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# Development of new simple molecular probes of DNA bulged structures

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**Abstract**—NCSi-gb is a neocarzinostatin chromophore (NCS-chrom) metabolite which binds strongly to certain two-base DNA bulges. Compared with previously reported NCSi-gb analogues, a new analogue with a different aminoglycoside position was synthesized, and it showed strong fluorescence and improved binding and sequence selectivity to DNA bulges. The N-dimethylated form of this analogue had a similar binding pattern, and it competitively inhibited bulge-specific cleavage by NCS-chrom.


Bulged structures of nucleic acid are involved in many biological processes.[1] They were proposed as intermediates in RNA splicing, frame-shift mutagenesis, intercalator-induced mutagenesis, and imperfect homologous recombination.[2] They were also implicated as binding motifs for the regulatory proteins involved in viral replication including the TAR region of HIV-1.[3] Therefore, bulge-binding agents could have important therapeutic potential. So far, NCSi-gb, a wedge-shaped spirolactone containing molecule (Fig. 1), is the most promising binding agent for DNA containing a two-base bulge.[4] It was produced from the spontaneous cyclization of NCS-chrom, an enediyne antitumor antibiotic, in the absence of DNA and thiols.[5] The NMR structure of the complex containing a DNA two-base bulge and NCSi-gb has been solved.[6] Binding of NCSi-gb to the DNA two-base bulge involves major groove recognition by the drug aminoglycoside moiety and tight fitting of the wedge-shaped aglycon moiety in the triangular prism pocket formed by the two looped-out bulge bases and the neighboring base pairs, with which the drug ring systems stack.[6]

However, NCSi-gb is labile due to its lactone ring system and possesses appendages that limit its nucleic acid-binding properties.[4] Hence, readily available DDI (**1**) was designed and synthesized as an analogue of NCSi-gb that might evolve into a potential drug candidate that selectively binds two-base bulges.[4] As in the case of NCSi-gb, **1** contains a wedge-shaped aglycon moiety consisting of two aromatic ring systems held rigidly by a spirocyclic ring with a right-handed twist of 35°.[4] The aminoglycoside moiety of **1** is linked to the connecting spirocyclic ring instead of to an aromatic ring system in NCSi-gb. Similarly, an analogue of **1**, but having a leucine instead of the aminosugar attached to the spirocyclic ring, has been reported to interact with bulged DNA.[7] The binding of **1** to the best known bulge substrate is about 10 times weaker than that of NCSi-gb (entry 1, Table 1 and Fig. 2).[4] The binding of **1** to DNA bulges is much less sequence specific than that of NCSi-gb (entry 3-7 vs 1, Table 1 and Fig. 2).[4]

NMR structures of complexes containing a two-base DNA bulge and **1** or a diastereomer of **1** have been solved.[8,9] Contrary to the situation with NCSi-gb, the aminoglycoside moiety of **1** is positioned in the minor groove, and it blocks the intercalation of the dihydronaphthalenone moiety into the bulge site so that it too remains mainly in the minor groove.[6,8] The difference in the binding mode, likely accounting for the lower specificity and tightness of binding of **1** for the two-base bulge site, was attributed to the difference in the aminoglycoside positions.[8] Hence, analogue **2**, in which the aminoglycoside moiety is linked to the three-ring system, was prepared.[10] Analogue **2** has shown bulge binding ability comparable with that of **1** (Table 1).[10] The binding between

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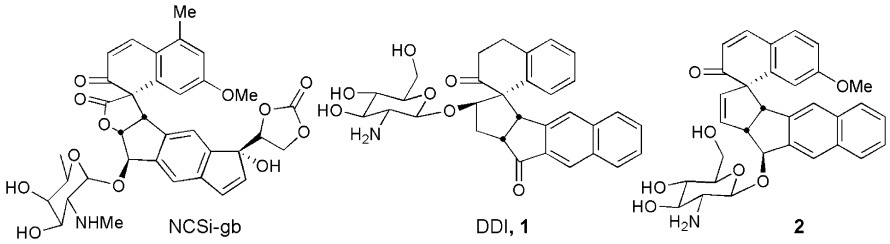



