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A new set of orthogonal-protecting groups for oligosaccharide synthesis on a polymeric support

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Abstract

The hydroxyl-protecting groups, levulinoyl (Lev) and 9-fluorenylmethoxycarbonyl (Fmoc), and the amino-protecting group 2,2,2-trichloroethoxycarbonyl (Troc), offer an ideal set of orthogonal-protecting groups which are compatible with oligosaccharide synthesis on a methylpolyethyleneglycol (MPEG) support using a *p*-alkyloxybenzyl-type linker.

1. Introduction

The development of new protecting groups and efficient glycosylation protocols and strategies make it possible to efficiently execute multi-step synthetic sequences leading to complex oligosaccharides.[1] Despite these important developments, oligosaccharide synthesis is still a time consuming process, and even in the hands of an experienced researcher, the preparation of di-, tri-, and tetrasaccharides may take between several months to a year.

A number of research groups are attempting to increase the efficiency and convenience of oligosaccharide synthesis by employing polymer-supported procedures in combination with combinatorial approaches.[2] In addition, the use of a set of orthogonal-protecting groups and linkers enables common building blocks to be used for the synthesis of a wide range of oligosaccharides. For example, Wong and co-workers proposed that the chloroacetyl (ClAc), *p*-methoxybenzyl (PMB), levulinoyl (Lev), and *tert*-butyldiphenylsilyl (TBDPS) offer an attractive set of orthogonal-protecting groups.[3] It was shown that a monosaccharide substituted with these four protecting groups can be used for the synthesis of a large number of trisaccharides.

As part of a project to prepare combinatorial saccharide libraries on polymeric support, we required a set of orthogonal hydroxyl- and amino-protecting groups. In addition, this set of protecting groups has to be compatible with a linker, which enables easy attachment and cleavage from a polymeric support.

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Here, we report that the hydroxyl-protecting groups, levulinoyl (Lev) and 9-fluorenylmethoxycarbonyl (Fmoc), and the amino-protecting group 2,2,2-trichloroethoxycarbonyl (Troc), offer an ideal set of orthogonal protecting groups. It is also shown that these functionalities are compatible with a *p*-alkoxybenzyl-type linker for immobilization to a methylpolyethyleneglycol (MPEG) support.

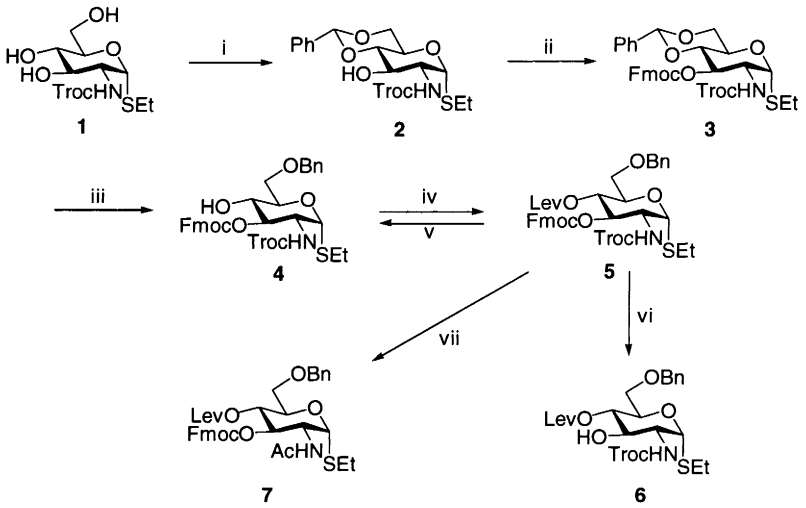
2. Result and discussion

In carbohydrate chemistry, the phthalimido group is the most commonly used amino-protecting group. However, this functionality can only be cleaved under relatively harsh conditions, making it incompatible with many hydroxyl-protecting functionalities. Also, a C-2 phthalimido group significantly reduces the glycosyl accepting properties of a neighboring 3-hydroxyl.[4] Therefore, the Troc was selected as an alternative amino-protecting group. It can be removed under relatively mild conditions[5] with activated Zn and, furthermore, glycosyl donors protected with an *N*-Troc functionality are generally more reactive than similar derivatives substituted with a phthalimido group.[6] The Lev and Fmoc were selected as temporary hydroxyl-protecting groups. The Fmoc group is a well-established amino-protecting group often used in peptide synthesis. It is exceptionally stable under acidic conditions, but sensitive towards mild bases (ammonia, piperidine, morpholine). Surprisingly few reports[7] deal with the Fmoc as a hydroxyl-protecting group. It is to be expected that a mild and hindered base will cleave the Fmoc group without affecting the Lev functionality. On the other hand, the Lev group can be cleaved with bidentate nucleophiles such as hydrazine buffered with acetic acid. It was anticipated that this almost neutral reagent would not affect the Fmoc group. Several other hydroxyl-protecting groups were considered but dismissed. For example, the ClAc is not compatible with the removal of the *N*-Troc functionality.

