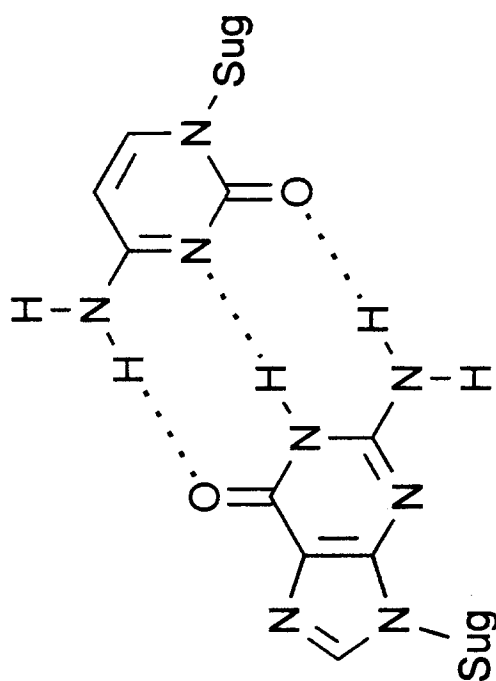
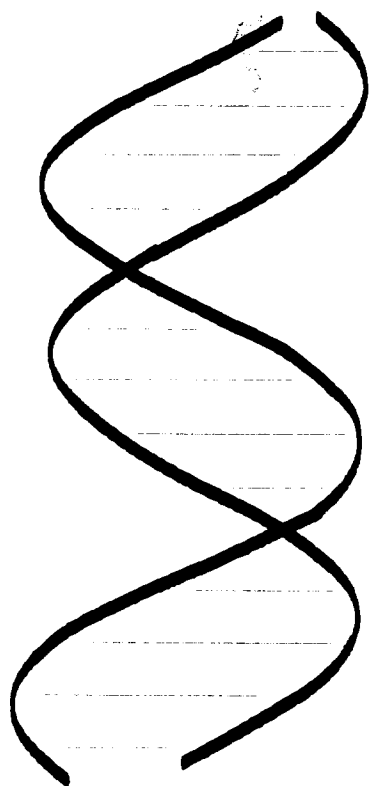
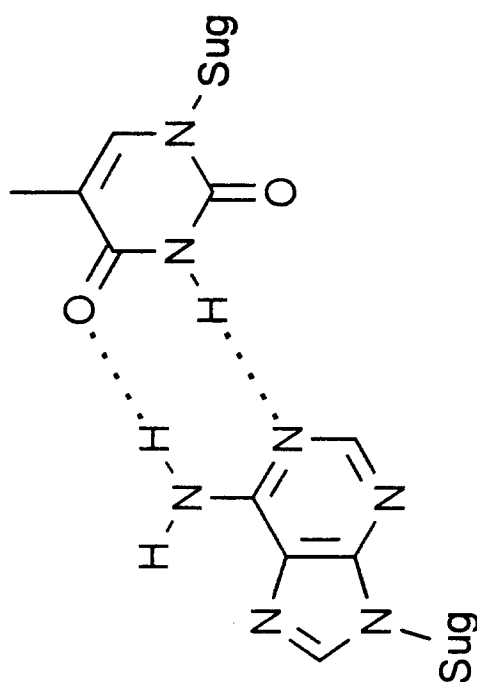


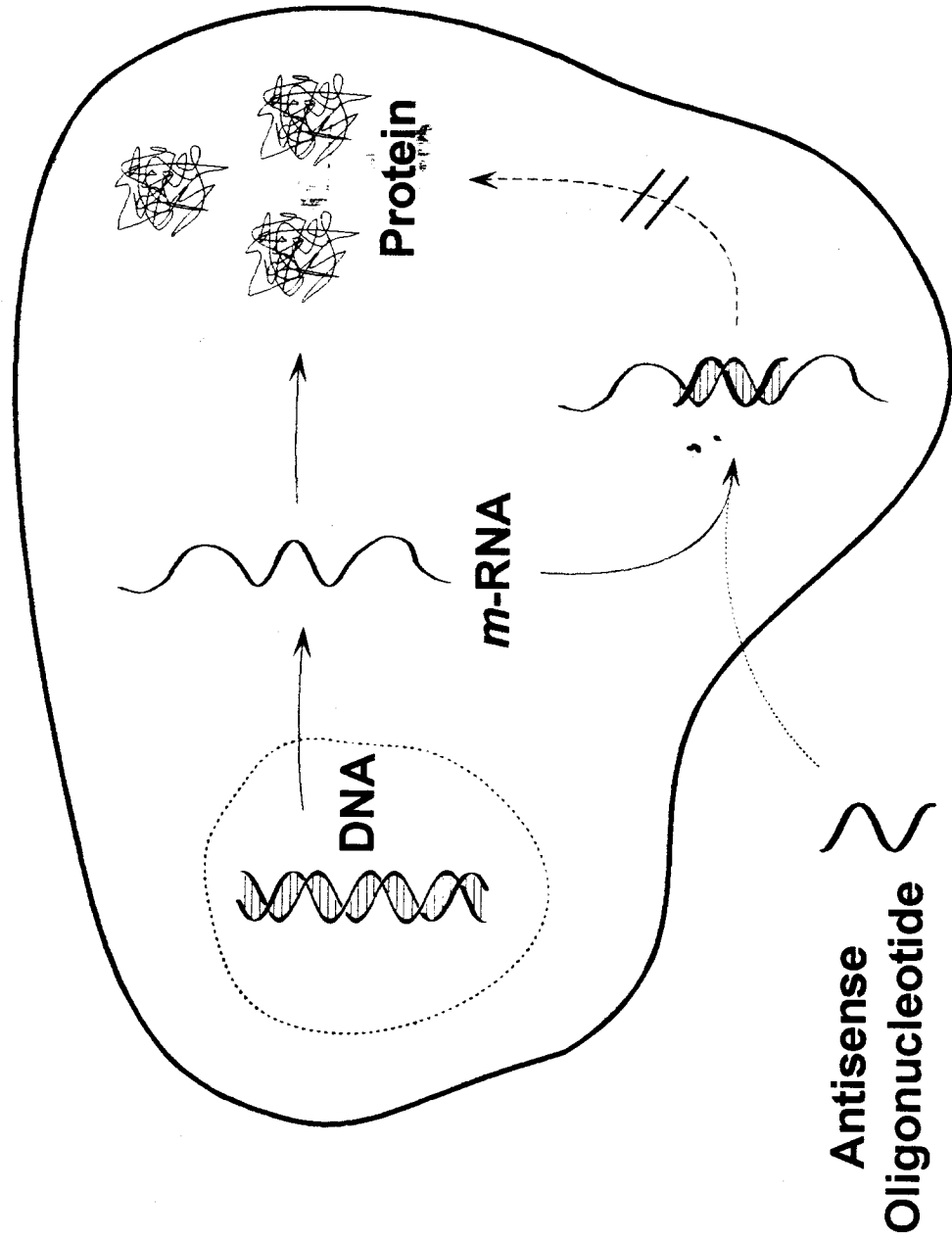


C-G Basepair



A-T Basepair

Antisense Strategy



*Binding Affinity
and Selectivity*

*Nuclease
Resistance*

*Target Site
Accessibility*

Cellular Uptake

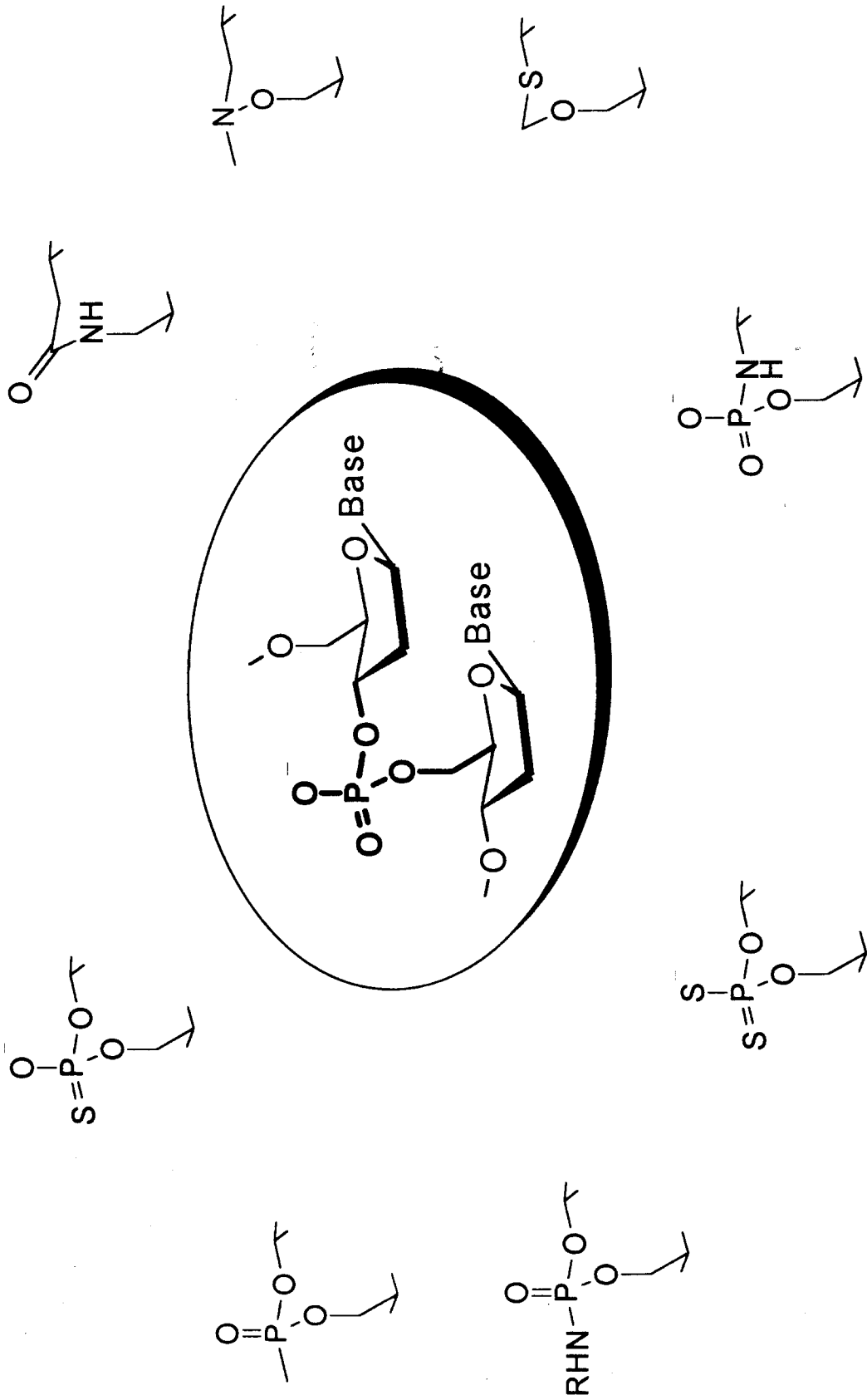


m-RNA Cleavage

Technical Feasibility

Bioavailability, Pharmacokinetics

Backbone Modifications



Evaluation of Sugar and Backbone Modifications

(100 mM Na⁺, 10 mM PO₄^{x-} pH 7.0, 0.1 mM EDTA, 4 mM oligo)

