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Total synthesis of antimuscarinic alkaloid, (±)-TAN1251A

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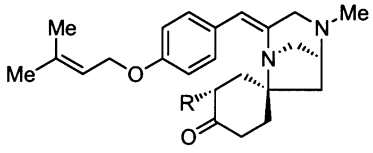
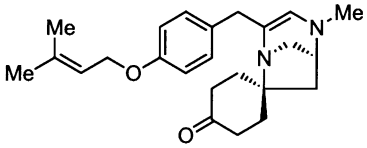
Abstract—The total synthesis of (±)-TAN1251A possessing an antimuscarinic activity was achieved. Carboxylic acid (**1**) was converted into carbamate (**3**) through a sequence of alkylation and Curtius rearrangement. After a few functional group interconversions of **3**, the corresponding amine (**9**) was converted into an azaspiro molecule (**18**) through cyclization and installation of a C_2 unit. Hydrolysis of **18** followed by lactam formation afforded tricyclic compound (**20**). Coupling of **20** with aldehyde (**22**) gave two epimeric adducts, (**23A**) and (**23B**), which were converted into TAN1251A by four steps.

1. Introduction

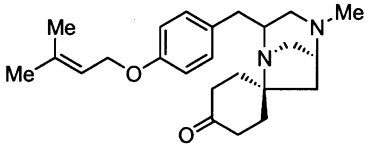
TAN1251 A, B, C and D are alkaloids isolated from a culture of *Penicillium thomii* RA-89.[1] These compounds show the inhibition activity toward a muscarinic acetylcholine receptor. In particular, the affinity of TAN1251B is stronger than that of atropine. TAN1251A shows selective inhibition toward the M_1 subtype of the muscarinic receptor. The structures involve unique tricyclic skeleton that consists of a 1,4-diazabicyclo[3.2.1]octane ring and a cyclohexanone ring through a spiro bond. In the case of TAN1251A and B, the skeleton is linked to a long side chain through a Z-double bond. The unique structure and characteristic biological property attracted the interest of organic chemist. This report describes full explanation for our total synthesis of racemic TAN1251A.[2,3]



TAN1251A (R = H)
TAN1251B (R = OH)



TAN1251C



TAN1251D

2. Results and discussion

Scheme 1 shows the retrosynthetic analysis of TAN1251A. The target molecule should be obtained by aldol reaction of tricyclic lactam (**20**) with aromatic aldehyde and subsequent functional group interconversions. The key intermediate **20** might be prepared from an azaspirocyclic molecule (**10**) via installation of an acetic acid unit. Disconnection of the pyrrolidine ring in **10** leads to carbamate (**3**). Miller et al. have reported the efficient conversion of cyclohexylcarboxylic acid into cyclohexylamine methylcarbamate through a sequence of alkylation and Curtius rearrangement.[4] Thus, we selected carboxylic acid (**1**) as a starting material.

Compound **1** was synthesized from *p*-hydroxybenzoic acid according to the reported procedure.[5] The first subject was the introduction of an alkyl chain and an amino group into the cyclohexanone ring. Treatment of **1** with two equivalents of lithium diisopropylamide (LDA) and allyl bromide gave the desired product (**2**) in high yield. Curtius rearrangement of **2** showed a satisfactory result in the case of using diphenylphosphoryl azide (DPPA). Treatment of **2** with DPPA in refluxing benzene followed by addition of benzyl alcohol gave urethane compound **3** in 65% yield.[4,6] Next, we carried out the construction of a pyrrolidine ring. Our initial

Keywords: TAN1251A; antimuscarinic activity; aldol reaction; Curtius rearrangement.

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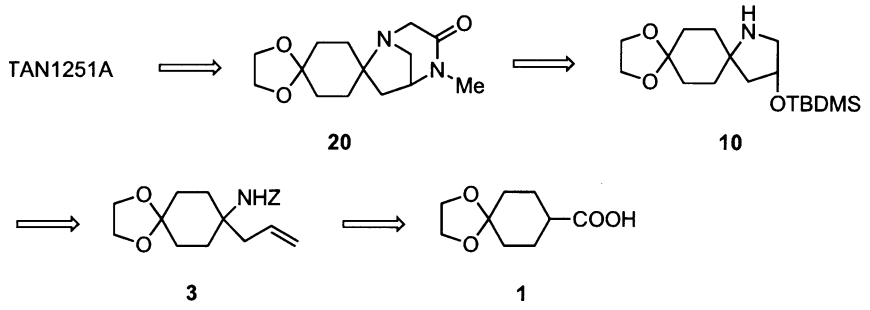
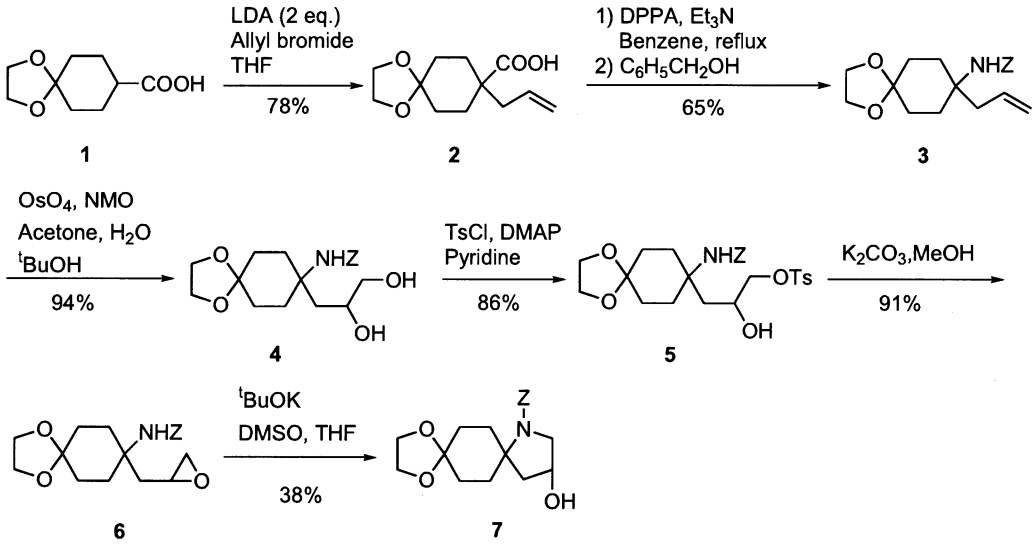


Scheme 1.

