Labeled Amino Acids

DOI: 10.1002/ange.200600346

Conformationally Rigid Trifluoromethyl-Substituted α-Amino Acid Designed for Peptide Structure Analysis by Solid-State ^{19}F NMR Spectroscopy

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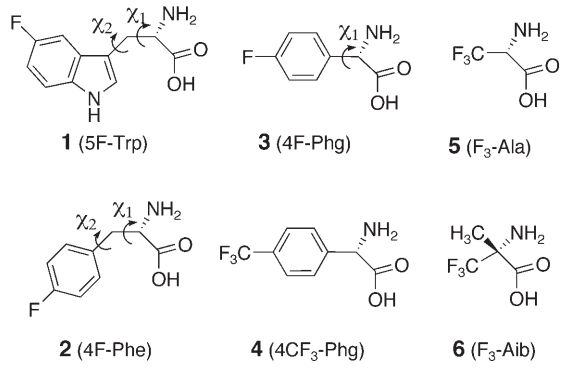
The incorporation of specifically fluorine-labeled L-α-amino acids (FAAs) into polypeptides is a prerequisite for analyzing them by ^{19}F NMR spectroscopy. The advantages of observing this isotope arise from its high sensitivity, the absence of biological background signals, its wide range of chemical shifts, and strong dipolar interactions.[1,2] An FAA is usually incorporated into a peptide by solid-phase peptide synthesis (SPPS), though some other methods have also been developed.[1,3] In most NMR applications the FAAs simply serve to probe qualitative changes in the local environment.[1] Only recently, FAAs have been incorporated into membrane-bound peptides to determine explicit structural parameters in terms of orientational constraints and/or interatomic distances by solid-state ^{19}F NMR spectrosopy.[4,5] These solid-state NMR studies are particularly informative about membrane-active peptides, as they can reveal their conformation, alignment, and dynamic behavior in a lipid bilayer under quasi-native conditions.[4] However, only a limited number of FAAs are applicable to this approach, as they must meet certain structural requirements.[5] All of the compounds (**1**–**6**) used in the past face certain drawbacks in terms of either their structural interpretation and/or their chemical incorporation into peptides.

Here we report the design, synthesis, and characterization of a novel FAA that avoids the problems of **1**–**6** and is ideally suited as a label for structure analysis of membrane-bound peptides by solid-state ^{19}F NMR spectroscopy. The principles of its design are based on the known advantages and disadvantages of the previously used FAAs,[4,5] given that the following criteria have to be satisfied. First, the ^{19}F reporter group has to be rigidly attached to the peptide backbone in a well-defined position with respect to the molecular framework. Any conformational flexibility of the side chain around χ_1 and χ_2 would make the structural interpretation of the NMR parameters ambiguous, as is the case for **1** and **2**.[4d,5,6] In **3** and **4** the position of the ^{19}F reporter group is well-defined, but analysis of the chemical shift anisotropy of the single ^{19}F substituent in **3** must still take into account the value of χ_1.[4c,e,5,7] Fast rotation of the CF_3 group in **4** renders all ^{19}F interactions axially symmetric and colinear with the C_α–C_β bond, which makes it ideal for orientational analysis.[4a,b,5] While the chemical shift of a monofluorinated FAA is difficult to reference,[2,5,8] this problem does not arise when the absolute dipolar splitting of a CF_3 group is analyzed.[4a,b,5]

It is also important that the FAA should not disturb the structure and function of a peptide. Finally, the FAA should



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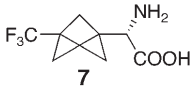
be chemically stable and sufficiently reactive, and should maintain its L configuration during peptide synthesis. Even under optimized conditions of Fmoc-SPPS the previously used FAAs were found to racemize (**3, 4**), to eliminate HF (**5**), or to be highly unreactive (**6**).[5,7]

In search of a rigid L-amino acid that would satisfy all of the above-mentioned criteria, we focused on the bicyclo-[1.1.1]pentane system. Placing CF_3 and aminocarboxylate moieties at the bridgehead positions of this skeleton yields the ^{19}F-labeled amino acid **7** (3-(trifluoromethyl)bicyclopent-[1.1.1]-1-ylglycine, CF_3-Bpg) as the target. The C_α–C_β and C–CF_3 bonds in **7** are colinear; the side chain can rotate only around these bonds. In contrast to **3** and **4**, in **7** the CF_3 group and the α-carbon atom are separated by a saturated bicyclic cage. This should make **7** less prone to racemization, since the transmission of electronic effects across the bicyclo-[1.1.1]pentane cage is far less pronounced than through an aromatic ring.[9] Neither degradation by the loss of HF (as in **5**) nor an unusually low reactivity of the aminocarboxylate moiety as a result of steric hindrance (as in **6**), are expected for **7** under SPPS conditions.



