Synthesis of a Hybrid Molecule Containing Neocarzinostatin Chromophore Analogue and Minor Groove Binder

Masahiko Tokuda, Kenshu Fujiwara, Takuya Gomibuchi, and Masahiro Hirama*

Department of Chemistry, Faculty of Science, Tohoku University, Sendai 980, Japan

Motonari Uesugi and Yukio Sugiura

Institute for Chemical Research, Kyoto University, Uji, Kyoto 611, Japan

Abstract: A designed hybrid (1) of neocarzinostatin chromophore model and netropsin-type minor groove binder was synthesized and its improved ability of DNA cleavage has been demonstrated.

Design and synthesis of new DNA cleaving molecules are currently a topic of intense research investigations.¹ We have synthesized neocarzinostatin chromophore analogues such as 3-5 and have disclosed their mode of aromatization leading to generation of carbon radicals.² Their DNA cleaving abilities,^{2b} however, are proven to be not so high as those of neocarzinostatin³ and the related enediyne antibiotics.⁴ To improve the DNA cutting ability and render the higher base- and site-selectivities we have designed hybrid molecules containing the DNA cleaving moiety^{2b} and the DNA binder, represented by 2. In this communication we disclose a synthesis of the hybrid 1 possessing a netropsin type minor groove binder.^{1,5,6}



After considerable preliminary experiments we found that the 10 membered ring dienediynone 9 possessing an active ester is a versatile intermediate for preparing the hybrids such as 1. Synthesis of 9 was accomplished from the known racemic intermediate 6^{2a} in 5 steps (Scheme I): Triethylsilyl group of 6 was removed selectively with alkaline MeOH (68%). To the resulting alcohol 7 succinic acid linker was attached, condensed with p-nitrophenol, and t-butyldimethylsilyl ether was hydrolized to afford 8 in 68% overall yield. Swern oxidation of 8 with excess reagents² gave unstable 9⁷ (80% yield). This key intermediate was coupled with netropsin analogue 10, prepared from N-methyl pyrrole according to Shibuya's method,⁸ leading to the racemic hybrid 1 as a pale yellow oil in 55% yield after silica gel chromatography (eluent: EtOH to 3% Et₃N/EtOH).^{7,9}



Sc	he	m	e	I
----	----	---	---	---

Synthesis of the optically active (S)-1 was also achieved as shown in Scheme II. Readily available (dl)-11¹⁰ was converted to acetate (dl)-12 by conventional manner (5 steps, 76% yield). This t-butyldiphenyl-silyl (TBPS) ether monoacetate (dl)-12 turned out to be a good substrate for enzymatic asymmetric hydrolysis by the lipoprotein lipase Amano III[®] to afford the optically active alcohol 13 { $[\alpha]_D^{24}$, +0.72° (c 1.2, CHCl₃), 3S, 5R : 97% ee} and (3R, 5S)-12 { $[\alpha]_D^{24}$ +20.4° (c 0.89, CHCl₃), 81% ee} in 45% and 53% yield, respectively.¹¹ Oxidation and bromination of 13 gave 14 { $[\alpha]_D^{26}$ +30.2° (c 1.0, CHCl₃), 52% yield for 2 steps} and 1,2-addition of propargyl magnesium bromide proceeded smoothly to afford 15 { $[\alpha]_D^{28}$ +24.1° (c 1.1, CHCl₃)} in 94% yield as a 12:1 diastereomeric mixture. To remove the protecting group of the primary alcohol selectively at the later stage, the TBPS group of 15 was replaced by the triethylsilyl (TES) and the resulting TES ether 16 {83% yield for 2 steps, $[\alpha]_D^{26}$ +24.0° (c 1.0, CHCl₃)} was transformed to (S)-1 { $[\alpha]_D^{26}$ +4.2° (c 1.0, CHCl₃)} via (12R)-6 { $[\alpha]_D^{27}$ +62.1° (c 1.0, CHCl₃)} as described for the racemic series^{2a} (vide supra).

Thiol triggering aromatization of 1 was caused smoothly by addition of 3 equivalent of methyl thioglycolate in a degassed ethanol solution at 25°C without addition of an external base² and completed within 40 min, yielding the indane derivative 18 in 64% yield probably through mercapto hydrogen abstraction from the excess thiol by diradical intermediate 17 (Scheme III).^{2a}

The racemic hybrid 1 cleaved the covalently closed supercoiled pBR322 DNA (form I) to the open circular DNA (form II) and the linear DNA (form III) more effectively than 3 and 4 as shown in Figure 1.

