Pergamon

Tetrahedron Vol. 50, No. 5, pp. 1327-1340, 1994

0040-4020/94 $6.00+0.00

0040-4020(93)E0203-R

Synthesis and Binding of Simple Neocarzinostatin Chromophore Analogues to the Apoprotein

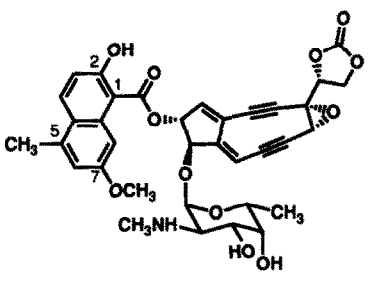
Kazunobu Takahashi, Toshiyuki Tanaka, Toshio Suzuki, and Masahiro Hirama*

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

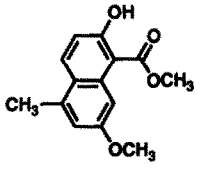
Abstract: Synthetic analogues (**3-5, 8-10**) of neocarzinostatin chromophore (**1**) are found to bind to the apoprotein with high affinity. Their binding energies suggest that the naphthoate moiety of **1** is essential for the binding, and that the C5-CH_3 and C7-OCH_3 groups are necessary for the high affinity.

INTRODUCTION

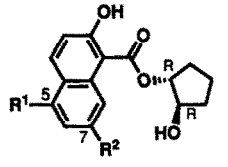
A labile nonprotein chromophore (**1**)[1] of neocarzinostatin (NCS)[2], a potent antitumor antibiotic complex, binds to NCS apoprotein tightly[3] and specifically.[4] Consequently **1** is stabilized toward light[5] and heat. The



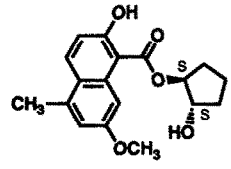
1



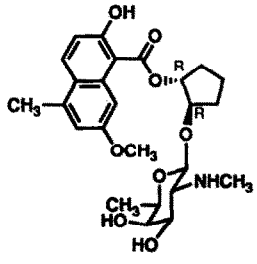
2



3 : $R^1 = CH_3$, $R^2 = OCH_3$
4 : $R^1 = CH_3$, $R^2 = OH$
5 : $R^1 = H$, $R^2 = OCH_3$
6 : $R^1 = CH_3$, $R^2 = H$
7 : $R^1 = H$, $R^2 = H$

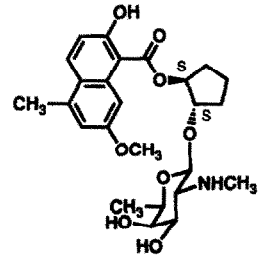


8



9

11 (α-glycoside)



10

12 (α-glycoside)

binding structure and the stabilizing interactions preventing **1** from decomposing are intriguing subjects in terms of molecular recognition between the protein and the bioactive small molecule.[6,7] Edo *et al.* reported interesting competitive binding experiments: addition of a 30-fold excess β-naphthol to the acetate buffer solution of NCS (1:1) complex caused a complete release of **1**, but a large excess (5000-fold) *D*-galactosamine was needed. Moreover, they estimated maximum dissociation constants (K_d) for methyl 2-hydroxy-7-methoxy-5-methyl-1-naphthoate (**2**) of 1 x 10^{-5} M and for **1** of 7.0 x 10^{-6} M.[3b] Our recent studies on the three-dimensional structure of NCS complex by computer modeling[6] and nuclear magnetic resonance (NMR) analysis[7] revealed that the naphthoate moiety of **1** is placed on the bottom of the hydrophobic binding pocket. These results suggested that the β-hydroxy-α-naphthoate moiety is essential for binding to the apoprotein. The NMR analysis of the NCS complex indicated a precise binding structure and several possible interactions operating between the naphthoate of **1** and the amino acid residues as illustrated in Fig. 1: two hydrogen-bondings between C7-O and OγH of Ser98, and between C2-O and Nε1H of Trp39; CH-π interaction of C7-OCH_3 with the aromatic ring of Phe52; CH···O type hydrogen-bonding between C6-H and the backbone carbonyl of Gly96; Van der Waals interaction of C5-CH_3 with the β-methylene of Gln94.[7]

There may be two approaches to evaluate these interactions through the apoprotein-ligand binding experiments. One is to prepare the point-mutated apoproteins at the above residues, to which **1** would be bound with a distinct affinity. The other is to design and synthesize analogues of **1** useful for clarifying the binding interactions toward the intact NCS apoprotein. Our study takes the latter approach. Since it is difficult to manipulate **1** through ester or glycoside hydrolysis due to its extreme instability, we have synthesized various stable analogues of **1**. In this paper, we focus our attention on the naphthoate moiety and report the syntheses of several naphthoate monoester derivatives (**3**-**10**) of optically active *trans*-1,2-cyclopentanediol and their binding affinities to NCS apoprotein by means of fluorescence quenching titration, leading to the evaluation of the contribution of the substituents on the naphthalene ring toward the affinity for the apoprotein.

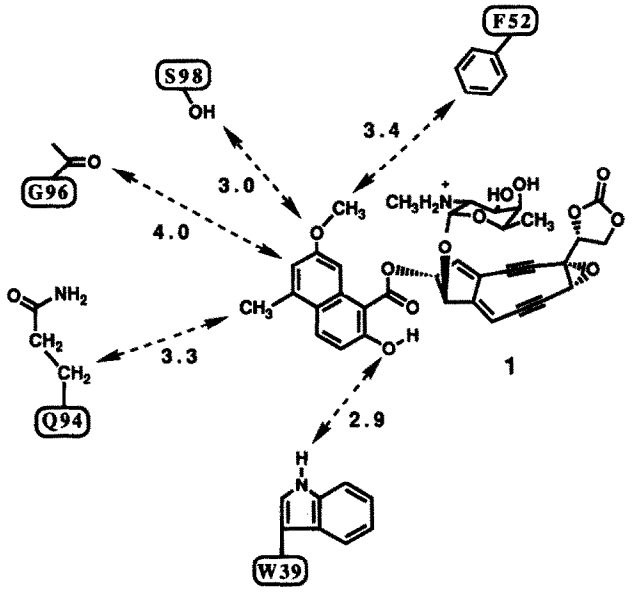


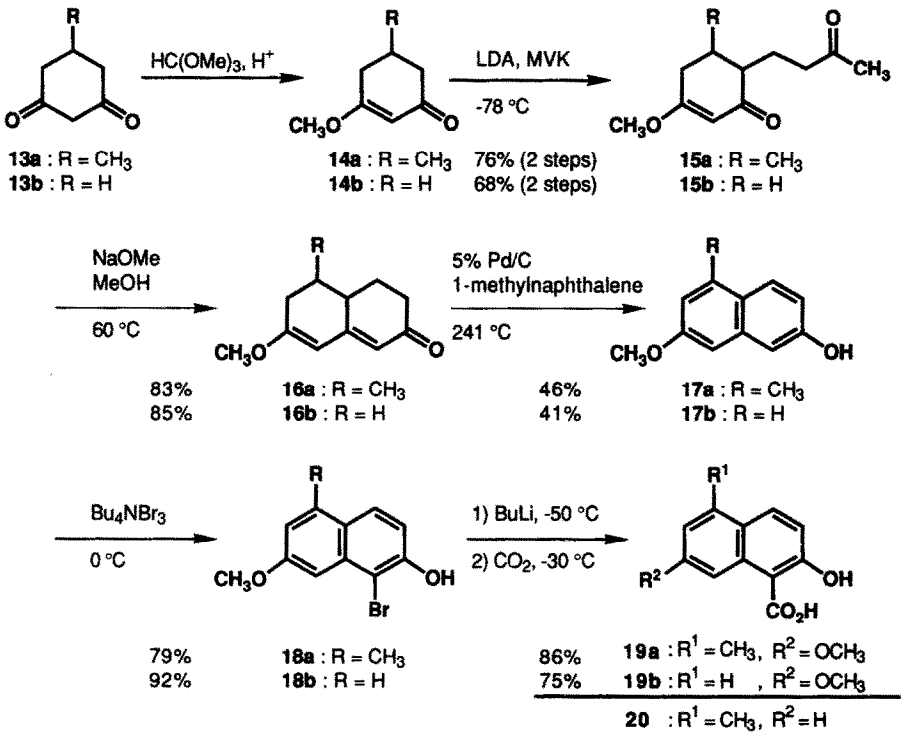
Fig. 1. Binding interactions between the naphthoate moiety of **1** and the amino acid residues of the apoprotein implicated by NMR analysis of NCS complex;[7] the possible interactions are indicated by arrows. The number is an interatomic distance between the heavy atoms of the interacting groups.

