Published in final edited form as:

Nucleosides Nucleotides Nucleic Acids. 2009 November ; 28(11): 1076–1094. doi:
10.1080/15257770903368385.

Synthesis of Guanosine and Deoxyguanosine Phosphoramidites with Cross-linkable Thioalkyl Tethers for Direct Incorporation into RNA and DNA

Xiaorong Hou, Gang Wang, Barbara L. Gaffney, and Roger A. Jones *

Department of Chemistry and Chemical Biology, 610 Taylor Road, Rutgers, The State University of New Jersey, Piscataway New Jersey 08854

Abstract

We describe the synthesis of protected phosphoramidites of deoxyriboguanosine and guanosine derivatives containing a thiopropyl tether at the guanine N2 (**7a,b**) for site-specific crosslinking from the minor groove of either DNA or RNA to a thiol of a protein or another nucleic acid. The thiol is initially protected as a *tert*-butyl disulfide that is stable during oligonucleotide synthesis. While the completed oligonucleotide is still attached to the support, or after purification, the *tert*-butyl thiol can readily be removed or replaced by thioethylamine or 5-thio-2-nitrobenzoic acid, that have more favorable cross-linking rates.

Keywords

DNA; RNA; guanosine; thioalkyl tether; disulfide crosslink

INTRODUCTION

Useful methods for site-specific disulfide crosslinking of nucleic acid fragments to themselves or to proteins were developed by Verdine¹ and Glick.² These approaches have proven to be particularly valuable for enhancing structural studies, because otherwise heterogeneous populations can thereby be constrained into functionally relevant positions.^{3–8} Disulfide crosslinked complexes of oligonucleotides have also helped to understand enzymatic reactions,^{9–11} probe ribozyme function,^{12,13} increase stability,^{14–19} delineate folding pathways and dynamics,^{20–23} and prepare conjugates.²⁴

The ‘convertible nucleoside’ approach was developed by Verdine for introducing a thioalkyl tether in DNA²⁵ and RNA²⁶ following oligonucleotide synthesis. Among other sites, the method was used for the N2 of guanosine by displacement of a 2-fluoro in a modified inosine.²⁷ This post-synthetic displacement strategy is particularly valuable when a variety of linkers are to be used with a given oligonucleotide, but suffers from having a low yield step at the end of the synthesis. When large amounts of a single tethered oligonucleotide are required, direct incorporation of a modified nucleoside already containing the tether may be more efficient. This approach was developed by Glick for the N3 of uridine/thymidine and the C5/2′O of cytidine.^{28–30} We report here procedures for the synthesis of protected phosphoramidites of deoxyriboguanosine and guanosine derivatives containing a thiopropyl tether at the guanine N2 (**7a,b**), for crosslinking from the minor groove of either DNA or RNA to proteins or other nucleic acids. Although the tether described here contains three methylenes, a shorter or longer

one could easily be used. Further, the thiol is initially protected as a *tert*-butyl disulfide, shown by Glick to be stable during oligonucleotide synthesis.³⁰ While the completed oligonucleotide is still attached to the support, or after purification, the *tert*-butyl thiol can readily be removed or replaced by other disulfides that have more favorable cross-linking rates.

RESULTS AND DISCUSSION

In our procedure (Scheme 1), the thioalkylamine disulfide is introduced directly into the nucleoside by displacement of a 2-fluoro, rather than after oligonucleotide synthesis as in Verdine's strategy.²⁷ Preparation of the deoxyribo- and ribo-2-fluoroinosine intermediates (**4a,b**) in good yield requires prior protection of the O6 by a Mitsunobu alkylation. Rather than the *p*-nitrophenylethyl group that needs an additional deprotection step,²⁷ we have used the acid-labile trimethylsilylethyl group described by Harris.³¹ It is sufficiently stable to survive synthesis of the amidite, but is removed without consequence during the detritylation steps of oligonucleotide synthesis. We introduce it here with transient protection of the hydroxyls.³² Deoxyguanosine (**1a**) or guanosine (**1b**) is first treated with trimethylsilyl imidazole to give **2a,b**, followed by reaction with trimethylsilylethanol, diisopropylazodicarboxylate, and triphenylphosphine. After removal of the sugar trimethylsilyl (TMS) groups in dilute NH₃, purification by normal phase chromatography gives **3a** in 88% yield from **1a**, and crystallization gives **3b** in 91% yield from **1b**. The subsequent non-aqueous diazotization using *tert*-butyl nitrite and pyridine·HF, first reported by Robins,³³ converts the amino to a fluoro and gives **4a,b** in 85–88% yield.

For the deoxyribo compound, protection of the 5' hydroxyl with the standard dimethoxytrityl group gives **5** in 67% yield and provides a convenient lipophilic handle for workup after the subsequent displacement of the fluoro with 3-aminopropyl-*tert*-butyl disulfide, made from its hydrochloride **10**, whose synthesis is described below. This displacement takes place in pyridine over 3 days at 60 °C to give **6** in 81% yield. Synthesis of the final phosphoramidite uses 2-cyanoethyl (*N,N,N',N'*-tetraisopropyl)phosphordiamidite activated with pyridinium trifluoroacetate³⁴ and gives **7a** in 61% yield.

For the ribo compound, we found it necessary for displacement of the fluoro to be done before 2' protection with the *tert*-butyldimethylsilyl (TBS) group to avoid cleavage of the TBS by the released fluoride, and **8** is produced in 92% yield. We next introduce the TBS group at the 2' hydroxyl, following a procedure reported by Beigelman that uses temporary protection of the 3' and 5' hydroxyls as a di-*tert*-butylsilylene.³⁵ Subsequent dimethoxytritylation gives **9** in 85% yield from **8**, and the final phosphoramidite, **7b**, is made as for **7a** in 80% yield.

Synthesis of the *tert*-butyl disulfide protected thiopropylamine hydrochloride (**10**) is shown in Scheme 2 and starts with treatment of 3-chloropropylammonium chloride (**11**) with sodium thiosulfate to give **12**, followed by treatment with I₂ to give **13**, as described by Doi and Musker.³⁶ We find that purification of the crude disulfide (**13**) using a falling film distillation apparatus with toluene as the refluxing solvent gives a much better yield (80%) than standard vacuum distillation. Reduction with dithiothreitol (DTT) then gives the free thioalkylamine (**14**). Protection as the *tert*-butyl disulfide is done by a modified version of a procedure described by Ellman,³⁷ using diisopropyl-1-(*tert*-butylthio)-1,2-hydrazinedicarboxylate (**15**) made from diisopropylazodicarboxylate and *tert*-butyl thiol according to Wünsch *et al.*³⁸

Using standard phosphoramidite chemistry with **7a** at single positions and commercial amidites elsewhere, we have made a DNA 19mer (**16a**) and a DNA 20mer (**16b**), each with one deoxyguanosine containing a *tert*-butyl disulfide protected thiopropyl tether at the N2. The couplings for the modified amidite **7a** gave yields similar to those with the commercial amidites. Following standard HPLC purification, desalting, and conversion to the sodium form,

we obtained 56 μmol of the pure DNA 19mer in 80% yield. The DNA 20mer was left on the support for further modification.

