

Figure 1a

a

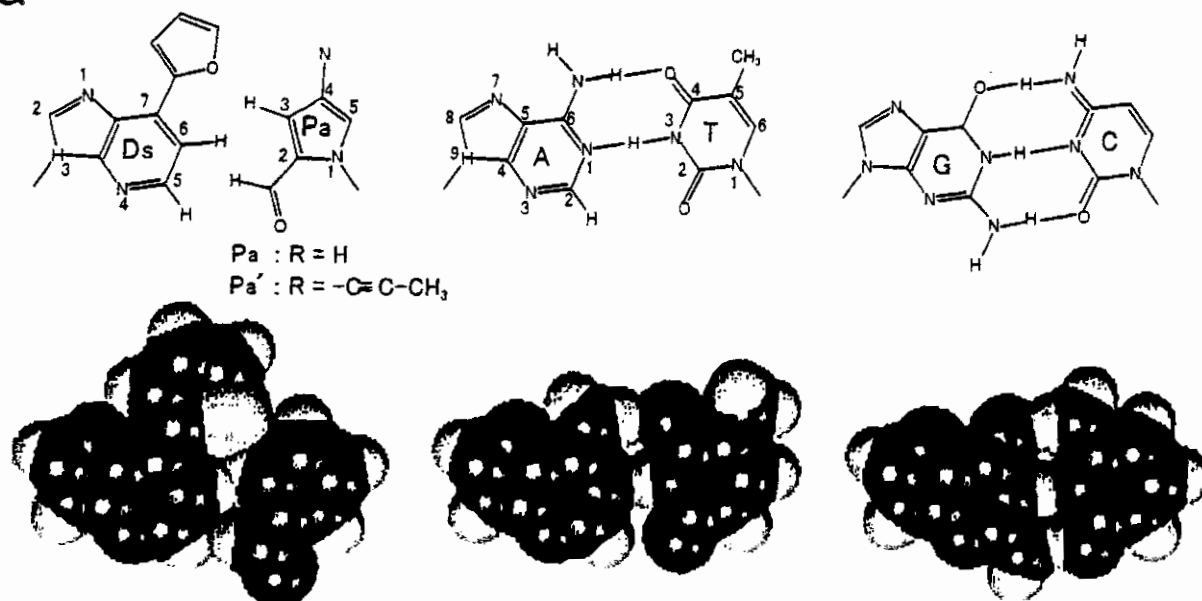
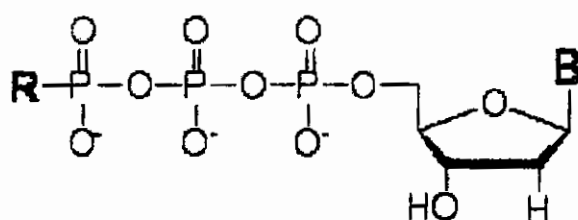


Figure 1b

b



Unmodified dNTP: R=OH
 Modified dNTP (dNTP_N): R=NH₂

Figure 1c

c

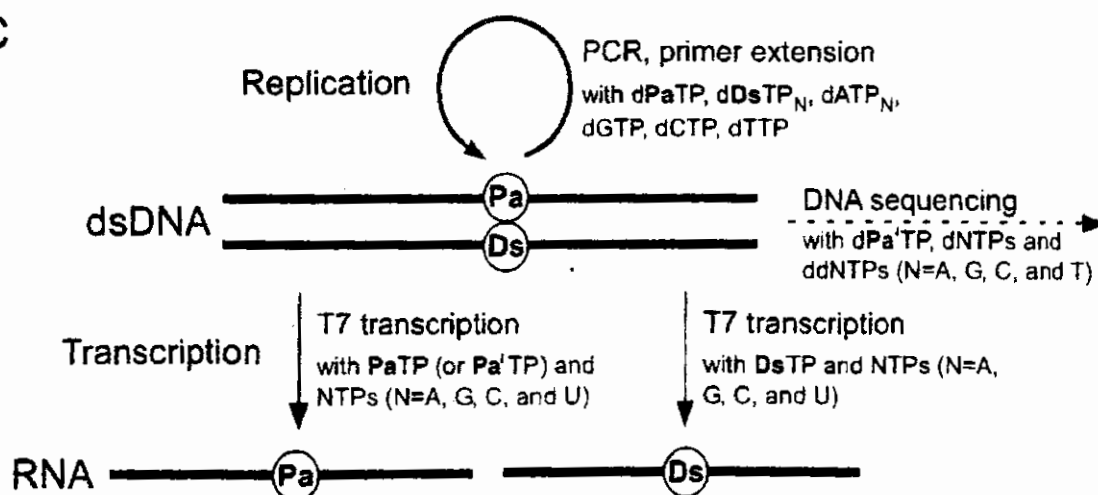


Figure 2a

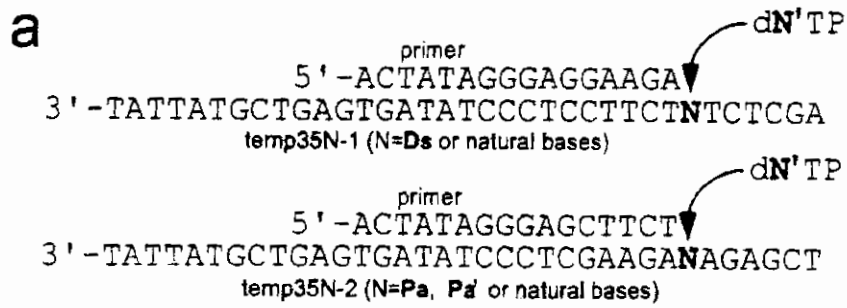


Figure 2b

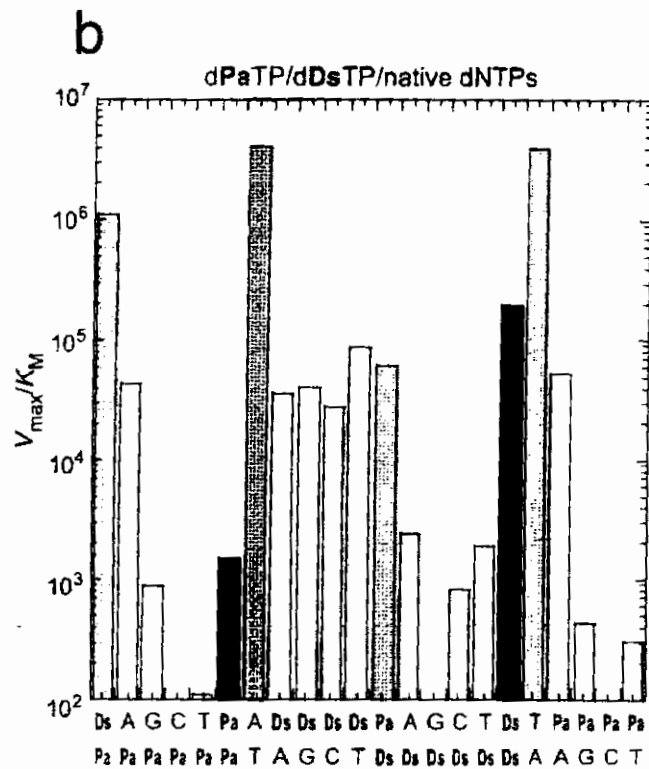


Figure 2c

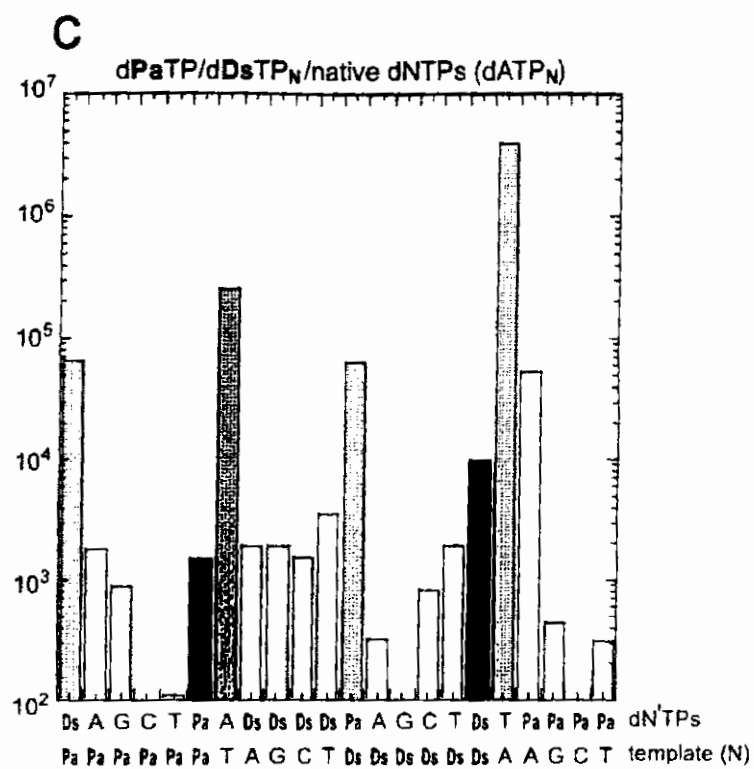


Figure 2d

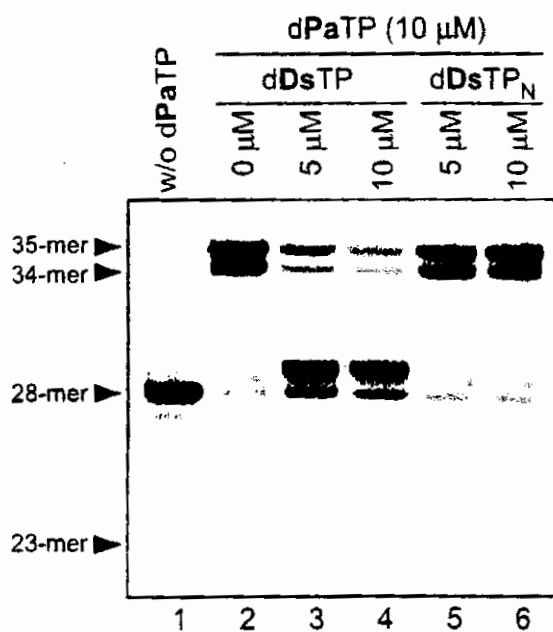
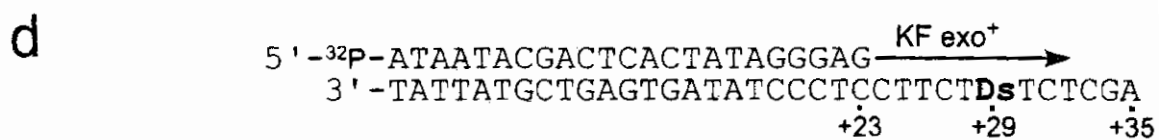


Figure 3d

d PCR 10 cycles, Sequencing with dPa' TP

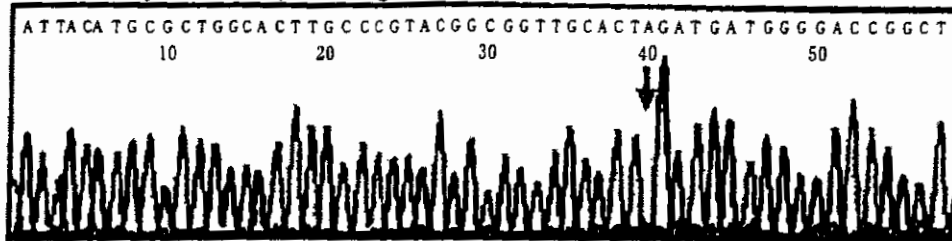


Figure 3e

e PCR 10+10 cycles, Sequencing with dPa' TP

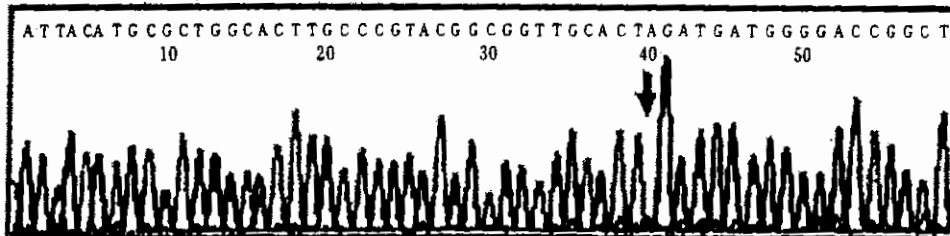


Figure 3f

f PCR 10 cycles without the Ds & Pa substrates. Sequencing with dPa' TP

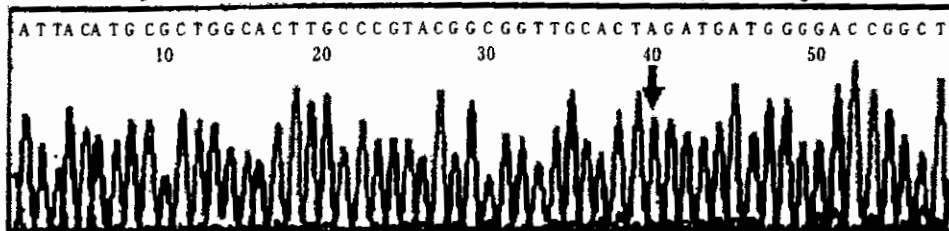


Figure 3g

g PCR 0 cycle, Sequencing without dPa' TP



Figure 3h

h PCR 10 cycles, Sequencing without dPa' TP

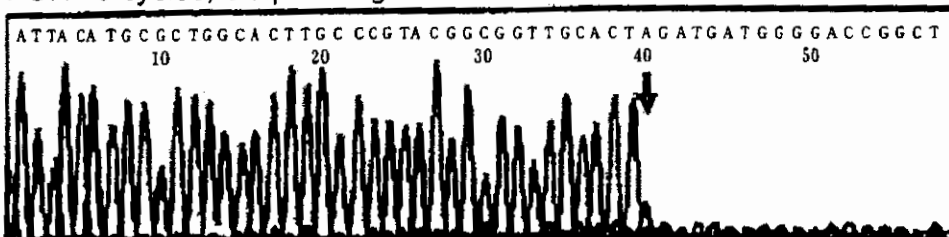


Figure 3i

i PCR 10+10 cycles, Sequencing without dPa' TP

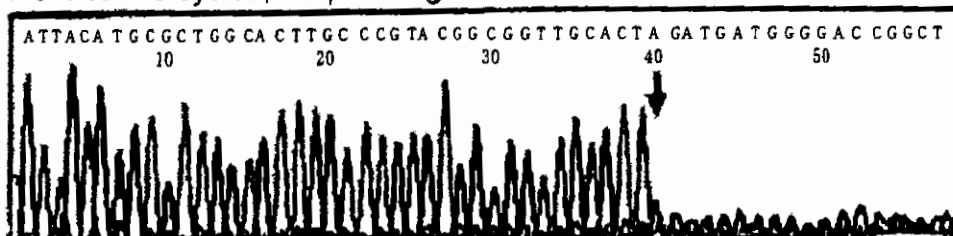


Figure 4a

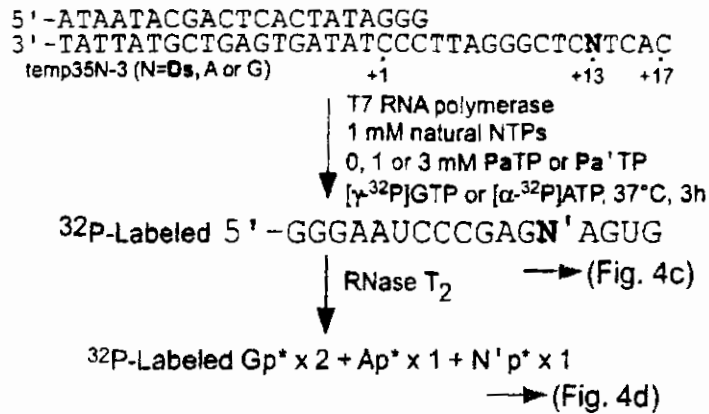
a

Figure 4b

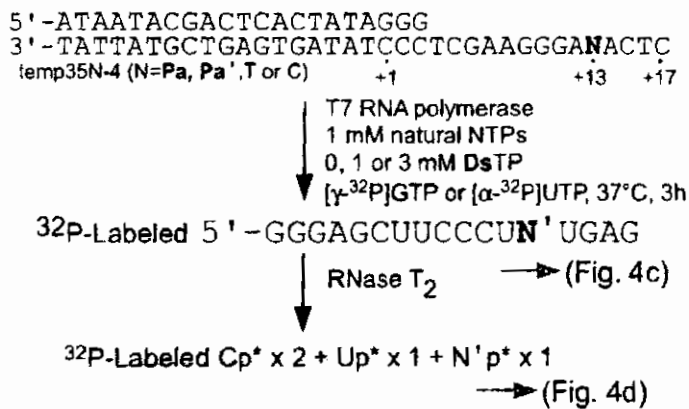
b

Figure 4c

C

Template (N)	Ds		A		Pa		Pa'		C
Unnatural NPT (1 mM)	Pa	Pa'	none	none	Ds	none	Ds	none	none
Relative yield (%)	29	28	8	100	91	30	71	33	100

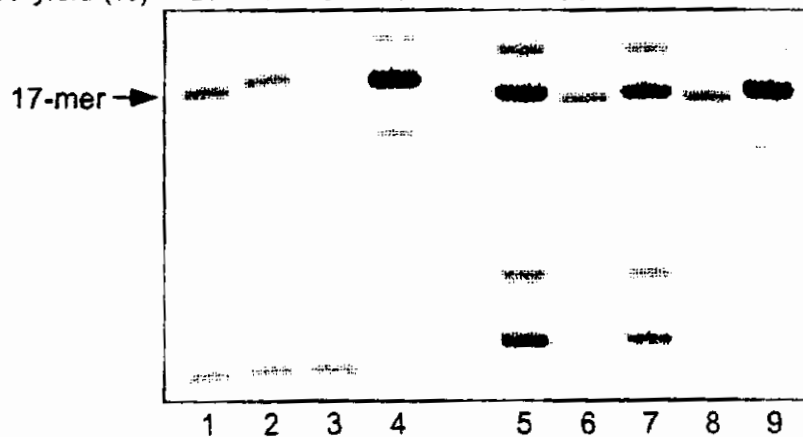


Figure 4d

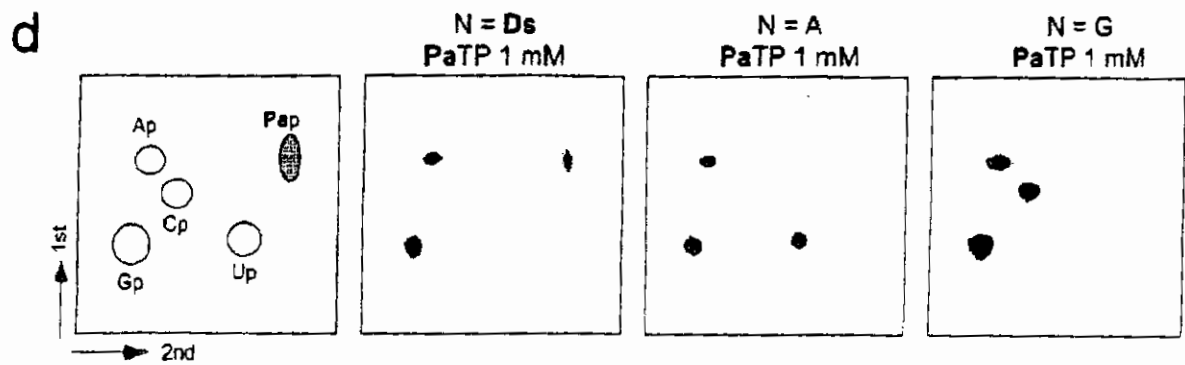


Figure 4e

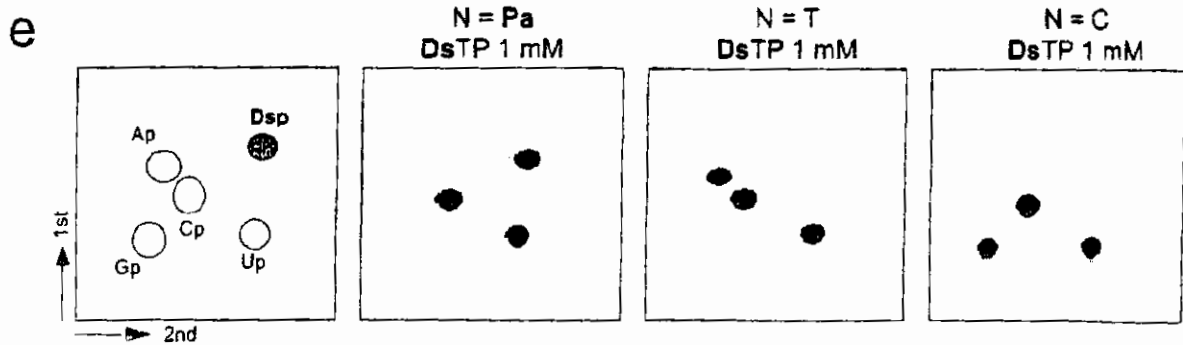


Figure 5a

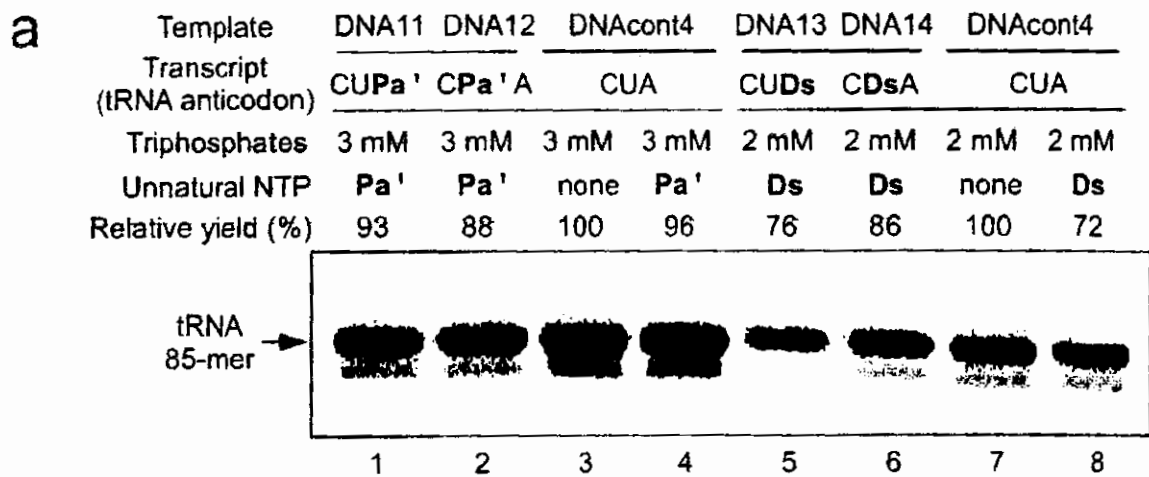


Figure 5b

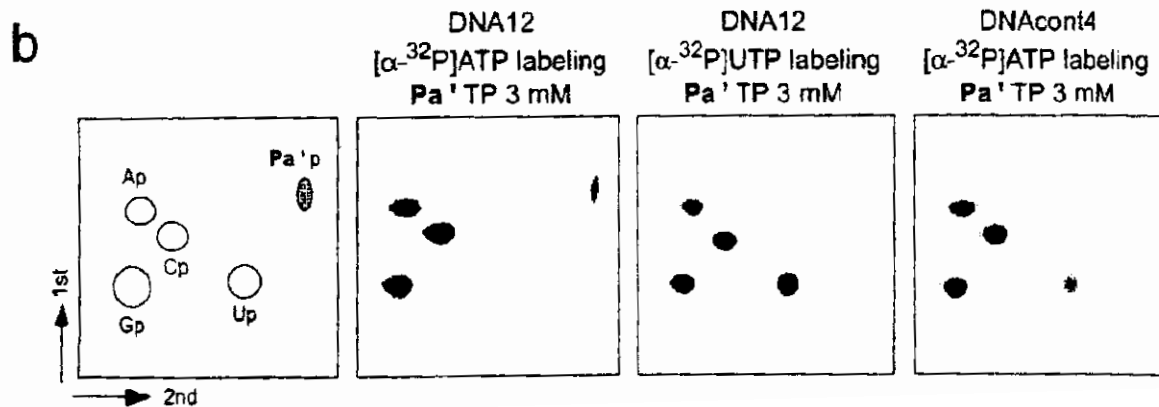


Figure 5c

C

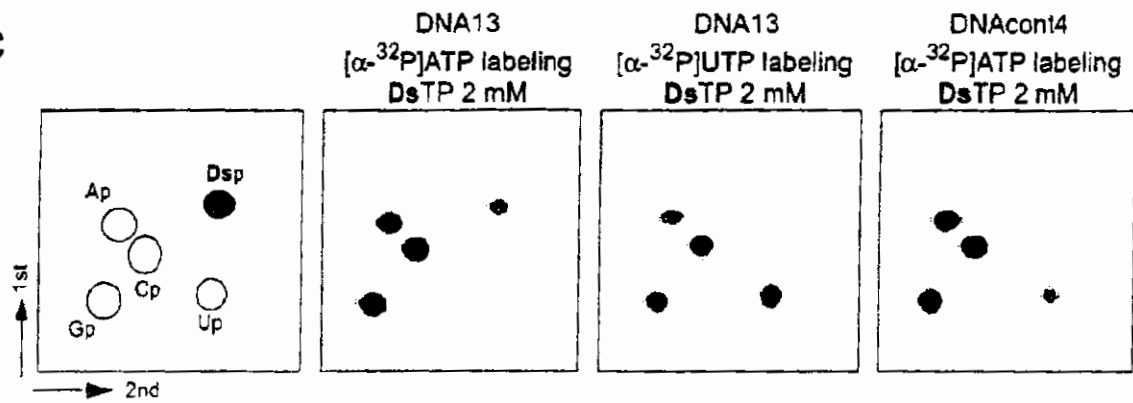


Figure 5d

d

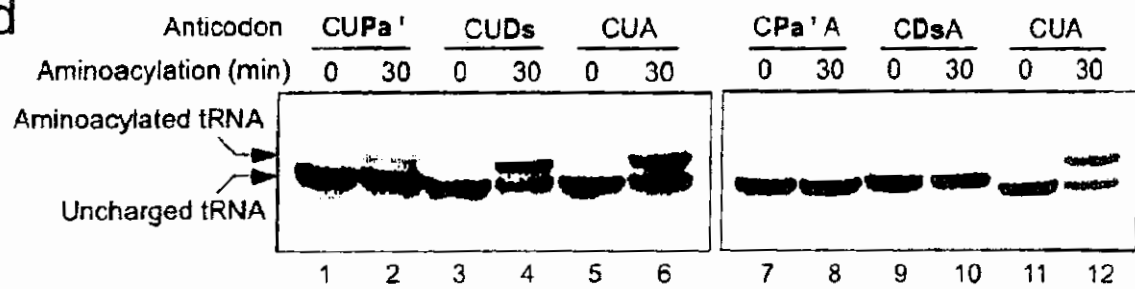
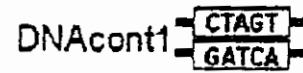
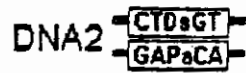
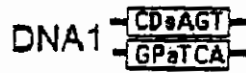
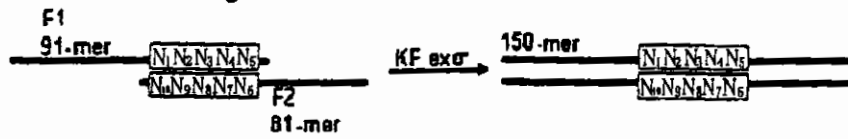
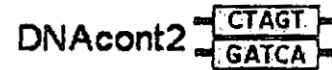
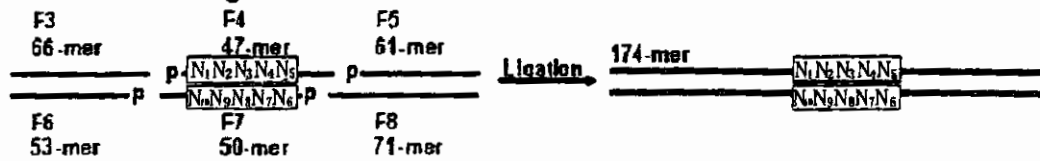


Figure 6

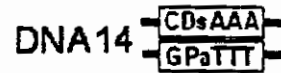
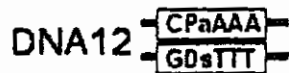
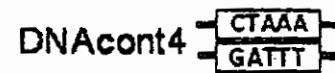
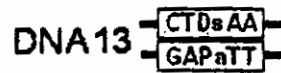
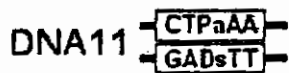
150-mer dsDNA fragments



174-mer dsDNA fragments



104-mer dsDNA fragments



174-mer dsDNA fragments

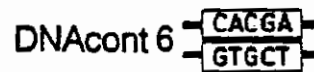
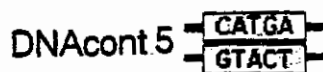
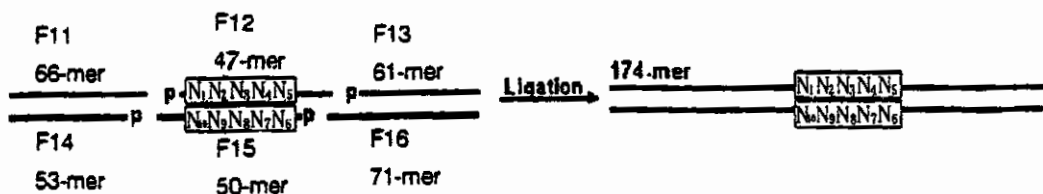


Figure 7a

Sequences of chemically synthesized DNA fragments.