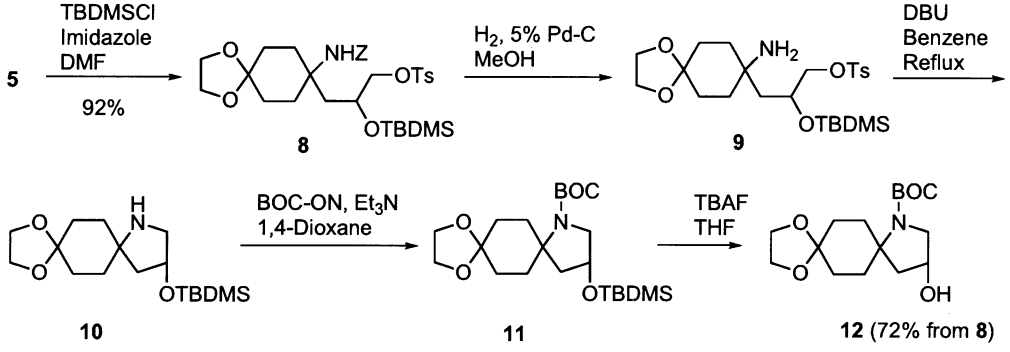


Scheme 2.

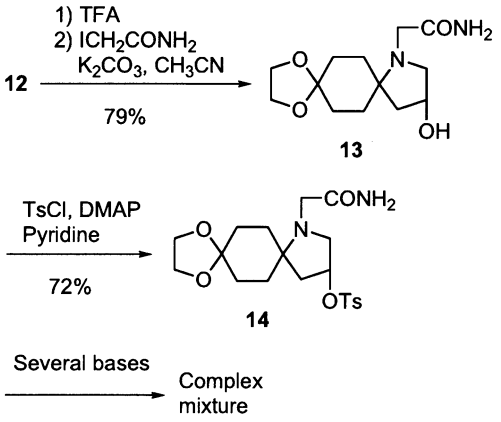
plan led us to select the ring formation of epoxide (**6**) as a key step. Treatment of **3** with a catalytic amount of osmium tetroxide and an equivalent of *N*-methylmorpholine *N*-oxide (NMO) as oxidants gave diol (**4**) in high yield. When **4** was subjected to tosylation by using one equivalent of *p*-toluenesulfonyl chloride (TsCl), primary alcohol was selectively tosylated to give **5** in 86% yield. Treatment of **5** with K_2CO_3 in methanol afforded the desired product **6** in 91% yield. The cyclization of **6** upon treatment with potassium *tert*-butoxide in THF and DMSO afforded cyclic compound (**7**) in 38% yield. The cyclization was carried out by other procedures, but there was no increase in yield (Scheme 2).

The difficulty in the cyclization might be attributed to the poor nucleophilicity of carbamate nitrogen and the disadvantageous cyclization mode in view of Baldwin's rule.[7] Thus, aminotosylate **9** was expected to be more suited for the construction of a pyrrolidine ring. Silylation of **5** afforded **8** in 92% yield. Catalytic hydrogenolysis of **8** proceeded smoothly to give **9**. Without purification, **9** was excellently cyclized by treatment with 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) in refluxing benzene. Cyclized compound **10** was further protected by a BOC group, and the resulting product was desilylated to give alcohol (**12**) in 72% yield from **8** (Scheme 3).

The most important step in our synthesis of TAN1251A was the construction of a piperazine ring. Our first attempt at

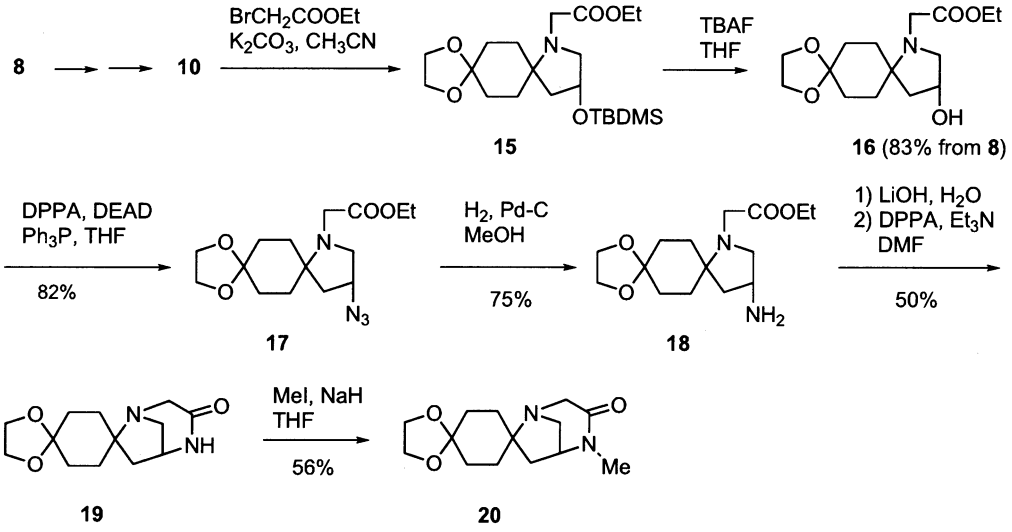


Scheme 3.



Scheme 4.

Aldol reaction of **20** with aldehyde (**22**)[10] was carried out by using LDA in THF at −78°C to form two adducts (**23A** (less polar)) (36%) and (**23B** (more polar)) (49%). These compounds were easily separated by column chromatography with silica gel. The ^{1}H NMR of **23A** showed a benzylic proton peak at 4.96 ppm (d, J=7.8 Hz) and two bridgehead methylene proton peaks at 3.12 ppm (d, J=12.5 Hz) and 2.76 ppm (d, J=12.5 Hz), while that of **23B** exhibited a benzylic proton peak at 4.77 ppm (d, J=7.3 Hz) and two bridgehead methylene proton peaks at 3.29 ppm (d, J=12.0 Hz) and 2.82 ppm (d, J=12.0 Hz). Their configurations at the α position of the amide group were assigned by observation of NOEs between the bridgehead proton peak and the benzylic proton peak. It was concluded that **23A** and **23B** were epimers at the bezylic position. The result demonstrated that **22** attacked enolate



Scheme 5.

constructing a piperazine ring was the cyclization of tosylate (**14**). Deprotection of **12** with trifluoroacetic acid (TFA) followed by alkylation with iodoacetamide under alkaline conditions gave an *N*-alkylated product (**13**). Treatment of **13** with TsCl provided **14** in 72% yield. Unfortunately, cyclization of **14** by treatment with several bases did not give any desired compound (Scheme 4).

The construction of a piperazine ring was successfully achieved by the following procedure. Without purification, **10** was alkylated with ethyl bromoacetate in the presence of potassium carbonate, and the resulting ester (**15**) was desilylated to give alcohol (**16**) in high yield. Treatment of **16** with DPPA[8] followed by hydrogenolysis provided amine (**18**). Hydrolysis of **18** with lithium hydroxide followed by cyclization with DPPA and Et_3N in DMF afforded the desired product (**19**) in 50% overall yield.[9] Treatment of **19** with methyl iodide in the presence of sodium hydride provided methylated compound (**20**) in 56% yield. The low yield of **20** would be attributed to competitive methylation of tertiary amine undertaking decomposition of the skeleton (Scheme 5).