RESULTS

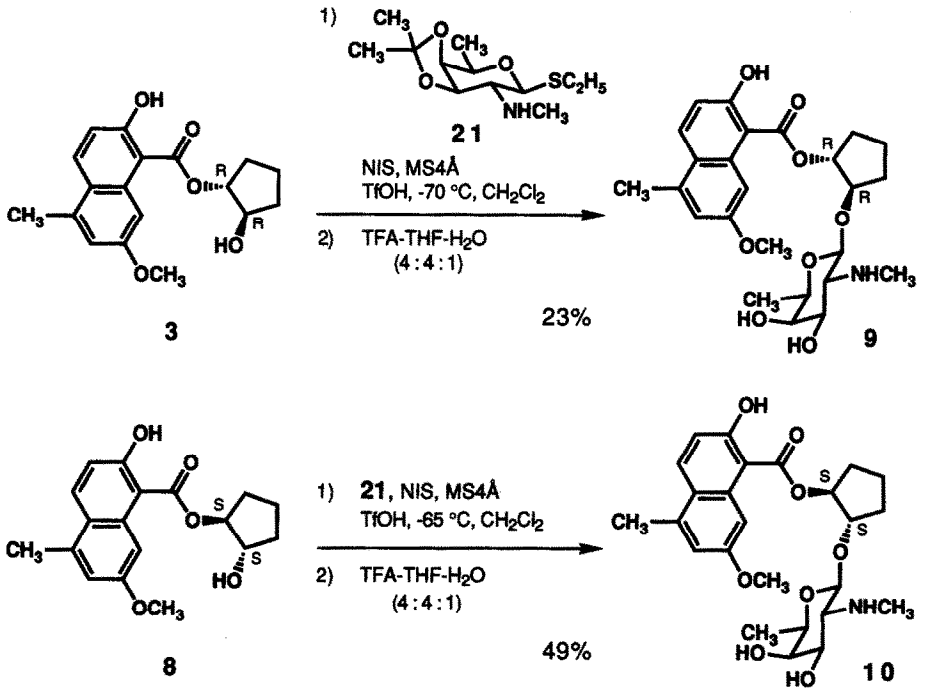
Syntheses of model compounds

Our simple and efficient synthesis of 2-hydroxy-7-methoxy-5-methyl-1-naphthoic acid (**19a**) is outlined in Scheme 1.[8] This synthetic strategy is very useful to synthesize the related 2-hydroxy-1-naphthoic acid derivatives such as 2-hydroxy-7-methoxy-1-naphthoic acid (**19b**) (Scheme 1). Demethoxy derivative **20** was also synthesized in the same way starting from 5-methyl-2-cyclohexen-1-one. These acids were condensed with (*R,R*)-1,2-cyclopentanediol[9] of 97% ee by 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) to afford the mono-esters **3**, **5**, and **6**. The enantiomeric **8** was also synthesized from **19a** and (*S,S*)-1,2-cyclopentanediol[9] of 79% ee. The diol derivative **4** was obtained in high yield by direct demethylation of the corresponding methyl ether **3** with BBr_3.[10] The derivative with no substitution at C5 and C7 (**7**) was prepared from commercially available 2-hydroxy-1-naphthoic acid.

Moreover, we tried to synthesize glycoside analogues **11** and **12** with the natural α-stereochemistry from **3** and **8** by developing a good way to couple these alcohols with an appropriate *N*-methyl-*D*-fucosamine donor directly without protection of the *N*-methylamino group. We envisioned that the neighboring group participation of the *N*-methylamino group of such a donor in the glycosidation can be inhibited in the presence of excess strong acids or silylating agents through *in situ* formation of the ammonium salt. Activation of thioglycoside **21**[11] with a stoichiometric amount of NIS/TfOH[12] or NIS/TESOTf in CH_2Cl_2[12a] realized the glycosidation in reasonable yields, but their stereochemistry was controlled in the undesired way. The β-glycosides **9** (23%) and **10** (49%) were obtained stereoselectively from **3** and **8**, respectively. Stereoselective α-glycosidation methodology for **21** and other donors is currently under investigation.



Scheme 1. Simple entry for the syntheses of various 5,7-substituted 2-hydroxy-1-naphthoic acids (**19-20**).



Fluorescence quenching titration

Edo[3b] and Goldberg[3a] reported that the intensity of fluorescence emission at 448 nm from **1** due to the naphthoate moiety was decreased and the maximum shifted to 420 nm by interaction with the apoprotein, and estimated the dissociation constant (*Kd*) for NCS complex of 7.0 x 10^{-6} M in 0.1 M acetate buffer (pH 4.0)[3b] or 2.0 x 10^{-8} M in 20% methanol-20 mM Tris buffer (pH 8.0).[3a,13] Excitation and emission maxima of the model compounds (5.0 x 10^{-6} M) in 5% methanol-0.1 M ammonium acetate buffer (pH 4.0)[14] are listed in Table 1. Addition of the apoprotein (1.0 x 10^{-5} to 1.5 x 10^{-4} M) to these solutions of the fluorophores[15] caused the similar decreases of the fluorescence intensities except for compound **4** as shown in Fig. 2 and the shifts of the maxima (Table 1). In case of compound **4**, the fluorescence intensity increased by the addition of the apoprotein. Minimum association constants ($Ka = 1/Kd$) of the fluorophores to the apoprotein were estimated according to Edo's procedure[3b] utilizing Kondo's eq (1),[16] provided that (i) a fluorophore forms a 1:1 complex with apoprotein, (ii) $C_0^P >> C^{PL}$, and that (iii) the fluorescence intensity decrement (or increment in case of **4**) is proportional to the concentration of the complex ($\Delta F = \alpha C^{PL}$). The linear relationships between $1/\Delta F$ and $1/C_0^P$ for **3**-**5** and **8**-**10** (Fig. 3) supported their formation of a 1:1 complex with the apoprotein. Association constants (*Ka*) calculated from the slopes and the intercepts of these linear plots are listed in Table 2. The values for the C7-deoxy analogues **6** and **7** could not be estimated because of their weak fluorescence intensities (about one twentieth of those of the other fluorophores).

$$\frac{1}{\Delta F} = \frac{1}{Ka} \cdot \frac{1}{\alpha \cdot C_0^L} \cdot \frac{1}{C_0^P} + \frac{1}{\alpha \cdot C_0^L} \qquad (1)$$

α : proportionality constant

C_0^L : total concentration of a fluorophore

C_0^P : total concentration of apoprotein

C^{PL} : concentration of a fluorophore-apoprotein complex

Table 1. Excitation and Emission Maxima of **3-10** (5 x 10^{-6} M).

	3	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Excitation maximum (nm)	330	333	330	340	335	330	330	330
Emission maximum (nm)	447	~450sh[a]	432	413	394	447	445	447
Emission maximum after excess addition of apoprotein (nm)	437	445	427	-[b]	-[b]	437	435	442

[a] This shoulder was used for fluorescence quenching titration, because it was raised substantially to become one of the main peaks as apoprotein was added, although the intensities of two main peaks appeared originally at 525 nm and 545 nm did not increase appreciably. [b] Too weak to be assigned.

