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Use of Controlled Pore Glass in Solid Phase Oligosaccharide Synthesis. Application to the Semiautomated Synthesis of a Glyconucleotide Conjugate

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Abstract : Three polymeric supports (polystyrene, Tentagel and controlled pore glass) have been tested for solid phase synthesis of oligosaccharides based on the trichloroacetimidate methodology. Controlled pore glass has been found to yield satisfactory results with TMSOTf as the glycosylation promoter. An application to the on-line preparation of a glyconucleotide is also reported.

Solid phase synthesis of oligosaccharides constitutes a basic problem of modern carbohydrate chemistry and despite the recent developments[1] a number of problems associated with this approach are still unsolved. The choice of glycosyl donors and of suitable transient protecting groups is currently under investigation in order to find the most efficient strategy leading to stereoselective, high yielding and fast reactions. However solid phase synthesis has already found noteworthy applications in combinatorial chemistry.[2] Another important goal related to this methodology is the preparation of glycoconjugates with oligopeptides[3] and oligonucleotides by an approach allowing the construction of both the saccharidic and non-saccharidic moieties by a single solid phase synthesis ("on-line solid phase synthesis").[4] To this purpose the choice of a support and of a chemical strategy compatible with both the moieties of the hybrid chain anchored to the matrix is decisive

We have recently reported[5] that satisfying yields can be obtained using the copolymer of polystyrene and polyethyleneglycol, commercially known as Tentagel® (TG), in stereoselective solid-phase glycosylations based on the use of the imidate strategy. In this paper we wish to report the extension of our investigation to other supports such as polystyrene (PS) and controlled pore glass (CPG), the improvement in the glycoside bond formation and the first example, to the best our knowledge, of the synthesis of a saccharo-nucleotide conjugate by a semi-automated solid phase "on-line" approach, in which both trichloroacetimidate and phosphoramidite[6] chemistry are employed.

Up to now PS has been the most investigated solid support in the synthesis of oligosaccharides[1,2b] and actually this resin presents favourable features such as chemical inertness and low cost. On the other hand

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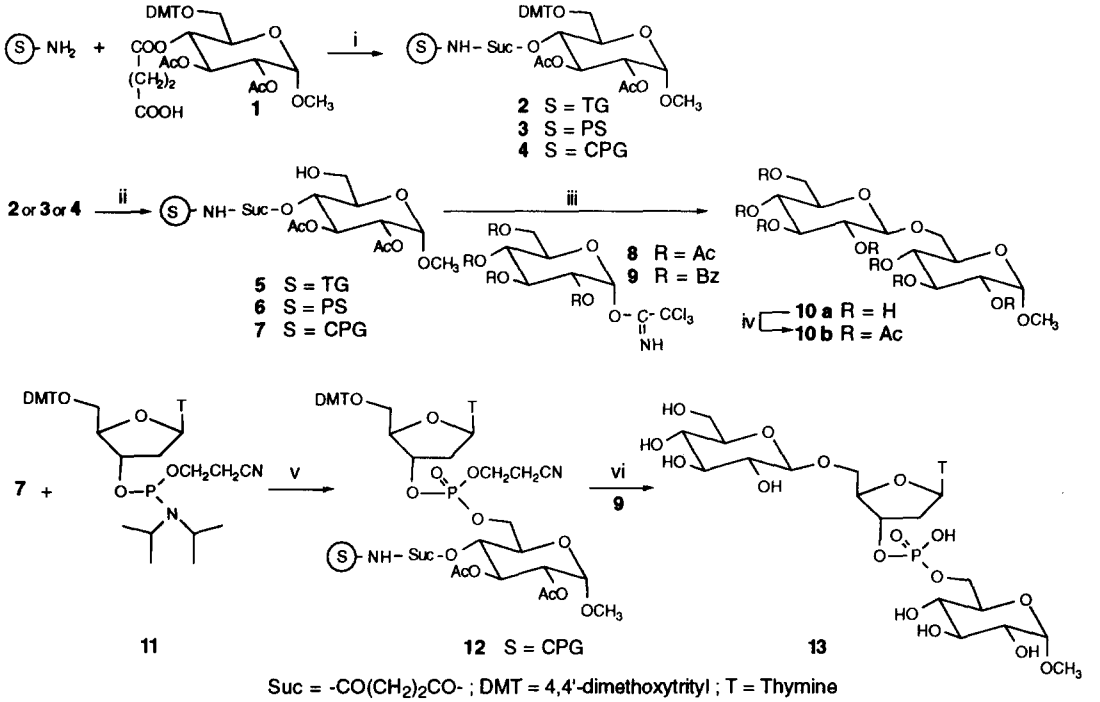
CPG, commonly used in the preparation of oligonucleotides, looks attractive due to rigid structure allowing fast washings but is an almost unexplored support in the field of oligosaccharides.[7] For our comparative studies the first sugar unit, a suitable α-D-methylglucopyranoside[8] **1** (Scheme) was anchored to the solid supports through a succinic bridge involving the C-4 hydroxyl group of the sugar residue and the native amino functions of the resins.[9] Following the previously reported procedure[5] the obtained supports **2**, **3**, **4** were capped on the unreacted amino functions and deprotected at C-6 to afford **5**, **6** and **7**, respectively.[10]



