DOI: 10.1002/ejoc.201100934

Synthesis of Potassium (2*R*)-2-*O*-α-D-Mannopyranosyl-(1→2)-α-D-glucopyranosyl-2,3-dihydroxypropanoate: A Naturally Compatible Solute

Eva C. Lourenço[a] and M. Rita Ventura*[a]

Keywords: Glycosylation / Hypersolutes / Natural products / Thioglycosides / Solid-phase synthesis

An expedient synthesis of the potassium salt of (2*R*)-2-*O*-α-D-mannopyranosyl-(1→2)-α-D-glucopyranosyl-2,3-dihydroxypropanoic acid (MGG)—a recently isolated, rare, compatible solute—was accomplished. A bis-acetal-protected thioglucoside, 6-OTBDPS, with a 2-OH group was used as the acceptor in the first glycosylation reaction with tetraacetylmannosyl trichloroacetimidate, and as the donor in the glycosylation reaction with the glycerate derivative. The α anomer was the only product of both glycosylation reactions, as expected for the formation of the α-mannoside. The formation of the 1,2-*cis* glucoside was more challenging.

Introduction

Compatible solutes are low-molecular-mass organic compounds that accumulate in the cytoplasm of many halophilic or halotolerant organisms to counterbalance the osmotic pressure of the external medium.[1] Hyperthermophiles accumulate compatible solutes that exhibit thermoprotection against heat denaturation of a variety of proteins.[2,3] The thermostabilization properties of hypersolutes have enormous potential for industrial and health-related applications.

In contrast with the neutral or zwitterionic nature of the solutes commonly found in mesophiles, organisms with optimal growth temperatures above 60 °C accumulate mainly negatively charged compounds, such as α-D-mannosyl-(1→2)-D-glycerate (MG), α-D-glucosyl-(1→2)-D-glycerate (GG), α-D-glucosyl-(1→6)-α-D-glucosyl-(1→2)-D-glycerate (GGG) (Figure 1) and di-*myo*-inositol-1,3′-phosphate (DIP). The trisaccharide GGG, which was isolated from hyperthermophile *Persephonella marina*, has recently been synthesized, along with related GG (Figure 1).[4]

A new solute, the structure of which was established to be (2*R*)-2-*O*-α-D-mannopyranosyl-(1→2)-α-D-glucopyranosyl-2,3-dihydroxypropanoate [**1**, α-D-mannopyranosyl-(1→2)-α-D-glucopyranosyl-(1→2)-D-glycerate (MGG)], was isolated from the thermophilic bacteria *Petrotoga miotherma*[5] and *Petrotoga mobilis*[6] (Figure 1). MGG is a rare solute and it serves as an osmolyte during low-level osmotic adaptation in *Ptg. miotherma*, but it is not used against oxidative or heat stress by the cell.[5] In contrast, in *Ptg. mobilis*, MGG is the major compatible solute under osmotic and thermal stresses.[5] It was shown that MGG

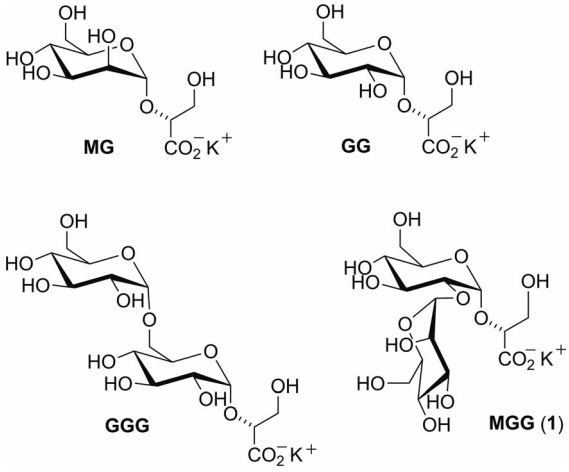


Figure 1. Natural glycerate solutes.

was an efficient protector of pig-heart malate dehydrogenase against heat inactivation and freeze-drying and has been patented for the stabilization and preservation of biomaterials.[5]

This solute is available in very small quantities from its natural sources and chemical synthesis was considered the best route to obtain **1** in larger amounts.

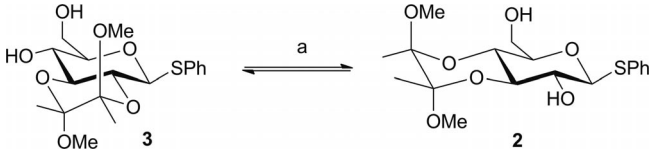
Results and Discussion

During the analysis of the structure of **1**, two immediate critical issues emerged: the need for the C-2 hydroxy group of glucose to be free[7] to form a glycosidic bond with mannose and the stereoselective formation of the 1,2-*cis* glucosidic bond with glycerate. Additionally, we hoped to perform the synthesis on a solid support to avoid the need for difficult and time-consuming separations, such as silica and solvent-consuming chromatographies. A two-way strategy was

[a] Instituto de Tecnologia Química e Biológica,
Universidade Nova de Lisboa,
Apartado 127, 2780-901 Oeiras, Portugal
E-mail: rventura@itqb.unl.pt

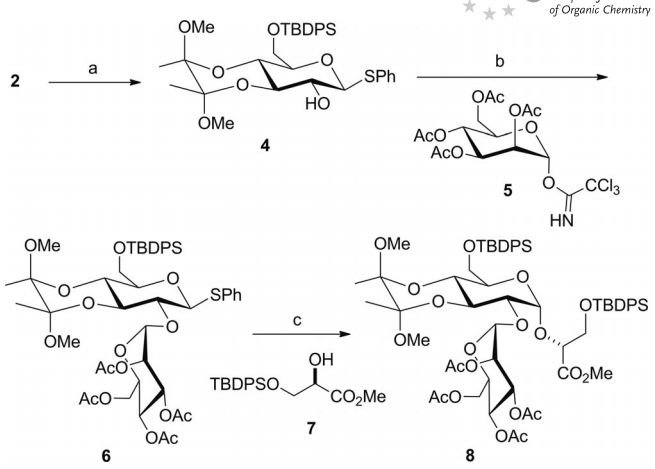
considered to be suitable; therefore, we needed to prepare a donor/acceptor with the 6-OH of the glucose unit differentially exposed to attach this donor/acceptor to a suitable resin. However, a solution-phase synthesis was carried out to test the strategy and to later compare this strategy with the solid-phase one.

