The Direct Synthesis of c-AMP Derivatives and Selective 3',5'-Hydroxy Group Protection of Adenosine

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The direct synthesis of a c-AMP derivative by reaction of N^6,N^6-bis-TCBOC-adenosine† with TCB-chlorophosphite-*N*,*N*-diisopropylamidite and subsequent oxidation is described, as well as the analogous preparation of a thymidine derivative. The N^6,N^6-bis-TCBOC adenosine 3',5'-cyclophosphite is formed in practically quantitative yield by highly selective 5'-phosphitylation in DMF at −30°C and subsequent cyclization by NTP in boiling acetonitrile. The c-AMP derivative results from oxidation of the cyclic phosphite with 3-(2,4-dichlorophenyl)-2-tosyloxaziridine.

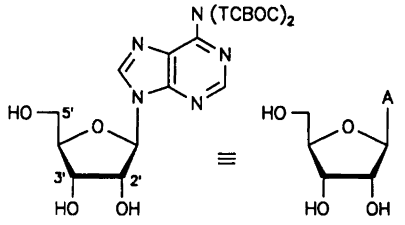
Dedicated to Professor Salo Gronowitz on the occasion of his 65th birthday.

When the unprotected hydroxy groups of the ribose moiety of ribonucleoside derivatives such as **1** react with bifunctional reagents, ring closure occurs preferentially between the hydroxy groups at the 2'- and 3'-positions.[1] Ring formation involving the 3'- and 5'-hydroxy groups will only be favoured, if a bifunctional reagent is used whose primary attack occurs with a high degree of selectivity at the 5'-hydroxy group.

Reagents with considerable steric bulk react more rapidly with primary alcohols than with secondary alcohols and are therefore good candidates for selective reactions at the 5'-hydroxy group of nucleoside derivatives. Such 5'-selectivity is exploited in the preparation of 5'-protected nucleoside derivatives that are needed in the synthesis of oligonucleotides.[2]

In the synthesis of DNA and RNA fragments protection of the 5'-hydroxy group of nucleoside derivatives is necessary. As a rule, this 5'-hydroxy group is protected by bulky reagents that selectively attack the primary 5'-hydroxy group of nucleoside derivatives in the presence of free secondary hydroxy groups at the 2'- and 3'-positions. The trityl[3] and pixyl[4] groups and some of their derivatives as well as the bis-TCB-phosphate groups[5] are such bulky 5'-protective groups that can be selectively introduced.

The synthesis of c-AMP derivatives[8] and related compounds is generally accomplished by forming the respective 3',5'-cyclophosphates by intramolecular cyclization of activated 5'-phosphonucleotides or by reaction of nucleoside derivatives with bifunctional phosphorylating reagents.[9]



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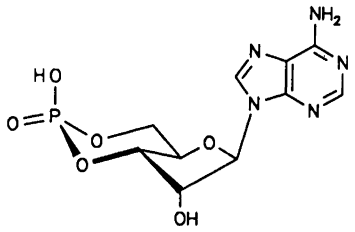

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In 1959, Lipkin *et al.*[10] observed the formation of c-AMP **2** on treatment of ATP with $Ba(OH)_2$. Two years later, Khorana *et al.*[11] obtained 3',5'-cyclic ribonucleosides from the 5'-ribonucleotides by treatment with DCC in dilute solution. In 1966, Borden and Smith[12] reported on the syntheses of nucleoside 3',5'-cyclophosphates from 5'-*O*'-(4-nitrophenyl) nucleoside phosphates by treatment with strong base.

Mukaiyama and Hashimoto[13] cyclized nucleoside 5'-phosphates with triphenylphosphine and 2,2'-dipyridyl disulfide. In 1975, Taguchi and Mushika[14] described the preparation of nucleoside 3',5'-cyclophosphates from unprotected nucleosides with 2-(*N*,*N*-dimethylamino)-4-nitrophenylphosphate and DCC in boiling pyridine.

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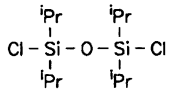
† *Abbreviations*: NPT: 5-(4-nitrophenyl)tetrazole; TCB: 2,2,2-trichloro-*tert*-butyl;[6] TCBOC: TCB-oxycarbonyl;[7] TIPDSi: tetraisopropyl-dichlorosiloxane.

