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# Stimulation on DNA triplet repeat strand slippage synthesis by the designed spirocycles

Zhen Xi,* Di Ouyang† and Hong-Tao Mu†

*State Key Laboratory of Elemento-Organic Chemistry, Department of Chemical Biology, Nankai University, Tianjin 300071, China*

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**Abstract**—The designed simpler chiral spirocyclic helical compounds that mimic the molecular architecture of the DNA bulge binder NCSi-gb have been prepared. It has been found that the synthesized spirocyclic compounds have strong stimulation effect on DNA slippage synthesis. Their stimulation activities on DNA strand slippage suggest that they may bind to or induce the formation of a non Watson–Crick structure during in vitro replication of DNA triplet repeats.
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Several human hereditary neurodegenerative and neuromuscular disease genes are associated with the expansion of triplet repeats.[1] The formation of abnormal structure has been proposed as a part of the mechanism for large-scale expansion of this kind of triplet repeats during DNA replication.[2] Bulged structures have been implicated as intermediates in the slippage synthesis of DNA associated with the unstable expansion of triplet repeats.[3–6]

It has already been demonstrated that intercalators, such as ethidium bromide,[7] 2-acylamino-1,8-naphthyridine,[8] tylophorine B,[9] and a number of antitumor antibiotics[10,11] have increased binding affinity for sites on duplex DNA carrying simple bulge (one or more unpaired bases). Of special interest, the enediyne antitumor antibiotic neocarzinostatin chromophore (NCS-chrom) induces a highly efficient site-specific strand cleavage at either single or two-base bulges depending on the particular mode of activation of the drug.[11–13] After a general base-catalyzed spirolactonization and intramolecular rearrangement, NCS-chrom undergoes spontaneous cyclization to form NCSi-gb (Fig. 1A),[14,15] a more stable isostructural mimic of the DNA-cleaving species, which specifically targets the bulged DNA rather than the duplex form[13,16,17] at submicromolar concentration,[18] and the solution-phase NMR studies have also confirmed that the binding to the bulge emanates from the unique molecular geometry of the agent.[16,19] Molecular studies have revealed that the affinity of NCSi-gb to bind DNA bulges is mostly dependent on the spirocyclic ring junction with an appropriate angle, pendant amino sugar group that enhances binding at the bulged site, and two discrete aromatic moieties for π-stacking that mimic the base pairing. Some synthetic molecules, which are mimics of NCSi-gb, exhibited high affinity to the bulged structures in nucleic acid[20–24] and enhanced the repeat nucleotide slippage during in vitro DNA synthesis.[23,25] Of them, compound DDI (Fig. 1B) displayed quite a strong enhancement in various repeat DNA strand slippage syntheses. Given the rationality of such selective binding, it has prompted effort to design and synthesize the simpler analogues of DDI with a spirocyclic backbone, and to explore the specificity of bulge bindings

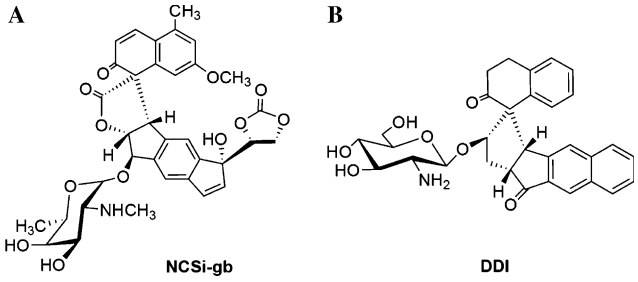


**Figure 1.** Structure of natural and synthetic spirocyclic compounds. (A) DNA bulge-specific compound derived from NCS-chrom upon base catalysis. (B) Synthetic compound mimicking (A), which showed selectivity for binding to DNA bulge site,[21] and strongly enhanced the repeat nucleotide slippage during in vitro DNA synthesis.[25]

*Keywords*: Neocarzinostatin; Synthesis; Triplet repeat; DNA Slippage.
* Corresponding author. Tel./fax: +86 22 23504782; e-mail: zhenxi@nankai.edu.cn
† These authors have contributed equally to this work.

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and the stimulation in DNA strand slippage synthesis in vitro.