Butane-2,3-bis-acetals have been used in chiral memory studies with tartaric acid[8] and extensively used to protect vicinal diequatorial hydroxy groups in sugars.[9,10] An easily separable 1:1 mixture of bisacetals **2** and **3** was formed (Scheme 1)[11,12] by treating phenylthioglucopyranoside[13] with 2,2,3,3-tetramethoxybutane (TMB). It was possible to recycle isomer **3** by heating it in methanol at reflux with catalytic CSA overnight; this again afforded a 1:1 mixture of isomers, increasing the overall yield of **2** to 70% (Scheme 1).

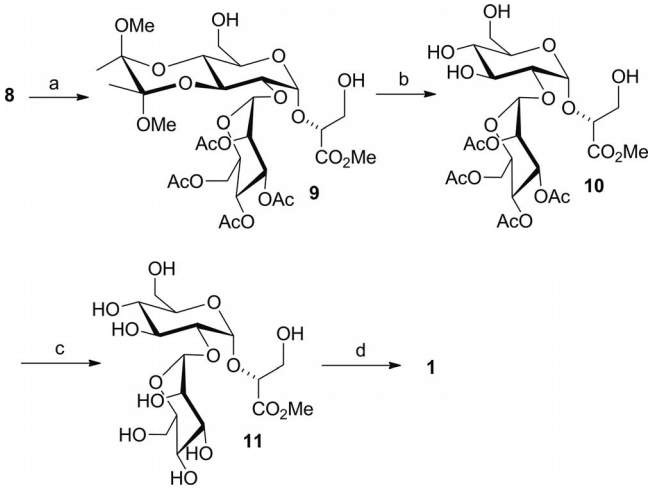


Scheme 1. Reagents and conditions: (a) Camphorsulfonic acid (CSA), MeOH, Δ, 91%.

Selective silylation of the primary hydroxy group of **2** by using *tert*-butyldiphenylsilyl chloride (TBDPSCl) and imidazole afforded **4** in 89% yield (Scheme 2). A glycosylation reaction between thioglucoside **4** as the acceptor and mannose trichloroacetimidate **5**, using TMSOTf as the promoter,[14] afforded exclusively, as expected, the corresponding α-mannoside **6** in 58% yield. The second glycosylation reaction, using disaccharide **6** as the donor and glycerate **7** by employing NIS/TfOH as the activating system,[12] was also highly selective and afforded α-glucoside **8** in 60% yield (Scheme 2). It has been reported that the α selectivity of glucosylations was increased when bulky substituents, such as TBDPS, were introduced at the 6-position of the glucosyl donor.[15] The main reason for the moderate yields was the partial hydrolysis of the acetal under the glycosylation conditions, which we were unable to avoid.



Scheme 2. Reagents and conditions: (a) TBDPSCl, imidazole, DMF, r.t., 89%; (b) trimethylsilyl trifluoromethanesulfonate (TMSOTf), CH_2Cl_2, –20 °C, 58%; (c) *N*-iodosuccinimide (NIS), trifluoromethanesulfonic acid (TfOH), 4 Å molecular sieves, CH_2Cl_2, 0 °C, 60%.



Scheme 3. Reagents and conditions: (a) TBAF, THF, r.t., 71%; (b) TFA/CH_2Cl_2/H_2O, r.t., 60%; (c) MeONa, MeOH, r.t., quant. (d) KOH, H_2O, r.t., quant.

Selective fluorolysis of the silyl ether of **8** with tetrabutylammonium fluoride (TBAF; 71%), followed by cleavage of the acetal with trifluoroacetic acid (TFA) in dichloromethane/water (60%), methanolysis of the acetates of the mannosyl unit, and hydrolysis of the methyl ester of the glycerate afforded **1** (Scheme 3).

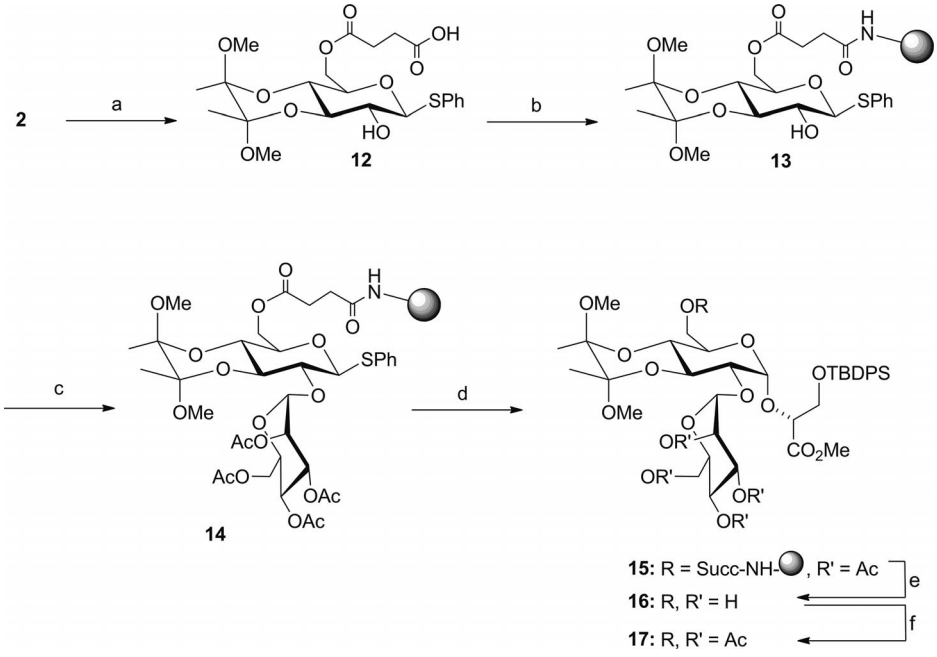
The NMR spectroscopic data for the synthesized product were identical to those described in the literature[5] for natural MGG (Table 1).

