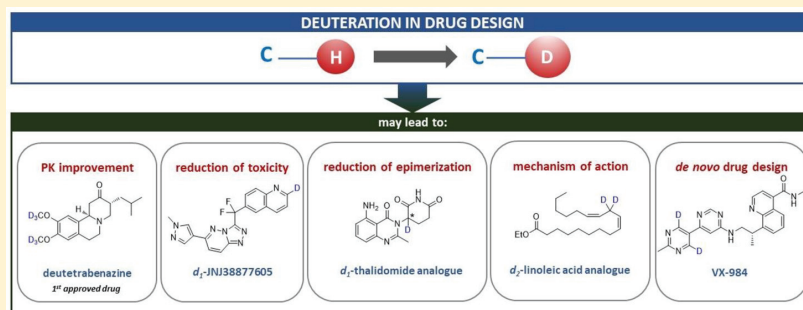


Applications of Deuterium in Medicinal Chemistry

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ABSTRACT: The use of deuteriation in medicinal chemistry has exploded in the past years, and the FDA has recently approved the first deuterium-labeled drug. Precision deuteriation goes beyond the pure and simple amelioration of the pharmacokinetic parameters of a drug and might provide an opportunity when facing problems in terms of metabolism-mediated toxicity, drug interactions, and low bioactivation. The use of deuterium is even broader, offering the opportunity to lower the degree of epimerization, reduce the dose of coadministered boosters, and discover compounds where deuterium is the basis for the mechanism of action. Nevertheless, designing, synthesizing, and developing a successful deuterated drug is far from straightforward, and the translation from concept to practice is often unpredictable. This Perspective provides an overview of the recent developments of deuteriation, with a focus on deuterated clinical candidates, and highlights both opportunities and challenges of this strategy.

INTRODUCTION

Deuterium is a rare, stable, nonradioactive isotope of hydrogen, which differs from protium by a single neutron. It is produced in high amounts in stars and can be found on Earth with a natural abundance of 0.0156% of all the naturally occurring hydrogen in the oceans.¹

Deuterium was first described by Harold Urey in 1932,² the same scientist that in the 1950s came up with the landmark Miller–Urey experiment,³ demonstrating that spark discharge through a simulated reducing atmosphere yields amino acids from a primordial inorganic soup. In 2017, Cooper et al. capitalized on Urey's discoveries⁴ and compared the products of a “deuterated world”, containing fully deuterated starting materials, with the standard “hydrogenated world”. Remarkably, the authors observed the production of unique species in the deuterated soup, confirming that the presence of this isotope in the natural world leads to additional complexity in the chemical space. Its incorporation in place of protium has been exploited in many different disciplines in life sciences, including proteomics, metabolomics, and diagnostics.⁵

This Perspective aims at providing a snapshot of the potential of deuterium in medicinal chemistry. We have mainly limited our efforts to peer-reviewed papers in order to base our reporting on unbiased results, although we acknowledge that, given the industrial interest in the field, the patent literature and other online resources (such as poster abstracts and

company Web sites) also provide additional clues on the applications of deuterated compounds. These resources, though, present biases, and we have therefore used them sparingly. We have concentrated, where possible, on recent examples, and we refer the reader to other excellent papers⁶ and book chapters⁷ that had reviewed earlier work.

How Does Deuterium Differ from Protium from a Medicinal Chemistry Viewpoint? Compared to protium (that at times we will refer to as hydrogen, H), deuterium (D) displays a smaller molar volume (by 0.140 cm³/mol per atom), is less lipophilic ($\Delta \log P_{\text{oct}} = -0.006$), and might display a slightly different pK_a.⁸ More importantly, C–D bonds are shorter (by 0.005 Å) and at times more stable to oxidative processes. Indeed, D has a 2-fold higher mass than H, leading to a reduced vibrational stretching frequency of the C–D bond compared to the C–H bond and, therefore, a lower ground-state energy. It follows that the activation energy required for reaching the transition state for bond cleavage is greater for C–D than C–H and the reaction rate (represented by rate constant *k*) is slower ($k_{\text{H}} > k_{\text{D}}$).

The difference in stability of isotopically substituted molecules is referred to as the primary kinetic isotopic effect (KIE),⁹ which for deuterium can be defined as the deuterium

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kinetic isotope effect (DKIE). DKIE is quantified as the ratio of the rate constants for the reaction (k_H/k_D) and typically ranges from 1 (for reactions where deuterium has no effect) to 7, with the theoretical limit being 9,¹⁰ even though DKIEs lower than 1¹¹ or up to 16¹² have been reported. Obviously, given that enzyme-catalyzed transformations are multistep, to observe high DKIEs, it is necessary that the C–H cleavage step is at least partially rate-limiting.

It must be acknowledged that in other kinetic models the quantum-mechanical tunneling is invoked to explain a secondary DKIE.¹³ While this is usually much smaller in magnitude than the primary effect (typically 1.1–1.2), this mechanism can lead to significantly larger effects.¹⁴

Despite the above differences, the substitution of protium with deuterium represents the most conservative example of isosteric replacement.⁸ Indeed, deuterium keeps 3D surface, shape, and steric flexibility unaltered compared to protium. It follows that deuterium-modified compounds usually retain biochemical potency and selectivity. Even when differences are reported, they are usually minor (although not necessarily irrelevant) as in the case of d_5 -sildenafil,¹⁵ which has been reported to be 2- to 3-fold more selective than the parent compound for phosphodiesterase V versus II and VI. In the present manuscript, we will use the term parent to indicate the nondeuterated compound.

Historical Timeline of Deuterated Compounds in the Medicinal Chemistry Field. The very first examples of deuterium incorporation in bioactive compounds date back to the 1960s, when two independent groups reported a decreased metabolism for d_2 -tyramine¹⁶ and d_3 -morphine¹⁷ compared to the parent compounds. In the following decade, deuterium substitution was exploited with the aim to lower oxidative clearance of halothane¹⁸ and fludalanine,¹⁹ with a consequent decrease in liver and central nervous system toxicity, respectively.

For many years thereafter, deuterated compounds have been used as internal standards for bioanalytical methods,²⁰ a current and important medicinal chemistry application that will not be covered by this review.

Deuteration in drug design did not attract significant interest until the first decade of the new millennium, when several companies that presented deuterated compounds as their core business rose to the attention of the scientific and investment community (e.g., Concert Pharmaceuticals, Deutera Pharmaceuticals, DeuteRx, Protia, Auspex, Retrotope). Acquisitions or licensing deals exceeding 7 billion dollars have been reported since 2014, demonstrating the attractiveness of the field.²¹

In April 2017, deutetabenazine (**1**, Figure 1) was approved by the FDA for the treatment of choreas associated with Huntington's disease and for tardive dyskinesias,²² the same indications of the parent drug, tetrabenazine, which is also on the market. This represented a milestone per se for the field and gave courage to the industrial world as deutetabenazine was allowed to submit as a new chemical entity (providing exclusivity advantages to the manufacturer). Moreover, deutetabenazine was approved via a hybrid regulatory pathway (S05(b)(2)) that allowed the applicant to rely in part on data from the parent compound. Approval of deutetabenazine relied on a placebo-controlled trial, and it is therefore difficult to ascertain its added clinical value compared to tetrabenazine, although it presents a reduced number of daily administrations. It has been suggested that tolerability of the deuterated form is improved over the parent

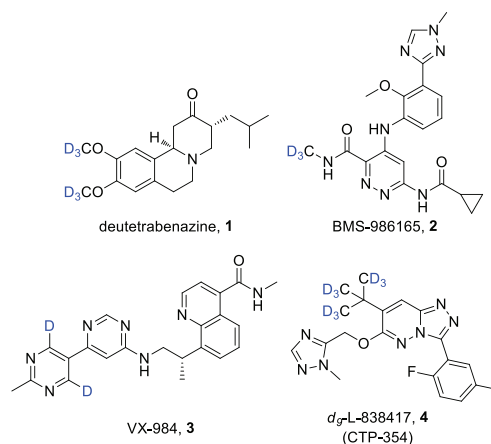


Figure 1. Structures of deutetabenazine, BMS-986165, VX-984, and CTP-354.

compound,²³ although others have pointed out that indirect comparisons are unreliable and dependent on the statistical strategy used²⁴ and that only head-to-head trials would answer the question on the real added value of the new compound. It should be noticed that at least another deuterated-compound-containing-medicine (d_6 -dextromethorphan/quinidine) has received fast track designation by the FDA, but the medicine has not yet reached regulatory approval.²⁵

Both examples above represent putative improvements of medicines already on the market, in a manner that is referred to as deuterium switch. Yet deuterium has also been incorporated in the early stages of the drug discovery process, giving rise to deuterated bioactive compounds that do not have counterparts on the market. This is exemplified by two molecules that have reached clinical trials, BMS-986165 and VX-984 (**2** and **3**, Figure 1). The former is a potent (K_i of 0.02 nM) and selective (as determined against other 265 kinases and pseudokinases) inhibitor of tyrosine kinase 2 (Tyk2) and has shown its efficacy in a number of preclinical models.²⁶ Encouraging data in a placebo-controlled clinical trial on psoriasis have recently been published.²⁷ VX-984 is a selective DNA-dependent protein kinase (DNA-PK) inhibitor²⁸ and has completed a phase I trial for the treatment of recurrent or metastatic endometrial cancer.²⁹ It is interesting to note that in the very recent paper in the *New England Journal of Medicine* reporting the clinical efficacy of BMS-986165 there is no mention about its deuterated nature,²⁷ suggesting that this modification is now considered common practice in drug development.

