

# Synthesis of deuterium-labelled isotopomer of deferasirox

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A d<sub>4</sub>-labeled isotopomer of deferasirox was synthesized as internal standard for use in a LC/mass spectroscopy (MS)/MS method developed for the simultaneous quantitative determination of deferasirox in human serum. d<sub>4</sub>-deferasirox was synthesized from d<sub>8</sub>-toluene.

**Keywords:** deferasirox; deuterium

## Introduction

Deferasirox, marketed as Exjade,<sup>1</sup> is a rationally designed<sup>2</sup> oral iron chelator. Its main use is to reduce chronic iron overload in patients who are receiving long-term blood transfusions for conditions such as beta-thalassemia and other chronic anemias.<sup>2,3</sup> It is the first oral medication approved in the USA for this purpose.<sup>4</sup> Two molecules of deferasirox are capable of binding to one atom of iron that is subsequently eliminated by fecal excretion. Its low molecular weight and high lipophilicity allow the drug to be taken orally. There are many properties of deferasirox that make it an attractive agent for use in other oxidative-stress-related diseases.

In a project aiming at the development of a bioanalytical method based on high-performance liquid chromatography (LC) with electrospray tandem mass spectrometry (MS/MS) for the simultaneous quantitative determination of deferasirox in human serum, deuterium-labeled deferasirox was required. While stable isotope-labeled compounds exhibit the same stability, extraction, and chromatographic behavior as their nonlabeled counterpart, their different molecular weights make them distinguishable in LC/MS/MS from the nonlabeled counterpart. Therefore, isotopically labeled compounds are ideal internal standards in LC/MS/MS assays used for the quantitation of drugs in biological matrices.

## Results and discussion

The benzoic acid site of deferasirox appears to be appropriate for the labeling with deuterium because of the ready availability of toluene-d<sub>8</sub> (Schemes 1–3).

4-Nitrotoluene-d<sub>7</sub> (**5**) was obtained in good yields by nitration of toluene-d<sub>8</sub> (**4**) in the presence of zeolite catalyst ZS-5 and nitric acid. The oxidation of 4-nitrotoluene-d<sub>7</sub> (**5**) was carried out using potassium permanganate to afford 4-nitrobenzoic acid-d<sub>4</sub>. 4-Nitrobenzoic acid-d<sub>4</sub> (**6**) was reduced to 4-aminobenzoic acid-d<sub>4</sub> (**7**) by *in situ* generated hydrogen gas from ammonium formate in the presence of palladium catalyst. The reaction was carried out under microwave irradiation. 4-Aminobenzoic acid-d<sub>4</sub> (**7**) was converted to its hydrazine via diazotization and simultaneous reduction by stannous chloride to afford 4-hydrazinebenzoic acid-d<sub>4</sub> (**8**). 4-Hydrazinebenzoic

acid-d<sub>4</sub> (**8**) was condensed with 2-(2-hydroxyphenyl)-4H-1,3-benzoxazin-4-one (**3**) under microwave irradiation to afford deferasirox-d<sub>4</sub> (**9**) having isotopic purity of 99%.

## Experimental procedures

### Materials and methods

The analytical HPLC verification was carried out on an Agilent HPLC system (Make: 1290 infinity). UV detection was at 242 nm. The mobile phase consisted of A (water with 0.1% trifluoroacetic acid (TFA)), B (acetonitrile with 0.1% TFA), gradient: 0–5 min 0%, B, 5–30 min 0–50% B, 30–30.1 min 50–100% B, and hold to 40 min. Precoated thin-layer chromatography (TLC) sheets (silica gel 60F254) were obtained from Merck. Toluene-d<sub>8</sub> was purchased from Alfa Aesar Chemical. All other reagents were purchased from Sigma-Aldrich.

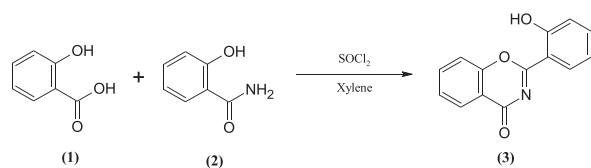
### Preparation of 2-(2-hydroxyphenyl)-4H-1,3-benzoxazin-4-one (**3**)