F1 (91-mer)

5'-GAAATTAATACGACTCACTATAGGGTTAACTTTAAGAAGGAGATATACCATG
GGCTCCAAGAAGCCGGTCCCCATCATN₁N₂N₃N₄N₅GCAACCGC

F2 (81-mer)

5'-TTTCACACAGGAAACAGCTATGACCCGGGTATTACATGCGCTGGCACTTGC
CCGTACGGCGGTTGCN₆N₇N₈N₉N₁₀ATGATGGGG

F3 (66-mer)

5'-GAAATTAATACGACTCACTATAGGGTTAACTTTAAGAAGGAGATATACCATG
GGCTCCAAGAAGCC

F4 (47-mer)

5'-pGGTCCCCATCATN₁N₂N₃N₄N₅GCAACCGCCGTACGGGCAAGACGCAGCGCG

F5 (61-mer)

5'-pGGGGTTCTCATCATCATCATCATTAATAACCCGGGTCATAGCTGTTTCC
TGTGTGAAA

F6 (71-mer)

5'-TTTCACACAGGAAACAGCTATGACCCGGGTATTAATGATGATGATGATGAT
GAGAACCCCCGCGCTGCGT

F7 (50-mer)

5'-pCTTGCCCGTACGGCGGTTGCN₆N₇N₈N₉N₁₀ATGATGGGGACCGGCTTCTTGGA
GC

F8 (53-mer)

5'-pCCATGGTATATCTCCTTCTTAAAGTTAACCCTATAGTGAGTCGTATTAATTT
C

F9 (67-mer)

5'-GATAATACGACTCACTATAGGTGGGGTTCCCGAGCGGCCAAAGGGAGCAGA
CTN₁N₂N₃N₄N₅TCTGCCGTC

F10 (60-mer)

5'-TmGmGTGGTGGGGGAAGGATTCGAACCTTCGAAGTCTGTGACGGCAGAN₆N₇
N₈N₉N₁₀AGTCTGCTC (Tm=2'-O-methylthymidine, Gm=2'-O-methylguanosine)

Primer 1 (5'-primer for sequencing and PCR, 25-mer)

5'-GAAATTAATACGACTCACTATAGGG

Primer 2 (3'-primer for sequencing and PCR, 24-mer)

5'-TTTCACACAGGAAACAGCTATGAC

Figure 7b

Sequences of chemically synthesized DNA fragments.

F11 (66-mer)

5'-GAAATTAATACGACTCACTATAGGGCCAACCAGAAGAAGGAGACAGACCAA
GGGCACCAAGAAGCC

F12 (47-mer)

5'-pGGACCCCAACAA CANGAGCAACCGCCGAACGGGCAAGACGCAGCGCG

F13 (61-mer)

5'-pGGGGAACACAACAGCACCAACAGCACCAACAACCCGGGACAAAGCAGCAA
CCAGGGAGAAA

F14 (53-mer)

5'-CCTTGGTCTGTCTCCTTCTTCTGGTTGGCCCTATAGTGAGTCGTATTAATTTC

F15 (50-mer)

5'-pCTTGCCCGTTCGGCGGTTGCTCNTGTTGTTGGGGTCCGGCTTCTTGGTG
C

F16 (71-mer)

5'-TTTCTCCCTGGTTGCTGCTTTGTCCCGGGTTGTTGGTGCTGTTGGTGCTGTTG
TGTTCCCCCGCGCTGCGT

Figure 8

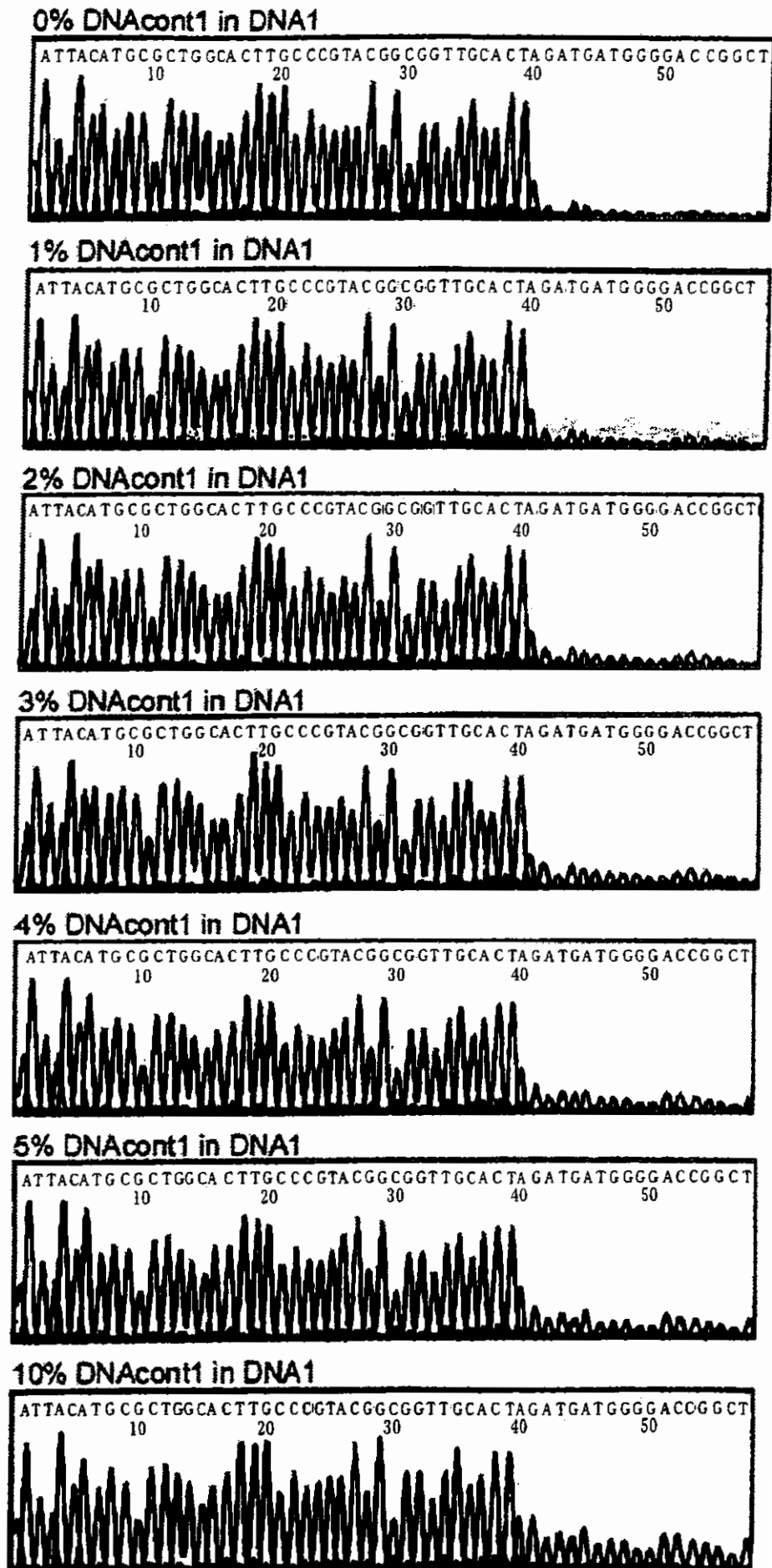
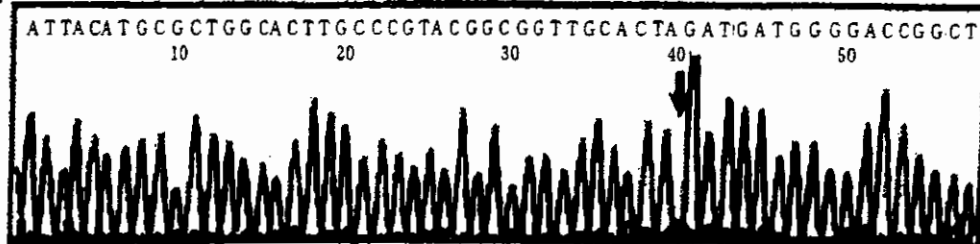


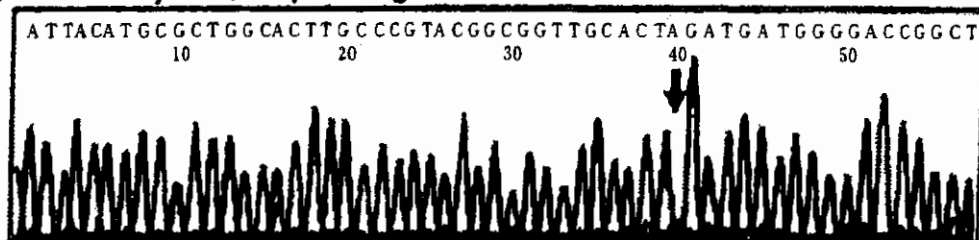
Figure 9

DNA1: PCR 300 μ M dDsTP_N, dPaTP, dATP, dGTP, dCTP, dTTP

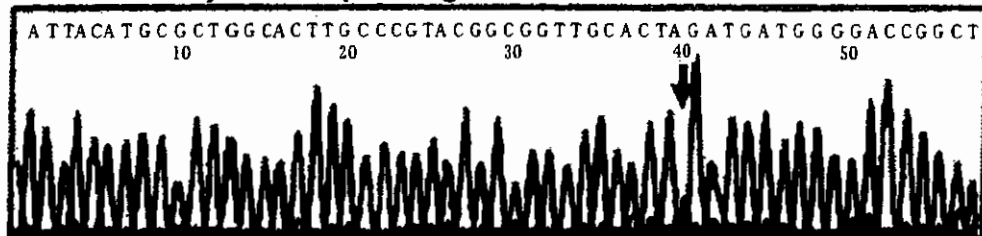
a PCR 0 cycle, Sequencing with dPa' TP



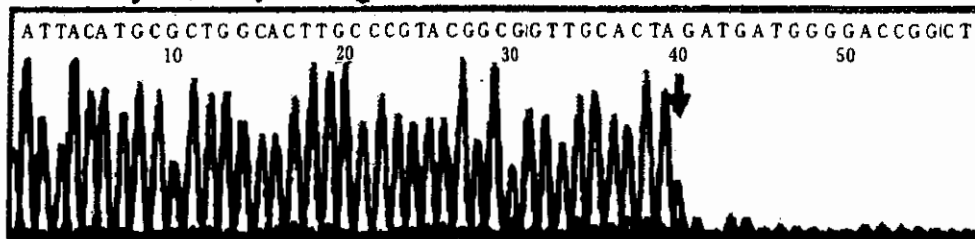
b PCR 10 cycles, Sequencing with dPa' TP



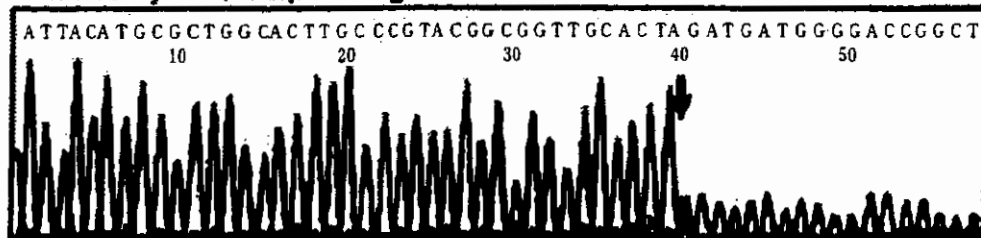
c PCR 10+10 cycles, Sequencing with dPa' TP



d PCR 0 cycle, Sequencing without dPa' TP



e PCR 10 cycles, Sequencing without dPa' TP



f PCR 10+10 cycles, Sequencing without dPa' TP



Figure 11

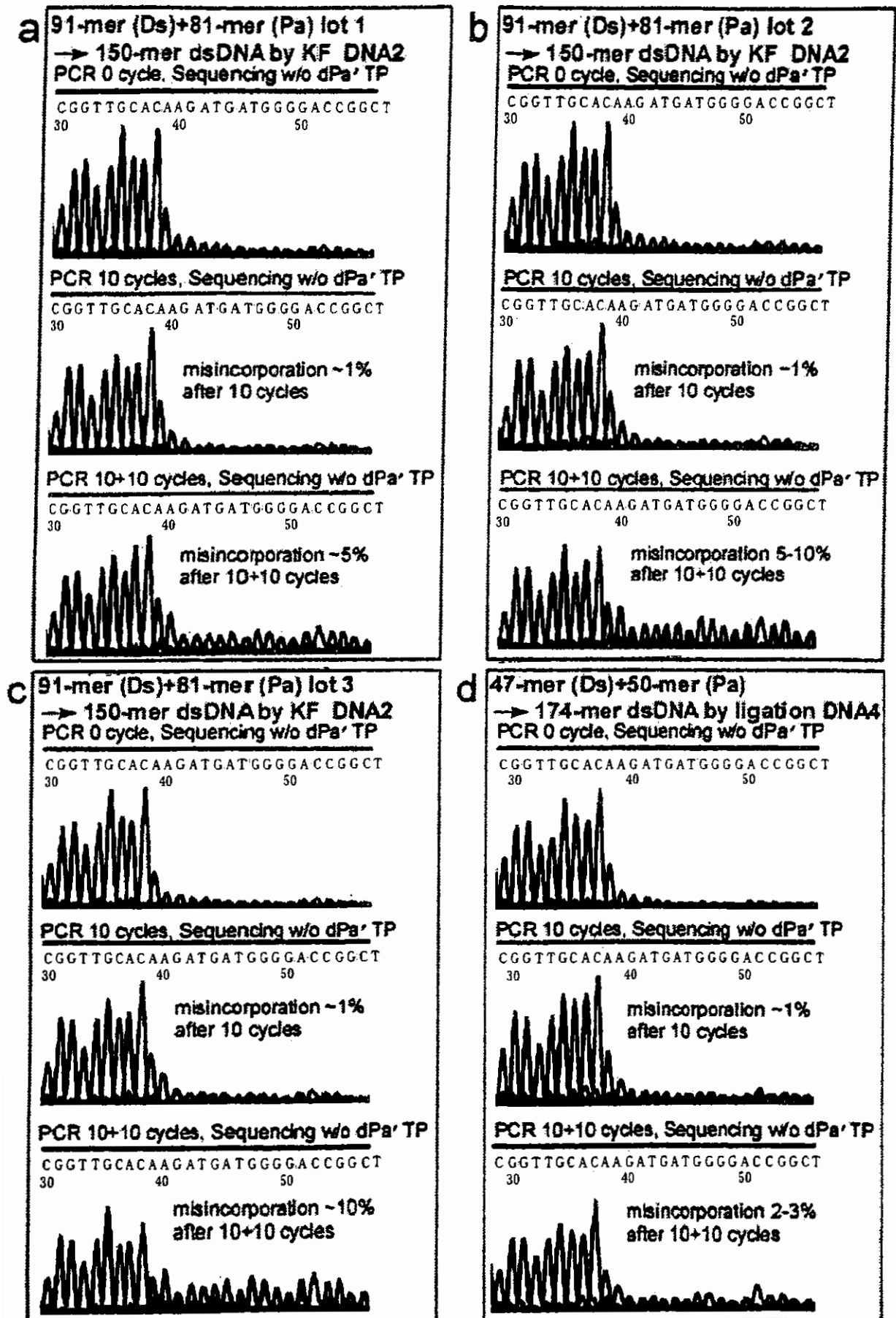


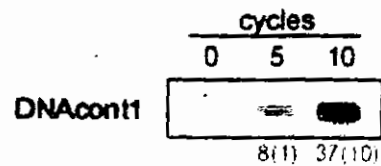
Figure 12

Agarose Gel for DNA1 and DNAcont1 (150 bp)

[94°C 30 sec - 45°C 30 sec - 65°C 4 min] x 10 cycles (Vent DNA polymerase 4 units/100 µl)
 300 µM dATP_N, dD₃TP_N, dPaTP, dGTP, dCTP, dTTP



[94°C 30 sec - 45°C 30 sec - 72°C 1 min] x 10 cycles (Vent DNA polymerase 1 unit/100 µl)
 200 µM dATP, dGTP, dCTP, dTTP

**Agarose Gel for DNA3-10 (174 bp)**

[94°C 30 sec - 45°C 30 sec - 65°C 4 min] x 10 cycles (Vent DNA polymerase 4 units/100 µl)
 300 µM dATP_N, dD₃TP_N, dPaTP, dGTP, dCTP, dTTP

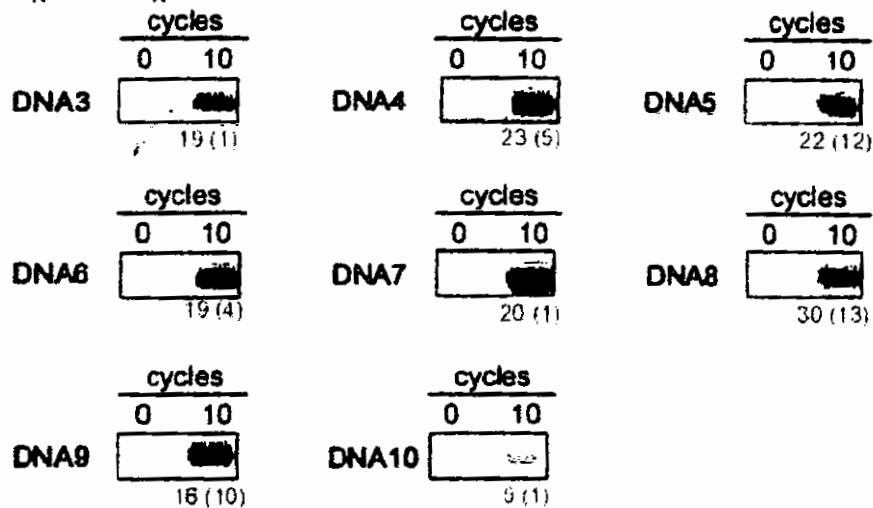


Figure 13

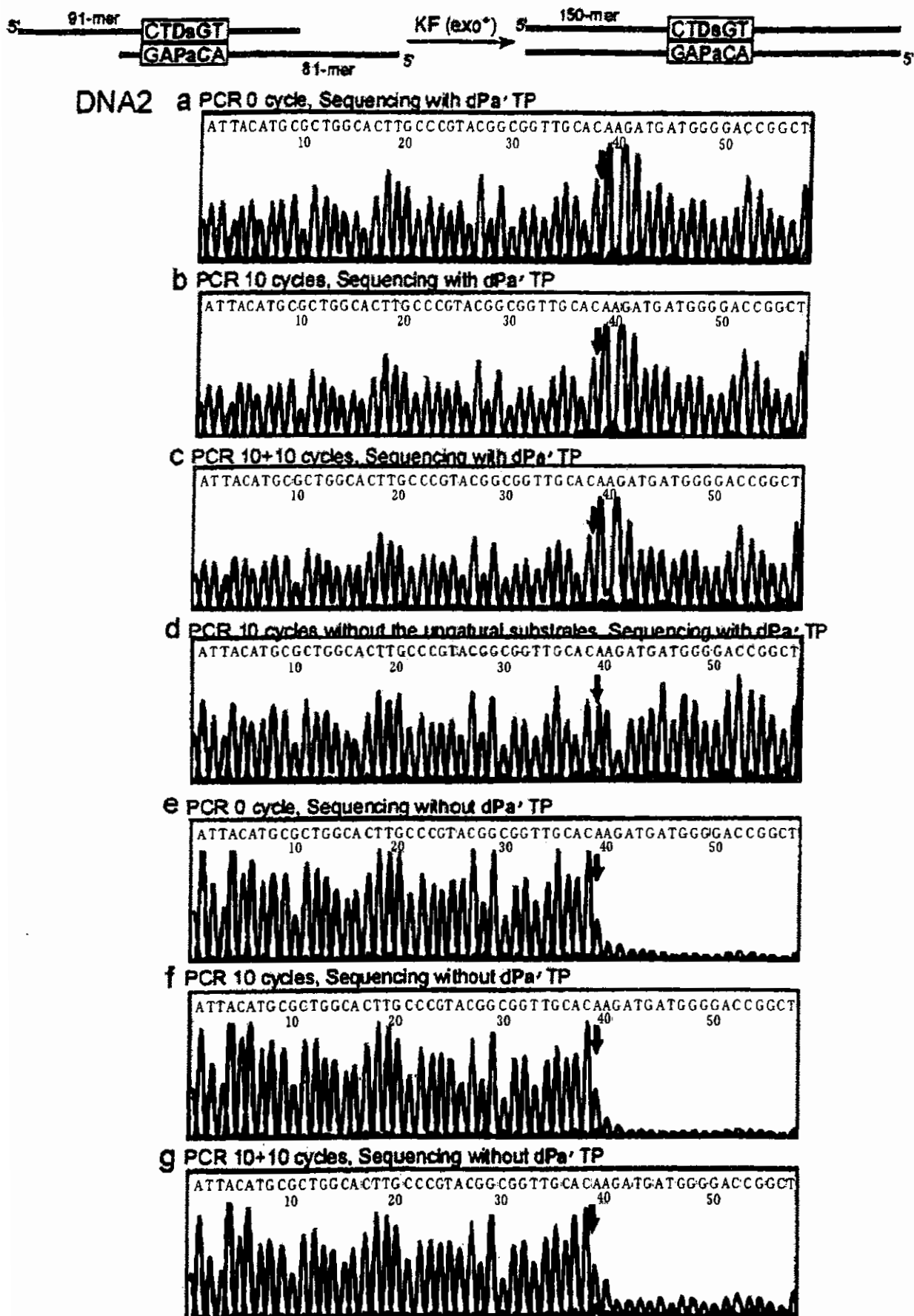
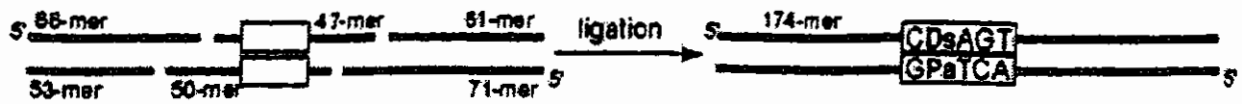
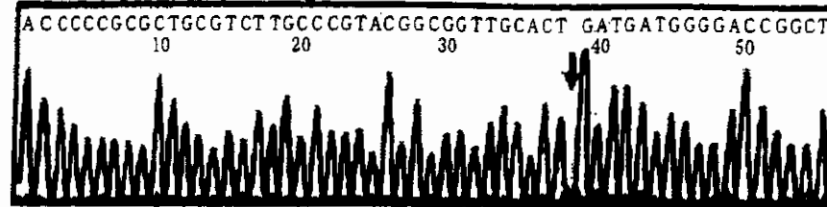


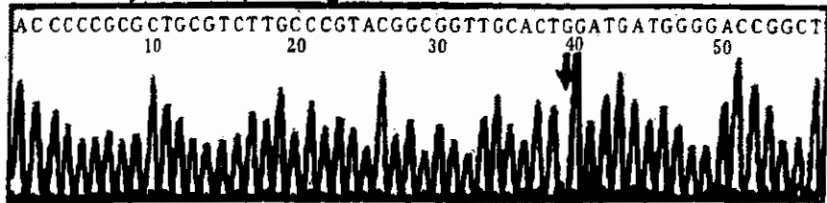
Figure 14



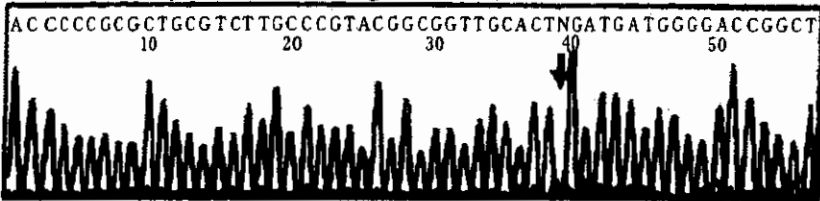
DNA3 a PCR 0 cycle, Sequencing with dPa' TP



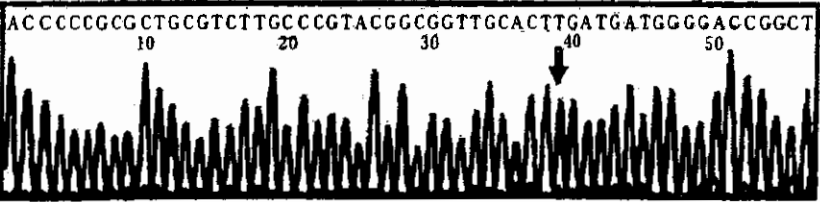
b PCR 10 cycles, Sequencing with dPa' TP



c PCR 10+10 cycles, Sequencing with dPa' TP



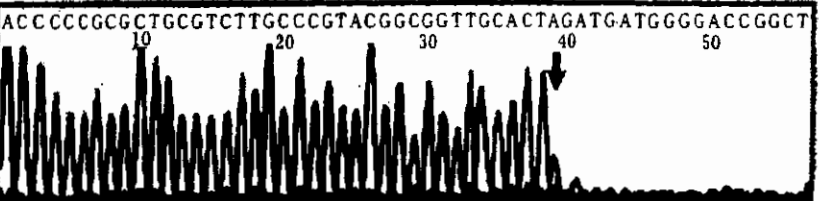
d PCR 10 cycles without the unnatural substrates, Sequencing with dPa' TP



e PCR 0 cycle, Sequencing without dPa' TP



f PCR 10 cycles, Sequencing without dPa' TP



g PCR 10+10 cycles, Sequencing without dPa' TP

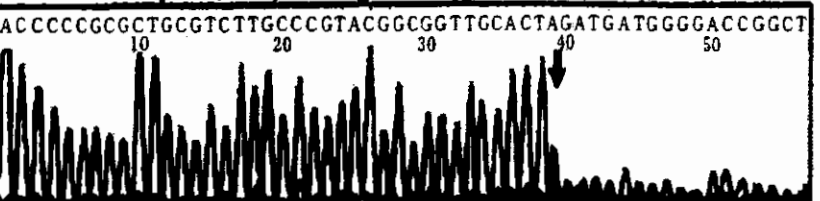
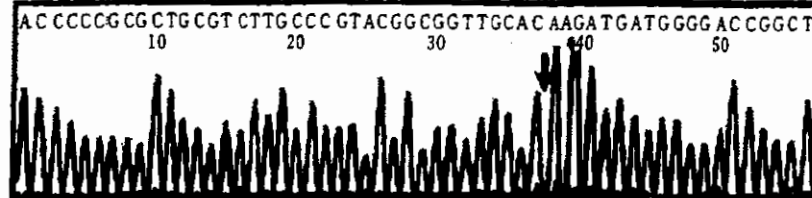