The *tert*-butyl thiol protecting group is not an efficient leaving group for crosslinking. Therefore, we have developed procedures to conveniently remove it by reduction to give the free thiol (**17a**, DNA), or exchange it to other disulfides, such as the moderately reactive 2-thioethylamine (**19a**, DNA)^{4,15} or the even more reactive 5-thio-2-nitrobenzoic acid (TNB) (**18a**, DNA) (Scheme 3).^{39,40} The TNB disulfide has been shown to be the most reactive of these three alternatives, and as a consequence may not display sufficient selectivity when several crosslinking sites are available.⁴⁰ However, for cases requiring forced crosslinking to a single site, the high reactivity of TNB may be desirable.³⁹ Although the thioethylamine is often convenient, occasionally it may be too unreactive.⁴⁰ The free thiol requires air oxidation to crosslink with another thiol.^{9,14,20,27}

For DNA, the free thiol **17a** is generated by treatment of **16a** with dithiothreitol (DTT) at pH 8 for 3 hours at 40 °C, followed by HPLC purification. The TNB disulfide, **18a**, is made by treating the free thiol, **17a**, with commercially available 5,5'-dithiobis(2-nitrobenzoic acid) at pH 8 for 30 min at room temperature, followed by HPLC purification. Although the ethylamine disulfide, **19a**, can be made by a similar two-step method, we found it can be prepared more conveniently and in higher yield in a one-flask procedure by adding a mixture of cysteamine hydrochloride (2-aminoethanethiol hydrochloride) and cystamine dihydrochloride (2,2'-diaminodiethyl disulfide dihydrochloride, both commercially available), to **16a** at pH 8 for 2 hours at 40 °C. Most of the product forms directly from the aminoethanethiol, but the small amount of free thiol that is generated is converted to **19a** by reaction with cystamine. HPLC purification then gives pure **19a**.

We also found that the *tert*-butyl thiol protecting group can be exchanged to the aminoethanethiol while the DNA is still attached to the solid phase. Support containing the DNA 20mer is shaken at room temperature with an aqueous solution of cysteamine hydrochloride and cystamine dihydrochloride at pH 8. After 2 days, the excess reagents are easily washed away, and the DNA can then be cleaved from the support, deprotected and purified as usual, to give pure **19b**.

We also made a RNA 23mer, **16c**, with one guanosine containing a *tert*-butyl disulfide protected thiopropyl tether, using **7b**. Using the same methods described for DNA, **16c** can be converted to the free thiol, **17c**, the TNB disulfide, **18c**, and the ethylamine disulfide, **19c**.

CONCLUSION

We have demonstrated that direct synthesis of both DNA and RNA fragments containing guanine N2 thioalkyl tethers is an efficient approach to the preparation of cross-linkable oligonucleotides. The tether is protected as a stable *tert*-butyl disulfide during oligonucleotide synthesis and is subsequently converted to more reactive groups for crosslinking to proteins or other nucleic acids. For many applications, the ethylamine disulfide may be ideal, but either the TNB group or the free thiol may be appropriate under some circumstances. This approach is particularly well suited to experiments requiring large amounts of oligonucleotides with a given linker.

EXPERIMENTAL

General Methods

Analytical reverse phase HPLC was carried out on a Waters Alliance system using XTerra MS or Atlantis dC18 columns, with 0.1M triethylammonium acetate (TEAA, pH=6.8) and

CH₃CN gradients. A Waters Micromass LCZ spectrometer was used for ESI-MS. Semi-preparative HPLC of the oligonucleotides was performed on a Waters Nova-Pak HR C18 19×300 mm column. Oligonucleotides were analyzed by anion exchange HPLC using a Dionex NucleoPac PA-100 column on a Biocad Sprint Perfusion Chromatography System using gradients of LiClO₄ with 0.02 M CH₃COOLi in 10% CH₃CN in water. Pure oligonucleotides were characterized by ESI-MS, using MassLynx software to deconvolute the spectra of multiply charged ions. The amounts of DNA and RNA were measured on an AVIV 14DS UV spectrophotometer at 260 nm using calculated extinction coefficients. ¹H NMR spectra were acquired either on a Varian Mercury 300 MHz or a Varian Unity 400 MHz NMR spectrometer. ³¹P NMR spectra were acquired on the Varian Mercury and referenced to neat phosphoric acid at 0.0 ppm.

O⁶-(Trimethylsilylethyl)-2'-deoxyguanosine (3a)

2'-Deoxyguanosine hydrate, **1a**, (1.43 g, 5.0 mmol) was dried by co-evaporation with 1,4-dioxane and then suspended in 70 mL 1,4-dioxane. Trimethylsilyl imidazole (2.0 mL, 13.6 mmol, 2.7 equiv) was added by syringe to the stirred suspension under N₂. This protection step was complete in 30 min. Triphenylphosphine (6.55 g, 25.0 mmol, 5.0 equiv), trimethylsilylethanol (3.6 mL, 22.5 mmol, 5.0 equiv) and diethylazodicarboxylate (3.97 mL, 25.2 mmol, 5.0 equiv) were added to the reaction mixture under N₂, which turned a clear orange. This Mitsunobu reaction was complete in 1 h. A mixture of 20 mL conc aq NH₃ and 35 mL water was added to the reaction mixture, which was stirred overnight. Water (~200 mL) was added, and the solution was extracted 3× with methylene chloride. The combined organic layers were concentrated and purified by normal phase chromatography using a gradient of 0–10% methanol in methylene chloride. Fractions with pure product were combined, concentrated to a foam, and dried in a vacuum desiccator over P₂O₅ to give 1.62 g of **3a** (4.41 mmol, 88% from **1a**). The mass of **3a** was confirmed by ESI-MS in negative mode as *m/z* (M-H+CH₃COOH) 426.28 (calculated for C₁₅H₂₄N₅O₄Si·C₂H₄O₂: 426.52). UV λ_{max} 248, 281 nm. ¹H NMR (DMSO) δ 8.05 (s, 1H), 6.34 (s, 2H), 6.20 (t, J = 7.0 Hz, 1H), 5.28–5.25 (m, 1H), 5.01 (t, J = 5.4 Hz, 1H), 4.51–4.47 (m, 2H), 4.37–4.31 (m, 1H), 3.83–3.80 (m, 1H), 3.59–3.47 (m, 2H), 2.62–2.53 (m, 1H), 2.23–2.17 (m, 1H), 1.14–1.10 (m, 2H), 0.05 (s, 9H).

