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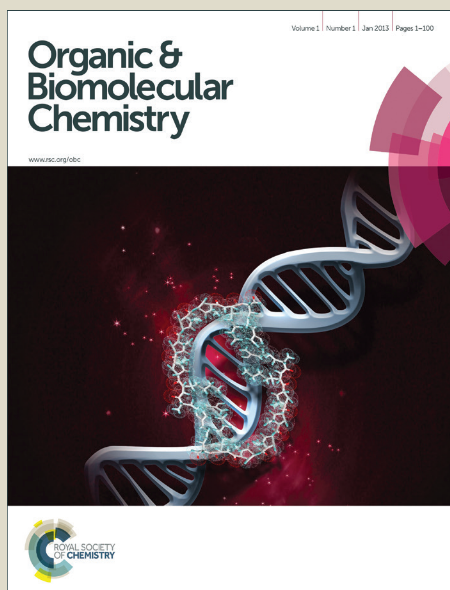

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Practical synthesis of natural plant-growth regulator 2-azahypoxanthine, its derivatives, and biotin-labeled probes

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We describe a practical, large-scale synthesis of the "fairy-ring" plant-growth regulator 2-azahypoxanthine (AHX), and its biologically active hydroxyl metabolite (AOH) and riboside derivative (AHXr). AHXr, a biosynthetic intermediate, was synthesized from inosine via a biomimetic route. Biotinylated derivatives of AHX and AHXr were also synthesized as probes for mechanistic studies.

"Fairy rings" arising from fungus-stimulated plant growth occur worldwide, and were first reported in 1675, as reviewed in Nature in 1884. [1] In 2010, we reported that, in the case of the fungus *Lepista sordida*, the "fairy" is a plant growth stimulator, which we identified as 2-azahypoxanthine (AHX: **1**). [2] AHX exhibited growth-regulating activity towards not only turf grass, but also other plants tested, even from different families. Furthermore, this compound increased the seed yields of rice and wheat in pot-growth and field experiments,[2] suggesting that it might find practical application in agriculture. We also showed that 2-aza-8-oxohypoxanthine (AOH: **3**) is a common, biologically active metabolite of AHX (**1**) in plants (Figure 1).[3]

AHX (**1**) was chemically synthesized from 5-aminoimidazole-4-carboxamide (AICA **2**; an intermediate in the purine metabolic pathway in animals, plants, and microorganisms), and converted into AOH (**3**) by xanthine oxidase-mediated reaction. We hypothesized that plants themselves produce AHX (**1**) and AOH (**3**) through a similar pathway, and indeed, we demonstrated that endogenous AHX (**1**) and AOH (**3**) are formed via the purine pathway in plants.[3] We considered that riboside and/or ribotide derivatives of AICA (**5**, **7**) and AHX (**4**, **6**) might also be involved in the biosynthetic pathway. Further, biotinylated derivatives of AHX and AHX riboside (AHXr; **4**) are of interest as potential tools for mechanistic studies to identify receptor(s) and related proteins. Herein we report efficient synthetic routes to AHX (**2**), AOH (**3**), AHXr (**4**) and also biotinylated derivatives of AHX and AHXr.

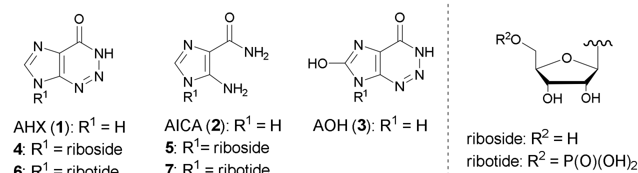

Figure 1. Structures of AHX derivatives.

First, we wished to develop a practical synthesis of AHX (**1**) and AOH (**3**). Although synthesis of **1** has already been reported,[4] optimization for large scale-preparation is necessary to obtain sufficient material for field experiments. As shown in Scheme 1, the synthesis was commenced with inexpensive **2**·HCl. Upon treatment of **2** with sodium nitrite under acidic conditions, the desired diazonium formation proceeded smoothly to provide diazoimidazole carboxamide (DICA: **8**). Although a hundred-gram scale AHX (**1**) was prepared according to the reported procedure (method A), [4] we found that the triazine ring of **1** can be constructed by heating **8** in methanol at 60 °C (method B). Furthermore method B was more superior than method B in terms of yield as well as handling. By means of this transformation, together with activated carbon treatment, **1** was obtained on an almost deca-gram scale. Conversion of **1** to **3** was carried out by enzymatic oxidation with xanthine oxidase.[5] Purification of **3** was also accomplished by recrystallization, and chromatographic purification was unnecessary (see ESI for the details).

