Total synthesis of [$^{13}C_2$]-labelled phytosiderophores of the mugineic and avenic acid families

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Abstract:

We herein report the synthesis of $^{13}C_2$-labelled natural products from the mugineic acid and avenic acid family. These phytosiderophores ("plant iron carriers") are built up from non-proteinogenic amino acids and play a key role in micronutrient uptake in gramineous plants. In this work two central building blocks are prepared from labelled starting materials ($^{13}C_2$-bromoacetic acid, $^{13}C_2$-glycine) and further employed in our recently reported divergent, branched synthetic strategy delivering eight isotopically labelled phytosiderophores. The required labelled building blocks ($^{13}C_2$-L-allylglycine and a related hydroxylated derivative), were prepared *via* enantioselective phase-transfer catalysis and enantio- and diastereoselective aldol condensation with a chiral auxiliary respectively, both potentially valuable themselves for other synthetic routes towards labelled (natural) products.

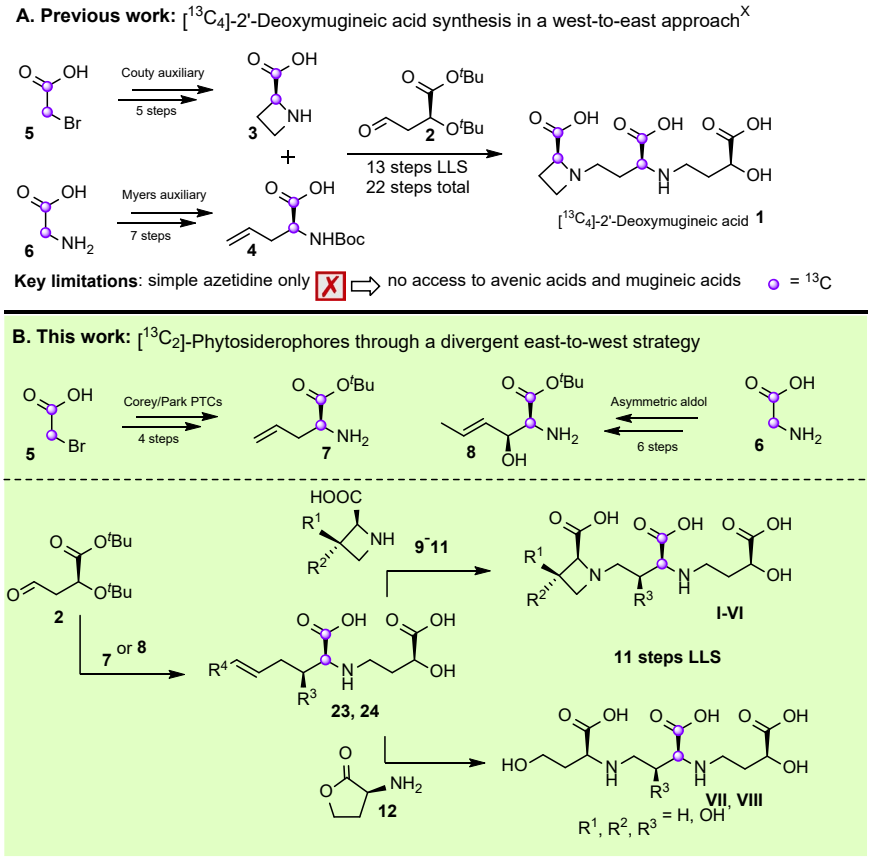
Introduction:

Essential micronutrients are extremely important for the survival of all living beings, and encompass substances like vitamins, amino acids or minerals. In humans, micronutrient malnutrition causes impaired growth and cognitive development as well as low disease resistance.[1] The development of micronutrient rich plant crops is considered the most cost-effective and sustainable approach to battle this 'hidden hunger'. [2] Thus, understanding the mechanisms involved in plant micronutrient acquisition is a pre-requisite for successful plant breeding programs.

