SYNTHESIS OF DESIGNED BLEOMYCIN MODELS WITH DIFFERENT DNA RECOGNITION SITES

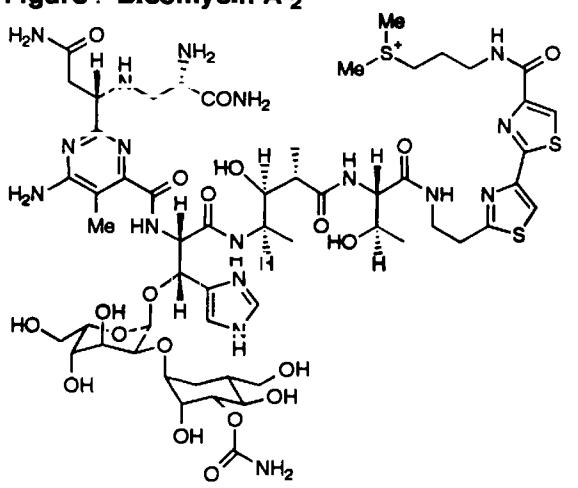
Liren Huang, James C. Quada, Jr. and J. William Lown*

Department of Chemistry
University of Alberta, Edmonton, Alberta, T6G 2G2 Canada

Abstract: The design and synthesis are described of compounds **1b** - **1f**, that are functional models for the antitumor antibiotic bleomycin. These models contain peptidic heterocyclic moieties with various DNA sequence selectivities that have been incorporated as DNA binding sites. The multi-functional heterocyclic unit AMPHIS serves as the metal complexing subunit and chiral 1,2-*trans*-disubstituted cyclopropane are employed as linkers.

It has been reasonable to conclude that DNA sequence selectivity is an important factor contributing to the cytotoxic potency of several chemotherapeutic agents derived from natural sources such as calicheamicins (1), CC-1065 (2) and bleomycin (3). Therefore there is increasing attention being paid to the development of DNA sequence-selective or specific agents for targeting genomic DNA (4). There is also current interest in the design and synthesis of hybrids conjugating DNA cleaving cores which simulate the functions of natural products, with for example, information-reading vectors, so called " lexitropsins" capable of binding to double-stranded DNA with sequence selectivities (5).

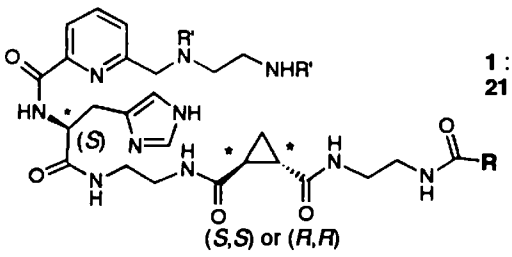
Figure : Bleomycin A_2



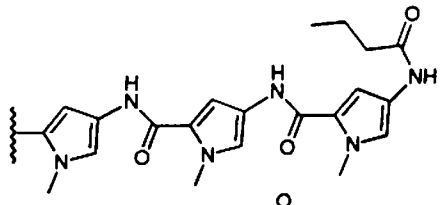
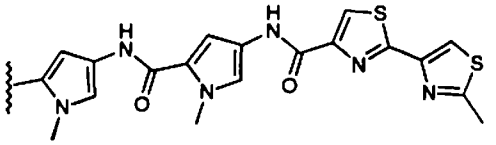
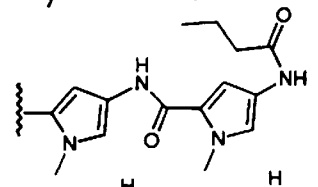
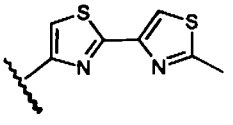
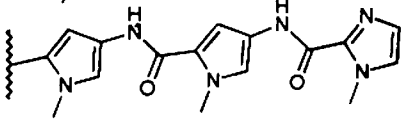
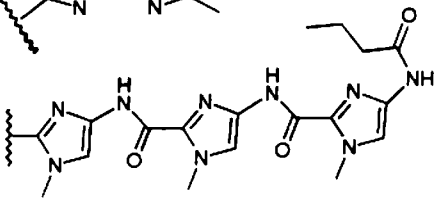
The bleomycins (BLM), especially bleomycin A_2 which is one component of the clinically used congener mixture of BLM, have attracted considerable interest both synthetically and biologically (Figure) (3). The therapeutic effect of BLM is believed to arise from its ability to cause cleavage of double-stranded DNA specifically at 5'-GC-3' and 5'-GT-3' in the presence of Fe(II) ion and molecular oxygen. The bithiazole moiety in the side-chain is believed to contribute to the site specificity. This recognition has stimulated studies to design BLM analogs by altering the DNA recognition moiety to incorporate oligonucleotide (6), protein (7) and lexitropsins. Ohno et al. synthesized BLM models which incorporated a distamycin moiety as a DNA recognition element (8). We have previously reported a series of synthetic models for BLM where the designed metal-chelating subunit "AMPHIS" and distamycin moiety were connected by appropriate linkers. Especially effective amongst such compounds was **1a** tethered by chiral 1,2-*trans*-disubstituted cyclopropane units which

