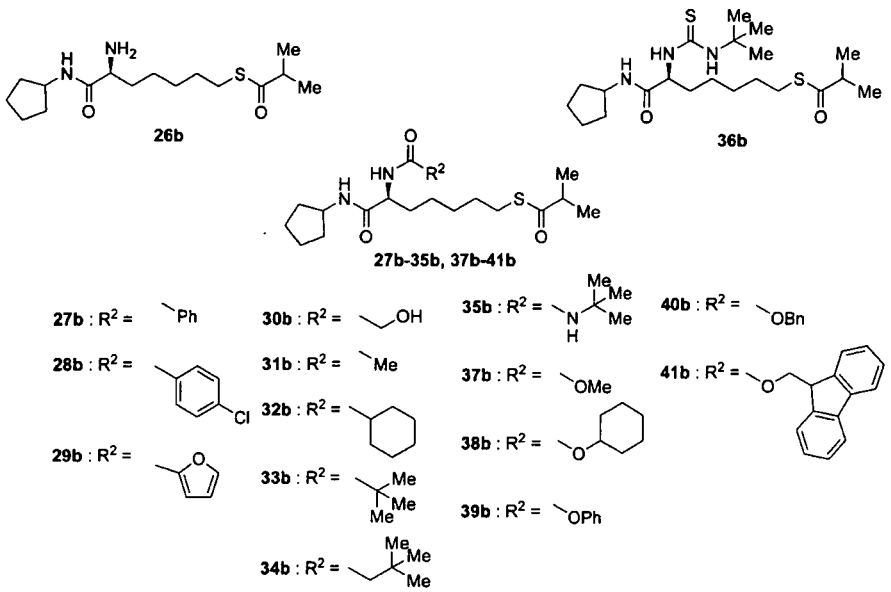


Figure 3. Structures of **26b**–**41b**.

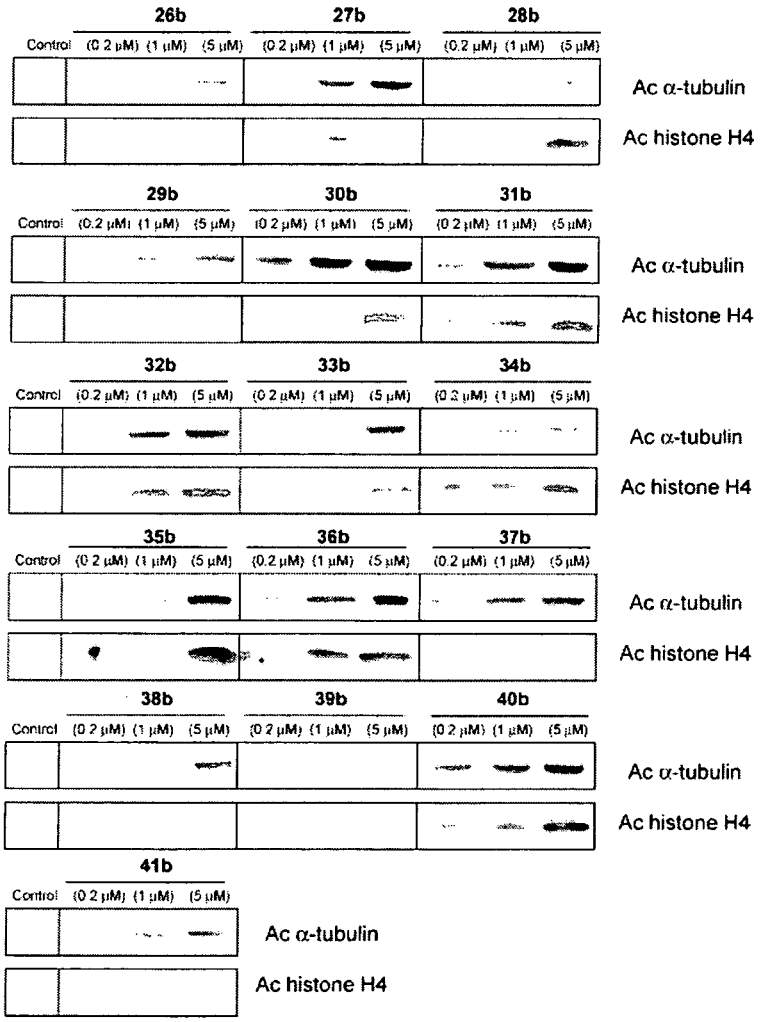
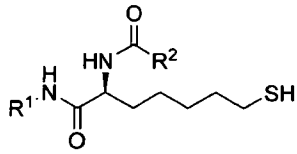


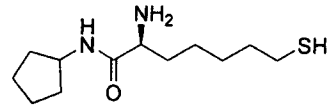
Figure 4. Western blot detection of acetylated α-tubulin and acetylated histone H4 levels in HCT116 cells after 8 h treatment with **26b**–**41b**.

evaluated these two compounds. While compounds **35b** and **36b** sustained α-tubulin acetylation activity, selectivity was significantly reduced. These results highlighted the importance of the carbamate group in α-tubulin/histone acetylation selectivity.

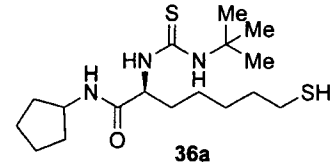
Table 1. In Vitro HDAC1-, HDAC4-, and HDAC6-Inhibitory Activities of **13a**, **15a**–**20a**, **26a**–**28a**, **31a**, **33a**, **35a**–**38a**, and **40a**[a]



13a, 15a-20a, 27a, 28a, 30a, 33a, 35a, 37a, 38a, 40a



26a



36a

cmpd	R^1	R^2	IC_{50} (nM) HDAC1	HDAC4	HDAC6	selectivity HDAC1/HDAC6	HDAC4/HDAC6
1			21	34	81	0.26	0.42
5			ND[c]	ND	ND	4[b]	4[b]
7			48	32	41	1.2	0.78
13a	3-biphenyl	-O*t*-Bu	62	38	54	1.2	0.70
15a	3-quinolinyl	-O*t*-Bu	51	33	32	1.6	1.0
16a	cyclopentyl	-O*t*-Bu	1210	1030	29	42	36
17a	cyclohexyl	-O*t*-Bu	1270	1140	36	35	32
18a	cycloheptyl	-O*t*-Bu	900	840	23	39	37
19a	-*t*-Bu	-O*t*-Bu	3000	1900	71	42	26
20a	1-adamantyl	-O*t*-Bu	3800	4200	82	46	51
26a			52 300	31 200	3860	14	8.1
27a	cyclopentyl	-Ph	560	280	190	3.0	1.5
28a	cyclopentyl	4-chlorophenyl	1430	650	510	2.8	1.3
30a	cyclopentyl	$-CH_2OH$	1980	970	150	13	6.5
33a	cyclopentyl	-*t*-Bu	1430	730	430	3.3	1.7
35a	cyclopentyl	-NH*t*-Bu	460	310	100	4.6	3.1
36a			400	220	170	2.4	1.3
37a	cyclopentyl	-OMe	2320	1160	120	19	9.7
38a	cyclopentyl	cyclohexyloxy	1030	580	120	8.6	4.8
40a	cyclopentyl	-OBn	130	130	78	1.7	1.7

[a] Values are means of at least three experiments. [b] Taken from the literature (ref 22). [c] ND = No data.

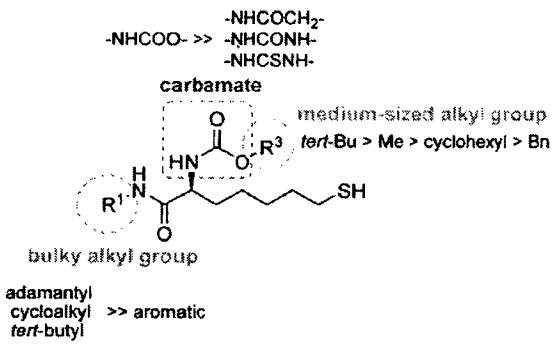


Figure 5. Structure–selectivity relationship.

Next, we converted the *tert*-butyl group of **16b** to other functional groups (**37b**–**41b**). While methyl group (**37b**) retained the α-tubulin acetylation activity and selectivity to some extent, larger alkyl groups (**38b**, **40b**, and **41b**) and a phenyl group (**39b**) caused a decrease in either selectivity or potency. Medium-sized alkyl groups such as the *tert*-butyl group seem to be preferred as the substituents attached to the carbamate. As a result, the *tert*-butoxy group (**16b**) was the best R^2 group choice for the selective accumulation of acetylated α-tubulin in cells.

To confirm HDAC6 selectivity and to examine the structure–selectivity relationship for this series of inhibitors, we performed in vitro enzyme assays using HDAC1, HDAC4, and HDAC6. A selected set of thiols was prepared and evaluated for enzyme inhibition activity. The results of the enzyme assays are shown in Table 1. Compound **1** was chosen as a reference compound. We initially tested compound **7** and compounds with various R^1 groups (**13a**, **15a**, and **16a**–**20a**). Consistent with the results of Western blotting, compound **7** and aromatic compounds **13a** and **15a** did not discriminate among HDAC1, HDAC4, and HDAC6. The strong HDAC1 inhibitory activity of **13a** and **15a** is consistent with the results observed in similar aromatic group-containing HDAC inhibitors.[21] The HDAC6 inhibitory activity of compounds **16a**–**20a**, in which R^1 = a bulky alkyl group, was similar to or greater than that of **1** (IC_{50} of 81 nM, **16a** 29 nM, **17a** 36 nM, **18a** 23 nM, **19a** 71 nM, **20a** 82 nM). Furthermore, while **1** inhibited HDAC1 and HDAC4 rather than HDAC6 (HDAC1 IC_{50}/HDAC6 IC_{50} = 0.26; HDAC4 IC_{50}/HDAC6 IC_{50} = 0.42), compounds **16a**–**20a** efficiently inhibited HDAC6 in preference to HDAC1 and HDAC4 (HDAC1 IC_{50}/HDAC6 IC_{50} = 35–46; HDAC4 IC_{50}/HDAC6 IC_{50} = 26–51). The HDAC6 selectivity of compounds **16a**–**20a** was much higher than that of **5**, which showed only about a 4-fold selectivity for HDAC6 over HDAC1 and HDAC 4 in enzyme assays.[22] In addition, in the case of compounds with various R^2 groups (**26a**–**28a**, **30a**, **33a**, **35a**–**38a**, and **40a**), a reasonable correlation between cellular and enzyme assay data was observed. Amine **26a** did not show strong HDAC inhibition, and aromatic amides **27a** and **28a** and aliphatic amides **30a** and **33a** were nonselective inhibitors as compared with **16a**. Conversion of the carbamate of **16a** to urea (**35a**) and thiourea (**36a**) significantly reduced the HDAC6 selectivity. As for carbamate derivatives, methyl carbamate **37a** exerted a moderate level of HDAC6 selectivity (HDAC1 IC_{50}/HDAC6 IC_{50} = 19; HDAC4 IC_{50}/HDAC6 IC_{50} = 9.7), whereas cyclopentyl **38a** and benzyl **40a** showed a decrease in HDAC6 selectivity as compared with that of **16a** and **37a**. As a result, compounds **16a**–**20a**, in which R^1 = a bulky alkyl group and R^2 = a *tert*-butoxy group, were the most potent and selective HDAC6 inhibitors.

As shown in Figure 5, the results of Western blotting and enzyme assays clarified the structural requirements for HDAC6-selective inhibition. For R^1, bulky alkyl groups such as

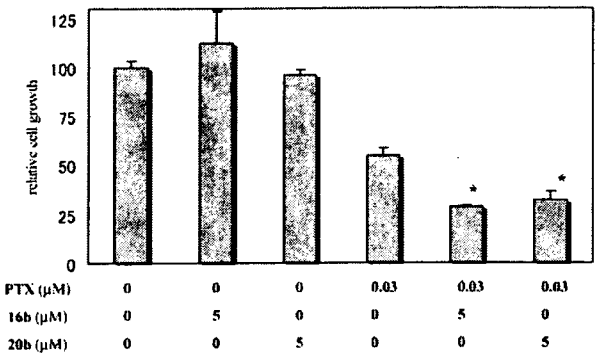


Figure 6. Cell growth inhibition of HCT116 cells using different combinations of HDAC6 inhibitor **16b** or **20b** and paclitaxel (PTX). $^{*}P < 0.05$; ANOVA and Bonferroni-type multiple t test results indicated differences between PTX (0.03 μM) and a combination of PTX (0.03 μM) with **16b** (5 μM) and between PTX (0.03 μM) and a combination of PTX (0.03 μM) with **20b** (5 μM).

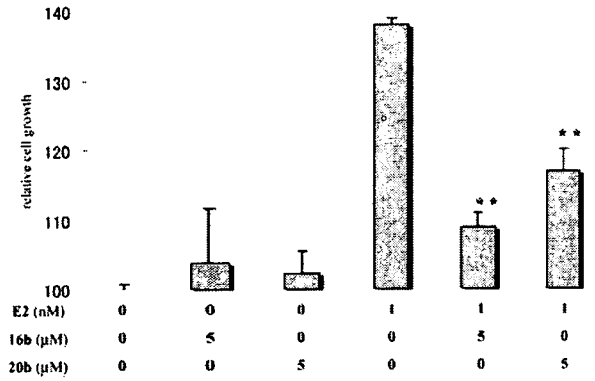


Figure 7. Cell growth inhibition of MCF-7 cells stimulated with estradiol (E2) using HDAC6 inhibitor **16b** or **20b**. $^{**}P < 0.01$; ANOVA and Bonferroni-type multiple t test results indicated differences between E2 (1 nM) and a combination of E2 (1 nM) with **16b** (5 μM) and between E2 (1 nM) and a combination of E2 (1 nM) with **20b** (5 μM).

adamantyl and cycloalkyl are preferred. The carbamate structure is also important for HDAC6-selective inhibition. For R^3, medium-sized alkyl groups such as *tert*-butyl are suitable. Because there is no information at present on the three-dimensional structure of HDAC6, the reason for the high HDAC6 selectivity of compounds **16**–**20** is unclear, but it is likely that HDAC6 has at least two hydrophobic pockets where a bulky R^1 group and a medium-sized R^3 group could be placed, as well as an amino acid residue, which could interact with the carbamate group, whereas the other HDAC isoforms lack the pockets and amino acid residue.

To explore the potential of HDAC6-selective inhibitors as anticancer drugs, we first tested compounds **16b** and **20b**, the most selective and active compounds in this series, by means of a cancer cell growth inhibition assay using human colon cancer HCT116 cells. However, they were found to be inactive up to a concentration of 5 μM above which they displayed distinct α-tubulin acetylation on Western blot analysis (Figure 2). We next evaluated whether our HDAC6-selective inhibitors could act synergistically with paclitaxel (PTX) in growth inhibition assays using HCT116 cells. Because PTX exerts its anticancer effect by stabilizing microtubules, where acetylated α-tubulins are most abundant,[5b,c,23] we considered that the combination of our HDAC6-selective inhibitors and PTX might cause a synergistic inhibition of cancer cell growth. As shown in Figure 6, 5 μM of **16b** or **20b** did not show any activity as a single agent, whereas treatment with 0.03 μM of PTX reduced the cell growth by approximately 50%. As expected, however, a combination of 5 μM of **16b** or **20b** and 0.03 μM of PTX reduced cell growth by approximately 70%, suggesting that HDAC6-selective inhibitors have potential as drug candidates when used in combination with PTX.

We also evaluated the effect of HDAC6-selective inhibitors on ERα-positive breast cancer MCF-7 cells. It has been reported that estrogen stimulation of MCF-7 cells increases expression of HDAC6 and enhances cell motility.[7] This report suggested to us that HDAC6 may be associated with the growth of ERα-positive breast cancer cells treated with estrogen. We therefore examined whether HDAC6-selective inhibitors could inhibit growth of MCF-7 cells stimulated with this hormone (Figure 7). As with HCT116 cells, the growth of MCF-7 cells was not influenced by treatment with 5 μM of **16b** or **20b**, whereas treatment with 1 nM of 17β-estradiol (E2)-induced cell growth by approximately 40%. Interestingly, treatment with 5 μM of **16b** or **20b** significantly blocked the growth of MCF-7 cells stimulated with 1 nM of E2. These results suggested that HDAC6 is involved in the growth of ERα-positive breast cancer cells and that HDAC6-selective inhibitors may be useful in the treatment of ERα-positive breast cancer.

Conclusion

We have identified novel HDAC6-selective inhibitors whose designs were based on the structure of HDAC6-selective substrate **10**. Compounds **16**–**20** showed high HDAC6 selectivity in both cellular and enzyme assays and investigation of the structure–selectivity relationship revealed that the presence of a bulky alkyl group, such as adamantyl and cycloalkyl, and a *tert*-butylcarbamate group in these compounds is important for HDAC6-selective inhibition. In biological experiments, the combination of compound **16b** or **20b** and PTX caused a synergistic inhibition of cancer cell growth. The synergistic effect of these HDAC6-selective inhibitors may allow for the reduction of the PTX dosage with consequently fewer side effects. Compounds **16b** and **20b** also showed growth inhibition of MCF-7 cells stimulated by estrogen. Inhibition of ERα-positive breast cancer cell growth by HDAC6-selective inhibitors suggests that these inhibitors may be effective as antibreast cancer drugs. We believe that the findings presented here will provide a basis for constructing new tools for probing the biology of HDAC6 and uncovering new strategies for cancer treatments.

Experimental Section

Chemistry. Melting points were determined using a Yanagimoto micromelting point apparatus or a Büchi 545 melting point apparatus and were left uncorrected. Proton nuclear magnetic resonance spectra (^{1}H NMR) and carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded JEOL JNM-LA500, JEOL JNM-A500 or BRUKER AVANCE600 spectrometer in solvent as indicated. Chemical shifts (δ) are reported in parts per million relative to the internal standard tetramethylsilane. Elemental analysis was performed with a Yanaco CHN CORDER NT-5 analyzer, and all values were within ±0.4% of the calculated values. High-resolution mass spectra (HRMS) and fast atom bombardment (FAB) were recorded on a JEOL JMS-SX102A mass spectrometer. GC-MS analyses were performed on a Shimadzu GCMS-QP2010. Reagents and solvents were purchased from Aldrich, Tokyo Kasei Kogyo, Wako Pure Chemical Industries, and Kanto Kagaku and used without purification. Flash column chromatography was performed using silica gel 60 (particle size 0.046–0.063 mm) supplied by Merck.

(*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-oxo-7-[2-oxo-4-(trifluoromethyl)-2*H*-chromen-7-ylamino]heptyl 2-Methylpropanethioate (11b). Step 1: Preparation of (*S*)-7-Bromo-2-*tert*-butoxycarbonylaminoheptanoic Acid (43). To a solution of (*S*)-2-amino-7-bromoheptanoic acid (**42**; 2.01 g, 8.97 mmol) and Et_3N (5 mL, 67.7 mmol) in THF/H_2O (20 mL/40 mL) was added a solution of $(Boc)_2O$ (3.90 g, 17.9 mmol) in THF (20 mL), and the mixture was stirred overnight at room temperature. The reaction mixture was poured into 2 N aqueous NaOH (15 mL), and the whole mixture was washed with $CHCl_3$. To the separated aqueous layer was added 10% aqueous citric acid (15 mL) and was extracted with AcOEt. The organic layer was washed with water and brine and dried over Na_2SO_4. Filtration and concentration in vacuo gave 2.77 g (95%) of **43** as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.02 (1H, d, J = 7.0 Hz), 4.31 (1H, s), 3.40 (2H, t, J = 6.7 Hz), 1.87 (1H, m), 1.72−1.65 (1H, m), 1.87 (2H, quintet, J = 7.1 Hz), 1.52−1.43 (13H, m).

