# Trifluoroacetophenone Derivatives as Amino Acid Selective Ionophores for the Potentiometric Determination of Phenylalanine**

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The development of artificial receptors/ionophores for the selective recognition of biologically important species has attracted much attention because of their possible application to chemical sensors, such as ion-selective electrodes (ISEs) or optical chemosensors (optodes).[1] So far, many ionophores for cations or anions have been developed and applied to ion sensing.[2] On the other hand, molecular recognition and determination of amino acids by artificial ionophores is still challenging because of their highly hydrophilic character. Because amino acids are essential for the human body,[3] several analytical methods for the determination of amino acids have been developed.[4] However, simpler and more inexpensive devices are required, and a potentiometric sensor seems to be a promising candidate for this purpose. Although there are several examples of ionophores which can bind amino acids in the zwitterionic form through multiple-point binding,[5] charged species have to be transported into a lipophilic membrane from the water phase to allow the potentiometric detection with carrier-based ISEs. Because amino acids exist in the cationic form in acidic solutions, most studies of ionophore-based ISEs have evaluated the ammonium form. This approach results in a cationic response, in which the more lipophilic amino acid esters are measured instead of the amino acid itself.[6] It was recently reported that $Mn^{III}$ porphyrin[7] and lead phthalocyanine[8] can be used as ionophores for underivatized cysteine- and histidine-selective electrodes, respectively. However, to our knowledge, there is no report on the rational design and synthesis of artificial ionophores for potentiometric determination of underivatized amino acids. Here we report on the characteristics of trifluoroacetophenone derivatives as neutral ionophores for ISEs. It was shown that electrodes based on preorganized tripodal ionophores with trifluoroacetophenone moieties respond to phenylalanine with a monoanionic Nernstian response.

Trifluoroacetophenone is known to form adducts with amines or alcohols,[9] and almost all ionophores for carbonate are trifluoroacetophenone derivatives.[1b,10,11] To bind the amino and the carboxylate groups of an amino acid in the anionic form simultaneously, more than two trifluoroacetophenone moieties are included in one molecule. Thus, the tripodal ionophores **1**–**3**, with a hexasubstituted benzene ring as a spacer,[12,13] were designed and synthesized, together with a reference compound **4**. As illustrated in Figure 1b, it is expected that two of the three functional groups participate in binding with the amino and the carboxylate groups of phenylalanine and, additionally, the benzene ring of phenylalanine is suitably accomodated in the hydrophobic cavity formed by three benzene rings of the binding arms and the spacer benzene ring. The characteristics of the ISEs based on these neutral ionophores combined with a cationic additive (tridodecylmethylammonium chloride; TDDMACl) were examined under the conditions reported in reference [11e], in which the condition for carbonate-selective ionophores was optimized. In the first stage, the relationship between ionophore structure and the carbonate selectivity was examined (see Supporting Information). All of the ISEs based on ionophores **1**–**4** (see Figure 1a) exhibited almost the same response curves to carbonate with a theoretical Nernstian slope ($\approx -30$ mV/decade). Because the observed carbonate selectivity was in the order of $\mathbf{3} > \mathbf{4} > \mathbf{2} > \mathbf{1}$, it was concluded that the binding ability to carbonate is mainly influenced not

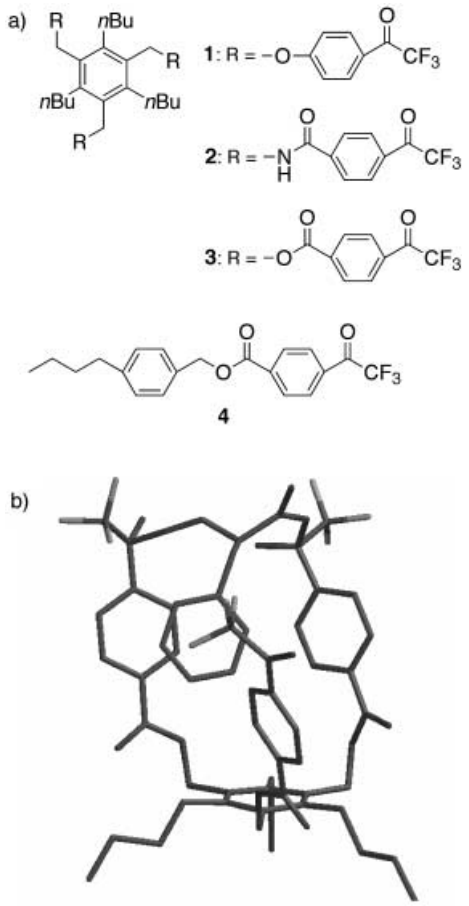


Figure 1. a) Chemical structures of tripodal ionophores **1**–**3** and reference compound **4**. b) Proposed structure of the complex of ionophore **3** with the anionic form of phenylalanine. The structure was optimized by use of AM1 calculations. Hydrogen atoms are omitted for clarity.