Table: (*S,S*) and (*R,R*) - 1 synthesized

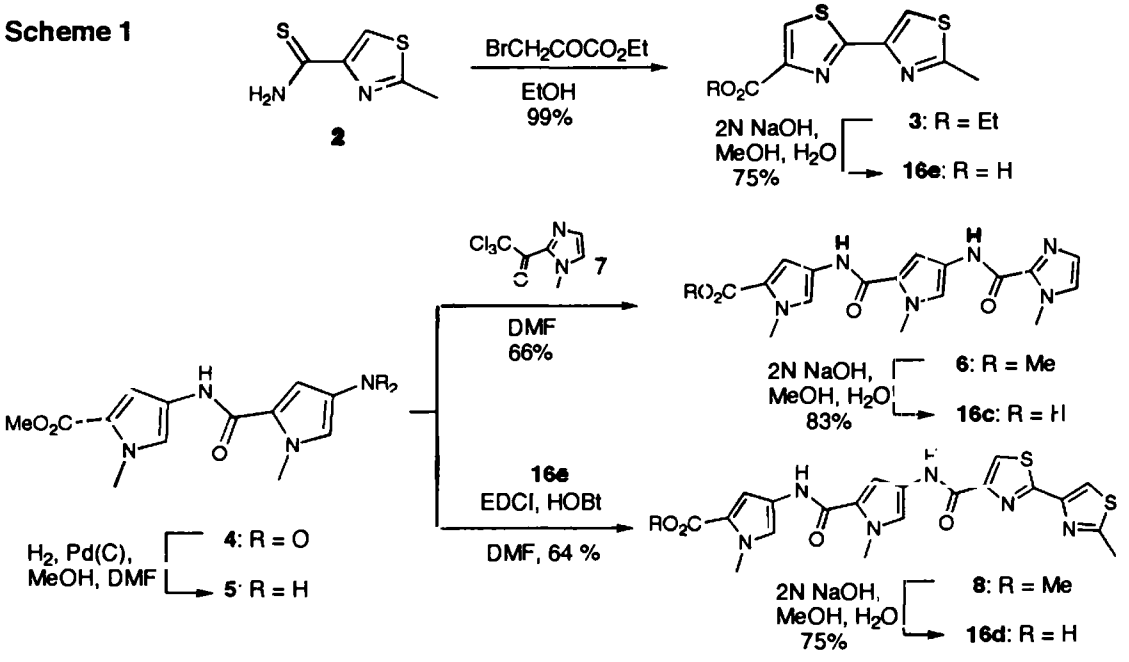
Site selectivity changes from AT-rich region to GC-rich region in the order of **a** - **f**.