For the solid-supported synthesis of **1**, our strategy consisted of attaching the thioglucoside to a Tentagel MB NH_2 resin through the formation of an amide bond between the carboxylic acid of the hemisuccinic acid moiety at C-6 and the amino group of the Tentagel resin.[16–18] The hemisuccinate was selectively formed at the C-6 hydroxy of diol **2** by using succinic anhydride to afford **12** in good yield (90%; Scheme 4) and left the hydroxy group at C-2 free. Attachment of **12** to Tentagel MB-NH_2 resin was successfully accomplished by using standard carbodiimide coupling. Resin-bound **13** was characterized by high-resolution magic angle spinning (HR-MAS) NMR spectroscopy experiments. This technique was an excellent way of monitoring the solid-supported synthesis by the analysis of high-quality ^1H, ^{13}C, and HSQC NMR spectra. From the dry-weight difference, an initial loading of 0.32 mmol g^{-1} of resin was estimated.

The glycosylation reactions were performed with polymer-bound glucoside **13**, as indicated in Scheme 4, and we obtained immobilized fully protected **15**. Both glycosylation reactions afforded the corresponding α products, which was expected in the first reaction. For subsequent formation of the 1,2-*cis* glucoside **15**, the succinate ester exerted the same orienting effect as the acetate ester in the 6-position of the thioglucoside donor.[19] In these two sequential glycosyl-

Table 1. Comparison of ^{13}C NMR chemical shifts for the synthetic potassium salt of **1** with data from the natural product.[4]

	^{13}C, δ [ppm]														
	α(1→2)mannosyl moiety						α(1→2)glucosyl moiety						Glyceryl moiety		
	C-1	C-2	C-3	C-4	C-5	C-6	C-1	C-2	C-3	C-4	C-5	C-6	C-1	C-2	C-3
Natural	99.90	72.53	72.88	69.33	75.47	63.50	96.60	76.76	73.85	72.24	74.62	63.30	179.2	81.0	65.7
Synthetic	100.1	72.7	73.1	70.6	75.7	63.9	96.8	76.9	74.1	72.5	74.8	63.5	179.5	81.3	65.9



Scheme 4. Reagents and conditions: (a) Succinic anhydride, *i*Pr_2NEt, 4-dimethylaminopyridine (DMAP), CH_2Cl_2, r.t., 90%; (b) Tentagel MB-NH_2, diisopropylcarbodiimide (DIC), 1-hydroxybenzotriazole (HOBt), CH_2Cl_2, r.t.; (c) **5**, TMSOTf, CH_2Cl_2, –20 °C; (d) **7**, NIS, TfOH, 4 Å molecular sieves, CH_2Cl_2, 0 °C; (e) MeONa, MeOH, r.t.; (f) Ac_2O, pyridine, DMAP, r.t., quant.

ations no additional purification was needed and capping with acetate made no difference to the overall efficiency, the excess of reagents were simply filtered and washed off the Tentagel-bound products.

Cleavage from the resin was accomplished by using sodium methoxide in methanol, with simultaneous hydrolysis of the acetate groups of the mannose moiety (Scheme 4). To better purify and analyze the product obtained from the resin, the free hydroxy groups were acetylated to give peracetate **17**. After chromatographic purification, the spectroscopic data obtained confirmed the success of the polymer-bound glycosylations but an overall yield of 18%, based on calculated immobilized **12**, was disappointing.

Conclusions

A solution-phase synthesis of the natural solute **1** has been achieved with an overall yield of 15%. Differentiation of the 2-OH (acceptor) group of the glucose moiety was accomplished in just two steps through the dioxane bis-acetal, followed by selective protection of the more reactive 6-OH group. Different activating groups were used to carry out glycosylation reactions in the presence of the other groups. A similar solid-phase synthesis of **1** was carried out to compare with the product obtained through the solution-phase method by using a bidirectional (non-linear) approach, whereby the central glucose moiety was attached to the resin such that the donor and acceptor atoms were free to react with their counterparts under adequate conditions. A partially deprotected product was obtained in 18% overall yield by this method.

Experimental Section

General: ^1H NMR spectra were obtained with a Bruker Avance II+ 400 MHz spectrometer at 400 MHz in $CDCl_3$ or D_2O with chemical shift values (δ) in ppm downfield from tetramethylsilane in the case of $CDCl_3$, and ^{13}C NMR spectra were obtained at 100.61 MHz in $CDCl_3$ or D_2O. Assignments are supported by 2D correlation NMR spectroscopy studies. Preparative chromatographic separations were carried out by using medium-pressure column chromatography with Merck 60 H silica gel. Analytical TLC was performed on aluminum-backed Merck 60 F_{254} silica gel plates. Specific rotations ($[\alpha]_D^{20}$) were measured by using a Perkin–Elmer 241 automatic polarimeter. Reagents and solvents were purified and dried according to ref.[20] All of the reactions were carried out under an inert atmosphere (argon), except when the solvents were not dried.

