Importance of Solvent-Bridged Structures of Fluorinated Diphenylalanines. Synthesis, Detailed NMR Analysis, and Rotational Profiles of Phe(2-F)-Phe(2-F), Phe(2-F)-Phe, and Phe-Phe(2-F)

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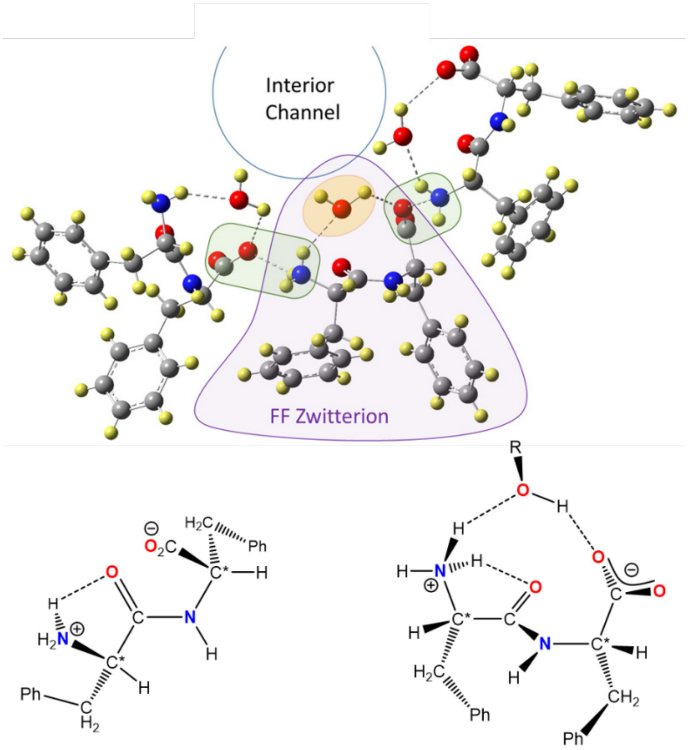
Abstract

The crystal structure of *L*-phenylalanyl *L*-phenylalanine (Phe-Phe, FF, a.k.a. diphenylalanine) is not merely non-centrosymmetric, but it is highly dipole parallel aligned. It is for this reason that FF is a non-linear optical (NLO) material and exhibits strong second harmonic generation (SHG). Enhancement of the SHG response by *ortho* fluorination was demonstrated. Crystallization is non-trivial and learning about the zwitterion structures in solution is important for the rational improvement of the crystallization process. Here we present an NMR study of di-fluorinated FF (Phe(2-F)-Phe(2-F)) and of mono-fluorinated FF isomers (Phe(2-F)-Phe and Phe-Phe(2-F)). The dipeptides were prepared by solid phase synthesis and purified by HPLC. Their ^{1}H- and ^{13}C-NMR spectra were measured in partially deuterated water (10% D_2O) and 2D-NMR techniques were employed for signal assignments. The unambiguous assignments are reported of all chemical shifts for the aliphatic H and C atoms and of the C atoms of the carboxylate, the amide-carbonyl, the CF carbons, and of every arene C atom in each phenyl ring. The dipeptides are *trans* amides and intramolecular hydrogen bonding between the ammonium group and the amide carbonyl restricts the $H_3N{-}CH{-}C(O)$ geometry. We explored the rotational profile of the diphenylalanines as a function of the $\tau = \angle(C{-}N{-}C{-}CO_2)$ dihedral angle at the SMD(B3LYP/6-31G*) level without and with specific hydration and report the associated Karplus curves $J(\theta)$ *vs*. $\theta = \angle(H{-}N{-}C{-}H)$. The rotational profiles show that a maximum of three stationary structures and relative conformer stabilities of the free diphenylalanines show that the conformation found in the crystal, **M1** is the least stable among the three, **M3** > **M2** ≫ **M1**. Specific water solvation makes all the difference and adds a large competitive advantage to the water-bridged ion pair **M1a**. In fact, **M1a** becomes the most stable and dominant conformation for the parent diphenylalanine and mono1 F-FF and **M1a** becomes competitive with **M3c** for mono2 F-FF and di F-FF. Implications are discussed regarding the importance of the conformational pre-organization of diphenylalanines in solution and the facility for their crystallization.

1. Introduction

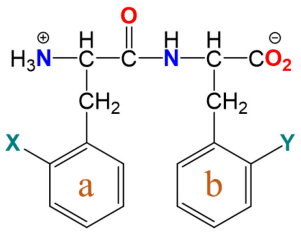
Non-linear optical (NLO) materials alter some aspect of incident light such as the plane of polarization or the frequency,[1] and these materials are ubiquitous in daily life.[2] Second harmonic generation (SHG) is the most important property of NLO materials, that is, the phenomenon that the materials emit light with twice the frequency of the incident light.[3,4] Non-centrosymmetry is a requirement for a materials to exhibit SHG activity.[5] SHG materials play essential roles in the fields of optical signal processing, optical limiting systems, parametric oscillators, and data storage.[6] Many traditional SHG materials are inorganic materials, but organic materials are becoming more important as NLO materials.[7,8] Organic NLO materials typically are based on non-centrosymmetric, conjugated donor-acceptor molecules.[9,10] Biological materials[11] peptides play an increasing role as NLO materials, because the intrinsic chirality of the amino acids ensures non-centrosymmetry.[12,13]

Scheme 1. Stereochemistry of FF and ROH-bridged FF



Phenylalanyl phenylalanine (Phe-Phe, FF, a.k.a. diphenylalanine) is a zwitterion (Scheme 1) and self-assembles into nano materials that exhibit SHG. The crystal structure of FF is not merely chiral because of the intrinsic chirality of any amino acid, but it is highly dipole aligned.[14,15] In the crystal structure, six FF zwitterions form a helical ring around the "interior channel" and the stacking of such rings forms a nanotube.[16] The immediate environment of one FF zwitterion is shown in Scheme 1 based on the crystal structure data. Contact ion pairs are formed between neighboring FF zwitterions (green highlight in Scheme 1). It is one of the characteristic features of the crystal structure that the interior channel contains crystal water, which stabilizes the zwitterions by formation of the intramolecular water-separated ion pairs (orange highlight in Scheme 1). We will quantify the stabilization afforded by the formation of the water-separated ion pair. All the carbonyl groups of the FF amide backbone are pointing in the same direction and result in the polar alignment in the entire nanotube. Diphenylalanine has been applied successfully for the fabrication of drug delivery systems,[17] optical waveguides,[18] and antibacterial agents.[19] The self-assembled FF nanotubes can be used as chiral sensing platform[20] and as molds for metal nanowires.[21] A variety of modified diphenylalanines have been studied because of the simple synthesis of FF and the ease of its chemical modification.[22-24]

Scheme 2. Structures of FF, Isomers of Mono F-FF, and Di F-FF



FF: X = H, Y = H
Mono1 F-FF: X = F, Y = H
Mono2 F-FF: X = H, Y = F
Di F-FF: X = F, Y = F

We have been interested in studying the effects of fluorination on the properties of FF. We have demonstrated the successful improvement of the SHG signal intensity by replacing an *ortho* H with a fluorine atom in both benzene rings, di F-FF in Scheme 2.[25,26] To study the mechanism of

this SHG enhancement, we wanted to expand the scope of our study to include the mono-fluorinated FF molecules. In the present paper, we present a comparative study of di F-FF, mono1 F-FF, and mono2 F-FF (Scheme 2). Only one benzene ring is *ortho*-fluorinated in the isomers mono1 F-FF and mono2 F-FF. In mono1 F-FF (Phe(2-F)-Phe), only the benzene of the phenylalanine at the *N*-terminus is fluorinated, and in mono2 F-FF (Phe-Phe(2-F)), it is the benzene close to the *C*-terminus that is fluorinated. The dipeptides were prepared by solid phase synthesis and their purity and identity was established by LC-MS analysis. A variety of one- and two-dimensional NMR spectroscopic techniques were applied to obtain complete assignments of their ^{1}H- and ^{13}C-NMR signals.

The presented NMR measurements in principle can discriminate between structural options in the accessible conformational space, but such mapping is not trivial because each of the diphenylalanines may occupy a vast conformational space (Scheme 1, left). However, there are a few reasonable constraints to allow a first analysis of the structural chemistry in solution. Each dipeptide will be a *trans* amide with ∠(O=C–N–H) ≈ 180° and the ammonium group will engage in hydrogen bonding with the amide carbonyl restricting the H–C–C(O)–N–H geometry. The zwitterion is a frustrated ion pair in that its ammonium group and the carboxylate group cannot approach each to form a stable contact ion pair $(H_2N–H)^{+}\cdots(OCO)^{-}$. Because of this frustration, the carboxylate will prefer a position that allows for the formation of a solvent separated ion pair $(H_2N–H)^{+}\cdots O(R)–H\cdots(OCO)^{-}$. The bridging by water (R = H) or alcohol (R = alkyl) in the solvent separated ion pair imposes strong constraints on the H–N–C–H geometry (Scheme 1, right). Thus, we include an extensive computational study of the rotational profiles about the N–C bonds of the parent diphenylalanine and the three fluorinated derivatives. Karplus analysis of the structures along the rotational profiles show that the $^{3}J_{HNCH}$ coupling constants do not differentiate between possible conformations. However, the computed thermochemistry shows that the inclusion of the specific solvation is key to adequately assess the relative importance of the N–C conformations.

2. Synthesis and Characterization

2.1. Synthesis of Fluorinated Diphenylalanine

All three peptides were prepared manually in a reaction vessel for peptide synthesis on 2.5 g of 2-chlorotrityl (2-ClTrt) chloride resin.

Synthesis of di F-FF: 1.2 g (3 mMol) of Fmoc-*L*-Phe(2-F)-OH were added to the resin together with 1.7 ml (10 mMol) of *N*,*N*-diisopropylethylamine (DIPEA). The reaction was left to proceed for 1 hour and then repeated. Capping of the resin was then performed with MeOH (5 min, 15 ml) and the loading of the resin was experimentally shown to be ≈ 0.6 mMol/g by HPLC based quantitative Fmoc evaluation test. Fmoc deprotection was achieved by treatment with 20% piperidine in DMF for 20 min, repeated twice.

2.4 g (6 mMol) of the second protected amino acid, Fmoc-*L*-Phe(2-F)-OH was reacted with 3.4 ml of DIPEA (20 mMol) and subsequently with 2 g (5.5 mMol) of 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) for 5 min to provide the corresponding activated ester. This activated ester was reacted in situ with the peptidyl resin for 1 hour. The same coupling procedure was repeated once to afford the protected dipeptide on the resin. Capping of unreacted amino groups of the first phenylalanine residue by acylation was achieved by reaction with 5% Ac_2O and DIPEA for 5 min. Final Fmoc deprotection was performed as above to obtain the desired dipeptide on the resin.

Acid-catalyzed ester hydrolysis was used to cleave the peptide from the resin and involved treatment with 10% trifluoroacetic acid (TFA) in presence of water and triisopropylsilane (TIPS) scavengers (both 5%) in dichloromethane (DCM). After 45 min of reaction, the reaction mixture was filtered and evaporated by nitrogen to almost dryness before to be diluted with 50% water and acetonitrile and lyophilized overnight to obtain 500 mg of crude dipeptide. Crude dipeptide identity was confirmed by LC-MS analysis and its preparative purification by MS-assisted flash chromatography yielded 158 mg of 93% pure H_2N-Phe(2-F)-Phe(2-F)-COOH.

