Synthesis and separation of diastereomers of *O*-(2,2,2-trifluoroethyl)-3′,5′-dinucleoside phosphates

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Received August 10, 1993

WEIDE LUO, ELENA ATRAZHEVA, NANCY FREGEAU, WILLIAM H. GMEINER, and J. WILLIAM LOWN. Can. J. Chem. **72**, 1548 (1994).

The synthesis, diastereomeric separation, and characterization are described for a series of novel *O*-(2,2,2-trifluoroethyl)-3′,5′-dinucleoside phosphates, required for incorporation into antisense probes in the magnetic resonance imaging investigation of biodistribution. Preliminary assignment of the absolute configuration of the *R*p and *S*p diastereomers is made on the basis of ^{31}P NMR spectra.

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On décrit la synthèse, la séparation des diastéréoisomères et la caractérisation d'une série de nouveaux phosphates de *O*-(2,2,2-trifluoroéthyl)-3′,5′-dinucléosides requis pour l'incorporation dans des sondes pour une étude MRI de biodistribution. Faisant appel à la RMN du ^{31}P, on fait une attribution préliminaire de la configuration absolue des diastéréoisomères *R*p et *S*p.

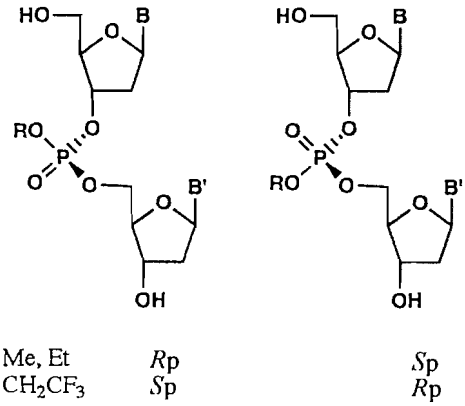
[Traduit par la Rédaction]

Introduction

Synthetic oligonucleotides that have backbone modifications are useful as model compounds for studying DNA structure and dynamics (1, 2), and as probes for elucidating specific interactions of DNA with proteins and enzymes (3–5). Phosphoric triesters have long been studied as models of these modifications. The phosphoric triester linkage in oligonucleotides can be constructed by oxidative substitution of dinucleoside H-phosphonates with an alcohol (6), transesterification of dinucleoside phosphoric triesters (7), or alkylation of dinucleoside phosphoric diesters. As an example of the latter, two *O*-alkyl-5,5′-dinucleoside phosphates have been synthesized as potential anti-AIDS reagents (8).

The phosphoric triester linkage possesses a chiral centre at the phosphorus atom. The absolute configuration can be defined in the manner of Cahn et al. (9), by inspection of the four groups about the phosphorus atom. The *R*p and *S*p isomers of dinucleoside monophosphate methyl, ethyl, or trifluoroethyl triesters, so defined, are shown in Fig. 1. The stereochemistry of alkylated DNA phosphate groups has been shown to be of biochemical significance (10, 11). Stereochemistry is also clearly important when such modified DNAs are used as probes in structural studies or for antisense applications. Because dinucleoside monophosphate alkyl triesters are almost always produced as a mixture of diastereomers by various preparative methods, the separation of the diastereomers is required. This has often proved to be difficult.

We require certain trifluoroethyl phosphate triester derivatives of nucleosides to extend our studies on the application of ^{19}F NMR in magnetic resonance imaging (MRI) determination of biodistribution of antisense probes (12). In this paper, we present the synthesis of dinucleoside monophosphate 2,2,2-trifluoroethyl triesters, including dinucleotides of TT, TC, TA, CA, AT, and AA, by the phosphite-triester method and separation of the *S*p and *R*p diastereomers. Their absolute configurations were tentatively assigned by comparison of chemical shifts of ^{31}P NMR signals between diastereomers.



R = Me, Et *R*p *S*p
R = CH_2CF_3 *S*p *R*p

FIG. 1. Designation of *R*p and *S*p configurations of diastereomeric *O*-(2,2,2-trifluoroethyl)-3′,5′-inucleoside phosphates and the related methyl and ethyl derivatives.

Results and discussion

To avoid the difficulty of separation of diastereomeric mixtures of phosphoric triesters, we initially carried out the synthetic route as outlined in Scheme 1, in which the key intermediates, dinucleoside H-phosphonates **1**, were subjected to separation of P-diastereomers by flash chromatography on silica gel (13). The next steps to the target molecules (**4**) (sulfurization, alkylation of dinucleoside phosphothioates **2** with 2,2,2-trifluoroethyl iodide, and the oxidation of the resulting phosphoric thiotriesters **3** with *m*-chloroperbenzoic acid) were expected to be stereospecific (14). In the event, our attempts to generate *O*-(2,2,2-trifluoroethyl)dinucleoside phosphothioates **3** from compounds **2** by alkylation in the presence of bases such as pyridine, triethylamine, DABCO, and *N*,*N*-diisopropylethylamine were unsuccessful. The reason for the failure of alkylation is not clear. However, it is possible owing to the lower nucleophilicity of 2,2,2-trifluoroethyl iodide.

The synthesis of the dinucleotides by the phosphite-triester method, which has been applied in DNA solid-phase synthesis, proceeded in two steps: the preparation of deoxyribonucleoside-3′-*O*-(*N*,*N*-di-*iso*-propylamino)phosphoramidites (**5**, **6**, and **7**) and their coupling reactions with 3′-*O*-protected deoxyri-

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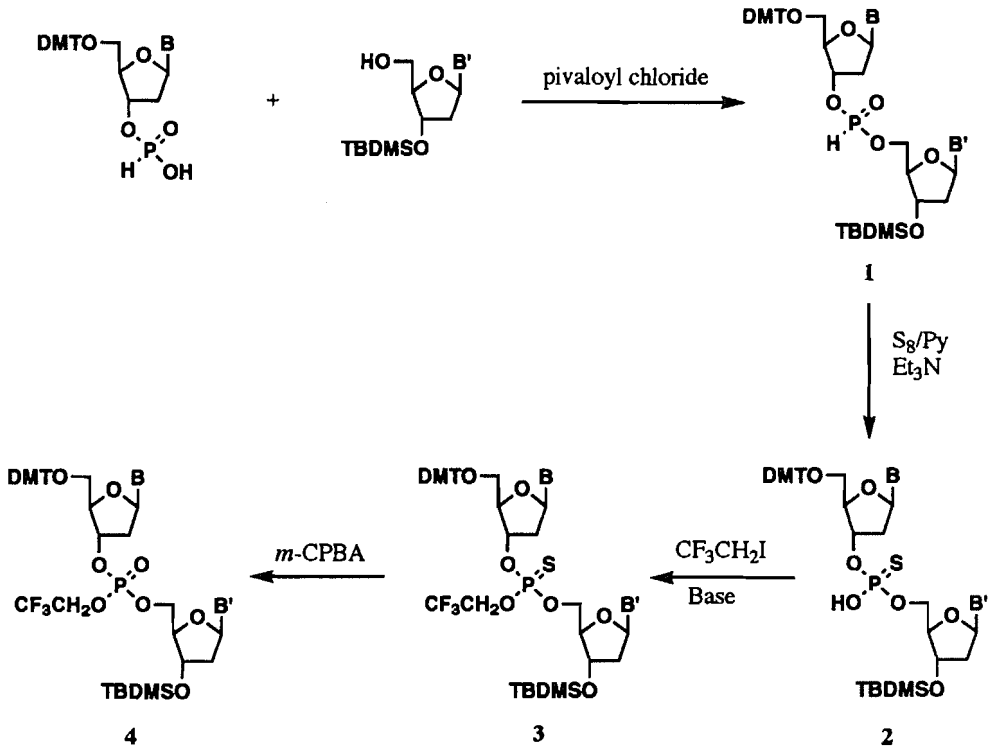


SCHEME 1. Outline of attempted synthesis of the desired *O*-(2,2,2-trifluoroethyl)-3′,5′-dinucleoside phosphates via and dinucleoside H-phosphonates.

