Pergamon

Tetrahedron 58 (2002) 521–530

TETRAHEDRON

Synthesis and biological evaluation of oligosaccharides related to the molecule signals in plant defence and the *Rhizobium*-legume symbiosis

I. Robina,[a,*] S. Gómez-Bujedo,[a] J. G. Fernández-Bolaños,[a] J. Fuentes[a] and H. P. Spaink[b]

[a]*Departamento de Química Orgánica, Facultad de Química, Universidad de Sevilla, Apartado 553, E-41071 Sevilla, Spain*
[b]*Institute of Molecular Plants Sciences, Leiden University, Wassenaarseweg 64, 2333 Al. Leiden, The Netherlands*

Received 20 July 2001; revised 28 September 2001; accepted 5 November 2001

Abstract—The synthesis of tetrasaccharides related to the chitinoligosaccharides involved in plant defence and *Rhizobium*-legume symbiosis is reported. One and two central GlcNAc residues of the chitin backbone have been replaced by Glc units. A biological evaluation using *Catharanthus roseaus* assay indicates that the replacement of the two central units produces a total loss of activity. However, the replacement of the GlcNAc moiety close to the reducing end by Glc in the chitin tetramer, does not reduce its activity.

1. Introduction

Chitin oligosaccharides are important biological signal molecules because they play a role in plant defence response and because they constitute the backbone of the rhizobial nodulations factors of nitrogen fixation. Nitrogen fixation is a natural process, which is exclusive to some prokaryote microorganisms and consists of the reduction of atmospheric nitrogen into ammonium ion, which is assimilated by plants. The most generally extended nitrogen fixation is a symbiotic process which is provoked by Gram-negative bacteria of the Rhizobia family and among them, five kinds, *Rhizobium*, *Sinorhizobium*, *Bradyrhizobium*, *Mesorhizobium* and *Azorhizobium*. These bacteria are able to establish a symbiosis with legume plants leading to the formation of certain organs called nodules in which nitrogen is fixed. In return, bacteria obtain sources of carbon, in general products of vegetal photosynthesis. This symbiotic association is probably one of the most important and well-known of all plant–microbe interactions.[1] In the symbiotic process, the plant controls the bacteria by secreting flavonoids, which if they are recognised by the bacteria, put into action the transcription of the nodulation genes (Nod-genes) that induce codified proteins (Nod-proteins), which are enzymes involved in the biosynthesis and releasing of specific signal molecules called nodulation factors (Nod-factors). These Nod-factors, also called LCOs, are lipochitinoligosaccharides, which, on being recognised by the plant, induce the formation of the nodules. The symbiosis is specific: one type of *Rhizobium* infects one or several types of leguminous plant, and the two partners exchange low molecular weight signal molecules.

These LCOs are always lipooligosaccharides with β(1→4) linkages, generally with a tri-, tetra- or pentasaccharide backbone of *N*-acetylglucosamine, decorated at both ends with a variety of substituents: acetyl, carbamoyl, sulphate or another sugar.[2] The biological activity is highly specific,[3] the substituents decorating the oligosaccharide backbone being mediators in that specificity. As an exception to these LCOs in which the base backbone is formed by GlcNAc units, in 1996, Promé and co-workers[4] described another signal molecule secreted by *Sinorhizobium fredii* which has a pentasaccharide structure with a central unit of Glc and four units of GlcNAc.

It is important to point out that in some cases it has been possible to initiate the nodulation process in the absence of the bacteria, by adding or injecting these LCOs in very low concentrations (10^{-6}–10^{-12} M) into the roots.[5]

The important role of these molecules in nitrogen fixation, in the study of signal exchange process, host specificity and their natural scarcity, has encouraged several groups to perform their chemical synthesis and in the last few years several strategies for the preparation of these LCOs[6] have been described. In order to clarify the basis of the structure–activity relationships, synthetic analogues[7] have also been reported.

At the same time, a rapid and sensitive bioassay for chitin oligosaccharides has been used[8] employing suspension-cultured plant cells of *Catharantus roseus*. This plant cell

Keywords: *Rhizobium*; chitin; chitooligosaccharides; LCO.
* Corresponding author. Fax: +34-95-4624960; e-mail: robina@us.es

0040–4020/02/$ - see front matter
PII: S0040-4020(01)01148-6

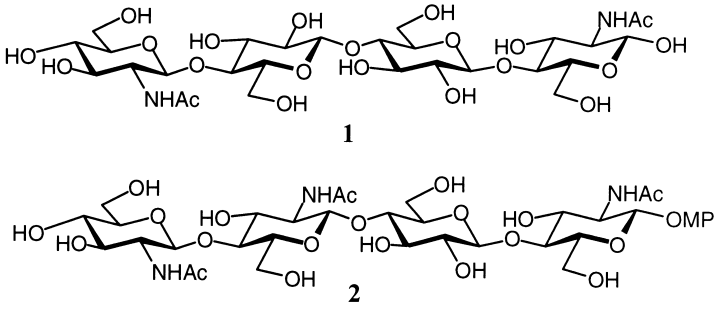
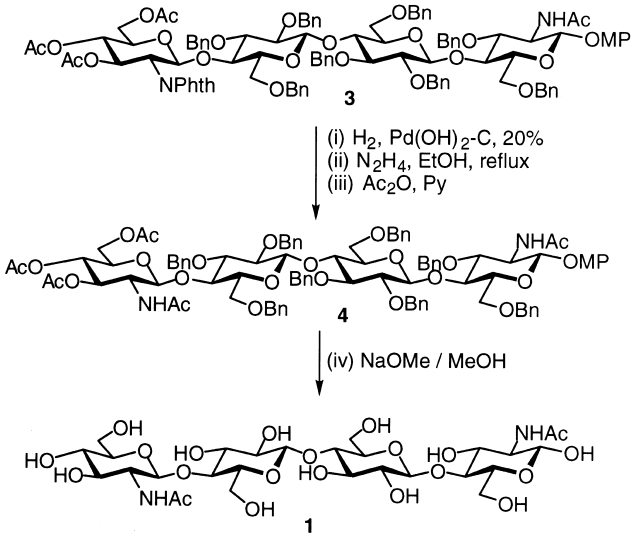


Figure 1.



Scheme 1.

suspension can respond to chitin tetraose in quantities as low as 20 fmol. This biological activity probably relates to a function of COs in plant defence mechanisms. With respect to the biological action of LCOs it should be pointed out that the fatty acyl moiety is important for transport of the LCO inside the plant tissue.[9]

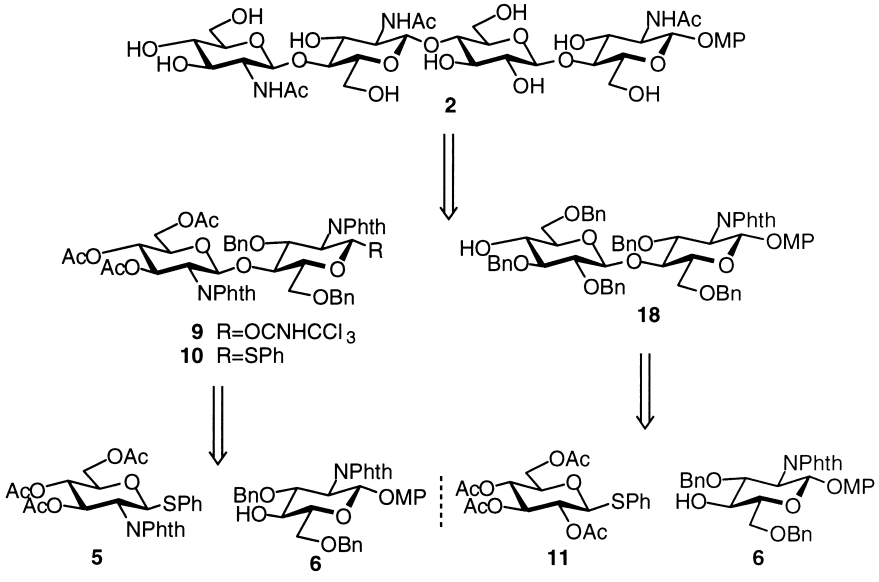
With the aim of investigating the role of the chitin backbone in the biological activity, in this paper we describe the synthesis and biological evaluation of tetrasaccharides **1** and **2** (Fig. 1). These are analogues of the LCOs involved in the *Rhizobium*-legume symbiosis, where two or one central units, respectively, of GlcNAc have been substituted by Glc units.

