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Synthesis and spectroscopic characterization of binaphthol aminosugars for stimulation of DNA strand slippage synthesis

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ABSTRACT

Expansion of DNA repeat sequences is associated with many human genetic diseases. Bulged DNA structures have been implicated as intermediates in DNA slippage within the DNA repeat regions. Two new binaphthol aminosugars were first synthesized as DNA bulge binders to study the triplet repeat expansion due to the wedge-shaped structure of 1,1′-bi-2-naphthol. Both compounds were structurally characterized by 1- and 2-D NMR. They showed remarkable fluorescence enhancement when binding with bulge DNA and they exhibited stimulation for ATT·AAT trinucleotide repeat DNA sequence slippage synthesis.

1. Introduction

Bulge structure in nucleic acid has been shown to play a significant biological role in protein binding recognition,[1] frameshift mutation,[2] imperfect homologous recombination by repair enzymes,[3] naturally occurring antisense RNA,[4] and expansion of triplet repeats during DNA synthesis.[5] Triplet repeat expansion is believed to be caused by the unstable nature of triplet repeats sequence.[6] Till now, a number of human neurodegenerative diseases including Huntington's disease, Friederich's ataxia, and fragile X syndrome have been reported to be caused by triplet repeat expansion.[7]

We are interested in small molecule that can control triplet repeat expansion. As far as we know, there are two types of small molecule that have been reported to have slippage synthesis property in vitro (Scheme 1). One class is some hairpin structure binding ligands such as DAPI (4′,6-diamidino-2-phenylindole), which was reported to induce ATT trinucleotide repeats strand to form mismatched hairpin structure.[8] The other class is a series of bulge structure binding molecule designed from the decomposed product of NCS-chrom. NCS-chrom is a bicyclic enediyne antibiotic.[9] One of its decomposed products NCSi-gb shows bulge binding selectivity and its solution structure with bulge DNA has been reported.[10] It's believed that the selectivity comes from the wedge shape of spirocyclic part, which fits into DNA bulge pocket. The aminosugar part binds in the groove to give more binding affinity. Some other similar spirocyclic molecules have been synthesized and reported, such as DDI (double-decker intercalator) and ent-DDI.[11] These molecules are more stable compared with NCSi-gb and show DNA slippage synthesis property in vitro. The molecule may cause bulge structure formation during that time triplet repeats strand expanded by polymerase.[11i]

2,2′-Binaphthol (BINOL) has two independent aromatic rings with a wedge-shaped structure, which are widely used in industry for both stoichiometric and catalytic asymmetric reactions.[12] Herein, binaphthol aminosugars shown in Scheme 1 have been chosen for the study based on: (1) their luminescent properties to permit fluorescent binding studies; (2) aminosugar moiety to enhance its aqueous solubility and binding to the phosphate backbone at the bulged site; (3) a convenient synthesis from BINOL; (4) their possible biochemical behaviors with potential metabolism of BINOL in vivo.

2. Results and discussion

As shown in Scheme 2, *S*-BINOL was converted to its monomethyl ether *S*-**5**.[13] Then *S*-**5** reacted with 2-chloroethanol in DMF in the presence of K_2CO_3 to give the monohydroxy intermediate *S*-**3**, which was further treated with compound **7**. After column gel purification, all protecting groups were removed by sodium methoxide in methanol to give *S*-**1**. *R*-**2** was prepared by the same method to prepare compound **1** starting with *R*-BINOL. Compounds

