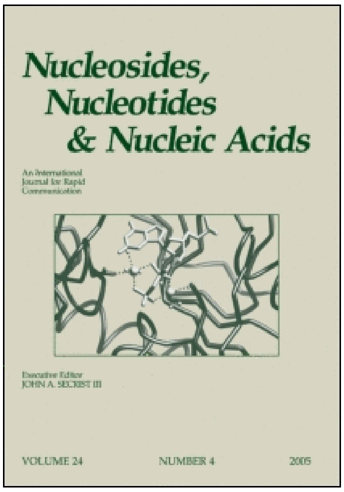

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A Reinvestigation of Sulfenyl Groups as Amino Protecting Groups for the Synthesis of Oligonucleotides on Solid Support by Phosphoramidite Chemistry

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A REINVESTIGATION OF SULFENYL GROUPS AS AMINO PROTECTING GROUPS FOR THE SYNTHESIS OF OLIGONUCLEOTIDES ON SOLID SUPPORT BY PHOSPHORAMIDITE CHEMISTRY[†]

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ABSTRACT: Although sulfenyl groups as protectors of heterocyclic amines of nucleosides appeared satisfactory during the synthesis of DNA and RNA *via* the phosphotriester approach, their usefulness in automated synthesis of oligonucleotides using phosphoramidite chemistry has not been investigated. Herein, we examined the stability and efficiency of 2-nitrophenylsulfenyl- and tritylsulfenyl-nucleosides upon the conditions applied in oligonucleotide synthesis by the phosphoramidite approach.

Antisense oligonucleotides have opened perspectives of new therapies for the treatment of various diseases[1-3]. Oligonucleotides hybridize in a sequence-specific manner mRNA or DNA targets and subsequently inhibit gene expression. Natural oligonucleotides suffer from some limitations, such as their rapid degradation by extra- and intra-cellular nucleases[4] and their poor cellular uptake.

Nuclease resistant oligonucleotides have been obtained by the replacement of the natural phosphodiester linkages by isoelectric phosphorothioate, non-ionic methylphosphonate and phosphoramidate linkages or by dephosphono linkers. Other

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[†] This article is dedicated to the memory of Professor Tsujiaki Hata

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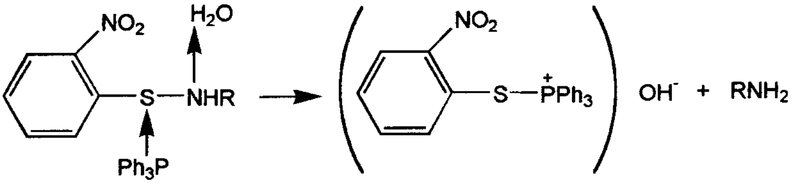
nuclease resistant oligonucleotides i.e. phosphotriesters[5] and primary phosphoramidates[6] are sensitive under the base conditions (concentrated aqueous NH_3, 5 h, 55°C) necessary for removal of the common heterocyclic *N*-acyl protecting groups and release of the oligonucleotide from the regular succinyl anchored solid support. The synthesis of such oligonucleotide analogs requires amino-protecting groups which could be removed readily under milder conditions.

Recently, we reported the synthesis of oligodeoxynucleoside phosphoramidates[7] using the highly base-labile tert-butylphenoxyacetyl[8] group for the *N*-protection of nucleoside bases. However, the final base treatment (saturated NH_3 in methanol, 2 h, room temp), even milder than the standard treatment, will not allow the synthesis of longer sensitive oligomers. Alternatively, the use of the pent-4-enoyl group removable by treatment with iodine[9,10] was proposed as nucleobase protecting group and allowed the synthesis of various base-sensitive dimer and trimer analogs[11,12]. But, no evidence was provided that iodine treatment alone was able to remove quantitatively every pent-4-enoyl group present on oligonucleotides[13].

Therefore, there is a need for new *N*-protecting groups which could be employed for the routine synthesis on solid support of base-sensitive oligonucleotide analogs. Among several possibilities, use of sulfenamides as protection for the exocyclic amino functions of the nucleobases appears at first promising. Particularly, the (2-nitrophenyl)sulfenyl (Nps) and the triphenylmethanesulfenyl (Trs) groups which were developed originally in peptide synthesis[14] have been used in oligonucleotide synthesis by the phosphotriester approach as well[15].

