HETEROCYCLES, Vol. 99, No. 1, 2019, pp. 521 - 533. © 2019 The Japan Institute of Heterocyclic Chemistry
Received, 3rd September, 2018, Accepted, 15th October, 2018, Published online, 26th December, 2018
DOI: 10.3987/COM-18-S(F)49

IDENTIFICATION OF TARGET PROTEIN FOR BATZELLADINES AS CD4†

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Abstract – Batzelladines are a family of polycyclic guanidine alkaloids. Among the congeners, batzelladines A-E were reported to inhibit the interaction of human immunodeficiency virus (HIV) gp120 protein with human CD4. Here, we designed a batzelladine photoaffinity probe bearing trifluoromethyl-3*H*-diazirine and biotin groups, and employed it to establish the identity of the target protein of batzelladines as CD4.

INTRODUCTION

Batzelladines are polycyclic guanidine alkaloids, and at least 15 congeners have been reported.[1-3] Among them, batzelladines A-E were reported to inhibit the interaction between the human immunodeficiency virus (HIV) envelope glycoprotein gp120 and the extracellular domain of human CD4 receptor protein.[1a] Because of the potential of batzelladines to prevent virus infection, identification of their target protein is crucial in order to establish their mode of action. We have previously reported syntheses of (+)-batzelladine A (**1**)[4a] and (-)-batzelladine D (**2**)[4b] based upon a successive nitrone 1,3-dipolar cycloaddition strategy. In addition, the target protein for these compounds was investigated by using immobilized CD4 and gp120 affinity gels bearing synthetic batzelladines A (**1**) and D (**2**).[5] The results suggested that the target protein of these alkaloids is CD4. As a next step, we aimed to confirm these preliminary observations by employing a chemical-biology approach, using a photoaffinity chemical probe. In this article, we describe the synthesis of the batzelladine photoaffinity probe **8**, and its application to confirm the identity of the target protein of batzelladines.

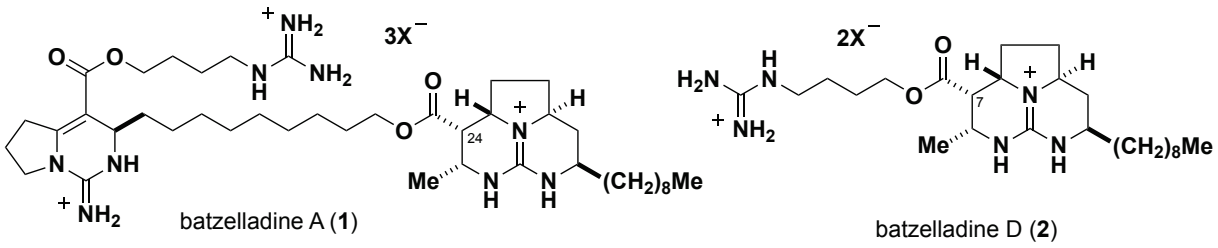


Figure 1. Structures of batzelladine A (**1**) and batzelladine D (**2**)

RESULTS AND DISCUSSION

Design of the photoaffinity probe

To design the chemical probe, we first needed to find a suitable position for the installation of a photoactivatable group in batzelladine without affecting the inhibitory activity.[6] We have previously examined the structure-activity relationship studies with small library of synthetic intermediates of batzelladines bearing bicyclic guanidine, **3**-**7**.[7] Those compounds did not significantly inhibit gp120 binding to CD4 at the concentration of 100 μM in ELISA-based assay.[8] On the other hand, 24-*epi*-batzelladine A and 7-*epi* isomer of **2** showed inhibitory activity,[7] thus, both the tricyclic guanidine and the linear guanidine moieties should be mandatory for the inhibitory activity.

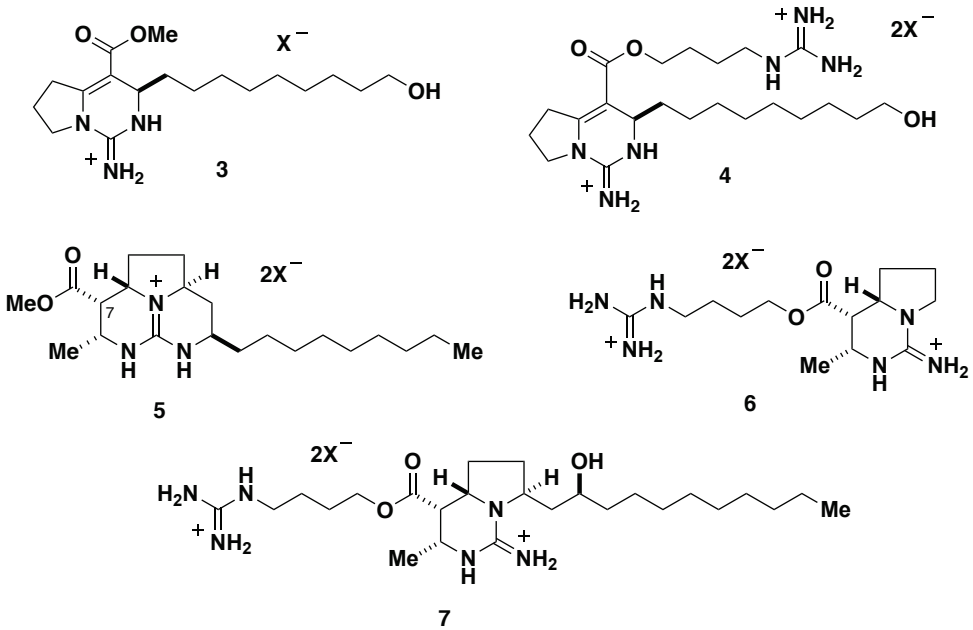


Figure 2. Structures of batzelladines synthesized for stricture-activity relationship studies

On the basis of these observations, we newly designed compound **8** as a baztelladine photoaffinity probe bearing trifluoromethyl-3*H*-diazirine and biotin groups, based upon the structure of batzelladine D (**2**).

