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Towards the Chemoenzymatic Synthesis Of Lipid A

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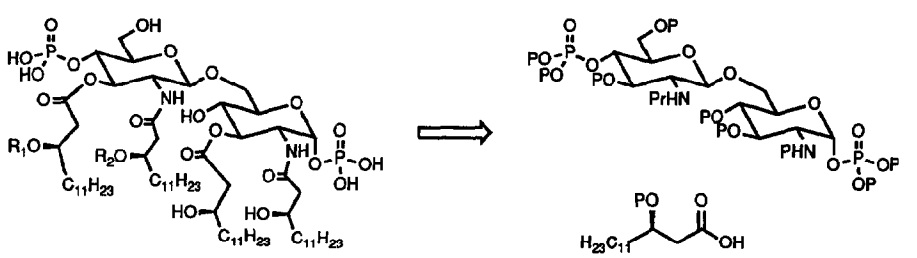
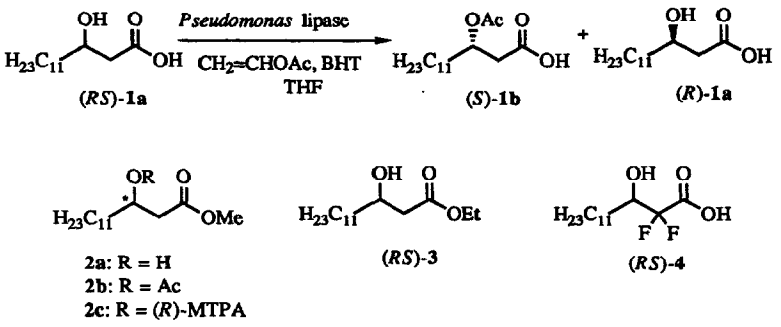
Abstract: New procedures have been developed for the synthesis of the two impotant components of lipid A; one is the lipase-catalyzed resolution of 3-hydroxytetradecanoic acid in tetrahydrofuran using vinyl acetate and the other is the synthesis of the disaccharide moiety using glycosylphosphite as the glycosylating reagent.

Lipid A is a lipophilic part of the cell surface lipopolysaccharides (LPS, endotoxin) of Gram-negative bacteria. It is responsible for the expression of most of the biological activities of the LPS e.g. endotoxicity, adjuvanticity and antitumor activity[1-3]. Lipid A from many different bacterial species share a common basic structure composed of a β (1-6) linked D-glucosamine disaccharide moiety. This disaccharide is phosphorylated at hydroxyls 1 and 4' and acylated at positions 2, 2', 3 and 3', usually with (R)-hydroxy- and/or (R)-acyloxy fatty acids. Most common among the acids is 3-(R)-hydroxytetradecanoic acid. Lipid A has been synthesized convergently from the corresponding monosaccharide and fatty acid moieties[4,5](Fig. 1).



$R_1 = COC_{13}H_{27}$; $R_2 = COC_{11}H_{23}$; P = Protecting group

Fig 1. Lipid A from *Escherichia coli* and its retrosynthetic analysis

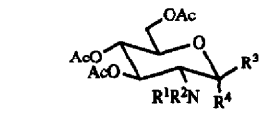


Scheme 1

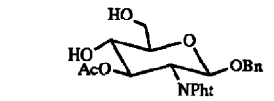
While a number of procedures for the preparation of optically active forms of 3-hydroxytetradecanoic acid has been reported, the practical preparation still depends on the classical preferential crystallization of a diastereomeric amine salt (e.g. D-α-methyl-β-phenethylamine salt[6], dehydroabietylamine salt[7,8]). Another matter of interest is the coupling of the two glucosamine residues, a reaction that usually is achieved using the Koenigs-Knorr method[9-12] with heavy metal salts as promoter. These salts are good promoters but toxic and therefore not suitable for bulk preparations. A recent publication uses zinc triflate as an alternative promoter for the glucosyl bromide in the synthesis of lipid A analogs[13]. As a part of our interest in the development of enzymatic or chemical/ enzymatic methods for the synthesis of biologically active molecules, we describe here our new approach towards the synthesis of the disaccharide and the fatty acid portions of lipid A.

