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Synthesis and Characterization of the 7-(4-Aminomethyl-1*H*-1,2,3-triazol-1-yl) Analogue of Kabiramide C

Chutima Petchprayoon,[†] Khanit Suwanborirux,*[,†] Reagan Miller,[‡] Tomoyo Sakata,[§] and Gerard Marriott*[,§]

Bioactive Marine Natural Products Chemistry Research Unit (BMNCU), Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Pathumwan, Bangkok 10330, Thailand, Department of Chemistry, University of Wisconsin, 1101 University Avenue, Madison, Wisconsin 53706, and Department of Physiology, University of Wisconsin, 1300 University Avenue, Madison, Wisconsin 53706

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The 7-(4-aminomethyl-1*H*-1,2,3-triazol-1-yl) analogue of kabiramide C (**5**) was synthesized by using the Mitsunobu reaction and 1,3-dipolar cycloaddition. This compound and the intermediate compounds **2** and **4** were shown to bind tightly to G-actin in a 1:1 complex and exhibited the same degree of cytotoxicity as **1**. Compound **5** serves as a key intermediate for the synthesis of actin-directed optical probes and drugs.

Actin is a highly conserved and abundant protein that plays essential roles in cytokinesis, cell motility, and vesicle transport. Actin exists in a dynamic equilibrium between a monomeric state (G-actin) and a polymeric state (F-actin). In physiological salt solutions, actin exists predominantly as F-actin, with G-actin being maintained only near the critical concentration of 0.2 μM. This equilibrium is considerably shifted in living cells, where almost half of the 200 μM actin exists as G-actin. The shift in equilibrium is brought about by the binding of different actin-binding proteins found within cells. These binding proteins exert a range of activities on actin including sequestering of G-actin, capping of the (+)-end of the actin filament, and accelerating filament disassembly.[1] A major challenge in cell biology is to understand how these actin-binding protein activities regulate actin filament dynamics and cell motility.

Pharmacological drugs that target sites on actin such as cytochalasin and latrunculin have played an important role in our understanding of actin function in cells.[2] More recently we have shown that some actin-targeted macrolide drugs, the family of compounds typified by kabiramide C (KabC), function as unregulated biomimetics of actin filament (+)-end-capping proteins.[3,4] These studies suggested that optical and chemical probes based on KabC could provide new information on the regulation of actin filament dynamics in living cells. An analysis of the high-resolution structure of the KabC−G-actin complex shows that optical probes linked to the tail region in the KabC molecule (Figure 1) would prevent the drug from binding to actin, and indeed, a previous study showed mixed results for the binding of mycalolide B and kabiramide D derivatives of biotin.[5] We argue that the 7-hydroxyl group of KabC would be a far more suitable site because it does not engage in direct contacts with actin and should therefore not interfere with actin binding.[3] However we are unaware of any studies on the chemistry of the 7-hydroxyl group of KabC, although the 5-amino and 5-hydroxy groups are found naturally in other trisoxazole macrolides, such as halishigamide A and jaspisamide A, respectively.[4] As part of our approach to the design and synthesis of functional optical probes of KabC, we elected to introduce an amino group to the 7-position since this would allow a facile route for the preparation of a myriad of KabC probes using commercially available activated carboxylic esters.

* To whom correspondence should be addressed. (K.S.) Tel: (662) 2188363. Fax: (662) 2545195. E-mail: skhanit@chula.ac.th. (G.M.) Tel: (608) 262-6309. Fax: (608) 265-5512. E-mail: GM@physiology.wisc.edu.
† Chulalongkorn University.
‡ Department of Chemistry, University of Wisconsin−Madison.
§ Department of Physiology, University of Wisconsin−Madison.

