ORGANIC LETTERS

1999
Vol. 1, No. 9
1375−1377

# Synthesis and DNA Binding of Spirocyclic Model Compounds Related to the Neocarzinostatin Chromophore

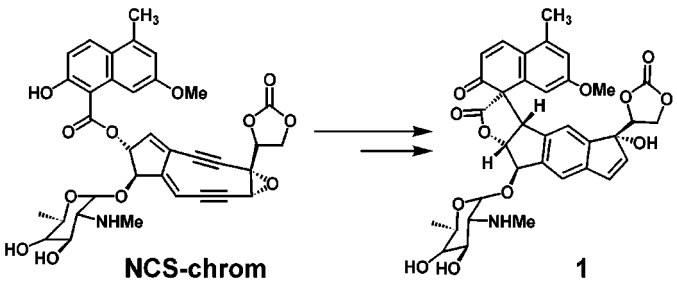
**Zhen Xi,[†] Graham B. Jones,*[,‡] Ghassan Qabaja,[‡] Justin Wright,[‡] Francis Johnson,[§] and Irving H. Goldberg*[,†]**

*Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115, Bioorganic and Medicinal Chemistry Laboratories, Department of Chemistry, Northeastern University, Boston, Massachusetts 02115, and Department of Pharmacological Sciences, State University of New York, Stony Brook, New York 11794*

*grjones@lynx.neu.edu*

**Received August 10, 1999**

## ABSTRACT




**Spirocyclic model compounds which mimic the molecular architecture of one of the decomposition products of the antitumor agent NCS-chrom have been synthesized. These readily accessible molecules bind with remarkable efficiency to bulged DNA oligonucleotides, offering potential for the design of therapeutic agents.**

The discovery of the enediyne class of antitumor antibiotics has stimulated considerable interest in both the biological and chemical community because of their unprecedented chemical composition and exceptional biological profiles.[1] The enediynes themselves are prodrugs which exert many of their biological effects via the generation of chemically reactive diyl radicals. In the case of the earliest enediyne to be discovered, NCS-chrom, it has been determined that one of the base-catalyzed cycloaromatization products is spirolactone **1** and that this product shows remarkable affinity for DNA containing a two-nucleotide bulge.[2] DNA bulges have been implicated as binding motifs for regulatory proteins and as targets for repair enzymes, rendering them potentially important therapeutic targets.[2a] In an effort to study these interactions in greater detail, we decided to pursue the chemical synthesis of accessible analogues of **1** in order to identify the minimum effective pharmacophore capable of recognizing these DNA targets. A key feature of **1** which is presumed to play a vital role in DNA recognition is the right-handed helical twist imposed by the spirocyclic junction. Scrutinizing this parameter using semiempirical PM3 calculations with a range of potential structural isosteres suggested that a simple mimic of this system is afforded by spiro-alcohol **2**. The candidacy of **2** was further strengthened

[†] Harvard Medical School.
[‡] Northeastern University.
[§] State University of New York.

(1) Xi, Z.; Goldberg, I. H. In *Comprehensive Natural Products Chemistry*; Barton, D. H. R., Nakanishi, K., Eds.; Elsevier Science, Oxford, 1999; Vol. 7, 553−592.

(2) (a) Stassinopoulos, A.; Ji, J.; Gao, X.; Goldberg, I. H. *Science* **1996**, *272*, 1943−1946. (b) Xi, Z.; Mao, Q.; Goldberg, I. H. *Biochemistry* **1999**, 38, 4342−4354.

10.1021/ol9909280 CCC: $18.00 
**Published on Web 10/03/1999**

by retrosynthetic analysis, where an aldol-type disconnection leads to enolate **3** (Figure 1). Functional group transforma-

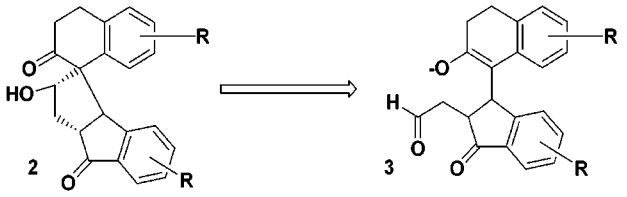
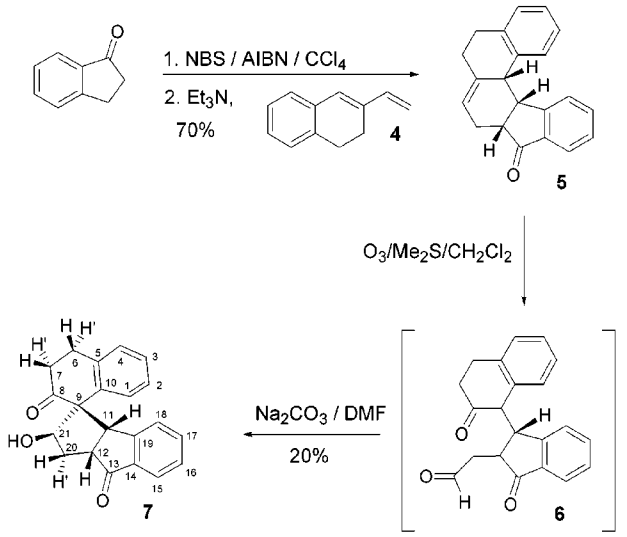


