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A practical semi-synthesis of tautomycin using a hydrolysate of natural tautomycin

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Abstract—A useful and brief semi-synthesis of tautomycin, which can be supplied a large quantity, has been achieved through the coupling reaction of the C21–C26 segment (an epoxide) with the C1–C20 segment (a dithiane), which was derived from the degradation of natural tautomycin, toward the conformational analysis of the C21–C26 moiety and the exploration of structure–activity relationships.

1. Introduction

Tautomycin (**1**) was isolated from *Streptomyces spiroverticillatus* in 1987 by Isono and co-workers as an antibiotics with strong antifungal activity against *Sclerotinia sclerotiorum*,[1] and **1** shows a specific inhibitor of protein phosphatase 1 and 2A.[2] It has been reported that PP1 or PP2A were inhibited by natural products such as okadaic acid, calyculin, and microcystin-LR.[3] These three compounds inhibit PP2A much more strongly than PP1, on the other hand, tautomycin **1** inhibits PP1 more selectively than PP2A.[3]These activities promoted the synthetic chemists, and four groups have reported the four different total syntheses of tautomycin including this research group in 1995.[4–10] Structure–activity relationship was reported from various sources with natural and synthetic derivatives.[5–8] We have been studying the structural relationship from view point of the molecular shape of **1** and the protein-phosphatase inhibitory activity. Initially **1** was regarded as a tautomeric compound and named accordingly,[1] later it became apparent that **1** exists in two forms in equilibrium between acid anhydride (**1a**) and diacid forms (**1b**).[4] In 1996, we separated the 2 forms **1a** and **1b** by HPLC and proved that **1a** did not show any inhibitory activity and that only diacid **1b** was truly active.[6j] Some other groups confirmed the importance of the anhydride moiety.[8b,11] Furthermore, we recently showed the importance of the hydrophobic spiroketal moiety of tautomycin in selective inhibition of PP; okadaic acid–tautomycin hybrid compound, which have right-end segment of okadaic acid (C28–C38 spiroketal structure) and left-hand segment of tautomycin (C17–C7′) resulting in PP2A selective inhibitory activity.[6k] We assumed that the conformation of C21–C26 moiety of **1** might bear key issues of the inhibitory activity. In order to confirm this issue, we have to prepare several compounds labeled and/or analogous to tautomycin. Total synthesis approach would not always suitable for such purposes, even though we have already accomplished the total synthesis.[6e,i] During the course of structural studies of tautomycin, Ubukata et al. reported that an unsaturated ketone **3** resulted as alkaline hydrolysate of **1** under weakly basic conditions (MeOH, 20% Cs_2CO_3, pH 9).[4] Because of the fact that tautomycin is a fermentation product, we already utilized this hydrolysate **3** for the semi-syntheses of several unnatural tautomycin analogs, which were all inactive.[6j] In order to prepare the labeled tautomycin itself, we searched for an alternative and practical semi-synthetic route starting from **3**. We have recently prepared the 100%-^{13}C-labeled tautomycin at the 18, 19, 21, and 22-positions for the purpose of elucidation of the dynamics and conformation of C17–C26 moiety through total synthesis pathway (Tsuboi and Isobe et al., unpublished result). This method would help us to prepare the required tautomycin analogs as well for modification of the maleic segment. Here we have established a practical synthetic pathway to tautomycin from the enone **3** along this line (Fig. 1).

2. Synthesis of C1–C20 segment

Tautomycin (**1**) was purified with an ODS column by a modified procedure (see Section 5) of the method described previously.[1] Treatment of **1** with dil. cesium carbonate (Cs_2CO_3) in MeOH at pH 9 provided the degradation product **3**.[4] We proposed this reaction mechanism to include

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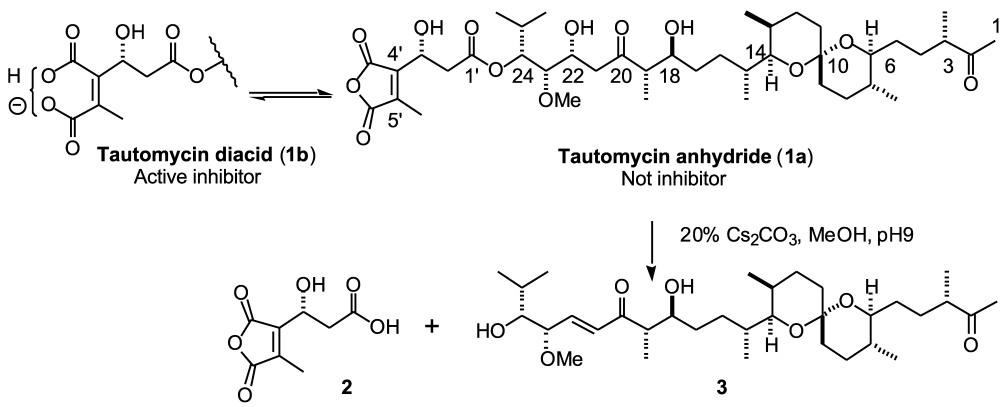
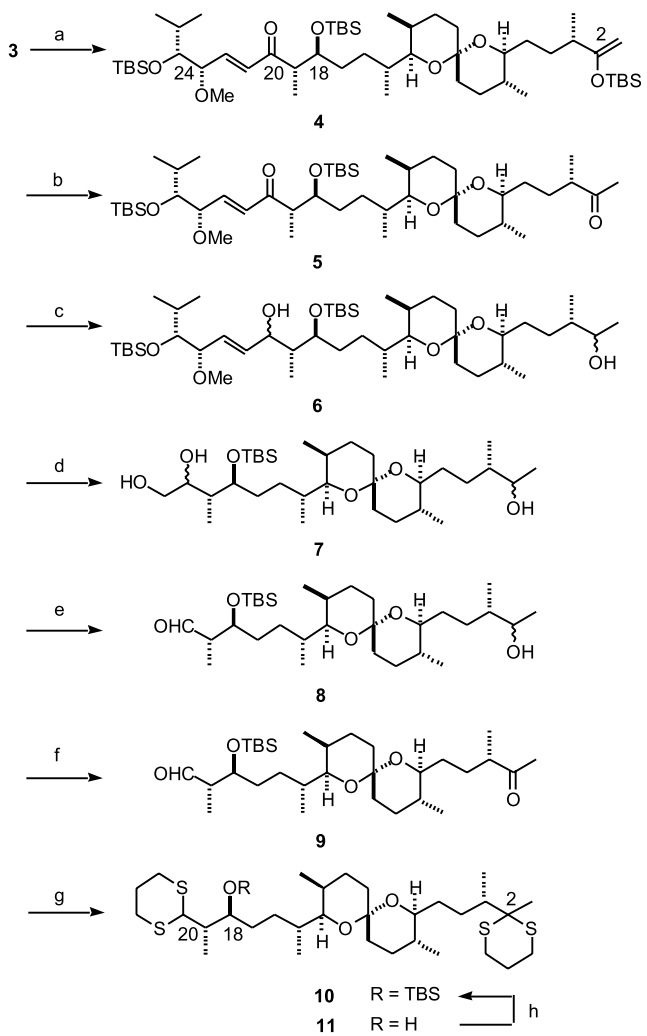


Figure 1.

trans-esterification and concomitant elimination to give the enone **3**.[6j] The synthesis of converting it into segment C1–20 (compound **10**) is summarized in Scheme 1. The protection of the hydroxyl group of **3** by treatment with

