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# Syntheses and bioactivities of tricyclic pyrones

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**Abstract**—In search of compounds that ameliorate the toxicity of amyloid-β (Aβ) peptides, new derivatives of tricyclic pyrones (**1**–**7**) were synthesized and their biological activities evaluated. The carboxylic ester and amide derivatives **1**–**4** were synthesized from a selective carboxylation of C3 methyl of (5a*S*,7*S*)-{7-Isopropenyl-3-methyl-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-*b*][1]benzopyran (**8**) with LDA followed by benzyl chloroformate or carbon dioxide to provide ester **1** and carboxylic acid **9**, respectively. Three isomeric tricyclic pyrone, **5**–**7**, containing adenine moiety at C7 side chain were synthesized from the alkylation of mesylate **13** with adenine, and displacement of chloropurine **15** with amine **14**. Although C3-benzyloxycarbonylmethyl analogs **1**–**3** have marginal ACAT and CETP activities, their modified aspartate analog **4** and C3-methyl-C7-(*N*3-adeninyl)-2-propyl analog **6** show a significant effect in protecting against neuron-cell death from the toxicity of intracellular accumulation of Aβ or Aβ-containing C-terminal fragments (CTF) of amyloid β precursor protein (APP). *N*9-Adenine analog **5** is 20-fold less effective than *N*3-adenine derivative **6** in the protection of neuron-cell death induced by Aβ, while *N*10-adenine analog **7** was inactive. As a result of this study, compounds **4** and **6** will well serve as lead compounds for further studies of the mechanism of action of Aβ-and CTF-induced neuron-cell death, studies which should enhance the future development of new drugs for the prevention and treatment of AD. 

## 1. Introduction

The pathogenetic events leading to Alzheimer's disease (AD) may reside in the production and deposition of amyloid-β (Aβ) peptides.[1] Aβ peptides are 39–42-amino acid hydrophobic polypeptides derived from a trans-membrane glycoprotein, amyloid β precursor protein (APP). Based on the bioactivities of pyripyropene A,[2] a potent ACAT (acyl-coenzyme A: cholesterol O-acyltrans-ferase inhibitor, and arisugacin,[3] an acetylcholinesterase inhibitor, a number of tricyclic pyrone analogs was synthesized and their bio-activities were studied.[4,5] A recent report on the modulating effect of ACAT on the generation of Aβ peptides[6] supports such a possibility, that tricyclic pyrone (TP) analogs might prevent Aβ-induced cellular toxicity. Those encouraging results[5] prompted us to prepare a number of new derivatives of tricyclic pyrones containing carboxyl, aspartate, and adenine moieties. We have also investigated the unusual reactivity of adenine towards alkyl methanesulfonate. Furthermore, we have determined the biological activities of new TPs (Fig. 1) including ACAT and CETP (cholesteryl ester transfer protein) inhibitory activities, anticancer activity, and the rescue of cultured APP C99-expressing neurons from cell death.

## 2. Results and discussion

### 2.1. Syntheses

Based on the synthesis of an ACAT inhibitor, pyripyropene A (a tetracyclic pyranopyrone),[2] several tricyclic pyrano-pyrones containing various functionalities (Fig. 1) were synthesized. A common intermediate, compound **8** (an optically pure compound),[4] was used in the synthesis of **1**∼**7** (Schemes 1 and 2) by selective functionalizations of C3 and C7 side chains. A regioselective deprotonation of the C3 methyl group of TP **8** can be carried out with a strong base such as lithium diisopropylamide (LDA) or *n*-BuLi. Hence, treatment of **8** with LDA followed by benzyl chloroformate gave a 46% yield (based on reacted **8**) of **1** with 37% recovery of **8** (Scheme 1). Apparently, the anion of **8** deprotonates α-CH of the benzyl ester function of product **1** to give **8** and anion of **1**. However, in the reaction

*Keywords*: Alzheimer's disease; amyloid-β peptides; ACAT inhibitors; CETP inhibitors; tricyclic pyrones; APP C99-induced cell death.

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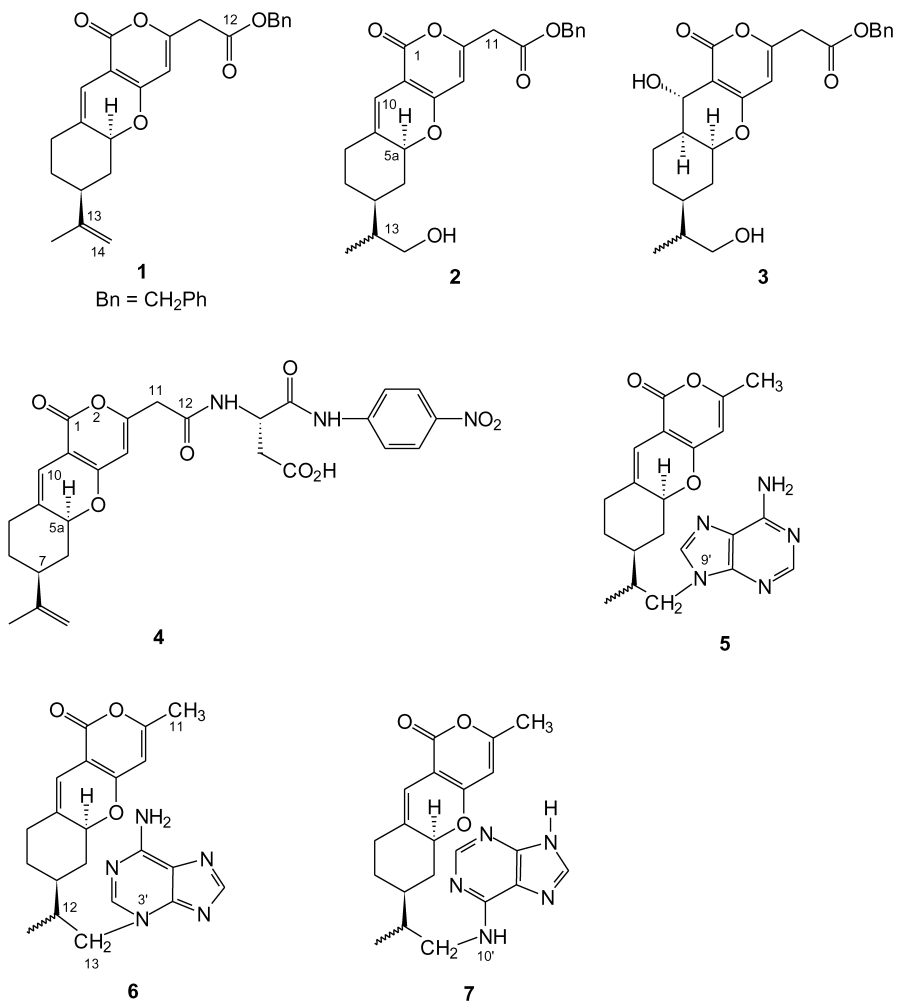
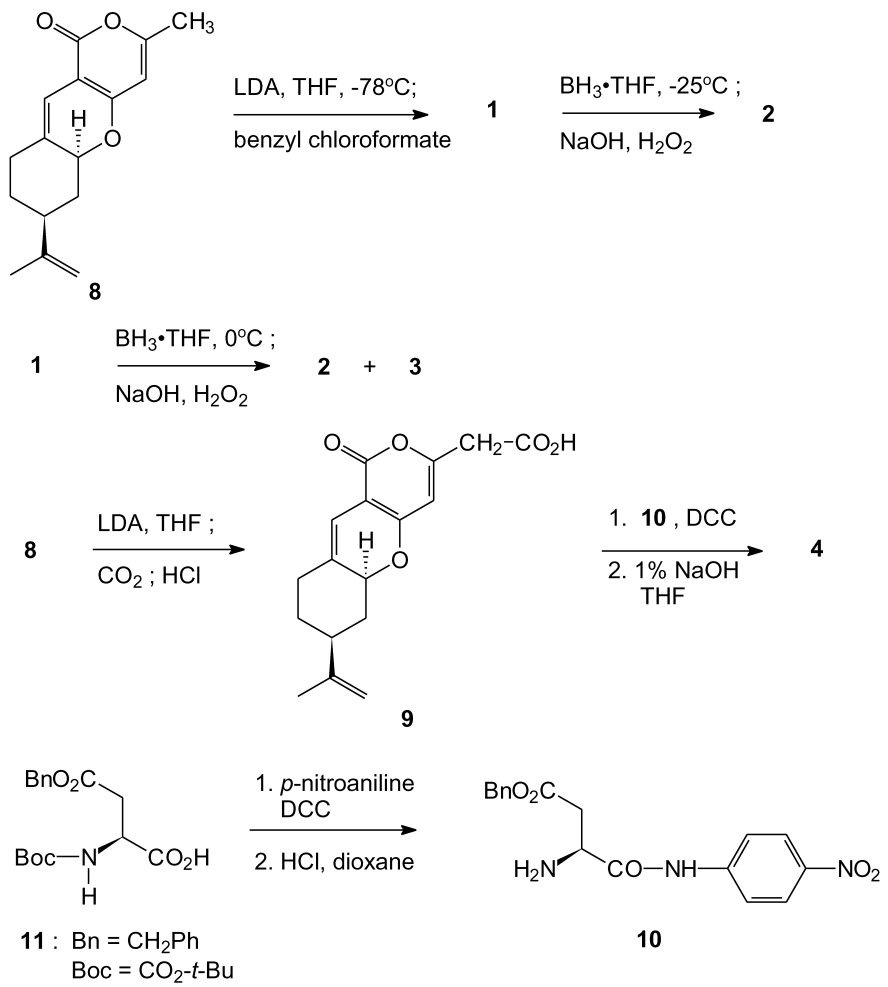



