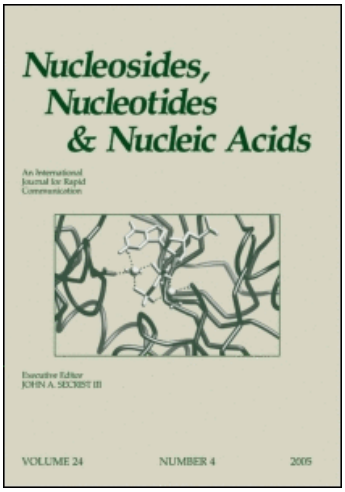


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis and Application of Isosteric Purine 2'-Deoxyribofuranosides

Frank Seela[a]; Uwe Bindig[a]; Hansjürgen Driller[a]; Wilhelm Herdering[a]; Klaus Kaiser[a]; Andreas Kehne[a]; Helmut Rosemeyer[a]; Herbert Steker[a]
[a] Laauboratorium für Organische und Bioorganische Chemie, Fachbereich Biologie/Chemie, Universitat Osnabriack, Osnabriick, West Germany

To cite this Article Seela, Frank , Bindig, Uwe , Driller, Hansjürgen , Herdering, Wilhelm , Kaiser, Klaus , Kehne, Andreas , Rosemeyer, Helmut and Steker, Herbert(1987) 'Synthesis and Application of Isosteric Purine 2'-Deoxyribofuranosides', Nucleosides, Nucleotides and Nucleic Acids, 6: 1, 11 — 23
To link to this Article: DOI: 10.1080/07328318708056176
URL: http://dx.doi.org/10.1080/07328318708056176

NUCLEOSIDES & NUCLEOTIDES, 6(1&2), 11-23 (1987)

SYNTHESIS AND APPLICATION OF ISOSTERIC PURINE 2'-DEOXYRIBOFURANOSIDES

Frank Seela *, Uwe Bindig, Hansjürgen Driller, Wilhelm Herdering, Klaus Kaiser, Andreas Kehne, Helmut Rosemeyer, and Herbert Steker.

Laboratorium für Organische und Bioorganische Chemie, Fachbereich Biologie/Chemie, Universität Osnabrück, D-4500 Osnabrück, West Germany

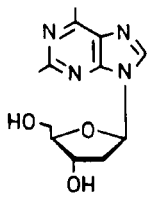
ABSTRACT. - The diastereoselective synthesis of several pyrrolo[2,3-d]- and pyrazolo[3,4-d]pyrimidine 2'-deoxyribofuranosides employing 1-chloro-2-deoxy-3,5-di-O-(p-toluoyl)-α-D-erythropentofuranose and the nucleobase anion, generated by liquid-liquid or solid-liquid phase-transfer catalysis, is described. Appropriately protected phosphoramidites of 8-aza-7-deaza-2'-deoxyadenosine and 2'-deoxytubercidin were prepared and employed in solid-phase synthesis of palindromic DNA-fragments. The replacement of dA residues by deoxytubercidin within the Eco RI recognition site d(GAATTC) of the dodecamer d(GTAGAATTCTAC) gave evidence for purine N-7 binding to the endodeoxyribonuclease. The interpretation of similar experiments carried out on d(CGCGAATTCGCG) was obscured because of hairpin formation.

Most of the naturally occurring DNA contains the 4 nucleobases adenine, guanine, cytosine, and thymine which are attached to the sugar-phosphate backbone. However, in DNA of bacteriophages rare nucleobases have been found [1]. Apart from these naturally occurring modified 2'-deoxyribonucleosides we became interested in synthetic DNA constituents which occupy similar space as the natural ones but have an altered pattern of nitrogens within the heterocyclic system. Our efforts were directed towards the synthesis of isosteric purine 2'-deoxyribofuranosides [2], their incorporation into short stretches of DNA, and the interaction of these oligomers with enzymes [3].