To demonstrate the versatility of a proposed set of orthogonal protecting groups, compound **5** was prepared and the Troc, Fmoc and Lev groups were selectively removed (Scheme 1). Thus, the readily available *N*-Troc-protected thioglycoside **1**[8] was treated with benzaldehyde dimethyl acetal and a catalytic amount of camphorsulfonic acid (CSA) in acetonitrile to give 4,6-*O*-benzylidene-protected derivative **2** in 87% yield.[9] The Fmoc group was introduced by reaction **2** with FmocCl in pyridine and the desired compound **3** was afforded in 94% yield. Regioselective cleavage of the benzylidene acetal of **3** using $NaCNBH_3$ and 1 M solution of HCl(g) in diethyl ether[10] almost exclusively gave thioglycoside **4** in a yield of 94%. Treatment of compound **4** with levulinic acid and DCC in the presence of catalytic amount of DMAP provided the fully protected compound **5** in a quantitative yield (98%).[11]

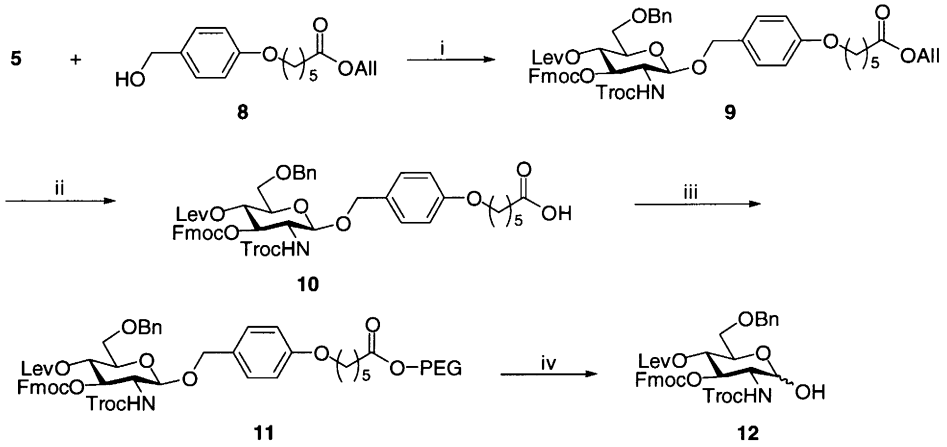
With key compound **5** in hand, attention was turned to examining the orthogonality of three chosen protecting groups (Fmoc, Lev, and *N*-Troc). It was anticipated that treatment with hydrazine acetate[11] in $MeOH/CH_2Cl_2$ would cleave the Lev without affecting the Fmoc group. Indeed, compound **5** was cleanly converted into **4** within 30 min and no loss of the Fmoc group was observed. The Fmoc group proved to be stable even after prolonged reaction times (up to 24 h). It was expected that triethylamine as a non-nucleophilic base would remove the Fmoc group without affecting the other functionalities. Treatment of **5** with 20% Et_3N in CH_2Cl_2 cleaved the Fmoc group and gave compound **6** in a quantitative yield. Finally, reductive cleavage of the *N*-Troc with zinc in acetic acid, followed by *N*-acetylation with acetic anhydride in pyridine, afforded the *N*-acetylated thioglycoside **7** in a yield of 86% (Scheme 1). Under these reaction conditions, both the Lev and Fmoc group remained intact.

