Design, Synthesis, and Discovery of a Novel CCR1 Antagonist

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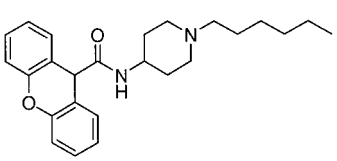
The CC chemokines may play an important role in the pathogenesis of chronic inflammatory diseases including rheumatoid arthritis, and their effects are thought to be mediated through CCR1 receptors. Several nonpeptide CCR1 receptor antagonists that showed high affinity for human CCR1 receptors have been identified; however, their effectiveness in animal models of inflammatory diseases has been scarcely demonstrated, probably due to species selectivity of the antagonists. To elucidate the pathophysiological role of CCR1 receptors in murine models of disease, we looked for a potent antagonist for both murine and human CCR1 receptors. Screening of our chemical collection for inhibition of ^{125}I-MIP-1α binding to human CCR1 receptors transfected in CHO cells led to the identification of xanthene-9-carboxamide **1a** as the lead compound. Derivatization of **1a** by quaternarizing the piperidine nitrogen with various alkyl groups and by installing substituents into the xanthene moiety dramatically improved the inhibitory activity against both human and murine CCR1 receptors. As a result, **2q-1** showing IC_{50} values of 0.9 and 5.8 nM for human and murine CCR1 receptors, respectively, was discovered. This compound is the first murine CCR1 receptor antagonist and may be a useful tool for clarifying the role of CCR1 receptors in murine models of disease.

Introduction

Chemokines, constituting a large family of chemotactic cytokines, are thought to be proinflammatory molecules implicated in the recruitment and activation of leukocytes in various diseases such as rheumatoid arthritis, multiple sclerosis, and asthma.[1,2] Chemokines are largely classified into two subfamilies, CXC or α-chemokines and CC or *β*-chemokines, based on the position of the first cysteine pair of their four conserved cysteines.[3] The specific effects of chemokines are mediated by their receptor which belongs to a family of the seven transmembrane G-protein-coupled receptors (GPCR). A total of 18 chemokine receptors including CCR1−11, CXCR1−5, XCR1, and CX_3CR1 receptors are known to date.

Among the chemokines, MIP-1α (macrophage inflammatory protein-1α) and RANTES (regulated on activation normal T-cell expressed and secreted), known as ligands for CCR1 receptors, may play an important role in chronic inflammatory diseases such as rheumatoid arthritis[4] and multiple sclerosis.[5] For example, it was reported that treatment of antibodies to RANTES resulted in a great reduction in clinical scores compared to the scores of untreated animals in a rat adjuvant-induced arthritis model.[6] Furthermore, RANTES protein and mRNA were reported to be upregulated in the synovial fibroblasts of patients with rheumatoid arthritis.[7] Taken together, these findings suggested that selective antagonists for CCR1 receptors may be an attractive therapeutic target for chronic inflammatory diseases.

Several nonpeptide CCR1 receptor antagonists that showed high affinity for human CCR1 receptors have been identified by pharmaceutical companies such as Berlex[8] and Takeda.[9] Recently, species selectivity of the CCR1 antagonist was reported, especially between humans and mice.[10] This species selectivity may complicate the demonstration of efficacy of a CCR1 antagonist in animal models of disease. Therefore, we decided to seek a potent antagonist for both mouse and human CCR1 receptors, to determine the pathophysiological role(s) of CCR1 receptors in murine models of disease.



1a **Berlex compound**

A screening of our chemical collection of compounds for percent inhibition at 1 *μ*M for ^{125}I-MIP-1α binding to human CCR1 receptors transfected in CHO led to the discovery of xanthenecarboxamide **1a** with an IC_{50} value of 510 nM as a lead compound. In this paper, we describe the design, synthesis, and structure−activity relationship (SAR) of xanthenecarboxamide derivatives on the binding affinity for mouse and human CCR1 receptors, based on the structure of the lead compound **1a**.

Results and Discussion

Chemistry. General methods for the synthesis of compounds **1a**−**n** and **2a**−**r** are outlined in Scheme 1. Acylation of the 4-aminopiperidine derivative **3** with an acid using the WSC−HOBT method, followed by deprotection of the Boc group under acidic conditions and

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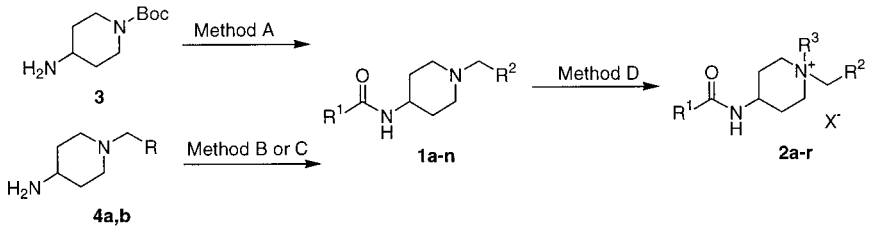
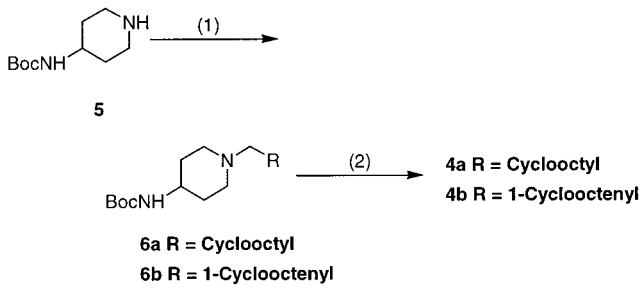
Scheme 1[a]



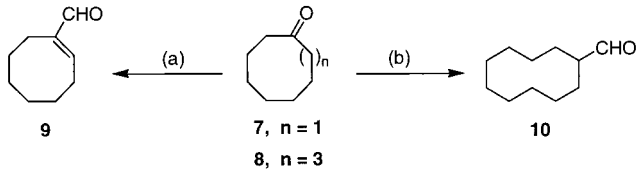
[a] Reagents: Method A − (1) R^1CO_2H, WSC−HCl, HOBt, Et_3N; (2) HCl−MeOH; (3) R^2CHO, $NaBH(OAc)_3$; Method B − R^1CO_2H, WSC−HCl, HOBt, Et_3N; Method C − R^1CO_2H, CDI; Method D − R^3X.

Scheme 2[a]



[a] Reagents: (1) RCHO, $NaBH(OAc)_3$; (2) HCl−MeOH.

Scheme 3[a]



[a] Reagents: (a) (1) *p*-$TsNHNH_2$, (2) *n*-BuLi, DMF; (b) (1) *n*-BuLi, $(EtO)_2POCH_2NC$, (2) HCl.

subsequent reductive *N*-alkylation with an appropriate aldehyde, gave compound **1** in a 60−70% yield (method A). Alternatively, compound **1** was also synthesized from amine **4** that was commercially available or easily prepared as shown in Scheme 2 (method B or C). Most of the aldehydes used for the preparation of compound **1** were commercially available. Cycloalkylcarboxaldehydes such as 1-cyclooctenyl- and cyclodecanylcarboxaldehyde were prepared as shown in Scheme 3. Cyclooctanone tosylhydrazone was treated with *n*-BuLi and subsequently reacted with DMF to afford **8** in 53% yield. Cyclodecanylcarboxaldehyde was obtained from cyclodecanone by reaction with lithium diethyl (isocyanomethyl)phosphonate and subsequent acidic hydrolysis.

Compound **1** was quaternarized with an appropriate alkyl halide to provide the quaternary ammonium derivative **2** (method D) as a mixture of two isomers (*cis* and *trans*) attributed to the 4-substituted piperidinium structure in a ratio of ~2:1, which was evaluated in the binding assay without separation. Compound **2q**, the most potent compound, was separated by silica gel column chromatography to give a major isomer (**2q-1**, 59%) and a minor isomer (**2q-2**, 32%).