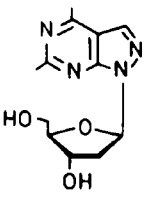
Among the isosteric purine 2'-deoxyribofuranosides with the same N-1/N-3 pattern as regular purine nucleosides - a prerequisite for specific Watson-Crick base pairing in DNA-duplexes - our interest was focussed towards pyrrolo[2,3-d] pyrimidine-, pyrazolo[3,4-d]pyrimidine-, and imidazo[1,2-a]-s-triazine 2'-deoxyribofuranosides (Fig. 1).

There are several problems which came across during the synthesis of isosteric purine 2'-deoxyribofuranosides. They arise from the glycosylation reaction which is neither regio- nor diastereospecific under conditions formerly employed. Similar to the formation of N-7 and N-9 purine nucleosides glycosylation of pyrazolo[3,4-d]pyrimidines give rise to a non-regioisomeric reaction [4]. From the structure of pyrrolo[2,3-d]pyrimidines one can expect a regiospecific glycosylation in the five-membered ring. However, the low nucleophilicity of the pyrrolo nitrogen directs glycosylation towards the pyrimidine moiety if the neutral nucleobase is employed.

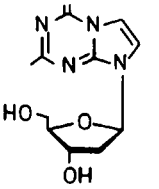
2'-DEOXYRIBOFURANOSIDES WITH ISOMERIC FIVE-MEMBERED RING SYSTEMS



Purine

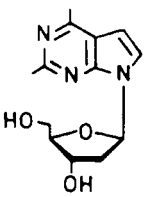


Pyrazolo[3,4-d]pyrimidine

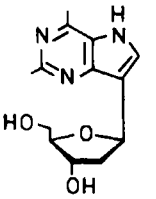


Imidazo[1,2-a]-s-triazine

2'-DEOXYRIBOFURANOSIDES LACKING ONE IMIDAZOLE NITROGEN

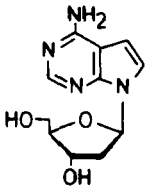


Pyrrolo[2,3-d]pyrimidine

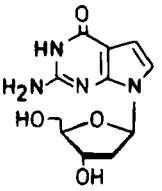


Pyrrolo[3,2-d]pyrimidine

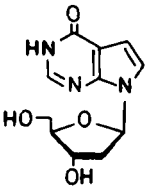
Figure 1.



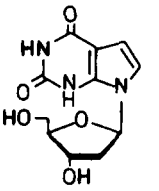
1



2

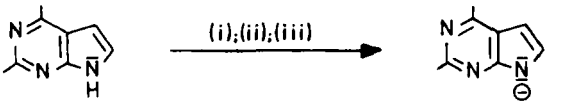


3



4

In the proceed of the synthesis of isosteric pyrrolo-[2,3-d]pyrimidine 2'-deoxyribofuranosides[5-8], of which examples are depicted in the formulas 1-4, it became apparent that the glycosylation reaction was regioselective if the nucleobase anion 6 was formed as an intermediate. This is a strong nucleophile which is formed from 5 by the action of strong bases either under liquid-liquid (i) or solid-liquid (ii) phase-transfer conditions (PTC) or in aprotic solvents (iii). The use of nucleobase anions or nucleobase ion pairs in nucleoside synthesis goes back to earlier observations of A. Holy [9] and T. Goto [10] who employed nucleobase sodium salts in ribonucleoside synthesis. Phase-transfer glycosylation of nucleobase anions has been developed in our laboratory [11].