The removal of the Nps protecting group could be achieved under neutral reductive conditions using triphenylphosphine (or tributylphosphine) and water[16] as shown in scheme 1. These mild conditions (tri-*n*-butylphosphine in dioxane/water, 20 min at room temp.) were employed by van Boom et al.[17] to remove the Nps groups from nucleopeptides. However, although this protecting group appeared satisfactory during the synthesis of DNA and RNA *via* the phosphotriester approach[17,18,19], its usefulness in the synthesis of nucleic acid analogs on solid support *via* the phosphoramidite approach and its subsequent deprotection with tributylphosphine/water, has not been reported to our knowledge[20].



SCHEME 1

As an alternative to the Nps protecting group, the Trs group[21] has been reported as protecting group of deoxycytidine, deoxyadenosine and guanosine derivatives[22]. The use of the Trs in oligonucleotide chemistry was limited to the protection of the *N*-3 uracil residue[23,24] for the synthesis in solution of oligouridylates *via* the phosphotriester approach[23]. This protecting group was found stable under a variety of deprotection conditions that are used in oligoribonucleotide chemistry, particularly during the acidic hydrolysis of dimethoxytrityl ethers and was easily removed by oxidation with aqueous iodine. Additionally, this protecting group was found stable under various anhydrous oxidations[25].

In relation with our prooligonucleotide approach[26,27], our effort is focused on the synthesis of these base-sensitive phosphotriester analogs on solid support using the phosphoramidite chemistry. For this purpose, protecting groups of the nucleobase exocyclic amines, which could be removed under mild non-basic and non-nucleophilic conditions, are required. We investigated the potentiality of the Nps and Trs sulfenyl protecting groups for the synthesis of such base-sensitive oligonucleotides on solid support *via* the phosphoramidite approach.

Sulfenyl-protected nucleosides were therefore synthesized and their compatibility under the experimental conditions used during the automatic solid phase synthesis of oligonucleotides was carefully studied.

Evaluation of the Nps protection

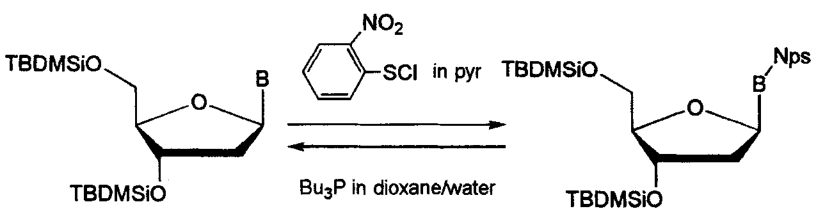
The Nps group was introduced on 2'-deoxycytidine, 2'-deoxyadenosine and 2'-deoxyguanosine by reaction of the 2-nitrophenylsulfenyl chloride with the corresponding 5',3'-di-*O*-tertbutyldimethylsilyl nucleosides **1**, **2** and **3** to give the 5',3'-di-*O*-TBDMSi derivatives of 4-*N*-(2-nitrophenylsulfenyl) deoxycytidine **4**, 6-*N*-(2-nitrophenylsulfenyl) deoxyadenosine **5** and 2-*N*-(2-nitrophenylsulfenyl) deoxyguanosine **6**, as yellow powders with 90%, 60% and 55 % yields, respectively (SCHEME 2).

First, it was necessary to check if the Nps protecting group could be removed from every protected nucleoside by neutral tributylphosphine treatment. These experiments were performed by reaction of Nps protected nucleosides **4**, **5** and **6** with 5 molar equivalents of Bu_3P in dioxane/water (9/1, v/v). Reactions were complete in 5 to 10 min affording, as judged by tlc analysis, quantitative conversion to the deprotected nucleosides **1**, **2** and **3**, respectively (SCHEME 2). Discoloration of the starting yellow solutions resulting from the removal of the Nps group was observed during these reactions, as previously reported in the literature[17].