The synthesis of spiroalcohol **1** was carried out according to the Ref. 20 by some modification. Structural assignment of this unusual spirocycle **1** was made using 2D NMR[20] and also the stereochemistry was confirmed unambiguously by X-ray analysis (Fig. 2).[26]

Though compound **1** could be separated into its enantiomer pairs via chiral chromatography, we elected to perform diastereomer separation following aminoglycosylation. An aminoglucose was selected as the pendant amino sugar for reasons of simplicity and economy. Thus, activated glycosyl donor **2** was subjected to coupling with racemic **1** to give a mixture of diastereomers **3** and **4**. They were separated by silica gel chromatography and then subjected to global deprotection under basic condition in one step to give diastereomers **5**[27] and **6**[28] (Scheme 1). Both compounds were used to test the stimulation ability on triplet repeat-strand slippage during DNA replication by DNA polymerase I based system.

The in vitro expansion assays provided a convenient method to examine compounds as potential probes to study the mechanism of DNA slippage itself, and helped in understanding the molecular basis of the human hereditary diseases.[6,29–31] DNA slippage synthesis was measured using the primer/template oligomers of $(ATT)_3$ and $(AAT)_5$ in a reaction catalyzed by the Klenow fragment of *Escherichia coli*. DNA polymerase I lacking 3′–5′ exonuclease activity. Both dose-dependency and time course of expansion of the triplet repeat AAT and stimulation effect on DNA in vitro synthesis by diastereomers **5** and **6** were tested. In Figure 3 is shown the extension of the AAT triplet repeat after reaction for 12 h and the resolution of the products on a denaturing polyacrylamide gel. The percentage of band intensities that exceed 26-mer marker in each lane on the gel by using Image J program is listed in Table 1. The concentration of **5** and **6** was varied from 0.1 to 200 μM, while the incubation course ranged from 12 to 72 h.

The synthetic DNA primer and template were used directly without further purification. In the control reaction (Fig. 3), the 9-mer primer was extended to a

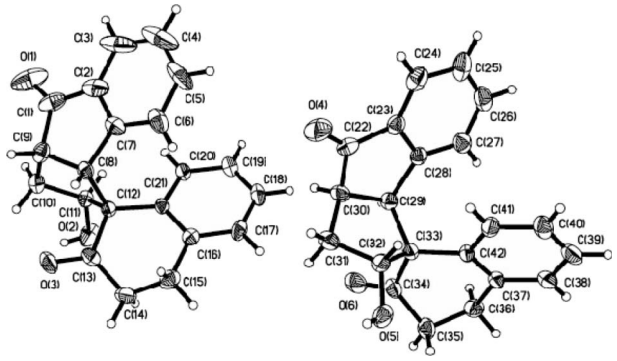
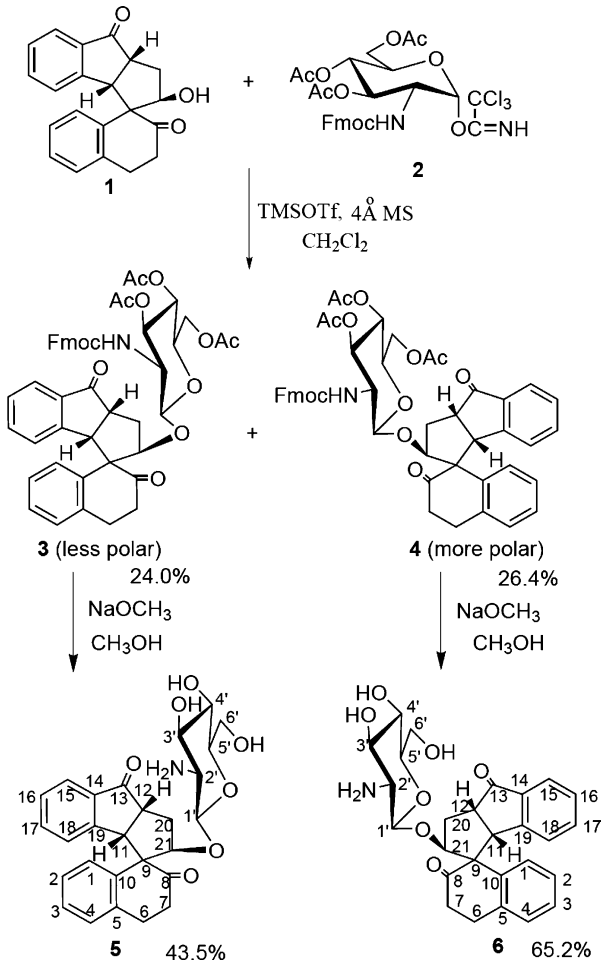


