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Synthesis of photoaffinity probes of tautomycin

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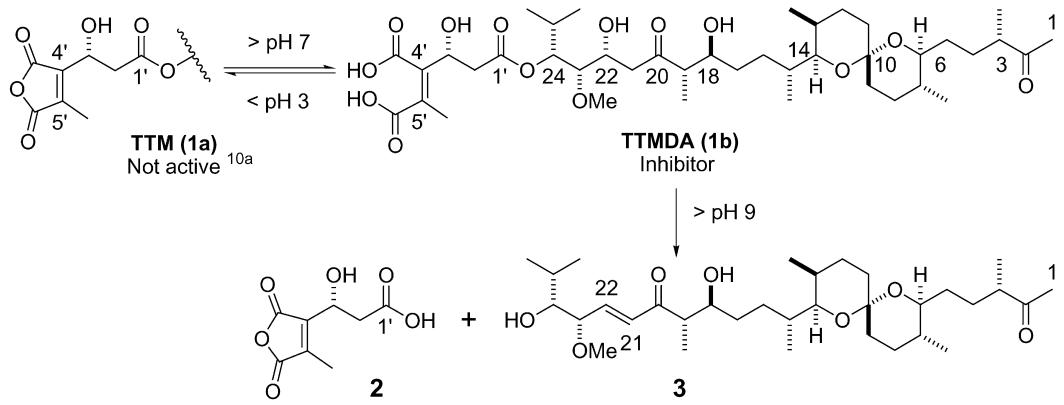
Abstract—Two types of photoaffinity probe, which possesses a benzophenone or a diazirine photophore on the 2-position of tautomycin, were prepared in order to prove the details of binding site to PP1. These photoaffinity probes were designed on the basis of the structure–activity relationship; thus, the diacid moiety is indispensable. The selective introduction of photolabeling units on the 2-position of tautomycin was achieved through the 2-oxime of tautomycin diacid.

1. Introduction

Tautomycin (TTM, **1**) was isolated in 1987,[1] structurally elucidated in 1990 by Isono (Scheme 1),[2] and shows a specific inhibitor of protein phosphatase (PP) 1 and 2A.[3] It has been reported that PP1 or PP2A were inhibited by natural products such as okadaic acid, calyculin, and microcystin–LR.[4] Each of these three compounds inhibits PP2A much more strongly than does PP1, which TTM inhibits PP1 more selectively than PP2A.[4] In 1995, the X-ray crystallographic structure of the complex of PP1–microcystin–LR provided the details of the interaction between the protein and the toxin.[5] The X-ray structures were reported for PP1 complexes of okadaic acid (2001)[6] and calyculin (2002).[7] However, the X-ray structure of PP1–TTM complex is not yet available, probably due to the nature of TTM. Herein, we describe the synthesis of two types of photoaffinity probe (**4**, **5**, **6**, and **7**), which possesses a benzophenone or a diazirine photophore on the 2-position of TTM in order to study the binding site to PP1 (Scheme 2). These photoaffinity probes were designed according to the structure–activity relationship reported in the various sources with natural and synthetic derivatives:[8–11] (i) Active inhibitor is not the anhydride (**1a**), but the dicarboxylic acids form (**1b**).[10a,11a] (ii) The hydroxyl groups at the C22 and the C′3 are also indispensable for its bioactivity.[10b,11a] (iii) The hydrophobic spiroketal moiety contributes significantly to the selective inhibition of PP.[10b] Thus, we have been introducing the photolabeling units into the 2-position of TTM for the current studies.

Under mild alkaline conditions (CH_3CN, 3% $NaHCO_3$, pH 8), TTM (**1**) exists predominantly as the diacid form (TTMDA, **1b**), which is the active inhibitor. These two



Scheme 1. Tautomycin (TTM) and tautomycin diacid (TTMDA).