Secondly, the stability of the Nps-protected nucleosides was checked in several conditions applied in a standard oligonucleotide synthesis by the phosphoramidite approach[15]:

Detritylation conditions: Chattopadhyaya et al. have reported that Nps-nucleosides are stable upon acidic conditions such acetic acid/water 80/20 or 5% toluenesulfonic acid in $EtOH/CHCl_3$ 98/2[28]. Stability in 3% trichloro- or dichloro-acetic acid in CH_2Cl_2 under conditions which are commonly used in DNA syntheses has never been investigated. With dC analog, traces of Nps-deprotected nucleoside were detected after 15 min of trichloroacetic acid treatment at room temperature but only after 8 hours with dichloroacetic acid. Furthermore, traces of the free Nps-protected base resulting from the depurination of the dA analog were detected after 15 min in 3% trichloroacetic acid and only after 8 hours in 3% dichloroacetic acid. The treatment with 3% dichloroacetic acid appears compatible with an oligonucleotide synthesis on solid support[15] where a typical detritylation treatment, on a 1 μmole scale, lasts 1 min per nucleotide incorporation. The dG derivative was found resistant upon the trichloroacetic acid treatment.

Oxidation conditions: All the Nps-protected nucleosides remained intact after 2 hours treatment in aqueous oxidative conditions i.e. 0.1 M I_2 in H_2O/pyr/THF (1/10/40), as well as in anhydrous conditions i.e. 1.1 M tBuOOH in CH_2Cl_2[29,30]. This stability is compatible with the conditions required for the oxidation of a phosphite triester in a phosphotriester linkage during an oligonucleotide synthesis (25 sec of iodine treatment per cycle).



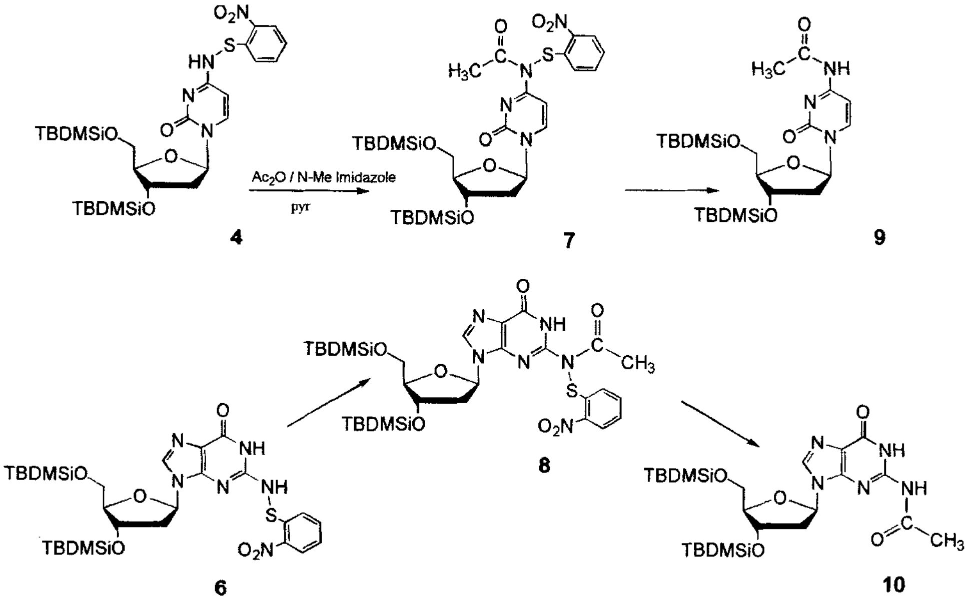
1, B = C
2, B = A
3, B = G

4, B-Nps = 4-N-Nps C
5, B-Nps = 6-N-Nps A
6, B-Nps = 2-N-Nps G

SCHEME 2

Capping and coupling steps: The capping step is generally performed with a mixture of Ac_2O/THF/2,6-lutidine (1/8/1, v:v:v) and 10% *N*-methyl imidazole/THF for 20 sec per cycle incorporation (1 μmole scale). Upon such conditions, only 5',3'-di-*O*-TBDMSi Nps-protected dA **5** was stable. No detectable degradation was observed after 19 hr treatment. For dC and dG derivatives **4** and **6**, tlc analysis of the reaction mixtures revealed complete conversion of the starting nucleosides into yellow compounds of higher Rf in 5 min and 30 min, respectively. After 5 min reaction with dC, attempt to isolate this later compound was performed. A small amount of a relatively unstable product was characterized by ^{1}H NMR as 5',3'-di-*O*-TBDMSi-4-*N*-acetyl 4-*N*-Nps dC **7** and resulted from the acetylation of dC nucleobase (SCHEME 3). It is likely that same reaction occurred for dG derivative **6** affording 5',3'-di-*O*-TBDMSi-2-*N*-acetyl-2-*N*-Nps dG **8**. When the reaction time was extended to 20 hr, loss of the Nps protection was observed resulting in the formation of lower Rf compounds identified as 4-*N*-acetyl-5',3'-di-*O*-TBDMSi dC **9** and 2-*N*-acetyl-5',3'-di-*O*-TBDMSi dG **10** (SCHEME 3). This indicates that the amino functions of the Nps-blocked dC and dG nucleosides are still somewhat nucleophilic and that the common Ac_2O capping treatment cannot be applied in combination with use of Nps-protected nucleosides.

