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# Synthetic Studies on Quinocarcin and Its Related Compounds. 5.[1, 2] Synthesis and Antitumor Activity of Various Structural Types of Quinocarcin Congeners

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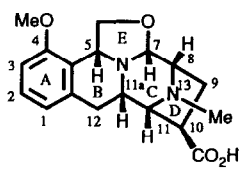
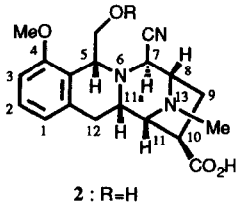
*Key words: quinocarcin, quinocarcin congeners, cytotoxicity, antitumor activity, structure-activity relationships*

***Abstract:*** *The antitumor activity of various structural types of quinocarcin congeners which had been previously prepared in the course of our synthetic studies or were originally synthesized by employing novel synthetic schemes, was primarily evaluated by in vitro cytotoxicity assay against P388 murine leukemia cells. Several compounds exhibiting prominent cytotoxicity were selected, and further subjected to in vitro cytotoxicity assay against HeLa S3 cells and to in vivo antitumor activity assay against P388 murine leukemia. These studies obviously disclosed novel aspects of the structure-activity relationships of quinocarcin congeners.*

(–)-Quinocarcin (**1**), isolated from the culture broth of *Streptomyces melanovinaceus* exhibits notable antitumor activity against several strains of solid mammalian carcinomas. DX-52-1 (**2**), the more stable semisynthetic 7-cyano congener of **1**, still retains significant antitumor activity.[2a]



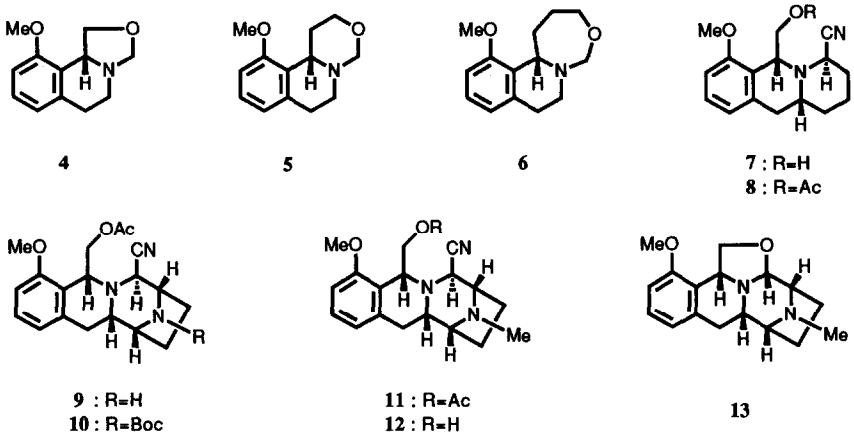

**1**




**2** : R=H
**3** : R=Ac

As reported in the preceding papers,[2a-d] we have succeeded in the total synthesis of enantiomeric pairs of **1** as well as various structural types of quinocarcin congeners such as the ABE ring systems (**4**, *ent*-**4**, **5**, *ent*-**5**, **6**, and *ent*-**6**), the ABC ring systems (**7**, *ent*-**7**, **8**, and *ent*-**8**), the ABCD ring systems (**9**, *ent*-**9**, **10**, *ent*-**10**, **11**, *ent*-**11**, **12**, and *ent*-**12**), and the ABCDE ring system (**13** and *ent*-**13**) (**Figure 1**). The explored synthetic scheme features the diastereoselective reduction of 3,4-dihydroisoquinoline or isoquinoline derivatives, wherein each enantiomer of 4-*O*-benzyl-2,3-*O*-isopropylidenethreose is employed as a common chiral auxiliary.[2a,b,d]

**Figure 1**




With completion of these total syntheses, we next investigated the structure-activity relationships of quinocarcin congeners.[7] Antitumor activity of various structural types of quinocarcin congeners which had been previously synthesized in the course of our studies on the total synthesis of **1**, or were originally synthesized by employing novel synthetic schemes starting with naturally occurring **1**, were primarily evaluated by *in vitro* cytotoxicity assay against P388 murine leukemia cells. Subsequently, several compounds exhibiting prominent cytotoxicity against P388 murine leukemia cells were selected and further subjected to both *in vitro* cytotoxicity assay against HeLa $S_3$ cells and *in vivo* antitumor activity assay against P388 murine leukemia. These studies obviously revealed various aspects of the structure-activity relationships of quinocarcin congeners, holding promise for both investigating the mode of action of **1** and designing novel quinocarcin congeners exhibiting characteristic profiles. In the fifth part of this series of papers, we wish to report full details of these studies on the structure-activity relationships.[1a-e]

## Results and Discussions

### 1. *In Vitro* Cytotoxicity Assay of Enantiomeric Pairs of Various Structural Types of Quinocarcin Congeners Against P388 Murine Leukemia Cells

Enantiomeric pairs of various structural types of quinocarcin congeners such as the ABE ring systems (**4**, *ent*-**4**, **5**, *ent*-**5**, **6**, and *ent*-**6**), the ABC ring systems (**7**, *ent*-**7**, **8**, and *ent*-**8**), the ABCD ring systems (**9**, *ent*-**9**, **10**, *ent*-**10**, **11**, *ent*-**11**, **12**, and *ent*-**12**), and the ABCDE ring system (10-decarboxyquinocarcin) (**13** and *ent*-**13**) pictured in **Figure 1** were first subjected to *in vitro* cytotoxicity assay against P388 murine leukemia cells along with **1**, **2**, **3** and their antipodes (*ent*-**1**, *ent*-**2**, and *ent*-**3**). As described in the preceding papers, enantiomeric pairs of these compounds had been prepared in the course of our project directed at the total synthesis of **1**. $IC_{50}$ values collected are shown in **Table 1**. These results clearly disclosed that 10-decarboxyquinocarcin (**13**) and its 7-cyano congeners (**11** and **12**) were $10^{1\text{-}3}$ times more cytotoxic than the corresponding 10-carboxy compounds (**1**, **3**, and **2**). It is also noteworthy that **1**, **2**,

**Table 1.** *In Vitro* Cytotoxicity of Various Structural Types of Quinocarcin Congeners Against P388 Murine Leukemia Cells

| Compound | $IC_{50}$ (μg/ml)[a] | Compound | $IC_{50}$ (μg/ml)[a] |
|---|---|---|---|
| **1** | $3.3 \times 10^{-2}$ | *ent*-**1** | 3.2 |
| **2** | $3.6 \times 10^{-2}$ | *ent*-**2** | 5.1 |
| **3** | $1.0 \times 10^{-1}$ | *ent*-**3** | >100 |
| **4** | 4.5 | *ent*-**4** | 4.5 |
| **5** | $6.6 \times 10^{-1}$ | *ent*-**5** | $6.6 \times 10^{-1}$ |
| **6** | $6.8 \times 10^{-1}$ | *ent*-**6** | $6.8 \times 10^{-1}$ |
| **7** | 13 | *ent*-**7** | 8.4 |
| **8** | 1.1 | *ent*-**8** | 6.6 |
| **9** | $3.0 \times 10^{-1}$ | *ent*-**9** | >4.4 |
| **10** | 1.5 | *ent*-**10** | 3.4 |
| **11** | $2.0 \times 10^{-4}$ | *ent*-**11** | >3.6 |
| **12** | $8.2 \times 10^{-4}$ | *ent*-**12** | >3.1 |
| **13** | $3.9 \times 10^{-3}$ | *ent*-**13** | 34 |

a) Concentration required for 50% inhibition of the cell growth after incubation for 96 h at 37°C (initial cell density : $1 \times 10^4$ cells/ml).

