Solid-Phase Synthesis of Oligosaccharides: Construction of a Dodecasaccharide**

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The synthesis of combinatorial libraries[1] of organic compounds for biological screening remains one of the severest bottlenecks in drug discovery and development. We have recently disclosed a reiterative approach[2] to solid-phase synthesis of oligosaccharides[3] that used thioglycosides as the carbohydrate donors and a photolabile linker,[4] and culminated in the total synthesis of HPE, a heptasaccharide phytoalexin elicitor. Despite its efficiency, this methodology[2] suffers from a) the presence of both α- and β-anomers at every cleavage stage and b) the need to reactivate the cleavage product prior to its possible reincorporation into the growing oligosaccharide chain on the solid phase. Herein we wish to report new solid-phase synthetic technology which provides solutions to both problems by incorporating an appropriate spacer between the photolabile linker and the anomeric position of the first glycoside, and which appears ideal for block-type oligosaccharide synthesis (Figure 1). The new technology allowed the construction of the stereochemically homogeneous dodecasaccharide **1** (see Scheme 2) which, to our knowledge, represents the largest oligosaccharide to be constructed on solid phase thus far. By virtue of its generality, this method could prove highly enabling for the construction of large and diverse libraries of oligosaccharides (see Figure 1).

The key elements of this new synthetic technology are demonstrated in Scheme 1. Thus, 5-hydroxy-2-nitrobenzaldehyde was loaded onto Merrifield resin in the presence of Cs_2CO_3 and nBu$_4$NI, and the product was reduced with $NaBH_4$ to afford resin **6** (98% yield over 2 steps).[5] The β-phenolic ester **7** was obtained from thioglycoside **A**[6] by reaction with 4-benzyloxybenzoic acid in the presence of NIS[7] (84%; for abbreviations, see legends in schemes), followed by hydrogenation of the benzyl protecting group (72%). Attachment of **7** to the solid support **6** by a Mitsunobu[8] reaction furnished conjugate **8** (100%),[5, 9] and removal of the silyl group with HF·py afforded conjugate **9** (100%). Structure **8** offers, by design, a) exclusively β-stereochemistry at the anomeric center, which is maintained throughout the synthetic scheme; b) the possibility of cleavage to a stereochemically homogeneous product **7** (ideal for analysis purposes and further elaboration); and c) the opportunity to cleave from the resin with simultaneous first-stage activation[10] to a thioglycoside. The latter reactions were

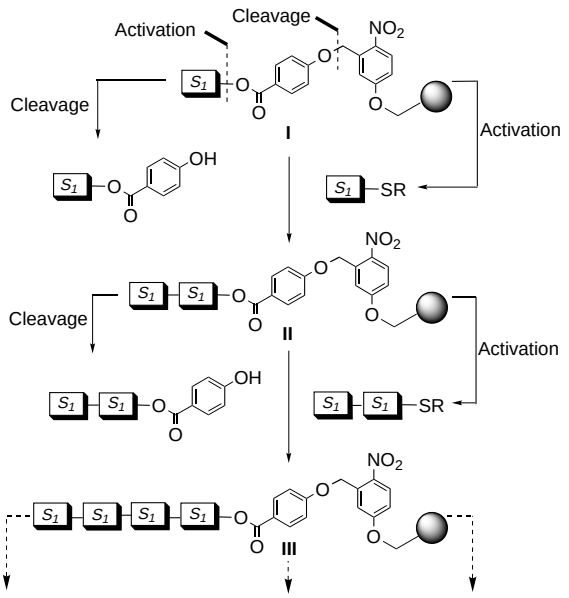
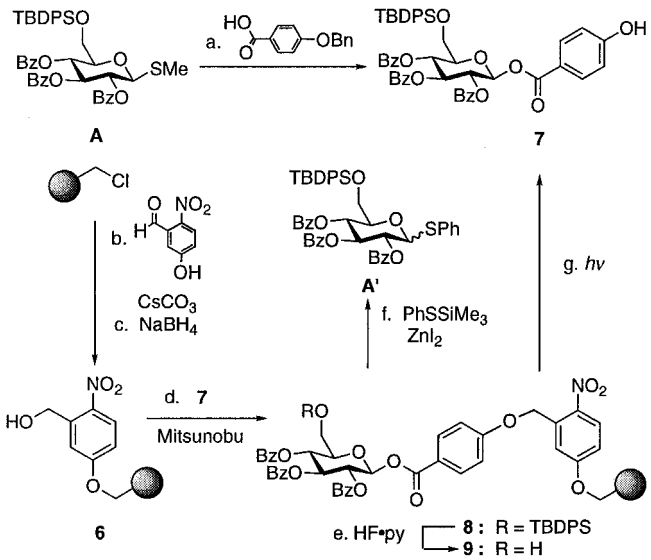