In DMF only the nucleoside 2′,3′-cyclophosphates are formed. Generally, the synthesis of nucleoside 3′,5′-alkylcyclophosphates by alkylation of nucleoside 3′,5′-cyclophosphates does not proceed in a satisfactory yield. Hitherto published syntheses of nucleoside 3′,5′-alkyl cyclophosphates from unprotected or incompletely protected nucleosides all proceed in low overall yields, and the intermediate products require cumbersome purification operations.[15]

Thymidine can be converted into 3′,5′-cyclophosphites by reaction with phosphite amidites.[16] Despite low yields, this type of synthesis is of interest, because the axial and equatorial stereoisomers of the 3′-5′-cyclophosphites are formed with a high degree of stereoselectivity (up to 95:5). These 3′,5′-cyclophosphites can be converted into a wide variety of thymidine derivatives.[17] The one-pot synthesis of nucleoside 3′,5′-thiocyclophosphates by reaction of nucleoside derivatives with thiophosphoryl chloride in ca. 30% yield was reported in 1988.[18]

Not only the 5′-hydroxy group, but also the 2′-hydroxy group of ribonucleosides must be protected in oligonucleotide syntheses. This is most conveniently achieved by the procedure of Markiewicz *et al.*[19] Here the bulky bifunctional TIPDSi reagent **3** is used for the selective temporary protection of the 3′- and 5′-hydroxy groups of ribonucleoside derivatives. Thus the 2′-hydroxy groups can be exclusively protected with a suitable protecting group.[20,21] Subsequently, a 2′-protected ribonucleoside derivative is obtained by deprotection of the 3′- and 5′-hydroxy groups. Owing to its steric bulk the TIPDSi reagent attacks the primary 5′-hydroxy group first and then forms a seven-membered ring by reacting at the 3′-hydroxy groups. An analogous reaction at the 2′-hydroxy group would require the formation of a nine-membered ring, which is much less favourable.[22]

Owing to its 'orthogonality'[23] to almost all other protective groups and its cleavability under extremely mild conditions, the TCB group is attractive as a protective group for phosphite and phosphate groups in oligonucleotide syntheses.[24,25] In order to exploit TCB protection in oligonucleotide syntheses by the phosphite amidite method,[26] the TCB-chloro-*N*,*N*-diisopropylamido phosphite **4**[27] was prepared and tested as a ring-forming phosphitylating agent.



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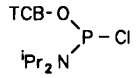
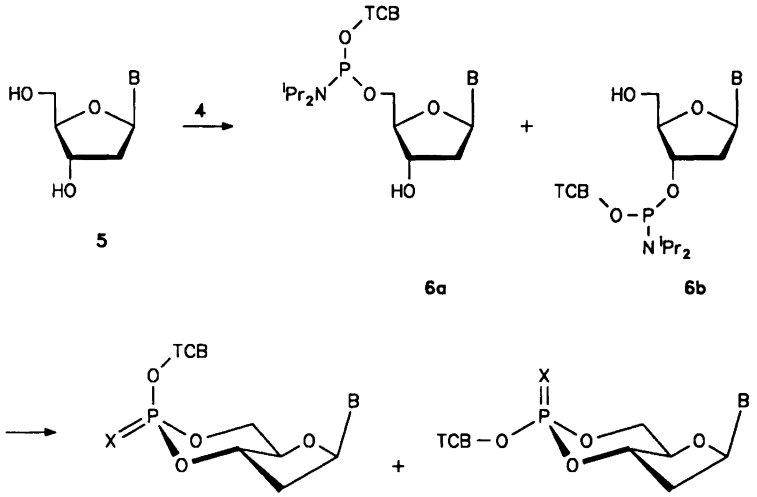


4

Because of its steric bulk, the use of **4** seemed promising for the selective protection of the 3′- and 5′-hydroxy groups of ribonucleoside derivatives, in analogy to the method of Markiewicz.[19]

