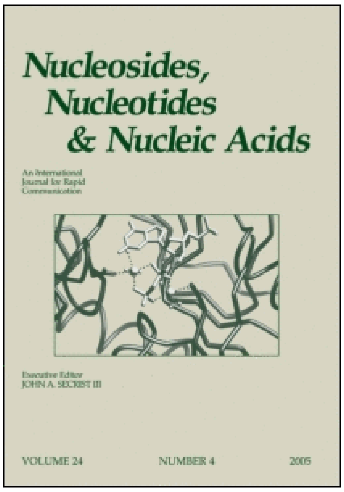

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PREDICTABLE AND REPRODUCIBLE YIELDS IN THE ANCHORING OF DMT-NUCLEOSIDE-SUCCINATES TO HIGHLY LOADED AMINOALKYL-POLYSTYRENE RESINS

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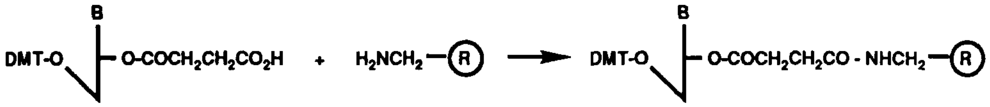
Abstract. Coupling of DMT-nucleoside-succinates[1] onto aminomethyl-polystyrene has been fine tuned in order to allow the preparation of DMT-nucleoside-resins with the desired substitution degree in reproducible yields.

The increasing demand for oligonucleotides in order to study their application as therapeutic agents[2] as well as for biological and structural studies has prompted many laboratories to develop or optimise methodologies for the large scale production of such biopolymers. Our research group has directed its efforts at making the phosphite triester approach compatible with polystyrene solid supports[3] and two different companies have recently developed instruments for the large scale synthesis of oligonucleotides on polystyrene-derived matrixes[4]. Polystyrene has the advantage over the commonly used controlled pore glass that it allows much higher loadings to be obtained and therefore higher concentrations but lower excesses of the phosphoramidites can be used to reduce the cost of the synthesis[5]. For this purpose, we have been interested in the preparation of DMT-nucleoside-resins of different and precise substitution degrees in order to evaluate their usefulness in the large-scale solid-phase synthesis of oligonucleotides.

Commercially available aminoalkyl-polystyrene resins are usually too highly loaded (0.5-1.5 mmol/g), and therefore only part of the available polymeric amino groups must be functionalised with the corresponding DMT-nucleoside-succinate. We have found that when a carboxylic acid derivative (either a DMT-nucleoside-succinate, a peptide or an amino acid derivative) is coupled onto an aminoalkyl-resin using an excess of amino groups with respect to the carboxylic acid, only part of the acid becomes anchored to the support and that yields vary from one experiment to another. We decided to undertake a systematic study of the coupling of DMT-nucleoside-succinate onto the solid matrix (which has often been claimed to be long and tedious[6,7]) in order to attempt to find a

Table 1. Extent of functionalisation of polymeric amino groups using different amounts of DMT-T-succinate and coupling reagents. (0.1 g of H_2N-R containing 0.66 mmol NH_2/g resin, 1.5 mL of solvent, 2 h, r.t.)



	Molar ratio of reagents					% reacted amino groups
Entry	DMT-T-succ.	DCC	HOBt	DMAP	H_2N-Ⓡ	(% reacted nucleoside)
1	0.125	0.125	-	-	1	2 (16)
2	0.25	0.25	-	-	1	8 (32)
3	0.35	0.35	-	-	1	12 (36)
4	0.50	0.50	-	-	1	27 (54)
5	0.75	0.75	-	-	1	37 (50)
6	0.125	0.125	0.125	-	1	4 (36)
7	0.25	0.25	0.25	-	1	12 (48)
8	0.35	0.35	0.35	-	1	21 (61)
9	0.50	0.50	0.50	-	1	35 (70)
10	0.75	0.75	0.75	-	1	50 (67)
11	0.25	0.25	0.25	0.125	1	19 (76)
12	0.25	0.25	0.25	0.25	1	8 (32)
13	0.50	0.50	0.50	0.25	1	32 (65)
14	0.50	0.50	0.50	0.50	1	33 (66)
15	0.75	0.75	0.75	0.375	1	69 (92)
16	0.75	0.75	0.75	0.75	1	20 (27)