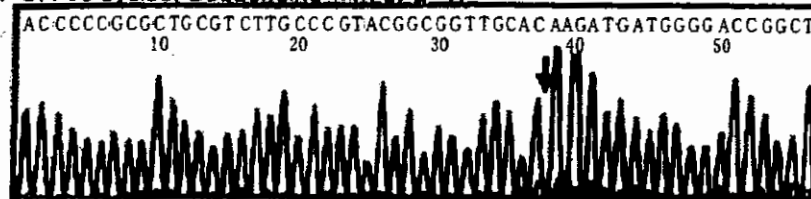
Figure 15



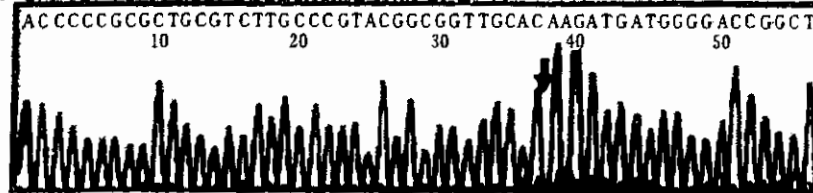
DNA4 a PCR 0 cycle. Sequencing with dPa' TP



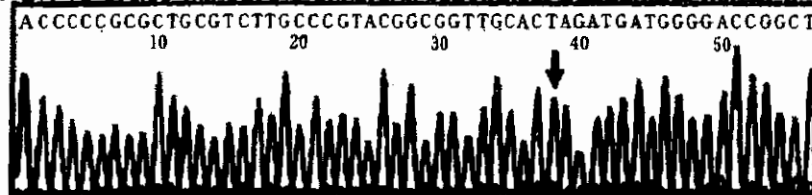
b PCR 10 cycles. Sequencing with dPa' TP



c PCR 10+10 cycles. Sequencing with dPa' TP



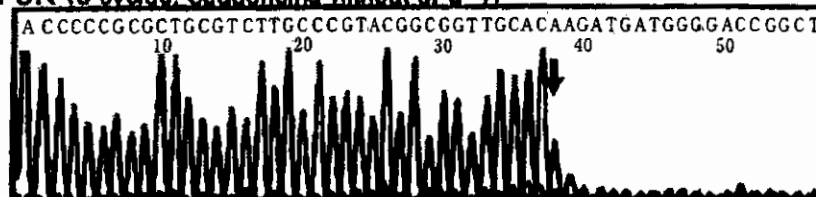
d PCR 10 cycles without the unnatural substrates. Sequencing with dPa' TP



e PCR 0 cycle. Sequencing without dPa' TP



f PCR 10 cycles. Sequencing without dPa' TP



g PCR 10+10 cycles. Sequencing without dPa' TP

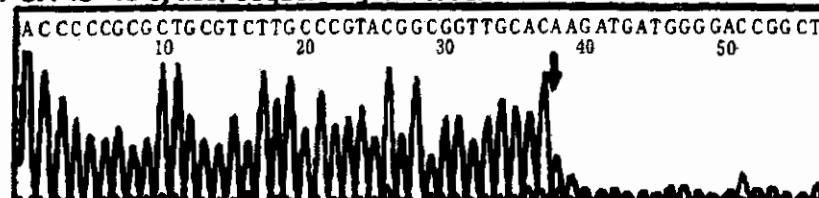
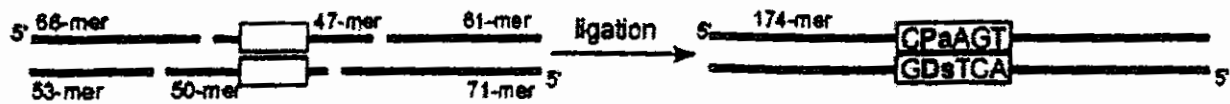
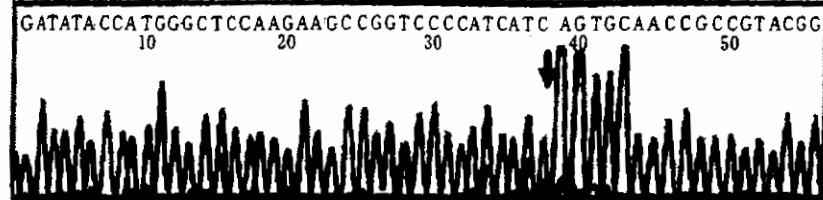


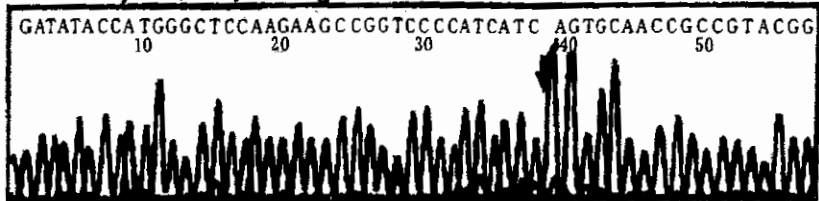
Figure 16



DNA5 a PCR 0 cycle, Sequencing with dPa' TP



b PCR 10 cycles, Sequencing with dPa' TP



c PCR 10 cycles without the unnatural substrates, Sequencing with dPa' TP



d PCR 0 cycle, Sequencing without dPa' TP



e PCR 10 cycles, Sequencing without dPa' TP

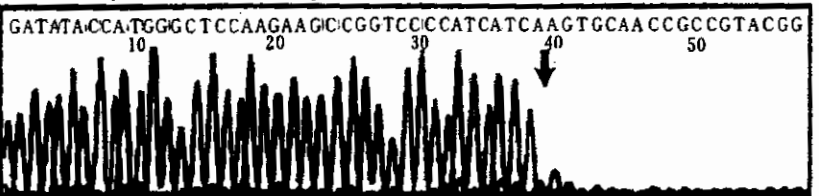
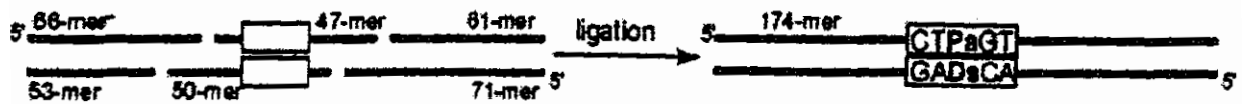
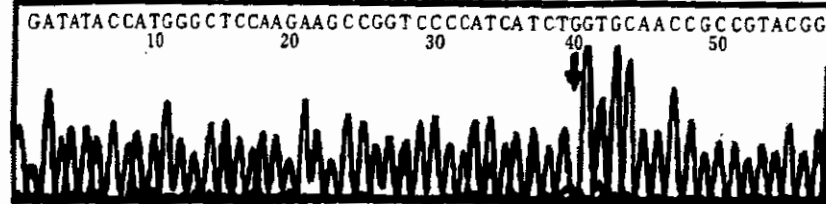


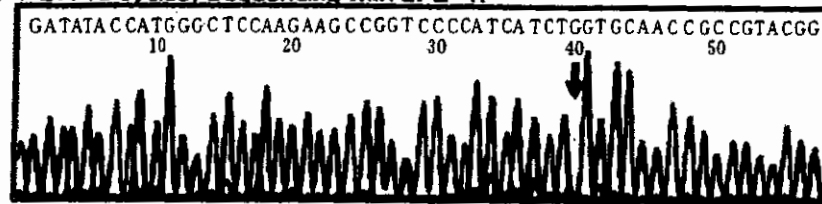
Figure 17



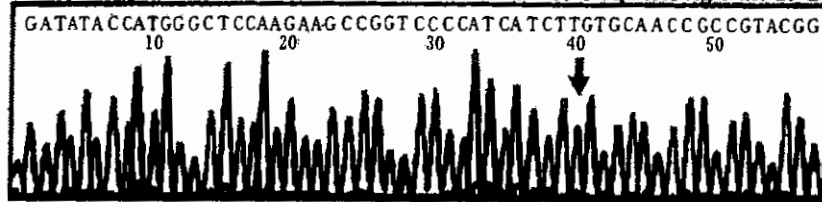
DNA6 a PCR 0 cycle, Sequencing with dPa' TP



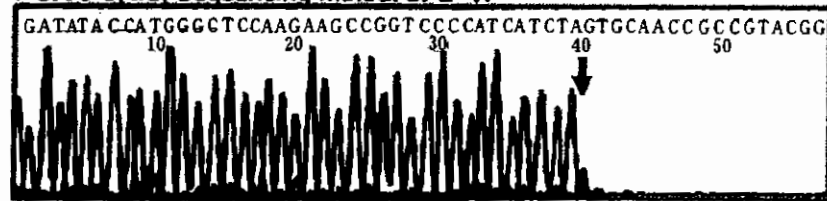
b PCR 10 cycles, Sequencing with dPa' TP



c PCR 10 cycles without the unnatural substrates, Sequencing with dPa' TP



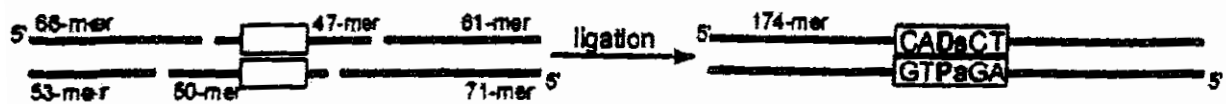
d PCR 0 cycle, Sequencing without dPa' TP



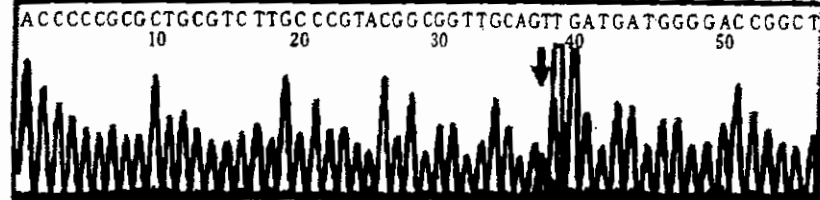
e PCR 10 cycles, Sequencing without dPa' TP



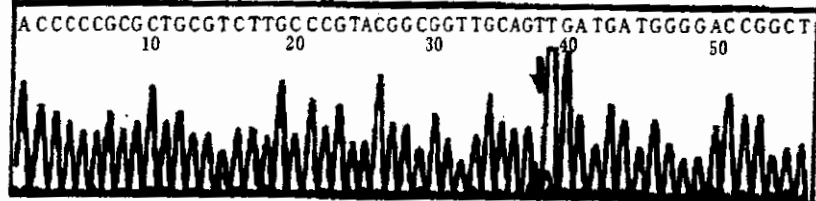
Figure 18



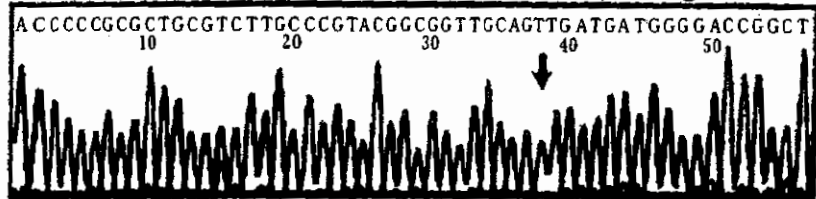
DNA7 a PCR 0 cycle, Sequencing with dPa' TP



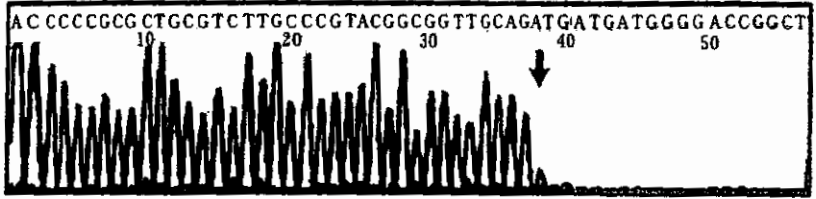
b PCR 10 cycles, Sequencing with dPa' TP



c PCR 10 cycles without the unnatural substrates, Sequencing with dPa' TP



d PCR 0 cycle, Sequencing without dPa' TP



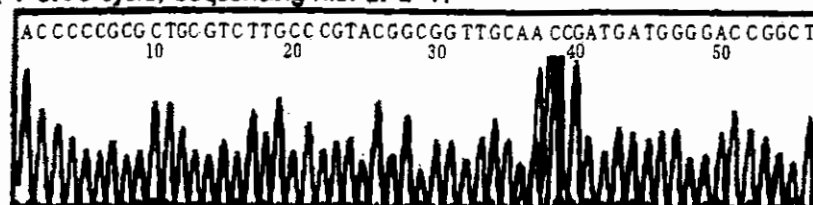
e PCR 10 cycles, Sequencing without dPa' TP



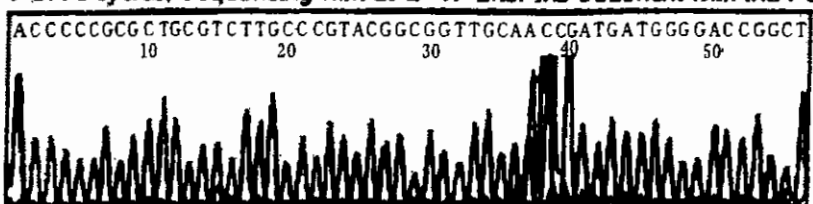
Figure 19



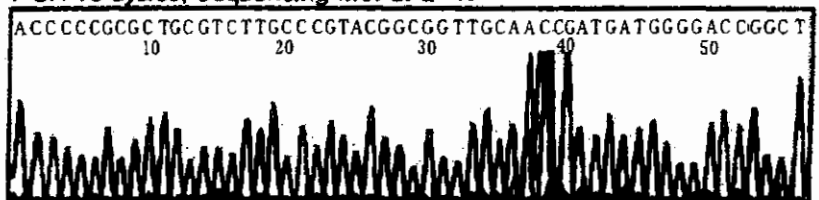
DNA8 a PCR 0 cycle, Sequencing with dPa' TP



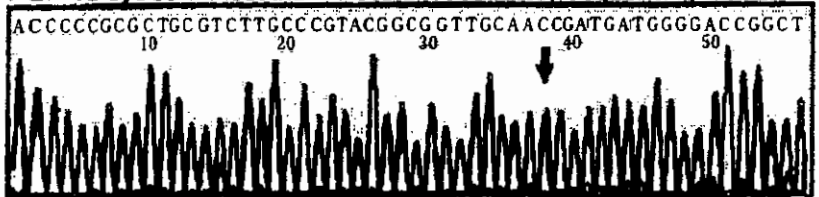
b PCR 0 cycles, Sequencing with dPa' TP after the treatment with the PCR buffer



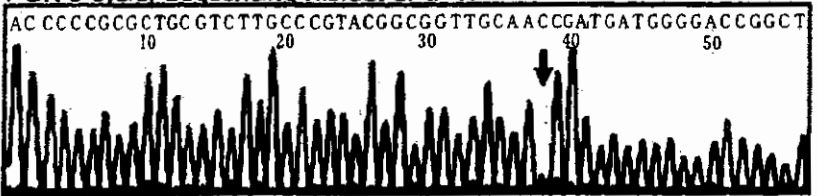
c PCR 10 cycles, Sequencing with dPa' TP



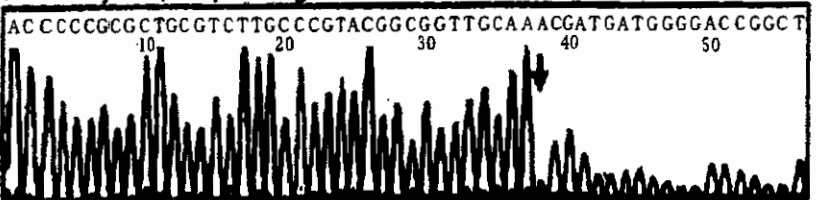
d PCR 10 cycles without the unnatural substrates, Sequencing with dPa' TP



e PCR 0 cycle, Sequencing without dPa' TP



f PCR 0 cycles, Sequencing without dPa' TP after the treatment with the PCR buffer



g PCR 10 cycles, Sequencing without dPa' TP

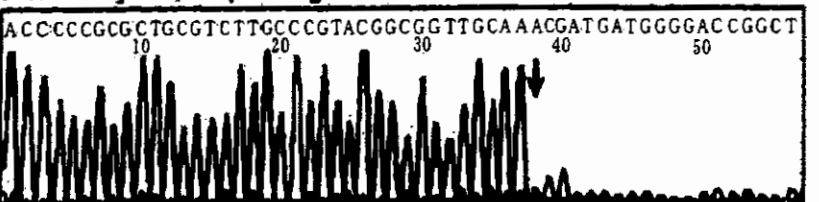
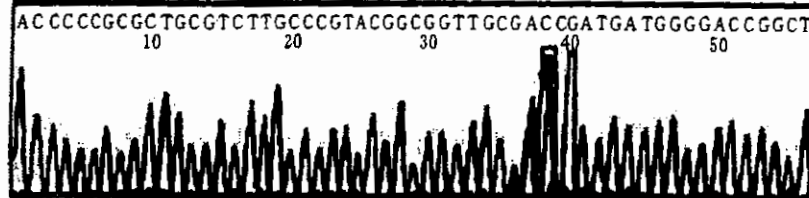


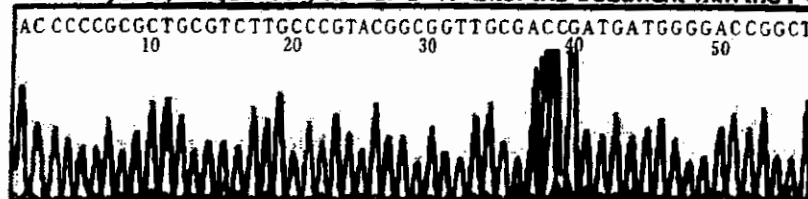
Figure 20



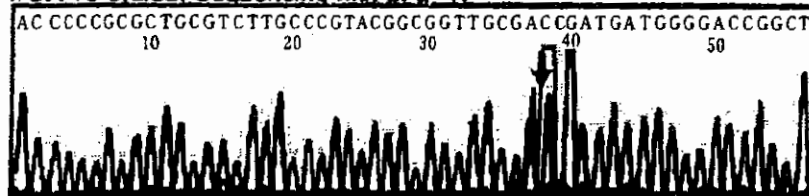
DNA9 a PCR 0 cycle, Sequencing with dPa' TP



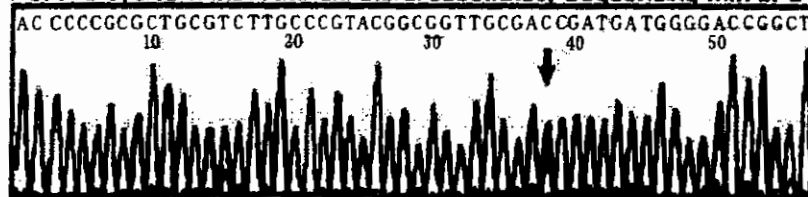
b PCR 0 cycles, Sequencing with dPa' TP after the treatment with the PCR buffer



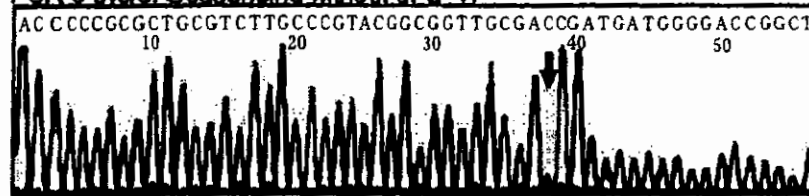
c PCR 10 cycles, Sequencing with dPa' TP



d PCR 10 cycles without the unnatural substrates, Sequencing with dPa' TP



e PCR 0 cycle, Sequencing without dPa' TP



f PCR 0 cycles, Sequencing without dPa' TP after the treatment with the PCR buffer



g PCR 10 cycles, Sequencing without dPa' TP

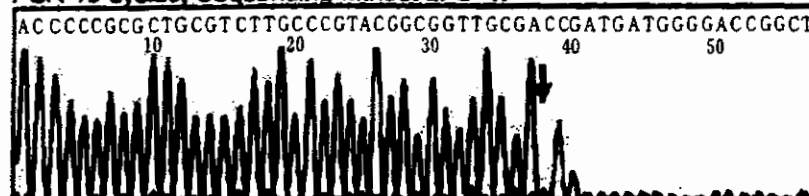


Figure 24a

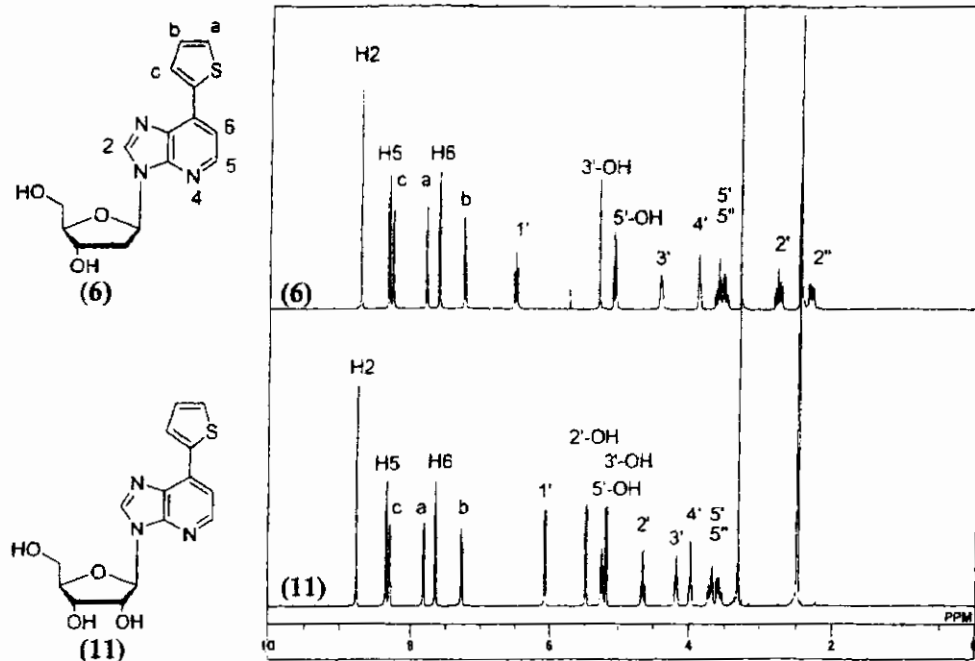
 ^1H NMR (270MHz, $\text{DMSO}-d_6$) spectra of compound 6 and 11.

Figure 24b

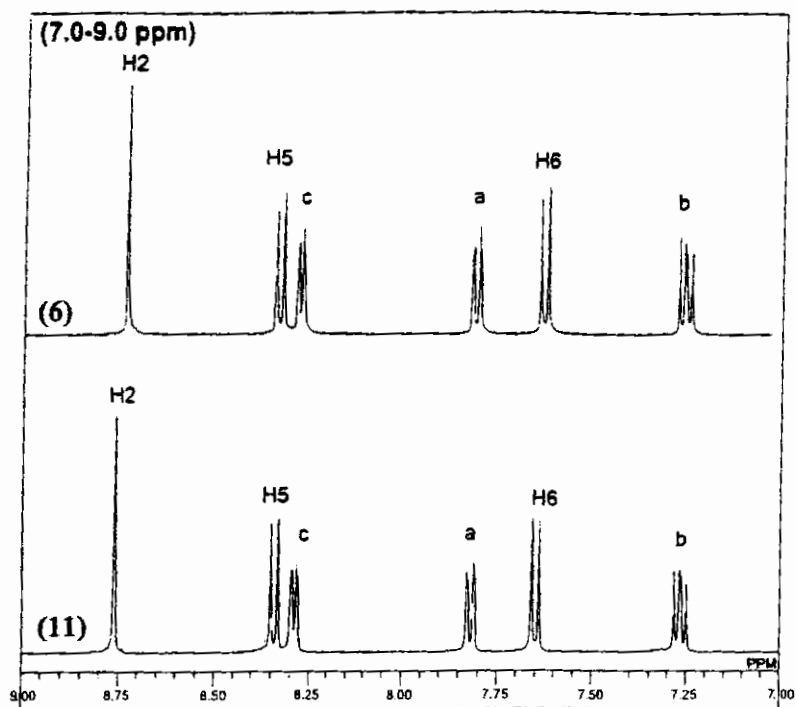
 ^1H NMR (270 MHz, $\text{DMSO}-d_6$) spectra of compound 6 and 11.