**Figure 1.** Structures of NCSi-gb, DDI (**1**) and **2**.

**Table 1.** Dissociation constant (μM) of DNA binding (Fig. 2) by the drugs determined via emission spectra ($\lambda_{exc}$ 313 nm; $\lambda_{emm}$ 460 nm for **13a**, **13b**, and **14**) at 5 °C

| Entry | Sequence code | NCSi-gb | 1 | 2 | 13a | 13b | 14 |
|---|---|---|---|---|---|---|---|
| 1 | HT3AGTT | 0.033 | 0.46 | 0.55 | 0.36 (0.03)[a] | 1.2 (0.1) | 0.28 (0.05) |
| 2 | HT3AT | 10 | 7.6 | 9.55 | 16 (2) | 25 (1) | 16 (2) |
| 3 | HT3AGT | 20.6 | 1.75 | 7.71 | 14 (2) | | 14 (3) |
| 4 | HT3AGCTT | 0.71 | 3.04 | | 2.6 (0.3) | | 6.4 (0.4) |
| 5 | HT3AGTA | 12.5 | 2.67 | 1.84 | 6.8 (0.6) | | 12 (2) |
| 6 | HT3TGTA | 22.2 | 3.26 | | 8.7 (0.4) | | 12 (2) |
| 7 | HT3GGTA | 12.9 | 5.0 | | 6.4 (0.5) | | 8.7 (1) |
| 8 | HT3GGTT | 0.064 | 1.03 | | 2.5 (0.8) | | 0.93 (0.01) |
| 9 | HT3GGTC | 0.416 | 2.74 | | 3.2 (0.6) | | 4.5 (1.5) |
| 10 | HT3CGTC | 0.5 | 1.48 | | 1.8 (0.2) | | 2.8 (0.5) |
| 11 | DA12:BA14 | 2.18 | 2.54 | 3.18 | 1.0 (0.1) | | 1.6 (0.1) |
| 12 | DA12:DAc12 | 307 | 32.8 | 100 | 22 (7) | | 36 (3) |

[a] Values in parentheses represent standard deviation.

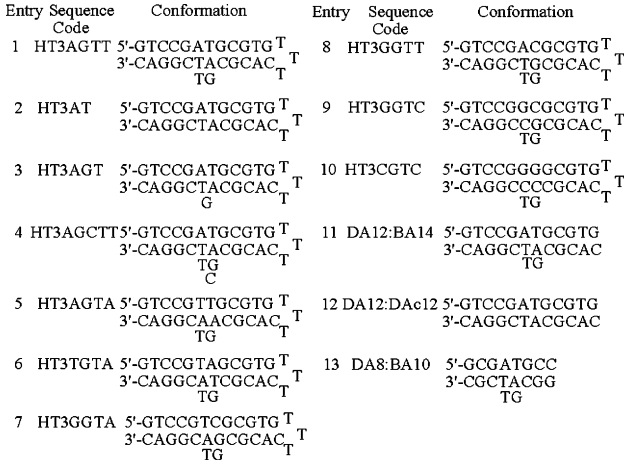
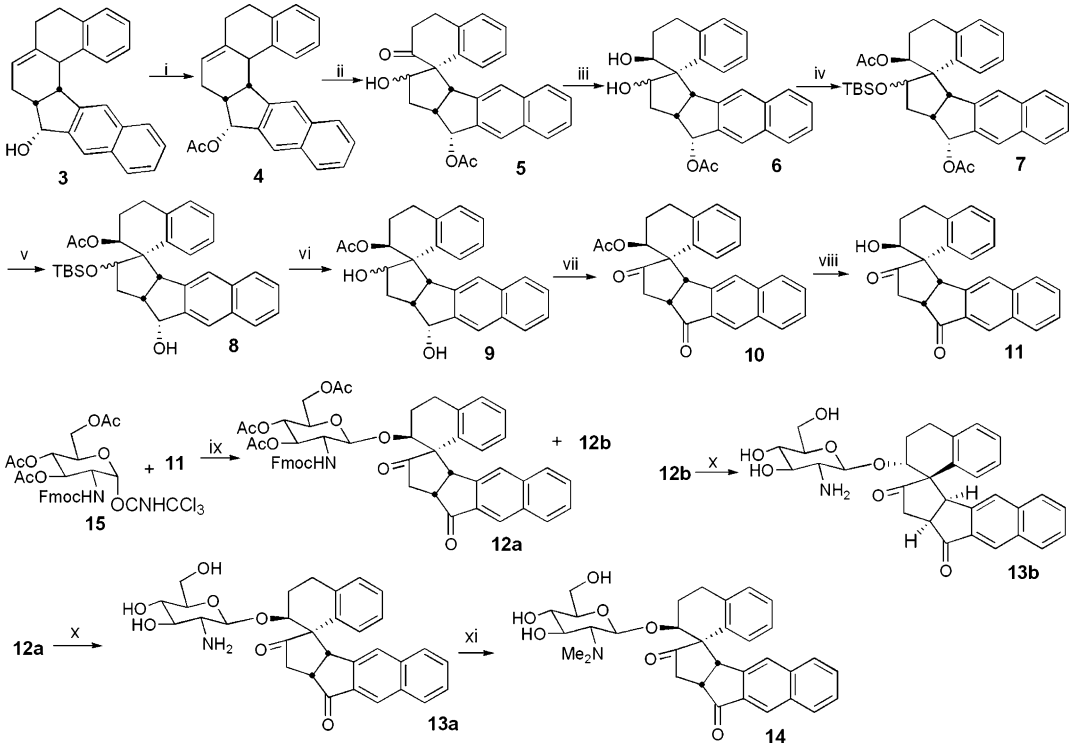