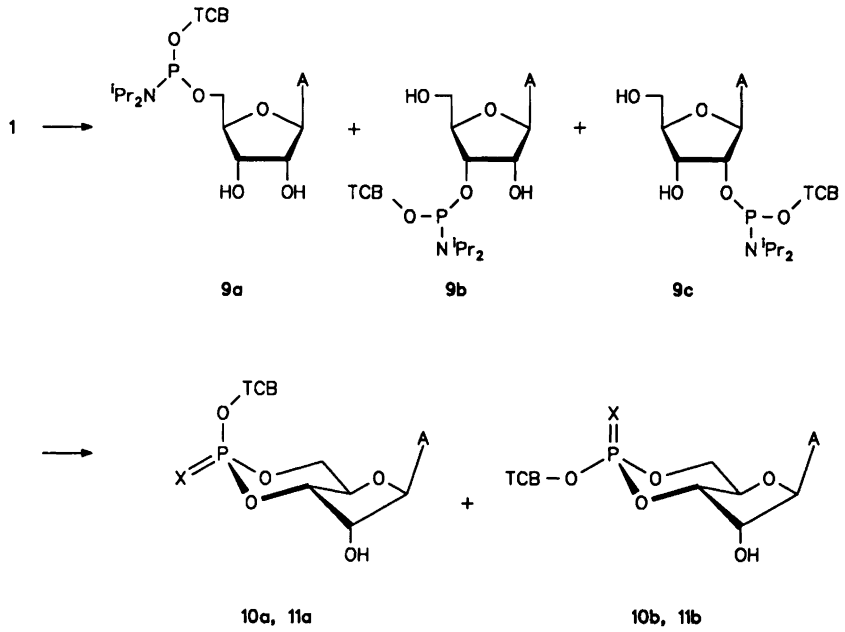
The ability of **4** to form rings was tested and the influence of the reaction conditions on the yields of these reactions was investigated in model experiments with TCBOC-thymidine **5**. Also, some analogues of **4** were subjected to similar experiments. **4** was found to be the most promising reagent for the selective formation of the 3′,5′-cyclophosphite derivatives from suitably protected ribonucleosides.[28] The reaction of **5** with **4** to form a mixture of **6a** and **6b**, their subsequent cyclization into a mixture of the stereoisomers **7a** and **7b** and also the conversion of the mixture of **7a** and **7b** into **8a** and **8b** were investigated. In one of the experiments, a mixture of **6a** and **6b** (77% vs. 23%) was formed in a total yield of 47%. When **6a** and **6b** are activated by NPT[29] the cyclization into **7a** and **7b** proceeds smoothly. Activation by 1*H*-tetrazole or pyridine hydrochloride is less effective. We have not yet determined whether or not **6a** and **6b** form **7a** and **7b** in different relative amounts.



5 **6a** **6b**

7a, 8a **7b, 8b**

(**7**: X = electron pair; **8**: X = O; B = N–TCBOC–thymine)



(10 : X = electron pair; 11: X = O; A like in 1)

Note that in such syntheses of 3',5'-cyclophosphites and phosphates of deoxynucleosides the primary reaction may take place at any one of the hydroxy groups, whereas 3',5'-cyclic derivatives only result from the ribonucleosides, if the primary attack occurs at the 5'-hydroxy group.

For the selective 3',5'-phosphitylation of ribonucleoside derivatives by **4**, **1** was selected as the model compound.

For the synthesis of the 3',5'-cyclophosphites, a highly selective 5'-phosphitylation like **1** → **9a** is of paramount importance. The course of the reaction of **1** with **4** in the presence of ethyldiisopropylamine yields the monophosphitylation products **9a**, **9b** and **9c**; the relative amounts of **9a** depend strongly on the choice of reaction conditions (see Table 1). The yields of **9a** were determined by ^{31}P NMR as the relative amounts of the 5'-phosphite (**9a**) vs. the amounts of all phosphites (**9a–c**, **10a** and **10b**, its 2',3'-isomer and the diphosphitylation products of **1**).

When **9a** is treated with an excess of NPT in boiling acetonitrile, it forms a mixture of the stereoisomers **10a** and **10b** in quantitative yield. The 3',5'-cyclophosphates **11a** and **11b** can be obtained from **10a** and **10b** in quantitative yield by oxidation with 3-(2,4-dichlorophenyl)-

Table 1. Influence of the reaction conditions on the synthesis of **9a** from **1** and **4**.

