

# Synthesis of ferrocene-modified oligonucleotides using a ferrocene-dT phosphoramidite

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## Introduction

Ferrocene (Fc) and its derivatives are attractive electrochemical probes for nucleic acid analysis because of their stability and convenient synthetic chemistry. Early examples of Fc labelling have utilised the conjugation of carboxy-Fc to 5'-amino-modified oligos.<sup>1</sup> Internal post-synthetic labelling of DNA probes has been obtained by reaction with ferrocenecarboxaldehyde or aminoferrocene.<sup>2</sup> For direct incorporation into oligonucleotides, Fc phosphoramidites<sup>3</sup> and monomers with a ferrocenyl moiety linked to position 5 of 2'-dU<sup>4(a)</sup> and dC<sup>4(a,b)</sup> or the 2' sugar position of dA and dC<sup>5</sup> have been described, as has on-column derivatisation of I-dU with ferrocenyl propargylamide.<sup>6</sup> Methods using redox tagging have also been employed.<sup>7</sup>

Recently Brisset and co-workers<sup>8</sup> have described the synthesis and use of abasic Fc-modified phosphoramidites, including the preparation of Fc-modified phosphorothioates<sup>9</sup> however, to our knowledge, these are not commercially available. In any case, reported coupling efficiencies and oligo synthesis yields are relatively low.

FRIZ Biochem have published details of a chip-based electrochemically detected DNA assay on gold electrodes incorporating multiple Fcs.<sup>10</sup> This is based on signalling oligos (20-70mers) with four Fc moieties at the 5' end. These are presently made by an on-column derivatisation process whereby, after synthesis of the oligo sequence, four Fmoc-protected amino-modifier amidites are added. The Fmoc groups (and the phosphate protecting groups) are cleaved by DBU and dimethylamine and the ferrocene acetic acid is coupled to the partially deprotected oligo using HBTU as coupling reagent (2-3h). This process, however, does have drawbacks. Aside from the inconvenience of the post-synthetic methodology, yields are relatively low and depend strongly on the length of the sequence. The process conditions are harsh and are also very susceptible to moisture.

To provide a robust phosphoramidite for direct incorporation into oligos we chose a structure (item **2167**) analogous to our current dT products (amino, dabcyll, biotin, fluorescein etc). This both simplifies its synthesis and imparts the benefits of having a nucleobasic structure consistent with natural DNA-sugar-phosphate backbone. Further,

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3 (a) M. Wiessler and D. Schutte, European Patent WO9709337 (1997); (b) T.Chunlin, US Patent Application US2009/0155795 A1 (2009).

4 (a) **Uridine-conjugated ferrocene DNA oligonucleotides: Unexpected cyclization reaction of the uridine base**, C.J. Yu, H. Yowanto, Y. Wan, T.J. Meade, Y. Chong, M. Strong, L.H. Donilon, J.F. Kayyem, M. Gozin and G.F. Blackburn, *J. Am. Chem. Soc.*, **122**, 6767-6768, 2000; (b) **Ferrocene-modified pyrimidine nucleosides: synthesis, structure and electrochemistry**, H. Song, X. Li, Y. Long, G. Schatte and H.-B. Kraatz, *Dalton Trans.*, 4696-4701, 2006.

5 **2'-Ribose-ferrocene oligonucleotides for electronic detection of nucleic acids**, C.J. Yu, H. Wang, Y. Wan, H. Yowanto, J.C. Kim, L.H. Donilon, C. Tao, M. Strong and Y. Chong, *J. Org. Chem.*, **66**, 2937-2942, 2001.

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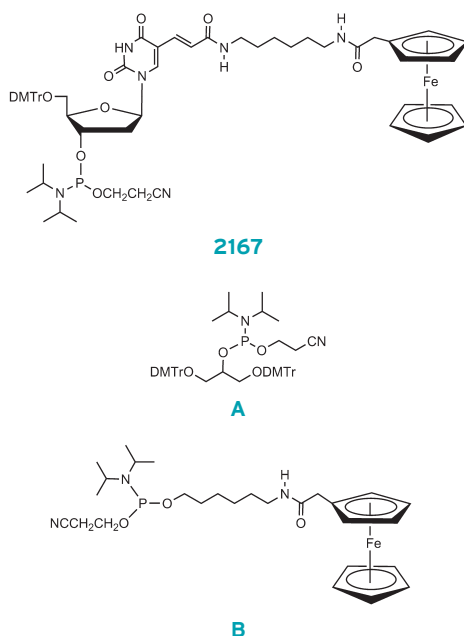
8 (a) **Automated synthesis of new ferrocenyl-modified oligonucleotides: study of their properties in solution**, A.E. Navarro, N. Spinelli, C. Moustrou, C. Chaix, B. Mandrand and H. Brisset, *Nucleic Acids Research*, **32**, 5310-5319, 2004; (b) **Supported synthesis of ferrocene modified oligonucleotides as new electroactive DNA probes**, A.-E. Navarro, N. Spinelli, C. Chaix, C. Moustrou, B. Mandrand and H. Brisset, *Bioorg. Med. Chem. Lett.*, **14**, 2439-2441, 2004; (c) C. Chaix-Bauvais et al, US Patent Application US2005/0038234 A1 (2005).