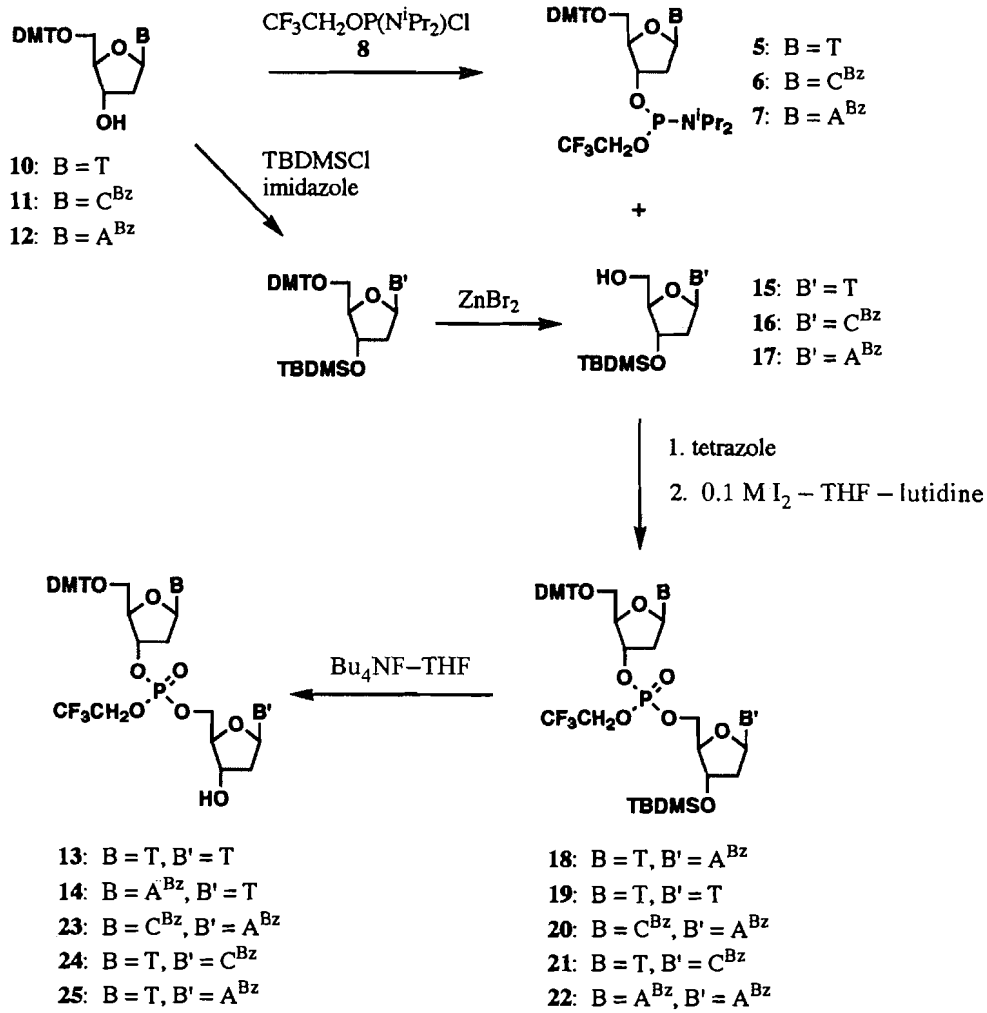
bonucleosides under the catalysis of tetrazole followed by oxidation with iodine solution, as outlined in Scheme 2.

The phosphitylating reagent (**8**) was prepared in a similar manner as described for chloro-*N,N*-diisopropylamino methoxyphosphine (15). Phosphorus trichloride was allowed to react with 1 equivalent of 2,2,2-trifluoroethanol at low temperature for 5 h. The crude product of the reaction contained unreacted phosphorus trichloride, 2,2,2-trifluoroethyl phosphodichloridite (**9**), di-2,2,2-trifluoroethyl phosphochloridite, and tri(2,2,2-trifluoroethoxy)phosphine, which could be detected by their ^{31}P NMR signals (δ 219.0, 180.8, 166.0, and 139.3, respectively). The desired product **9** was isolated from the mixture by two successive fractional distillations in a yield of 31%. The purity of the compound obtained was proven by its ^{31}P NMR spectrum, in which only one signal at δ 180.8 was observed. The compound **8** was then prepared by the reaction of **9** with 2 equivalents of diisopropylamine in a solution of dichloromethane at low temperature in a yield of 70%. This reagent is extremely sensitive to moisture and air, but can be stored at −30°C for ex-tended periods (at least 2 months) under an argon atmosphere.

The 5′-protected nucleosides (**10**, **11**, and **12**) were prepared by reported methods (15). The syntheses of **5**, **6**, and **7** from **10**, **11**, and **12** with phosphitylating agent **8**, according to the procedure for their methyl analogues described by McBride and Caruthers (16), proceeded normally with yields of 94, 87, and 80%, respectively. Each of the products was an approximately 1:1 mixture of the *S*p and *R*p diastereomers, as indicated by ^{31}P NMR and ^{19}F NMR analyses.

Compounds **5** and **7** were initially used to couple directly to unprotected thymidine under the catalysis of tetrazole. After treatment with the oxidizing reagent *tert*-butyl hydrogen peroxide, dinucleotides **13** and **14** were obtained in yields of 42 and 31%, respectively. To improve the yield, we considered that protection on the 3′-hydroxy group in the deoxyribonucleoside might be beneficial. *tert*-Butyldimethylsilyl was the group of choice as the blocking functionality in this case. Compounds **15**, **16**, and **17** (17) were synthesized from **10**, **11**, and **12** by silylation with *tert*-butyldimethylsilyl chloride under the catalytic action of imidazole, followed by treatment with zinc bromide in dichloromethane to remove the 5′-dimethoxytrityl group (15). The coupling reaction of **5** and **17** (1:1 equiv.) with tetrazole as a catalyst was carried out at room temperature in acetonitrile for 4 h. The resulting dinucleoside phosphite triester was oxidized without isolation by a 0.1 M solution of iodine in a mixture of tetrahydrofuran, 2,6-lutidine, and water (40:10:1 by volume). The desired product **18** was obtained in 91% yield as a 1:1 mixture of diastereomers. By following a similar procedure, dinucleotides **19**, **20**, **21**, and **22** were also successfully prepared from appropriate nucleosides in good yields (85, 77, 93, and 77%, respectively). Removal of the silyl protecting group in each of the dinucleosides was achieved by treatment of the silyl ethers **18**, **19**, **20**, and **21** with tetrabutylammonium fluoride in tetrahydrofuran, affording compounds **25**, **13**, **23**, and **24**, respectively, in good yields.

The separations of the diastereomers, however, proved to be difficult. In the TLC analysis for these dinucleotides, only two of the compounds, **13** and **20**, showed detectable differences in R_f values between the diastereomers when the TLC plates were developed by a mixture of acetic acid and ethyl acetate (4:96, v/v). Therefore, the diastereomeric mixtures of **13** and **20** were separated by flash chromatography on silica gel eluted with the same solvent system to afford fast-moving diastereomers **13*a*** and **20*a*** and then the slowmoving **13*b*** and **20*b*** with good recovery yields (70–80 or 30–40% of each diastereomer). Other diastereomeric mixtures of dinucleotides, such as **14**, **19**, **22**, **24**, and **25**, were partially separated by flash chromatography on silica gel eluted with a gradually increasing proportion of methanol in chloroform solution. The optimum concentrations of



SCHEME 2. Outline of alternative and successful synthesis and separation of diastereomeric *O*-(2,2,2-trifluoroethyl)-3′,5′-dinucleoside phosphates via the 3′-*O*-(*N*,*N*-diisopropylamino)phosphoramidites.

TABLE 1. Separation and ^{31}P NMR data of the diastereomers

Dinucleotide	No.	Eluting solvent in separation	^{31}P NMR δ of *R*p, *S*p diastereomer (fast, slow moving)	^{31}P NMR Solvent
TT	**13**	C	−1.46/−1.60	Py-d_5
AT	**14**	2–4.5% B	−2.71/−2.87	CD_3OD
TT	**19**	1–3% B	−2.26/−2.33	$CDCl_3$
CA	**20**	C	−2.28/−2.35	$CDCl_3$
AA	**22**	0.5–0.75% B	−2.20/−2.44	$CDCl_3$
TC	**24**	2–4.5% B	−1.34/−1.37	Py-d_5
TA	**25**	2–4.5% B	−1.67/−1.78	Py-d_5

NOTE: Solvent B, methanol in chloroform; solvent C, 4% acetic acid in ethyl acetate.

methanol for separation of the individual diastereomeric mixtures, as well as ^{31}P NMR data of the separated diastereomers, are listed in Table 1. The separations of each pair of diastereomers were followed by ^{31}P NMR analysis. As an example, the ^{31}P NMR spectra of separated diastereomers **22***a* and **22***b* are shown in Fig. 2. Each diastereomer in each pair showed very similar ^{1}H NMR spectra. The largest differences in chemical shift were usually observed for the base aromatic protons as shown in Fig. 3.