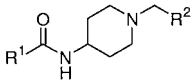
Biological Properties. Compounds **1a**−**n** and **2a**−**r** were screened for their inhibitory activity against ^{125}I-MIP-1α binding to both human and mouse CCR1 receptors. The selected compound was examined for its functional antagonist activity in U937 cells transfected with mouse and human CCR1 receptors, respectively.

Optimization of the lead compound **1a** was initiated by substituting the *n*-hexyl group on the piperidine nitrogen with cycloalkyl or arylmethyl groups. Replacement with a cyclohexyl (**1b**), cyclooctyl (**1c**), or cyclodecanyl (**1d**) group resulted in improvement in the binding affinity for human CCR1 receptors, especially in the case of **1c,** which showed approximately 3-fold higher binding affinity compared with **1a**. By contrast, replacement with aromatic groups, such as benzyl (**1e**) and 2-naphthylmethyl (**1f**) led to a substantial loss of affinity. Unfortunately, binding affinity of the compounds for mouse CCR1 receptors was not detected, indicating that there is high species selectivity of this xanthenecarboxamide class of compounds between human and mouse CCR1 receptors. Next, the cyclooctyl moiety on the piperidinyl side chain of **1c** being fixed, replacement of the xanthene moiety was performed to explore an alternative pharmacophore. When the xanthen-9-yl moiety of **1c** was replaced with an anthracen-9-yl one, the resulting compound **1g** lost potency. Substitution of this moiety with a diphenylmethyl (**1h**) or diphenylmethyl(hydroxy)methyl (**1i**) group resulted in an approximately 10-fold reduction of binding affinity for human CCR1 receptors. Interestingly, a 2,7-dichrolo- (**1j**) or 2,7-dibromoxanthen-9-yl (**1k**) group significantly reduced potency. These results indicated that the xanthen-9-yl moiety was optimal as the acid segment of **1c**.

Comparison of the binding affinity of **1c** with that of the Berlex compound in our binding assay system revealed that the Berlex compound was more active than **1c** and that both compounds still possessed species selectivity between humans and mice.

We hypothesized that the piperidine nitrogen in this series of compounds was recognized as a cation binding site that was close to the hydrophobic site recognizing the cycloalkyl moiety in the CCR1 receptor antagonist binding pocket.[8,11] Therefore, a quaternary ammonium group could be used to replace the tertiary piperidine nitrogen. Following up this hypothesis, the quaternary ammonium compound **2a** was prepared and evaluated in the binding assay. Interestingly, **2a** showed greatly enhanced binding affinity not only to human CCR1 but also to mouse CCR1 receptors, with IC_{50} values of 14 and 2100 nM, respectively. Further investigation of the substitution of the methyl group with other alkyl groups on the quaternary ammonium nitrogen in **2a** was performed to optimize this moiety. Replacement of the methyl group of **2a** with an ethyl (**2b**), *n*-propyl (**2c**), *n*-butyl (**2d**), or 2-propenyl (**2e**) group suggested that **2c** was optimal for binding affinity to both human and mouse CCR1 receptors. Substitution with a benzyl group led to a 26-fold decrease in binding affinity to human receptors while maintaining the binding to

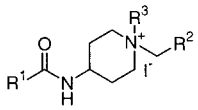
Table 1. Binding Affinity to Human and Mouse CCR1 Receptors[a]



compd	R^1	R^2	% yield (method)	binding affinity: IC_{50} (nM) hCCR1	mCCR1
1a	9-xanthenyl	*n*-pentyl	81 (A)	510	>10000
1b	9-xanthenyl	cyclohexyl	76 (A)	200	>10000
1c	9-xanthenyl	cyclooctyl	50 (B)	140	>10000
1d	9-xanthenyl	cyclodecanyl	93 (A)	800	>10000
1e	9-xanthenyl	phenyl	94 (B)	>10000	>10000
1f	9-xanthenyl	2-naphthyl	51 (A)	>10000	>10000
1g	9-anthracenyl	cyclooctyl	49 (B)	>10000	>10000
1h	diphenylmethyl	cyclooctyl	78 (B)	1100	>10000
1i	diphenylhydroxymethyl	cyclooctyl	45 (C)	1600	>10000
1j	2,7-dichloro-9-xanthenyl	cyclooctyl	83 (C)	>10000	>10000
1k	2,7-dibromo-9-xanthenyl	cyclooctyl	68 (C)	5100	>10000
1l	9-xanthenyl	1-cyclooctenyl	73 (B)	51	590
1m	2,7-dichloro-9-xanthenyl	1-cyclooctenyl	82 (C)	240	1900
1n	2,7-dibromo-9-xanthenyl	1-cyclooctenyl	83 (C)	150	2500
Berlex compound				48	5000

[a] The IC_{50} value of each compound is the mean of three assays.

Table 2. Binding Affinity to Human and Mouse CCR1 Receptors[a]



2a-2m; R^2 = Cyclooctyl, **2n-2r**; R^2 = 1-Cyclooctenyl

compd	R^1	R^3	% yield	binding affinity: IC_{50} (nM) hCCR1	mCCR1
2a	9-xanthenyl	methyl	50	14	2100
2b	9-xanthenyl	ethyl	50	5.2	660
2c	9-xanthenyl	*n*-propyl	62	3.6	270
2d	9-xanthenyl	*n*-butyl	6	5.0	380
2e[b]	9-xanthenyl	allyl	21	9.7	670
2f[b]	9-xanthenyl	benzyl	80	370	1300
2g	9-anthracenyl	methyl	65	1800	>10000
2h	diphenylmethyl	methyl	70	800	>10000
2i	diphenylhydroxymethyl	methyl	55	2100	>10000
2j	2,7-dichloro-9-xanthenyl	methyl	57	17	710
2k	2,7-dibromo-9-xanthenyl	methyl	80	3.9	240
2l	2,7-dibromo-9-xanthenyl	ethyl	75	3.3	140
2m	2,7-dibromo-9-xanthenyl	*n*-propyl	9	3.1	140
2n	9-xanthenyl	methyl	75	2.5	350
2o	9-xanthenyl	ethyl	39	2.0	63
2p	9-xanthenyl	*n*-propyl	10	2.8	270
2q	2,7-dichloro-9-xanthenyl	ethyl	91	1.2	12
2q-1	2,7-dichloro-9-xanthenyl	ethyl	53	0.9	5.8
2q-2	2,7-dichloro-9-xanthenyl	ethyl	29	47	740
2r	2,7-dibromo-9-xanthenyl	ethyl	52	1.9	12

[a] The IC_{50} value of each compound is the mean of three assays. [b] The counteranion of the compound was Br^-.

mouse receptors. The substituents on the quaternary ammonium nitrogen of **2a** being fixed, the xanthene moiety was again replaced with an anthracen-9-yl (**2g**), diphenylmethyl (**2h**), or diphenyl(hydroxy)methyl (**2i**). This derivatization considerably lowered the binding affinities. Unlike the tertiary amine **1n**, a 2,7-dibromoxanathen-9-yl group (**2k**) enhanced the binding affinity to both human and mouse CCR1 receptors. As the substituent on the quaternary ammonium nitrogen of **2k**, an *n*-propyl group (**2m**) seemed optimal for human receptors. Although **2m** possessed highly potent binding affinity for human receptors, its affinity for mouse CCR1 receptors was insufficient.

