Supplementary Data

Expanding the Repertoire of Pyrrolidyl PNA analogues for DNA/RNA Hybridization Selectivity: Aminoethylpyrrolidinone PNA(*aepone*-PNA)

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CONTENTS

Experimental Section:

General. Reagents were purchased from Lancaster, UK. DMF was dried by vacuum distillation over P_2O_5, THF refluxed over sodium, pyridine refluxed over KOH then CaH, CH_2Cl_2 refluxed over CaH and CH_3CN refluxed over CaH then distilled under anhydrous conditions. TLC were done using pre-coated silica gel plates 1.0554 DC-Alufolien 20 x 20 cm Kieselgel 60 F_{254} from Merck. Optical rotation of compounds was recorded on ADP220 Bellingham + Staneley polarimeter. ^{1}H NMR (200 MHz) and ^{13}C NMR were recorded in solvent $CDCl_3$ and values are quoted in δ ppm. Mass spectra were recorded using ESI (electron spray Ionization) on Finnigan MAT and MALDI-TOF using Micromass.

1-(N-Boc-aminoethyl)–4R-O-mesyl-5-one-2S-proline methyl ester (**3**)**.** To a vigorous stirred solution of compound **2** (380 mg, 0.95 mmol) in CH_3CN : CCl_4 (1:1, 10 mL), an aqueous solution (7.5 mL) of $NaIO_4$ (2.0 g, 9.08 mmole) and $RuCl_3$ (catalytic amount, 0.02 mmol) was added. After 30 min, the reaction was quenched by addition of isopropyl alcohol or 20% of aqueous solution (10 mL) of $NaHSO_3$ and stirred for another 20 min and the reaction mixture was concentrated under vacuum. The residue was taken into ethyl acetate (20 mL) and washed with water, the organic extract dried over Na_2SO_4 and concentrated to dryness. The resultant product was purified by column chromatography to obtain **3** as solid. Yield: 177 mg (45%). **3** was crystallized in mixture CH_2CL_2 and MeOH. $[\alpha]^{30}_D$ +80.8 (c 0.47, $CHCl_3$), ν_{max} cm^{-1} 1747, 1731, 1714, 1693, 1681, ^{1}H NMR, δ 5.3 (dd, J = 5.6, J = 5.4, 1H), 4.8 (bs, 1H), 4.5 (m,1H), 3.9 (s,3H), 3.8 (m,1H), 3.4 (s, 3H), 3.2 (m, 2H), 3.1 (m, 2H), 2.8 (m, 1H), 1.4 (s, 9H). ^{13}C NMR $CDCl_3$ δ 170.8, 169.4, 155.8, 79.3, 75.5, 56.3, 52.6, 42.9, 39.3, 37.4, 30.0, 28.1, (m/z): 380 (M^+).

1-(N-boc-aminoethyl)-4S-(thymin-1-yl)-5-one-2S-proline methyl ester (**4**). **3** (200 mg, 0.52 mmol), Thymine (80 mg, 0.63 mmol), K_2CO_3 (86.9 mg, 0.63 mmol) and catalytic amount of 18-crown-6 (54 mg, 0.15 mmol) in dry DMF (5 mL) were stirred at 65 ^{0}C overnight under N_2 atmosphere. The solvent was evaporated off and the residue was purified by chromatography (3%

MeOH in CH_2Cl_2) to obtain **4** as white foam. Yield: (65 mg, 30.0%), $[\alpha]^{25}_D$ -21.6 (c 0.6 , $CHCl_3$), ν_{max} cm^{-1} 1731, 1701, 1514, ^1H NMR, δ 9.2 (bs,1H), 7.2 (bs,1H), 5.25 (bs,1H), 5.0 (m,1H), 4.5 (m,1H), 3.9 (s,3H), 3.5(m,2H), 3.25 (m,2H), 2.5 (m,1H), 2.0 (s,3H), 1.9 (m,1H), 1.49 (s,9H), ^{13}C NMR ($CDCl_3$) δ 171.4, 170.0, 164.0, 156.2, 151.0, 139.3, 137.4, 111.6, 79.5, 56.6, 52.7, 43.1, 37.9, 29.1, 28.2, 12.1, m/z 410.0 (M^+).

1-(N-boc-aminoethyl)-4S-(N^4-benzyloxycarbonylcytosin-1-yl)-5-one-2S-proline methyl ester (**5**). Compound **3** was used to obtain **5** as foam by similar procedure as used for **4.** Yield: (36.0%), $[\alpha]^{25}_D$ +5.0 (c 0.8 , $CHCl_3$), ν_{max} cm^{-1} 1749, 1706, 1685, ^1H NMR, δ 7.5 (m,1H), 7.5 (s,5H), 6.5 (m,1H), 5.3 (s,4H), 3.15 (bs,5H), 2.0 (m,1H), 1.48 (s,9H), ^{13}C NMR ($CDCL_3$) δ 172.0, 170.0, 162.6, 159.4, 156.1, 152.4, 148.4, 134.9, 128.3, 99.5, 67.5, 57.7, 56.6, 52.4, 43.0, 37.7, 28.8, 28.1, m/z 530.0 (M^+).

1-(N-boc-aminoethyl)-4S-(N^6-benzoyladenin-9-yl)-5-one-2S-proline methyl ester (6). Compound 3 was used to obtain **6** as foam by same procedure as used for **4**. Yield (57%) of **6** as white foam. $[\alpha]^{25}_D$ + (c , $CHCl_3$), ν_{max} cm^{-1} 1708, 1610, ^1H NMR, δ 8.8 (m,1H), 8.2 (m, 1H), 8.0 (m, 1H), 7.5 (m, 3H), 5.5 (m, 1H), 5.0 (m, 1H), 3.9 (s, 3H), 3.7 (m, 1H), 3.4 (m, 2H), 3.2 (m, 2H), 2.8 (m, 2H), 1.49 (s, 9H), ^{13}C NMR ($CDCl_3$) δ 171.0, 169.0, 158.9, 155.8, 152.1, 149.6, 142.6, 141.6, 133.5, 132.9, 128.4, 127.7, 95.8, 79.3, 57.5, 54.3, 52.6, 43.1, 37.4, 30.1, 28, m/z 523 (M^+).

1-(N-boc-aminoethyl)-4S-(2-amino-6-chloropurin-9-yl)-5-one-2S-proline methyl ester (7). Compound **3** was used to obtain **7** by procedure similar to that for **4.** Yield: (45.0 %), $[\alpha]^{25}_D$ +10.0 (c 0.2, $CHCl_3$), ν_{max} cm^{-1} 1714, 1706, 1610.45, ^1H NMR (200 MHz, $CDCL_3$) δ 7.6-7.9 (m, 1H), 5.4(m, 1H), 5.2 (m, 2H), 4.5 (m, 1H), 3.8 (bs, 4H), 3.5 (m, 2H), 3.2 (m, 2H), 2.7 (m, 2H), 1.5(s, 9H), ^{13}C NMR($CDCl_3$) δ 171.0, 170.0, 158.9, 155.9, 142.4 141.6, 125.2, 95.9, 79.9,57.79, 52.71, 43.0, 73.4, 30.0, 29.2, 28.1, m/z 453 (M^+).

General protocol for solid phase synthesis of PNA.

Synthesis of PNA was carried out using BOC-β-alanine derivatized Merrifield resin (Pharmecia) (0.15 mmol/g substitution). The synthesis cycle was as follows: deprotection: 50% TFA in DCM (15 min), wash with DCM, DMF and DCM , neutralize (5% DIEA in DCM), wash DCM, DMF and DCM), coupling (**4b**/HOBT/HBTU/DIEA in DMF, 4 eq, 1.5 h) capping (10% Ac_2O/Pyridine in DCM), wash (DCM, DMF and DCM). Deprotection and amide Coupling reaction was monitored by Kaiser's test. The purity of PNA was ascertained on an analytical RP C18 column.

8. H_2N-T-T-T-T-T-T-T-T-β-ala-COOH (*aeg*-T_8)

9. H_2N-T-T-T-T-T-T-T-**t**-β-ala-COOH

M_r (MALDI-TOF) found 2232.0, calcd. For M =2230

10. H_2N-T-T-T-**t**-T-T-T-**t**-β-ala-COOH

M_r (MALDI-TOF) found 2244.0, calcd. For M =2243

11. H_2N-**t-t-t-t-t-t-t-t**-β-ala-COOH (*aepone*-$\mathbf{t}_8$)

M_r (MALDI-TOF) found 2315.0, calcd. For M =2315

12 H_2N-*t-t-t-t-t-t-t-t*-β-ala-COOH (*aep*-$\boldsymbol{t}_8$)

DNA was synthesized using standard procedure on automated synthesizer.

