A General and Highly Efficient Solid Phase Synthesis of Oligosaccharides. Total Synthesis of a Heptasaccharide Phytoalexin Elicitor (HPE)

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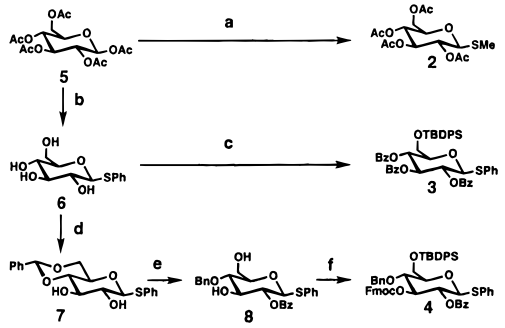
Received October 4, 1996

Combinatorial chemistry imposes new demands on the solid phase synthesis of organic molecules. Of particular interest to glycobiology and medicine is versatile and practical methodology for the construction of oligosaccharides of higher molecular complexity and in a combinatorial fashion. Although several methods for solid phase oligosaccharide synthesis have been reported,[1] new technologies that advance the field are still very much in demand. In this paper, we describe new synthetic technology for the construction of complex oligosaccharides on a solid support and its application to the synthesis of the heptasaccharide phytoalexin elicitor (HPE, **1**).[2,3] The described chemistry combines (a) application of phenolic polystyrene in solid phase oligosaccharide synthesis, (b) synthesis and utilization of a new photolabile linker in solid phase synthesis; (c) a number of versatile carbohydrate building blocks for potential applications in combinatorial chemistry, and (d) the largest branched oligosaccharide to be constructed on solid phase from monosaccharide units and in reiterative fashion.

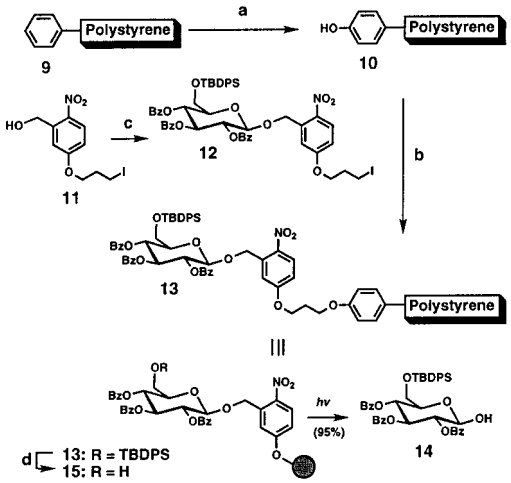
For the purposes of the present technology, monosaccharide building blocks **2**−**4**[4] (Scheme 1) were designed to allow, through neighboring group participation, selective β-glycoside bond formations at positions C-1 (unit **2**), C-6 (unit **3**), and C-3 (unit **4**). Their construction is summarized in Scheme 1.

Scheme 1.[a] Preparation of Monosaccharide Units **2**−**4**



[a] Reagents and conditions: (a) MeSH (excess), $SnCl_4$ (0.7 equiv), CH_2Cl_2, −20 °C, 3 h, 85%; (b) i. PhSH (1.1 equiv), $SnCl_4$ (0.7 equiv), CH_2Cl_2, 0 °C, 4 h, 82%; ii. K_2CO_3 (0.3 equiv), THF/MeOH 1:1 (v/v), 25 °C, 15 h, 98%; (c) i. *t*-$BuPh_2SiCl$ (1.5 equiv), imidazole (2.0 equiv), DMF, 25 °C, 3 h, 94%; ii. PhCOCl (4.0 equiv), Et_3N (8.0 equiv), 4-DMAP (0.2 equiv), THF, 50 °C, 15 h, 92%; (d) $PhCH(OMe)_2$ (2.0 equiv), CSA (0.5 equiv), benzene, reflux, 12 h, 96%; (e) *t*-$BuMe_2SiCl$ (1.2 equiv), imidazole (1.5 equiv), DMF, 0 °C, 12 h, 88%; ii. PhCOCl (1.5 equiv), Et_3N (3 equiv), 4-DMAP (0.5 equiv), CH_2Cl_2, 15 h, 96%; iii. BH_3·Me_3N (40 equiv), $AlCl_3$ (4 equiv), 4 Å MS, CH_2Cl_2/Et_2O 5:2 (v/v), 0 °C, 5 h; iv. 2 N HCl in MeOH, 25 °C, 3 h, 85% (two steps); (f) i. *t*-$BuPh_2SiCl$ (1.5 equiv), imidazole (2.0 equiv), DMF, 25 °C, 3 h, 96%; ii. Fmoc-Cl (3.0 equiv), pyridine, 25 °C, 1 h, 93%. 4-DMAP = 4-(dimethylamino)pyridine; CSA = (±)-10-camphorsulfonic acid; Fmoc-Cl = 9-fluorenylmethyl chloroformate.

