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Solid Phase Glycosidation of Oligonucleotides

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Abstract

CPG-bound nucleosides (deoxyguanosine excepted) can be stereoselectively glycosylated in high yield at their 5'-hydroxyl by a "disarmed" trichloroacetimidate donor in the presence of stoichiometric amounts of TMSOTf (5 eq.) and in short reaction times. These results allowed the solid-phase synthesis of an oligonucleotide functionalized at both ends with sugar residues.

Keywords: Solid-phase synthesis; Nucleosides; Oligonucleotides; Glycosidation.

Solid phase synthesis of oligosaccharides constitutes an important topic of modern carbohydrate chemistry. The importance of this approach, demonstrated by the increasing number of published contributions [1], is well established in the field of combinatorial chemistry [2]. Recently, we have reported [3], together with Schmidt [4], the convenient use of Controlled Pore Glass (CPG) as a matrix for solid phase glycosidations. Although in our hands successful glycosidations involving "disarmed" donors [5] were performed only by using stoichiometric amounts of acid promoter, we were able to accomplish efficiently on CPG the first preparation of a glyconucleotide conjugate (containing a residue of thymidine) by an "on-line" semiautomated solid phase approach. Thus, we have shown the potential of this support for the synthesis of glycoconjugates belonging to an interesting class of modified oligonucleotides possessing a promising and hitherto almost unexplored role as antisense [6] and antigene agents [7]. In this paper we wish to report further investigations aimed at establishing the scope and the limitations of this synthetic strategy. For this purpose we have investigated the compatibility of the other natural nucleosides to the conditions required to achieve glycosidation, being well aware of the lability of the N-glycosidic bonds under acidic conditions, mainly in the case of the purine nucleosides. Furthermore, we have tested the propensity of polymer-bound nucleosides to be glycosylated at their 5'-hydroxy function.

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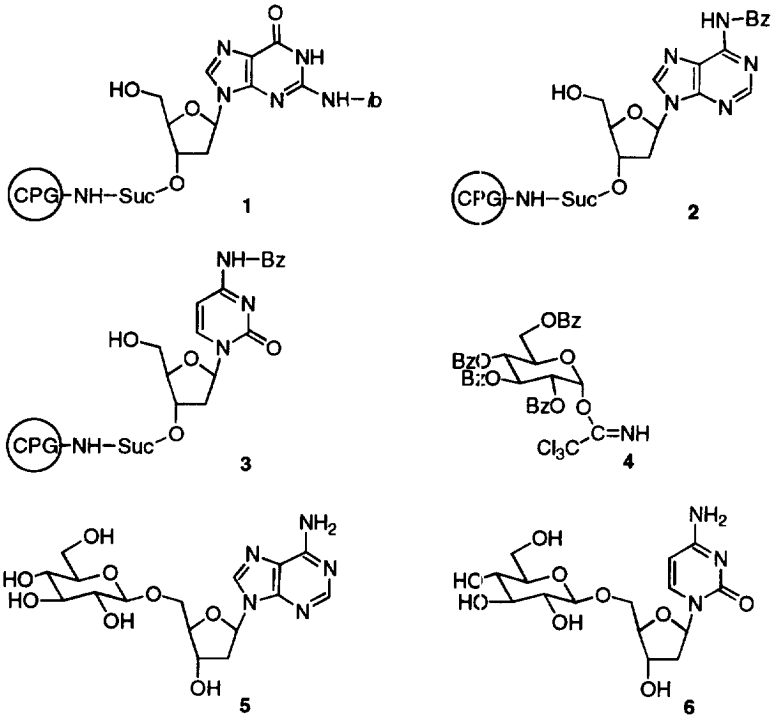
Chart



CPG: Controlled Pore Glass. Suc: -CO(CH$_2$)$_2$-CO-. *ib*: isobutyryl; Bz: benzoyl.

Firstly, acidic conditions which may be tolerated by the purine N-glycosidic bond were sought. We observed that the treatment of commercially available CPG-bound N^2-isobutyryl-2'-deoxyguanosine (dG) **1** (Chart) and N^4-benzoyl-2'-deoxyadenine (dA) **2** (Chart) with 2.5 equivalents of trimethylsilyl trifluoromethanesulfonate (TMSOTf), 25 eq of the imidate donor **4** (Chart) and 4Å MS in CH_2Cl_2/cyclohexane 1:1 for 3 hours or more resulted in a partial to complete depurination of the nucleosides as observed by NMR analysis of products detached from the resin (32% aq. ammonia, 6h, 60 °C). Interestingly, beside depurination, a high level of β-glucosylation was observed for the deoxyribose residue which had lost the base.[1] In an attempt to preserve the stability of the purine nucleoside the solid-phase glucosylations of **1** and **2** were performed in the presence of 5 equivalents of TMSOTf and shortening the reaction time to one hour. Under these conditions polymer bound dG **1** was still found to undergo depurination. On the other hand acceptor **2** turned out to be stable and, moreover, it was efficiently glycosylated, despite the decreased reaction time, yielding 81% of **5**[2] (Chart) after detachment and deprotection with 32% aq. ammonia. The observed greater stability of the

