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NEW REGIOSPECIFIC SYNTHESIS OF *BRANCHED* TETRA-, NONA- & DECA-RNA MODELLING THE LARIAT FORMED IN RNA SPLICING REACTIONS

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Abstract: *Convergent syntheses of branched tetraribonucleotide* ***39****, nonaribonucleotide* ***40*** *and decaribonucleotide* ***41****, modelling the lariat of pre-mRNA processing reaction, are reported. The first key step in the present strategy involves the condensation of the phosphodiester blocks* ***1****,* ***5*** *or* ***15*** *with the 3′,5′-dihydroxy-6-N-benzoyl-2′-O-pixyl(9-phenylxanthen-9-yl)adenosine* ***29*** *to give* ***30a*** *(65%),* ***31a*** *(63%) or* ***32a*** *(70%). Chemospecific phosphorylation at 3′-OH of these intermediates afforded the intermediates* ***30b*** *(92%),* ***31b*** *(83%) or* ***32b*** *(80%) which were treated with mild acid to achieve a regiospecific removal of the 2′-O-pixyl group to give compounds* ***30c*** *(74%),* ***31c*** *(86%) or* ***32c*** *(85%). The second key step involved the introduction of biscyanoethylphosphotriester moiety to the 2′-OH of the branch-point adenosine in* ***30c****,* ***31c*** *or* ***32c*** *in one single step using (biscyanoethoxy)-(diisopropylamino)phosphine to give the crucial branch-point building blocks* ***30d*** *(46%),* ***31d*** *(64%) or* ***32d*** *(62%) with two dissimilar vicinal phosphates at 2′- and 3′- of the branch-point. These blocks were then converted to the fully protected intermediates* ***33a*** *(59%) [****30d****+****28*** *→* ***33a****],* ***34a*** *(69%) [****31d****+****19*** *→* ***34a****] and* ***35a*** *(56%) [****32d****+****19*** *→* ***35a****], and were subsequently treated with a bulky tertiary amine to give the branch-point 2′-cyanoethylphosphodiester blocks* ***33b*** *(64%),* ***34b*** *(67%) and* ***35b*** *(68%). These were then condensed in the usual way with the appropriate 5′-hydroxy block (****27*** *or* ***25****) to give the fully protected branched oligomers* ***36*** *(69%) [****33b****+****27*** *→* ***36****],* ***37*** *(67%) [****34b****+****25*** *→* ***37****] and* ***38*** *(63%) [****35b****+****25*** *→* ***38****]. These oligomers were then deprotected in the usual manner to give the final branched oligoribonucleotides* ***39****,* ***40*** *and* ***41*** *in 62%, 21% and 21% yields respectively. Detailed 500 MHz* ^{1}*H-NMR and 202.4 MHz* ^{31}*P-NMR studies on* ***39****,* ***40*** *and* ***41*** *have unequivocally established their purities. Detailed spectroscopic studies such as COSY, HOHAHA & NOESY have also clearly established the structural integrity of the synthetic target compounds.*

The precise deletion of intervening sequences (introns) and the ligation of coding sequences (exons) in a pre-mRNA is termed as RNA splicing which regulates the expression of many genes in all eukaryotes as well as in some eubacteria and archaebacteria[1]. Group I splicing[2] shows RNA catalyzed intron excission in a bimolecular process where guanosine and Mg^{2+} are necessary elements for autocatalysis. Both Group II and the nuclear mRNA splicing[3] however involves formation of the lariat intermediate and subsequent ligation of two introns and excission of the lariat intron. The lariat intermediate has adenosine as the branch-point which is 2′→5′phosphodiester linked to guanosine, 3→5′ phosphodiester linked to either a uridine or cytidine residue while the 5′→3′phosphate ester is always either uridine or adenosine. Considerable attention has been directed to the synthesis of the branched oligoribonucleotides[4a-n], mainly owing to the inherent chemical problems encountered in the introduction of the 2′→5′ and 3′→5′ phosphodiester bonds regiospecifically at the vicinal hydroxy groups of the branch-point adenosine. We have reported regiospecific synthesis of branched trimeric[5a-d] and tetrameric[5e,f], pentameric[5g] and heptameric[5g] ribonucleotides, and have determined their solution conformations[5b,c,h-s] by high-field NMR spectroscopy. These studies showed for the first time that the conformation of the branch-point in a given branched oligoribonucleotide is entirely dictated by the neighbouring nucleobases. The predominant conformational feature of the branched triribonucleotide core[5b,c,h,j,l] is comprised of unnatural stacking between 2′→5′ linked nucleobases and the 3′-pyrimidine residue is free and apart from the branch-point adenosine while the conformation of a branched tetraribonucleotide resembles a distorted A-RNA helix fragment[5k-n] which is easily recognized by an enzyme[4d,6]. Pentameric branched RNA was found to mimic the conformation of the trimeric branched RNA, while the heptameric and tetrameric branched RNA showed close structural resemblance which is reminiscent of A-RNA helix. Clearly a systematic conformational studies

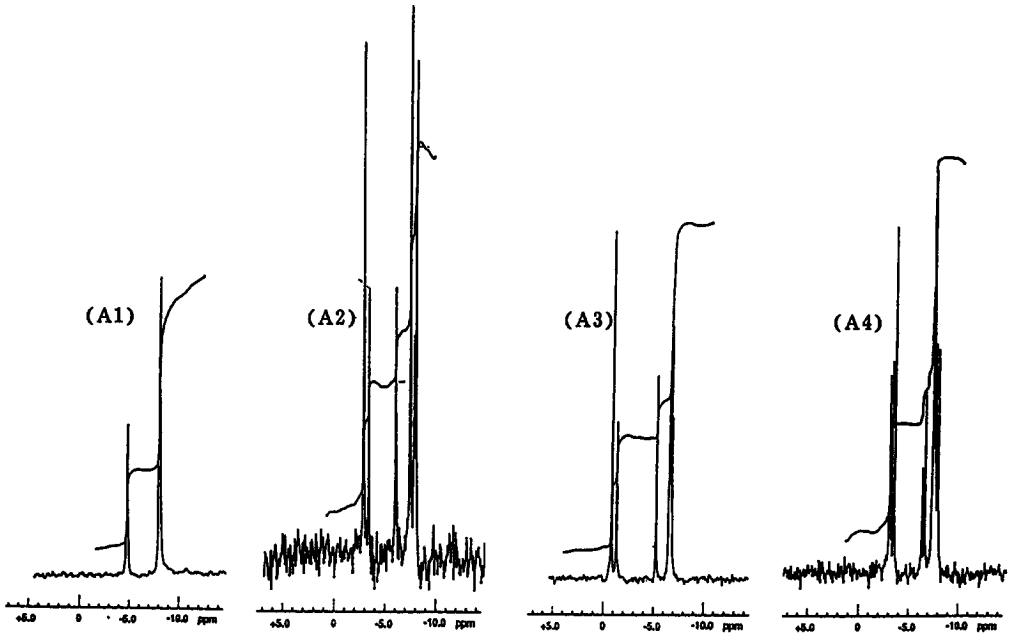


Fig. 1: ^{31}P-NMR spectra [36 MHz] of **30d** (Panel: A1) in $CDCl_3$+ CD_3OD, **33a** (Panel: A2) in **33b** (Panel: A3) in $CDCl_3$ and **36** (Panel: A4) in $CDCl_3$ at 22 °C.

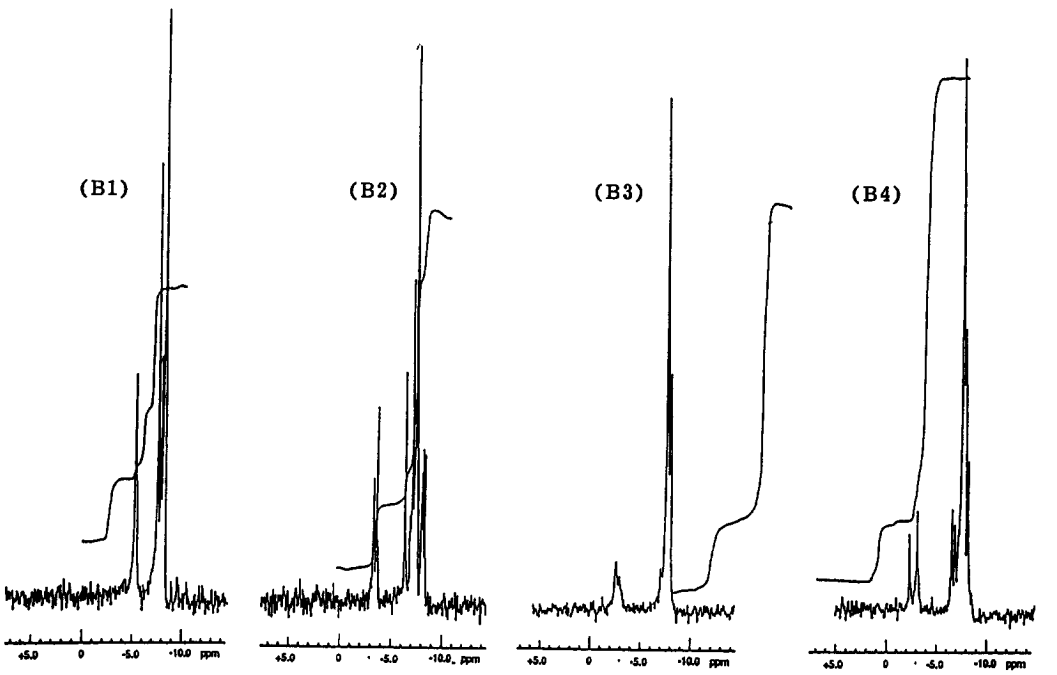
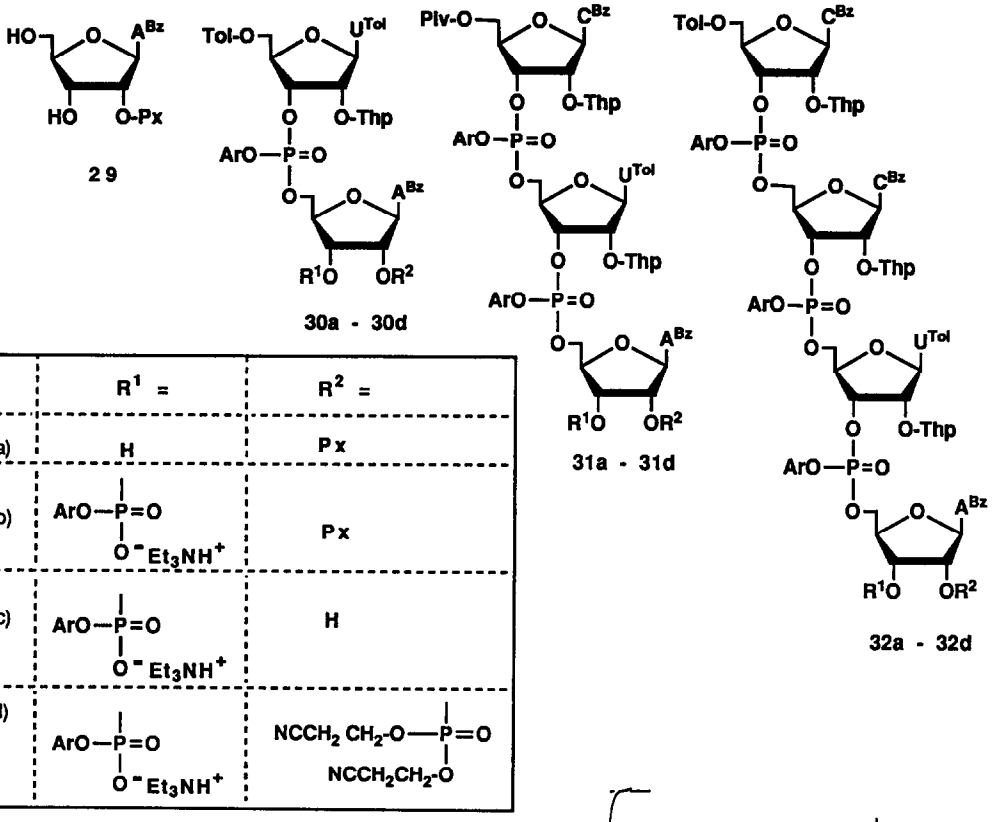


Fig. 2: ^{31}P-NMR spectra [36 MHz] of **31d** (Panel: B1) in $CDCl_3$+ CD_3OD, **34a** (Panel: B2) in **34b** (Panel: B3) in $CDCl_3$ and **37** (Panel: B4) in $CDCl_3$ at 22 °C.



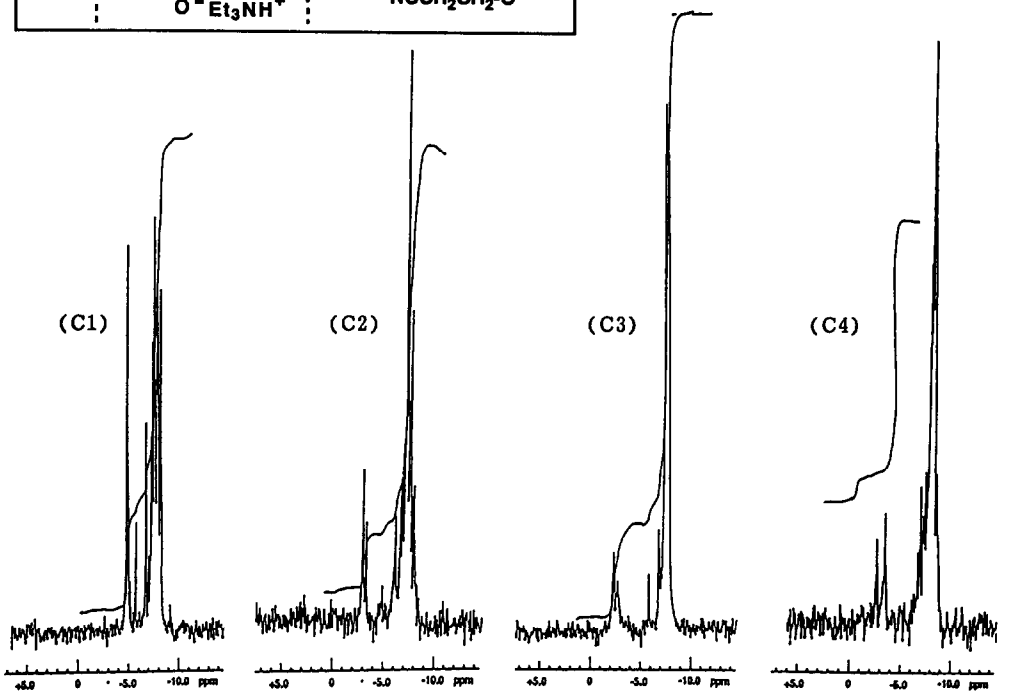


Fig. 3: ^{31}P-NMR spectra [36 MHz] of **32d** (Panel: C1) in $CDCl_3$+ CD_3OD, **35a** (Panel: C2) in **35b** (Panel: C3) in $CDCl_3$ + CD_3OD and **38** (Panel: C4) in $CDCl_3$ + CD_3OD at 22 °C.

with additional ribonucleotide residues in 2′→5′, 3′→ 5′ and 5′→3′ termini of the branched triribonucleotide core is necessary in order to mimic the conformation of a naturally-occurring lariat, and to understand the energetic and conformational consequences of the lariat formation in the Group II splicing reaction. A clear emphasis in these efforts is to develop efficient synthetic procedures which would produce larger branched oligoribonucleotide in a *pure state* for spectroscopic studies. All of the hitherto published procedures for synthesis of branched RNAs, except for a few[4a,f,k], have the common feature of introduction of the second phosphate ester bond by reacting an active phosphorylating species with the branch-point hydroxyl group which is vicinal to the first phosphodiester linkage. This is clearly so because it has been frequently shown that the internucleotide phospho*triester* linkage in oligoribonucleotides is cleaved by the vicinal 2′-hydroxyl function under mild acidic conditions[7] and is also very labile under neutral conditions[8,9]. On the other hand, a phosphodiester linkage of the natural oligoribonucleotide is stable under both neutral and mildly acidic conditions[10]. Thus, an internucleotidyl phosphodiester function allows the introduction of the second phosphate to the vicinal hydroxyl group to create the 2′→5 and 3′→5′ linked branch-point in a regiospecific manner. Efforts in this laboratory and elsewhere to synthesize various branched RNA have shown that the procedures developed hitherto are only suitable for the synthesis of the shorter branched RNAs (trimer to heptamer). Synthesis of branched heptaribonucleotide[5g] presents basically two disadvantages when it is employed for synthesis of longer branched RNAs such as **40** and **41**: *(i)* the procedure turns out to be uneconomical because it requires at least *three fold* excess of expensive oligomeric 5′-phosphoramidite blocks over the branch-point 2′-hydroxy phosphodiester block[5g]. Furthermore the reactivity of a phosphoramidite moiety might be expected to decrease upon increase of the oligomeric chain to which it is attached, turning the condensation more sluggish. *(ii)* Although the synthesis of the reported 6-N-benzoyl-5′-O-[(9-anisoyl)xanthen-9-yl]adenosine-2′-(o-chlorophenyl)phosphate block[5g] in a pure form is quite feasible, its final purification presents itself as a laborious task owing to the presence of excess n-tetrabutylammonium fluoride used for the removal of the 3′-disilyl chain in the preceeding reaction step[5g]. Contamination by this reagent causes side reactions owing to the cyclization of the 2′-phosphodiester function with the vicinal 3′-hydroxyl moiety during amidite coupling[5g]. Therefore a careful separation has to be excersised. Point *(ii)* perhaps illustrates the somewhat more unstable nature of O-aryl-O-alkylphosphodiester linkages towards fluoride ions compared to that of the O,O-dialkylphosphodiester linkages as in oligoribonucleotides[4d,e,j,5a,c,e]. In our present attempt to design an improved procedure, we have recognized and taken advantage of several useful features that have emerged in some of the published procedures: *(i)* use of the easily accessible 6-N-benzoyl-2′-O-pixyl[(9-phenylxanthen)-9-yl]adenosine **29**[11] as a key intermediate for extension of the chain in the 5′→3′, 3′→5′ and 2′→5′ directions, *(ii)* highly regioselective phosphorylation[11,14,24] of the 5′-hydroxyl function in a 3′,5′-dihydroxy-nucleoside block such as 6-N-benzoyl-2′-O-pixyladenosine **29**[11] with an appropriate 3′-phosphodiester block such as **1**, **5** or **15** in presence of MSNT, *(iii)* the regiospecific removal of the 2′-O-pixyl group at the branch-point over the 2′-O-Thp (tetrahydropyranyl) group(s) in **30b**, **31b** or **32b**, under mild acidic condition, to give **30c**, **31c** or **32c**, respectively, *(iv)* the introduction of the biscyanoethylphosphotriester moiety at the 2′-hydroxyl group at the branch-point, vicinal to 3′-O-(2-chlorophenyl)phosphodiester moiety as in **30c**, **31c** or **32c** to give **30d**, **31d** or **32d** respectively, by the reaction with an excess of biscyanoethylphosphoroamidite and tetrazole, which saved the use of an excess of oligomeric block, which is expensive and laborius to make, for extension at 2′→5′ direction from the branch-point[4k]. In the development of a new synthetic strategy for the preparation of larger branch-RNAs, these points enabled us *(i)* to use a minimum number of orthogonal protecting groups around the branch-point during the addition of various building blocks for chain extensions at 5′→3′, 3′→5′ and 2′→5′

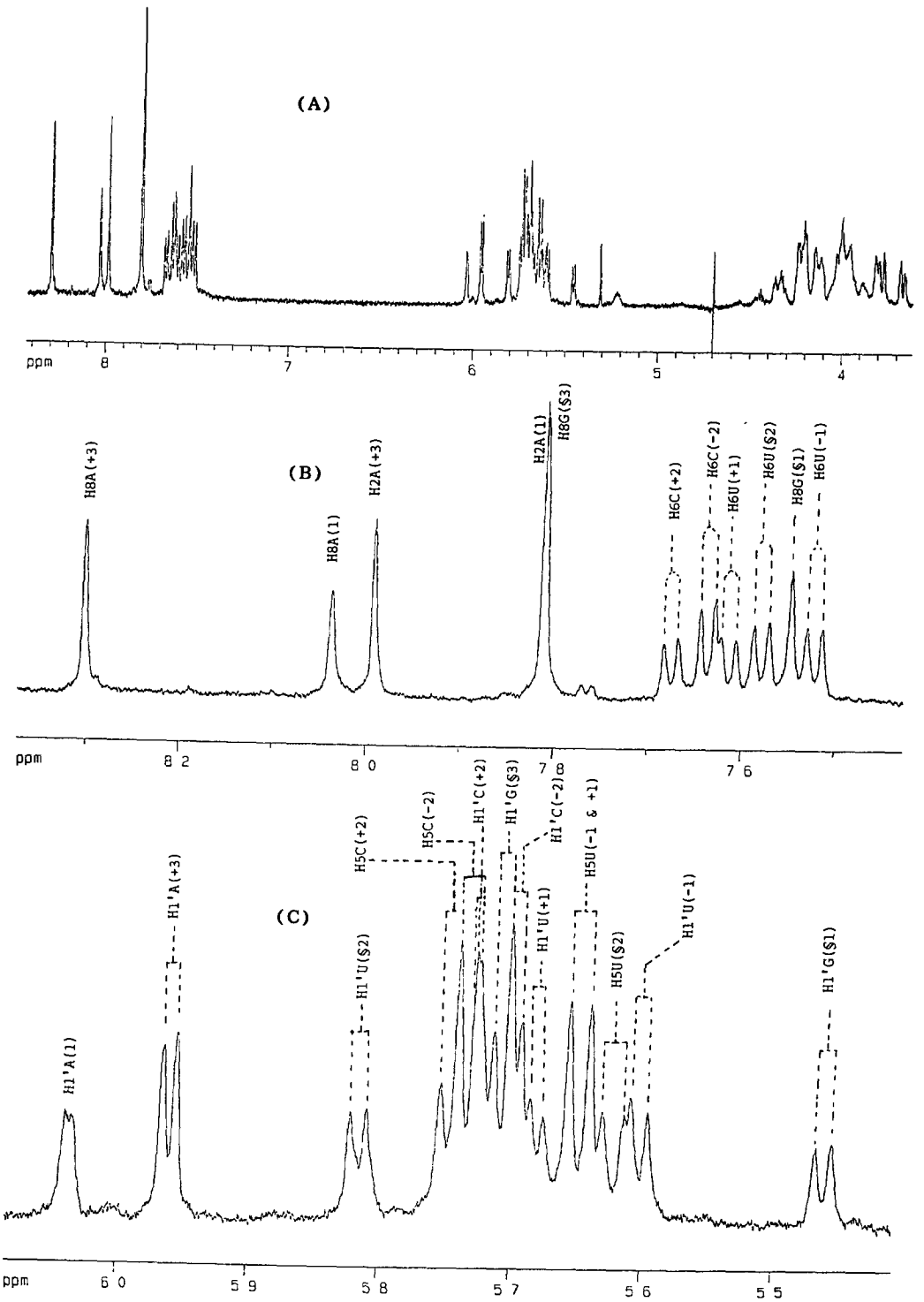


Fig. 4: Panel A shows the one Dimensional ^{1}H-NMR spectrum of branched Nona-RNA **40** at 500 MHz at 292 K (3.5 mM, DOH as internal reference at $\delta = 4.7$ ppm). Panel B & C show the expansion of the aromatic region and the anomeric region, respectively, with full assignments based on COSY, HOHAHA, ROESY and NOESY experiments.