Synthesis of mono F-FF: The syntheses of both mono1 and mono2 F-FF were performed in complete analogy to the procedure described for di F-FF and details are provided in the Supporting Information.

LC-MS Analysis of Dipeptides: The purity and identity of each dipeptide was established by LC-MS analysis. In supporting information, we provide the LC chromatogram and the ESI mass spectrum for each dipeptide. The molecular ions appear at m/z = 348.97 (di F-FF) and at m/z = 331 (mono F-FF).

2.2. NMR Measurements of Fluorinated Diphenylalanine

NMR data of the F-FF molecules was collected on a Bruker 600 MHz NMR spectrometer. All measurements were performed in partially deuterated water (10% D_2O and 90% H_2O). ^{1}H-NMR chemical shifts δ are reported in ppm relative to TMS and data in parentheses lists the signal multiplicity (d = doublet, t = triplet, q = quartet, and m = multiplet), integrated signal intensity in H equivalents, and coupling constant information. ^{13}C-NMR chemical shifts δ are reported in ppm relative to TMS. ^{19}F-NMR chemical shifts δ are reported in ppm relative to $CFCl_3$ and the internal standard trifluoroacetic acid was used and set to δ = -76.50 ppm and data in parentheses lists the signal multiplicity and assignment. Several two-dimensional NMR techniques were employed, and these include total correlation spectroscopy (TOCSY), heteronuclear single-quantum correlation spectroscopy (HSQC), heteronuclear multi-bond correlation spectroscopy (HMBC), and nuclear Overhauser effect spectroscopy (NOESY). H-H TOCSY cross terms inform about three-bond coupling between hydrogens.[27] C-H HSQC detects correlation between carbons directly attached hydrogens,[28] and C-H HMBC gives signals for carbons and hydrogens that are separated by 2 to 4 bonds.[29] H-H NOESY informs about hydrogen-hydrogen interactions through space.[30]

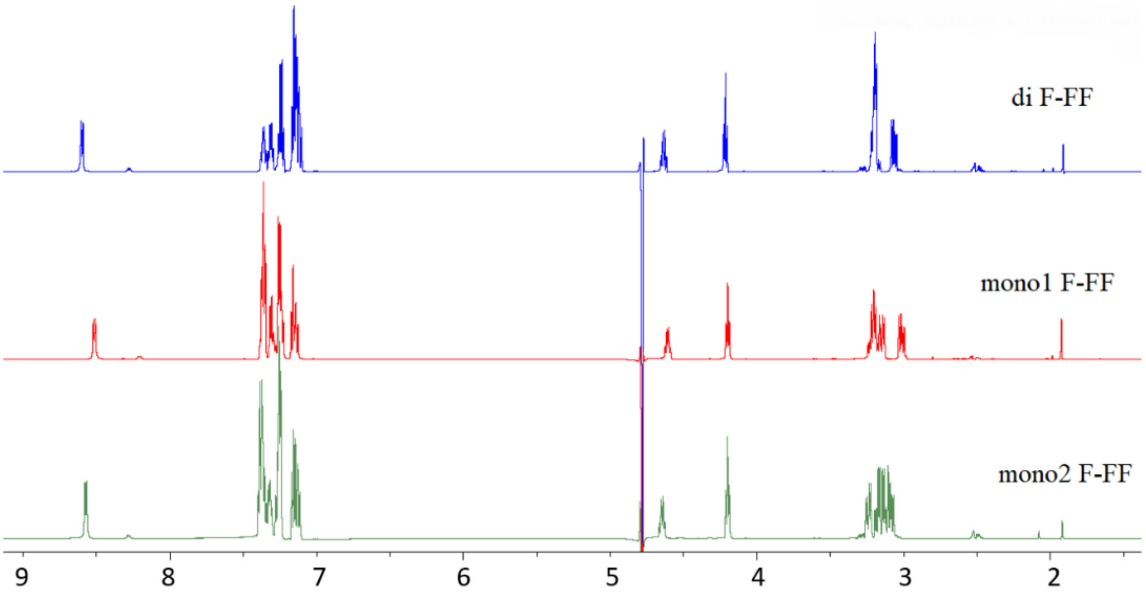


Figure 1. Measured ^{1}H-NMR spectra of di F-FF, mono1 F-FF and mono2 F-FF.

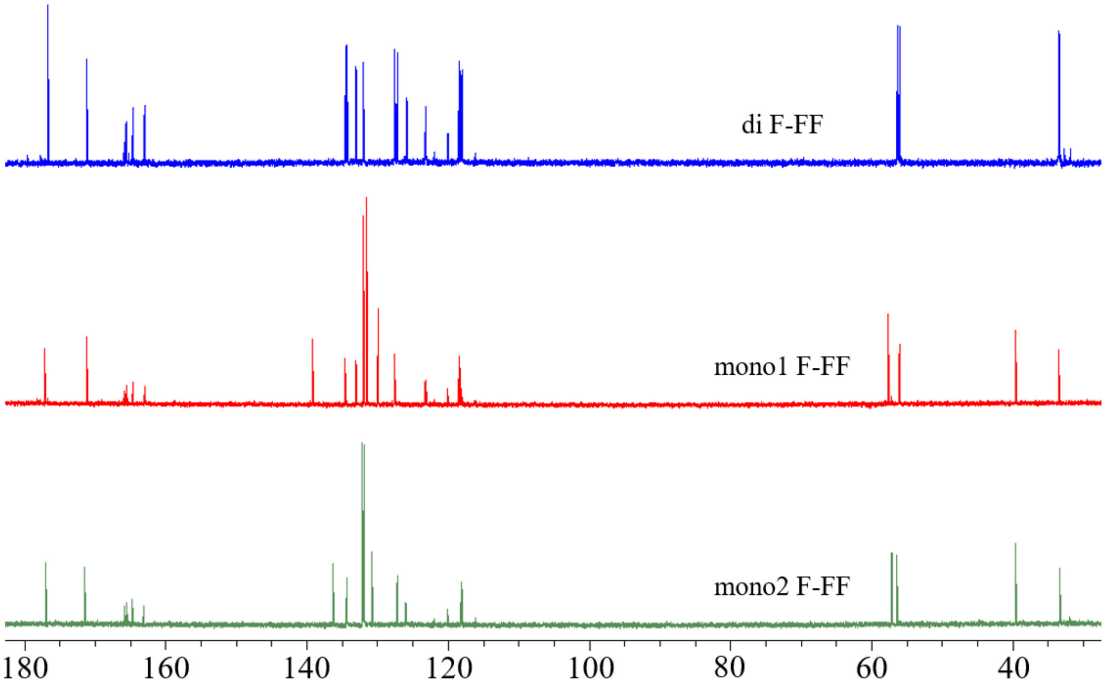


Figure 2. Measured ^{13}C-NMR spectra of di F-FF, mono1 F-FF and mono2 F-FF.

The experimental ^{1}H- and ^{13}C-NMR spectra, respectively, of di F-FF, mono1 F-FF, and mono2 F-FF are shown in Figures 1 and 2, respectively. The experimental ^{19}F-NMR spectra are shown in Figure S6, Figure S12, and Figure S18. We measured many 2D-NMR spectra, and they are only provided in the supporting information. There are two quartet signals caused by the TFA impurity at about 166 ppm and 120 ppm with the coupling constant being 35 Hz and 292.1 Hz, respectively.

2.3. Computational Methods

Potential energy surface analyses were performed at the SMD(B3LYP/6-31G*) level, that is, the B3LYP/6-31G* theoretical level[31] was employed in conjunction with the Universal Solvation Model (SMD[32]) which we have employed successfully in the context of heterocyclic chemistry for an extensive range of solvent.[33,34] NMR spin-spin coupling constants were computed at the SMD(B3LYP/6-31G*) level and SMD(B3LYP/6-311+G(2d,p)) level [35] with the Gauge-Independent Atomic Orbital (GIAO) method.[36,37] In addition, the minima **M1** and **M1a** were also optimized at the MP2/6-31G* level.[38,39] The calculations were performed with Gaussian 16, Revision A.03.[40]

For each minimum optimized with B3LYP, we report in Table S1 the total energy (E, in a.u.), vibrational zero-point energy (VZPE, in kcal/mol), thermal energy (TE, in kcal/mol), and molecular entropies S (total entropy S_{tot} and translational entropy S_{trans}, in cal mol^{-1} K^{-1}). Both Gibbs free energy ΔG and Helmholtz free energy ΔA are reported to describe the reaction thermochemistry. Because $\Delta G = \Delta A + \Delta(pV)$ and the water binding reaction is taking place in condensed phase where $\Delta(pV) \approx 0$, the ΔA value is a better estimate for the reaction energy.

In addition, the Wertz[41] correction in Equation 1 estimates the translational entropy of condensed phase systems based on their gas phase entropies.

$$^{w}S_{trans} = 0.54\, S_{trans} + 6.578 \qquad \text{(Eq. 1)}$$

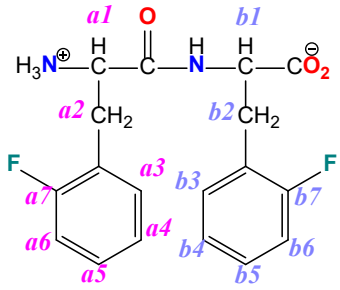
We apply this correction to the calculated translational entropy for each molecule because the translational component is the most affected by the transition from gas phase to solution. The Wertz-corrected Helmholtz free energy $\Delta^{w}A = \Delta H - T\bullet{}^{w}S_{tot}$ values are our best estimation to describe the reaction thermochemistry. For each minimum optimized with MP2, we report in Table S2 the same thermochemistry data.

3. Analysis of the Aliphatic Regions of the NMR Spectra

3.1. Atom Labeling and Complete Assignments of H-NMR and C-NMR

The labeling of the H and C atoms is shown in Scheme 3. The Phe group that is close to the NH_3^+ end of FF is labeled as *a* part and the Phe group that is close to the COO^- end is labeled as *b* part, respectively.

Scheme 3. Labeling of F-FF used for the NMR Assignment



The complete assignment of every NMR signal is shown in Table 1 for H-NMR and F-NMR spectra and Table 2 for C-NMR spectra. These assignments will be justified below.

