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Development of New Nucleic Acid Photoaffinity Probes: Synthesis of 4-thiothymine Labelled Nucleoside Analogues

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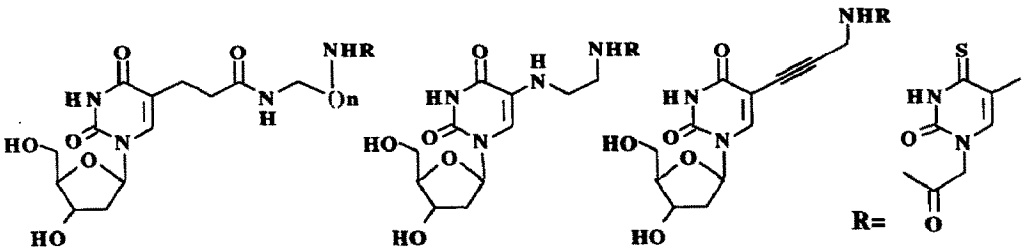
Key words: nucleoside, photoaffinity probe, nucleic acid, 4-thiothymine

Abstract: The new nucleic acid photoaffinity probes **1a**, **1b**, **2** and **3** in which a 4-thiothymine is linked at the end of a variable chain introduced at the C-5 position of deoxyuridine have been constructed.

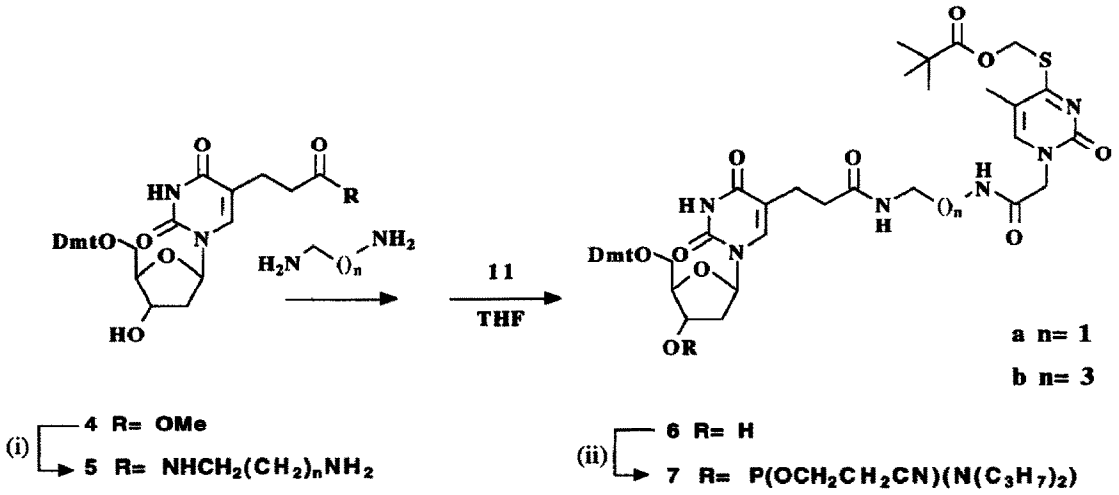
A variety of techniques can be used to obtain structural informations within nucleic acid assemblies. In this respect photoaffinity labelling is a method of choice to reveal tertiary interactions in such systems. The best results are obtained when using an intrinsic photoactivable probe the introduction of which provokes only minor structural perturbations. This probe should also exhibit high photoreactivity towards nucleotide residues and side reactions should be kept minimal[1].



Herein we propose the use of 4-thiothymine which was shown on simple models to undergo efficient photocross-linking reactions with nucleic acid bases[2]. As a

crosslinking reagent, 4-thiothymine offers several advantages: stability in the absence of light, selective excitation, labelling specificity.

We have designed a series of 2'-deoxyuridine derivatives (**1-3**) to be incorporated into oligonucleotides which we expect to offer a broad spectrum of potential applications. They contain a chain of variable length and flexibility at the C-5 position of this nucleoside. In each case a primary amine is present at the end of the chain to permit the attachment of *N*-1-carboxymethyl-4-thiothymine **8**. An interesting characteristics of such selectively photoactivable oligonucleotides containing **1-3** at variable positions resides in their potential capacity to hybridize to their complementary sequence with the probing residue located in the major groove of the corresponding helix.