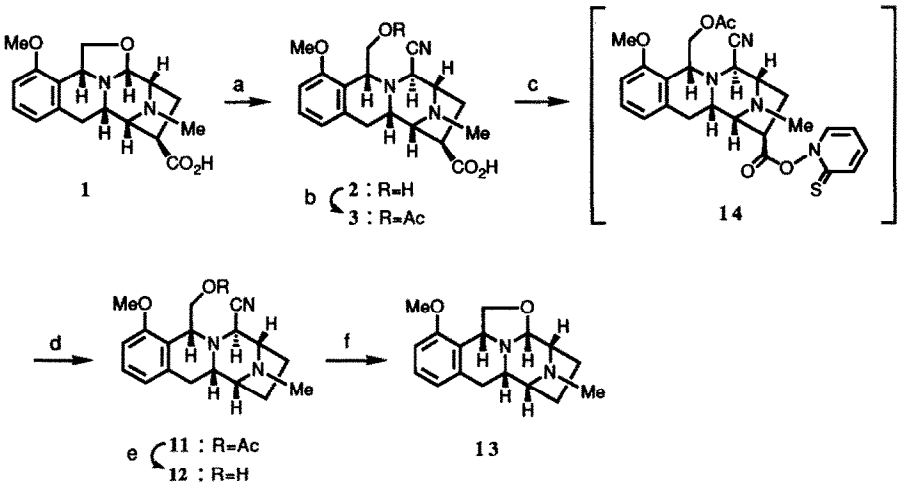
**3**, **11**, **12**, and **13** bearing natural absolute configurations were found to be $10^{2-4}$ times more cytotoxic than the corresponding enantiomers (*ent*-**1**, *ent*-**2**, *ent*-**3**, *ent*-**11**, *ent*-**12**, and *ent*-**13**) possessing unnatural absolute configurations. The $N_{13}$-H derivative **9** and the $N_{13}$-Boc derivative **10** were considerably inferior to the corresponding $N_{13}$-Me derivative **11**. The compounds (**4**, **7**, and **8**) consisting of the partial structures showed no potent cytotoxicity. The ABE ring systems (**5** and **6**) in which the E ring of **4** was replaced with six- and seven-membered rings, respectively, turned out to be one order of magnitude more cytotoxic than five-membered **4**.

These primary results obviously indicate that all the carbon framework (the ABCDE ring system or the ABCD ring system bearing the 7-cyano group) with natural absolute configuration is indispensable for significant cytotoxicity. Removal of the $N_{13}$-methyl group caused almost complete loss of inhibitory activity regardless of the presence of all the carbon framework with natural absolute configuration, suggesting that the $N_{13}$-Me group plays an important role to exhibit pronounced cytotoxicity. Furthermore, it appears that the $C_{10}$-carboxyl group is not responsible for the potent cytotoxicity of quinocarcin congeners. Among the compounds tested, **11**, **12**, and **13** were found to be most promising. Consequently, further evaluation of antitumor activity was performed using these compounds (*vide infra*).

## 2. Synthesis and *In Vitro* Cytotoxicity of Various 10-Substituted Quinocarcin Congeners

First, in order to evaluate characteristics of *in vivo* antitumor activity of 10-decarboxyquinocarcin (**13**) and its 7-cyano congeners (**11** and **12**), a novel preparation method was sought which could afford these compounds more expeditiously than the total synthesis achieved by us.[2d] We have found that **11**, **12**, and

**Scheme 1**

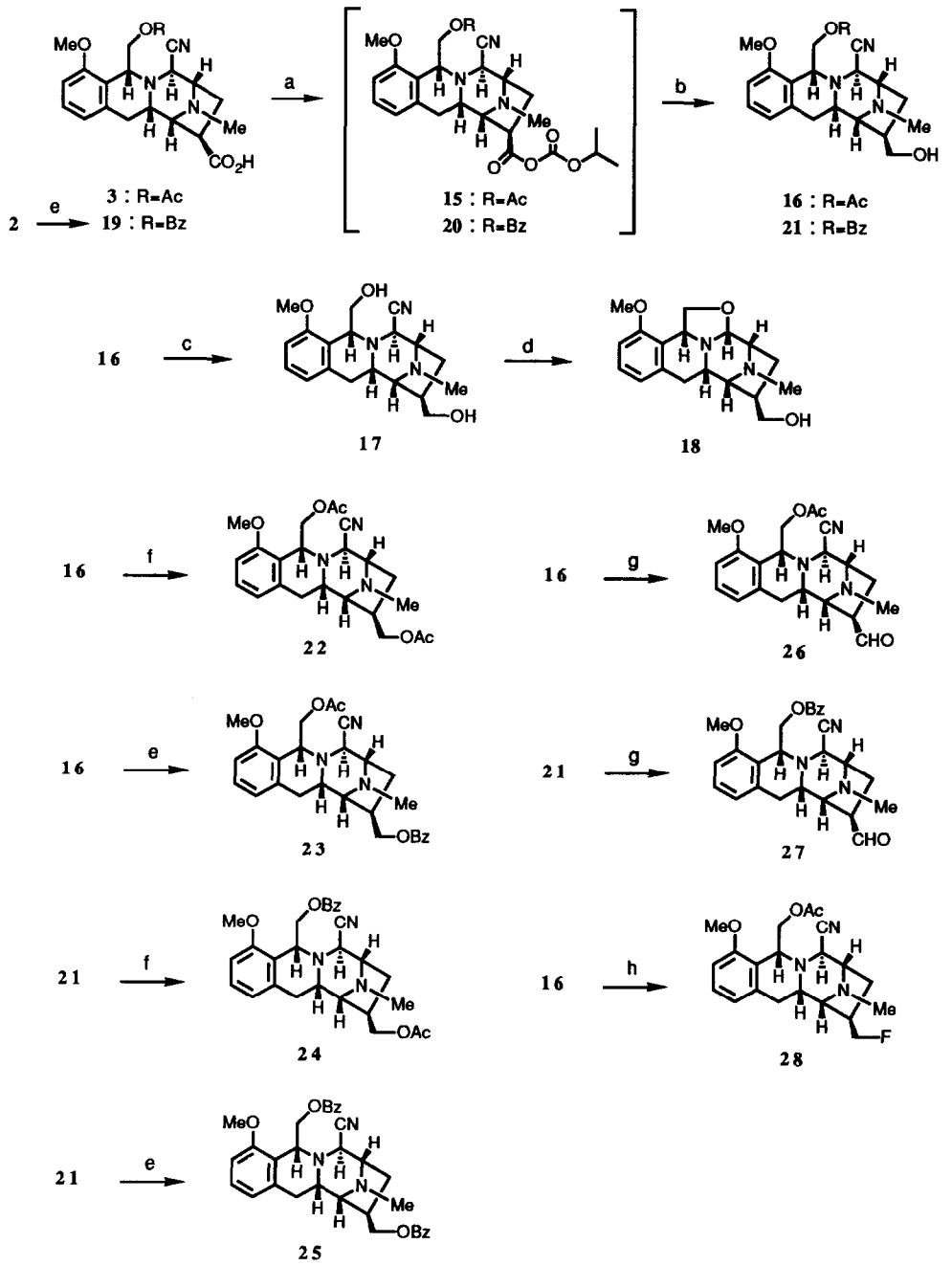



***reagents and conditions:*** a) NaCN, $NaHCO_3$, $H_2O$, rt, 95% b) $Ac_2O$, DMAP, Py, rt, 64% c) 2-mercaptopyridine-*N*-oxide, DCC, DMAP, benzene, reflux d) $^nBu_3SnH$, AIBN, benzene, reflux, 65% (2 steps) e) 1M NaOH, MeOH, rt, 98% f) $AgNO_3$, MeOH, rt, 81%

**13** can be synthesized in a straightforward manner starting with naturally occurring **1** by employing the Barton radical decarboxylation[8] as a key step. A large quantity of **1** is readily available from the culture broth of *Streptomyces melanovinaceus*.[9] As shown in **Scheme 1**, treatment of **1** with sodium cyanide according to the reported method,[7a] provided the amino nitrile, DX-52-1 (**2**), which was further acetylated to furnish acetate **3** in 61 % overall yield from **1**. Crucial decarboxylation of **3** turned out to be effected by employing the protocol of Barton.[8] Thus, **3** was initially esterified with 2-mercaptopyridine-*N*-oxide in the presence of 1,3-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) in refluxing benzene to afford the corresponding 2-thiopyridon-1-yl ester **14**. Without isolation, **14** was immediately subject to radical decarboxylation using α,α'-azobisisobutyronitrile (AIBN) and tributyltin hydride, giving rise to the 10-decarboxy derivative **11** in 65% overall yield from **3**. Saponification of **11** followed by treatment of the resulting alcohol **12** with silver nitrate in methanol according to the reported method,[7a] gave **13** in 79% yield from **11**. The compounds (**11**, **12**, and **13**) showed identical spectral properties (IR, $^1$H-NMR, MS) with those of authentic samples prepared in the preceding paper.[2d] With large quantities of **11**, **12**, and **13** in hand, investigations aiming at characterizing *in vivo* antitumor activity of these compounds were undertaken (*vide infra*).