A further use of deuteration that is emerging is the salvaging of compounds that had shown weaknesses in their non-deuterated form. For example, the GABA_A modulator L-838417 did not advance to the clinical stage due to its poor pharmacokinetic profile.³⁰ Its d_6 -deuterated analogue, CTP-354 (**4**, Figure 1), was shown to have comparable binding affinity, subunit specificity, and pharmacological activity, displaying such improved pharmacokinetics in rodents that a phase I clinical trial was initiated.³¹ CTP-354 represents therefore an example where deuteration is used to rescue a drug.³² This is of extreme interest as it suggests that deuterium might allow revitalization of abandoned drug programs.

DEUTERATION AND DRUG METABOLISM

The most straightforward application of deuterium substitution is to slow down drug metabolism, especially cytochrome P450 (CYP450)-mediated transformations.³³ Among the myriad of reactions catalyzed by this superfamily, high DKIEs are usually displayed by dealkylations of ethers and amides (>2), while amine N-dealkylation is less affected (<2), even if the rationale for this is still a subject of debate. Regarding the oxidation of alkyl groups, the magnitude of DKIE heavily depends on the rate-limiting step in a particular reaction. Last, DKIE is close to 1 for those reactions, such as aryl hydroxylations, epoxidations, and heteroatom oxygenations, in which no C–H bond is broken during enzyme catalysis, and therefore a precision deuteration can rely only on a secondary kinetic isotope effect.^{33b,c}

The effect of deuterium incorporation on oxidizing transformations catalyzed by other enzymes has also received attention. For example, aldehyde oxidase (AO) is involved in the oxidation of the formyl carbon of aldehydes as its name implies but is also involved in the oxidation of α -carbons next to nitrogens in heteroaromatic ring systems (e.g., pyridines, pyrimidines, benzimidazoles) through a C–H bond cleavage as the rate-limiting step. Therefore, it can be targeted by precision deuteration, as first described for carbazeran and zonisporide.³⁴ A more recent and advanced example is given by the already described VX-984 (3, Figure 1), where deuterium incorporation mitigates the AO-driven metabolism.²⁸

The development of d_3 -L-DOPA, SD-1077 (5, Figure 2), has clearly demonstrated that also reactions catalyzed by mono-

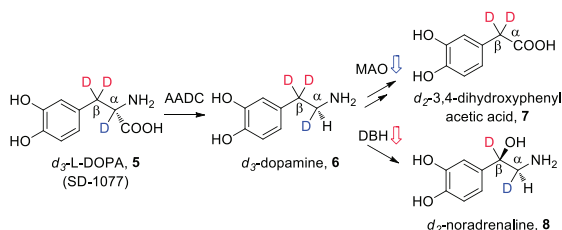


Figure 2. d_3 -L-DOPA and its metabolites.

amine oxidases (MAO) and dopamine β -hydroxylases (DBH) can be affected. After decarboxylation of d_3 -L-DOPA 5 by aromatic L-amino acid decarboxylase (AADC), the metabolism of d_3 -dopamine 6 can be fine-tuned by deuteration. Indeed, incorporation of deuterium at the α -carbon slows down MAO activity and the formation of d_2 -3,4-dihydroxyphenylacetic acid 7, while at the β -carbon it attenuates DBH functionality with consequent reductions of d_2 -noradrenaline 8. The result is that d_3 -L-DOPA is a more potent anti-kinetic compared to L-DOPA in a rodent model of Parkinson's disease, without increasing dyskinesias.³⁵ Given that this modification should affect dopamine metabolism at synapses, it is not surprising that the recently published phase I clinical trial³⁶ highlighted comparable peripheral pharmacokinetic (PK) and safety following a single oral dose of SD-1077/carbidopa and L-DOPA/carbidopa in healthy subjects, without indication of a major shift in the main systemic metabolic pathways.

The activity of other members of the amine oxidase family also appears to be affected by deuteration. This is exemplified by d_2 -AVE5638, a tryptase inhibitor³⁷ metabolized by

semicarbazide sensitive amine oxidase, and d_2 -lysine, which is a substrate for lysyl oxidase.³⁸

This change in metabolic rate of deuterated drugs may be capitalized upon to modify the profile of bioactive molecules in a range of different aspects:

(a) Optimization of the Pharmacokinetic Properties.

It is not surprising that ample literature is devoted to deuteration as a means to improve the PK profile of drugs. To give an overview of the effect of deuteration on PK, we have collected some recent examples where deuterated and parent compound were tested side-by-side (Table 1, Figure 3). Most experiments in the literature have a parallel group design, in which separate animals/humans are administered either the deuterated or the nondeuterated compound. Yet it is interesting to note that there are examples in which the same animal/individual is administered both compounds simultaneously, given the possibility to trace separately the two species analytically. This latter strategy reduces inter- and intraindividual variability and allows a reduction in the number of subjects/animals (n) required per study. Generalizing, it can be observed that when deuteration is successful, it results in an increased AUC and a reduced clearance, $C_{\max}$ and $t_{1/2}$ are usually increased, while $T_{\max}$ is less affected. Changes are observed in both enteral and parenteral administrations, and bioavailability is at times affected (presumably when the first-pass effect is of relevance).

A range of different competing effects might mask or even reverse the consequences of deuteration, such as different rate-limiting steps in enzyme mechanisms, alternative metabolic routes,³³ or competing nonmetabolic elimination mechanisms³⁴ rendering the translation of in vitro results to in vivo challenging and highly unpredictable. The case of rofecoxib exemplifies this unpredictability.³⁹ Indeed, d_5 -rofecoxib (9, Figure 4) showed a $C_{\max}$ of 4181 $\mu\text{g/L}$ compared to a $C_{\max}$ of 2416 $\mu\text{g/L}$ for the nondeuterated compound in male rats after 10 mg/kg dose (po), but the two isotopologs exhibited identical half-life values (4.29 h versus 4.18 h). The increase in $C_{\max}$ is surprising as deuteration of the aromatic ring should in principle not afford substantial improvements due to a primary DKIE since CYP450-mediated aromatic oxidations are known to occur without cleavage of the C–H/D bond.^{33b,c}

In stark contrast to rofecoxib, the effect of precision deuteration on recently reported selective ligands for GABA_A receptor $\alpha 6$ subunits (10, Figure 4), which display key soft positions susceptible to O-demethylation, showed a significant increase in half-life, an almost 2-fold increase in maximal brain concentration in rats, but no effect on systemic $C_{\max}$.⁴⁰ Importantly, in this example, the prolongation of the half-life was predictable in vitro, where metabolic stability was about tripled for the d_3 -form in both human and mouse liver microsomes compared to the nondeuterated compound (8.7 and 10.5 h, respectively, compared to 3 h for the nondeuterated).

d_3 -Imatinib (11, Figure 4), despite being significantly less susceptible to N-demethylation upon incubation with human liver microsomes, when intravenously administered to rats inexplicably does not show any increased exposure compared to the parent drug.⁴¹ The results could also be explained by a reduced N-demethylation of imatinib in rats compared to humans, highlighting the difficulty in translating PK across species. Indeed, deuteration can lead to different results across different species, as is the norm in metabolism. This is exemplified in a very thorough PK investigation performed on

Table 1. In Vivo Pharmacokinetic Properties of Selected Compounds and Related Deuterated Forms^a