**Figure 1.**

with carbon dioxide (vide infra), this deprotonation by the anion of **8** is precluded by its faster reaction with the carbon dioxide. Chemo-selective hydroboration of **1** with 1 equiv. of $BH_3$·THF at −25°C for 14 h followed by oxidation with 0.5% NaOH/30% $H_2O_2$ at 0°C gave a 69% yield (based on reacted **1**) of alcohols **2** along with 26% recovery of **1**. The less hindered C13 double bond was selectively reacted in the presence of C9a double bond. Based on the $^{13}C$ NMR spectrum of **2**, two diastereomers at C13 were formed in a ratio of 1:1 and were inseparable by silica gel column chromatography and HPLC (normal phase; **2**, **3**, **5**, and **6**). Hence, mixtures of these diastereomers were used for biological evaluation. A dihydroxylation was resulted when the hydroboration reaction was carried out at 0°C. Yields of 32% of **3** and 14% of **2** were isolated. Again, column chromatography afforded only an inseparable mixture of two C13 diastereomers, **3**. No other stereoisomers at C9a and C10 of **3** were detected. The C9a,10 stereochemistry of **3** was tentatively assigned assuming that borane approaches the C9a–C10 double bond of **2** from the less hindered α face (same side as C5a–H). Amide **4** was synthesized in 90% overall yield from pyrone **8** by (1) treatment with LDA in THF followed by carboxylation with carbon dioxide to give acid **9** (92% yield), and (2) coupling of **9** with amine **10** in a solution of *N*,*N*′-dicyclohexylcarbodiimide (DCC) in dichloromethane followed by basic hydrolysis with aqueous NaOH. Amine **10** was prepared in 85% overall yield from the coupling of *N*-(*t*-butyloxycarbonyl)-L-aspartic acid 4-benzyl ester (**11**) with *p*-nitroaniline in a solution of DCC in dichloromethane followed by acidic hydrolysis of the *t*-butoxycarbonyl protecting group with HCl.

Since the isopropenyl group of TPs can be selectively hydroxylated (vide supra), functional group transformation of the resulting hydroxyl group would provide a number of TP analogs. Hence, treatment of **8** with 1 equiv. of $BH_3$·THF at −25°C followed by oxidation with NaOH and $H_2O_2$ gave an 82% yield (based on reacted **8**) of an inseparable mixture of two C-12 diastereomers, **12**, and a 26% recovery of **8** (Scheme 2). $^{13}C$ NMR spectrum of **12** revealed the two diastereomers to be present in equal amounts. Mesylation of **12** with triethylamine and methanesulfonyl chloride gave mesylate **13** in a 94% yield. Treatment of mesylate **13** with adenine and sodium hydride in DMF at 80°C for 20 h produced **5** and **6** in a ratio of 4:1. Compounds **5** and **6** were separated by silica gel column chromatography. The corresponding N7′-analog was not detected under these reaction conditions. The regiochemistry of these two isomeric products was assigned based on the comparison of the $^1H$ NMR resonances of C2′H and C8′H of the adenine moiety with the corresponding values reported.[7] Moreover, 2D NOESY experiments showed a correlation between




**Scheme 1.**

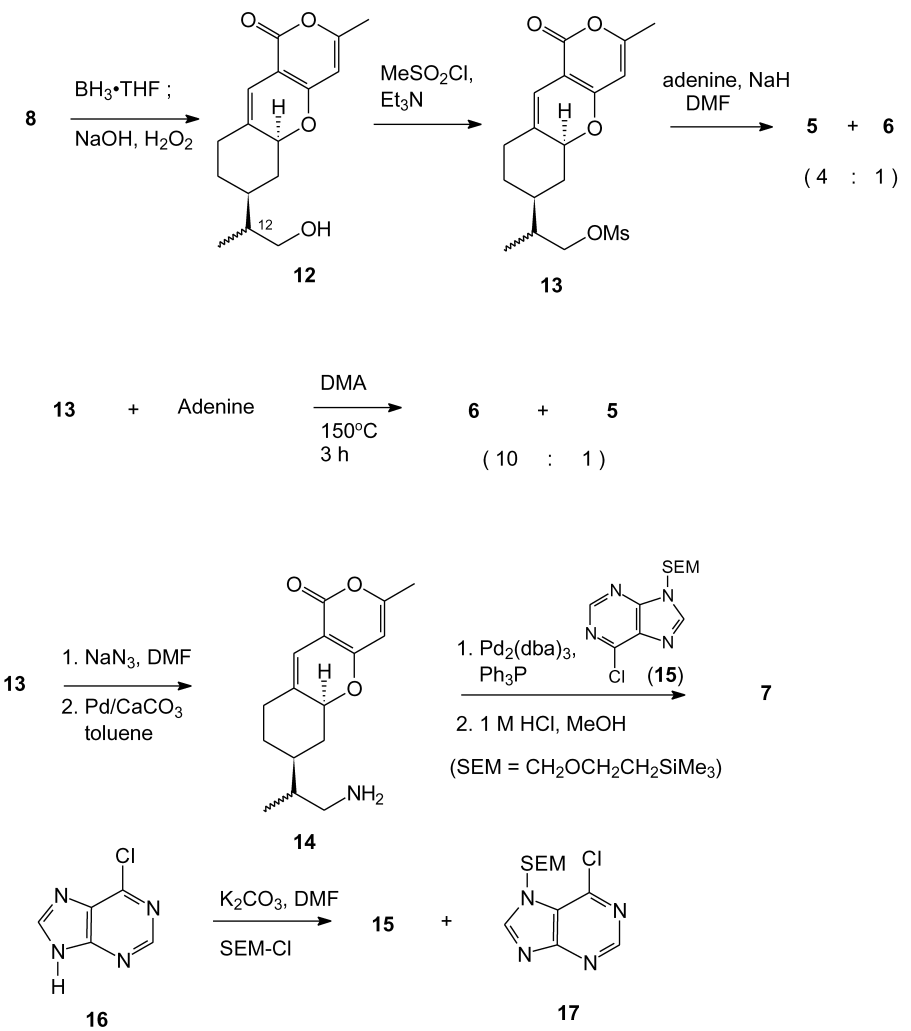
C8′-H ($\delta$ 7.78 ppm) and $CH_2N$ ($\delta$ 4.01 ppm) of **5** and between C8′-H ($\delta$ 7.98 ppm) and $CH_2N$ ($\delta$ 4.08 ppm) of **6**.