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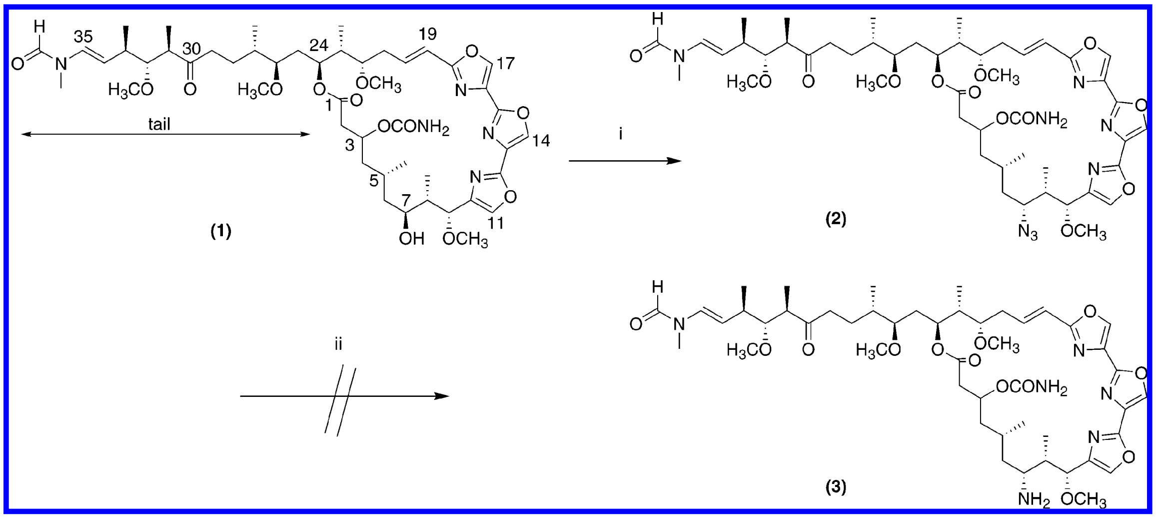

Figure 1. Synthesis of 7-aminokabiramide C (**3**): (i) HN_3, PPh_3, DIAD, dry THF, rt; (ii) $SnCl_2 \cdot 2H_2O$, MeOH, rt or PPh_3, THF/H_2O, rt or PPh_3, THF, NH_4OH/H_2O, rt or $NaBH_4$, THF/MeOH, rt.

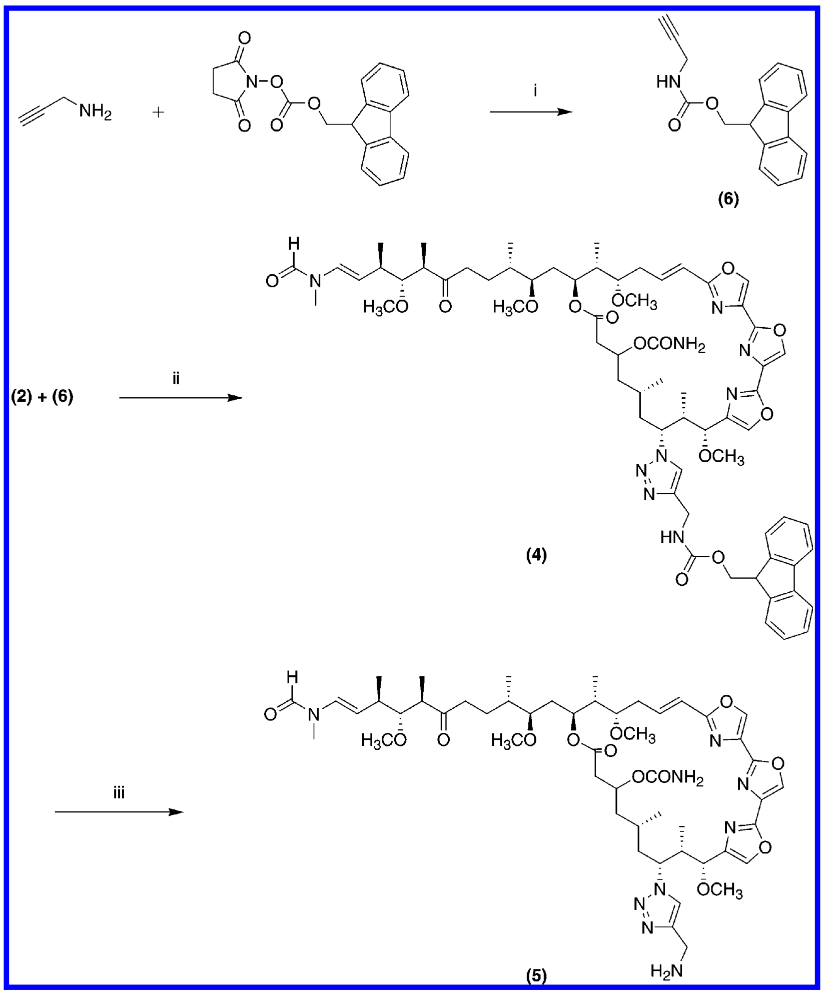

Figure 2. Synthesis of 7-(4-aminomethyl-1*H*-1,2,3-triazol-1-yl)kabiramide C (**5**): (i) THF, 0 °C → rt; (ii) MeOH/H_2O, Et_3N, CuI, rt; (iii) 20% v/v piperidine in dry CH_2Cl_2, rt.