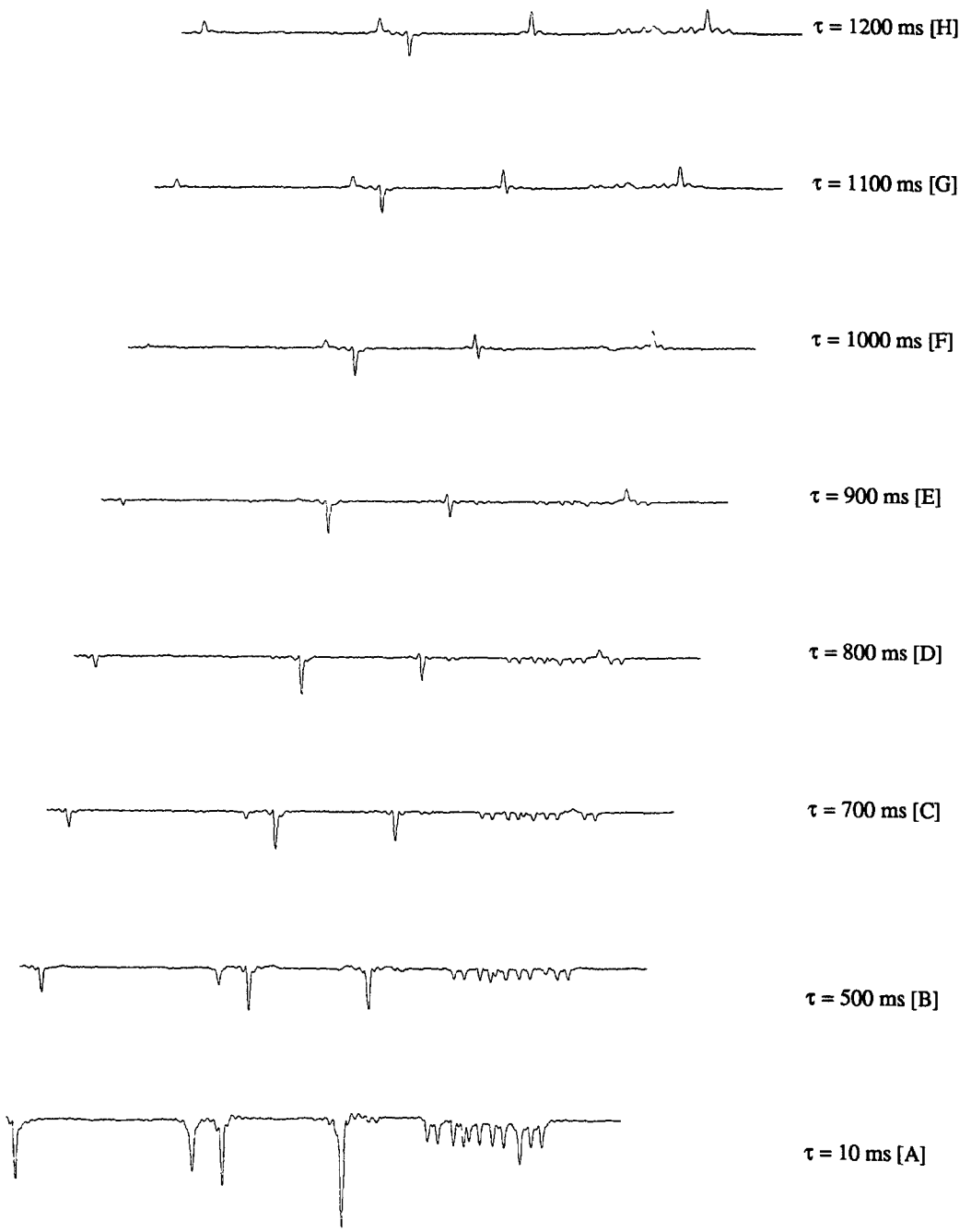

τ = 1200 ms [H]
τ = 1100 ms [G]
τ = 1000 ms [F]
τ = 900 ms [E]
τ = 800 ms [D]
τ = 700 ms [C]
τ = 500 ms [B]
τ = 10 ms [A]

Fig. 5a

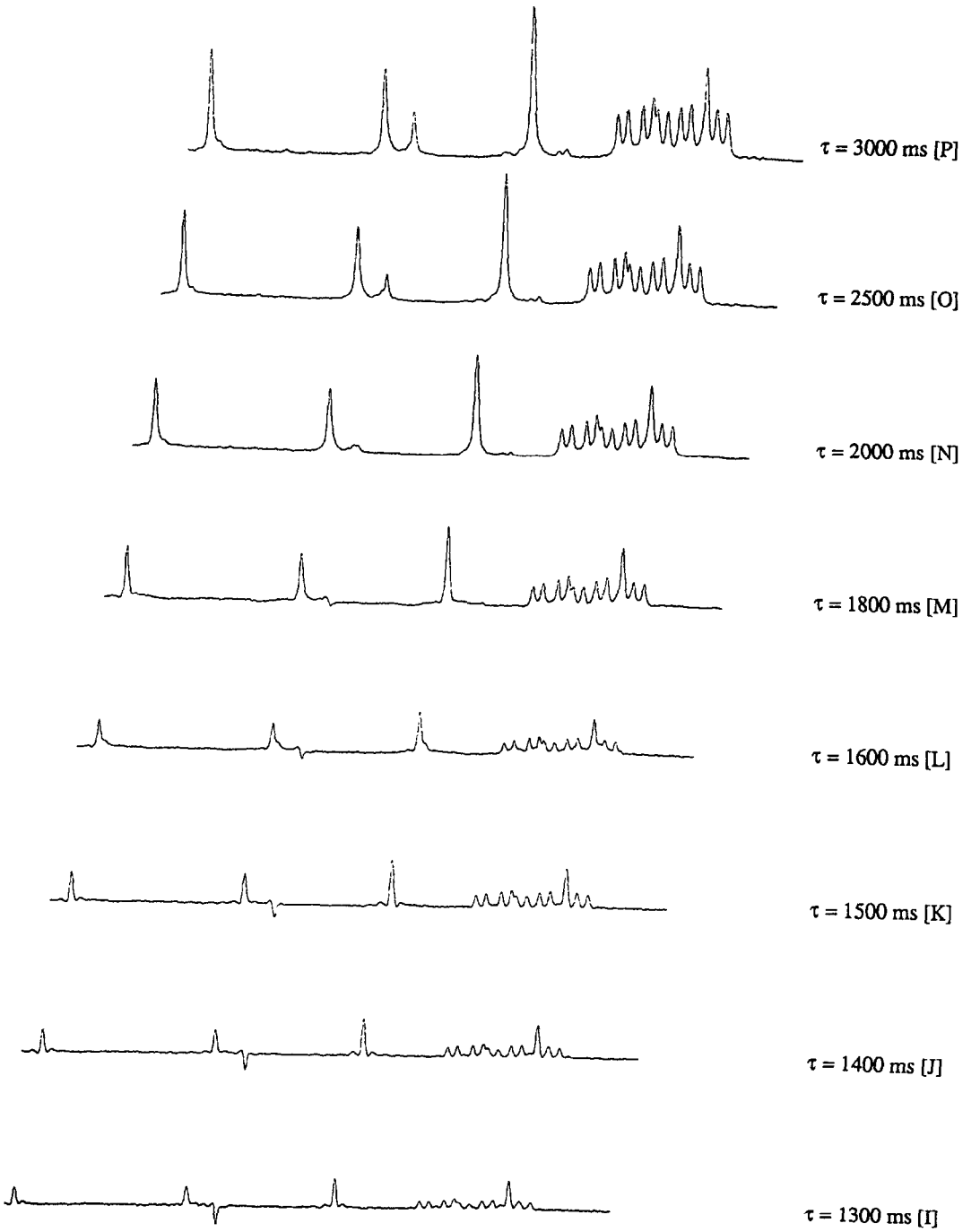


Fig. 5b

Figs. 5a & 5b: Results from an inversion recovery sequence experiment (64 scans, 16K data points, Sweep width 4166.6 Hz at 500.139 MHz at 292 K) of the aromatic region (δ 8.35 - 7.4) of the branched Nona-RNA **40** (see text for specific τ values for the T_1 of various aromatic protons).

directions, thus minimizing the problem of complementarity amongst the protecting groups, and *(ii)* to use only 5′-hydroxy monomeric or oligomeric blocks for extension in the 3′→5′ and 2′→5′ directions, thus avoiding the use of expensive 5′-phosphoramidite or 5′-H-phosphonate blocks[5d-f].

The synthesis of branched oligoribonucleotides 39, 40 and 41. We report in this paper a successful procedure for the synthesis of larger branched oligo-RNA which has not been possible hitherto. This new procedure have allowed us to assemble the branched oligo-RNA **39, 40** and **41** in high yield and in pure form enabling us to perform high resolution NMR spectroscopy. These oligomers corresponds to the sequence at the branch-site of the Group II intron bl1 from yeast mitochondria[12]. Synthesis of all protected precursors leading to branched tetramer **39**, nonamer **40** and decamer **41** have been outlined herein. The (5′→3′)-, (3′→5′)- and (2′→5′)-monomeric and -oligomeric blocks **1, 5** and **15** have been synthesized separately, employing normal phosphotriester chemistry[13] prior to the actual assembly of the branched oligo-RNAs. As the first step in the assembly of the branched oligomers, the 3′-phosphodiester blocks **1, 5** and **15** were condensed with the 3′,5′-dihydroxy block **29**[11] using 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole (MSNT)[14,15] as a condensing agent under conditions normally employed in the phosphotriester approach[13] to give the key 3′-hydroxy blocks **30a** [δ^{31}P = -7.47, -7.98], **31a** [δ^{31}P = -7.39 to -8.11] and **32a** [δ^{31}P = -4.56, -4.91, (-6.71 to -9.01)] in 65, 63 and 70 % yields respectively. The regiospecific condensation of **1, 5** or **15** with **29** was confirmed by ^{1}H-NMR spectroscopy by identification of the H3′A resonances centered at ~3.30 ppm which is characteristic for this proton in a 3′-hydroxy-2′-O-pixyl protected adenosine block[11]. Further evidence for this regiospecific coupling was corroborated by the fact that compounds **30a, 31a** and **32a** upon treatment with o-chlorophenylphosphoro-bis-(1,2,4-triazolide)[16] were found to transform specifically to the corresponding 3′-phosphodiester blocks **30b** [δ^{31}P = -6.15, -6.20 *(3′-phosphodiester)*, -7.64, -7.84 *(3′→5′-phosphotriester)*], **31b** [δ^{31}P = -6.17, -6.25, -6.32 *(3′-phosphodiester)*, -7.42 to -7.84, *(3′→5′-phosphotriester)*] and **32b** [δ^{31}P = -4.96, -5.20 *(3′-phosphodiester)*, -6.37 to -8.54, *(3′→5′-phosphotriester)*] in 92, 83 and 80 % yields respectively. In the ^{1}H-NMR spectrum for each product, **30b, 31b**, and **32b**, the H3′A resonances moved downfield by 1.5-1.7 ppms. Depixylation of **30b, 31b** or **32b** was carried out using trichloroacetic acid in 2% CH_3OH-CH_2Cl_2 at 0 °C[17] to give the 3′-phosphodiester-2′-hydroxy blocks **30c** [δ^{31}P = -7.91], **31c** [δ^{31}P = -7.39, -7.64, -7.81, -7.93, - 8.08] and **32c** [δ^{31}P = -5.49, -5.78, -6.25, (-6.71 to -8.28)] in 74, 86 and 85 % yields, respectively, obtained directly by precipitation from diethylether-hexane after work up and drying. The product in each case was completely homogenous on TLC (system D) and ^{1}H-NMR spectroscopy revealed the total absence of branch-point 2′-O-pixyl resonances (~6.80-6.15 ppm) and also that the resonances from the 2′-O-Thp groups had remained intact (~3.90-3.35, ~1.45-1.50 ppm). For the protection of 2′-OH group(s) of the nucleoside residue(s) at the 5′-terminal end of the branch-point adenosine, we chose the more acid-stable tetrahydropyranyl (Thp) protecting group to ensure 100% regiospecific removal of the 2′-O-pixyl group under mild acidic condition[17]. This particular choice of complementary 2′-acid labile protecting groups (pixyl and Thp) played a critical role in avoiding complications which arise owing to the partial removal of the 2′-protecting group from the 5′-terminal residues during the deprotection of the 2′-O-pixyl at the branch-point. The method for the introduction of the biscyanoethylphosphotriester moiety at the branch-point hydroxyl vicinal to a phosphodiester moiety as devised by Hata *et al* [4k] or to a 3′-hydroxy function in a partially protected 2′-deoxynucleoside block as devised in a recent work by Sekine *et al* [18] are both multistep procedures. We found that this phosphotriester function can be easily introduced to the branch-point *in one single-step* using (biscyanoethoxy)-(diisopropylamino)phosphine[19] by standard amidite chemistry[20].

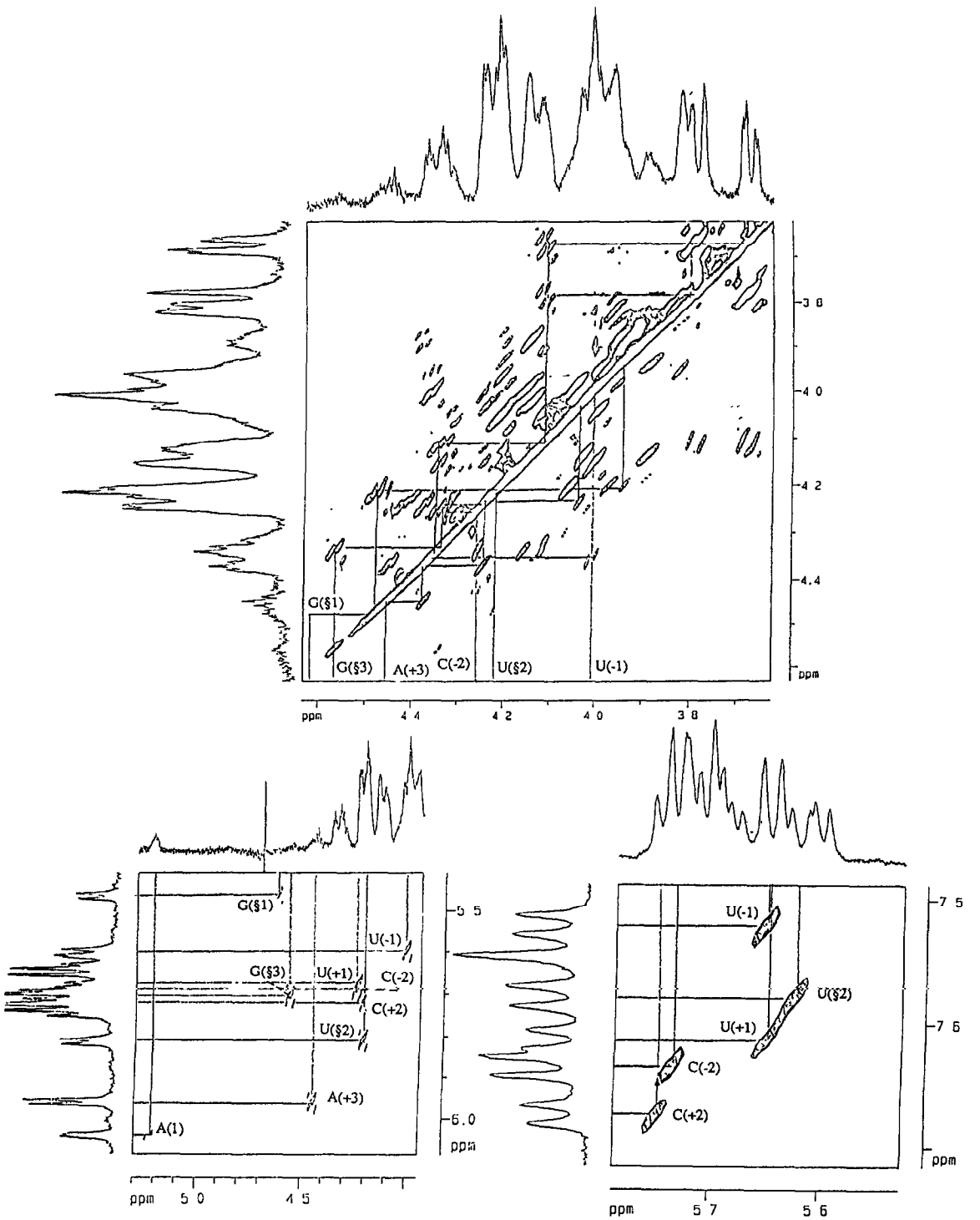


Fig. 6: Expansion of the 500 MHz COSY spectrum of branched Nona-RNA **40** at 292 K (3.5 mM) [2K data points, 512 experiments, 32 scans, zero-filled to 2K x 1K, processed by window function sine 3]

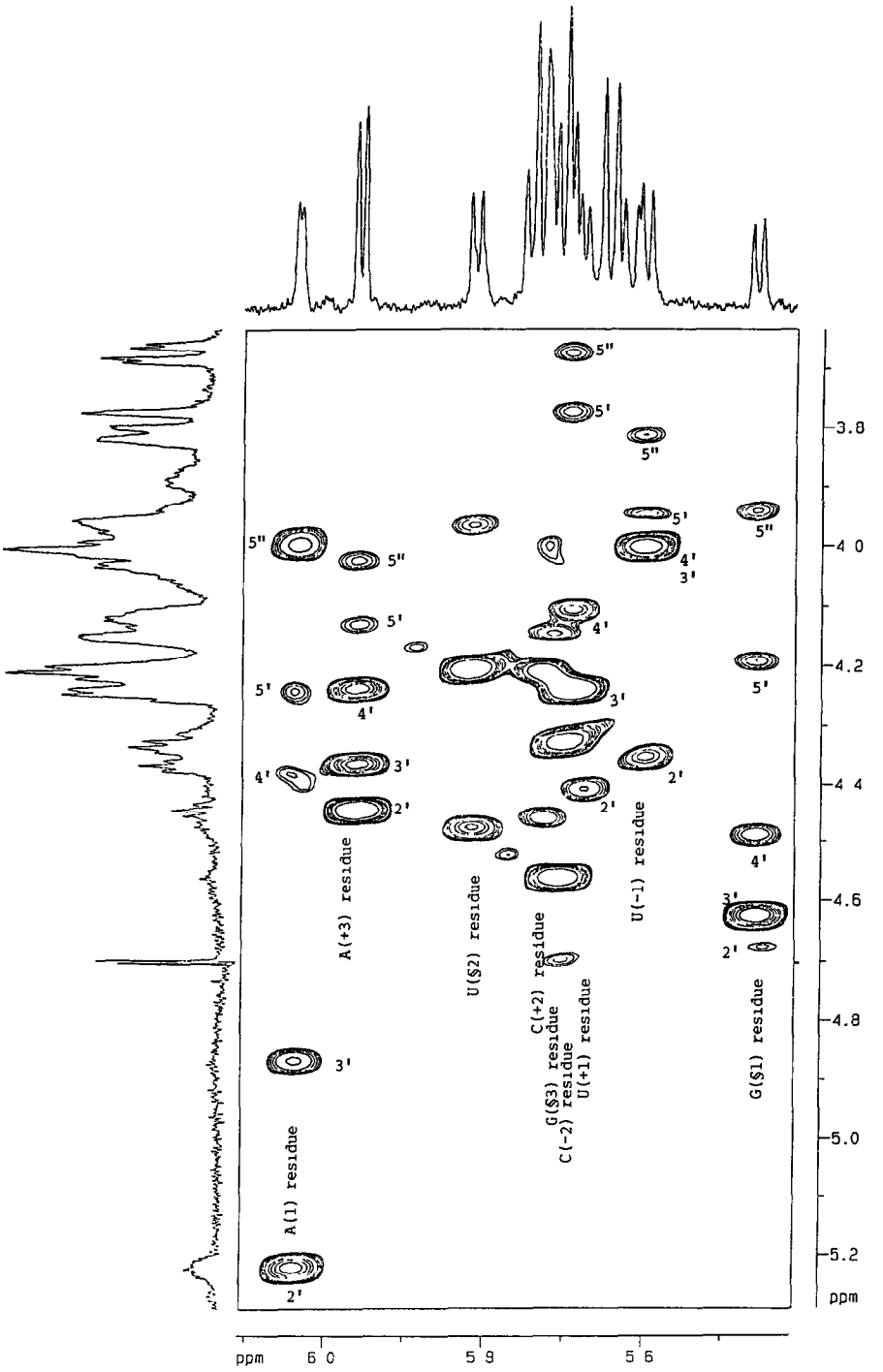


Fig. 7: Expansion of the 500 MHz homonuclear Hartmann-Hahn spectrum of branched Nona-RNA **40** at 292 K (3.5 mM) [2K data points, 256 experiments, 64 scans, zero-filled to 2K x 1K, processed by window function Qsine 4 in both F1 & F2 directions].

