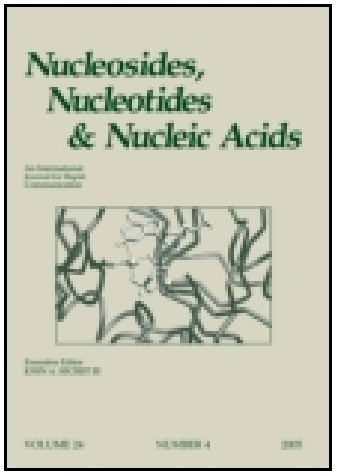

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A Biotin Phosphoramidite Reagent for the Automated Synthesis of 5′-Biotinylated Oligonucleotides

Zhengyun Zhao [a] & John Ackroyd [a]
[a] Cruachem Limited, Todd Campus, West of Scotland Science Park , Acre Road, Glasgow, G20 OUA
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A BIOTIN PHOSPHORAMIDITE REAGENT FOR THE AUTOMATED SYNTHESIS OF 5'-BIOTINYLATED OLIGONUCLEOTIDES

Zhengyun Zhao and John Ackroyd*

Cruachem Limited, Todd Campus, West of Scotland Science Park, Acre Road, Glasgow G20 0UA

Abstract A bis(DMTr)biotin phosphoramidite containing serine and 6-aminohexanol moieties was prepared by a multiple-step reaction, and used successfully in the solid phase synthesis of 5'-biotinylated oligonucleotides.

The use of biotin labelled oligonucleotides as a valuable tool in molecular biology is primarily due to the strong binding between biotin and avidin or streptavidin, it has consequently been used as a reporter group.[1] Chemically this type of oligonucleotide can be readily achieved by a so-called «biotin derivatised phosphoramidite» on machine aided synthesis.[2-7] Here we wish to report an alternative bis(DMTr) biotin phosphoramidite with serine and a six carbon chain as a linker.[8] The distance of the potential extension from biotin is an 8-carbon chain, which may be useful for applications involving affinity selection of nucleic acid and protein complexes.[9] The second DMTr group introduced on biotin N-1 position greatly increases the solubility of the molecule in organic solvents such as acetonitrile.

The synthesis of this biotin amidite (**9**), see FIG 1, was commenced from commercially available biotin (**1**) which was reacted with *N*-hydroxysuccinimide (NHS) (1.2eq.) in the presence of *N,N'*-dicyclohexylcarbodiimide (DCC) (1.2eq.) in DMF to yield biotin NHS ester (**2**) in 75% yield.[10] The ester **(2)** was then coupled with D,L-serine methyl ester (1.1eq.), diisopropylethylamine (DIPEA) (1.1eq) functioning as an HCl scavenger, in

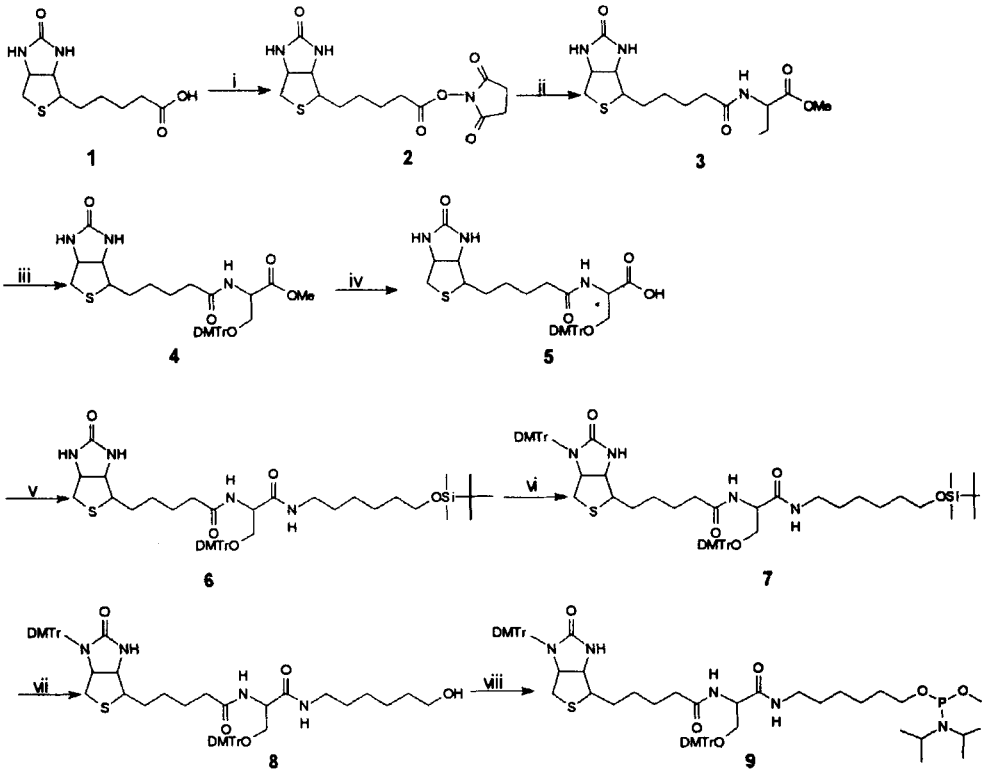

FIG 1 i. NHS, DCC, DMF ii. D,L-serine methyl ester hydrochloride, DIPEA, DMF iii. DMTr-Cl, pyridine iv. (a) 1N aq. NaOH/MeOH. (b) 1N aq. AcOH v. 6-amino-tBDMS-hexanol, DEC, pyridine vi. DMTr-Cl, DMAP, pyridine vii. 1M TBAF/THF viii. 2-cyanoethyl bis(diisopropylamino)phosphine and disopropylammonium tetrazolide

DMF to yield *N*-biotinyl serine methyl ester (**3**). Without isolation, the crude material (**3**) was tritylated with 4,4'-dimethoxytrityl chloride (DMTr-Cl) (2eq.) in pyridine to give *N*-biotinyl-*O*-DMTr-serine methyl ester (**4**) in 72% yield starting from (**2**).

