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AN EFFICIENT AND STEREOSELECTIVE SYNTHESIS OF 2',5'-OLIGO-(S_P)-THIOADENYLATES [1]

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Abstract- 2',5'-Phosphorothioato-oligoadenylates have been synthesized with S_P-stereoselectivity by using hydrogenphosphonate methodology followed by oxidative sulphurization.

INTRODUCTION

The 2'-5' internucleotidic bond is an essential element of oligonucleotides involved in a mechanism of cellular regulation, called "2-5 A system", thought to mediate the antiviral action of interferon[2]. Modified analogues of 2',5'-oligoadenylates aim to interact with the 2-5 A system addressing to new approaches to antiviral and antitumoral therapy. Particularly our efforts were directed to find inhibitors of 2'-phosphodiesterase, the specific enzyme responsible for the degradation of 2',5'-oligoadenylates[3], in order to increase their endogenous level and hence selectively potentiate their action in virus-infected cells where they are able to block the viral protein synthesis. In this contest we were looking for molecules that could bind to 2'-phosphodiesterase but not be processed. 2',5'-Di- and triadenylate phosphorothioates of S_P configuration seemed to be reasonable candidates, this configuration being known to be very resistant to the enzyme action.

In the last period the researchers involved in oligonucleotide synthesis and applications are getting more and more awareness of the need for the stereoselective formation of modified internucleotide bonds. Indeed modified oligonucleotides are gaining importance for new therapeutical approaches. Often modifications of the internucleoside group generate a new stereogenicity at the phosphorus atom and this stereogenicity can influence the biological activity. So it is appreciable how much the stereochemistry control is desirable in the formation of modified internucleotidic bonds.

In recent years, many syntheses of analogues of 2-5 A molecule (pppA2'p5'A2'p5'A)

and its core (A2'p5'A2'p5'A) have been performed.[4-12] In particular Nelson et al.[11] and Pfleiderer et al.[12] have published the synthesis of dimer and trimer adenylates containing 2'-5' phosphorothioate linkages via phosphite triester and phosphoramidite approaches, respectively, followed by oxidative sulphurization. Van Boom et al.[13] have prepared individual diastereomers of 2-5 A core analogues containing one phosphorothioate linkage, via a modified hydroxybenzotriazole phosphotriester method. Unfortunately none of these approaches is stereoselective, all of them yielding mixtures of R_P and S_P diastereomers in a ratio close to 1:1. Up to date the only known stereoselective synthesis of these compounds is the enzymatic synthesis (by 2-5 A synthetase with α-thioATP) giving the R_P configuration[14]. There are few other phosphorothioate syntheses with some selectivity for R_P[15,16] or S_P[16-18], but none applied to 2',5'-oligonucleotides.

In this paper we wish to report an efficient and stereoselective synthesis of S_P dimer and S_P,S_P trimer adenylates with 2'-5' phosphorothioate linkages, via hydrogenphosphonate method, followed by thiooxidation, starting from appropriately protected ribonucleosides. Oligonucleotide synthesis using nucleoside hydrogenphosphonates has been reported[19]. This method has been used for the preparation of oligodeoxyribonucleotides[20] and oligoribonucleotides[21] as well as for the synthesis of phosphoramidate and phosphothioate analogues of DNA[22], but never in the case of 2'-5' linkages and in relation to stereoselectivity.

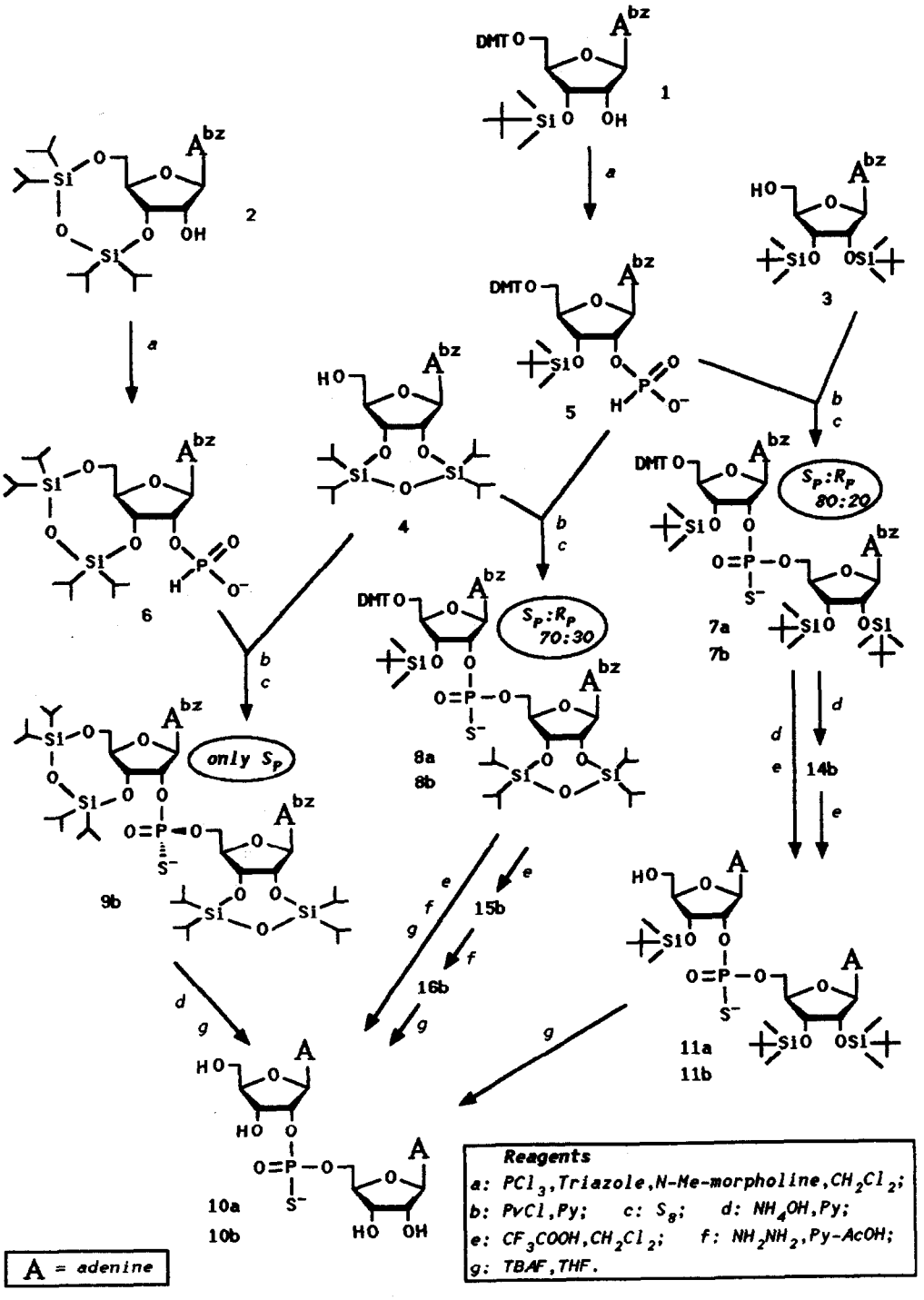
RESULTS

The 2',5'-oligo-thioadenylates have been prepared starting from properly protected adenosine analogues, one having free the 2'-hydroxyl group (like 1 and 2) and the other having the 2' and 3' positions protected and the 5'-hydroxyl group available for reaction (like 3 and 4). An important step in the synthetic design is the appropriate choice of protecting groups. In a recent work Strömberg et al.[23] have studied the stability of t.butyldimethylsilyl group towards usual conditions of deprotection in RNA synthesis via the hydrogenphosphonate approach. Thus, we decided to use the t.butyldimethylsilyl group for the protection of the secondary hydroxylic functions in ribonucleotides, which is compatible with the dimethoxytrityl group in the 5' position and the 1,1,3,3-tetraisopropyl-1,3-disiloxanediyl group for the simultaneous protection of 5',3' or 2',3' positions by cycle formation.