Thus, the several times augmentation in DNA cutting ability by combining the netropsin-type DNA binding unit to the chromophore analog has been demonstrated. The precise mechanism, the effect of stereochemical difference, and the site selectivity of the DNA cleavage will be reported in due course.



Scheme III

Acknowledgement. This work was supported by the Ministry of Education, Science and Culture, Japan (Grant-in-Aid for Scientific Research on Priority Areas), and by the Mitsubishi, the CIBA-GEIGY, the Shorai, and the TERUMO Science Foundations.



Figure 1. Agarose gel electrophoretic patterns of ethidium bromide stained pBR322 DNA, after treatment with NCS chromophore models (0.32 mM) in the presence of methyl thioglycolate (10 mM) at 37°C for 18 h (pH 7.5). Lane 1: DNA alone, lane 2: 4, lane 3: 1, lane 4: 3.

REFERENCES AND NOTES

- 1. (a) Dervan, P. B. Science (Washington, D.C.) 1986, 232, 464. (b) Nicolaou, K. C.; Dai, W.-M. Angew. Chem. Int. Ed. Engl. 1991, 30, 1387.
- (a) Hirama, M.; Tokuda, M.; Fujiwara, K. Synlett, 1991, 651. (b) Hirama, M.; Gomibuchi, T.; Fujiwara, K.; Sugiura, Y.; Uesugi, M. J. Am. Chem. Soc. 1991, 113, 9851. (c) Hirama, M. J. Synth. Org. Chem. Jpn. 1991, 49, 1032.
- 3. Lee, S. H.; Goldberg, I. H. Biochemistry, 1989, 28, 1019, references therein.
- 4. Nicolaou, K. C.; Dai, W.-M.; Tsay, S.-C.; Estevez, V. A.; Wrasidlo, W. Science, 1992, 256, 1172, references therein.
- 5. Kopka, M. L.; Yoon, C.; Goodsell, D.; Pjura, P.; Dickerson, R. E. Proc. Natl. Acad. Sci. USA, 1985, 82, .1376.
- 6. Otsuka, M.; Masuda, T.; Haupt, A.; Ohno, M.; Shiraki, T.; Sugiura, Y.; Maeda, K. J. Am. Chem. Soc. 1990, 112, 838.
- 7. Be stored in solution in refrigerator.
- 8. Nishiwaki, E.; Tanaka, S.; Lee, H.; Shibuya, M. Heterocycles, 1988, 27, 1945.
- 9. ¹H NMR (400 MHz, CDCl₃): δ 1.29 (6H, s), 1.69 (1H, dddd, J=14.0, 8.0, 6.8, 6.2 Hz), 1.74 (2H, quint., J=6.3 Hz), 1.80 (1H, dtd, J=14.0, 6.2, 6.0 Hz), 2.30 (6H, s), 2.37 (1H, dt, J=17.8, 2.1 Hz), 2.46 (2H, t, J=6.3 Hz), 2.60 (1H, d, J=13.0 Hz), 2.63 (1H, d, J=13.0 Hz), 2.64 (2H, m), 2.75 (2H, m), 2.91 (1H, ddd, J=17.8, 7.0, 2.0 Hz), 2.96 (1H, m), 3.45 (2H, br q, J=6.3 Hz), 3.86 (3H, s), 3.89 (3H, s), 4.12 (1H, ddd, J=11.0, 6.8, 6.0 Hz), 4.17 (1H, dt, J=11.0, 6.2 Hz), 5.49 (1H, m), 6.46 (1H, d, J=1.9 Hz), 6.47 (1H, m), 6.50 (1H, d, J=1.9 Hz), 7.01 (1H, d, J=1.9 Hz), 7.19 (1H, d, J=1.9 Hz), 7.73 (1H, br t, J=5 Hz), 7.76 (1H, br s), 7.97 (1H, br s); IR (film): 3296, 2942, 2168, 1736, 1651, 1582, 1537, 1466, 1437, 1406, 1261, 913, 731 cm⁻¹; HRMS (FAB): Calcd. for C_{38H47}O₆N₆ 683.3457, Found 683.3569 (M+H).
- 10. Drian, C. L.; Greene, A. E. J. Am. Chem. Soc. 1982, 104, 5473.
- 11. The enantiomeric excess was determined by HPLC analysis using Chiralcel OD column (Daicel Co., hexane/i-PrOH=250:1) for the corresponding benzoate, and the absolute configuration was assigned by conversion to known 4-(butoxycarbonylmethyl)cyclopent-2-en-1-one¹⁰ and comparison of $[\alpha]_D$.

(Received in Japan 24 August 1992)