Figure 1. General block-type solid-phase strategy for the construction of complex oligosaccharides with a photolabile linker and thioglycoside donors.



Scheme 1. Synthesis of photolabile linker, loading and cleavage. Reagents and conditions: a) i. 4-benzyloxybenzoic acid (1.3 equiv), NIS (1.2 equiv), TfOH (cat.), CH_2Cl_2, 25 °C, 2 h, 84%; ii. HCO_2NH_4 (10.0 equiv), 10% Pd-C, MeOH/THF (1/1, v/v), 25 °C, 2.5 h, 72%; b) 5-hydroxy-2-nitrobenzaldehyde (4.0 equiv), Cs_2CO_3 (4.0 equiv), nBu$_4$NI (0.1 equiv), DMF, 80 °C, 12 h; c) $NaBH_4$ (5.0 equiv), THF/EtOH (95/5, v/v), 0 °C, 4 h, 98%; d) **7** (3.0 equiv), nBu$_3$P (3.0 equiv), DIAD (3.0 equiv), iPr$_2$NEt (3.0 equiv), THF, 25 °C, 6 h, 100%; e) HF·py, THF, 25 °C, 15 h; f) $PhSSiMe_3$ (6.0 equiv), ZnI_2 (2.5 equiv), nBu$_4$NI (1.0 equiv), $ClCH_2CH_2Cl$, 40–45 °C, 3 h, 92%; g) $h\nu$, THF, 25 °C, 6 h, 95%. Bn = benzyl; Bz = benzoyl; DIAD = diisopropyl azodicarboxylate; py = pyridine; NIS = *N*-iodosuccinimide; TfOH = trifluoromethanesulfonic acid.