Scheme 2.[a] Synthesis of Polystyrene-Bound Monosaccharides **13** and **15**



[a] Reagents and conditions: (a) i. *n*-BuLi (1.3 equiv), cyclohexane, 65 °C, 4 h; ii. O_2, cyclohexane, 25 °C, 2 h; iii. PPh_3 (2.0 equiv) THF, 25 °C, 12 h; (b) **12** (2.0 equiv), Cs_2CO_3 (2.0 equiv), DMF, 25 °C, 30 h, >90%; (c) **3** (1.0 equiv), **11** (1.5 equiv), DMTST (4.0 equiv), 4 Å MS, CH_2Cl_2, 25 °C, 4 h, 95%; (d) 30% hydrogen fluoride·pyridine, THF, 25 °C, 15 h, >98%. DMTST = (dimethylthio)methylsulfonium triflate; TBDMS = Si-*t*-$BuMe_2$.

Polystyrene (**9**, Scheme 2) was functionalized to phenolic polystyrene (**10**) by the sequential action of *n*-BuLi, oxygen, and PPh_3.[5,6] As a linker,[7] a readily available *o*-nitrobenzyl ether tether was utilized for its ease of attachment and cleavage. Thus, commercially available 5-hydroxy-2-nitrobenzaldehyde was reacted with 1,3-diiodopropane in the presence of Cs_2CO_3 in

(1) For a review of solid phase oligosaccharide synthesis up to 1980, see: (a) Fréchet, J. M. J. *Polymer-Supported Reactions in Organic Synthesis*; Wiley: New York, 1980, Chapter 8, p 407. For more recent advances in solid phase synthesis of oligosaccharides, see: (b) Veeneman, G. H.; Notermans, S.; Liskamp, R. M. J.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1987**, *28*, 6695. (c) Danishefsky, S. J.; McClure, K. F.; Randolph, J. T.; Ruggeri, R. B. *Science* **1993**, *260*, 1307. (d) Randolph, J. T.; Danishefsky, S. J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1470. (e) Yan, L.; Taylor, C. M.; Goodnow, R., Jr.; Kahne, D. *J. Am. Chem. Soc.* **1994**, *116*, 6953. (f) Schulster, M.; Wang, P.; Paulson, J. C.; Wong C.-H. *J. Am. Chem. Soc.* **1994**, *116*, 1135. (g) Randolph, J. T.; McClure, K. F.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1995**, *117*, 5712. (h) Rademann, J.; Schmidt, R. R. *Tetrahedron Lett.* **1996**, *23*, 3989. Adinolfi, M.; Barone, G.; De Napoli, L.; Iadonisi, A.; Piccialli, G. *Tetrahedron Lett.* **1996**, *28*, 5007. For oligosaccharide synthesis on soluble polymers, see: (i) Douglas, S. P.; Whitfield, D. M.; Krepinsky, J. J. *J. Am. Chem. Soc.* **1991**, *113*, 5095. (j) Whitfield, D. M.; Douglas, S. P.; Krepinsky, J. J. *Tetrahedron Lett.* **1992**, *33*, 6795. (k) Verduyn, R.; van der Klein, P. A. M.; Douwes, M.; van der Marel, G. A.; van Boom, J. H. *Recl. Trav. Chim. Pays-Bas* **1993**, *112*, 464. (l) Douglas, S. P.; Whitfield, D. M.; Krepinsky, J. J. *J. Am. Chem. Soc.* **1995**, *117*, 2116.