Results and Discussion

KabC (**1**) was isolated as a white amorphous solid from the sponge *Pachastrissa nux* collected from Sichang Island, Thailand. The ^1H NMR spectrum showed some characteristic peaks of this class of compound in the downfield region such as three proton signals of oxazole rings at δ 8.09 (H-14), 8.03 (H-17), and 7.57 (H-11) ppm and *N*-formamide proton signals at δ 8.27 and 8.06 ppm in the ratio 2:1 due to the two geometrical forms. The ^1H NMR data of our KabC were identical with KabC data previously reported in the literature.[6]

KabC was converted to 7-azido KabC (**2**) via the Mitsunobu reaction[7] by using hydrazoic acid as nucleophile in the presence of triphenylphosphine (PPh_3) and diisopropyl azadicarboxylate (DIAD; Figure 1). The reaction proceeded smoothly using the small molecule hydrazoic acid but failed using the more conventional reagent diphenylphosphoryl azide (DPPA),[8] presumably because of steric

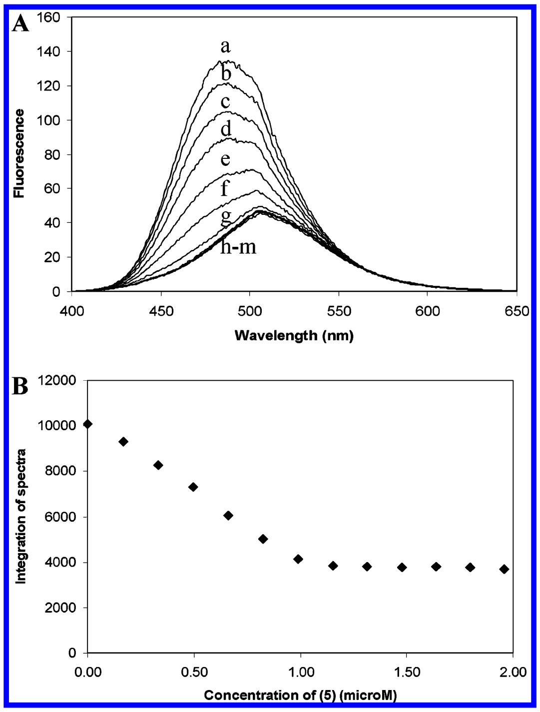

Figure 3. (A) Stoichiometric binding of 1 μM Prodan−G-actin in G-buffer with **5** at final concentrations of 0 μM (a), 0.17 μM (b), 0.33 μM (c), 0.50 μM (d), 0.66 μM (e), 0.83 μM (f), 0.99 μM (g), 1.15 μM (h), 1.32 μM (i), 1.48 μM (j), 1.64 μM (k), 1.80 μM (l), and 1.96 μM (m). (B) Plot of the integrated intensity in (A) against concentration of **5**.

hindrance. Attempts to prepare 7-amino KabC (**3**) from **2** by using various methods[9] such as $SnCl_2 \cdot 2H_2O$, PPh_3/THF/H_2O, and PPh_3/THF/NH_4OH were unsuccessful. We speculate that these reactions also failed because of steric hindrance at this position. In addition, reduction of **2** with $NaBH_4$[9d] caused the complete decomposition of **2**. These findings prompted the synthesis of a 7-modified KabC derivative harboring an amino group at the end of a longer linker group. For this approach, we coupled **2** with 3-aminopropyne using the 1,3-dipolar cycloaddition reaction[10] to create the 1,2,3-triazole derivative. The commercially available 3-aminopropyne was protected as 3-(fluoren-9-ylmethoxycarbonyl)aminopropyne (**6**) by using *N*-(9*H*-fluoren-9-ylmethoxycarbonyloxy)succinimide (FmocNHS). Compound **2** was reacted with **6** in the presence of a catalytic amount of copper iodide (CuI) and triethylamine (Et_3N) to afford 7-[4-*N*-(9*H*-fluoren-9-ylmethoxycarbonyl)aminomethyl-1,2,3-triazol-1-yl]-KabC (**4**). The presence of the catalytic amount of Cu(I) salt in 1,3-dipolar cycloaddition of terminal alkyne to azide not only catalyzed the reaction but also improved the regioselectivity to give the 1,4-substituted 1,2,3-triazole.[11] The structure of this compound was confirmed by the presence of aromatic proton signals of Fmoc in the ^{1}H NMR spectrum. Finally, deprotection of Fmoc with 20% piperidine in dry CH_2Cl_2 gave 7-(4-aminomethyl-1*H*-1,2,3-triazol-1-yl)kabiramide C (**5**) (Figure 2). The ^{1}H NMR spectrum confirmed the presence of the triazole proton signal at δ 7.49 and methylene proton signal at δ 4.01 ppm.