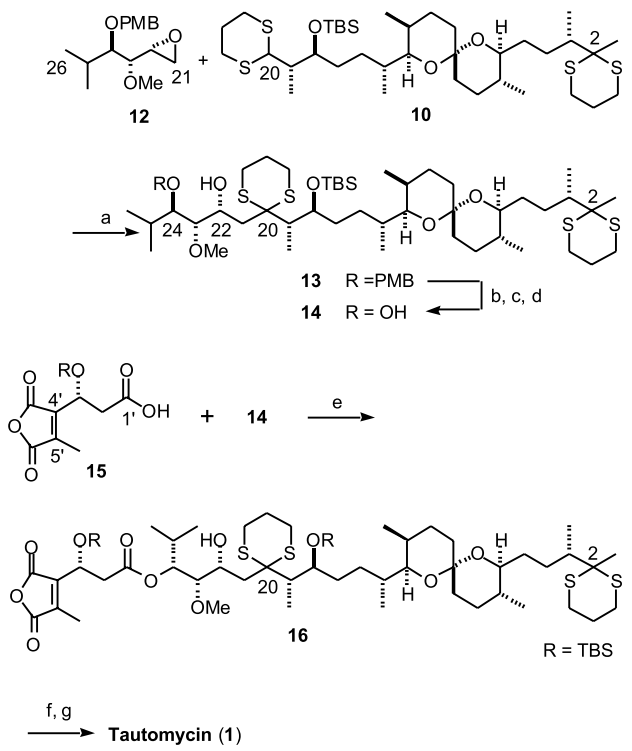


Scheme 1. *Reagents and conditions*: (a) TBSOTf, 2,6-lutidine, −10°C, 10 min, then room temperature, 40 min; (b) AcOH–THF–H_2O=3:1:1, room temperature, 12 h, 90% (2 steps); (c) $NaBH_4$, MeOH, 0°C, 30 min, 85%; (d) O_3/CH_2Cl_2, −78°C, 20 min, then $NaBH_4$/MeOH, −78°C to room temperature, 76%; (e) $NaIO_4$, THF–H_2O=4:1, 0°C, 30 min, then room temperature, 30 min, quant.; (f) PCC, MS 4 Å, room temperature, 1.5 h, 83%; (g) $HS(CH_2)_3SH/BF_3{\cdot}OEt_2$, 12 mM, 0°C, 1 h, then room temperature, 24 h, 59%, **11**, 38%; (h) TBSOTf, 2,6-lutidine, 0°C, 30 min, 99%.

t-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) and 2,6-lutidine gave the silyl vinyl ether **4**, which was successively treated with dil. acetic acid (AcOH) in THF at room temperature for 12 h. Under these conditions, the silyl vinyl ether moiety was selectively cleaved to give **5**. Reduction of **5** with sodium borohydride ($NaBH_4$) in MeOH led to exclusive 1,2-reduction to give a diastereomeric mixture (C2 and C20) of the allylic alcohol **6**. We performed oxidative cleavage of the allylic alcohol under Lemieux–Johnson condition.[12] No oxidative cleavage, however, was observed under various conditions. Dihydroxylation with osmium tetroxide (OsO_4) did not occur under a condition using 4-(dimethylamino)pyridine (DMAP) (used for the purpose of in situ generation of more reactive OsO_4-DMAP).[13] It may be due to strong steric congestion around the olefin. Fortunately, treatment of the allylic alcohol with ozone worked well in CH_2Cl_2 at −78°C, which was followed by reductive cleavage of the resulting ozonide with $NaBH_4$ to provide an diastereomeric mixture of the triol **7** in 76% yield. The 1,2-glycol moiety of **7** was cleaved with sodium metaperiodate ($NaIO_4$), which was successively treated with pyridinium chlorochromate (PCC) in the presence of molecular sieves 4 Å to furnish the C1–C20 segment. Thioketalization at the C20 and C2 positions furnished the protected C1–C20 segment **10** in 59% yield. No epimerization occurred at the C3 stereogenic center by employing the similar conditions as our previous synthesis of tautomycin.[6i] Since TBS group at C18 was partly deprotected, the resulting alcohol **11** was re-protected as a TBS ether **10** in 99% yield.

3. Semi-synthesis of tautomycin

The segment coupling is summarized in Scheme 2. The coupling reaction between the anion of dithiane **10** and the epoxide **12** was the key element of our synthetic program, and it had previously been defined as Segment B1 in our total synthesis.[6i] Treatment of the dithiane **10** with *t*-butyllithium (^{t}BuLi) at −43°C for 1 h in 20% hexamethylphosphoramide (HMPA)/THF gave the corresponding dithiane carbanion, which was confirmed by reddish color. To this solution was added the C21–C26 segment **12**[6g] at −78°C, and the temperature was allowed to warm to −50°C, then maintained at −50°C for 1 h. After work-up, the coupling product **13** was separated by silica gel column chromatography and isolated in 87% yield. The excess



Scheme 2. *Reagents and conditions*: (a) *t*-BuLi, 20% THF–HMPA, −50 to −43°C, 1 h, then **12**, −78 to −50°C, 1 h, 87%; (b) Ac_2O, pyridine, DMAP, room temperature, 12 h; (c) DDQ, CH_2Cl_2–H_2O=10:1, room temperature, 1 h; (d) MeOH, $CaCO_3$, room temperature, 7 h, 76% (3 steps); (e) $Cl_3C_6H_2COCl$, Et_3N, DMAP, 84%; (f) $(HF)_x$·Py, THF, room temperature, 12 h; (g) $Hg(ClO_4)_2$, $CaCO_3$, CH_3CN, H_2O, room temperature, 3 min, 69% (2 steps).

C1–C20 segment **10** was recovered in 43% yield. The protective *p*-methoxybenzyl (PMB) group of **13** was removed in 3 steps; thus, (i) acetylation at the C22 hydroxyl group, (ii) oxidative cleavage of the PMB group with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ),[14] and (iii) hydrolysis of the temporary acetate to provide the diol **14** in 76% overall yield. This compound was identical with the authentic compound, which had been prepared, during the course of the total synthesis under the similar condition.[6i]

Selective esterification of **14** with the maleic segment **15**[6c] under Yamaguchi condition[15] proceeded expectedly to afford the desired product **16** in 88% yield as reported previously.[6i] Two-step deprotection of **16** was achieved to remove TBS groups with poly(hydrogen fluoride)pyridine complex ($(HF)_x$·Py)[16] and to cleave the two dithioketals using mercury perchlorate ($Hg(ClO_4)_2$) in aqueous acetonitrile.[17] The semi-synthetic tautomycin was obtained in 69% yield in two steps. The synthetic material was proved to be identical in all respects ($[\alpha]_D$, ^1H and ^{13}C NMR, IR, MS) with natural tautomycin.

4. Conclusion

A useful and alternative synthetic route of tautomycin has been established through the coupling reaction of the epoxide **12** (C21–C26 segment) with the dithiane anion of **10** (C1–C20 segment) in 16% overall yield in 16 steps from natural tautomycin (**1**), which was available through fermentation in 10 gram scale. Further synthetic studies are in progress with respect to ^{13}C incorporated tautomycins. And the corresponding bioorganic studies including PP1γ (protein phosphatase) will be published elsewhere.

5. Experimental

5.1. General methods

Infrared spectra (IR) were recorded on a JASCO FT/IR-8300 spectrophotometer and are reported in wave number (cm^{-1}). Proton nuclear magnetic resonance (^1H NMR) spectra were measured with a BRUKER ARX-400 or an AMX-600 spectrometers. Chemical shifts are reported in parts per million (ppm) relative to the residual undeuterated $CHCl_3$ (δ=7.26 ppm) as an internal standard. Data are reported as follows; chemical shift, integration, multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, qn=quintet, sext=sextet, oct=octet, br=broadened, m=multiplet), coupling constant(s), and assignment, respectively. Carbon nuclear magnetic resonance (^{13}C NMR) spectra were measured with a BRUKER ARX-400 or an AMX-600 spectrometers. Chemical shifts are reported in ppm using $CDCl_3$ (δ=77.0 ppm) as an internal standard. 2D NMR (COSY, HMQC, and HMBC) were measured with a BRUKER ARX-400 or AMX-600 spectrometers. Tautomycin numbering corresponding to the front page is employed for assignment of ^1H NMR. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. MS spectra were measured utilizing a Micromass Q-TOF mass spectrometer equipped with a Z-spray type ESI source and are reported in *m*/*z*. Low-resolution mass spectra (FAB) were recorded on a JEOL M Station and reported in *m*/*z*. Elemental analyses was performed by Mr S. Kitamura in Analytical Laboratory at Bioagricultural Sciences, Nagoya University to whom the authors gratefully acknowledge. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm silica gel coated glass plates $60F_{254}$ using UV light as visualizing agent and 12.molybdo(VI)phosphoric acid *n*-hydrate or *p*-anisaldehyde solution and heated as developing agents. Silica gel 60 (particle size 0.063–0.2 mm ASTM) was used for open-column chromatography. Dry THF was distilled from potassium metal with benzophenone indicator under N_2 atmosphere. Dry CH_2Cl_2 was distilled from CaH_2 under N_2 atmosphere. Pyridine and Et_3N were dried over anhydrous KOH. BF_3·OEt_2 was distilled from CaH_2. All other commercially available reagents were used as received.

