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Synthesis of stable isotope labelled internal standards for drug–drug interaction (DDI) studies

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In memory of Mireille Denoux

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ABSTRACT

The syntheses of stable isotope labelled internal standards of important CYP-isoform selective probes, like testosterone **1**, diclofenac **3**, midazolam **5**, and dextromethorphan **7**, as well as their corresponding hydroxylated metabolites 6β-hydroxytestosterone **2**, 4′-hydroxydiclofenac **4**, 1′-hydroxymidazolam **6** and dextrorphan **8** are reported. Microwave-enhanced H/D-exchange reactions applying either acid, base, or homogeneous and heterogeneous transition metal catalysis, or combinations thereof proved to be highly efficient for direct deuterium labelling of the above mentioned probes. Compared to conventional stepwise synthetic approaches, the combination of H/D exchange and biotransformation provides the potential for considerable time- and cost savings, in particular for the synthesis of the stable isotope labelled internal standards of 4′-hydroxydiclofenac **4** and 1′-hydroxymidazolam **6**.

1. Introduction

Drug–drug interactions can significantly influence the therapeutic effect of drugs and thus may also increase the risk of adverse effects. Consequently, in the course of pharmaceutical development a number of drug–drug-interaction (DDI) studies are performed in order to investigate and understand the DDI potential of new drug candidates.[1] Particular attention is paid to the cytochrome P450 (CYP) enzyme family because certain subclasses, most notably CYP1, CYP2, and CYP3, predominate in human drug biotransformation.[2] For each of these cytochrome P450 subtypes, specific isoform-selective substrates are now accepted as standards,[3] whose metabolism serves as a measure for DDI investigations of new drug candidates (Fig. 1).[4]

Typically, the potential of a new chemical entity to inhibit or to induce the formation of a specific metabolite from these benchmark substances is evaluated. Generally concentrations of both the probe compound and the metabolite formed are determined by LC–MS/MS methods.[5] In order to avoid the effect of ion-suppression and hence to facilitate quantification, Obach et al.[6] reported a validated method of CYP inhibition screening by using stable isotope labelled versions of each CYP probe and its major metabolite as internal standards. Usually, internal standards can be prepared with very high isotope abundance starting from commercially available labelled precursors by conventional synthesis. However, for stable labelled testosterone **1**,[7] diclofenac **3**,[8] midazolam **5**,[9] and dextromethorphan **7**,[10] and their respective CYP subtype-specific metabolites 6β-hydroxytestosterone **2**,[11] 4′-hydroxydiclofenac **4**,[12] 1′-hydroxymidazolam **6**,[9] and dextrorphan **8**,[10] only time consuming, low yielding multi-step syntheses have been reported so far. Fortunately, improved methods of H/D exchange which have evolved during the last ten years provided a possibility of circumventing long and expensive conventional linear syntheses, since they can be carried out directly on the target molecule or an advanced intermediate.[13] In spite of recent methodological improvements,[14] H/D exchange still often leads either to insufficient deuterium incorporation, results in a very broad isotope cluster, or leads to decomposition of the compound.[15] The drastic reaction conditions of existing H/D exchange methods often require balancing high deuterium incorporations with substrate decomposition and consequently a very careful optimization of the reaction conditions is needed for a given substrate. In this paper we report recent efforts aimed at developing optimized conditions for H/D-exchange labelling of the above mentioned metabolites. In combination with biocatalytic methods for direct selective hydroxylation, this method proved to be a highly efficient

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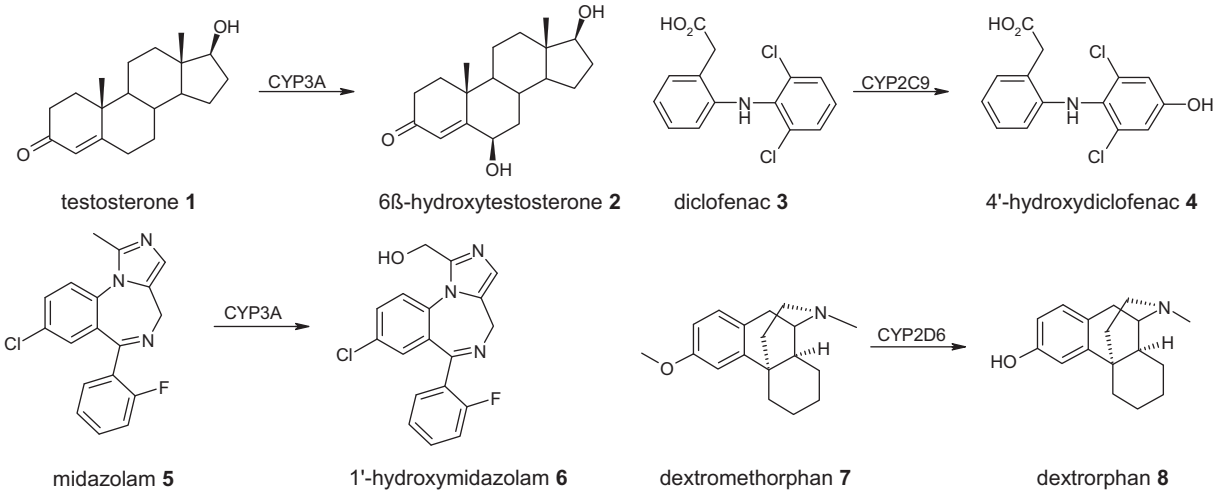


Figure 1. CYP450 substrates and their corresponding metabolites.

and inexpensive approach for the preparation of stable labelled internal standards of key CYP450 probes for in vitro DDI investigations.

2. Results and discussion

The testosterone-6β-hydroxylase assay is a very well established marker for the determination of CYP3A activity.[6,16] For stable isotope introduction into the steroid skeleton of **1** or other steroidal analogues, in addition to a classical stereoselective total synthesis, semi-synthetic approaches applying H/D-exchange have also been reported.[7,17] For the latter, appropriate steroidal precursors such as 4-androsten-3,17-dione, 1,4-androsta-dien-3,17-dione or 5-androsten-3-ol-17-one were labelled by applying conventional base-catalyzed H/D-exchange and the products subsequently subjected to further chemical transformation to give labelled **1**.[11,18]

Our screening revealed that two different H/D-exchange methods are applicable, both starting directly from commercially available testosterone **1**. A base-catalysed H/D-exchange (method A) using aq NaOD under conventional heating conditions led to complete exchange of the five enolizable hydrogen atoms adjacent to the α,β-unsaturated carbonyl group within 3 h.[19] This H/D-exchange reaction could be even further accelerated under microwave heating conditions. In a sealed tube at a temperature of 120 °C five deuterium atoms were incorporated with only one minute of reaction time (Scheme 1). The reaction proceeded without the formation of any by-products and pure testosterone-[D_5] **1a** was isolated in quantitative yield.

The second method (method B) applied to the labelling of testosterone was a heterogeneous Pd-catalysed H/D-exchange with an $NaBD_4$-activated catalyst.[20] The reaction was performed in a sealed tube in the microwave slightly above the melting point of testosterone (155 °C)[21] at 160 °C (Scheme 1). At lower temperatures (130–140 °C) an insufficient incorporation of deuterium was observed, most likely due to the low solubility of testosterone in D_2O. Alternatively, with THF-d_8 as co-solvent a comparable deuterium uptake was reached at 140 °C. This heterogeneous H/D-exchange afforded deuterium labelled testosterone as a mixture of isotopologues ranging from M+2 to M+12 with the M+6 isotopologue being the most abundant (Fig. 2).

Similar results for both the base and heterogeneous palladium-catalysed H/D-exchange reaction were obtained with other steroids, for example, androstendione or androstadiene. However,

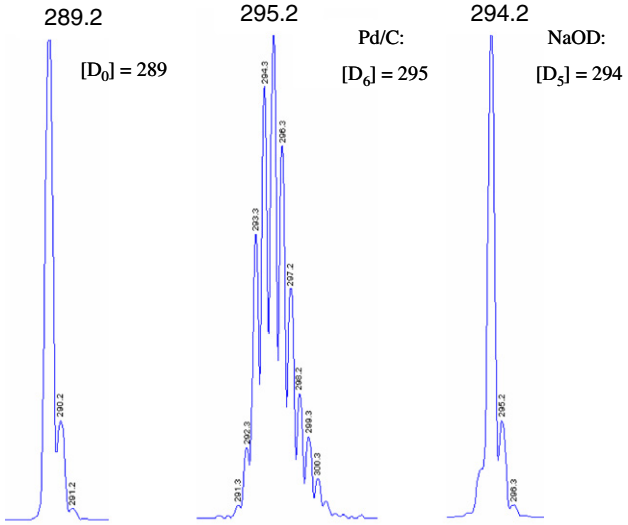
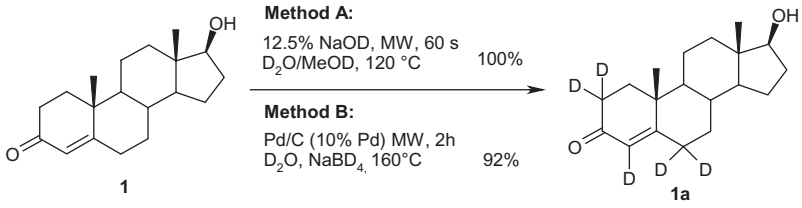


Figure 2. MS molecular ion cluster of testosterone **1** and deuterated testosterone **1a**.



