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Solid Phase Synthesis of 2',5'-Oligoadenylates Containing 3'-Fluorinated Ribose§

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Abstract: 2',5'-phosphodiester bond-linked oligoadenylate trimers with 3'-fluoro-3'deoxyadenosine residues incorporated at specific positions of the nucleotide sequence were synthesized by the solid phase phosphite triester (phosphoramidite) method. The syntheses were in the 2' to 5' direction and were performed manually using commercially available microcolumns. The oligonucleotides were 5'-end phosphorylated on the support before deprotection.

The series of 2',5'-phosphodiester bond linked small oligoadenylates of the general formula ppp5'A2'(p5'A)_n ($n\geq 1$) is called 2-5A¹. With the exception of the dimeric member of this group, these 2',5'-oligonucleotides are potent inhibitors of translation and also can play a role in the degradation of mRNA and rRNA. Their mechanism of action is mediated exclusively through the activation of a latent 2-5A-dependent endonuclease^{2,3}. It appears that each individual nucleotide residue of 2-5A may assume a fundamentally different role in binding to and activation of the 2-5A-dependent RNase. Several sites of strong interaction of the endonuclease with its oligonucleotide activator have been uncovered to date⁴. These include the polyphosphate or monophosphate (depending on the source of the enzyme) of the 5'-terminus, the purine $N1/N^{6}$ amino molety of the adenosine residues of the 5'-terminal (first) and the third nucleotide, the purine N7 of the first (5'-terminus) and third residues, and the 3'-hydroxyl group of the second nucleotide of 2-5A⁴⁻⁸. Furthermore, there is a vital dependence on the nature of the phosphodiester linkage: a 3',5'-linkage isomer of 2-5A was orders of magnitude less active as a translational inhibitor 9.

Previous studies⁷ have shown that only the 3'-hydroxyl group of the second from the 5'terminus of 2-5A is needed for effective activation of the 2-5A dependent nuclease. To evaluate in

[§]Preliminary reports of portions of this work were presented at the Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression: Therapeutic Implications meeting, Rockville, MD, 18-21 June 1989 and at the 199th National Meeting of the American Chemical Society, Boston, MA, 22-27 April 1991.

more detail the contribution of the 3'-OH to the binding and activation of RNase L by the oligonucleotide ligand, we have synthesized analogues in which the 3'-hydroxyl group of one or more specific nucleotide residues of 2-5A trimer have been replaced by fluorine. The rationale for replacing the OH and H groups of biologically significant molecules by fluorine have been extensively reviewed¹⁰. Fluorine closely mimics hydrogen with respect to steric requirements (the van der Waals' radii of F: 1.35Å; of H: 1.1Å), but it strongly differs from hydrogen in electronegative character. Replacement by fluorine eliminates hydrogen bond donation as an interactive mechanism, but due to the great electronegativity of fluorine, fluorine still can function as a hydrogen bond receptor, albeit not as effectively as oxygen because of it's reduced tendency to share its non-bonded electrons ¹¹. Furthermore, the conformation of a fluorine compound often resembles that of the parent hydroxyl derivative rather than that of the hydrogen analogue, as was shown by NMR studies ¹²⁻¹⁴.

Herein we report on the synthesis of 2-5A analogues containing fluorinated ribose by a solid phase phosphite triester (phosphoramidite) methodology as modified for the preparation of oligoribonucleotides with 2',5'-phosphodiester bonds¹⁵. The biological activity of the fluorinated 2-5A analogues has been described elsewhere¹⁶.

