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Synthesis and NMR Binding Study of a Chiral Spirocyclic Helical Analogue of a Natural DNA Bulge Binder

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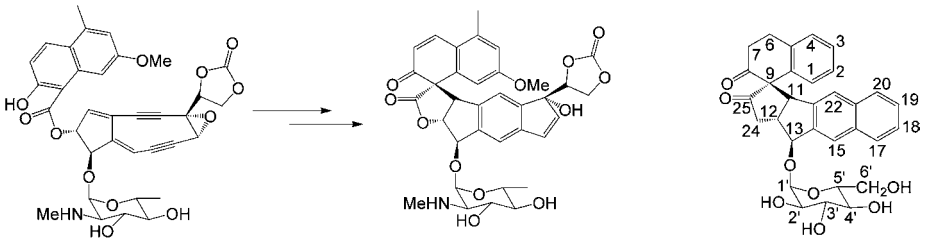
ABSTRACT



Synthesis of chiral spirocyclic helical compounds which mimic the molecular architecture of the potent DNA bulge binder obtained from the antitumor agent NCS-chrom has been accomplished. Structural analysis of the compounds by CD and NMR is presented. NMR titration study indicates binding of P,α-helimer (1d) at a two-base bulge site in a DNA oligomer, providing insight to the design of agents as specific probes of a bulged structure in nucleic acids.

The helix represents an important example of conformational chirality that is ubiquitous in the structure of biomolecules and is also observed in small molecules.[1] The conformational asymmetry in the host and/or guest molecule plays an important role in molecular recognition. One such example that we recently studied in our laboratory is wedge-shaped spirolactone **1**, formed in the base-catalyzed cyclo-aromatization of the neocarzinostatin chromophore (NCS-chrom), a potent antitumor antibiotic that has been shown to have very high and specific affinity for DNA containing a two-nucleotide bulge.[2] The prospect of selective recognition of a DNA bulge by a small molecule could have important therapeutic potential, as DNA-bulged structures have been shown to have significant biological roles.[3] Unique helix-shaped molecular geometry that fits tightly in the triangular prism binding pocket formed by the two looped out bases at the bulge site was found to be responsible for the activity of **1**. The orientation of ring systems, transposed approximately 60° by the spirolactone, and the right-handed ∼35° twist renders the molecule, **1**, a helical shape.[4] On the basis of spectroscopic and molecular modeling studies, it was concluded that the key features required for specific bulge

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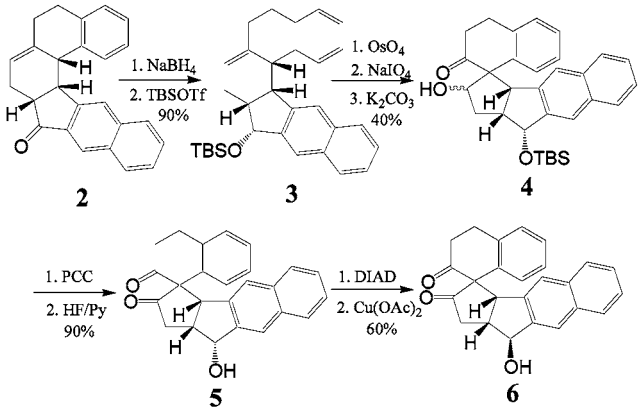
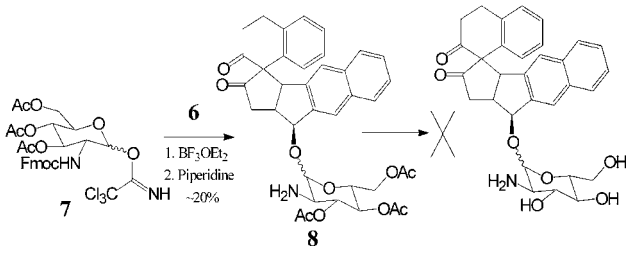
Scheme 1. Synthesis of Spirocyclic Diketo 13-Endo Alcohol Aglycon **6**



binding are (1) two independent aromatic π systems, (2) a spirocyclic ring junction capable of offsetting the systems by 30−40°, and (3) a pendant aminosugar moiety to enhance binding to the sugar phosphate backbone at the bulged site.[4] In our attempts to design and synthesize structural mimics of **1** with the potential of therapeutic use, we previously prepared a number of spirocyclic compounds.[5] Fluorescence binding and NMR structural studies showed that these compounds, unlike **1** which is a major groove binder, bind through the minor groove and are approximately 10 times less potent and selective toward the bulges.[5,6] The apparent reason for lower affinity is that the aminoglycoside moiety, unlike natural product **1,** is attached to C-25 of aglycon by a β-linkage rather than at C-13 by an α-glycoside linkage. In this regard, we pursued the synthesis of a stable structural mimic of **1**, a diketospirocyclic glycoside with a right-handed helix and sugar moiety attached at C-13 by α-glycoside linkage **1d**.