The above-described experiments demonstrate that the Lev, Fmoc, and Troc groups can be selectively cleaved in the presence of each other to give the expected products in high yields. Encouraged by these results, the orthogonally protected **5** was used for immobilization to a soluble polymeric support (Scheme 2). *N*-Iodosuccinimide/trimethylsilyl trifluoromethanesulfonate (NIS/TMSOTf)[12] mediated coupling of



Scheme 1. *Reagents and conditions*: (i) dimethyl benzaldehyde acetal, CSA, MeCN, 87%; (ii) FmocCl, Py, 94%; (iii) $NaCNBH_3$, HCl/Et_2O, TMF, MS 3 Å, 94%; (iv) LevOH, DCC, DMAP (cat.), DCM, 98%; (v) $NH_2NH_2 \cdot HOAc$, MeOH:DCM (1:10, v/v), 99%; (vi) Et_3N (20%), DCM, 99%; (vii) Zn, HOAc then Ac_2O, Py, 86%

thioglycosyl donor **5** with the *p*-alkyloxybenzyl-type linker **8**[13] gave compound **9** in a 75% yield. Only the β-anomer was formed due to the presence of *N*-Troc as a neighboring participating group. The allyl ester **9** was converted into the carboxylic acid **10** by heating in a mixture of THF:ethanol:water in the presence of a catalytic amount of $Pd(PPh_3)_4$.[14] The success of this reaction demonstrates that the three protecting groups are compatible with transition metal-catalyzed cleavage of an allyl ester. Derivative **10** with immobilized onto MPEG (M.W. 5000) by ester bond formation using a standard procedure[15] to give polymer bound **11**. The oligosaccharide attached to **11** could be released from the polymeric support by cleavage of the *p*-alkyloxybenzyl-type linker with 10% TFA to give hemiacetal **12** in a high yield.[13] As expected, the Lev, Fmoc, and Troc groups were stable under this reaction condition.



Scheme 2. *Reagents and conditions*: (i) NIS/TMSOTf, MS 4 Å, DCM, 0°C, 75%; (ii) 10% $Pd(PPh_3)_4$, THF:EtOH:H_2O (9:9:1, v/v/v), 60°C, 93%; (iii) MPEG (M.W. 5000), DCC, DMAP (cat.), DCM; (iv) 10% TFA, DCM, 85%

In conclusion, we have described an orthogonal set of protecting groups for amino-sugars. The Lev, Fmoc, and *N*-Troc groups can each be readily introduced using standard procedures and selectively and rapidly be removed without affecting the others. Furthermore, the three protecting groups are compatible

with glycosylations and cleavage of allyl esters and a *p*-alkyloxybenzyl-type linker. The new set of orthogonal-protecting groups will be an important tool for the rapid synthesis of large collections of oligosaccharides.

3. Experimental

3.1. General methods

Chemicals were purchased from Aldrich and Fluka, and used without further purification. Molecular sieves were activated at 350°C in vacuo for 3 h. All solvents were distilled from the appropriate drying agents; dichloromethane and toluene were distilled from P_2O_5 and stored over 4 Å molecular sieves. Diethyl ether and THF were distilled from CaH_2, redistilled from $LiAlH_4$, and stored over sodium wire. Pyridine and acetonitrile were distilled from CaH_2 and stored over 4 Å molecular sieves. Methanol was distilled from sodium and stored over 4 Å molecular sieves. All the reactions were performed under anhydrous conditions and monitored by TLC on Kieselgel 60 F_{254} (Merck). Detection was by examination under UV light (254 nm) and by charring with 10% sulfuric acid in methanol. Column chromatography was performed on silica gel (Merck, mesh 70–230). Extracts were concentrated under reduced pressure at <40°C (bath). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Merc300 spectrometer and a Varian Inova500 spectrometer equipped with Sun workstations. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$; chemical shifts (δ) are given in ppm relative to solvent peaks (^{1}H, δ=7.26; ^{13}C, δ=77.3) as internal standard. Negative ion matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectra were recorded using an HP-MALDI instrument using gentisic acid as a matrix. Optical rotations were measured on a Jasco P-1020 polarimeter, and $[\alpha]_D$-values are given in units of deg cm^3 g^{-1}.