compd	enzymatic reaction	in vivo model	ROA	deuterated form (dose, mg/kg)/parent compd (dose, mg/kg)	pharmacokinetic parameters (expressed as fold change)					
					C_{max}	T_{max}	$t_{1/2}$	AUC	clearance	bioavailability
L-DOPA/carbidopa ³⁶	MAO and DBH oxidation	human	po	SD-1077 (5) + L-DOPA (150.0)/parent (150.0)	0.91 ≈	1.00 ≈	0.91 ≈	0.90 ≈		
rofecoxib ^{37,39}	aryl hydroxylation	rat	po	d ₅ -rofecoxib (9) (10.0)/parent (10.0)	1.73 ↑		1.00 ≈	1.78 ↑		
α6β2/3γ2 GABA _A R selective ligands ⁴⁰	O-dealkylation	rat	ip	d ₃ (10) (10.0)/parent (10.0)	0.88 ≈	1.67 ↑	2.34 ↑↑	0.84 ≈		
imatinib ⁴¹	amine N-dealkylation	rat	iv	d ₃ -imatinib (11) (1.0)/parent (1.0)			0.91 ≈	0.94 ≈	1.12 ≈	
Aurora kinase inhibitors ⁴²	alkyl oxidation	mouse	po	d ₂ (12) (50.0)/parent (50.0)				3.82 ↑↑		0.62 ↓
			iv	d ₂ (12) (3.0)/parent (3.0)				6.56 ↑↑↑	0.16 ↓↓↓	
		rat	po	d ₂ (12) (30.0)/parent (30.0)				9.00 ↑↑↑		1.74 ↑
			iv	d ₂ (12) (10.0)/parent (10.0)				5.19 ↑↑↑	0.19 ↓↓↓	
		dog	po	d ₂ (12) (10.0)/parent (10.0)				1.19 ≈		1.65 ↑
			iv	d ₂ (12) (1.0)/parent (0.3)				22.5 ↑↑↑	0.14 ↓↓↓	
		monkey	po	d ₂ (12) (30.0)/parent (30.0)				3.00 ↑↑		1.00 ≈
			iv	d ₂ (12) (1.0)/parent (0.3)				13.57 ↑↑↑	0.23 ↓↓↓	
		rat	po	d ₃ (13) (1.0)/(14) (1.0)	1.56 ↑	0.89 ≈	1.08 ≈	1.08 ≈		
			iv	d ₃ (13) (3.0)/(14) (3.0)	0.91 ≈	2.13 ↑↑	1.79 ↑	1.31 ↑		
dapagliflozin ⁴³	O-dealkylation	rat	iv	d ₃ (13) (1.0)/(14) (1.0)			1.29 ≈	1.09 ≈	0.90 ≈	
JNJ38877605 ⁴⁴	AO oxidation	monkey	po	d ₁ -JNJ38877605 (15) (10.0)/parent (10.0)	1.56 ↑	1.33 ↑	0.78 ≈	1.88 ↑		
4,6-benzylidene-D-glucose ⁴⁵	reduction	mice	po	d ₁ -4,6-benzylidene-D-glucose (16) (164.0)/parent (272.0)			0.86 ≈	0.74 ≈	1.00 ≈	1.07 ≈
				d ₁ -4,6-benzylidene-D-glucose (16) (218.0)/parent (272.0)			1.00 ≈	0.96 ≈	0.75 ≈	1.07 ≈
				d ₁ -4,6-benzylidene-D-glucose (16) (264.0)/parent (272.0)			0.93 ≈	1.26 ≈	0.90 ≈	1.14 ≈
				d ₁ -4,6-benzylidene-D-glucose (16) (85.0)/parent (85.0)			1.12 ≈	1.44 ↑	1.12 ≈	1.45 ↑
tivozanib ⁴⁶	O-dealkylation	rat	po	d ₆ -tivozanib (17) (2.5)/parent (2.5)	1.30 ≈	1.04 ≈	1.19 ≈	1.50 ↑		
apalutamide ⁴⁷	amide N-dealkylation	mouse	po	d ₃ -apalutamide (18) (10.0)/parent (10.0)	1.35 ↑	0.67 ↓	5.47 ↑↑↑	1.43 ↑		
		rat	po	d ₃ -apalutamide (18) (10.0)/parent (10.0)	1.79 ↑	1.0 ≈	1.20 ≈	1.95 ↑		
brecanavir ⁴⁸	multiple reactions	rat	iv	parent + RTV (1.0 + 4.0)/parent (1.0)			11.0 ↑↑↑	11.73 ↑↑↑	0.08 ↓↓↓	
				d ₁₉ -brecanavir (19) (1.0)/parent (1.0)			1.0 ≈	0.89 ≈	0.91 ≈	
				d ₁₆ -brecanavir (20) (1.0)/parent (1.0)			1.0 ≈	0.97 ≈	0.98 ≈	
enzalutamide ^{49,50}	N-dealkylation	mouse	po	d ₃ -enzalutamide (21) (10.0)/parent (10.0)	0.77 ≈	0.80 ≈	1.93 ↑	1.29 ≈		
		rat ^e	po	d ₃ -enzalutamide (21) (10.0)/parent (10.0)	1.35 ↑	2.35 ↑↑	1.54 ↑	2.03 ↑↑		
NVS-CRF38 ^{d,51}	O-dealkylation	rat	po	d ₃ -NVS-CRF38 (22) (10.0)/parent (10.0)	1.49 ↑	1.62 ↑	1.60 ↑	2.57 ↑↑		
ivacaftor ⁵²	alkyl oxidations	rat	po	d ₉ -ivacaftor (CTP-656, 23) (10.0)/parent (10.0)	1.03 ≈		1.26 ≈	1.09 ≈		
				d ₁₈ -ivacaftor (24) (10.0)/parent (10.0)	1.29 ≈		1.41 ↑	1.42 ↑		
		dog	po	d ₉ -ivacaftor (CTP-656, 23) (10.0)/parent (10.0)	1.54 ↑		1.29 ≈	1.69 ↑		
				d ₁₈ -ivacaftor (24) (10.0)/parent (10.0)	1.34 ↑		1.02 ≈	1.36 ↑		
nerispiridine ^{c,37}	multiple reactions	rat	po	d ₁₄ -nerispiridine (25) (20.0)/parent (20.0)	1.18 ≈	0.50 ↓	1.08 ≈	0.91 ≈		

^aLegend: ≈, fold-changes values between 0.70 and 1.30; ↑, fold-change values between 1.31 and 2.00; ↑↑, fold-change values between 2.01 and 4.00; ↑↑↑, fold-change values of >4.00; ↓, fold-change values between 0.50 and 0.70; ↓↓, fold-change values between 0.25 and 0.49; ↓↓↓: fold-change values of <0.25; ip, intraperitoneal administration; iv, intravenous administration; po, per os; ROA, route of administration; RTV, ritonavir.

^bDifferent doses were tested. ^cDeuterated compounds other than the reported ones were tested. ^dRoutes of administrations other than po were

Table 1. continued

assessed. ^cThe parent compound and its deuterated form were simultaneously administered in order to minimize individual differences between animals.

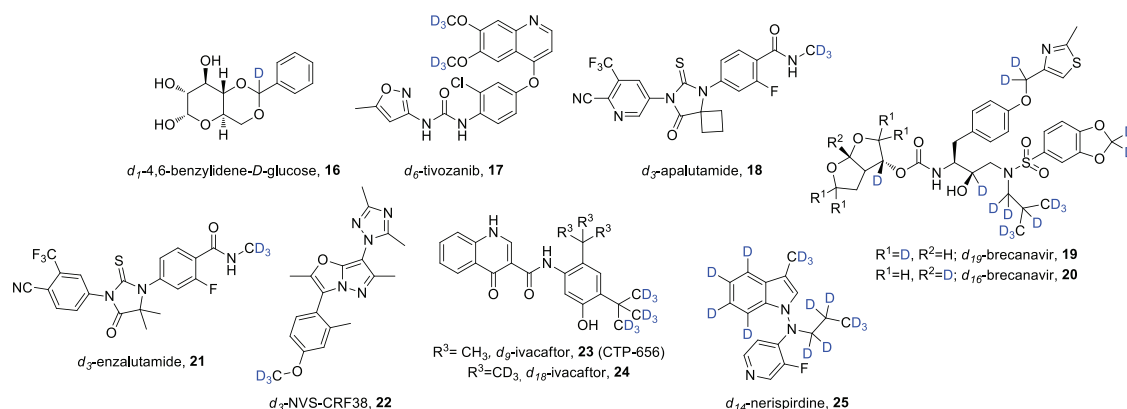
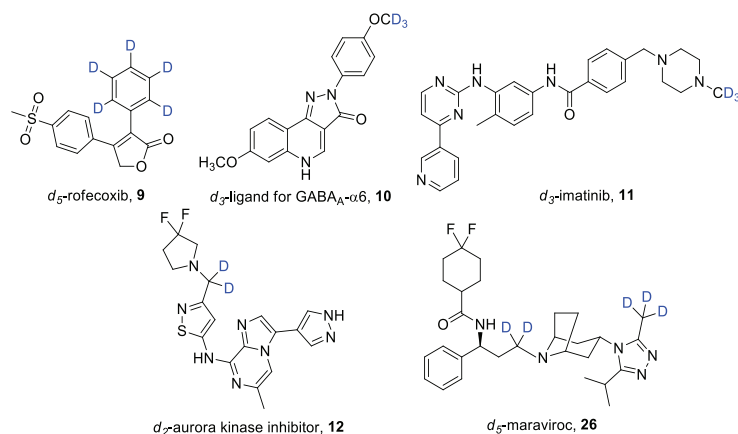


Figure 3. Structures of molecules from Table 1 not shown elsewhere.

Figure 4. Structures of *d*₅-rofecoxib, *d*₃-ligand for GABA_A-α6, *d*₃-imatinib, *d*₂-aurora kinase inhibitor, and *d*₅-maraviroc.

imidazo[1,2-*a*]pyrazine-based inhibitors of aurora kinase (12, Figure 4). Here, the simultaneous replacement of hydrogens at two known soft-spots of a lead compound by two deuterium and two fluorine atoms enabled the identification of an orally available compound where increase in drug exposure was reached in four different species (rats, mice, dogs, and monkeys). Yet, as it can be observed in Table 1, differences between deuterated and nondeuterated forms varied considerably, both qualitatively and quantitatively, across species,⁴² suggesting that deuterium does not mitigate the risk of metabolic differences between species.

Among the reasons for which the effect of deuteration is not always predictable is the possibility that the drug may undergo a metabolic switch; i.e., the incorporation of deuterium at one site successfully reduces metabolism on that site but enhances metabolism at another site. This is well exemplified by maraviroc, a negative allosteric modulator of CCR5 for the treatment of HIV infections. In this instance, deuteration at the methyl group (26, Figure 4) switches metabolism to N-dealkylation and paradoxically accelerates in vitro microsomal clearance (*t*_{1/2} in human liver microsomes; 97.2 versus 45.5

min). Therefore, in this specific case, simultaneous deuteration at both sites of metabolism is necessary to see a significant decline in microsomal turnover (HLM *t*_{1/2} = 145.5 min).^{7b,54} Unfortunately, no in vivo data are available.

