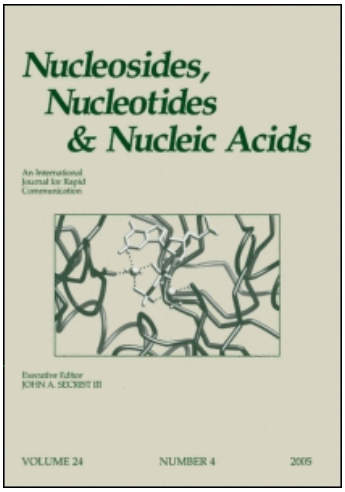

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A New Model for the Comparison of the Efficiency and Selectivity of Photoreactive Groups Towards the Nucleic Acid and Protein Functional Groups

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A NEW MODEL FOR THE COMPARISON OF THE EFFICIENCY AND SELECTIVITY OF PHOTOREACTIVE GROUPS TOWARDS THE NUCLEIC ACID AND PROTEIN FUNCTIONAL GROUPS

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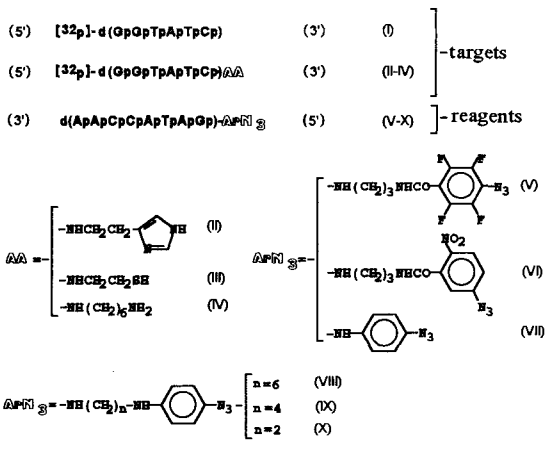
Abstract: A new model for the comparison of the efficiency and selectivity of photoreactive groups towards the nucleic acid and protein functional groups is described. It was shown that reagents carrying *p*-azidoaniline residue are the most efficient and selective for the modiication of the side radicals of amino acids.

Chemical modification, especially affinity labeling is the most powerful tool to study molecular organization of complicated supramolecular structures which at present can't be studied by powerful physical method such as X-ray crystallography and NMR spectroscopy. Among chemical reagents photoreactive groups are most attractive since they open the possibility to be carried out in the millisecond range of the events thus promising to be useful for the experimental study of the molecular dynamics of the processes in such systems. However, the chemistry of photomodification especially for proteins is poorly studied [1].

As a first step to deeper understanding of chemistry of protein photomodification we have designed a special model permitting to compare the efficiency of different photoreactive groups towards side radicals typical of amino acids within proteins. For this goal photoreactive group and side radical were attached, respectively, to 5'- and 3'-phosphate of two complementary oligonucleotides the formers serving as reagents, and the latters as the targets. In the duplex formed by the reagent and the target reacting moiety are drawn together thus raising the efficiency of reaction. The derivatives used are presented in the scheme. The binding was carried out



using the approach elaborated by one of the authors based upon activation of the terminal phosphate with the mixture of triphenylphosphine and dipyridyldisulfide in the presence of N,N-dimethylaminopyridine. The oligonucleotide derivative of the latter may be isolated and used as an efficient phosphorylating reagent for respective amines [2, 3]. The targets were labelled by traditional reaction with [γ-^{32}P] ATP in the presence of polynucleotide kinase. The reaction mixture contained 10^{-5}M of both oligonucleotide derivatives in 0.16M NaCl, 0.02M Na_2HPO_4, pH=8.9. Irradiation was carried out by the filtered light of the mercury lamp at 300-365nm. The irradiated samples were subjected to anion exchange chromatography in 7M urea to provide dissociation of the duplexes. In all cases the appearance of new products with the retention time exceeding that of the starting target was observed. These products are considered as the result of cross-linking. Their yields were estimated as the ratio of radioactivity of the respective fractions to the total radioactivity.

The results of cross-linking are presented in the table.

Targets & reagents	The covalent adducts yields, %					
	(V)	(VI)	(VII)	(VIII)	(IX)	(X)
(I)	23	0	0	0	0	0
(II)		24			25	
(III)	26	22	63	40	68	62
(IV)				67	43	

Considering the data presented it should be taken into account that according to our previous data [4] reagent (V) cross-links with the target lacking any additional residue and the reaction level contains both the reaction with oligonucleotide moiety and amino acid side radical. It is seen that p-azidaniline residue is the most efficient for the modification of the side radicals of amino acid and respective derivatives may be recommended for modification of proteins and protein components of supramolecular sructures.

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