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STEREOSELECTIVE SYNTHESIS OF DINUCLEOSIDE PHOSPHATE ARYL ESTERS BY USING NEW CONDENSING REAGENTS, ARENESULFONYL 5-(PYRIDIN-2-YL)-TETRAZOLE[1]

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Abstract—Deoxyadenylyl-(3'-5')-phosphoro-*o*-chlorophenyl deoxyadenosine having the *Sp* configuration was synthesised stereoselectively by condensing N,5'-bis-dimethoxytrityldeoxyadenosine 3'-(*o*-chlorophenyl)phosphate with N,3'-bis-dimethoxytrityldeoxyadenosine using 1-2,4,6-triisopropylbenzene-5-(pyridin-2-yl)tetrazole as the activating reagent followed by dedimethoxytritylation. The absolute configuration of this diastereomer was determined spectroscopically by comparison of two corresponding diastereomers which were prepared by using a conventional condensing reagent and isolated by chromatography on silica gel. Their nuclear Overhauser effect in proton magnetic resonances and the circular dichroism of these two diastereoisomers could only be explained if the product obtained by the stereoselective synthesis had *Sp* configuration. Our results also suggest that phosphotriesters of this type exist in solution as an equilibrium of folded and extended forms.

The phosphotriester method for oligonucleotide syntheses has been improved by the introduction of phenyl derivatives as protecting groups for internucleotide linkages.[2] These triester intermediates are sufficiently stable during reactions and isolation processes. However, diastereoisomers which arise from phosphorus chirality give complex products. We have previously reported that one of the diastereoisomers of dinucleoside phosphate *o*-chlorophenyl esters could be synthesised[3] stereospecifically by using 1-(arenesulfonyl)-5-(pyridin-2-yl)-tetrazole[4] as the activating reagent for nucleoside phosphoroaryl esters. In the present paper, we describe the stereoselective synthesis of a phosphotriester and the absolute configuration of the product, *Sp*-deoxyadenylyl-(3'-5')-(*o*-chlorophenyl)deoxyadenosine (dAp(Ar)A). Mechanisms for the activation of phosphodiesters with arenesulfonyl azolides have been proposed by several investigators[5] but the details of active intermediates are still to be resolved. We have explained some steps in the condensation reaction by using the new condensing reagent in this report. Spectral data for the two diastereoisomers of dAp(Ar)A which were obtained by using conventional condensing reagents have been used to estimate the absolute configuration of *Sp*-dAp(Ar)A.

Preparation of the two diastereoisomers of deoxyadenylyl-(3'-5')-(o-chlorophenyl)deoxyadenosine (**11**)

The triester (**11**) was synthesised by condensation of monomers bearing dimethoxytrityl groups as illustrated in Chart 1. Two types of deoxyadenosine derivative (**5** and **8**) were used as intermediates. N,5'-O-bis-dimethoxytrityldeoxyadenosine (**5**) was prepared by protection of the 3'-hydroxyl function with t-butyldimethylsilyl ether. The fully protected nucleoside (**4**) was obtained by N-dimethoxytritylation of 5'-O-dimethoxytrityl-3'-O-t-butyldimethylsilyldeoxyadenosine (**3**) and the N,5'-protected nucleoside (**5**) was synthesised by removal of the silyl group with tetrabutylammonium fluoride (TBAF). The 5'-unblocked deoxyadenosine (**8**) was prepared via 5'-O-t-butyldimethylsilyldeoxyadenosine (**6**). The 5'-protected deoxyadenosine (**6**) was dimethoxytritylated to give the bis-dimethoxytritylated product (**7**) which was converted to N,3'-O-bis-dimethoxytrityldeoxyadenosine (**8**) by treatment with TBAF. The dinucleoside monophosphate triester (**10**) was synthesised by condensation of **8** with N,5'-O-bis-dimethoxytrityldeoxyadenosine 3'-O-(*o*-chlorophenyl)phosphate (**9**) which was prepared by phosphorylation of **5** with *o*-chlorophenyl phosphoroditriazolide. 1 - (Mesitylene - 2 - sulfonyl) - 3 - nitro - 1,2,4 - triazole was used as the condensing reagent. Two diastereoisomers were detected by thin layer chromatography (TLC) on silica gel. The protected triester with a high R_f value (**10-h**) was separated from the product with a low R_f value (**10-l**) by column chromatography on silica gel. **10-h** and **10-l** were deblocked with 80% AcOH separately and the products were named as **11-h** and **11-l**, respectively.

