Hyperpolarization of a bisfluorinated phenylalanine derivative using PHIP and examination of the interaction with β-cyclodextrin

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Purpose

Amino acids are highly relevant in biological pathways and for protein and enzyme structures or for the synthesis of neurotransmitter. An essential amino acid is L-phenylalanine (Phe). It is hydroxylated to tyrosine, which is necessary for synthesis of e.g. GABA, dopamine and catecholamine. Additionally, Phe is used for protein biosynthesis. In the case of phenylketonuria, L-Phe was metabolized to phenylpyruvate. The use of fluorinated amino acids are of great interest in medical chemistry and diagnostics especially for the investigation of amino acid metabolism, protein structures and protein-ligand interactions.[1] Very low natural abundance in living organisms and the similarity of ^{19}F with ^{1}H with respect to bond length and van der Waals radius makes fluorine ideally suited for ^{19}F-MRS and MRI. The disadvantage of low spin density *in vivo* which results in low signals can be overcome by hyperpolarization methods such as Parahydrogen Induced Polarization (PHIP).[2,3,4] Here, we present the hyperpolarization of a bisfluorinated phenylalanine derivative and studies of the interaction between Phe and β-cyclodextrin.

Methods

The hyperpolarization of (2E)-2-acetamido-3-(2,5-difluorophenyl)acrylic acid (fig. 1) was realized with about 50 % enriched parahydrogen (6 bar pressure) in different vented solvents in presence of Rh(I) based catalysts. Directly after hydrogenation in earth field (ALTADENA), a ^{1}H NMR spectrum was detected by using a single pulse experiment with a 45° excitation pulse on a Bruker wide bore 300 MHz spectrometer. Additionally, ^{19}F NMR spectra were measured under same reaction conditions, with a 90° excitation pulse. The obtained signal enhancements (SEs) were calculated from signal-to-noise ratios of the thermal and the hyperpolarized spectra. The influence of the presence of β-cyclodextrin to the ^{19}F chemical shifts was examined in D_2O.

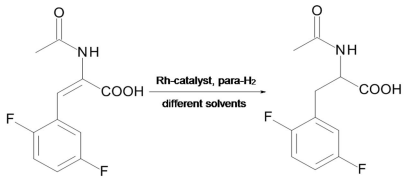

Figure 1: Reaction scheme of the hydrogenation of (2E)-2-acetamido-3-(2,5-difluorophenyl)-acrylic acid to the fluorinated phenylalanine derivative.

Results

The single-scan ^{1}H NMR spectrum recorded directly after hydrogenation (fig. 2, blue spectrum) shows three enhanced signals which can be attributed to the added hydrogen atoms at 4.8 ppm (CH), 3.3/3.0 ppm (CH_2) and 7.6-6.8 ppm (aromatic protons). The signal of the new CH group at 4,8 ppm has a SE of 68. Smaller SEs were calculated for the CH_2 group (SE=36), the aromatic protons (SE=9). The ^{1}H NMR spectrum in thermal equilibrium after hydrogenation is presented below (fig. 2, red spectrum). ^{19}F-NMR spectra of the precursor molecule (fig. 3 blue spectra) show two signals (a and b) in acetone-d_6 and CD_3OD. After hydrogenation (middle spectra), the product signals were shifted. The fluorine in ortho position (negative phase) has a higher chemical shift sensitivity but smaller SE. The fluorine in meta position (in-phase signal) has a slightly larger SE but a smaller chemical shift dependency. The small signal enhancements of SE = 3.8 and 2.6 could be increased by using field cycling. The different SE can be explained by a comparison of the T_1 relaxation times. In pure water both signals of the precursor have the same chemical shift while the signals of the bisfluorinated phenylalanine derivative are separated (figure 4A). The addition of β-cyclodextrin leads to a change of the chemical shifts until the concentration (mmol) of guest and host molecule are equal.

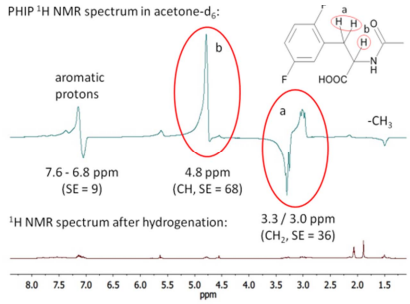

Figure 2: ^{1}H NMR spectra measured in acetone-d_6: hyperpolarized fluorinated phenylalanine derivative (above) and its spectrum in thermal equilibrium (below).

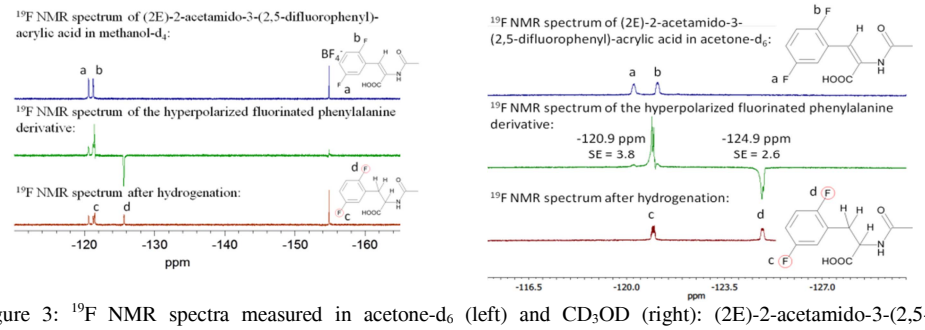

Figure 3: ^{19}F NMR spectra measured in acetone-d_6 (left) and CD_3OD (right): (2E)-2-acetamido-3-(2,5-difluorophenyl)-acrylic acid (above), hyperpolarized fluorinated phenylalanine derivative (middle) and its spectrum in thermal equilibrium (below).

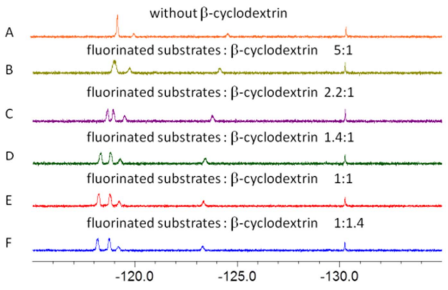

Figure 4: ^{19}F NMR spectra in D_2O show the interaction between the precursor respectively the product and β-cyclodextrin.

Discussion

For the first time, the hyperpolarization of a fluorinated amino acid was presented exemplarily for a phenylalanine derivative in different solvents. The ^{19}F NMR spectra demonstrated a polarization transfer from ^{1}H to ^{19}F. Therefore, tree different Rh-catalysts were used. The high sensitivity of the ^{19}F chemical shifts was demonstrated by changing the polarity of the solvent and additionally by examination the interaction with β-cyclodextrin. These aspects are very important in analytical MR studies of biological systems.

References

[1] Ojima I, Fluorine in Medical Chemistry and Chemical Biology,1.Ed.,Wiley-Blackwell:Chichester, 2009. [2] Bhattacharya P, et al., J Magn Reson **2007**, 186, 150 -155. [3] Bargon J, et al., Proc Intl Soc Mag Reson Med **2006**, 14, 2549. [4] Soon PC, et al., Chem Commun **2013**, 49, 5304-5306.