**Figure 1.** Retrosynthetic analysis of spirocyclic mimic.

tions applied to **2** would offer access to a wide variety of analogues and also provide a platform to modulate stereochemical bias.

Accordingly, the synthesis of model compound **7** was initiated from indanone (Scheme 1). Benzylic bromination

**Scheme 1.** Synthesis of Pentacyclic Model Compound




followed by in situ elimination allowed Diels−Alder cycloaddition with diene **4**, in turn prepared from acetyl tetralone,[3] giving pentacyclic alkene **5** in moderate yield.[4] Ozonolytic cleavage produced aldehyde **6**, which was subjected to enolizing conditions. Spiro-alcohol **7**, the result of a 5-*exo*-trig attack on the aldehyde, was recovered in moderate yield from a complex mixture. Structural assignment of this unusual spirocycle was made using 2D NMR, which confirmed the *syn* stereochemistry about the 5,5 ring junction and also indicated an *endo* configuration between the two aromatic systems. The hydroxyl group at C21 is assigned *exo* to the ring system (*syn* to the carbonyl group at C8) on the basis of the observed strong ROSEY interaction between H-1 and H-21.[5] Preliminary evaluation of **7** binding to DNA containing a two-nucleotide bulge using competition experiments against **1** revealed a $K_d$ of 50 $\mu$m (Table 1).[6]

**Table 1.** Affinity of NCS-Chrom Cascade Mimics for Bulged DNA Sequences

| compd | excitation (nm) | emission (nm) | bulge[b] $K_d$ ($\mu$m) | duplex[c] $K_d$ ($\mu$m) |
|---|---|---|---|---|
| **1** | 390 | 500 | 2.2 | 307 |
| ±-**7** | nd[a] | nd[a] | ≈50[a] | |
| ±-**13** | 310 | 450 | 25 | 90 |
| *ent*$_1$-**13** | 310 | 450 | 35 | 89 |
| *ent*$_2$-**13** | 310 | 450 | 17 | 90 |
| ±-**12** | 310 | 450 | >500 | |

[a] Competition assay against **1**.
[b] Bulged DNA: **5'-GTCCGATGCGTG-3'** / **3'-CAGGCTACGCAC-5'** / **TG**
[c] Duplex DNA: **5'-GTCCGATGCGTG-3'** / **3'-CAGGCTACGCAC-5'**

Unfortunately, inadequate fluorescence precluded quantitative analysis using conventional protocols, thus necessitating the introduction of additional chromophores to the molecule.

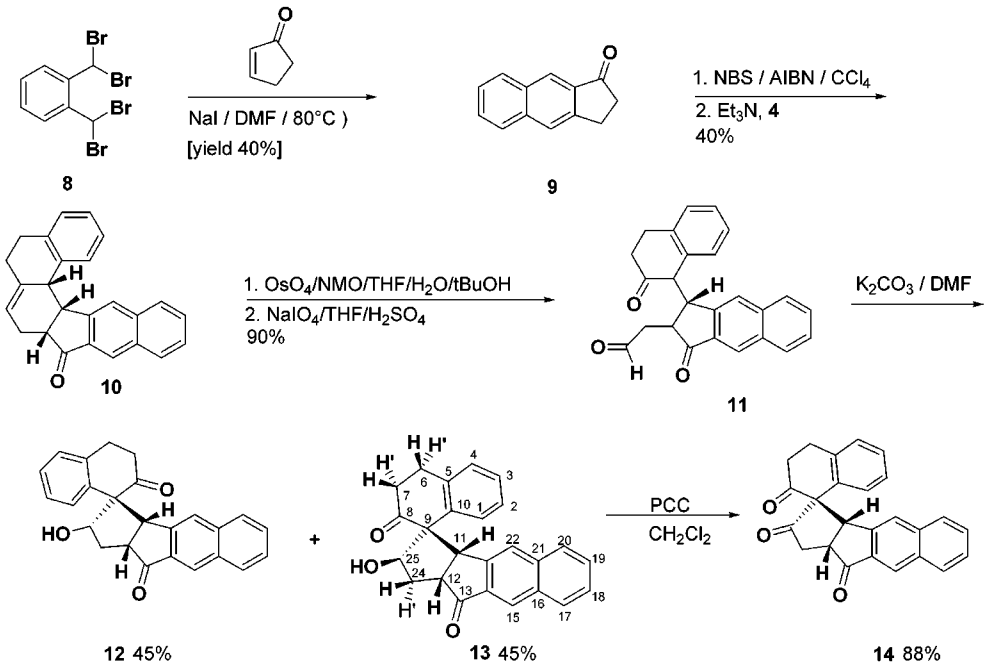
(3) Minuti, L.; Taticchi, A.; Gacs-Baitz, E.; Marrocchi, A. *Tetrahedron* **1995**, *51*, 8953−8958.

