Fluorescent signalling of the brain neurotransmitter γ-aminobutyric acid and related amino acid zwitterions

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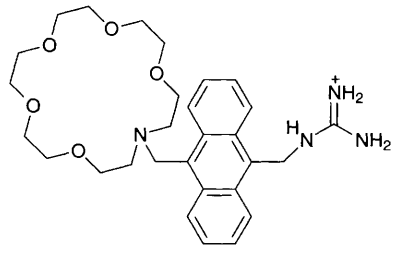
Fluorescent PET (photoinduced electron transfer) sensor 1 with monoaza-18-crown-6 ether and guanidinium receptor units shows a significant fluorescence enhancement with γ-aminobutyric acid (GABA) in mixed aqueous solution.

The field of fluorescent sensors has developed to the point that a range of inorganic cations can now be targeted successfully.[1] PET has been a particularly valuable design principle in this regard[2] and even some inorganic anions can be accommodated in sensing schemes.[3] In spite of their structural complexity, several classes of organic species have also succumbed. Among neutral organics, steroids and sugars are prominent.[4] Organic cations and anions are represented by α,ω-alkanediammonium, nucleotides and acetylcholine.[5] We are not aware of any fluorescent sensors (*i.e.* nondestructive monitors) which directly target zwitterionic species.[6] We now present a fluorescent PET sensor **1** for GABA and related amino acid zwitterions, particularly important since GABA is a principal neurotransmitter in the brain.[7]

Sensor **1** consists of monoaza-18-crown-6 ether, a reasonable receptor for the ammonium terminal[8] of GABA, while a guanidinium unit serves as a receptor for the carboxylate end.[8b,9] Importantly, an azacrown ether unit can engage in PET with an anthracene fluorophore positioned nearby and ammonium ion binding can cause fluorescence recovery.[5b] The anthracene unit also serves as a rigid backbone between the two receptor units to confer a degree of linear recognition when various α,ω-amino acid zwitterions are presented to **1** (Fig. 1). The arrangement of the various components in **1** follows the format of Schmidtchen's ditopic receptor which binds GABA zwitterion in preference to ammonium ions.[10] Scheme 1 outlines the synthesis of **1**.†

The fluorescence of dialkylaminomethyl anthracenes is known to be 'switched on' with protons and **1** is no exception. **1** shows a fluorescence enhancement factor (FE) of 70 and a pK_a of 7.4. Therefore, amino acid binding has been studied at pH 9.5 to minimize interference by protons, while maintaining the amino acids largely in their zwitterionic form. Methanol–water (3 : 2, *v*/*v*) was used as the solvent to permit significant host–guest binding *via* hydrogen bonding and ion pairing while allowing us to approach physiological conditions with higher generations of **1**. Several amino acid zwitterions cause useful fluorescence enhancements in **1**, with negligible change in the emission band shape and wavelength as expected for a fluorescent PET sensor.[2a,b] The dependence of fluorescence intensity upon amino acid concentration was analysed according to the Benesi–Hildebrand equation[11] and the binding constants (β) obtained are reported in Table 1 along with the corresponding fluorescence enhancement factors (FE). The linear recognition capability of sensor **1** is evident from the patterns of both these parameters. If we focus on the biologically important zwitterions, it is notable that **1** responds



1

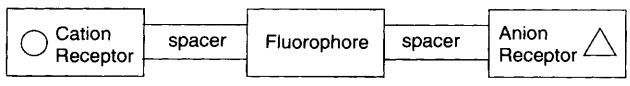
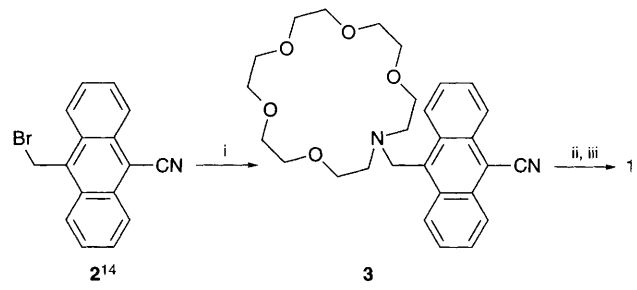


Fig. 1



2[14] **3**

Scheme 1 *Reagents and conditions*: i, monoaza-18-crown-6 ether, Na_2CO_3, C_6H_6; ii, BH_3·Me_2S, THF; iii, 3,5-dimethylpyrazole-1-carboxyamidine nitrate, Et_3N, THF[15]