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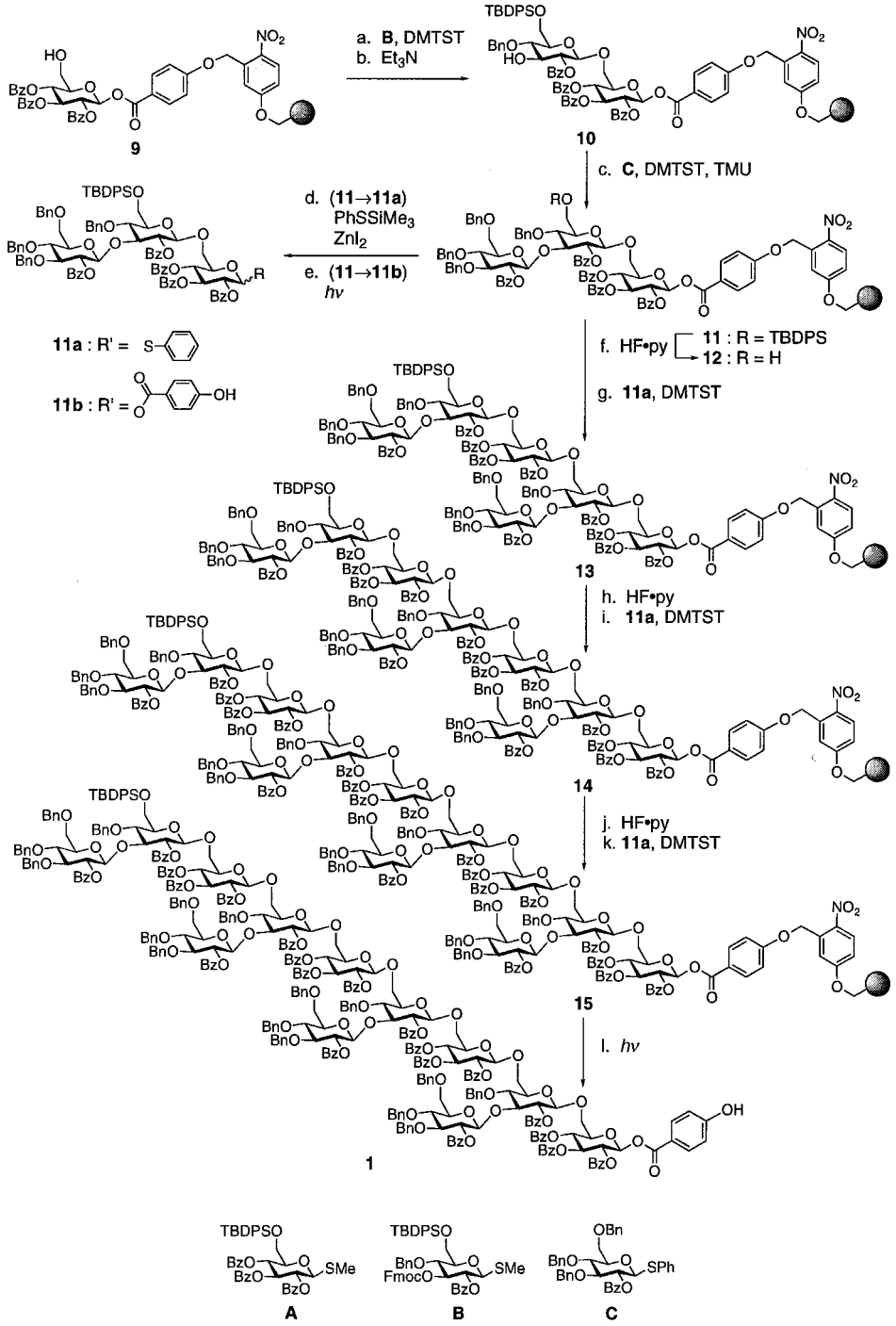
demonstrated by formation of compounds **7** (*hν*, THF, 25 °C, 95 %) and **A′**[11] ($PhSSiMe_3$, ZnI_2, nBu_4NI, 92 %),[12] respectively (Scheme 1).

With the principle established, we proceeded to demonstrate its potential by targeting the dodecasaccharide **1** (Scheme 2) related to the phytoalexin elicitor family.[13] Scheme 2 summarizes the chemistry leading to the successful synthesis of this complex oligosaccharide. Thus, coupling of **9** to donor **B** with the activator DMTST[14] and Fmoc removal (Et_3N) afforded the new conjugate **10** (90 % from **9**).[15] Attachment of the third building block, thioglycoside **C**, onto the resin required both DMTST and tetramethyl urea, and furnished **11** (78 %), from which the silyl group was removed by exposure to HF · py in THF to afford **12**. Cleavage of the trisaccharide from polymer **11** under photolytic conditions (*hν*, THF, 25 °C) generated exclusively the *β*-anomer of **11b** in 63 % overall yield from **8** (Scheme 1). The protected trisaccharide was cleaved from the polymer **11** with concomitant activation by exposure to $PhSSiMe_3/ZnI_2/nBu_4NI$ at 40–45 °C, furnishing phenylthioglycoside donor **11a** (76 % yield). Building block **11a** was then loaded onto the polymer **12** by glycosidation with the DMTST method, affording the hexasaccharide-polymer conjugate **13** after desilylation(61 % from **11**). Reiteration of the process with trisaccharide **11a** led to dodecasaccharide conjugate **15** via intermediate **14**. Finally, cleavage from the resin was induced by photolysis (*hν*, THF, 25 °C), furnishing the targeted oligosaccharide **1**[16] as a single stereochemical isomer (*ca* 10 % overall yield[17] of isolated product from **8,** Scheme 1).