2. Results and discussion

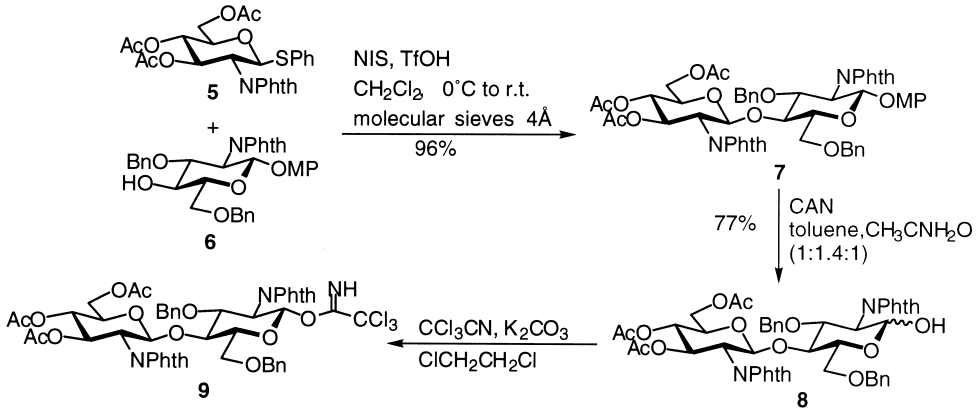
The synthesis of tetrasaccharide **1** has been performed from **3**[7b] (Scheme 1) after hydrogenolysis at rt in the presence of $Pd(OH)_2$ and hydrazinolysis in refluxing ethanol followed by acetylation and Zemplén de-*O*-acetylation.

The preparation of compound **2**, was achieved by a convergent synthesis where the tetrasaccharide backbone is formed by coupling the glycosyl acceptor **18** and the glycosyl donors **9** and **10**. These two disaccharides were obtained from the monosaccharides **5**, **6** and **11** as shown in the retrosynthetic analysis (Scheme 2).

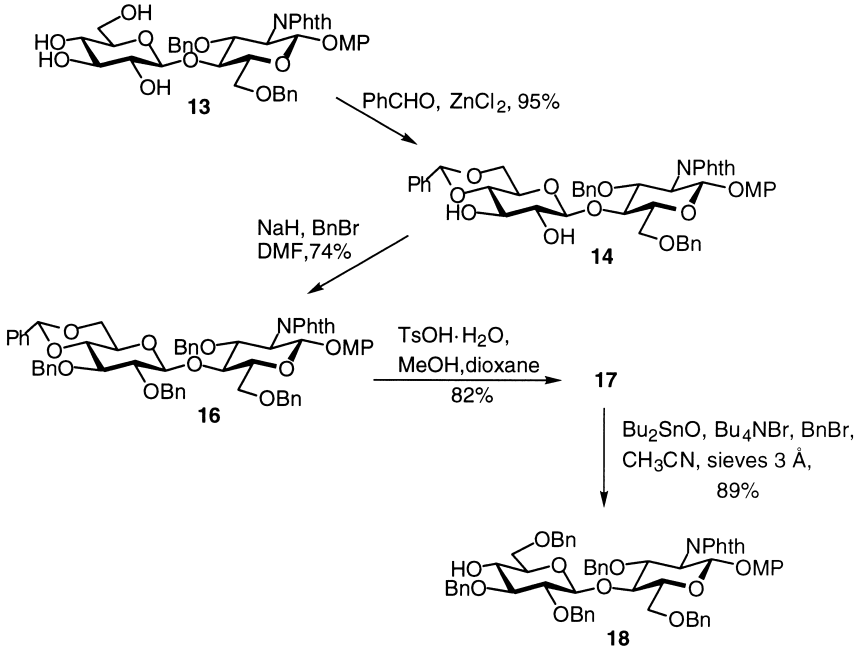
For the synthesis of the trichloroacetimidate **9** (Scheme 3), the corresponding disaccharide **7** was obtained in 96% yield by coupling of **5** and **6** in the presence of NIS and TfOH.



Scheme 2.



Scheme 3.



Scheme 4.

Same reaction but using phenylsulfinyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-β-D-glucopyranoside[10] as glycosyl donor and Tf_2O as promotor gave **7** in 42% yield (see Section 3). Anomeric deprotection with CAN followed by reaction with trichloroacetonitrile gave **9** in 77% overall yield.

The synthesis of the acceptor **18** (Scheme 4) was performed starting from compound **13**[11] previously prepared from **11** and **6**. Benzylidenation of **13** with benzaldehyde and zinc chloride afforded **14** in 95% yield; subsequent treatment with benzyl bromide and NaH gave **16** in 74% yield. It is worth noting that when an excess of NaH is used, the cleavage of one of the amido bonds of the phthalimido group took place and the formation of compound **15** (Fig. 2) is observed formed by basic hydrolysis and *O*- and *N*-benzylation. Acetal hydrolysis (82%) of **16** followed by regioselective benzylation (89%) via tin derivatives[12] rendered the acceptor.

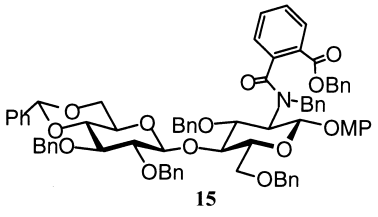
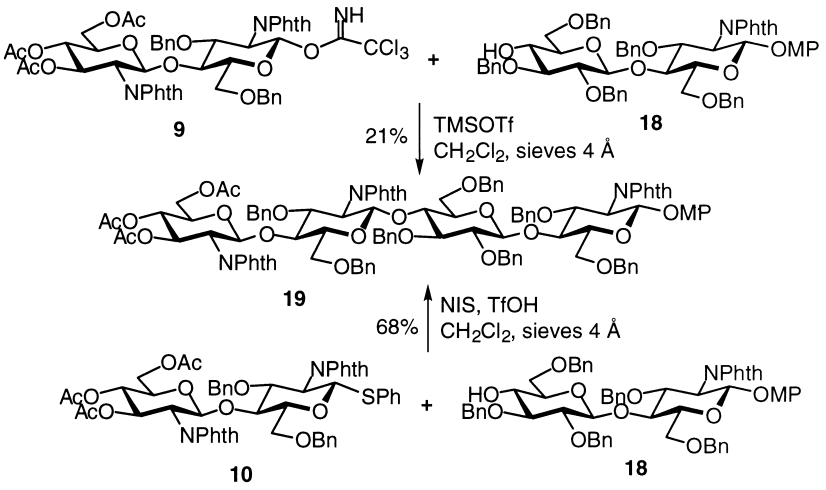


Figure 2.

The coupling reaction between **9** and **18** promoted by TMSOTf gives tetrasaccharide **19** in only 21% yield (54% with respect the consumed acceptor) (Scheme 5). When using the thioglycoside **10**, which was obtained from **9** and thiophenol (83%), for the coupling reaction, the yield increased up to 68%.

The preparation of the unprotected tetrasaccharide **2** was carried out by treatment of **19** with ethylenediamine in ethanol under reflux giving the corresponding amino-tetrasaccharide, which was isolated after acetylation to give **20**. Hydrogenolysis in the presence of $Pd(OH)_2$ gave

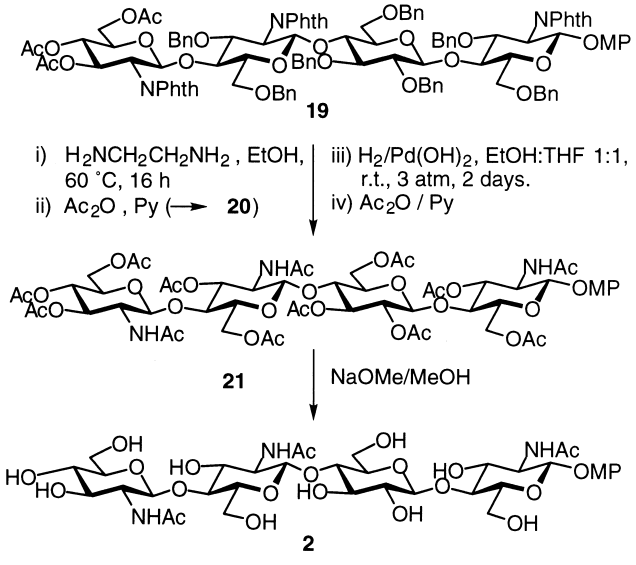


Scheme 5.

2, which was purified via **21** by acetylation and Zemplém de-*O*-acetylation (Scheme 6)

The structures of compounds **1**, **2**, **4**–**19** are supported by analytical, NMR and MS spectroscopic data.

The biological activity of the tetrasaccharides **1** and **2** has been tested in the *C. roseus* assay.[8] This test, which is probably related to a plant defence response, is a much more sensitive assay for biological function than the previously described microtargeting assay in leguminous roots.[13] Furthermore, in contrast to the microtargeting assay (where an *O*-acetyl moiety on the non-reducing terminus is needed), no additional substitutions of the CO backbone is needed for biological activity with *C. roseus*. This test is a preliminary probe before the nodulation assay where the curling of the roots, necessary for the nodulation itself and nitrogen fixation, is analysed. Compound **1** was non-active; however, compound **2**, working in a low concentration of 10 nM, induces alkalinization similar to the chitin tetramer that is very active in this type of experiment. So replacing one GlcNAc moiety by Glc in the chitin tetramer does not reduce activity, but the replacement of two GlcNAc moieties produces a total loss of activity.



Scheme 6.