5 6

(i) PTC(liquid-liquid), CH_2Cl_2/50 % aq. NaOH/Bu_4NHSO_4

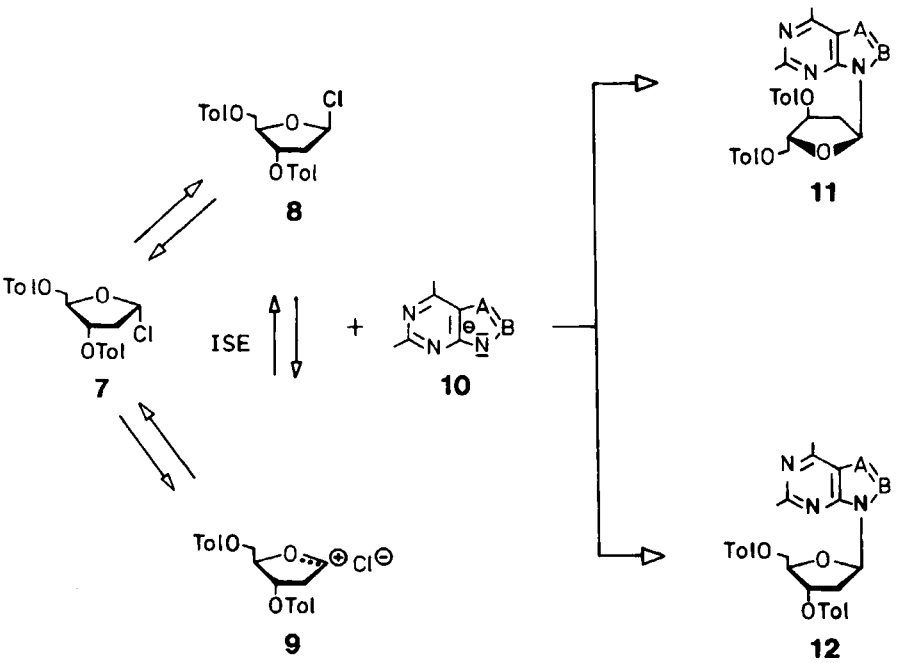
(ii) PTC(solid-liquid), CH_3CN/KOH/$(CH_3OCH_2CH_2OCH_2CH_2)_3N$

(iii) CH_3CN/NaH

The generation of a nucleobase anion was also advantageous with respect to the stereochemical course of the glycosylation reaction. In contrast to 2-benzoylated or 2-acylated D-ribofuranosyl chlorides of which the 2-substituent can assist the glycosylation reaction by neighbouring group participation [12], 2-deoxyribofuranosyl halides cannot form such acyloxonium ions which direct the nucleobase in trans-configuration. On the other hand it is established that the crystalline halogenose 7 which is the source of the sugar

moiety in these 2'-deoxyribonucleosides syntheses has α-D-configuration [13,14].

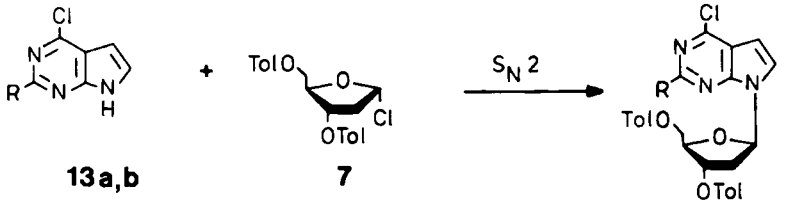
It has been reported earlier that the α-halogenose 7 equilibrates in solution to the ß-anomer 8 [14,15,2]. This equilibration is catalysed by many reagents which have been formerly used during 2'-deoxyribonucleoside synthesis. Moreover, in solvents of high polarity the halogenose equilibration may occur via the ion-pair 9 already in the absence of catalysts.



ISE: in situ equilibration

Due to the equilibration process mixtures of the anomers 11,12 are formed if reaction conditions are chosen which give the sugar moiety time to equilibrate. This process can be avoided, if the nucleobase anion 10 is employed, which is a highly reactive species ensuring a rapid glycosylation before equilibration. The exclusive formation of a ß-D-deoxyribofuranoside is then the result of a S_N2 displacement reaction [6,14].

The glycosylation of 4-chloropyrrolo[2,3-d]pyrimidine (13a) or 2-amino-4-chloropyrrolo[2,3-d]pyrimidine (13b) - key-intermediates of the nucleosides 1 and 2 - with the halogenose 7, which was carried out under different conditions, may demonstrate the utility of glycosylation condition employing the nucleobase anion.