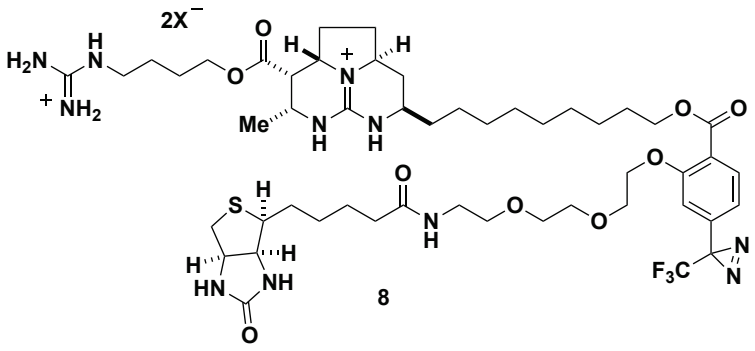
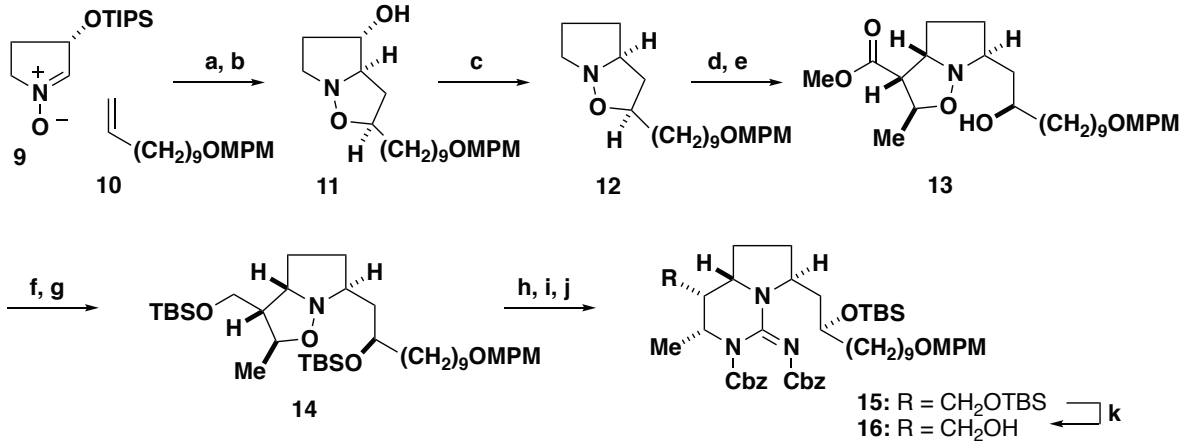


Figure 3. Structure of baztelladine photoaffinity probe **8**

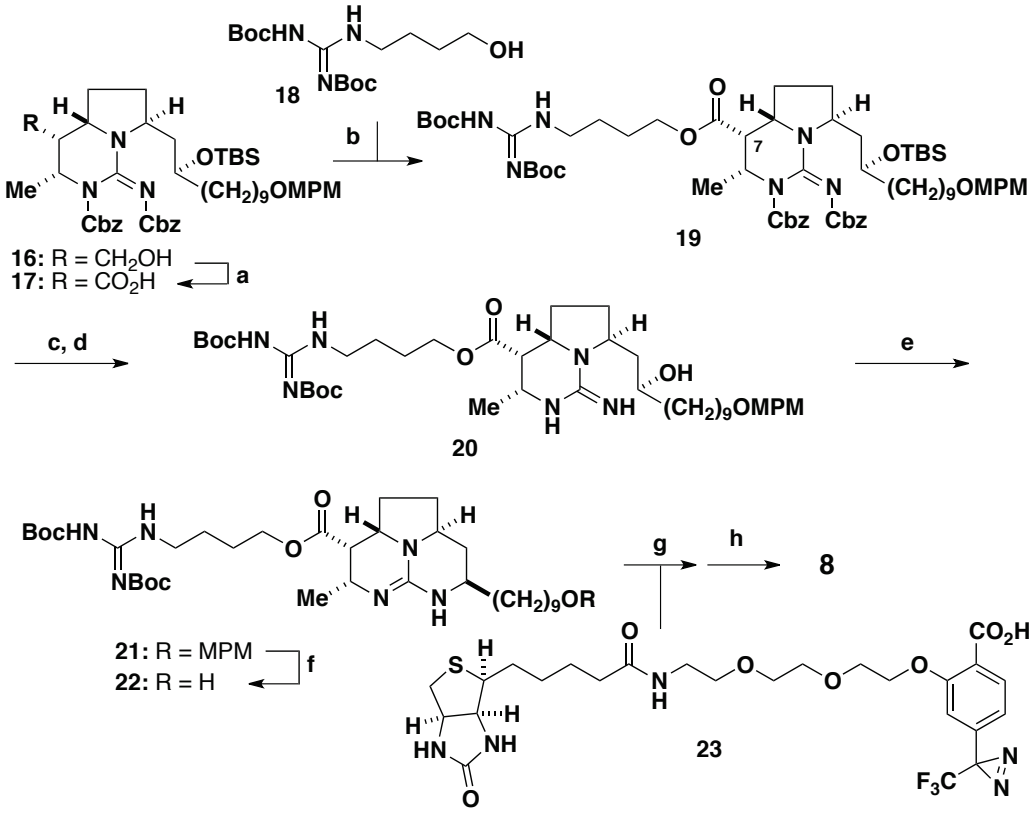
Synthesis of photoaffinity probe 8

Synthesis of **8** was carried out based upon the procedure developed by our group.[9] Firstly, carboxylic acid **17** was synthesized from chiral nitrone **9**,[9b] which was derived from malic acid (Scheme 1). 1,3-Dipolar cycloaddition of the chiral nitrone **9** and 1-undecene derivative **10** gave the isoxazolidine, whose triisopropylsilyl ether group was removed by CsF to give **11**. Then, the hydroxy group was reductively removed under Barton-McCombie conditions to give isoxazolidine **12**. Oxidation of isoxazolidine **12** with *m*CPBA, and a second 1,3-dipolar reaction with methyl crotonate gave isoxazolidine **13** stereoselectively in 57% yield from **12**. The ester group in **13** was then reduced to alcohol with $LiAlH_4$, and the two hydroxy groups were protected as TBS ethers by reaction with TBS chloride in the presence of imidazole to give **14** in 81% yield (2 steps).