Figure 24c

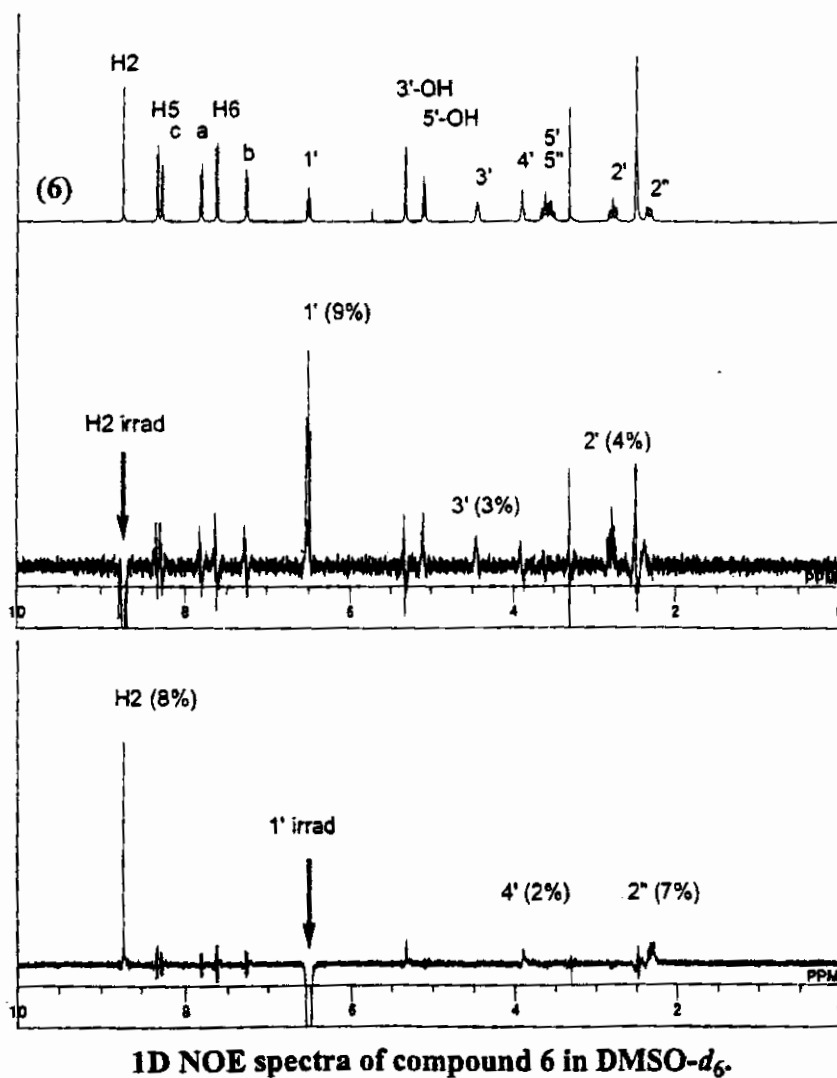


Figure 24d

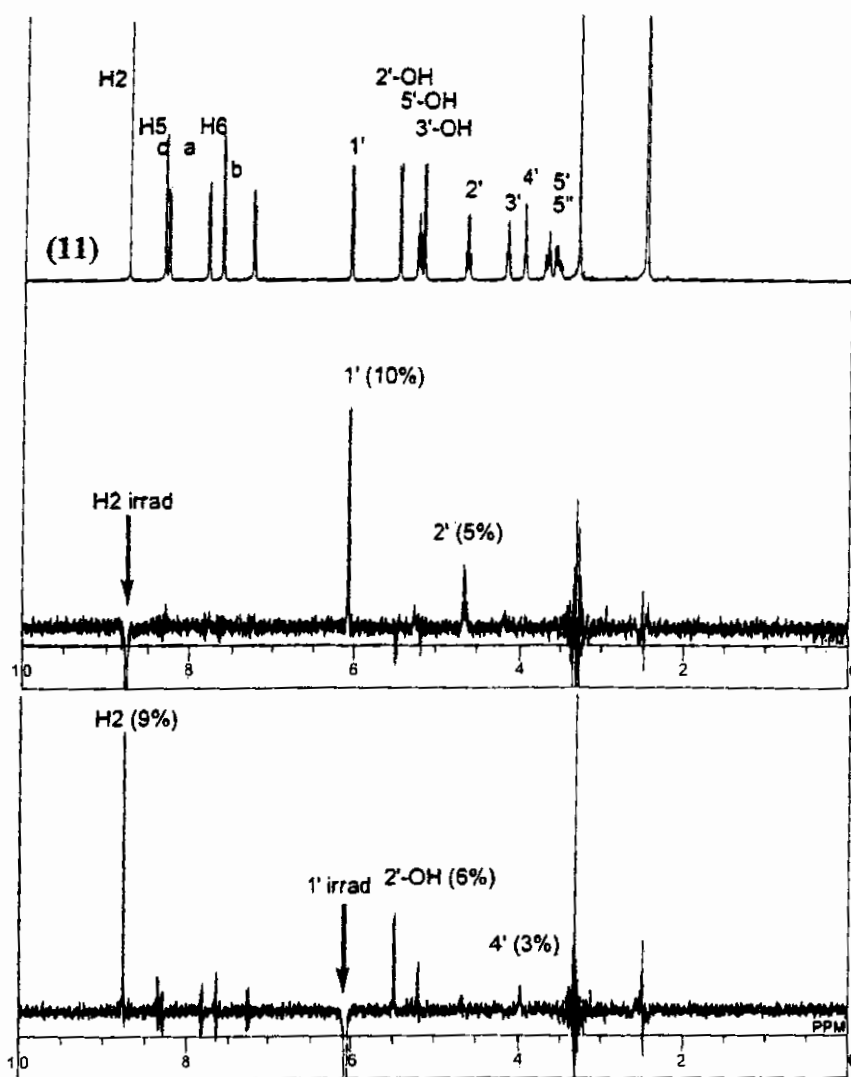
1D NOE spectra of compound 11 in DMSO- d_6 .

Figure 24e

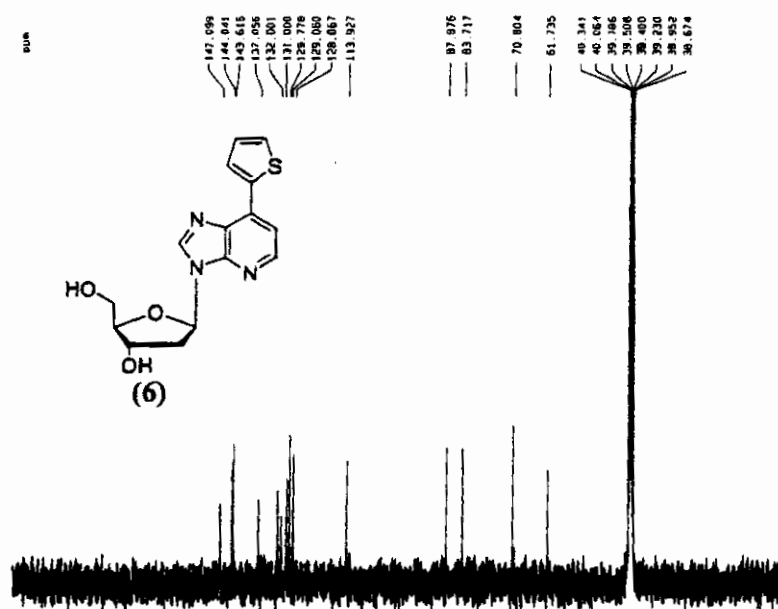
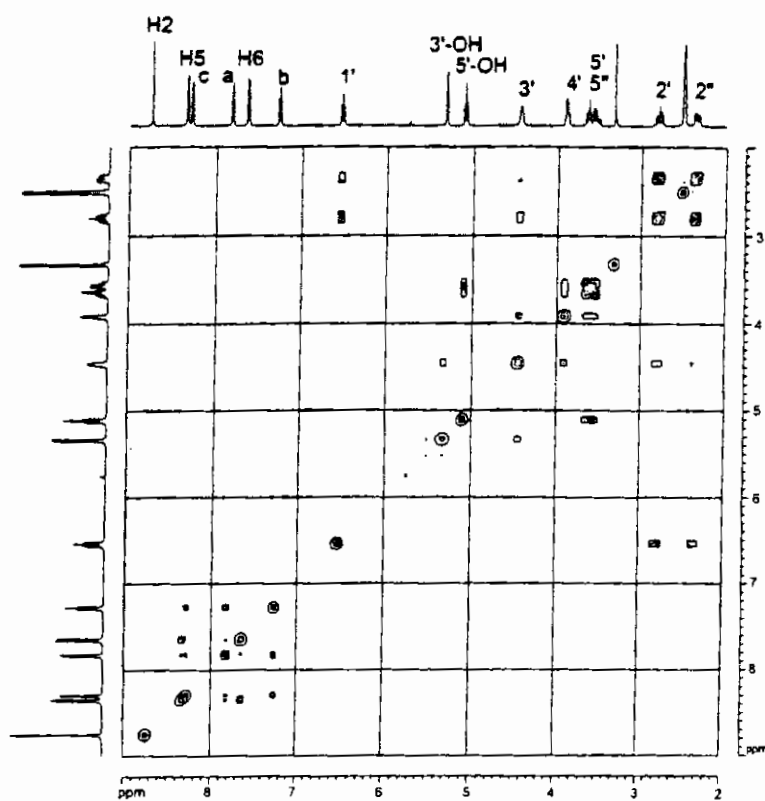
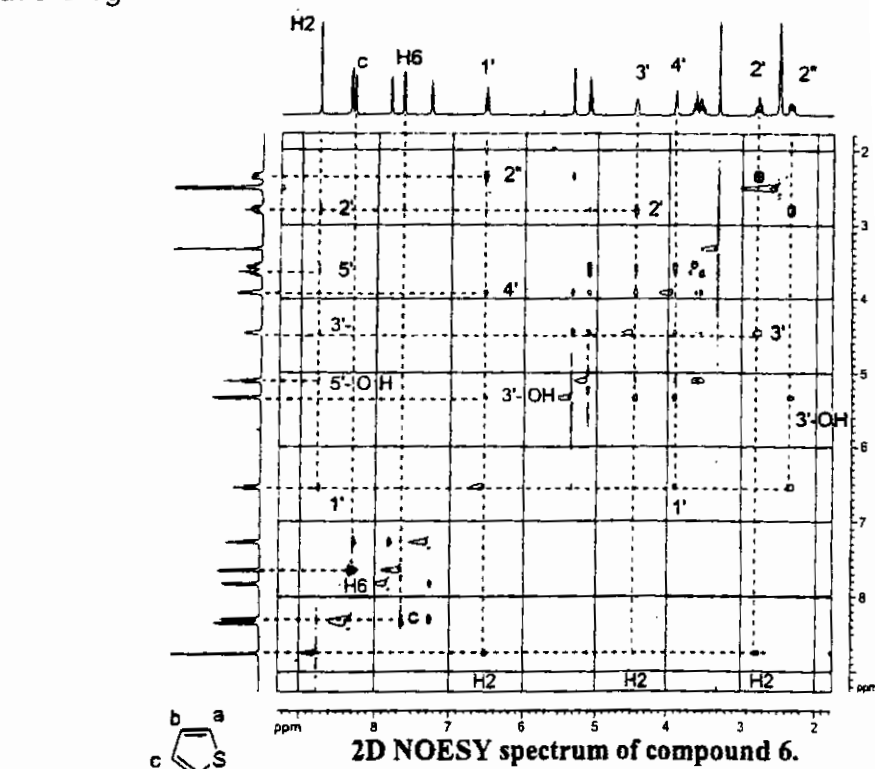
 ^{13}C NMR (75MHz, DMSO- d_6) spectrum of compound 6.

Figure 24f



2D COSY spectrum of compound 6.

Figure 24g



2D NOESY spectrum of compound 6.

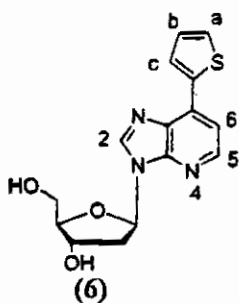


Figure 24j

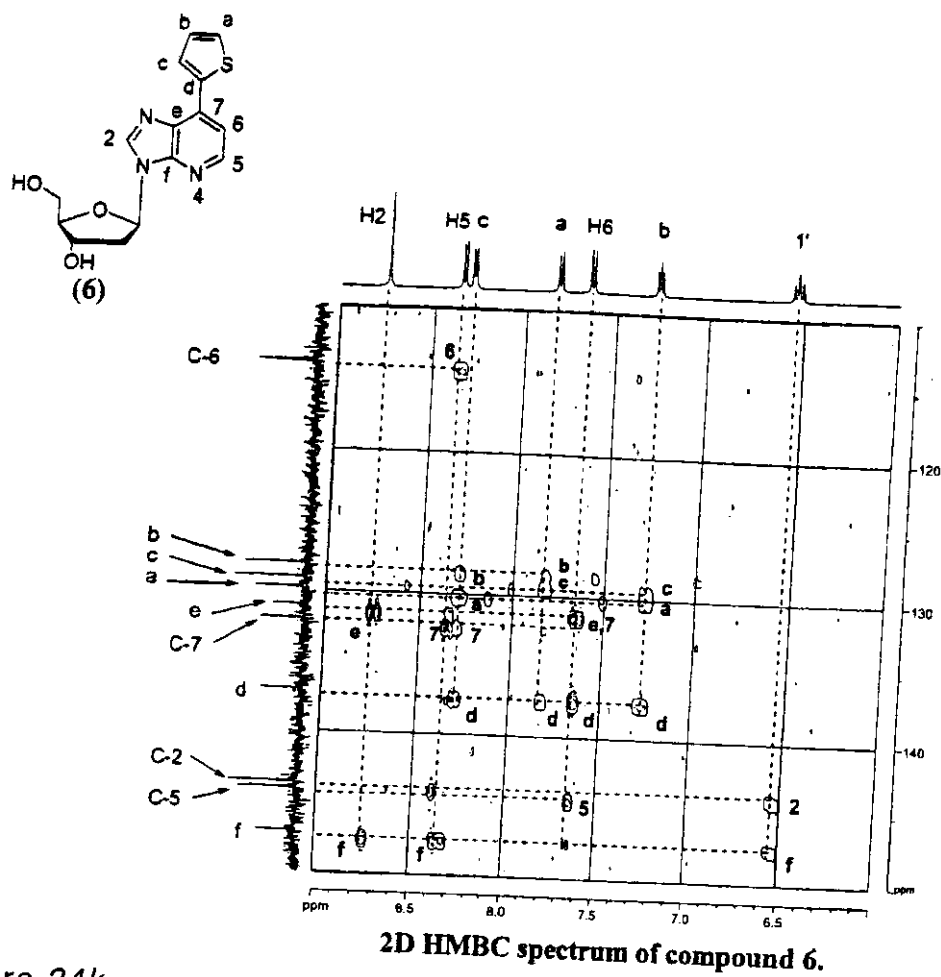


Figure 24k

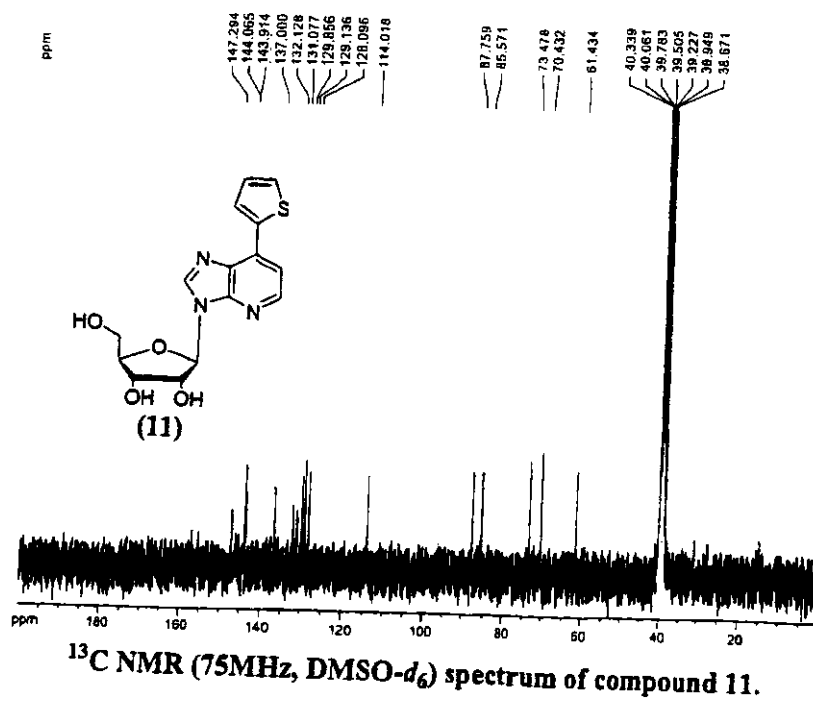
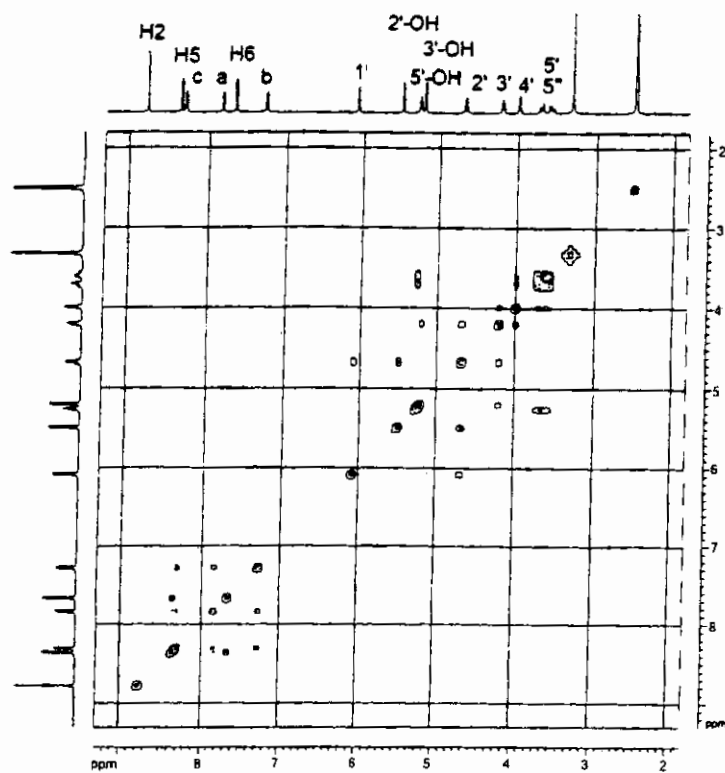
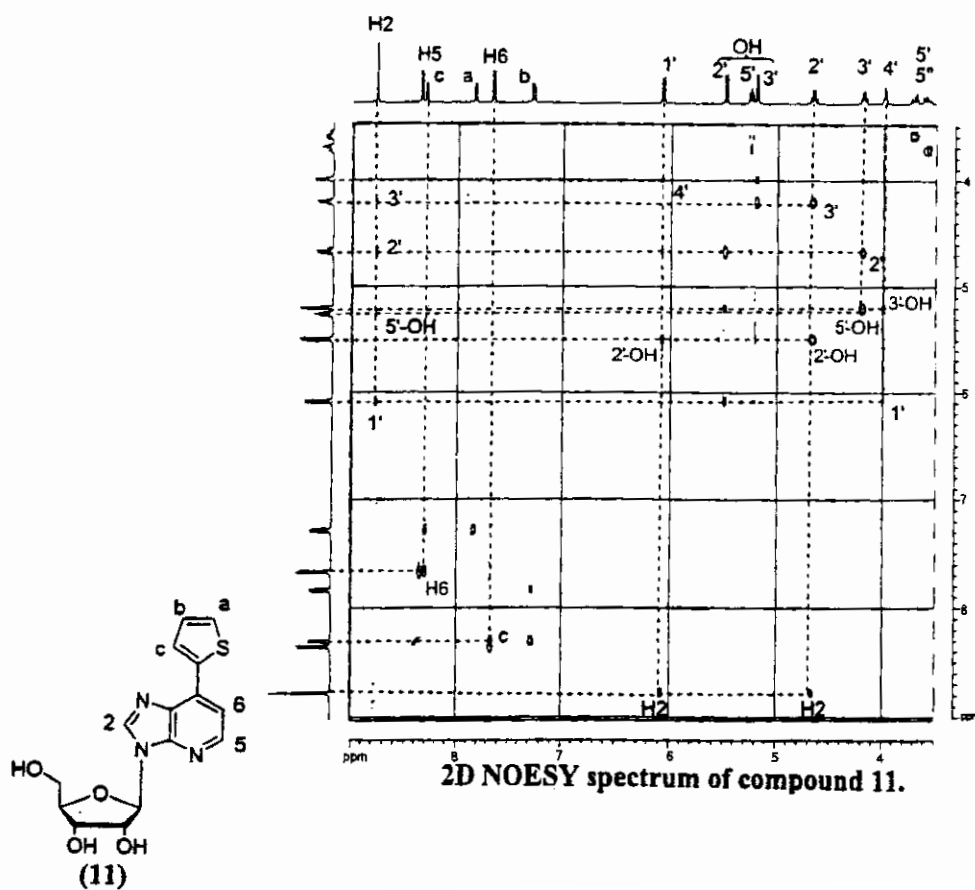


Figure 24l



2D COSY spectrum of compound 11.

Figure 24m



2D NOESY spectrum of compound 11.