This example, though, suggests that for molecules displaying more than one oxidizable hotspot, a possible strategy is to initially perdeuterate all these positions, profile the in vivo PK to observe any potential benefit, and then pinpoint any metabolic soft-spot sensitive to deuterium incorporation by selectively reverting deuterium back to hydrogen. Yet metabolic switches do not always occur. An example of this circumstance is the work by Sun and collaborators that aimed at optimizing dapagliflozin, a SGLT2 inhibitor for the treatment of type 2 diabetes. Given that there were worries regarding a possible hepatotoxicity related to the formation of methine-quinones, a traditional medicinal chemistry approach coupled to deuteration of selected compounds was performed in order to increase metabolic stability and reduce the possibility of forming these toxic metabolites. Indeed, the single substitution of the soft-spot -OCH₂CH₃ in dapagliflozin with -OCD₃ (13, Figure 5) was sufficient to significantly

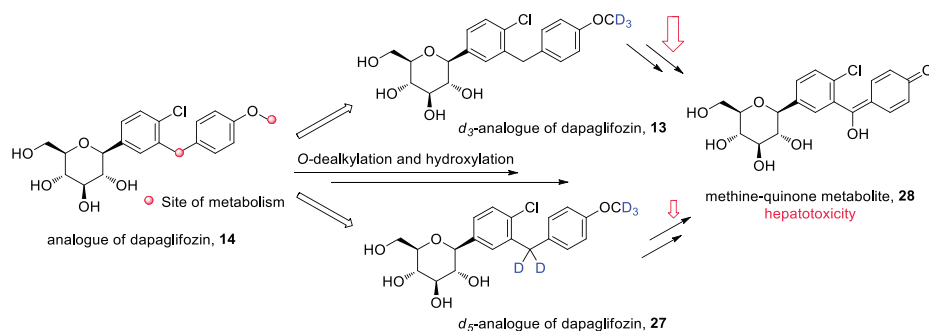


Figure 5. Deuteration of dapagliflozin analogues.

improve the PK profile and reduce the formation of metabolites (28) of possible toxicological worry, although other soft-spots were present.⁴³ No improvements, or a slight detrimental effect, were instead observed when further deuteration was performed, suggesting that more is not always better (i.e., $t_{1/2}$ of 6.34 h for d_3 versus 5.66 h for d_5 , 27, Figure 5).

Many retroviral agents have metabolism as their Achilles' heel, and some of these compounds are purposely coadministered with CYP enzyme inhibitors, known as boosters, to prolong the systemic exposure. Yet the literature is particularly scarce on this issue, suggesting that the deuterium strategy might not have been successful in many instances. We have retrieved a patent application of d_{15} -atazanavir, named CTP-518 (29, Figure 6), and it appears that phase I clinical trials

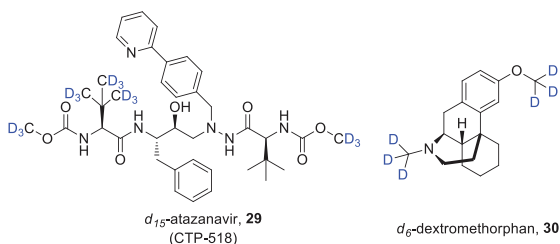


Figure 6. d_{15} -Atazanavir and d_6 -dextromethorphan.

have been completed for this compound.⁵⁵ d_{15} -Atazanavir has been reported to display an average 52% increase of half-life compared to atazanavir in primates. Whether this will be clinically relevant is yet to be determined.

A very similar strategy has led to the development of a fixed combination (AVP-786) of d_6 -dextromethorphan (30, Figure 6) and quinidine for agitation in Alzheimer's disease. This new combination is currently undergoing phase III clinical trials and represents a deuterium switch of a previously approved medication, Nuedexta, for pseudobulbar affect. Quinidine acts

as a booster, reducing the first-pass metabolism of dextromethorphan to its O-demethylated metabolite and increasing the plasma half-life. An improved half-life of d_6 -dextromethorphan requires a lower dose of quinidine, therefore theoretically sparing patients of some of the unwanted side effects of the latter drug.²⁵

Noteworthy, deuterium can be also used for the prolongation of drug release and polymer biodegradation. The first report describing the use of the isotope in a drug delivery system has been very recently published.⁵⁶ More specifically, a drug is covalently attached via a carbamate to hydrogel microspheres using a β -eliminative linker, which is also installed into cross-links of the polymer to trigger degelation after drug release. After subcutaneous injection of the conjugate, the drug is slowly released into the systemic circulation, followed by degradation of the hydrogel. The incorporation of deuterium in the α -carbon of the linker (31, Figure 7), because of a significant DKIE ($k_H/k_D = 2.5$ – 3.5), is able to significantly extend drug release, in vivo elimination of the drug (i.e., octreotide), and in vivo biodegradation of the hydrogel, compared to hydrogen-containing counterparts. Such finding might open up a completely novel avenue for deuterium in pharmaceutical technology.

(b) Deuterium-Induced Reduction of Toxicity. Independent of PK parameters, deuteration of a soft-spot can be capitalized upon to reduce the formation of unwanted metabolites, as well as to increase the formation of desirable active metabolites, a phenomenon named metabolic shunting. In the past, a plethora of examples where deuterium-mediated metabolic shunting exerted protective effects against side effects that hampered the use of nondeuterated drugs have been reported. These include d_3 -nevirapine,⁵⁷ characterized by reduced incidence and severity of skin rashes compared to nevirapine, d_1 -efavirenz, featuring an attenuated nephrotoxicity in rats,⁵⁸ and [d_5 -ethyl]-tamoxifen, endowed with a reduced genotoxicity compared to tamoxifen.⁵⁹

Recently, it has been reported that incorporation of deuterium at the α and α' carbons in ifosfamide (35, Figure

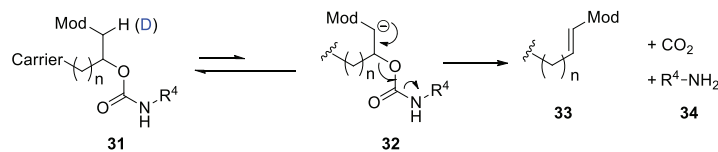


Figure 7. Deuteration applied to drug release and polymer biodegradation.

8) results in enhanced hydroxylation at the 4' position, while reducing N-dechlorination for all CYPs tested, compared to

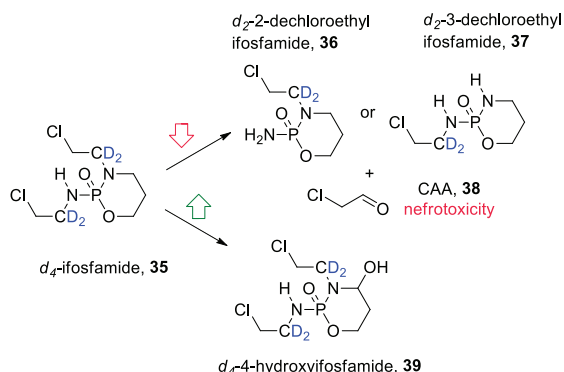


Figure 8. Metabolic pathway of d_4 -ifosfamide.

the nondeuterated drug.⁶⁰ The metabolic shunting favoring the 4-hydroxylation over N-dechlorination is noteworthy, as the latter leads to more toxic and less efficacious metabolites, such as chloroacetaldehyde 38, responsible for the ifosfamide-specific nephrotoxicity, suggesting that the deuterium-induced metabolic shunting might improve the therapeutic index of this chemotherapeutic agent.

Precision deuteration has enabled the reduction of nephrotoxicity also in the case of JNJ38877605 (15, Figure 9), a c-Met inhibitor, whose clinical study was terminated due to the poor solubility of its AO metabolites that are responsible for renal toxicity. In order to lower the AO-driven metabolism, two strategies were pursued. The first one, which eventually led to volitinib (40, Figure 9), which is currently in clinical trials, was the substitution of the quinoline substructure with an imidazo[1,2-*a*]pyridine.⁶¹ Yet a parallel deuterium approach was also undertaken. Indeed, incorporation of deuterium at the 2-position of quinoline ring (15, Figure 9) resulted in retention of biological activity, an improved stability toward AO, and a reduction in the formation of the two metabolites in plasma (41 and 42, Figure 9), together with an increased oral exposure (1.88-fold AUC and 1.56-fold C_{max}) compared to the nondeuterated analogue.⁴⁴ This led to the demonstration of higher efficacy/potency in a cancer xenograft model. It is interesting to note that other substitutions, for example, with a fluorine atom, would not have been possible as they would

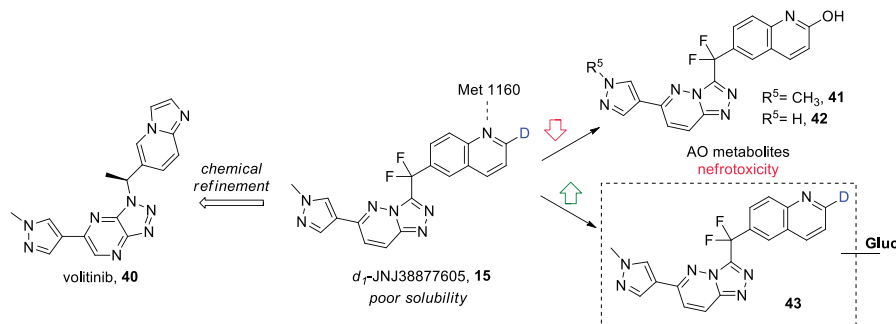


Figure 9. Metabolic pathway of d_1 -JNJ38877605.

have hampered a key hydrogen bond between the quinoline nitrogen atom and Met1160 of the kinase.