Alternatively, diethyl *N,N*-diisopropyl phosphoramidite in the presence of tetrazole[31] has been used as capping reagent in the synthesis of oligonucleotides with the phosphoramidite chemistry. The stability of the Nps-protected nucleosides in the presence of 0.1 M diethyl *N,N*-diisopropyl phosphoramidite and 0.45 M tetrazole was



SCHEME 3

studied. Moreover, this treatment will give us information about the behavior of the Nps-protected nucleosides under the conditions used during a coupling step. Under these conditions, the total removal of Nps group of dC analog **4** occurred in less than 15 min to give compound **1**. Although occurring at a slower rate (reactions were not complete after 10 hours), dA and dG derivatives **5** and **6** were deprotected to give compounds **2** and **3**, respectively. Tetrazole alone was unable to induce such deprotection. By comparison with the previous acetylation reactions, these deprotections could result from the reaction of the phosphitylating agent with the Nps-amino function to give unstable phosphoramidite derivatives. This could explain the high rate of dC reaction, since cytosine is the most reactive of the nucleoside bases towards phosphitylating agents[32,33]. These phosphoramidites were not detected by tlc analysis. We hypothesize that the subsequent release of the Nps group and phosphityl groups afforded **1**, **2** and **3**. This is not compatible with a standard oligonucleotide synthesis by the phosphoramidite approach where the oxidation step follows the coupling step and will give a stable phosphoramidate from the unstable phosphoramidite.

As observed for the alpha-amino group of the Nps-blocked amino acids[34], the nitrogen atom of Nps-protected exocyclic amines of nucleosides remain sufficiently nucleophilic so that electrophilic species like phosphitylating and acylating reagents react with them. Therefore the Nps group appears inappropriate for the amino protection of nucleobases in an oligonucleotide synthesis by the phosphoramidite approach.

Evaluation of the Trs protection

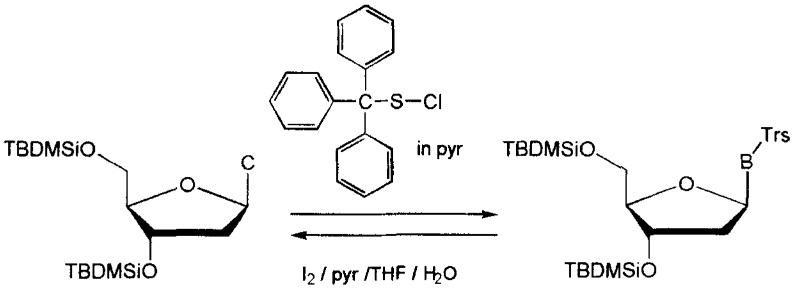
As compared to the Nps, the Trs group was found to render the nitrogen atom nonbasic and relatively nonnucleophilic[21]. Moreover, its stability to aqueous acids and bases and to reducing agents has been already demonstrated[21]. This protecting group is removed very easily by oxidation with aqueous iodine but is stable to various anhydrous oxidations[25].

This protecting group has been introduced on the exocyclic amino functions of dA, dC and rG derivatives using a two-phase reaction (Na_2CO_3-CH_2Cl_2)[22]. However the Trs group do not have general use as exocyclic amino-protecting group of nucleosides since it was shown by Sekine and Seio[22] that sulfenylation of 5',3'-Di-*O*-TBDMSi dA **2** resulted in the formation of 5',3'-Di-*O*-TBDMSi-N^1-Trs dA as the major product along with its regioisomer 6-*N*-Trs. Despite this drawback, we wanted here to see if this group will be more suitable than the Nps group for the protection for the nucleophilic amino-function of dC.

The tritylsulfenyl derivatives of dC **11** was prepared from the 5',3'-di-*O*-TBDMSi nucleoside **1** by reaction with triphenylmethanesulfenyl chloride in pyridine with 53% yield (SCHEME 4)[35]. As expected, the Trs group was readily removed from **11** in less than 10 min upon treatment with 0.2 M I_2 in pyr/H_2O/THF but was stable after 2 hours in the presence of 1.1 M tBuOOH in CH_2Cl_2. This is compatible with the oxidation step during oligonucleotide synthesis[29,30].