**Figure 2.** Structures of synthetic oligonucleotides.

DNA bulge structures and NCSi-gb or its analogues was measured using fluorescence. The fluorescent chromophore of **1**, the naphthyl indanone, was sacrificed in **2** in forming the glycoside bond. To maintain the fluorescent properties of NCSi-gb and **1**, a methoxynaphthalenone system was included in **2** as the fluorescent chromophore, via a complicated synthetic process. However, **2** suffers from the drawback of relatively weak fluorescence. Its fluorescence is more than 100 times weaker than that of **1**, and is about 40 times weaker than that of NCSi-gb, limiting its usefulness as a general probe for DNA bulged structures. In the binding studies using fluorescence, the concentration of the fluorescent compound has to be comparable to or smaller than the dissociation constant. If other analogues with the same aglycon structure as **2** have a much tighter binding with bulged DNA structures, their concentrations have to be low. As a result, the fluorescence intensity will be low, and binding studies will be difficult.

Analogue **13a** (Scheme 1) was, therefore, designed and prepared. Hoping for a stronger bulge affinity than **1**, the aminoglycoside moiety of **13a** is linked to the two-ring aromatic system instead of the spirocyclic ring. The fluorescent chromophore of **1**, naphthyl indanone, was preserved to allow for strong fluorescence. The methoxynaphthalenone system is avoided, allowing a more simple synthesis than **2**. Another difference between NCSi-gb and analogues **1** and **2** is that NCSi-gb is N-methylated. Compound **14** (Scheme 1) was prepared by N-methylation of **13a** to study the effect of N-methylation on bulge binding.

Compound **3** was prepared according to a known procedure.[10] Acylation of **3** gave **4**. Hydroxylation of **4** with $OsO_4$/NMO and subsequent oxidative cleavage with $NaIO_4/H_2SO_4$ followed by aldol closure gave **5**.[4,10] Compound **6**, obtained from reduction of **5** with $NaBH_4$, was selectively protected by *t*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) and $Ac_2O$ to give **7**. Selective deprotection of **7** with $K_2CO_3$/methanol followed by deprotection with TBAF gave **9**. Compound **10** was obtained from oxidation of **9** and its deprotection with $Et_3N$ gave **11**. Crystals of **11** were obtained by solvent diffusion of hexanes into a $CH_2Cl_2$ solution of **11**. Structure of **11**, determined by X-ray diffraction



