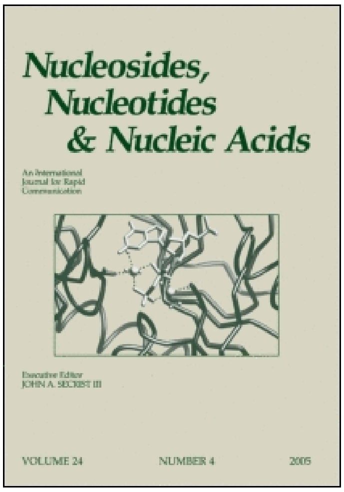

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Artur Burdzy[a]; Bohdan Skalski[a]; Ewa Biał[b]; Andrzej Kowalewski[a]; Stefan Paszyc[a]; Rys2ard W. Adamiak[b]
[a] Faculty of Chemistry, Adam Mickiewicz University, Poznań [b] Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland

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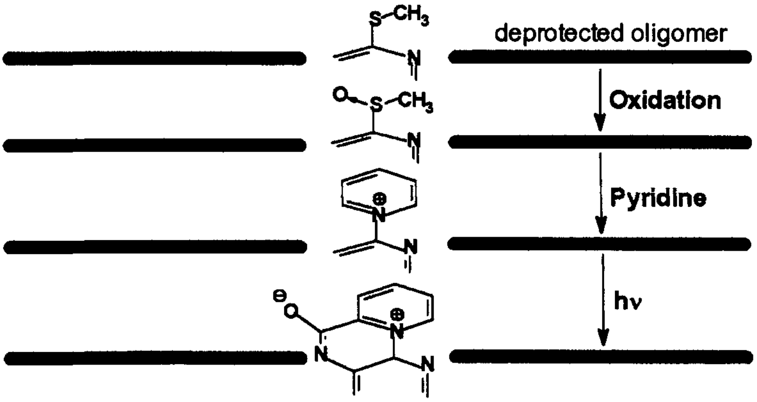
POSTSYNTHETIC TRANSFORMATIONS OF OLIGODEOXYNUCLEOTIDES ORIGINATED AT 6-METHYLTHIO-PURINE SITE

Artur Burdzy[1], Bohdan Skalski[1], Ewa Biała[2], Andrzej. Kowalewski[1],
Stefan Paszyc[1] and Ryszard.W. Adamiak*[2],
[1]*Faculty of Chemistry, Adam Mickiewicz University, Poznań,*
[2]*Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland.*

Abstract : New route to oligodeoxynucleotides labeled with fluorescent luminarine was explored. Regioselective oxidation of 6-methylthio-purines to 6-methylsulphoxides reactive toward pyridine was achieved. Upon UV irradiation of 6-pyridinium-purines oligonucleotide cleavage instead of phototransformation to luminarine was observed.

Since years we have been interested in postsynthetic reactions of oligonucleotides[1,2] which appeared to be an attractive route to requested modifications in RNA and DNA fragments. In this communication the sequence specific introduction of highly fluorescent luminarine fluorophore[3] *via* regioselective photochemical transformation of oligodeoxynucleotide containing 6-pyridinium purine residue was



SCHEME

further[4] explored (SCHEME). Since the 6-pyridinium residue is difficult to form and isolate by selective O^6-phosphorylations of respective hypoxanthine lactam system[5] on oligomer level we have decided to start from 6-methylthiopurine site which after oxidation should deliver 6-methylsulphoxide species reactive toward pyridine.

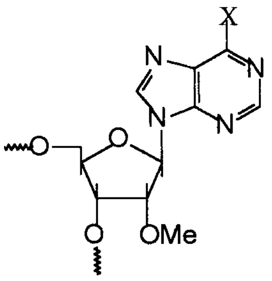
RESULTS AND DISCUSSION

Preparation of substrates. 6-Methylthiopurine riboside is stable towards both standard iodine oxidation and ammonolysis conditions to be applied during oligonucleotide synthesis and deprotection. 3'-(2-Cyanoethyl, N,N-diisopropylamino-phosphoramidite derived from 5'-O-DMT,2'-O-methyl-6-methylthiopurine riboside was prepared[6] and applied for the solid-phase synthesis of model oligodeoxynucleotides **1a-6a**.