Even though mineral micronutrients, like Fe, Cu or Zn are abundant in the earth's crust, low solubility limits the availability for plants due to differences in soil mineral composition and pH. Thus, plants have established different strategies to increase their uptake of these ions. With regard to Fe, two acquisition mechanisms have evolved. All non-gramineous plants rely on a multistep process including acidifying the rhizosphere, reducing Fe(III) to Fe(II) via enzymes on the cell wall and complexation of the Fe(II) followed by uptake into the cell (strategy I). [3] In contrast, all gramineous plants synthesize so-called phytosiderophores (PS) that are secreted into the surrounding soil (so-called rhizosphere where complexation of insoluble Fe(III) can take place. The metal-PS complex is transported back into the cell where the metal ion is liberated. [4] The biosynthesis and secretion of PS is upregulated if the plant experiences micronutrient deficiency. [5]

In order to further elucidate the micronutrient acquisition system of these gramineous plants, PS are required as analytical tools in natural form to develop sensitive analytical approaches to allow accurate detection of PS in plant and soil samples [6] (Spiridon et al., manuscript in preparation) and to study the micronutrient mobilization efficiency of PS in different soils.[7] Furthermore, isotopically labelled forms of natural compounds are of particular importance in biological research as they allow to trace compounds of interest through biological systems and to determine process rates.[8] Additionally, labelled compounds serve as internal standards significantly improving accuracy of quantification approaches [9]. In the context of a multidisciplinary project our group established an efficient, divergent synthesis of non-labelled PS. [10] In this work we extended the developed methodology to produce $^{13}C_2$-labelled PS in

an affordable and efficient manner. It constitutes a second-generation synthesis of these important natural products in labelled form, moving from our earlier disclosed singular synthesis of $^{13}C_4$-deoxymugineic acid [11] to a divergent approach towards eight different $^{13}C_2$-labelled PS from the mugineic acid family (I-VI) and the avenic acid family (VII, VIII).



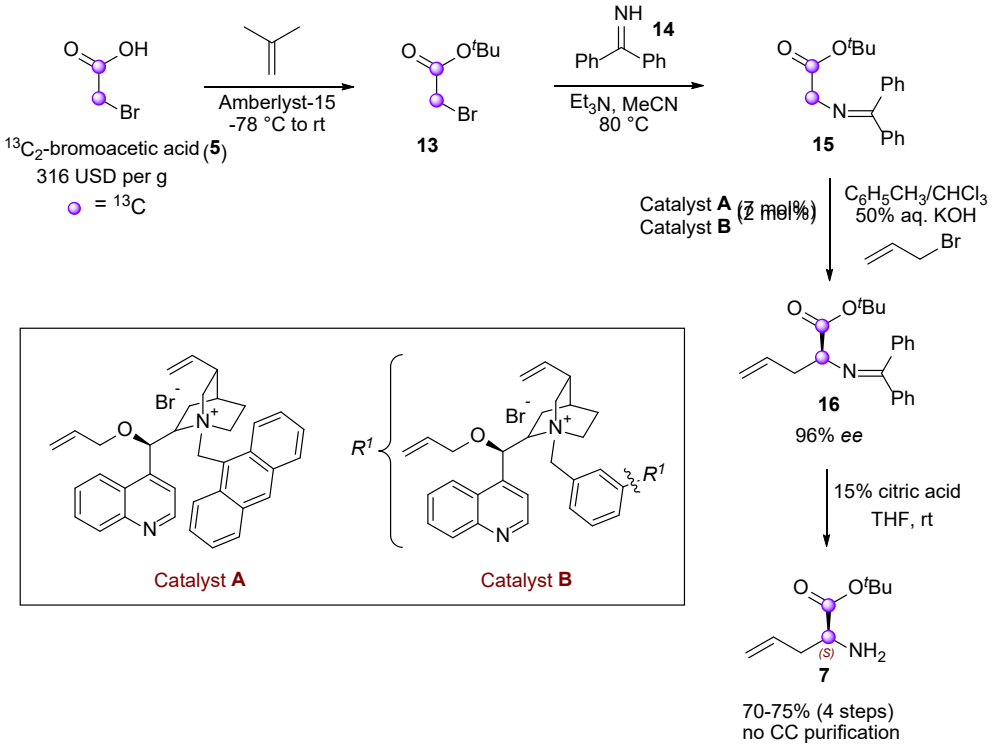
Scheme 1: Convergent approach towards 8 phytosiderophores with labels on middle building blocks

Based on results by our collaborators [6-9] the middle fragment of the targets was identified as a suitable, highly conserved, site for labelled atoms to be introduced. Relating this to the previously described synthetic strategy, the problem can be focused on the preparation of middle building blocks **7** and **8** with ^{13}C-isotope labels at C-1 and C-2 (see Scheme 1). For hydroxy-containing building block **8** a modified version of our own synthesis can be carried out starting from $^{13}C_2$-glycine **6** instead of standard glycine [10]. The allylglycine subunit **7** could be traced back to $^{13}C_2$-bromoacetic acid **5** and obtained by a classic phase-transfer catalyzed asymmetric amino acid synthesis [12-13].

