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Biocatalytic synthesis of (2S)-5,5,5-trifluoroleucine and improved resolution into (2S,4S) and (2S,4R) diastereoisomers

Hernan Biava *, Nediljko Budisa

Department of Biocatalysis, Institute of Chemistry, Technical University Berlin, Strasse des 17. Juni 135, 10623 Berlin, Germany

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ABSTRACT

The introduction of fluorinated amino acid carrying a CF_3 moiety in therapeutical agents and protein engineering requires the accessibility of highly pure chiral samples in order to correctly understand the effect of fluorination on bioactivity. Here we report an easy enzymatic approach for the synthesis of (2S)-5,5,5-trifluoroleucine and its subsequent resolution into its (2S,4S) and (2S,4R) diastereoisomers. This strategy stands for a sustainable synthesis and enhanced resolution by means of alternative protecting groups for the amino/acid functionalities than that previously reported.

The introduction of amino acid analogs carrying a CF_3 moiety into the structure of therapeutical agents, proteins, and peptides has been extensively applied as a tool for improving their activity and stability.[1,2] The enhanced pharmacological properties are generally explained in terms of an increased hydrophobicity of the trifluoromethyl group when compared to the methyl group, which most probably improves diffusion through cell membranes and resistance to hydrolysis by endogenous proteases.

Highly fluorinated amino acids have been additionally exploited in rational protein design, by introducing CF_3 substituents into the hydrophobic core of proteins and peptides.[3,4] In principle, this modification would lead to an enhanced stability of the folded form of the biopolymer in presence of organic solvents or high temperature conditions (so-called 'fluorous effect').[5,6] These are both greatly desired properties for biocatalytical purposes.

Due to their attractive applications, the synthesis of fluorinated amino acids varying the number and position of the CF_3 substituent has retained a significant role in the scientific community for several decades.[7] With the purpose of correctly pondering the outcome of fluorination into bioactivity, it is imperative to control the stereochemistry at the substituted carbon atoms. The amino acid (2S)-5,5,5-trifluoroleucine (TFLeu, **1**), which can exist as a mixture of two diastereoisomers **1a** and **1b** (Fig. 1), is a typical example of the many efforts devoted to stereospecific synthesis or preparative resolution from commercial racemic sources.[8–14]

Even though a number of synthetic pathways for the stereoisomers of TFLeu have been published, these methodologies are complex, require multiple steps, or employ expensive chiral auxiliaries, making their application unfeasible for bigger scale purposes. Therefore, chromatographic resolution after derivatization of commercial racemic mixtures is still the most practical method for preparing pure samples for **1a** and **1b** isomers.

Here we report an easy chemoenzymatic approach for the synthesis of (2S)-TFLeu followed by its subsequent resolution into the (2S,4S) and (2S,4R) diastereoisomers. This synthetic strategy provides ready access to the S stereochemistry at the Cα avoiding resolution of N-acetyl derivates by acylases and subsequent necessity for R-Cα isomers recycling.[15] The introduction of alternative protecting groups for the amino/acid functionalities allows a more efficient separation by flash column chromatography on silica gel than that previously reported.[12,13]

The synthesis of TFLeu on a milligram scale using *Alcaligenes faecalis* whole cells has been previously reported.[11] This procedure makes use of an α-keto ester and L-glutamic acid as amino donor for a transamination reaction. Another biocatalytic reductive amination for the synthesis of (S)-5,5,5,5′,5′,5′-hexafluoroleucine and (S)-5,5,5,5′,5′-tetrafluoroleucine has been accomplished at gram

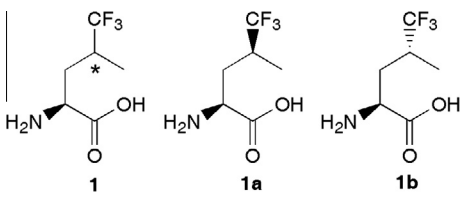

Figure 1. Chemical structure of (2S)-5,5,5-trifluoroleucine (**1**), (2S,4S)-5,5,5-trifluoroleucine (**1a**), and (2S,4R)-5,5,5-trifluoroleucine (**1b**). The asterisk in structure **1** indicates unresolved stereochemistry.

* Corresponding author. Tel.: +49 30 314 23661; fax: +49 30 314 28279.
E-mail address: biava@chem.tu-berlin.de (H. Biava).

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scale, employing phenylalanine dehydrogenase, NH_4^+ and NADH as reducing agent.[16,17] The latter method requires fewer steps for the obtention of the α-keto ester intermediates and therefore we examined the possibility to extend this methodology for the synthesis of **1**.

For this purpose, 1,1,1-trifluoroacetone was reacted with the ylide **3** in a Wittig reaction to give the unsaturated pyruvate ester **4** (Scheme 1).[18] No contamination with side-products was detected after vacuum distillation, whether the reaction was performed in THF, toluene, or benzene, contrary to the observation made by Chiu and Chen.[16] As previously reported,[19] the reaction is highly diastereoselective toward the E isomer (E:Z >95:5 judged from ^{1}H NMR spectra). The stereochemistry of the product was unambiguously assigned as E by ^{1}H{^{19}F} NOE experiments (Fig. S1, Supplementary data).[20,21]

Taking advantage of the high diasteroselectivty observed for the synthesis of **4**, we envisioned the likelihood for enantioselective hydrogenation of the double bond in order to control the stereochemistry at the second chiral center at this step of the synthesis. For this purpose, we inspected (S,S′,R,R′)-TangPhos (cyclooctadiene)rhodium(I)TBF[22–24] and [((4S,5S)-Cy2-Ubaphox)Ir(COD)]BARF[25–27] as possible chiral catalysts (Fig. S2, Supplementary data). Both metal complexes have been successfully employed for the enantioselective hydrogenation of related substrates, with good ee and acceptable yields. However, despite the variety of conditions assayed, such as various solvents, catalyst loading, temperature, reaction time, H_2 pressure, etc., all experiments resulted in a racemic mixture of the desired product plus a variable amount of the over-hydrogenated alcohol, as judged by chiral CG–MS and NMR analysis of the reaction products. The formation of the alcohol as by-product seems to be attributable to the presence of multiple electron-withdrawing groups and the conjugated carbonyl, which makes the double bond in **4** particularly electron deficient. We decided then to solve this problem in a further step by resolution of the final diastereoisomeric mixture.