Unfortunately, the sulfenyl derivative of dC **11** was not stable in 3% trichloro- or dichloro-acetic acid in CH_2Cl_2. Under both conditions, removal of the sulfenyl group was complete in less than 1.5 hours which makes it not compatible with the detritylation step occurring in oligonucleotide synthesis.

Use of sulfenyl groups as protections of heterocyclic amines of nucleosides appeared satisfactory during the synthesis of DNA and RNA *via* the phosphotriester approach in solution. We have studied their potential usefulness in automated synthesis of



1 **11**, B-Trs = 4-N-Trs C

SCHEME 4

oligonucleotides with phosphoramidite chemistry. Sulfenamides derivatives of regular deoxynucleosides were prepared. The stability of *N*-(2-nitrophenylsulfenyl)- and *N*-tritylsulfenyl-nucleosides upon the conditions applied in oligonucleotide synthesis by the phosphoramidite approach was evaluated. We showed that the exocyclic amino functions of the Nps-blocked nucleosides are still nucleophilic enough to react with electrophilic species like phosphytilating and acylating reagents. Considering the Trs group, its easy removal from dC derivative in acidic conditions is not compatible with the detritylation step occurring in oligonucleotide synthesis. Thus, these sulfenyl groups cannot act as efficient protecting groups in an oligonucleotide synthesis on solid support with the phosphoramidite approach. Evaluation of other non-base labile protecting groups, needed for the synthesis of oligonucleotide prodrugs[27] is underway.

EXPERIMENTAL

Material and methods. Thin layer chromatography (tlc) was carried out on 60 PF_{254} silica gel coated aluminia sheets (Merck) in the solvent systems: A) 5% MeOH in CH_2Cl_2, B) 10% MeOH in CH_2Cl_2, C) 20% MeOH in CH_2Cl_2 and D) 2.5% MeOH in CH_2Cl_2. Column chromatography was performed on Kieselgel 60 (40 μm-63 μm) from Merck. Elution of the compounds was performed with a stepwise gradient of methanol (0 to 5%) in CH_2Cl_2. ^{1}H-NMR spectra were recorded at 250 MHz on a Brucker AC-250-P instrument. The chemical shifts are described as δ values in ppm relative to $CHCl_3$ shift (7,24 ppm) as internal reference. Mass spectra were obtained on a Jeol JMS DX

300 Mass spectrometer under FAB conditions (matrix: 3-nitro-benzyl alcohol or glycerol-thioglycerol (50/50: v/v), Xenon bombardment). Solvents (pyridine, THF, dioxane) were dried and purified before use according to standard procedures. Diethyl *N,N*-diisopropyl phosphoramidite was purchased from Chemgenes Corporation. Detritylation solution (« Deblock solution »: DCA 3% in CH_2Cl_2) was purchased from Glen Research. Capping solutions (« Cap A »: THF/2,6 lutidine/Ac_2O (8 :1 :1), « Cap B »: 10% MeIm in THF), oxidative solution (iodine-water-pyridine-THF; 0.1M, 1 :10 :40) and detritylation solution (« deblock solution »: TCA 3% in CH_2Cl_2) were purchased from Perseptive Biosystems.

General Procedure for the Synthesis of 3',5'-Di-*O-tert*-butyldimethylsilyl-*N*-(2-nitrophenylsulfenyl)-2'-deoxynucleosides. 3',5'-Di-*O-tert*-butyldimethylsilyl 2'-deoxy nucleoside **1**, **2** or **3** (0.2 mmol) was coevaporated three times with anhydrous pyridine and then dissolved in anhydrous pyridine (10 ml). 2-nitrophenylsulfenyl chloride (0.3 mmol, 58.6 mg) was added and after 18h stirring, tlc analysis indicated that the reaction was complete. After extraction with CH_2Cl_2 (30 ml), the organic layer was washed with water (2x30 ml), dried (Na_2SO_4), concentrated, co-evaporated with toluene (to remove remaining pyridine) and the residue was purified by column chromatography by applying a 0-5% gradient of methanol in CH_2Cl_2. Pure fractions were pooled and the solvents removed by evaporation. The residue was dissolved in dioxane and lyophilized to give a yellow powder.