1: T (6X-Pu) T
2: C (6X-Pu) C
3: G (6X-Pu) G
4: A (6X-Pu) A
5: (6X-Pu) T
6: CTCCT (6X-Pu) TCCCT

7: AGGGAAAGGAG

a: X=MeS
b: X=MeS→O
c: X=Pyr+



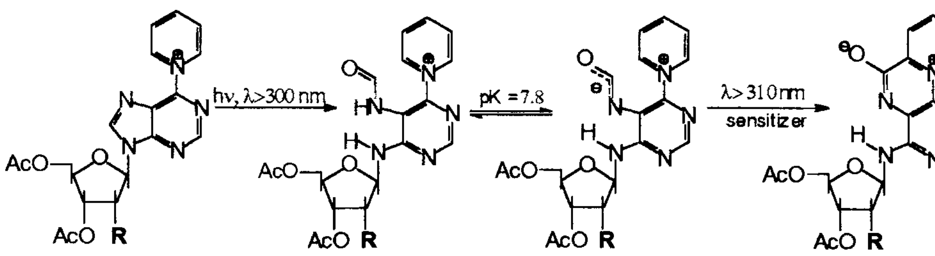
2'-O-Methyl group was chosen to facilitate further experiments in the ribo series. 11-Mer of sequence AGGGAAAGGAG (**7**) complementary to **6a** was also prepared. All oligomers were obtained using trityl-on protocol, deprotected and purified by HPLC[7] and desalted on Sephadex G10.

Oxidation of 6-methylthiopurines into 6-methylsulphoxides on oligomer level. Action of several oxidants operating in the aqueous medium on trimers **1a-4a** was checked in order to obtain the respective 6-methysulphoxides with no side-reactions on other nucleobases. Aqueous bromine previously used for oligopurine oligomers[8] as the oxidation reagent led to partial bromination of pyrimidine residues. Magnesium salt of monoperoxyphtalic acid (Aldrich) appeared to be the oxidant of choice. Oligonucleotides **1a-6a** were treated with aqueous solution of this reagent at final concentrations 2×10^{-3}M. In all cases transformation into 6-methylsulphoxides **1b-6b** was quantitative using substrate/oxidant molar ratio 1:1 and within 2 hr at room temp., as checked by HPLC - (see TABLE)[9].

In the case of trimers **2b** and **4b**, both diastereoisomers of 6-methylsulphoxide species were clearly separated. Larger excess of oxidizing reagent transforms both diastereoisomers into one product unreactive towards pyridine, most probably related 6-methylsulphone. All oligonucleotides **1b-6b** were enough stable to withstand HPLC isolation on Resolve C18 column and desalting. Interestingly, oxidation, although less efficient, takes place also in the duplex formed *via* association of **6a** and **7**. Using 4 molar excess of magnesium salt of monoperoxyphtalic acid after 3 hrs in 10°C the 40% conversion to methyl sulphoxide species was detected by HPLC.

Nucleophilic substitution of 6-methylsulphoxide group with pyridine. On monomer level 6-methylsulphoxide group is substituted with pyridine under anhydrous conditions with low yield forming dark-brown mixtures. However, when 1,2,4-triazole was added in equimolar (to substrate) amount, the reaction proceeds very efficiently forming related 6-pyridinium derivative with high yield. In aqueous solutions the best results have been obtained using 80% aq. pyridine carefully acidified to pH 6.7 with HCl aq. Oligonucleotides containing 6-methylsulphoxide residue **1b-6b** react with pyridine under this conditions with formation of blue-fluorescent derivatives **1c-6c** (λEm=433.5nm) bearing 6-pyridinium group in almost quantitative yield. **1c-6c** were isolated by HPLC on Resolve C18 column and Sephadex G10 desalting. Duplex formed from HPLC pure 6-metylsulphoxide-oligomer **6b** and **7** when treated with 60% aq. pyridine (pH of reaction mixture adjusted to 6.7) at 10°C for 24 hr led to 6-pyridinium substituted oligomer in 30% yield as checked by Zincke reaction UV test[5].

Light induced transformations of **1c-6c**. Photoreactivity of 6-pyridinium-purine residues on monomer level with formation of highly fluorescent nucleosides of luminarosine group (λEm=525nm)[3,10,11] was subjected to detailed mechanistic studies[12].



R= H, OAc, OMe, OThp

Those results prompted us to investigate the photochemical reactivity of 6-pyridinium purine residues on oligonucleotide level for sequences **1c-6c** and duplex **6c+7** as an attractive complementary route to applying the 3'-phosphoramidite of 2'-O-methyl-luminarosine as building block[11] for oligonucleotide synthesis.

