Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron
Tetrahedron 63 (2007) 2593–2603

Synthesis of the second generation photoaffinity probes of tautomycin

Magne O. Sydnes and Minoru Isobe*

Laboratory of Organic Chemistry, Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan

Received 20 January 2007; accepted 23 January 2007
Available online 25 January 2007

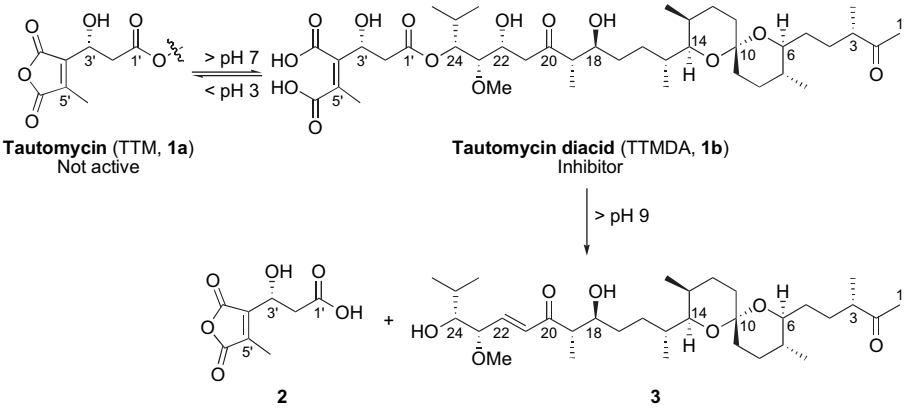
Abstract—Five photoaffinity probes of tautomycin, which possess an aromatic azide with linker attached to the 2-position of tautomycin, were prepared in order to study the binding site of tautomycin with protein phosphatase 1γ. The photoaffinity probes were synthesized by selectively introducing the photolabeling units onto the 2-position of tautomycin by using oxime chemistry.
© 2007 Elsevier Ltd. All rights reserved.

1. Introduction

Tautomycin (TTM, **1**, Scheme 1) was first isolated in 1987[1] and structurally elucidated in 1990 by Isono et al.[2] TTM was found to be a specific inhibitor of protein phosphatase (PP) 1 and 2A, which are two of in total four major enzymes that dephosphorylate serine/threonine residues of proteins in eukaryotic cells.[3] Three other natural products, namely microcystin-LR (**4**), okadaic acid (**5**), and calyculin A (**6**) (see Fig. 1), have also been found to inhibit PP1 and PP2A.[4] These three compounds are stronger inhibitors of PP2A than PP1, while TTM was found to inhibit PP1 more selectively than PP2A.[4]

The X-ray crystallographic structure of the complex of PP1 and the three later natural products, namely microcystin-LR,[5] okadaic acid,[6] and calyculin A,[7] have provided detailed information of the interaction between the protein and the toxins. However, no such X-ray crystallography data has yet been reported for the PP1–TTM complex. An artifact probably caused by the non-crystalline nature of TTM.

Our group has for some time been involved in work toward the elucidation of the binding site of PP1–TTM. We recently reported the synthesis of five photoaffinity probes of tautomycin, which possessed a benzophenone or a diazirine photophore attached to the 2-position of the natural product.[8]



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

Keywords: Tautomycin; Photoaffinity probe; Photolabeling; Protein phosphatase.
* Corresponding author. Tel.: +81 52 789 4109; e-mail: isobem@agr.nagoya-u.ac.jp

0040–4020/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2007.01.047

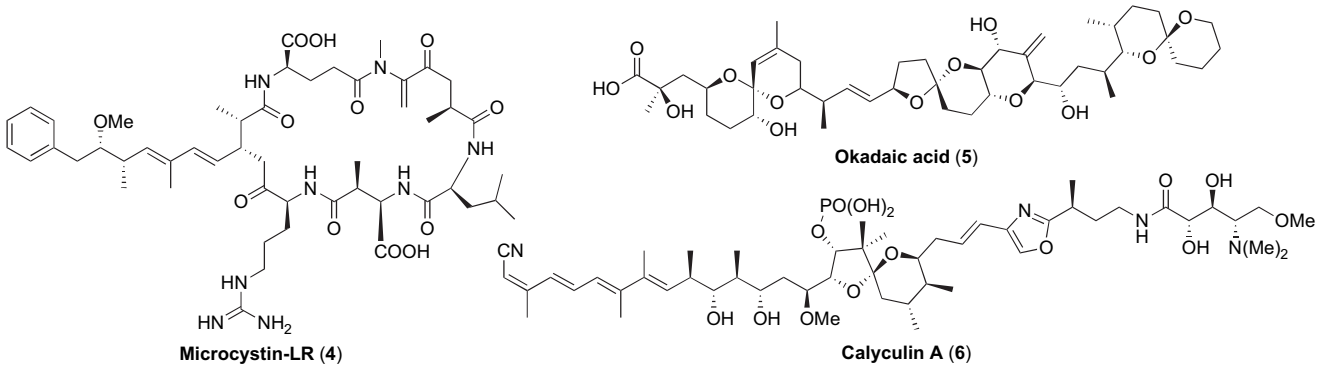


Figure 1. The structure of microcystin-LR (**4**), okadaic acid (**5**), and calyculin A (**6**).

A synthesis of ^{13}C-labeled tautomycin has also been achieved and the interaction between PP1 and the natural product has been studied by ^{13}C NMR.[9] However, these studies have not, as of yet, enabled us to elucidate the binding site for TTM with PP1, mainly due to low chemical yield for the photochemical reaction.

Herein, we report the synthesis of a series of new photoaffinity probes of tautomycin (compounds **7**–**9**), which possess an aryl fluoro azide photophore[10,11] attached to the 2-position of TTM through linkers with various length and functionality (Scheme 2). These compounds will be used in order to study the binding interaction of TTM–PP1 using different MS techniques. Photoaffinity probe **9** also incorporates a disulfide bond, that can be cleaved by a number of reducing agents including dithiothreitol (DTT),[12] thus reducing the molecular weight of the bound photoaffinity probes with PP1 making it more suitable for MS analysis.

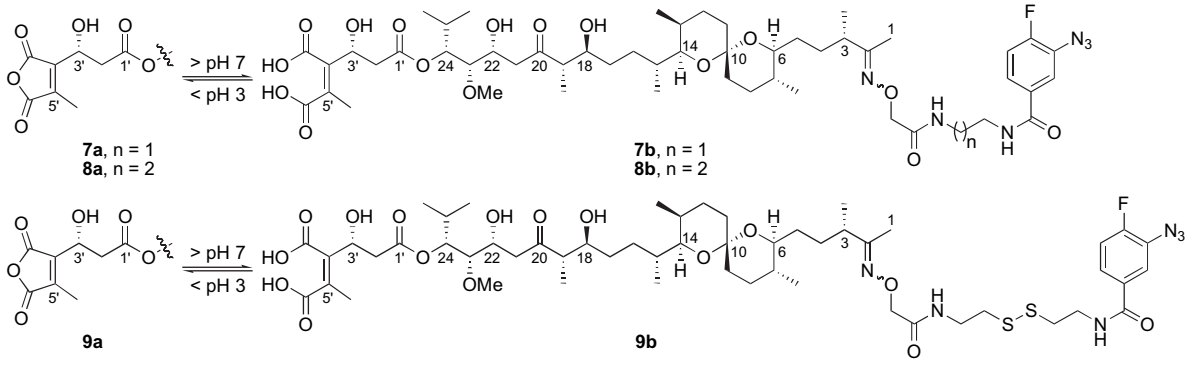
The first generation photoaffinity probes[8] and the photoaffinity probes described herein were designed taking into account the structure–activity relationship reported for natural and synthetic derivatives of TTM.[13–16] Briefly, the active inhibitor is the dicarboxylic acid form of TTM (**1b**, TTMDA) and not the anhydride form (**1a**) (see Scheme 1).[15a,16a] The hydroxyl groups at C22 and C3′ are also necessary for the bioactivity[15b,16a] and the hydrophobic spiroketal moiety contributes significantly to the selective inhibition of PP.[15b] Thus, we have introduced the photolabeling units into the 2-position of TTM in order to retain the activity of the natural product.

TTM (**1**) exists predominantly as the diacid (TTMDA, **1b**) under mild alkaline conditions (CH_3CN, 3% $NaHCO_3$, pH 8).[15a] However, under alkaline conditions (MeOH, 20% Cs_2CO_3, pH 9) TTM (**1**) is unstable and undergoes trans-esterification followed by elimination resulting in the formation of acid **2** and alcohol **3** (Scheme 1).[2,15a] Basic conditions should therefore be avoided when introducing the photolabeling unit into tautomycin. From our previous work[8a] we knew that an oxime linker would work well since the coupling with TTM (**1a**) can be achieved at pH 6.[17]

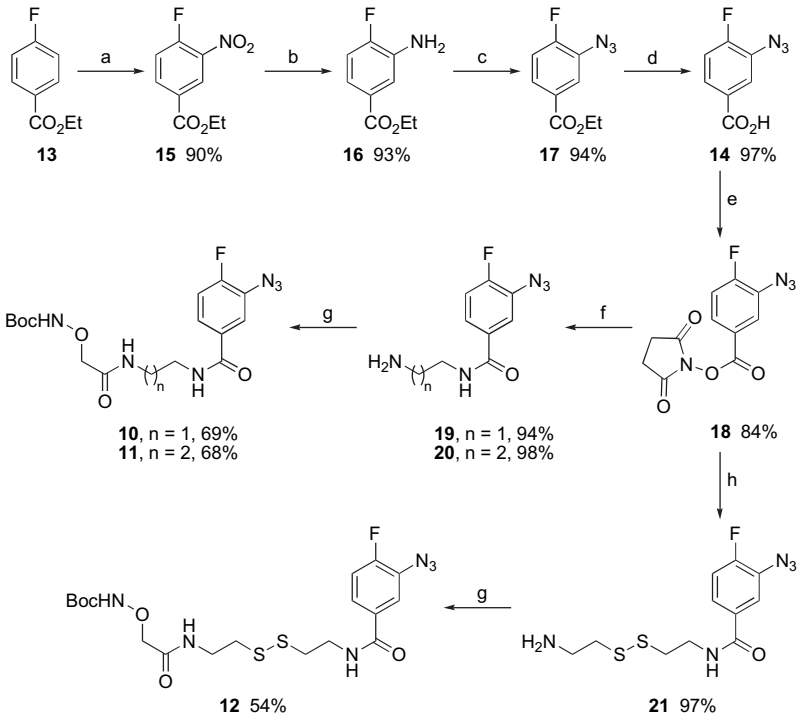
2. Synthesis of the photolabeling units containing the azide photophore

Synthesis of the photolabeling units **10**–**12** started with ethyl 4-fluorobenzoate (**13**), which was converted to acid **14** over four steps using chemistry well established within the group.[18] The specifics of this chemistry is outlined in Scheme 3 and started with nitration of ethyl 4-fluorobenzoate giving compound **15** in good yield (90%). The nitro group within the latter compound was then reduced to the amine upon treatment with hydrogen over Pd/C (10%), thus affording the corresponding amine **16** in 93% yield. Diazotization of substrate **16** with sodium nitrite, followed by Sandmeyer reaction with sodium azide utilizing a procedure reported by Pinney and Katzenellenbogen[19] gave the desired compound **17** in excellent yield (94%). Hydrolysis of the ester functionality within the former compound gave the desired acid **14** in 97% yield.