The use of glycosyl phosphites as glycosylation reagents has recently been studied at our laboratory in a successful synthesis of sialic acid derivatives[14]. This method is here extended to the β (1-6) coupling of the lipid A backbone. The fatty acid 3-(*R*)-hydroxytetradecanoic acid is prepared via a lipase-catalyzed enantioselective acylation (Scheme 1).

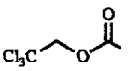
We chose the lipase-catalyzed kinetic resolution[15,16] of (±)-**1a**, because of the accessibility and inexpensiveness of both the starting material and the tool for the optical resolution. The procedure for the preparation of starting material[17] was further improved, to provide more than ten grams of pure (±)-**1a** without any chromatographic separation. Thus, (±)-**1a** was treated with *Pseudomonas* lipase (lipase PS-30, Amano) in a mixture of vinyl acetate and THF in the presence of the polymerization inhibitor, di-*t*-butyl *p*-cresol (BHT)[18] at 60°C. A smooth preferential acetylation of (*S*)-**1a** was observed and the reaction almost completed within 24 h. The products were separated after conversion to the corresponding methyl ester to give **2b** (50.0%, 51.6% conversion) and **2a** (46.9%, 88%*e.e.*; determined by NMR spectrum of the (*R*-)-(+)-methoxy-α-(trifluoromethyl)phenylacetyl[19] ester **2c**). The E value[20] was estimated to be 16.0. When the related ethyl ester (±)-**3** was used as the substrate, the E value was lowered to 2.9, indicating that the bulkiness around the carbonyl portion has an effect on the enantioselectivity of the lipase-catalyzed resolution. This result is consistent with those obtained so far in the case of α-hydroxy acids and their esters[21]. The successful resolution prompted us to apply this method to another related substrate (±)-**4**, which has been reported as the key-intermediate for the synthesis of a fluorinated analog of lipid A[22]. To our disappointment, however, no reaction could be observed under the same reaction conditions. It is probable that α,α-disubstituted fluorine

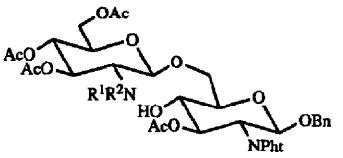


5 R^1= R^2= Phthalimido, R^3, R^4=H, OH
6 R^1= H, R^2= Troc, R^3, R^4=H, OH
7 R^1= R^2= Phthalimido, R^3= $OP(OBn)_2$, R^4=H
8 R^1= H, R^2= Troc, R^3= H, R^4= $OP(OBn)_2$



9

Troc = 



10 R^1= R^2= Phthalimido
11 R^1= H, R^2= Troc

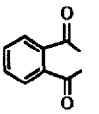
Pht = 

Fig. 2

atoms have a strong electron withdrawing effect, which lowered the reactivity of the β-hydroxyl group in **4**. However, hydrolysis of the *O*-acyl derivative of **4** in aqueous solution with the lipase may be feasible.

A gram-scale resolution of (±)-**1a** based on the transesterification was then carried out at 60-65°C for 36 h. The reaction using 8.0 g of substrate worked well, and the desired product in highly pure state (37.3%, 98%*e.e.*) was successfully obtained after recrystallization from hexane. We found that a higher enantioselectivity and simpler product separation was achieved with the use of the free acid (*RS*)-**1a** instead of its carboxylic acid ester.