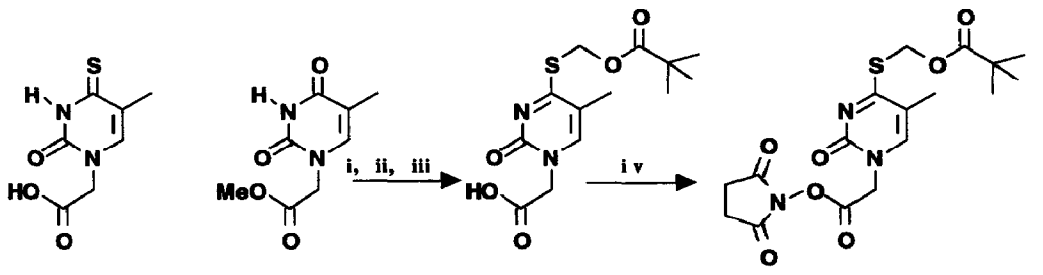


Scheme 1: (i) neat, r.t., overnight. (ii) 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite, *N,N*-diisopropylethylamine, CH_2Cl_2, r. t., 2-3h.

In order to allow their introduction into oligonucleotides by application of the standard solid phase procedure of DNA synthesis, the corresponding nucleosides were appropriately derivatized[3]. Accordingly, the 5'- and 3'-hydroxyl functions were dimethoxytritylated and phosphitylated, respectively whereas the thiocarbonyl group of 4-thiothymine was masked by *S*-pivaloyloxymethylation[4] to give phosphoramidites **7a**, **7b**, **13c** and **15c**.

Two routes were utilized to synthesize the desired nucleoside derivatives. In the cases of **7a** and **7b** (Scheme 1) we used a well established procedure to obtain the known methyl ester **4**[5] which in the presence of 1,2-diaminoethane or 1,4-diaminobutane gave the 5-substituted deoxyuridine derivatives **5a**[5] and **5b**, respectively. Then these nucleosides were combined in 60% yield with the active ester **11** which was best obtained *via* hydroxysuccinylation of **10**, starting from the readily available *N*-1-carboxymethylthymine **9**[6], when proceeding under the conditions

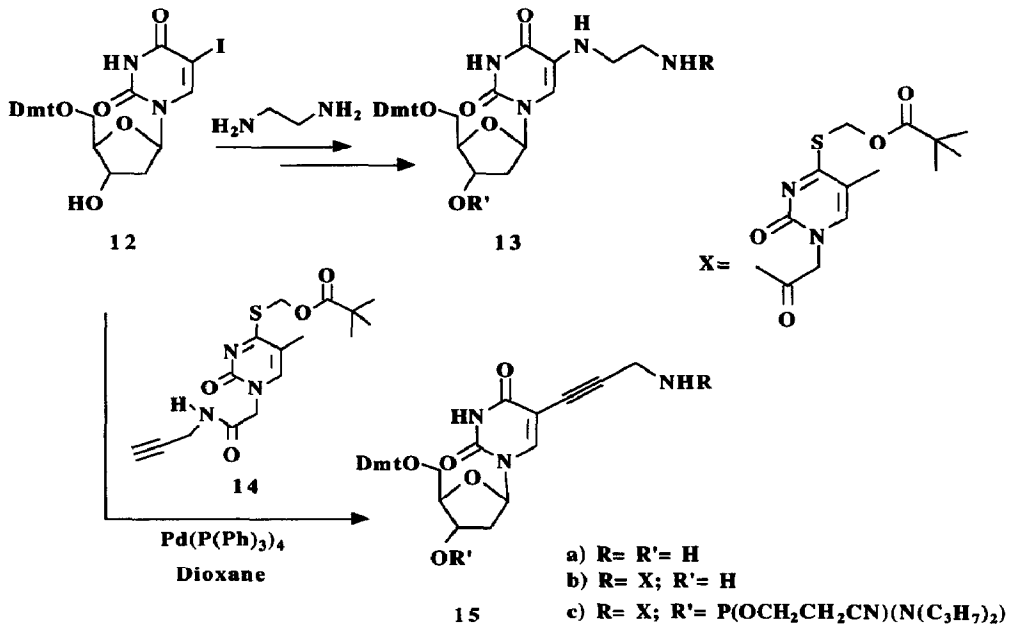
indicated in Scheme 2. The resulting nucleosides **6a** (FAB m/z 963, M+Na$^+$) and **6b** (FAB m/z 991, M+Na$^+$) were subsequently treated with 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite to prepare phosphoramidites **7a** (^{31}P δ: 149.5-149.3 ppm) and **7b** (^{31}P δ: 149.7-149.5 ppm).



8 **9** **10** **11**

Scheme 2: (i) P_2S_5, dioxane, reflux (81%). (ii) Aqueous NaOH, rt, (86%). (iii) chloromethylpivalate, K_2CO_3, H_2O, DME, rt, 48h (74%). (iv) N-hydroxysuccinimide, DCC, THF.

