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Synthesis of RGD Analogs as Potential Vectors for Targeted Drug Delivery

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RGD analogs bind to integrin receptors with high affinity and therefore have the potential to be used as vectors for the targeted delivery of pharmaceutical agents to designated sites. Critical to this application is the ability to synthesize RGD analogs with different side chain functional groups that allow for the ready tethering of pharmaceutical agents without sacrificing their affinity for the target receptor significantly. A series of RGD analogs intended to be used as delivery vectors of pharmaceutical agents were prepared and evaluated for their ability to inhibit platelet aggregation by binding to glycoprotein IIb/IIIa. Among them, compound 11 showed the lowest IC_{50} against platelets activated by ADP. It was found that such RGD analogs could tolerate side chain modification fairly well with various functional groups attached such as amide, amine, ester, protected amine and poly(ethylene glycol). The fact that the compound with a side chain modification of poly(ethylene glycol) retained high affinity for glycoprotein IIb/IIIa (IC_{50} 150 nM) suggests the feasibility of tethering fairly large pharmaceutical agents to such RGD analogs without significant sacrifice of their affinity to the intended receptor.

INTRODUCTION

The tripeptide sequence Arginine–Glycine–Aspartic (RGD) acid is a universal recognition motif through which cells interact with extracellular proteins (*1*). The integrin family of receptors is composed of over 20 permutations of alpha and beta subunits that together form transmembrane cell surface receptors (*2,3*). Many integrins recognize the RGD sequence, although other peptide sequences are also targets for these receptors. Integrin receptor–ligand interactions "integrate" the cell cytoplasm with the extracellular environment and are involved in a variety of disease processes including thrombosis, tumor growth and metastases and inflammatory states (*2,3*).

The multiple roles of integrins have led to the development of RGD peptides or peptide mimetics to block integrin-mediated activities. The greatest success in this arena has been the development of inhibitors of $\alpha_{IIb}\beta_3$ (or GP IIb/IIIa), the fibrinogen receptor on platelets. Three agents are now approved for the treatment of acute

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coronary ischemic syndromes and/or for use during percutaneous coronary interventions (*4*). One of these (abciximab) is a chimeric humanized antibody Fab fragment to GP IIb/IIIa. Eptifibatide is a cyclical L-homoarginine–glycine–aspartic acid-containing peptide and tirofiban (MK-383) is a peptidomimetic (*5*).

The success of these agents and the high levels of platelet surface expression of the fibrinogen receptor have prompted studies that target GP IIb/IIIa for other goals. The ability of RGD peptides to serve as a thrombus imaging agent has been tested in early studies. RGD peptides, coupled to iron oxide particles or technetium-99^m, allows for thrombus imaging by magnetic resonance (*6*) or nuclear scintigraphy (*7*), respectively. Furthermore, red blood cells conjugated with RGD ligands have been envisioned as potential substitutes for platelets (*8*), thereby overcoming the limitations of platelet transfusion therapy. Other integrins have also been the target of RGD-based strategies. Radiolabeled RGD peptides, directed at the vitronectin receptor ($\alpha_v\beta_3$) have been used to image tumors or tumor neovasculature in animal models (*9–11*). RGD peptides that bind to $\alpha_v\beta_3$ have also been used to transiently increase the permeability of tumor vasculature, thereby allowing a higher fractional uptake of a radiolabeled monoclonal antibody directed at a tumor (*12*). RGD peptides conjugated with polylysine have been designed as delivery vehicles for enhancing the efficiency of transfecting cells with recombinant DNA (*13–15*). Finally, RGD peptides have been used in tissue engineering applications to covalently modify biomaterial surfaces, thereby enhancing cell attachment (*16–20*).

These and other applications suggest that RGD-based imaging and targeting strategies will be used in a variety of clinical arenas in which integrin receptors play a dominant role. Although RGD peptides have comprised the bulk of the earliest effort, future work is likely to focus on peptidomimetics, which have several advantages over peptide-based approaches. As an example, the arginine residue in short peptides is known to be a target of cellular methylases, with the resultant methylarginyl group lacking binding activity for some integrins (*21*). This modification could limit long-term applications of peptide-containing compounds.

Imaging or targeting strategies that are based on RGD peptidomimetic compounds will likely require that an appropriate functional group be incorporated for tethering of the active agent. As a first step towards our goal of designing integrin-targeting compounds, we have synthesized a series of 10 analogs of MK-383, a GP IIb/IIIa inhibitor (*22*), and the affinities of these analogs for GP IIb/IIIa have been tested by determining their abilities to inhibit platelet aggregation. The analogs differ in containing either amide, amine, ester, protected amine, or polyethylene glycol-containing side chains of differing sizes. It was found that these functional groups could be introduced while retaining platelet inhibitory IC_{50}s in the nanomolar range. These analogs represent novel base compounds for designing targeting or imaging strategies directed at the integrin GP IIb/IIIa and set the stage for a general integrin targeting strategy using one or more of these functional groups.

EXPERIMENTAL

General methods. ^1H NMR and ^{13}C NMR spectra were recorded at 300 MHz. Mass spectral analyses were conducted by the Mass Spectrometry Laboratory for Biotechnology at North Carolina State University. Elemental analyses were performed

by Atlantic Microlab Inc. UV spectra were recorded on Shimadzu 1601 UV-Visible spectrophotometer. Column chromatography was performed using silica gel (230–400 mesh) from Aldrich. Chromatotron plates were made using silica gel (Merck, TLC grade 7749, with gypsum binder and fluorescent indicator) from Aldrich. Unless stated otherwise all starting materials were commercially available and all solvents and reagents were used without purification with the exceptions of THF and CH_2Cl_2. THF was distilled from sodium/benzophenone. CH_2Cl_2 was previously dried over $CaCl_2$ and then distilled from CaH_2.

*Affinity of modified RGD analogs (**2–11**) toward GPIIb/IIIa as determined by platelet aggregation (25).* After informed consent, blood was obtained from healthy donors taking no medications. Platelet rich plasma (PRP) was prepared by centrifuging the blood at 200*g* for 10 min. After removing the PRP, the sample was centrifuged again at 1200*g* for 10 min to obtain platelet-poor plasma that was used as the optical blank. The platelet aggregation assay is based on the fact that the platelet suspension scatters light and that increased transmission of light occurs after aggregation. Since interference with light transmission depends more on the number of particles rather than the size of particles, platelet aggregation results in an increase in light transmission. Aggregation assays were performed under standard conditions using a Chrono-Log Lumi-Aggregometer (Havertown, PA), at 37°C with a magnetic stirbar (rate 1000 rpm) with monitoring for 5 min after the addition of agonist. Platelet aggregation was stimulated by ADP at 20 μM final concentration. The IC_{50} of the compound was obtained by determining the concentration that reduced the maximum slope (percentage light transmittance/min) of aggregation by 50%.

*4-(4-Pyridinyl) butyl chloride (**13**) (24).* 4-Picoline (16.763 g, 0.180 mol) was dissolved in 82 ml of dry THF and the solution was cooled to −78°C with an acetone-dry ice bath. *n*-Butyllithium (79.19 ml, 0.198 mol) was added slowly while keeping the internal temperature below −50°C. The addition took over 1.5 h, giving an orange solution with precipitates. The reaction was allowed to warm to room temperature and then stirred at 40–45°C for 2 h. THF (82 ml) was added to dissolve the 4-picolyllithium slurry to give a deep orange solution. The solution was cooled down to 0°C and carefully added into the solution of 1-bromo-3-chloropropane (18.69 ml, 0.189 mol) in THF (30 ml) at −78°C. During the addition, the temperature was kept below −65°C. The reaction was allowed to gradually warm up to 0°C and then worked up by adding 150 ml H_2O. A yellow solution was obtained. The organic layer was separated and the aqueous layer was extracted with 2 × 50 ml EtOAc. All the organic layers were combined and dried over $MgSO_4$. Then it was concentrated and the residue was purified with flash chromatography using hexanes/ethyl acetate (1:1.5) to give an orange oil **13** (28.0089 g, 92%): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.50 (d, 2H, J = 4.86 Hz), 7.13 (d, 2H, J = 4.95 Hz), 3.56 (t, 2H, J = 5.19 Hz), 2.65 (t, 2H, J = 3.3 Hz).

*4-(4-Piperidinyl) butyl chloride (**14**).* The reaction flask was flushed with Ar first. Then acetic acid (7.5 ml), platinum(IV) oxide (0.75 g), and the solution of **13** (15 g, 0.088 mol) in acetic acid (7.5ml) were added sequentially. The black suspension was subjected to 60 psi H_2 in a Parr instrument for 72 h at room temperature. The catalyst was filtered off and the filtrate was concentrated to afford compound **14** as an acetate

salt (100%): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.54 (t, 2H, J = 6.30 Hz), 3.39 (d, 2H, J = 11.7 Hz), 2.84 (t, 2H, J = 12.2 Hz), 1.74–1.87 (m, 4H), 1.31–1.49 (m, 7H).

*4-(4-Chloro-butyl)-piperidine-1-carboxylic acid benzyl ester (**15**).* The acetate salt of compound **14** (5 g) was dissolved in 200 ml EtOAc. This organic solution was washed with 15% aqueous K_2CO_3 solution (2 × 50 ml). The organic layer was dried over $MgSO_4$ and concentrated to give a yellow oil (3.5389 g, 21.20 mmol). This oil was dried on the oil pump for 0.5 h and then dissolved in acetonitrile (90 ml). Pyridine (4.88 ml, 60.32 mmol) and benzyl chloroformate (4.5 ml, 30.16 mmol) were added sequentially into the solution at 0°C. The reaction was stirred at 0°C for 0.5 h and then at room temperature for 24 h. The solution was concentrated and the residue was dissolved in EtOAc (300 ml). The organic solution was washed with 1N HCl (3 × 50 ml), brine (50 ml) and dried over $MgSO_4$. EtOAc was then evaporated to give a yellow oil. This yellow oil was chromatographed on a silica gel column with hexanes/EtOAc (5:1) to give a colorless oil **32** (4.4253 g, 71%): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.26–7.36 (m, 5H), 5.12 (s, 2H), 4.11–4.17 (m, 2H), 3.54 (t, 2H, J = 6.6 Hz), 2.72–2.79 (m, 2H), 1.66–1.81 (m, 4H), 1.09–1.51 (m, 7H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 154.8, 136.8, 128.1, 127.5, 127.4, 66.5, 44.6, 43.9, 35.4, 32.4, 31.7, 23.6; MS/FAB *m/z* (MH^+) Calcd for $C_{17}H_{24}O_2NCl$ 310.16, found 310.2. Anal. Calcd for $C_{17}H_{24}O_2NCl$: C, 65.90; H, 7.81; N, 4.52. Found: C, 65.65; H, 7.92; N, 4.38.

*4-{4-[4-(2-tert-Butoxycarbonylamino-2-carboxy-ethyl)-phenoxy]-butyl}-piperidine-1-carboxylic acid benzyl ester (**16**) (22).* Under the protection of Ar, 60% disperse NaH was washed with hexanes four times to give a pale powder. The powder was dried on oil pump for 0.5 h. Then it was added into DMF (4.5 ml). *N*-Boc-tyrosine (3.6288 g, 12.9 mmol) was also dissolved in DMF (15 ml) and was added dropwise to the suspension of NaH (928.8 mg, 38.7 mmol) over 0.5 h at 0°C. The suspension was stirred for 1 h at 0°C. Then compound **15** (4.0000 g, 12.9 mmol) in 15 ml DMF was added into the suspension at 0°C over 0.5 h, followed by the addition of Kl (0.4283 g, 2.58 mmol). The reaction was stirred at 0°C for 1 h and then at 40–45°C for 43 h to give a dark solution with white precipitates. DMF was removed and EtOAc (150 ml) was added into the residue. The organic solution was acidified to pH 4 with 10% $KHSO_4$ (30 ml). The organic layer was separated and the aqueous layer was extracted again with EtOAc (100 ml). The combined organic layers were washed with brine (2 × 20 ml), dried over $MgSO_4$, and concentrated to an oil. The crude product was chromatographed on a silica gel column with hexanes/EtOAc (2:1) to give compound **16** (5.8390 g, 82%): ^{1}H-NMR (300 MHz, $CDCl_3$), δ 7.30–7.37 (m, 5H), 7.08 (d, 2H, J = 9.0 Hz), 6.82 (d, 2H, J = 8.1 Hz), 5.12 (s, 2H), 4.90–4.95 (m, 1H), 4.50–4.60 (m, 1H), 4.11–4.17 (m, 2H), 3.94 (t, 2H, J = 6.2 Hz), 3.00–3.15 (m, 2H), 2.65–2.80 (m, 2H), 1.65–1.78 (m, 4H), 1.07–1.50 (m, 7H), 1,42 (s, 9H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 175.7, 158.4, 155.7, 137.1, 130.6, 128.7, 128.1, 127.9, 114.8, 80.4, 67.9, 67.3, 54.6, 44.5, 37.1, 36.3, 36.0, 32.3, 29.6, 28.5, 23.3; MS/FAB *m/z* (MH^+) calcd for $C_{31}H_{42}O_7N_2$ 555.3, found 555.4. Anal. calcd for $C_{31}H_{42}O_7N_2$: C, 67.13; H, 7.63; N, 5.05. Found: C, 66.87; H, 7.72; N, 4.92.