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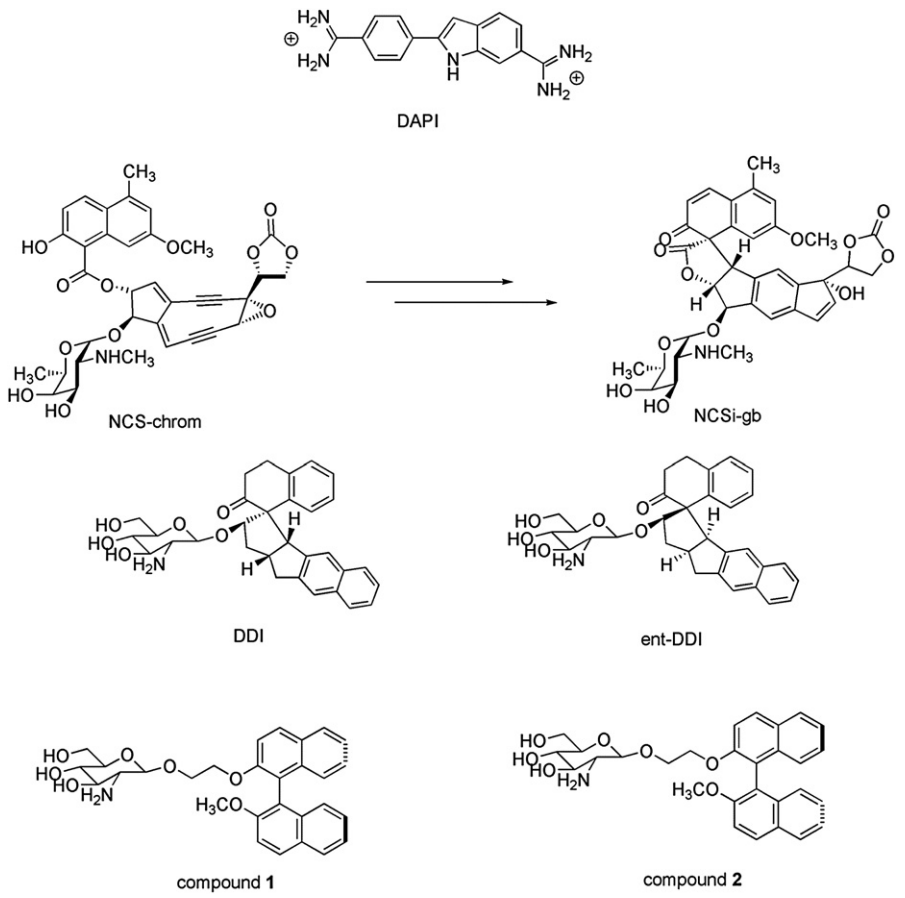
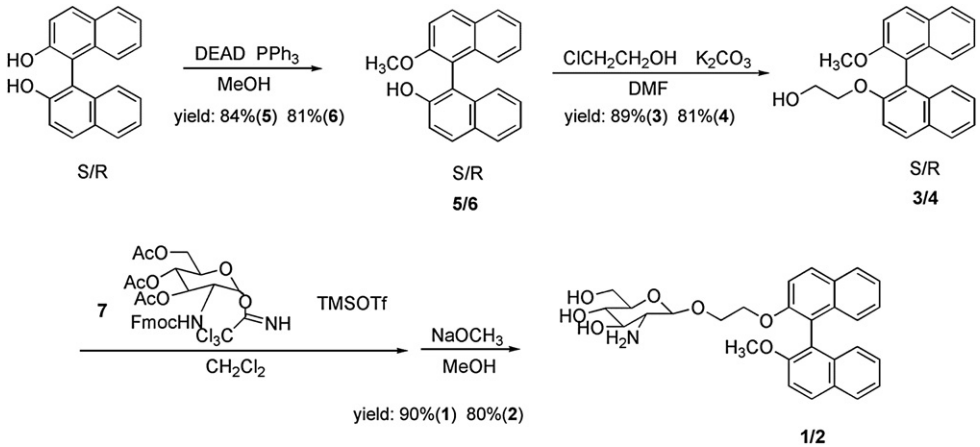


Scheme 1. Molecular structures mentioned in Section 1.

1 and **2** were characterized by a combination of 1- and 2-D NMR spectroscopy, including COSY, HSQC, HMBC, and ROESY experiments, in conjunction with mass spectrometry (See Fig. 1 and Supplementary data). Isomeric purity of **1** and **2** was higher than 95% by NMR. The 8-hydrogen NMR signal show at least 0.2 ppm higher field than other hydrogen of the naphthalene, which confirmed the wedge-shaped structure of these compounds in proton solvent.[11]

Compounds **1** and **2** exhibited weak fluorescence at the excitation wavelength of 310 nm in phosphates buffer. This phenomenon may result from the intermolecular photoinduced-electron-transfer (PET) process between the binaphthalene group and the amino alcohols.[14] The fluorescence maximum is at about 400 nm. In order to determine the bulge binding property of the molecule, we examined the fluorescence change of the molecule binding to different DNA sequences. The fluorescence intensity was enhanced when the molecule bound to DNA bulge structure. Figure 2 shows the fluorescence spectrum of compound **1**, in which the fluorescence intensity increased with the concentration increase of three-nucleotide bulged hairpin DNA. The enhancement may result from the cooperative effect of the prevention of intermolecular PET, conformation change of binaphthalene, and π–π stacking with DNA base pairs. The duplex DNA of similar sequence with no bulge or loop structures showed only about one-third enhancement of the maximum enhancement of bulge or loop DNA at similar DNA concentration.