Phenyl 6-*O*-*tert*-Butyldiphenylsilyl-3,4-di-*O*-(2,3-dimethoxybutane-2,3-diyl)-1-thio-β-D-glucopyranoside (4): TBDPSCl (1.28 mL,

4.92 mmol) was added to a solution of **2**[10] (0.634 g, 1.64 mmol) in dry DMF (5 mL) at room temperature, followed by imidazole (0.391 g, 5.74 mmol). After 12 h at room temperature, the reaction was quenched with H_2O (5 mL), extracted with CH_2Cl_2 (3 × 5 mL), and the combined organic phases were dried ($MgSO_4$) and concentrated. Purification by flash column chromatography on silica gel (30:70 EtOAc/hexane) afforded the product **4** as a white solid (0.908 g, 89%). $[\alpha]_D^{20}$ = +34.1 (*c* = 0.17, CH_2Cl_2). M.p. 69.2–71.4 °C. FTIR (film): $\tilde{\nu}$ = 3496 (O–H) cm^{-1}. ^1H NMR ($CDCl_3$): δ = 7.67–7.72 (m, 4 H), 7.57–7.55 (m, 2 H), 7.42–7.31 (m, 6 H), 7.23–7.19 (m, 3 H), 4.57 [d, *J* = 9.3 Hz, 1 H, 1-H(β)], 3.99–3.90 (m, 2 H), 3.82–3.73 (m, 2 H), 3.59–3.52 (m, 2 H), 3.31 (s, 3 H), 3.19 (s, 3 H), 1.33 (s, 3 H), 1.28 (s, 3 H), 1.06 (s, 9 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 135.8, 135.5, 133.7, 133.1, 132.8, 131.9, 129.6, 129.5, 129.0, 127.9, 127.66, 127.63, 99.7, 99.4, 88.5 [C-1(β)], 78.9, 73.7, 69.1, 64.9, 61.9, 48.2, 48.0, 26.9, 19.3, 17.7, 17.6 ppm. $C_{34}H_{44}O_7SSi$ (624.86): calcd. C 65.35, H 7.10, S 5.13; found C 65.00, H 6.86, S 5.01.

Phenyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→2)-6-*O*-*tert*-butyldiphenylsilyl-3,4-di-*O*-(2,3-dimethoxybutane-2,3-diyl)-1-thio-β-D-glucopyranoside (6): Acceptor **4** (0.254 g, 0.41 mmol) was added to a solution of trichloroacetamidate **5** (0.250 g, 0.51 mmol) in dry CH_2Cl_2 (4 mL). The solution was cooled to –20 °C and TMSOTf (92.30 μL, 0.51 mmol) was slowly added. When the reaction was completed, a saturated aqueous solution of $NaHCO_3$ (2 mL) was added, followed by extractions with CH_2Cl_2. The combined organic phases were dried ($MgSO_4$) and concentrated. The residue was purified by flash column chromatography on silica gel (20:80 EtOAc/hexane) to afford **6** as a white foam (0.237 g, 58%). $[\alpha]_D^{20}$ = +54.15 (*c* = 1.30, CH_2Cl_2). FTIR (film): $\tilde{\nu}$ = 1752 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$): δ = 7.73–7.70 (m, 4 H), 7.55–7.53 (m, 2 H), 7.43–7.19 (m, 9 H), 5.51 [s, 1 H, 1-H(α) mannose], 5.35–5.33 (m, 3 H), 4.73–4.72 (m, 1 H), 4.65 [d, *J* = 8.7 Hz, 1 H, 1-H(β) glucose], 4.29 (dd, *J* = 3.6, 12.0 Hz, 1 H), 4.18–4.15 (m, 1 H), 3.95–3.88 (m, 2 H), 3.79–3.76 (m, 3 H), 3.32 (s, 3 H), 3.17 (s, 3 H), 2.20 (s, 3 H), 2.12 (s, 3 H), 2.01 (s, 3 H), 1.99 (s, 3 H), 1.26 (s, 3 H), 1.25 (s, 3 H), 1.06 (s, 9 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 170.8, 169.9, 169.8, 169.6, 135.8, 135.5, 133.9, 133.6, 133.0, 131.9, 131.5, 129.6, 129.5, 129.0, 127.6, 127.5, 99.9, 99.3, 97.1 [C-1(α) mannose], 88.7 [C-1(β) glucose], 78.5, 73.4, 72.8, 69.2, 69.1, 68.6, 66.0, 65.1, 62.0, 48.0, 47.9, 26.8, 20.9, 20.71, 20.70, 20.6, 19.3, 17.6, 17.3 ppm. $C_{48}H_{62}O_{16}SSi$ (955.15): calcd. C 60.36, H 6.54; found C 60.50, H 6.40.