2-Fluoro-O⁶-(trimethylsilylethyl)-2'-deoxyinosine (4a)

At –30 °C under argon, 10 mL anhydrous pyridine, 5 mL anhydrous toluene and 11 mL 70% HF·pyridine (423 mmol, 211 equiv) were mixed well and then added to **3a** (0.74 g, 2 mmol). *Tert*-butyl nitrite (0.8 mL, 6.7 mmol, 3.4 equiv) was added dropwise by syringe to the stirred reaction mixture. This non-aqueous diazotization was complete in 2 h. The excess HF was then quenched by slowly pouring the reaction mixture into a stirred solution of 30 g K₂CO₃ in 50 mL water in an ice bath. The solution was extracted 3× with ethyl acetate. The combined organic layers were concentrated and purified by normal phase chromatography using a gradient of 0–10% methanol in methylene chloride. Fractions with pure product were combined, concentrated to a foam, and dried in a vacuum desiccator over P₂O₅ to afford 0.63 g of **4a** (1.7 mmol, 85%). The mass of **4a** was confirmed by ESI-MS in negative mode as *m/z* (M-H+CH₃COOH) 429.15 (calculated for C₁₅H₂₂FN₄O₄Si·C₂H₄O₂: 429.49). UV λ_{max} 255 nm. ¹H NMR (CDCl₃) δ 8.00 (s, 1H), 6.36–6.32 (m, 1H), 4.93–4.91 (m, 1H), 4.82–4.79 (m, 1H), 4.72–4.67 (m, 2H), 4.22–4.19 (m, 1H), 4.01–3.96 (m, 1H), 3.86–3.78 (m, 1H), 2.98–2.90 (m, 1H), 2.81–2.76 (m, 1H), 2.42–2.35 (m, 1H), 1.29–1.24 (m, 2H), 0.10 (s, 9H).

5'-O-(4,4'-Dimethoxytrityl)-2-fluoro-O⁶-(trimethylsilylethyl)-2'-deoxyinosine (5)

Compound **4a** (0.75 g, 2.0 mmol) was dried by co-evaporation with pyridine. After being dissolved in 30 mL anhydrous pyridine, 4, 4'-dimethoxytrityl chloride (0.87 g, 2.56 mmol, 1.3 equiv) was added under N₂, and the mixture was stirred for 3 h. The reaction was quenched

with aqueous methanol followed by 3×50 mL extractions with ethyl ether. The combined organic layers were concentrated and purified by normal phase chromatography using a gradient of 0–5% methanol in methylene chloride (containing 0.5% pyridine). Fractions with pure product were combined, concentrated to a foam, and dried in a vacuum desiccator over P₂O₅ to afford 0.91 g of **5** (1.35 mmol, 67%). The mass of **5** was confirmed by ESI-MS in negative mode as *m/z* (M-H+CH₃COOH) 731.46 (calculated for C₃₆H₄₀FN₄O₆Si·C₂H₄O₂: 731.86). ¹H NMR (CDCl₃) δ 8.00 (s, 1H), 7.42–6.75 (m, 13H), 6.38 (t, *J* = 6.7 Hz, 1H), 4.72–4.62 (m, 3H), 4.16–4.09 (m, 1H), 3.79 (s, 6H), 3.50–3.30 (m, 2H), 2.82–2.68 (m, 1H), 2.61–2.45 (m, 1H), 1.32–1.20 (m, 2H), 0.11 (s, 9H).

5'-O-(4,4'-Dimethoxytrityl)-N²-(*tert*-butyldithiopropyl)-O⁶-(trimethylsilylethyl)-2'-deoxyinosine (**6**)

3-Aminopropyl-*tert*-butyl disulfide hydrochloride (**10**, 0.691 g, 3.20 mmol, 4.0 equiv) was dissolved in 10 mL aq 1M NaOH and then extracted 3× with 30 mL methylene chloride. The combined organic layers were concentrated and mixed with 25 mL anhydrous pyridine and 0.2 mL triethylamine. Compound **5** (0.54 g, 0.80 mmol) in 20 mL pyridine was added under N₂. After being stirred at 60 °C for 3 days, the reaction mixture was concentrated to an oil and purified by normal phase chromatography using a gradient of 25%–60% ethyl acetate (containing 0.5% pyridine) in hexane. Fractions with pure product were combined, concentrated to a foam, and dried in a vacuum desiccator over P₂O₅ to afford 0.54 g of **6** (0.65 mmol, 81%). The mass of **6** was confirmed by ESI-MS in negative mode as *m/z* (M-H+CH₃COOH) 890.55 (calculated for C₄₃H₅₆N₅O₆S₂Si·C₂H₄O₂: 891.20). UV λ_{max} 235, 285 nm. ¹H NMR (CDCl₃) δ 7.65 (s, 1H), 6.79–7.42 (m, 13H), 6.30 (t, *J* = 6.7 Hz, 1H), 4.87 (br s, 1H), 4.66 (br s, 1H), 4.60–4.52 (m, 2H), 4.11–4.07 (m, 1H), 3.78 (s, 6H), 3.50–3.39 (m, 3H), 3.36–3.30 (m, 1H), 2.90–2.80 (m, 1H), 2.78–2.70 (m, 2H), 2.49–2.41 (m, 1H), 2.30–2.26 (m, 1H), 2.00–1.90 (m, 2H), 1.33 (s, 9H), 1.27–1.20 (m, 2H), 0.09 (s, 9H).

5'-O-(4,4'-Dimethoxytrityl)-N²-(*tert*-butyldithiopropyl)-O⁶-(trimethylsilylethyl)-3'-O-[(2-cyanoethoxyl)-(N,N-diisopropylamino)]phosphinyl-2'-deoxyguanosine (**7a**)

Under argon, 2-cyanoethyl (N,N,N',N'-tetraisopropyl)phosphordiamidite (0.36 mL, 1.13 mmol, 1.6 equiv) was added by syringe to **6** (0.58 g, 0.70 mmol) in 10 mL methylene chloride. Pyridinium trifluoroacetate (0.24 g, 1.24 mmol, 1.8 equiv) dissolved in 15 mL methylene chloride was then added by syringe to the stirred mixture in an ice bath. The reaction was complete in 4 h. The solution was then concentrated and purified by normal phase chromatography using a gradient of 15–60% ethyl acetate (containing 0.5% pyridine) in hexane. Fractions with pure product were combined, concentrated to a foam, and dried in a vacuum desiccator over P₂O₅ to afford 0.44 g of **7a** (0.43 mmol, 61%). The mass of **7a** was confirmed by ESI-MS in positive mode as *m/z* (M+H) 1033.44 (calculated for C₅₂H₇₅N₇O₇PS₂Si: 1033.39). UV λ_{max} 233, 285 nm. ³¹P NMR (CDCl₃) δ 149.2, 149.5.