The binding of compounds **2**, **4**, and **5** to Prodan−G-actin was confirmed by measuring the change in the fluorescence emission spectrum of Prodan−G-actin as a function of drug concentration. The data shown in Figure 3A,B show that each KabC derivative was capable of tightly binding G-actin with the same 1:1 stoichiometry as KabC.[3−5] The toxicity of **5** on human cervix carcinoma (HeLa) cells was evaluated by treating the medium bathing HeLa cells with varying amounts of **5** (0, 10, 100, and 1000 nM) for 16 h. Cells treated with **5** at a concentration of 1 μM died within 16 h, whereas lower concentrations of drug (10 and 100 nM) caused defects in cytokinesis and loss of cell−cell contacts. Control cells treated with the same volume of methanol were mainly mononucleate and healthy (Supporting Information).

Experimental Section

General Experimental Procedures. All chemicals used in this work were purchased from Sigma-Aldrich Corporation. Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS), and penicillin-streptomycin were purchased from Invitrogen. Acrylodan was purchased from Molecular Probes. THF was dried over benzophenone and sodium, and CH_2Cl_2 was passed through Al_2O_3 (activity I) and dried over CaH_2 before use. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. UV spectra were determined with a Shimadzu UV-1601PC UV−visible spectrophotometer. IR spectra were obtained from a Perkin-Elmer 2000 FT-IR spectrometer and a Mattson Polaris FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively, on a Varian INOVA-500 spectrometer and at 300 and 75 MHz on a Bruker AC-300 spectrometer using TMS as an internal standard. Mass spectra were recorded on a Micromass LCT or Micromass AutoSpec. Fluorescence emission spectra were recorded on an SLM-Aminco AB2 fluorometer.

Animal Material. The marine sponge was collected at the depth of 20−25 feet from Sichang Island, Chon Buri Province, Thailand, in February 2003. The sponge was identified as *Pachastrissa nux* by Dr. John N. A. Hooper of Queensland Museum, Brisbane, Australia. A voucher specimen (QM G320223) has been deposited at the museum.

Extraction and Isolation. The sponge (15 kg, wet wt) was homogenized and exhaustively extracted with MeOH (11 L × 4). After filtration and concentration, the residue was partitioned with EtOAc. The EtOAc layer was evaporated to obtain the residue, which was dissolved in MeOH and partitioned with hexane. The MeOH extract was concentrated to a give residue (11.47 g), which was further chromatographed on a SiO_2 gel vacuum column (step-gradient of $CHCl_3$ and MeOH), a Sephadex LH-20 column (hexane/$CHCl_3$/MeOH, 2:1:1), a SiO_2 gel flash column ($CHCl_3$/MeOH, 25:1), and a preparative HPLC column (ODS, MeOH/H_2O, 78:22) to give kabiramide C (1.61 g, 0.01% w/w, wet wt).