Initially, identification of the structure of **6** was difficult since it is a previously unreported compound, and reports in the literature often do not mention N-3 alkylation under basic conditions.[8] To verify its structure, a different method to prepare it was sought, and an adenine-tricyclic pyrone isomer, **7**, was also synthesized to compare the spectroscopic data and as well as the bioactivity data. A reported method for the selective N3-alkylation of adenine[9] was followed. Treatment of mesylate **13** with adenine in *N*,*N*-dimethylacetamide (DMA) at 150°C without base provided compound **6** (43% yield) as the major product along with a small amount of **5** (4% yield).

The synthesis of N10′-adenine derivative **7** was achieved from a palladium-mediated displacement reaction of amine **14** with chloropurine.[10] Amine **14** was prepared from the displacement of mesylate **13** with sodium azide in DMF at 60°C (83% yield) followed by hydrogenation with 10% Pd/$CaCO_3$ in toluene under 1 atm of hydrogen at 25°C (78% yield). N-10′ Adenine derivative **7** was synthesized via palladium-mediated displacement of 6-chloro-9-[2-(trimethylsilyl)ethoxy]methyl-1*H*-purine (**15**)[10] and amine **14** in the presence of 0.1 equiv. of $Pd_2(dba)_3$, 0.4 equiv. of triphenylphosphine and 1.5 equiv. of potassium carbonate in toluene under reflux (25% yield) followed by removal of the protecting group by treatment with 1 M HCl in methanol at 50°C (100% yield). Purine **15** (61% yield)[10] was prepared from the alkylation of 6-chloro-1*H*-purine (**16**) with potassium carbonate and 1-chloromethoxy-2-trimethylsilylethane (SEM-Cl) in DMF at 25°C, which also provided 6-chloro-7-[2-(trimethylsilyl)ethoxy]methyl-1*H*-purine (**17**; 14% yield). The regiochemistry of purine **15** and its C-7 isomer **17** were determined from 2D NOESY experiments in which their C8-H ($\delta$ 8.24 and 8.36 ppm, respectively) show correlation with their $CH_2N$ ($\delta$ 5.62 and 5.80 ppm, respectively).

## 2.2. Biological activity results

ACAT mediates the esterification of intracellular cholesterol and is believed to play a key role in lipoprotein metabolism and atherogenesis.[11] Studies of the inhibition of ACAT with tricyclic pyrone analogs were carried out first; the TP results are summarized in Table 1. Esters **1**–**3** showed marginal activity, with $IC_{50}$ values of 157, 310, and 150 μM, respectively. Under the same conditions, the $IC_{50}$ value of pyripyropene A was 0.1 μM. The inhibition of CETP with these three compounds was then studied. CETP, a hydrophobic neutral glycoprotein, mediates the transfer of cholesteryl ester from high-density lipoprotein to low-density lipoprotein.[12] It is encouraging to find that the $IC_{50}$




**Scheme 2.**

value of compound **3** was 75 μM, while the values of compounds **1** and **2** were >100 μM. Based on these studies, we synthesized and evaluated a number of amides derived from the attachment of natural amino acid at C12 of **1** and the modification at the isopropenyl side chain such as with a linkage of an adenine[13] moiety at C14, i.e. compounds **5**–**7**. The *N*-(*p*-nitrophenyl)aspartamide portion of **4** was attached in order to study whether **4** was a substrate of caspase **3**.[14] The in vitro study of **4** with caspase **3** showed no release of *p*-nitroaniline, which indicated that **4** is not a substrate. However, to our delight, compounds **4** and **6** (code named CP2)[5] protected from death MC65 cells that conditionally expressed with a partial APP fusion protein (amino-17 residues+carboxy-99 residues[15] in the absence of tetracycline.[5] The $EC_{50}$ (effective concentration at 50%) values of **4** and **6** were 2.0 and 0.15 μM, respectively. Surprisingly, N-9′ analog **5**, a regioisomer of **6**, was 20-fold less active ($EC_{50}$=3.0 μM) than **6**, while N-10′ analog **7**, another regioisomer of **6** was inactive. It is presently uncertain why **6** is the most active compound, but it may be associated with the water solubility of N3′-adenine **6**, in contrast to that of the N9′- and N10′ derivatives **5** and **7**, or their position of substitution on the adenine ring. Adenine itself shows no activity. Since some tricyclic pyrones such as

**Table 1**. Biological activities of various tricyclic pyrones

| Compounds | $IC_{50}$, ACAT | $IC_{50}$, CETP | $IC_{50}$, L1210 leukemic cells | $EC_{50}$, protection of APP C99-induced cell death |
|---|---|---|---|---|
| **1** | 157 μM | >100 μM | – | – |
| **2** | 310 μM | >100 μM | – | – |
| **3** | 150 μM | 75 μM | – | – |
| **4** | 168 μM | >180 μM | – | 2.0 μM |
| **5** | – | – | 30 μM | 3.0 μM |
| **6** | >500 μM | >250 μM | – | 0.15 μM |
| **7** | – | – | – | >3.0 μM |
| **8** | 167 μM | – | – | >10 μM |
| Pyripyropene A | 0.1 μM | – | >100 μM | – |
| Adenine | – | – | – | >10 μM |

H10, {3-(3-pyridyl)-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano-[4,3-*b*][1]benzopyran},[16] show intense anticancer activity, the cytotoxicity of adenine conjugated tricyclic pyrone was examined. Compound **5** is cytotoxic to L1210 tumor cells, with $IC_{50}$=30 μM. The inability of **5** to decrease tumor-cell viability at 1–25 μM may be ascribed to its top pyrone A-ring, which possesses a methyl substituent at C3 and not a 3-pyridyl substituent, which is essential for the antitumor activity of the TP analogs.[16]

## 3. Conclusions

Various tricyclic pyrones containing ester and amide side chains at C3 were synthesized from a regioselective deprotonation of C3 methyl group followed by carbonylation with carbon dioxide or benzyl chloroformate. Tricyclic pyrones containing alkyl side chain at C7 possessing an adenine moiety were also synthesized by a chemoselective hydroboration of C7 isopropenyl group followed by functional group transformation. Chemical modification of the functionalized tricyclic pyrones could lead to a library of analogs for biological evaluation. Compared with the moderate ACAT and CETP activities of TP analogs possessing an ester side chain at C3 of the pyrone ring (i.e. compounds **1**–**3**), $EC_{50}$ values of compounds **4** and **6** are 2.0 and 0.15 μM, respectively, in the protection of neuron-cell death from the toxicity of intracellular accumulation of Aβ or Aβ-containing C-terminal fragments (CTF) of APP. Thus, compounds **4** and **6** serve as lead compounds for mechanistic studies now being carried out on intracellular Aβ and CTF induced neuron-cell death. These studies should enhance the future development of new drugs for the prevention and treatment of AD.