Diels−Alder product **2**, synthesized as described previously,[5] was reduced with $NaBH_4$, and the resulting alcohol was protected with TBSOTf to yield **3**. Oxidation of the double bond followed by base-catalyzed spiro-aldol cyclization gave hydroxyketone **4** in good yield, isolated as a mixture of the endo isomer accompanied by ∼35% of its exo isomer. The endo isomer was oxidized and further deprotected to yield spirocyclic diketo *endo*-alcohol, **5**. In a modified Mitsunobu reaction **5** was esterified with picolinic acid, and the resulting ester was cleaved under essentially neutral conditions using $Cu(OAc)_2$ and methanol to yield the desired spirocyclic diketo *exo*-alcohol[7] **6** (Scheme 1). Trichloroacetimidate **7**, obtained from the aminoglucoside, was coupled with **6**, and the products were subjected to Fmoc deprotection to give mixture of diastereomers **8** (data not shown). Unfortunately, all the attempts of further global

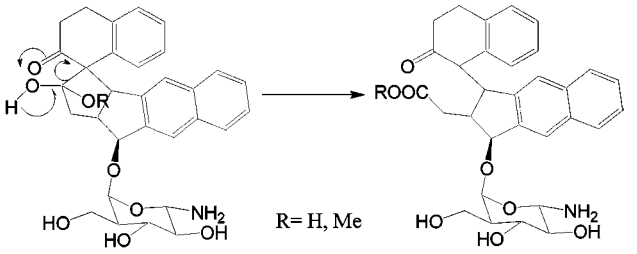
Scheme 2. Glycosylation of Aglycon **6** with Acetyl-Protected Aminoglycoside



acetyl deprotection using NaOMe, Et_3N, and Mg/MeOH were unsuccessful (Scheme 2).

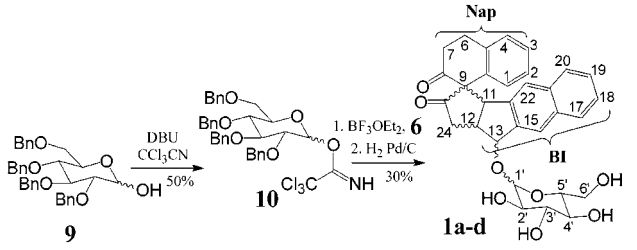
The probable reason is thought to be that the deacetylation reaction conditions catalyze the retroaldol-type ring opening (Scheme 3).

Scheme 3. Proposed Retroaldol-Type Ring-Opening Mechanism



To overcome this problem, we chose easily available benzyl-protected glycoside **9**. Trichloroacetimidate **10**, synthesized from **9**, was coupled with **6** in good yields. The global benzyl deprotection was easily carried out by reductive cleavage of the benzyl protecting groups using H_2 Pd/C[8] (Scheme 4). The crude products were purified on silica gel

Scheme 4. Glycosylation of Aglycon **6** Using Benzyl-Protected Glycoside



and by reversed-phase HPLC. HPLC chromatography indicated the presence of two peaks in a ∼70:30 ratio. ^{1}H NMR analysis of the separated peaks indicated the presence of a

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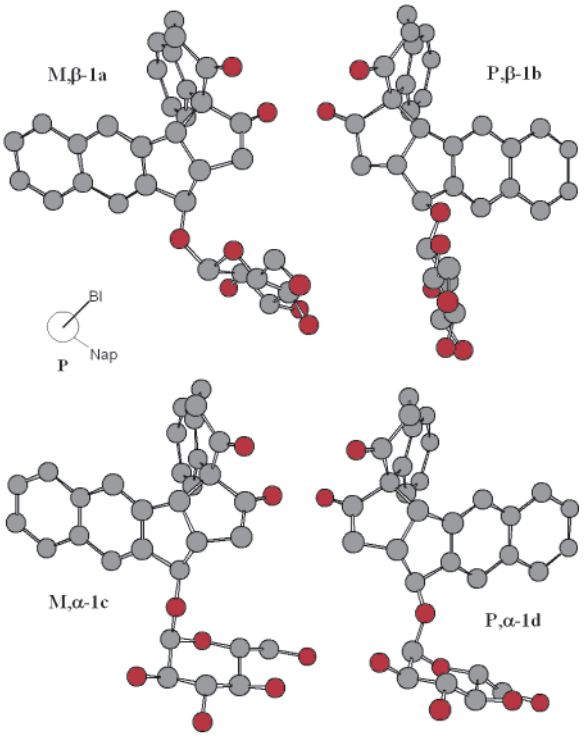