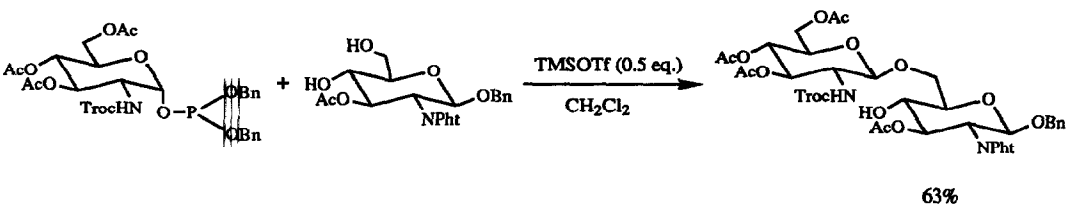
For the investigation of the coupling reaction, two model compounds were used, dibenzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl phosphite (**7**) and dibenzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl phosphite (**8**) (~5% of the β-anomer was also included). The glycosylations were performed at low temperatures in methylene chloride or acetonitrile as solvents and with different orders of addition of the reacting species. The results showed that the order of addition was the most important factor. When mixing the phosphite and acceptor first and then adding the trimethylsilyl triflate (TMSOTf) the yield of disaccharide was very low (< 10%). Addition of the phosphite last increased the yields significantly. The best result was obtained in 63% yield when the N-Troc protected glycosyl phosphite was used in the presence of 1 equivalent of acceptor (**9**) and 0.5 equivalents of TMSOTf in methylene chloride at -78°C (Scheme 2). Based on this and some other glycosylation reactions with glycosyl phosphites, it indicates that TMSOTf may form a TMS derivative of the acceptor first which in the prescence of triflic acid then reacts with the donor phosphite to form the glycosyl bond.

Treatment of **5** or **6** with *N, N*-diethylphosphoramidite[23] and 1-*H*-tetrazole gave the phosphites **7** and **8** in 67 and 88% yield respectively. For the glycosylation, compound **9** was dissolved in acetonitrile/ methylene chloride containing molecular sieves (4Å). The solution was cooled to 40°/ -76°C and 0.5 equiv. TMSOTf

Table 1. Different conditions used in glycosylation

Donor	Equiv	Temp(°C)	Solvent	TMSOTf eq.	Product (yield %)
7	1	-43	CH_3CN	0.5	**10** (45)
7	1	-78	CH_2Cl_2	0.5	**10** (49)
8	1	-43	CH_3CN	0.5	**11** (32)
8	1	-78	CH_2Cl_2	0.5	**11** (63)

was added. After stirring for 10 min. the phosphite, dissolved in the appropriate solvent, was added and the reaction mixture was allowed to attain room temperature during 40 min. The β-glucosides **10** and **11** were isolated after work-up and silica gel chromatography. No α-glycosylation or α or β 1-4 linked disaccharides were observed. In summary, this report describes new procedures for the preparation of the two important components of lipid A: lipase-catalyzed resolution of the hydroxy fatty acid and glycosyl phosphite-mediated synthesis of the disaccharide. These methods may find use in the total synthesis of lipid A and analogs.



63%

Scheme 2

Experimental

General methods: The enzyme was purchased from Amano. All ^1H NMR spectra were taken with a Brueker 250 MHz or 400 MHz instruments. The 250 MHz instrument was generally used unless otherwise indicated. ^{13}C NMR were recorded at 62.5. The solvent used was $CDCl_3$ (0.03% v/v TMS). Optical rotations were measured with a Perkin-Elmer 141 polarimeter in a 1 mL, 1.001 cm pathlength cell.

Large-scale preparation of (±)-1a.

The acylation of Meldrum's acid was carried out according to the reported procedure[17]. To a solution of Meldrum's acid (23.0 g, 0.16 mol) and pyridine (25.6 g) in CH_2Cl_2 (200 mL) was added dropwise dodecanoyl chloride (38.5 g) with ice-cooling. After stirring at room temperature for 3 h, the mixture was washed successively with water, hydrochloric acid (10%) three times, and water. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The trace of CH_2Cl_2 was removed by azeotropic distillation with methanol *in vacuo* several times. The residue was dissolved in methanol (300 mL), and the mixture was

