Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 20, pp. 2617-2622, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)10030-0

SYNTHESIS AND EVALUATION OF THE HYBRID MOLECULES POSSESSING DNA-CLEAVING ACTIVITY

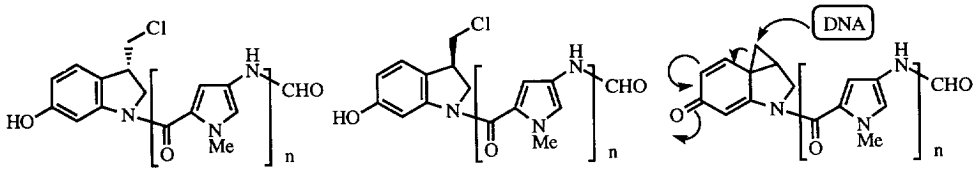
Kozo Shishido,*[a] Shigenori Haruna,[a] Chisato Yamamura,[a] Hiromi Iitsuka,[b] Hisao Nemoto,[b] Yasuo Shinohara[b] and Masayuki Shibuya*[b]

[a] Institute for Medicinal Resources, University of Tokushima, Sho-machi 1, Tokushima 770, Japan

[b] Faculty of Pharmaceutical Sciences, University of Tokushima, Sho-machi 1, Tokushima 770, Japan

Abstract: The design and synthesis of enantiomerically enriched hybrid molecules, **1a-c** and **2a-c**, have been accomplished by employing the lipase-mediated asymmetric acetylation of prochiral diol **9** as the key step. Evaluation of their DNA-cleaving activity has revealed the unnatural type of enantiomer **2a-c** to be more potent than **1a-c** with natural configuration.

The design and synthesis of artificial agents, which possess DNA-cleaving activity, have become an increasingly valuable approach for developing novel types of antitumor drugs.[1] During the course of our studies directed towards the exploration of potent and sequence-specific DNA-cleaving agents based on the natural product,[2] we intended to synthesize the enantiomeric hybrid molecules, **1a-c** and **2a-c**, and to evaluate their activity and sequence-specificity. Our designed molecule is constituted of both a DNA-alkylating structural unit and the moiety responsible for the sequence-selective recognition of DNA.[3] As the alkylating part, we chose a chemically stable 3-chloromethyl-6-hydroxy-2,3-dihydroindole,[4] which is a penultimate intermediate for the pharmacophore moiety in **3** commonly present in CC-1065 and in duocarmycins.[5] On the other hand, the different sized pyrrole amides **5a-c** found in lexitropsins were selected as the DNA minor groove binder.[6] (Fig. 1)



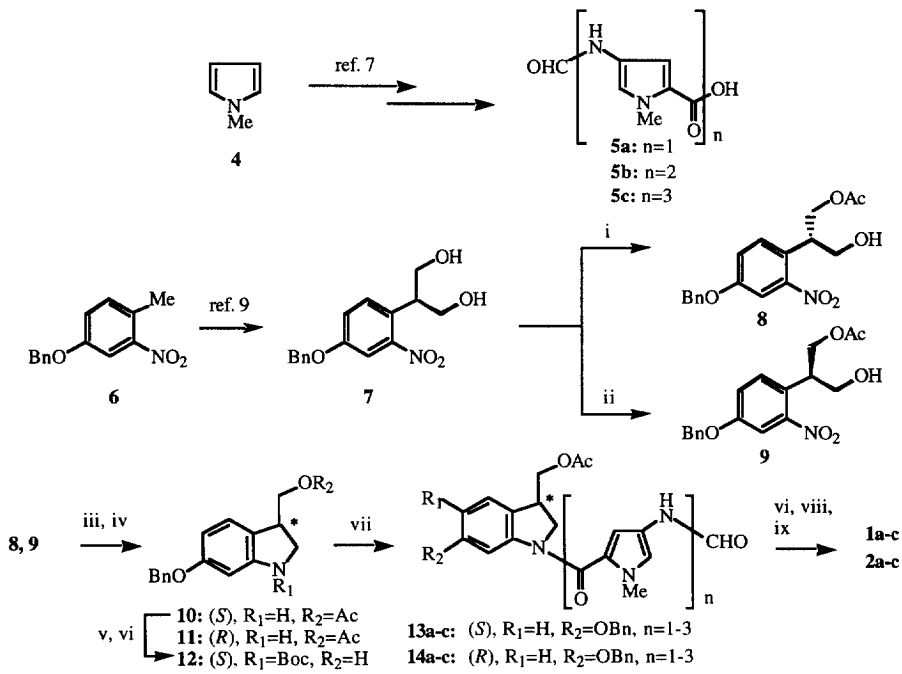
1a-c: n=1-3 **2a-c:** n=1-3 **3a-c:** n=1-3

