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Solid Phase Synthesis of Oligosaccharides

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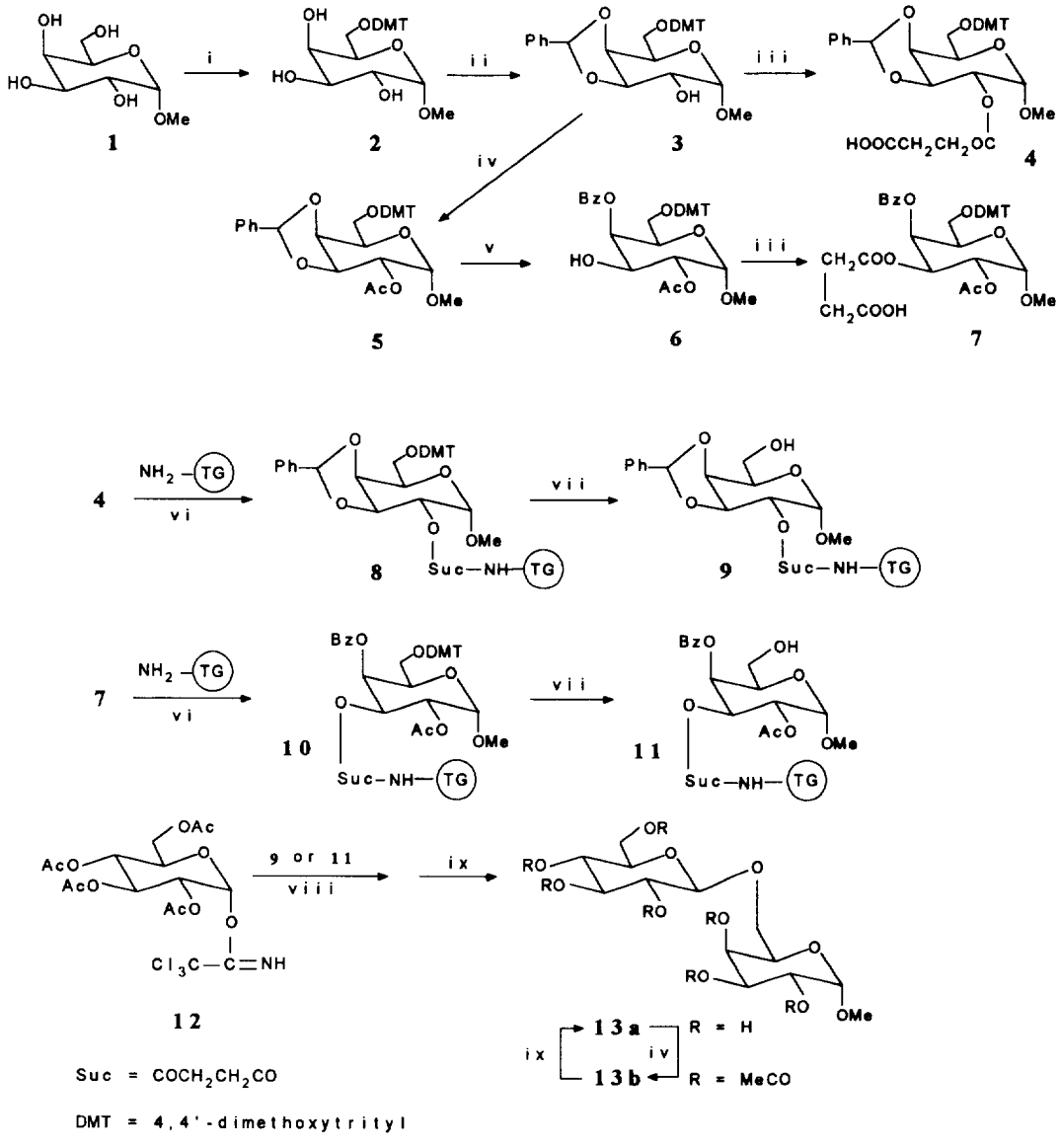
Abstract: An efficient and stereocontrolled solid-phase chemical synthesis of oligosaccarides using a new type of insoluble support (a copolymer of polyethylene glycol and polystyrene) is described. Several Lewis acids were tested as activators of the trichloroacetimidate **12**, used as glycosyl donor, in the dimer **13** formation. Copyright © 1996 Elsevier Science Ltd