A non-enantioselective hydrogenation of substrate **4** under low H_2 pressure and Pd/C as catalyst gave the corresponding reduced α-keto ester **5** in 82% yield.[28] Interestingly, $TiCl_{3(aq)}$ did not perform this reaction successfully, albeit it has been employed by Chiu for the reduction of the hexafluoro analog.[16] This result supports author's statement that the number and position of fluorine atom strongly affects reactivity for these types of molecules.

In a further step, we studied the ability of the enzyme phenylalanine dehydrogenase (EC 1.4.1.20) to act on substrate **5** which has a marked polarity difference when compared to the substrates assessed by Chiu (Scheme 1). After hydrolysis in the presence of Na_2CO_3 to generate the free salt, an enzymatic amino reduction catalyzed by phenylalanine dehydrogenase at pH 8.5 was performed, in the presence of NADH as reducing agent. Interestingly, both enantiomers of **5** were evenly processed by the enzyme, as

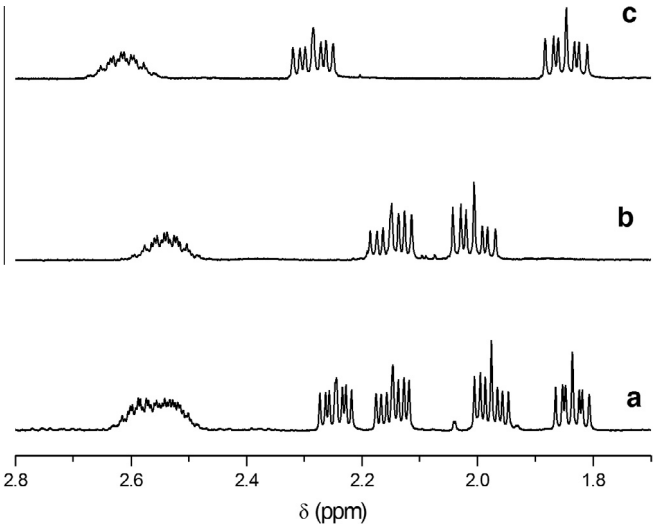


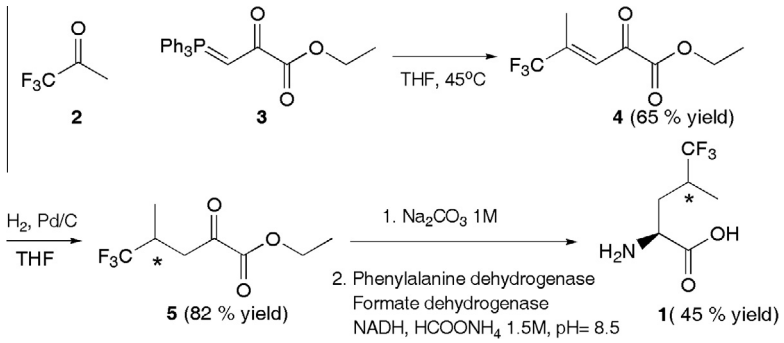
Figure 2. ^{1}H NMR in the δ = 2.8–1.7 ppm window, showing diastereotopic hydrogen atoms for compounds (a) **1**; (b) **1a**; and (c) **1b**.

it can be seen on the ^{1}H NMR spectra for the resulting product after purification by ion-exchange chromatography (Fig. 2a).[29] The spectral pattern corresponds to 1:1 mixture of the two isomers of (2S)-trifluoroleucine. This result evidences once again the enzymatic versatility of phenylalanine dehydrogenase to act on a wide range of substrates, with different sizes and polarities. In order to make the synthesis viable in terms of regeneration of the expensive NADH, we employed in addition formate dehydrogenase (EC 232.844.2) to regenerate NADH, with concomitant oxidation of the formate in the buffer.

When confined to a dialysis membrane, both enzymes could be reused several times, without no evident loss in the activity or the enantioselectivity, making this synthesis an excellent alternative to the methodologies published by Matsumura et al.[11]

The enantioselectivity of the enzymatic reductive amination was assessed by derivatization of new synthesized (2S)-5,5,5-trifluoroleucine with (2R)-2-methoxy-2-trifluoromethylphenylacetyl chloride (R-(−)-Mosher's acid chloride, MTPA).[30–32] The ^{1}H NMR of the derivatized sample is shown in Figure 3. Only two peaks which correspond to OCH_3 groups of the amide derived from diasteroisomers (2S,4S) and (2S,4R) plus unreacted MTPA were detected, which confirms the enantioselectivity of the enzymatic reaction.

As previously stated, two related methodologies have been reported for the resolution of all four isomers of 5,5,5-triflouroleucine.[12,13] These procedures involved conversion of the racemic commercial samples into the N-Boc-5,5,5-trifluoroleucine t-butyl



Scheme 1. Biocatalytic synthesis of (2S)-5,5,5-trifluoroleucine. Yields correspond to individual steps.