Step 2: Preparation of (*S*)-*tert*-Butyl 7-Bromo-1-oxo-1-(2-oxo-4-(trifluoromethyl)-2*H*-chromen-7-ylamino)heptan-2-ylcarbamate (11c). To a solution of 7-amino-4-(trifluoromethyl)coumarin (688 mg, 3.00 mmol) and **43** (973 mg, 3.00 mmol) obtained above in dry pyridine (18 mL) was added phosphoryl chloride (750 μL, 2.97 mmol) at −15 °C, and the solution was stirred at −15 °C for 15 min. The solution was poured into water, and the solution was extracted with AcOEt. The AcOEt layer was separated, washed with saturated aqueous $NaHCO_3$, 10% aqueous citric acid, and brine, and dried over Na_2SO_4. Filtration, concentration in vacuo, and purification by silica gel flash column chromatography (AcOEt/*n*-hexane = 1/3) gave 922 mg (57%) of **11c** as a colorless solid: ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.02 (1H, s), 7.80 (1H, d, J = 1.8 Hz), 7.62 (1H, d, J = 7.9 Hz), 7.46 (1H, d, J = 9.1 Hz), 6.67 (1H, s), 4.97 (1H, s), 4.19 (1H, s), 3.41 (2H, t, J = 6.7 Hz), 2.02−1.96 (1H, m), 1.88 (2H, quintet, J = 7.0 Hz), 1.71−1.60 (1H, m), 1.48 (9H, s), 1.52−1.35 (4H, m).

Step 3: Preparation of (*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-oxo-7-[2-oxo-4-(trifluoromethyl)-2*H*-chromen-7-ylamino]heptyl 2-methylpropanethioate (11b). To a solution of sodium hydrosulfide (4.29 g, 77.9 mmol) in MeOH (50 mL) was added *iso*-butyryl chloride (4 mL, 37.2 mmol) and stirred at 0 °C for 1 h. Then, the solution was stirred at room temperature for 2 h. After that, the reaction mixture was poured into water and extracted with AcOEt. The extract was washed with 1 N aqueous HCl and brine and dried over Na_2SO_4. Filtration and evaporation in vacuo gave 2.29 g (59%) of thioisobutyric acid as a yellow oil. To a solution of **11c** (443 mg, 0.827 mmol) obtained above in EtOH (10 mL) was added a solution of thioisobutyric acid (455 mg, 4.27 mmol) in EtOH (5 mL) and triethylamine (1 mL, 13.6 mmol), and the mixture was stirred overnight at room temperature. The reaction mixture was poured into water, and the mixture was extracted with AcOEt. The AcOEt layer was separated, washed with 10% aqueous citric acid and brine, and dried over Na_2SO_4. Filtration, concentration in vacuo, purification by silica gel flash column chromatography (AcOEt/*n*-hexane = 1/4), and recrystallization from AcOEt/*n*-hexane gave 250 mg (54%) of **11b** as colorless crystals: mp 163−165 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.07 (1H, s), 7.81 (1H, s), 7.62 (1H, d, J = 7.9 Hz), 7.41 (1H, d, J = 7.3 Hz), 6.68 (1H, s), 5.06 (1H, s), 4.18 (1H, broad s), 2.84 (2H, t, J = 7.8 Hz), 2.73 (1H, quintet, J = 6.9 Hz), 1.99−1.95 (1H, m), 1.70−1.65 (1H, m), 1.60−1.54 (2H, m), 1.47 (9H, s), 1.45−1.40 (4H, m), 1.18 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.59, 171.00, 159.10, 155.24, 142.34, 128.50, 125.83, 122.40, 116.18, 113.84, 109.36, 107.51, 81.26, 77.24, 43.14, 29.32, 28.31, 28.20, 25.12, 19.42; Anal. ($C_{26}H_{33}F_3N_2O_6S\cdot 1/4H_2O$) C, H, N.

Compounds **12b**−**15b** were prepared from **43** and an appropriate amine using the procedure described for **11b**.

(*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-oxo-7-(phenylamino)heptyl 2-Methylpropanethioate (12b). Yield 9%; yellow oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 8.22 (1H, s), 7.52 (2H, d, J = 8.0 Hz), 7.31 (2H, t, J = 7.8), 7.10 (1H, t, J = 7.5 Hz), 5.04 (1H, s), 4.17 (1H, s), 2.84 (2H, t, J = 7.3 Hz), 2.73 (1H, quintet, J = 7.0 Hz), 1.96−1.92 (1H, m), 1.65−1.55 (3H, m), 1.50−1.45 (13H, m), 1.19 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.45, 170.26, 129.00, 124.38, 119.87, 77.22, 43.12, 29.36, 28.31, 28.25, 25.13, 19.42; MS (EI) *m/z* 422 (M^+); HRMS calcd for $C_{22}H_{34}O_4N_2S$, 422.224; found, 422.225.

(*S*)-*S*-7-(Biphenyl-3-ylamino)-6-(*tert*-butoxycarbonyl)-7-oxoheptyl 2-Methylpropanethioate (13b). Yield 6%; yellow oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 8.24 (1H, broad s), 7.79 (1H, s), 7.58 (2H, d, J = 7.6 Hz), 7.48 (1H, d, J = 7.9 Hz), 7.44−7.34 (5H, m), 4.98 (1H, s), 4.18 (1H, s), 2.84 (2H, t, J = 7.3 Hz), 2.72 (1H, quintet, J = 7.0 Hz), 2.00−1.95 (1H, m), 1.71−1.65 (1H, m), 1.60−1.54 (2H, m), 1.45−1.40 (13H, m), 1.18 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.43, 170.44, 142.12, 140.66, 129.35, 128.73, 127.47, 127.20, 123.16, 118.68, 81.28, 77.24, 43.13, 29.36, 28.33, 28.26, 25.16, 19.43; MS (EI) *m/z* 498 (M^+); HRMS calcd for $C_{28}H_{38}O_4N_2S$, 498.255; found, 498.255.

(*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-oxo-7-(4-phenylthiazol-2-ylamino)heptyl 2-Methylpropanethioate (14b). Yield 28%; yellow oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 9.97 (1H, broad s), 7.82 (2H, d, J = 7.0 Hz), 7.41 (2H, t, J = 7.6 Hz), 7.32 (1H, d, J = 7.3 Hz), 7.14 (1H, s), 5.05 (1H, d, J = 7.5 Hz), 4.37 (1H, s), 2.83 (2H, t, J = 7.3 Hz), 2.73 (1H, quintet, J = 7.0 Hz), 2.00−1.95 (1H, m), 1.71−1.65 (1H, m), 1.60−1.54 (2H, m), 1.48 (9H, s), 1.45−1.40 (4H, m), 1.18 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.42, 170.36, 157.27, 150.08, 134.34, 128.72, 128.05, 126.12, 107.95, 80.94, 77.24, 54.61, 43.12, 31.75, 29.29, 28.30, 28.20, 25.00, 19.42; MS (EI) *m/z* 505 (M^+); HRMS calcd for $C_{25}H_{35}O_4N_3S_2$, 505.207; found, 505.214.

(*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-oxo-7-(quinolin-3-ylamino)-heptyl 2-Methylpropanethioate (15b). Yield 3%; yellow oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 8.76 (1H, s), 8.74 (2H, s), 8.03 (1H, d, J = 8.8 Hz), 7.88 (1H, d, J = 7.9 Hz), 7.62 (1H, d, J = 7.2 Hz), 7.53 (1H, d, J = 7.3 Hz), 5.01 (1H, s), 4.25 (1H, s), 2.85 (2H, t, J = 7.5 Hz), 2.73 (1H, quintet, J = 7.0 Hz), 2.04−1.95 (1H, m), 1.71−1.65 (1H, m), 1.60−1.54 (2H, m), 1.50−1.45 (13H, m), 1.19 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.59, 171.08, 143.97, 131.40, 128.94, 128.29, 128.17, 127.74, 127.25, 123.88, 77.22, 43.14, 29.33, 28.31, 28.18, 25.13, 19.43; MS (EI) *m/z* 473 (M^+); HRMS calcd for $C_{25}H_{35}O_4N_3S$, 473.235; found, 473.234.

(*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate (16b). Step 1: Preparation of (*S*)-*tert*-Butyl 7-Bromo-1-(cyclopentylamino)-1-oxoheptan-2-ylcarbamate (16c). To a solution of **43** (2.49 g, 7.68 mmol) obtained above in THF (12 mL) were added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI; 2.22 g, 11.6 mmol), 1-hydroxy-1*H*-benzotriazole monohydrate (HOBt·H_2O; 1.77 g, 11.6 mmol), and cyclopentylamine (781 μL, 7.92 mmol), and the mixture was stirred overnight at room temperature. The reaction mixture was poured into water and was extracted with AcOEt. The AcOEt layer was separated, washed with 10% aqueous citric acid, water, saturated $NaHCO_3$, and brine, and dried over Na_2SO_4. Filtration, concentration in vacuo, and purification by silica gel flash column chromatography (AcOEt/*n*-hexane = 2/5) gave 2.28 g (76%) of **16c** as a colorless solid: ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.97 (1H, d, J = 7.6 Hz), 4.98 (1H, s), 4.18 (1H, sextet, J = 7.0 Hz), 3.95 (1H, s), 3.40 (2H, t, J = 6.7 Hz), 1.97 (2H, m), 1.90−1.78 (3H, m), 1.71−1.53 (5H, m), 1.49−1.30 (15H, m).

Step 2: Preparation of (*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate (16b). To a solution of sodium hydrosulfide (426 mg, 7.60 mmol) in EtOH (12 mL) was added *iso*-butyryl chloride (820 μL, 7.62 mmol) dropwise at 0 °C, and the mixture was stirred at room temperature for 1 h. Then, to the mixture was added a solution of **16c** (296 mg, 0.756 mmol) in EtOH (7 mL) and Et_3N (1 mL, 13.6 mmol), and the resulting mixture was stirred overnight at room temperature. The reaction mixture was poured into water and was extracted with AcOEt. The AcOEt layer was separated, washed with brine, and dried over Na_2SO_4. Filtration, concentration in vacuo, and purification by silica gel flash column chromatography (AcOEt/*n*-hexane = 1/4) gave 314 mg (100%) of **16b** as a yellow oil: ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.97 (1H, d, J = 7.6 Hz), 4.97 (1H, s),

4.18 (1H, sextet, J = 6.9 Hz), 3.94 (1H, s), 2.83 (2H, t, J = 7.2 Hz), 2.73 (1H, septet, J = 6.9 Hz), 1.97−1.94 (2H, m), 1.83−1.78 (1H, m), 1.68−1.53 (5H, m), 1.44 (9H, s), 1.47−1.37 (8H, m), 1.19 (6H, t, J = 6.7 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.29, 170.59, 155.72, 77.23, 50.37, 43.11, 35.04, 34.95, 29.40, 28.43, 28.32, 28.30, 27.99, 25.06, 24.36, 19.42; Anal. ($C_{21}H_{38}N_2O_4S\cdot 1/3H_2O$) C, H, N.

Compounds **17b** and **19b−25b** were prepared from **43** and an appropriate amine using the procedure described for **16b**.

(*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-(cyclohexylamino)-7-oxoheptyl 2-Methylpropanethioate (17b). Yield 19%; yellow oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.89 (1H, d, J = 8.2 Hz), 4.99 (1H, s), 3.94 (1H, s), 3.75 (1H, s), 2.83 (2H, t, J = 7.4 Hz), 2.73 (1H, quintet, J = 7.0 Hz), 1.90−1.78 (3H, m), 1.72−1.68 (2H, m), 1.65−1.50 (4H, m), 1.47−1.28 (15H, m), 1.21−1.10 (9H, m); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.35, 170.87, 155.69, 77.23, 48.13, 43.11, 33.04, 29.39, 28.41, 28.32, 25.49, 25.03, 24.72, 19.42; MS (EI) m/z 428 (M^+); HRMS calcd for $C_{22}H_{40}O_4N_2S$, 428.270; found, 428.276.

(*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-(*tert*-butylamino)-7-oxoheptyl 2-Methylpropanethioate (19b). Yield 33%; yellow oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.80 (1H, s), 4.97 (1H, s), 3.88 (1H, s), 2.83 (2H, t, J = 7.3 Hz), 2.73 (1H, septet, J = 6.9 Hz), 1.81−1.74 (1H, m), 1.59−1.50 (2H, m), 1.48−1.40 (11H, m), 1.39−1.34 (11H, m), 1.18 (6H, d, J = 6.7 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.29, 171.11, 77.23, 51.32, 43.11, 32.33, 29.41, 28.71, 28.46, 28.31, 25.02, 19.42; Anal. ($C_{20}H_{38}N_2O_4S\cdot 2/3H_2O$) C, H, N.

(*S*)-*S*-7-(Adamant-1-ylamino)-6-(*tert*-Butoxycarbonyl)-7-oxoheptyl 2-Methylpropanethioate (20b). Yield 66%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm), 5.62 (1H, s), 4.99 (1H, m), 3.88 (1H, m), 2.83 (2H, t, J = 7.3 Hz), 2.72 (1H, septet, J = 6.7 Hz), 2.07 (3H, s), 1.98 (6H, s), 1.77 (1H, sextet, J = 7.0 Hz), 1.67 (6H,s), 1.56−1.30 (16H, m), 1.18 (6H, d, J = 6.7 Hz); ^{13}C NMR ($CDCl_3$,500 MHz, δ, ppm), 204.27, 170.87, 155.69, 79.90, 55.01, 43.12, 41.58, 36.33, 36.20, 32.51, 29.55, 29.50, 29.43, 28.49, 28.34, 25.00, 19.42; MS (FAB) m/z 481 (MH^+); Anal. ($C_{26}H_{44}N_2O_4S$) C, H, N.

(*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-(2,3-dihydro-1*H*-inden-2-ylamino)-7-oxoheptyl 2-Methylpropanethioate (21b). Yield 87%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 7.23−7.14 (4H, m), 6.24 (1H, d, J = 7.6 Hz), 4.94 (1H, m), 4.71 (1H, m), 3.94 (1H, m), 3.30 (2H, m), 2.76 (4H, m), 2.69 (2H, septet, J = 6.7 Hz), 1.79 (1H, m), 1.58−1.51 (3H, m), 1.48−1.51 (13H, m), 1.18 (6H, d, J = 6.7 Hz); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 204.32, 171.76, 155.68, 140.71, 126.81, 124.79, 80.09, 54.54, 50.57, 43.11, 40.09, 39.99, 32.24, 29.70, 28.37, 28.28, 25.05, 19.41; MS (FAB) m/z 463 (MH^+); Anal. ($C_{25}H_{38}N_2O_4S\cdot 1/2\ H_2O$) C, H, N.

(*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-(2-hydroxyethylamino)-7-oxoheptyl 2-Methylpropanethioate (22b). Yield 46%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 6.43 (1H, m), 4.98 (1H, m), 3.99 (1H, m), 3.72 (2H, q, J = 4.8 Hz), 3.43 (2H, m), 2.61 (1H, broad s) 1.82 (1H, m), 1.57−1.30 (16H, m), 1.18 (6H, d, J = 6.7 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.68, 173.06, 155.57, 80.37, 61.96, 54.89, 43.13, 42.34, 32.12, 29.35, 28.32, 28.26, 28.39, 28.18, 24.95, 19.42; MS (FAB) m/z 391 (MH^+); Anal. ($C_{18}H_{34}N_2O_5S\cdot 1/2H_2O$) C, H, N.

(*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-(2-methoxyethylamino)-7-oxoheptyl 2-Methylpropanethioate (23b). Yield 72%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 6.30 (1H, s), 4.98 (1H, m), 4.02 (1H, m), 3.45 (4H, m), 3.35 (3H, m), 2.83 (2H, t, J = 7.3 Hz), 2.72 (1H, septet, J = 6.9 Hz), 1.82 (1H, sextet, J = 7.0 Hz), 1.60−1.55 (2H, m), 1.50−1.29 (14H, m), 1.18 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.28, 172.02, 155.61, 80.02, 71.06, 58.77, 54.59, 43.12, 39.20, 32.57, 29.42, 28.43, 28.32, 25.03, 19.41; MS (FAB) m/z 495 (MH^+); Anal. ($C_{19}H_{36}N_2O_5S\cdot 1/3H_2O$) C, H, N.

(*S*)-*S*-7-Amino-6-(*tert*-butoxycarbonyl)-7-oxoheptyl 2-Methylpropanethioate (24b). Yield 36%; yellow oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 6.04 (1H, broad s), 5.36 (1H, broad s), 4.97 (1H, m), 4.10 (1H, m), 2.83 (2H, t, J = 7.6 Hz), 2.72 (1H, septet, J = 6.7 Hz), 1.84 (1H, m), 1.60−1.31 (16H, m), 1.18 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 204.37, 174.46, 155.74, 80.23, 54.03, 43.13, 32.10, 29.38, 28.33, 28.26, 25.94, 19.33; MS (FAB) m/z 347 (MH^+); Anal. ($C_{16}H_{30}N_2O_4S\cdot 1/3H_2O$) C, H, N.

(*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-oxo-7-(pyrrolidin-1-yl)heptyl 2-Methylpropanethioate (25b). Yield 74%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.32 (1H, d, J = 8.5 Hz), 4.40 (1H, m), 3.63 (1H, m), 3.53 (1H, m), 3.41 (2H, m), 2.82 (2H, t, J = 7.3 Hz), 2.72 (2H, quintet, J = 6.7 Hz), 1.97 (2H, quintet, J = 6.7 Hz), 1.87 (2H, m), 1.66 (1H, m), 1.60−1.29 (16H, m), 1.18 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 170.86, 155.58, 79.48, 51.84, 46.45, 45.94, 33.83, 33.11, 28.40, 28.10, 26.07, 24.48, 24.17; MS (FAB) m/z 401 (MH^+); MS (EI) m/z 400 (M^+); HRMS calcd for $C_{20}H_{36}N_2O_4S$, 400.240; found, 400.240.

(*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-(cycloheptylamino)-7-oxoheptyl 2-Methylpropanethioate (18b). Compound **18b** was prepared from **43** using the procedure described for **11b** (step 2) and **16b** (step 2) in 63% yield: yellow oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.98 (1H, d, J = 8.2 Hz), 4.98 (1H, s), 3.95−3.91 (2H, m), 2.83 (2H, t, J = 7.3 Hz), 2.73 (1H, septet, J = 7.0 Hz), 1.88−1.87 (2H, m), 1.81−1.78 (1H, m), 1.61−1.36 (26H, m), 1.18 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.31, 171.16, 77.23, 60.40, 50.37, 43.11, 32.27, 29.40, 28.32, 28.30, 27.99, 25.06, 24.03, 21.06, 19.42, 14.21; Anal. ($C_{23}H_{42}N_2O_4S\cdot 1/2H_2O$) C, H, N.

(*S*)-*S*-6-Amino-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate Hydrochloride (26b·HCl). To a solution of **16b** (962 mg, 2.32 mmol) in AcOEt (12 mL) was added 4 N HCl/AcOEt (6 mL), and the mixture was stirred at room temperature for 2 h. Then, the solvent was removed in vacuo to give 813 mg (100%) of **26b**·HCl as a colorless oil: ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm) 8.59 (1H, d, J = 7.3 Hz), 8.19 (3H, broad s), 4.03 (1H, sextet, J = 6.7 Hz), 3.67 (1H, m), 2.81 (2H, t, J = 7.3 Hz), 2.73 (1H, septet, J = 6.7 Hz), 1.85−1.76 (2H, m), 1.69−1.58 (4H, m), 1.52−1.20 (10H, m), 1.10 (6H, t, J = 7.0 Hz); ^{13}C NMR (DMSO-d_6, 500 MHz, δ, ppm) 203.17, 167.70, 52.14, 50.55, 42.40, 32.32, 31.83, 30.87, 28.82, 27.61, 27.59, 23.60, 23.38, 23.33, 19.09; MS (FAB) m/z 315 (MH^+ − HCl); Anal. ($C_{16}H_{30}N_2O_2S\cdot HCl\cdot H_2O$) C, H, N.