(2) For isolation and biological activity, see: (a) Sharp, J. K.; Valent, B.; Albersheim, P. *J. Biol. Chem.* **1984**, *259*, 11312. Sharp, J. K.; Valent, B.; Albersheim, P. *J. Biol. Chem.* **1984**, *259*, 11321. For a review in this field, see: (b) Darvill, A.; Augur, C.; Bergmann, C.; Carlson, R. W.; Cheong, J.-J.; Eberhard, S.; Hahn, M. G.; Ló, V.-M.; Marfà, V.; Meyer, B.; Mohnen, D.; O'Niell, M. A.; Spiro, M. D.; van Halbeek, H.; York, W. S.; Albersheim, P. *Glycobiology* **1992**, *2*, 181.

(3) For previous syntheses, see: (a) Ossowski, B. P.; Pilotti, Å.; Garegg, P. J.; Lindberg, B. *Angew. Chem., Int. Ed. Engl.* **1983**, *10*, 793. (b) Ossowski, B. P.; Pilotti, Å.; Garegg, P. J.; Lindberg, B. *J. Biol. Chem.* **1984**, *259*, 11337. (c) Fügedi, P.; Birberg, W.; Garegg, P. J.; Pilotti, Å. *Carbohydr. Res.* **1987**, *164*, 297. (d) Fügedi, P.; Garegg, P. J.; Kvarnström, I.; Svansson, L. *Carbohydr. Chem.* **1988**, *7*, 389. (e) Birberg, W.; Fügedi, P.; Garegg, P. J.; Pilotti, Å. *J. Carbohydr. Res.* **1989**, *8*, 47. (f) Lorentzen, J. P.; Helpap, B.; Oswald, L. *Angew. Chem., Int. Ed. Engl.* **1991**, *12*, 1681. (g) Hong, N.; Ogawa, T. *Tetrahedron Lett.* **1990**, *31*, 3179. (h) Verduyn, R.; van der Klein, P. A. M.; Douwes, M.; van der Marel, G. A.; van Boom, J. H. *Recl. Trav. Chim. Pays-Bas* **1993**, *112*, 464.

(4) All new compounds exhibited satisfactory spectral and exact mass data. Yields refer to spectroscopically and chromatographically homogeneous materials.

(5) Fréchet, J. M. J.; de Smet, M.; Farral, J. M. *Polymer* **1979**, *20*, 675.

(6) Although only *p*-substituted polystyrene is shown, it is estimated that the phenolic polystyrene **10** contains both *p*- and *m*-hydroxyphenyl rings. The functionalization was performed to the extent of 0.25−1.0 mmol/g, and the subsequent chemistry was carried out using a 0.25 mmol/g phenolic polystyrene.

S0002-7863(96)03482-8 CCC: $14.00

DMF and then directly reduced with $NaBH_4$ to afford iodobenzyl alcohol **11** (92% overall yield, Scheme 2), which served admirably its intended purposes.