The suitably protected starting nucleosides 1, 2, 3 and 4 were synthesized by literature procedures[24,25]. In turn the 2'-hydrogenphosphonates monomers 5 and 6 were prepared from N^6-benzoyl-3'-O-(t.butyldimethylsilyl)-5'-O-(dimethoxytrityl)adenosine 1 and from N^6-benzoyl-3',5'-O-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)adenosine 2 in 90% and 96% yield respectively, by using phosphorus trichloride, N-methylmorpholine and triazole procedure as described previously for deoxyribonucleosides by Matteucci[20a] (scheme 1).

Coupling reactions were accomplished by using pivaloyl chloride or adamantoyl chloride as condensing agent at room temperature, followed by oxidative sulphurization

SCHEME 1


1
2
3
4
5
6
7a
7b
8a
8b
9b
10a
10b
11a
11b
14b
15b
16b
only S_p
S_p:R_p 80:20
S_p:R_p 70:30
A = adenine
Reagents
a: PCl_3, Triazole, N-Me-morpholine, CH_2Cl_2;
b: PvCl, Py; c: S_8; d: NH_4OH, Py;
e: CF_3COOH, CH_2Cl_2; f: NH_2NH_2, Py-AcOH;
g: TBAF, THF.

with elemental sulphur (scheme 1). Three cases differing for the combination of the protective groups at the proper ribosidic hydroxyl groups showed a marked prevalence of the S_P diastereomer but in different extent.

In the first example, coupling between the 2'-hydrogenphosphonate **5** and 5'-OH monomer **3** afforded the two diastereomers **7a** (R_P isomer) and **7b** (S_P isomer)[26] in 90% yield and ratio 20:80. The diastereomers can be independently converted to completely deprotected dimers by usual procedures in order to establish the correct stereochemistry at phosphorus centre.

The same coupling reaction was performed between **5** and **4** to give the two diastereomers **8a** (R_P isomer) and **8b** (S_P isomer)[26] in 80% yield and ratio 30:70.

Finally the condensation between **6** and **4** gave only the diastereomer **9b** (S_P isomer)[26] in 63% yield.

The phosphorothioate formation by the present methodology actually involves two steps: the internucleosidic coupling to an H-phosphonate derivative and a subsequent thiooxidation. It has been recently proved by F. Seela and U. Kretschmer[27] that the thiooxidation of separated H-phosphonate diastereomers is a stereospecific process. This finding leads the conclusion that in our case of asymmetric induction the stereoselectivity actually takes place at the level of the first new stereogenic centre formation, namely at the level of the H-phosphonate diester formation. At the same time of our findings another group (Stawinski, Strömberg and Thelin) also evidentiated the stereoselectivity of H-phosphonate formation in the route to phosphorothioates for the 3'-5' internucleotidic bond in the "ribo" series[28].

In order to determine the actual stereochemistry at the phosphorus atom, separate diastereomers were deprotected in the usual way: 1) debenzoylation by treatment with concentrated ammonium hydroxide in pyridine or with hydrazine in pyridine-acetic acid, 2) detritylation with trifluoroacetic acid in methylene chloride (not for isomer **9b**) and 3) desilylation by reaction with tetrabutylammonium fluoride in tetrahydrofuran. After final purification by reverse phase chromatography on RP8 and treatment with ionic exchange resin (Dowex Na^+ form) the fully deprotected phosphorothioate dimers **10a** and **10b** were isolated in good yield (54-80% depending on the substrates), pure by HPLC and ^{31}P NMR analysis.

Dimer **10a** : ^{31}P NMR (D_2O) δ = 57.69 ; HPLC analysis[29] : Rt 6.5 min.

Dimer **10b** : ^{31}P NMR (D_2O) δ = 56.25 ; HPLC analysis[29] : Rt 7.0 min.

The phosphorus configuration in **10a** and **10b** was determined by enzymatic studies. It has been established that Snake Venom Phosphodiesterase (SVPD) shows stereoselective hydrolytic activity towards phosphorothioate dimer of R_P configuration[11,30c]. Treatment of the deprotected phosphorothioate dimer **10a**, derived from the protected analogues **7a** or **8a**, with Snake Venom Phosphodiesterase yielded adenosine and adenosine 5'-monothiophosphate in a molar ratio of 1:1 analyzed by HPLC; the half life was 3 hours. On the contrary the stereomer **10b**, derived from the protected analogues **7b**, **8b** or **9b** , was not a substrate for Snake Venom

Phosphodiesterase under these conditions. These results have indicated the R_P configuration for the phosphorothioate dimer **10a** and its protected analogues **7a** and **8a**. Hence the S_P configuration has been assigned to the phosphorothioate dimer **10b** and its protected analogues **7b, 8b, 9b and 11b.** Furthermore, ^{31}P NMR and HPLC analyses are in accordance with the literature data[11,30,31].

In principle further elongation of the oligomer could be endowed with different characteristics of stereoselectivity. Indeed they came out to be the same at least in the step to the trimer and for one combination of protecting groups. The scheme 2 outlines the synthesis of the trimer phosphorothioate that has been performed starting from the 5'-OH unprotected dimer of S_P configuration **11b**, which derived from **7b** (S_P isomer) after debenzoylation and detritylation as shown in scheme 1.

Condensation of the resulting phosphorothioate **11b** with the 2'-hydrogenphosphonate **5** as described above, followed by oxidation with elemental sulphur, afforded the two diastereomers **12a** (R_P configuration at the new stereogenic centre) and **12b** (S_P configuration at the new stereogenic centre)[26] in 85% yield and ratio 20:80. Each of the diastereomers **12a** and **12b** was deprotected through debenzoylation, detritylation and desilylation using standard procedures as mentioned above and the final trimers were purified by reverse phase chromatography on RP8 followed by treatment with ionic exchange resin (Dowex Na^+ form). The fully deprotected phosphorothioate trimers **13a** and **13b** were isolated as sodium salts in 73% and 78% yield respectively, pure by HPLC and ^{31}P NMR analysis.

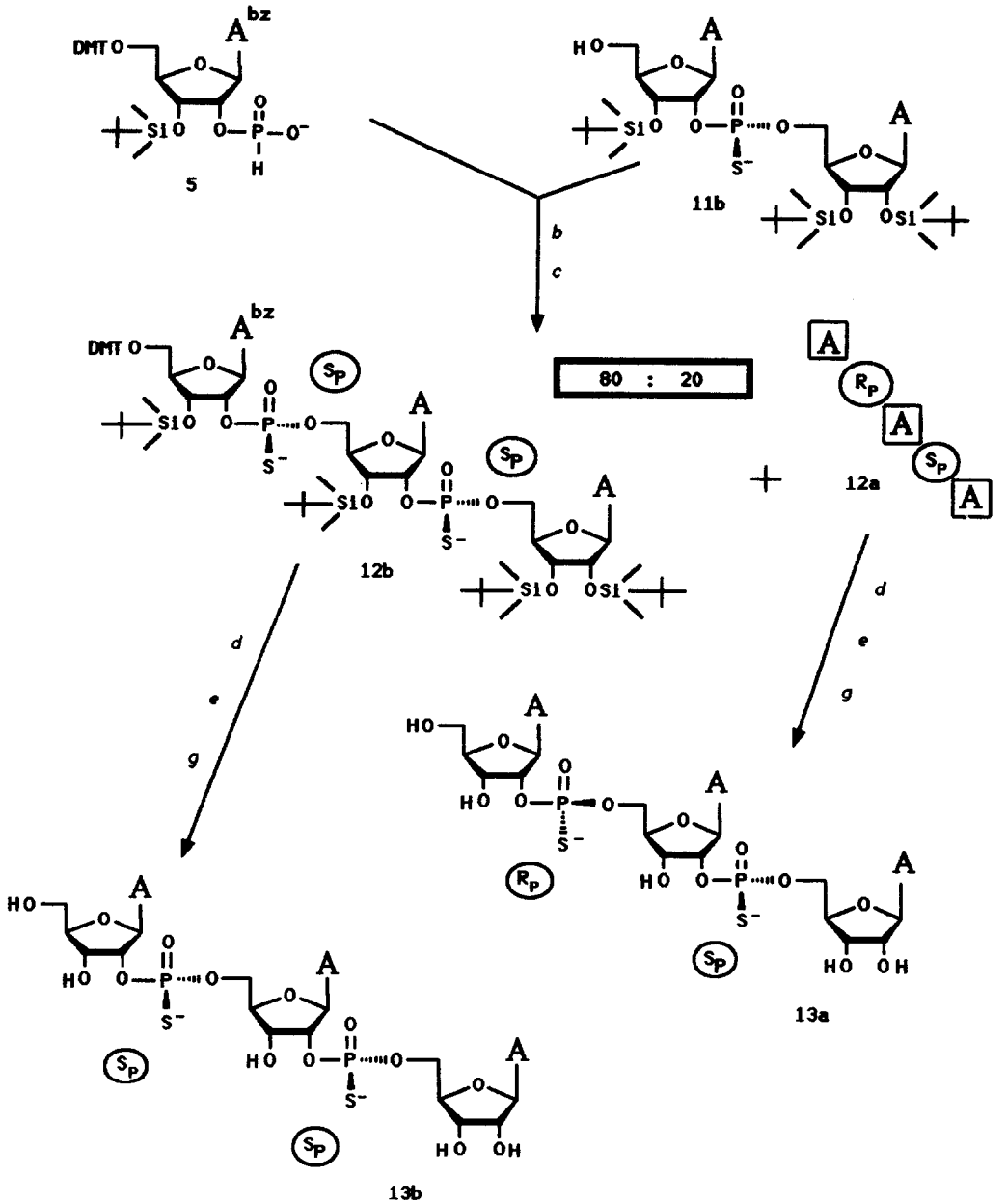
Trimer **13a** : ^{31}P NMR (D_2O) δ = 57.54 , 56.60 ; HPLC analysis[32]: Rt 4.0 min.