Keywords: Tautomycin; Photoaffinity probe; Protein phosphatase.
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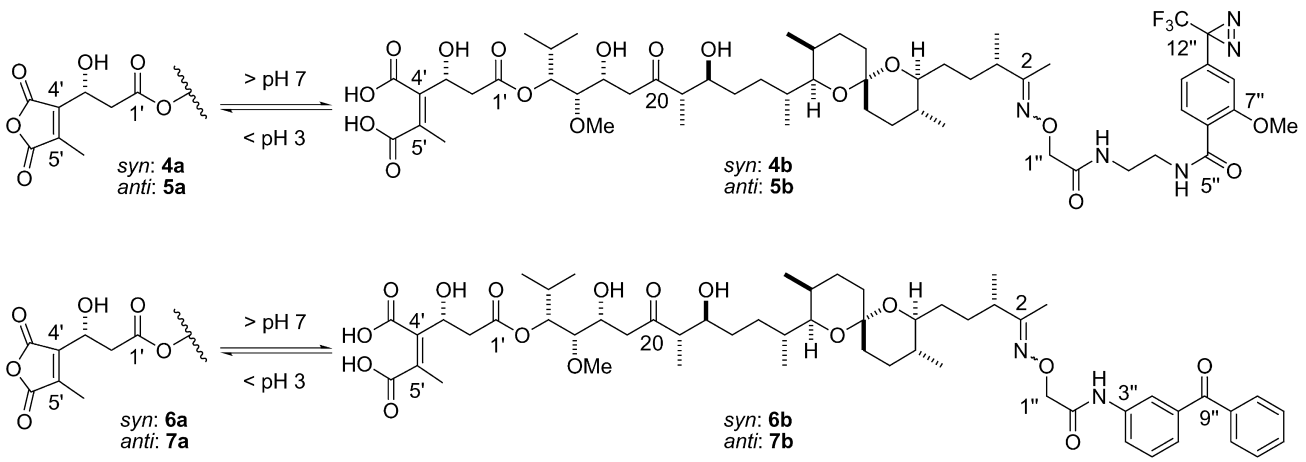
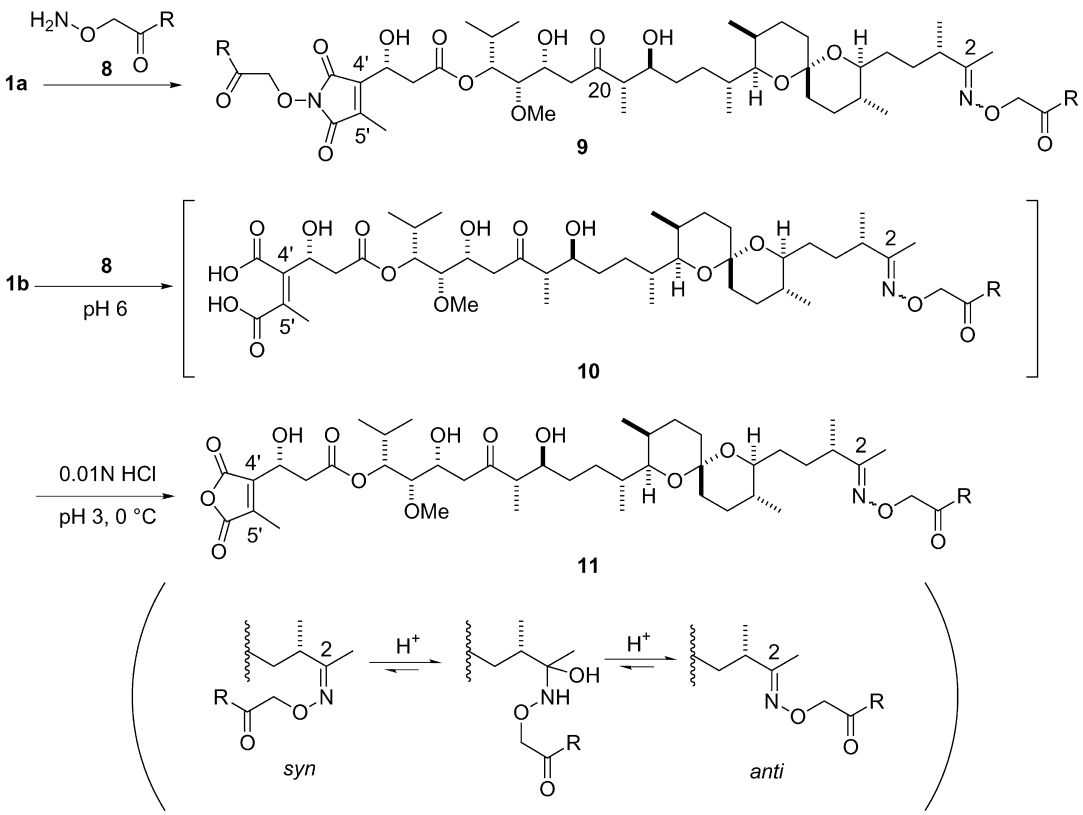
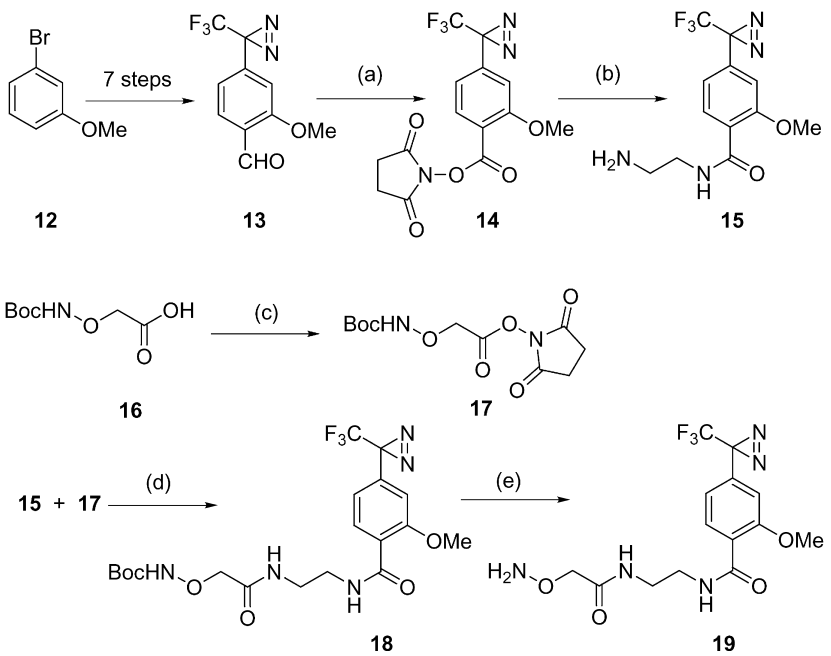


Scheme 2. Photoaffinity probes.

species (TTM and TTMDA) were separable with a short column on HPLC (see Section 7).[10a] However, **1** exhibits an unstable nature under alkaline conditions (MeOH, 20% Cs_2CO_3, pH 9) to cause elimination of C22 hydroxy group (**3**, Scheme 1).[2] This mechanism is suggested to include a trans-esterification before the elimination.[10a] Introduction of photolabeling unit under such a basic condition should be avoided. Therefore, the oxime linkage was selected as a suitable one, because its coupling reaction with **1** could be achieved at pH 6.[12] However, the coupling reaction of **1a** with aminooxy compound **8** raise another serious problem: thus, **8** reacted not only with one of the carbonyl groups, but also with the anhydride moiety of **1** to give the product **9** (Scheme 3). To avoid these problems, we selected the diacid **1b**, for the coupling reaction at pH 6.[10a] The reaction mixture was immediately acidified with 0.01 N hydrochloric acid (HCl) at 0 °C. Under these conditions, The aminooxy compound **8** rapidly receives protonation to oxiammonium salt and loses the nucleophilicity. On the other hand, the diacid moiety in the product **10** was relatively slowly converted into the anhydride oximes **11**, which were separated by HPLC with, for example, MeOH containing 0.1% TFA. During the acidification, the *syn* oxime isomer isomerized to the corresponding *anti* oxime isomer, the latter being is thermodynamically more stable. When the reaction mixture is neutralized, it should be noted that the aminooxy compound **8** recovered the nucleophilicity react with the anhydride moiety of **11**. Therefore, the short column is required to remove the oxiammonium salt from the reaction mixture (see Section 7).



Scheme 3. The strategy for the introduction of photolabeling units.



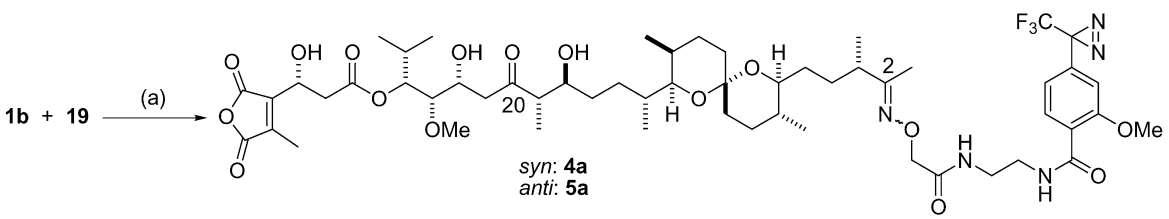
Scheme 4. Synthesis of photolabeling unit bearing the diazirine photophore: (a) (i) $^{n}Bu_4NMnO_4$, pyridine, room temperature, 1 h; (ii) *N*-hydroxysuccinimide, EDC·HCl, DMF, room temperature, 1 h, 74% in two steps; (b) ethylenediamine, MeOH, 0 °C, 7 min, 94%; (c) *N*-hydroxysuccinimide, EDC·HCl, DMF, room temperature, 36 h, 78%; (d) Et_3N, DMF, room temperature, 30 min, 84%; (e) TFA/CH_2Cl_2 (1:1), 0 °C, 30 min.