O⁶-(Trimethylsilylethyl)-guanosine (**3b**)

Guanosine hydrate, **1b** (1.42 g, 4.7 mmol) was dried by co-evaporation with 1,4-dioxane and then suspended in 70 mL 1,4-dioxane. Trimethylsilyl imidazole (2.2 mL, 15 mmol, 3.2 equiv) was added by syringe to the stirred suspension under N₂. This protection step was complete in 1 h. Triphenylphosphine (6.5 g, 25 mmol, 5.3 equiv), trimethylsilylethanol (7.2 mL, 50 mmol, 10.6 equiv) and diisopropylazodicarboxylate (4.9 mL, 31 mmol, 6.6 equiv) were added to the reaction mixture under N₂, which turned a clear orange. This Mitsunobu reaction was complete in 2 h. A mixture of 20 mL conc aq NH₃ and 35 mL water was added to the reaction, which was stirred overnight. Water (~200 mL) was added, and the solution was extracted 3× with methylene chloride. The combined organic layers were concentrated and 20 mL dioxane was added. The solution was then concentrated to 15 mL and left overnight to allow the

triphenylphosphine oxide to crystallize. After the triphenylphosphine oxide was removed by filtration, the solution was concentrated again and the product was crystallized from 20 mL ethyl acetate and methylene chloride (9:1) to afford 1.65 g of **3b** (4.30 mmol, 91%). The mass of **3b** was confirmed by ESI-MS in negative mode as m/z (M-H) 382.36 (calculated for $C_{15}H_{24}N_5O_5Si$: 382.47). UV λ_{max} 248, 282 nm. 1H NMR (DMSO) δ 8.06 (s, 1H), 6.35 (s, 2H), 5.76 (d, J = 6.0 Hz, 1H), 5.38 (d, J = 6.0 Hz, 1H), 5.11 (d, J = 4.8 Hz, 1H), 5.09 (t, J = 5.2 Hz, 1H), 4.55–4.42 (m, 3H), 4.10–4.07 (m, 1H), 3.89–3.86 (m, 1H), 3.65–3.45 (m, 2H), 1.16–1.10 (m, 2H), 0.06 (s, 9H).

2-Fluoro- O^6 -(trimethylsilylethyl)-inosine (**4b**)

At $-30^\circ C$ under argon, 8 mL anhydrous pyridine, 3 mL anhydrous toluene and 11 mL 70% HF-pyridine (423 mmol, 235 equiv) were mixed well and then added to **3b** (0.69 g, 1.8 mmol). *Tert*-butyl nitrite (0.70 mL, 5.9 mmol, 3.3 equiv) was added dropwise by syringe to the stirred reaction mixture. This non-aqueous diazotization was complete in 2 h. The excess HF was then quenched by slowly pouring the reaction mixture into a stirred solution of 30 g K_2CO_3 in 50 mL water in an ice bath. The solution was extracted three times with 100 mL ethyl acetate. The combined organic layers were concentrated and purified by normal phase chromatography using a gradient of 0–10% methanol in methylene chloride (with 0.5% triethylamine). Fractions with pure product were combined, concentrated to a white solid, and dried in a vacuum desiccator over P_2O_5 to afford 0.610 g of **4b** (1.58 mmol, 88%). The mass of **4b** was confirmed by ESI-MS in negative mode as m/z (M-H) 385.40 (calculated for $C_{15}H_{22}FN_4O_5Si$: 385.44). UV λ_{max} 256 nm. 1H NMR ($CDCl_3$) δ 7.81 (s, 1H), 6.23–6.16 (br s, 1H), 5.77 (d, J = 7.6 Hz, 1H), 5.13–5.07 (br s), 5.03–4.95 (br, 1H), 4.68–4.50 (m, 3H), 4.00–3.90 (m, 1H), 3.85–3.76 (m, 1H), 3.38–3.31 (m, 1H), 3.17–3.06 (m, 1H), 1.43–1.32 (m, 2H), 0.12 (s, 9H).

N^2 -(*tert*-Butyldithiopropyl)- O^6 -(trimethylsilylethyl)-guanosine (**8**)

3-Aminopropyl-*tert*-butyl disulfide hydrochloride (**10**) (0.86g, 4.0 mmol, 2.0 equiv) was dissolved in 10 mL aq 1M NaOH and then extracted 3 $\times$ with 30 mL methylene chloride. The combined organic layers were concentrated and mixed with 20 mL anhydrous pyridine and 1 mL triethylamine. Compound **4b** (0.77 g, 2.0 mmol) in 20 mL pyridine was added under N_2 . After being stirred at $60^\circ C$ for 3 days, the reaction mixture was concentrated to an oil and purified by normal phase chromatography using a gradient of 0–8% methanol (containing 0.5% pyridine) in methylene chloride. Fractions with pure product were combined, concentrated to a foam, and dried in a vacuum desiccator over P_2O_5 to afford 1.01 g of **8** (1.85 mmol, 92%). The mass of **8** was confirmed by ESI-MS in negative mode as m/z (M-H+ CH_3COOH) 604.54 (calculated for $C_{22}H_{38}N_5O_5S_2Si-C_2H_4O_2$: 604.83). UV λ_{max} 253, 292 nm. 1H NMR ($CDCl_3$) δ 7.30 (s, 1H), 5.63 (d, J = 7.6 Hz, 1H), 5.25 (br s, 1H), 5.00 (br s, 1H), 4.64–4.32 (m, 4H), 3.95–3.91 (m, 1H), 3.77–3.71 (m, 1H), 3.65–3.54 (m, 1H), 3.48–3.36 (m, 1H), 2.77 (t, J = 7.0 Hz, 2H), 2.03–1.92 (m, 2H), 1.34 (s, 9H), 1.38–1.18 (m, 2H), 0.11 (s, 9H).

2'-*tert*-Butyldimethylsilyl-5'- O -(4,4'-dimethoxytrityl)- N^2 -(*tert*-butyldithiopropyl)- O^6 -(trimethylsilylethyl)-guanosine (**9**)

Compound **8** (1.09 g, 2.0 mmol) was dissolved in 30 mL pyridine and di-*tert*-butylsilyl ditriflate (0.72 mL, 2.0 mmol, 1.0 equiv) was added dropwise over 5 min with stirring at $0^\circ C$. The solution was stirred at $0^\circ C$ for 30 min, and imidazole (0.68 g, 10 mmol, 5.0 equiv) was then added. The mixture was stirred for 5 min at $0^\circ C$ and then for 25 min at room temperature. *tert*-Butyldimethylchlorosilane (0.362 g, 2.40 mmol, 1.2 equiv) was added and the reaction was stirred at $60^\circ C$ for 2 h. The solution was concentrated to an oil and used in the next step without further purification. The mass of the crude 2'-*tert*-butyldimethylsilyl-3',5'-di-*tert*-butylsilylene intermediate was confirmed by ESI-MS in positive mode as m/z (M+H) 800.62 (calculated for $C_{36}H_{70}N_5O_5S_2Si_3$: 801.36).

Hydrogen fluoride-pyridine (1 mL, 38.5 mmol, 19.2 equiv) was chilled and carefully diluted with cold pyridine (8 mL). The resulting solution was added slowly to a stirred solution of the intermediate described above in 10 mL dichloromethane at 0 °C. After 1 h, the reaction mixture was washed twice with saturated aq NaHCO₃. It was then concentrated to an oil and used in the following reaction without further purification.