(*S*)-*S*-6-Benzamido-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate (27b). To a suspension of **26b**·HCl (250 mg, 0.712 mmol) and Et_3N (1 mL, 13.6 mmol) in CH_2Cl_2 (2 mL) was added a solution of benzoyl chloride (248 μL mg, 2.13 mmol) in CH_2Cl_2 (3 mL) dropwise. The reaction mixture was poured into water and was extracted with AOEt. The AcOEt layer was separated, washed with 10% aqueous citric acid and brine, and dried over Na_2SO_4. Filtration, evaporation of the solvent in vacuo, and purification by flash column chromatography (AcOEt/*n*-hexane = 1/4 to 1/2) gave a crude solid. The solid was recrystallized from AcOEt/*n*-hexane and collected by filtration to give 208 mg (70%) of **27b** as colorless crystals: mp 116−117 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 7.78 (2H, m), 7.51 (1H, t, J = 7.3 Hz), 7.43 (2H, t, J = 7.6 Hz), 6.89 (1H, d, J = 7.9 Hz), 6.24 (1H, d, J = 7.6 Hz), 4.57 (1H, q, J = 7.3 Hz), 4.19 (1H, sextet, J = 7.0 Hz), 2.83 (2H, m), 2.72 (1H, septet, J = 6.7 Hz), 2.02−1.90 (3H, m), 1.80−1.65 (7H, m), 1.45−1.35 (6H, m), 1.18 (6H, dd, J = 6.9, 1.2 Hz); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 204.36, 170.98, 167.24, 133.95, 131.73, 128.57, 127.06, 53.50, 51.34, 43.09, 33.10, 32.94, 32.57, 29.35, 28.35, 28.20, 24.85, 23.75, 23.72, 19.40; MS (EI) m/z 418 (M^+); HRMS calcd for $C_{23}H_{34}N_2O_3S$, 418.229; found, 418.229; Anal. ($C_{23}H_{34}N_2O_3S$) C, H, N.

Compounds **28b** and **29b** were prepared from **26b**·HCl and an appropriate acid chloride using the procedure described for **27b**.

(*S*)-*S*-6-(4-Chlorobenzamido)-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate (28b). Yield 95%; mp 119−121 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 7.74 (2H, d, J = 8.5 Hz), 7.40 (2H, d, J = 8.5 Hz), 6.89 (1H, d, J = 7.9 Hz), 6.04 (1H, d, J = 7.6 Hz), 4.51 (1H, q, J = 7.0 Hz), 4.20 (1H, sextet, J = 7.0 Hz), 2.82 (2H, m), 2.72 (1H, septet, J = 7.0 Hz), 2.04−1.87 (3H, m), 1.80−1.53 (9H, m), 1.40 (6H, m), 1.18 (6H, dd, J = 7.0, 1.5 Hz); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 204.48, 170.88, 166.13,

138.03, 132.32, 128.85, 128.55, 53.37, 51.44, 43.13, 33.13, 32.97, 32.64, 29.64, 28.25, 28.13, 24.76, 23.75, 23.72, 19.43, 19.41; Anal. ($C_{23}H_{33}ClN_2O_3S$) C, H, N.

(*S*)-*S*-7-(Cyclopentylamino)-6-(furan-2-carboxamido)-7-oxoheptyl 2-Methylpropanethioate (29b). Yield 37%; mp 88−89 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 7.46 (1H, d, J = 0.9 Hz), 7.11 (1H, d, J = 3.6 Hz), 6.88 (1H, d, J = 7.9 Hz), 6.50 (1H, dd, J = 3.5, 1.8 Hz), 6.03 (1H, d, J = 7.0 Hz), 4.46 (1H, q, J = 7.6 Hz), 4.19 (1H, sextet, J = 6.7 Hz), 2.84 (2H, td, J = 7.5, 2.1 Hz), 2.72 (1H, septet, J = 7.0 Hz), 2.02−1.87 (3H, m), 1.74−1.53 (7H, m), 1.45−1.34 (6H, m), 1.18 (6H, dd, J = 7.0, 0.9 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.39, 170.66, 158.20, 147.48, 144.28, 114.66, 112.15, 52.83, 51.39, 43.13, 33.13, 32.98, 32.43, 29.37, 28.35, 28.26, 24.93, 23.76, 23.73, 19.42; MS (EI) *m/z* 408 (M^+); HRMS calcd for $C_{21}H_{32}N_2O_4S$, 408.208; found, 408.209; Anal. ($C_{21}H_{32}N_2O_4S$) C, H, N.

Compounds **30b** and **32b**−**34b** were prepared from **26b**·HCl and an appropriate carboxylic acid using the procedure described for **16b** (step 1).

(*S*)-*S*-7-(Cyclopentylamino)-6-(2-hydroxyacetamido)-7-oxoheptyl 2-Methylpropanethioate (30b). Yield 28%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 6.97 (1H, d, J = 8.2 Hz), 5.99 (1H, d, J = 7.3 Hz), 4.34 (1H, m), 4.21−4.13 (3H, m), 2.91 (1H, broad s), 2.83 (2H, m), 2.74 (1H, septet, J = 7.0 Hz), 1.98 (2H, m), 1.85 (1H, m), 1.71−1.53 (7H, m), 1.44−1.31 (6H, m), 1.18 (6H, dd, J = 7.0, 1.4 Hz); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 204.91, 171.85, 170.86, 62.21, 52.78, 51.38, 43.14, 33.08, 32.92, 32.25, 29.28, 29.24, 29.06, 28.04, 24.73, 23.74, 19.44, 19.42; MS (EI) *m/z* 372 (M^+); HRMS calcd for $C_{18}H_{32}N_2O_4S$, 372.208; found, 372.208.

(*S*)-*S*-6-(Cyclohexanecarboxamido)-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate (32b). Yield 62%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 6.34 (2H, m), 4.29 (1H, q, J = 6.7 Hz), 4.16 (1H, sextet, J = 7.0 Hz), 2.82 (2H, m), 2.73 (1H, septet, J = 7.0 Hz), 2.10 (1H, tt, J = 7.0, 3.3 Hz), 1.94 (2H, m), 1.90−1.75 (5H, m), 1.70−1.56 (9H, m), 1.49−1.22 (10H, m), 1.18 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.39, 176.22, 171.14, 52.75, 51.22, 45.42, 43.13, 33.07, 33.01, 32.03, 29.70, 29.35, 28.33, 28.22, 25.72, 25.68, 25.64, 24.87, 23.74, 19.42; MS (EI) *m/z* 424 (M^+); HRMS calcd for $C_{23}H_{40}N_2O_3S$, 424.276; found, 424.275.

(*S*)-*S*-7-(Cyclopentylamino)-7-oxo-6-pivalamidoheptyl 2-Methylpropanethioate (33b). Yield 62%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 6.21 (1H, d, J = 7.6 Hz), 6.08 (1H, d, J = 7.3 Hz), 4.29 (1H, q, J = 6.7 Hz), 4.17 (1H, sextet, J = 7.0 Hz), 2.82 (2H, m), 2.72 (1H, septet, J = 7.0 Hz), 1.96 (2H, m), 1.83 (1H, m), 1.70−1.53 (7H, m), 1.42−1.29 (6H, m), 1.20 (9H, m), 1.18 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.34, 178.62, 171.17, 52.89, 51.22, 43.13, 38.75, 33.07, 31.98, 29.37, 28.36, 28.23, 27.48, 24.90, 23.74, 23.69, 19.41; MS (EI) *m/z* 398 (M^+); HRMS calcd for $C_{21}H_{38}N_2O_3S$, 398.260; found, 398.261.

(*S*)-*S*-7-(Cyclopentylamino)-6-(3,3-dimethylbutanamido)-7-oxoheptyl 2-Methylpropanethioate (34b). Yield 59%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 6.21 (1H, d, J = 7.3 Hz), 6.05 (1H, d, J = 8.2 Hz), 4.30 (1H, q, J = 6.7 Hz), 4.16 (1H, sextet, J = 6.9 Hz), 2.81 (2H, m), 2.73 (1H, septet, J = 6.7 Hz), 2.07 (2H, m), 1.96 (2H, m), 1.80 (1H, m), 1.70−1.52 (7H, m), 1.42−1.30 (6H, m), 1.18 (6H, d, J = 6.7 Hz), 1.02 (9H, s); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.41, 171.89, 171.10, 53.02, 52.94, 51.23, 50.46, 43.13, 33.09, 32.97, 32.34, 31.90, 30.99, 29.85, 29.33, 28.31, 28.19, 23.75, 23.73, 19.43, 19.42; MS (EI) *m/z* 412 (M^+); HRMS calcd for $C_{22}H_{40}N_2O_3S$, 412.276; found, 412.276.

(*S*)-*S*-6-Acetamido-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate (31b). To a solution of **26b**·HCl (102 mg, 0.291 mmol) and a catalytic amount of DMAP in CH_2Cl_2 (3 mL) were added acetic acid anhydride (55 μL, 0.582 mmol) and Et_3N (200 μL, 2.72 mmol), and the mixture was stirred at room temperature for 4 h. The reaction mixture was poured into water and was extracted with AcOEt. The AcOEt layer was separated, washed with brine, and dried over Na_2SO_4. Filtration, evaporation of the solvent in vacuo, and purification by silica gel flash column chromatography (AcOEt/*n*-hexane = 3/1 to AcOEt only) gave a crude solid. The solid was recrystallized from AcOEt/*n*-hexane and collected by filtration to give 54 mg (52%) of **31b** as colorless crystals: mp 130−131 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 6.10 (1H, d, J = 8.2 Hz), 5.94 (1H, d, J = 7.6 Hz), 4.28 (1H, q, J = 7.3 Hz), 4.17 (1H, sextet, J = 7.3 Hz), 2.82 (2H, m), 2.73 (1H, septet, J = 6.7 Hz), 2.02−1.93 (5H, m), 1.80 (1H, m), 1.74−1.49 (7H, m), 1.46−1.29 (6H, m), 1.18 (6H, dd, J = 7.0, 0.9 Hz); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 204.46, 170.02, 170.02, 53.17, 51.30, 43.14, 33.12, 32.97, 32.34, 29.32, 28.26, 28.16, 24.77, 23.73, 23.71, 23.26, 19.43; MS (EI) *m/z* 356 (M^+); HRMS calcd for $C_{18}H_{32}N_2O_3S$, 356.213; found, 356.213; Anal. ($C_{18}H_{32}N_2O_3S$) C, H, N.

(*S*)-*S*-6-(3-*tert*-Butylureido)-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate (35b). To a solution of **26b**·HCl (99 mg, 0.282 mmol) and Et_3N (400 μL, 5.44 mmol) in CH_2Cl_2 (4 mL) was added *tert*-butyl isocyanate (132 μL, 1.14 mmol), and the mixture was stirred at room temperature for 6 h. The reaction mixture was poured into water and was extracted with AcOEt. The AcOEt layer was separated, washed with brine, and dried over Na_2SO_4. Filtration, evaporation of the solvent in vacuo, and purification by silica gel flash column chromatography (AcOEt/*n*-hexane = 1/1) gave 89 mg (76%) of **35b** as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 6.27 (1H, d, J = 7.9 Hz), 4.81 (1H, d, J = 7.9 Hz), 4.48 (1H, s), 4.16 (1H, sextet, J = 6.7 Hz), 4.07 (1H, q, J = 5.8 Hz), 2.92−2.70 (3H, m), 1.94 (2H, m), 1.76 (1H, m), 1.72−1.55 (7H, m), 1.43−1.25 (15H, m), 1.18 (6H, dd, J = 6.7, 0.9 Hz); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 204.82, 172.39, 157.10, 53.85, 51.12, 50.57, 43.14, 33.08, 33.00, 32.07, 29.43, 29.22, 28.21, 28.12, 24.78, 23.73, 23.69, 19.46, 19.44; MS (EI) *m/z* 413 (M^+); HRMS calcd for $C_{21}H_{39}N_3O_3S$, 413.271; found, 413.273.

(*S*)-*S*-6-(3-*tert*-Butylthioureido)-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate (36b). Compound **36b** was prepared from **26b**·HCl and *tert*-thioisocyanate using the procedure described for **35b** in 24% yield: colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 6.53 (1H, d, J = 7.0 Hz), 6.09 (1H, m), 5.97 (1H, d, J = 7.0 Hz), 4.85 (1H, m), 4.19 (1H, sextet, J = 7.0 Hz), 2.82 (2H, m), 2.73 (1H, septet, J = 7.0 Hz), 1.98 (3H, m), 1.78−1.52 (7H, m), 1.48−1.29 (15H, m), 1.19 (6H, dd, J = 7.0, 1.5 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.47, 179.78, 170.93, 58.73, 52.94, 51.53, 43.12, 33.10, 32.94, 32.64, 29.46, 29.34, 28.33, 28.15, 24.21, 23.69, 23.64, 19.44, 19.42; MS (EI) *m/z* 429 (M^+); HRMS calcd for $C_{21}H_{39}N_3O_2S_2$, 429.248; found, 429.248.

(*S*)-*S*-7-(Cyclopentylamino)-6-(methoxycarbonyl)-7-oxoheptyl 2-Methylpropanethioate (37b). To a solution of **26b**·HCl (177 mg, 0.504 mmol) in CH_2Cl_2 (3 mL) were added methyl chloroformate (39 μL, 0.505 mmol) and Et_3N (500 μL, 6.80 mmol), and the mixture was stirred at room temperature for 15 min. The reaction mixture was poured into water and was extracted with AcOEt. The AcOEt layer was separated, washed with brine, and dried over Na_2SO_4. Filtration, evaporation of the solvent in vacuo, and purification by silica gel flash column chromatography (AcOEt/*n*-hexane = 1/2 to AcOEt only) gave a crude solid. The solid was recrystallized from AcOEt/*n*-hexane and collected by filtration to give 138 mg (74%) of **37b** as colorless crystals: mp 88−89 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.90 (1H, d, J = 6.7 Hz), 5.24 (1H, broad s), 4.18 (1H, sextet, J = 7.0 Hz), 4.02 (1H, m), 3.68 (3H, s), 2.82 (2H, td, J = 7.6, 2.4 Hz), 2.73 (1H, septet, J = 7.0 Hz), 1.89 (2H, m), 1.81 (1H, m), 1.73−1.53 (7H, m), 1.42−1.31 (6H, m), 1.18 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.38, 171.08, 156.86, 54.99, 52.38, 51.29, 43.13, 33.12, 33.01, 32.61, 29.71, 29.36, 28.30, 28.23, 24.82, 23.74, 19.42; MS (EI) *m/z* 372 (M^+); HRMS calcd for $C_{18}H_{32}N_2O_4S$, 372.208; found, 372.208; Anal. ($C_{18}H_{32}N_2O_4S$) C, H, N.

Compounds **39b**−**41b** were prepared from **26b**·HCl and an appropriate chloroformate using the procedure described for **37b**.

(*S*)-*S*-7-(Cyclopentylamino)-7-oxo-6-(phenoxycarbonyl)heptyl 2-Methylpropanethioate (39b). Yield 17%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 7.36 (2H, t, J = 7.9 Hz), 7.20 (1H, t, J = 7.6 Hz), 7.12 (2H, d, J = 7.9 Hz), 5.85 (1H, d, J = 7.0 Hz), 5.66 (1H, d, J = 8.2 Hz), 4.22 (1H, sextet, J = 7.3 Hz), 4.09

(1H, q, J = 7.3 Hz), 2.83 (2H, td, J = 7.0, 2.4 Hz), 2.73 (1H, septet, J = 7.0 Hz), 1.99 (2H, m), 1.86 (1H, m), 1.72−1.53 (7H, m), 1.46−1.36 (6H, m), 1.18 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.42, 170.69, 154.82, 150.92, 129.33, 125.47, 121.54, 55.06, 51.42, 43.14, 33.15, 33.02, 32.71, 29.37, 28.26, 28.21, 24.76, 23.74, 19.42; MS (FAB) *m/z* 435 (MH^+); Anal. ($C_{23}H_{34}N_2O_4S$) C, H, N.

(*S*)-*S*-6-(Benzyloxycarbonyl)-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate (40b). Yield 24%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 7.35 (5H, m), 5.89 (1H, m), 5.31 (1H, m), 5.10 (2H, s), 4.17 (1H, sextet, J = 6.7 Hz), 4.04 (1H, m), 2.82 (2H, td, J = 7.2, 2.1 Hz), 2.72 (1H, septet, J = 7.0 Hz), 1.94 (2H, s), 1.81 (1H, m), 1.70−1.51 (7H, m), 1.42−1.23 (6H, m), 1.17 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 204.39, 170.98, 156.19, 136.27, 128.56, 128.22, 128.06, 67.04, 55.00, 51.28, 43.12, 33.09, 32.99, 32.52, 29.36, 28.30, 28.23, 24.84, 23.71, 19.42; MS (EI) *m/z* 448 (M^+); HRMS calcd for $C_{24}H_{36}N_2O_4S$, 448.240; found, 448.239; Anal. ($C_{24}H_{36}N_2O_4S$) C, H, N.

(*S*)-*S*-6-[{(9*H*-Fluoren-9-yl)methoxy}carbonyl]-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate (41b). Yield 11%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 7.76 (2H, d, J = 7.6 Hz), 7.58 (2H, d, J = 7.6 Hz), 7.40 (2H, t, J = 7.6 Hz), 7.31 (2H, td, J = 7.7, 0.9 Hz), 5.82 (1H, m), 5.34 (1H, d, J = 7.7 Hz), 4.40 (2H, m), 4.23−4.15 (1H, m), 4.02 (1H, m), 2.84 (2H, m), 2.73 (1H, septet, J = 6.7 Hz), 1.97 (2H, m), 1.82 (1H, m), 1.68−1.52 (7H, m), 1.45−1.25 (6H, m), 1.18 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.38, 170.95, 156.19, 143.80, 141.35, 127.75, 127.10, 125.04, 120.02, 120.00, 67.01, 54.99, 51.32, 47.21, 43.14, 33.12, 33.02, 32.59, 29.37, 28.28, 28.23, 24.80, 23.74, 23.72, 19.42; MS (FAB) *m/z* 537 (MH^+); Anal. ($C_{31}H_{40}N_2O_4S\cdot 1/3H_2O$) C, H, N.

(*S*)-*S*-6-(Cyclohexyloxycarbonyl)-7-(cyclopentylamino)-7-oxoheptyl 2-Methylpropanethioate (38b). To a solution of triphosgene (654 mg, 2.20 mmol) in dry Et_2O (4 mL) was added dropwise a mixture of cyclohexanol (682 μL, 6.46 mmol) and pyridine (626 μL, 7.74 mmol) at −78 °C. The mixture was stirred for 1 h at −78 °C and then 1.5 h at room temperature. The reaction mixture was poured into 1 N aqueous HCl and was extracted with AcOEt, washed with brine, and dried over Na_2SO_4. Filtration and evaporation in vacuo gave a colorless oil. To a solution of **26b**·HCl (94 mg, 0.268 mmol) and Et_3N (300 μL, 4.08 mmol) in CH_2Cl_2 (4 mL) was added the colorless oil, and the mixture was stirred at room temperature for 13 h. The reaction mixture was poured into water and was extracted with AcOEt. The AcOEt layer was separated, washed with brine, and dried over Na_2SO_4. Filtration, evaporation of the solvent in vacuo, and purification by silica gel flash column chromatography (AcOEt/*n*-hexane = 1/3) gave 30 mg (26%) of **38b** as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.91 (1H, d, J = 7.3 Hz), 5.08 (1H, m), 4.61 (1H, m), 4.18 (1H, sextet, J = 7.6 Hz), 3.98 (1H, m), 2.82 (2H, td, J = 7.0, 1.8 Hz), 2.73 (1H, septet, J = 7.0 Hz), 1.98 (2H, m), 1.84 (3H, m), 1.75−1.50 (11H, m), 1.37 (10H, m), 1.18 (6H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 204.38, 171.20, 63.46, 54.80, 51.20, 43.11, 33.11, 33.03, 32.41, 31.92, 29.37, 28.34, 28.25, 25.36, 24.92, 23.72, 23.70, 23.69, 19.42; MS (FAB) *m/z* 441 (MH^+); MS (EI) *m/z* 440 (M^+); HRMS calcd for $C_{23}H_{40}N_2O_4S$, 440.271; found, 440.270.

