

## Synthesis of deuterated benzyladenine and its application as a surrogate

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### ABSTRACT

Palladium on carbon-ethylenediamine complex [Pd/C(en)] catalyzed deuteration of *N*<sup>6</sup>-benzyladenine-*d*<sub>5</sub>, which is a plant growth regulator, to introduce 5 deuterium atoms, while use of Pd/C as a catalyst led to a complete removal of *N*<sup>6</sup>-benzyl group. The corresponding deuterated *N*<sup>6</sup>-benzyladenine was successfully used as a surrogate compound for the quantitative analysis of residual benzyladenine in crops using LC/MS/MS

### INTRODUCTION

Benzyladenine (*N*<sup>6</sup>-benzyladenine) has been used as a plant growth regulator of vegetables, grain and fruits. Although radiolabeled benzyladenine was prepared as a surrogate in quantitative analysis of residual agrochemical, the employment of stable isotope-labeled benzyladenine is desired for safety reasons. We recently reported an efficient

and chemoselective hydrogen-deuterium (H–D) exchange reaction on a benzylic carbon using Pd/C as a heterogeneous catalyst and deuterium oxide (D<sub>2</sub>O) as a deuterium source in the presence of a catalytic amount of hydrogen gas at room temperature.<sup>1,2</sup> We also found that the application of heat could enhance the catalytic activity toward the H–D exchange reaction of various compounds including base moieties of nucleosides (Figure 1)<sup>3–5</sup> and use of Pt/C instead of Pd/C was effectively promoted the H–D exchange reaction on aromatic rings.<sup>6,7</sup> In this symposium, we present an efficient synthesis of deuterated *N*<sup>6</sup>-benzyladenine avoiding the hydrogenolysis of benzyl group and its application as a surrogate compound toward the quantitative analysis.

### RESULTS AND DISCUSSION

We first investigated the H–D exchange reaction of benzyladenine at room temperature using 10% Pd/C as a catalyst, while no deuteration on the purine ring was observed (Table 1, entry 1). When the reaction was carried out at 110 °C, a complete and undesirable removal of the *N*-benzyl group took place to give the corresponding adenine as the sole product (entry 2). The hydrogenolysis

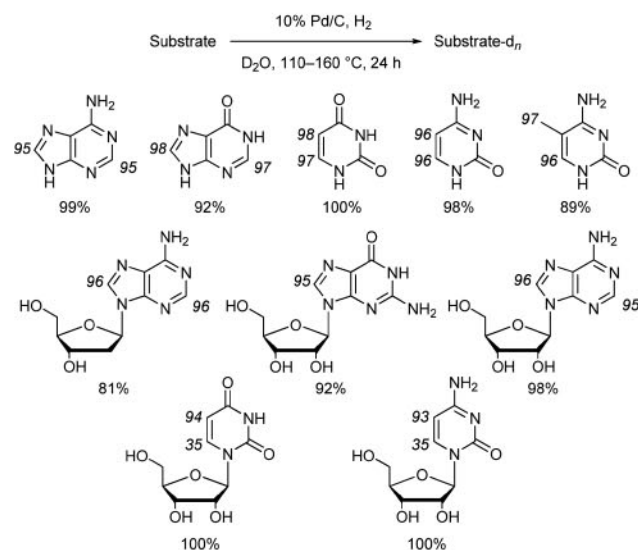


Fig. 1 Pd/C-catalyzed deuteration of nucleobases and nucleosides. The italic numbers indicate the deuterium efficiency.

Table 1 H–D exchange reaction of *N*<sup>6</sup>-benzyladenine

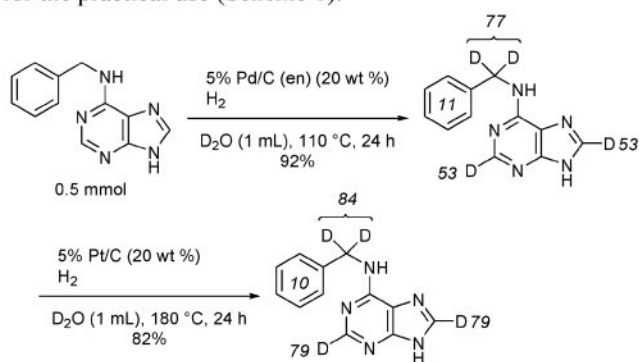
| entry | catalyst    | temp. (°C) | D content (%) <sup>a</sup> |    |                  |                  | yield (%) |
|-------|-------------|------------|----------------------------|----|------------------|------------------|-----------|
|       |             |            | Ph                         | Bn | 2-D <sup>b</sup> | 8-D <sup>b</sup> |           |
| 1     | 10% Pd/C    | rt         | 21                         | 27 | 0                | 0                | 42        |
| 2     | 10% Pd/C    | 110        | debenzylation              |    |                  |                  | 0         |
| 3     | 5% Pt/C     | rt         | 0                          | 20 | 18               | 18               | 86        |
| 4     | 5% Pt/C     | 110        | 9                          | 33 | 37               | 37               | 60        |
| 5     | 5% Pt/C     | 180        | 9                          | 28 | 54               | 54               | 29        |
| 6     | 5% Pd/C(en) | 110        | 11                         | 73 | 53               | 53               | 92        |

<sup>a</sup> Determined by <sup>1</sup>H NMR.