Acid **14** was then converted to the corresponding activated ester **18** by using a method analogous to the one reported previously by us.[8a] By such means, and after a simple purification by flash chromatography, compound **18** could be isolated in 84% yield. Substrate **18** was then converted to



Scheme 2. Photoaffinity probes.



Scheme 3. Synthesis of the photolabeling units (compounds **10**–**12**). Reagents and conditions: (a) HNO_3, H_2SO_4, 0 °C, 45 min; (b) H_2, Pd/C (10%), EtOH, rt, 23 h; (c) $NaNO_2$, NaN_3, TFA, 0 °C, 10 min; (d) 2 N KOH/EtOH solution, Et_2O, 0 °C, 15 min; (e) *N*-hydroxysuccinimide, EDC·HCl, DMF, rt, 4.5 h; (f) 1,2-diaminoethane for n=1 and 1,3-diaminopropane for n=2, MeOH, 0 °C, 7 min; (g) *N-tert*-butoxycarbonyl-aminooxy acetic acid, EDC·HCl, CH_2Cl_2, THF, rt, overnight; (h) 2,2′-diaminoethyl disulfide dihydrochloride, Et_3N, MeOH, 0 °C, 40 min.

amines **19** and **20** upon treatment with 1,2-diaminoethane and 1,3-diaminopropane, respectively (Scheme 3).[8a] This gave, after purification, the two compounds **19** (94%) and **20** (98%) in excellent yields. The two amines were then coupled with *N-tert*-butoxycarbonyl-aminooxy acetic acid using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl) as the coupling reagent giving protected amines **10** and **11** in 69% and 68% yield, respectively. Finally the third photolabeling unit, namely compound **12**, was prepared in two steps from activated ester **18**. The free amine **21** (97% yield) was first formed using a slightly modified procedure compared to the one used previously for the synthesis of compounds **19** and **20** (see Section 5 for details) followed by coupling with *N-tert*-butoxycarbonyl-aminooxy acetic acid using the same method as previously described. This gave substrate **12** in 54% yield after a simple purification on silica. Despite the low yield for the final coupling reaction, namely the formation of compound **12**, the synthetic strategy outlined here still provides the desired photolabeling unit in a serviceable yield.

The photo-reactivity of compounds **10**–**12** was tested at this stage in order to verify their activity before attaching them to tautomycin. All three substrates worked satisfactory and resulted in C–H insertion (compounds **22**–**24** in Fig. 2) when irradiated in 2,2,2-trifluoroethanol as evident from extensive ESI-Q-TOF-MS and -MS/MS analysis.[20]

Next the product mixture from the photolysis experiment of disulfide **12** was concentrated and ca. half of the product mixture (ca. 0.3 mg) was treated with excess DTT in acetonitrile at room temperature for 16 h. This experiment was executed in order to verify if the disulfide bond within this

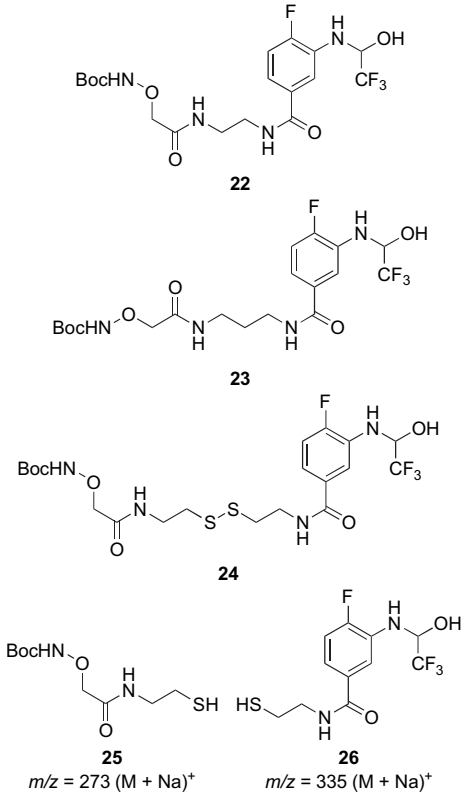


Figure 2. Photolysis products (**22**–**24**) and structure of the two compounds (**25** and **26**) formed upon treating disulfide **24** with DTT in acetonitrile.

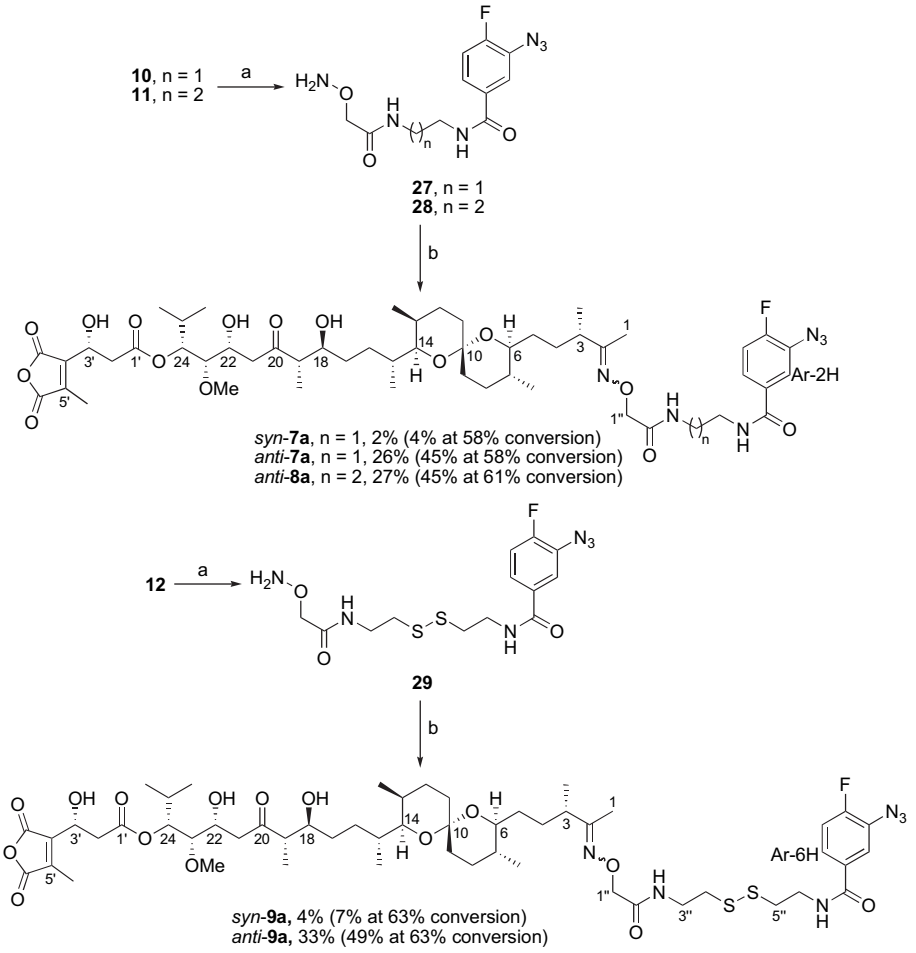
compound could easily be cleaved under such conditions. The resulting product mixture was analyzed by ESI-Q-TOF-MS, which revealed that no starting material remained. Furthermore, a strong signal at *m/z* 273 [(M+Na)$^+$] and a weak signal at *m/z* 335 [(M+Na)$^+$] corresponding to the masses of the two expected products, namely compounds **25** and **26** (Fig. 2), further proved the positive outcome of this reaction. Moreover, strong signals at *m/z* 217 [(M−C_4H_8+Na)$^+$] and *m/z* 173 [(M−$C_5H_8O_2$+Na)$^+$] resulting from fragmentation of amine **25**, which corresponds to fragmentation patterns associated with Boc-protected amines, serve as further proof of successful formation of the two expected reaction products.

3. Synthesis of the photoaffinity probes bearing the azide photophore

After proving that our photolabeling units (compounds **10**–**12**) would undergo the desired photochemical reaction focus could now shift toward the formation of the desired photoaffinity probes. This was implemented using the same strategy as in our previous work[8a] and the details are outlined in Scheme 4. The protection group within substrates **10**, **11**, and **12** was removed upon treatment with trifluoroacetic acid (TFA) in dichloromethane (Scheme 4). Thus, the corresponding aminooxy compounds **27**–**29** formed were used in the next step without further purification.

Tautomycin diacid (**1b**) for the synthesis of the photoaffinity probes was obtained by converting TTM (**1a**) to TTMDA (**1b**) by treating acetonitrile/water (4:1) solution of the anhydride **1a** with aqueous $NaHCO_3$ according to our previously reported method.[8a] A solution of aminooxy **27** in *N*,*N*-dimethylacetoamide (DMA)/H_2O (1:1), which prior to addition had been adjusted to pH 6 by adding base (0.1 N NaOH solution), was then added to a solution of TTMDA (**1b**) in DMA/H_2O (1:1) at room temperature. The resulting reaction mixture was stirred in the dark for 49 h before being poured into a ice-cooled solution of 1.0 N hydrochloric acid/acetonitrile (1:1) (the solution obtained after addition to acid had pH 3). The ice bath was then removed and the reaction mixture was stirred at room temperature for 13.5 h. After a simple work-up, the crude product was purified by HPLC giving *syn*-**7a** and *anti*-**7b** in 2% (4% yield at 58% conversion) and 26% (45% yield at 58% conversion) yield, respectively, together with some recovered starting material (42%).