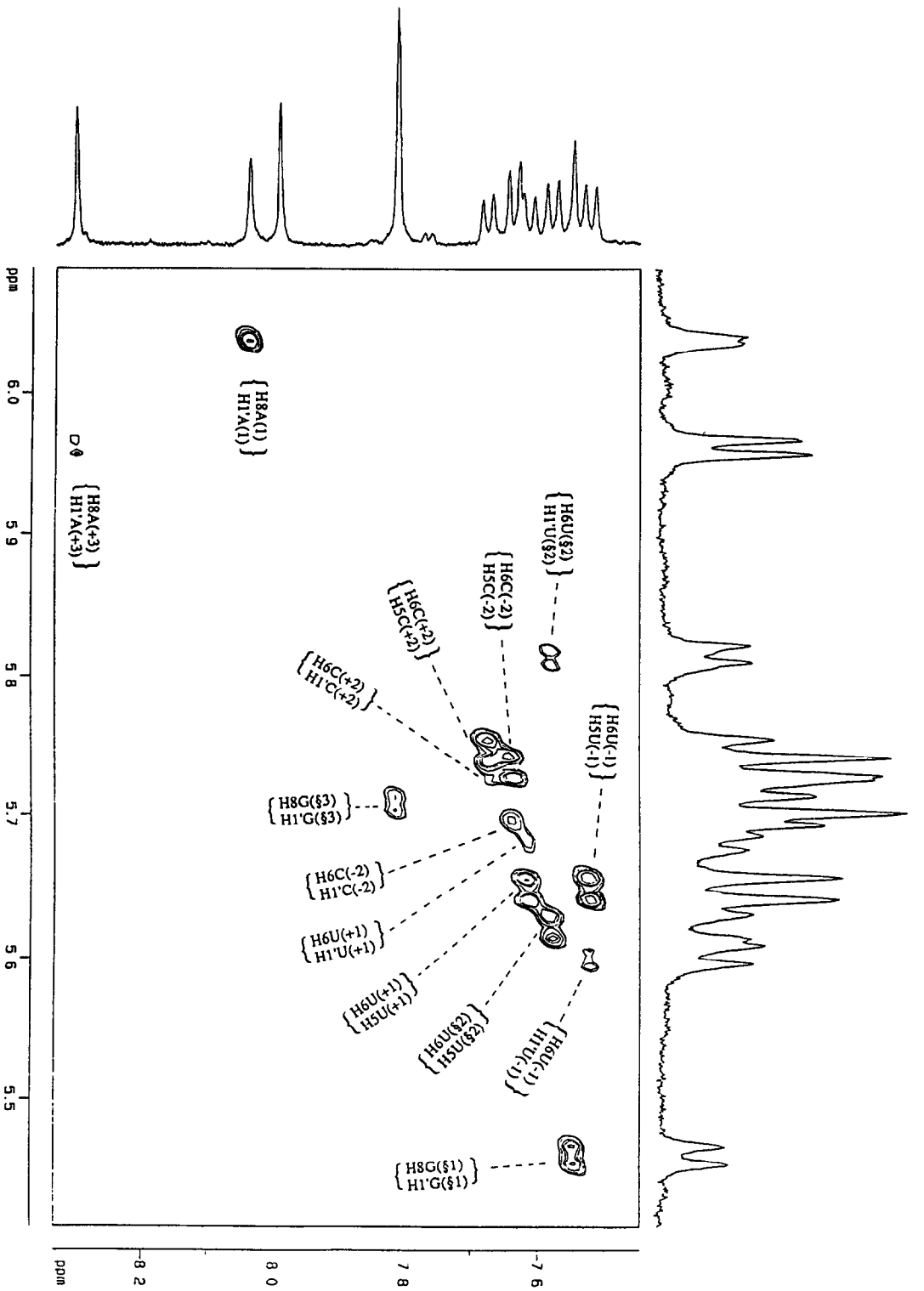
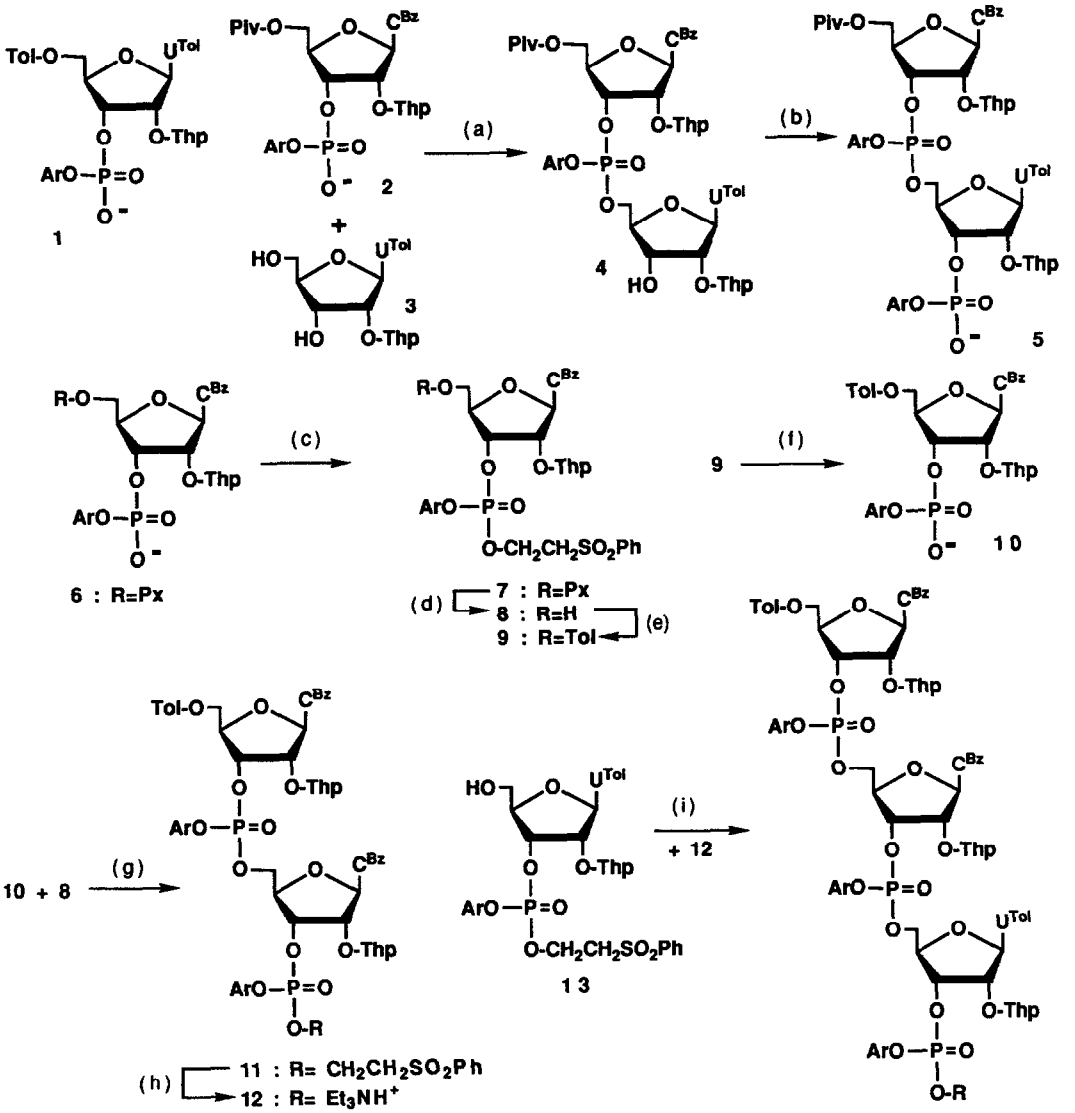


Fig. 8: NOESY spectrum of branched Nona-RNA **40** at 292 K (3.5 mM, mixing time 900 ms). 2K x 256 experiments (each experiment consist of 72 scans) were performed for the acquisition of the spectrum. The matrix was zero-filled to 2K x 1K, and then processed by the window function Qsine 4 in both F1 and F2 directions.



(a) (2) (1.1 eq.), (3) (1 eq.), MSNT (3.3 eq.), pyridine, 40 min RT; (b) o-chlorophenylphosphoro-bis-(1,2,4-triazolide) (1.5 eq.), pyridine / MeCN 40 min RT, (c) $PhSO_2CH_2CH_2OH$ (1.5 eq.), MSNT (2.5 eq.), pyridine, 45 min RT, (d) CCl_3CO_2H (10 eq.), 4% EtOH / $CHCl_3$, 3 h, 0°C; (e) TolCl (1.3 eq.), pyridine, 3 h RT, (f) Et_3N (20 eq.), pyridine, 45 min RT; (g) (10) (1 eq.), (8) (0.85 eq.), MSNT (3 eq.), pyridine, 50 min, RT; (h) Et_3N (20 eq.), pyridine, 45 min RT, (i) (12) (1 eq.), (13) (1.2 eq.), MSNT (4.7 eq.), pyridine, 20 min RT; (j) Et_3N (20 eq.), pyridine, 45 min RT

Abbreviations: Ar = 2-Chlorophenyl; Px = 9-phenylxanthen-9-yl; MDMP = 1,5-Dicarbomethoxy-3-methoxypentan-3-yl; Fmoc = 9-fluorenylmethoxycarbonyl; G^{NP} = N^2-(t-butylbenzoyl-O^4-(2-nitrophenyl)guanine-9-yl; G^{NP} = N^2-(t-butylbenzoyl-O^4-(2-nitrophenyl)guanine-9-yl; G^{Tbb} = N^2-(t-butylbenzoyl)guanine-9-yl; C^{Bz} = N^4-benzoylcytosine, U^{Tol} = N^3-(4-toluoyl)uracil-1-yl; Piv = Pivaloyl; A = 9-adeninyl; C = 1-cytosinyl; G = 9-guaninyl; U = 1-uracilyl

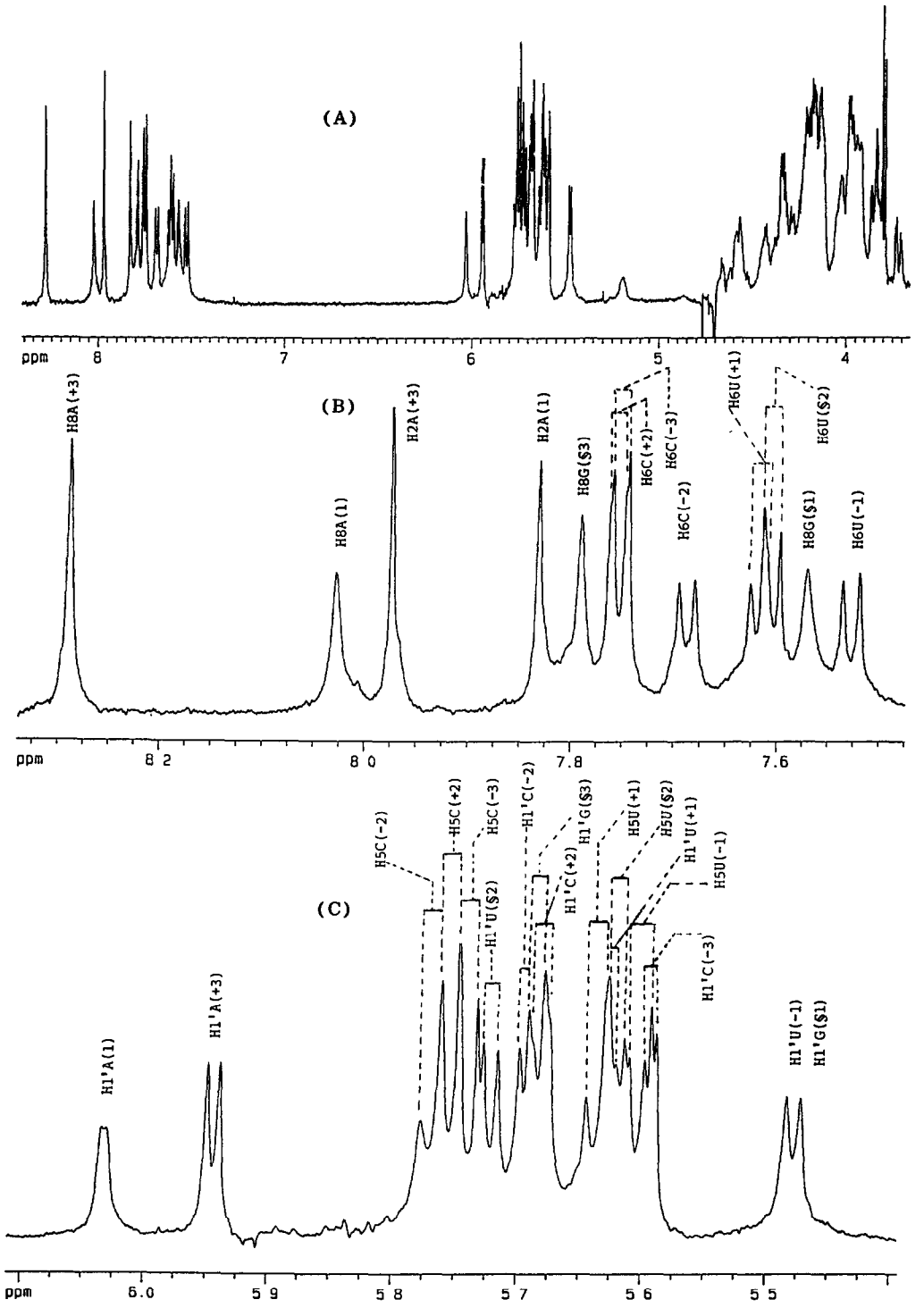
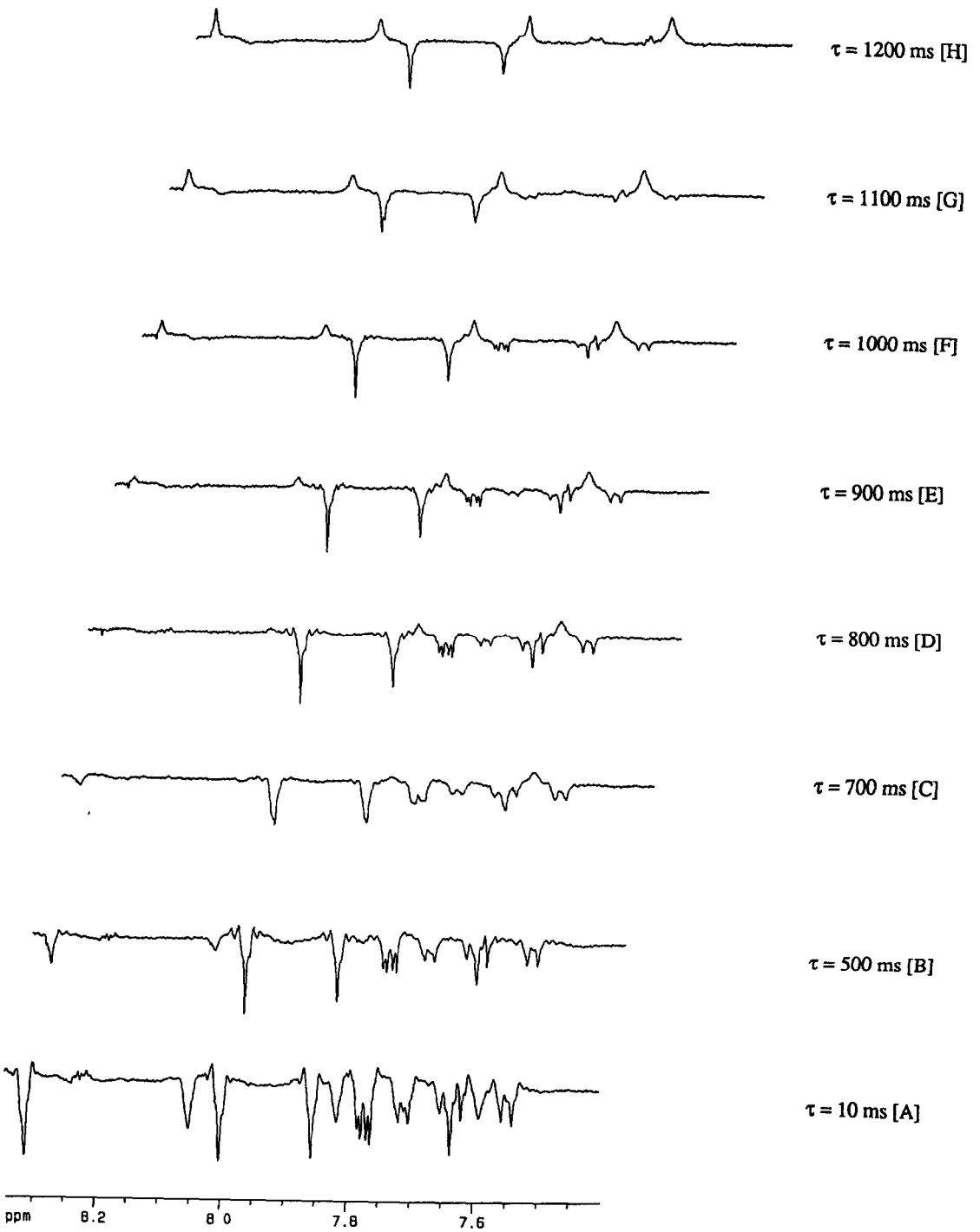


Fig. 10: Panel A shows the one dimensional ^{1}H-NMR spectrum of branched Deca-RNA **41** at 500 MHz at 292 K (2.4 mM, DOH as internal reference at $\delta = 4.7$ ppm). Panel B & C show the expansion of the aromatic region and the anomeric region with full assignments based on COSY, HOHAHA, ROESY and NOESY experiments.

