

Neocarzinostatin Chromophore: Synthesis and Structure of Naphthalenecarboxylic Acid Moiety¹⁾

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Methyl 2-hydroxy-5-methoxy-7-methyl-1-naphthalenecarboxylate (**1**), which was previously reported as the structure of degradation product obtained from neocarzinostatin chromophore, was synthesized and was proved not to be the true structure. The structure was revised as methyl 2-hydroxy-7-methoxy-5-methyl-1-naphthalenecarboxylate (**2**) by its total synthesis.

KEY WORDS: Neocarzinostatin chromophore/ Structure revision/
Naphthalenecarboxylic acid/ Naphthalene synthesis

An antitumor antibiotic neocarzinostatin (NCS) was isolated from culture filtrate of *Streptomyces carzinostaticus* var. F-41 by Ishida *et al.*²⁾ in 1965. It has been reported that NCS inhibits DNA synthesis and cell division, causes single-strand breaks in linear duplex or superhelical DNA *in vitro*, and induces DNA repair synthesis. NCS also causes DNA strand scission *in vivo*, and considerable evidence indicates that DNA damage is the primary result of its activity *in vivo*.³⁾ Clinically, NCS has been used for the treatment of patients with acute leukemia,⁴⁾ gastric cancer,⁵⁾ and pancreas cancer.⁶⁾ NCS consists of a protein and a tightly but noncovalently bound chromophore⁷⁾ which can be separated from the protein.

The isolated NCS chromophore, which has empirical formula $C_{35}H_{33}NO_{12}$, is chemically very unstable and possesses the full biological activity of NCS.⁸⁾ The apoprotein which was sequenced previously (MW 10,700)⁹⁾ was thought to be the active drug and recently revised as shown in Fig. 1.¹⁰⁾ The apoprotein serves as a carrier for the chromophore and acts to stabilize it and to control its release for interaction with target DNA. The mode of action of NCS chromophore on DNA has not been clarified yet, but the consequences so far obtained from wide investigation about that^{8,11)} can be summarized as follows: (i) NCS chromophore introduces single-strand breaks almost exclusively at thymidylate and adenylate residue in DNA in a reaction stimulated greatly by a thiol compound and dependent on oxygen. (ii) A radical form of the drug generated by thiol addition to the chromophore abstracts a hydrogen from the 5'-carbon of deoxyribose in DNA to form a carbon-centered radical which could then react with oxygen to give a peroxyl radical and ultimately, in the presence of thiol, the nucleoside 5'-aldehyde. (iii) The abstracted hydrogen is cova-

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Neocarzinostatin Chromophore

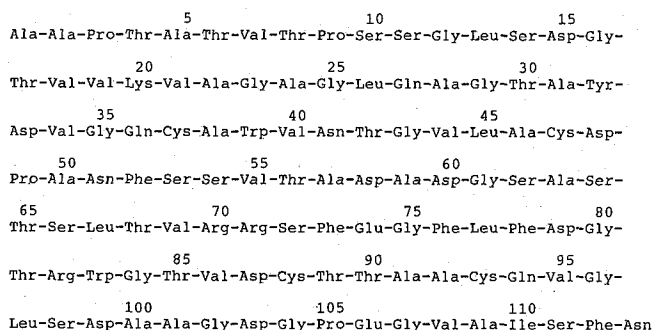


Fig. 1. Primary structure of neocarzinostatin protein⁽¹⁰⁾

lently bound to the NCS chromophore, and molecular oxygen is the only source of the 5'-aldehydic oxygen. (iv) Presumably, these two lesions (sugar-oxidation and adduct formation) share a common precursor having some form of oxygen linkage between chromophore and C-5' of deoxyribose.