Prep. No.	**4**/equiv.	**1**/equiv.	$EtNiPr_2$/equiv.	Solvent	*T*/°C	*t*/h	Relative yield of **9a** (%)[a]
1	1	1	1	CH_2Cl_2	−20	24	41
2	1	1	1	CH_2Cl_2	−70/23[b]	4/20	30
3	1	1	4	CH_2Cl_2	−70/23	2/15	48
4	1	2	2	CH_2Cl_2	23	20	56
5	1	2	2	CH_2Cl_2	23	3	60[c]
6	1.2	1	7	CH_2Cl_2	0	2.5	27[d]
7	2	1	20	CH_2Cl_2	−20/10	1/1	68[e]
8	1[f]	1	4	CH_2Cl_2	23	24	60
9	1[f]	1	2	CH_2Cl_2	23	15	60
10	1	1	1	CH_3CN	23	24	38
11	1.2	1	10	CH_3CN	−40/23	5/20	61
12	1.2	1	12	DMF	−30	19	93[g]
13[h]	1	1.1	12	DMF	−30	20	>98

[a] Relative yield of **9a** based on the ratio of the ^{31}P NMR integrals of **9a** and the 2'- and 3'-phosphitylated products from **1**. [b] Addition of **4** and reaction for the indicated time at the lower temperature, subsequent reaction at the higher temperature. [c] Incomplete reaction. [d] Also contains products of multiple phosphitylation. [e] Scavenged with MeOH at ca. 50% reaction of **1**. [f] Solution of **1** added to solid **4**. [g] According to a scavenging experiment with MeOH, **1** reacted completely. When the reaction mixture is not analyzed immediately, the relative yield of **9a** decreases, owing to the ensuing formation of **10** that is catalyzed by the diisopropylethylamine hydrochloride. [h] Micossi, A., M. Sc. Thesis, Techn. Univ. München, in preparation.

2-tosyloxaziridine;[31] **11a** and **11b** can be separated by chromatography. The latter products can be prepared in an overall yield of 45% from **1** and **4**.

The NMR data suffice to prove that **11** is the 3′,5′-cyclophosphate. In the ^{1}H NMR spectrum the sharp singlet of H-1′ is characteristic of anomers of adenosinyl 3′,5′-cyclophosphates. This has been observed for more than 200 c-AMPs.[32] The assignment of the axial and equatorial configurations to **11a** and **11b** is possible by ^{31}P NMR spectroscopy.

According to a comparison[33] of X-ray crystallographic structures[17,34] and ^{31}P NMR of some nucleoside 3′,5′-alkylcyclophosphates, the ^{31}P chemical shifts of the axial stereoisomers always appear at higher field than those of the equatorial stereoisomers.[35]

The extraordinarily high value of the ^{31}P coupling constant, $^3J_{PH} \geqslant 20$ Hz, of the axial triester indicates that the six-membered 3′,5′-cyclophosphate ring has a chair conformation. In this conformation one CH_2 proton (H-5″) is antiperiplanar (*trans*) to the phosphorus atom, while the other CH_2 proton (H-5′) and H-3′ are synclinal (*gauche*) to the phosphorus atom. It follows from the Karplus equation that the coupling constants $^3J_{POCH}$ assume very small values for a dihedral angle POH of ca. 90°, and very high values for dihedral angles of ca. 180°C.[36]

The use of **11a** and **11b** for the synthesis of 2′-protected ribonucleoside derivatives is still under investigation. In 1962, Khorana *et al.*[3b] prepared 2′-tetrahydropyranyluridine 3′-phosphate from the corresponding 3′,5′-cyclophosphate.

The synthesis of **11a** and **11b** as well as related compounds is also of substantial interest for the preparation of c-AMP and its analogues, because it is still no trivial matter to synthetize pure c-AMP and its derivatives on a preparative scale.

Experimental

Instruments and materials. ^{1}H NMR: Jeol JNM PMX 60 (60 MHz); Bruker WP 200 (200 MHz); Bruker AM 360 (360.1 MHz); δ (ppm); $SiMe_4$ int. standard; J(Hz). ^{13}C NMR: Jeol JNM FX90 (22.6 MHz); Bruker AM 360 (90.56 MHz); proton decoupling; δ (ppm) vs. $SiMe_4$ (0.0 ppm); signals of CH_2 and quaternary C identified by DEPT experiments ($\theta = 135°$). ^{31}P NMR: Jeol JNM FX90 (36.43 MHz); Bruker AM 250 (101.26 MHz); Bruker AM 360 (145.79 MHz). IR: Perkin Elmer 177 and 257; KBr; ν (cm^{-1}). M.p. uncorrected. Flash chromatography: silica gel 60, 15–40 μm Merck and 20–45 μm Amicon; DC: silica gel 60 F_{254}, Merck on aluminium foil.