EXPERIMENTAL

Ultraviolet spectra were recorded on a Varian DMS 200 UV-Visible spectrophotometer. The ¹H NMR spectra were determined with a Varian VXR 300 MHz spectrophotometer with TMS as internal standard; for ³¹P NMR spectra 5% H3PO4 was used as external standard. Precoated Uniplate[™] silica gel GHLF plates (Analtech, Inc.) were used for TLC. Column chromatography was performed on silicagel 60 (Fluka Chemika). Reverse phase high performance liquid chromatography on a Beckman Ultraspere ODS column (4.6mm x 25cm, flow rate: 1mL/min.) was executed with a Beckman System Gold HPLC with two model 112 pumps and a Knauer variable wavelength monitor operating at 260 nm. Ion-exchange column chromatography on DEAE-Sephadex A-25 (Pharmacia LKB) was carried out at 4°C using various concentrations of triethylammonium bicarbonate (TEAB), pH 7.5, as an elution buffer. A stock solution of TEAB (1M) was prepared by introducing carbon dioxide in a 1 M solution of triethylamine in water at 4°C. Buffer was removed by repeated coevaporation with water and finally with anhydrous ethanol. Long chain alkylamine controlled pore glass (LCAA-CPG, pore size 500Å) support was obtained from Pierce Chemical Company (Rockland, IL). Adenosine, anhydrous solvents (Sure/Seal[™]) and reagents used in the solid phase synthesis were purchased from Aldrich Chemical Company. The syntheses were performed manually in commercially available microcolums (Applied Biosystems, Inc.) as described by Uznanski et al. 17. Snake venom phosphodiesterase was a product of Cooper Biomedical, alkaline phosphatase was obtained from Sigma (St. Louis, MO).

Preparation of Fully Protected Monomers

5'-O-Dimethoxytrityl-N⁶-benzoyladenosine was prepared by 5'-tritylation and transient protection of OH groups by trimethylsilyl chloride for N-benzoylation¹⁸. Sugar hydroxyl groups of adenosine were protected with the *tert* - butyldimethylsilyl protecting group according to the method of Ogilvie *et al.* ¹⁹. The 2'-silyl isomer was utilized for derivatization of the solid support

via a succinate linkage, while the 3'-silyl isomer was converted into the 2'-O-(β -cyanoethyl)-N,N-diisopropyl phosphoramidite as described by Usman *et al.* ²⁰. Derivatization of 3'-fluoro-3'-deoxyadenosine for solid phase oligoribonucleotide synthesis has not been previously reported, so the preparation of the fully protected monomers **4**,**5** is detailed here.

N⁶-Benzoyl-3'-fluoro-3'-deoxyadenosine (2)

A portion of 538.5 mg (2 mmol) of 3'-fluoro-3'-deoxyadenosine^{21, 22} (dried by coevaporation with anhydrous pyridine) in 20 mL of anhydrous pyridine was cooled to 0°C in an ice-bath and 1.25 mL (20 mmol) of trimethylchlorosilane (Fluka) was added. After stirring at 0° for 30 min, benzoyl chloride (Fluka, 0.58 mL, 5 mmol) was added. The colored reaction mixture was stirred at room temperature for 2 h. After cooling (ice-bath) 4 mL of cold water was added with stirring at 0° for 15 min, then 4 mL of concentrated ammonia was added with additional stirring at 0° for 30 min. After evaporation the mixture was purified on silica gel using ethyl acetate first, then ethyl acetate-methanol (95:5) as an eluent. Crystallization from MeOH afforded 531 mg (1.42 mmol, 71%) of the title compound as a colorless amorphous powder. TLC in ethyl acetate-methanol (95:5): Rf = 0.5. CI MS (NH₃) 374 (M+H)+. ¹H NMR (CDCl₃ + DMSO-d₆ (20% v/v)) δ : 3.72 (m, H-5', H-5"); 4.35 (broadened d, JH-4',F = 27.8 Hz); 4.90 (m, H-2'); 5.03 (dd, J = 4.2 Hz, J₃',F = 54.6 Hz, H-3'); 5.72 (m, 2'-OH, 5'-OH); 5.99 (d, J = 7.9 Hz, H-1'); 7.45 (m, 3H, Ar-H); 7.98 (d, J = 7.4Hz, 2H, Ar-H); 8.28 (s, H-2); 8.61 (s, H-8); 10.41 (s, 6-NH).