The *syn* and *anti*-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) was exerted by the oxime moiety.[21] In the



Scheme 4. Synthesis of the photoaffinity probes. Reagents and conditions: (a) TFA, CH_2Cl_2, 0 °C, 2–4.5 h; (b) DMA/H_2O (1:1), rt, 48–49 h then 1 N HCl/CH_3CN (1:1), 0 °C–rt, 13.5–17 h (see Section 5 for specifics).

syn isomer (*syn*-**7a**) the H-3 chemical shift (δ 3.36 ppm) was down-field by 0.98 ppm in comparison with the corresponding H-3 chemical shift (δ 2.38 ppm) for the *anti* isomer (*anti*-**7a**). Furthermore, the H-1 proton is more shielded by 0.11 ppm in the *syn* isomer (δ 1.76 ppm) than the corresponding signal in the *anti* isomer (δ 1.87 ppm).

The acid treatment used at the final stage of the reaction serves two purposes: It converts the product(s) from the diacid form to the anhydride form, thus simplifying purification by HPLC (the diacid form has almost the same retention time as the solvent shock on a reversed phase column), and it also protonates the aminooxy compound, which is present in excess in the reaction mixture. The latter is necessary in order to avoid the aminooxy reacting with the anhydride functionality, thus forming compound(s) with two photoprobes attached.[8a] The acid treatment also causes the *syn*-oxime to isomerize to the corresponding *anti*-oxime isomer, which is the thermodynamic most stable form of the two.

The remaining two aminooxy compounds (**28** and **29**) were converted to the corresponding photoaffinity probes by similar means. Thus forming the desired compounds *anti*-**8a**, *syn*-**9a**, and *anti*-**9a** in 27% (45% at 61% conversion), 4% (7% at 63% conversion), and 33% (49% at 63% conversion) yields, respectively. In the second experiment we could see that also the *syn* product (*syn*-**8a**) was formed prior to acid treatment, as evident from HPLC analysis. However, after acid treatment only the *anti* product could be detected and isolated. Furthermore, we did not isolate any products resulting from reaction with the carbonyl functionality at C20 in any of these experiments, a result that is most likely due to the steric congestion around this ketone.

4. Conclusion

The synthesis of five photoaffinity probes (*syn*-**7a**, *anti*-**7a**, *anti*-**8a**, *syn*-**9a**, and *anti*-**9a**), which possess an aromatic azide photophore attached to the 2-position of tautomycin, has been accomplished through the selective reaction of photolabeling units (**27**–**29**) with tautomycin diacid (**1b**). Efforts directed toward quantifying the PP1γ inhibitory activity of these photoaffinity probes as well as studies directed toward elucidating the binding site with PP1 are now in progress. Analysis of modified protein and peptides derived from the later study will be executed by using matrix assisted laser desorption ionization (MALDI)-TOF-MS as well as nano-HPLC-ESI-Q-TOF-MS and -MS/MS. The outcome of these studies will be reported in due course.

5. Experimental

5.1. General experimental

Melting points were measured on a Yanaco MP-S3 melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded on a JASCO FT/IR-6100 spectrophotometer and are reported in wave number (cm^{-1}). UV spectra were recorded on a JASCO V-570 UV/VIS/NIR spectrophotometer. Proton (^1H) and carbon (^{13}C) NMR spectra were recorded on either a Bruker ARX-400 operating at 400 MHz for proton and 101 MHz for carbon, a JEOL JNM-A600 operating at 600 MHz for proton or a Bruker AMX2600 operating at 151 MHz for carbon. Chemical shifts were recorded as δ values in parts per million (ppm) using tetramethylsilane (δ=0.00 ppm) and residual chloroform (δ=77.0 ppm) as internal standard for proton and carbon NMR, respectively. The assignment of signals in various ^1H NMR spectra was assisted by conducting a range of 2D NMR experiments (COSY, NOESY, ROESY, and HOHAHA). Fluorine (^{19}F) NMR spectra were recorded on a JEOL JNM-A400 operating at 376 MHz for fluorine and spectra were referenced externally to 1,1,1-trifluorotoluene at 0.00 ppm. Low- and high-resolution mass spectra, EI and FAB, were recorded on a JEOL JMN-700. ESI mass spectra were recorded on a Q-TOF mass spectrometer (Micromass, Manchester, UK) equipped with a Z-spray type ESI source. Optical rotations were measured with a JASCO P-1010-GT polarimeter at the sodium D-line (589 nm) and the concentrations (*c*) (g/100 mL) indicated using spectroscopic grade $CHCl_3$. Elemental analyses were performed by Mr. S. Kitamura in the Analytical Laboratory at Bioagricultural Sciences, Nagoya University. Unless otherwise noted, the reaction flask was wrapped with aluminum foil in order to protect the reaction from light, and non-aqueous reactions were carried out under an 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 12-molybdo-(VI)phosphoric acid *n*-hydrate, *p*-anisaldehyde or basic $KMnO_4$ solution followed by heating as developing agents. Silica gel 60 (particle size 0.063–0.2 mm ASTM) was used for flash chromatography. Tautomycin (**1**) was purified according to our previously published procedure.[22] The progress of the coupling reaction between tautomycin and the photolabeling units was monitored by HPLC using a Develosil C30-UG-5 column (4.6×250 mm i.d.), CH_3CN/H_2O 4:1 with a flow rate of 1.0 mL/min, detected at 254 nm.

5.1.1. Ethyl 4-fluoro-3-nitrobenzoate (15). HNO_3 (1.5 mL of a 60% solution) was added dropwise over a 5 min period to a stirred solution of ethyl 4-fluorobenzoate (**13**) (3.03 g, 18.02 mmol) in H_2SO_4 (13 mL) at 0 °C. The resulting reaction mixture was then stirred at 0 °C for 45 min before being diluted with EtOAc (15 mL) and poured into ice water (30 mL). The water phase was extracted with EtOAc (3×30 mL) and the combined organic fractions were washed with water (2×30 mL) and brine (1×30 mL) before being dried (Na_2SO_4). Filtration and concentration in vacuo gave a yellow oil, which was subjected to flash chromatography (silica, hexane/Et_2O 3:2 elution). Concentration of the relevant fractions (R_f 0.36) gave 3.45 g (90%) of the title compound **15**[23] as a light-yellow solid, mp 43.5–44 °C. IR ν_{max} 1726, 1618, 1543, 1353, 1285, 1112 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.73 (dd, *J*=2.2 and 7.0 Hz, 1H, Ar-2*H*), 8.32 (ddd, *J*=2.2, 4.2, and 8.8 Hz, 1H, Ar-5*H*), 7.38 (dd, *J*=8.8 and 10.0 Hz, 1H, Ar-6*H*), 4.44 (q, *J*=7.2 Hz, 2H, CH_2), 1.43 (t, *J*=7.2 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 163.6, 158.0 (d, J_{C-F}=272 Hz), 136.5 (d, J_{C-F}=9 Hz), 127.8, 127.6 (3), 127.6 (0), 118.7 (d, J_{C-F}=22 Hz), 62.1, 14.2; ^{19}F NMR ($CDCl_3$, 376 MHz) δ −48.1; MS (EI+) *m*/*z* 213 ($M^{+\bullet}$, 16%), 185 (60), 168 (100), 122 (67), 94 (50), 83 (14).

5.1.2. Ethyl 3-amino-4-fluorobenzoate (16). 10% Pd/C (183 mg) was added to a solution of ethyl 4-fluoro-3-nitrobenzoate (**15**) (3.42 g, 16.04 mmol) in EtOH (100 mL) at room temperature. The reaction mixture was then stirred vigorously under an atmosphere of H_2 for 23 h before being filtered through a plug of Celite and washed after with EtOH (2×10 mL). Concentration of the filtrate under reduced pressure gave a dark orange oil, which was subjected to flash chromatography (silica, hexane/Et_2O 2:1 elution). Concentration of the relevant fractions (R_f 0.24) gave 2.73 g (93%) of ethyl 3-amino-4-fluorobenzoate (**16**)[24] as a yellow oil. IR ν_{max} 3470, 3373, 2983, 1711, 1631, 1596, 1514, 1442, 1310, 1254, 1203, 1102, 765 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.50 (dd, J=2.2 and 8.8 Hz, 1H, Ar-2*H*), 7.42 (ddd, J=2.2, 4.8, and 8.4 Hz, 1H, Ar-5*H*), 7.02 (dd, J=8.4 and 10.8 Hz, 1H, Ar-6*H*), 4.35 (q, J=7.2 Hz, 2H, CH_2), 3.78 (br s, 2H, NH_2), 1.38 (t, J=7.2 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 166.1, 154.3 (d, J_{C-F}= 247 Hz), 134.6 (d, J_{C-F}=14 Hz), 127.0 (d, J_{C-F}=3 Hz), 120.5 (d, J_{C-F}=8 Hz), 118.1 (d, J_{C-F}=5 Hz), 115.1 (d, J_{C-F}=19 Hz), 61.0, 14.3; ^{19}F NMR ($CDCl_3$, 376 MHz) δ −65.9; MS (EI+) *m/z* 183 ($M^{+\bullet}$, 90%), 155 (38), 138 (100), 110 (41), 83 (17).