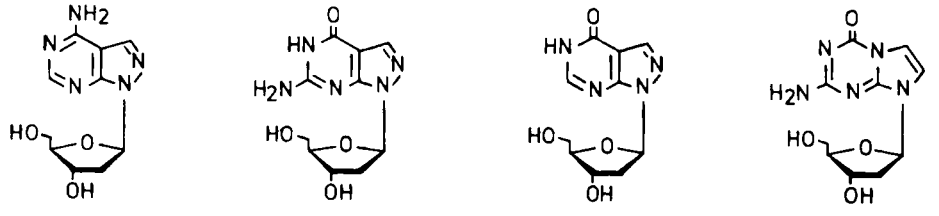


13a,b **7** **14a,b**

a : R = H, b : R = NH_2

Reaction Conditions	Yield (%) of Crystalline 14a	14b
(i) PTC: liq.-liq., vibromixing, r.t. (CH_2Cl_2/50% aq.NaOH, 13a or 13b /Bu_4NHSO_4, 10:1).	63	45
(ii) PTC: sol.-liq.,stirring, r.t. (CH_3CN/sol.KOH, 13a or 13b/ $CH_3O(CH_2CH_2O)_3CH_2CH_2)_3N$ [16], 10:1)	81	65
(iii) CH_3CN-NaH (stirring, heat).	66, 71 [17]	

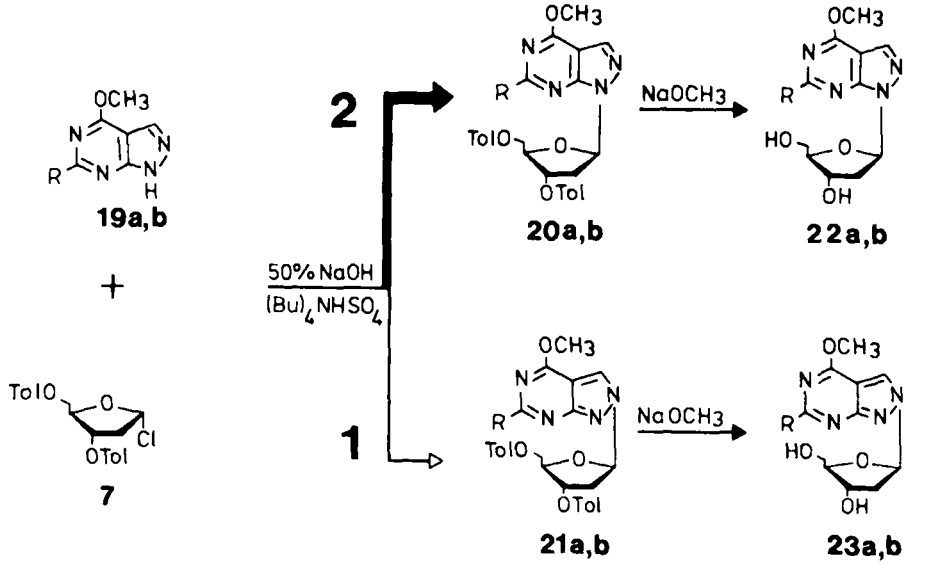
Employing liquid-liquid phase-transfer techniques the isosteric 2'-deoxyribofuranosides 15-18 have been prepared which contain either a pyrazolo[3,4-d]pyrimidine [18-20] or an imidazo[1,2-a]-s-triazine system.