1 : R' = H
21: R' = Boc

Hybrids 1	R	Hybrids 1	R
a		d	
b		e	
c		f	

Scheme 1

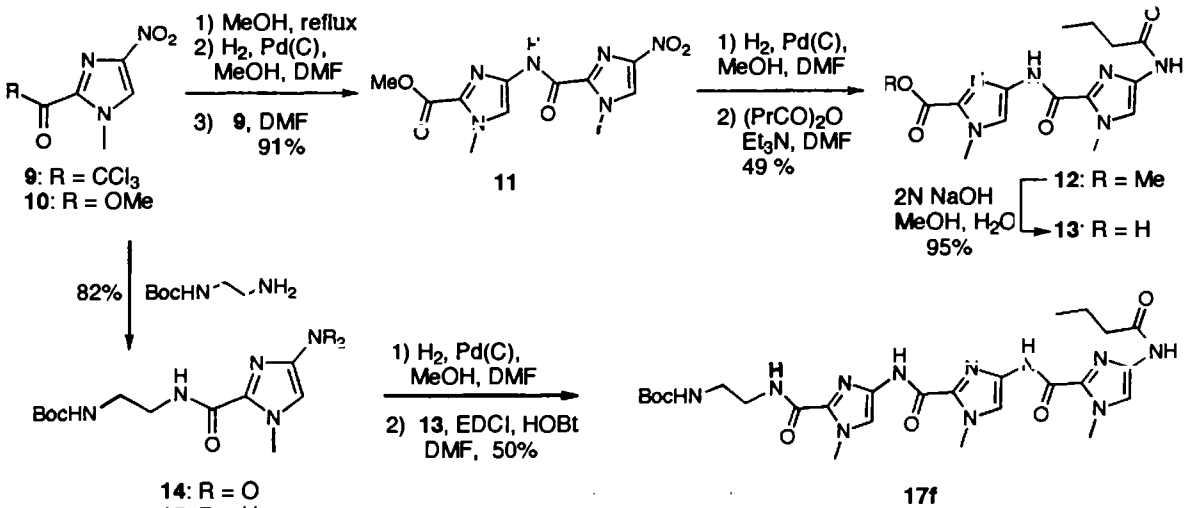


show promise in their cleaving activities against supercoiled DNA (9). Further studies by means of autocleavage reactions revealed that the selectivities may be solely attributed to the DNA binding vectors (10). We report herein further synthetic studies based on these findings in the design and synthesis of BLM models in which are incorporated lexitropsin moieties other than distamycin.

Recent progress in the design of lexitropsins have made it possible to synthesize DNA-binding vectors with diverse site selectivities in 4 -5 base-pair binding site. For instance, structural modification of netropsin and distamycin by replacement of N-methylpyrrole units with a increasing numbers of other five membered aromatic heterocyclic systems such as N-methylimidazole and thiazole led to the lexitropsins with a increasing capacity to recognize and accept GC sites (4). We have chosen the oligopeptide carrier moieties (R in Table) **b** - **f**, reported by Dervan (11), Lown (12) and Henichart (13) et al. by the criterion that the selectivities change gradually from AT-rich region to CG-rich region.

The DNA binding vectors **16c** - **e** were synthesized as shown in Scheme **1**. Condensation of commercially available thiocarboxamide **2** with ethyl bromopyruvate in ethanol at ambient temperature provided bithiazole **3** quantitatively. Subsequent hydrolysis of **3** afforded the corresponding acid **16e** in a yield of 75%. Reactions of the bipyrrole moiety **5**, derived from the catalytic hydrogenation of the nitro- derivative **4** (9), with imidazole **7** (14) and **16e**, followed by hydrolysis gave rise to **16c** and **16d**, respectively, in good yield.

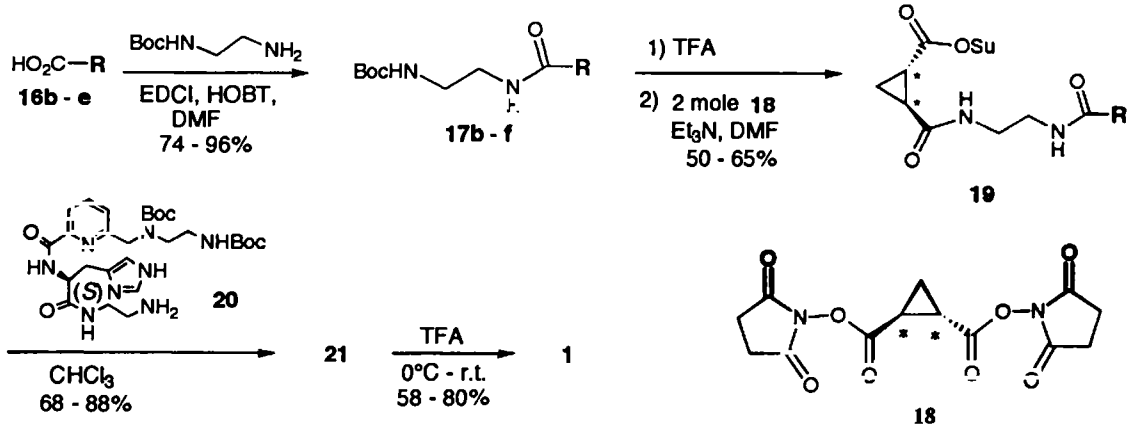
Compound **17f** was synthesized by a different procedure to avoid handling of triimidazole intermediates.(Scheme 2) Reduction of **10** derived from the reflux of **9** (14) in methanol, followed by condensation with **9** provided the diimidazole unit **11** in a yield of 91%. Similar reduction, subsequent acylation with butyric anhydride in DMF and hydrolysis resulted in acid **13**. Coupling reaction of **13** with **15**, a reduction product of **14** derived from condensation of **9** with N-Boc ethylenediamine, in the presence of EDCI, HOBt, provided **17f** in 50% yield.