In light of the results collected by *in vitro* cytotoxicity assay against P388 murine leukemia described above, it was of interest to examine antitumor activity of quinocarcin congeners (**16**, **17**, **18**, **21**, **22**, **23**, **24**, **25**, **26**, **27**, and **28**) bearing various functionalities at their C$_{10}$-positions. Therefore, we next addressed on the synthesis of these compounds as shown in **Scheme 2**. Thus, the mixed acid anhydride **15** derived by treatment of **3** with isopropyl chloroformate in the presence of triethylamine was allowed to react with sodium borohydride, providing alcohol **16** in 81% yield from **3**. After saponification of **16**,

**Scheme 2**




***reagents and conditions:*** a) $ClCO_2{}^iPr$, $Et_3N$, THF, 0°C b) $NaBH_4$, THF-$H_2O$, rt, 81% (2 steps) for **16**, 90% (2 steps) for **21** c) 1M NaOH, MeOH, rt, 99% d) $AgNO_3$, MeOH, rt, 71% e) BzCl, Py, 0°C, 33% for **19**, 99% for **23**, 84% for **25** f) $Ac_2O$, Py, DMAP, rt, 98% for **22**, 68% for **24** g) $(COCl)_2$, DMSO, $CH_2Cl_2$, -78°C; $Et_3N$, 88% for **26**, 85% for **27** h) DAST, $CH_2Cl_2$, rt, 48%

**Table 2.** *In Vitro* Cytotoxicity of Various 10-Substituted Quinocarcin Congeners Against P388 Murine Leukemia Cells

| Compound | $IC_{50}$ (μg/ml)[a] | Compound | $IC_{50}$ (μg/ml)[a] |
|---|---|---|---|
| **1** | $3.3 \times 10^{-2}$ | **23** | $5.6 \times 10^{-3}$ |
| **2** | $3.6 \times 10^{-2}$ | **24** | $3.1 \times 10^{-2}$ |
| **16** | $3.4 \times 10^{-3}$ | **25** | $3.1 \times 10^{-2}$ |
| **17** | $3.2 \times 10^{-3}$ | **26** | $3.2 \times 10^{-2}$ |
| **18** | $7.2 \times 10^{-3}$ | **27** | $3.0 \times 10^{-3}$ |
| **21** | $1.0 \times 10^{-5}$ | **28** | $1.6 \times 10^{-2}$ |
| **22** | $1.3 \times 10^{-3}$ | | |

a) See the footnote a) in **Table 1**.

further treating of the resulting diol **17** with silver nitrate gave the desired 10-hydroxymethyl derivative **18**. By employing the reaction sequence similar to that described for the preparation of acetate **16**, the benzoate **21** was prepared starting with **2** *via* carboxylic acid **19** and mixed anhydride **20**. The 10-acetoxymethyl derivatives **22** and **24** were prepared by acetylation of **16** and **21**, respectively. Benzoylation of **16** and **21** cleanly provided the corresponding 10-benzoyloxymethyl derivatives **23** and **25**. The 10-formyl derivatives **26** and **27** were synthesized by Swern oxidation of **16** and **21**, respectively. Furthermore, treatment of **16** with diethylaminosulfur trifluoride (DAST) provided the 10-fluoromethyl derivative **28**.

With various 10-substituted quinocarcin congeners (**16**, **17**, **18**, **21**, **22**, **23**, **24**, **25**, **26**, **27**, and **28**) in hand, *in vitro* cytotoxicity of these compounds against P388 murine leukemia was investigated in a similar manner to that described above. $IC_{50}$ values collected are shown in **Table 2**. From these results, it was revealed that almost all of these congeners exhibit superior cytotoxicity to **1**. It is noteworthy that the cytotoxicity of **21** is $10^3$ times more potent than that of **1**. Taking into account both the potent cytotoxicity and chemical stability, **18**, **21**, and **24** were further subjected to *in vivo* antitumor activity assay (*vide infra*).

### 3. Antitumor Activity of Various Highly Cytotoxic Quinocarcin Congeners

The antitumor activity of highly cytotoxic quinocarcin congeners (**11**, **12**, **13**, **18**, **21**, and **28**) primarily screened with *in vitro* cytotoxicity assay against P388 murine leukemia cells, was further evaluated by both growth inhibition against HeLa $S_3$ cells (*in vitro*) and increase of life span (ILS) by single and five daily administrations for mice implanted with P388 murine leukemia cells (*in vivo*). Results shown in **Table 3** disclosed that **11**, **12**, **13**, **18**, and **21** exhibit the activity superior to that of **1**. The cytotoxicity of **28** was approximately 4 times less than that of **1**. As shown in **Table 4**, *in vivo* experiments revealed that all of the tested compounds except for **28** show appreciable antitumor activity in single administration or in five daily administrations, while they are a little less effective than **1**. Only marginal antitumor activity was observed for **28**. These results obviously suggest that the $C_{10}$-carboxy group is not always indispensable

**Table 3.** *In Vitro* Cytotoxicity of Quinocarcin Congeners Against HeLa S3 Cells

| Compound | $IC_{50}$ (μg/ml)[a] | Compound | $IC_{50}$ (μg/ml)[a] |
|---|---|---|---|
| 1 | $1.0 \times 10^{-1}$ | 18 | $4.1 \times 10^{-2}$ |
| 11 | $6.4 \times 10^{-2}$ | 21 | $3.6 \times 10^{-2}$ |
| 12 | $7.5 \times 10^{-2}$ | 28 | $5.0 \times 10^{-1}$ |
| 13 | $5.4 \times 10^{-2}$ | | |

a) Concentration required for 50% inhibition of the cell growth after incubation for 72 h at 37°C (initial cell density : $8 \times 10^3$ cells/ml).

**Table 4.** *In Vivo* Antitumor Activity of Quinocarcin Congeners Against P388 Murine Leukemia Cells

| Compound | P388 ip-ip[a] | | | |
|---|---|---|---|---|
| | optimal dose (mg/kg) x 1 | ILS[b] (%) | optimal dose (mg/kg) x 5 | ILS[b] (%) |
| 1 | 25.0 | 33-39 | 6.25 | 67-85 |
| 11 | 12.5 | 6 | 12.5 | 33 |
| 12 | 12.5 | 8 | 12.5 | 29 |
| 13 | 6.25 | 20 | 3.13 | 37 |
| 18 | 3.13 | 17 | 6.25 | 61 |
| 21 | 25.0 | 27 | 3.13 | 21 |
| 28 | 25.0 | 12 | 12.5 | 13 |

a) CD2F1 mice (5 mice/group) were implanted intraperitoneally (i.p.) with $1 \times 10^6$ cells, and a sample was dosed i.p. on day 1 and days 1-5.
b) Percent increase of life span calculated (T/C-1) x 100, where T and C are mean survival days of treated and control mice, respectively.

for potent antitumor activity of quinocarcin congeners.

## Conclusion

Summarizing the results of *in vitro* cytotoxicity and *in vivo* antitumor activity assay for various structural types of quinocarcin congeners described above, it appears evident that (i) all the carbon framework (the ABCDE ring system or the ABCD ring system bearing the 7-cyano group) with natural absolute configurations is indispensable for significant cytotoxicity, wherein the absolute configuration inherent in **1** might provide a key structural feature for molecular recognition by DNA , (ii) the *N*13-Me group plays an important role to exhibit prominent inhibitory activity, and (iii) the C10-carboxyl group is not always necessary for potent antitumor activity. These studies on the structure-activity relationships should hold promise for both investigating the mode of action of **1** and designing novel quinocarcin congeners which can exhibit characteristic profiles.