stirred and heated under reflux overnight, then concentrated *in vacuo*. The residue was dissolved in ethanol (450 mL), and to this solution $NaBH_4$ (2.36 g) was added portionwise with stirring and ice-cooling. After stirring at room temperature for 30 min, aqueous NaOH solution (10N, 25 mL) was added and the mixture was stirred at room temperature for 30 min, and for another 30 min with ice-cooling. The precipitates were collected by filtration and washed successively with ethanol and ether. The precipitates were then dissolved in hot water (900 mL) and its pH was adjusted to 2 by the addition of conc. hydrochloric acid. The precipitates were finely divided by applying sonication and collected by filtration, and washed alternatively with water and hexane, then dried *in vacuo* for 1 h. The solid was dissolved in THF and treated with small amount of Na_2SO_4, activated charcoal and Celite, then filtered through filter paper. A small amount of silica gel was added to the filtrate and mixed well to remove the trace of charcoal, then filtered again. The filtrate was concentrated *in vacuo* and the residue was recrystallized from hot hexane (400 mL). The collected crystals were washed with cold hexane and dried *in vacuo* to give (±)-**1a** (25.44 g, 65% from Meldrum's acid), mp 78.0-78.5°C; ^{1}H NMR δ 0.88 (3H, t, J = 7.0 Hz, ω-CH_3), 1.20-1.60 (20H, m), 2.48 (1H, dd, J = 8.9, 16.6 Hz, H-2), 2.58 (1H, dd, J = 3.1, 16.6 Hz, H-2'), 4.03 (1H, m, H-3). The ^{1}H NMR spectrum was identical with that reported previously for optically active one.[24]

Lipase-catalyzed acetylation of (±)-1a.

To a solution of (±)-**1a** (100 mg, 0.43 mmol) and BHT (1 mg) in a mixture of vinyl acetate (1 mL) and THF (dried over molecular sieves 4A, 1 mL) was added lipase PS-30 (66 mg), and the mixture was stirred with heating at 60°C for 24 h. After cooling, the mixture was filtered and the filtrate was concentrated *in vacuo* and the residue was treated with ethereal soln of diazomethane. The residue was chromatographed on silica gel (5 g). Elution with hexane/ether (10/1) afforded (*S*)-**2b** (61.5 mg, 50.0%), $[\alpha]^{25}_D$ -1.3 (*c* 3.08, $CHCl_3$); ^{1}H NMR δ 0.88 (3H, t, J = 7.0 Hz, ω-CH_3), 1.20-1.60 (20H, m), 2.04 (3H, s, acetyl), 2.534 (1H, dd, J = 5.5, 16.4 Hz, H-2), 2.59 (1H, dd, J = 7.3, 15.3 Hz, H-2'), 3.68 (3H, s, $COOCH_3$), 5.20 (1H, m, H-3). Further elution with hexane-ether (5/1) gave (*R*)-**2a** (49.6 mg, 46.9%), $[\alpha]^{26}_D$ -11.1 (*c* 2.53, $CHCl_3$) [lit.[24] $[\alpha]_D$ -18.5 (*c* 1.05, $CHCl_3$)]; ^{1}H NMR δ 0.88 (3H, t, J = 7.0 Hz, ω-CH_3), 1.20-1.60 (20H, m), 2.41 (1H, dd, J = 9.0, 16.4 Hz, H-2), 2.52 (1H, dd, J = 7.1, 16.4 Hz, H-2'), 3.72 (3H, s, CO_2CH_3), 4.00 (1H, m, H-3). A small portion was converted to (*S*)-MTPA ester **2c**, ^{1}H NMR δ 3.53 [3H, q, $J_{H\text{-}F}$ = 1.2 Hz, $C(CF_3)OCH_3$, 90%], 3.55 [3H, q, $J_{H\text{-}F}$ = 1.2 Hz, $C(CF_3)OCH_3$, 10%], 3.59 (3H, s, CO_2CH_3, 90%), 3.66 (3H, s, CO_2CH_3, 10%).

