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Design and Synthesis of DNA Cleaving Bleomycin Models: 1,2-trans-Disubstituted Cyclopropane Units as Novel Linkers

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Abstract. The design and synthesis of 1a - e, functional models for bleomycin are described in which AMPHIS, a metal-complexing model of bleomycin, and a distamycin moiety are connected with a series of linkers. Studies on the rates of DNA breakage and a mobility assay of DNA cleavage ability show that trans-1,2-disubstituted cyclopropane units are the best linkers amongst those examined.

An important objective in modern bioorganic and medicinal chemistry concerns the design of synthetic models that mimic various aspects of biologically active molecules. Detailed study of such models could lead to development of better chemotherapeutic agents, novel artificial enzymes and molecular biological and diagnostic tools. In recent years, considerable interest has been concentrated in the development of sequence-selective vectors for targeting genomic DNA. We have long been engaged in the design, synthesis and mechanistic studies of DNA minor groove binding molecules. Recent study found that bis-netropsins in which two DNA-binding netropsin motifs are connected by optically pure C_2 -symmetric linkers, especially 1,2-trans-cyclopropanedicarboxamide show efficient DNA binding.¹ Further studies including footprinting and two-dimensional NMR methods revealed that the cyclopropane linkers permit the two netropsin units to match the natural right-handed twist of the base pairs along the minor groove of DNA.²

The bleomycins (BLM), a family of glycopeptide antitumor antibiotics, especially bleomycin A_2 which is the main constituent of the clinically used mixture of BLM,³ have attracted considerable current interest both synthetically and biologically (Figure 1). The therapeutic effect of BLM is believed to arise from its ability to cause sequence-selective cleavage of DNA in the presence of Fe(II) ion.⁴ Umezawa, Hecht and Ohno's studies indicated that the length and the chiralities on the linker in bleomycin are important factors in the design of bleomycin analogs.⁵ We have reported the first part of our studies on design, synthesis and DNA sequence selective cleavage studies of a simplified synthetic functional model of bleomycin, 1f-h, in which a distamycin moiety was used as an alternative DNA binding domain.⁶ We report herein our studies on hybrids in which AMPHIS-distamycin was connected with certain chiral linker moieties to enhance isohelical binding.

If we consider bleomycin as a bidentate molecule, a linker with C_2 -symmetry should also increase DNA binding affinities. Thus, we designed 1a, and 1b in which a pair of enantiomers of 1,2-trans-cyclopropanedicarboxylic acid derivatives were used as linkers (Figure 1). For comparison, we also designed 1d and 1e, in which (S) and (R) alanine were incorporated to replace the $V\gamma$ - CH_3 that has been reported to be essential for the orientation of the reactive center,^{7,5} and 1c, a linker without chirality but with the same length as in 1d and 1e.

Condensation of diester (1S,2S) or (1R, 2R) - 2⁸ with 4⁶ in chloroform provided the active monoester