## Experimental

*General.* All melting points were determined with a Yamato MP-21 micro melting point apparatus and are uncorrected. Measurements of optical rotations were performed with a Horiba SEPA-200 automatic digital polarimeter. $^1$H-NMR spectra were measured with a Hitachi R-90H (90MHz) and a Brucker AM-400 (400MHz) spectrometer.The chemical shifts were expressed in ppm using tetramethylsilane (δ=0) and/or residual solvents such as chloroform (δ=7.25) and benzene (δ=7.20) as internal standards. The following abbreviations are used: singlet (s), doublet (d), triplet (t), quartet (q), quintet (quint), sixtet (sxt), multiplet (m), and broad (br). Infrared (IR) spectral measurements were carried out with a JASCO A-202 and a JASCO FT/IR-5300 spectrometer. Low resolution mass (MS) spectra were taken with a Hitachi RMU-6MG spectrometer, and high resolution mass (HRMS) spectra were obtained on a Hitachi M-80A spectrometer. Routine monitoring of reactions was carried out using Merck 60 F254 silica gel, glass-supported TLC plates. Flash column chromatography was performed with indicated solvents on Wakogel C-300. Solvents and commercial reagents were dried and purified before use. Ether and tetrahydrofuran were distilled from sodium benzophenone ketyl and dichloromethane was distilled from calcium hydride under argon.

**(5*R*,7*R*,8*S*,10*R*,11*R*,11a*S*)-5-Acetoxymethyl-7-cyano-4-methoxy-13-methyl-5,7,8,9,10,11,11a,12-octahydro-8,11-iminoazepino[1,2-*b*]isoquinoline-10-carboxylic acid (3)**

Acetic anhydride (3.94 ml, 42 mmol) was added dropwise to a stirred solution of **2**[7a] (3.73 g, 10 mmol) in pyridine (50 ml) containing a catalytic amount of 4-dimethylaminopyridine (50.0 mg, 0.41 mmol) at room temperature under argon. After 12 h, the mixture was concentrated *in vacuo* to give a residue, which was purified by column chromatography (hexane-ethyl acetate, 1:2) to give **3** (2.67 g, 64%) as a colorless caramel. [α]D$^{20}$ +17.3° (c 0.37, CHCl3) [lit.,[1d,2d] [α]D$^{20}$ +18.4° (c 0.38, CHCl3)]. The IR, $^1$H-NMR, and mass specra of this sample were identical with those reported in the preceding paper.[2d]

**(5*R*,7*R*,8*S*,11*R*,11a*S*)-5-Acetoxymethyl-7-cyano-4-methoxy-13-methyl-5,7,8,9,10,11,11a,12-octahydro-8,11-iminoazepino[1,2-*b*]isoquinoline (11)**

2-Mercaptopyridine *N*-oxide (153 mg, 1.2 mmol), 1,3-dicyclohexylcarbodiimide (DCC) (310 mg, 1.5 mmol) and 4-dimethylaminopyridine (183 mg, 1.5 mmol) were added successively to a stirred solution of **3** (398 mg, 1.0 mmol) in benzene (20 ml) at room temperature, and the mixture was heated at reflux for 2 h under argon. A solution of α,α'-azobisisobutyronitrile (AIBN) (10 mg, 61 μmol) and tributyltin hydride (0.81 ml, 3.0 mmol) in benzene (10 ml) was added dropwise over 10 min, and the resulting mixture was further heated at reflux for 2 h under argon. After cooling, the mixture was diluted with ethyl acetate (150 ml), and the organic layer was washed with water and brine, then dried over Na2SO4. Concentration of the solvent *in vacuo* gave a residue, which was purified by column chromatography (hexane-ethyl acetate, 2:1) to give **11** (230 mg, 65%) as a white solid. Recrystallization from hexane-ethyl acetate gave an analytical sample of **11** as colorless prisms, mp 62-63 °C [lit.,[2d] mp 61-62.5 °C] and [α]D$^{20}$ +16.7° (c 0.89, CHCl3) [lit.,[1d,2d] [α]D$^{20}$ +16.4° (c 0.23, CHCl3)]. The IR, $^1$H-NMR, and mass specra of this sample were identical with those reported in the preceding paper.[2d]

**(5*R*,7*R*,8*S*,11*R*,11a*S*)-7-Cyano-5-hydroxymethyl-4-methoxy-13-methyl-5,7,8,9,10,11,11a,12-octahydro-8,11-iminoazepino[1,2-*b*]isoquinoline (10-decarboxy-DX-52-1) (12)**

1M Sodium hydroxide (4.61 ml, 4.6 mmol) was added dropwise to a stirred solution of **11** (545 mg, 1.5 mmol) in methanol (13 ml) at room temperature. After 2 h, the mixture was diluted with ethyl acetate (150 ml), and the organic layer was washed with brine and dried over Na2SO4. Concentration of the solvent *in vacuo* gave a residue, which was purified by column chromatography (hexane-ethyl acetate, 1:1) to give **11** (471 mg, 98%) as a colorless amorphous powder. [α]D$^{20}$ +25.9° (c 1.03, CHCl3) [lit.,[1d,2d] [α]D$^{20}$ +27.3° (c 0.13, CHCl3)]. The IR, $^1$H-NMR, and mass specra of this sample were identical with those reported in the preceding paper.[2d]

**(5*R*,7*R*,8*S*,11*R*,11a*S*)-4-Methoxy-13-methyl-5,6,7,8,9,10,11,11a,12-nonahydro-8,11-iminoazepino[1,2-*b*]isoquinolino[1,2-*c*]oxazole (10-Decarboxyquinocarcin) (13)**

Silver nitrate (91.4 mg, 0.53 mmol) was added to a stirred solution of **12** (153 mg, 0.49 mmol) in methanol (18 ml) at room temperature under argon. After 1 h, the mixture was concentrated *in vacuo* to give a residue, which was purified by column chromatography (ethyl acetate-methanol, 3:1) to give **13** (113 mg, 81%) as a colorless amorphous powder. [α]D$^{20}$ -13.2° (c 1.03, MeOH) [lit.,[1d,2d] [α]D$^{20}$ -13.0° (c 0.23, MeOH)]. The IR, $^1$H-NMR, and mass specra of this sample were identical with those reported in the preceding paper.[2d]

**(5*R*,7*R*,8*S*,10*R*,11*R*,11a*S*)-5-Acetoxymethyl-7-cyano-10-hydroxymethyl-4-methoxy-13-methyl-5,7,8,9,10,11,11a,12-octahydro-8,11-iminoazepino[1,2-*b*]isoquinoline (16)**

Isopropyl chloroformate (0.499 ml, 3.6 mmol) was added dropwise to a stirred solution of **3** (652 mg, 1.6 mmol) in dry tetrahydrofuran (28 ml) containing triethylamine (0.501 ml, 3.6 mmol) at 0°C under argon. After 2 h, a solution of sodium borohydride (309 mg, 8.2 mmol) in water (4 ml) was added dropwise, and the resulting mixture was further stirred for 1 h at room temperature. The mixture was diluted with ethyl acetate (180 ml), and the organic layer was washed with water and brine, then

dried over Na2SO4. Concentration of the solvent *in vacuo* gave a residue, which was purified by column chromatography (hexane-ethyl acetate, 1:2) to give **16** (510 mg, 81%) as a colorless amorphous powder. $[\alpha]D^{20}$ +29.2° (c 1.28, CHCl3) [lit.,[1d,2d] $[\alpha]D^{20}$ +28.8° (c 0.63, CHCl3]. The IR, $^1$H-NMR, and mass specra of this sample were identical with those reported in the preceding paper.[2d]

**(5*R*,7*R*,8*S*,10*R*,11*R*,11a*S*)-7-Cyano-5,10-di(hydroxymethyl)-4-methoxy-13-methyl-5,7,8,9,10,11,11a,12-octahydro-8,11-iminoazepino[1,2-*b*]isoquinoline (17)**