Figure 1

Syntheses of the Hybrid Molecules and Their DNA-Cleaving Activities

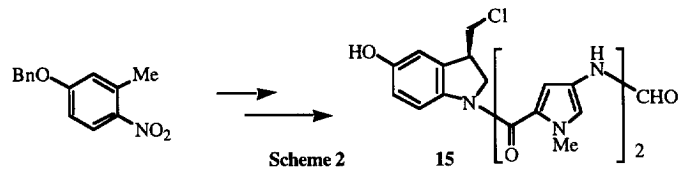
Preparation of three types of pyrrole carboxylic acids **5a-c** was achieved as shown in Scheme 1 by the procedure developed in our laboratories,[7] while both enantiomers of the alkylating part were synthesized by employing the lipase-mediated asymmetric acetylation[8] of the prochiral diol **7**,[9] which was readily derived

from 4-benzyloxy-2-nitrotoluene **6**. A variety of lipases was examined to search for the optimum conditions. Of these, Lipase AK-catalyzed conditions using vinyl acetate as an acyl donor in *tert*-butyl methyl ether proved to be the best choice and *S*-monoacetate **8** was obtained. On the contrary, for the *R*-isomer **9**, PPL-catalyzed conditions[10] produced the best result. Thus, the enantiomerically enriched monoacetates **8** and **9** were obtained in 88% and 98% yield, respectively. The enantiomeric excesses were 98% and 88%, respectively, as determined by ^{1}HNMR analysis of their MTPA ester derivatives. Although the absolute configuration of both enantiomers could not be elucidated at this stage, the determination was made by the following conversion. Independent mesylation of **8** and **9** followed by catalytic hydrogenation in the presence of triethylamine provided the indolines, **10** and **11**.[11] To confirm the absolute structure, the acetate **10** was treated with di-*tert*-butyl dicarbonate to give the N-Boc derivative which was then hydrolized to afford the alcohol **12** in 33% overall yield from **8**. The specific rotation of **12** thus obtained showed +24.1 (for the *R*-**12**,[12] $[\alpha]_D$ -25.1) and the absolute configuration of **8** was unambiguously established to be *S*. Condensation of **10** and **11** with **5a-c** was accomplished by using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDCI•HCl) to provide the coupled amides, **13a-c** and **14a-c**, in moderate yields. Finally, sequential alkaline hydrolysis, chlorination and debenzylation produced the requisite hybrid molecules, **1a-c** and **2a-c**. (Scheme 1)



Scheme 1. ***Reagents and Conditions***: i, Lipase AK, vinyl acetate, ^{t}BuOMe; 88%; ii, PPL, vinyl acetate, Et_2O; 98%; iii, MsCl, Et_3N, CH_2Cl_2; iv, H_2, PtO_2, Et_3N, THF; v, $(Boc)_2O$, Et_3N, CH_2Cl_2; vi, LiOH, THF:H_2O (3:1); 33% from **8**; vii, **5a-c**, EDCI•HCl, DMF; for **13a**, 54%; for **13b**, 45%; for **13c**, 24% from **8**; for **14a**, 32%; for **14b**, 67%; for **14c**, 67% from **9**; viii, Ph_3P, CCl_4, CH_2Cl_2; ix, BBr_3, CH_2Cl_2; for **1a**, 65% from **13a**; for **1b**, 21% from **13b**; for **1c**, 3% from **13c**; for **2a**, 13% form **14a**; for **2b**, 19% from **14b**; for **2c**, 4% from **14c**.