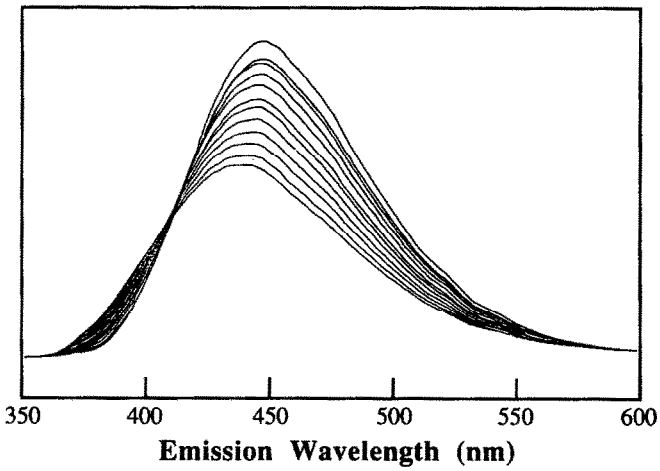


Fig. 2. Fluorescence spectra of **3** (5.0 x 10^{-6} M). Concentration of apoprotein, from top to bottom: 0, 1.0 x 10^{-5}, 1.5 x 10^{-5}, 2.0 x 10^{-5}, 3.0 x 10^{-5}, 4.0 x 10^{-5}, 5.0 x 10^{-5}, 6.5 x 10^{-5}, 8.0 x 10^{-5}, 1.0 x 10^{-4}, 1.25 x 10^{-4}, and 1.5 x 10^{-4} M.

Table 2. Minimum Association Constants and Binding Energies for **3-5**, **8-10**, and **1** to Apoprotein, Estimated by Fluorescence Quenching Titration.

	3	**4**	**5**	**8**	**9**	**10**	**1**[a]
Ka (M^{-1})	1.3 x 10^4	4.2 x 10^3	4.0 x 10^3	1.5 x 10^4	2.0 x 10^4	1.6 x 10^5	ca. 3 x 10^6
ΔG (KJ/mol)	-23.5	-20.7	-20.5	-23.8	-24.5	-29.7	ca. -37

[a] *Ka* for **1** estimated by competitive binding: see text.

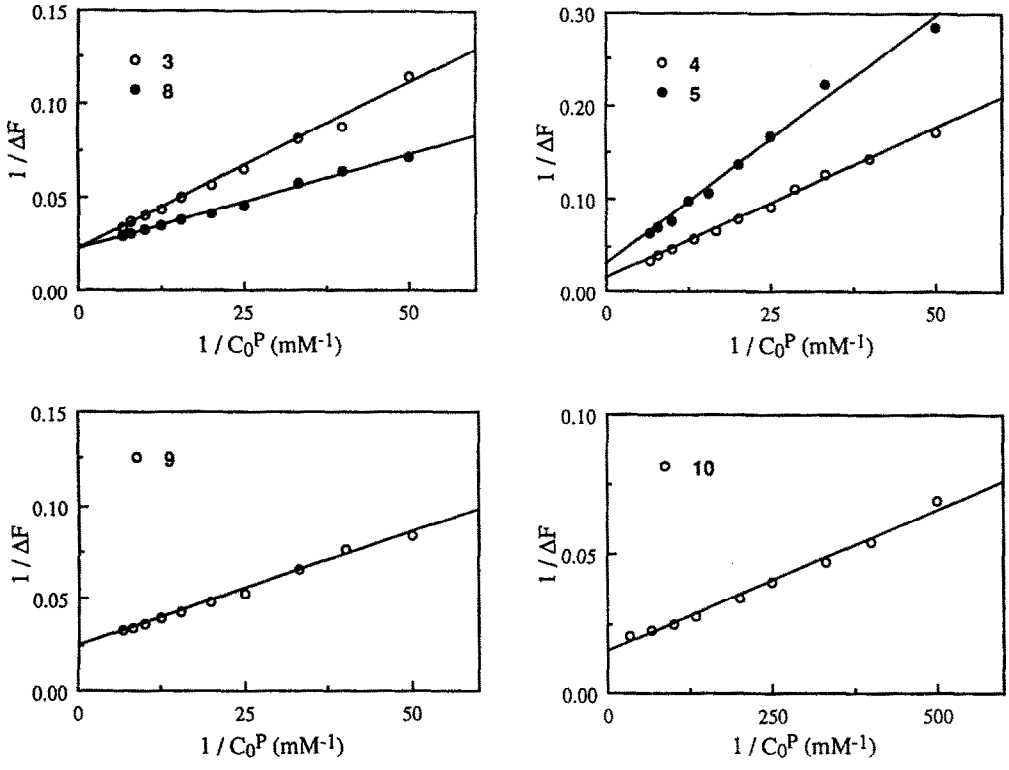


Fig. 3. Fluorescence quenching titration of **3**-**5** and **8**-**10** (5.0×10^{-6} M) by apoprotein.

Table 3. Competitive Binding of **9** or **10** with **1** to Apoprotein.

Model Compound (1.0×10^{-3} M)	Incubation Time (h)	Release of **1**[a,b] (%)
9	3	2
9	24	13
9	48	23
10	3	12
10	24	37
10	48	50
-[c]	24	3
-[c]	48	4

[a] Percentage of free apoprotein detected by HPLC.

[b] Initial concentration of NCS complex (**1**-apoprotein): 1.0×10^{-4} M.

[c] Model compounds not added.

Competitive binding assay

Estimation of *Ka* for NCS complex (**1**-apoprotein) in the same solvent system as above is necessary for comparison, since *Ka* is highly dependent upon solvent and pH.[3,13] However, the fluorescence intensity of **1** was approximately one twentieth of those of the fluorophores, and its binding to the apoprotein turned out to be so tight that eq (1) was no longer useful for the estimation of *Ka* or *Kd* for **1**. Therefore, the binding constant calculation reported for NCS complex ($Kd = 7.0 \times 10^{-6}$ M) according to the above method[3b] might be inaccurate or overestimated at least.

Therefore, we carried out a competitive binding experiment using **9** and **10** to estimate *Ka* for **1**. Solution of **9** or **10** (1.0×10^{-3} M) and NCS complex (1.0×10^{-4} M) in 5% methanol-0.1 M ammonium acetate buffer (pH 4.0) was stirred at 37 °C in the dark. As shown in Table 3, considerable amount of free apoprotein was detected by high performance liquid chromatography (HPLC) after one or two days when **9** or **10** was added, whereas no free apoprotein appeared essentially in the absence of **9** and **10**. These suggest that **9** and **10** bind competitively to the apoprotein and that *Ka* for **1** is $\geq 3 \times 10^6$ M^{-1} on the assumption that equilibrium was reached after 48 h (Table 2), although we cannot rule out the conformational change of the protein and the resulting release of **1** in the presence of excess **9** or **10**.

DISCUSSION

While the bindings of the naphthoates **3** and **8** are still 200-fold weaker than the natural chromophore (**1**), their binding energies reach to approximately 64% of the total binding energy of **1**. This supports the previous speculation[6,7] that the naphthoate moiety of **1** is mainly responsible for the high affinity of **1** toward NCS apoprotein. There is virtually no increase of *Ka* for the glycoside **9** compared with the alcohol **3**. On the other hand, the glycoside **10** has a 10-fold higher affinity than the alcohol **8** and the glycoside **9**. The analogues **3** and **9** should have the natural type-(*R*,*R*)-cyclopentanediol configuration,[1b] and **8** and **10** have the unnatural (*S*,*S*). The reason for this interesting difference should be discussed after the corresponding α-glycosides **11** and **12** become available in our hand.

The binding affinity of the demethyl analogue **5** is approximately one third of that of **3**, supporting the Van der Waals interaction between C5-CH_3 and the β-methylene of Gln94.

While the demethylated diol **4** also showed a lower affinity than **3**, **4** should be able to retain the hydrogen-bonding with Ser98. So, the *Ka* difference between **4** and **3** appears to correspond to the loss of CH-π interaction between C7-OCH_3 and the nearby aromatic ring of Phe52.[7] This CH-π interaction could be then estimated as approximately -2.8 KJ/mol. This value is very reasonable when considering the maximum stabilization energy of -3.5 KJ/mol for the methane-benzene CH-π interaction calculated by CNDO/2.[17] Unfortunately, the analogous **6** and **7** with no oxygen functionality at C7 exhibited too weak fluorescences to estimate the binding affinities through the apoprotein-quenching titration. Therefore, to discuss the contribution of C7-oxygen as well as C2 hydroxy group, other techniques to measure the binding affinity are needed.