Scheme 1. H/D-exchange of testosterone **1**.

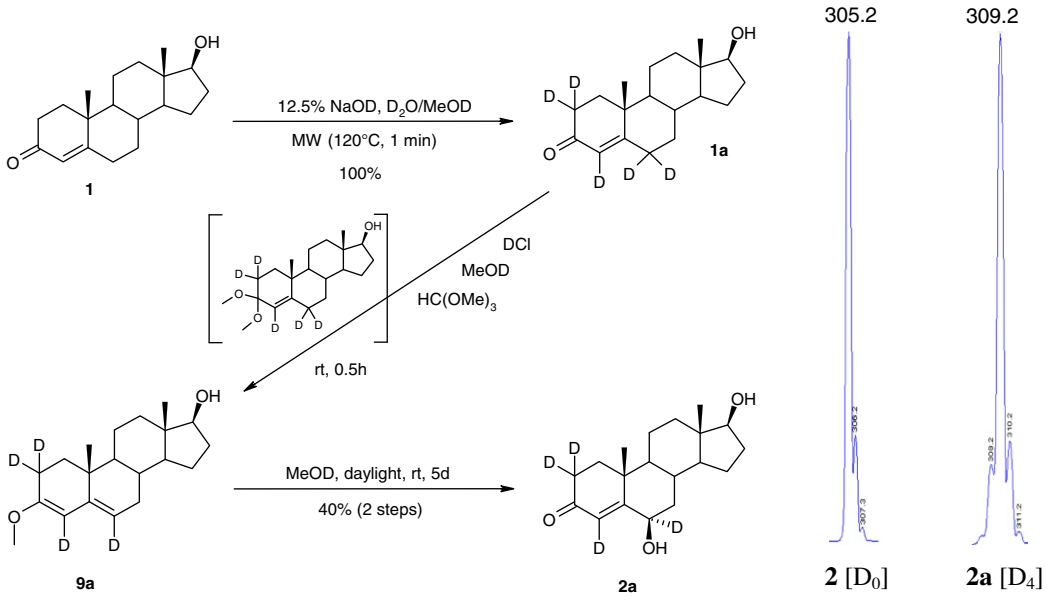


Figure 3. Synthesis of 6β-hydroxytestosterone-[D_4] **2a** and MS molecular ion cluster of 6β-hydroxytestosterone **2** and 6β-hydroxytestosterone-[D_4] **2a**.

deuterium labelling of 6β-hydroxytestosterone **2** using either of these two methods resulted in almost no deuterium incorporation and high levels of decomposition of the steroid. Therefore, we decided to perform a conventional synthesis of the 6β-hydroxy metabolite starting from testosterone **1**. (See fig 3)

Testosterone-[2,2,4,6,6-D_5] **1a** was enolized under standard conditions using deuterated reagents to prevent acid-catalyzed back exchange (Fig. 3). Subsequently, the deuterated, crude 3,5-dien-3-ol methyl ether **9a** was subjected to an autoxidition reaction,[22,23] which afforded the 6α- and 6β-hydroxytestosterone in a ratio of 1:10 with little by-product formation. The autoxidation was carried out by exposing the methanolic reaction mixture to bright daylight. In the dark, the reaction proceeded very slowly, while irradiation with a 150 W UV-lamp resulted in formation of a large number of by-products. Bubbling molecular oxygen through the reaction mixture during the autoxidation improved neither the speed nor the β-stereoselectivity of the oxidation. The influence of the temperature on the autoxidation process was not investigated. Finally, the two epimers were purified and separated from each other by means of reversed phase HPLC yielding the desired 6β-hydroxytestosterone-[2,2,4,6α-D_4] **2a** (Fig. 3) in an overall yield of 40%.

The diclofenac-4′-hydroxylase assay has been developed for measurement of CYP2C9 activity.[6,24] Due to the presence of two chlorine atoms, at least a five mass unit difference is required in order to use a stable labelled version of **3** as an internal MS standard. The synthesis of stable isotope labelled diclofenac **3a** reported here uses a combination of acid-catalysed and homogeneous metal-catalysed H/D-exchange reactions, resulting in a stepwise introduction of seven deuterium atoms into the molecule.

In the first step of the reaction sequence, concentrated deuterium chloride served as deuterium source and induced conversion of non-labelled diclofenac **3** to the indolone derivative **10a**. The product was subjected twice to these conditions in order to increase deuterium introduction. Thus, about four D-atoms were introduced in the positions indicated in Figure 4. The subsequent

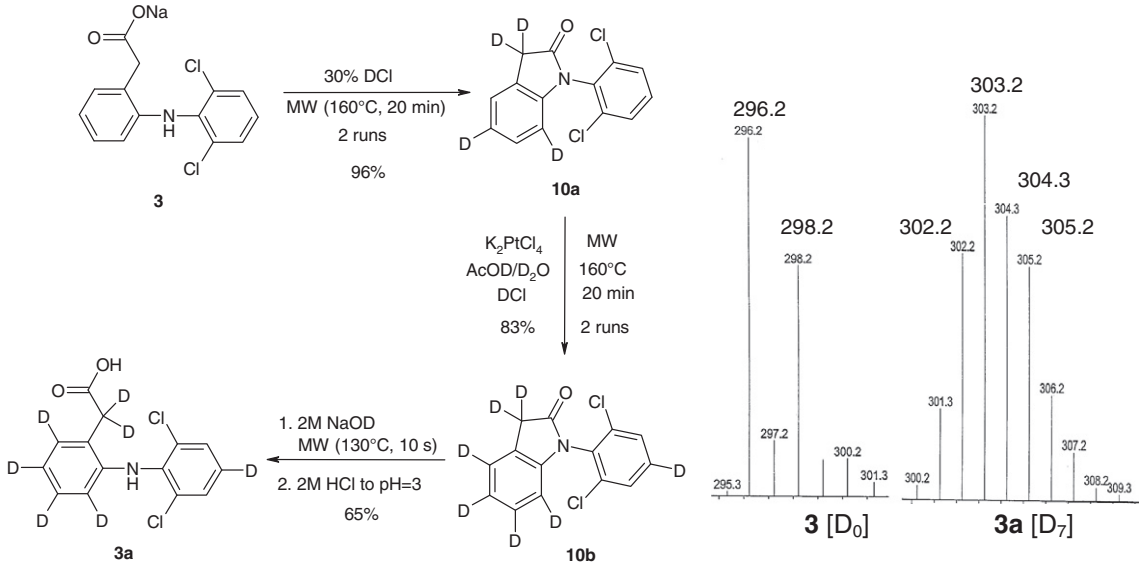


Figure 4. Microwave-assisted synthesis of diclofenac-[D_7] **3a** and MS molecular ion cluster of the diclofenac **3** and diclofenac-[D_7] **3a**.

