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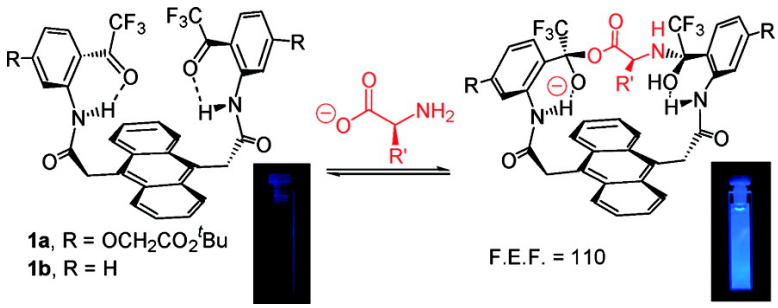


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# A Rational Approach to Fluorescence "Turn-On" Sensing of α-Amino-carboxylates

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Fluorescence sensing of anions has attracted continuing interest given that a variety of anion molecules are involved in chemical and biological systems.[1] A number of fluorescence anion sensors have been developed based on different disciplines in the sensor design. However, the development of fluorescence turn-on type sensors for anions of biological importance remains as a challenging issue, because in many cases fluorescence quenching rather than enhancement has been observed.[2] In a few cases fluorescence enhancement results from anion sensing.[3] Such fluorescence sensors, however, give only marginal enhancement in the fluorescence emission with rare exceptions[4] and may not represent fluorescence "turn-on" sensors if we consider the real meaning of the words. Therefore, it would be highly desirable to devise a fluorescence turn-on sensor for anions based on a new discipline, in addition to the known approaches. We report herein the rational approach to the fluorescence turn-on sensor for anions, particularly α-amino carboxylates, anionic forms of α-amino acids that are key constituents of proteins.

Recently we demonstrated that a trifluoroacetophenone derivative containing an H-bonding donor such as a sulfonamide group at the ortho position gives fluorescence enhancement rather than quenching upon exposure to anions such as cyanide and acetate ions.[5] The H-bonding donor stabilizes the anionic carbonyl carbon adducts through intramolecular H-bonding, which seems to suppress otherwise possible quenching processes by anionic species as well as increases the conformational rigidity of the adducts, thereby resulting in the fluorescence enhancement. Fluorescence modulation in anion sensing by the intramolecular H-bonding was supported by the fact that fluorescence quenching resulted when the H-bonding donor was absent.[5] The previous system, however, showed only several times enhancement in the fluorescence in the case of acetate ion. We reasoned that a proper combination of a fluorophore and a linker that connects the binding site to the fluorophore may lead to a fluorescence turn-on sensor for anions such as amino-carboxylates. Underlying rationale is as follows: Our anion recognition motif is based on the trifluoroacetyl carbonyl group, which may act as a fluorescence killer[6] as well as a binding motif toward anions such as carboxylates.[7] Upon forming an adduct as a result of nucleophilic attack of the anion at the carbonyl carbon, the quenching energy level may be perturbed and hence the emission from the $\pi-\pi^*$ transition of the fluorophore would be restored. A fluorescent sensor composed of an aromatic fluorophore and keto groups as the binding sites for metal cations has been used;[8] however, its extension to anion sensing has not been realized yet, because carbonyl groups poorly coordinate anions.

On the basis of preliminary studies, we have found that anthracene-based bis(*o*-trifluoroacetylcarboxanilide) **1a** shows remarkable fluorescence turn-on properties toward amino-carboxylates. The *o*-trifluoroacetylcarboxanilde (TFACA) moiety is also

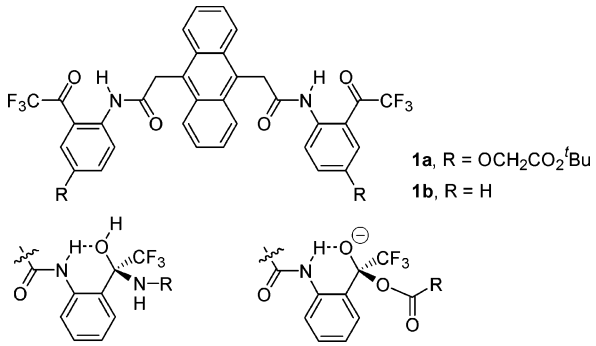


***Figure 1.*** Structures of bis(TFACA)s **1a** and **1b** and the binding modes between the TFACA moiety and amine or carboxylate groups.

an efficient recognition motif for amines in their neutral forms and gives fluorescence enhancement[9] even though amines are known to be fluorescence quenchers[6] (Figure 1).

An energy-minimized structure of **1b**[10] showed that the TFACA binding sites are standing over the anthracene plane, ready to accommodate an α-amino carboxylate between them (Supporting Information): The distance between the two trifluoroacetyl carbonyl carbons in **1b** was found to be 5.22 Å and that of its glycinate complex was 5.48 Å. In contrast, a minimized structure for *β*-aminopropionate gave a large structural change, which suggested selective recognition of α-amino-carboxylates over other homologues such as *β*- and *γ*-analogues might be possible.

UV−vis absorption spectra of **1a** measured in acetonitrile displayed absorption maxima characteristic to the anthracene moiety. When excited at 358 nm, **1a** emitted very weak fluorescence (quantum yield, $\Phi_F = 0.0078$), indicating that the TFACA moiety acts as the fluorescence quencher.[11] When **1a** was subjected to fluorescence titrations against increasing amounts of glycinate (as $Bu_4N^+$-salt) at $\mu$M scales in acetonitrile, fluorescence emission increased steadily up to the equivalent point, *with a large fluorescence enhancement factor of 110*. Other α-amino acids gave similar enhancement because their α-substituents are away from the cavity of the cyclic adducts and little affect the adduct formation. The fluorescence titration of **1a** toward glycinate gave a linear relationship between the intensity and concentration of the analyte, suggesting its usefulness in quantifying α-amino acids (Figure 2).