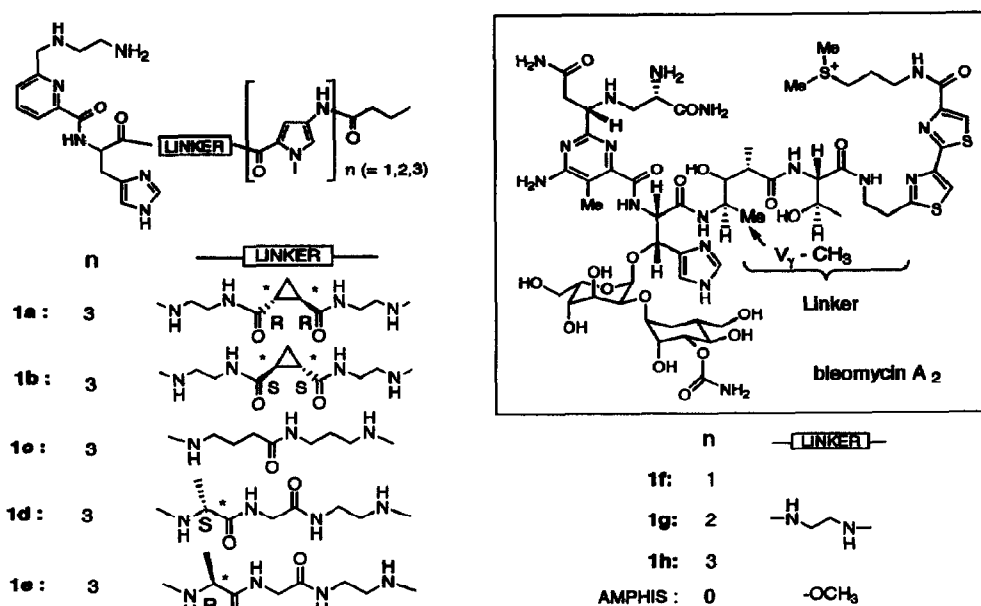
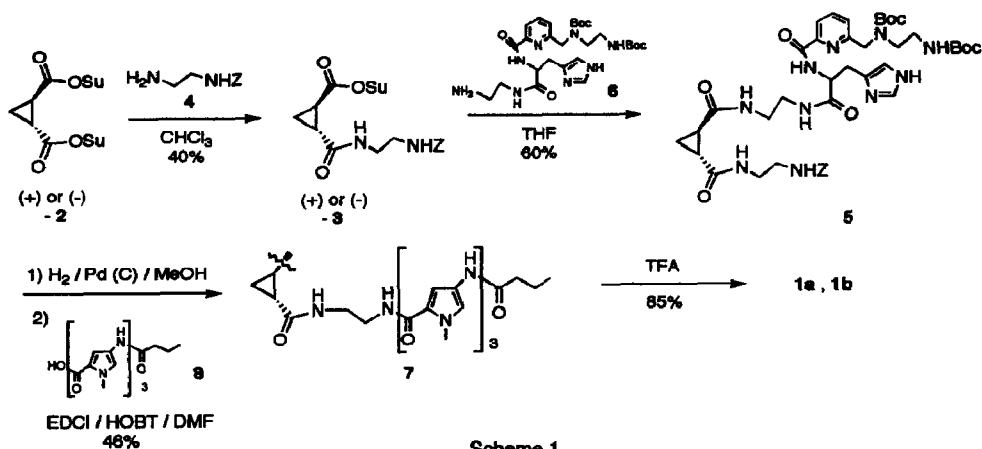


Figure 1

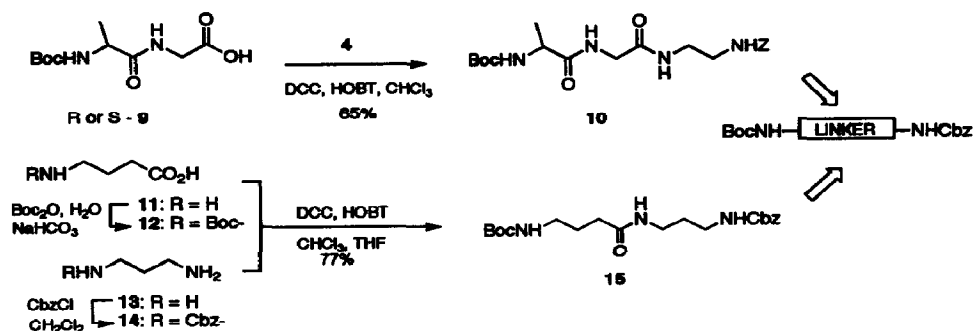


Scheme 1

(1*S*,2*S*) or (1*R*,2*R*) - **3** in a yield of 40% (Scheme 1). Coupling of **3** with "warhead" **6**⁶ in tetrahydrofuran afforded **5** in 60% yield. Removal of the carbonyl benzyloxy group by hydrogenation in the presence of 10% Pd(C) in methanol and subsequent coupling with the distamycin unit **8**⁶ in the presence of EDCI and HOBT in dimethylformamide gave **7** in a yield of 46%. Deprotection of **7** in trifluoroacetic acid at 0°C, followed by the purification on Amberlite XAD-2 resin resulted in **1a**, and **1b** in approximately 85% yield.⁹

