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Synthesis of a complete set of L-difluorophenylalanines, L-(F_2)Phe, as molecular explorers for the CH/π interaction between peptide ligand and receptor

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Abstract

A complete set of difluorophenylalanines in the L-configuration [L-(F_2)Phe] (namely, L-(2,3-F_2)Phe, L-(2,4-F_2)Phe, L-(2,5-F_2)Phe, L-(2,6-F_2)Phe, L-(3,4-F_2)Phe, L-(3,5-F_2)Phe) was prepared and incorporated into the thrombin receptor-tethered ligand peptide SFLLRNP to identify the phenyl hydrogens of the Phe-2 residue involved in the CH/π receptor interaction.

Keywords: amino acids; amino acid derivatives; fluorine; fluorine compounds; molecular recognition; peptides; polypeptides.

1. Introduction

Phenylalanine (Phe), an aromatic amino acid, is often crucially important in biologically active peptides to elicit intrinsic activity, and on that occasion, the side-chain phenyl group plays an essential role in the ligand–receptor interaction.[1] The molecular mechanism of this so-called hydrophobic interaction, however, has never been elucidated in detail. When Phe interacts with the alkyl side chains of amino acids Leu, Ile, Val, or Ala, the Phe–phenyl π system would function as a hydrogen acceptor. This interaction is denoted as a CH/π interaction, the concept of which has recently been established by Nishio et al.[2] When Phe interacts with aromatic amino acids such as Phe, Tyr, Trp, and His, Phe–phenyl should be involved in two different types of π–π interactions, i.e., the face-to-face π–π stacking interaction and the edge-to-face CH/π interaction. To better understand the molecular mechanism of peptide interactions involving the Phe residue, differentiation of these Phe–phenyl π interactions is requisite. We have postulated that

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these π–π interactions can apparently be distinguished by replacing fluorophenylalanines for the Phe residue.

Fluorine can substitute the benzene hydrogens (CHs) without changing the atomic size, and thus it is highly likely that multiple fluorine replacements of benzene hydrogens could clarify whether essential hydrogens exist on Phe–phenyl or whether the π system is required for the interaction with the receptor. For fluorophenylalanines (F_n)Phe, there are 19 isomers in total (three of (F_1)Phe, six of (F_2)Phe, six of (F_3)Phe, three of (F_4)Phe, and one of (F_5)Phe), and only several of them such as mono- and pentafluorophenylalanines have been prepared in optically pure states. Most of the di-, and tetrafluorinated derivatives were synthesized as racemates, and almost no data have been reported for trifluorinated derivatives.[3] In the present study, we synthesized a complete set of optically pure L- and D-difluorinated phenylalanines, difluorophenylalanines [(F_2)Phe] (namely, (2,3-F_2)Phe, (2,4-F_2)Phe, (2,5-F_2)Phe, (2,6-F_2)Phe, (3,4-F_2)Phe and (3,5-F_2)Phe) (Fig. 1). Furthermore, we incorporated all six isomers of L-(F_2)Phe, respectively, into a thrombin receptor-tethered ligand peptide S/Phe/LLRNP to clarify the specific edge-to-face CH/π interaction.[4]

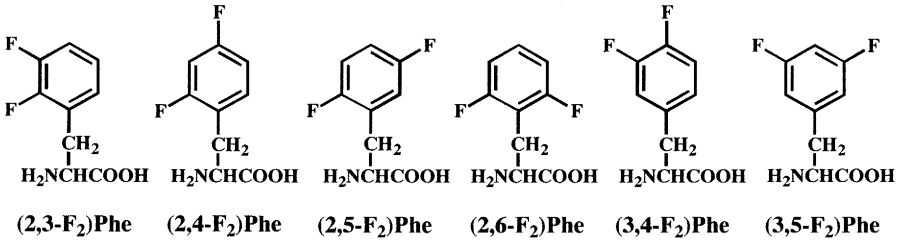

Fig. 1. The side chain structure of difluorophenylalanines [(F_2)Phe]