2. Synthesis of photolabeling unit bearing the diazirine photophore

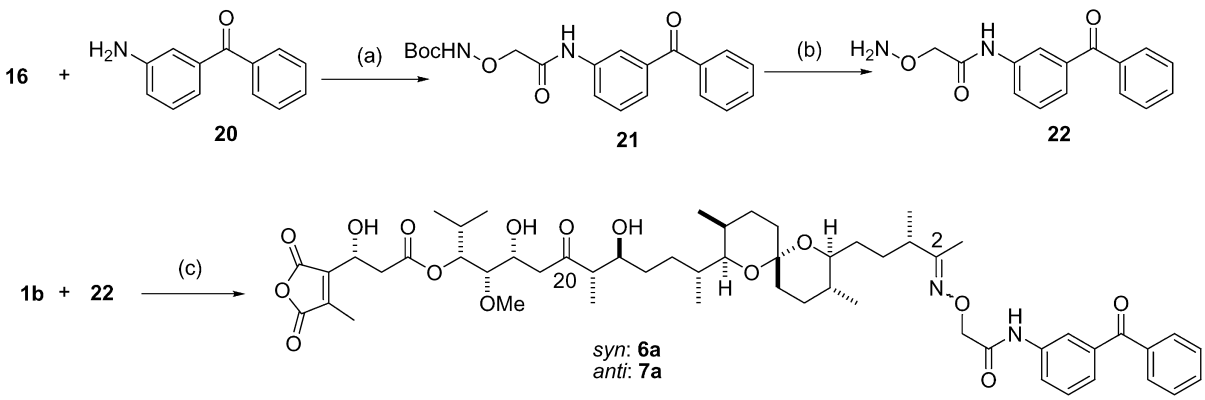
Synthesis of the photolabeling unit (**19**) is illustrated in Scheme 4. According to the method by Hatanaka,[13] we synthesized 2-methoxy-4-(3-trifluoromethyl-3*H*-diazirin-3-yl) benzaldehyde **13** in 34% overall yield from a commercially available 3-bromoanisole **12**. The oxidation of **13** with *n*-tetrabutylammonium permanganate ($^{n}Bu_4MnO_4$)[14] in pyridine was followed by activation of the resulting carboxylic acid with *N*-hydroxysuccinimide in the presence of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC·HCl) to provide the activated ester **14**. In order to insert a spacer between TTMDA **1b** and photolabeling function, **14** was first connected with the ethylenediamine, to provide the amine **15**. Secondly, **15** was connected with the activated ester **17** in the presence of triethylamine (Et_3N) to furnish the protected photolabeling unit **18**. Finally the protective *t*-butoxycarbonyl (Boc) group of **18** was removed with TFA in CH_2Cl_2 to afford the aminooxy compound **19**, which was used in the coupling reaction with **1b** without further purification.

3. Synthesis of photoaffinity probe bearing the diazirine photophore

The synthesis of the diazirine photoaffinity probe (**4a**, **5a**) is summarized in Scheme 5. The coupling reaction of **1b** with photolabeling unit (**19**) was carried out in 50% *N*,*N*′-dimethylacetamide (DMA)/H_2O at pH 6, which was successively acidified with 0.01 N HCl to furnish the corresponding *E*/*Z* regioisomeric mixture of the photoaffinity probe (**4a**, **5a**). No oxime was found on 20-ketone under this condition presumably due to heavily steric congestion around the 20-ketone. The reaction mixture was separated by HPLC (ODS column, CH_3CN/H_2O) to give the pure *Z* isomer **4a** (1.3 mg, 6% in two steps), and the pure *E* isomer **5a** (6.8 mg, 31% in two steps). The *Z* or *E* oxime configurations were determined from ^{1}H NMR spectroscopy on the basis of Karabatsos report, thus, the deshielding effect (N–O group) or shielding effect (N lone pair) exerted by the oxime moiety.[15] In the *Z* isomer **4a** the H-3 chemical shift (δ 3.35 ppm) was down-field by 0.98 ppm in comparison with the corresponding H-3 chemical shift (δ 2.37 ppm) for the *E* isomer **5a**. On the other hand, the H-1 is more shielded by 0.14 ppm in the *Z* isomer **4a** (δ 1.73 ppm)



Scheme 5. Synthesis of photoaffinity probe bearing the diazirine photophore: (a) 50% DMA/H_2O, pH 6, room temperature, 36.5 h, then 0.01 N HCl, pH 3, room temperature, 4 h, *Z*/*E* (1:4), 37% (two steps).



Scheme 6. Synthesis of photoaffinity probe bearing the benzophenone photophore: (a) EDC·HCl, CH_2Cl_2, room temperature, 1 h, 72%; (b) TFA/CH_2Cl_2 (1:1), 0 °C, 3 h; (c) 50% DMA/H_2O, pH 6, room temperature, 48 h, then 0.01 N HCl, pH 3, room temperature, 3 h, *Z*/*E* (1:4), 47% (two steps).

than the corresponding signal in the *E* isomer **5a** (δ 1.87 ppm).

4. Synthesis of photoaffinity probe bearing the benzophenone photophore

The synthesis of the photoaffinity probe bearing the benzophenone photophore (**6a**, **7a**) is summarized in Scheme 6. Treatment of 3-aminobenzophenone (**20**) with the compound **16** in the presence of EDC·HCl furnished the protected photolabeling unit **21**. The Boc group was removed with TFA/CH_2Cl_2 to provide the aminooxy compound **22**. The coupling reaction of **1b** with photolabeling unit (**22**) was carried out under the similar condition as above, to furnish the corresponding *E*/*Z* regioisomeric mixture of the photoaffinity probe (**6a**, **7a**). The reaction mixture was separated by HPLC to give the pure *Z* isomer **6a** (1.6 mg, 10% in two steps), and the pure *E* isomer **7a** (6.0 mg, 37% in two steps).

The PP1γ inhibitory activity of these photoaffinity probes were measured by the firefly bioluminescence system, which have already established in our laboratry.[16] These photoaffinity probes showed high inhibitory activity for PP1 in the range of K_i=10–200 nM (**1b**: 4.5 nM).[17]

5. Photoreactive experiment

Photochemical reaction was examined with **4a**; one of the synthesized diazirine photoaffinity probes. A solution of **4a** (126 μM) in CH_3CN was irradiated with UV lamp (365 nm, FI-5L) at room temperature. The reaction was monitored in the different photoirradiation time from 0 to 7 min by the ESI (electrospray ionization)-Q-TOF (tandem quadrupole/orthogonal-acceleration time-of-flight) MS. According to the time, a peak increased at *m*/*z* 1137 corresponding to the acetonitrile adduct structure being an equivalent of the possible structure **23** [**4a**-N_2+CH_3CN]$^+$ ion (Scheme 7).[18] This result indicates involvement the carbene which would react with PP1γ by the irradiation of UV light for 7 min.