Results and discussion:

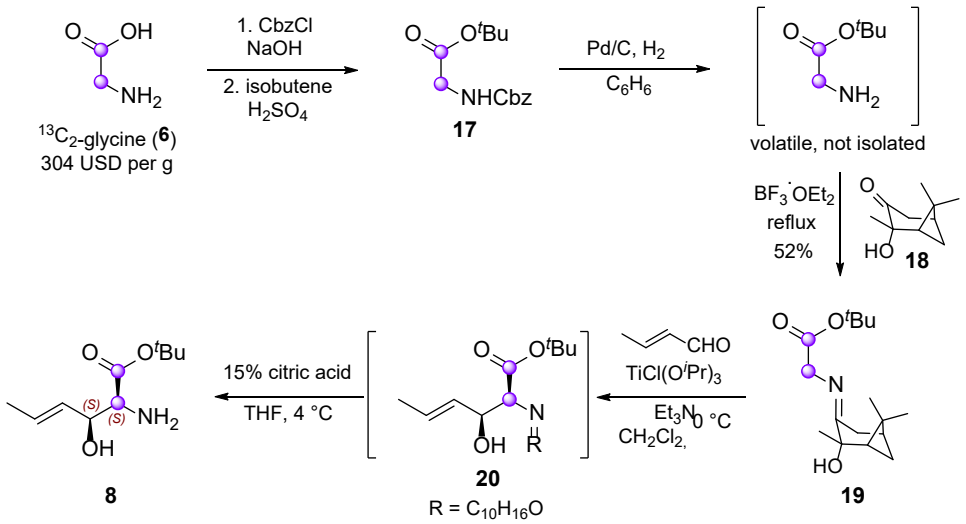
The synthesis of *tert*-butyl $^{13}C_2$-L-allylglycine **7** commenced with protection of the carboxylic acid moiety of $^{13}C_2$-bromoacetic acid **5** by treatment with condensed isobutene and Amberlyst 15 (see Scheme 1), according to a previously published procedure [14]. The protected product **13** was obtained in quantitative yield and subjected to the next step without further purification. The imine-group required for the alkylation reaction was introduced by nucleophilic displacement of the bromine with benzophenone imine **14** in acetonitrile. The product **15** was isolated in quantitative yield and good purity (ca. 85-90%) with the main impurity consisting of hydrolyzed benzophenone. The crude product was subjected to phase-transfer catalyzed alkylation under conditions developed by Jew, Park and coworkers using a novel mixture of PTCs **A** and **B** developed by Jew/Park [13e] and Corey/Lygo [13k-m]

respectively. The use of this mixture of catalysts was crucial to obtain a speedy conversion (4 hours) while retaining excellent enantioselectivity (96% *ee*). The resulting allylglycine imine **16** was finally hydrolyzed with aqueous citric acid providing $^{13}C_2$-L-allylglycine **7** in 70-75% yield over 4 steps. The purity of the product after acid/base extraction was already very good and thus did not require any additional chromatographic purification. The isotopically enriched compound **7** was isolated in a comparable purity to our previous non-labelled synthesis starting from unprotected allylglycine [10].