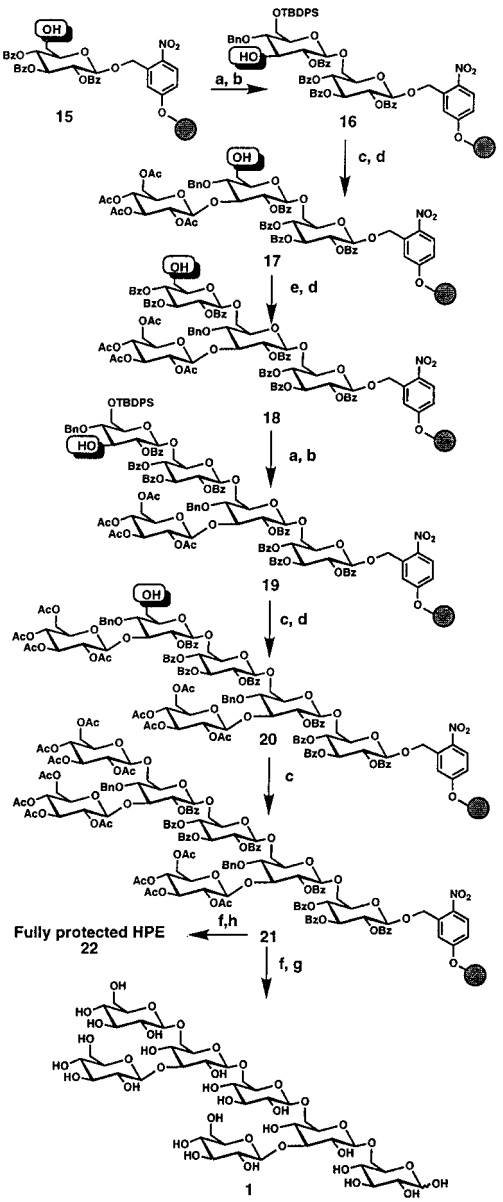
Glycosidation of **11** with carbohydrate unit **3** in the presence of dimethylthiomethylsulfonium triflate ($Me_2S^+SMeTfO^-$ = DMTST)[8] proceeded in 95% yield to afford exclusively β-glycoside **12**[9] (Scheme 2). The monosaccharide **12** was then attached to phenolic polystyrene (**10**) via its linker by the action of Cs_2CO_3 in DMF at 25 °C to afford conjugate **13** in >90% yield based on mass gain of the polymer. Efficient cleavage of the carbohydrate fragment from the resin was demonstrated by irradiation of **13** in THF at 25 °C to afford monosaccharide **14** in 95% yield.[10]

Having established the feasibility and efficiency of the solid phase chemistry, attention was then turned to the construction of the complex HPE oligosaccharide (**1**). The heptasaccharide **1** has been isolated from mycelial walls of *Phytophthora megasperma* f. sp. *glycinea* and shown to exhibit potent phytoalexin elicitor activity with nanomolar binding properties toward its receptor.[2] Previous syntheses[3] of this compound involve either solution chemistry or block synthesis using a soluble polymer (PEG).[3h] The present strategy required not only suitable protecting groups on each monosaccharide unit but also a convenient glycosidation method with high degree of β-selectivity. The protecting groups shown on **2**−**4** (Scheme 1) served admirably in terms of ease of attachment and removal, survivability under the reaction conditions, and orthogonality, whereas the thioglycoside−DMTST glycosidation method[8] proved both rapid and efficient.

The total synthesis of HPE (**1**) by the present solid phase technology was carried out as follows. Fluoride-induced liberation of the primary hydroxyl group in **13** furnished **15** (Scheme 2), onto which was attached monosaccharide **4** carrying the Fmoc protecting group at the C-3 position (Scheme 3). Removal of the latter group with Et_3N in CH_2Cl_2 proceeded smoothly and efficiently furnishing **16**[11] (>95%, calculated after photocleavage and based on loading of **13**). Coupling of **16** with monosaccharide **2** followed by fluoride-induced desilylation gave **17**, which was converted to **18** by addition of monosaccharide **3** and desilylation. Reiteration of these procedures allowed the growth of the branched oligosaccharide on the solid support in the desired direction until heptasaccharide **21** was reached via compounds **20** (addition of unit **4**) and **19** (addition of unit **2**). Photolytic cleavage of the heptasaccharide from the resin (**21**), followed by acetylation of the anomeric hydroxyl group, furnished a mixture of fully protected α- and β-heptasaccharides (**22**, *ca.* 20% overall yield from **13**), whose HPLC, ^{1}H NMR, and mass spectroscopic properties were consistent with the expected structure. The targeted heptasaccharide (**1**, mixture of α- and β-anomers) was obtained from **21** by photocleavage, followed by treatment with NaOMe in MeOH and hydrogenolysis (H_2, Pd-C, MeOH, *ca*. 95% for two steps). All reactions were carried out at ambient temperatures.