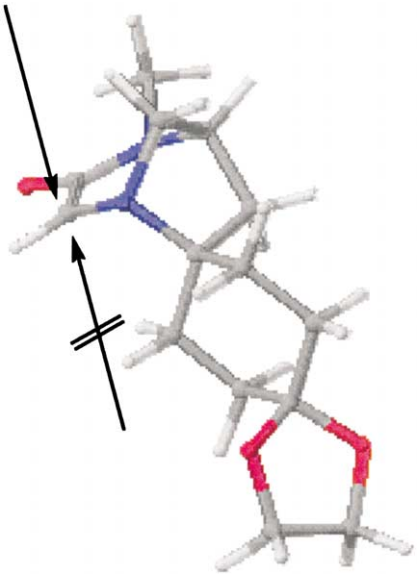


Figure 1. The optimized structure of the enolate anion **21**. Black, carbon; white, hydrogen; blue, nitrogen, red, oxygen.

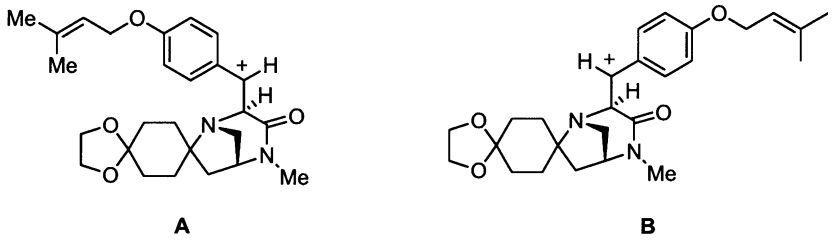
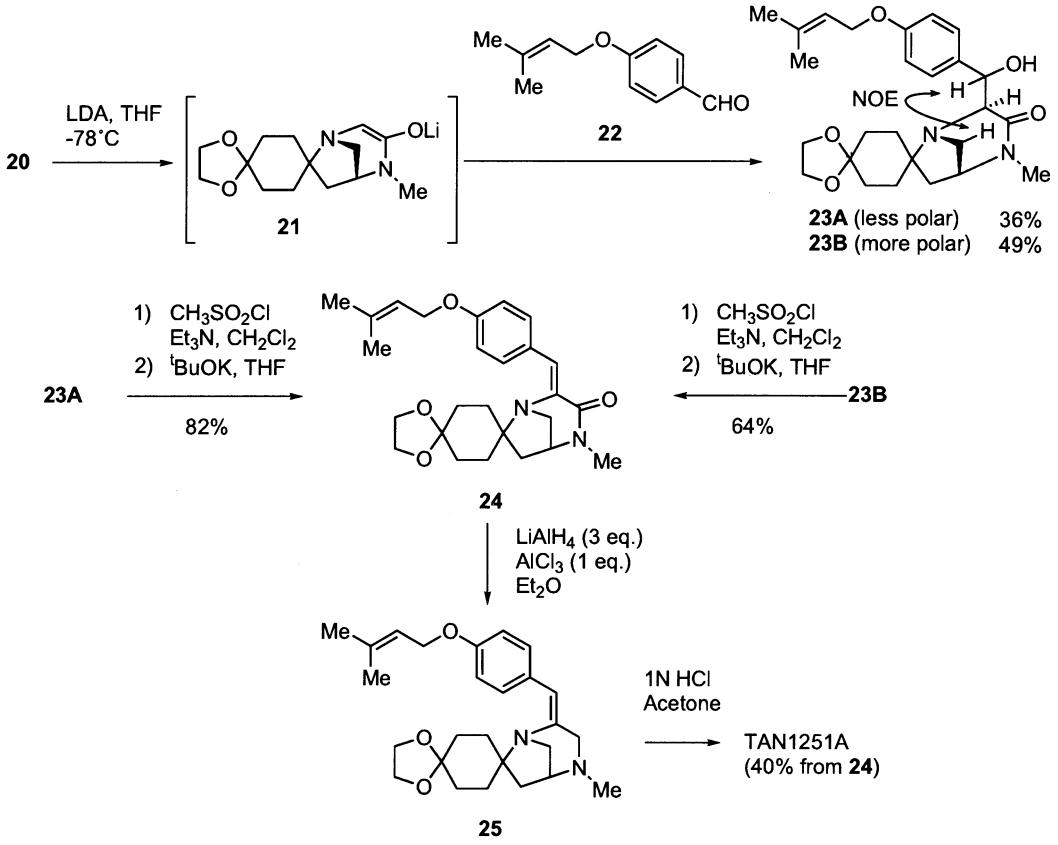


Figure 2. (A) Δ Heat of formation=0 kcal/mol; (B) Δ heat of formation=4.7 kcal/mol.



Scheme 6.

(**21**) from the β-face. The conformation of **21** was optimized by semi-empirical molecular orbital calculation using AM1 hamiltonian as shown in Fig. 1.[11] The stereoselectivity might be due to a shielding of the α-face of **21** by the cyclohexanone ring. The configuration at the benzylic position of the respective compounds was not determined. Interestingly, mesylation of **23A** and **23B** followed by treatment with ^{t}BuOK gave enone (**24**)[12] in 82 and 64% yield, respectively. The stereochemical result of these elimination should be due to the pass way including a benzylic cation (E1 elimination). Semi-empirical molecular orbital calculation using AM1 hamiltonian showed that cation **A** is more stable than cation **B** (Fig. 2).[11] Reduction of **24** with AlH_3 prepared from $LiAlH_4$ and $AlCl_3$ provided enamine (**25**),[13] which was deprotected by hydrolysis with 1N HCl in acetone to afford TAN-1251A in 40% yield from **24**. Spectroscopic data of the synthetic material were identical with those of the natural product (Scheme 6).[1]

3. Experimental

3.1. General methods

The melting points were determined on Yanagimoto micro melting point apparatus and were uncorrected. IR spectra were recorded on JASCO IRA-2 spectrometer. NMR spectra were recorded on a JEOL GX-270 spectrometer using tetramethylsilane as an internal standard. Chemical shifts are given in ppm. The following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad. MS spectra were measured on a Hitachi M-2000 spectrometer. Column chromatography was carried out on Merck's Silica gel 60 (70–230 mesh ASTM) or Wako's Florisil (100–200 mesh). All experiments except for dihydroxylation of **3** and hydrolyses of **18** and **25** were performed under anhydrous conditions under Ar atmosphere.