The solvents (except CH_2Cl_2) were dried by standard methods and stored over molecular sieves (3 or 4 Å); CH_2Cl_2 was dried with Siccapent. The nucleosides were azeotropically dried with pyridine and toluene.

N^6,N^6-Bis-TCBOC-adenosine (**1**). At 0°C 13.15 ml (104 mmol) trimethylchlorosilane were slowly added from a dropping funnel to a stirred solution of 5.57 g (20.8 mmol) adenosine in 200 ml pyridine. After 3 h 12.0 g (50 mmol) TCBOC-chloride were added. Stirring was continued for 3 h at room temperature. Subsequently, the reaction mixture was poured into 300 ml ice-water. The product was extracted three times with 150 ml CH_2Cl_2. After drying and evaporation, the residue was dissolved in 300 ml ethyl acetate and treated for 15 min with 200 ml of a 1:1 (v/v) mixture of methanol–hydrochloric acid (6.4% by wt.). The solution was washed five times with sat. aqueous NaCl and evaporated. The residue was dissolved in 35 ml CH_2Cl_2 and poured into 600 ml stirred *n*-hexane. The precipitate was collected by suction filtration and dried *in vacuo*. The product crystallized from MeOH–H_2O as needles.

Yield: 12.76 g (91%); $R_f = 0.37$ (CH_2Cl_2–MeOH 9:1 v/v). M.p. 200–201°C. Found: C 35.87; H 3.57; N 10.43. Calc. for $C_{20}H_{23}Cl_6N_5O_8$: C 35.63; H 3.44; N 10.39. ^{1}H NMR ($CDCl_3$): 1.91 (s, 12 H, CH_3, TCBOC), 3.45 (d, $J = 2.5$, 1 H, 3′OH), 3.74 (d, $J = 6.5$, 1 H, 2′OH), 3.81 (ddd, $J_{gem} = 12.7$, $J_{5'',5'OH} = 10.7$, $J_{5'',4'} < 2$, 1 H, H-5″), 4.03 (ddd, $J_{gem} = 12.7$, $J_{5',4'} < 2$, $J_{5',5'OH} < 2$, 1 H, H-5′), 4.38 (m, $J_{4',3'}$, $J_{4',5'}$, $J_{4',5''} < 2$, 1 H, H-4′), 4.52 (m, 1 H, H-3′), 4.89 (ddd, $J_{2',1'} = 6.7$, $J_{2',3'} = 5.0$, $J_{2',2'OH} = 6.5$, 1 H, H-2′), 5.06 (dd, $J_{5'OH,5'} < 2$, $J_{5'OH,5''} = 10.7$, 1 H, 5′OH), 5.94 (d, $J_{1',2'} = 6.7$, 1 H, H-1′), 8.26 (s, 1 H, H-2), 8.87 (s, 1 H, H-8). ^{13}C NMR ($CDCl_3$): 21.19, 21.21 (CH_3, TCBOC), 62.88 (C-5′), 72.07 (C-3′), 74.36 (C-2′), 87.65 (C-4′), 91.30 (C-1′), 91.53 (C-1, TCBOC), 104.84 (CCl_3, TCBOC), 131.72 (C-5), 145.51, 151.69 (C-2, C-8), 147.23 (CO, TCBOC), 149.17, 152.66 (C-4, C-6). IR (KBr): 1800, 1610, 1580, 1290, 1140, 1110, 795 cm^{-1}.

N^3-TCBOC-thymidine (**5**). At 0°C 18.9 ml (150 mmol) chlorotrimethylsilane were added to 7.27 g (30 mmol) thymidine in 250 ml pyridine. After 1 h 14.40 g (60 mmol) TCBOC chloride were added; the reaction mixture was left for 24 h and worked up as described in the above preparation.