(*S*)-*tert*-Butyl 1-(Cyclopentylamino)-7-mercapto-1-oxoheptan-2-ylcarbamate (16a). Step 1: Preparation of (*S*)-*S*-6-(*tert*-Butoxycarbonyl)-7-(cyclopentylamino)-7-oxoheptyl Ethanethioate (16d). A solution of **16c** (410 mg, 1.05 mmol) obtained above and KSAc (183 mg, 1.60 mmol) in EtOH (3 mL) was stirred overnight at room temperature. The reaction mixture was evaporated in vacuo. The residue was subjected to silica gel flash column chromatography (AcOEt/*n*-hexane = 1/4) to give 303 mg (75%) of **16d** as a yellow solid: ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.97 (1H, d, J = 7.3 Hz), 4.97 (1H, s), 4.18 (1H, m), 3.94 (1H, m), 2.85 (2H, t, J = 7.3 Hz), 2.32 (3H, s), 1.97−1.96 (2H, m), 1.81−1.80 (1H, m), 1.67−1.55 (8H, m), 1.44 (9H, s), 1.39−1.36 (5H, m).

Step 2: Preparation of (*S*)-*tert*-Butyl 1-(Cyclopentylamino)-7-mercapto-1-oxoheptan-2-ylcarbamate (16a). To a solution of **16d** (300 mg, 0.778 mmol) obtained above in EtOH (5 mL) was added 2 N aqueous NaOH (2 mL, 4.00 mmol), and the solution was stirred at room temperature for 10 min. The reaction mixture was poured into water and was extracted with AcOEt. The AcOEt layer was separated, washed with water and brine, and dried over Na_2SO_4. Filtration, concentration in vacuo, and purification by silica gel flash column chromatography (AcOEt/*n*-hexane = 1/4) gave 200 mg (74%) of **16a** as a colorless solid. The solid (106 mg) was recrystallized from AcOEt/*n*-hexane to give 60 mg of **16a** as colorless crystals: mp 108−110 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.93 (1H, d, J = 7.0 Hz), 4.95 (1H, s), 4.19−4.16 (1H, m), 3.94 (1H, s), 2.52 (2H, q, J = 7.3 Hz), 1.98−1.96 (2H, m), 1.85−1.80 (1H, m), 1.67−1.52 (7H, m), 1.44 (9H, s), 1.42−1.31 (7H, m); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 171.89, 77.23, 48.04, 36.96, 33.79, 33.31, 28.65, 28.04, 25.68, 25.56, 24.88, 24.56; Anal. ($C_{17}H_{32}N_2O_3S$) C, H, N.

Compounds **13a**, **15a**, and **17a**−**20a** were prepared from the corresponding bromide using the procedure described for **16a**.

(*S*)-*tert*-Butyl 1-(Biphenyl-3-ylamino)-7-mercapto-1-oxoheptan-2-ylcarbamate (13a). Yield 34%; colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 8.23 (1H, broad s), 7.79 (1H, s), 7.58 (2H, d, J = 7.3 Hz), 7.48 (1H, d, J = 7.9 Hz), 7.44−7.33 (5H, m), 4.97 (1H, s), 4.18 (1H, s), 2.53 (2H, q, J = 7.3 Hz), 2.00−1.96 (1H, m), 1.71−1.59 (3H, m), 1.47−1.43 (13H, m), 1.33 (1H, t, J = 7.8 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 170.39, 156.27, 142.14, 140.64, 138.12, 129.35, 128.73, 127.48, 127.18, 123.18, 118.65, 118.61, 80.71, 55.27, 33.70, 30.93, 28.33, 27.99, 25.25, 24.46; MS (EI) *m/z* 428 (M^+); HRMS calcd for $C_{24}H_{34}N_2O_3S$, 428.213; found, 428.213.

(*S*)-*tert*-Butyl 7-Mercapto-1-oxo-1-(quinolin-3-ylamino)heptan-2-ylcarbamate (15a). Yield 39%; yellow oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 8.79 (1H, broad s), 8.74 (2H, s), 8.03 (1H, d, J = 8.5 Hz), 7.78 (1H, d, J = 7.9 Hz), 7.62 (1H, t, J = 7.0 Hz), 7.52 (1H, t, J = 7.8 Hz), 5.00 (1H, d, J = 6.7 Hz), 4.26 (1H, m), 2.53 (2H, q, J = 7.6 Hz), 2.00 (1H, m), 1.73−1.63 (3H, m), 1.50−1.36 (13H, m), 1.34 (1H, t, J = 7.9 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 171.17, 145.18, 143.89, 131.37, 128.97, 128.20, 128.07, 127.68, 127.14, 123.66, 81.13, 77.23, 33.65, 30.94, 28.35, 27.97, 25.35, 24.43; MS (EI) *m/z* 403 (M^+); HRMS calcd for $C_{21}H_{29}N_3O_3S$, 403.193; found, 403.194.

(*S*)-*tert*-Butyl 1-(cyclohexylamino)-7-mercapto-1-oxoheptan-2-ylcarbamate (17a). Yield 72%; mp 125−127 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.85 (1H, d, J = 7.9 Hz), 4.96 (1H, s), 3.95 (1H, s), 3.75 (1H, s), 2.51 (2H, q, J = 7.4 Hz), 1.90−1.78 (3H, m), 1.72−1.68 (2H, m), 1.64−1.56 (4H, m), 1.44 (9H, s), 1.43−1.31 (7H, m), 1.19−1.13 (3H, m); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 170.84, 77.23, 48.13, 33.74, 33.08, 32.93, 30.94, 28.33, 27.99, 25.49, 25.07, 24.71, 24.46; MS (EI) *m/z* 358 (M^+); HRMS calcd for $C_{18}H_{34}O_3N_2S$, 358.229; found, 358.229; Anal. ($C_{18}H_{34}N_2O_3S$) C, H, N.

(*S*)-*tert*-Butyl 1-(Cycloheptylamino)-7-mercapto-1-oxoheptan-2-ylcarbamate (18a). Yield 62%; mp 76−78 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.92 (1H, d, J = 7.6 Hz), 4.96 (1H, s), 3.95−3.91 (2H, m), 2.52 (2H, q, J = 7.3 Hz), 1.88−1.79 (3H, m), 1.62−1.58 (7H, m), 1.54−1.33 (20H, m); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 170.55, 77.21, 54.60, 50.38, 35.06, 34.96, 33.74, 32.34, 28.33, 27.99, 25.08, 24.47, 24.02; Anal. ($C_{18}H_{34}N_2O_3S$) C, H, N.

(*S*)-*tert*-Butyl 1-(*tert*-Butylamino)-7-mercapto-1-oxoheptan-2-ylcarbamate (19a). Yield 58%; mp 90−91 °C; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.78 (1H, s), 4.97 (1H, m), 3.89 (1H, m), 2.52 (2H, q, J = 7.6 Hz), 1.79 (1H, sextet, J = 8.2 Hz), 1.66−1.50 (3H, m), 1.49−1.38 (11H, m), 1.36−1.30 (12H, m); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 171.05, 155.69, 79.90, 54.91, 51.26, 33.68, 32.36, 28.66, 28.26, 27.94, 24.96, 24.39; MS (FAB) *m/z* 333 (MH^+); Anal. ($C_{16}H_{32}N_2O_3S$) C, H, N.

(*S*)-*tert*-Butyl 1-(Adamant-1-ylamino)-7-mercapto-1-oxoheptan-2-ylcarbamate (20a). Yield 41%; colorless oil; ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.60 (1H, s), 4.97 (1H, m), 3.88 (1H, m), 2.81 (2H, q, J = 7.3 Hz), 2.07 (3H, s), 1.98 (6H, d, J = 2.7 Hz), 1.78 (1H, sextet, J = 7.8 Hz), 1.67 (3H, s), 1.65−1.30 (17H, m); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 170.87, 155.71, 79.93,

54.98, 41.60, 36.32, 33.76, 32.60, 30.92, 29.55, 29.43, 29.22, 28.35, 28.02, 25.00, 24.47; MS (FAB) *m/z* 411 (MH^+); Anal. ($C_{22}H_{38}N_2O_3S \cdot 1/4H_2O$) C, H, N.

(*S*)-2-Amino-*N*-cyclopentyl-7-mercaptoheptanamide Hydrochloride (26a·HCl). Compound **26a**·HCl was prepared from **16a** using the procedure described for **26b**·HCl in 94% yield: colorless oil; ^1H NMR (DMSO-d_6, 500 MHz, δ, ppm) 8.43 (1H, d, J = 7.3 Hz), 8.13 (3H, broad s), 4.03 (1H, sextet, J = 6.7 Hz), 3.65 (1H, t, J = 6.7 Hz), 2.46 (2H, q, J = 7.3 Hz), 2.28 (1H, t, J = 7.6 Hz), 1.82 (2H, m), 1.66 (4H, m), 1.58−1.23 (10H, m); ^{13}C NMR (DMSO-d_6, 500 MHz, δ, ppm) 167.65, 52.08, 50.46, 32.89, 32.24, 31.75, 30.85, 27.08, 23.50, 23.41, 23.30, 23.24; MS (FAB) *m/z* 245 (MH^+ − HCl); Anal. ($C_{12}H_{24}N_2OS \cdot HCl \cdot 3/4H_2O$) C, H, N.

Compounds **27a** and **28a** were prepared from **16d** using the procedure described for **26b**·HCl, **27b**, and **16a** (step 2).

(*S*)-*N*-(1-(Cyclopentylamino)-7-mercapto-1-oxoheptan-2-yl)-benzamide (27a). Yield 69%; mp 171−172 °C; ^1H NMR ($CDCl_3$, 500 MHz, δ, ppm) 7.79 (2H, m), 7.52 (1H, t, J = 7.3 Hz), 7.44 (2H, t, J = 7.6 Hz), 6.79 (1H, d, J = 8.2 Hz), 6.01 (1H, d, J = 7.0 Hz), 4.54 (1H, q, J = 7.0 Hz), 4.19 (1H, sextet, J = 6.7 Hz), 2.51 (2H, q, J = 7.6 Hz), 1.98 (3H, m), 1.77−1.56 (7H, m), 1.48−1.35 (6H, m), 1.32 (1H, t, J = 7.6 Hz); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 170.94, 167.23, 133.94, 131.81, 128.65, 127.04, 53.50, 51.41, 33.71, 33.17, 33.00, 32.76, 28.04, 25.01, 24.46, 23.75, 23.72; MS (EI) *m/z* 348 (M^+); HRMS calcd for $C_{19}H_{28}N_2O_2S$, 348.187; found, 348.187; Anal. ($C_{19}H_{28}N_2O_2S$) C, H, N.

(*S*)-4-Chloro-*N*-[1-(cyclopentylamino)-7-mercapto-1-oxoheptan-2-yl]benzamide (28a). Yield 54%; mp 178−180 °C; ^1H NMR ($CDCl_3$, 500 MHz, δ, ppm) 7.73 (2H, d, J = 8.5 Hz), 7.41 (2H, d, J = 8.5 Hz), 6.84 (1H, d, J = 7.6 Hz), 5.95 (1H, d, J = 7.3 Hz), 4.53 (1H, q, J = 7.0 Hz), 4.20 (1H, sextet, J = 6.7 Hz), 2.51 (2H, q J = 7.0 Hz), 1.96 (3H, m), 1.74−1.54 (7H, m), 1.41 (6H, m), 1.32 (1H, t, J = 7.9 Hz); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 170.84, 166.10, 138.08, 132.29, 128.88, 128.51, 53.55, 51.44, 33.66, 33.17, 32.96, 32.91, 28.01, 24.94, 24.45, 23.74, 23.71; Anal. ($C_{19}H_{27}ClN_2O_2S$) C, H, N.

Compounds **30a** and **33a** were prepared from **16d** using the procedure described for **26b**·HCl, **16b** (step 1), and **16a** (step 2).

(*S*)-*N*-Cyclopentyl-2-(2-hydroxyacetamido)-7-mercaptoheptanamide (30a). Yield 55%; mp 85−86 °C; ^1H NMR ($CDCl_3$, 500 MHz, δ, ppm) 6.93 (1H, d, J = 8.5 Hz), 5.90 (1H, d, J = 6.7 Hz), 4.34 (1H, q, J = 7.9 Hz), 4.21−4.10 (3H, m), 2.69 (1H, t, J = 5.7 Hz), 2.51 (2H, q, J = 7.3 Hz), 1.97 (2H, m), 1.83 (1H, m), 1.72−1.53 (7H, m), 1.46−1.30 (7H, m); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 171.82, 170.99, 62.16, 52.97, 51.40, 33.65, 33.10, 32.89, 32.48, 27.93, 25.02, 24.45, 23.73; MS (EI) *m/z* 302 (M^+); HRMS calcd for $C_{14}H_{26}N_2O_3S$, 302.166; found, 302.167; Anal. ($C_{14}H_{26}N_2O_3S$) C, H, N.

(*S*)-*N*-Cyclopentyl-7-mercapto-2-pivalamidoheptanamide (33a). Yield 60%; mp 134−139 °C; ^1H NMR ($CDCl_3$, 500 MHz, δ, ppm) 6.20 (1H, d, J = 7.9 Hz), 6.08 (1H, d, J = 7.3 Hz), 4.30 (1H, q, J = 7.3 Hz), 4.17 (1H, sextet, J = 6.7 Hz), 2.51 (2H, q, J = 7.3 Hz), 1.96 (2H, m), 1.83 (1H, m), 1.71−1.55 (8H, m), 1.45−1.25 (6H, m), 1.20 (9H, s); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 178.60, 171.13, 52.83, 51.22, 38.75, 33.70, 33.08, 33.03, 32.13, 28.01, 27.47, 24.97, 24.45, 23.73, 23.68; MS (EI) *m/z* 328 (M^+); HRMS calcd for $C_{17}H_{32}N_2O_2S$, 328.218; found, 328.219; Anal. ($C_{17}H_{32}N_2O_2S$) C, H, N.

Compounds **35a** and **36a** were prepared from **16d** using the procedure described for **26b**·HCl, **35b**, and **16a** (step 2).

(*S*)-1-*tert*-Butyl-3-[1-(cyclopentylamino)-7-mercapto-1-oxoheptan-2-yl]urea (35a). Yield 71%; mp 188−191 °C; ^1H NMR ($CDCl_3$, 500 MHz, δ, ppm) 6.19 (1H, d, J = 6.7 Hz), 4.92 (1H, d, J = 8.5 Hz), 4.49 (1H, s), 4.16 (1H, sextet, J = 7.3 Hz), 4.09 (1H, q, J = 7.6 Hz), 2.52 (2H, q, J = 7.6 Hz), 1.94 (2H, m), 1.76 (1H, m), 1.72−1.50 (7H, m), 1.46−1.22 (16H, m); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 172.45, 157.02, 53.83, 51.16, 50.57, 33.75, 33.10, 32.98, 32.64, 29.44, 28.07, 25.20, 24.47, 23.72, 23.67; Anal. ($C_{17}H_{33}N_3O_2S \cdot 1/6H_2O$) C, H, N.

(*S*)-1-*tert*-Butyl-3-[1-(cyclopentylamino)-7-mercapto-1-oxoheptan-2-yl]thiourea (36a). Yield 25%; mp 125−127 °C; ^1H NMR ($CDCl_3$, 500 MHz, δ, ppm) 6.48 (1H, d, J = 7.6 Hz), 6.06 (1H, s), 5.85 (1H, d, J = 7.6 Hz), 4.87 (1H, m), 4.19 (1H, sextet, J = 7.0 Hz), 2.50 (2H, q, J = 7.0 Hz), 2.02 (3H, m), 1.76−1.58 (7H, m), 1.48−1.36 (16H, m); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 179.92, 171.32, 58.04, 53.03, 51.49, 33.71, 33.10, 32.87, 32.74, 29.42, 28.08, 24.50, 24.45, 23.70, 23.66; MS (EI) *m/z* 359 (M^+); HRMS calcd for $C_{17}H_{33}N_3O_2S$, 359.207; found, 359.205; Anal. ($C_{17}H_{33}N_3O_2S$) C, H, N.

Compounds **37a** and **40a** were prepared from **16d** using the procedure described for **26b**·HCl, **37b**, and **16a** (step 2).

(*S*)-Methyl 1-(Cyclopentylamino)-7-mercapto-1-oxoheptan-2-ylcarbamate (37a). Yield 89%; mp 81−82 °C; ^1H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.79 (1H, d, J = 7.0 Hz), 5.18 (1H, m), 4.18 (1H, sextet, J = 6.7 Hz), 4.02 (1H, m), 3.68 (3H, s), 2.51 (2H, q, J = 7.6 Hz), 1.98 (2H, m), 1.81 (1H, m), 1.71−1.53 (7H, m), 1.46−1.30 (7H, m); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 171.02, 54.97, 52.41, 51.30, 33.68, 33.14, 33.03, 32.73, 27.93, 24.91, 24.45, 23.71; MS (EI) *m/z* 302 (M^+); HRMS calcd for $C_{14}H_{26}N_2O_3S$, 302.166; found, 302.169; Anal. ($C_{14}H_{26}N_2O_3S$) C, H, N.

(*S*)-Benzyl 1-(Cyclopentylamino)-7-mercapto-1-oxoheptan-2-ylcarbamate (40a). Yield 77%; mp 119−122 °C; ^1H NMR ($CDCl_3$, 500 MHz, δ, ppm) 7.35 (5H, m), 5.80 (1H, m), 5.27 (1H, m), 5.11 (2H, s), 4.17 (1H, q, J = 6.7 Hz), 4.03 (1H, m), 2.50 (2H, q, J = 7.3 Hz), 1.96 (2H, s), 1.84 (1H, m), 1.70−1.53 (7H, m), 1.45−1.26 (7H, m); ^{13}C NMR ($CDCl_3$, 500 MHz, δ, ppm) 170.98, 156.18, 136.22, 128.57, 128.24, 128.06, 67.04, 54.17, 51.26, 33.68, 33.11, 32.98, 32.66, 27.93, 24.92, 24.44, 23.71; MS (EI) *m/z* 378 (M^+); HRMS calcd for $C_{20}H_{30}N_2O_3S$, 378.198; found, 378.197; Anal. ($C_{20}H_{30}N_2O_3S \cdot 1/3H_2O$) C, H, N.

(*S*)-Cyclohexyl 1-(Cyclopentylamino)-7-mercapto-1-oxoheptan-2-ylcarbamate (38a). Compound **38a** was prepared from **16d** using the procedure described for **26b**·HCl, **38b**, and **16a** (step 2) in 78% yield: mp 110−111 °C; ^1H NMR ($CDCl_3$, 500 MHz, δ, ppm) 5.85 (1H, m), 5.06 (1H, m), 4.62 (1H, m), 4.18 (1H, sextet, J = 7.0 Hz), 3.99 (1H, m), 2.51 (2H, q, J = 7.0 Hz), 1.97 (2H, m), 1.85 (3H, m), 1.74−1.46 (11H, m), 1.45−1.30 (11H, m); ^{13}C NMR ($CDCl_3$, 600 MHz, δ, ppm) 171.21, 156.06, 73.63, 54.75, 51.20, 33.70, 33.12, 33.03, 32.53, 31.91, 29.70, 27.96, 25.35, 24.99, 24.46, 23.76, 23.71, 23.39; MS (FAB) *m/z* 371 (MH^+); Anal. ($C_{19}H_{34}N_2O_3S \cdot 1/4H_2O$) C, H, N.

Biology. Western Blot Analysis. Human colon cancer HCT116 cells were purchased from American Type Culture Collection (ATCC, Manassas, VA) and cultured in McCoy5A culture medium containing penicillin and streptomycin, which was supplemented with fetal bovine serum as described in the ATCC instructions. HCT-116 cells (5×10^5) were treated for 8 h with samples at the indicated concentrations in 10% FBS supplemented with McCoy's 5A medium and were then collected and extracted with SDS buffer. Protein concentrations of the lysates were determined using a Bradford protein assay kit (Bio-Rad Laboratories) with which equivalent amounts of protein from each lysate were resolved in 15% SDS-polyacrylamide gels and then transferred onto nitrocellulose membranes (Bio-Rad Laboratories). After blocking for 30 min with Tris-buffered saline (TBS) containing 3% skimmed milk, the transblotted membranes were incubated overnight at 4 °C with hyperacetylated histone H4 antibody (Upstate Biotechnology; 1:4000 dilution), acetylated α-tubulin antibody (SIGMA; 1:4000 dilution), or β-actin antibody (Abcam; 1:500 dilution) in TBS containing 3% skimmed milk. After probing with the primary antibody, the membrane was washed twice with water and then incubated with goat, antirabbit, or antimouse IgG-horseradish peroxidase conjugates (diluted 1:5000) for 2 h at room temperature and washed twice more with water. The immunoblots were visualized by enhanced chemiluminescence.