5.1.3. Ethyl 3-azido-4-fluorobenzoate (17). $NaNO_2$ (2.03 g, 29.42 mmol) followed by NaN_3 (2.87 g, 44.14 mmol) was added to a stirred solution of amine **16** (2.68 g, 14.63 mmol) in trifluoroacetic acid (30 mL) maintained at 0 °C. The reaction mixture was stirred at 0 °C for 10 min before being diluted with Et_2O (20 mL) and the resulting solution was poured into ice water (30 mL). The water phase was extracted with Et_2O (2×30 mL) and the combined organic fractions were washed with water (2×20 mL) and brine (1×20 mL) before being dried (Na_2SO_4). Concentration under reduced pressure gave a dark orange oil, which was subjected to flash chromatography (silica, hexane→hexane/Et_2O 9:1→8:2 gradient elution). Concentration of the relevant fractions (R_f 0.5 in hexane/Et_2O 8:2) gave 2.89 g (94%) of azide **17** as a orange oil. IR ν_{max} 2123, 1724, 1603, 1506, 1418, 1306, 1418, 1306, 1253, 1109, 763 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.80 (ddd, J=2.0, 4.8, and 8.4 Hz, 1H, Ar-5*H*), 7.78 (dd, J=2.0 and 8.4 Hz, 1H, Ar-2*H*), 7.14 (dd, J=8.4 and 10.4 Hz, 1H, Ar-6*H*), 4.38 (q, J=7.2 Hz, 2H, CH_2), 1.40 (t, J=7.2 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 164.8, 157.4 (d, J_{C-F}=257 Hz), 128.4 (d, J_{C-F}=11 Hz), 127.7 (d, J_{C-F}= 4 Hz), 127.4 (d, J_{C-F}=8 Hz), 122.5 (d, J_{C-F}=2 Hz), 116.6 (d, J_{C-F}=20 Hz), 61.4, 14.2; ^{19}F NMR ($CDCl_3$, 376 MHz) δ −56.6; MS (EI+) *m/z* 209 ($M^{+\bullet}$, 33%), 181 (90), 164 (39), 152 (71), 124 (90), 108 (100), 97 (78), 81 (28), 57 (24); HRMS (EI+) found: $M^{+\bullet}$, 209.0625. $C_9H_8FN_3O_2$ requires $M^{+\bullet}$, 209.0601. Anal. Found: C 51.68, H 3.75, N 19.92%. $C_9H_8FN_3O_2$ requires: C 51.68, H 3.85, N 20.09%.

5.1.4. 3-Azido-4-fluorobenzoic acid (14). A solution of KOH/EtOH (10 mL of a 2 N solution) was added dropwise to a stirred solution of ester **17** (2.83 g, 13.53 mmol) in Et_2O (20 mL) maintained at 0 °C. The reaction mixture was then stirred at 0 °C for 15 min before being allowed to heat to room temperature. After stirring at room temperature for 5 h the reaction mixture was cooled to 0 °C and acidified to pH 1 with HCl (1 N aq solution). The resulting solution was extracted with EtOAc (3×30 mL) and the combined organic phases were washed with water (1×30 mL) before being dried (Na_2SO_4). Filtration and concentration under reduced pressure gave 2.37 g (97%) of the title acid **14**[25] as a yellow solid, mp 156–157 °C (hexane/Et_2O). IR ν_{max} 2922 (br), 2132, 1704, 1437, 1290, 1084, 962, 763 cm^{-1}; ^1H NMR ($CDCl_3$+two drops of DMSO-d_6, 400 MHz) δ 8.23–7.86 (br s, 1H, COO*H*), 7.85–7.81 (m, 2H, Ar-2*H* and Ar-5*H*), 7.15 (d, 1H, J=9.2 and 10.4 Hz, Ar-6*H*); ^{13}C NMR ($CDCl_3$+two drops of DMSO-d_6, 101 MHz) δ 167.1, 157.3 (d, J_{C-F}=257 Hz), 128.2 (d, J_{C-F}=11 Hz), 127.9 (d, J_{C-F}=4 Hz), 127.7 (d, J_{C-F}=8 Hz), 122.8 (d, J_{C-F}=2 Hz), 116.6 (d, J_{C-F}=20 Hz); ^{19}F NMR ($CDCl_3$+two drops of DMSO-d_6, 376 MHz) δ −54.7; MS (EI+) *m/z* 181 ($M^{+\bullet}$, 25%), 153 (72), 138 (18), 124 (26), 108 (30), 97 (100), 82 (35), 70 (26), 57 (17); HRMS (EI+) found: $M^{+\bullet}$, 181.0317. $C_7H_4FN_3O_2$ requires $M^{+\bullet}$, 181.0288.

5.1.5. 3-Azido-4-fluorobenzoic acid 2,5-dioxopyrrolidin-1-yl ester (18). A solution of EDC·HCl (2.74 g, 14.29 mmol) in DMF (200 mL) was added to a stirred solution of acid **14** (2.34 g, 12.92 mmol) and *N*-hydroxysuccinimide (1.57 g, 13.64 mmol) in DMF (50 mL) at room temperature. The reaction mixture was then stirred at room temperature for 4.5 h before being concentrated under reduced pressure. The residue thus obtained was dissolved in EtOAc (60 mL) and washed with water (2×20 mL) and brine (1×20 mL) before being dried (Na_2SO_4). Filtration and concentration under reduced pressure gave a yellow oil, which was subjected to flash chromatography (silica, hexane/EtOAc 4:1→3:1→2:1→1:1 gradient elution). Concentration of the relevant fractions (R_f 0.5 in hexane/EtOAc 1:1) gave 3.01 g (84%) of the title compound **18** as an off-white solid, mp 135–136 °C. IR ν_{max} 2119, 1768, 1735, 1596, 1506, 1415, 1368, 1315, 1203, 1071, 1017, 645 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.90 (ddd, J=2.4, 4.4, and 8.4 Hz, 1H, Ar-5*H*), 7.85 (dd, J=2.4 and 8.4 Hz, 1H, Ar-2*H*), 7.24 (dd, J=8.4 and 10.4 Hz, 1H, Ar-6*H*), 2.90 (s, 4H, 2×CH_2); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 169.0, 160.3, 158.6 (d, J_{C-F}=260 Hz), 129.3 (d, J_{C-F}= 11 Hz), 128.5 (d, J_{C-F}=9 Hz), 123.5 (d, J_{C-F}=3 Hz), 122.2 (d, J_{C-F}=4 Hz), 117.4 (d, J_{C-F}=20 Hz), 25.6; ^{19}F NMR ($CDCl_3$, 376 MHz) δ −52.2; MS (EI+) *m/z* 278 ($M^{+\bullet}$, 22%), 250 (47), 164 (98), 136 (27), 108 (100), 81 (22), 57 (19); HRMS (EI+) found: $M^{+\bullet}$, 278.0443. $C_{11}H_7FN_4O_4$ requires $M^{+\bullet}$, 278.0451. Anal. Found: C 47.59, H 2.31, N 19.85%. $C_{11}H_7FN_4O_4$ requires: C 47.49, H 2.54, N 20.14%.

5.1.6. *N*-(2-Amino-ethyl)-3-azido-4-fluorobenzamide (19). 1,2-Diaminoethane (2.30 mL, 34.37 mmol) was added to a stirred solution of activated ester **18** (193.1 mg, 0.694 mmol) in MeOH (200 mL) maintained at 0 °C. The reaction mixture was stirred for 7 min at 0 °C before being concentrated under reduced pressure and the residue thus obtained was purified by flash chromatography [silica, CH_2Cl_2/MeOH/ammonia solution (28% aq solution) 95:4.25:0.75 elution]. Evaporation of the relevant fractions (R_f 0.07) gave 146.2 mg (94%) of the title compound **19** as a yellow oil, which slowly solidified upon standing, mp 117–118 °C. IR ν_{max} 3294, 2124, 1643, 1597, 1548, 1502, 1323, 1232, 1092, 964 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.58 (dd, J=2.4 and 8.8 Hz, 1H, Ar-2*H*), 7.53 (ddd, J=2.4, 4.4, and 8.8 Hz, 1H, Ar-5*H*), 7.26 (br s, 1H, N*H*), 7.10 (dd, J=8.8 and 10.4 Hz, 1H, Ar-6*H*), 3.47 (q,

J=5.6 Hz, 2H, CH_2), 2.94 (t, J=5.6 Hz, 2H, CH_2), 1.83 (br s, 2H, NH_2); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 165.8, 156.4 (d, J_{C-F}=255 Hz), 131.7 (d, J_{C-F}=4 Hz), 128.3 (d, J_{C-F}=11 Hz), 124.3 (d, J_{C-F}=7 Hz), 120.4 (d, J_{C-F}=2 Hz), 116.5 (d, J_{C-F}=20 Hz), 42.3, 41.0; ^{19}F NMR ($CDCl_3$, 376 MHz) δ −59.0; MS (EI+) m/z 223 ($M^{+\bullet}$, 3%), 194 (97), 181 (45), 166 (83), 137 (98), 109 (99), 108 (100), 82 (61), 81 (57), 57 (47); HRMS (EI+) found: $M^{+\bullet}$, 223.0828. $C_9H_{10}FN_5O$ requires $M^{+\bullet}$, 223.0869. Anal. Found: C 48.53, H 4.42, N 31.34%. $C_9H_{10}FN_5O$ requires: C 48.43, H 4.52, N 31.38%.

5.1.7. *N*-(3-Amino-propyl)-3-azido-4-fluorobenzamide (20). 1,3-Diaminopropane (2.40 mL, 28.83 mmol) was added to a stirred solution of activated ester **18** (165.0 mg, 0.593 mmol) in MeOH (165 mL) maintained at 0 °C. The reaction mixture was stirred for 7 min at 0 °C before being concentrated in vacuo. The residue thus obtained was subjected to flash chromatography [silica, CH_2Cl_2/MeOH/ammonia solution (28% aq solution) 95:4.25:0.75 elution]. Concentration of the relevant fractions (R_f 0.08) gave 137.8 mg (98%) of the desired amine **20** as a yellow oil, which solidified upon standing to a waxy solid, mp 37–40 °C. IR ν_{max} 3280, 2125, 1642, 1597, 1549, 1503, 1323, 1091, 963 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.26 (br s, 1H, NH), 7.60 (dd, J=2.4 and 8.4 Hz, 1H, Ar-2H), 7.53 (ddd, J=2.4, 4.4, and 8.4 Hz, 1H, Ar-5H), 7.11 (dd, J=8.4 and 10.4 Hz, 1H, Ar-6H), 3.57 (q, J=6.0 Hz, 2H, CH_2), 2.95 (br s, 2H, NH_2), 1.74 (quintet, J=6.0 Hz, 4H, CH_2); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 165.3, 156.3 (d, J_{C-F}=255 Hz), 131.9 (d, J_{C-F}=3 Hz), 128.3 (d, J_{C-F}=12 Hz), 124.3 (d, J_{C-F}=9 Hz), 120.4 (d, J_{C-F}=2 Hz), 116.6 (d, J_{C-F}=20 Hz), 41.0, 40.0, 30.6; ^{19}F NMR ($CDCl_3$, 376 MHz) δ −59.5; MS (EI+) m/z 237 ($M^{+\bullet}$, 38%), 220 (12), 209 (22), 194 (35), 192 (35), 167 (28), 151 (27), 138 (70), 137 (50), 108 (100), 97 (15), 82 (18), 81 (17), 57 (32), 56 (34); HRMS (EI+) found: $M^{+\bullet}$, 237.1038. $C_{10}H_{12}FN_5O$ requires $M^{+\bullet}$, 237.1026. Anal. Found: C 50.73, H 5.37, N 29.59%. $C_{10}H_{12}FN_5O$ requires: C 50.63, H 5.10, N 29.52%.