**Scheme 1.** Chemical synthesis of spirocyclic analogues. Reagents and conditions: (i) $Ac_2O$, DMAP, pyridine, 0 °C, 12 h, 96%; (ii) 1—$OsO_4$ (cat), NMO (1.1 equiv), 12 h, 2—$HIO_4$, 6 h, 3—$K_2CO_3$, DMF, 0 °C, 30 min, 55%; (iii) $NaBH_4$, 0 °C, 5 min, 75%; (iv) 1—TBSOTf (1 equiv), 2,6-lutidine, −30 °C, 2 h, 2—$Ac_2O$, DMAP, pyridine, 12 h, 82%; (v) $K_2CO_3$, methanol, 1 h, 93%; (vi) TBAF, 96 h, 64%; (vii) PCC, 4 h, 95%; (viii) $Et_3N$, methanol, 4 °C, 24 h, 41%; (ix) triflic acid (cat), **15** (3 equiv), −30 °C, 3 h, **12a**, 28%, **12b**, 41%; (x) 1—piperidine, 45 min, 2—$K_2CO_3$, methanol, 2 h, **13a**, 75%, **13b**, 73 %; (xi) MeI, $K_2CO_3$, 5 h, 87%.

analysis, is shown in Figure 3.[11] The X-ray structure illustrates the position of the hydroxyl group in **11** as well as the endo relationship of the two aromatic rings. Like the aglycon moiety of NSCi-gb or **1**, **11** is wedge-shaped consisting of two aromatic ring systems held rigidly by a spirocyclic ring with a similar angle of right-handed twist.[4]

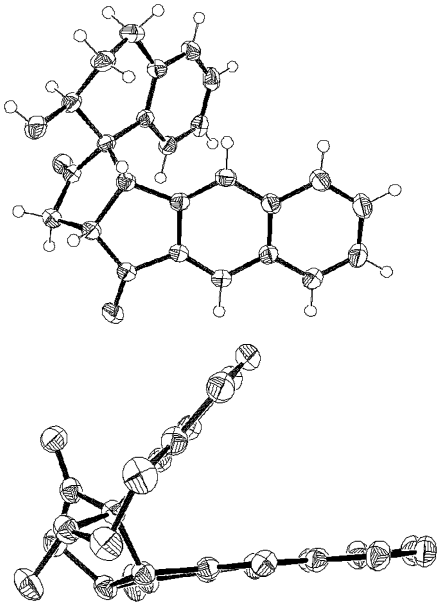


**Figure 3.** ORTEP drawings of **11** showing thermal ellipsoids at 50% probability. Hydrogen atoms have been removed for clarity in the side view.

Coupling of **11** with **15**[4] gave two diastereomers **12a** and **12b**.[4] Subsequent deprotection was carried out according to the reported procedure to obtain **13a** and **13b**.[4] The relative stereochemistry of **13a** and **13b** was determined by their CD spectra (Fig. 4). The CD spectrum of **13a** was similar to that of DDI, indicating that

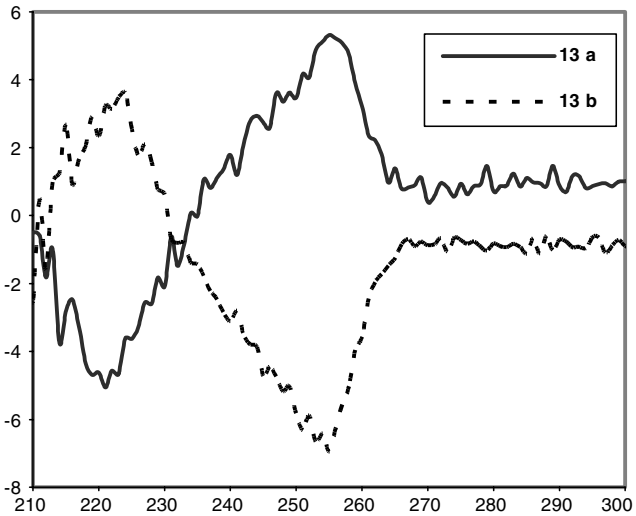



**Figure 4.** CD spectra of **13a** and **13b**.

**13a** is an analogue of NCSi-gb.[4] Methylation of **13a** with $CH_3I/K_2CO_3$ results in **14**.

The fluorescence intensities of **13a**, **13b** or **14** ($\lambda_{exc}$ 313 nm; $\lambda_{emm}$ 460 nm for **13a**, **13b**, and **14**) are strong, with the same relative fluorescence as **1**. Fluorescence quenching was observed when certain DNA bulges were added to a solution of **13a**, **13b** or **14**. These fluorescence changes were used to measure the binding constants for selected oligonucleotides and **13a**, **13b** or **14**.[4] The results are shown in Table 1. The dissociation constants of NCSi-gb, **1** and **2** with DNA bulges are included for comparison.[4,10]