3.2. Ethyl 6-O-benzyl-3-O-(9-fluorenylmethoxycarbonyl)-2-deoxy-2-[[(2,2,2-trichloroethoxy)carbonyl] amino]-1-thio-α-D-glucopyranoside **4**

A solution of hydrogen chloride in diethyl ether (1 M, 23 ml) was added to a solution of benzylidene acetal **3** (1.3 g, 1.84 mmol) and sodium cyanoborohydride (1.45 g, 23.0 mmol) in THF (20 ml) containing 3 Å molecular sieves (500 mg). The mixture was stirred until the evolution of gas had ceased. The reaction mixture was filtered and the filtrate was co-evaporated with methanol (3×15 ml). The residue was diluted with dichloromethane (50 ml) and washed successively with water (2×20 ml) and brine (15 ml). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The resulting syrup was applied to a column of silica gel which was eluted with ethyl acetate:hexane (1:3, v/v) to give **4** as a white foam (1.22 g, 94%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.78–7.31 (m, 13H, Ar-H), 5.42 (2d, 2H, H-1, N*H*, $J_{1,2}$=5.1 Hz, $J_{NH,2}$=9.8 Hz), 4.87 (dd, 1H, H-3, $J_{2,3}$=11.1 Hz, $J_{3,4}$=9.4 Hz), 4.62 (2AB q, PhCH_2, J_{AB}=12.0 Hz, OCH_2CCl$_3$, J_{AB}=12.0 Hz), 4.43 (dd, 1H, C*H*aO (Fmoc), *J*=7.3 Hz, 10.3 Hz), 4.37 (dd, 1H, C*H*bO (Fmoc), *J*=7.7, 10.3 Hz), 4.31 (ddd, 1H, H-2), 4.27–4.23 (m, 2H, H-5, H-9), 3.93 (t, 1H, H-4, $J_{4,5}$=9.4 Hz), 3.85 (dd, 1H, H-6a, $J_{5,6a}$=4.3 Hz, $J_{6a,6b}$=10.7 Hz), 3.74 (dd, 1H, H-6b, $J_{5,6b}$=4.3 Hz), 2.83 (br s, 1H, O*H*), 2.72-2.60 (m, 2H, SCH_2), 1.30 (t, 3H, SCH$_2$CH_3, *J*=7.3 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.0, 154.2, 143.4, 143.2, 141.5, 128.7, 128.1, 127.9, 127.4, 125,4, 120.3, 95.8, 84.9, 78.2, 74.9, 74.0, 71.1, 70.9, 70.5, 69.9, 54.5, 46.9, 26.1, 15.5. $[\alpha]_D^{25}$ +51.5 (*c* 0.3, CH_2Cl_2). MALDI-TOF MS: *m/z* 732 [M+Na]$^+$. Anal. calcd for $C_{33}H_{34}Cl_3NO_8S$: C, 55.74; H, 4.82; N, 1.97; found: C, 55.60; H, 4.84; N, 1.91.

3.3. *Ethyl 6-O-benzyl-3-O-(9-fluorenylmethoxycarbonyl)-4-O-levulinoyl-2-deoxy-2-[[(2,2,2-trichloroethoxy)carbonyl]amino]-1-thio-α-D-glucopyranoside* **5**

DCC (1.78 g, 8.65 mmol) and DMAP (3 mg) in dichloromethane (2 ml) were added to a stirred solution of compound **4** (1.22 g, 1.73 mmol) and levulinic acid (1.78 ml, 17.3 mmol) in dichloromethane (20 ml). The reaction mixture became instantaneously cloudy. After stirring for 1 h, TLC analysis (ethyl acetate:hexane, 1:2, v/v) indicated a complete conversion of the starting material. The precipitated urea was removed by filtration and the filtrate was diluted with dichloromethane (30 ml), which was washed with water (2×20 ml) and brine (20 ml), and dried ($MgSO_4$). The organic layer was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (eluent: ethyl acetate:hexane, 1:3, v/v) to give **5** (1.37g, 98%) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.78–7.28 (m, 13H, Ar-H), 5.48 (d, 1H, H-1, $J_{1,2}$=5.1 Hz), 5.35 (d, 1H, N*H*, $J_{NH,2}$=9.8 Hz), 5.33 (t, 1H, H-4, $J_{3,4}$= $J_{4,5}$=9.8 Hz), 5.01 (dd, 1H, H-3, $J_{2,3}$=9.4 Hz,), 4.61 (AB q, PhCH_2, J_{AB}=12.0 Hz), 4.56 (AB q, OCH_2CCl_3, J_{AB}=12.0 Hz), 4.45–4.37 (m, 4H, H-2, H-5, CH_2O (Fmoc)), 4.26 (t, 1H, H-9 (Fmoc), J=7.7 Hz), 3.66 (dd, 1H, H-6a, $J_{5,6a}$=3.0 Hz, $J_{6a,6b}$=11.1 Hz), 3.62 (dd, 1H, H-6b, $J_{5,6b}$=4.3 Hz), 2.72–2.58 (m, 4H, COCH_2CH_2, SCH_2), 2.41 (t, 2H, COCH_2CH_2, J=6.9 Hz), 2.05 (s, 3H, CH_3CO), 1.31 (t, 3H, SCH_2CH_3, J=7.3 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 206.0, 171.3, 155.4, 154.0, 143.5, 143.3, 141.4, 138.0, 128.5, 128.1, 127.8, 127.4, 127.5, 120.2, 95.9, 84.6, 75.8, 74.9, 73.9, 71.0, 70.9, 69.1, 68.7, 54.7, 46.8, 38.1, 29.9, 28.2, 26.0, 15.4. $[\alpha]_D^{25}$ +24.3 (*c* 0.4, CH_2Cl_2). MALDI-TOF MS: *m/z* 830 $[M+Na]^+$. Anal. calcd for $C_{38}H_{40}Cl_3NO_{10}S$: C, 56.41; H, 4.98; N, 1.73; found: C, 56.26; H, 4.92; N, 1.77.