Figure 24n

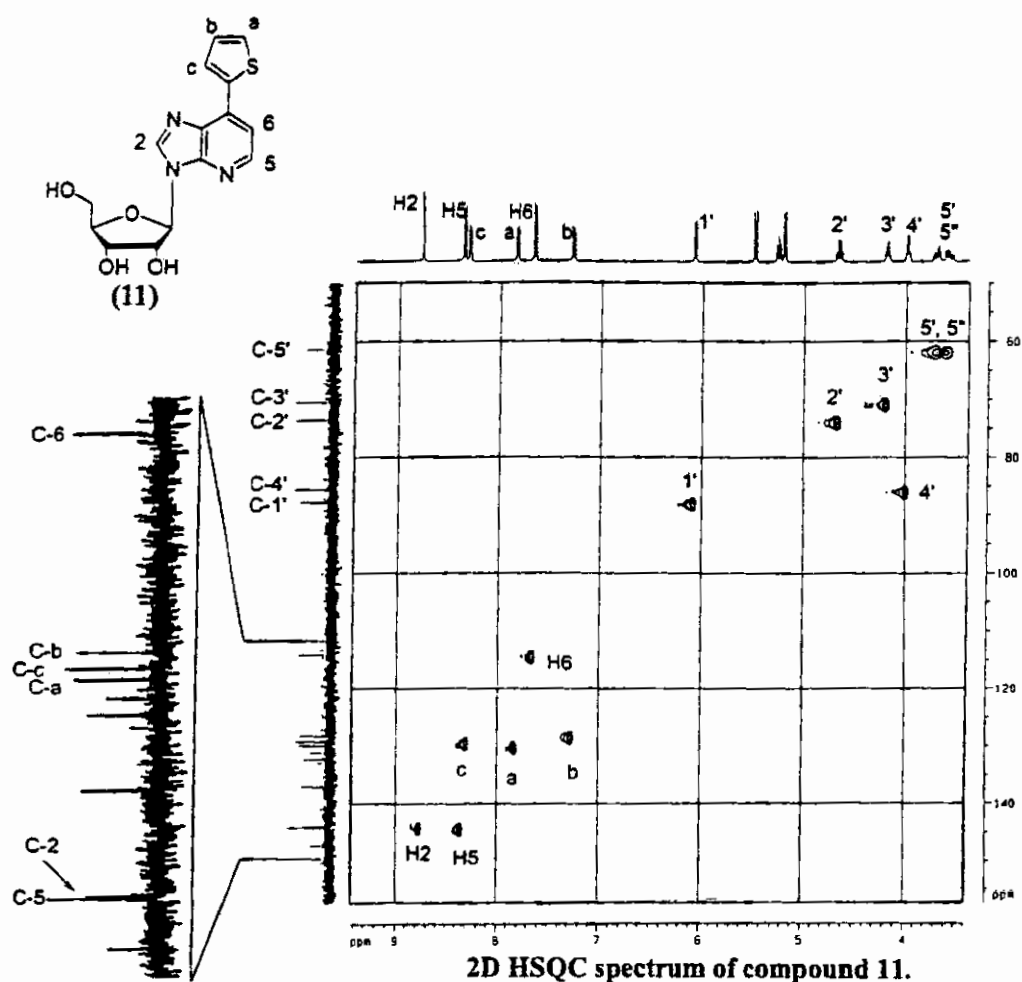


Figure 24o

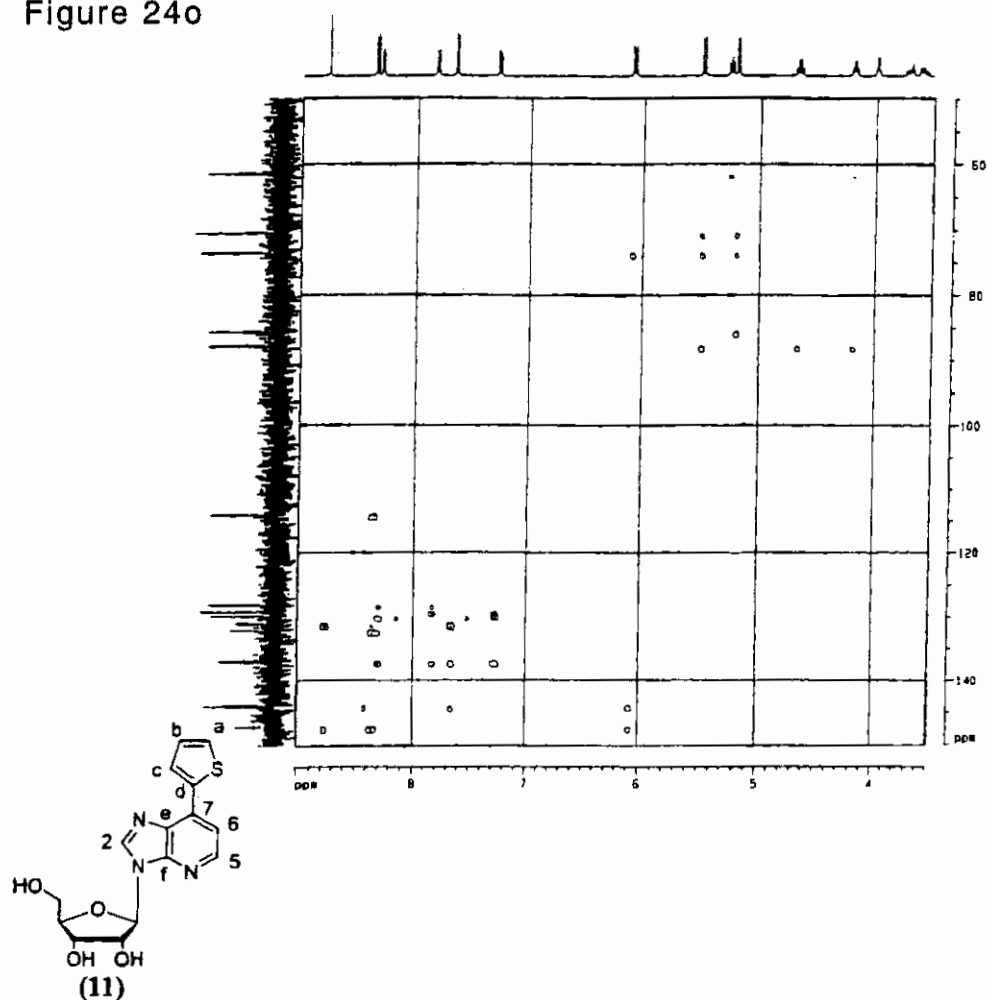


Figure 24p

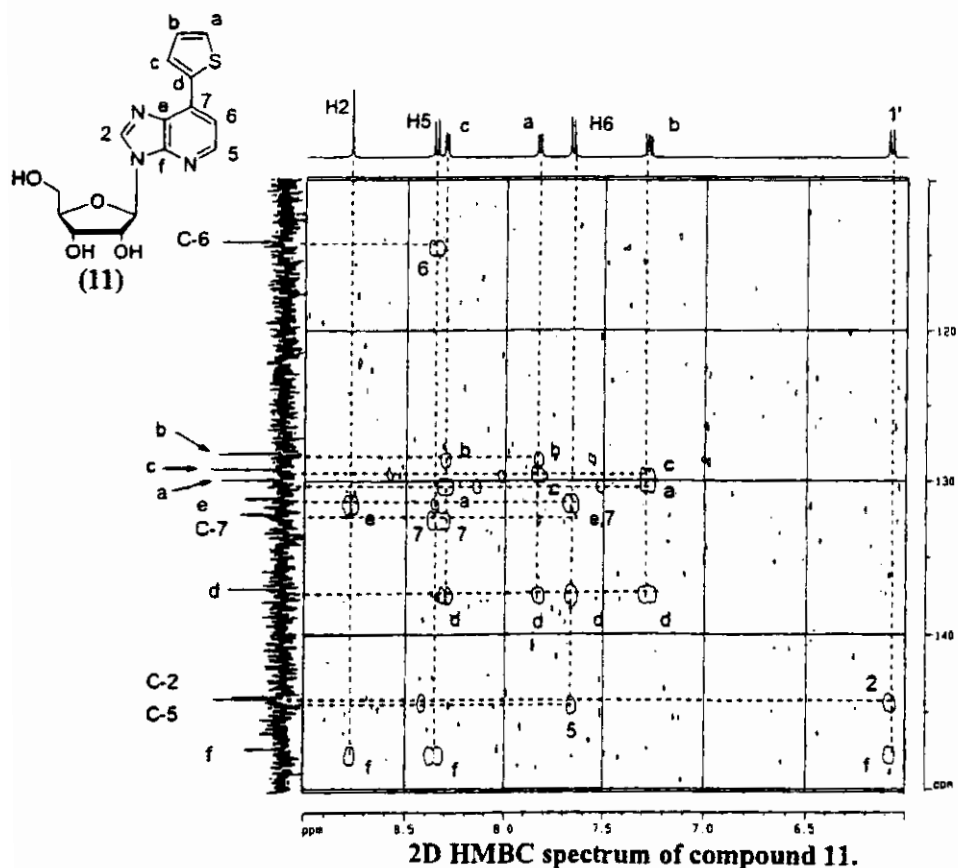


Figure 25a

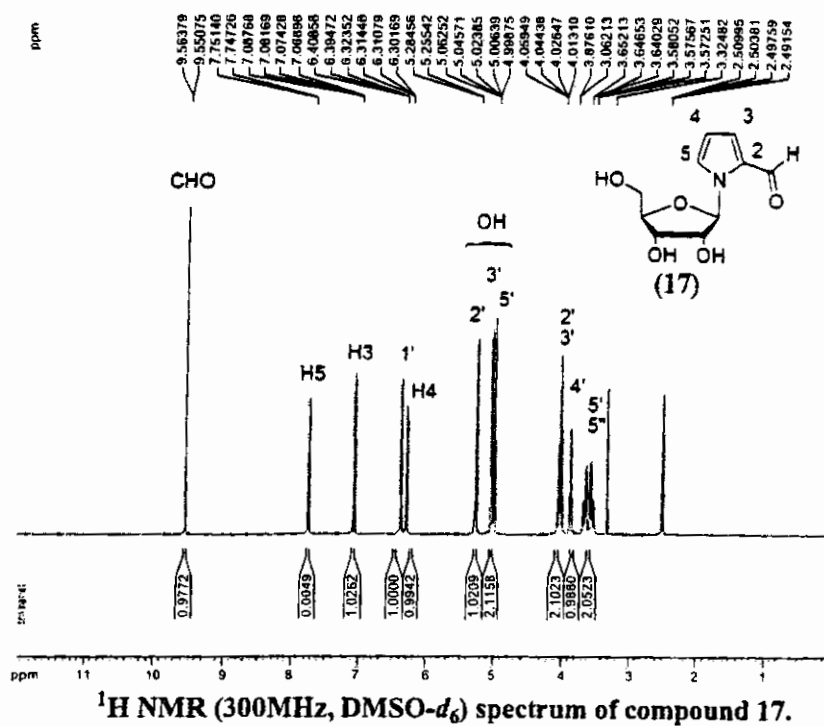


Figure 25b

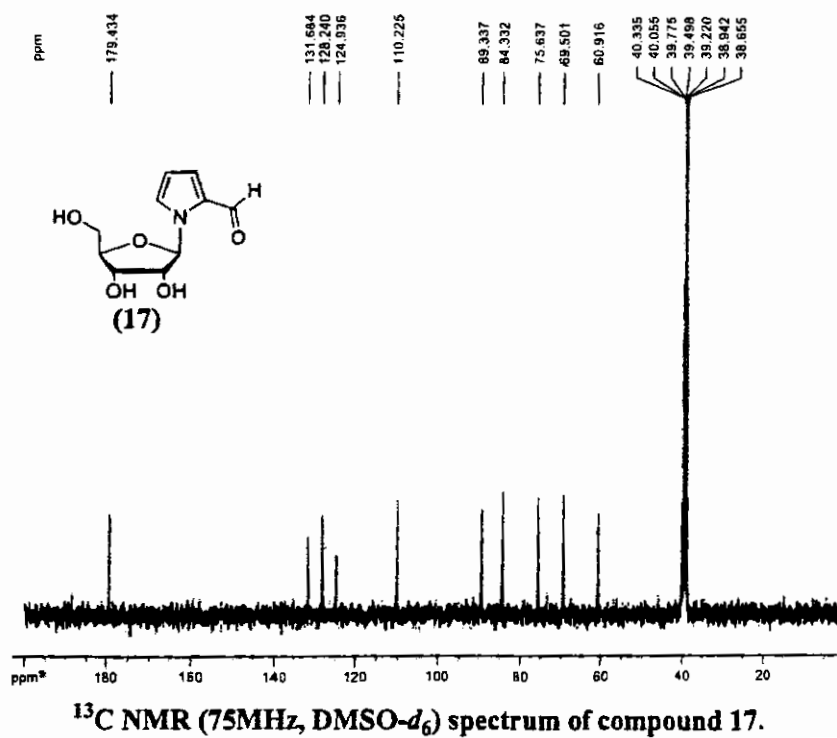


Figure 25c

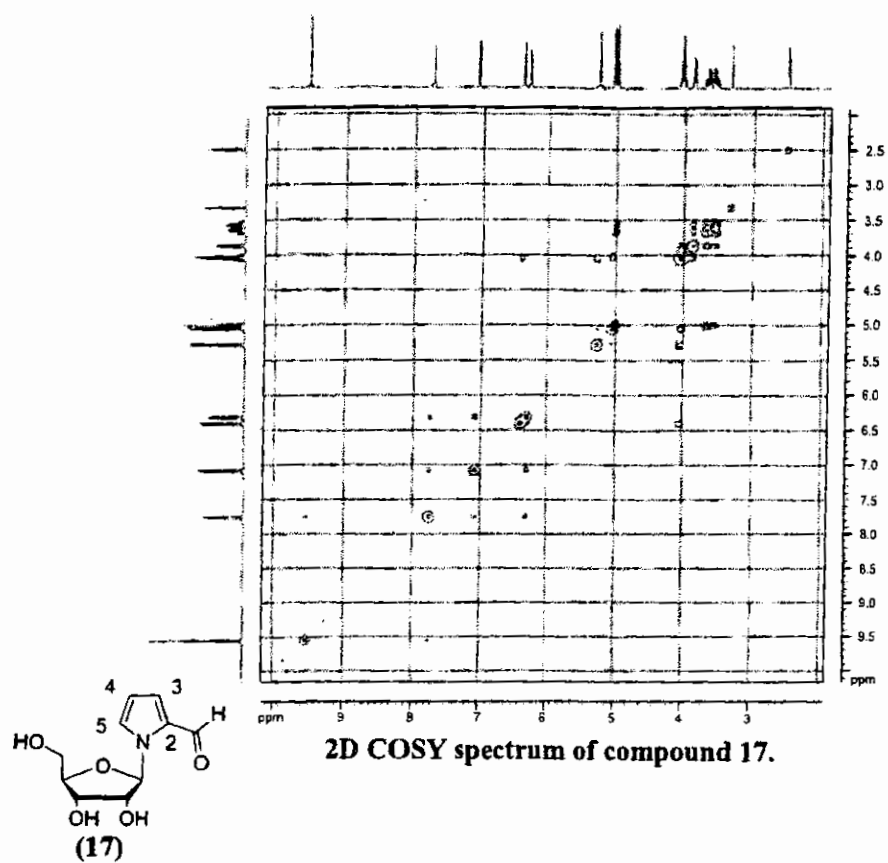


Figure 25d

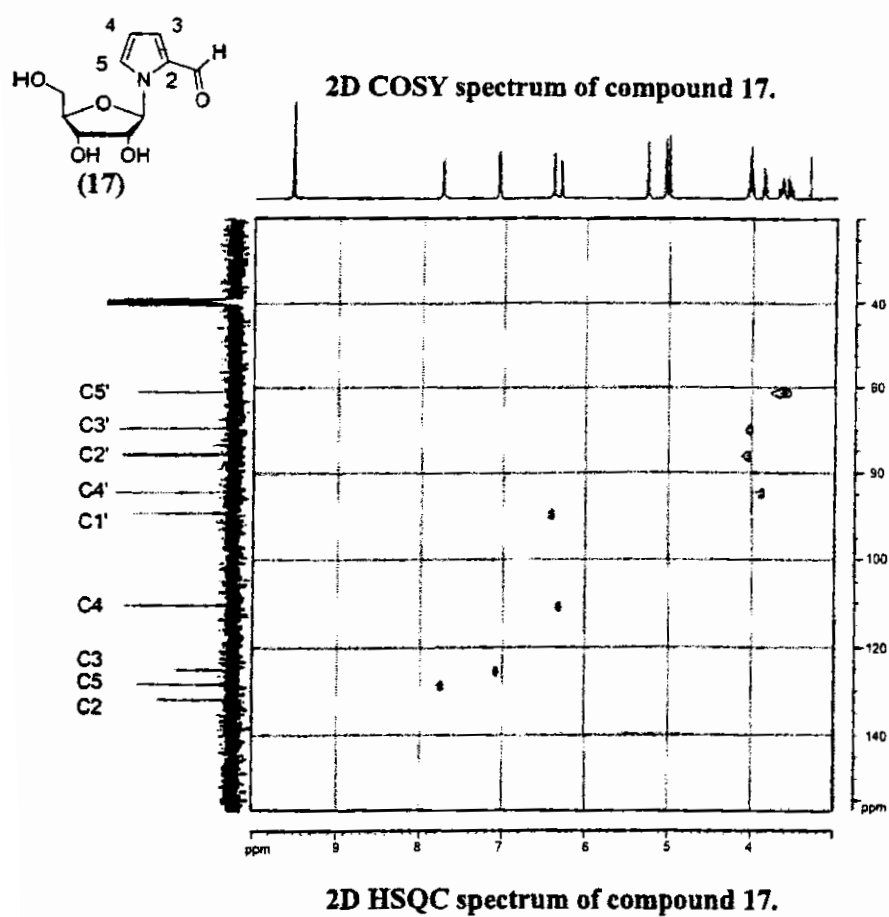


Figure 25e

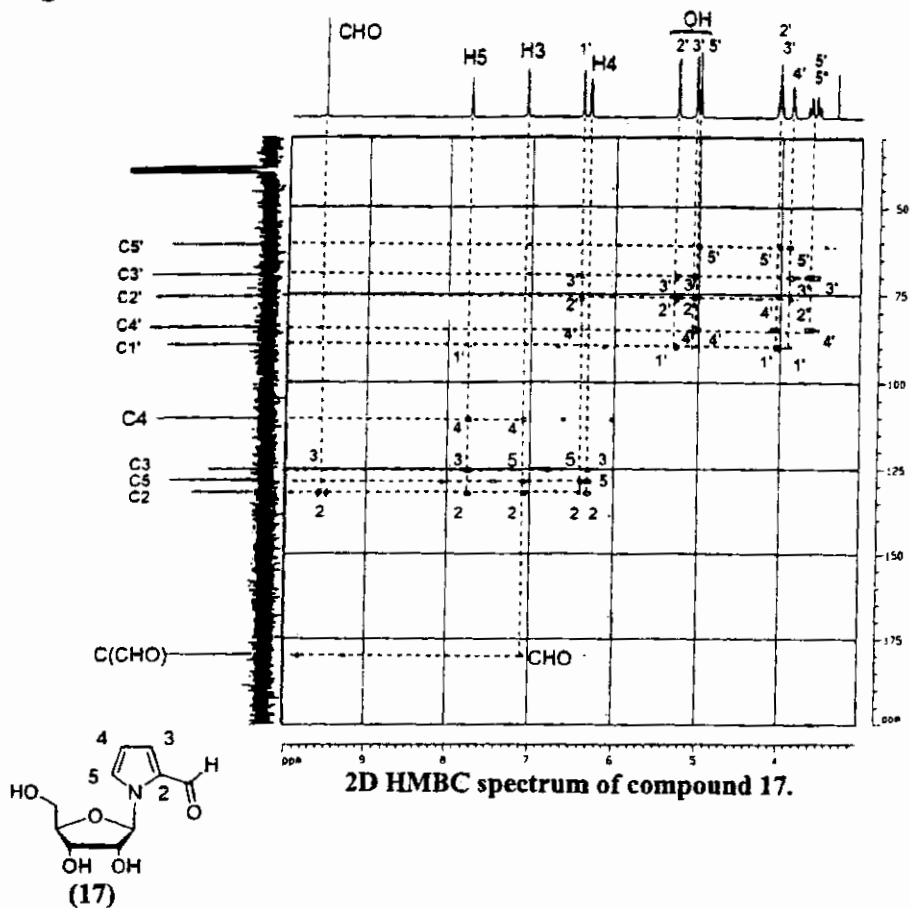


Figure 25f

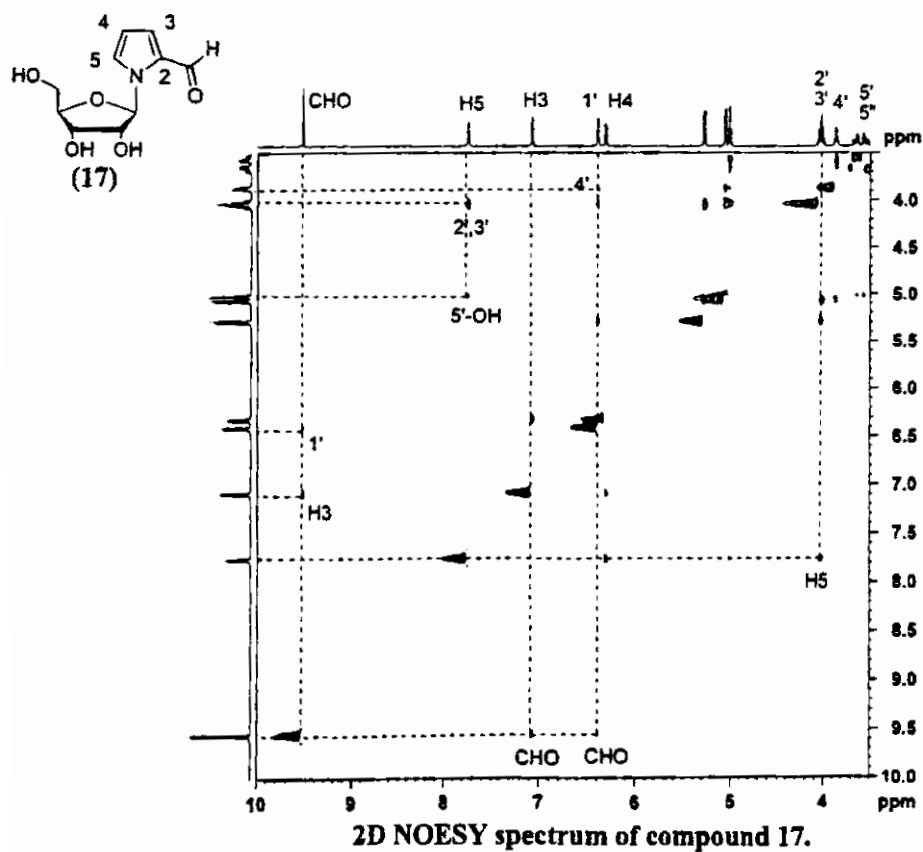


Figure 25g

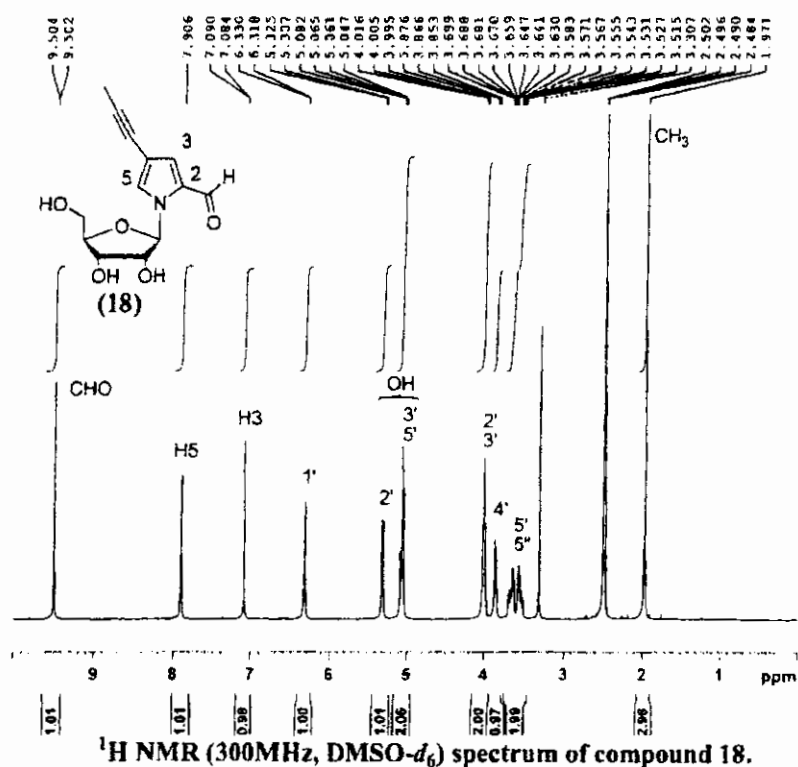


Figure 25h

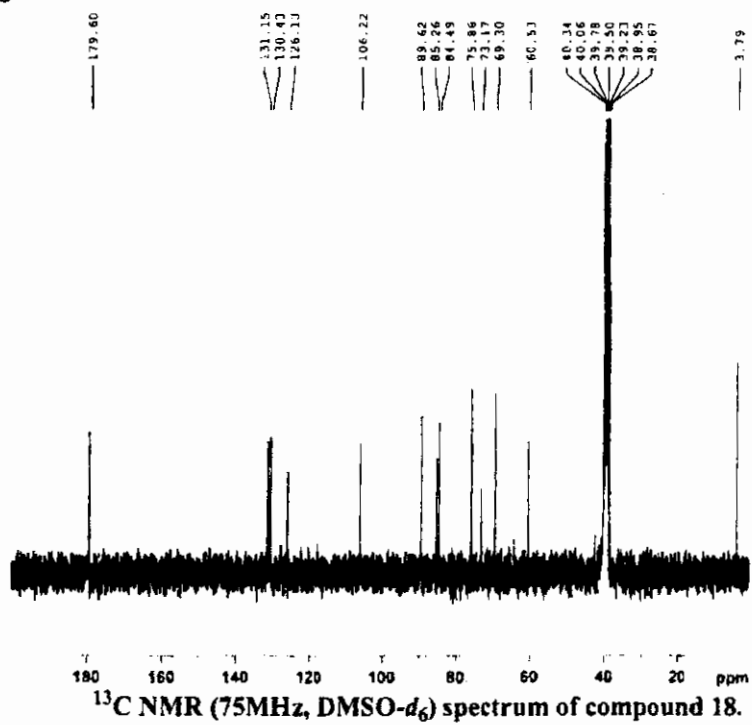


Figure 25i

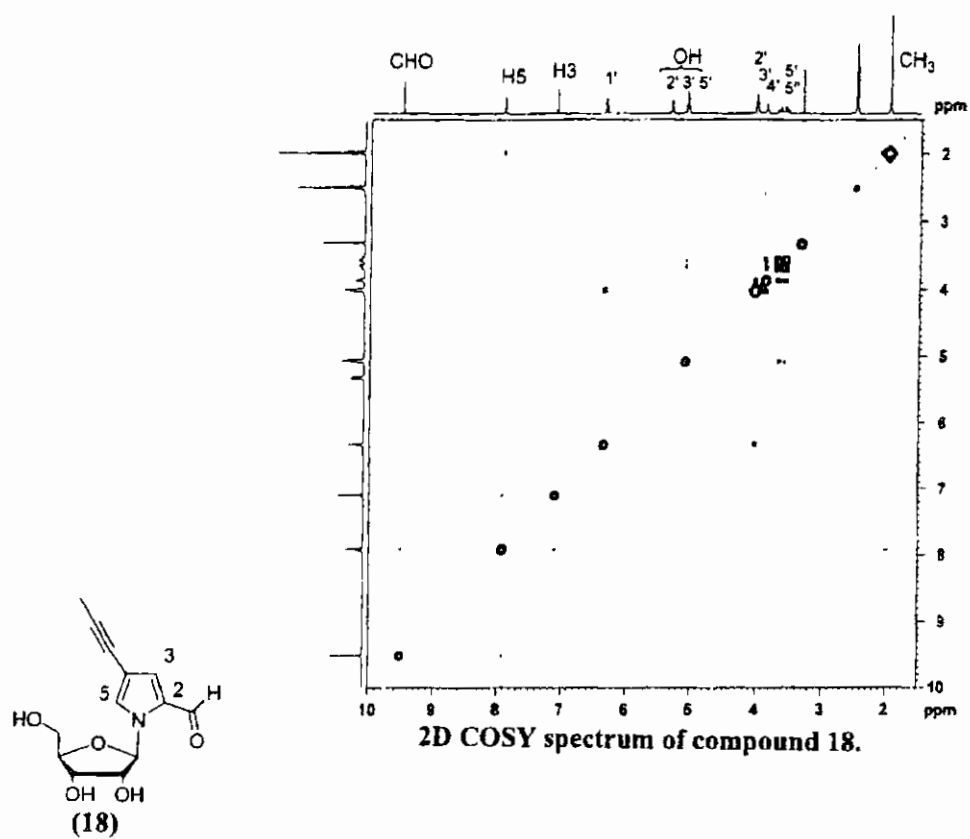


Figure 25j

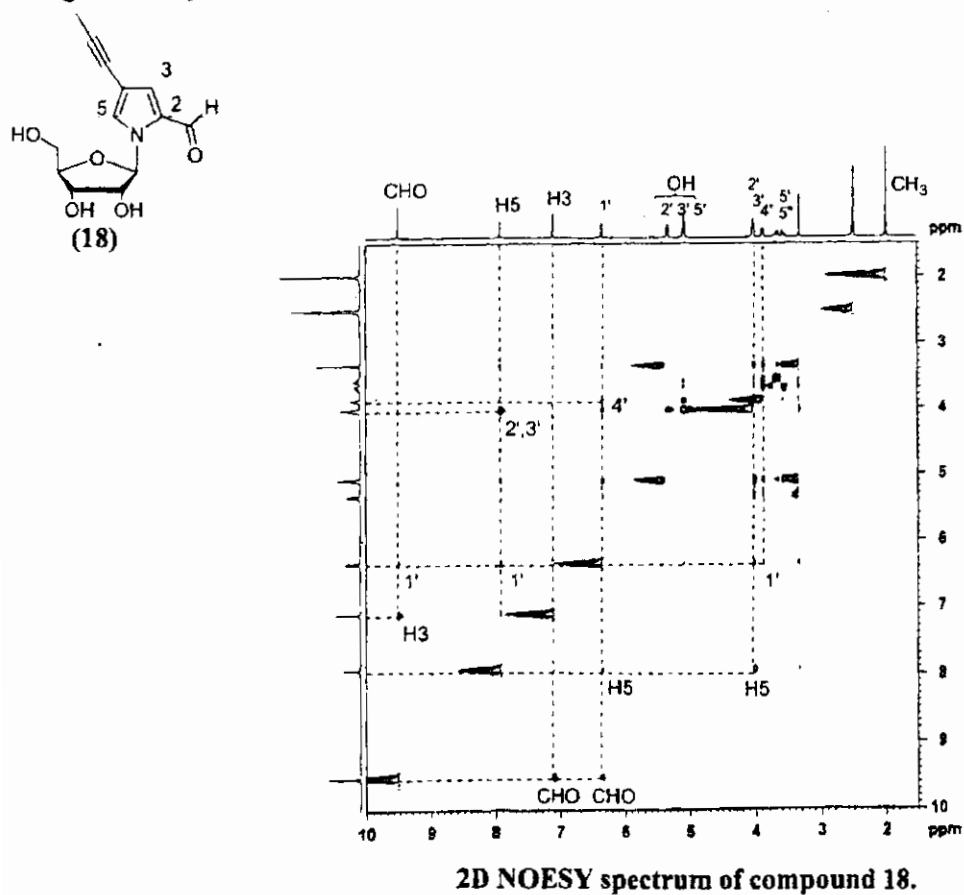


Figure 25k

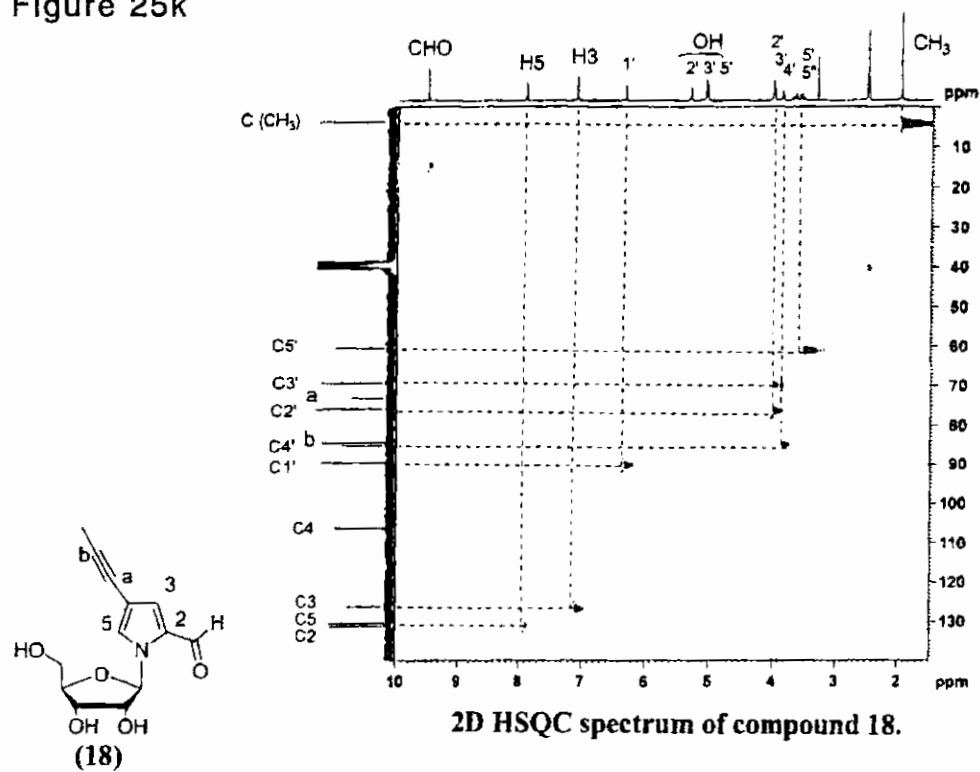


Figure 25l

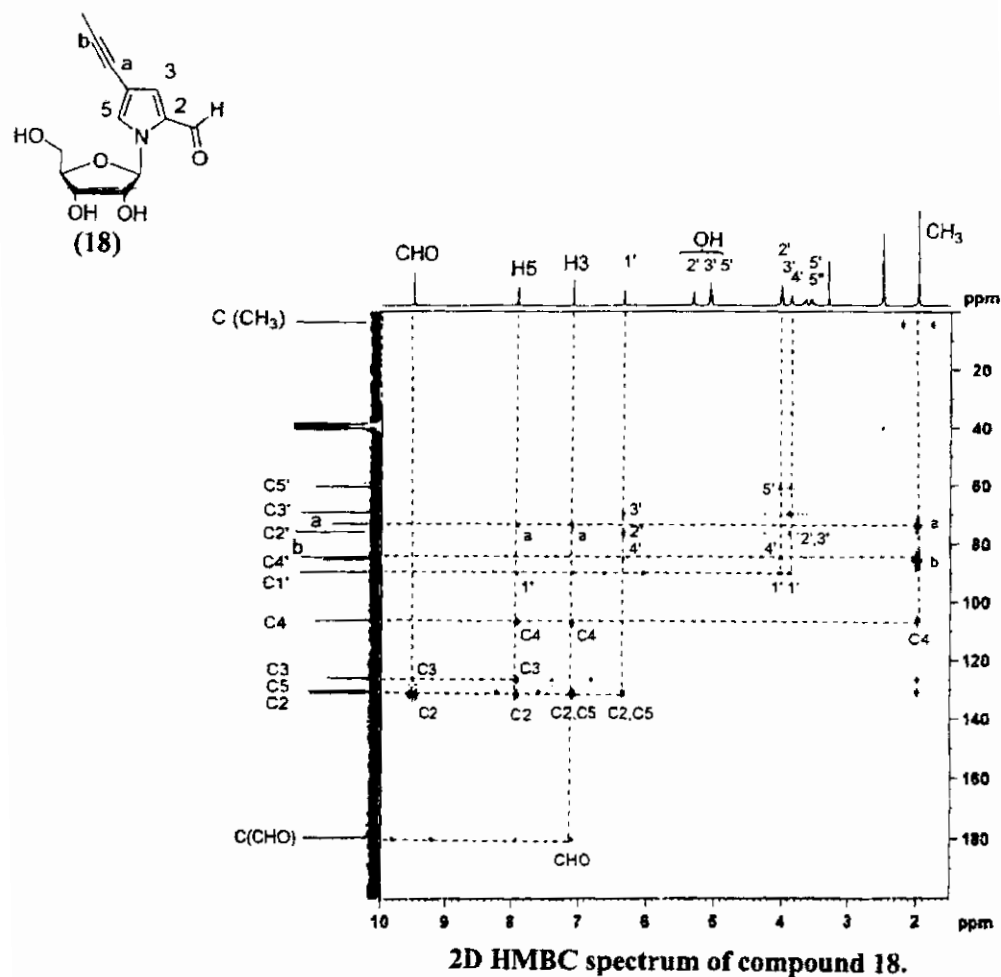


Figure 25m

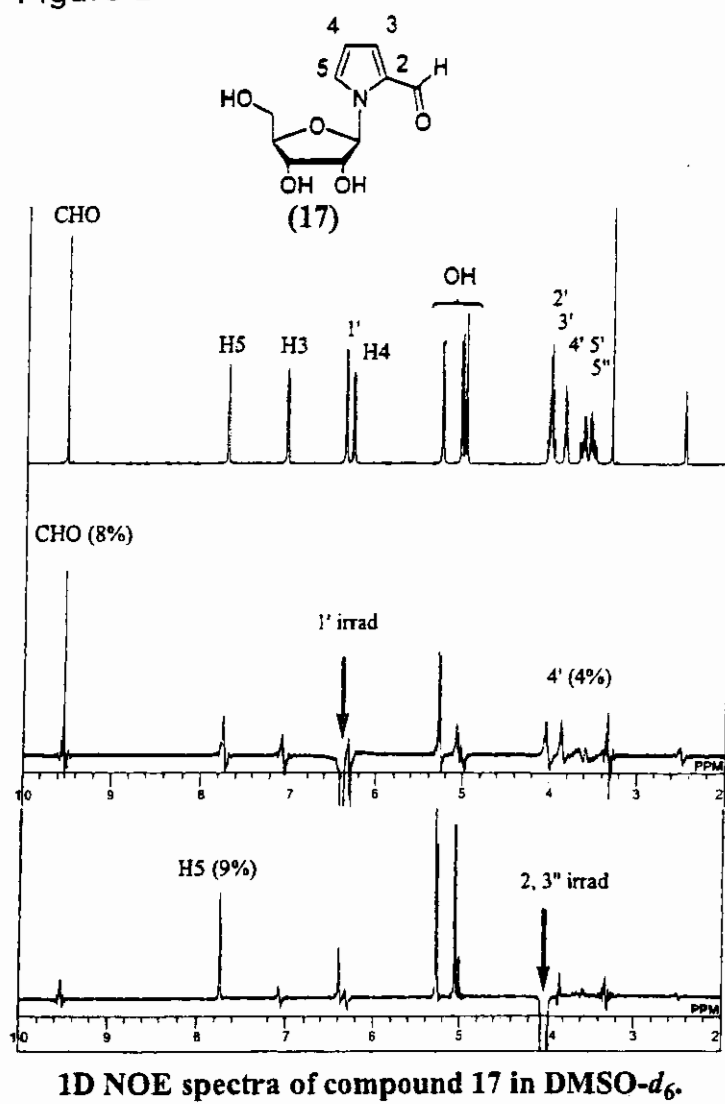


Figure 25n

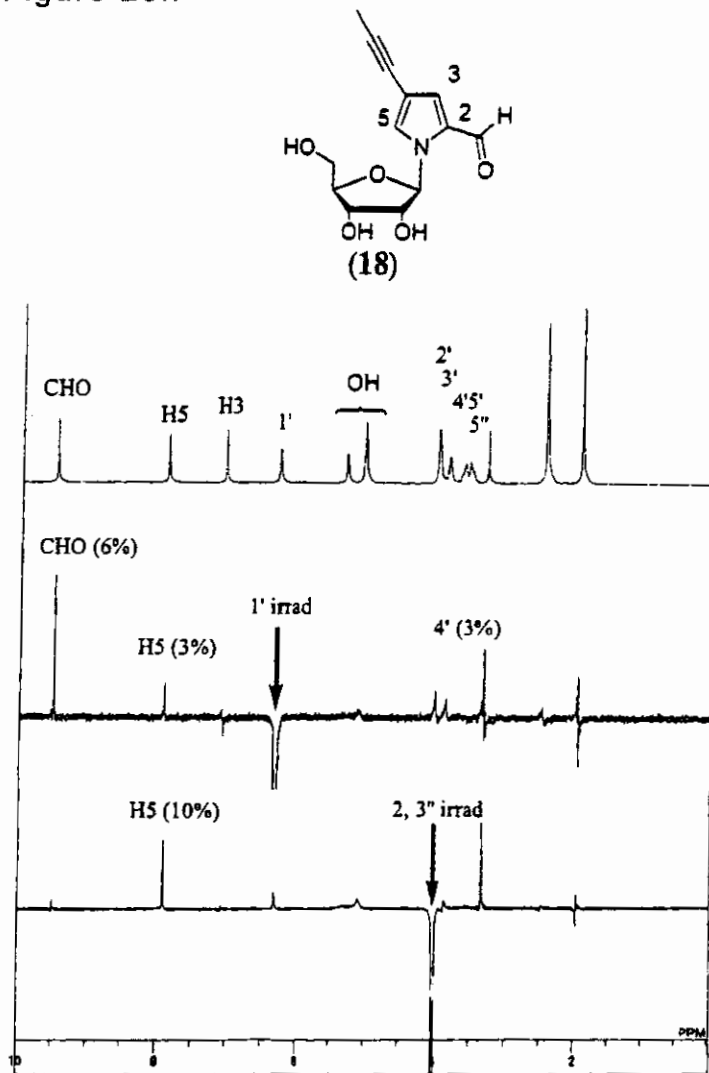
1D NOE spectra of compound 18 in DMSO- d_6 .