straightforward method for accomplishing this. We also have to set up the reaction conditions under which DMT-nucleoside-resins can be obtained with a predictable substitution degree and in a reproducible way, without having to remove aliquots of resin at different reaction times until the desired loading (f, mmol DMT/g resin) is obtained[8,9].

We have carried out the anchoring of 5'-DMT-thymidine-3'-succinate onto aminomethyl-poly(styrene-*co*-1%-divinylbenzene) in the presence of either i) DCC ii) DCC/HOBt or iii) DCC/HOBt/DMAP. All experiments were carried out with *ca.* 100 mg of aminomethyl-polystyrene (Novabiochem, f=0.66 mmol NH_2/g resin[10]), in 1.5 mL of solvent[11] (DCM or a 14/1 mixture of DCM/DMF when HOBt was used) for 2 hours at room temperature. Results are presented in Table 1.

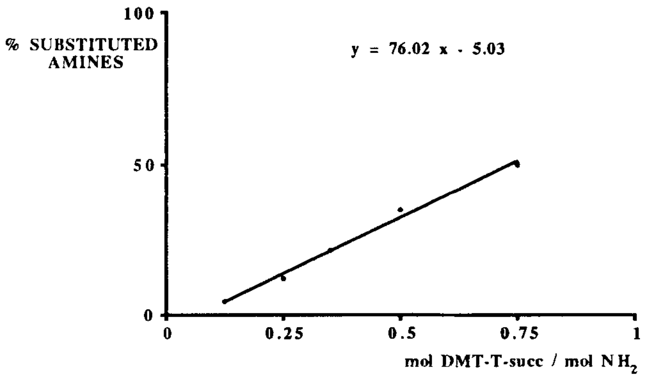


Figure 1. Linear relationship obtained in DCC/HOBt-mediated couplings between the percentage of reacted amines and the ratio mol nucleoside/mol NH_2-R.

The main conclusions that can be drawn from the above data are the following:

i) Both DCC and DCC/HOBt mediated couplings show a linear increase in yield with the amount of DMT-nucleoside-succinate used but, for a given amount of nucleoside, yields are better with DCC/HOBt (entries 6 to 10) than with DCC only (entries 1 to 5).

ii) When couplings are carried out with DCC/HOBt/DMAP (entries 11 to 16), yields are generally worse when DMAP is present in equimolar amount with respect to the other reagents than when it is present in half-molar ratio (compare entries 11-12, 13-14 and 15-16) but, although the yields are sometimes better than with DCC/HOBt, they do not show a linear increase with the amount of nucleoside used. Moreover, the data in Table I do not reflect the fact that it was difficult to obtain reproducible yields in couplings carried out with DMAP under the same experimental conditions.

iii) It should be emphasised that when using a smaller ratio of nucleoside with respect to polymeric amines, not only the amount of reacted amines is obviously smaller, but there is also a decrease in the yield of anchored nucleoside with respect to the amount used. In other words, the smaller the ratio of nucleoside/polymeric amines, the lower the effective utilisation of the nucleoside reagent used. These results may be related to the fact that when using smaller amounts of reagents their concentrations also decrease since the reaction volume is kept constant.

In summary, we consider that the best choice is to use DCC/HOBt for the anchoring of DMT-nucleoside-succinates since reasonably good yields and reproducible results are obtained. Under the described conditions the extent of functionalisation of the resin expressed as percentage of reacted amines is linearly dependent on the amount of DMT-nucleoside-succinate/DCC/HOBt used as shown in Fig.1.