Table 1. ^{1}H-NMR and ^{19}F-NMR Chemical Shifts (in ppm) of Fluorinated Diphenylalanines

Molecules	Ha1	Ha2	Ha2'	Ha3	Ha4	Ha5	Ha6	NH	Fa
Di F_FF	4.21	3.19	3.19	7.24	7.12	7.36	7.15	8.59	-118.93
Mono1 F_FF	4.19	3.20	3.20	7.24	7.16	7.35	7.15	8.51	-118.88
Mono2 F_FF	4.19	3.15	3.50	7.24	7.35	7.24	7.37	8.57	
	Hb1	Hb2	Hb2'(n)	Hb3	Hb4	Hb5	Hb6	H7	Fb
Di F_FF	4.62	3.06	3.17	7.24	7.14	7.31	7.16		-119.28
Mono1 F_FF	4.60	3.14	3.01	7.24	7.35	7.30	7.35	7.24	
Mono2 F_FF	4.64	3.24	3.08	7.15	7.12	7.31	7.26	7.37	-119.25

Table 2. ^{13}C-NMR Chemical Shifts (in ppm) of Fluorinated Diphenylalanines

Molecules	Ca1	Ca2	Ca3	Ca4	Ca5	Ca6	Ca7	Ca8	C=O
Di F_FF	55.9	33.3	118.2	118.0	132.9	134.4	163.0	123.1	171.1
Mono1 F_FF	55.9	33.3	127.5	132.9	134.5	118.2	163.7	123.1	171.1
Mono2 F_FF	57.0	39.5	131.8	132.0	130.7	132.0	131.8	136.2	171.4
	Cb1	Cb2	Cb3	Cb4	Cb5	Cb6	Cb7	Cb8	COO^-
Di F_FF	56.2	33.3	127.4	127.1	131.9	134.2	164.5	125.6	176.6
Mono1 F_FF	57.5	39.4	131.4	131.9	129.8	131.9	131.4	139.1	177.0
Mono2 F_FF	56.3	33.2	127.1	118.0	131.9	134.3	163.8	125.8	176.9

The standard report of NMR assignments is shown below. All coupling constants refer to $^{3}J_{\text{H-H}}$ unless specified otherwise. The NMR calculations show that $J_{(\text{H-F})}$ coupling constants are positive except for $^{5}J_{(\text{H4-F})}$, which is negative and very small in magnitude. We measured the three $J_{\text{C-F}}$ coupling constants $^{2}J_{\text{C6-F}}$, $^{1}J_{\text{C7-F}}$ and $^{2}J_{\text{C8-F}}$, and NMR calculations show that the $^{1}J_{\text{C7-F}}$ values are negative, while the others are positive.

Di F-FF: ^{1}H-NMR: δ_{H} 8.60 (0.8H, d, J = 7.6 Hz, NH), 7.36 (1H, ddd, $J_1 \approx J_2 \approx {}^{4}J_{3(\text{H-F})}$ = 7.3 Hz, Ha5), 7.31 (1H, ddd, $J_1 \approx J_2 \approx {}^{4}J_{3(\text{H-F})}$ = 6.9 Hz, Hb5), 7.24 (2H, m, Ha3 and Hb3), 7.09 – 7.16 (4H, m, Ha4, Hb4, Ha6, and Hb6), 4.62 (1H, ddd, $J_1 \approx J_2 \approx J_3$ = 7.5 Hz, Hb1), 4.21 (1H, dd, $J_1 \approx J_2$ = 7.1 Hz, Ha1), 3.16 – 3.22 (3H, m, Ha2, Ha2', and Hb2'), 3.04 – 3.08 (1H, dd, ^{2}J = 13.7 Hz, J = 7.9, Hb2).

^{13}C-NMR: δ_C 33.3 (Ca2 and Cb2), 55.9 (Ca1), 56.2 (Cb1), 118.0 (1C, d, $^2J_{C\text{-}F}$ = 21.6 Hz, Ca6), 118.2 (1C, d, $^2J_{C\text{-}F}$ = 21.6 Hz, Cb6), 123.1 (1C, d, $^2J_{C\text{-}F}$ = 15.8 Hz, Ca8), 125.6 (1C, d, $^2J_{C\text{-}F}$ = 15.8 Hz, Cb8), 127.1 (Ca4), 127.4 (Cb4), 131.9 (Cb5), 132.95 (Ca5), 134.2 (Cb3), 134.4 (Ca3), 163.82 (2C, d, $^1J_{C\text{-}F}$ = 243.1 Hz, Ca7 and Cb7), 171.1 (C=O), 176.62 (COO^-).

^{19}F-NMR: δ_F -119.28 (1F, m, Fb), -118.93 (1F, m, Fa).

Mono1 F-FF: ^{1}H-NMR: δ_H 8.51 (0.8H, d, J = 7.7 Hz, NH), 7.35 (3H, m, J = 7.0 Hz, Ha5, Hb4, and Hb6), 7.27 – 7.31 (1H, m, Hb5), 7.22 – 7.25 (3H, m, Ha3, Hb3, and H7), 7.16 (1H, dd, $J_1 \approx J_2$ = 7.9 Hz, Ha4), 7.15 (1H, d, J = 9.4 Hz, Ha6), 4.60 (0.7H, ddd, $J_1 \approx J_2 \approx J_3$ = 7.3 Hz, Hb1), 4.19 (1H, dd, $J_1 \approx J_2$ = 6.9 Hz, Ha1), 3.12 – 3.23 (3H, m, Hb2, Ha2, and Ha2'), 3.01 (1H, dd, $^2J_1$ = 14.0 Hz, J_2 = 8.1 Hz, Hb2n).

^{13}C-NMR: δ_C 33.3 (Ca2), 39.4 (Hb2), 55.9 (Ca1), 57.5 (Cb1), 118.2 (1C, d, $^2J_{C\text{-}F}$ = 21.6 Hz, Ca6), 123.1 (1C, $^2J_{C\text{-}F}$ = 15.6 Hz, Ca8), 127.5 (Ca3), 129.8 (Cb5), 131.4 (Cb3 and Cb7), 131.9 (Cb4 and Cb6), 134.5 (Ca5), 139.1 (Cb8), 163.7 (d, $^1J_{C\text{-}F}$ = 243.9 Hz, Ca7), 171.1 (C=O), 177.08 (COO^-).

^{19}F-NMR: δ_F -118.88 (1F, m, Fa).

Mono2 F-FF: ^{1}H-NMR: δ_H 8.57 (0.8H, d, J = 7.4 Hz, NH), 7.33 – 7.39 (3H, m, Ha4, Ha6, and Ha5), 7.31 (1H, m, Hb5), 7.24 – 7.27 (3H, m, Hb3, Ha3, and H7), 7.11 – 7.16 (2H, m, Hb6 and Hb4), 4.64 (0.7H, ddd, $J_1 \approx J_2 \approx J_3$ = 7.2 Hz, Hb1), 4.19 (1H, dd, $J_1 \approx J_2$ = 7.0 Hz, Ha1), 3.22 – 3.25 (1H, m, ^2J = 14.1 Hz, J = 6.4 Hz, Hb2), 3.06 – 3.19 (3H, m, Ha2, Ha2', and Hb2').

^{13}C-NMR: δ_C 33.2 (Cb2), 39.5 (Ca2), 56.3 (Cb1), 57.0 (Ca1), 118.0 (d, 1C, $^2J_{C\text{-}F}$ = 21.65 Hz, Cb6), 123.13 (d, $^2J_{C\text{-}F}$ = 15.8 Hz, Cb8), 127.1 (Cb4), 130.7 (Ca5), 131.8 (Ca3 and Ca7), 131.9 (Cb5) 132.0 (Ca4 and Ca6), 134.3 (Cb3), 139.17 (Ha8), 163.8 (d, $^1J_{C\text{-}F}$ = 243.5Hz, Cb7), 171.4 (C=O), 176.9 (COO^-).

^{19}F-NMR: δ_F -119.25 (1F, m, Fb).

3.2. Analysis of the Dipeptide Backbone: Sequence

The ^{1}H-NMR spectra of the three compounds are shown in Figure 1. For all three di-phenylalanine, the peak with the chemical shift at about 8.5 ppm is the amide NH signal, and it gives rise to a doublet because of coupling to the proximate CH hydrogen. The ammonium hydrogens do not show up in the spectra as expected because of their fast exchange with water. The chemical shifts of the NH hydrogens in di F-FF and mono2 F-FF are virtually the same (fluorinated *b*-phenyl) while the chemical shift in mono1 F-FF is slightly lower (non-fluorinated *b*-phenyl).

In di F-FF, the peaks of the two backbone CH hydrogens show up in the range of 4.0 to 4.7 ppm and this expanded region is shown in Figure 3a. Both CH hydrogens couple to the adjacent diastereotopic methylene hydrogens and the CH hydrogen of the *C*-terminal amino acid (*b*-CH) also couples to the amide-NH. The CH hydrogen that is more upfield is assigned to the *a*-CH hydrogen and gives rise to a triplet-like signal because the coupling constants with the methylene-Hs are very similar ($J \approx 7.1$ Hz). The downfield CH hydrogen is assigned to the *b*-CH hydrogen and gives rise to a quartet-like pattern for the *ddd* system. The distances between the four peaks of this "quartet" are 7.1, 7.5, and 7.7 Hz, respectively, and close examination of the peak shapes shows shoulders. This multiplet is defined by three coupling constants between Hb1 with the amide NH ($J_1 = 7.7$ Hz) and with the two methylene hydrogens Hb2 (J_2) and Hb2' (J_3). The J_1 value was determined from the NH signal and the J_2 and J_3 values cannot be extracted by analysis of this multiplet.

The centers of the Ha1 signals in mono1 F-FF (4.19 ppm) and mono2 F-FF (4.19 ppm) both appear at slightly lower chemical shifts compared to di F-FF (4.21 ppm). In contrast, the center between the major peaks of the Hb1 signal in mono1 F-FF (4.60 ppm) appears at slightly lower chemical shift compared to di F-FF (4.62 ppm), whereas the center of the Hb1 signal in mono2 F-FF (4.64