Scheme 2. Synthesis of compounds **1** and **2** from enantiomerically pure 1,1′-bi-2-naphthol.

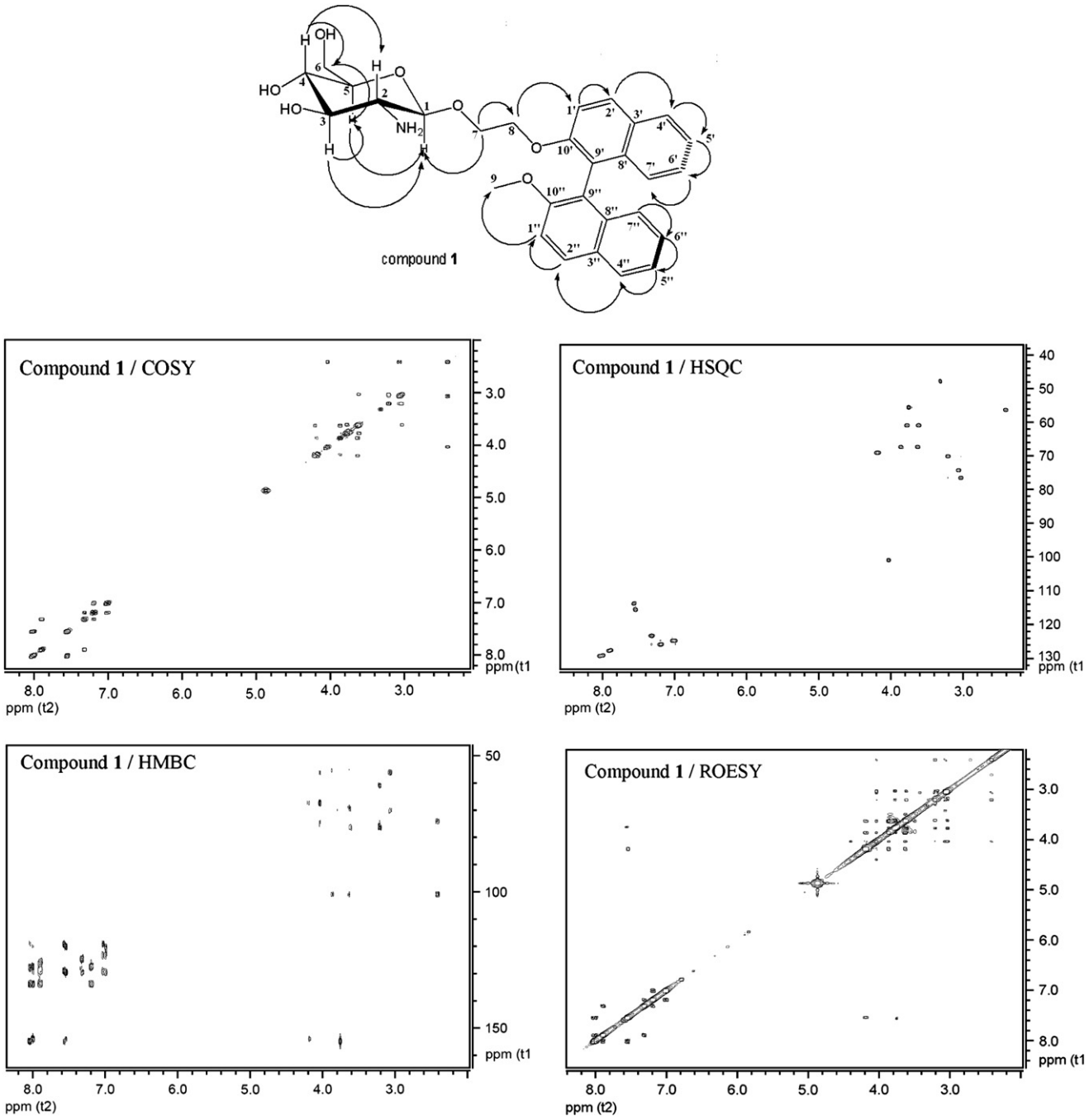


Figure 1. COSY, HSQC, HMBC, and ROESY of **1**. There are strong ROESY interactions between H-1 and H-3, H-1 and H-5, H-3 and H-5, H-2 and H-4, H-4 and H-6, H-6 and H-5, H-1 and H-7. These signals confirm the conformation of the sugar part.