Methyl 3-*O*-*tert*-Butyldiphenylsilyl-(2*R*)-2-*O*-[2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→2)-6-*O*-*tert*-butyldiphenylsilyl-3,4-di-*O*-(2,3-dimethoxybutane-2,3-diyl)-1-*O*-α-D-glucopyranosyl]-2,3-dihydroxypropanoate (8): A suspension of thioglycoside **6** (0.387 g, 0.40 mmol), glycerate **7** (0.143 g, 0.40 mmol) and 4 Å molecular sieves in CH_2Cl_2 (3 mL) was stirred at room temperature for 1 h. The solution was cooled to 0 °C and *N*-iodosuccinimide (0.099 g, 0.44 mmol) and TMSOTf (36 μL, 0.20 mmol) were added. After all of the starting material had been consumed, a 10% aqueous solution of $Na_2S_2O_3$ (2 mL) and a saturated aqueous solution of $NaHCO_3$ (1 mL) were added and the mixture was extracted with CH_2Cl_2; the combined organic phases were dried ($MgSO_4$), filtered and the solvent was removed in vacuo. The crude product was purified by column chromatography (30:70 EtOAc/hexane) to afford glycoside **8** as a colorless viscous foam (0.286 g, 60%). $[\alpha]_D^{20}$ = +83.3 (*c* = 1.00, CH_2Cl_2). FTIR (film): $\tilde{\nu}$ = 1641, 1753 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$): δ = 7.68–7.62 (m, 8 H), 7.42–7.30 (m, 12 H), 5.46 (dd, *J* = 3.4, 9.9 Hz, 1 H), 5.40–5.39 (m), 5.29 (t, *J* = 10.0 Hz, 1 H), 5.20 [d, *J* = 1.4 Hz, 1 H, C-1(α) mannose], 5.13 [d, *J* = 3.8 Hz, 1 H, C-1(α) glucose], 4.52–4.48 (m, 1 H), 4.37 (dd, *J* = 5.2, 3.8 Hz), 4.29–4.18 (m, 3 H), 3.97 (dd, *J* = 5.4, 10.5 Hz, 1 H), 3.91–3.83 (m, 2 H), 3.80–3.71 (m, 4 H), 3.72 (s, 3 H), 3.28 (s, 3 H), 3.17 (s, 3 H), 2.17 (s, 3 H), 2.13 (s, 3 H), 2.04 (s, 3 H), 1.97 (s, 3 H), 1.27 (s, 6 H), 1.01 (s, 9 H), 1.00 (s, 9 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 170.7, 170.3, 169.8, 169.6, 169.5, 135.8, 135.6, 135.5, 135.4, 133.7, 133.2, 132.7, 129.8, 129.7, 129.6, 129.5, 127.8, 127.7, 127.5, 127.4, 99.6, 99.5, 94.6 [C-1(α) mannose], 93.9 [C-1(α) glucose], 74.6, 70.9, 70.4, 69.6, 69.1, 68.5, 67.8, 66.3, 65.8, 64.4, 62.3, 61.7, 51.9, 47.9, 47.8, 26.7, 26.5, 20.9, 20.7, 20.69, 20.67 19.3, 19.1, 17.6, 17.6 ppm. $C_{62}H_{82}O_{20}Si_2$ (1203.49): calcd. C 61.88, H 6.87; found C 61.90, H 6.75.

Methyl (2*R*)-2-*O*-[2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4-di-*O*-(2,3-dimethoxybutane-2,3-diyl)-1-*O*-α-D-glucopyranosyl]-2,3-dihydroxypropanoate (9): TBAF (1 M in THF; 0.22 mL, 0.22 mmol) was added to a solution of **8** (0.246 g, 0.20 mmol) in THF (3 mL) at room temperature. The reaction mixture was stirred for 3 h and then water was added. The mixture was extracted with EtOAc, dried ($MgSO_4$) and concentrated to give a yellow viscous residue. Purification by column chromatography (EtOAc) afforded product **9** as a viscous colorless foam (0.103 g, 71%). $[\alpha]_D^{20}$ = +155.2 (*c* = 1.82, CH_2Cl_2). FTIR (film): $\tilde{\nu}$ = 3463 (O–H), 1747, 1636 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$): δ = 5.39–5.37 (m, 1 H), 5.35–5.28 (m, 2 H), 5.19 [d, *J* = 3.8 Hz, 1 H, C-1(α) glucose], 5.13 [s, 1 H, C-1(α) mannose], 4.50–4.48 (m, 1 H), 4.39–4.36 (m, 1 H), 4.26–4.17 (m, 2 H), 4.10 (t, *J* = 10.0 Hz, 1 H), 3.95–3.81 (m, 5 H), 3.75–3.67 (m, 2 H), 3.73 (s, 3 H), 3.34 (s, 3 H), 3.24 (s, 3 H), 2.17 (s, 3 H), 2.13 (s, 3 H), 2.04 (s, 3 H), 1.98 (s, 3 H), 1.30 (s, 3 H), 1.26 (s, 3 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 170.7, 170.1, 169.9, 169.74, 169.70, 99.8, 99.7, 94.9 [C-1(α) mannose], 94.6 [C-1(α) glucose], 75.4, 71.4, 70.0, 69.3, 69.2, 68.7, 67.4, 66.0, 65.8, 63.3, 62.1, 60.9, 52.2, 47.9, 47.8, 20.8, 20.7, 17.6, 17.5 ppm. HRMS: calcd. for $C_{30}H_{46}O_{20}Na^+$ [M^+ + Na] 749.2475; found 749.2451.

Methyl (2*R*)-2-*O*-[2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→2)-1-*O*-α-D-glucopyranosyl]-2,3-dihydroxypropanoate (10): A mixture of TFA/H_2O (5:1) (0.8 mL) was added to a solution of **9** (0.093 g, 0.13 mmol) in CH_2Cl_2 (1 mL) at room temperature. The reaction mixture was stirred until all of the starting material had been consumed and then it was concentrated to give a viscous residue. Purification by column chromatography (1:4, MeOH/CH_2Cl_2) afforded product **10** as a viscous colorless foam (0.055 g, 70%). $[\alpha]_D^{20}$ = +72.17 (*c* = 0.46, EtOH). FTIR (film): $\tilde{\nu}$ = 3460 (O–H), 1748 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$): δ = 5.43–5.42 (m, 1 H), 5.38 (dd, *J* = 3.2, 10.0 Hz, 1 H), 5.26 (t, *J* = 10.0 Hz, 1 H), 5.20 [d, *J* = 3.6 Hz, 1 H, 1-H(α) glucose], 5.17 [d, *J* = 1.4 Hz, 1 H, 1-H(α) mannose], 4.48 (t, *J* = 4.2 Hz, 1 H), 4.46–4.39 (m, 2 H), 4.15–4.12 (m, 1 H), 3.88–3.68 (m, 7 H), 3.69 (s, 3 H), 3.43 (t, *J* = 9.4 Hz, 1 H), 2.18 (s, 3 H), 2.09 (s, 3 H), 2.06 (s, 3 H), 1.98 (s, 3 H) ppm. ^{13}C NMR ($CDCl_3$):δ = 173.7, 173.1, 172.8, 172.7, 172.0, 95.4 [C-1(α) mannose], 94.8 [C-1(α) glucose], 75.9, 75.4, 72.2, 70.9, 69.7, 69.3, 68.4, 65.5, 62.3, 61.8, 60.3, 52.7, 20.1, 20.0 ppm. HRMS: calcd. for $C_{24}H_{36}O_{18}Na^+$ [M^+ + Na] 635.1794; found 635.2451.

