# A New Base-stable Linker for Solid-phase Oligonucleotide Synthesis

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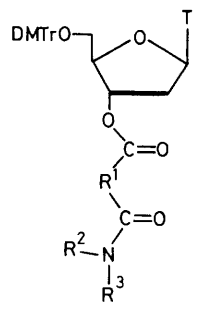
The succinyl-sarcosyl linker (**1b**), which is stable to prolonged contact with 1,8-diazabicyclo[5.4.0]undec-7-ene but cleaves rapidly with aqueous ammonia, is of potential value in solid-phase oligonucleotide synthesis.

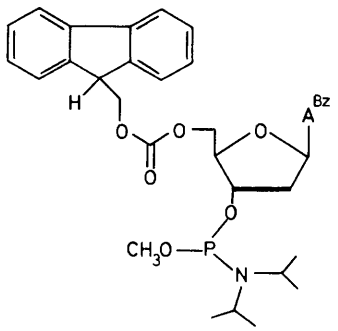
The DMTr† group is not ideal for the protection of the 5′-hydroxy function of deoxyribose in solid-phase DNA synthesis[1,2] as acidic deprotection at successive cycles gives rise to depurination, particularly at deoxyadenosine residues.[3,4] This is not a serious problem in small scale synthesis as the acidic treatment is very brief (*ca.* 30 s) but it is a limiting factor in large-scale work when a deprotection time of around 3 min is necessary. In addition, some uncommon nucleosides such as 2-amino-deoxyadenosine are acutely sensitive to acid-catalysed depurination and cannot be used routinely.[3] When the 5′-DMTr-group is used in RNA synthesis in conjunction with acid-labile THP 2′-OH protection, it has been shown that repeated TCA treatment leads to significant loss of THP and subsequent 3′ to 2′ phosphoryl migration.[5]

Clearly there is a need for an alternative 5′-hydroxy protecting group for certain applications in oligonucleotide synthesis. Recent advances[6,7] indicate that the 5′-FMOC group can be removed by brief treatment in a suitable base, offering the possibility of a completely acid-free protocol. Unfortunately, the linker conventionally used to attach the oligonucleotide to the support matrix (**1a**) is unstable to DBU and 1% cleavage occurs at each FMOC-deprotection step.[6]

† *Abbreviations*: DMTr = 4,4′-dimethoxytrityl; FMOC = 9-fluorenylmethoxycarbonyl; LCAA = long chain alkylamino; CPG = Corning porous glass (controlled pore glass); DBU = 1,8-diazabicyclo(5.5.0)undec-7-ene; DMAP = 4-dimethylaminopyridine; dT = thymidine; $A^{Bz}$ = $N^6$-benzoyladenine; THP = tetrahydropyran-2-yl; DCCI = dicyclohexylcarbodiimide; TCA = trichloroacetic acid.



(1)



(2)

| | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| a | $-CH_2CH_2-$ | Me | LCAA·CPG |
| b | $-CH_2CH_2-$ | Me | $CH_2CONH$·LCAA·CPG |
| c | $-C_6H_4$-*o*- | H | $CH_2CO_2Et$ |
| d | $-CH_2CH_2-$ | H | $CH_2CO_2Et$ |
| e | $-CH_2CH_2-$ | Me | $CH_2CO_2Et$ |
| f | $-CH_2CH_2CH_2-$ | H | $CH_2CO_2Et$ |

Piperidine and morpholine can be used as alternatives,[7] but although (**1a**) is more stable to these, FMOC-deprotection is much slower. The obvious incompatibility of base-labile 5′-protecting groups and (**1a**) prompted us to design a resin–oligonucleotide linker that is resistant to DBU. We have found that the linker (**1b**) suffers less than 5% cleavage after overnight treatment with a 10% solution of DBU in dry dichloromethane or toluene.‡ Under the same conditions scission of (**1a**) is complete in less than 1 h, presumably owing to a mechanism involving deprotonation of the amide nitrogen followed by intramolecular nucleophilic displacement at the ester carbonyl group. Although (**1b**) is stable to DBU, it is hydrolysed in less than 1 h at room temperature by concentrated aqueous ammonia, the standard reagent for removal of an oligonucleotide from the solid support.

The properties of the linkers were demonstrated using the model compounds (**1d**), (**1e**), and (**1f**) where reactions could be conveniently monitored by t.l.c. The ester group of (**1d**) is cleaved by 10% DBU in dry dichloromethane in 5 min whereas under the same conditions (**1e**) is essentially unchanged after 24 h and compound (**1f**) is cleaved slowly (5 h). Cleavage of (**1f**) can be assumed to proceed by an intramolecular cyclisation and the formation of a 6-membered ring. An analogous mechanism for (**1d**) produces a 5-membered ring (an *N*-substituted succinimide) and will therefore take place more readily.§ Additional support for this mechanism comes from studies on the phthaloyl linker (**1c**). When the solid support is treated with 10% DBU, liberation of the nucleoside is extremely fast owing to the rigid conformation imposed by the aromatic ring favouring the intramolecular reaction (50% cleavage in 3.5 min). Hydrolysis of the phthaloyl linker with concentrated aqueous ammonia is relatively slow (35 min).