3.4. *Procedure to remove levulinoyl on compound* **5**

A solution of hydrazine acetate (18 mg, 0.2 mmol) in methanol (0.5 ml) was added to a solution of thioglycoside **5** (162 mg, 0.2 mmol) in dichloromethane (5 ml) and the resulting reaction mixture was stirred for 30 min. The solvents were removed under reduced pressure and the residue was purified by silica gel column chromatography (eluent: ethyl acetate:hexanes, 1:2, v/v) to give product **4** (142 mg, 99%) as a white foam.

3.5. *Ethyl 6-O-benzyl-4-O-levulinoyl-2-deoxy-2-[[(2,2,2-trichloroethoxy)carbonyl] amino]-1-thio-α-D-glucopyranoside* **6**

Triethylamine (0.5 ml) was added to a solution of compound **5** (81 mg, 0.1 mmol) in dichloromethane (2 ml). After stirring for 2 h, TLC analysis (ethyl acetate:hexanes, 1:2, v/v) indicated the completion of the reaction. The reaction mixture was concentrated under reduced pressure and the crude product was purified by silica gel column chromatography (eluent: ethyl acetate:hexanes, 1:2, v/v) to give **6** as a white foam (59 mg, 99%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.35–7.26 (m, 5H, Ar-H), 5.53 (d, 1H, H-1, $J_{1,2}$=4.9 Hz), 5.35 (d, 1H, N*H*, $J_{NH,2}$=7.5 Hz), 5.03 (t, 1H, H-4, $J_{3,4}$= $J_{4,5}$=9.3 Hz), 4.74 (AB q, PhCH_2, J_{AB}=11.9 Hz), 4.54 (AB q, OCH_2CCl_3, J_{AB}=11.9 Hz), 4.31 (ddd, 1H, H-5, $J_{5,6a}$=$J_{5,6b}$=3.5 Hz), 4.12 (ddd, 1H, H-2), 3.73 (t, 1H, H-3, $J_{2,3}$=9.3 Hz), 3.60 (d, 2H, H-6a, H-6b), 2.78–2.73 (m, 2H, COCH_2CH_2), 2.71–2.59 (m, 2H, SCH_2), 2.54–2.39 (m, 2H, COCH_2CH_2), 2.17 (s, 3H, CH_3CO), 1.30 (t, 3H, SCH_2CH_3, J=7.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 207.5, 172.6, 154.7, 138.1, 128.5, 128.0, 127.8, 95.6, 84.6, 75.0, 73.8, 72.7, 71.9, 69.9, 68.8, 56.0, 38.6, 30.1, 28.4, 26.1, 15.5. $[\alpha]_D^{25}$ +50.3 (*c* 0.3, CH_2Cl_2). MALDI-TOF MS: *m/z* 608 $[M+Na]^+$. Anal. calcd for $C_{23}H_{30}Cl_3NO_8S$: C, 47.07; H, 5.15; N, 2.39; found: C, 46.85; H, 5.01; N, 2.36.

