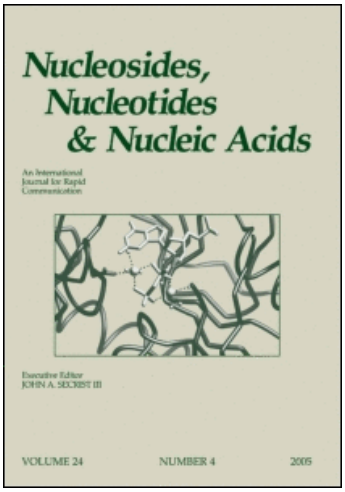

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Mielewczyk, Sllawornir , Dominiak, Grażyna , Gdaniec, Zofia , Krzymańska-Olejnik, Edyta , Adamiak, Ryszard W. and Skalskiy, Bohdan(1991) 'Deoxyluminarosine: New, Photochemically Prepared Fluorophore for the Sequence-Specific Oligonucleotide Labelling', Nucleosides, Nucleotides and Nucleic Acids, 10: 1, 263 — 267
To link to this Article: DOI: 10.1080/07328319108046456
URL: http://dx.doi.org/10.1080/07328319108046456

NUCLEOSIDES & NUCLEOTIDES, 10(1-3), 263-267 (1991)

DEOXYLUMINAROSINE: NEW, PHOTOCHEMICALLY PREPARED FLUOROPHORE FOR THE SEQUENCE-SPECIFIC OLIGONUCLEOTIDE LABELLING#

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Abstract: Photochemical preparation of green-yellow light emitting fluorophore, deoxyluminarosine 4, and routes of its sequence-specific introduction into oligonucleotide chain, are outlined.

INTRODUCTION

Fluorescent labelling of nucleic acids have recently received much attention in studies aimed at the non-isotopic DNA detection /1,2/ and automated sequencing /3-5/. In contrast, applications of luminescence methods to study nucleic acids stereodynamics and interactions are still restricted due to both limited choice of fluorescent conformational probes and difficulties in course of their sequence-specific introduction into oligonucleotide chain. Among fluorescent nucleoside derivatives which might serve as structural and/or conformational probes only those emitting blue light have been offered. Hypermodified nucleoside,wybutine intrinsic probe of some tRNAs /6/, ethenoadenine /7/ and 2-aminopurine /8/ are the most important examples. Thus, the need to design new nucleoside fluorophores of suitable photophysical properties is evident.

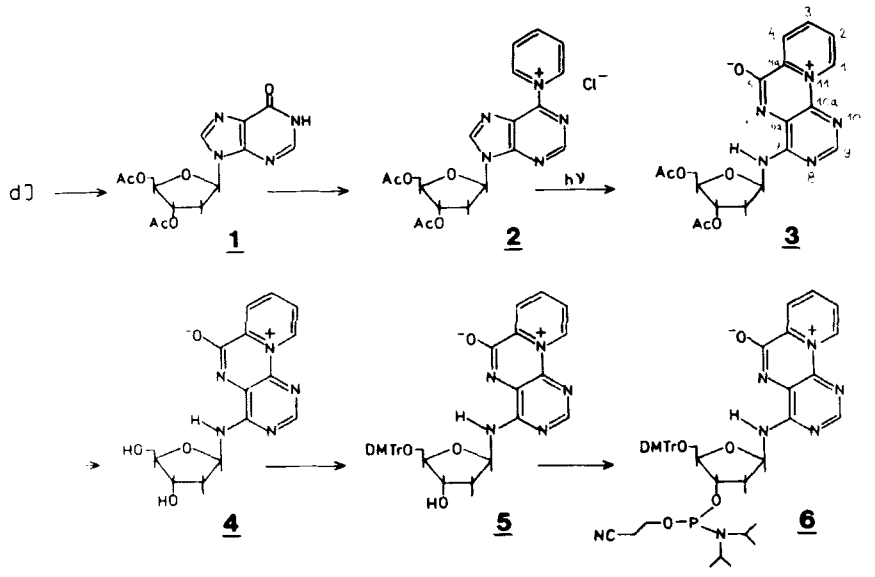
Phosphorylation of 2',3',5'-tri-O-acetylinosine in pyridine led to N-[9(2',3',5'-tri-O-acetylnebularin-6-yl]pyridinium chloride /9/ which, when subjected to visible light illumination, converts into highly fluorescent 7-(2',3',5'-tri-O-acetylribofuranosylamino)-pyrido[2,1-h]pteridin-11-ium-5-olate, termed O-acetylluminarosine (λ Em 530nm, φ 0.65) /10,11/

