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Design and Synthesis of Novel ^{19}F-Amino Acid: A Promising ^{19}F NMR Label for Peptide Studies

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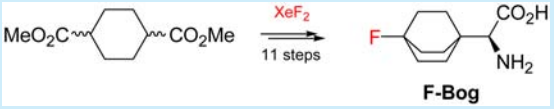
Supporting Information

ABSTRACT: Novel aliphatic ^{19}F-substituted amino acid was designed as a ^{19}F NMR label for peptide studies. The synthesis was performed in 11 steps and 9% overall yield from a commercially available starting material. The key transformation was a decarboxylative fluorination of an aliphatic carboxylic acid with XeF_2 in C_6F_6.



Solid-state ^{19}F NMR spectroscopy is a useful technique to study membrane-active peptides under their natural conditions in lipid bilayers.[1] In this method, scientists first selectively incorporate a suitable fluorine-containing amino acid (^{19}F label) into the peptide of interest. Several very strict criteria to an effective ^{19}F label exist, however. The amino acid must (a) be conformationally constrained to place the ^{19}F reporter in a well-defined position to aminocarboxylic moiety; (b) be compatible with the peptide synthesis; and (c) not change structure and function of the peptide.[1]

^{19}F labels bearing either CF_3[2] or CF_2 groups[3] have been used in a number of peptide studies. Mono-F-substituted amino acids, however, have received less attention. Recent validation of (4-fluorophenyl)glycine (**1**) (Figure 1) as a ^{19}F label,[4] for example, revealed several drawbacks. First, the amino acid **1** completely racemized during the peptide synthesis, making thereby an assignment of the stereoconfiguration of the obtained epimeric peptides (*R* or *S*) extremely problematic. In addition, replacing the residues of *aliphatic* amino acids (Ala, Val, Ile, Leu) with *aromatic* compound **1** changed the structure/function of some peptides. It is not surprising, therefore, that amino acid **1** has not received wide popularity in peptide studies as a ^{19}F label.

In this work, we have rationally designed and synthesized an aliphatic analogue of **1** which is less prone to racemization—the amino acid **2** (F-Bog).

Design

Amino acid **1** racemized during the peptide synthesis because of the structural features contributing to the stability of the corresponding enolate ion, an adjacent benzene ring, and efficient electron-withdrawing effect of the fluorine atom easily transmitted via the benzene ring to the aminocarboxylate moiety. Therefore, we came up with an idea of replacing the benzene ring with its aliphatic isostere. In drug discovery, chemists commonly use three saturated bioisosters of benzene: bicyclo[1.1.1]-pentane,[5,6] bicyclo[2.2.2]octane,[7] and cubane.[8] All three compounds have collinear alignment of the substituents and similar C−C distances (Figure 1). In this work, we report our studies on the bicyclo[2.2.2]octane skeleton.[9] We envisioned that such replacement should make the resulting aliphatic amino acid **2** less prone to racemization than **1** due to the absence of the stabilizing effect of the benzene ring on the enolate ion.

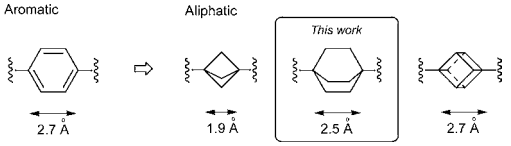
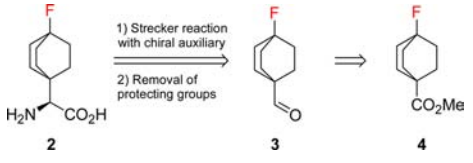


Figure 1. Isosteric cores: benzene and bicyclo[2.2.2]octane.

Retrosynthetic Analysis

We planned to synthesize the chiral amino acid **2** from the corresponding aldehyde **3** using the Strecker reaction with a chiral auxiliary (chiral amine) followed by a removal of the protecting groups (Scheme 1). We envisioned that aldehyde **3**, in turn, could be prepared from the known ester **4**.