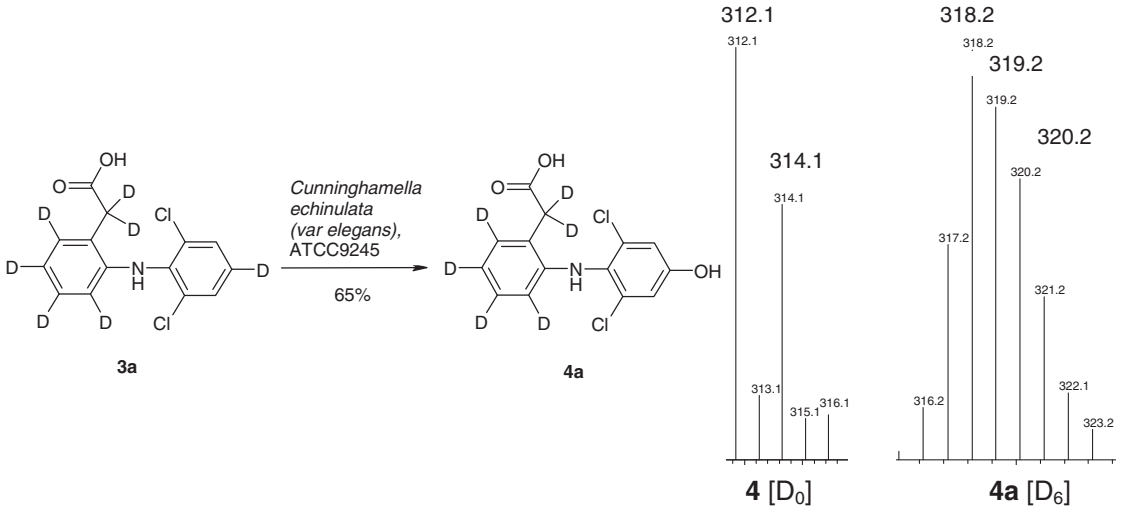


Figure 5. Biocatalytical preparation 4′-hydroxydiclofenac-[D_6] **4a** and MS molecular ion cluster of the 4′-hydroxydiclofenac **4** and 4′-hydroxydiclofenac-[D_6] **4a**.

platinum-catalysed H/D-exchange, which was also run twice, introduced another three deuterium atoms to give the indolone **10b**. Changing the order of the two H/D-exchanges resulted in an almost identical isotope pattern as depicted in Figure 4. Finally, ring opening was achieved under basic conditions. All three synthesis steps were performed under microwave irradiation, providing diclofenac-[D_7] **3a** in an overall yield of 51%, a considerable gain in efficiency compared to reported syntheses.[8] NMR and MS analysis clearly showed that the final compound **3a** was obtained as a mixture of isotopologues with the [D_7]-isotopologue being the most abundant.

Unfortunately, the application of similar H/D exchange conditions or variations thereof to the deuteration of 4′-hydroxydiclofenac **4** failed due to decomposition or insufficient deuterium incorporation into the molecule. Therefore, we considered a direct hydroxylation starting from **3a** as an alternative approach for the preparation of deuterated **4**. The direct bioconversion[25] of unlabelled **3** to the corresponding hydroxyl metabolite **4** has been published already.[26] However, the interesting question was whether an isotope effect would be observed during metabolisation, the C–D bond present in **3a** at the site of metabolic oxidation being 1.2–1.5 kcal/mol more stable than the C–H bond.[27] The exploitation of the kinetic isotope effect in the development of 'deuterated drugs' and related potential beneficial effects such as enhanced efficacy, improved safety profile and reduced levels of toxic metabolites have been intensively discussed.[28]

In a short screening of our microbial strain collection, *Cunninghamella echinulata (var elegans)*, ATCC9245 catalyzed both the direct hydroxylation of **3** and **3a** without major differences in the product spectrum and the expected isotope pattern for deuterated **4a**. As expected the conversion of deuterated diclofenac **3a** to its hydroxylated derivative **4a** was slightly lower than the biotransformation of unlabelled **3**. However, the same yield of 65% was obtained for both substrates, with the limiting factor being a difficult extraction/purification from the brew (Fig. 5). Compared to published methods for the synthesis of stable labeled standards of **4**[12] the combination of H/D exchange and microbial biotransformation proved to be highly efficient and 4′-hydroxydiclofenac-[D_6] **4a** was obtained in only four steps from **3**, up to a scale of several hundreds of milligrams.

A midazolam 1′-hydroxylase enzyme assay has been validated as an analytical method for CYP3A activity determination.[6] Both midazolam **5** and its major metabolite 1′-hydroxymidazolam **6** are therefore needed as stable isotope labelled compounds, for use as internal standards during these assays. Despite its long-standing application as an active pharmaceutical ingredient, literature on the synthesis of midazolam and its derivatives is rather sparse and mainly based on variants of the original synthesis from Walser et al.[29,30] In addition, the access to stable isotope labelled midazolam has been reported, but the route described is long and low yielding.[9] The chemical synthesis of 1′-hydroxymidazolam is described in the literature; however some discussion around the reproducibility of the procedure has been noted.[31]

Access to 1-hyrdoxymidazolam **6** from midazolam using biotransformation has been described by several groups.[32,33] A recent publication by Roy et al. underlines the preparative character of this biocatalytic approach.[34] Our strategy for preparing stable isotope labelled 1-hydroxymidazolam **6b** was therefore based on combining the H/D-exchange protocol with oxidative biotransformation to introduce the hydroxy group. In the first step we needed to find conditions under which a sufficient number of hydrogen atoms could be exchanged for deuterium, so as to reduce signal overlap in the mass spectrum. H/D-Exchange on commercially available midazolam **5** was our first choice to access the labelled drug.

Unfortunately, for the synthesis of stable labelled midazolam **5a**, none of our standard microwave enhanced H/D-exchange reactions gave sufficient deuterium incorporation. Heterogeneous catalysis using Pd/C activated by $NaBD_4$ led to good deuterium incorporation but with the chlorine atom being concomitantly lost. Little or no exchange was observed under homogeneous Garnett conditions[35] using K_2PtCl_4 as the catalyst with microwave heating at 160 °C. Furthermore, use of $RhCl_3$ as catalyst led to almost complete reduction of the imine double bond, a side reaction which could not be suppressed even by stirring the pre-activated catalyst for 30 min prior to substrate addition to remove excess D_2.

However, Pt/C proved to be efficient for the exchange of hydrogen atoms of **5**. Between four and up to 13 deuterium atoms could be incorporated into midazolam **5** using 5 mol % Pt/C catalyst (5% Pt content), pre-activated with $NaBD_4$, for 6 h at 160 °C in the microwave (Fig. 6). The isotopic distribution turned out to be Gaussian, as expected, the most abundant isotopologue being [D_8] (Fig. 6). Again, after catalyst activation stirring under argon was necessary prior to midazolam addition in order to prevent reduction as a side reaction. The crude reaction mixture was filtered and purified by column chromatography to afford

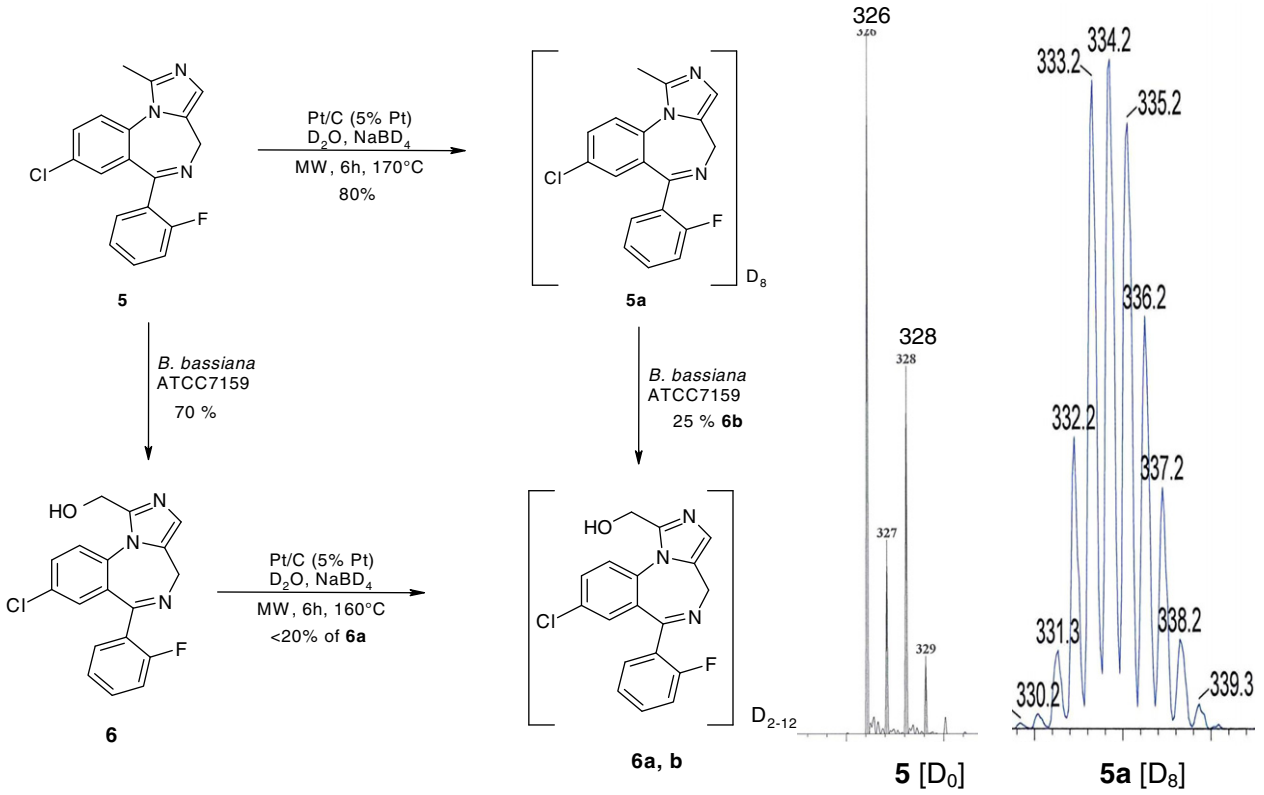


Figure 6. Transformation of midazolam **5** into midazolam-[D_8] **5a** and 1-hydroxymidazolam **6** and labelled **6a**, **b** and MS molecular ion cluster and deuterium distribution of midazolam **5** and [D_8]-midazolam **5a**.