Enzyme Assays. The inhibitory activities of the test compounds against partially purified HDAC1, HDAC4, and HDAC6 were assayed according to a method reported in ref 14c.

Cell Growth Inhibition Assay. Human colon cancer HCT116 cells and ERα-positive breast cancer MCF-7 cells, which were purchased from American Type Culture Collection (ATCC, Manassas, VA), were cultured in McCoy5A and Dulbecco's Modified

Eagle's Medium (DMEM) containing penicillin and streptomycin, which was supplemented with fetal bovine serum as described in the ATCC instructions, respectively. HCT116 and MCF-7 cells were plated in 96-well plates at initial densities of 5000 (HCT116) or 1500 (MCF-7) cells/well (50 μL/well) and incubated at 37 °C. After 24 h, cells were exposed to test compounds by adding solutions (50 μL/well) of compounds at various concentrations in McCoy5A (HCT116) and DMEM (MCF-7) medium at 37 °C at 5% CO_2 for 72 h. The mixtures were then treated with 10 μL of alamarBlue, and cells were further incubated at 37 °C for 3 h. The fluorescence in each well was measured on a fluorometric plate reader, with excitation set at 530 nm and emission detection set at 590 nm, and the percentage of cell growth was calculated from the fluorescence readings.

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Supporting Information Available: Results of the elemental analysis of **11b**, **16b**, **18b**−**24b**, **26b**−**29b**, **31b**, **37b**, **39b**−**41b**, **16a**−**20a**, **26a**−**28a**, **30a**, **33a**, **35a**−**38a**, and **40a** are reported. This material is available free of charge via the Internet at http://pubs.acs.org.

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New Series of Antiprion Compounds: Pyrazolone Derivatives Have the Potent Activity of Inhibiting Protease-Resistant Prion Protein Accumulation

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Abstract: To find effective antiprion compounds, we synthesized and evaluated various pyrazolone derivatives. Seven of 19 compounds showed inhibition of PrP-res accumulation and the remarkably active compound **13** showed an IC_{50} value of 3 nM in both ScN2a and F3 cell lines. Findings from studies on physicochemical and biochemical properties suggest that the action mechanism of these compounds does not correlate with any antioxidant activities, any of hydroxyl radical scavenging activities, or any SOD-like activities.

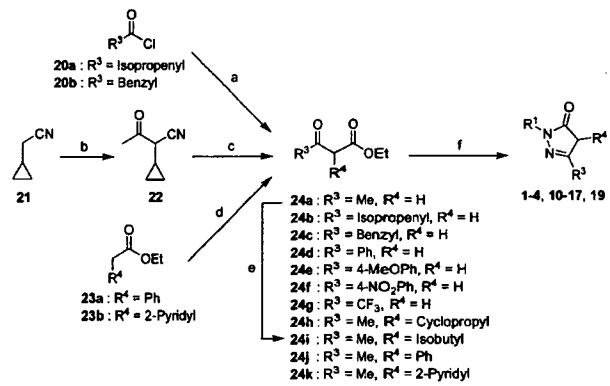
Prion diseases or transmissible spongiform encephalopathyies (TSEs[a]) are invariably fatal neurodegenerative diseases that include Creutzfeldt−Jakob disease (CJD), Gerstmann−Sträussler−Scheinker syndrome (GSS), familial fatal insomnia (FFI), and kuru in humans, scrapie in sheep and goats, chronic wasting disease (CWD) in deer and elk, and bovine spongiform encephalopathy (BSE) in cattle. These diseases are characterized by deposition of the protease-resistant isoform of prion protein (PrP^{Sc}), which is thought to be the main component responsible for the pathogenesis. PrP^{Sc} is known to be an abnormally folded β-rich conformation of cellular prion protein (PrP^{C}) and is resistant to digestion with proteinase K.[1]

Natural and constitutive prion protein, PrP^{C}, is a GPI-anchored membrane glycoprotein. The biological significance of this protein is unclear, but it is reported that the N-terminal octapeptide repeat region of PrP^{C} binds several copper ions with a femtomolar dissociation range (K_d).[2,3] PrP^{C} has copper-dependent superoxide dismutase (SOD) activity[4] and may also be involved in copper uptake into cells.[5,6] Recently, there has been increasing interest in the role of copper in prion diseases.[7,8] In 2003, it was reported that a copper chelator, D-penicillamine, delayed the onset of prion disease in infected mice and suggested that chelator-based therapy might attenuate the disease.[9] Copper has been implicated in the pathogenesis of prion disease, but numerous studies have only succeeded in demonstrating the complexity of the effects of copper on the development of prion diseases, and it remains unclear whether this ion promotes or inhibits disease progression.

Although there are no suitable therapies for this disorder, outbreaks of variant CJD and iatrogenic CJD through the use of cadaveric growth hormone or dural grafts in younger people have necessitated their development. Furthermore, screening for antiprion compounds in a cell culture model of prion disease has led to the identification of many antiprion compounds,[10] such as quinoline derivatives,[11,12] Congo red and analogues,[13,14] and 2-aminopyridine-3,5-dicarbonitrile compounds;[15] however, their activity is thought to be insufficient to develop therapeutic agents.

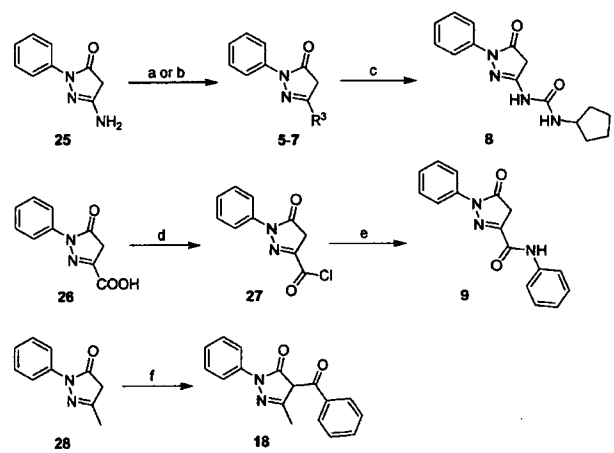
Recently, a new pyrazolone compound, edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one, also known as MCI-186), has been developed as a medical drug for brain ischemia[16,17] and has also been reported to be effective for myocardial ischemia.[18] In this

Scheme 1. Synthesis of Pyrazolone Derivatives **1**−**4**, **10**−**17**, and **19**[a]



[a] Reagants and conditions: (a) (i) malonic acid monoethyl ester potassium salt, $MgCl_2$, Et_3N, MeCN; (ii) 2 M HCl aq, 0 °C, 39−43%; (b) (i) LDA, THF; (ii) Ac_2O, THF, −78 °C, 78%; (c) (i) AcCl, EtOH; (ii) c-HCl, EtOH, 40 °C, 89%; (d) (i) NaH, THF, 60 °C; (ii) Ac_2O, THF, rt, 7−51%; (e) (i) NaOEt, EtOH; (ii) isobutyl iodide, THF, 80 °C, 43%; (f) R^1NHNH_2, EtOH or AcOH, reflux, 11−85%.

Scheme 2. Synthesis of Pyrazolone Derivatives **5**−**9**, **18**[a]



[a] Reagants and conditions: (a) for **5** and **6**, R^1OCOCl, pyridine, 50 °C, 16−23%; (b) for **7**, benzoyl chloride, dioxane, rt, 15%; (c) **6**, cyclopentylamine, xylene reflux, 42%; (d) oxalyl chloride, DMF, CH_2Cl_2; (e) aniline, CH_2Cl_2, rt, 74% (two steps); (f) benzoyl chloride, $Ca(OH)_2$, dioxane, reflux, 79%.

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[a] Abbreviations: TSEs, transmissible spongiform encephalopathies; CJD, Creutzfeldt−Jakob disease; GSS, Gerstmann−Sträussler−Scheinker syndrome; FFI, familial fatal insomnia; CWD, chronic wasting disease; BSE, bovine spongiform encephalopathy; PrP^{Sc}, infectious conformational form of prion protein; PrP^{C}, normal cellular prion protein; GPI, glycosylphosphatidylinositol; SOD, superoxide dismutase; PrP-res, protease-resistant form of prion protein; RML, Rocky Mountain Laboratory; PBS, phosphate-buffered saline; Tris, tris(hydroxymethyl) aminomethane; SDS, sodium dodecyl sulfate.

Table 1. Inhibition of PrP-res Accumulation in ScN2a Cells and F3 Cells, Oxidation Potentials (E_{pa}), and Hydroxyl Radical Scavenging Activity

cmpd	R^1	R^3	R^4	inhibition PrP-res IC_{50}[a,c,d] (nM) ScN2a cells	inhibition PrP-res IC_{50}[a,c,d] (nM) F3 cells	E_{pa}[e,f] (mV)	pH[g]	scavenging activity IC_{50} (mM)[h]
edaravone	Ph	CH_3	H	>1000	N.E.[b]	483	7.0	0.25
1	cyclohexyl	CH_3	H	13	25	549	7.4	
2	4-CH_3OPh–	CH_3	H	N.E.[b]	N.E.[b]	678	7.8	
3	4-ClPh–	CH_3	H	0.5	N.E.[b]	473	7.4	
4	Ph	isopropenyl	H	158	794	387	7.4	
5	Ph	CH_3OCONH–	H	6	501	454	7.8	
6	Ph	PhOCONH–	H	N.E.[b]	N.E.[b]	397	7.0	0.38
7	Ph	PhCONH–	H	2000	1260	458	7.8	0.22
8	Ph	cyclopentylNHCONH–	H	126	158	372	7.8	
9	Ph	PhNHCO–	H	398	1580	478	7.8	
10	Ph	$PhCH_2$–	H	N.E.[b]	N.E.[b]	269	>8.0	
11	Ph	Ph	H	N.E.[b]	N.E.[b]	397	7.6	
12	Ph	4-CH_3OPh–	H	N.E.[b]	N.E.[b]	397	7.8	
13	Ph	4-NO_2Ph–	H	3	3	419	7.4	0.09
14	Ph	CF_3	H	398	631	673	7.6	0.81
15	Ph	CH_3	cyclopropyl	N.E.[b]	N.E.[b]	275	7.8	0.72
16	Ph	CH_3	isobutyl	N.E.[b]	16	262	>8.0	
17	Ph	CH_3	Ph	40	1	227	7.6	0.79
18	Ph	CH_3	PhCO–	6	1000	640	7.0	0.61
19	Ph	CH_3	2-pyridyl	79	631	403	>8.0	

[a] IC_{50}, concentration of a compound causing 50% inhibition of PrP-res accumulation relative to the control. [b] N.E., no effect. [c] At the concentration range for antiprion activity assay (10^{-10}–10^{-7} M for **2**, **3**, **11**, **12**, and **17** and 10^{-10}–10^{-6} M for the others), no cytotoxicity was observed against both of the two cell lines (Supporting Information). [d] In our system, IC_{50} values of quinine and quinidine in ScN2a cells are 10 μM and 5 μM, respectively. Those values are consistent with a previously report.[11] [e] Conditions for measurement: 10 mM sample in 50 mM NaCl; working electrode, Pt; reference electrode, Ag^+/AgCl; counter electrode, Pt; scan speed, 50mV/s; scan range, −0.2 to 1.0 V. [f] Oxidation potentials were expressed versus Ag^+/AgCl. [g] Oxidation properties (E_{pa}) were measured at indicated pH because of their poor solubility in acidic and neutral aqueous solutions. [h] Conditions for measurement: a mixture of 25 mM H_2O_2, 25 mM DMPO, and a compound was irradiated with UV. ESR spectrometer parameters were as follows: microwave power, 10 mW; modulation width, 0.063 mT; time constant, 0.03 s; sweep width, 7.5 mT; sweep time, 1 min; gain, 320.

study, we focused on and explored the pyrazolone compounds derived from edaravone, as antiprion agents, and found new and highly active antiprion compounds.

Our initial goal was to prepare a small focused library of edaravone derivatives. The preparation of pyrazolone compounds was achieved by refluxing the corresponding β-ketoester and hydrazine compound in ethanol or acetic acid. β-Ketoesters **24b,c,h–k**, which are not commercially available, were synthesized from acyl chlorides **20a,b**, nitrile **21**, ethyl acetoacetate **24a**, or ethyl esters **23a,b** (Scheme 1). Treatment of amine **25** with chloroformic acid ester or benzoyl chloride gave carbamates **5** or **6** or amide **7** (Scheme 2). Carbamate **6** was then converted to urea **8** by treatment with cyclopentylamine. Amide **9** was synthesized from carboxylic acid **26** via acyl chloride **27**. Compound **18** was prepared from edaravone **28** with benzoyl chloride in the presence of $Ca(OH)_2$.

The antiprion activity of each compound was evaluated as the ability to inhibit the accumulation of the abnormal protease-resistant form of prion protein (PrP-res), as described in previous reports.[11,19,20] In this study, two types of prion-infected mouse neuroblastoma (N2a) cell lines, ScN2a and F3, were used. N2a cells that were infected with the RML strain are called ScN2a,[21] and N2a#58 cells that were infected with the Fukuoka-1 strain are called F3. N2a#58 cells are known to express five times more normal PrP than N2a cells. Both ScN2a cells and F3 cells were grown in six-well culture plates in Opti-MEM (Invitrogen) supplemented with 10% fetal bovine serum. Compounds were added at the designated concentration to the medium when cells were passaged at 10% confluency. The cells were allowed to grow to confluence (3 or 4 days) and lysed with lysis buffer (0.5% sodium deoxycholate, 0.5% Nonidet P-40, and PBS). The lysates were digested with 10 μg/mL proteinase K for 30 min at 37 °C and centrifuged at 15 000 rpm for 5 min at 24 °C with GLASSFOG (Q-bio gene, CA). The pellets were resuspended in sample loading buffer and boiled. Samples were separated by electrophoresis on 15% Tris-glycine-SDS-polyacryl-amide gel and electroblotted. PrP-res was detected using an antibody, SAF83 (1:5000; SPI-Bio, Montigny-le-Bretonneux, France), followed by an alkaline phosphatases-conjugated secondary antibody. Immunoreactive signals were visualized using CDP-Star detection reagent (Amersham Biosciences Corp., NJ) and were analyzed densitometrically. At least three independent experiments were performed to determine the IC_{50} value of each compound.

The original lead compound, edaravone, showed weak antiprion activity in ScN2a cells. The pyrazolone compounds **3** and **16** were effective in one of two cell lines (Table 1). Compounds **1**, **4**, **5**, **7**, **8**, **9**, **13**, **14**, **17**, **18**, and **19** inhibited PrP-res accumulation in both ScN2a cells and F3 cells, but the others did not (within a nontoxic dose range). Among the synthesized pyrazolone derivatives, 3-(4-nitrophenyl) compound **13** showed the highest activity for inhibiting PrP-res accumulation (IC_{50} = 3 nM), which is 130 times more active than quinacrine (IC_{50} = 400 nM)[19] and was one of the most potent compounds reported so far.[12,14] Although there are no reports that pyrazolone derivatives inhibit PrP-res accumulation in

prion-infected cells, compounds having a pyrazolone ring might be a new series of antiprion activity substances.

Because various types of compounds, such as 1-cyclohexyl compound **1**, 3-isopropenyl compound **4**, 3-(4-nitrophenyl) compound **13**, and 4-benzoyl compound **18**, showed relatively high antiprion activity, the position and class of substituents were not directly correlated with the activity of inhibiting PrP-res accumulation; therefore, we searched for the properties of synthesized compounds.

We had previously determined the oxidation potential and hydroxyl radical scavenging activity of edaravone-related derivatives.[22] Briefly, one-electron oxidation potentials (E_{pa}) of all synthesized derivatives were measured in a 50 mM NaCl solution by cyclic voltammetry (CV). Oxidation currents were observed with all the tested compounds but were irreversible, probably because the one-electron oxidation products were unstable and converted to degraded compounds as reported.[23] Because of the poor solubility of several derivatives in the neutral aqueous solution, the solutions were slightly basified using aqueous NaOH to solubilize these compounds.

Although the derivatives showed a wide variety of oxidation potentials (Table 1), no correlations were observed between oxidation potentials and antiprion activity.

Radical scavenging activity, which is known as the main action of edaravone as a brain-protecting drug,[23] was evaluated using the electron spin resonance (ESR) spin-trapping method with 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) as a spin trap.[22] Hydroxyl radicals were generated by UV irradiation (200 mJ/cm^2) of hydrogen peroxide solution containing DMPO and edaravone derivatives. The inhibitory effect of the derivatives on the formation of hydroxyl radical adducts of DMPO was used as a measure of radical scavenging activity.

IC_{50} values were determined for 7 of 19 derivatives with diverse antiprion activity (**6**, **7**, **13**, **14**, **15**, **17**, and **18**). Compounds **15** and **17** exhibited efficient inhibition of the hydroxyl radical adduct formation to a similar extent, but **15** did not inhibit PrP-res accumulation in contrast with **17**, which showed antiprion activity in the nanomolar range. It was found that there is poor correlation between hydroxyl radical scavenging activity and antiprion activity.

Recently, Fukuuchi et al. found that compounds that have copper-selective chelating ability and whose copper complexes have high SOD-like activity are candidates for antiprion drugs.[24] For example, D-penicillamine has been reported to show moderate antiprion activity[9] and its copper complex exhibits SOD-like activity with an IC_{50} value of 28 μM.[24] The copper complex of 2,2′-biquinoline, whose IC_{50} value of antiprion activity has been reported to be 5 nM,[24] also exhibits SOD-like activity, with an IC_{50} value of 3 μM.[24] We therefore considered if our compounds might show SOD-like activity itself or in the form of a copper complex.

To investigate this idea, we first examined whether the synthesized derivatives could chelate with Cu(II). The chelation study was carried out using Job's method.[25] Solutions of each compound and $Cu(ClO_4)_2$ at a ratio (compound/Cu(II)) of 1:0 to 0:1 were prepared in 95% ethanol, and absorption spectra were measured. Spectrophotometric complexation studies showed that **1**, **15**, and **18** bound with Cu(II) at a 2:1 ratio and **6**, **7**, and **16** bound with Cu(II) at a 1:1 ratio. Compounds **9**, **14**, and edaravone showed no spectral changes in the presence of Cu(II) (**18**, Figure 1A; others, data not shown). It was unclear whether other compounds, such as **3**, **13**, and **17**, can bind with Cu(II) because they showed little spectral shift in the presence of Cu(II) (**13**, Figure 1B; others, data not shown).

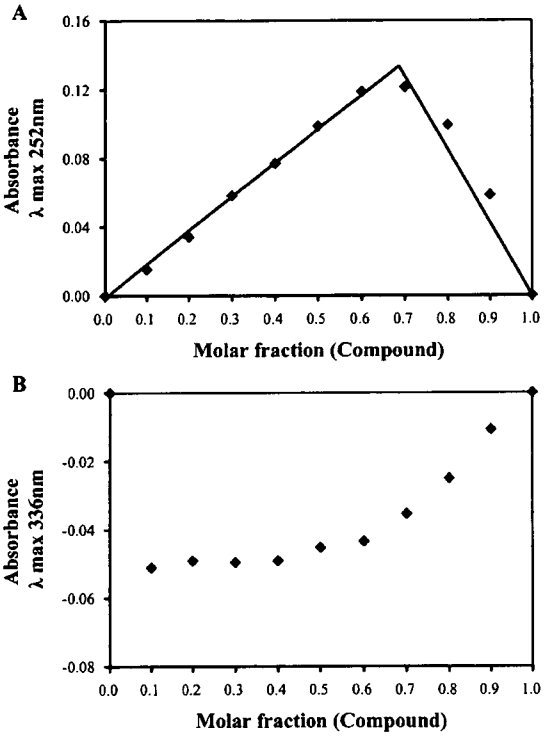
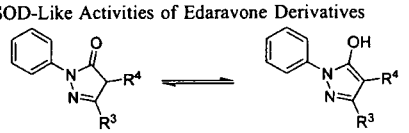


Figure 1. Continuous variation plots for compound **18** and Cu(II) (A) and compound **13** and Cu(II) (B). A: A 2:1 binding ratio between compound **18** and Cu(II); B: binding of compound **13** to Cu(II) was uncertain. Plots were obtained by Job's method in ethanol solution.