The oil formed above was dried by co-evaporation with pyridine. After being dissolved in 20 mL anhydrous pyridine, 4,4'-dimethoxytrityl chloride (0.745 g, 2.2 mmol, 1.1 equiv) was added at 0 °C under N₂, and the reaction was stirred at 0 °C overnight. The mixture was poured into aq NaHCO₃, and then extracted 3× with methylene chloride. The combined organic layers were concentrated and purified by normal phase chromatography using a gradient of 0–5% acetone in methylene chloride (containing 0.5% pyridine). Fractions with pure product were combined, concentrated to a foam, and dried in a vacuum desiccator over P₂O₅ to afford 1.63 g of **9** (1.7 mmol, 85% from **8**). The mass of **9** was confirmed by ESI-MS in negative mode as *m/z* (M-H) 960.64 (calculated for C₄₉H₇₀N₅O₇S₂Si₂: 961.41). UV λ_{max} 236, 284 nm. ¹H NMR (CDCl₃) δ 7.70 (s, 1H), 7.47–6.78 (m, 13H), 5.88–5.83 (d, J = 5.2 Hz, 1H), 5.07 (br s, 1H), 4.64–4.54 (m, 3H), 4.39–4.32 (m, 1H), 4.23–4.19 (m, 1H), 3.78 (s, 6H), 3.52–3.24 (m, 3H), 2.78–2.74 (m, 1H), 2.65–2.55 (m, 2H), 1.86–1.76 (m, 2H), 1.32 (s, 9H), 1.34–1.22 (m, 2H), 0.85 (s, 9H), 0.09 (s, 9H), 0.01 (s, 3H), –0.15 (s, 3H).

2'-O-(*tert*-Butyldimethylsilyl)-5'-O-(4,4'-dimethoxytrityl)-N²-(*tert*-butyldithiopropyl)-O⁶-(trimethylsilylethyl)-3'-O-[(2-cyanoethoxy)-(N,N-diisopropylamino)]phosphinyl guanosine (7b**)**

Under argon, 2-cyanoethyl (*N,N,N',N'*-tetraisopropyl)phosphordiamidite (0.32 mL, 1.0 mmol, 2.0 equiv) was added by syringe to **9** (0.48 g, 0.50 mmol) in 10 mL methylene chloride. Pyridinium trifluoroacetate (0.20 g, 1.0 mmol, 2.0 equiv) dissolved in 15 mL methylene chloride was then added by syringe to the stirred mixture in an ice bath. The reaction was complete in 4 h. This solution was then concentrated and purified by normal phase chromatography using a gradient of 10–50% ethyl acetate (containing 0.5% pyridine) in hexane. Fractions with pure product were combined, concentrated to a foam, and dried in a vacuum desiccator over P₂O₅ to afford 0.47 g of **7b** (0.40 mmol, 80%). The mass of **7b** was confirmed by ESI-MS in positive mode as *m/z* (M+H) 1164.04 (calculated for C₅₈H₈₉N₇O₈PS₂Si₂: 1163.65). UV λ_{max} 233, 285 nm. ³¹P NMR (CDCl₃) δ 149.5, 151.7.

3,3'-Dithiobispropylamine (13**)**

Five batches of crude reagent were prepared individually as described here, and then purified together. 3-Chloropropylamine hydrochloride (**11**) (2.8 g, 21.5 mmol) and sodium thiosulfate (3.44 g, 21.8 mmol, 1.01 equiv) were dissolved in 80 mL aq methanol (50%) and refluxed overnight to afford the thiosulfate intermediate **12**. Iodine (2.7 g, 10.6 mmol, 0.49 equiv) in methanol was slowly added to the refluxing solution through an addition funnel over 10 h. The reaction mixture was concentrated, dissolved in 15 mL 6 N aq NaOH, and then extracted three times with methylene chloride. The methylene chloride layers were concentrated under vacuum. The five batches of crude reagent were then combined and placed in the addition funnel of a falling film distillation apparatus (Aldrich), with toluene as the refluxing solvent. The product **13** (7.75 g, 43 mmol, 80%) was collected as a clear liquid. ¹H NMR (CDCl₃) δ 2.29 (t, J = 7.1 Hz, 4H), 2.14 (t, J = 6.8 Hz, 4H), 1.38 (br, 4H), 1.22 (m, 4H).

3-Aminopropylthiol hydrochloride (14**)**

Dithiothreitol (2.15 g, 13.9 mmol, 1.3 equiv) was added to an aq solution of **13** (1.95 g, 10.8 mmol). Conc HCl (1.7 mL) was added to adjust the pH to 6. The reaction mixture was shaken at room temperature for 2 d, concentrated to an oil, and used below without purification.

Diisopropyl-1-(*tert*-butylthio)-1,2-hydrazinedicarboxylate (15)

2-Methyl-2-propanethiol (4.5 mL, 40 mmol) and diisopropyl azodicarboxylate (8.0 mL, 40.6 mmol, 1.0 equiv) were dissolved in 100 mL anhydrous ethyl ether under N₂. Sodium methoxide (0.2 mL) in methanol was added. The orange color disappeared after 5 min, and the reaction mixture was concentrated to an oil and used below without purification.

3-Aminopropyl-*tert*-butyl disulfide hydrochloride (10)

Compound **14** was dissolved in 50 mL argon-degassed DMF and then added dropwise to a solution of **15** in 5 mL anhydrous triethylamine and 50 mL argon-degassed DMF. After the reaction was stirred for 1 day, 25 mL DMF was added, the mixture was stirred overnight, and 1.0 mL triethylamine was then added. The reaction mixture was filtered and concentrated to an oil. The crude product was precipitated in ethyl ether acidified with HCl and purified by normal phase chromatography using a gradient of methanol in methylene chloride to afford 2.96 g of **10** (13.7 mmol, 64%). ¹H NMR of free amine (CDCl₃) δ 8.0 (br, 2H), 3.16 (t, J = 7.2 Hz, 2H), 2.82 (t, J = 7.0 Hz, 2H), 2.19 (m, 2H), 1.32 (s, 9H).

DNA Synthesis (16a, 16b)

The DNA 19mer, d[CAGTCCCTGTTCGG(G*)CGCC], **16a**, and DNA 20mer, d[ACAGTCCCTGTTCGG(G*)CGCC], **16b**, where G* is deoxyguanosine with the *tert*-butyl disulfide protected thiopropyl tether, were both synthesized using commercial phosphoramidites on an Amersham Oligopilot II synthesizer. The modified phosphoramidite **7a**, along with standard deoxynucleoside phosphoramidites and deoxycytidine solid support (deoxycytidine with acetyl protection) from Glen Research, were used. The scale of the syntheses was 65–70 μmol. Three equivalents of 0.1 M solutions of amidites in anhydrous CH₃CN were used in each coupling, along with a mixture of 0.22 M pyridinium trifluoroacetate and 0.11 M *N*-methyl imidazole as activators. After synthesis, the DNA 19mer was cleaved from the support and deprotected in conc aq NH₃. It was then purified by reverse phase HPLC using 0.1 M TEAA and CH₃CN, first with the DMT group on, and again after its removal, to give 56 μmol (80%) pure **16a**. The mass of the DNA 19mer with the *tert*-butyl disulfide protected tether (**16a**) was confirmed by ESI-MS as 5916 (calculated 5919). The *tert*-butyl thiol protecting group was converted to other forms as described below. The mass of a small sample of the DNA 20mer with the *tert*-butyl disulfide protected tether (**16b**) was confirmed by ESI-MS as 6232 (calculated 6232). For most of the DNA 20mer, the *tert*-butyl thiol protecting group was converted to other forms prior to removal from the support, as described below. The calculated extinction coefficient for the DNA 19mer is 164,000 M⁻¹ cm⁻¹, and that for the DNA 20mer is 178,000 M⁻¹ cm⁻¹.