In recent years the development of an efficient and yet simple procedure for the synthesis of oligosaccharides has been a major goal of carbohydrate chemistry. This arises from the important role that these macromolecules and their conjugates play in a variety of biological processes.[1]

A number of methods for the synthesis of oligosaccharides in solution has been developed in the years which involve stereocontrolled and high yield reactions.[2] Chemical and enzymatic syntheses of oligosaccharides on soluble[3] or insoluble[4] polymeric supports have also been reported. However, at this time, the solid phase methodologies cannot be considered competitive with the solution methods. Nevertheless, the well known advantages associated with the use of an insoluble polymeric support (reduction of time consuming work-up and possibility of a complete automation of the synthetic process) justify further efforts aimed at improving the solid phase strategy.

We report here the results of our studies on glycoside bond formation by a solid phase approach in the synthesis of dimer D-Glc*p*-(1β→6)-D-Gal*p*-α-OMe (**13**) using the functionalised supports **9** and **11** as glycosyl acceptors and the O-glucosyl trichloroacetimidate **12** as glycosyl donor. O-Glycosyl trichloroacetimidates are widly used in glycosylation reactions,[2] and often afford high yields with some stereocontrol under appropriate conditions and with a suitable O-2-substituent on the donor. Hovewer, in our knowledge they have never been used in the glycosidic bond formation on an insoluble polymeric matrix. We tested as solid support the copolymer of polyethylene glycol and polystyrene commercially known as Tentagel.[5] In this support, already in use in oligopeptide[6] and oligonucleotide synthesis,[7] the high mobility of the polyethylene glycol chains (m.w. 3000) assures an easy accessibility of the reagents to the reactive groups of the polymer.

In our synthetic strategy the first sugar unit, a suitable methyl α-D-galactopyranoside derivative **4**[8] (or **7**[9]) was anchored to the solid support through a succinic bridge, involving the C-2 (or C-3) hydroxyl group of the sugar residue and the native amino function of the Tentagel support (0.24 meq/g, TG-NH_2, Scheme). Compounds **4** and **7** were synthesized starting from methyl α-D-galactopyranoside **1**, following the pathways **1**→ **4** and **3** → **7**, respectively.[10] As a transient protecting group for the C-6 hydroxyl functions of **4** and **7** we chose the dimethoxytrityl (DMT) group which ensured an easy spectrophotometric determination of the sugar loading on the polymer and a fast removal procedure. The functionalization[11] of the Tentagel was performed



Scheme: i) DMTCl (1.3 eq.), pyridine, Et_3N, r. t., 16 h; ii) $PhCHBr_2$ (1.2 eq.), pyridine, Et_3N, DMAP (cat.), 100°, 2 h; iii) succinic anhydride, (1.5 eq), DMAP (1.7 eq), pyridine, r. t., 15 h; iv) Ac_2O, pyridine, r. t. , 6 h; v) NBS (1.4 eq), AIBN (cat.), H_2O (80 eq.), CCl_4, 77°, 3 h; vi) DCCI (3.5 eq), pyridine r. t. , 16 h.; vii) $CHCl_2COOH/CH_2Cl_2$, 2 % (w/w), 2 ml, three times, 1 min each; viii) glycosidic bond formation, see Table; ix) NH_4OH (32 %), r.t., 4 h.

by addition of a pyridine solution of **4** (or 7) to the polymer in the presence of dicyclohexylcarbodiimide (DCCI), thus obtaining support **8** (or **10**). The incorporation yields of the sugar material were estimated by quantitative spectrophotometric measurement of the DMT cation released by acidic treatment of a weighed amount of the support and resulted in both cases to be in the range 0.15-0.17 meq/g.

After washings, the supports were treated with a solution of pyridine/Ac_2O in order to cap the unreacted amino functions on the polymer. Finally, the derivatised supports **8** and **10** were completely deprotected at the C-6 hydroxy functions (dichloroacetic acid/CH_2Cl_2, 2%, w/w) affording **9** and **11**, respectively, which were dried *in vacuo* and stored in the presence of P_2O_5. Compounds **9** and **11** were tested as glycosyl acceptors in coupling experiments with trichloroacetimidate **12**[12] using different activators (triflic acid,[13] TMS-triflate,[14] $BF_3{\cdot}OEt_2$,[14] $LiClO_4$[15]), see Table.