3.1.1. 8-(2′-Propenyl)-1,4-dioxaspiro[4.5]decane-8-carboxylic acid (2). To a solution of diisopropylamine (16.6 g, 164 mmol) in THF (150 ml) were successively added 1.63 M-BuLi in hexane (102 ml, 166 mmol) and a solution of **1** (12.4 g, 66.7 mmol) in THF (40 ml) at 0°C. The mixture was stirred for 1 h at room temperature. After the mixture was cooled to 0°C, allyl bromide (13.5 ml, 156 mmol) was added. The reaction mixture was further stirred for 2 h at room temperature, then quenched with saturated aqueous NH_4Cl, and extracted with AcOEt. The extract was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluted with AcOEt to give **2** (11.8 g, 52.2 mmol, 78%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.40–2.60 (8H, m), 2.32 (2H, d, J=7.4 Hz), 3.94 (4H, s), 5.02–5.14 (2H, m), 5.75 (1H, m). IR (neat) cm^{-1}: 3600–2400, 1700, 1640. EI-MS *m/z*: 226 (M^+), 211, 198, 184, 169, 157, 149, 139, 126. HR-MS *m/z*: calcd for $C_{12}H_{18}O_4$ 226.1204; found 226.1222.

3.1.2. 8-Phenylmethoxycarbonylamino-8-(2′-propenyl)-1,4-dioxaspiro[4.5]decane (3). A mixture of **2** (5.00 g, 22.1 mmol), triethylamine (7.7 ml, 55.3 mmol), diphenylphosphoryl azide (7.2 ml, 33.2 mmol) and benzene (70 ml) was refluxed for 30 min. After addition of benzyl alcohol (22.9 ml, 221 mmol) at room temperature, the reaction mixture was further refluxed for 5 h and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluted with hexane/AcOEt (5:1) to give **3** (4.72 g, 14.3 mmol, 65%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.58–1.71 (6H, m), 2.02–2.09 (2H, m), 2.48 (2H, d, J=7.4 Hz), 3.93 (4H, s), 4.53 (1H, s), 4.99–5.09 (4H, m), 5.67 (1H, ddt, J=16.8, 10.2, 7.4 Hz), 7.26–7.42 (5H, m). IR (neat) cm^{-1}: 3300, 1725, 1710, 1640. EI-MS *m/z*: 331 (M^+), 290, 246. HR-MS *m/z*: calcd for $C_{19}H_{25}NO_4$ 331.1782; found 331.1754.

3.1.3. 8-(2′,3′-Dihydroxypropyl)-8-phenylmethoxycarbonylamino-1,4-dioxaspiro[4.5]decane (4). To a solution of **3** (4.12 g, 12.4 mmol) in acetone (3.5 ml) and H_2O (7.5 ml) were successively added *N*-methylmorpholine *N*-oxide (2.05 g, 17.5 mmol) and 0.02 M solution of osmium tetroxide in *tert*-BuOH (3.2 ml, 0.064 mmol). The mixture was stirred at room temperature for 5 h, then quenched with saturated aqueous $NaHSO_3$ and extracted with AcOEt. The extract was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluted with AcOEt to give **4** (4.28 g, 11.7 mmol, 94%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.50–1.81 (10H, m), 2.09–2.18 (1H, m), 2.22–2.35 (1H, m), 3.39 (1H, dd, J=10.9, 7.9 Hz), 3.52 (1H, dd, J=10.9, 3.6 Hz), 3.82–3.94 (5H, m), 4.79 (1H, br s), 5.05 (s, 2 H), 7.31–7.38 (m, 5 H). ^{13}C NMR ($CDCl_3$) δ: 30.3 (t), 30.5 (t), 32.8 (t), 33.3 (t), 41 (t), 54 (s), 64.2 (t), 64.3 (t), 66 (t), 67 (t), 69 (d), 108 (s), 128.0 (t×2), 128.1 (t), 128.5 (t×2), 136 (s), 156 (s). IR ($CHCl_3$) cm^{-1}: 3400, 1710. EI-MS *m/z*: 365 (M^+), 101. HR-MS *m/z*: calcd for $C_{19}H_{27}NO_6$ 365.1837; found 365.1802.

3.1.4. 8-(2′,3′-Epoxypropyl)-8-phenylmethoxycarbonylamino-1,4-dioxaspiro[4.5]decane (6). To a solution of **4** (3.66 g, 10.0 mmol) in pyridine (22 ml) were successively added *p*-toluenesulfonyl chloride (2.30 g, 12.1 mmol) and 4-(*N*,*N*-dimethylamino)pyridine (122 mg, 1.00 mmol). The mixture was stirred at room temperature for 4 h, then quenched with saturated aqueous NaCl, and extracted with AcOEt. The extract was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluted with hexane/AcOEt (1:2) to give **5** (4.46 g, 8.59 mmol, 86%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.51–1.88 (8H, m), 2.07–2.24 (2H, m), 2.45 (3H, s), 2.55 (1H, br s), 3.81–4.10 (7H, m), 4.76 (1H, br s), 5.02 (2H, s), 7.28–7.39 (7H, m), 7.78 (2H, d, J=8.4 Hz). ^{13}C NMR ($CDCl_3$) δ: 21 (q), 30.3 (t), 30.4 (t), 32.7 (t), 33.3 (t), 41 (t), 53 (s), 64.2 (t), 64.3 (t), 66.3 (d), 66.4 (t), 74 (t), 102 (s), 128.0 (d×4), 128.1 (d), 128.5 (d×2), 130 (d×2), 133 (s), 136 (s), 145 (s), 155 (s). IR ($CHCl_3$) cm^{-1}: 3450, 1720, 1600. EI-MS *m/z*: 347 (M^+−TsOH), 101, 91. To a solution of **5** (11.1 g, 21.4 mmol) in MeOH (40 ml) was added K_2CO_3 (3.90 g). The mixture was stirred for 1 h at room temperature, then concentrated under reduced pressure, quenched with saturated aqueous NH_4Cl, and extracted with AcOEt. The extract was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluted with hexane/AcOEt (1:1) to give **6** (6.75 g, 19.5 mmol, 91%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.52–1.82 (8H, m), 2.07–2.33 (2H, m), 2.40 (1H, dd, J=5.1, 2.8 Hz), 2.70 (1H, dd, J=5.1, 4.1 Hz), 2.96 (1H, m), 3.94 (4H, s), 4.74 (1H, br s), 5.06 (2H, s), 7.28–7.42 (5H, m). IR (neat) cm^{-1}: 3350, 1720, 1705. HR-MS *m/z*: calcd for $C_{19}H_{25}NO_5$ 347.1731; found 347.1720.