EXPERIMENTAL

General methods

Melting points were measured on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-370 polarimeter. Infrared (IR) spectra were recorded on a JASCO FT/IR-7000 spectrophotometer. Proton and carbon NMR spectra were measured on Varian Gemini-200, JEOL GX-400, or Bruker AM-600 spectrometers. For proton spectra, chemical shifts are reported in parts per million (ppm) relative to an internal reference of tetramethylsilane (δ 0.00) for solvent $CDCl_3$ and relative to the CDH_2OH peak at 3.30 ppm for solvent CD_3OD. For carbon spectra, the reference is 77.0 ppm for $CDCl_3$. Elemental analyses were performed on a Yanaco CHN corder MT-5. Low- and high-resolution mass spectra (LRMS and HRMS) were obtained using Hitachi M-52 and JEOL HX-110 mass spectrometers, respectively.

Tetrahydrofuran (THF) was distilled from sodium metal/benzophenone ketyl just prior to use; MeOH and CH_2Cl_2 were distilled from Mg turnings and CaH_2, respectively. Analytical and preparative thin layer chromatography (TLC) was performed by using Merck Kieselgel 60 F_{254} glass plates precoated with a 0.25 mm and 0.50 mm layer of silica gel, respectively. Flash column chromatography was carried out using Merck Kieselgel 60 (230-400 mesh) silica gel.

Synthesis and characterization of compounds

3-Methoxy-5-methyl-2-cyclohexen-1-one (14a).[18] To a solution of commercially available 5-methylcyclohexane-1,3-dione (**13a**) (38.5 g, 305 mmol) in MeOH (600 mL) was added trimethyl orthoformate (33.4 mL, 305 mmol) and *p*-toluenesulfonic acid monohydrate (200 mg), and the resulting solution was stirred for 25 h at room temperature. After concentrated to 100 mL under reduced pressure, the reaction mixture was diluted with water (200 mL) and then extracted with CH_2Cl_2 (3 x 200 mL). The combined organic layers were dried over $MgSO_4$, concentrated under reduced pressure, and purified on silica gel flash column chromatography (hexane/AcOEt = 1/1) to give 40.5 g (95%) of **14a**: colorless needles; mp 46 °C (hexane); IR (KBr) ν 3446, 2954, 1657, 1609, 1383, 1226, 1143, 1007, 605, 551, 480, 418 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.08 (3H, d, J = 6.2 Hz), 2.04 (1H, dd, J = 16.5, 11.5 Hz), 2.15 (1H, ddd, J = 16.5, 10.5, 1.2 Hz), 2.23 (1H, ttq, J = 11.5, 6.2, 4.0 Hz), 2.42 (1H, bddd, J = 16.5, 4.0, 1.5 Hz), 2.43 (1H, bddd, J = 16.5, 4.0, 1.5 Hz), 3.69 (3H, s), 5.36 (1H, d, J = 1.2 Hz). Anal. Calcd for $C_8H_{12}O_2$: C, 68.55; H, 8.63. Found: C, 68.41; H, 8.59.

3-Methoxy-5-methyl-6-(3-oxobutyl)-2-cyclohexen-1-one (15a). To a solution of $(i\text{-Pr})_2NH$ (4.81 mL, 34.3 mmol) in THF (230 mL) was slowly added BuLi (21.5 mL of a 1.6 M solution in hexane, 34.4 mmol) at 0 °C under argon, and the mixture was stirred at 0 °C for 1 h. To this lithium diisopropylamide (LDA) solution, a solution of **14a** (4.38 g, 31.2 mmol) in THF (20 mL) was added dropwise at -78 °C via cannula over 30 min. After stirring for 1 h at the same temperature, 3-buten-2-one (MVK, 2.60 mL, 31.2 mmol) was added to the mixture, and stirring was continued for 40 min. Water (300 mL) was added at the temperature, and the mixture was extracted with Et_2O (2 x 200 mL) and then with AcOEt (2 x 200 mL). The combined organic extracts were washed with satd. aq. $NaHCO_3$, dried over $MgSO_4$, and concentrated under reduced pressure. Flash column chromatography (hexane/AcOEt = 1/1) gave 5.27 g (80%) of **15a**: pale yellow oil; IR (film) ν 3460, 2934, 1715, 1651, 1613, 1444, 1383, 1224, 1164, 1007, 839, 553 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.09 (3H, d, J = 6.5 Hz), 1.70-1.85 (2H, m), 1.98-2.03 (1H, m), 2.06 (1H, dtq, J = 11.3, 5.0, 6.5 Hz), 2.15 (3H, s), 2.17 (1H, dd, J = 17.5, 7.4 Hz), 2.34-2.45 (1H, m), 2.49-2.55 (1H, m), 2.58 (1H, dd, J = 17.5, 5.0 Hz), 3.69 (3H, s), 5.30 (1H, s). LRMS (EI, 25 eV, 100 °C) *m/z*(%) 210(5), 153(19), 140(67), 139(15), 137(16), 125(100), 98(71).

7-Methoxy-5-methyl-4,4a,5,6-tetrahydro-2(3*H*)-naphthalenone (16a). A solution of **15a** (42.3 g, 201 mmol) in MeOH (100 mL) was added to NaOMe solution [Na (11.6 g, 504 mmol)/MeOH (109 mL)] and refluxed for 1 h 15 min under argon. The mixture was diluted with water (200 mL) at room temperature, and acetic acid was added until the solution was neutralized. The mixture was extracted with CH_2Cl_2 (4 x 200 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. Flash column chromatography (hexane/AcOEt = 1/1) gave 31.9 g (83%) of **16a**: pale yellow prisms; mp 81 °C (haxane-AcOEt); IR (KBr) ν 2966, 2926, 1651, 1605, 1458, 1444, 1388, 1325, 1292, 1236, 1174, 1143, 1006, 984, 917, 884 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.09 (3H, d, J = 6.6 Hz), 1.48-1.60 (1H, m), 1.69 (1H, dtq, J = 11.7, 6.6, 5.0 Hz), 2.06 (1H, bddt, J = 11.7, 4.0, 2.0 Hz), 2.15 (1H, bddd, J = 17.0, 11.7, 1.7 Hz), 2.26-2.39 (2H, m), 2.29 (1H, dd, J = 17.0, 5.0 Hz), 2.48-2.54 (1H, m), 3.70 (3H, s), 5.41 (1H, bd, J = 1.5 Hz), 5.74 (1H, bd, J = 1.2 Hz). Anal. Calcd for $C_{12}H_{16}O_2$: C, 74.97; H, 8.39. Found: C, 74.87; H, 8.33.

7-Methoxy-5-methyl-2-naphthol (17a). A mixture of **16a** (19.6 g, 102 mmol), 1-methylnaphthalene (400 mL, bp 241-243 °C), and 5% Pd/C (10.0 g, Kawaken Chemical Co. Ltd.) was refluxed for 1 h 15 min under nitrogen. After cooling to room temperature, the mixture was filtered through Celite pad. The filtrate was diluted with Et_2O (300 mL) and washed with 0.5 N NaOH (5 x 150 mL). When the combined aqueous layers were cooled at 0 °C and acidified to pH 1 with conc. HCl, colorless crystals appeared. The mixture was extracted with CH_2Cl_2 (4 x 300 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. Flash column chromatography (hexane/AcOEt = 3/1) gave 8.76 g (46%) of **17a**: colorless needles; mp 132-133 °C (CH_2Cl_2); IR (KBr) ν 3268, 1630, 1470, 1402, 1238, 1220, 1166, 1050, 859 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 2.60 (3H, s), 3.88 (3H, s), 5.10 (1H, bs), 6.84 (2H, s), 6.96 (1H, dd, J = 8.9, 2.6 Hz), 7.06 (1H, d, J = 2.6 Hz), 7.80 (1H, d, J = 8.9 Hz). HRMS (EI, 70 eV) *m/z* calcd. for $C_{12}H_{12}O_2$: 188.0837; found 188.0830.