*4-{4-[4-(2-Benzyloxycarbonyl-2-tert-butoxycarbonylamino-ethyl)-phenoxy]-butyl}-piperidine-1-carboxylic acid benzyl ester (**17**).* 1,3-Dicyclohexylcarbodiimide (0.4667 g, 2.26 mmol) was added to the solution of **16** (0.9625 g, 1.74 mmol) in CH_2Cl_2 (1 ml) at 0°C. The reaction was stirred for 10 min with white precipitates

formed. Then benzyl alcohol (0.4328 g, 4.00 mmol) and 4-dimethylaminopyridine (0.0425 g, 0.35 mmol) were added sequentially. The solution was stirred at 0°C for 1.5 h, then at room temperature for 4.5 h. The resulted suspension was put in the freezer overnight. The white precipitates that formed were filtered off and the filtrate was concentrated. The residue was dissolved in EtOAc (25 ml) and the organic solution was washed with 1N HCl (2 × 10 ml), saturated $NaHCO_3$ (2 × 10 ml) and brine (10 ml). The organic layer was dried over $MgSO_4$ and then concentrated to give an oil. The crude oil was chromatographed on a silica gel column with hexanes/EtOAc (1.5:1) to give compound **17** (0.9167 g, 80%): ^{1}H-NMR (300 MHz, $CDCl_3$), δ 7.28–7.35 (m, 10H), 6.93 (d, 2H, J = 8.1 Hz), 6.74 (d, 2H, J = 8.2 Hz), 5.07–5.15 (m, 4H), 4.90–5.00 (m, 1H), 4.50–4.60 (m, 1H), 4.10–4.30 (m, 2H), 3.90 (t, 2H, J = 6.2 Hz), 3.02 (d, 2H, J = 5.0 Hz), 2.76 (m, 2H), 1.64–1.77 (m, 4H), 1.01–1.47 (m, 7H), 1.41 (s, 9H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 172.0, 158.3, 155.5, 155.3, 137.2, 135.4, 130.5, 128.7, 128.6, 128.5, 128.1, 128.0, 127.8, 114.7, 80.0, 67.9, 67.2, 67.1, 54.7, 44.4, 37.6, 36.4, 36.0, 32.9, 32.3, 29.6, 28.5, 23.3; MS/FAB *m/z* (MH^+) calcd for $C_{38}H_{48}O_7N_2$ 645.4, found 645.5. Anal. calcd for $C_{38}H_{48}O_7N_2$: C, 70.78; H, 7.50; N, 4.34. Found: C, 70.60; H, 7.56; N, 4.42.

*4-(4-{4-[2-Benzyloxycarbonyl-2-(4-carboxy-benzenesulfonylamino)-ethyl]-phenoxy}-butyl)-piperidine-1-carboxylic acid benzyl ester (**18**).* Compound **17** (111.5 mg, 0.17 mmol) was dissolved in 25% TFA solution in CH_2Cl_2 (6.0 ml) and stirred at room temperature for 1 h. The solution was concentrated and the residue was dissolved in 3.0 ml CH_2Cl_2. TEA (1.0 ml) was added and the resulted solution was stirred for 5 min. Then it was concentrated and dried on oil pump for 0.5 h. The residue was dissolved in 1.5 ml CH_3CN and 1 mL H_2O. To this solution, K_2CO_3 (143.5 mg, 1.04 mmol) was added. Then 4-(chlorosulfonic)benzoic acid (76.5 mg, 0.35 mmol) was added in portions at 0°C over 15 min. After the addition, the solution was stirred at 0°C for 0.5 h and at room temperature for 1 h. Another one equivalent of 4-(chlorosulfonic)benzoic acid (38.2 mg, 0.17 mmol) was added in portions at 0°C. The reaction was stirred at 0°C for 0.5 h and at room temperature for another 3.5 h. The solution was acidified to pH 1 with 1 N HCl. Then it was concentrated and the residue was dissolved in 100 ml EtOAc. The organic solution was washed with 1N HCl (20 ml) and brine (20 ml). The organic layer was dried over $MgSO_4$ and concentrated. The crude product was purified on chromatotron with hexanes/EtOAc (3:1) to give an oil **18** (60.7 mg, 48.4%): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.08 (d, 2H, J = 8.2 Hz), 7.78 (d, 2H, J = 8.3 Hz), 7.17–7.35 (m, 10H), 5.23 (d, 1H, J = 9.3 Hz), 5.13 (s, 2H), 4.93 (s, 2H), 4.15–4.27 (m, 3H), 3.89 (t, 2H, J = 6.2 Hz), 2.97–3.00 (m, 2H), 2.70–2.90 (m, 2H), 1.50–1.77 (m, 4H), 1.15–1.75 (m, 7H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 170.9, 158.5, 155.7, 144.5, 137.0, 135.3, 134.7, 133.4, 130.9, 130.6, 128.8, 128.6, 128.1, 128.0, 127.3, 126.5, 114.7, 67.9, 67.7, 67.3, 57.2, 44.5, 38.7, 36.3, 36.0, 32.2, 29.6, 23.3; HRMS/FAB *m/z* (MH^+) calcd for $C_{40}H_{44}O_9N_2S$ 729.2876, found 729.2832.

*4-(4-{4-[2-Benzyloxycarbonyl-2-(4-pentanoyl-benzenesulfonylamino)-ethyl]-phenoxy}-butyl)-piperidine-1-carboxylic acid benzyl ester (**19**).* Compound **18** (28.0 mg, 0.04 mmol) was dissolved in 1.0 ml CH_2Cl_2. EDC (8.0 mg, 0.04 mmol) was added and the reaction was stirred for 10 min to give a cloudy solution. Then HOBT (5.2 mg, 0.04 mmol) was added and the reaction was stirred for 30 min. *n*-Butyl amine

(7 μm, 0.07 mmol) and DMAP (1.0 mg, 0.008 mmol) were added sequentially at 0°C. The solution was stirred at 0°C for 0.5 h and then at room temperature for 12 h. The precipitates were filtered off and the filtrate was concentrated. The residue was dissolved in 20 ml EtOAc. The organic solution was washed with 10% citric acid (2 × 5 ml), saturated Na_2CO_3 (2 × 5 ml), and brine (5 ml). Then the organic layer was dried over $MgSO_4$ and concentrated. The crude product was purified on chromatotron with hexanes/EtOAc (1:1) to give compound **19** (30.0 mg, 99%): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.71–7.78 (m, 4H), 7.15–7.35 (m, 10H), 6.87 (d, 2H, J = 8.5 Hz), 6.68 (d, 2H, J = 8.4 Hz), 6.14–6.18 (m, 1H), 5.29 (d, 1H, J = 9.2 Hz), 5.12 (s, 2H), 4.87–4.95 (m, 2H), 4.11–4.22 (m, 3H), 3.89 (t, 2H, J = 6.4 Hz), 3.46 (q, 2H, J = 6.9 Hz), 2.97 (d, 2H, J = 5.2 Hz), 2.72–2.80 (m, 2H), 1.05–1.78 (m, 15H), 0.97 (t, 3H, J = 7.3 Hz); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 170.7, 166.1, 158.6, 155.6, 142.4, 139.0, 134.2, 134.8, 131.0, 130.7, 128.9, 128.7, 128.1, 128.1, 127.8, 127.6, 126.7, 126.6, 114.8, 68.1, 67.8, 67.2, 57.0, 44.5, 40.3, 38.8, 36.4, 36.1, 32.3, 31.9, 29.6, 23.4, 20.4, 14.0; HRMS/FAB m/z (MH^+) calcd for $C_{44}H_{53}O_8N_3S$ 784.3632, found 784.3668. Anal. calcd for $C_{44}H_{53}O_8N_3S$: C, 67.41; H, 6.81; N, 5.36. Found: C, 67.68; H, 7.06; N, 5.18.

*2-(4-Pentanoyl-benzenesulfonylamino)-3-[4-(4-piperidin-4-yl-butoxy)-phenyl]-propionic acid (**2**).* Compound **19** (30.0 mg, 0.04 mmol) was dissolved in CH_3OH (2.0 ml) and DMF (0.4 ml). This clear solution was flushed with Ar for 10 min and then 10% Pd/C (4.4 mg) was added. The black suspension was flushed with Ar for 10 min and then it was subjected to hydrogen balloon at room temperature for 1 h. The catalyst was filtered off and the filtrate was concentrated to give **2** (20.0 mg, 100%): ^{1}H-NMR (300 MHz, CD_3OD) δ 7.76–7.85 (dd, 4H, J = 8.7 Hz), 7.07 (d, 2H, J = 8.1 Hz), 6.70 (d, 2H, J = 8.7 Hz), 3.95 (t, 2H, J = 6.3 Hz), 3.79 (m, 1H), 3.38 (t, 2H, J = 7.3 Hz), 2.83–2.96 (m, 4H), 1.28–1.94 (m, 15H), 0.97 (t, 3H, J = 7.2 Hz); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 169.0, 159.1, 144.4, 139.4, 131.8, 130.9, 129.0, 128.3, 115.4, 68.9, 61.1, 56.0, 45.4, 41.1, 39.8, 36.7, 32.6, 30.4, 30.1, 24.0, 21.3, 14.4; HRMS/FAB m/z (MH^+) calcd for $C_{29}H_{41}N_3O_6S$ 560.2794, found 560.2830.

*4-(4-Chloro-butyl)-piperidine-1-carboxylic acid tert-butyl ester (**20**).* Compound **14** was dissolved in EtOAc and the organic solution was washed with 15% aqueous K_2CO_3 solution to give a pale yellow oil in a similar manner as that for **15**. This oil (0.7320 g, 4.29 mmol) was dissolved in CH_2Cl_2 (10 ml) and TEA (0.90 ml, 6.44 mmol) was added. At 0°C di-tert-butyl dicarbonate (1.1230 g, 4.29 mmol) was added. The reaction was allowed to warm up to room temperature and stirred for 20 h. The solution was concentrated and the residue was dissolved in EtOAc. The organic solution was washed with brine twice, dried over $MgSO_4$ and concentrated. The crude product was purified on a silica gel column with hexanes/EtOAc (5:1) to give a pale yellow oil **20** (0.9483 g, 80%): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 4.05–4.09 (bd, 2H), 3.54 (t, 2H, J = 6.7 Hz), 2.66 (t, 2H, J = 12.4 Hz), 1.72–1.79 (m, 2H), 1.61–1.68 (m, 2H), 1.45 (s, 9H), 1.21–1.30 (m, 2H), 1.04–1.15 (m, 2H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 155.0, 79.0, 45.0, 44.0, 35.8, 35.7, 32.7, 28.4, 27.4, 24.0; MS/FAB m/z (MH^+) calcd for $C_{14}H_{26}ClNO_2$ 276.2, found 276.2. Anal. calcd for $C_{14}H_{26}ClNO_2$: C, 60.96; H, 9.50; N, 5.08. Found: C, 61.08; H, 9.52; N, 4.99.