Another report, even if at an early stage of development, describes the potential reduction of the gut motility side effects associated with erythromycin B.⁶² In acidic aqueous solution, erythromycin exists in equilibrium with its 6,9-enol ether, which is likely to be responsible for the activity on gut motility. The incorporation of deuterium into position 8 (44, Figure 10) led to a significant reduction of the acid-catalyzed enol

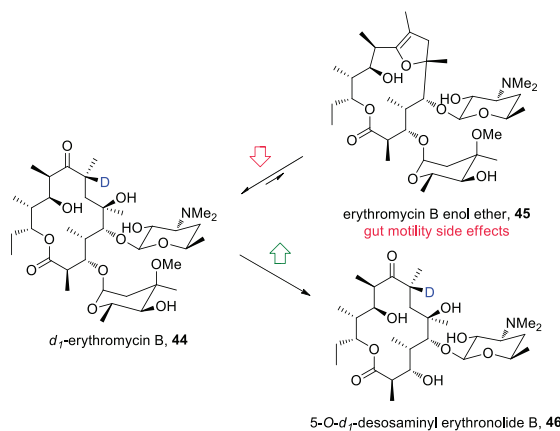


Figure 10. Metabolic pathway of d_1 -erythromycin B.

ether (45) formation and did not compromise the antibacterial effect, although the decrease of the gut motility effects in vivo was not demonstrated.

The potential of the work on tramadol is just as surprising. Briefly, tramadol is rapidly metabolized to its *O*-desmethyl metabolite, which, unlike the parent compound, displays important activity on opioid receptors. In vitro, d_9 -tramadol (47, Figure 11) shows a 5- to 10-fold higher metabolic

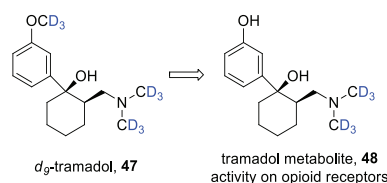


Figure 11. d_9 -Tramadol and its *O*-desmethyl metabolite.

stability. Despite this, when intraperitoneally administered in a rat tail-flick model at the same dose, tramadol and d_9 -tramadol did not show any difference in the extent and duration of analgesia.⁶³ While of interest, these results may have a number of different explanations, which have not been elucidated further by the authors. Indeed, tramadol is often referred to as a prodrug, and therefore it would have been expected that the effect of d_9 -tramadol should have been blunted. Given that this is not the case, it might reflect differences between *in vitro* and *in vivo* metabolism or an effect of tramadol itself on norepinephrine and serotonin reuptake, a reported action of this drug as well as a known mechanism to achieve an analgesic effect (e.g., duloxetine, venlafaxine). This latter explanation would mean that the two drugs exert similar overall effects via separate mechanisms. This possibility is worth exploring in the future, as it would suggest d_9 -tramadol is a strong analgesic not acting on opioid receptors and therefore devoid of opioid-related side effects. Unfortunately, the authors did not characterize the deuterated form of the *O*-desmethyl metabolite (48, Figure 11) *in vivo*, as this might have yielded further data. While inconclusive, this example suggests in principle that a further application of deuteration would be to modify drug to metabolite ratio to eventually exert different clinical effects.

Last, precision deuteration may also be used in a paradoxical manner to reduce drug–drug interactions, which may indirectly cause significant side effects. CYP2D6 is inhibited by paroxetine (49) as it first catalyzes the oxidation of the methylenedioxy carbon (50, Figure 12) and then forms an

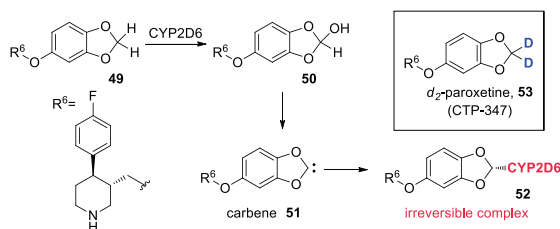


Figure 12. Irreversible complex with CYP2D6 of paroxetine and structure of d_2 -paroxetine.

irreversible complex 52 with the carbene species 51 formed after dehydration. The incorporation of deuterium at this hot-spot (d_2 -paroxetine 53, CTP-347) attenuates this pathway, both in *in vitro* and in *in vivo* settings. Multidose PK in humans demonstrates that deuteration increases the rate of metabolism by reducing CYP2D6 inactivation and, at the same time, reduces possible drug–drug interactions, as exemplified when dextromethorphan was used as a selective probe for CYP2D6 activity.⁶⁴

(c) Deuterium-Induced Increase of Bioactivation.

Clopidogrel is an antithrombotic prodrug that requires the CYP450-mediated thiophene 2-activation for its bioactivation. The observed clinical variability and resistance have been, in part, associated with low bioactivation efficiency, which in turn has been attributed to extensive metabolism, including oxidation, of the nonactivating piperidine motif. Perdeuteration of the piperidine substructure potentiated the prodrug activation percentage *in vitro* (19.3% of bioactivation percentage for d_6 -clopidogrel 55 versus 14.5% for clopidogrel 54) and led to a higher inhibitory activity against ADP-induced

platelet aggregation in a rat model, thereby suggesting that deuteration of competing soft-spots in prodrugs may be a possible strategy (Figure 13).⁶⁵

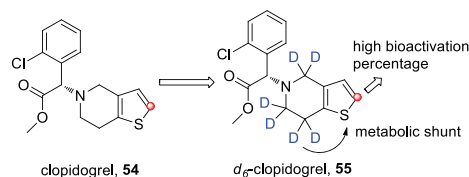


Figure 13. Clopidogrel and d_6 -clopidogrel.

(d) Deuterium To Stabilize Chemically Unstable Stereoisomers. Since 1992, many compounds originally developed as racemates have been separated, evaluated, and developed as single preferred enantiomers, the so-called eutomers.⁶⁶ This approach, known as chiral switching, has resulted in several drugs marketed as pure enantiomers. Nevertheless, for a cluster of compounds chiral switching is impossible, due to the rapid interconversion of the stereoisomers *in vitro* and/or *in vivo*. In this setting, the substitution of the acidic proton with deuterium at the chiral center of a single stereoisomer might decrease the rate of atom abstraction and afford a good strategy to stabilize chemically unstable stereoisomers.

This approach was initially demonstrated in 2009, with the selective deuteration of telaprevir (56, Figure 14).⁶⁷ The chiral

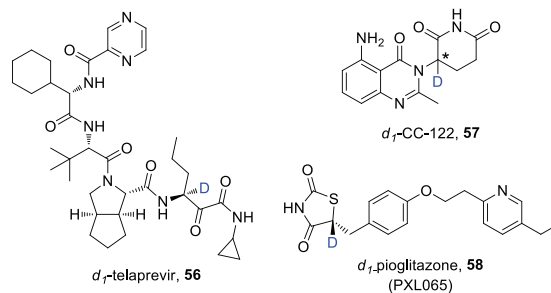


Figure 14. d_1 -Telaprevir, d_1 -CC-122 and d_1 -pioglitazone.

center next to the α -ketoamide in 56 (*S*-configuration) is prone to epimerization through proton exchange at alkaline pH via an enol tautomer, and the resulting (*R*)-diastereoisomer is approximately 30-fold less active against HCV proteases. Selective incorporation of deuterium at the chiral center was proven to decrease the rate of epimerization (with a DKIE ranging from 4 to 7), to enhance *in vitro* pH and plasma stability, and to reduce epimerization in preclinical models.

Similarly, thalidomide bears a chiral center with an exchangeable hydrogen, and the ignominious teratogenic effect is attributed mainly to the (*S*)-enantiomer.⁶⁸ The administration of the single (*R*)-enantiomer does not overcome teratogenicity due to the considerable and rapid epimerization under physiological conditions. Deuteration was shown to be partly effective at overcoming this drawback as (*R*)- d_1 -thalidomide was shown to be 5 times more stable than (*R*)-thalidomide toward epimerization in various buffer solutions, although biological characterization of the deuterated form is not provided.⁶⁹

Given the importance that thalidomide derivatives (e.g., lenalidomide, pomalidomide, CC-11006, CC-220) have in blood cancers and may have in inflammatory diseases, a similar strategy was applied for the generation of further analogues, including CC-122 (57, Figure 14).⁷⁰ The authors show that deuterium stabilizes the configuration of the chiral center and that the (–)-deuterated enantiomer is the preferred stereoisomer for its *in vitro* anti-inflammatory and *in vivo* antitumorigenic effects, leading to an approach named as deuterium enabled chiral switching.

Pioglitazone is a racemic mixture of two interconverting enantiomers. The ability of deuterium to stabilize the preferred (*R*)-enantiomer of pioglitazone (58, Figure 14) has also been suggested,⁷¹ although no full paper has been published.