Potter and co-workers (18–20) have examined ^{31}P NMR spectra for a variety of dinucleoside methyl phosphotriesters of known configuration, and in all cases it was observed that the *S*p isomer resonates at lower field than the *R*p isomer. Similarly, Summers et al. (21) and Weinfeld et al. (7) found the signal from the *S*p isomer of ethylated internucleotide phosphate at a lower field than the equivalent signal from the *R*p isomer. Although dinucleoside 2,2,2-trifluoroethyl phosphotriesters have not been reported hitherto, ^{31}P NMR is our method of

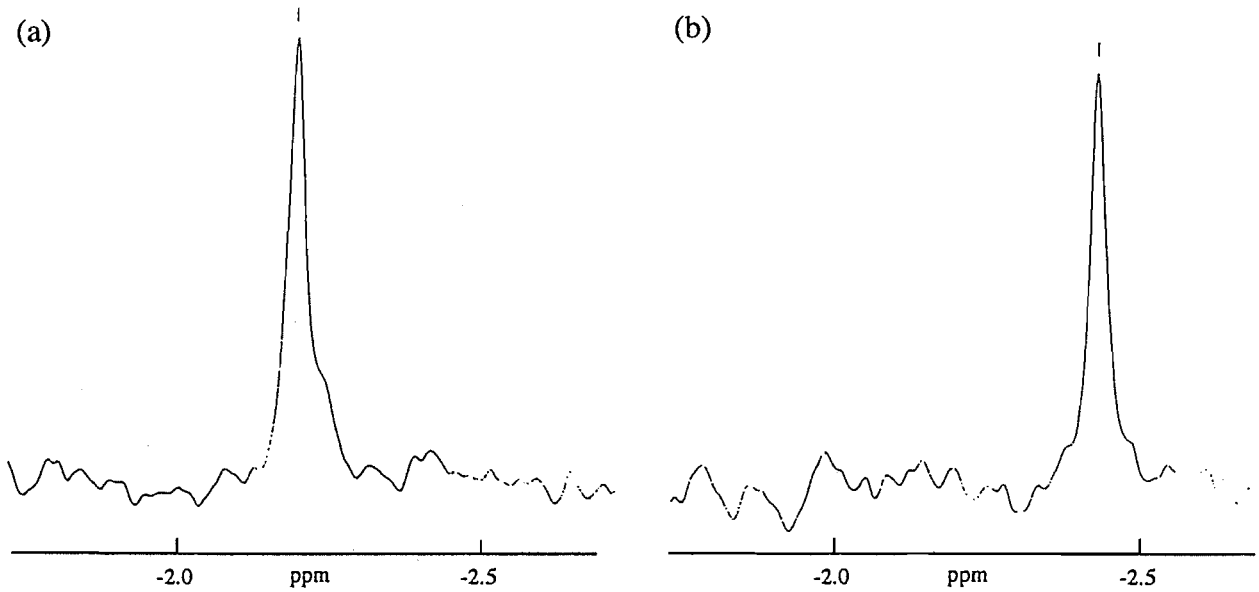


FIG. 2. ^{31}P NMR spectra of separated diastereomers **22***a* (*a*) and **22***b* (*b*).

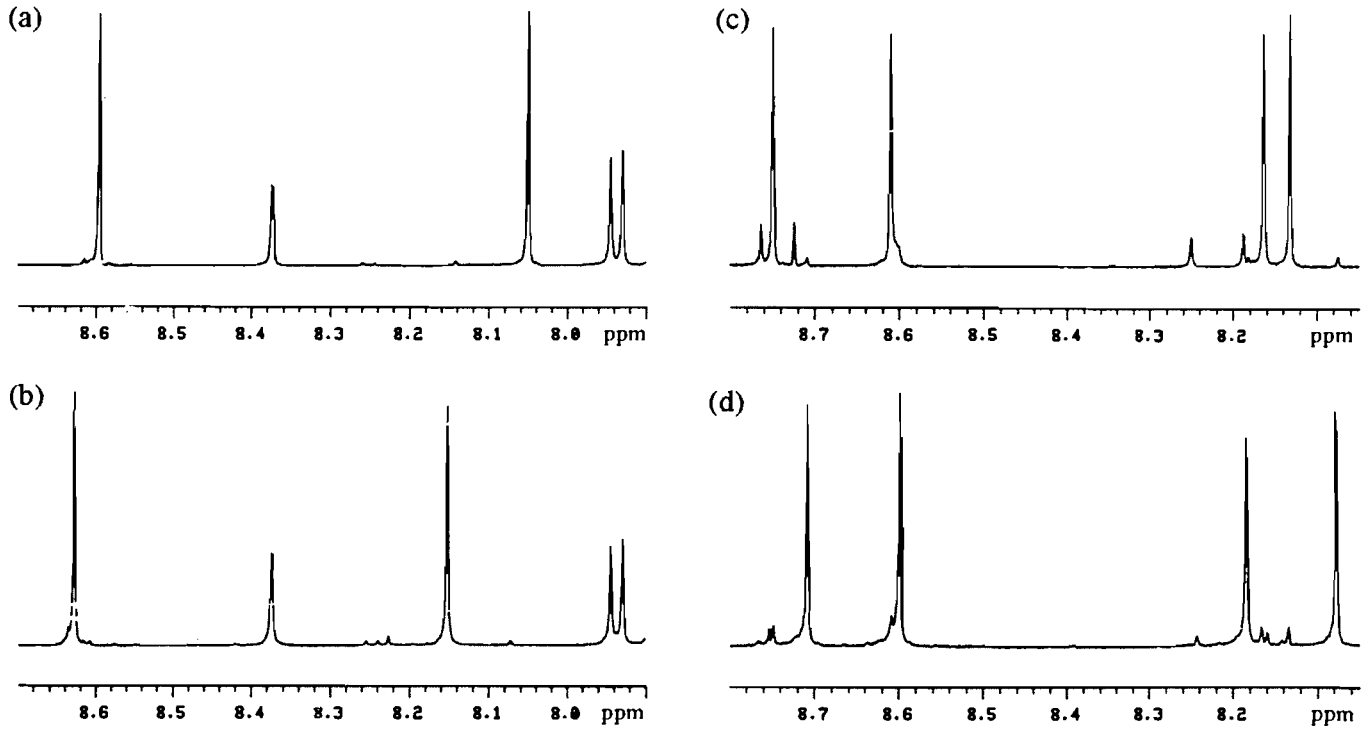


FIG. 3. Comparison of the ^1H NMR spectra of the base aromatic protons of **20***a* (*a*) and **20***b* (*b*) in $CDCl_3$–Py-d_5 (5:1), and **22***a* (*c*) and **22***b* (*d*) in $CDCl_3$.

choice for an initial examination of the absolute configuration about the phosphorus chiral centre. Considering that the weighting order of the four groups about the phosphorus atom in dinucleoside 2,2,2-trifluoroethyl phosphotriesters is different from that in the methyl or ethyl analogues, the faster moving isomers, from which the ^{31}P signal appears at lower field, are tentatively assigned as *R*p isomers and the slower moving isomers are assigned as *S*p. Studies directed towards a more definitive assignment of phosphorus configuration and towards the incorporation of diastereomeric *O*-(2,2,2-trifluoroethyl)-3′,5′-dinucleoside phosphates into specific antisense probes for MRI experiments are ongoing and will be reported in due course.

Experimental

General methods

NMR spectra were recorded on Bruker WH-200 or WH-400 spectrometers. ^1H, ^{19}F, and ^{31}P spectra were referenced to internal tetramethylsilane, external 50% trichlorofluoromethane in deuterium chloroform, and external 85% phosphoric acid in deuterium oxide, respectively. High-resolution mass spectra (HREIMS) and fast-atom bombardment mass spectra (FABMS) were recorded using A.E.I. model MS50 or MS9 mass spectrometers.

Acetonitrile and dichloromethane were distilled from phosphorus pentoxide, chloroform from anhydrous sodium carbonate, and pyridine from potassium hydroxide, toluene-4-sulfonyl chloride, or calcium hydride. Flash chromatography was performed with silica gel, 230–400 mesh ASTM. Merck silica gel plates (60 F_{254}) were used for analytical TLC, and the spots were examined with UV light and 1 N hydrochloric acid spray. Solvent systems for TLC development were as follows: A, Et_3N–EtOAc–CH_2Cl_2 (10:45:45); B, CH_3OH–$CHCl_3$ (1:9); C, AcOH–EtOAc (4:96). The concentrations of all solvent systems were determined by volume.