*4-{4-[4-(2-Benzyloxycarbonylamino-2-tert-butoxycarbonyl-ethyl)-phenoxy]-butyl}-piperidine-1-carboxylic acid tert-butyl ester (**21**).* NaH (0.27 g, 6.67 mmol, 60%

dispersion in mineral oil) was placed in a 100 ml dry flask, then washed with 5 ml hexanes. Dry DMF (2 ml) was added into the above flask, then the flask was cooled to 0°C with an ice-bath. A solution of Boc-*N*-tyrosine *t*-butyl ester (2.20 g, 5.63 mmol) in dry DMF was added dropwise into the suspension. After the reaction mixture was stirred at 0°C for an additional hour, a solution of Boc-*N*-chloride (1.55 g, 5.63 mmol) was added following by addition of KI (0.187 g, 1.13 mmol). The reaction mixture was continued stirring at RT for 26 h. The solvent was evaporated under reduced pressure to give an oil, which was dissolved in 180 mL of ethyl acetate. The organic layer was washed with 1 N HCl aqueous solution (2 × 35 ml) and brine (35 ml), dried ($MgSO_4$), and concentrated to give an oil. The residue was purified on silica gel using ethyl acetate/hexanes (1/5) to afford a colorless oil (2.47 g, 72%). ^{1}H-NMR ($CDCl_3$) δ 7.34 (s, 5H), 7.03 (d, 2H, J = 8.4 Hz), 6.78 (d, 2H, J = 8.4 Hz), 5.20 (d, 1H, J = 7.9 Hz), 5.10 (s, 2H), 4.49 (dd, 1H, J = 6.1, 13.5 Hz), 4.09 (m, 2H), 3.91 (t, 2H, J = 6.3 Hz), 3.01 (d, 2H, J = 5.5 Hz), 2.67 (brt, 2H, J = 12.3 Hz), 1.61–1.80 (m, 4H), 1.52 (m, 2H), 1.45 (s, 9H), 1.41 (s, 9H), 1.24–1.33 (m, 3H), 1.01–1.15 (2H, m). ^{13}C-NMR ($CDCl_3$) δ 170.8, 158.3, 155.8, 155.0, 136.6, 130.6, 129.6, 128.2, 128.0, 114.6, 82.3, 79.3, 67.9, 66.9, 55.5, 44.2, 37.6, 36.4, 36.1, 32.3, 29.6, 28.6, 28.1, 23.3. MS/FAB *m/z* (MH^+) calcd for $C_{35}H_{50}N_2O_7$ 611.4, found 611.4. Anal. calcd for $C_{35}H_{50}N_2O_7$: C, 68.83; H, 8.25; N, 4.59. Found: C, 68.81; H, 8.35; H, 4.61.

*4-{4-[4-(2-Amino-2-tert-butoxycarbonyl-ethyl)-phenoxy]-butyl}-piperidine-1-carboxylic acid tert-butyl ester (**22**).* A solution of **21** (1.25 g, 2.05 mmol) in 40 ml of methanol in the presence of 10% Pd-C (100 mg) was hydrogenated at hydrogen atmosphere for 7 h. The catalyst was filtered out and washed with methanol (2 × 8 ml). The solvent was evaporated to give a slightly gray solid (0.939 g, 96%). The product was used directly for the next step reaction without purification. ^{1}H NMR ($CDCl_3$) δ 7.11 (d, 2H, J = 8.4 Hz), 6.82 (d, 2H, J = 8.4 Hz), 4.08 (brs, 2H), 3.93 (t, 2H, J = 6.3 Hz), 3.56 (dd, 1H, J = 5.5, 7.5 Hz), 3.48 (brs, 2H), 3.97 (dd, 1H, J = 5.5, 13.7 Hz), 2.77 (dd, 1H, J = 7.5, 13.7 Hz), 2.66 (brt, 2H, J = 12.3 Hz), 1.75 (m, 2H), 1.60–1.71 (m, 3H), 1.45 (s, 9H), 1.44 (s, 9H), 1.23–1.38 (m, 4H), 1.07 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 174.6, 158.1, 155.1, 130.5, 129.6, 114.6, 81.3, 79.3, 68.0, 56.6, 44.3, 40.5, 36.4, 36.2, 32.4, 29.7, 28.7, 28.2, 23.4.

*4-(4-{4-[2-tert-Butoxycarbonyl-2-(4-carboxy-benzenesulfonylamino)-ethyl]-phenoxy}-butyl)-piperidine-1-carboxylic acid tert-butyl ester (**23**).* To a solution of compound **22** (218 mg, 0.46 mmol) and K_2CO_3 (381 mg, 2.76 mmol) in 4 ml of CH_3CN and 2 ml of H_2O at 0°C was added 4-(chlorosulfonyl)benzoic acid (202 mg, 0.92 mmol) in portions during 25 min. The reaction mixture was stirred at 0°C for 1 h, keeping pH > 9. An additional of K_2CO_3 (127 mg, 0.92 mmol) was added, then 4-(chlorosulfonyl)benzoic acid (101 mg, 0.46 mmol) was added in portions during 15 min, keeping pH > 9. The mixture was stirred at 0°C for 1.5 h, then 4-(chlorosulfonyl)benzoic acid (101 mg, 0.46 mmol) was added in portions during 1 h. The reaction mixture was continued stirring at 10–20°C for 2 h. The reaction was acidified to pH 1 using 1 N HCl. The reaction mixture was extracted with ethyl acetate (2 × 60 ml). The combined organic fractions were dried over anhydrous $MgSO_4$, and concentrated under reduced pressure to give an oil. The residue was purified on a chromatatron (1 mm) with MeOH and CH_2Cl_2 containing 0.1% HOAc) to afford a white solid (167

mg, 55%). ^{1}H NMR ($CDCl_3$) δ 14.26 (brs, 1H), 8.12 (d, 2H, J = 8.3 Hz), 7.82 (d, 2H, J = 8.3 Hz), 7.01 (d, 2H, J = 8.4 Hz), 6.73 (d, 2H, J = 8.4 Hz), 5.73 (d, 1H, J = 9.2 Hz), 4.09 (dd, 2H, J = 5.9, 15.1 Hz), 3.89 (t, 2H, J = 6.2 Hz), 2.95 (m, 2H), 2.69 (brs, 2H, J = 12.2 Hz), 1.65–1.79 (m, 4H), 1.46 (s, 9H), 1.30–1.54 (m, 5H), 1.25 (s, 9H), 1.02–1.18 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 170.3, 169.5, 158.4, 155.3, 144.8, 133.3, 130.7, 127.3, 127.1, 114.6, 83.1, 79.8, 67.9, 57.6, 44.3, 38.7, 36.3, 36.0, 32.3, 29.5, 28.6, 27.9. MS/FAB m/z (MH^+) calcd for $C_{34}H_{48}N_2O_9S$ 661.3, found 661.3. Anal. calcd for $C_{34}H_{48}N_2O_9S$: C, 61.80; H, 7.32; N, 4.24. Found: C, 61.92; H, 7.31; H, 4.05.

*Protected RGD analog **24**.* To a solution of **23** (22 mg, 0.033 mmol) in 1 ml of dry CH_2Cl_2 at 0°C under argon atmosphere was added DCC (7.5 mg, 0.036 mmol). After stirring for 1–2 min, HOBt (5 mg, 0.036 mmol) was added. After 10 min, 3-(dimethylamino)propylamine (4 μl, 0.033 mmol) and DMAP (5 mg, 0.036 mmol) were added. The reaction was stirred at 0°C for 1.5 h and then RT for 3 h. The white precipitate was filtered out and the solvent was removed under reduced pressure to give an oil. The residue was redissolved in 80 ml of ethyl acetate, then was washed with water (2 × 15 ml), saturated $NaHCO_3$ (15 ml), and brine (15 ml), dried over anhydrous $MgSO_4$, and concentrated to give an oil. The oil was purified on a chromatatron (1 mm) with MeOH and CH_2Cl_2 (1/50 then 1/30 with 0.1% TEA) to afford an oil (18 mg, 72%). ^{1}H NMR ($CDCl_3$) δ 8.91 (brs, 1H), 7.87 (d, 2H, J = 8.3 Hz), 7.82 (d, 2H, J = 8.3 Hz), 7.02 (d, 2H, J = 8.4 Hz), 6.76 (d, 2H, J = 8.4 Hz), 4.06 (m, 2H), 3.91 (t, 2H, J = 6.3 Hz), 3.57 (m, 2H), 2.95–3.03 (m, 4H), 2.67 (m, 2H), 2.60 (t, 2H, J = 5.7 Hz), 2.36 (s, 6H), 1.64–1.84 (m, 6H), 1.45 (s, 9H), 1.39–1.51 (m, 2H), 1.25–1.34 (m, 3H), 1.22 (s, 9H), 1.04–1.11 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 169.9, 165.9, 158.4, 155.0, 142.5, 138.0, 130.8, 128.2, 127.4, 127.1, 114.6, 82.9, 79.3, 68.0, 57.3, 56.7, 46.0, 43.8, 38.7, 38.1, 36.4, 36.1, 32.3, 29.8, 29.6, 28.6, 27.9, 24.4. HRMS/FAB m/z (MH^+) calcd for $C_{39}H_{60}N_4O_8S$ 745.4210, found 745.4228.

*RGD analog (**3**).* Compound **24** (13 mg, 0.017 mmol) was treated with 40% TFA in dry CH_2Cl_2 (2 ml) at RT for 2.5 h. The solvents were evaporated *in vacuo* to afford an oil (12 mg, 86%). ^{1}H NMR ($CDCl_3$) δ 7.90 (d, 2H, J = 8.0 Hz), 7.80 (d, 2H, J = 8.0 Hz), 7.07 (d, 2H, J = 8.0 Hz), 6.75 (d, 2H, J = 8.0 Hz), 4.13 (m, 1H), 3.94 (t, 2H, J = 5.9 Hz), 3.50 (t, 2H, J = 6.5 Hz), 3.36 (m, 4H), 3.16 (t, 2H, J = 7.6 Hz), 3.04 (m, 1H), 2.91 (m, 1H), 2.87 (s, 6H), 2.07 (m, 2H), 1.94 (m, 2H), 1.73–1.79 (m, 3H), 1.51 (m, 2H), 1.38 (m, 2H), 0.88 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 174.2, 169.4, 159.6, 145.4, 138.7, 131.6, 129.8, 129.1, 128.3, 115.6, 68.9, 59.2, 56.9, 45.5, 43.7, 39.3, 37.9, 36.9, 35.0, 30.5, 30.2, 26.3, 24.1.

*Protected RGD analog (**25**).* To a solution of **23** (130 mg, 0.197 mmol) in 0.5 ml of dry CH_2Cl_2 at 0°C under argon atmosphere was added EDC (41 mg, 0.216 mmol). After stirring for 1–2 min, HOBt (40 mg, 0.294 mmol) was added. After 10 min, 1,4-bis(3-aminopropyl)piperazine (5 μl, 0.024 mmol) and DMAP (26 mg, 0.216 mmol) were added. After stirring for 10 min, 10 μl (0.050 mmol) of 1,4-bis(3-aminopropyl)piperazine was added. After 30 min, an additional of 1,4-bis(3-aminopropyl)piperazine (5 μl, 0.024 mmol) was added. The reaction was continued stirring for 1 h at 0°C and RT for 4 h. The solvent was removed under reduced pressure to give an oil, which was redissolved in 80 ml of ethyl acetate, washed with saturated $NaHCO_3$ (2 × 15 ml), dried over anhydrous $MgSO_4$, and concentrated to give an

oil. The oil was purified on a chromatatron (1 mm) with MeOH and CH_2Cl_2 (1/30 then 1/20) to afford a white solid (105 mg, 72%). ^{1}H NMR ($CDCl_3$) δ 8.23 (brs, 2H), 7.87 (d, 4H, J = 8.2 Hz), 7.81 (d, 4H, J = 8.2 Hz), 7.00 (d, 4H, J = 8.1 Hz), 6.74 (d, 4H, J = 8.1 Hz), 5.59 (brs, 2H), 5.30 (s, 2H), 4.07 (t, 4H, J = 5.4 Hz), 3.89 (t, 4H, J = 5.7 Hz), 3.53 (m, 4H), 2.93 (m, 4H), 2.50–2.70 (m, 12H), 1.64–1.76 (m, 12H), 1.37–1.53 (m, 6H), 1.45 (s, 18H), 1.24 (s, 18H), 1.22–1.32 (m, 6H), 1.01–1.14 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 170.0, 165.9, 158.4, 155.0, 142.5, 138.9, 130.7, 128.0, 127.3, 127.1, 114.6, 83.0, 80.0, 68.0, 58.0, 57.4, 52.9, 44.2, 40.6, 38.8, 36.4, 36.1, 32.3, 30.0, 28.6, 27.9, 24.3, 23.2. HRMS/FAB m/z (MH^+) calcd for $C_{78}H_{116}N_8O_{16}S_2$ 1,485.80, found 1,485.80.