Kabiramide C (1): white amorphous solid; $[\alpha]^{23}_{D}$ +8.33° (*c* 0.047, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 247 (4.37) nm; IR (film) ν_{max} 3455, 2929, 1716, 1653 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.27 (0.7H, s, NCOH-35), 8.09 (1H, s, H-14), 8.06 (0.3H, s, NCOH-35), 8.03 (1H, s, H-17), 7.57 (1H, d, J = 0.53 Hz, H-11), 7.45 (1H, ddd, J = 5.78, 9.69, 15.78, H-20), 7.11 (0.3H, d, J = 14.60 Hz, H-35), 6.45 (0.7H, d, J = 14.60 Hz, H-35), 6.28 (1H, d, J = 15.78 Hz, H-19), 5.30 (1H, m, H-3), 5.14 (1H, m, H-24), 5.09 (1H, m, H-34), 4.79 (1H, br s, H-9), 3.82 (1H, m, H-7), 3.65 (1H, m, H-22), 3.44 (3H, s, OCH_3-9), 3.42 (3H, s, OCH_3-22), 3.33 (3H, s, OCH_3-32), 3.31 (3H, s, OCH_3-26), 3.24 (1H, H-32), 3.02 (1H, s, NCH_3-35), 3.06 (2H, s, NCH_3-35), 2.98 (1H, H-26), 2.90−2.30 (8H), 2.13 (1H, m, H-8), 2.00−1.20 (11H), 1.14 (3H, d, J = 7.21 Hz, CH_3-33), 0.98 (3H, d, J = 6.70 Hz, CH_3-8), 0.92 (3H, d, J = 6.47 Hz, CH_3-5), 0.90 (3H, d, J = 6.89 Hz, CH_3-31), 0.86 (3H, d, J = 6.92 Hz, CH_3-23), 0.81 (3H, d, J = 6.59 Hz, CH_3-27); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 213.79 (C), 213.70 (C), 171.21 (C), 162.78 (C), 161.86 (CH), 160.59 (CH), 156.96 (C), 156.06 (C), 155.07 (C), 141.62 (CH), 141.03 (C), 137.02 (CH), 136.71 (CH), 135.44 (CH), 130.80 (C), 129.59 (C), 128.49 (CH), 124.48 (CH), 115.28 (CH), 112.93 (CH), 111.16 (CH), 87.22 (CH), 87.14 (CH), 81.86 (CH), 79.03 (CH), 78.24 (CH), 73.84 (CH), 73.08 (CH), 69.13 (CH), 61.24 (CH_3), 57.76 (CH_3), 57.52 (CH_3), 57.37 (CH_3), 49.07 (CH), 48.98 (CH), 44.76 (CH_2), 43.55 (CH_2), 42.83 (CH_2), 42.33 (CH_2), 42.26 (CH_2), 40.41 (CH), 37.58 (CH), 37.52 (CH), 37.38 (CH), 34.49 (CH), 33.88 (CH_2), 33.10 (CH_3), 32.86 (CH_2), 27.59 (CH_3), 24.99 (CH), 24.89 (CH_2), 19.36 (CH_3), 18.40 (CH_3), 15.56 (CH_3), 13.58 (CH_3), 10.88 (CH_3), 8.52 (CH_3); ESIMS *m/z* [M + Na]$^+$ 964.4866 (calcd for $C_{48}H_{71}N_5O_{14}Na$, 964.4895).

7-Azidokabiramide C (2). A paste of sodium azide (260 mg, 4 mmol) in H_2O (260 μL) was stirred on an ice bath, and benzene (1.6 mL) was added. Concentrated sulfuric acid (106 μL, 2 mmol) was carefully added dropwise to the reaction mixture. After stirring for 10 min, the organic layer containing hydrazoic acid was separated and dried over anhydrous $MgSO_4$. To the mixture of KabC (286 mg, 304 μmol) and PPh_3 (158 mg, 602 μmol) in dry THF (3 mL) stirred on an ice bath under a nitrogen atmosphere was added the solution of hydrazoic acid in benzene (300 μL). After stirring for 15 min, DIAD (117 μL, 604 μmol) was added dropwise over 1 min to the reaction mixture. The reaction mixture was allowed to warm to room temperature and stirred for 4 h. The reaction mixture was concentrated and purified by a Sephadex LH-20 column (hexane/CH_2Cl_2/MeOH, 2:1:1) and preparative SiO_2 gel TLC (CH_2Cl_2/MeOH, 20:1) to give **2** as a white amorphous solid (119 mg, 40.46%): $[\alpha]^{23}_D$ −6.27° (*c* 0.047, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 247 (4.12) nm; IR ($CHCl_3$) ν_{max} 3464, 3347, 3166, 3020−2935, 2104, 1720, 1657 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.27 (0.7H, s, NCOH-35), 8.10 (1H, s, H-14), 8.08 (0.3H, s, NCOH-35), 8.04 (1H, s, H-17), 7.61 (1H, d, *J* = 1.18 Hz, H-11), 7.51 (1H, ddd, *J* = 5.89, 9.99, 16.00, H-20), 7.13 (0.3H, d, *J* = 14.30 Hz, H-35), 6.46 (0.7H, d, *J* = 14.30 Hz, H-35), 6.29 (1H, d, *J* = 16.00 Hz, H-19), 5.31 (1H, m, H-3), 5.14 (1H, m, H-24), 5.10 (1H, m, H-34), 4.42 (1H, br s, H-9), 3.72 (1H, m, H-22), 3.52 (3H, s, OCH_3-9), 3.52−3.44 (1H, H-7), 3.44 (3H, s, OCH_3-22), 3.34 (3H, s, OCH_3-32), 3.33 (3H, s, OCH_3-26), 3.34−3.32 (1H, H-32), 3.08 (1H, s, NCH_3-35), 3.04 (2H, s, NCH_3-35), 2.99 (1H, H-26), 2.90−2.20 (8H), 2.00−1.20 (12H), 1.16 (3H, d, *J* = 7.07 Hz, CH_3-33), 1.03 (3H, d, *J* = 6.10 Hz, CH_3-8), 0.95−0.81 (12H, 4 × CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 214.16 (C), 214.07 (C), 171.57 (C), 163.13 (C), 162.08(CH), 160.80 (CH), 157.19 (C), 156.28 (C), 155.50 (C), 142.35 (C), 142.04 (CH), 137.11 (CH), 136.70 (CH), 135.52 (CH), 131.00 (C), 129.80 (C), 128.66 (CH), 124.66 (CH), 115.31 (CH), 113.02 (CH), 111.22 (CH), 87.28 (CH), 87.21 (CH), 82.39 (CH), 81.95 (CH), 78.98 (CH), 73.87 (CH), 69.25 (CH), 64.42 (CH), 61.28 (CH_3), 58.50 (CH_3), 57.77 (CH_3), 57.32 (CH_3), 49.05 (CH), 48.97 (CH), 42.89 (CH_2), 42.46 (CH_2), 42.34 (CH_2), 42.26 (CH_2), 40.36 (CH), 39.51 (CH_2), 38.56 (CH), 37.54 (CH), 37.35 (CH), 34.47 (CH), 34.42 (CH), 33.65 (CH_2), 33.03 (CH_3), 32.81 (CH_2), 27.51 (CH_3), 25.56 (CH), 24.81 (CH_2), 20.92 (CH_3), 19.27 (CH_3), 15.45 (CH_3), 13.48 (CH_3), 8.43 (CH_3), 6.23 (CH_3); ESIMS *m/z* [M + Na]$^+$ 989.4691 (calcd for $C_{48}H_{70}N_8O_{13}Na$, 989.4690).