*2,2,2-Trifluoroethyl phosphodichloridite (**9**)*

2,2,2-Trifluoroethanol (97.3 g, 70 mL, 0.97 mol) contained in a pressure-equalizing dropping funnel was added dropwise over 2 h into magnetically stirred phosphorus trichloride (136.9 g, 87 mL, 1.00 mol) at −20°C, contained in a 250-mL three-necked flask vented via an air condenser equipped with a calcium chloride drying tube. During the addition, the temperature of the Dry Ice – acetone bath was maintained between −20 and −15°C. After the addition was completed, the temperature was allowed to increase to −5°C and maintained for 4 h. Then the bath was removed, and the mixture was allowed to warm up to room temperature with stirring (1 h) and set aside overnight in a fume hood to degas. The homogeneous liquid was fractionated by distillation at atmospheric pressure using a Vigreux column (300–400 mm long). The fraction boiling from 85 to 110°C was refractionated. The pure product (bp 94°C/700 Torr, 64 g, 0.30 mol) was obtained in 31% yield. ^{1}H NMR ($CDCl_3$) δ: 4.45 (dq, J = 8, 7.5 Hz); ^{31}P NMR ($CDCl_3$) δ: 180.84.

*Chloro-*N,N*-diisopropylamino-(2,2,2-trifluoroethoxy)phosphine (**8**)*

Under an argon atmosphere, a solution of diisopropylamine (17.83 g, 0.176 mol) in dichloromethane (40 mL), contained in a 100-mL addition funnel equipped with a calcium chloride drying tube, was added to a magnetically stirred solution of **9** (17.74 g, 0.088 mol) in dichloromethane (50 mL) in a 250-mL three-necked flask at −20°C. During the addition, the temperature of the Dry Ice – acetone bath was maintained between −20 and −10°C. Then the mixture was allowed to warm up slowly to room temperature over 3 h. The white precipitate of diisopropylamine hydrochloride was removed rapidly by vacuum filtration. The filtrate was concentrated by distillation at atmospheric pressure. The residue was fractionated under reduced pressure and the product (**8**, bp 73°C/11 Torr, 16.35 g, 0.0616 mol) was obtained in 70% yield. ^{1}H NMR ($CDCl_3$) δ: 4.04 (dq, 2H, J = 8, 8 Hz, CH_2), 3.74 (dh, 2H, J = 12, 7 Hz, CH), 1.5 (m, 12H, CH_3); ^{31}P NMR ($CDCl_3$) δ: 180.00; ^{19}F NMR ($CDCl_3$) δ: −75.25 (dq, J = 7, 8 Hz); HREIMS *m/e*: 267.0576, 265.0608 ($C_{H816}{}^{37}ClF_3NOP$ and $C_{H816}{}^{35}ClF_3NOP$, 5.19 and 11.41).

*General procedure for preparation of deoxyribonucleoside-3′-*O*-(*N,N*-diisopropylamino)-(2,2,2-trifluoroethyl)phosphoramidites*

Under an argon atmosphere, phosphitylating reagent **8** (1.65 mL, 1.99 g, 7.5 mmol) was injected over 10 s into a magnetically stirred solution of 5′-dimethoxytrityldeoxyribonucleoside **10**, **11**, or **12** (5.0 mmol) and diisopropylethylamine (2.58 g, 2.91 mL, 20.0 mmol) in dichloromethane (15 mL) at room temperature. The mixture was stirred for 20 min, ethanol (0.5 mL) was then added, and the mixture was stirred for 5 min to quench excess phosphitylating reagent. The reaction mixture was transferred to a 250-mL separatory funnel, diluted with a mixture of triethylamine and ethyl acetate (1:20, 100 mL), and washed with 10% aqueous sodium carbonate (2 × 60 mL) and then with saturated aqueous sodium chloride (60 mL). The organic phase was dried over anhydrous sodium sulfate, filtered, and evaporated to a foam under reduced pressure. The crude product was purified by flash chromatography using a mixture of triethylamine, ethyl acetate, dichloromethane, and petroleum ether in the ratio of 1:10:10:20 as eluent. The desired product was obtained in the yield of 80–95%.

*(R*p*,S*p*)-5′-*O*-(4,4′-Dimethoxytrityl)thymidine-3′-*O*-(*N,N*-diisopropylamino)-(2,2,2-trifluoroethyl)phosphoramidites (**5**)*

From **10** (5.0 mmol), **5** (3.64 g, 4.70 mmol) was isolated in 94% yield. TLC (silica gel, solvent A) R_f 0.80; ^{1}H NMR ($CDCl_3$) δ: 8.10 (bs, 1H, NH), 7.67 and 7.58 (s, 1H, H6), 7.4–7.2 (m, 9H, ArH), 6.83 (d, 4H, J = 8 Hz, ArH), 6.40 (m, 1H, H1′), 4.66 (m, 1H, H3′), 4.20 and 4.14 (m, 1H, H4′), 3.98 (m, 2H, CF_3CH_2), 3.80 (s, 6H, CH_3O), 3.60 (m, 2H, $CH(CH_3)_2$), 3.50 and 3.33 (m, 1H each, H5′ and H5″), 2.50 and 2.33 (m, 2H, H2′ and H2″), 1.40 and 1.42 (s, 3H, 5-CH_3), 1.20 (m, 12H, $CH(CH_3)_2$); ^{31}P NMR ($CDCl_3$) δ: 152.89 and 152.62 (1:1, q, J = 6 Hz); ^{19}F NMR ($CDCl_3$) δ: −75.10 and −75.28 (1:1, dt, J = 6, 9 Hz); FABMS *m/e*: 774 ($M + H^+$).

*(R*p*,S*p*)-N^4-Benzoyl-5′-(4,4′-dimethoxytrityl)-2′-deoxycytidine-3′-*O*-(N,N-diisopropylamino)-(2,2,2-trifluoroethyl)phosphoramidites (**6**)*

From **11** (5.0 mmol), **6** (3.75 g, 4.35 mmol) was isolated in 87% yield. TLC (silica gel, solvent A) R_f 0.75; ^{1}H NMR ($CDCl_3$) δ: 9.10 (bs, 1H, NH), 8.35 and 8.30 (d, 1H, J = 7 Hz, H6), 7.90 (d, 2H, J = 8 Hz, BzH), 7.62 (t, 1H, J = 8 Hz, BzH), 7.52 (t, 2H, J = 8 Hz, BzH), 7.42 (d, 1H, J = 7 Hz, H5), 7.3 (m, 9H, ArH), 6.88 (d, 4H, J = 8 Hz, ArH), 6.32 and 6.30 (dd, 1H, J = 6, 6 Hz, H1′), 4.66 (m, 1H, H3′), 4.28 and 4.24 (m, 1H, H4′), 3.97 and 3.87 (ddq, 1H each, J = 12, 8, 8 Hz, CF_3CH_2), 3.81 (s, 6H, CH_3O), 3.63 (m, 2H, $CH(CH_3)_2$), 3.50 and 3.43 (m, 1H each, H5′ and H5″), 2.80 and 2.33 (m, 2H, H2′ and H2″), 1.20 (m, 12H, $CH(CH_3)_2$); ^{31}P NMR ($CDCl_3$) δ: 153.07 and 152.66 (1:1, q, J = 6 Hz); ^{19}F NMR ($CDCl_3$) δ: −75.05 and −75.04 (1:1, dt, J = 6, 9 Hz); FABMS *m/e*: 863 ($M + H^+$).

*(R*p*,S*p*)-N^6-Benzoyl-5′-(4,4′-dimethoxytrityl)-2′-deoxyadenosine-3′-*O*-(N,N-diisopropylamino)-(2,2,2-trifluoroethyl)-phosphoramidites (**7**)*

From **12** (5.0 mmol), **7** (4.0 mmol) was isolated in 80% yield. TLC (silica gel, solvent A) R_f 0.80; ^{1}H NMR ($CDCl_3$) δ: 9.10 (bs, 1H, NH), 8.70 (s, 1H, H2), 8.20 and 8.15 (s, 1H, H8), 8.00 (d, 2H, J = 8 Hz, BzH), 7.65 (t, 1H, J = 8 Hz, BzH), 7.50 (t, 2H, J = 8 Hz, BzH), 7.4–7.2 (m, 9H, ArH), 6.75 (d, 4H, J = 8 Hz, ArH), 6.48 (m, 1H, H1′), 4.77 (m, 1H, H3′), 4.28 and 4.32 (m, 1H, H4′), 3.82 and 3.80 (dq, 1H each, J = 9, 9 Hz, CF_3CH_2), 3.74 (s, 6H, CH_3O), 3.60 (m, 2H, $CH(CH_3)_2$), 3.40 and 3.31 (m, 1H each, H5′ and H5″), 2.92 and 2.66 (m, 2H, H2′ and H2″), 1.20 (m, 12 H, $CH(CH_3)_2$); ^{31}P NMR ($CDCl_3$) δ: 152.90 and 152.70 (1:1, q, J = 6 Hz); ^{19}F NMR ($CDCl_3$) δ: −75.07 and −75.08 (1:1, dt, J = 6, 9 Hz); FABMS *m/e*: 909 and 807 ($M + Na^+$ and $M + H^+$).