From the change of fluorescence intensity we could calculate the dissociation constant K_d value using the equation $i/i_0=1+(\Delta i/2i_0)([T_0]+[\mathrm{DNA}]+K_d-(([T_0]+[\mathrm{DNA}]+K_d)^2-4[T_0][\mathrm{DNA}])^{1/2})$,wherein $[T_0]$ is the initial concentration of the fluorescent, i is the intensity of the sample, i_0 is the initial intensity of the sample, and Δi is the total change in intensity per compound unit from the free state to total binding state.[15] Some K_d values of the molecule with different DNA sequences are listed in Table 1. Compounds **1** and **2** showed moderate binding affinity with bulge structure, which was comparable with three-nucleotide loop. We used far more excessive DNA to ensure that only one compound binds to single DNA. The type of nucleotide and size of bulge structure influence binding strength distinctly. Three-nucleotide bulge showed relatively better affinity than one-nucleotide and two-nucleotide bulge. For example, the K_d values of compound **1** are over 100 μM for hairpin, one-nucleotide or two-nucleotide bulge DNA, but only a half for AAT bulge containing hairpin DNA. Compound **2** showed slightly better binding strength than compound **1** especially for small bulge structure and the two compounds had different behavior on changing the nucleotides in the bulge. This result demonstrated that this kind of molecule needs space large enough to fit in the bulge pocket and the flexibility of the binaphthalene made the two molecules less discriminated.

As bulge has been postulated as intermediate in DNA slippage synthesis involving templates with nucleotide repeats, we examined the influence of **1** and **2** on the triplet repeat expansion in vitro. The AAT·ATT trinucleotide repeats distribution in different types of genomic sequences and frequently in introns suggesting an

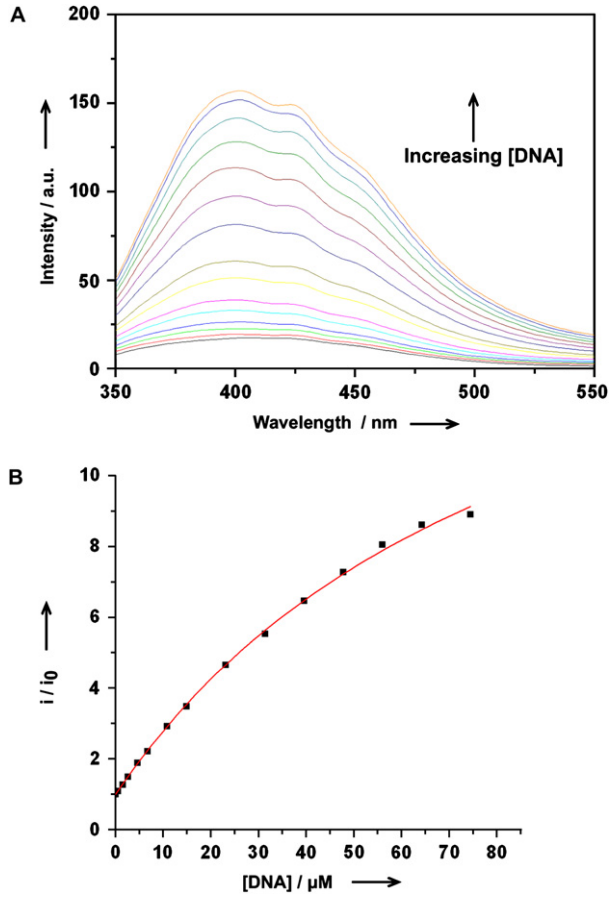


Figure 2. Fluorescence enhancing spectra of compound **1** by ATT bulge contained hairpin DNA (CTG CGA TGC GTG TTT CAC GCA ATT TCG GAC). (A) DNA concentration from 0, 0.51, 1.54, 2.57, 4.62, 6.68, 10.79, 14.90, 23.12, 31.34, 39.56, 47.78, 56.00, 64.22 to 74.49 μM; containing 0.5 μM compound **1**, 10 mM phosphate buffer, pH 7. (B) The fluorescence ratio i/i_0 as a function of the DNA concentration.