DNA 19mer with propylthiol tether (17a)

Dithiothreitol (0.0117 g, 0.076 mmol, 178 eq) was dissolved in 2 mL water, and the pH was adjusted to 8 with conc aq NH₃. Purified DNA 19mer with the *tert*-butyl disulfide protected tether (**16a**, 70 OD) was dissolved in this solution and heated at 40 °C for 3 hours. After cooling, it was purified by reverse phase HPLC using 0.1 M TEAA and CH₃CN. Fractions containing pure **17a** were lyophilized and desalted by reverse phase HPLC using 0.1 M NH₄HCO₃ and CH₃CN. The NH₄⁺ form of **17a** was converted to the Na⁺ form on a Dowex X50 column, with a final yield of 45 OD (64%). The mass of **17a** was confirmed by ESI-MS as 5827 (calculated 5831).

DNA 19mer with nitrobenzoic acid disulfide tether (18a)

5,5'-Dithiobis(2-nitrobenzoic acid) (0.121 g, 0.305 mmol, 626 eq) was dissolved in 200 mL 0.1 M potassium phosphate (pH=8). Purified DNA 19mer with the propylthiol tether (**17a**, 80 OD) was dissolved in 1 mL of this solution. After 30 min at room temperature, it was purified

by reverse phase HPLC using 0.1 M TEAA and CH₃CN. Fractions containing pure **18a** were lyophilized and desalted by reverse phase HPLC using 0.1 M NH₄HCO₃ and CH₃CN. The NH₄⁺ form of **18a** was converted to the Na⁺ form on a Dowex X50 column, with a final yield of 55 OD (69%). The mass of **18a** was confirmed by ESI-MS as 6026 (calculated 6028).

DNA 19mer with ethylamine disulfide tether (**19a**), one-step method

Cysteamine hydrochloride (2-aminoethanethiol hydrochloride, 0.069 g, 0.61 mmol, 1000 eq) and 0.028 g cystamine dihydrochloride (2,2'-diaminodiethyl disulfide dihydrochloride, 0.12 mmol, 200 eq) were dissolved in 1 mL water, and the pH was adjusted to 8 with conc aq NH₃. Purified DNA 19mer with the *tert*-butyl disulfide protected tether (**16a**, 100 OD) was dissolved in this solution and heated at 40 °C for 2 hr. After cooling, it was purified by reverse phase HPLC using 0.1 M TEAA and CH₃CN. Fractions containing pure **19a** were lyophilized and desalted by reverse phase HPLC using 0.1 M NH₄HCO₃ and CH₃CN. The NH₄⁺ form of **19a** was converted to the Na⁺ form on a Dowex X50 column, with a final yield of 75 OD (75%). The mass of **19a** was confirmed by ESI-MS as 5903 (calculated 5906).

DNA 19mer with ethylamine disulfide tether (**19a**), two-step method

Cystamine dihydrochloride (0.0137 g, 0.061 mmol, 100 eq) was dissolved in 2 mL of 0.1 M potassium phosphate (pH=8). Purified DNA 19mer with the propylthiol tether (**17a**, 100 OD) was dissolved in 1 mL of this solution and heated at 40 °C for 1 hr. After cooling, it was purified by reverse phase HPLC using 0.1 M TEAA and CH₃CN. Fractions containing pure **19a** were lyophilized and desalted by reverse phase HPLC using 0.1 M NH₄HCO₃ and CH₃CN. The NH₄⁺ form of **19a** was converted to the Na⁺ form on a Dowex X50 column, with a final yield of 70 OD (70%).

DNA 20mer with ethylamine disulfide tether (**19b**)

After DNA synthesis, cysteamine hydrochloride (2-aminoethanethiol hydrochloride, 0.170 g, 1.50 mmol, 258 eq) and 0.330 g cystamine dihydrochloride (2,2'-diaminodiethyl disulfide dihydrochloride, 1.47 mmol, 253 eq) were dissolved in 4.0 mL water, and the pH was adjusted to 8 with conc aq NH₃. This solution was then added to 0.2 g of the solid support with the fully protected DNA 20mer attached (29 μmol/g) in a 50 mL centrifuge tube that was then shaken at room temperature for 2 days. The supernatant was removed after centrifugation, and the solid support was washed with water three times. Conc aq NH₃ was then added to the solid support to cleave the DNA and deprotect it, leaving the DMT group attached. After 2 days at room temperature, the mixture was filtered and the support washed with 25 mL additional conc aq NH₃. The filtrates were combined and concentrated in a SpeedVac. The crude product was then purified twice by reverse phase HPLC using 0.1 M TEAA and CH₃CN, before and again after detritylation using 0.4 M acetic acid at pH 3.5 for 20 minutes. Fractions containing pure **19b** were lyophilized and desalted by reverse phase HPLC using 0.1 M NH₄HCO₃ and CH₃CN. The NH₄⁺ form of **19b** was converted to the Na⁺ form on a Dowex X50 column, with a final yield of 340 OD (33%). The mass of **19b** was confirmed by ESI-MS as 6218 (calculated 6219).

RNA 23mer Synthesis (**16c**)

The RNA 23mer, [AGCAGUGGCG(G*)CCGAACAGGGAC] (**16c**), where G* is guanosine with the *tert*-butyl disulfide protected thiopropyl tether, was synthesized by the standard phosphoramidite method on a 58 μmol scale. The modified phosphoramidite **7b**, along with standard phosphoramidites with 2'-tom protection (cytidine with acetyl protection) from Glen Research, were used. The procedures were similar to those described above for DNA, except that 6 equiv of amidites were used. After the synthesis, 2.0 g of the solid support with the RNA attached (27.6 μmol/g) was treated with 5 mL 40% aq methylamine at 65 °C for 10 minutes to

partially deprotect it and cleave it from the support. After filtration, the support was rinsed with 50 mL aq ethanol, and the combined filtrates were concentrated in a Speed-Vac. The RNA was desilylated using a mixture of 9.4 mL 1-methylpyrrolidinone (NMP), 6.3 mL triethylamine trihydrofluoride (TEA-3HF), and 4.7 mL dry triethylamine (TEA) at 65 °C for 2 hours.⁴¹ A fluoride scavenger (isopropoxytrimethylsilane, 30 mL) was used,⁴² and the RNA was isolated by ethyl ether precipitation. It was then purified twice by reverse phase HPLC using 0.1 M TEAA and CH₃CN, before and again after detritylation using 0.4 M acetic acid at pH 3.5 for 20 minutes. Fractions containing pure **16c** were lyophilized and desalted by reverse phase HPLC using 0.1 M NH₄HCO₃ and CH₃CN. The NH₄⁺ form of **16c** was converted to the Na⁺ form on a Dowex X50 column, with a final yield of 1850 OD (15%). The mass of **16c** was confirmed by ESI-MS as 7664 (calculated 7665). The calculated extinction coefficient for the RNA 23mer is 229,000 M⁻¹ cm⁻¹.