Stereoselective synthesis of the triester **11-l**

N - 5' - O - Bis - dimethoxytrityldeoxyadenosine 3'-O-(*o*-chlorophenyl)phosphate (**9**) and the 5'-hydroxyl component (**8**) were condensed using 1-(2,4,6-triisopropylbenzenesulfonyl) - 5 - (pyridin - 2 - yl) tetrazole. The major product which travelled with **10-l** was observed by TLC on silica gel. A trace of a faster moving compound was also detected and its amount was estimated as less than 5%. In order to investigate the effect of 5-(pyridin-2-yl)tetrazole in the selective formation of the triester (**10-l**), a mixture of triisopropylbenzenesulfonyl chloride (TPS) and a threefold excess of 5-(pyridin-2-yl)tetrazole was used to condense the phosphodiester component (**9**) and the nucleoside (**8**). Formation of the two diastereomers was confirmed by TLC. Previously, Seth and Jay[5] reported that the presence of a threefold excess of tetrazole markedly enhanced the condensation of a phos-

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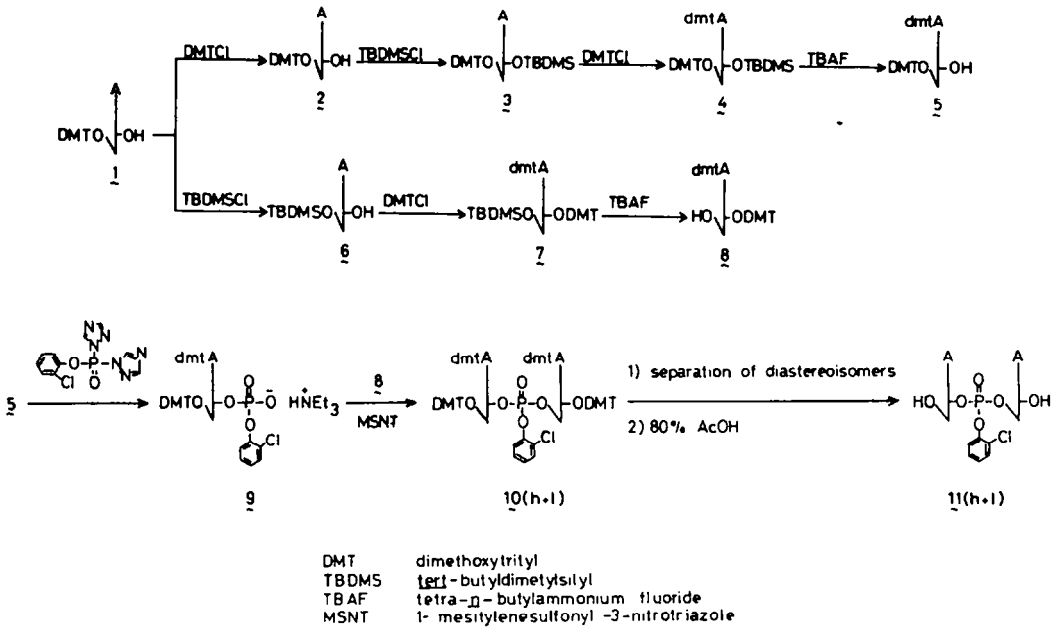

Chart 1.

phodiester and a nucleoside with TPS. They proposed that successive conversion to an active intermediate phosphorotetrazolide occurred. The present results also suggest the formation of these types of intermediate. It seems that the selective reaction occurred during formation of sulfonic anhydride (12).

Absolute conformations of **11-h** *and* **11-l**

^{31}P-NMR of **11-h** and **11-l** indicated appreciable differences between the spectra of the two isomers (Fig. 1). ^{1}H-NMR chemical shifts of the two triesters are listed in Table 1. Nuclear Overhauser effects (NOE) of the isomers were investigated to elucidate possible models. When aromatic protons of the lower isomer (**11-l**) at 7.2 ppm were irradiated, negative NOEs were observed at signals for the H—$C_{1'}$ and H—$C_{4'}$ on the 5'dAp (Fig. 2a). However the higher isomer (**11-h**) did not show any change on the same treatment. Irradiation of the other aromatic protons at 7.5 ppm resulted in an NOE at H—C_8 of the deoxyadenosine of **11-l**. No effect was observed with **11-h** (Fig. 2b).

These data are explicable if the absolute configurations of **11-h** and **11-l** are *Rp* and *Sp*, respectively. Coupling constants between H1' and H2' ($J_{1'2'}$) and between H1' and H2" ($J_{1'2''}$), as shown in Table 1, show that the C2'-*endo* conformation of the furanose ring is predominant[6] in both sugars of **11-l**. The result of NOE experiments indicate that irradiated protons on the *o*-chlorophenyl residue in **11-l** are close to the H—$C_{1'}$ and H—$C_{4'}$ of dAp and H—C_8 of -pdA at 40°.