important biological role[16] and the expansion has been observed during DNA replication in vitro.[17] Our experiment was carried out by the Klenow fragment of DNA polymerase at 23 °C using $(AAT)_5$ as template and $(ATT)_3$ as primer (Fig. 3A).[11] The primer was 5′-^{32}P-end-labeled using [γ-^{32}P]ATP and ploynucleotide kinase. The experiment result was analyzed by electrophoretic mobility shift. Figure 3B shows the resolution of the products on a denaturing gel. In the control reaction (lane 3), the 9-*mer* was expanded to longer length, which indicated the slippage occurring during synthesis and the slippage could be restrained by doxorubicin, a known slippage synthesis inhibitor (lane 4).[12] In the presence of compound **1** or **2** the slippage synthesis was enhanced distinctly. Lanes 5–8 and 10–13 show the product of reactions in the presence of **2** or **1** at different concentration. At low concentration the slippage length grew with increasing concentration of **1** or **2**. The best concentration for both compounds was at about 100 μM and the longest band exceeded 42 bp (base pair) for 12 h. Higher concentration led to slippage length drop. We believe that this phenomenon is caused by high concentration of the free compound that inhibited the dissociation process of the binding compound. Note that the expanded bands at length 18, 21, and 24 bp in the control and 24, 27, and 30 bp, etc. in the drug added samples were stronger than the others, indicating that the in vitro DNA strand slippage syntheses of $(ATT)_3/(AAT)_5$ were mainly occurred by triplet step expansion. This may be due to the relative stability of complementary structure formed by the two triplet strands. The slippage enhancement by **1** or **2** could also be restrained by doxorubicin (lane 14 and lane 9), which confirmed our assumption that drug–DNA intermediate caused the enhancement of slippage synthesis. Comparing the electrophoresis with the binding affinity, we believe that the trinucleotide repeats DNA slippage synthesis caused by small molecule is a dynamic equilibrium, so molecule with moderate binding affinity can effectively stimulate slippage synthesis. The slippage stimulation effect by **2** was better than that of **1**, presumably due to the conformational difference of **1** and **2**. This effect is associated with the binding ability of the modified BINOL with bulge DNA as revealed by fluorescent titration result. Compound **2** has a right-handed naphthalene helix, which possesses the geometry for mimicking DNA helix, showing better binding with DNA.

3. Conclusions

In summary, based on the previous investigation of DNA binders such as NCSi-gb and DDI,[11] we have designed and synthesized two

Table 1
Dissociation constants of **1** and **2** for selected sequences[a]

Entry	DNA sequence	Compound **1** (μM)	Compound **2** (μM)
1	5′ G T C C G A T G C G T G T T 3′ C A G G C T A C G C A C T T	120±5	107±12
2	5′ G T C C G A T G C G T G T T 3′ C A G G C T A C G C A C T T G	106±4	74±6
3	5′ G T C C G A T G C G T G T T 3′ C A G G C T A C G C A C T T T G	119±8	60±4
4	5′ G T C C G A T G C G T G T T 3′ C A G G C T A C G C A C T T T A T	89±5	47±5
5	5′ G T C C G A T G C G T G T T 3′ C A G G C T A C G C A C T T T A A	54±4	59±6

[a] Fluorescence studies were conducted using Varian Cary Eclipse spectrophotometer at 20 °C phosphate buffer (10 mM, pH 7, NaCl 50 mM). Emission spectra were obtained in the range 350–550 nm upon excitation at 310 nm. Emission reading at 400 nm was imported in binding calculation. Dissociation constant was derived from curve-fitting.

