Stereoselective preparation of (R_P)-8-hetaryladenosine-3′,5′-cyclic phosphorothioic acids†

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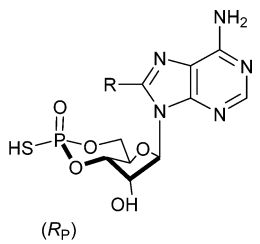
Cyclic adenosine monophosphate (cAMP) has been converted into its 8-bromo derivative and 2′*O*-TBDMS protected before activation of the phosphoric acid moiety with a reagent generated *in situ* from oxalyl chloride and DMF. Further reactions with primary amines furnished corresponding phosphoramidates with high stereoselectivity at the phosphorus atom. Cross-coupling reactions with the 8-bromopurine yielded 8-hetaryl derivatives. X-Ray analyses showed the amidates to possess the (S_P)-configuration. Carbon disulfide effected thiylation under strongly basic conditions stereospecifically provided the (R_P)-phosphorothioic acids.

Introduction

The naturally occurring purine cyclic monophosphates, cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), are important secondary messengers, regulating a wide range of cell functions in response to various hormones.[1,2] In one group of cAMP analogues, one of the oxygen atoms pendant from the phosphorus atom has been replaced by a sulfur atom. The new stereogenic center created at the phosphorus atom provides an (R_P)- and an (S_P)-adenosine-3′,5′-cyclic monophosphorothioic acid (cAMPS) diastereomer, which differ in the pharmacological effect they cause at the level of the effector, protein kinase A, where (R_P)-cAMPS acts as a competitive antagonist and (S_P)-cAMPS acts as an agonist.[3,4] Substitution in cAMPS at the purine 8-position may affect bioactivity as demonstrated for some 8-thia, -oxa and -aza derivatives.[5,6] cAMPS derivatives with a carbon substituent in the purine 8-position are of interest as immune stimulating agents.[7] Considerable efforts have been devoted to studies of 8-halogeno derivatives,[5,8–10] and the 8-bromo analogue has been proposed as an immune stimulant in HIV treatment.[10,11]

cAMPS derivatives carrying a carbon substituent in the 8-position were not available when the present work was initiated. We report a stereoselective and convenient preparative synthesis of members of the (R_P)-cAMPS family of cAMP analogues carrying a carbon substituent in the 8-position. The methodology involves stereoselective thiation at the phosphorus atom and introduction of a carbon substituent into the purine 8-position in cAMPS derivatives. The general structure for the target molecules is shown in Fig. 1, structure **A**.



Structure A

Fig. 1 Target molecules.

Target molecules

Reports are available on the preparation of the parent cAMPS compound (structure **A**, R = H) and a few 8-hetero-substituted derivatives. In most cases, adenosine has served as a substrate for thiophosphorylation at the 5′-OH group by thiophosphoryl chloride and a subsequent alkali hydroxide treatment to effect cyclisation. The steric induction by this approach was poor, however, and the diastereoisomers were separated by extensive chromatographic operations.[12] Other cyclothiophosphorylation reactions reported also show a low degree of stereoselectivity and require chromatographic separation steps for the isolation of the diastereomers.[13,14] The (R_P)-diastereomer of cAMPS, however, has been prepared enzymatically from the corresponding nucleoside 5′*O*-(1-thiotriphosphate).[15] A more promising approach appeared to be the use of cAMP as a substrate. The latter was converted into a phosphoryl chloride in an Apple type reaction using Ph_3P–CCl_4, and the product reacted with amines to form phosphoramidates. The reaction sequence proceeded with low diastereoselectivity, however, and the diastereomers were separated by chromatography, and each isomer was separately converted into the corresponding phosphorothioic acid in a Stec reaction with carbon disulfide under basic conditions.[16] The thiation proceeded with retention of the configuration.[16–19] We have adapted this approach for our synthetic work.

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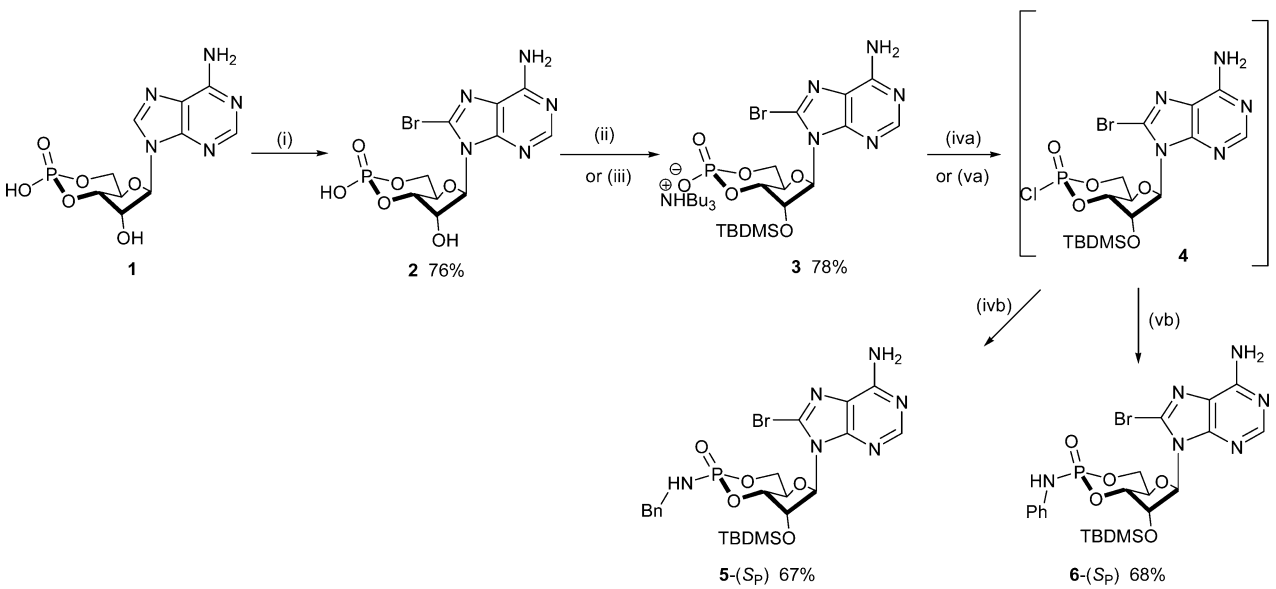
Results and discussion

The main challenge has been to achieve stereochemical control in the construction of the new configurational center at the phosphorus atom by way of phosphoramidate formation. All stereoselective processes detailed below involve an amidate intermediate. The amidate must be formed from a primary amine in order to have an amidate N–H proton available for abstraction and metallation in the thiylation step (*vide infra*). Once the amidate has been constructed, the *P–N* bond is cleaved intramolecularly by a sulfur nucleophile with retention of the true configuration at the phosphorus atom. There is, however, an apparent change in the expression of the configuration at the phosphorus atom because of the nomenclature priority rules. Deprotonation of an amide hydrogen with a strong base provides a metallated amido-nitrogen which reacts with carbon disulfide. A sulfur atom in the intermediate becomes a nucleophile and a cyclisation reaction subsequently occurs where a sulfur atom adds to the phosphorus atom with a concurrent cleavage of the *P–N* bond. This process generates a phosphorothioic acid in a stereocontrolled manner with retention of the true configuration.

Ready variations in the nature of the 8-substituent in the target molecules would suggest a common substrate after the introduction of the stereochemistry at phosphorus (Scheme 1). Convenient substrates were 8-bromo and 8-chloro derivatives. The 8-bromo derivative was available from cAMP by bromination reactions.[20] In this work bromination conditions were adapted for larger scale operation, and the reaction was carried out in a concentrated solution with two equivalents of sodium acetate and neat bromine. Under these conditions, the 8-Br-cAMP was precipitated as formed with continuous removal of the HBr generated, thereby maintaining a constant pH in the solution. These reaction conditions have repeatedly yielded 70–80% product in work on a "100 g scale". Before amidation, the bromo substrate **2** was protected at the 2′-OH substituent with a bulky silyl group for two major reasons. Firstly, chemical interference with other functional groups was to be avoided under the conditions employed in the construction of the phosphoryl chloride **4** or an equivalent. Secondly, the hydrophobic and bulky silyl group was expected to facilitate isolation of the thioic acid after the thiation reaction by precipitation from the complex aqueous mixture (*vide infra*).