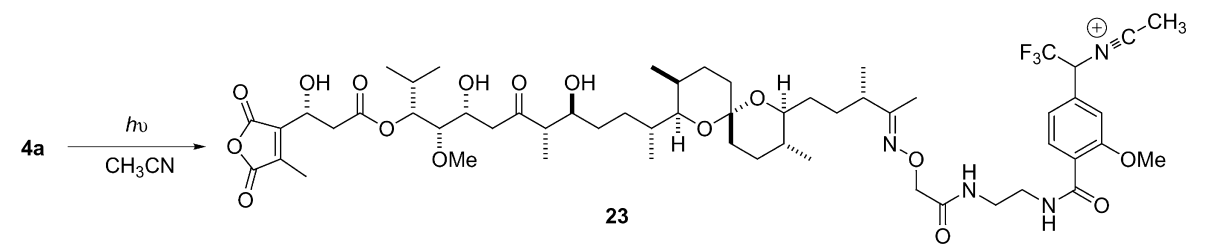
6. Conclusion

Two types of the photoaffinity probe (**4a**, **5a**, **6a**, and **7a**), which possesses a benzophenone or a diazirine photophore on the 2-position of tautomycin, has been accomplished through the selective reaction of the photolabeling units (**19** and **22**) with tautomycin diacid (**1b**). Further studies are in progress in order to detect of modified protein and peptides using matrix assisted laser desorption ionization (MALDI)-TOF-MS as well as HPLC-ESI-Q-TOF-MS and MS/MS.

7. Experimental

7.1. General methods

Melting points were recorded on a Yanaco MP-S3 melting point apparatus. Infrared spectra (IR) were recorded on a JASCO FT/IR-8300 spectrophotometer and are reported in wave number (cm^{-1}). Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a BRUKER AMX-600 (600 MHz), or an ARX-400 (400 MHz), and/or an Avance-400 (400 MHz) spectrometers. Chemical shifts are reported in perts per million (ppm) using tetramethylsilane (δ=0.00 ppm) and dimethylslufoxide-d_6 (δ=2.49 ppm) as an internal standard. Data are reported as follows; chemical



Scheme 7. Photochemical reaction of **4a**.

shift, multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, sext=sextet, br=broad, m=multiplet), coupling constant(s), and assignment, respectively. Carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a BRUKER AMX-600 (150 MHz), an ARX-400 (100 MHz), and/or an Avance-400 (100 MHz) spectrometers. Chemical shifts are reported in ppm using $CDCl_3$ (δ=77.0 ppm) and dimethylslufoxide-d_6 (δ=39.7 ppm) as an internal standard. Data are reported as follows; chemical shift, multiplicity (q=quartet), coupling constant(s), and assignment, respectively. Fluoro nuclear magnetic resonance (^{19}F NMR) spectra were recorded on an Avance-400 (376.5 MHz) spectrometer. Chemical shifts are reported in ppm using $CFCl_3$ (δ=0.00 ppm) as an external standard. 2D NMR (COSY, HMBC, HMQC, and HSQC) spectra were measured at a BRUKER AMX-600 (600 MHz), or a BRUKER ARX-400 (400 MHz) and/or an Avance-400 (400 MHz) spectrometers. Tautomycin and photoaffinity probes numbering corresponding to the front page are employed for assignment of ^{1}H NMR. MS spectra were measured utilizing a Q-TOF mass spectrometer (Micromass, Manchester, UK) equipped with a Z-spray type ESI source. HPLC analyses were performed with combination of Jasco PU-980 pump, a Rheodyne model 7125 sample injector, a Jasco VL-611 Variable loop injector, a Jasco UV-970 UV/VIS detector, and a Jasco 807-IT integrator. Elemental analyses were performed by Mr S. Kitamura in Analytical Laboratory at Bioagricultural Sciences, Nagoya University to whom the authors gratefully acknowledge. Unless otherwise noted, the reaction flask was wrapped with aluminium foil to protect from light, and non-aqueous reactions were carried out under nitrogen or argon atmosphere. 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 12molybdo(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 CH_2Cl_2 was distilled from CaH_2 under nitrogen atmosphere. Pyridine and Et_3N were dried over anhydrous KOH pellets. All other commercially available reagents were used as received.

Tautomycin (**1**) was kindly provided by Dr. K. Isono (ex-Riken Institute) and further purified by the procedure of the method described previously with slight modification.[19]

7.1.1. Tautomycin diacid (TTMDA, 1b). TTM (**1a**, 24 mg, 30.4 mmol) was dissolved in 1.4 ml of 80% CH_3CN/H_2O, and adjusted to pH 8 with aqueous $NaHCO_3$ (28 mg/ml). After being stirred for 5 h at room temperature, a major compound was detected by HPLC (Develosil ODS-UG-5 (i.d. 4.6×250 mm), 85% CH_3CN/H_2O, 1.0 ml/min, UV 254 nm, and T_r=2.4 min). The reaction mixture was neutralized with 0.01 N HCl at 0 °C, concentrated and then purified by HPLC (Develosil ODS-10/20 (i.d. 4.6×50 mm), 70% CH_3CN/H_2O, 1.0 ml/min, UV 254 nm, and T_r=0.6 min). The fraction was evaporated and lyophilized to give TTMDA (**1b**, 23 mg, 95%) as a white powder.

7.1.2. 2-Methoxy-4-(3-trifluoromethyl-3*H*-diazirin-3-yl)-benzoic acid 2,5-dioxopyrrolidin-1-yl ester (14). To a solution of benzaldehyde **13** (0.75 g, 3.06 mmol) in pyridine (2.0 ml) was added a solution of *n*-tetrabutylammonium permanganate[14] (1.66 g, 3.06 mmol) in pyridine (10.2 ml). After being stirred for 1 h at room temperature, the reaction mixture was diluted with water, acidified with 1 N HCl to pH 3, and saturated aqueous $NaHSO_3$ was added until all the MnO_2 precipitates dissolved. The colorless solution was extracted with Et_2O and the organic layer was combined, washed with sat. $CuSO_4$, water. The combined organic phase was dried over Na_2SO_4 and then concentrated under reduced pressure to afford the crude aromatic acid (0.77 g). To a solution of the crude acid (352 mg, 1.35 mmol) in DMF (10.0 ml) was added *N*-hydroxysuccinimide (164 mg, 1.42 mmol), a solution of EDC·HCl (286 mg, 1.49 mmol) in DMF (24.0 ml) at room temperature. After being stirred for 1 h at room temperature, the solvent was evaporated in vacuo. The reaction mixture was extracted with CH_2Cl_2 (×3) and the organic layer was combined, and washed with water (×3) and brine. The combined organic phase was dried over Na_2SO_4. After evaporation of the solvent, the residue was purified by column chromatography (silica gel 8.0 g, ethyl acetate/hexane=1:2, then 2:1) to give the activated ester **14** (357 mg, 74% in two steps) as a yellow oil.

