# SYNTHESIS AND EVALUATION OF ENANTIOMERICALLY PURE AZINOMYCIN-LEXITROPSIN HYBRID MOLECULES WITH DNA-CLEAVING ACTIVITY†

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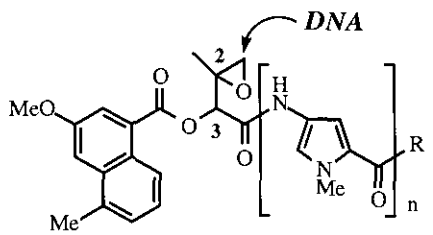
**Abstract** - Several enantiomerically enriched azinomycin-lexitropsin hybrid molecules, (**1a-c**), (**2a-c**), (**3a-c**) and (**12**), were synthesized and their DNA-cleaving activities were evaluated. Of these, the compound (**3c**) with natural configuration was proved to exhibit the strongest activity.

During the course of our investigations directed towards the development of artificial and potent DNA-cleaving agents based on natural products,[1] we designed the enantiomerically pure azinomycin-lexitropsin hybrid molecules (**1a-c**), (**2a-c**), (**3a-c**) and (**12**). These are constituted of both a DNA-alkylating part,[2] the left-hand segment of azinomycins,[3] and the pyrrole amide moieties, which are responsible for the sequence-selective recognition of DNA, found in lexitropsins.[4] In this paper, we report the synthesis of the hybrid molecules and the evaluation of their DNA-cleaving activity. (Figure 1)

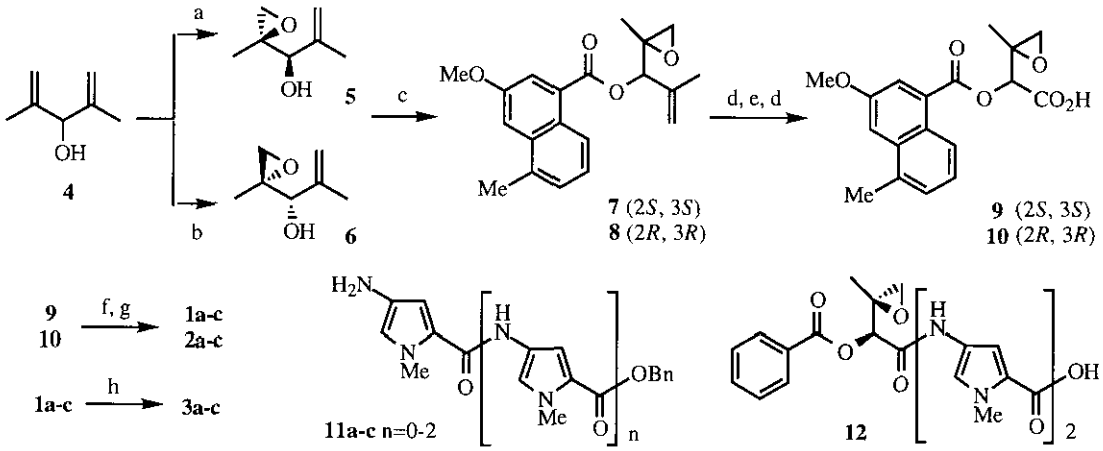



**1a-c** R=OH, (*2S, 3S*), n=1-3
**2a-c** R=OH, (*2R, 3R*), n=1-3
**3a-c** R=$NH(CH_2)_3NMe_2$, (*2S, 3S*), *n=1*

**Figure 1**

According to the procedure described in the previous paper,[3a] the enatiomeric carboxylic acids (**9**) and (**10**) corresponding to the left-hand segment of azinomycins were synthesized as shown in Scheme 1. Sharpless asymmetric epoxidation of prochiral diisopropenylcarbiol (**4**) using D-(-)- and L-(+)-diisopropyl tartrates provided (*S*, *R*)- (**5**) and (*R*, *S*)-epoxy alcohols (**6**),[5] respectively, which were condensed with