Yield: 10.49 g (78.5%); $R_f = 0.35$ (CH_2Cl_2–MeOH 9:1 v/v); m.p. 90–91°C. Found: C 40.14, H 4.52, N 5.97. Calc. for $C_{15}H_{19}Cl_3N_2O_7$: C 40.42, H. 4.30, N 6.29. ^{1}H NMR ($CDCl_3$): 1.92 (s, 3 H, C-5-CH_3), 2.08 (s, 6 H, CH_3, TCBOC), 2.33 (m, 2 H, H-2′), 2.80 (wide, 2 H, OH), 3.85 (m, 2 H, H-5′), 3.98 (m, 1 H, H-4′), 4.52 (m, 1 H, H-3′), 6.19 (dd, $J = 6.7$, 1 H, H-1′), 7.54 (s, 1 H, H-6). ^{13}C NMR ($CDCl_3$): 12.60 (C-5-CH_3), 20.96 (CH_3, TCBOC), 40.28 (C-2′), 62.24 (C-5′), 71.35 (C-3′), 86.40, 87.07, (C-1′, C-4′), 93.21 (C-1, TCBOC), 104.50 (CCl_3, TCBOC), 110.82 (C-5), 136.36 (C-6), 147.46, 148.46 (CO, TCBOC, C-4), 161.14 (C-2).

5′-O- and 3′-O-(N^3-TCBOC-thymidinyl)-N,N-diisopropylamino-TCB-phosphites (**6a** *and* **6b**). At 23°C 0.35 g (1.02 mmol) [427] and 0.35 ml (2.04 mmol) ethyldiisopropylamine in 8.0 ml CH_2Cl_2 were added to a stirred solution of 0.91 g (2.04 mmol) **5** in 10.0 ml CH_2Cl_2. After

24 h, 50 ml ethyl acetate were added. The solution was washed with sat. aqueous NaCl, dried over $MgSO_4$ and evaporated. Unchanged **5** was removed from the residue (1.17 g) by flash chromatography (CH_2Cl_2–MeOH gradient, 0–2%).

Yield: 0.36 g (47%); $R_f = 0.56$, 0.65 (CH_2Cl_2–MeOH 96:4 v/v). ^{31}P NMR ($CDCl_3$): +140.2, +140.4 (77% diastereomers of **6a**), +141.8, +142.6 (23%, diastereomers of **6b**; $J_{POCH} = 11$, $J_{PNCH} = 11$). ^1H NMR ($CDCl_3$): 1.20 (m, 12 H, CH_3, iPr_2N); 1.80 (s, 6 H, CH_3, TCB), 1.95 (s, 3 H, C-5-CH_3), 2.10 (s, 6 H, CH_3, TCBOC), 2.33 (m, 2 H, H-2'), 3.10–4.60 (m, 7 H, CH, iPr_2N, deoxyribose-H), 6.32 (m, 1 H, H-1'), 7.58, 7.73 (2 s, 1 H, H-6).

*TCB-3'-O,5'-O-thymidinyl-cyclophosphites (***7a** *and* **7b***) and -cyclophosphates (***8a** *and* **8b***).* The solution of 0.36 g (0.48 mmol) of **6** from the above preparation in 10 ml acetonitrile was added to a boiling solution of 0.27 g (1.44 mmol) NPT in 10 ml acetonitrile. After 10 min the solvent was evaporated off, and the residue was examined by ^{31}P NMR spectroscopy. ^{31}P NMR ($CDCl_3$): +115.0 (61% **7a**), +121.5 (39% **7b**).

The solution of 0.17 g (0.48 mmol) 3-(2,4-dichlorophenyl)-2-tosyloxaziridine[31] in 10 ml CH_2Cl_2 was added to the aforementioned product at room temperature. After 5 min the reaction mixture was filtered and the filtrate was diluted with *n*-hexane at room temperature and filtered again. The product (**8a** + **8b**) precipitated on being cooled to −70°C. According to ^{31}P NMR spectroscopy this crude product did not contain any other phosphorus compounds; further purification of these compounds required flash chromatography. $R_f = 0.85$, 0.90 (CH_2Cl_2–MeOH 9:1 v/v). ^{31}P NMR ($CDCl_3$): −13.2 (d, $J_{PH} = 23.2$, 61% **8a**), −10.6 (dd, $J_{PH} = 12.2$, 8.5, 39% **8b**).

