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Convenient Synthesis of NCS–Chromophore Metabolite Isosteres: Binding Agents for Bulged DNA Microenvironments

Yiqing Lin[†] and Graham B. Jones*

Bioorganic and Medicinal Chemistry Laboratories, Department of Chemistry and Chemical Biology, Northeastern University, Boston, Massachusetts 02115

Geum-Sook Hwang,[†] Lizzy Kappen, and Irving H. Goldberg

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachussetts 02215

gr.jones@neu.edu

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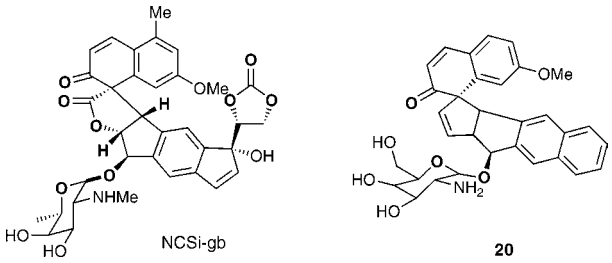
ABSTRACT



A designed molecule with capacity to bind DNA bulges (20) has been prepared from readily available starting materials. The spirocyclic template was modeled on a metabolite of neocarzinostatin chromophore (NCSi-gb) and is equipped with functionality to enable convenient bioassay. Preliminary studies confirm binding at specific bulged sequences and induction of polymerase-mediated slippage events. The target compound offers a convenient means to study affinity for unique bulged motifs and for use as a molecular biology reagent.

Enediyne antibiotics have been the focus of intense research over the past decade due to their observed antitumoral activity and unique modes of action.[1] Clinical candidates have now emerged fueling additional interest in the molecular mechanisms of this intriguing class, whose activity is derived in part from an ability to induce strand breaks in duplex DNA.[2] Recent studies on the neocarzinostatin chromophore [NCS-chrom], however, have revealed an alternate target and mode of action.[3] Specifically, the NCS-chrom phenoxide ion has been shown to undergo a general base-catalyzed spirolactonization and intramolecular rearrangement to eneyne allene **1**, whose biological target is bulged DNA as opposed to the duplex form.[3] Following cycloaromatization of **1**, which proceeds via diradical intermediates capable of cleaving bulged DNA, metabolite NCSi-gb is formed. Molecular studies have revealed that the affinity of NCSi-gb to bind DNA bulges is dependent on numerous features, including the spirocyclic ring junction, pendant aminosugar group, and two discrete aromatic moieties for π-stacking.[3] Since bulged

* Fax: 617-373-8795.
[†] These authors contributed equally to this work.

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(3) Stassinopoulos, A.; Ji, J.; Gao, S.; Goldberg, I. H. *Science* **1996**, *272*, 1943−1946. Kappen, L. S.; Goldberg, I. H. *Science* **1993**, *261*, 1319−1321. Xi, Z.; Mao, Q. K.; Goldberg, I. H. *Biochemistry* **1999**, *38*, 4342−4354.

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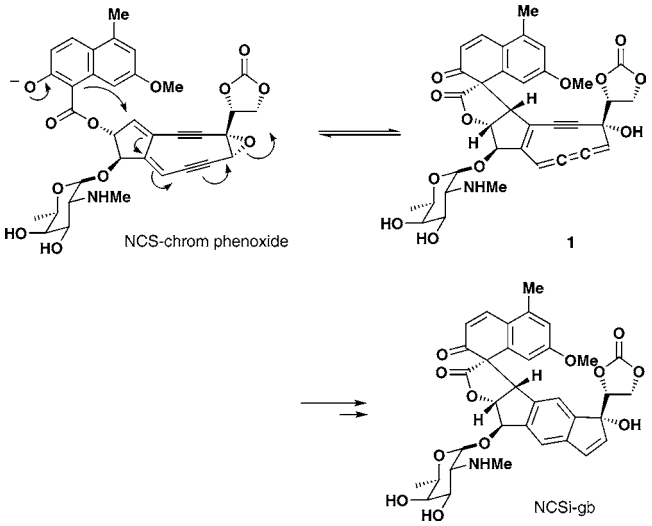
structures in nucleic acids are of general biological significance (proposed as intermediates in RNA splicing, frameshift mutagenesis, intercalator-induced mutagenesis, and imperfect homologous recombination and as binding motifs for regulatory proteins),[4] designed small molecules with bulge affinity have become sought after targets.



