positive charge. The more stabilizing a β-substituent, the less is the demand for charge delocalization into the aromatic ring.

The para carbon chemical shifts in 1-mesitylvinyl cation (**1**) (181.0 ppm) and 1-mesitylethyl cation (**9**) (179.6 ppm) are similar. The electron demand is thus about the same, demonstrating that both cations are stabilized by σ bond interaction with the β-substituent as compared to mesitylmethyl cation (**10**) (189.6 ppm), which lacks a β-substituent.

A pronounced upfield shift of the para carbon (10–12 ppm) is observed for the β-silyl vinyl cations **4–7** relative to the silyl-free cations **1–3**, indicating a decrease in electron demand when the β-substituent is changed from β-H or β-alkyl to a β-silyl group. This shows that β-C–Si hyperconjugation is more efficient than β-C–H or β-C–C hyperconjugation. In fact the similar para carbon shift of the silyl-substituted cations **4–7** (168–170 ppm) to that of the 1-mesitylallenyl cation **8** (165.9 ppm), which in addition to α-aryl conjugation enjoys β-allyl resonance stabilization, shows that hyperconjugative interaction of a β-C–Si σ bond with the "vacant" 2p orbital on C^+ in **4–7** is about as efficient in dispersing the positive charge as β-π conjugation in **8**.

In conclusion we have prepared the first persistent α-aryl vinyl cations by protonation of alkynes in superacids. The NMR spectroscopic data of the β-silyl-substituted vinyl cations give experimental proof for the hyperconjugative charge delocalizing ability of β-silyl groups, and the comparison with silyl-free analogs demonstrates the magnitude of the β-silyl effect. The results are in accord with IGLO chemical shift calculations on model cations.

Acknowledgment. This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

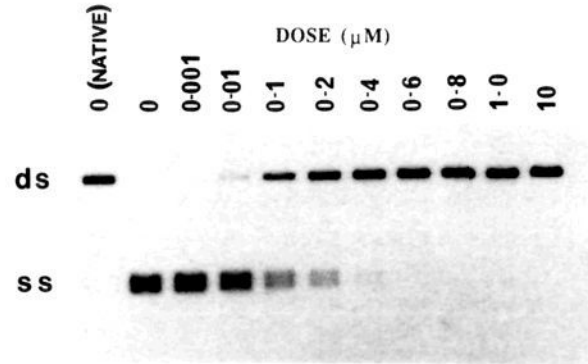

Figure 1. Autoradiograph of a neutral agarose gel showing DNA interstrand cross-linking by **7** in linear ^{32}P end-labeled pBR322 DNA. Drug reactions (2 h at 37 °C) were in 25 mM triethanolamine/1 mM EDTA pH 7.2 buffer with 10 ng of DNA in a final volume of 50 μL. Reaction was terminated by addition of an equal volume of 0.6 M sodium acetate, 20 mM EDTA, and 100 μg/mL tRNA, and the DNA precipitated with ethanol. Dried pellets were taken up in strand separation buffer (30% w/w DMSO in 1mM EDTA). Denaturation for 2 min at 90 °C was followed by immediate chilling in an ice–water bath. Electrophoresis was carried out on 0.8% w/v submerged horizontal agarose gels at 40 V for 16 h with tris-acetate running buffer. Double-stranded (ds) and single-stranded (ss) DNA were quantitated by laser densitometry.

Table I. In Vitro Cytotoxicity of **7** and **8**[a]

IC_{50} (μM)	L1210	ADJ/PC6	CH1
7 (DSB-120)	0.01	0.0005	0.003
8 (DC-81)	0.38	0.33	0.1

[a] IC_{50} is the dose (μM) for 50% growth inhibition compared to solvent controls. Drugs were dissolved in DMSO to provide a final concentration of 0.05% DMSO. Incubation times (37 °C) were as follows: L1210, 3 days; ADJ/PC6, 4 days; CH1, 9 days.