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by the number of binding sites, but rather by the electronic nature of the connecting unit (ester > amide > ether).[14] On the other hand, the ISEs based on tripodal ionophores **1**–**3** exhibited a Nernstian response to the anionic form of phenylalanine (≈ −59 mV/decade) whereas the ISE based on **4** exhibited only a weak response in the relatively high concentration range, as shown in Figure 2.[15] These results are coincident with the expected molecular design; it is necessary

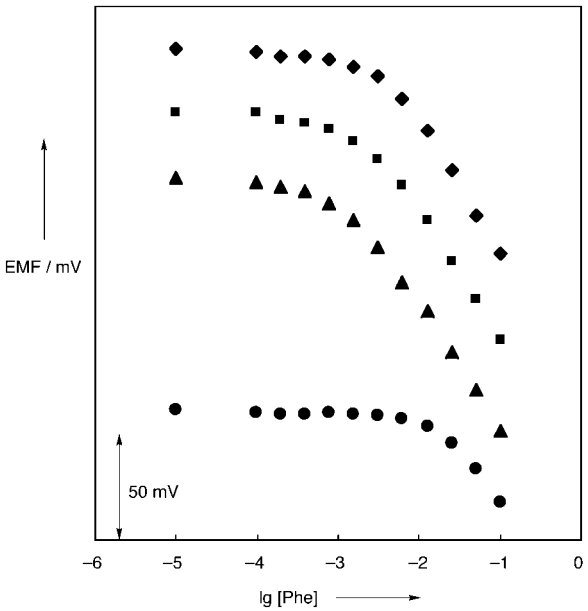


Figure 2. EMF response curves of the electrodes based on ionophores **1** (◆), **2** (■), **3** (▲), and **4** (●) to phenylalanine.

to introduce more than two binding sites in one molecule to interact effectively with the amino acids in the aqueous phase. Moreover, it should be pointed out that the electrode based on TDDMACl without addition of ionophore showed little response towards relatively lipophilic phenylalanine, apparently because of the strong hydration of the amino acid. The gain in selectivity caused by the ionophore was two orders of magnitude, with the electrode responding to the amino acid from $10^{-4}$ M and $10^{-2}$ M for the membrane with and without ionophore, respectively. The electrodes based on neutral ionophores without cationic additive did not show any response towards phenylalanine.

The selectivity coefficients of the electrodes for essential amino acids are summarized in Table 1. Although all electrodes showed phenylalanine selectivity among the amino acids examined,[16] it should be noted that the selectivity of the ISEs based on tripodal ionophores **1**–**3** is better than that of the ISE based on **4**. Therefore, it is concluded that the observed selectivity is not only caused by the lipophilicity of the phenylalanine itself, but also by the preorganized shape of the tripodal ionophores with a hydrophobic pocket. Among the electrodes based on **1**–**3**, ISEs based on **3** are superior to the others, probably because of the electron-withdrawing ability of the connecting ester groups, as observed in the carbonate determination. The phenylalanine selectivity towards several representative inorganic anions was also investigated, and the

Table 1. Potentiometric selectivity coefficients (log $K^{pot}_{Phe,J}$) for ISEs based on ionophores **1**–**4** for essential amino acids.[a]

| Amino acids | **1** | **2** | **3** | **4** |
|---|---|---|---|---|
| glycine | −1.1 | −1.2 | −1.7 | −0.4 |
| alanine | −1.2 | −1.3 | −1.7 | −0.4 |
| valine | −1.1 | −1.2 | −1.3 | −0.7 |
| leucine | −0.8 | −0.7 | −0.9 | −0.6 |
| isoleucine | −0.9 | −0.7 | −0.9 | −0.7 |
| serine | −1.3 | −1.5 | −1.7 | −0.6 |
| threonine | −1.2 | −1.4 | −1.7 | −0.6 |
| methionine | −0.8 | −0.7 | −0.9 | −0.6 |
| proline | −1.3 | −1.6 | −1.8 | −0.7 |
| histidine | −1.4 | −1.4 | −1.8 | −0.8 |
| lysine | −1.2 | −1.5 | −1.9 | −0.8 |
| arginine | −1.3 | −1.4 | −1.7 | −0.6 |
| aspartic acid | −1.0 | −0.9 | −1.4 | −0.3 |
| asparagine | −1.4 | −1.4 | −1.8 | −0.7 |
| glutamic acid | −1.2 | −0.9 | −1.6 | −0.5 |
| glutamine | −1.1 | −1.0 | −1.7 | −0.4 |

[a] The selectivity coefficients were calculated from the response potentials to 0.1 M sample solutions using the separate solution method (SSM).

best result was obtained for the ISE based on **3**. We found that the electrode has good selectivity over carbonate or other anions, though the interference of lipophilic perchlorate is still high (log $K^{pot}_{Phe,J}$: $ClO_4^-$ +0.61, $CO_3^{2-}$ −0.99, $I^-$ −0.83, $Br^-$ −1.64, $Cl^-$ −1.71, $HPO_4^{2-}$ −2.34). Thus, we have demonstrated an example of rationally designed artificial ionophores for ISEs that respond to underivatized phenylalanine with a theoretical Nernstian response.