5'-O-(N⁶,N⁶-Bis-TCBOC-adenosinyl)-N,N-diisopropyl-amino-TCB-phosphite (**9a**). At −30°C 1.45 g (4.24 mmol) **4** in 10.0 ml DMF were added from a cooled dropping funnel to a stirred solution of 2.40 g (3.56 mmol) **1** and 7.33 ml (42.4 mmol) ethyldiisopropylamine in 15.0 ml DMF. After 19 h complete reaction was verified by reacting a sample with MeOH; unless the reaction is complete, iPr_2N-P(OTCB)OMe is found by ^{31}P NMR spectroscopy at +141.6 ppm. The reaction mixture was evaporated *in vacuo* (0.02 Torr), and the residue was dissolved in 300 ml ethyl acetate. The solution was washed five times with 50 ml 15% aqueous NaCl, dried over Na_2SO_4 and evaporated to yield 3.70 g of a yellowish amorphous residue. According to TLC this residue did not contain **1**; the ^{31}P NMR spectrum indicated a ratio of 93.4% 5'-phosphitylation (+140.4 and +140.8 ppm) to 3.5% 3'-phosphitylation (+141.7 and 142.1 ppm) and 3.1% 2'-phosphitylation (+145.2 and +145.9 ppm).

Pure **9a** was obtained from this crude product by gradient chromatography eluting with CH_2Cl_2, Et_3N (1%), MeOH (0–3%) on silica gel Merck (15–40 µm). $R_f = 0.67$ (CH_2Cl_2–MeOH 9:1 v/v). ^{31}P NMR ($CDCl_3$): +140.4, +140.8. ^1H NMR ($CDCl_3$): 1.16–1.22 (m, 12 H, CH_3, iPr_2N), 1.75, 1.77 (2 s, 6 H, CH_3, TCB), 1.91 (s, 12 H, CH_3, TCBOC), 3.66 (dsept, $J_{PH} = 10.7$, $J = 6.6$, 2 H, CH, iPr_2N), 3.80–3.97 (m, 2 H, H-5'), 4.39 (m, 1 H, H-4'), 4.49 (m, 1 H, H-3'), 4.59 (m, 1 H, H-2'), 6.13, 6.19 (2 d, $J = 6.7$, 1 H, H-1'), 8.47, 8.55 (2 s, 1 H, H-2), 8.87, 8.88 (2 s, 1 H, H-8). ^{13}C NMR ($CDCl_3$): 21.15, 21.20 (CH_3, TCBOC), 24.07–24.21, 24.57–24.83 (CH_3, TCB and iPr_2N), 43.28–43.51 (CH, iPr_2N), 62.36, 62.49 (1 d each, $J_{PC} = 10.1$ or 13.0, C-5'), 71.41 (C-3'), 75.59, 75.77 (C-2'), 84.77–85.08 (C-4'), 89.38, 89.92 (C-1'), 91.33, 91.36 (C-1, TCB, TCBOC), 104.86 (CCl_3, TCBOC), 107.91 (CCl_3, TCB), 130.62, 130.75 (C-5), 144.19, 151.84 (C-2, C-8), 147.34 (CO, TCBOC), 148.47, 148.54, 153.13, 153.31 (C-4, C-6).

3'-O,5'-O-N⁶,N⁶-Bis-TCBOC-adenosinyl-TCB-cyclophosphite (**10**). Within 5 min, the above crude product in 50 ml acetonitrile was added to a stirred boiling solution of 2.04 g (10.7 mmol) NPT in 50 ml acetonitrile. After 5 min of reflux, the reaction mixture was cooled to room temperature and evaporated *in vacuo*. The residue was treated with 100 ml CH_2Cl_2 and the filtered solution was evaporated *in vacuo*. According to ^{31}P NMR spectroscopy the residue consisted mainly of **10a** and **10b** and a minor amount of other phosphates.

When pure **9a** (obtained by chromatography) was subjected to an analogous cyclization by NPT, **10a** and **10b** were formed exclusively. $R_f = 0.75$, 0.81 (**10a** + **10b**; CH_2Cl_2–MeOH 9:1 v/v). ^{31}P NMR ($CDCl_3$): +115.4 (d, $J_{PH} = 8.6$, **10a**), +122.0 (d, $J_{PH} = 9.8$, **10b**). ^1H NMR ($CDCl_3$): 1.90 (m, 18 H, CH_3, TCB, TCBOC), 3.90–5.40 (m, 6 H, ribose-H), 6.02 (s, 1 H, H-1'), 8.21 (s, 1 H, H-2), 8.88, 8.91 (both 1 s, 1 H, H-8).

