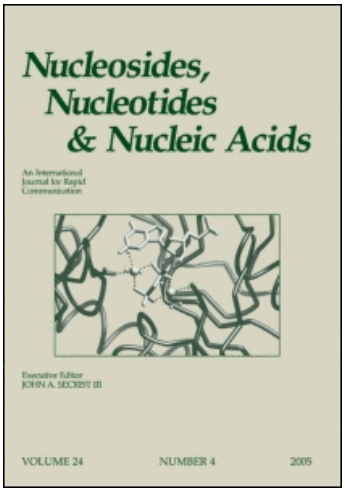

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DNA-Fragments Resistant to Endodeoxyribonuclease Hydrolysis PCR-Amplification using 7-Deaza-2'-deoxyguanosine Triphosphate in Place of dGTP

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DNA-FRAGMENTS RESISTANT TO ENDODEOXYRIBONUCLEASE HYDROLYSIS: PCR-AMPLIFICATION USING 7-DEAZA-2'-DEOXYGUANOSINE TRIPHOSPHATE IN PLACE OF dGTP

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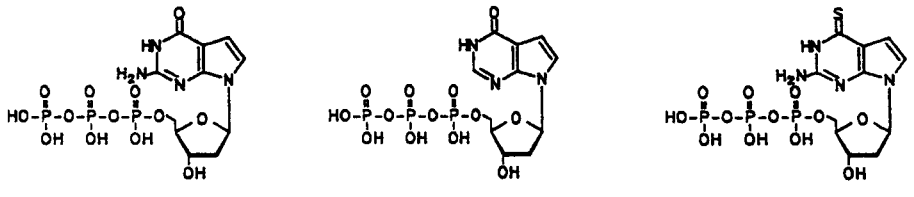
Abstract: 7-Deaza-2'-deoxyguanosine triphosphate was used instead of dGTP during PCR-amplification of a pUC 18 fragment of 501 base pairs. The oligomer containing c^7G_d was protected from hydrolysis by various endodeoxyribonucleases when the 7-deazapurine nucleoside was localized at the 3'- or the 5'-position of the cleavage site.

7-Deaza-2'-deoxyguanosine triphosphate (c^7GTP_d, 1) is a substrate of several DNA-polymerases, such as the DNA polymerase I (Klenow fragment)[1,2], reverse transcriptase and Taq polymerase[3]. It can improve dideoxysequencing as it avoids problems of secondary structure formation. In the following we describe the amplification and properties of a pUC 18[4] fragment (501-mer, scheme 2) with Taq polymerase employing 1, c^7ITP_d (2) and $c^7s^6GTP_d$ (3) instead of GTP.

From the plasmid pUC 18 the 501-mer (Scheme 2) was amplified with Taq-polymerase using the primers I and II. Besides the three regular nucleotides (dATP, TTP, and dCTP) dGTP was replaced by c^7GTP_d (1). 30 Amplification cycles were carried out.



(1) (2) (3)

Scheme 1.

5'd(ACCGCACAGATGCGTAAGGAgAAAATACCgCATCAggCgCCATTCgCCATTCAggCTgCg
CAACTgTTgggAAgggCgATCggTgCgggCCTCTTCgCTATTACgCCAgCTggCgAAAgggggATgTg
CTgCAAggCgATTAAgTTgggTAACgCCAgggTTTTCCCAgTCACgACgTTgTAAAACgACggCCAg
T gCCA/AgCTTgCATgCCTgCA/ggTCgACTCTAgAg/gATC/CCC/gggT/ACCgAgCTCg/AATTCg
TAATCATggTCATAgCTgTTTCCTgTgTgAAATTgTTATCCgCTCACAATTCCACACAACATAC
gAgCCggAAgCATAAAgTgTAAAgCCTggggTgCCTAATgAgTgAgCTAACTCACATTAATTgCgT
TgCgCTCACTgCCCgCTTTCCAgTCgggAAACCTgTCgTgCCAgCTgCATTAATgAATCggCCAAC
gCgCggggAgAggCggTTgCgTATTTgggCgCTCTTCCgCTTCCTCgC) 3'

Primer I : 5' d(ACCGCACAGATGCGTAAGG) g = c^7G_d residue
Primer II: 5' d(GCGAGGAAGCGGAAGAGCG)

Scheme 2. 501-mer (fragment from 200-700 of the + strand of pCU 18).[4]

Table. Phosphodiester Hydrolysis of the 501-mer (pUC18 Fragment, Scheme 2) by Various Endodeoxyribonucleases.

Endo-nuclease	Recognition Sequence	Cleavage Position	Oligomer Hydrolysis	Oligomeric Products
Hind III	**A/AGCTT**	**399/400**	**(+)**	200 and 301
EcoR I	**G/AATTC**	**450/451**	**(-)**	251 and 250
Pst I	CTGCA/G	415/416	(-)	216 and 285
BamH I	G/GATCC	429/430	(-)	230 and 271
Sma I	CCC/GGG	412/413	(-)	213 and 288
Rsa I	GT/AC	440/441	(+)	241 and 260

According to the table DNA is protected from endodeoxyribonuclease hydrolysis when c^7G_d replaces dG directly at the 3' or 5' position of the phosphodiester moiety at the cleavage site. In cases where c^7G_d was localized at other positions of the recognition sequence or outsite of it (data not shown) hydrolysis occurred. Further experiments which make use of this phenomenon are under investigation.

The amplified ds DNA-fragment contains c^7G_d (g) in both strands. The 5' ends formed by the primers I or II are unmodified. The T_m value was not significantly lower (88°C) as that one of the parent oligomer (90°C). Identical CD-spectra prooved B-DNA structure.

We have also synthesized compounds 2 (λ_{max} = 258 nm ; ^{31}P-NMR (D_2O; pH 7.0): δ = -10.11 ppm (d, J = 19 Hz, P_α); -21.52 ppm (t, J = 19 Hz, P_β); -7,19 ppm (d, J = 19 Hz; P_γ)) and 3 (λ_{max} = 236 nm, 271 nm, 344 nm ; ^{31}P-NMR (D_2O; pH 7.0): δ = -10.29 ppm (d, J = 20 Hz, P_α); -21.42 ppm (t, J = 20 Hz, P_β); -6.62 ppm (d, J = 20 Hz; P_γ)) and have tried to incorporate them into DNA. Amplification was not detected due to unfavourable substrate properties or a reduced complex formation with ethidium bromide.

It has been reported earlier that phosphodiester hydrolysis of short oligonucleotides can be suppressed when c^7G_d replaces dG within the recognition sequence of EcoR I[5]. As the DNA-fragment of scheme 1 contains recognition sequences of about 50 restriction enzymes it is an ideal probe to study the influence of c^7G_d on the regioselective phosphodiester hydrolysis. Hydrolysis experiments with a few representative restriction enzymes are depicted in the Table.

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