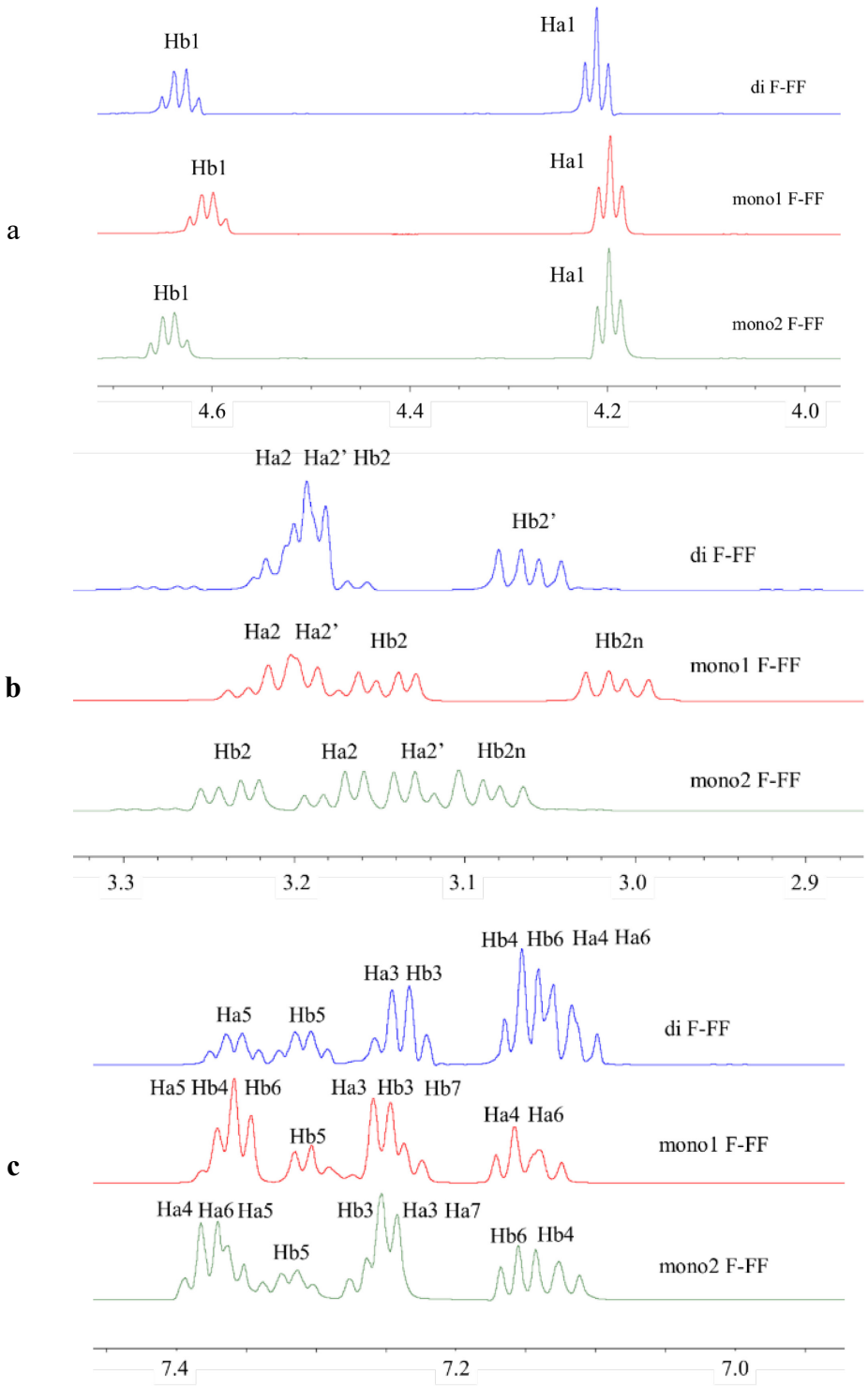


Figure 3. ^{1}H-NMR spectra of di F-FF, mono1 F-FF and mono2 F-FF. a: Expanded backbone CH region. b: Expanded methylene region. c: Expanded aromatic region.

ppm) is shifted in the opposite direction. Phenyl fluorination is expected to increase the chemical shifts of the methylene hydrogens because of inductive effects. The absence of fluorine in the *b* moiety of mono1 F-FF explains the lower chemical shift of Hb1 compared to di F-FF. Following this simple logic, one may expect δ(Ha1, mono2 F-FF) < δ(Ha1, di F-FF), while the signals of the fluorinated moieties should be about the same; δ(Hb1, mono2 F-FF) ≈ δ(Hb1, di F-FF) and δ(Ha1, mono1 F-FF) ≈ δ(Ha1, di F-FF). Clearly, these chemical shifts are not governed by fluorination alone, but also reflect changes in the relative orientation of the phenyl groups.

The splitting patterns of the CH hydrogens in the mono F-FF molecules are very similar to those of di F-FF. The *a*-CH hydrogen couples with the two diastereotopic methylene hydrogens and give rise to a triplet-like signal. Assuming that the coupling constants are very similar, we find $J \approx 6.9$ Hz (mono1) and $J \approx 7.0$ Hz (mono2). As with the *b*-CH hydrogen signal of di F-FF, we can only determine the one coupling constant with the amide NH; $J_{(NH\text{-}CH)} = 7.3$ Hz in mono1 F-FF and $J_{(NH\text{-}CH)} = 7.2$ Hz in mono2 F-FF.

3.3. Assignment and Splitting Analysis of the Methylene Region in ^{1}H-NMR Spectra

The assignment of the remaining NMR signals was performed with the help of 2D-NMR spectroscopy. The assignments of the methylene Hs shown in Figure 3b were made based on the TOCSY spectra (Figure S7, S13, and S19). For example, the CH hydrogen that is correlated to the amide NH signal in the TOCSY spectrum was assigned to the Hb1 atom. The CH_2 hydrogen signals that are correlated to Hb1 were assigned as the Hb2 and Hb2' methylene hydrogens.

Each CH_2 group should give rise to two doublets of doublets (dd) in the H-NMR spectra because of the proximity of the chiral centers. As shown in Figure 3b, one CH_2 hydrogen gives rise to a clear *dd* splitting pattern without overlap; Hb2' in di F-FF, Hb2n in mono1 F-FF, and Hb2 in mono2 F-FF. These signals allowed for the extraction of the two coupling constants $^2J_{(H\text{-}H)}$ and $^3J_{(CH2\text{-}CH)}$ listed above. The two hydrogens from *a*-CH_2 have similar chemical shifts and thus form

a broad multiplet, which appears at about 3.2 ppm for di F-FF and mono1 F-FF, and more upfield for mono2 F-FF. That is because *a*-CH_2 is attached to a fluorinated phenyl ring in di F-FF and mono1 F-FF, and to a non-fluorinated phenyl ring in mono2 F-FF. The two *a*-CH_2 hydrogens signals are too close to distinguish, so they are labelled as Ha2 and Ha2' and assigned the same chemical shifts as shown in Table 1.

The two *b*-CH_2 hydrogens afford very different peaks: one is always significant more downfield than the other in all three FF compounds. In both mono F-FF, the more upfield hydrogen has a stronger NOESY signal with the backbone amide H, indicating this *b*-CH_2 hydrogen's close proximity to the amide NH group. This hydrogen is labeled as Hb2n in Table 1. In di F-FF, one *b*-CH_2 hydrogen signal is overlapping with the two *a*-CH_2 hydrogen signals, making it impossible to compare the intensities of the NOESY cross peaks between the two *b*-CH_2 signals and the NH signals. So these CH_2 hydrogens are labeled as Hb2 and Hb2' without differentiating them.

3.4. Chemical Shift Analysis of the Aliphatic Region in ^{13}C-NMR Spectra

The full ^{13}C-NMR spectra of the three compounds are shown in Figure 2. The aliphatic C atoms were assigned according to the HSQC spectra (Figure S8, S14, S20) and the results are shown in Table 2.

The peaks of the two CH_2 carbons appear in the range of 30 to 40 ppm. All the carbon signals of the methylene groups attached to a fluorinated phenyl ring appear at about 33.3 ppm. In the mono F-FF, the methylene groups attached to the non-fluorinated phenyl ring appear at the higher chemical shifts of about 39.5 ppm. The two CH carbons show up at about 56 ppm, and in mono F-FF, the CH carbon of the fluorinated Phe caused the signals that are slightly more upfield.

In all three compounds, the two most downfield peaks are carbonyl carbon signals (177 ppm for carboxylate carbon and 171 ppm for amide-C).

4. Analysis of the Aromatic Regions of the NMR Spectra

In the following discussion, we refer to the benzene positions just as in non-fluorinated diphenylalanine. Therefore, the arene-C attached to the methylene group is the *ipso* carbon and fluorination occurs in the *ortho* position.

4.1. ^{1}H-NMR Assignment and Splitting Analysis of the Aromatic Region

The assignments of the aromatic Hs are much more difficult because there are overlapping signals in the H-NMR spectra (Figure 3c). We first identified the two *ipso* carbons according to the HSQC spectra (Figures S8, S14, S20) and then used the HMBC spectra (Figures S9, S15, and S21) to classify the H signals into the two benzene rings. For example, the *ipso*-C in benzene *a* has an HMBC signal with the peak at 7.23 ppm in di F-FF, so we assigned that signal to Ha4. The Ca4 signal was then identified easily with the help of the HSQC spectrum. The H signal that is correlated with the *a*-CH_2 carbon in the HMBC spectrum is assigned as the *ortho*-H (Ha3). And the remaining aromatic Hs in benzene *a* were assigned based on their H-H TOCSY signals with Ha4 and Ha3, respectively, and their H-C HMBC cross peaks with Ca4 and Ca3, respectively.

In di F-FF, the two most downfield signal groups are caused by the *para* H atoms. Each *para* H5 couples with the two neighboring H4 and H6 atoms and shows long-rang coupling with the *ortho* F atom. The value of $^{4}J_{\text{H-F}}$ (about 5 Hz) is usually larger than $^{4}J_{\text{H-H}}$ (2-3 Hz),[42] so it is more important to consider the coupling between H5 and F than between H5 and H3. The presence of fluorine strongly suggested that $^{3}J_{\text{H5-H4}}$ and $^{3}J_{\text{H5-H6}}$ would be different. Therefore, we expected a *ddd* splitting pattern for each H5 signal, which would yield the J_1, J_2 and J_3 coupling constants using the standard analysis of the *ddd* system.[43] To our surprise, however, a quartet-like pattern is observed, which indicates that $^{3}J_{\text{H5-H4}} \approx {}^{3}J_{\text{H5-H6}} \approx {}^{4}J_{\text{H5-F}}$ (cf., CH splitting pattern in section 3.2).

The quartet-like signal in the center of the aromatic region is due to the H3 hydrogens. Each H3 signal is expected to cause a *dd* pattern because of coupling with H4 and F, but the signal actually

presents as a false triplet ($^3J_{H3\text{-}H4} \approx {}^4J_{H3\text{-}F}$). The observed quartet-like signal group results from overlap of the two false triplets caused by H3a and H3b, respectively. The distinction between Ha3 and Hb3 is not possible. The chemical shifts at the maximum of any overlapping peak does not inform about the precise chemical shifts of the underlying bands and, hence, there are limits to the accuracy of the extracted *J* values. For the four *meta*-Hs, we expect a *dd* pattern for each H4 atom and each H6 atom. As can be seen in Figure 3c, the resulting signals from both benzene rings overlap in a non-tractable fashion.

In mono F-FFs, the presence of fluorine in only one of the benzene rings causes one additional signal for H7 and major shifts of the *meta* H4 and *meta* H6 signals in the non-fluorinated benzene. The chemical shift of the new H7 signal should be the same as for the H3 signal and the spectra of mono1 F-FF and mono 2 F-FF show H7 to overlap with the H3 region. In mono1 F-FF, the most downfield signal contains *para* H from *a*-Ph^F (Ha5) as expected. The Ha5 signal overlaps with the two *meta* Hs from *b*-Ph, Hb4 and Hb6. In fluorinated benzene, the most electron deficient centers are C7 (*ipso* relative to F) and C3 and C5 (*meta* relative to F). The *meta* Hs (H4, H6) are the most electron rich positions because they are *ortho* or *para* relative to the fluorine and benefit from the charge alternation caused by the fluorine substituent.[44] In the *b*-Ph of mono1 F-FF, the *meta* Hs no longer benefit from that charge alternation, they are less shielded and their peaks appear more downfield. The assignment of δ(H4, H6) > δ(H5) is in agreement with the published NMR spectrum of non-fluorinated phenylalanine.[45] The most downfield multiplet signal contains one *ddd* signal from Ha5 and two *dd* signals from Hb4 and Hb6. The complexity of the signal group does not even allow the extraction of precise chemical shifts.

The second most downfield signal group is due to the *para* H from *b*-Ph (Hb5). The chemical shift of Hb5 is very similar irrespective as to whether the ring is fluorinated (di F-FF, mono2 F-FF) or non-fluorinated (mono1 F-FF). We did not expect this outcome and we cannot offer an explanation either. The Hb5 hydrogen should be coupled with the two neighboring *meta* Hs and should form a triplet if the *meta* hydrogens (H4 and H6) are magnetically equivalent. We do not observe a

triplet and therefore must conclude that the *meta* hydrogens are not equivalent, possibly because of arene-arene interactions. The peaks in the region of $7.20 < \delta < 7.28$ ppm contain three *ortho* Hs. In fluorinated benzene, Ha3 should give rise to one *dd* signal, and in a simple benzyl derivative, Hb3 and Hb7 should afford one doublet. The complicated multiplet structure of that region again indicates intramolecular arene-arene interactions.