Scheme 1. Synthesis of carboxylic acid **17**. Reagents and conditions; (a) toluene, 90 °C; (b) CsF, EtOH, 90 °C, 72% (2 steps); (c) TCDI, THF; then, *n*-Bu_3SnH, AIBN, toluene, 74%; (d) *m*CPBA, CH_2Cl_2, 0 °C; (e) methyl crotonate, toluene, 110 °C, 57% (2 steps); (f) $LiAlH_4$, Et_2O; (g) TBSCl, imidazole, 81% (2 steps); (h) $Pd(OH)_2$-C, H_2, pyrrolidine, EtOH; (i) bis-Cbz-2-methyl-2-thiopseudourea, $HgCl_2$, Et_3N, DMF, 67% (2 steps); (j) DEAD, PPh_3, toluene, 43%; (k) TBAF, THF, 74%.

Next, selective reduction of the N-O bond in **14** was examined. Under usual hydrogenolysis conditions in the presence of Pd/C or $Pd(OH)_2/C$ in ethanol, the MPM group was simultaneously deprotected. On the other hand, the N-O bond was selectively reduced under hydrogen in the presence of $Pd(OH)_2/C$ catalyst and a catalytic amount of pyrrolidine in ethanol, affording the corresponding secondary amine, i.e., 2,5-disubstituted pyrrolidine. The resulting amine was reacted with bis-Cbz- 2-methyl-2-thiopseudourea followed by formation of the bicyclic guanidine under the Mitsunobu conditions to give **15** in 29% yield from **14**. Then, the primary silyl ether in **15** was selectively deprotected with TBAF to give alcohol **16** in 74%.



Scheme 2. Synthesis of photoaffinity probe **8**. Reagents and conditions; (a) CrO_3, H_2SO_4, acetone, 0 °C; (b) **18**, EDCI, DMAP, CH_2Cl_2, 0 °C, 42% (2 steps); (c) HF-Py, THF, 0 °C, 97%; (d) $Pd(OH)_2$-C, H_2, EtOH, rt; (e) DEAD, PPh_3, toluene, rt, 29% (2 steps); (f) DDQ, CH_2Cl_2, H_2O; (g) **23**, DEAD, PPh_3, THF, rt; (h) TFA, CH_2Cl_2, rt, 31% (3 steps).

Batzelladine photoaffinity probe **8** was synthesized from **16** as illustrated in Scheme 2. The primary alcohol in **16** was oxidized to carboxylic acid **17** by Jones oxidation. Then, esterification of carboxylic acid **17** with guanidine alcohol **18** took place with EDCI in the presence of DMAP at 0 °C, and the ester **19** was obtained in 42% yield (2 steps). After deprotection of the TBS ether and Cbz group with HF-Py and hydrogen in the presence of 10% Pd-C, respectively, the resulting guanidine **20** was subjected to the Mitsunobu conditions to give tricyclic guanidine **21**. The MPM group was selectively removed with DDQ

to give primary alcohol **22**, which was subsequently subjected to esterification with aziridine-biotin-conjugated carboxylic acid **23**,[10] followed by removal of the Boc groups with TFA to give photoaffinity probe **8** in 31% yield from **21**.

Photoaffinity labelling experiment with 8

With the photoaffinity probe **8** in hand, we examined its ability to photolabel CD4 or gp120. Each protein was irradiated in the presence of probe **8** and the reaction mixture was analyzed by SDS-PAGE, followed by transfer to a nitrocellulose membrane, and Western blotting with chemiluminescence detection. Figure 4 (a) shows that photoaffinity labelling of CD4 was greater than that of gp120. This result indicates that probe **8** has higher affinity for CD4 than gp120. Next, inhibition of the labelling by synthetic batzelladine alkaloids A (**1**)[4a] and D (**2**),[4b] as well as guanidine hydrochloride, was examined (Figure 2(b)). In the presence of batzelladine A (**1**) and D (**2**), lower levels of labelling were observed (Figure 4(b), lane 5, 6) compared to the case with no additive (lane 4). Biologically more potent batzelladine A (**1**)[1a] showed higher inhibitory activity (lane 5). Guanidine hydrochloride showed much weaker inhibition (lane 7). Collectively, these results indicate that probe **8** binds to CD4 protein; further this binding is inhibited by batzelladine alkaloids **1** and **2** in line with their inhibitory activity. This inhibition might not simply be due to ionic interaction, considering the very low inhibitory activity of guanidine hydrochloride. The polycyclic core structure of batzelladine and an appropriate distance between the guanidine moieties in the molecule may be important for the inhibitory activity.

