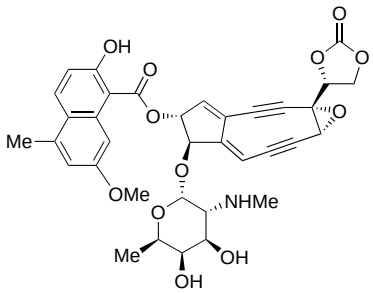
[13] For recent synthetic advances from our laboratory in the area of O-linked glycopeptides see W. Glunz, S. Hintermann, J. B. Schwarz, S. D. Kuduk, X.-T. Chen, L. J. Williams, D. Sames, S. J. Danishefsky, V. Kudryashov, K. O. Lloyd, *J. Am. Chem. Soc.* **1999**, *121*, 10636, and references therein.
[14] R. D. Cummings in *Glycoconjugates* (Eds.: H. J. Allen, E. C. Kisailus), Marcel Dekker, New York, **1992**, chap. 10, p. 333.
[15] For the general strategy we employ in reaching the "high mannose" core system, see P. H. Seeberger, P. F. Cirillo, S. Hu, X. Beebe, M. T. Bilodeau, S. J. Danishefsky, *Enantiomer J. Stereochem.* **1996**, *1*, 311.
[16] S. J. Danishefsky, M. T. Bilodeau, *Angew. Chem.* **1996**, *108*, 1482; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1380.
[17] S. J. Danishefsky, S. Hu, P. F. Cirillo, M. Eckhardt, P. H. Seeberger, *Chem. Eur. J.* **1997**, *3*, 1617.
[18] see: D. A. Griffith, S. J. Danishefsky, *J. Am. Chem. Soc.* **1990**, *112*, 5811.
[19] E. N. Cain, L. L. Welling, *Tetrahedron Lett.* **1975**, *16*, 1353.
[20] L. M. Likhosherstov, O. S. Novikova, V. A. Derevitskaja, N. K. Kochetkov, *Carb. Res.* **1986**, *146*, C1; S. T. Cohen-Anisfeld, P. T. Lansbury, Jr., *J. Org. Chem.* **1990**, *55*, 5560.
[21] S. T. Cohen-Anisfeld, P. T. Lansbury, Jr., *J. Am. Chem. Soc.* **1993**, *115*, 10531.
[22] Since our yield is far from quantitative, we cannot rule out the possibility of aspartimide formation, or even products arising from N-acylation by the α-carboxy group of the Asn residue via such an intermediate. This same issue, which can be raised with respect to the previous cases,[21] requires further detailed study.
[23] A. I. Meyers, D. L. Comins, D. M. Roland, R. Henning, K. Shimizu, *J. Am. Chem. Soc.* **1979**, *101*, 7104.
[24] Prepared by San San Yi, Microchemistry Laboratory, Sloan-Kettering Institute for Cancer Research.
[25] PE Sciex API-100.

Chemical Synthesis and DNA Photocleavage of the Intercalator–Carbohydrate Hybrid Moiety of the Neocarzinostatin Chromophore**

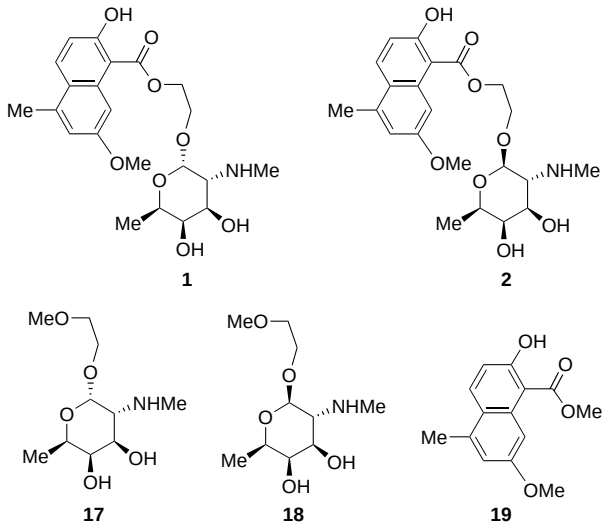
Kazunobu Toshima,* Shigeki Takai, Yutaka Maeda, Ryusuke Takano, and Shuichi Matsumura