*(R*p*,S*p*)-5′-*O*-(4,4′-Dimethoxytrityl)thymidylyl-3′,5′-N^6-benzoyl-3′-*O*-[(1,1-dimethylethyl)dimethylsilyl]-2′-deoxyadenosine (2,2,2-trifluoroethyl)phosphates (**18**)*

Under an argon atmosphere, phosphoramidite **5** (1.70 g, 2.2 mmol) and tetrazole (0.28 g, 4.06 mmol) were added to a magnetically stirred solution of **17** (0.938 g, 2.00 mmol) in acetonitrile (50 mL). The mixture was stirred at room temperature for 4 h and then 0.10 M iodine solution in a mixture of tetrahydrofuran, 2,6-lutidine, and water (40:10:1 by volume, 22 mL) was added. The mixture was stirred for 10 min and then transferred to a 250-mL separatory funnel and diluted with ethyl acetate (100 mL). The liquid was washed with saturated aqueous sodium bicarbonate (2 × 60 mL) and then with saturated aqueous sodium chloride (60 mL). The aqueous phases were extracted with ethyl acetate (2 × 50 mL). The organic phases were combined, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to a foam (2.76 g). The crude residue was subjected to flash chromatography with 3% solution of methanol in chloroform as eluent, giving dinucleotides **18** as a 1:1 mixture of two diastereomers (2.11 g, 1.82 mmol, 91% yield). TLC (silica gel, solvent B) R_f 0.52; ^{1}H NMR ($CDCl_3$) δ: 9.10 (bs, 1H, NH), 8.75 and 8.44 (m, 1H each, H2 and H8), 8.22 and 8.20 (s, 1H, H6), 8.00 (m, 2H, BzH), 7.60 (m, 1H, BzH), 7.50 (m, 2H, BzH), 7.36–7.20 (m, 9H, ArH), 6.82 (m, 4H, ArH), 6.52 (dd, 0.5H, H1′), 6.45 and 6.43 (m, 1.5H, H1′), 5.12 (m, 1H, H3′), 4.70 (m, 1H, H3′), 4.4–4.1 (m, 6H, H4′, H4′, CF_3CH_2, H5′, and H5″), 3.77 (s, 6 H, CH_3O), 3.47 and 3.33 (m, 1H each, H5′ and H5″), 2.91, 2.57, 2.49, and 2.38 (m, 1H each, H2′ and H2″), 1.37 and 1.35 (s, 3H, 5-CH_3), 0.91 and 0.92 (s, 9H, $C(CH_3)_3$), 0.12 (s, 6H,

$Si(CH_3)_2$); ^{31}P NMR ($CDCl_3$) δ: −1.90 and −2.42 (1:1); ^{19}F NMR ($CDCl_3$) δ: −75.27 and −75.37 (1:1, t, J = 8 Hz); FABMS *m/e*: 1180 and 1158 (M + Na^+ and M + H^+).

*(Rp,Sp)-5′-O-(4,4′-Dimethoxytrityl)thymidylyl-3′,5′-N^6-benzoyl-2′-deoxyadenosine (2,2,2-trifluoroethyl)phosphates (**25***a* and **25***b*)*

To a solution of **18** (310 mg, 0.272 mmol) in tetrahydrofuran (5 mL) was added 1 M tetrabutylammonium fluoride solution in tetrahydrofuran (containing 5% water, 1 mL). The mixture was stirred at room temperature for 20 min and then concentrated under reduced pressure. The residue was subjected to flash chromatography on silica gel with 0–6% methanol in chloroform. Products **25***a* and **25***b* (260 mg, 0.249 mmol) were obtained in 92% yield as a 1:1 mixture of two diastereomers: ^{31}P NMR (Py-d_5) δ: −1.67 and −1.78; FABMS *m/e*: 1066 and 1044 (M + Na^+ and M + H^+). The isomers were partially separated by further flash chromatography on silica gel, eluted with 2–4.5% methanol in chloroform. The percentage of methanol was increased at the rate of 0.25%/200 mL. With TLC analysis, compounds **25***a* and **25***b* were distributed in 30 of 60 eluted fractions. The first 5 fractions afforded 90% pure isomer **25***a* (25 mg). TLC (silica gel, solvent B) R_f 0.30; ^1H NMR (Py-d_5) δ: 13.3 and 12.3 (bs, 1H each, NH), 8.97 and 8.89 (s, 1H each, H2 and H8), 8.54 (s, 1H, H6), 8.28 (d, 2H, BzH), 7.68 (m, 3H, BzH), 7.55–7.36 (m, 8H, ArH), 7.27 (t, 1H, J = 8 Hz, ArH), 7.00 (d, 4H, J = 8 Hz, ArH), 6.90–6.80 (m, 2H, H1′), 5.60 (m, 1H, H3′), 5.10 (m, 1H, H3′), 4.82 (m, 2H, CF_3CH_2), 4.70 (m, 2H, H5′ and H5″), 4.64 (m, 1H, H4′), 4.56 (m, 1H, H4′), 3.67 (s, 6H, CH_3O), 3.63 (m, 2H, H5′ and H5″), 3.17 (ddd, 1H, J = 13, 6.5, 6.5 Hz, H2′ or H2″), 2.87 (m, 1H, H2′ or H2″), 2.75 (m, 2H, H2′ and H2″), 1.70 (s, 3H, 5-CH_3); ^{31}P NMR (Py-d_5) δ: −1.67; ^{19}F NMR (Py-d_5) δ: −75.01 (t, J = 8 Hz). The last 5 fractions gave the other isomer (**25***b*, 92% pure, 20 mg). TLC (silica gel, solvent B) R_f 0.30; ^1H NMR (Py-d_5) δ: 13.3 and 12.3 (bs, 1H each, NH), 8.96 and 8.84 (s, 1H each, H2 and H8), 8.54 (s, 1H, H6), 8.30 (d, 2H, BzH), 7.68 (m, 3H, BzH), 7.55–7.36 (m, 8H, ArH), 7.27 (t, 1H, J = 8 Hz, ArH), 7.00 (d, 4H, J = 8 Hz, ArH), 6.90–6.80 (m, 2H, H1′), 5.60 (m, 1H, H3′), 5.10 (m, 1H, H3′), 4.79 (m, 2H, CF_3CH_2), 4.72 (m, 2H, H5′ and H5″), 4.65 (m, 1H, H4′), 4.54 (m, 1H, H4′), 3.67 (s, 6H, CH_3O), 3.63 (m, 2H, H5′ and H5″), 3.19 (ddd, 1H, J = 13, 6.5, 6.5 Hz, H2′), 2.87 (m, 1H, H2′ or H2″), 2.75 (m, 2H, H2′ or H2″), 1.70 (s, 3H, 5-CH_3); ^{31}P NMR (Py-d_5) δ: −1.78; ^{19}F NMR (Py-d_5) δ: −75.00 (t, J = 8 Hz). From other fractions, the diastereomeric mixture of **25***a* and **25***b* (182 mg) was recovered.

*(Rp,Sp)-5′-O-(4,4′-Dimethoxytrityl)thymidylyl-3′,5′-3′-O-[(1,1-dimethylethyl)dimethylsilyl]thymidine 2,2,2-trifluoroethyl)-phosphates (**19**a and **19**b)*