3',5'-Di-*O-tert*-butyldimethylsilyl-4-*N*-(2-nitrophenylsulfenyl)-2'-deoxycytidine (4). Yield: 90%. Rf: 0.34 (system A). ^1H NMR ($CDCl_3$): δ ppm: 8.28 (1H, m, Nps), 8.04 (1H, d, H6, J 7.5Hz), 7.59-7.52 (2H, m, Nps), 7.30 (1H, m, Nps), 6.20 (1H, m, H1'), 6.14 (1H, d, H5, J 7.5Hz), 4.30 (1H, m, H3'), 3.86 (1H, m, H4'), 3.82-3.68 (2H, m, H5', H5''), 2.41 and 2.02 (2H, 2 m, H2' and H2''), 0.81 (18H, 2s, $C(CH_3)_3$), 0.00 (12H, 2s, $Si(CH_3)_2$). MS (FAB, pos. Mode): *m/z* $[M+H]^+$ 609, $[M\text{-}Nps+H]^+$ 456, $[base]^+$ 112, $[base+Nps]^+$ 265, (FAB, neg. Mode): *m/z* $[M\text{-}H]^-$ 607.

3',5'-Di-*O-tert*-butyldimethylsilyl-6-*N*-(2-nitrophenylsulfenyl)-2'-deoxyadenosine (5). Yield: 60%. Rf: 0.64 (system A). ^1H NMR ($CDCl_3$): δ ppm: 8.43 (1H, s, H8), 8.23 (1H, m, Nps), 8.17 (1H, s, H2), 7.39 (2H, m, Nps), 7.2 (1H, m, Nps), 6.37 (1H, m, H1'), 4.53 (1H, m, H3'), 3.97 (1H, m, H4'), 3.78-3.67 (2H, m, H5', H5''), 2.59 and 2.35 (2H, 2 m, H2'and H2''), 0.81 (18H, 2s, $C(CH_3)_3$), -0.01 (12H, 2s, $Si(CH_3)_2$). MS

(FAB, pos. Mode): *m/z* $[M+H]^+$ 633, $[M\text{-}Nps +H]^+$ 480, $[base]^+$ 136, $[base+Nps]^+$ 289, (FAB, neg. Mode): *m/z* $[M\text{-}H]^-$ 631.

3',5'-Di-*O-tert*-butyldimethylsilyl-2-*N*-(2-nitrophenylsulfenyl)-2'-deoxyguanosine (6). Yield: 55%, Rf: 0.34 (system B). ^1H NMR ($CDCl_3$): δ ppm: 8.29 (1H, m, Nps), 7.77 (1H, s, H8), 7.57 (2H, m, Nps), 7.24 (1H, m, Nps), 6.13 (1H, m, H1'), 4.41 (1H, m, H3'), 3.89 (1H, m, H4'), 3.69 (2H, m, H5', H5''), 2.39 and 2.20 (2H, 2 m, H2'and H2''), 0.87 (18H, 2s, $C(CH_3)_3$), 0.06 (12H, 2s, $Si(CH_3)_2$). MS (FAB, pos. Mode): *m/z* $[M+H]^+$ 649, (FAB, neg. Mode): *m/z* $[M\text{-}H]^-$ 647.

General Procedure for the Deprotection of 3',5'-Di-*O-tert*-butyldimethylsilyl-*N*-(2-nitrophenylsulfenyl)-2'-deoxynucleosides. 3',5'-Di-*O-tert*-butyldimethylsilyl-*N*-(2-nitrophenylsulfenyl)-2'-deoxynucleoside **4**, **5** or **6** (3 μmol, ≈ 2 mg) was dissolved in dioxane/water (9/1, v/v, 1 ml) and treated with tri-n-butylphosphine (15 μmol, ≈ 5 μl). After 5 to 10 min at 20°C, tlc analysis indicated the complete conversion of **4** to **1**, **5** to **2** and **6** to **3** and the formation of a yellow byproduct (Rf 0.9, system A).

Stability of Nps-nucleosides towards Detritylation Conditions. 3',5'-Di-*O-tert*-butyldimethylsilyl-*N*-(2-nitrophenylsulfenyl)-2'-deoxynucleoside **4**, **5** or **6** (3 μmol, ≈ 2 mg) was treated with a 3% trichloroacetic acid in CH_2Cl_2 (500 μl) or with 3% dichloroacetic acid in CH_2Cl_2 (500 μl). The reaction was followed by tlc analysis (System A).