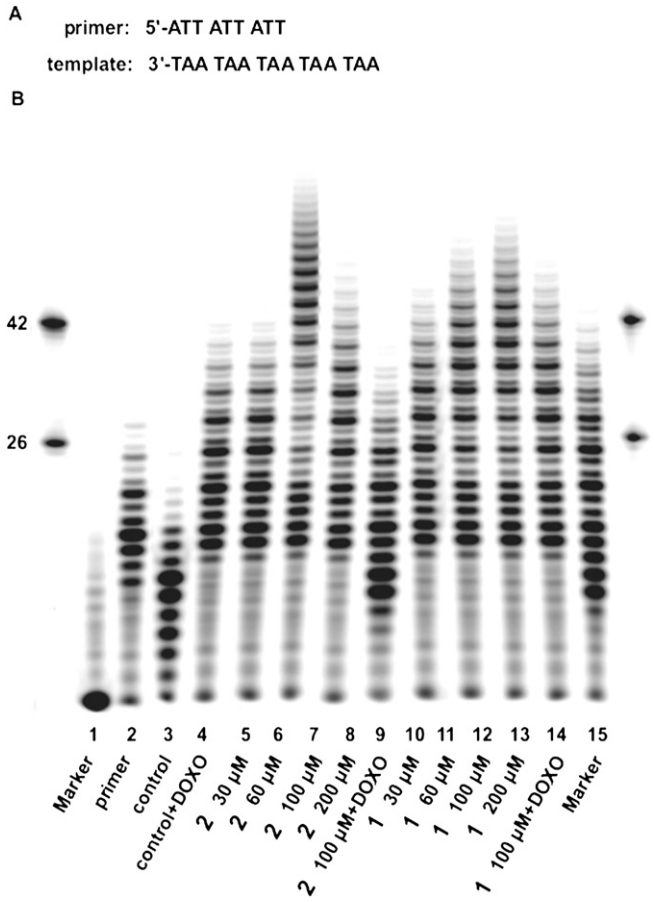


Figure 3. Effect of compounds **1** and **2** on the expansion of the trinucleotide repeat. (A) The sequence of primer–template duplex employed in the experiment. (B) The primer expansion affected by compounds **1** and **2**. A standard reaction (23 °C, 12 h) containing 5′-^{32}P-end-labeled $(ATT)_3$ and unlabeled template $(AAT)_5$ was catalyzed by the Klenow fragment. Lane 1 and 15 are markers, 42 bp (top) and 26 bp (bottom); lane 2 is the reaction without Klenow fragment; lane 3 is the reaction with 2% DMSO as control; lane 4 is the control reaction mixed with doxorubicin; lanes 5–8 is reaction in the presence of **2** from 30, 60, 100 to 200 μM and lane 9 is **2** (100 μM) with doxorubicin; lanes 10–13 is reaction in the presence of **1** from 30, 60, 100 to 200 μM and lane 14 is **1** (100 μM) with doxorubicin. In all of the reactions the doxorubicin concentration was 40 μM. The products were resolved on a 15% sequence gel.

new binaphthol derivatives **1** and **2**. They showed DNA bulge structure binding selectivity with moderate binding affinity, and behaved as fluorescence enhancer when interaction taking place. Considering the 3D structural features of NCSi-gb and compound **2** (Fig. 4), three similar facets are: (1) both compounds have two independent aromatic rings; (2) the dihedral angle between the aromatic rings is 30–50° with a wedge-shaped structure; (3) there is a pendant aminosugar for enhancing the binding ability with DNA backbone. It seems that the prerequisite for selective motif binding may be the steric similarity between small molecules that interact with DNA motif. Both **1** and **2** can cause DNA slippage synthesis, which gives us a useful tool to study triplet repeat expansion. The further development of new kind of DNA/RNA bugle binders is currently underway.

4. Experimental

4.1. General

Compound **7** was prepared according to the previous method.[20] All other chemical reagents and solvents were commercially available and were used without further purification. NMR spectra

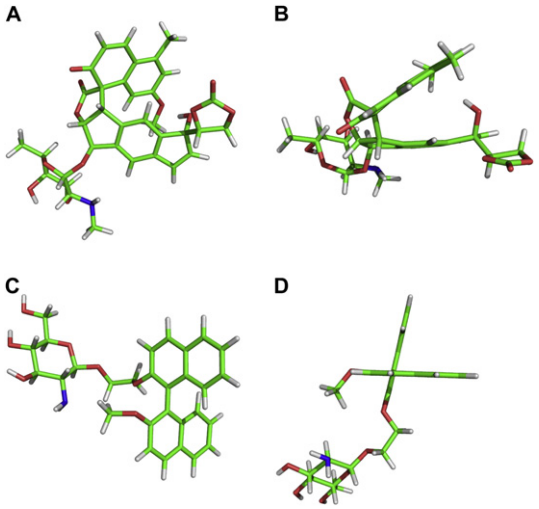


Figure 4. Comparison of the stereo structures of NCSi-gb (A, B) and compound **2** (C, D) from face and side view. The 3D structures of the NCSi-gb and **2** were generated using the InsightII molecular modeling program.[18] The structures' energy minimization was performed using the CVFF force field[19] with a 0.001 kcal/mol energy gradient convergence criterion by InsightII/Discover package. Graphics were generated using PyMOL (http://pymol.sourceforge.net).

were obtained on either a Varian Mercury Plus 400 MHz spectrometer or a Bruker Avance 600 MHz spectrometer. Mass spectra were obtained either on a Finnigan LCQ Advantage mass spectrometer (ESI). High-resolution mass spectra (HRMS) were obtained on a Varian QFT-ESI mass spectrometer. Solvents were distilled from the appropriate drying agents before use. All reactions were monitored by TLC on silica gel GF254 (0.5 mm). Spots were detected under UV light. Flash column chromatography was carried out on silica gel H (400 mesh, Qingdao, China) or silica gel (200–300 mesh, Qingdao, China). Fluorescence studies were conducted using Varian Cary Eclipse spectrophotometer at phosphate buffer (10 mM, pH 7, NaCl 50 mM). Emission spectra were obtained in the range 350–550 nm upon excitation at 310 nm. Emission reading at 400 nm was imported in binding calculation. Dissociation constant was derived from curve-fitting. The Klenow fragment of *Escherichia coli* DNA polymerase I (KF) was purchased from New England BioLabs Inc, and T4 ploynucleotide kinase was purchased from TAKARA BIOTECHNOLOGY.