3.6. Ethyl 2-deoxy-2-acetamido-6-O-benzyl-3-O-(9-fluorenylmethoxycarbonyl)-4-O-levulinoyl-1-thio-α-D-glucopyranoside **7**

Compound **5** (81 mg, 0.1 mmol) was dissolved in acetic acid (2 ml) and zinc (nano-size activated powder, 99.9%, 200 mg) was added. After stirring for 1 h, TLC analysis (ethyl acetate:hexanes, 1:2, v/v) showed that the starting material was consumed. The reaction mixture was filtered through short pad of silica gel (eluent: ethyl acetate) and the filtrate was concentrated under reduced pressure. The residue was dissolved in a mixture of pyridine (3 ml) and acetic anhydride (2.5 ml). After stirring the reaction mixture for 0.5 h, it was co-concentrated with toluene (3×15 ml) and the residue purified by silica gel column chromatography (eluent: ethyl acetate:hexanes, 2:3, v/v) to give **7** as a white foam (58 mg, 86%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.78–7.28 (m, 13H, Ar-H), 5.75 (d, 1H, N*H*, $J_{NH,2}$=8.7 Hz), 5.51 (d, 1H, H-1, $J_{1,2}$=5.5 Hz), 5.29 (t, 1H, H-4, $J_{3,4}$= $J_{4,5}$=9.6 Hz), 4.98 (dd, 1H, H-3, $J_{2,3}$=11.5 Hz,), 4.64 (ddd, 1H, H-2), 4.55 (AB q, PhCH_2, J_{AB}=11.9 Hz), 4.40–4.36 (m, 3H, H-5, CH_2O (Fmoc)), 4.28 (t, 1H, H-9 (Fmoc), J=7.8 Hz), 3.64 (dd, 1H, H-6a, $J_{5,6a}$=2.7 Hz, $J_{6a,6b}$=11.0 Hz), 3.61 (dd, 1H, H-6b, $J_{5,6b}$=5.1 Hz), 2.71–2.58 (m, 4H, COCH_2CH$_2$, SCH_2), 2.42 (t, 2H, COCH$_2$CH_2, J=6.9 Hz), 2.06 (s, 3H, CH_3CO), 1.90 (s, 3H, NHCOCH_3), 1.31 (t, 3H, SCH$_2$CH_3, J=7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 206.2, 171,3, 169.9, 155.7, 143.5, 141.4, 138.1, 128.5, 128,1, 128.0, 127.8, 127.5, 127.4, 125.4, 120.2, 84.2, 75.9, 73.8, 70.9, 69.9, 69.2, 68.9, 52.8, 46.8, 38.1, 30.1, 28.2, 25.7, 23.7, 15.4. $[\alpha]_D^{25}$ +18.8 (*c* 0.8, CH_2Cl_2). MALDI-TOF MS: *m/z* 698 $[M+Na]^+$. Anal. calcd for $C_{37}H_{41}NO_9S$: C, 65.76; H, 6.12; N, 2.07; found: C, 65.58; H, 6.14; N, 2.11.

3.7. 4-Alloxycarbonylpentoxybenzyl 6-O-benzyl-3-O-(9-fluorenylmethoxycarbonyl)-4-O-levulinoyl-2-deoxy-2-[[(2,2,2-trichloroethoxy)carbonyl]amino]-β-D-glucopyranoside **9**

A solution of **5** (162 mg, 0.2 mmol) and **8** (61 mg, 0.22 mmol) in dichloromethane (4 ml) was stirred in the presence of 4 Å molecular sieves (100 mg, powdered) for 15 min. The mixture was cooled (0°C) and NIS (50 mg, 0.22 mmol) and TMSOTf (4 μl, 22 μmol) were added. After 5 min, TLC analysis (ethyl acetate:hexanes, 1:1, v/v) showed that compound **5** was consumed. The reaction mixture was diluted with dichloromethane (50 ml), filtered, and the filtrate was washed successively with aqueous sodium thiosulfate (15%, 2×25 ml), water (20 ml), and brine (15 ml). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated in vacuo. Purification of the residue by silica gel column chromatography (eluent: ethyl acetate:hexanes, 1:2, v/v) gave **9** as an amorphous solid (154 mg, 75%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.77–6.83 (m, 17H, Ar-H), 5.98–5.90 (m, 1H, C*H*=CH$_2$), 5.36–5.25 (m, 2H, CH=CH_2), 5.22–5.16 (m, 2H, H-3, H-4), 5.09 (br s, 1H, N*H*), 4.87–4.57 (m, 9H, H-1, PhCH_2, OCH_2CCl$_3$, CH_2CH=CH$_2$, CH_2O (Fmoc)), 4.27 (t, 1H, H-9 (Fmoc), J=7.6 Hz), 3.97 (t, 2H, PhOCH_2CH$_2$, J=6.4 Hz), 3.75–3.64 (m, 4H, H-2, H-5, H-6a, H-6b), 2.59 (t, 2H, COCH_2CH$_2$, J=5.2 Hz), 2.42–2.38 (m, 4H, COCH$_2$CH_2, CH_2COOAll), 2.04 (s, 3H, CH_3CO), 1.85–1.50 (m, 6H, OCH$_2$CH_2CH_2CH_2CH$_2$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 205.9, 173.4, 171.5, 159.1, 155.1, 154.0, 143.5, 141.4, 138.1, 132.4, 130.0, 128.7, 128.5, 128.1, 128.0, 127.9, 127.4, 125.5, 120.2, 118.4, 114,7, 100.3, 98.7, 95.6, 76.4, 74.7, 73.9, 73.6, 70.8, 69.8, 69.3, 67.9, 65.3, 56.8, 46.8, 38.0, 34.5, 29.9, 29.3, 28.1, 26.0, 25.0. $[\alpha]_D^{25}$ −4.6 (*c* 0.2, CH_2Cl_2). MALDI-TOF MS: *m/z* 1046 $[M+Na]^+$. Anal. calcd for $C_{52}H_{56}Cl_3NO_{14}$: C, 60.91; H, 5.50; N, 1.37; found: C, 60.98; H, 5.66; N, 1.32.