Stability of Nps-nucleosides towards Oxidation. 3',5'-Di-*O-tert*-butyldimethylsilyl-*N*-(2-nitrophenylsulfenyl)-2'-deoxynucleoside **4**, **5** and **6** (3 μmol, ≈ 2 mg) was treated with a 0.1M solution of iodine in water-pyridine-THF (1 :1 : 40, 500 μl) or with a 1.1 M solution of tBuOOH in CH_2Cl_2 (500 μl). The reactions were followed by tlc analysis (System A).

Stability of Nps-nucleosides towards Capping Conditions. 3',5'-Di-*O-tert*-butyldimethylsilyl-*N*-(2-nitrophenylsulfenyl)-2'-deoxynucleoside **4**, **5** or **6** (3 μmol, ≈ 2 mg) was treated with a mixture of Ac_2O/THF/2,6-lutidine (« Cap A », 250 μl) and 10% *N*-methylimidazole in THF (« Cap B », 250 μl). The reaction was followed by tlc analysis (System A, D).

3',5'-Di-*O-tert*-butyldimethylsilyl-4-*N*-acetyl-4-*N*-(2-nitrophenylsulfenyl)-2'-deoxycytidine (7). Compound **4** (4.5 μmol, 30 mg) was treated with commercial solutions of Ac_2O/THF/2,6-lutidine (2.5 ml) and 10% *N*-methylimidazole in THF (2.5 ml). After 5 min, tlc analysis indicated complete disappearance of starting product **4** (system A, Rf 0.34) and formation of a yellow derivative with higher Rf (System A, Rf 0.74). 1/3 of the reaction mixture was withdrawn and extracted with CH_2Cl_2 (30 ml). The organic layer was washed with water (2x30 ml), dried (Na_2SO_4), concentrated and the residue was purified by column chromatography by applying a stepwise gradient of 0-1% methanol in CH_2Cl_2 to give 5',3'-Di-*O*-TBDMSi-4-*N*-Ac-4-*N*-Nps dC 7. ^{1}H NMR ($CDCl_3$): δ ppm: 8.45 (1H, d, H6, J 7.4Hz), 8.36 (1H, m, Nps), 7.65 (1H, m, Nps), 7.38 (2H, m, Nps), 6.76 (1H, d, H5, J 7.4Hz), 6.22 (1H, m, H1'), 4.41 (1H, m, H3'), 3.99 (1H, m, H4'), 3.95-3.76 (2H, m, H5', H5''), 2.57 (3H, s, $COCH_3$), 2.37 and 2.18 (2H, 2m, H2' and H2''), 0.91 (18H, 2s, $C(CH_3)_3$), 0.1 (12H, 2s, $Si(CH_3)_2$).

4-*N*-acetyl-3',5'-Di-*O*-*tert*-butyldimethylsilyl-2'-deoxycytidine (9). The remaining 2/3 of the previous reaction mixture were stirred at room temperature for 20h and tlc analysis indicated the formation of a lower Rf compound (System A, Rf 0.29). After extraction with CH_2Cl_2 (30 ml), the organic layer was washed with water (2x30 ml), dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography by applying a 0-5% gradient of methanol in CH_2Cl_2. Fractions containing pure material were evaporated to dryness to give compound **9**. Yield: 82%. ^{1}H NMR ($CDCl_3$): δ ppm: 9.37 (1H, broad s, NH base), 8.34 (1H, d, H6, J 7.4Hz), 7.33 (1H, d, H5, J 7.4Hz), 6.17 (1H, m, H1'), 4.33 (1H, m, H3'), 3.91 (1H, m, H4'), 3.77-3.63 (2H, m, H5', H5''), 2.48 and 2.22 (2H, 2m, H2' and H2''), 2.26 (3H, s, $COCH_3$), 0.85 (18H, 2s, $C(CH_3)_3$), 0.12 (12H, 2s, $Si(CH_3)_2$).