3. Experimental

3.1. General

Melting points are uncorrected. Optical rotations were measured at 22±1°C for solutions in dichloromethane or methanol. ^{1}H NMR spectra (300 and 500 MHz) were obtained for solutions in $CDCl_3$ or D_2O; *J* values are given in Hz. The FABMS spectra were measured with a KRATOS MS-80RFA instrument. Ions were produced by a beam of xenon atoms (6–7 KeV) using a matrix consisting of glycerol or thioglycerol and NaI as salt, $(CsI)_{37}Cs$ was used as reference. TLC was performed on Silica Gel HF_{254} (Merck), with detection by UV light or charring with H_2SO_4. Silica Gel 60 (Merck, 230 mesh) was used for preparative chromatography. Biogel P2 (biorad) column (42×2.6 cm^2) was used with water as eluent and carbohydrates were detected using a refractive index detector.

3.1.1. *p*-Methoxyphenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoyl-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (4). A mixture of *p*-methoxyphenyl (2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (**3**) (150 mg, 0.08 mmol) and $Pd(OH)_2$–C (20%, 125 mg) in ethanol–THF (1:1, 75 mL) was hydrogenated at 5 atm and at rt for 48 h. Then the reaction mixture was filtered through celite and evaporated to dryness to afford a residue (78 mg) that presented FABMS: *m/z* 958 [100%, $(M+Na)^+$] and that was used without purification in the

next step. To a solution of the crude product in absolute ethanol (20 mL), $N_2H_4{\cdot}H_2O$ (0.3 mL) was added. The mixture was heated under reflux for 3 days, evaporated in vacuo and coevaporated several times with toluene and ethanol. Pyridine (1 mL) and acetic anhydride were added at 0°C to the residue. After 24 h at rt, the solvents were evaporated in vacuo and coevaporated with toluene and ethanol to dryness. Column chromatography of the residue on silica gel (dichloromethane–acetone, 60:1→20:1 and toluene–acetone, 2:1) gave **4** (58 mg, 52%) as a white solid, which crystallised from acetone had mp 248–250°C; $[\alpha]_D=-21°$ (*c* 1, dichloromethane).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.92–6.77 (m, 4H, $C_6H_4OCH_3$), 5.92 (d, 1H, J=8.9 Hz, NH), 5.82 (d, 1H, J=9.2 Hz, NH), 4.94† (d, 1H, $J_{1,2}$=7.4 Hz, H-1), 4.55† (d, 1H, $J_{1''',2'''}$=8.2 Hz, H-1‴), 4.49† (d, 1H, $J_{1'',2''}$=7.8 Hz, H-1″), 4.45† (d, 1H, $J_{1',2'}$=7.8 Hz, H-1′), 5.22–4.80, 4.50–3.90, 3.80–3.60 (3m, sugar ring protons), 3.76 (s, 3H, $C_6H_4OCH_3$), 2.15, 2.14, 2.08, 2.08, 2.05, 2.04, 2.03, 2.01, 2.00, 1.99, 1.99, 1.98, 1.94 (s, 3H each, $COCH_3$). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 170.9–169.2, (13C, $COCH_3$), 155.3 (C-4 de $C_6H_4OCH_3$), 150.9 (C-1 de $C_6H_4OCH_3$), 118.2, 114.4 (4C, $C_6H_4OCH_3$), 100.8, 100.6, 100.3, 99.8 (4C, C-1, C-1′, C-1″, C-1‴), 76.1, 75.6, 75.6, 72.9, 72.7, 72.6, 72.6, 72.3, 72.1, 71.8, 71.6, 71.6, 71.5 (13C, sugar moiety), 67.9 (C-4‴), 62.2, 61.9, 61.7, 61.5 (4C, C-6, C-6′, C-6″, C-6‴), 55.5 ($C_6H_4OCH_3$) 54.8, 53.0 (2C, C-2, C-2‴) 20.5–20.4 (13$COCH_3$). FABMS: *m/z* 1339 [100%, $(M+Na)^+$]. HR-FABMS: Found $(M+Na)^+$ 1339.4327. $C_{57}H_{76}N_2O_{33}Na$ requires 1339.4228.

3.1.2. *p*-Methoxyphenyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (1). To a solution of *p*-methoxyphenyl (2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (**4**) (23 mg, 0.02 mmol) in dry methanol (6 mL), NaOMe/MeOH (1 M, 0.2 mL) was added. The reaction mixture was left at rt for 3 h, made neutral with Amberlist IR-120 (H^+), filtered and concentrated to dryness. The residue was purified on Biogel P-2 to give **1** (13 mg, 76%) as an amorphous solid.

^{1}H NMR (500 MHz, D_2O) δ 7.06–6.96 (m, 4H, $C_6H_4OCH_3$), 5.06† (d, 1H, $J_{1,2}$=8.6 Hz, H-1), 4.58† (m, 2H, H-1′, H-1″), 4.52† (d, 1H, $J_{1''',2'''}$=8.0 Hz, H-1‴), 3.39–3.35 (m, 24H, sugar moiety), 3.81 (s, 3H, $C_6H_4OCH_3$), 2.07, 2.04 (s, 3H each, $COCH_3$). ^{13}C NMR (125.7 MHz, D_2O) δ 176.0, 175.7 (2C, $COCH_3$), 156.0 (C-4 de $C_6H_4OCH_3$), 152.2 (C-1 de $C_6H_4OCH_3$), 119.5, 116.2 (4C, $C_6H_4OCH_3$), 103.5, 103.4, 102.6, 101.7 (4C, C-1, C-1′, C-1″, C-1‴), 80.3, 79.6, 79.3 (3C, C-4, C-4′, C-4″), 77.0, 76.1, 76.0, 75.7, 75.3, 75.2, 74.6, 74.1, 73.9, 73.2, 70.9 (11C, sugar moiety), 61.7, 61.2, 60.1, 60.1 (4C, C-6, C-6′, C-6″, C-6‴), 57.0, 56.7, 56.4 (3C, $C_6H_4OCH_3$, C-2, C-2‴), 23.3, 23.3 (2$COCH_3$). FABMS: *m/z* 877 [100%, $(M+Na)^+$]. HR-FABMS: Found $(M+Na)^+$ 877.3061. $C_{35}H_{54}N_2O_{22}Na$ requires 877.3066.

† Interchangeable signals.

3.1.3. *p*-Methoxyphenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (7). *Procedure (a)*: To a solution of phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**5**) (620 mg, 1.18 mmol) and *p*-methoxyphenyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**6**) (500 mg, 0.84 mmol) in dry dichloromethane (5 mL) with 4 Å molecular sieves, *N*-iodosuccinimide (755 mg, 1.40 mmol) was added under argon. The reaction mixture was stirred at rt for 30 min and then cooled at 0°C. A solution of TfOH (115 μL) of a solution of 25 μL TfOH in dichloromethane (1 mL) was added. The reaction mixture was stirred at 0°C for 1.5 h, then diluted with dichloromethane (15 mL), filtered through celite and washed with saturated aq $NaHCO_3$, aq $Na_2S_2O_3$ 10% and water; dried (Na_2SO_4) and evaporated in vacuo. Column chromatography of the residue on silica gel (ether–petroleum ether, 2:1) gave **7** (813 mg, 96%) as a white solid, which crystallised from ether had mp 110–112°C; $[\alpha]_D=+13°$ (*c* 0.8, dichloromethane).