5'-(4,4'-O-Dimethoxytrityl)-N⁶-benzoyl-3'-fluoro-3'-deoxyadenosine (3)

A mixture of 500 mg (1.34 mmol) of N⁶-benzoyl-3'-fluoro-3'-deoxyadenosine (dried by coevaporation with anhydrous pyridine) and 550 mg (1.62 mmol) of 4,4'-dimethoxytrityl chloride (Aldrich) in 10 mL of anhydrous pyridine was stirred at room temperature until TLC-analysis showed the disappearance of the starting material (2 h). Methanol (0.5 mL) was added. After evaporation, the residue was taken up in 10 mL of water and extracted twice with 10 mL of ether. The organic layer was dried over Na₂SO₄, solvent was evaporated and the residue purified by column chromatography in the presence of 0.2% of pyridine using first dichloromethane, then CH₂Cl₂-MeOH (98:2) to yield 616 mg (0.91 mmol, 68%) of the title compound as a pale yellow foam. TLC in chloroform-methanol (95:5): R_f = 0.66. ¹H NMR [CDCl₃ + pyridine-d₅ (1% v/v)] δ : 3.22 and 3.40 (2 x dd, J = 3.2 Hz, J = 10.7 Hz, H-5', H-5''); 3.65 (s, 6H, CH₃O-); 4.45 (broadened d, J4', F = 26.9 Hz, H-4'); 5.06 (m, H-2'); 5.06 (dd, J = 4.4 Hz, J₃', F = 55.1 Hz, H-3'); 6.02 (d, J = 7.2 Hz, H-1'); 6.64-7.21 (m, 13H, dimethoxytrityl); 7.44 (m, 3H, Ar-H); 7.70 (broadened d, J = 7.1 Hz, 2'-OH); 7.91 (d, J = 7.2, 2H, Ar-H); 8.12 (s, H-2); 8.61 (s, H-8); 9.02 (bs, 6-NH).

5'-(4,4'-O-Dimethoxytrityl)-N⁶-benzoyl-3'-fluoro-3'-deoxyadenosine-2'-O-(ßcyanoethyl)-N,N-diisopropyl phosphoramidite (<u>4</u>)

To a stirred solution of 405 mg (0.6 mmol) of 5'-(4,4'-O-dimethoxytrityl)-N⁶-benzoyl-3'fluoro-3'-deoxyadenosine and 42 mg (0.6 mmol) of tetrazole (Aldrich) in 10 mL of dry dichloromethane (distilled from CaH₂), 180.8 mg (0.6 mmol) of ß-cyanoethyl)-N,N,N',N'tetraisopropyl phosphorodiamidite (Aldrich) was added with a syringe. After stirring for 1 h at room temperature under a nitrogen atmosphere, the reaction mixture was evaporated and purified by flash column chromatography using benzene-hexane-triethylamine (7:2:1) to yield 352 mg (0.4 mmol, 67%) of the title compound as a colorless foam. TLC in benzene-hexane-TEA (7:2:1): $R_f = 0.46$ and 0.34 for the diastereomers, respectively. ³¹P NMR (CDCl₃ + 1% pyridine-d₅) δ : 152.2 ppm (1P) and 151.4, 151.3 ppm (1P) Jp-F: 9.1 Hz.

5'-(4,4'-O-Dimethoxytrityl)-N⁶-benzoyl-3'-fluoro-3'-deoxyadenosine-2'-Osuccinate (<u>5</u>)