3.1.5. Dispiro[1,3-dioxolane-2,1′-cyclohexane-4′,5″-3-hydroxy-1-phenylmethoxycarbonylpyrrolidine] (7). To a solution of potassium *tert*-butoxide (43.0 mg, 0.384 mmol) in THF (0.25 ml) and dimethylsulfoxide (0.25 ml) was added a solution of **6** (86.5 mg, 0.249 mmol) in THF (0.5 ml). The reaction mixture was stirred for 5.5 h at room temperature, then quenched with saturated aqueous NH_4Cl, and extracted with AcOEt. The extract was washed with saturated aqueous NaCl and dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on florisil eluted with hexane/AcOEt (1:1) to give **7** (33.0 mg, 0.095 mmol, 38%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.40–1.78 (8H, m), 1.90–2.06 (2H, m), 2.19 (1H, td, J=12.4, 4.4 Hz), 3.64 (1H, dd, J=12.2, 5.4 Hz), 3.80 (1H, dd, J=12.2, 3.0 Hz), 3.92–4.00 (4H, m), 4.33 (1H, m), 5.10 (2H, s), 7.28–7.42 (5H, m). IR (neat) cm^{-1}: 3350, 1670. EI-MS *m/z*: 347 (M^+), 101, 91. HR-MS *m/z*: calcd for $C_{19}H_{25}NO_5$ 347.1731; found 347.1718.

3.1.6. 8-(2′-*tert*-Butyldimethylsilyloxy-3′-tosyloxypropyl)-8-phenylmethoxycarbonylamino-1,4-dioxaspiro[4.5]decane (8). To a solution of **5** (1.59 g, 3.06 mmol) in DMF (1.5 ml) were successively added imidazole (417 mg, 6.13 mmol) and *tert*-butyldimethylsilyl chloride (553 mg, 3.67 mmol). The mixture was stirred at room temperature for 4.5 h, then quenched with saturated aqueous NaCl, and extracted with AcOEt. The extract was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluted with hexane/AcOEt (3:1) to give **8** (1.78 g, 2.81 mmol, 92%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 0.01 (3H, s), 0.02 (3H, s), 0.81 (9H, s), 1.58–1.63 (6H, m), 1.75 (1H, dd, J=14.8,

5.9 Hz), 2.02 (1H, dd, J=14.8, 5.1 Hz), 2.06–2.18 (2H, m), 2.43 (3H, s), 3.78–4.01 (7H, m), 4.67 (1H, br s), 4.99 (1H, d, J=11.9 Hz), 5.05 (1H, d, J=11.9 Hz), 7.25–7.37 (7H, m), 7.77 (2H, d, J=8.3 Hz). ^{13}C NMR ($CDCl_3$) δ: −4.6 (q), −4.3 (q), 18 (q), 22 (q), 26 (q×3), 30.35 (t), 30.43 (t), 32.4 (t), 32.9 (t), 42 (t), 53 (s), 64.2 (t), 64.3 (t), 66 (t), 68 (d), 74 (t), 108 (s), 128.0 (d×2), 128.1 (d×2), 128.3 (d), 128.5 (d×2), 130 (d×2), 136 (s), 145 (s), 154 (s). IR ($CHCl_3$) cm^{-1}: 3450, 1720, 1600. EI-MS m/z: 633 (M^+), 405, 229. HR-MS m/z: calcd for $C_{32}H_{47}NO_8SSi$ 633.2789; found 633.2787.

3.1.7. Dispiro[1-*tert*-butoxycarbonyl-3-hydroxypyrrolidine-5,1′-cyclohexane-4′,2″-1,3-dioxolane] (12). A mixture of **8** (3.00 g, 4.74 mmol), 5% Pd–C (70 mg) and MeOH (30 ml) was stirred under H_2 atmosphere for 3 h. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to give crude **9** (2.40 g). To a solution of **9** in benzene (25 ml) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (2.3 ml, 15.4 mmol). The mixture was refluxed for 3.5 h, then quenched with saturated aqueous $NaHCO_3$ and extracted with AcOEt. The extract was washed with saturated aqueous NaCl, dried over $MgSO_4$ and concentrated under reduced pressure to give crude **10** (1.66 g). To a solution of **10** in dioxane (10 ml) were successively added triethylamine (1.3 ml, 9.34 mmol) and 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile (2.30 g, 9.35 mmol). The reaction mixture was stirred at room temperature for 3.5 h, then quenched with saturated aqueous NaCl and extracted with AcOEt. The extract was dried over $MgSO_4$ and concentrated under reduced pressure to give crude **11** (4.25 g). After rough purification by column chromatography on silica gel, **11** was dissolved in THF. To the solution was added 1 M tetrabutylammonium fluoride in THF (4.8 ml, 4.8 mmol). The reaction mixture was stirred at room temperature for 15 h, then quenched with saturated aqueous NH_4Cl and extracted with AcOEt. The extract was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluted with AcOEt to give **12** (1.07 g, 3.42 mmol, 72% from **8**) as a white crystalline solid, mp 213–214°C (AcOEt). ^{1}H NMR ($CDCl_3$) δ: 1.22–1.80 (8H, m), 1.49 (9H, s), 2.09 (2H, m), 2.70 (1H, br s), 3.40–3.70 (2H, m), 3.93 (4H, s), 4.35 (1H, m). IR ($CHCl_3$) cm^{-1}: 1670. EI-MS m/z: 313 (M^+), 257, 101. HR-MS m/z: calcd for $C_{16}H_{27}NO_5$ 313.1887; found 313.1861.

3.1.8. Dispiro[1-aminocarbonylmethyl-3-hydroxypyrrolidine-5,1′-cyclohexane-4′,2″-1,3-dioxolane] (13). A mixture of **12** (96.0 mg, 0.307 mmol) and trifluoroacetic acid (0.36 ml) was stirred at 0°C for 30 min, and then concentrated under reduced pressure to give crude amine. To a solution of the amine crude in acetonitrile (2 ml) were successively added potassium carbonate (129 mg, 0.935 mmol) and iodoacetamide (86.0 mg, 0.465 mmol). The mixture was stirred at room temperature, then quenched with ethanol, filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluted with dichloromethane/ethanol/ammonia (10:1:1) to give **13** (65.5 mg, 0.243 mmol, 79%) as a white crystalline solid, mp 210–211°C (AcOEt). ^{1}H NMR ($CDCl_3$) δ: 1.28–1.84 (10H, m), 2.22 (1H, dd, J=13.7, 7.4 Hz), 2.98–3.07 (2H, m), 3.29 (1H, d, 17.0 Hz), 3.93 (4H, s), 4.41 (1H, m), 5.45 (1H, br s), 7.19 (1H, br s). IR ($CHCl_3$) cm^{-1}: 1680. EI-MS m/z: 270 (M^+), 226. HR-MS m/z: calcd for $C_{13}H_{22}N_2O_4$ 270.1577; found 270.1567.