The chemical structure of NCS chromophore has been investigated by Tohoku,⁽¹²⁾ and Harvard⁽¹³⁾ groups independently and they proposed that it consists of four subcomponents, 2-hydroxy-5-methoxy-7-methylnaphthoate, 2, 6-dideoxy-2-methylaminogalactose, five membered cyclic carbonate ring, and epoxy ring, all of which are linked to a 12-carbon subunit. (Fig. 2)⁽¹³⁾

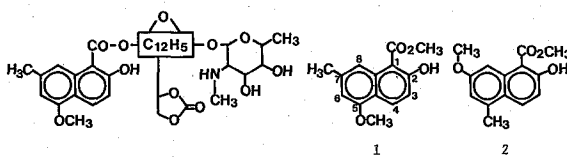
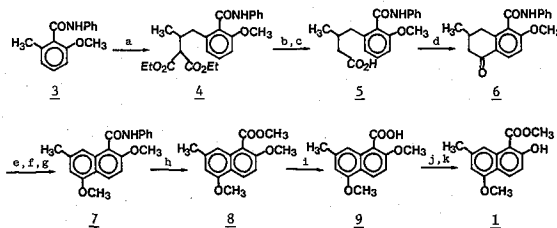


Fig. 2. Proposed structure of NCS chromophore (I.H. Goldberg)⁽¹³⁾

The structure assignment by NMR analysis is difficult because of the instability and the high degree of unsaturation of the C₁₂-subunit. Thus, we decided to elucidate the structure by synthetic approach. As the first target in a strategy on the total synthesis of NCS chromophore, we chose the naphthalenecarboxylate **1**^(12a) because



Scheme 1.

a. 2.1 equiv *n*-butyllithium/TMEDA/diethyl ethylidenemalonate/THF/ -78° /86%. **b.** 5% aq. NaOH/ 90° /1 h/95%. **c.** $140-150^{\circ}$ /1 h/98%. **d.** 2 equiv $(\text{CF}_3\text{CO})_2\text{O}$ /benzene/reflux/5 min/92%. **e.** 2 equiv $\text{CuBr}_2/\text{AcOEt}-\text{CHCl}_3$ (1:1)/reflux/2 h/99%. **f.** K_2CO_3 /acetone/reflux/95%. **g.** K_2CO_3 /1.3 equiv $(\text{CH}_3\text{O})_2\text{SO}_2$ /cat. $(\text{C}_4\text{H}_9)_4\text{NHSO}_4$ /reflux/1 h/88%. **h.** 20% $\text{BF}_3\text{-MeOH}$ / $110-120^{\circ}$ /3 h/72%. **i.** 10% NaOH in $\text{H}_2\text{O}-\text{DMSO}$ (1:1)/ 120° /99%. **j.** 4 equiv $\text{BCl}_3/\text{CH}_2\text{Cl}_2$ / $-78^{\circ} \rightarrow 0^{\circ}$ /0.5 h/93%. **k.** CH_2N_2 /ether/89%.

it was only compound isolated from the degradation products of NCS and NCS chromophore and characterized.

The *o*-toluamide **3**, synthesized from 2-methoxy-6-methylbenzoic acid¹⁴⁾ in two steps [(i) SOCl_2 , 80° , 1 h, (ii) PhNH_2 , CH_2Cl_2 , 0° -room temp, 40 min], was metallated with LDA and allowed to react with diethyl ethylenemalonate¹⁵⁾ to give the diester **4**. Hydrolysis of the diester **4** followed by the thermal decarboxylation afforded the acid **5** in good yield. The cyclization of the acid using 2 equiv of trifluoroacetic anhydride in benzene at refluxing temperature provided the ketone **6**. The bromination of compound **6** with copper (II) bromide followed by debromination of the diastereomeric mixture of the monobromide with potassium carbonate gave the naphthol compound which was then methylated with dimethylsulfate in the presence of catalytic tetrabutylammonium hydrogen sulfate to afford the desired naphthalene compound **7**. Methanolysis of the amide group was carried out by heating at 110 – 120° in 20% BF_3 -MeOH solution to give the methyl ester **8**. Hydrolysis of the ester **8** with 10% sodium hydroxide solution in H_2O -DMSO (1:1) at 120° gave the carboxylic acid **9**. Finally, selective deprotection of the methoxy group adjacent to the methoxy-carbonyl group of **9** using 4 equiv of boron trichloride in dichloromethane at low temperature followed by methylation with diazomethane afforded the desired naphthol **1**. Surprisingly, the reported data for the degradation product of NCS chromophore and derivatives synthesized from it^{12a)} were markedly different from those of our synthetic samples (**1**, **8**, and **9**). Table 1 summarizes NMR spectral data on **1**, **8**, and **9** with those reported for natural naphthalene derivatives. We reinterpreted the reported spectral data in comparison with ours, and speculated that the naphthalenecarboxylate obtained from NCS chromophore is the regioisomer of **1** and has the structure **2**. Thus, we decided to confirm the structure by the total synthesis of **2**. The synthetic pathway is summarized in Scheme 2.