Table 2. SOD-Like Activities of Edaravone Derivatives



cmpd	R^3	R^4	SOD-like activity[a] (%) cmpd	with Cu(II)[b]
edaravone	CH_3	H	0.6	5.7
6	PhOCONH—	H	5.6	11.6
7	PhCONH—	H	9.2	3.2
13	4-NO_2Ph—	H	1.8	11.9
14	CF_3	H	13.4	7.4
15	CH_3	cyclopropyl	15.9	35.4

[a] Activity, percentage of inhibition of WST-1 tetrazorium formation by a compound at 1 mM. [b] All compounds were measured at 1 mM and 2 mM of Cu(II).

Findings from these experiments suggest that copper-chelating ability was not essential for antiprion activity, as previously reported.[24,26]

SOD-like activity of synthesized compounds (edaravone, **6**, **7**, **13**, **14**, and **15**) was measured in vitro using SOD-like assay kit-WST (Dojindo Laboratories, Kumamoto, Japan). This method is a xanthine-based photometric assay using tetrazolium salt WST-1. SOD-like activities of derivatives were evaluated at 1 mM (Table 2). Although it was uncertain whether some compounds, such as **13**, bind with Cu(II), SOD-like activities of derivatives in the presence of Cu(II) were also investigated using a solution of 1 mM $Cu(ClO_4)_2$ and 0.5 mM compound in a 0.9% NaCl solution. Because it is known that Cu(II) has superoxide scavenging activity, we also evaluated the SOD-

like activity of Cu(II) itself. A total of 1 mM of Cu(II) inhibited WST-1 formazan formation by 6.7%.

For all measured compounds, SOD-like activity was found to be very weak. Furthermore, because nonantiprion compounds, such as **6** and **15**, showed comparable activity with **13** and were more effective than compounds **7** and **14**, SOD-like activity may not be correlated with antiprion activity of these compounds.

In conclusion, we found that some pyrazolone compounds derivatized from edaravone have the ability to inhibit the accumulation of PrP-res, and 3-(4-nitrophenyl) compound **13** had remarkable activity (IC_{50} = 3 nM). To obtain information about their action mechanism, we investigated their oxidation potentials, copper-complexing, and SOD-like activity. Findings from these experiments suggest that these properties have little correlation with activity.

Further active antiprion derivatives and the mechanistic studies are under investigation.

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Supporting Information Available: Experimental details of the synthesis and characterization data for all compounds and analytical methodologies. This information is available free of charge via the Internet at http://pubs.acs.org.

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Synthesis and Evaluation of 2-Nonylaminopyridine Derivatives as PPAR Ligands

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To find novel PPAR ligands, we prepared several 3-{3 or 4-[2-(nonylpyridin-2-ylamino)ethoxy]phenyl}-propanoic acid derivatives which were designed based on the structure of our previous PPARγ ligand 1. In PPAR binding affinity assays, compound 4, which had an ethoxy group at the C-2 position of the propanoic acid of 1, showed selective binding affinity for PPARγ. Compound 3, with an ethyl group at the C-2 position, was found to be a PPARα/γ dual ligand. Compound 6, the meta isomer of 1, has been shown to be a PPARα ligand. The introduction of methyl (7) and ethyl (8) groups to the C-2 position of the propanoic acid of 6 further improved PPARα-binding potency. In cell-based transactivation assay, compounds 3 and 4 showed dual-agonist activity toward PPARα and PPARγ. Compound 6 was found to be a triple agonist and compound 8 proved to be a selective PPARα agonist. In the human hypodermic preadipocyte differentiation test, it was demonstrated that the maximal activity of compounds 3 and 4 was higher than that of rosiglitazone.

Key words peroxisome proliferator-activated receptor (PPAR); agonist; selectivity

The peroxisome proliferator-activated receptors (PPARα, PPARγ and PPARδ) are a set of ligand-activated transcription factors in the nuclear hormone receptor superfamily.[1—4] These receptors regulate the expression of a large number of genes involved in lipid metabolism and energy balance by binding to a DNA sequence termed PPAR response elements.[5] The PPARγ is predominantly expressed in adipose tissues, and plays a pivotal role in adipose differentiation, and the regulation of glucose and lipid homeostasis. The clinically useful thiazolidinedione (TZD) class of insulin sensitizers such as rosiglitazone[6] and pioglitazone[7] (Fig. 1) are potent PPARγ agonists used in the treatment of Type 2 diabetes. TZDs are known to improve insulin resistance, which is a key underlying feature of Type 2 diabetes[8]; however, the use of TZDs has been limited because of their serious side effects such as hepatic toxicity, weight gain and edema. Meanwhile, PPARα is highly expressed in metabolically active tissues such as the liver, heart and muscle, and regulates lipid homeostasis. PPARα agonists such as clofibrate (Fig. 1) have demonstrated the ability to reduce serum triglyceride and increase HDL cholesterol levels,[9] and are being utilized as hypolipidemic agents. In addition, recent studies revealed that dual agonists of PPARα/γ decrease the free triglyceride plasma concentration and increase plasma HDL concentration in an insulin-resistant animal model.[10,11] Thus, many groups have ongoing research programs to identify more potent and less toxic PPARα agonists, PPARγ agonists and PPARα/γ dual agonists.

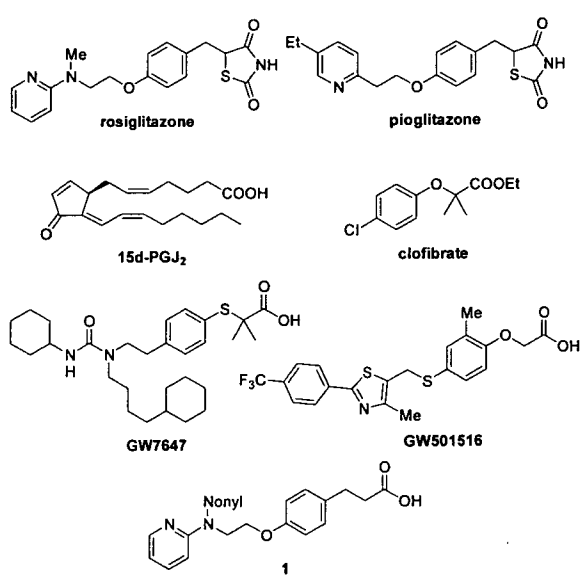
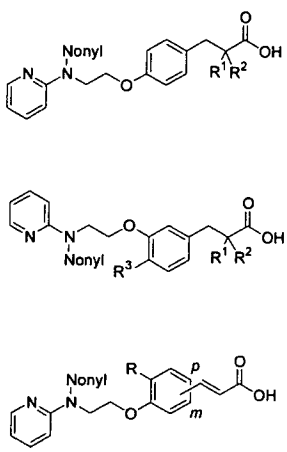


Fig. 1. Structures of Rosiglitazone, Pioglitazone, 15d-PGJ_2, Clofibrate, GW7647, GW501516 and Compound 1

We previously reported compound **1** (Fig. 1), which was designed based on the structure of rosiglitazone and 15d-PGJ_2,[12,13] as a potent PPARγ ligand.[14] To find more potent PPARγ agonists and novel PPARα agonists, we chose compound **1** as the lead structure, because recent reports indicated that PPARγ affinity can be increased by the introduction of substituents into the C-2 position of propanoic acid,[15—19] and minor structural modifications can convert PPAR subtype selectivity.[20—22] In this article, we report the synthesis, binding affinity and biological activity of PPAR ligands based on the structure of compound **1**.

Chemistry The compounds prepared for this study are shown in Fig. 2, and the routes used for synthesis are shown in Charts 1—3. Chart 1 shows the preparation of compounds **1**, **3**, **4**, **6—10**, **12** and **13**. The 2-nonylaminopyridine **18** was prepared by the method of Buchwald[23]: treatment of *n*-nonylamine **17** with 2-bromopyridine, $Pd_2(DBA)_3$, BINAP, and *t*-BuONa in toluene at 105 °C. *p*-Hydroxybenzaldehyde **19a**, *m*-hydroxybenzaldehyde **19b** and isovaniline **19c** were allowed to react with 1,2-dibromoethane to give ethers **20a—c**. The Horner–Wadsworth–Emmons reaction[24] was applied to the conversion of **20a—c** into acrylic acid ethyl esters **21a—**

j. The double bonds of **21a—j** were hydrogenated to yield compounds **22a—j**. Coupling between 2-nonylaminopyridine **18** and propanoic acid ethyl esters **22a—j** afforded *N*-(2-pyridinyl)-*N*-nonylpropanoic acid ethyl esters **23a—j**. The subsequent hydrolysis of **23a—j** gave the desired carboxylic acids **1**, **3**, **4**, **6—10**, **12** and **13**.



1 : R^1 = H, R^2 = H
2 : R^1 = Me, R^2 = H
3 : R^1 = Et, R^2 = H
4 : R^1 = OEt, R^2 = H
5 : R^1 = Me, R^2 = Me

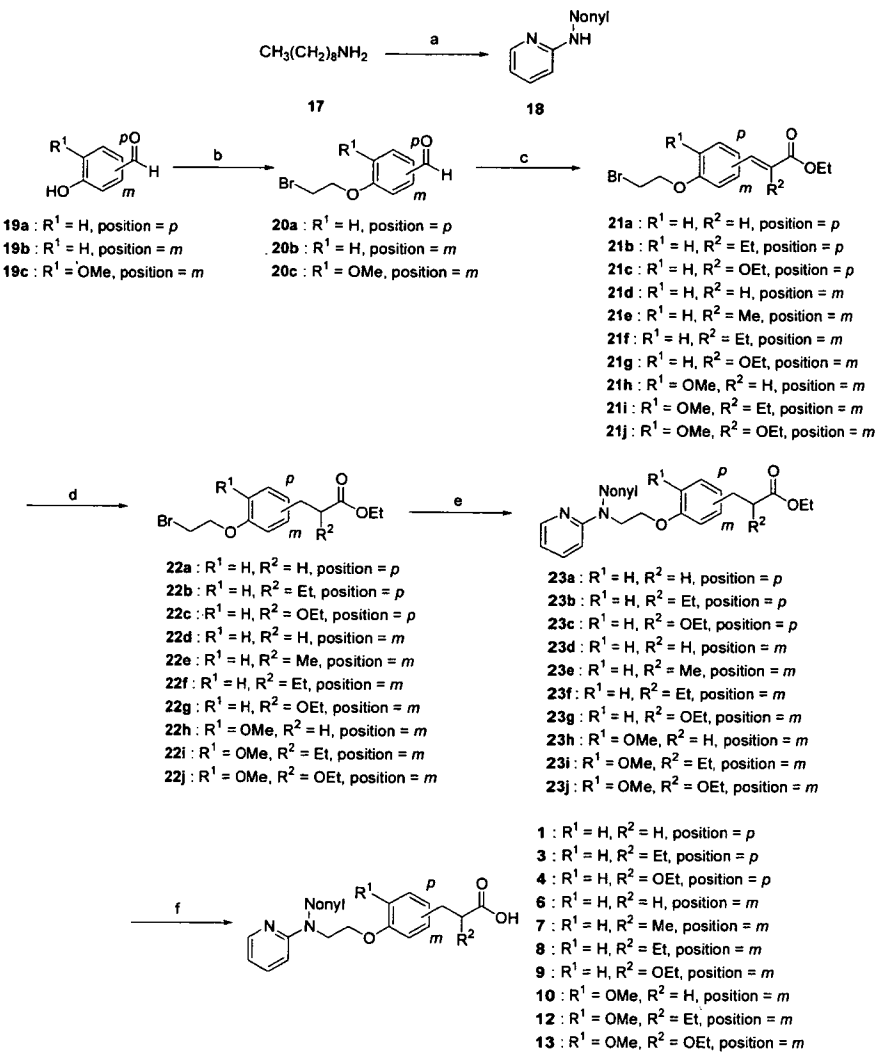
6 : R^1 = H, R^2 = H, R^3 = H
7 : R^1 = Me, R^2 = H, R^3 = H
8 : R^1 = Et, R^2 = H, R^3 = H
9 : R^1 = OEt, R^2 = H, R^3 = H
10 : R^1 = H, R^2 = H, R^3 = OMe
11 : R^1 = Me, R^2 = H, R^3 = OMe
12 : R^1 = Et, R^2 = H, R^3 = OMe
13 : R^1 = OEt, R^2 = H, R^3 = OMe
14 : R^1 = Me, R^2 = Me, R^3 = OMe

15 : R = H, position = *p*
16 : R = OMe, position = *m*

Fig. 2. Structures of Compounds **1—16**

The preparation of compounds **2**, **5**, **11** and **14**, which have one or two methyl groups at the C-2 position of propanoic acid, is outlined in Chart 2. Aldehydes **20a** and **20c** were reduced by $NaBH_4$ and allowed to react with acetic anhydride to give **25a** and **25b**. Compounds **25a** and **25b** were treated with 1-methoxy-1-trimethylsilyloxypropene or dimethylketene methyltrimethylsilyl acetal in the presence of magnesium perchlorate in anhydrous CH_2Cl_2 to give esters **26a—d**.[25] Coupling between 2-nonylaminopyridine **18** and propanoic acid methyl esters **26a—d** afforded *N*-(2-pyridinyl)-*N*-nonyl compounds **27a—d** and subsequent hydrolysis gave carboxylic acids **2**, **5**, **11** and **14**.

Preparation of the acrylic acid derivatives **15** and **16** is shown in Chart 3. Acrylic acid ethyl esters **21a** and **21h** were allowed to react with 2-nonylaminopyridine **18** to give compounds **28a** and **28b**. Treatment of **28a** and **28b** with aqueous NaOH gave *N*-(2-pyridinyl)-*N*-nonyl acrylic acids **15** and **16**.



19a : R^1 = H, position = *p*
19b : R^1 = H, position = *m*
19c : R^1 = OMe, position = *m*

20a : R^1 = H, position = *p*
20b : R^1 = H, position = *m*
20c : R^1 = OMe, position = *m*

21a : R^1 = H, R^2 = H, position = *p*
21b : R^1 = H, R^2 = Et, position = *p*
21c : R^1 = H, R^2 = OEt, position = *p*
21d : R^1 = H, R^2 = H, position = *m*
21e : R^1 = H, R^2 = Me, position = *m*
21f : R^1 = H, R^2 = Et, position = *m*
21g : R^1 = H, R^2 = OEt, position = *m*
21h : R^1 = OMe, R^2 = H, position = *m*
21i : R^1 = OMe, R^2 = Et, position = *m*
21j : R^1 = OMe, R^2 = OEt, position = *m*

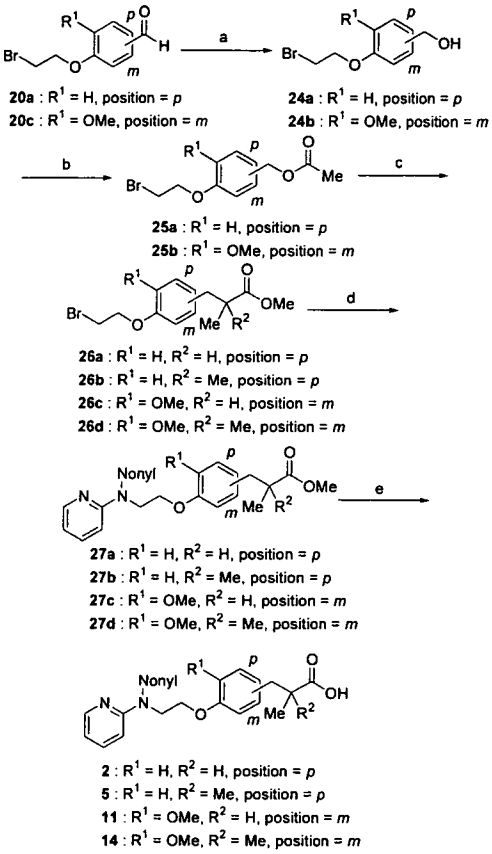
22a : R^1 = H, R^2 = H, position = *p*
22b : R^1 = H, R^2 = Et, position = *p*
22c : R^1 = H, R^2 = OEt, position = *p*
22d : R^1 = H, R^2 = H, position = *m*
22e : R^1 = H, R^2 = Me, position = *m*
22f : R^1 = H, R^2 = Et, position = *m*
22g : R^1 = H, R^2 = OEt, position = *m*
22h : R^1 = OMe, R^2 = H, position = *m*
22i : R^1 = OMe, R^2 = Et, position = *m*
22j : R^1 = OMe, R^2 = OEt, position = *m*

23a : R^1 = H, R^2 = H, position = *p*
23b : R^1 = H, R^2 = Et, position = *p*
23c : R^1 = H, R^2 = OEt, position = *p*
23d : R^1 = H, R^2 = H, position = *m*
23e : R^1 = H, R^2 = Me, position = *m*
23f : R^1 = H, R^2 = Et, position = *m*
23g : R^1 = H, R^2 = OEt, position = *m*
23h : R^1 = OMe, R^2 = H, position = *m*
23i : R^1 = OMe, R^2 = Et, position = *m*
23j : R^1 = OMe, R^2 = OEt, position = *m*

1 : R^1 = H, R^2 = H, position = *p*
3 : R^1 = H, R^2 = Et, position = *p*
4 : R^1 = H, R^2 = OEt, position = *p*
6 : R^1 = H, R^2 = H, position = *m*
7 : R^1 = H, R^2 = Me, position = *m*
8 : R^1 = H, R^2 = Et, position = *m*
9 : R^1 = H, R^2 = OEt, position = *m*
10 : R^1 = OMe, R^2 = H, position = *m*
12 : R^1 = OMe, R^2 = Et, position = *m*
13 : R^1 = OMe, R^2 = OEt, position = *m*

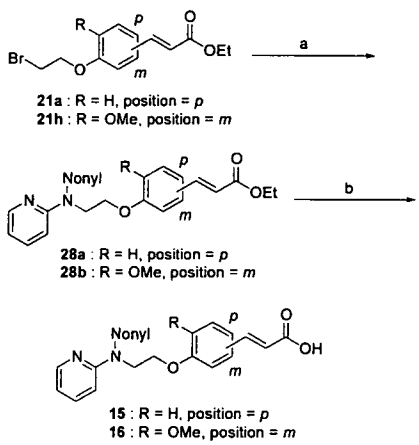
(a) 2-bromopyridine, $Pd_2(DBA)_3$, BINAP, *t*-BuOH, toluene, 105 °C, 70%; (b) 1,2-dibromoethane, Cs_2CO_3, THF, 65 °C, 33—57%; (c) $(EtO)_2P(O)CH(R)CO_2Et$, NaH, anhydrous THF, 0 °C to rt, 47—95%; (d) H_2, Pd/C, EtOH, 79—97%; (e) **18**, Et_3N, KI, 105 °C, 9—17%; (f) 2 N aq. NaOH, EtOH, THF, rt, 82—100%.

Chart 1



(a) $NaBH_4$, EtOH, rt, 81—95%; (b) Ac_2O, DMAP, rt, 96—97%; (c) 1-methoxy-1-trimethylsilyloxypropene, or dimethylketene methyltrimethylsilyl acetal, $Mg(ClO_4)_2$, rt, 90—94%; (d) **18**, Et_3N, KI, 105 °C, 5—13%; (e) 2 N aq. NaOH, EtOH, rt, 75—97%.

Chart 2



(a) **18**, Et_3N, KI, 105 °C, 9—13%; (b) 2 N aq. NaOH, EtOH, THF, rt, 82—99%.

Chart 3

Results and Discussion

The binding affinity of the compounds for PPARs was evaluated with the CoA-BAP System (Microsystems).[26] In this system, alkaline phosphatase (AP) activity is directly proportional to the affinity of the ligands for PPARs. The abilities of compounds **1**—**16** to bind PPARα, PPARγ and PPARδ were evaluated and the results are shown in Figs. 3, 4 and 5, respectively. GW7647[27] (PPARα), rosiglitazone[6] (PPARγ) and GW501516[28] (PPARδ) were used as reference compounds (Fig. 1).