7

The synthesis of **7** commences from **8** and proceeds via [1.1.1]propellane **9**[10,11] as the key intermediate (Scheme 1). Addition of CF_3I at the "inverted" carbon atoms of **9** led to compound **10**,[12] which was treated directly with *t*BuLi to generate the carbanion and then with methylformate to produce aldehyde **11**. A mixture of compound **11** and the corresponding hemiacetal **12** was used in the Strecker synthesis with (*R*)-α-phenylglycinol as the chiral auxiliary.[13] Products **13** and **14** were separated chromatographically. The X-ray crystal structure of **14**[14] confirmed the absolute configuration at the newly formed chiral center to be *S* (Figure 1).

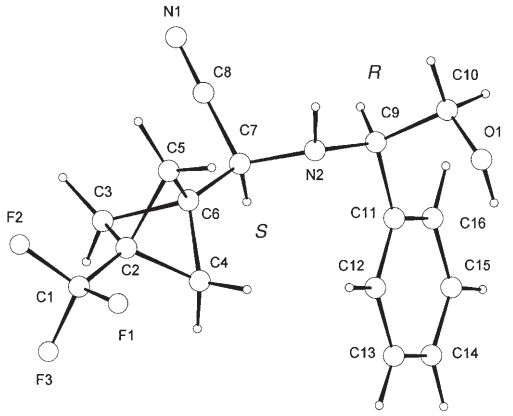
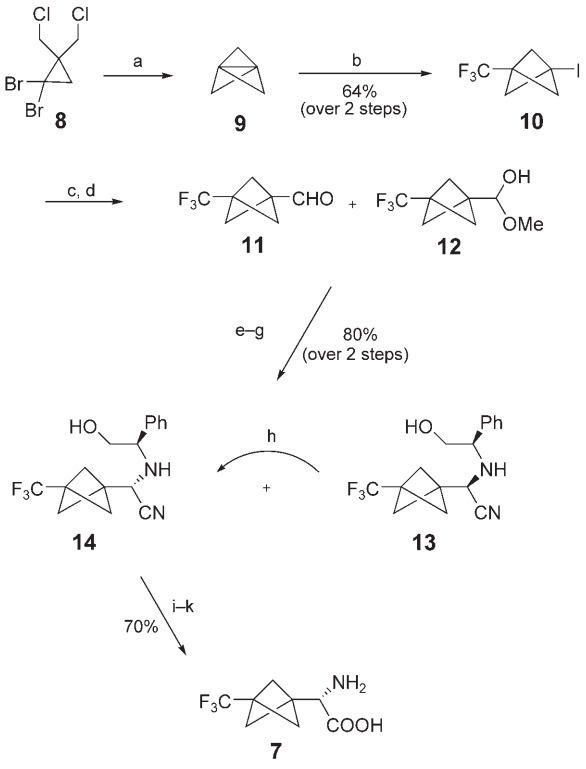


Figure 1. Molecular structure of **14**.



Scheme 1. Reagents and conditions: a) MeLi, pentane, −78 °C, 0.5 h; b) CF_3I, pentane, RT, 20 h; c) *t*BuLi, Et_2O, −78 °C, 1 h; d) CH_3OCHO, Et_2O, −78 °C→RT, 3 h; e) (*R*)-α-phenylglycinol, CH_2Cl_2, RT, 2 h; f) $(CH_3)_3SiCN$, RT, 10 h; g) chromatographic separation; h) MeOH, reflux, 3 h; i) $Pb(OAc)_4$, CH_2Cl_2, 0 °C, 5 min; j) 6 M HCl, reflux, 2 h; k) chromatography on Dowex-50.

Monitoring the asymmetric Strecker reaction by HPLC we found that the initial **14**/**13** ratio was 1:1. However, equilibration of purified **13** in MeOH led to isomerization, giving a **14**/**13** ratio of 4:1. Repetition of the isomerization–separation procedure provided **14** in 80 % yield. The desired L amino acid **7** was obtained from **14** by cleavage of the PhCH–CH_2OH bond and hydrolysis of the intermediate Schiff base. Compound **7** was optically pure (> 95 % *ee*), as evidenced by the ^{1}H NMR spectrum of the corresponding methyl ester in the presence of a chiral lanthanide shift reagent.