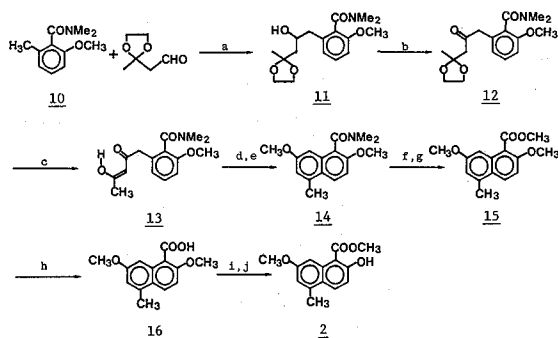
Table 1. NMR spectral data on **1**, **8**, and **9***1

	CO_2CH_3	$\text{C}_2\text{-OCH}_3$	$\text{C}_3\text{-H}$	$\text{C}_4\text{-H}$	$\text{C}_5\text{-OCH}_3$	$\text{C}_6\text{-H}$	$\text{C}_7\text{-CH}_3$	$\text{C}_8\text{-H}$
1	4.07	—	7.04, d J=9.2	8.35, d J=9.2	3.94	6.57, d J=1.1	2.50, m	8.07, m
	(4.10)	—	(7.01)	(8.01)	(3.92)	(6.90)	(2.63)	(8.09)
9	—	4.01*2	7.16, d J=9.5	8.32, dd J=0.7, 9.5	3.96*2	6.55, d J=1.0	2.50, m	7.57, m
		(4.06)*2	(7.12)	(8.02)	(3.90)*2	(6.90)	(2.63)	(7.74)
8	3.97	3.91*2	7.07, d J=9.5	8.17, dd J=0.7, 9.5	3.93*2	6.43, d J=1.0	2.44, m	6.95, m
	(3.89)	(3.65)	(6.91)	(7.77)	(3.82)	(6.73)	(2.57)	(6.73)

*1 Determined with a JEOL JNM-FX200 instrument in CDCl_3 (**1**, **9**) and $\text{CCl}_4/\text{CDCl}_3$ (3:1) (**8**) at sample concentrations of 4% w/v. Reported chemical shifts^{12a)} for the corresponding natural products are shown in parentheses. *2 Assignments may be reversed.

Metallation of the *o*-toluamide **10**, which was synthesized by the similar procedure as that for **3**, with LDA followed by quenching with 2-methyl-2-(2-oxoethyl)-1, 3-

Neocarzinostatin Chromophore



Scheme 2

a. 1.5 equiv LDA/THF/ -78° /0.5 h/98%. **b.** 4 equiv CrO_3 /8 equiv pyr./4 equiv Ac_2O / CH_2Cl_2 /RT/2 h/83%. **c.** 10% aq. HCl /acetone/RT/3 h/88%. **d.** conc H_2SO_4 /RT/12 h/45%. **e.** K_2CO_3 /1.3 equiv $(\text{CH}_3\text{O})_2\text{SO}_2$ /cat. $(\text{C}_4\text{H}_9)_4\text{NHSO}_4$ /reflux/1 h/92%. **f.** FSO_3CH_3 /RT/1 h/100%. **g.** 1N $\text{NaOH}/\text{CH}_3\text{CN}/50^{\circ}/10 \text{ min}/31\%$ (66% recovery of **14**). **h.** 10% NaOH in H_2O -DMSO (1:1)/ 120° /1 h/99%. **i.** 4 equiv $\text{BCl}_3/\text{CH}_2\text{Cl}_2/-78 \rightarrow 0^{\circ}/0.5 \text{ h}/87\%$. **j.** CH_3N_2 /ether/55%.