RNA Binding Affinity

TTTTtCTCTCTCTCT

TTTTt-tCTCTCTCTCT

CTCGTACttttCCGGTCC

CTCGTACtt-tCCGGTCC

GCGtttttttttGCG

GCGtt-tt-tt-tGCG

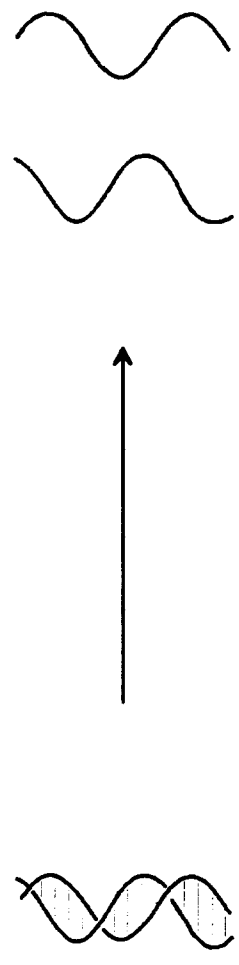
ttttt^mt^mt^mt^mt^mt^mT

ttttt^mt^mt^mt^mt^mT

Nuclease Resistance

CGACTATGCAAttC

CGACTATGCAAtt-tC



Relative
Hypochromicity

0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0

5'-tt tt tc tc tc tc tcT-3'

A

B

C

0

10

20

30

40

50

60

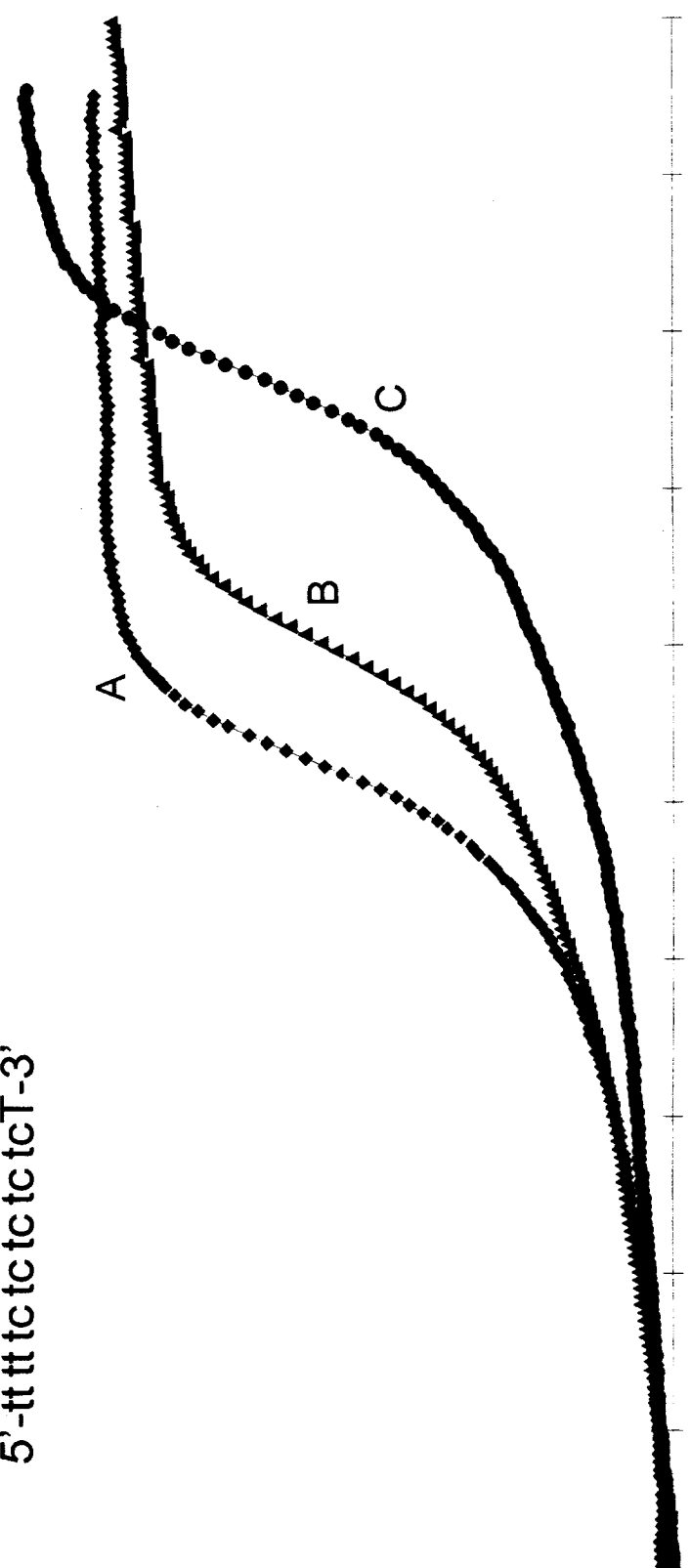
70

80

90

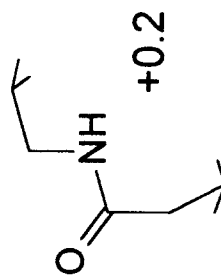
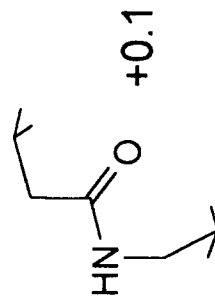
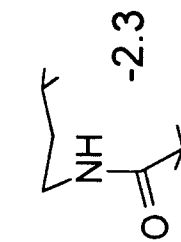
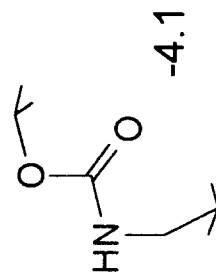
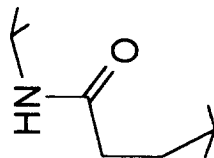
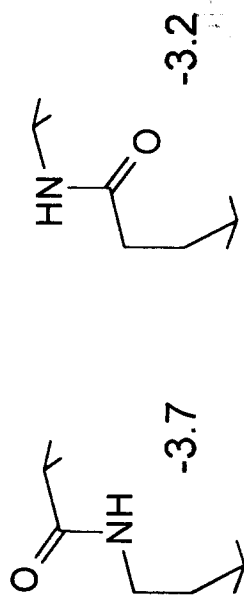
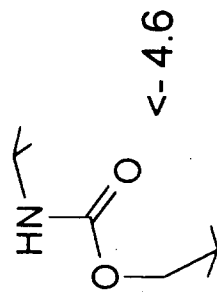
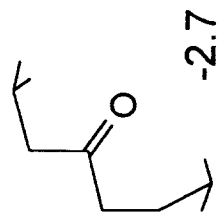
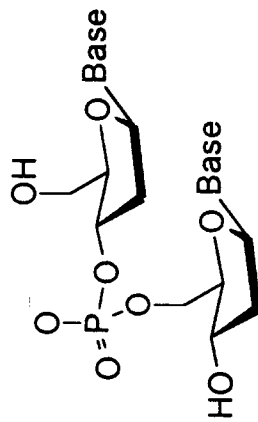
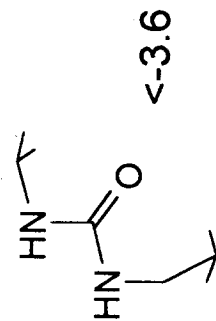
100

Temperature (°C)