Considering these observations, Corey–Pauling–Koltun (CPK) models were built, which suggested that only the *Sp* configuration could reasonably take such a conformation (Figs 3a and b). The *Sp* form can take a relatively better stacked form than is possible for the *Rp* form (Fig. 3a). It is difficult for the *Rp* isomer to take a conformation which is consistent with the NOE results observed for **11-l** (Fig. 3b). It is most probable that **11-l** has the *Sp* configuration. Therefore **11-h** is assumed to have the *Rp* configuration.

Michels and Schlimme[7] synthesised and separated two diastereoisomers of fully protected dApA. They concluded that the higher and lower isomers could be assigned to *Rp* and *Sp*, respectively, on the basis of chemical shift consideration for base protons. However, our results offer more definitive information on the stereochemistry of phosphotriesters since sugar protons are completely assigned and significant NOEs were observed.

Recently Potter *et al.* analysed the stereochemistry of the methylesters of dTpT,[8] UpA[9] and dGpA[10] on the

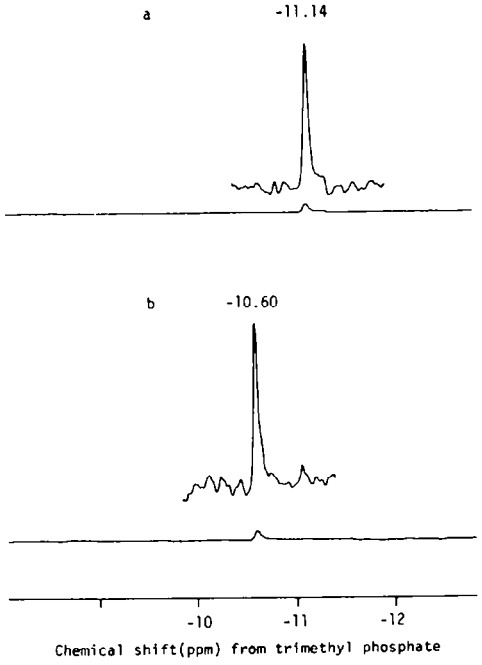

Fig. 1. ^{31}P-NMR spectra of **11-h** (a) and **11-l** (b).

Table 1. ^{1}H-NMR Chemical shifts and coupling constants of **11-h** and **11-l**

Compound	Residue	Chemical shifts (ppm from DSS)										Coupling constants (Hz)	
		1′	2′	2″	3′	4′	5′	5″	2	8	Ar-H	J1′2′	J1′2″
11-h	dAp-	6.15	2.53	2.66	5.28	4.28	3.71	3.71	8.02	8.15		8.5	5.5
	-pdA	6.38	2.83	2.65	4.70	4.33	4.56	4.51	8.08	8.16		5.9	5.9
	Ar[a]										7.21–7.24 (3H) 7.49–7.50 (1H)		
11-l	dAp-	6.25	2.51	2.65	5.20	4.23	3.67	3.67	7.95	8.16		8.8	5.1
	-pdA	6.40	2.94	2.67	4.81	4.34	—[b]	4.58	8.16	8.24		6.6	6.6
	Ar[a]										7.15–7.24 (3H) 7.48–7.50 (1H)		

[a] *o*-Chlorophenyl.
[b] Merged with HDO.