dioxolane¹⁶⁾ provided the amide alcohol **11**. In the NMR spectrum of **11**, a set of signals of the two conformational isomers arising from restricted rotation about the amide bond, was observed. These two isomers could not be isolated, but two spots were observed on TLC at room temperature. Oxidation of **11** with chromium trioxide-pyridine complex and acetic anhydride¹⁷⁾ gave the keto amide **12**, which was hydrolyzed in acidic conditions to afford the enolated β -diketone **13**. Cyclization of **13** with conc. sulfuric acid provided the naphthol, which upon methylation with potassium carbonate and dimethylsulfate in acetone afforded the dimethyl ether **14**. After several unsuccessful attempts to hydrolyze **14** [(a) 50% KOH , H_2O /DMSO, 170° , 12 h, (b) 60% HClO_4 , 130° , 12 h, etc.], the ester **15** was obtained in 31% yield (66% recovery of **14**) by methylation with methyl fluorosulfonate followed by alkaline hydrolysis of the intermediate imidate salt.

This type of hydrolysis of imidate salt was investigated stereoelectronically by Deslongchamps¹⁸⁾ and well established, but unfortunately, the ester **15** could not be obtained predominantly by hydrolysis under various conditions. Presumably, because of the steric hindrance, predominant conformer of the hemiorthoamide tetrahedral intermediate is that favored to produce the starting amide. Alkaline hydrolysis of the ester **15** afforded the carboxylic acid **16** in good yield. Selective demethylation of **16** with boron trichloride produced the hydroxy acid which was methylated with diazomethane to afford the hydroxy ester **2**. Compound **2** was found to be identical with the degradation product of NCS by direct comparison (mp, ^1H NMR, IR, MS, TLC). Compound **15** and **16** were also identical spectroscopically with the derivatives of the above ester obtained from NCS chromophore.^{12a)} Thus, the structure of the naphthalenecarboxylic acid derivative from NCS chromophore was concluded to be methyl 2-hydroxy-7-methoxy-5-methyl-1-naphthalenecarboxylate **2**.

Recently, the total structure of NCS chromophore having a bicyclo[7.3.0]dodecadiene ring system was proposed by Edo *et al.*¹⁹⁾ as shown in Fig. 3. In spite of

the lack of stereochemistry, this structure has significant features which are of considerable biological interest. For the elucidation of structural and biological problems, the total synthesis of possible stereo isomers of NCS chromophore and its analogues is very important and now in progress in our laboratory.

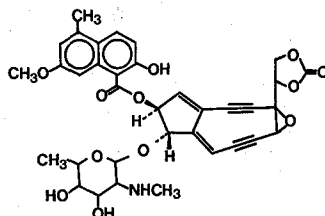


Fig. 3.

ACKNOWLEDGEMENTS

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EXPERIMENTAL

IR spectra were obtained with a JASCO DS-701G spectrometer, NMR spectra were determined with a JEOL JNM-PS100 spectrometer (100 MHz) and a JEOL JMS FX200 spectrometer (200 MHz) using TMS as an internal reference. Mass spectra were recorded on a JEOL JMS-D 300 mass spectrometer generally at 20 eV using a direct insertion probe. Mps were determined by the capillary method and are uncorrected.

All experiments were carried out under argon atmosphere. Usual work-up refers to addition of a reaction mixture to a mixture of excess ice and AcOEt, phase separation, re-extraction of the aqueous phase, washing of the combined organic layers with brine, drying the organic extracts over MgSO₄, filtration, and evaporation of the solvents under reduced pressure at 20–35°. For column chromatography, Wako C-300 silica gel was employed.