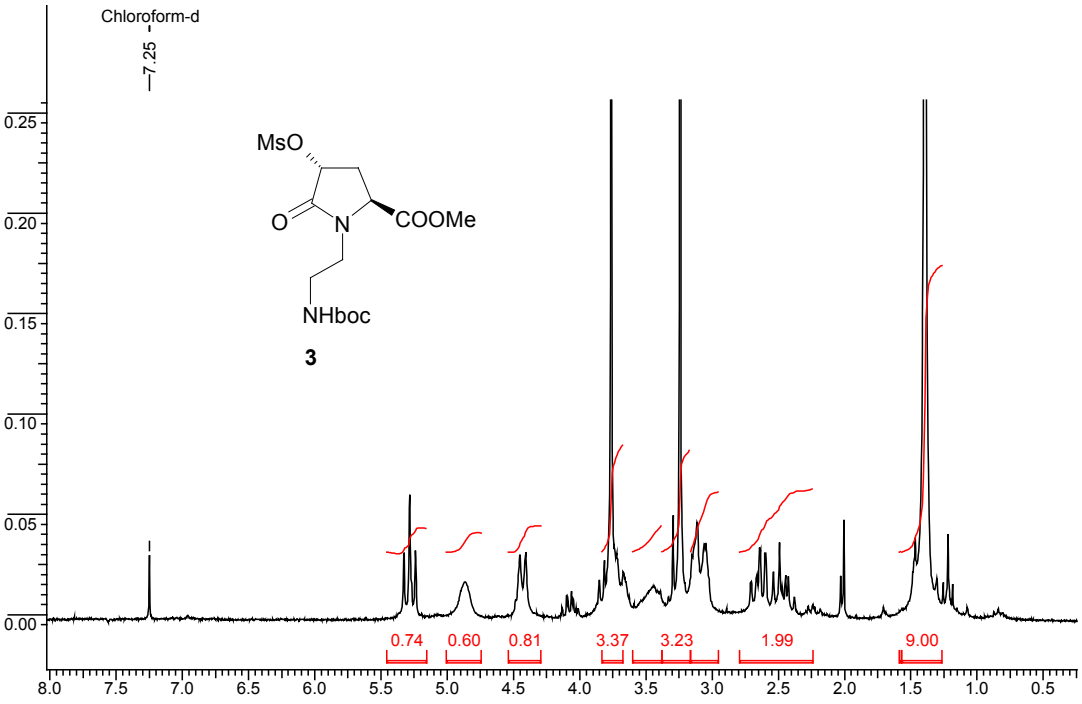
13 d(GCAAAAAAAACG) (DNA)

UV-T_m experiments.

UV melting experiments were performed on Lambda-35 UV Spectrometer (Perkin-Elmer) equipped with a thermal melt system, PTP-6 Peltier Temperature Programmer with water circulator Thermohake K20. The sample for Tm measurement was prepared by mixing calculated amount of stock oligonucleotide and PNA solutions together in 2 mL of sodium phosphate buffer (pH 7.1). The samples 2 mL were transferred to quartz cell and sealed with Teflon stopper after degassing with nitrogen gas for 15 min and equilibrated at the starting temperature for at least 30 min. The OD

at 260 nm was recorded in steps from 10-85 ^{o}C with temperature increment of 0.2 ^{o}C/min. The results were normalized and analysis of data was performed on using Origin 5.0 (Microsoft Corp.).

PMR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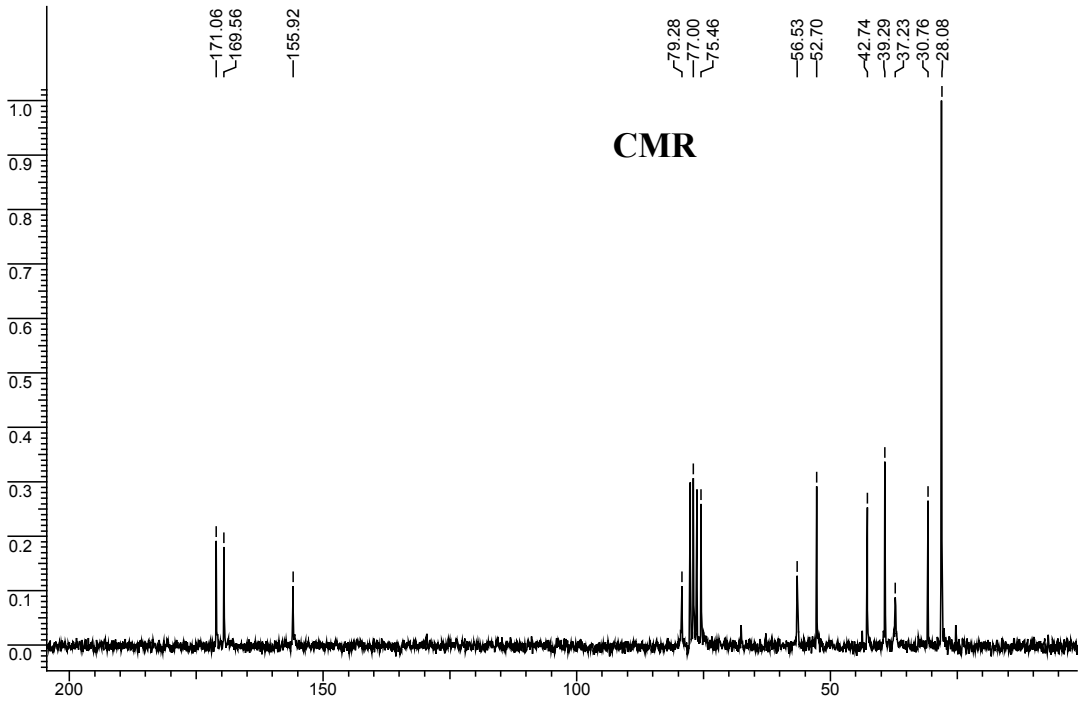
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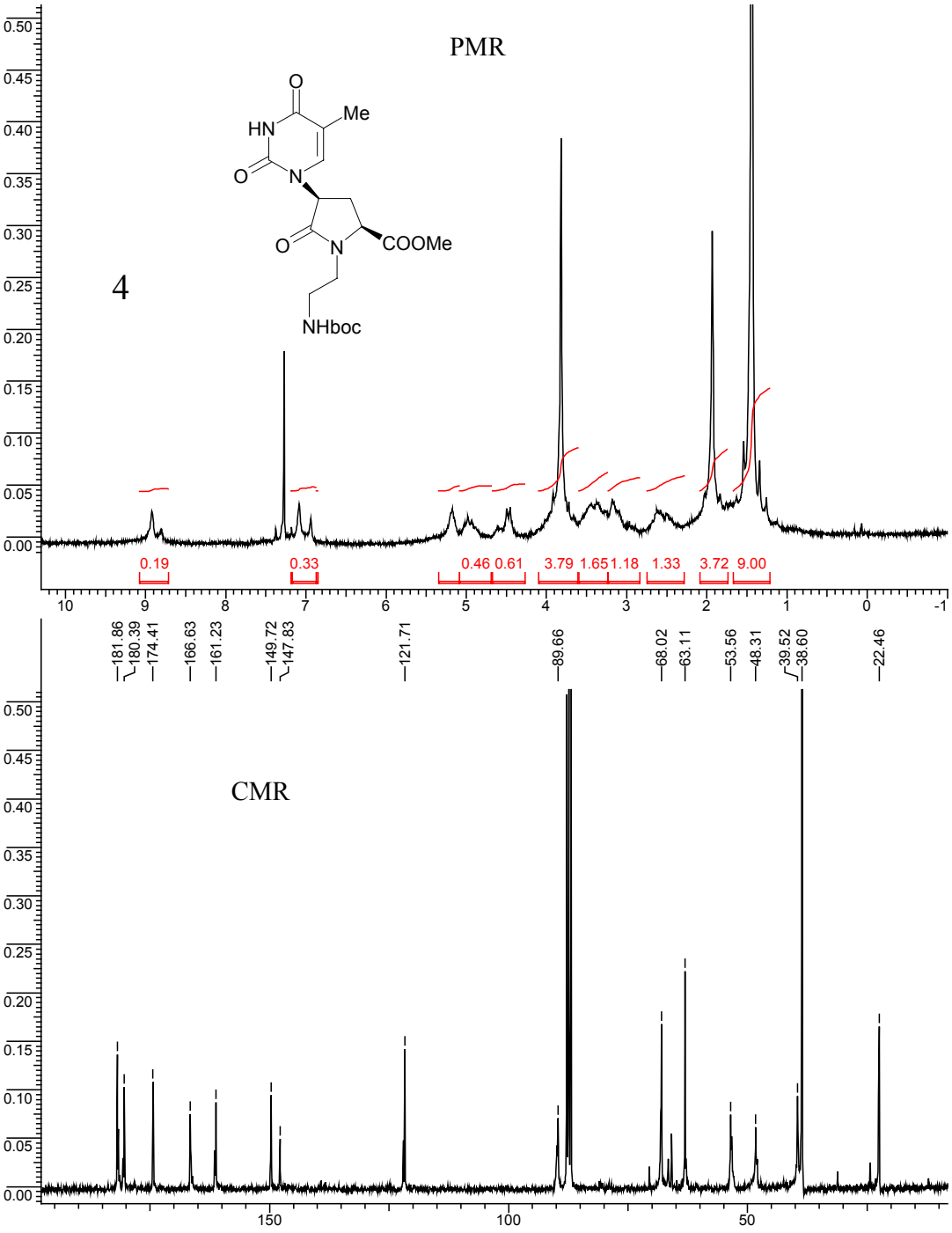
CMR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4
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HN
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N
O
N
COOMe
NHboc
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0.33
0.46 0.61
3.79 1.65 1.18
1.33
3.72
9.00
181.86
180.39
174.41
166.63
161.23
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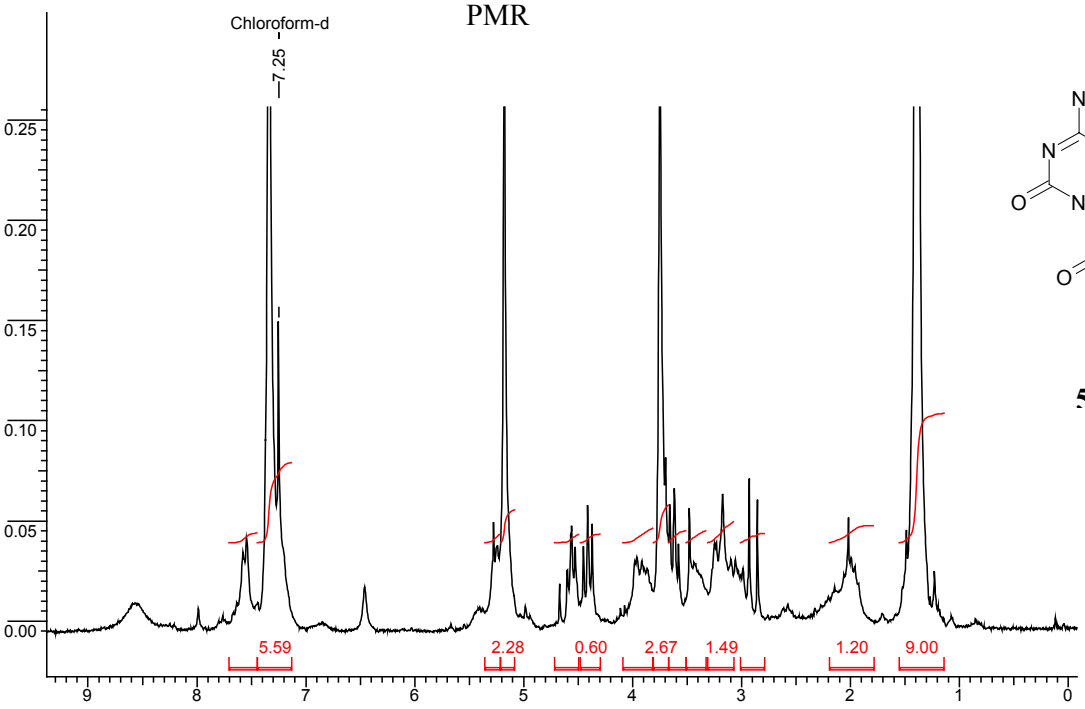
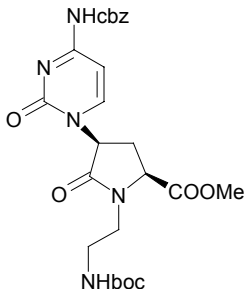
22.46
CMR