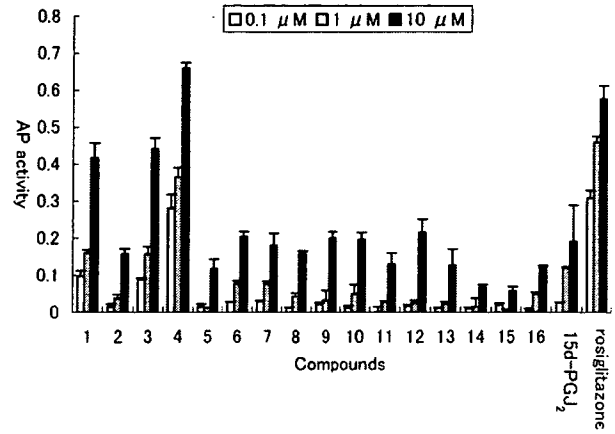


Fig. 3. Binding Affinity for PPARγ of Compounds **1**—**16** at 0.1, 1.0 and 10 μM

Values are the means of at least three experiments.

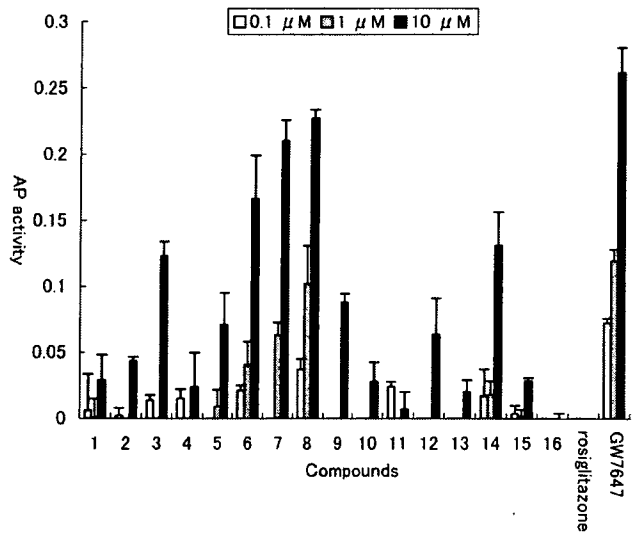


Fig. 4. Binding Affinity for PPARα of Compounds **1**—**16** at 0.1, 1.0 and 10 μM

Values are the means of at least three experiments.

The lead compound **1** showed relatively high affinity for PPARγ and little affinity for PPARα and PPARδ (Fig. 3—5). We initially examined the effect of substituents at the C-2 position of the propanoic acid of **1**, because it has been reported that the introduction of an alkyl or an alkoxy group at this position increases the activity of PPARγ and PPARα.[15—19] Among compound **1**, methyl **2**, ethyl **3**, ethoxy **4**, and dimethyl **5**, compound **4** showed the strongest affinity for PPARγ, so compound **4** was founded to be a potent and selective PPARγ ligand (Figs. 3—5). In addition, the PPARγ affinity of compound **3** was comparable to that of compound **1**, whereas compound **3** displayed strong affinity for PPARα as compared with **1** (Figs. 3, 4).

Next, we investigated the PPARs affinity of compound **6**, the *meta* isomer of compound **1**.[29] Compound **6** showed

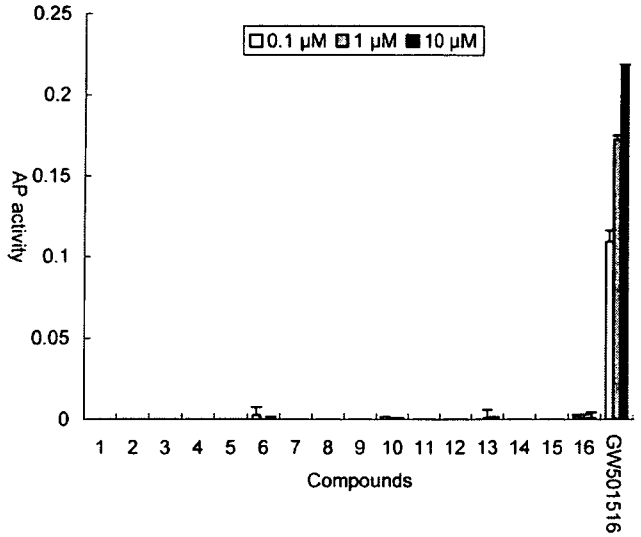


Fig. 5. Binding Affinity for PPARδ of Compounds **1**—**16** at 0.1, 1.0 and 10 μM

Values are the means of at least three experiments.

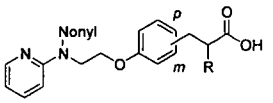
much higher affinity for PPARα than **1** (Fig. 4). Furthermore, the affinity for PPARγ of **6** was lower than that of **1** (Fig. 3), and compound **6** exhibited little affinity for PPARδ (Fig. 5). To find more potent PPARα ligands, we prepared and tested compounds **7**, **8** and **9** in which a methyl, ethyl and ethoxy group was substituted at the C-2 position of the propanoic acid of **6**, respectively. Compounds **7** and **8** showed strong affinity for PPARα and compound **8** displayed slightly weak affinity for PPARγ as compared with the parent compound **6**. The effect of the introduction of a methoxy group at the C-4 position of the benzene ring of **6** was also investigated; however, compounds **10**—**14** showed no pronounced affinity for PPARs as compared to compound **6** (Figs. 3—5).

The acrylic acid derivatives **15** and **16** did not show notable affinity for PPARs (Figs. 3—5).

Based on the findings in the binding assay, we next investigated the PPAR transactivation activity of compounds **1**, **3**, **4**, **6**, **7**, and **8** by reporter gene assay[30] (Table 1). We initially tested the activity of compound **1**, which did not show significant transcriptional activity for PPARα, δ, and γ. We then examined the activity of compounds **3** and **4**, which have an ethyl or ethoxy group at the C-2 position of the propanoic acid of **1**. As expected from the binding assay, compound **3** was a PPARα/γ dual agonist. Although the binding assay indicated that compound **4** is a selective PPARγ ligand, it showed potent dual-agonist activity toward PPARα and PPARγ. Compound **6**, the *meta* isomer of compound **1**, was found to be a triple agonist. Compound **7**, which had an introduced methyl group at the C-2 position of the propanoic acid moiety of **6**, improved the EC_{50} values and selectivity for PPARα and PPARγ, and the introduction of ethyl group (compound **8**) increased the activity and selectivity for PPARα. Among these compounds, compound **8** showed the highest selectivity for PPARα.

As compounds **3** and **4** were found to have relatively high PPARγ agonist activity in our study, we used them for further evaluation. Since it has been reported that the activation

Table 1. *In Vitro* Functional PPAR Transactivation Activity of Compounds



Compound	R	Position	EC_{50}[a] (μM)		
			PPARγ	PPARδ	PPARα
1	H	*p*	>10	>10	>10
3	Et	*p*	2.62	>10	3.68
4	OEt	*p*	0.32	>10	0.74
6	H	*m*	7.76	6.42	3.20
7	Me	*m*	4.72	>10	2.65
8	Et	*m*	>8	>10	1.81

a) Compounds were screened for agonist activity on PPAR-GAL4 chimeric receptors in transiently transfected HEK-293 cells as described. EC_{50} value is the molar concentration of the test compound that affords 50% of maximal reporter activity.[33]

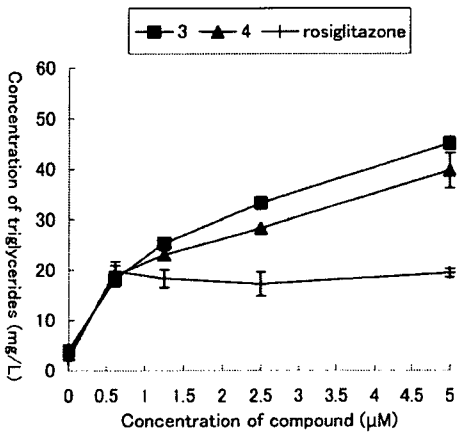


Fig. 6. Accumulation of Fatty Acid in Human Preadipocytes by Compounds 3, 4 and Rosiglitazone

Values are the means of at least three experiments.

of PPARγ enhances adipocyte differentiation[31] and increases insulin sensitivity, compounds **3** and **4** were subjected to a human hypodermic preadipocyte differentiation test.[32] The accumulation of triglycerides in the cells was observed after the administration of compounds **3** and **4** at concentrations of 0, 0.613, 1.25, 2.5 and 5 μM, and the activity of compounds **3** and **4** was found to be higher than that of rosiglitazone at 1 μM and higher concentrations (Fig. 6).

Conclusion

To find novel PPAR ligands, we prepared several 2-nonylaminopyridine derivatives which were designed based on the structure of a PPARγ ligand **1**. In PPAR binding affinity assays, compound **4**, which had an ethoxy group at the C-2 position of the propanoic acid of **1**, showed high binding affinity for PPARγ and little affinity for PPARα and PPARδ. Compound **3**, which had an ethyl group at the C-2 position of propanoic acid, was found to be a PPARα/γ dual ligand. Compound **6**, the *meta* isomer of **1**, has been shown to be a PPARα ligand. The introduction of methyl (**7**) and ethyl (**8**) groups at the C-2 position of the propanoic acid of **6** further improved PPARα-binding potency. In cell-based transactivation assay, compounds **3** and **4** showed dual-agonist activity toward PPARα and PPARγ. Compound **6** was found to be a

triple agonist and compound **8** proved to be a selective PPARα agonist. In the human hypodermic preadipocyte differentiation test, it was demonstrated that the activity of compounds **3** and **4** was higher than that of rosiglitazone. The findings of this study will help provide an effective agent for Type 2 diabetes and hyperlipidemia.

Experimental

Melting points were determined using a Yanagimoto micro melting point apparatus or a Büchi 545 melting point apparatus and were left uncorrected. Proton nuclear magnetic resonance spectra (^{1}H-NMR) were recorded on a JEOL JNM-LA500 spectrometer in solvent as indicated. Chemical shifts (δ) were reported in parts per million relative to the internal standard tetramethylsilane. High-resolution mass spectra (HR-MS) were recorded on a JEOL JMS-SX102A mass spectrometer. Reagents and solvents were purchased from Aldrich, Tokyo Kasei Kogyo, Wako Pure Chemical Industries, and Kanto Kagaku, and used without purification. Flash column chromatography was performed using Silica Gel 60 (particle size 0.046—0.063 mm) supplied by Merck.

2-Nonylaminopyridine (18) To a solution of 2-bromopyridine (0.9 ml, 9.20 mmol) in 10 ml of anhydrous toluene were added *n*-nonylamine (**17**, 10.0 ml, 55.2 mmol), $Pd_2(DBA)_3$ (0.169 g, 0.18 mmol), *rac*-BINAP (0.229 g, 0.37 mmol) and sodium *tert*-butoxide (1.24 g, 12.9 mmol). The reaction mixture was stirred at 105 °C for 3 d under Ar, and poured into water. The whole was extracted with AcOEt. The AcOEt layer was separated, washed with water and brine, and dried over Na_2SO_4. The solvent was removed by evaporation *in vacuo*. Purification by silica gel flash chromatography (AcOEt/*n*-hexane=1/5) gave 1.43 g (70%) of **18** as a yellow solid: ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.07 (1H, dd, J=4.9, 1.5 Hz), 7.41 (1H, ddd, J=8.5, 6.7, 1.8 Hz), 6.55 (1H, dd, J=6.7, 5.5 Hz), 6.37 (1H, d, J=8.2 Hz), 4.45 (1H, m), 3.24 (2H, m), 1.65—1.59 (2H, m), 1.41—1.27 (12H, m), 0.88 (3H, t, J=6.9 Hz).

4-(2-Bromoethoxy)benzaldehyde (20a) To a solution of *p*-hydroxybenzaldehyde (**19a**, 1.25 g, 10.0 mmol) in 13 ml of THF were added Cs_2CO_3 (4.28 g, 13.0 mmol) and 1,2-dibromoethane (1.76 ml, 20.0 mmol). The mixture was stirred at 65 °C for 14 h. The mixture was poured into 2 N aqueous NaOH. The mixture was extracted with AcOEt. The AcOEt layer was separated, washed with water and brine, and dried over Na_2SO_4. The solvent was removed by evaporation *in vacuo*. Purification by silica gel flash chromatography (AcOEt/*n*-hexane=1/3) gave 0.758 g (33%) of **21a** as a light yellow solid: ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 9.90 (1H, s), 7.85 (2H, d, J=8.5 Hz), 7.02 (2H, d, J=8.8 Hz), 4.38 (2H, t, J=6.1 Hz), 3.67 (2H, t, J=6.1 Hz).

3-[4-(2-Bromoethoxy)phenyl]acrylic Acid Ethyl Ester (21a) To a suspension of NaH (191 mg, 4.78 mmol) in 4 ml of anhydrous THF was added dropwise a solution of ethyl diethylphosphono acetate (1.07 ml, 5.21 mmol) in 5 ml of anhydrous THF at 0 °C under Ar. The mixture was stirred for 1 h at 0 °C. To the mixture was added dropwise a solution of **20a** (916 mg, 4.00 mmol) in 8 ml of anhydrous THF. The reaction mixture was stirred for 16 h at 0 °C. The reaction mixture was poured into ice-water and extracted with AcOEt. The AcOEt layer was separated, washed with brine, and dried over Na_2SO_4. The solvent was removed by evaporation *in vacuo*. Purification by silica gel flash chromatography (AcOEt/*n*-hexane=1/5) gave 901 mg (76%) of **20a** as a white solid: ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 7.64 (1H, d, J=15.8 Hz), 7.48 (2H, d, J=8.8 Hz), 6.92 (2H, d, J=8.5 Hz), 6.32 (1H, d, J=15.8 Hz), 4.32 (2H, t, J=6.1 Hz), 4.26 (2H, q, J=7.3 Hz), 3.65 (2H, t, J=6.1 Hz), 1.34 (3H, t, J=7.0 Hz).

3-[4-(2-Bromoethoxy)phenyl]propanoic Acid Ethyl Ester (22a) To a solution of **21a** (445 mg, 1.49 mmol) in 5 ml of EtOH was added 64 mg of 7% Pd/C. The reaction mixture was stirred for 1 d under H_2 at room temperature. The catalyst was removed by filtration, and the solvent was removed by evaporation *in vacuo*. 359 mg (80%) of **22a** was obtained as a light yellow oil: ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 7.12 (2H, dt, J=8.8, 3.0 Hz), 6.83 (2H, dt, J=8.5, 3.0 Hz), 4.26 (2H, t, J=6.1 Hz), 4.12 (2H, q, J=7.0 Hz), 3.62 (2H, t, J=6.1 Hz), 2.89 (2H, t, J=7.6 Hz), 2.58 (2H, t, J=8.2 Hz), 1.23 (3H, t, J=7.0 Hz).

3-{4-[2-(Nonyl-pyridin-2-ylamino)ethoxy]phenyl}propanoic Acid Ethyl Ester (23a) To a solution of **22a** (0.343 g, 1.14 mmol) in 1 ml of THF were added 2-nonylaminopyridine (1.01 g, 4.56 mmol), KI (0.189 g, 1.14 mmol) and Et_3N (0.45 ml, 2.30 mmol). The reaction mixture was stirred for 28 h at 105 °C. The reaction mixture was poured into water and extracted with AcOEt. The AcOEt layer was separated, washed with water and brine, and dried over Na_2SO_4. Purification by silica gel flash chromatography (toluene : $CHCl_3$: AcOEt=45 : 5 : 3) gave 55 mg (11%) of **23a** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.13 (1H, ddd, J=4.8, 1.8, 0.9 Hz), 7.40 (1H, ddd, J=8.8, 7.0, 1.8 Hz), 7.09 (2H, d, J=8.5 Hz), 6.83 (2H, dt, J=8.5, 2.7 Hz), 6.50 (2H, m), 4.14 (2H, t, J=6.1 Hz), 4.11 (2H, q, J=7.3 Hz), 3.91 (2H, t, J=6.1 Hz), 3.47 (2H, t, J=7.9 Hz), 2.87 (2H, t, J=7.6 Hz), 2.57 (2H, t, J=7.9 Hz), 1.66—1.59 (2H, m), 1.36—1.20 (12H, m), 1.23 (3H, t, J=7.0 Hz), 0.88 (3H, t, J=6.7 Hz); HR-MS Calcd for $C_{27}H_{40}N_2O_3$ 440.3039, Found 440.3036.

3-{4-[2-(Nonylpyridin-2-ylamino)ethoxy]phenyl}propanoic Acid (1) (Chart 1) To a solution of **23a** (49 mg, 0.119 mmol) in 1.0 ml of THF and 1.0 ml of EtOH was added a solution of 2 N aqueous NaOH (0.60 ml, 1.19 mmol). The reaction mixture was stirred for 1 d at room temperature, and neutralized to pH 7 with 2 N aqueous HCl. The mixture was subjected to silica gel flash chromatography ($CHCl_3$/MeOH=19/1) to give 44 mg (90%) of **1** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.14 (1H, dd J=4.9, 1.2 Hz), 7.45 (1H, t, J=7.1 Hz), 7.10 (2H, d, J=8.5 Hz), 6.81 (2H, d, J=8.5 Hz), 6.54 (2H, m), 4.12 (2H, t, J=5.6 Hz), 3.93 (2H, t, J=5.6 Hz), 3.50 (2H, t, J=7.9 Hz), 2.88 (2H, t, J=7.8 Hz), 2.61 (2H, t, J=7.8 Hz), 1.66—1.62 (2H, m), 1.35—1.25 (12H, m), 0.88 (3H, t, J=6.8 Hz); MS (EI) *m/z*: 412 (M^+); HR-MS Calcd for $C_{25}H_{36}N_2O_3$ 412.2727, Found 412.2726.

2-{4-[2-(Nonylpyridin-2-ylamino)ethoxy]benzyl}butyric Acid (3) Compound **3** was prepared from **19a** using the procedure described for **1** in 2.5% yield. In the step of the synthesis of **21b**, 2-(diethoxyphosphoryl)butyric acid ethyl ester was used instead of ethyl diethylphosphono acetate: colorless oil; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.11 (1H, ddd, J=4.9, 1.8, 0.9 Hz), 7.41 (1H, ddd, J=8.8, 7.0, 1.8 Hz), 7.08 (2H, d, J=8.5 Hz), 6.79 (2H, d, J=8.5 Hz), 6.50 (1H, dd, J=7.0, 4.8 Hz), 6.48 (1H, d, J=7.9 Hz), 4.05 (1H, m), 3.99 (1H, m), 3.85 (2H, m), 3.46 (2H, t, J=7.9 Hz), 2.88 (1H, dd, J=13.7, 8.5 Hz), 2.70 (1H, dd, J=13.7, 6.4 Hz), 2.58 (1H, m), 1.68—1.55 (4H, m), 1.31—1.21 (12H, m), 0.96 (3H, t, J=7.6 Hz), 0.88 (3H, t, J=6.7 Hz); HR-MS Calcd for $C_{27}H_{40}N_2O_3$ 440.3039, Found 440.3029.

2-Ethoxy-3-{4-[2-(nonylpyridin-2-ylamino)ethoxy]phenyl}propanoic Acid (4) Compound **4** was prepared from **19a** using the procedure described for **1** in 2.1% yield. In the step of the synthesis of **21c**, 2-(diethoxyphosphoryl)ethoxyacetic acid ethyl ether was used instead of ethyl diethylphosphono acetate: colorless oil; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.12 (1H, dd, J=4.9, 1.2 Hz), 7.43 (1H, ddd, J=8.8, 7.0, 1.8 Hz), 7.28 (2H, d, J=8.5 Hz), 6.80 (2H, d, J=8.5 Hz), 6.51 (2H, m), 4.05 (2H, m), 3.90 (2H, m), 3.60 (1H, m), 3.47 (2H, t, J=7.6 Hz), 3.05 (1H, dd, J=14.0, 4.5 Hz), 2.94 (1H, dd, 14.3, 7.6 Hz), 1.65—1.59 (2H, m), 1.35—1.20 (12H, m), 1.17 (3H, t, J=7.0 Hz), 1.17 (3H, t, J=7.0 Hz), 0.88 (3H, t, J=7.0 Hz); HR-MS Calcd for $C_{27}H_{40}N_2O_4$ 456.2988, Found 456.2999.