As described above, the SAR of the xanthenecarboxamide class of compounds revealed that the cyclooctyl moiety also played an important role in increasing the binding potency to human CCR1 receptors and that the putative hydrophobic site close to the cationic recognition site in the binding pocket of CCR1 receptors seemed stringent. To further explore an alternative substituent to the cyclooctyl moiety, a 1-cyclooctenyl group was designed. The resulting compounds **1l**−**n** exhibited unexpectedly enhanced binding affinity to mouse receptors, compared with **1c**. Particularly, **1l** was found to be most potent in the tertiary amine series. Quaternarization of **1l** by a methyl (**2n**), ethyl (**2o**), or *n*-propyl (**2p**) group revealed that an ethyl group appeared optimal on the piperidinium nitrogen. Quaternarization of **1m**,**n** by an ethyl group led to compounds **2q**,**r**, which showed high binding affinity for both human and mouse receptors. In paticular, **2q** seemed most attractive, showing IC_{50} values of 1.2 and 12 nM for human and mouse CCR1 receptors, respectively. Since the quaternary ammonium derivatives were a mixure of the two isomers attributed to the 4-substituted piperidinium structure, **2q** was separated chromatographically to provide **2q-1**[12] and **2q-2**, which were evaluated for their binding affinity. The more active isomer **2q-1** showed IC_{50} values of 0.9 and 5.8 nM for human and mouse CCR1 receptors, respectively, while another isomer **2q-2** was 50- and 120-fold less active than **2q-1** for these receptors, respectively.

To determine whether the compound **2q-1** is also a functional antagonist for CCR1 receptors, we measured its ability to inhibit the MIP-1α-induced Ca^{2+} response in U937 cells expressing human or mouse CCR1 receptors. The IC_{50} values of **2q-1** were 0.73 and 21 nM for inhibiting the MIP-1α-induced Ca^{2+} response in these cells expressing human and mouse CCR1 receptors, respectively, indicating that **2q-1** is a potent functional CCR1 receptor antagonist.

Finally, the selectivity of **2q-1** over some other chemokine receptors was examined. Interestingly, **2q-1** was found to be a potent human CCR3 receptor antagonist because it inhibited ^{125}I-Eotaxin binding to human CCR3 receptors with an IC_{50} value of 0.58 nM. However, **2q-1** showed high selectivity for other CCR receptors

Table 3. In Vitro Selectivity of **2q-1** against Some Chemokine Receptors

receptor	IC_{50} (nM)	receptor	IC_{50} (nM)
hCCR1	0.90 0.73 (Ca^{2+} response)	hCCR2b	>1000
mCCR1	5.8 21 (Ca^{2+} response)	hCCR4[a]	>10000
hCCR3	0.58 6.1 (Ca^{2+} response)	hCCR5	>1000
mCCR3	460 350 (Ca^{2+} response)	hCXCR1,2[b]	>1000
		hCX_3CR1[c]	>10000

[a] Tarc-induced increases in intracellular Ca^{2+} concentrations in KU812 cells. [b] ^{125}I-Interleukin-8 binding to human neutrophil membranes. [c] Fractalkine-induced increases in Ca^{2+} concentrations in HEL cells.

as shown in Table 3. These results suggested a high degree of sequence homology between the binding sites of human CCR1 and CCR3 receptors.[13]

Conclusion

The initial screening of our compound libraries for inhibitory activity against ^{125}I-MIP-1α binding to human CCR1 receptors led to the identification of **1a** as a lead compound. Derivatization of **1a** focusing on substitution on the piperidine nitrogen and installment of substituents into the xanthene group resulted in the discovery of **2q-1** showing potent antagonist activity against not only human but also mouse CCR1 receptors. Therefore, **2q-1** may be a useful tool for clarifying the involvement of CCR1 receptors in mouse disease models.

Experimental Section

Materials and Methods. All reagents and solvents were of commercial quality and were used without further purification unless otherwise noted. Melting points were determined with a Yanaco MP micromelting point apparatus and were not corrected. ^{1}H NMR spectra were recorded on a Varian VXR 300 spectrometer with tetramethylsilane as an internal standard. Mass spectrometry was performed with a JEOL JMS-SX102A spectrometer. Elemental analyses were performed on an EA-1108 FISONS Instruments CHNSO analyzer and the results were within 0.4% of calculated values. TLC was done with Merck Kieselgel F_{254} precoated plates. Silica gel column chromatography was carried out on Merck silica gel 60 (mesh 63−200 nm). Carboxylic acid fragments used for preparation of the compounds shown in Tables 1 and 2 were commercially available or were prepared using standard literature procedures.

General Method A. *N*-(1-*n*-Hexylpiperidin-4-yl)xanthene-9-carboxamide (1a). (i) *N*-(1-*tert*-Butyloxycarbonylpiperidin-4-yl)xanthene-9-carboxamide. To a stirred solution of xanthene-9-carboxylic acid (400 mg, 1.77 mmol) and 4-amino-1-*tert*-butoxycarbonylpiperidine[14] (350 mg, 1.75 mmol) in $CHCl_3$ (20 mL) were added 1-hydroxybenzotriazole (350 mg, 2.59 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (500 mg, 2.61 mmol) at room temperature. After stirring for 20 h, the reaction mixture was diluted with EtOAc, washed with saturated $NaHCO_3$ solution, 10% citric acid solution, and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (3% MeOH in $CHCl_3$) to give *N*-(1-*tert*-butyloxycarbonylpiperidin-4-yl)xanthene-9-carboxamide (546 mg, 76%) as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 0.99−1.15 (m, 2H), 1.40 (s, 9H), 1.65−1.76 (m, 2H), 2.70−2.86 (m, 2H), 3.67−3.94 (m, 3H), 4.87 (s, 1H), 5.09 (d, J = 7.8 Hz, 1H), 7.03−7.17 (m, 4H), 7.26−7.41 (m, 4H); HRMS calcd for $C_{24}H_{29}N_2O_4$ $(M + H)^+$ 409.2127, found 409.2122.

(ii) *N*-(Piperidin-4-yl)xanthene-9-carboxamide. A solution of *N*-(1-*tert*-butyloxycarbonylpiperidin-4-yl)xanthene-9-carboxamide (510 mg, 1.25 mmol) in 10% HCl−MeOH (10 mL) was stirred at room temperature for 20 h. The mixture was concentrated in vacuo, and the residue was adjusted to pH 9 with saturated $NaHCO_3$ and extracted with $CHCl_3$. The organic layer was dried ($MgSO_4$) and concentrated in vacuo to give *N*-(piperidin-4-yl)xanthene-9-carboxamide (546 mg, 76%) as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 0.98−1.15 (m, 2H), 1.66−1.80 (m, 2H), 2.51−2.65 (m, 2H), 2.80−2.95 (m, 2H), 3.66−3.82 (m, 1H), 4.86 (s, 1H), 5.12 (d, J = 8.0 Hz, 1H), 7.05−7.16 (m, 4H), 7.25−7.45 (m, 4H); HRMS calcd for $C_{19}H_{21}N_2O_2$ $(M + H)^+$ 309.1603, found 309.1597.

(iii) To a solution of *N*-(piperidin-4-yl)xanthene-9-carboxamide (100 mg, 0.32 mol) in CH_2Cl_2 (5.0 mL) were added *n*-hexanal (60 mL, 0.50 mmol) and $NaBH(OAc)_3$ (100 mg, 0.47 mmol) at room temperature, and the mixture was stirred for 20 h. After the addition of saturated $NaHCO_3$ solution, the mixture was extracted with $CHCl_3$. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by silica gel column chromatography (3−5% MeOH in $CHCl_3$) and triturated with *i*-PrOH to give **1a** (103 mg, 81%) as a colorless solid: mp 213-214 °C; ^{1}H NMR ($CDCl_3$) δ 0.85 (t, J = 7.0 Hz, 3H), 1.15−1.90 (m, 12H), 1.95−2.75 (m, 6H), 3.60−3.77 (m, 1H), 4.82 (s, 1H), 5.15 (d, J = 5.7 Hz, 1H), 7.05−7.40 (m, 8H); HRMS calcd for $C_{25}H_{33}N_2O_2$ $(M + H)^+$ 393.2542, found 393.2530. Anal. ($C_{25}H_{32}N_2O_2 \cdot 0.33H_2O$) C, H, N.