Procedure (b): To a solution of *p*-methoxyphenyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**6**) (100 mg, 0.17 mmol) and 2,6-di-*terc*-butyl-4-methylpyridine (42 mg, 0.2 mmol) in dry dichloromethane (0.5 mL) with 4 Å molecular sieves, a solution of Tf_2O (32 μL, 0.2 mmol) in dichloromethane (1 mL) at −30°C under argon, was added. The mixture was stirred for 10 min, and then a solution of phenylsulfinyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-β-D-glucopyranoside (108 mg, 0.2 mmol) in dry dichloromethane (1 mL) was dropped. The reaction mixture was warmed to −20°C and stirred for 6.5 h. The reaction mixture was diluted with dichloromethane (15 mL), filtered through celite and washed with saturated aq $NaHCO_3$ and water; dried (Na_2SO_4) and evaporated in vacuo. Purification as above gave **7** (71 mg, 42%, 60% with respect to the consumed acceptor) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93–7.56 (m, 8H, 2Phth), 7.36–6.85 (m, 10H, 2Ph), 6.74–6.60 (m, 4H, $C_6H_4OCH_3$), 5.81 (dd, 1H, $J_{2',3'}$=10.6 Hz, $J_{3',4'}$=9.1 Hz, H-3′), 5.54 (d, 1H, $J_{1',2'}$=8.4 Hz, H-1′), 5.46 (d, 1H, $J_{1,2}$=8.3 Hz, H-1), 5.14 (dd, 1H, $J_{4',5'}$=9.7 Hz, H-4′), 4.86, 4.50 (d, 1H each, $^2J_{H,H}$=12.5 Hz, CH_2Ph), 4.56, 4.50 (d, 1H each, $^2J_{H,H}$=11.8 Hz, CH_2Ph), 4.35 (m, 2H, H-2, H-2′), 4.30 (m, 1H, H-3), 4.27 (m, 1H, H-4), 4.23 (m, 1H, $J_{5',6'a}$=4.3 Hz, $J_{6'a,6'b}$=12.3 Hz, H-6′a), 3.66 (s, 3H, $C_6H_4OCH_3$), 3.64 (m, 1H, H-6a), 3.51 (m, 1H, H-6b), 3.50 (m, 1H, $J_{5',6'b}$=2.0 Hz, H-5′), 3.43 (m, 1H, H-5), 2.96 (dd, 1H, H-6′b), 2.01, 1.99, 1.86 (s, 3H each, $COCH_3$). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 170.6, 170.0, 169.4 (3C, $COCH_3$), 168.0–166.0 (4CO, 2Phth), 155.1 (C-4 de $C_6H_4OCH_3$), 150.6 (C-1 de $C_6H_4OCH_3$), 138.3–114.1 (28C aromatic), 97.4 (C-1′), 96.8 (C-1), 77.1 (C-4), 76.1 (C-3), 74.4 (C-5), 74.2, 72.6 (2C, CH_2Ph), 71.4 (C-5′), 70.5 (C-3′), 68.7 (C-4′), 67.8 (C-6), 61.4 (C-6′), 55.4 ($C_6H_4OCH_3$), 55.4, 55.1 (3C, C-2, C-2′, C-5), 20.5, 20.5, 20.3 (3C, $COCH_3$). FABMS: *m/z* 1035 [100%, $(M+Na)^+$]. Anal. Calcd for $C_{55}H_{52}N_2O_{17}$: C, 65.21; H, 5.17; N, 2.76. Found C, 65.04; H, 5.38; N, 2.76.

3.1.4. 3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-D-glucopyranose (8). To a solution of *p*-methoxyphenyl

(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido)-β-D-glucopyranoside (**7**) (813 mg, 0.80 mmol) in toluene–acetonitrile–water (1:1.4:1, 3 mL), CAN (4.4 g, 8.0 mmol) was added. The reaction mixture was stirred at rt for 1.5 h, then diluted with dichloromethane and washed successively with saturated aq NaCl, saturated aq $NaHCO_3$ and water; dried (Na_2SO_4) and evaporated in vacuo. Column chromatograhy of the residue on silica gel (ether–petroleum ether, 3:1) gave **8** (561 mg, 77%;) in a ratio α–β=1:6. $[\alpha]_D$=0° (*c* 1, dichloromethane).

^{1}H NMR, β anomer (300 MHz, $CDCl_3$) δ 7.93–7.65 (m, 8H, 2Phth), 7.47–6.84 (m, 10H, 2CH_2Ph), 5.78 (dd, 1H, $J_{2',3'}$=10.7 Hz, $J_{3',4'}$=9.0 Hz, H-3′), 5.52 (d, 1H, $J_{1',2'}$=8.3 Hz, H-1′), 5.17 (d, 1H, $J_{1,2}$=8.3 Hz, H-1), 5.12 (dd, 1H, $J_{4',5'}$=10.0 Hz, H-4′), 4.84, 4.47 (d, 1H each, $^2J_{H,H}$=12.5 Hz, CH_2Ph), 4.58 (m, 2H, CH_2Ph), 4.33 (dd, 1H, H-2′), 4.27–4.24 (m, 2H, H-3, H-4), 4.22 (m, 1H, $J_{5',6'a}$=4.2 Hz, $J_{6'a,6'b}$=12.4 Hz, H-6′a), 4.01 (dd, 1H, $J_{2,3}$=10.4 Hz, H-2), 3.95 (dd, 1H, $J_{5',6'b}$=2.2 Hz, H-6′b), 3.60 (dd, 1H, $J_{5,6a}$=1.3 Hz, $J_{6a,6b}$=11.2 Hz, H-6a), 3.49 (m, 1H, H-6b), 3.48 (m, 1H, H-5′), 3.44 (m, 1H, H-5), 2.01, 1.97, 1.84 (s, 3H each, COCH_3). ^{13}C NMR, β anomer (75.4 MHz, $CDCl_3$) δ 170.5, 170.0, 169.4 (3C, *C*OCH$_3$), 167.8 (4CO, 2Phth), 138.4–123.1 (24C aromatic), 96.6 (C-1′), 92.6 (C-1), 76.2 (C-3), 75.7 (C-4), 74.3 (C-5), 74.0, 72.7 (2C, *C*H$_2$Ph), 71.4 (C-5′), 70.5 (C-3′), 68.6 (C-4′), 68.0 (C-6), 61.4 (C-6′), 57.4 (C-2), 55.1 (C-2′), 20.5, 20.3, 19.6 (3C, CO*C*H$_3$). FABMS: *m/z* 929 [100%, (M+Na)$^+$]. HR-FABMS: Found (M+Na)$^+$ 929.2761. $C_{49}H_{51}NO_{17}Na$ requires 929.2745.

3.1.5. 3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (9). To a solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-D-glucopyranose (**8**) (350 mg, 0.39 mmol) in dry 1,2-dichloroethane (4 mL), K_2CO_3 (240 mg, 1.74 mmol) and freshly distilled Cl_3CCN (0.21 mL, 2.08 mmol) were added. The reaction mixture was left at rt for 20 h, then filtered and evaporated to dryness. The crude product **9** (399 mg) was used without purification in the next step.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.43 (s, 1H, N*H*), 7.93–7.76 (m, 8H, 2Phth), 7.44–6.83 (m, 10H, 2CH_2Ph), 6.24 (d, 1H, $J_{1,2}$=8.7 Hz, H-1), 5.81 (dd, 1H, $J_{2',3'}$=10.7 Hz, $J_{3',4'}$=9.0 Hz, H-3′), 5.54 (d, 1H, $J_{1',2'}$=8.4 Hz, H-1′), 5.13 (dd, 1H, $J_{4',5'}$=10.1 Hz, H-4′), 4.85, 4.50 (d, 1H each, $^2J_{H,H}$=12.5 Hz, CH_2Ph), 4.62, 4.56 (d, 1H each, $^2J_{H,H}$=11.5 Hz, CH_2Ph), 4.44–4.30 (m, 4H, H-2′, H-2, H-3, H-4), 4.22 (m, 1H, $J_{5',6'a}$=4.1 Hz, $J_{6'a,6'b}$=12.3 Hz, H-6′a), 3.66 (m, 1H, H-6a), 3.55 (m, 1H, H-5), 3.52 (m, 1H, H-6b), 3.45 (ddd, 1H, $J_{5',6'a}$=2.2 Hz, H-5′), 3.94 (dd, 1H, H-6′b), 2.01, 1.99, 1.86 (s, 3H each, COCH_3). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 170.6, 169.9, 169.4 (3C, *C*OCH$_3$), 167.3 (CO, Phth), 160.7 (C=NH), 138.2–123.1 (24C aromatic), 96.5 (C-1′), 93.7 (C-1), 76.1 (C-3), 75.4, 75.2 (2C, C-4, C-5), 74.1, 72.6 (2C, *C*H$_2$Ph), 71.4 (C-5′), 70.4 (C-3′), 68.7 (C-4′), 67.5 (C-6), 61.4 (C-6′), 55.0 (C-2′), 54.3 (C-2), 20.8, 20.5, 20.3 (3CO*C*H$_3$). FABMS: *m/z* 1074 [100%, (M+Na)$^+$].