Figure 26

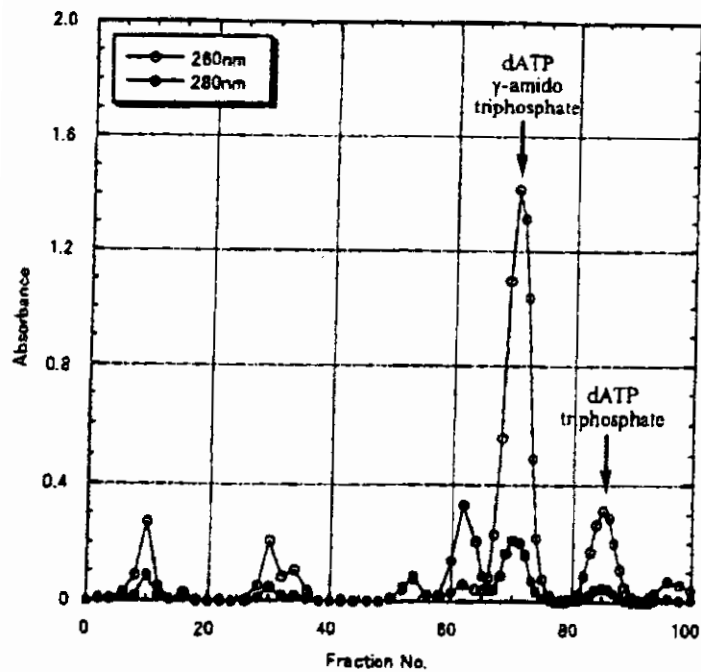
Figure. DEAE Sephadex ion-exchange column elution pattern of γ -amidotriphosphate of deoxyadenosine.

Figure 27

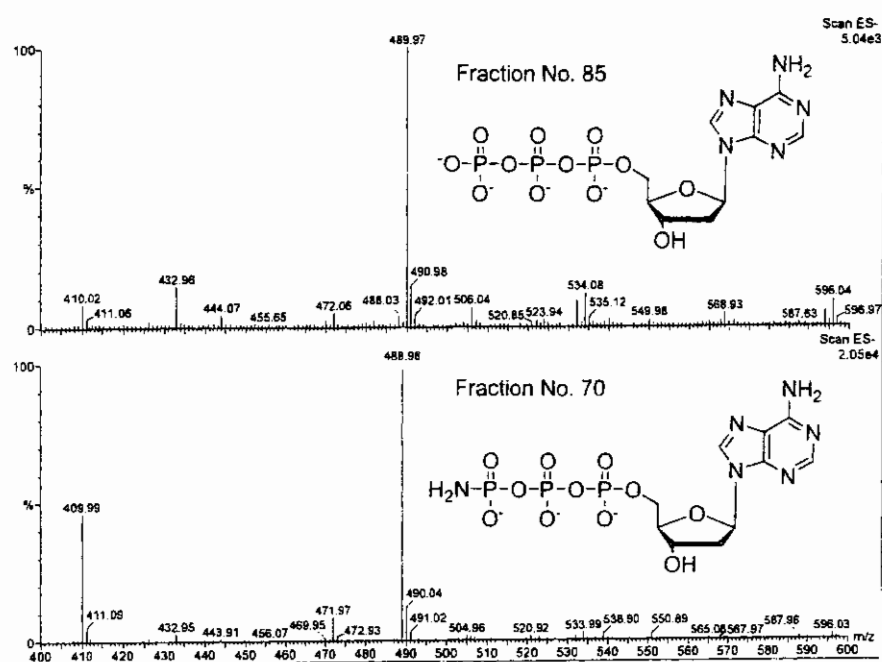
Figure. ESI-mass spectra of γ -amidotriphosphate of deoxyadenosine.

Figure 28a

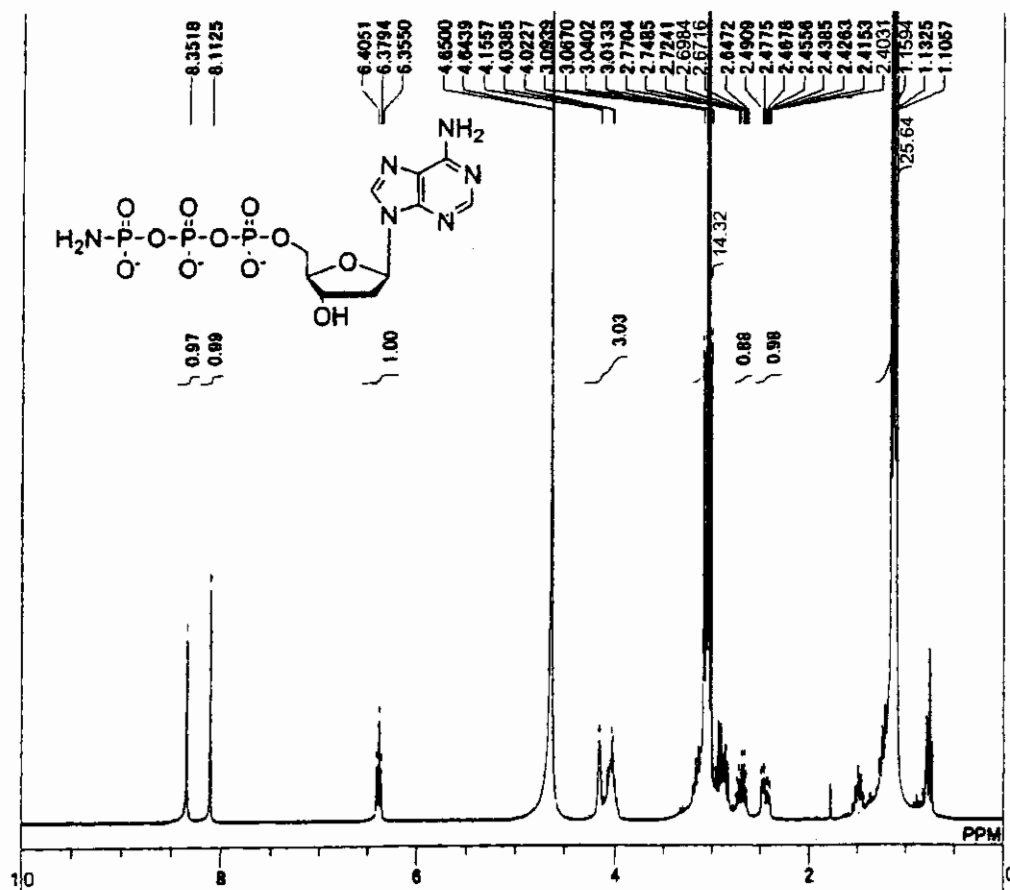
 ^1H NMR (270MHz, D_2O) spectrum of γ -amidotriphosphate of deoxyadenosine.

Figure 28b

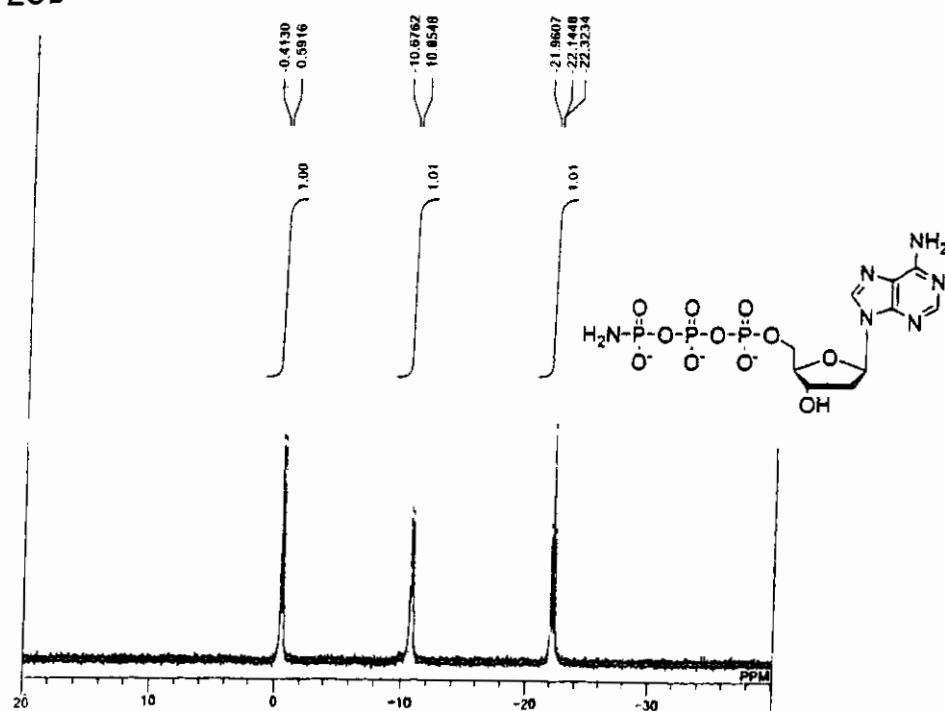
 ^{31}P NMR (109MHz, D_2O) spectrum of γ -amidotriphosphate of deoxyadenosine.

Figure 29a

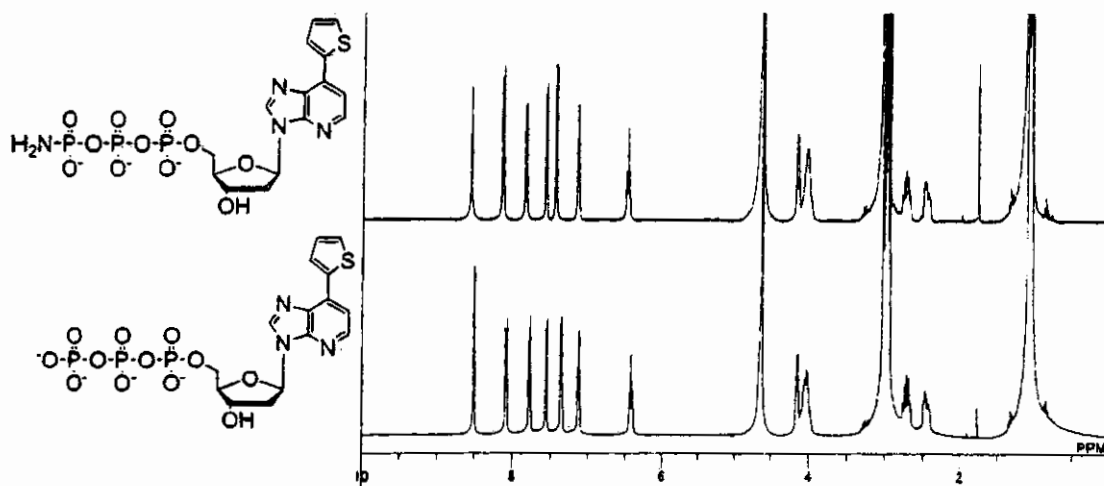
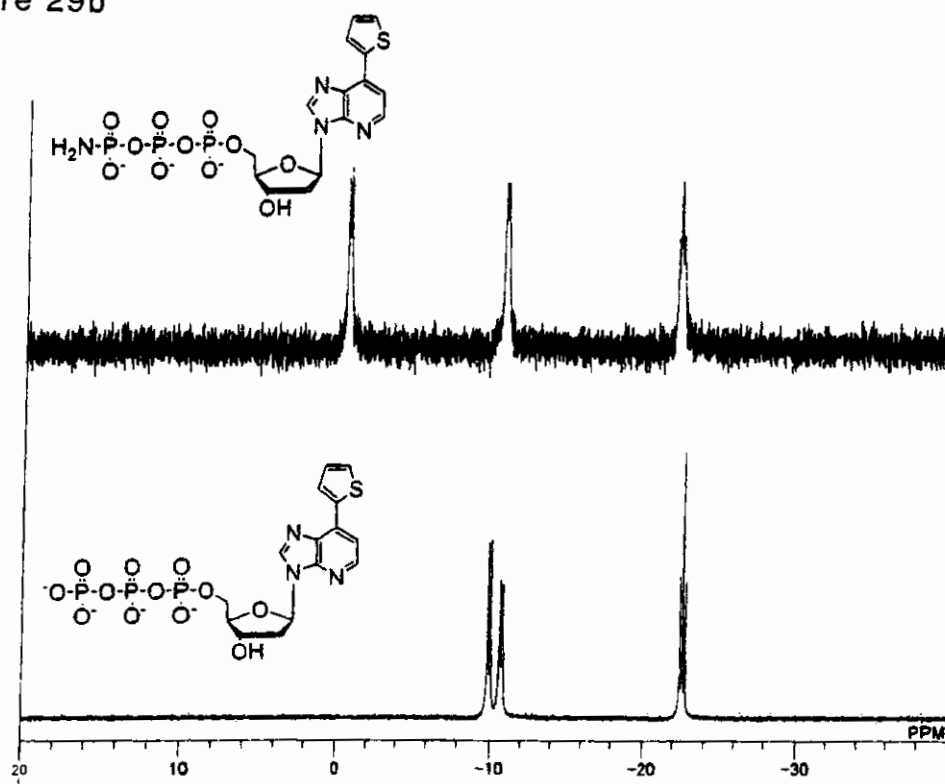
 ^1H NMR (270MHz, D_2O) spectra of γ -amidotriphosphate (upper) and triphosphate (lower) of 7-(2-Thienyl)-3-(2-deoxy- β -D-ribofuranosyl)-3H-imidazo[4,5-b]pyridine.

Figure 29b



^{31}P NMR (109MHz, D_2O) spectra of γ -amidotriphosphate (upper) and triphosphate (lower) of 7-(2-Thienyl)-3-(2-deoxy- β -D-ribofuranosyl)-3H-imidazo[4,5-b]pyridine.