15 **16** **17** **18**

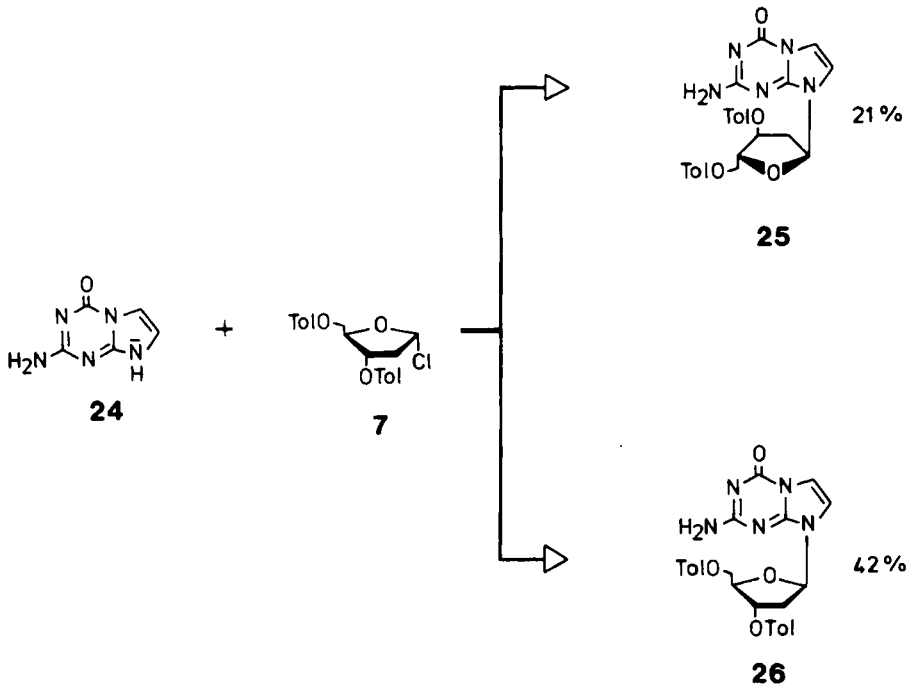
Reaction of the pyrazolo[3,4-d]pyrimidines 19a or 19b with the halogenose 7 yielded 20a or 20b in a diastereo-

selective reaction. However, N-1/N-2 charge delocalization of the nucleobase anion gave the N-2 glycosylation products 21a and 21b additionally to the N-1 compounds. The ratio of the N-1 vs. N-2 glycosylation products, based on the isolated materials, was 2:1. Sugar deprotection of 20a,b and 21a,b with $NaOCH_3$/MeOH yielded the nucleosides 22a,b as well as the regioisomers 23a,b. Nucleophilic displacement of the methoxy group of 22a with KOH gave the deoxynucleoside 17, whereas 15 was formed, when the reaction was carried out in conc. ammonia. The deoxyguanosine isostere 16 was obtained from 22b on a similar route described for 17.



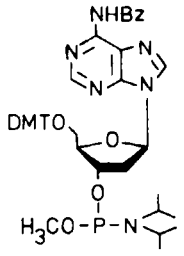
a : R = H, b : R = NH_2

The synthesis of the isosteric imidazo[1,2-a]-s-triazine 2'-deoxynucleoside 18 (5-aza-7-deaza-2'-deoxyguanosine) was encountered with difficulties due to the low solubility of the nucleobase 24 [21] in organic media. As a consequence, solid-liquid phase-transfer glycosylation as well as glycosylation in the presence of sodium hydride failed.

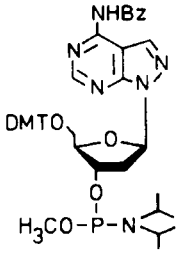


Employing CH_2Cl_2/10 % aq. K_2CO_3 in the presence of Bu_4NHSO_4 as catalyst gave the protected nucleoside 26 and its α-anomer 25 in a 2:1-ratio (63% total yield). The formation of the α-anomer could not be circumvented due to the unfavourable partition of 24 between the organic and the aqueous phase. This resulted in a prolongated reaction time which gave the halogenose 7 time to equilibrate. Deprotection of 26 with MeOH/NH_3 yielded 18 which was isolated crystalline. Its structure was confirmed by homo- and heteronuclear J-coupled ^{1}H- and ^{13}C-NMR spectra.