Dedicated to Prof. Colin B.Reese on the occasion of his 60th birthday.

We now communicate on the photochemical synthesis of deoxy-analogue -deoxyluminarosine,4 -and two routes of its, sequence-specific, chemical introduction into oligonucleotide chain.

RESULTS AND DISCUSSION

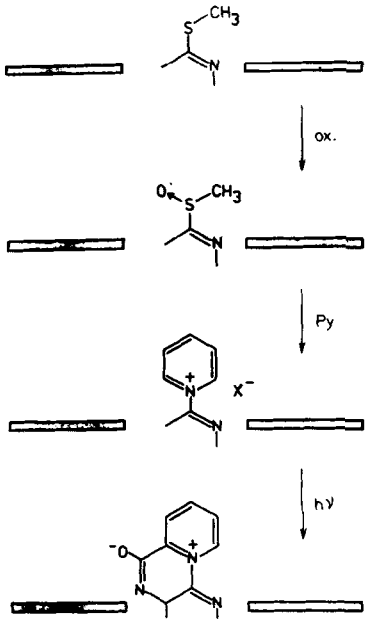
Deoxyluminarosine 4. 3',5'-Di-O-acetyldeoxyinosine 1 was subjected to pyridine-assisted phosphorylation with 4-ClPhOPOCl$_2$ acc. to general procedure /9/ in order to obtain ,labile /12/, N-(3',5'-di-O-acetyldeoxy nebularin-6-yl)pyridinium chloride 2 /13/ as the aqueous solution. 10^{-3}M solution of 2, adjusted to pH 7.0 with $NaHCO_3$ aq., was placed in narrow 2-liter glass cylinders and exposed to sunlight. Formation of the photoproduct with intense green-yellow fluorescence was monitored either by UV (decrease of absorption at λ 280 nm, characteristic of 2, and concominant formation of new absorption band at λ 424 nm) or fluorescence (decrease in the emmission of 2 at λ 424 nm and appearence of new one at λ 527 nm). Aqueous solution was extracted with chloroform, organic layer concentrated and partitioned on silica gel column using methanol gradient in chloroform to obtain 7-(3',5'-di-O-acetyl-β-D-deoxy ribofuranosylamino)-pyrido[2,1-h]pteridin-11-ium-5-olate 3 /14/ as a foam (30% yield). NMR measurements reveal no α⟷β anomerisation of 3 under neutral conditions. The X-ray structure of its aglycone, 7-amino-pyrido [2,1-h]pteridin-11-ium-5-olate (luminarine /10/), has been solved /15/. Heterocyclic betaine system of luminarine is acid-stable but shows some lability under basic conditions /10,11/. To achieve mild de-O-acetyla-



tion of 3 into deoxyluminarosine 4 anhyd. methanol containing triethylamine (5%) was used. Different batches of 4 /16/ isolated (70%) by chromatography on silica gel (gradient of methanol in chloroform) contain, as detected by HPLC, 5-20% of α-anomer. Such preparations of 4 were used for further experiments.