DNA-cleaving activities of the synthesized **1** and **2** were assayed with supercoiled plasmid Col E1 (0.25 μg) in Tris-EDTA buffer (pH 7.8) at 37 °C.[2] DNA strand cleavage was estimated on agarose gels by conversion of the covalently closed circular (Form I) DNA initially open circular (Form II) and finally to linear duplex form (Form III). After electrophoresis, each DNA was quantitated by ethidium bromide staining and densiometry. While the hybrid molecules **1a-c** with natural configuration showed moderate DNA-cleaving activity, the unnatural counterparts **2a-c** unexpectedly[13] exhibited potent activities depending upon the length of the binding moiety,[14] drug concentrations and reaction times as shown in Fig. 2. Since the regioisomer *R*-**15**, which was derived analogously from 3-benzyloxy-2-nitorotoluene, did not show any activity, it was concluded that the possibility of direct alkylation of DNA at the chlorine-bearing carbon should be ruled out and the intervention of the dienone **3**, which may be generated via the facile cyclopropane ring closure under the conditions of assay,[15] was strongly suggested. (Scheme 2)



Scheme 2 **15**

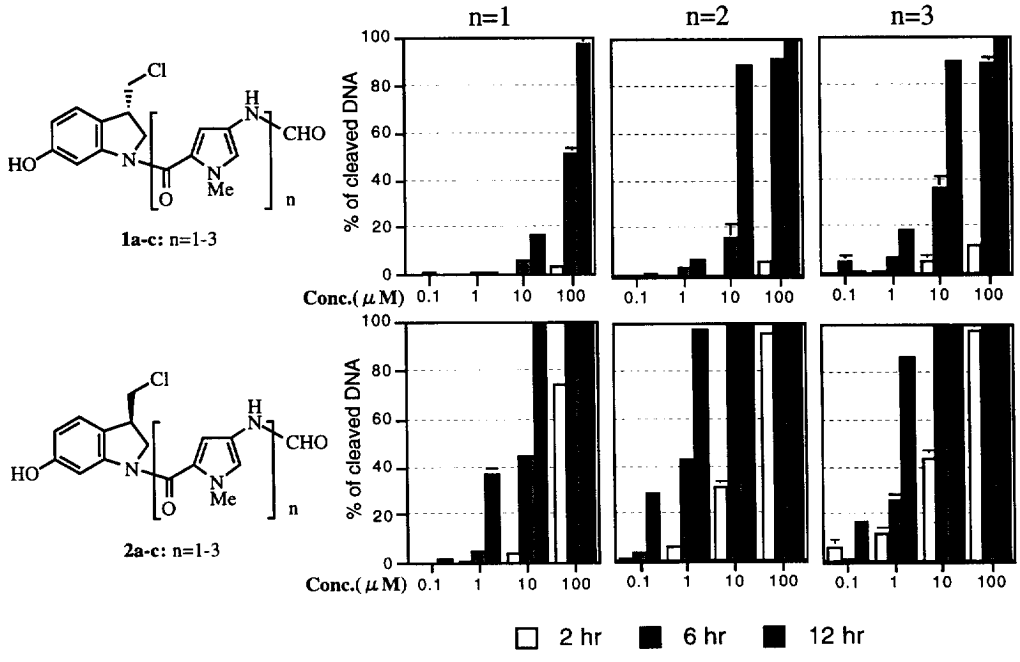


Figure 2. DNA-cleaving activity of **1a-c** and **2a-c** using supercoiled plasmid Col E1 (0.25 μg) in Tris-EDTA buffer (pH 7.8) at 37 °C.

