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A Direct and Efficient Method for Derivatisation of Solid Supports for Oligonucleotide Synthesis

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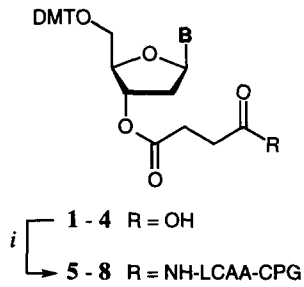
Abstract: A direct, efficient and general method which utilises commercially available coupling reagents has been developed for derivatisation of LCAA-CPG solid supports for oligonucleotide synthesis giving loadings of up to, and greater than, 50 μmol.g^{-1}.

Automated synthesis on LCAA-CPG[1] solid supports using nucleoside phosphoramidites[2] provides one of the most direct methods for the preparation of oligonucleotides and their PNA[3] chimeras[4] for biological applications. Protection strategies for the amino functions of the DNA bases and the phosphorus centres of oligonucleotide backbones attract sustained interest. Procedures designed to simplify the synthesis of modified solid supports have also been the focus of attention and a number of solid support derivatisation methods now exists[5a-j]. One of the most efficient of these which has been reported recently[6] involves attachment of commercially available nucleoside 3'-*O*-succinates to LCAA-CPG in the presence of TPP/DTNP as coupling agents.

Our interest in the synthesis of oligonucleotides for antisense and antigene applications prompted us to investigate a method of general applicability to maximise nucleoside loadings on LCAA-CPG solid supports relying on commercially available coupling reagents and without the need for rigorously anhydrous conditions. The solid support derivatisation method which we describe here[7] is both direct and efficient involving treatment of LCAA-CPG with a solution of the appropriate nucleoside-3'-*O*-succinate[8] containing DCC, DhbtOH and DMAP in DMF. Derivatised solid supports **5** to **8** were thus prepared from 5'-*O*-DMT-protected nucleoside 3'-*O*-succinates **1** to **4** (Figure 1). Any unreacted amine groups on the solid support were then capped through acetylation following the standard procedure[5j]. This method gives excellent loadings of between 49 and 54 μmol.g^{-1} as determined by the standard DMT assay procedure[5j] (Figure 1). The likely mechanism involved in the DCC/DhbtOH solid support derivatisation method presumably involves attachment of the nucleoside 3'-*O*-succinate, firstly to DCC, followed by transformation of this adduct into an active ester on reaction with DhbtOH with concomitant displacement of DCU. Reaction of the intermediate nucleoside 3'-*O*-succinate Dhbt active ester with LCAA-CPG attaches the nucleoside succinate to the solid support on formation of an amide bond. Excellent coupling yields were obtained when the derivatised solid



	Succinate B =		Loading/ μmol.g^{-1}
1	G^{iBu}	5	50
2	C^{Bz}	6	53
3	A^{Bz}	7	54
4	T	8	49

Figure 1. Solid support derivatisation using nucleoside 3'-*O*-succinates **1** to **4**. Reagents and conditions: *i* DCC, DhbtOH, DMAP, H_2N-LCAA-CPG, DMF, 24 h, room temperature.

supports **5** to **8** were employed in automated synthesis of oligonucleotides **9** to **12** using phosphoramidite chemistry[8,9] (Table 1). Treatment of the solid supported oligonucleotides with NH_3 (aq) at 55 °C during 16 h facilitated removal of protecting groups and detachment of the oligonucleotide products **9** to **12** from the solid supports. The oligonucleotide sequences **9** to **12** were then subjected to reversed-phase HPLC analysis[10] and gave retention times (t_Rs) of between 15.5 and 16.5 min (Table 1). Co-injection of **9** to **12** made from commercially available LCAA-CPG solid supports[8], with **9** to **12** made from supports produced by the DCC/DhbtOH method, showed the respective sequences made by both methods to be identical. In order to prove conclusively that no inadvertent chemical modifications had occurred during oligonucleotide synthesis, sequences **9** to **12** made using supports **5** to **8**, were analysed by negative ion electrospray mass spectrometry[11]. The molecular masses (M), masses of monosodium adducts (M+Na-H) and disodium adducts (M+2Na-2H, data not shown) were calculated from the mass-to-charge (m/z) ratios of the multiply charged ions observed and were consistently within ±0.01% of their expected values (Table 1). Using 5'-*O*-DMT-dA^{Bz}-3'-*O*-succinate **3** as a representative example, we examined the time course for the formation of derivatised LCAA-CPG solid support **7**. Figure 2 illustrates that solid support loadings of between 25 and

	Sequence	t_R/min	Species	Found	Calculated	ΔM
9	5'-dCGTTTAAG-3'	16.5	M	2423.59	2423.45	0.14
			M+Na-H	2445.54	2445.43	0.11
10	5'-dCGTTTAAC-3'	15.8	M	2383.76	2383.44	0.32
			M+Na-H	2405.70	2405.42	0.28
11	5'-dCGTTTAAA-3'	16.0	M	2407.68	2407.45	0.23
			M+Na-H	2429.68	2429.43	0.25
12	5'-dCGTTTAAT-3'	15.5	M	2398.70	2398.43	0.27
			M+Na-H	2420.74	2420.42	0.32

Table 1. Reversed-phase HPLC[10] and negative ion electrospray mass analysis[11] of oligonucleotides **9** to **12**.