## 4. Experimental

### 4.1. General methods

Nuclear magnetic resonance spectra were obtained at 400 MHz for $^1$H and 100 MHz for $^{13}$C in deuteriochloroform, unless otherwise indicated. Mass spectra were taken from a Hewlett–Packard 5890A Series II, GC–MS and a Bruker Esquire 3000 Plus electrospray ionization mass spectrometer. FAB spectra were taken by using Xe beam (8 kV) and *m*-nitrobenzyl alcohol as matrix. High-resolution Mass spectra were taken from an IonSpec HiResMALDI mass spectrometer using 2,5-dihydroxybenzoic acid as a matrix. (5a*S*,7*S*)-{7-Isopropenyl-3-methyl-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-*b*][1]benzopyran (**8**), an optically pure compound, was prepared as described.[4] $Pd_2(dba)_3$ was prepared from palladium chloride, *trans*,*trans*-1,5-diphenyl-1,4-pentadien-3-one (dibenzylidene acetone), and sodium acetate in methanol.[17] *N*-(*t*-Butyloxycarbonyl)-L-aspartic acid 4-benzyl ester, adenine, 6-chloro-1*H*-purine, and 1-(chloromethoxy)-2-(trimethylsilyl)ethane (SEM-Cl) were purchased from Aldrich Chem. Co.

The reported procedures for the inhibitory activities of ACAT,[18] CETP,[19] L1210 tumor cells,[16] and APP C99-induced cell death[5] were followed.

**4.1.1. Benzyl (5a*S*,7*S*)-{7-isopropenyl-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-*b*][1]benzopyran-3-yl}acetate (1).** To a cold (−10°C) solution of 0.68 mL (4.8 mmol) of diisopropylamine in 20 mL of diethyl ether under argon was added 3.00 mL (4.8 mmol; 1.6 M solution in hexanes) of *n*-butyllithium via syringe, and the solution was stirred for 1 h. In another flask, a solution of 0.50 g (1.9 mmol) of **8** in 20 mL of THF under argon was cooled to −78°C. The LDA solution was added to the pyrone solution at −78°C via cannula, followed by 0.67 mL (3.9 mmol) of HMPA. After 3 h of stirring, a cold (−78°C) solution of 0.55 mL (3.9 mmol) of benzyl chloroformate in 20 mL of THF was added via cannula, and stirred for 2 h. The reaction solution was diluted with 40 mL of aqueous $NaHCO_3$, and extracted three times with dichloromethane. The combined organic layer was washed with 40 mL of brine, dried ($MgSO_4$), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and ether as eluant to give 0.22 g (46% yield; based on reacted **8**) of **1** and 0.19 g (38% recovery) of **8**. Mp 114–116°C; $[\alpha]_D^{23}$=−28° (*c* 1.5, $CHCl_3$); $^1$H NMR δ 7.38–7.31 (m, 5H, Ar), 6.08 (s, 1H, C10-H), 5.91 (s, 1H, C4-H), 5.28 (s, 2H, $CH_2O$), 5.12 (dd, *J*=1.2, 5.2 Hz, 1H, C5a–H), 4.75 (s, 1H, =$CH_2$), 4.72 (s, 1H, =$CH_2$), 3.50 (s, 2H, $CH_2CO$), 2.49~2.45 (m, 1H), 2.21~2.01 (m, 3H), 1.86–1.70 (m, 2H), 1.73 (s, 3H, Me), 1.34–1.25 (m, 1H); $^{13}$C NMR δ 167.5, 162.6, 161.9, 156.2, 147.8, 135.2, 133.2, 128.7, 128.6, 128.4, 109.8, 109.3, 102.2, 98.8, 79.5, 67.5, 43.4, 39.9, 39.4, 32.4, 31.9, 20.8. Anal. calcd for $C_{24}H_{24}O_5$: C, 73.45; H, 6.16. Found: C, 73.74; H, 6.50.

**4.1.2. Benzyl (5a*S*,7*S*)-{7-[(1*R*)- and (1*S*)-2-hydroxy-1-methylethyl]-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-*b*][1]benzopyran-3-yl}acetate (2).** A solution of 0.115 g (0.29 mmol) of **1** and 0.30 mL (0.30 mmol) of $BH_3$·THF complex (1.0 M in THF) in 5 mL of THF was kept at −25°C for 14 h. The solution was warmed to 0°C, 2 mL (0.3 mmol) of 0.5% aqueous NaOH solution and 2 mL (17.6 mmol) of 30% hydrogen peroxide were added, and the mixture was stirred at 0°C for 6 h. The mixture was then neutralized with 6N HCl, diluted with 30 mL of water, and extracted three times with dichloromethane. The combined extract was washed with brine, dried ($MgSO_4$), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluant to give 0.061 g (69% yield; based on reacted **1**) of **2** as a mixture of two diastereomers at C13, **2R** and **2S**, and 0.030 g (26% recovery) of **1**. Mp 108–110°C; $^1$H NMR δ 7.39~7.31 (m, 5H, Ar), 6.06 (s, 1H), 5.91 (s, 1H), 5.17 (s, 2H, $OCH_2$), 5.10 (dd, *J*=10.8, 5.6 Hz, 1H, C5a–H), 3.61–3.52 (m, 2H, $CH_2OH$), 3.50 (s, 2H, $CH_2CO$), 2.46 (dd, *J*=14.0, 1.2 Hz, 1H), 2.13–1.96 (m, 2H), 1.73–1.11 (m, 5H), 0.91 (d, *J*=2 Hz, 3H, Me); $^{13}$C NMR δ 167.6, 162.7, 162.1, 156.2, 135.2, 133.8, 128.8, 128.6, 128.5, 109.0, 102.3, 98.9, 79.9, 79.8, 67.6, 65.8, 40.1, 40.0, 39.5, 39.4, 37.4, 37.3, 37.1, 32.6, 32.4, 31.2, 30.5, 28.7, 13.3 (Me for a diastereomer), 13.2 (Me for another diastereomer). Anal. calcd for $C_{24}H_{26}O_6$·$0.5H_2O$: C, 68.65; H, 6.49. Found: C, 69.22; H, 6.54.

**4.1.3. Benzyl (5a*S*,7*S*,9a*S*,10*S*) -{10-hydroxy-7-[(1*R*) and (1*S*)-2-hydroxy-1-methylethyl]-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-*b*][1]benzopyran-3-yl}acetate**

**(3).** A solution of 0.10 g (0.26 mmol) of **1** and 0.26 mL (0.26 mmol) of $BH_3$·THF complex (1.0 M in THF) in 3 mL of THF was kept at −20°C for 3 h and then at 0°C for 12 h. To it, 2 mL of 0.5% aqueous NaOH solution and 2 mL of 30% hydrogen peroxide were added, and the mixture was stirred at 0°C for 4 h. The mixture was then neutralized with 6N HCl, diluted with 10 mL of water, and extracted three times with dichloromethane. The combined extract was washed with brine, dried ($MgSO_4$), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluant to give 15 mg (14% yield) of **2** and 36 mg (32% yield) of **3**. $^1$H NMR 7.36 (m, 5H, Ar), 5.99 (s, C4H), 5.18 (s, $CH_2O$), 4.66 (dd, $J$=8.8, 3.2 Hz, 1H, C10H), 4.46 (m, 1H, C5aH), 4.26 (bs, 1H, OH), 3.58 (m, 2H, $CH_2O$), 3.53 (s, 2H, $CH_2CO$), 2.21 (m, 1H), 2.14 (bs, 1H, OH), 1.78–1.33 (a serious of m, 8H), 0.93 (d, $J$=7 Hz, 3H, Me of a diastereomer), 0.90 (d, $J$=7 Hz, 3H, Me of another diasteromer). $^{13}$C NMR (2 diastereomers at C13) $\delta$ 167.3, 164.6, 163.4, 156.6, 135.0, 128.7 (2C, Ar), 128.4 (2C, Ar), 103.0, 100.9, 94.0, 67.5, 65.9, 59.5, 39.5, 39.1, 37.9, 31.6, 29.6, 24.0, 22.3, 13.7, 13.5. Anal. calcd for $C_{24}H_{28}O_7$·0.5$H_2O$: C, 65.89; H, 6.68. Found: C, 66.17; H, 7.26.