Introduction of deoxyluminarosine 4 into oligonucleotide chain. 5'-O-Dimethoxytrityldeoxyluminarosine 5 obtained (70%) in a usual manner was 3'-phosphitylated to give fluorescent (2-cyanoethyl)phosphoramidite 6 /17/ (75%, lyophilisate from benzene) containing ca. 20% of α-anomer. Due to pronounced reactivity of luminarine toward conc. ammonia, typical procedure for automated DNA synthesis had to be modified (introduction of more labile protecting groups, ammonia step at room temp.). Using 6, the following oligodeoxynucleotides have been synthesized and purified by HPLC (data not shown): dLCCCAGTCACGACGTT, dLGTAAAACGACGGCCA -(M-13 sequencing primers); dL$(CAC)_5$ -(DNA-fingerprinting); dL$(T)_9$, $(A)_5$dL$(A)_5$, AGGAAdLAAGGA -(structural studies). Deoxyluminarosine-containing oligomers exhibit strong fluorescent bands during PAGE analysis /18/.

Sequence-specific introduction of luminarine fluorophore via regioselective photochemical modification of a precursor-oligonucleotide This concept is based on regioselective transformation of 6-methylthiopurine residue,placed within deprotected oligonucleotide, into 6-methylsulphoxide and conversion of the latter to 6-pyridinium derivative. Its irradiation with visible-light should generate fluorescent luminarine on oligomer level. Hexamer (2'-O-Me-L)$(dA)_5$ was obtained accordingly. Photochemical transformation of precursor-oligonucleotide bearing at 5'-end 6-pyridinium cation (λ Em 425 nm) into (2'-O-Me)luminarosine-labelled one (λ Em 528 nm) was monitored by spectrofluorymetry. Till present, Br_2aq. (phosphate, pH 6.5) was used for oxidation. Due to side-reactivity of pyrimidines this route, by now, cannot be offered as a general one. Further work is in progress.



Acknowledgements. Authors thanks Ms. Danuta Więckowska for technical assistance and Dr Ewa Biała for making of 2'-O-methyl-6-methylthionebularine derivative avaiable. This work was supported by Polish Academy of Sciences within project CPBR 3.13.4.2.1.