Tautomycin (**1**) was kindly provided by ex-Professor K. Isono (Tokai University) and further purified by the procedure of the method described previously with slight modification. The purification system of tautomycin is shown in Figure 2.

5.1.1. Purification of natural tautomycin (1). A crude tautomycin **1** (550 g) was suspended CH_2Cl_2 (300 ml) and filtrated through Celite pad. The filtrate was concentrated to give a dark brown oil (423 g). This oil was roughly purified by silica gel column chromatography (4 kg). It was then eluted with CH_2Cl_2 (8 l) followed by 5% MeOH/CH_2Cl_2 (24 l) and 30% MeOH/CH_2Cl_2 (16 l). 5% MeOH/CH_2Cl_2

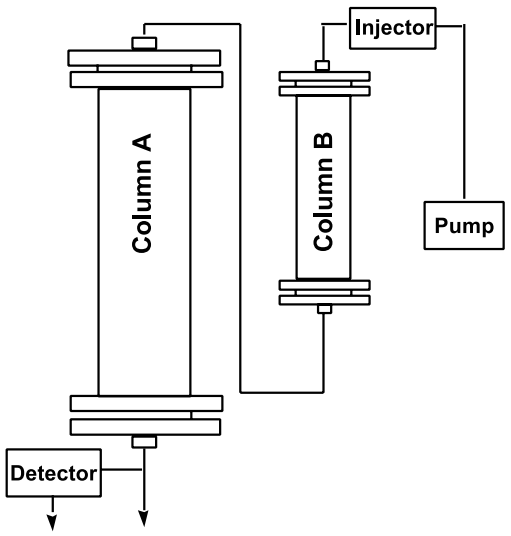


Figure 2. Purification system of tautomycin: Column A: a stainless column (50×500 mm, 981 ml) (Nomura Chemical) was packed with Cosmosil $75C_{18}$-OPN. Column B: a stainless steel column (20×250 mm, 78.5 ml) (Nomura Chemical) was packed with Cosmosil $75C_{18}$-OPN to which was made stick crude tautomycin. Pump: Waters 170 P-1 (100 ml/min) (Waters). Injector: Waters Prep LC 3000 System (Waters). Detector: Waters 490E (254 nm) (Waters). Recorder: UNICORDER U-228 (Nippon Denshi Kagaku).

fraction was concentrated in vacuum to give a brown oil (220 g). To a solution of an aliquot (10.22 g) in CH_2Cl_2 was added ODS silica gel (Cosmosil $75C_{18}$-OPN, 26 g), and the suspension were concentrated to dryness. The adsorbates were dry-packed in column (20×250 mm) which equipped with ODS-HPLC column (50×500 mm), and then eluted with 60% MeOH–H_2O (9.0 l), 70% MeOH–H_2O (7.0 l) and 100% MeOH (3.5 l). The 70% fraction was evaporated below 30°C to removed the MeOH, and adjusted to pH 3 by 1N HCl. The suspension was extracted with CH_2Cl_2 (×3), and the organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure to give tautomycin (**1**) (2.52 g). **1**: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.80 (3H, d, J=6.5 Hz, 7-CH_3), 0.89 (3H, d, J=7.0 Hz, 13-CH_3), 0.97 (3H, d, J=7.0 Hz, 25-CH_3), 0.98 (3H, d, J=7.0 Hz, 25-CH_3), 1.00 (3H, d, J=6.5 Hz, 15-CH_3), 1.10 (3H, d, J=7.0 Hz, 3-CH_3), 1.11 (3H, d, J=7.5 Hz, 19-CH_3), 1.20–1.70 (17H, m, H-4a, 4b, 5a, 5b, 7, 8a, 8b, 9a, 9b, 11a, 11b, 12a, 15, 16a, 16b, 17a, 17b), 1.83 (1H, m, H-13), 2.01 (1H, m, H-12b), 2.11 (1H, m, H-25), 2.15 (3H, s, H-1), 2.27 (3H, d, J=1.5 Hz, 5′-CH_3), 2.46 (1H, br s, OH), 2.53 (1H, sext, J=7.0 Hz, H-3), 2.67 (1H, qn, J=7.5 Hz, H-19), 2.67 (1H, dd, J=17.5, 4.0 Hz, H-21a), 2.77 (1H, dd, J=16.5, 10.0 Hz, H-2′a), 2.92 (1H, dd, J=16.5, 3.0 Hz, H-2'b), 2.99 (1H, dd, J=17.5, 8.5 Hz, H-21b), 3.16 (1H, dt, J=10.0, 2.0 Hz, H-6), 3.20 (1H, br s, OH), 3.27 (1H, dd, J=6.0, 2.0 Hz, H-23), 3.27 (1H, dd, J=10.0, 2.0 Hz, H-14), 3.44 (3H, s, OCH_3), 3.70 (1H, br t, J=7.0 Hz, H-18), 4.35 (1H, m, H-22), 4.54 (1H, br s, OH), 5.10 (1H, t, J=6.0 Hz, H-24), 5.21 (1H, br d, J=10.0 Hz, H-3′). ^{13}C NMR ($CDCl_3$, 150.9 MHz) δ 10.2, 11.0, 13.7, 16.2, 16.7, 17.9, 18.0, 19.4, 26.8, 27.4, 27.6, 28.1, 28.1, 28.7, 29.1, 30.2, 30.7, 31.4, 34.8, 34.8, 36.0, 41.0, 45.8, 47.3, 52.4, 59.1, 63.9, 66.4, 74.3, 74.3, 74.8, 76.5, 80.6, 95.7, 142.1, 143.0, 164.8, 165.8, 169.5, 213.1, 215.3. IR (KBr): 3444, 2934, 1768, 1709, 1460, 1381, 1257, 1100, 987, 909, 732 cm^{-1}. ESI Q-TOF MS calcd for $C_{41}H_{66}NaO_{13}^+$ $[M+Na]^+$ 789.4401, found 789.4387.