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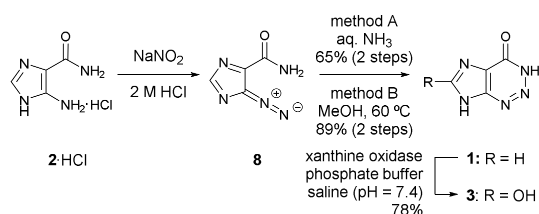
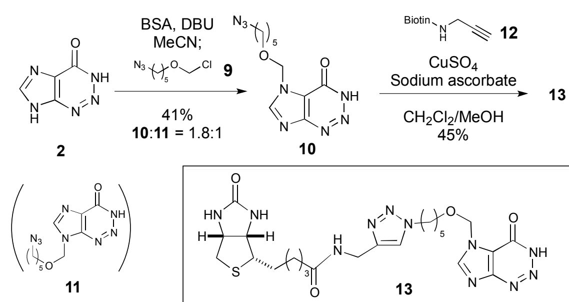


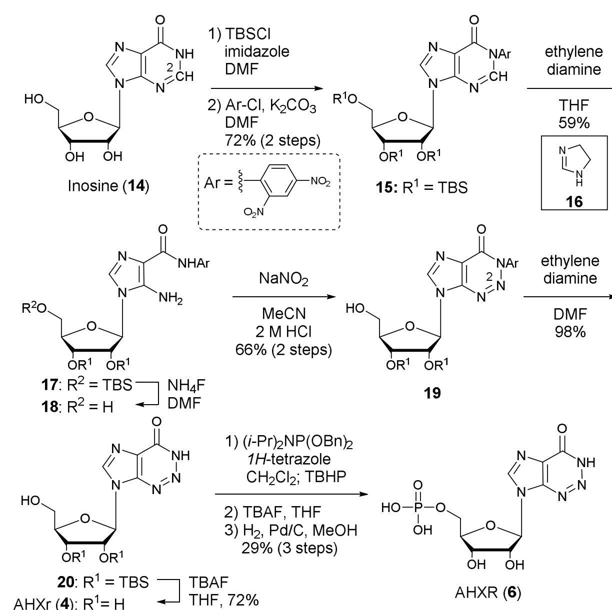
Scheme 1. Practical synthesis of AHX (**1**) and AOH (**3**).

With the desired biologically active natural products **1** and **3** in hand, we turned our attention to the synthesis of derivatives that would be suitable as probes for mechanistic studies, following on from our previous work.[6] During studies of polyphenols such as catechins[7] and flavones,[8] we had found that a terminal amine or azide group is favorable for facile incorporation of a probe moiety without the need for protection of the phenol group.[7b, 8a] Thus, we decided to incorporate a terminal azide group onto AHX (**1**). However, direct alkylation of **1** with alkyl halide did not proceed smoothly. In order to enhance the reactivity of the linker, we designed the probe precursor **10**, in which the linker group is connected through the acetal group. Alkylation reaction was performed after *in situ* protection of the nitrogen of imidazole and amide with a TMS group by treatment with *N*,*O*-bis(trimethylsilyl)acetamide (BSA), as shown in Scheme 2. Upon treatment of **1** with 1-azido-5-(chloromethyloxy)pentane **9** in the presence of DBU and BSA, the desired reaction proceeded smoothly to provide **10** and **11** as a 1.8 : 1 mixture. After separation of each regioisomer, the azide **10** was used as the AHX probe precursor. Hüisgen condensation reaction[9] of azide **10** and acetylene **12** containing a biotin group in the presence of $CuSO_4$ and sodium ascorbate proceeded smoothly to afford **13**.[10] Considering the convenience of the Hüisgen reaction, this synthetic strategy should be applicable to incorporation of a wide range of functional units, such as a fluorescent moiety for imaging or a carrier protein for synthesis of an immunogen.[7c] We found that the coupling reaction did not require a tedious purification step, although purification of biotin-containing probes is sometimes troublesome.



Scheme 2. Hüisgen reaction of azide **10** and alkyne **12**.