3.1.6. Phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (10). To a mixture of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**9**) (165 mg, 0.157 mmol) and thiophenol (240 μL, 2.35 mmol) with 4 Å molecular sieves in 1,2-dichloroethane (3 mL) at 0°C under argon, 32 μL (0.016 mmol) of a solution of 50 μL TMSOTf, in 1,2-dichloroethane (1 mL) was added. The mixture was stirred at rt for 16 h, and then diluted with dichloromethane and washed with saturated aq $NaHCO_3$ and water; dried (Na_2SO_4) and evaporated to dryness. Column chromatography of the residue on silica gel (ether–petroleum ether, 1:1) gave **10** (130 mg, 83%) as a white solid that crystallised from ether had mp 100–102°C; $[\alpha]_D$=+11° (*c* 1.8, dichloromethane).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92–7.69 (m, 8H, 2Phth), 7.52–6.82 (m, 15H, 2CH_2Ph, Ph), 5.79 (dd, 1H, $J_{2',3'}$=10.7 Hz, $J_{3',4'}$=9.1 Hz, H-3′), 5.52 (d, 1H, $J_{1',2'}$=8.4 Hz, H-1′), 5.34 (d, 1H, $J_{1,2}$=10.1 Hz, H-1), 5.11 (dd, 1H, $J_{4',5'}$=10.1 Hz, H-4′), 4.85, 4.46 (d, 1H each, $^2J_{H,H}$=12.5 Hz, CH_2Ph), 4.56, 4.50 (d, 1H each, $^2J_{H,H}$=11.7 Hz, CH_2Ph), 4.32 (dd, 1H, H-2′), 4.22 (m, 1H, $J_{5',6'a}$=4.2 Hz, $J_{6'a,6'b}$=12.3 Hz, H-6′a), 4.21–4.18 (m, 3H, H-2, H-3, H-4), 3.60 (dd, 1H, $J_{5,6a}$=1.2 Hz, $J_{6a,6b}$=11.2 Hz, H-6a), 3.51 (m, 1H, H-5′), 3.47 (m, 1H, H-6b), 3.40 (m, 1H, H-5), 3.93 (dd, 1H, H-6′b), 1.99, 1.93, 1.83 (s, 3H each, COCH_3). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 170.5, 169.9, 169.3 (3C, *C*OCH$_3$), 167.8–167.1 (4CO, 2Phth), 138.3–123.1 (30C aromatic), 96.8 (C-1′), 83.3 (C-1), 77.8, 76.0 (2C, C-3, C-4), 78.6 (C-5), 74.4, 72.7 (2C, *C*H$_2$Ph), 71.5 (C-5′), 70.6 (C-3′), 68.9 (C-4′), 68.2 (C-6), 61.5 (C-6′), 55.3 (C-2′), 54.7 (C-2), 20.5, 20.5, 20.5 (3CO*C*H$_3$). FABMS: *m/z* 1021 [100%, (M+Na)$^+$]. HR-FABMS: Found (M+Na)$^+$ 1021.2863. $C_{54}H_{50}N_2O_{15}NaS$: requires 1021.2829.

3.1.7. *p*-Methoxyphenyl 4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (14). To a solution of *p*-methoxyphenyl β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside[10] (**13**) (820 mg, 1.08 mmol) in freshly distilled benzaldehyde (4 mL) dry $ZnCl_2$ (295 mg, 2.17 mmol) was added. The reaction mixture was stirred at rt for 16 h and then washed with water (3×30 mL). Petroleum ether was added to the organic layer and a white solid was obtained which was filtered off and washed successively with water, petroleum ether and diethyl ether to give a residue that was finally purified by column chromatography on silica gel (dichloromethane–acetone, 30:1→15:1) to give **14** (910 mg, 95%) as a white solid, that crystallised from acetone had mp 110–112°C; $[\alpha]_D$=+42° (*c* 0.9, dichloromethane).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12–6.88 (m, 19H, Ph, 2CH_2Ph, Phth), 6.82–6.67 (m, 4H, C_6H_4OCH$_3$), 5.60 (d, 1H, $J_{1,2}$=8.3 Hz, H-1), 5.46 (s, 1H, C*H*Ph), 4.82, 4.46 (d, 1H each, $^2J_{H,H}$=12.2 Hz, CH_2Ph), 4.78, 4.62 (d, 1H each, $^2J_{H,H}$=12.0 Hz, CH_2Ph), 4.72 (d, 1H, $J_{1',2'}$=7.8 Hz, H-1′), 4.44 (m, 2H, H-2, H-3), 4.24 (m, 1H, H-4), 4.16 (dd, 1H, $J_{5',6'a}$=5.0 Hz, $J_{6'a,6'b}$=10.3 Hz, H-6′a), 4.05 (dd, 1H, $J_{5,6a}$=3.4 Hz, $J_{6a,6b}$=11.6 Hz, H-6a), 3.86 (dd, 1H, $J_{5,6b}$=2.1 Hz,

H-6b), 3.73 (m, 1H, H-5), 3.71 (dd, 1H, $J_{2',3'}$=8.9 Hz, $J_{3',4'}$=9.5 Hz, H-3′), 3.71 (s, 3H, $C_6H_4OCH_3$), 3.56 (t, 1H, $J_{5',6'b}$=10.3 Hz, H-6′b), 3.48 (t, 1H, $J_{4',5'}$=8.9 Hz, H-4′), 3.48 (dd, 1H, H-2′), 3.24 (td, 1H, H-5′). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 167.7, 167.7 (2CO, Phth), 155.3 (C-4, $C_6H_4OCH_3$), 150.6 (C-1, $C_6H_4OCH_3$), 138.1–114.2 (28C aromatic), 103.4 (C-1′), 101.7 (*C*HPh), 97.7 (C-1), 78.7 (C-4), 78.1 (C-3), 74.6 (C-5), 65.5 (C-5′), 74.7, 73.2 (2C, CH_2Ph), 73.5 (C-3′), 68.7 (C-4′), 67.9(C-6), 68.5 (C-6′), 75.4 (C-2′), 55.6 (C-2), 55.5 ($C_6H_4OCH_3$). FABMS: *m/z* 868 [100%, $(M+Na)^+$]. Anal. Calcd for $C_{48}H_{47}NO_{13}$: C, 68.15; H, 5.60; N, 1.66. Found C, 68.21; H, 5.98; N, 1.67.

3.1.8. *p*-Methoxyphenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (16). To a solution of *p*-methoxyphenyl (4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**14**) (412 mg, 0.488 mmol) in dry DMF (2 mL), NaH (80%) (89 mg, 2.93 mmol) was added. The mixture was stirred at rt for 15 min, cooled to 0°C and then BnBr (521 μL, 4.39 mmol) was added. The reaction mixture was stirred for 24 h at rt, diluted with dichloromethane and filtered through celite. The filtrate was washed successively with, saturated aq $NaHCO_3$ and water; dried (Na_2SO_4) and evaporated to dryness. Column chromatography of the residue (toluene–acetone, 15:1) gave **16** (370 mg, 74%) as a white solid that crystallised from acetone had mp 68–70°C; $[\alpha]_D$=+14° (*c*0.9, dichloromethane).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69 (m, 4H, Phth), 7.51–6.91 (m, 25H, 5Ph), 6.84–6.68 (m, 4H, $C_6H_4OCH_3$), 5.61 (d, 1H, $J_{1,2}$=8.5 Hz, H-1), 5.49 (s, 1H, C*H*Ph), 4.93, 4.79 (d, 1H each, $^2J_{H,H}$=11.3 Hz, CH_2Ph), 4.87, 4.83 (d, 1H each, $^2J_{H,H}$=11.1 Hz, CH_2Ph), 4.81, 4.47 (d, 1H each, $^2J_{H,H}$=12.3 Hz, CH_2Ph), 4.59 (d, 1H, $J_{1',2'}$=7.8 Hz, H-1′), 4.58, 4.40 (d, 1H each, $^2J_{H,H}$=12.0 Hz, CH_2Ph), 4.43 (dd, 1H, $J_{2,3}$=10.7 Hz, H-2), 4.34 (dd, 1H, $J_{3,4}$=8.5 Hz, H-3), 4.28 (dd, 1H, $J_{5',6'a}$=4.9 Hz, $J_{6'a,6'b}$=10.5 Hz, H-6′a), 4.16 (dd, 1H, $J_{4,5}$=9.9 Hz, H-4), 3.89 (dd, 1H, $J_{5,6a}$=4.2 Hz, $J_{6a,6b}$=11.1 Hz, H-6a), 3.75 (dd, 1H, $J_{5,6b}$=1.6 Hz, H-6b), 3.71 (s, 3H, $C_6H_4OCH_3$), 3.67 (dd, 1H, $J_{2',3'}$=8.7 Hz, $J_{3',4'}$=9.3 Hz, H-3′), 3.62 (ddd, 1H, H-5), 3.59 (t, 1H, $J_{4',5'}$=9.3 Hz, H-4′), 3.48 (t, 1H, $J_{5',6'b}$=10.5 Hz, H-6′b), 3.42 (dd, 1H, H-2′), 3.23 (ddd, 1H, H-5′). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 155.3 (C-4, $C_6H_4OCH_3$), 150.9 (C-1, $C_6H_4OCH_3$), 138.5–114.3 (40C aromatic), 103.0 (C-1′), 101.1 (*C*HPh), 97.7 (C-1), 82.6 (C-2′), 81.1 (C-3′), 81.1 (C-4′), 78.1 (C-4), 77.2 (C-3), 75.3 (C-5), 75.6, 74.9, 74.4, 73.2 (4C, CH_2Ph), 68.7 (C-6′), 67.7 (C-6), 65.7 (C-5′), 55.5 (C-2), 55.5 ($C_6H_4OCH_3$). FABMS: *m/z* 1048 [70%, $(M+Na)^+$]. Anal. Calcd for $C_{62}H_{59}NO_{13}$: C, 72.57; H, 5.79; N, 1.36. Found: C, 72.28; H, 5.98; N, 1.44.