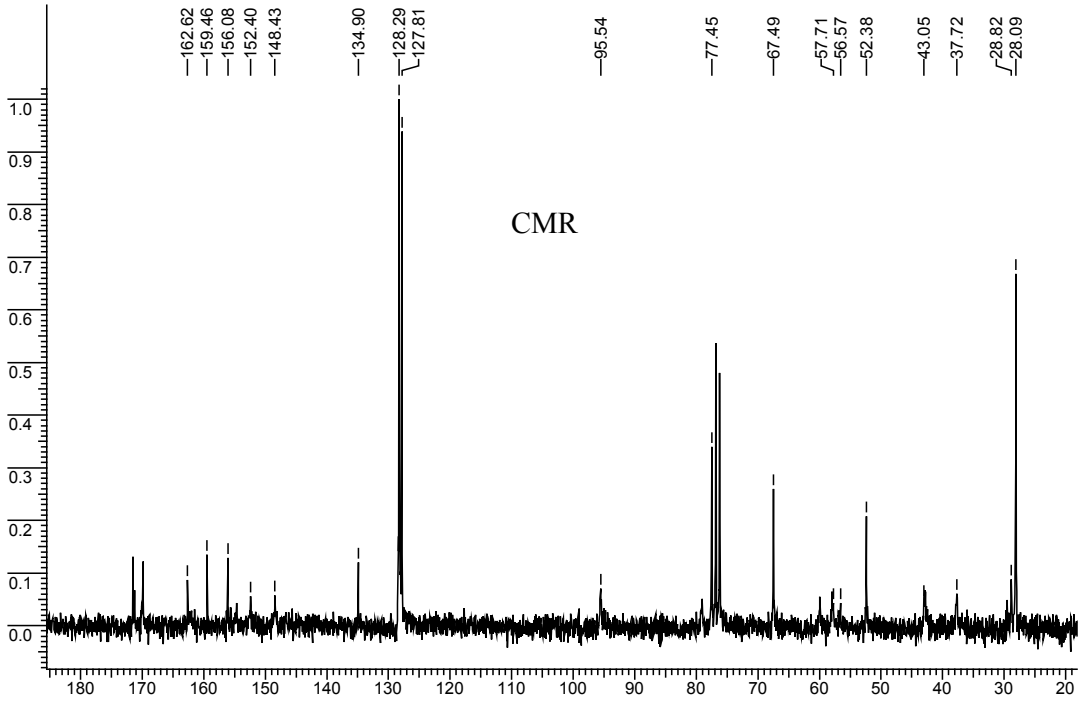
PMR





5

NKS12



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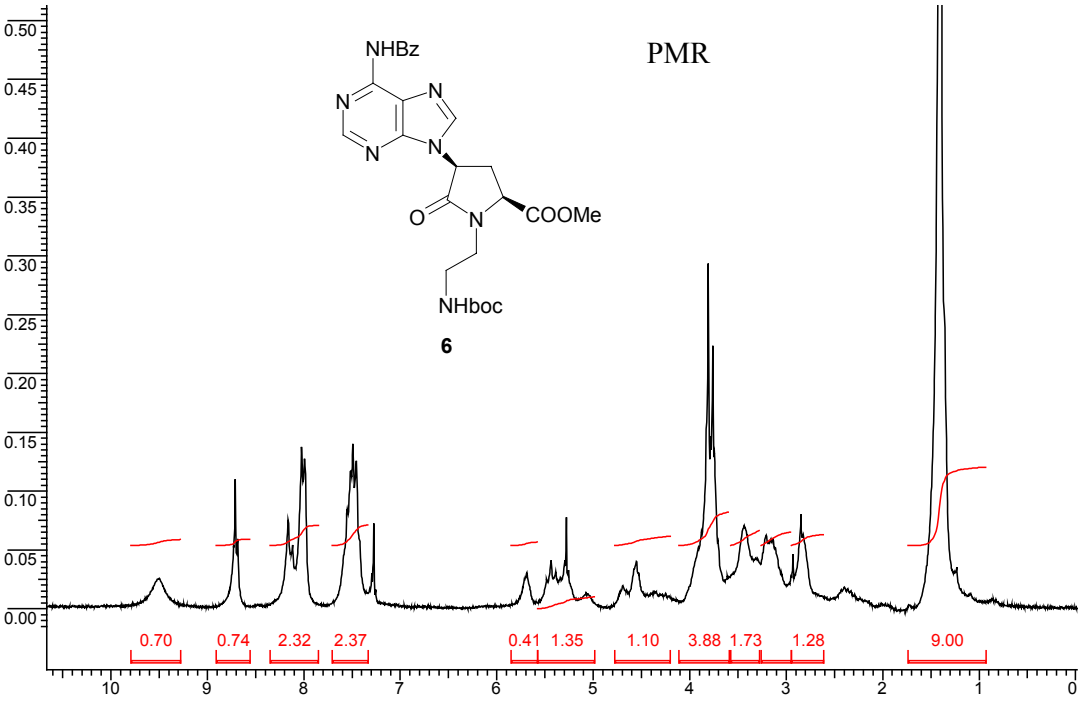
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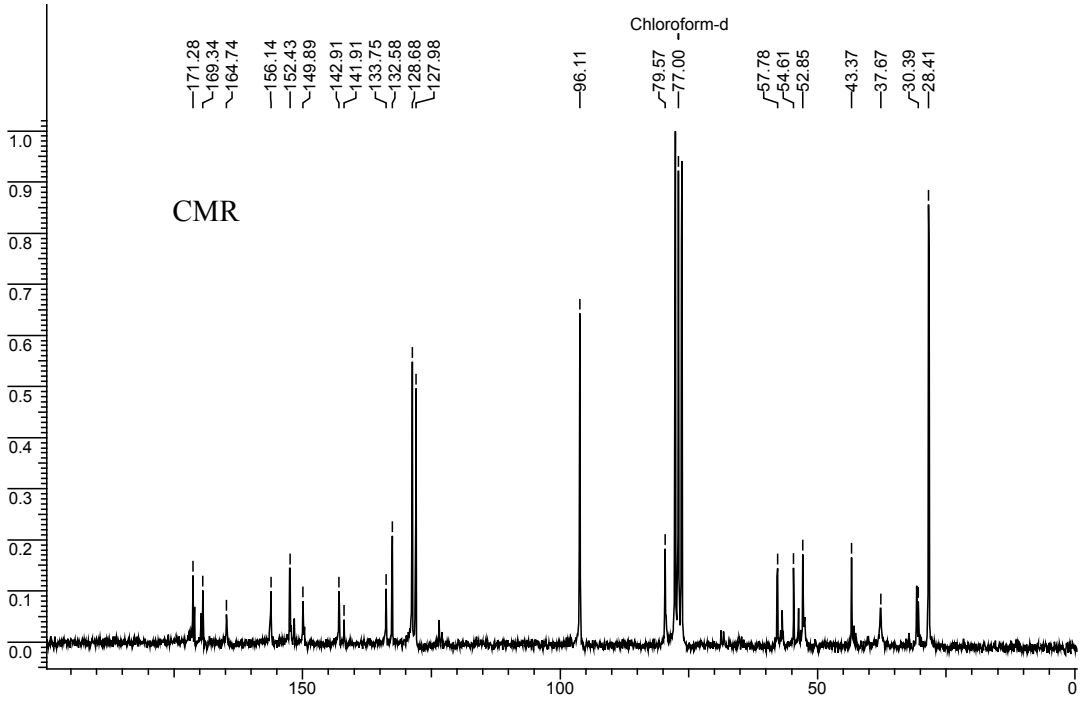
9.00