midazolam-[D_8] **5a** in >80% yield. A detailed NMR analysis revealed the exchanged positions (Fig. 7) and indicated the preference for exchange of the aliphatic protons in position 4 of the benzo[e]azulene ring.

Having found satisfactory conditions for deuterium incorporation on midazolam **5**, we applied the Pt/C-system to its metabolite, 1-hydroxymidazolam **6**. The latter was obtained by biotransformation using *Beauveria bassiana* (ATCC7159) in 70%

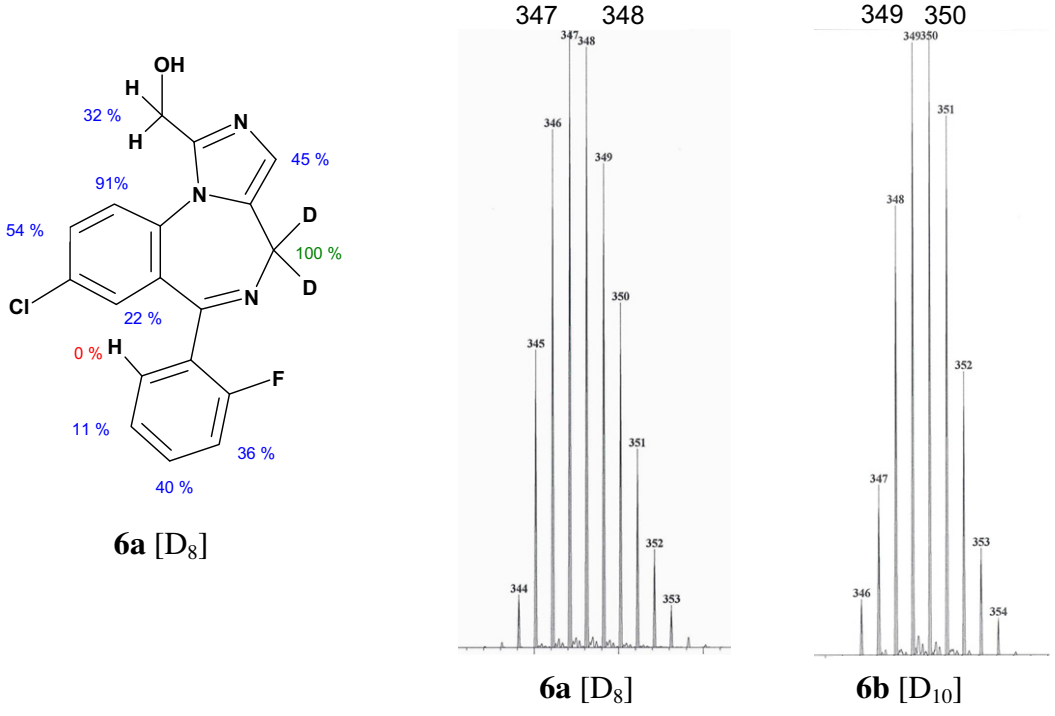


Figure 7. MS molecular ion cluster and deuterium distribution of 1-hydroxymidazolam **6a & 6b**.

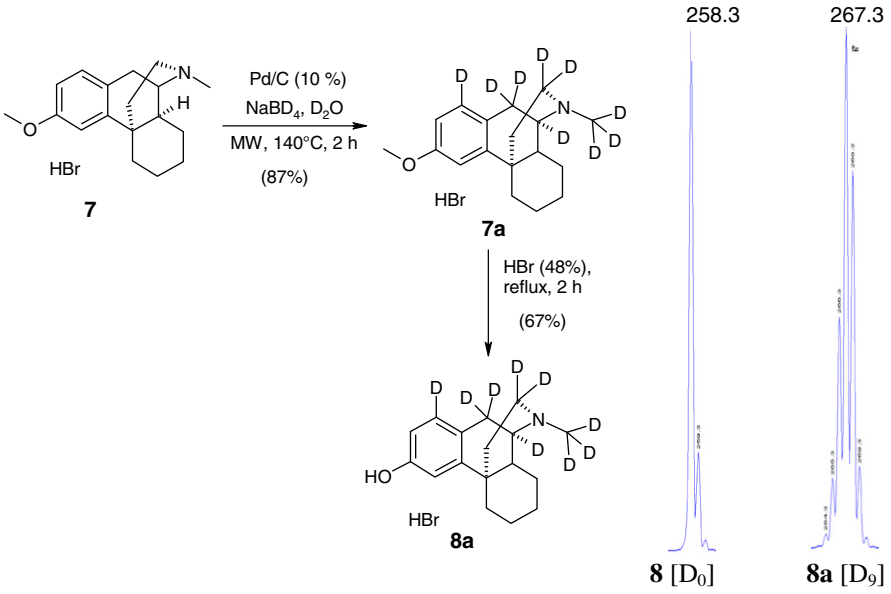


Figure 8. Microwave-assisted synthesis of dextromethorphan-[D_9] **7a** and dextrorphan-[D_9] **8a**.

yield. Unfortunately, 1-hydroxymidazolam **6** turned out to being rather temperature-sensitive. Even though the deuterium incorporation worked well above 160 °C, only a low (<20%) yield of **6b** could be isolated**.** The exchange reaction was accompanied by extensive degradation of 1-hydroxymidazolam, a serious drawback to this approach.

We therefore turned our attention towards the formation of isotopically labelled 1-hydroxymidazolam **6b** by biotransformation starting from the H/D-exchanged midazolam **5a**. Since all three methyl protons had been exchanged we anticipated some change in the outcome of the microbiological hydroxylation due the above mentioned kinetic isotope effects. In fact, instead of the 70% yield for the unlabelled midazolam **5**, the hydroxylation of labelled midazolam **5a** employing *B. bassiana* led to a reduced yield of 25% of **6b**. However, unreacted [D_8]-midazolam **5a** could be recovered from the fermentation reaction.

Nevertheless, both approaches led to sufficiently labelled 1-hydroxymidazolam to serve as internal standard for the interaction studies.

A dextrorphan **8** enzyme assay is one important method used to determine CYP2D6 activity[6] The stable isotope labelled dextrorphan **8a**, which is applied as internal standard for this assay, was synthesised in two reaction steps from readily available dextromethorphan **7** by a heterogeneous H/D exchange reaction and subsequent acidic cleavage (Fig. 8).

A suspension of hydrobromide **7** and $NaBD_4$-activated Pd/C catalyst in D_2O was heated in a sealed tube in the microwave at 140 °C for 2 h.[20] The H/D exchange occurred predominantly at the aliphatic positions and after crystallisation the deuterated dextrormethorphan was obtained in 87% yield as a mixture of isotopologues with the [D_{10}]-isotopologue being the most abundant. Finally the *O*-methyl group was removed by heating the deuterated intermediate in concd HBr. After purification, the dextrorphan-[D_9] hydrobromide **8a** was obtained in 67% yield.

3. Conclusion

We have developed highly efficient H/D exchange protocols for the synthesis of stable labelled internal standards of key CYP probes such as testosterone **1**, diclofenac **3**, midazolam **5** and dextromethorphan **7**, and their corresponding metabolites 6β-hydroxytestosterone **2**, 4′-hydroxydiclofenac **4**, 1′-hydroxymidazolam **6**, and dextrorphan **8**. Microbial biotransformations have been applied for selective oxy-functionalization of diclofenac-[D_6] **3a** and midazolam-[D_8] **5a** resulting in a more efficient process for the preparation of internal MS standards of 4′-hydroxydiclofenac **4**, 1′-hydroxymidazolam **6** compared to published methods.