**Figure 2.** ORTEP of spiroalcohol **1**.




**Scheme 1.** Synthesis of chiral spirocyclic helical compounds containing aminosugar.

length obviously below 26 nucleotides (lanes 2 and 3), the size of which was more than the combined length of the primer and the template, thus it is indicative of the occurrence of slippage in the in vitro DNA synthesis process. The addition of **5** and **6** both stimulated the in vitro synthesis of triplet repeat AAT to generate products much longer (lanes 5–9 for **5**, lanes 12–16 for **6**) than those in the control (lanes 2 and 3). That is, in the presence of **5** and **6**, the radioactivity in the bands corresponding to the long fragments steadily increased with increase of dosage, concomitant with a decrease in products shorter than 26 nucleotides in length. At the lowest concentration shown in Figure 3 (10 μM), the stimulated slippage of ATT synthesis by **5** was enhanced to the extent that over 4% of the band intensities in the lane was longer than 26 nucleotides at 12 h; whereas, **6** had a weaker effect.[32] At the highest concentration used (200 μM), over 25% of the band intensities was longer than the 26-mer by **5** and over 20% by **6** (Table 1). This showed that **5** and **6** had stimulated in vitro DNA slippage synthesis of AAT with dose dependency. At the concentration of 100 μM (at which, **5** and **6** strongly affect DNA slippage), 2-deoxy-2-aminoglucose used here did not affect DNA slippage; also, at the concentration of 1.0, 10, 100, 200, and 500 μM, the aglycon **1** did not affect DNA slippage (see supporting information).

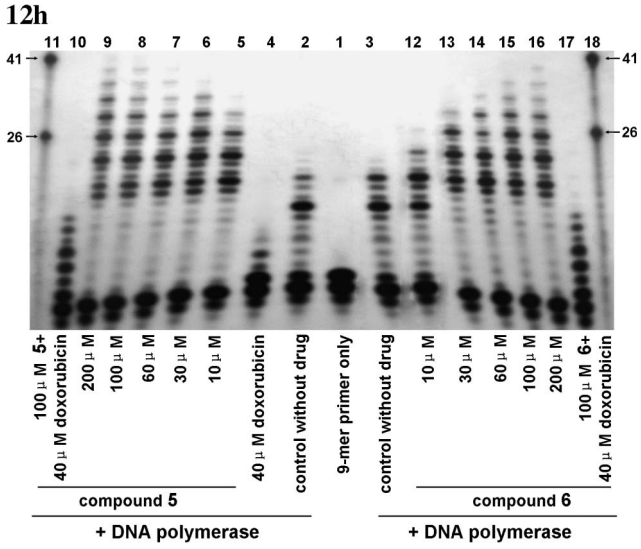



**Figure 3.** Dose-dependency of expansion of triplet repeat AAT and stimulation effect by compounds **5** and **6**. Compound **5** or **6** was dissolved in DMSO. DNA primer $(ATT)_3$ was 5′-$^{32}$P-end-labeled using [γ-$^{32}$P]ATP and T4 polynucleotide kinase. The labeled primer was used directly without further purification. A mixture of 5′-$^{32}$P-end-labeled primer and unlabeled template, generally in equimolar concentrations, was annealed by heating to 95 °C followed by slow cooling to room temperature. The annealed mixture of $(ATT)_3/(AAT)_5$ (4 μM) in 50 mM Tris–HCl, pH 7.5, was supplied with 15 mM $MgCl_2$, 4 mM dithiothreitol, 1 mM dATP and dTTP, and compound **5** or **6** before starting the synthesis by the addition of enzyme. The final concentration of DMSO was 2% in the assay. The enzyme was at a level of 0.032 unit per microliter of the reaction, with a DNA to enzyme molar ratio of 140. After incubation at 23 °C for the times indicated, 80% formamide containing 100 mM EDTA and marker dyes were added to the reaction mixtures at 1:1 volume and were loaded for analysis on a 15% polyacrylamide sequencing gel. The gels were exposed to X-ray film. The film was developed, fixed, and pictures taken by digital camera. Lane 1: the control reaction system to which no DNA polymerase was added. Lanes 2 and 3: control lacking compound **5** or **6** received an equal volume of DMSO. The arrows in the figure point to the size markers of 26 and 41 nucleotides in length.