Using a similar procedure as for **18**, from **5** (2.38 g, 3.08 mmol) and **15** (0.98 g, 2.75 mmol), **19** (2.31 g, 2.21 mmol, 80% yield) was obtained as a 1:1 mixture of two diastereomers, which were partially separated in the manner as described for **25***a* and **25***b* with 1–3% methanol in chloroform as eluent. Compound **19***a*: TLC (silica gel, solvent B) R_f 0.50; ^1H NMR ($CDCl_3$) δ: 9.10 (bs, 2H, NH), 7.50 (m, 1H, H6), 7.40–7.10 (m, 10H, H6 and ArH), 6.83 (d, 4H, J = 8 Hz, ArH), 6.18 (m, 2H, H1′), 5.15 (m, 1H, H3′), 4.4–4.1 (m, 5H, H3′, CF_3CH_2, H5′, and H5″), 3.95 (m, 2H, H4′), 3.75 (s, 6H, CH_3O), 3.52 and 3.40 (m, 1H each, H5′ and H5″), 2.60, 2.50, 2.40 and 2.20 (m, 1H each, H2′ and H2″), 1.85 and 1.45 (d, 3H each, J = 1 Hz, 5-CH_3), 0.90 (s, 9H, $C(CH_3)_3$), 0.05 (s, 6H, $Si(CH_3)_2$); ^{31}P NMR ($CDCl_3$) δ: −2.26; ^{19}F NMR ($CDCl_3$) δ: −75.24 (t, J = 8 Hz); FABMS *m/e*: 1067 (M + Na^+). Compound **19***b*: TLC (silica gel, solvent B) R_f 0.50; ^1H NMR ($CDCl_3$) δ: 9.10 (bs, 2H, NH), 7.50 (m, 1H, H6), 7.40–7.10 (m, 10H, H6 and ArH), 6.82 (d, 4H, J = 8 Hz, ArH), 6.43 (dd, 1H, J = 9, 5 Hz, H1′), 6.16 (t, 1H, J = 7 Hz, H1′), 5.15 (m, 1H, H3′), 4.4–4.1 (m, 5H, H3′, CF_3CH_2, H5′, and H5″), 3.95 (m, 2H, H4′), 3.75 (s, 6H, CH_3O), 3.52 and 3.35 (m, 1H each, H5′ and H5″), 2.60, 2.50, 2.40, and 2.20 (m, 1H each, H2′ and H2″), 1.85 and 1.40 (d, 3H each, J = 1 Hz, 5-CH_3), 0.90 (s, 9H, $C(CH_3)_3$), 0.05 (s, 6H, $Si(CH_3)_2$); ^{31}P NMR ($CDCl_3$) δ: −2.26; ^{19}F NMR ($CDCl_3$) δ: −75.24 (t, J = 8 Hz); FABMS *m/e*: 1067 (M + Na^+).

*(Rp,Sp)-5′-O-(4,4′-Dimethoxytrityl)thymidylyl-3′,5′-thymidine (2,2,2-trifluoroethyl)phosphates (**13**a and **13**b)*

Desilylation method

Using the desilylation procedure described for **18**, **19** (0.47 g, 0.45 mmol) gave an 85% yield of a 1:1 diastereomeric mixture of **13***a* and **13***b* (0.36 g, 0.38 mmol). The mixture was separated by flash chromatography on silica gel, eluted with 4% acetic acid in ethyl acetate, giving 18% of **13***a*, 13% of diastereomeric mixture, and 20% of **13***b*. Compound **13***a*: TLC (silica gel, solvent B) R_f 0.27 and TLC (silica gel, solvent C) R_f 0.29; ^1H NMR (Py-d_5) δ: 13.25 (bs, 2H, NH), 7.75–7.24 (m, 11H, H6 and ArH), 7.02 (d, 4H, J = 9 Hz, ArH), 6.83 (m, 2H, H1′), 5.70 (m, 1H, H3′), 4.92 (p, 2H, CF_3CH_2), 4.80 (m, 1H, H3′), 4.65 (m, 3H, H4′, H5′, and H5″), 4.45 (m, 1H, H4′), 3.75 (s, 6H, CH_3O), 3.67 (m, 2H, H5′ and H5″), 2.86 and 2.54 (m, 2H each, H2′ and H2″), 2.05 and 1.70 (d, 3H each, J = 1 Hz, 5-CH_3); ^{31}P NMR (Py-d_5) δ: −1.46; ^{19}F NMR (Py-d_5) δ: −74.99 (t, J = 8 Hz); FABMS *m/e*: 953 (M + Na^+). Compound **13***b*: TLC (silica gel, solvent B) R_f 0.27 and TLC (silica gel, solvent C) R_f 0.20; ^1H NMR (Py-d_5) δ: 13.2 (bs, 2H, NH), 7.7–7.2 (m, 11H, H6 and ArH), 7.00 (d, 4H, J = 9 Hz, ArH), 6.90–6.78 (m, 2H, H1′), 5.70 (m, 1H, H3′), 4.85 (m, 3H, H3′ and CF_3CH_2), 4.70–4.45 (m, 4H, H4′, H5′, and H5″), 3.7 (s, 6H, CH_3O), 3.67 (m, 2H, H5′ and H5″), 2.91 and 2.62 (m, 2H each, H2′ and H2″), 2.05 and 1.67 (d, 3H each, J = 1 Hz, 5-CH_3); ^{31}P NMR (Py-d_5) δ: −1.60; ^{19}F NMR (Py-d_5) δ: −74.98 (t, J = 8 Hz); FABMS *m/e*: 953 (M + Na^+).

Direct method

Thymidine (245 mg, 1.00 mmol) was dried by coevaporation with acetonitrile and pyridine (5:1, 2 × 5 mL) under reduced pressure. Under an argon atmosphere, the thymidine was dissolved in pyridine (3 mL) and a solution of **5** (512 mg, 0.661 mmol) in acetonitrile (10 mL), which was saturated with tetrazole, was added by syringe. The mixture was stirred at room temperature overnight. *tert*-Butyl hydrogen peroxide solution in 2,2,4-trimethylpentane (3 M, 0.33 mL) was added. After stirring for 10 min, the mixture was poured into aqueous saturated sodium chloride (30 mL) and extracted with chloroform (2 × 40 mL). The extracts were combined, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was subjected to flash chromatography on silica gel. Elution with 6% methanol in chloroform gave products **13***a* and **13***b* as a 1:1 diastereomeric mixture (258 mg, 0.278 mmol, 42% yield).

*(Rp,Sp)-N^6-Benzoyl-5′-(4,4′-dimethoxytrityl)-2′-deoxyadenoylyl-3′,5′-thymidine (2,2,2-trifluoroethyl)phosphates (**14**a and **14**b)*

Using a similar procedure as described for **13** (direct method) from **7** (886 mg, 1.0 mmol) and thymidine (290 mg, 1.2 mmol), **14** (318 mg, 0.31 mmol, 31% yield) was obtained as a 1:1 mixture of diastereomers. The mixture was partially separated in the manner as described for **25**, giving **14***a* and **14***b*. Compound **14***a*: TLC (silica gel, solvent B) R_f 0.32; ^1H NMR (CD_3OD) δ: 13.3 and 10.3 (bs, 1H each, NH), 8.57 (s, 1H, H2), 8.46 (s, 1H, H8), 8.07 (d, 2H, J = 8 Hz, BzH), 7.65 (d, 1H, J = 8 Hz, BzH), 7.55 (t, 2H, J = 8 Hz, BzH), 7.42 (q, 1H, J = 1 Hz, H6), 7.37–7.12 (m, 7H, ArH), 6.78 (d, 4H, J = 8 Hz, ArH), 6.53 and 6.21 (t, 1H each, J = 6.5 Hz, H1′), 5.48 (m, 1H, H3′), 4.63 (dq, 2H, J = 8, 8 Hz, CF_3CH_2), 4.48–4.33 (m, 4H, H3′, H5′, H5″, and H4′), 4.05 (m, 1H, H4′), 3.73 (s, 6H, CH_3O), 3.40 (m, 2H, H5′ and H5″), 2.90–2.80 and 2.28–2.23 (m, 2H each, H2′ and H2″), 1.81 (s, 3H, 5-CH_3); ^{31}P NMR (CD_3OD) δ: −2.71; ^{19}F NMR (CD_3OD) δ: −76.88 (t, J = 8 Hz); FABMS *m/e*: 1066 (M + Na^+). Compound **14***b*: TLC (silica gel, solvent B) R_f 0.32; ^1H NMR (CD_3OD) δ: 13.3 and 10.3 (bs, 1H each, NH), 8.56 (s, 1H, H2), 8.43 (s, 1H, H8), 8.07 (d, 2H, J = 8 Hz, BzH), 7.65 (d, 1H, J = 8 Hz, BzH), 7.55 (t, 2H, J = 8 Hz, BzH), 7.47 (q, 1H, J = 1 Hz, H6), 7.37–7.12 (m, 7H, ArH), 6.75 (d, 4H, J = 8 Hz, ArH), 6.52 and 6.20 (t, 1H each, J = 6.5 Hz, H1′), 5.42 (m, 1H, H3′), 4.59 (dq, 2H, J = 8, 8 Hz, CF_3CH_2), 4.48–4.33 (m, 4H, H3′, H5′, H5″, and H4′), 4.05 (m, 1H, H4′), 3.73 (s, 6H, CH_3O), 3.40 (m, 2H, H5′ and H5″), 2.90–2.80 and 2.28–2.23 (m, 2H each, H2′ and H2″), 1.80 (s, 3H, 5-CH_3); ^{31}P NMR (CD_3OD) δ: −2.87; ^{19}F NMR (CD_3OD) δ: −76.85 (t, J = 8 Hz); FABMS *m/e*: 1066 (M + Na^+).