Rational Design of a Highly Efficient Irreversible DNA Interstrand Cross-Linking Agent Based on the Pyrrolobenzodiazepine Ring System

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Received December 18, 1991

Many DNA cross-linking agents with significant antitumor activity[1] are GC site-specific, which may contribute to their potency, as it has been established that a number of oncogenes, including c-Ha-*ras*, contain highly GC-rich regions.[2] Most known cross-linking agents are of sufficient size to recognize only two or three base pairs, and extension of this limited sequence recognition is of interest, as such agents may have the potential to produce irreparable cross-links at precisely defined genomic locations.[3] Furthermore, clinically-useful cross-linking agents such as the nitrogen mustards alkylate within the major groove of DNA whereas, with few exceptions,[4–6] the biological consequences of minor groove cross-linking have been relatively unexplored. We report here the synthesis of a pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) bifunctional alkylating agent, DSB-120 (**7**), that forms an irreversible interstrand cross-link between two guanine bases within the minor groove via their exocyclic N2 atoms.[7] According to molecular modeling and NMR studies, it spans six base pairs, actively recognizing a central 5′-GATC sequence. It is one of the most efficient DNA cross-linking agents known and is significantly cytotoxic toward tumor cells in vitro.

The PBD antitumor antibiotics monoalkylate the exocyclic N2 of guanine in the minor groove of DNA via their electrophilic

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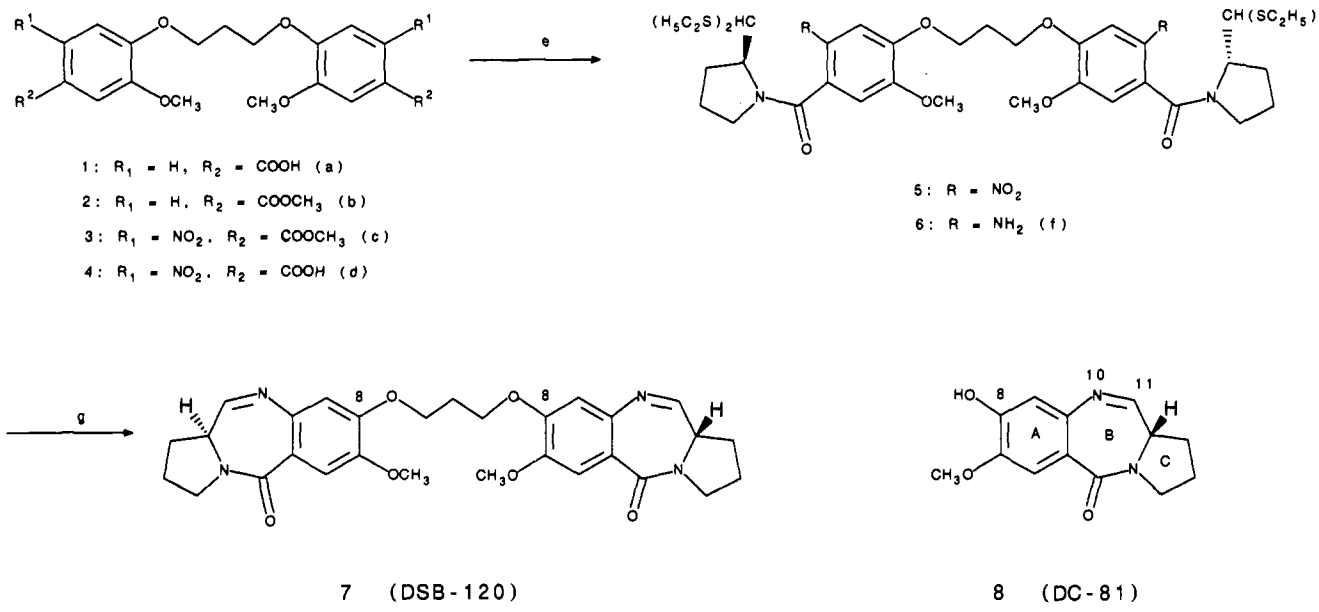
Scheme I[a]



[a] (a) Vanillic acid/$I(CH_2)_3I$/aqueous NaOH/THF; (b) dimethyl sulfate/K_2CO_3/acetone; (c) $SnCl_4/HNO_3/CH_2Cl_2$ (−20 °C); (d) aqueous NaOH/THF; (e) $(COCl)_2$/THF/Et_3N/H_2O/(2*S*)-pyrrolidine-2-carbaldehyde diethyl thioacetal;[12] (f) $SnCl_2 \cdot 2H_2O$/MeOH; (g) $HgCl_2/CaCO_3/CH_3CN/H_2O$.