**Table 1.** Dose–response and time course for compounds **5** and **6** in DNA in vitro slippage synthesis of triplet repeat AAT

| Fragments longer than 26-mer (%) | 0 | 10 | 30 | 60 | 100 | 200 |
|---|---|---|---|---|---|---|
| *Compound **5** (μM)* | | | | | | |
| 12 h | 0 | 4.5 | 12.1 | 18.4 | 21.3 | 25.7 |
| 24 h | 0 | 33.3 | 36.0 | 42.0 | 43.7 | 45.6 |
| 36 h | 0 | 40.1 | 44.2 | 46.8 | 48.6 | 50.9 |
| 48 h | 0 | 44.5 | 49.6 | 55.5 | 61.1 | 63.6 |
| 72 h | 0 | 49.1 | 58.6 | 78.3 | 85.1 | 84.9 |
| *Compound **6** (μM)* | | | | | | |
| 12 h | 0 | 0.8 | 6.9 | 12.9 | 17.5 | 20.0 |
| 24 h | 0 | 4.1 | 14.0 | 28.4 | 36.1 | 39.0 |
| 36 h | 0 | 6.3 | 30.2 | 42.1 | 45.9 | 44.7 |
| 48 h | 0 | 6.9 | 32.8 | 46.7 | 50.2 | 50.7 |
| 72 h | 0 | 7.8 | 42.9 | 54.2 | 63.5 | 66.4 |

Data were taken from the experiments described in Figure 3. After gel analysis of the products, the band intensities of each lane were quantitated using Image J program. The data showed dose and time-dependent increase in the length of the DNA replication products.

The effects of **5** and **6** on ATT expansion also exhibited time-dependent increase in the length of the slippage products. As the incubation time extended, the fragments longer than 26-mer increased gradually, the effects of **5** and **6** on the in vitro DNA slippage synthesis became stronger (Table 1). At the low concentration used (10 μM), as for **5**, the percentage of band intensities in the lane longer than 26 nucleotides was 4%, 33%, 40%, 44%, and 49% at 12, 24, 36, 48 and 72 h, respectively; whereas, for **6**, the percentage was 0%, 4%, 6%, 7%, and 8% at 12, 24, 36, 48, and 72 h, respectively. At the same time, when the highest level was used (200 μM), the percentage of the band intensities longer than 26 nucleotides turned to 25%, 45%, 50%, 63%, and 85% at 12, 24, 36, 48 and 72 h, respectively, for **5**; and 20%, 39%, 44%, 50%, and 66% at 12, 24, 36, 48, and 72 h, respectively, for **6**. On the contrary, in the control reaction, there was not any slippage band exceeded 26-mer during the whole time course. At 72 h, the extension of ATT almost reached saturation at higher concentrations (100 and 200 μM) for both **5** and **6** (Table 1), respectively.

Interestingly, there was a hierarchy of intensities of the three bands, each apparently separated by two nucleotides, which was faithfully repeated every three nucleotides throughout the lane. This band spacing appeared to reflect the triplet repeat unit. The addition of **5** and **6** did not influence this pattern. The effect of other DNA-binding agents on DNA slippage synthesis was also examined. Doxorubicin, an anthracycline glycoside that intercalates between DNA base pairs,[33] inhibited ATT expansion (lane 4, Fig. 3). When both compound **5** or **6** and doxorubicin were present, the inhibition was reversed to some extent at the experimental concentration (lanes 10 or 17, Fig. 3). It is possible that the duplex regions involved in the binding of doxorubicin are disrupted by drug-induced bulge formation and consequent Watson–Crick hydrogen bond breakage associated with strand slippage, leading to its dissociation from the DNA.[25]

In the primer extension reaction with short oligonucleotides, the repetitive sequence and the transient DNA structure formed during DNA strand synthesis by polymerase are two important intrinsic factors. DDI, preferentially bound to bulges of two to three unpaired bases in DNA, enhanced the slippage process by stabilizing an unusual DNA structure or inducing bulge formation.[25] The designed small molecules **5** and **6**, simpler analogues to DDI, also exhibited a similar enhancement in DNA strand slippage. Unlike the NCS-chrom derivative, DDI binds to the bulge site via the minor groove.[34] The two synthetic compounds reported here possessed the geometry for mimicking the helix bases with spirolactone moiety containing two-arene units.[24] The spiro-alcohol backbone of **5** and **6**, with one benzene ring less compared to DDI, adopted the wedge-shaped configuration that was necessary to achieve binding to DNA bulges, and its simple amino sugar derivative of the hydroxy group on spirocycle showed affinity for bulged sequences.[15–17,21,35] The handedness of the aglycon part of **5** was analogous to DDI, and it displayed better

stimulation effect than that of **6**. The smaller skeleton of **5** and **6** with the amino sugar attached to the same position on the spiroalcohol as DDI did not influence the intercalation of aromatic moieties into the base pair of the double stranded DNA with simple bulges through π-stacking. The possible difference may be in the mode or extent during the intercalation. The precise DNA structure that the compounds bound to and the detailed mechanism for the stimulation of DNA slippage remain to be further clarified.

## Acknowledgments

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## Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.11.082.