(e) Deuterating Compounds as Experimental Strategies To Elucidate Mechanisms. Deuteration can also be used as a means to expand knowledge, for example, to test the contribution of a metabolite to the action of a particular drug, and work performed on ketamine (KET, 59, Figure 15)

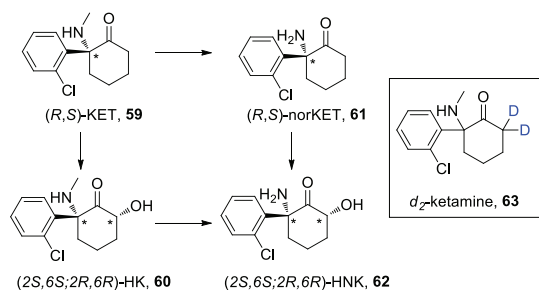


Figure 15. Ketamine, its metabolites, and d_2 -ketamine.

provides an example of this. This drug is known for its short-acting anesthetic effect, but it has recently been suggested that a single subhallucinogenic dose of ketamine leads to a rapid antidepressant effect. This effect starts 4 h after intravenous administration and lasts for 1–2 weeks.⁷² To verify the mechanism of the antidepressant activity, the levels of ketamine and its metabolites, such as (2*S*,6*S*;2*R*,6*R*)-hydroxyketamine (HK, 60), norketamine (nor-KET, 61), and (2*S*,6*S*;2*R*,6*R*)-hydroxynorketamine (HNK, 62, i.e., the major metabolite found in plasma and brain), were monitored in the brains of female and male rodents. Indeed, the antidepressant activity seems to be more marked in female than in male. Surprisingly, in the brain of females 3-fold higher levels of HNK than in male were discovered, suggesting that this metabolite was responsible for the antidepressant activity. Indeed, the synthesis of d_2 -KET 63, which does not affect overall ketamine levels in brain but leads to a significantly lower levels HNK, had no antidepressant effects.⁷³

(f) PET Tracers. A field in which deuterium switch has gained increasing attention is represented by PET tracers, which have the great potential to both detect disease-related biochemical changes and serve as biomarkers during the development of potential drugs because of the presence of a radionuclide in their structure (commonly ^{18}F or ^{11}C). On the other hand, the development of PET tracers is challenging, and success or failure is determined by numerous physicochemical and pharmacological factors among which metabolism is of great relevance. PET imaging agents need to display fast clearance, but not too fast, and precision deuteration can

represent a viable solution in achieving the best compromise, improving *in vivo* behavior of the parent radioligand.

Among the early examples is the peripheral benzodiazepine receptor (PBR) radioligand [^{18}F]-64 reported in 2005.⁷⁴ As PBR density is increased in injured brains and in tumors, PBR imaging might be useful for visualizing the distribution of the receptor in these regions. The authors started from [^{18}F]-64, a potent ligand for PBR that, after *in vivo* administration, was rapidly metabolized by defluorination to form [^{18}F] F^- . This species is largely accumulated in bones, decreasing the effective signal and the sensitivity of PET image in brain. Deuterium incorporation would decrease the rate of defluorination, where the cleavage of the C–D is the rate-limiting step. Despite the $T_{1/2}$ (calculated as $\ln 2/\text{decay constant}$) in mouse plasma remaining almost the same (2.575 min for d_2 -[^{18}F]-64 versus 2.367 min for [^{18}F]-64), the $T_{1/2}$ in mouse brain was more than 60 min for d_2 -[^{18}F]-64, whereas that of [^{18}F]-64 was 2.227 min, with decreased radioactivity levels in mouse bones (Figure 16). The authors suggest that this is related to the level

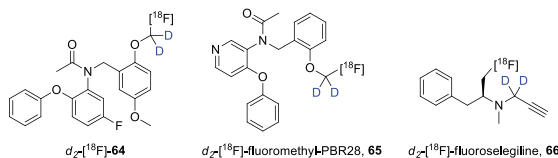


Figure 16. Structures of selected PET tracers.

of enzymes that in plasma is much higher than in brain. Unfortunately, after d_2 -[^{18}F]-64 injection, PET images of monkey revealed that there was higher radioactivity in skull compared to the brain, highlighting that d_2 -[^{18}F]-64 is rapidly defluorinated to form [^{18}F] F^- .

Similarly, the translocator protein (TSPO) is overexpressed in ischemic brain injury and degenerative brain diseases, and for this reason it has become an attractive biomarker to visualize neuroinflammation. Among the TSPO selective radiotracers developed so far, [^{18}F]-fluoromethyl-PBR28 shows promising profile in terms of *in vitro* biological properties, but it is affected by a low metabolic stability due to defluorination.⁷⁵ The corresponding deuterated analogue, d_2 -[^{18}F]-fluoromethyl-PBR28 65 (Figure 16), while retaining the same *in vitro* biological properties, shows higher organ uptake (except for the skull), enhanced *in vivo* stability and reaches the highest target-to-background ratio, an index commonly used in the field, at an early imaging time (3.8-fold versus 3.0-fold for the nondeuterated compound).⁷⁶

Both of these examples suggest that deuterium substitution could be an effective strategy to reduce defluorination rate and improve the stability of fluoromethoxy groups.

In a similar manner as for TSPO, deuteration has recently found application in the development of agents able to visualize the *in vivo* activity of MAO-B. MAO-B system is up-regulated in neuroinflammatory processes, and radiolabeled MAO-B inhibitors may serve as biomarkers in neuroinflammation and neurodegeneration (e.g., Alzheimer and Parkinson diseases, epilepsy). d_2 -[^{18}F]-Fluoroselegiline 66 (Figure 16) is a radiolabeled MAO-B inhibitor developed as a molecular imaging biomarker for the quantification of the enzyme activity in brain.⁷⁷ Its nondeuterated counterpart binds too quickly and irreversibly to the enzyme, and therefore, the distribution in tissue is limited by blood flow rather than by

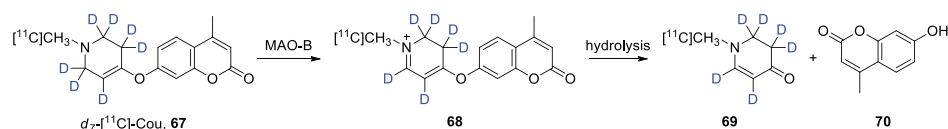
Figure 17. Metabolic pathway of d_7 - $[^{11}\text{C}]$ -Cou.

Table 2. Synthetic Approaches Used for the Most Advanced Deuterated Candidates

compd	synthetic approach	deuterium source ^b
deutetrabenazine, 1	deuterated pool	CD_3OD
d_9 -ivacaftor, 23 (CTP-656)	deuterated pool	D_2SO_4 , d_{10} - <i>tert</i> -butanol
d_6 -dextromethorphan, 30 (+quinidine, AVP-786)	deuterated pool	CD_3I
d_2 -linoleic acid ethyl ester, 92 (RT001)	deuterated pool	d_2 -paraformaldehyde
d_4 -sodium oxibate, 81 (JZP-386)	deuterated pool	d_4 -succinic anhydride, CH_3OD
d_3 -sorafenib, 106 (donafenib)	deuterated pool	d_3 -methyl amine
d_5 -apremilast, 83 (CTP-730) ^a	deuterated pool	d_5 -bromoethane
d_{15} -atazanavir, 29 (CTP-518)	deuterated pool	d_9 -2-chloro-2-methylpropane, CD_3OD
BMS-986165, 2	deuterated pool	d_3 -methylamine hydrochloride
d_9 -L-838417, 4 (CTP-354)	deuterated pool	d_9 -trimethylpivalic acid
d_7 -tolperisone, ^a 71 (BDD-10103)	deuterated pool	d_8 -toluene
d_9 -venlafaxine, 72 (SD-254)	deuterated pool	CD_3I
VX-984, 3	X/D exchange	d_2 -formic acid, Pd, CD_3OD
d_3 -vitamin A, 93 (ALK-001)	H/D exchange	D_2O , NaOD
CTP-499, 105	H/D exchange	D_2O , K_2CO_3
d_3 -L-DOPA, 5 (SD-1077) ^a	H/D exchange	DCl , D_2O , CD_3OD
d_1 -pioglitazone, 58 (PXL065)	H/D exchange	d_6 -DMSO, CD_3OD
d_9 -ruxolitinib, 82 (CTP-543) ^a	H/D exchange and deuterated pool	d_8 -1,4-dibromobutane, D_2O , NaOD
d_2 -paroxetine, 53 (CTP-347)	H/D exchange and deuterated pool	D_2O , CD_2Cl_2

^aThe structure has not been disclosed yet. The reported synthetic approach and the deuterium source are putative. ^bThe deuterium sources are those reported in patent literature. The synthetic approaches used for industrial productions may be different.

MAO-B levels.⁷⁸ As the MAO-B catalyzed cleavage of the C–H bond in the propargyl moiety is the rate-limiting step in the retention of the radioligand in brain, the authors suggested that the substitution of H with D would have reduced the rate of cleavage and, therefore, the affinity to MAO-B, ultimately improving the sensitivity. Indeed, the deuterated tracer showed a less irreversible behavior and a decreased rate of trapping in the monkey brain with an improved sensitivity. Furthermore, deuterium incorporation increased the stability in monkey blood plasma.

While in the above example the deuteration approach has proven to be successful, very recently it has been reported that incorporation of deuterium in the metabolic trapping agent $[^{11}\text{C}]$ -Cou 67 (Figure 17) did not afford the expected results. $[^{11}\text{C}]$ -Cou undergoes a MAO-B oxidation followed by hydrolysis, and the resulting metabolite is trapped in the brain. Similar to d_2 - $[^{18}\text{F}]$ -fluoroselegiline 66, the rapid trapping rate makes it difficult to discriminate between regions of high and low MAO enzymatic activity. Incorporation of deuterium in the tetrahydropyridine ring did not reduce the rate of trapping in brain tissues, despite strong literature precedent demonstrating a significant DKIE (3.55) on MPTP and similar substructures.⁷⁹ Indeed, no effect of deuterium in slowing the MAO-B mediated oxidation step and reducing the rate of trapping in brain tissues was seen, neither in *in vitro* nor in *in vivo* PK studies.⁸⁰ This can be explained by considering the masking of the DKIE by the second rate-limiting hydrolytic step leading to 69 and 70, in the overall metabolism of this radiotracer.