<sup>b</sup> <sup>1</sup>H NMR signals for the positions of 2 and 8 positions were not assigned and the numbers shown were averaged.

of benzylamino moiety was tolerated at room temperature by the replacement of the catalyst with 5% Pt/C (entry 3). Raising the temperature improved the deuterium efficiency although the partial debenzoylation was accompanied (entries 4 and 5).

We previously developed a Pd/C-ethylenediamine complex [Pd/C(en)], which possesses no catalytic activity toward the hydrogenation of various reducible functionalities including benzyl ethers and benzyl alcohols.<sup>8,9</sup> The Pd/C(en) was then employed as the catalyst. As the result, the *N*<sup>6</sup>-debenzoylation was efficiently suppressed during the deuteration of benzyladenine at 110 °C with moderate deuterium contents (entry 6). The obtained deuterated benzyladenine was again stirred in D<sub>2</sub>O at 180 °C together with 5% Pt/C under H<sub>2</sub> atmosphere. The deuterium efficiency was slightly improved, but not enough for the practical use (Scheme 1).



**Scheme 1** Stepwise deuteration of *N*<sup>6</sup>-benzyladenine

We next examined the combined use of 5% Pd/C(en) and 5% Pt/C (Table 2, entries 1 and 2). The deuterium efficiency was improved at 110 °C compared with single use of either 5% Pt/C or 5% Pd/C(en) (Table 2, entry 1 vs. Table 1, entries 4 and 6, respectively). Increase of the reaction temperature to 180 °C remarkably promoted the

**Table 2** Pd/C(en)-catalyzed H–D exchange reaction of *N*<sup>6</sup>-benzyladenine

| entry          | catalyst              | temp. (°C) | D content (%) <sup>a</sup> |    |                  |                  | yield (%) |
|----------------|-----------------------|------------|----------------------------|----|------------------|------------------|-----------|
|                |                       |            | Ph                         | Bn | 2-D <sup>b</sup> | 8-D <sup>b</sup> |           |
| 1              | 5% Pd/C(en) + 5% Pt/C | 110        | 10                         | 71 | 75               | 75               | 82        |
| 2              | 5% Pd/C(en) + 5% Pt/C | 180        | 20                         | 96 | 88               | 88               | 70        |
| 3 <sup>c</sup> | 5% Pd/C(en)           | 180        | 17                         | 97 | 94               | 94               | 47        |

<sup>a</sup> Determined by <sup>1</sup>H NMR.

<sup>b</sup> <sup>1</sup>H NMR signals for the positions of 2 and 8 positions were not assigned and the numbers shown were averaged.

<sup>c</sup> 30% of the substrate weight of 5% Pd/C(en) were used.

H–D exchange reaction (Table 2, entry 2). We finally found that the independent use of 5% Pd/C(en) (30% of the substrate weight) at 180 °C led to a good formation of highly deuterated *N*<sup>6</sup>-benzyladenine (entry 3).

The deuterated benzyladenine which possesses five deuterium atoms within the molecule was successfully used for the development of new analytical method of residual benzyladenine in the crops. A 1:1 mixed solution of deuterated and non-deuterated benzyladenines in MeOH was sprayed on the surface of fifteen crops including cabbage, onion, rice, potato, banana, and lemon. The agrochemicals were extracted from crops with acetonitrile, and their quantities in the extracts were measured using LC/MS/MS without further purification. It was found that the agrochemicals could be detected within the concentration of 0.25–0.50 ng/g (crops) and quantitatively measured.

## CONCLUSION

We have developed an efficient method to satisfactorily deuterate *N*<sup>6</sup>-benzyladenine with minimal removal of *N*<sup>6</sup>-benzyl group using the Pd/C(en)-D<sub>2</sub>O-H<sub>2</sub> system. One of the most distinctive features of the method is no use of deuterium gas as a deuterium source. The novel analytical method to quantify the residual benzyladenine in crops has also been developed using deuterated benzyladenine as a surrogate by means of LC/MS/MS.

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