To examine the suitability of **7** as a ^{19}F-labeled amino acid for the analysis of peptides by NMR spectroscopy (^{19}F NMR label), we first compared its calculated properties[15] with those of the nonpolar proteinogenic amino acids and the aforementioned ^{19}F-labeled compounds. The shape and steric volume of **7** resemble closely the corresponding values of Leu, Ile, Met, Trp, and Phe. The similarity to Pro, Val, and Ala is poorer. The nonaromatic character of **7** constitutes its main difference to Trp and Phe. If one considers the values of octanol/water partition coefficients, a substitution of Leu, Ile, or Met by **7** would cause minimal change in the mean hydrophobicity, which is an important prerequisite for the proper folding of a polypeptide.[16] According to all parameters it is apparent that **7** is closer to the natural nonpolar amino acids than any of the previously used phenylglycine derivatives (**3, 4**).

To address the suitability of **7** experimentally, the new FAA was incorporated in the 21-mer sequence of the antimicrobial peptide PGLa in the very same way as **3** and **4** had been previously used as ^{19}F NMR labels.[4a,b,h] The substituted positions were Ile 9 (**15**), Ala 10 (**16**), Ile 13 (**17**), and Ala 14 (**18**). Previously, the FAAs **3** and **4** had racemized

completely under the SPPS conditions used to produce PGLa.[4a,b,5,7] Now, all of the synthetic peptides containing **7** were obtained as single diastereomers, as shown by HPLC/MS. It is known that PGLa assembles as an α helix when bound to a lipid bilayer and permeabilizes bacterial membranes.[4a,b] The biological activity of **15**–**18** was tested by bacterial-growth inhibition assays, which can respond strongly to single-point mutations (see the Supporting Information). This proved that none of the labeled analogues display any functional deviations from the wild-type peptide, suggesting that the new building block **7** did not induce any structural perturbations. Structure analysis of the labeled peptides **15**–**18** by circular dichroism proved that no distortion was induced by the new unit in any of the positions examined (see Figure 2).

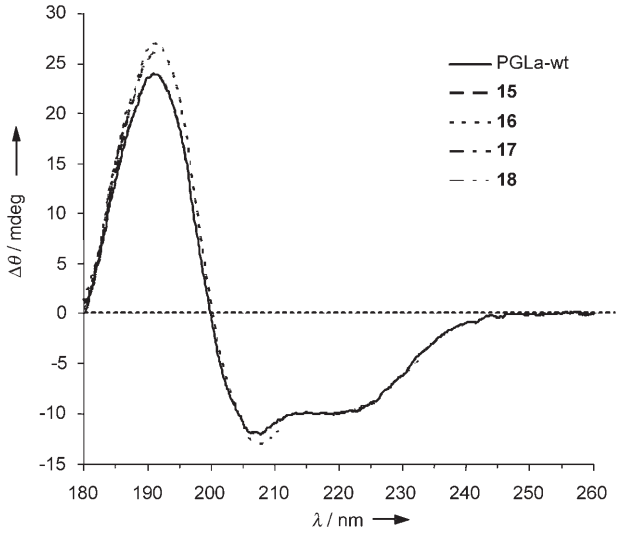


Figure 2. Circular dichroism spectra of the PGLa wild-type peptide and its L-CF_3-Bpg-labeled analogues **15**–**18** in the presence of sodium dodecyl sulfate micelles (5 mmol), normalized to the peptide concentration.

Finally, we reconstituted the labeled peptides into mechanically oriented phospholipid bilayers for a comprehensive solid-state ^{19}F NMR analysis. In brief, **15**–**18** behaved the same way as the PGLa analogues that had been previously labeled with **3** and **4**.[4a,b,5,7] Namely, 1) they bind to and uniformly align in 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) bilayers; 2) they rotate around the bilayer normal; 3) they realign in a concentration-dependent manner; and 4) for a given concentration the labeled positions exhibit the expected dipolar coupling and sign compatible with the known α-helical conformation. Therefore, we conclude that CF_3-Bpg satisfies all chemical and conformational requirements as a good labeling unit for peptide structure analysis by solid-state ^{19}F NMR spectroscopy.

In summary, we have synthesized the novel conformationally rigid fluorinated L α-amino acid **7** (CF_3-Bpg). The overall yield of the synthesis is 35 %, which makes it attractive for an intermediate-scale production of **7**.[17] This amino acid was designed as a label for membrane-bound peptides and can be used to study their alignment, structure, and dynamics by means of solid-state ^{19}F NMR spectroscopy.

Received: January 26, 2006
Revised: May 15, 2006
Published online: July 25, 2006

Keywords: amino acids · fluorine · membrane-active peptides · NMR spectroscopy

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