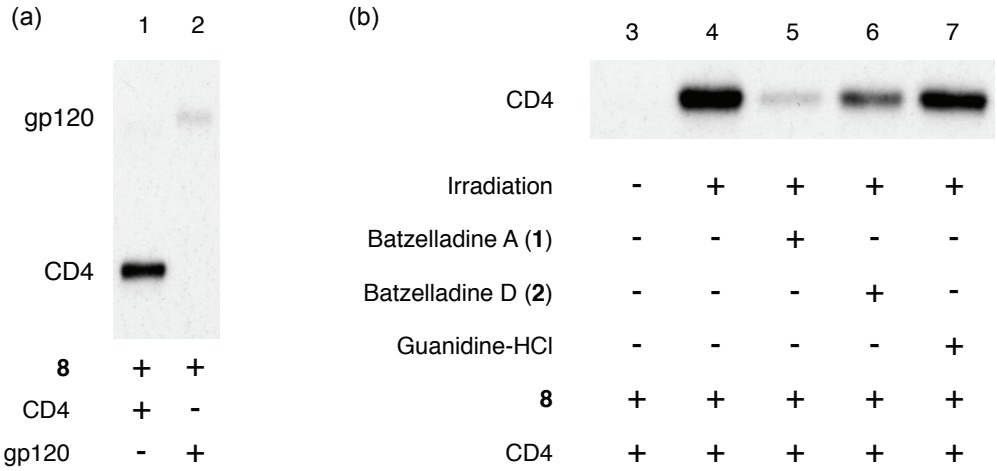


Figure 4. (a) SDS-PAGE analysis of CD4 (5 μM), gp120 (4 μM) in the presence (+) and absence (–) of photoaffinity probe **8** (0.85 μM); (b) SDS-PAGE analysis of CD4 (5 μM) in the presence (+) and absence (–) of photoirradiation, batzelladine A (**1**) (85 μM), batzelladine D (**2**) (85 μM), guanidine-HCl (85 μM), and photoaffinity probe **8** (0.85 μM).

In summary, we have designed and synthesized a batzelladine photoaffinity probe **8** based upon the SAR results for batzelladine derivatives. Binding experiments of probe **8** successfully confirmed the identity of the target protein of batzelladines as human CD4 receptor protein.

EXPERIMENTAL

General procedure: Flash chromatography was performed on silica gel 60 (spherical, particle size 0.040-0.100 mm; Kanto Chemical Co. Inc.). Optical rotations were measured on a JASCO DIP polarimeter 370, using the sodium D line. IR spectra were measured with a JASCO VALOR-III FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on JEOL JNM-ECP 500 (500 MHz). The spectra are referenced internally according to the residual solvent signals of $CDCl_3$ (^{1}H NMR; δ = 7.26 ppm, ^{13}C NMR; δ = 77.0 ppm). Data for ^{1}H NMR are reported as follows: chemical shift (δ, ppm) multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; m, multiplet; br, broad), integration, coupling constant (Hz). Data for ^{13}C NMR are reported in terms of chemical shift (δ, ppm). Mass spectra were recorded on a JEOL JMA-HX100 spectrometer.

Isoxazolidine **11**

A mixture of nitrone **9** (3.11 g, 12.0 mmol) and olefin **10** (10.5 g, 36.0 mmol) in toluene (30 mL) was heated at 110 °C for 4 h. After cooling, the reaction mixture was concentrated *in vacuo* and roughly chromatographed (5:1 hexane-EtOAc to 3:1 hexane-EtOAc) to afford crude oxazolidine. The mixture of crude oxazolidine and CsF (5.47 g, 36.0 mmol) in EtOH (30 mL) was refluxed at 90 °C for 15 h. After cooling, the solution was poured into H_2O and extracted with CH_2Cl_2 twice. The solution was dried over $MgSO_4$ and concentrated *in vacuo*. The resulting residue was purified by flash chromatography (100:1 CH_2Cl_2-MeOH to 9:1 CH_2Cl_2-MeOH) to give **11** (3.40 g, 8.63 mmol, 72% overall) as a clear oil. Spectral data for **11**: $[\alpha]^{22}_{D}$ –26 (*c* 0.9, $CHCl_3$); IR (neat) 3150, 2930, 2852, 1612, 1513, 1243 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25 (d, *J* = 8.6 Hz, 2H), 6.87 (d, *J* = 8.6 Hz, 2H), 4.42 (s, 2H), 4.09 (m, 1H), 3.91 (m, 1H), 3.79 (s, 3H), 3.57 (m, 1H), 3.43–3.37 (m, 1H), 3.42 (t, *J* = 6.8 Hz, 2H), 3.14 (m, 1H), 2.16–2.02 (m, 3H), 1.71 (m, 1H), 1.65–1.52 (m, 3H), 1.50–1.20 (m, 13H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.0, 130.7, 129.2, 113.7, 77.7, 73.3, 72.5, 70.2, 55.3, 55.2, 40.2, 33.8, 33.7, 29.7, 29.5, 29.4, 26.3, 26.1 ppm; HRMS (FAB, MH^+) calcd for $C_{23}H_{38}NO_4$ 392.2801, found 392.2823.

Isoxazolidine **12**

To a solution of alcohol **11** (2.90 g, 7.41 mmol) in THF (100 mL) was added thiocarbonyldiimidazole (1.98 g, 11.1 mmol). After stirring for 7 h at 60 °C, the reaction mixture was filtered through a short pad of silica to give crude xanthate as a yellow oil. To a solution of the xanthate in toluene (70 mL) were

added *n*-Bu_3SnH (4.0 mL, 14.8 mmol) and AIBN (122 mg, 0.74 mmol), and the mixture was stirred at 100 °C for 40 min. The reaction mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (2:1 hexane-EtOAc to 1:2 hexane-EtOAc) to afford isoxazolidine **12** (2.02 g, 5.38 mmol, 73% overall). Spectral data for **12**: $[\alpha]^{22}_D$ –26 (*c* 1.4, $CHCl_3$); IR (neat) 2929, 2854, 1613, 1513, 1248 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.25 (d, J = 8.6 Hz, 2H), 6.87 (d, J = 8.6 Hz, 2H), 4.42 (s, 2H), 4.00 (m, 1H), 3.79 (s, 3H), 3.71 (m, 1H), 3.42 (t, J = 6.8 Hz, 2H), 3.12 (m, 1H), 2.07–1.82 (m, 4H), 1.71–1.20 (m, 18H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.0, 130.8, 129.2, 113.7, 76.5, 72.4, 70.2, 64.9, 57.1, 55.2, 42.5, 34.0, 31.7, 29.7, 29.6, 29.4, 26.4, 26.1, 24.3 ppm; HRMS (FAB, MH^+) calcd for $C_{23}H_{38}NO_3$ 376.2852, found 376.2857.