‡ The resin was functionalised as follows: FMOC-sarcosine (10 equiv.) and DCCI (5 equiv.) were added to Pierce long-chain alkylamino CPG in a mixture of DMF and dichloromethane. Removal of the FMOC-group with piperidine in DMF followed by coupling of the sarcosine methylamino group to 5′-DMTr thymidine 3′-*O*-succinate (10 equiv.) in the presence of DCCI (5 equiv.) gave a loading of 20 mequiv. of 5′-DMTrdt/g of dry resin. (DMTr cation assayed colorimetrically at 490 nm).

§ Compounds (**1d**), (**1c**) and (**1f**) were characterised by f.a.b. mass spectrometry, $^1$H n.m.r., and elemental analysis.

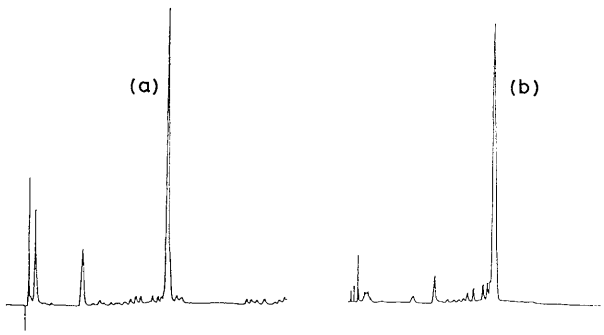



**Figure 1.** Reverse-phase h.p.l.c.: (a) $d(A)_8$DMTr; (b) $d(A)_8$ FMOC.

Solid-phase DNA synthesis¶ was carried out using the linker (**1b**) and 5′-DMTr-β-cyanoethyl phosphoramidite monomers. The oligonucleotides produced were indistinguishable in terms of purity and yield from those synthesised using linker (**1a**) (Partisil-SAX ion-exchange and C8-reverse-phase h.p.l.c. analysis).

We have demonstrated the value of the sarcosyl linker in conjunction with the 5′-FMOC protected monomer (**2**) by synthesising $d(A)_8$.‖ The reverse-phase h.p.l.c. chromatograms of the crude product and a sample of $d(A)_8$ prepared by standard methodology (Figure 1) indicate that both methods produce excellent quality DNA. The choice of capping agent in the FMOC cycle is crucial, as acetic anhydride and DMAP produce some cleavage of the FMOC group. Replacement of DMAP with *N*-methylimidazole gives cleaner synthesis but the best results were obtained using trimethylsilyl chloride (1 min).[8] A more serious problem occurs when unprotected thymidines are exposed to DBU. Deprotonation of the N(3) atom occurs, giving rise to a rapid reaction with the adjacent phosphotriester and the formation of an *N*(3)-methyl thymine residue. Thus, if $d(T)_6$ is prepared by standard methods using the 5′-DMTr-dT methoxy phosphoramidite and the linker (**1b**), and the fully protected resin-bound oligonucleotide is treated with 10% DBU in dichloromethane for 10 min, after which time it is liberated from the resin with aqueous ammonia, a mixture of several products is obtained. It can be shown by reverse-phase h.p.l.c. that authentic $d(T)_6$ is only a minor component. Interestingly, anion-exchange h.p.l.c. failed to separate the mixture and the single peak was shown to coelute with authentic $d(T)_6$. The base-promoted methylation of thymidine has been reported previously by Jones.[9] Thymine base protection should clearly be employed in the 5′-FMOC DNA synthesis protocol. A number of suitable protecting groups have been developed.[10]

In conclusion, we suggest that the resin-oligonucleotide linker (**1b**) in combination with 5′-FMOC or similar base-labile protecting groups will provide a viable acid-free protocol for solid-phase oligonucleotide synthesis. The value of this combination in RNA synthesis has recently been demonstrated.[11]

¶ Solid-phase oligonucleotide synthesis was carried out on an Applied Biosystems 380B DNA-synthesiser, using the standard 0.2 μmol β-cyanoethyl or methyl phosphoramidite cycle. The following sequences were prepared using oligonucleotide-resin-linkers (**1a**) and (**1b**) and DMTr monomers.

$d(T)_{15}$
d(GAAGAACTGTAGAGTCGGT)
d(TCGACAGTTCAGATCGCGAT)
$d(A)_8$

‖ 10% DBU in dichloromethane was used to replace TCA but otherwise the standard methoxy-phosphoramidite cycle was used.

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