Dedicated to Professor Kuniaki Tatsuta on the occasion of his 60th birthday

The development of photochemical DNA-cleaving agents, which effectively cleave DNA by irradiation at a specific wavelength under mild conditions and without any additives such as metals and reducing agents, is very interesting from a chemical and biological standpoint and offers considerable potential in medicine.[1] Indeed, photodynamic therapy using a photosensitizing drug has recently emerged as a promising modality against cancer and allied diseases.[2] Sugiura et al.[3] first demonstrated the light-induced DNA cleavage by the antitumor enediyne antibiotic neocarzinostatin[4] and Hirama et al.[5] reported the photoinduced cycloaromatization of the neocarzinostatin chromophore (Scheme 1), which is responsible for the DNA-cleavage activity of neocarzinostatin. In this context, we anticipated that if the intercalator–carbohydrate hybrid moiety of the neocarzinostatin chromophore, without its enediyne moiety, interacts with DNA and if the C=O bond in the hybrid generates a photoexcited $^3(n\text{-}\pi^*)$ radical-like[5] state by photoirradiation, then the intercalator–carbohydrate hybrid moiety of the enediyne-free neocarzinostatin chromophore could be capable of DNA cleavage. Herein, we report the chemical synthesis and DNA-photocleavage properties of the intercalator–carbohydrate hybrids[6, 7] **1** and **2**, which correspond to the intercalator and the carbohydrate moieties of the enediyne antibiotic, neocarzinostatin (Scheme 2).



Scheme 1. Neocarzinostatin chromophore.



Scheme 2. The intercalator–carbohydrate hybrids and their components.

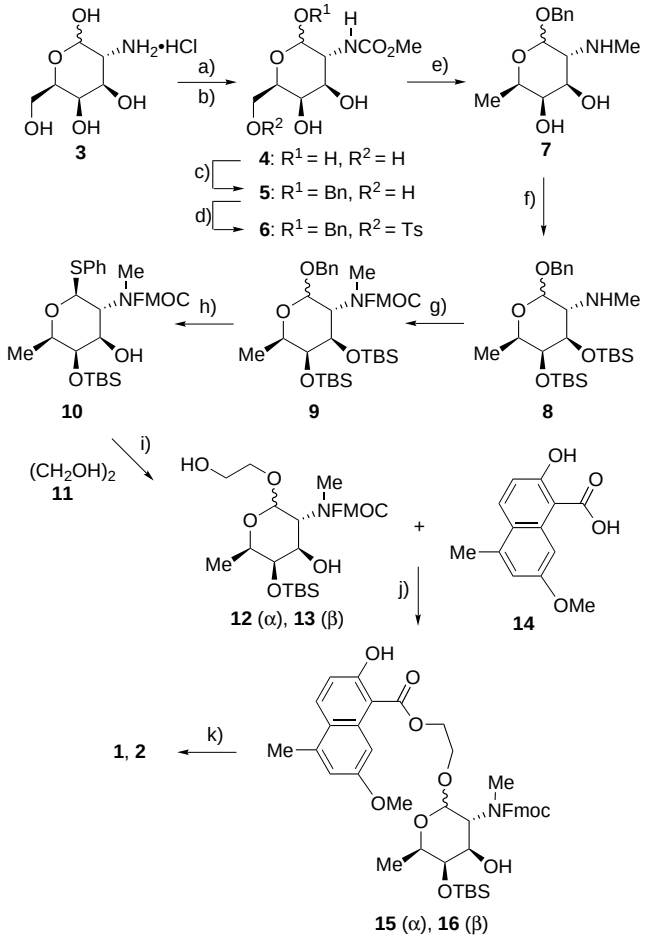
To confirm our hypothesis, we designed and synthesized **1** and **2**, in which the aromatic and sugar moiety of the neocarzinostatin chromophore were linked by only an ethylene glycol unit to each other. Compounds **1** and **2** are the anomers of each other. Their synthesis began with the

[*] Prof. Dr. K. Toshima, S. Takai, Y. Maeda, R. Takano, Prof. Dr. S. Matsumura
Department of Applied Chemistry
Faculty of Science and Technology
Keio University
3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223-8522 (Japan)
Fax: (+81) 45-566-1576
E-mail: toshima@applc.keio.ac.jp

[**] This research was partially supported by a Grant-in-Aid for Encouragement of Young Scientists from the Ministry of Education, Science, Sports, and Culture, Japan, and a research grant of Keio University Special Grant-in-Aid for Innovative Collaborative Research Projects.