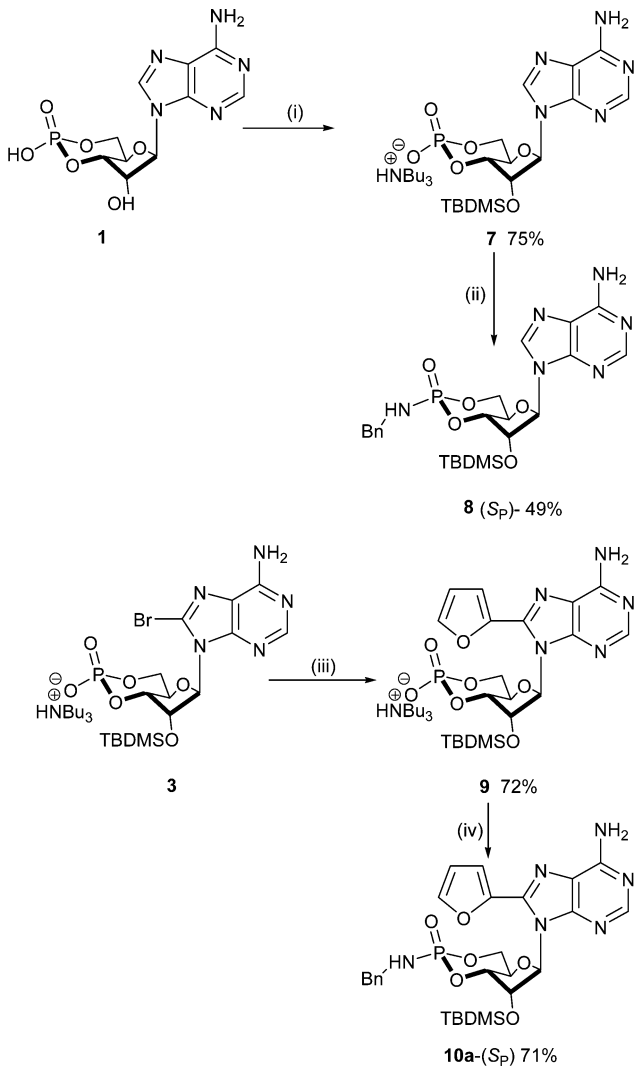
A practical problem may be experienced in the work with cyclic nucleotides because of low solubility in organic solvents. The problem was overcome for the 8-Br-cAMP which was converted into its tri-*n*-butylammonium salt before silylation, since the tri-*n*-butylammonium salt is soluble in dry DMF. The reactions with *tert*-butyldimethylsilyl (TBDMS) chloride in DMF in the presence of imidazole as base at 20–60 °C, furnished the silyl protected compound **3**. The bromine in the 8-position may be displaced by another halide ion, and therefore the silylation was effected under relatively mild conditions. This chloride exchange problem could also be avoided by using *tert*-butyldimethylsilyl triflate (TBDMSOTf) as a silylating reagent instead of TBDMSiCl. Conditions for the amidation step have been varied, and in our best procedure oxalyl chloride has been used in the presence of DMF in THF or dichloromethane at low temperature (−60–−20 °C). The actual chlorinating agent of phosphorus is probably the Vilsmeyer reagent, (chloromethylene)dimethylammonium chloride, which is generated *in situ* from oxalyl chloride and DMF. The stereochemistry of an intermediate phosphoryl chloride **4**, or an equivalent, has not been investigated. The crude halide was used directly in the amidation step with primary amines. Benzylamine provided the corresponding amidate. Only the (S_P)-isomer **5** was detected. Aniline provided the *N*-phenyl phosphoramidate (S_P)-**6**. Sometimes a small amount of the other diastereomer was present but was readily removed during the purification process. The intermediate phosphoryl chloride in the reaction may be a



Scheme 1 *Reagents and reaction conditions:* (i) Br_2, NaOAc, H_2O, rt, 24 h, (ii) TBDMS-Cl, imidazole, DMF, 60 °C, 20 h, (iii) TBDMS-triflate, imidazole, CH_2Cl_2, rt, 20 h, (iv) (a) $(COCl)_2$, DMF, CH_2Cl_2, −60 °C–rt, 30 min, (b) $BnNH_2$, CH_2Cl_2, −60 °C–rt, 2.5 h, (v) (a) $(COCl)_2$, DMF, CH_2Cl_2, −20 °C 1 h. (b) $PhNH_2$, rt, 3 h.

stereochemical mixture. The main signal in ^{31}P NMR of the mixture was seen at −1.73 ppm. Selective formation of the amidate with (S_P)-configuration from a mixture of acid chlorides would require some configurational equilibrations. In the (S_P)-isomer, the benzylamino or anilino group in the amidates occupies an equatorial position as shown by single crystal X-ray analyses (*vide infra*).

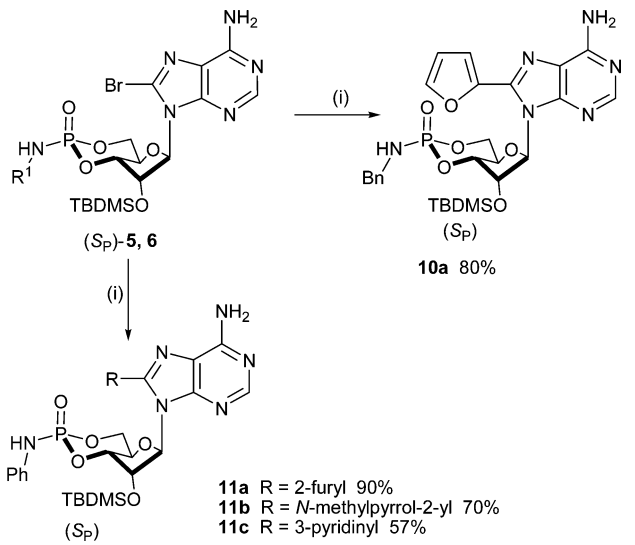
Any important influence of an 8-substituent in the heterocycle on the amination selectivity at the phosphorus atom was investigated. A derivative without any 8-substituent was required and was synthesised as shown in Scheme 2. cAMP (**1**) was silyl-protected, converted to its tri-*n*-butylammonium salt **7**, and amidated as above (Scheme 2). Only the (S_P)-stereoisomer of the benzylamidate **8** was obtained. In a similar way, any stereochemical directing effect from an 8-carbon substituent was investigated using a furyl substrate **9**. The latter was available from the 8-bromo-cAMP tri-*n*-butylammonium salt **3**, which was cross-coupled by Pd-catalysis under Stille conditions in DMF where the ammonium salt is soluble. Chlorination and amidation of substrate **9** proceeded to furnish one stereoisomer, *viz* compound (S_P)-**10a**. These experiments indicate that the nature of an 8-substituent is not the main cause of the stereoselectivity. The finding is rationalised by the large distance between the 8-substituent and the phosphorus atom where the reaction occurs. A common feature of all these substrates for amination is a bulky 2′-silyloxy group which presumably will favour a *quasi*-equatorial position with a flattening of the five-membered ribose ring and subsequent conformational changes in the annulated six-membered phosphorus ring, which eventually lead to the (S_P)-amidate with the amino group in an equatorial position.



1 **7** 75% **8** (S_P)- 49% **3** **9** 72% **10a**-(S_P) 71%

Scheme 2 *Reagents and reaction conditions:* (i) *N*-*n*Bu_3, TBDMS-Cl, imidazole, CH_2Cl_2, rt, 20 h, (ii) (a) $(COCl)_2$, DMF, CH_2Cl_2, −20 °C, 1 h, (b) $BnNH_2$, CH_2Cl_2, −20 °C–rt, 3 h, (iii) 2-(*n*-Bu_3Sn)furan, $Pd(OAc)_2$, PPh_3, DMF, 85 °C, 5 h, (iv) (a) $(COCl)_2$, DMF, CH_2Cl_2, 0 °C–rt, (b) $BnNH_2$, CH_2Cl, −20 °C–rt, 2 h.

The amidates are neutral molecules which are soluble in several common organic solvents. Hence these molecules are appropriate for cross-coupling reactions in the 8-position. DMF was a good solvent except for the preparation of the pyridinyl derivate **11c** when *N*-methylpyrrolidone (NMP) was a better choice for solubility reasons. Cross-coupling reactions were effected by palladium catalysis under Stille conditions and provided high yields of the 8-hetaryl substituted products **10** and **11** as shown in Scheme 3 (see the ESI†).



Scheme 3 *Reagents and reaction conditions:* (i) *n*-Bu_3Sn-R, $Pd(OAc)_2$, PPh_3; **10a** DMF, 85 °C, 2.5 h; **11a** DMF, 80 °C, 1 h; **11b** DMF, 90 °C, 4 h; **11c** NMP, 110 °C, 10 h.

The (S_P)-configuration assigned to the amidates has been ascertained by a single crystal X-ray structure analysis of the 2-furyl derivatives **10a** and **11a**. The X-ray data show that both the alkyl **10a** and the aryl **11a** amidates possess the same (S_P)-configuration at the phosphorus atom (Figs. 2 and 3). The configuration in the furyl derivatives **10a** and **11a** is the same as in the bromo-amidates **5** and **6** because the cross-coupling reaction does not interfere with the configuration at the phosphorus atom. The ORTEP plots of the crystalline structures **10a** and **11a** are shown in Fig. 2 and Fig. 3, respectively.

The crystal structures of the *N*-benzyl and the *N*-phenyl amidates are closely similar. The amino group occupies an equatorial position. The bulky silyloxy group tends to reduce steric repulsions in a quasiplanar conformation by a flattening of the ribose ring.

For the thiylation reaction in the benzyl series, *n*-BuLi or LDA was used, initially at −78 °C. Sodium or potassium *tert*-butoxide

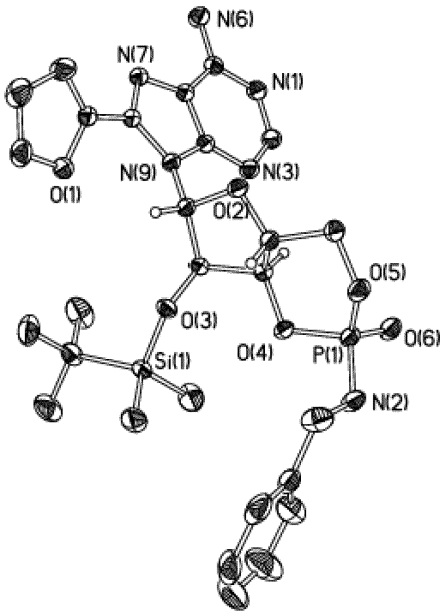


Fig. 2 ORTEP plot of compound **10a**. Ellipsoids are shown at 50% probability. For clarity only the hydrogen atoms at the stereogenic centers are shown.