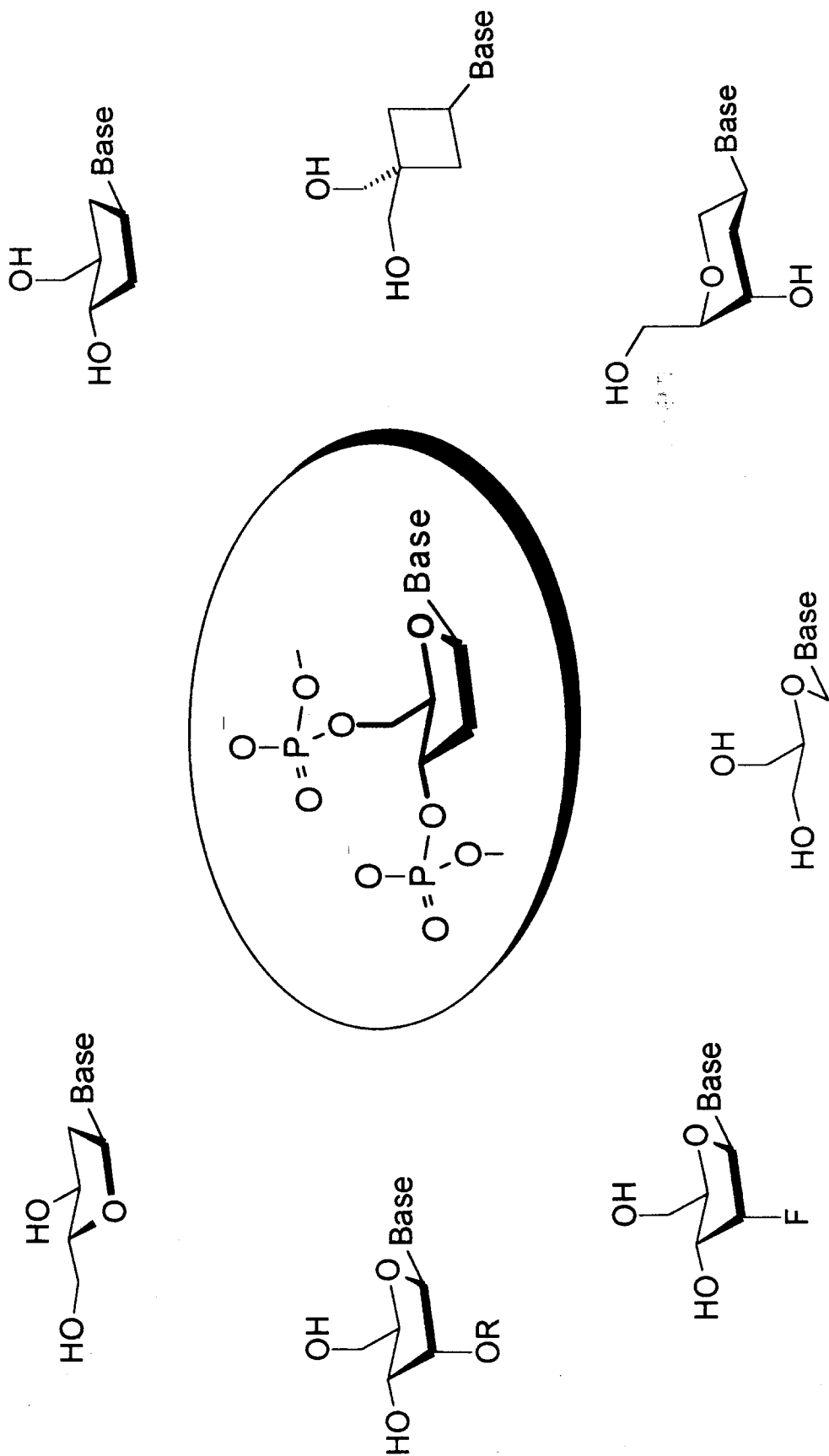


Backbone Replacements of Phosphodiester

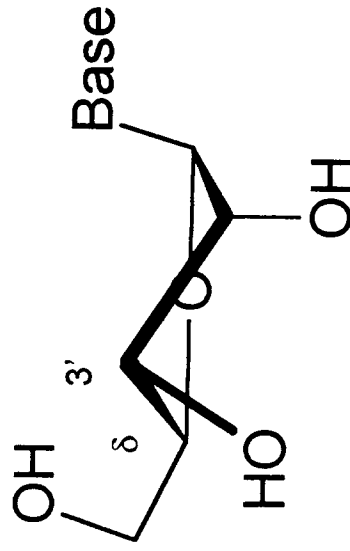
(average ΔT_m values in $^{\circ}\text{C}$ / modification)



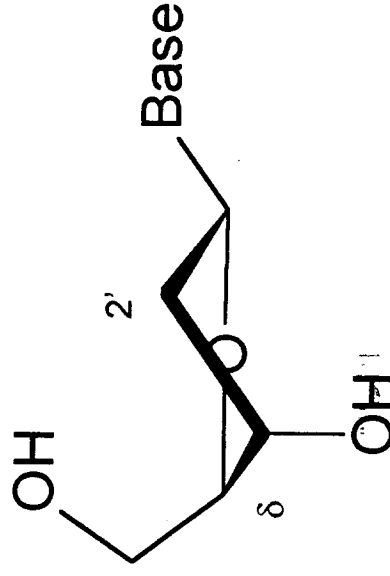
Sugar Modifications



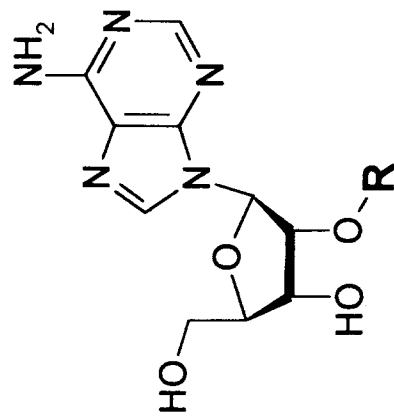
Typical Sugar Conformations of A- and B-Type Nucleic Acids



C_3' -endo (C_2' -exo)
(A-RNA)



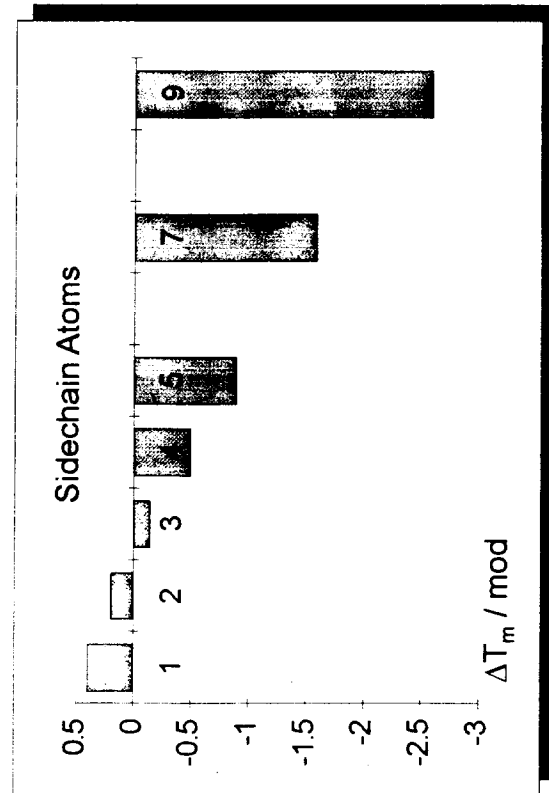
C_2' -endo (C_3' -exo)
(B-DNA)



a CCG a GG a TC a TGTCGT a CGC
 GG a CCGG aa GGT a CG a G
 CTCGTACC a TTCCGGTCC
 G a GCTCCC a GGC

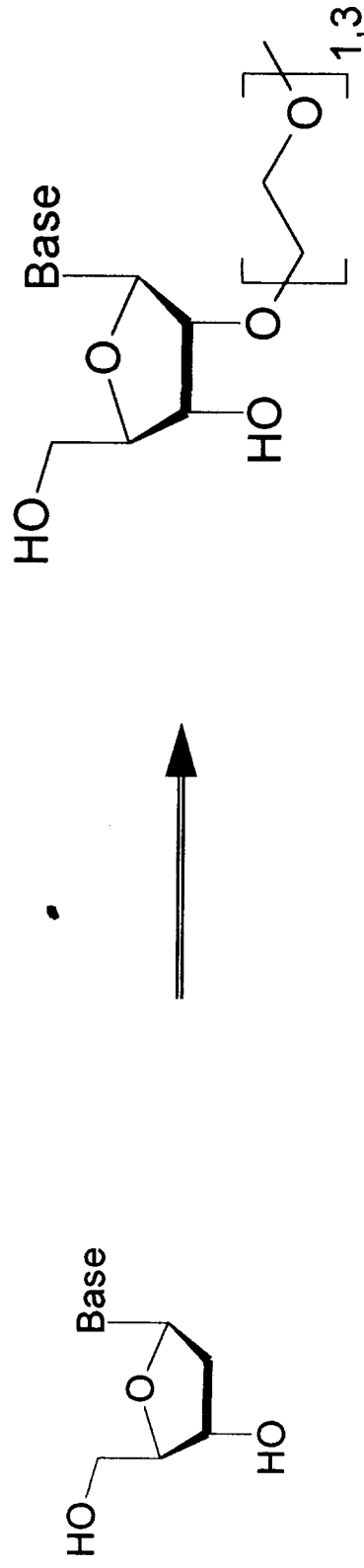
ΔT_m / modification ($^{\circ}\text{C}$)

R = CH ₃	+ 0.4
R = CH ₂ CH ₃	+ 0.2
R = CH ₂ CHCH ₂	- 0.1
R = (CH ₂) ₂ CH ₃	- 0.15
R = (CH ₂) ₃ CH ₃	- 0.5
R = (CH ₂) ₄ CH ₃	- 0.9
R = CH ₂ C ₆ H ₅	- 1.6
R = (CH ₂) ₈ CH ₃	- 2.6



E. Lesnik, C. Guinosso, A. Kawasaki, H. Sasnor, M. Zounes, L. Cummins, D. Ecker, D. Cook, and S. Freier;
Biochemistry **32** (1993), 7832.

2'-O-Alkoxy Ribonucleosides as Promising Sugar Modifications



High binding affinity
Increased stability *in vitro*
No diastereomeric mixtures
Ease of synthesis

$\Delta T_m / \text{mod. (}^\circ\text{C)}$ rel. stability

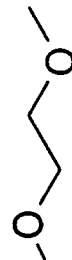
8

+1.3



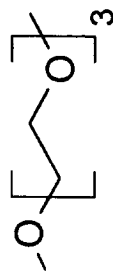
24

+1.4



>48

+1.4



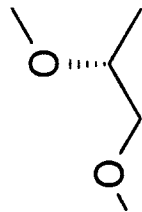
16

-0.1



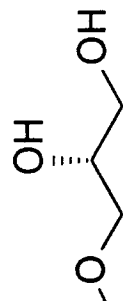
-

+1.6



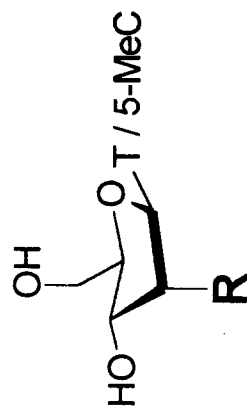
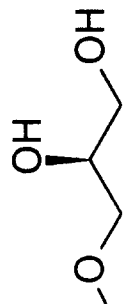
-

+1.5

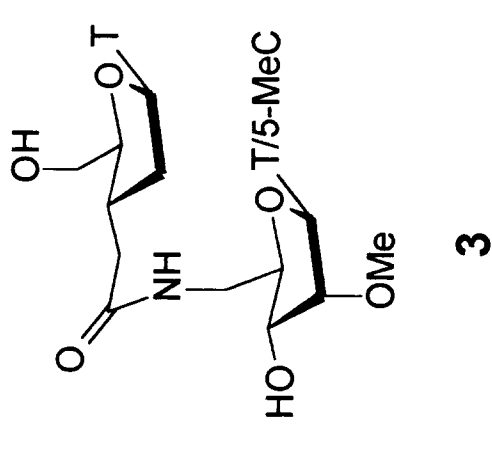
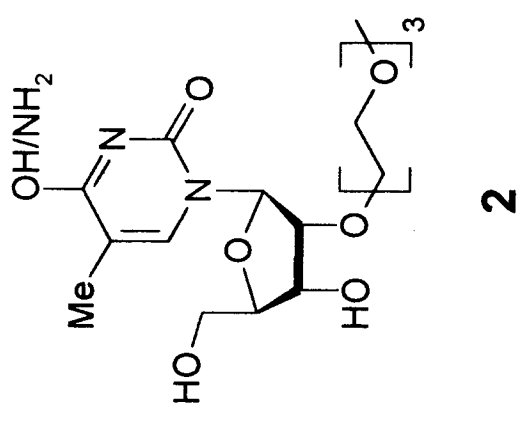
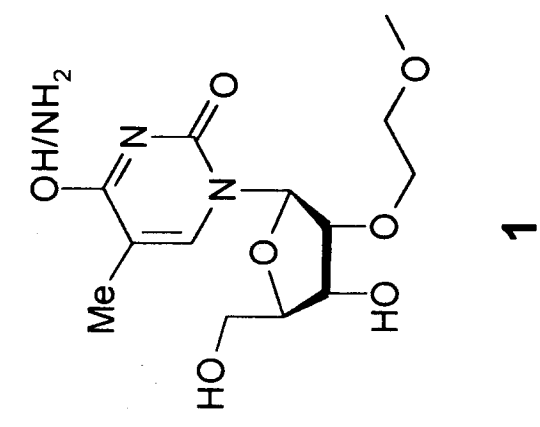