 © WILEY-VCH Verlag GmbH, D-69451 Weinheim, 2000 0044-8249/00/11220-3802 $ 17.50+.50/0

conversion of D-galactosamine hydrochloride (**3**) into the benzyl glycoside **6** in four standard steps (Scheme 3). After separation of the pyranosides **6** and the corresponding furanosides by column chromatography at this stage, the lithium aluminum hydride (LAH) reduction of **6** gave **7**, which



Scheme 3. Synthesis of **1** and **2**. a) IRA-400 resin, H_2O; b) $ClCO_2Me$ (2.0 equiv), iPr_2NEt (4.0 equiv), MeOH, RT, 14 h; c) $BF_3 \cdot Et_2O$ (0.15 equiv), BnOH, 70 °C, 16 h; d) TsCl (1.5 equiv), Py, RT, 5 h, 38% from **3;** e) LAH (4.0 equiv), THF, reflux, 14 h, 91%; f) TBSOTf (3.0 equiv), 2,6-lutidine (4.0 equiv), 40 °C, 16 h, 88%; g) FMOCCl (1.0 equiv), K_2CO_3 (2.0 equiv), THF/H_2O, RT, 0.5 h, 95%; h) $BF_3 \cdot Et_2O$ (1.5 equiv), PhSH, RT, 5 h, 68%; i) NBS (1.5 equiv), MS 4Å, MeCN, 0 °C, 1 h, 35% for **12**, 62% for **13;** j) WSCD · HCl (2.0 equiv), CH_2Cl_2, 64% for **15**, 60% for **16**; h) TBAF (5.0 equiv), THF, RT, 1 h, 90% for **1**, 78% for **2**.

was subjected to protection with *tert*-butyldimethylsilyl (TBS) and 9-fluorenylmethoxycarbonyl (FMOC) groups to afford **9** in high overall yield. Conversion of the benzyl glycoside **9** into the phenylthio glycoside **10** accompanied by deprotection of the TBS group at the C-3 position was next carried out using PhSH and $BF_3 \cdot OEt_2$. The glycosidation of **10** (1.0 equiv) with ethylene glycol **11** (5.0 equiv) using *N*-bromosuccinimide (NBS)[8] in the presence of molecular sieves (MS 4Å) in MeCN gave both the α-glycoside **12** and the β-glycoside **13** in 97% yield in a ratio of 1:1.8. After their separation by column chromatography, **12** (1.0 equiv) and **13** (1.0 equiv) were esterified with the carboxylic acid **14**[9] (2.0 equiv) using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSCD · HCl) in CH_2Cl_2 to give the hybrids **15** and **16** in 64% and 60% yields, respectively. Finally, the deprotection of the TBS and FMOC groups in **15** and **16** using tetrabutylammonium fluoride (TBAF) in THF furnished the intercalator–carbohydrate hybrids **1** and **2**, respectively.

The photoinduced DNA-cleavage activities of the intercalator–carbohydrate hybrids **1** and **2**, along with the components of these hybrids **17**–**19**,[10] were assayed using covalently closed supercoiled ΦX174 DNA (Form I). As noted in Figure 1, and, as expected, the intercalator–carbohydrate

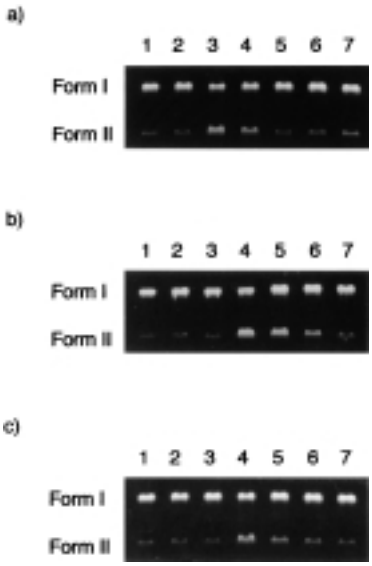


Figure 1. Photocleavage of supercoiled ΦX174DNA. ΦX174DNA (50 μM per base pair) was incubated with various compounds in 19% acetonitrile and 1% dimethyl sulfoxide in Tris/HCl buffer (pH 7.5, 50 mM) at 25 °C for 2 h under UV irradiation (365 nm, 15 W) from a lamp placed at 10 cm from the mixture. The product was analyzed by gel electrophoresis (0.9% agarose gel, ethidium bromide stain): a) lane 1, DNA alone; lane 2, DNA with UV; lanes 3–7, compounds **1**, **2**, **17**, **18**, and **19** (500 μM), respectively; b) lane 1, DNA alone; lane 2, DNA with UV; lane 3, DNA + **1** (500 μM) without UV; lanes 4–7, **1** (500), **1** (100), **1** (20), and **1** (5 μM), respectively; c) lane 1, DNA alone; lane 2, DNA with UV; lane 3, DNA + **2** (500 μM) without UV; lanes 4–7, **2** (500), **2** (100), **2** (20), and **2** (5 μM), respectively.

