Pergamon

Tetrahedron Letters 43 (2002) 7243–7245

TETRAHEDRON LETTERS

Fluororeceptor for zwitterionic form amino acids in aqueous methanol solution

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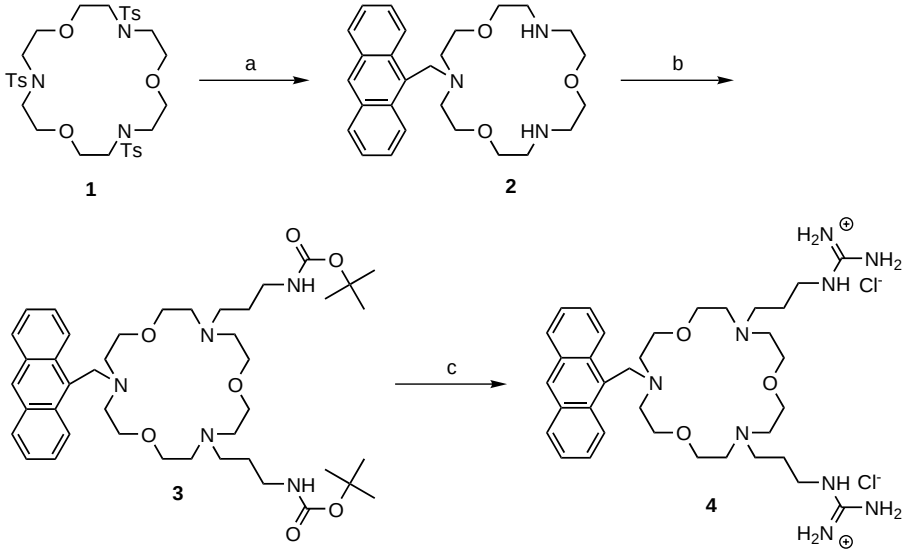
Received 19 June 2002; revised 23 July 2002; accepted 26 July 2002

Abstract—A fluorescent receptor for underivatized amino acids was developed. The receptor based on triaza-18-crown-6 ether combined with two guanidinium groups could bind several biologically important amino acids in aqueous methanol solution and showed fluorescence enhancement response by a PET (photoinduced electron transfer) mechanism.

The development of artificial chromo/fluororeceptors for the selective recognition of biologically important species has attracted much attention because of their possible application as molecular probes[1] or components of optical chemical sensors such as optodes.[2] However, compared to the large number of chromo/fluororeceptors for cations or anions,[3,4] the development of artificial receptors for amino acids is quite limited and still a challenging theme due to their highly hydrophilic character. In order to recognize the zwitter-



Scheme 1. *Reagents and conditions*: (a) (i) $LiAlH_4$, THF, reflux; (ii) 9-chloromethylanthracene, Cs_2CO_3, CH_3CN, 60°C, 22%; (b) 3-bromopropylcarbamic acid *tert*-butyl ester, Cs_2CO_3, CH_3CN, 70°C, 18%; (c) (i) CF_3COOH, rt.; (ii) 1*H*-pyrazole-1-carboxamidine, $(i\text{-Pr})_2NEt$, DMF, 11%.

Keywords: fluororeceptor; amino acids; multipoint binding; photoinduced electron transfer.

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0040-4039/02/$ - see front matter
PII: S0040-4039(02)01618-0

ionic form of an amino acid effectively, simultaneous binding of the ammonium and the carboxylate groups is required. In most examples reported so far, crown ethers were chosen as ammonium binding sites,[5–10] and quaternary ammonium,[5] guanidinium,[6–8] or metals[9–12] were used as carboxylate binding sites. In view of the design of effective amino acid receptors, the combination of stronger binding sites in one molecule is an important factor. In this work, we selected triaza-18-crown-6 ether as one of the strongest ammonium binding sites,[13] connected to two guanidinium groups as carboxylate binding sites,[14] and an anthracene unit as a fluorescent reporter moiety to construct a new fluororeceptor for amino acid sensing allowing multipoint recognition. It was demonstrated that the fluororeceptor responds to several amino acids in aqueous methanol solution by PET-induced enhancement of the fluorescence emission.

The new fluororeceptor **4** was synthesized as shown in Scheme 1 and its fluorescence response towards several essential amino acids, γ-aminobutyric acid (GABA), and *n*-butylammonium was evaluated and compared with that of receptors **2** and **3**, obtained as intermediates during the synthesis.[15] The structural characteristics for each of the three receptors are as follows: receptor **2** has no carboxylate binding site and the triazacrown ether consists of two secondary nitrogen atoms and a tertiary nitrogen atom which is connected to the anthracene moiety via one methylene group; the crown structure of receptor **3** consists of three tertiary nitrogen atoms but has no carboxylate binding sites; receptor **4** has two guanidinium groups and its crown structure consists of three tertiary nitrogen atoms. In order to confirm the design of receptor **4** having multipoint recognition sites, the complex structure of **4** with GABA was estimated based on a molecular modeling study.[16] The calculation result supports a 1:1 complex formation, where the triazacrown ring binds the ammonium terminal and two guanidinium groups capture the opposite carboxylate terminal.