5.1.8. *N*-[2-(2-Aminooxy-acetylamino)-ethyl]-*N*-*tert*-butoxycarbonyl-3-azido-4-fluorobenzamide (10). EDC·HCl (27.0 mg, 0.141 mmol) was added to a stirred solution of amine **19** (21.3 mg, 0.0954 mmol) and *N*-*tert*-butoxycarbonyl-aminooxy acetic acid (20.9 mg, 0.1093 mmol) in CH_2Cl_2 (2.0 mL) and THF (0.2 mL) at room temperature. The resulting reaction mixture was stirred at room temperature overnight before being diluted with CH_2Cl_2 (20 mL), washed with water (1×10 mL) and brine (1×10 mL), and dried over Na_2SO_4. Filtration and concentration gave a yellow oil, which was subjected to flash chromatography (silica, EtOAc/Et_3N 99.8:0.2 elution). Concentration of the relevant fractions (R_f 0.4 in EtOAc) gave 25.9 mg (69%) of the title compound **10** as a colorless oil. UV (MeOH): λ_{max} (log ε) 286 (3.12). IR ν_{max} 3302 (br), 2979, 2934, 2125, 1725, 1655, 1601, 1550, 1503, 1325, 1288, 1258, 1237, 1166, 1115 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.58 (br s, 1H, NH), 7.93 (br s, 1H, NH), 7.65 (dd, J=2.0 and 8.0 Hz, 1H, Ar-2H), 7.64 (br s, 1H, NH), 7.57 (ddd, J=2.0, 4.4, and 8.8 Hz, 1H, Ar-5H), 7.12 (dd, J=8.8 and 10.4 Hz, 1H, Ar-6H), 4.35 (s, 2H, CH_2), 3.59 (m, 4H, CH_2), 1.46 [s, 9H, C(CH_3)$_3$]; ^{13}C NMR ($CDCl_3$, 101 MHz) δ 170.7, 165.9, 158.0, 156.5 (d, J_{C-F}=255 Hz), 131.4 (d, J_{C-F}=4 Hz), 128.5 (d, J_{C-F}=11 Hz), 124.5 (d, J_{C-F}=8 Hz), 120.7 (d, J_{C-F}=2 Hz), 116.7 (d, J_{C-F}=20 Hz), 83.4, 76.4, 41.8, 38.9, 28.1; ^{19}F NMR ($CDCl_3$, 376 MHz) δ −59.1; MS (FAB+) m/z 397 (M^++H, 5%), 297 (14); HRMS (FAB+) found: M^++H, 397.1663. $C_{16}H_{22}FN_6O_5$ requires M^++H, 397.1636. Anal. Found: C 48.48, H 5.01, N 20.90%. $C_{16}H_{21}FN_6O_5$ requires: C 48.48, H 5.34, N 21.20%.

5.1.9. *N*-[3-(2-Aminooxy-acetylamino)-propyl]-*N*-*tert*-butoxycarbonyl-3-azido-4-fluorobenzamide (11). EDC·HCl (39.2 mg, 0.204 mmol) was added to a stirred solution of amine **20** (31.7 mg, 0.134 mmol) and *N*-*tert*-butoxycarbonyl-aminooxy acetic acid (30.1 mg, 0.157 mmol) in CH_2Cl_2 (2.7 mL) and THF (0.3 mL) at room temperature. The resulting reaction mixture was stirred at room temperature overnight before being diluted with CH_2Cl_2 (20 mL), washed with water (1×10 mL) and brine (1×10 mL), and dried over Na_2SO_4. Filtration and concentration gave a yellow oil, which was subjected to flash chromatography (silica, EtOAc/hexane/Et_3N 69.8:30:0.2→79.8:20:0.2 gradient elution). Concentration of the relevant fractions (R_f 0.4 in EtOAc/hexane 8:2) gave 37.4 mg (68%) of the title compound **11** as a colorless oil. UV (MeOH): λ_{max} (log ε) 284 (3.29); IR ν_{max} 3334 (br), 2962, 2926, 2855, 2123, 1691, 1652, 1600, 1542, 1504, 1322, 1260, 1168, 1092 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.47 (br s, 1H, NH), 7.96 (s, 1H, NH), 7.75 (br t, J=5.6 Hz, 1H, NH), 7.72 (dd, J=2.2 and 8.0 Hz, 1H, Ar-2H), 7.62 (ddd, J=2.2, 4.4, and 8.4 Hz, 1H, Ar-5H), 7.14 (dd, J=8.4 and 10.6 Hz, 1H, Ar-6H), 4.36 (s, 2H, CH_2), 3.45 (quintet, J=6.0 Hz, 2×CH_2), 1.78 (quintet, J=6.0 Hz, 2H, CH_2), 1.48 [s, 9H, C(CH_3)$_3$]; ^{13}C NMR ($CDCl_3$, 101 MHz) δ 170.3, 165.4, 158.0, 156.4 (d, J_{C-F}=255 Hz), 131.7 (d, J_{C-F}=3 Hz), 128.4 (d, J_{C-F}=12 Hz), 124.3 (d, J_{C-F}=8 Hz), 120.7 (d, J_{C-F}=2 Hz), 116.6 (d, J_{C-F}=19 Hz), 83.3, 76.1, 35.7, 35.5, 29.0, 28.0; ^{19}F NMR ($CDCl_3$, 376 MHz) δ −59.4; MS (FAB+) m/z 411 (M^++H, 13%), 311 (29); HRMS (FAB+) found: M^++H, 411.1785. $C_{17}H_{24}FN_6O_5$ requires M^++H, 411.1792.

5.1.10. *N*-(2-Aminoethyl disulfide 2′-ethyl)-3-azido-4-fluorobenzamide (21). A solution of 2,2′-diaminoethyl disulfide dihydrochloride (1.07 g, 4.75 mmol) in MeOH (20 mL), which prior to addition had been treated with triethylamine (1.32 mL, 9.47 mmol), was added to a stirred solution of activated ester **18** (53.1 mg, 0.191 mmol) in MeOH (80 mL) maintained at 0 °C. The reaction mixture was then stirred at 0 °C for 40 min before being concentrated under reduced pressure. The resulting residue was subjected to flash chromatography [silica, CH_2Cl_2/MeOH/ammonia solution (28% aq solution) 95:4.25:0.75 elution]. Concentration of the appropriate fractions (R_f 0.1) gave 58.6 mg (97%) of the title compound **21** as a viscous yellow oil. IR ν_{max} 3292 (br), 2925, 2123, 1644, 1599, 1549, 1502, 1323, 1230 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.58 (dd, J=2.0 and 8.0 Hz, 1H, Ar-2H), 7.51 (ddd, J=2.0, 4.4, and 8.8 Hz, 1H, Ar-5H), 7.14 (dd, J=8.8 and 10.4 Hz, 1H, Ar-6H), 6.91 (br s, 1H), 3.79 (apparent q, J=6.0 Hz, 2H, CH_2), 3.04 (apparent br s, 2H, CH_2), 2.92 (t, J=6.2 Hz, CH_2), 2.80 (t, J=6.2 Hz, CH_2), 1.73 (br s, 2H, NH_2); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 165.8, 156.6 (d, J_{C-F}=255 Hz), 131.5 (d, J_{C-F}=4 Hz), 128.6 (d, J_{C-F}=11 Hz), 124.3 (d, J_{C-F}=8 Hz), 120.5 (d, J_{C-F}=2 Hz), 116.8 (d, J_{C-F}=19 Hz), 42.2, 40.6, 39.0, 37.6; ^{19}F NMR ($CDCl_3$,

376 MHz) δ −58.6; MS (FAB+) *m/z* 316 (M^++H, 100%); HRMS (FAB+) found: M^++H, 316.0723. $C_{11}H_{15}FN_5OS_2$ requires M^++H, 316.0702. Anal. Found: C 42.71, H 4.34, N 20.79%. $C_{11}H_{14}FN_5OS_2 \cdot 1/4C_4H_8O_2$ requires: C 42.72, H 4.78, N 20.76%.

5.1.11. *N*-[2-(Aminooxy-acetylamino)-2-aminoethyl disulfide 2′-ethyl]-*N*-*tert*-butoxycarbonyl-3-azido-4-fluorobenzamide (12). EDC·HCl (27.1 mg, 0.141 mmol) was added to a stirred solution of amine **21** (29.3 mg, 0.093 mmol) and *N*-*tert*-butoxycarbonyl-aminooxy acetic acid (30.5 mg, 0.160 mmol) in CH_2Cl_2 (2.0 mL) and THF (0.4 mL) at room temperature. The resulting reaction mixture was stirred at room temperature overnight before being diluted with CH_2Cl_2 (20 mL), washed with water (1×10 mL) and brine (1×10 mL), and dried (Na_2SO_4). Filtration and concentration gave a yellow oil, which was subjected to flash chromatography (silica, hexane/EtOAc/Et_3N 70:29.8:0.2→50:49.8:0.2 gradient elution). Concentration of the relevant fractions (R_f 0.45 in hexane/EtOAc 1:1) gave 24.5 mg (54%) of the title compound **12** as a colorless oil. UV (MeOH): λ_{max} (log ε) 284 (3.30); IR ν_{max} 3293 (br), 2978, 2927, 2122, 1724, 1652, 1600, 1548, 1502, 1322, 1288, 1257, 1233, 1164, 1114 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.55 (br s, 1H, N*H*), 7.92 (s, 1H, N*H*), 7.70 (dd, *J*=2.0 and 8.0 Hz, 1H, Ar-2*H*), 7.63 (ddd, *J*=2.0, 4.6, and 8.4 Hz, 1H, Ar-5*H*), 7.56 (br t, *J*=5.4 Hz, 1H, N*H*), 7.13 (dd, *J*=8.4 and 10.4 Hz, 1H, Ar-6*H*), 4.33 (s, 2H, CH_2), 3.74 (q, *J*=6.0 Hz, CH_2), 3.67 (q, *J*=6.8 Hz, CH_2), 3.01 (t, *J*=6.0 Hz, CH_2), 2.87 (t, *J*=6.8 Hz, CH_2), 1.48 [s, 9H, C(CH_3)$_3$]; ^{13}C NMR ($CDCl_3$, 101 MHz) δ 169.6, 165.9, 157.9, 156.4 (d, J_{C-F}=255 Hz), 131.4 (d, J_{C-F}=4 Hz), 128.3 (d, J_{C-F}=12 Hz), 124.9 (d, J_{C-F}=8 Hz), 120.6 (d, J_{C-F}=2 Hz), 116.6 (d, J_{C-F}=20 Hz), 83.3, 76.2, 39.4, 38.8, 38.3, 37.2, 28.1; ^{19}F NMR ($CDCl_3$, 376 MHz) δ −59.0; MS (FAB+) *m/z* 489 (M^++H, 12%), 389 (18); HRMS (FAB+) found: M^++H, 489.1382. $C_{18}H_{26}FN_6O_5S_2$ requires M^++H, 489.1390.