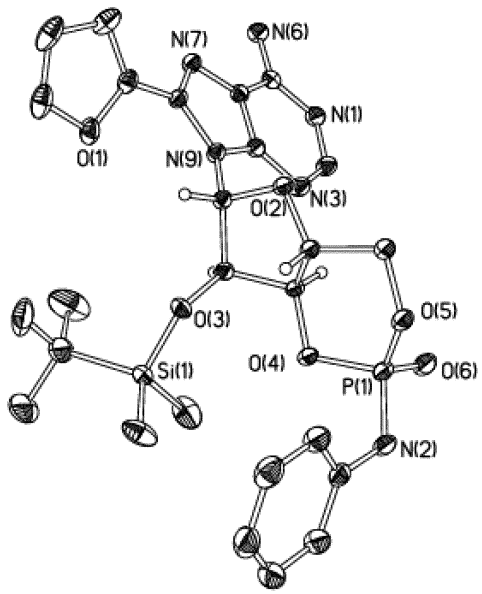
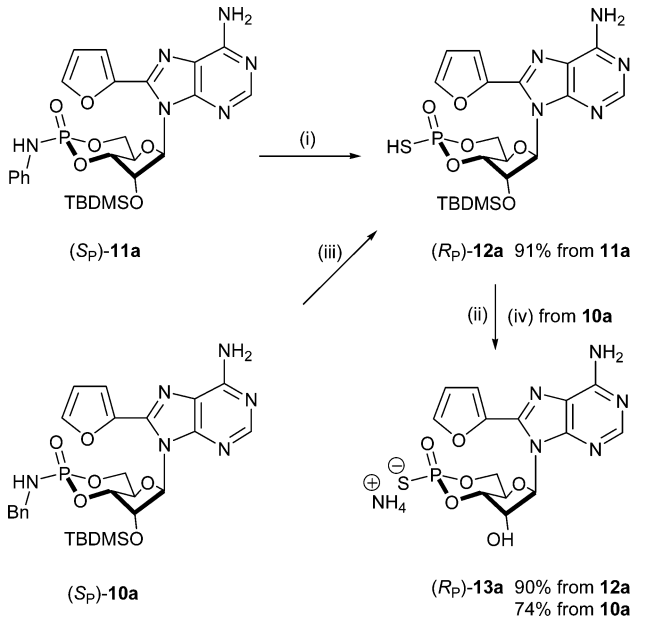


Fig. 3 ORTEP plot of compound **11a**. Ellipsoids are shown at 50% probability. For clarity only the hydrogen atoms at the stereogenic centers are shown.

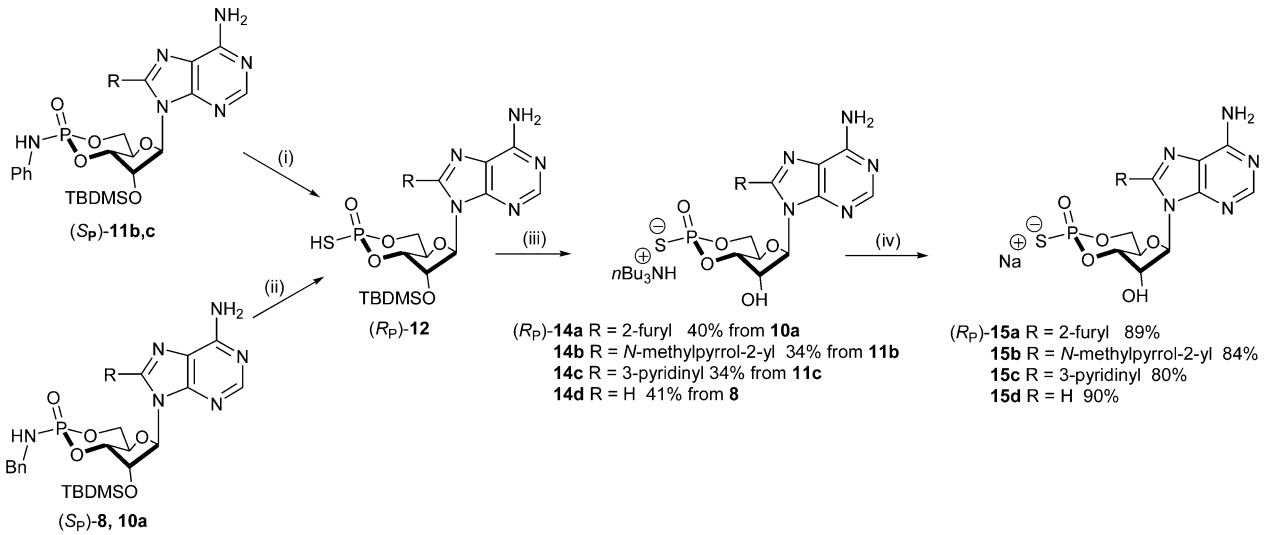


Scheme 4 *Reagents and reaction conditions:* (i) *t*-BuOK, THF, rt, CS_2, 3 h, (ii) NH_4F, DMF, rt, 5 d, (iii) (a) *n*-BuLi, THF, −78 °C, (b) CS_2, rt, 3 h, (iv) NH_4F, 40 °C, 48 h.

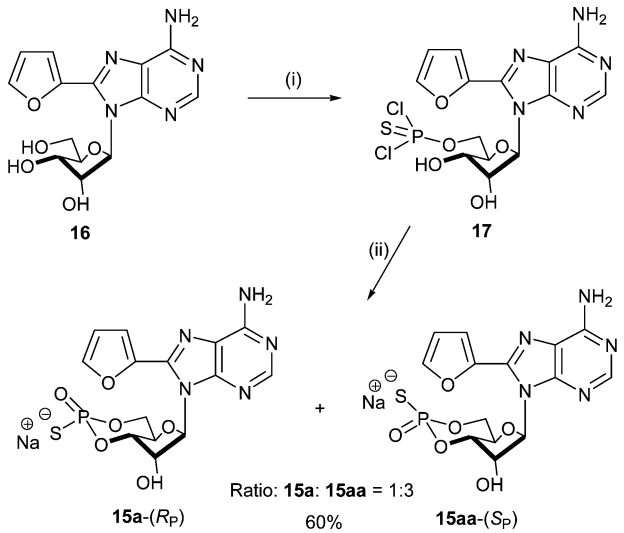
was used for proton abstraction in the phenyl series **11** at room temperature. The 6-amino group in the purine did not appear to cause any adverse reactions. The thiations of the amidates by means of carbon disulfide (the Stec reaction),[21] were stereospecific.

The reaction sequence for the 8-furyl *N*-benzyl- and *N*-phenylamidates **10a** and **11a** is shown in Scheme 4. The thiylation product **12a** is the same from both substrates. The silyl function was removed by ammonium fluoride, and the product was isolated as the ammonium salt **13a**. More conveniently, the phosphorothioic acid was isolated as its tri-*n*-butylammonium salt. After desilylation as shown in Scheme 5, the ammonia was displaced from the initial ammonium salt *in situ* by addition of tri-*n*-butylamine (see the ESI†). The tri-*n*-butylammonium salts are soluble in several organic solvents and are readily purified by preparative chromatography. Further conversion of the purified tri-*n*-butylammonium salts to sodium salts **15** were effected by sodium hydroxide in a methanol solution.

The overall reaction sequence is remarkably chemo- and stereoselective. Only one signal in the ^{31}P NMR spectra was present. cAMPS, the parent molecule, was first synthesised by Eckstein *et al.*,[22] and the configurational assignment of cAMPS was first established by Stec *et al.*[23] The ^{31}P NMR chemical shifts for cAMPS as sodium salt in aqueous solution were reported as 54.81 ppm, for the (S_P)-, and as 56.37, for the (R_P)-, isomer.[24] The value recorded for the (R_P)-isomer **15d** was 57.02 ppm in D_2O, and the chemical shifts for the sodium salts **15** were in the range 56.4–57.0 ppm in D_2O and 58.1–58.4 ppm in MeOH-d_4. The ^{31}P NMR signals from the tri-*n*-butylammonium salts **13b**, **14c** and **14d** (recorded in $CDCl_3$), appeared in the range 55.7–58.2 ppm. The resonances of the (S_P)-*N*-benzyl amidates **5** and **10a** in $CDCl_3$ were at 8.0–8.3 ppm, which differed significantly from the (S_P)-*N*-phenylamidates **11a–c** at 2.5–3.1 ppm. The ^{31}P NMR shifts for the phosphoric acid derivatives as tri-*n*-butylammonium salts **3**, **7** and **9** were in the range −1.1–−2.0 ppm. Thus, the phosphorus NMR shifts are useful diagnostic tools for structure assignments. The ^{31}P NMR shift for (R_P)-cAMPS as sodium salt in D_2O is found at a lower chemical field than for its (S_P)-cAMPS diastereomer.[24] In the correlation of the configuration at the phosphorus atom in the thioic acids by ^{31}P NMR shifts, spectroscopic data from both stereoisomers of a phosphorothioic acid would be required. A new synthetic approach had to be devised for making both diastereomers available (Scheme 6). This pathway is inferior to the one outlined above for the preparation of pure (R_P)-isomers, but from the mixture of stereoisomers prepared, as in Scheme 6, the (S_P)-isomer became available. The substrate was



Scheme 5 *Reagents and reaction conditions:* (i) *t*-BuOK, THF, rt, CS_2, 3 h, (ii) (a) *n*-BuLi, THF, −78 °C, 10 min, (b) CS_2, rt, 3 h, (iii) (a) NH_4F, DMF, rt, 5 d, (b) *n*-Bu_3N, DMF, rt, (iv) (a) NaOH, MeOH, rt, (b) hexane.