5.1.2. Enone (3). To a solution of tautomycin **1** (30.0 mg, 0.039 mmol) in MeOH (2.0 ml) was added dropwise 20% Cs_2CO_3 solution (about 3.2 ml) until the mixture solution reached pH 9. After being stirred for 3 h at 0°C, the reaction mixture was allowed to warm to room temperature and stirred for 2 h. While the reaction went on, the solution was kept to pH 9. The solution was adjusted to pH 5 with 1N HCl, and the MeOH was evaporated off. The resulting mixture was extracted with AcOEt (×3), washed with water (×3) and brine. The combined organic extracts were dried over Na_2SO_4 and then concentrated to give a brown oil (30.1 mg). Purification by column chromatography (silica gel 2 g, ether/hexane=2/1) gave the pure enone **3** (20.3 mg, 92%) as a colorless oil. **3**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.79 (3H, d, J=6.5 Hz, 7-CH_3), 0.88 (3H, d, J=7.5 Hz, 13-CH_3), 0.90 (3H, d, J=7.0 Hz, 25-CH_3), 0.99 (3H, d, J=6.5 Hz, 15-CH_3), 0.99 (3H, d, J=7.0 Hz, H-26), 1.09 (3H, d, J=7.0 Hz, 3-CH_3), 1.18 (3H, d, J=7.0 Hz, 19-CH_3), 1.20–1.73 (18H, m, H-4a, 4b, 5a, 5b, 7, 8a, 8b, 9a, 9b, 11a, 11b, 12a, 15, 16a, 16b, 17a, 17b, 25), 1.84 (1H, m, H-13), 2.01 (1H, m, H-12b), 2.14 (3H, s, H-1), 2.29 (1H, br s, OH), 2.53 (1H, sext, J=7.0 Hz, H-3), 2.76 (1H, br s, OH), 2.94 (1H, dq, J=7.5, 7.0 Hz, H-19), 3.16 (1H, td, J=10.0, 2.0 Hz, H-6), 3.26 (1H, dd, J=10.0, 2.5 Hz, H-14), 3.33 (3H, s, 23-OCH_3), 3.48 (1H, dd, J=7.5, 4.5 Hz, H-24), 3.69 (1H, m, H-18), 3.81 (1H, ddd, J=7.0, 4.5, 1.5 Hz, H-23), 6.34 (1H, dd, J=16.0, 1.0 Hz, H-21), 6.82 (1H, dd, J=16.0, 7.0 Hz, H-22). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 10.9, 14.7, 16.1, 16.7, 17.9, 18.2, 18.9, 26.8, 27.6, 27.9, 28.1, 28.2, 29.0, 29.7, 29.8, 30.2, 30.6, 31.7, 34.8, 34.9, 36.1, 47.3, 48.6, 57.1, 74.2, 75.0, 77.8, 82.1, 95.6, 132.0, 142.7, 204.4, 212.5. $[\alpha]_D^{26}=-19.3°$ (c 0.21, $CHCl_3$). IR (KBr): 3447, 2932, 2363, 1706, 1629, 1458, 1375, 1255, 1231, 1180, 1099, 1066, 987, 875 cm^{-1}. FAB-MS (positive p-nitrobenzyl alcohol): m/z 567 ($C_{33}H_{58}O_7$, $[M+H]^+$), 549 ($[M+H-H_2O]^+$). Anal. calcd for $C_{33}H_{58}O_7$: C, 69.86; H, 10.41. Found: C, 69.93; H, 10.31.

5.1.3. TBS ether (5). To a solution of enone **3** (1.96 g, 3.46 mmol) dissolved in CH_2Cl_2 (20 ml) were added 2,6-lutidine (6.05 ml, 51.9 mmol) and TBSOTf (5.17 ml, 22.5 mmol) at −20°C under N_2 atmosphere. After being stirred for 10 min, the reaction mixture was allowed to warm to room temperature and stirred for 30 min. The reaction mixture was poured into sat. NH_4Cl and extracted with CH_2Cl_2 (×3) washed with water (×3) and brine. The combined organic phase was dried over Na_2SO_4 and then concentrated under reduced pressure to afford the silyl vinyl ether **4** (4.50 g).

To a solution of **4** (4.50 g) dissolved in THF (1 ml) were added AcOH (12 ml), H_2O (4 ml) and THF (3 ml) at room temperature. After being stirred for 12 h at room temperature, the reaction mixture was poured into sat. $NaHCO_3$ and extracted with CH_2Cl_2 (×3). The combined organic phase was washed with water (×3) and brine, dried over Na_2SO_4 and then concentrated to give the oil (4.57 g). Purification by column chromatography (silica gel 250 g, ether/hexane=1/6) gave the pure **5** (2.47 g, 90%) as a colorless oil. **5**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.01 (3H, s, CH_3Si),

0.04 (3H, s, CH_3Si), 0.04 (3H, s, CH_3Si), 0.05 (3H, s, CH_3Si), 0.80 (3H, d, J=6.5 Hz, 7-CH_3), 0.85 (9H, s, SiC(CH_3)$_3$), 0.88 (3H, d, J=7.5 Hz, 13-CH_3), 0.88 (9H, s, SiC(CH_3)$_3$), 0.90 (3H, d, J=7.0 Hz, 25-CH_3), 0.91 (3H, d, J=7.0 Hz, H-26), 1.01 (3H, d, J=7.0 Hz, 15-CH_3), 1.09 (3H, d, J=7.0 Hz, 3-CH_3), 0.98 (3H, d, J=7.0 Hz, 19-CH_3), 1.20–1.70 (17H, m, H-4a, 4b, 5a, 5b, 7, 8a, 8b, 9a, 9b, 11a, 11b, 12a, 15, 16a, 16b, 17a, 17b), 1.74 (1H, m, H-25), 1.84 (1H, m, H-13), 2.02 (1H, m, H-12b), 2.14 (3H, s, H-1), 2.56 (1H, sext, J=7.0 Hz, H-3), 3.01 (1H, qn, J=7.0 Hz, H-19), 3.16 (1H, td, J=9.5, 2.0 Hz, H-6), 3.25 (1H, dd, J=10.5, 2.5 Hz, H-14), 3.27 (3H, s, 23-OCH_3), 3.51 (1H, dd, J=6.0, 4.0 Hz, H-24), 3.71 (1H, ddd, J=7.0, 4.0, 1.0 Hz, H-23), 4.01 (1H, m, H-18), 6.32 (1H, dd, J=16.0, 1.0 Hz, H-21), 6.75 (1H, dd, J=16.0, 7.0 Hz, H-22). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ −4.8, −4.5, −4.4, −3.8, 10.9, 12.4, 16.0, 16.8, 18.0, 18.3, 19.6, 25.8, 25.9, 26.1, 26.7, 27.6, 28.0, 28.1, 29.1, 30.2, 30.4, 30.5, 31.3, 34.8, 34.9, 36.1, 47.3, 49.0, 56.9, 73.4, 74.3, 75.0, 79.6, 83.0, 95.6, 131.5, 143.5, 202.4, 212.7. $[\alpha]_D^{27}$=18.8° (c 0.33, $CHCl_3$). IR (KBr): 2931, 2362, 1717, 1636, 1464, 1362, 1255, 1086, 987, 939, 836, 775 cm^{-1}. Anal. calcd for $C_{45}H_{86}O_7Si_2$: C, 67.86; H, 11.08. Found: C, 67.96; H, 10.90.

5.1.4. Allylic alcohol (6). To a solution of **5** (2.64 g, 3.31 mmol) in MeOH (25 ml) was added $NaBH_4$ (752 mg, 19.88 mmol) at 0°C under N_2 atmosphere. After being stirred for 30 min, the reaction mixture was poured into 1N HCl. After the organic solvent was evaporated, the resulting mixture was extracted with AcOEt (×3). The combined organic phase was washed with water (×3) and brine, dried over Na_2SO_4 and then concentrated under reduced pressure to afford the crude **6** (2.37 g). Purification by column chromatography (silica gel 150 g, ether/hexane=1/4) gave the epimeric mixture **6** (2.24 g, 85%) as a colorless oil. **6**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.04 (3H, s, CH_3Si), 0.06 (3H, s, CH_3Si), 0.07 (3H, s, CH_3Si), 0.08 (3H, s, CH_3Si), 0.82 (3H, d, J=7.0 Hz, 7-CH_3), 0.82 (3H, d, J=7.0 Hz, 19-CH_3), 0.88 (3H, d, J=7.0 Hz, 13-CH_3), 0.88 (3H, d, J=7.0 Hz, 3-CH_3), 0.89 (3H, d, J=7.0 Hz, 25-CH_3), 0.89 (9H, s, SiC(CH_3)$_3$), 0.90 (9H, s, SiC(CH_3)$_3$), 0.91 (3H, d, J=7.0 Hz, H-26), 1.00 (3H, d, J=7.0 Hz, 15-CH_3), 1.13 (1.7H, d, J=7.0 Hz, H-1), 1.15 (1.3H, d, J=7.0 Hz, H-1), 1.20–1.84 (20H, m), 1.72 (1H, oct, J=7.0 Hz, H-25), 1.84 (1H, m, H-13), 2.04 (1H, m, H-12b), 2.54 (1H, br s, OH), 3.19 (0.57H, td, J=10.0, 2.0 Hz, H-6), 3.20 (0.43H, td, J=10.0, 2.0 Hz, H-6), 3.22 (3H, s, 23-OCH_3), 3.29 (0.57H, dd, J=10.0, 2.5 Hz, H-14), 3.30 (0.43H, dd, J=10.0, 2.5 Hz, H-14), 3.45 (1H, dd, J=4.0, 7.0 Hz, H-24), 3.55 (1H, dt, J=4.0, 7.0 Hz, H-23), 3.64 (0.57H, dt, J=6.5, 7.0 Hz, H-2), 3.72 (0.43H, dd, J=4.0, 7.0 Hz, H-2), 3.82 (1H, m, H-18), 4.02 (1H, dd J=8.0, 6.0 Hz, H-20), 5.61 (1H, dd, J=16.0, 7.0 Hz, H-22), 5.66 (1H, dd, J=16.0, 6.0 Hz, H-21). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ −4.7, −4.5, −4.2, −3.6, 10.9, 10.9, 12.5, 14.3, 14.8, 16.7, 18.0, 18.1, 18.5, 18.8, 19.7, 19.7, 20.3, 25.9, 26.2, 26.8, 27.2, 27.2, 27.6, 27.6, 28.2, 28.6, 29.8, 30.2, 30.3, 30.5, 31.1, 34.9, 35.0, 35.0, 36.2, 39.7, 40.0, 43.5, 55.9, 65.8, 71.4, 71.4, 74.4, 74.5, 75.0, 75.1, 75.1, 75.2, 79.8, 79.8, 83.7, 95.7, 95.7, 96.2, 129.1, 136.3, 136.3. $[\alpha]_D^{26}$=8.0° (c 0.12, $CHCl_3$). IR (KBr): 3448, 2929, 2363, 1458, 1254, 1070, 835, 775 cm^{-1}. Anal. calcd for $C_{45}H_{90}O_7Si_2$: C, 67.63; H, 11.38. Found: C, 67.61; H, 11.35.