7.1.3. Compound 14. IR (KBr) λ_{max} 1774, 1740, 1297, 1274, 1194, 827 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.89 (4H, s, COC*H*$_2$C*H*$_2$CO), 3.93 (3H, s, 2-OC*H*$_3$), 6.72 (1H, br s, Ar-*H*), 6.87 (1H, br d, *J*=8.4 Hz, Ar-*H*), 8.05 (1H, d, *J*=8.4 Hz, Ar-*H*). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 25.7, 28.5 (q, *J*=40.7 Hz), 56.3, 110.0, 115.6, 118.2, 122.3 (q, *J*=273.1 Hz), 133.2, 136.9, 159.7, 160.4, 169.1. Anal. Calcd for $C_{14}H_{10}F_3N_3O_5$: C, 47.07; H, 2.82; N, 11.76. Found: C, 47.07; H, 2.77; N, 11.63.

7.1.4. *N*-(2-Amino-ethyl)-2-methoxy-4-(3-trifluoromethyl-3*H*-diazirin-3-yl)-benzamide (15). To a solution of the activated ester **14** (103 mg, 0.29 mmol) in MeOH (85 ml) was added ethylenediamine (863 mg, 14.36 mmol) at 0 °C. After being stirred for 7 min, the reaction mixture was concentrated to one-tenth of its original volume. The residue was purified by column chromatography (Silica Gel 60 N (spherical, neutral), 10 g, CH_2Cl_2/MeOH/*i*-PrNH$_2$=20:1:0.3) to give the compound **15** (82 mg, 94%) as a yellow oil.

7.1.5. Compound 15. IR (KBr) λ_{max} 3386, 1647, 1613, 1541, 1260, 1154, 829 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.93 (2H, m, NH$_2$C*H*$_2$C*H*$_2$NH), 3.52 (2H, m, NH$_2$C*H*$_2$C*H*$_2$-NH), 3.98 (3H, s, 2-OC*H*$_3$), 6.71 (1H, d, *J*=1.8 Hz, Ar-*H*), 6.90 (1H, br d, *J*=8.4 Hz, Ar-*H*), 8.07 (1H, s, CON*H*), 8.21 (1H, d, *J*=8.4 Hz, Ar-*H*). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.3 (q, *J*=40.4 Hz), 41.3, 42.6, 56.0, 109.2, 119.1, 121.8 (q, *J*=273.1 Hz), 123.0, 132.7, 133.4, 157.3, 164.3. ESI-Q-TOF-MS Calcd for $C_{12}H_{14}F_3N_4O_2^+$ 303.1069 ([M+H]$^+$). Found 303.1050.

7.1.6. 2-*tert*-Butoxycarbonylaminoxy-acetic acid 2,5-dioxo-pyrrolidin-1-yl ester (17). To a solution of *N*-*tert*-butoxycarbonyl-aminooxy acetic acid **16** (34 mg, 0.18 mmol) in DMF (0.7 ml) was added *N*-hydroxysuccinimide (25 mg, 0.22 mmol) and then a solution of EDC·HCl (41 mg, 0.22 mmol) in DMF (2.0 ml) at room temperature.

After being stirred for 36 h at room temperature, the solvent was evaporated in vacuo. The residual mixture was extracted with AcOEt (×3). The combined organic phase was washed with water (×3), brine, and dried over Na_2SO_4. After evaporation of the solvent, the residue was purified by column chromatography (silica gel 1.5 g, ethyl acetate/hexane=1:1, then ethyl acetate) to give the activated ester **17** (40 mg, 78%) as a colorless oil.

7.1.7. Compound 17. IR (KBr) λ_{max} 3280, 2983, 1741, 1371, 1253, 1208, 1163 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.49 (9H, s, C(C*H*$_3$)$_3$), 2.88 (4H, s, COC*H*$_2$C*H*$_2$CO), 4.78 (2H, s, H-2), 7.80 (1H, s, CON*H*). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 25.3, 28.1, 70.8, 82.7, 156.3, 165.0, 168.7. ESI-Q-TOF-MS calcd for $C_{11}H_{16}N_2NaO_7^+$ 311.0855 ([M+Na]$^+$). Found 311.0913.

7.1.8. *N*-[2-(2-Aminooxy-acetylamino)-ethyl]-*N*-*tert*-butoxycarbonyl-2-methoxy-4-(3-trifluoromethyl-3*H*-diazirin-3-yl)-benzamide (18). A solution of compound **15** (23 mg, 0.08 mmol) in DMF (0.2 ml) was mixed with Et_3N (19 mg, 0.19 mmol) at 0 °C and a solution of compound **17** (32 mg, 0.12 mmol) in DMF (1.2 ml) at room temperature. After being stirred for 30 min at room temperature, the solution was evaporated in vacuo. The reaction mixture was extracted with CH_2Cl_2 (×3). The combined organic phase was washed with water (×3) and brine, dried over Na_2SO_4. After evaporation of the solvent, the residue was purified by column chromatography on silica gel (silica gel 1.0 g, ethyl acetate/hexane=1:1, then ethyl acetate) to give the protected photolabeling unit **18** (31 mg, 84%) as a colorless oil.

7.1.9. Compound 18. IR (KBr) λ_{max} 3380, 1725, 1653, 1540, 1160, 851 cm^{-1}. ^1H NMR ($CDCl_3$, 600 MHz) δ 1.43 (9H, s, C(C*H*$_3$)$_3$), 3.57 (2H, m, NHC*H*$_2$C*H*$_2$NH), 3.65 (2H, m, NHC*H*$_2$C*H*$_2$NH), 3.98 (3H, s, OC*H*$_3$), 4.32 (2H, s, COC*H*$_2$ONH), 6.70 (1H, br s, Ar-*H*), 6.89 (1H, br d, *J*=8.2 Hz, Ar-*H*), 8.20 (4H, m, Ar-*H*, amides-N*H*). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 28.01, 28.33 (q, *J*=40.5 Hz), 39.17, 39.96, 56.07, 76.08, 82.75, 109.24, 119.13, 121.83 (q, *J*=273.0 Hz), 122.50, 132.78, 133.76, 157.56, 157.60, 165.20, 169.58. Anal. Calcd for $C_{19}H_{24}F_3N_5O_4$: C, 47.07; H, 2.82; N, 11.76. Found: C, 47.07; H, 2.77; N, 11.63.

7.1.10. Photoaffinity probe bearing the diazirine photophore (4a, 5a). To a solution of **18** (20.9 mg, 43.96 μmol) in CH_2Cl_2 (1.2 ml) was slowly added TFA (1.2 ml) at 0 °C. After being stirred for 30 min the reaction mixture was evaporated. To remove of TFA, the residue was dissolved in water and concentrated under reduced pressure to provide the aminooxy compound **19**, which was used in the coupling reaction with **1b** without purification.