REFERENCES

1. Urdea,M.S.,Warner,B.D.,Running,J.A.,Stempien,M.,Clyne,J. and Horn,T. (1988) Nucleic Acids Res., 16, 4937
2. Morrison,L.E.,Halder,T.C. and Stols,L..(1989) Analytical Biochem., 183, 231
3. Smith,L.M.,Sanders,J.,Kaiser,R.,Hughes,P.,Dodd,C.,Connel,C.,Heiner,C Kent,S. and Hood,L. (1986) Nature, 321, 674
4. Ansorge,W.,Sproat,B.,Stegeman,J.,Schwager,C. and Zenke,M. (1987) Nucleic Acids Res., 15, 4593
5. Prober,J.M.,Trainor,G.L.,Dam,R.J.,Hobbs,F.W.,Robertson,C.W.,Cocuzza, A.J.,Jensen,M.J. and Baumeister,K. (1987) Science, 238, 336
6. Adamiak,R.W.,Górnicki,P., (1985) Prog.Nucleic Acids Res. Mol. Biol., 32, p.27. W.E.Cohn Ed. Academic Press, New York
7. Leonard N.J., (1984) CRC Crit. Rev. Biochem., 15, 125,
8. Nordlund,T.M.,Andersson,S.,Nilsson,N.,Rigler,R.,Gyaslund,A. and McLaughlin,L.W. (1989) Biochemistry, 28, 9095
9. Adamiak,R.W.,Biała,E.and Skalski,B.(1985) Nucleic Acids Res.,13,2989
10. Skalski,B.,Bartoszewicz,J.,Paszyc,S.,Gdaniec,Z. and Adamiak,R.W. (1987) Tetrahedron, 43, 3955
11. Skalski,B.,Paszyc,S.,Adamiak,R.W.,Steer,R.P. and Verrall,R.F. (1989) J.Chem.Soc.Perkin Trans. II, 1691
12. Seela,F.,Herdering,W. and Kehne,A. (1987) Helv.Chim.Acta, 70, 1649
13. 2. H NMR (90 MHz, D_2O): δ(ppm) 10.05 (d,2,J=7.1Hz,H-α), 9.17 (s,2, H-2), 8.95 (t,1,J=7.0Hz,H-γ), 8.95 (s,1,H-8), 8.43 (t,2,J=7.1 Hz,H-β), 6.74 (t,1,J=6.8Hz,H-1'), 5.58 (m,1,H-3'), 4.56 (m,1,H-4'), 4.43 (m,2,H-5',5''), 3.38-2.73 (m,2,H-2'), 2.17,2.03 (s,6,Ac); C13 NMR (22.5 MHz, D_2O): δ(ppm) 174.35, 174.18 (CO-Ac), 155.82 (C-4), 152.84 (C-2), 151.11 (C-γ), 149.04 (C-8), 147.26 (C-6), 144.06 (C-α), 129.22 (C-β), 126.24 (C-5), 86.20 (C-4'), 83.38 (C-1'), 75.15 (C-3'), 64.69 (C-5'), 36.84 (C-2'), 21.18, 20.97 (CH_3-Ac)
14. 3. UV-VIS (H_2O) λ_{max} (ε): 265 (16000),344 (3700),424 (11000); Fluorescence (H_2O) λ_{Em} 527 nm.
H NMR (90 MHz, $CDCl_3$): δ(ppm) 10.19 (d,1,J=6.6Hz,H-1), 9.08 (d, 1,J=8.3Hz,H-4), 8.47 (t,1,J-7.3Hz,H-3), 8.36 (s,1,H-9), 8.10 (t,1, J=8.1Hz,H-2), 7.68 (d,1,J=9.52Hz, -NH), 6.35 (td,1,J=9.5Hz,5.8Hz, H-1'), 5.29 (m,1,H-3'), 4.23 (m-br,3,H-4',H-5',5''),2.45-2.27 (m,2, H-2'), 2.18, 2.10 (s,6,OAc); C13 NMR (22.5 MHz, $CDCl_3$): δ(ppm) 170.66, 170.34 (CO-Ac), 160.38 (C-5), 158.31 (C-7), 149.04 (C-9), 141.35 (C-3), 138.32 (C-4a), 131,71 (C-10a), 131.01 (C-1), 127.54 126.29 (C-2), 125.16 (C-6a), 81.6 (C-1',C-4'), 75.04 (C-3'), 64.26 (C-5'), 37.82 (C-2'), 20.97 (CH_3-Ac).
15. Adamiak,D.A,Surma,K.,Gawron,M. and Skalski,B., paper in this volume
16. 4. pure β-anomer, UV-VIS and fluorescence spectra as for 3.
H NMR (90MHz, D_2O): δ(ppm) 9.9 (d,1,J=6.6Hz, H-1), 8.61 (m,2, H-3,H-4), 8.29 (td,1,J=6.6;2.4Hz,H-2), 8.0 (s,1,H-9), 5.95 (t,1, J=6.8Hz,H-1'), 4.46 (m,1,H-3'), 4.01 (m,1,H-4'), 3.74 (m,2,H-5', 5''), 2.39 (m,2,H-2',2'').

17. 6 P31 NMR (36.2 MHz, ext. 85% H_3PO_4): δ(ppm) 147.58, 147.46 (α-anomer), 146.85, 145.76 (β-anomer).
18. It is interesting to note that estimated quantum yield of fluorescence for 5'-deoxyluminarosine within dLCCCGTTCGTCACGTA (φ 0.60,pH 7.5 using fluorescein in 0.1 NaOH as a standard)is virtually the same as for monomeric luminarosine /11/. On the other hand, for the fluorescein-labelled linker-containing oligomer F/\/N(CAC)5 /19/, a value φ 0.25 was estimated under similar conditions.
19. Krzymańska-Olejnik,E. and Adamiak,R.W., paper in this volume.