5.1.12. Synthesis of photoaffinity probes *syn*-7a and *anti*-7a. Trifluoroacetic acid (1.2 mL) was added dropwise over a 10 min period to a stirred solution of amine **10** (19.0 mg, 0.0479 mmol) in CH_2Cl_2 (1.2 mL) maintained at 0 °C. The resulting reaction mixture was stirred at 0 °C for 2 h before being concentrated under reduced pressure. In order to remove trace amounts of TFA the residue was dissolved in water (5.0 mL) and concentrated in vacuo thus providing aminooxy **27** (R_f 0.34 in EtOAc). The crude product was used directly in the coupling reaction with tautomycin **1b** without further purification. MS (ESI+) *m/z* 297 [(M+H)$^+$].

A solution of aminooxy **27** in DMA/H_2O (0.8 mL of a 1:1 solution), which carefully had been adjusted to pH 6 with NaOH solution (0.1 N aq solution), was added to a stirred solution of tautomycin **1b**[26] (17.1 mg, 0.0218 mmol) in DMA/H_2O (2.0 mL of a 1:1 solution) at room temperature. The resulting reaction mixture was stirred in the dark at room temperature for 49 h before being poured into an ice-cooled solution of HCl/CH_3CN (3.0 mL of a 1 N HCl aq/CH_3CN 1:1 solution) and stirred vigorously for 10 min. The ice bath was then removed and stirring was continued at room temperature for 13.5 h. The resulting reaction mixture was extracted with $CHCl_3$ (3×15 mL) and the combined organic fractions were concentrated under reduced pressure. The crude yellow oil was subjected to HPLC purification [Develosil C30-UG-5 (4.6×250 mm i.d.), CH_3CN/H_2O 4:1, 1 mL/min, 254 nm] thus affording three fractions, A–C.

Concentration of fraction A (t_R 12.55 min) gave 7.1 mg (42% recovery) of TTM (**1a**), which was identical, in all respects, with an authentic sample.

Concentration of fraction B (t_R 14.79 min) gave 0.5 mg [2% (4% at 58% conversion)] of compound *syn*-**7a** as a white solid, mp 63–65 °C. $[\alpha]_D^{22}$ −33.9 (*c* 0.04, $CHCl_3$); IR ν_{max} 3340 (br), 2960, 2929, 2871, 2126, 1766, 1654, 1547, 1261, 1229, 1096, 1076, 757 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 7.64 (dd, *J*=2.2 and 7.9 Hz, 1H, Ar-2*H*), 7.59 (br s, 1H, N*H*), 7.52 (ddd, *J*=2.2, 4.4, and 8.6 Hz, 1H, Ar-5*H*), 7.14 (dd, *J*=8.6 and 10.4 Hz, 1H, Ar-6*H*), 6.78 (apparent t, *J*=4.4 Hz, 1H, N*H*), 5.20 (apparent br d, *J*=9.2 Hz, 1H, H-3′), 5.09 (t, *J*=6.2 Hz, 1H, H-24), 4.63 (br s, 1H, 3′-OH), 4.48 (s, 2H, H-1″), 4.36 (m, 1H, H-22), 3.75–3.45 (m, 7H, H-18, H-3″, H-4″, 18-OH, 22-OH), 3.44 (s, 3H, 23-OCH_3), 3.36 (apparent q, *J*=7.3 Hz, 1H, H-3), 3.27 (dd, *J*=2.1 and 5.7 Hz, 1H, H-23), 3.23 (dd, *J*=2.0 and 10.3 Hz, 1H, H-14), 3.19 (dt, *J*=2.4 and 8.3 Hz, 1H, H-6), 2.99 (dd, *J*=8.5 and 17.5 Hz, 1H, H-21b), 2.92 (dd, *J*=3.4 and 16.2 Hz, 1H, H-2′b), 2.78 (dd, *J*=9.8 and 16.2 Hz, 1H, H-2′a), 2.69–2.65 (m, 2H, H-19, H-21a), 2.26 (d, *J*=1.3 Hz, 3H, 5′-CH_3), 2.10 (m, 1H, H-25), 2.02–1.95 (m, 1H, H-12b), 1.87–1.23 (m, 19H), 1.76 (s, 3H, H-1), 1.10 (d, *J*=7.0 Hz, 3H, 19-CH_3), 1.06 (d, *J*=7.0 Hz, 3H, 3-CH_3), 0.99 (d, *J*=6.0 Hz, 3H, 15-CH_3), 0.98 (d, *J*=6.6 Hz, 3H, H-26), 0.96 (d, *J*=7.0 Hz, 3H, 25-CH_3), 0.88 (d, *J*=7.0 Hz, 3H, 13-CH_3), 0.82 (d, *J*=6.3 Hz, 3H, 7-CH_3); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −58.9; HRMS (ESI+) found: [(M+H)$^+$], 1045.5518. $C_{52}H_{78}FN_6O_{15}$ requires [(M+H)$^+$], 1045.5509.

Concentration of fraction C (t_R 16.58 min) gave 5.9 mg [26% (45% at 58% conversion)] of compound *anti*-**7a** as white solid, mp 77–79 °C. $[\alpha]_D^{23}$ −7.7 (*c* 0.12, $CHCl_3$); IR ν_{max} 3335 (br), 2960, 2929, 2876, 2126, 1766, 1645, 1542, 1497, 1261, 1229, 1096, 757 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 7.63 (dd, *J*=2.2 and 7.9 Hz, 1H, Ar-2*H*), 7.54–7.51 (m, 2H, Ar-5*H*, N*H*), 7.15 (dd, *J*=8.6 and 10.4 Hz, 1H, Ar-6*H*), 6.86 (apparent t, *J*=5.5 Hz, 1H, N*H*), 5.20 (br d, *J*=10.1 Hz, 1H, H-3′), 5.09 (t, *J*=6.2 Hz, 1H, H-24), 4.73 (br s, 1H, 3′-OH), 4.47 (s, 2H, H-1″), 4.38 (m, 1H, H-22), 3.71 (dt, *J*=2.2 and 8.6 Hz, 1H, H-18), 3.59–3.49 (m, 6H, H-3″, H-4″, 18-OH, 22-OH), 3.44 (s, 3H, 23-OCH_3), 3.28 (d, *J*=2.4 and 11.9 Hz, 1H, H-23), 3.27 (d, *J*=2.0 and 6.0 Hz, 1H, H-14), 3.16 (apparent t, *J*=9.5 Hz, 1H, H-6), 2.99 (dd, *J*=8.6 and 17.0 Hz, 1H, H-21b), 2.91 (dd, *J*=3.5 and 16.1 Hz, 1H, H-2′b), 2.77 (dd, *J*=9.7 and 16.1 Hz, 1H, H-2′a), 2.70–2.66 (m, 2H, H-21a, H-19), 2.38 (m, 1H, H-3), 2.25 (d, *J*=1.3 Hz, 3H, 5′-CH_3), 2.10 (m, 1H, H-25), 2.02–1.95 (m, 1H, H-12b), 1.87 (s, 3H, H-1), 1.81 (m, 1H, H-13), 1.72–1.22 (m, 17H), 1.07 (d, *J*=7.0 Hz, 3H, 19-CH_3), 1.05 (d, *J*=7.0 Hz, 3H, 3-CH_3), 0.99 (d, *J*=6.4 Hz, 3H, 15-CH_3), 0.97 (d, *J*=6.8 Hz, 3H, H-26), 0.96 (d, *J*=6.8 Hz, 3H, 25-CH_3), 0.86 (d, *J*=7.0 Hz, 3H, 13-CH_3), 0.81 (d, *J*=6.4 Hz, 3H, 7-CH_3); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 215.1, 172.8, 169.4, 165.9, 164.7, 164.5, 157.4, 142.8, 142.1, 131.2, 124.3 (d, J_{C-F}=8 Hz),

120.6, 116.7 (d, J_{C-F}=20 Hz), 95.5, 80.8, 74.8, 74.1, 73.5, 72.3, 66.4, 63.8, 59.0, 52.5, 45.8, 41.4, 40.8, 39.2, 38.8, 36.0, 34.9 (4), 34.9 (0), 31.6, 30.3, 30.1, 29.4, 28.6, 28.0, 27.6, 27.2, 26.7, 19.3, 17.8, 17.6, 17.1, 16.7, 13.4, 11.3, 10.9, 10.0 (three signals obscured or overlapping); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −58.7; HRMS (ESI+) found: [(M+H)$^+$], 1045.5505. $C_{52}H_{78}FN_6O_{15}$ requires [(M+H)$^+$], 1045.5509.