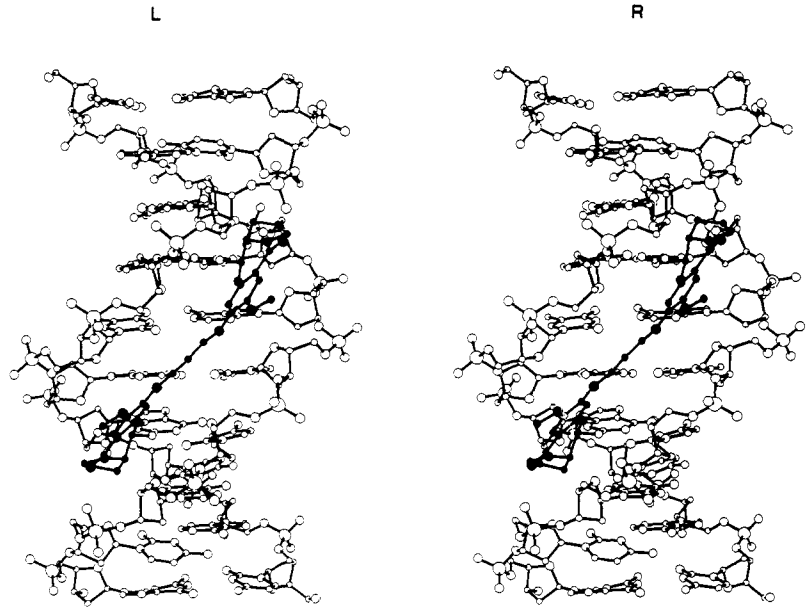


Figure 2. Stereoview of the cross-linked d(CICGATCICG)$_2$–PBD dimer adduct following X-PLOR refinement. The dimer **7** (shown in bold) is located in the minor groove of B-DNA, spanning bases C3–I8, and is bound covalently to G4 bases on adjacent strands. All H-atoms have been removed for clarity.

N10–C11 imine (or equivalent) moieties,[8] the preferred bonding sequence involving 5′-PuGPu motifs.[9] Our objective was to tether two PBD units through their C8 positions via a flexible 1,3-propanediyldioxy ether linkage,[7] with a view to extending the number of spanned base pairs, thus enhancing DNA sequence selectivity. Molecular modeling suggested that C8-linked dimers have greater isohelicity with the minor groove of DNA compared to the C7-linked dimers reported by Suggs.[6a] C8 dimers of this type should have the potential to form intra- or interstrand DNA cross-links, in addition to adducts resulting from monoalkylation.[3] The natural product DC-81 (**8**) was chosen as the monomer PBD unit,[10] and an efficient synthetic pathway was developed (Scheme

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I) to provide the target dimer **7** as a stable yellow oil[11] in 8% overall yield.

DNA-binding of **7** was initially observed through thermal denaturation studies with calf thymus DNA[13] (ΔT_m >15.1 °C for a 5:1 ratio of DNAp:ligand at 37 °C for 18 h). Cross-linking efficiency was investigated using an agarose gel electrophoresis assay[14] based on the principle that, following complete denaturation of linear pBR322 DNA, an interstrand cross-link results in renaturation to the double-stranded form in a neutral gel. The results indicate that **7** is a remarkably efficient cross-linking agent (Figure 1). After 2 h at 37 °C, cross-linking is measurable down to at least 0.01 μM (drug:nucleotide ratio = 0.025), with >90% cross-linking at 0.4 μM (drug:nucleotide ratio = 1.0). In the same assay, it is 50-fold more effective than the major groove cross-linkers mechlorethamine and cisplatin,[14] approximately 300 times more efficient than melphalan,[14] and similar in efficiency to rigid CC-1065 dimers such as U77779.[5c] However, in contrast to the weaker-binding C7-linked PBD dimer,[6,15,16] no reversibility of cross-linking was observed after incubation for 16 h at 37 °C and pH 4-10. In addition, compound **7** is highly cytotoxic, and comparison with the monomer **8** (Table I) emphasizes the biological consequences of DNA cross-linking.