-

+1.5



P. Martin



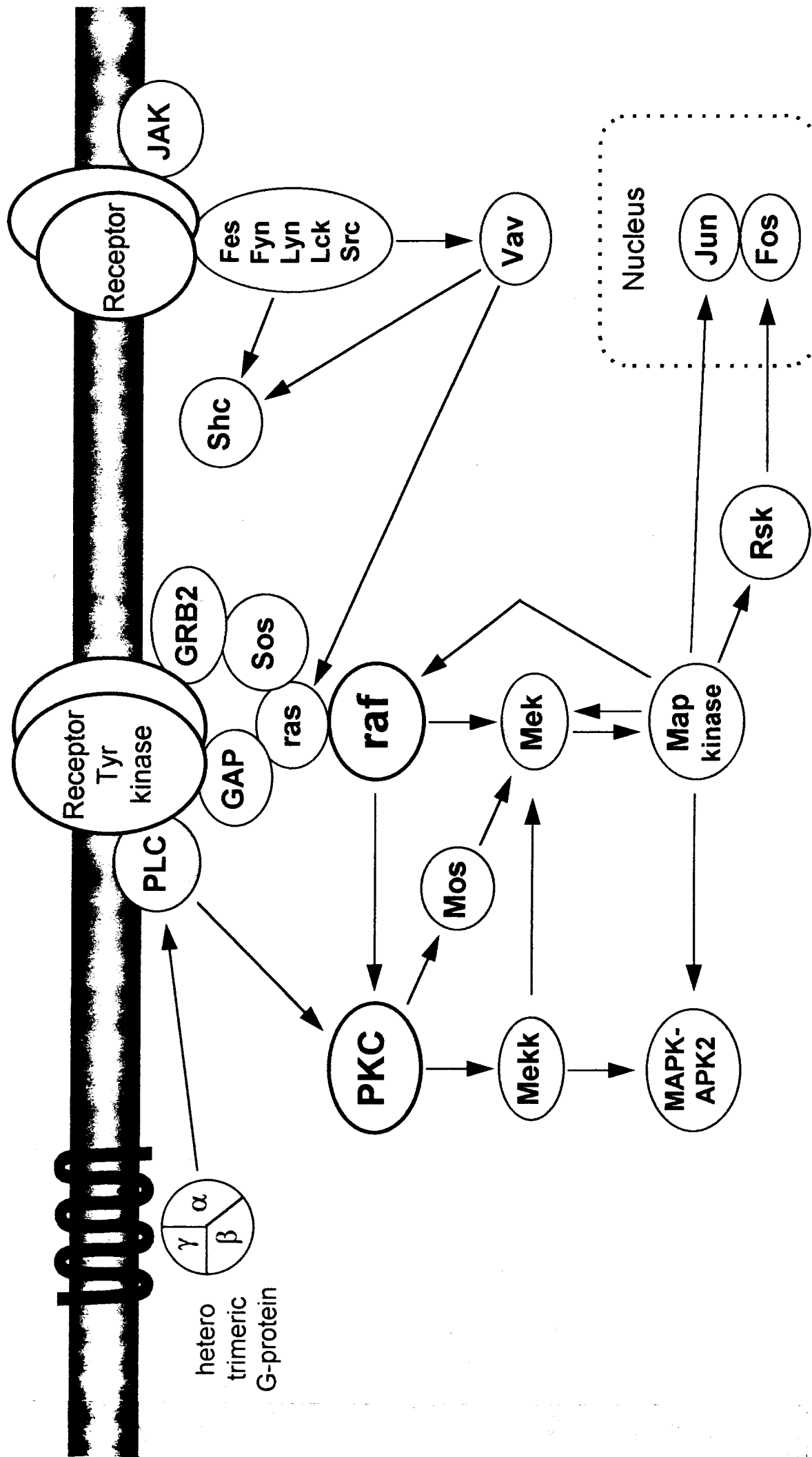
	T_m (°C)	ΔT_m / mod.	$\Delta\Delta G_{37}$ (kcal / mol)
wildtype (DNA)	53	-	-13.6
1	77	1.7	-22.0
2	77	1.7	-22.3
3	75	3.1	-22.4

5'-tt tc tc tc tc T

NMDA, Angiotensin II
Endothelin, Vasopressin
Bradykinin, f-Met-Leu-Phe

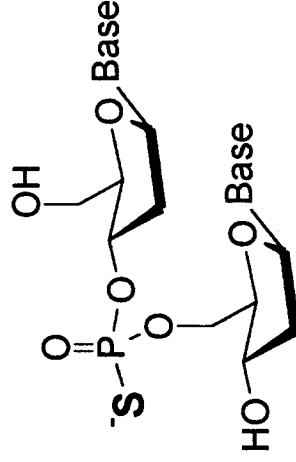
Epidermal, Nerve, and
Platelet-derived
growth factors

Interleukines-1, 2, 5
T-cell antigen

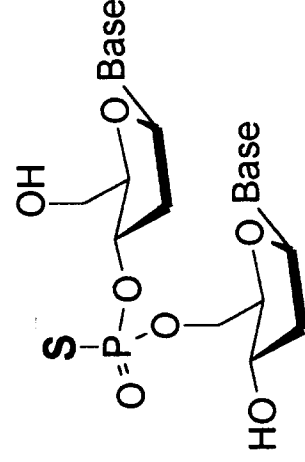


Phosphorothioate Oligonucleotides

- + Ease of synthesis
- + Biological and chemical resistance
- + RNase H mediated RNA-cleavage
- + Beneficial pharmacokinetics

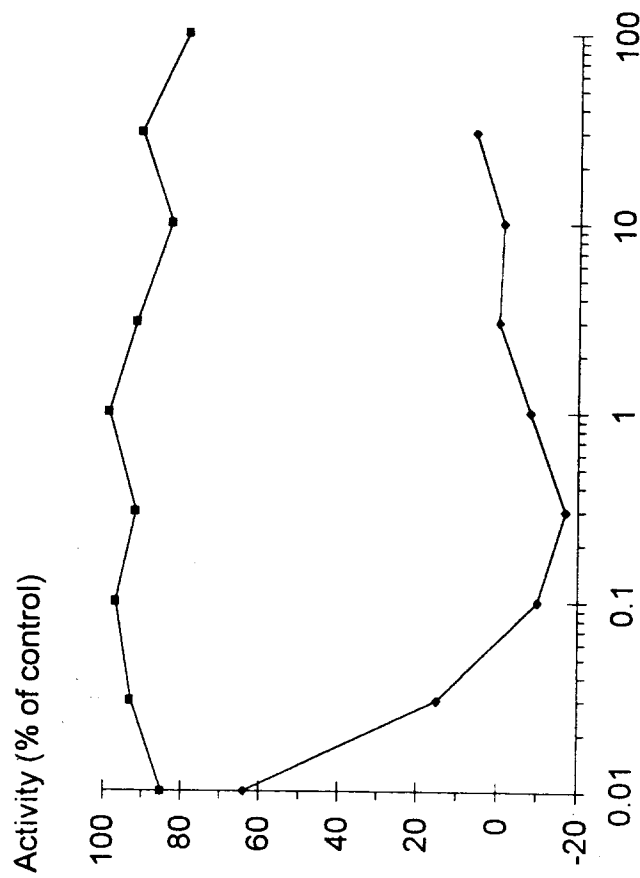


and

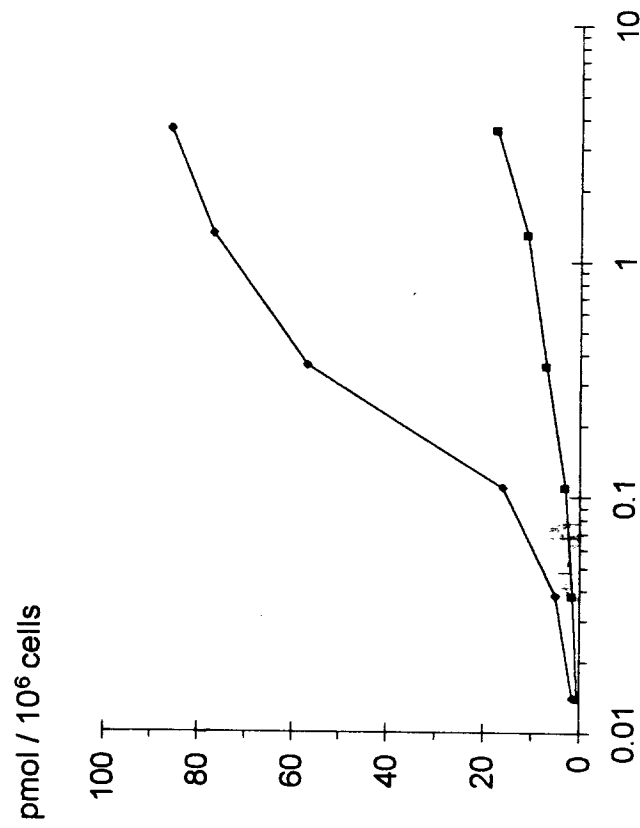


- Diastereomeric mixtures
- Non-specific protein binding
- Moderate decrease of RNA-binding affinity

Inhibition of ICAM-1 Expression

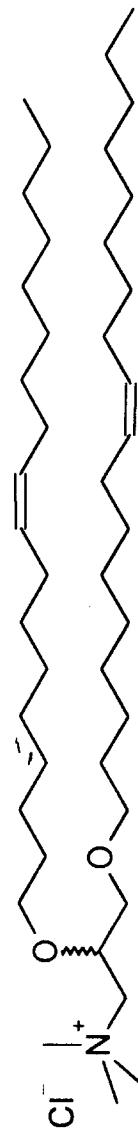


Cellular Uptake (HUVEC-Cells)