Table : results of the glycoside syntheses

Entry	Support	Glycosylation promoter	Solvent	Yield %[a]	Reaction time (h)
1	**9**	Triflic acid (0.1 eq)[b]	CH_2Cl_2/cyclohexane (2:1)	55[d]	2
2	**9**	TMS-triflate (0.1 eq)[b]	CH_2Cl_2/cyclohexane (2:1	55[d]	2
3	**9**	$BF_3{\cdot}OEt_2$ (0.1 eq)[b]	CH_2Cl_2/cyclohexane (2:1)	52[d]	2
4	**9**	$LiClO_4$[c]	CH_2Cl_2	3-5	48
5	**11**	Triflic acid (0.1 eq)[b]	CH_2Cl_2/cyclohexane (2:1)	50(60)[e]	2
6	**11**	TMS-triflate (0.1 eq)[b]	CH_2Cl_2/cyclohexane (2:1)	45(55)[e]	2
7	**11**	$BF_3{\cdot}OEt_2$ (0.3 eq)[b]	CH_2Cl_2/cyclohexane (2:1)	70(90)[e]	2
8	**11**	$LiClO_4$[c]	CH_2Cl_2	3-5	48

[a] Determined by integration of the OMe signals[16] in the ^1H NMR spectra of the crude detached material. [b] On the respect to **12**. [c] 1 M in CH_2Cl_2. [d] Loss of benzylidene protecting group. [e] The highest yield, parenthesis, was obtained performing twice the coupling reaction on the support.

In a typical coupling procedure, 50 mg (7.5 μmol) of support **9** (or **11**) were swollen in CH_2Cl_2 under argon atmosphere and then treated with a solution of **12** (0.15 mmol) in the reaction solvent (1.5 ml), followed by addition of the imidate activator. The resin was gently shaken for the coupling time (see Table) at room temperature, washed with CH_2Cl_2, CH_3OH and then treated with aq. conc. ammonia (32%, 5 h , r.t.). The products detached from the resin were analysed by ^1H NMR spectroscopy[16] in order to estimate the yields of glycoside bond formation and resolved in their peracetylated form by silica gel TLC. The isolated **13b** (identified by ^1H NMR spectroscopy[17]) was successively hydrolysed to the desired dimer **13a**[17] by treatment with aq. conc. ammonia.

As expected, all coupling reactions resulted to be totally stereospecific, only trans glycosidic bond being formed. The results obtained indicate that the yields of the glycoside bond formation are affected by the nature of the imidate activator used, (see Table), in contrast with the data so far reported in the literature for similar synthesis performed in solution.[14-15] Analysing our data in detail, the best yields of the desired dimer **13** were obtained when the synthesis was performed on support **11** with $BF_3{\cdot}Et_2O$ (entry 7). In the case of support **9**, the use of acidic conditions (entries 1-3) led to an almost complete loss of the benzylidene protecting group. When $LiClO_4$, which has been successfully exploited[15] for solution reaction, was used to activate the trichloroacetimidate function (entry 4 and 8), only poor yields of the target compound **13a** were detected (3-5%), even after prolonged reaction time (48 h). Moreover, it was found that, when the addition of the glycosylating agent was repeated twice on support **11**, a sensible increase in the coupling yields (up to 90 %) was correspondly observed (entry 7).

In conclusion, the functionalised support **11** associated with the trichloroacetimidate **12** was shown to be suitable for the stereocontrolled solid-phase synthesis of oligosaccharides, giving acceptable yields in the reported preliminary experiments aimed at the preparation of the dimer **13**. The introduction of the copolymer

of polyethylene glycol and polystyrene as new insoluble polymeric support in the oligosaccharide synthesis seems to be promising also in the light of exploiting it in chemoenzymatic synthetic methods, where the hydrophilicity and the mobility of its polyethylene glycol spacer arms can play a crucial positive role. Future efforts will be addressed to a protocol for the preparation of longer and/or branched oligomers.