5.1.5. Triol (7). To a solution of **6** (270 mg, 0.34 mmol) in CH_2Cl_2 (25 ml) was bubbled ozone until the color of solution was blue (50 V, 30 min) at −78°C. After nitrogen purging, to the reaction mixture was added $NaBH_4$ (63.9 mg, 1.69 mmol) in MeOH (5 ml) at −78°C at N_2 atmosphere. After being stirred for 90 min, the reaction mixture was poured into 1N HCl. After the organic solvent was evaporated, the resulting mixture was extracted with AcOEt (×3). The combined organic phase was washed with water (×3) and brine, dried over Na_2SO_4 and then concentrated under reduced pressure to afford the crude **7**. Purification by column chromatography (silica gel 10 g, ether/hexane=2/1) gave the epimeric mixture **7** (146 g, 76%) as a colorless oil. **7**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.09 (3H, s, CH_3Si), 0.09 (3H, s, CH_3Si), 0.82 (3H, d, J=7.0 Hz, 7-CH_3), 0.86 (3H, d, J=7.0 Hz, 19-CH_3), 0.87 (3H, d, J=7.0 Hz, 13-CH_3), 0.89 (3H, d, J=6.5 Hz, 3-CH_3), 0.90 (9H, s, SiC(CH_3)$_3$), 1.00 (3H, d, J=7.0 Hz, 15-CH_3), 1.14 (1.7H, d, J=6.5 Hz, H-1), 1.15 (1.3H, d, J=6.5 Hz, H-1), 1.20–1.74 (19H, m), 1.83 (3H, m, H-13, OH×2), 2.03 (1H, m, H-12b), 3.19 (0.57H, td, J=10.0, 2.0 Hz, H-6), 3.19 (0.43H, td, J=10.0, 2.0 Hz, H-6), 3.31 (0.57H, dd, J=10.0, 2.5 Hz, H-14), 3.31 (0.43H, dd, J=10.0, 2.5 Hz, H-14), 3.48 (1H, ddd, J=11.0, 6.5, 2.0 Hz, H-18), 3.58–3.78 (5H, m, H-2, 19, 20, 21a, 21b). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ −4.6, −4.2, 10.9, 10.9, 12.7, 12.7, 14.1, 14.7, 16.5, 18.0, 18.1, 19.7, 20.2, 25.9, 26.8, 27.2, 27.6, 27.6, 28.2, 28.3, 28.6, 29.9, 30.2, 30.6, 30.6, 30.8, 31.0, 34.7, 34.7, 35.0, 35.0, 36.1, 36.1, 39.7, 40.0, 40.0, 64.7, 64.7, 71.6, 71.7, 73.9, 74.4, 74.6, 74.8, 76.5, 76.6, 95.7, 95.7. $[\alpha]_D^{27}$=−34.4° (c 0.18, $CHCl_3$). IR (KBr): 3421, 2928, 2360, 1653, 1560, 1457, 1255, 1058, 835, 775 cm^{-1}. Anal. calcd for $C_{32}H_{64}O_6Si$: C, 67.08; H, 11.26. Found: C, 66.89; H, 11.35.

5.1.6. Alcohol (8). To a solution of **7** (1.20 g, 2.09 mmol) in THF (40 ml) was added to $NaIO_4$ (1.34 g, 6.28 mmol) in H_2O (10 ml) at 0°C. After stirring for 45 min, the reaction mixture was allowed to warm to room temperature and stirred for 30 min. After the solvent was evaporated, the reaction mixture was extracted with AcOEt (×3), washed with water (×3) and brine. The combined organic phase were dried over Na_2SO_4 and then concentrated to give a colorless oil. Purification by column chromatography (silica gel 60 g, ether/hexane=1/2) gave the epimeric mixture **8** (1.14 g, quant.) as a colorless oil. **8**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.06 (3H, s, CH_3Si), 0.06 (3H, s, CH_3Si), 0.82 (3H, d, J=7.0 Hz, 7-CH_3), 0.88 (3H, d, J=7.0 Hz, 13-CH_3), 0.88 (9H, s, SiC(CH_3)$_3$), 0.90 (3H, d, J=6.5 Hz, 3-CH_3), 0.99 (3H, d, J=7.0 Hz, 15-CH_3), 1.09 (3H, d, J=7.0 Hz, 19-CH_3), 1.14 (1.7H, d, J=7.0 Hz, H-1), 1.15 (1.3H, d, J=7.0 Hz, H-1), 1.20–1.74 (19H, m), 1.81 (1H, m, H-13), 2.03 (1H, m, H-12b), 2.50 (1H, qdd, J=7.0, 5.0, 2.0 Hz, H-19), 3.18 (0.57H, td, J=10.0, 2.0 Hz, H-6), 3.19 (0.43H, td, J=10.0, 2.0 Hz, H-6), 3.29 (0.57H, dd, J=10.0, 2.5 Hz, H-14), 3.29 (0.57H, dd, J=10.0, 2.5 Hz, H-14), 3.65 (0.57H, dt, J=6.5, 7.0 Hz, H-2), 3.73 (0.43H, dd, J=4.0, 7.0 Hz, H-2), 3.90 (1H, q, J=5.0 Hz, H-18), 9.75 (1H, d, J=2.0 Hz, H-20). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ −4.7, −4.2, 10.5, 10.9, 14.2, 14.8, 16.6, 18.0, 19.7, 20.4, 25.8, 26.7, 26.8, 27.6, 27.6, 28.1, 28.6, 29.8, 30.2, 30.2, 30.4, 31.7, 31.8, 34.7, 34.8, 34.9, 36.1, 39.7, 40.0, 51.0, 71.3, 71.4, 73.7, 74.5, 74.5, 74.8, 74.8, 95.7, 95.7, 205.1. $[\alpha]_D^{26}$=−23.5° (c 0.18, $CHCl_3$). IR (KBr): 3462, 2956, 2932, 2860, 1726,

1459, 1383, 1255, 1099, 1006, 988, 837, 775 cm^{-1}. Anal. calcd for $C_{31}H_{60}O_5Si$: C, 68.74; H, 11.43. Found: C, 68.84; H, 11.18.