To a solution of 270.3 mg (0.4 mmol) of 5'-(4,4'-O-dimethoxytrityl)-N⁶-benzoyl-3'fluoro-3'-deoxyadenosine (dried by coevaporation with anhydrous pyridine) in 2.4 mL of anhydrous pyridine containing 4-dimethylaminopyridine (24.4 mg, 0.2 mmol), succinic anhydride (120.8 mg, 1.2 mmol, Fluka) was added. The yellow solution was stirred for 36 h at room temperature and monitored by TLC using dichloromethane-methanol (95:5). No starting material was left. After evaporation the residual pyridine was removed by coevaporation with dry toluene (3 x 5 mL). The residue was dissolved in 15 mL of chloroform and washed with saturated NaCl solution (3 x 15 mL) and then with water (1x15 mL). The organic layer was dried over CaCl₂, evaporated and purified by column chromatography in the presence of 0.2% of pyridine using a stepwise gradient of dichloromethane-methanol from 95:5 to 9:1 yielding 240 mg (0.31 mmol, 77%) of the title compound as a pale yellow amorphous powder. TLC in ethyl acetate-acetonewater (5:10:1) containing 0.5% pyridine $R_f = 0.61$. ¹H NMR (CDCl₃ + 1% pyridine-d₅) δ : 2.46 (broad s, CH2CH2); 3.19 and 3.63 (2 x m, H-5', H-5"); 3.63 (s, 6H, CH3O-); 4.38 (broadened d, J4', F = 26.9 Hz, H-4'); 5.29 (broad d, J3', F = 57.9 Hz, H-3'); 5.98 (m, H-2'); 6.23 (broad s, H-1'); 6.64-7.25 (m, 13H, dimethoxytrityl); 7.30 (m, 3H, Ar-H); 7.84 (m, 2H, Ar-H); 8.10 (s, H-2); 8.47 (s, H-8); 9.20 (bs, 6-NH).

Derivatization of the solid support

A mixture of 100 μ mol of fully protected 3'-fluoro-3'-deoxyadenosine 2'-succinate (5) (for adenosine the 3'-succinate was used), 500 mg of pre-activated LCAA-CPG²³, 6 mg (50 μ mol) of DMAP, 40 μ L of TEA and 191 mg (1 μ mol) of N-(dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (Fluka) in 5 mL of anhydrous pyridine was first sonicated for 2 min, and then shaken at room temperature for 2 d. After shaking, the solid support was filtered off and washed successively with pyridine, methanol and chloroform and then dried under vacuum over P2O5. The unreacted sites were then capped using 100 μ l of acetic anhydride in 1.5 ml of anhydrous pyridine. After shaking for 30 min, the CPG was filtered off and washed successively with pyridine, methanol and then dried under vacuum over P2O5. Colorimetric dimethoxytrityl assay²⁴ indicated a loading of 35 μ mol/g for 3'-fluoro-3'-deoxyadenosine and 59 μ mol/g for adenosine, respectively.

Solid phase synthesis of oligonucleotides

Oligonucleotides were synthesized by the phosphoramidite method²⁵ using β -cyanoethyl as the phosphate protecting group²⁶. The syntheses were performed manually in commercially available microcolumns using adaptors and gas-tight syringes¹⁷ on a 1-2 µmol scale depending on the solid support loading. The chain elongation was carried out in the 2' to 5' direction. The synthetic cycle used was as follows: detritylation, 2% TCA in dichloromethane; washing, 2%

pyridine in CH₃CN, followed by CH₃CN, then dry N₂; coupling, 0.5 M tetrazole & 0.2 M phosphoramidite in CH₃CN; washing with CH₃CN, then dry N₂; oxidation with 0.1 M I₂ in THF-lutidine-H₂O (7:2:1); washing with CH₃CN, then dry N₂; capping with 6.5% DMAP in THF (w/v) and Ac₂O-2,6-lutidine (2:3); then washing with CH₃CN followed by dry N₂. Syntheses were monitored by colorimetric dimethoxytrityl assay²⁴. After the desired sequence had been obtained, the 5'-terminal nucleoside was converted to the 5'-phosphate by reaction with bis-(β-cyanoethyl)-N,N-diisopropylphosphoramidite using tetrazole as catalyst and subsequent oxidation with iodine/water^{27,28}.