RNA 23mer with propylthiol tether (**17c**)

Dithiothreitol (0.0374 g, 0.242 mmol, 278 eq) was dissolved in 2 mL water, and the pH was adjusted to 8 with conc aq NH₃. Purified RNA 23mer with the *tert*-butyl disulfide protected tether (**16c**, 200 OD) was dissolved in this solution and heated at 40 °C for 3 hours. After cooling, it was purified by reverse phase HPLC using 0.1 M TEAA and CH₃CN. Fractions containing pure **17c** were lyophilized and desalted by reverse phase HPLC using 0.1 M NH₄HCO₃ and CH₃CN. The NH₄⁺ form of **17c** was converted to the Na⁺ form on a Dowex X50 column, with a final yield of 160 OD (80%). The mass of **17c** was confirmed by ESI-MS as 7575 (calculated 7577).

RNA 23mer with nitrobenzoic acid disulfide tether (**18c**)

5,5'-Dithiobis(2-nitrobenzoic acid) (0.25 g, 0.63 mmol, 1800 eq) was dissolved in 200 mL 0.1 M potassium phosphate (pH=8). Purified RNA 23mer with the propylthiol tether (**17c**, 80 OD) was dissolved in 1 mL of this solution. After 30 min at room temperature, it was purified by reverse phase HPLC using 0.1 M TEAA and CH₃CN. Fractions containing pure **18c** were lyophilized and desalted by reverse phase HPLC using 0.1 M NH₄HCO₃ and CH₃CN. The NH₄⁺ form of **18c** was converted to the Na⁺ form on a Dowex X50 column, with a final yield of 40 OD (50%). The mass of **18c** was confirmed by ESI-MS as 7772 (calculated 7774).

RNA 23mer with ethylamine disulfide tether (**19c**), one-step method

Cysteamine hydrochloride (2-aminoethanethiol hydrochloride, 0.20 g, 1.76 mmol, 8080 eq) and 0.050 g cystamine dihydrochloride (2,2'-diaminodiethyl disulfide dihydrochloride, 0.22 mmol, 1020 eq) were dissolved in 1 mL water, and the pH was adjusted to 8 with conc aq NH₃. Purified RNA 23mer with the *tert*-butyl disulfide protected tether (**16c**, 50 OD) was dissolved in this solution and heated at 40 °C for 2 hr. After cooling, it was purified by reverse phase HPLC using 0.1 M TEAA and CH₃CN. Fractions containing pure **19c** were lyophilized and desalted by reverse phase HPLC using 0.1 M NH₄HCO₃ and CH₃CN. The NH₄⁺ form of **19c** was converted to the Na⁺ form on a Dowex X50 column, with a final yield of 35 OD (70%). The mass of **19c** was confirmed by ESI-MS as 7650 (calc 7652).

RNA 23mer with ethylamine disulfide tether (**19c**), two-step method

Cystamine dihydrochloride (0.0187 g, 0.083 mmol, 190 eq) was dissolved in 2 mL of 0.1 M potassium phosphate (pH=8). Purified RNA 23mer with the propylthiol tether (**17c**, 100 OD) was dissolved in 1 mL of this solution and heated at 40 °C for 1 hr. After cooling, it was purified by reverse phase HPLC using 0.1 M TEAA and CH₃CN. Fractions containing pure **19c** were lyophilized and desalted by reverse phase HPLC using 0.1 M NH₄HCO₃ and CH₃CN. The NH₄⁺ form of **19c** was converted to the Na⁺ form on a Dowex X50 column, with a final yield of 55 OD (55%).

Acknowledgments

This work was supported by NIH GM66671.

References

1. Verdine GL, Norman DPG. Covalent trapping of protein-DNA complexes. *Annual Review of Biochemistry* 2003;72:337–366.
2. Glick GD. Design, synthesis, and analysis of conformationally constrained nucleic acids. *Biopolymers (Nucleic Acid Sciences)* 1998;48:83–96. [PubMed: 9846126]
3. Cain RJ, Zwietering ERP, Glick GD. Solution structure of a DNA hairpin and its disulfide cross-linked analog. *Nucleic Acids Research* 1995;23:2153–2160. [PubMed: 7610043]
4. Huang H, Chopra R, Verdine GL, Harrison SC. Structure of a covalently trapped catalytic complex of HIV-1 reverse transcriptase: implications for drug resistance. *Science* 1998;282:1669–1675. [PubMed: 9831551]
5. He C, Verdine GL. Trapping distinct structural states of a protein/DNA interaction through disulfide crosslinking. *Chemistry & Biology* 2002;9:1297–1303. [PubMed: 12498882]
6. Mishina Y, He C. Probing the structure and function of the *Escherichia coli* DNA alkylation repair AlkB protein through chemical cross-linking. *Journal of the American Chemical Society* 2003;125:8730–8731. [PubMed: 12862460]
7. Tuske S, Sarafianos SG, Clark J, AD, Ding J, Naeger LK, White KL, Miller MD, Gibbs CS, Boyer PL, Clark P, Wang G, Gaffney BL, Jones RA, Jerina DM, Hughes SH, Arnold E. Structures of HIV-1 RT-DNA complexes before and after incorporation of the anti-AIDS drug tenofovir. *Nature Structural & Molecular Biology* 2004;11:469–474.
8. Banerjee A, Santos WL, Verdine GL. Structure of a DNA glycosylase searching for lesions. *Science* 2006;311:1153–1157. [PubMed: 16497933]
9. Allerson CR, Verdine GL. Synthesis and biochemical evaluation of RNA containing an intrahelical disulfide crosslink. *Chemistry & Biology* 1995;2:667–675. [PubMed: 9383473]
10. van Aalten DMF, Erlanson DA, Verdine GL, Joshua-Tor L. A structural snapshot of base-pair opening in DNA. *Proceedings of the National Academy of Sciences U S A* 1999;96:11809–11814.
11. Krummel DAP, Kent O, MacMillan AM, Altman S. Evidence for helical unwinding of an RNA substrate by the RNA enzyme RNase P: use of an interstrand disulfide crosslink in substrate. *Journal of Molecular Biology* 2000;295:1113–1118. [PubMed: 10653690]
12. Sigurdsson ST, Tuschl T, Eckstein F. Probing RNA tertiary structure: interhelical crosslinking of the hammerhead ribozyme. *RNA* 1995;1:575–583. [PubMed: 7489517]
13. Earnshaw DJ, Masquida B, Muller S, Sigurdsson ST, Eckstein F, Westhof E, Gait MJ. Inter-domain cross-linking and molecular modelling of the hairpin ribozyme. *Journal of Molecular Biology* 1997;274:197–212. [PubMed: 9398527]
14. Glick GD, Osborne SE, Knitt DS, Marino JP Jr. Trapping and isolation of an alternate DNA conformation. *Journal of the American Chemical Society* 1992;114:5447–5448.
15. Ferentz AE, Keating TA, Verdine GL. Synthesis and characterization of disulfide cross-linked oligonucleotides. *Journal of the American Chemical Society* 1993;115:9006–9014.
16. Chaudhuri NC, Kool ET. Very high affinity DNA recognition by bicyclic and cross-linked oligonucleotides. *Journal of the American Chemical Society* 1995;117:10434–10442.
17. Osborne SE, Cain RJ, Glick GD. Structure and dynamics of disulfide cross-linked DNA triple helices. *Journal of the American Chemical Society* 1997;119:1171–1182.
18. Alefelder S, Sigurdsson ST. Interstrand disulfide cross-linking of internal sugar residues in duplex RNA. *Bioorganic & Medicinal Chemistry* 2000;8:269–273. [PubMed: 10968286]
19. Wilds CJ, Noronha AM, Robidoux S, Miller PS. Mismatch-aligned N3T-alkyl-N3T interstrand cross-linked DNA: synthesis and characterization of duplexes with interstrand cross-links of variable lengths. *Journal of the American Chemical Society* 2004;126:9257–9265. [PubMed: 15281815]
20. Goodwin JT, Osborne SE, Scholle EJ, Glick GD. Design, synthesis, and analysis of yeast tRNA analogs possessing intra- and interhelical disulfide cross-links. *Journal of the American Chemical Society* 1996;118:5207–5215.