5.1.7. C1–C20 segment (9). To a solution of **8** (530 mg, 0.98 mmol) in CH_2Cl_2 (10 ml) was added PCC (634 mg, 2.94 mmol) and a powdered molecular sieves 4 Å (795 mg) at room temperature. After being stirred for 2 h, the reaction mixture was diluted with ether, filtered through a pad of Celite and then concentrated under reduced pressure to afford **9**. Purification by column chromatography (silica gel 50 g, ether/hexane=1/5) gave the pure **9** (896 mg, 83%) as a colorless oil. **9**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.06 (3H, s, CH_3Si), 0.07 (3H, s, CH_3Si), 0.80 (3H, d, J=7.0 Hz, 7-CH_3), 0.88 (3H, d, J=7.0 Hz, 13-CH_3), 0.88 (9H, s, SiC(CH_3)$_3$), 0.99 (3H, d, J=6.5 Hz, 15-CH_3), 1.09 (3H, d, J=7.0 Hz, 3-CH_3), 1.09 (3H, d, J=7.0 Hz, 19-CH_3), 1.20–1.74 (17H, m, 4a, 4b, 5a, 5b, 7, 8a, 8b, 9a, 9b, 11a, 11b, 12a, 15, 16a, 16b, 17a, 17b), 1.82 (1H, m, H-13), 2.02 (1H, m, H-12b), 2.14 (3H, s, H-1), 2.50 (1H, qdd, J=7.0, 5.0, 2.0 Hz, H-19), 2.53 (1H, qd, J=13.5, 7.0 Hz, H-3), 3.16 (1H, td, J=10.0, 2.0 Hz, H-6), 3.25 (1H, dd, J=10.0, 2.5 Hz, H-14), 3.90 (1H, q, J=5.0 Hz, H-18), 9.74 (1H, d, J=2.0 Hz, H-20). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ −4.7, −4.2, 10.1, 10.5, 10.9, 16.1, 16.7, 18.0, 25.7, 26.7, 27.6, 28.0, 28.1, 29.1, 30.2, 30.6, 31.6, 34.8, 34.8, 36.1, 47.3, 51.0, 73.6, 74.3, 74.9, 95.6, 204.9, 212.7. $[\alpha]_D^{27}$=−5.6° (c 0.15, $CHCl_3$). IR (KBr): 2931, 2859, 1717, 1458, 1378, 1255, 987, 837, 775 cm^{-1}. Anal. calcd for $C_{31}H_{58}O_5Si$: C, 68.95; H, 11.04. Found: C, 69.09; H, 10.85.

5.1.8. Protected C1–C20 segment (10). To a solution of **9** (220 mg, 0.41 mmol) and 1,3-propanedithiol (0.49 ml, 4.9 mmol) in CH_2Cl_2 (0.2 ml) was added boron trifluoride etherate (15.5 μl, 0.122 mmol) in CH_2Cl_2 (10 ml) at 0°C under N_2 atmosphere. After being stirred for 1 h, the cooling bath was removed and stirring was continued for 24 h. The reaction mixture was poured into sat. $NaHCO_3$ and extracted with Et_2O (×3). The combined organic phase was washed with water (×3) and brine, dried over Na_2SO_4 and then concentrated in vacuum. The residue was purified by column chromatography (silica gel, ether/hexane=1/6) to afford protected **10** (173 mg, 59%) and **11** (94.4 mg, 38%). **10**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.07 (3H, s, CH_3Si), 0.11 (3H, s, CH_3Si), 0.82 (3H, d, J=7.0 Hz, 7-CH_3), 0.87 (3H, d, J=7.0 Hz, 13-CH_3), 0.90 (9H, s, SiC(CH_3)$_3$), 1.00 (3H, d, J=7.0 Hz, 19-CH_3), 1.01 (3H, d, J=7.0 Hz, 15-CH_3), 1.10 (3H, d, J=7.0 Hz, 3-CH_3), 1.20–2.15 (25H, m, H-3, 4a, 4b, 5a, 5b, 7, 8a, 8b, 9a, 9b, 11a, 11b, 12a, 12b, 13, 15, 16a, 16b, 17a, 17b, 19, SCH$_2$CH_2CH$_2$S×2), 1.60 (3H, s, H-1), 2.74–2.96 (8H, m, SCH_2CH$_2$CH_2S×2), 3.22 (1H, td, J=9.5, 2.5 Hz, H-6), 3.36 (1H, dd, J=10.0, 2.5 Hz, H-14), 3.84 (1H, m, H-18), 4.31 (1H, d, J=5.0 Hz, H-20). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ −4.7, −4.1, 10.9, 12.1, 14.3, 16.9, 18.0, 18.1, 23.5, 25.4, 25.6, 25.9, 26.3, 26.4, 26.7, 27.5, 27.7, 28.3, 29.4, 30.3, 30.4, 31.3, 32.0, 34.8, 35.0, 36.1, 42.0, 43.3, 51.9, 54.8, 72.2, 74.5, 74.7, 95.6. $[\alpha]_D^{27}$=−35.7° (c 0.22, $CHCl_3$). IR (KBr): 2954, 2857, 2363, 1459, 1382, 1255, 1091, 986, 939, 836, 775 cm^{-1}. Anal. calcd for $C_{37}H_{70}O_3S_4Si$: C, 61.66; H, 10.08. Found: C, 61.78; H, 9.81. **11**: ^{1}H NMR^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82 (3H, d, J=7.0 Hz, 7-CH_3), 0.87 (3H, d, J=7.0 Hz, 13-CH_3), 1.02 (3H, d, J=7.0 Hz, 15-CH_3), 1.05 (3H, d, J=7.0 Hz, 19-CH_3), 1.09 (3H, d, J=7.0 Hz, 3-CH_3), 1.20–2.14 (26H, m, 3, 4a, 4b, 5a, 5b, 7, 8a, 8b, 9a, 9b, 11a, 11b, 12a, 12b, 13, 15, 16a, 16b, 17a, 17b, 19, SCH$_2$CH_2CH$_2$S×2, OH), 2.73–3.02 (8H, m, SCH_2CH$_2$CH_2S×2), 3.21 (1H, td, J=9.5, 2.5 Hz, H-6), 3.38 (1H, dd, J=10.0, 2.5 Hz, H-14), 3.56 (1H, m, H-18), 4.53 (1H, d, J=5.0 Hz, H-20). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 10.9, 13.6, 14.2, 16.6, 18.0, 23.5, 25.6, 26.3, 26.4, 26.8, 27.4, 27.5, 28.3, 30.3, 30.8, 31.1, 31.4, 31.9, 34.7, 35.0, 36.1, 41.9, 44.0, 52.3, 54.9, 73.6, 74.4, 74.6, 95.6. $[\alpha]_D^{26}$=−56.9° (c 0.33, $CHCl_3$). IR (KBr): 3483, 2951, 2860, 2364, 1718, 1457, 1381, 1230, 1096, 986, 876 cm^{-1}. Anal. calcd for $C_{31}H_{56}O_3S_4$: C, 61.41; H, 9.57. Found: C, 61.54; H, 9.33.

5.1.9. Protected C1–C20 segment (10). To a solution of **11** (150 mg, 0.25 mmol) in CH_2Cl_2 (5 ml) was added 2,6-lutidine (0.14 ml, 1.24 mmol) and TBSOTf (0.14 ml, 0.62 mmol) at −20°C under N_2 atmosphere. After being stirred for 10 min, the reaction mixture was allowed to warm to room temperature and stirred for 30 min. The mixture was poured into sat. NH_4Cl aq. and extracted with CH_2Cl_2 (×3), washed with water (×3) and brine. The combined organic phase was dried and then concentrated under reduced pressure to afford the crude oil (240 mg). Purification by column chromatography (silica gel 20 g, ether/hexane=1/10) gave the pure **10** (176 mg, 99%) as a colorless oil.