7-[4-*N*-(9*H*-Fluoren-9-ylmethoxycarbonyl)aminomethyl-1,2,3-triazol-1-yl]kabiramide C (4). Compound **2** (119 mg, 123 μmol) was dissolved in a small volume of MeOH followed by water (6 mL). To the suspension of **2** were added compound **6** (51 mg, 184 μmol), Et_3N (35 μL, 251 μmol), and Cu(I)I (3 mg, 16 μmol). The reaction mixture was stirred at room temperature for 1 h and then extracted with EtOAc (6 mL × 4). The combined extracts were washed with brine, dried, and concentrated to give a crude product, which was purified by preparative SiO_2 gel TLC (CH_2Cl_2/MeOH, 20:1) to give **4** as a white amorphous solid (97 mg, 63.41%): $[\alpha]^{23}_D$ +3.06° (*c* 0.032, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 255 (4.49), 299 (3.82) nm; IR ($CHCl_3$) ν_{max} 3453, 3348, 3072, 3006−2883, 1721, 1655, 1513 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.29 (0.7H, s, NCOH-35), 8.07 (1H, s, H-14), 8.07 (0.3H, s, NCOH-35), 8.03 (1H, s, H-17), 7.76 (2H, d, *J* = 7.16 Hz, Fmoc H-4 and H-5), 7.58 (2H, d, *J* = 7.16 Hz, Fmoc H-1 and H-8), 7.57 (1H, s, H-11), 7.53 (1H, m, H-20), 7.41 (1H, s, triazole H-5), 7.40 (2H, t, *J* = 7.16 Hz, Fmoc H-3 and H-6), 7.30 (2H, t, *J* = 7.16 Hz, Fmoc H-2 and H-7), 7.13 (0.3H, d, *J* = 14.58 Hz, H-35), 6.46 (0.7H, d, *J* = 14.58 Hz, H-35), 6.29 (1H, d, *J* = 16.27 Hz, H-19), 5.47 (1H, m, NH), 5.31 (1H, m, H-3), 5.14 (1H, m, H-24), 5.12 (1H, m, H-34), 4.53 (3H, H-9 and NH*CH₂*), 4.40 (2H, d, *J* = 7.15 Hz, Fmoc CH_2), 4.22 (1H, t, *J* = 7.15 Hz, Fmoc H-9), 3.71 (1H, m, H-22), 3.49−3.43 (1H, H-7), 3.43 (3H, s, OCH_3-22), 3.34 (3H, s, OCH_3-32), 3.32 (3H, s, OCH_3-26), 3.34−3.32 (1H, H-32), 3.09 (3H, s, OCH_3-9), 3.08 (1H, s, NCH_3-35), 3.04 (2H, s, NCH_3-35), 3.00 (1H, H-26), 2.83−2.30 (8H), 2.15−1.20 (12H), 1.16 (3H, d, *J* = 6.52 Hz, CH_3-33), 1.03 (3H, d, *J* = 5.92 Hz, CH_3-8), 0.95−0.83 (12H, 4 × CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 214.20 (C), 214.11 (C), 171.68 (C), 163.19 (C), 162.13 (CH), 160.85 (CH), 157.09 (C), 156.36 (C), 156.36 (C), 155.48 (C), 144.53 (C), 143.81 (C), 143.81 (C), 141.93 (CH), 141.93 (C), 141.19 (C), 141.19 (C), 137.37 (CH), 136.94 (CH), 135.48 (CH), 130.94 (C), 129.73 (C), 128.70 (CH), 127.63 (CH), 127.63 (CH), 126.97 (CH), 126.97 (CH), 125.03 (CH), 125.03 (CH), 124.68 (CH), 121.27 (CH), 119.91 (CH), 119.91 (CH), 115.63 (CH), 113.06 (CH), 111.28 (CH), 87.33 CH), 87.24 (CH), 81.89 (CH), 80.71 (CH), 79.40 (CH), 73.90 (CH), 69.48 (CH), 66.89 (CH_2), 62.23 (CH), 61.32 (CH_3), 58.09 (CH_3), 57.93 (CH_3), 57.38 (CH_3), 49.12 (CH), 49.03 (CH), 47.11 (CH), 42.59 (CH_2), 42.37 (CH_2), 41.84 (CH_2), 40.24 (CH), 39.89 (CH_2), 39.20 (CH), 37.59 (CH), 37.37 (CH), 36.66 (CH_2), 34.35 (CH), 33.67 (CH_2), 33.08 (CH_3), 32.28 (CH_2), 27.56 (CH_3), 26.15 (CH), 24.72 (CH_2), 20.96 (CH_3), 19.31 (CH_3), 15.53 (CH_3), 13.54 (CH_3), 8.64 (CH_3), 6.81 (CH_3); ESIMS *m/z* [M + Na]$^+$ 1266.6033 (calcd for $C_{66}H_{85}N_9O_{15}Na$, 1266.6063).