5.1.13. Synthesis of photoaffinity probe *anti*-8a. Trifluoroacetic acid (1.2 mL) was added dropwise over a 10 min period to a stirred solution of amine **11** (19.7 mg, 0.048 mmol) in CH_2Cl_2 (1.2 mL) maintained at 0 °C. The resulting reaction mixture was stirred at 0 °C for 4.5 h before being concentrated under reduced pressure. In order to remove trace amounts of TFA the residue was dissolved in water (5.0 mL) and concentrated in vacuo thus providing aminooxy **28** (R_f 0.29 in EtOAc/hexane 4:1). The crude product was used directly in the coupling reaction with tautomycin **1b** without further purification. MS (ESI+) *m*/*z* 311 [(M+H)$^+$].

A solution of aminooxy **28** in DMA/H_2O (0.8 mL of a 1:1 solution), which carefully had been adjusted to pH 6 with NaOH solution (0.1 N aq solution), was added to a stirred solution of tautomycin **1b**[26] (17.1 mg, 0.0218 mmol) in DMA/H_2O (2.0 mL of a 1:1 solution) at room temperature. The resulting reaction mixture was stirred in the dark at room temperature for 48 h before being poured into an ice-cooled solution of HCl/CH_3CN (3.0 mL of a 1 N HCl aq/CH_3CN 1:1 solution). The resulting reaction mixture was stirred vigorously for 10 min at 0 °C before the stirring was continued at room temperature in the dark for 13.5 h. The reaction mixture was then extracted with $CHCl_3$ (3×15 mL) and the combined organic phases were concentrated under reduced pressure. The resulting yellow oil was purified by HPLC [Develosil C30-UG-5 (4.6×250 mm i.d.), CH_3CN/H_2O 4:1, 1 mL/min, 254 nm] thus affording two fractions, A and B.

Concentration of fraction A (t_R 12.55 min) gave 6.6 mg (39% recovery) of TTM (**1a**), which was identical, in all respects, with an authentic sample.

Concentration of fraction B (t_R 18.95 min) gave 6.3 mg [27% (45% at 61% conversion)] of compound *anti*-**8a** as a white solid, mp 94–96 °C. $[\alpha]_D^{23}$ −4.1 (*c* 0.07, $CHCl_3$); IR ν_{max} 3407 (br), 2960, 2929, 2858, 2122, 1725, 1658, 1640, 1560, 1461, 1261, 1073, 801 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.68 (dd, *J*=2.2 and 7.8 Hz, 1H, Ar-2*H*), 7.59 (ddd, *J*=2.2, 4.4, and 8.7 Hz, 1H, Ar-5*H*), 7.51 (apparent t, *J*=5.9 Hz, 1H, N*H*), 7.16 (dd, *J*=8.7 and 10.3 Hz, 1H, Ar-6*H*), 6.70 (apparent t, *J*=6.2 Hz, 1H, N*H*), 5.20 (br d, *J*=10.3 Hz, 1H, H-3′), 5.09 (t, *J*=6.2 Hz, 1H, H-24), 4.52 (s, 1H, 3′-OH), 4.50 (s, 2H, H-1″), 4.35 (m, 1H, H-22), 3.72 (dt, *J*=2.2 and 8.6 Hz, 1H, H-18), 3.60–3.40 (m, 6H, H-3″, H-5″, 18-OH, 22-OH), 3.44 (s, 3H, 23-OC*H*$_3$), 3.27 (dd, *J*=1.8 and 11.0 Hz, 1H, H-23), 3.26 (dd, *J*=2.0 and 6.3 Hz, 1H, H-14), 3.17 (apparent t, *J*=10.0 Hz, 1H, H-6), 2.98 (dd, *J*=8.5 and 17.4 Hz, 1H, H-21b), 2.92 (dd, *J*=3.3 and 16.2 Hz, 1H, H-2′b), 2.77 (dd, *J*=9.7 and 16.2 Hz, 1H, H-2′a), 2.70–2.66 (m, 2H, H-21a, H-19), 2.41 (apparent q, *J*=6.7 Hz, 1H, H-3), 2.26 (s, 3H, 5′-C*H*$_3$), 2.10 (sextet, *J*=6.6 Hz, 1H, H-25), 2.02–1.95 (m, 1H, H-12b),1.89 (s, 3H, H-1), 1.82 (m, 1H, H-13), 1.74 (quintet, *J*=6.0 Hz, 2H, H-4″), 1.69–1.25 (m, 17H), 1.07 (0) (d, *J*=7.0 Hz, 3H, 19-C*H*$_3$), 1.06 (7) (d, *J*=7.1 Hz, 3H, 3-C*H*$_3$), 0.99 (d, *J*=6.4 Hz, 3H, 15-C*H*$_3$), 0.97 (d, *J*=6.8 Hz, 3H, H-26), 0.95 (d, *J*=7.0 Hz, 3H, 25-C*H*$_3$), 0.86 (d, *J*=7.0 Hz, 3H, 13-C*H*$_3$), 0.81 (d, *J*=6.4 Hz, 3H, 7-C*H*$_3$); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 215.2, 169.4, 165.5, 164.4, 142.9, 142.1, 124.2, 120.7, 116.7 (d, J_{C-F}=20 Hz), 95.5, 80.6, 74.8, 74.0, 73.5, 72.2, 66.3, 63.8, 59.0, 52.3, 45.9, 40.9, 38.9, 36.0, 35.8, 35.4, 35.0, 34.8, 31.7, 30.3, 30.1, 29.5, 29.3, 28.6, 28.0, 27.5, 27.0, 26.7, 19.3, 17.9, 17.8, 17.3, 16.8, 13.5, 11.5 (nine signals obscured or overlapping); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −59.1; HRMS (ESI+) found: [(M+H)$^+$], 1059.5601. $C_{53}H_{80}FN_6O_{15}$ requires [(M+H)$^+$], 1059.5666.

5.1.14. Synthesis of photoaffinity probes *syn*-9a and *anti*-9a. Trifluoroacetic acid (1.2 mL) was added dropwise over a 10 min period to a stirred solution of amine **12** (22.6 mg, 0.046 mmol) in CH_2Cl_2 (1.2 mL) maintained at 0 °C. The resulting reaction mixture was stirred at 0 °C for 2 h before being concentrated under reduced pressure. In order to remove trace amounts of TFA the residue was dissolved in water (5.0 mL) and concentrated in vacuo thus providing aminooxy **29** (R_f 0.6 in EtOAc). The crude product was used directly in the coupling reaction with tautomycin **1b** without further purification. MS (ESI+) *m*/*z* 389 [(M+H)$^+$].

A solution of aminooxy **29** in DMA/H_2O (0.8 mL of a 1:1 solution), which carefully had been adjusted to pH 6 with NaOH solution (0.1 N aq solution), was added to a stirred solution of tautomycin **1b**[26] (17.1 mg, 0.0218 mmol) in DMA/H_2O (2.0 mL of a 1:1 solution) at room temperature. The resulting reaction mixture was stirred in the dark at room temperature for 48 h before being poured into an ice-cooled solution of HCl/CH_3CN (3.0 mL of a 1 N HCl aq/CH_3CN 1:1 solution). The resulting reaction mixture was stirred vigorously for 10 min at 0 °C before the stirring was continued at room temperature in the dark for 17 h. The reaction mixture was then extracted with $CHCl_3$ (3×15 mL) and the combined organic phases were concentrated under reduced pressure. The resulting yellow oil was purified by HPLC [Develosil C30-UG-5 (4.6×250 mm i.d.), CH_3CN/H_2O 4:1, 1 mL/min, 254 nm] thus affording three fractions, A–C.

Concentration of fraction A (t_R 12.55 min) gave 5.5 mg (33% recovery) of TTM (**1a**), which was identical, in all respects, with an authentic sample.

Concentration of fraction B (t_R 21.98 min) gave 1.1 mg [4% (7% at 63% conversion)] of compound *syn*-**9a** as a white solid, mp 132–134 °C. $[\alpha]_D^{24}$ −44.4 (*c* 0.01, $CHCl_3$); IR ν_{max} 3349 (br), 2960, 2929, 2876, 2126, 1766, 1658, 1542, 1502, 1261, 1229, 1096, 1073, 757 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.69 (dd, *J*=2.2 and 7.8 Hz, 1H, Ar-2*H*), 7.61 (ddd, *J*=2.2, 4.4, and 8.6 Hz, 1H, Ar-5*H*), 7.33 (br s, 1H, N*H*), 7.14 (dd, *J*=8.6 and 10.4 Hz, 1H, Ar-6*H*), 6.68 (t, *J*=4.4 Hz, 1H, N*H*), 5.20 (apparent br d, *J*=9.2 Hz, 1H, H-3′), 5.09 (t, *J*=6.1 Hz, 1H, H-24), 4.56 (br s, 1H, 3′-OH), 4.47 (s, 2H, H-1″), 4.35 (m, 1H, H-22), 3.77–3.44 (m, 7H, H-3″, H-6″, H-18, 18-OH, 22-OH), 3.44 (s, 3H, 23-OC*H*$_3$), 3.37 (apparent q, *J*=7.3 Hz, 1H,

H-3), 3.27 (dd, J=2.1 and 5.7 Hz, 1H, H-23), 3.23 (dd, J=2.0 and 9.9 Hz, 1H, H-14), 3.19 (m, 1H, H-6), 3.01–2.97 (m, 3H, H-5″, H-21b), 2.92 (dd, J=3.5 and 16.3 Hz, 1H, H-2′b), 2.85 (t, J=7.0 Hz, 2H, H-4″), 2.78 (dd, J=9.8 and 16.3 Hz, 1H, H-2′a), 2.67–2.63 (m, 2H, H-19, H-21a), 2.27 (d, J=1.3 Hz, 3H, 5′-CH_3), 2.10 (m, 1H, H-25), 2.02–1.95 (m, 1H, H-12b), 1.85 (m, 1H, H-13), 1.80 (s, 3H, H-1), 1.65–1.25 (m, 17H), 1.10 (d, J=7.1 Hz, 3H, 19-CH_3), 1.07 (d, J=7.0 Hz, 3H, 3-CH_3), 0.99 (d, J=6.6 Hz, 3H, 15-CH_3), 0.98 (d, J=6.8 Hz, 3H, H-26), 0.97 (d, J=6.8 Hz, 3H, 25-CH_3), 0.89 (d, J=7.0 Hz, 3H, 13-CH_3), 0.82 (d, J=6.6 Hz, 3H, 7-CH_3); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −58.8; HRMS (ESI+) found: [(M+H)$^+$], 1137.5349. $C_{54}H_{82}FN_6O_{15}S_2$ requires [(M+H)$^+$], 1137.5264.