*RGD analog (**4**).* Compound **25** (25 mg, 0.017 mmol) was treated with 40% TFA in CH_2Cl_2 (3 ml) at RT under argon for 4 h. The solvents were evaporated under reduced pressure to give a solid (27 mg, 98%). ^{1}H NMR ($CDCl_3$) δ 7.87 (d, 4H, J = 8.3 Hz), 7.75 (d, 4H, J = 8.3 Hz), 7.03 (d, 4H, J = 8.6 Hz), 6.72 (d, 4H, J = 8.6 Hz), 4.03 (dd, 2H, J = 5.4, 8.4 Hz), 3.93 (t, 4H, J = 6.2 Hz), 3.50 (t, 4H, J = 6.5 Hz), 3.31–3.40 (m, 12H), 3.12 (t, 4H, J = 7.2 Hz), 2.90–3.03 (m, 6H), 2.78 (dd, 2H, J = 8.6, 13.8 Hz), 1.93–2.05 (m, 8H), 1.76 (m, 4H), 1.48–1.63 (m, 6H), 1.29–1.42 (m, 8H). ^{13}C NMR ($CDCl_3$) δ 174.3, 169.2, 159.6, 145.3, 138.8, 131.6, 129.8, 129.0, 128.3, 115.6, 68.9, 59.3, 55.9, 51.4, 45.5, 39.2, 38.4, 36.9, 35.0, 30.5, 30.2, 26.1, 24.1. HRMS/FAB m/z (MH^+) calcd for $C_{60}H_{84}N_8O_{12}S_2$ 1173.5728, found 1173.5723.

*Protected RGD analog (**26**).* To a solution of **18** (42 mg, 0.058 mmol) in 2 ml of dry CH_2Cl_2 at 0°C under argon atmosphere was added EDC (12 mg, 0.0634 mmol). After stirring for 1–2 min, HOBt (12 mg, 0.0634 mmol) was added. After 30 min, poly(ethylene glycol (5 μl, 0.019 mmol) and DMAP (8 mg, 0.0634 mmol) were added. After the reaction was stirred at 0°C for 50 min, an additional of poly(ethylene glycol) (2.7 μl, 0.01 mmol) was added. The reaction was continued stirring at 0°C for 1.5 h then RT for 4 h. The solvent was removed under reduced pressure to give an oil. The oil was purified on a chromatatron (1 mm) with MeOH and CH_2Cl_2 (1/20) to afford an oil (41 mg, 82%). ^{1}H NMR ($CDCl_3$) δ 8.06 (d, 4H, J = 8.0 Hz), 7.89 (d, 4H, J = 8.0 Hz), 7.13–7.37 (m, 20H), 6.88 (d, 4H, J = 8.4 Hz), 6.69 (d, 4H, J = 8.4 Hz), 5.25 (m, 2H), 5.12 (s, 4H), 4.89 (s, 4H), 4.50 (brs, 4H), 4.23 (m, 6H), 3.88 (t, 4H, J = 6.3 Hz), 3.64–3.68 (m, 22H), 2.99 (d, 4H, J = 5.6 Hz), 2.76 (brt, 4H, J = 11.2 Hz), 1.10–1.77 (m, 22H).

*RGD analog (**5**).* A solution of **26** (40 mg, 0.023 mmol) in 2 ml of methanol in the presence of 10% Pd-C (20 mg) was hydrogenated at hydrogen atmosphere for 5.5 h. The catalyst was filtered out and washed with methanol (2 × 4 ml). The solvent was evaporated to give an oil (28 mg, 95%). ^{1}H NMR (CD_3OD) 8.01 (d, 4H, J = 8.0 Hz), 7.74 (4H, J = 8.0 Hz), 7.01 (d, 4H, J = 8.4 Hz), 6.68 (d, 4H, J = 8.4 Hz), 4.50 (brs, 4H), 4.20 (m, 6H), 3.86–3.96 (m, 10H), 3.64–3.70 (m, 16H), 2.65–3.18 (m, 8H), 1.10–1.92 (m, 22H). HRMS/FAB m/z (MH^+) calcd for $C_{30}H_{44}N_4O_6S$ 589.3060, found 589.3073.

*(2-Chlorosulfonyl-ethyl)-carbamic acid benzyl ester (**29**) (26–30).* Taurine (1.4600 g, 11.70 mmol) was dissolved in 1 N NaOH (11.7 ml, 11.70 mmol). Benzyl chloroformate (2.0 ml, 14 mmol) and 1 N NaOH (14.0 ml, 14 mmol) were added simultaneously at 0°C. The reaction was stirred at 0°C for 0.5 h and at room temperature for

2 h. There was some oil formed in the solution. The solution was washed with EtOAc three times. Then the aqueous layer was concentrated to give a white solid. The white solid was converted into tetrabutylammonium salt **28** using 40 W % tetrabutylammonium hydroxide.

To this salt **28** (0.50 mmol), 2 ml CH_2Cl_2, 40 μl of DMF and triphosgene (148.3 mg, 0.25 mmol) were added sequentially. The suspension was stirred at room temperature for 1 h and then concentrated. The residue was eluted through a small silica gel with EtOAc and the eluant was concentrated to give a yellow oil. This crude product was purified on chromatotron using hexanes/EtOAc (1:1) to give compound **29** (102.5 mg, 74% for 2 steps): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.34 (bs, 5H), 5.42 (bs, 1H), 5.12 (bs, 2H), 3.81–3.87 (m, 4H).

*Potassium; 4-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-butane-1-sulfonate (**32**) (31).* Phthalimide potassium derivative (1.6300 g, 8.81 mmol) and 1,4-butane sultone (0.75 ml, 7.34 mmol) were mixed in DMF (20 ml) and stirred at 80°C for 2 h. DMF was removed and the white residue was dissolved in water. The aqueous solution was washed with EtOAc twice. The aqueous layer was concentrated to give a white solid **32**. This white solid was used to next step directly without further purification: ^{1}H-NMR (300 MHz, D_2O) δ 7.82–7.87 (m, 4H), 3.73 (t, 2H, J = 5.7 Hz), 2.98 (t, 2H, J = 6.8 Hz), 1.80–1.82 (m, 4H).

*(4-Chlorosulfonyl-butyl)-carbamic acid benzyl ester (**34**) (32,33).* Compound **32** (459.2 mmol, 1.43 mmol) was dissolved in concentrated HCl (4.5 ml) and the solution was stirred at 107°C for 19 h. The gray precipitate that formed was filtered off and the filtrate was washed with EtOAc three times. The aqueous layer was concentrated to give a yellow solid. This yellow solid was dissolved in 1 N NaOH (3.0 ml). Benzyl chloroformate (410 μl, 2.86 mmol) and 1 N NaOH (3.0 ml) were added simultaneously at 0°C. The reaction was stirred at 0°C for 0.5 h and at room temperature for 2 h. The aqueous solution was washed with EtOAc three times and then concentrated to give a white solid **33**: ^{1}H-NMR (300 MHz, D_2O) δ 7.47 (s, 5H), 5.16 (s, 2H), 3.20 (t, 2H, J = 6.2 Hz), 2.95 (t, 2H, J = 7.4 Hz), 1.77–1.82 (m, 2H), 1.63–1.68 (m, 2H).

To compound **33** (4.67 mmol), CH_2Cl_2 (27.0 ml), DMF (460 μl) and thionyl chloride (681 μl, 9.33 mmol) were added sequentially. The suspension was stirred at room temperature for 3 h and then concentrated. The residue was eluted through a small silica gel plug using EtOAc. The eluant was concentrated and the residue was purified on chromatotron using hexanes/EtOAc (1:1) to give compound **34** (924.2 mg, 68% for 3 steps): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.35 (bs, 5H), 5.10 (s, 2H), 4.83 (bs, 1H), 3.71 (t, 2H, 7.5 Hz), 3.23–3.29 (m, 2H), 2.02–2.12 (m, 2H), 1.62–1.76 (m, 2H).

*2-(6-Bromo-hexyl)-isoindole-1, 3-dione (**35**) (33).* Phthalimide potassium derivative (11.9473 g, 64.50 mmol) was mixed with 1,6-dibromohexane (31.4734 g, 129.00 mmol) in DMF (150 ml). The reaction was stirred at room temperature for 18 h. Acetone (150 ml) was added to precipitate to KBr. The white precipitate was filtered off and the filtrate was concentrated. The residue was purified on a silica gel column using a gradient solvent system of hexanes/EtOAc (50:1 to 10:1 and finally to 5:1) to give a white solid **35** (14.0899 g, 70%): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.83–7.86 (m, 2H), 7.70–7.72 (m, 2H), 3.69 (t, 2H, J = 7.1 Hz), 3.42 (t, 2H, J = 6.7 Hz), 1.83–1.88 (m, 2H), 1.70–1.72 (m, 2H), 1.37–1.52 (m, 4H).

*(6-Chlorosulfonyl-hexyl)-carbamic acid benzyl ester (**37**) (34,35).* Compound **35** (2.5000 g, 8.06 mmol) and Na_2SO_3 (2.0329 g, 16.13 mmol) were dissolved in H_2O (48.0 ml) and 95% EtOH (30.0 ml). The reaction was stirred at 95°C for 20 h and then concentrated. The residue was dissolved in H_2O and the resulted aqueous solution was washed with EtOAc three times. The aqueous layer was then concentrated to give a solid. Concentrated HCl (25.4 ml, 306.28 mmol) was added and the reaction was stirred at 110°C for 18 h. The resulted precipitates were filtered off and the filtrate was washed with EtOAc three times. The aqueous layer was concentrated to give a yellow solid. This yellow solid was dissolved in 1 N NaOH (16.1 ml, 16.12 mmol). Benzyl chloroformate (2.9 ml, 20.15 mmol) and 1 N NaOH (20.2 ml, 20.15 mmol) were added simultaneously at 0°C. The reaction was stirred at 0°C for 0.5 h and at room temperature for 2 h. The aqueous solution was washed with EtOAc three times and the aqueous layer was concentrated to give compound **36**: ^{1}H-NMR (300 MHz, D_2O) δ 7.47 (s, 5H), 5.15 (s, 2H), 3.14–3.16 (m, 2H), 2.90–2.95 (m, 2H), 1.55–1.75 (m, 2H), 1.35–1.55 (m, 6H).

To compound **36** (4.03 mmol), CH_2Cl_2 (40.0 ml), DMF (400 μl), and thionyl chloride (588 μl, 8.06 mmol) were added sequentially. The suspension was stirred at room temperature for 5 h and then concentrated. The residue was eluted through a small silica gel plug using EtOAc. The eluant was concentrated and the residue was purified on chromatotron using hexanes/EtOAc (1:1) to give compound **37** (491.3 mg, 37% for 4 steps): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.36 (bs, 5H), 5.10 (s, 2H), 4.70–4.73 (m, 1H), 3.65 (t, 2H, 7.7 Hz), 3.16–3.24 (m, 2H), 1.99–2.04 (m, 2H), 1.39–1.59 (m, 6H).

*4-{4-[4-(2-Benzyloxycarbonylamino-2-carboxy-ethyl)-phenoxy]-butyl}-piperidine-1-carboxylic acid tert-butyl ester (**39**) (22).* NaH (3.6000 g, 60% disperse) was washed with hexanes four times to give a gray powder NaH (2.5320g, 105.50 mmol). Anhydrous DMF (10 ml) was added. The solution of Z-Tyr-OH (8.8307 g, 28.00 mmol) in DMF (38.0 ml) was added to the suspension of NaH at 0°C over 0.5 h. The mixture was stirred at 0°C for 1 h to give a yellow green solution with sticky residue in it. The solution of **20** (7.7242 g, 28.0 mmol) in DMF (38 ml) was added into the reaction at 0°C over 0.5 h, followed by the addition of KI (0.9290 g, 5.6 mmol). The reaction mixture was stirred at 0°C for 1 h and at 40–45°C for 72 h. DMF was removed and the residue was dissolved in EtOAc. The organic solution was acidified to pH 1 with 1 N HCl. The aqueous layer was extracted with EtOAc twice. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated to give an oil. The crude product was purified on a silica gel column with a gradient solvent system of hexanes/EtOAc/HOAc (4:1:0.1 to 2:1:0.1 and finally to 1:1:0.1) to give compound **39** (6.2252 g, 53%): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.34 (bs, 5H), 7.05 (d, 2H, J = 8.4 Hz), 6.79 (d, 2H, J = 8.5 Hz), 5.19 (bd, 1H), 5.11 (s, 2H), 4.63–4.66 (bd, 1H), 4.04 (bd, 1H), 3.93 (t, 2H, J = 6.2 Hz), 3.08–3.12 (m, 2H), 2.60–2.70 (m, 2H), 1.62–1.77 (m, 4H), 1.45 (s, 9H), 1.04–1.50 (m, 7H).

*4-{4-[4-(2-Benzyloxycarbonylamino-2-methoxycarbonyl-ethyl)-phenoxy]-butyl}-piperidine-1-carboxylic acid tert-butyl ester (**40**) (22).* Compound **39** (8.1610 g, 14.7 mmol) was dissolved in DMF (57 ml). Cs_2CO_3 (2.3985 g, 7.4 mmol) was added. The reaction was stirred at room temperature for 1.5 h, followed by the addition of MeI (2.0898 g, 14.7 mmol). Then the reaction was stirred at room temperature for 18 h.