7-(4-Aminomethyl-1*H*-1,2,3-triazol-1-yl)kabiramide C (5). To remove the Fmoc protecting group, compound **4** (97 mg, 78 μmol) was treated with 20% v/v piperidine in dry CH_2Cl_2 (2 mL) for 20 min. Water (3 mL) was added, and the reaction mixture was extracted with CH_2Cl_2 (3 mL × 4). The organic layers were combined, washed with brine, dried, and concentrated to give a residue, which was purified by preparative SiO_2 gel TLC (CH_2Cl_2/MeOH, 20:3) to give **5** as a white amorphous solid (25 mg, 30.76%): $[\alpha]^{23}_D$ −3.87° (*c* 0.012, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 246 (4.12); IR ($CHCl_3$) ν_{max} 3464, 3352, 3072−2884, 1722, 1655 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.29 (0.7H, s, NCOH-35), 8.10 (1H, s, H-14), 8.07 (0.3H, s, NCOH-35), 8.06 (1H, s, H-17), 7.64 (1H, s, H-11), 7.51 (1H, m, H-20), 7.49 (s, 1H, triazole H-5), 7.13 (0.3H, d, *J* = 14.20 Hz, H-35), 6.46 (0.7H, d, *J* = 14.20 Hz, H-35), 6.31 (1H, d, *J* = 15.41 Hz, H-19), 5.30 (1H, m, H-3), 5.13 (1H, m, H-24), 5.10 (1H, m, H-34), 4.48 (1H, br s, H-9), 4.01 (2H, s, NH_2*CH₂*), 3.65 (1H, m, H-22), 3.49−3.44 (1H, H-7), 3.43 (3H, s, OCH_3-22), 3.35 (3H, s, OCH_3-32), 3.33 (3H, s, OCH_3-26), 3.35−3.30 (1H, H-32), 3.15 (3H, s, OCH_3-9), 3.08 (1H, s, NCH_3-35), 3.04 (2H, s, NCH_3-35), 2.99 (1H, H-26), 2.83−2.30 (8H), 2.10−1.20 (12H), 1.16 (3H, d, *J* = 6.49 Hz, CH_3-33), 1.01 (3H, CH_3-8), 0.95−0.83 (12H, 4 × CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 214.25 (C), 214.15 (C), 171.67 (C), 163.26 (C), 162.16 (CH), 160.87 (CH), 157.14 (C), 156.44 (C), 155.52 (C), 142.28 (C), 142.28 (C), 141.82 (CH), 137.36 (CH), 136.98 (CH), 135.44 (CH), 131.11 (C), 129.86 (C), 128.75 (CH), 124.74 (CH), 120.33 (CH), 115.70 (CH), 113.11 (CH), 111.32 (CH), 87.38 (CH), 87.28 (CH), 81.96 (CH), 80.81 (CH), 79.48 (CH), 73.94 (CH), 69.52 (CH), 62.19 (CH), 61.36 (CH_3), 58.29 (CH_3), 57.97 (CH_3), 57.43 (CH_3), 49.16 (CH), 49.07 (CH), 42.64 (CH_2), 42.43 (CH_2), 42.38 (CH_2), 41.97 (CH_2), 40.38 (CH), 40.12 (CH_2), 39.58 (CH), 37.43 (CH), 37.32 (CH), 34.41 (CH), 33.77 (CH_2), 33.12 (CH_3), 32.40 (CH_2), 27.60 (CH_3), 26.46 (CH), 24.77 (CH_2), 21.72 (CH_3), 19.35 (CH_3), 15.56 (CH_3), 13.57 (CH_3), 8.44 (CH_3), 6.97 (CH_3); ESIMS *m/z* [M + H]$^+$ 1022.5574 (calcd for $C_{51}H_{76}N_9O_{13}$, 1022.5562).

