoyl groups), 1602 (νC=C, ar). Anal. Calcd for $C_{29}H_{25}NO_{10}$ (547.51): C, 63.61; H, 4.60; N, 2.56. Found: C, 63.82; H, 4.77; N, 2.38.

Phthalimidomethyl 2,3-di-O-*acetyl-4,6-di*-O-*benzoyl*-α-D-*mannopyranoside* (**9b**).—To the dihydroxy compound **9a** (0.095 g, 0.17 mmol) in dry pyridine (2.0 mL), in a 10 mL round-bottom flask and cooled to 0 °C. Ac_2O (1.0 mL) was added. Stirring at rt overnight gave the di-*O*-acetyl derivative (TLC, R_f 0.65). Purification was achieved by column chromatography over silica gel. Elution with 1:9 EtOAc–hexane gave pure **9b** (0.10 g, 91%); $[\alpha]_D^{25}$ $+35° \pm 2$ (*c* 1.0, $CHCl_3$); R_f 0.65; IR (KBr): 1781.2 (ν_{as}CO of phthalimide group), 1753.2 (ν_{s}CO of phthalimide part), 1726.1 (νCO of acetyl and benzoyl groups) and 1602.7 cm^{-1} (C=C, Ar); ^{1}H NMR ($CDCl_3$): δ 1.87 (s, 3 H, CH_3–CO), 2.15 (s, 3 H, CH_3–CO), 4.32–4.52 (m, 3 H, H-5, H-6, H-6′), 5.21 (d, 1 H, *J* 2.10 Hz, H-1), 5.28 and 5.37 (2d, 2 H, *J* 10.2 Hz, O–CH_2–N), 5.28 (dd, 1 H, *J* 3.3, *J* 1.8 Hz, H-2), 5.56 (dd, 1 H, *J* 10.20, *J* 3.30 Hz, H-3), 5.73 (t, 1 H, *J* 10.1 Hz, H-4), 7.34–7.46 (m, 4 H, meta protons of benzoyl group), 7.49–7.60 (m, 2 H, para protons of benzoyl group), 7.79 (dd, 2 H, *J* 5.4, *J* 3.0 Hz, H-5′, H-6′), 7.93 (dd, 2 H, *J* 5.4, *J* 3.0 Hz, H-4′, H-7′), 8.01 (m, 4 H, ortho protons of benzoyl group). Anal. Calcd for $C_{33}H_{29}NO_{12}$ (631.56): C, 62.75; H, 4.63; N, 2.21. Found: C, 62.71; H, 4.91; N, 2.23.

Hypolipidemic activity tests

Drug administration. The compounds were suspended in 1% carboxymethylcellulose and administered orally in the morning[8] at either 10 or 20 mg/kg per day for 2 week periods to normolipidaemic male Swiss White mice (age about 3 months, body weight 30–32 g) by using an intubation needle. Periodic animal body weights were obtained during the experiment and expressed as a percentage of the animal's weight on day 0.

Lipids analysis. Blood was collected by retro-orbital plexus into EDTA-containing tubes (1 mg/mL, disodium salt), on days 0 and 16, and the plasma was separated by centrifugation at 2500*g* for 10 min at 4 °C. Plasma cholesterol was determined by the CHOD-PAP method, an enzymatic assay[9] for photometric determination, using the enzymes cholesterol esterase, cholesterol oxidase and peroxidase contained in E. Merck test 1.14830.0001 Ecoline 25 reagents (Diagnostica–E. Merck KGaA, Darmstadt, Germany), according to the manufacturer's instructions. Plasma triglycerides (E. Merck test 1.19706.0001 System Multi-Test) were also measured enzymatically (GPO-PAP method)[10] by a combination of the reactions catalyzed by lipase, glycerokinase, glycerol phosphate oxidase and peroxidase.

Statistics. All groups had ten animals and each determination was processed before and after the drug treatment. The results are expressed as mean ± S.D. and we used paired Student-'*t*' test. In all cases, $P < 0.05$ was used as the criterion of statistic significance.

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