3-{3-[2-(Nonylpyridin-2-ylamino)ethoxy]phenyl}propanoic Acid (6) Compound **6** was prepared from **19b** using the procedure described for **1** in 7.7% yield: colorless oil; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.14 (1H, ddd, J=4.9, 2.0, 0.8 Hz), 7.42 (1H, ddd, J=8.5, 6.7, 2.1 Hz), 7.18 (1H, t, J=7.9 Hz), 6.75 (3H, m), 6.5 (2H, m), 4.15 (2H, t, J=6.1 Hz), 3.91 (2H, t, J=5.8 Hz), 3.47 (2H, t, J=7.6 Hz), 2.91 (2H, t, J=7.6 Hz), 2.65 (2H, t, J=7.9 Hz), 1.65—1.61 (2H, m), 1.35—1.25 (12H, m), 0.88 (3H, t, J=6.7 Hz); HR-MS Calcd for $C_{25}H_{36}N_2O_3$ 412.2726, Found 412.2729.

2-Methyl-3-{3-[2-(nonylpyridin-2-ylamino)ethoxy]phenyl}propanoic Acid (7) Compound **7** was prepared from **19b** using the procedure described for **1** in 3.5% yield. In the step of the synthesis of **21e**, 2-(diethoxyphosphoryl)propanoic acid ethyl ester was used instead of ethyl diethylphosphono acetate: light yellow oil; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.14 (1H, ddd, J=4.8, 1.8, 0.9 Hz), 7.42 (1H, ddd, J=8.8, 6.7, 2.1 Hz), 7.17 (1H, t, J=7.9 Hz), 6.74—6.78 (3H, m), 6.52 (1H, m), 6.50 (1H, d, J=8.8 Hz), 4.14 (2H, t, J=6.1 Hz), 3.90 (2H, m), 3.46 (3H, t, J=7.6 Hz), 3.02 (1H, dd, J=13.4, 6.7 Hz), 2.75 (1H, m), 2.64 (1H, dd, J=13.4, 7.6 Hz), 1.66—1.58 (2H, m), 1.35—1.25 (12H, m), 1.17 (3H, d, J=7.0 Hz), 0.88 (3H, t, J=7.3 Hz); HR-MS Calcd for $C_{26}H_{38}N_2O_3$ 426.2882, Found; M^1 426.2877.

2-{3-[2-(Nonylpyridin-2-ylamino)ethoxy]benzyl}butyric Acid (8) Compound **8** was prepared from **19b** using the procedure described for **1** in 4.9% yield. In the step of the synthesis of **21f**, 2-(diethoxyphosphoryl)butyric acid ethyl ester was used instead of ethyl diethylphosphono acetate: yellow oil; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.13 (1H, ddd, J =5.2, 1.2 Hz), 7.42 (1H, ddd, J=8.8, 6.7, 2.1 Hz), 7.15 (1H, t, J=7.9 Hz), 6.71—6.79 (3H, m), 6.52 (1H, dd, J=6.7, 5.2 Hz), 6.50 (1H, d, J=8.8 Hz), 4.12 (2H, t, J=5.5 Hz), 3.83—3.94 (2H, m), 3.46 (3H, t, J=7.6 Hz), 2.93 (1H, dd, J=13.7, 8.2 Hz), 2.72 (1H, dd, J=13.7, 6.7 Hz), 2.60 (1H, m), 1.69—1.55 (4H, m), 1.35—1.25 (12H, m), 0.96 (3H, t, J=7.3 Hz), 0.82 (3H, t, J=7.0 Hz); HR-MS Calcd for $C_{27}H_{40}N_2O_3$ 440.3039, Found 440.3043.

2-Ethoxy-3-{3-[2-(nonylpyridin-2-ylamino)ethoxy]phenyl}propanoic Acid (9) Compound **9** was prepared from **19b** using the procedure described for **1** in 2.5% yield. In the step of the synthesis of **21g**, 2-(diethoxyphosphoryl)ethoxyacetic acid ethyl ether was used instead of ethyl diethylphosphono acetate: yellow oil; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.13 (1H, dd, J=4.9, 1.2 Hz), 7.44 (1H, ddd, J=8.8, 7.0, 1.8 Hz), 7.17 (1H, t, J=7.6 Hz), 6.83 (2H, m), 6.77 (1H, dd, J=7.9, 1.8 Hz), 6.53 (2H, m), 4.15 (2H, t, J=5.5 Hz), 4.07 (1H, dd, J=7.3, 4.9 Hz), 3.90 (2H, m), 3.59 (1H, m), 3.47 (2H, t, J=7.9 Hz), 3.43 (1H, m), 3.09 (1H, dd, J=13.7, 4.6 Hz), 2.98 (1H, dd, J=13.7, 7.3 Hz), 1.67—1.59 (2H, m), 1.36—1.23 (12H, m), 1.16 (3H, t, J=7.0 Hz), 0.88 (3H, t, J=6.7 Hz); HR-MS Calcd for $C_{27}H_{40}N_2O_4$ 456.2988, Found 456.2985.

3-{4-Methoxy-3-[2-(nonylpyridin-2-ylamino)ethoxy]phenyl}propanoic Acid (10) Compound **10** was prepared from **19c** using the procedure described for **1** in 2.7% yield: yellow oil; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.16 (1H, ddd, J=4.9, 1.8, 0.9 Hz), 7.43 (1H, ddd, J=8.8, 7.0, 1.8 Hz), 6.93 (1H, d, J=1.5 Hz), 6.79 (1H, d, J=8.2 Hz), 6.74 (1H, dd, J=8.2, 1.8 Hz), 6.53 (1H, dd, J=7.0, 5.2 Hz), 6.51 (1H, d, J=8.8 Hz), 4.24 (2H, t, J=6.7 Hz), 3.94 (2H, t, J=6.4 Hz), 3.83 (3H, s), 3.45 (2H, t, J=7.6 Hz), 2.88 (2H, t, J=7.6 Hz), 2.61 (2H, t, J=7.6 Hz), 1.64—1.58 (2H, m), 1.31—1.25 (12H, m), 0.88 (3H, t, J=7.0 Hz); HR-MS Calcd for $C_{26}H_{38}N_2O_4$ 442.2832, Found 442.2846.

2-{4-Methoxy-3-[2-(nonylpyridin-2-ylamino)ethoxy]benzyl}butyric Acid (12) Compound **12** was prepared from **19c** using the procedure described for **1** in 3.0% yield. In the step of the synthesis of **21i**, 2-(diethoxyphosphoryl)butyric acid ethyl ester was used instead of ethyl diethylphosphono acetate: yellow oil; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.16 (1H, ddd, J=4.8, 1.8, 0.6 Hz), 7.43 (1H, ddd, J=9.1, 7.3, 2.1 Hz), 6.91 (1H, d, J=1.8 Hz), 6.76 (1H, d, J=8.2 Hz), 6.71 (1H, dd, J=8.2, 2.1 Hz), 6.53 (1H, dd, J=6.4, 5.1 Hz), 6.50 (1H, d, J=8.8 Hz), 4.23 (2H, m), 3.98 (2H, m), 3.86 (1H, m), 3.83 (3H, s), 3.43 (2H, m), 2.87 (1H, dd, J=13.7, 8.5 Hz), 2.71 (1H, dd, J=13.6, 6.1 Hz), 2.56—2.50 (1H, m), 1.70—1.51 (4H, m), 1.34—1.23 (12H, m), 0.95 (3H, t, J=7.0 Hz), 0.88 (3H, t, J=7.0 Hz); HR-MS Calcd for $C_{28}H_{42}N_2O_4$ 470.3145, Found 470.3142.

2-Ethoxy-3-{4-methoxy-3-[2-(nonylpyridin-2-ylamino)ethoxy]phenyl-propanoic Acid (13) Compound **13** was prepared from **19c** using the procedure described for **1** in 3.8% yield. In the step of the synthesis of **21j**, 2-(diethoxyphosphoryl)ethoxyacetic acid ethyl ether was used instead of ethyl diethylphosphono acetate: yellow oil; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.13 (1H, dd, J=4.9, 1.8 Hz), 7.45 (1H, ddd, J=8.8, 7.0, 1.8 Hz), 6.96 (1H, s), 6.78 (2H, m), 6.54 (1H, dd, J=6.7, 5.5 Hz), 6.52 (1H, d, J=8.5 Hz), 4.25 (2H, m), 4.04 (1H, m), 3.93 (2H, m), 3.83 (3H, s), 3.59 (1H, m), 3.44 (2H, t, J=7.6 Hz), 3.05 (1H, dd, J=13.7, 4.9 Hz), 2.97 (1H, dd, J=13.7, 6.4 Hz), 1.65—1.58 (2H, m), 1.35—1.21 (12H, br), 1.17 (3H, t, J=7.0 Hz), 0.88 (3H, t, J=6.7 Hz); HR-MS Calcd for $C_{28}H_{42}N_2O_5$ 486.3094, Found 486.3111.

{4-(2-Bromoethoxy)phenyl}methanol (24a) To a solution of **20a** (890 mg, 3.89 mmol) in 10 ml of EtOH was added $NaBH_4$ (163 mg, 3.89 mmol). The reaction mixture was stirred for 6 h at room temperature. The reaction mixture was poured into water, and extracted with AcOEt. The AcOEt layer was separated, washed with water and brine, and dried over Na_2SO_4. The solvent was removed by evaporation *in vacuo*. 763 mg (81%) of **24a** was obtained as a white solid: ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 7.30 (2H, d, J=8.5 Hz), 6.91 (2H, d, J=8.8 Hz), 4.62 (2H, d, J=5.1 Hz), 4.29 (2H, t, J=6.1 Hz), 3.64 (2H, t, J=6.4 Hz), 1.58 (1H, t, J=5.5 Hz).

Acetic Acid 4-(2-Bromoethoxy)benzyl Ester (25a) To a solution of **24a** (2.31 g, 10.0 mmol) in 20 ml of CH_2Cl_2 were added Ac_2O (3.10 ml, 30.0 mmol) and a catalytic amount of DMAP. The reaction mixture was stirred for 1 d at room temperature and diluted with AcOEt. The AcOEt solution was washed with water and brine, and dried over Na_2SO_4. Purification by silica gel flash chromatography (AcOEt/*n*-hexane =1/5) gave 2.62 g (96%) of **25a** as a light yellow oil: ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 7.29 (2H, d, J=8.8 Hz), 6.90 (2H, d, J=8.8 Hz), 5.04 (2H, s), 4.28 (2H, t, J=6.1 Hz), 3.63 (2H, t, J=6.1 Hz), 2.07 (3H, s).

3-{4-(2-Bromoethoxy)phenyl}-2-methylpropionic Acid Methyl Ester (26a) To a solution of **25a** (0.948 g, 3.47 mmol) and 1-methoxy-1-trimethylsilyloxypropene (1.00 g, 6.24 mmol) in 35 ml of anhydrous CH_2Cl_2 was added magnesium perchlorate (0.129 g, 0.694 mmol) under Ar. The reaction mixture was stirred for 1 d at room temperature. The reaction mixture was diluted with CH_2Cl_2. The CH_2Cl_2 solution was washed with water and brine, and dried over Na_2SO_4. The solvent was removed by evaporation *in vacuo*. Purification by silica gel flash chromatography (AcOEt/*n*-hexane=1/10) gave 0.935 g (90%) of **26a** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 7.07 (2H, dt, J=8.5, 3.0 Hz), 6.82 (2H, dt, J=8.5, 3.0 Hz), 4.26 (2H, t, J=6.4 Hz), 3.63 (3H, s), 3.62 (2H, t, J=6.1 Hz), 2.95 (1H, dd, J=13.4, 7.1 Hz), 2.69 (1H, m), 2.61 (1H, dd, J=13.4, 7.6 Hz), 1.14 (3H, d, J=7.0 Hz).

3-{4-[2-(Nonylpyridin-2-ylamino)ethoxy]phenyl}-2,2-dimethylpropanoic Acid Methyl Ester (27a) Compound **27a** was prepared from **26a** using the procedure described for **23a** in 11% yield: ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.13 (1H, ddd, J=4.9, 1.8, 0.9 Hz), 7.41 (1H, ddd, J=8.8, 7.0, 2.1 Hz), 7.04 (2H, d, J=8.5 Hz), 6.82 (2H, dt, J=8.5, 1.8 Hz), 6.5 (2H, m), 4.14 (2H, t, J=6.1 Hz), 3.91 (2H, t, J=5.8 Hz), 3.63 (3H, s), 3.47 (2H, t, J=7.9 Hz), 2.94 (1H, dd, J=13.4, 6.7 Hz), 2.68 (1H, m), 2.59 (1H, dd, J=13.4, 7.6 Hz), 1.64—1.57 (2H, m), 1.35—1.25 (12H, m), 1.13 (3H, d, J=7.0 Hz), 0.88 (3H, t, J=7.2 Hz); HR-MS Calcd for $C_{27}H_{40}N_2O_3$ 440.3039, Found 440.3046.

3-{4-[2-(Nonylpyridin-2-ylamino)ethoxy]phenyl}-2-methylpropanoic Acid (2) Compound **2** was prepared from **27a** using the procedure described for **1** in 97% yield: colorless oil; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.13 (2H, d, J=4.8 Hz), 7.41 (1H, ddd, J=8.8, 6.7, 1.8 Hz), 7.08 (2H, d, J=8.5 Hz), 6.82 (2H, d, J=8.5 Hz), 6.5 (2H, m), 4.12 (2H, m), 3.90 (2H, t, J=5.8 Hz), 3.47 (2H, t, J=7.9 Hz), 2.98 (1H, dd, J=13.4, 7.0 Hz), 2.73 (1H, m), 2.63 (1H, dd, J=13.4, 7.6 Hz), 1.65—1.58 (2H, br), 1.35—1.25 (12H, m), 1.17 (3H, d, J=6.7 Hz), 0.88 (3H, t, J=7.0 Hz); HR-MS Calcd for $C_{26}H_{38}N_2O_3$ 426.2882, Found 426.2889.

3-{4-[2-(Nonylpyridin-2-ylamino)ethoxy]phenyl}-2,2-dimethylpropanoic Acid (5) Compound **5** was prepared from **20a** using the procedure described for **2** in 4.2% yield. In the step of the synthesis of **26b**, dimethylketene methyltrimethylsilyl acetal was used instead of 1-methoxy-1-trimethylsilyloxypropene: light yellow oil; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.14 (2H, dd, J=4.9, 1.5 Hz), 7.41 (1H, ddd, J=8.8, 6.7, 1.8 Hz), 7.05 (2H, d, J=8.5 Hz), 6.80 (2H, d, J=8.5 Hz), 6.50 (2H, m), 4.12 (2H, t, J=5.8 Hz), 3.90 (2H, t, J=5.8 Hz), 3.47 (2H, t, J=7.6 Hz), 2.81 (2H, s), 1.65—1.59 (2H, m), 1.35—1.25 (12H, m), 1.17 (6H, s), 0.88 (3H, t, J=7.0 Hz); HR-MS Calcd for $C_{27}H_{40}N_2O_3$ 440.3039, Found 440.3040.

3-{4-Methoxy-3-[2-(Nonylpyridin-2-ylamino)ethoxy]phenyl}-2-methylpropanoic Acid (11) Compound **11** was prepared from **20c** using the procedure described for **2** in 3.3% yield: colorless oil; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.16 (1H, ddd, J=4.9, 1.8, 0.9 Hz), 7.44 (1H, ddd, J=8.8, 6.7, 1.8 Hz), 6.95 (1H, d, J=1.8 Hz), 6.78 (1H, d, J=7.9 Hz), 6.71 (1H, dd, J=8.2, 1.8 Hz), 6.54 (1H, dd, J=7.0, 5.1 Hz), 6.50 (1H, d, J=8.8 Hz), 4.25 (2H, m), 3.92 (2H, m), 3.83 (3H, s), 3.43 (2H, t, J=7.3 Hz), 2.92 (1H, m), 2.70 (2H, m), 1.64—1.54 (2H, m), 1.31—1.20 (12H, m), 1.16 (3H, d, J=6.7 Hz), 0.88 (3H, t, J=6.7 Hz); HR-MS Calcd for $C_{27}H_{40}N_2O_4$ 456.2988, Found 456.3007.

3-{4-Methoxy-3-[2-(Nonylpyridin-2-ylamino)ethoxy]phenyl}-2,2-dimethylpropanoic Acid (14) Compound **14** was prepared from **20c** using the procedure described for **2** in 8.4% yield. In the step of the synthesis of **26d**, dimethylketene methyltrimethylsilyl acetal was used instead of 1-methoxy-1-trimethylsilyloxypropene: colorless oil; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.16 (1H, ddd, J=5.1, 1.8, 0.6 Hz), 7.45 (1H, ddd, J=8.8, 7.0, 1.8 Hz), 7.00 (1H, d, J=2.1 Hz), 6.76 (1H, d, J=8.2 Hz), 6.68 (1H, dd, J=8.2, 1.8 Hz), 6.55 (1H, dd, J=7.0, 5.2 Hz), 6.50 (1H, d, J=8.8 Hz), 4.27 (2H, t, J=7.0 Hz), 3.90 (2H, t, J=7.3 Hz), 3.84 (3H, s), 3.40 (2H, t, J=7.6 Hz), 2.79 (2H, s), 1.63—1.57 (2H, m), 1.35—1.25 (12H, m), 1.20 (6H, s), 0.88 (3H, t, J=7.0 Hz); HR-MS Calcd for $C_{28}H_{42}N_2O_4$ 470.3145, Found 470.3144.

3-{4-[3-(Nonylpyridin-2-ylamino)propyl]phenyl}acrylic Acid Ethyl Ester (28a) (Chart 3) Compound **28a** was prepared from **21a** using the procedure described for **23a** in 13% yield: ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.14 (1H, ddd, J=4.8, 2.1, 0.9 Hz), 7.63 (1H, d, J=16.2 Hz), 7.45 (2H, d, J=8.8 Hz), 7.42 (1H, ddd, J=8.8, 7.0, 1.8 Hz), 6.91 (2H, d, J=8.5 Hz), 6.52 (1H, dd, J=7.0, 5.5 Hz), 6.50 (1H, d, J=8.8 Hz), 6.30 (1H, d, J=15.8 Hz), 4.25 (2H, q, J=7.0 Hz), 4.21 (2H, t, J=5.8 Hz), 3.94 (2H, t, J=5.8 Hz), 3.46 (2H, t, J=7.9 Hz), 1.64—1.60 (2H, m), 1.36—1.30 (3H, t, J=7.0 Hz), 1.33—1.23 (12H, br), 0.88 (3H, t, J=7.0 Hz); HR-MS Calcd for $C_{27}H_{38}N_2O_3$ 438.2882, Found 438.2855.

3-{4-[3-(Nonylpyridin-2-ylamino)propyl]phenyl}acrylic Acid (15) To a solution of **28a** (84 mg, 0.192 mmol) in 2 ml of THF and 2 ml of EtOH was added a solution of 2 N aqueous NaOH (0.29 ml, 0.580 mmol). The reaction mixture was stirred for 18 h at room temperature, and neutralized to pH 7 with aqueous 2 N HCl. Purification by silica gel flash chromatography ($CHCl_3$/MeOH=19/1) gave 78 mg (99%) of white solid. The 41 mg of the white solid was recrystallized from AcOEt–*n*-hexane and collected by filtration to give 22 mg (52%) of **15** as a white solid: mp 142—143 °C; ^{1}H-NMR ($CDCl_3$, 500 MHz, δ: ppm) 8.15 (1H, dd, J=4.9, 1.8 Hz), 7.70 (1H, d, J=15.9 Hz), 7.47 (2H, d, J=8.8 Hz), 7.43 (1H, ddd, J=8.8, 7.0, 1.8 Hz), 6.93 (1H, d, J=8.8 Hz), 6.53 (1H, dd, J=6.4, 5.5 Hz), 6.50 (1H, d,