Scheme 2. Solid-phase synthesis of dodecasaccharide **1** with the key donors **A**–**C**. Reagents and conditions: a) **B** (3.0 equiv), DMTST (12.0 equiv), 4 Å MS, CH_2Cl_2, 25 °C, 12 h; b) Et_3N/THF (1/4, v/v), 25 °C, 1 h, 90 % over 2 steps; c) **C** (3.0 equiv), DMTST (12.0 equiv), TMU (12.0 equiv), 4 Å MS, CH_2Cl_2, 25 °C, 12 h, 78 %; d) $PhSSiMe_3$ (6.0 equiv), ZnI_2 (2.5 equiv), nBu_4NI (1.0 equiv), $ClCH_2CH_2Cl$, 40–45 °C, 2 h, 76 %; e) *hν*, THF, 25 °C, 15 h, 63 % over 5 steps from **8**; f) HF · py, THF, 25 °C, 15 h; g) **11a** (3.0 equiv), DMTST (12.0 equiv), 4 Å MS, CH_2Cl_2, 25 °C, 12 h, 61 % over 2 steps; h) HF · py, THF, 25 °C, 15 h; i) **11a** (3.0 equiv), DMTST (12.0 equiv), 4 Å MS, CH_2Cl_2, 25 °C, 12 h, 56 % over 2 steps; j) HF · py, THF, 25 °C, 15 h; k) **11a** (3.0 equiv), DMTST (12.0 equiv), 4 Å MS, CH_2Cl_2, 25 °C, 12 h, 54 % over 2 steps; l) *hν*, THF, 25 °C, 20 h, 10 % overall yield from **8**. Bn = benzyl; Bz = benzoyl; DMTST = (dimethylthio)methylsulfonium triflate; py = pyridine; TBDPS = *t*-butyldiphenylsilyl; TMU = tetramethyl urea; MS = molecular sieves.

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Table 1. Selected physical properties of compounds **1**, **11a**, and **11b**.

11a (β-anomer): $R_f = 0.49$ (silica gel, ethyl acetate/*n*-pentane 1/2); $[\alpha]_D^{22} = +29.8$ ($c = 1.00$, $CHCl_3$); IR (thin film): $\tilde{\nu} = 2926$, 2857, 1732, 1602, 1453, 1364, 1264, 1094, 1070, 1027, 847, 748, 709 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 7.96 - 7.00$ (m, 60 H, Ar-H), 5.66 (t, $J = 9.0$ Hz, 1 H), 5.28 – 5.21 (m, 2 H), 5.15 – 5.09 (m, 3 H), 4.86 (d, $J = 8.0$ Hz, 1 H), 4.73 (d, $J = 10.5$ Hz, 1 H), 4.65 (d, $J = 10.0$ Hz, 1 H), 4.59 – 4.39 (m, 7 H), 4.25 (t, $J = 8.5$ Hz, 1 H), 3.83 – 3.76 (m, 5 H), 3.72 – 3.66 (m, 3 H), 3.59 – 3.55 (m, 2 H), 3.52 – 3.50 (m, 1 H), 3.38 – 3.36 (m, 1 H), 0.98 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta = 165.8$, 165.4, 165.1, 164.5, 136.9, 138.6, 138.0, 137.7, 137.5, 135.8, 135.5, 133.7, 133.4, 133.1, 132.9, 132.8, 132.6, 132.1, 129.9, 129.8, 129.8, 129.7, 129.6, 129.6, 129.4, 129.0, 128.8, 128.7, 128.5, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 128.1, 128.0, 127.9, 127.6, 127.6, 127.5, 127.4, 127.3, 100.9, 100.1, 85.5, 83.0, 79.5, 78.5, 78.3, 75.9, 75.7, 75.6, 75.1, 75.0, 74.9, 74.1, 74.0, 73.9, 73.5, 70.8, 69.2, 68.7, 67.9, 62.5, 26.8, 19.8; ES^+ MS: calcd for $C_{103}H_{98}O_{20}SSiNa$ [M+Na^+] 1739, found 1739.