Hydrolysis of (**4**) by treatment with 1N aqueous sodium hydroxide solution (2 eq.) in methanol, followed by careful neutralisation with 1N aqueous acetic acid (2 eq.), yielded the corresponding acid (**5**) in 94%. Condensation of (**5**) with 6-amino-tBDMS-hexanol[2] (2 eq.) was carried out in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide

(DEC) (2eq.) in pyridine to provide *N*-biotinyl-*O*-DMTr-6-tBDMS-hexanol serine amide (**6**) in 61% yield.

The further tritylation of (**6**) with DMTr-Cl (2.4eq.) and dimethylaminopyridine (DMAP) at elevated temperature gave rise to N-1 tritylated product *N*-(*N*-1-DMTr-biotinyl)-*O*-DMTr-6-tBDMS-hexanol serine amide (**7**) in 86% yield.[2] The desilylation was carried out with 1M tetrabutylammonium fluoride (TBAF) (5eq.) in THF for 1 hour to yield the alcohol *N*-(N-1-DMTr-biotinyl)-*O*-DMTr-6-hydroxyhexyl serine amide (**8**) in 86% yield.

Finally, phosphitylation by a modification of the general method of Caruthers[11] with 2-cyanoethyl bis(diisopropylamino)phosphine (1.5eq) and diisopropylammonium tetrazolide (0.5eq.) as a catalyst in dichloromethane (DCM) produced the title phosphoramidite compound (**9**) in 73% yield. A 0.1M solution of (**9**) in acetonitrile was used on a Cruachem PS250 DNA synthesiser for the preparation of 5'-Biotin-T_7 with trityl-off option on a 0.1 μmol scale. The coupling efficiency was 97.4% judged by trityl cation released. Further experiments are underway to assess the product as a multi-biotin label of oligonucleotides. There is also an opportunity for the chemistry to be extended to the preparation of analogous amidites where serine is replaced by other amino acids.

EXPERIMENTAL

Bis(DMTr) Biotinylserine CEPA (**9**)

2-Cyanoethyl bis(diisopropylamino)phosphine (27.1g, 90mmol) and diisopropylammonium tetrazolide (5.16g, 30mmol) were added to (**8**) (62.1g, 60mmol) in dry DCM (500ml) and the mixture was stirred at r.t. (2h). The reaction mixture was washed with saturated aqueous $NaHCO_3$ (2x500ml), followed by NaCl (2x500ml). The organic layer was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was redissolved in DCM (100ml) and precipitated into hexane (2x2000ml). The residue was then purified by a short silica gel column eluted with 1-2% MeOH/DCM, after evaporation and freeze drying to yield the title product (**9**) 54.0g as a foam (72%).

^{31}P n.m.r. ($CDCl_3$): 147.1(s), ^1H-nmr ($CDCl_3$) δ1.08-1.25(19H, m), δ1.43-1.57(6H, m, $3CH_2$), δ1.96-2.16(4H, m, $2CH_2$), δ2.26-2.33(2H, m), δ2.50(2H, m, CH_2S), δ3.00-

3.07(4H, m), δ3.28-3.58(6H, m, $3OCH_2$), δ3.65-3.67(12H, m, $4OCH_3$), δ4.21-4.30(2H, m, 2CHN), δ4.65(1H, m, NCHCO), δ5.97(1H, d, NH), δ6.51(1H, dd, NH), δ6.64(8H, m, ArH), δ6.86(1H, t, NH), δ7.03-7.32(18H, m, ArH). MS 1234.7 (M^+-1) (FAB) (1235.55).

REFERENCES

1 E. A. Bayer and M. Wilchek, *Methods Biochem Anal.*, **1980**, *26*, 1-45.

2 R. T. Pon, *Tetrahedron Lett.*, **1991**, *32*, 1715-1718.

3 K. Misiura, I. Durrant, M. R. Evans and M. J. Gait, *Nucleic Acids Res.*, **1990**, *18*, 4345-4354.

4 A. M. Alves, D. Holland and M. D. Edge, *Tetrahedron Lett.*, **1989**, *30*, 3089-3092.

5 A. J. Cocuzza, *Tetrahedron Lett.*, **1989**, *30*, 6287-6290.

6 J. Olejnik, E. K. Olejnik and K. J. Rothschild, *Nucleic Acids Res.*, **1996**, *24*, 361-366.

7 U. Pieles, B. S. Sproat and G. M. Lamm, *Nucleic Acids Res.*, **1990**, *18*, 4355-4360.

8 Peninsula Laboratories Inc. produces a similar compound, however there are no published procedures.

9 S. M. L Barabino, B. S Sproat, U. Ryder, B. J. Blencowe, and A. I. Lamond, *EMBO J.*, **1989**, *8*, 4171-4178.

10 E. A. Bayer and M. Wilchek, *Methods Enzymol.*, **1974**, *34*, 265-267.

11 A. D. Barone, J. Y. Tang and M. H. Caruthers, *Nucleic Acids Res.*, **1984**, *12*, 4051-4061.