Methyl (2*R*)-2-*O*-[α-D-Mannopyranosyl-(1→2)-1-*O*-α-D-glucopyranosyl]-2,3-dihydroxypropanoate (11): A 1 N solution of NaOMe (34 μL, 0.03 mmol) in MeOH was added to a stirred solution of **10** (0.036 g, 0.06 mmol) in MeOH (1 mL) at 0 °C. After 1 h, previously activated Dowex-H^+ resin was added until the pH of the reaction mixture reached 7. After filtration with MeOH and water, the solvent was removed in vacuo to yield **11** as a viscous colorless foam (0.026 g, quantitative). $[\alpha]_D^{20}$ = +135.61 (*c* = 2.03, H_2O). ^1H NMR (D_2O): δ = 5.26 [d, *J* = 3.4 Hz, 1 H, 1-H(α) glucose], 5.09 [d, *J* = 1.3 Hz, 1 H, 1-H(α) mannose], 4.52 (t, *J* = 3.8 Hz, 1 H), 4.03 (q, *J* = 1.6 Hz, 1 H), 3.91–3.90 (m, 2 H), 3.87–3.63 (m, 10 H), 3.77 (s, 3

H), 3.45 (t, J = 9.0 Hz, 1 H) ppm. ^{13}C NMR (D_2O): δ = 174.6, 99.6 [C-1(α) mannose], 96.8 [C-1(α) glucose], 77.9, 76.3, 75.2, 74.7, 73.5, 72.8, 72.4, 71.8, 69.1, 64.9, 63.4, 62.9, 55.3 ppm. HRMS: calcd. for $C_{16}H_{28}O_{14}Na^+$ [M^+ + Na] 467.1371; found 467.1372.

Potassium (2*R*)-2-*O*-α-D-Mannopyranosyl-(1→2)-α-D-glucopyranosyl-2,3-dihydroxypropanoate (1): A solution of 2 M KOH (320 μL) was added to a stirred solution of ester **11** (0.026 g, 0.06 mmol) in H_2O (1 mL). After all of the starting material had been consumed, the pH was adjusted to 7 with 10% HCl and the solvent was evaporated to afford **1** as a viscous colorless foam (0.027 g, quantitative). The NMR spectroscopic data were identical to those of a natural sample. $[\alpha]_D^{20}$ = +67.76 (c = 0.85, H_2O). FTIR (film): $\tilde{\nu}$ = 3315, 1655 cm^{-1}. ^{1}H NMR (D_2O): δ = 5.12 [d, J = 3.4 Hz, 1 H, 1-H(α) glucose], 5.06 [d, J = 1.12 Hz, 1 H, 1-H(α) mannose], 4.13 (dd, J = 3.2, 7.0 Hz, 1 H), 4.04–4.02 (m, 1 H), 3.85–3.58 (m, 12 H), 3.39 (t, J = 9.3 Hz, 1 H) ppm. ^{13}C NMR (D_2O): δ = 176.5, 97.1 [C-1(α) mannose], 93.8 [C-1(α) glucose], 78.3, 73.9, 72.7, 71.8, 71.1, 70.1, 69.7, 69.5, 66.6, 62.9, 60.9, 60.5 ppm. HRMS: calcd. for $C_{15}H_{26}O_{14}K^+$ [M^+ + H] 469.09541; found 469.0954.

6-*O*-[Phenyl-3,4-di-*O*-(2,3-dimethoxybutane-2,3-diyl)-1-thio-β-D-glucopyranosyl] Succinate (12): Succinic anhydride (0.041 g, 0.41 mmol), followed by diisopropylethylamine (171 μL, 0.98 mmol) and a catalytic amount of DMAP were added to a solution of **2** (0.160 g, 0.41 mmol) in CH_2Cl_2 (2 mL) at 0 °C. The reaction mixture was stirred as the temperature was allowed to rise to room temperature. After 3 h, the reaction was quenched and washed with H_2O. The aqueous phase was extracted with AcOEt (3 × 5 mL) and the combined organic phases were dried ($MgSO_4$) and concentrated. Purification by column chromatography (80:20 EtOAc/hexane) afforded product **12** as a viscous colorless oil (0.180 g, 90%). $[\alpha]_D^{20}$ = +110.5 (c = 0.19, CH_2Cl_2). FTIR (film): $\tilde{\nu}$ = 3488 (O–H), 1736 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 7.56–7.53 (m, 2 H), 7.33–7.29 (m, 3 H), 4.52 [d, J = 9.3 Hz, 1 H, 1-H(β)], 4.48 (dd, J = 1.9, 11.8 Hz, 1 H), 4.21 (dd, J = 5.4, 11.9 Hz, 1 H), 3.77–3.71 (m, 2 H), 3.59 (t, J = 9.8 Hz, 1 H), 3.48 (t, J = 9.32 Hz, 1 H), 3.30 (s, 3 H), 3.21 (s, 3 H), 2.72–2.63 (m, 4 H), 1.32 (s, 3 H), 1.28 (s, 3 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 176.6, 171.8, 133.5, 130.9, 128.9, 128.5, 99.8, 99.7, 88.0 [C-1(β)], 75.7, 73.3, 69.0, 65.6, 62.8, 48.05, 48.02, 28.7, 28.6, 17.6, 17.5 ppm. HRMS: calcd. for $C_{22}H_{30}O_{10}SNa^+$ [M^+ + Na] 509.1452; found 509.1454.