Xylene (15 ml) and salicylic acid (**1**; 10 g, 0.072 mol) were added in a round-bottom flask equipped with a mechanical stirrer and thermocouple. Thionyl chloride (8.7 g, 0.073 mol) was added at 10–15°C. After addition of thionyl chloride, the reaction mixture was stirred at 10–15°C for 30 min. The reaction mixture was heated at 35–40°C for 1 h. A solution of salicylamide (**2**; 10 g, 0.072 mol) in xylene (20 ml) was added to the reaction mixture at 25–30°C. After addition, the reaction mixture was gradually heated at 120°C and stirred for 2 h. After completion of the reaction, excess of xylene was distilled out. Methanol (20 ml) was added to the reaction mixture at 70–80°C and stirred for 1 h. The reaction mixture was gradually cooled to 25–30°C. The solid was collected by filtration and washed with methanol then dried under vacuum at 55–60°C to give 2-(2-hydroxyphenyl)-4H-1,3-benzoxazin-4-one (**3**) as a white powder (16.5 g, 95%). m.p. 239°C. ESI MS *m/z* [M + H]<sup>+</sup> 240.0.

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**Scheme 1.** Preparation of 2-(2-hydroxyphenyl)-4H-1,3-benzoxazin-4-one.

### Preparation of 4-nitrotoluene-d<sub>7</sub> (5)

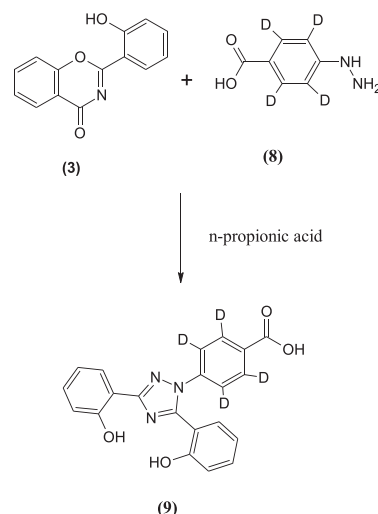
Toluene-d<sub>8</sub> (**4**; 20 g, 0.2 mol) was added to a round-bottom flask equipped with a magnetic stirrer cooled in an ice bath. A precooled mixture of 65% HNO<sub>3</sub> (20 ml) and 98% H<sub>2</sub>SO<sub>4</sub> (20 ml) was added dropwise over 40 min to the vigorously stirred mixture at 5–10°C. The mixture was stirred for 3 h at 14°C. Stirring was continued for an additional hour at 25°C. The mixture was poured onto ice water (300 ml) and extracted for three times with ether (50 ml). The combined organic layers were successively washed with a solution of 5% NaHCO<sub>3</sub> (50 ml) and twice with water (50 ml). Removal of the solvent under vacuum yielded an oil. The crude oil was crystallized with chilled *n*-hexane to obtain light-yellow-colored solid of 4-nitrotoluene-d<sub>7</sub> (**5**; 12.6 g, 42%). m.p. 49–51°C. ESI MS *m/z* [M + H]<sup>+</sup> 145.09.

### Preparation of 4-nitrobenzoic acid-d<sub>4</sub> (6)

A solution was prepared by dissolving potassium permanganate (29 g, 0.18 mol) in boiling water (150 ml). Water (70 ml), *p*-nitrotoluene-d<sub>4</sub> (**5**; 14.4 g, 0.1 mol), and magnesium sulfate heptahydrate (29 g) were added to a round-bottom glass flask equipped with a magnetic stirring bar and reflux condenser, and the mixture was stirred vigorously and heated at 85°C. To the stirred reaction mixture, the solution of potassium permanganate was added slowly over 30 min at 85°C. The reaction mixture was maintained at 85°C for 2 h. The mixture was allowed to cool down to 25–30°C; the brown precipitate formed was filtered off. Ethanol (20 ml) was added to the filtrate and the mixture again heated to 85–90°C for 45 min. The reaction mixture was cooled to 10°C, and pH was adjusted to 3–4 using 20% H<sub>2</sub>SO<sub>4</sub> (200 ml). The mixture was stirred for 1 h at 10°C and filtered. The solid was washed with water (4 ml) and dried in a desiccator affording pale-yellow-colored solid of 4-nitrobenzoic acid-d<sub>4</sub> (**6**; 10 g, 58%). m.p. 237°C.

