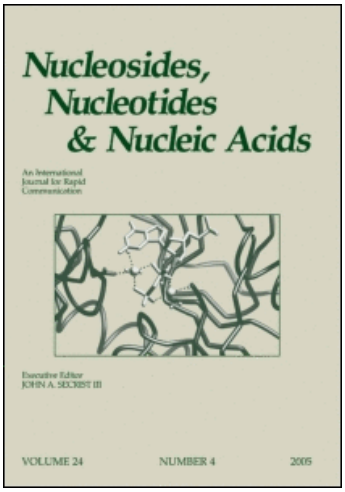

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A Novel Approach to Sequence Specific Cross-Linking in Oligonucleotides

R. Cosstick[a]; M. E. Douglas[a]
[a] The Department of Chemistry, The University of Liverpool, Liverpool, U.K.

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A NOVEL APPROACH TO SEQUENCE SPECIFIC CROSS-LINKING IN OLIGONUCLEOTIDES

R. Cosstick * and M.E. Douglas
The Department of Chemistry, The University of Liverpool, Liverpool L69 3BX . U.K.

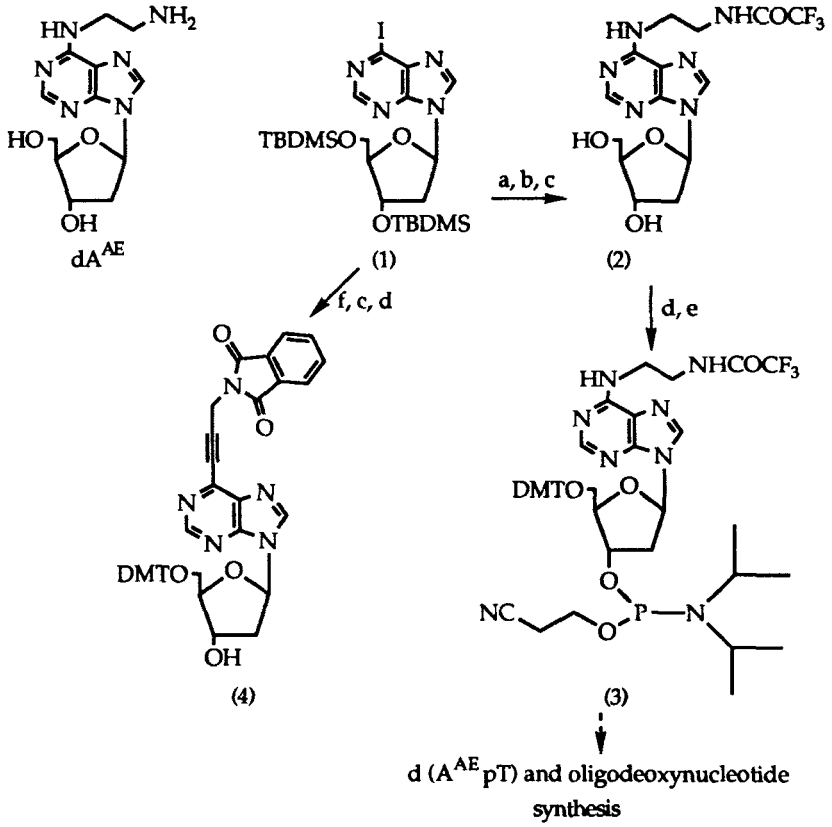
Abstract. The incorporation of 6-*N*-(2-aminoethyl)-2'-deoxyadenosine (dA^{AE}) into a dinucleotide using a phosphoramidite intermediate is described. The study serves as a model investigation for the incorporation of (1) into oligodeoxynucleotides to examine their potential for forming sequence specific interstrand cross-links.

Recently we have become interested in the synthesis of stabilised nucleic acid duplexes in which the two strands are "stapled" together at predetermined sites through a covalent interstrand cross-link. These "stapled" oligonucleotides would appear to have several useful and interesting applications such as: (i) stabilising cruciforms and other thermodynamically unfavourable structures; (ii) to discern the effects of a site specific cross-link on the processing of DNA (the efficacy of bifunctional alkylating agents such as 2-haloethylnitrosoureas as anticancer drugs against L1210 leukemia correlates with the extent of DNA cross-linking[1]) and (iii) the synthesis of highly stable oligonucleotide duplexes that could be used in affinity chromatography. There are comparatively few published studies on sequence specific cross-linking. However, notable work has been reported by Webb and Matteucci[2] which involves the incorporation of 4-*N*,4-*N*-ethanodeoxycytidine into an oligodeoxynucleotide as an electrophilic base analogue that can cross-link to an opposing deoxycytidine residue. More recently Pieles *et al.*[3] have developed a cross-linking procedure which uses oligodeoxynucleotides derivatised with a psoralen intercalator which is able to undergo a cycloaddition reaction with a neighbouring pyrimidine residue in the opposite strand.

Our approach to sequence specific cross-linking relies on the incorporation of dA^{AE} into an oligodeoxynucleotide so that upon hybridisation to a target DNA or RNA sequence the nucleophilic amino group is brought into close proximity with a pyrimidine residue in the complementary strand. Bisulphite which is known to add across the 5,6-double bond in pyrimidines activates the 4-position to nucleophilic attack by the amino function to bring about cross-linking. Detailed studies have revealed that only single stranded regions of nucleic acids undergo reaction with bisulphite and since, for example, a cytosine base opposite a 6-*N*-

modified adenine base cannot be involved in a Watson-Crick hydrogen bond it is predicted that only this specific cytosine residue would be activated.

The synthesis of a suitably protected phosphoramidite derivative of dA^{AE}, starting from the 6-iodopurine nucleoside (1), is shown below. The use of palladium coupling chemistry to prepare the intermediate (4) potentially enables the incorporation of nucleosides in which a 3-aminopropyl group is attached directly to the 6-position of the purine through a C-C bond.



Reagents: a; ethylenediamine, triethylamine, CH_3CN, Δ: b; $CF_3COSC_2H_5$, CH_3OH, : c; tetrabutylammonium fluoride, THF: d; DMTCl, 4-(dimethylamino)pyridine, triethylamine, pyridine: e; 2-cyanoethyl-*N*,*N*-di-isopropylaminochlorophosphine, *N*,*N*-di-isopropylethylamine, CH_2Cl_2: f; *N*-(propargyl)phthalimide, $(Ph_3P)_2PdCl_2$, CuI, sodium acetate, CH_3OH, Δ. TBDMS = t-butyldimethylsilyl; DMT = 4,4'-dimethoxytrityl.

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