The solution was concentrated and the residue was dissolved in EtOAc. The organic solution was washed with saturated $NaHCO_3$ twice and brine once, dried over $MgSO_4$, and concentrated to give a clear oil. The crude product was eluted through a silica gel plug with EtOAc. Then the eluant was concentrated to give product **40** (8.3379 g, 100%): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.34 (bs, 5H), 6.99 (d, 2H, J = 8.2 Hz), 6.78 (d, 2H, J = 8.2 Hz), 5.14–5.19 (m, 1H), 5.10 (s, 2H), 4.60–4.63 (m, 1H), 4.05–4.07 (m, 2H), 3.91 (t, 2H, J = 6.2 Hz), 3.71 (s, 3H), 3.04 (bs, 2H), 2.66 (bt, 2H, J = 12.7 Hz), 1.63–1.77 (m, 4H), 1.45 (s, 9H), 1.10–1.54 (m, 7H).

*4-{4-[4-(2-Amino-2-methoxycarbonyl-ethyl)-phenoxy]-butyl}-piperidine-1-carboxylic acid tert-butyl ester (**41**).* Compound **40** (786.5 mg, 1.38 mmol) was dissolved in CH_3OH (16 ml). To this solution 10% Pd/C (157.3 mg) was added. The suspension was flushed with Ar for 10 min and was subjected to hydrogen balloon for 1 h. The catalyst was filtered off and the filtrate was concentrated to give a clear oil **41** (552.7 mg, 92%): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.09 (d, 2H, J = 8.3 Hz), 6.83 (d, 2H, J = 8.3 Hz), 4.05–4.09 (m, 2H), 3.93 (t, 2H, J = 6.3 Hz), 3.72 (s, 3H), 3.03 (dd, 1H, J_1 = 13.6 Hz, J_2 = 5.0 Hz), 2.82 (dd, 1H, J_1 = 13.6 Hz, J_2 = 7.7 Hz), 2.66 (bt, 2H, J = 12.3 Hz), 1.64–1.78 (m, 4H), 1.45 (s, 9H), 1.06–1.54 (m, 7H).

*4-(4-{4-[2-(2-Benzyloxycarbonylamino-ethanesulfonylamino)-2-methoxycarbonyl-ethyl]-phenoxy}-butyl)-piperidine-1-carboxylic acid tert-butyl ester (**42**) (22).* Compound **41** (791.9 mg, 1.82 mmol) was dissolved in EtOAc (20 ml), followed by the addition of $NaHCO_3$ (612.6 mg, 7.29 mmol). The solution of **29** (757.7 mg, 2.74 mmol) in EtOAc (25 ml) was added and the suspension was stirred at 40–45°C for 43 h. The resulted precipitates were filtered off. The filtrate was washed with 1 N HCl twice and brine once, dried over $MgSO_4$, and concentrated. The crude product was purified on a silica gel column using a gradient solvent system of hexanes/EtOAc (3:1 to 1:1) to give compound **42** (1.0559 g, 86%): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.34 (bs, 5H), 7.06 (d, 2H, J = 8.4 Hz), 6.82 (d, 2H, J = 8.4 Hz), 5.36–5.38 (m, 1H), 5.08 (s, 2H), 4.92–4.95 (m, 1H), 4.31–4.36 (m, 1H), 4.05–4.08 (m, 2H), 3.91 (t, 2H, J = 6.3 Hz), 3.77 (s, 3H), 3.52 (t, 2H, J = 5.5 Hz), 3.09 (dd, 1H, J_1 = 14.0 Hz, J_2 = 5.2 Hz), 2.87–3.03 (m, 3H), 2.66 (t, 2H, J = 12.0 Hz), 1.05–1.77 (m, 11H), 1.45 (s, 9H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 172.1, 158.6, 155.0, 136.5, 130.6, 128.6, 128.3, 128.2, 127.2, 114.9, 79.3, 68.0, 67.0, 57.4, 53.5, 52.9, 44.2, 38.6, 36.4, 36.1, 35.9, 32.3, 29.6, 28.6, 23.3; HRMS/FAB m/z (MH^+) calcd for $C_{34}H_{49}N_3O_9S$ 676.3268, found 676.3229. Anal. calcd for $C_{34}H_{49}N_3O_9S$: C, 60.42; H, 7.31; N, 6.22. Found: C, 60.51; H, 7.18; N, 6.13.

*4-(4-{4-[2-(4-Benzyloxycarbonylamino-butane-1-sulfonylamino)-2-methoxycarbonylethyl]-phenoxy}-butyl)-piperidine-1-carboxylic acid tert-butyl ester (**43**) (22).* Compound **41** (811.1 mg, 1.87 mmol) was dissolved in EtOAc (23 ml), followed by the addition of $NaHCO_3$ (627.7 mg, 7.47 mmol). The solution of **34** (924.2 mg, 3.02 mmol) in EtOAc (23 ml) was added and the suspension was stirred at 40–45°C for 65 h. The resulted precipitates were filtered off. The filtrate was washed with 1 N HCl twice and brine once, dried over $MgSO_4$, and concentrated. The crude product was purified on a silica gel column using a gradient solvent system of hexanes/EtOAc (3:1 to 1:1) to give compound **43** (766.4 mg, 58%): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.34 (bs, 5H), 7.07 (d, 2H, J = 8.2 Hz), 6.82 (d, 2H, J = 8.3 Hz), 5.08 (s, 2H), 4.97 (d, 2H, J = 9.2 Hz), 4.29–4.31 (m, 1H), 4.06–4.08 (m, 2H), 3.91 (t, 2H, J = 6.2

Hz), 3.75 (s 3H), 3.04–3.14 (m, 3H), 2.93 (dd, 1H, J_1 = 14.0 Hz, J_2 = 7.4 Hz), 2.62–2.77 (m, 5H), 1.59–1.77 (m, 8H), 1.45 (s, 9H), 1.06–1.59 (m, 7H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 172.4, 158.6, 156.6, 155.1, 136.7, 130.7, 128.7, 128.3, 127.4, 114.9, 79.4, 68.1, 66.8, 57.5, 53.3, 52.9, 44.5, 40.4, 38.8, 36.4, 36.1, 32.3, 29.6, 28.7, 23.3, 20.9; HRMS/FAB *m/z* (MH^+) calcd for $C_{36}H_{53}N_3O_9S$ 704.3581, found 704.3610. Anal. calcd for $C_{36}H_{53}N_3O_9S$: C, 61.43; H, 7.59; N, 5.97. Found: C, 61.00; H, 7.37; N, 5.84.

*4-(4-{4-[2-(6-Benzyloxycarbonylamino-hexane-1-sulfonylamino)-2-carboxy-ethyl]-phenoxy}-butyl)-piperidine-1-carboxylic acid tert-butyl ester (**44**).* Compound **41** (524.1 mg, 1.21 mmol) was dissolved in EtOAc (10 ml), followed by the addition of Na_2CO_3 (511.8 mg, 4.83 mmol). The solution of **37** (491.3 mg, 1.47 mmol) in EtOAc (20 ml) was added and the suspension was stirred at 40–45 °C for 72 h. The resulted precipitates were filtered off. The filtrate was washed with 1 N HCl twice and brine once, dried over $MgSO_4$, and concentrated. The crude product was purified on a silica gel column using a gradient solvent system of hexanes/EtOAc (3:1 to 1:1) to give compound **44** (528.6 mg, 60%): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.35 (brs, 5H), 7.06 (d, 2H, J = 8.4 Hz), 6.82 (d, 2H, J = 8.4 Hz), 5.09 (s, 2H), 4.77–4.79 (m, 1H), 4.73 (d, 1H, J = 9.1 Hz), 4.31–4.35 (m, 1H), 3.92 (t, 2H, J = 6.3 Hz), 3.76 (s, 3H), 3.14–3.20 (m, 2H), 3.08 (dd, 1H, J_1 = 13.9 Hz, J_2 = 5.1 Hz), 2.95 (dd, 1H, J_1 = 14.0 Hz, J_2 = 7.1 Hz), 2.62–2.78 (m, 4H), 1.06–1.77 (m, 19H), 1.45 (s, 9H), ^{13}C-NMR (300 MHz, $CDCl_3$) δ 172.3, 158.7, 156.6, 155.1, 136.8, 130.7, 128.7, 128.3, 127.3, 115.0, 79.4, 68.1, 66.8, 57.4, 53.9, 52.9, 44.3, 41.0, 39.0, 36.5, 36.2, 32.4, 29.8, 29.7, 28.7, 28.0, 26.3, 23.5, 23.4; HRMS/FAB *m/z* (MH^+) calcd for $C_{38}H_{57}N_3O_9S$ 732.3894, found 732.3888. Anal. calcd for $C_{38}H_{57}N_3O_9S$: C, 62.36; H, 7.85; N, 5.74. Found: C, 62.34; H, 7.78; N, 5.57.

*4-(4-{4-[2-(2-Benzyloxycarbonylamino-ethanesulfonylamino)-2-carboxy-ethyl]-phenoxy}-butyl)-piperidine-1-carboxylic acid tert-butyl ester (**45**).* Compound **42** (77.4 mg, 0.12 mmol) and LiOH (13.7 mg, 0.57 mmol) were dissolved in 3 ml of THF/CH_3OH/H_2O (1:1:1). The reaction was stirred at room temperature for 18 h and then concentrated. The residue was acidified with 1 N HCl and the aqueous solution was extracted with EtOAc three times. The combined organic layers were washed with brine once, dried over $MgSO_4$, and concentrated. The crude product was purified on a silica gel column with a gradient solvent system of hexanes/EtOAc/HOAc (2:1:0.1 to 1:1:0.1) to give compound **45** (65.7 mg, 86%): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.34 (brs, 5H), 7.12 (d, 2H, J = 8.1 Hz), 6.81 (d, 2H, J = 8.1 Hz), 5.43–5.47 (m, 1H), 5.13–5.15 (m, 1H), 5.08 (s, 2H), 4.34–4.41 (m, 1H), 3.99–4.06 (m, 2H), 3.92 (t, 2H, J = 6.2 Hz), 3.45–3.56 (m, 2H), 3.13 (dd, 1H, J_1 = 14.1 Hz, J_2 = 5.1 Hz), 2.93–3.08 (m, 3H), 2.60–2.70 (m, 2H), 1.61–1.78 (m, 4H), 1.45 (s, 9H), 0.98–1.51 (m, 7H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 174.4, 158.6, 156.8, 155.3, 148.0, 138.6, 136.4, 130.8, 128.7, 128.4. 128.3, 127.5, 114.9, 79.8, 68.0, 67.3, 57.4, 53.3, 44.3, 38.4, 36.3, 36.1, 32.3, 29.6, 28.7, 23.3; HRMS/FAB *m/z* (MH^+) calcd for $C_{33}H_{47}N_3O_9S$ 662.3111, found 662.3135.

*4-(4-{4-[2-(4-Benzyloxycarbonylamino-butane-1-sulfonylamino)-2-carboxy-ethyl]-phenoxy}-butyl)-piperidine-1-carboxylic acid tert-butyl ester (**46**).* Compound **43** (684.4 mg, 0.97 mmol) and LiOH (116.53 mg, 4.87 mmol) were dissolved in 24 ml of THF/CH_3OH/H_2O (1:1:1). The reaction was stirred at room temperature for 18

h and then concentrated. The residue was acidified with 1 N HCl and the aqueous solution was extracted with EtOAc three times. The combined organic layers were washed with brine once, dried over $MgSO_4$, and concentrated. The crude product was purified on a silica gel column with a gradient solvent system of hexanes/EtOAc/HOAc (2:1:0.1 to 1:1:0.1) to give compound **46** (626.7 mg, 93%): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.33 (brs, 5H), 7.13 (d, 2H, J = 7.8 Hz), 6.79 (d, 2H, J = 7.5 Hz), 5.15–5.30 (m, 2H), 5.08 (s, 2H), 4.31–4.32 (m, 1H), 4.04–4.07(m, 2H), 3.89 (bs, 2H), 3.09–3.10 (m, 3H), 2.93–2.96 (m, 1H), 2.62–2.75 (m, 4H), 1.04–1.73 (m, 15H), 1.45 (s, 9H); ^{13}C-NMR (300 MHz, $CDCl_3$) δ 174.5, 158.5, 156.9, 155.3, 136.6, 130.9, 128.7, 128.3, 127.8, 114.9, 79.7, 68.0, 67.1, 57.4, 53.2, 44.3, 40.5, 38.5, 36.3, 36.1, 32.3, 29.6, 28.7, 23.3, 20.8; HRMS/FAB *m/z* (MH^+) calcd for $C_{35}H_{51}N_3O_9S$ 690.3424, found 690.3447. Anal. calcd for $C_{35}H_{51}N_3O_9S$: C, 60.94; H, 7.45; N, 6.09. Found: C, 59.87; H, 7.55; N, 5.66.