Trimer **13b** : ^{31}P NMR (D_2O) δ = 56.56 , 56.33 ; HPLC analysis[32]: Rt 4.5 min.

The absolute configuration of **13a** and **13b** was determined by using cellular lysates containing 2'-phosphodiesterase (2'-PDE)[30a,c], the above mentioned specific exoribonuclease which cleaves 2',5'-oligonucleotides from the 2'/3'-terminus. Trimer **13a**, derived from **12a**, was a substrate for 2'-PDE yielding the R_P dimer and adenosine 5'-monophosphorothioate; trimer **13b** , derived from **12b**, was not a substrate for the enzyme. This experiment indicates that the trimer **13a** (and hence the protected derivative **12a**) has the R_P,S_P configuration and the trimer **13b** (and hence the protected derivative **12b**) has the S_P,S_P configuration. Furthermore, ^{31}P NMR and HPLC analyses are in accordance with the literature data[11,30,31].

The stereoselective formation of S_P diastereomer in these cases can be tentatively ascribed to the steric effect of the 3' protecting group close to the reaction centre, exerting its influence on the first interaction of the phosphonate ion with the activating agent (pivaloyl or adamantoyl chloride) that could be the key step for determining the stereoselectivity. According to this hypothesis the degree of stereoselectivity should be more influenced by the term acting as phosphonyl donor rather than by the nucleophile acting as phosphonyl acceptor, and this is actually the case. Indeed the formation of the dimer and the elongation to trimer show the same stereoselectivity using the same phosphorylated partner. Furthermore working at the 2' position allows to block 5' and 3'

SCHEME 2


5
11b
b
c
80 : 20
12a
12b
d
e
g
13a
13b
A = adenine
A = adenosine
Reagents
b: Adamantoyl Chloride, Py; c: S8;
d: NH4OH,Py; e: CF3COOH,CH2Cl2;
g: TBAF,THF.

positions in a more rigid structure and this seems to enhance the S_P stereoselectivity further.

The synthesized compounds showed some biological activity that will be reported in detail elsewhere. Indeed the S_P dimer phosphorothioate, but not the R_P diastereomer, is able to potentiate the interferon action and poly-IC activity in viral infection of cultured cells.

In conclusion this synthetic approach has the advantage to use starting materials (2'-hydrogenphosphonate ribonucleosides) that are stable, easy to prepare and handle. Coupling reactions occur in very good yields and with a remarkable degree of stereoselectivity. Another feature of the synthesis is that the hydrogenphosphonate coupling is compatible with the presence of free amino groups and unprotected phosphorothioate moiety (scheme 2). Indeed the approach *via* hydrogenphosphonate seems to be a method of choice for the chemical synthesis of oligoribonucleotide phosphorothioates particularly whether a defined phosphorus configuration is required, like S_P configuration in the case of 2',5'-oligoribonucleotides.

EXPERIMENTAL

^1H NMR spectra were recorded using VARIAN VXR 200 and VXR 400 spectrometers. ^{31}P NMR spectra were recorded on a VARIAN VXR 200 spectrometer. Coupling costants are measured in Hertz. NMR data are given indicating with A, B, C, respectively the first, the second, and the third ribose ring from the 2' end to the 5' end of the oligonucleotide molecule. Mass spectra were recorded using a VARIAN Mat-311A spectrometer. Column chromatography was performed on Carlo Erba silica gel 60 (230-400 mesh). Reverse phase column chromatography was performed on Merck LiChroprep RP8 (230-400 mesh). HPLC was performed using Beckman Sistem Gold with Partisphere Whatman column. Analytical thin layer chromatography was performed using pre-coated glass-backed plates (Merck Kieselgel 60 F_{254}) and visualised by ultraviolet light and acidic ammonium molibdate (IV) solution. Snake venum phosphodiesterase (SVPD from Crotalus durissus) for enzymatic hydrolysis was purchased from Boehringer.

N^6-Benzoyl-3'-O-(t.butyldimethylsilyl)-5'-O-(dimethoxytrityl)adenosine-2'-(hydrogen phosphonate), triethylammonium salt (5)

1,2,4-Triazole (8.76 g, 126.8 mmol) was added to a stirred solution of phosphorus trichloride (3.32 ml, 38 mmol) and N-methylmorpholine (42 ml, 380 mmol) in anhydrous methylene chloride (300 ml), at room temperature. After 30 minutes the reaction mixture was cooled to 0°C and N^6-benzoyl-3'-O-(t.butyldimethylsilyl)-5'-O-(dimethoxytrityl)-adenosine (1) (6.0 g, 7.62 mmol), (dried by coevaporation with acetonitrile) in anhydrous methylene chloride (40 ml) was added dropwise over 20 minutes, stirred for 10 minutes, poured into 1.0 M aqueous triethylammonium hydrogencarbonate (TEAB, pH 8.5), shaken and separated. The aqueous phase was extracted with methylene chloride and the combined organic phase was dried (Na_2SO_4) and concentrated. Purification by silica gel column chromatography (eluent: methylene chloride/methanol/triethylamine 90:10:0.2) followed by TEAB extraction gave the title compound (5) (6.53 g, 90% yield).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 9.1 (bs, 1H, NHCOPh); 8.74, 8.37 (two s, 2H, adenine H's); 8.0-6.7 (m, 18H, aromatic H's); 6.89 (d, J = 626.9 Hz, 1H, PH); 6.32 (d, J = 5.7 Hz, 1H, H1'); 5.39 (ddd, J = 4.3, 5.7, 10.3 Hz, 1H, H2'); 4.61 (dd, J = 4.0, 4.3 Hz, 1H, H3'); 4.25 (dt, J = 4.0, 4.4 Hz, 1H, H4'); 3.76 (s, 6H, two OCH_3); 3.43, 3.28 (two dd, J = 4.4, 10.5 Hz, 2H, $CH_2$5'); 0.86 (s, 9H, $SiC(CH_3)_3$); 0.13, 0.05 (two s, 6H, $Si(CH_3)_2$); + triethylammonium signals.

^{31}P NMR (81 MHz, DMSO-d_6): δ = 1.86 (two d, J = 10.3, 626.9 Hz) (H_3PO_4 as external reference).

FAB-MS : m/z 852 ($[M+H]^+$).