Deprotection and purification

The oligonucleotides synthesized were cleaved from the solid support by treatment with a mixture of concentrated ammonia and ethanol (3:1, v/v) at room temperature for 2 h. The resulting solutions were further incubated for 6 h at 55°C to remove the N⁶-benzoyl protecting groups as well as the β -cyanoethyl phosphate protecting groups. Finally, the 3'-O-(*tert*-butyldimethylsilyl) protection of the adenosine moieties was removed by treating the oligomers with 1M tetrabutylammonium fluoride in THF at room temperature for 4-6 h²⁰, ²⁹. The synthesized oligonucleotides were purified by ion-exchange chromatography using DEAE-Sephadex column eluted with a linear gradient of 0.0-0.5M TEAB buffer (pH 7.5). Final desalting was achieved by reverse phase C18 SEP-PAK cartridges (Waters Millipore) using methanol/water = 1:1 (v/v) as eluent.

Determination of hypochromicities of oligonucleotides

The appropriate oligonucleotide (0.5 A₂₆₀ unit) in 200 mM Tris-HCl, pH 8.5 containing 50 mM MgCl₂ was digested by adding snake venom phosphodiesterase (0.5 U, 0.5 μ L) until a constant A₂₆₀ value was obtained. The total volume of the reaction mixture was 1 mL. The hypochromicity values were determined by the ratio A_{initial}/A_{final} and the oligonucleotide extinction coefficients calculated by multiplying the respective hypochromicity values by the sum of the constituent nucleoside micromolar extinction coefficients at 260 nm.

Characterization of oligonucleotides

The appropriate oligonucleotide (1 A₂₆₀ unit) in 10 mM Tris-HCl, pH 8.5 containing 10 mM Mg(OAc)₂ was digested by adding snake venom phosphodiesterase (0.3 U, 0.3 μ L) for 2 h at 37°C. The total volume of the reaction mixture was 100 μ L. Alkaline phosphatase (0.5 U, 1.7 μ L) then was added. After a further 2 h incubation, the sample was analyzed by reverse phase HPLC using an elution program of 0-50% buffer B into buffer A over 30 min followed by a linear gradient of 50-100% buffer B over 5 min followed by100% buffer B for 10 min. Buffer A was 20 mM ammonium phosphate pH 7.0 (Condition I) or 60 mM potassium dihydrogen phosphate + 5 mM PIC A reagent (Waters), final pH 5.0 (Condition II). For both conditions, buffer B was 50% methanol-water (v/v). The nucleoside compositions were calculated from the peak area integrals by comparison with that of a standard nucleoside mixture.

RESULTS AND DISCUSSION

The structures of fluorinated 2',5'-oligoadenylates prepared for this study are presented in Figure 1. Oligonucleotides were synthesized by the phosphoramidite method²⁵ as modified



Figure 1. Structure of the 2',5'-linked 3'-fluoro-oligoadenylates.

for the preparation of oligoribonucleotides with 2',5'-phosphodiester bonds¹⁵. We have used benzoyl and B-cyanoethyl²⁶ as protection for the N⁶-amino and phosphate groups, respectively. For protection of the sugar hydroxyl groups of adenosine, we have utilized the tertbutyldimethylsilyl protecting group 2^{9-31} . The reaction sequence for the 3'-fluoro-3'deoxyadenosine building blocks, 5'-(4,4'-O-dimethoxytrityl)-N⁶-benzoyl-3'-fluoro-3'deoxyadenosine-2'-O-(B-cyanoethyl)-N,N-diisopropyl phosphoramidite (4) and 5'-(4,4'-Odimethoxytrityl)-N⁶-benzoyl-3'-fluoro-3'-deoxyadenosine-2'-O-succinate (5), was straightforward and can be described as follows. Using transient protection compound 1, 3'-fluoro-3'deoxyadenosine, was first N⁶-benzoylated giving N⁶-benzoyl-3'-fluoro-3'-deoxyadenosine (2) in 71% yield. Subsequent reaction of the primary hydroxyl group with 4,4'-dimethoxytrityl chloride afforded 3, 5'-(4,4'-O-dimethoxytrityl)-N⁶-benzoyl-3'-fluoro-3'-deoxyadenosine (3) in 68% yield. Compound 3 having the necessary 2'-OH free was either phosphitylated yielding 4. 5'-(4,4'-O-dimethoxytrityl)-N⁶-benzoyl-3'-fluoro-3'-deoxyadenosine-2'-O-(B-cyanoethyl)-N,Ndiisopropyl phosphoramidite in 67% yield as the fully protected monomer for chain elongation or 3 was converted into compound 5, 5'-(4,4'-O-dimethoxytrityl)-N⁶-benzoyl-3'-fluoro-3'deoxyadenosine-2'-O-succinate ($\underline{5}$), in a good yield (77%). The building block $\underline{5}$ was then utilized for the derivatization of the solid support via succinate linkage with a loading of 35 µmol/g for 3'fluoro-3'-deoxyadenosine.