3-methoxy-5-methylnaphthalene-1-carboxylic acid[6] with the aid of DCC to give the esters (**7**) and (**8**). Oxidative cleavage of the double bond in **7** and **8** with catalytic amount of osmium tetroxide and sodium metaperiodate (Lemieux-Johnson oxidation) followed by treatment of the resulting methyl ketones with lithium hexamethyldisilazide and methyl chloroformate provided the enol carbonates,[7] which were again exposed to the conditions of Lemieux-Johnson oxidation to give the carboxylic acids (**9**) and (**10**). Condensation of the enantiomeric carboxylic acids thus obtained with the pyrroles (**11a-c**), prepared by the procedure developed in our laboratories,[8] was accomplished by using benzotriazol-1-yloxytripyrrolidino-phosphonium hexafluorophosphate (PyBOP) and hydroxybenzotriazole (HOBT) in the presence of triethylamine to afford the coupled amides, which were debenzylated to give the enantiomeric hybrid molecles (**1a-c**) and (**2a-c**). Other hybrid compounds (**3a-c**), which contain the dimethylaminopropyl appendage[9] found in distamycin A at the terminal position of the minor groove binder, were prepared by treatment of **1a-c** with 3-dimethylaminopropylamine, PyBOP, HOBT and triethylamine. (Scheme 1)




**Scheme 1.** *Reagents and Conditions:* a, D-(-)-DIPT, $Ti(O^iPr)_4$, TBHP, 38%; b, L-(+)-DIPT, $Ti(O^iPr)_4$, TBHP, 31%; c, 3-methoxy-5-methylnaphthalene-1-carboxylic acid, DCC, 4-DMAP, 89% for **7**, 47% for **8**; d, $OsO_4$, $NaIO_4$, 36% for **9** (3 steps), 35% for **10** (3 steps); e, $LiN(TMS)_2$, $ClCO_2Me$; f, **11a-c**, PyBOP, HOBT, $Et_3N$; g, $H_2$, 10% Pd-C, 86% for **1a**, 81% for **1b**, 78% for **1c**, 15% for **2a**, 47% for **2b**, 55% for **2c**; h, $NH_2(CH_2)_3NMe_2$, PyBOP, HOBT, $Et_3N$, 88% for **3a**, 87% for **3b**, 97% for **3c**.

DNA-cleaving activities of the synthesized **1a-c**, **2a-c**, **3a-c** and **12** (*vide infra*) were assayed with supercoiled plasmid Col E1 (0.25 mg) in Tris-EDTA buffer (pH 7.8) at 37 °C.[10] 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. As shown in Figure 2, the hybrid molecules (**1a-c**) with natural configuration at the two stereogenic centers showed more potent activities than the compounds (**2a-c**) with unnatural configuration depending upon the length of the binding moiety, drug concentration and reaction times. In the case of **3a-c**, remarkable enhancement of the activity was observed even at lower concentration of drugs as shown in Figure 3. To evaluate the need of a naphthyl moiety for