To a solution of **1b** (15.5 mg, 19.75 μmol) in 50% DMA/H_2O (2.0 ml) was added a solution of **19** in 50% DMA/H_2O (0.6 ml), which had preliminarily and carefully adjusted to pH 6 with 0.1 N NaOH. The progress of reaction was monitored by HPLC (Develosil ODS-UG-5 (i.d. 4.6×250 mm), 85% CH_3CN/H_2O, 1.0 ml/min, and UV 254 nm). After stirring magnetically for 36.5 h at room temperature, the reaction mixture was poured into a cold 0.01 N HCl solution, and stirring for 4 h at room temperature. The two regioisomeric (*E* and *Z*) oximes were detected by HPLC (Develosil ODS-UG-5 (i.d. 4.6×250 mm), 85% CH_3CN/H_2O, 1.0 ml/min, UV 254 nm), the *Z* isomer was the earlier elute (T_r=13 min) and the *E* isomer was the later elute (T_r=15 min). The reaction mixture was separated by short column chromatography on silica gel (Develosil ODS-10/20, CH_3CN/H_2O=1:1, then 3:1) to remove the oxiammonium salt. The fractions were concentrated, and separated by HPLC (Develosil ODS-UG-5 (i.d. 10.0×250 mm), 85% CH_3CN, 4.0 ml/min, T_r=14, 17 min) to give the pure *Z* isomer **4a** (1.3 mg, 6% in two steps) as a colorless oil, and the pure *E* isomer **5a** (6.8 mg, 31% in two steps) as a colorless oil. The total yield was in the range of 30–51% on repeated runs.

7.1.11. Compound 4a. IR (KBr) λ_{msax} 3854, 1767, 1647, 1540, 1259, 1162 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 0.80–1.72 (17H, m), 0.80 (3H, d, *J*=6.7 Hz, 7-C*H*$_3$), 0.88 (3H, d, *J*=7.0 Hz, 13-C*H*$_3$), 0.96 (3H, d, *J*=6.0 Hz, 25-C*H*$_3$), 0.98 (3H, d, *J*=6.0 Hz, H-26), 0.98 (3H, d, *J*=6.5 Hz, 15-C*H*$_3$), 1.04 (3H, d, *J*=7.0 Hz, 3-C*H*$_3$), 1.09 (3H, d, *J*=7.0 Hz, 19-C*H*$_3$), 1.73 (3H, s, H-1), 1.82 (1H, m, H-13), 1.99 (1H, m, H-12b), 2.11 (1H, sext, *J*=5.9 Hz, H-25), 2.26 (3H, d, *J*=1.2 Hz, 5′-C*H*$_3$), 2.66 (1H, m, H-19), 2.67 (1H, dd, *J*=17.4, 4.2 Hz, H-21a), 2.77 (1H, dd, *J*=16.2, 9.8 Hz, H-2′a), 2.92 (1H, dd, *J*=16.4, 3.6 Hz, H-2′b), 2.98 (1H, dd, *J*=17.5, 8.6 Hz, H-21b), 3.17 (1H, m, H-6), 3.23 (1H, dd, *J*=10.0, 2.2 Hz, H-14), 3.27 (1H, dd, *J*=6.0, 2.0 Hz, H-23), 3.35 (1H, q, *J*=7.2 Hz, H-3), 3.44 (3H, s, 23-OC*H*$_3$), 3.49–3.62 (4H, m, H-3″, 4″), 3.72 (1H, m, H-18), 3.99 (3H, s, 7″-OC*H*$_3$), 4.36 (1H, m, H-22), 4.46 (2H, s, H-1″), 4.60–4.65 (1H, br m, 3′-OH), 5.09 (1H, dd, *J*=6.2, 6.0 Hz, H-24), 5.20 (1H, m, H-3′), 6.70 (1H, d, *J*=1.2 Hz, H-8″), 6.74 (1H, m, CON*H*CH$_2$), 6.89 (1H, ddd, *J*=8.2, 1.0, 0.5 Hz, H-10″), 8.12 (1H, m, CH$_2$N*H*COAr), 8.17 (1H, d, *J*=8.0 Hz, H-11″). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 10.1, 11.0, 13.6, 15.3, 16.8, 17.0, 17.9, 18.0, 19.4, 26.7, 27.2, 27.7, 28.1, 28.7, 29.4, 30.2, 30.7, 31.5, 32.3, 34.6, 34.9, 36.1, 39.0, 40.1, 40.9, 45.7, 52.4, 56.1, 59.1, 63.9, 66.5, 72.3, 74.1, 74.4, 75.0, 76.5, 80.7, 95.7, 109.3, 119.1, 122.4, 132.9, 133.9, 142.2, 142.9, 157.6, 164.8, 165.0, 165.1, 165.8, 169.5, 171.3, 215.2. ^{19}F NMR ($CDCl_3$, 376.5 MHz) δ -64.83. ESI-Q-TOF-MS Calcd for $C_{55}H_{81}F_3N_5O_{16}^+$ 1124.5630 ([M+H]$^+$). Found 1124.5548.

7.1.12. Compound 5a. IR (KBr) λ_{max} 3417, 1768, 1653, 1540, 1258, 1160 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 0.75–1.85 (17H, m), 0.89 (3H, d, *J*=6.8 Hz, 7-C*H*$_3$), 0.87 (3H, d, *J*=7.0 Hz, 13-C*H*$_3$), 0.96 (3H, d, *J*=5.0 Hz, 25-C*H*$_3$), 0.97 (3H, d, *J*=5.0 Hz, H-26), 0.97 (3H, d, *J*=6.5 Hz, 15-C*H*$_3$), 1.02 (3H, d, *J*=7.0 Hz, 3-C*H*$_3$), 1.06 (3H, d, *J*=7.0 Hz, 19-C*H*$_3$), 1.80–1.85 (1H, m, H-13), 1.87 (3H, s, H-1), 1.95–2.00 (1H, m, H-12b), 2.11 (1H, m, H-25), 2.26 (3H, d, *J*=1.4 Hz, 5′-C*H*$_3$), 2.37 (1H, q, *J*=7.0 Hz, H-3), 2.68 (1H, dd, *J*=8.0, 7.2 Hz, H-19), 2.72 (1H, dd, *J*=17.4, 4.6 Hz, H-21a), 2.76 (1H, dd, *J*=16.0, 9.8 Hz, H-2′a), 2.90 (1H, dd, *J*=16.0, 3.5 Hz, H-2′b), 2.97 (1H, dd, *J*=17.4, 8.0 Hz, H-21b), 3.16 (1H, td, *J*=9.6, 1.2 Hz, H-6), 3.28 (1H, dd, *J*=5.4, 2.0 Hz, H-14), 3.30 (1H, d, *J*=2.5 Hz, H-23), 3.40–3.64 (6H, m, H-3″, 4″, 18-OH, 22-OH), 3.44 (3H, s, 23-OC*H*$_3$), 3.70 (1H, m, H-18), 3.99 (3H, s, 7″-OC*H*$_3$), 4.38 (1H, m, H-22), 4.45 (2H, s, H-1″), 4.77 (1H, m, 3′-OH), 5.09 (1H, t, *J*=6.2 Hz, H-24), 5.20 (1H, m, H-3′), 6.70 (1H, d, *J*=1.8 Hz, H-8″), 6.91 (1H, dd, *J*=8.4, 1.8 Hz, H-10″), 7.03