Scheme 1. Retrosynthetic Analysis of Amino Acid 2



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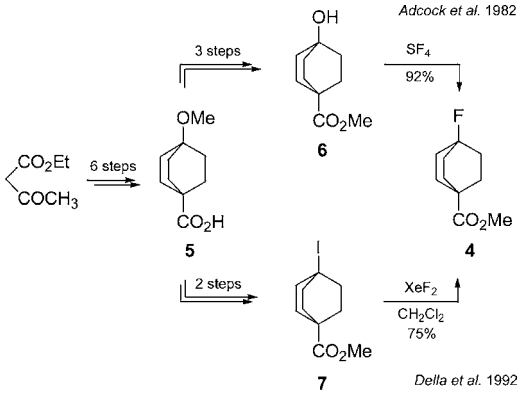
Synthesis

In 1982, Adcock et al. synthesized **4** from ethyl acetoacetate via acid **5** in 10 steps (Scheme 2).[10] The final fluorination of alcohol **6**

Scheme 2. Literature Syntheses of Compound 4.[10,12]

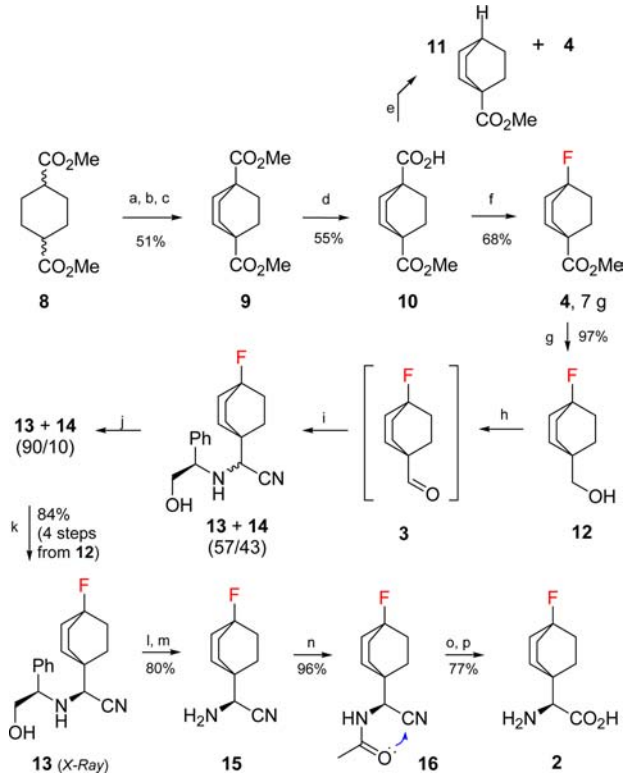


was performed with SF_4. The use of highly toxic gas SF_4, however, limits the applicability of this method. In addition, storage and handling of SF_4 is forbidden in some European countries and in the United States.[11] Later, Della et al. performed an alternative synthesis of compound **4** from ethyl acetoacetate, but still in nine steps.[12] The key transformation was a reaction of iodide **7** with XeF_2. We decided, therefore, to develop a shorter synthetic strategy to **4** from the same starting materials.

We started the synthesis of **4** from the commercially available diester **8** as a mixture of isomers (Scheme 3). Double alkylation of **8** with $BrCH_2CH_2Cl$ using LDA/HMPA afforded the bicyclic derivative **9** in 72% yield following the literature procedure.[8a,13] Monohydrolysis of the ester group in **9** using 1 equiv of NaOH in methanol/water mixture gave acid **10** in 55% yield.[14] Finally, the crucial step, conversion of the carboxylic group in **10** into the fluorine atom with XeF_2,[15] was attempted. The reaction in CH_2Cl_2 afforded a mixture of the target fluoride **4** and the side product **11**, however.[6] The source of the hydrogen atom in formation of **11** was presumably the solvent. To prevent incorporation of the hydrogen atom into the product, we decided to attempt the reaction in a polyfluorinated solvent. In fact, the reaction of **10** with XeF_2 in C_6F_6 gave **4** as the sole product in 68% yield after sublimation. Formation of the side compound **11** was not observed. The developed transformation was scalable, and we synthesized ca. 7 g of pure ester **4**.