Scheme 6 *Reagents and reaction conditions:* (i) $PSCl_3$, pyridine, −8 °C, 90 min, (ii) (a) NaOH, MeCN–H_2O (50%), rt, (b) solid CO_2.

8-(2-furyl)adenosine[25] **16** which was reacted with thiophosphoryl chloride in dry pyridine to furnish a thiophosphoryl intermediate **17**. For this reaction to proceed well, it is essential that both the solvent and reactants have been well dried. Base treatment of the phosphorylated product in the cold provided the crude sodium salt of a diastereomeric mixture of the (R_P)- and (S_P)-isomers **15** in the ratio 1 : 3. Thus this approach favours preparation of the (S_P)-isomer. The ^{31}P NMR shift values of the 8-furyl diastereomers as sodium salts in D_2O were at 55.45 ppm for the (S_P)-isomer **15aa** and at 56.20 ppm for the (R_P)-isomer **15a** as compared with the values 54.81 ppm for (S_P)-**15d** and 56.37 for (R_P)-**15d**.[24] Thus the (S_P)-configuration of the amidates, as determined by the X-ray analysis, can be correlated with the (R_P)-configuration in the phosphorothioic acid series by phosphorus NMR shift values.

The bulky silyl protecting group is important for the isolation of the first formed phosphorothioic acid **12** because it renders the product insoluble and assists in the precipitation of the thiated product **12** from a complex aqueous mixture in the work up of the reaction. The purity of the product, thus obtained, was often sufficient for direct silyl deprotection with fluorides such as ammonium fluoride. With ammonium fluoride in DMF at room temperature, the reaction was slow and required 3–5 days. With cesium fluoride, however, the reaction in the cold required only a few hours, but the product was less pure.

Conclusion

A method for stereocontrolled preparation of 8-carbon substituted (R_P)-adenosine-3′,5′-cyclic phosphorothioic acids has become available. Stereochemistry at phosphorus was introduced by stereoselective amidation. Phosphoramidates with the (S_P)-configuration have been converted by thiylation into phosphorothioic acids with the (R_P)-configuration. cAMP has been efficiently brominated to provide 8-bromo-cAMP which, in a reaction sequence, has been cross-coupled under Pd-catalysis to provide 8-hetaryl derivatives as substrates for 8-hetaryl-cAMPS derivatives. The cAMP substrates were 2*O*′-protected by a bulky silyl group. The nature of the purine 8-substituent did not exert any marked effect on the stereoselectivity in the amidation.

Experimental

^1H NMR spectra were recorded in $CDCl_3$ or DMSO at 200, 300 and 500 MHz with Bruker DPX 200, 300 and 500 instruments. The ^{13}C NMR spectra were recorded at 75, 100 and 150 MHz. Chemical shifts are reported in ppm using $CHCl_3$ (7.24 ppm) and $CDCl_3$ (77 ppm) as references, and in DMSO, 2.49 ppm in ^1H NMR and 39.5 ppm in ^{13}C NMR. The ^{31}P spectra were recorded in $CDCl_3$, MeOH-d_4 or D_2O at 81 MHz or 121 MHz with a Bruker DPX 200 or 300 instrument with 85% H_3PO_4 as an external reference. Mass spectra were recorded at 70 eV with a Fisons VG Prospectrometer. The spectra are presented as *m*/*z* (% relative intensity). Electrospray spectra were obtained with a Micromass QTOF 2 W spectrometer with electrospray ionisation quadrupole time of flight.

THF was distilled from sodium–benzophenone. Dichloromethane and tri-*n*-butylamine were distilled from calcium hydride. Flash chromatography on silica gel was carried out on Merck kieselgel 60 (230–400).

X-Ray crystallographic analysis for compounds 10a and 11a‡

X-Ray data were collected on a Siemens SMART CCD diffractometer[26] using graphite monochromated Mo$K\alpha$ radiation ($\lambda = 0.71073$ Å). Data collection method: ω-scan, range 0.6°, crystal to detector distance 5 cm. Data reduction and cell determination were carried out with the SAINT and XPREP programs.[26] Absorption corrections were applied by the use of the SADABS program.[27] The structures were determined and refined using the SHELXTL program package.[28] The non-hydrogen atoms were refined with anisotropic thermal parameters, hydrogen atoms were located from difference Fourier maps and refined with isotropic thermal parameters.

Crystal data for $C_{27}H_{35}N_6O_6PSi$ (10a). $M = 598.67$, orthorhombic, $P2_12_12_1$, $a = 10.853(1)$, $b = 13.388(1)$, $c = 20.924(1)$ Å, $V = 3040.3(1)$ Å^3, $Z = 4$, $D_x = 1.308$ Mg m^{-3}, $\mu = 0.180$ mm^{-1}, $T = 120(2)$ K, measured 57613 reflections in 2θ range 3.6–72.6°, $R_{int} = 0.033$. 510 parameters refined against 14434 F^2, $R = 0.032$ for $I_o > 2\sigma(I_o)$ and 0.042 for all data.

Crystal data for $C_{26}H_{33}N_6O_6PSi$ (11a). $M = 584.64$, orthorhombic, $P2_12_12_1$, $a = 10.913(1)$, $b = 13.523(1)$, $c = 20.290(1)$ Å, $V = 2994.3(1)$ Å^3, $Z = 4$, $D_x = 1.297$ Mg m^{-3}, $\mu = 0.181$ mm^{-1}, $T = 105(2)$K, measured 39032 reflections in 2θ range 3.6–56.6°, $R_{int} = 0.031$. 493 parameters refined against 7452 F^2, $R_1 = 0.024$ for $I_o > 2\sigma(I_o)$ and 0.025 for all data.

8-Bromoadenosine-3′,5′-cyclic phosphoric acid (2)

Bromine (15.4 mL, 0.30 mol) was added with stirring to a solution of cAMP (98.8 g, 0.30 mol) and sodium acetate trihydrate (81.6 g, 0.60 mol) in water (1.5 L) over 1 h at room temperature. Sodium sulfite was added slowly after 24 h until disappearance of the dark red colour. The precipitate was collected by filtration, the solid washed with water, with 2-propanol and diethyl ether, and the product was dried at reduced pressure. The product was dispersed in water (500 mL) and dissolved by slow addition of sodium bicarbonate (1 eq.). When all the material had dissolved, small portions of sodium sulfite were added to remove the dark red colour of the solution. Precipitation of the product was effected by dropwise addition of 1.0 M hydrobromic acid under vigorous stirring. The precipitate was collected, washed with water, 2-propanol, and diethyl ether, and the bright yellow powder dried under high vacuum, yield 92.0 g (76%). ^{1}H NMR analysis was in accordance with the literature data.[20]

8-Bromo-2′*O*-(*tert*-butyldimethylsilyl)adenosine-3′,5′-cyclic phosphoric acid tri-*n*-butylammonium salt (3)

Method (i). TBDMS-Cl (2.72 g, 18 mmol) was added to a solution of 8-Br-cAMP (**2**) as tri-*n*-butylammonium salt (7.0 g, 11.8 mmol) and imidazole (2.45 g, 36 mmol) in DMF (30 mL) at room temperature. The mixture was stirred under argon at 60 °C for 20 h. The solvent was removed at reduced pressure, the residual material was suspended in water (150 mL), and was stirred for 30 min to hydrolyse any silylated phosphoric acid. Then 1 M HBr (*ca.* 12 mL to pH 2–3) was added to the filtrate. The precipitate was filtered off, washed with water, and dried under vacuum. The acid was suspended in MeOH (80 mL) and *n*-Bu_3N (5 mL) was added. The mixture was stirred at room temperature until a clear solution was obtained (30 min). The solvent was distilled off, and the residual salt product was dried under vacuum over P_2O_5 before being used as such in the subsequent reaction, yield 6.60 g (78%) of a white solid. HRMS (Electrospray, TOF ES$^-$): M 520.0427. Calc. for $C_{16}H_{24}BrN_5O_6PSi$: 520.0422. ^{31}P NMR ($CDCl_3$, 81 MHz): δ −1.36; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.01 (3H, s, Si-CH_3), 0.03 (3H, s, Si-CH_3), 0.83 (9H, s, Si-*t*Bu), 0.87 (9H, t), 1.28 (6H, m), 1.53 (6H, m), 2.79 (6H, m), 3.90 (2H, m), 4.11 (1H, m), 5.02 (2H, m), 7.53 (2H, s, NH_2), 8.15 (1H, s, H-2); ^{13}C NMR (DMSO-d_6, 75 MHz): δ −5.3, −4.7, 13.6, 18.0, 19.6, 25.6, 25.7, 51.9, 65.4, 72.3, 72.4, 76.2, 94.3, 119.2, 126.5, 150.0, 153.2, 155.0.

Method (ii). TBDMS-triflate (4.75 g, 4.13 mL, 18 mmol) was added to a suspension of 8-Br-cAMP (**2**) as tri-*n*-butylammonium salt (5.3 g, 8.9 mmol) and imidazole (1.72 g, 27 mmol) in CH_2Cl_2 (40 mL) at room temperature, and the mixture stirred under argon for 20 h. The solvent was removed, the residual material suspended in water (120 mL), stirred for 30 min to hydrolyse any silylated phosphoric acid. Then 1 M HBr (*ca.* 10 mL) was added to the filtrate to pH 2–3. The precipitate was filtered off, washed with water, and dried at reduced pressure. The acid was suspended in MeOH (60 mL) and *n*-Bu_3N (4 mL) was added. The mixture was stirred at room temperature until a clear solution was obtained (30 min), and the solvent distilled off. The residual product was dried at reduced pressure over P_2O_5. The crude product was used as such in the subsequent reaction, yield 4.93 g (78%) of a white solid salt. Physical data as above.