Methyl ester **13**

To a solution of isoxazolidine **12** (2.02 g, 5.38 mmol) in CH_2Cl_2 (50 mL) was added *m*CPBA (1.59 g, 7.53 mmol) at –10 °C. After stirring for 20 min, large excess of $Ca(OH)_2$ was added and the resulting mixture was filtered through a pad of Celite and eluted with CH_2Cl_2. The filtrate was concentrated *in vacuo* to give nitrone as a clear oil. The mixture of crude nitrone and methyl crotonate (2.83 mL, 26.9 mmol) in toluene (30 mL) was stirred at 90 °C for 18 h. After cooling, the reaction mixture was concentrated under reduced pressure and the residue was purified by flash chromatography (2:1 hexane-EtOAc; 1:1 hexane-EtOAc) to give methyl ester **13** (1.51 g, 3.07 mmol, 57% overall) as a clear oil. Spectral data for **13**: $[\alpha]^{22}_D$ –69 (*c* 1.2, $CHCl_3$); IR (neat) 3377, 3316, 2931, 2919, 2850, 1727, 1613, 1518, 1464, 1438 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.26 (d, J = 8.6 Hz, 2H), 6.87 (d, J = 8.6 Hz, 2H), 4.43 (s, 2H), 4.30 (m, 1H), 4.06 (m, 1H), 3.91 (m, 1H), 3.80 (s, 3H), 3.72 (s, 3H), 3.43 (t, J = 6.8 Hz, 2H), 3.36 (m, 1H), 3.04 (t, J = 9.8 Hz, 1H), 1.94–1.82 (m, 2H), 1.75 (m, 1H), 1.62–1.20 (m, 19H), 1.35 (d, J = 14.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4, 159.0, 130.8, 129.2, 113.7, 72.4, 71.2, 70.2, 69.0, 65.3, 57.4, 55.2, 51.8, 39.3, 37.6, 29.72, 29.7, 29.52, 29.5, 29.4, 28.8, 27.3, 26.1, 25.7, 16.6 ppm; HRMS (FAB, MH^+) calcd for $C_{28}H_{46}NO_6$ 492.3325, found 492.3322.

Bis-TBS ether **14**

To a suspension of $LiAlH_4$ (175 mg, 4.61 mmol) in Et_2O (15 mL), a solution of methyl ester **13** (1.51 g, 3.07 mmol) in Et_2O (15 mL) was added slowly at 0 °C. After stirring for 30 min at the same temperature, the reaction was quenched by sequential addition of H_2O (100 μL), 2.0 M NaOH aq. (100 μL), and H_2O (300 μL). $MgSO_4$ was added and the resulting mixture was stirred for 20 min, filtered through Celite, and the eluent was concentrated to afford a residue that was used for the next reaction without further purification. To a solution of the crude diol and imidazole (1.25 g, 18.4 mmol) in CH_2Cl_2 (30 mL) was added TBSCl (1.39 g, 9.21 mmol) and stirred at room temperature for 20 min. The reaction was quenched

by the addition of H_2O and extracted with ethyl EtOAc twice. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The resulting oil was purified on silica gel (hexane; 20:1 hexane-EtOAc) to give bis TBS ether **14** (1.70 g, 2.49 mmol, 81% overall) as a colorless oil. Spectral data for **14**: $[\alpha]^{22}_D$ –50 (*c* 1.0, $CHCl_3$); IR (neat) 2928, 2855, 1614, 1513, 1471, 1463 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.26 (d, *J* = 8.6 Hz, 2H), 6.73 (d, *J* = 8.6 Hz, 2H), 4.43 (s, 2H), 3.91 (m, 1H), 3.83-3.63 (m, 4H), 3.80 (s, 3H), 3.43 (t, *J* = 6.8 Hz, 2H), 3.12 (m, 1H), 2.33 (m, 1H), 1.95 (m, 1H), 1.76–1.20 (m, 22H), 0.88 (s, 18H), 0.06 (s, 6H), 0.05 (S, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.0, 130.8, 129.2, 113.7, 72.9, 72.5, 70.5, 70.2, 66.8, 65.2, 60.5, 55.2, 54.2, 43.0, 38.0, 31.1, 29.9, 29.7, 29.6, 29.52, 29.5, 24.7, 17.6, –4.3, –4.2, –5.5, –5.6 ppm; HRMS (FAB, MH^+) calcd for $C_{39}H_{74}NO_5Si$ 692.5106, found 692.5096.