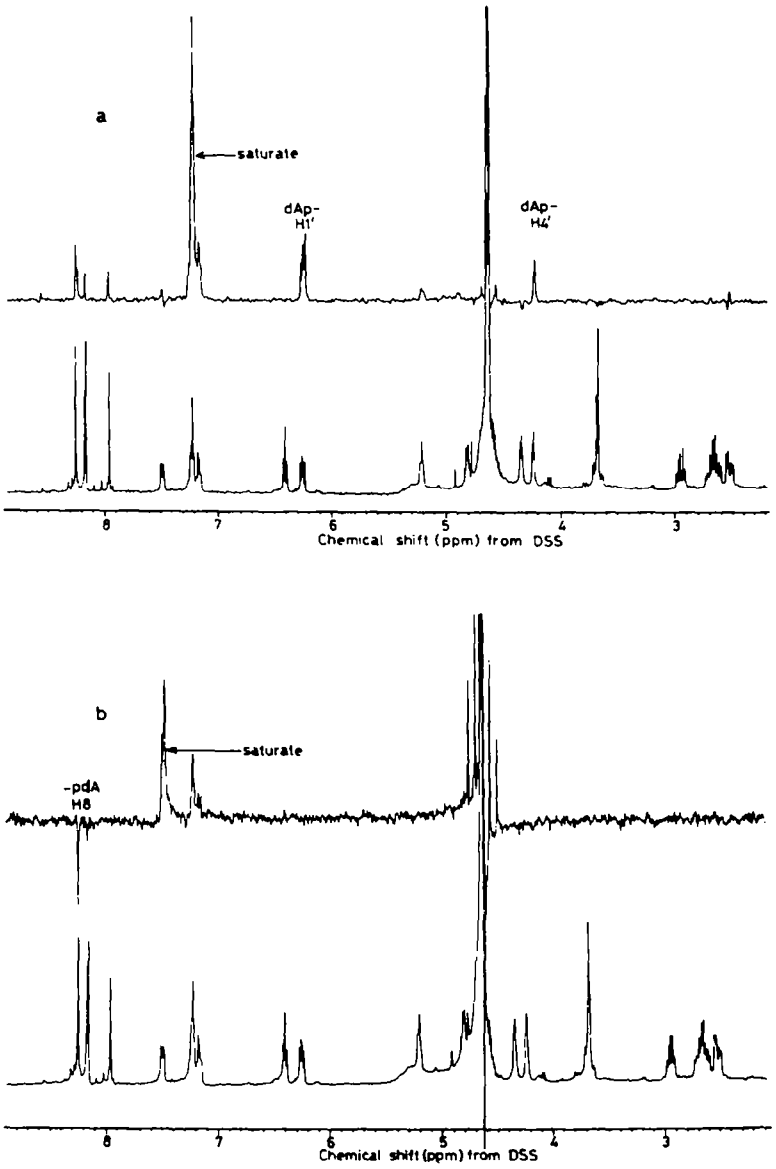

Fig. 2. ^{1}H-NMR spectra of **11-l**. The difference spectra after saturation of *o*-chlorophenyl protons at 7.22 ppm (a) and 7.49 ppm (b), respectively. For details see Experimental.

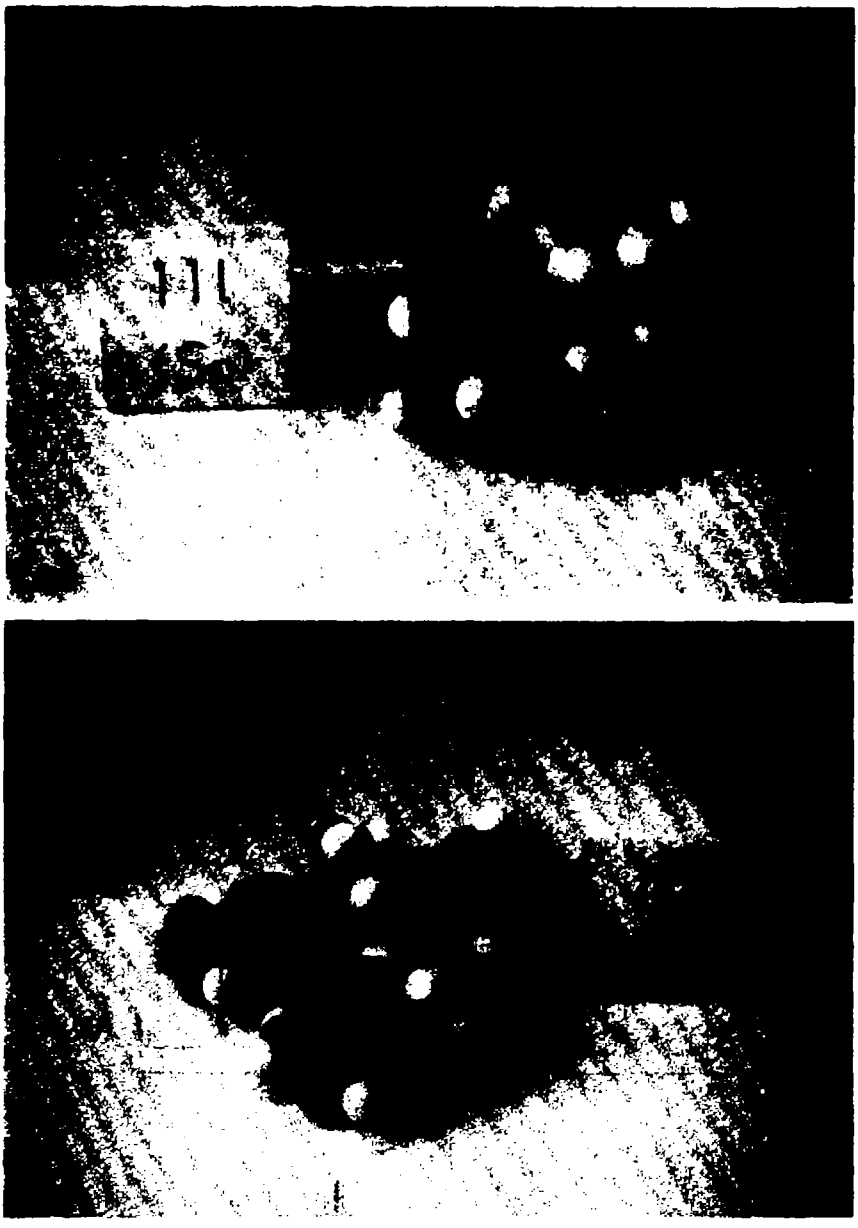

Fig. 3. CPK model for the dimer with *Sp* (a) and *Rp* (b) configuration.

basis of ^{18}O-isotope effects in ^{31}P-NMR. They demonstrated that the ^{31}P-NMR resonance at the lower field could be assigned to the *Sp* isomer, and the one at the higher field to the *Rp* isomer. These results are consistent with our tentative ^{31}P-NMR assignments on the isomers of dApA *o*-chlorophenyl ester.