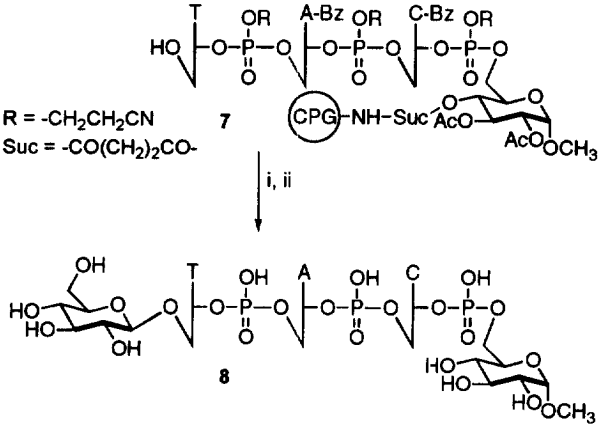
[1] An intense doublet at δ 4.50 (J = 8 Hz) displayed in the ^{1}H NMR spectrum of the detached products suggests that β-glucosylation of the deoxy ribose unit occurred extensively. However the structure of this undesired β-glucoside was not determined.

[2] Selected ^{1}H NMR data of **5**: δ 8.46 and 8.33 (2H, 2 x s, dA H-2 and H-8), 6.57 (1H, t, J = 6.3 Hz, dA H-1'), 4.43 (1H, d, J = 8.2 Hz, Glc*p* H-1), 4.35 (1H, m, dA H-3'), 2.69 and 2.60 (2H, 2 x m, dA H-2').

polymer-bound dA **2** with respect to the dG **1** was quite surprising the former usually being considered the most labile nucleoside under acidic conditions [8]. As expected polymer bound N^4-benzoyl-2'-deoxycytidine **3** was completely stable in the same conditions and was also efficiently glycosylated by the donor **4** providing **6**[3] (Chart) (83% yield) after detachment and deprotection. Use of less promoter (2-3 equivalents) for an hour resulted in a decrease of the glycosidation yields in all cases.

In the light of these results we decided to test the efficiency of this glycosidation approach in the synthesis of a longer conjugate containing dC, dA and thymidine (T).

Scheme 1-Synthesis of conjugate 8



i) **4** (25 eq), TMSOTf (5 eq), CH_2Cl_2/cyclohexane 1:1, 4Å MS, 1h, rt; ii) 32% aq ammonia, 60°C, 6h.

Thus the polymer bound 3'-glucotrinucleotide **7** (Scheme 1) was prepared through an automated standard phosphoramidite procedure [9] starting from CPG suitably functionalized with a protected methyl α-glucopyranoside [3]. The hybrid **7** obtained was then submitted to the previously optimized glucosylation procedure with **4**. NMR and HPLC[4] analysis of the detached products indicated a 71% yield in the coupling step (without iteration) and once again no degradation products were detected.[5]

In summary we have shown that high yielding solid-phase glycosidation of nucleoside acceptors can be carried out in one hour in the presence of stoichiometric amounts of TMSOTf (5 eq.). The short reaction time should allow iteration of the coupling step in order to increase further the yields. Although this methodology appears still limited by the incompatibility of deoxyguanosine to the conditions required to achieve successful glycosidations, it could already

[3] Selected ^{1}H NMR data of **6**: δ 7.96 and 6.10 (2H, 2 x d, $J_{5,6}$ = 7.6 Hz, dC H-5 and H-6), 6.34 (1H, t, J = 6.5 Hz, dC H-1'), 4.58 (1H, m, dC H-4'), 4.56 (1H, d, J = 8.0 Hz, Glc*p* H-1), 4.27 (1H, m, dC H-3'), 2.51 and 2.40 (2H, 2 x m, dC H-2').

[4] HPLC analysis (UV detector , λ = 260 nm) and purification were performed on a reverse phase column (Whatman C-18 partisphere). 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 **7** (detached and deprotected): 19.8 min. Retention time of **8**: 18.8 min.

[5] Selected ^{1}H NMR data of **8**: δ 8.52 and 8.27 (2H, 2 x s, dA H-2 and H-8), 7.88 and 6.00 (2H, 2 x d, J = 7.4 Hz, dC H-5 and H-6), 7.53 (1H, s, thymidine H-6), 6.48, 6.37, 6.15 (3H, 3 x t, dA, dC and thymidine H-1'), 4.52 (1H, d, J = 8.0 Hz, β-Glc*p* H-1), 3.50 (3H, s, $-OCH_3$), 1.98 (3H, bs, thymidine 5-CH_3).

be profitably exploited for the preparation of biologically interesting oligonucleotides conjugated with sugars. In this regard, the synthesis of polypyrimidine oligonucleotides functionalized at the extremities with saccharidic residues is currently under way in our laboratory in order to evaluate their capability to form triplex structures [7].

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