3.1.9. *p*-Methoxyphenyl (2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-(*N*-benzyl-*N*-benzyloxycarbonylbenzoyl)amino-2-deoxy-β-D-glucopyranoside (15). To a solution of *p*-methoxyphenyl (4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**14**) (50 mg, 0.059 mmol) in dry DMF (1 mL), NaH (80%) (71 mg, 2.36 mmol) was added. The mixture was stirred at rt for 15 min, cooled to 0°C and then BnBr (280 μL, 2.36 mmol) was added. The reaction mixture was stirred for 24 h at rt, diluted with dichloromethane and filtered through celite. The filtrate was washed successively with, saturated aq $NaHCO_3$ and water, dried (Na_2SO_4) and evaporated to drynesss. Column chromatography of the residue (toluene–acetone, 40:1) gave **17** (48 mg, 80%) as a white solid that crystallised from acetone had mp 66–68°C; $[\alpha]_D$=-4° (*c* 0.9, dichloromethane).

^{1}H NMR (300 MHz, $CDCl_3$, 318 K) δ 7.99–6.79 (m, 39H, Phth, Ph, $C_6H_4OCH_3$), 6.25 (m, 1H, H-1), 5.42 (s, 1H, C*H*Ph), 5.33–5.06 (m, 4H, $2CH_2Ph$), 4.91, 4.77 (d, 1H each, $^2J_{H,H}$=11.4 Hz, CH_2Ph), 4.86, 4.81 (d, 1H each, $^2J_{H,H}$=11.1 Hz, CH_2Ph), 4.59 (m, 2H, H-1′, H-2), 4.56, 4.38 (d, 1H each, $^2J_{H,H}$=12.0 Hz, CH_2Ph), 4.55 (s, 2H, CH_2Ph), 4.15 (dd, 1H, $J_{5',6'a}$=4.5 Hz, $J_{6'a',6'b}$=10.0 Hz, H-6′a), 4.07 (t, 1H, $J_{3,4}$=$J_{4,5}$=9.2 Hz, H-4), 3.85 (dd, 1H, $J_{5,6a}$=4.5 Hz, $J_{6a,6b}$=10.0 Hz, H-6a), 3.75 (s, 3H, $C_6H_4OCH_3$), 3.69 (m, 3H, H-3, H-5, H-6b), 3.62 (t, 1H, $J_{2',3'}$=$J_{3',4'}$=8.9 Hz, H-3′), 3.52 (t, 1H, $J_{4',5'}$=8.9 Hz, H-4′), 3.39 (t, 1H, H-2′), 3.28 (t, 1H, $J_{5',6'b}$=4.5 Hz, H-6′b), 3.18 (td, 1H, H-5′). ^{13}C NMR (75.4 MHz, $CDCl_3$) y δ 172.8, 164.8 (2CO, Phth), 154.8 (C-4, $C_6H_4OCH_3$), 151.4 (C-1, $C_6H_4OCH_3$), 138.9–114.2 (46C aromatic), 102.7 (C-1) 100.9 (*C*HPh), 97.8 (C-1′), 82.6 (C-2′), 81.5 (C-4′), 80.8 (C-3′), 78.6 (C-4), 78.0, 75.5, 74.8, 73.8, 73.1 (5C, CH_2Ph), 74.6 (C-5′),75.2 (C-5), 68.5 (C-6′), 67.7 (C-6), 66.7 (C-3), 64.4 (NCH_2Ph), 56.4 (C-2), 55.5 ($C_6H_4OCH_3$). FABMS: *m/z* 1246 [100%, $(M+Na)^+$]. Anal. Calcd for $C_{76}H_{73}NO_{14}$: C, 74.55; H, 5.90; N, 1.14. Found: C, 74.05; H, 5.95; N, 1.22.

3.1.10. *p*-Methoxyphenyl 2,3-di-*O*-benzyl-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (17). To a solution of *p*-methoxyphenyl (2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**16**) (350 mg, 0.34 mmol) in MeOH–dioxane (1:1, 10 mL), TsOH·H_2O (7 mg, 0.03 mmol) was added. The reaction mixture was left to stand at 65°C for 15 min and at 85°C for 3 h, then made neutral with Et_3N and evaporated to dryness. Column chromatography of the residue on silica gel (ether–petroleum ether, 5:1) gave **17** (263 mg, 82%) as a white solid that crystallised from ether had mp 64–66°C; $[\alpha]_D$=+16° (*c* 1, dichloromethane).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68 (m, 4H, Phth), 7.38–6.92 (m, 20H, $4CH_2Ph$), 6.85–6.68 (m, 4H, $C_6H_4OCH_3$), 5.63 (d, 1H, $J_{1,2}$=8.4 Hz, H-1), 4.96, 4.72 (d, 1H each, $^2J_{H,H}$=11.5 Hz, CH_2Ph), 4.91, 4.79 (d, 1H each, $^2J_{H,H}$=11.2 Hz, CH_2Ph), 4.84, 4.49 (d, 1H each, $^2J_{H,H}$=12.2 Hz, CH_2Ph), 4.61, 4.45 (d, 1H each, $^2J_{H,H}$=12.0 Hz, CH_2Ph), 4.55 (d, 1H, $J_{1',2'}$=7.5 Hz, H-1′), 4.45 (dd, $J_{2,3}$=10.7 Hz, H-2), 4.37 (dd, $J_{3,4}$=8.5 Hz, H-3), 4.13 (dd, 1H, $J_{4,5}$=9.9 Hz, H-4), 3.87 (dd, 1H, $J_{5,6a}$=4.3 Hz, $J_{6a,6b}$=11.0 Hz, H-6a), 3.79 (dd, 1H, $J_{5,6b}$=1.5 Hz, H-6b), 3.76 (m, 1H, $J_{5',6'a}$=3.3 Hz, $J_{6'a,6'b}$=12.2 Hz, H-6′a), 3.71 (s, 3H, $C_6H_4OCH_3$), 3.67 (m, 1H, H-5), 3.48 (t, 1H, $J_{4',5'}$=9.3 Hz, H-4′), 3.46 (t, 1H, $J_{5',6'b}$=5.6 Hz, H-6′b), 3.37 (m, 2H, H-2′, H-3′), 3.20 (ddd, 1H, H-5′). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 167.8, 167.8 (2CO, Phth), 155.4 (C-4, $C_6H_4OCH_3$), 150.9

(C-1, $C_6H_4OCH_3$), 138.5–114.4 (34C aromatic), 102.7 (C-1′), 97.7 (C-1), 84.4 (C-3′), 82.5 (C-2′), 78.0 (C-4), 77.2 (C-3), 75.4, 75.0, 74.6, 73.3 (4C, CH_2Ph), 75.2 (C-5), 74.6 (C-5′), 70.8 (C-4′), 67.8 (C-6), 62.5 (C-6′), 55.6 (C-2), 55.5 ($C_6H_4OCH_3$). FABMS: *m/z* 960 [100%, $(M+Na)^+$].

3.1.11. *p*-Methoxyphenyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (18). A mixture of *p*-methoxyphenyl 2,3-di-*O*-benzyl-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**17**) (277 mg, 0.30 mmol), Bu_2SnO (149 mg, 0.59 mmol), Bu_4NBr (153 mg, 0.47 mmol) and BnBr (0.26 mL, 2.19 mmol) in freshly distilled acetonitrile (150 mL) was heated under reflux with a Soxhlet apparatus containing 3 Å molecular sieves for 3 days. Then the reaction mixture was cooled to rt, Et_3N (10 mL) was added and the reaction mixture was stirred at rt for 30 min to destroy the excess of benzyl bromide and then concentrated to dryness. The residue was dissolved in ethyl acetate (20 mL) and saturated aq $NaHCO_3$ was added and stirred for 3 h, filtered and the organic layer washed with saturated aq KCl, dried (Na_2SO_4) and evaporated. Column chromatography of the residue on silica gel (ether–petroleum ether, 1:1→;10:1) gave **18** (272 mg, 89%) as a white solid that crystallised from ether–petroleum ether had mp 46–48°C; $[\alpha]_D=+22°$ (*c* 1, dichloromethane).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.67 (m, 4H, Phth), 7.36–6.98 (m, 25H, $5CH_2Ph$), 6.84–6.67 (m, 4H, $C_6H_4OCH_3$), 5.60 (d, 1H, $J_{1,2}$=8.5 Hz, H-1), 4.97, 4.83 (d, 1H each, $^2J_{H,H}$=12.5 Hz, CH_2Ph), 4.90, 4.79 (d, 1H each, $^2J_{H,H}$=11.6 Hz, CH_2Ph), 4.87, 4.82 (d, 1H each, $^2J_{H,H}$=11.6 Hz, CH_2Ph), 4.59, 4.53 (d, 1H each, $^2J_{H,H}$=12.1 Hz, CH_2Ph), 4.53 (d, 1H, $J_{1',2'}$=7.5 Hz, H-1′), 4.49, 4.44 (d, 1H each, $^2J_{H,H}$=11.6 Hz, CH_2Ph), 4.43 (dd, 1H, $J_{2,3}$=9.1 Hz, H-2), 4.33 (dd, 1H, $J_{3,4}$=8.5 Hz, H-3), 4.13 (dd, 1H, $J_{4,5}$=9.9 Hz, H-4), 3.87 (dd, 1H, $J_{5,6a}$=4.3 Hz, $J_{6a,6b}$=11.0 Hz, H-6a), 3.77 (dd, 1H, $J_{5,6b}$=1.7 Hz, H-6b), 3.70 (s, 3H, $C_6H_4OCH_3$), 3.64 (dd, 1H, $J_{5',6'a}$=4.7 Hz, $J_{6'a,6'b}$=10.3 Hz, H-6′a), 3.64 (m, 1H, $J_{3',4'}=J_{4',5'}$=9.1 Hz, H-4′), 3.63 (m, 1H, H-5), 3.60 (dd, 1H, $J_{5',6'b}$=5.0 Hz, H-6′b), 3.41 (t, 1H, $J_{2',3'}$=9.1 Hz, H-3′), 3.38 (t, 1H, H-2′), 3.32 (dt, 1H, H-5). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 155.4 (C-4 de $C_6H_4OCH_3$), 151.0 (C-1 de $C_6H_4OCH_3$), 138.8–114.4 (40C aromatic), 102.7 (C-1′), 97.7 (C-1), 84.4 (C-3′), 82.3 (C-2′), 78.0 (C-4), 77.2 (C-3), 75.5 (C-5), 75.4–70.7 (5C, CH_2Ph), 73.3 (C-5′), 72.9 (C-4′), 70.7 (C-6′), 67.9 (C-6), 55.5 ($C_6H_4OCH_3$), 55.7 (C-2). FABMS: *m/z* 1050 [100%, $(M+Na)^+$]. HR-FABMS: Found $(M+Na)^+$ 1050.4049. $C_{110}H_{105}N_3O_{28}$: requires 1050.4040.