The following compounds were prepared in a manner similar to the procedure described for **1a** using *N*-(piperidin-4-yl)-xanthene-9-carboxamide and an appropriate aldehyde.

***N*-[1-(Cyclohexylmethyl)piperidin-4-yl]xanthene-9-carboxamide (1b).** This was prepared from cyclohexylcarboxaldehyde (76%): mp 219−222 °C; ^{1}H NMR ($CDCl_3$) δ 0.77−0.84 (m, 2H), 1.12−1.26 (m, 4H), 1.34−1.39 (m, 1H), 1.57−1.75 (m, 10H), 1.89−1.96 (m, 2H), 2.51−2.56 (m, 2H), 3.63−3.68 (m, 1H), 4.84 (s, 1H), 5.08 (d, J = 5.9 Hz, 1H), 7.08−7.14 (m, 4H), 7.25−7.31 (m, 2H), 7.36−7.40 (m, 2H); HRMS calcd for $C_{26}H_{33}N_2O_2$ $(M + H)^+$ 405.2542, found 405.2526. Anal. ($C_{26}H_{32}N_2O_2 \cdot 0.5H_2O$) C, H, N.

***N*-[1-(Cyclodecanylmethyl)piperidin-4-yl]xanthene-9-carboxamide (1d).** This was prepared from cyclodecanylcarboxaldehyde **(10)** (93%): mp 191−193 °C; ^{1}H NMR ($CDCl_3$) δ 1.11−1.80 (m, 23H), 1.85−2.08 (m, 4H), 2.46−2.65 (m, 2H), 3.56−3.73 (m, 1H), 4.84 (s, 1H), 5.09 (d, J = 7.2 Hz, 1H), 7.05−7.16 (m, 4H), 7.24−7.33 (m, 2H), 7.35−7.42 (m, 2H); HRMS calcd for $C_{30}H_{41}N_2O_2$ $(M + H)^+$ 461.3168, found 461.3173. Anal. ($C_{30}H_{40}N_2O_2 \cdot 0.1{}^i$PrOH) C, H, N.

***N*-[1-(2-Naphthylmethyl)piperidin-4-yl]xanthene-9-carboxamide (1f).** This was prepared from 2-naphthaldehyde (51%): mp 218−220 °C; ^{1}H NMR ($CDCl_3$) δ 1.12−1.32 (m, 2H), 1.67−1.80 (m, 2H), 1.98−2.16 (m, 2H), 2.50−2.72 (m, 2H), 3.55 (s, 2H), 3.59−3.80 (m, 1H), 4.84 (s, 1H), 5.10 (d, J = 7.7 Hz, 1H), 7.00−7.85 (m, 15H); HRMS calcd for $C_{30}H_{29}N_2O_2$ $(M + H)^+$ 449.2229, found 449.2227. Anal. ($C_{30}H_{28}N_2O_2$) C, H, N.

General Method B. *N*-(1-Benzylpiperidin-4-yl)xanthene-9-carboxamide (1e). To a stirred solution of xanthene-9-carboxylic acid (3.0 g, 13.3 mmol) and 4-amino-1-benzylpiperidine (2.7 mL, 13.2 mmol) in DMF (60 mL) were added 1-hydroxybenzotriazole (3.0 mg, 19.6 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (3.8 g, 19.8 mmol) and triethylamine (3.7 mL, 34.1 mmol), and the mixture was stirred at room temperature for 20 h. The solvent was removed in vacuo, and the residue was diluted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ solution and water, dried ($MgSO_4$), and concentrated in vacuo. The residue was triturated with *i*-PrOH to give **1e** (5.0 g, 94%) as a colorless solid: mp 212−214 °C; ^{1}H NMR ($CDCl_3$) δ 1.12−1.28 (m, 2H), 1.67−1.79 (m, 2H), 1.94−2.09 (m, 2H), 2.50−2.68 (m, 2H), 3.39 (s, 2H), 3.59−3.76 (m, 1H), 4.84 (s, 1H), 5.08 (d, J = 7.6 Hz, 1H), 7.00−7.41 (m, 13H); HRMS calcd for $C_{26}H_{27}N_2O_2$ $(M + H)^+$ 399.2073, found 399.2079. Anal. ($C_{26}H_{26}N_2O_2 \cdot 0.5H_2O$) C, H, N.

The following compounds were prepared in a manner similar to general method B using the amine **4a** or **4b** and an appropriate acid.

***N*-[1-(Cyclooctylmethyl)piperidin-4-yl]xanthene-9-carboxamide (1c).** This was prepared from xanthene-9-carboxylic acid and **4a** (50%): mp 212−215 °C; ^{1}H NMR ($CDCl_3$) δ 1.11−1.74 (m, 19H), 1.89−1.97 (m, 4H), 2.54−2.58 (m, 2H), 3.64−3.66 (m, 1H), 4.84 (s, 1H), 5.10 (d, J = 8.1 Hz, 1H), 7.08−7.14 (m, 4H), 7.26−7.33 (m, 2H), 7.37−7.40 (m, 2H); HRMS calcd for $C_{28}H_{37}N_2O_2$ (M + H)$^+$ 433.2855, found 433.2839. Anal. ($C_{28}H_{36}N_2O_2\cdot 0.33H_2O$) C, H, N.

***N*-[1-(Cyclooctylmethyl)piperidin-4-yl]anthracene-9-carboxamide (1g).** This was prepared from anthracene-9-carboxylic acid and **4a** (49%): mp 186−189 °C; ^{1}H NMR ($CDCl_3$) δ 1.12−1.95 (m, 17H), 2.02−2.40 (m, 6H), 2.75−3.00 (m, 2H), 4.25−4.45 (m, 1H), 5.96 (d, J = 7.1 Hz, 1H), 7.35−7.65 (m, 4H), 7.88−8.18 (m, 4H), 8.48 (s, 1H); HRMS calcd for $C_{29}H_{37}N_2O$ (M+H)$^+$ 429.2906, found 429.2906. Anal. ($C_{29}H_{36}N_2O\cdot 0.5H_2O$) C, H, N.

***N*-[1-(Cyclooctylmethyl)piperidin-4-yl]-2,2-diphenylacetamide (1h).** This was prepared from 2,2-diphenylacetic acid and **4a** (78%): mp 136−137 °C; ^{1}H NMR ($CDCl_3$) δ 1.11−1.98 (m, 19H), 1.98−2.15 (m, 4H), 2.63−2.79 (m, 2H), 3.78−3.95 (m, 1H), 4.90 (s, 1H), 5.44 (d, J = 8.0 Hz, 1H), 7.18−7.35 (m, 10H); HRMS calcd for $C_{28}H_{39}N_2O$ (M + H)$^+$ 419.3062, found 419.3060. Anal. ($C_{28}H_{38}N_2O$) C, H, N.

***N*-[1-(1-Cyclooctenylmethyl)piperidin-4-yl]xanthene-9-carboxamide (1l).** This was prepared from xanthene-9-carboxylic acid and **4b** (73%): mp 196−198 °C; ^{1}H NMR ($CDCl_3$) δ 1.08−1.27 (m, 2H), 1.33−1.52 (m, 8H), 1.62−1.78 (m, 2H), 1.82−1.99 (m, 2H), 2.00−2.20 (m, 4H), 2.44−2.65 (m, 2H), 2.71 (s, 2H), 3.58−3.75 (m, 1H), 4.84 (s, 1H), 5.10 (d, J = 8.1 Hz, 1H), 5.40 (t, J = 8.2 Hz, 1H), 7.10 (t, J = 7.4 Hz, 2H), 7.13 (d, J = 7.4 Hz, 2H), 7.29 (dd, J = 7.4, 7.8 Hz, 2H), 7.38 (d, J = 7.8 Hz, 2H); HRMS calcd for $C_{28}H_{35}N_2O_2$ (M + H)$^+$ 431.2699, found 431.2678. Anal. ($C_{28}H_{34}N_2O_2$) C, H, N.