Table 2. Comparison between desired and experimental results obtained in the preparation of different DMT-nucleoside-resins using DCC/HOBt as coupling reagents (0.81 mmol NH_2/g resin, DCM/DMF 14:1, 15 mL solvent/g resin, 2 h, r.t.).

Nucleoside	mol nucl./mol NH_2-Ⓡ	% reacted amines		f (mmol DMT/g resin)	
		desired	obtained	desired	obtained
T	0.225	13	14	0.10	0.11
G^{ibu}	0.31	18	16	0.15	0.13
C^{bz}	0.39	25	25	0.20	0.20
T	0.45	29	27	0.24	0.22
C^{bz}	0.58	40	42	0.33	0.34

The definitive test of the method was to see whether using the linear relationship shown in Fig.1 were we able to prepare DMT-nucleoside-resins with the desired extent of loading, starting from another commercial polystyrene resin (Sigma, 0.81 mmol NH_2/g resin), using larger amounts of support (up to 0.5 g) for preparative purposes and anchoring not only thymidine derivatives but also other nucleosides. Results are summarised in Table 2.

These results confirm that we have been able to fine-tune the experimental conditions for the anchoring of any DMT-nucleoside-succinate to aminomethyl-polystyrene in order to allow the desired loadings of DMT-nucleoside-resin to be obtained within 10% error.

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REFERENCES

(1) Abbreviations: DMT : 4,4'dimethoxytrityl; DCC : dicyclohexylcarbodiimide; HOBt : 1-hydroxybenzotriazole; DMAP : 4-dimethylaminopyridine; DCM : dichloromethane; DMF : dimethylformamide; Fmoc : 9-fluorenylmethoxycarbonyl

(2) Uhlman, E.; Peyman, A. *Chem. Revs.* **1990**, *90*, 543-584.

(3) Bardella, F.; Giralt, E.; Pedroso, E. *Tetrahedron Lett.* **1990**, *31*, 6231-6234.

(4) Communications presented at the "International Conference on Nucleic Acid Medical Applications", Cancun (México), January 26-30 (1993), by L. Holmberg (Pharmacia LKB, Sweden) and P. Wright and A. Andrus (Applied Biosystems Inc., USA).

(5) Montserrat, F.X.; Eritja, R.; Grandas, A.; Pedroso, E.; manuscript in preparation.

(6) Pon, R.T.; Ussman, N.; Ogilvie, K.K. *BioTechniques* **1988**, *6*, 768-775.

(7) Damha, M.J.; Giannaris, P.A.; Zabarylo, S.V. *Nucleic Acids Res.* **1990**, *18*, 3813-3821.

(8) Ito, H.; Ike, Y.; Ikuta, S.; Itakura, K. *Nucleic Acids Res.* **1982**, *10*, 1755-1769.

(9) Coupling yields were determined by spectrophotometric quantitation of the dimethoxytrityl cation released after acidic treatment of a sample of DMT-nucleoside-resin, as described by Atkinson, T. and Smith, M. *Oligonucleotide Synthesis: A Practical Approach*, Gait, M.J. editor, IRL Press, Oxford, 1984; pp 48.

(10) The substitution degree of commercial aminomethyl-resins was redetermined by carrying out a quantitative coupling (until a negative ninhydrin test was obtained) of an Fmoc-amino acid onto the resin (10 fold excess of amino acid and DCC in DCM/DMF, 1h, r.t.) and determining spectrophotometrically the amount of 9-fluorenylmethylpiperidine formed after piperidine treatment of an aliquot of Fmoc-amino acid-resin (see Grandas, A.; Jorba, X.; Giralt, E; Pedroso, E. *Int. J. Peptide Protein Res.* **1989**, *33*, 386-390).

(11) This is the volume of solvent that is needed to properly swell the resin without unnecessarily diluting the reagents.

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