4. Experimental

4.1. Synthesis

All reagents were of commercial quality and were used as received. Sodium diclofenac and D_2O (99% D) were purchased from ABCR, testosterone from Merck, midazolam hydrochloride from Apin, potassium tetrachloroplatinate (47% Pt), $NaBD_4$ (98% D), MeOD (99% D), and CH_3COOD (98% D), from Acros, NaOD (30% solution in D_2O, 99+% D), DCl (35% solution in D_2O, 99+% D), and DCl (4 M solution in 1,4-dioxane) from Sigma–Aldrich, and Pd/C (10% Pd) and Pt/C (5% Pt) from Heraeus. Reactions were monitored by TLC on aluminium sheets coated with silica gel containing fluorescence indicator (silica gel 60 F_{254}, from Merck KGaA) or by LC–MS (Agilent 1100 Series; column: Symmetry C18, 5 μm, 4.6 × 50 mm; mass detector: LC/MSD SL). Purifications by column chromatography were carried out on silica gel 60 (0.063–0.2 mm) from Merck KGaA with the described eluents. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX 500 (500 MHz) or on a Bruker Avance 600 (600 MHz) nuclear magnetic resonance spectrometer. ^{1}H NMR data were compared with literature data or checked against an authentic sample of the corresponding unlabelled compound. MS spectra were recorded on a Bruker Esquire 3000 or on a Shimadzu IT-TOF mass spectrometer. Microwave reactions were performed with the Initiator system from Biotage or Explorer system from CEM. Final HPLC controls were performed on either Waters Alliance 2695, Agilent 1200 Series or Dionex Summit HPLC systems, each equipped with DAD or variable wavelength UV detectors, respectively. The relative deuterium distributions are reported without consideration of 13C and 37Cl contributions in the calculation.

4.2. Cultivation of microorganisms

Screening of our microbial strain collection: Based on literature precedent and prior experience, 10–35 microorganisms were chosen among a variety of commercially available fungi and bacterial species and were incubated with (labelled or unlabelled) midazolam and diclofenac.

Here are listed the most commonly used fungi of our screening: Absidia cylindrospora MMP 1569; *Aspergillus niger* ATCC 9142; *Beauveria sulfurescens* ATCC 7159; *Cunninghamella baineiri* ATCC 9244; *Cunninghamella echinulata* NRRL 3655; *Mortierella isabellina* MMP 108; *Mucor circinelloides* CBS 108-16; *Rhizopus arrhizus* ATCC 11145; *Cunninghamella echinulata (var. elegans)* ATCC 9245; *Beauveria bassiana* DSM 875.

The most efficient strain was selected to be used for the preparative bioconversions.

Cunninghamella echinulata (var elegans) ATCC 9245 and Beauveria bassiana ATCC 7159 were maintained on agar slants at 4 °C. The medium for filamentous fungi cultures contained 10 g corn steep liquor (Solulys®, Roquette, Lestrem, France), 30 g D-(+)-glucose, 0.5 g $MgSO_4 \cdot 7H_2O$, 2 g $NaNO_3$, 0.02 g $FeSO_4 \cdot 7H_2O$, 0.5 g KCl, 1 g KH_2PO_4, 2 g K_2HPO_4 in 1 L of deionised water (final pH 6.5). Media were sterilized by autoclaving at 121 °C for 20 min. After inoculation with glycerol spore suspensions, the liquid media were incubated in conical flasks at 28 °C with orbital shaking (200 rpm) for 60–72 h.

4.2.1. Androst-4-en-17β-ol-3-one-[2,2,4,6,6-D_5] (testosterone-[D_5]) (1a)

In a 50-ml round-bottom flask 3.0 g (10.4 mmol) testosterone **1** were dissolved in 25 ml MeOD and the solvent was removed in vacuo. The pre deuterium-exchanged steroid was divided into four portions and each portion was dissolved in 12 ml MeOD/D_2O (5:1, v/v) in a Biotage microwave vial. Under argon, 3 ml NaOD (12.5% in D_2O) were added to each, and the vials tightly sealed and heated to 120 °C for 1 min under microwave irradiation. The basic solutions were neutralised with DCl (10% in D_2O) and combined. After evaporation of most of the MeOD, the aqueous phase was extracted twice with 25 ml of dichloromethane. The combined organic extracts were washed with 25 ml water, dried over anhydrous sodium sulphate and the solvent removed. Yield: 3.04 g (10.4 mmol, 100%) colourless solid, mp 154–155 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 4.44 (d, ^3J(H,H) = 4.8 Hz, 1H,), 3.43 (dt, ^3J(H,H) = 8.5 Hz, ^3J(H,H) = 4.9 Hz, 1H), 1.95 (d, ^2J(H,H) = 13.4 Hz, 1H), 1.83 (m, 1H), 1.77–1.74 (m, 2H), 1.57 (d, ^2J(H,H) = 13.2 Hz, 1H), 1.54–1.47 (m, 3H), 1.40–1.31 (m, 2H), 1.20 (dq, ^3J(H,H) = 5.9 Hz, ^2J(H,H) = 12.2 Hz, 1H), 1.14 (s, 3H), 0.97 (dt, ^3J(H,H) = 12.2 Hz, ^3J(H,H) = 4.1 Hz, 1H), 0.92–0.83 (m, 3H), 0.68 (s, 3H) ppm; Proton signals at δ = 5.62, 2.36, 2.21 and 2.12 ppm disappeared; ^{13}C NMR (125 MHz, DMSO-d_6) δ = 198.0, 170.9, 122.8 (t, ^1J(C,D) = 23.7 Hz), 79.8, 53.5, 50.0, 42.4, 38.1, 36.3, 35.1, 35.0, 32.9, 31.1, 29.8, 23.0, 20.1, 17.0, 11.2 ppm. TLC (DCM/MeOH 25:1 v/v): R_f = 0.59; LC–MS: R_t = 7.75 min; LC–MS ESI *m*/*z* (%): 316.2 $[M+Na]^+$ (18), 294.2 $[M+H]^+$ (100); Deuterium distribution (%): D_5 (80) D_4 (18), D_3 (2).

4.2.2. Androst-4-en-6β,17β-diol-3-one-[2,2,4,6α-D_4] (6β-hydroxytestosterone-[D_4]) (2a)

Under an argon atmosphere 0.2 ml (0.8 mmol) DCl (4 M solution in 1,4-dioxane) were added to a solution of 3.0 g (10.2 mmol) **1a** and 5 ml (45.6 mmol) trimethyl orthoformate dissolved in 25 ml dry 1,4-dioxane and 6 ml dry MeOD. The mixture was stirred for 30 min at room temperature and quenched by the dropwise addition of 0.1 ml pyridine. After adding 50 ml water the aqueous phase was extracted three times with 50 ml dichloromethane. The combined organic phases were dried over anhydrous sodium sulphate and the solvent removed under reduced pressure to give crude testosterone-[2,2,4,6-D_4]-3,5-dienol-3-methyl ether **9a**.