3.1.9. Dispiro[1-aminocarbonylmethyl-3-tosyloxypyrrolidine-5,1′-cyclohexane-4′,2″-1,3-dioxolane] (14). To a solution of **13** (50.0 mg. 0.185 mmol) in pyridine (1 ml) were successively added tosyl chloride (353 mg, 1.85 mmol) and dimethylaminopyridine (22.6 mg, 0.185 mmol). The reaction mixture was stirred at room temperature for 4.5 h, and then quenched with saturated aqueous NaCl and extracted with AcOEt. The extract was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluted with hexane/AcOEt (1:1) to give **14** (56.3 mg, 0.133 mmol, 72%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.25–1.62 (6H, m), 1.64–1.80 (4H, m), 2.08 (2H, m), 2.46 (3H, s), 3.04 (1H, dd, J=10.9, 3.1 Hz), 3.24 (1H, dd, J=10.9, 5.9 Hz), 3.53 (1H, d, J=17.5 Hz), 3.61 (1H, d, J=17.5 Hz), 3.92 (4H, s), 5.01 (1H, m), 7.36 (2H, d, J=8.5 Hz), 7.79 (2H, d, J=8.5 Hz). EI-MS m/z: 406 (M^+-H_2O). HR-MS m/z: calcd for $C_{20}H_{26}N_2O_5S$ 406.1561; found 406.1594.

3.1.10. Dispiro[1,3-dioxolane-2,1′-cyclohexane-4′,5″-1-ethoxycarbonylmethyl-3-hydroxypyrrolidine] (16). Crude amine **10** (1.32 g) was prepared from **8** (2.45 g, 3.87 mmol) according to the already described method. To a solution of **10** in acetonitrile (20 ml) were successively added potassium carbonate (1.61 g, 11.7 mmol)) and ethyl bromoacetate (1.3 ml, 11.8 mmol). The reaction mixture was stirred at room temperature for 2.5 h, then quenched with saturated aqueous NaCl, extracted with AcOEt. The extract was dried over $MgSO_4$ and concentrated under reduced pressure to give crude product **15** (1.98 g). To a solution of **15** in THF (1 ml) was added 1 M tetrabutylammonium fluoride in THF (5.8 ml, 5.80 mmol). The reaction mixture was stirred at room temperature for 15 h, and then quenched with saturated aqueous NH_4Cl. To the mixture was added potassium sodium (+)-tartrate tetrahydrate (Rochelle salt). The mixture was extracted with AcOEt. The extract was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluted with AcOEt to give **16** (964 mg, 3.22 mmol, 83% from **8**) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.27 (3H, t, J=7.1 Hz), 1.52–1.88 (9H, m), 2.08 (1H, dd, J=14.0, 6.9 Hz), 2.36 (1H, br s), 2.94–2.99 (1H, m), 3.10 (1H, dd, J=9.7, 5.0 Hz), 3.27 (1H, d, J=16.8 Hz), 3.45 (1H, d, J=16.8 Hz), 3.93 (4H, s), 4.16 (2H, q, J=7.1 Hz), 4.30 (1H, m). ^{13}C NMR ($CDCl_3$) δ 14 (q), 29 (t), 31.7 (t), 32.2 (t), 32.8 (t), 44 (t), 49 (t), 60 (t), 61 (t), 62 (s), 64.1 (t), 64.3 (t), 70 (d), 108 (s), 172 (s). IR (neat) cm^{-1}: 3400, 1730. EI-MS m/z: 299 (M^+), 256, 198. HR-MS m/z: calcd for $C_{15}H_{25}NO_5$ 299.1731; found 299.1761.

3.1.11. Dispiro[3-azide-1-ethoxycarbonylmethylpyrrolidine-5,1′-cyclohexane-4′,2″-1,3-dioxolane] (17). To a solution of **16** (347 mg, 1.16 mmol) in THF (4 ml) were successively added triphenylphosphine (456 mg, 1.74 mmol), diethyl azodicarboxylate (0.27 ml, 1.74 mmol) and diphenylphosphoryl azide (0.37 ml, 1.74 mmol). The

reaction mixture was stirred at room temperature for 2 h, and then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluted with hexane/AcOEt (3:1) to give **17** (308 mg, 0.951 mmol, 82%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.26 (3H, t, J=7.1 Hz), 1.54–1.79 (8H, m), 1.90 (1H, dd, J=13.7, 4.0 Hz), 2.16 (1H, dd, J=13.7, 8.4 Hz), 2.95 (1H, dd, J=10.1, 4.5 Hz), 3.27–3.40 (3H, m), 3.93 (4H, s), 4.03 (1H, m), 4.16 (2H, q, J=7.1 Hz). ^{13}C NMR ($CDCl_3$) δ: 14 (q), 29.5 (t), 30.2 (t), 33 (t×2), 40 (t), 49 (t), 57 (t), 58 (d), 61 (t), 63 (s), 64.2 (t), 64.3 (t), 108 (s), 171 (s). IR (neat) cm^{-1}: 2100, 1740. EI-MS m/z: 324 (M^+), 251, 101. HR-MS m/z: calcd for $C_{15}H_{24}N_4O_4$ 324.1769; found 324.1777.

3.1.12. Dispiro[3-amino-1-ethoxycarbonylmethylpyrrolidine-5,1′-cyclohexane-4′,2″-1,3-dioxolane] (18). A mixture of **17** (166 mg, 0.512 mmol), 5% Pd–C (trace amount) and MeOH (2 ml) was stirred under H_2 atmosphere for 1.5 h. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluted with chloroform which was saturated by ammonia/methanol (9:1) to give **18** (114 mg, 0.383 mmol, 75%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ: 1.26 (3H, t, J=7.1 Hz), 1.31–1.79 (11H, m), 2.23 (1H, dd, J=13.2, 8.2 Hz), 2.76 (1H, dd, J=9.1, 4.6 Hz), 3.09 (1H, dd, J=9.1, 6.6 Hz), 3.23 (1H, d, J=16.7 Hz), 3.40 (1H, d, J=16.7 Hz), 3.45–3.54 (1H, m), 3.93 (4H, m), 4.16 (2H, q, J=7.1 Hz). ^{13}C NMR ($CDCl_3$) δ: 14 (q), 29 (t), 31.7 (t), 32.4 (t), 32.6 (t), 45 (t), 49.0 (d), 49.5 (t), 60.4 (t), 60.9 (t), 63 (s), 64.1 (t), 64.2 (t), 108 (s), 172 (s). IR (neat) cm^{-1}: 3350, 1740. EI-MS m/z: 298 (M^+), 225, 197. HR-MS m/z: calcd for $C_{15}H_{26}N_2O_4$ 298.1891; found 298.1917.

