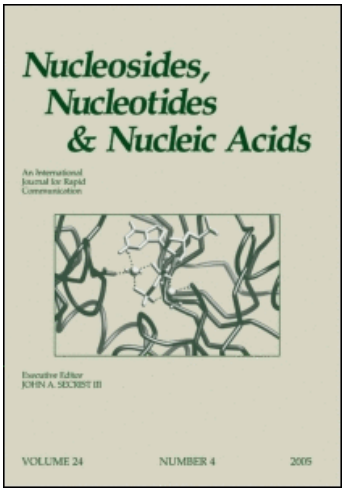

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γ-Aminobutyric Acid as Enzymolabile Groups for the Pro-oligonucleotide Approach

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Γ-AMINOBUTYRIC ACID AS ENZYMOLABILE GROUPS FOR THE PRO-OLIGONUCLEOTIDE APPROACH

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ABSTRACT : γ-aminobutyric acid derivatives were evaluated as biolabile protecting groups of the internucleoside backbone of a dimer phosphorothioate, in the context of the pro-oligonucleotide approach.

After the promising results of the pro-nucleotide approach using S-AcylThioEthyl (SATE) groups (1), we have developed the pro-oligonucleotide approach (2). However, we have noticed that the removal of the SATE groups by carboxyesterases was not efficient for the poorly soluble most lipophilic pro-oligonucleotides (3). That prompted us to investigate the possibility to introduce positive charges in pro-oligonucleotides to obtain, less lipophilic and more soluble compounds.

We evaluated γ-aminobutyric acid derivatives (**FIG.**) as new enzymolabile protecting groups of the phosphate backbone since these compounds have been shown to increase the biodisponibility of the butyric acid (4). The iodide derivatives (R-I) of the γ-trimethylaminobutyric and γ-aminobutyric acids were synthesized and used to alkylate the dithymidine phosphorothioate diester to give 1 and 2 with 50% and 65% yield respectively.

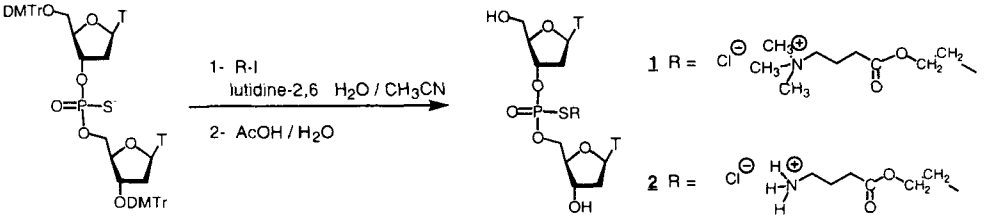

FIGURE : Synthesis of the phosphorothiolate triester 1 and 2.

TABLE : Half-lives of the phosphorothiolate triesters

	1	2	3
Human Serum	7.6 h.	2.8 h.	8 min.
Cell extracts	22.2 h.	15.1 h.	< 5 min.
Phosphate Buffer	26 h.	17 h.	7 h
Pig Liver Esterase	26 h.	10 h.	< 5 min.

Stability of 1 and 2 were studied by HPLC in human serum, cell extracts and with pig liver esterase (PLE) (**TABLE**). As a reference, we indicated the half-lives of the dithymidine phosphorothiolate triester 3 bearing a MeSATE group (R=CH3C(O)SCH2CH2-).

In each media, 1 and 2 were hydrolyzed in the corresponding dinucleoside phosphodiester which supports either a chemical or an enzymatic path of hydrolysis.

It is interesting to note that 1 and 2 are more stable than 3 in human serum by a factor of 55 and 20 respectively. However, the rate of decomposition of 1 and 2 is slow in cell extracts compare to the rate of decomposition of 3. Hydrolysis of 1 and 2 seems to take place *via* a chemical path. We investigated further their substrate character for carboxyesterases using a non specific enzyme, the pig liver esterase (PLE). The comparison of the half-lives data obtained for 1 and 2 in phosphate buffer and in presence of PLE indicates clearly that 1 is not substrate of these carboxyesterases while 2 is a poor substrate of these enzymes (**TABLE**).

Thus, 1 and 2 are neither good substrates of carboxyesterases, contained in cell extract nor with PLE. Although, these compounds are more stable than their SATE analogs in human serum. This property could be very useful in a chimeric pro-oligonucleotide, where the flanks would be composed of the corresponding 1 or 2, and the window would be a SATE protected phosphodiester or phosphorothioate diester. The envisioned advantage would be a dramatic increase in solubility of the pro-oligonucleotides.

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