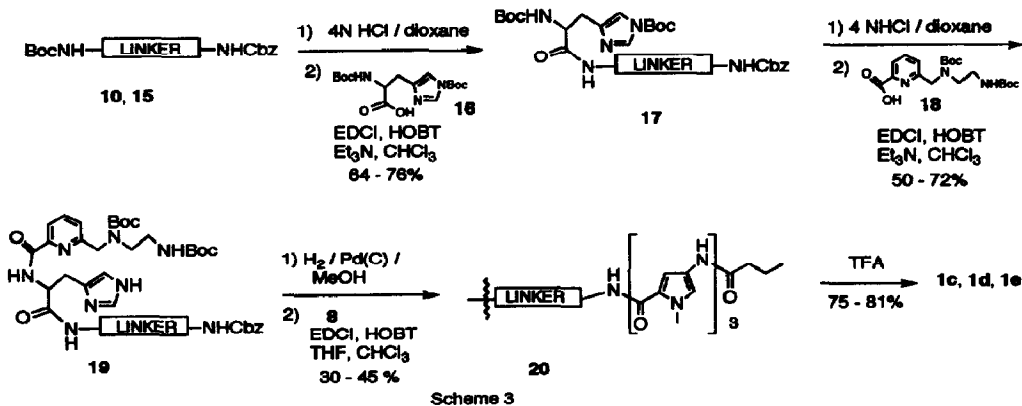
Compounds **1c** - **1e** are synthesized in a different approach. Coupling reaction of peptides (R) or (S) - **9** with **4** in the presence of DCC and HOBT in chloroform afforded (R) or (S) - **10** in 65% yield (Scheme 2). Similar coupling reaction of **12** with **14**, derived from the condensation of 1,3-diaminopropane **13** with benzyl

chloroformate in chloroform gave linker **15** in 77% yield. The protected linkers **10** and **15** were then stirred in 4N HCl dioxane solution at 0°C to remove Boc group and the free amines coupled with protected histidine



Scheme 2

to afford the intermediates **17** in 64 - 76% yield (Scheme 3). Deprotection of **17** and coupling of the free amines with **18**⁶ under the same conditions provided the protected reactive center - linker hybrids **19** in 50 - 72% yield. Removal of the carbonyl benzyloxy group by hydrogenation and coupling of the free amine with **8** under the conditions of EDCI and HOBT in THF and chloroform gave **20** in 30 - 45% yield. Finally, deprotection and purification by the same method as **1a** and **1b** afforded the hybrids **1c**, **1d** and **1e** in 75 - 81% yield.⁹



Scheme 3

A study of the kinetics of the cleavage of supercoiled covalently closed circular DNA by **1a** - **1b** in the presence of Fe(II) ion and reductants such as DTT was conducted by an ethidium binding assay.¹⁰ The relative rates of the PM-2 DNA cleavage by the Fe(II)-hybrids complexes at 80 μ M are **1a**, **1b** > **1c** > **1d**, **1e** > **1h**. The results are consistent to Hecht's report that a certain critical length of the linker is necessary for bleomycin to cleave DNA efficiently.^{5a} Independent evidence of the DNA cleavage was obtained by agarose gel electrophoresis experiments. After incubation of the complexes with PM-2 DNA and DTT for 25 min., the reaction mixtures were loaded on the gel. Under the experimental conditions, all of the complexes converted CCC DNA (form I) to OC DNA (form II) and **1a** and **1b** were apparently more efficient than the others. Detailed studies of the mechanism and sequence selectivity of DNA cleavage will be reported in due course.

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9. All new compounds gave consistent spectral and analytical data. For **1a**: R_f (AcOH:*n*-BuOH:H₂O = 1:1:1): 0.38; $[\alpha]^{20}_D$: -14.14° (c 1.45, MeOH); FT-IR (CH₂Cl₂ cast): 3284 (br.), 2961 (m), 2935 (m), 1652 (s), 1576 (s), 1539 (s), 1533 (s), 1457 (m), 1436 (m), 1349 (w), 1262 (w), 1205 (w), 766 (w) cm⁻¹; ¹H-NMR (DMSO-*d*₆:CD₃OD = 5:1) δ : 7.95 - 7.84 (m, 2H), 7.60 (m, 1H), 7.55 (s, 1H), 7.22 (s, 1H), 7.19 (s, 1H), 7.15 (s, 1H), 7.03 (s, 1H), 6.86 (s, 2H), 6.81 (s, 1H), 4.58 (m, 1H), 3.88 (s, 2H), 3.84 (s, 3H), 3.82 (s, 3H), 3.78 (s, 3H), 3.23 - 2.98 (m, 10H), 2.77 (m, 2H), 2.66 (m, 2H), 2.20 (t, *J* = 7.5 Hz, 2H), 1.94 (m, 2H), 1.58 (sex., *J* = 7.5 Hz, 2H), 1.04 (m, 2H), 0.87 (t, *J* = 7.5 Hz, 3H); FABHRMS *m/e*: calcd. for C₄₆H₆₀N₁₆O₈H: 965.4858, found: 965.4830.
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