*4-(4-{4-[2-(6-Benzyloxycarbonylamino-hexane-1-sulfonylamino)-2-carboxy-ethyl]-phenoxy}-butyl)-piperidine-1-carboxylic acid tert-butyl ester (**47**).* Compound **44** (420.8 mg, 0.57 mmol) and LiOH (68.9 mg, 2.88 mmol) were dissolved in 14.1 ml of THF/CH_3OH/H_2O (1:1:1). The reaction was stirred at room temperature for 18 h and then concentrated. The residue was acidified with 1 N HCl and the aqueous solution was extracted with EtOAc three times. The combined organic solutions were washed with brine once, dried over $MgSO_4$, and concentrated. The crude product was purified on a silica gel column with a gradient solvent system of hexanes/EtOAc/HOAc (2:1:0.1 to 1:1:0.1) to give compound **47** (358.0 mg, 87%): ^{1}H-NMR (300 Mhz, $CDCl_3$) δ 7.33 (bs, 5H), 7.13 (d, 2H, J = 6.9 Hz), 6.79 (d, 2H, J = 7.5 Hz), 5.14–5.43 (m, 2H), 5.08 (s, 2H), 4.26–4.27 (m, 1H), 4.04–4.07 (m, 2H), 3.89 (bs, 2H), 3.12 (bs, 3H), 2.89–2.90 (m, 1H), 2.62–2.70 (m, 4H), 1.04–1.72 (m, 19H), 1.45 (s, 9H); ^{13}C-NMR (330 MHz, $CDCl_3$) δ 176.3, 158.3, 156.9, 155.2, 136.6, 130.7 128.6, 128.1, 114.7, 79.7, 67.9, 66.8, 57.6, 53.6, 44.2, 40.9, 38.4, 36.2, 35.9, 32.2, 29.5, 28.5, 27.6, 26.0, 23.2, 23.0, HRMS/FAB *m/z* (MH^+) calcd for $C_{37}H_{55}N_3O_9S$ 718.3737, found 718.3726.

*2-(2-Benzyloxycarbonylamino-ethanesulfonylamino)-3-[4-(4-piperidin-4-yl-butoxy)-phenyl]-propionic acid (**6**).* Compound **45** (300.0 mg, 0.45 mmol) was dissolved in CH_2Cl_2 (6.7 ml). TFA (2.2 ml, 29.03 mmol) in CH_2Cl_2 (6.7 mL) was added at 0°C. The reaction was stirred at 0 °C for 10 min and at room temperature for 1 h. The solution was concentrated and dried on oil pump to give compound **6** as a TFA salt (357.2 mg, 140%): ^{1}H-NMR (300 MHz, CD_3OD) δ 7.32 (brs, 5H), 7.17 (d, 2H, J = 8.1 Hz), 6.83 (d, 2H, J = 8.1 Hz), 5.09 (s, 2H), 4.17–4.21 (m, 1H), 3.91 (t, 2H, J = 5.8 Hz), 3.32 (bd, 4H), 3.11 (dd, 1H, J_1 = 13.2 Hz, J_2 = 3.9 Hz), 2.79–2.93 (m, 5H), 1.85–1.90 (m, 2H), 1.69–1.73 (m, 2H), 1.42–1.51 (m, 3H), 1.30–1.39 (m, 4H); ^{13}C-NMR (300 MHz, CD_3OD) δ 175.0, 159.7, 158.6, 138.3, 131.8, 130.2, 129.6, 129.2, 128.9, 115.7, 68.9, 67.7, 59.3, 53.7, 45.4, 39.2, 36.8, 34.8, 30.4, 30.1, 24.0; HRMS/FAB *m/z* (MH^+) calcd for $C_{28}H_{39}N_3O_7S$ 562.2584, found 562.2612.

*2-(4-Benzyloxycarbonylamino-butane-1-sulfonylamino)-3-[4-(4-piperidin-4-yl-butoxy)-phenyl]-propionic acid (**7**).* Compound **46** (100.0 mg, 0.15 mmol) was dissolved in CH_2Cl_2 (2.1 ml). TFA (715 μl, 9.28 mmol) in CH_2Cl_2 (2.2 ml) was added at 0°C. The reaction was stirred at 0°C for 10 min and at room temperature for 1 h. The solution was concentrated and dried on oil pump to give compound **7** as a TFA

salt: ^{1}H-NMR (300 MHz, CD_3OD) δ 7.28–7.34 (m, 5H), 7.19 (d, 2H, J = 9.0 Hz), 6.85 (d, 2H, J = 8.1 Hz), 5.06 (s, 2H), 4.13 (dd, 1H, J_1 = 9.6 Hz, J_2 = 4.5 Hz), 3.94 (t, 2H, J = 5.9 Hz), 3.31–3.36 (m, 2H), 3.13 (dd, 1H, J_1 = 13.8 Hz, J_2 = 5.1 Hz), 3.03 (t, 2H, J = 6.2 Hz), 2.87–2.96 (m, 2H), 2.78–2.82 (m, 1H), 2.64–2.67 (m, 2H), 1.88–1.93 (m, 2H), 1.72–1.76 (m, 2H), 1.47–1.57 (m, 5H), 1.32–1.45 (m, 6H); ^{13}C-NMR (300 MHz, CD_3OD) δ 175.2, 159.7, 159.0, 139.0, 131.8, 130.5, 129.6, 129.1, 128.8, 115.7, 68.9, 67.5, 59.5, 54.1, 45.5, 41.3, 39.2, 36.9, 34.9, 30.5, 30.1, 29.6, 24.1, 22.0; HRMS/FAB m/z (MH^+) calcd for $C_{30}H_{43}N_3O_7S$ 590.2900, found 590.2914.

*2-(6-Benzyloxycarbonylamino-hexane-1-sulfonylamino)-3-[4-(4-piperidin-4-yl-butoxy)-phenyl]-propionic acid (**8**).* Compound **47** (78.0 mg, 0.11 mmol) was dissolved in CH_2Cl_2 (1.6 ml). TFA (536 μl, 6.96 mmol) in CH_2Cl_2 (1.6 ml) was added at 0°C. The reaction was stirred at 0°C for 10 min and at room temperature for 1 h. The solution was concentrated and dried on oil pump to give compound **8** as a TFA salt: ^{1}H-NMR (300 MHz, CD_3OD) δ 7.28–7.35 (m, 5H), 7.18 (d, 2H, J = 8.7 Hz), 6.85 (d, 2H, J = 8.7 Hz), 5.07 (s, 2H), 4.11 (dd, 1H, J_1 = 9.6 Hz, J_2 = 4.5 Hz), 3.94 (t, 2H, J = 6.2 Hz), 3.32–3.37 (bd, 2H), 3.07–3.15 (m, 3H), 2.89–2.97 (m, 3H), 2.89–2.97 (m, 2H), 2.77 (dd, 1H, J_1 = 13.8 Hz, J_2 = 10.2 Hz), 2.62 (t, 2H, J = 7.4 Hz), 1.89–1.99 (m, 2H), 1.71–1.80 (m, 2H), 1.33–1.59 (m, 11H), 1.20–1.30 (m, 4H); ^{13}C-NMR (300 MHz, CD_3OD) δ 159.7, 159.0, 138.6, 131.8, 130.6, 129.6, 129.1, 128.9, 115.7, 68.9, 67.4, 59.8, 54.4, 45.4, 41.8, 39.1, 36.9, 34.9, 30.5, 30.2, 29.0, 27.4, 24.5, 24.1; HRMS/FAB m/z (MH^+) calcd for $C_{32}H_{47}N_3O_7S$ 618.3213, found 618.3248.

*2-(2-Amino-esthanesulfonylamino)-3-[4-(4-piperidin-4-yl-butoxy)-phenyl]-propionic acid (**9**).* Compound **6** (127.3 mg, 0.23 mmol) was dissolved in CH_3OH (2.5 ml) and 3 drops of HOAc were added. Under Ar 10% Pd/C (25.5 mg) was added. The black suspension was subjected to H_2 balloon at room temperature for 1 h. The catalyst was filtered off and the filtrate was concentrated to give compound **9** as an acetic acid salt (111.3 mg, 115%): ^{1}H-NMR (300 MHz, CD_3OD) δ 7.19 (d, 2H, J = 8.1 Hz), 6.86 (d, 2H, J = 8.7 Hz), 4.23–4.26 (m, 1H), 3.96 (t, 2H, J = 6.3 Hz), 3.31–3.38 (m, 2H), 3.12–3.22 (m, 5H), 2.84–2.98 (m, 3H), 2.02 (s, HOAc), 1.91–2.00 (m, 2H), 1.72–1.81 (m, 2H), 1.47–1.60 (m, 3H), 1.28–1.44 (m, 4H); ^{13}C-NMR (300 MHz, CD_3OD) δ 175.4, 159.7, 131.7, 130.0, 115.7, 68.9, 50.9, 45.4, 39.0, 36.8, 35.6, 34.9, 30.5, 30.1, 24.0; HRMS/FAB m/z (MH^+) calcd for $C_{20}H_{33}N_3O_5S$ 428.2219, found 428.2234.

*2-(4-Amino-butane-1-sulfonylamino)-3-[4-(4-piperidin-4-yl-butoxy)-phenyl]-propionic acid (**10**).* Compound **7** (85.5 mg, 0.15 mmol) was dissolved in CH_3OH (1.6 ml). Under Ar 10% Pd/C (17.1 mg) was added and the black suspension was flushed with Ar for 10 min. Then the reaction was subjected to H_2 balloon at room temperature for 1 h. The catalyst was filtered off and the filtrate was concentrated to give compound **10** as a TFA salt (63.8 mg, 97% for two steps): ^{1}H-NMR (300 MHz, CD_3OD) δ 7.19 (d, 2H, J = 7.5 Hz), 6.82 (d, 2H, J = 7.2 Hz), 3.93–3.97 (m, 3H), 3.30–3.35 (m, 2H), 3.03–3.07 (m, 1H), 2.67–2.95 (m, 7H), 1.89–1.94 (m, 2H), 1.67–1.77 (m, 6H), 1.49–1.60 (m, 3H), 1.28–1.44 (m, 4H); ^{13}C-NMR (300 MHz, CD_3OD) δ 178.0, 159.4, 131.9, 131.3, 115.5, 68.9, 61.3, 52.7, 45.4, 40.0, 39.9, 36.9, 34.9, 30.5, 30.2, 27.1, 24.0, 21.7; HRMS/FAB m/z (MH^+) calcd for $C_{22}H_{37}N_3O_5S$ 456.2532, found 456.2545.

*2-(6-Amino-hexane-1-sulfonylamino)-3-[4-(4-piperidin-4-yl-butoxy)-phenyl]-propionic acid (**11**).* Compound **8** (85.8 mg, 0.14 mmol) was dissolved in CH_3OH (1.6 ml). Under Ar 10% Pd/C (34.2 mg) was added, followed by the addition of two drops of HOAc. The black suspension was flushed with Ar for 10 min and then subjected to H_2 balloon at room temperature for 1.5 h. The catalyst was filtered off and the filtrate was concentrated to give compound **11** as an acetic acid salt (68.7 mg, 97% for two steps): ^{1}H-NMR (300 MHz, CD_3OD) δ 7.19 (d, 2H, J = 8.1 Hz), 6.83 (d, 2H, J = 8.1 Hz), 3.93–4.01 (m, 3H), 3.30–3.37 (m, 2H), 3.05–3.08 (m, 1H), 2.67–2.97 (m, 7H), 1.99 (s, HOAc), 1.90–1.94 (m, 2H), 1.71–1.80 (m, 2H), 1.44–1.62 (m, 6H), 1.28–1.42 (m, 9H); ^{13}C-NMR (300 MHz, CD_3OD) δ 159.5, 131.9, 131.1, 115.6, 68.9, 53.7, 45.4, 40.7, 40.0, 36.9, 34.9, 30.5, 30.1, 28.6, 28.2, 26.8, 24.2, 24.0; HRMS/FAB m/z (MH^+) calcd for $C_{24}H_{41}N_3O_5S$ 484.2845, found 484.2851.