The spectral characteristics of receptors **2**–**4** were examined in aqueous methanol solution adjusted to pH 9.5 with Me_4NOH.[17] The absorption spectra of receptors **2**–**4** showed no changes upon the addition of guests or upon pH changes. On the other hand, an increase in the fluorescence intensity was observed under the same circumstances, supporting the assumption of a PET mechanism for the fluorescence response. Fluorescence spectra of receptors **3** and **4** are shown in Fig. 1. The fluorescence response characteristics of receptor **2** were essentially the same as those of receptor **3**. Namely, receptors **2** and **3** showed little or no response towards α-amino acids except for lysine, which has a higher pK_a value (10.53) due to its alkyl ammonium terminal. Since these two receptors bear no carboxylate binding site, guest binding relies only on the triazacrown structure that can bind the ammonium group. No response towards most α-amino acids is ascribed to the strong hydration of these guests in mixed aqueous solution, relatively lower pK_a values, and the attenuation of the charge density of the ammonium unit by the α-carboxylate anion.[8] On the other hand, receptor **4** having two guanidinium groups, apparently responds to glycine as shown in Fig. 1(b). Moreover, it should be noted that GABA and lysine result in stronger fluorescent enhancement than *n*-$BuNH_3^+$.

Table 1 summarizes the fluorescence enhancement factors (FE) of receptors **2**–**4**. The order of FE values of **2** and **3** is *n*-$BuNH_3^+$>lysine>GABA, which is consistent with the order of their pK_a values. It is shown that the nature of the nitrogen atoms (secondary or tertiary) of the triazacrown structure or the side arms of the receptor do not affect the selectivity. On the contrary, the order of FE values of receptor **4** is GABA>lysine>*n*-$BuNH_3^+$, which is opposite to those of **2** and **3**. Because GABA and lysine have both carboxylate and alkyl ammonium groups, it is concluded that receptor **4** binds the ammonium and the carboxylate moieties simultaneously to form a 1:1 complex. The inversion of the

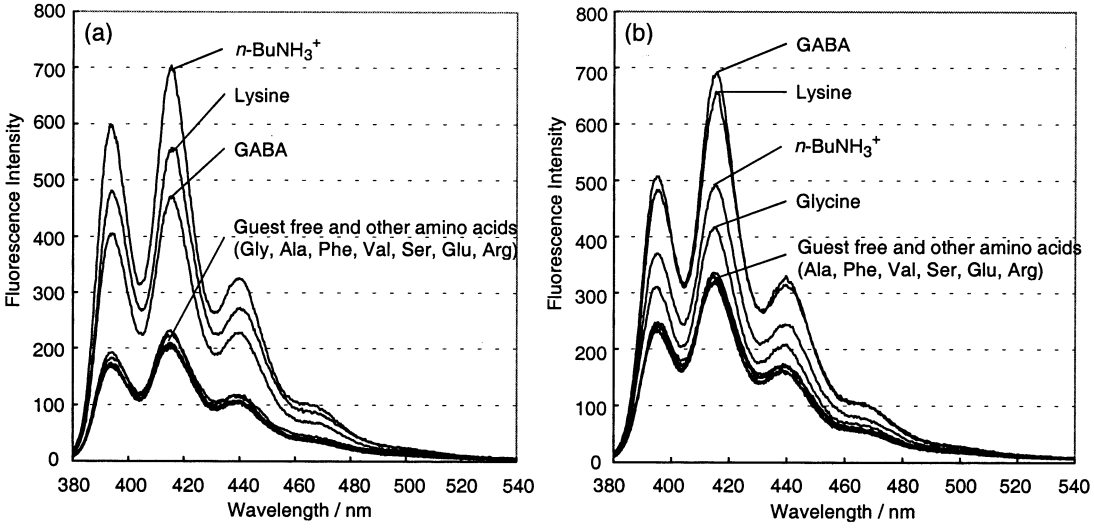

Figure 1. Fluorescence spectra of (a) receptor **3** and (b) receptor **4** in the presence of several guests in H_2O/MeOH = 1/2 (v/v). [receptor] = 3.0×10^{-5} M. [guest] = 0.1 M. Excitation wavelength: 366 nm.

Table 1. Fluorescence enhancement factors (FE) of receptors **2**–**4** with several guests[a]

Guest[b] (pK_a)[c]	**2**	**3**	**4**
Glycine (9.8)	1.0	1.0	1.3
GABA (10.40)	2.6	2.2	2.2
Lysine (10.53)	2.7	2.5	2.1
n-$BuNH_3^+$ (10.8)	3.3	3.1	1.6

[a] All samples were measured in H_2O/MeOH = 1/2 (v/v) adjusted to pH 9.5 with Me_4NOH. [receptor] = 3.0×10^{-5} M. [guest] = 0.1 M. Excitation wavelength: 366 nm. FE values were calculated as the ratio of fluorescence intensity in the presence and in the absence of each guest. The reported FE values were calculated by averaging the values obtained from the three peaks at 395, 415, and 440 nm.