CD spectra of **11-h** *and* **11-l**

The CD spectra of the two diastereoisomers of the triester **11-h** and **11-l** were measured at different temperature as shown in Figs 4(a) and (b) and compared to that of the corresponding diester dApA (Fig. 4c). The triesters showed a smaller molecular ellipticity value $[\theta]$ than dApA at 20° but they seemed to have a right-handed B-form like structure. The slower travelling isomer (**11-l**) which was identical to the one obtained by the stereoselective synthesis showed a larger $[\theta]$ value at low temperature. On the other hand, the faster travelling isomer (**11-h**) showed no temperature dependency. This may mean that **11-l** adopts a base–base stacked form at lower temperature while the other isomer (**11-h**) exists as an unstacked form.

The CPK model building study also suggested that it is difficult for the *Rp* isomer to take a stacked conformation. This is consistent with the NOE results for **11-h** which is assigned as the *Rp* isomer.

CONCLUSION

The spectroscopic data discussed above are explicable if the absolute configurations of **11-h** and **11-l** are *Rp* and *Sp*. Based on the stereochemistry discussed above and the mechanism for the phosphotriester condensation reaction proposed by Seth and Jay,[5] the

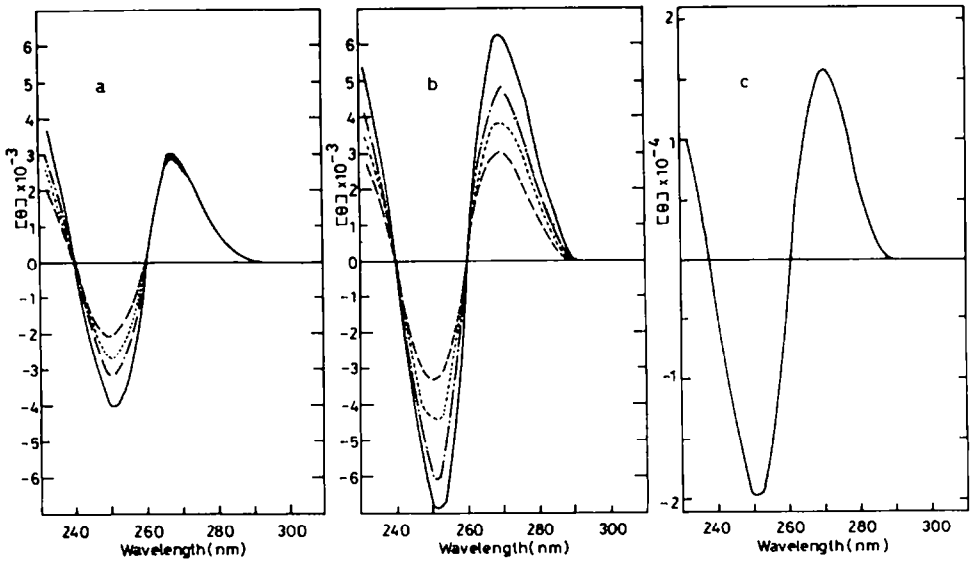

Fig. 4. CD spectra of dimers: (a) **11-h**; (b) **11-l**; (c) dApA at 20°. For (a) and (b), spectra were measured at 0°, ——; 20°, — · — · —; 40°, - - - - -; 60°, — —.

stereoselective reaction mechanism could be postulated. Since **10-l**, which was obtained stereoselectively by using TPSPy, was converted to **11-l**, **10-l** is considered to have the *Sp* configuration. By using TPS and 5-(pyridin-2-yl)tetrazole as condensing reagents, two isomers were obtained. This may mean that the stereoselective reaction occurred at the stage of formation of the mixed anhydride (Chart 2). In Chart 2, the assumed reaction mechanism is illustrated. Our proposed mechanism is as follows. When phosphate–sulfate mixed anhydride is formed, the *pro-S* oxygen undergoes selective sulfonation. The *pro-R* oxygen may not undergo sulfonation, since it may interact, in the transition state, with the nitrogen of the pyridine moiety of the condensing reagent by means of a triethylammonium ion. Mixed anhydride formation is followed in turn by two successive substitutions at phosphorus by tetrazole then by the 5′-hydroxyl group of the sugar. Since each substitution proceeds with inversion of configuration at phosphorus, the final product possesses the *Sp* configuration.