^{1}HNKS10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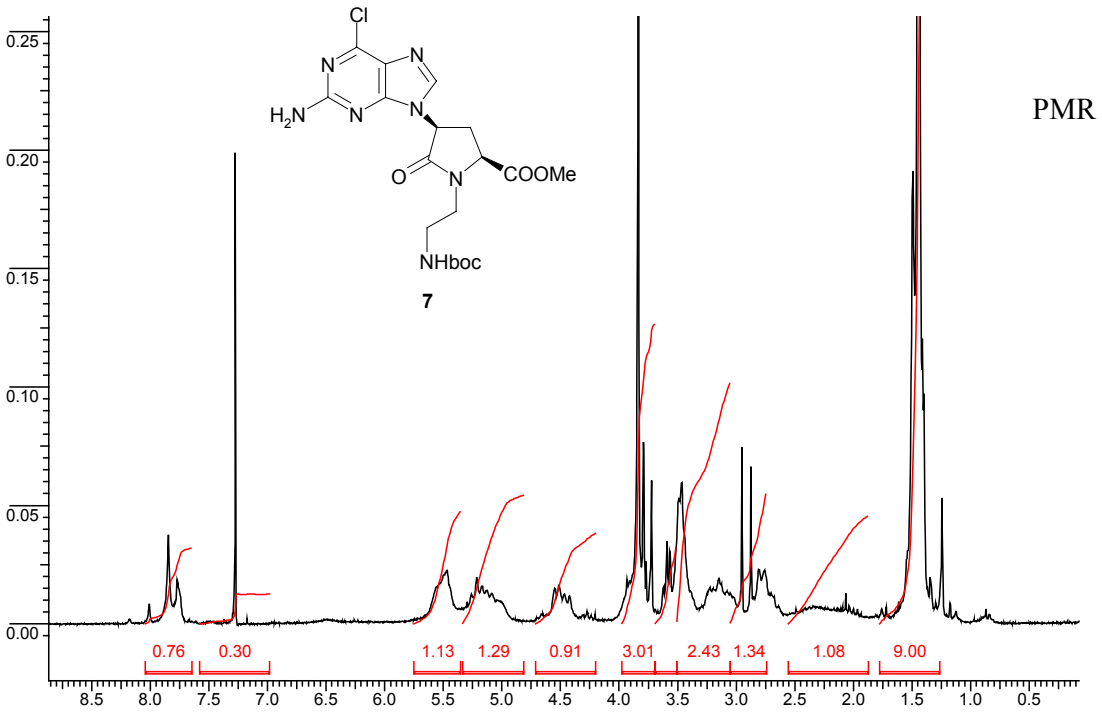
CMR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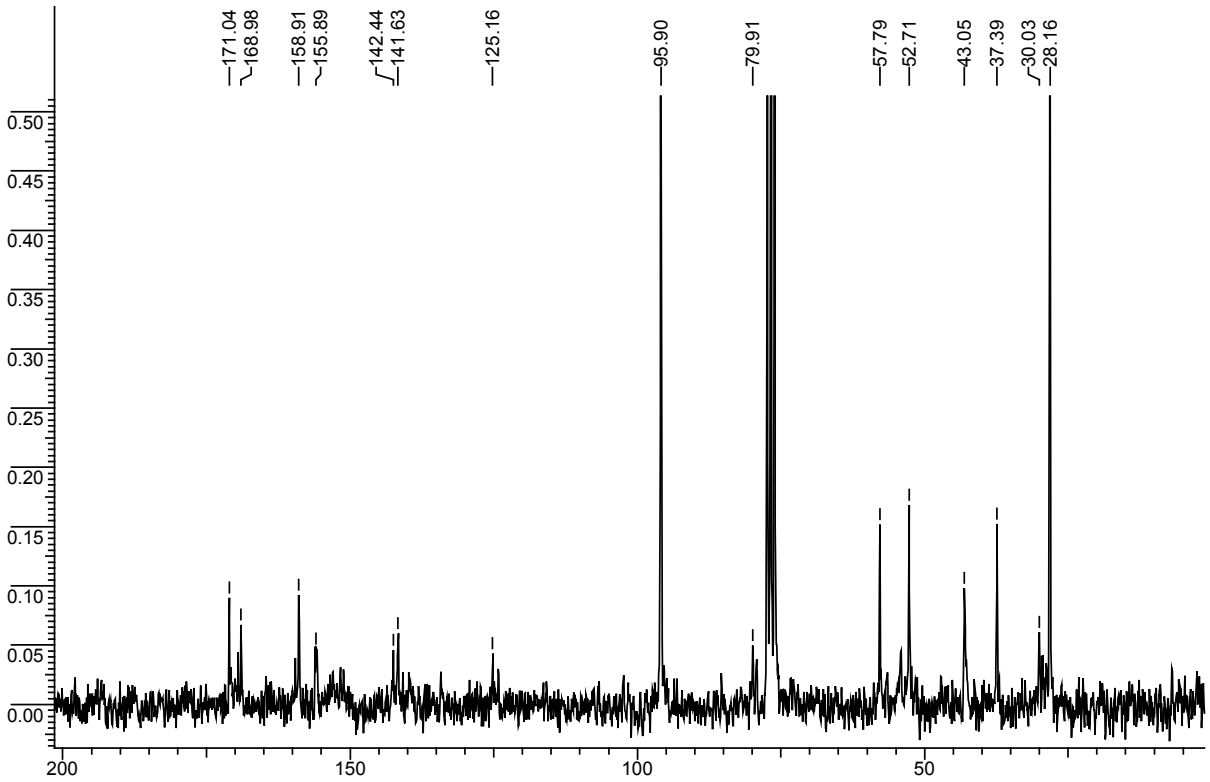
1.34
1.08
9.00