hybrids **1** and **2** (500 μM) cleaved the DNA and caused a single strand break, leading to the nicked, open-circular DNA (Form II) by photoirradiation with long wavelength UV light (365 nm), while **17**–**19** did not show DNA cleavage activity under the same conditions. These results clearly indicate a new and interesting fact, that the intercalator–carbohydrate hybrid moiety of the neocarzinostatin chromophore, without its enediyne moiety, induced photocleavage of the DNA. The importance of the hybrid structure, as constructed from the intercalator and the carbohydrate, towards DNA cleavage was confirmed. It was also confirmed that no DNA cleavage by **1** and **2** was observed in the absence of light. Furthermore, the DNA photocleavage of **1** was stronger than that of its anomer **2**, as **1** cleaved the DNA in concentrations over 20 μM at 25 °C. The cleavage site specificity of the **1** and **2** was also analyzed according to the Sanger protocol.[11] These results, shown in Figure 2, clearly show the identical high guanine selectivity. Furthermore, these results also indicated that the

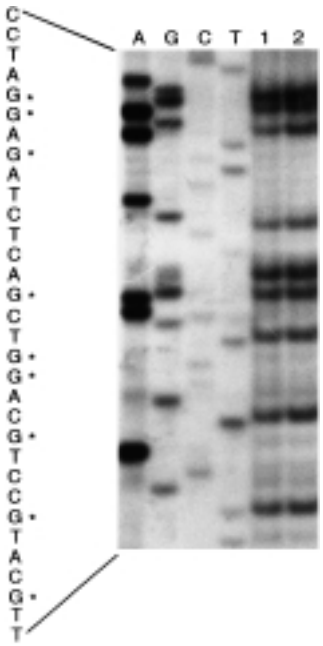


Figure 2. Autoradiogram of polyacrylamide/urea (12 %/8 M) slab gel electrophoresis for sequence analysis. The 5′-end-labeled M13mp18 DNA was cleaved by the hybrids at pH 7.5 and 25 °C for 2 h under irradiation, as described in Figure 1. Bases 48 – 79 are shown: lanes A, G, C, and T; Sanger A, G, C, and T reactions, respectively; lanes 1 and 2; **1** and **2** (500 μM), respectively.

cleavage site selectivity of **1** and **2** was different from that of natural neocarzinostatin chromophore.[3]

Received: December 14, 1999 [Z14390]
Revised: May 17, 2000

[1] B. Armitage, *Chem. Rev.* **1998**, *98*, 1171 – 1200.
[2] A. R. Morgan, *Curr. Med. Chem.* **1995**, *2*, 604 – 615; R. Bonnett, *Chem. Soc. Rev.* **1995**, 19 – 33; *Photodynamic Therapy: Basic Principles and Clinical Applications*, (Eds.: R. W. Henderson, T. J. Dougherty), Marcel Dekker, New York, **1992**.
[3] Y. Uesawa, J. Kuwahara, Y. Sugiura, *Biochem. Biophys. Res. Commun.* **1989**, *164*, 903 – 911.
[4] For a review of neocarzinostatin, see: I. H. Goldberg, *Acc. Chem. Res.* **1991**, *24*, 191 – 198.
[5] T. Gomibuchi, M. Hirama, *J. Antibiot.* **1995**, *48*, 738 – 740.
[6] K. Takahashi, T. Tanaka, T. Suzuki, M. Hirama, *Tetrahedron* **1994**, *50*, 1327 – 1340.
[7] Total synthesis of the neocarzinostatin chromophore, see: A. G. Myers, J. Liang, M. Hammond, P. M. Harrington, Y. Wu, E. Y. Kuo, *J. Am. Chem. Soc.* **1998**, *120*, 5319 – 5320.
[8] K. C. Nicolaou, S. P. Seitz, D. P. Papahatjis, *J. Am. Chem. Soc.* **1983**, *105*, 2430 – 2434.
[9] M. Shibuya, K. Toyooka, S. Kubota, *Tetrahedron Lett.* **1984**, *25*, 1171 – 1174; K. Shishido, A. Yamashita, K. Horoya, K. Fukumoto, T. Kametani, *Tetrahedron Lett.* **1989**, *30*, 111 – 112; K. Takahashi, T. Suzuki, M. Hirama, *Tetrahedron Lett.* **1992**, *33*, 4603 – 4604; A. G. Myers, V. Subramanian, M. Hammond, *Tetrahedron Lett.* **1996**, *37*, 587 – 590.
[10] These compounds were obtained by ways similar to that for the hybrids **1** and **2**.
[11] F. Sanger, S. Nicklen, A. R. Coulsen, *Proc. Natl. Acad. Sci. USA* **1977**, *74*, 5463 – 5467. Since the Sanger sequencing reactions result in base incorporation, cleavage at nucleotide *N* (sequencing) represents cleavage site by the agent or the Maxam – Gilbert reaction at $N+1$. See also: D. L. Boger, S. A. Munk, H. Zarrinmayeh, T. Ishizaki, J. Haught, M. Bina, *Tetrahedron* **1991**, *47*, 2661 – 2682.

