# Photoactivation of Neocarzinostatin Chromophore: Photo-Induced Cycloaromatization *via* Naphthoate Participation

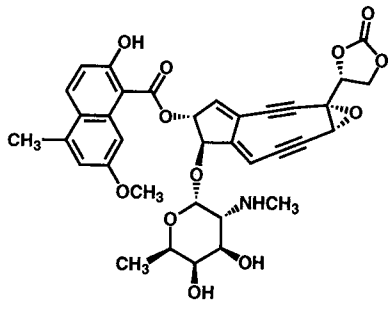
Sir:

Neocarzinostatin chromophore (**1**), responsible for the biological activity of antitumor antibiotic neocarzinostatin,[1~5] is labile even toward ambient light when released from the apoprotein.[6],† Recently we proposed that a central pathway of photochemical decomposition of **1** would be a Norrish Type II fragmentation of naphthoate ester leading to the formation of naphthoic acid (**2**) and a putative unstable fulvene derivative, mainly based on the model experiments.[7] Here, we describe an another important pathway, in which photoactivated **1** undergoes a new mode of cycloaromatization *via* naphthoate participation without thiol trigger affording an indacene derivative (**3**). Moreover, the highly effective DNA cleavage by this photoactivation of **1** is demonstrated.

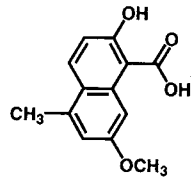
A deoxygenated solution of **1** (1 mM) in 0.1 M $CH_3CO_2H$/$CH_3OH$ containing 1,4-cyclohexadiene (300 mM) and a small amount of water was exposed to weak near-ultraviolet light of wave length 350~380 nm (BLAK-RAY: 6W UVL-56, UVP, Inc.) at 7°C for 5 hours. Since the primary photochemical products appeared to be labile and change to more stable compounds, the reaction mixture was further stirred in the dark at 7°C for 4 days, giving rise to **2**[7,8] and a new compound (**3**) (see spectral data on page 739) in ~19% and ~28% yield, respectively, as major products. (Isolation of these products was performed by HPLC (MERCK Purospher RP-18 (5 $\mu$m) 250 × 20 mm, a linear gradient: methanol - water - formic acid = 20 : 80 : 5→95 : 5 : 5, 4 ml/minute).) Neither **2** nor **3** were produced in the dark. The photoactivation of **1** in 0.1 M $CD_3CO_2D/CD_3OD$ resulted in the formation of bis-deuterated indacene (**4**). The structure of the new indacene derivative (**3**) was unambiguously assigned by the spectroscopic methods. The migration of the naphthoate to C-12 is apparent from the large deshielding of H-12 ($\delta$ 6.49) instead of H-11 ($\delta$ 4.76). Since the coupling constants of the protons at C-10, C-11, and C-12 varied considerably by the groups attached to the hydroxyls (SUZUKI, T.; M. OYAKE & M. HIRAMA, unpublished results),[9] NOE experiments were carefully carried out and established the cis relationship between H-11 and H-12: NOEs were observed between H-11 and H-12, and between H-10 and H-8″.

The product **2** appeared to support the Norrish Type II fragmentation of **1**.[7] Furthermore, the new product **3** strongly indicated the existance of an alternative photodecomposition pathway *via* the intramolecular rearrangement of the activated naphthoate as shown in scheme 1. The naphthoate migration to C-12 with the concomitant epoxide opening is likely to occur from the photo-excited triplet state which may have a radical character, as the C-12 center of **1** is prone to react with the oxy radicals as well as the ordinary nucleophiles such as thiols,[9] although we can not rule out an another possibility *via* the photo-enol intermediate (**5**).[7] The ketene acetal with ene-yne-cumulene structure (**6**) thus formed should undergo the cycloaromatization giving the biradical intermediate **7**, which leads to indacene **8** through hydrogen abstraction from the media.[3~5] Slow hydrolysis of the ketene acetal (**8**) should provide **3** as well as **2**. The selective formation of **3** would be due to the steric interaction with the C-10 glycoside.