3.1.13. Dispiro[1,4-diazabicyclo[3.2.1]octan-3-one-7,1′-cyclohexane-4′,2″-1,3-dioxolane] (19). To a solution of **18** (552 mg, 1.85 mmol) in H_2O (7 ml) was added lithium hydroxide (66.7 mg, 2.78 mmol). The reaction mixture was stirred at room temperature for 40 min, and then concentrated under reduced pressure to give crude amino acid (625 mg). To a solution of the crude in dimethylformamide (40 ml) was added triethylamine (0.77 ml, 5.55 mmol). After being stirred for 10 min, diphenylphosphoryl azide (1.2 ml, 5.55 mmol) was added and the resulting reaction mixture was further stirred at room temperature for 1.5 h, and then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluted with chloroform/methanol (30:1), which was saturated by ammonia, to give **19** (234 mg, 0.929 mmol, 50%) as a white crystalline solid, mp 182–183.5°C (AcOEt–benzene). ^{1}H NMR ($CDCl_3$) δ: 1.53–1.96 (10H, m), 3.05 (1H, d, J=12.2 Hz), 3.18 (1H, dd, J=12.2, 2.5 Hz), 3.51 (1H, d, J=18.8 Hz), 3.61 (1H, d, J=18.8 Hz), 3.79 (1H, br s), 3.95 (4H, s), 7.12 (1H, br s). ^{13}C NMR ($CDCl_3$) δ: 31.8 (t), 32.6 (t), 32.8 (t), 36 (t), 48 (t), 53 (d), 55 (t), 56 (t), 64.1 (s), 64.22 (t), 64.26 (t), 108 (s), 171 (s). IR ($CHCl_3$) cm^{-1}: 3250, 1660. EI-MS m/z: 252 (M^+), 166, 83. HR-MS m/z: calcd for $C_{13}H_{20}N_2O_3$ 252.1473; found 252.1472. Anal. calcd for $C_{13}H_{20}N_2O_3$: C 61.88, H 7.99, N 11.10. Found: C 62.07, H 8.08, N 11.14.

3.1.14. Dispiro[1,3-dioxolane-2,1′-cyclohexane-4′,7″-4-methyl-1,4-diazabicyclo[3.2.1]octan-3-one] (20). To a solution of 55% sodium hydride (35 mg, 0.802 mmol) in THF (4 ml) were successively added a solution of **19** (142 mg, 0.563 mmol) in THF (2 ml) and iodomethane (0.08 ml, 1.3 mmol) at 0°C. The reaction mixture was further stirred at room temperature for 1.5 h, then quenched with H_2O and extracted with $CHCl_3$. The extract was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluted with chloroform/methanol (30:1), which was saturated by ammonia, to give **20** (84.1 mg, 0.316 mmol, 56%) as white crystalline solid, mp 128–130°C (AcOEt–hexane). ^{1}H NMR ($CDCl_3$) δ: 1.49–1.93 (10H, m), 2.90 (3H, s), 3.05 (1H, d, J=12.2 Hz), 3.24 (1H, dd, J=12.2, 2.6 Hz), 3.48 (1H, d, J=18.8 Hz), 3.59 (1H, d, J=18.8 Hz), 3.60–3.63 (1H, m), 3.94 (4H, s). ^{13}C NMR ($CDCl_3$) δ: 31.8 (t), 32.5 (t), 32.7 (t), 33.3 (q), 36 (t), 45 (t), 55 (t), 56 (t), 60 (d), 64.1 (s), 64.20 (t), 64.24 (t), 108 (s), 168 (s). IR ($CHCl_3$) cm^{-1}: 1630. EI-MS m/z: 266 (M^+), 235, 113. HR-MS m/z: calcd for $C_{14}H_{22}N_2O_3$ 266.1629; found 266.1634. Anal. calcd for $C_{14}H_{22}N_2O_3$: C 63.14, H 8.33, N 10.52. Found: C 63.39, H 8.38, N 10.45.

3.1.15. Aldol reaction of 20 with 22. To a solution of **20** (102 mg, 0.383 mmol) in THF (5 ml) was added dropwise 0.2 M solution of lithium diisopropylamide in THF (3.8 ml, 0.760 mmol). After being further stirred at −78°C for 20 min, a solution of **22** (144 mg, 0.758 mmol) in THF (2 ml) was added dropwise to the reaction mixture. After being further stirred at −78°C for 20 min, the mixture was quenched with saturated aqueous NaCl and extracted with $CHCl_3$. The extract was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on florisil eluted with hexane/AcOEt (3:1) to give **23A** (less polar, 62.9 mg, 0.138 mmol, 36%) and **23B** (more polar, 85.7 mg, 0.188 mmol, 49%).

23A: Mp 149–150°C (AcOEt–hexane). ^{1}H NMR ($CDCl_3$) δ: 1.23–1.86 (10H, m), 1.74 (3H, s), 1.79 (3H, s), 2.77 (1H, d, J=12.7 Hz), 2.93 (3H, s), 3.13 (1H, d, J=12.7 Hz), 3.42 (1H, d, J=8.1 Hz), 3.56 (1H, m), 3.83 (4H, s), 4.51 (2H, d, J=6.6 Hz), 4.96 (1H, d, J=8.1 Hz), 5.24 (1H, br s, OH), 5.51 (1H, m), 6.88 (2H, d, J=8.8 Hz), 7.32 (2H, d, J=8.8 Hz). ^{13}C NMR ($CDCl_3$) δ 18 (q), 26 (q), 31.5 (t), 31.8 (t), 32.3 (t), 33.5 (q), 36.0 (t), 47 (t), 52 (t), 60 (d), 64.0 (t), 64.1 (t), 64.4 (s), 64.9 (t), 66 (d), 74 (d), 108 (s), 114.1 (d), 114.3 (d), 120 (d), 128 (d×2), 134 (s), 138 (s), 158 (s), 170 (s). IR ($CHCl_3$) cm^{-1}: 3300, 1610. EI-MS m/z: 457 (M^++1), 266. HR-MS m/z: calcd for $C_{26}H_{36}N_2O_5$+H 457.2622; found 457.2691. Anal. calcd for $C_{26}H_{36}N_2O_5$: C 68.40, H 7.95, N 6.14. Found: C 68.38, H 8.14, N 6.06.