RESULTS AND DISCUSSION

The design. As stated earlier, there have been many literature reports of the development of RGD analogs as potential GP IIb/IIIa antagonists. One prominent example is MK-383 (the active ingredient of Tirofiban, Fig. 1), which has been approved by the U.S. Food and Drug Administration for the treatment of myocardial infarction and unstable angina pectoris. This RGD analog offers a good lead for the development of drug delivery vectors to target GP IIb/IIIa. The modification of MK-383 to suit the need of targeted drug delivery requires the incorporation of a functional group which (1) can allow for the tethering of a pharmaceutical reagent and (2) would not affect the binding affinity to the GP IIb/IIIa receptor. For the tethering of a pharmaceutical reagent to an RGD analog, it is desirable to have a functional group that can be easily conjugated to a handle on the pharmaceutical agent to be delivered. A carboxyl group should allow for the tethering of compounds with an amino or hydroxyl group through amide or ester formation, respectively. On the other hand, an amino group should allow for the tethering of a compound with a carboxyl group through amide bond formation. It is known that the terminal carboxyl and amino groups of an RGD analog are essential for their affinity (*22*). Modification of these two ionizable function groups would most likely lead to the abolishment of the biological activity. Therefore, an additional carboxyl or amino group needs to be introduced for the tethering of the pharmaceutical agents to be delivered. By examining the structure of MK-383, the side chain sulfonamide moiety appears to be the only site that could be modified without reducing its affinity for GP IIb/IIIa. Based on this consideration, we have designed a series of 10 modified MK-383 analogs (Fig. 2) that have side chains containing ester, amide, amine, protected amine, and poly(ethylene glycol). These analogs will help us to examine the effect of side-chain subunits with different

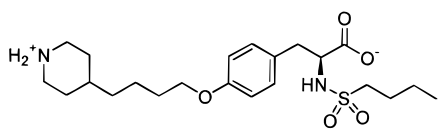


FIG. 1. MK-383 (Tirofiban), 1, IC_{50} = 11 nM.

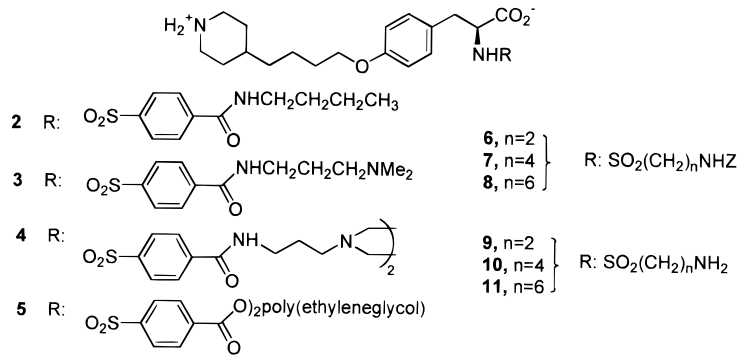
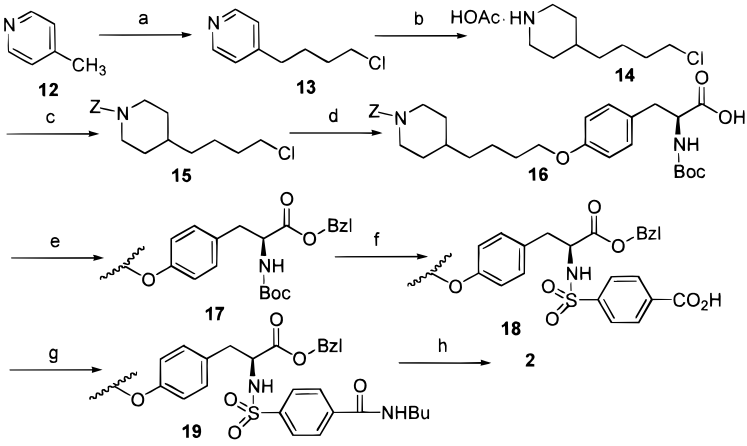


FIG. 2. RGD analogs designed.

structural features on their affinity for GP IIb/IIIa. The comparison of compounds **2** and **3** will allow us to examine the effect of an additional positive charge (a protonated tertiary amine). The comparison of **3** and **4** will allow us to examine the effect of increased molecular weight since **4** is the dimer of **3**. Compound **5** has a poly(ethylene glycol) unit bridging two RGD analog molecules. The synthesis of this molecule will allow us to examine the effect of tethering a macromolecule to such a RGD analog on its affinity to GP IIb/IIIa. The series of compounds **6**, **7**, and **8** allow for the examination of the effect of side chain length on their binding affinities. The series of **9**, **10**, and **11** serves the same purpose. By comparing the series containing **6**, **7**, and **8** and the series containing **9**, **10**, and **11**, we would be able to examine the effect of masking the positive charge of the protonated amine on the side chain.

Synthesis. The synthesis of the RGD analog backbone started with the alkylation of 4-methylpyridine (**12**) followed by hydrogenation to give **13** using modified literature procedures (Scheme 1) (*23*,*24*). Different catalysts were examined in order to reduce the pyridine ring of **13** to a piperidine ring (**14**). Among them (Pd/C, Pt/C, and PtO_2) only PtO_2 worked very well giving 100% yield. Then the secondary amine of **14** was protected with a Z group using benzyl chloroformate to give compound **15**. Reactions using NaH/DMF as base to couple L-tyrosine and alkyl bromide/chloride have been reported without racemization (*23*,*24*). Using the same conditions, compound **16** was obtained in 82% yield. The carboxylic acid of **16** was protected as a benzyl ester (**17**). After removal of the Boc group, the free amine was coupled to commercially available 4-(chlorosulfonyl) benzoic acid to give **18** in 49% yield (Scheme 1). In this reaction a mixed solvent system (CH_3CN/H_2O) was used in order to get a homogeneous reaction to improve the yield. Compound **18** was further coupled with *n*-butyl amine to give compound **19**. Then both protecting groups of compound **19** were cleaved by hydrogenation to give RGD analog **2**. Similar procedures were followed for the synthesis of RGD analogs **3**, **4**, and **5** (Scheme 2).

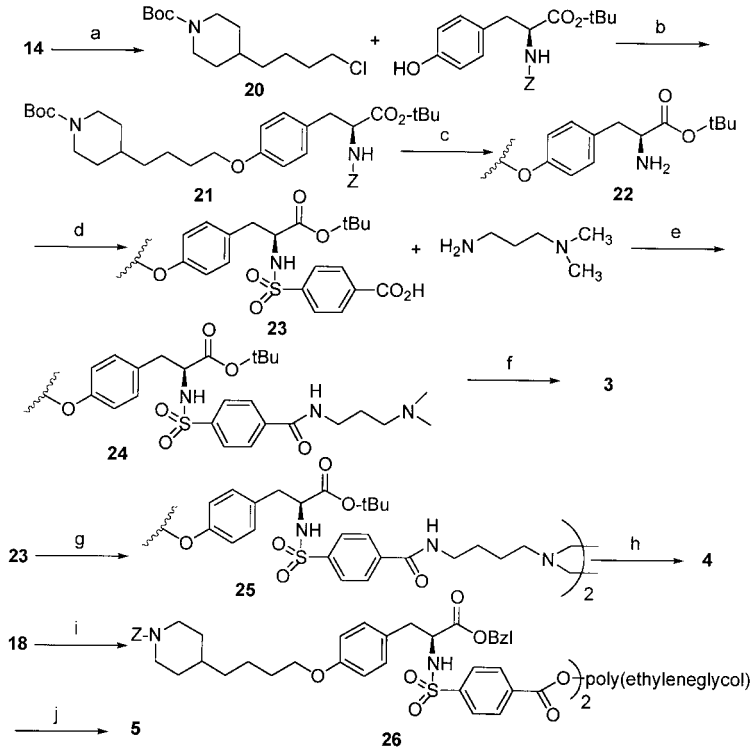
The synthesis of RGD analogs with different alkyl chain linkers (**6**–**11**) followed essentially the same routes (Schemes 3 and 4). The synthesis of the side chain linkers **29**, **34**, and **37** are illustrated in Scheme 3. Starting with taurine (**27**), the primary amino group was first protected with a Z group. This was followed by treatment with



SCHEME 1. Synthetic route for RGD analog **2.** Conditions: (a)(I) n-BuLi, −78 °C; (II) 40–45 °C, 2 h; (III) $Br(CH_2)_3Cl$, −65 °C-r.t.; 92%; (b) PtO_2, H_2, HOAc, 60 psi, 100%; (c) (I) aq. K_2CO_3; (II) benzyl chloroformate, pyridine, CH_3CN, 87%; (d) *N*-Boc-L-tyrosine, NaH, DMF, Kl, 82%; (e) benzyl alcohol, DCC, DMAP, 80%; (f) (I) TFA, CH_2Cl_2; (II) 4-(chlorosulfonyl) benzoic acid, K_2CO_3, CH_3CN, H_2O, 49%; (g) *n*-butylamine, EDC, HOBT, DMAP, CH_2Cl_2, 99%; (h) Pd/C, H_2, 100%.

triphosgene to give the sulfonyl chloride of the two-carbon linker **29** in 75%. The synthesis of the four-carbon linker started with the reaction of potassium phthalimide with sulfonate **31** to give **32**. Then the phthalimide protecting group was removed and replaced with a Z group because of its mild cleavage conditions. Attempts were made without success to convert **33** to its corresponding sulfonyl chloride (**34**) using triphosgene, which worked well with the synthesis of two-carbon linker **29**. Then we studied the reactions using thionyl chloride under various conditions and found that the best yield was obtained under dilute conditions (2 eq of thionyl chloride in CH_2Cl_2) at room temperature giving compound **34** in three steps with 68% overall yield. Similar strategy was followed for the synthesis of the six-carbon linker **37**. First, potassium phthalimide was reacted with 1,6-dibromohexane to give **35**. Then the phthalimide protecting group was removed and replaced with a Z group to give **36**, which was converted to the corresponding sulfonyl chloride **37** by reacting with thionyl chloride.

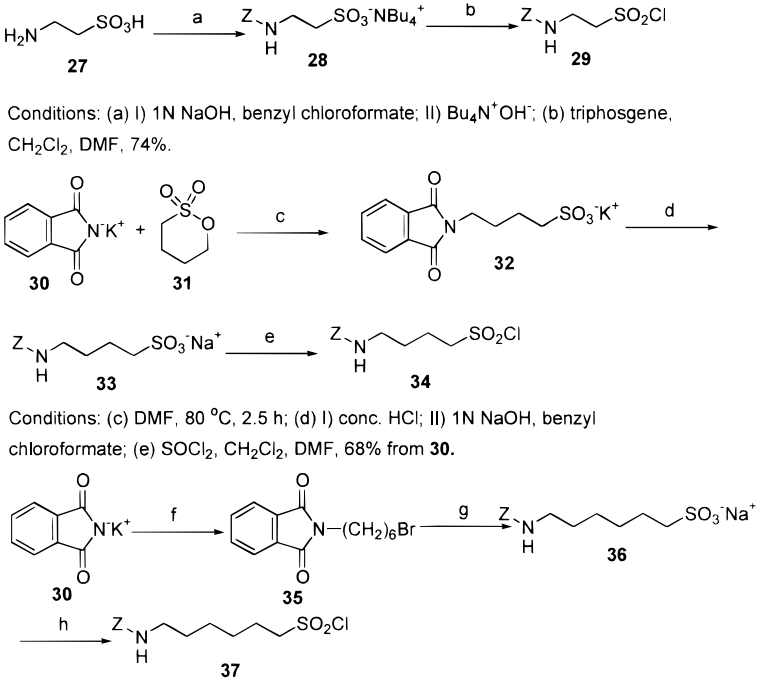
The synthesis of the key intermediate **41** for RGD analogs **6** to **11** was similar to that of compound **22**. As shown in Scheme 5, **41** was obtained in 66% yield starting from **38**. This key intermediate was coupled with the respective side chain linkers (**29**, **34**, and **37**) using $NaHCO_3$ or Na_2CO_3 in EtOAc to give compounds **42–44**. The yields ranged from 58 to 86%, depending on the activity of different sulfonyl chloride. It was found that a longer carbon chain between the amino group and the sulfonyl group resulted in a diminished reactivity of the corresponding sulfonyl chloride. Then the methyl group, Z group and Boc group were cleaved using standard conditions (Scheme 5) to give the desired RGD analogs **6** to **11**.