Thus, compound **30c**, **31c** or **32c** was treated with (biscyanoethoxy)-(diisopropylamino)phosphine in ten fold excess together with 30 equivalents of tetrazole in dimethylformamide-acetonitrile for 40 min followed by aqueous iodine oxidation to give the 3′-phosphodiester-2′-biscyanoethylphosphotriester blocks **30d**, **31d** or **32d** in 46, 64 and 62% isolated yields respectively. Two distinct groups of phosphate resonances in ^{31}P-NMR can be observed in each spectrum of all the cyanoethylphosphate containing oligomers (i.e **30d**, **31d**, **32d**, **33a**, **33b**, **34a**, **34b**, **35a**, **35b**, **36**, **37** and **38**). Resonance due to the cyanoethylphosphate moieties (Ce-P) are observable at a lower field (between ~-2.0 and -5.0 ppm) than those are from o-chlorophenylphosphates (ClPh-P) which absorb at a higher field (between ~-6.0 and -8.0 ppm). Simple integration of these two groups of phosphate resonances reveals the outcome of each reaction step [see panels A1-A4 (Figure 1), panels B1-B4 (Figure 2), panels C1-C4 (Figure 3)]. Thus, the ratio between the integrals of the two groups of phosphate resonances corresponding to compounds **30d**, **31d** and **32d** are as expected 1: 2 (panel A1, figure 1), 1: 3 (panel B1, figure 2) and 1: 4 (panel C1, figure 3), respectively. The specific down-field shift of the branch-point anomeric proton (H1′A) in the ^{1}H-NMR spectrum (δ~6.05-6.15 → δ~6.30-6.40 ppm) upon introduction of the 2′-O-cyanoethylphosphate moiety further confirms that the regiospecific coupling of the amidite reagent to the 2′-hydroxyl function has indeed been achieved. The down field resonance of H1′A is observed in all the ^{1}H-NMR spectra of the subsequent 2′-O-cyanoethylphosphate containing oligomers (i.e **30d**, **31d**, **32d**, **33a**, **33b**, **34a**, **34b**, **35a**, **35b**, **36**, **37** and **38**). These observations actually substantiates that all the reaction steps prior to and including the amidite reaction had been carried out in the expected manner. In the next step, normal MSNT condensation reactions were carried out with compounds **30d**, **31d** or **32d** and 5′-hydroxy blocks **19** or **28** to yield the fully protected oligomers **33a** (59 %), **34a** (69 %) and **35a** (56 %) [**30d+28** → **33a** (δ^{31}P: panel A2, Ce-P : ClPh-P = 1: 2), **31d+19** → **34a** (δ^{31}P: panel B2, Ce-P : ClPh-P = 1: 4), and **32d+19** → **35a** (δ^{31}P: panel C2, Ce-P : ClPh-P = 1: 5)]. Compounds **33a**, **34a** and **35a** were treated with diisopropylethylamine (25 fold excess) in pyridine at 40 °C for ~2h[4k] to give the 2′-cyanoethylphosphodiester blocks **33b** [δ^{31}P: panel A3, Ce-P : ClPh-P = 1: 2], **34b** [δ^{31}P: panel B3, Ce-P : ClPh-P = 1: 4] and **35b** [δ^{31}P: panel C3, Ce-P : ClPh-P = 1: 5] in 64, 67 and 68 % yields, respectively. These blocks were then subjected to a final MSNT mediated condensation with the 5′-hydroxy blocks **27** or **25** to give the fully protected branched oligomers **33b+27** → **36** [δ^{31}P: panel A4, Ce-P: ClPh-P = 1: 2], **34b+25** → **37** [δ^{31}P: panel B4, Ce-P : ClPh-P = 1: 7] and **35b+25** → **38** [δ^{31}P: panel C4, Ce-P: ClPh-P = 1: 8] in 69, 67 and 63% yields, respectively. These fully protected branched-RNAs **36**, **37** and **38** were then deprotected and the products purified in the usual manner to finally give pure fractions of **39**, **40** and **41** (see experimental section for details). The isolated yields of pure compound were 62 % (288 A_{260} units), 21% (599 A_{260} units) and 21% (263 A_{260} units) respectively. The total yield of deprotection for **40** and **41** amounted to ~35% and ~34% respectively judging from a HPLC profile of the crude deprotection mixture for each compound. The strategy presented herein for the syntheses of branched RNA is general in nature which should find use to produce branched oligoribonucleotides of various lengths in a feasible and economical way using easily accessible and stable reagents, starting materials and intermediates. A branched tridecamer belonging to same branch-site Group II intron bl1 sequence is currently being assembled in this laboratory.

Structure elucidation of branched nona-RNA 40 & deca-RNA 41 by 500 MHz NMR spectroscopy. *Aromatic protons:* The assignment of the H-6 and H-5 of the pyrimidine moieties were based on their distinctive coupling constants: H-6 and H-5 of uridine appear as a doublet of ~ 8.1 Hz while H-6 and H-5 of cytidine appear as a doublet of ~ 7.6 Hz. One dimensional ^{1}H-NMR spectra at 500 MHz showed that the three uracil protons and two cytosine protons for branched nona-RNA $C_{[-2]}U_{[-1]}A_{[1]}{}^{G_{[\$1]}U_{[\$2]}G_{[\$3]}}_{U_{[+1]}C_{[+2]}A_{[+3]}}$ (**40**) occurred

Fig. 11a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τ = 1100 ms [G]
τ = 1000 ms [F]
τ = 900 ms [E]
τ = 800 ms [D]
τ = 700 ms [C]
τ = 500 ms [B]
τ = 10 ms [A]
ppm
8.2
8.0
7.8
7.6

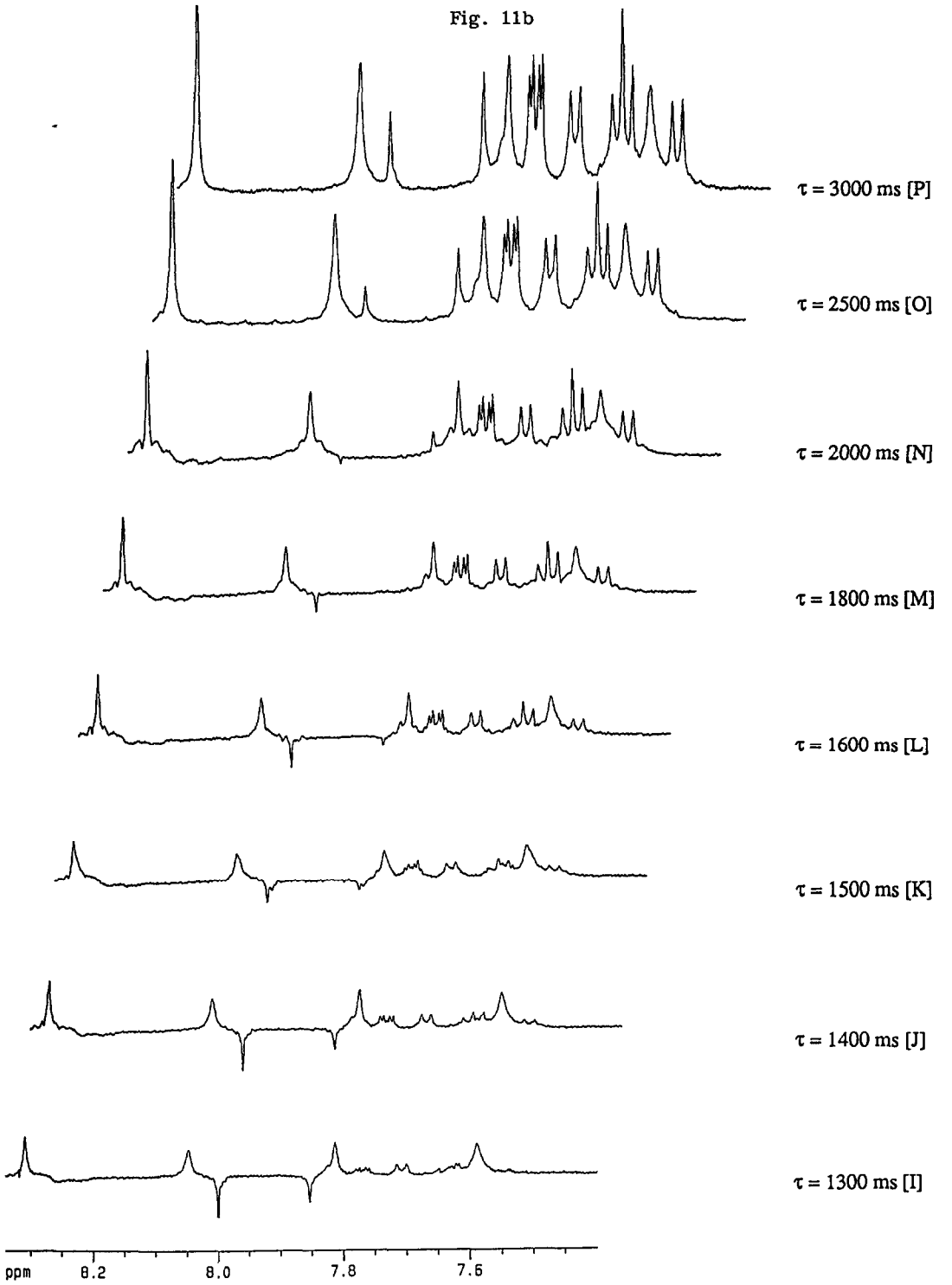
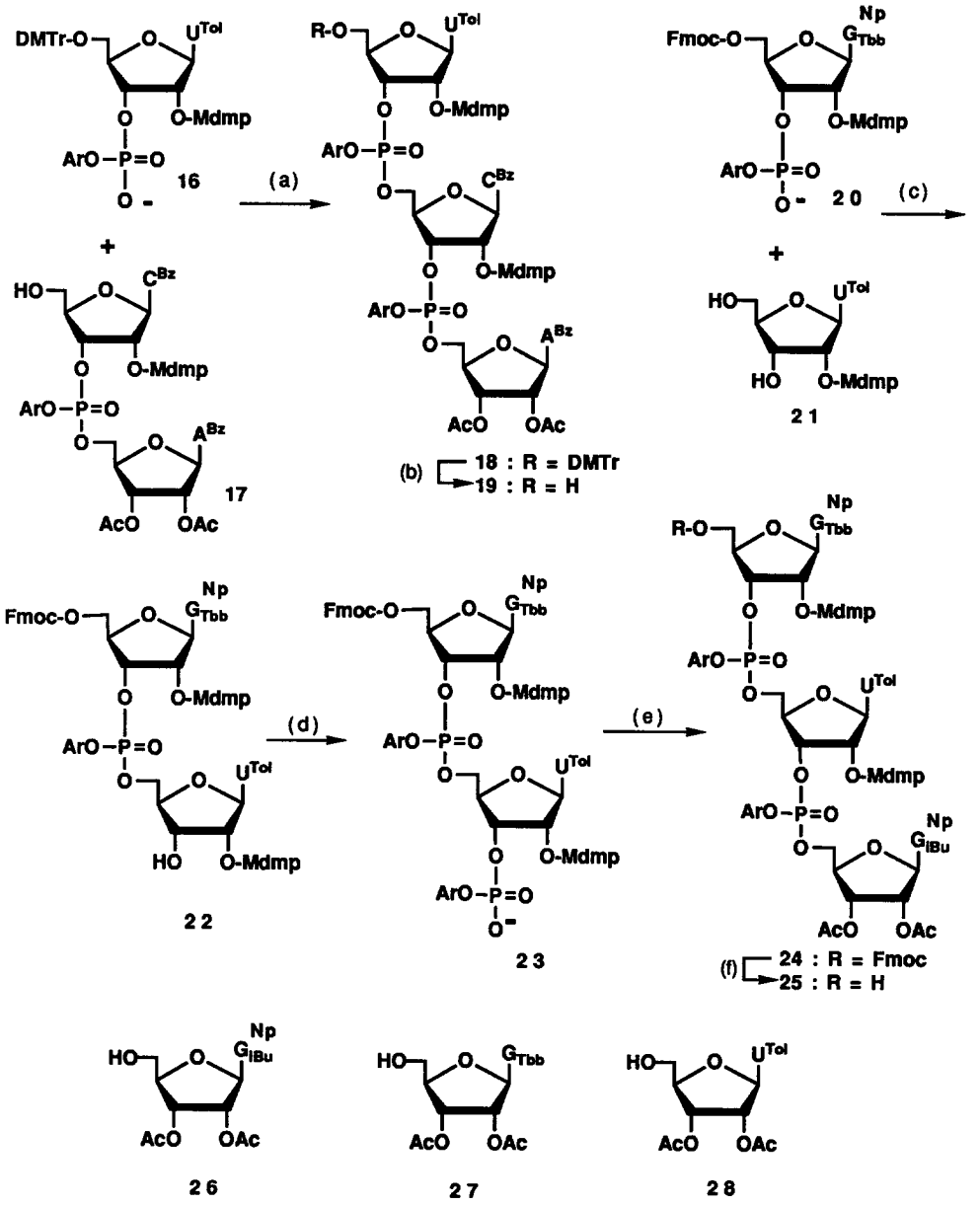


Fig. 11a & 11b: Results from an inversion recovery sequence experiment (64 scans, 16K data points, Sweep width 4166.6 Hz at 500.139 MHz at 292 K) of the aromatic region (δ 8.35 - 7.4) of the branched Deca-RNA **41** (see text for specific τ values for the T_1 of various aromatic protons).

(Fig. 4) at δ 7.676 (d, $J_{5,6}$ = 7.6 Hz, 1H, $C_{[+2]}$), 7.637 (d, $J_{5,6}$ = 7.6 Hz, 1H, $C_{[-3]}$), 7.614 (d, $J_{5,6}$ = 8.1 Hz, 1H, $U_{[+1]}$), 7.580 (d, $J_{5,6}$ = 8.1 Hz, 1H, $U_{[\$2]}$) and 7.527 (d, $J_{5,6}$ = 8.1 Hz, 1H $U_{[-1]}$). For the branched deca-RNA $C_{[-3]}C_{[-2]}U_{[-1]}A_{[1]}{}^{G_{[\$1]}U_{[\$2]}G_{[\$3]}}_{U_{[+1]}C_{[+2]}A_{[+3]}}$ (41), ^{1}H-NMR spectra at 500 MHz showed (Fig. 10) three uracil and



(a) (16) (1 eq.), (17) (0 75 eq.), MSNT (2.24 eq.), pyridine, 40 min RT; (b) TsOH . H_2O (8.7 eq.), MeOH / CH_2Cl_2, 20 min, 0°C; (c) (20) (1 eq.), (21) (0.92 eq.), MSNT (3 eq.), pyridine, 40 min RT; (d) o-chlorophenylphosphoro-bis-(1,2,4-triazolide) (1.5 eq.), pyridine / MeCN, 40 min RT; (e) (26) (0.91 eq.), MSNT (3 eq.), pyridine, 40 min RT; (f) Et_3N (20 eq.), pyridine, 1 h RT.

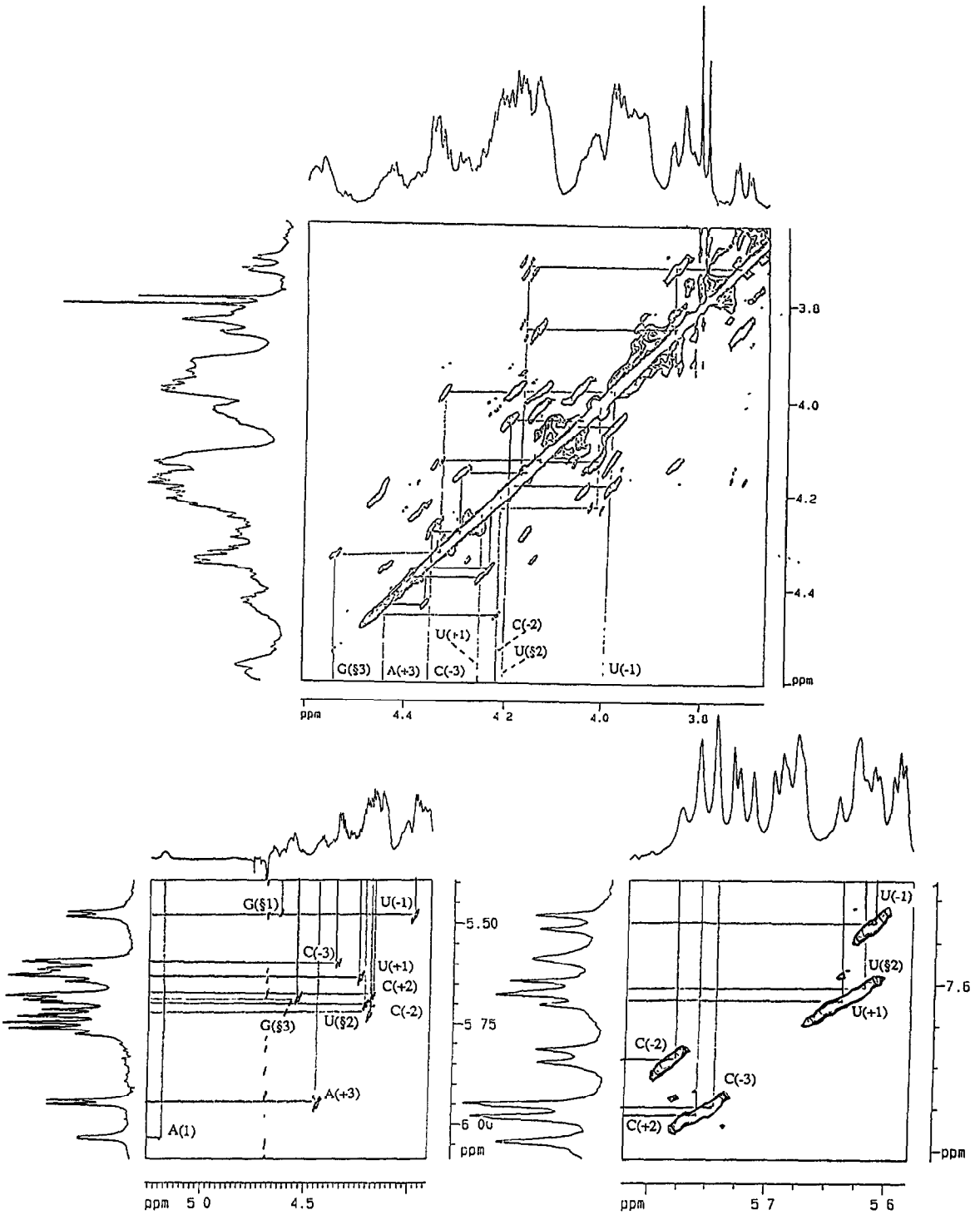
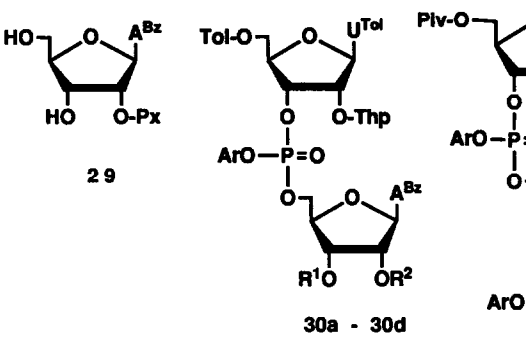
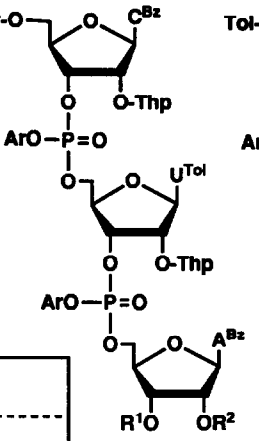


Fig. 12: Expansion of the 500 MHz COSY spectrum of branched Deca-RNA **41** at 292 K (2.4 mM) [2K data points, 512 experiments, 32 scans, zero-filled to 2K x 1K, processed by window function sine 3 in both F1 and F2 directions].