(S_P)-8-Bromoadenosine-2′*O*-(*tert*-butyldimethylsilyl)-3′,5′-cyclic *N*-benzylphosphoramidate (5)

A solution of 8-bromadenosine-2′*O*-(*tert*-butyldimethylsilyl)-3′,5′-cyclic phosphoric acid tri-*n*-butylammonium salt (**3**) (7.070 g, 10.0 mmol) in CH_2Cl_2 (70 mL) was added dropwise to a solution of oxalyl chloride (5.5 mL, 11.0 mmol, 2 M in CH_2Cl_2) and DMF (0.58 mL, 3.6 mmol) in CH_2Cl_2 (70 mL) at −60 °C. Benzylamine (40 mmol, 4.37 mL), (dried over calcium hydride), was added after 30 min, and the mixture was stirred at −60 °C for 10 min and at room temperature for 2 h, diluted with $CHCl_3$ (100 mL) and washed with saturated $NaHCO_3$(aq.) (2 × 50 mL). The organic phase was dried ($MgSO_4$), the solvent was distilled off and the residue was purified by flash chromatography on silica gel using CH_2Cl_2–MeOH (3 : 97 and 5 : 95); yield 3.00 g (49%) of a white solid. The product was dissolved in CH_2Cl_2 and reprecipitated as a white powder on slow addition of hexane, mp slow decomp. 190–200 °C (brown–black remains no mp <300 °C). Electrospray (TOF MS ES$^+$): 611.2/613.2; ^{31}P NMR ($CDCl_3$, 121 MHz): δ 8.29; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.011 (3H, s, Si-CH_3), 0.014 (3H, s, Si-CH_3), 0.82 (9H, s, Si-*t*Bu), 3.81–3.90, (1H, m, NH), 4.12–4.21 (3H, m), 4.39–4.68 (3H, m), 4.97 (1H, d, *J* 5.1 Hz), 5.54–5.60 (1H, m), 5.88 (1H, s), 6.50 (2H, br s, NH_2), 7.19–7.33 (5H, m), 8.04 (1H, s, H-2); ^{13}C NMR ($CDCl_3$, 75 MHz): δ −5.1, −4.7, 18.1,

‡ CCDC reference numbers 625282 (**11a**) and 625283 (**10a**). For crystallographic data in CIF or other electronic format see DOI: 10.1039/b702403g

25.6, 45.3, 68.2 (*J*p 6.9 Hz), 71.3 (*J*p 4.2 Hz), 73.0 (*J*p 8.3 Hz), 76.3 (*J*p 3.6 Hz), 94.7, 120.1, 127.0, 127.4, 128.5, 138.7, 139.7(*J*p 6.5 Hz), 150.4, 153.5, 154.4.

(S_P)-8-Bromoadenosine-2′*O*-(*tert*-butyldimethylsilyl)-3′,5′-cyclic *N*-phenylphosphoramidate (6)

A solution of dry DMF (0.289 g, 3.96 mmol) in dry THF (20 mL) under argon gas was cooled to 0 °C and oxalyl chloride in dichloromethane (2 mL, 2 M, 4 mmol) was added slowly. The cooling bath was removed and the suspension was left with stirring at room temperature for 30 min. The reaction mixture was cooled to −20 °C and added to a solution of tri-*n*-butylammonium 8-bromoadenosine-2′*O*-TBDMS-3,5-cyclic monophosphate (**3**) (2.55 g, 3.60 mmol) in dry dichloromethane (8 mL). The mixture was stirred at this temperature for 1 h, and was allowed to reach room temperature before dry aniline (3.35 g, 36 mmol) was added. The aniline had previously been dried (calcium hydride). After 3 h, the turbid reaction mixture was diluted to 100 mL with dichloromethane and washed with cold, saturated sodium hydrogen carbonate (3 × 25 mL). The organic phase was dried ($MgSO_4$), the solvent was removed at reduced pressure, and the residual material was added slowly with vigorous stirring to cyclohexane (100 mL). The precipitate was dried and subjected to flash chromatography on silica gel using 7% methanol in dichloromethane, yield 1.46 g (68%). ^{31}P NMR (DMSO-d_6, 81 MHz): δ 2.28; ^{1}H NMR (DMSO-d_6, 300 MHz): δ −0.09 (3H, s, Si-CH_3), 0.02 (3H, s, Si-CH_3), 0.79 (9H, s, Si-*t*Bu), 4.4 (2H, m), 4.65 (1H, m), 5.12 (1H, d), 5.52 (1H, m), 5.92 (1H, s), 6.93 (1H, t), 7.10 (2H, d), 7.20 (2H, t), 7.56 (2H, s, NH_2), 8.23 (1H, s, H-2), 8.55 (1H, d, Ar-NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ −5.2–5.0, 17.8, 24.9, 68.2, 70.4, 72.3, 76.1, 92.9, 117.7, 118.5, 121.8, 128.9, 135.9, 139.5, 149.7, 153.4, 155.1.

2′*O*-(*tert*-Butyldimethylsilyl)adenosine-3′,5′-cyclic phosphoric acid tri-*n*-butylammonium salt (7)

TBDMS-triflate (8.04 mL, 35 mmol) was added to a suspension of cAMP (**1**) as tri-*n*-butylammonium salt (8.99 g, 17.5 mmol) and imidazole (3.57 g, 52.5 mmol) in CH_2Cl_2 (60 mL) at room temperature. The mixture was stirred at room temperature under argon for 20 h. The solvent was removed, and the residual material suspended in water (120 mL) and stirred for 30 min to hydrolyse any silylated phosphoric acid. Then 1 M HBr was added to the filtrate to pH 2–3. The precipitate was filtered off, and was washed with water and dried under vacuum. The acid was suspended in MeOH (60 mL) and *n*-Bu_3N (5 mL) was added. The mixture was stirred at room temperature until a clear solution was obtained (30 min). The solvent was distilled off, and the residual product was dried under vacuum over P_2O_5 before being used as such in the subsequent reaction, yield 8.24 g (75%) of a white salt.

^{31}P ($CDCl_3$, 121 MHz): δ −2.01; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.01 (6H, s, 2 × Si-Me), 0.79 (9H, s, Si-*t*Bu), 0.79–0.85 (9H, t), 1.16–134 (6H, m), 1.49–1.65 (6H, m), 2.83–2.91 (6H, m), 4.14–4.27 (3H, m), 4.50–4.61 (2H, m,), 5.89 (1H, s), 6.47 (2H, br s, NH_2), 7.81 (1H, s,), 8.16 (1H, s, H-2); ^{13}C NMR ($CDCl_3$, 50 Mz): δ −4.6, −4.2, 13.9, 18,6, 20.3, 25.7, 26.1, 52.7, 67.5, 72.6, 74.9, 76.2, 93.3, 118.8, 138.6, 149.5, 153.5, 156.2.

(S_P)-2′*O*-(*tert*-Butyldimethylsilyl)adenosine-3′,5′-cyclic *N*-benzylphosphoramidate (8)

Dry DMF (0.385 mL, 5.00 mmol) in dry CH_2Cl_2 (40 mL) was placed under an atmosphere of argon gas and cooled to 0 °C before oxalyl chloride (4 mL, 2 M in CH_2Cl_2, 8.14 mmol) was added slowly. The cooling bath was removed and the suspension left to stir at room temperature for 30 min. The reaction mixture was cooled to −20 °C, and a solution of 2′*O*-(*tert*-butyldimethylsilyl)adenosine-3′,5′-cyclic phosphoric acid tri-*n*-butylammonium salt (**7**) (4.650 g, 7.4 mmol) in dry dichloromethane (30 ml) was added through a Teflon tube. The mixture was stirred at this temperature for 1 h, and benzylamine (3.2 mL, 29 mmol) (dried over CaH_2) was added. The cooling bath was removed after 1 h and stirring was continued at room temperature for 2 h before the turbid reaction mixture was filtered. Dichloromethane (120 mL) was added to the filtrate before extraction with cold, saturated sodium hydrogen carbonate (2 × 50 mL). The organic phase was dried ($MgSO_4$), the solvent was removed at reduced pressure, and the residual material subjected to flash chromatography on silica gel using 10% methanol in CH_2Cl_2; yield 1.92 g (49%) of a white solid. The product was dissolved in CH_2Cl_2 and reprecipitated as a white powder on slow addition of hexane, mp slow decomp. 200–210 °C (brown–black remains, no mp <300 °C). HRMS (Electrospray, TOF ES$^+$): 533.2076. Calc. for $C_{23}H_{33}N_6O_5PSi + H^+$: 533.2092. ^{31}P ($CDCl_3$, 121 MHz): δ 7.92; ^{1}H NMR ($CDCl_3$, 200 MHz): δ −0.05 and 0.0 (6H, 2s, 2 × Si-Me), 0.76 (9H, s, Si-*t*Bu), 3.97–4.03 (2H, m), 4.17–4.22 (2H, m), 4.35–4.56 (2H, m), 4.64 (1H, d, *J* 5.1 Hz), 4.96–5.08 (1H, m), 5.83 (2H, br s, NH_2), 5.86 (1H, s), 7.13–7.20 (5H, m), 7.88 (1H, s), 8.12 (1H, s, H-2); ^{13}C NMR (MeOH, 75 Mz): δ −1.65, −1.48, 17.3, 21.5, 48.5, 69.4, 72.0, 74.55, 77.5, 96.6, 122.8, 130.5, 130.9, 131.9, 142.1, 142.4, 152.4, 156.7, 159.1.

