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- [7] Crystal data for **3**: C₇₂H₉₆N₂₄O₁₈Pd₃·2CH₃OH, *M*_r = 1968.98, orthorhombic, space group *Pnma* (no. 62), *a* = 27.6315(9), *b* = 22.1045(7), *c* = 17.7133(6) Å, *V* = 10818.9(6) Å³, *Z* = 4, *T* = 23.0 °C, *ρ*_{calcd} = 1.209 g cm⁻³, *μ*(MoK_α) = 5.59 cm⁻¹, MoK_α radiation (*λ* = 0.71069 Å), *R* = 0.090, *R*_w = 0.253. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-160516. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
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Directing Topoisomerase I Mediated DNA Cleavage to Specific Sites by Camptothecin Tethered to Minor- and Major-Groove Ligands**

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Hairpin polyamides containing pyrrole- and imidazole-carboxamide units represent convenient tools to target specific sequences in DNA.^[1] These compounds form dimeric motifs that fit snugly into the minor groove of B-DNA and recognize defined sequences through the formation of an array of hydrogen bonds and van der Waals contacts. They provide a paradigm for the design of small molecules that regulate genes.^[2]

The sequence-recognition properties of hairpin polyamides can be exploited for directing DNA-cleaving and -alkylating agents to particular sequences.^[3–7] By analogy, we reasoned that they could also be used to direct the action of DNA-cleaving proteins such as topoisomerases. These ubiquitous

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enzymes regulate DNA topology in cells and therefore are essential for many vital processes, such as replication, transcription, and chromosome segregation.^[8] Topoisomerases I and II both represent privileged targets for a number of cytotoxic anticancer drugs.^[9] In particular, the camptothecins (CPT), which include the clinically available derivatives topotecan and irinotecan, are the most potent inhibitors capable of stimulating DNA single strand cleavage by topoisomerase I at a variety of sites, essentially at T–G sites.^[10, 11]

DNA cleavage by topoisomerase I can be targeted to specific sequences by the linkage of topoisomerase inhibitors to sequence-recognition elements, such as oligonucleotides that form triple helices^[12–14] or small molecules related to the antibiotics netropsin and distamycin.^[3, 15, 16] The limited sequence-recognition properties of these antibiotics and analogues that form 1:1 drug:DNA complexes restrict their use as DNA-cleaving delivery systems. No sequence specificity was reported for the cleavage by the conjugates with the camptothecin derivatives.^[15] Here we present an alternative approach to direct camptothecin to specific sites in DNA and to introduce DNA breaks at a precise location by making use of the recognition of the target sequence by hairpin polyamide ligands that bind to the minor groove of DNA in a sequence-specific manner.^[1] We have compared these conjugates to camptothecin that is covalently linked to triplex-forming oligonucleotides that bind in the major groove at oligopyrimidine · oligopurine sequences.

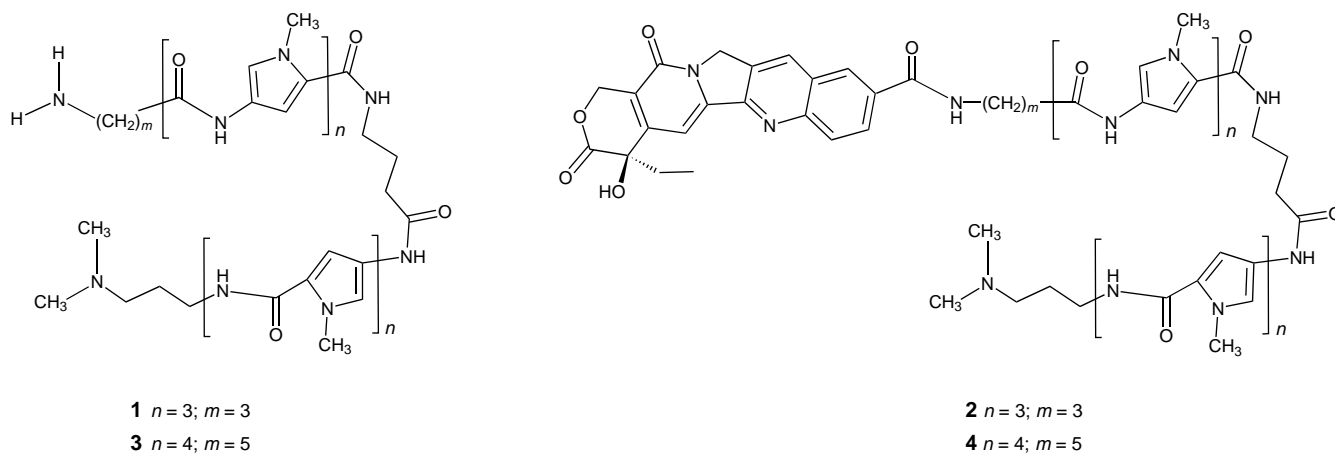
Two hairpin polyamides, each containing two series of three or four *N*-methylpyrrolicarboxamide units that form antiparallel dimers, were synthesized as previously described.^[17] Compounds **1** and **3** were linked to 10-carboxycamptothecin through aminoalkyl side chains of different lengths. The two minor-groove binder/camptothecin (MGB–CPT) conjugates **2** and **4** contain the same dimethylaminopropylamino cationic side chain on the C-terminal pyrrole heterocycle and an identical γ -aminobutyrate residue between the two oligopyrrole branches, but they differ in the number of *N*-methylated pyrrole units (2×3 or 2×4 pyrrole rings) and the nature of the alkyl connector (propyl in **2** or pentyl in **4**) between the MGB moiety and the topoisomerase I inhibitor. The coupling procedure recently described for the synthesis of oligonucleotide-aminoalkyl-CPT conjugates was employed.^[12, 13] Briefly,