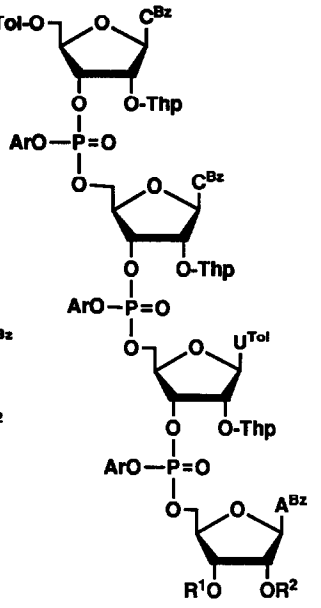


29

30a - 30d



31a - 31d



32a - 32d

	R^1 =	R^2 =
(a)	H	Px
(b)	ArO—P(=O)(O⁻ Et_3NH^+)—	Px
(c)	ArO—P(=O)(O⁻ Et_3NH^+)—	H
(d)	ArO—P(=O)(O⁻ Et_3NH^+)—	$NCCH_2CH_2$-O—P(=O)(O-CH_2CH_2CN)—

and cytosine protons at δ 7.53 (d, $J_{5,6}$ = 7.6 Hz, 1H, $C_{[-2]}$), 7.61 (d, $J_{5,6}$ = 7.6 Hz, 1H, $C_{[-3]}$), 7.62 (d, $J_{5,6}$ = 7.6 Hz, 1H, $C_{[+2]}$), 7.69 (d, $J_{5,6}$ = 8.1 Hz, 1H, $U_{[-1]}$), 7.75 (d, $J_{5,6}$ = 8.1 Hz, 2H, $U_{[+1]}$ & $U_{[§2]}$). Comparison of chemical shifts of H2 and H8 protons of adenine and H8 proton of guanine in $C_{[-2]}U_{[-1]}A_{[1]}{}^{G_{[§1]}U_{[§2]}G_{[§3]}}_{U_{[+1]}C_{[+2]}A_{[+3]}}$ (**40**) [δ 8.303 (1H, H8A(+3)), 8.03 7 (1H, H8A(1)), 7.992 (1H, H2A(+3)), and 7.811 (2H, H2A(1) & H8G(§2))] and in $C_{[-3]}C_{[-2]}U_{[-1]}A_{[1]}{}^{G_{[§1]}U_{[§2]}G_{[§3]}}_{U_{[+1]}C_{[+2]}A_{[+3]}}$ (**41**) [δ 8.29 (1H, H8A(+3)), 8.03 (1H, H8A(1)), 7.97 (1H, H2A(+3)), and 7.83 (2H, H2A(1) & H8G(§2))] with those of the assignments made in our earlier work on branched heptameric-RNA[5g,q,s], CUA^{GU}_{UC}, again suggested that the assignments shown in figures Figs. 4 & 10 are correct. Inversion recovery experiments (Figs. 5a,5b & 11a,11b) further enabled us to clearly distinguish between the H2 and H8 protons of two adenine and two H8 of two guanine residues in **40** and **41**. This showed that for branched nonamer $C_{[-2]}U_{[-1]}A_{[1]}{}^{G_{[§1]}U_{[§2]}G_{[§3]}}_{U_{[+1]}C_{[+2]}A_{[+3]}}$ (**40**), 3′-terminal H8A[+3] (δ 8.303) & H2A[+3] (δ 7.992) has a T_1 at τ = ~900 ms & 1800 ms, respectively; branch-point H8A[1] (δ 8.03) has a T_1 at τ = ~800 ms ; & H2A[1] (δ 7.811) and 2′-terminal H8G[§1] (δ 7.811) have identical chemical shifts and therefore precise T_1 measurements were not possible; 2′→5′-linked H8G[§1] (δ 7.547) has a T_1 at τ = ~600 ms. For branched decamer $C_{[-3]}C_{[-2]}U_{[-1]}A_{[1]}{}^{G_{[§1]}U_{[§2]}G_{[§3]}}_{U_{[+1]}C_{[+2]}A_{[+3]}}$ (**41**), 3′-terminal H8A[+3] (δ 8.29) & H2A[+3] (δ 7.97) has a T_1 at τ = ~800 ms & 2000 ms, respectively; branch-point H8A[1] (δ 8.03) & H2A[1] (δ 7.83) has a T_1 at τ = ~750 ms &

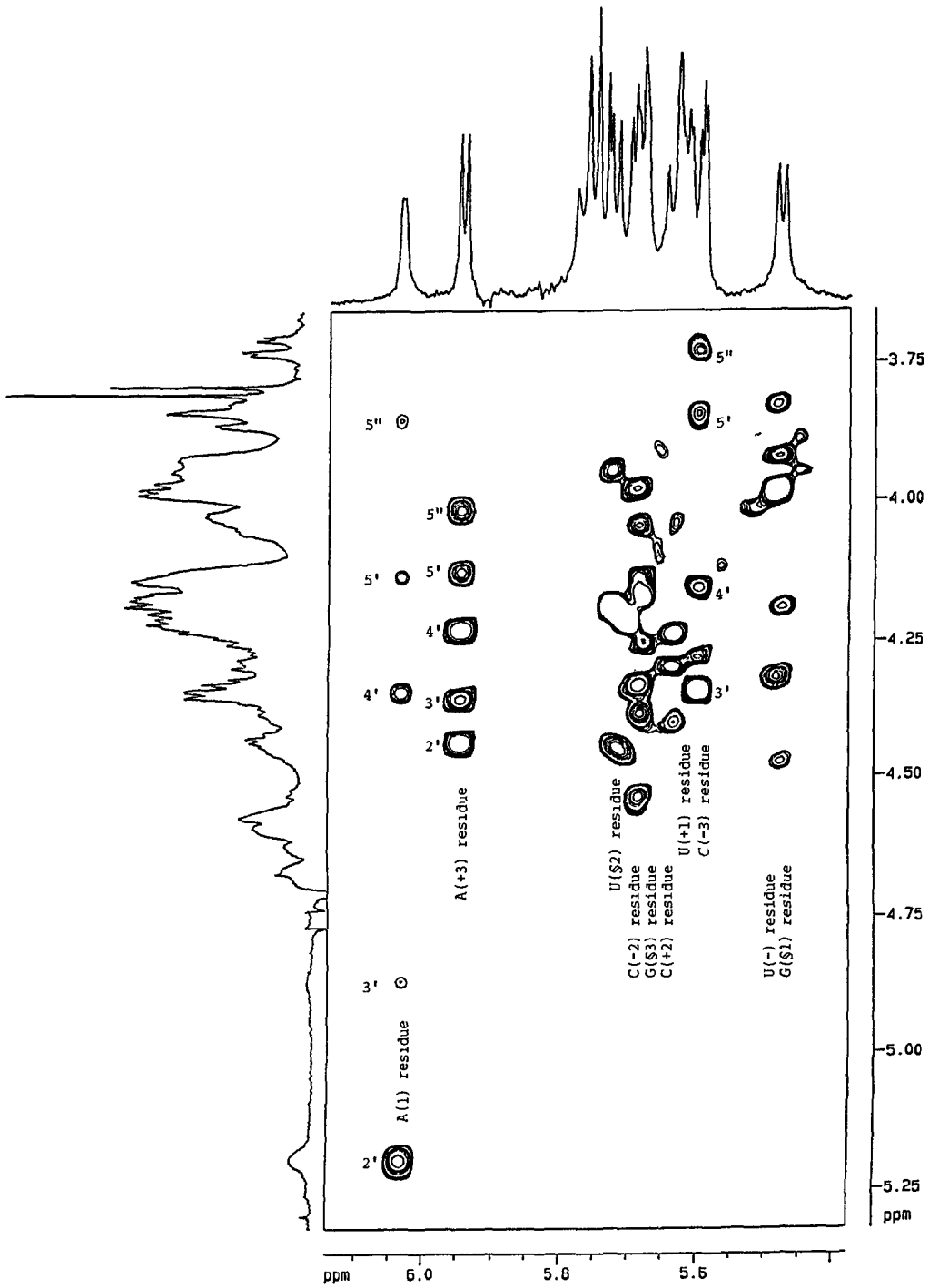
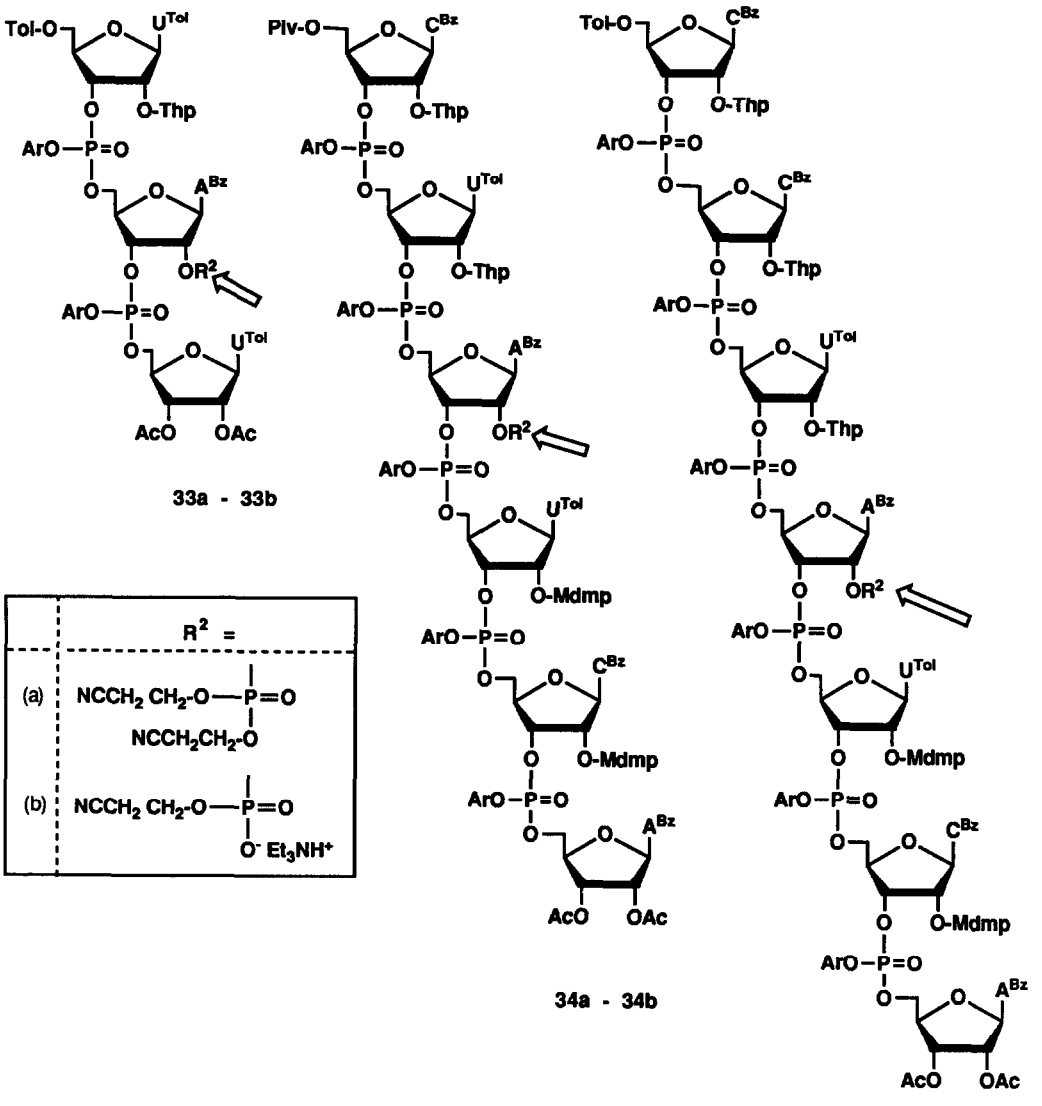


Fig. 13: Expansion of the 500 MHz homonuclear Hartmann-Hahn spectrum of branched Deca-RNA **41** at 292 K (2.4 mM) [2K data points, 256 experiments, 64 scans, zero-filled to 2K x 1K, processed by window function Qsine 4 in both F1 & F2 directions].

1700 ms, respectively; 2′-terminal H8G[§1] (δ 7.79) has a T_1 at τ = ~600 ms; 2′→5′-linked H8G[§1] (δ 7.57) has a T_1 at τ = ~500 ms.

Sugar protons & Phosphate resonances: The sugar protons in nona-RNA $C_{[-2]}U_{[-1]}A_{[1]}{}^{G_{[§1]}U_{[§2]}G_{[§3]}}_{U_{[+1]}C_{[+2]}A_{[+3]}}$ (**40**)



and deca-RNA, $C_{[-3]}C_{[-2]}U_{[-1]}A_{[1]}{}^{G_{[§1]}U_{[§2]}G_{[§3]}}_{U_{[+1]}C_{[+2]}A_{[+3]}}$ (**41**), were assigned from the measurements and interpretation of several 2D NMR spectra. The resonances of the sugar protons in **40** and **41** were assigned using a COSY 45 experiment in which the second 90° pulse is replaced by a 45° pulse to achieve better resolution of the cross peaks close to the diagonal (Figs. 6 & 12). To resolve some ambiguities in assignment due to overlap of absorptions in the H3', H4' and H5'/ H5" region, homonuclear Hartmann-Hahn (HOHAHA) experiments were

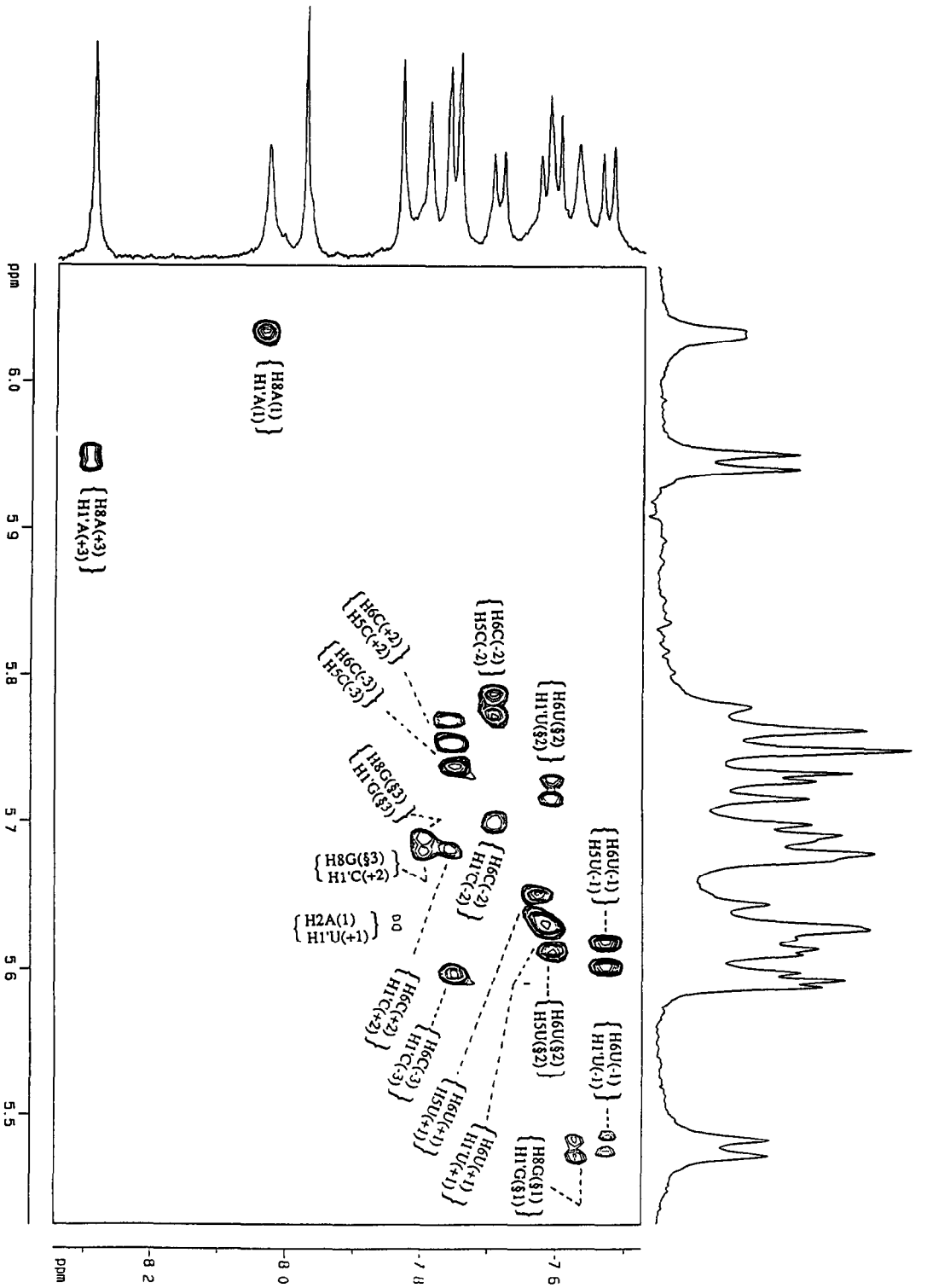


Fig. 14: NOESY spectrum of Branched Deca-RNA **41** at 292 K (2.4 mM, mixing time 900 ms). 2K x 256 experiments (each experiment contained 72 scans) were performed for the acquisition of the spectrum. The matrix was zero-filled to 2K x 1K, and then processed by the window function Qsine 4 in both F1 and F2 directions.

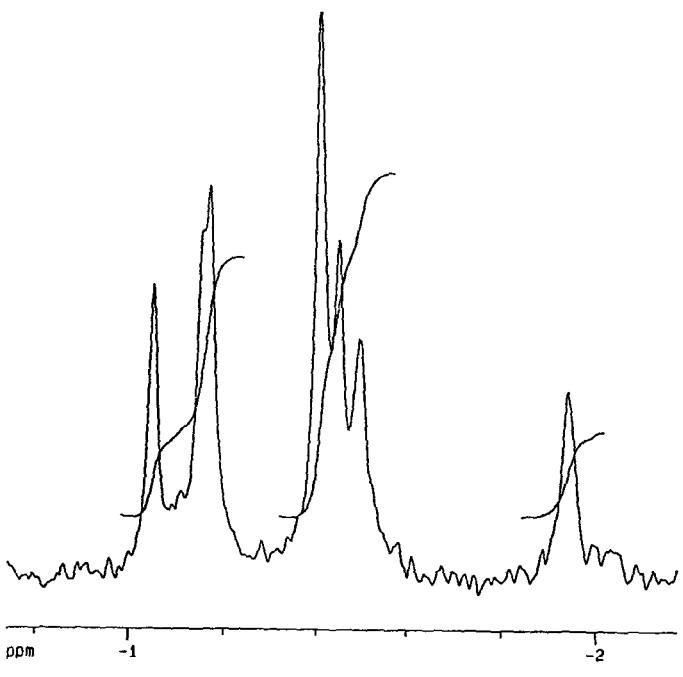


Fig. 9: 202.45 MHz ^{1}H-decoupled ^{31}P-NMR spectrum of branched Nona-RNA **40** as its Na^{+} salt in $^{2}H_2O$ at 292 K.