3.1.12. *p*-Methoxyphenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (19). *Procedure (1)*: To a solution of *p*-methoxyphenyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**18**) (25 mg, 0.024 mmol) and 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**9**) (33 mg, 0.032 mmol) in dry 1,2-dichloroethane (2.5 mL) with 4 Å molecular sieves, 10 μL (2.4 μmol) of a solution of 42 μL of TMSOTf in 1 mL of 1,2-dichloroethane was added dropwise at 0°C and under argon. The reaction mixture was stirred at 0°C for 10 min and then left warmed in rt. After 24 h, the reaction mixture was diluted with dichloromethane and washed successively with saturated aq $NaHCO_3$, saturated aq NaCl and water, dried (Na_2SO_4) and evaporated to dryness. Column chromatography of the residue (ether–petroleum ether, 2:1) gave **19** (10 mg, 21, 54% with respect the consumed acceptor).

Procedure (2): A mixture of *p*-methoxyphenyl (2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**18**) (90 mg, 0.088 mmol), phenyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**10**) (122 mg, 0.123 mmol) and NIS (188 mg, 0.350 mmol) in dry dichloromethane (2 mL) with 4 Å molecular sieves was stirred at rt for 30 min. The mixture was then cooled to 0°C and 70 μL (0.018 mmol) of a solution of TfOH (20 μL) in dichloromethane (1 mL) was added dropwise under argon. The reaction mixture was stirred for 1 h at 0°C, then diluted with dichloromethane and washed successively with saturated aq $NaHCO_3$, aq $Na_2S_2O_3$ (10%) and water; dried (Na_2SO_4) and evaporated to dryness. Column chromatography of the residue on silica gel (ether–petroleum ether 2:1) gave **19** (115 mg, 68, 88% on respect the consumed acceptor) as a white solid mp 80–82°C; $[\alpha]_D=-3°$ (*c* 1, dichloromethane).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93–7.59 (m, 12H, 3Phth), 7.35–6.68 (m, 35H, Ph), 6.78–6.31 (m, 4H, $C_6H_4OCH_3$),5.73 (dd, 1H, $J_{3''',4'''}$=9.1 Hz, $J_{4''',5'''}$=10.7 Hz, H-4‴), 5.72 (dd, 1H, $J_{2''',3'''}$=10.6 Hz, H-3‴), 5.52† (d, 1H, $J_{1,2}$=8.5 Hz, H-1), 5.45‡ (d, 1H, $J_{1''',2'''}$=8.4 Hz, H-1‴), 5.12† (d, 1H, $J_{1'',2''}$=8.0 Hz, H-1″), 4.96, 4.85 (d, 1H each, $^2J_{H,H}$=11.8 Hz, CH_2Ph), 4.21 (d, 1H, $J_{1',2'}$=7.7 Hz, H-1′), 4.75–2.8 (m, 30H, 12C*H*Ph, 18H sugar ring), 3.67 (s, 3H, $C_6H_4OCH_3$), 1.98, 1.92, 1.82 (s, 3H each, $COCH_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 170.5, 169.9, 169.3, (3C, $COCH_3$), 158.4–114.3 (66C aromatic), 102.4 (C-1′), 97.6 (C-1), 96.9 (C-1‴), 96.9 (C-1″), 83.0, 82.3 (C-2′, C-3′), 77.7–68.9 (11C sugar ring, 7C CH_2Ph), 67.5, 67.5, 67.5 (3C, C-6, C-6′, C-6″), 61.5 (C-6‴), 56.5, 55.6, 55.5 (3C, C-2, C-2″, C-2‴), 55.3 ($C_6H_4OCH_3$), 20.4, 20.4, 20.2 ($3COCH_3$). FABMS: *m/z* 1938 [100%, $(M+Na)^+$]. HR-FABMS: Found $(M+Na)^+$ 1938.6889. $C_{110}H_{105}N_3O_{28}$: requires 1938.6782.

3.1.13. *p*-Methoxyphenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (20). To a solution of *p*-methoxyphenyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**19**) (70 mg, 0.037 mmol) in ethanol (3 mL) 1,2-diaminoethane (0.37 mL, 5.48 mmol) was added under argon. The reaction

mixture was heated at 60°C for 16 h and then concentrated. The residue was dissolved at 0°C in pyridine (1 mL) and acetic anhydride (1 mL) and warmed in rt. After 24 h, the reaction mixture was evaporated to dryness. Column chromatography of the residue on silica gel (toluene–acetone, 5:1→1:1) gave **20** (46 mg, 75%) as an oil. $[\alpha]_D=-35°$ (*c* 0.2, dichloromethane).

^{1}H NMR (300 MHz, $CDCl_3$) 7.48–7.23 (m, 35H, $2CH_2Ph$), 6.97–6.64 (m, 4H, $C_6H_4OCH_3$), 5.78 (d, 1H, $J_{H2,NH}$= 12.4 Hz, NH), 5.69 (d, 1H, $J_{H2,NH}$=15.4 Hz, NH), 5.37 (d, 1H, $J_{H2,NH}$=11.4 Hz, NH), 4.95–3.28 (m, 42H, $7CH_2Ph$, 28H sugar ring), 3.75 (s, 3H, $C_6H_4OCH_3$), 2.02, 2.00, 1.96, (s, 3H each, $COCH_3$), 1.80, 1.75, 1.72 (s, 3H each, $NHCOCH_3$). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 170.8, 170.5, 170.3, 170.1, 169.9, 169.1 (6C, $COCH_3$), 155.0 (C-4 of $C_6H_4OCH_3$), 151.2 (C-1 of $C_6H_4OCH_3$), 139.4–137.7, 128.6–127.3 (42C aromatic), 118.3, 118.3, 114.3, 114.3 (4C, $C_6H_4OCH_3$), 102.6, 101.0, 99.1, 98.6 (4C, C-1, C-1′, C-1″, C-1‴), 83.1, 82.5, 79.7, 77.5, 77.4, 77.3, 76.8, 75.0, 74.8, 74.4, 74.4, 74.3, 73.4, 73.4, 73.1, 73.1, 72.7, 72.4, 71.4, 69.1 ($7CH_2Ph$, 13C sugar ring), 69.1, 68.4, 68.1 (3C, C-6, C-6′, C-6″), 61.5 (C-6‴), 55.5 ($C_6H_4OCH_3$), 54.9, 54.2, 53.8 (3C, C-2, C-2″, C-2‴), 23.3, 23.1, 22.9, 20.5 20.5, 20.5 ($6COCH_3$). FABMS: *m/z* 1674 [100%, $(M+Na)^+$]. HR-FABMS: Found $(M+Na)^+$ 1674.6936. $C_{92}H_{105}N_3O_{25}Na$ requires: 1674.6934

3.1.14. *p*-Methoxyphenyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1(4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1(4)-β-D-glucopyranosyl-(1(4)-2-acetamido-2-deoxy-β-D-glucopyranoside (2). A mixture of *p*-methoxyphenyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1(4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1(4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl-(1(4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (**20**) (46 mg, 0.03 mmol) and $Pd(OH)_2$–C (20%, 100 mg) in ethanol (60 mL) was hydrogenated at 3 atm and rt for 24 h. Then filtered through celite and concentrated. The residue (20 mg) was conventionally acetylated with acetic anhydride and pyridine. The obtained crude product containing *p*-methoxyphenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (**21**), FABMS: *m/z* 1338 [20%, $(M+Na)^+$], was deprotected under Zemplén conditions with NaOMe in MeOH 1 M, made neutral with Amberlite IR-120 (H^+) and evaporated to dryness. Purification of the residue on biogel P-2 gave **2** (3 mg, 12%) as an amorphous solid.