In mono2 F-FF, the most downfield signals are due to the *para* H and the two *meta* Hs from the non-fluorinated *a*-Ph. The two *meta* Hs move downfield because they are more electron poor in the non-fluorinated benzene, just like with the *b*-Ph in mono1 F-FF. The two *meta* Hs should show two *dd* signals and the *para* H should show one triplet signal, and all of these peaks are overlapping. The second most downfield multiplet is caused by the *para* H in *b*-Ph. The signals in the two upfield regions are analogous to mono1 F-FF. Instead of *ortho* hydrogens Ha3, Hb3 and Hb7 in mono1 F-FF, there is now a similar multiplet due to Hb3, Ha3, and Ha7 in mono2 F-FF. Instead of *meta* hydrogens Ha4 and Ha6 in mono1 F-FF, there is now a similar multiplet due to Hb4 and Hb6 in mono2 F-FF.

4.2. ^{13}C-NMR Assignments of the Aromatic Region

There are really two aromatic regions in the carbon spectra: the region that contains fluorinated carbons (Figure 4a) and the region of the non-fluorinated carbons (Figure 4b).

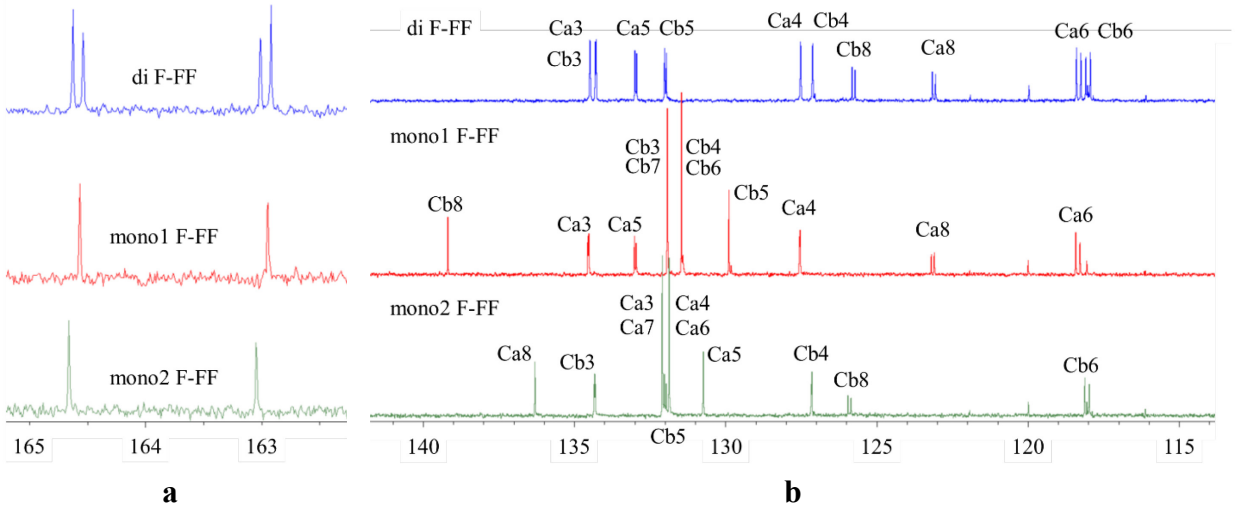


Figure 4. Expanded aromatic region of measured ^{13}C-NMR spectra of di F-FF, mono1 F-FF and mono2 F-FF: a. Fluorinated carbons. b. Non-fluorinated carbons.

The peak at about 165 ppm is due to the aromatic carbon that is attached to the F atom and the signal is split by ^{19}F (spin ½) to doublets with $^{1}J_{C\text{-}F} \approx 243$ Hz, which agrees with the reported $^{1}J_{CF}$ in 2-fluoro-*DL*-phenylalanine.[46] In mono F-FF, there is only one fluorinated carbon and it gives rise to one doublet (J = 242.9 Hz in mono1 F-FF and J = 242.2 Hz in mono2 F-FF). In di F-FF, however, there are two fluorinated carbons, giving rise to two overlapping doublets. Comparison of the distances between the four peaks confirms that the first and the third peaks belong to one doublet and the second and the fourth peaks belong to the other. Furthermore, the chemical shift and coupling constant of the more downfield di F-FF carbon signal (δ = 163.82 ppm, J = 242.5 Hz) are very similar to mono2 F-FF carbon signal (δ = 163.85 ppm, J = 242.2 Hz). And the characteristics of the more upfield di F-FF carbon signal (δ = 163.73 ppm, J = 243.0 Hz) are very close to the respective values of mono1 F-FF (δ = 163.75 ppm, J = 242.9 Hz). Thus, it can be concluded that the more downfield signal is due to C7b and the more upfield signal is due to C7a in di F-FF.

The signals of the non-fluorinated aromatic carbons show up in the range of 115 and 165 ppm. In a fluorinated benzene ring, the most upfield peaks should be expected for the *ipso* carbon (C8) and

the *meta* carbons (C4 and C6), because these positions are *meta* and *para* relative to the F substituent and therefore most shielded. In the di F-FF NMR spectrum, the most upfield aromatic signals are caused by two C6, followed by two C8 and two C4. Both the C8 and C6 signals are split by the neighboring F atoms with $^{2}J_{C\text{-}F}$ coupling constants of approximately 15.7 Hz and 21.6 Hz, respectively, in agreement with the reported NMR data of fluorinated phenylalanine.[45] Compared to di F-FF, the *ipso* and *meta* carbon in the non-fluorinated phenyl ring of mono F-FF would be less shielded, thus more downfield. And that is why the chemical shifts of the *meta* carbons (C6b, C4b) and the *ipso* carbon (C8b) in mono1 F-FF as well as those of the *meta* carbons (C4a, C6a) and the *ipso* carbon (C8a) in mono2 F-FF are more downfield than they are in di F-FF.

In analogy, the most de-shielded positions are the *ortho* and *para* positions in the fluorinated benzene ring. So, the most downfield signals in di F-FF are caused by the two C3 and two C5 atoms. The chemical shifts of these carbons in non-fluorinated phenylalanine moiety are more upfield, and that is the reason for the upfield shift of the signals of the *ortho* carbons (C3b, C7b) and of the *para* carbon (C5b) in mono1 F-FF, and of the signals of the *ortho* carbons (C3a, C7a) and of the *para* carbon (C5a) in mono2 F-FF.

5. Conformational Preference about the NH–CH Bond

5.1. NH–CHb Rotamers and Specific Solvation

We computed the rotational profiles about the NH–CHb bond for FF, mono1 F-FF, mono2 F-FF, and di F-FF. Beginning with the conformation found in the crystal structure of the parent compound FF,[16] we determined the rotational profile by driving the $\tau = \angle(C\text{–}N\text{–}C\text{–}CO_2)$ dihedral angle, and rotational profiles were determined in each case for the dipeptide itself and the aggregate formed with one specific solvation water. The resulting rotational profiles are shown in Figure 5 for the four systems. The molecular models of the NH–CHb bond conformers are shown in Figure 6 and Figure 7 for the parent FF and di F-FF, respectively, and the molecular models of the conformers of the mono1 F-FF and the mono2 F-FF are provided in the Figure S22 and Figure

S23. In the top and bottom rows of figures, molecular models are shown of the conformer structures without and with specific solvation.

The conformations denoted as **M1** and **M1a** most closely resemble the ion pair structure found in the crystal structure of parent FF, and these two structures are necessary to quantify the stabilization afforded by the formation of the water-separated ion pair in crystals. While our focus is with **M1** and **M1a**, we recognize the possible formation of a contact ion pair **M1-CIP** and of neutral dipeptides **M1-N1**, **M1-N2**, and **M1-N3** and included the structures in the potential energy surface analyses for the parent diphenylalanine and the three fluorinated structures. Having localized **M1-CIP** for a given dipeptide, we then optimized **M1-N1**, the structure resulting by proton transfer from the ammonium group to the carboxylate group to form a neutral dipeptide with an $H_2N\cdots$HOCO hydrogen bond involving a *cis* carboxylic acid with $\angle$(H–O–C=O) ≈ 180°. In addition, we optimized local minima for the neutral structures **M1-N2** and **M1-N3** containing a *trans* carboxylic acid with $\angle$(H–O–C=O) ≈ 0° and with the potential for HNH···HOCO or HNH···OCOH hydrogen bonding, respectively. Molecular models of these sets of four structures are shown in Figure S24a to Figure S24d along with their relative energies ΔE and ΔG with respect to the most stable minimum **M3**. Cartesian coordinates of all stationary structures are collected in the Supporting Information.

Rotation about the NH–CHb bond is of the sp^2-sp^3 type, which features at most six idealized conformations **A**-**F** with $\tau = \pm 30°, \pm 90°$, and $\pm 150°$, as shown in the top row of Scheme 4. In each of the conformations **A**-**F**, one substituent of the sp^3 carbon is placed perpendicular to the OC–NH plane. However, there are at most three minima **M1**-**M3** along the rotational profiles and they are schematically shown in the bottom row of Scheme 4. At least one of the large substituents is placed in the privileged position perpendicular to the OC–NH plane and hence structures **C** and **F** do not exist. Structure of type **E** do not exist because of the steric interference between the two Ph groups. Intramolecular non-bonded interactions (*vide infra*) cause the substantial deviations of the τ values from the idealized conformations. In addition, the backbone nitrogen features a minor degree of

pyramidalization, and we measured the improper dihedral angle φ = ∠(C–N–C–H(N)). The values of τ and φ of all the minima are summarized in Table 3. The expected values of τ are shown in the parentheses and the expected values of φ are 180° if the N is flat. In the last row, we calculated the difference of the expected values and the actual values. In fact, in the case of the parent FF molecule and of mono1 F-FF, an **M2** type structure does not exist as a local minimum and the **M2**-like structures shown in Figure 5 and Figure S22 were computed at the fixed τ values given in the figures. The specific water solvent in **M1a** bridges between the NH_3^+ and the H bond acceptor CO_2^-. In conformer **M2** and **M3**, more than one option for the aggregate formation may exist and those will be referred to as **b**- and **c**-types.