Following qualitative SAR analysis of NCSi-gb, we designed and synthesized spiro alcohol **2**, the first lead compound for library design.[5] Crystallographic analysis of the core supported the design philosophy, the molecule adopting the wedge-shape necessary to achieve binding to bulges (Figure 1). Simple aminosugar derivatives of the spirohydroxy group showed good affinity for bulged sequences, but a number of shortcomings led us to refine our design criterion viz. **2**.[5] Specifically, we desired (1) additional functionality to permit (fluorescence) binding studies, (2) installation of the aminosugar in the appropriate location, and (3) a more thermodynamically stable platform, as the spiro alcohol was prone to retro-aldol reaction on decomposition. With these factors in mind, a synthesis was devised to a functional core. 7-Methoxy tetralone was acetylated (**3**) and then converted to diene **4** (Scheme 1).[6] Intermolecular cycloaddition with dienophile **5** (produced from tetrabromo-*o*-xylene) gave adduct **6** in good yield. Reduction of the keto group at the locus of the desired aminosugar (**7**) and subsequent inversion and masking (**8**) allowed us to use previously reported oxidative cleavage followed by aldol chemistry to form spirocycles **9** for subsequent manipulation.[5]

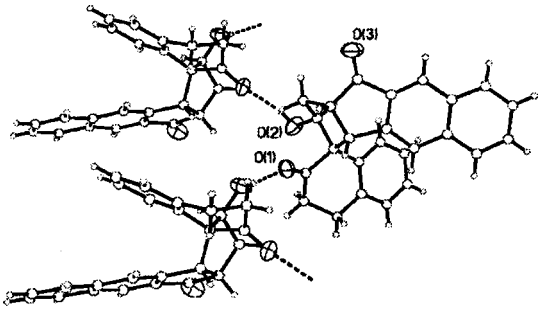
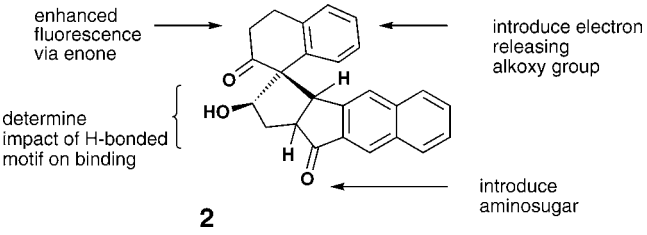
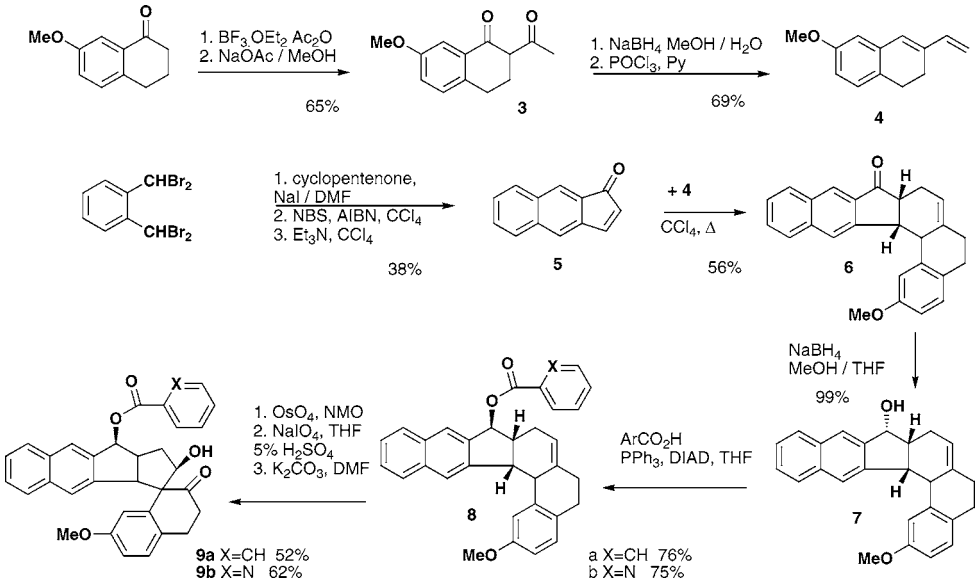