The crude dienol methyl ether **9a** was dissolved in 50 ml MeOD and stirred while irradiating with a lamp emitting artificial daylight for 5 days. After all the starting material had been consumed, the solvent was evaporated, the residue taken up in 50 ml MeOH and the solvent evaporated again. The crude product was purified by column chromatography (DCM/MeOH = 25:1, v/v) to afford the pure mixture of α- and β-epimers. Subsequently, the two epimers were separated by preparative HPLC in reversed phase mode (column: Phenomenex Luna C18, 10 μm, 250 × 50 mm, isocratic program, eluent: acetonitril/water = 24:76, flow: 110 ml/min, R_t (α) = 18.8 min, R_t(β) = 24.0 min). The combined HPLC fractions were concentrated to low volume and the product isolated by lyophilisation. Yield: 1.25 g (4.05 mmol, 40%) colourless, amorphous solid. A final HPLC control resulted in a purity of 99.8% by area. Conditions: column: Merck LiChrosphere 100 RP18e, 125 × 4 mm, 5 μm. Eluent: water/acetonitrile, 80:20 (v/v). Flow rate: 1 ml/min. UV detection wavelength: 240 nm. Injection volume: 10 μl. Sample solvent/concentration: 0.4 mg/ml in eluent. Column temperature: 20 °C. Sampler temperature: 5 °C. Retention time: 12.5 min; ^{1}H NMR (500 MHz, DMSO-d_6) δ = 5.03 (s, 1H), 4.44 (d, ^3J(H,H) = 4.8 Hz, 1H), 3.44 (dt, ^3J(H,H) = 8.5 Hz, ^3J(H,H) = 4.9 Hz, 1H), 1.93 (d, ^2J(H,H) = 13.2 Hz, 1H), 1.90–1.75 (m, 4H), 1.57 (d, ^2J(H,H) = 13.2 Hz, 1H), 1.55–1.48 (m, 2H), 1.43–1.33 (m, 2H), 1.29 (s, 3H), 1.18 (dq, ^3J(H,H) = 5.9 Hz, ^2J(H,H) = 12.2 Hz, 1H), 1.07 (t, ^2J(H,H) = 12.8 Hz, 1H), 0.97 (dt, ^3J(H,H) = 4.1 Hz, ^2J(H,H) = 12.2 Hz, 1H), 0.92–0.80 (m, 2H), 0.69 (s, 3H) ppm; Proton signals at δ = 5.65, 4.15, 2.43 and 2.18 ppm disappeared; ^{13}C NMR (125 MHz, DMSO-d_6) δ = 199.3, 169.0, 124.7 (t, ^1J(C,D) = 24.6 Hz), 79.9, 70.5 (t, ^1J(C,D) = 20.9 Hz), 53.4, 49.9, 42.5, 38.3, 37.5, 36.4, 36.3, 33.2, 29.8, 29.4, 23.0, 20.2, 19.9, 11.2 ppm. TLC (DCM/MeOH 25:1 v/v): R_f = 0.24. MS ESI *m*/*z* (%): 331.2 $[M+Na]^+$ (7), 309.2 $[M+H]^+$ (100); Deuterium distribution (%): D_4 (76) D_3 (19), D_2 (5).

4.2.3. Androst-4-en-6α,17β-diol-3-one-[2,2,4,6β-D_4] (6α-hydroxytestosterone-[D_4]) (2b)

Isolated as by-product from the synthesis of the β-epimer. Yield: 160 mg (0.52 mmol, 5%) colourless, amorphous solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 5.10 (s, 1H), 4.45 (d, ^3J(H,H) = 4.9 Hz, 1H), 3.44 (dt, ^3J(H,H) = 8.5 Hz, ^3J(H,H) = 4.9 Hz, 1H), 1.97–1.93 (m, 2H), 1.84 (m, 1H), 1.75 (dt, ^3J(H,H) = 3.0 Hz, ^2J(H,H) = 12.4 Hz, 1H,), 1.64–1.47 (m, 4H), 1.39–1.28 (m, 2H), 1.22 (dq, ^3J(H,H) = 5.8 Hz, ^2J(H,H) = 12.1 Hz), 1.12 (s, 3H), 0.97 (dt, ^3J(H,H) = 4.0 Hz, ^2J(H,H) = 12.2 Hz), 0.95–0.87 (m, 2H), 0.82 (dt, ^3J(H,H) = 8.0 Hz, ^3J(H,H) = 3.0 Hz, 1H), 0.67 (s, 3H) ppm; Proton signals at δ = 5.96, 4.17, 2.38 and 2.14 ppm disappeared; ^{13}C NMR (125 MHz, DMSO-d_6) δ = 198.2, 172.8, 119.0 (t, ^1J(C,D) = 25.7 Hz), 79.8, 66.2 (t, ^1J(C,D) = 19.1 Hz, 53.5, 49.8, 42.4, 40.8, 38.5, 36.2, 35.7, 33.6, 32.8, 29.8, 23.0, 20.3, 17.8, 11.2 ppm. TLC (DCM/MeOH 25:1 v/v): R_f = 0.19. MS ESI *m*/*z* (%): 331.2 $[M+Na]^+$ (31), 309.2 $[M+H]^+$ (100); Deuterium distribution (%): D_4 (79) D_3 (17), D_2 (4).

4.2.4. 1-(2,6-Dichlorophenyl)-1,3-dihydro-indol-2-one-[3,3,5,7-D_4] (10a)

In a Biotage microwave vial, 1.5 g (4.62 mmol) sodium diclofenac **3** was suspended in 2 ml D_2O and 12 ml DCl (35% in D_2O). The vial was tightly sealed and heated in the microwave at 160 °C for 20 min. After cooling, the mixture was transferred into a separating funnel with 25 ml dichloromethane. 25 ml water was added and the phases separated. The aqueous phase was extracted twice with 25 ml dichloromethane. The combined organic phases were dried over anhydrous sodium sulphate and filtered. The solvent was reduced to a small volume, transferred into another microwave vial and evaporated under a stream of nitrogen. This exchange procedure was repeated once. Yield: 1.25 g

(4.43 mmol, 96%) slightly orange solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 7.75 (d, ^3J(H,H) = 8.1 Hz), 7.61 (dd, ^3J(H,H) = 8.5 Hz, ^3J(H,H) = 8.6 Hz), 7.38 (s, 0.9H), 7.20 (s, 0.9H,), 6.38 (d, ^3J(H,H) = 7.4 Hz, 0.1H), 3.86 (s, 0.1H) ppm; Proton signals at δ = 7.09 ppm disappeared. LC–MS ESI *m*/*z* (%): 304.0 $[M+Na]^+$ (16), 282.0 $[M+H]^+$ (100); Deuterium distribution (%): D_4 (91) D_3 (9).

4.2.5. 1-(2,6-Dichlorophenyl-[4-D])-1,3-dihydro-indol-2-one-[3,3,4,5,6,7-D_6] (10b)

Under argon 1.25 g (4.43 mmol) **10a** was dissolved in 12 ml AcOD and 1.5 ml D_2O in a Biotage microwave vial, with the addition of 0.37 ml DCl (35% in D_2O; 4.43 mmol) and 196 mg K_2PtCl_4 (0.22 mmol, dissolved in 1.5 ml D_2O). The vial was tightly sealed and heated in the microwave at 160 °C for 20 min. After cooling, the mixture was transferred into a separating funnel with 25 ml dichloromethane. 25 ml water was added and the phases separated. The aqueous phase was extracted twice with 25 ml dichloromethane. The combined organic phases were dried over anhydrous sodium sulfate and filtered. Subsequently, the solvent was reduced to a small volume, transferred into another microwave vial and evaporated under a stream of nitrogen. This exchange procedure was repeated once. Yield: 1.05 g (3.68 mmol, 83%) orange solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 7.75 (d, ^3J(H,H) = 8.1 Hz, 2H), 7.61 (t, ^3J(H,H) = 8.6 Hz, 0.1H,), 7.38 (s, 0.1H), 7.20 (s, 0.1H), 3.86 (s, 0.1H) ppm; Proton signals at δ = 7.09, 6.38 ppm disappeared. LC–MS ESI *m*/*z* (%): 307.0 $[M+Na]^+$ (12), 285.0 $[M+H]^+$ (100); Deuterium distribution (%):D_7 (74) D_6 (18), D_5 (7), D_4 (1).

4.2.6. [2-(2,6-Dichloro-phenylamino-[4-D])-phenyl-[3,4,5,6-D_4]]-essigsäure-[1,1-D_2] (diclofenac-[D_7]) (3a)

Under argon, 7 ml MeOD and 3.3 ml NaOD (2 M in D_2O) were added to 0.9 g (3.16 mmol) **10b**, the Biotage microwave vial sealed and the mixture irradiated in the microwave at 130 °C for 10 s. MeOD was removed and the remaining suspension taken up in 50 ml water. The pH was adjusted to 3–4 by dropwise addition of 2 M HCl and the precipitated solid isolated by filtration. The crude product was recrystallised from *tert*-butylmethyl ether/heptane (1:1, v/v). Yield: 0.62 g (2.04 mmol, 65%) colourless solid. A final HPLC control resulted in a purity of 99.7% by area. Conditions: column: Waters SunFire C18, 150 × 3 mm, 3.5 μm. Eluent A: 0.1% formic acid (aq, v/v). Eluent B: 0.1% formic acid in acetonitrile (v/v). Flow rate: 0,5 ml/min. UV detection wavelength: 280 nm. Injection volume: 10 μl. Sample solvent/concentration: 0.5 mg/ml in acetonitrile. Column temperature: 30 °C. Sampler temperature: 8 °C. Gradient profile: 0 min/30% eluent B, 10 min/90% eluent B, 14 min/90% eluent B, 15 min/30% eluent B, 20 min/30% eluent B. Retention time: 9.8 min; ^{1}H NMR (500 MHz, DMSO-d_6) δ = 12.67 (s, 1H, COOH), 7.52 (s, 2H), 7.22 (s, 1H, NH), 7.20 (s, 0.1H), 7.18 (t, ^3J(H,H) = 8.5 Hz, 0.1H), 7.06 (s, 0.1H) ppm; Proton signals at δ = 6.86 (5-H), 6.29 (3-H), 3.68 (–CH_2) ppm disappeared; ^{13}C NMR (125 MHz, DMSO-d_6) δ = 173.3 (C=O), 142.6, 137.1, 130.4 (t, ^1J(C,D) = 22.1 Hz), 130.0, 129.0, 127.0 (t, ^1J(C,D) = 23.0 Hz), 125.3 (t, ^1J(C,D) = 21.5 Hz), 123.7, 120.3 (t, ^1J(C,D) = 21.3 Hz), 115.5 (t, ^1J(C,D) = 21.3 Hz), 37.4 (m,) ppm; MS ESI (%)*m*/*z*: 324.9 $[M+Na]^+$ (25), 302.9 $[M+H]^+$ (100), 284.8 $[M-H_2O]^+$ (8), 256.8 $[M-HCO_2H]^+$ (6); Deuterium distribution (%): D_7 (79) D_6 (15), D_5 (5), D_4 (1).