1-Bromo-7-methoxy-5-methyl-2-naphthol (18a). To a solution of **17a** (11.0 g, 58.4 mmol) in CH_2Cl_2-MeOH (3 : 2, 500 mL) was added a solution of Bu_4NBr_3 (28.1 g, 58.4 mmol) in CH_2Cl_2-MeOH (3 : 2, 100 mL) at 0 °C under argon. After stirring for 5.5 h at 0 °C, water (400 mL) was added. The mixture was extracted with CH_2Cl_2 (4 x 250 mL), and the combined organic extracts were dried over $MgSO_4$. Concentration of the organic layers under reduced pressure followed by flash column chromatography (CH_2Cl_2/MeOH = 100/1) gave 12.4 g (79%) of **18a**: colorless prisms; mp 138-139 °C; IR (KBr) ν 3420, 2944, 1624, 1516, 1466, 1435, 1398, 1212, 1178, 1060, 1000, 866, 830, 806, 762 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 2.62 (3H, s), 3.94 (3H, s), 5.86 (1H, s), 6.89 (1H, dd, J = 2.4, 0.9 Hz), 7.13 (1H, d, J = 9.1 Hz), 7.21 (1H, d, J = 2.4 Hz), 7.81 (1H, d, J = 9.1 Hz). HRMS (EI, 70 eV) *m/z* calcd. for $C_{12}H_{11}O_2Br$: 265.9942; found 265.9941.

2-Hydroxy-7-methoxy-5-methyl-1-naphthoic acid (19a). To a solution of **18a** (8.76 g, 32.8 mmol) in THF (331 mL) was slowly added BuLi (50.0 mL of a 1.6 M solution in hexane, 80.0 mmol) at -50 °C under argon. The solution turned green after the mixture was stirred for 40 min at -30 °C. Then, CO_2 gas was bubbled through the solution for 15 min, and the solution turned deep green. The mixture was diluted with CH_2Cl_2 (200 mL) and extracted with satd. aq. $NaHCO_3$ (4 x 150 mL). When the combined aqueous layers were acidified to pH 1 with conc. HCl, colorless crystals appeared. The mixture was extracted with $CHCl_3$ (4 x 200 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure to afford 6.55 g (86%) of **19a**: pale yellow prisms; mp 136-138 °C ($CHCl_3$); IR (KBr) ν 3440, 2924, 1632, 1441, 1400, 1288, 1247, 1232, 1104, 1079, 1025, 851 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 2.65 (3H, s), 3.95 (3H, s), 6.93 (1H, dd, J = 2.4, 0.9 Hz), 7.06 (1H, d, J = 9.2 Hz), 8.11 (1H, d, J = 9.2 Hz), 8.23 (1H, bd, J = 2.8 Hz), 12.10 (1H, s). HRMS (EI, 70 eV) *m/z* calcd. for $C_{13}H_{12}O_4$: 232.0736; found 232.0732.

3-Methoxy-2-cyclohexen-1-one (14b): colorless oil; IR (neat) ν 3460, 2950, 1647, 1605, 1458, 1431, 1381, 1352, 1330, 1228, 1187, 1137, 1004, 963, 863, 826, 760, 596 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.99 (2H, tt, J = 6.7, 6.4 Hz), 2.35 (2H, t, J = 6.7 Hz), 2.42 (2H, bt, J = 6.4 Hz), 3.70 (3H, s), 5.38 (1H, bs). HRMS (EI, 70 eV) *m/z* calcd. for $C_7H_{10}O_2$: 126.0681; found 126.0682.

3-Methoxy-6-(3-oxobutyl)-2-cyclohexen-1-one (15b): colorless oil; IR (neat) ν 3472, 2944, 1715, 1653, 1609, 1456, 1381, 1230, 1199, 1172, 984, 835 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.68-1.80 (2H, m), 1.95-2.05 (2H, m), 2.16 (3H, s), 2.22 (1H, dddd, J = 10.0, 7.4, 5.8, 4.7 Hz), 2.45 (2H, t, J = 6.0 Hz), 2.58 (2H, t, J = 7.4 Hz), 3.68 (3H, s), 5.32 (1H, s).

7-Methoxy-4,4a,5,6-tetrahydro-2(3*H*)-naphthalenone (16b): pale yellow needles; mp 99-100 °C (hexane-AcOEt); IR (KBr) ν 3450, 2936, 1644, 1609, 1576, 1460, 1446, 1383, 1330, 1263, 1249, 1205, 1172, 1143, 1019, 978, 880 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.55 (1H, dq, J = 13.0, 5.0 Hz), 1.72 (1H,

dddd, J = 14.0, 13.0, 11.8, 5.0 Hz), 1.95 (1H, bddt, J = 13.0, 5.0, 2.0 Hz), 2.08 (1H, bddt, J = 13.0, 4.6, 2.5 Hz), 2.29 (1H, ddd, J = 17.7, 5.0, 2.0 Hz), 2.40 (1H, ddd, J = 17.2, 14.0, 5.0 Hz), 2.40-2.54 (3H, m), 3.71 (3H, s), 5.44 (1H, d, J = 1.2 Hz), 5.75 (1H, bs). HRMS (EI, 70 eV) *m/z* calcd. for $C_{11}H_{14}O_2$: 178.0994; found 178.0989.

7-Methoxy-2-naphthol (17b): colorless plates; mp 120 °C (hexane-AcOEt); IR (KBr) ν 3244, 1634, 1613, 1518, 1481, 1450, 1388, 1352, 1282, 1209, 1183, 1160, 1031, 859, 830, 793, 466 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 3.91 (3H, s), 5.04 (1H, bs), 6.94 (1H, dd, J = 7.5, 2.3 Hz), 6.99 (1H, dd, J = 9.2, 2.3 Hz), 6.99 (1H, bd, J = 2.3 Hz), 7.06 (1H, bd, J = 2.3 Hz), 7.66 (1H, bd, J = 9.2 Hz), 7.66 (1H, bd, J = 7.5 Hz). HRMS (EI, 70 eV) *m/z* calcd. for $C_{11}H_{10}O_2$: 174.0681; found 174.0672.

1-Bromo-7-methoxy-2-naphthol (18b): colorless powder; mp 92-93 °C (hexane-AcOEt); IR (KBr) ν 3424, 1624, 1514, 1464, 1427, 1381, 1222, 1168, 1143, 839 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 3.97 (3H, s), 5.88 (1H, s), 7.04 (1H, dd, J = 8.8, 2.5 Hz), 7.12 (1H, d, J = 8.8 Hz), 7.33 (1H, bd, J = 2.5 Hz), 7.66 (1H, bd, J = 8.5 Hz), 7.67 (1H, bd, J = 8.5 Hz). HRMS (EI, 70 eV) *m/z* calcd. for $C_{11}H_9O_2Br$: 251.9786; found 251.9797.

2-Hydroxy-7-methoxy-1-naphthoic acid (19b): colorless needles; mp 144-145 °C ($CHCl_3$); IR (KBr) ν 3442, 2854, 1622, 1518, 1446, 1425, 1301, 1241, 1226, 1205, 845, 806 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 3.94 (3H, s), 7.02 (1H, d, J = 9.0 Hz), 7.04 (1H, dd, J = 8.8, 2.4 Hz), 7.66 (1H, d, J = 9.0 Hz), 7.86 (1H, bd, J = 8.8 Hz), 8.29 (1H, bd, J = 2.4 Hz), 12.27 (1H, s). HRMS (EI, 70 eV) *m/z* calcd. for $C_{12}H_{10}O_4$: 218.0579; found 218.0559.

2-Hydroxy-5-methyl-1-naphthoic acid (20): ^1H NMR (200 MHz, $CDCl_3$) δ 2.69 (3H, s), 7.22 (1H, d, J = 9.3 Hz), 7.25 (1H, bd, J = 7.0 Hz), 7.49 (1H, dd, J = 9.0, 7.0 Hz), 8.22 (1H, bd, J = 9.3 Hz), 8.78 (1H, bd, J = 9.0 Hz), 12.05 (1H, s).