Synthesis of Resin-Bound 13: Thioglucoside **12** (0.130 g, 0.27 mmol) was dissolved in CH_2Cl_2 (2 mL) and cooled to 0 °C. HOBt (0.036 g, 0.27 mmol) was added, followed by DIC (0.041 mL, 0.37 mmol), and the solution was stirred at 0 °C for 10 min. The reaction mixture was transferred by cannula to Tentagel MB-NH_2 resin (0.601 g, 0.24 mmol) swelled in CH_2Cl_2 (2 mL), and the reaction was shaken at room temperature for 6 h. The resin was filtered, and rinsed three times with DMF, MeOH and CH_2Cl_2, and dried in vacuo over phosphorus pentoxide. HR-MAS ^{1}H NMR spectroscopy indicated successful coupling, with a theoretical resin loading of 0.32 mmol g^{-1}, as calculated from the resin weight gain.

Synthesis of Resin-Bound 14: Resin-bound thioglucoside acceptor **13** (0.711 g, 0.23 mmol) was swelled in CH_2Cl_2 (2 mL) with 4 Å molecular sieves. Donor **5** (0.340 g, 0.69 mmol) was dissolved in CH_2Cl_2, transferred by cannula to the reaction flask and shaken for 1 h. The reaction was cooled to –20 °C and TMSOTf (0.049 mL, 0.27 mmol) was added. The solution phase was filtered after 15 min and the resin washed three times with DMF, MeOH, Et_2O and CH_2Cl_2. The resin was dried in vacuo over phosphorus pentoxide. HR-MAS ^{1}H NMR spectroscopy indicated successful coupling, with a theoretical resin loading of 0.28 mmol g^{-1}, as calculated from the resin weight gain.

Synthesis of Resin-Bound 15: Resin-bound donor **14** (0.350 g, 0.10 mmol) was swelled in CH_2Cl_2 (2 mL) with 4 Å molecular sieves. Acceptor **7** (0.280 g, 0.78 mmol) was dissolved in CH_2Cl_2, transferred by cannula to the reaction flask and the reaction mixture was shaken at room temperature for 1 h. The reaction was cooled to –20 °C, NIS (0.037 g, 0.12 mmol) and TMSOTf (0.013 mL, 0.07 mmol) was added, and the reaction shaken at –20 °C for min. The solution phase was filtered out and the resin was washed three times with DMF, MeOH, Et_2O and CH_2Cl_2. The resin was dried in vacuo over phosphorus pentoxide. HR-MAS ^{1}H NMR spectroscopy indicated successful coupling with a theoretical resin loading of 0.186 mmol g^{-1}, as calculated from resin weight gain.

Cleavage from the Resin−Methyl 3-*O*-*tert*-Butyldiphenylsilyl-(2*R*)-2-*O*-[α-D-mannopyranosyl-(1→2)-3,4-di-*O*-(2,3-dimethoxybutane-2,3-diyl)-1-*O*-α-D-glucopyranosyl]-2,3-dihydroxypropanoate (16): Resin **15** (0.430 g) was swelled in CH_2Cl_2 for 30 min and then suspended in MeOH. A 1 N solution of NaOMe (0.6 equiv.) was added and the reaction was stirred for 5 h at room temperature. The resin was filtered and washed six times with CH_2Cl_2 and MeOH. The solution phase was treated with previously activated Dowex-H^+, filtered with MeOH and concentrated under reduced pressure. Purification was accomplished by preparative TLC (1:9, MeOH/CH_2Cl_2) to give the desired product **16** (2 mg, 18%). This product was very polar and for further characterization it was acetylated to afford **17**. Compound **16**: ^{1}H NMR ($CDCl_3$): δ = 7.68–7.64 (m, 4 H), 7.42–7.39 (m, 6 H), 5.16–5.12 [m, 2 H, 1-H(α) mannose and glucose], 4.41–4.39 (m, 1 H), 4.15–3.58 (m, 14 H), 3.75 (s, 3 H), 3.21 (s, 3 H), 3.17 (s, 3 H), 1.26 (s, 3 H), 1.22 (s, 3 H), 1.02 (s, 9 H) ppm.