5.1.10. Alcohol (13). To a solution of **10** (264 mg, 0.37 mmol), which was dried in advance with toluene-azeotrope, and HMPA (0.19 ml 1.10 mmol) in dry THF (0.7 ml) was added dropwise t-BuLi (1.45 M solution in hexane, 0.31 ml, 0.44 mmol) at −50°C under Ar atmosphere, then allowed to warm to −43°C. After stirring for 90 min, the reaction mixture was allowed to cool at −78°C. A solution of epoxide **12** (25.7 mg, 0.092 mmol) in THF (0.4 ml) was added to the reaction mixture at −78°C, and allowed to warm to −50°C. After stirring for 1 h, the reaction mixture was poured into sat. NH_4Cl aq. and extracted with Et_2O (×3). The combined organic phase was washed with water (×3) and brine, dried over Na_2SO_4, and then concentrated in vacuum. The residue was purified by column chromatography (silica gel 25 g, ether/hexane=1/4) to afford the coupling product **13** (73.0 mg, 87%) and recovered dithiane **10** (82 mg, 43%). **13**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.09 (6H, s, CH_3Si), 0.81 (3H, d, J=7.0 Hz, 7-CH_3), 0.84 (9H, s, SiC(CH_3)$_3$), 0.86 (3H, d, J=7.0 Hz, 13-CH_3), 0.98 (3H, d, J=7.0 Hz, H-26), 0.99 (3H, d, J=7.0 Hz, 25-CH_3), 1.02 (3H, d, J=7.0 Hz, 15-CH_3), 1.09 (3H, d, J=7.0 Hz, 3-CH_3), 1.09 (3H, d, J=7.0 Hz, 19-CH_3), 1.20–2.12 (25H, m, H-3, 4a, 4b, 5a, 5b, 7, 8a, 8b, 9a, 9b, 11a, 11b, 12a, 12b, 13, 15, 16a, 16b, 17a, 17b, 25, SCH$_2$CH_2CH$_2$S×2), 1.58 (3H, s, H-1), 2.20 (1H, qd, J=7.0, 2.0 Hz, H-19), 2.25 (1H, dd, J=15.5, 2.0 Hz, H-21a), 2.64 (1H, dd, J=15.5, 8.0 Hz, H-21b), 2.60–2.90 (6H, m, SCH_2CH$_2$CH_2S), 2.97 (1H, ddd, J=11.5, 14.5, 3.0 Hz, one of SCH_2CH$_2$CH$_2$S), 3.10 (1H, dd, J=6.0, 1.5 Hz, H-23) 3.14 (1H, ddd, J=11.5, 14.5, 3.0 Hz, one of SCH_2CH$_2$CH$_2$S), 3.25 (1H, td, J=9.5, 2.5 Hz, H-6), 3.36 (1H, dd, J=10.0, 2.5 Hz, H-14), 3.51 (3H, s, OCH_3), 3.54 (1H, dd, J=6.0, 5.0 Hz, H-24), 3.79 (3H, s, Ar–OCH_3), 3.99 (1H, br d, J=2.5 Hz, OH), 4.19 (1H, m, H-22), 4.25 (1H, m,

H-18), 4.51 (1H, d, J=11.0 Hz, one of CH_2Ar), 4.72 (1H, d, J=11.0 Hz, one of CH_2Ar), 6.83 (1H, dd, J=9.5, 2.5 Hz, one of ArH), 6.84 (1H, dd, J=9.5, 2.5 Hz, one of ArH), 7.25 (1H, dd, J=9.5, 2.5 Hz, one of ArH), 7.26 (1H, dd, J=9.5, 2.5 Hz, one of ArH). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ −4.5, −3.8, 8.2, 10.8, 14.2, 16.8, 17.6, 17.9, 18.0, 20.4, 23.6, 25.1, 25.6, 25.8, 26.1, 26.2, 26.5, 26.7, 27.4, 27.5, 28.2, 29.3, 29.5, 30.3, 31.3, 31.7, 34.8, 35.2, 36.1, 40.4, 41.4, 46.9, 54.8, 55.2, 55.8, 59.5, 67.9, 73.4, 74.3, 74.5, 75.3, 82.9, 84.3, 95.6, 113.6, 129.3, 130.7, 159.0. $[\alpha]_D^{26}$=−38.1° (c 0.24, $CHCl_3$). IR (KBr): 3464, 2958, 2928, 2856, 1615, 1515, 1463, 1383, 1251, 1174, 1099, 941, 834, 775 cm^{-1}. Anal. calcd for $C_{53}H_{94}O_7S_4Si$: C, 63.68; H, 9.48. Found: C, 63.70; H, 9.35.

5.1.11. Diol (14). To a solution of **13** (37 mg, 0.037 mmol) in Ac_2O (0.5 ml) and pyridine (1.0 ml) was added DMAP (1.0 mg, 8.19 μmol) at room temperature. After being stirred overnight, the reaction mixture was poured into H_2O and extracted with CH_2Cl_2 (x 3). The combined organic phase was washed with aqueous $CuSO_4{\cdot}5H_2O$ (×3), water (×3) and brine, dried over Na_2SO_4, and concentrated to give the crude acetylated compound.

To a solution of the crude acetylated compound in CH_2Cl_2 (2.0 ml) and H_2O (0.2 ml) was added DDQ (13.6 mg 0.06 mmol) in toluene (0.1 ml) and CH_2Cl_2 (0.2 ml) at room temperature. After stirring for 1 h, the reaction mixture was poured into sat. $NaHCO_3$ aq. and then extracted with CH_2Cl_2 (×3). The combined organic phase was washed with water (×3) and brine, dried over Na_2SO_4 and then concentrated in vacuum to give a crude deprotected compound.

To a solution of the crude deprotected compound in MeOH (1.5 ml) was added $CaCO_3$ (30 mg, 0.217 mmol) at room temperature. After stirring for 7 h, the reaction mixture was poured into sat. NH_4Cl aq. and then extracted with AcOEt (×3). The combined organic phase was washed with water (×3) and brine, dried over Na_2SO_4, and then concentrated in vacuum. The residue was purified by column chromatography (silica gel 1.5 g, ether/hexane=1/3) to afford **14** (24.7 mg, 76%). **14**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.07 (3H, s, CH_3Si), 0.10 (3H, s, CH_3Si), 0.82 (3H, d, J=7.0 Hz, 7-CH_3), 0.88 (3H, d, J=7.0 Hz, 13-CH_3), 0.89 (9H, s, SiC(CH_3)$_3$), 0.97 (3H, d, J=7.0 Hz, H-26), 1.01 (3H, d, J=7.0 Hz, 25-CH_3), 1.03 (3H, d, J=7.0 Hz, 15-CH_3), 1.07 (3H, d, J=7.0 Hz, 19-CH_3), 1.10 (3H, d, J=7.0 Hz, 3-CH_3), 1.20–2.15 (25H, m, H-3, 4a, 4b, 5a, 5b, 7, 8a, 8b, 9a, 9b, 11a, 11b, 12a, 12b, 13, 15, 16a, 16b, 17a, 17b, 25, SCH$_2$CH_2CH$_2$S×2), 1.59 (3H, s, H-1), 2.24 (1H, ddd, J=16.0, 9.0, 1.0 Hz, H-21a), 2.25 (1H, qd, J=7.0, 4.5 Hz, H-19), 2.66–2.92 (6H, m, SCH_2CH$_2$CH_2S), 2.78 (1H, dd, J=16.0, 9.0 Hz, H-21b), 3.03 (1H, ddd, J=11.0, 14.5, 3.0 Hz, one of SCH_2CH$_2$CH$_2$S), 3.16 (1H, dd, J=7.0, 3.0 Hz, H-23), 3.17 (1H, ddd, J=12.0, 14.5, 3.0 Hz, one of SCH_2CH$_2$CH$_2$S), 3.26 (1H, td, J=9.5, 2.5 Hz, H-6), 3.38 (1H, dd, J=10.0, 2.5 Hz, H-14), 3.42 (1H, br s, OH), 3.45 (3H, s, OCH_3), 3.63 (1H, m, H-24), 4.24 (2H, m, H-22, OH), 4.32 (1H, m, H-18). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ −4.3, −3.8, 7.9, 10.8, 14.2, 16.9, 17.0, 18.0, 19.6, 23.6, 25.0, 25.6, 25.9, 26.3, 26.3, 26.7, 26.8, 27.5, 27.6, 28.3, 29.4, 29.7, 30.4, 31.5, 31.8, 34.8, 35.2, 36.1, 38.5, 41.4, 46.8, 54.9, 55.6, 58.2, 68.6, 73.8, 74.4, 75.3, 75.5, 81.6, 95.7. $[\alpha]_D^{26}$=−35.8° (c 0.13, $CHCl_3$). IR (KBr): 3448, 2929, 2360, 2343, 1718, 1462, 1256, 1099, 834, 774 cm^{-1}. ESI Q-TOF MS calcd for $C_{45}H_{86}NaO_6S_4Si^+$ $[M+Na]^+$ 901.4974, found 901.4932.