**4.1.4. (5a*S*,7*S*)-{7-Isopropenyl-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-*b*][1]-benzopyran-3-yl}acetic acid (9).** To a cold (−10°C) solution of 0.27 mL (1.90 mmol) of diisopropylamine in 5 mL of diethyl ether under argon was added 1.20 mL (1.90 mmol; 1.6 M solution in hexanes) of *n*-butyllithium via syringe, and the solution was stirred for 1 h. In another flask, a solution of 0.25 g (0.97 mmol) of **8** in 5 mL of THF under argon was cooled to −78°C. To it, the LDA solution was added via cannula, stirred for 2 h, and carbon dioxide gas was then introduced. The color of the blue anion changed to brownish color. The reaction mixture was stirred for 30 min, 20 mL of aqueous $NaHCO_3$ was added, and extracted with diethyl ether three times. The aqueous layer was acidified with 6N HCl, extracted three times with dichloromethane. The combined dichloromethane layers were washed with water, brine, dried ($MgSO_4$), and concentrated to give 0.271 g (92% yield) of **9**. This material was used in the next step without further purification. Compound **9** undergoes decarboxylation when subjected to silica gel column chromatography to give **8**. $[\alpha]_D^{23}$=+22° (*c* 1.0, $CHCl_3$); MS, EI *m*/*z* 258 (M−$CO_2$), 189, 176; $^1$H NMR $\delta$ 6.09 (s, 1H), 5.95 (s, 1H), 5.13 (m, 1H), 4.75 (s, 1H, =$CH_2$), 4.72 (s, 1H, =$CH_2$), 3.51 (s, 2H, $CH_2CO$), 2.51–1.21 (a series of m, 7H), 1.73 (s, 3H, Me); $^{13}$C NMR $\delta$ 171.0, 163.2, 162.8, 156.5, 147.8, 133.6, 110.0, 109.2, 102.6, 98.9, 79.8, 43.5, 40.0, 39.3, 32.6, 32.1, 20.9. HRMS calcd for $C_{17}H_{19}O_5$ (M+1) 303.1457, found 303.1400.

**4.1.5. (3*S*)-3-{[(5a′*S*,7′*S*)-7-Isopropenyl-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyran[4,3-*b*][1]benzopyran-3-yl]methylcarbamoyl}-4-oxo-4-(4-nitrophenylamino)-butanoic acid (4).** A mixture of 35 mg (0.12 mmol) of **9**, 44 mg (0.12 mmol) of **10**, and 36 mg (0.18 mmol) of DCC in 5 mL of dichloromethane under argon was stirred at 25°C for 20 h. The reaction mixture was filtered, and the filtrate was concentrated, and column chromatographed on silica gel using a gradient mixture of hexane, dichloromethane and methanol as eluant to give 72 mg (90% yield) of the benzyl ester protected amide. A solution of 40 mg (0.06 mmol) of the above amide and 1 mL of 1% aqueous NaOH in 2 mL of THF was stirred at 25°C for 1 h. The solution was neutralized with 4N HCl, concentrated, and the resulting solid was washed with dichloromethane (to remove benzyl alcohol) and dried under vacuum to give 34 mg (100% yield) of **4**. Mp 234–236°C; $[\alpha]_D^{23}$=+23.1° (*c* 1.2, $CHCl_3$); MS (electrospray), *m*/*z* 537 ($M^+$), 535 (M−2), 518 (100%, M−1−$H_2O$). $^1$H NMR (DMSO-$d_6$) $\delta$ 8.19 (d, $J$=9.6 Hz, 1H, Ar), 7.85 (d, $J$=9.6 Hz, 1H, Ar), 6.11 (s, 1H, C4H), 5.94 (s, 1H, C 10H), 4.75 (s, 1H, =$CH_2$), 4.72 (s, 1H, =$CH_2$), 4.05 (m, 1H, CHN), 3.24 (s, 2H, $CH_2CO$), 2.10 (m, 2H, $CH_2CO$), 1.90–1.02 (a series of m, 7H), 1.70 (s, 3H, Me); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 173.9, 170.8, 169.8, 165.4, 162.0, 157.0, 148.1, 145.6, 141.9, 133.1, 128.2, 124.9, 118.8, 109.9, 100.6, 90.3, 67.9, 55.8, 49.9, 34.7, 31.5, 29.0, 25.4, 20.6, 17.2. HRMS calcd for $C_{27}H_{27}N_3O_9$ 537.1748, found 537.1742.

**4.1.6. Benzyl (*S*)-3-amino-4-oxo-4-(4-nitrophenylamino)-butanoate (10).** To a solution of 0.214 g (1.55 mmol) of *p*-nitroaniline and 0.50 g (1.55 mmol) of *N*-(*t*-butyloxycarbonyl)-L-aspartic acid 4-benzyl ester (**11**) in 10 mL of dichloromethane at 25°C under argon, was added a solution of 0.383 g (1.86 mmol) of DCC in 20 mL of dichloromethane via cannula. The reaction mixture was stirred for 24 h, filtered, concentrated, and column chromatographed on silica gel using a mixture of hexane, methanol, and dichloromethane (40:4:1) as eluant to give 0.45 g of the amide (85% yield; based on reacted *p*-nitroaniline) and 0.05 g (23% recovery) of *p*-nitroaniline. Mp 47–50°C; $[\alpha]_D^{20}$=−36.5° (*c* 1.3, $CHCl_3$); $^1$H NMR $\delta$ 9.18 (s, 1H, NH), 8.20 (d, $J$=7 Hz, 2H, Ar), 7.66 (d, $J$=7 Hz, 2H, Ar), 7.36 (s, 5H, Ar), 5.86 (s, 1H, NH), 5.20 (d, $J$=12.4 Hz, 1H, $OCH_2$), 5.16 (d, $J$=12.4 Hz, 1H, $OCH_2$), 4.67 (m, 1H, CHN), 3.09 (dd, $J$=17, 4 Hz, 1H, $CH_2CO$), 2.82 (dd, $J$=17, 6 Hz, 1H, $CH_2CO$), 1.49 (s, 9H, *t*-Bu); $^{13}$C NMR $\delta$, 171.6, 169.4, 153.1, 143.4, 135.2, 128.6, 128.5, 128.47, 128.2, 125.0, 119.2, 67.1, 65.8, 51.6, 35.4, 28.2. A solution of 0.15 g (0.34 mmol) of the above amide and 0.85 mL (3.39 mmol) of 4 M HCl in dioxane was stirred at 25°C for 2 h, neutralized with 10 mL of aqueous $NaHCO_3$, and extracted three times with dichloromethane. The combined organic layer was washed with brine, dried ($MgSO_4$), and concentrated to give 0.122 g of **10** (100% yield): $[\alpha]_D^{22}$=−19° (*c* 0.15, $CHCl_3$; $^1$H NMR $\delta$ 10.00 (s, 1H, CONH), 8.22 (d, $J$=7 Hz, 2H, Ar), 7.75 (d, $J$=7 Hz, 2H, Ar), 7.34 (s, 5H, Ar), 5.18 (d, $J$=12.4 Hz 1H, $OCH_2$), 5.14 (d, $J$=12.4 Hz, 1H, $OCH_2$), 3.83 (dd, $J$=6.8, 4.4 Hz, 1H, CHN), 3.02 (dd, $J$=17, 4.4 Hz, 1H, $CH_2$), 2.96 (dd, $J$=17, 6.8 Hz, 1H, $CH_2$); $^{13}$C NMR $\delta$, 174.4, 172.0, 143.4, 135.1, 128.7, 128.5, 128.4, 128.3, 125.1, 118.9, 66.9, 52.4, 38.9. HRMS calcd for $C_{17}H_{18}N_3O_5$ (M+1) 344.1169, found 344.1150.