Treatments of **16** (125 mg, 0.32 mmol) in a similar manner to that described for the preparation of **12** from **11** gave **17** (110mg, 99%) as a colorless amorphous powder after purification by column chromatography (hexane-ethyl acetate: 1:3). $[\alpha]D^{20}$ +101° (c 0.23, CHCl3). IR (KBr): 3490, 2930, 2900, 2830, 2350, 1710, 1660, 1580, 1460, 1260, 1070 cm$^{-1}$. $^1$H-NMR (400 MHz, CD3OD) δ: 1.84 (1H, ddd, J=13.0, 6.5, 6.2 Hz, C9-H), 2.10 (1H, dd, J=13.0, 9.1 Hz, C9-H), 2.49 (1H, dd, J=13.9, 2.4 Hz, C12-H), 2.51 (3H, s, NMe), 2.63 (1H, dddd, J=9.1, 8.8, 7.6, 6.2 Hz, C10-H), 2.66 (1H, dd, J=13.9, 11.1 Hz, C12-H), 2.91 (1H, dd, J=11.1, 2.4 Hz, C11a-H), 2.99 (1H, s, C11-H), 3.42 (1H, dd, J=10.6, 6.9 Hz, C5-C*H*2OH), 3.43 (1H, dd, J=6.5, 2.7 Hz, C8-H), 3.56 (1H, dd, J=10.6, 8.8 Hz, C10-C*H*2OH), 3.62 (1H, dd, J=10.6, 7.6 Hz, C10-C*H*2OH), 3.76 (1H, dd, J=10.6, 2.6 Hz, C5-C*H*2OH), 3.83 (3H, s, ArO*Me*), 4.20 (1H, dd, J=6.9, 2.6 Hz, C5-H), 4.30 (1H, d, J=2.7 Hz, C7-H), 6.72 (1H, d, J=8.0 Hz, C3-H), 6.82 (1H, d, J=8.0 Hz, C1-H), 7.14 (1H, t, J=8.0 Hz, C2-H). EIMS m/z: 343 ($M^+$), 312 [(M-CH2OAc)$^+$]. HRMS calcd for C19H25N3O3 ($M^+$): 343.1894. Found: 343.1919.

**(5*R*,7*R*,8*S*,10*R*,11*R*,11a*S*)-10-Hydroxymethyl-4-methoxy-13-methyl-5,6,7,8,9,10,11,11a,12-nonahydro-8,11-iminoazepino[1,2-*b*]isoquinolino[1,2-*c*]oxazole (18)**

Treatments of **17** (380 mg, 0.11 mmol) in a similar manner to that described for the preparation of **13** from **12** gave **18** (249mg, 71%) as a colorless amorphous powder after purification by column chromatography (chloroform: methanol=3:1). $[\alpha]D^{20}$ -14.3° (c 0.41, MeOH). IR (KBr): 3480, 2930, 1470, 1380, 1260, 1090, 1055 cm$^{-1}$. $^1$H-NMR (400 MHz, CD3OD) δ: 2.04 (1H, ddd, J=13.9, 6.9, 6.4 Hz, C9-H), 2.44 (1H, dd, J=13.9, 9.9 Hz, C9-H), 2.56 (1H, dd, J=14.6, 2.7 Hz, C12-H), 2.74 (1H, dd, J=14.6, 12.0 Hz, C12-H), 2.95 (3H, s, NMe), 3.00-3.05 (1H, m, C10-H), 3.39 (1H, dd, J=10.8, 7.2 Hz, C*H*2OH), 3.49 (1H, dd, J=10.8, 2.0 Hz, C*H*2OH), 3.61-3.75 (3H, m, C*H*2O and C8-H), 3.73-3.78 (1H, m, C11-H), 3.83 (3H, s, ArO*Me*), 4.08-4.13 (1H, m, C11a-H), 4.60 4.60 (1H, dd, J=7.1, 2.8 Hz, C5-H), 4.63 (1H, d, J=3.0 Hz, C7-H), 6.76 (1H, d, J=7.6 Hz, C3-H), 6.85 (1H, d, J=7.6 Hz, C1-H), 7.18 (1H, t, J=7.6 Hz, C2-H). EIMS m/z: 287 ($M^+$-CHO).

**(5*R*,7*R*,8*S*,10*R*,11*R*,11a*S*)-5-Benzoyloxymethyl-7-cyano-4-methoxy-13-methyl-5,7,8,9,10,11,11a,12-octahydro-8,11-iminoazepino[1,2-*b*]isoquinoline-10-carboxylic acid (19)**

Benzoyl chloride (0.54 ml, 4.6 mmol) was added dropwise to a stirred solution of **2**[7a] (410 mg, 1.1 mmol) in pyridine (15 ml) at 0°C under argon. After 2 h, water (30 ml) was added. The mixture was diluted with ethyl acetate (150 ml) and the organic layer was dried over Na2SO4. Concentration of the solvent *in vacuo* gave a residue, which was purified by column chromatography (hexane-ethyl acetate, 1:2) to give **19** (175 mg, 33%) as a colorless amorphous powder. $[\alpha]D^{20}$ -5.7° (c 0.33, CHCl3). IR (CHCl3): 3440, 2900, 2830, 1710, 1590, 1470, 1380, 1340, 1260, 1230, 1130, 1080, 1060, 1040 cm$^{-1}$. $^1$H-NMR (400 MHz, C6D6) δ: 1.81 (1H, dd, J=13.3, 9.6 Hz, C9-H), 2.07 (3H, s, NMe), 2.17 (1H, dd, J=14.6, 1.8 Hz, C12-H), 2.29 (1H, dd, J=14.6, 11.5 Hz, C12-H), 2.44 (1H, dd, J=13.3, 11.8 Hz, C9-H), 2.68-2.73 (1H, m, C8-H), 2.77-2.83 (1H, m, C10-H), 3.02 (1H, dt, J=11.5, 1.8 Hz, C11a-H), 3.11 (3H, s, ArO*Me*), 3.14 (1H, br s, C11-H), 3.52 (1H, d, J=2.7 Hz, C7-H), 4.38 (1H, dd, J=10.9, 4.0 Hz, C*H*2OBz), 4.73 (1H, dd, J=4.0, 3.3 Hz, C5-H), 4.78 (1H, dd, J=10.9, 3.3 Hz, C*H*2OBz), 6.34 (1H, d, J=8.3 Hz, C3-H), 6.57 (1H, d, J=8.3 Hz, C1-H), 7.00 (1H, t, J=8.3 Hz, C2-H), 6.95-7.05 (3H, m, aromatic protons), 8.0 (2H, dd, J=8.3 Hz, aromatic protons). EIMS m/z: 461 ($M^+$), 435 [(M-CN)$^+$], 326 [(M-CH2OBz)$^+$]. HRMS calcd for C26H27N3O5 ($M^+$): 461.1984. Found: 461.1947.

**(5*R*,7*R*,8*S*,10*R*,11*R*,11a*S*)-5-Benzoyloxymethyl-7-cyano-10-hydroxymethyl-4-methoxy-13-methyl-5,7,8,9,10,11,11a,12-octahydro-8,11-iminoazepino[1,2-*b*]isoquinoline (21)**

Treatments of **19** (162 mg, 0.35 mmol) in a similar manner to that described for the preparation of **16** from **3** gave **21** (141 mg, 90%) as a colorless amorphous powder after purification by column chromatography (hexane-ethyl acetate, 1:2). $[\alpha]D^{20}$ +1.0° (c 0.75, CHCl3). IR (neat): 3500, 2930, 2830, 2270, 1710, 1590, 1470, 1450, 1260, 1090, 1060 cm$^{-1}$. $^1$H-NMR (400 MHz, C6D6) δ: 1.10 (1H, br s, OH), 1.34 (1H, ddd, J=12.7, 6.3, 6.0 Hz, C9-H), 1.70 (1H, dd, J=12.7, 9.0 Hz, C9-H), 2.02 (1H, dddd, J=9.0, 7.7, 6.4, 6.0 Hz, C10-H), 2.08 (1H, dd, J=14.9, 2.3 Hz, C12-H), 2.23 (3H, s, NMe), 2.45 (1H, dd, J=14.9, 12.0 Hz, C12-H), 2.53 (1H, s, C11-H), 2.80 (1H, dd, J=6.3, 2.4 Hz, C8-H), 3.08 (1H, dd, J=12.0, 2.3 Hz, C11a-H), 3.12 (1H, dd, J=10.0, 7.7 Hz, C*H*2OH), 3.13 (3H, s, ArO*Me*), 3.17 (1H, dd, J=10.0, 6.4 Hz, C*H*2OH), 3.56 (1H, d, J=2.4 Hz, C7-H), 4.42 (1H, dd, J=10.9, 4.0 Hz, C*H*2OBz), 4.77 (1H, dd, J=4.0, 3.2 Hz, C5-H), 4.84 (1H, dd, J=10.9, 3.0 Hz, C*H*2OBz), 6.37 (1H, d, J=8.0 Hz, C3-H), 6.63 (1H, d, J=8.0 Hz, C1-H), 6.94-7.08 (3H, m, aromatic protons), 7.04 (1H, t, J=8.0 Hz, C2-H), 7.98-8.04 (2H, m, aromatic protons). EIMS m/z: 447 ($M^+$), 312 [(M-CH2OBz)$^+$]. HRMS calcd for C26H29N3O4 ($M^+$): 447.2156. Found: 447.2156.