4.2. Preparation of compounds 3 and 4

Compound **5** or **6** (300 mg, 1 mmol), 2-chloroethanol (162 mg, 2 mmol), and K_2CO_3 (270 mg, 2 mmol) were stirred in 10 mL DMF at 90 °C for 10 h. The mixture was diluted with ethyl acetate (100 mL), which was washed with water (50 mL) and brine (4×20 mL). The organic layer was separated and dried with Na_2SO_4. After the solvent was removed, the residual oil was purified by chromatography (eluted by ethyl acetate) to give **3** (309 mg, 89%) or **4** (280 mg, 81%).

*4.2.1. Compound **3***

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.91 (t, J=6.5 Hz, 1H), 3.56–3.59 (m, 2H), 3.76 (s, 3H), 4.09–4.11 (m, 1H), 4.18–4.20 (m, 1H), 7.12 (t, J=10.0 Hz, 2H), 7.23 (t, J=6.9 Hz, 2H), 7.34 (dd, J=6.5, 11.8 Hz, 2H), 7.42 (d, J=9.0 Hz, 1H), 7.47 (d, J=9.0 Hz, 1H), 7.88 (d, J=8.1 Hz, 2H), 7.98 (t, J=7.8 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 56.96, 61.18, 71.16, 114.21, 115.64, 119.41, 120.35, 123.75, 123.85, 125.00, 125.31, 126.41, 126.59, 127.95, 128.05, 129.29, 129.50, 129.63, 133.80, 133.94, 153.65, 154.53. MS (ESI) m/z $(C_{23}H_{20}O_3Na)^+$: calcd 367.13, found 367.32.

4.2.2. Compound ***4***

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.91 (t, J=6.5 Hz, 1H), 3.56–3.59 (m, 2H), 3.76 (s, 3H), 4.09–4.11 (m, 1H), 4.18–4.20 (m, 1H), 7.12 (t, J=10.0 Hz, 2H), 7.24 (t, J=7.0 Hz, 2H), 7.34 (dd, J=6.3, 11.7 Hz, 2H), 7.42 (d, J=9.3 Hz, 1H), 7.47 (d, J=7.8 Hz, 1H), 7.88 (d, J=7.8 Hz, 2H), 7.99 (t, J=7.8 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 56.96, 61.18, 71.16, 114.21, 115.64, 119.41, 120.35, 123.76, 123.85, 125.00, 125.31, 126.41, 126.58, 127.95, 128.04, 129.29, 129.50, 129.63, 133.82, 133.94, 153.67, 154.53. MS (ESI) m/z $(C_{23}H_{20}O_3Na)^+$: calcd 367.13, found 367.26.

4.3. Preparation of compounds 1 and 2

Compound **3** or **4** (30 mg, 0.087 mmol), compound **7** (114 mg, 0.174 mmol), and 4 Å molecule sieves (200 mg) were stirred in dry CH_2Cl_2 (2 mL) at room temperature for 30 min. Then the mixture was cooled to −20 °C and TMSOTf (30 μL, 0.2 M in CH_2Cl_2) was added. After stirring for 30 min at low temperature, the mixture was warmed to room temperature and stirred for 3 h. Then CH_2Cl_2 (20 mL) was added. The organic layer was washed with saturated $NaHCO_3$ (10 mL) and dried with Na_2SO_4. After the solvent was removed, the residual oil was purified by chromatography to give the intermediate as a syrup. The syrup was dissolved in MeOH solution of NaOMe (10 mL, 0.2 M) and stirred overnight. The mixture was diluted with $CHCl_3$ (20 mL), then washed with brine (2×5 mL). The organic layer was separated and dried with Na_2SO_4. After the solvent was removed, the residual oil was purified by chromatography (eluted by CH_3OH) to give **1** (40 mg, 90%) or **2** (35 mg, 80%).