For a preparative-scale experiment, the reaction was carried out by using 8.0 g of the substrate, for 36 h at 65°C, and under the slow continuous stream of N_2 gas to purge the acetaldehyde formed concomitantly with the proceeding of the reaction. The crude product after removal of the enzyme was recrystallized from hexane (80 mL) at room temperature. The mother liquor after the recovery of crystalline material was concentrated, and the oily residue was crystallized from cold hexane (4°C, 40 mL) overnight. The combined crystals thus obtained (*ca.* 3.0 g) were further recrystallized from cold hexane (50 mL) to give (*R*)-**1a** (2.88 g, 37.3%), mp 72.0-72.5°C; (lit.[6] mp 73-74°C, lit.[17] mp 72.0°C, lit.[24] mp 71.0-71.5°C); $[\alpha]^{25}_D$ 15.1 (*c* 1.06, $CHCl_3$) [lit.[6] $[\alpha]^{25}_D$ -16 (*c* 2, $CHCl_3$), lit.[17] $[\alpha]^{26}_D$ 16.2 (*c* 1, $CHCl_3$), lit.[24] $[\alpha]^{22}_D$ -16.° (*c* 1.00, $CHCl_3$)]. Its NMR spectrum was identical with that of (±)-**1a**. This was treated with diazomethane, subsequently by MTPA-Cl in pyridine to give (*R*)-**2c**; ^{1}H NMR δ 3.59 (3H, s, CO_2CH_3, 99%), 3.66 (3H, s, CO_2CH_3, 1%).

Dibenzyl 3-*O*-acetyl-6-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl phosphite (7). -Dibenzyl *N,N*-diethylphosphamidate (146 mg, 0.46 mmol) was added dropwise to a solution of 5 (100 mg, 0.23 mmol) and 1-*H*-tetrazol (65 mg, 0.93 mmol) in tetrahydrofuran (3 mL) under argon atmosphere, and the mixture was stirred for 2 h at room temperature. Dichloromethane (10 mL) was added to the mixture, and the organic phase was washed with ice-cold dilute HCl, aqueous $NaHCO_3$ and ice-water and dried over anhydrous sodium sulfate. The solution was evaporated in vacuo to give a crude material, which was chromatographed on a silica-gel column (hexane-ethyl acetate 3:1) to give 7 (140 mg, 88%). ^{1}H-NMR ($CDCl_3$) δ: 5.97(1H, t, J 8.4 Hz, H-1), 5.89(1H, dd, J 9.2, 10.7 Hz, H-3), 5.22(1H, dd, J 9.3, 10.0 Hz, H-4), 4.46(1H, dd, J 8.4, 10.7 Hz, H-2).

Dibenzyl 3-*O*-acetyl-6-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl phosphite (8). -Dibenzyl N,N-diethylphosphamidate (527 mg, 1.66 mmol) was added dropwise to a solution of **6** (200 mg, 0.42 mmol) and 1-*H*-tetrazol (116 mg, 1.66 mmol) in tetrahydrofuran (3 mL) under argon atmosphere, and the mixture was stirred for 2 h at room temperature. Dichloromethane (10 mL) was added to the mixture, and the organic phase was washed with ice-cold dilute HCl, aqueous $NaHCO_3$ and ice-water and dried over anhydrous sodium sulfate. The solution was evaporated in vacuo to give a crude material, which was chromatographed on a silica-gel column (hexane-ethyl acetate 2:1) to give 8 (225 mg, 67%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.01(3H, s, C*H*$_3$CO), 2.02(6H, s 2xC*H*$_3$CO), 3.89(1H, dd, J 2.2, 12.4, H-6a), 4.00(1H, m, H-5), 4.08(1H, dt, J 3.4, 10.4 Hz, H-2), 4.13(1H, dd, J 4.1, 12.5 Hz, H-6b), 4.53(1H, d, J 12.0 Hz), 4.76(1H, d, J 11.7 Hz), 4.87-4-96(4H, m), 5.09(1H, t, J 9.8 Hz, H-4), 5.16-5.25(2H, m, H-3, N*H*), 5.59(1H, dd, J 3.4, 7.7 Hz, H-1).