(1*R*,2*R*)-2-(2-Hydroxy-7-methoxy-5-methyl-1-naphthalenecarbonyloxy)-1-cyclopentanol (3). To a solution of **19a** (20.1 mg, 0.0866 mmol) and (*R,R*)-1,2-cyclopentanediol[9] (89.1 mg, 0.872 mmol) in CH_2Cl_2 (0.86 mL) was added EDC (22.7 mg, 0.118 mmol) at 0 °C under argon. After stirring the mixture for 2 h at 0 °C, AcOEt (10 mL) and 1 M HCl (10 mL) were added, and the mixture was extracted with AcOEt (4 x 10 mL). The combined AcOEt layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified on flash column chromatography (hexane/AcOEt = 4/1) to afford 22.9 mg (84%) of **3**: colorless prisms; mp 98-100 °C (hexane-CH_2Cl_2); $[\alpha]^{22}_D$ -36.4° (c 0.720, $CHCl_3$); IR (KBr) ν 3446, 2962, 1647, 1618, 1412, 1379, 1251, 1209, 1035, 847 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 1.76 (1H, ddt, J = 15.0, 7.8, 5.5 Hz), 1.87 (1H, dq, J = 13.5, 7.5 Hz), 1.93 (1H, dtt, J = 13.5, 8.0, 5.5 Hz), 2.02 (1H, ddt, J = 13.5, 7.0, 5.0 Hz), 2.15 (1H, dq, J = 14.0, 7.0 Hz), 2.36 (1H, dq, J = 14.0, 7.0 Hz), 2.63 (3H, s), 3.90 (3H, s), 4.37 (1H, bddd, J = 7.0, 5.0, 3.2 Hz), 5.24 (1H, ddd, J = 7.3, 4.0, 3.2 Hz), 6.89 (1H, d, J = 1.5 Hz), 7.04 (1H, d, J = 9.0 Hz), 8.04 (1H, d, J = 9.0 Hz), 8.05 (1H, d, J = 1.5 Hz), 11.73 (1H, s). HRMS (EI, 70 eV) *m/z* calcd. for $C_{18}H_{20}O_5$: 316.1311; found 316.1311.

(1*R*,2*R*)-2-(2,7-Dihydroxy-5-methyl-1-naphthalenecarbonyloxy)-1-cyclopentanol (4). To a solution of **3** (13.6 mg, 0.0430 mmol) in CH_2Cl_2 (0.5 mL) was added BBr_3 (20.5 μL, 0.217 mmol) at -78 °C under argon. After stirring for 40 min at -50 °C, the reaction was quenched by the addition of satd. aq. $NaHCO_3$ (3 mL). The mixture was extracted with CH_2Cl_2 (3 x 5 mL), and the combined organic extracts were dried over $MgSO_4$. Evaporation of the solvent under reduced pressure gave 10.9 mg (84%) of crude **4**, which was recrystallized from hexane and AcOEt to give **4** (6.2 mg, 49%): colorless needles; mp 184-185 °C (hexane-AcOEt); $[\alpha]^{22}_D$ -55.9° (c 0.53, MeOH); IR (KBr) ν 3430, 2956, 1630, 1615, 1456, 1429, 1381,

1321, 1255, 1207, 1073, 1056, 859, 818 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.60-2.47 (6H, m), 2.64 (3H, s), 4.37-4.47 (1H, m), 5.16-5.27 (1H, m), 5.58 (1H, bs), 6.86 (1H, d, J = 2.5 Hz), 7.03 (1H, d, J = 8.8 Hz), 8.01 (1H, d, J = 2.5 Hz), 8.04 (1H, d, J = 8.8 Hz), 12.08 (1H, s). HRMS (EI, 70 eV) *m/z* calcd. for $C_{17}H_{18}O_5$: 302.1154; found 302.1156.

(1*R*,2*R*)-2-(2-Hydroxy-7-methoxy-1-naphthalenecarbonyloxy)-1-cyclopentanol (5). To a solution of **19b** (49.7 mg, 0.228 mmol) and (*R*,*R*)-1,2-cyclopentanediol[9] (123 mg, 1.20 mmol) in CH_2Cl_2-THF (1 : 1, 2.4 mL) was added EDC (48.1 mg, 0.251 mmol) at 0 °C under argon. After stirring for 3 h at 0 °C, AcOEt (5 mL) and 1 M HCl (5 mL) were added. The mixture was extracted with AcOEt (5 x 5 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. Flash column chromatography of the residue (hexane/AcOEt = 5/2) gave 57.1 mg (83%) of **5**: colorless needles; mp 81-82 °C (hexane-AcOEt); $[\alpha]^{23}_D$ -36.0° (c 0.820, $CHCl_3$); IR (KBr) ν 3498, 2960, 1651, 1624, 1518, 1456, 1429, 1386, 1222, 1207, 1135, 1031, 851, 835 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.71-2.44 (6H, m), 3.92 (3H, s), 4.34-4.43 (1H, m), 5.20-5.30 (1H, m), 7.02 (1H, d, J = 8.8 Hz), 7.03 (1H, dd, J = 8.8, 2.4 Hz), 7.66 (1H, d, J = 8.8 Hz), 7.84 (1H, d, J = 8.8 Hz), 8.19 (1H, bd, J = 2.4 Hz), 12.33 (1H, s). HRMS (EI, 70 eV) *m/z* calcd. for $C_{17}H_{18}O_5$: 302.1155; found 302.1158.

(1*R*,2*R*)-2-(2-Hydroxy-5-methyl-1-naphthalenecarbonyloxy)-1-cyclopentanol (6): ^1H NMR (200 MHz, $CDCl_3$) δ 1.66-2.45 (6H, m), 2.68 (3H, s), 4.34-4.45 (1H, m), 5.22-5.32 (1H, m), 7.21 (1H, d, J = 9.3 Hz), 7.22 (1H, bd, J = 6.7 Hz), 7.43 (1H, dd, J = 8.8, 6.7 Hz), 8.16 (1H, bd, J = 9.3 Hz), 8.57 (1H, bd, J = 8.8 Hz), 12.05 (1H, s).

(1*R*,2*R*)-2-(2-Hydroxy-1-naphthalenecarbonyloxy)-1-cyclopentanol (7): colorless prisms; mp 65-66 °C (hexane-$CHCl_3$); $[\alpha]^{21}_D$ -23.8° (c 0.726, $CHCl_3$); IR (KBr) ν 3492, 2950, 1647, 1466, 1379, 1338, 1209, 830, 748 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.65-2.45 (6H, m), 2.70 (1H, bs), 4.35-4.46 (1H, m), 5.21-5.31 (1H, m), 7.17 (1H, d, J = 9.0 Hz), 7.37 (1H, ddd, J = 8.0, 6.9, 1.2 Hz), 7.55 (1H, ddd, J = 8.7, 6.9, 1.6 Hz), 7.76 (1H, dd, J = 8.0, 1.6 Hz), 7.91 (1H, bd, J = 9.0 Hz), 8.66-8.74 (1H, m), 12.23 (1H, s). LRMS (EI, 70 eV) *m/z*(%) 272(21), 188(30), 171(42), 170(100), 138(13), 115(22), 114(14).

(1*S*,2*S*)-2-(2-Hydroxy-7-methoxy-5-methyl-1-naphthalenecarbonyloxy)-1-cyclopentanol (8). To a solution of **19a** (74.8 mg, 0.322 mmol) and (*S*,*S*)-1,2-cyclopentanediol[9] (101 mg, 0.992 mmol) in CH_2Cl_2-THF (1 : 1, 3.2 mL) was added EDC (68.1 mg, 0.355 mmol) at 0 °C under argon. After stirring for 3 h at 0 °C, EDC (12.2 mg, 0.0636 mmol) was added, and the reaction was continued for 1.5 h at the same temperature. The mixture was diluted with CH_2Cl_2 (10 mL) and 1 M HCl (10 mL), and extracted with CH_2Cl_2 (5 x 10 mL). The combined organic extracts were dried over $MgSO_4$, concentrated under reduced pressure, and purified on flash column chromatography (hexane/AcOEt = 2/1) to give 75.2 mg (74%) of **8**: colorless prisms; mp 97-99 °C (hexane-CH_2Cl_2); $[\alpha]^{22}_D$ +39.7° (c 0.710, $CHCl_3$); IR (KBr) ν 3438, 2960, 1647, 1618, 1458, 1412, 1379, 1319, 1267, 1249, 1209, 1152, 1033, 940, 847, 816 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 1.76 (1H, ddt, J = 15.0, 7.8, 5.5 Hz), 1.87 (1H, dq, J = 13.5, 7.5 Hz), 1.93 (1H, dtt, J = 13.5, 8.0, 5.5 Hz), 2.02 (1H, ddt, J = 13.5, 7.0, 5.0 Hz), 2.15 (1H, dq, J = 14.0, 7.0 Hz), 2.36 (1H, dq, J = 14.0, 7.0 Hz), 2.63 (3H, s), 3.90 (3H, s), 4.37 (1H, bddd, J = 7.0, 5.0, 3.2 Hz), 5.24 (1H, ddd, J = 7.3, 4.0, 3.2 Hz), 6.89 (1H, d, J = 1.5 Hz), 7.04 (1H, d, J = 9.0 Hz), 8.04 (1H, d, J = 9.0 Hz), 8.05 (1H, d, J = 1.5 Hz), 11.73 (1H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 20.70 (CH_3), 22.64 (CH_2), 31.19 (CH_2), 33.55 (CH_2), 55.65 (CH_3), 79.00 (CH), 86.30 (CH), 104.30 (CH), 105.11 (C), 116.81 (CH), 117.22 (CH), 123.77 (C), 133.48 (CH), 134.86 (C), 137.73 (C), 160.13 (C), 165.51 (C), 173.97 (C). HRMS (EI, 70 eV) *m/z* calcd. for $C_{18}H_{20}O_5$: 316.1311; found 316.1313.