Next, we performed reduction of ester **4** with $LiAlH_4$ into alcohol **12**. Oxidation of the hydroxylic group in **12** using $Py{\cdot}SO_3$ complex in DMSO afforded the volatile aldehyde **3** that was used in the next step without isolation. To perform the planned Strecker synthesis with a chiral auxiliary, we focused our attention at the known one, α-phenylglycinol.[16] Because of the presence of polar hydroxy group, the diastereomeric Strecker products can often be separated by column chromatography on silica gel more efficiently compared with the corresponding derivatives of α-phenylethylamine. Unfortunately, Strecker reaction of aldehyde **3** with (*R*)-α-phenylglycinol gave a mixture of stereoisomers **13**/**14** in an almost equimolar ratio of 57/43. We noticed, however, that at room temperature in MeOH this ratio slowly changed in a favor of **13**. Obviously, **14** underwent isomerization into **13**. Presumably, this process occurred via the MeOH-separated contact ion pair of **14**.[17] Absence of isomerization in less polar hexane supported this suggestion. We found that the isomerization proceeded faster at higher temperature: after heating a

Scheme 3. Synthesis of Amino Acid 4[a]



[a]Key: (a) LDA (1.2 equiv), HMPA (4 equiv), THF, −78 °C; (b) $BrCH_2CH_2Cl$ (1.1 equiv), −78 °C; (c) LDA (1.2 equiv), THF, −78 °C; (d) NaOH (1.0 equiv), MeOH/H_2O (2/1), 20 °C; (e) XeF_2 (1.5 equiv), CH_2Cl_2, 0 °C; (f) XeF_2 (1.5 equiv), C_6F_6, reflux; (g) $LiAlH_4$ (2.5 equiv), THF, reflux; (h) $SO_3{\cdot}Py$ (3.6 equiv), NEt_3 (3.0 equiv), CH_2Cl_2, DMSO, −10 → 20 °C; (i) (*R*)-2-phenylglycinol (1.0 equiv), Me_3SiCN (3.0 equiv), CH_2Cl_2/MeOH (1/1), 0 °C; (j) MeOH, reflux; (k) column chromatography; (l) $Pb_2(OAc)_4$ (1.5 equiv), CH_2Cl_2/MeOH (2/1), 0 °C; (m) 2 N HCl, reflux; (n) Ac_2O (1.5 equiv), CH_2Cl_2, 20 °C; (o) 6 N HCl, reflux; (p) cation-exchange chromatography on KU-2 resin.

solution of **13**/**14** in MeOH at reflux for 3 h, the isomerization reached the thermodynamic equilibrium of **13**/**14** = 90/10. This ratio did not change after the subsequent heating. At this point, we isolated the needed major isomer **13** from the reaction mixture by column chromatography on silica gel in 84% overall yield (from **12** in four steps). The needed (*S*)-stereoconfiguration of the newly formed chiral center in **13** was confirmed by X-ray crystallographic analysis (Figure 2). Oxidative cleavage of the chiral auxiliary in **13** with $Pb(OAc)_4$ in CH_2Cl_2 followed by the acidic hydrolysis of the intermediate Schiff base gave nitrile **15**. Unexpectedly, we were not able to hydrolyze the nitrile group in **15** under acidic conditions: the reaction did not proceed even in 40% aq HBr under reflux (only the intact starting material was isolated). Apparently, the bulky bicyclo[2.2.2]-octyl substituent in **15** hampered the reaction. To overcome this problem, we decided to attempt an acidic hydrolysis of N-acetylated derivative **16** with the intent that the carbonyl group would facilitate the reaction via intramolecular anchimeric interaction with the nitrile (Scheme 3, blue arrow). In the event, acylation of **15** with Ac_2O smoothly proceeded in CH_2Cl_2 without base at room temperature to give **16**. As expected, hydrolysis of **16** proceeded easily in 6 N HCl during 2 h to give the target amino acid **2** in 77% yield after ion-exchange chromatography.