(1H, t, J=5.0 Hz, CONHCH$_2$), 8.13 (1H, m, CH$_2$NHCOAr), 8.16 (1H, d, J=8.4 Hz, H-11″). ^{13}C NMR (CDCl$_3$, 100 MHz) δ 10.1, 11.0, 11.3, 13.5, 16.8, 17.0, 17.2, 18.0, 19.4, 26.8, 27.2, 27.6, 28.1, 28.7, 29.4, 30.2, 30.3, 31.8, 35.0, 35.1, 36.1, 38.6, 39.7, 39.8, 41.0, 46.1, 52.5, 56.2, 59.1, 63.8, 66.4, 72.4, 73.4, 74.1, 74.8, 76.5, 80.7, 95.5, 109.3, 119.2, 121.9 (q, J=273.0 Hz), 122.2, 133.0, 134.0, 142.3, 142.9, 157.6, 164.3, 164.8, 165.3, 165.9, 169.4, 171.6, 215.3. ^{19}F NMR (CDCl$_3$, 376.5 MHz) δ -64.83. ESI-Q-TOF-MS Calcd for $C_{55}H_{81}F_3N_5O_{16}^+$ 1124.5630 ([M+H]$^+$). Found 1124.5658.

7.1.13. 2-(*N*-*tert*-Butoxycarbonyl-aminooxy)-*N*-(3-benzoyl-phenyl)-acetamide (21). To a solution of *N*-*tert*-butoxycarbonyl-aminooxy acetic acid (**16**, 101 mg, 0.53 mmol) in CH$_2$Cl$_2$ was added 3-aminobenzophenone (**20**, 219 mg, 1.06 mmol) and EDC·HCl (253 mg, 1.32 mmol). The mixture was stirred for 1 h at room temperature, diluted with CH$_2$Cl$_2$ and washed with 5% NaHCO$_3$ aqueous solution. The organic layer was dried over Na$_2$SO$_4$. After evaporation of the solvent, the residue was purified by column chromatography (silica gel 2.0 g, ethyl acetate/hexane=1:3, then 1:1) to give **21** (140.0 mg, 72%) as a colorless solid.

7.1.14. Compound 21. Mp 129–130 °C (as white tiny needles from ether-hexane). IR (KBr) λ_{max} 3262, 2980, 2934, 1725, 1663, 1591, 1559, 1486, 1448, 1321, 1286, 1256, 1163, 1114, 721 cm^{-1}. ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.45 (9H, s, C(CH$_3$)$_3$), 4.43 (2H, s, COCH$_2$O), 7.46 (1H, dd, J=8.0, 5.5 Hz, Ar-*H*), 7.48 (2H, ddd, J=8.0, 5.5, 2.0 Hz, Ar-*H*), 7.55–7.59 (2H, m, Ar-*H*), 7.82 (2H, m, Ar-*H*), 8.01 (1H, ddd, J=8.0, 2.0, 1.0 Hz, Ar-*H*), 8.06 (1H, t, J=2.0 Hz, Ar-*H*), 8.10 (1H, s, CONH), 10.52 (1H, s, CH$_2$ONH). ^{13}C NMR (100.6 MHz, CDCl$_3$) δ 28.0, 76.6, 83.6, 121.5, 123.8, 125.7, 128.2, 128.9, 130.0, 132.4, 137.4, 137.9, 138.2, 158.5, 167.5, 169.3. Anal. Calcd for $C_{20}H_{22}N_2O_5$: C, 64.85; H, 5.99, N, 7.56. Found: C, 64.86; H, 5.95, N; 7.52.

7.1.15. Photoaffinity probe bearing the benzophenone photophore (6a, 7a). To a solution of **21** (12.0 mg, 32.40 μmol) in CH$_2$Cl$_2$ (0.8 ml) was slowly added TFA (0.8 ml) at 0 °C. After being stirred for 3 h, the reaction mixture was evaporated. To remove TFA, the residue was dissolved in water and concentrated under reduced pressure to provide the aminooxy compound **22**, which was used in the coupling reaction with **1b** without purification.

To a solution of **1b** (12.7 mg, 16.08 μmol) in 50% DMA/H$_2$O (0.1 ml) was added a solution of **22** in 50% DMA/H$_2$O (0.8 ml). This mixture was carefully maintained at pH 6 with 0.1 N NaOH. The progress of reaction was monitored by HPLC (Develosil ODS-UG-5 (i.d. 4.6×250 mm), 85% CH$_3$CN/H$_2$O, 1.0 ml/min, and UV 254 nm). After stirring magnetically for 48 h at room temperature, the reaction mixture was poured into a cold 0.01 N HCl solution, and stirring magnetically for 3 h at room temperature. The two regioisomeric (*E* and *Z*) oximes were detected by HPLC (Develosil ODS-UG-5 (i.d. 4.6×250 mm), 85% CH$_3$CN/H$_2$O, 1.0 ml/min, and UV 254 nm), the *Z* isomer eluted at 12 min, while the *E* isomer eluted at 14 min. The reaction mixture was separated by short column chromatography on silica gel (Develosil ODS-10/20, CH$_3$CN/H$_2$O=1:1, then 3:1) to remove the oxiammonium salt, the fractions were concentrated, and separated by HPLC (Develosil ODS-UG-5 (i.d. 10.0×250 mm), 85% CH$_3$CN/H$_2$O, 4.0 ml/min, T_r=15, 17 min) to give the pure *Z* isomer **6a** (1.6 mg, 10% in two steps) as a light yellow oil, and the pure *E* isomer **7a** (6.0 mg, 37% in two steps) as a light yellow oil. The total yield was in the range of 30–56% on repeated runs. The *Z* or *E* configurations for the above reported oximes were assessed by ^{1}H NMR spectroscopy. In the *Z* isomer **6a** the H-3 chemical shift (3.41 ppm) is down-field by 0.94 ppm in comparison with the corresponding H-3 chemical shift (2.47 ppm) for the *E* isomer **7a**. Conversely, the H-1 is more shielded (0.1 ppm) in the *Z* isomer **6a** (1.83 ppm) than the corresponding signal in the *E* isomer **7a** (1.93 ppm).