Figure 30a

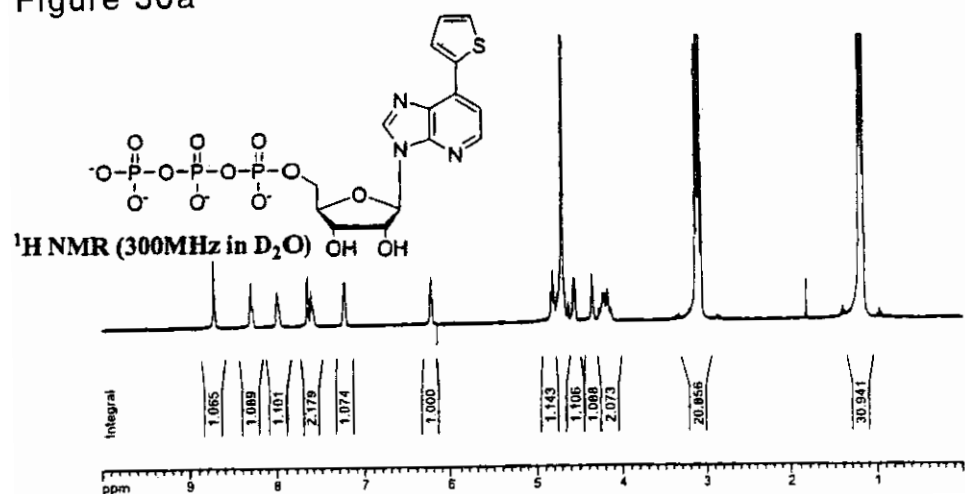


Figure 30b

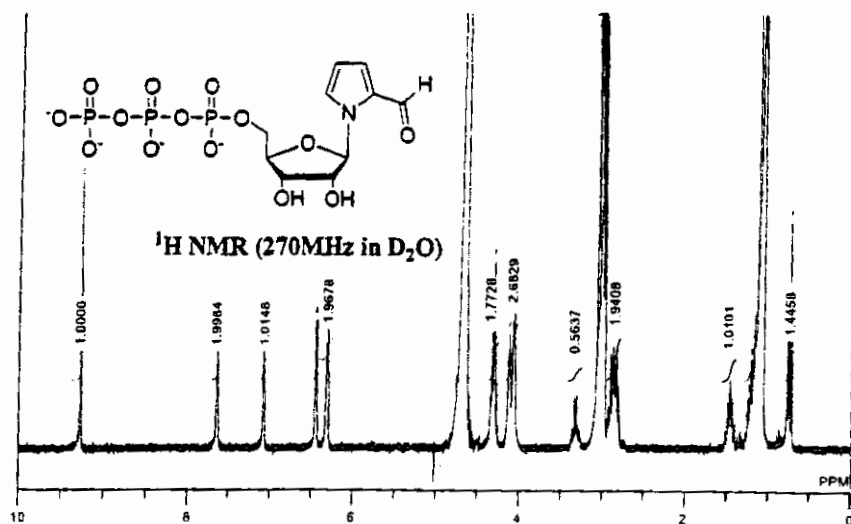


Figure 30c

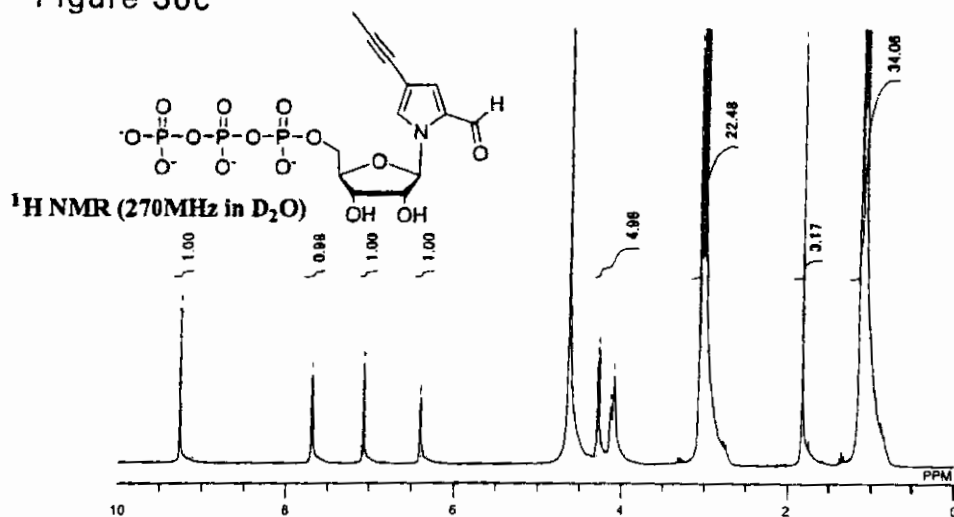


Figure 31a

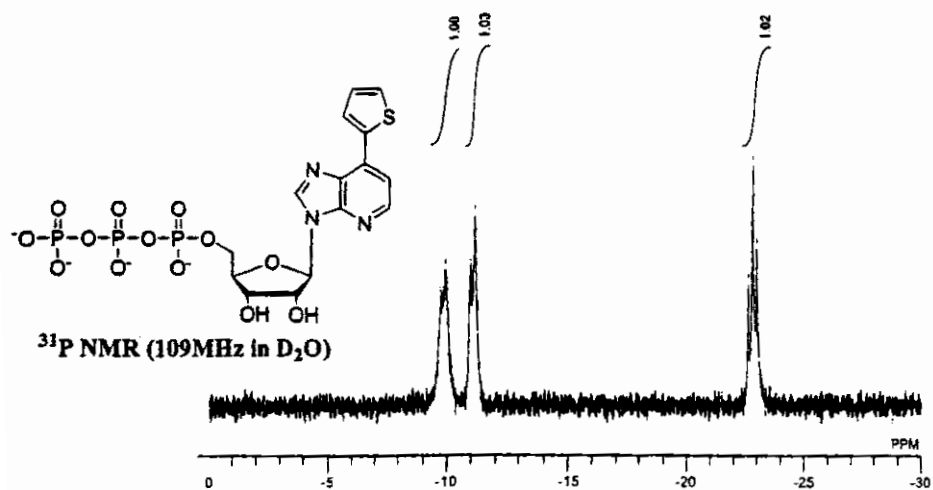


Figure 31b

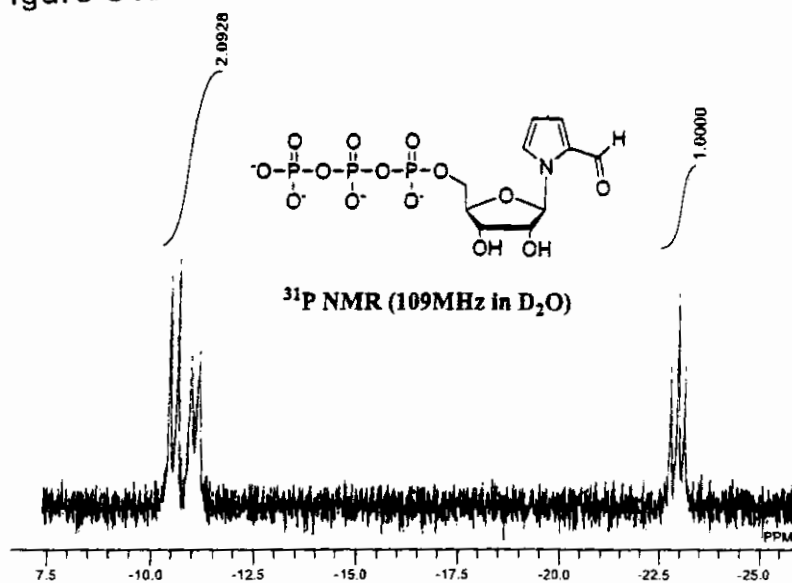


Figure 31c

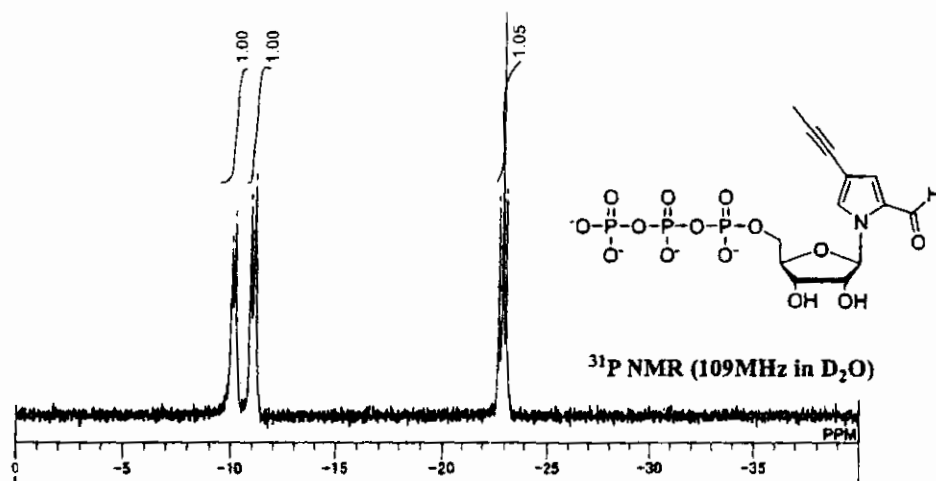


Figure 32

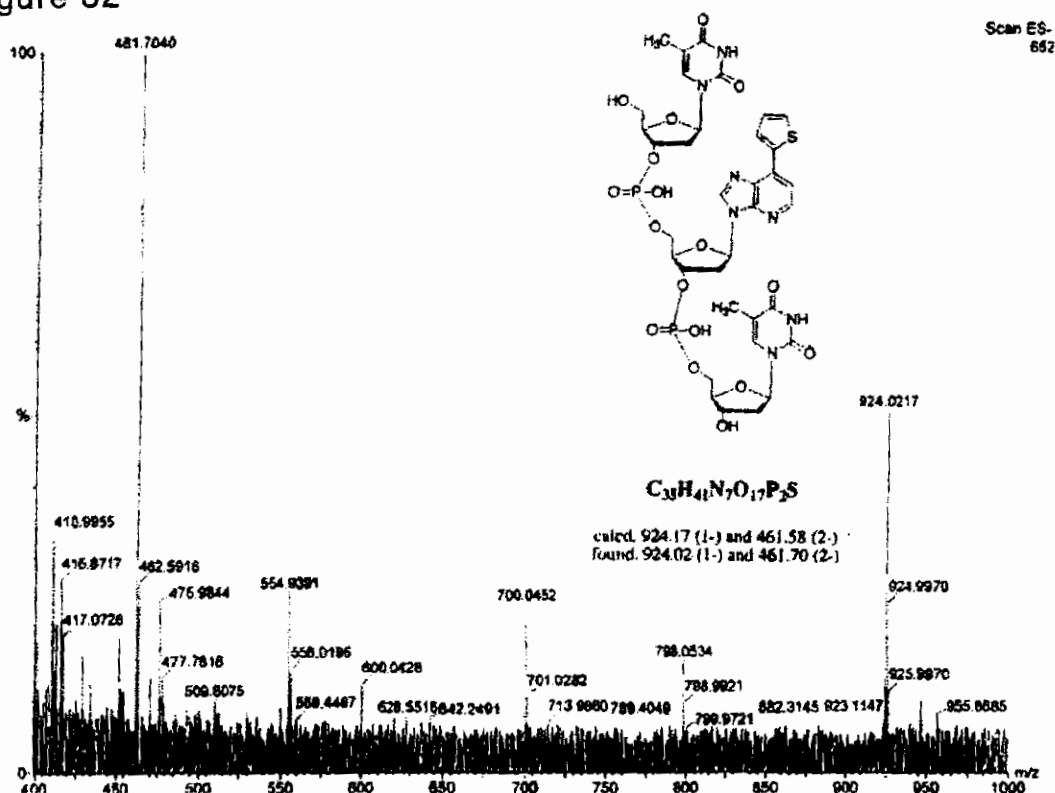


Figure 33

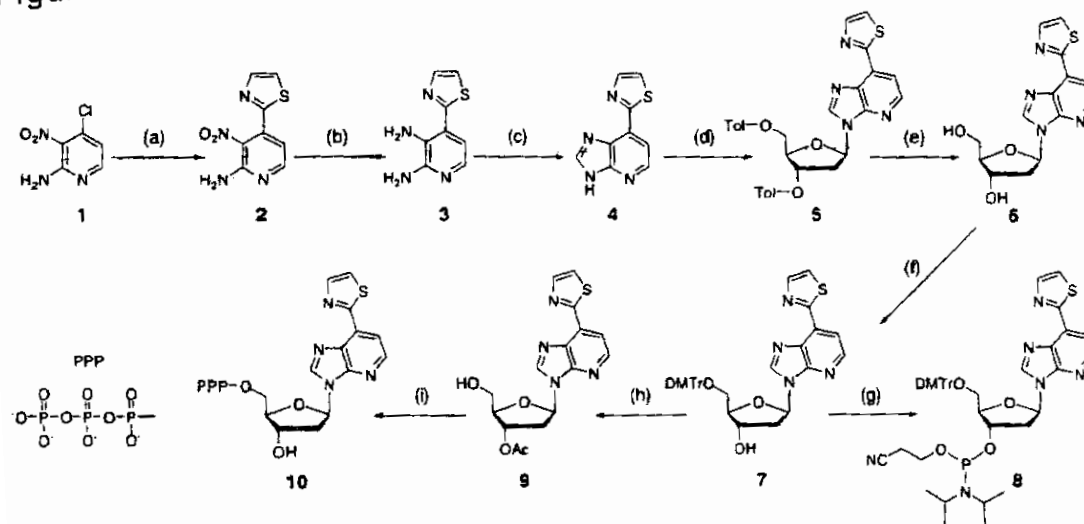


Figure 34

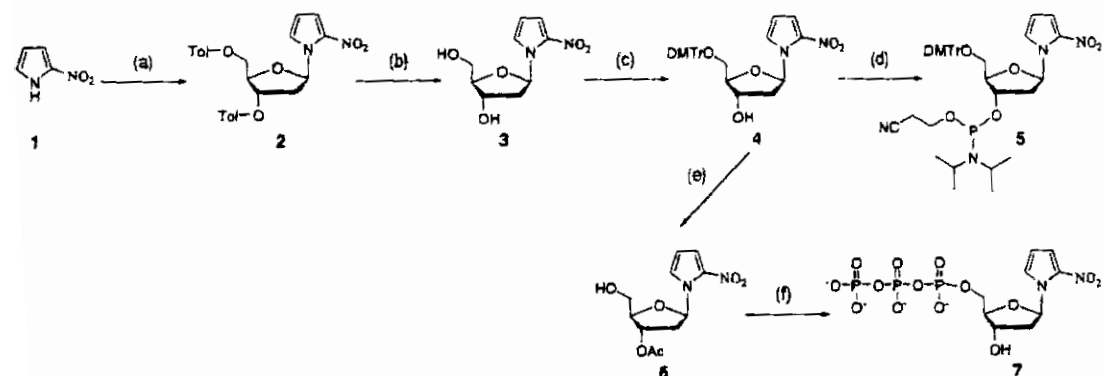


Figure 35

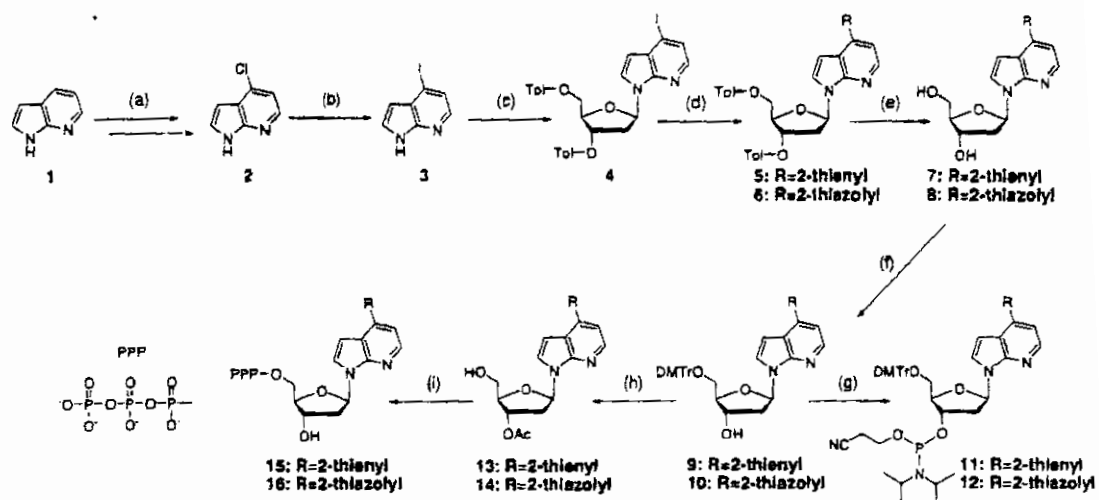


Figure 36

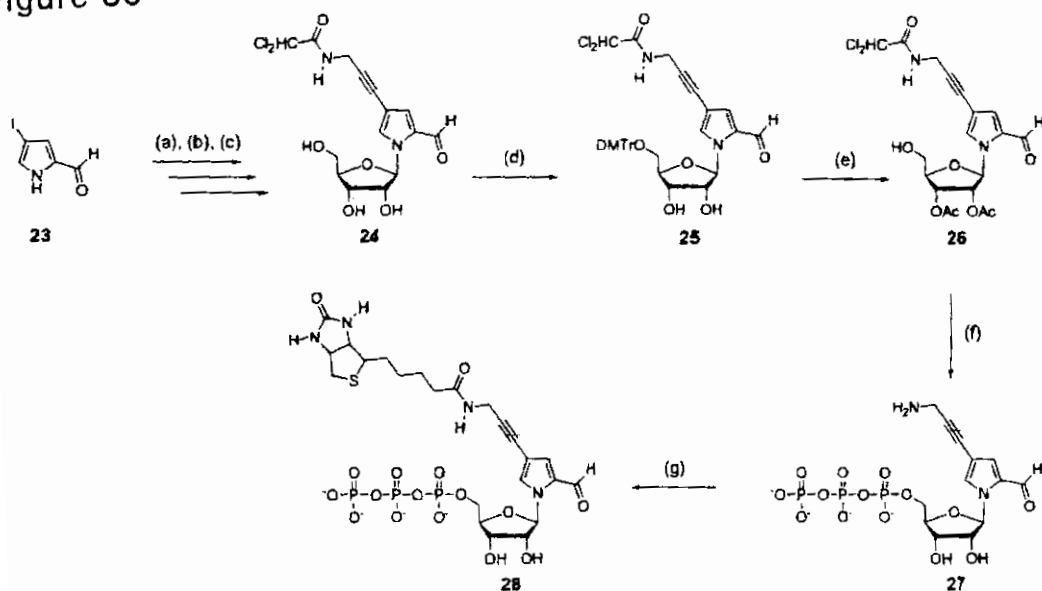


Figure 38b

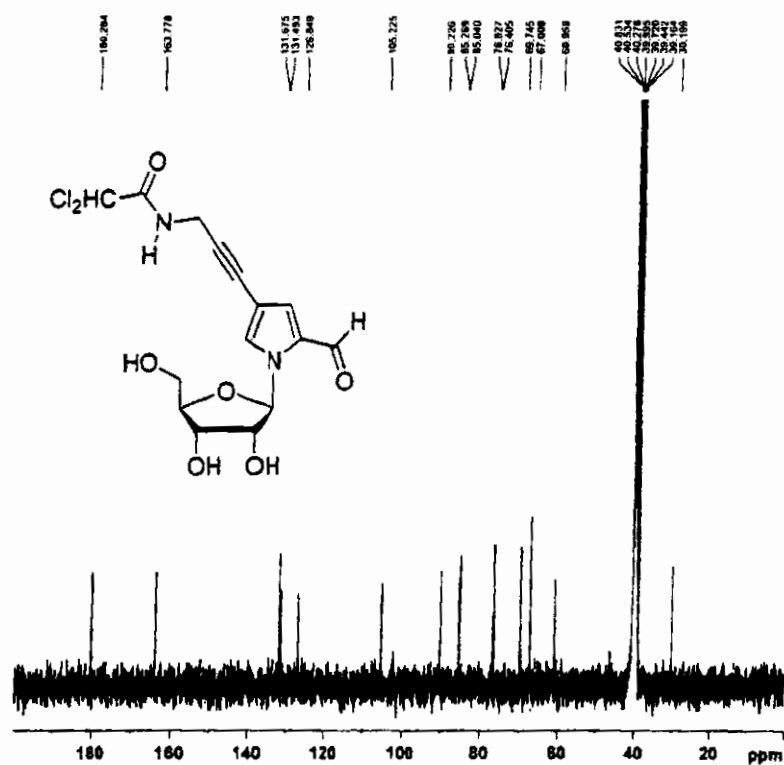
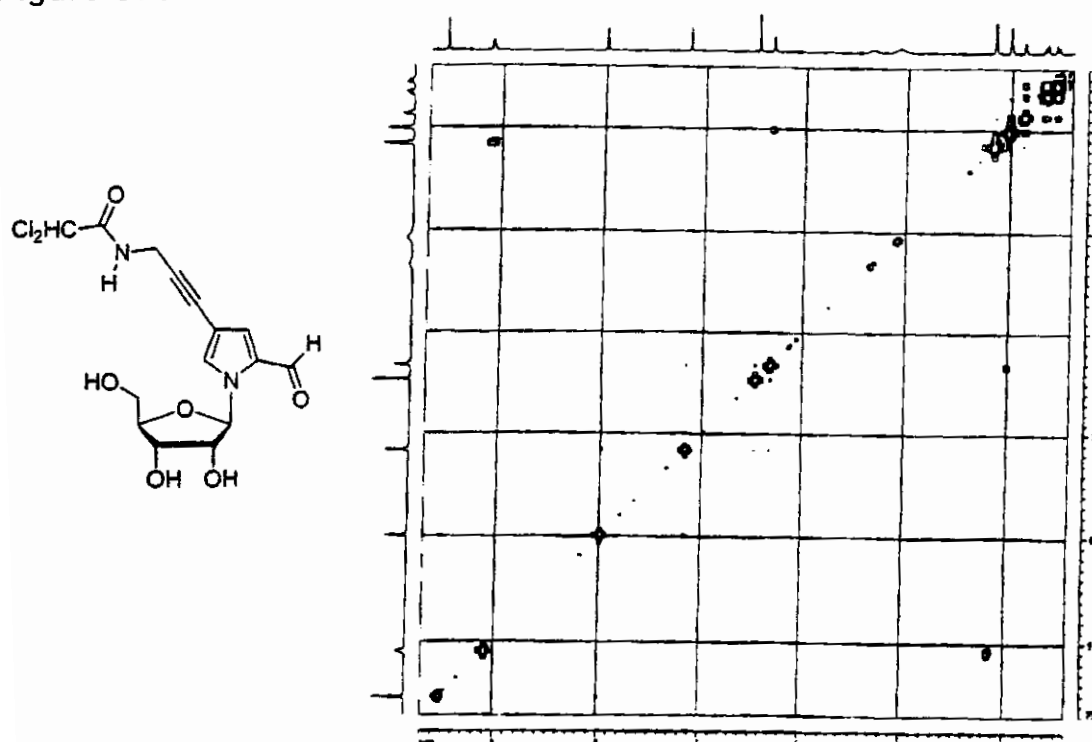
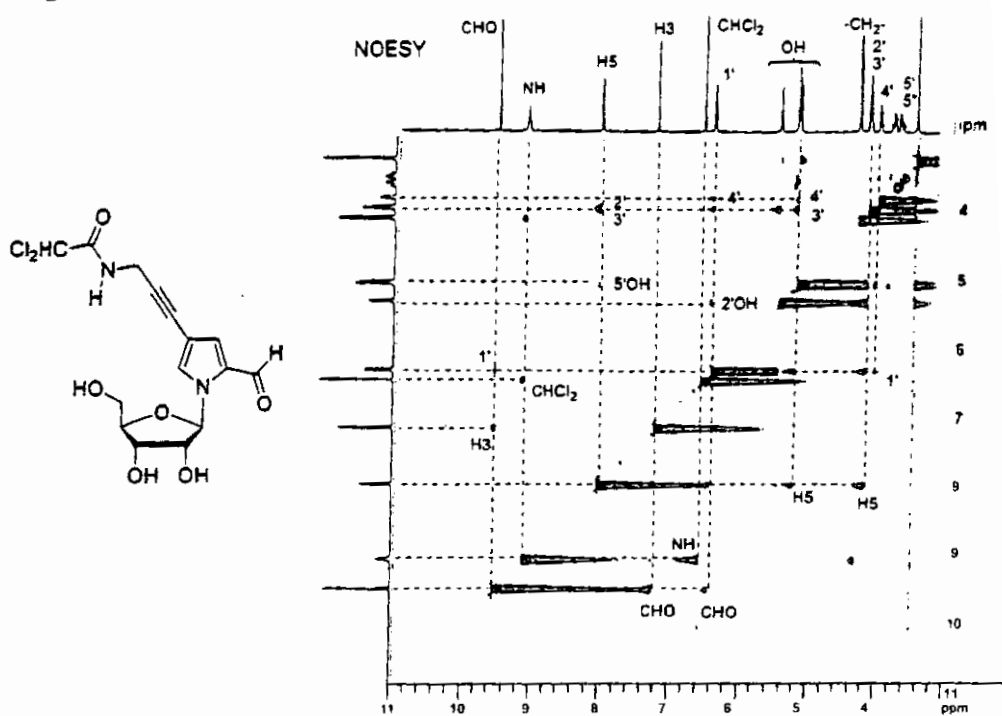
¹³C NMR (75 MHz, DMSO-*d*₆) spectrum of compound 24.

Figure 38c



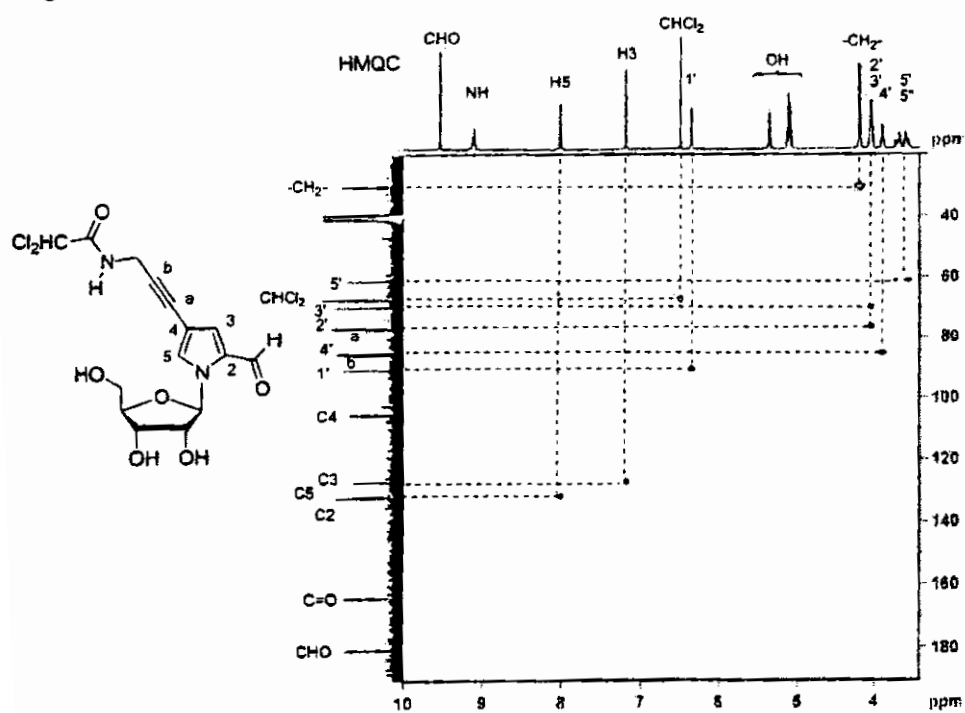
2D COSY spectrum of compound 24.

Figure 38d



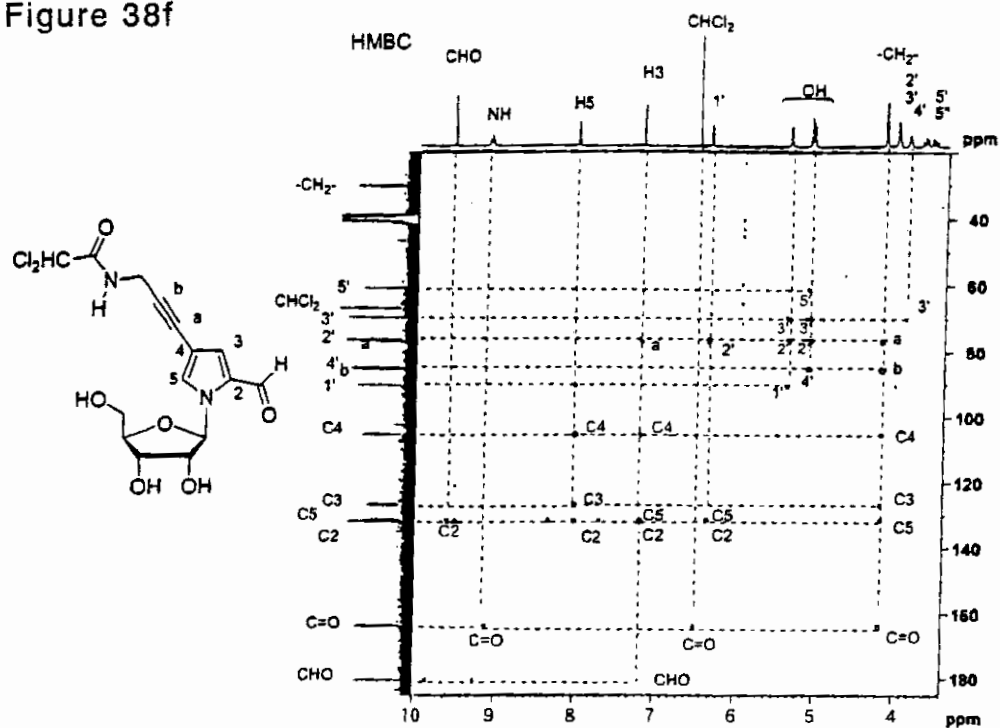
2D NOESY spectrum of compound 24.

Figure 38e



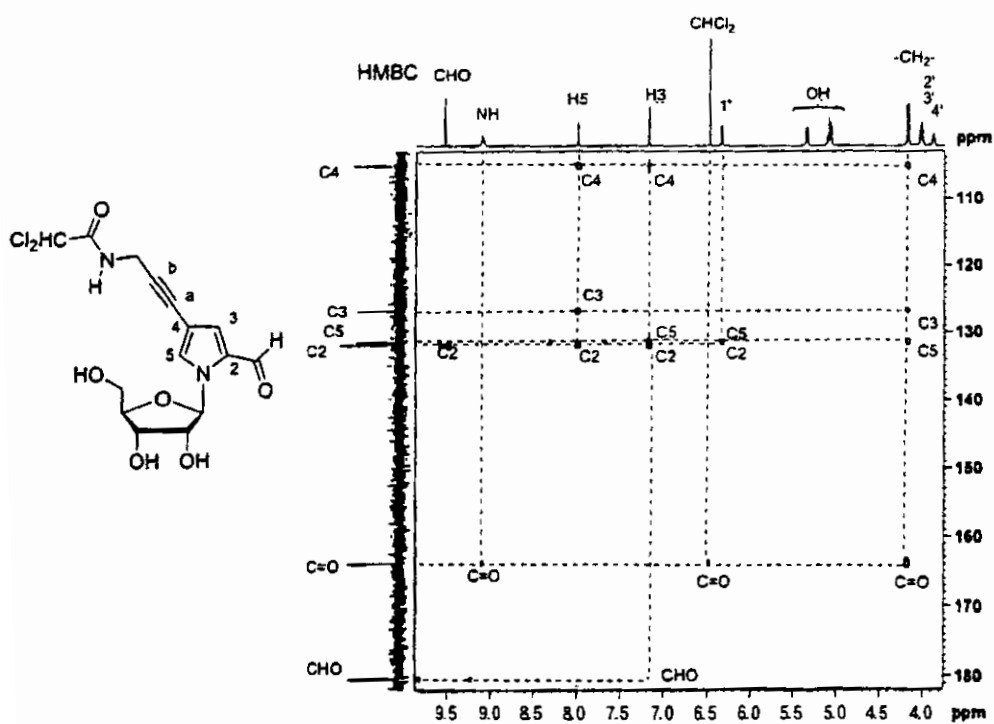
2D HMQC spectrum of compound 24.

Figure 38f



2D HMBC spectrum of compound 24.

Figure 38g



2D HMBC spectrum of compound 24.

Figure 39

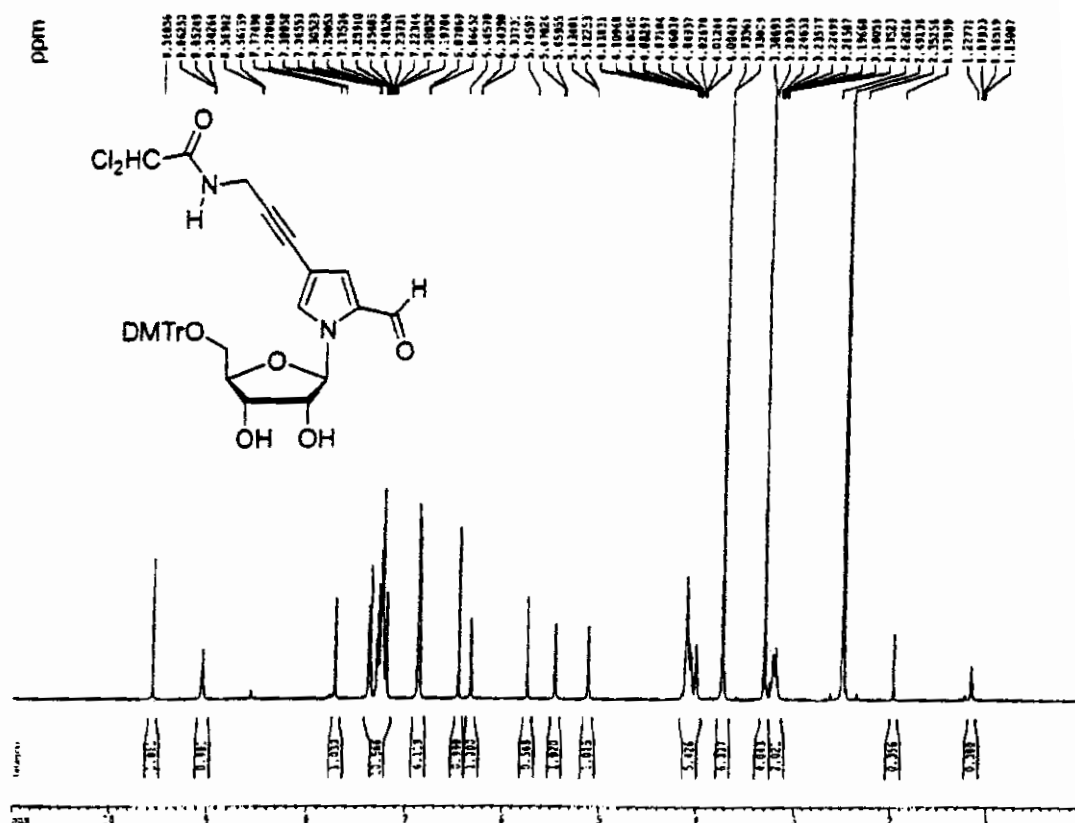
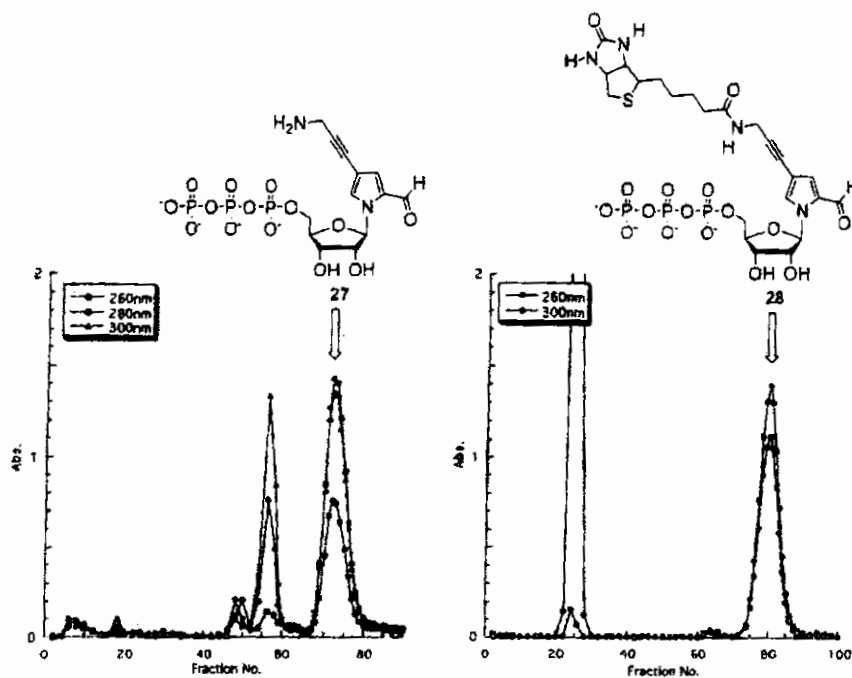


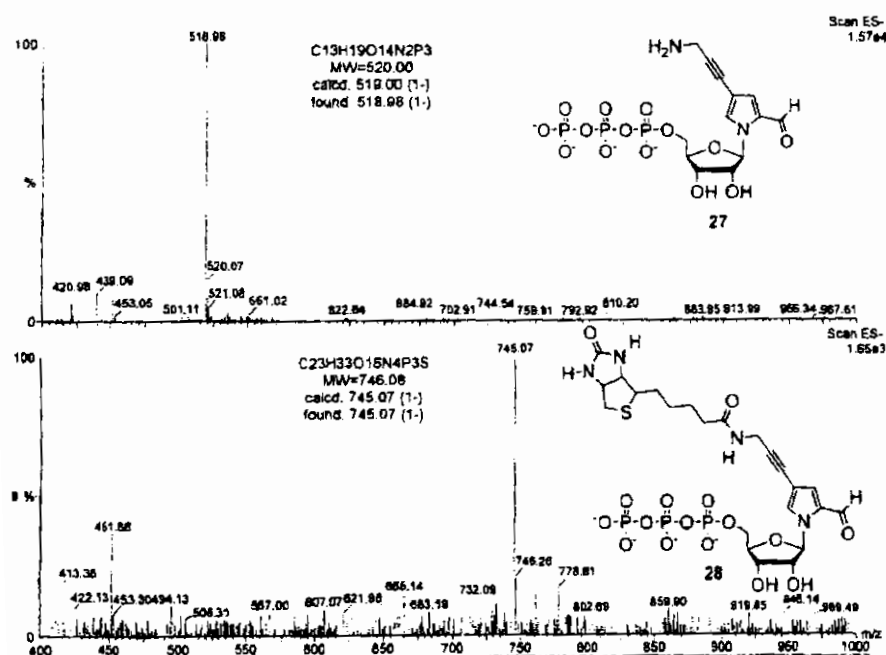
Figure 41

S2-6. DEAE Sephadex ion-exchange column elution patterns, ESI-MS spectra, and NMR spectra of 5'-triphosphates.