**(5*R*,7*R*,8*S*,10*R*,11*R*,11a*S*)-5,10-Di(acetoxymethyl)-7-cyano-10-hydroxymethyl-4-methoxy-13-methyl-5,7,8,9,10,11,11a,12-octahydro-8,11-iminoazepino[1,2-*b*]isoquinoline (22)**

Treatments of **16** (52.2 mg, 0.14 mmol) in a similar manner to that described for the preparation of **3** from **1**[7a] gave **22** (57.0 mg, 98%) as a white amorphous powder after purification by column chromatography (hexane-ethyl acetate, 2:3). $[\alpha]D^{20}$ +16.2° (c 1.18, CHCl3). IR (neat): 2930, 2830, 2250, 1735, 1590, 1470, 1380, 1260, 1230, 1040 $cm^{-1}$. $^1$H-NMR (400 MHz, C6D6) δ: 1.34 (1H, ddd, J=12.9, 6.4, 6.1 Hz, C9-H), 1.65 (3H, s, Ac), 1.68 (1H, dd, J=12.9, 9.1 Hz, C9-H), 1.69 (3H, s, Ac), 2.08 (1H, dd, J=14.7, 2.4 Hz, C12-H), 2.20 (3H, s, NMe), 2.44 (1H, dd, J=14.7, 10.6 Hz, C12-H), 2.47 (1H, dddd, J=9.1, 8.5, 8.0, 6.1 Hz, C10-H), 2.48 (1H, s, C11-H), 2.81 (1H, dd, J=6.4, 2.6 Hz, C8-H), 3.07 (1H, dd, J=10.6, 2.4 Hz, C11a-H), 3.13 (3H, s, ArO*Me*), 3.44 (1H, d, J=2.6 Hz, C7-H), 3.86 (1H, dd, J=10.7, 8.5 Hz, C10-C*H*2OAc), 3.94 (1H, dd, J=10.7, 8.0 Hz, C10-C*H*2OAc), 4.08 (1H, dd, J=11.5, 4.8 Hz, C5-C*H*2OAc), 4.59 (1H, dd, J=11.5, 3.0 Hz, C5-C*H*2OAc), 4.60 (1H, dd, J=4.8, 3.0 Hz, C5-H), 6.33 (1H, d, J=8.0 Hz, C3-H), 6.60 (1H, d, J=8.0 Hz, C1-H), 7.01 (1H, t, J=8.0 Hz, C2-H). EIMS m/z: 427 ($M^+$), 401 [(M-CN)$^+$], 354 [(M-CH2OAc)$^+$]. HRMS calcd for C23H29N3O5 ($M^+$): 427.2104. Found: 427.2104.

**(5*R*,7*R*,8*S*,10*R*,11*R*,11a*S*)-5-Acetoxymethyl-10-benzoyloxymethyl-7-cyano-4-methoxy-13-methyl-5,7,8,9,10,11,11a,12-octahydro-8,11-iminoazepino[1,2-*b*]isoquinoline (23)**

Treatments of **16** (60.0 mg, 0.16 mmol) in a similar manner to that described for the preparation of **19** from **2** gave **23** (75.2 mg, 99%) as a white amorphous powder after purification by column chromatography (hexane-ethyl acetate, 1:1). $[\alpha]D^{20}$ +14.8° (c 1.23, CHCl3). IR (neat): 2940, 2830, 2220, 1730, 1715, 1590, 1470, 1450, 1380, 1260, 1230, 1100, 1040 $cm^{-1}$. $^1$H-NMR (400 MHz, C6D6) δ: 1.38 (1H, ddd, J=12.9, 6.3, 6.1 Hz, C9-H), 1.65 (3H, s, Ac), 1.73 (1H, dd, J=12.9, 8.9 Hz, C9-H), 2.08 (1H, dd, J=14.5, 2.3 Hz, C12-H), 2.23 (3H, s, NMe), 2.50 (1H, dd, J=14.5, 12.0 Hz, C12-H), 2.58 (1H, s, C11-H), 2.61 (1H, dddd, J=9.7, 8.9, 7.8, 6.1 Hz, C10-H), 2.83 (1H, dd, J=6.3, 2.6 Hz, C8-H), 3.07 (1H, dd, J=12.0, 2.3 Hz, C11a-H), 3.12 (3H, s, ArO*Me*), 3.48 (1H, d, J=2.6 Hz, C7-H), 4.09 (1H, dd, J=11.5, 4.7 Hz, C*H*2OAc), 4.10 (1H, dd, J=10.6, 9.7 Hz, C*H*2OBz), 4.18 (1H, dd, J=10.6, 7.8 Hz, C*H*2OBz), 4.59 (1H, dd, J=11.5, 3.0 Hz, C*H*2OAc), 4.60 (1H, dd, J=4.7, 3.0 Hz, C5-H), 6.32 (1H, d, J=8.0 Hz, C3-H), 6.52 (1H, d, J=8.0 Hz, C1-H), 6.98 (1H, t, J=8.0 Hz, C2-H), 7.06-7.13 (3H, m, aromatic protons), 8.14-8.20 (2H, m, aromatic protons). EIMS m/z: 489 ($M^+$), 463 [(M-CN)$^+$], 416 [(M-CH2OAc)$^+$]. HRMS calcd for C28H31N3O5 ($M^+$): 489.2261. Found: 489.2250.

**(5*R*,7*R*,8*S*,10*R*,11*R*,11a*S*)-10-Acetoxymethyl-5-benzoyloxymethyl-7-cyano-4-methoxy-13-methyl-5,7,8,9,10,11,11a,12-octahydro-8,11-iminoazepino[1,2-*b*]isoquinoline (24)**

Treatments of **21** (55.0 mg, 0.12 mmol) in a similar manner to that described for the preparation of **3** from **1**[7a] gave **24** (40.9 mg, 68%) as a white amorphous powder after purification by column chromatography (hexane-ethyl acetate, 1:1). $[\alpha]D^{20}$ -7.7° (c 0.33, CHCl3). IR (neat): 2940, 2900, 2830, 2220, 1740, 1720, 1590, 1470, 1450, 1380, 1260, 1110, 1100 $cm^{-1}$. $^1$H-NMR (400 MHz, C6D6) δ: 1.29 (1H, ddd, J=13.0, 6.3, 5.8 Hz, C9-H), 1.62 (3H, s, Ac), 1.73 (1H, dd, J=13.0, 9.0 Hz, C9-H), 2.04 (1H, dd, J=14.8, 2.3 Hz, C12-H), 2.15 (3H, s, NMe), 2.24 (1H, dddd, J=9.0, 8.2, 7.9, 5.8 Hz, C10-H), 2.40 (1H, s, C11-H), 2.44 (1H, dd, J=14.8, 11.9 Hz, C12-H), 2.76 (1H, dd, J=6.3, 2.6 Hz, C8-H), 3.06 (1H, dd, J=11.9, 2.3 Hz, C11a-H), 3.13 (3H, s, ArO*Me*), 3.52 (1H, d, J=2.6 Hz, C7-H), 3.76 (1H, dd, J=10.7, 8.2 Hz, C*H*2OAc), 3.79 (1H, dd, J=10.7, 7.9 Hz, C*H*2OAc), 4.43 (1H, dd, J=11.0, 3.6 Hz, C*H*2OAc), 4.73 (1H, dd, J=3.6, 3.1 Hz, C5-H), 4.82 (1H, dd, J=11.0, 3.1 Hz, C*H*2OBz), 6.61 (1H, d, J=8.0 Hz, C3-H), 6.61 (1H, d, J=8.0 Hz, C1-H), 6.93-7.07 (4H, m, C2-H and aromatic protons), 7.97-8.03 (2H, m, aromatic protons). EIMS m/z: 489 ($M^+$), 463 [(M-CN)$^+$], 354 [(M-CH2OBz)$^+$]. HRMS calcd for C28H31N3O5 ($M^+$): 489.2262. Found: 489.2270.