4.3.1. Compound ***1***

^{1}H NMR (600 MHz, CD_3OD): δ 2.42 (dd, J=8.3, 10.0 Hz, 1H), 3.03 (ddd, J=2.2, 5.5, 9.5 Hz, 1H), 3.08 (t, J=9.5 Hz, 1H), 3.22 (t, J=9.21 Hz, 1H), 3.61–3.65 (m, 2H), 3.76 (s, 3H), 3.78 (dd, J=2.1, 11.9 Hz, 1H), 3.87 (dt, J=4.1, 11.5 Hz, 1H), 4.04 (d, J=8.1 Hz, 1H), 4.16–4.23 (m, 2H), 7.00 (d, J=8.5 Hz, 1H), 7.03 (d, J=8.6 Hz, 1H), 7.19 (t, J=8.9 Hz, 1H), 7.21 (t, J=8.6 Hz, 1H), 7.31–7.34 (m, 2H), 7.55 (d, J=9.1 Hz, 1H), 7.57 (d, J=9.2 Hz, 1H), 7.89 (d, J=8.2 Hz, 1H), 7.92 (d, J=8.2 Hz, 1H), 8.00 (d, J=9.1 Hz, 1H), 8.09 (d, J=9.1 Hz, 1H). ^{13}C NMR (150 MHz, CD_3OD): δ 55.67, 56.43, 61.00, 67.44, 69.16, 70.23, 74.40, 76.65, 101.09, 113.86, 115.62, 119.29, 120.32, 123.29, 123.45, 124.77, 125.88, 125.98, 127.63, 127.83, 129.16, 129.29, 129.34, 129.76, 133.89, 133.96, 154.14, 154.96. HRMS (ESI) m/z $(C_{29}H_{32}NO_7)^+$: calcd 506.2179, found 506.3762.

4.3.2. Compound ***2***

^{1}H NMR (600 MHz, CD_3OD): δ 2.51 (t, J=9.0 Hz, 1H), 3.03–3.05 (m, 1H), 3.21–3.26 (m, 1H), 3.58 (ddd, J=3.3, 7.4, 11.2 Hz, 1H), 3.61 (dd, J=5.37, 11.9 Hz, 1H), 3.75 (dd, J=2.1, 12.3 Hz, 1H), 3.77 (s, 3H), 3.86 (dt, J=4.1, 11.7 Hz, 1H), 4.10 (d, J=8.1 Hz, 1H), 4.13 (dt, J=4.1, 11.2 Hz, 1H), 4.27 (ddd, J=3.6, 7.4, 10.9 Hz, 1H), 7.02 (d, J=9.0 Hz, 1H), 7.03 (d, J=9.0 Hz, 1H), 7.21 (t, J=7.5 Hz, 2H), 7.33 (t, J=7.5 Hz, 2H), 7.56 (d, J=9.1 Hz, 1H), 7.58 (d, J=9.2 Hz, 1H), 7.90 (t, J=8.13 Hz, 2H), 8.01 (d, J=9.0 Hz, 1H), 8.05 (d, J=9.1 Hz, 1H). ^{13}C NMR (150 MHz, CD_3OD): δ 55.83, 56.44, 60.92, 67.40, 69.23, 70.22, 74.24, 76.69, 100.94, 113.94, 115.78, 119.45, 120.40, 123.33, 123.52, 124.76, 125.91, 125.97, 127.63, 127.74, 129.21, 129.38, 129.82, 133.91, 154.11, 154.79. HRMS (ESI) m/z $(C_{29}H_{32}NO_7)^+$: calcd 506.2179, found 506.3788.

4.4. DNA polymerase assays

Reactions were carried out in 15 μL at pH 7.5 containing 10 mM Tris–HCl, 7 mM $MgCl_2$, 0.1 mM dithiothreitol, 4 μM each of the primer and template, 1 mM each of dATP, dTTP, and the Klenow fragment of DNA polymerase I. The enzyme was at a level of 0.02 unit per microliter of the reaction. A mixture of 5′-^{32}P-end-labeled primer and unlabeled template, generally in equimolar concentrations, was annealed by heating in Tris–HCl, pH 7.5, and $MgCl_2$ to 95 °C followed by slowly cooling to room temperature. The concentrations of the components at the annealing stage were 30–50% higher than those in the final assay to accommodate the dilution resulting from the addition of the rest of the components in the subsequent stage. Following addition of dithiothreitol and deoxynucleoside triphosphates to the annealed mixture, it was distributed for assays. The compound to be tested was added as a solution in dimethyl sulfoxide. Controls lacking the compound received an equal volume of dimethyl sulfoxide, the final concentration of which was 2% in the assay. The reaction was started by the addition of the enzyme. The incubation was at room temperature (23 °C). The reaction was terminated by the addition of EDTA to a final concentration of 50 mM after 12 h. The products were resolved on a 15% sequence gel.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.04.047.

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