**4.1.7. (5a*S*,7*S*)-7-[(1*R*) and (1*S*)-2-Hydroxy-1-methylethyl]-3-methyl-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-*b*][1]benzopyran (12).** To a cold solution (−25°C) of 0.50 g (1.94 mmol) of **8** in 10 mL of THF under argon, was added 1.94 mL (1.94 mmol) of $BH_3$·THF complex (1.0 M in THF). After stirring the solution at −25°C for 10 h, 14 mL (2.1 mmol) of 0.5% aqueous NaOH and 4 mL of 30% hydrogen peroxide were added at 0°C. The solution

was stirred for 4 h, diluted with 50 mL of water, and extracted three times with dichloromethane. The combined organic layer was washed with 40 mL of brine, dried ($MgSO_4$), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluants to give 0.326 g (82% yield; based on reacted **8**) of **12** as a mixture of two diastereomers at C12 (1:1; based on $^{13}C$ NMR spectrum) and 0.130 g (26% recovery) of **8**. $^1H$ NMR $\delta$ 6.08 (s, 1H, C4H), 5.71 (s, 1H, C10H), 5.07 (t, $J$=5.2 Hz, 1H, C5aH), 3.62–3.52 (m, 2H, $CH_2O$), 2.46 (m, 1H), 2.19 (s, 3H, Me), 2.13–1.99 (m, 2H), 1.73–1.51 (m, 3H), 1.19–1.12 (m, 2H), 0.92 (d, $J$=7 Hz, 3H, Me); $^{13}C$ NMR (two diastereomers) $\delta$ 163.5, 162.8, 161.6, 133.0, 109.0, 100.0, 97.4, 79.7, 79.6, 65.6, 39.9, 39.8, 39.4, 37.2, 37.1, 36.9, 32.4, 32.3, 31.1, 30.4, 28.5, 20.1, 13.2 (Me for a diastereomer), 13.1 (Me for another diastereomer). Anal. calcd for $C_{16}H_{20}O_4$: C, 69.55; H, 7.29. Found: C, 69.26; H, 7.03.

**4.1.8. (5a*S*,7*S*)-3-Methyl-7-[(1*R*) and (1*S*)- 2-(methanesulfonyloxy)-1-methylethyl]-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-*b*][1]benzopyran (13).** To a cold (0°C) solution of 50 mg (0.18 mmol) of **12** in 5 mL of dichloromethane under argon, were added 0.08 mL (0.54 mmol) of triethylamine and 0.02 mL (0.27 mmol) of methanesulfonyl chloride. The solution was stirred for 3 h, diluted with 30 mL of water, and extracted three times with dichloromethane. The combined dichloromethane layer was washed with saturated aqueous $NaHCO_3$, and brine, dried ($MgSO_4$), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and ether as eluants to give 60 mg (94% yield) of **13** as a mixture of two diastereomers (1:1; based on $^{13}C$ NMR spectrum). $^1H$ NMR $\delta$ 6.08 (s, 1H, C4H), 5.71 (s, 1H, C10H), 5.06 (m, 1H, CHO), 4.18–4.08 (m, 2H, $CH_2O$), 3.03 (s, 3H, MeS), 2.49 (d, $J$=2.8 Hz, 1H), 2.19 (s, 3H, Me), 2.14–1.11 (m, 7H), 0.98 (d, $J$=6.8 Hz, 3H, Me); $^{13}C$ NMR $\delta$ 163.2, 162.4, 161.7, 132.1, 109.6, 105.2, 99.8, 79.2, 79.1, 72.3, 38.9, 37.5, 37.4, 37.3, 37.2, 36.9, 32.2, 32.1, 30.8, 28.6, 20.2, 13.3 (Me for a diastereomer), 13.2 (Me for another diastereomer). Anal. calcd for $C_{17}H_{22}O_6S$: C, 57.61; H, 6.26. Found: C, 57.65; H, 6.43.

**4.1.9. (5a*S*,7*S*)-7-[(1*R*) and (1*S*)-2-(*N*9-Adenyl)-1-methylethyl]-3-methyl-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-*b*][1]benzopyran (5) and (5a*S*,7*S*)-7-[(1*R*) and (1*S*)- 2-(*N*3-adenyl)-1-methylethyl]-3-methyl-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-*b*][1]benzopyran (6).** To a solution of 0.008 g (0.34 mmol) of NaH in 3 mL of DMF under argon was added 0.046 g (0.34 mmol) of adenine at 25°C, and the solution was stirred for 1 h. This solution was then added to a solution of 0.110 g (0.30 mmol) of **13** in 3 mL of DMF via cannula. The reaction solution was stirred at 80°C for 20 h. The solvent, DMF, was removed under vacuum at 50°C, 10 mL of dichloromethane was added to the residue, and the dichloromethane was removed via a pipette (to remove trace of DMF and tricyclic pyrone by-product **12**). To the resulting solids, 20 mL of ethanol was added, filtered, and the filtrate was concentrated, and column chromatographed on silica gel using a gradient mixture of dichloromethane and methanol as eluant to give 0.026 g (21% yield) of **5** (less polar) and 0.006 g (5% yield) of **6** (more polar). Compound **5** (less polar; two diastereomers at C12): mp 228–230°C; MS, electrospray, $m/z$ 394 (M+1; 100%), 259 (M−adenine), 136 (adenine); $^1H$ NMR $\delta$ 8.36 (s, 1H, C2′H), 7.78 (s, 1H, C8′H), 6.09 (s, 1H, C4H), 5.89 (bs, 2H, $NH_2$; disappeared when 1 drop of $D_2O$ was added), 5.72 (s, 1H, C10H), 5.01 (m, 1H, C5aH), 4.24 (dd, $J$=14, 7 Hz, 1H, CHN), 4.01 (dd, $J$=14, 7 Hz, 1H, CHN), 2.5–1.2 (a serious of m, 8H), 2.19 (s, 3H, Me), 0.90 (d, $J$=7 Hz, 3H, Me); $^{13}C$ NMR $\delta$ 163.4, 162.7, 161.9, 155.6 (adenine moiety), 153.5 (adenine moiety), 150.6 (adenine moiety), 140.9 (adenine moiety), 132.1, 119.8 (adenine moiety), 109.9, 99.9, 97.5, 79.4, 79.2, 47.9, 39.3, 38.4, 38.3, 38.1, 38.0, 36.2, 32.3, 32.1, 31.1, 27.8, 20.3, 13.8. Anal. calcd for $C_{21}H_{23}N_5O_3$: C, 64.11; H, 5.89. Found: C, 63.80; H, 5.93.

Compound **6** (more polar; 2 diastereomers at C12): MS, electrospray, $m/z$ 394 (M+1, 100%), 259 (M−adenine), 136 (adenine); $^1H$ NMR $\delta$ 8.07 (s, C8′H of adenine), 7.98 and 7.97 (2 s, 1H, C2′H of adenine; 2 diastereomers), 6.10 (s, 1H, C10H), 5.72 and 5.71 (2s, 1H, C4H), 5.02 (m, 1H, C5aH), 4.50 (dd, $J$=14, 7 Hz, 1H, CHN), 4.08 (2dd, $J$=14, 8 Hz, 1H, CHN; 2 diastereomers), 2.46 (m, 2H), 2.20 and 2.19 (2s, 3H, Me; 2 diastereomers), 2.10–1.22 (a series of m, 6H), 0.91 (d, $J$=7.0 Hz, 3H, Me). $^{13}C$ NMR (2 diastereomers) $\delta$ 163.2 and 163.1, 162.4, 161.7, 154.4, 154.0, 150.7, 142.3, 131.7 and 131.6, 121.0, 199.8, 99.7, 97.3, 79.0, 78.8, 54.5 and 54.4, 38.9, 38.1 and 38.0, 37.1 and 36.9, 36.1, 32.0 and 31.9, 30.7, 27.6, 20.1, 13.3 and 13.2. HRMS calcd for $C_{21}H_{24}N_5O_3$ (M+H) 394.1881, found 394.1875. Anal. calcd for $C_{21}H_{23}N_5O_3{\cdot}2H_2O$: C, 58.73; H, 6.34. Found: C, 59.20; H, 6.18.