Based on the proposed biosynthetic pathway of AHXr (**4**) and AHX-ribotide (AHXR: **6**), we next planned to synthesize **4** and **6** from inexpensive inosine (**14**) as shown in Scheme 3.[11] Although conversion of the carbon atom at C-2 position of **14** into the nitrogen atom of **4** seemed to be a challenging task, a similar aminolysis of the pyrimidine ring of inosine (**14**) has been reported.[12] After protection of the hydroxyl groups of **14** with a TBS group, incorporation of a 2,4-dinitrophenyl group on the amide nitrogen was carried out via nucleophilic aromatic substitution reaction of 1-chloro-2,4-dinitrobenzene to give **15**. Upon treatment of **15** with ethylenediamine in THF, the desired aminolysis reaction on the imidamide ring proceeded smoothly to give **17** with release of dihydro-imidazole **16**.[12] After selective deprotection of the TBS ether on the primary alcohol of **17** by treatment with NH_4F, construction of the triazine ring of **19** was performed by treatment of **18** with sodium nitrite and hydrochloric acid, as employed in the preparation of **1**. Removal of the 2,4-dinitrophenyl protecting group of **19** was carried out by treatment with ethylenediamine as a nucleophile. In the original report,[12] treatment of **15** with ethylenediamine in DMF caused simultaneous ring opening reaction and dinitrobenzene cleavage reaction to afford the $-CONH_2$ derivative. However, the 2,4-dinitrophenyl group played a key role in the efficient preparation of **4**, **6** and the probe precursor **23**, and a selective aminolysis reaction of **15** was needed. Chemoselective reaction of **15** was accomplished by changing the solvent to THF from DMF. Finally, TBAF-mediated deprotection of the TBS groups of **20** provided AHXr (**4**).[10] On the other hand, AHXR (**6**) was synthesized by incorporation of phosphate ester into **20** by the phosphoramidite method,[13] followed by removal of the TBS groups and the benzyl group to give **6**.[10]



Scheme 3. Preparation of AHXr (**4**) and AHXR (**6**).

With an efficient synthetic method for AHXr (**4**) in hand, we turned our attention to the synthesis of a biotin-labeled AHXr probe compound, starting from the synthetic intermediate **19**. Since AHXr (**4**) is a biosynthetic precursor of AHX (**2**) in plants, the biotinylated AHXr probe should enable the identification of proteins involved in the biosynthesis of AHX. Since the ribose unit of the AHXr probe (**24**) can serve as a hydrophilic linker unit of the AHX probe, a efficient and flexible synthesis of **24** would be useful. As shown in Scheme 4, alkylation reaction of **19** with **9** proceeded smoothly to provide **21**. Utilizing a similar method to that used for preparation of

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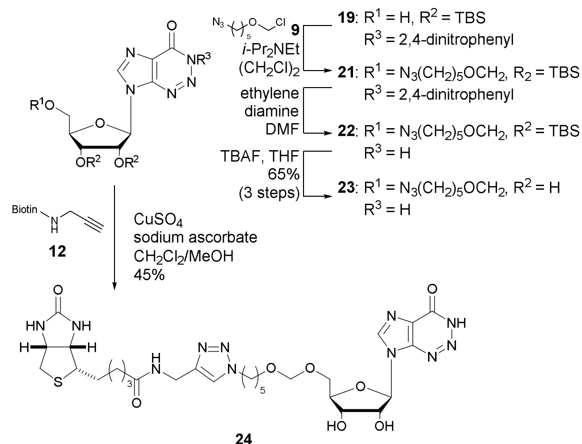
4, removal of the 2,4-dinitrophenyl group and the TBS group of **21** provided a reactive probe precursor **23**. Next, coupling with biotin was examined. Upon treatment of azide **23** and acetylene **12** under the conditions employed for preparation of **13**, the desired Hüisgen reaction proceeded smoothly to provide **24**[10] without any need for purification. This coupling reaction is compatible with the reactive hydroxyl group and amide group. Synthesis of other kinds of probe molecules from **10** and **23** is in progress in our laboratories, and the details will be reported in due course.



Scheme 4. Synthesis of biotin-labelled AHXr probe **24**.

Conclusion

We have developed a practical, large-scale synthesis of the plant-growth regulator AHX (**1**), as well as an efficient synthetic route for AOH (**3**), AHXr (**4**) and AHXR (**6**), respectively. Biotin-labeled derivatives of AHX (**13**) and AHXr (**24**) were also synthesized. The probe precursors **10** and **23** should also be suitable for preparation of other probe molecules.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental Procedures and NMR spectral data and bioassay of **4**, **6**, **13** and **24**. See DOI: 10.1039/c000000x/

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