## *Experimental Section*

Electrode preparation and EMF measurements: PVC matrix-based membranes were prepared from a mixture of ionophore (3 wt %), membrane solvent bis(2-ethylhexyl)sebacate (DOS; 67 wt %), PVC (30 wt %), and tridodecylmethylammonium chloride (TDDMACl; 50 mol %, relative to the ionophore). The components dissolved in THF were poured into a vial which was placed on a hotplate. Slow evaporation of THF at 40 °C gave ion-sensitive membranes of approximately 100 μm thickness. A 6 mm diameter circle was cut from a prepared membrane and placed on the tip of a PVC ion-selective-electrode body assembly (Liquid Electrode Membrane Kit, DKK Co., Ltd., Tokyo, Japan). The prepared electrodes were immersed in 0.1 M aqueous NaCl solution for more than 24 h for preconditioning before use. The external reference electrode was a double-junction-type Ag–AgCl electrode (HS-305DS, Toa Electronics, Ltd., Tokyo, Japan). The electrode potential (emf) measurements were performed at 25 ± 0.5 °C by using the electrochemical cell system, Ag | AgCl | saturated KCl | 0.3 M $NH_4NO_3$ | | sample solution | ISE membrane | 0.1 M NaCl | AgCl | Ag.

All sample solutions were prepared with 0.1 M Tris–$H_2SO_4$ buffer solution (Tris = tris(hydroxymethyl)aminomethane) at pH 8.6. The pH values of 0.1 M glutamic acid and aspartic acid in 0.1 M Tris solution were 6.10 and 5.83, respectively, and were adjusted to pH 8.6 using NaOH. The selectivity coefficients $K^{pot}_{I,J}$ where I stands for the primary ion ($PhCH_2CH(NH_2)COO^-$ for Table 1 and $CO_3^{2-}$ for Table S1 in the Supporting Information) and J for the interfering ion, were calculated from the response potentials using the separate-solution method (SSM; [I] = [J] = 0.1 M), according to the recommendations of IUPAC[17] and JIS.[18]

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[15] At pH 8.6, the concentration of the anionic form of phenylalanine is calculated to be 24% of the total concentration, as the $pK_{a2}$ value of phenylalanine is 9.1.
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## Combinatorial and Rational Strategies To Develop Nonpeptidic α4β7-Integrin Antagonists from Cyclic Peptides

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The development of low-molecular-weight, nonpeptidic, and orally available drugs starting from biologically active peptides or proteins is one of the great challenges in medicinal chemistry. Herein we present an example in which a biologically active tripeptide has been reduced to a dipeptide in which the activity has been retained. New concepts in the treatment of inflammatory diseases have recently emerged.[1] One promising target to treat inflammatory diseases in the intestine is the inhibition of the α4β7-integrin/MAdCAM-1 interaction (MAdCAM-1 = mucosal addressin cell-adhesion molecule 1) interaction.[2] In contrast to other cell adhesion molecules, MAdCAM-1 is expressed only on a few cell types.[3,4] The interference of the α4β7-integrin/MAdCAM-1 interaction, which selectively mediates lymphocyte recruitment to the mucosa-associated lymphoid tissues of the intestine, is not expected to affect other parts of the immune system. As a consequence, no systemic side effects should occur during this therapy. The potential value of this therapy has already been shown with antibodies in animal models of colitis.[5,6] Recently, we and others reported the synthesis of peptidic α4β7-integrin antagonists that contain the Leu-Asp-Thr (LDT) recognition sequence for α4β7-integrins.[7–10] In our previous work we showed that the amide bond between $Thr^4$ and $Asp^5$ in the cyclic peptide *cyclo*-(Phe[1]-Leu[2]-Asp[3]-Thr[4]-Asp[5]-D-Pro[6]) was not essential. Furthermore, $Thr^4$ could be substituted with Val without loss of biological activity. Therefore, we synthesized a library of cyclic hexapeptides of the general formula *cyclo*-(Phe-Leu-Asp-Xaa-Asp-D-Pro). The biological evaluation of the corresponding compounds with a cell adhesion assay showed that when Xaa = phenylalanine (see **1**) or phenylglycine (see **2**) the biological activity towards inhibiting the α4β7-integrin/MAdCAM-1 interaction was maintained (Table 1). Based on these results and on the results of Shroff et al.,[9,10] we developed a library illustrated in Figure 1. Four different building blocks **A**, **B**, **C,** and **D** were used. Starting from isoquinoline-3-carbonyl-Leu-Asp-Thr-OH (**3**)[10] only one building block was changed each time. The resulting library was consequently not completely combinatorial. The synthesis of these compounds was performed step-by-step in parallel on solid supports, according to the Fmoc-strategy.[11] The incorporation of fluoroaromatics as

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