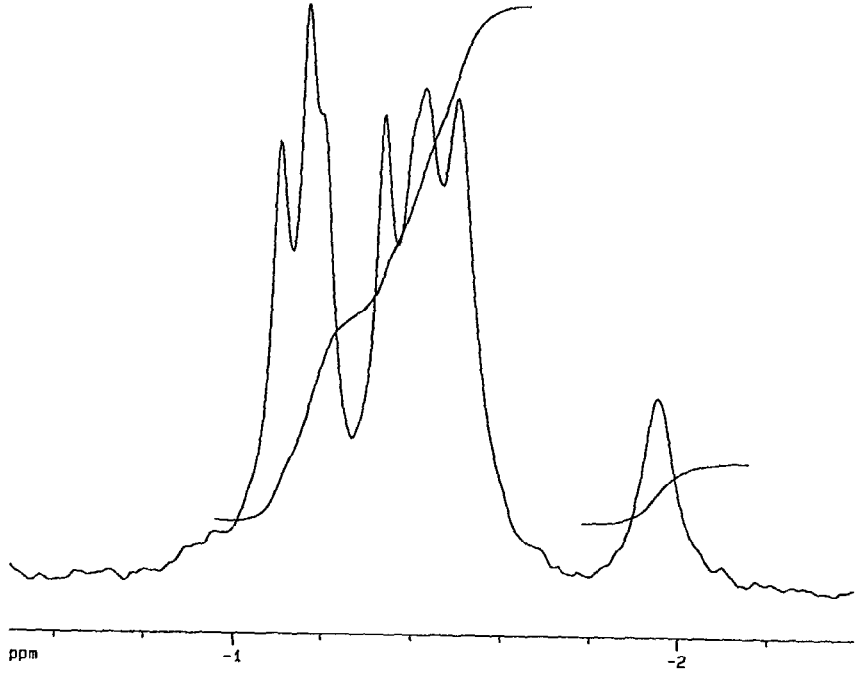
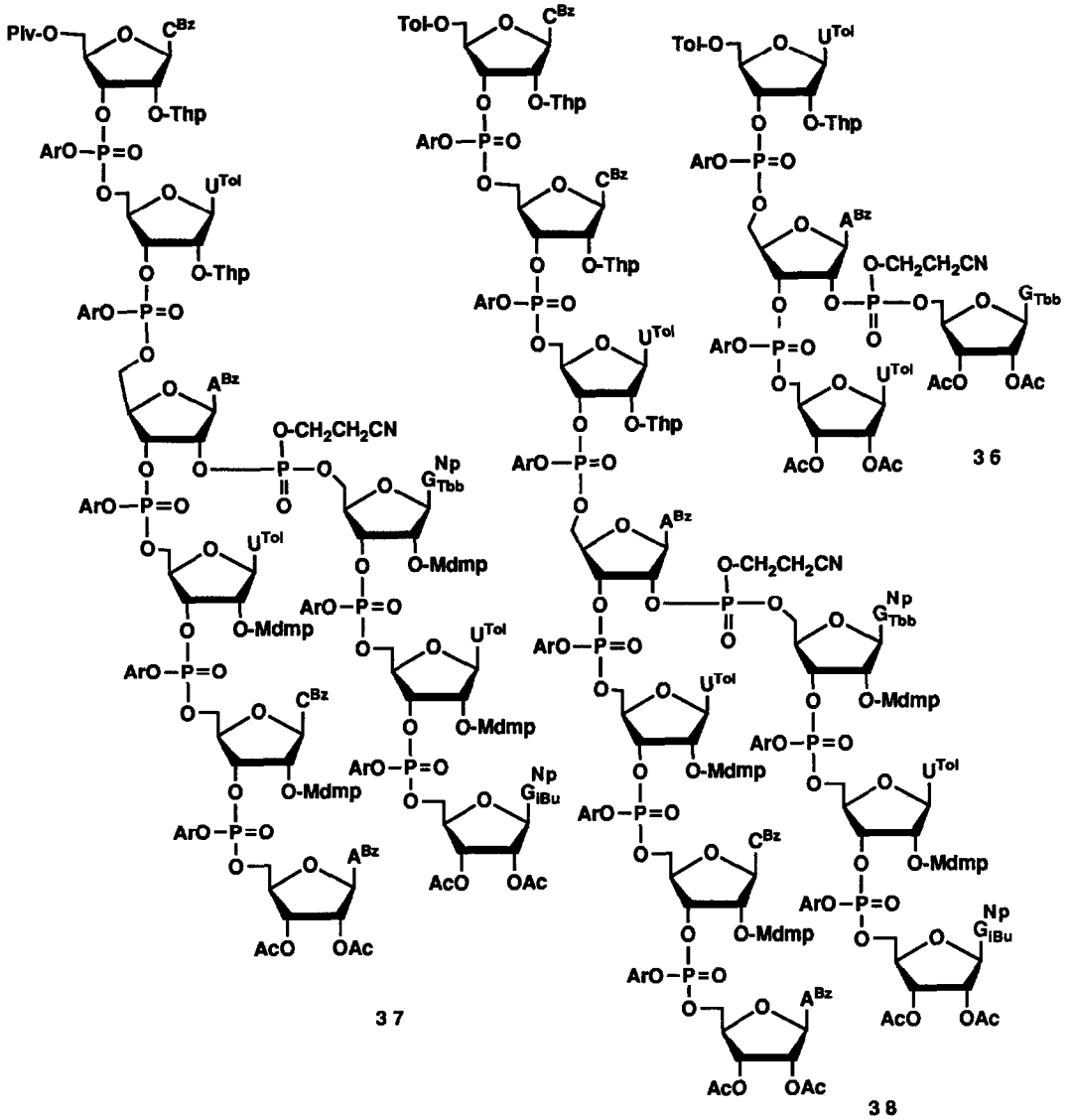


Fig. 15: 202.45 MHz ^{1}H-decoupled ^{31}P-NMR spectrum of Branched Deca-RNA **41** as its Na^{+} salt in $^{2}H_2O$ at 292 K.

also performed (Figs. 7 & 13). In the anomeric region, clear distinction between H1′ and the H5 of pyrimidine protons were made both by COSY 45 and HOHAHA spectra, and assignments are shown in the spectra (Figs. 6, 7, 12 & 13). The branch-point $A_{[1]}$ residue was easily assigned by the characteristic downfield shift of its H2' and H3' protons due to the presence of 2′→5′ and 3′→5′ phosphodiester residues which in turn allowed us to identify the nucleoside residues attached to the 2′- and 3′-end of the branch-point. The H1' of $A_{[1]}$ is the most downfield signal (6.03 ppm) of all anomeric protons. The 5'-terminal $C_{[-2]}$ sugar-residue in **40** and $C_{[-3]}$ sugar-



residue in **41** were identified by the absence of their coupling with phosphorus, and by the upfield shift of their H5'/ H5" protons in HOHAHA, which in turn allowed us to identify its full J-network in the HOHAHA spectrum. NOESY spectrum at 900 ms mixing time for both **40** (Fig. 8) and **41** (Fig. 14) were used to assign

the H1′ to its respective H6 in uridine/cytidine residues, or H1′ to its own H8 of adenosine or guanosine moieties unequivocally. ^{31}P-NMR of **40** at 202.45 MHz in D_2O showed (cAMP was used as the external reference at δ = -2.1 ppm) seven phosphate absorptions: δ = -1.939 [2′→5′-linked G[ii]], -1.492, -1.4445, -1.404, -1.666, -1.152, -1.048 (Fig. 9). Integration of the phosphate absorptions with respect to the resonance at δ = -1.939 for 2′→5′-linked G[ii] residue showed that each absorption integrates for one phosphate except for the resonance at δ = -1.404 which integrated for two overlapping phosphate resonances. Thus the spectroscopic evidence of the presence of nine pentoses, eight phosphates, two cytosines, three uracils, two adenines and two guanine residues in the branched nona-RNA **40** unambiguously corroborates its structure. ^{31}P-NMR of branched deca-RNA **41**, on the other hand, under identical measurement conditions showed (Fig. 15) eight phosphate absorptions: δ = -1.95 [2′→5′-linked G[ii]], -1.5, -1.426, -1.405, -1.335, -1.196, -1.164, -1.098. Integration of the phosphate absorptions with respect to the resonance at δ = -1.95 showed that each absorption integrates for one phosphate residue except for the resonance at δ = -1.164 which integrated for two phosphates due to the superimposition of signals. Thus the above spectroscopic evidence corroborate the presence of ten pentoses, nine phosphates, three cytosines, three uracils, two adenines and two guanine residues in the branched deca-RNA **41** which clearly establish the unequivocal proof substantiating its structural integrity. Work is now in progress in this laboratory to determine the solution structures of branched nona-RNA **40** and deca-RNA **41** by 500 and 600 MHz NMR spectroscopy.

EXPERIMENTAL

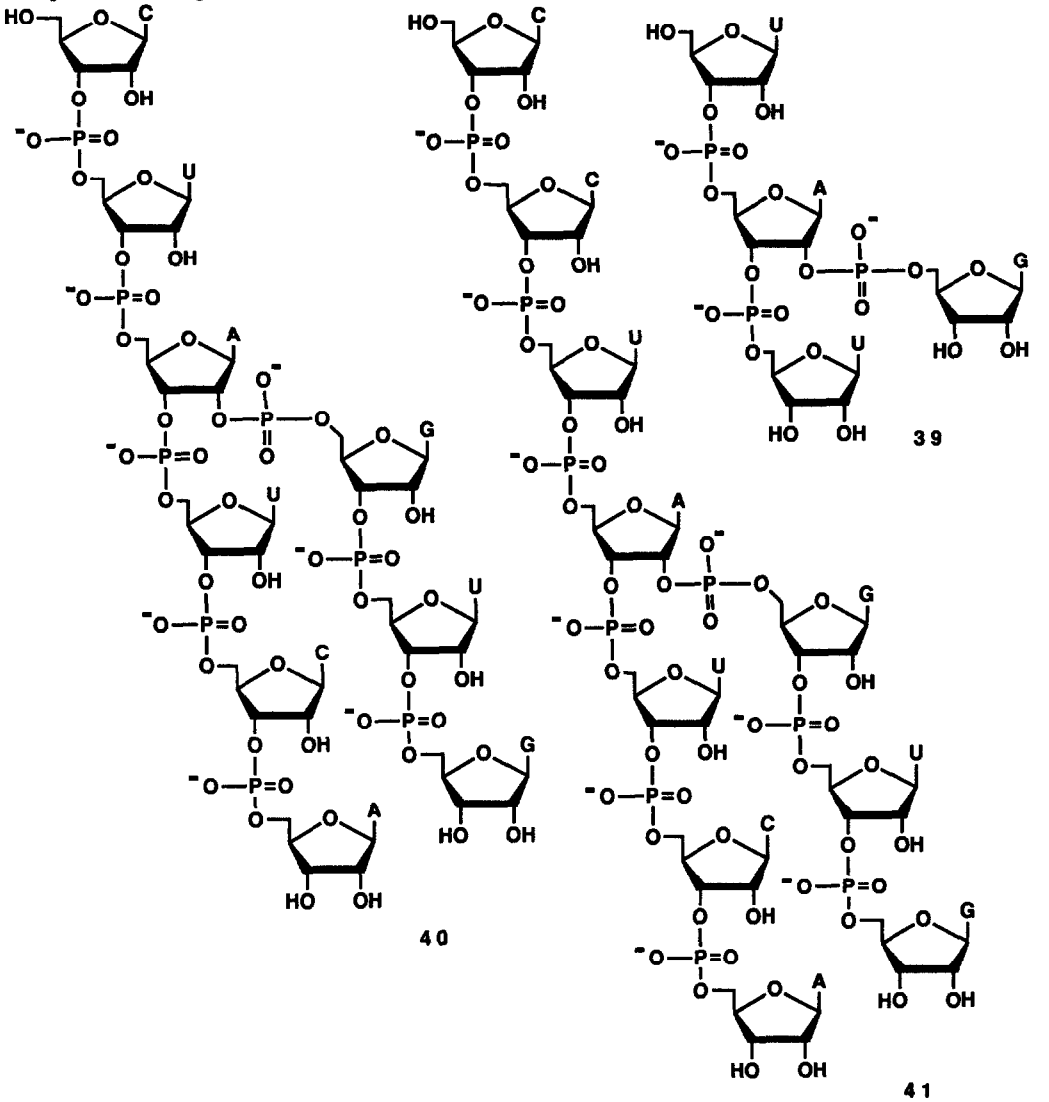
^{1}H-NMR spectra were recorded in δ scale with Jeol FX 90 Q and Bruker AMX-500 spectrometers at 90 and 500 MHz respectively, using TMS or H_2O (set at 4.7 ppm) as internal standards. ^{31}P-NMR spectra were recorded at 36 and 202 MHz in the same solvent using 85 % phosphoric acid or cAMP as external standard. TLC was carried out using pre-coated silica gel F_{254} plates in the following dichloromethane-methanol mixtures: (A) 98: 2 (v/v), (B) 95: 5 (v/v), (C) 90: 10 (v/v), (D) 80: 20 (v/v). Dry pyridine was obtained by distillations over CaH, p-TsCl, and KOH. Acetonitrile was distilled from P_2O_5 under argon. Dimethylformamide was distilled over CaH. The column chromatographic separations were carried out using Merck G 60 silica gel. DEAE-Sephadex A-25 from Pharmacia was used for the ion exchange chromatography. NH_4HCO_3 (BDH Chemicals Ltd Poole England) was used for the ion exchange buffers. 2′-O-Thp derivatives of all nucleosides used in this work were separated and used in a diastereomerically pure form which are designated as "Low R_f" or "High R_f" subsequently in the following experimental section.

Compound 4: 4-N-benzoyl-5′-O-pivaloyl-2′-O-tetrahydropyranylcytidine-3′-O-triethylammonium(2-chloro phenyl)phosphate[13] (High R_f) (2) (855,3 mg, 1.06 mmol) was condensed with 2′-O-tetrahydropyranyl-N-3-toluoyl-uridine[13,21] (Low R_f) **3** (431.3 mg, 0.965 mmol) in presence of 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole (MSNT) (941 mg, 3.18 mmol) in dry pyridine (6ml / mmol) for 40 min at room temperature. Aqueous ammonium bicarbonate work up (partition between concentrated ammonium bicarbonate solution and dichloromethane) followed by silica gel column chromatography (0-2 % EtOH / CH_2Cl_2) afforded **4.** Yield: 523 mg (48%); R_f: 0.61 (C); ^{1}H-NMR($CDCl_3$) (High R_f phosphotriester isomer): 8.88 (br, 1H) NH; 8.04-7.15 (m, 16H) arom., CH-5, CH-6& UH-6; 6.16 (d, $J_{1',2'}$= 4.64Hz, 1H) UH-1′; 6.02 (d, $J_{1',2'}$= 3.91Hz,1H) CH-1′; 5.74 (d, J = 8.06Hz, 1H); 5.10-4.20 (m, 12H) sugar protons + tetrahydropyranyl-; 4.02-3.34 (m, 4H) tetrahydropyranyl-; 2.40 (s, 3H) -COPhCH_3; 1.95-1.41 (m, 12H) tetrahydropyranyl-; 1.25 (s, 9H) *t*-butylCO-; ^{31}P-NMR($CDCl_3$): -7.01(High R_f phosphotriester isomer), -7.67 (Low R_f phosphotriester isomer) ppm.

Compound 5: Compound **4** (523 mg 0.46 mmol) in dry pyridine (4 ml) was treated with 0.2 M acetonitrile solution of o-chlorophenylphosphoro-bis-(1,2,4-triazolide) (3.46 ml, 0.69 mmol) for 40 min. Aqueous ammonium bicarbonate work up followed by silica gel column chromatography (2-6% EtOH / CH_2Cl_2) afforded **5.** Yield: 527 mg (80%); R_f: 0.69 (D); ^{1}H-NMR ($CDCl_3$) (High + Low R_f phosphotriester isomers): 8.77 (br, 1H) NH; 8.15-6.84 (m, 20H) arom., CH-5, CH-6 & UH-6; 6.09 (m, 2H) CH-1′, UH-1′; 5.78 (d, J = 8.30 Hz, 1H) UH-5 (Low R_f phosphotriester isomer); 5.73 (d, J = 8.06Hz, 1H) UH-5 (High R_f phosphotriester isomer); 5.10-4.16 (m, 12H) sugar protons + tetrahydropyranyl-; 4.01-3.32 (m, 4H) tetrahydropyranyl-; 2.98 (q, 6H) CH_2 of triethylammonium; 2.41 (s, 3H) -COPhCH_3; 1.95-1.41 (m, 12H) tetrahydropyranyl-; 1.24 (s, 9H) *t*-butylCO-; 1.23 (t, 9H) CH_3 of triethylammonium; ^{31}P-NMR ($CDCl_3$)(High + Low R_f phosphotriester isomers): -6.08, -6.17, -7.20, -7.52 ppm. **Compound 7:** N-4-benzoyl-5′-O-pixyl-2′-

O-tetrahydropyranylcytidine-3′-O-triethylammonium(2-chloro phenyl)phosphate[13] **6** (High R_f) (2.72 g, 2.78 mmol) was co-evaporated with dry pyridine then dissolved in of the same solvent (27 ml). Phenylsulfonylethanol (0.78 ml, 4.17 mmol) was added followed by addition of MSNT (2.06g, 6.95 mmol) and the mixture stirred for 45 min. Aqueous ammonium bicarbonate work up and column chromatography gave compound **7**. Yield: 2.65 g (91 %); R_f: 0.47 (B); ^{1}H-NMR ($CDCl_3$ + DABCO): 8.36 (m, 1H) H-6; 7.98-6.90 (m, 29H) arom. & H-5; 6.13 (d,d, 1H) H-1′; 5.02 (m, 2H) H-3′, H-2′; 4.78-4.20(m, 4H) H-4′, tetrahydropyranyl- & $PhSO_2CH_2CH_2O$-; 3.92-3.03 (m, 6H) H-5′, H-5″, tetrahydropyranyl-, $PhSO_2CH_2CH_2O$-; 1.54 (m, 6H) tetrahydropyranyl-; ^{31}P-NMR ($CDCl_3$ + DABCO): -7.47, -8.40 ppm.

Compound 8: Compound **7** (2.65 g, 2.53 mmol) was dissolved in chloroform (135 ml) and cooled to 0 °C.



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To this solution, 0.2M trichloroacetic acid in 4% EtOH / $CHCl_3$ (135 ml) was added and stirred for 3h at 0°C. Aqueous ammonium bicarbonate work up and silica gel column chromatography afforded the 5′-hydroxy triester **8**. Yield: 1.44 g (72 %); R_f: 0.44 (C); ($CDCl_3$): 8.40 (d, J = 7.16Hz, 1H) H-6; 7.94-7.04 (m, 16H) arom. & H-5; 5.90 (d,d, 1H) H-1′; 5.20-4.85 (m, 2H) H-3′, H-2′; 4.78-4.20 (m, 6H) H-4′, H-5′, H-5″, tetrahydropyranyl- & $PhSO_2CH_2CH_2O$-; 3.95 (m, 2H) , tetrahydropyranyl-; 3.52 (t, 2H) $PhSO_2CH_2CH_2O$-; 1.54 (m, 6H) tetrahydropyranyl-; ^{31}P-NMR ($CDCl_3$): -7.01, -7.32 ppm. **Compound 9:** After repeated co-