### Preparation of *p*-aminobenzoic acid-d<sub>4</sub> (7)

The 4-nitrobenzoic acid-d<sub>4</sub> (**6**; 10 g, 0.06 mol) and methanol (100 ml) were added to a round-bottom flask equipped with a magnetic stirring bar and reflux condenser. The flask was placed in a CEM Discover™ synthesizer. Ammonium formate (10 g, 0.16 mol) and 10% Pd/C, 50% water wet (1 g), were added to the solution. The reaction mixture was exposed to microwave irradiation at temperature of 90–100°C at an initial power of 120 W for 20 min. The progress of the reaction was monitored

**Scheme 3.** Preparation of Deferasirox-d<sub>4</sub>.

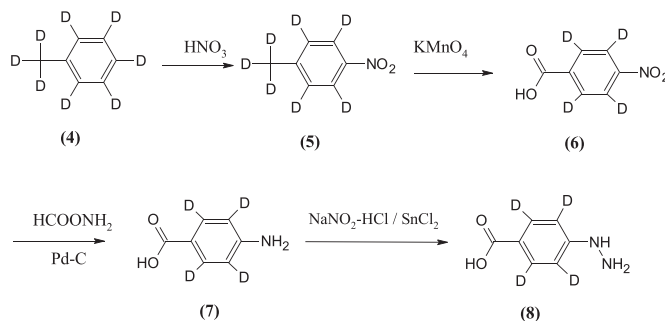
using TLC. After completion of reaction, the Pd/C was filtered off by using Celite, and the solvent was removed under vacuum and dried in a desiccator affording a gray-white solid of *p*-aminobenzoic acid-d<sub>4</sub> (**7**; 7.8 g, 95%). m.p. 187–189°C. ESI MS *m/z* [M + H]<sup>+</sup> 142.13.

### Preparation of 4-hydrazinobenzoic acid-d<sub>4</sub> (8)

*p*-Aminobenzoic acid-d<sub>4</sub> (**7**; 6 g, 0.04 mol), 30.0 ml water, and 30.0 ml concentrated HCl were added to a round-bottom glass flask equipped with a magnetic stirring bar cooled by an ice bath. A 25.0-ml, 20% aqueous NaNO<sub>2</sub> solution was added maintaining temperature at 0–10°C, and the resulting mixture was stirred for 0.5 h. A mixture of SnCl<sub>2</sub>·2H<sub>2</sub>O (20.0 g, 0.105 mmol) and concentrated HCl (40 ml) was then added to the reaction mixture maintaining temperature at 0–10°C, followed by stirring in the ice bath for 0.5 h. The solid was collected by filtration and dried to give 4-hydrazinobenzoic acid-d<sub>4</sub> (**8**) as a white powder (7.8 g, 98%). m.p. 220°C. ESI MS *m/z* [M + H]<sup>+</sup> 157.15.

### Preparation of 4-[3,5-bis(2-hydroxyphenyl)-1H-1,2,4-triazol-1-yl]-[2,3,5,6-<sup>2</sup>H<sub>4</sub>] benzoic acid (deferasirox d<sub>4</sub>; 10)

*n*-Propionic acid (30 ml) was added to a round-bottom glass flask equipped with a magnetic stirring bar and reflux condenser. The flask was placed in a CEM Discover™ synthesizer. 2-(2-Hydroxyphenyl)-4H-1,3-benzoxazin-4-one (**3**; 6 g, 0.39 mol) and 4-hydrazinobenzoic acid (**5**; 4.5 g, 0.3 mol) were added to the reaction mixture and exposed to microwave irradiation at a temperature of 100°C at an initial power 120 W for 60 min. The progress of the reaction was monitored using

**Scheme 2.** Preparation of 4-hydrazinobenzoic acid-d<sub>4</sub>.

TLC. After completion of reaction, ethyl acetate (40 ml) was added to the reaction mixture, and the suspension was stirred for 30 min maintaining the temperature at 15–20°C. The solid was collected by filtration and dried to give 4-[3,5-bis(2-hydroxyphenyl)-1*H*-1,2,4-triazol-1-yl]-[2,3,5,6-<sup>2</sup>H<sub>4</sub>]benzoic acid, deferasirox d<sub>4</sub>, (**10**; 8.25 g, 98%). HPLC purity 99.4%. Isotopic purity 99%. m.p. 260°C. ESI MS *m/z* [M + H]<sup>+</sup> 378.1. <sup>1</sup>H-NMR (400 MHz, d<sub>6</sub>-dimethyl sulfoxide) δ: 13.02 (br, s, COOH), 10.05 (br, s, -OH), 8.04 (dd, 1H, Ar-H), 7.53–7.51 (dd, 1H, Ar-H), 7.39–7.32 (m, 2H, Ar-H), and 6.99–6.84 (m, 4H, Ar-H).

## Conclusion

We have developed a simple and efficient process for the preparation of deuterium-labeled deferasirox. The main advantages of these procedures are excellent chemical and isotopic purity, highly reproducible yields, facile processing, and low cost.

## Acknowledgements

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## Conflict of Interest

The authors did not report any conflict of interest.

## References

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