DNA Alkylation Property

The DNA alkylation reaction employing the drug **2b** was examined with the singly 5' end-labeled fragment derived from pUC 19 plasmid (ca. 240 bps). After treatment of the labeled DNA samples with a range of drug concentrations at 37 °C for 2h, DNA fragments were recovered by ethanol precipitation. DNA samples thus obtained were denatured in solutions containing formamide at 95 °C for 5 min and subjected to electrophoresis with Sanger sequence ladder as standards. From the autoradiography shown in Fig. 3, it was clarified that the strand cleavage took place in the A-T rich regions depending upon the concentrations of the drug, and the site of alkylation was found to be mainly adenine.[15a, 16] The results thus obtained from the sequencing studies were consistent with those of duocarmycins. It is also noteworthy that the alkylated DNA with **2b** was smoothly led to the strand cleavage without any thermal treatments. (Figure 3)

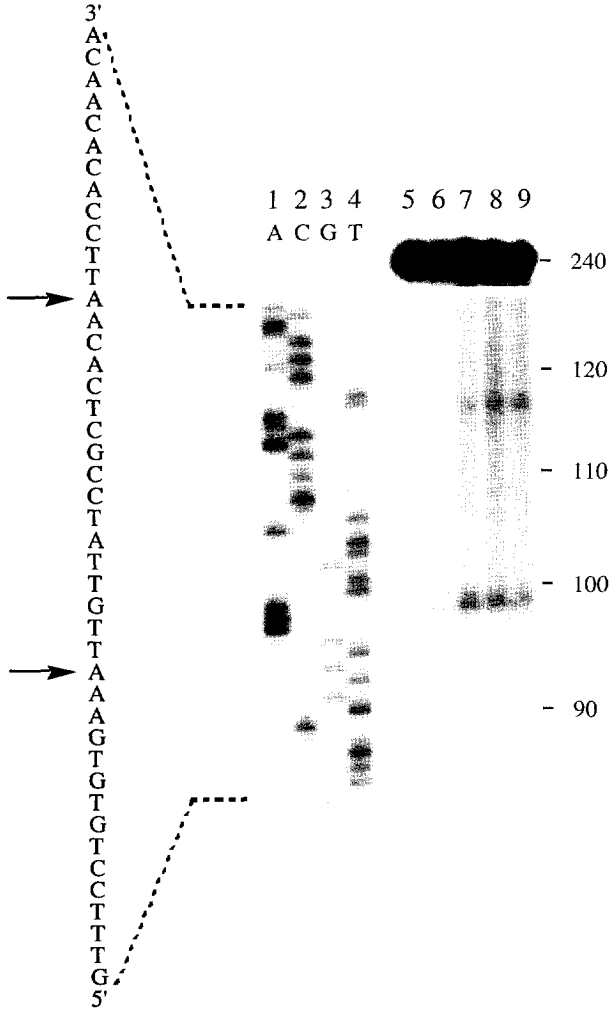
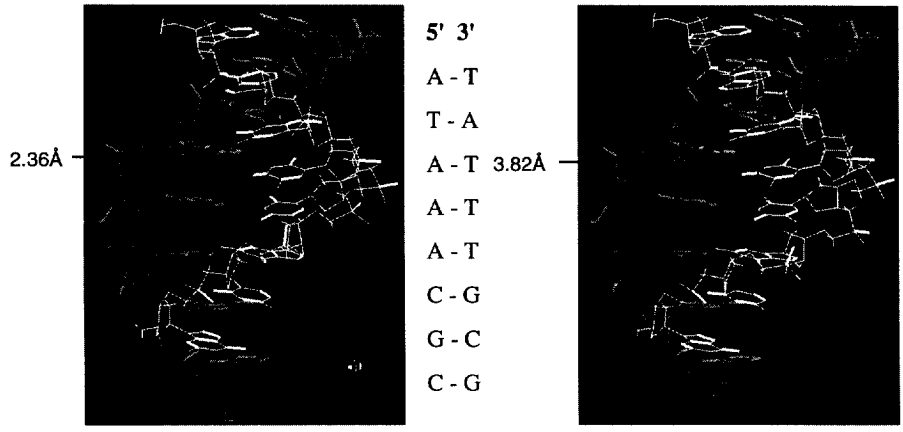