evaporation with dry pyridine, compound **8** (0.79 g, 1.0 mmol) was dissolved in the same solvent (10 ml) and 4-toluoyl chloride (0.17 ml, 1.3 mmol) was added, and the reaction solution was stirred for 3 h at room temperature. The reaction mixture was worked up in the usual way and the product was purified by short column chromatography. Yield: 0.87 g (96 %); R_f: 0.61 & 0.68 (C); ^{1}H-NMR ($CDCl_3$): 8.11-6.96 (m, 21H) arom. & H-6, H-5; 6.04 (d,d, 1H) H-1′; 5.06 (m, 2H) H-3′, H-2′; 4.78-4.28(m, 6H) H-4′, H-5′, H-5′′, tetrahydropyranyl- & $PhSO_2CH_2\mathit{CH_2}O$-; 3.84 (m, 1H) , tetrahydropyranyl-; 3.53 (m, 3H) $PhSO_2\mathit{CH_2}CH_2O$- & tetrahydropyranyl-; 2.40 (s, 3H) $COPhCH_3$; 1.54 (m, 6H) tetrahydropyranyl-; ^{31}P-NMR ($CDCl_3$): -7.40, -8.26 ppm. **Compound 10:** Compound **9** (0.87 g, 0.95 mmol) was dissolved in dry pyridine (10 ml) then dry triethylamine (2.65 ml, 19.12 mmol) was added and the mixture stirred for 45 min. Volatile materials were evaporated and the resulting foam was subjected to short column chromatography with 1% triethylamine in CH_2Cl_2 as co-eluent. The toluene co-evaporated residue was precipitated from cold hexane to give **10.** Yield: 0.76 g (91 %); R_f: 0.49 (D); ^{1}H-NMR ($CDCl_3$): 8.11 (d, J = 7.16Hz, 1H) H-6; 7.94-7.01 (m, 14H) arom. & H-5; 6.00 (s, 1H) H-1′; 5.17 (m, 1H) H-3′; 4.86-4.41(m, 5H) H-2′,H-4′, H-5′, H-5′′& tetrahydropyranyl-; 3.84 (m, 1H) , tetrahydropyranyl-; 3.49 (m, 1H) tetrahydropyranyl-; 2.84 (q, 6H) CH_2 of triethylammonium; 2.45 (s, 3H) $COPhCH_3$; 1.63 (m, 6H) tetrahydropyranyl-; 1.17 (t, 9H) CH_3 of triethylammonium; ^{31}P-NMR ($CDCl_3$): -5.86 ppm. **Compound 11:** Compound **10** (0.76 g, 0.91 mmol) and **8** (0.61 g, 0.77 mmol) were condensed together in presence of MSNT (0.81 g, 2.73 mmol) in dry pyridine (10ml) for 50 min. Usual work up and silica gel column chromatography gave **11**(0.99 g, 85 %); R_f: 0.64 (C); ^{1}H-NMR ($CDCl_3$): 8.21-6.96 (m, 32H) arom. & 2 x H-6, 2 x H-5; 6.11-5.88 (m, 2H) 2 x H-1′; 5.19-4.28 (m, 12H) sugar protons, tetrahydropyranyl- & $PhSO_2CH_2\mathit{CH_2}O$-; 3.83 (m, 1H) , tetrahydropyranyl-; 3.51 (m, 3H) $PhSO_2\mathit{CH_2}CH_2O$- & tetrahydropyranyl-; 2.42 (s, 3H) $COPhCH_3$; 1.54 (m, 12H) tetrahydropyranyl-; ^{31}P-NMR ($CDCl_3$): -5.91, -6.64, -7.49, -7.67, -7.81, -8.03, -8.11, -8.15 ppm. **Compound 12:** Compound **11** (0.98 g, 0.65 mmol) was treated with triethylamine (1.79 ml, 13.0 mmol) in pyridine (6.4 ml) for 45 min. Usual work up followed by short column chromatography to afford **12** (0.84 g, 89 %); R_f: 0.44 (D); ^{1}H-NMR ($CDCl_3$): 8.11-7.20 (m, 26H) arom. & 2 x H-6, 2 x H-5; 6.14-5.94 (m, 2H) 2 x H-1′; 5.19-4.32 (m, 12H) sugar protons & tetrahydropyranyl-; 3.87 (m, 1H) , tetrahydropyranyl-; 3.45 (m, 1H) tetrahydropyranyl-; 2.93 (q, 6H) CH_2 of triethylammonium; 2.42 (s, 3H) $COPhCH_3$; 1.54 (m, 12H) tetrahydropyranyl-; 1.21 (t, 9H) CH_3 of triethylammonium; ^{31}P-NMR ($CDCl_3$): -5.81, -5.98, -6.49, - 7.42 ppm. **Compound 14**: Compound **12** (0.84 g, 0.58 mmol) and **13** (low R_f) (0.56 g, 0.7 mmol) were condensed together in presence of MSNT (0.81 g, 2.73 mmol) in dry pyridine (6 ml) for 20 min. Usual work up and silica gel column chromatography afforded compound **14** (1.18 g, 95 %); R_f: 0.53 (C); ^{1}H-NMR ($CDCl_3$): 8.21-6.96 (m, 41H) arom. & 2 x CH-6, 2 x CH-5 & UH-6; 6.11-5.78 (m, 4H) 3 x H-1′ & UH-5; 5.19-4.28 (m, 20H) sugar protons, tetrahydropyranyl- & $PhSO_2CH_2\mathit{CH_2}O$-; 3.83 (m, 3H) , tetrahydropyranyl-; 3.51 (m, 6H) $PhSO_2\mathit{CH_2}CH_2O$- & tetrahydropyranyl-; 2.41 (s, 6H) 2 x $COPhCH_3$; 1.54 (m, 18H) tetrahydropyranyl-; ^{31}P-NMR ($CDCl_3$): -4.54,-4.78, [-6.69 to -8.91] ppm. **Compound 15**: Compound **14** (1.18 g, 0.55 mmol) was treated with dry triethylamine (1.53 ml, 11 mmol) in dry pyridine (5.5 ml) for 45 min. Usual work up and silica gel column chromatography afforded **15** (0.98 g, 85 %); R_f: 0.71 (D); ^{1}H-NMR ($CDCl_3$): 8.11-6.93 (m, 35H) arom. & 2 x CH-6, 2 x CH-5 & UH-6; 6.15-5.77 (m, 4H) 3 x H-1′ & UH-5; 5.19-4.28 (m, 18H) sugar protons & tetrahydropyranyl-; 3.87 (m, 3H) tetrahydropyranyl-; 3.45 (m, 3H) tetrahydropyranyl-; 2.94 (q, 6H) CH_2 of triethylammonium; 2.41 (s, 6H) $COPhCH_3$; 1.56 (m, 18H) tetrahydropyranyl-; 1.20 (t, 9H) CH_3 of triethylammonium; ^{31}P-NMR ($CDCl_3$): -5.00, -6.18, -6.40, -6.71, -7.25, -7.35, -7.47, -7.64, -8.13 ppm. **Compound 18** : 2′-O-(1,5-dicarbomethoxy-3-methoxypentan-3-yl)-5′-O-(4,4′-dimethoxytrityl)-3-N-toluoyl uridine-3′-O-triethylammonium (2-chlorophenyl) phosphate[22] **16** (229 mg, 2 mmol) was condensed with dimer **17** (173 mg, 1.49 mmol) in presence of MSNT (132 mg, 4.47 mmol) in the usual way. Silica gel column chromatography (0-3% EtOH, 0.25% pyridine / CH_2Cl_2) afforded 2.96 g (91%) of **18.** R_f: 0.56 (C); ^{1}H-NMR ($CDCl_3$): 9.29 (br, 1H) NH; 8.78 (s, 1H) AH-8; 8.26 (s, 1H) AH-2; 8.21-6.79 (m, 40H) arom. & CH-6, CH-5 & UH-6; 6.34-5.72 (m, 4H) 3 x H-1′& UH-5; 5.19-4.28 (m, 15H) sugar protons ; 3.68 (s, 6H) 2 x -OCH_3; 3.50 (m, 12H) 4 x -CO_2CH_3; 3.22, 2.96 (2 x s, 6H) 2 x -OCH_3; 2.38 (s, 3H) $COPhCH_3$; 2.13, 2.04 (2 x s, 6H) 2 x -$COCH_3$; ^{31}P-NMR ($CDCl_3$): -6.88, -7.15, -7.20, -7.32, -7.76, -7.86 ppm. **Compound 19:** The 5′-O-dimethoxytrityl group was removed from **18** according to a known procedure[17], using p-toluenesulfonic acid monohydrate as acid catalyst. Yield 1.34 g (52%); R_f: 0.53 (C); ^{1}H-NMR ($CDCl_3$): 9.30 (br, 1H) NH; 8.81 (s, 1H) AH-8; 8.25 (s, 1H) AH-2; 8.09-6.99 (m, 27H) arom. & CH-6, CH-5 & UH-6; 6.30 (d, $J_{1',2'}$= 4.88Hz, 1H) UH-1′; 6.06-5.73 (m, 3H) 2 x H-1′& UH-5; 5.25-4.35 (m, 15H) sugar protons ; 3.54 (m, 12H) 4 x -CO_2CH_3; 3.10, 2.98 (2 x s, 6H) 2 x -OCH_3; 2.40 (s, 3H) $COPhCH_3$; 2.16, 2.07 (2 x s, 6H) 2 x -$COCH_3$; ^{31}P-NMR ($CDCl_3$+CD_3OD): -6.93, -7.30, -7.71, -7.81 ppm. **Compound 22:** 5′-O-fluorenylmethoxycarbonyl-2′-O-(1,5-dicarbomethoxy-3-methoxypentan-3-yl)-2-N-(t-butylbenzoyl)-6-O-(2-nitrophenyl)guanosine-3′-O-triethylammonium(2-chloro phenyl)phosphate[23] **20** (544 mg, 0.43 mmol) was condensed with 2′-O-(1,5-dicarbomethoxy-3-methoxy-2-

pentenyl)-N-3-toluoyluridine[17] **21** (225 mg, 0.396 mmol) in presence of MSNT (382 mg, 1.29 mmol) in the usual way. Silica gel column chromatography (0-2% EtOH, 0.25% pyridine / CH_2Cl_2) afforded **19** (527 mg, 80%). R_f: 0.60 (C); ^{1}H-NMR ($CDCl_3$): 8.26, 8.21 (2 x s, 1H) GH-8; 7.89-7.21 (m, 23H) arom. & UH-6; 6.24 (m, 1H) UH-1′; 6.05 -5.72 (m, 2H) GH-1′& UH-5; 4.77-4.19 (m, 13H) sugar protons & fluorenylmethoxy- ; 3.61, 3.60, 3.59, 3.56, 3.53, 3.49 (7 x s, 12H) 4 x -CO_2CH_3; 3.19, 3.15 (2 x s, 3H) -OCH_3 (U_{Mdmp}); 2.85, 2.77 (2 x s, 3H) -OCH_3 (G_{Mdmp}); 2.39 (s, 3H) $COPhCH_3$; 1.27 (s, 9H) *t*-butylPhCO- ; ^{31}P-NMR ($CDCl_3$): -6.59, -7.42 ppm. **Compound 23:** Compound **22** (436 mg, 0.254 mmol) was treated with 0.2 M acetonitrile solution of o-chlorophenylphosphoro-bis-(1,2,4-triazolide) (1.91 ml, 0.381 mmol) in dry pyridine for 40 min. Usual work up and silica gel column chromatography afforded 448 mg (92%) of **20**. R_f: 0.71 (D); ^{1}H-NMR ($CDCl_3$): 8.20, 8.17 (2 x s, 1H) GH-8; 7.84-6.83 (m, 23H) arom. & UH-6; 6.26 (m, 1H) UH-1′; 6.11 -5.76 (m, 2H) GH-1′& UH-5; 5.36 (m, 1H) UH-3′; 4.96-4.11 (m, 12H) sugar protons & fluorenylmethoxy- ; 3.68, 3.58, 3.53, 3.47, 3.42 (4 x s, 12H) 4 x -CO_2CH_3; 3.17, 3.09, 3.04, 2.95 (4 x s, 6H) 2 x -OCH_3; 2.39 (s, 3H) $COPhCH_3$; 1.23 (s, 9H) *t*-butylPhCO-; ^{31}P-NMR ($CDCl_3$+CD_3OD): -6.69, -7.13, -7.81 ppm. **Compound 24:** Compound **23** (380 mg, 0.198 mmol) was condensed with 2′,3′-di-O-acetyl-N-2-isobutyryl-6-O-(2-nitrophenyl)guanosine **26** (100 mg, 0.180 mmol) in presence of MSNT (176 mg, 0.594 mmol) in the usual way. After normal column separation **24** (360 mg, 81%) was collected. R_f: 0.57 (C); ^{1}H-NMR ($CDCl_3$): 8.62-7.21 (m, 41H) arom. & 2 x GH-8 & UH-6; 6.43-5.68 (m, 6H) anomeric protons, $G_{3énd}$H-2′, $G_{3énd}$H-3′& UH-5; 5.38 (m, 1H) UH-3′; 5.08-4.15 (m, 16H) sugar protons & fluorenylmethoxy- ; 3.67, 3.61, 3.57, 3.55, 3.54, 3.50, 3.47 (6 x s, 12H) 4 x -CO_2CH_3; 3.17, 3.12, 3.05, 2.92 (4 x s, 3H) -OCH_3 (U_{Mdmp}); 2.74 (m, 3H) -OCH_3 (G_{Mdmp}); 2.41 (s, 3H) $COPhCH_3$; 2.07 (m, 6H) 2 x -$COCH_3$; 1.23 (s, 9H) *t*-butylPhCO-; 0.96 (m, 6H) isobutylCO-; ^{31}P-NMR ($CDCl_3$): -6.13, -6.66, -7.30, -7.49, -7.64, -8.13, -8.25 ppm. **Compound 25:** Compound **24** (360 mg, 0.146 mmol) was treated with dry triethylamine (406 ml, 2.92 mmol) in dry pyridine (1.35 ml) and stirred for 1h at room temperature. The reaction mixture was evaporated and co-evaporated with toluene. Silica gel column chromatography (0.5-4% EtOH / CH_2Cl_2) afforded 285 mg (87%) of **25**. R_f: 0.48, 0.45 (C); ^{1}H-NMR ($CDCl_3$): 8.46-7.07 (m, 33H) arom. & 2 x GH-8 & UH-6; 6.22-5.56 (m, 6H) anomeric protons, $G_{3énd}$H-2′, $G_{3énd}$H-3′& UH-5; 5.40-4.34 (m, 13H) sugar protons ; 3.57(m, 12H) 4 x -CO_2CH_3; 3.18, 3.14, 3.07, 2.88 (4 x s, 3H) -OCH_3 (U_{Mdmp}); 2.69, 2.63 (2 x s, 3H) -OCH_3 (G_{Mdmp}); 2.42 (s, 3H) $COPhCH_3$; 2.09 (m, 6H) 2 x -$COCH_3$; 1.31 (s, 9H) *t*-butylPhCO-; 0.96 (m, 6H) isobutylCO-; ^{31}P-NMR ($CDCl_3$): -6.49, -6.81, -7.30, -7.45, -8.03, -8.15, -8.47 ppm. **Synthesis of compounds 30a, 31a and 32a.** *A general procedure:* A mixture of the 3′-phosphodiester block **1, 5** or **15** (0.234 -0.369 mmol, 1 equiv) and 6-N-benzoyl-2′-O-pixyladenosine[11] **29** (0.95 eq.) was co-evaporated with dry pyridine and redissolved in dry pyridine (6ml / mmol). (MSNT) (3 equiv) was added and the reaction mixture was stirred for 40 min at room temperature. The mixture was poured into concentrated ammonium bicarbonate solution and extracted with dichloromethane three times. The pyridine- free gum obtained after toluene co-evaporation of the organic residue was then purified by short silica gel column chromatography. The silica gel was prewashed with 1% Et_3N / CH_2Cl_2, followed by wash with pure CH_2Cl_2 and then EtOH-CH_2Cl_2 gradients were used as mobile phases (0-2% EtOH, 0.25% pyridine) to give the 3′-hydroxy blocks **30a, 31a** or **32a** as white powders after co-evaporation with toluene and cyclohexane. **Compound 30a**: Yield 197 mg (65 %); R_f: 0.70 (C); ^{1}H-NMR ($CDCl_3$+2,6-lutidine): 9.01 (br, 1H) NH; 8.62, 8.59 (2 x s, 1H) AH-8; 8.10-6.45 (m, 27H) arom., AH-2 & UH-6; 6.08-5.87 (m, 2H) AH-1′, UH-1′; 5.62 (d, J = 8.06Hz, 1H) UH-5; 4.81 (m, 1H) AH-2′; 5.14 (m, 1H) UH-3′; 4.66 (m, 1H) tetrahydropyranyl-; 4.60-4.20 (m, 8H) UH-2′,-4′,-5′, -5′′, AH-2′,-4′, -5′, -5′′; 3.50 (m, 2H) tetrahydropyranyl-; 3.33 (m,1H) AH-3′; 2.40 (s, 6H) 2 x -$COPhCH_3$; 1.54 (m, 6H) tetrahydropyranyl-; ^{31}P-NMR ($CDCl_3$+2,6-lutidine): -7.47, -7.98 ppm. **Compound 31a:** Yield 427 mg (63 %); R_f: 0.61 (C); ^{1}H-NMR ($CDCl_3$+2,6-lutidine): 9.07 (br, 1H) NH; 8.59, 8.54 (2 x s, 1H) AH-8; 8.10-6.28 (m, 39H) arom., AH-2, CH-5, CH-6 & UH-5; 6.11-5.68 (m, 4H) anomeric protons & UH-5; 5.03-4.19 (m, 17H) sugar protons + tetrahydropyranyl-; 3.79-3.47 (m, 4H) tetrahydropyranyl-; 3.34 (m, 1H) AH-3′; 2.40 (s, 3H) -$COPhCH_3$; 1,47 (m, 12H) tetrahydropyranyl-; 1.23 (s, 9H) *t*-butylCO-; ^{31}P-NMR ($CDCl_3$+2,6-lutidine): [-7.39 to -8.11] ppm. **Compound 32a:** Yield 369 mg (70 %); R_f: 0.74 (C); ^{1}H-NMR ($CDCl_3$+2,6-lutidine): 9.09 (br, 1H) NH; 8.54 (s, 1H) AH-8; 6.14-5.76 (m, 5H) anomeric protons + UH-5; 3.79-3.40 (m, 6H) tetrahydropyranyl-; 3.32 (m, 1H) AH-3′; 2.40 (s, 6H) 2 x -$COPhCH_3$; 1,46 (m, 18H) tetrahydropyranyl-; ^{31}P-NMR ($CDCl_3$+2,6-lutidine): -4.56, -4.91, [-6.71 to -9.01] ppm. **Synthesis of compounds 30b, 31b and 32b.** *A general procedure:* A 0.2 M acetonitrile solution of o-chlorophenylphosphoro-bis-(1,2,4-triazolide) (3 equiv) was added to a solution of **30a, 31a** or **32a,** (1 equiv) in dry pyridine (10ml / mmol). The reaction mixture was stirred at room temperature for 40 min and after normal aqueous ammonium bicarbonate work up, the organic residue was purified by short silica gel column chromatography. The silica gel was prewashed with 1 % Et_3N / CH_2Cl_2, followed by wash with pure CH_2Cl_2 and then EtOH-CH_2Cl_2 gradients were used as mobile phases (2-6 % EtOH, 1 % pyridine) to give the 3′-phosphodiester blocks **30b, 31b** or **32b** as white powders after co-evaporation with toluene and cyclohexane. **Compound 30b**: Yield 219 mg (92 %); R_f: 0.78 (C); ^{1}H-

NMR ($CDCl_3$+2,6-lutidine): 9.05 (br, 1H) NH; 8.61, 8.58 (2 x s, 1H) AH-8; 8.11-6.31 (m, 31H) arom., AH-2 & UH-6; 6.23-5.91 (m, 2H) AH-1′, UH-1′; 5.59 (d, J = 8.06Hz, 1H) UH-5; 5.05 (m, 2H) AH-3′, UH-3′; 4.79-4.19 (m, 9H) UH-2′,-4′,-5′, -5′′& AH-2′,-4′, -5′, -5′′& tetrahydropyranyl-; 3.49 (m, 2H) tetrahydropyranyl-; 3.01 (q, J = 7.57Hz, 6H) CH_2 of triethyl ammonium; 2.40 (s, 6H) 2 x -$COPhCH_3$; 1.52 (m, 6H) tetrahydropyranyl-; 1.29 (t, J = 7.57Hz, 9H) CH_3 of triethyl ammonium; ^{31}P-NMR ($CDCl_3$+2,6-lutidine): -6.15, -6.20, -7.64, -7.84 ppm. **Compound 31b:** Yield 409 mg (83 %); R_f: 0.38 (C); ^{1}H-NMR($CDCl_3$ + 2,6-lutidine): 9.09 (br, 1H) NH; 8.61, 8.59, 8.55, 8.53 (4 x s, 1H) AH-8; 5.73 (d, J = 8.55Hz, 1H) UH-5; 3.92-3.32 (m, 4H) tetrahydropyranyl-; 2.99 (q, 6H) CH_2 of triethylammonium; 2.41 (s, 3H) -$COPhCH_3$; 1.47 (m, 12H) tetrahydropyranyl-; 1.22 (s, 9H) *t*-butylCO-; 1.24 (t, 9H) CH_3 of triethylammonium; ^{31}P-NMR ($CDCl_3$+2,6-lutidine): -6.17, -6.25, -6.32, [-7.42 to -7.84] ppm. **Compound 32b:** Yield 330 mg (80 %); R_f: 0.36, 0.31 (C); ^{1}H-NMR ($CDCl_3$+2,6-lutidine): 9.09 (br, 1H) NH; 8.55 (m, 1H) AH-8; 6.18-5.78 (m, 5H) anomeric protons + UH-5; 3.90-3.30 (m, 6H) tetrahydropyranyl-; 2.98 (q, 6H) CH_2 of triethylammonium; 2.40 (s, 6H) 2 x -$COPhCH_3$; 1,46 (m, 18H) tetrahydropyranyl-; 1.24 (t, 9H) CH_3 of triethylammonium; ^{31}P-NMR ($CDCl_3$+2,6-lutidine): -4.96, -5.20, [-6.37 to -8.54] ppm. **Synthesis of compounds 30c, 31c and 32c.** *A general procedure:* The 3′-phosphodiester block **30b, 31b** or **32b**, (1 equiv), was dissolved in 2 % EtOH-CH_2Cl_2 and chilled to 0 °C in an ice bath. Trichloroacetic acid (TCA) (10 equiv) was dissolved in the second half of the 2 % EtOH-CH_2Cl_2 solution and chilled to 0 °C prior to pouring it into the diester solution. The final concentration of TCA was 0.055 M. In the case of **30b** the depixylation was finished within 8 min. In the cases of **31b** and **32b** the reaction time was 45-60 min as followed by TLC. After complete reaction the solution was poured into 0.2 M ammonium bicarbonate solution which was saturated with ammonium chloride and slightly acidified with dry ice and the aqueous phase (pH ~6.5) was extracted with dichloromethane three times. The organic phase was dried in magnesium sulfate and filtered and evaporated. The residue was dissolved in a small amount of dichloromethane and pipetted into a diethylether-hexane solution (100 ml) (2:1 v/v). The precipitate was centrifuged, the supernatant was decanted and the white pixyl free solid was dried *in vacuo* . **Compound 30c:** Yield 140 mg (74 %); R_f: 0.45 (D); ^{1}H-NMR ($CDCl_3$+CD_3OD): 8.70 (br, 1H) AH-8; 8.31-6.70 (m, 18H) arom., AH-2 & UH-6; 6.16-5.94 (m, 2H) AH-1′, UH-1′; 5.60 (d, J = 8.06Hz, 1H) UH-5; 5.10 (m, 2H) AH-3′, UH-3′; 4.64-4.29 (m, 9H) UH-2′,-4′,-5′, -5′′& AH-2′,-4′, -5′, -5′′& tetrahydropyranyl-; 3.50 (m, 2H) tetrahydropyranyl-; 3.08 (q, 6H) CH_2 of triethyl ammonium; 2.38 (s, 6H) 2 x -$COPhCH_3$; 1.48 (m, 6H) tetrahydropyranyl-; 1.31 (t, 9H) CH_3 of triethyl ammonium; ^{31}P-NMR ($CDCl_3$+CD_3OD): -7.91 ppm. **Compound 31c:** Yield 318 mg (86 %); R_f: 0.53 (D); ^{1}H-NMR($CDCl_3$+CD_3OD): 8.67 (br, 1H) AH-8; 6.08 (m, 3H) anomeric protons; 5.72 (d, J = 8.05Hz, 1H) UH-5; 3.92-3.32 (m, 4H) tetrahydropyranyl-; 3.10 (q, 6H) CH_2 of triethylammonium; 2.39 (s, 3H) -$COPhCH_3$; 1.47 (m, 12H) tetrahydropyranyl-; 1.22 (s, 9H) *t*-butylCO; 1.24 (t, 9H) CH_3 of triethylammonium; ^{31}P-NMR ($CDCl_3$+CD_3OD): -7.39, -7.64, -7.81, -7.93,- 8.08 ppm. **Compound 32c:** Yield 251 mg (85 %); R_f: 0.71 (D); ^{1}H-NMR ($CDCl_3$+CD_3OD): 8.67 (s, 1H) AH-8; 8.25 (m, 1H) AH-2; 6.21-5.70 (m, 5H) anomeric protons + UH-5; 3.90-3.30 (m, 6H) tetrahydropyranyl-; 2.37 (s, 6H) 2 x -$COPhCH_3$; 1,44 (m, 18H) tetrahydropyranyl-; ^{31}P-NMR ($CDCl_3$+CD_3OD): -5.49, -5.78, -6.25, [-6.71 to -8.28] ppm. **Synthesis of compounds 30d, 31d and 32d.** *A general procedure:* Bis(2-cyanoethoxy)- (diisopropylamino)phosphine[19] (10 equiv) was weighed into a dry 25 ml round bottomed flask and dry 15 % dimethylformamide / acetonitrile solution was added (corresponding to ~1ml / 0.9 mmol tetrazole) under argon (argon balloon). Then dry sublimed tetrazole (30 equiv) was added under stirring and rapidly went into solution followed by a quick formation of a precipitate. After 3 min stirring solid 2′-hydroxy-3′-phosphodiester block **30c, 31c** or **32c** was added to the colorless suspension and the clear reaction solution was then stirred for 40 min at room temperature under argon. A solution of 0.1 M I_2 / tetrahydrofuran / pyridine / H_2O (7:2:1 v/v/v) (10.5 equiv of I_2) was added and the reaction solution was stirred for 15 min and was then poured into 0.1 M sodium thiosulfate / concentrated ammonium bicarbonate solution and extracted three times with dichloromethane. The pyridine free gum obtained after toluene co-evaporation of the organic residue was then purified by short silica gel column chromatography. EtOH-CH_2Cl_2 gradients were used as mobile phases (2-9 % EtOH, with 1 % increments) to finally give the 2′-phosphodiester-3′- biscyanoethoxyphosphotriester blocks **30d, 31d** or **32d** as white powders after co-evaporation with toluene and cyclohexane. **Compound (30d):** Yield 68 mg (46 %); R_f: 0.51 (D); ^{1}H-NMR ($CDCl_3$+CD_3OD): 8.70 (s, 1H) AH-8; 8.32, 8.27 (2 x s, 1H) AH-2; 8.10-6.94 (m, 17H) arom., & UH-6; 6.31 (s, 1H) A*H-1′; 5.98 (m, 1H) UH-1′; 5.65 (m, 1H) AH-2′; 5.62 (d, J = 8.05Hz, 1H) UH-5; 5.23 (m, 2H) AH-3′, UH-3′; 4.62-4.19 (m, 12H) UH-2′,-4′,-5′, -5′′& AH-4′, -5′, -5′′& tetrahydropyranyl-& 2 x -OCH_2CH_2CN; 3.47 (m, 2H) tetrahydropyranyl-; 2.79 (m, 4H) 2 x -OCH_2CH_2CN; 2.38 (s, 6H) 2 x -$COPhCH_3$; 1.46 (m, 6H) tetrahydropyranyl-; ^{31}P-NMR ($CDCl_3$+CD_3OD): -4.88, -8.11 ppm. **Compound (31d):** Yield 214 mg (64 %); R_f: 0.56 (D); ^{1}H-NMR($CDCl_3$+CD_3OD): 8.68 (s, 1H) AH-8; 8.28 (s, 1H) AH-2; 6.34 (s, 1H) A*H-1′; 6.05 (m, 2H) CH-1′, UH-1′; 5.74 (d, J = 8.06Hz, 1H) UH-5; 3.80-3.26 (m, 4H) tetrahydropyranyl-; 2.79 (t, 4H) 2 x -OCH_2CH_2CN; 2.41 (s, 3H) -$COPhCH_3$; 1.47 (m, 12H)