PMR

CMR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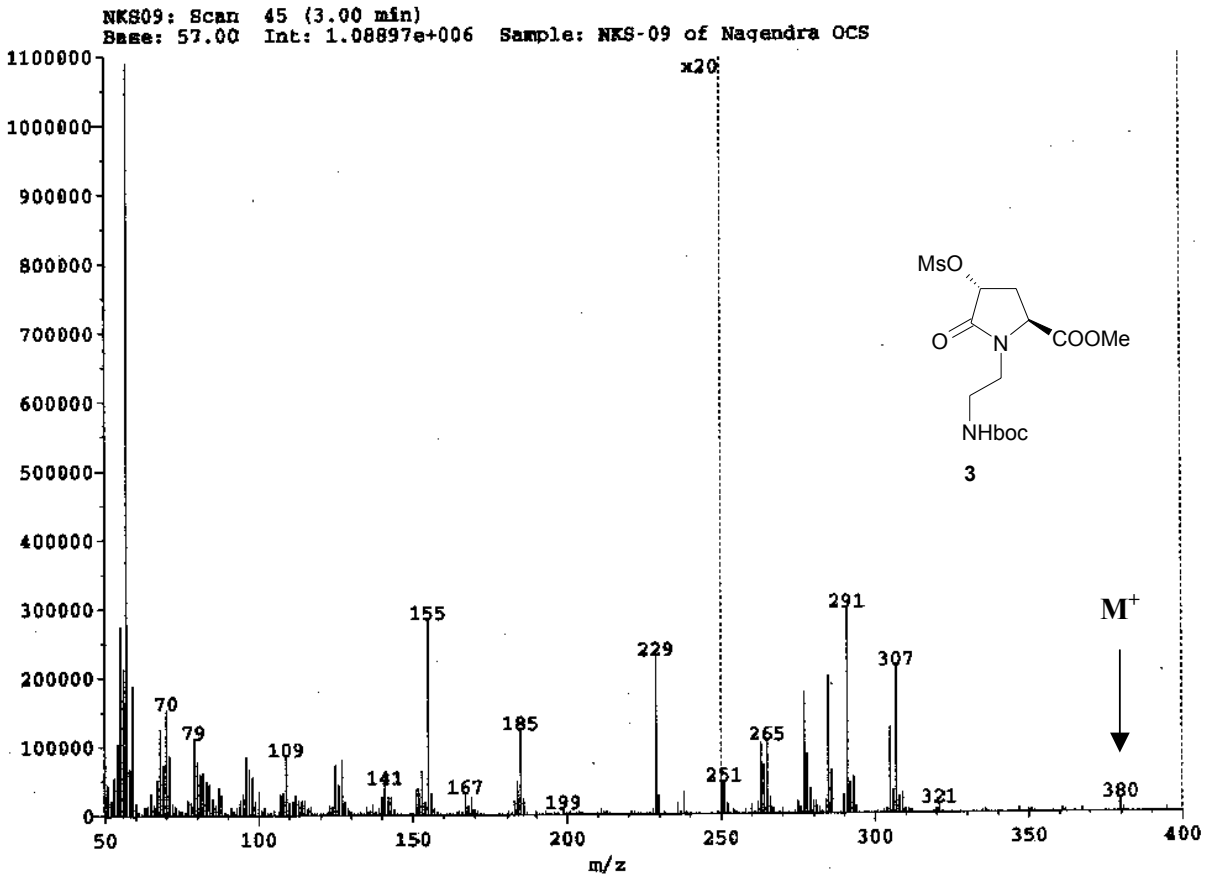
37.39
30.03
28.16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O
N
COOMe
NHboc
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M+
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229
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79
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141
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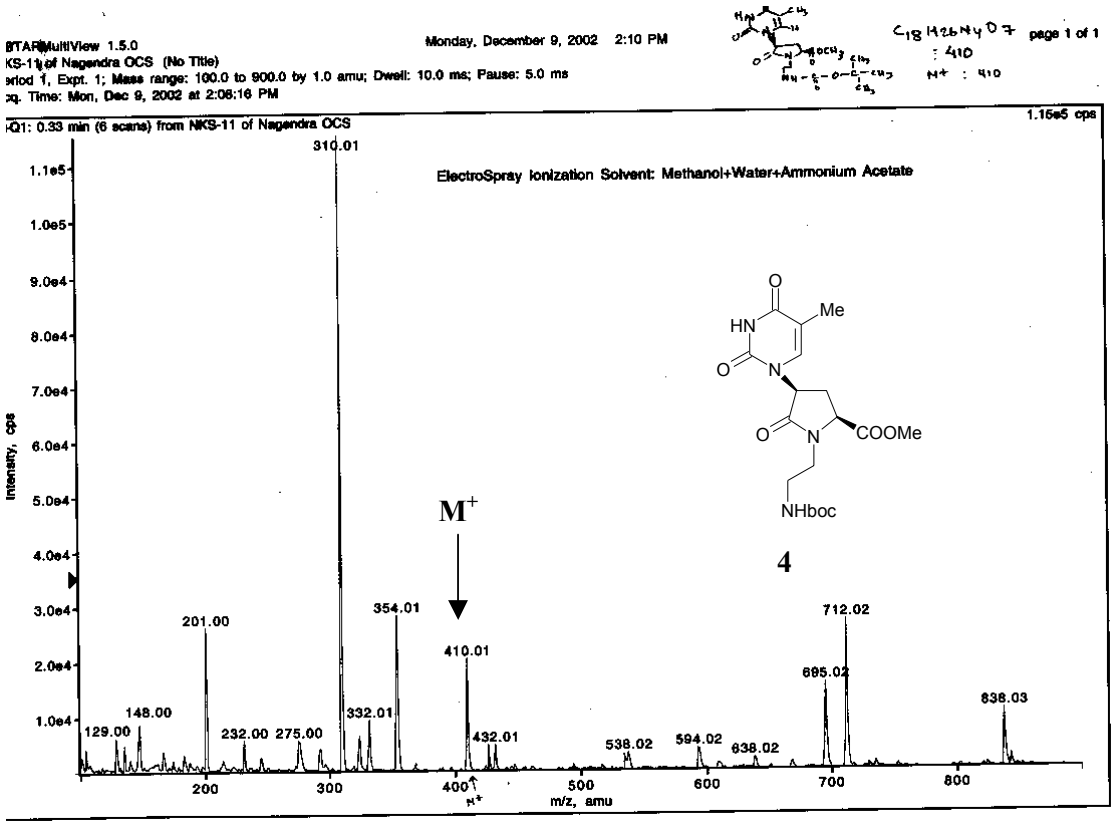
321
380
m/z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Monday, December 9, 2002 2:10 PM
C18H26N4O7 page 1 of 1
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M+ : 410
KS-11 of Nagendra OCS (No Title)
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Intensity, cps
m/z, amu
M+
4
HN
Me
O
N
COOMe
NHboc
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201.00
410.01
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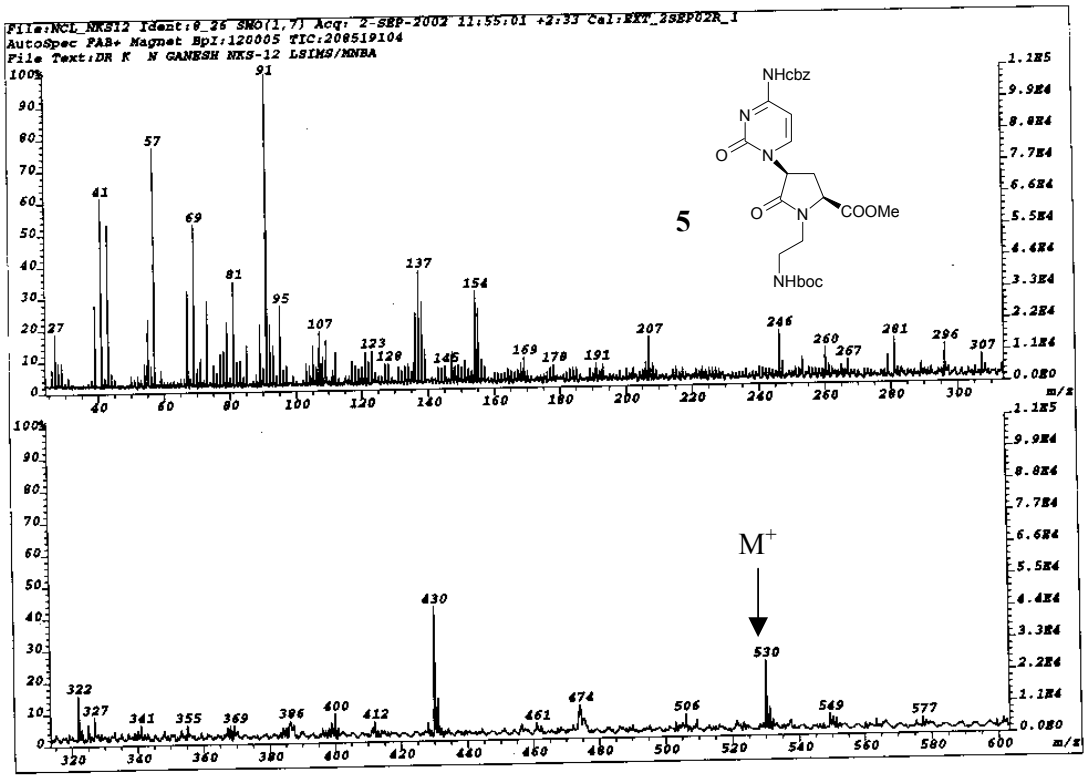
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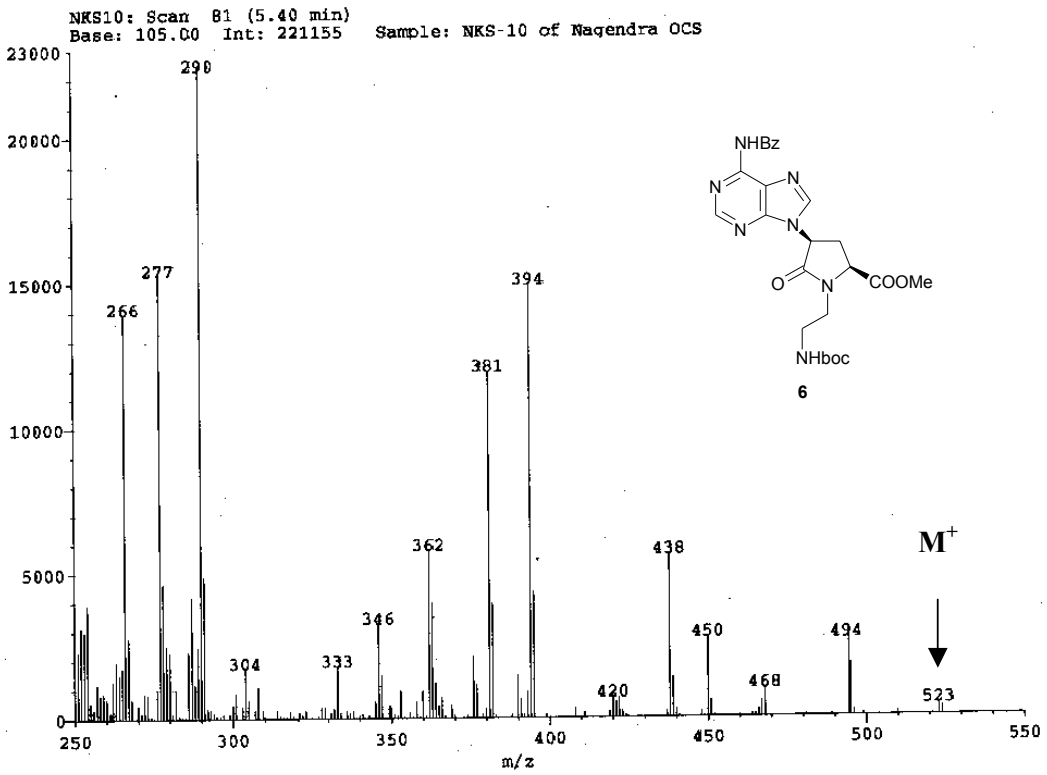
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350
400
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500
550
m/z
290
277
266
394
381
362
438
346
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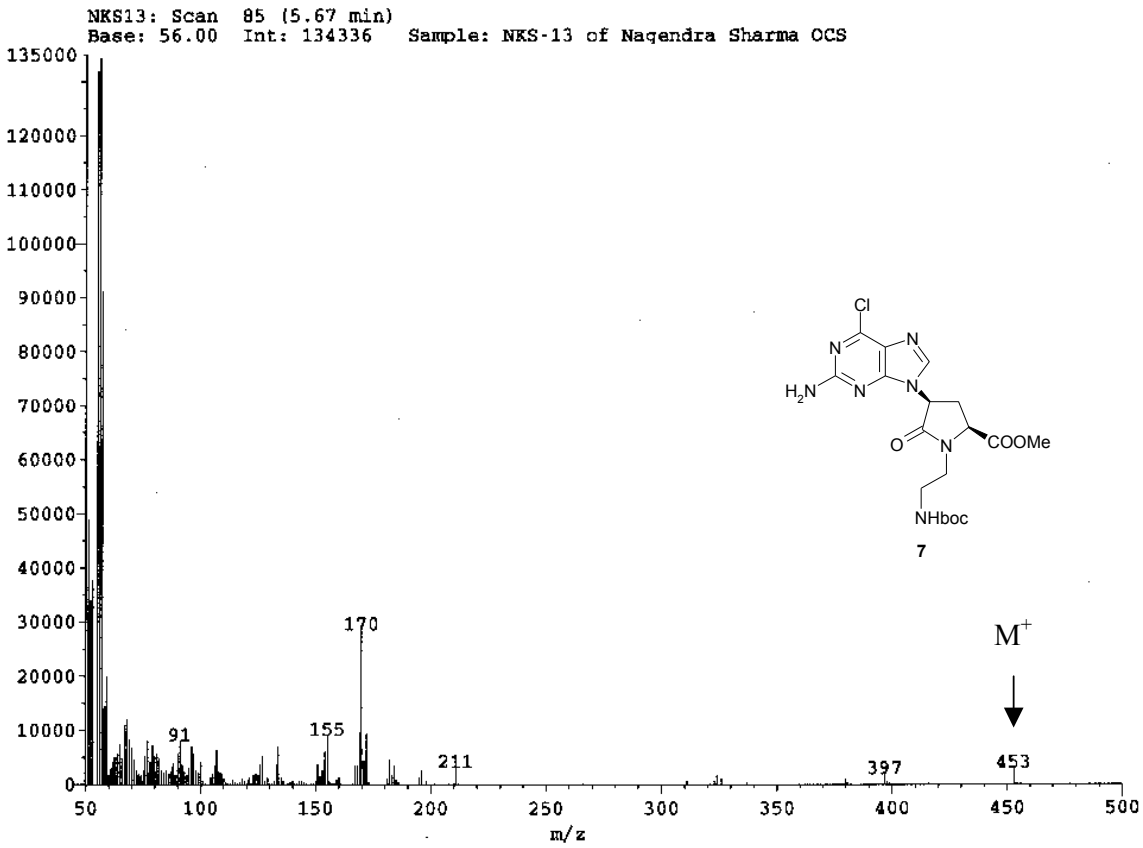
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60000
50000
40000
30000
20000
10000
0
50
100
150
200
250
300
350
400
450
500
m/z
91
155
170
211
397
453
M+
Cl
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N
N
N
H2N
O
N
COOMe
NHboc
7