**4.1.10. Synthesis of compound 6 from adenine in DMA.** A solution of 0.21 g (0.59 mmol) of mesylate **13** and 80 mg (0.59 mmol) of adenine in 3 mL of DMA (freshly distilled from $CaCl_2$ under reduced pressure) was heated at 150°C for 3 h. DMA was removed under reduced pressure (70°C/0.5 mm Hg), and the residue of the distillation was triturated with 5 mL of dichloromethane. To the residue, 50 mg (0.59 mmol) of $NaHCO_3$ and 3 mL of ethanol were added, and the mixture was subjected to a silica gel column chromatography using a gradient mixture of dichloromethane and ethanol as eluant to give 0.10 g (43% yield) of **6** and 0.01 g (4% yield) of **5**.

**4.1.11. (5a*S*,7*S*)-7-[(1*R*) and (1*S*)-2-Azido-1-methylethyl]-3-methyl-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-*b*][1]benzopyran (18).** A solution of 0.70 g (2.0 mmol) of mesylate **13** and 0.26 g (4.0 mmol) of sodium azide in 20 mL of DMF under argon was stirred at 60°C for 16 h. The solution was cooled, diluted with ether, washed twice with water, and brine, dried ($MgSO_4$), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and ethyl acetate to give 0.50 g (83% yield) of **18** and 60 mg (9% recovery) of **13**. Compound **18** (2 diastereomers at C12): mp 87–88°C; MS, $m/z$ 301 ($M^+$); $^1H$ NMR $\delta$ 6.09 (s, 1H, C4H), 5.71 (s, 1H, C10H), 5.10 (m, 1H, C5aH), 3.30 (dd, $J$=13, 6 Hz, 1H, CHN), 3.23 (dd, $J$=13, 7 Hz, 1H, CHN), 2.48 (d, $J$=14 Hz, 1H), 2.19 (s, 3H, Me), 2.10–1.95 (m, 2H), 1.7–1.5 (m, 4H), 1.27–1.08 (m, 1H), 0.95 (d, $J$=7 Hz, 3H, Me); $^{13}C$ NMR $\delta$ 163.4, 162.7, 161.8, 132.4, 109.6, 99.9, 97.5, 79.5 and 79.4 (C5a, 2 isomers), 55.5, 39.2, 38.2 and 38.15 (2 isomers), 37.8 and

37.78 (2 isomers), 37.0, 32.3 and 32.2 (2 isomers), 30.9, 28.6, 20.3, 14.5. Anal. calcd for $C_{16}H_{19}N_3O_3$: C, 63.77; H, 6.35. Found: C, 63.99; H, 6.51.

**4.1.12. (5a*S*,7*S*)-7-[(1*R*) and (1*S*)-2-Amino-1-methylethyl)-3-methyl-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-*b*][1]benzopyran (14).** A solution of 0.42 g (1.4 mmol) of azide **18** and 1.0 g of 10% $Pd/CaCO_3$ in toluene was stirred under 1 atm. of hydrogen at 25°C for 14 h. The mixture was filtered through a short silica gel column using ethyl acetate, $CHCl_3/CH_3OH$ (2:1), and then $CHCl_3/CH_3OH/NH_4OH$ (2:1:0.01) as eluants to give 0.30 g (78% yield) of amine **14** (2 diastereomers at C12). $^1$H NMR $\delta$ 6.07 (s, 1H, C4H), 5.71 (s, 1H, C10H), 5.07 (m, 1H, C5aH), 2.73 (m, 1H, 2 isomer, CHN), 2.58 (m, 1H, 2 isomers, CHN), 2.46 (d, $J$=13 Hz, 1H), 2.19 (s, 3H, Me), 2.15–1.10 (m, 7H), 0.91 (d, $J$=7 Hz, 3H, Me); $^{13}$C NMR $\delta$ 163.4, 162.7, 161.7, 132.9, 109.3, 100.0, 97.5, 79.8 and 79.7 (2 isomers), 66.0, 46.0 and 45.9 (2 isomers), 40.9, 39.1, 38.5 and 38.4 (2 isomers), 37.1, 32.6 and 32.4 (2 isomers), 31.3, 31.2, 28.6, 20.3, 15.5, 14.3. HRMS calcd for $C_{17}H_{22}NO_3$ (M+1) 276.1601, found 276.1610.

**4.1.13. 6-Chloro-9-[(2-trimethylsilylethoxy)methyl]-1*H*-purine (15).**[15] A solution of 0.31 g (2.0 mmol) of 6-chloro-1*H*-purine and 0.83 g (6.0 mmol) of potassium carbonate in 15 mL of DMF was stirred at 25°C for 20 min. under argon. To it, 0.53 mL (3.0 mmol) of 1-(chloromethoxy)-2-(trimethylsilyl)ethane (SEM-Cl) was added via syringe, the solution was stirred for 9 h, filtered through Celite, and the filtrate was diluted with diethyl ether, washed with water, and brine, dried ($MgSO_4$), concentrated, and column chromatographed on silica gel using a gradient mixture of hexane and ethyl acetate to give 0.35 g (61% yield) of **15** and 0.08 g (14% yield) of 6-chloro-3-[(2-trimethylsilylethoxy)methyl]-1*H*-purine (**17**). Compound **15**: mp 33–34°C; $^1$H NMR $\delta$ 8.70 (s, 1H), 8.24 (s, 1H), 5.62 (s, 2H, $NCH_2O$), 3.60 (t, $J$=8 Hz, 2H, $CH_2O$), 0.94 (t, $J$=8 Hz, 2H, $CH_2Si$), −0.12 (s, 9H, MeSi); $^{13}$C NMR $\delta$ 152.4, 152.2, 151.3, 145.4, 131.5, 72.7 (CN), 67.8 (CO), 17.8, −1.4 (CSi).

6-*Chloro*-7-[(2-*trimethylsilylethoxy*)*methyl*]-1*H*-*purine* (**17**). Mp 80–81°C; $^1$H NMR $\delta$ 8.93 (s, 1H), 8.36 (s, 1H), 5.80 (s, 2H, $NCH_2O$), 3.61 (t, $J$=8 Hz, 2H, $CH_2O$), 0.94 (t, $J$=8 Hz, 2H, $CH_2Si$), −0.02 (s, 9H, MeSi); $^{13}$C NMR $\delta$ 162.5, 153.0, 149.3, 143.7, 122.5, 75.9 (CN), 67.2 (CO), 17.9, −1.3 (CSi). Anal. calcd for $C_{11}H_{17}ClN_4OSi$: C, 46.39; H, 6.02. Found: C, 46.52; H, 6.08.