Michael Reaction of Lithiated *o*-Toluamide (3). To a soln of N-phenyl-2-methoxy-6-methylbenzamide **3** (15.51 g) and N, N, N', N'-tetramethylethylenediamine (15.69 g, 2.1 equiv) in THF (400 ml) at –78°, 75 ml (2.1 equiv) of 1.8 M soln of n-BuLi in n-hexane was added over a period of 20 min and then warmed up to 0°. After stirring at 0° for 1 h, the mixture was cooled again to –78°. Diethyl ethylidenemalonate (13.66 g, 1.1 equiv) in THF (50 ml) was added and the soln was allowed to stir for a further 30 min at –78°. The reaction was quenched by adding AcOH (16.56 ml). The usual work-up gave the crude product which was purified by the column chromatography on silica gel provided 23.630 g (86%) of the diester **4** as a colorless solid. Recrystallization of **4** from i-Pr₂O gave an analytical sample, mp

84–85°. IR(CHCl₃) ν 3420, 1725, 1675, and 1598; NMR(CDCl₃) δ 0.97 (3H, d, J=6.5), 1.15 (3H, t, J=7), 1.21 (3H, t, J=7), 2.50–2.29 (3H, m), 3.27 (1H, d, J=7.5), 3.78 (3H, s), 3.97 (2H, q, J=7), 4.10 (2H, q, J=7), 6.70–7.75 (8H, m), and 7.93 (1H, br, NH). (Found: C, 67.70; H, 6.86; N, 3.51. C₂₄H₂₉NO₆ requires: C, 67.43; H, 6.84; N, 3.28%).

N-Phenyl-2-(3-carboxy-2-methylpropyl)-6-methoxybenzamide (5). The diester **4** (27 g) was added to a soln of NaOH (7.58 g, 3 equiv) in H₂O (160 ml) and the reaction mixture was stirred at 90° for 1 h. The resulting soln was cooled in ice, acidified with conc HCl, and the usual work-up giving 22.3 g (95% yield) of the diacid as a colorless solid. The diacid, without further purification, was heated at 140–150° for 1 h and the resulting monoacid was purified by column chromatography to give 19.66 g (98% yield) of **5**. Recrystallization of **5** from 20% i-Pr₂O/CHCl₃ gave an analytical sample, mp 155–156°. IR(CHCl₃) ν 3420, 1715, 1675, and 1598; NMR(CDCl₃) δ 0.95 (3H, d, J=6.5), 2.00–2.60 (3H, m), 2.65 (2H, d, J=7), 3.77 (3H, s), 6.70–7.85 (9H, m), and 9.46 (1H, br, COOH). (Found: C, 69.80; H, 6.15; N, 4.34. C₁₉H₂₁NO₄ requires; C, 69.70; H, 6.47; N, 4.28%).

N-Phenyl-5-oxo-2-methoxy-7-methyl-5,6,7,8-tetrahydro-1-naphthalene-carboxamide (6). To a soln of **5** (8.27 g) in benzene (40 ml), was added (CF₃CO)₂O (3.93 ml, 1.1 equiv) and the soln was refluxed for 5 min. Evaporation of the soln under reduced pressure and the crystalline residue obtained was purified by recrystallization from CH₂Cl₂/i-Pr₂O (1:5) to give colorless plates of **6** (7.190 g, 92% yield), mp 219–220°. IR(CHCl₃) ν 3420, 1678, and 1587; NMR(CDCl₃) δ 1.09 (3H, d, J=5.5), 2.00–3.20 (4H, m), 3.87 (3H, s), 6.95 (1H, d, J=9), 7.00–7.85 (5H, m), 8.05 (1H, d, J=9), and 10.02 (1H, br). (Found: C, 74.05; H, 6.27; N, 4.37. C₁₉H₁₉NO₃ requires; C, 73.76; H, 6.19; N, 4.53%).