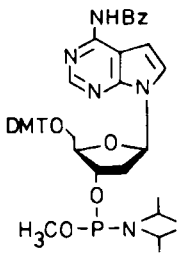
Isosteric pyrrolo[2,3-d]pyrimidine or pyrazolo[3,4-d] pyrimidine 2'-deoxyribofuranosides are ideal probes to study protein/DNA recognition [3, 22]. This is due to their altered nitrogen pattern, in particular to the lack of N-7 which is a potential hydrogen bond acceptor within the major groove of DNA. Since modern strategies of oligonucleotide synthesis, in particular the use of polymeric matrices and the application of phosphittriester methodology [23], the rapid preparation of isosterically modified DNA-fragmentscould be tackled. We have prepared the phosphoramidites 28 and 29, which are isosteric to the parent purine compound 27.



27



28



29

The phosphoramidite 28, derived from 8-aza-7-deaza-2'-deoxyadenosine, (15) as well as 29 [24] being a derivative of 2'-deoxytubercidin (1), were then employed in oligonucleotide synthesis of palindromic DNA fragments, which are recognized by endodeoxyribonucleases. The following oligomers have been prepared [25]:

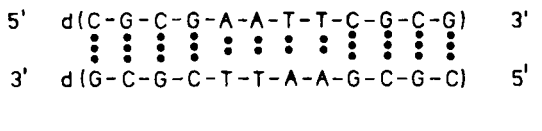
d(CTGGATCCAC)	30	d(GGAATTCC)	33
d(CTGGc^7z^8ATCCAC	31	d(GGc^7AATTCC)	34
d(CTGGATCCc^7z^8AC)	32	d(GGAc^7ATTCC)	35
		d(GG$c^7A c^7$ATTCC)	36
d(CGCGAATTCGCG)	37	d(GTAGAATTCTAC)	41
d(CGCGc^7AATTCGCG)	38	d(GTAGc^7AATTCTAC)	42
d(CGCGAc^7ATTCGCG)	39	d(GTAGAc^7ATTCTAC)	43
d(CGCG$c^7A c^7$ATTCGCG)	40	d(GTAG$c^7A c^7$ATTCTAC)	44
		d(GTc^7AGAATTCTAC)	45
		d(GTAGAATTCTc^7AC)	46

Whereas the compounds 31 and 32 represent oligomers with an isosterically altered recognition sequence of the endodeoxyribonuclease Bam H1, the octamers 34-36, and the dodecamers 38-40, as well as 42-46 contain the altered recognition site of the endodeoxyribonuclease Eco RI.

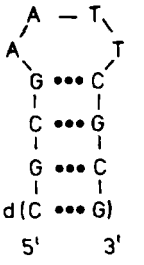
Next, the oligomers containing 2'-deoxytubercidin (dc^7A) were characterized by their T_m-values measured UV-spectrophotometrically. At low salt and low oligomer concentration different melting profiles at 260nm and 280nm were observed for the "Dickerson" oligomer 37 as well as for compounds 38-40. Moreover, anomalous chromatographic proper-

ties, giving rise to 2 peaks of identical nucleoside content in the HPLC profiles were detected. The intensity of these peaks was strongly dependent on the concentration of the oligomer 37 employed in HPLC chromatography. Finally, the T_m-values measured at 260nm were strongly dependent on the oligomer concentration whereas those measured at 280nm were almost independent. From that it can be concluded that the dodecamers form hairpins (48) beside duplexes (47) in particular at low salt and low oligomer concentration [26]. These results were in agreement with the extraordinary slow cleavage rate of the oligomers 37-40 by the endodeoxyribonuclease Eco RI.

In contrast to the sequences with d(GC)-alternating flanking regions 37-40 the dodecamers with "random" sites flanking the Eco RI recognition site showed a regular behaviour in their physicochemical properties indicating that only duplexes are formed under that conditions.