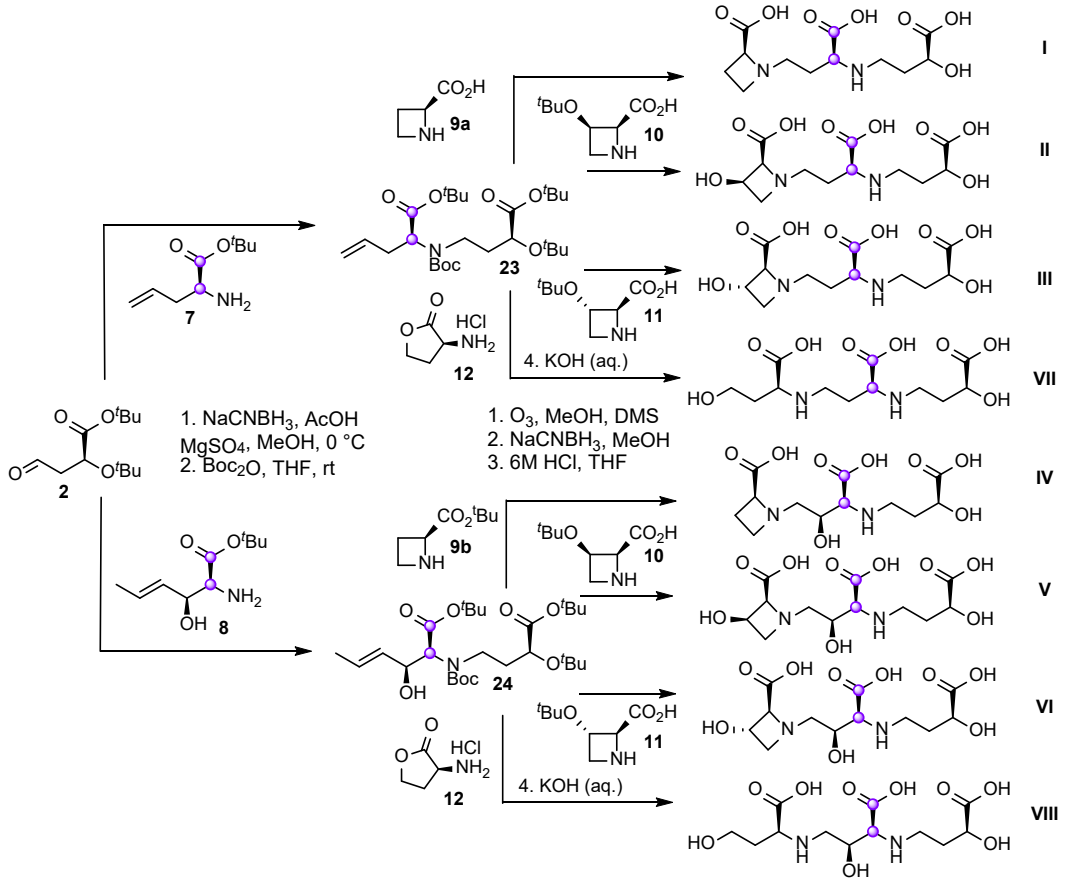
Scheme 2: Synthesis of $^{13}C_2$-(L)-allylglycine **7** from $^{13}C_2$-bromoacetic acid **5**.

Our published procedure for the preparation of the second labelled building block **8** had to be adapted to accommodate the use of commercially available starting material $^{13}C_2$-glycine **6** (see Scheme 2) instead of tert-butyl glycinate hydrochloride which was utilized in the synthesis of the unlabelled material. Direct installation of the required *tert*-butylester could not be accomplished reproducibly. Thus, the amino functionality in **6** was protected as a benzyl carbamate under standard conditions [15] in 70% yield. Then the introduction of the *tert*-butyl protecting group on the C-terminus was achieved with isobutylene under acid catalysis to give **17**. Subsequently, the Cbz protecting group was cleaved *via* catalytic hydrogenation. This step was conducted in benzene as a solvent to allow the resulting solution (after filtration of the catalyst) to be directly used in the attachment of the chiral auxiliary, hydroxypinanone **18**, furnishing imine **19** in 52% yield over 2 steps. It is important to note that imine **19** is not stable for prolonged periods of time and should be used immediately after purification. We observed that, through hydrolysis, pinanone **18** was the most abundant impurity. Finally, the titanium mediated aldol reaction [16] was carried out as described in earlier work to furnish the $^{13}C_2$-labelled aminoalcohol **8** after cleavage of the auxiliary in acceptable yields and purities. The remaining five building blocks **2** and **9-12** required for the synthesis were prepared as previously described [10] or purchased from commercial sources.



Scheme 3: Synthesis of labelled aminoalcohol **8** from $^{13}C_2$-glycine **6**

With all building blocks in hand assembly of the target molecules was straightforward and fully analogous to our recently published unified approach toward the PS in their isotopically natural form [10].