N-Phenyl-2,5-dimethoxy-7-methyl-1-naphthalenecarboxamide (7). A suspension of copper(II) bromide (6.3 g, 2 equiv) in a soln of **6** (4.363 g) in AcOEt (26 ml) and CHCl₃ (26 ml) was stirred magnetically under reflux for 2 h. During the time, the black CuBr₂ disappeared and the color of the soln turned from green to amber. The mixture was filtered through a filter paper and the filter cake was washed with AcOEt. The filtrate was diluted with AcOEt and the usual work-up gave crude bromide. Chromatography on silica gel provided 5.42 g (99% yield) of the bromide as a colorless oil. To a soln of above bromide (1.02 g) in acetone (30 ml), 436 mg (1.2 equiv) of K₂CO₃ was added and stirred under reflux for 1 h. After filtration, the residual solid was washed thoroughly with AcOEt. The combined filtrate and washings were worked-up as usual to give the crude naphthol (765 mg) as an oil. The resulting naphthol, without further purification was used for the next step. A soln of the naphthol (765 mg) was added to a suspension of K₂CO₃ (654 mg, 1.9 equiv), dimethyl sulfate (408 mg, 1.3 equiv), and (n-Bu)₄NHSO₄ (100 mg) in acetone (6 ml) and refluxed under stirring for 1 h. The usual work-up gave 820 mg of an oil which was purified by column chromatography to give 705 mg (88% yield) of **7** as a colorless solid. Recrystallization of **7** from MeOH/CHCl₃ gave an analytical sample, mp 213–214°. IR(CHCl₃) ν 3420, 1675, and 1598; NMR(DMSO-d₆) δ 2.37, 3.85, 3.91 (3×3H, s), 6.66, 7.02 (2×1H, m), 6.90–7.90 (5H, m), 7.35, (1H, d, J=9.5), 8.16 (1H, d,

$J=9.5$), and 10.41 (1H, br, NH). (Found: C, 74.67; H, 5.91; N, 4.36. $C_{20}H_{19}NO_3$ requires; C, 74.76; H, 5.96; N, 4.36%).

Methyl 2, 5-Dimethoxy-7-methyl-1-naphthalenecarboxylate (8). A soln of **7** (1 g) in 20% BF_3 in MeOH (20 ml) was heated at 211–120° (bath temp) for 3 h. The usual work-up of the cooled soln gave an oil which was purified by silica gel chromatography followed by recrystallization from 20% $CHCl_3/i-Pr_2O$ to provide 810 mg (72% yield) of **8**, mp 118–119°. IR($CHCl_3$) ν 1723, 1679, 1583, and 1270. (Found; C, 69.20; H, 6.22. $C_{15}H_{16}O_4$ requires; C, 69.21; H, 6.20%).

2, 5-Dimethoxy-7-methyl-1-naphthalenecarboxylic Acid (9). To a soln of **8** (780 mg) in DMSO (6 ml), 20% aqueous NaOH soln (3 ml, 5 equiv) was added and then heated at 120° (bath temp) for 1 h. The mixture was cooled to room temp and then acidified with 1 N HCl. The usual work-up gave an oil which was purified by column chromatography to give 730 mg (99% yield) of **9** as a colorless solid. Recrystallization of **9** from 20% $i-Pr_2O/CHCl_3$ gave an analytical sample, mp 141–142°. IR($CHCl_3$) ν 1722, 1628, 1597, and 1583. (Found; C, 68.24; H, 5.78. $C_{14}H_{14}O_4$ requires; C, 68.28; H, 5.73%).

Methyl 2-Hydroxy-5-methoxy-7-methyl-1-naphthalenecarboxylate (1). To a stirred soln of **9** (100 mg) in 1.5 ml of CH_2Cl_2 , a soln of 25% BCl_3 in CH_2Cl_2 (0.76 ml, 4 equiv) was added at -78° and then warmed up to 0° for 1 h, the reaction mixture was added to a mixture of excess ice and AcOEt. The crude substance obtained by the usual work up was used for the following step without purification. A soln of the hydroxy acid obtained above in diethyl ether (3 ml), was added excess diazomethane in diethyl ether (3 ml). After 5 min, excess of diazomethane and diethyl ether was removed under reduced pressure to give a solid, which was purified by recrystallization from $CH_2Cl_2/i-Pr_2O$ (1:5) to give colorless plates of **1** (83 mg, 83% yield from **9**), mp 141–142°. IR($CHCl_3$) ν 1657(sh), 1648, 1623, and 1272. (Found; C, 68.20; H, 5.70. $C_{14}H_{14}O_4$ requires; C, 68.28; H, 5.73%).