[b] Other amino acids examined (Ala, Phe, Val, Ser, Glu, Arg) showed little or no fluorescence response.

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selectivity pattern is explained as follows: n-$BuNH_3^+$ has no carboxylate group and is therefore bound less strongly through monotopic interaction. The small preference for GABA over lysine is explained by the sterical hindrance around the α-amino group of lysine. The small fluorescence response of glycine compared to the lack of response of other α-amino acids is also ascribed to similar sterical reasons.

In contrast to extraction experiments, which reflect the lipophilicity of the amino acids, this is a rare example of a fluororeceptor that can respond to glycine with fluorescence enhancement. Although receptor **4** also responds to alkaline metal cations (Na^+, FE = 1.4; K^+, FE = 2.3), it is demonstrated that the combination of a triazacrown and two charged guanidinium groups enables the receptor to bind biologically important glycine, lysine, and GABA in aqueous media with fluorescence enhancement response. The receptor is expected to be useful as an indicator for amino acids in aqueous solution or as a component of an optode membrane.

Acknowledgements

D.C. gratefully acknowledges a research fellowship granted by the Science and Technology Agency of Japan (STA).

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14. In the case of thioureas, which are structurally similar to the guanidinium group and act as anion binding site, it is known that a bis-thiourea forms the stronger 1:1 complex than a mono-thiourea: Nishizawa, S.; Bühlmann, P.; Iwao, M.; Umezawa, Y. *Tetrahedron Lett.* **1995**, *36*, 6483–6486.
15. Spectral data of receptors **2**–**4**. **2**: ^{1}H NMR ($CDCl_3$) δ 2.18 (br, NH protons overlapped the residual H_2O), 2.73 (t, J = 4.8 Hz, 4H), 2.78 (t, J = 4.6 Hz, 4H), 2.90 (t, J = 5.7 Hz, 4H), 3.49 (t, J = 4.8 Hz, 4H), 3.54 (t, J = 5.7 Hz, 4H), 3.60 (t, J = 4.6 Hz, 4H), 4.56 (s, 2H), 7.42–7.55 (m, 4H), 7.99 (d, J = 8.3 Hz, 2H), 8.40 (s, 1H), 8.54 (d, J = 10.0 Hz, 2H); ESI-MS 191 ($C_{14}H_9CH_2^+$), 261 ($M-C_{14}H_9CH_2^+ + 2H^+$), 453 ($M+H^+$); λ_{max}/nm (H_2O/MeOH = 1/2) (ε/M^{-1} cm^{-1}) 332 (2700), 348 (5200), 366 (8000), 386 (7700). **3**: ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 18H), 1.57–1.63 (m, 4H), 2.50 (t, J = 6.0 Hz, 4H), 2.70 (br, 8H), 2.87 (t, J = 5.5 Hz, 4H), 3.15–3.17 (m, 4H), 3.46–3.55 (m, 12H), 4.56 (s, 2H), 5.50 (br, 2H), 7.42–7.52 (m, 4H), 7.99 (dd, J = 8.4, 1.6 Hz, 2H), 8.40 (s, 1H), 8.54 (d, J = 8.6 Hz, 2H); ESI-MS 191 ($C_{14}H_9CH_2^+$), 577 ($M-C_{14}H_9CH_2^+ + 2H^+$), 767 ($M+H^+$); λ_{max}/nm (H_2O/MeOH = 1/2) (ε/M^{-1} cm^{-1}) 332 (2300), 348 (4300), 366 (6600), 386 (6300). **4**: ^{1}H NMR (D_2O) δ 1.53 (br, 4H), 2.47–3.38 (m, 32H), 4.6 ($C_{14}H_9CH_2$ protons overlapped the residual solvent peak), 7.43–7.51 (m, 4H), 8.00 (d, J = 8.6 Hz, 2H), 8.19 (d, J = 8.8 Hz, 2H), 8.53 (s, 1H); ESI-MS 191 ($C_{14}H_9CH_2^+$), 231 ($M^{2+}-C_{14}H_9CH_2^+$), 326 (M^{2+}); λ_{max}/nm (H_2O/MeOH = 1/2) (ε/M^{-1} cm^{-1}) 332 (6200), 348 (12200), 366 (18800), 386 (17900).
16. The AM1 calculation was performed using PC-SPARTAN ver. 1.0.1 program package; Wavefunction, Inc., USA.
17. pH response of receptors **2**–**4** was examined in the range of pH 7.0 to pH 10.0. Down to pH 9.5, the response to protons is essentially negligible.