5.1.12. Ester (16). To a solution of Segment A **15** (7.9 mg, 0.025 mmol) and Et_3N (7.0 μl, 0.05 mmol) in toluene (1 ml) was added 2,4,6-trichlorobenzoyl chloride (7.0 μl, 0.045 mmol) at room temperature. After stirring for 5 h, a solution of **14** (16.8 mg, 0.019 mmol) in toluene (1 ml) and DMAP (4.2 mg, 0.034 mmol) in toluene (0.5 ml) were added to reaction mixture at room temperature. After being stirred for 30 min at room temperature, the mixture was diluted with water, adjusted to pH 3 by 1N HCl and then extracted with Et_2O (×3). The combined organic phase was washed with water (×3) and brine, dried over Na_2SO_4 and then concentrated in vacuum. The residue was purified by column chromatography (silica gel, ether/hexane=1/3) to provide **16** (18.9 mg, 84%). **16**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.02 (3H, s, SiCH_3), 0.06 (3H, s, SiCH_3), 0.07 (3H, s, SiCH_3), 0.12 (3H, s, SiCH_3), 0.82 (3H, d, J=7.0 Hz, 7-CH_3), 0.85 (3H, d, J=6.5 Hz, 13-CH_3), 0.85 (9H, s, SiC(CH_3)$_3$), 0.87 (9H, s, SiC(CH_3)$_3$), 0.92 (3H, d, J=7.0 Hz, 26-CH_3), 0.93 (3H, d, J=7.0 Hz, 25-CH_3), 1.03 (3H, d, J=6.5 Hz, 15-CH_3), 1.04 (3H, d, J=7.0 Hz, 19-CH_3), 1.10 (3H, d, J=7.0 Hz, 3-CH_3), 1.09–2.22 (26H, m, H-3, 4a, 4b, 5a, 5b, 7, 8a, 8b, 9a, 9b, 11a, 11b, 12a, 12b, 13, 15, 16a, 16b, 17a, 17b, 19, 25, SCH$_2$CH_2CH$_2$S×2), 1.59 (3H, s, H-1), 2.22 (3H, s, 5′-CH_3), 2.60–3.13 (12H, m, H-2′a, H-2′b, H-21a, H-21a, SCH_2CH$_2$CH_2S×2), 3.20 (1H, dd, J=7.0, 3.0 Hz, H-23), 3.26 (1H, td, J=9.0, 3.0 Hz, H-6), 3.37 (1H, dd, J=10.0, 2.0 Hz, H-14), 3.44 (4H, s, OCH_3, OH), 4.04 (1H, m, H-22), 4.22 (1H, m, H-18), 5.09 (1H, dd, J=7.0, 5.0 Hz, H-24), 5.16 (1H, dd, J=7.0, 6.0 Hz, H-3′). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ −5.2, −4.9, −4.3, −3.8, 7.7, 10.1, 10.9, 14.2, 15.3, 16.8, 16.9, 18.0, 18.0, 19.7, 23.7, 25.1, 25.6, 25.9, 26.0, 26.3, 26.6, 26.8, 27.5, 27.6, 28.4, 28.4, 29.5, 30.4, 31.4, 31.8, 34.8, 35.3, 36.2, 39.2, 41.2, 41.5, 46.7, 54.9, 55.7, 59.4, 65.8, 63.0, 67.6, 73.8, 74.4, 75.3, 76.0, 82.0, 95.6, 142.4, 143.5, 164.1, 165.9, 169.3. $[\alpha]_D^{28}$=−15.4° (c 0.18, $CHCl_3$). IR (KBr): 3438, 1771, 1736, 1464, 1378, 1255, 1100, 912, 836, 778 cm^{-1}. ESI Q-TOF MS calcd for $C_{59}H_{106}NaO_{11}S_4Si_2^+$ $[M+Na]^+$ 1197.6054, found 1197.6084.

5.1.13. Tautomycin (1). To a solution of **16** (17.9 mg, 0.015 mmol) in THF (1 ml), which was transferred into a teflon vial was added pyridinium poly(hydrogen fluoride) (12 drops) at room temperature. After being stirred for 12 h, the reaction mixture was neutralized by sat. $NaHCO_3$ aq. to pH 3 and then extracted with ether (×3). The combined organic phase was washed with water (×3) and brine, dried and then concentrated in vacuum.

To a solution of the residue in acetonitrile (0.8 ml) and water (0.08 ml) was added mercury (II) perchlorate trihydrate (20.4 mg, 0.045 mmol) and calcium carbonate (7.5 mg, 0.075 mmol) at room temperature. After being stirred for 3 min, the mixture was diluted with ether, filtered through SuperCel®, and then concentrated to give a crude oil. The residue was purified by column chromatography (silica gel 0.8 g, ethyl acetate/hexane=1/2) to afford

synthetic tautomycin **1** (8.0 mg, 69%). **1**: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.80 (3H, d, J=6.5 Hz, 7-CH_3), 0.89 (3H, d, J=7.0 Hz, 13-CH_3), 0.97 (3H, d, J=7.0 Hz, 25-CH_3), 0.98 (3H, d, J=7.0 Hz, 25-CH_3), 1.00 (3H, d, J=6.5 Hz, 15-CH_3), 1.10 (3H, d, J=7.0 Hz, 3-CH_3), 1.11 (3H, d, J=7.5 Hz, 19-CH_3), 1.20–1.70 (17H, m, H-4a, 4b, 5a, 5b, 7, 8a, 8b, 9a, 9b, 11a, 11b, 12a, 15, 16a, 16b, 17a, 17b), 1.83 (1H, m, H-13), 2.01 (1H, m, H-12b), 2.11 (1H, m, H-25), 2.15 (3H, s, H-1), 2.27 (3H, d, J=1.5 Hz, 5′-CH_3), 2.53 (1H, sext, J=7.0 Hz, H-3), 2.67 (1H, m, H-19), 2.67 (1H, dd, J=17.5, 4.0 Hz, H-21b), 2.78 (1H, dd, J=16.5, 10.0 Hz, H-2′a), 2.92 (1H, dd, J=16.5, 3.0 Hz, H-2′b), 2.99 (1H, dd, J=17.5, 8.5 Hz, H-21b), 3.16 (1H, td, J=10.0, 2.5 Hz, H-6), 3.28 (1H, dd, J=6.0, 2.0 Hz, H-23), 3.28 (1H, dd, J=10.0, 2.0 Hz, H-14), 3.44 (3H, s, OCH_3), 3.70 (1H, td, J=8.0, 3.0 Hz, H-18), 4.35 (1H, ddd, J=8.0, 4.0, 2.0 Hz, H-22), 5.10 (1H, t, J=6.0 Hz, H-24), 5.21 (1H, m, H-3′). ^{13}C NMR ($CDCl_3$, 150.9 MHz) δ 10.1, 11.0, 13.7, 16.2, 16.7, 17.9, 18.0, 19.4, 26.8, 27.4, 27.7, 28.1, 28.1, 28.7, 29.1, 30.2, 30.7, 31.5, 34.8, 34.8, 36.1, 41.0, 45.8, 47.3, 52.4, 59.1, 63.9, 66.4, 74.3, 74.3, 74.8, 76.5, 80.6, 95.7, 142.1, 142.9, 164.8, 165.8, 169.6, 213.1, 215.3. $[\alpha]_D^{27}$=4.4° (c 0.32, $CHCl_3$). Lit. $[\alpha]_D^{25}$=3.4° (c 1, $CHCl_3$). IR (KBr): 3447, 2928, 2360, 1769, 1716, 1457, 1379, 1260, 1098, 908, 803, 733, 628 cm^{-1}. ESI Q-TOF MS calcd for $C_{41}H_{66}NaO_{13}^+$ $[M+Na]^+$ 789.4401, found 789.4387.

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