4.2.7. [2-(2,6-Dichloro-4-hydroxyphenylamino)-phenyl-[3,4,5,6-D_4]]-essigsäure-[1,1-D_2] (4′-hydroxydiclofenac-[D_6]) (4a)

Cunninghamella echinulata ATCC 9245 was grown at 28 °C for 65 h in four 1 L conical flasks each containing 300 mL of liquid medium. 1 g of -[D_7] diclofenac **3a**, solubilised in 12 mL of acetone/ethanol/water 50/33.33/16.67 was dispensed into the four flasks. After 7 days of biotransformation, the reaction was stopped, no starting material being observed by HPLC/UV. The cells were filtered and extracted twice with a mixture of ethyl acetate and methanol (9/1). The supernatant was acidified to pH 3 with 1 N HCl, then extracted three times with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuo. The residue (3 g) was pre-purified by column chromatography on silica gel 150 g 40–63 μm (eluant (CH_2Cl_2/CH_3OH/acetic acid = 97/3/0.5)). Fractions containing the desired phenol were combined and concentrated to give 1 g of material which was purified on silica gel (150 g, 15–40 μm (eluent, (CH_2Cl_2/CH_3OH = 99/1–98/2)) the pure fractions were combined and concentrated to give 695 mg of a white solid. Yield: 695 mg (2.18 mmol, 65%) A final HPLC control resulted in a purity of 99.6% area. Conditions: column: Phenomenex Kinetex C18, 150 × 4.6 mm, 2.6 μm. Eluent A: 0.1% formic acid (aq)/acetonitrile, 90:10 (v/v). Eluent B: 0.1% formic acid (aq) /acetonitrile, 10:90 (v/v). Flow rate: 1 ml/min. UV detection wavelength: 280 nm. Injection volume: 10 μl. Sample solvent/concentration: 0.5 mg/ml in acetonitrile. Column temperature: 30 °C. Sampler temperature: 8 °C. Gradient profile: 0 min/20% eluent B, 20 min/90% eluent B, 24 min/90% eluent B, 25 min/20% eluent B, 30 min/20% eluent B. Retention time: 9.0 min; ^{1}H NMR (600 MHz, DMSO-d_6) δ = 12.5 (br s, 1H), 10.2 (s, 1H), 7.1 (s, 0.2H), 6.97 (s, 0.2H), 6.93 (s, 2H), 6.71 (s, 0.2H), 6.09 (s, 0.05H), 3.58 (s, 0.2H) ppm; ^{13}C NMR (150 MHz, DMSO-d_6) δ = 173.1 (C=O), 155.4, 144.0, 133.0, 130.6, 128.1, 127.3, 121.6, 118.8, 115.8, 113.2, 37.4 ppm; ESI- *m*/*z* (%): 316 $[M-H]^-$ (100); Deuterium distribution (%): D_5 (12), D_6 (26), D_7 (23), D_8 (22), D_9 (11), D_{10} (6).

4.2.8. [D_8]-8-Chloro-6-(2-fluoro-phenyl)-1-methyl-4*H*-2,5,10b-triaza-benzo[e]azulene (midazolam-[D_8]) (5a)

Into a 10 ml microwave vial, charged with argon were placed 46 mg Pt/C (5% Pt, Sigma–Aldrich), 2 mL D_2O and 57 mg (4.1 equiv) $NaBD_4$. To avoid imine reduction, the activated catalyst was stirred at RT for 10 min to eliminate the produced D_2, then 122 mg (0.34 mmol) **5** hydrochloride were added while stirring. After refilling with argon, the vial was sealed and heated to 165 °C for 6 h in the microwave. The reaction mixture was then cooled down to RT and the catalyst filtered off, the filtrate evaporated to dryness, dissolved in dichloromethane, and the organic phasewashed with water. The crude product, obtained after drying over Na_2SO_4, filtration and evaporation, was purified by flash-chromatography on pre-packed silica (Merck 25 g, 15 μ) using CH_2Cl_2/MeOH (3/1 v/v) as eluent to give 95 mg of **5a** midazolam-D_8. (Yield: 0.29 mmol, 80%). A final HPLC control resulted in a purity of 95% area. Conditions: column: Kromasil C18, 250 × 4.6 mm, 5 μm. Eluent A: ammonium actetate (10 mM)/acetic acid at pH 4.6/acetonitrile (95/5 v/v). Eluent B: acetonitrile. Flow rate: 1 ml/min. UV detection wavelength: 254 nm. Injection volume: 20 μl. Sample solvent/concentration: 1 mg/ml in acetonitrile/methanol. Column temperature: 30 °C. Gradient profile: 0 min/10% eluent B, 30 min/50% eluent B, 32 min/50% eluent B, 35 min/80% eluent B, 40 min/80% eluent B, 42 min/10% eluent B, 50 min/10% eluent B. Retention time: 29 min; ^{1}H NMR (600 MHz, CD_2Cl_2): δ = 7.65 (t, *J* = 7.6, 1.25H), 7.62 (d, *J* = 7.5, 0.5H), 7.61 (dd, *J* = 8.6 and 2.4, 0.5H), 7.45 (d, *J* = 8.7, 1.3H), 7.31 (d, *J* = 1.9, 1.0H), 7.27 (d, *J* = 7.6, 0.8H), 7.06 (d, *J* = 10.6, 1.1H), 6.92 (s, 0.27H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ = 164.3, 160 (d, *J* = 50), 144.0, 134.2, 133.8, 132.7, 132.0, 130.9, 129.5, 127.5, 125.6, 124.3, 123.5, 116.1, 45.4 (m), 14.3 (m) ppm; ESI *m*/*z* (isotopic distribution): 330–339 $[M+H^+]$; major isotopologue at *m*/*z* 334; Deuterium distribution (%): D_5 (2), D_6 (10), D_7 (21), D_8 (25), D_9 (20), D_{10} (15), D_{11} (4), D_{12} (3).

4.2.9. [D_5]-[8-Chloro-6-(2-fluoro-phenyl)-4*H*-2,5,10b-triaza-benzo[e]azulen-1-yl]-methanol (1′-hydroxymidazolam-[D_5]) (6a)

Into a 10 ml microwave vial, charged with argon were placed 12 mg Pt/C (5% Pt, Sigma–Aldrich), 2 mL D_2O and 42 mg (4 equiv)