Oligonucleotides **1c-6c** have been subjected to UV irradiation above 310 nm and under conditions optimized for various monomeric 6-pyridinium salts. In all cases no characteristic rise of yellow-green fluorescence of luminarine derivatives was detected by HPLC with fluorescence detection. The cleavage of oligonucleotides was observed instead. In case of trimers **1c-4c** an initial reaction mixture contained modified dimer(s) of unknown structure bearing no blue-fluorescence characteristic for 6-pyridinium-purines and nucleotides. Oligonucleotide **6c** underwent cleavage as well with formation of the respective pentamers bearing dC and T. Irradiation of duplex formed from pure oligomers **6c** and **7** at 5°C led to cleavage of modified strand in a similar manner . Complementary oligonucleotide **7** remained intact.

Conclusions. Although studies directed to the elucidation of DNA cleavage mechanism will be continued it is clear that this route cannot be applied for preparation of oligonucleotides labeled with the luminarine fluorophore. Since both oxidation step and subsequent substitution with pyridine proceeded with quantitative yields, we are investigating the use of those modifications for further nucleophilic reactions.

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REFERENCES AND NOTES:

1. Adamiak, R.W., Biała, E., Grześkowiak, K., Kierzek, R., Kraszewski, A., Markiewicz, W.T., Okupniak, J., Stawiński, J. and Wiewiórowski, M., Nucleic Acids Res. (1978), 5, 1889 .
2. Świderski, P., Antkowiak, W.Z. and Adamiak, R.W., Nucleosides & Nucleotides (1991), 10, 589.
3. Skalski, B., Bartoszewicz, J. , Paszyc, S. Gdaniec Z. and Adamiak, R.W. Tetrahedron (1987), 43, 3955.
4. Mielewczyk, S. , Dominiak, G. , Gdaniec, Z. , Krzymańska-Olejnik, E., Adamiak, R.W. and Skalski, B., Nucleosides & Nucleotides (1991), 10, 263 .
5. Adamiak, R.W., Biała, E. and Skalski, B., Nucleic Acids Res. (1985), 13, 2989.
6. ^{31}P NMR spectra (CDCl$_3$, δ ppm): - 151.15, 150.35.
7. HPLC. Waters 600E instrument equipped with diode-array detector and fluorescence detector was used in this studies. For the initial purification of 5'-O-DMTr oligos: NovaPak C18 cartridge, 25-50% acetonitrile gradient in 0.1M ammonium acetate; deprotected oligos were purified to homogeneity on μBondaPak C18 column in 5-15% acetonitrile gradient in the same buffer.
8. Swiderski, P., Dembek, P., Antkowiak, W.Z., Biała, E. and Adamiak, R.W. , Nucleic Acid Symph.Ser., (1987), No 18, 105 .

9. TABLE. HPLC (Waters, NovaPak C18 column, flow 0.8 ml/min) retention times data [min] for oligomers studied using specified acetonitrile gradient in 0.1M ammonium acetate buffer.

Deoxy-oligomer	**a**: X=MeS	**b**: X=MeS->O	**c**: X=Pyr+
1: T(6X-Pu)T	18.2	14.7	19.5
2: C(6X-Pu)C	13.1	6.8, 7.8	14.3
3: G(6X-Pu)G	17.2	14.6	17.9
4: A(6X-Pu)A	17.5	14.7, 15.1	18.6
5: (6X-Pu)T$_5$	17.3	6.7	7.6
6: CTCCT(6X-Pu)TCCCT	16.6	15.9	16.7

Oligomers **1-4**, gradient: 7-14% for 12[min], then isocratic. Oligomers **5**, gradient:5-15% for 12[min], then isocratic. Oligomers **6**, gradient:7-20% 12[min], then isocratic.

10. Skalski, B., Paszyc, S., Adamiak, R.W., Steer, R.P and Verrall, R.E., J.Chem.Soc. Perkin Trans. II, (1989), 1691.
11. Burdzy, A., Skalski, B., Paszyc, S., Gdaniec, Z., Popenda, M. and Adamiak, R.W., manuscript in preparation.
12. Skalski, B., Steer, R.P., Verrall, R.E., J.Am.Chem.Soc. (1991), 113, 1756 .