3'-O,5'-O-N⁶,N⁶-Bis-TCBOC-adenosinyl-TCB-cyclophosphate (**11**). At room temperature 1.46 g (4.24 mmol) of 3-(2,4-dichlorophenyl)-2-tosyloxaziridine[31] was added to a stirred solution of 4.21 g of the crude phosphites **10a** and **10b** that contained a minor relative amount of NPT (see above) in 50 ml CH_2Cl_2. When the exothermic reaction was complete, the reaction mixture was subjected to flash chromatography. After removal of residual oxaziridine and its reaction product, the corresponding *N*-tosylimine, by chromatography with pure CH_2Cl_2, the axial and equatorial cyclophosphates **11a** and **11b** were separated in a 0.5–1.5% MeOH–CH_2Cl_2 gradient.

Total yield of **11a** and **11b**: 1.43 g (45%, based on **1**); $R_f = 0.55$ (**11a**), 0.61 (**11b**) (CH_2Cl_2–MeOH 9:1 v/v).

^{31}P NMR of **11a**: ($CDCl_3$): −12.5 (d, $J_{PH} = 20.8$). ^1H NMR ($CDCl_3$): 1.89, 1.92 (2 s, 12 H, CH_3, TCBOC), 2.03, 2.04 (2 s, 6 H, CH_3, TCB), 4.45–4.56 (m, 2 H, H-4', H-5'), 4.66 (dm, $J_{5'',P} = 23.2$, 1 H, H-5''), 5.00 (d, $J_{2',3'} = 4.8$, 1 H, H-2'), 5.34 (broad, 1 H, 2'OH), 5.71 (ddd, $J_{3',2'} = 4.8$, $J_{3',4'} = 8.0$, $J_{3',P} < 1$, 1 H, H-3'), 6.08 (s, 1 H, H-1'), 8.24 (s, 1 H, H-2), 8.87 (s, 1 H, H-8). ^{13}C NMR ($CDCl_3$): 21.07, 21.21 (CH_3, TCBOC), 23.52, 23.68 (CH_3, TCB), 70.28 (d, $J_{PC} = 8.7$, C-5'), 70.64 (d, $J_{PC} = 4.8$, C-4'), 72.05 (d, $J_{PC} = 8.4$, C-2'), 79.24 (d,

J_{PC} = 5.8, C-3'), 90.89 (d, J_{PC} = 5.0, C-1, TCB), 91.30 (C-1, TCBOC), 92.94 (C-1'), 104.85 (CCl_3, TCBOC), 105.69 (d, J_{PC} = 16.0, CCl_3, TCB), 131.05 (C-5), 145.79, 152.35 (C-2, C-8), 147.08 (CO, TCBOC), 148.91, 152.83 (C-4, C-6).

^{31}P NMR of **11b** ($CDCl_3$): −9.4 (dd, J_{PH} = 14.7, 4.9). ^{1}H NMR ($CDCl_3$): 1.87, 1.90 (2 s, 12 H, CH_3, TCBOC), 2.00 (s, 6 H, CH_3, TCB), 4.33–4.48, 4.68–4.80 (2 m, 4 H, H-4', H-5', H-5'', 2'OH), 5.00 (d, $J_{2',3'}$ = 4.8, 1 H, H-2'), 5.59 (dd, $J_{3',2'}$ = 4.8, $J_{3',4'}$ = 9.6, 1 H, H-3'), 6.12 (s, 1 H, H-1'), 8.25 (s, 1 H, H-2), 8.92 (s, 1 H, H-8). ^{13}C NMR ($CDCl_3$): 21.13, 21.20 (CH_3, TCBOC), 23.23, 23.74 (CH_3, TCB), 69.85 (d, J_{PC} = 8.9, C-5'), 70.14 (d, J_{PC} = 10.8, C-4'), 71.83 (d, J_{PC} = 7.3, C-2'), 78.55 (d, J_{PC} = 4.7, C-3'), 91.10 (d, J_{PC} = 4.8, C-1, TCB), 91.35 (C-1, TCBOC), 92.94 (C-1'), 104.81 (CCl_3, TCBOC), 105.44 (d, J_{PC} = 16.5, CCl_3, TCB), 130.87 (C-5), 145.39, 152.53 (C-2, C-8), 147.14 (CO, TCBOC), 148.87, 152.90 (C-4, C-6).

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