N^6-Benzoyl-3',5'-O-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)adenosine-2'-(hydrogen phosphonate), triethylammonium salt (6)

1,2,4-Triazole (11.5 g, 167.5 mmol) was added to a stirred solution of phosphorus trichloride (4.36 ml, 50 mmol) and N-methylmorpholine (53.7 ml, 500 mmol) in anhydrous methylene chloride (500 ml) at room temperature. After 30 minutes the reaction mixture was cooled to 0°C and N^6-benzoyl-3',5'-O-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl) adenosine (6.13 g, 10 mmol) (2), (dried by evaporation with pyridine) in anhydrous methylene chloride (130 ml) was added dropwise over 20 minutes, then kept stirring for 10 minutes more. The reaction mixture was quenched by adding 1.0 M aqueous triethylammonium hydrogen carbonate (400 ml), then shaken and separated. The aqueous phase was extracted with methylene chloride and the combined organic layer was dried (Na_2SO_4) and evaporated under reduced pressure. Purification of the crude was performed by silica gel colum chromatography (eluent: ethyl acetate/methanol/ triethylamine 10:1:0.2) followed by TEAB extraction to obtain the title compound (7.5 g, 96% yield).

(R_P) and (S_P)-N^6-Benzoyl-3'-O-(t.butyldimethylsilyl)-5'-O-(dimethoxytrityl-P-thio adenylyl-(2'-5')-N^6-benzoyl-2',3'-O-bis(t.butyldimethylsilyl)adenosine, triethylammonium salt (7a and 7b)

N^6-Benzoyl-3'-O-(t.butyldimethylsilyl)-5'-O-(dimethoxytrityl)adenosine-2'-(hydrogen phosphonate), triethylammonium salt (5) (6.36 g, 6.67 mmol) and N^6-benzoyl-2',3'-O-bis(t.butyldimethylsilyl)adenosine (3) (4.0 g, 6.67 mol) were first coevaporated three times with anhydrous pyridine, then dissolved in anhydrous pyridine (50 ml). Pivaloyl chloride (2.05 ml, 16.67 mmol) was added and the resulting solution was stirred at room temperature for 45 minutes. Sulphur (2.4 g) and after 3 hours triethylamine (30 ml) were added. The reaction mixture was stirred at room temperature for 30 minutes, then the solvent was evaporated. The residue was diluted with water and extracted with methylene chloride. The combined extracts were dried (Na_2SO_4) and concentrated. Purification and separation of the diastereoisomers 7 were accomplished by silica gel column chromatography (eluent: methylene chloride/methanol/triethylamine 90:3:0.2). The high Rf (R_P) (1.87 g; TLC: Rf 0.33, methylene chloride/methanol/triethylamine, 90:5:0.2) and low Rf (S_P) (7.51 g; TLC: Rf 0.28, methylene chloride/methanol/triethylamine, 90:5:0.2) diastereomers gave a combined yield of 9.38 g (90%) as white solids.

High Rf diastereomer (R_p) (7a)

^{1}H NMR (200 MHz, DMSO-d_6): δ = 11.12, 11.11 (two s, 2H, NHCOPh); 9.03, 8.69, 8.63, 8.61 (four s, 4H, adenine H's); 8.0-6.7 (m, 23H, aromatic H's); 6.36 (d, J = 4.5 Hz, 1H, H1'B); 6.06 (d, J = 7.5 Hz, 1H, H1'A); 5.55 (m, 1H, H2'B); 5.0-4.9 (m, 2H, H3'B, H2'A); 4.35 (d, J = 4.6 Hz, 1H, H3'A); 4.04 (m, 1H, H4'B); 3.95 (m, 1H, H4'A); 3.8-3.6 (m, 2H, $CH_2$5'A); 3.66 (s, 6H, two OCH_3); 3.3-3.0 (m, 2H, $CH_2$5'B); 0.88, 0.81, 0.59 (three s, 27H, $SiC(CH_3)_3$); 0.16, 0.12, 0.05, -0.14, -0.54 (five s, 18H, three $Si(CH_3)_2$); + triethylammonium signals.

FAB-MS : m/z 1463.6 ([M-H]$^-$).

Low Rf diastereomer (S_p) (7b)

^{1}H NMR (200 MHz, DMSO-d_6): δ = 8.92, 8.69, 8.65, 8.63 (four s, 4H, adenine H's); 8.1-6.7 (m, 23H, aromatic H's); 6.37 (d, J = 4.0 Hz, 1H, H1'B); 6.06 (d, J = 7.5 Hz, 1H, H1'A); 5.55 (m, 1H, H2'B); 4.93 (m, 2H, H2'A, H3'B); 4.47 (d, J = 4.4 Hz, 1H, H3'A); 4.1-3.8 (m, 4H, H4'A, H4'B, $CH_2$5'A); 3.68 (s, 6H, two OCH_3); 3.4-3.3 (m, 2H, $CH_2$5'B); 0.88, 0.79, 0.62 (three s, 27H, $SiC(CH_3)_3$); 0.14, 0.09, 0.07, -0.12, -0.48 (five s, 18H, three $Si(CH_3)_2$); + triethylammonium signals.

FAB-MS : m/z 1463.5 ([M-H]$^-$).

(R_p) and (S_p)-N^6-Benzoyl-3'-O-(t.butyldimethylsilyl)-5'-O-(dimethoxytrityl)-P-thio adenylyl-(2'-5')-N^6-benzoyl-2',3'-O-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)adenosine, triethylammonium salt (8a and 8b)

N^6-Benzoyl-3'-O-(t.butyldimethylsilyl)-5'-O-(dimethoxytrityl)adenosine-2'-(hydrogen phosphonate), triethylammonium salt (5) (3.8 g, 4.0 mmol) and N^6-benzoyl-2',3'-O-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)adenosine (4) (2.45 g, 4.0 mmol) were rendered anhydrous by evaporation of added dry pyridine and dissolved in the same solvent (35 ml) and then pivaloyl chloride (12.9 ml, 10.0 mmol) was added dropwise. After 30 minutes at room temperature sulphur (10 eq) was added and the mixture was stirred for 2.5 hours. The reaction mixture was quenched by adding triethylamine (5 ml), evaporated under reduced pressure and the residue was dissolved in ethyl acetate. The organic layer was washed with water and evaporated under vacuum. Purification and separation of the diastereomers 8 were accomplished by silica gel column chromatography eluting with a stepwise gradient from ethyl acetate to ethyl acetate/methanol 95:5. The high Rf (R_p) (1.4 g, TLC Rf 0.47, ethyl acetate/methanol 95:5) and the low Rf (S_p) (3.26 g, TLC Rf 0.33 ethyl acetate/methanol 95:5) diastereomers gave a combined yield of 4.66 g (80%) as white solids.

High Rf diastereomer (R_p) (8a)

^{1}H NMR (200 MHz, DMSO-d_6): δ = 11.2-11.1 (m, 2H, two NHCOPh); 8.9-8.6 (m, 4H, adenine H's); 8.1-6.7 (m, 23H, aromatic H's); 6.34 (d, J = 4.2 Hz, 1H, H1'B); 6.12 (d, J = 5.5 Hz, 1H, H1'A); 5.51 (m, 1H, H2'B); 5.12 (m, 1H, H2'A); 4.92 (m, 1H, H3'B); 4.67 (m, 1H, H3'A); 4.1-4.0 (m, 4H, H4'A, H4'B, $CH_2$5'A); 3.67 (s, 6H, two OCH_3); 1.3-0.8 (m, 37H, four $SiCH(CH_3)_2$, $SiC(CH_3)_3$); 0.13, 0.10 (two s, 6H, $Si(CH_3)_2$); + triethylammonium signals.

FAB-MS: m/z 1477.7 ([M-H]$^-$).

Low Rf diastereomer (S_P) (8b)

^{1}H NMR (200 MHz, DMSO-d_6): δ = 11.19, 11.14 (two s, 2H, NHCOPh); 8.89, 8.71, 8.64 (three s, 4H, adenine H's); 8.0-6.7 (m, 23H, aromatic H's); 6.36 (d, J = 4.0 Hz, 1H, H1'B); 6.10 (d, J = 6.4 Hz, 1H, H1'A); 5.53 (m, 1H, H2'B); 5.13 (dd, J = 4.8, 6.4 Hz, 1H, H2'A); 4.94 (m, 1H, H3'B); 4.76 (m, 1H, H3'A); 4.2-3.7 (m, 4H, H4'A, H4'B, $CH_2$5'A); 3.68 (s, 6H, two OCH_3); 3.3-3.0 (m, 2H, $CH_2$5'B); 1.2-0.8 (m, 37H, $SiC(CH_3)_3$, four $SiCH(CH_3)_2$); 0.10, 0.06 (two s, 6H, $Si(CH_3)_2$); + triethylammonium signals.

