

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

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## COMMUNICATION

# 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.<sup>1</sup> 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**).<sup>2</sup> 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,<sup>2</sup> 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).<sup>3</sup>

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.<sup>3</sup> 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.

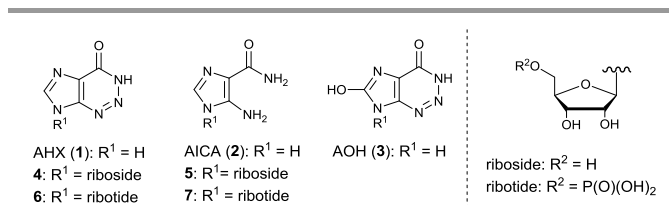
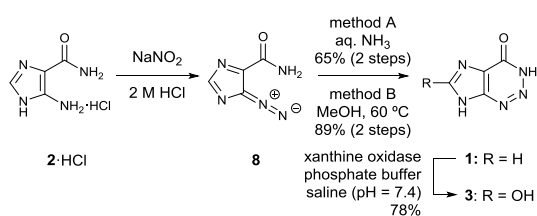


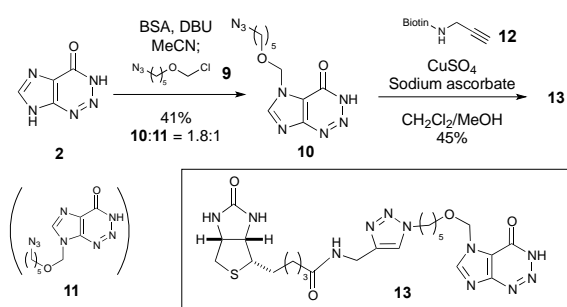
Figure 1. Structures of AHX derivatives.

First, we wished to develop a practical synthesis of AHX (**1**) and AOH (**3**). Although synthesis of **1** has already been reported,<sup>4</sup> 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),<sup>4</sup> 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 A 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.<sup>5</sup> Purification of **3** was also accomplished by recrystallization, and chromatographic purification was unnecessary (see ESI for the details).



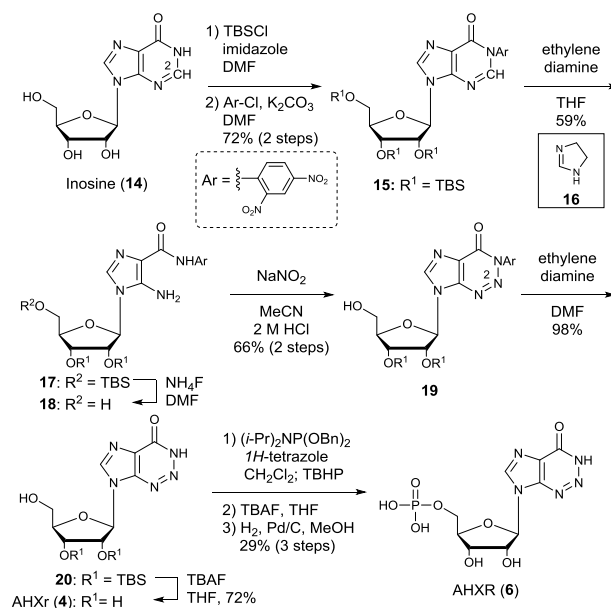
Scheme 1. Practical synthesis of AHX (1) and AOH (3).

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.<sup>6</sup> During studies of polyphenols such as catechins<sup>7</sup> and flavones,<sup>8</sup> 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.<sup>7b, 8a</sup> 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-(chloromethoxy)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. Huisgen condensation reaction<sup>9</sup> of azide **10** and acetylene **12** containing a biotin group in the presence of CuSO<sub>4</sub> and sodium ascorbate proceeded smoothly to afford **13**.<sup>10</sup> Considering the convenience of the Huisgen 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.<sup>7c</sup> We found that the coupling reaction did not require a tedious purification step, although purification of biotin-containing probes is sometimes troublesome.

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

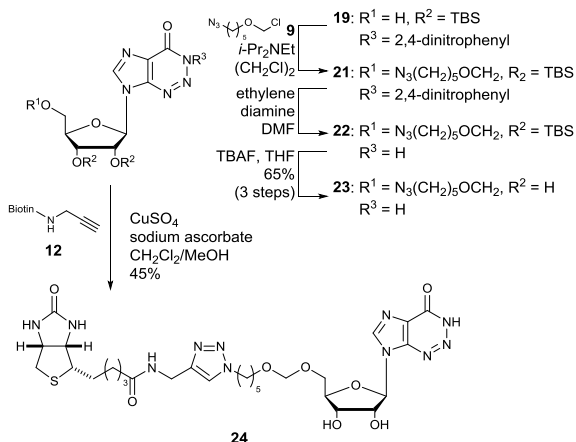
Based on the proposed biosynthetic pathway of AHXr (**4**) and AHX-ribose (AHXR: **6**), we next planned to synthesize **4** and **6** from inexpensive inosine (**14**) as shown in Scheme 3.<sup>11</sup> Although conversion of the carbon atom at C-2 position of **14** into the nitrogen atom of **4** seemed to be a challenging task, a similar aminolysis reaction of the pyrimidine ring of inosine (**14**) has been reported.<sup>12</sup> 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**.<sup>12</sup> After selective deprotection of the TBS ether on the primary alcohol of **17** by treatment with NH<sub>4</sub>F, 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,<sup>12</sup> treatment of **15** with ethylenediamine in DMF caused simultaneous ring opening reaction and dinitrobenzene cleavage reaction to afford the -CONH<sub>2</sub> 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**).<sup>10</sup> On the other hand, AHXR (**6**) was synthesized by incorporation of phosphate ester into **20** by the phosphoramidite method,<sup>13</sup> followed by removal of the TBS groups and the benzyl group to give **6**.<sup>10</sup>

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

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 Huisgen reaction proceeded smoothly to provide **24**<sup>10</sup> 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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- Interestingly, biotin probes **13** and **24**, as well as AHXr (**4**) and AHXR (**6**), possessed comparable biological activity to natural AHX (**1**). For details, see the ESI.
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