(4) All new compounds gave appropriate spectroscopic and analytical data. **7:** $^1$H NMR (300 MHz, $CDCl_3$) 7.70 (d, 7.2 Hz, 1H, H-15), 7.29 (d, 7.5 Hz, 1H, H-4), 7.28 (t, 7.2 Hz, 1H, H-16), 7.21 (dt, 7.5 Hz, 1.2 Hz, 1H, H-3), 7.16 (dt, 7.5 Hz, 1.5 Hz, 1H, H-2), 6.95 (t, 7.2 Hz, 1H, H-17), 6.48 (d, 7.5 Hz, 1H, H-1), 6.14 (dd, 7.5 Hz, 0.9 Hz, 1H, H-18), 4.29 (dd, 11.1 Hz, 7.5, Hz, 1H, H-21), 4.23 (d, 7.2 Hz, 1H, H-11), 3.43 (ddd, 5.7 Hz, 12.6 Hz, 15.9 Hz, 1H, H-6), 3.21 (ddd, 2.1 Hz, 7.5 Hz, 11.1 Hz, 1H, H-12), 3.15 (ddd, 2.4 Hz, 6.6 Hz, 15.9 Hz, 1H, H-6′), 2.97 (ddd, 2.4 Hz, 5.7 Hz, 16.5 Hz, 1H, H-7), 2.80 (s, 1H) OH, 2.77 (ddd, 6.6 Hz, 12.6 Hz, 16.5 Hz, 1H, H-7′), 2.62 (dt, 11.1 Hz, 12.9 Hz, 1H, H-20), 2.51 (ddd, 2.1 Hz, 7.5 Hz, 12.9 Hz, 1H, H-20′). $^{13}$C NMR: 213.8 (C-13), 208.2 (C-8), 152.7 (C-14), 138.4 (C-5), 137.7 (C-19), 135.4 (C-10), 134.2 (C-2), 128.4 (C-1), 128.2 (C-16), 128.1 (C-4), 127.9 (C-18), 127.6 (C-3), 126.7 (C-17), 123.6 (C-15), 76.4 (C-21), 63.4 (C-9), 48.4 (C-12), 47.2 (C-11), 38.7 (C-7), 34.0 (C-20), 28.7 (C-6). All assignments were supported by 2D NMR analysis such as COSY, ROSEY, HMBC, and HMQC. MS: calcd for $C_{21}H_{18}O_3$ 318; found *m/e* 319.18 $(M + H)^+$, 307.11, 289.12, 154.31, 136.32. **13:** $^1$H NMR (500 MHz, $CDCl_3$) 8.28 (s, 1H, H-15), 7.93 (m, 1H, H-17), 7.46 (m, 2H, H-18, H-20), 7.37 (t, 1H, H-3), 7.34 (ddd, 1H, H-4), 7.25 (t, 7.25 Hz, 1H, H-2), 6.87 (t, 8 Hz, 1H, H-19), 6.55 (d, 7.5 Hz, 1H, H-1), 6.43 (s, 1H, H-22), 4.41 (d, 8 Hz, 1H, H-11), 4.33 (ddd, 1H, H-25), 3.58 (ddd, 6.5 Hz, 12.5 Hz, 16 Hz, 1H, H-6), 3.36 (ddd, 1H, H-12), 3.32 (ddd, 2.0 Hz, 12.5 Hz, 1H, H-6′), 3.06 (ddd, 2.0 Hz, 6.0 Hz, 16.5 Hz, 1H, H-7), 2.82 (ddd, 12.5 Hz, 1H, H-7′), 2.75 (s, 1H, OH), 2.74 (dd, 11.5 Hz, 12.5 Hz, 1H, H-24), 2.60 (ddd, 1.5 Hz, 7.5 Hz, 1H, H-24′). $^{13}$C NMR: 213.9, 208.5, 145.1, 138.6, 136.9, 135.5, 135.0, 132.6, 130.3, 128.8, 128.7, 128.3, 128.2, 127.9, 127.1, 127.0, 126.9, 124.5, 75.3, 64.6, 48.0, 46.5, 38.6, 35.2, 28.0. MS: calcd for$C_{25}H_{20}O_3$ 368; found *m/e* 369.2 $(M + H)^+$, 223.1, 195.1, 147.1, 99.1; calcd for $C_{25}H_{20}O_3$ 368.1412, found 368.1411.