2. Experiments and results

The synthesis and optical resolution of difluorophenylalanines were carried out by the method shown in Fig. 2.[5] Six different difluorobenzyl bromides **1** (120 mmol) (JRD Fluorochemicals Ltd, Surrey, UK) were coupled with diethyl acetamidomalonate (100 mmol), respectively, in abs. EtOH which dissolved Na (120 mmol) or NaOEt (120 mmol) by refluxing for 2 h. After filtration and evaporation, the residue was treated with cold water to collect the precipitate, which was recrystallized from EtOH–water to afford diethyl [difluorophenylmethyl]acetamidomalonate **2** (av. yield, 90%) (Table 1). Diethyl ester **2** (70 mmol) was then subjected to simultaneous saponification in MeOH (200 ml)/2 M NaOH (280 mmol) at 40°C for 3 h. The resulting malonic acid derivatives **3** were liberated by 6 M HCl and extracted with ethyl acetate. Unlike non-fluorinated derivatives in the literature,[5] **3** appeared to be quite unstable, and thus the residue of evaporation was directly refluxed in *para*-xylene for 2 h. This afforded decarboxylated acetyl-DL-difluorophenylalanines (Ac-DL-(F_2)Phe, **4**) in very high yield (av. yield, 92%) (Table 1).

Ac-DL-(F_2)Phe was digested with *Aspergillus genus* acylase (Tokyo Chemical Industry) to resolve into Ac-D-(F_2)Phe and L-(F_2)Phe. To a solution (pH 8.0) of Ac-DL-(F_2)Phe (50 mmol) in 0.2 M NaOH (275 ml) was added acylase (400 mg) in 0.2 M AcONa, and the solution was incubated at 38°C for 48 h. After removal of the enzyme by Norit, Ac-D-(F_2)Phe was extracted with EtOAc from the aqueous solution (pH 3.0) and purified by recrystallization (av. yield, 91%). The liberated free amino acid L-(F_2)Phe was collected and purified by applying to a column (3.8×22 cm) of Dowex 50X8 (H^+ form) eluted with 2 M NH_4OH (av. yield after recrystallization, 92%). Optical purity of L-(F_2)Phe was confirmed by high-

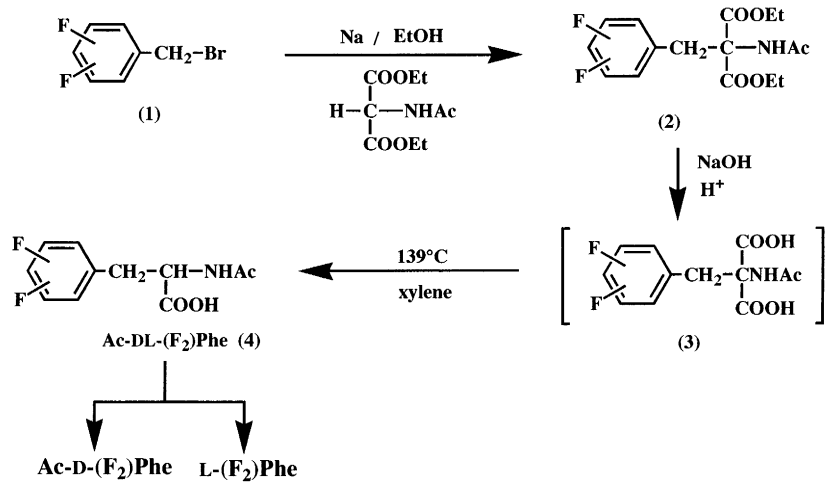

Fig. 2. Synthetic route of difluorophenylalanines [(F_2)Phe]

Table 1
The melting point of compounds related to difluorophenylalanines

	Malonate **2**[1)]	Ac-DL-(F_2)Phe	Ac-D-(F_2)Phe	L-(F_2)Phe	Boc-L-(F_2)Phe
2,3-F_2	152[2)]	170	178	214[3)]	112
2,4-F_2	132	148	152	222[3)]	83
2,5-F_2	137	150	171	212[3)]	117
2,6-F_2	102	181	182	200[3)]	99
3,4-F_2	145	137	144	204[3)]	83
3,5-F_2	132	161	158	216[3)]	67

The data of elemental analyses indicated that the values analyzed are consistent with those calculated.
[1)] diethyl [difluorophenylmethyl]acetamidomalonate. [2)] The values indicate the mean with ± 1°C. [3)] decomp.

performance liquid chromatography (HPLC) using a chiral column SUMICHIRAL OA 5000 (0.46×15 cm, 5 mm; Sumika Chemical Analysis Service, Osaka). The analytical data are shown in Tables 1 and 2.