The rendering of HPE (**1**) and its variants as readily available materials should facilitate biological investigations in the field.

Scheme 3.[a] Solid Phase Synthesis of HPE (**1**)



[a] Reagents and conditions: (a) **4** (3.0 equiv), DMTST (12 equiv), 4 Å MS, CH_2Cl_2, 25 °C, 15 h; (b) 20% Et_3N in CH_2Cl_2, 25 °C, 5 h; (c) **2** (4.0 equiv), DMTST (16 equiv), 4 Å MS, CH_2Cl_2, 25 °C, 30 h; (d) 30% hydrogen fluoride·pyridine, THF, 25 °C, 15 h; (e) **3** (3 equiv), DMTST (12 equiv), 4 Å MS, CH_2Cl_2, 25 °C, 15 h; (f) *hν*, THF, 25 °C, ca. 20% overall yield from **13**; (g) i. NaOMe, THF/MeOH 1:2 (v/v) 25 °C, 15 h; ii. H_2 (1 atm), Pd/C cat., MeOH, 25 °C, 12 h, 95% two steps; (h) Ac_2O (10 equiv), 4-DMAP (1.0 equiv), Et_3N (20 equiv), THF, 25 °C, 15 h, 100%.

Furthermore, the described solid phase chemistry and variations thereof should prove useful in both combinatorial synthesis of oligosaccharide libraries and complex oligosaccharide construction.

Acknowledgment. We thank Drs. Dee Huang and Gary Siuzdak for their superb NMR and mass spectroscopic assistance and the Ministerio de Educación y Ciencia (Spain) for a postdoctoral fellowship (J.P.). This program was financially supported by the National Institutes of Health, The Skaggs Institute for Chemical Biology, Merck, DuPont Merck, Amgen, and Hoffmann La Roche.

Supporting Information Available: Selected procedures and data for compounds (and/or derivatives of) **2**−**4**, **11**, **12**, **14**, **16** (off the resin), **17** (off the resin), **22**, and **1** (19 pages). See any current masthead page for ordering and Internet access instructions.

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(7) (a) For a review of photolabile protecting groups, see: Pillai, R. V. N. *Organic Photochemistry* Padwa, A., Ed.; Marcel Dekker: New York, 1987; Vol. 9, p 225. (b) Zehavi, U.; Amit, B.; Patchornik, A. *J. Org. Chem.* **1972**, *14*, 2281. (c) Holmes, C. P.; Jones, D. G. *J. Org. Chem.* **1995**, *60*, 2319. (d) Venkatesan, H.; Greenberg, M. M. *J. Org. Chem.* **1996**, *61*, 525.

(8) Fügedi, P.; Garegg, P. J. *Carbohydr. Res.* **1986**, *149*, 9.

(9) The purity and exclusive β-stereochemistry of **12** was confirmed by HPLC and ^{1}H NMR spectroscopy (H-1, δ 4.99, J = 7.5 Hz, see Supporting Information).

(10) Typically, 5 mg of resin is sufficient to provide, after photolysis, enough material for HPLC and mass spectroscopic analysis at each step.

(11) The homogeneity and β-stereochemistry for the **1** → **6** and **1** → **3** glycosidations was confirmed by photolytic cleavage, followed by acetylation, HPLC, and ^{1}H NMR spectroscopy for the disaccharide and trisaccharide compounds (see Supporting Information). The stereochemistry of the higher oligosaccharides obtained by simple reiteration of the same **1** → **6** and **1** → **3** glycosidations was assumed to be the same as that of their dimeric and trimeric counterparts, whereas their homogeneity was verified by HPLC analysis (see Supporting Information).