EXPERIMENTAL

M.ps were determined on a Yanagimoto micro m.p. apparatus and are uncorrected. TLC was performed on plates

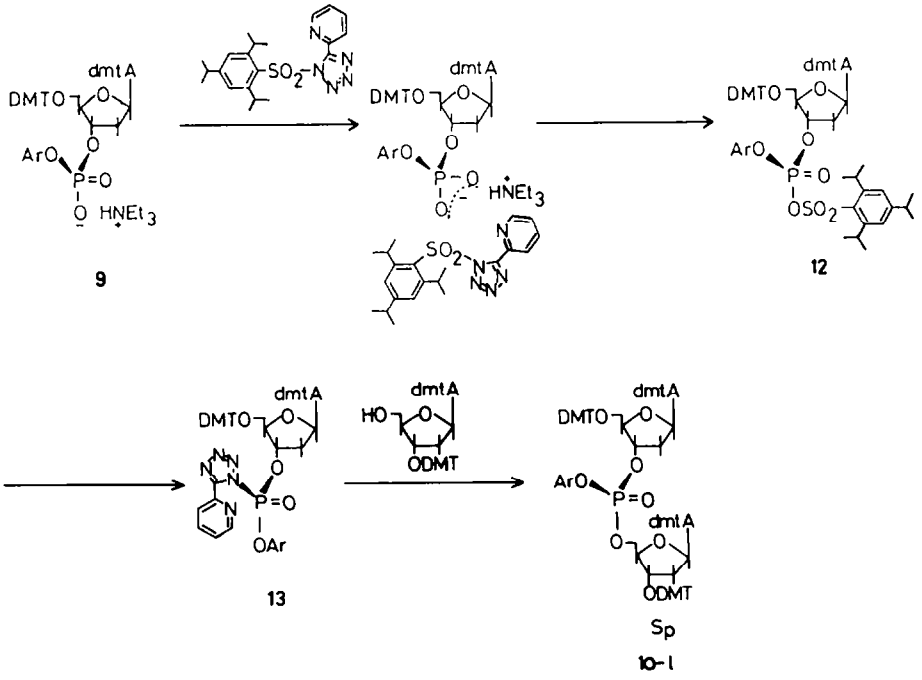

Chart 2.

of silica gel (Kieselgel $60F_{254}$, Merck) using a mixture of $CHCl_3$ and MeOH. For reversed phase thin layer chromatography (RTLC), silanised silica gel, high performance thin layer chromatography (HPTLC) RP-2 or RP-18 F_{254} was used with a mixture of acetone–H_2O. For columns, silica gel (60H, Merck) was packed with $CHCl_3$ and elution was carried out with a mixture of $CHCl_3$ and MeOH. For reversed phase column chromatography, alkylated silica gel (C-18, 35–105 μ, Waters) was used with aqueous acetone.

CD spectra were recorded on a JASCO J-500A spectropolarimeter. The molar ellipticity, $[\theta]$, is presented in terms of per base residue values. The molar absorption coefficient, ε, was determined by quantitative analysis of phosphorus according to the procedure of Chen *et al.*[11] CD samples were adjusted to $1A_{260}$ per 1 ml of 10 mM of sodium-cacodylate buffer.

NMR experiments were performed on a Bruker WM-360 wb spectrometer (360 MHz for ^1H and 145.8 MHz for ^{31}P) with data accumulated in the Fourier transform mode. The ^1H chemical shifts were determined relative to internal 2-methyl-2-propanol, which had in turn been referenced to sodium 3-(trimethylsilyl)-propane-sulfonate (DSS). The ^{31}P chemical shifts were determined relative to external trimethyl phosphate. Nuclear Overhauser effects (NOE) were measured by applying a 2 s single-frequency preirradiation pulse prior to data acquisition with 2 ms delay. Negative NOE's are presented as positive peaks in the difference spectra. NMR samples ($20A_{260}$) were lyophilised three times with 99.85% D_2O and the final solutions were made up in 0.4 ml of 99.95% D_2O. ^1H-NMR spectra were measured at 40° and ^{31}P at 45°.