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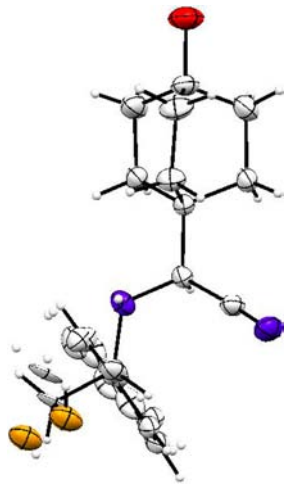
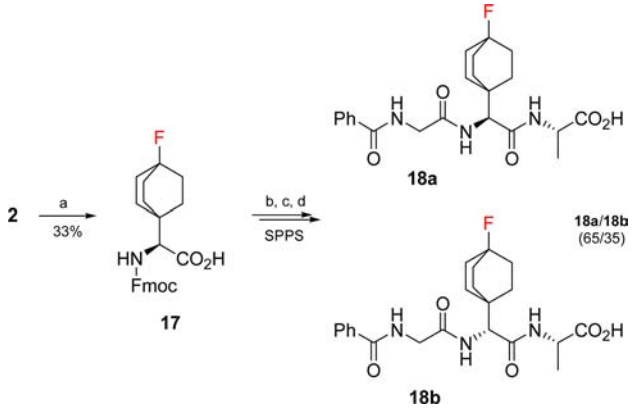


Figure 2. Molecular structure of compound **13** according to X-ray diffraction data (F, red; N, blue; O, orange).[18]

Finally, we wanted to check whether amino acid **2** would indeed be more stable to racemization than **1**. Having observed that acidic hydrolysis of **15** was not effective, we feared that sterically restricted amino acid **2** would not be compatible with the standard protocol of solid phase peptide synthesis (SPPS). Therefore, we first synthesized an Fmoc-protected derivative **17** (Scheme 4), and attempted next the synthesis of simple

Scheme 4[a]



[a]Reagents and conditions: (a) FmocCl (1.1 equiv), K_2CO_3 (2.0 equiv), dioxane/water, 20 °C); solid-phase peptide synthesis (starting from alanine preloaded 2-chlorotrityl resin), **17** (1.2 equiv), HOBt (1.2 equiv), PyBOP (1.2 equiv), DIPEA (2.4 equiv); (c) 20 °C; TFA, 20 °C; (d) HPLC separation.

tripeptide **18** (Bz-Gly-**2**-Ala-OH) by SPPS. L-Alanine-preloaded 2-chlorotrityl resin and PyBOP as a coupling reagent were used. Neither low activity nor degradation of **17** was observed, as the both -C and -N termini were completely coupled during the synthesis. However, two epimeric peptides **18a**/**18b** were isolated from the reaction mixture by HPLC. Although the benzene ring in **1** was replaced by a saturated core in **2**, the electron-withdrawing influence of a fluorine atom still caused the racemization.[19] However, in strict contrast to **1**, amino acid **2** racemized only partially (**18a**/**18b** = 65/35) solving thereby the major problem: determination of stereoconfiguration of the obtained peptides. Obviously, the major isomer **18a** contained the residue of (*S*)-**2**, while the minor one, **18b**, contained the residue of (*R*)-**2**.

In summary, we have synthesized novel conformationally rigid fluorinated α-amino acid **2**. This compound was rationally designed as an aliphatic analogue of the prone-to-racemization ^{19}F label **1**. In fact, amino acid **2** was compatible with the standard protocol of SPPS and racemized only partially, therefore solving the problem of determination of stereoconfiguration of the obtained epimeric peptides. This makes amino acid **2** an attractive ^{19}F label to replace the residues of aliphatic amino acids (Val, Ile, Leu) in peptides for their study via ^{19}F NMR. Incorporation of **2** into naturally occurring peptides for subsequent NMR studies is ongoing.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and copies of NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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