Methyl 3-*O*-*tert*-Butyldiphenylsilyl-(2*R*)-2-*O*-[2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→2)-6-*O*-acetyl-3,4-di-*O*-(2,3-dimethoxybutane-2,3-diyl)-1-*O*-α-D-glucopyranosyl]-2,3-dihydroxypropanoate (17): Acetic anhydride (5 μL, 5.3×10^{-2} mmol) and a catalytic quantity of DMAP were added to a solution of **16** (2 mg, 2.5×10^{-3} mmol) in pyridine (0.5 mL) at room temperature. After 12 h at room temperature, the reaction was quenched with H_2O (1 mL), extracted with EtOAc (3 × 2 mL) and the combined organic phases were dried ($MgSO_4$) and concentrated. Purification by flash column chromatography on silica gel (30:70 EtOAc/hexane) afforded product **17** as viscous colorless oil (3 mg, 100%). $[\alpha]_D^{20}$ = +101.5 (c = 1.99, CH_2Cl_2). FTIR (film): $\tilde{\nu}$ = 1747 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 7.70–7.66 (m, 4 H), 7.43–7.42 (m, 6 H), 5.45 (dd, J = 3.4, 9.9 Hz, 1 H), 5.39–5.37 (m, 1 H), 5.30 (t, J = 10.0 Hz, 1 H), 5.18 [d, J = 3.7 Hz, 1 H, C-1(α) glucose], 5.16 [d, J = 0.96 Hz, 1 H, C-1(α) mannose], 4.50–4.46 (m, 1 H), 4.44 (dd, J = 6.0, 3.6 Hz, 1 H), 4.23–4.10 (m, 5 H), 4.07–4.00 (m, 2 H), 3.93–3.88 (m, 2 H), 3.71–3.64 (m, 1 H), 3.68 (s, 3 H), 3.29 (s, 3 H), 3.19 (s, 3 H), 2.17 (s, 3 H), 2.13 (s, 3 H), 2.04 (s, 3 H), 2.03 (s, 3 H), 1.97 (s, 3 H), 1.27 (s, 3 H), 1.26 (s, 3 H), 1.04 (s, 9 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 170.7, 170.6, 170.0, 169.8, 169.7, 169.6, 135.6, 135.5, 133.1, 132.8, 129.8, 129.7, 127.9, 127.86, 127.84, 99.8, 99.7, 94.7, 94.2, 75.0, 70.8, 69.4, 69.1, 68.5, 67.5, 67.4, 66.2, 66.0, 64.3, 62.1, 62.0, 60.3, 52.0, 47.8, 47.7, 26.6, 20.9, 20.76, 20.71, 20.70, 20.6, 19.1, 17.6, 17.5, 14.1 ppm.

Acknowledgments

We wish to acknowledge the analysis department at the ITQB for providing elemental analyses. We acknowledge the generous financial support provided by Fundação para a Ciência e Tecnologia (FCT) PTDC/BIO/70806/2006. E. L. acknowledges the FCT for a

Ph.D. grant SFRH/47702/2008. The NMR spectrometers are part of the National NMR Network and were purchased in the framework of the National Program for Scientific Re-equipment, contract REDE/1517/RMN/2005, with funds from POCI 2010 (FEDER) and FCT.

[1] M. S. da Costa, H. Santos, E. A. Galinski, *Adv. Biochem. Eng./Biotechnol.* **1998**, *61*,117–153.
[2] H. Santos, M. S. da Costa, *Environm. Microbiol.* **2002**, *4*, 501–509.
[3] A. Ramos, N. D. H. Raven, R. J. Sharp, S. Bartolucci, M. Rossi, R. Cannio, J. Lebbink, J. Van Der Oost, W. M. De Vos, H. Santos, *Appl. Environm. Microbiol.* **1997**, *63*, 4020–4025.
[4] E. C. Lourenço, M. R. Ventura, C. D. Maycock, *Carbohydr. Res.* **2009**, *344*, 2073–2078.
[5] C. D. Jorge, P. Lamosa, H. Santos, *FEBS J.* **2007**, *274*, 3120–3127. H. Santos, P. Lamosa, C. D. Jorge, M. S. Costa, European Patent EP1616005B1; WO 2004/094631.
[6] C. Fernandes, V. Mendes, J. Costa, N. Empadinhas, C. Jorge, P. Lamosa, H. Santos, M. S. da Costa, *J. Bacteriol.* **2010**, *192*, 1624–1633.
[7] H. Imai, T. Oishi, T. Kikuchi, M. Hirama, *Tetrahedron* **2000**, *56*, 8451–8459.
[8] A. J. Burke, C. D. Maycock, M. R. Ventura, *Org. Biomol. Chem.* **2006**, *4*, 2361–2363.
[9] J.-L. Montchamp, F. Tian, M. E. Hart, J. W. Frost, *J. Org. Chem.* **1996**, *61*, 3897–3899.
[10] S. V. Ley, D. K. Baeschlin, D. J. Dixon, A. C. Foster, S. J. Ince, H. W. M. Priepke, D. J. Reynolds, *Chem. Rev.* **2001**, *101*, 53–80.
[11] D. Crich, P. Jayalath, *J. Org. Chem.* **2005**, *70*, 7252–7259.
[12] M. Kort, R. P. M. Valentijn, G. A. van der Marel, J. H. van Boom, *Tetrahedron Lett.* **1997**, *38*, 7629–7632.
[13] D. Crich, C. Weiling, *J. Org. Chem.* **1999**, *64*, 4926–4930.
[14] R. R. Schmidt, J. Michel, *Angew. Chem.* **1980**, *92*, 763; *Angew. Chem. Int. Ed. Engl.* **1980**, *19*, 731–733.
[15] H. Tokimoto, Y. Fujimoto, K. Fukase, S. Kusumoto, *Tetrahedron: Asymmetry* **2005**, *16*, 441–447.
[16] T. Zhu, G.-J. Boons, *Angew. Chem.* **1998**, *110*, 2000; *Angew. Chem. Int. Ed.* **1998**, *37*, 1898–1900.
[17] D. K. Hunt, P. H. Seeberger, *Org. Lett.* **2002**, *4*, 2751–2754.
[18] M. Adinolfi, G. Barone, L. De Napoli, A. Iadonisi, G. Piccialli, *Tetrahedron Lett.* **1996**, *37*, 5007–5010.
[19] E. Lourenço, M. R. Ventura, *Carbohydr. Res.* **2011**, *346*, 163–168.
[20] W. L. F. Armarego, C. L. L. Chai in *Purification of Laboratory Chemicals*, 5th Ed., Elsevier, **2003**.

Received: June 28, 2011
Published Online: September 19, 2011