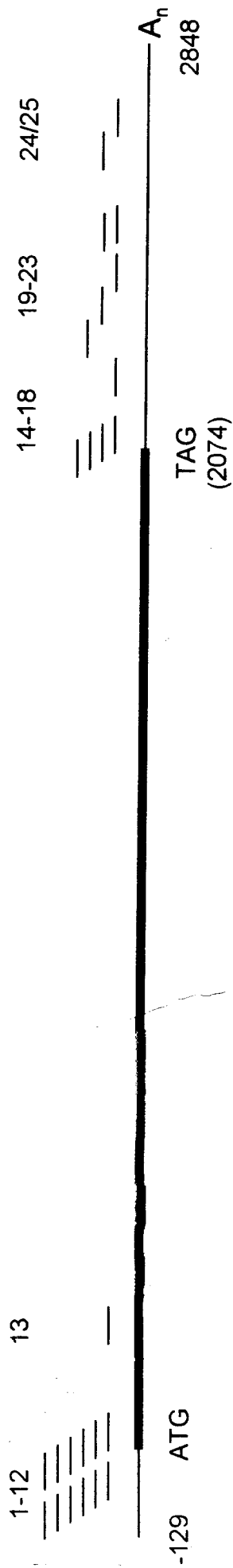


with

without



Reduction of c-raf *m*-RNA Expression in T24 Cells (500 nM, 24 h)

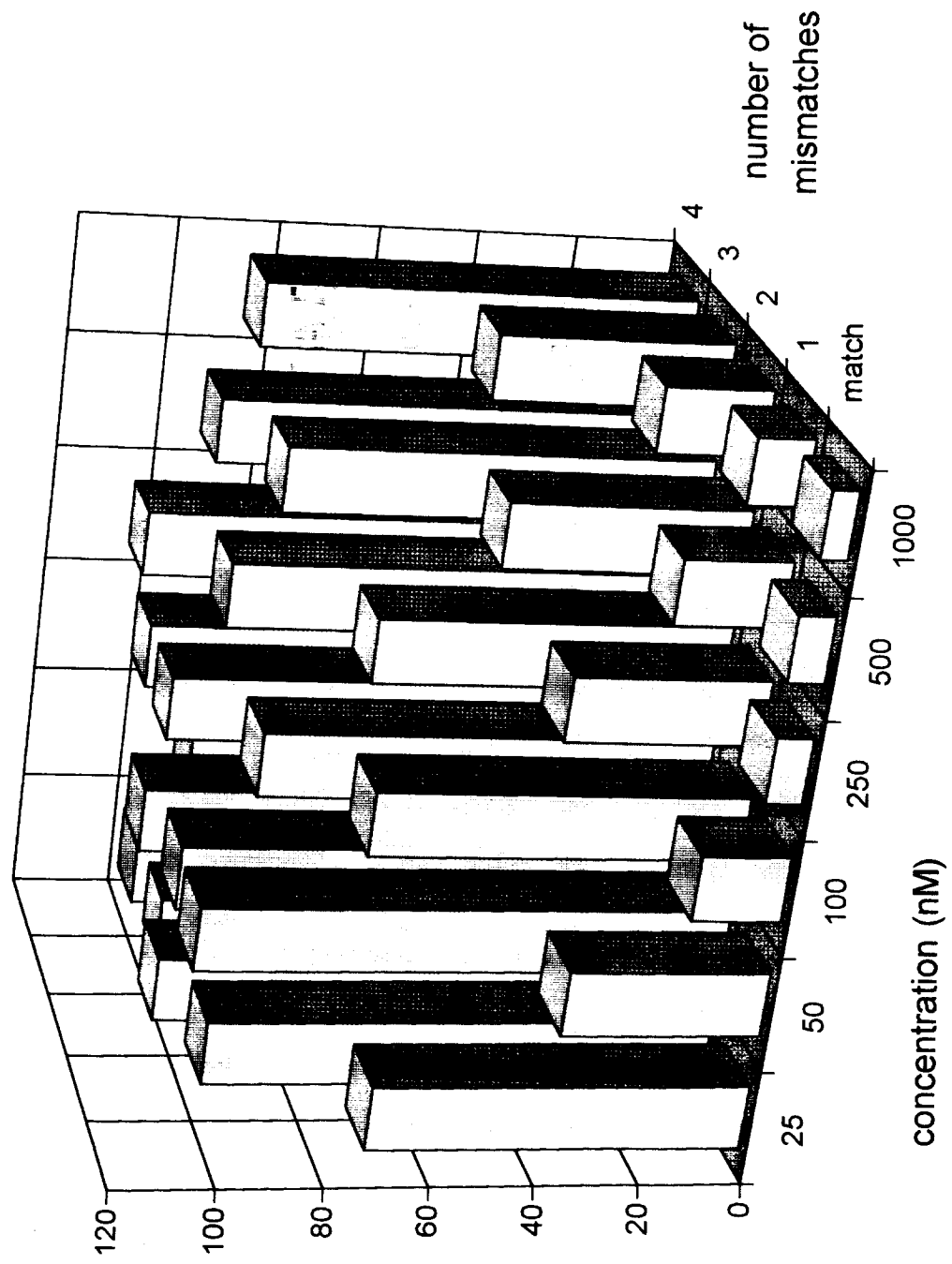


21: CGP 69846 A (IS/S-5132; 1355-2374, 5'-TCCCGCCCTGTGACATGCATT)

CGP 69846 A Mismatch Series (1-7)

Mismatches	Sequence	T _m (°C)
-	5'-TCCCGCCTGTGACATGCATT-3'	62
1	5'-TCCCGCCTGCGACATGCATT-3'	56
2	5'-TCCCGCCTGCTACATGCATT-3'	47
3	5'-TCCCGCCTACTACATGCATT-3'	42
4	5'-TCCCGCCTACTTCATGCATT-3'	36
5	5'-TCCCGCCCACCTTCATGCATT-3'	27
6	5'-TCCCGCCCACCTTGATGCATT-3'	--
7	5'-TCCCGCGCACTTGATGCATT-3'	--

Reduction of c-raf *m*-RNA Expression in T24 Cells (24 h)



Modified Antisense Oligonucleotides (*c-rat* Kinase)

CGP 69846A

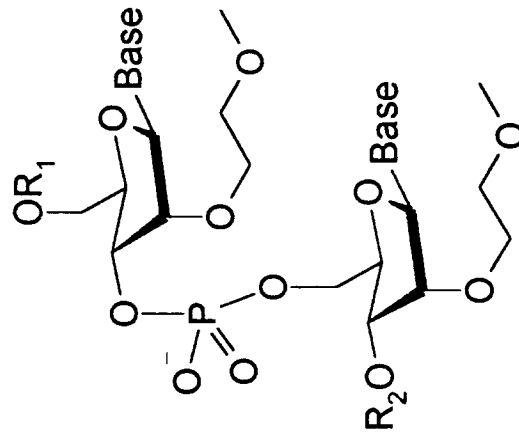
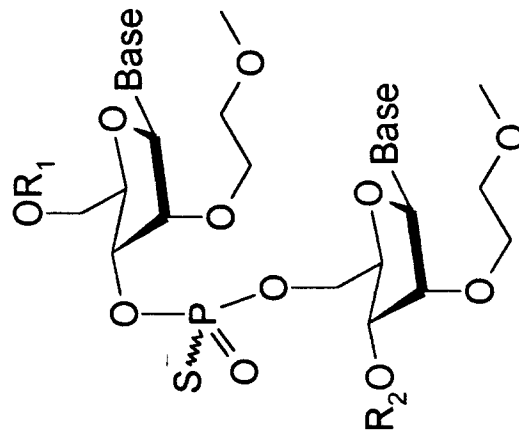
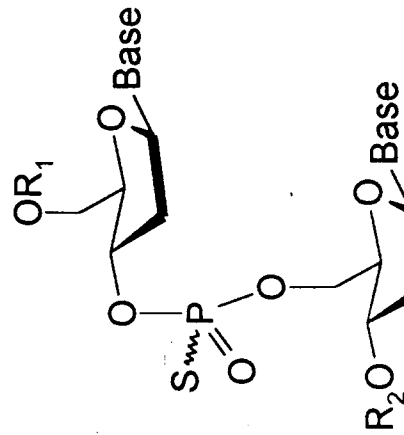
TCCCGCCTGTGACATGCATT