R K N GANESH
KS-12
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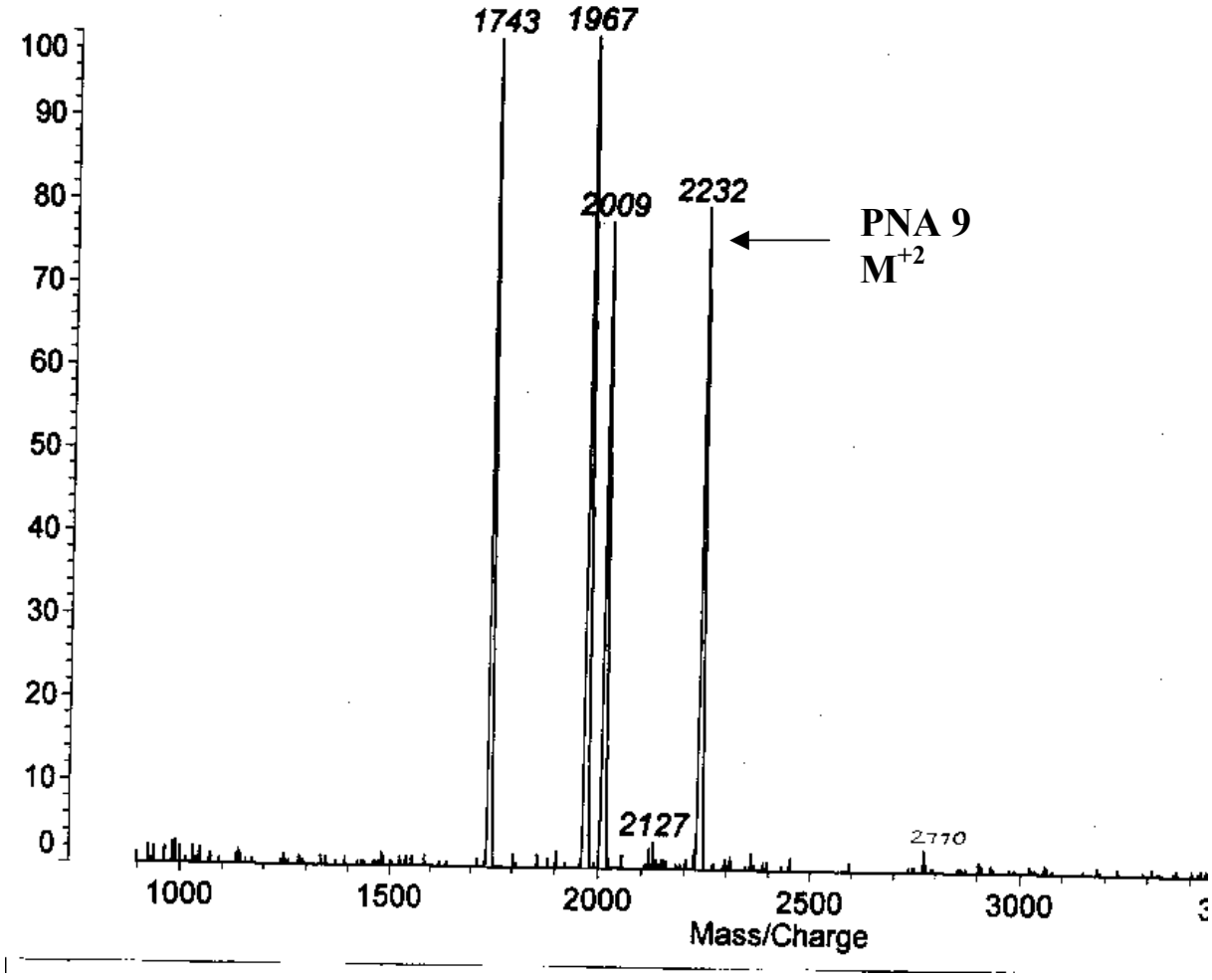

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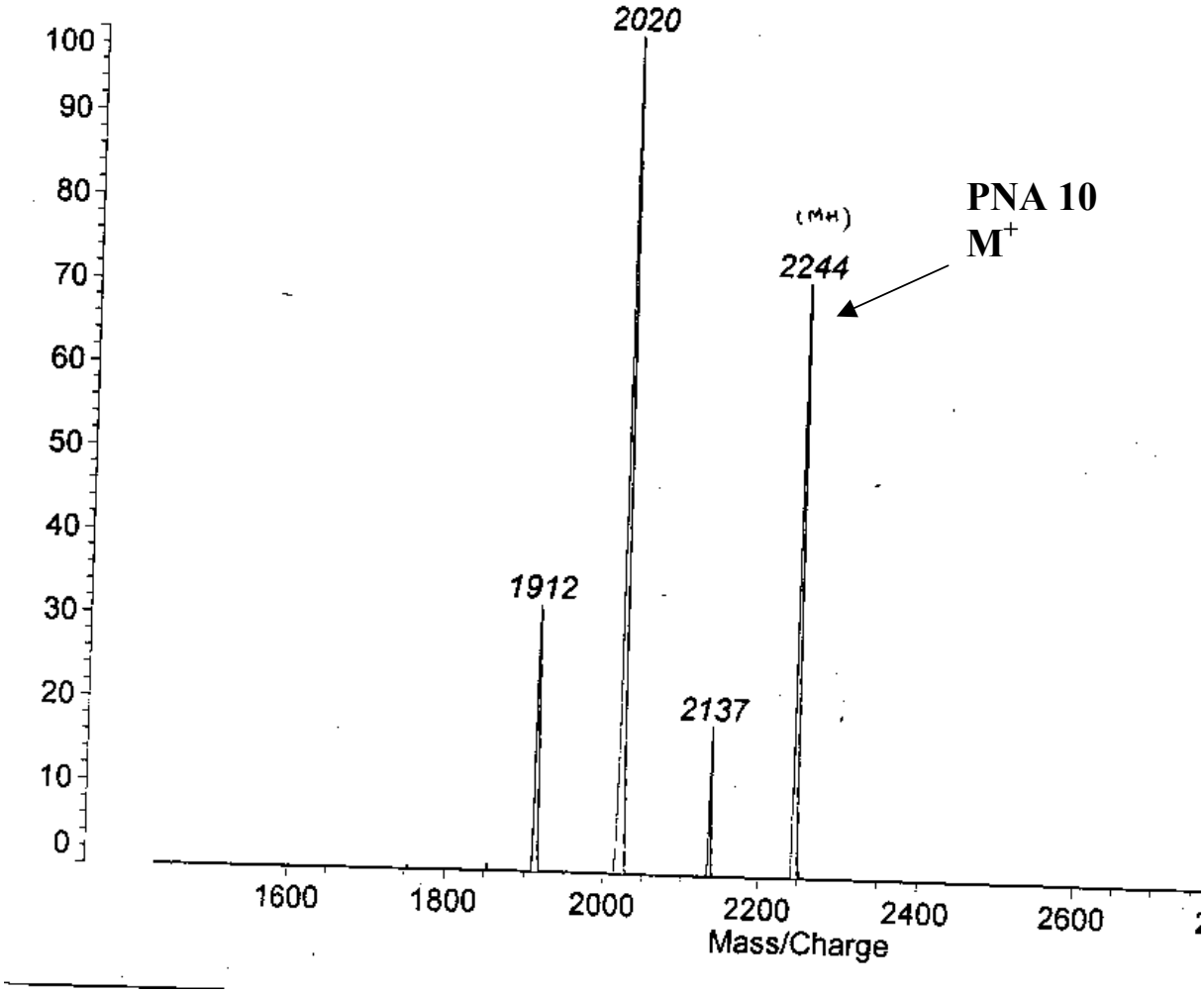