General Method C. *N*-[1-(Cyclooctylmethyl)piperidin-4-yl]-2,7-dichloroxanthene-9-carboxamide (1j). A solution of 2,7-dichloroxanthene-9-carboxylic acid[15] (500 mg, 1.69 mmol) and 1,1′-carbonyldiimidazole (330 mg, 2.04 mmol) in THF (20 mL) were stirred at room temperature for 1 h. To this mixture was added **4a** (500 mg, 1.69 mmol), and the mixture was stirred for 12 h. The mixture was diluted with EtOAc and washed with saturated $NaHCO_3$ solution and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by silica gel column chromatography (3% MeOH in $CHCl_3$), and triturated with *i*-PrOH to give **1j** (700 mg, 83%) as a colorless solid: mp 217−220 °C; ^{1}H NMR ($CDCl_3$) δ 1.15−2.13 (m, 23H), 2.52−2.70 (m, 2H), 3.69−3.75 (m, 1H), 4.73 (s, 1H), 5.11 (d, J = 8.8 Hz, 1H), 7.07 (d, J = 8.6 Hz, 2H), 7.27 (dd, J = 2.7, 8.6 Hz, 2H), 7.35 (d, J = 2.7 Hz, 2H); HRMS calcd for $C_{28}H_{35}N_2O_2{}^{35}Cl_2$ (M + H)$^+$ 501.2076, found 501.2074. Anal. ($C_{28}H_{34}N_2O_2Cl_2$) C, H, N.

The following compounds were prepared in a manner similar to the procedure described for **1j** using the amine **4a** or **4b** and appropriate acid.

***N*-[1-(Cyclooctylmethyl)piperidin-4-yl]-2,2-diphenyl-2-hydroxyacetamide (1i).** This was prepared from 2,2-diphenyl-2-hydroxyacetic acid and **4a** (45%): mp 146−148 °C; ^{1}H NMR ($CDCl_3$) δ 1.10−1.99 (m, 19H), 1.99−2.22 (m, 4H), 2.63−2.78 (m, 2H), 3.75−3.92 (m, 1H), 3.82−4.15 (m, 1H), 6.18 (d, J = 7.9 Hz, 1H), 7.26−7.49 (m, 10H); HRMS calcd for $C_{28}H_{39}N_2O_2$ (M + H)$^+$ 435.3012, found 435.3010. Anal. ($C_{28}H_{38}N_2O_2$) C, H, N.

***N*-(1-Cyclooctylmethylpiperidin-4-yl)-2,7-dibromoxanthene-9-carboxamide (1k).** This was prepared from 2,7-dibromoxanthene-9-carboxylic acid[16] and **4a** (68%): mp 237−240 °C; ^{1}H NMR ($CDCl_3$) δ 1.09−1.95 (m, 19H), 1.95−2.07 (m, 4H), 2.55−2.69 (m, 2H), 3.60−3.78 (m, 1H), 4.73 (s, 1H), 5.12 (d, J = 8.0 Hz, 1H), 7.01 (d, J = 8.6 Hz, 2H), 7.41 (dd, J = 2.3, 8.6 Hz, 2H), 7.50 (d, J = 2.3 Hz, 2H); HRMS calcd for $C_{28}H_{35}N_2O_2{}^{79}Br_2$ (M + H)$^+$ 589.1065, found 589.1081. Anal. ($C_{28}H_{34}N_2O_2Br_2$) C, H, N.

***N*-[1-(1-Cyclooctenylmethyl)piperidin-4-yl]-2,7-dichloroxanthene-9-carboxamide (1m).** This was prepared from 2,7-dichloroxanthene-9-carboxylic acid and **4b** (82%): mp 212−213 °C; ^{1}H NMR ($CDCl_3$) δ 1.15−1.35 (m, 2H), 1.35−1.85 (m, 10H), 1.85−2.03 (m, 2H), 2.05−2.17 (m, 4H), 2.50−2.68 (m, 2H), 2.75 (s, 2H), 3.60−3.77 (m, 1H), 4.74 (s, 1H), 5.12 (d, J = 8.6 Hz, 1H), 5.43 (t, J = 7.5 Hz, 1H), 7.07 (d, J = 8.7 Hz, 2H), 7.27 (dd, J = 2.6, 8.7 Hz, 2H), 7.36 (d, J = 2.6 Hz, 2H); HRMS calcd for $C_{28}H_{33}N_2O_2{}^{35}Cl_2$ (M + H)$^+$ 499.1919, found 499.1917. Anal. ($C_{28}H_{32}N_2O_2Cl_2$) C, H, N.

***N*-[1-(1-Cyclooctenylmethyl)piperidin-4-yl]-2,7-dibromoxanthene-9-carboxamide (1n).** This was prepared from 2,7-dibromoxanthene-9-carboxylic acid and **4b** (83%): mp 220−223 °C; ^{1}H NMR ($CDCl_3$) δ 1.15−1.35 (m, 2H), 1.35−1.52 (m, 8H), 1.70−1.84 (m, 2H), 1.86−2.02 (m, 2H), 2.03−2.20 (m, 4H), 2.49−2.68 (m, 2H), 2.74 (s, 2H), 3.60−3.75 (m, 1H), 4.73 (s, 1H), 5.11 (d, J = 7.9 Hz, 1H), 5.43 (t, J = 8.8 Hz, 1H), 7.01 (d, J = 8.8 Hz, 2H), 7.41 (dd, J = 2.5, 8.8 Hz, 2H), 7.49 (d, J = 2.5 Hz, 2H); HRMS calcd for $C_{28}H_{33}N_2O_2{}^{79}Br_2$ (M + H)$^+$ 587.0909, found 587.0914. Anal. ($C_{28}H_{32}N_2O_2Br_2$) C, H, N.

General Method D. 1-Cyclooctylmethyl-1-methyl-4-(xanthene-9-carboxamido)piperidinium Iodide (2a). A mixture of **1c** (167 mg, 386 mmol) and iodomethane (5.0 mL) was stirred at room temperature for 20 h. The solvent was removed in vacuo, and the residue was purified by silica gel column chromatography (3−10% MeOH in $CHCl_3$), and trituated with *i*-PrOH to give **2a** (110 mg, 50%) as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 1.37−1.81 (m, 19H), 1.86−2.04 (m, 2H), 2.14−2.49 (m, 2H), 3.23 (s, 3H), 3.40−3.69 (m, 2H), 3.98−4.26 (m, 1H), 5.17 & 5.41 (s, 1H), 6.90−7.60 (m, 8H), 8.25−8.52 (m, 1H); HRMS calcd for $C_{29}H_{39}N_2O_2$ (M − I)$^+$ 447.3012, found 447.3001. Anal. ($C_{29}H_{39}N_2O_2I\cdot 0.33H_2O$) C, H, N.

The following compounds were prepared in a manner similar to the procedure described for **2a**.