the 10-carboxyl group of the camptothecin derivative was first treated with *N*-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide to provide the corresponding active ester, which was then treated with the minor-groove binder **1** or **3**. The resulting MGB–CPT hybrid molecules **2** and **4** were separated from the remaining camptothecin molecules by successive precipitations from a mixture of DMSO and acetonitrile (1:4). The final compounds were characterized by UV spectroscopy and mass spectrometry.

The MGB–CPT conjugates were incubated with a 324 base pair (bp) ³²P-labeled DNA fragment prior to the addition of topoisomerase I to initiate the DNA cleavage reactions. Control experiments were performed in parallel with camptothecin alone and with the MGBs **1** and **3**, as well as with the noncovalent association of camptothecin plus each of the two MGBs (CPT + **1** and CPT + **3**). In all cases the DNA samples were treated with sodium dodecylsulfate (SDS) and proteinase K to remove any covalently bound protein and then resolved on an 8% polyacrylamide sequencing gel under denaturing conditions so as to identify the sequence of the drug-induced topoisomerase I cleavage sites. A typical example of a gel is shown in Figure 1a and a summary map showing the position of the main cleavage sites induced by CPT (asterisks) is presented in Figure 2. The positions of the AT-rich MGB-binding sites (boxed sequences in Figure 2) were inferred from DNase I footprinting experiments performed with the same *Eco*RI-*Pvu*II 324-bp restriction fragment (³²P-labeled at the 3'-end of the *Eco*RI site) incubated with the untethered polyamides **1** and **3** at 3 μ M (data not shown). This particular DNA fragment contains a 24 nucleotide long oligopyrimidine · oligopurine tract used previously for triple helix formation.^[12, 13] It was selected because it contained several CPT-sensitive sites, some of which are close to short AT tracts, thus providing exquisite binding sites for oligopyrrole polyamides such as compounds **1** and **3**. In addition it allowed us to compare the cleavage efficacy and selectivity of minor-groove and major-groove ligands conjugated to CPT.

With camptothecin alone, the position of seven cleavage sites (a–g) were determined with nucleotide resolution by various electrophoresis experiments (Figure 2).

As shown in Figure 1, the covalent linkage of a MGB to the CPT chromophore exerts a drastic effect on the sequence