**(5*R*,7*R*,8*S*,10*R*,11*R*,11a*S*)-5,10-Di(benzoyloxymethyl)-7-cyano-10-hydroxymethyl-4-methoxy-13-methyl-5,7,8,9,10,11,11a,12-octahydro-8,11-iminoazepino[1,2-*b*]isoquinoline (25)**

Treatments of **21** (61.0 mg, 0.14 mmol) in a similar manner to that described for the preparation of **19** from **2** gave **25** (62.7 mg, 84%) as a white amorphous powder after purification by column chromatography (hexane-ethyl acetate, 1:1). $[\alpha]D^{20}$ -5.8° (c 0.24, CHCl3). IR (neat): 2930, 2830, 2220, 1710, 1590, 1470, 1450, 1310, 1270, 1110, 1070 $cm^{-1}$. $^1$H-NMR (400 MHz, C6D6) δ: 1.34 (1H, ddd, J=13.0, 6.3, 5.9 Hz, C9-H), 1.79 (1H, dd, J=13.0, 9.0 Hz, C9-H), 2.03 (1H, dd, J=14.8, 2.4 Hz, C12-H), 2.17 (3H, s, NMe), 2.37 (1H, dddd, J=9.0, 8.8, 7.8, 5.9 Hz, C10-H), 2.47 (1H, s, C11-H), 2.48 (1H, dd, J=14.8, 11.9 Hz, C12-H), 2.78 (1H, dd, J=6.3, 2.5 Hz, C8-H), 3.06 (1H, dd, J=11.9, 2.4 Hz, C11a-H), 3.13 (3H, s, ArO*Me*), 3.54 (1H, d, J=2.5 Hz, C7-H), 3.96 (1H, dd, J=10.6, 7.8 Hz, C10-C*H*2OBz), 4.01 (1H, dd, J=10.6, 8.8 Hz, C10-C*H*2OBz), 4.42 (1H, dd, J=11.0, 3.6 Hz, C5-C*H*2OBz), 4.73 (1H, dd, J=3.6, 3.1 Hz, C5-H), 4.87 (1H, dd, J=11.0, 3.1 Hz, C5-C*H*2OBz), 6.37 (1H, d, J=8.0 Hz, C3-H), 6.57 (1H, d, J=8.0 Hz, C1-H), 7.06 (1H, t, J=8.0 Hz, C2-H), 6.90-7.20 (6H, m, aromatic protons), 7.98-8.04 (2H, m, aromatic protons), 8.06-8.13 (2H, m, aromatic protons). EIMS m/z: 551 ($M^+$), 525 [(M-CN)$^+$], 416 [(M-CH2OBz)$^+$]. HRMS calcd for C33H33N3O5 ($M^+$): 551.2417. Found: 551.2413.

**(5*R*,7*R*,8*S*,10*R*,11*R*,11a*S*)-5-Acetoxymethyl-7-cyano-10-formyl-4-methoxy-13-methyl-5,7,8,9,10,11,11a,12-octahydro-8,11-iminoazepino[1,2-*b*]isoquinoline (26)**

Dimethyl sulfoxide (54.1 μl, 0.76 mmol) in dry dichloromethane (1 ml) was added dropwise to a stirred solution of oxalyl chloride (49.4 μl, 0.57 mmol) in dry dichloromethane (3 ml) at -78°C under argon. After 10 min, a solution of **16** (109 mg, 0.28 mmol) in dry dichloromethane (4 ml) was added slowly, and stirring was continued for 15 min at -78°C. After addition of triethylamine (0.118 ml, 0.85 mmol), the mixture was gradually warmed up to -25°C and further stirred for 30 min. The resulting mixture was diluted with water (2 ml) and extracted with ethyl acetate (60 ml). The extract was washed with brine and dried over Na2SO4. Concentration of the solvent *in vacuo* gave a residue, which was purified by column chromatography (hexane-ethyl acetate, 5:1→ 1:1) to give **26** (95.4 mg, 88%) as a colorless amorphous powder. $[\alpha]D^{20}$ +46.4° (c 0.54, CHCl3). IR (neat): 2930, 2830, 2710, 2220, 1730, 1720, 1590, 1470, 1450, 1440, 1380, 1290, 1260, 1230, 1180, 1140, 1090, 1070, 1040 $cm^{-1}$. $^1$H-NMR (400 MHz, C6D6) δ: 1.51 (1H, dd, J=13.0, 9.6 Hz, C9-H), 1.53 (3H, s, Ac), 1.79 (3H, s, NMe), 1.84-1.96 (1H, m, C9-H), 2.05 (1H, dd, J=14.5, 2.1 Hz, C12-H), 2.22 (1H, dd, J=14.5, 12.0 Hz, C12-H), 2.53 (1H, dd, J=9.8, 5.5 Hz, C10-H), 2.70 (1H, dt, J=8.6, 2.9 Hz, C8-H), 2.93 (1H, s, C11-H), 3.00 (1H, dt, J=11.6, 2.0 Hz, C11a-H), 3.13 (3H, s, ArO*Me*), 3.48 (1H, d, J=2.9 Hz, C7-H), 4.05 (1H, dd, J=10.8, 5.4 Hz, C*H*2OAc), 4.42 (1H, dd, J=10.8, 3.0 Hz, C*H*2OAc), 4.59 (1H, dd, J=5.4, 3.0 Hz, C5-H), 6.32 (1H, d, J=8.2 Hz, C3-H), 6.56 (1H, d, J=8.2 Hz, C1-H), 7.00 (1H, t, J=8.2 Hz, C2-H), 9.33 (1H, s, CHO). EIMS m/z: 383 ($M^+$), 357 [(M-CN)$^+$], 310 [(M-CH2OAc)$^+$]. HRMS calcd for C21H25N3O4 ($M^+$): 383.1844. Found: 383.1856.

**(5*R*,7*R*,8*S*,10*R*,11*R*,11a*S*)-5-Benzoyloxymethyl-7-cyano-10-formyl-4-methoxy-13-methyl-5,7,8,9,10,11,11a,12-octahydro-8,11-iminoazepino[1,2-*b*]isoquinoline (27)**

Treatments of **21** (43.8 mg, 98 μmol) in a similar manner to that described for the preparation of **26** from **16** gave **27** (37 mg, 85%) as a colorless amorphous powder after purification by column chromatography (hexane-ethyl acetate, 5:1→ 1:1). $[\alpha]D^{20}$ +2.1° (c 0.83, CHCl3). IR (neat): 3010, 2940, 2900, 2830, 2700, 2220, 1710, 1590, 1470, 1460, 1270, 1110, 1100, 1070 $cm^{-1}$. $^1$H-NMR (400 MHz, C6D6) δ: 1.63 (1H, dd, J=13.2, 9.8 Hz, C9-H), 1.73 (3H, s, NMe), 1.82 (1H, ddd, J=13.2, 6.3, 5.6 Hz, C9-H), 2.04 (1H, dd, J=14.8, 2.4 Hz, C12-H), 2.26 (1H, dd, J=14.8, 11.9 Hz, C12-H), 2.40 (1H, dd, J=9.8, 5.6 Hz, C10-H), 2.62 (1H, dd, J=6.3, 2.8 Hz, C8-H), 2.86 (1H, s, C11-H), 3.00 (1H, dd, J=11.9, 2.4 Hz, C11a-H), 3.12 (3H, s, ArO*Me*), 3.54 (1H, d, J=2.8 Hz, C7-H), 4.36 (1H, dd, J=10.9, 4.2 Hz, C*H*2OBz), 4.72 (1H, dd, J=4.2, 3.1 Hz, C5-H), 4.78 (1H, dd, J=10.9, 3.1 Hz, C*H*2OBz), 6.36 (1H, d, J=8.0 Hz, C3-H), 6.59 (1H, d, J=8.0 Hz, C1-H), 6.93-7.05 (3H, m, aromatic protons), 7.03 (1H, t, J=8.0 Hz, C2-H), 7.94-7.99 (2H, m, aromatic protons), 9.16 (1H, s, CHO). EIMS m/z: 445 ($M^+$), 419 [(M-CN)$^+$], 310 [(M-CH2OBz)$^+$]. HRMS calcd for C26H27N3O4 ($M^+$): 445.1997. Found: 445.1999.