Scheme 2

Coupling of **16b** (9) and **16c** - e with N-Boc ethylenediamine in the presence of EDCI, HOBt in DMF provided **17**d- **e**, respectively, in the yields in the range of 74 - 96% (Scheme 3). Deprotection of **17**, followed by reaction with excess of (1*S*,2*S*)- and (1*R*,2*R*)- *N*-succinimidyl cyclopropanedicarboxylate **18**, which was prepared by a literature method (15), provided both of the (*S*,*S*) and (*R*,*R*) diastereomers of **19b** - **f** in modest yields. The reaction of the active monoesters **19** with the metal-chelating domain **20** (9) afforded the protected

hybrids **21** in yields of 68 - 88%. Deprotection of **21** with trifluoroacetic acid at 0°C to room temperature for 30 minutes, followed by gel filtration on Amberlite XAD-2 resulted in the final product **1** (16) (Scheme 3).



Scheme 3

A study of the cleavage of supercoiled covalently closed circular DNA by (*S,S*) and (*R,R*) - **1a - f** in the presence of Fe(II) ion was conducted by agarose gel electrophoresis experiment. After incubation of the Fe(II) complexes at 20 - 80 μM with PM-2 DNA and 1,4-dithiothreitol for 30 minutes, the reaction mixtures were loaded on 1% gel. Under the experiment conditions, all of the complexes converted CCC DNA (form I) to OC (form II) DNA. Detailed studies of the mechanism and selectivity of DNA cleavage including autocleavage reactions via autoradiography, and cytotoxicity will be reported in due course.

Acknowledgement

This work was supported by a grant (to J.W.L.) from the Natural Sciences and Engineering Research Council of Canada.

References

(1) K. C. Nicolaou, and W. -M. Dai, Angew. Chem. Int. Ed. Engl. 30, 1387 (1991)

(2) M. A. Warpehoski, and L. H. Hurley, Chem. Res. Toxicol. 1, 315 (1988)

(3) Reviews: a) S. M. Hecht, Acc. Chem. Res. 19, 383 (1986); b) J. Stubbe, and J. W. Kozarich, Chem. Rev. 87, 1107 (1987); c) S. M. Hecht, Bioconjugate Chem. 5, 513 (1994); d) L. Huang, J. C. Quada, Jr., and J. W. Lown, Current Med. Chem., in press

(4) Reviews: a) J. W. Lown, Chemtracts: Org. Chem. 6, 205 (1993); b) J. W. Lown, Antiviral Research 17, 179 (1992); c) J. W. Lown, Anti-Cancer Drug Design 3, 25 (1988); d) J. W. Lown, In Molecular Basis of Specificity in Nucleic Acid-Drug Interactions, B. Pullman, and J. Jortner (Ed.), Kluwer Acad. Publishers: Dordrecht, Netherlands, 1990, p 103

(5) For example: a) T. M. Bregant, J. Groppe, and R. D. Little, J. Am. Chem. Soc. 116, 3635 (1994); b) M. F. Semmelhack, and J. J. Gallagher, J. Org. Chem. 59, 4357 (1994); c) S. T. Sigurdsson, S. M. Rink, and P. B. Hopkins, J. Am. Chem. Soc. 115, 12633 (1993)

(6) V. F. Zarytova, D. S. Sergeyev, and T. S. Godovikova, Bioconjugate Chem. 4, 189 (1993)

(7) M. G. Oakley, K. D. Turnbull, and P. B. Dervan, Bioconjugate Chem. 5, 242 (1994)

(8) a) M. Otsuka, T. Masuda, A. Haupt, M. Ohno, T. Shiraki, Y. Sugiura, and K. Maeda, J. Am. Chem. Soc. 112, 838 (1990); b) T. Owa, A. Haupt, M. Otsuka, S. Kobayashi, N. Tomioka, A. Itai, M. Ohno, T. Shiraki, M. Uesugi, Y. Sugiura, and K. Maeda, Tetrahedron 48, 1193 (1992)

(9) a) L.Huang, R. Morgan, and J. W. Lown, BioMed. Chem. Lett. 3, 1751 (1993) b) L. Huang, J. C. Quada, Jr., and J. W. Lown, Tetrahedron Lett. 35, 5323 (1994); c) L. Huang, and J. W. Lown, Heterocycles, 1995, in press

(10) a) L. Huang, J. C. Quada, Jr., and J. W. Lown, Bioconjugate Chem. 1995, in press; b) L. Huang, J. C. Quada, Jr., and J. W. Lown, BioMed. Chem. (1995), in press