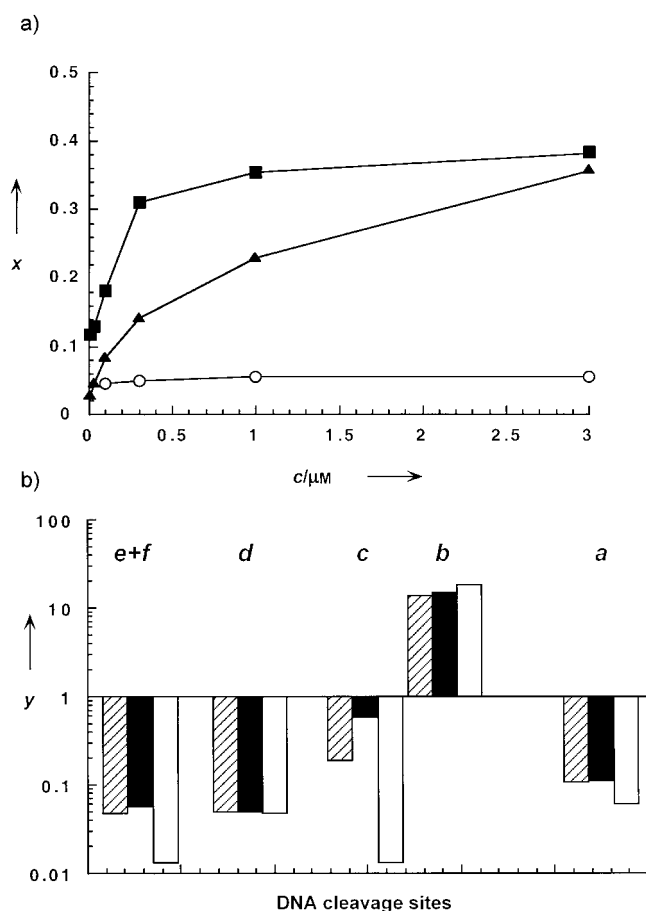


Figure 3. a) Quantification of the intensity of topoisomerase I mediated cleavage (x) at site b is reported as a function of the concentration (μM) for free camptothecin (\circ), hybrid **2** (\blacktriangle), and **4** (\blacksquare). x = fraction of cleaved DNA. b) Intensities of the cleavage observed in the presence of conjugates MGB-CPT **2**, **4**, and TFO-CPT ($3 \mu\text{M}$ each) are indicated by hatched, black, and white bars, respectively. The extent of the topoisomerase I mediated cleavage refers to the ratio between the intensity of the cleavage in the presence of the conjugate and that in the presence of free CPT (y) and is reported on a logarithmic scale. The position of the cleavage sites is indicated. y = ratio of cleavage intensity for conjugated drug versus free drug.

single site in the DNA fragment; cleavage at all the other sites disappeared upon conjugation of the CPT to the DNA ligand. Some cleavage at site c was still observed, since we used conjugate **4** for the comparison, which is in contrast to the conjugate TFO-CPT. It should be noted that the linkers were different for the two conjugates (Figure 1b). However, even though the CPT moiety is brought from the major-groove side of DNA by the TFO and from the minor-groove side by the MGB, both approaches provide a suitable route to guide a cytotoxic agent to a selected sequence in DNA (Figure 3b). The main advantage of MGB-CPT over TFO-CPT conjugates is that the binding of MGBs is not restricted to oligopyrimidine · oligopurine DNA sequences as observed with TFOs. In addition, MGBs are expected to be more cell permeable than TFOs and, therefore, might be more suitable for cell studies.

In summary, prominent cutting at site b attests that the covalent linkage of a hairpin polyamide to camptothecin provides an efficient system to direct DNA cleavage mediated

by topoisomerase I to specific sites. Hairpin polyamide/camptothecin conjugates represent a new category of single-strand-breaking artificial nucleases that could provide a basis for potential therapeutic applications.

Experimental Section

Synthesis of the CPT-MGB conjugates: 10-Carboxycamptothecin (0.005 mmol) was activated with a small excess of *N*-hydroxysuccinimide (0.007 mmol) in the presence of dicyclohexylcarbodiimide (0.007 mmol) for 8 h at room temperature in dry dimethylformamide (DMF). The camptothecin ester was then precipitated by diethyl ether, dissolved in DMF, and added to a solution of **1** or **3** (at 0.001 mmol) and triethylamine (4 μL) in dimethyl sulfoxide (DMSO; 50 μL) and left to stand at room temperature in the dark for 8 h. The product was isolated by precipitation with deionized water (1.5 mL). The pellet was dried, dissolved again in DMSO, and precipitated twice with 80% acetonitrile, then dried, and then dissolved in DMSO for subsequent biochemical experiments.

The resulting conjugate **2** or **4** was characterized by UV spectroscopy and by mass spectrometry. The $A_{379}:A_{310}$ ratio demonstrated a 1:1 stoichiometry of camptothecin to the minor groove binder in the conjugate. MS (matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF)); found: 1381.7 (calcd: 1382.6) for **2**.

DNase I footprinting experiments: The protocol previously described was used.^[18] A 324-bp 3'-end radiolabeled DNA fragment was prepared as described in ref. [13]. The DNA was incubated with the conjugate at room temperature for 6 h.

Topoisomerase I cleavage assays were performed using the calf thymus enzyme at room temperature as described in refs. [13, 14].

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