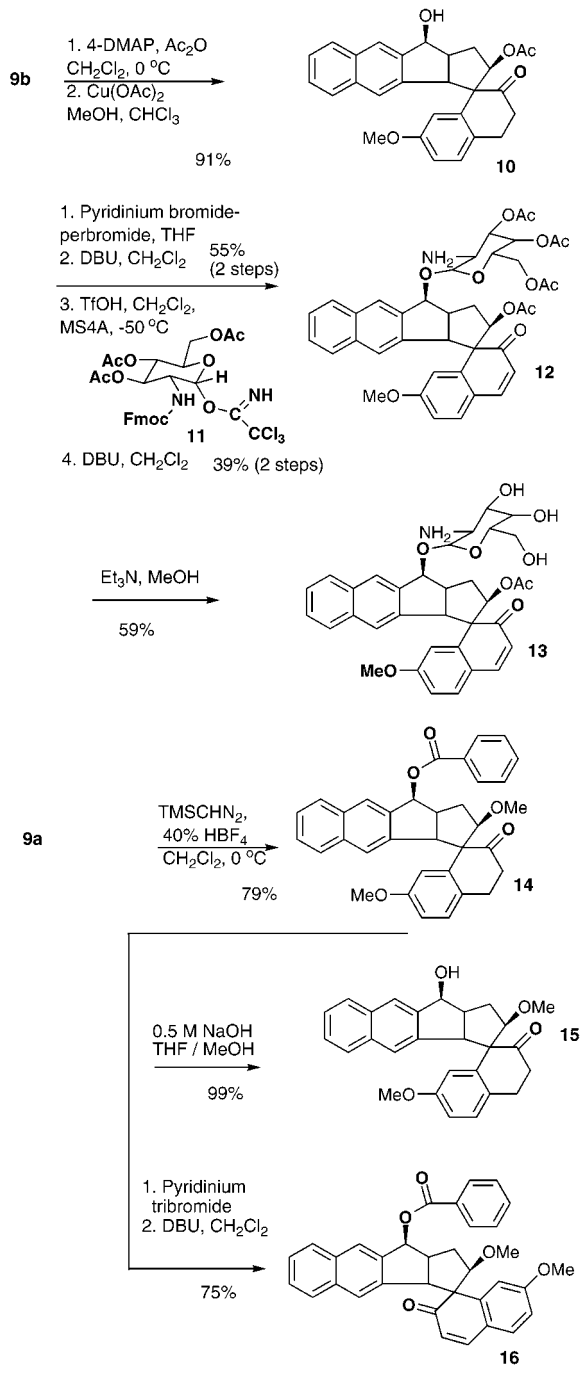
Figure 1.