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3NK
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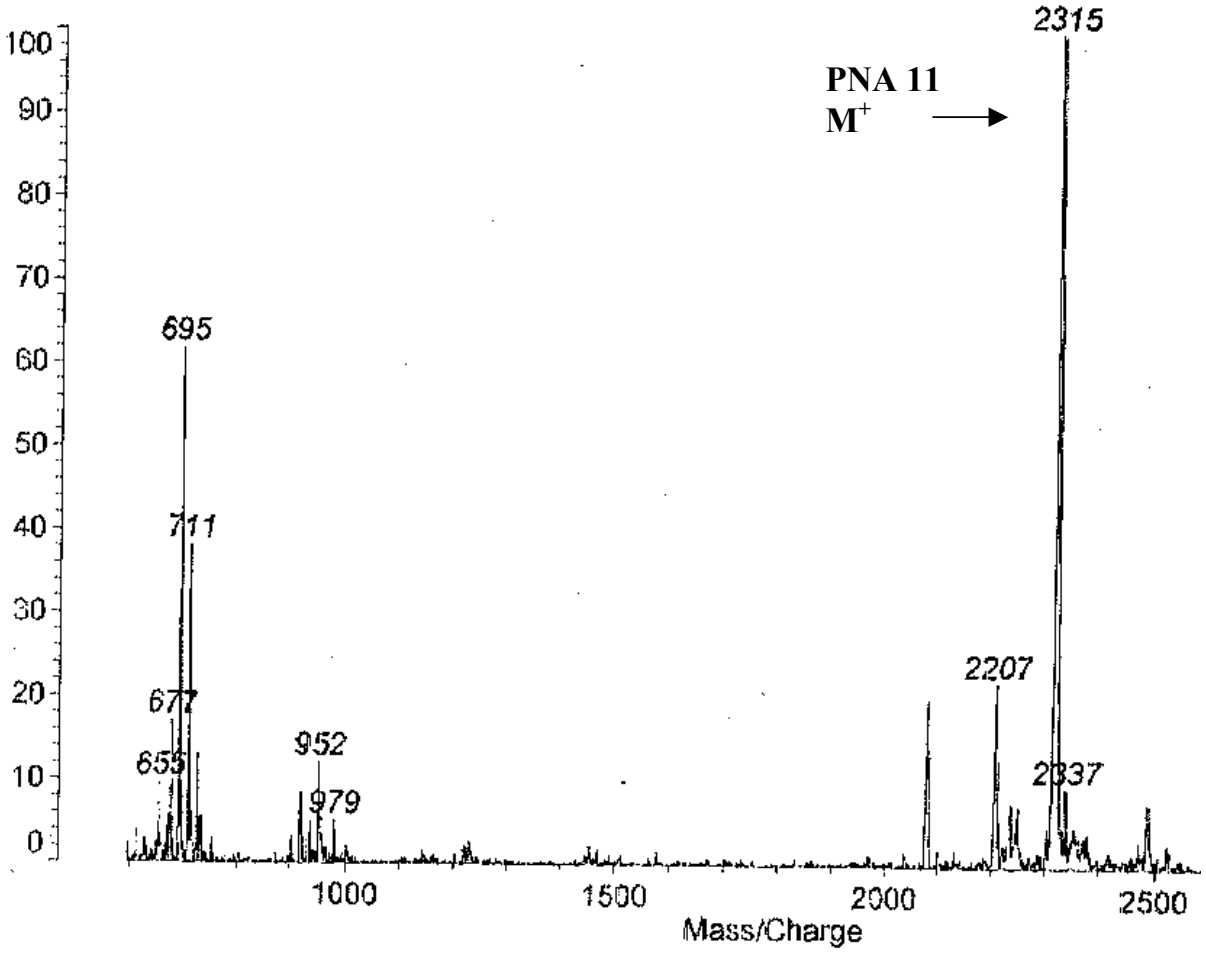
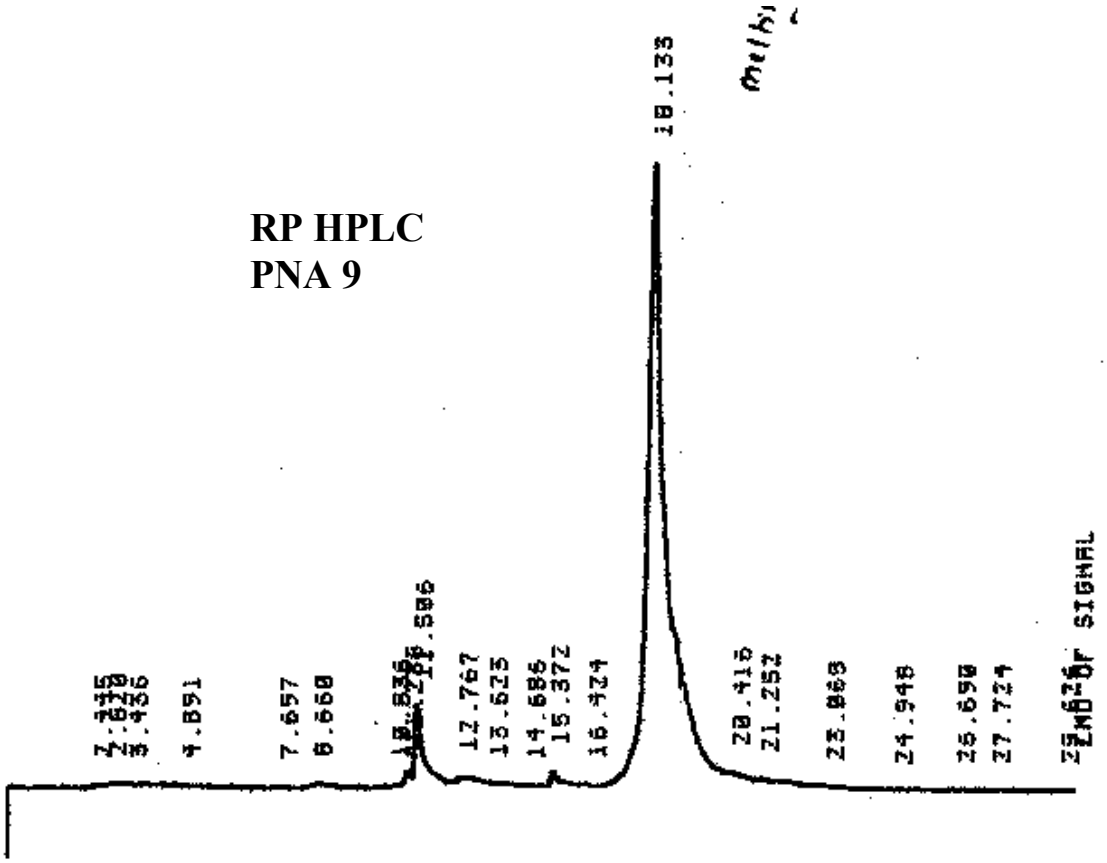