3.8. 4-Hydroxylcarbonylpentoxybenzyl 6-O-benzyl-3-O-(9-fluorenylmethoxycarbonyl)-4-O-levulinoyl-2-deoxy-2-[[(2,2,2-trichloroethoxy)carbonyl]amino]-β-D-glucopyranoside **10**

$Pd(PPh_3)_4$ (12 mg, 0.01 mmol) was added to a solution of compound **9** (103 mg, 0.1 mmol) in ethanol (2 ml), THF (2 ml), and water (0.2 ml). After stirring the reaction mixture for 2 h at 60°C,

TLC analysis (ethyl acetate:hexanes, 1:1, v/v) indicated the completion of the reaction. The reaction was concentrated to dryness and the crude product was subjected to a column of silica gel (eluent: MeOH:dichloromethane, 2:100, v/v) to give compound **10** as a colorless syrup (91 mg, 93%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.78–6.84 (m, 17H, Ar-H), 5.22–5.10 (m, 3H, H-3, H-4, N*H*), 4.86–4.56 (m, 7H, H-1, PhC*H*$_2$, OC*H*$_2$CCl$_3$, C*H*$_2$O (Fmoc)), 4.26 (t, 1H, H-9 (Fmoc), *J*=7.6 Hz), 3.97 (t, 2H, PhOC*H*$_2$CH$_2$, *J*=6.4 Hz), 3.75–3.63 (m, 4H, H-2, H-5, H-6a, H-6b), 2.58 (t, 2H, COC*H*$_2$CH$_2$, *J*=5.2 Hz), 2.42–2.32 (m, 4H, COCH$_2$C*H*$_2$, C*H*$_2$COOH), 2.02 (s, 3H, C*H*$_3$CO), 1.85–1.44 (m, 6H, OCH$_2$C*H*$_2$C*H*$_2$C*H*$_2$CH$_2$). MALDI-TOF MS: *m/z* 1006 $[M+Na]^+$. Anal. calcd for $C_{49}H_{52}Cl_3NO_{14}$: C, 59.73; H, 5.32; N, 1.42; found: C, 59.58; H, 5.36; N, 1.46.

3.9. Immobilization of compound *10* on MPEG

A mixture of MPEG (1 g, 0.2 mmol) and compound **10** (196 mg, 0.2 mmol) was dried over P_2O_5 at high vacuum for 12 h. This mixture was dissolved in dichloromethane (10 ml), and a catalytic amount of DMAP (3 mg), followed by DCC (45 mg, 0.22 mmol), was added. The solution became cloudy within 15 min and was stirred overnight at room temperature. The precipitated urea was removed by filtration, washed with dichloromethane, and the combined filtrates were concentrated to dryness. The residue was dissolved in dichloromethane (8 ml), cooled (0°C), and *tert*-butyl methyl ether (100 ml) was added with vigorous stirring. The precipitate was washed with diethyl ether (2×10 ml) and cold ethanol (2×10 ml), and then further purified by recrystallization from ethanol.

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