*(Rp,Sp)-N^4-Benzoyl-5′-(4,4′-dimethoxytrityl)-2′-deoxycytidylyl-3′,5′-N^6-benzoyl-3′-O-[(1,1dimethylethyl)dimethylsilyl]-2′-deoxyadenosine (2,2,2-trifluoroethyl)phosphates (**20**a and **20**b)*

Using a similar procedure as described for **18**, from **6** (2.62 g, 3.04 mmol) and **17** (1.23 g, 2.62 mmol), **20** (2.52 g, 2.02 mmol, 77% yield) was obtained as a 1:1 mixture of two diastereomers. The mixture (500 mg) was separated by flash chromatography on silica gel, eluted with 4% acetic acid in ethyl acetate, giving **20***a* (180 mg, 36%) and **20***b* (216 mg, 43%). Compound **20***a*: TLC (silica gel, solvent B) R_f 0.61 and TLC (silica gel, solvent C) R_f 0.32; ^{1}H NMR ($CDCl_3$) δ: 9.25 (bs, 1H, NH), 8.67 and 8.24 (s, 1H each, H2 and H8), 8.10 (d, 1H, J = 7.5 Hz, H6), 8.05 and 7.93 (dd, 2H each, J = 8, 1 Hz, BzH), 7.55 and 7.50 (td, 1H each, J = 8, 1 Hz, BzH), 7.45 and 7.42 (t, 2H each, BzH), 7.36–7.19 (m, 10H, ArH and H5), 6.95 (dd, 4H, J = 8, 1 Hz, ArH), 6.46 and 6.31 (t, 1H each, J = 7.5 Hz, H1′), 5.11 (m, 1H, H3′), 4.71 (ddd, 1H, J = 6, 4.5, 4.5 Hz, H3′), 4.43–4.24 and 4.16 (m, 6H, H4′, CF_3CH_2, H5′, and H5″), 3.72 (s, 6H, CH_3O), 3.44 (m, 2H, H5′ and H5″), 2.99–2.91 (m, 2H, H2′ and (or) H2″), 2.46 (ddd, 1H, J = 14, 5.5, 4.5 Hz, H2′ or H2″), 2.36 (m, 1H, H2′ or H2″), 0.93 (s, 9H, $C(CH_3)_3$), 0.15 (s, 6H, $Si(CH_3)_2$); ^{31}P NMR ($CDCl_3$) δ: −2.28; ^{19}F NMR ($CDCl_3$) δ: −75.23 (t, J = 8 Hz); FABMS *m/e*: 1269 (M + Na^+). Compound **20***b*: TLC (silica gel, solvent B) R_f 0.61 and TLC (silica gel, solvent C) R_f 0.22; ^{1}H NMR ($CDCl_3$) δ: 9.25 (bs, 1H, NH), 8.78 and 8.30 (s, 1H each, H2 and H8), 8.10 (d, 1H, J = 7.5 Hz, H6), 8.00 and 7.90 (dd, 2H each, J = 8, 1 Hz, BzH), 7.50 and 7.46 (td, 1H each, J = 8, 1 Hz, BzH), 7.40 and 7.36 (t, 2H each, BzH), 7.34–7.16 (m, 10H, ArH and H5), 6.81 (dd, 4H, J = 8, 1 Hz, ArH), 6.45 and 6.28 (t, 1H each, J = 7.5 Hz, H1′), 5.13 (m, 1H, H3′), 4.73 (ddd, 1H, J = 6, 4.5, 4.5 Hz, H3′), 4.39–4.18 and 4.16 (m, 6 H, H4′, CF_3CH_2, H5′, and H5″), 3.68 (s, 6H, CH_3O), 3.42 and 5.38 (dd, 1H each, J = 11, 3.5 Hz, H5′ and H5″), 2.96 (ddd, 1H, J = 13, 6.5, 6.5 Hz, H2′ or H2″), 2.91 (ddd, 1H, J = 14, 5.5, 2.5 Hz, H2′ or H2″), 2.43 (ddd, 1H, J = 14, 5.5, 4.5 Hz, H2′ or H2″), 2.32 (m, 1H, H2′ or H2″), 0.90 (s, 9H, $C(CH_3)_3$), 0.10 (s, 6H, $Si(CH_3)_2$); ^{31}P NMR ($CDCl_3$) δ: −2.35; ^{19}F NMR ($CDCl_3$) δ: −75.29 (t, J = 8 Hz); FABMS *m/e*: 1269 (M + Na^+).

*(Rp,Sp)-N^4-Benzoyl-5′-(4,4′-dimethoxytrityl)-2′-deoxycytidylyl-3′,5′-N^6-benzoyl-2′-deoxyadenosine (2,2,2-trifluoroethyl)phosphates (**23**a and **23**b)*

Using the desilylation procedure described for **18**, **20***a* (70 mg, 0.056 mmol) gave **23***a* (30 mg, 0.027 mmol, 48% yield). TLC (silica gel, solvent B) R_f 0.30 and TLC (silica gel, solvent C) R_f 0.35; ^{1}H NMR (Py-d_5) δ: 13.5 and 12.3 (bs, 1H each, NH), 8.71 and 8.52 (s, 1H each, H2 and H8), 8.09 (d, 1H, J = 7.5 Hz, H6), 8.05 and 7.94 (dd, 2H each, J = 8, 1 Hz, BzH), 7.41–7.03 (m, 16H, ArH and H5), 6.75 (dd, 4H, J = 8, 1 Hz, ArH), 6.50 and 6.30 (t, 1H each, J = 6.5 Hz, H1′), 5.27 (m, 1H, H3′), 4.87 (m, 1H, H3′), 4.55–4.35 (m, 6H, H4′, CF_3CH_2, H5′, and H5″), 3.49 (s, 6H, CH_3O), 3.41 (m, 2H, H5′ and H5″), 2.97 (ddd, 1H, J = 14, 6.5, 6 Hz, H2′ or H2″), 2.92 (ddd, 1H, J = 14, 6.5, 2.5 Hz, H2′ or H2″), 2.53 (ddd, 1H, J = 14, 6.5, 4.5 Hz, H2′ or H2″), 2.43 (ddd, 1H, J = 14, 6.5, 7 Hz, H2′ or H2″); ^{31}P NMR (Py-d_5) δ: −1.76; ^{19}F NMR (Py-d_5) δ: −75.39 (t, J = 8 Hz); FABMS *m/e*: 1155 (M + Na^+).

Using the desilylation procedure described for **18**, **20***b* (200 mg, 0.161 mmol) gave 47% yield of **23***b* (85 mg, 0.075 mmol). TLC (silica gel, solvent B) R_f 0.30 and TLC (silica gel, solvent C) R_f 0.35; ^{1}H NMR (Py-d_5) δ: 13.5 and 12.3 (bs, 1H each, NH), 8.73 and 8.57 (s, 1H each, H2 and H8), 8.09 (d, 1H, J = 7.5 Hz, H6), 8.05 and 7.94 (dd, 2H each, J = 8, 1 Hz, BzH), 7.41–7.03 (m, 16H, ArH and H5), 6.75 (dd, 4H, J = 8, 1 Hz, ArH), 6.60 and 6.39 (t, 1H each, J = 6.5 Hz, H1′), 5.27 (m, 1H, H3′), 4.87 (m, 1 H, H3′), 4.55–4.35 (m, 6H, H4′, CF_3CH_2, H5′, and H5″), 3.49 (s, 6H, CH_3O), 3.41 (m, 2H, H5′ and H5″), 2.97 (ddd, 1H, J = 14, 6.5, 6 Hz, H2′ or H2″), 2.92 (ddd, 1H, J = 14, 6.5, 2.5 Hz, H2′ or H2″), 2.53 (ddd, 1H, J = 14, 6.5, 4.5 Hz, H2′ or H2″), 2.43 (ddd, 1H, J = 14, 6.5, 7 Hz, H2′ or H2″); ^{31}P NMR (Py-d_5) δ: −1.86; ^{19}F NMR (Py-d_5) δ: −75.36 (t, J = 8 Hz); FABMS *m/e*: 1155 (M + Na^+).