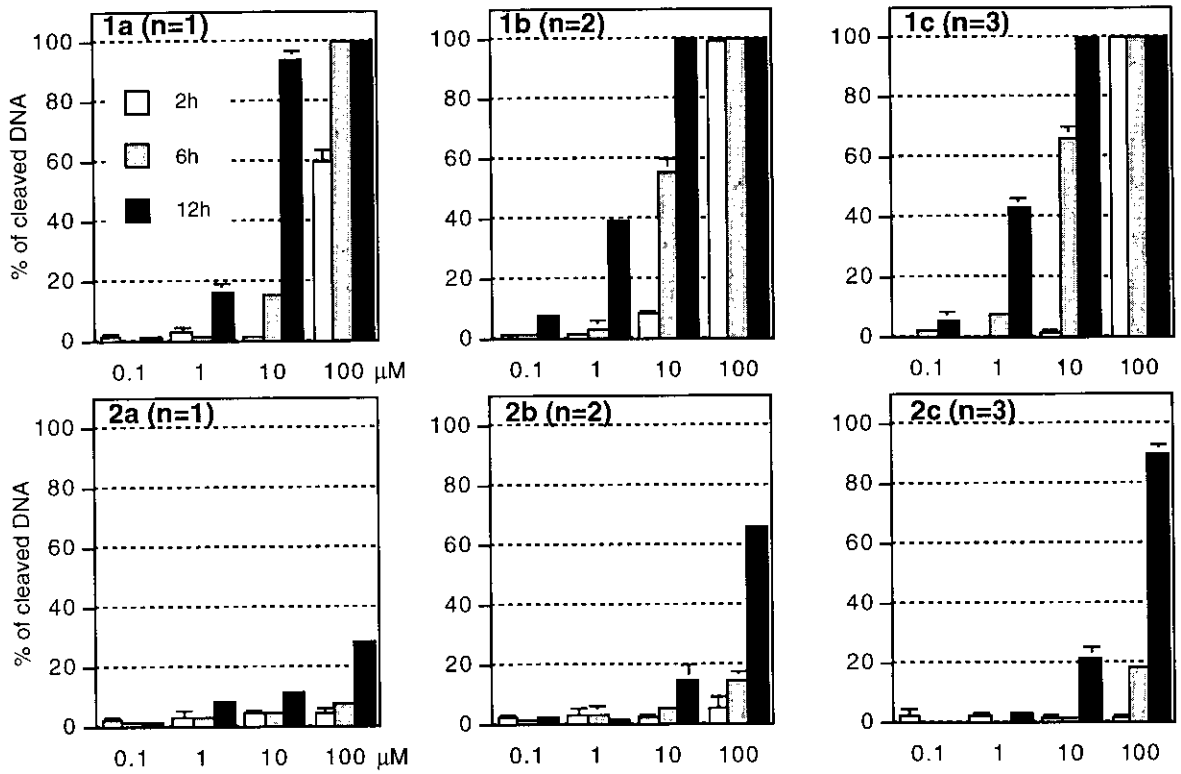



**Figure 2.** DNA-cleaving activity of **1a-c** and **2a-c**.

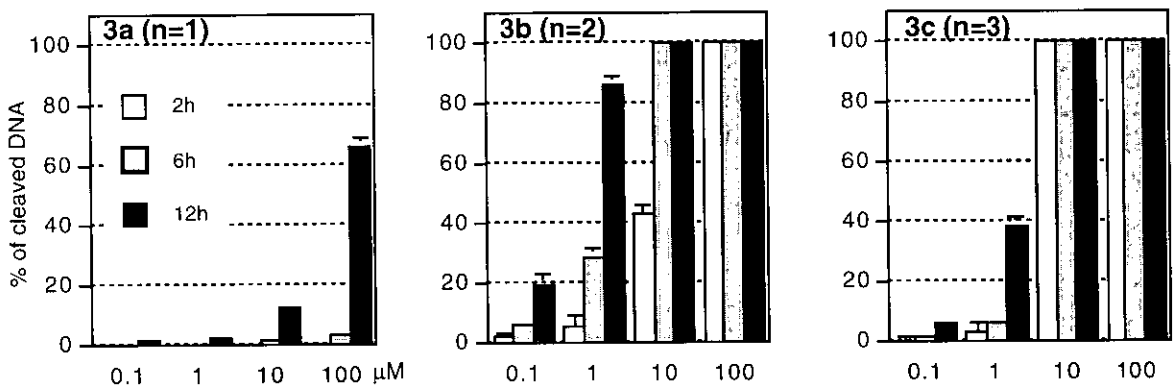



**Figure 3.** DNA-cleaving activity of **3a-c**.

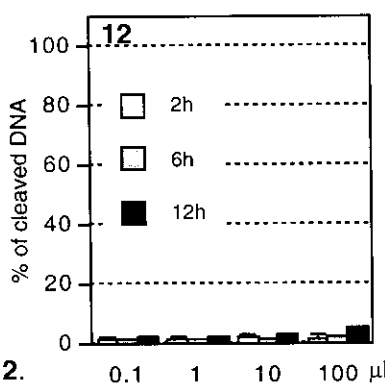



**Figure 4.**
DNA-cleaving activity of **12**.

the activity, the benzoate (**12**) with natural configuration was synthesized, starting from benzoic acid, in 16% overall yield for 6 steps in the same way as described for **1** and **2**. Since it scarcely exhibited the activity at any concentration, the naphthyl group proved to be essential. (Figure 4)

From these experimentations, it has been clarified that the hybrid molecule (**3c**) shows the strongest DNA-cleaving activity of the compounds synthesized. From the structural point of view, both the left-hand segment of azinomycins with natural configuration and the trimeric pyrrole amide moiety with a dimethylaminopropyl appendage as the DNA-binding domain are indispensable for the appearane of potent activity.

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## REFERENCES

† Dedicated to Dr. Bernhard Witkop on the occasion of his 80th birthday.

1. K. Shishido, S. Haruna, C. Yamamura, H. Iitsuka, H. Nemoto, Y. Shinohara, and M. Shibuya, *Bioorg. & Med. Chem. Lett.*, 1997, **7**, 2617.
2. A. Terawaki and J. Greenberg, *Nature*, 1996, **209**, 481; J. W. Lown and K. C. Majumdar, *Can. J. Biochem.*, 1977, **55**, 630; R. W. Armstrong, M. E. Slvati, M. Nguyen, *J. Am. Chem. Soc.*, 1992, **114**, 3144.