23B: Mp 152–153°C (AcOEt–hexane). ^{1}H NMR ($CDCl_3$) δ: 1.50–1.94 (10H, m), 1.73 (3H, s), 1.79 (3H, s), 2.84 (1H, d, J=12.5 Hz), 2.86 (3H, s), 3.28 (1H, dd, J=12.5, 1.7 Hz), 3.57–3.60 (2H, m), 3.94 (4H, s), 4.49 (2H, d, J=6.6 Hz), 4.76 (1H, d, J=7.6 Hz), 4.97 (1H, br s), 5.50 (1H, m), 6.89 (2H, d, J=8.6 Hz), 7.29 (2H, d, J=8.6 Hz). ^{13}C NMR ($CDCl_3$) δ: 18 (q), 26 (q), 32.1 (t), 32.39 (t), 32.42 (t), 33.4 (q), 36.0 (t), 45 (t), 52 (t), 60 (d), 64.26 (t), 64.34 (t), 64.45 (s), 64.7 (t), 66 (d), 74 (d), 108 (s), 114 (d×2), 120 (d), 129 (d×2), 134 (s), 138 (s), 159 (s), 168 (s). IR ($CHCl_3$) cm^{-1}: 3350, 1640, 1610. EI-MS m/z: 457 (M^++1), 266. HR-MS m/z: calcd for $C_{26}H_{36}N_2O_5$+H

457.2622; found 457.2720. Anal. calcd for $C_{26}H_{36}N_2O_5$: C 68.40, H 7.95, N 6.14. Found: C 68.15, H 8.10, N 6.04.

3.1.16. Dehydration of 23A and 23B. To a solution of **23A** (17.9 mg, 39.3 μmol) in dichloromethane (1 ml) were successively added triethylamine (0.055 ml, 0.392 mmol) and methanesulfonyl chloride (0.018 ml, 0.233 mmol) at −78°C. After being further stirred at 0°C for 1 h, the reaction was quenched with saturated aqueous NaCl and extracted with $CHCl_3$. The extract was dried over $MgSO_4$ and concentrated under reduced pressure. To a solution of the crude (43.6 mg) compound in THF (1 ml) was added potassium *tert*-butoxide (26 mg, 0.232 mmol) at 0°C. After being further stirred at 0°C for 1 h, the mixture was quenched with saturated aqueous NaCl and extracted with $CHCl_3$. The extract was dried over $MgSO_4$ and concentrated under reduced pressure. Recrystallization of the residue from AcOEt–hexane gave **24** (14.1 mg, 32.2 μmol, 82%) as white crystalline solid.

To a solution of **23B** (20.6 mg, 45.2 μmol) in dichloromethane (1 ml) were successively added triethylamine (0.066 ml, 0.477 mmol) and methanesulfonyl chloride (0.02 ml, 0.259 mmol) at −78°C. After being further stirred at 0°C for 3 h, the reaction was quenched with saturated aqueous NaCl and extracted with $CHCl_3$. The extract was dried over $MgSO_4$ and concentrated under reduced pressure. To a solution of the residue (27 mg) in THF (1 ml) was added potassium *tert*-butoxide (30 mg, 0.268 mmol) at 0°C. After being further stirred at 0°C for 30 min, the mixture was quenched with saturated aqueous NaCl and extracted with $CHCl_3$. The extract was dried over $MgSO_4$ and concentrated under reduced pressure. Recrystallization of the residue from AcOEt–hexane gave **24** (12.6 mg, 28.8 μmol, 64%) as white crystalline solid.

Mp 217–219°C (AcOEt–hexane). ^{1}H NMR ($CDCl_3$) δ: 1.40–2.07 (10H, m), 1.75 (3H, s), 1.80 (3H, s), 3.01 (3H, s), 3.12 (1H, dd, J=12.2, 2.2 Hz), 3.29 (1H, dd, J=12.2, 2.0 Hz), 3.72 (1H, m), 3.77–3.89 (4H, m), 4.54 (2H, d, J=6.6 Hz), 5.50 (1H, m), 6.90 (2H, d, J=9.0 Hz), 7.36 (1H, s), 8.08 (2H, d, J=9.0 Hz). ^{13}C NMR ($CDCl_3$) δ: 18 (q), 26 (q), 30 (t), 32.0 (t), 32.4 (t), 32.6 (t), 34 (q), 36 (t), 58 (t), 61 (d), 64.2 (t), 64.3 (t), 64.7 (t), 66 (s), 74 (d), 108 (s), 114 (d×2), 120 (d), 128 (s), 129 (d), 133 (d×2), 137.6 (s), 138.2 (s), 159 (s), 163 (s). IR ($CHCl_3$) cm^{-1}: 1650, 1600. EI-MS *m/z*: 438 (M^+), 370, 216. HR-MS *m/z*: calcd for $C_{26}H_{34}N_2O_4$ 438.2517; found 438.2487. Anal. calcd for $C_{26}H_{34}N_2O_4$: C 71.21, H 7.81, N 6.39. Found: C 71.23, H 7.98, N 6.34.

3.1.17. Preparation of TAN1251A. To a mixture of lithium aluminium hydride (10.0 mg, 263 μmol) and Et_2O (0.5 ml) was added aluminium chloride (12.0 mg, 89.9 μmol) and resulting mixture was further stirred at 0°C for 10 min. To the reaction mixture was added a solution of **24** (10.4 mg, 23.7 μmol) in Et_2O (0.5 ml). After being stirred at room temperature for 1 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with $CHCl_3$. The extract was dried over $MgSO_4$ and concentrated under reduced pressure to give crude **25** (5.2 mg). To a solution of the crude **25** in acetone (0.3 ml) was added 1N HCl (0.1 ml). The whole was further stirred at room temperature for 2 h, and then quenched with saturated aqueous $NaHCO_3$ and extracted with $CHCl_3$. The extract was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluted with chloroform/methanol (30:1), which was saturated by ammonia, to give TAN1251A (3.6 mg, 9.47 μmol, 40% from **24**) as a pale yellow oil. ^{1}H NMR ($CDCl_3$) δ: 1.48 (1H, dd, J=13.9, 5.6 Hz), 3.29 (1H, dd), 1.74 (3H, s), 1.79 (3H, s), 1.83–2.19 (8H, m), 2.19 (3H, s), 2.77 (1H, m), 2.92–3.04 (2H, m), 3.25–3.34 (3H, m), 4.47 (2H, d, J=6.8 Hz), 5.49 (1H, m), 6.02 (1H, br s), 6.80 (2H, d, J=8.8 Hz), 7.76 (2H, d, J=8.8 Hz). ^{13}C NMR ($CDCl_3$) δ: 18.17 (q), 25.79 (q), 32.18 (t), 34.48 (t), 38.20 (t), 38.54 (t), 38.61 (t), 42.51 (q), 55.72 (t), 58.53 (t), 61.15 (d), 64.01 (s), 64.62 (t), 114.03 (d×2), 119.65 (d), 122.82 (d), 128.53 (s), 130.69 (d×2), 138.12 (s), 141.59 (s), 157.83 (s), 211.97 (s). IR ($CHCl_3$) cm^{-1}: 1700, 1600. EI-MS *m/z*: 380 (M^+), 270, 201. HR-MS *m/z*: calcd for $C_{24}H_{32}N_2O_2$ 380.2462; found 380.2457. These spectroscopic data were identical with those of the natural product.

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