Suc = -CO(CH$_2$)$_2$CO- ; DMT = 4,4'-dimethoxytrityl ; T = Thymine

Scheme. **i)** DCC/Py; **ii)** a. capping: Ac_2O/Py; b.10% Cl_2HCCO_2H/CH_2Cl_2; **iii)** a. promoter (see Table); b. 32% aq. ammonia; **iv)** Ac_2O/Py; **v)** a. tetrazole, CH_3CN; b. I_2; c. capping: Ac_2O/Py; d. 10% Cl_2HCCO_2H/CH_2Cl_2; **vi)** a. TMSOTf; b. 32% aq. ammonia, 60 °C.

Polymer bound glycosyl acceptor **5** (**6** or **7**) was reacted with the peracetylated (**8**) or perbenzoylated (**9**) glucosyltrichloroacetimidate in the presence of a Lewis acid ($BF_3{\cdot}OEt_2$ or TMSOTf (trimethylsilyltriflate)) as the glycosylation promoter.

In a typical experiment (see Table), 50 mg (8 μmol) of support **5** were swollen in CH_2Cl_2 under argon atmosphere and then treated with a 1:1 solution of CH_2Cl_2/cyclohexane (1 mL) containing **8** or **9**. After five minutes a suitable amount of the promoter (0.1 M solution in CH_2Cl_2) was added to the gently stirred suspension (the same procedure was adopted for 8 μmol of support **6** or **7**). After 3 hours the resin was filtered, washed with CH_2Cl_2 and CH_3OH and then treated with 32% aq. ammonia (5 h, r.t. for TG and CPG) or with a 1:1 solution of dioxane/32% aq. ammonia (5 h, 60 °C for PS). Detached products were acetylated and then analyzed by ^1H NMR ($CDCl_3$) in order to evaluate the glycosylaton yield by integrating the methoxy

protons signals of the monomer (δ = 3.41) and of the dimer **10b**[11] (δ = 3.39) having the expected β configuration at the produced glycosidic bond.

Table. Results of solid phase synthesis of **10a**

Entry	Support	Donor (eq)	Promoter (eq)	Yield %
1	**5**	**8** (25)	TMSOTf (5)	45
2	**5**	**8** (25)	$BF_3{\cdot}OEt_2$ (5)	60
3	**6**	**8** (25)	TMSOTf (5)	45
4	**6**	**8** (25)	$BF_3{\cdot}OEt_2$ (5)	35
5	7	**8** (50)	TMSOTf (1.2)	55
6	7	**8** (50)	TMSOTf (2.5)	65
7	7	**8** (100)	$BF_3{\cdot}OEt_2$ (2.5)	60
8	**5**	**9** (25)	TMSOTf (5)	50
9	**5**	**9** (25)	$BF_3{\cdot}OEt_2$ (5)	65
10	**6**	**9** (25)	TMSOTf (5)	90
11	7	**9** (25)	TMSOTf (2.5)	95