FAB-MS: m/z 1477.9 ([M-H]$^-$).

N^6-Benzoyl-3',5'-O-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)-(S_P)-P-thioadenylyl-(2'-5')-N^6-benzoyl-2',3'-O-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)adenosine, triethyl ammonium salt (9b)

N^6-Benzoyl-3',5'-O-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)adenosine-2'-(hydrogen phosphonate), triethylammonium salt (6) (2.3 g, 2.96 mmol) and N^6-benzoyl-2',3'-O-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)adenosine (4) (1.9 g, 2.96 mmol) were coevaporated three times with dry pyridine, then dissolved in the same solvent (20 ml). Pivaloyl chloride (0.91 ml, 7.4 mmol) was added dropwise and the reaction mixture was stirred under nitrogen atmosphere for 30 minutes. Sulphur (10 eq) was added and the mixture was stirred overnight. The reaction was quenched by adding triethylamine (15 ml), evaporated under reduced pressure and the residue was dissolved in methylene chloride. The organic layer was washed several times with water, dried (Na_2SO_4) and evaporated under vacuum. Purification performed by silica gel column chromatography eluting with a stepwise gradient from ethyl acetate to ethyl acetate/methanol 95:5 gave the title compound as white solid (2.7 g, 63% yield).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 9.07, 8.99 (two bs, 2H, NHCOPh); 8.82, 8.72, 8.68, 8.24 (four s, 4H, adenine H's); 7.99 (d, J = 7.0 Hz, 4H, aromatic H's ortho to CONH); 7.6-7.4 (m, 6H, aromatic H's meta and para to CONH); 6.34 (s, 1H, H1'B); 6.2 (d, J = 4.3 Hz, 1H, H1'A); 5.41 (dd, J = 5.3, 11.6 Hz, 1H, H2'B); 5.12 (m, 1H, H3'B); 4.86 (dd, J = 4.3, 4.3 Hz, 1H, H2'A); 4.61 (dd, J = 4.3, 4.3 Hz, 1H, H3'A); 4.37 (m, 1H, CH(H)5'A); 4.28 (m, 1H, H4'A); 4.2-3.9 (m, 4H, CH(H)5'A, $CH_2$5'B, H4'B); 1.3-0.9 (m, 56H, eight $SiCH(CH_3)_2$); + triethylammonium signals.

FAB-MS: m/z 1304 ([M-H]$^-$).

3'-O-(t.Butyldimethylsilyl)-(R_P)-P-thioadenylyl-(2'-5')-2',3'-O-bis(t.butyldimethyl silyl)adenosine, triethylammonium salt (11a)

A suspension of N^6-benzoyl-3'-O-(t.butyldimethylsilyl)-5'-O-(dimethoxytrityl)-(R_P)-P-thioadenylyl-(2'-5')-N^6-benzoyl-2',3'-O-bis(t.butyldimethylsilyl)adenosine, triethyl ammonium salt (7a) (1.0 g, 0.64 mmol) in 30% aqueous ammonia (120 ml) and pyridine (10 ml) was stirred at room temperature for 48 hours. The reaction mixture was concentrated, diluted with methylene chloride (50 ml), and a solution of trifluoroacetic acid (0.6 ml) in methylene chloride (10 ml) at 0°C was added. After 5 minutes the reaction mixture was poured into 1.0 N aqueous triethylammonium hydrogencarbonate (100 ml). The organic phase was separated and the aqueous phase was extracted 3 times

with methylene chloride. The combined extracts were dried (Na_2SO_4) and concentrated. The residue was purified by silica gel column chromatography (eluent: methylene chloride/methanol/triethylamine 90:10:0.2) to give the title compound (**11a**) (572 mg, 85% overall yield).

^1H NMR (200 MHz, DMSO-d_6): δ = 8.53, 8.28, 8.09, 8.03 (four s, 4H, adenine H's); 7.20 (bs, 4H, two NH_2); 6.06 (d, J = 6.6 Hz, 1H, H1'B); 5.86 (d, J = 7.6 Hz, 1H, H1'A); 5.48 (m, 1H, OH); 5.33 (ddd, J = 4.8, 6.6, 12.1 Hz, 1H, H2'B); 4.82 (dd, J = 4.4, 7.6 Hz, 1H, H2'A); 4.59 (dd, J = 2.3, 4.8 Hz, 1H, H3'B); 4.3 (d, J = 4.4 Hz, 1H, H3'A); 3.93 (m, 1H, H4'B); 3.79 (m, 1H, H4'A); 3.7-3.2 (m, 4H, $CH_2$5'B, $CH_2$5'A); 0.89, 0.87, 0.59 (three s, 27H, $SiC(CH_3)_3$); 0.18, 0.03, -0.13, -0.53 (four s, 18H, three $Si(CH_3)_2$); + triethylammonium signals.

FAB-MS: m/z 955 ($[M+H]^+$).

(R_p)-P-thioadenylyl-(2'-5')-adenosine, sodium salt (10a) from 11a

A 1.0 N tetrabutylammonium fluoride solution in tetrahydrofuran (1 ml) was added to a solution of 3'-O-(t.butyldimethylsilyl)-(R_p)-P-thioadenylyl-(2'-5')-2',3'-O-bis(t.butyl dimethylsilyl)adenosine, triethylammonium salt (**11a**) (120 mg, 0.114 mmol) in tetrahydrofuran/pyridine (4:1, 6 ml). The reaction mixture was stirred for 3 hours, then concentrated and diluted with water. The aqueous phase was extracted 3 times with methylene chloride then concentrated. The residue was purified by reverse phase chromatography on RP8 column (eluent: stepwise gradient of methanol from 0 to 20% in water) and passed through a column of Dowex 50W x 8, Na^+ form. Evaporation of the solvent gave the title compound (**10a**) (61 mg, 85% yield) as a white solid. HPLC analysis: RT 6.5 min [A = 0.2 M ammonium acetate, B = methanol, 3-20% B, 10 min, hold 20% B].

^1H NMR (200 MHz, DMSO-d_6): δ = 8.42, 8.29, 8.12, 8.11 (four s, 4H, adenine H's); 7.15, 7.08 (two bs, 4H, NH_2); 6.02 (d, J = 6.0 Hz, 1H, H1'B); 5.89 (d, J = 6.1 Hz, 1H, H1'A); 5.11 (ddd, J = 5.9, 6.0, 11.4 Hz, 1H, H2'B); 4.55 (dd, J = 5.3, 6.1 Hz, 1H, H2'A); 4.43 (dd, J = 3.1, 5.9 Hz, 1H, H3'B); 4.14 (dd, J = 3.2, 5.3 Hz, 1H, H3'A); 4.0-3.9 (m, 2H, H4'A, H4'B); 3.9-3.7 (m, 2H, $CH_2$5'A); 3.7-3.5 (m, 2H, $CH_2$5'B).

^{31}P NMR (81 MHz, D_2O): δ = 57.69 (85% H_3PO_4 as external reference).

FAB-MS: m/z 635 ($[M+H]^+$).

3'-O-(t.Butyldimethylsilyl)-5'-O-(dimethoxytrityl)-(S_p)-P-thioadenylyl-(2'-5')-2',3'-O-bis(t.butyldimethylsilyl)adenosine, triethylammonium salt (14b)

A suspension of N^6-benzoyl-3'-O-(t.butyldimethylsilyl)-5'-O-(dimethoxytrityl)-(S_p)-P-thioadenylyl-(2'-5')-N^6-benzoyl-2',3'-O-bis(t.butyldimethylsilyl)adenosine, triethylammonium salt (**7b**) (3.0 g, 1.91 mmol) in 30% aqueous ammonia (300 ml) and pyridine (30 ml) was stirred at room temperature for 48 hours. The reaction mixture was concentrated and purified by silica gel column chromatography eluting with methylene chloride/methanol/triethylamine, 90:10:0.2 to give the title compound (**14b**) (2.34 g, 90% yield).