8-(2-Furyl)adenosine-2′*O*-(*tert*-butyldimethylsilyl)-3′,5′-cyclic phosphoric acid tri-*n*-butylammonium salt (9)

A solution of $Pd(OAc)_2$ (0.170 g, 0.76 mmol) and PPh_3 (0.420 g, 1.6 mmol) in dry, degassed DMF (20 mL) was stirred at 50 °C until the solution had turned dark red. A solution of 8-bromoadenosine-2′*O*-(*tert*-butyldimethylsilyl)-3′,5′-cyclic phosphoric acid tri-*n*-butylammonium salt (**3**) (2.700 g, 3.8 mmol) in DMF (10 mL) together with 2-(tri-*n*-butylstannyl)furan (1.7 g, 4.77 mmol) was added. The reaction mixture was stirred at 90 °C for 5 h. The DMF was removed at reduced pressure, and the residual material was subjected to flash chromatography on silica gel using CH_2Cl_2–MeOH–NBu_3 (100 : 7.5 : 1). The coupled product was isolated as a white solid material which contained traces of organotin residues which were effectively removed by dissolution in CH_2Cl_2 and precipitation by hexane, yield 1.8 g (72%). HRMS (Electrospray, TOF ES$^-$): 508.1407. Calc. for $[C_{20}H_{27}N_5O_7PSi]^-$: 508.1422. ^{31}P ($CDCl_3$, 121 MHz): δ −1.1; ^{1}H NMR ($CDCl_3$, 200 MHz): δ −0.05 and 0.05 (6H, 2 s, 2 × Si-Me), 0.77 (9H, s, Si-*t*Bu), 0.88–0.95 (9H, t), 1.29–1.40 (6H, m), 1.53–1.71 (6H, m), 2.87–2.96 (6H, m), 4.18–4.34 (3H, m,), 5.08 (1H, d, *J* 5.1 Hz), 5.39–5.44 (1H, m), 6.07 (2H, br s, NH_2), 6.19 (1H, s), 6.55–6.58 (1H, m), 7.08–7.10 (1H, m), 7.61–7.62 (1H, m), 8.28 (1H, s, H-2); ^{13}C NMR ($CDCl_3$, 50 Mz): −5.3, −4.3, 13.6, 18.2,

20.1, 25.1, 25.7, 51.7, 67.5, 72.6, 74.9, 76.2, 94.4, 111.9, 113.9, 119.7, 142.1, 143.4, 144.9, 150.5, 153.1, 155.2.

(S_P)-2′*O*-(*tert*-Butyldimethylsilyl)-8-(2-furyl)adenosine-3′,5′-cyclic *N*-benzyl phosphoramidate (10a)

(i) By amidation of the cyclic phosphoric acid tri-*n*-butylammonium salt 9. Dry DMF (0.1 mL, 1.3 mmol) in dry CH_2Cl_2 (20 mL) was placed under an atmosphere of argon gas and cooled to 0 °C before oxalyl chloride (1 mL, 2 M in CH_2Cl_2, 2 mmol) was added slowly. The cooling bath was removed and the suspension stirred at room temperature for 30 min, cooled to −20 °C and a solution of 8-(2-furyl)adenosine-2′*O*-(*tert*-butyldimethylsilyl)-3′,5′-cyclic phosphoric acid tri-*n*-butylammonium salt (**9**) (1.2 g, 1.75 mmol) in dry CH_2Cl_2 (15 mL) was added through a Teflon tube. The mixture was stirred at this temperature for 1 h, dry (CaH_2) benzylamine (0.76 mL, 7 mmol) added, and the mixture was stirred at room temperature for 2 h when the turbid reaction mixture was filtered. Then CH_2Cl_2 (120 mL) was added to the filtrate, the solution shaken and the solvent removed at reduced pressure. The residual material was subjected to flash chromatography on silica gel using 4% methanol in CH_2Cl_2; yield 0.745 g (71%) of a white solid. The product was dissolved in CH_2Cl_2 and reprecipitated as a white powder on slow addition of hexane, mp slow decomp. 190–200 °C (brown–black remains, no mp <300 °C). Electrospray MS (TOF MS ES$^+$): 599.2; ^{31}P ($CDCl_3$, 121 MHz): δ 7.94; ^{1}H NMR ($CDCl_3$): δ −0.05 and 0.02 (6H, 2s, 2 × CH_3), 0.78 (9H, s, $C(CH_3)_3$), 3.61–3.72 (1H, m, NH), 4.13–4.23 (3H, m), 4.35–4.59 (2H, m), 5.11 (1H, d, *J* 5.2 Hz), 5.65–5.73 (1H, m), 6.07 (2H, br s, NH_2), 6.30 (1H, s), 6.58–6.61 (1H, m), 7.10–7.13 (1H, m), 7.28–7.33 (5H, m), 7.61–7.62 (1H, m), 8.26 (1H, s, H-2); ^{13}C NMR ($CDCl_3$, 75 Mz): δ −5.3, −4.6, 18.05, 25.5, 45.4, 68.4, 71.3, 73.1, 77.2, 94.2, 112.1, 114.1, 119.7, 127.1, 127.5, 128.6, 138.7, 141.8, 143.5, 144.9, 150.3, 153.2, 155.2.

(ii) By Pd-catalysed cross-coupling from the benzylphosphoramidate 5. A solution of $Pd(OAc)_2$ (60 mg, 0.27 mmol) and PPh_3 (142 mg, 0.54 mmol) in DMF (8 mL) under argon was stirred at 50 °C for 15 min before 2-(tri-*n*-butylstannyl)furan (0.63 ml, 2.0 mmol) was added. Subsequently, a solution of (S_P)-8-bromoadenosine-2′*O*-(*tert*-butyldimethylsilyl)-3′,5′-cyclic *N*-benzylphosphoramidate (**5**) (800 mg, 1.3 mmol) in DMF (10 mL) was added. The mixture was stirred at 85 °C for 2.5 h. The solvent was evaporated off, and the residue was purified by flash chromatography on silica gel using CH_2Cl_2–MeOH (3 : 97 and 5 : 95); yield 636 mg (80%) of a white solid.

(S_P)-8-(2-Furyl)adenosine-2′*O*-(*tert*-butyldimethylsilyl)-3′,5′-cyclic *N*-phenylphosphoramidate (11a)

A solution of $Pd(OAc)_2$ (99 mg, 0.37 mmol) and PPh_3 (253 mg, 0.81 mmol) in DMF (15 mL) under argon was stirred at 50 °C for 15 min when the mixture had become reddish brown. A solution of tri-*n*-butyl(2-furyl)stannane (1.4 mL, 4.4 mmol) was added, followed by a solution of (S_P)-8-bromadenosine-2′*O*-(*tert*-butyldimethylsilyl)-3′,5′-cyclic *N*-phenylphosphoramidate (**6**) (2.2 g, 3.7 mmol) in DMF (10 mL). The mixture was stirred at 80 °C for 1 h. The solvent was evaporated, and the residue subjected to flash chromatography on silica gel using CH_2Cl_2–MeOH (3 : 97 and 5 : 95); yield 1.93 g (90%) of a white solid. HRMS (Electrospray, TOF ES$^+$): M 585.2023. Calc. for $C_{26}H_{34}N_6O_6PSi$: 585.2041. ^{31}P NMR ($CDCl_3$, 81 MHz): δ 3.06; ^{1}H NMR (DMSO-d_6, 300 MHz): δ −0.14 (3H, s, Si-CH_3), −0.11 (3H, s, Si-CH_3), 0.70 (9H, s, Si-*t*Bu), 4.4–4.5 (2H), m, 4.65 (1H, dm), 5.19 (1H, d), 5.65 (1H, m), 6.31 (1H, s), 6.77 (1H, dd), 6.93 (1H, t), 7.1–7.2 (5H, m), 7.58 (2H, br s), 8.00 (1H, d), 8.27 (1H, s), 8.56 (1H, d); ^{13}C NMR (DMSO-d_6, 75 MHz): δ −5.6, −5.0, 17.7, 25.3, 68.3, 70.2, 72.4, 76.3, 93.6, 112.3, 113.9, 118.45, 121.8, 128.9, 139.6, 140.3, 143.2, 145.6, 149.8, 153.4, 156.1.

(S_P)-2′*O*-(*tert*-Butyldimethylsilyl)-8-(*N*-methylpyrrol-2-yl)adenosine-3′,5′-cyclic *N*-phenylphosphoramidate (11b)

Compound **11b** was prepared as above from (S_P)-8-bromoadenosine-2′*O*-(*tert*-butyldimethylsilyl)-3′,5′-cyclic-*N*-phenylphosphoramidate (**6**) in 70% yield. HRMS (electrospray, TOF ES$^+$): M + H 598.2339. Calc. for [$C_{27}H_{36}N_7O_5PSi$ + H$^+$]: 598.2357. ^{31}P NMR ($CDCl_3$), 121 MHz): δ 2.54 ppm.