The synthetic cycle used for the solid phase oligonucleotides synthesis is described in the experimental section. With an extended coupling time (up to 8 min) average coupling efficiencies for 3'-fluoro-3'-deoxyadenosine were 95-98 % and for adenosine 93-95 % as determined by colorimetric quantitation of the dimethoxytrityl fractions ²⁴.

These stepwise yields were somewhat lower than those of generally accepted in automatic DNA syntheses. One explanation is the steric hindrance generated by the bulky 3'-O-tert -

Oligomer	Retention time (min)	Condition	$e (M^{-1} cm^{-1} x 10^3)$
pdAflpApA	15.88	I	37.4
pApdAflpA	15.96	Ι	34.8
pApApdAfl	31.14	II	36.4
pdÅflpdAflpA	20.49	I	36.2
pdAflpApdÅfl	33.95	Π	36.1
pdAflpdAflpdAfl	33.47	п	38.2

 Table 1.

 HPLC Behavior and Molar Extinction Coefficients of 2',5'-Linked Oligonucleotides^a.

Abbreviation: dAfl, 3'-fluoro-3'-deoxyadenosine. a For Conditions (I or II) see Experimental.

butyldimethylsilyl group of the adenosine residues on the coupling rates of the corresponding phosphoramidite monomers during chain elongation. Using competitive phosphitylation experiments Kierzek *et al.* have studied the steric aspects of the 2'-protective groups on the synthesis of oligoribonucleotides by the phosphoramidite approach and have shown a strong dependence on the steric hindrance of the 2'-substituents both for the 3'-phosphitylation and condensation steps³². On the other hand, in the case of 3'-fluoro-3'-deoxyadenosine moieties, direct steric influence seems unlikely based on the similarity of the van der Waals radii of hydrogen (1.1Å) and fluorine (1.35Å). A more plausible explanation is the decreased reactivity of the modified 2'-phosphoramidites by the electron-withdrawing inductive effect of fluorine³³. A similar observation was made during the solid phase synthesis of oligodeoxyribonucleotides containing the anti-cancer drug 2',2'-difluoro-2'-deoxycytidine³⁴.

In order to study the binding of the fluorinated 2'5'-oligoadenylates to RNase L the 5'terminal monophosphate functions were required³⁵. The 5'-end phosphorylation could be easily achieved by the reaction with bis-(β -cyanoethyl)-N,N-diisopropylphosphoramidite in the coupling step of the synthetic cycle with a shorter reaction time (4 min) followed by oxidation with iodine/water ^{27,28}. The oligomer 5'-phosphates were then removed from the solid support and were deprotected as described in the Experimental part. Final yields, after purification and desalting, for the fluorinated 2',5'-oligoadenylates were between 65-81%. All oligonucleotides were pure, as judged by analytical HPLC.