Benzyl 3-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (9). -A solution of benzyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside[25] (15.68 g, 2.98 mmol) in methanol (50 mL) was treated with sodium methoxide (1.6 mL of a 25% solution in methanol). After 1 h the solution was neutralised with Dowex 50 H^+ resin, filtered and concentrated. The residue was dissolved in acetone (180 mL), 2,2-dimethoxypropane (180 mL) and a catalytic amount of *p*-toluenesulfonic acid was added and the solution was stirred over night. The precipitated residue was filtered off, and to the filtratet was added aq $NaHCO_3$, this was stirred, filtered and concentrated *in vacuo* . The residue was diluted with chloroform and washed with brine, dried (Na_2SO_4), filtered, concentrated and crystallized from chloroform- toluene to give benzyl 2-deoxy-4,6-O-isopropylidene-2-phthalimido-β-D-glucopyranoside (10.41 g, 79%). M. p. 205 °C ^{1}H-NMR ($CDCl_3$) δ: 1.42, 1.52(3H each, s,C*H*$_3$C), 3.45(1H, dt, J 5.4, 9.8, H-5), 3.65(1H, t, J 9.2, H-4), 3.86(1H, t, J 10.4, H-6a), 4.00(1H, dd, J 5.5, 10.8, H-6b), 4.23(1H, dd, J 8.5, 10.4, H-2), 4.45(1H, dd, J 8.9, 10.4, H-3), 4.49(1H, d, J 12.3, PhC*H*$_2$), 4.81(1H, d, J 12.3, PhC*H*$_2$), 5.22(1H, d, J 8.4, H-1), 7.00-7.08(5H), 7.68-7.76(4H). HRMS : Calc. for $C_{24}H_{25}NO_7Cs$ ($M+Cs^+$) 572.0685, found 572.0691.
Benzyl 2-deoxy-4,6-O-isopropylidene-2-phthalimido-β-D-glucopyranoside (1.00 g, 2.28 mmol) was stirred in pyridine-acetic anhydride (30 mL, 2:1) for 2.5 h. The solution was evaported *in vacuo*, coevaporated twice with toluene, dissolved in 60% aqueous acetic acid (40 mL) and stirred at 60 °C for 20 min. The solution was cooled, evaporated and subjected to column chromatography (ethyl acetate) to give **9** (739 mg, 72%).

^{1}H-NMR ($CDCl_3$) δ: 1.94(3H , s,CH_3CO), 2.13(1H, m, 6-OH), 2.96(1H, d, J 5.2 Hz, 2-OH), 3.63(1H, m, H-5), 3.80(1H, dd J 4.9, 9.3, H-4), 3.88(1H, m, H-6a), 3.99(1H, m, H-6b), 4.28(1H, dd, J 8.5, 10.7, H-2), 4.57(1H, d, J 12.2, PhCH_2), 4.82(1H, d, J 12.3, PhCH_2), 5.41x(1H, d, J 8.5, H-1), 5.65(1H, dd, J 8.9, 10.7, H-3), 7.00-7.14(5H), 7.71-8.80(4H). HRMS : Calc. for $C_{23}H_{23}NO_8Cs$ (M+Cs^+),574.0478, found 574.0478.