(S_P)-2′*O*-(*tert*-Butyldimethylsilyl)-8-(3-pyridinyl)adenosine-3′,5′-cyclic *N*-phenylphosphoramidate (11c)

A solution of $Pd(OAc)_2$ (37 mg, 0.166 mmol) and PPh_3 (91 mg, 0.348 mmol) in NMP (4 mL) under argon was stirred at 50 °C for 30 min when the solution had turned dark red. A solution of (S_P)-8-bromoadenosine-2′*O*-(*tert*-butyldimethylsilyl)-3′,5′-cyclic *N*-phenylphosphoramidate (**6**) (0.500 g, 0.83 mmol) in NMP (2 mL) and 3-(tri-*n*-butylstannyl)pyridine (0.610 g, 1.66 mmol) was added. The reaction mixture was stirred at 110 °C for 10 h before the NMP was removed at reduced pressure. The residual material subjected to flash chromatography on silica gel using 7.5% MeOH in CH_2Cl_2. The product was a white solid contaminated with traces of organotin residues which were removed by dissolution of the coupling product in CH_2Cl_2 and reprecipitation by hexane, yield 0.280 g (57%); HRMS (electrospray, TOF ES$^+$): M + H 596.2211. Calc. for [$C_{27}H_{34}N_7O_5PSi$ + H$^+$]: 596.2201. ^{31}P NMR ($CDCl_3$, 121 MHz): δ 2.73 ppm, ^{1}H NMR ($CDCl_3$, 300 MHz): δ −0.16 (3H, s, CH_3), −0.15 (3H, s, CH_3), 0.60 (9H, s, $C(CH_3)_3$), 4.30–4.43 (1H, m, H-4′), 4.60–4.68 (2H, m, OCH_2), 5.15 (1H, d, *J* 5.2 Hz, H-2′), 5.69 (1H, s, H-1′), 5.75–5.82 (1H, m, H-3′), 6.37 (2H, br s, NH_2), 6.58 (1H, d, *J* 9.2 Hz, NH), 6.99–7.10 (3H, m, 3 × H-Ph), 7.17–7.24 (2H, t, *J* 7.4 Hz, 2 × H-Ph), 7.42–7.48 (1H, m, H-pyr), 8.02–8.06 (1H, m, H-pyr), 8.37 (1H, s, H-2), 8.76–8.79 (1H, m, H-pyr), 8.97 (1H, d, *J* 1.7 Hz, H-pyr); ^{13}C NMR ($CDCl_3$, 75 MHz): δ −5.5 and −4.8 (2 × CH_3), 18.0 (Si-C), 25.4 (3 × CH_3), 68.9 (d, *J* 6.8 Hz, OCH_2), 71.3 (d, *J* 4.5 Hz, CH-4′), 73.3 (d. *J* 8.8 Hz, CH-2′), 77.5 (d, *J* 3.8 Hz, CH-3′), 94.2 (CH-1′), 119.4, 119.5, 119.6, 122.9, 123.5, 125.0, 129.1, 129.1, 136.8, 138.5, 148.1, 149.8, 150.3, 151.4, 153.5, 155.9.

(R_P)-2′*O*-(*tert*-Butyldimethylsilyl)-8-(2-furyl)adenosine-3′,5′-cyclic phosphorothioic acid (12a)

A 1.0 M solution of *t*-BuOK in THF (3.6 mL, 3.6 mmol) was added to a solution of (S_P)-2′*O*-(*tert*-butyldimethylsilyl)-8-(2-furyl)adenosine-3′,5′-cyclic *N*-phenylphosphoramidate (**11a**) (1.51 g, 2.6 mmol) in THF (30 mL) at room temperature. The mixture was stirred at this temperature for 1 h before CS_2 (0.47 mL, 7.8 mmol) was added. The mixture was stirred for another 3 h at room temperature, the volume of the solvent was reduced to

about 10 mL, and hexane (90 mL) was added. The precipitate was collected by filtration, suspended in water (55 mL), and 1.2 M HCl (9 mL) was added. The product was collected by filtration, washed with water and dried, yield 1.23 g (91%) of a light tan solid. HRMS (Electrospray, TOF ES$^-$): M 526.1326. Calc. for $C_{20}H_{29}N_5O_6PSSi$: 526.1339. ^{31}P NMR (DMSO-d_6, 81 MHz): δ 58.4; ^{1}H NMR (DMSO-d_6, 300 MHz): δ −0.03 (3H, s, Si-CH_3), 0.04 (3H, s, Si-CH_3), 0.79 (9H, s, Si-*t*-Bu), 4.0–4.5 (3H, m), 5.06 (1H, d), 5.34 (1H, m), 6.21 (1H, s), 6.79 (1H, q), 7.18 (1H, d), 8.02 (1H, s), 8.2 (2H, br s), 8.33 (1H, s); ^{13}C NMR (DMSO-d_6, 75 MHz): δ −5.4, −4.6, 17.9, 25.5, 67.7, 71.1, 72.8, 75.9, 93.2, 112.4, 114.4, 119.1, 141.3, 142.8, 146.0, 149.4, 150.2, 153.7

(R_P)-8-(2-Furyl)adenosine-3′,5′-cyclic phosphorothioic acid ammonium salt (13a)

(i) Prepared from the phosphorothioic acid 12a. A solution of (R_P)-2′*O*-(*tert*-butyldimethylsilyl)-8-(2-furyl)adenosine-3′,5′-cyclic phosphorothioic acid (**12a**) (1.15 g, 2.7 mmol) and NH_4F (225 mg, 6.0 mmol) in DMF (10 mL) under argon was stirred at room temperature for 5 d. Subsequently, TMSOMe (1 mL) was added and the stirring was continued for 24 h. The solvent was distilled off at reduced pressure, the crude product was suspended in MeOH (10 mL), and Et_2O (80 mL) added. The light tan-coloured solid was filtered off and dried, yield 847 mg (90%). HRMS (Electrospray, TOF ES$^-$): M 410.0330. Calc. for $C_{14}H_{13}N_5O_6PS$: 410.0329. ^{31}P NMR (DMSO-d_6, 81 MHz): δ 54.3; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 4.0–4.2 (4H, m), 5.03 (1H, d), 5.14 (1H, m), 6.02 (1H, s), 6.76 (1H, dd), 7.13 (1H, d), 8.01 (1H, d), 8.21 (1H, s); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 66.15, 75.3, 75.4, 76.9, 92.8, 112.3, 113.8, 118.9, 141.2, 143.1, 145.8, 149.8, 152.7, 155.6.

(ii) Prepared from the *N*-benzylamidate 10a. A 1.6 M solution of *n*-BuLi in hexane (0.25 mL, 0.39 mmol) was added to a solution of (S_P)-2′*O*-(*tert*-butyldimethylsilyl)-8-(2-furyl)adenosine-3′,5′-cyclic *N*-benzylphosphoramidate (**10a**) (200 mg, 0.33 mmol) in THF (4 mL) at −78 °C. The mixture was stirred for 10 min at this temperature before carbon disulfide (0.06 ml, 1.0 mmol) was added and the cooling bath removed. The mixture was stirred for 3 h at room temperature. The volume of the solvent was reduced to about 1 mL before hexane (40 mL) was added. The precipitate was collected and dissolved in dry DMF (2 mL), and ammonium fluoride (75 mg, 2.0 mmol) added. The mixture was stirred under argon at 40 °C for 48 h. The solvent was distilled off and the crude product purified by flash chromatography on silica gel using iPrOH–EtOAc–H_2O–NH_3(aq.) (7 : 7 : 1 : 1); yield: 106 mg (74%) of a white solid. Spectroscopic data for this compound is given in the alternative preparation described above.

(R_P)-8-(2-Furyl)adenosine-3′,5′-cyclic phosphorothioc acid tri-*n*-butylammonium salt (14a)

A 1.6 M solution of *n*-BuLi in hexane (1.87 mL, 3.0 mmol) was added to a solution of (S_P)-8-(2-furyl)adenosine-2′*O*-*tert*-butyldimethylsilyl)-3′,5′-cyclic *N*-benzylphosphoramidate **10a** (1.650 g, 2.75 mmol) in dry THF (25 mL) at −78 °C. The mixture was stirred under argon at this temperature for 10 min before CS_2 (0.49 mL, 8.25 mmol) was added. The cooling bath was removed after 10 min. The reaction mixture was stirred at room temperature for 3 h before the solvent was partially removed at reduced pressure. Addition of hexane to the residual solution precipitated a solid, which was filtered off and dissolved in water (10 mL). Then 1 M HBr was bubbled into the solution at 0 °C to reach pH 2–3. The resulting (R_P)-8-(2-furyl)adenosine-2′*O*-(*tert*-butyldimethylsilyl)-3′,5′-cyclic phosphorothioic acid **12a** was collected by filtration and dried overnight at high vacuum.

The dried phosphorothioic acid **12a** (1.050 g, 2.0 mmol) was dissolved in DMF (10 mL). Then NH_4F (0.520 g, 14 mmol) was added, and the mixture was stirred at room temperature for 5 d and filtered. Then *n*-Bu_3N (0.740 g, 4.0 mmol) was added until a clear solution resulted, and the solvent was removed at reduced pressure. The residue was washed with hexane to remove the excess of *n*-Bu_3N before being subjected to flash chromatography on silica gel using CH_2Cl_2–CH_3OH–*n*-Bu_3N (100 : 5 : 1). The ammonium salt, which contained some *n*-Bu_3N, was further purified by dissolution in CH_2Cl_2 and reprecipitation by hexane addition, yield 0.656 g (40% from **10a**) HRMS (Electrospray, TOF ES$^-$): M 410.0332. Calc. for $[C_{14}H_{13}N_5O_6PS]^-$: 526.0339. ^{31}P ($CDCl_3$, 121 MHz) δ 56.66; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.93 (9H, t, *J* 7.2 Hz), 1.35–1.48 (6H, m), 1.69–1.71 (6H, m), 2.91–3.04 (6H, m), 4.35–4.53 (3H, m), 5.15 (1H, d, *J* 5.2 Hz), 5.6–5.79 (1H, m), 5.81 (2H, br s), 6.24 (1H, s), 6.59–6.63 (1H, m), 7.11–7.13 (1H, m,), 7.62–7.63 (1H, m), 8.21 (1H, s, H-2); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 13.6, 21.1, 25.2, 51.9, 67.2, 71.7, 72.5, 77.5, 94.3, 113.2, 115.3, 120.1, 143.4, 144.4, 146.8, 151.4, 154.0, 156.9.