Table 1 Parameters derived from the fluorescence enhancement of **1** with various organic guests[a]

Guest	Binding constant (β)/dm³ mol⁻¹	Fluorescence enhancement factor (FE)[e]
$H_3N^+CH_2CO_2^-$	—[d]	1.2
$H_3N^+(CH_2)_2CO_2^-$	17	1.9
$H_3N^+(CH_2)_3CO_2^-$	36	2.2
$H_3N^+(CH_2)_4CO_2^-$	84	3.5
$H_3N^+(CH_2)_5CO_2^-$	54	3.1
$H_3N^+(CH_2)_7CO_2^-$	44	3.1
$H_3N^+CH(CO_2^-)(CH_2)_2CO_2^-$	—[d]	1.1
$Me(CH_2)_2NH_3^+$[b]	79	2.3
$MeCO_2^-$[c]	—[d]	1.1

[a] 10^{-5} M Sensor **1** in MeOH–H_2O (3 : 2, *v*/*v*) at pH 9.5 maintained with 10^{-3} M Me_3N and adjusted with Me_4NOH and HCl. 10^{-2} M Me_4NCl was used to minimize ionic strength variations. The absorption spectroscopic parameters of **1** are: Absorption maxima = 393, 372 and 354 nm. Extinction coefficients = 8600, 8600 and 5200 dm³ mol⁻¹ cm⁻¹ respectively. The fluorescence spectroscopic parameters of **1** are: Excitation wavelength = 372 nm, Emission wavelength = 424 nm (other peaks at 402 and 449 nm), Quantum yield (when 'ion-free') = 0.011%. The average uncertainty in β values is 15%. [b] Counter ion = Cl^-. [c] Counter ion = Me_4N^+. [d] Fluorescence response is too small to determine β. [e] [Guest] = 0.1 M. Solubility difficulties arise beyond this point in several cases.

well to GABA while its physiological precursor glutamic acid gives essentially no response. The binding of GABA by **1** is also seen by ^{1}H NMR spectroscopy [in $CDCl_3$–CD_3OD (3 : 2 *v*/*v*) the multiplet for β-methylene protons of GABA shifts from δ 1.87 to 1.13 in the presence of **1** due to the paramagnetic shielding by the anthracene π-system]. The attenuation of the charge density of the ammonium unit by the α-carboxylate anion can contribute to the poor performance of glutamic acid. Glycine, which is also a neurotransmitter, elicits a poor response from **1**, probably due to the above effect and also due to its inability to span the distance between the receptor units in **1**. Thus, **1** shows interesting selectivity characteristics for GABA monitoring even at this early stage of its design. The excision of the guanidinium unit from **1** while preserving the electron density conditions at the azacrown nitrogen can be achieved in the model compound **3**. **3** has a proton sensitivity of fluorescence (FE = 80, pK_a = 7.0) similar to **1**. However, **3** yields no measurable fluorescence response to GABA. So **1** consists of the minimal set of components to result in useful GABA sensing according to the present approach. However, the significant fluorescence responses found with propyl ammonium, sodium (FE = 0.7, log β = 1.5) and potassium (FE = 1.5, log β = 3.3) ions need to be suppressed in higher generations of **1** by employing two PET-active receptors rather than one.[12] Also, the availability of newer receptors which bind GABA strongly and selectively in water[13] will influence future sensor designs.

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Footnote

† ^{1}H NMR [$CDCl_3$/CD_3OD (5 : 1)] data for **1**. NO_3^-: δ 7.50–8.54 (m, 8 H, ArH), 5.37 [s, 2 H, $H_2NC(N^+H_2)NHCH_2Ar$], 4.50 [s, 2 H, $H_2NC(N^+H_2)CH_2ArCH_2$], 3.54–3.76 (m, 20 H, OCH_2), 3.41 (t, 4 H, NCH_2). ^{13}C NMR (Me_2SO-[$^2H_6$]) for **1**. NO_3^-: δ 156.5, 132.7, 130.6, 129.5, 126.6, 126.1, 125.4, 123.9, 70.0, 69.9, 69.8, 69.6, 69.4, 69.3, 69.0, 66.7, 53.1 and 51.1.

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