*(Rp,Sp)-5′-O-(4,4′-Dimethoxytrityl)thymidylyl-3′,5′-N^4-benzoyl-3′-O-[(1,1-dimethylethyl)-dimethylsilyl]-2′-deoxycytidine (2,2,2-trifluoroethyl)phosphates (**21**a and **21**b)*

Using a similar procedure as described for **18**, from **5** (722 mg, 0.94 mmol) and **16** (380 mg, 0.85 mmol), **21** (900 mg, 0.794 mmol, 93% yield) was obtained as a 1:1 mixture of two diastereomers. TLC (silica gel, solvent B) R_f 0.57; ^{1}H NMR ($CDCl_3$) δ: 8.75 and 8.72 (bs, 1H, NH), 8.62 (m, 2H, NH and TH6), 8.03 and 7.99 (d, 1H, J = 8 Hz, C-H6), 7.82 and 7.89 (d, 1H, J = 8 Hz, H5), 7.86 (d, 2H, J = 8 Hz, BzH), 7.70 (t, 1H, J = 8 Hz, BzH), 7.61 (t, 2H, J = 8 Hz, BzH), 7.55 (m, 3H, ArH), 7.30 (m, 6H, ArH), 6.83 (m, 4H, ArH), 6.43 and 6.41 (t, 1H, J = 5.5 Hz, H1′), 6.21 (m, 1H, H1′), 5.16 (m, 1H, H3′), 4.50–1.40 (m, 7H, H3′, H4′, CF_3CH_2, H5′, and H5″), 3.78 (s, 6H, CH_3O), 3.54 and 3.39 (m, 2H, H5′ and H5″), 2.70–2.30 and 2.19 (m, 4H, H2′ and H2″), 1.42 and 1.41 (s, 3H, 5-CH_3), 0.88 and 0.89 (s, 9H, $C(CH_3)_3$), 0.08 and 0.07 (s, 6H, $Si(CH_3)_2$); ^{31}P NMR ($CDCl_3$) δ: −1.93 and −1.97 (1:1); ^{19}F NMR ($CDCl_3$) δ: −75.18 and −75.29 (1:1, t, J = 8 Hz); FABMS *m/e*: 1156 (M + Na^+).

*(Rp,Sp)-5′-O-(4,4′-Dimethoxytrityl)thymidylyl-3′,5′-N^4-benzoyl-2′-deoxycytidine (2,2,2-trifluoroethyl)phosphates (**24**a and **24**b)*

Using the desilylation procedure described for **18**, **21** (480 mg, 0.420 mmol) gave 79% yield of a 1:1 diastereomeric mixture (**24***a* and **24***b*, 340 mg, 0.334 mmol), which was partially separated in the same manner as described for **25**, giving **24***a* and **24***b*. Compound **24***a*: TLC (silica gel, solvent B) R_f 0.33; ^{1}H NMR (Py-d_5) δ: 13.3 and 12.3 (bs, 1H each, NH), 8.40 (d, 1H, J = 8 Hz, C-H6), 8.20 (s, 1H, T-H6), 8.20 (d, 2H, J = 8 Hz, BzH), 7.78 (d, 1H, J = 8 Hz, BzH), 7.73 (t, 2H, J = 8 Hz, BzH), 7.72 (d, 1H, J = 8 Hz, H5), 7.61 (t, 2H, J = 8 Hz, BzH), 7.56–7.30 (m, 9H, ArH), 7.01 (d, 4H, J = 8 Hz, ArH), 6.90 and 6.80 (t, 1H each, J = 6 Hz, H1′), 5.72 (m, 1H, H3′), 4.98 (m, 2H, CF_3CH_2), 4.83 (m, 1H, H3′), 4.72 (m, 2H, H5′ and H5″), 4.68 and 4.60 (m, 1H each, H4′), 3.69 (s, 6H, CH_3O), 3.62 (m, 2H, H5′ and H5″), 2.98–2.80 (m, 3H, H2′ and H2″), 2.56 (ddd, 1H, J = 14, 7, 6 Hz, H2′ or H2″), 1.70 (s, 3H, 5-CH_3); ^{31}P NMR (Py-d_5) δ: −1.34; ^{19}F NMR (Py-d_5) δ: −74.87 (t, J = 8 Hz); FABMS *m/e*: 1042 (M + Na^+). Compound **24***b*: TLC (silica gel, solvent B) R_f 0.33; ^{1}H NMR (Py-d_5) δ: 13.3 and 12.3 (bs, 1H each, NH), 8.47 (d, 1H, J = 8 Hz, C-H6), 8.20 (s, 1H, T-H6), 8.20 (d, 2H, J = 8 Hz, BzH), 7.78 (d, 1H, J = 8 Hz, BzH), 7.73 (t, 2H, J = 8 Hz, BzH), 7.72 (d, 1H, J = 8 Hz, H5), 7.61 (t, 2H, J = 8 Hz, BzH), 7.56–7.30 (m, 9H, ArH), 7.01 (d, 4H, J = 8 Hz, ArH), 6.90 and 6.80 (t, 1H each, J = 6 Hz, H1′), 5.72 (m, 1H, H3′), 4.98 (m, 2H, CF_3CH_2), 4.84 (m, 1H, H3′), 4.76 (m, 2H, H5′ and H5″), 4.63 (m, 2H, H4′), 3.69 (s, 6H, CH_3O), 3.62 (m, 2H, H5′ and H5″), 2.98–2.80 (m, 3H, H2′ and H2″), 2.59 (ddd, 1H, J = 14, 7, 6 Hz, H2′ or H2″), 1.70 (s, 3H, 5-CH_3); ^{31}P NMR (Py-d_5) δ: −1.37; ^{19}F NMR (Py-d_5) δ: −74.88 (t, J = 8 Hz); FABMS *m/e*: 1042 (M + Na^+).

*(Rp,Sp)-N^6-Benzoyl-5′-(4,4′-dimethoxytrityl)-2′-deoxyadenoylyl-3′,5′-N^6-benzoyl-3′-O-[(1,1-dimethylethyl)dimethylsilyl]-2′-deoxyadenosine (2,2,2-trifluoroethyl)phosphates (**22**a and **22**b)*

Using a similar procedure as described for **18**, from **7** (443 mg, 0.50 mmol) and **17** (233 mg, 0.50 mmol), **22** (479 mg, 0.377 mmol, 77% yield) was obtained as a 1:1 mixture of two diastereomers, which was partially separated in a similar manner as used for **25** with 0.5–0.75% methanol in chloroform as eluent, giving **22***a* and **22***b*. Compound **22***a*: TLC (silica gel, solvent B) R_f 0.43; ^{1}H NMR ($CDCl_3$) δ: 13.3 and 12.3 (bs, 2H, NH), 8.73, 8.60, 8.17 and 8.13 (s, 1H each, H2 and H8), 8.00 and 7.95 (d, 2H each, J = 8 Hz, BzH), 7.68 (t, 1H, J = 8 Hz, BzH), 7.60–7.13 (m, 14H, ArH), 6.75 (d, 4H, J = 8 Hz, ArH), 6.45 (dd, 1H, J = 9, 6 Hz, H1′), 6.43 (t, 1H, J = 6.5 Hz, H1′), 5.24 (m, 1H, H3′), 4.71 (m, 1H, H3′), 4.42–4.20 and 4.17 (m, 6H, H4′, CF_3CH_2, H5′, and H5″), 3.72 (s, 6H, CH_3O), 3.40 and 3.36 (dd, 1H each, J = 10, 4 Hz, H5′ and H5″), 3.12, 2.92, 2.48, and 2.73 (m, 1H each, H2′ and H2″), 0.92 (s, 9H, $C(CH_3)_3$), 0.12 (s, 6H, $Si(CH_3)_2$); ^{31}P NMR ($CDCl_3$) δ: −2.20; ^{19}F NMR ($CDCl_3$) δ: −75.23 (t, J = 8 Hz); FABMS *m/z*: 1293 (M + Na^+). Compound **22***b*: TLC (silica gel, solvent B) R_f 0.43; ^{1}H NMR ($CDCl_3$) δ: 13.3 and 12.3 (bs, 2H, NH), 8.70, 8.59, 8.19 and 8.09 (s, 1H each, H2 and H8), 8.00 and 7.95 (d, 2H each, J = 8 Hz, BzH), 7.68 (t, 1H, J = 8 Hz, BzH), 7.60–7.13 (m, 14H, ArH), 6.75 (d, 4H, J = 8 Hz, ArH), 6.41 (dd, 1H, J = 9, 6 Hz, H1′), 6.44 (t, 1H, J = 6.5 Hz, H1′), 5.24 (m, 1H, H3′), 4.71 (m, 1H, H3′), 4.42–4.20 and 4.17 (m, 6H, H4′, CF_3CH_2, H5′, and H5″), 3.74 (s, 6H, CH_3O), 3.40 and 3.36 (dd,

1H each, J = 10, 4 Hz, H5′ and H5″), 3.06, 2.95, 2.48, and 2.48 (m, 1H each, H2′ and H2″), 0.92 (s, 9H, $C(CH_3)_3$), 0.12 (s, 6H, $Si(CH_3)_2$); ^{31}P NMR ($CDCl_3$) δ: −2.44; ^{19}F NMR ($CDCl_3$) δ: −75.29 (t, J = 8 Hz); FABMS *m/e*: 1293 ($M + Na^+$).

Acknowledgments

This work was supported by a grant (J.W.L.) from the Natural Sciences and Engineering Research Council of Canada and by the Department of Chemistry of the University of Alberta.

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