**4.1.14. (5a*S*,7*S*)-3-Methyl-7-{(1*R*) and (1*S*)-2-[(*N*9-trimethylsilylethoxymethyl)-*N*10-adenyl]-1-methylethyl}-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-*b*][1]benzopyran (19).** A mixture of 56 mg (0.20 mmol) of amine **14**, 114 mg (0.40 mmol) of purine **15**, 18 mg (0.02 mmol) of $Pd_2(dba)_3$, 21 mg (0.08 mmol) of $Ph_3P$, and 41 mg (0.30 mmol) of $K_2CO_3$ in 5 mL of toluene was heated in a sealed tube at 140°C. After stirring for 5 h, the mixture was cooled to 25°C, diluted with water, and extracted three times with diethyl ether. The combined ether layer was washed with water, and brine, dried ($MgSO_4$), concentrated, and column chromatographed on silica gel using a gradient mixture of dichloromethane and ethyl acetate as eluant to give 17 mg (16% yield) of **19** (2 diastereomers at C12). $^1$H NMR $\delta$ 8.40 (s, 1H), 7.89 (s, 1H), 6.07 (s, 1H, C4H), 5.81 (bs, 1H, NH), 5.70 (s, 1H, C10H), 5.56 (s, 2H, $NCH_2O$), 5.03 (m, 1H, C5aH), 3.64 (m, 1H, CHN), 3.60 (t, $J$=8 Hz, 2H, $CH_2O$), 3.50 (m, 1H, CHN), 2.46 (d, $J$=12 Hz, 1H), 2.18 (s, 3H, Me), 2.10–1.2 (a serious of m, 7H), 0.93 (d, $J$=7 Hz, 3H, Me; 2 diastereomers as indicated by 2 sets of doublet), 0.92 (t, $J$=8 Hz, 2H, $CH_2Si$, 2 diastereomers as indicated by 2 sets of triplet), −0.05 (s, 9H, MeSi); $^{13}$C NMR $\delta$ 163.5, 162.7, 161.8, 155.3, 153.8, 140.1, 132.6, 109.5, 100.0, 97.6, 79.7 and 79.5 (C5a; 2 diastereomers), 72.2, 67.4, 39.5, 38.4 and 38.3 (2 diastereomers), 38.1, 36.7, 32.5 and 32.3 (2 diastereomers), 31.3, 28.3, 20.3, 17.9, 14.3 and 14.2 (2 diastereomers), −1.2. HRMS calcd for $C_{27}H_{38}N_5O_4Si^+$ ($M+H^+$) 524.2695, found 524.2292.

**4.1.15. (5a*S*,7*S*)- 7-[(1*R*) and (1*S*)-2-(*N*10-Adenyl)-1-methylethyl]-3-methyl-1*H*,7*H*-5a,6,8,9-tetrahydro-1-oxopyrano[4,3-*b*][1]benzopyran (7).** A solution of 4 mg (0.008 mmol) of SEM-adenine **19** in 1 mL each of 1 *N* HCl and methanol was heated at 50°C for 6 h and cooled to 25°C. The solution was neutralized with saturated aqueous $NaHCO_3$, extracted with ethyl acetate, and the organic layer was washed with brine, dried ($MgSO_4$), concentrated, and column chromatographed on silica gel column using a mixture of $CHCl_3$/MeOH (10:1) as eluant to give 3.0 mg (97% yield) of **7**. $^1$H NMR $\delta$ 8.44 (s, 1H), 8.00 (s, 1H), 6.3 (bs, 1H, NH), 6.08 (s, 1H, C4H), 5.72 (s, 1H, C10H), 5.05 (m, 1H, C5aH), 3.75 (m, 1H, CHN), 3.57 (m, 1H, CHN), 2.47 (d, $J$=14 Hz, 1H), 2.19 (s, 3H, Me), 2.10–1.2 (a serious of m, 7H), 1.01 (d, $J$=7 Hz, 3H, Me); $^{13}$C NMR $\delta$ 163.5, 162.8, 161.8, 152.8, 138.3 (2 C), 133.7, 132.6, 131.6, 109.5, 100.0, 97.6, 79.7 and 79.5 (2 isomers at C12), 39.5, 38.5, 38.3, 38.1, 36.7, 32.3, 31.3, 29.9, 28.4, 20.3, 14.3 and 14.2 (2 isomers). Anal. calcd for $C_{21}H_{23}N_5O_3$: C, 64.11; H, 5.89. Found: C, 64.35; H, 6.10.

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## References

1. For recent reviews: (a) Sambamurti, K.; Greig, N. H.; Debomoy, K. *Neuro Mol. Med.* **2002**, *1*, 1–31. (b) Carson, J. A.; Turner, A. J. *J. Neurochem.* **2002**, *81*, 1–8.
2. Omura, S.; Tomoda, H.; Kim, Y. K.; Nishida, H. *J. Antibiot.* **1993**, *46*, 1168–1169.
3. Omura, S.; Kuno, F.; Otoguro, K.; Sunazuka, T.; Shiomi, K.; Masuma, R.; Iwai, Y. *J. Antiobiot.* **1995**, *48*, 745–746.

4. Hua, D. H.; Chen, Y.; Sin, H.-S.; Maroto, M. J.; Robinson, P. D.; Newell, S. W.; Perchellet, E. M.; Ladesich, J. B.; Freeman, J. A.; Perchellet, J.-P.; Chiang, P. K. *J. Org. Chem.* **1997**, *62*, 6888–6896.
5. Jin, L.-W.; Hua, D. H.; Shie, F.-S.; Maezawa, I.; Sopher, B.; Martin, G. M. *J. Mol. Neurosci.* **2002**, *19*, 57–61.
6. Puglielli, L.; Konopka, G.; Pack-Chung, E.; Ingano, L. A. M.; Berezovska, O.; Hyman, B. T.; Chang, T. Y.; Tanzi, R. E.; Kovacs, D. M. *Nature Cell Biol.* **2001**, *3*, 905–912.
7. (a) Barbuch, R.; Curran, T. T.; Hay, D. A.; Vaz, R. J. *Tetrahedron* **1997**, *53*, 7181–7190. (b) Magnin, G. C.; Dauvergne, J.; Burger, A.; Biellman, J. F. *Tetrahedron Lett.* **1996**, *37*, 7833–7836.
8. Borcherding, D. R.; Scholtz, S. A.; Borchardt, R. T. *J. Org. Chem.* **1987**, *52*, 5457–5461.
9. Leonard, N. J.; Fujii, T. *J. Am. Chem. Soc.* **1963**, *85*, 3719.
10. Although compound **15** has been reported,[10a] spectral data is not available, moreover, the formation of N7-SEM derivative, the minor isomer, was not mentioned[10a] (a) Chida, N.; Suzuki, T.; Tanaka, S.; Yamada, I. *Tetrahedron Lett.* **1999**, *40*, 2573–2576. (b) Gundersoen, L.; Bakkestuen, A. K.; Aasen, A. J.; Overas, H.; Rise, F. *Tetrahedron* **1994**, *50*, 9743–9756.
11. For a review: Suckling, K. E.; Stange, E. F. *J. Lipid Res.* **1985**, *26*, 647–671.
12. For a review: Tall, A. R. *J. Lipid Res.* **1993**, *34*, 1255–1274.
13. Adenine containing carbocycles possess antitumor and antiviral activities: (a) Hayashi, M.; Yaginuma, S.; Muto, N.; Tsujino, N. *Nucleic Acid Res. Symp. Ser.* **1980**, *8*, s65–s67. (b) Yaginuma, S.; Muto, N.; Tsujino, M.; Sudata, Y.; Hayashi, M.; Otani, M. *J. Antibiot.* **1981**, *34*, 359–366. (c) De Clercq, E. *Antimicrob. Agents Chemother.* **1985**, *28*, 84–89.
14. For a review: Villa, P.; Kaufmann, S. H.; Earnshaw, W. C. *Trends Biochem. Sci.* **1997**, *22*, 388–393.
15. Sopher, B. L.; Fukuchi, K.; Smith, A. C.; Leppig, K. A.; Furlong, C. E.; Martin, G. M. *Brain Res. Mol. Brain Res.* **1994**, *26*, 207–217.
16. Perchellet, E. M.; Ladesich, J. B.; Magill, M. J.; Chen, Y.; Hua, D. H.; Perchellet, J. P. *Anti-Cancer Drugs* **1999**, *10*, 489–504.
17. Ukai, T.; Kawazura, H.; Ishii, Y.; Bonnet, J. J.; Ibers, J. A. *J. Organomet. Chem.* **1974**, *65*, 253–266.
18. Tomoda, H.; Nishida, H.; Masuma, R.; Cao, J.; Okuda, S.; Omura, S. *J. Antibiot.* **1991**, *44*, 136–143.
19. Tomoda, H.; Tabata, N.; Masuma, R.; Si, S.-Y.; Omura, S. *J. Antibiot.* **1998**, *51*, 618–623.