Concentration of fraction C (t_R 23.48 min) gave 8.2 mg [33% (49% at 63% conversion)] of compound *anti*-**9a** as a white solid, mp 50–51 °C. $[\alpha]_D^{24}$ −0.6 (c 0.27, $CHCl_3$); IR ν_{max} 3362 (br), 2965, 2929, 2876, 2126, 1766, 1654, 1542, 1502, 1261, 1229, 1096, 757 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.66 (dd, J=2.2 and 7.9 Hz, 1H, Ar-2*H*), 7.58 (ddd, J=2.2, 4.4, and 8.6 Hz, 1H, Ar-5*H*), 7.15 (1) (t, J=5.5 Hz, 1H, N*H*), 7.15 (0) (dd, J=8.6 and 10.3 Hz, 1H, Ar-6*H*), 6.69 (t, J=6.0 Hz, 1H, N*H*), 5.21 (br d, J=8.2 Hz, 1H, H-3′), 5.09 (t, J=6.2 Hz, 1H, H-24), 4.66 (br s, 1H, 3′-OH), 4.47 (d, J=2.6 Hz, 2H, H-1″), 4.35 (m, 1H, H-22), 3.75 (q, J=6.0 Hz, 2H, H-3″), 3.72–3.60 (m, 4H, H-6″, H-18, 18-OH), 3.44 (s, 3H, 23-OCH_3), 3.38–3.20 (m, 3H, H-23, H-14, 22-OH), 3.17 (apparent t, J=9.8 Hz, 1H, H-6), 2.99–2.95 (m, 3H, H-5″, H-21b), 2.91 (dd, J=3.3 and 16.1 Hz, 1H, H-2′b), 2.84 (t, J=6.7 Hz, 2H, H-4″), 2.77 (dd, J=9.8 and 16.1 Hz, 1H, H-2′a), 2.71–2.65 (m, 2H, H-21a, H-19), 2.41 (apparent q, J=6.8 Hz, 1H, H-3), 2.27 (d, J=1.3 Hz, 3H, 5′-CH_3), 2.10 (sextet, J=6.7 Hz, 1H, H-25), 2.02–1.95 (m, 1H, H-12b), 1.85 (s, 3H, H-1), 1.85–1.23 (m, 18H), 1.08 (d, J=7.1 Hz, 3H, 19-CH_3), 1.07 (d, J=7.0 Hz, 3H, 3-CH_3), 1.00 (d, J=6.4 Hz, 3H, 15-CH_3), 0.98 (d, J=6.8 Hz, 3H, H-26), 0.96 (d, J=6.8 Hz, 3H, 25-CH_3), 0.88 (d, J=7.0 Hz, 3H, 13-CH_3), 0.81 (d, J=6.6 Hz, 3H, 7-CH_3); ^{13}C NMR ($CDCl_3$, 151 MHz) δ 215.2, 171.2, 169.4, 165.8, 164.8, 164.4, 156.5 (d, J_{C-F}=255 Hz), 142.9, 142.1, 131.3, 128.4, 124.6 (d, J_{C-F}=8 Hz), 120.6, 116.7 (d, J_{C-F}=20 Hz), 95.5, 80.6, 74.8, 74.0, 73.6, 72.3, 66.3, 63.8, 59.0, 52.4, 45.9, 40.9, 39.2, 38.8, 38.3, 37.8, 37.0, 36.0, 35.0, 34.9, 31.7, 30.3, 30.1, 29.4, 28.6, 28.0, 27.5, 27.2, 26.7, 19.3, 18.2, 17.9, 17.8, 17.3, 16.8, 13.5, 11.3, 10.9, 10.1 (one signal was obscured or overlapping); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −58.7; HRMS (ESI+) found: [(M+H)$^+$], 1137.5283. $C_{54}H_{82}FN_6O_{15}S_2$ requires [(M+H)$^+$], 1137.5264.

Acknowledgements

Financial support from Grant-in-aid for Specially Promoted Research (16002007) from the Ministry of Education, Sports, Science and Technology (MEXT), Japan is gratefully acknowledged. M.O.S. is grateful for the provision of an Inoue Fellowship from the Inoue Foundation for Science. We thank Dr. M. Kuse and Mr. K. Koga for acquisition of certain MS and NMR data, respectively, and Mr. I. Doi for expert assistance with the operation of ESI-Q-TOF-MS. We would also like to thank Dr. K. Isono (ex-Riken Institute) and Kaken Phermaceutical Co. Ltd. for kindly providing a sample of crude tautomycin.

References and notes

1. Cheng, X.-C.; Kihara, T.; Kusakabe, H.; Magae, J.; Kobayashi, Y.; Fang, R.-P.; Ni, Z.-F.; Shen, Y.-C.; Ko, K.; Yamaguchi, I.; Isono, K. *J. Antibiot.* **1987**, *40*, 907–909.
2. (a) Ubukata, M.; Cheng, X.-C.; Isono, K. *J. Chem. Soc., Chem. Commun.* **1990**, 244–246; (b) Cheng, X.-C.; Ubukata, M.; Isono, K. *J. Antibiot.* **1990**, *43*, 809–819; (c) Ubukata, M.; Cheng, X.-C.; Isobe, M.; Isono, K. *J. Chem. Soc., Perkin Trans. 1* **1993**, 617–624.
3. (a) Cohen, P. *Annu. Rev. Biochem.* **1989**, *58*, 453–508; (b) Shenolikar, S.; Nairn, A. C. *Adv. Second Messenger Phosphoprotein Res.* **1991**, *23*, 1–121.
4. Takai, A.; Sasaki, K.; Nagai, H.; Mieskes, G.; Isobe, M.; Isono, K.; Yasumoto, T. *Biochem. J.* **1995**, *306*, 657–665.
5. Goldberg, J.; Huang, H.; Kwon, Y.; Greengard, P.; Nairn, A. C.; Kuriyan, J. *Nature* **1995**, *376*, 745–753.
6. Maynes, J. T.; Bateman, K. S.; Cherney, M. M.; Das, A. K.; Luu, H. A.; Holmes, C. F. B.; James, M. N. G. *J. Biol. Chem.* **2001**, *276*, 44078–44082.
7. Kita, A.; Matsunaga, S.; Takai, A.; Kataiwa, H.; Wakimoto, T.; Fusetani, N.; Isobe, M.; Miki, K. *Structure* **2002**, *10*, 715–724.
8. (a) Kurono, M.; Shimomura, A.; Isobe, M. *Tetrahedron* **2004**, *60*, 1773–1780; (b) Kurono, M.; Isobe, M. *Chem. Lett.* **2004**, *33*, 452–453.
9. Isobe, M.; Kurono, M.; Tsuboi, K.; Takai, A. *Chem. Asian J.*, in press.
10. Poe, R.; Schnapp, K.; Young, M. J. T.; Grayzar, J.; Platz, M. S. *J. Am. Chem. Soc.* **1992**, *114*, 5054–5067.
11. Karney, W. L.; Borden, T. *J. Am. Chem. Soc.* **1997**, *119*, 3347–3350.
12. Li, H.; Liu, Y.; Fang, K.; Nakanishi, K. *Chem. Commun.* **1999**, 365–366.
13. For Isono's structure–activity investigations, see: Nishiyama, U.; Ubukata, M.; Magae, J.; Kataoka, T.; Erdodi, F.; Hartshorne, D. J.; Isono, K.; Nagai, K.; Osada, H. *Biosci. Biotechnol. Biochem.* **1996**, *60*, 103–107.
14. For Oikawa and Ichihara's structure–activity investigations, see: (a) Kawamura, T.; Matsuzawa, S.; Mizuno, Y.; Kikuchi, K.; Oikawa, H.; Oikawa, M.; Ubukata, M.; Ichihara, A. *Biochem. Pharmacol.* **1998**, *55*, 995–1003; (b) Oikawa, H. *Curr. Med. Chem.* **2002**, *9*, 2033–2054.
15. For Isobe's structure–activity investigations, see: (a) Sugiyama, Y.; Ohtani, I. I.; Isobe, M.; Takai, A.; Ubukata, M.; Isono, K. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 3–8; (b) Takai, A.; Tsuboi, K.; Koyasu, M.; Isobe, M. *Biochem. J.* **2000**, *350*, 81–88.
16. For Chamberlin's structure–activity investigations, see: (a) Liu, W.; Sheppeck, J. E., II; Colby, D. A.; Huang, H.-B.; Nairn, A. C.; Chamberlin, A. R. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1597–1600; (b) Colby, D. A.; Liu, W.; Sheppeck, J. E., II; Huang, H.-B.; Nairn, A. C.; Chamberlin, A. R. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1601–1605.
17. (a) Jencks, W. P. *Prog. Phys. Org. Chem.* **1964**, *2*, 63–128; (b) Shao, J.; Tam, J. P. *J. Am. Chem. Soc.* **1995**, *117*, 3893–3899.
18. Kuse, M.; Doi, I.; Kondo, N.; Kageyama, Y.; Isobe, M. *Tetrahedron* **2005**, *61*, 5754–5762.

19. Pinney, K. G.; Katzenellenbogen, J. A. *J. Org. Chem.* **1991**, *56*, 3125–3133.
20. The details of these studies are the subject of a separate publication, which is in preparation.
21. (a) Karabatsos, G. J.; Taller, R. A.; Vane, F. M. *J. Am. Chem. Soc.* **1963**, *85*, 2327–2328; (b) Karabatsos, G. J.; His, N. *Tetrahedron* **1967**, *23*, 1079–1095; (c) Karabatsos, G. J.; Taller, R. A. *Tetrahedron* **1968**, *24*, 3347–3360.
22. Kurono, M.; Isobe, M. *Tetrahedron* **2003**, *59*, 9609–9617.
23. Ethyl 4-fluoro-3-nitrobenzoate is also available from several chemical suppliers.
24. Hopff, H.; Valkanas, G. *J. Org. Chem.* **1962**, *27*, 2923–2924.
25. Cogan, D. A.; Hao, M.-H.; Qian, K. C.; Swinamer, A. D. U.S. Patent 2004102492, 2004; *Chem. Abstr.* **2004**, *141*, 7121.
26. TTMDA (**1b**) was obtained by treating TTM (**1a**) with base according to our previously reported method. For details see Ref. 8a.