1-Cyclooctylmethyl-1-ethyl-4-(xanthene-9-carboxamido)piperidinium Iodide (2b). This was prepared from **1c** and iodoethane (50%, colorless solid): ^{1}H NMR ($CDCl_3$) δ 1.18−2.03 (m, 24H), 2.15−2.51 (m, 2H), 3.05−3.79 (m, 4H), 3.85−4.30 (m, 1H), 5.18 & 5.42 (s, 1H), 6.80−7.60 (m, 8H), 8.33 & 8.55 (d, J = 7.7 Hz, 1H); HRMS calcd for $C_{30}H_{41}N_2O_2$ (M − I)$^+$ 461.3168, found 461.3172. Anal. ($C_{30}H_{41}N_2O_2I$) C, H, N.

1-Cyclooctylmethyl-1-*n*-propyl-4-(xanthene-9-carboxamido)piperidinium Iodide (2c). This was prepared from **1c** and 1-iodopropane (62%, colorless solid): ^{1}H NMR (CD_3OD) δ 1.03 & 1.05 (t, J = 7.3 Hz, 3H), 1.30−2.20 (m, 21H), 3.05−3.65 (m, 8H), 3.85−3.98 (m, 1H), 4.56 (s, 1H), 7.06−7.45 (m, 8H); HRMS calcd for $C_{31}H_{43}N_2O_2$ (M − I)$^+$ 475.3325, found 475.3329. Anal. ($C_{31}H_{43}N_2O_2I$) C, H, N.

1-*n*-Butyl-1-cyclooctylmethyl-4-(xanthene-9-carboxamido)piperidinium Iodide (2d). This was prepared from **1c** and 1-iodobutane (6%, colorless solid): ^{1}H NMR (CD_3OD) δ 1.01 & 1.03 (t, J = 7.3 Hz, 3H), 1.09−2.09 (m, 23H), 3.14−3.68 (m, 8H), 3.85−4.00 (m, 1H), 4.94 & 4.97 (s, 1H), 7.15−7.40 (m, 8H); HRMS calcd for $C_{32}H_{45}N_2O_2$ (M − I)$^+$ 489.3481, found 489.3493. Anal. ($C_{32}H_{45}N_2O_2I\cdot 0.33H_2O$) C, H, N.

1-Allyl-1-cyclooctylmethyl-4-(xanthene-9-carboxamido)piperidinium Bromide (2e). This was prepared from **1c** and 3-bromopropene (21%, colorless solid): ^{1}H NMR ($CDCl_3$) δ 1.22−2.41 (m, 19H), 3.00−4.05 (m, 9H), 5.29 & 5.47 (s, 1H), 5.65−6.10 (m, 3H), 6.80−7.80 (m, 8H), 9.15 & 9.52 (d, J = 8.5 Hz, 1H); HRMS calcd for $C_{31}H_{41}N_2O_2$ (M − Br)$^+$ 473.3168, found 473.3171. Anal. ($C_{31}H_{41}N_2O_2Br$) C, H, N.

1-Benzyl-1-cyclooctylmethyl-4-(xanthene-9-carboxamido)piperidinium Bromide(2f). This was prepared from **1c** and benzyl bromide (80%, colorless solid): ^{1}H NMR (CD_3OD) δ 1.25−1.42 (m, 19H), 3.00−4.05 (m, 7H), 4.59 & 4.74 (s, 2H), 5.00 (s, 1H), 7.00−7.38 (m, 8H), 7.43−7.65 (m, 5H): HRMS calcd for $C_{35}H_{43}N_2O_2$ (M − Br)$^+$ 523.3325, found 523.3315. Anal. ($C_{35}H_{43}N_2O_2Br\cdot 0.5H_2O$) C, H, N.

1-Cyclooctylmethyl-1-methyl-4-(anthracene-9-carboxamido)piperidinium Iodide (2g). This was prepared from **1g** and iodomethane (65%, colorless solid): ^{1}H NMR (DMSO-d_6) δ 1.30−2.40 (m, 19H), 3.01 & 3.11 (s, 3H), 3.13−3.35 (m, 2H), 3.45−3.52 (m, 4H), 4.30−4.50 (m, 1H), 7.50−7.64 (m, 4H), 7.90−8.19 (m, 4H), 8.69 (s, 1H), 8.80−8.90 (m, 1H); HRMS calcd for $C_{30}H_{39}N_2O$ (M − I)$^+$ 443.3062, found 443.3066. Anal. ($C_{30}H_{39}N_2OI$) C, H, N.

1-Cyclooctylmethyl-1-methyl-4-(2,2-diphenylacetamido)piperidinium Iodide (2h). This was prepared from **1h** and iodomethane (70%, colorless solid): ^{1}H NMR (DMSO-d_6) δ 1.31−2.19 (m, 19H), 2.99 & 3.03 (s, 3H), 3.15−3.52 (m, 6H), 3.75−3.95 (m, 1H), 4.93 & 4.95 (s, 1H), 7.15−7.36 (m, 10H), 8.27 & 8.33 (d, J = 7.4 Hz, 1H); HRMS calcd for $C_{29}H_{41}N_2O$ $(M - I)^+$ 433.3219, found 433.3215. Anal. ($C_{29}H_{41}N_2OI$) C, H, N.

1-Cyclooctylmethyl-1-methyl-4-(2,2-diphenyl-2-hydroxyacetamido)piperidinium Iodide (2i). This was prepared from **1i** and iodomethane (55%, colorless solid): ^{1}H NMR (DMSO-d_6) δ 1.20−2.20 (m, 19H), 3.03 (s, 3H), 3.10−3.54 (m, 6H), 3.85−4.05 (m, 1H), 6.81 & 6.82 (s, 1H), 7.20−7.48 (m, 10H), 8.20 & 8.29 (d, J = 8.9 Hz, 1H); HRMS calcd for $C_{29}H_{41}N_2O_2$ $(M - I)^+$ 449.3168, found 449.3165. Anal. ($C_{29}H_{41}N_2O_2I$) C, H, N.

1-Cyclooctylmethyl-1-methyl-4-(2,7-dichloroxanthene-9-carboxamido)piperidinium Iodide (2j). This was prepared from **1j** and iodomethane (57%, colorless solid): ^{1}H NMR (DMSO-d_6) δ 1.20−2.18 (m, 19H), 3.02 (s, 3H), 3.11−3.53 (m, 6H), 3.65−3.83 (m, 1H), 4.91 (s, 1H), 7.16−7.47 (m, 6H), 8.41 (d, J = 5.9 Hz, 1H); HRMS calcd for $C_{29}H_{37}N_2O_2{}^{35}Cl_2$ $(M - I)^+$ 515.2232, found 515.2236. Anal. ($C_{29}H_{37}N_2O_2Cl_2I\cdot 1.0H_2O\cdot 1.0$*i*-PrOH) C, H, N.

1-Cyclooctylmethyl-1-methyl-4-(2,7-dibromoxanthene-9-carboxamido)piperidinium Iodide (2k). This was prepared from **1k** and iodomethane (80%, colorless solid): ^{1}H NMR (CD_3OD) δ 1.24−2.25 (m, 19H), 3.10 & 3.12 (s, 3H), 3.20−3.65 (m, 6H), 3.80−3.96 (m, 1H), 4.88 (s, 1H), 7.05−7.55 (m, 6H); HRMS calcd for $C_{29}H_{37}N_2O_2{}^{79}Br_2$ $(M - I)^+$ 603.1222, found 603.1218. Anal. ($C_{29}H_{37}N_2O_2Br_2I$) C, H, N.