tetrahydropyranyl-; 1.23 (s, 9H) *t*-butylCO-; ^{31}P-NMR ($CDCl_3$+CD_3OD): -5.32, -5.52, [-7.57 to -8.23] ppm. **Compound 32d:** Yield 160 mg (62 %); R_f: 0.74 (D); ^{1}H-NMR ($CDCl_3$+CD_3OD): 8.66 (s, 1H) AH-8; 8.30 (s, 1H) AH-2; 6.33 (s, 1H) A*H-1´; 6.06-5.73 (m, 5H) anomeric protons + UH-5; 3.90-3.30 (m, 6H) tetrahydropyranyl-; 2.79 (t, 4H) 2 x -OCH_2CH_2CN; 2.40 (s, 6H) 2 x -$COPhCH_3$; 1,44 (m, 18H) tetrahydropyranyl-; ^{31}P-NMR ($CDCl_3$+CD_3OD): [-5.05 to -5.98], [-6.98 to -8.40] ppm. **Synthesis of compounds 33a, 34a** and **35a.** *A general procedure:* A mixture of [**30d** (1 equiv)+**28** (1.5 equiv)], [**31d** (1 equiv)+**19** (1.5 equiv)] or [**32d** (1 equiv)+**19** (1.5 equiv)] was co-evaporated with dry pyridine and redissolved in dry pyridine (6ml / mmol). (MSNT) (7 equiv) was added and the reaction mixture was stirred for 6 h at room temperature. Aqueous ammonium bicarbonate work up followed by silica gel column chromatography of the organic residue using 0-5 % EtOH-CH_2Cl_2 (1 % increments) gradients as mobile phases gave the fully protected oligomers **33a, 34a** or **35a** as white powders after co-evaporation with toluene and cyclohexane. **Compound 33a:** Yield 51 mg (59 %); R_f: 0.63 (C); ^{1}H-NMR ($CDCl_3$): 9.11 (br, 1H) NH; 8.78 (s, 1H) AH-8; 8.49-7.15 (m, 28H) arom., AH-2 & 2 x UH-6; 6.39 (s, 1H) A*H-1´; 6.14-5.57 (m, 4H) 2 x UH-1´, 2 x UH-5; 4.25 (m, 4H) 2 x -OCH_2CH_2CN 3.52 (m, 2H) tetrahydropyranyl-; 2.67 (t, 4H) 2 x -OCH_2CH_2CN; ; 2.41 (s, 9H) 3 x -$COPhCH_3$; 2.05, 2.03 (2 x s, 6H) 2 x -$COCH_3$; 1.46 (m, 6H) tetrahydropyranyl-; ^{31}P-NMR ($CDCl_3$): -2.88, -3.42, -6.07, -7.49, -7.91, -8.08 ppm. **Compound 34a:** Yield 273 mg (69 %); R_f: 0.54 (C); ^{1}H-NMR($CDCl_3$): 8.79, 8.73, 8.69 (3 x s, 2H) 2 x AH-8; 6.45 (m, 1H) A*H-1´; 4.22 (m, 4H) 2 x -OCH_2CH_2CN; 3.51 (m, 12H) 4 x -CO_2CH_3; 3.13, 3.09, 2.97 (3 x s, 6H) 2 x -OCH_3; 2.63 (m, 4H) 2 x -OCH_2CH_2CN; 2.39 (s, 6H) 2 x -$COPhCH_3$; 2.13, 2.04 (2 x s, 6H) 2 x -$COCH_3$; 1.42 (m, 12H) tetrahydropyranyl-; 1.23 (s, 9H) *t*-butylCO-; ^{31}P-NMR ($CDCl_3$): -3.32, -3.30, -3.37, -3.54, -3.69, [-6.29 to -8.28] ppm. **Compound 35a:** Yield 150 mg (56 %); R_f: 0.44 (C); ^{1}H-NMR ($CDCl_3$): 9.29 (br, 5H) NH; 8.83, 8.77, 8.67 (3 x s, 2H) 2 x AH-8; 8.34 (m, 2H) 2 x AH-2; 6.30 (m, 1H) A*H-1´; 6.06-5.67 (m, 8H) anomeric protons + 2 x UH-5; 4.22 (m, 4H) 2 x -OCH_2CH_2CN; 3.55 (m, 12H) 4 x -CO_2CH_3; 3.11, 2.98 (2 x s, 6H) 2 x -OCH_3; 2.63 (m, 4H) 2 x -OCH_2CH_2CN; 2.40 (s, 9H) 3 x -$COPhCH_3$; 2.16, 2.13, 2.08, 2.04 (4 x s, 6H) 2 x -$COCH_3$; 1,44 (m, 18H) tetrahydropyranyl-; ^{31}P-NMR ($CDCl_3$): -3.08, -3.17, -3.44, [-6.98 to -8.40] ppm. **Synthesis of compounds 33b, 34b** and **35b.** *A general procedure.* The fully protected oligomer block **33a, 34a** or **35a** was co-evaporated with dry pyridine and redissolved in dry pyridine. The solution was warmed to 40 °C in an oil bath and then dry diisopropylethylamine (25 equiv) was added and the solution (30 % amine in pyridine) was stirred for 2h. The reaction solution was then evaporated and co-evaportated with toluene. The organic residue was purified by short silica gel column chromatography using EtOH-CH_2Cl_2 gradients as mobile phases (2-9 % EtOH, 1 % increments) to give the diester blocks **33b, 34b** or **35b** as white powders after co-evaporation with toluene and cyclohexane. **Compound (33b):** Yield 34 mg (64 %); R_f: 0.52, 0.46 (D); ^{1}H-NMR ($CDCl_3$): 9.13 (br, 1H) NH; 8.75 (s, 1H) AH-8; 8.43-7.06 (m, 28H) arom., AH-2 & 2 x UH-6; 6.39 (m, 1H) A*H-1´; 3.81 (m, 2H) -OCH_2CH_2CN 3.59 (m, 2H) tetrahydropyranyl-; 2.92 (q, 6H) CH_2 of triethyl ammonium; 2.54 (t, 2H) -OCH_2CH_2CN; 2.41 (s, 9H) 3 x -$COPhCH_3$; 2.09, 2.07, 2.04, 2.00 (4 x s, 6H) 2 x -$COCH_3$; 1.50 (m, 6H) tetrahydropyranyl-;1.18 (t, 9H) CH_3 of triethyl ammonium; ^{31}P-NMR ($CDCl_3$): -1.76, -2.25, -2.31, -6.25, [-7.49 to -7.86] ppm. **Compound (34b):** Yield 182 mg (67 %); R_f: 0.52, 0.44 (C); ^{1}H-NMR($CDCl_3$+CD_3OD): 8.76, 8.62 (2 x s, 2H) 2 x AH-8; 6.31 (m, 1H) A*H-1´; 4.25 (m, 2H) -OCH_2CH_2CN; 3.53 (m, 12H) 4 x -CO_2CH_3; 3.13, 3.08, 2.93 (3 x s, 6H) 2 x -OCH_3; 2.63 (m, 2H) -OCH_2CH_2CN; 2.39 (s, 6H) 2 x -$COPhCH_3$; 2.13, 2.04 (2 x s, 6H) 2 x -$COCH_3$; 1.42 (m, 12H) tetrahydropyranyl-; 1.22 (s, 9H) *t*-butylCO-; ^{31}P-NMR ($CDCl_3$+CD_3OD): -2.59, -2.98 [-7.10 to -8.06] ppm. **Compound (35b):** Yield 105 mg (68 %); R_f: 0.37, 0.30 (C); ^{1}H-NMR ($CDCl_3$+CD_3OD): 8.77, 8.62 (2 x s, 2H) 2 x AH-8; 8.33 (m, 2H) 2 x AH-2; 6.30 (m, 1H) A*H-1´; 6.10-5.67 (m, 8H) anomeric protons + 2 x UH-5; 4.30 (m, 2H) -OCH_2CH_2CN; 3.52 (m, 12H) 4 x -CO_2CH_3; 3.13, 3.07, 2.93 (3 x s, 6H) 2 x -OCH_3; 2.63 (m, 2H) -OCH_2CH_2CN; 2.39 (s, 9H) 3 x -$COPhCH_3$; 2.12, 2.03 (2 x s, 6H) 2 x -$COCH_3$; 1,44 (m, 18H) tetrahydropyranyl-; ^{31}P-NMR ($CDCl_3$+CD_3OD): -2.42, -2.91, [-6.03 to -7.91] ppm. **Synthesis of compounds 36, 37** and **38.** *A general procedure.* A mixture of [**33b** (1 equiv) + **27** (1.5 equiv)], [**34b** (1 equiv) + **25** (1.5 equiv)] or [**35b** (1 equiv) + **25** (1.5 equiv)] was co-evaporated with dry pyridine and redissolved in dry pyridine (6ml / mmol). (MSNT) (7 equiv) was added and the reaction mixture was stirred for 6 h at room temperature. Aqueous ammonium bicarbonate work up followed by silica gel column chromatography of the organic residue using 0-5 % EtOH-CH_2Cl_2 (1 % increments) gradients as mobile phases gave the fully protected oligomers **36, 37** or **38** as white powders after co-evaporation with toluene and cyclohexane. **Compound 36:** Yield 28 mg (69 %); R_f: 0.50 (C); ^{1}H-NMR ($CDCl_3$): 9.20 (br, 1H) NH; 8.71-7.13 (m, 17H) arom., AH-8, AH-2, GH-8 & 2 x UH-6; 6.36-5.54 (m, 6H) anomeric protons+2 x UH-5; 3.50 (m, 2H) tetrahydropyranyl-; 2.71 (m, 2H) -OCH_2CH_2CN; 2.40 (s, 9H) 3 x -$COPhCH_3$; 2.14, 2.06, 2.05, 2.02 (4 x s, 12H) 4 x -$COCH_3$; 1.52 (m, 6H) tetrahydropyranyl-; 1.31 (s, 9H)*t*-butylPhCO-; ^{31}P-NMR ($CDCl_3$): [-3.25 to -3.76, (m)], [-6.52 to -8.20] ppm. **Compound 37:** Yield 190 mg (67 %); R_f: 0.56 (C); ^{1}H-

NMR($CDCl_3$): 8.78, (s, 2H) 2 x AH-8; 6.31 (m, 1H) A*H-1´; 4.25 (m, 2H) -OCH_2CH_2CN; 3.50 (m, 24H) 8 x -CO_2CH_3; 3.05 (m, 12H) 4 x -OCH_3; 2.61 (m, 2H) -OCH_2CH_2CN; 2.38 (s, 9H) 3 x -COPhCH_3; 2.12, 2.09, 2.05, 2.03 (4 x s, 12H) 4 x -$COCH_3$; 1.42 (m, 12H) tetrahydropyranyl-; 1.23 (s, 9H) *t*-butylCO-; 1.21 (s, 9H) t-butylPhCO-;1.10-0.85 (m, 6H) isobutylCO-; ^{31}P-NMR ($CDCl_3$): -2.37, -2.44, -3.17, [-6.57 to -8.28] ppm. **Compound 38:** Yield 89 mg (63 %); R_f: 0.35 (C); ^{1}H-NMR ($CDCl_3$+CD_3OD): 8.77 (s, 1H) AH-8; 8.63 (m, 2H) AH-8; 8.35 (m, 2H) 2 x AH-2; 6.30 (m, 1H) A*H-1´; 6.10-5.67 (m, 12H) anomeric protons + 3 x UH-5; 4.25 (m, 2H) -OCH_2CH_2CN; 3.51 (m, 24H) 8 x -CO_2CH_3; 3.05 (m, 12H) 4 x -OCH_3; 2.67 (m, 2H) -OCH_2CH_2 CN; 2.40 (s, 12H) 4 x -COPhCH_3; 2.14, 2.04 (2 x s, 12H) 4 x -$COCH_3$; 1.24, 1.22 (2 x s, 9H) *t*-butylPhCO-; 1,44 (m, 18H) tetrahydropyranyl-; 0.96 (m, 6H) isobutylCO-; ^{31}P-NMR ($CDCl_3$+CD_3OD): -2.34, -3.17, [-6.42 to -8.39] ppm. **Deprotection of 36 to 39:** Compound **36** (25.6 mg, 10.6 μmol) was dissolved in dioxane : water (8:2 v/v) (3.5 ml) solution. To the solution *syn*-4-nitrobenzaldoxime (56.8 mg, 0.34 mmol) and 1,1,3,3-tetramethylguanidine (38.5 μl, 0.308 mmol) were added. After 48 h stirring at room temperature the solvents were removed by evaporation *in vacuo* and concentrated ammonia (20 ml, d = 0.9) was added. The reaction mixture was stirred for 7 days at room temperature and was then evaporated and co-evaporated with distilled water. The residue was treated with 80 % aqeous acetic acid (20 ml) for 24 h at room temperature. After evaporation and co-evaporation with distilled water the residue was dissolved in 20 ml distilled water and extracted with diethylether. The aqueous phase was evaporated and the residue redissolved in 0.001 M ammonium bicarbonate buffer (pH 7.8) and applied to a DEAE-Sephadex A-25 column (2 x 15 cm, HCO_3^- form). The column was eluted with a linear gradient 0.001 M - 0.5 M ammonium bicarbonate solution (1000 ml / 1000 ml, pH 7.5). Appropriate fractions were pooled, evaporated and co-evaporated with distilled water a few times to remove the salt. The title compound was obtained as a sodium salt by elution through a Dowex column (1 x 20 cm, Na^+ form) with distilled water. The collected water solution was evaporated and the residue was redissolved in a small amount of distilled water and lyophilized. Yield 288 A_{260} units (62 %). ^{1}H- and ^{31}P-NMR spectra proved to be identical with the ^{1}H- and ^{31}P-NMR spectra of a tetramer with the same nucleotide sequence and which was synthezised according to another synthetic route developed in our laboratory[5e]. **Deprotection of 37 to 40:** Compound **37** (190 mg, 30.95 μmol) was deprotected in the same way as for **36** (*syn*-4-nitroaldoxime (360 mg, 2.17 mmol), 1,1,3,3-tetramethylguanidine (240 μl, 1.92 mmol) in 10 ml dioxane-water solution, 45 ml concentrated ammonia, 30 ml 80 % acetic acid). 0.001 - 0.35 M (500 ml / 500 ml), 0.35M - 0.7M (500 ml / 500 ml) and 0.7M - 0.8M (500 ml / 500 ml) ammonium bicarbonate gradients were used for the elution of the DEAE-Sephadex A-25 column (2 x 25 cm, HCO_3^- form). The center part (599 A_{260} units) of the product peak was collected and sodium exchanged and high resolution proton and phosphorous nmr spectroscopy ((Figs. 4-9)) proved it to have > 98% purity. Yield : 599 A_{260} units (21%). **Deprotection of 38 to 41:** Compound **38** (87 mg, 12.8 μmol) was deprotected in the same way as for **36** (*syn*-4-nitroaldoxime (171 mg, 1.03 mmol), 1,1,3,3-tetramethylguanidine (116 μl, 0.924 mmol) in 4.2 ml dioxane-water solution, 20 ml concentrated ammonia, 20 ml 80 % acetic acid). 0.001 - 0.5 M (500 ml / 500 ml), 0.5M - 0.8M (500 ml / 500 ml) ammonium bicarbonate gradients were used for the elution of the first DEAE-Sephadex A-25 column (2 x 15 cm, HCO_3^- form). The middle part (293 A_{260} units) of the major peak was collected and applied to a second DEAE-Sephadex A-25 column (2 x 15 cm, HCO_3^- form) and 0.001 - 0.5 M (500 ml / 500 ml), 0.5 - 0.65 M (500 ml / 500 ml), 0.65 - 0.75 M (500 ml / 500 ml) ammonium bicarbonate gradients were used for the elution. The center part (171 A_{260} units) of the broad symmetrical peak was collected and sodium exchanged and its 100 % purity was confirmed by high resolution proton and phosphorous nmr spectroscopy (Figs. 10-15).The remaining product containing fractions from both column separations were pooled together applied to a third DEAE-Sephadex A-25 column (2 x 15 cm, HCO_3^- form) using the same gradients as for the second column. From this column another 92 A_{260} units of pure product was collected. Total yield of pure product 263 A_{260} units (21 %).

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