Figure 1. Energy-minimized (mm2) molecular structures of **1a**−**d**.

mixture that was further purified by chiral HPLC to yield two sets of helimers **1a**,[9] **1b**[10] and **1c**,[11] **1d**[12] (Figure 1). Structural assignments of these helimers were made using 2D NMR.

Chemical shifts of anomeric proton of **1a**, **1b**, **1c**, and **1d** were 4.54 (d, J = 7.0 Hz), 4.66 (d, J = 7.5 Hz), 5.09 (d, J = 3.78 Hz), and 5.19 (d, J = 3.66 Hz) ppm, respectively. NMR data for **1a** and **1b** are characteristic of β-anomeric protons and for **1c** and **1d** are of α. The handedness of the

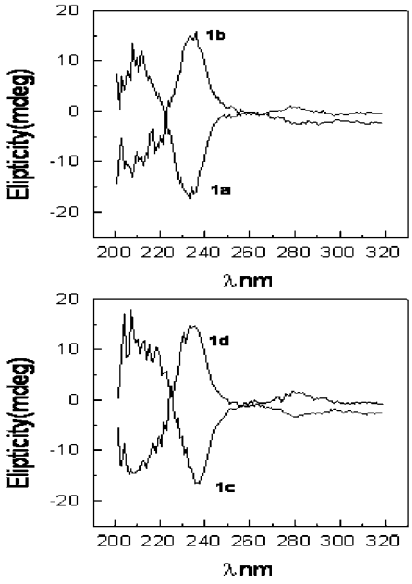

Figure 2. CD spectra of **1a**−**d**.

aglycon part of the helimers was determined by circular dichroism (CD) spectroscopy (Figure 2).

The CD spectrum of **1a**−**d** in acetonitrile displayed absorptions at 209, 235, and 280 (weak). Accordingly, the CD of **1a** and **1c** in acetonitrile showed a positive cotton effect (CE) at 209, 280 and a negative CE at 235. **1b** and **1d** showed positive CE at 235 and negative CE at 209, 280, which is complementary to **1a** and **1c**. All four helimers showed crossover at 223 and 269 nm. The peaks at 209, 235, and 280 nm are CEs associated with the corresponding π to π^* transitions in the UV spectra. The positive CD spectra for **1b** and **1d** suggests the helix with right-handedness and hence P conformation, while **1a** and **1c**, with left-handedness has M configuration.[1] On the basis of this analysis, it was confirmed that **1d** has a right-handed aglycon helix and the glucopyran is attached to it by an α-glycoside linkage, indicating that **1d** is an exact structural mimic of NCSi-gb, **1**. Therefore, we used **1d** for further binding studies with bulged DNA.

Since **1d** is weakly fluorescent, its binding to the two-base bulge-containing DNA oligomer (Figure 3) was better

(9) **1a**: ^{1}H NMR (CD_3CN) δ 8.04 (s, 1H, H-15), 7.94 (d, 1H, J = 8 Hz, H-17), 7.45−7.53 (m, 3H, H-18,19,20), 7.33−7.38 (m, 2H, H-3,4), 6.99 (t, 1H, H-2), 6.46 (d, 1H, J = 7 Hz, H-1), 6.21 (s, 1H, H-22), 5.39 (s, 1H, H-13), 4.81 (d, 1H, J = 7 Hz, H-11), 4.53 (d, 1H, J = 7 Hz, H-1′), 3.95 (d, 1H, J = 9 Hz, H-6′), 3.78 (dd, 1H, J = 4.5, 15.5 Hz, H-6′), 3.55 (m, 2H, H-6b,12), 3.45 (m, 1H, H-5′), 3.36 (m, 2H, H-3′,4′), 3.23 (m, 2H, H-2′,6a), 3.07 (m, 2H, H-7b,24b), 2.72 (ddd, 1H, J = 5.5, 6, 6.5 Hz, H-7a), 3.43 (dd, 1H, J = 8, 19 Hz, H-24a); ^{13}CNMR (CD_3CN) δ 215.76, 210.93, 147.74, 140.26, 137.59, 135.59, 131.82, 130.53, 128.74, 127.97, 127.82, 127.76, 127.55, 126.28, 126.14, 125.92, 125.75, 125.72, 100.91, 78.09, 76.58, 76.32, 71.78, 70.53, 61.85, 52.53, 45.49, 42.49, 37.86, 27.33, 24.52; MS calcd 553.1838, obsd 553.1878.