The structure of each modified oligoadenylate was confirmed by NMR spectra of the intermediates, HPLC behavior, and analysis of enzymatic hydrolysis products. For the latter, the oligomers were subjected to complete digestion with snake venom phosphodiesterase, followed by alkaline phosphatase digestion. The products obtained were analyzed by HPLC, and the determined 3'-fluoro-3'-deoxyadenosine/ adenosine ratios were found to be consistent with the proposed sequences. The molar extinction coefficients were calculated for all oligonucleotides from the increase in UV absorbance obtained after the enzymatic digestion to the constituent nucleosides (Table 1).

To summarize, solid-phase synthesis using phophoramidite chemistry provides a good yield of 3'-fluoro-3'-deoxyadenosine 2',5'-oligonucleotides as homo-oligonucleotides or as mixed

sequence-specific oligonucleotides with adenosine itself. The oligonucleotides containing 3'fluoro-3'-deoxyadenosine residues presented here have been used successfully as probes of hydrogen bonding in enzymes of the 2-5A system¹⁶. The facile preparation of 2',5'oligoadenylates and their analogues is of particular interest in light of the recent description of 2-5A-antisense composite oligonucleotides which provide a technology for the targeted destruction of RNA and a new potential for oligonucleotide therapeutics 36-39.

REFERENCES

- Kerr, I. M. and Brown, R. E. (1978) Proc. Natl. Acad. Sci. U.S.A. 73, 256-260. 1.
- 2. Torrence, P. F. (1985) In Biological Response Modifiers-New Approaches to Disease Intervention (Torrence, P. F., ed.) Academic Press, New York, pp. 77-105.
- 3. Johnston, M. I. and Torrence, P. F. (1984) In Interferons: Mechanism of Production and Action (Friedman, R. M., ed.) Elsevier, Amsterdam, Vol. 3, pp. 189-298 .
- 4. Torrence, P. F., Imai, J., Jamoulle, J.-C. and Lesiak, K. (1986) Chem. Scripta, 26, 191-197.
- 5. Luxembourg, A. (1988) Bull. Inst. Pasteur, 86, 373-417.
- 6.
- Jamoulle, J.-C., Lesiak, K. and Torrence, P. F. (1986) *Biochem.* 26, 376-383. Torrence, P. F., D. Brozda, D. Alster, R. Charubala and W. Pfleiderer (1988) *J. Biol.* 7. Chem. 263, 1131-1139.
- Dong, B., Xu, L., Zhou, A., Hassel, B. A., Lee, X., Torrence, P. F. and Silverman, 8. R. H. (1994) J. Biol. Chem. 269, 14153-14158.
- 9. Lesiak, K., Imai, J., Floyd-Smith, G. and Torrence, P. F. (1983) J. Biol. Chem. 258, 1131-1139.
- Kirk, K. L. (1991) Biochemistry of the Elements: Biochemistry of Halogenated 10. Organic Compounds, Vol 9B, Plenum Press, New York.
- Pauling, L. (1980) In The Nature of the Chemical Bond, Cornell University Press, Ithaca, 11. N. Y. 3rd ed., p.460.
- Blandin, M., Son, T-D., Catlin, J. C. and Guschlbauer, W. (1974) Biochem . 12. Biophys. Acta, 361, 249-256.
- Lipnick, R. L. and Fissekis, J. D. (1980) Biochem. Biophys. Acta 608, 96-102. 13.
- Glaudemans, C. P. J., Lerner, L., Daves, G. D., Kovac, P., Venable, R. and Bax, A. 14. (1990) Biochem. 29, 10906-10911.
- Lesiak, K., Uznanski, B. and Torrence, P., F. (1989) Nucleosides & Nucleotides, 8, 15. 1055-1056.
- Kovács, T., Pabuccuoglu, A., Lesiak, K. and Torrence, P. F. (1993) Bioorg. Chem. 16. 21, 192-208.
- Uznanski, B., Koziolkiewicz, M., Stec, W. J., Zon, G., Shinozuka, K. and Marzili, L. 17. G. (1986) Chem. Scripta, 26, 221-224.
- Jones, R. A. (1984) In Oligonucleotide Synthesis-A Practical Approach (Gait, M. J., ed.) 18. IRL Press, Oxford, pp. 23-34.
- Ogilvie, K. K., Beaucage, S. L., Schifman, A. L., Theriault, N. Y. and Sadana, K. L. 19. (1978) Can. J. Chem. 56, 2768-2780.
- Usman, N., Ogilvie, K. K., Jiang, M.-Y. and Cedergren, R. J. (1987) J. Am. Chem. 20. Soc. 109, 7845-7854.
- Herdewijn, P., Van Aerschot, A. and Kerremans, L. (1989) Nucleosides & Nucleotides, 21. **8,** 65-96.
- 22. Van Aerschot, A., Herdewijn, P., Janssen, M., Cools, M. and De Clercq, E. (1989) Antiviral Res. 12, 133-150.
- Pon, R.T., Usman, N. and Ogilvie, K. K. (1988) BioTechniques, 6, 768-775. 23.
- 24. Atkinson, T. and Smith, M. (1984) In Oligonucleotide Synthesis-A Practical Approach (Gait, M. J., ed.) IRL Press, Oxford, pp.35-81.
- 25. Beaucage, S.L. and Iyer, R.P. (1992) Tetrahedron, 48, 2223-2311 and references cited therein.
- Sinha, N.D., Biernat, J., McManus, J. and Köster, H. (1984) Nucl. Acids Res., 12, 26. 4539-4557.
- Uhlmann, E. and Engels, J. (1986) Tetrahedron Lett., 27, 1023-1026. 27.
- 28. Bannwarth, W. and Trzeciak, A. (1987) Helv. Chim. Acta, 70, 175-186.
- 29. Scaringe, S. A., Francklyn, C. and Usman, N. (1990) Nucl. Acids Res., 18, 5433-5441.