21. Erlanson DA, Glover JNM, Verdine GL. Disulfide cross-linking as a mechanistic probe for the B to Z transition in DNA. *Journal of the American Chemical Society* 1997;119:6927–6928.
22. Cohen SB, Cech TR. A quantitative study of the flexibility contributed to RNA structures by nicks and single-stranded gaps. *RNA* 1998;4:1179–1185. [PubMed: 9769093]
23. Cain RJ, Glick GD. Use of cross-links to study the conformational dynamics of triplex DNA. *Biochemistry* 1998;37:1456–1464. [PubMed: 9477975]
24. Gottschling D, Seliger H, Tarrason G, Piulats J, Eritja R. Synthesis of oligodeoxynucleotides containing N⁴-mercaptoethylcytosine and their use in the preparation of oligonucleotide-peptide conjugates carrying *c-myc* Tag-sequence. *Bioconjugate Chemistry* 1998;9:831–837. [PubMed: 9815178]
25. MacMillan AM, Verdine GL. Engineering tethered DNA molecules by the convertible nucleoside approach. *Tetrahedron* 1991;47:2603–2616.
26. Allerson CR, Chen SL, Verdine GL. A chemical method for site-specific modification of RNA: the convertible nucleoside approach. *Journal of the American Chemical Society* 1997;119:7423–7433.
27. Erlanson DA, Chen L, Verdine GL. DNA methylation through a locally unpaired intermediate. *Journal of the American Chemical Society* 1993;115:12583–12584.
28. Glick GD. Synthesis of a conformationally restricted DNA hairpin. *Journal of Organic Chemistry* 1991;56:6746–6747.
29. Goodwin JT, Glick GD. Incorporation of alkylthio chains at C-5 of deoxyuridine. *Tetrahedron Letters* 1993;34:5549–5552.
30. Goodwin JT, Glick GD. Synthesis of a disulfide stabilized RNA hairpin. *Tetrahedron Letters* 1994;35:1647–1650.
31. DeCorte BL, Tsarouhtsis D, Kuchimanchi S, Cooper MD, Horton P, Harris CM, Harris TM. Improved strategies for postoligomerization synthesis of oligodeoxynucleotides bearing structurally defined adducts at the N2 position of deoxyguanosine. *Chemical Research in Toxicology* 1996;9:630–637. [PubMed: 8728509]
32. Gao X, Gaffney BL, Hadden S, Jones RA. Transient protection. 2. One-flask synthesis of 6-O-[(4-nitrophenyl)ethyl]-2'-deoxyguanosine nucleosides. *Journal of Organic Chemistry* 1986;51:755–758.
33. Robins MJ, Uznanski B. Nucleic acid related compounds. 34. Non-aqueous diazotization with *tert*-butyl nitrite. Introduction of fluorine, chlorine, and bromine at C-2 of purine nucleosides. *Canadian Journal of Chemistry* 1981;59:2608–2611.
34. Sanghvi YS, Guo Z, Pfundheller HM, Converso A. Improved process for the preparation of nucleosidic phosphoramidites using a safer and cheaper activator. *Organic Process Research & Development* 2000;4:175–181.
35. Serebryany V, Beigelman L. An efficient preparation of protected ribonucleosides for phosphoramidite RNA synthesis. *Tetrahedron Letters* 2002;43:1983–1985.
36. Doi JT, Musker WK. A general mechanism for the oxidative cleavage of amine disulfides and cystine in aqueous iodine. Isolation of cyclic sulfinamides. *Journal of Organic Chemistry* 1985;50:1–4.
37. Virgilio AA, Ellman JA. Simultaneous solid-phase synthesis of β -turn mimetics incorporating side-chain functionality. *Journal of the American Chemical Society* 1994;116:11580–11581.
38. Wunsch E, Moroder L, Romani S. 1-(*tert*-Butylthio)-1,2-hydrazinedicarboxylic acid derivatives. *Hope-Seyler's Z Physiol Chem* 1982;363:1461–1464. [PubMed: 7160826]
39. Wolfe SA, Ferentz AE, Grantcharova V, Churchill MEA, Verdine GL. Modifying the helical structure of DNA by design: recruitment of an architecture-specific protein to an enforced DNA bend. *Chemistry & Biology* 1995;2:213–221. [PubMed: 9383423]
40. Gao K, Butler SL, Bushman F. Human immunodeficiency virus type 1 integrase: arrangement of protein domains in active cDNA complexes. *EMBO Journal* 2001;20:3565–3576. [PubMed: 11432843]
41. Wincott F, DeRenzo A, Shaffer C, Grimm S, Tracz D, Workman C, Sweedler D, Gonzalez C, Scaringe S, Usman N. Synthesis, deprotection, analysis and purification of RNA and ribozymes. *Nucleic Acids Research* 1995;23:2677–2684. [PubMed: 7544462]
42. Song Q, Jones RA. Use of silyl ethers as fluoride scavengers in RNA synthesis. *Tetrahedron Letters* 1999;40:4653–4654.

Scheme 1.

Scheme 2.

Scheme 3.