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as the Fc-modification is on the 5-position of the pyrimidine, natural base-pairing to dA will still take place.

Recently, FRIZ have tested this product along with an abasic alternative in comparison with their standard method. Firstly, the test oligo was modified at the 5'-end twice with a doubly-branching amidite (**A**), allowing four subsequent insertions of the Fc amidite (**B**). Secondly, the test oligo was directly modified at the 5'-end with four additions of the Fc-dT amidite.



## Experimental

A 31mer (GCG GTC GAC GTT CGG GGT GTA GCG GTC GTC G) was used as the test sequence. All syntheses were carried out on an ABI 394 on a 200nmol scale. 0.25M ETT Activator was used, as were Ac-dC and dmF-dG allowing for cleavage & deprotection with AMA at 65°C for 10min. The on-column derivatisation was carried out as described above.

100µl of 0.1M acetonitrile amidite solutions were used per coupling, using a coupling time for 600s for the branching method and 120s for Fc-dT. The latter was synthesised DMT OFF.

Preparative HPLC of the oligos were run on a ReproSil-Pur C18-AQ 5µm, 100 x 8mm column at 3ml/min, using solvent system A: 0.1M triethylammonium acetate, pH 7.3, B: 75% acetonitrile, 25% 0.1M triethylammonium acetate, pH 7.3 (the % B is shown in red in traces opposite).

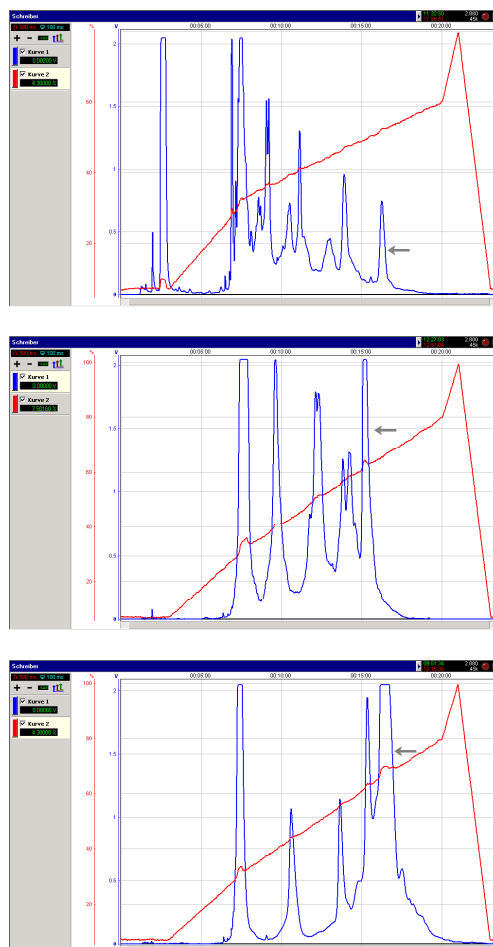
In the handling of ferrocene oligos, speed-vac times were kept short (no overnight drying) and the speed-vac vented with argon. Aqueous solutions were also purged with argon to remove oxygen.

## Results & Discussion

For each of the three methods under scrutiny, the preparative HPLC traces are shown opposite.

The amount of modified oligonucleotide (in nmol) obtained after HPLC was calculated as thus:

Method	Quantity/nmol
On-column	6.5
Brancher	17.2
Fc-dT	42.4



Preparative HPLC traces of oligos prepared by on-column derivatisation (top), branching amidite (middle), and Fc-dT (bottom). The product peak is marked with an ←.

It is clear that in using the ferrocene-dT amidite a significant increase in yield was obtained.

For initial quality monitoring, we have also measured the coupling efficiency of the Fc-dT, using a simple FCT<sub>6</sub> sequence with a 15min coupling time, as >99% by HPLC.

The above results suggest that use of Fc-dT is the best method to incorporate several ferrocene moieties into an oligonucleotide, not only regarding the overall yield, but also when considering its ease of use in comparison with both the complexity of synthesis and handling of competing methods.

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## Further Information

For up to date ordering and protocol information please see [www.linktech.co.uk](http://www.linktech.co.uk), e-mail us at [sales@linktech.co.uk](mailto:sales@linktech.co.uk) or call +44(0)1698 849911.