Scheme 1. Preparation of Multiply Substituted Spirocyclic Building Blocks

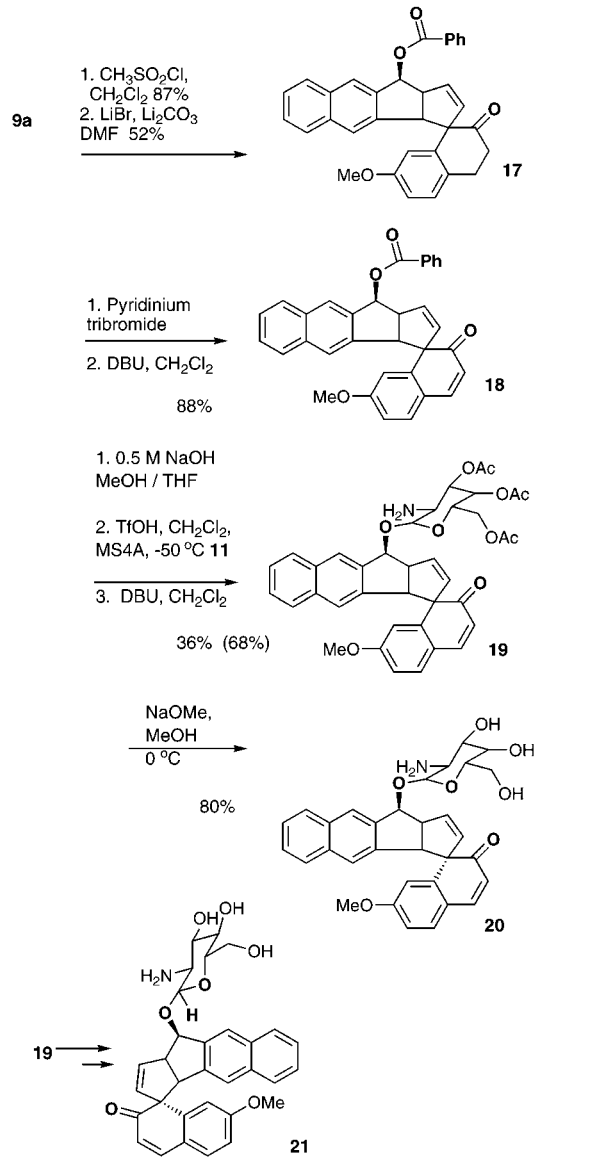


Scheme 2. Attempted Routes to Aminoglycosylated Enone Core



We initially elected to mask the spirohydroxy group as the corresponding acetate, which allowed liberation of the benzylic alcohol (**10**) (Scheme 2). Installation of the desired enone group proceeded smoothly, as did aminoglycosylation with **11**[7] to produce advanced substrate **12**. Though the Fmoc group was removed without incident, all attempts to isolate the product from global deacetylation failed, resulting in isolation of **13** and associated decomposition products.

Scheme 3. Alkene Variant to Aminoglucosyl Enone



Thwarted by this obstacle, an alternate masking group was introduced. Specifically, **9a** was methylated to **14**, which allowed unmasking of the benzoate ester to give **15**; however, all attempts to brominate α to the ketone resulted in decomposition. Suspecting that the free benzyl alcohol was playing a role, we investigated enone generation on the masked substrate. However, in all cases the only product isolated was *inverted* stereoisomer **16**, suggesting retro-aldol reaction followed by reclosure onto a methylketonium ion.[8]

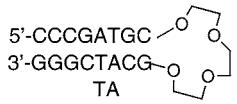
(4) Singer, R. H. *Science* **1998**, *280*, 696−697. Perutz, M. F. *Curr. Opin. Struct. Biol.* **1996**, *6*, 848−858.

(5) Xi, Z.; Hwang, G.-S.; Goldberg, I. H.; Harris, J. L.; Pennington, W. T.; Fouad, F. S.; Qabaja, G.; Wright, J. M.; Jones, G. B. *Chem. Biol.* **2002**, *9*, 925−931.

(6) Satisfactory spectroscopic and analytical data were obtained for all new compounds.

(7) Produced from α-aminoglucose: FmocCl, $NaHCO_3$, dioxane, water, 1 h, and then Ac_2O, Py, 12 h (91%); N_2H_4, HOAc, DMF, 50 min, and then Cl_3CCN, DBU, CH_2Cl_2, 45 min (28%). Compound **12** was isolated as a single diastereomer following separation after glycosylation. For coupling methodology, see: Schmidt, R. R.; Michel, J. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 731−732.

Table 1. Dissociation Constant (μM) of Spirocyclic Alkenes **20** for Selected Sequences[a]

		NCSi-gb	**20**	**21**
1	5'-GTCCGATGCGTG 3'-CAGGCTACGCAC	307	~100	
2	5'-GTCCGATGCGTG T 3'-CAGGCTACGCAC T T	10	9.55	13.5
3	5'-GTCCGATGCGTG 3'-CAGGCTACGCAC TG	2.18	3.18	8.55
4	5'-GTCCGATGCGTG T 3'-CAGGCTACGCAC T TG	0.033	0.55	1.81
5	5'-GTCCGATGCGTG T 3'-CAGGCTACGCAC T G	20.6	7.71	
6		0.081	0.84	
7	5'-GTCCGTTGCGTG T 3'-CAGGCAACGCAC T TG	22.2	1.84	