RP HPLC
PNA 9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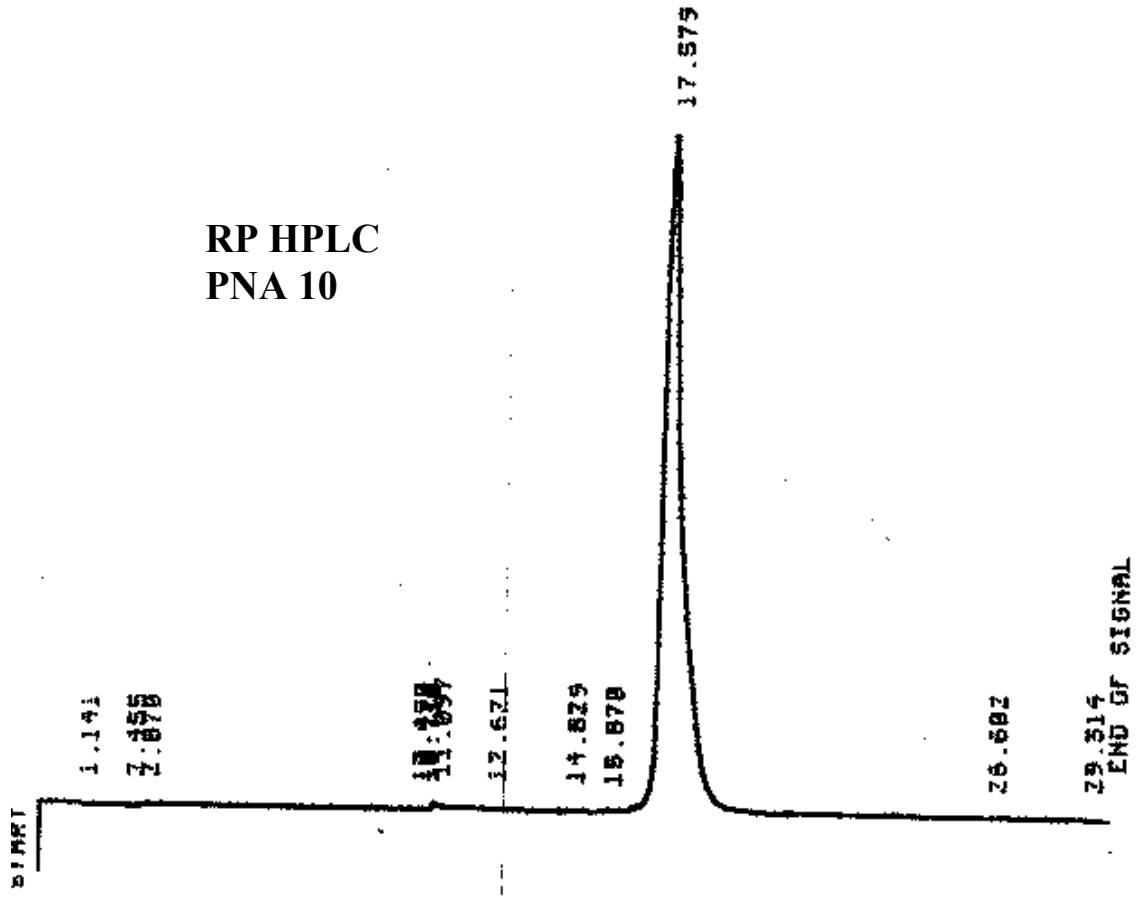
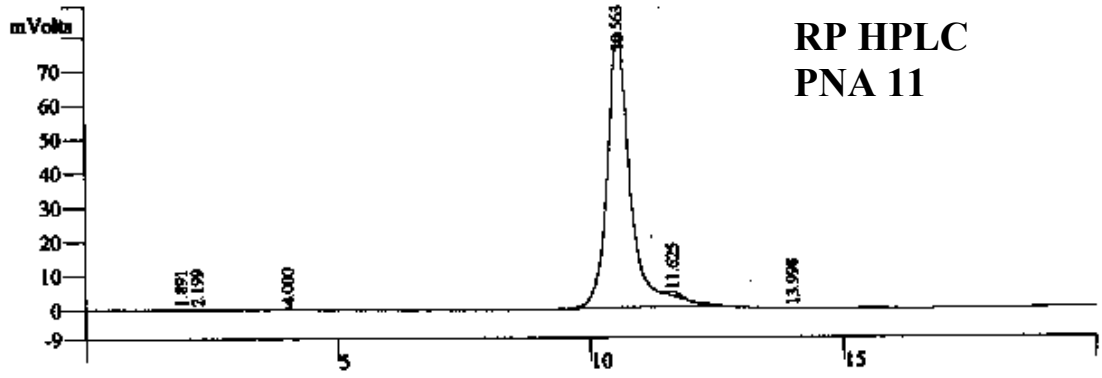
SIGNAL

RP HPLC
PNA 10



RP HPLC
PNA 11



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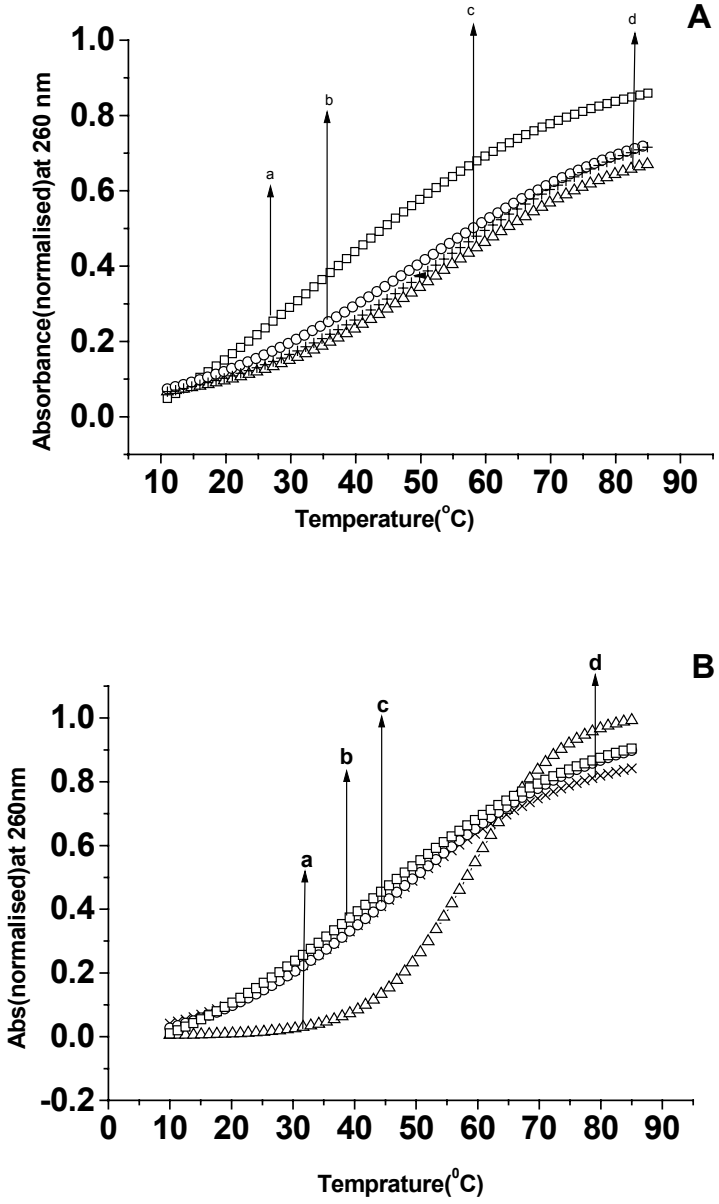
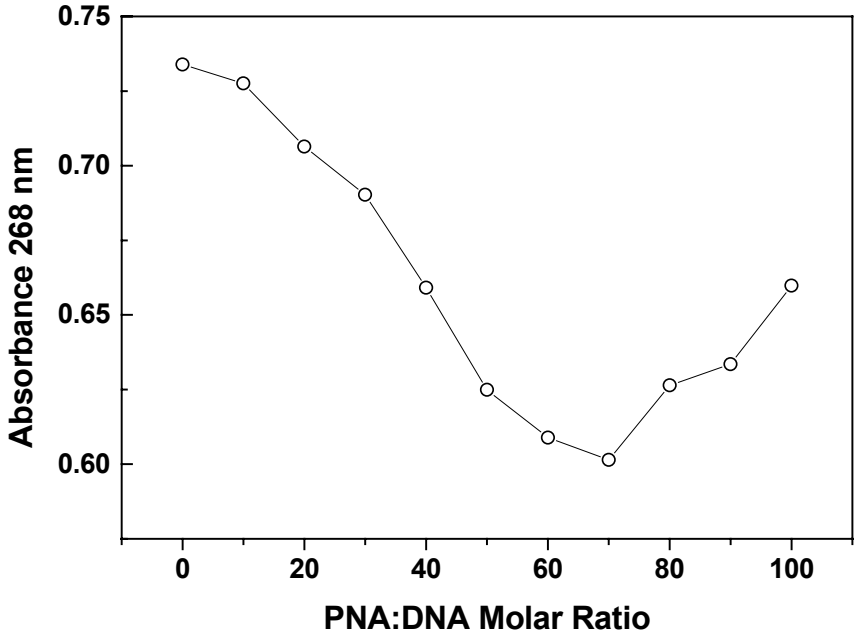
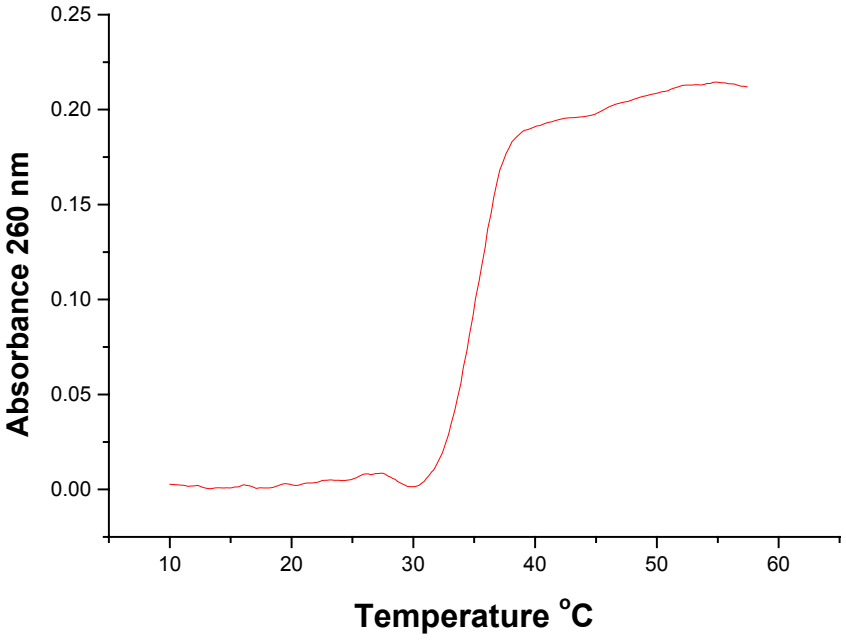


Figure 2. Melting absorbance (260nm)-temperature profiles, A. PNA:DNA **13** hybrids and B. PNA:poly **rA** hybrids a. **8** b.**9** c.**10** d.**11**



Job's plot for aepone-PNA 11: DNA 13, indicating 2:1 binding



Melting profile for PNA 12: poly rA