$NaBD_4$. To avoid imine reduction, the activated catalyst was stirred at RT for 10 min to eliminate the produced D_2, then 89 mg (0.26 mmol) **6** were added while stirring. After refilling with argon, the vial was sealed and heated to 165 °C for 2 h in the microwave. The reaction mixture was then cooled down to RT, the catalyst filtered off, and the filtrate was evaporated to dryness. The crude product was purified by flash-chromatography on pre-packed silica (Merck 10 g, 15 μ) using CH_2Cl_2/MeOH (3/1 v/v) containing 10% of concd NH_3– in water) as eluent to give 26 mg of white solid (28% yield); A final HPLC control resulted in a purity of 95% by area. Conditions: column: Kromasil C18, 250 × 4.6 mm, 3 μm. Eluent A: ammonium actetate (10 mM)/acetic acid at pH 3.2. Eluent B: acetonitrile. Flow rate: 1 ml/min. UV detection wavelength: 254 nm. Injection volume: 20 μl. Sample solvent/concentration: 1 mg/ml in acetonitrile/methanol. Column temperature: 30 °C. Gradient profile: 0 min/10% eluent B, 30 min/50% eluent B, 32 min/80% eluent B, 35 min/10% eluent B, 40 min/10% eluent B. Retention time: 23 min; ^{1}H NMR (600 MHz, D_6-DMSO): δ = 8.10 (d, *J* = 4.7, 0.1 H), 7.79 (dd, *J* = 6.3 and 1.7, 0.4H), 7.79 (d, *J* = 1.8, 0.06H), 7.55 (dt, *J* = 14.9 and 1.2, 1H), 7.53–7.51 (m, 0.6H), 7.29 (t, *J* = 7.5, 0.9H), 7.22 (dd, *J* = 1.0 & 0.8, 0.8H), 7.20–7.18 (m, 0.6H), 5.65 (q, *J* = 5.6, OH, 0.9H), 4.71 (dt, *J* = 13.1 & 5.1, 07H), 4.32 (dt, *J* = 12.9, 5.8, 07H) ppm; ESI *m*/*z* (%): 347 (100) [M+H$^+$], Deuterium distribution (%): D_2 (2), D_3 (9), D_4 (17), D_5 (21), D_6 (18), D_7 (14), D_8 (10), D_9 (5), D_{10} (3), D_{11} (1)

4.2.10. [D_8]-[8-Chloro-6-(2-fluoro-phenyl)-4*H*-2,5,10b-triaza-benzo[e]azulen-1-yl]-methanol (1′-hydroxymidazolam-[D_8]) (6b)

Beauveria bassiana ATCC 7159 was grown at 28 °C for 65 h in a 250 mL conical flask containing 100 mL of liquid medium. 50 mg of [D_6]-Midazolam **5a**, dissolved in a minimal amount of ethanol/water was added to the flask. The transformation was monitored by HPLC/UV, and after ten days the biotransformation was stopped. The cells were filtered and washed twice with a mixture of ethyl acetate and methanol (9/1) to extract organic product. The supernatant was extracted three times with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by column chromatography on silica gel (27 g, 15–40 μm) using dichloromethane/methanol as solvent (CH_2Cl_2/CH_3OH = 100/0–95/5), the pure fractions were combined and concentrated to give 12 mg of a white solid (0.035 mmol, 25% yield). A final HPLC control resulted in a purity of >95% by area. Conditions: column: Kromasil C18, 250 × 4.6 mm, 3 μm. Eluent A: ammonium actetate (10 mM)/acetic acid at pH 3.2. Eluent B: acetonitrile. Flow rate: 1 ml/min. UV detection wavelength: 254 nm. Injection volume: 20 μl. Sample solvent/concentration: 1 mg/ml in acetonitrile/methanol. Column temperature: 30 °C. Gradient profile: 0 min/10% eluent B, 30 min/50% eluent B, 32 min/80% eluent B, 35 min/10% eluent B, 40 min/10% eluent B. Retention time: 23 min; ^{1}H NMR (600 MHz, DMSO-d_6) δ = 8.10 (t, *J* = 8.69, 0.3H), 7.79 (d, *J* = 8.69, 0.11H), 7.55 (t, *J* = 7.67, 0.45H), 7.53 (m, 0.1H), 7.29 (mbr, 1.0H), 7.19 (mbr, 1.2H), 7.13 (mbr, 0.6H), 6.97 (s, 0.2H),5.56 (s, 0.4H) ppm; ESI- *m*/*z* (%): 350 [M−H]$^+$ (100); Deuterium distribution (%): D_4 (2), D_5 (5), D_6 (15), D_7 (21), D_8 (23) D_9 (19), D_{10} (10), D_{11} (4), D_{12} (1).

4.2.11. [D_{10}]-(9*S*,13*S*,14*S*)-3-Methoxy-17-methylmorphinan (Dextromethorphan-[D_{10}]) (7a)

Into a microwave vial filled with argon were placed 352 mg (1.00 mmol) hydrobromide **7**, 35 mg Pd/C (10% Pd; Heraeus K209), 10 mg (20 mol %) $NaBD_4$ and 6 ml D_2O. The mixture was stirred for approximately 30 s, the vial was sealed and heated in the microwave to 140 °C for 2 h. The mixture was cooled to room temperature and 3 ml acetonitrile were added. The catalyst was filtered off and the filtrate evaporated to dryness. The residue was purified by recrystallisation from methanol/water (1:1 v/v). Yield: 314 mg (0.87 mmol, 87%) colourless solid. A final HPLC control resulted in a purity of 99.2% by area. Conditions: column: Thermo Hypersil Gold, 150 × 3 mm, 3 μm. Eluent A: 0.1% formic acid (aq, v/v). Eluent B: 0.1% formic acid in acetonitrile (v/v). Flow rate: 0,5 ml/min. UV detection wavelength: 210 nm. Injection volume: 10 μl. Sample solvent/concentration: 1 mg/ml in water. Column temperature: 20 °C. Sampler temperature: 20 °C. Gradient profile: 0 min/10% eluent B, 15 min/95% eluent B, 20 min/95% eluent B, 22 min/10% eluent B, 25 min/10% eluent B. Retention time: 11.5 min; LC–MS purity (254 nm): 98%; ^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.70 (br s, 1H), 7.23 (d, 1H, disappeared), 6.84 (d, *J* = 2.3 Hz, 2H, one disappeared), 3.73 (s, 3H), 3.61 (m, 2H), 3.19–3.06 (m, 2H, disappeared), 2.98–2.92 (m, 2H, disappeared), 2.65 (s, 3H, NC*H*$_3$, disappeared), 2.20–1.80 (m, 2H, one disappeared), 1.50–0.91 (m, 7H, one disappeared); ^{13}C NMR (125 MHz, DMSO-d_6) δ = 158.5, 138.7, 128.6, 125.7, 112.1, 110.7, 58.7, 55.0, 47.0, 41.7, 39.0, 35.2, 34.7, 34.2, 25.4, 25.3, 24.9, 21.4 ppm; ESI *m*/*z* (%): 281 [M+H]$^+$ (100); Deuterium distribution (%): D_7 (3), D_8 (10), D_9 (24), D_{10} (39), D_{11} (17), D_{12} (5), D_{13} (2).

4.2.12. [D_9] (+)-17-Methyl-9a,13a14a-morphinan-3-ol (Dextrorphan-[D_9]) (8a)

314 mg (0.87 mmol) dextromethorphan-[D_{10}] hydrobromide (**7a**) was suspended in 5 ml HBr (48% in water) and heated to 115 °C (bath temperature) for 2 h. Reaction control by LC–MS showed complete conversion. The reaction mixture was poured onto 5 g of crushed ice and the pH was adjusted to 10 by addition of saturated sodium carbonate solution. The aqueous phase was extracted three times with 20 ml ethyl acetate. The combined organic layers were washed with 10 ml brine and dried over sodium sulphate. After evaporation of solvents under vacuum the crude product was purified by HPLC (LUNA C18 column, water/acetonitrile; gradient program) to give 201 mg (0.58 mmol) of **8a** hydrobromide. Yield: 201 mg (0.58 mmol, 67%) light orange solid; LC–MS purity (254 nm): 98%. A final HPLC control resulted in a purity of 98.8% area. Conditions: as for Dextromethorphan-[D_{10}] (**7a**): Retention time: 9.2 min. ^{1}H NMR (500 MHz, DMSO-d_6) δ = 9.70 (br s, 1H), 7.16 (d, *J* = 8.2 Hz, 1H), 6.71 (d, *J* = 2.3 Hz, 1H), 6.75–6.70 (m, 1H), 3.61 (m, 2H), 3.19–3.06 (m, 2H, disappeared), 2.98–2.92 (m, 2H, disappeared), 2.65 (s, 3H, NC*H*$_3$, disappeared), 2.41–2.30 (m, 3H, one disappeared), 2.20–1.80 (m, 2H, one disappeared), 1.50–0.91 (m, 5H); ^{13}C NMR (125 MHz, DMSO-d_6) δ = 156.5, 138.5, 129.2, 123.8, 114.0, 111.4, 64.9, 58.8, 46.3, 41.7, 35.0, 34.8, 25.3, 25.2, 21.4, 21.4, 15.1 ppm; MS ESI *m*/*z* (%): 267 [M+H]$^+$ (100). Deuterium distribution (%): D_7 (5), D_8 (19), D_9 (38), D_{10} (30), D_{11} (8).

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Supplementary data

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