In due course, we achieved, for the first time, the synthesis of all six isomers of optically pure L- and D-difluorophenylalanines (F_2)Phe. Prudechenko[6] reported the synthesis of five DL-(F_2)Phe racemates, except for DL-(2,3-F_2)Phe, by the azlactone method, in which difluorinated benzaldehydes were coupled with benzoylglycine. Although all six isomers of DL-(F_2)Phe racemates are now commercially available (JRD), none of the optically pure stereoisomers of L- or D-(F_2)Phe are available. For peptide synthesis, however, amino-protected derivatives of optically active amino acids usually in the L-configuration are required, and among the L-(F_2)Phe isomers only Boc and Fmoc derivatives of L-(3,4-F_2)Phe and L-(3,5-F_2)Phe are available from several commercial sources. Thus, in this study, we prepared Boc derivatives of all of the L-(F_2)Phe isomers with di-*tert*-butyldicarbonate (av. yield, 94%). These Boc-L-(F_2)Phe isomers were used for the syntheses of thrombin receptor-tethered ligand peptide SFLLRNP (denoted by one-letter amino acid codes), in which L-(F_2)Phe isomers were incorporated into position 2 to replace Phe(=F) essential for the receptor activation.

Peptides were synthesized by the manual solid-phase synthesis method. Coupling reactions (0.1 mmol) were carried out with 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) in the presence of 1-hydroxybenzotriazole (HOBt) for 30 min. After HF treatment, the peptides were purified by Sephadex G-15 followed by preparative reversed-phase HPLC (Cica–Merck, LiChros-

Table 2
Optical properties of difluorophenylalanines and their derivatives

	Retention time in HPLC[1]		Optical rotation $[\alpha]_D^{20}$		
	L-(F_2)Phe	D-(F_2)Phe[2]	Ac-D-(F_2)Phe[3]	L-(F_2)Phe[4]	Boc-L-(F_2)Phe[3]
2,3-F_2	22.6	39.1	–27.6°	–18.4°	+3.6°
2,4-F_2	15.7	22.1	–22.4°	–9.6°	–2.3°
2,5-F_2	12.4	18.0	–25.1°	–14.4°	–4.8°
2,6-F_2	7.7	11.0	–13.5°	+2.9°	–12.0°
3,4-F_2	26.1	42.2	–27.6°	–28.6°	+4.7°
3,5-F_2	17.1	23.9	–24.5°	–15.6°	–2.1°

[1] The data of retention time (min) were obtained on a chiral column SUMICHIRAL OA 5000 (0.46 x 15 cm, 5 mm). [2] D-(F_2)Phe for this HPLC analysis was obtained from Ac-D-(F_2)Phe by the treatment with 4 M HCl-dioxane (1:1, v/v) under reflux for 5 hr (av. yield, 95%). [3] *c* 1.0, MeOH. [4] *c* 1.0, H_2O.

pher RP-18 (e) (5 μ): 25×250 mm). The total yield of peptides from the resin was av. 43%. The purity was verified by analytical HPLC and amino acid analyses. All the peptides synthesized were evaluated in the assay for human platelet aggregation by the standard turbidimetric procedure using a hema tracer.

It was found that any replacement of the phenyl hydrogens at the *ortho* and *meta* positions diminishes the activity considerably (6–35% activity of parent SFLLRNP). This finding, together with previous assay results,[3] clarified an important aspect of the structure, which led to the conclusion that Phe-2-phenyl of S/Phe/LLRNP is in the edge-to-face CH/π interaction with the receptor aromatic group, utilizing the benzene ring-edge along with hydrogens at positions 5 and 6 (Fig. 3). Furthermore, the high activity (140–150%) of L-(2,4-F_2)Phe- and L-(3,4-F_2)Phe-containing S/Phe/LLRNP analogs confirmed the previous result that the fluorine atom at position 4 increases biological activity.[3,7] This fluorine appears to increase the acidity of the hydrogen at adjacent position 5, resulting in a reinforcement of the CH/π interaction and consequently in enhancement of biological activity. Collectively, benzene hydrogen/fluorine replacement was found to enable an effective structural examination of the Phe residue, i.e., to identify the hydrogens in the CH/π interaction, and to strengthen the CH/π interaction. Fluorophenylalanines would substantiate the structural examination to elucidate the functional roles of the Phe residue in biologically active peptides.

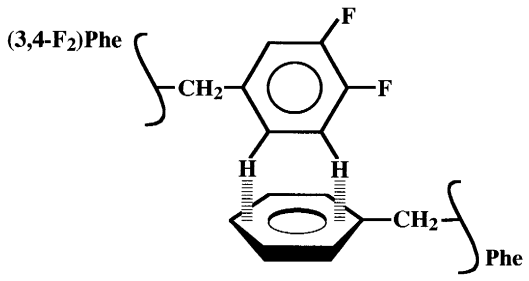

Fig. 3. Putative edge-to-face CH/π interaction between the peptide ligand (3,4-F_2)Phe–phenyl and the receptor Phe–phenyl groups

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