CGP 71849A

TCCCGCCTGTGACATGCATT

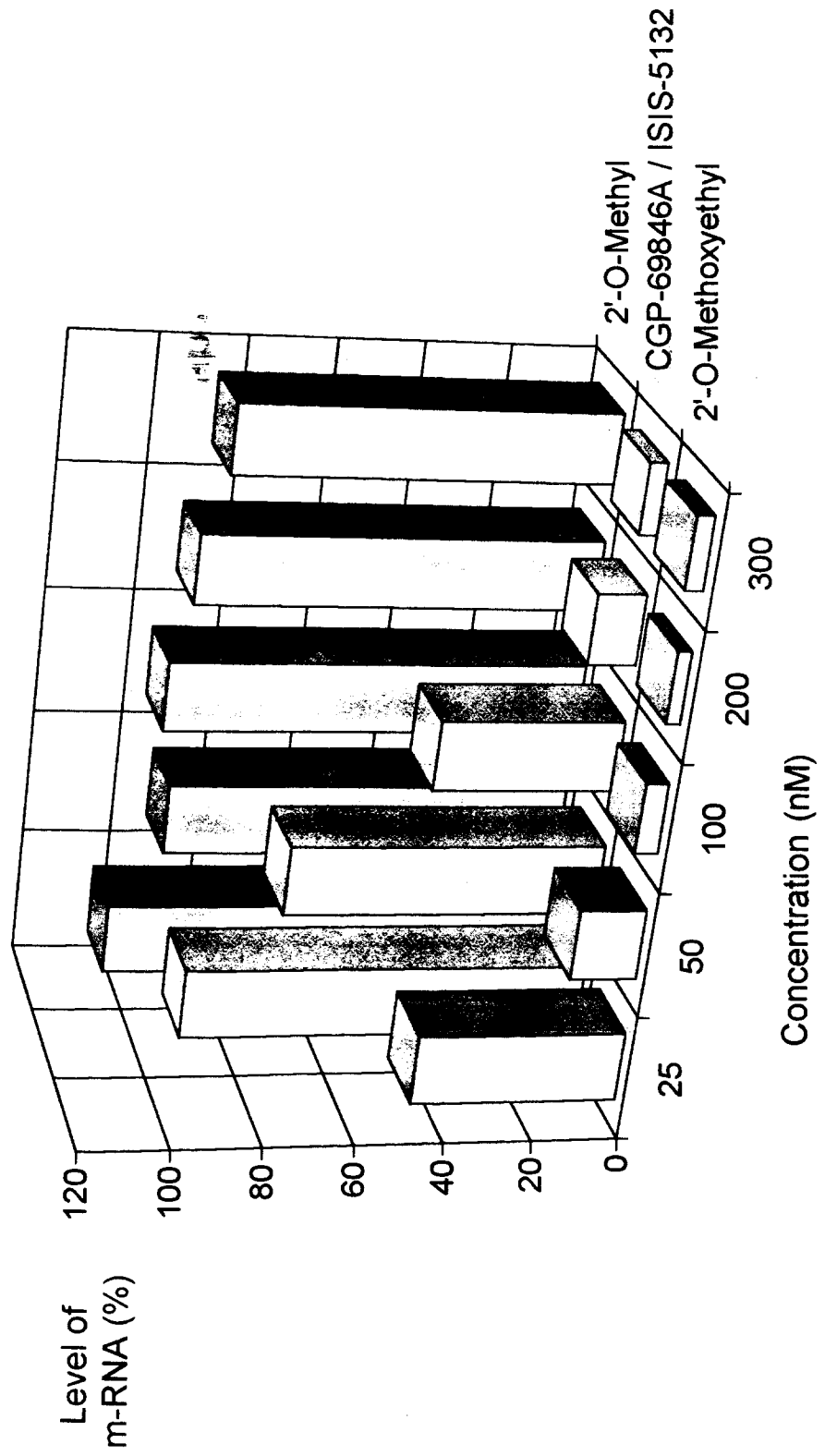
CGP 69845A

TCCCGCCTGTGACATGCATT

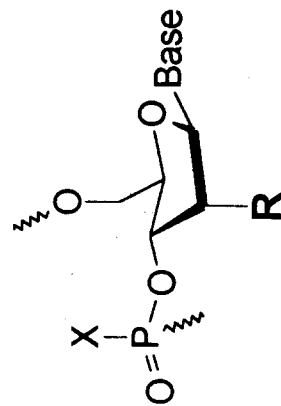


Reduction of C-raf *m*-RNA Expression in T24 Cells (24 h)

TpCpCpCpGpC
sCsTsGsTsGsAsCsAs
TpGpCpApTpT



Reduction of c-raf *m*-RNA Expression in T24 Cells (24 h)



TpCpCpCpGpC

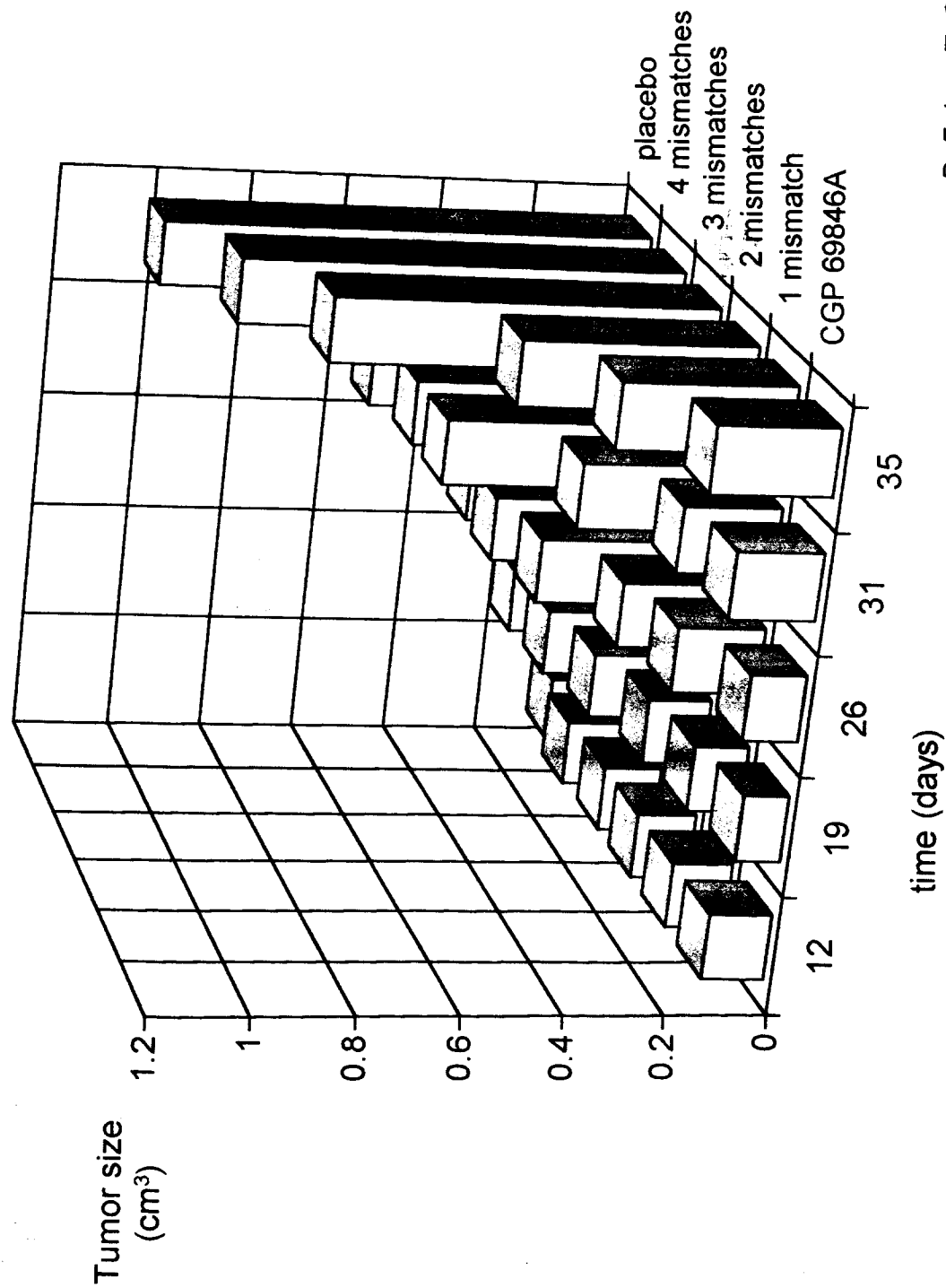
sCsTsGsTsGsAsCsAs

TpGpCpApTpT

	Full PS (X = S)		PS-gap / PO-wings (X = O)	
R	T_m (°C) ^{c)}	IC ₅₀ (nM)	T_m (°C) ^{c)}	IC ₅₀ (nM) stability ^{d)}
H	62	90	65	- 0.05
OCH ₃	70	40	74	>500 0.19
OCH ₂ CH ₂ OCH ₃	76	20	78	20 1.18

Growth of s.c. Transplanted Tumors in Female Balb/c Nude Mice

(daily 6 mg/kg of CGP-69846A or mismatched analogs; human lung carcinoma A549)



Growth of s.c. Transplanted Tumors in Female Balb/c Nude Mice

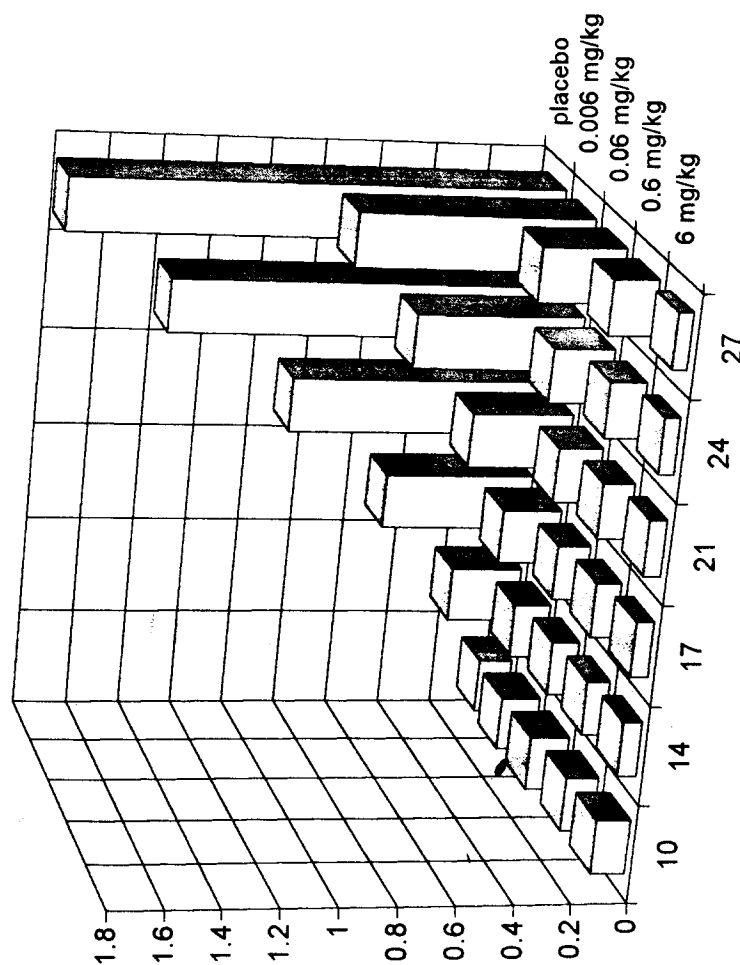
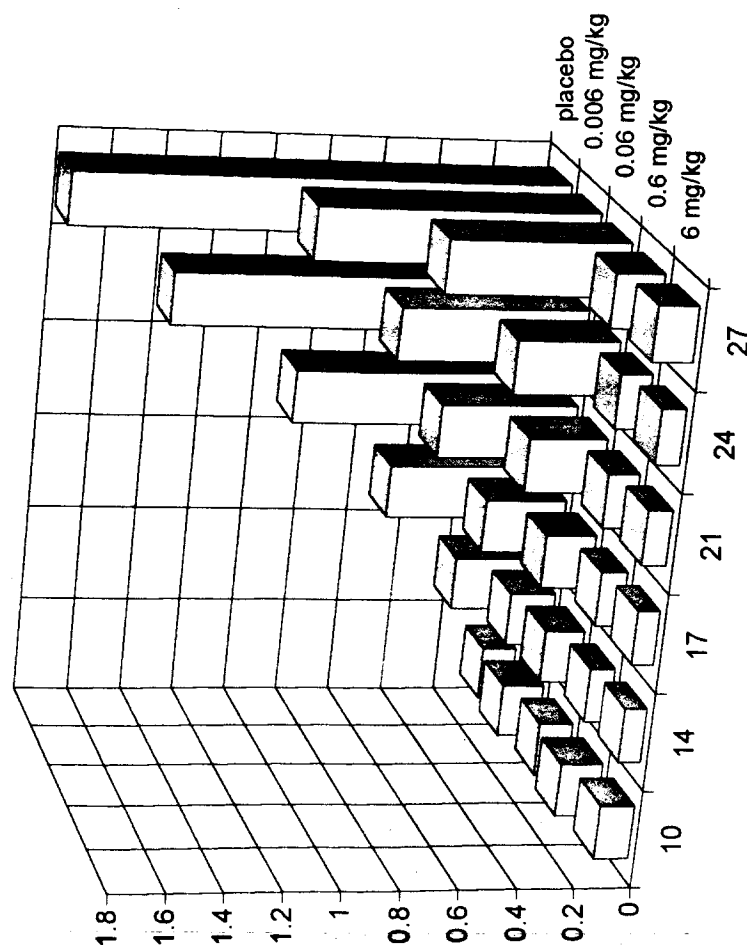
(3'-untranslated region of C-raf kinase, CGP 69846 A, human lung carcinoma A549)