^{1}H NMR (500 MHz, D_2O) δ 7.07–6.97 (m, 4H, $C_6H_4OCH_3$), 5.06† (d, 1H, $J_{1,2}$=8.6 Hz, H-1), 4.58† (m, 3H, H-1′, H-1″, H-1‴), 4.01–3.35 (m, 24H, sugar ring), 3.82 (s, 3H, $C_6H_4OCH_3$), 2.07, 2.06 2.04 (s, 3H each, $COCH_3$). ^{13}C NMR (125.7 MHz, D_2O) δ 176.0, 175.7, 175.7 (3C, $COCH_3$), 156.0 (C-4, $C_6H_4OCH_3$), 152.2 (C-1, $C_6H_4OCH_3$), 119.5, 119.5, 116.2, 116.2 (4C, $C_6H_4OCH_3$), 103.4, 102.6, 102.4, 101.7 (4C, C-1, C-1′, C-1″, C-1‴), 80.4, 80.0, 79.4 (3C, C-4, C-4′, C-4″) 77.0, 76.1, 75.7, 75.6, 75.2, 74.6, 74.0, 73.3, 73.2 (9C sugar ring), 70.9 (C-4‴), 61.7, 61.2, 60.1, 60.1 (4C, C-6, C-6′, C-6″, C-6‴), 56.9, 56.7, 56.4, 56.2 (4C, $C_6H_4OCH_3$, C-2, C-2″, C-2‴), 23.3, 23.3, 23.3 ($3COCH_3$). FABMS: *m/z* 918 [100%, $(M+Na)^+$]. HR-FABMS: Found $(M+Na)^+$ 918.3333. $C_{37}H_{57}N_3O_{22}Na$ requires 918.3331.

Acknowledgements

We thank the Dirección General de Investigación Científica y Técnica of Spain (Grant No PB 97-0730) and Junta de Andalucía (FQM 134) for financial support. This work is part of the European COST D13 Program, working group COST D13/0001/98.

References

1. Denarié, J.; Debelle, F.; Promé, J.-C. *Ann. Rev. Biochem.* **1996**, *65*, 503–535.
2. (a) Spaink, H. P.; Sheely, D. M.; van Brussel, A. A. N.; Glushka, J.; York, W. S.; Tak, T.; Geiger, O.; Kennedy, E. P.; Reinhold, V. N.; Lugtenberg, B. J. J. *Nature* **1991**, *354*, 125–130. (b) Schultze, M.; Quiclet-Sire, B.; Kondorosi, É.; Virelizier, H.; Glushka, J. N.; Endre, G.; Géro, S. D.; Kondorosi, A. *Proc. Natl Acad. Sci. USA* **1992**, *89*, 192–196. (c) Poupot, R.; Martínez-Romero, E.; Promé, J.-C. *Biochemistry* **1993**, *32*, 10430–10435. (d) Sanjuan, J.; Carlson, R. W.; Spaink, H. P.; Bhat, U. R.; Barbour, W. M.; Glushka, J.; Stacey, G. *Proc. Natl Acad. Sci. USA* **1992**, *89*, 8789–8793. (e) Price, N. P.; Relic, B.; Talmont, F.; Lewin, A.; Promé, D.; Pueppke, S. G.; Maillet, F.; Dénairé, J.; Promé, J.-C.; Broughton, W. J. *Mol. Microbiol.* **1992**, *6*, 3574–3584. (f) Mergaert, P.; van Montagu, M.; Promé, J.-C.; Holsters, M. *Proc. Natl Acad. Sci. USA* **1993**, *90*, 1551–1555. (g) Bec-Ferté, M. P.; Krishman, H. B.; Promé, D.; Savagnac, A.; Peuppke, S. G.; Promé, J.-C. *Biochemistry* **1994**, *33*, 11782–11788.
3. Demont, N.; Roche, P.; Aurelle, H.; Talmont, F.; Promé, J. C.; Price, N. P. J.; Relic, B.; Broughton, W. J.; Débellé, F.; Ardourel, M. Y.; Maillet, F.; Rosenberg, C.; Truchet, G.; Dénairé, J. *Advances in Molecular Genetics of Plant Microbe Interactions*; Daniels, M. J., Downie, J. A., Osbourn, A. E., Eds.; Kluwer: Dororecht, The Netherlands, 1992; pp 133–141.
4. Bec-Ferté, M. P.; Krishman, H. B.; Promé, D.; Savagnac, A.; Peuppke, S. G.; Promé, J.-C. *FEBS Lett.* **1996**, 273–279.
5. (a) Lerouge, P.; Roche, P.; Faucher, C.; Maillet, F.; Truchet, G.; Promé, J. C.; Denarié, J. *Nature* **1990**, *344*, 781–784. (b) Spaink, H. P.; Sheely, D. M.; Van Brussel, A. A. N.; Glushka, J.; York, W. S.; Tak, T.; Geiger, O.; Kennedy, E. P.; Reinhold, V. N.; Lugtenberg, B. J. J. *Nature* **1991**, *354*, 125–130. (c) Schultze, M.; Quiclet-Sire, B.; Kondorosi, E.; Virelizier, H.; Glushka, J.; Endre, G.; Gero, S. D.; Kondorosi, A. *Proc. Natl Acad. Sci. USA* **1992**, *89*, 193–196.
6. For the synthesis of the molecule signal secreted by *Rhizobium meliloti*, see: (a) Nicolaou, K. C.; Bockorich, N. J.; Carcanague, D. R.; Hummel, C. W.; Even, L. F. *J. Am. Chem. Soc.* **1992**, *114*, 8701–8703. (b) Wang, L.-X.; Li, Q.; Wang, Q.-W.; Hui, Y.-Z. *Tetrahedron Lett.* **1993**, *34*, 7763–7767. (c) Ikeshita, S.; Sakamoto, A.; Nakahara, Y.; Nakahara, Y.; Ogawa, T. *Tetrahedron Lett.* **1994**, *35*, 3123–3126. (d) Tailler, D.; Jacquinet, J.-C.; Beau, J.-M. *J. Chem. Soc., Chem. Commun.* **1994**, 1827–1828. For the molecule signal secreted by *Bradyrhizobium japonicum* see: Ikeshita, S.;

Nakahara, Y.; Ogawa, T. *Glycoconjugate J.* **1994**, *11*, 257–267. For the synthesis of *Rhizobium fredii* see: Debenham, J. S.; Rodebaugh, R.; Fraser-Reid, B. *J. Org. Chem.* **1997**, *62*, 4591–4600.

7. (a) Robina, I.; López-Barba, E.; Jiménez-Barbero, J.; Martín-Pastor, M.; Fuentes, J. *Tetrahedron: Asymmetry* **1997**, *8*, 1207–1224. (b) Robina, I.; López-Barba, E.; Fuentes, J. *Tetrahedron* **1996**, *52*, 10771–10784. (c) Auzanneau, F.-I.; Mialon, M.; Promé, D.; Promé, J.-C.; Gelas, J. *J. Org. Chem.* **1998**, *63*, 6460–6465. (d) Auzanneau, F.-I.; Bennis, K.; Fanton, E.; Promé, D.; Promé, J.-C.; Gelas, J. *J. Chem. Soc., Perkin Trans. 1* **1998**, 3629–3636.
8. Bakkers, J.; Semino, C. E.; Stroband, H.; Kijne, J. W.; Robbins, P. W.; Spaink, H. *Proc. Natl Acad. Sci. USA* **1997**, *94*, 7982–7986.
9. Spaink, H. P.; Bloemberg, G. V.; Wijfjes, A. H. M.; Ritsema, T.; Geiger, O.; López-Lara, I. M.; Harteveld, M.; Kafetzopoulos, D.; van Brussel, A. A. N.; Kijne, J. W.; Lugtenberg, B. J. J.; Van der Drift, K. M. G. M.; Thomas-Oates, J. E.; Potrykus, I.; Sautter, C. *Advances in Molecular Genetics of Plant–Microbe Interactions*; Daniels, M. J., Downie, J. A., Osbourn, A. E., Eds.; Kluwer: Dororecht, The Netherlands, 1994; pp 91–98.
10. Robina, I.; Gómez-Bujedo, S.; Fernádez-Bolaños, J. G.; del Pozo, L.; Demange, R.; Picasso, S.; Vogel, P. J. *Carbohydr. Lett.* **2000**, *3*, 389–396.
11. Robina, I.; Gómez-Bujedo, S.; Fernández-Bolaños, J. G.; Fuentes, J. *Synth. Commun.* **1998**, *28*, 2379–2397.
12. Robina, I.; López-Barba, E.; Fuentes, J. *Synth. Commun.* **1996**, *26*, 2847–2856.
13. Boots, K. J. M.; van Brussel, A. A. N.; Tak, T.; Spaink, H. P.; Kijne, J. W. *Mol. Plant–Microbe Interact.* **1999**, *12*, 839–844.