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26. CCDC 272413 contains the supplementary crystallographic data for compound 1. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.
27. Compound **5**: $^{1}$H NMR (300 MHz, $CD_3OD$): $\delta$ 7.74 (d, $J$ = 7.6 Hz, 1H, H-15), 7.38 (t, $J$ = 7.5 Hz, 1H, H-16), 7.34 (d, $J$ = 9.1 Hz, 1H, H-4), 7.25 (t, $J$ = 7.5 Hz, 1H, H-17), 7.20 (t, $J$ = 7.5 Hz, 1H, H-3), 6.84 (t, $J$ = 7.6 Hz, 1H, H-2), 6.35 (d, $J$ = 7.9 Hz, 1H, H-1), 6.25 (d, $J$ = 7.8 Hz, 1H, H-18), 4.76 (dd, $J$ = 10.1,7.2 Hz, 1H, H21), 4.38 (d, $J$ = 7.4 Hz, 1H, H-11), 4.24 (d, $J$ = 8.0 Hz, 1H, H-1′), 3.50–3.40 (m, 3H, H-12,H-6,-6′), 3.35–3.18 (m, 4H, H-6a, 6b, 4′, 3′), 3.11–2.97 (m, 2H, H-7a, 5′), 2.86–2.61 (m, 3H, H-7b, 20a, 20b), 2.49 (dd, $J$ = 9.5, 7.9 Hz, 1H, H-2′). $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 213.70, 208.69, 153.91, 137.93, 137.51, 136.20, 134.03, 128.90, 128.58, 127.95, 126.92, 125.47, 123.44, 99.89, 86.97, 77.19, 75.67, 69.89, 62.84, 60.87, 50.65, 49.75, 40.71, 31.35, 29.67, 28.92. MS (ESI, positive): Anal. found: *m/e* 480.1 $(M+1)^+$; Calcd. for $C_{27}H_{29}NO_7$ 479.
28. Compound **6**: $^{1}$H-NMR (300 MHz, $CD_3OD$): $\delta$ 7.69 (d, $J$ = 7.4 Hz, 1H, H-15), 7.34 (t, $J$ = 7.7 Hz, 1H, H-16), 7.31 (d, $J$ = 7.7 Hz, 1H, H-4), 7.21 (t, $J$ = 7.6 Hz, 1H, H-17), 7.17 (t, $J$ = 7.4 Hz, 1H, H-3), 6.82 (t, $J$ = 7.4 Hz, 1H, H-2), 6.27 (d, $J$ = 7.9 Hz, 1H, H-1), 6.17 (d, $J$ = 7.6 Hz, 1H, H-18), 4.63 (dd, $J$ = 9.9, 7.6 Hz, 1H, H-21), 4.19 (d, $J$ = 7.3 Hz, 1H, H-11), 3.97 (d, $J$ = 7.9 Hz, 1H, H-1′), 3.80 (d, $J$ = 11.7 Hz, 1H, H-6′), 3.63 (dd, $J$ = 11.8, 5.4 Hz, 1H, H-6′), 3.41–3.16 (m, 4H, H-12, 6a, 6b,4′), 3.03–2.97 (m, 2H, H-5′, 3′), 2.89-2.51 (m, 4H, H-7a, 7b, 20a, 20b), 2.41 (dd, $J$ = 9.6, 8.1 Hz, 1H, H-2′). $^{13}$C-NMR (75 MHz, $CD_3OD$): $\delta$ 214.61, 210.76, 155.48, 139.98, 138.91, 137.59, 135.28, 130.23, 130.10, 129.31, 129.22, 128.30, 126.97, 124.16, 104.39, 87.10, 78.07, 77.43, 71.50, 64.08, 62.62, 58.42, 51.56, 51.53, 42.72, 33.87, 29.94. MS (ESI, positive): Anal. found: *m/e* 480.1 $(M+1)^+$; Calcd. for $C_{27}H_{29}NO_7$ 479.
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32. The lowest concentration tested for **5** and **6** is 0.1 μM. It has been found that the minimum effective concentration for stimulating DNA slippage is 5.0 μM for **5** and 10 μM for **6**. No obvious stimulation of DNA slippage was observed at the concentrations of 0.1, 0.5, 1.0 μM for **5**, and 0.1, 0.5, 1.0, and 5.0 μM for **6**. At the concentration of 10, 100, 500, and 1000 μM (at 100 μM, **5** and **6** strongly affect DNA slippage), 2-deoxy-2-aminoglucose used here did not affect DNA slippage; also, at the concentration of

1.0, 10, 100, 200, and 500 μM, the aglycon **1** did not affect DNA slippage (see supporting information for the detail).
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