DEAE Sephadex ion-exchange column elution patterns of compound 27 and 28.

Figure 42



ESI-mass spectra of compound 27 (upper) and 28 (lower).

Figure 43a

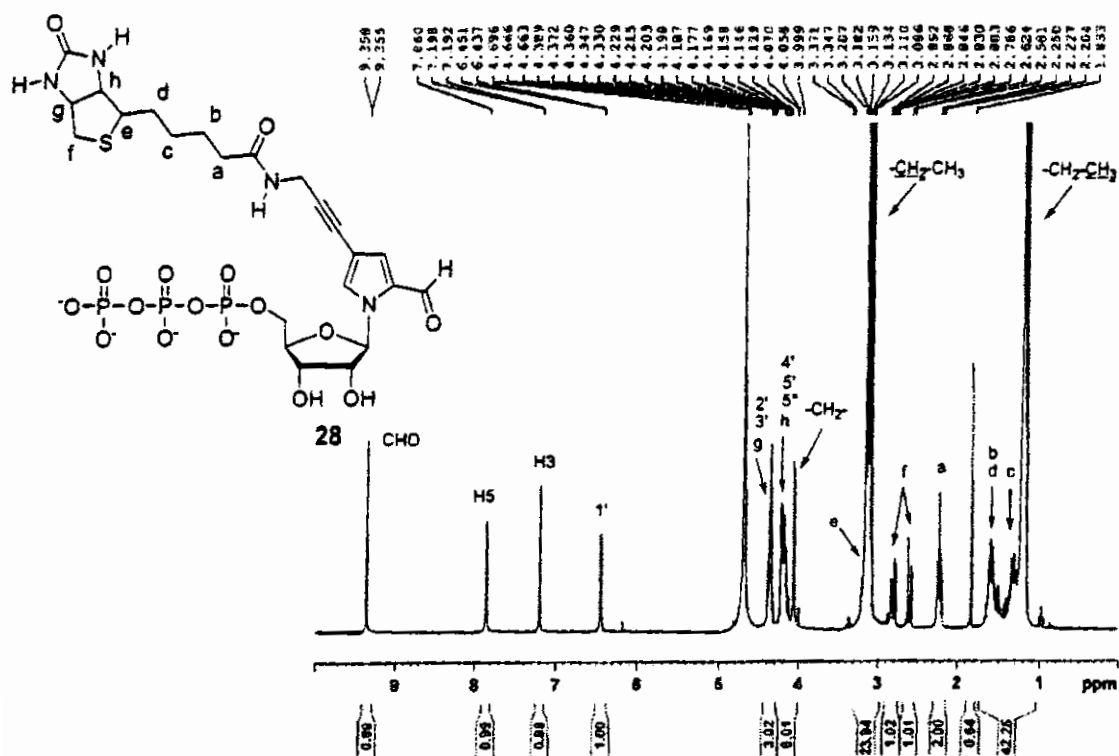
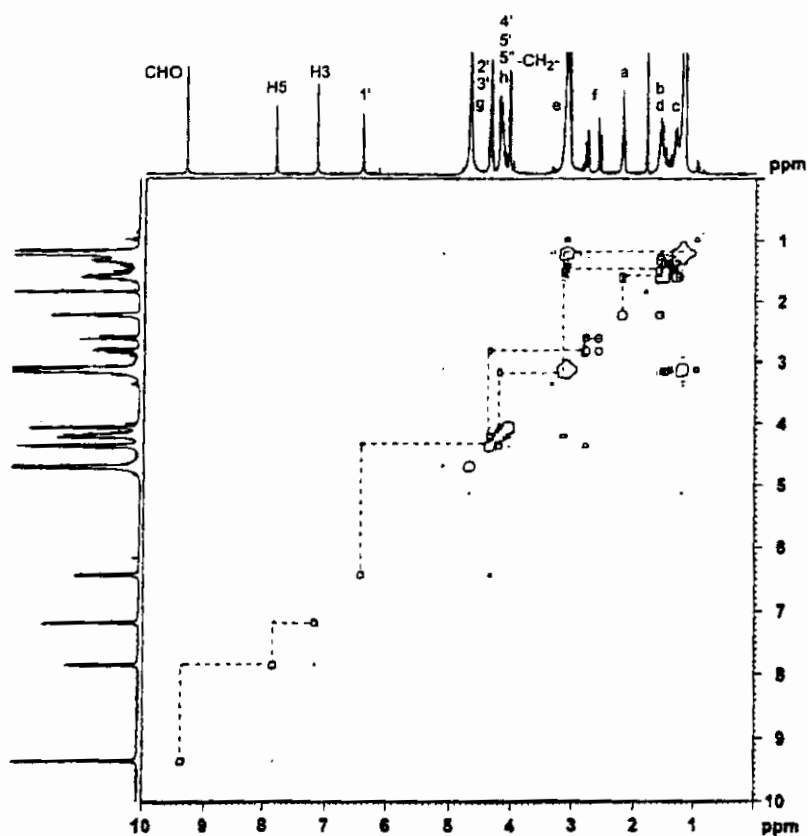
¹H NMR (300 MHz, D₂O) spectrum of compound 28.

Figure 43b



2D COSY spectrum of compound 28.

Figure 43c

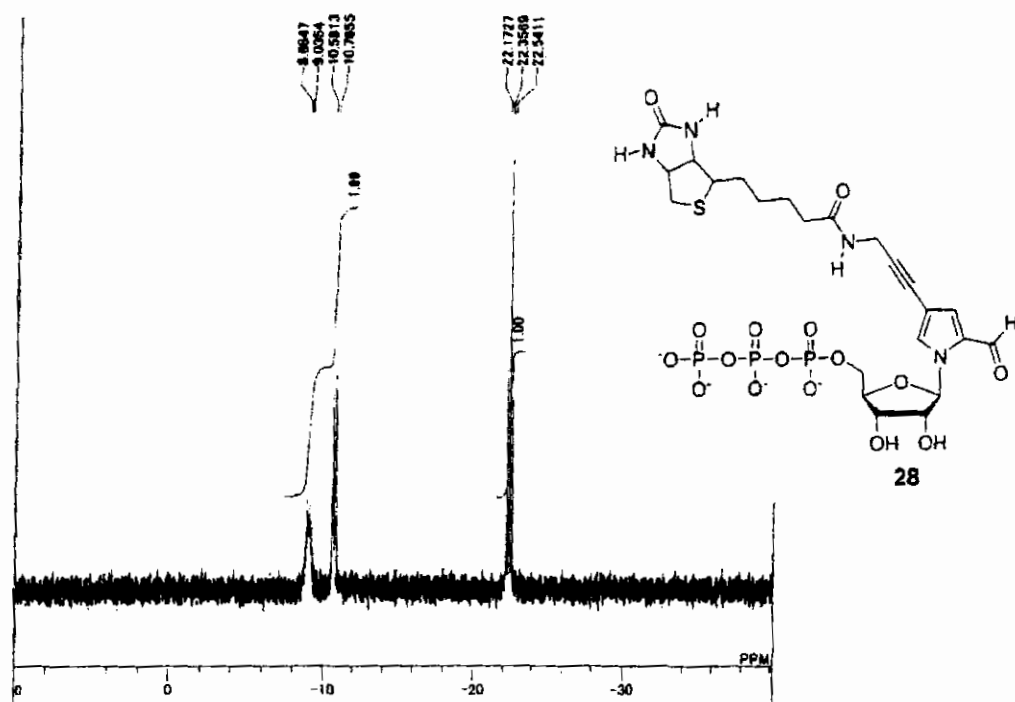
 ^{31}P NMR (109 MHz, D_2O) spectrum of compound **28**.

Figure 44a

a

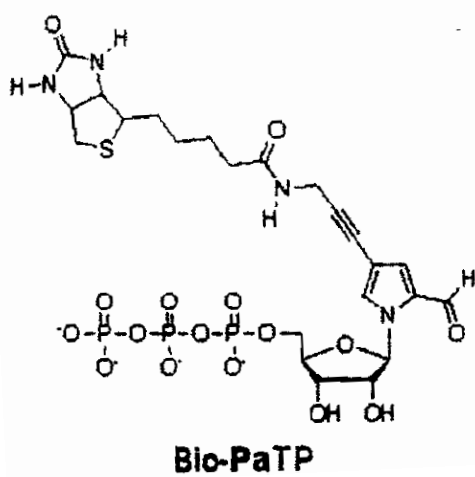


Figure 44b

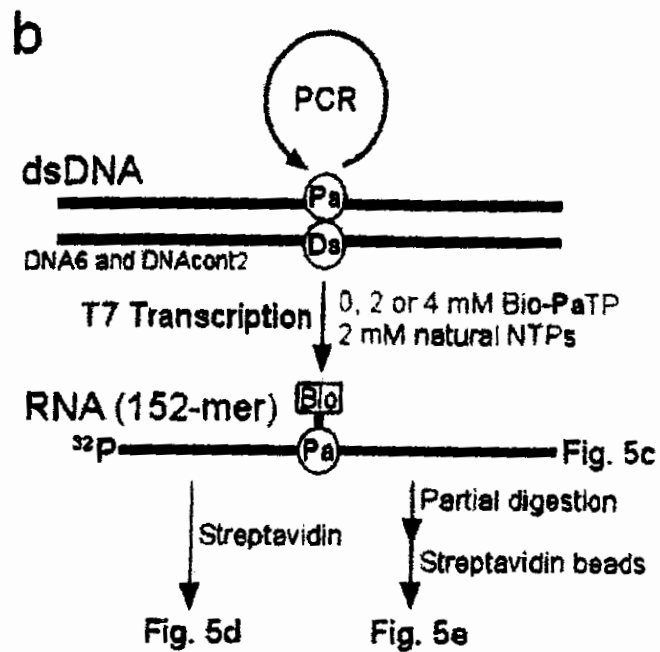


Figure 44c

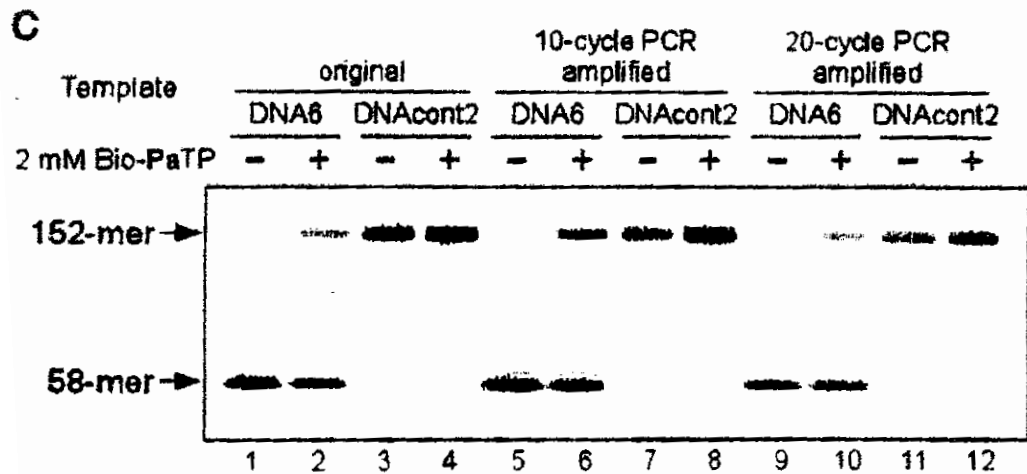


Figure 44d

d

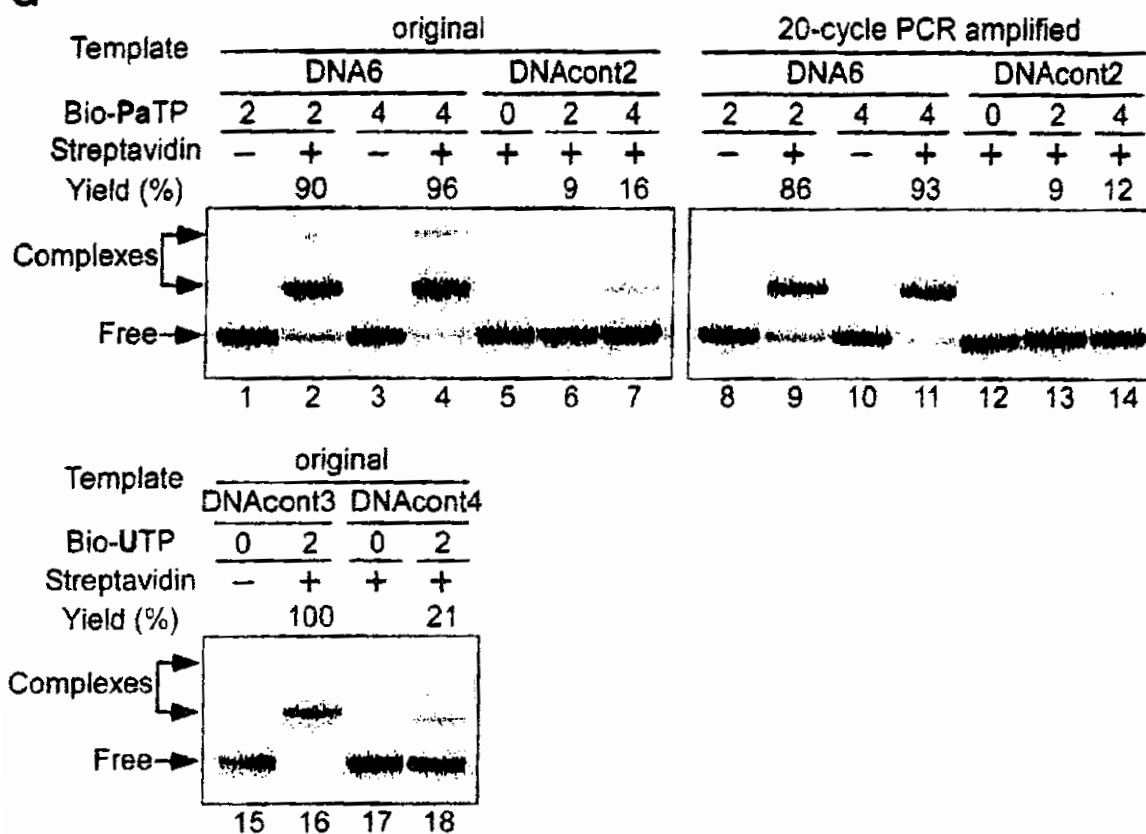


Figure 44e

e

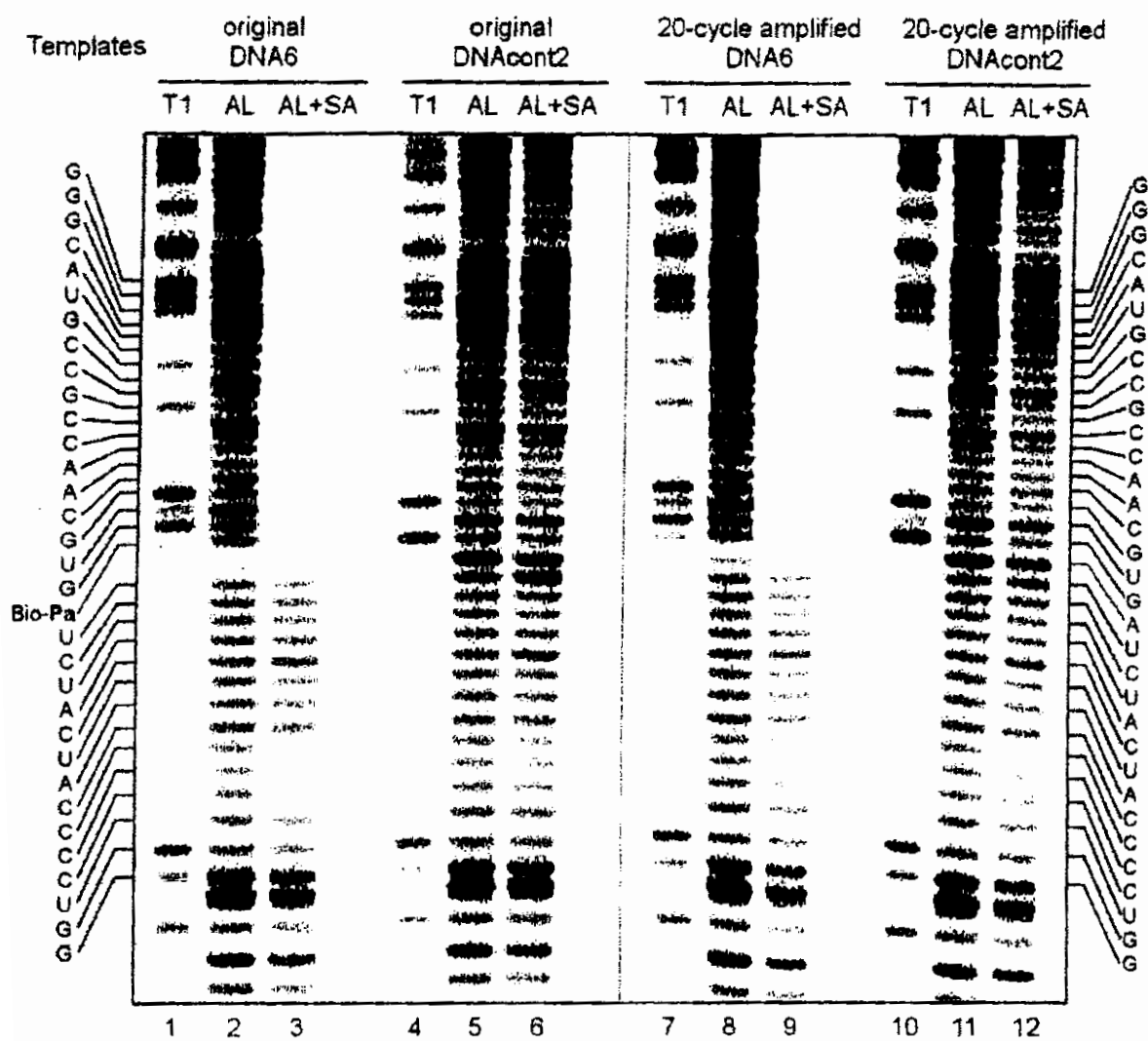


Figure 47

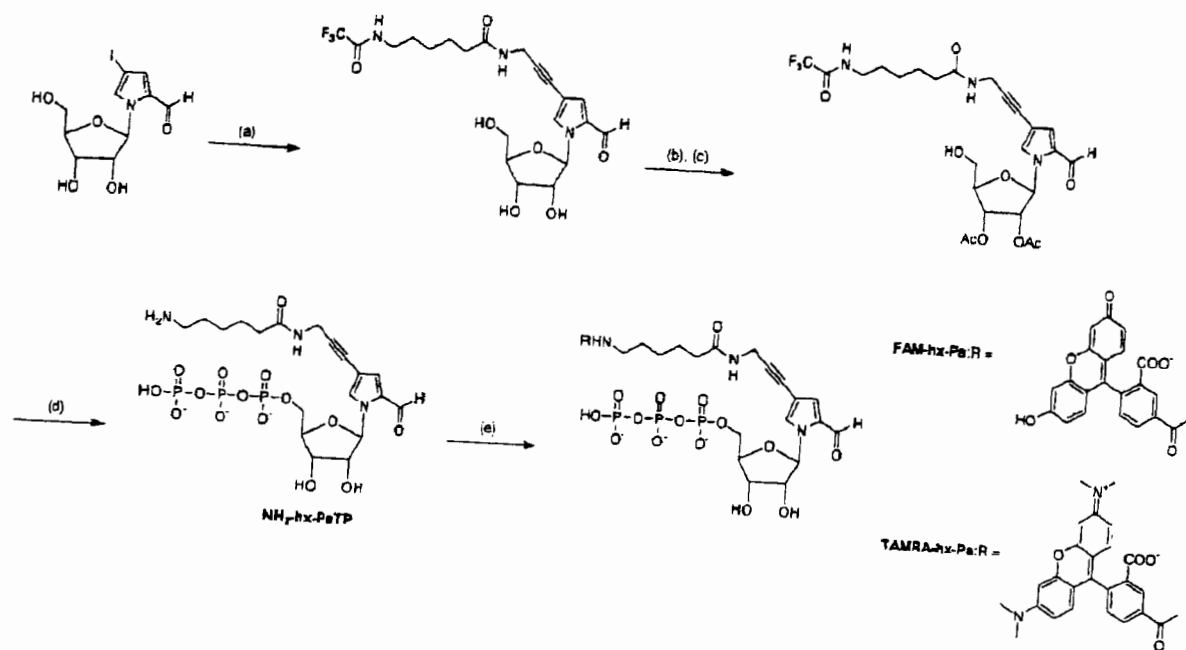
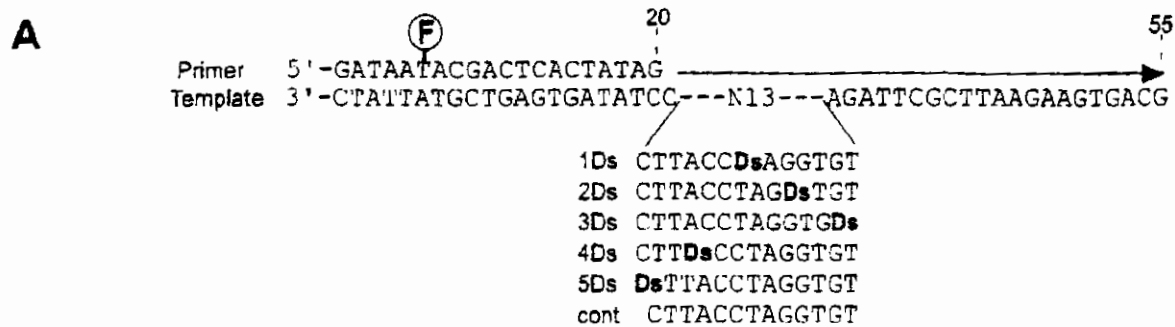
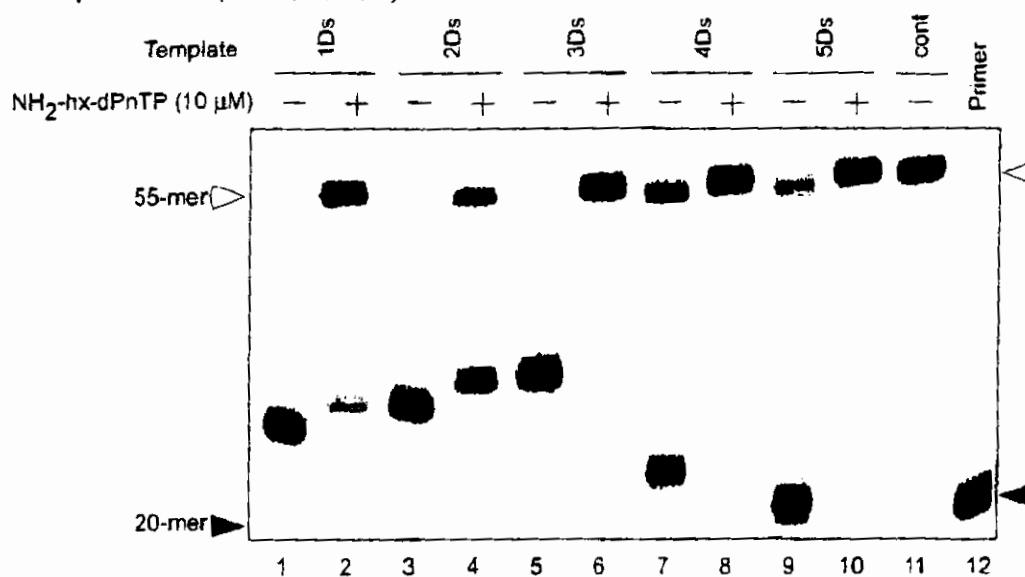


Figure 48

**B** 100 μ M dNTPs (N = A, G, C, T)**C** 200 μ M dNTPs (N = A, G, C, T)

Template	4Ds	
NH ₂ -hx-dPnTP (10 μ M)	-	+
FAM-hx-dPnTP (10 μ M)	-	+

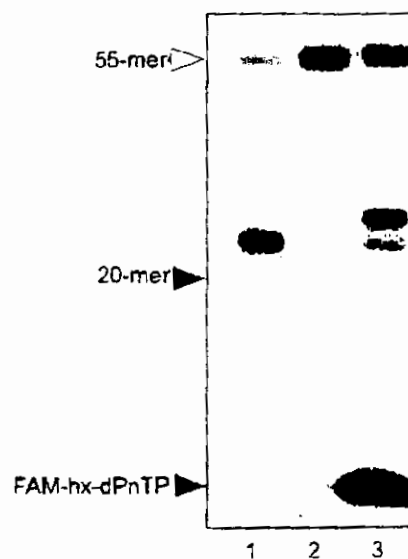


Figure 49