(R_P)-8-(*N*-Methylpyrrol-2-yl)adenosine-3′,5′-cyclic phosphorothioic acid tri-*n*-butylammonium salt (14b)

A mixture of (S_P)-2′*O*-(*tert*-butyldimethylsilyl)-8-(*N*-methylpyrrol-2-yl)adenosine-3′,5′-cyclic *N*-phenylphosphoramidate (**11b**) (0.300 g, 0.5 mmol) in dry THF (6 mL) and potassium *tert*-butoxide (0.62 mL, 0.62 mmol, 1 M in THF) was stirred under argon at room temperature for 1 h before carbon disulfide (0.09 ml, 1.5 mmol) was added. The reaction mixture was stirred at room temperature for 3 h. Most of the solvent was distilled off at reduced pressure. Addition of hexane gave a solid precipitate which was dissolved in water (9 mL), and 1.2 M HCl (1.25 mL) was added at 0 °C. The precipitate was the silylated (R_P)-8-(*N*-methylpyrrol-2-yl)adenosine-3′,5′-cyclic phosphorothioic acid (**12b**). The product was well dried *in vacuo* before the solid (0.170 g, 0.3 mmol) was dissolved in DMF (1.5 mL) under argon, and ammonium fluoride (0.075 g, 2.0 mmol) was added. The reaction mixture was stirred at room temperature for 5 d before tri-*n*-butylamine (0.111 g, 0.6 mmol) was added. A clear solution resulted. The volatile materials were removed at reduced pressure, the residue was triturated with hexane to remove any tri-*n*-butylamine and the residual material was subjected to flash chromatography on silica gel using CH_2Cl_2–MeOH–*n*Bu_3N (100 : 10 : 1). The tri-*n*-butylammonium salt, which contained some free tri-*n*-butylamine, was further purified by dissolution in CH_2Cl_2 and precipitation with hexane, yield 0.105 g (34%) of a white solid. HRMS (electrospray, TOF ES$^-$]: M-NHBu_3 423.0647. Calc. for $C_{15}H_{16}N_6O_5PS^-$): 423.0646. ^{31}P NMR ($CDCl_3$, 121 MHz): 55.68; ^{1}H NMR ($CDCl_3$, 300 MHz, MeOH-d_4): δ 0.93 (9H, t, *J* 7.3 Hz, 3 × CH_3), 1.29–1.41 (6H, m, 3 × CH_2), 1.55–1.65 (6H, m, 3 × CH_2), 2.98–3.04 (6H, m, 3 × CH_2), 3.78 (3H, s, N-CH_3), 4.12–4.24 (1H, m, H-4′), 4.25–4.30 (2H, m, OCH_2), 4.96 (1H, d, *J* 5.3 Hz,

H-2′), 5.49 5.57 (1H, m, H-3′), 5.94 (1H, s, H-1′), 6.25–6.28 (1H, m, H-pyr), 6.60–6.62 (1H, m, H-pyr), 6.93–6.95 (1H, m, H-pyr), 8.13 (1H, s, H-2); ^{13}C NMR (MeOH-d_4, 75 MHz): δ 13.9 (3 × CH_3), 20.9 (3 × CH_2), 26.9 (3 × CH_2), 35.7 (N-CH_3), 54.0 (3 × CH_2), 68.5 (d, J 9.8 Hz, OCH_2), 72.8 (d, J 7.6 Hz, CH-4′), 73.1 (d. J 5.7 Hz, CH-2′), 77.7 (d, J 6.6 Hz, CH-3′), 94.2 (CH-1′), 109.4, 120.1, 121.1, 128.4, 146.3, 151.2, 153.6, 156.8.

(R_P)-8-(3-Pyridinyl)adenosine-3′,5′-cyclic phosphorothioic acid tri-*n*-butylammonium salt (14c)

Compound **14c** was prepared as above from (S_P)-2′*O*-(*tert*-butyldimethylsilyl)-8-(3-pyridinyl)adenosine-3′,5′-cyclic *N*-phenylphosphoramidate (**11c**) in 34%. HRMS (electrospray, TOF ES$^-$): M-NHBu$_3$ 421.0492. Calc. for $[C_{15}H_{14}N_6O_5PS]^-$: 421.0489. ^{31}P ($CDCl_3$, 121 MHz): δ 57.17.

(R_P)-Adenosine-3′,5′-cyclic phosphorothioic acid tri-*n*-butylammonium salt (14d)

Compound **14d** was prepared as described for **14a** from (S_P)-2′*O*-(*tert*-butyldimethylsilyl)adenosine-3′,5′-cyclic *N*-benzylphosphoramidate (**8**) in 41%. HRMS (electrospray, TOF ES$^-$): 344.0231. Calc. for $[C_{10}H_{11}N_5O_5PS]^-$: 344.0224. ^{31}P NMR (MeOH-d_4, 121 MHz): δ 58.16.

(R_P)-8-(2-Furyl)adenosine-3′,5′-cyclic phosphorothioic acid sodium salt (15a)

(*R*p)–8-(2-Furyl)adenosine-3′,5′-cyclic phosphorothioic acid tri-*n*butylammonium salt (**14a**) (0.600 g, 1.00 mmol) was dissolved in 0.1 M NaOH in MeOH (10 mL). The sodium salt was precipitated by addition of diethyl ether and collected by filtration, yield 0.389 g (89%) of a white, solid material. HRMS (electrospray, TOF ES$^-$): 410.0320 Calc. for $[C_{14}H_{13}N_5O_6PS]^-$: 410.0329). ^{31}P (MeOH-d_4, 121 MHz): δ 58.05; ^{31}P (D_2O, 121 MHz): δ 56.44; ^{1}H NMR (MeOH-d_4, 200 MHz): 4.25–4.39 (3H, m), 5.15 (1H, d, J 5.2 Hz), 5.45–5.54 (1H, m), 6.24 (1H, s), 6.68–6.71 (1H, m), 7.19–7.21 (1H, m,), 7.82–7.83 (1H, m,), 8.20 (1H, s, H-2); ^{13}C NMR (MeOH-d_4, 75 MHz): δ 68.5, 72.6, 73.2, 77.6, 94.3, 113.2, 115.3, 120.1, 143.4, 144.4, 146.8, 151.4, 154.0, 156.9.

(R_P)-8-(*N*-Methylpyrrol-2-yl)adenosine-3′,5′-cyclic phosphorothioic acid sodium salt (15b)

Compound **15b** was prepared as above from (R_P)-8-(*N*-methylpyrrol-2-yl)-3′,5′-cyclic phosphorothioic acid tri-*n*butylammonium salt (**14b**) in 84% yield. HRMS (electrospray, TOF ES$^-$): M − Na 423.0630. Calc. for $[C_{15}H_{16}N_6O_5PS]^-$: 423.0646. ^{31}P NMR (MeOH-d_4) δ 58.06; (D_2O): δ 56.42.

(R_P)-8-(3-Pyridinyl)adenosine-3′,5′-cyclic phosphorothioic acid sodium salt (15c)

Compound **15c** was prepared from (R_P)-8-(3-pyridinyl)adenosine-3′,5′-cyclic phosphorothioic acid tri-*n*-butylammonium salt (**14c**) as above in 80% yield. HRMS (electrospray, TOF ES$^-$): M − Na 421.0495. Calc. for $[C_{15}H_{14}N_6O_5PS]$: 421.0489. ^{31}P (MeOH-d_4, 121 MHz): δ 58.08 ppm.

(R_P)-8-Adenosine-3′,5′-cyclic phosphorothioic acid sodium salt (15d)

Compound **15d** was prepared from (R_P)-adenosine-3′,5′-cyclic phosphorothioic acid tri-*n*-butylammonium salt (**14d**) 90% yield. HRMS (electrospray, TOF ES$^-$): M − Na 344.0211. Calc. for $[C_{10}H_{11}N_5O_5PS]^-$: 344.0224. ^{31}P NMR (MeOH-d_4, 121 MHz): δ 58.39. ^{31}P NMR (D_2O, 121 MHz): δ 57.02.

Diastereomeric synthesis: (R_P)- and (S_P)-8-(2-furyl)adenosine-3′,5′-cyclic phosphorothioic acid sodium salt (15a) and (15aa)

8-Furyladenosine[22] (**16**) (0.333g, 1 mmol) was azeotropically dried in pyridine under reflux. The dried material was dissolved in dry pyridine (20 mL), and thiophosphoryl chloride (0.169 g, 1 mmol) in dry pyridine (5 mL) was added over 10 min to the reaction mixture under argon at −8 °C. The mixture was stirred at this temperature for 90 min when a single signal at 58.4 ppm in the ^{31}P NMR spectrum showed full conversion to the cyclic thiophosphoryl chloride. The reaction mixture was subsequently added to a vigorously stirred solution of sodium hydroxide (0.240 g, 6 mmol) in MeCN–H_2O (50%, 40 mL) at room temperature, and the mixture rapidly poured onto crushed dry ice. When the evolution of CO_2 had ceased, the solvents were removed under reduced pressure with a bath temperature not exceeding 35 °C. The solid precipitate was washed repeatedly with THF and dissolved in DMF (7 mL) and the solution filtered. Evaporation of the solvent at reduced pressure furnished the crude diastereomeric mixture as a solid sodium salt (0.26 g, 60%) in a 1 : 3 ratio of the (R_P)- and (S_P)-diastereomers. The diastereomers could be separated by preparative reversed phase chromatography using H_2O–MeOH–HCO_2H (80 : 20 : 0.5). ^{31}P NMR (D_2O, 81 MHz): (R_P)-isomer 56.20 ppm, (S_P)-isomer 55.45 ppm).

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