47



48

The aim of the enzymatic studies was the detection of N-7 as binding sites of the two dA residues within the recognition sequence d(GAATTC) towards the endodeoxyribonuclease Eco RI. For this purpose the dodecamers 41-46 were investigated with respect to enzymatic cleavage. At a temperature of 30°C the cleavage of the parent oligomer d(GTAGAATTCTAC) was complete within 55 min (Fig. 2).
As one can see from figure 2 the incorporation of dc^7A within the recognition site reduces the cleavage rate strongly, whereas the replacement of dA by dc^7A in the flanking region

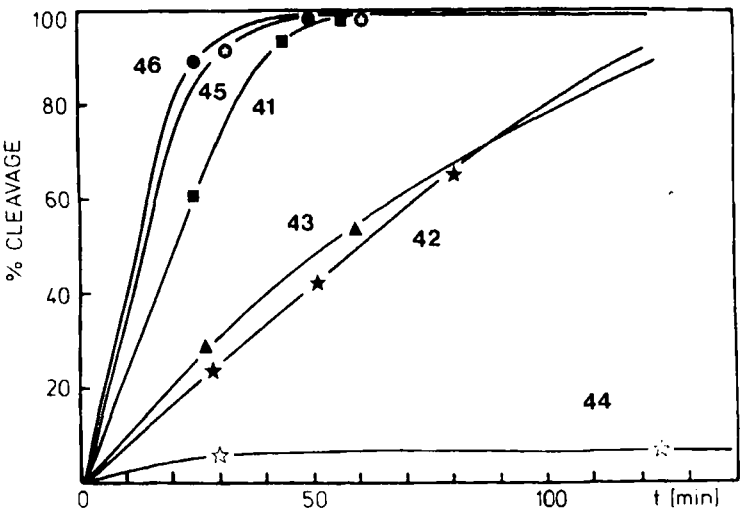
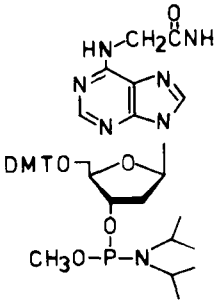


Figure 2. Cleavage of the oligomers 41-46 with the endodeoxyribonuclease Eco RI in 10 mM TRIS-HCl buffer, pH 7.5, containing 80 mM NaCl, 20 mM $MgCl_2$, 10 µM dimeric oligomer, and 0.7 µM dimeric enzyme. Incubation temperature was 30°C. The reaction was monitored by RP-18 HPLC. The percentage of cleavage was calculated on the basis of the uncleaved oligomer.

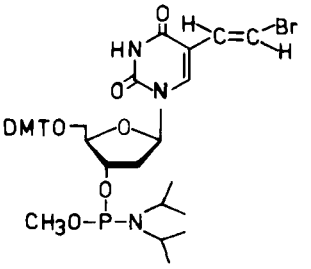
even enhances the cleavage velocity. From these experiments it was concluded that both purine-7 nitrogens of the dA residues within the recognition site are important proton acceptors for the endodeoxyribonuclease Eco RI. These data support recent findings of Rosenberg et al. [27] derived from the solid state. However, a structural alteration of the duplex by dc^7A cannot be completely ruled out.

Recently we have extended our work from the incorporation of isosteric purine 2'-deoxyribofuranosides into oligonucleotides to naturally occurring rare DNA constituents or 2'-deoxynucleosides which are incorporated in vitro into high-molecular-weight DNA.

From the rare DNA constituent of the phage Mu [28] as well as from the antivirally active nucleoside (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU) [29] the appropriately protected phosphoramidites 49 and 50 have been prepared.



49



50

d(Å-T-Å-T-Å-T-Å-T-Å-T-Å-T) 51

d(Ů-A-Ů-A-Ů-A-Ů-A-Ů-A-Ů-A) 52

Employing common phosphite methodology compounds 49 and 50 were used during solid-phase synthesis of the oligonucleotides 51 and 52, in which dA residues are replaced by (N^6-carboxyaminomethyl)-2'-deoxyadenosine (dÅ) or dT residues by BVDU (dŮ). The effect of these 2'-deoxynucleoside analogues on secondary and tertiary structure of short DNA fragments is under investigation.