3-(Fluoren-9-ylmethoxycarbonyl)aminopropyne (6). A suspension of *N*-(9*H*-fluoren-9-ylmethoxycarbonyloxy)succinimide (168 mg, 0.5 mmol) in dry THF was cooled with an ice bath, and 3-aminopropyne (propargylamine, 35 μL, 0.55 mmol) was added dropwise. The reaction mixture was stirred and allowed to warm to room temperature over 2 h. The solvent was removed under reduced pressure to give a residue. The residue was dissolved in EtOAc and washed with water. The organic layer was dried and concentrated. Recrystallization from EtOAc gave **6** as white needles (102 mg, 73.65%): UV (MeOH) λ_{max} (log ϵ) 266 (4.24), 290 (3.65), 300 (3.74) nm; IR ($CHCl_3$) ν_{max} 3453, 3307, 3066−2954, 1725, 1510 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.77 (2H, d, *J* = 7.42 Hz, Fmoc H-4 and H-5), 7.59 (2H, d, *J* = 7.42 Hz, Fmoc H-1 and H-8), 7.41 (2H, dt, *J* = 0.83, 7.42 Hz, Fmoc H-3 and H-6), 7.32 (2H, dt, *J* = 1.31, 7.42 Hz, Fmoc H-2 and H-7), 4.95 (1H, NH), 4.44 (2H, d, *J* = 6.69 Hz, Fmoc CH_2), 4.23 (1H, t, *J* = 6.69 Hz, Fmoc H-9), 4.01 (2H, dd, *J* = 2.37, 5.39 Hz, H-3), 2.26 (1H, t, *J* = 2.37 Hz, H-1); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.86 (C), 143.70 (C), 143.70 (C), 141.22 (C), 141.22 (C), 127.66 (CH), 127.66 (CH), 126.99 (CH), 126.99 (CH), 124.95 (CH), 124.95 (CH), 119.93

(CH), 119.93 (CH), 79.59 (C), 71.62 (CH), 66.97 (CH_2), 47.03 (CH), 30.75 (CH_2); EIMS *m/z* $[M]^+$ 277.1114 (calcd for $C_{18}H_{15}NO_2$, 277.1103).

G-Actin Binding Assay. G-Actin was prepared from rabbit muscle as described by Spudich and Watt.[12] Actin was labeled with the thiol probe, Acrylodan, as described by Marriott et al.[13] For the G-actin binding study, Prodan−G-actin was diluted to 1 μM in G-buffer[14] and then titrated with increasing amounts of the test compound from 0.17 to 2 μM. Fluorescence emission spectra were conducted by exciting Prodan at 385 nm and recording the emission between 400 and 650 nm.

Cell Culture. HeLa cells were cultured in DMEM supplemented with 10% v/v heat-activated FBS, 1% v/v penicillin (10 000 unit/mL), and streptomycin (10 mg/mL) at 37 °C in a humidified atmosphere containing 5% CO_2. Compound **5** was dissolved in MeOH and diluted to 10, 100, and 1000 nM in culture medium immediately prior to use. The final concentration of MeOH in this experiment was 0.1% v/v and was nontoxic to the cells. Phase contrast images of control and drug-treated cells were recorded after 16 h.

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Supporting Information Available: ^{1}H NMR spectra of compounds **1**, **2**, **4**, and **5**. Images of cells treated with varying amounts of compound **5** are also shown. These materials are available free of charge via the Internet at http://pubs.acs.org.

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(14) G-buffer contains 5 mM Tris, 0.1 mM $CaCl_2$, 0.2 mM ATP, and 1 mM dithiothreitol (DTT).

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