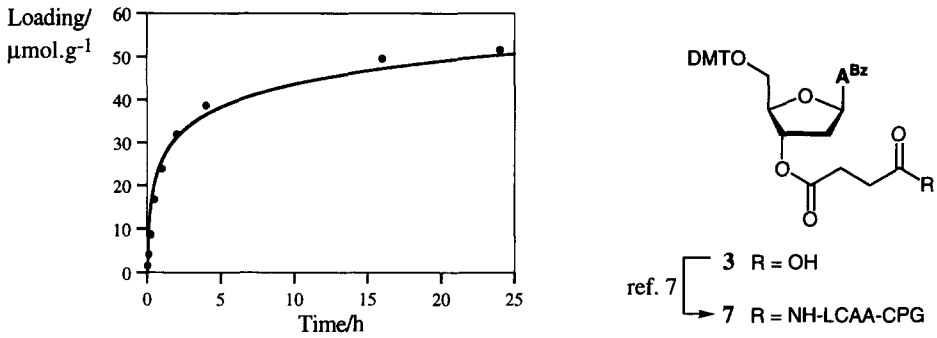


Figure 2. Time course of the derivatisation of LCAA-CPG solid support using 5'-*O*-DMT-dA^{Bz}-3'-*O*-succinate **3**.

30 μmol.g^{-1}, ideal for the synthesis of both medium and long oligonucleotides, were achieved within 90 min. If the derivatisation reaction was allowed to proceed further (15 to 24 h), excellent loadings of up to, and greater than, 50 μmol.g^{-1} could be achieved. Such high nucleoside loadings are ideal for synthesis of short and antisense oligonucleotides. We recently described synthesis[12] of a fluoride labile SLCPG silica solid support **17** and demonstrated its usefulness for the preparation of base labile oligonucleotides and their conjugates[13]. To illustrate the generality of the DCC/DhbtOH solid support derivatisation procedure, we exploited this method to improve upon our chemical synthesis of SLCPG. Succinate **13** was prepared by reacting 6-*O*-DMT-hexan-1,6-diol with succinic anhydride in the presence of DMAP. LCAA-CPG was then derivatised with succinate **13** using the DCC/DhbtOH coupling conditions[7] to give the modified solid support **14** with an excellent loading of 68 μmol.g^{-1} as determined by the standard DMT assay procedure[5j]. Brief treatment of **14** with 3% dichloroacetic acid in CH_2Cl_2 unmasked the primary hydroxyl function giving **15** which, on reaction with the known[14] triflate **16**, gave SLCPG **17** with a superior nucleoside loading of 40 μmol.g^{-1} on the solid support, compared to 27 μmol.g^{-1} from a longer, alternative route[13]. The DCC/DhbtOH solid support derivatisation method which we have described is both direct and efficient giving moderate to good solid support loadings within 90 minutes and excellent solid support loadings within 24 h.

Figure 3. Improved synthesis of SLCPG **17** (40 μmol.g^{-1}) via DCC/DhbtOH mediated coupling of **13** to LCAA-CPG (68 μmol.g^{-1}). Reagents and conditions: *i*, acetic anhydride, DMAP, 2,6-lutidine; *ii*, ref. 5j; *iii*, **15**, ref. 14.

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References and Notes

1. Abbreviations: DCC (*N,N'*-Dicyclohexylcarbodiimide), DCU (*N,N'*-Dicyclohexylurea), DhbtOH (3,4-Dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine), DMAP (4-Dimethylaminopyridine), DMF (Dimethylformamide), DTNP (2,2'-Dithiobis-(5-nitropyridine)), LCAA-CPG (Long Chain Alkyl Amine-Controlled Pore Glass), PNA (Peptidic Nucleic Acid), SLCPG (Silyl-Linked CPG), TEAA (Triethylammonium acetate), TPP (Triphenylphosphine).
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7. General method: To LCAA-CPG (200 mg) was added a solution of the appropriate succinate (70 μmol), DCC (21 mg, 102 μmol), DMAP (14 mg, 120 μmol) and DhbtOH (16.7 mg, 102 μmol) in DMF (4 mL) and the mixture shaken gently overnight. After 24 h the product solution was filtered and the solid support washed successively with DMF (5 x 1.5 mL), methanol (5 x 1.5 mL) and diethyl ether (5 x 1.5 mL). Any unreacted amino groups were capped by the standard acetylation procedure[5j].
8. Nucleoside-3'-*O*-succinates (dG^{iBu}, dC^{Bz}, dA^{Bz}, dT) and LCAA-CPG silica were purchased from the Sigma Chemical Company. Cyanoethylphosphoramidites (dG^{iBu}, dC^{Bz}, dA^{Bz}, dT) and conventional, derivatised LCAA-CPG supports were purchased from Cruachem.
9. User's Manual for Beckman 1000 DNA Synthesiser, 1994.
10. Gradient elution HPLC, with flow rate 1 $mL.min^{-1}$, was performed using an HPLC Technology C18 Reversed Phase Column (250 x 4.6 mm) with solvent system A mixed with solvent system B (0-40%) during 20 min, then with B (60%) during a further 10 min. A was composed of 1 M aqueous TEAA (10%) and MeCN (2%) at pH 7.0 and B was composed of 1 M aqueous TEAA (10%) and MeCN (80%) at pH 7.0.
11. Negative ion electrospray analysis was performed on Hewlett-Packard HP 5989B MS Engine apparatus using a HP 59987A API-Electrospray LC/MS interface with oligonucleotide samples dissolved in weakly basic NH_3 (aq) solution.
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