^1H NMR (200 MHz, DMSO-d_6): δ = 8.68, 8.28, 8.19, 8.15 (four s, 4H, adenine H's); 7.4-6.7 (m, 13H, aromatic H's); 6.33 (d, J = 4.6 Hz, 1H, H1'B); 6.10 (d, J = 4.9 Hz, 1H,

H1'A); 5.56 (m, 1H, H2'B); 4.86 (m, 1H, H2'A); 4.57 (m, 1H, H3'B); 4.3-4.1 (m, 4H, H4'A, H4'B, $CH_2$5'A); 3.73 (s, 6H, two OCH_3); 3.5-3.2 (m, 2H, $CH_2$5'B); 0.89, 0.85, 0.77 (three s, 27H, $SiC(CH_3)_3$); 0.18, 0.10, 0.07, 0.04 (four s, 18H, three $Si(CH_3)_2$); + triethylammonium signals.

FAB-MS (sodium salt): m/z 1255.4 ($[M-Na]^-$).

3'-O-(t.Butyldimethylsilyl)-(S_P)-P-thioadenylyl-(2'-5')-2',3'-O-bis(t.butyldimethyl silyl)adenosine, triethylammonium salt (11b)

A solution of trifluoroacetic acid (1.8 ml) in methylene chloride (60 ml) was added to a solution of 3'-O-(t.butyldimethylsilyl)-5'-O-(dimethoxytrityl)-(S_P)-P-thioadenylyl -(2'-5')-2',3'-O-bis(t.butyldimethylsilyl)adenosine, triethylammonium salt (**14b**) (2.34 g, 1.72 mmol) in methylene chloride (120 ml), at 0°C. After 5 minutes the reaction mixture was poured into 1.0 N aqueous triethylammonium hydrogencarbonate (200 ml). The organic phase was separated and the aqueous phase was extracted three times with methylene chloride. The combined extracts were dried (Na_2SO_4) and concentrated. The residue was purified by silica gel column chromatography eluting with methylene chloride/ methanol/triethylamine, 90:10:0.2 to give the title compound (**11b**) (1.72 g, 95% yield).

^{1}H NMR (200 MHz, DMSO-d_6): δ = 8.45, 8.26, 8.09, 8.03 (four s, 4H, adenine H's); 7.22, 7.20 (two bs, 4H, NH_2); 6.06 (d, J = 6.4 Hz, 1H, H1'B); 5.87 (d, J = 7.6 Hz, 1H, H1'A); 5.48 (m, 1H, OH); 5.31 (m, 1H, H2'B); 4.79 (dd, J = 4.5, 7.6 Hz, 1H, H2'A); 4.57 (m, 1H, H3'B); 4.47 (d, J = 4.5 Hz, 1H, H3'A); 3.9-3.6 (m, 4H, H4'A, H4'B, $CH_2$5'A); 3.6-3.4 (m, 2H, $CH_2$5'B); 0.88, 0.87, 0.59 (three s, 27H, $SiC(CH_3)_3$); 0.17, 0.05, -0.13, -0.5 (four s, 18H, three $Si(CH_3)_2$); + triethylammonium signals.

FAB-MS: m/z 955 ($[M+H]^+$).

(S_P)-P-Thioadenylyl-(2'-5')-adenosine, sodium salt (10b) from 11b

A 1.0 N tetrabutylammonium fluoride solution in tetrahydrofuran (2.5 ml) was added to a solution of 3'-O-(t.butyldimethylsilyl)-(S_P)-P-thioadenylyl-(2'-5')-2',3'-O-bis (t.butyldimethylsilyl)adenosine, triethylammonium salt (**11b**) (380 mg, 0.36 mmol) in tetrahydrofuran/pyridine (10 ml, 4:1). The reaction mixture was stirred at room temperature for 3 hours, then concentrated and diluted with water. The aqueous phase was extracted 3 times with methylene chloride then concentrated. The residue was purified by reverse phase chromatography on RP8 column (eluent: stepwise gradient of methanol from 0 to 20% in water) and passed through a column of Dowex 50W x 8, Na^+ form. Evaporation of the solvent gave the title compound (**10b**) (205 mg, 90% yield) as a white solid. HPLC analysis: RT 7.0 min [A = 0.2 M ammonium acetate, B = methanol, 3-20% B, 10 min, hold 20% B].

^{1}H NMR (200 MHz, DMSO-d_6): δ = 8.41, 8.27, 8.12, 8.08 (four s, 4H, adenine H's); 7.11, 7.08 (two bs, 4H, NH_2); 6.02 (d, J = 5.9 Hz, 1H, H1'B); 5.87 (d, J = 6.2 Hz, 1H, H1'A); 5.12 (ddd, J = 4.6, 5.9, 10.0 Hz, 1H, H2'B); 4.52 (dd, J = 5.1, 6.2 Hz, 1H, H2'A); 4.41 (dd, J = 4.1, 4.6 Hz, 1H, H3'B); 4.15 (dd, J = 2.9, 5.1 Hz, 1H, H3'A); 3.88 (m, 2H, H4'A, H4'B); 3.9-3.7 (m, 2H, $CH_2$5'A); 3.63 (dd, J = 3.0, 12.2 Hz, 1H, C<u>H</u>(H)5'B); 3.51 (dd, J = 4.1, 12.2

Hz, 1H, CH(H)5'B).

^{31}P NMR (81 MHz, D_2O): δ = 56.25 (85% H_3PO_4 as external reference).

FAB-MS: m/z 635 ([M+H]$^+$).

(R_P)-P-thioadenylyl-(2'-5')-adenosine, sodium salt (10a) from 8a

Trifluoroacetic acid (1.25 ml) was added dropwise to an ice cooled solution of N^6-benzoyl-3'-O-(t.butyldimethylsilyl)-5'-O-(dimethoxytrityl)-(R_P)-P-thioadenylyl -(2'-5')-N^6-benzoyl-2',3'-O-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)adenosine, triethylammonium salt (8a) (1.0 g, 0.635 mmol) in methylene chloride (50 ml). After 30 minutes at 0°C the reaction was over and 1.0 N aqueous triethylammonium hydrogencarbonate (50 ml) was added. The organic layer was washed 3 times with water, dried (Na_2SO_4) and evaporated under reduced pressure. The residue was dissolved in hydrazine buffer (0.5 M hydrazine in pyridine/acetic acid 3:2, 23 ml) and the reaction mixture was kept at room temperature for 16 hours. The solution was cooled to 0°C, acetylacetone was added (2.85 ml), the solution was evaporated in vacuum, and the residue was partitioned between water and methylene chloride. The organic layer was washed with water, dried (Na_2SO_4), evaporated under reduced pressure and coevaporated with toluene. A 1.0 N tetrabutylammonium fluoride solution in tetrahydrofuran (3 ml) was added to a solution of the residue in tetrahydrofuran/pyridine (3:1, 12 ml). The reaction mixture was stirred at room temperature for 3 hours, then concentrated and diluted with water. The aqueous phase was extracted 3 times with methylene chloride then concentrated. The residue was purified by reverse phase chromatography on RP8 column (eluent: stepwise gradient of methanol from 0 to 20% in water) and passed through a column of Dowex 50W x 8, Na^+ form. Evaporation of the solvent gave the title compound (10a) (215 mg, 54% overall yield) as a white solid. Analytical data are the same as reported for the preparation of R_P dimer from 11a.

N^6-benzoyl-3'-O-(t.butyldimethylsilyl)-(S_P)-P-thioadenylyl-(2'-5')-N^6-benzoyl-2',3'-O-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)adenosine, triethylammonium salt (15b)

Trifluoroacetic acid (2.5 ml) was added dropwise to an ice cooled solution of N^6-benzoyl-3'-O-(t.butyldimethylsilyl)-5'-O-(dimethoxytrityl)-(S_P)-P-thioadenylyl -(2'-5')-N^6-benzoyl-2',3'-O-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)adenosine, triethylammonium salt (8b) (1.94 g, 1.23 mmol) in methylene chloride (100 ml). After 30 minutes at 0°C the reaction was over and 1.0 N aqueous triethylammonium hydrogencarbonate (100 ml) was added. The organic layer was washed 3 times with water, dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by silica gel column chromatography eluting with ethyl acetate/methanol 95:5 to obtain the title compound (15b) as white foam (1.49 g, 95% yield).