- Wang, Y. Y., Lyttle, M. H. and Borer, P. N. (1990) Nucl. Acids Res., 18, 3347- 3352. 30.
- 31.
- Wu, T. and Ogilvie, K. K. (1990) J. Org. Chem., 55, 4717-4724. Kierzek, R., Rozek, M. and Markiewicz, W. T. (1987) Nucl. Acids Res. Symp.Ser., 32. No.18, 201-204.
- 33. Bergstrom, D. E. and Swartling, D. J. (1988) In Fluorine-Containing Molecules: Structure, Reactivity, Synthesis and Applications (Liebman, J. F. and Greenberg, A., eds.) VCH Publishers, Inc. N. Y., pp.259-308. Richardson, F. C., Richardson, K. K., Kroin, J. S. and Hertel, L. W. (1992) Nucl.
- 34. Acids Res., 20, 1763-1768.
- Torrence, P. F., Imai, J., Lesiak, K., Jamoulle, J.-C. and Sawai, H. (1984) J. Med. 35. Chem., 27, 726-733. Torrence, P. F., Maitra. R. K., Lesiak, K., Khamnei, S., Zhou, A. and Silverman, R.
- 36. H. (1993) Proc. Natl. Acad. Sci. USA 90, 1300-1304.
- 37. Lesiak, K., Khamnei, S. and Torrence, P. F. (1993) Bioconjugate Chem. 4, 467-472.
- Maran, A., Maitra, R. K., Kumar, A., Dong, B., Xiao, W., Li, G., Williams, B. R. G., Torrence, P. F. and Silverman, R. H. (1994) *Science*, **265**, 789-792. Torrence, P. F., Xiao, W., Li, G., Lesiak, K., Khamnei, S., Maran, A., Maitra, R., 38.
- 39. Dong, B. and Silverman, R. H. (1994) Carbohydrates: Synthetic Methods and Applications in Antisense Therapeutics (D. Cook and P. Sanghvi, eds.) ACS Symposium Series, in press.

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