Benzyl 3-*O*-acetyl-6-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside (10). -A solution of 7 (140 mg, 0.22 mmol) in methylene chloride (1 mL) was added to a solution of 9 (99 mg, 0.23 mmol), TMSOTf (22 μL, 0.12 mmol) and molecular sieves 3Å in methylene chloride (acetonitrile) (2 mL) at -78 °C(-40 °C). After 3 h when the solution had attained room temperature the reaction was quenched with saturated aq $NaHCO_3$ diluted with methylene chloride and washed with saturated $NaHCO_3$. The organic solvent were dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was chromatographed on silica gel (hexane-ethyl acetate 2:3) to provide benzyl 6-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside (97 mg, 49 %(69 mg, 40%)) as a colourless syrup.
The same procedure was used with acetonitrile as solvent. The amounts there were 100 mg (0.23 mmol) acceptor, 140 mg (0.20 mmol) phosphite, and 24 μL, (0.11 mmol) TMSOTf at -40 °C giving 69 mg (40%) 7. $[\alpha]^{25}_D$ -21.2 (*c* 0.8, $CHCl_3$). NMR ($CDCl_3$) ^{13}C δ: 20.4, 20.6, 20.7(*C*H_3CO), 54.4(C-2 overlap), 61.7, 2x68.7, 70.2, 70.5, 70.6, 71.9, 73.2, 74.9(C-3, 4, 5, 6, 3', 4', 5', 6', Ph*C*H_2), 96.6, 98.3, 123.4-136.8(aromatic C), 167.5, 169.4, 170.1, 170.8, 171.2(C=O). ^{1}H δ: 1.84, 1.88, 2.04, 2.13(3H each, s,CH_3CO), 3.01(1H, OH), 3.47(1H, t, J 9.3 Hz, H-4), 3.65-3.71(1H, m, H-5), 3.79-3.91(2H, H-5', H-6), 4.10-4.35(4H, H-6, 2xH-6', PhCH_2,), 4.12(1H, dd, J 8.5, 10.7 Hz, H-2), 4.41(1H, dd, J 8.5, 10.7 Hz, H-2'), 4.56(1H, d, J 12.2 Hz, PhCH_2), 5.21(1H, t, J 9.6 Hz, H-4'), 5.23(1H, d, J 8.6 Hz, H-1), 5.55(1H, dd, J 8.8, 10.6 Hz, H-3), 5.56(1H, d, J 8.5 Hz, H-1'), 5.82(1H, dd, J 9.2, 10.6 Hz, H-3'), 6.97-7.11(5H, phenyl), 7.62-7.79(8H, phthalimido). HRMS : Calc. for $C_{43}H_{42}N_2O_{17}Cs$ (M+Cs^+) 991.1538, found 991.1546.

Benzyl 3-*O*-acetyl-6-*O*-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamido)-β-D-glucopyranosyl]-2-deoxy-2-phthalimido-β-D-glucopyranoside (11). -A solution of **8** (124 mg, 0.17 mmol) in methylene chloride (1 mL) was added to a solution of **9** (74 mg, 0.17 mmol), TMSOTf (17 μL 0.09 mmol) and molecular sieves 3Å in methylene chloride (2 mL) at -78°C. After 3 h when the solution had attained room temperature the reaction was quenched with saturated aq $NaHCO_3$ diluted with more methylene chloride and washed with saturated $NaHCO_3$. The organic solvent were dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was chromatographed on silica gel (hexane-ethyl acetate 2:3) to provide benzyl 6-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamido)-β-D-glucopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside (97 mg, 63 %) as a colourless syrup.
The same procedure was used with acetonitrile as solvent. The amounts there were 60 mg (0.14 mmol) acceptor, 101 mg (0.14 mmol) phosphite, and 14 μL, (0.07 mmol) TMSOTf at -40 °C giving 40 mg (32%) 6. $[\alpha]^{25}_D$ -24.7 (*c* 0.8, $CHCl_3$). NMR ($CDCl_3$) ^{13}C δ: 20.6, 20.7(*C*H_3CO),, 54.6, 55.9(C-2) 61.9, 2x68.5, 69.8, 71.1, 71.7, 72.0, 73.2, 74.4, 74.8(C-3, 4, 5, 6, 3', 4', 5', 6', Ph*C*H_2, *C*H_2Cl_3), 95.3(*C* Cl_3), 97.3, 101.1, 123.4-137.0(aromatic C), 154.3(N*HCO*$_2$), 167.6, 169.4, 170.6, 170.8, 171.1(C=O), ^{1}H δ: 5.93(1H, anomeric J 8.6

Hz), 5.37(1H, anomeric J 8.2 Hz). HRMS : Calc. for $C_{38}H_{41}Cl_3N_2O_{17}Cs$ ($M+Cs^+$) 1035.0525 found 1035.0558.

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