Scheme 4. Newman Projections of Idealized and Actual Conformations about the NH–CHb Bond

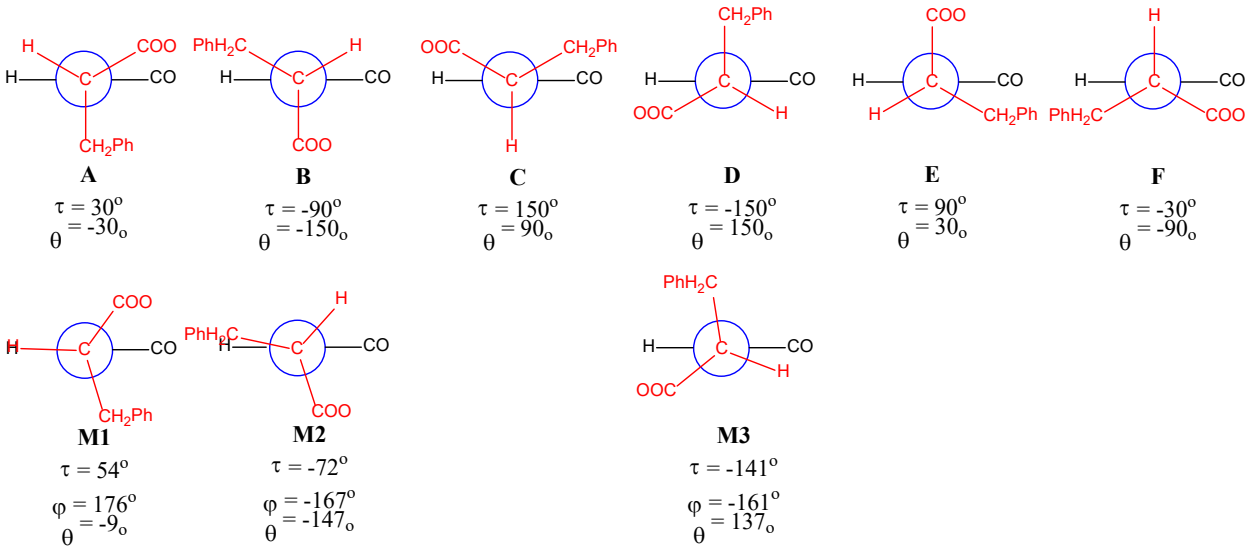


Table 3. Dihedral Angle of Minima Along the Rotational Profile (°)

	M1 Type		**M2** Type		**M3** Type	
	τ (30)	φ	τ (-90)	φ	τ (-150)	φ
FF	57	159	--	--	-133	-154
FFWB[a]	54	168	-70	-164	-134	-156
Mono1 F-FF	61	152	--	--	-139	-159
Mono1 F-FF WB	54	168	-73	-160	-135	-156
Mono2 F-FF	53	177	-73	-170	-134	-154
Mono2 F-FF WB	55	174	-72	-170	-140	-157
Di F-FF	54	176	-72	-167	-141	-161
Di F-FFWB	55	172	-69	-166	-146	-164
AD[b]	25	12	19	14	12	22

a) WB: water bridge, referring to the structures with one solvent water.
b) AD: averaged difference from the expected value.

The rotational profile of FF is shown in Figure 5a as a dark blue dashed line and features two minima, **M1** (τ = 57°) and **M3** (τ = -133°) shown in the top row of Figure 6. Even though **M1** is the preferred conformation in the crystal structure, **M3** is 4.4 kcal/mol more stable than **M1**. The rotational profile of FF computed with an extra molecule of water included as a specific solvent molecule is more complicated because the solvation mode changes along the rotational profile. The conformation found in the crystal structure is perfectly set up for a water molecule to bridge the frustrated ion pair in minimum **M1a** (τ = 54°), that is, the water engages the H bond donor NH_3^+ and the H bond acceptor CO_2^- (**a**-type). Changes in the dihedral angle τ trace the light blue solid rotational profile of Figure 5a. In the region τ ≈ 0° the distance between the NH_3^+ and the CO_2^- groups becomes too long for **a**-type water bridging, and the **b**-type of specific solvation starts to compete. In this **b**-type mode, the specific water molecule retains the stronger H bond to the NH_3^+ group and forms a second H bond with the carbonyl-O acceptor. The red solid rotational profile of Figure 5a is the segment where the **b**-type is preferred and contains minima **M2b** (τ = -70°) and **M3b** (τ = -134°). Molecular models of **M1a**, **M2b**, and **M3b** are shown in bottom row of Figure 6.

The resulting rotational profile of mono1 F-FF, mono2 F-FF and di F-FF are similar and are shown in Figures 5b-d as dark blue dashed lines and features two (mono1 F-FF) or three minima (mono2 F-FF and di F-FF). Molecular models of these minima are shown in Figure 6 for di F-FF and Figures S19 and S20 for mono1 F-FF and mono2 F-FF, respectively. The following structural discussion focuses on di F-FF and similar considerations apply to the mono substituted species.

For di F-FF, while conformation **M1** (τ = 53.8°) is preferred in the crystal structure, minima **M2** (τ = -73.0°) and **M3** (τ = -141.1°) are more stable by 2.77 and 3.40 kcal/mol, respectively. Careful inspection of the rotational profile shows a discontinuity at $\tau \approx 90°$ because of a change in the preferred arene-arene interaction. The rotational profile of di F-FF computed with one molecule of water included as a specific solvent molecule is shown in solid lines in Figure 5d. The light blue demonstrates the conformation found in the crystal structure where the water engages the H bond donor NH_3^+ and the H bond acceptor CO_2^- (**a**-type) with the minimum **M1a** (τ = 55.4°). And the orange line represents the **b**-type mode where the specific water molecule retains the stronger H bond to the NH_3^+ group and forms a second H bond with the carbonyl-O acceptor, with the minima **M2b** (τ = -69.5°) and **M3b** (τ = -139.6°). The rotational profile includes a third coordination mode (**c**-type) with water bridging between the always engaged H bond donor NH_3^+ and an F atom serving as a H bond acceptor. This coordination mode is traced out by the green solid segment of the rotational profile, and it is preferred in the region $\tau \approx -95°$ to $\tau \approx -180°$. Minimum **M3c** (τ = -145.6°, Figure 6) is 1.39 kcal/mol more stable than **M3b**.

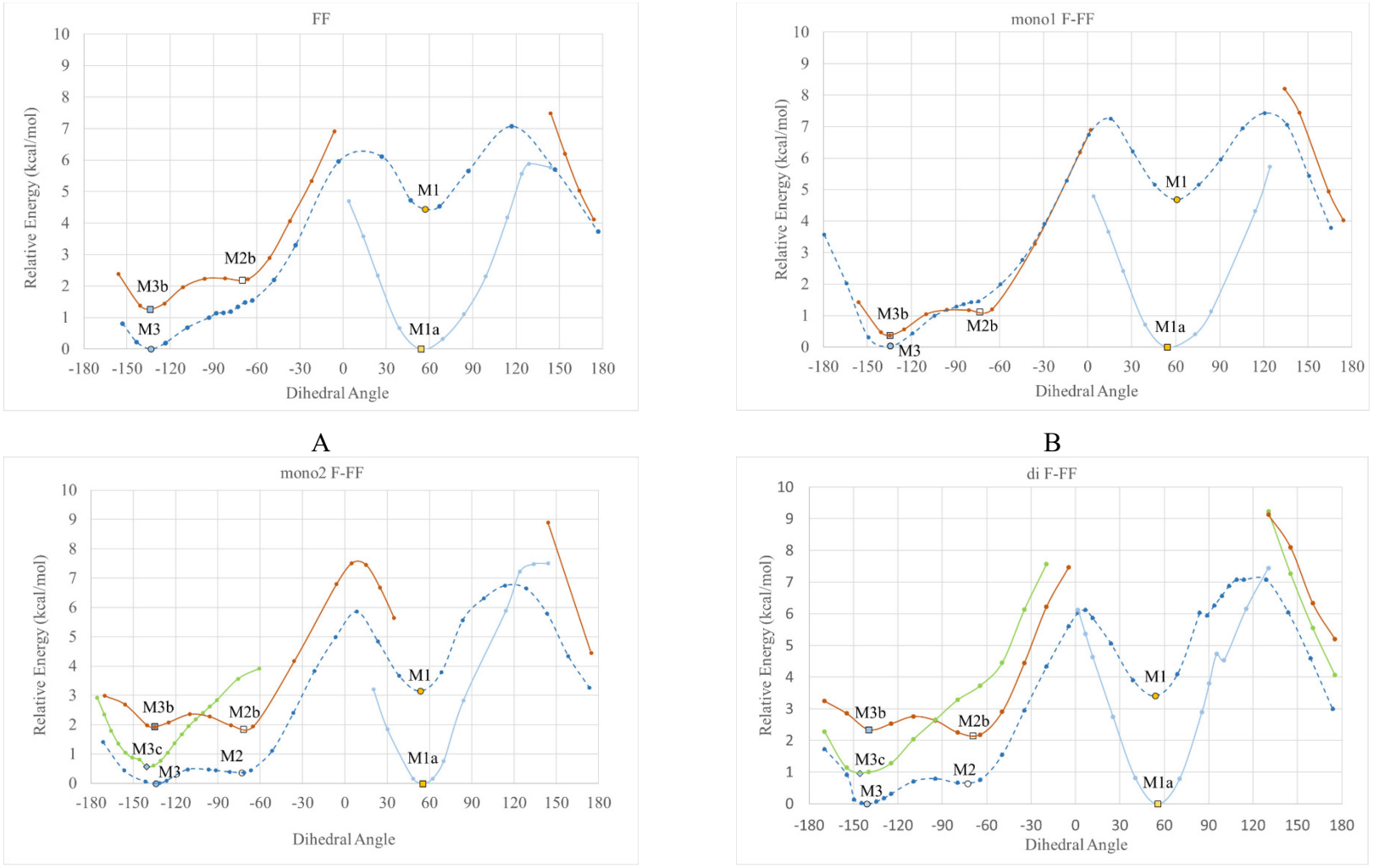


Figure 5. Rotational profiles for FF (A), mono1 F-FF (B), mono2 F-FF (C) and di F-FF (D) without and with water bridging computed at the SMD(B3LYP/6-31G*) level. In each case, the rotational profile computed without specific water solvation is shown as the dark blue dashed line. The rotational profile with specific solvation is more complicated because of varying modes of specific solvation and all parts of the profiles are shown in solid lines. See texts for details.

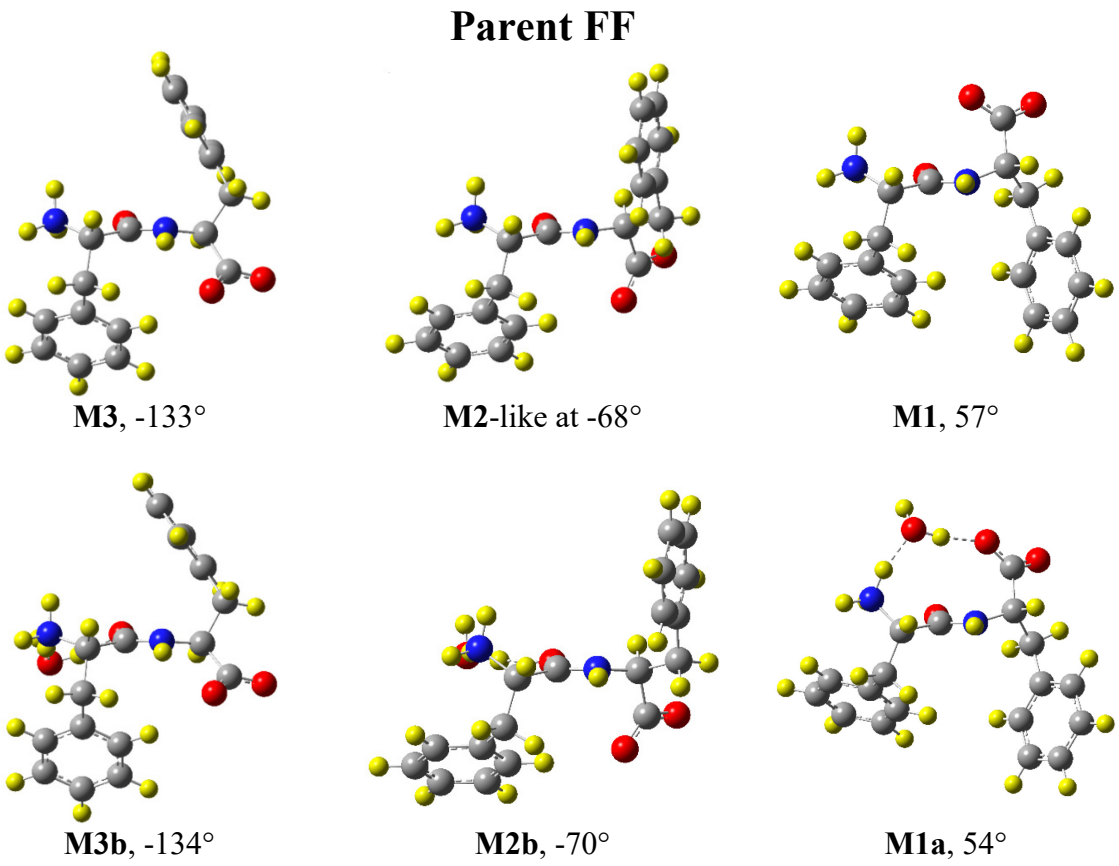


Figure 6. Minima of parent FF. In each case, the unbridged structures are shown on top and the bridged structures are on bottom. See text for explanation of nomenclature.