**(5*R*,7*R*,8*S*,10*R*,11*R*,11a*S*)-5-Acetoxymethyl-7-cyano-10-fluoromethyl-4-methoxy-13-methyl-5,7,8,9,10,11,11a,12-octahydro-8,11-iminoazepino[1,2-*b*]isoquinoline (28)**

Diethylaminosulfur trifluoride (DAST) (79.0 μl, 0.60 mmol) was added to a stirred solution of **16** (76.1 mg, 0.20 mmol) in dry dichloromethane (5 ml) at room temperature under argon. After 1 h, the mixture was diluted with dichloromethane (40 ml), the organic layer was washed with water and brine, then dried over Na2SO4. Concentration of the solvent *in vacuo* gave a residue, which was purified by column chromatography (hexane-ethyl acetate, 2:1) to give **28** (36.7 mg, 48%) as a pale yellow amorphous powder. $[\alpha]D^{20}$ +12.7° (c 0.74, CHCl3). IR (neat): 2930, 2830, 2710, 2220, 1730, 1580, 1470, 1450, 1430, 1370, 1290, 1260, 1240, 1180, 1140, 1090, 1040 $cm^{-1}$. $^1$H-NMR (400 MHz, C6D6) δ: 1.35-1.52 (2H, m, C9-H2), 1.59 (3H, s, Ac), 1.88 (1H, dd, J=14.7, 2.2 Hz, C12-H), 2.12 (3H, s, NMe), 2.25-2.30 (2H, m, C11-H and C12-H), 2.53-2.57 (1H, m, C8-H), 2.98-3.02 (1H, m, C11a-H), 3.13 (3H, s, ArO*Me*), 3.12-3.17 (1H, m, C10-H), 3.53 (1H, d, J=2.2 Hz, C7-H), 4.10 (1H, dd, J=11.0, 6.7 Hz, C*H*2OAc), 4.25 (1H, dd, J=11.0, 3.2 Hz, C*H*2OAc), 4.59 (1H, dd, J=6.7, 3.2 Hz, C5-H), 5.38-5.61 (1H, m, CH2F), 6.33-6.60 (1H, m, CH2F), 6.32 (1H, d, J=8.2 Hz, C3-H), 6.55 (1H, d, J=8.2 Hz, C1-H), 7.01 (1H, t, J=8.2 Hz, C2-H). EIMS m/z: 387 ($M^+$), 361 [(M-CN)$^+$], 314 [(M-CH2OAc)$^+$]. HRMS calcd for C21H26FN3O3 ($M^+$): 387.1957. Found: 387.1963.

**Evaluation of *in vitro* cytotoxicity and *in vivo* antitumor activity**

Murine P388 cells ($1x10^4$/ml) were seeded in the RPMI-1640 medium containing 10% fetal bovine serum and 0.1 mg/ml of kanamycin. Compounds to be tested were added in graded concentrations, and the cultures were incubated for 96 h at 37°C in a humidified atmosphere of 5% carbon dioxide. The tumor cells were counted by MTT method,[10] and the IC50 value (concentration required for 50% inhibition of the cell growth) was determined by means of the growth carve.

HeLa S3 cells ($8x10^3$/ml) were seeded in the Eagle's minimal essential medium containing 10% fetal bovine serum and 0.06 mg/ml of kanamycin. Graded concentrations of compounds were added 24 h after the cell had been seeded. After cultivation for 72 h at 37°C in a humidified atmosphere of 5% carbon dioxide, the IC50 value was determined by neutral red dye uptake method.[11]

Murine P388 cells ($1x10^6$/mouse) were implanted intraperitoneally (i.p.) into 6-week-old male mice divided into groups each consisting of five test mice. Administration (i.p.) of compounds to be tested was started the day after tumor implantation. Antitumor efficacy was expressed by increase of life span (ILS) calculated by (T/C-1)x100, wherein T and C are mean survival days of treated and control mice, respectively.

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**References and Notes**

1. Parts of this series of papers have been the subject of five preliminary communications: a) Saito, S.; Matsuda, F.; Terashima, S., *Tetrahedron Lett.*, **1988**, *29*, 6301. b) Saito, S.; Tanaka, K.; Nakatani, K.; Matsuda, F.; Terashima, S., *ibid.*, **1989**, *30*, 7423. c) Katoh, T.; Nagata, Y.; Kobayashi, Y.; Arai, K.; Minami, J.; Terashima, S., *ibid.*, **1993**, *34*, 5743. d) Katoh, T.; Kirihara, M.; Nagata, Y.; Kobayashi, Y.; Arai, K.; Minami, J.; Terashima, S., *ibid.*, **1993**, *34*, 5747. e) Katoh, T.; Kirihara, M.; Yoshino, T.; Terashima, S., *ibid.*, **1993**, *34*, 5751.
2. a) Part 1: Saito, S.; Tamura, O.; Kobayashi, Y.; Matsuda, F.; Katoh, T.; Terashima, S., *Tetrahedron*, the preceding paper. b) Part 2: Saito, S.; Tanaka, K.; Nakatani, K.; Matsuda, F.; Katoh, T.; Terashima, S., *Tetrahedron*, the preceding paper. c) Part 3: Katoh, T.; Nagata, Y.; Kobayashi, Y.; Arai, K.; Minami, J.; Terashima, S., *Tetrahedron*, the preceding paper. d) Part 4: Katoh, T.; Kirihara, M.; Nagata, Y.; Kobayashi, Y; Arai, K.; Minami, J.; Terashima, S., *Tetrahedron*, the preceding paper.
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6. Present address: Department of Chemistry, Faculty of Science, Hokkaido University, Sapporo 006, Japan.
7. For chemical modifications of the A ring of **1** and their biological evaluation, see, a) Saito, H.; Kobayashi, S.; Uosaki, Y.; Sato, A.; Fujimoto, K.; Miyoshi, K.; Morimoto, A.; Hirata, T., *Chem. Pharm. Bull.*, **1990**, *38*, 1278. b) Saito, H.; Sato, A.; Ashizawa, T.; Morimoto, M.; Hirata, T., *Chem. Pharm. Bull.*, **1990**, *38*, 3202. c) Saito, H.; Hirata, T.; Fujimoto, K.; Ashizawa, T.; Morimoto, M.; Sato, A, *J. Med. Chem.*, **1991**, *34*, 1959.
8. a) Barton, D. H. R., Crich, D., Motherwell, W. B., *J. Chem. Soc. Chem. Commun.*, **1983**, 939. b) *Idem*, *Tetrahedron*, **1987**, *43*, 4321. c) Barton, D. H. R., *Aldrichimica Acta*, **1990**, *23*, 3.
9. Personal communication from Dr. T. Hirata, Kyowa Hakko Kogyo Co., Ltd..
10. Alley, M. C.; Scudiero, D. A.; Monk, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. L.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R., *Cancer Res.*, **1988**, *48*, 589.
11. Morimoto, M.; Ashizawa, T.; Ohno, H.; Azuma, M.; Kobayashi, E.; Okabe, M.; Gomi, K.; Kono, M.; Saitoh, Y.; Kanda, Y.; Arai, H.; Sato, A.; Kasai, M.; Tsuruo, T., *Cancer Res.*, **1991**, *51*, 110.

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