N, N-Dimethyl-2-(4-oxo-2-hydroxypentyl)-6-methoxybenzamide ethylene acetal (11). A soln of **10** (10.23 g) in THF (100 ml) was added dropwise over a period of 15 min at -78° to a freshly prepared soln of LDA made from $n-BuLi$ (45 ml of a 1.80 M soln in n -hexane, 1.53 equiv) and $i-Pr_2NH$ (8.196 g, 1.53 equiv) in THF (100 ml). The resulting soln was kept at -78° for a period of 1 h and then, a soln of 2-methyl-2-(2-oxoethyl)-1,3-dioxolane¹⁶⁾ (8.267 g, 1.2 equiv) in THF (50 ml) was added over 10 min. After the mixture was stirred for 30 min at -78° , the reaction was quenched by the addition of AcOH (9.27 ml, 3.06 equiv). The usual work-up gave 17.85 g of an oil which was purified by column chromatography to give 16.74 g (98% yield) of **11** as a colorless oil. IR($CHCl_3$) ν 3500, 1620, 1600, and 1583. NMR ($CDCl_3$, of a set of signals of the two conformational isomers obtained with 200 MHz NMR, only singlet signals are shown.) δ 1.33, 1.36 (C-Me), 2.80, 2.83 (N-Me), 3.125, 3.133 (N-Me), 3.798, 3.802 (O-Me), 3.97, and 3.98 (dioxolane); MS m/e 323.1711. Calcd. for $C_{17}H_{25}NO_5$: 323.1733 (M^+).

Oxidation of (11). To a soln of pyridine (28.764 g, 8 equiv) in CH_2Cl_2 (600 ml), CrO_3 (18.181 g, 4 equiv) was added and stirred at room temp for 15 min. To the stirred soln, a soln of **11** (14.7 g) in CH_2Cl_2 (50 ml) was added in one portion,

and immediately, Ac_2O (17.19 ml, 4 equiv) was added. The resulting soln was stirred for 1.5 h at room temp. The mixture was poured into ice water and extracted with AcOEt . The extract was washed successively with 5% NaOH , 5% HCl , sat. NaHCO_3 , and brine, then dried, filtered, and concentrated. The residue was chromatographed on silica gel to give the ketone **12** (12.07 g, 83% yield) as a colorless oil. $\text{IR}(\text{CHCl}_3) \nu$ 1712 and 1622; $\text{NMR}(\text{CDCl}_3) \delta$ 1.40 (3H, s), 2.78 (2H+3H, s), 3.05 (3H, s), 3.79 (3H, s), 3.95 (4H, s), 3.70 (1H, d, $J=17$), 3.90 (1H, d, $J=17$), 6.70 (1H, d, $J=7.5$), 6.79 (1H, d, $J=9$), and 7.23 (1H, dd, $J=7.5$ and 9); MS m/e 321.1548. Calcd. for $\text{C}_{17}\text{H}_{23}\text{NO}_5$: 321.1576.

N, N-Dimethyl-2-(2-oxo-4-hydroxy-3-pentenyl)-6-methoxybenzamide (13).

A soln of **12** (10 g) in acetone (100 ml) was added 10% aqueous HCl (200 ml) and the reaction mixture was stirred at room temp for 3 h. The soln was concentrated *in vacuo* to half of its original volume and the usual work-up gave 8.05 g of crude **13**. Chromatography on silica gel provided 6.510 g of **13** (75% yield) as a colorless oil. $\text{IR}(\text{CHCl}_3) \nu$ 1624; $\text{NMR}(\text{CDCl}_3) \delta$ 2.00, 2.72, 3.03, 3.76 (4 $\times$ 3H, s), 3.42 (1H, d, $J=15$), 3.63 (1H, d, $J=15$), 5.48 (1H, s), 6.78 (1H, d, $J=7.5$), 6.85 (1H, d, $J=7.5$) and 7.25 (1H, t, $J=7.5$); MS m/e 277.1290. Calcd. for $\text{C}_{15}\text{H}_{19}\text{NO}_4$: 277.1314 (M^+).

N, N-Dimethyl-2,7-dimethoxy-5-methyl-1-naphthalenecarboxamide (14).