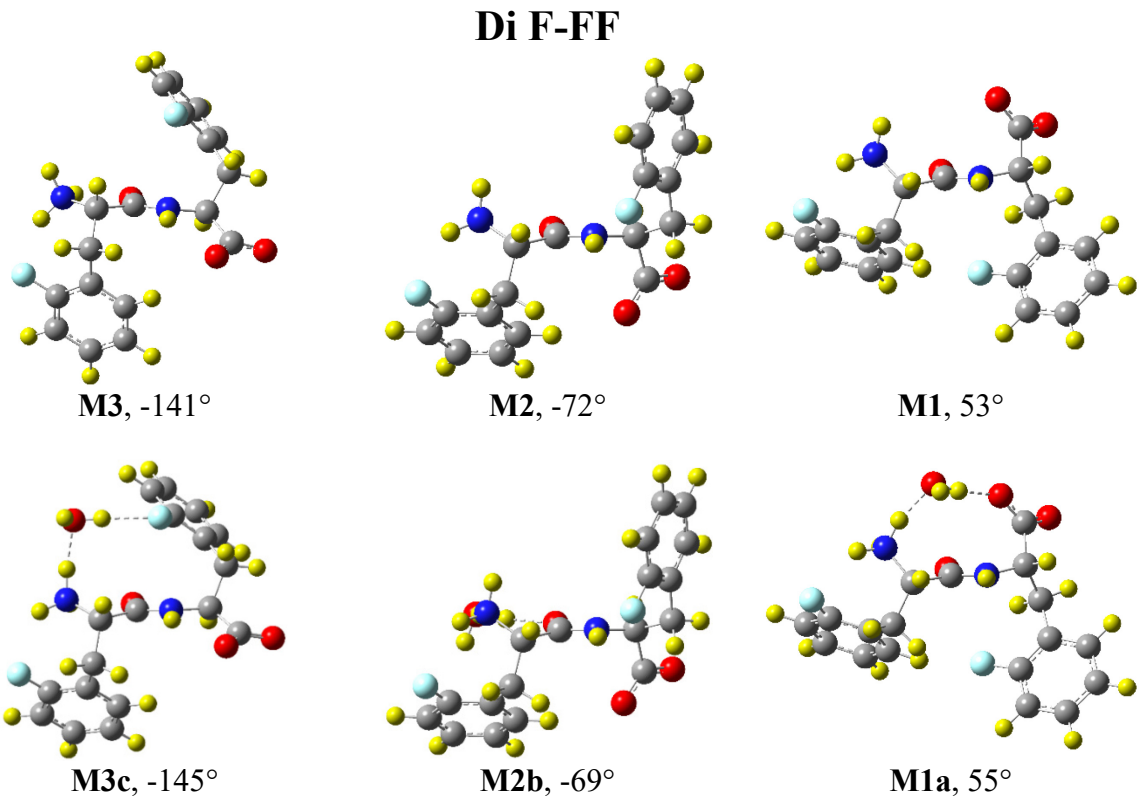


Figure 7. Minima of parent di F-FF. In each case, the unbridged structures are shown on top and the bridged structures are on bottom. See text for explanation of nomenclature.

The rotational profiles of Figure 5 demonstrate that the water-bridged ion pair **M1a** is greatly stabilized compared to the unbridged structure **M1**, and this is true for all four diphenylalanines. Conformational preference energies ΔE and ΔG are listed in Table 4 with and without the specific water solvation. While the conformational preference energies ΔE would suggest the **M1a** structure to be the most stable conformation for all four diphenylalanines, the ΔG values indicated that the **M3c** structurers are preferred for mono2 F-FF (ΔG = -0.5 kcal/mol) and di F-FF (ΔG = -1.32 kcal/mol).

Our finding of the water-bridged structure **M1a** being the most stable structure of the parent diphenylalanine resolves the apparent discrepancy between the known conformation of the water-bridged FF in its crystal structure, and the solution structure **M1** of FF. Moreover, our results

suggest that the conformation of the water-bridged FF in the crystal structure is not caused by crystal packing, but rather that the crystal structure is the results of pre-organization of the solution structure by specific solvation.

The ΔG values for the conformational energy allow for the calculation of the equilibrium constants K = [**Mx** type]/[**M1** type] (Table 4). Note that the K values for the structures *without* specific water solvation are in the hundreds or thousands, while the K values of structures *with* specific water solvation are magnitudes lower, which indicates that the competitiveness of **M1** type structures because of the specific water solvation. We also list in Table 4 the conformational preference ratio (CPR) for the **M1** type structures, which are simply the reciprocal values of K; CPR = $1/K$ = [**M1** type]/[**Mx** type]. The CPR values show a decline of relative **M1a** concentration with fluorination and this decline is most pronounced for mono2 F-FF and di F-FF. If pre-organization of the solution structure by specific solvation is needed for crystallization of the type observed for FF, the chances to crystallize mono1 F-FF are at least ten-fold higher compared to the other fluorinated diphenylalanines.

These calculations suggest that water bridging can change the preferred conformation in solution. And they also provide an explanation why crystals of fluorinated FF are very difficult to obtain. In FF, it is obvious that **M1** with water bridging is the dominant structure compared to **M2** and **M3** type. We have studied water bridging in other contexts extensively[47-49] and found that the engagement of the bridging water molecule in two hydrogen bonding interactions synergistically enhances both. Therefore, one has every reason to assume that the bridging water molecule would be present in clusters of **M1** with more specific water molecules.

Table 4. Conformational Preference Energies[a]

With specific water solvation					Without specific water solvation			
Molecule	ΔE	ΔG	K[b]	CPR[c]	Molecule	ΔE	ΔG	K[b]
FF								
M2b *v.* **M1a**	2.19	2.63	0.01	84.5				
M3c *v.* **M1a**	1.26	1.65	0.06	16.2	**M3** *vs.* **M1**	-4.45	-5.07	5180
Mono1 F-FF								
M2b *v.* **M1a**	1.12	1.23	0.13	8.0				
M3c *v.* **M1a**	0.38	1.00	0.19	5.4	**M3** *vs.* **M1**	-4.68	-4.35	1538
Mono2 F-FF								
M2b *v.* **M1a**	1.84	2.29	0.02	47.6	**M2** *vs.* **M1**	-2.79	-3.60	434
M3c *v.* **M1a**	0.81	-0.46	2.17	0.5	**M3** *vs.* **M1**	-3.15	-5.10	5449
Di F-FF								
M2b *v.* **M1a**	2.15	1.99	0.03	28.7	**M2** *vs.* **M1**	-2.77	-3.08	180
M3c *v.* **M1a**	0.96	-1.32	9.27	0.1	**M3** *vs.* **M1**	-3.41	-3.21	225

a) Relative energies in kcal/mol.
b) Equilibrium constant K for **M1** type → **Mx** type computed with $\Delta G = -RT \bullet \ln(K)$ at room temperature.
c) Conformational preference ratio CPR = $1/K$ = [**M1** type]/[**Mx** type].

In Table 5, we report on the thermochemistry of several hydration reactions. If a conformation occurs without and with specific water solvation, we computed the hydration energy for the molecule in that conformation. The conformations **M1** and **M1a** most closely resemble the ion pair structure found in the crystal structure of parent FF and the energy of the reaction **M1** + H_2O → **M1a** quantifies the stabilization due to the formation of the water-separated ion pair in the conformation that occurs in the crystal. We report ΔE_{water}, ΔG_{water}, and $\Delta^W A_{water}$ to estimate the water binding energy. While the ΔG values are appropriate for discussion of conformational preference (because pV terms cancel), the accurate determination of hydration energies requires $\Delta^W A_{water}$ to properly account for translational entropy changes in solution and the absence of significant volume effects in solution. With the $\Delta^W A_{water}$ values, we computed the equilibrium constant K_{water} for the water adduct formation and the bridging ratio BR = [bridged]/[unbridged].

In the context of crystal engineering fluorinated derivatives of diphenylalanine, the bridging ratio BR = [**M1a**]/[**M1**] is the most relevant because it quantifies the advantage for conformer **M1a** provided by specific solvation. The BR values in Table 5 clearly show that the water bridged **M1a** structure dominates by more than 99% over **M1**. For corroboration, we optimized all **M1** and **M1a** structures at the correlated level SMD(MP2/6-31G*), their molecular models are shown in Figure S25 and very similar to their SMD(B3LYP/6-31G*) structures, and the thermochemistry for the reaction **M1** + H_2O → **M1a** at the correlated level confirms our conclusion.

Table 5. Hydration Energies of Conformers of FF and Fluorinated Derivatives at SMD(B3LYP/6-31G*)

Molecule	ΔE_{water}[a]	ΔG_{water}[a]	$\Delta^W A_{water}$[a]	K_{water}[b]	BR[c]
FF					
M1 + H_2O → **M1a**	-14.11	-3.26	-5.43	9507.66	522921.11
M3 + H_2O → **M3b**	-8.40	3.45	1.29	0.11	6.24
Mono1 F-FF					
M1 + H_2O → **M1a**	-13.94	-1.93	-4.1	1008.56	55470.82
M3 + H_2O → **M3b**	-8.90	3.42	1.25	0.12	6.68
Mono2 F-FF					
M1 + H_2O → **M1a**	-14.60	-3.20	-5.37	8592.45	472584.59
M2 + H_2O → **M2b**	-9.96	2.68	0.51	0.42	23.27
M3 + H_2O → **M3c**	-9.51	2.88	0.70	0.31	16.89
Di F-FF					
M1 + H_2O → **M1a**	-14.56	-1.67	-3.85	661.53	36384.13
M2 + H_2O → **M2b**	-9.65	3.39	1.22	0.13	7.03
M3 + H_2O → **M3c**	-10.19	0.22	-1.95	26.96	1482.55

a) Hydration energies in kcal/mol.
b) Equilibrium constant K_{water} computed with $\Delta^W A_{water} = -RT \cdot \ln(K_{water})$ at room temperature.
c) Bridging ratio BR = [bridged]/[unbridged] computed as product $K_{water} \cdot [H_2O]$.