(10) **1b**: ^{1}H NMR (CD_3CN) δ 8.05 (s, 1H, H-15), 7.91 (d, 1H, J = 8.5 Hz, H-17), 7.52−7.39 (m, 4H, H-3,18,19,20), 7.32 (d, 1H, J = 8 Hz, H-4), 7.02 (t, 1H, J = 7.5 Hz, H-2), 6.48 (d, 1H, J = 8 Hz, H-1), 6.03 (s, 1H, H-22), 5.39 (s, 1H, H-13), 4.79 (d, 1H, J = 7 Hz, H-11), 4.66 (d, 1H, J = 7.5 Hz, H-1′), 3.86 (d, 1H, J = 10.5 Hz, H-6′), 3.69 (dd, 1H, J = 5.75, 15 Hz, H-6′), 3.49−3.58 (m, 2H, H-6b,12), 3.38−3.43 (m, 2H, H-4′,5′), 3.31 (t, 1H, J = 9 Hz, H-2′), 3.23 (ddd, 1H, J = 1.5, 6, 15.8 Hz, H-6a), 3.12 (dd, 1H, J = 8, 9 Hz, H-3′), 3.03−3.08 (m, 2H, H-7b,24b), 2.9 (bs, 1H, OH), 2.67−2.74 (m, 1H, H-7a), 2.28 (dd, 1H, J = 9, 19.5 Hz, H-24a); ^{13}C NMR (CD_3CN) δ 211.38, 208.86, 145.46, 139.87, 138.53, 135.41, 134.62, 130.65, 128.76, 127.94, 127.91, 127.83, 127.68, 127.66, 126.11, 126.08, 125.88, 125.77, 107.79, 77.28, 76.36, 75.57, 73.74, 71.63, 61.06, 55.11, 52.12, 44.06, 37.72, 27.28, 24.55; MS calcd 553.1838, obsd 553.1902.

(11) **1c**: ^{1}H NMR (CD_3CN) 7.97 (s, 1H, H-15), 7.87 (d, 1H, J = 8.2 Hz, H-17), 7.45 (m, 2H, H-18,20), 7.40 (t, 1H, J = 7.88 Hz, H-3), 7.34 (t, 1H, J = 7.41 Hz, H-19), 7.29 (d, 1H, J = 7.88 Hz, H-4), 6.97 (t, 1H, J = 7.73 Hz, H-2), 6.43 (d, 1H, J = 7.57 Hz, H-1), 6.07 (s, 1H, H-22), 5.20 (s, 1H, H-13), 5.09 (d, 1H, J = 3.78 Hz, H-1′), 4.77 (d, 1H, J = 7.25 Hz, H-11), 3.83 (bd, 1H, J = 8.83 Hz, H-6′), 3.72 (bd, 2H, J = 8.51 Hz, H-6′,-12), 3.61 (m, 1H, H-3′), 3.50 (m, 2H, H-2′,5′), 3.31 (m, 2H, H-4′,6b), 3.17 (m, 1H, H-6a), 2.99 (m, 2H, H-7b, 24b), 2.65 (m, 1H, H-7a), 2.30 (dd, 1H, J = 8.51, 19.0 Hz, H-24a); MS calcd 553.1838, obsd 553.1.

(12) **1d**: ^{1}H NMR (CD_3CN) δ 7.99 (s, 1H, H-15), 7.87 (d, 1H, J = 8.24 Hz, H-17), 7.43 (m, 4H, H-3,18,19,20), 7.27 (d, 1H, J = 8.24 Hz, H-4), 6.99 (t, 1H, J = 7.62 Hz, H-2), 6.45 (d, 1H, J = 7.94 Hz, H-1), 5.95 (s, 1H, H-22), 5.25 (s, 1H, H-13), 5.19 (d, 1H, J = 3.66 Hz, H-1′), 4.79 (d, 1H, J = 7.02 Hz, H-11), 3.77 (bd, 1H, J = 11.6 Hz, H-6′), 3.63 (dd, 1H, J = 5.49 Hz, H-6′), 3.56 (m, 1H, H-3′), 3.46 (m, 2H, H-5′, 12), 3.35 (m, 2H, H-2′,4′), 3.26 (t, 1H, J = 9.0 Hz H-6b), 3.19 (m, 1H, H-6a), 3.01 (m, 2H, H-7b, 24b), 2.66 (m, 1H, H-7a), 2.18 (dd, 1H, J = 10.68, 20 Hz, H-24a); MS calcd 553.1838, obsd 553.1.