(11) a) W. S. Wade, M. Mrksich, and P. B. Dervan, J. Am. Chem. Soc. 114, 8783 (1992); b) M. Mrksich, W. S. Wade, T. J. Dwyer, B. H. Geierstanger, D. E. Wemmer, and P. B. Dervan, Proc. Natl. Acad. Sci. USA 89, 7586 (1992)

(12) a) D. Mrani, G. Gosselin, C. Bailly, R. Houssin, K. E. Rao, J. Zimmermann, J. Balzarini, E. D. Clercq, J. P. Henichart, J. L. Imbach, and J. W. Lown, Eur. J. Med. Chem. 27, 331 (1992); b) C. Bailly, P. Colson, C. Houssier, R. Houssin, D. Mrani, G. Gosselin, J. L. Imbach, M. J. Waring, J. W. Lown, and J. P. Henichart, Biochemistry 31, 8349 (1992)

(13) K. Krowicki, and J. W. Lown, J. Org. Chem. 52, 3493 (1987)

(14) E. Nishiwaki, S. Tanaka, H. Lee, M. Shibuya, Heterocycles 27, 1945 (1988)

(15) a) A. Misumi, K. Iwanaga, K. Furuta, and H. Yamamoto, J. Am. Chem. Soc. 107, 3343 (1985); b) K. Furuta, K. Iwanaga, and H. Yamatmoto, Org. Synth. 67, 76 (1989); c) R. Knorr, A. Trzeciak, W. Bannwarth, and D. Gillessen, Tetrahedron Lett. 30, 1927 (1989)

(16) All new compounds give satisfied IR, ^{1}H-NMR (300 MHz) and HRFABMS data. For (1*R*,2*R*)-**1d**: R_f (AcOH:*n*-BuOH:H_2O = 1:1:1): 0.28; $[\alpha]^{20}_D$ -19° (c 0.42, $CHCl_3$:MeOH = 7:3); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 10.23 (s, 1H, -CONH-), 9.97 (s, 1H, -CONH-), 8.95 (br, 1H, -CONH-), 8.42 (br, 1H, -CONH-), 8.38 (br, 1H, -CONH-), 8.34 (s, 1H), 8.21 (s, 1H), 8.16 (br, 1H, -CONH-), 8.06 (br, 1H, -CONH-), 7.93 (t, J = 7.0 Hz, 1H), 7.87 (d, J = 7.0 Hz, 1H), 7.63 (d, J = 7.0 Hz, 1H), 7.55 (s, 1H), 7.35 (s, 3H), 7.21 (s, 1H), 7.17 (s, 1H), 6.87 (s, 1H), 6.82 (s, 1H), 4.58 (m, 1H), 3.87 (s, 5H), 3.80 (s, 3H), 3.25 - 2.98 (m, 10H), 2.75 (s, 3H), 2.70 (t, J = 6.0 Hz, 2H), 2.60 (t, J = 6.0 Hz, 2H), 1.93 (m, 2H), 1.03 (m, 2H); HRFABMS m/e calcd for $C_{44}H_{52}N_{16}O_7S_2H$ (M^++H): 981.3725, found: 981.3677. For (1*S*,2*S*)-**1f**: R_f (AcOH:*n*-BuOH:H_2O = 1:1:1): 0.18; $[\alpha]^{20}_D$ +36° (c 0.22, $CHCl_3$:MeOH = 7:3); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 10.35 (s, 1H, -CONH-), 8.95 (br, 1H, -CONH-), 8.43 (br m, 1H, -CONH-), 8.33 (br m, 1H, -CONH-), 8.17 (br m, 1H, -CONH-), 7.93 (t, J = 7.5 Hz, 1H), 7.86 (d, J = 7.5 Hz, 1H), 7.65 (s, 1H), 7.62 (d, J = 7.5 Hz, 1H), 7.55 (s, 1H), 7.54 (s, 1H), 7.53 (s, 1H), 6.82 (s, 1H), 4.61 (m, 1H), 4.02 (s, 3H), 3.98 (s, 3H), 3.97 (s, 3H), 3.88 (s, 2H), 3.40 - 2.96 (m, 10H), 2.71 (t, J = 6.0 Hz, 2H), 2.60 (t, J = 6.0 Hz, 1H), 2.29 (t, J = 7.5 Hz, 1H), 1.92 (t, J = 7.0 Hz, 2H), 1.58 (sex, J = 7.5 Hz, 2H), 1.03 (m, 2H), 0.89 (t, J = 7.5 Hz, 3H); HRFABMS m/e calcd for $C_{43}H_{57}N_{19}O_8H$ (M^++H): 968.4716, found: 968.4736.

Received February 13, 1995