[a] Fluorescence quenching studies conducted using a SPEX Fluoromax-2 at 4 °C in phosphate buffer (10 mmol, pH 7.0). Emission spectra of **20** and **21** were obtained in the range 400−600 nm upon excitation at 385 nm. Emission reading at 490 nm was imported in binding calculation. Dissociation constant (k_d) was derived from curve-fitting (Kaleidagraph).[5]

Prompted by the retro-aldol issue, we made a decision to investigate the effects of eliminating the spiro alcohol group altogether. Though the crystal structure of **2** suggests that hydrogen bonding of this group contributes to secondary structure, the wedge-shaped template should persist on the basis of the spiro junction. Accordingly, elimination of the alcohol from **9a** to give alkene **17** was effected (Scheme 3). Conversion to enone **18** was uneventful as expected, and saponification followed by aminoglycosylation gave **19** and its stereoisomer in good yield. At this point, diastereomer resolution of stereoisomers at the spiro junction was possible, the desired isomer subjected to deprotection to give NCSi-gb mimic **20**. To provide a convenient control substrate, the spiro-inverted stereoisomer of **19** was subjected to identical conditions to give substrate **21** (75%). A preliminary analysis of binding against a panel of bulged oligonucleotides confirmed that **20** is as effective as other aminoglycosylated versions of **2** and in some cases is superior to NCSi-gb itself (Table 1).[9] The need for right-handed twist in order to satisfy molecular recognition in the bulged environment is evident by comparison to stereoisomer **21** (Table 1, entries 2−4). The results confirm that a hydrogen bonding group is not required at the spiro junction, greatly simplifying synthetic routes to this class of agent. Indeed, for some substrates the affinity of **20** is superior to the natural product metabolite (entries 5 and 7), suggesting adoption of unique architectures. Given the ease of access to **20**, we became interested to gauge effectiveness as a molecular biology tool. Bulged structures have been proposed as intermediates in DNA slippage synthesis involving the extension of nucleotide repeats. Given the possible involvement of the slippage process in the progression of certain neurological disorders (e.g., CAG repeats in Huntington's disease[10]), a readily available reagent capable of inducing slippage may have a number of uses. Accordingly, **20** was subjected to a slippage assay, which measures ^{32}P TMP uptake for DNA slippage synthesis dependent on primers/templates bearing simple sequence repeats, catalyzed by the Klenow fragment of DNA polymerase I. The results (Table 2) confirm that **20** can significantly promote slippage within the 5 h assay with >10-fold induction at 30 μM.[11]

Table 2. Effect of **20** on DNA Slippage Synthesis[a]

	^{32}P incorporated (CPM)	stimulation
control	38 664	
20 (10 μM)	89 101	2.3
20 (30 μM)	511 667	13.2

[a] DNA slippage synthesis was measured using the primer/template oligomers of thymidylate (20-mer) and deoxyadenylate (30-mer) in a reaction catalyzed by the Klenow fragment of DNA polymerase I.[10] The annealed mixture of T20/A30 (2 μM) in 50 mM Tris−HCl pH 7.5 was supplied with 5 mM magnesium, 4 mM dithiothreitol, 1 mM dATP, 0.5 mM TTP, and 1.5 μCi [α- ^{32}P]TTP before starting the synthesis by the addition of enzyme. Test compound was present from the beginning of the reaction. After incubation at 37° for 5 h. ^{32}P-TMP incorporated into acid-precipitable DNA products was determined, and data were reported relative to control.[11]

In summary, a versatile and efficient route to mimics of the natural product metabolite NCSi-gb has been developed. The lead molecule, available in eight steps from a readily available template, shows submicromolar and selective binding to bulged DNA targets and induces slippage at relevant concentrations, allowing for rational drug design to address this key molecular biology target. Of potential significance is the synthetic versatility of the vinyl group in **20**, which will allow design and assembly of diverse libraries for screening.[12]

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

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(8) Attemps to isolate the intermediates in this cascade were unsuccessful.

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