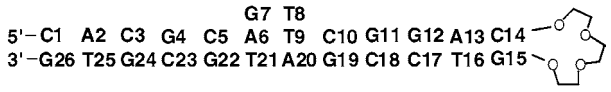

Figure 3. Structure of the bulged DNA oligomer.

monitored by 1D NMR spectroscopy in 10 mM phosphate buffer, pH 8, at 25 °C. The ^{1}H NMR spectra of the nonexchangeable protons of **1d**, free DNA oligomer, and **1d**−DNA complex are given in Figure 4A, B, and C, respectively. The formation of the **1d**−DNA complex is indicated by the changes in the chemical shifts of several residues upon addition of the **1d**. The effect is most evident for the methyl and aromatic base protons.

Assignments of the several proton resonances of the free DNA oligomer and **1d**−DNA complex were obtained by analysis of NOESY and TOCSY spectra. The peaks for the methyl protons of T8 and T21 that are either bulge residues or adjacent to them are downfield shifted upon complex formation, whereas the methyl protons for T16 and T25 that are residues far from the bulge site remain unchanged in the chemical shift. In addition, the base proton for A20H8 adjacent to bulge residues is shifted to the downfield region upon complex formation, as indicated in Figure 4. The protons of the DNA residues that are subjected to chemical shift changes by the binding of **1d** are similar to those by the binding of natural product, but the magnitudes of the chemical shift changes in the **1d**-bulged DNA complex are smaller than those in the natural product−bulge DNA complex, indicating that **1d** binds to the bulge site of DNA but the induced DNA conformational changes upon complex formation are different from those of the natural product. Similar studies with **1a**, which has the least favored conformation, did not show significant chemical shift changes in **1a**−bulged DNA complex (data not shown).

In conclusion, novel spirocyclic helimer analogues of cycloaromatization products of NCS-chrom have been synthesized. P,α-helimer(**1d**), which is a structural mimic of **1**, binds to bulged DNA at the bulge site. These results

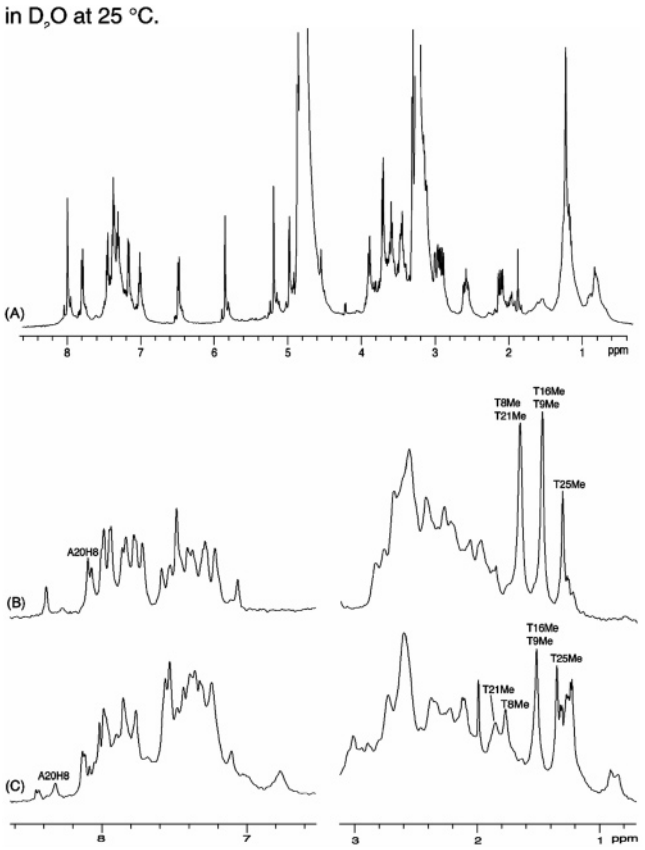

Figure 4. ^{1}H NMR spectra of (A) **1d** in CD_3OD, (B) free bulged DNA oligomer, and (C) the **1d**−bulged DNA complex in D_2O at 25 °C.

shed light on the design of small molecules as specific probes of bulged structures in nucleic acids.

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Supporting Information Available: Experimental material and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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