For **13a** and **14**, binding to a Watson Crick duplex (entry 12) or to a duplex with a loop (entry 2) is much weaker than that to a DNA duplex containing a two-base bulge (entry 1). This indicates that the two-base bulge, not the DNA duplex or the loop, is the preferred binding site. With the best binding substrate HT3AGTT (entry 1) **13a** and **14** showed slightly stronger binding than the previously reported synthetic analogues, **1** and **2**. A switch from pyrimidine to purine base 3′ to the bulge resulted in marked reduction in affinity for NCSi-gb (entry 5, 6, 7 versus 1).[4,10] Compared to **1** and **2**, which have lost much of the sequence-specificity,[4,10] **13a** and **14** showed improved sequence-specific affinity (entry 5, 6, 7 versus 1). Similar to NCSi-gb, **13a** and **14** bind to certain two-base bulges much more strongly than to a one-base bulge (entry 3 versus 1). These results are consistent with NMR structural studies on DNA bulge complexes containing NCSi-gb or **1** showing that the position of the aminoglycoside played an important role in DNA bulge binding.[6,8] The binding patterns of **13a** and **14** are similar, suggesting that N-methylation has a limited effect on bulge binding.

Having established the bulge-binding specificity of **14** it was also of interest to test its ability to compete with NCS-chrom (its activated radical form) and block bulge-specific cleavage by the latter. DNA duplex (Fig. 2, entry 13, 9 μM) having a single cleavage site at the bulge was treated with NCS-chrom (12 μM) as previously described.[12,13] DNA was preincubated with varying levels of **14** for 15 min on ice prior to the addition of NCS-chrom. Products from a 10 min reaction were separated on a 15% sequencing gel and the band intensities were quantitated on a phosphorimager. Figure 5 shows that **14** inhibited strand scission by NCS-chrom in a dose-dependent manner. Fifty percent inhibition was obtained at a concentration of **14** about 10-fold in excess of NCS-chrom. This result is compatible with the relative bulge-binding affinities of **14** and of NCSi-gb presented in Table 1. In control experiments, there was no inhibition by **14** of the cleavage of duplex DNA by thiol-activated NCS-chrom.

In summary, new strongly fluorescent spirocyclic analogues of NCSi-gb possessing aminoglycoside appendage on the two-ring system were synthesized. These compounds resemble the natural product in binding affinity and sequence selectivity for two-base DNA bulges and offer a template to explore additional bulge-binding compounds.

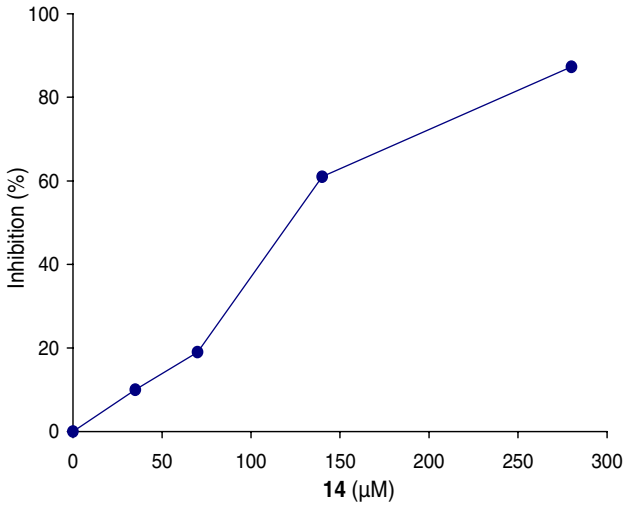



**Figure 5.** Effect of **14** on strand scission at a two-base DNA bulge by NCS-chrom. Twenty-nine percent of the DNA was cut by NCS-chrom alone.

## Acknowledgments

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.03.006.

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11. Crystallographic data (excluding structure factors) for compound **11** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 296872. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].
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