1-Cyclooctylmethyl-1-ethyl-4-(2,7-dibromoxanthene-9-carboxamido)piperidinium Iodide (2l). This was prepared from **1k** and iodoethane (75%, colorless solid): ^{1}H NMR ($CDCl_3$) δ 1.29 & 1.36 (t, J = 7.1 Hz, 3H), 1.38−2.50 (m, 19H), 3.21 & 3.58 (d, J = 4.3 Hz, 2H), 3.36−3.69 (m, 2H), 3.43 & 3.82 (q, J = 7.1 Hz, 2H), 3.88−4.41 (m, 3H), 5.39 & 5.57 (s, 1H), 6.91 & 6.92 (d, J = 8.7 Hz, 2H), 7.30 & 7.31 (dd, J = 2.4, 8.7 Hz, 2H), 7.55 & 7.61 (d, J = 2.4 Hz, 2H), 8.88 & 9.12 (d, J = 8.6 Hz, 1H); HRMS calcd for $C_{30}H_{39}N_2O_2{}^{79}Br_2$ $(M - I)^+$ 617.1378, found 613.1384. Anal. ($C_{30}H_{39}N_2O_2Br_2I$) C, H, N.

1-Cyclooctylmethyl-1-*n*-propyl-4-(2,7-dibromoxanthene-9-carboxamido)piperidinium Iodide (2m). This was prepared from **1k** and 1-iodopropane (9%, colorless solid): ^{1}H NMR ($CDCl_3$) δ 1.07 &1.13 (t, J = 7.1 Hz, 3H), 0.90−2.53 (m, 21H), 3.10−4.46 (m, 9H), 5.36 & 5.67 (s, 1H), 6.90 & 6.93 (d, J = 8.7 Hz, 2H), 7.29 & 7.31 (dd, J = 2.4, 8.7 Hz, 2H), 7.56 & 7.63 (d, J = 2.4 Hz, 2H), 8.84 & 9.04 (d, J = 8.4 Hz, 1H); HRMS calcd for $C_{31}H_{41}N_2O_2{}^{79}Br_2$ $(M - I)^+$ 631.1535, found 631.1539. Anal. ($C_{31}H_{41}N_2O_2Br_2I$) C, H, N.

1-(1-Cyclooctenylmethyl)-1-methyl-4-(xanthene-9-carboxamido)piperidinium Iodide (2n). This was prepared from **1l** and iodomethane (75%, colorless solid): ^{1}H NMR ($CDCl_3$) δ 1.30−2.49 (m, 16H), 2.90 & 3.13 (s, 3H), 3.31−3.68 (m, 4H), 3.82 & 4.18 (s, 2H), 3.92−4.30 (m, 1H), 5.14 & 5.42 (s, 1H), 5.99 & 6.12 (t, J = 8.3 Hz, 1H), 6.80−7.60 (m, 8H), 8.26 & 8.52 (d, J = 8.3 Hz, 1H); HRMS calcd for $C_{29}H_{37}N_2O_2$ $(M - I)^+$ 445.2855, found 445.2858. Anal. ($C_{29}H_{37}N_2O_2I\cdot 0.33H_2O$) C, H, N.

1-(1-Cyclooctenylmethyl)-1-ethyl-4-(xanthene-9-carboxamido)piperidinium iodide (2o). This was prepared from **1l** and iodoethane (39%, colorless solid): ^{1}H NMR ($CDCl_3$) δ 1.23 & 1.24 (t, J = 7.3 Hz, 3H), 1.35−2.50 (m, 16H), 3.23−4.32 (m, 7H), 3.70 & 4.08 (s, 2H), 5.11 & 5.44 (s, 1H), 5.96 & 6.15 (t, J = 8.2 Hz, 1H), 6.80−7.60 (m, 8H), 8.34 & 8.75 (d, J = 7.8 Hz, 1H); HRMS calcd for $C_{30}H_{39}N_2O_2$ $(M - I)^+$ 459.3012, found 459.3012. Anal. ($C_{30}H_{39}N_2O_2I$) C, H, N.

1-(1-Cyclooctenylmethyl)-1-*n*-propyl-4-(xanthene-9-carboxamido)piperidinium Iodide (2p). This was prepared from **1l** and 1-iodopropane (10%, colorless solid): ^{1}H NMR ($CDCl_3$) δ 1.02 & 1.08 (t, J = 6.7 Hz, 3H), 1.29−2.68 (m, 18H), 2.99−4.38 (m, 7H), 3.71 & 4.16 (m, 2H), 5.10 & 5.51 (s, 1H), 5.96 & 6.15 (t, J = 8.3 Hz, 1H), 6.92−7.58 (m, 8H), 8.31 & 8.72 (d, J = 8.4 Hz, 1H); HRMS calcd for $C_{31}H_{41}N_2O_2$ $(M - I)^+$ 473.3168, found 473.3183. Anal. ($C_{31}H_{41}N_2O_2I$) C, H, N.

1-(1-Cyclooctenylmethyl)-1-ethyl-4-(2,7-dichloroxanthene-9-carboxamido)piperidinium Iodide (2q). This was prepared from **1m** and iodoethane (91%, colorless solid): ^{1}H NMR ($CDCl_3$) δ 1.35 & 1.40 (t, J = 7.3 Hz, 3H), 1.25−1.74 (m, 8H), 1.96−2.60 (m, 8H), 3.22−3.86 (m, 6H), 3.82 & 4.24 (s, 2H), 4.15−4.40 (m, 1H), 5.28 & 5.69 (s, 1H), 6.07 & 6.26 (t, J = 8.2 Hz, 1H), 6.96 & 7.00 (d, J = 8.7 Hz, 2H), 7.16 (dd, J = 2.4, 8.7 Hz, 2H), 7.39 & 7.51 (d, J = 2.4 Hz, 2H), 8.80 & 9.08 (d, J = 7.8 Hz, 1H); HRMS calcd for $C_{30}H_{37}N_2O_2{}^{35}Cl_2$ $(M - I)^+$ 527.2232, found 527.2234. Anal. ($C_{30}H_{37}N_2O_2Cl_2I\cdot 0.5H_2O$) C, H, N.

Separation of Major Isomer 2q-1 and Minor Isomer 2q-2. 2q (5.9 g) was separated by silica gel column chromatography (30−50% acetone in $CHCl_3$) and triturated with *i*-PrOH to give major isomer **2q-1** (3.5 g, 59%) and minor isomer **2q-2** (1.9 g, 32%), respectively, as a colorless solid. **2q-1**: mp 148−150 °C; ^{1}H NMR ($CDCl_3$) δ 1.40 (t, J = 7.3 Hz, 3H), 1.25−1.67 (m, 8H), 1.96−2.60 (m, 8H), 3.55−3.86 (m, 6H), 3.82 (s, 2H), 4.15−4.30 (m, 1H), 5.28 (s, 1H), 6.07 (t, J = 8.2 Hz, 1H), 6.96 (d, J = 8.7 Hz, 2H), 7.16 (dd, J = 2.4, 8.7 Hz, 2H), 7.39 (d, J = 2.4 Hz, 2H), 8.80 (d, J = 7.8 Hz, 1H); HRMS calcd for $C_{30}H_{37}N_2O_2{}^{35}Cl_2$ $(M - I)^+$ 527.2232, found 527.2234. Anal. ($C_{30}H_{37}N_2O_2Cl_2I\cdot 0.5H_2O$) C, H, N. **2q-2**: mp 146−148 °C; ^{1}H NMR ($CDCl_3$) δ 1.35 (t, J = 7.1 Hz, 3H), 1.38−1.74 (m, 8H), 1.98−2.45 (m, 8H), 3.22−3.40 (m, 4H), 4.24 (s, 2H), 4.38−4.40 (m, 1H), 4.41−4.60 (m, 2H), 5.69 (s, 1H), 6.26 (t, J = 8.2 Hz, 1H), 7.00 (d, J = 8.7 Hz, 2H), 7.17 (dd, J = 2.5, 8.7 Hz, 2H), 7.51 (d, J = 2.5 Hz, 2H), 9.08 (d, J = 8.6 Hz, 1H); HRMS calcd for $C_{30}H_{37}N_2O_2{}^{35}Cl_2$ $(M - I)^+$ 527.2232, found 527.2234. Anal. ($C_{30}H_{37}N_2O_2Cl_2I\cdot 0.5H_2O$) C, H, N.