Bicyclic guanidine **15**

To a solution of bis TBS ether **14** (1.58 g, 2.32 mmol) and pyrrolidine (90 μL, 0.70 mmol) in EtOH (6.0 mL) was added $Pd(OH)_2$-C (100 mg), and the reaction mixture was stirred at room temperature under an atmosphere of hydrogen gas (balloon). After 7 h, the reaction mixture was filtered through Celite and eluted with EtOAc. The solution was concentrated under reduced pressure to give pyrrolidine. To a solution of the pyrrolidine, 1,3-bis(benzyloxycarbonyl)-2-methyl-2-thiopseudourea (998 mg, 2.78 mmol) and triethylamine (970 μL, 6.96 mmol) in DMF (20 mL) was added $HgCl_2$ (756 mg, 2.78 mmol), and the resulting mixture was stirred for 1 h. The reaction mixture was diluted with Et_2O, and filtered through a pad of Celite. The filtrate was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (hexane; 10:1 hexane-EtOAc, 6:1 hexane-EtOAc) to give the bis-Cbz protected guanidine (1.56 g, 1.55 mmol, 67% overall). To a solution of the bisguanidine (1.56 g, 1.55 mmol) and PPh_3 (622 mg, 2.37 mmol) in toluene (15 mL) was added DEAD (375 μL, 40% in toluene, 2.37 mmol). After stirring for 5 min, the reaction was quenched with H_2O (10 μL) and concentrated *in vacuo*. Purification of the residue by flash chromatography (hexane; 9:1 hexane-EtOAc) gave bicyclic guanidine **15** (671 mg, 0.680 mmol, 43%) as a clear oil. Spectral data for **15**: $[\alpha]^{23}_D$ –123 (*c* 1.5, $CHCl_3$); IR (neat) 2928, 2855, 1726, 1578, 1513, 1470, 1464 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.37-7.20 (m, 12H), 6.87 (d, *J* = 8.6 Hz, 2H), 5.17 (d, *J* = 12.4 Hz, 2H), 4.97 (d, *J* = 12.4 Hz, 2H), 4.92 (d, *J* = 12.4 Hz, 2H), 4.90 (d, *J* = 12.4 Hz, 2H), 4.63 (m, 1H), 4.43 (s, 2H), 4.04 (m, 1H), 3.84–3.76 (m, 1H), 3.80 (s, 3H), 3.65 (m, 3H), 3.42 (t, *J* = 6.8 Hz, 2H), 2.65–2.57 (m, 2H), 2.17 (m, 1H), 1.96 (m, 1H), 1.73–1.16 (m, 19H), 1.18 (d, *J* = 7.3 Hz, 3H), 0.87 (s, 18H), 0.05 (s, 6H), 0.04 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.34, 153.2, 150.5, 137.4, 135.7, 130.8, 129.2, 128.4, 128.2, 128.1, 128.0, 127.4, 113.7, 72.5, 70.6, 68.2, 66.8, 60.7, 57.7, 56.3, 55.3, 49.8, 42.9, 41.8, 37.0, 29.9, 29.8, 29.62, 29.6, 29.5, 28.5 ppm; HRMS (FAB, MH^+) calcd for $C_{56}H_{88}N_3O_8Si_2$ 986.6110, found 986.6093.

Alcohol **16**

TBAF hydrate (570 mg, 2.18 mmol) was added to a solution of bicyclic guanidine **15** (671 mg, 0.680 mmol) in THF (7.0 mL) at 0 °C and stirred at the temperature for 25 min. The reaction mixture was poured into saturated $NaHCO_3$ aq. and extracted with EtOAc twice. The combined organic layer was washed with saturated NH_4Cl aq., H_2O and brine. The solution was concentrated *in vacuo* and the residue was purified by silica gel column chromatography (4:1 hexane-EtOAc; 3:2 hexane-EtOAc) to afford alcohol **16** (441 mg, 0.506 mmol, 74%) as a clear oil. Spectral data for **16**: $[\alpha]^{24}_{D}$ –173 (*c* 0.87, $CHCl_3$); IR (neat) 3446, 2929, 2854, 1725, 1575, 1513 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.37-7.20 (m, 12H), 6.87 (d, *J* = 8.6 Hz, 2H), 5.19 (d, *J* = 12.4 Hz, 1H), 4.97 (d, *J* = 12.4 Hz, 1H), 4.91 (d, *J* = 12.4 Hz, 1H), 4.87 (d, *J* = 12.4 Hz, 1H), 4.64 (m, 1H), 4.42 (s, 2H), 4.06 (m, 1H), 3.82-3.66 (m, 4H), 3.80 (s, 3H), 3.42 (t, *J* = 6.8 Hz, 2H), 2.62 (m, 1H), 2.54 (ddd, *J* = 12.8, 7.3, 3.0 Hz, 1H), 2.18 (m, 1H), 2.02 (m, 1H), 1.73–1.18 (m, 19H), 1.21 (d, *J* = 6.8 Hz, 3H), 0.87 (s, 9H), 0.04 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.1, 153.2, 150.6, 137.3, 135.6, 130.8, 129.2, 128.4, 128.3, 128.1, 128.0, 127.5, 113.7, 72.5, 70.5, 70.3, 68.3, 66.9, 60.4, 57.7, 56.3, 55.3, 49.9, 42.8, 41.8, 36.9, 29.9, 29.8, 29.7, 29.6, 29.55, 29.5, 28.5, 26.2, 25.9, 24.4, 18.0, 14.5, –4.3, –4.4 ppm; HRMS (FAB, MH^+) calcd for $C_{50}H_{74}N_3O_8Si$ 872.5245, found 872.5247.

Ester **19**

To a solution of alcohol **16** (134 mg, 0.154 mmol) in acetone (2.0 mL) was added Jones reagent (20 drops) and stirred at 0 °C for 15 min. To the mixture was added excess of 2-propanol and poured into EtOAc. The solution was washed with brine four times and the aqueous layer was reextracted with EtOAc. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to give the crude carboxylic acid **17**. To a mixture of crude carboxylic acid **17** and alcohol **18** (72.2 mg, 0.218 mmol) in CH_2Cl_2 (1.5 mL) was added EDCI·HCl (88.6 mg, 0.462 mmol) and DMAP (2.0 mg, 0.016 mmol) at 0 °C. After stirring at the temperature for 3 h, the reaction mixture was poured into H_2O and extracted with Et_2O twice. The solution was washed with saturated $NaHCO_3$ aq. solution, brine, H_2O and dried over $MgSO_4$. The solution was evaporated and the residue was purified by silica gel column chromatography (8:4:1 CH_2Cl_2-hexane-EtOAc; 16:8:3 CH_2Cl_2-hexane-EtOAc) to afford compound **19** (77.6 mg, 0.065 mmol, 42%) as a clear oil. Spectral data for **19**: $[\alpha]^{26}_{D}$ –105 (*c* 1.4, $CHCl_3$); IR (neat) 2930, 2859, 1718, 1637, 1615 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 11.49 (br, 1H), 8.31 (be, 1H), 7.34-7.19 (m, 12H), 6.87 (d, *J* = 8.6 Hz, 2H), 5.14 (d, *J* = 12.4 Hz, 1H), 4.97 (d, *J* = 12.4 Hz, 1H), 4.90 (d, *J* = 12.4 Hz, 1H), 4.87 (d, *J* = 12.4 Hz, 1H), 4.50 (m, 1H), 4.42 (s, 2H), 4.12–4.02 (m, 3H), 3.85 (m, 2H), 3.79 (s, 3H), 3.42 (m, 4H), 3.21 (dd, *J* = 7.7, 4.7 Hz, 1H), 2.26 (m, 2H), 1.83 (m, 1H), 1.75–1.19 (m, 23H), 1.49 (s, 9H), 1.48 (s, 9H), 1.37 (d, *J* = 6.8 Hz, 3H), 0.86 (s, 9H), 0.04 (s, 3H), 0.03 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ

169.8, 163.6, 160.0, 156.1, 153.3, 152.9, 150.0, 137.2, 135.6, 130.8, 129.2, 128.3, 128.2, 128.15, 128.1, 128.0, 127.4, 113.7, 83.1, 79.2, 72.5, 71.0, 70.2, 68.1, 66.8, 64.8, 62.1, 58.5, 56.2, 55.2, 52.2, 50.1, 40.6, 40.2, 37.3, 29.9, 29.7, 29.6, 29.5, 28.8, 28.3, 28.0, 27.5, 26.2, 25.9, 25.8, 25.6, 24.4, 21.8, 18.0, 16.4, –4.3, –4.4 ppm; HRMS (FAB, MH^+) calcd for $C_{65}H_{99}N_6O_{13}Si$ 1199.7039, found 1199.7034.

Alcohol **20**

To a solution of **19** (77.6 mg 0.065 mmol) in THF (2.0 mL) in a polypropylene tube was added HF-Py. complex (200 μL) at 0 °C. After stirring at the temperature for 4 h, saturated $NaHCO_3$ aq. (500 μL) and solid $NaHCO_3$ (100 mg) was added at 0 °C. The mixture was dried over $MgSO_4$, filtered through a pad of Celite and washed with EtOAc. The solution was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (4:1 hexane-EtOAc; 2:1 hexane-EtOAc) to afford compound **20** (68.4 mg, 0.060 mmol, 97%) as a clear oil. Spectral data for **20**: $[\alpha]^{22}_D$ –87 (*c* 0.73, $CHCl_3$); IR (neat) 3331, 2982, 2931, 2859, 1723, 1639, 1613, 1574, 1513 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 11.48 (br, 1H), 8.30 (br, 1H), 7.30-7.18 (m, 12H), 6.87 (d, J = 8.6 Hz, 2H), 6.22 (m, 1H), 5.14 (d, J = 12.4 Hz, 1H), 5.01 (d, J = 12.8 Hz, 1H), 4.84 (d, J = 12.8 Hz, 1H), 4.78 (d, J = 12.4 Hz, 1H), 4.58 (m, 1H), 4.42 (s, 2H), 4.40 (m, 1H), 4.11 (m, 2H), 3.95 (m, 1H), 3.80 (s, 3H), 3.70 (t, J = 6.4 Hz, 2H), 3.51 (br, 1H), 3.47–3.35 (m, 4H), 3.21 (dd, J = 7.7, 3.9 Hz, 1H), 2.33 (m, 1H), 1.97 (m, 1H), 1.85 (m, 1H), 1.76–1.10 (m, 23H), 1.24 (d, J = 9.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.8, 163.5, 159.3, 159.0, 156.1, 153.6, 153.2, 152.6, 136.9, 135.0, 130.7, 129.1, 128.4, 128.1, 127.9, 127.8, 127.5, 127.4, 113.7, 83.1, 83.0, 79.2, 72.4, 70.2, 68.2, 66.3, 65.0, 62.0, 56.9, 56.4, 55.2, 53.1, 50.7, 44.3, 40.3, 40.1, 36.9, 29.9, 29.7, 29.6, 29.54, 29.5, 29.4, 28.7, 28.2, 28.0, 27.0, 26.2, 26.1, 26.0, 25.9, 25.7, 25.54, 25.5, 16.5 ppm; HRMS (FAB, MH^+) calcd for $C_{59}H_{85}N_6O_{13}$ 1085.6175, found 1085.6177.

Tricyclic guanidine **21**

To a solution of compound **20** (7.7 mg, 0.007 mmol) in EtOAc (1.0 mL) was added a catalytic amount of $Pd(OH)_2$. The reaction mixture was stirred at room temperature under an atmosphere of hydrogen gas (balloon) for 3 h, and the reaction mixture was filtered and washed with EtOAc. The solution was concentrated under reduced pressure to afford crude guanidine alcohol **22**. To a solution of the guanidine alcohol **22** and PPh_3 (3.4 mg, 0.013 mmol) in toluene (500 μL) was added DEAD (5.9 μL, 40% in toluene, 0.013 mmol). After stirring for 1 h, the reaction was quenched with one drop of H_2O and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (EtOAc; 100:1 $CHCl_3$-MeOH; 40:1 $CHCl_3$-MeOH, Chromatorex NH, Fuji Silysia Chemical ltd.) to give **21** (1.5 mg, 0.0019 mmol, 29%) as a viscous oil. Spectral data for **21**: $[\alpha]^{22}_D$ –50 (*c* 0.15, $CHCl_3$); IR (neat) 2920, 2850, 1732, 1717, 1637, 1615, 1577, 1569, 1558, 1541, 1508 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 11.49 (br, 1H), 8.33 (br, 1H),

7.26 (d, *J* = 8.6 Hz, 2H), 6.87 (d, *J* = 8.6 Hz, 2H), 4.43 (s, 2H), 4.13 (t, *J* = 6.4 Hz, 2H), 3.80 (s, 3H), 3.77 (m, 2H), 3.57–3.35 (m, 6H), 2.89 (m, 1H), 2.25 (m, 1H), 2.15 (m, 1H), 2.00 (s, 3H), 1.90–1.00 (m, 28H), 1.50 (s, 9H), 1.49 (s, 9H), 1.34 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.7, 170.1, 168.8, 156.2, 153.3, 150.7, 130.8, 129.2, 113.7, 83.2, 72.5, 70.2, 64.3, 56.1, 55.5, 55.3, 51.5, 48.7, 44.6, 40.3, 35.6, 33.4, 30.8, 29.7, 29.5, 29.4, 29.37, 28.4, 28.3, 28.1, 26.2, 26.0, 25.7, 25.3, 24.3, 18.1 ppm; HRMS (FAB, MH^+) calcd for $C_{43}H_{71}N_6O_8$ 799.5333, found 799.5331.