5.2. Coupling Constant $J_{NH\text{-}CH}$ as a Function of Dihedral Angle

It is clear that the computed NMR properties for any one minimum structure will not match the measured NMR data. Instead, fast rotations about the HN–CH, HC–CH_2Ph, and CH_2–Ph bonds occur, and the measured NMR data contain information about these dynamic processes. The structures-NMR relationship is nontrivial and requires mathematical approaches to deduce the best match of the NMR data and the solution structure(s), and such a study is in progress. One important aspect of the theoretical analysis of the NMR properties concerns the question regarding the relationship between the measured ^3J coupling constants and the NH–CHb rotational profiles and whether the Karplus curves can be used to distinguish between possible conformations.

After the study of the rotational profile clarified the relationship between the energy and $\tau = \angle$(C–N–C–CO_2), we then explored the NMR properties related to this dihedral angle. The dihedral angle $\theta = \angle$((N)H–N–C–Hb1) also describes the rotation along the same N–C bond, but with focus on the two hydrogen atoms, which have a measurable coupling constants ^3J, which are related to the dihedral angle θ via the Karplus equation $J(\theta) = A\cos^2(\theta) + B\cos(\theta) + C$. Thus, we calculated the NMR chemical shifts and spin-spin coupling constants of all the structures along the rotational profiles for all four structures (compare marks in Figure 5) and plotted their $^3J(\theta)$ against θ in Figure 8. Dark purple dashed lines were computed for the structures *without* water bridging and light purple solid lines refer to the structures *with* water bridging. Horizontal lines are included to indicate the measured ^3J values for each dipeptide from our own measurements (fluorinated dipeptides) and from literature (parent FF).[50] Furthermore, the minima are shown in yellow (**M1** type), white (**M2** type) and blue (**M3** type) markers. It is well known that the ^3J values are theoretical level dependent[51] and for di F-FF, we also calculated the *J* values with a better basis set at the level of SMD(B3LYP/6-311+G(2d,p))//SMD(B3LYP/6-31G*). The results are marked in Figure 8d (markers with red frame) and show an increase of the *J* values for all minima and further improvements in the theoretical level might result in still higher *J* values. Our focus is less on the absolute *J* values, but we are interested in the relative *J* values and their relation to conformation.

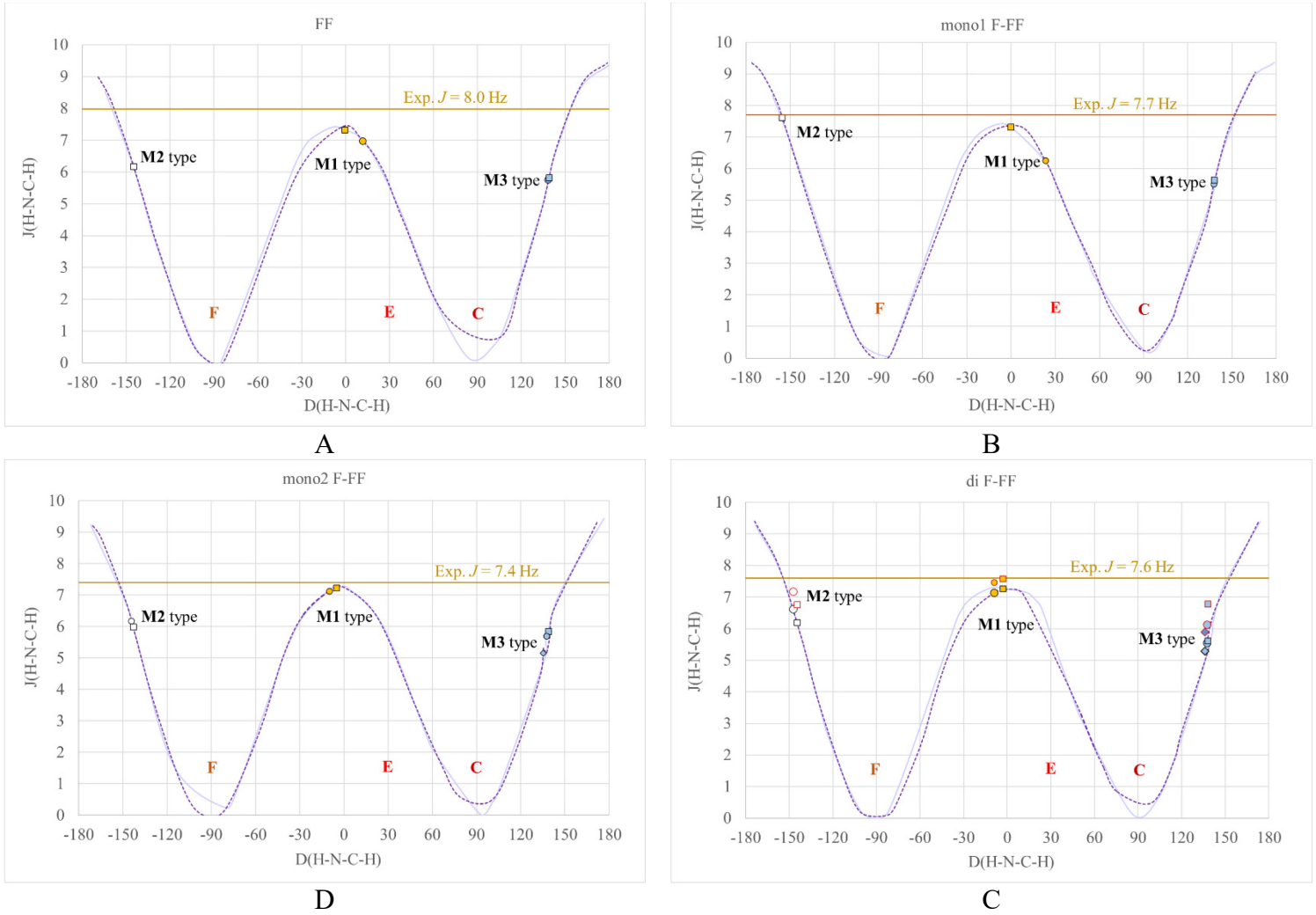


Figure 8. Karplus relationship of dihedral angle ∠(H–N–C–H) for FF (A), mono1 F-FF (B), mono2 F-FF (C) and di F-FF (D) without (dark purple dashed line) and with (light purple solid line) water bridging computed at the SMD(B3LYP/6-31G*) level. Yellow markers: **M1** type minima. White markers: **M2** type minima. Blue markers: **M3** type minima. Square markers: minima with water bridging. Round markers: minima without water bridging. Diamond markers: **M3c** minima.

Figure 8 shows the expected slightly asymmetric double-well curve for values -180° ≤ θ ≤ 180° with a large variation of $0 \leq J(\theta) \leq 10$ Hz. The minima of the Karplus curves occur for structures with conformations **C** (θ = 90°) and **F** (θ = -90°), see Scheme 4, and we have shown that the conformers with H(C) in the privileged position are not stationary structures. Putative structures with conformation **E** would be expected with θ values of about 30° and they also do not exist as local minima on the potential energy surface. As can be seen in Figure 8, the calculated *J* values of stationary structures **M1** to **M3** fall within a narrow range of 2 Hz and therefore *J* values do not inform about the conformation.

Conclusion

The three fluorinated diphenylalanines were synthesized by solid phase peptide synthesis, purified by flash chromatography, and dipeptide purity and identity were established by LC-MS analysis. The pure dipeptides were studied in detail in partially deuterated aqueous solution with one- and two-dimensional NMR spectroscopic techniques. The results of the extensive NMR study include the unambiguous assignments of all chemical shifts for the H and C atoms of the aliphatic backbone (*a*-CH, *b*-CH, NH, *a*-CH_2, *b*-CH_2) and the complete assignments of all chemical shifts of the C atoms of the carboxylate, the amide-carbonyl, the CF carbons, and of every arene C atom in each phenyl ring. In addition, the measurements allow for unambiguous determination of several H,H coupling constants ($^3J_{NH\text{-}CH}$, $^2J_{H\text{-}H}(CH_2)$, and $^3J_{b\text{-}CH\text{-}b\text{-}CH2}$) and C,F coupling constants ($^1J_{C\text{-}F}$, both $^2J_{C\text{-}F}$ for every fluorinated phenyl group). The aromatic Hs cannot be assigned based on the ^{1}H-NMR measurements alone; additional information would be required, for example, based on simulations of the observed splitting patterns. This highlights the significance of the C-NMR measurements to inform about the environments of both arenes.

The NMR analysis clearly shows one set of signals for each dipeptide. This finding does *not* imply that each peptide adopts only one structure and the computed NMR properties for any minimum structure is not expected to match the measured NMR data. Instead, fast rotations about the

NH–CH bond and as well as the HC–CH_2Ph and CH_2–Ph bonds occur and required the exploration of the NH–CH rotational profiles for FF and its fluorinated derivatives with the computational studies at the SMD(B3LYP/6-31G*) level. The rotational profiles were computed for the dipeptide themselves and for the aggregates formed by specific water solvation. Rotation about the NH–CHb bond is of the sp^2-sp^3 type and allows in principle for six conformations (**A**-**F**), and our results show that at most three conformational structures (**A**, **B**, and **D**) correspond to stationary structures (**M1**, **M2**, and **M3**).

The construction of the rotational profiles allowed for the computation of the associated Karplus curves for $^3J_{NHCH}$ using the GIAO method and they show the expected asymmetric double-well shape. The Karplus curves demonstrate similar *J* values for all computed stationary structures and do not allow any discrimination of conformational preferences. However, the analysis of the relative energies ΔE and ΔG of the stationary structures informs about the conformations. In the absence of specific solvation, the stability of the stationary structure follows the order **M3** > **M2** ≫ **M1**. In a stunning reversal of relative conformer stabilities, the specific water solvation makes all the difference and adds a large competitive advantage to the water-separated ion pair **M1a**. In fact, **M1a** becomes the most stable and dominant conformation for the parent diphenylalanine and mono1 F-FF and **M1a** becomes competitive with **M3c** for mono2 F-FF and di F-FF.

It is only with the inclusion of the specific solvation that the conformation found in crystals of FF becomes a competitive structure in solution, and this finding suggests that such pre-organization in solution might be an important factor in the crystallization of FF. If this hypothesis holds, the chances to crystallize mono1 F-FF are at least ten-fold higher compared to the other fluorinated diphenylalanines.

Associated Content

Supporting Information: Chromatogram and MS spectra, H-NMR, C-NMR, F-NMR, TOCSY, HSQC, and HMBC spectra of each fluorinated diphenylalanine. Table of energies and thermochemistry data of the stationary structures of FF and the fluorinated derivatives, two figures showing molecular models of stationary structure of mono fluorinated diphenylalanines, four figures showing the molecular models of stationary structure of the **M1-CIP**, **M1-N1**, **M1-N2**, and **M1-N3**, and one figure showing molecular model of MP2 optimized **M1** and **M1a** structures. Cartesian coordinates of all stationary structures. The Supporting Information (82 pages) is available free of charge at XXX

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Notes

The authors declare no competing financial interest.

Acknowledgements

This research was supported by the Missouri University of Science and Technology and, in part, by NSF grant #1665487. CA gratefully acknowledges support by an OURE scholarship. We thank Brian Jameson for measuring F-NMR spectra.

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