11b (β-anomer): $R_f = 0.23$ (silica gel, ethyl acetate/*n*-pentane 1/2); $[\alpha]_D^{22} = +10.1$ ($c = 1.00$, $CHCl_3$); IR (thin film): $\tilde{\nu} = 3387$, 3068, 2931, 1735, 1606, 1452, 1265, 1092, 1070, 1026, 744, 706 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 8.04 - 7.00$ (m, 57 H, Ar-H), 6.61 (d, 2 H, $J = 9.0$ Hz, Ar-H), 6.43 (bs, 1 H), 6.00 (d, $J = 7.5$ Hz, 1 H, H_A-1), 5.82 (t, $J = 9.0$ Hz, 1 H), 5.65 (t, $J = 9.0$ Hz, 1 H), 5.36 (t, $J = 9.5$ Hz, 1 H), 5.25 (t, $J = 9.5$ Hz, 1 H), 5.14 – 5.07 (m, 2 H), 4.88 (d, $J = 8.0$ Hz, 1 H), 4.74 (d, $J = 11.0$ Hz, 1 H), 4.58 – 4.38 (m, 7 H), 4.24 (t, $J = 8.0$, 1 H), 4.00 – 3.94 (m, 2 H), 3.85 – 3.78 (m, 3 H), 3.69 – 3.50 (m, 6 H), 3.30 (d, $J = 10.0$ Hz, 1 H), 0.95 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR (100.0 MHz, $CDCl_3$): $\delta = 165.6$, 165.5, 165.1, 164.9, 164.7, 164.6, 164.0, 163.7, 160.8, 138.6, 138.1, 137.7, 137.5, 135.7, 135.5, 133.5, 133.3, 133.2, 133.1, 133.0, 133.0, 132.6, 132.3, 129.9, 129.8, 129.7, 129.6, 129.5, 128.8, 128.7, 128.7, 128.4, 128.4, 128.3, 128.2, 128.1, 127.9, 127.6, 127.6, 127.5, 127.3, 121.1, 120.6, 115.5, 115.2, 100.5, 100.2, 92.5, 83.0, 79.5, 78.1, 75.8, 75.7, 75.4, 75.2, 75.0, 74.9, 74.2, 73.9, 73.6, 72.7, 71.7, 71.0, 70.5, 69.3, 69.2, 66.6, 62.7, 26.7, 19.1; ES^+ MS: calcd for $C_{104}H_{98}O_{23}SiNa$ [M+Na^+] 1766, found 1765; ES^- MS: calcd for $C_{104}H_{97}O_{23}Si$ [$M - H$]$^-$ 1741, found 1741.

1: $R_f = 0.42$ (silica gel, ethyl acetate/*n*-pentane 1/1); $[\alpha]_D^{22} = -7.50$ ($c = 0.20$, $CHCl_3$); IR (thin film): $\tilde{\nu} = 3404$, 3064, 2922, 2855, 1731, 1602, 1453, 1366, 1264, 1096, 1067, 1028, 746, 708 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 7.97 - 6.96$ (m, 192 H, Ar-H), 6.62 (d, $J = 8.5$ Hz, 2 H, Ar-H), 6.11 (d, $J = 7.5$ Hz, 1 H, H_{A1}-1), 5.85 – 5.76 (m, 4 H), 5.69 (dd, $J = 8.5$, 7.0 Hz, 1 H), 5.47 (t, $J = 9.5$ Hz, 1 H), 5.34 – 5.28 (m, 4 H), 5.25 – 5.03 (m, 12 H), 4.90 – 4.67 (m, 12 H), 4.57 – 4.23 (m, 25 H), 4.17 – 3.92 (m, 12 H), 3.86 – 3.36 (m, 32 H), 3.30 – 2.83 (m, 12 H), 0.87 (s, 9 H, $C(CH_3)_3$); MALDI MS: calcd for $C_{347}H_{320}O_{82}SiNa$ [M+Na^+] 5869, found 5869.

combinatorial libraries of oligosaccharides for chemical biology and other studies. Table 1

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[16] Although a complete spectral assignment of **1** is beyond the scope of this publication, the following key NMR signals are characteristic and diagnostic. A distinct signal for two of the aromatic protons of the spacer appears in the region of $\delta = 6.61 - 6.63$ and a doublet for the anomeric proton H_{A1} attached to the spacer shows at *ca* $\delta = 6.10$. The signals of the C_A-3 proton from sugar donor **A** of each trisaccharide subunit are consistently in the region of $\delta = 5.76 - 5.80$ as expected. As each trisaccharide subunit was attached, the number of C_A-3 proton signals increased by one respectively.

[17] Yields refer to isolated, spectroscopically homogenous materials.

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