TsCsCsGsCsCsTsGsTsGsAsCsAsTsGsCsAsTsT

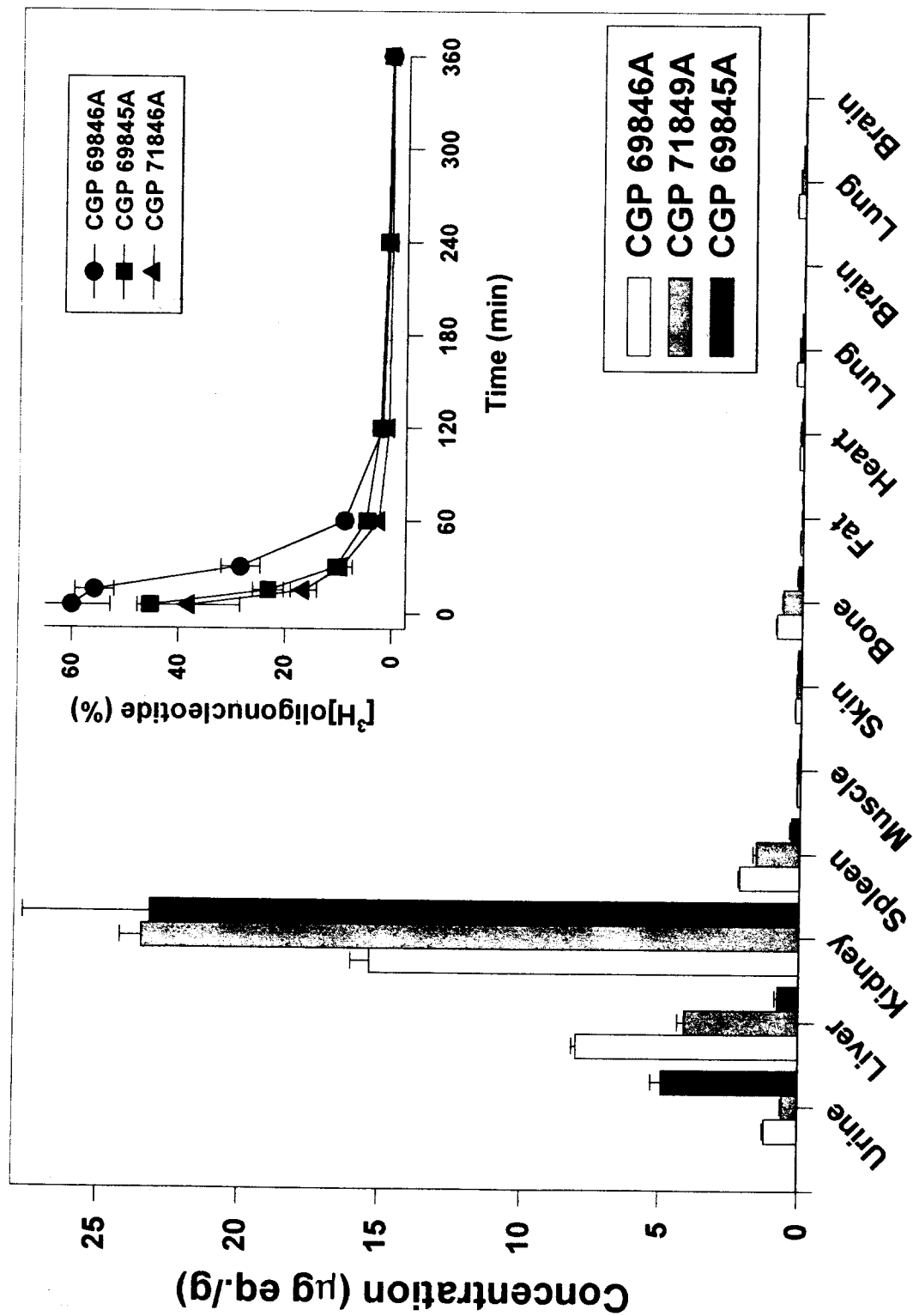
TpCpCpCpGpC

sCsTsGsTsGsAsCsAs

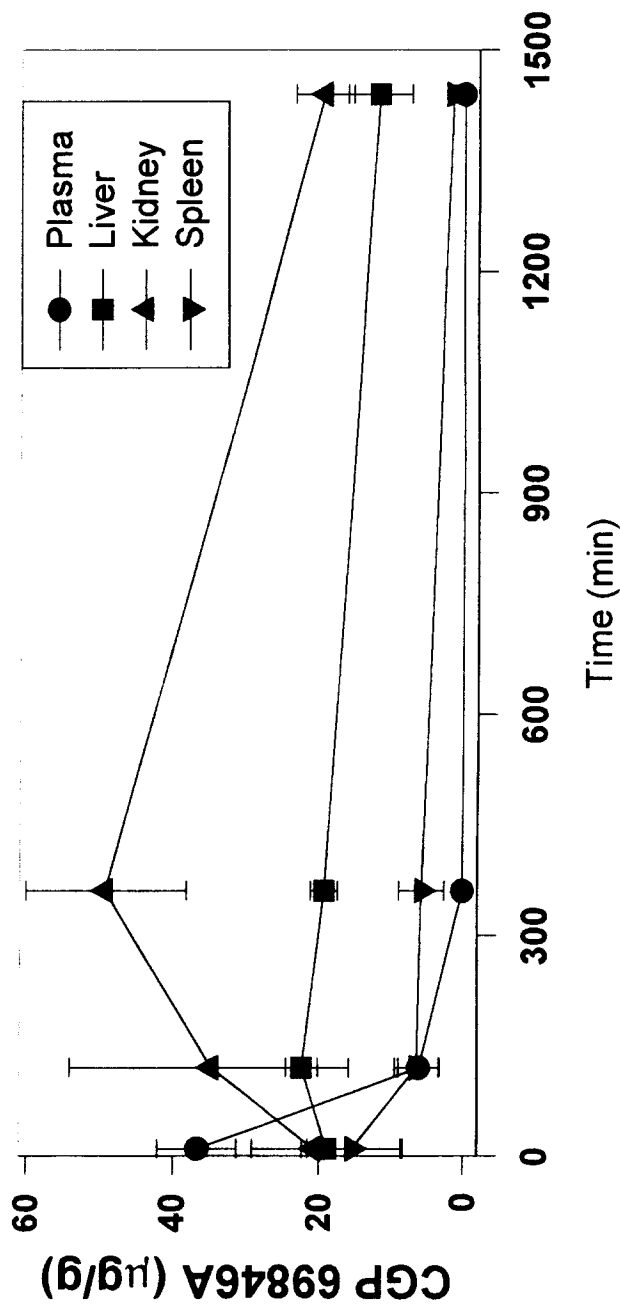
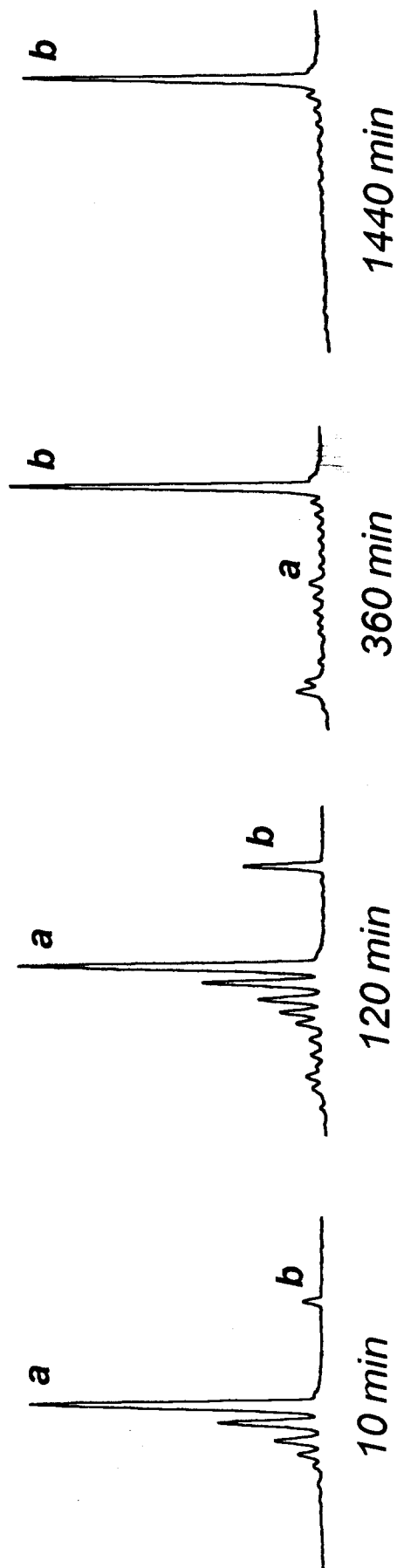
TpGpCpApTpT