Batzelladine D photoaffinity probe **8**

To a solution of **21** (3.8 mg, 0.0047 mmol) in a mixed solvent (CH_2Cl_2 : H_2O = 9 : 1, 300 μL) was added DDQ (2.7 mg, 0.012 mmol) at room temperature. After stirring for 1 h, 5 drops of saturated $NaHCO_3$ aq. were added and the resultant was dried over Na_2SO_4. The solution was diluted with EtOAc and filtered through a short pad of Chromatorex NH (Fuji Silysia Chemical ltd.). The filtrates were evaporated under reduced pressure to give a crude primary alcohol **22** (2.9 mg, 0.0043 mmol, 91%). Primary alcohol **22** (2.9 mg) and **23** (6.5 mg, 0.011 mmol) was co-evaporated with THF-MeOH and toluene. To a mixture were added PPh_3 (5.6 mg, 0.021 mmol) and THF (200 μL) then DEAD (9.8 μL, 40% in toluene, 0.021 mmol) at room temperature. After 30 min, 1 drop of H_2O was added and evaporated *in vacuo*. The resulting residue was purified on preparative TLC (Chromatorex NH, Fuji Silysia Chemical ltd.) and eluted with CH_2Cl_2-MeOH; 9:1. The solution was evaporated and dissolved in 50% TFA in CH_2Cl_2 (500 μL). After 30 min, the solution was concentrated under reduced pressure and purified by HPLC (PEGASIL-ODS 50% MeCN aq. 0.1% TFA) to give batzelladine D photoaffinity probe **8** (1.7 mg, 0.0013 mmol, 31% overall) as a viscous oil. Spectral data for **8**: $[\alpha]^{23}_D$ 2.3 (*c* 0.17, MeOH); IR (neat) 2926, 2859, 1579, 1442, 1207, 1185, 1137 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ 7.78 (d, *J* = 8.3 Hz, 1H), 6.96 (d, *J* = 7.9 Hz, 1H), 6.85 (s, 1H), 4.47 (m, 1H), 4.27 (m, 3H), 4.19 (m, 4H), 3.95 (m, 1H), 3.86 (m, 3H), 3.71 (m, 2H), 3.63 (m, 4H), 3.53 (m, 4H), 3.34 (m, 2H), 3.20 (m, 1H), 3.13 (m, 1H), 2.90 (dd, *J* = 12.5, 5.2 Hz, 1H), 2.69 (d, *J* = 5.2 Hz, 1H), 2.33 (m, 1H), 2.20 (m, 3H), 1.80–1.20 (m, 30H), 0.89 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 172.4, 170.6, 163.0, 160.5, 158.6, 151.5, 133.0, 124.0, 119.8, 112.8, 71.9, 71.3, 70.7, 70.6, 70.4, 66.5, 65.4, 63.3, 61.6, 57.8, 57.3, 57.0, 53.2, 49.9, 49.8, 45.5, 42.0, 41.0, 40.3, 37.0, 36.7, 34.2, 31.4, 30.8, 30.5, 30.3, 29.7, 29.5, 29.3, 27.1, 26.9, 26.8, 26.6, 26.2, 23.7, 22.1, 18.4 ppm; HRMS (FAB, MH^+) calcd for $C_{50}H_{77}F_3N_{11}O_9S$ 1064.5579, found 1064.5619.

Materials. Soluble recombinant hCD4 (Sf21-derived, carrier free) was purchased from Genzyme Techne (Minneapolis, MN), and recombinant gp120 (HIV-1 IIIB) was purchased from Immunodiagnostics (Woburn, MA).

Photoaffinity labeling (competition assay). A mixture of 200 ng of recombinant hCD4, 480 ng of gp120 and a 17 μM DMSO solution of photoaffinity probe (0.13 μL) in PBS buffer (pH 7.4, 5 μL) was incubated at 0 °C for 1 h in an open Eppendorf tube, and then irradiated for 20 min from above with a 450 W high-pressure Hg lamp (Model UM-452, Ushio) at 0 °C. After addition of NuPAGE LDS sample buffer (4×) (2.5 μL), 0.5 M DTT (1.0 μL) and H_2O (1.5 μL), the mixture was incubated for 10 min at 70 °C. The samples were electrophoresed in NuPAGE 4-12% Bis-Tris Gel (Invitrogen) and transferred to a nitrocellulose membrane (Schleicher & Schuell). The membrane was blocked for 1 h at room temperature with 3% skimmed milk in TBS-T (pH 7.6), washed with TBS-T three times, and incubated with horseradish peroxidase-conjugated streptavidin in TBS-T for 1 h (1:1000, Pierce). The blots were washed with TBS-T, and visualized with ECL (Amersham).

Photoaffinity labeling (labeling inhibition). A mixture of 200 ng of recombinant hCD4 and a 17 μM DMSO solution of photoaffinity probe (0.25 μL) in PBS buffer (5 μL) was incubated in the presence or absence of inhibitors at 0 °C for 1 h in an open Eppendorf tube, and then irradiated for 20 min from above with an 450 W high-pressure Hg lamp (Model UM-452, Ushio) at 0 °C. SDS-PAGE and Western blotting were performed as described above.

ACKNOWLEDGEMENTS

We thank Dr. Masami Suganuma (Saitama Cancer Center) for helpful discussion. This work was supported in part by a Grant-in-Aid for Scientific Research on Priority Areas (no. 17035025) from The Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

REFERENCES AND NOTES

†We would like to dedicate this paper to Professor Tohru Fukuyama on the occasion of his 70th birthday.

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