A soln of **13** (4 g) in conc H_2SO_4 (40 ml) was stirred at room temp for 12 h. The mixture was carefully poured into ice water saturated with NaHCO_3 and extracted with AcOEt . The extract was washed with brine, then dried, filtered and concentrated. The residue was chromatographed on silica gel to give the naphthol (1.680 g, 45% yield). To a stirred suspension of the above naphthol (1.680 g) and K_2CO_3 (1.791 g, 2 equiv) in acetone (20 ml), dimethyl sulfate (1.062 g, 1.3 equiv) and (n-Bu) $_4\text{NHSO}_4$ (100 mg) was added at room temp. The suspension was stirred under reflux for 2.5 h. The crude substance obtained by the usual work-up was chromatographed on silica gel giving **14** (1.630 g, 92% yield). Recrystallization of **14** from *i*- Pr_2O gave an analytical sample, mp 132–134°; $\text{IR}(\text{CHCl}_3) \nu$ 1623; $\text{NMR}(\text{CDCl}_3) \delta$ 2.57, 2.75, 3.20, 3.81, 3.88 (5 $\times$ 3H, s), 6.71 (1H, m), 6.84 (1H, m), 7.06 (1H, d, $J=9.5$), and 7.85 (1H, d, $J=9.5$). (Found: C, 70.01; H, 7.10; N, 5.23. $\text{C}_{16}\text{H}_{19}\text{NO}_3$ requires; C, 70.31; H, 7.01; N, 5.12%).

Methyl 2,7-Dimethoxy-5-methyl-1-naphthalenecarboxylate (15).

To a stirred soln of **14** (1.535 g) in CH_3CN (6 ml), FSO_3CH_3 (4 ml) was added at room temp. After stirring for 1 h, the soln was evaporated under reduced pressure and the residue obtained was taken up in CH_3CN (6 ml). To the stirred soln, 1 N NaOH (9 ml) was added and stirring was continued for 1 h at 50° (bath temp). The resulting mixture was cooled to 0° and the usual work-up gave 1.535 g of an oil, which was purified by silica gel column to provide 1.008 g (66% yield) of the starting amide **14**, and 457 mg (31% yield) of the ester **15**. Recrystallization of **15** from *i*- Pr_2O gave an analytical sample, mp 147–148° (lit.^{12a}) 151–151.5°. (Found: C, 68.99; H, 6.06. $\text{C}_{15}\text{H}_{16}\text{O}_4$ requires; C, 69.21; H, 6.20%). This compound was identical spectroscopically with reported degradation product of NCS chromophore.^{12a})

2,7-Dimethoxy-5-methyl-1-naphthalenecarboxylic Acid (16).

The same procedure as that for **9** gave **16** (380 mg, 99%) from the ester **15** (407 mg) and

aqueous 20% NaOH (1.56 ml) as colorless needles, mp 159–160° (lit.,^{12a}) 133–134°. (Found: C, 68.02; H, 5.56. $C_{14}H_{14}O_4$ requires; C, 68.28; H, 5.73%). This compound was identical spectroscopically with reported degradation product of NCS chromophore.^{12a})

Methyl 2-Hydroxy-7-methoxy-5-methyl-1-naphthalenecarboxylate (2).

The carboxylic acid **16** (300 mg) was treated by the same procedure as that for **9** to give 145 mg (48% yield) of **2**, mp 105–106° lit.,^{12a}) 104–105°; NMR($CDCl_3$, 200 MHz) δ 2.60, 3.90, 4.08 ($3 \times 3H$, s), 6.87 (1H, dd, $J=0.7$ and 2.6 Hz), 7.01 (1H, d, $J=9.2$), 8.00 (1H, d, $J=9.2$), 8.05 (1H, d, $J=2.6$), and 12.13 (1H, s). (Found: C, 68.13; H, 5.75. $C_{14}H_{14}O_4$ requires; C, 68.28; H, 5.73%). This compound was identical in all respects with the natural sample obtained from Tohoku university. Edo *et al.*^{12a}) reported that there was a nuclear Overhauser effect (18%) between the methoxyl group and the paramagnetically shifted member of the ortho coupled hydrogens. Our results were as follows. Irradiation of the methoxyl peak (δ 3.90) resulted in 25% increase in the intensity for C_8-H (δ 8.05) and 7% increase in that for C_6-H (δ 6.87), but no increase for C_4-H (δ 8.00).

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