Compounds **13c** and **15c** were elaborated starting from 5'-*O*-dimethoxytrityl-5-iododeoxyuridine **12** (Scheme 3). Thus, the new derivative **13a** was obtained when a solution of **12** in 1,2-diaminoethane was kept overnight at room temperature.



12 **13** **14** **15**

Scheme 3

The latter was combined as above with the active ester **11** to provide **13b** (Yield: 50%; FAB m/z 907, M+Na$^+$) which finally was converted into phosphoramidite **13c** (^{31}P δ: 149.5-149.1ppm). A more convergent route was defined to obtain compound **15c**. Treatment of propargyl amine with **11** gave amide **14** which, using the palladium(0) coupling procedure described by Hobbs[7], underwent a Heck type reaction with **14** providing derivative **15b** (Yield: 45%; FAB m/z 902, M+Na$^+$). The latter gave the expected phosphoramidite **15c** (^{31}P δ: 149.6-149.3ppm) after chlorophosphoramidite treatment as above.

Phosphoramidites **7a-b**, **13c** and **15c** have served to introduce the corresponding modified nucleosides into oligonucleotide probes. These photoactivable agents are more particularly designed to be used to reveal tertiary interactions within the catalytically active conformation of some ribozyme domains[8] as well as in other biologically important nucleic acid structures. Interestingly the cross-link data to be gathered from these photochemical experiments are currently used to introduce constraints in molecular modelling of hammerhead ribozyme systems[9]. It is noteworthy that these two complementary approaches are required to reconstruct a plausible structure[10].

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References

1) Shuster, D. I.; Probst, W. C. ; Ehrlich, G. K.; Sing, G. *Photochem. Photobiol.* **1989**, *47*, 785-804. Hanna, M. M. *Methods Enzymol.*, **1989**, *180*, 383-409.

2) Clivio, P.; Fourrey, J-L.; Gasche, J.; Favre, A. *J. Am. Chem. Soc.* **1991**, *113*, 5481-5483 and unpublished results from this laboratory. Favre, A. in *Bioorganic Photochemistry: Photochemistry and the Nucleic Acids*, Morrison H. ed, Wiley & Sons, New York **1990**, Vol 1, pp 379-425.

3) Reviews on functionalized oligonucleotides : Beaucage, S. L.; Rhadhakrishnan, P. I. *Tetrahedron*, **1993**, *49*, 1925-1963; *Ibid.*.**1993**, *49*, 6123-6194.

4) Clivio, P.; Fourrey, J-L., Gasche, J.; Audic, A.; Favre, A.; Perrin, C.; Woisard, A. *Tetrahedron Lett.* **1992**, *33*, 65-68. Clivio, P.; Fourrey, J-L., Gasche, J.; Favre, A. *J. Chem Soc , Perkin Trans.* **1992**, 2383-2388.

5) Dreyer, G. B.; Dervan, P. B. *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 968-972.

6) Compound **9** was prepared by methanol/H$^+$ treatment of the corresponding acid (Jones, A. S.; Lewis, P.; Withers, S. F. *Tetrahedron* **1973**, *29*, 2293-2296).

7) Selected examples of the Heck reaction in the 5-iodo-2'-deoxyuridine series: (a) Goodchild, J.; Porter, R. A.; Raper, R. H.; Sim, I. S.; Upton, R. M.; Viney, J.; Wadsworth, H. J. (b) *J. Med Chem.* **1983**, *26*, 1252-1257. Robins, M. J.; Barr, P. J. *J. Org. Chem.* **1983**, *48*, 1854-1862. (c) Hobbs Jr, F. W. *J. Org. Chem.* **1989**, *54*, 3420-3422. (d) Froehler, B. C.; Wadwani, S.; Terhorst, T. J.; Gerrard, S. J. *Tetrahedron Lett.* **1992**, *33*, 5307-5310.

8) Woisard, A.; Favre, A.; Clivio, P.; Gasche, J.; Fourrey, J-L. *J. Am. Chem. Soc.* **1992**, *114*, 1615-1617. Dos Santos, V. D.; Luiza, A-L.; Fourrey, J-L.; Favre, A. *Nucleic Acids Res.* **1993**, *21*, 201-207. Dos Santos, V. D.; Fourrey, J-L.; Favre, A. *Biochem. Biophys. Res. Commun.* **1993**, *190*, 377-385.

9) Laugaa, P. (personal communication).

10) Full experimental data and applications will be reported elsewhere.

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