2-*N*-acetyl-3',5'-Di-*O*-*tert*-butyldimethylsilyl-2'-deoxyguanosine (10). 3',5'-Di-*O*-*tert*-butyldimethylsilyl-2-*N*-(2-nitrophenylsulfenyl)-2'-deoxyguanosine **6** (0.26 mmol, 17 mg) was treated as described for the preparation of compound **9**. Yield: 75%. Rf 0.30 (System B). ^{1}H NMR ($CDCl_3$): δ ppm: 7.79 (1H, s, H8), 6.22 (1H, m, H1'), 4.44 (1H, m, H3'), 3.89 (1H, m, H4'), 3.70 (2H, m, H5', H5''), 2.40 and 2.23 (2H, 2 m, H2'and H2''), 2.30 (3H, s, $COCH_3$), 0.88 (18H, 2s, $C(CH_3)_3$), 0.07 (12H, 2s, $Si(CH_3)_2$).).

Stability of Nps-nucleosides in presence of Diethyl *N,N*-Diisopropyl Phosphoramidite and Tetrazole. 3',5'-Di-*O*-*tert*-butyldimethylsilyl-*N*-(2-nitrophenylsulfenyl)-2'-deoxynucleoside **4**, **5** or **6** (3 μmol, ≈ 2 mg) was treated with a mixture of 0.45 M solution of tetrazole in CH_3CN (0.45 ml) and 0.1 M solution of diethyl *N,N*-

diisopropyl phosphoramidite in CH_3CN (0.45 ml). The reaction was followed by tlc analysis (System A, D, B for **4**, **5** and **6** respectively).

Synthesis of 3',5'-Di-*O*-*tert*-butyldimethylsilyl-*N*-(triphenylmethanesulfenyl)-2'-deoxycytidine (11). 3',5'-Di-*O*-*tert*-butyldimethylsilyl-2'-deoxycytidine **1** (1 mmol) was coevaporated with anhydrous pyridine and subsequently dissolved in anhydrous pyridine (10 ml). Triphenylmethanesulfenyl chloride (2.5 mmol, 810 mg) was added and after 24h stirring, tlc analysis indicated that the reaction was complete. The reaction was quenched by addition of methanol/water (1/1, v/v, 1 ml). After addition of CH_2Cl_2 (50 ml), the organic layer was washed with 5% aqueous sodium hydrogen carbonate (50 ml) and water (50 ml), dried (Na_2SO_4), concentrated, co-evaporated with toluene (to remove remaining pyridine) and the residue was purified by column chromatography by applying a stepwise gradient of 0-2% methanol in CH_2Cl_2. Pure fractions were pooled and the solvents removed by evaporation. The residue was dissolved in dioxane and lyophilized to give **11** as a colorless powder. Yield: 53%, Rf 0.38 (System B). ^{1}H NMR in accord with the literature data[22]. MS (FAB, pos. Mode): *m/z* $[M+H]^+$ 730, $[Trityl]^+$ 243, $[base]^+$ 112, (FAB, neg. Mode): *m/z* $[M-H]^-$ 728.

Deprotection of 3',5'-Di-*O*-*tert*-butyldimethylsilyl-*N*-(triphenylmethanesulfenyl)-2'-deoxycytidine. 3',5'-Di-*O*-*tert*-butyldimethylsilyl-*N*-(triphenylme-thanesulfenyl)-2'-deoxycytidine **11** (6.6 µmol, ≈ 5 mg) was treated with 0.1 M iodine in water-pyridine-THF (1 :1 :40, 2.15 ml). After 9 min, tlc analysis (System B) indicated complete conversion of **11** to **1**.

Stability of 3',5'-Di-*O*-*tert*-butyldimethylsilyl-*N*-(triphenylmethanesulfenyl)-2'-deoxycytidine upon Acidic Conditions. 3',5'-Di-*O*-*tert*-butyldimethylsilyl-*N*-(trityl-methanesulfenyl)-2'-deoxycytidine **11** (9.4 µmol, ≈ 7 mg) was treated with a 3% trichloroacetic acid in CH_2Cl_2 (200 µl) or 3% dichloroacetic acid in CH_2Cl_2 (200 µl). The reaction was followed by tlc analysis (System B, C).

Stability of 3',5'-Di-*O*-*tert*-butyldimethylsilyl-*N*-(triphenylmethanesulfenyl)-2'-deoxycytidine upon 1.1 M tBuOOH in CH_2Cl_2. 3',5'-Di-*O*-*tert*-butyl dimethylsilyl-*N*-(tritylmethanesulfenyl)-2'-deoxycytidine **11** (9.6 µmol, ≈ 7 mg) was treated with 1.1M tBuOOH in CH_2Cl_2 (500 µl). The reaction was followed by tlc analysis (System B, C).

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