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8. **4**, ^{1}H NMR (270 MHz, $CDCl_3$) δ: 6.70-7.50 (18H, aromatic protons); 6.16 (1H, s, PhC*H*); 5.09 (1H,dd, $J_{2,1}$ = 3.3, $J_{2,3}$ = 8.2, H-2); 4.95 (1H, d, H-1); 4.66 (1H, dd, $J_{3,4}$ = 5.1, H-3); 4.35 (1H, dd, $J_{4,5}$ = 2.2, H-4); 4.05 (1H, dt, $J_{5,6a}$ = 6.3, $J_{5,6b}$ = 6.3, H-5); 3.79 and 3.78 (3H each, s's, PhOCH_3); 3.48 (2H, d, H_2-6); 3.44 (3H, s, 1-OCH_3); 2.76 (4H, m, succinic protons). HRMS (FAB), m/z $(M+1)^+$ 685.2653, calcd. 685.2649 for $C_{39}H_{40}O_{11}$ + H^+.
9. **7**, ^{1}H NMR (270 MHz, $CDCl_3$) δ: 8.00-6.60 (18H, aromatic protons); 5.82 (1H, dd, $J_{4,3}$ = 3.4, $J_{4,5}$ = 1.1, H-4); 5.52 (1H, dd, $J_{3,2}$ = 10.8, H-3); 5.19 (1H, dd, $J_{2,1}$ = 3.9, H-2); 5.02 (1H, d, H-1); 4.15 (1H, m, H-5); 3.72 and 3.73 (3H each, s's, PhOCH_3) 3.43 (3H, s, 1-OCH_3); 3.34 (1H, dd, $J_{6a,6b}$ = 9.1, $J_{6a,5}$ = 6.2, H-6a); 3.16 (1H, dd, H-6b); 2.60 (4H, m, succinic porotons); 2.07 (3H, s, MeCO). HRMS (FAB), m/z $(M+1)^+$ 743.2707, calcd. 743.2704 for $C_{41}H_{42}O_{13}$ + H^+.
10. All the structures of the synthetic intermediates were confirmed by ^{1}H and ^{13}C NMR spectroscopy.
3, HRMS (FAB), m/z $(MH)^+$ 585.2493, calcd. 585.2488 for $C_{35}H_{36}O_8$ + H^+.
6, HRMS (FAB), m/z $(MH)^+$ 643.2546, calcd. 643.2543 for $C_{37}H_{38}O_{10}$ + H^+.
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16. ^{1}H NMR (400 MHz, D_2O), methoxyl signals of **1** at δ 3.45 and of the dimer **13a** at δ 3.47.
17. **13a**, ^{1}H NMR (400 MHz, D_2O) anomeric protons at δ 4.54 (1H, d, $J_{1,2}$ = 7.8 Hz, β-Glc*p*); 4.72 (1H, bs, α-Gal*p*); methoxyl protons at δ 3.47 (3H, s). ^{13}C NMR as reported.[18]
13b, ^{1}H NMR (400 MHz, $CDCl_3$), Glc*p* protons at δ: 5.19 (1H, t, $J_{3,2}$ = 9.8, $J_{3,4}$ = 9.8, H-3); 5.07 (1H, t, $J_{4,5}$ = 9.8, H-4); 4.98 (1H, dd, $J_{2,1}$ =7.8, H-2); 4.55 (1H, d, H-1); 4.27 (1H, dd, $J_{6a,5}$ = 4.8, $J_{6a,6b}$ = 12.2, H-6a); 4.13 (1H, dd, $J_{6b,5}$ = 2.4, H-6b); 3.71 (1H, ddd, H-5); Gal*p* protons at δ: 5.42 (1H, dd, $J_{4,3}$ = 3.4, $J_{4,5}$ = 1.0 H-4); 5.33 (1H, dd, $J_{3,2}$ = 10.7, H-3); 5.13 (1H, dd, $J_{2,1}$ = 3.4, H-2); 4.98 (1H, d, H-1); 4.19 (1H, m, H-5); 3.79 (1H, dd, $J_{6a,5}$ = 4.4, $J_{6a,6b}$ = 10.7, H-6a); 3.66 (1H, dd, $J_{6b,5}$= 7.8, H-6b); 3.59 (3H, s, 1-OCH_3), 2.14, 2.10, 2.09, 2.04, 2.03, 2.00, 1.98 (3H each, s's, MeCO)
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