SYNTHETIC APPROACHES IN PRECISION DEUTERATION

In the synthesis of deuterium-labeled compounds, two main approaches can be explored: a conventional multistep synthesis that we name “deuterated pool” (DP) synthesis and an isotope exchange approach.

In the first case, the desired compound is afforded using conventional reactions through a multistep route, starting from commercially available and low molecular weight deuterium-labeled building blocks. Obviously, deuterated reagents are more expensive than the corresponding parent ones, but this is counterbalanced by the fact that deuterated pool synthesis ensures perfect regio- and chemoselectivity and almost 100% efficiency. A variety of deuterated reagents are commercially available from dedicated companies, such as C/D/N Isotopes, Cambridge Isotope Laboratories, and CombiPhos Catalysts. The source of all the deuterated starting reagents is deuterium oxide, produced from regular water by using Girdler sulfide process, an industrial procedure to enrich deuterium in water up to 20%. This mixture is then distilled to afford pure deuterium oxide, representing the only case in which it is possible to separate labeled compounds from the corresponding nondeuterated one. Notably, a company named Isowater (<https://www.isowater.com>) supplies heavy water to non-nuclear users in life sciences and high technology and is currently developing a new proprietary process for making D_2O .

In the isotope exchange approach, deuterium is inserted on the target molecule or on a late intermediate using a deuterium donor (usually D_2O or D_2 gas) by (i) reductive deuteration,

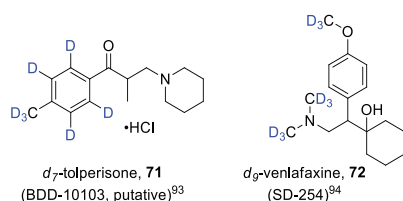
such as hydrogenation and borohydride reduction;⁸¹ (ii) X/D (halogen–deuterium) exchange via aromatic dehalogenation;⁸² or (iii) H/D (hydrogen–deuterium) exchange.⁸³ Alternative methods have started to appear in the literature concomitantly with the explosion of precision deuteration of bioactive compounds, and in particular, elegant photocatalytic deuteration methods have been very recently described.⁸⁴

Among all the methods belonging to the isotope exchange approach, the H/D exchange approach is the most exploited one and includes the pH-dependent H/D exchange⁸⁵ and the metal-catalyzed H/D exchange.⁸⁶

The pH-dependent H/D exchange is based on the enolization of specific groups in the substrate, requires a deuterium source, and can be acid- or base-catalyzed. In the first case, both Lewis and deuterated Brønsted acids can be used, while in the second case a deuterated or nondeuterated base is necessary. Moreover, for some pH-dependent H/D exchanges, acids and bases are unnecessary as deuterium oxide, due to its autoprotolysis process, is able to deuterate acidic positions.

Among the metal catalyzed H/D exchange procedures, both heterogeneous and homogeneous systems are reported.⁸⁷ In general, homogeneous catalysis offers a better selectivity in the incorporation at the different positions and milder reaction conditions, but conversely, it needs the preparation of complex catalysts and is usually associated with higher costs. A plethora of different catalysts (e.g., Ir,⁸⁸ Ru,⁸⁹ Pd,⁹⁰ Pt⁹¹) are reported in the literature for the H/D exchange of aromatic, aliphatic, allylic, vinylic, heteroaromatic positions or for the exchange of hydrogen adjacent to oxygen- and nitrogen-based directing groups.

Except for a few cases, the H/D exchange approach requires harsh experimental conditions, including the use of strong acids and bases and heavy metals. In the context of drug development, this might represent a big concern, in terms of both safety and tolerance toward functional groups, especially when the exchange is performed in the late-stage part of the synthetic route. Lower selectivity and efficiency are sometimes encountered, with the consequent necessity of reiterating the exchange process to achieve high deuterium enrichment. For these reasons, as shown in Table 2 and Figure 18, it is not



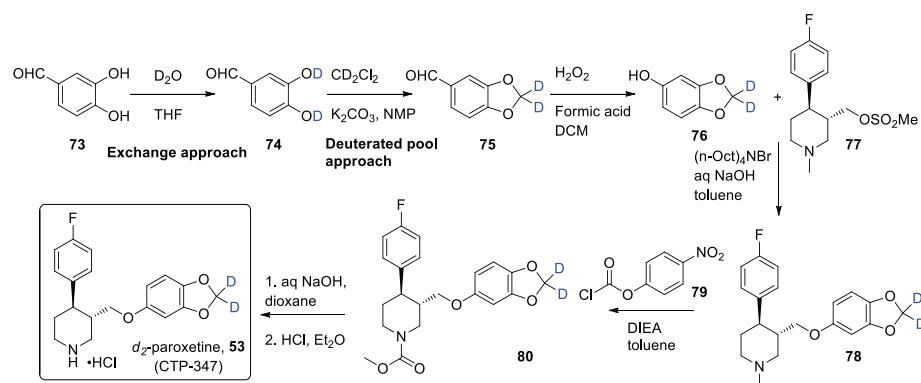


Figure 19. Synthesis of d_2 -paroxetine.

have not been included in Table 3 as trials relating to these molecules were not found in Clinicaltrials.gov, the unbiased source we chose to identify the molecules. Registration on Clinicaltrials.gov is mandatory only for those trials that enroll patients in the United States and is not required for phase I clinical candidates. To overcome this limitation, we searched company Web sites and we used search engines to compile a further list of deuterated drugs entered in clinical development (Table 4). Furthermore, it must be highlighted that pharmaceutical companies often adopt the policy of not disclosing chemical structures until late clinical development; hence we cannot exclude that some other deuterated compounds may have entered clinical development without being recognized as such.

Among the plethora of deuterated compounds that we identified, we arbitrarily describe below those compounds not extensively elucidated in previous reviews and for which we found literature that, in our opinion, represents successful examples of precision deuteration and that go beyond simple slowing down of metabolism.

Inhibition of Chemical Processes of Nutritional Agents. 1. *The Case of RT001.* Linoleic acid (**86**, Figure 21), the most abundant polyunsaturated fatty acid (PUFA) in humans,¹⁰⁸ displays an 18-carbon chain containing a bis-allylic methylene group ($-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}=\text{CH}-$). The C–H bonds in the methylene group therein are weak enough to be cleaved by reactive oxygen species (ROS), thus allowing the initiation of the lipoperoxidation process.¹⁰⁹ More precisely, when ROS rip hydrogens from susceptible bis-allylic methylene groups, resonance-stabilized free radicals **87** and **88** are formed. The latter, after having reacted with oxygen, are then transformed into lipid radicals, which trigger the lipid peroxidation chain reactions. 11,11- d_2 -Linoleic acid ethyl ester (RT001, **92**) has been developed on the basis that the bis-allylic methylene group could be stabilized by a double hydrogen–deuterium substitution, thereby reducing the progress of lipid peroxidation.

Given encouraging results in a number of in vitro and in vivo models of neurological degenerative disorders in which free radicals are thought to play a role,¹¹⁰ the sponsor (Retrotope) decided to test the compound in Friedreich's ataxia (FA),¹¹¹ which is a rare inherited disorder caused by a mutation of the gene encoding for frataxin (FXN). Mitochondrial abnormalities due to FXN mutations and consequent increased lipid peroxidation are thought to be relevant in FA and are the

rationale to test RT001.¹¹² Patients affected by FA, for which there is no approved medicine, suffer from progressive ataxia associated with areflexia, dysarthria, scoliosis, sensory deficits, cardiomyopathy, and increased odds of developing diabetes mellitus.¹¹³

In the recent phase I/II double blind, comparator-controlled trial (NCT02445794), the preliminary efficacy, safety, and PK properties of RT001 were tested in 19 FA adult patients that were randomized to receive either RT001 or nondeuterated linoleic acid for 28 days. The deuterated compound was shown to be safe, well-tolerated and to lead to an ameliorated motor capability in FA patients. However, this preliminary evidence, even if encouraging, must be interpreted with caution given the extremely limited sample size and the short duration of the study, which might overestimate the beneficial effects.¹¹¹ It should be noticed that the use of the nondeuterated compound as comparator makes the trial design informative.

Retrotope has more recently also sponsored a trial on infantile neuroaxonal dystrophy (NCT03570931), an ultrarare life-threatening neurodegenerative disorder.