Formation of the (1:1)-adduct between **1a** and glycinate was clearly evidenced by $^{19}$F NMR. The reversible adduct formation was slow compared to the NMR time scale, and thus both **1a** and its glycinate adduct exhibited distinct $^{19}$F peaks from each other, and only the adduct peaks appeared after the equivalent point (Supporting Information).

Frontier molecular orbitals (MOs) of **1b** and its glycinate adduct calculated show a stark difference between the two. In the case of **1b**, highest occupied molecular orbital−lowest unoccupied molecular orbital (HOMO−LUMO) excitation moves the electron density

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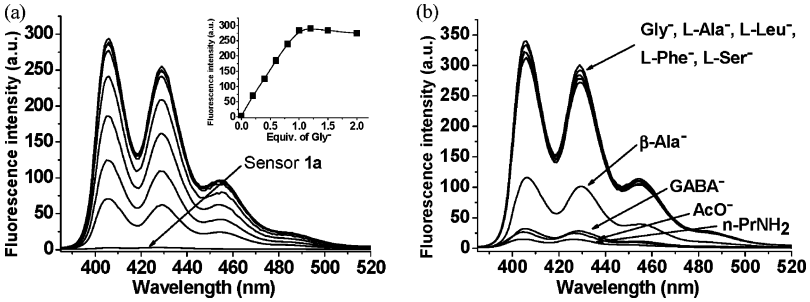


***Figure 2.*** (a) Fluorescence titration of **1a** (5.0 $\mu$M) with increasing amounts of $Gly^-$ ($\lambda_{ex}$ = 377 nm); (inset) dependence of fluorescence intensity with respect to [$Gly^-$]/[**1a**]; (b) collective intensity data for α-, $\beta$-, $\gamma$-amino acid anions (1.0 equiv), $AcO^-$ and *n*-$PrNH_2$ (2.0 equiv)

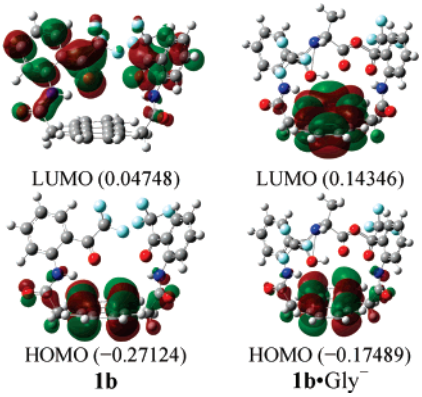


***Figure 3.*** The HOMO and LUMO orbitals of **1b** and its glycinate adduct.

distribution form the anthracene moiety to the TFACA, generating a charge-transfer fluorescence state that leads to fluorescence quenching. In contrast, in the case of **1b**·$Gly^-$, HOMO−LUMO excitation involves no such movement of the electron density that distributes mostly on the anthracene moiety; hence, the excited electrons would decay by emitting strong fluorescence. The calculated data corroborate the experimental results (Figure 3).

Quantum yields measured for a series of mixture between **1a** and glycinate increased as the complex being formed, supporting that the fluorescence enhancement is due to the adduct formation. The (1:1)-complex showed a high quantum yield ($\Phi_F$ = 0.84), suggesting a nearly complete restoration of the fluorescence from the 9,10-substituted anthracene moiety[12] upon adduct formation.

Isothermal titration calorimetry (ITC) carried out between **1a** and glycinate in acetonitrile provided thermodynamic parameters for the binding process. The integration data of the heat evolved upon titration showed an inflection point near the equivalent point, again supporting the (1:1)-adduct formation. The "dominant" binding interaction involved a favorable enthalpy change ($\Delta H^\circ = -2.0 \times 10^4$ cal/mol) accompanied with an unfavorable entropy change ($-T\Delta S^\circ = 1.0 \times 10^4$ cal/mol, $T$ = 303 K), from which a large association constant ($K_{ass} \approx 1.0 \times 10^7$ $M^{-1}$) was obtained (Supporting Information). The ITC data support the formation of the cyclic adduct through a cooperative binding by the amine and carboxylate functions. In the case of $\beta$-aminopropionate, a complex binding equilibrium was suggested, with a smaller association constant.

In summary, we have disclosed a rational approach to fluorescence turn-on sensing of amino carboxylates. The approach primarily relies on the perturbation of the quenching n−$\pi^*$ transition energy level of the carbonyl ionophore relative to the $\pi-\pi^*$ transition energy level of the fluorophore in the sensor, which has remained as an unexplored approach in the anion sensing so far. Our anthracene-based bis(trifluoroacetylcarboxanilide) sensor is structurally simple but selectively senses α-amino acids as their carboxylate forms over $\beta$- and $\gamma$-homologues by forming a cyclic adduct.

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**Supporting Information Available:** Details for the synthesis of **1a**, $^1H/^{19}F$ NMR and ITC titration data, and MO calculation and experimental results. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) Because of poor solubility of **1b** in most organic solvents, we used **1a** for sensing experiments, except for molecular modeling. Synthesis of water-soluble analogues of **1** and molecular sensing studies are under investigation.

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(12) The quantum yields of 9,10-dimethylanthracene are 0.63 and 0.98 in oxygen-containing and oxygen-free ethanol, respectively. See chapter 2 in ref 6c.

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