Alkane Carbonylation with Carbon Monoxide on Sulfated Zirconia: NMR Observation of Ketone and Carboxylic Acid Formation from Isobutane and CO**

Alexander G. Stepanov,* Mikhail V. Luzgin, Alexey V. Krasnoslobodtsev, Vera P. Shmachkova, and Nina S. Kotsarenko

Direct conversion of inert alkanes into carbonyl-containing organic compounds is an important goal for industrial organic chemistry. Alkanes can be carbonylated into carboxylic acids or aldehydes in superacidic HF/SbF_5 or CF_3SO_3H/SbF_5 systems.[1] However, environmental concerns require the use of more environmentally friendly solid catalysts. The strong acidity and exceptionally high activity of sulfated zirconia[2] (SZ) means it has received much attention as a potential catalyst for hydrocarbon conversion,[3] and especially for overcoming the chemical inertness of alkanes. The direct carbonylation of benzene with CO using a pure SZ as the solid acid catalyst has been reported recently.[4] However, saturated hydrocarbons were never reported to be involved in carbonylation reactions on SZ, only the inhibiting effect of carbon monoxide on linear alkane isomerization with SZ has been demonstrated.[5] Herein we report direct ^{13}C solid-state NMR spectroscopic measurements of the carbonylation of isobutane with CO, using a pure SZ as the solid acid catalyst.

Figure 1 displays the ^{13}C cross-polarization magic-angle spinning (CP/MAS) NMR spectra obtained after coadsorption of isobutane and CO on SZ and subsequent heating of the sample at 70 °C for 1 h. We rationalize these spectra in terms of selective formation of methyl isopropyl ketone (**5**) by pathways a), d), g) and/or b), c), g) in Scheme 1. If 2-^{13}C-labeled isobutane ([2-^{13}C]iC_4H_{10}, that is, isobutane labeled at the quaternary carbon atom) and unlabeled CO are coadsorbed, the following spectral features are observed (Figure 1 A): the intense signal at $\delta = 47.0$ arises from the labeled CH group of the isopropyl fragment of **5**, the weak signal at $\delta = 19.6$ is assigned to the unlabeled CH_3 group of the isopropyl fragment, while the signal at $\delta = 25.5$ arises from residual isobutane. The resonance signal of the other unlabeled methyl group of **5** is not seen because of its very low intensity. If unlabeled isobutane and ^{13}C-labeled carbon monoxide are coadsorbed (Figure 1 B), the resonance signal

[*] Dr. A. G. Stepanov, Dr. M. V. Luzgin, Dr. V. P. Shmachkova, Dr. N. S. Kotsarenko
Group of NMR Spectroscopy for Catalytic Hydrocarbon Conversion and Laboratory for Zeolites and Acid-Base Catalysis
Boreskov Institute of Catalysis
Siberian Branch of the Russian Academy of Sciences
Prospekt Akademika Lavrentieva 5, Novosibirsk 630090 (Russia)
Fax: (+7) 3832-34-3056
E-mail: a.g.stepanov@catalysis.nsk.su
A. V. Krasnoslobodtsev
Natural Science Department, Novosibirsk State University
ul. Pirogova 2, Novosibirsk 630090 (Russia)

[**] This work was supported by grant No. 99-03-32454 from the Russian Foundation for Basic Research (RFBR) and in part by a joint RFBR-INTAS grant (No. 95-0194). The authors sincerely thank Dr. V. N. Sidelnikov for GC-MS analysis.

 © WILEY-VCH Verlag GmbH, D-69451 Weinheim, 2000 0044-8249/00/11220-3804 $ 17.50+.50/0