^{1}H NMR (200 MHz, DMSO-d_6): δ = 11.19, 11.11 (two s, 2H, NHCOPh); 8.85, 8.71, 8.65, 8.63 (four s, 4H, adenine H's); 8.0-7.5 (m, 10H, aromatic H's); 6.19 (d, J = 6.5 Hz, 1H, H1'B); 6.07 (d, J = 6.2 Hz, 1H, H1'A); 5.43 (m, 1H, H2'B); 5.07 (dd, J = 4.6, 6.2 Hz, 1H, H2'A); 4.71 (m, 1H, H3'A); 4.56 (m, 1H, H3'B); 4.06 (m, 1H, H4'A); 3.95 (m, 1H, H4'B); 4.0-3.4 (m, 4H, $CH_2$5'B, $CH_2$5'A); 1.2-0.7 (m, 37H, $SiC(CH_3)_3$, four $SiCH(CH_3)_2$); 0.15 (s, 6H,

$Si(CH_3)_2$); + triethylammonium signals.

FAB-MS: m/z 1175.6 ([M-H]$^-$).

3'-O-(t.Butyldimethylsilyl)-(S_P)-P-thioadenylyl-(2'-5')-2',3'-O-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)adenosine, triethylammonium salt (16b)

N^6-Benzoyl-3'-O-(t.butyldimethylsilyl)-(S_P)-P-thioadenylyl-(2'-5')-N^6-benzoyl-2',3'-O-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)adenosine, triethylammonium salt (**15b**) (0.5 g, 0.38 mmol) was dissolved in hydrazine buffer (0.5 M hydrazine in pyridine/acetic acid 3:2, 15 ml) and the reaction mixture was kept at room temperature for 16 hours. The solution was cooled to 0°C, acetylacetone was added (1.57 ml), the solution was evaporated in vacuum, and the residue was partitioned between water and methylene chloride. The organic layer was washed with water, dried (Na_2SO_4), evaporated under reduced pressure and coevaporated with toluene. The crude was purified by silica gel column chromatography eluting with a stepwise gradient of methylene chloride/methanol from 85:15 to 70:30 to obtain the title compound (**16b**) (200 mg, 65% yield).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.48, 8.26, 8.12, 8.05 (four s, 4H, adenine H's); 7.29, 7.22 (two bs, 4H, NH_2); 6.06 (d, J = 6.4 Hz, 1H, H1'B); 5.93 (d, J = 6.7 Hz, 1H, H1'A); 5.47 (bs, 1H, OH); 5.28 (ddd, J = 4.5, 6.4, 10.0 Hz, 1H, H2'B); 5.02 (dd, J = 4.8, 6.7 Hz, 1H, H2'A); 4.73 (dd, J = 2.5, 4.8 Hz, 1H, H3'A); 4.56 (dd, J = 2.9, 4.5 Hz, 1H, H3'B); 4.01 (m, 1H, H4'A); 3.92 (m, 1H, H4'B); 3.9-3.4 (m, 4H, $CH_2$5'A, $CH_2$5'B); 1.2-0.9 (m, 28H, four $SiCH(CH_3)_2$); 0.88 (s, 9H, $SiC(CH_3)_3$); 0.14 (s, 6H, $Si(CH_3)_2$); + triethylammonium signals.

FAB-MS: m/z 991 ([M+H]$^+$).

(S_P)-P-Thioadenylyl-(2'-5')-adenosine, sodium salt (10b) from 16b

A 1.0 N tetrabutylammonium fluoride solution in tetrahydrofuran (1.4 ml) was added to a solution of 3'-O-(t.butyldimethylsilyl)-(S_P)-P-thioadenylyl-(2'-5')-2',3'-O-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)adenosine, triethylammonium salt (**16b**) (200 mg, 0.187 mmol) in tetrahydrofuran/pyridine (3:1, 5.6 ml). The reaction mixture was stirred at room temperature for 3 hours, then concentrated and diluted with water. The aqueous phase was extracted 3 times with methylene chloride then concentrated. The residue was purified by reverse phase chromatography on RP8 column eluting with a stepwise gradient of methanol from 0 to 20% in water and passed through a column of Dowex 50W x 8, Na^+ form. Evaporation of the solvent gave the title compound (**10b**) (104 mg, 88% yield) as a white solid. Analytical data are the same as reported for the preparation of S_P dimer from **11b**.

(S_P)-P-thioadenylyl-(2'-5')-adenosine, sodium salt (10b) from 9b

A 30% aqueous ammonia solution (19 ml) was added to a solution of N^6-benzoyl-3',5'-O-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)-(S_P)-P-thioadenylyl-(2'-5')-N^6-benzoyl-2',3'-O-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)adenosine, triethylammonium salt (**9b**) (0.20 g, 0.138 mmol) in dioxane (10 ml). The reaction mixture was kept stirring in a sealed vessel at 50°C for 4 hours then evaporated under reduced pressure. The residue, dissolved in pyridine/dioxane 1:4 (10 ml) was treated with tetrabutylammonium fluoride (trihydrate) (435 mg, 1.38 mmol) and stirred overnight. The reaction mixture was

quenched with water, and washed with methylene chloride. The aqueous phase was evaporated under reduced pressure. The crude was purified by reverse phase colomn chromatography performing a linear gradient from pure water to water/acetonitrile 85:15. The fractions containing the wished product were collected and passed through a column of Dowex 50W x 8, Na^+ form. The aqueous solution was lyophilized to obtain 70 mg (80% yield) of title compound as sodium salt. Analytical data are the same as reported for the preparation of S_P dimer from **11b**.

(S_P) and (R_P)-N^6-Benzoyl-3'-O-(t.butyldimethylsilyl)-5'-O-(dimethoxytrityl)-P-thio adenylyl-(2'-5')-3'-O-(t.butyldimethylsilyl)-(S_P)-P-thioadenylyl-(2'-5')-2',3'-O-bis(t.butyldi methylsilyl)adenosine, triethylammonium salt (12b and 12a)

N^6-Benzoyl-3'-O-(t.butyldimethylsilyl)-5'-O-(dimethoxytrityl)adenosine-2'-(hydrogen phosphonate), triethylammonium salt (5) (360 mg, 0.379 mmol) and 3'-O-(t.butyldi methylsilyl)-(S_P)-P-thioadenylyl-(2'-5')-2',3'-O-bis(t.butyldimethylsilyl)adenosine, triethyl ammonium salt (**11b**) (400 mg, 0.379 mmol) were first coevaporated 3 times with anhydrous pyridine, then dissolved in the same solvent (10 ml). Adamantoyl chloride (271 mg, 1.36 mmol) was added and the resulting solution was stirred at room temperature for 1 hour. Sulphur (150 mg) and after 3 hours triethylamine (3 ml) were added. The reaction mixture was stirred at room temperature for 30 minutes, then the solvent was evaporated. The residue was diluted with water and extracted with methylene chloride. The combined extracts were dried (Na_2SO_4) and concentrated. Purification and separation of the diastereomers (12) was accomplished by silica gel column chromatography eluting with methylene chloride/methanol/triethylamine 90:10:0.2. The high Rf (S_P,S_P) (492 mg; TLC: Rf 0.35, methylene chloride/methanol/triethylamine, 80:20:0.2) and low Rf (R_P,S_P) (123 mg; TLC: Rf 0.30, methylene chloride/methanol/ triethylamine, 80:20:0.2) diastereomers gave a combined yield of 650 mg (85% yield) as white solids.

High Rf diastereomer (S_P,S_P) (12b)

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.64, 8.61, 8.52, 8.25, 8.24 (five s, 6H, adenine H's); 8.1-6.7 (m, 18H, aromatic H's); 6.31 (d, J = 4.1 Hz, 1H, H1'B); 6.08 (d, J = 5.4 Hz, 1H, H1'C); 5.87 (d, J = 7.6 Hz, 1H, H1'A); 5.48 (m, 1H, H2'B); 5.18 (m, 1H, H2'C); 4.86 (m, 1H, H3'B); 4.82 (dd, J = 4.4, 7.6 Hz, 1H, H2'A); 4.57 (m, 1H, H3'C); 4.43 (m, 1H, H3'A); 4.03 (m, 1H, H4'B); 4.0-3.6 (m, 6H, H4'C, H4'A, $CH_2$5'A, $CH_2$5'B); 3.78 (s, 6H, two OCH_3); 3.5-3.3 (m, 2H, $CH_2$5'C); 0.94, 0.92, 0.84, 0.74 (four s, 36H, $SiC(CH_3)_3$); 0.24, 0.20, 0.15, 0.11, 0.09, -0.05 (six s, 24H, four $Si(CH_3)_2$); + triethylammonium signals.