1-(1-Cyclooctenylmethyl)-1-ethyl-4-(2,7-dibromoxanthene-9-carboxamido)piperidinium Iodide (2r). This was prepared from **1n** and iodoethane (52%, colorless solid): ^{1}H NMR ($CDCl_3$) δ 1.09−1.78 (m, 11H), 1.89−2.55 (m, 8H), 3.29−4.35 (m, 7H), 3.86 & 4.17 (s, 2H), 5.18 & 5.65 (s, 1H), 6.04 & 6.19 (t, J = 8.3 Hz, 1H), 6.86 & 6.89 (d, J = 8.7 Hz, 2H), 7.29 (dd, J = 2.3, 8.7 Hz, 2H), 7.47 & 7.62 (d, J = 2.3 Hz, 2H), 8.70 & 8.98 (d, J = 7.9 Hz, 1H); HRMS calcd for $C_{30}H_{37}N_2O_2{}^{79}Br_2$ $(M - I)^+$ 615.1222, found 615.1221. Anal. ($C_{30}H_{37}N_2O_2Br_2I$) C, H, N.

4-*tert*-Butoxycarbonylamino-1-(cyclooctylmethyl)piperidine (6a). This was prepared in a manner similar to the procedure described for **1a** using 4-*tert*-butoxycarbonylaminopiperidine[17] and cyclooctanecarboxaldehyde (95%, colorless solid): ^{1}H NMR ($CDCl_3$) δ 0.92−1.70 (m, 19H), 1.44 (s, 9H), 1.75−2.10 (m, 4H), 2.62−2.80 (m, 2H), 3.28−3.52 (m, 1H), 4.30−4.55 (m, 1H); HRMS calcd for $C_{19}H_{37}N_2O_2$ $(M + H)^+$ 325.2855, found 325.2853.

4-Amino-1-(cyclooctylmethyl)piperidine (4a). This was prepared in a manner similar to the procedure described for *N*-(piperidin-4-yl)xanthene-9-carboxamide using **6a** (88%, colorless oil): ^{1}H NMR ($CDCl_3$) δ 1.09−1.82 (m, 21H), 1.85−2.01 (m, 2H), 2.03−2.12 (m, 2H), 2.55−2.70 (m, 1H), 2.72−2.85 (m, 2H); HRMS calcd for $C_{14}H_{29}N_2$ $(M + H)^+$ 225.2331, found 225.2328.

4-*tert*-Butoxycarbonylamino-1-(1-cyclooctenylmethyl)-piperidine (6b). This was prepared in a manner similar to the procedure described for **1a** using 4-*tert*-butoxycarbonylaminopiperidine and **9** (88%, colorless solid): ^{1}H NMR ($CDCl_3$) δ 1.30−1.58 (m, 10H), 1.44 (s, 9H), 1.80−2.25 (m, 8H), 2.66−2.85 (m, 2H), 2.79 (s, 2H), 3.34−3.55 (m, 1H), 4.33−4.54 (m, 1H), 5.46 (t, J = 8.1 Hz, 1H); HRMS calcd for $C_{19}H_{35}N_2O_2$ $(M + H)^+$ 323.2699, found 323.2698.

4-Amino-1-(1-cyclooctenylmethyl)piperidine (4b). This was prepared in a manner similar to the procedure described for *N*-(piperidin-4-yl)xanthene-9-carboxamide using **6b** (96%, colorless oil): ^{1}H NMR ($CDCl_3$) δ 1.20−1.61 (m, 10H), 1.65−1.98 (m, 4H), 2.00−2.28 (m, 4H), 2.50−2.70 (m, 1H), 2.70−2.90 (m, 2H), 2.80 (s, 2H), 5.46 (t, J = 7.7 Hz, 1H); HRMS calcd for $C_{14}H_{27}N_2$ $(M + H)^+$ 223.2174, found 223.2175.

1-Cyclooctenylcarboxaldehyde (9). To a stirred suspension of cyclooctanone *p*-tolylsulfonylhydrazone[18] (12 g, 40.8

mmol) in *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (120 mL) was added 1.6 M of *n*-BuLi in hexane (100 mL, 160 mmol) at −55 to −45 °C under N_2. The resulting deep red solution was stirred at −45 °C for 0.5 h, and then allowed to warm to room temperature over a period of 1 h. When N_2 evolution had ceased, the mixture was cooled at 0 °C, and DMF (15 mL, 204 mmol) was added. After the mixture was stirred for 1 h, the reaction was quenched by adding water. The mixture was extracted with EtOAc, and the organic layer was washed with 2 N HCl and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by silica gel column chromatography (3% EtOAc in hexane) to give **9** (3.0 g, 53%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.39−1.74 (m, 8H), 2.38−2.51 (m, 4H), 6.72 (t, J = 8.2 Hz, 1H), 9.41 (s, 1H).

1-Cyclodecanylcarboxaldehyde (10). To a stirred solution of diethyl (isocyanomethyl)phosphonate (320 μL, 2.00 mmol) in THF (5.0 mL) was added 2.5 M *n*-BuLi in hexane (0.75 mL, 1.88 mmol) at −70 °C under N_2, and the resulting solution was stirred at the same temperature for 30 min. To this solution was added cyclodecanone (250 μL, 1.55 mmol), and the mixture was stirred for 30 min. The reaction was quenched by adding 1 N HCl, and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. To the residue in Et_2O (10 mL) was added concentrated HCl (10 mL), and the mixture was stirred for 12 h. The mixture was extracted with Et_2O, and the organic layer was washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by silica gel column chromatography (3% EtOAc in hexane) to give **10** (150 mg, 58%) as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.39−2.00 (m, 18H), 2.41−2.70 (m, 1H), 9.59−9.63 (m, 1H).

^{125}I-Chemokine Binding Study. The cell-based binding assays were performed in 96-well microplates in a total volume of 400 μL. CHO cells transfected with CCR receptors were detached by PBS (−) containing 2 mM EDTA and resuspended in binding buffer (Krebs-Linger phosphate buffer containing 0.1% BSA and 0.1% glucose). CHO cells (1×10^5 cells) were incubated with 50 pM ^{125}I-chemokine and an antagonist or an unlabeled chemokine for 1 h at 37 °C in the binding buffer to reach equilibrium. Nonspecific binding was determined in the presence of 100 nM of unlabeled chemokine. After incubation, the ice-cold binding buffer was added to the binding reaction. Then, the binding reaction was filtered by GF/C glass fiber filter (Whatman International Ltd., Maidstone, U.K.) presoaked with 1% polyethylenimine to reduce nonspecific binding to the glass filter. The radioactivity on the glass filter was determined with a gamma counter (COBRA 5002, Packard, Downers Grove, IL).

The CCR5 membrane binding studies were performed according to the manufacturer's instructions (NEN Life Science Products, Inc., Boston, MA).

Measurement of Intracellular Ca^{2+}. U937 cells transfected with CCR receptors were loaded with 1 μM Fura-2 acetoxymethyl ester (Molecular Probes Inc., Eugene, OR) for 30 min at 37 °C. After two washings, the cells were resuspended at a concentration of 1×10^6 cells/mL in Krebs-Henseleit-Hepes buffer containing 0.1% BSA. The cell suspension (500 μL) was transferred into cuvettes with constant stirring. Changes in fluorescence were monitored at 37 °C using a spectrophotometer (CAF-110, JASCO Corp., Tokyo, Japan) at excitation wavelengths of 340 and 380 nm and an emission wavelength of 510 nm. Calculation of Ca^{2+} concentration was performed using a K_d for Ca^{2+} binding of 224 nM. An antagonist was added to the cuvette 5 min prior to the addition of chemokine.

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