Extensive modeling studies of **7** with $d(CGYGXXCYCG)_2$ have suggested that spatial separation of the PBD units is optimal for spanning six base pairs with a preference for 5′-PuGATCPy or 5′-PyGATCPu sequences, and that it actively recognizes the embedded $d(GATC)_2$ sequence. The self-complementary 10-mer $d(CICGATCICG)_2$[17] was designed to investigate the interstrand cross-linking potential of **7**. ^{1}H-NMR examination of the 1:1 adduct showed that the duplex is cross-linked symmetrically via the minor groove N2 positions of the guanines, with 11(*S*),11′-(*S*)-stereochemistry in the ligand and minimal distortion of the helix. The model was subsequently refined at the all-atom level by an interproton NOE distance-restrained dynamic annealing procedure using X-PLOR 2.1[18] (Figure 2).

In summary, C8-linked PBD dimers of this class are highly efficient irreversible DNA cross-linking agents that may have potential application in cancer chemotherapy and as biochemical tools.

Acknowledgment. We thank the SERC Molecular Recognition Initiative (GR/F52675) and the Cancer Research Campaign for financial support and Professor Ken Harrap (ICR, Sutton, Surrey, U.K.) for the cytotoxicity data.

Registry No. 1, 3018-48-2; **2**, 130107-96-9; **3**, 140658-44-2; **4**, 140658-45-3; **5**, 140658-46-4; **6**, 140658-47-5; **7**, 140676-21-7; **8**, 89824-22-6; $d(CICGATCICG)_2$, 140658-48-6; $I(CH_2)_3I$, 627-31-6; vanillic acid, 121-34-6; (2*S*)-pyrrolidine-2-carbaldehyde diethyl thioacetal, 105089-88-1.

(11) Compound **7** is stable on storage under dry conditions: $[\alpha]^{25}_D$ +330° (*c* = 0.6, $CHCl_3$). All new compounds were characterized by standard spectroscopic methods.

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Formation of 3-Amino-5-hydroxybenzoic Acid, the Precursor of mC_7N Units in Ansamycin Antibiotics, by a New Variant of the Shikimate Pathway†

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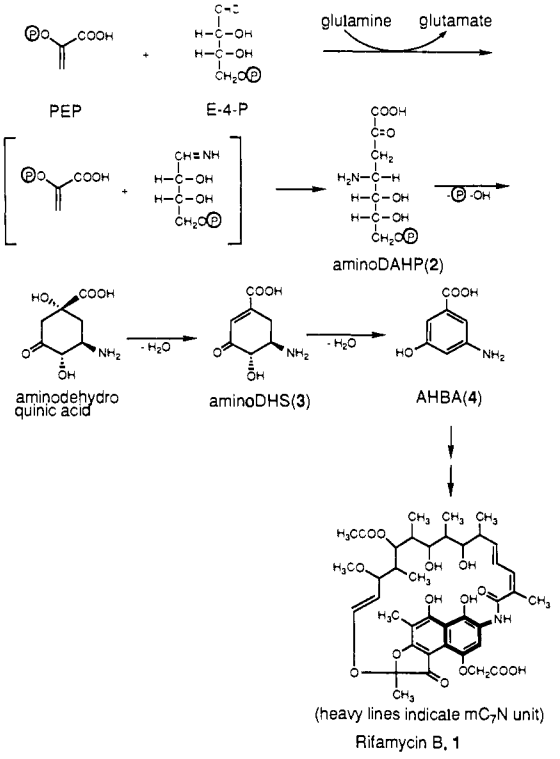
Received February 13, 1992

A variety of antibiotics, notably the ansamycins, e.g., rifamycin B[1] (**1**), and the mitomycins,[2] contain a biosynthetically unique moiety, called an mC_7N unit, consisting of a six-membered carbocyclic ring carrying an extra carbon and a nitrogen in a meta arrangement. Extensive tracer[2,3] and genetic[4] experiments have demonstrated the shikimate pathway origin of this mC_7N unit, although neither shikimic acid[3a,c,5,6] nor dehydroquinic acid[3d] were incorporated, and have identified 3-amino-5-hydroxybenzoic acid (AHBA, **4**) as its proximate precursor.[7] The nitrogen of the mC_7N unit is linked to the carbon corresponding to C-5, not C-3 of, for example, dehydroshikimic acid,[3d,8-11] and its origin, in the case of **1**, has been traced to the amide nitrogen of glutamine.[12] This and mechanistic considerations led us[10] to propose the pathway shown in Scheme I for the formation of **4**. The key

Scheme I



feature is the suggested operation of a modified DAHP synthase containing an additional protein subunit which binds and hy-

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