As illustrated in the Table, the most efficient activator of the peracetylated donor **8** resulted to be TMSOTf for glycosylations on PS **6** and CPG 7 supports (entries 3 and 6, respectively) and $BF_3{\cdot}OEt_2$ on the TG support **5** (entry 2). In addition, the use of the more stable[3] perbenzoylated glycosyl donor **9** resulted in a noteworthy increase of the coupling yields for PS and CPG resins **6** and 7 (over 90%, entries 10 and 11). Surprisingly, no remarkable improvement of the glycosylation yield was found for the TG resin **5** when imidate **9** was utilized (entries 8 and 9). The best result (95% yield, entry 11) was furnished by the CPG support which assures high coupling yields by using lower amounts of acidic promoter and not resorting to the iteration of the coupling reaction often required to increase solid phase glycosylation yields.[1a,1c,2a,5]

Owing to the observed favourable result provided by the CPG support and in view of our interest[12] in the synthesis of modified oligonucleotides with potential biological activity, we planned the possible use of 7 for the preparation of oligonucleotides functionalized at one or both the extremities with sugar units. These kinds of glycoconjugates are currently under investigation[13] for their potential properties as antisense reagents.[14]

In a preliminary experiment, 7 was used to prepare the hybrid trimer **13** (Scheme) employing both the phosphoramidite and the trichloroacetimidate methods. In this approach the polymer bound dimer **12** was quantitatively prepared reacting 5'-DMT-thymidine-3'-phosphoramidite **11** with 7 through an automated standard procedure.[6,15] The subsequent incorporation of the glucose unit was performed employing the imidate **9** according to the conditions of entry 11 (Table). The HPLC[16] analysis on the crude detached material indicated that the hybrid trimer **13** was obtained in an 88% yield. **13** was purified by HPLC and its structure was confirmed by ^{1}H NMR and FAB-MS.[17]

In conclusion, in this work CPG has been shown to act as a suitable support for the preparation of oligosaccharides and glyconucleotide conjugates. In addition, the decisive role often played by the protective groups of peracylated glycosyl trichloroacetimidates in carrying out high yielding and stereoselective solid-phase glycoside bond formation has been emphasized.

Work directed toward the preparation of longer glycoconjugates and oligosaccharides is currently underway.

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8. Prepared from commercially available 4,6-*O*-benzylidene-α-methyl-D-glucopyranoside through acetylation of 2-OH and 3-OH, removal of the benzylidene protecting group, regioselective dimethoxytritylation of 6-OH and succinylation of 4-OH (65 % overall yield).
9. Amino group loading of the supports: TG 0.22 mmol/g, PS (1% DVB) 1.18 mmol/g, CPG 30-35 μmol/g.
10. Obtained functionalizations: 0.14-0.18 mmol/g for **5**, 0.22-0.24 mmol/g for **6**, 25-30 μmol/g for **7**.
11. Isolated by silica gel TLC. Significative ^{1}H NMR (400 Mhz, $CDCl_3$) signals of **10b**: anomeric protons at δ 4.93 (1H, d, $J_{1,2}$= 3.6 Hz, α-Glc*p* H-1) and 4.56 (1H, d, $J_{1,2}$= 8.1 Hz, β-Glc*p* H-1); methoxyl protons at δ 3.39 (3H, s); AcO protons at δ 2.10, 2.07, 2.05, 2.03, 2.02, 2.01, 2.00 (singlets).
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15 The incorporation of the nucleotide was performed on a Millipore Cyclone Plus automated synthesizer.
16. HPLC analysis (UV detector, λ = 260 nm) and purification were performed on a reverse phase column (Whatman C18 partisphere, 12.5 x 0.5 cm, 5 μm); eluent: linear gradient (0-20% in 30 min) of CH_3CN in 0.1 M triethylammonium acetate, pH 7; flow 0.6 mL/min; retention time of **13**: 9.1 min.
17. Significant ^{1}H NMR signals of **13** (400 MHz, D_2O): δ 7.73 (1H, s, thymidine H-6), 6.38 (1H, t, $J_{1,2a} = J_{1,2b}$ = 6.5 Hz, thymidine H-1'), 4.95 (1H, m, thymidine H-3'), 4.54 (1H, d, $J_{1,2}$= 8.1 Hz, β-Glc*p* H-1), 3.44 (3H, s, -OCH_3), 1.92 (3H, s, thymidine 5-CH_3). FAB-MS 659 *m/z* $(M-H)^-$.