Figure 3. Strand cleavage of pUC 19 plasmid DNA at 37 °C for 2 h: lane 1-4, Sanger A, C, G, and T sequencing reactions; lane 5, control; lane 6, 1mM of **2b**; lane 7, 5 mM; lane 8, 10 mM; lane 9, 50 mM. Lengths of single strand DNA, in base, are shown to the right.

Molecular Modeling of the DNA Alkylation for Enantiomeric Drugs

To clarify the remarkable differences in the DNA-cleaving activities between two enantiomers **1** and **2**, the energy-minimized conformations of the interaction models (*R*-**3b** and *S*-**3b**)-DNA were compared as follows. The initial coordinates of *R*-**3b**/DNA and *S*-**3b**/DNA complexes for MM2" calculation in MacroModel ver. 4.5 [17] were prepared based on an X-ray coordinate of the netropsin/DNA complex[18] registered in The Protein Data Bank. After the coordinate of the complex was read into MacroModel software, some water around the complex and several nucleoside residues far from the netropsin moiety were deleted to simplify the energy minimization. (5'-ATAAACGC-3' and 3'-TATTTGCG-5' were retained) One terminus of netropsin (Me_2N-CH_2-CH_2-NH-) was deleted and the pharmacophore was introduced instead to give our designed molecules *R*-**3b** and *S*-**3b**, respectively. Except the pharmacophore, all coordinates of the atoms were constrained (value=4000) to fix DNA spirals and the dipyrrole moiety during the energy minimization with solvation treatment in water based on the assumption that these two enantiomers show similar affinity for DNA duplex because the affinity of dipyrrol moiety for DNA molecule was expected to be much stronger than that of pharmacophore moiety. As shown in Fig. 4, it would be presumed that the difference in cleaving activities can be attributed to the distinct difference in distance (3.82Å and 2.36Å) between the least substituted carbon of the activated cyclopropane and adenine-N3. (Figure 4)



R-**3b**/DNA *S*-**3b**/DNA

Figure 4

In these studies, it has been clarified that the hybrid molecule *R*-**2** possessing unnatural configuration shows more potent DNA-cleaving activity than the *S*-counterpart **1**. The site of alkyklation has been proved to be adenine by the sequencing studies. Additionally, the difference in activity of both enantiomers can be attributed to the proximity of the alkylated carbon to adenine-N3.

Acknowledgement: The authors are grateful to Dr. Yoshihiko Hirose, Amano Pharmaceutical Co., Ltd., for providing Lipases.