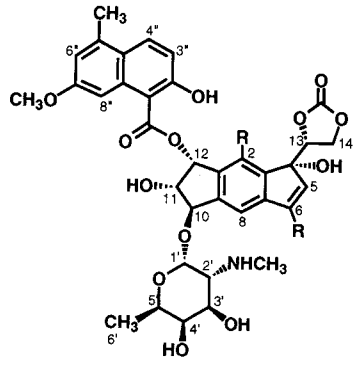
Then, DNA cleavage experiments by photoactivation[10] of **1** without thiol under the above weak near-ultraviolet light were performed. The mixture of **1** and



**1**



**2**

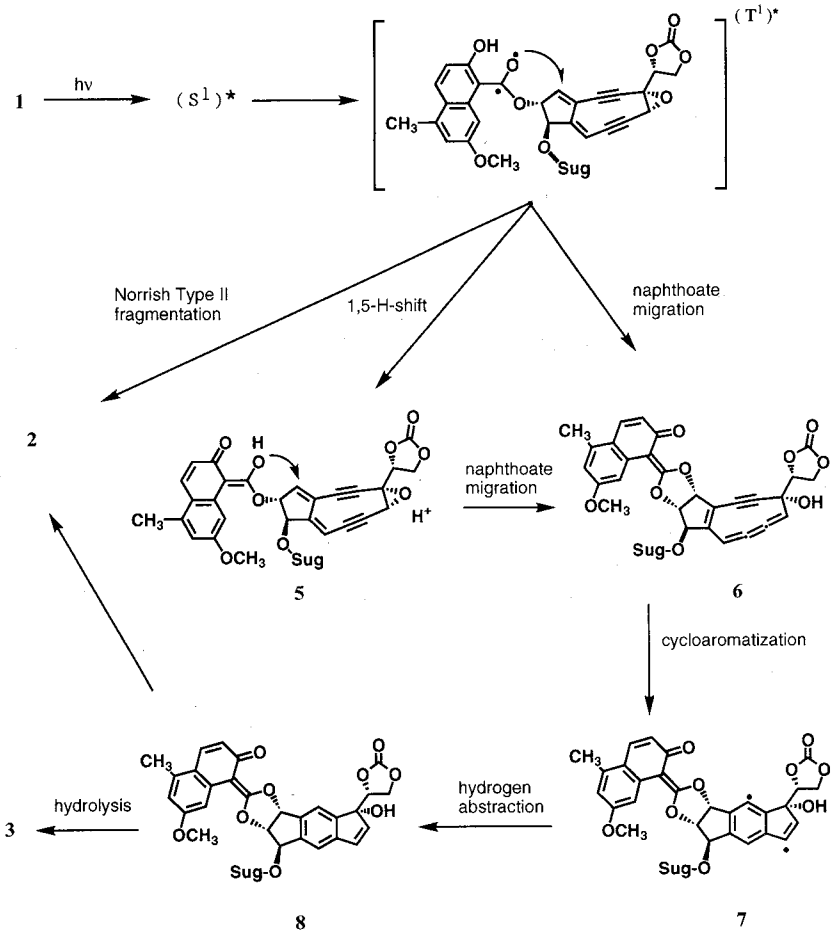


**3** R=H
**4** R=D (~100%)

† The first-order decay of **1** was accelerated four-times by ambient light, while complexation of **1** with the apoprotein retarded it by three-times (TANAKA, T., unpublished results).

Dedicated to Professor SATOSHI ŌMURA on the occasion of his 60th birthday.

Scheme 1. A proposed photodecomposition pathway yielding **2** and **3** from **1**.




pBR322 form I DNA (32 $\mu$M per base pair) was exposed to the light at 2°C for 2 hours in the various concentrations of **1**, in $CH_3CO_2H$-$CH_3OH$-Tris-HCl buffer (pH 7.9, 20 mM). The experiments analyzed by electrophoresis (1% agarose gel, ethidium bromide stain) indicated its potent DNA cleaving activity: at the 2.0 $\mu$M concentration of **1**, the complete change of form I (covalently closed supercoild DNA) to form II (open circular DNA) was demonstrated.