Scheme 4: Coupling of fragments and assembly of phytosiderophores **I-VIII**

Thus, aldehyde **2** was reacted in a reductive amination with labelled amines **7** and **8** providing desired amines **21** and **22** (not depicted in Scheme 4) in good yields. Subsequent Boc-

protection proceeded smoothly to yield **23** and **24**. These two protected key fragments were ozonolysed to reveal aldehyde functionalities. These crude aldehydes were in turn used in a second reductive amination with the four western building blocks **9-12** to give rise to the eight final compounds in protected form (not depicted, see ESI). These protected phytosiderophores were globally deprotected with 6M HCl to reveal the desired $^{13}C_2$-labelled target compounds **I-VI** in excellent yield. Additional lactone cleavage was which was performed with aqueous potassium hydroxide was applied for the deoxy avenic acid (**VII**) and avenic acid (**VIII**), respectively. [17] Final purification was based on passage over acidic Dowex resin and elution with ammonia.

Because the NMR spectra of these final compounds are highly pH-dependent the identity of the labelled compounds was demonstrated via phosphate buffered proton NMR and comparison to equally buffered naturally labelled reference materials. (See Figure 2 and ESI).

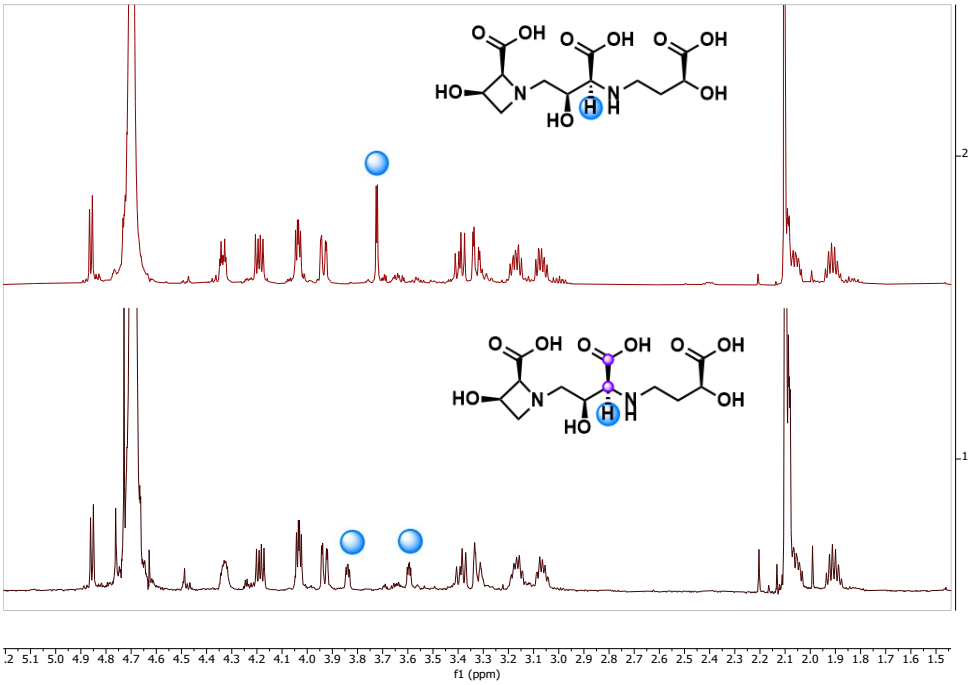


Figure 2: Comparison of ^{1}H-NMR spectra of final compound **V** with and without the $^{13}C_2$-label (Conditions: D_2O, pH = 6.0 phosphate buffer, 0.1 M, 10 mL containing 37 mg $Na_2HPO_4 \cdot 7\ H_2O$ and 119 mg $NaH_2PO_4 \cdot H_2O$)

Conclusion:

To summarise, a concise synthesis of $^{13}C_2$-labelled phytosiderophores was accomplished by employing state-of-the-art asymmetric methodology to install the required stereocenters in high selectivities. The obtained building blocks were reacted in our previously disclosed divergent synthetic approach to furnish the target compounds in 10 or 11 steps longest linear sequence and good yields overall. These precious materials will enable collaborating plant and soil scientists to study PS secretion patterns in grass crops as well as PS interaction with the soil matrix including microbial decomposition dynamics. Detailed insights into the mechanisms of micronutrient acquisition by plants will advance the development of micronutrient rich crops which is considered a key strategy to battle wide-spread mineral micronutrient deficiencies in human populations around the globe.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements:

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