References and Notes

1. Nielsen, P. E. *J. Mol. Recogn.* **1990**, *3*, 1-25; Sigman, D. S.; Chen, C. B. *Annu. Rev. Biochem.* **1990**, *59*, 207-236.
2. Nishiwaki, E.; Lee, H.; Matsumoto, T.; Toyooka, K.; Sakurai, H.; Shibuya, M. *Tetrahedron Lett.* **1990**, *31*, 1299-1302; Sakai, Y.; Bando, Y.; Shishido, K.; Shibuya, M. *Tetrahedron Lett.* **1992**, *33*, 957-960.
3. Baker, B. F.; Dervan, P. B. *J. Am. Chem. Soc.* **1989**, *111*, 2700-2712.
4. Boger, D. L.; Machiya, K. *J. Am. Chem. Soc.* **1992**, *114*, 10056-10058; Boger, D. L.; Palanki, M. S. S. *J.. Am. Chem. Soc.* **1992**, *114*, 9318-9327; Boger, D. L.; Machiya, K.; Hertzog, D. L.; Kitos, P. A.; Holmes, D. *J. Am. Chem. Soc.* **1993**, *115*, 9025-9036; Boger, D. L.; Han, N.; Tarby, C. M.; Boyce, C. W.; Cai, H.; Jin, Q. Kitos, P. A. *J. Org. Chem.* **1996**, *61*, 4894-4912.
5. For a pertinent review, see; Boger, D. L.; Johonson, D. S. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1438-1474.
6. Goodsell, D. S.; Ng, H. L.; Kopka, M. L.; Lown, J. W.; Dickerson, R. E. *Biochemistry* **1995**, *34*, 16654-16661; Lee, M.; Roldan, M. C.; Haskell, M. K.; McAdam, S. R.; Hartley, J. A. *J. Med. Chem.* **1994**, *37*, 1208-1213; Anneheim-Herbelin, G.; Perrée-Fauvet, M.; Gaudemer, A.; Helissey, P.; Giorgi-Renault, S.; Gresh, N. *Tetrahedron Lett.* **1993**, *34*, 7263-7266.
7. Nishiwaki, E.; Tanaka, S.; Lee, H.; Shibuya, M. *Heterocycles* **1988**, *27*, 1945-1952.
8. Guanti, G.; Narisano, T.; Podgorski, Thea, S.; Williams, A. *Tetrahedron* **1990**, *46*, 7081-7092; Kosaka, T.; Bando, T.; Shishido, K. *Chem. Commun.* **1997**, 1167-1168.
9. Warpehoski, M. A.; Gebhard, I.; Kelly, R. C.; Krueger, W. C.; Li, L. H.; McGovren, J. P.; Prairie, M. D.; Wicnienski, N.; Wierenga, W. *J. Med. Chem.* **1988**, *31*, 590-603.
10. The same conversion using PPL has been reported; Chênevert, R.; Courchesne, G. *Chem. Lett.* **1997**, 11-12.
11. Fukuda, Y.; Itoh, Y.; Nakatani, K.; Terashima, S. *Tetrahedron* **1994**, *50*, 2793-2808.
12. Boger, D. L.; Wysocki, R. J.; Ishizaki, T. *J. Am. Chem. Soc.* **1990**, *112*, 5230-5240.
13. Boger, D. L.; Yun, W.; Terashima, S.; Fukuda, Y.; Nakatani, K.; Kitos, P. A.; Jin, Q. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 759-765.
14. Boger, D. L.; Johnson, D. S.; Yun, W.; Tarby, C. M. *Bioorg. Med. Chem.* **1994**, *2*, 115-135; Boger, D. L.; Coleman, R. S.; Invergo, B. J.; Sakya, S. M.; Ishizaki, T.; Munk, S. A.; Zarrinmayeh, H.; Kitos, P. A.; Thompson, S. C. *J. Am. Chem. Soc.* **1990**, *112*, 4623-4632.
15. a) Boger, D. L.; Johnson, D. S.; Yun, W. *J. Am. Chem. Soc.* **1994**, *116*, 1635-1656. b) Boger, D. L.; Ishizaki, T.; H. Zarrinmayeh, Munk, S. A.; Kitos, P. A.; Suntornwat, O. *J. Am. Chem. Soc.* **1990**, *112*, 8961-8971.
16. Boger, D. L.; Ishizaki, T.; Zarrinmayeh, H. *J. Am. Chem. Soc.* **1991**, *113*, 6645-6649.
17. Still, W. C. Columbia University.
18. Gurskaya, G. V.; Grakhovskii, S. L.; Zhuze, A. L.; Gottikh, B. P. *Biochim. Biophys. Acta* **1979**, 336-342; Grokhovskii, S. L.; Zubarev, V. E. *Nucleic Acid Res.* **1991**, *19*, 257-264; Kopka, M. L.; Yoon, C.; Goodsell, D.; Pjura, P.; dickerson, R. E. *J. Mol. Biol.* **1985**, *183*, 553-563.

(*Received in Japan* 4 *August* 1997; *accepted* 11 *September* 1997)