^{31}P NMR (81 MHz, $CDCl_3$): δ = 58.52, 57.70 (85% H_3PO_4 as external reference).

FAB-MS: m/z 1819.8 ([M-H]$^-$).

Low Rf diastereomer (R_P,S_P) (12a)

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.65, 8.63, 8.53, 8.24 (four s, 6H, adenine H's); 8.1-6.8 (m, 18H, aromatic H's); 6.32 (d, J = 4.5 Hz, 1H, H1'B); 6.08 (d, J = 6.0 Hz, 1H, H1'C); 5.88 (d, J = 7.9 Hz, 1H, H1'A); 5.53 (m, 1H, H2'B); 5.20 (m, 1H, H2'C); 4.88 (t, J = 5.1 Hz, 1H, H3'B); 4.82 (dd, J = 4.4, 7.9 Hz, 1H, H2'A); 4.60 (m, 1H, H3'C); 4.43 (m, 1H,

H3'A); 4.01 (m, 1H, H4'B); 4.0-3.6 (m, 6H, H4'C, H4'A, $CH_2$5'A, $CH_2$5'B); 3.68 (s, 6H, two OCH_3); 3.3-3.0 (m, 2H, $CH_2$5'C); 0.86, 0.83, 0.73, 0.58 (four s, 36H, $SiC(CH_3)_3$); 0.14, 0.11, 0.05, 0.03, 0.02, -0.17, -0.52 (seven s, 24H, four $Si(CH_3)_2$); + triethylammonium signals.

FAB-MS: m/z 1819.5 ($[M-H]^-$).

(R_P)-P-Thioadenylyl-(2'-5')-(S_P)-P-thioadenylyl-(2'-5')-adenosine, sodium salt (13a)

A suspension of N^6-benzoyl-3'-O-(t.butyldimethylsilyl)-5'-O-(dimethoxytrityl)-(R_P)-P-thioadenylyl-(2'-5')-3'-O-(t.butyldimethylsilyl)-(S_P)-P-thioadenylyl-(2'-5')-2',3'-O-bis(t.butyldimethylsilyl)adenosine, triethylammonium salt (12a) (90 mg, 0.044 mmol) in 30% aqueous ammonia (10 ml) and pyridine (2 ml) was stirred at room temperature for 48 hours. The reaction mixture was concentrated then diluted with methylene chloride (7 ml) and treated with trifluoroacetic acid (0.1 ml) at 0°C. After 5 minutes the reaction mixture was poured into 1.0 N aqueous triethylammonium hydrogencarbonate (10 ml). The organic phase was separated and the aqueous phase was extracted 3 times with methylene chloride. The combined of extracts were dried (Na_2SO_4) and concentrated. The residue was diluted with a mixture tetrahydrofuran/pyridine (4:1, 2.5 ml) and treated with 1.0 N tetrabutylammonium fluoride solution in tetrahydrofuran (0.5 ml). The reaction mixture was stirred for 24 hours, then concentrated and diluted with water. The aqueous phase was extracted 3 times with methylene chloride then concentrated. The residue was purified by reverse phase chromatography on RP8 column eluting with a stepwise gradient of methanol from 0 to 20% in water and passed through a column of Dowex 50W x 8, Na^+ form. Evaporation of the solvent gave the title compound (13a) (32 mg, 73% overall yield). HPLC analysis: RT 4.0 min [A = 0.1 M ammonium acetate, B = acetonitrile/0.1 M ammonium acetate 80:20; 0-65% B, 18 min, hold 65% B].

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.45, 8.42, 8.38, 8.12, 8.11 (five s, 6H, adenine H's); 7.34, 7.27, 7.23 (three bs, 6H, NH_2); 6.07 (d, J = 6.7 Hz, 1H, H1'B); 6.04 (d, J = 6.0 Hz, 1H, H1'C); 5.89 (d, J = 6.7 Hz, 1H, H1'A); 5.65 (m, 4H, OH3'C, OH3'B, OH2'A, OH5'C); 5.4 (d, J = 3.5 Hz, 1H, OH3'A); 5.19 (m, 1H, H2'B); 5.07 (m, 1H, H2'C); 4.6 (m, 1H, H2'A); 4.42 (m, 2H, H3'C, H3'B); 4.23 (m, 1H, H3'A); 4.07 (m, 1H, H4'A); 4.01 (m, 1H, H4'C); 4.0-3.4 (m, 7H, H4'B, $CH_2$5'C, $CH_2$5'A, $CH_2$5'B).

^{31}P NMR (81 MHz, D_2O): δ = 57.54, 56.60 (85% H_3PO_4 as external reference).

FAB-MS: m/z 1002 ($[M+H]^+$).

(S_P)-P-Thioadenylyl-(2'-5')-(S_P)-P-thioadenylyl-(2'-5')-adenosine, sodium salt (13b)

A suspension of N^6-benzoyl-3'-O-(t.butyldimethylsilyl)-5'-O-(dimethoxytrityl)-(S_P)-P-thioadenylyl-(2'-5')-3'-O-(t.butyldimethylsilyl)-(S_P)-P-thioadenylyl-(2'-5')-2',3'-O-bis(t.butyldimethylsilyl)adenosine, triethylammonium salt (12b) (320 mg, 0.158 mmol) in 30% aqueous ammonia (30 ml) and pyridine (5 ml) was stirred at room temperature for 48 hours. The reaction mixture was concentrated then diluted with methylene chloride (20 ml) and treated with trifluoroacetic acid (0.2 ml) at 0°C. After 5 minutes the reaction mixture was poured into 1.0 N aqueous triethylammonium hydrogencarbonate (20 ml). The organic phase was separated and the aquoues phase was extracted 3 times with methylene chloride. The combined extracts were dried (Na_2SO_4) and concentrated. The

residue was diluted with a mixture tetrahydrofuran/pyridine (4:1, 5 ml) and treated with 1.0 N tetrabutylammonium fluoride solution in tetrahydrofuran (1.2 ml). The reaction mixture was stirred for 24 hours, then concentrated and diluted with water. The aqueous phase was washed 3 times with methylene chloride then concentrated. The residue was purified by reverse phase chromatography on RP8 column eluting with a stepwise gradient of methanol from 0 to 20% in water and passed through a column of Dowex 50W x 8, Na^+ form. Evaporation of the solvent gave the title compound (**13b**) (123 mg, 78% overall yield). HPLC analysis : RT 4.5 min [A = 0.1 M ammonium acetate, B = acetonitrile/0.1 M ammonium acetate 80:20; 0-65% B, 18 min, hold 65% B].

^1H NMR (400 MHz, DMSO-d_6): δ = 8.48, 8.45, 8.38, 8.12, 8.10 (five s, 6H, adenine H's); 7.28, 7.24 (two bs, 6H, three NH_2); 6.05 (m, 2H, H1'B, H1'C); 5.88 (d, J = 6.7 Hz, 1H, H1'A); 5.58 (t, J = 5.8 Hz, 1H, OH5'C); 5.54, 5.51, 5.29, 5.24 (four d, J = 6.3, 1.6, 4.1, 4.5 Hz, 4H, OH); 5.21 (m, 1H, H2'B); 5.11 (m, 1H, H2'C); 4.57 (m, 1H, H2'A); 4.47 (m, 1H, H3'C); 4.42 (m, 1H, H3'B); 4.18 (m, 1H, H3'A); 4.07 (m, 1H, H4'C); 4.02 (m, 1H, H4'A); 4.0-3.8 (m, 5H, H4'B, $CH_2$5'A, $CH_2$5'B); 3.58 (m, 2H, $CH_2$5'C).

^{31}P NMR (81 MHz, D_2O): δ = 56.56, 56.33 (85% H_3PO_4 as external reference).

FAB-MS: m/z 1002 ($[M+H]^+$).

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