ACKNOWLEDGMENTS

We thank Dr. Erik De Clercq for a generous gift of BVDU and Dr. A. Pingoud for a sample of endodeoxyribonuclease Eco RI. Financial support by the Deutsche Forschungsgemeinschaft and the Stiftung Volkswagenwerk is gratefully acknowledged.

REFERENCES

[1] R.A.J. Warren, Ann. Rev. Microbiol. 1980, 34, 137.

[2] F. Seela, H. Driller, A. Kehne, S. Menkhoff, J. Ott, and H.-D. Winkeler, Chemical Synthesis in Molecular Biology (H. Blöcker, R. Frank, and H.J. Fritz, eds.), Verlag Chemie, Weinheim, 1986.

[3] F. Seela, H. Driller, Nucl. Acids Res. 1986, 14, 2319.

[4] F. W. Lichtenthaler, E. Cuny, Chem. Ber. 1981, 114, 1610.

[5] F. Seela, A. Kehne, Liebigs Ann. Chem. 1983, 876.
[6] H.-D. Winkeler, F. Seela, J. Org. Chem. 1983, 48, 3119.
[7] F. Seela, S. Menkhoff, Liebigs Ann. Chem. 1985, 1360.
[8] F. Seela, H. Driller, U. Liman, Liebigs Ann. Chem. 1985, 312.
[9] A. Holy, F. Sorm, Collect Czech. Chem. Commun. 1969, 34, 3383.
[10] T. Kondo, T. Ohgi, T. Goto, Agric. Biol. Chem. 1977, 41, 1501.
[11] F. Seela, H.-D. Winkeler, Angew. Chem. Int. Ed. Engl. 1979, 18, 536.
[12] B. R. Baker, Ciba Found. Symp. Chem. Biol. Purines, 1956, p. 122.
[13] M. Hoffer, Chem. Ber. 1960, 93, 2777.
[14] M. P. Kotick, C. Szantay, T. J. Bardos, J. Org. Chem. 1969, 34, 3806.
[15] A. J. Hubbard, A. S. Jones, R. T. Walker, Nucl. Acids Res. 1984, 12 , 6827.
[16] G. Soula, J. Org. Chem. 1985, 50, 3717.
[17] Kazimierczuk, H. B. Cottam, G. R. Revankar, R. K. Robins, J. Amer. Chem. Soc. 1984, 106, 6379.
[18] F. Seela, H. Steker, Helv. Chim. Acta, 1985, 68, 563.
[19] F. Seela, H. Steker, Heterocycles 1985, 23, 2521.
[20] F. Seela, H. Steker, Helv. Chim. Acta, 1986, in press.
[21] S. H. Kim, D. G. Bartholomew, L. B. Allen, R. K. Robins, G. R. Revankar, P. Dea, J. Med. Chem. 1978, 21, 883.
[22] A. Ono, M. Sato, Y. Ohtani, T. Ueda, Nucl. Acids Res. 1984, 12, 8939.
[23] M. D. Matteucci, M. H. Caruthers, J. Am. Chem. Soc. 1981, 103, 3185.
[24] F. Seela, A. Kehne, Tetrahedron, 1985, 41, 5387.
[25] F. Seela, A. Kehne, Biochemistry, 1986, in press.
[26] L. A. Marky, K. S. Blumenfeld, S. Kozlowsky, K. J. Breslauer, Biopolymers, 1983, 22, 1247.
[27] J. M. Rosenberg, J. A. Mc Clarin, C. A. Frederick, B. C. Wang, H. W. Boyer, P. Greene, 1985, International Interdisciplinary Symposium on "The Biological Significance of Conformational Changes in DNA and DNA-Protein Complexes", Bielefeld, West-Germany.

[28] D. Swinton, S. Hattman, P. F. Crain, C.-S. Cheng, D. L. Smith, J. A. McCloskey, Proc. Natl. Acad. Sci, USA, 1983, 80, 7400.

[29] E. De Clercq, J. Descamps, P. De Somer, P. J. Barr, A. S. Jones, R. T. Walker, Proc. Natl. Acad. Sci, [USA], 1979, 76, 2947.