Very recently, FUCHS *et al.* suggested a possibility of an involvement of the hydroxy naphthoate ester moiety in the activation of **1**,[11] and indeed, HENSENS and GOLDBERG *et al.* reported the intramolecular carbon-alkylation activation pathway under the weakly basic conditions.[12,13] The present photo-induced cycloaromatization is an alternative naphthoate participation to activate **1** without thiols.

Spectral data of **3** and **4**. **3**: $^1$H NMR (600 MHz, $CD_3OD$) $\delta$ 1.37 (3H, d, $J$=6.0 Hz, C-6′), 2.56 (3H, s, $PhCH_3$), 2.62 (3H, s, $NHCH_3$), 3.48 (1H, br d, $J$=10.0 Hz, C-2′), 3.76 (1H, br d, $J$=2.5 Hz, C-4′), 3.91 (1H, br dd, $J$=10.0, 2.5 Hz, C-3′), 4.00 (3H, s, $PhOCH_3$), 4.20 (1H, br q, $J$=6.0 Hz, C-5′), 4.45 (1H, br dd, $J$=8.0, 6.0 Hz, C-14), 4.51 (1H, br dd, $J$=8.5, 8.0 Hz, C-14), 4.64 (1H, dd, $J$=8.5, 6.0 Hz, C-13), 4.76 (1H, d, $J$=7.0 Hz, C-11), 5.84 (1H, s, C-10), 5.93 (1H, s, C-1′), 6.29 (1H, d, $J$=5.5 Hz, C-5), 6.49 (1H, d, $J$=7.0 Hz, C-12), 6.84 (1H, d, $J$=9.0 Hz, C-3″), 6.84 (1H, br d, $J$=2.0 Hz, C-6″), 6.86 (1H, d, $J$=5.5 Hz, C-6), 7.14 (1H, br d, $J$=2.0 Hz, C-8″), 7.31 (1H, s, C-8), 7.77 (1H, d, $J$=9.0 Hz, C-4″), 7.84 (1H, s C-2); FTIR (neat film) $\nu$ 3372, 2930, 2364, 1804, 1734, 1626, 1468, 1402, 1255, 1170, 1087, 1029, 797, 739, 420 cm$^{-1}$; High resolution FABMS (calcd for $[M+H-(CO_2+H_2O)]^+$, $C_{34}H_{36}O_{10}N$: 618.2340; found: 618.2354). **4**: $^1$H NMR (600 MHz, $CD_3OD$) $\delta$ 1.37 (3H, d, $J$=6.6 Hz, C-6′), 2.56 (3H, s, $PhCH_3$), 2.80 (3H, s, $NHCH_3$), 3.57 (1H, m, C-2′), 3.76 (1H, br d, $J$=3.0 Hz, C-4′), 3.90 (1H, br dd, $J$=11.0, 3.0 Hz, C-3′), 4.00 (3H, s, $PhOCH_3$), 4.19 (1H, m, C-5′), 4.46 (1H, br dd, $J$=8.0, 6.0 Hz, C-14), 4.51 (1H, br dd, $J$=8.0, 8.0 Hz, C-14), 4.65 (1H, dd, $J$=8.0, 6.0Hz, C-13), 4.76 (1H, d, $J$=7.0 Hz, C-11), 5.84 (1H, s, C-10), 5.92 (1H, br d, $J$=2.8 Hz, C-1′), 6.29 (1H, s C-5), 6.50 (1H, d, $J$=7.0 Hz, C-12), 6.84 (1H, d, $J$=9.0 Hz, C-3″), 6.84 (1H, br d, $J$=2.0 Hz, C-6″), 7.14 (1H, br d, $J$=2.0 Hz, C-8″), 7.31 (1H, s, C-8), 7.77 (1H, d, $J$=9.0 Hz, C-4″).

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