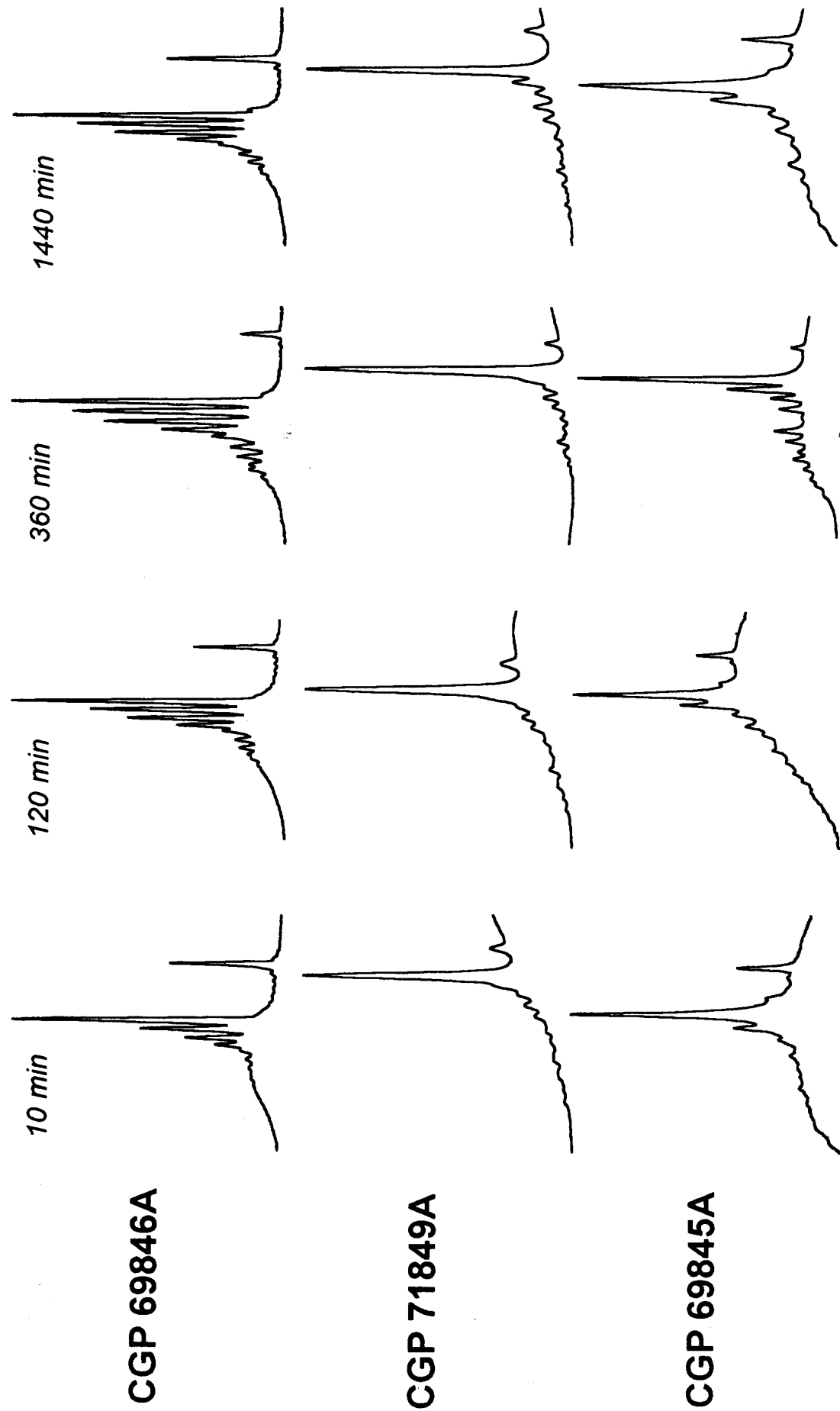
Disposition in rats (CGP 69846A, CGP 71849A and CGP 69845A)



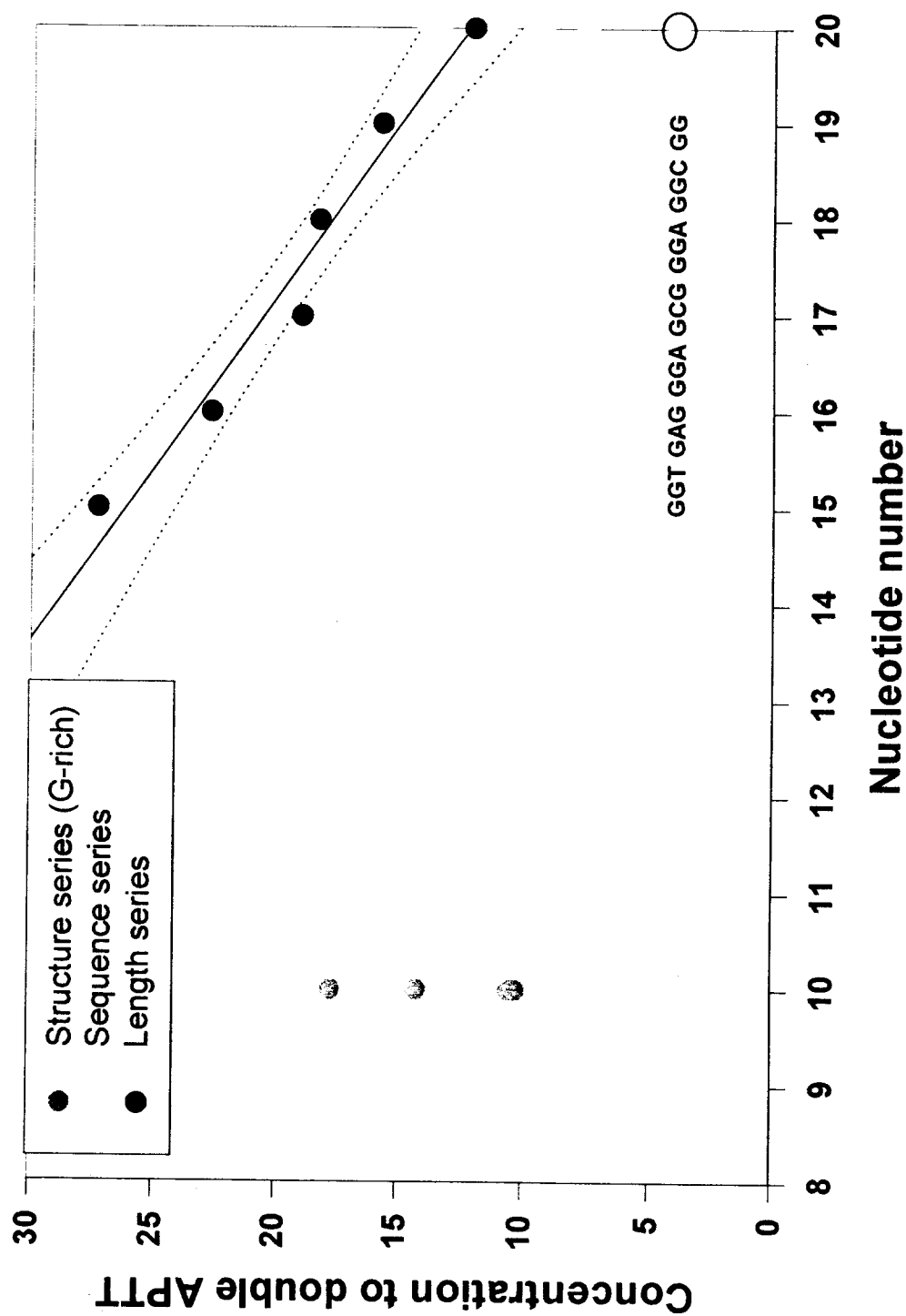
Plasma Kinetics and Metabolism of CGP 69846A in Rats



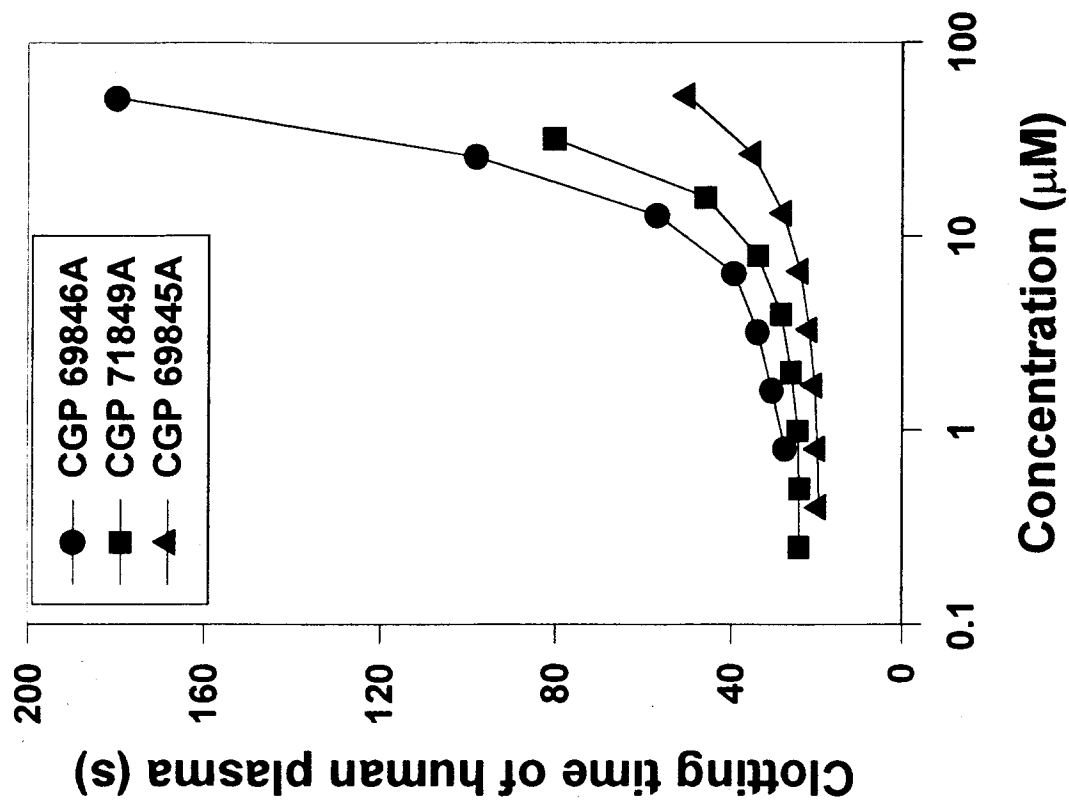
Metabolic Profile in Rat Kidneys



Anticoagulation Effects (length, sequence and G-T series)



In vitro Anticoagulation Effects



APTT¹⁾

CGP 69846A 12.1

CGP 71849A 16.6

CGP 69845A >53

1) Concentration of oligonucleotide required to double APTT

Modifications

K.H. Altmann
M.O. Bévierre
B. Cuenoud
A. De Mesmaeker
C. Garcia
C. Jouanno
J. Lebreton
C. Lesueur
R. Mah
P. Martin
C. Schmit
P. von Matt
A. Waldner
S. Wendeborn
E. Steiner / K. Auer

Oligonucleotide Synthesis / Analysis

W. Brill
R. Häner
D. Hüsken
F. Natt
U. Pieves
K. Skobridis
S. Freier (ISIS)

Biology (Basle)

D. Fabbro
T. Geiger
M. Müller

Biology (Horsham)

D. Bayley
S. Craig
P. Nicklin
J. Phillips

RNA Cleavage

J. Hall
R. Häner
D. Hüsken
U. Pieves

Biology (ISIS)

N. Dean
D. Ecker
B. Monia

Modelling: R. Wolf, V. Fritsch

NMR: M. Blommers, T. Winkler