(5) The highly unusual upfield shift of the H-1 (6.48 ppm) and H-18 (6.14 ppm) of **7** owing to the anisotropic effect of the two interacted aromatic rings, the observed COSY interaction between H-11 and -12, and the ROSEY interaction between H-1 and H-21 (strong), H-18 and H-11 (weak), H-11 and H-6 (strong), and H-11 and H-7 (weak) aided in the assignment of the stereochemistry of **7** as shown in Scheme 2. The stereochemistry in **13** was assigned similarly.

(6) Fluorescence quenching studies were carried out using SPEX Fluoro Max-2 at 5 °C in a 10 mM phosphate buffer (pH 7.5). Dissociation constant $K_d$ was derived from curve-fitting with Kaleidagraph, using the equation $i/i_0 = 1 + (\Delta i/2i_0)*([T_0] + [DNA] + K_d - (([T_0] + [DNA] + K_d)^2 - 4*[T_0][DNA])^{1/2}$, wherein $[T_0]$ is the initial concentration of the fluorescent probe, $i$ is the intensity of the sample, $i_0$ is the initial intensity of the sample, [DNA] is the concentration of the DNA, and $\Delta i$ is the total change in intensity per drug unit from the free state to the total binding state. The competition assay against **1** was used for the binding assay of **7**. The release of the bulge DNA-bound **1**, which is fluorescent (excitation at 390 nm, emission at 500 nm), was monitored by addition of **7**.

**Scheme 2.** Synthesis of Enhanced Model Compounds




After considering various strategies, it was decided to extend conjugation on the lower portion and pursue a naphthyl indanone analogue. Accordingly, ketone **9** was prepared by intermolecular cycloaddition of orthoquinodimethane (generated in situ from α,α,α′α′-tetrabromotoluene **8**) with cyclopentenone (Scheme 2). The ketone was elaborated into the corresponding hexacycle **10** by the previously established chemistry (Scheme 2), and methods for formation of required keto aldehyde **11** were investigated. Though ozonolysis followed by reductive workup did produce quantities of **11**, yields were variable, and the reaction did not scale-up well. These problems were overcome by using osmylation followed by oxidative cleavage, giving the desired product in excellent yield. Base-induced spiro-aldolization was then performed, which gave compounds **12** and **13** in good yields. As envisaged, the additional chromophore permitted quantitative analysis when subjected to assay using bulged DNA (Table 1).

In the case of **13**, the affinity of the racemate was comparable to that of **1**, suggesting that the geometry of the spirocyclic moiety mimics that of spirolactone **1** effectively. Separation of the enantiomers of **13** (C11/12) was possible using chiral HPLC, and the second eluting enantiomer was found to be a more effective DNA binder than the first, supporting a subtle stereochemical component to the interaction.[7] Oxidation of the racemate gave the corresponding triketone **14**, which should now allow a range of additional analogues of this readily accessible system to be explored.

In summary, a novel spirocyclization reaction has been applied to the synthesis of analogues of the cycloaromatization products of NCS-chrom. The designed species interact with bulged DNA at a remarkably low concentration, providing a readily available tool for the study of these important DNA microenvironments and throwing further light on the rich chemistry of the NCS family of enediynes.

**Acknowledgment.** We thank the NIGMS (RO1GM57123 to G.B.J. and RO1GM53793 to I.H.G.) for generous support of this project.

OL9909280

(7) Daicel OD column (20 cm × 4.5 cm) 10% IPA:90% hexanes eluent; 3 mL/min flow rate. $t_R(ent_1) = 20.5$ min, $t_R(ent_2) = 27.0$ min.