7.1.16. Compound 6a. IR (KBr) λ_{max} 3406, 2930, 1768, 1700, 1541, 1282, 1077, 987 cm^{-1}. ^{1}H NMR (600 MHz, CDCl$_3$) δ 0.79 (3H, d, J=6.8 Hz, 7-CH$_3$), 0.84–1.82 (19H, m), 0.87 (3H, d, J=7.0 Hz, 13-CH$_3$), 0.97 (3H, d, J=7.0 Hz, 25-CH$_3$), 0.97 (3H, d, J=7.0 Hz, H-26), 0.98 (3H, d, J=7.0 Hz, 15-CH$_3$), 1.09 (3H, d, J=7.1 Hz, 19-CH$_3$), 1.11 (3H, d, J=7.0 Hz, 3-CH$_3$), 1.83 (3H, s, H-1), 1.99 (1H, tt, J=10.0, 4.2 Hz, H-12b), 2.12 (1H, m, H-25), 2.26 (3H, d, J=0.8 Hz, 5′-CH$_3$), 2.54 (1H, br s, 18-OH), 2.66 (1H, m, H-19), 2.66 (1H, dd, J=17.3, 4.1 Hz, H-21a), 2.77 (1H, dd, J=16.3, 9.8 Hz, H-2′a), 2.92 (1H, dd, J=16.3, 3.2 Hz, H-2′b), 2.98 (1H, dd, J=17.5, 8.3 Hz, H-21b), 3.17 (1H, td, J=9.4, 2.5 Hz, H-6), 3.23 (1H, dd, J=10.0, 2.1 Hz, H-14), 3.27 (1H, dd, J=5.6, 2.0 Hz, H-23), 3.41 (1H, sext, J=7.0 Hz, H-3), 3.43 (3H, s, OCH$_3$), 3.70 (1H, td, J=8.0, 1.5 Hz, H-18), 4.36 (1H, m, H-22), 4.55 (1H, br s, 3′-OH), 4.59 (2H, s, H-1″), 5.09 (1H, t, J=6.0 Hz, H-24), 5.21 (1H, d, J=9.1 Hz, H-3′), 7.45 (1H, t, J=7.9 Hz, H-7″), 7.49 (1H, t, J=7.9 Hz, H-12″), 7.49 (1H, t, J=7.9 Hz, H-14″), 7.51 (1H, t, J=7.9 Hz, H-6″), 7.60 (1H, t, J=7.5 Hz, H-13″), 7.81 (1H, t, J=6.4 Hz, H-11″), 7.81 (1H, t, J=6.4 Hz, H-15″), 7.82 (1H, dd, J=2.0, 1.4 Hz, H-4″), 7.93 (1H, dd, J=7.5, 1.9 Hz, H-8″), 8.12 (1H, br s, CONHAr). ^{13}C NMR (150 MHz, CDCl$_3$) δ 10.2, 10.9, 13.7, 15.4, 16.9, 17.3, 18.0, 18.0, 19.4, 26.7, 27.3, 27.6, 28.0, 28.7, 29.5, 30.1, 30.8, 31.5, 32.5, 34.5, 34.9, 36.0, 41.0, 45.8, 52.4, 59.0, 63.9, 66.4, 72.6, 74.1, 74.4, 75.0, 76.4, 80.6, 95.7, 121.0, 123.9, 126.3, 128.3, 128.4, 129.0, 130.1, 130.1, 132.7, 137.2, 137.4, 138.4, 142.1, 143.0, 164.8, 165.8, 165.9, 168.9, 169.5, 196.2, 215.3. ESI-Q-TOF-MS calcd for $C_{56}H_{79}N_2O_{15}^+$ 1019.5480 ([M+H]$^+$). Found 1019.5478.

7.1.17. Compound 7a. IR (KBr) λ_{max} 3432, 2933, 1767, 1661, 1540, 1283, 1099, 918 cm^{-1}. ^{1}H NMR (600 MHz, CDCl$_3$) δ 0.77 (3H, d, J=6.8 Hz, 7-CH$_3$), 0.88–1.68 (19H, m), 0.79 (3H, d, J=7.0 Hz, 13-CH$_3$), 0.96 (3H, d, J=7.0 Hz, 25-CH$_3$), 0.97 (3H, d, J=7.1 Hz, H-26), 0.97 (3H, d, J=6.5 Hz, 15-CH$_3$), 1.07 (3H, d, J=7.0 Hz, 19-CH$_3$), 1.09 (3H, d, J=7.0 Hz, 3-CH$_3$), 1.82 (1H, tt, J=13.1, 5.5 Hz, H-12b), 1.93 (3H, s, H-1), 2.11 (1H, m, H-25), 2.26 (3H, d, J=0.8 Hz, 5′-CH$_3$), 2.47 (1H, sext, J=6.9 Hz, H-3), 2.69 (1H, m, H-19), 2.75 (1H, dd, J=17.5, 4.8 Hz, H-21a), 2.76 (1H, dd, J=16.3, 9.8 Hz, H-2′a), 2.92 (1H, dd, J=16.3, 3.1 Hz, H-2′b), 2.97 (1H, dd, J=17.5, 8.3 Hz, H-21b), 3.15 (1H, t, J=9.8 Hz, H-6), 3.24 (1H, dd, J=10.0, 2.2 Hz, H-14), 3.26 (1H, br s, 18-OH), 3.28 (1H, dd, J=5.6, 2.8 Hz, H-23), 3.44 (3H, s, OCH$_3$), 3.70 (1H, td, J=8.0, 2.0 Hz, H-18), 4.38 (1H, m, H-22), 4.55 (1H, d, J=16.2 Hz, H-1″a),

4.57 (1H, d, J=16.2 Hz, H-1″b), 4.65 (1H, br s, 3′-OH), 5.10 (1H, dd, J=6.8, 6.0 Hz, H-24), 5.21 (1H, d, J=9.1 Hz, H-3′), 7.45 (1H, t, J=7.9 Hz, H-7″), 7.49 (1H, t, J=7.9 Hz, H-12″), 7.49 (1H, t, J=7.9 Hz, H-14″), 7.50 (1H, t, J=7.9 Hz, H-6″), 7.60 (1H, t, J=7.5 Hz, H-13″), 7.80 (1H, m, H-11″), 7.80 (1H, m, H-15″), 7.80 (1H, m, H-4″), 7.98 (1H, dd, J=7.8, 1.9 Hz, H-8″), 8.14 (1H, br s, CON*H*Ar). ^{13}C NMR (150 MHz, $CDCl_3$) δ 10.1, 10.8, 11.5, 13.5, 16.8, 17.1, 18.0, 18.0, 19.4, 26.6, 27.1, 27.5, 28.0, 28.7, 29.3, 30.1, 30.1, 31.9, 35.0, 35.0, 36.0, 38.6, 41.0, 46.3, 52.3, 59.0, 63.8, 66.3, 72.6, 73.2, 74.2, 74.9, 76.4, 80.6, 95.5, 120.6, 123.5, 126.3, 128.3, 128.4, 129.0, 130.1, 130.1, 132.7, 137.2, 137.4, 138.4, 142.2, 142.9, 164.8, 165.5, 165.8, 169.3, 169.4, 196.1, 215.5. ESI-Q-TOF-MS calcd for $C_{56}H_{79}N_2O_{15}^+$ 1019.5480 ($[M+H]^+$). Found 1019.5416.

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