Ethyl 2,6-dideoxy-4,5-*O*-isopropylidene-2-methylamino-1-thio-β-*D*-galactopyranoside (21):[11] colorless needles; mp 91-92 °C (hexane); $[\alpha]^{27}_D$ -51.1° (c 0.520, $CHCl_3$); IR (KBr) ν 3420, 3336, 2988, 2938, 2860, 1483, 1450, 1379, 1267, 1247, 1220, 1176, 1141, 1079, 1054, 1033, 996, 982, 872, 793, 758 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.29 (3H, t, J = 7.4 Hz), 1.34 (3H, s), 1.39 (3H, d, J = 6.6 Hz), 1.51 (3H, s), 2.54 (3H, s), 2.58 (1H, dd, J = 10.0, 6.7 Hz), 2.71 (2H, dq, J = 7.4, 2.2 Hz), 3.80 (1H, dq, J = 6.6, 2.0 Hz), 3.98 (1H, dd, J = 5.2, 2.0 Hz), 4.04 (1H, dd, J = 6.7, 5.2 Hz), 4.26 (1H, d, J = 10.0 Hz). HRMS (EI, 70 eV) *m/z* calcd. for $C_{12}H_{23}O_3NS$: 261.1399; found 261.1398.

(1*R*,2*R*)-2-(2,6-Dideoxy-2-methylamino-β-*D*-galactopyranosyloxy)-1-cyclopentyl 2-hydroxy-7-methoxy-5-methyl-1-naphthalenecarboxylate (9). To a mixture of molecular sieves 4Å (MS4Å, 30.0 mg) and *N*-iodosuccinimide (NIS, 66.3 mg, 0.295 mmol) in CH_2Cl_2 (0.5 mL) was added trifluoromethanesufonic acid (TfOH, 20.0 μL, 0.226 mmol) at 0 °C under argon, and the resulted mixture was stirred for 2 h at the same temperature. After cooled to -70 °C, a solution of ethyl 2,6-dideoxy-4,5-*O*-isopropyliden-2-methylamino-1-thio-β-*D*-galactopyranoside **21** (34.3 mg, 0.131 mmol) in CH_2Cl_2 (0.6 mL) was added, and stirring was continued for 30 min. To this mixture a solution of **3** (50.9 mg, 0.161 mmol) in CH_2Cl_2 (0.6 mL) was added. After stirring for 1 h at -70 °C, a 1:1 mixture of 1 M aq. $Na_2S_2O_3$ and satd. aq. $NaHCO_3$ (10 mL) was added at the temperature, and the mixture was extracted with CH_2Cl_2 (2 x 10 mL). The combined organic layers were washed with satd. aq. NaCl and dried over $MgSO_4$. Concentration and flash column chromatography (AcOEt/MeOH = 20/1) gave 30.8 mg (46%) of the acetonide of **9**.

To a solution of the acetonide (62.2 mg, 0.121 mmol) in THF-H_2O (4 : 1, 2.0 mL) was added trifluoroacetic acid (TFA, 1.6 mL) at 0 °C, and the mixture was stirred for 2 h at the same temperature. The mixture was allowed to warm to room temperature and stirred for 5 h. The volatiles were evaporated under reduced pressure, and the residue was purified by a HPLC system using a YMC D-ODS-5 column (20.0 mm x 25 cm). The system consists of two JASCO 880-PU pumps, a JASCO 875-UV detector, and ERMA ERC-3311 degasser. The solvent A was MeOH : H_2O : HCO_2H = 20 : 80 : 5 and the solvent B was MeOH : H_2O : HCO_2H = 95 : 5 : 5. The gradient programmer was set as follows: a linear gradient of 0-75% B in 15 min, followed by 75-95% B in 40 min, and finally by 95-100% B in 5 min. The flow rate was 4.0 mL/min, and the eluate was monitored by ultraviolet absorption at 254 nm. The HPLC purification afforded 30.7 mg (49%) of **9**: colorless prisms; $[\alpha]^{27}_D$ -67.7° (c 0.598, MeOH); IR (KBr) ν 3424, 1650, 1620, 1468, 1412, 1379, 1249, 1205, 1094 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ 1.19 (3H, d, J = 6.5 Hz), 1.83-1.94 (4H, m), 2.16-2.24 (1H, m), 2.29-2.36 (1H, m), 2.61 (3H, s), 2.74 (3H, s), 2.94 (1H, bdd, J = 11.0, 8.2 Hz), 3.58 (1H, bd, J = 2.3 Hz), 3.62 (1H, bq, J = 6.5 Hz), 3.65 (1H, bdd, J = 10.8, 3.0 Hz), 3.88 (3H, s), 4.51-4.54 (1H, m), 4.67 (1H, d, J = 8.2 Hz), 5.70-5.73 (1H, m), 6.90 (1H, bs), 6.99 (1H, d, J = 9.0 Hz), 7.82 (1H, bd, J = 1.4 Hz), 8.06 (1H, d, J = 9.0 Hz), 8.52 (1H, bs). HRMS (FAB, matrix: *m*-nitrobenzylalcohol) calcd. for $C_{25}H_{34}O_8N$ (M + H): 476.2284; found 476.2283.

(1*S*,2*S*)-2-(2,6-Dideoxy-2-methylamino-β-*D*-galactopyranosyloxy)-1-cyclopentyl 2-hydroxy-7-methoxy-5-methyl-1-naphthalenecarboxylate (10). To a mixture of MS4Å (61.4 mg) and NIS (104 mg, 0.460 mmol) in CH_2Cl_2 (1.5 mL) was added TfOH (41.0 μL, 0.463 mmol) at 0 °C under argon, and the resulted mixture was stirred for 30 min at the same temperature. Then, to this mixture a solution of ethyl 2,6-dideoxy-4,5-*O*-isopropyliden-2-methylamino-1-thio-β-*D*-galactopyranoside **21** (60.2 mg, 0.230 mmol) and **8** (89.8 mg, 0.284 mmol) in CH_2Cl_2 (0.8 mL) was added at -65 °C. After stirring for 10 min at the same temperature, 1 M aq. $Na_2S_2O_3$ (5 mL) and satd. aq. $NaHCO_3$ (5 mL) were added, and extracted with CH_2Cl_2 (2 x 10 mL). The combined organic layers were washed with satd. aq. NaCl, dried over $MgSO_4$, and concentrated under reduced pressure. Purification of the residue by TLC (AcOEt/MeOH = 10/1) afforded 79.3 mg (67%) of the acetonide, which was deprotected as above to yield **10** (73%): colorless prisms; $[\alpha]^{28}_D$ +50.5° (c 0.592, MeOH); IR (KBr) ν 3344, 2940, 1715, 1644, 1618, 1458, 1412, 1381, 1323, 1267, 1247,

1209, 1178, 1152, 1069, 1038 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ 1.26 (3H, d, J = 6.3 Hz), 1.86-1.94 (3H, m), 1.94-2.00 (1H, m), 2.13-2.18 (1H, m), 2.29-2.36 (1H, m), 2.61 (3H, s), 2.82 (3H, s), 3.09 (1H, dd, J = 10.8, 8.3 Hz), 3.64 (1H, bd, J = 3.1 Hz), 3.66 (1H, bq, J = 6.3 Hz), 3.78 (1H, bdd, J = 10.8, 3.1 Hz), 3.87 (3H, s), 4.50-4.56 (1H, m), 4.91 (1H, d, J = 8.4 Hz), 5.54 (bdt, J = 7.0, 3.5 Hz), 6.90-6.91 (1H, m), 7.01 (1H, d, J = 9.0 Hz), 7.64 (1H, bd, J = 2.0 Hz), 8.04 (1H, d, J = 9.0 Hz), 8.44 (1H, bs). HRMS (FAB, matrix: *m*-nitrobenzylalcohol) calcd. for $C_{25}H_{34}O_8N$ (M + H): 476.2284; found 476.2277.