3. a) K. Shishido, T. Omodani, and M. Shibuya, *J. Chem. Soc., Perkin Trans. 1*, 1992, 2053. b) For recent reports on enantioselective syntheses of the epoxide fragment, see; H. J. Bryant, C. Y. Dardonville, T. J. Hodgkinson, M. Shipman, and A. M. Z. Slawin, *Synlett*, 1996, 973; R. S. Coleman, C. R. Sarko, and J. P. Gittinger, *Tetrahedron Lett.*, 1997, **38**, 5917; R. S. Coleman and J. D. McKinley, *Tetrahedron Lett.*, 1998, **39**, 3433.
4. B. F. Baker and P. B. Dervan, *J. Am. Chem. Soc.*, 1989, **111**, 2700; D. S. Goodsell, H. L. Ng, M. L. Kopka, J. W. Lown, and R. E. Dickerson, *Biochemistry*, 1995, **34**, 16654; M. Lee, M. C. Roldan, M. K. Haskell, S. R. McAdam, and J. A. Hartley, *J. Med. Chem.*, 1994, **37**, 1208; G. Anneheim-Herbelin, M. Perrée-Fauvet, A. Gaudemer, P. Helissey, S. Giorgi-Renault, and N. Gresh, *Tetrahedron Lett.*, 1993, **34**, 7263.
5. S. L. Schreiber, J. S. Schreiber, and D. B. Smith, *J. Am. Chem. Soc.*, 1987, **109**, 1525.
6. M. Shibuya, *Tetrahedron Lett.*, 1983, **24**, 1175.
7. Both enantiomeric carbonates were obtained as colorless prisms and the recrystallization from benzene made them optically pure.
8. E. Nishiwaki, S. Tanaka, H. Lee, and M. Shibuya, *Heterocycles*, 1988, **27**, 1945.
9. P. B. Schultz, J. S. Tayler, and P. B. Dervan, *J. Am. Chem. Soc.*, 1982, **104**, 6861.
10. E. Nishiwaki, H. Lee, T. Matsumoto, K. Toyooka, H. Sakurai, and M. Shibuya, *Tetrahedron Lett.*, 1990, **31**, 1299; Y. Sakai, Y. Bando, K. Shishido, and M. Shibuya, *Tetrahedron Lett.*, 1992, **33**, 957.

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