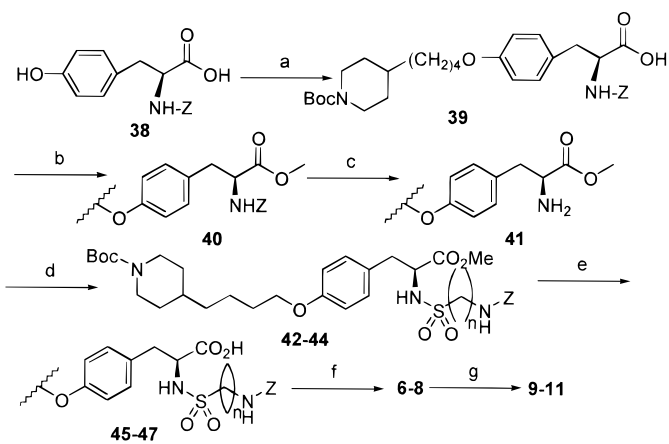
SCHEME 2. Synthesis of RGD analogs **3–5.** Conditions: (a) (I) aq. K_2CO_3; (II) Boc_2O, CH_2Cl_2, 80%; (b) NaH, Kl, DMF, 72%; (c) 10% Pd/C, H_2, 96%; (d) 4-(chlorosulfonyl)benzoic acid, K_2CO_3, CH_3CN, H_2O, 55%; (e) DCC, HOBt, DMAP, 72%; (f) 40% TFA; (g) 1,4-bis(3-aminopropyl)piperazine, DCC, HOBt, DMAP, 72%; (h) 40% TFA, 98%; (i) EDC, HOBt, DMAP, poly(ethylene glycol) (average MW ca. 300), 82%; (j) H_2/Pd-C, 95%.

BIOLOGICAL EVALUATION

Platelet aggregation assays to determine the binding affinity of the modified RGD analogs (**2–11**) toward GPIIb/IIIa were conducted using human volunteer blood samples (*25*). Platelets were activated with the addition ADP at 20 μM concentration. The concentration of the RGD analog that inhibited 50% of the platelet aggregation was signified as IC_{50}. The IC_{50}s of the modified RGD analogs are listed in Table 1. The only structural difference between compounds **2** (210 nM) and **3** (87 nM) is the added amino group in **3**, and yet compound **3** showed much higher affinity toward GPIIb/IIIa. This indicates that an ionizable amino group on the side chain helps to improve the binding affinity. Compounds **3** and **4** were designed to examine the effect of increased molecular weight. The IC_{50} of analog **4** (37 nM) was less than half of analog **3** (87 nM), as expected due to the doubling of the molecular weight and the number of RGD units in **4**. This indicates that increasing the molecular weight of such RGD analogs does not affect their binding to GPIIb/IIIa adversely. The conjugation of



SCHEME 3. Synthesis of side chain linkers **29, 34,** and **37.** Conditions: (f) 1,6-dibromohexane, DMF, 70%; (g) (I) Na_2SO_3, EtOH, H_2O; (II) conc. HCl; (III) 1 N NaOH, benzyl chloroformate; (h) $SOCl_2$, CH_2Cl_2, DMF, 37% for 4 steps.



SCHEME 4. Synthesis of RGD analogs **6–11.** Conditions: (a) NaH, Kl, DMF, **20,** 72%; (b) (I) Cs_2CO_3, DMF; (II) Mel, DMF, 100%; (c) H_2, Pd/C, CH_3OH, 92%; (d) **29, 34** or **37**, $NaHCO_3$ or Na_2CO_3, EtOAc, 45 °C, 58–86%; (e) LiOH, THF, CH_3OH, H_2O, 86–93%; (f) 25% TFA, CH_2Cl_2, DCM; (g) H_2, Pd/C, CH_3OH, 100% for two steps.

TABLE 1

Inhibition of Platelet Aggregation (IC_{50})

Compound	IC_{50} (nM)
2	210
3	87
4	37
5	150
6	180
7	180
8	300
9	100
10	150
11	50

a polymer such as poly(ethylene glycol) to the side chain of RGD analog (**5**) did not significantly affect the potency either (IC_{50}: 150 nM). This indicates the feasibility of tethering a fairly large pharmaceutical agent to an RGD analog for delivery purposes without sacrificing its high affinity for the target receptor. However, it should be noted that the dimeric RGD analog **5** with a polar poly(ethylene glycol) chain as a linker does not bind to GPIIb/IIIa as well as the analogous compound (**4**) with an alkylamine linker. This further indicates that an ionizable amino group on the side chain helps to improve the binding affinity. The side chain length also seemed to affect the affinity of these RGD analogs to GP IIb/IIIa to a degree. For example, with compounds **6–8**, the IC_{50} ranged from 180 to 300 nM. With compounds **9–11**, the IC_{50} ranged from 50 to 150 nM. The conversion of the free amines of compounds **9–11** to their protected form **6–8** did result in a noticeable effect on their binding affinity, again indicating the beneficial effect of the side chain amino group. The most significant is the comparison of **11** (IC_{50}: 50 nM) with **8** (IC_{50}: 300 nM). However, it is more important to note that all compounds showed sub-μM inhibitions of the GP IIb/IIIa binding. This indicates that in general RGD analog MK-383 tolerates side chain modification fairly well with various functional groups attached such as amide, amine, ester, protected amine and poly(ethylene glycol). Such information is very important for the further design of modified RGD analogs as vector for the delivery of pharmaceutical reagent.

CONCLUSIONS

A series of RGD analogs have been synthesized as potential delivery vectors of pharmaceutical agents. Their affinity for the target GPIIb/IIIa receptor has also been evaluated using platelet aggregation studies. It was found that such RGD analogs could tolerate side chain modifications fairly well with various functional groups attached such as amide, amine, ester, protected amine, and poly(ethylene glycol). The fact that side chain modification with poly(ethylene glycol) was tolerated well (IC_{50}: 150 nM) suggests the feasibility of tethering fairly large pharmaceutical agents to such RGD analogs without significant sacrifice of their affinity to the intended receptor.

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REFERENCES

1. Ruoslahti, E., and Pierchbacker, M. (1986) *Cell* **44,** 517–518.
2. Hynes, R. O. (1992) *Cell* **69,** 11–25.
3. Cheresh, D. (1993) *Adv. Mol. Cell. Biol.* **6,** 225–252.
4. Kong, D. F., Califf, R. M., Miller, D. P., Moliterno, D. J., White, H. D., Harrington, R. A., Tcheng, J. E., Lincoff, A., Hasselblad, V., and Topol, E. J. (1998) *Circulation* **98,** 2829–2835.
5. Bhatt, D. L., and Topol, E. J. (2000) *J. Am. Med. Assoc.* **284,** 1549–1558.
6. Johansson, L. O., Bjornerud, A., Ahlstrom, H. K., Ladd, D. L., and Fujii, D. K. (2001) *J. Magn. Res. Imag.* **13,** 615–618.
7. Pearson, D. A., ListerJames, J., McBride, W. J., Wilson, D. M., Martel, L. J., Civitello, E. R., and Dean, R. T. (1996) *J. Med. Chem.* **39,** 1372–1382.
8. Lee, D. H., and Blajchman, M. A. (2000) *Expert Opin. Investig. Drugs* **9,** 457–469.
9. Haubner, R., Wester, H. J., Burkhart, F., Senekowitsch-Schmidtke, R., Weber, W., Goodman, S. L., Kessler, H., and Schwaiger, M. (2001) *J. Nucl. Med.* **42,** 326–336.
10. Haubner, R., Wester, H. J., Weber, W. A., Mang, C., Ziegler, S. I., Goodman, S. L., Senekowitsch-Schmidtke, R., Kessler, H., and Schwaiger, M. (2001) *Cancer Res.* **61,** 1781–1785.
11. VanHagen, P. M., Breeman, W. A., Bernard, H. F., Schaar, M., Mooij, C. M., Srinivasan, A., Schmidt, M. A., Krenning, E. P., and deJong, M. (2000) *Inter. J. Cancer* **90,** 186–198.
12. DeNardo, S. J., Burke, P. A., Leigh, B. R., O'Donnell, R. T., Miers, L. A., Kroger, L. A., Goodman, S. L., Matzku, S., Jonczyk, A., Lamborn, K. R., and DeNardo, G. L. (2000) *Cancer Biother. Radiopharm.* **15,** 71–79.
13. Hart, S. L., Collins, L., Gustafsson, K., and Fabre, J. W. (1997) *Gene Ther.* **4,** 1225–1230.
14. Colin, M., Maurice, M., Trugnan, G., Kornprobst, M., Harbottle, R. P., Knight, A., Cooper, R. G., Miller, A. D., Capeau, J., Coutelle, C., and Brahimi-Horn, M. C. (2000) *Gene Ther.* **7,** 139–152.
15. Aris, A., and Villaverde, A. (2000) *Biochem. Biophys. Res. Commun.* **278,** 455–461.
16. De Giglio, E., Sabbatini, L., Colucci, S., and Zambonin, G. (2000) *J. Biomater. Sci.-Polym. Ed.* **11,** 1073–1083.
17. Marler, J. J., Guha, A., Rowley, J., Koka, R., Mooney, D., Upton, J., and Vacanti, J. P. (2000) *Plast. Reconstr. Surg.* **105,** 2049–2058.
18. LeBaron, R. G., and Athanasiou, K. A. (2000) *Tissue Eng.* **6,** 85–103.
19. Quirk, R. A., Chan, W. C., Davies, M. C., Tendler, S. J. B., and Shakesheff, K. M. (2001) *Biomaterials* **22,** 865–872.
20. McConachie, A., Newman, D., Tucci, M., Puckett, A., Tsao, A., Hughes, J., and Benghuzzi, H. (1999) *Biomed. Sci. Instrument.* **35,** 45–50.
21. Hyun, Y. L., Lew, D. B., Park, S. H., Kim, C. W., Paik, W. K., and Kim, S. (2000) *Biochem. J.* **348,** 573–578.
22. Egbertson, M. S., Chang, C. T. C., Duggan, M. E., Gould, R. J., Halczenko, W., Hartman, G. D., Laswell, W. L., Joseph J. Lynch, J., Lynch, R. J., Manno, P. D., Naylor, A. M., Prugh, J. D., Ramjit, D. R., Sitko, G. R., Smith, T. S., Turchi, L. M., and Zhang, G. (1994) *J. Med. Chem.* **37,** 2537–2551.
23. Hartman, G. D., Egbertson, M. S., Halczenko, W., Laswell, W. L., Duggan, M. E., Smith, R. L., Naylor, A. M., Manno, P. D., Lynch, R. J., Zhang, G., Chang, C. T.-C., and Gould, R. J. (1992) *J. Med. Chem.* **35,** 4640–4642.
24. Chung, J. Y. L., Zhao, D., Hughes, D. L., and Grabowski, E. J. J. (1993) *Tetrahedron* **49,** 5767–5776.
25. Born, G. (1964) *Nature* **194,** 927–933.
26. Reynolds, R. C., Crooks, P. A., Maddry, J. A., Akhtar, M. S., Montgonery, J. A., III (1999) *J. Org. Chem.* **57,** 2983–2985.
27. Paik, S., and White, E. H. (1996) *Tetrahedron Lett.* **37,** 4663–4666.
28. Marchand-Brynaert, J., Bouchet, M., Touillaux, R., Beauve, C., and Fastrez, J. (1996) *Tetrahedron* **4,** 5591–5605.

29. Bont, D. B. A. d., Dijkstra, G. D. H., Hartog, J. A. J. d., and Liskamp, R. M. J. (1996) *Bioorg. Med. Chem. Lett.* **6,** 3035–3040.
30. Carson, K. G., Schwender, C. F., Shroff, H. N., Cochran, N. A., Gallant, D. L., and Briskin, M. J. (1997) *Bioorg. Med. Chem. Lett.* **7,** 711–714.
31. Shue, H.-J., Chen, X., Blythin, D. J., Carruthers, N. I., Spitler, J. M., Wong, S.-C., Chapman, R. W., Rizzo, C., West, R., and She, H. S. (1996) *Bioorg. Med. Chem. Lett.* **6,** 1709–1714.
32. Helferich, V. B., and Otten, G. (1954) *J. Prakt. Chem.* **4,** 2–13.
33. Elslager, E. F., Moore, A. M., Short, F. W., Sullivan, M. J., and Tendick, F. H. (1957) *J. Am. Chem. Soc.* **79,** 4699–4703.
34. Johnson, T. J., and Jones, R. A. (1978) *Tetrahedron* **34,** 547–551.
35. Troyanskii, E. I., Lazareva, M. I., and Nikishin, G. I. (1986) *Bull. Acad. Sci. USSR Div. Chem. Sci. (Engl. Transl.)* **35,** 1428–1434.