Purification of apoprotein

NCS was kindly provided by Dr. Hideo Komatsu of POLA Pharmaceutical R & D Laboratory. It was suspended in 0.1 M acetic acid-methanol, and the suspension was stirred for 2.5 h at 0 °C in the dark. After centrifugation of 9,000 rpm for 20 min, the precipitate was collected and resuspended in 0.1 M acetic acid-methanol. After stirring for 2.5 h at 0 °C in the dark, the protein fraction was precipitated by centrifugation and dissolved in water. The protein solution was dialyzed against 0.02 M Tris-HCl buffer (pH 7.2) at 10 °C and purified by a HPLC system using a Pharmacia Hi Load Q Sepharose column. The system consists of two LC-7A pumps, a gradient programmer SCL-6B, a UV/VIS detector SPD-7AV, and a recorder C-R6A (Shimadzu). The solvent A (start buffer) was 0.02 M Tris-HCl buffer (pH 7.2) and the solvent B (limit buffer) was a start buffer containing 0.5 M NaCl. The gradient programmer was set as follows: a linear gradient of 0-20% B in 10 min, followed by 20-60% B in 40 min, and finally by 60-100% B in 20 min. The flow rate was 4.0 mL/min, and the eluate was monitored by ultraviolet absorption at 280 nm. The apoprotein fraction was dialyzed against water, followed by lyophilization to give pure apoprotein.

Purification of NCS

NCS was further purified to homogeneity by HPLC using a Pharmacia Mono Q HR 5/5 column in the dark according to Denklau *et al.*[19] with minor modifications. The HPLC system was identical with that used for the purification of apoprotein. The solvent A (start buffer) was 0.02 M ammonium acetate (pH 5.0) and the solvent B (limit buffer) was a start buffer containing 1.0 M NaCl. The gradient programmer was set as follows: a linear gradient of 0-3% B in 3 min, followed by 3-25% in 44 min, and finally by 25-100% in 3 min. The flow rate was 1.8 mL/min, and the eluate was monitored by ultraviolet absorption at 280 nm. The fraction containing NCS complex was dialyzed against 0.1 M ammonium acetate (pH 4.0) in the dark. The NCS concentration was determined by measuring the ultraviolet absorption at 280 nm, with an absorption coefficient $E^{1\%}_{1cm}$ of 9500.[20]

Fluorescence quenching titration

All measurements were performed on a Hitachi 850 spectrofluorometer. All samples contained 5.0 x 10^{-6} M of a model compound and 0-1.5 x 10^{-4} M of apoprotein in 5% methanol-0.1 M ammonium acetate buffer (pH 4.0). Fluorescence was measured immediately after sample preparation. Measurements were performed 3 or 4 times for each model compound, which allowed us to estimate the mean *Ka*. Excitation was carried out at 330 nm except for **4** at 333 nm.

Competitive binding assay

To a solution of NCS complex in 5% methanol-0.1 M ammonium acetate buffer (pH 4.0) was added a excess **9** or **10** in the same buffer, and the mixture was slowly stirred at 37 °C in the dark. The final concentration of NCS complex and model compounds were 1.0 x 10^{-4} M and 1.0 x 10^{-3} M, respectively. Amounts of apoprotein freed from NCS complex were measured by HPLC in the dark. The HPLC procedure was identical with that used for the purification of NCS except for the gradient pattern: a linear gradient of 0-3% B in 3 min, followed by 3-25% B in 11 min, and finally by 25-100% B in 3 min.

ACKNOWLEDGMENTS

We thank Dr. Hideo Komatsu, POLA Pharmaceutical R & D Laboratory, for the generous supply of NCS. NMR (400 MHz and 600 MHz), MS, and elemental analysis were performed through the auspices of the Instrumental Analysis Center for Chemistry, Faculty of Science, Tohoku University. This work was supported by the Ministry of Education, Science, and Culture, Japan, and by the Mitsubishi, TERUMO, Takeda, Fujisawa Science Foundations. A fellowship of the Japan Society for the Promotion of Science for Japanese Junior Scientists to K. T. is gratefully acknowledged.

REFERENCES AND NOTES

1. a) Edo, K.; Mizugaki, M.; Koide, Y.; Seto, H.; Furihata, K.; Otake, N.; Ishida, N. *Tetrahedron Lett.* **1985**, *26*, 331-334. b) Myers, A. G.; Proteau, P. J.; Handel, T. M. *J. Am. Chem. Soc.* **1988**, *110*, 7212-7214.
2. Ishida, N.; Miyazaki, K.; Kumagai, K.; Rikimaru, M. *J. Antibiot.* **1965**, *18*, 68-76.
3. a) Povirk, L. F.; Goldberg, I. H. *Biochemistry* **1980**, *19*, 4773-4780. b) Edo, K.; Saito, K.; Akiyama-Murai, Y.; Mizugaki, M.; Koide, Y.; Ishida, N. *J. Antibiot.* **1988**, *41*, 554-562.
4. a) Kappen, L. S.; Napier, M. A.; Goldberg, I. H.; Samy, T. S. A. *Biochemistry* **1980**, *19*, 4780-4785. b) Kappen, L. S.; Goldberg, I. H. *Biochemistry* **1980**, *19*, 4786-4790.
5. Gomibuchi, T.; Fujiwara, K.; Nehira, T.; Hirama, M. *Tetrahedron Lett.* **1993**, *34*, 5753-5756.
6. a) Ishiguro, M.; Imajo, S.; Hirama, M. *J. Med. Chem.* **1991**, *34*, 2366-2373. b) Tanaka, T.; Hirama, M.; Ueno, M.; Imajo, S.; Ishiguro, M.; Mizugaki, M.; Edo, K.; Komatsu, H. *Tetrahedron Lett.* **1991**, *32*, 3175-3178.
7. Tanaka, T.; Hirama, M.; Fujita, K.; Imajo, S.; Ishiguro, M. *J. Chem. Soc., Chem. Commun.* **1993**, 1205-1207.
8. Takahashi, K.; Suzuki, T.; Hirama, M. *Tetrahedron Lett.* **1992**, *33*, 4603-4604.
9. Xie, Z.-F.; Suemune, H.; Nakamura, I.; Sakai, K. *Chem. Pharm. Bull.* **1987**, *35*, 4454-4459.
10. McOmie, J. F. W.; Watts, M. L.; West, D. E. *Tetrahedron* **1968**, *24*, 2289-2292.
11. Prepared from *D*-galactosamine: the details will be reported separately.
12. a) Konradsson, P.; Udodong, U. E.; Fraser-Reid, B. *Tetrahedron Lett.* **1990**, *31*, 4313-4316. b) Veeneman, G. H.; van Leeuwen, S. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331-1334.
13. Without methanol present, $Kd \leq 1.0 \times 10^{-10}$ M in 20 mM Tris buffer (pH 8.0).[3a]
14. Methanol was necessary to dissolve model compounds.
15. Concentrations of fluorophores were kept constant.
16. Kondo, H.; Nakatani, H.; Hiromi, K. *J. Biochem.* **1976**, *79*, 393-405.
17. Nishio, M.; Hirota, M. *Tetrahedron* **1989**, *45*, 7201-7245.
18. Srikrishna, A.; Krishnan, K. *Tetrahedron Lett.* **1988**, *29*, 4995-4996.
19. Denklau, D.; Köhnlein, W.; Lüders, G.; Stellmach, J. *Z. Naturforsch.* **1983**, *38c*, 939-942.
20. Povirk, L. F.; Dattagupta, N.; Warf, B. C.; Goldberg, I. H. *Biochemistry* **1981**, *20*, 4007-4014.

(*Received* 2 *September* 1993; *accepted* 24 *November* 1993)