5'-O-Dimethoxytrityl deoxyadenosine (**2**)

Compound **2** was synthesised according to the procedure of Ti *et al.*[12]

3' - O - t - Butyldimethylsilyl - 5' - O - dimethoxytrityl deoxyadenosine (**3**)

To 6.15 g (11.1 mmol) of **2** and 2.26 g (33.3 mmol) of imidazole dried three times by evaporation of pyridine and dissolved in 11 ml of dry DMF was added 2.44 g (16.7 mmol) of TBDMSCl and the mixture was stirred at room temp for 1 hr. The mixture was then added dropwise to 150 ml of H_2O and the resulting mixture was stirred for 10 min. The gum was collected, dissolved in $CHCl_3$, and washed three times with sat $NaHCO_3$ aq, then twice with H_2O. The product **3** was separated by chromatography on silica gel (Kieselgel 60 H, 70 g, $\phi 7 \times 5.1$ cm) and crystallised from ether–hexane to yield 5.61 g (8.40 mmol) of **3** (75.7%). M.p. 151–153°. (Calc for $C_{37}H_{45}N_5O_5Si$: C, 66.54; H, 6.79; N, 10.49. Found: C, 66.32; H, 6.76; N, 10.35%.)

3' - O - t - Butyldimethylsilyl - N,5' - O - bisdimethoxytrityl deoxyadenosine (**4**)

To 4.94 g (7.40 mmol) of **3** dried three times by evaporation of pyridine and dissolved in 40 ml of dry pyridine were added 4.51 g (13.3 mmol) of dimethoxytrityl chloride and 22 mg (0.19 mmol) of 4-dimethylaminopyridine, and the mixture was stirred at room temp for 12 hr. A small amount of 0.1 M TEAB buffer was added with cooling, and the solvent was evaporated. The residue was dissolved in chloroform, washed with 0.1 M TEAB buffer, and twice back extracted with chloroform. The combined organic layers were washed twice with 0.1 M TEAB buffer. The product (**4**) was separated by reversed phase chromatography on C-18 silica gel ($\phi 5 \times 7$ cm). The eluent was evaporated, and to the residual gum was added a small amount of ethyl acetate followed by evaporation to dryness to yield 7.11 g (7.33 mmol) of **4** (quant.).

N,5'-O-Bisdimethoxytrityl deoxyadenosine (**5**)

To 10 ml of a 1 M soln of TBAF (in tetrahydrofuran) was added 7.11 g (7.33 mmol) of **4** and the mixture was stirred at room temp for 1.5 hr. The reaction mixture was diluted with 10 ml of pyridine–methanol–H_2O (3 : 1 : 1, v/v/v) and passed through a 44 ml Dowex 50 × 2 (pyridinium form) column. The resin was eluted with 130 ml of pyridine–MeOH–H_2O (3 : 1 : 1, v/v/v). To the combined eluents was added a small amount of Et_3N and the solvent was evaporated. The residual gum was dissolved in chloroform, washed twice with sat $NaHCO_3$ aq, then twice with H_2O. The organic layer was concentrated, and the crude product was precipitated with hexane. The product **5** was separated by reversed phase chromatography on C-18 silica gel ($\phi 5 \times 7$ cm), and precipitated with hexane from its soln in $CHCl_3$ to yield 5.45 g (6.37 mmol) of **5** (86.9%). M.p. 130–133° (dec.). ^1H-NMR ($CDCl_3$) δ 8.00 (s, 1H, H8), 7.90 (s, 1H, H2), 7.5–7.0 (m, 18H, phenyl H), 6.85–6.65 (m, 8H, phenyl H), 6.34 (t, 1H, H1'), 4.65–4.40 (br, 1H, H3'), 4.15–3.95 (m, 1H, H4'), 3.69 (s, 6H, —OCH_3), 3.67 (s, 6H, —OCH_3), 3.40–3.25 (m, 2H, H5'5"), 2.8–2.2 (m, 2, H2'2"). (Calc for $C_{52}H_{49}N_5O_7 \cdot 1/2\,H_2O$: 72.20; H, 5.85; N, 8.10. Found: C, 72.18; H, 5.92; N, 7.94%.)

5'-O-t-Butyldimethylsilyl deoxyadenosine (**6**)

Compound **6** was synthesised according to Ogilvie's procedure.[13]

5' - O - t - Butylmethylsilyl - N,3' - O - bisdimethoxytrityl deoxyadenosine (**7**)

To 1.89 g (5.16 mmol) of **6** dried twice by evaporation of pyridine and dissolved in 26 ml of dry pyridine were added 5.25 g (15.5 mmol) of dimethoxytrityl chloride and 16 mg (0.13 mmol) of 4-dimethylaminopyridine, and the mixture was stirred at room temp for 12 hr. The mixture was worked up in a similar manner to **4** to yield 4.60 g (4.74 mmol) of **7** (91.9%).

N,3'-O-Bisdimethoxytrityl deoxyadenosine (**8**)

To a 6 ml of 1 M soln of TBAF (in THF) was added 4.60 g (4.74 mmol) of **7** and the mixture was stirred at room temp for 1 hr. The mixture was worked up in a similar manner to **5** to yield 2.60 g (3.97 mmol) of **8** (83.8%). M.p. 143–146° (dec.). ^1H-NMR ($CDCl_3$) δ 7.93 (s, 1H, H8), 7.71 (s, 1H, H2), 7.45–7.05 (m, 18H, phenyl H), 6.9–6.7 (m, 8H, phenyl H), 6.19 (q, 1H, H1'), 4.57 (br, 1H, H3'), 4.04 (br, 1H, H4'), 3.73 (s, 6H, —OCH_3), 3.71 (s, 6H, —OCH_3), 3.26 (m, 2H, H5'H5"), 3.0–2.6 (m, 2H, H2'H2"). (Calc for $C_{52}H_{49}N_5O_7 \cdot 1/2\,H_2O$: C, 72.20; H, 5.85; N, 8.10. Found: C, 72.26; H, 5.69; N, 7.95%.)

Condensation of **8** *and* **9** *(by using MSNT)—synthesis of* **10** **(h + l)**

To 1.07 g (1.25 mmol) of **5** dried three times by evaporation of pyridine was added 1.75 mmol of *o*-chlorophenyl phosphoroditriazolide in 5.25 ml of dioxane and the mixture was stirred at room temp for 20 min. A small amount of 50% pyridine aq was added with cooling and the solvent was evaporated. The residual gum was dissolved in $CHCl_3$, washed with 0.1 M TEAB buffer, and twice back-washed with chloroform. The combined organic layers were washed five times with 0.1 M TEAB buffer, and the solvent was removed to yield **9** (gum, quant.).

To the gum of **9** was added 856 mg (1 mmol) of **8**, dried three times by evaporation of pyridine, and dissolved in 3 ml of dry pyridine. To the solution was added 741 mg (2.5 mmol) of 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole (MSNT) and the mixture was stirred at room temp for 15 min. A small amount of 50% pyridine aq was added with cooling and the solvent was evaporated. The residual gum was dissolved in chloroform, washed with 0.1 M TEAB buffer, and twice back-washed. The combined organic layers were washed three times with 0.1 M TEAB buffer, and concentrated to 10 ml. 3 ml of the $CHCl_3$ soln was applied to a column of silica gel (Kieselgel 60 H, 90 g, $\phi 3 \times 39.2$ cm). The products were precipitated with hexane from their solns in $CHCl_3$ to yield 160 mg (88.0 μmol) of **10-h**, 119 mg (63.3 μmol) of **10-l**, and 74 mg (39.2 μmol) of **10-(h + l)**.

R_f value (Kieselgel $CHCl_3$–MeOH = 40 : 1): **10-h**, 0.48; **10-l**, 0.39.

Conversion of **10-h** *to* **11-h**

To 40.4 mg (21.4 μmol) of **10-h** was added 1 ml of 80% AcOH and the mixture was stirred at room temp for 2 hr. AcOH was

evaporated followed by coevaporation with ethanol. The product (**11-h**) was separated by reversed phase chromatography on a column (ϕ 0.8 × 9.8 cm) of C-18 silica gel. Elution was performed with a linear gradient of acetonitrile (20–40%) in 0.1 M TEAA buffer to yield 177 A_{260} (6.44 μmol) of **11-h** (30.1%). M.p. 135° (dec.). R_f value (Kieselgel $CHCl_3$–MeOH = 10:2) 0.32 ε_{260} 2.79×10^4. (Calc for $C_{26}H_{28}ClN_{10}O_8P \cdot H_2O$; C, 45.06; H, 4.36; N, 20.21; Cl, 5.12. Found: C, 45.00; H, 4.10; N, 19.88; Cl, 5.21%.)

Conversion of **10-l** *to* **11-l**

To 42.4 mg (22.5 μmol) of **10-l** was added 1 ml of 80% AcOH and the mixture was stirred at room temp for 2 hr. The mixture was worked up as for **11-h** to yield $444A_{260}$ (16.1 μmol) of **11-l** (71.6%). M.p. 135° (dec.). R_f value (Kieselgel $CHCl_3$–MeOH = 10:2) 0.28 ε_{260} 2.68×10^4. (Calc for $C_{26}H_{28}ClN_{10}O_8P \cdot H_2O$; C, 45.06; H, 4.36; N, 20.21; Cl, 5.12. Found: C, 45.05; H, 4.15; N, 20.04; Cl, 5.23%.)

Condensation of **8** *and* **9** *(by using TPSPy)*

Using TPSPy as a condensing reagent, **8** and **9** were condensed as described above. **10-l** was obtained stereoselectively.

Condensation of **8** *and* **9** *(by using TPS and 5-(pyridin-2-yl)tetrazole*

Using TPS and 5-(pyridin-2-yl)tetrazole (2 and 6 equiv to **9**, respectively) as condensing reagents, **8** and **9** were condensed as described above. Two diastereoisomers of **10** (**10-h** and **10-l**) were obtained.

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