2. *ALK-001 in Stargardt's Disease.* Stargardt's disease, also known as juvenile macular degeneration, is an autosomal recessive macular dystrophy, in which there is an irreversible central visual loss arising in teenage years, which is primarily due to atrophy of the retinal pigment epithelium (RPE) and progressive loss of photoreceptors functionality. As for Friedrich's ataxia, no cure is at present available for Stargardt's disease.¹¹⁴

Stargardt's disease is attributable to mutations of the gene encoding for the ABCA4 protein (ATP-binding cassette, subfamily A, member 4), a transmembrane flippase expressed in retinal photoreceptors that transports retinaldehyde-PE Schiff base from the luminal side of the disc membrane to the outer side, thus ensuring photoreceptor homeostasis. If a defective functioning of ABCA4 protein exists, retinaldehyde-PE conjugates react to form vitamin A polyene dimers (N-retinylidene-N-retinylethanolamine **101** and all-*trans*-retinaldehyde dimer **102**, Figure 22), which, once deposited in the RPE, foster the production of lipofuscin, responsible for retinal degeneration.¹¹⁵

Since the rate-determining step in vitamin A dimerization is the cleavage of a C20 C–H bond in retinaldehyde-PE Schiff base, deuterium atoms were substituted to C20 hydrogens in order to slow vitamin A dimerization.¹¹⁶ Interestingly, the intraperitoneal administration of deuterated vitamin A (d_3 -

Table 3. Deuterated Compounds Entered in Clinical Development and Found via a Systematic Search on ClinicalTrials.gov⁴⁴

deuterated compd (synonyms)	parent compd	therapeutic indication	ClinicalTrials.gov identifier (year of data registration)	phase	status
deutetabenazine, 1 (<i>d</i> ₆ -tetabenazine; SD809; SD-809, TEV-50717)	tetabenazine	Chorea associated with Huntington disease	NCT01897896 (2013)	Phase III	Completed/ FDA approval
		Chorea associated with Huntington disease	NCT01795859 (2013)	Phase III	Completed/ FDA approval
		Tardive dyskinesia	NCT02195700 (2014)	Phase II/III	Completed/ FDA approval
		Tardive dyskinesia	NCT02198794 (2014)	Phase III	Active, not recruiting
		Tardive dyskinesia	NCT02291861 (2014)	Phase III	Completed/ FDA approval
		Tourette syndrome	NCT02674321 (2016)	Phase I	Completed
		Tourette syndrome	NCT03571256 (2018)	Phase III	Recruiting
		Tourette syndrome	NCT03567291 (2018)	Phase III	Recruiting
		Tourette syndrome	NCT03452943 (2018)	Phase II/III	Recruiting
		Tourette syndrome	NCT02534636 (2015)	Phase I	Completed
BMS-986165, 2	novel compound		NCT03004768 (2016)	Phase I	Completed
			NCT03044873 (2017)	Phase I	Completed
			NCT03262727 (2017)	Phase I	Completed
			NCT03254784 (2017)	Phase I	Completed
			NCT03419910 (2018)	Phase I	Completed
			NCT03541564 (2018)	Phase I	Completed
			NCT03660436 (2018)	Phase I	Recruiting
			NCT03402087 (2018)	Phase I	Completed
		Psoriasis	NCT02931838 (2016)	Phase II	Completed
		Psoriasis	NCT03624127 (2018)	Phase III	Recruiting
		Psoriasis	NCT03611751 (2018)	Phase III	Recruiting
		Systemic lupus erythematosus	NCT03252587 (2017)	Phase II	Recruiting
		Crohn's disease	NCT03599622 (2018)	Phase II	Recruiting
		Advanced solid tumors	NCT02644278 (2015)	Phase I	Completed
VX-984, 3 (M9831)	novel compound				
CTP-656, 23 (<i>d</i> ₆ -ivacaftor, VX-561, c-10358, c-10355)	ivacaftor		NCT02599792 (2015)	Phase I	Completed
			NCT02392702 (2015)	Phase I	Completed
			NCT02680249 (2016)	Phase I	Completed
		Cystic fibrosis	NCT02971839 (2016)	Phase II	Terminated
		Cystic fibrosis	NCT03227471 (2017)	Phase I/II	Completed
CTP-518, 29 (<i>d</i> ₁₅ -atazanavir)	atazanavir		NCT03224351 (2017)	Phase II	Completed
			NCT01458769 (2011)	Phase I	Completed
AVP-786 (<i>d</i> ₆ -dextromethorphan 30 + quinidine; CTP-786; <i>d</i> -DM; deudextromethorphan hydrobromide [<i>d</i> ₆ -DM 30]/quinidine sulfate [Q]; deuterated dextromethorphan plus ultra low dose quinidine; deuterated DM plus ultra low dose quinidine; deuterated-dextromethorphan; deuterated DM)	dextromethorphan		NCT01787747 (2013)	Phase I	Completed
			NCT02174822 (2014)	Phase I	Completed
			NCT02174835 (2014)	Phase I	Completed
		Major depressive disorder	NCT02153502 (2014)	Phase II	Completed
			NCT02336347 (2015)	Phase I	Completed
			NCT02402595 (2015)	Phase I	Completed
		Disinhibition syndrome	NCT02534038 (2015)	Phase II	Terminated
		Residual schizophrenia	NCT02477670 (2015)	Phase II	Completed
		Agitation in patients with dementia of the Alzheimer's type	NCT02446132 (2015)	Phase III	Recruiting
		Agitation in patients with dementia of the Alzheimer's type	NCT02442765 (2015)	Phase III	Recruiting
		Agitation in patients with dementia of the Alzheimer's type	NCT02442778 (2015)	Phase III	Recruiting
		Agitation in patients with dementia of the Alzheimer's type	NCT03393520 (2018)	Phase III	Recruiting

Table 3. continued

deuterated compd (synonyms)	parent compd	therapeutic indication	ClinicalTrials.gov identifier (year of data registration)	phase	status
JZP-386, 81 (<i>d</i> ₄ -sodium oxybate; C-10323) CTP-543, 82 (<i>d</i> ₈ -ruxolitinib)	sodium oxybate ruxolitinib	mentia of the Alzheimer's type			
		Neurobehavioral disinhibition	NCT03095066 (2017)	Phase II	Recruiting
		Intermittent explosive disorder	NCT03420222 (2018)	Phase II	Recruiting
			NCT02215499 (2014)	Phase I	Completed
			NCT02960945 (2016)	Phase I	Completed
CTP-730, 83 (<i>d</i> ₃ -apremilast)	apremilast		NCT02777008 (2016)	Phase I	Completed
		Alopecia areata	NCT03137381 (2017)	Phase II	Active, not recruiting
			NCT02239081 (2014)	Phase I	Completed
RT001, 92 (<i>d</i> ₂ -linoleic acid ethyl ester; 11,11-D2-ethyl linoleate; ethyl 11,11-D2-linoleate)	linoleic acid		NCT02404922 (2015)	Phase I	Completed
		Friedreich's ataxia	NCT02445794 (2015)	Phase I/II	Completed
		Infantile neuroaxonal dystrophy	NCT03570931 (2018)	Phase II/III	Not yet recruiting
ALK-001, 93 (<i>d</i> ₃ -vitamin A; C20-D3-retinyl acetate)	vitamin A		NCT02230228 (2014)	Phase I	Completed
			NCT02402660 (2015)	Phase II	Recruiting
		Stargardt disease	NCT01328821 (2011)	Phase I	Completed
CTP-499, 105 (PCS-499)	1-[(S)-5-hydroxyhexyl]-3,7-dimethylxanthine (HDX)				
		Nondialysis patients associated with moderate chronic kidney disease	NCT01460199 (2011)	Phase I	Completed
		Type 2 diabetic nephropathy	NCT01487109 (2011)	Phase II	Completed
		Advanced hepatocellular carcinoma	NCT02229071 (2014)	Phase I/II	Completed
		Advanced hepatocellular carcinoma	NCT02645981 (2016)	Phase II/III	Not recruiting
		Advanced gastric cancer	NCT02489214 (2015)	Phase I/II	Recruiting
		Advanced, inoperable esophageal cancer	NCT02489201 (2015)	Phase I/II	Recruiting
		Metastatic colorectal cancer	NCT02489916 (2015)	Phase I/II	Completed
		Metastatic colorectal cancer	NCT02870582 (2016)	Phase III	Recruiting
		Metastatic nasopharyngeal carcinoma	NCT02698111 (2016)	Phase I/II	Recruiting
		Thyroid cancer	NCT02870569 (2016)	Phase II	Active, not recruiting
		Differentiated thyroid cancer	NCT03602495 (2018)	Phase III	Recruiting
donafenib, 106 (<i>d</i> ₃ -sorafenib; CM 4307)	sorafenib				

^aThe list might not be complete as not all sponsors might have declared the deuterated nature of the investigational drug.

vitamin A **93**) in wild-type rodents with no defects in vitamin A processing was shown to diminish the biosynthesis of vitamin A dimers.¹¹⁵ Preclinical evidence subsequently confirmed that *d*₃-vitamin A, when orally administered as ALK001 (Alkeus Pharmaceuticals) in mouse models of Stargardt's disease, has the capability of both slowing vitamin A dimerization rate and lipofuscinogenesis.¹¹⁷ However, it must be highlighted that the preclinical demonstration of the efficacy of *d*₃-vitamin A in reducing dimerization of vitamin A and lipofuscin formation required a complete replacement of vitamin A with its deuterated form in mouse models,^{116,117} and hence, the translatability of these data to humans needs to be demonstrated.

To date, a phase I clinical trial (NCT02230228) has been conducted in order to assess the safety and PK properties of ALK001 in 40 healthy adults. The trial is complete, although no data are available, and a multicenter, randomized, placebo-controlled phase II study (NCT02402660) aimed at assessing the long-term safety and efficacy of ALK001 on the progression of Stargardt's disease in both pediatric and adult patients is now ongoing.

CTP-499: A Deuterated Metabolite of Pentoxifylline. Pentoxifylline (PTX **103**, Figure 23) is a synthetic dimethylxanthine derivative that acts as a nonselective phosphodiesterase inhibitor. Given its well-known hemorheologic properties, PTX has long been used to treat occlusive peripheral vascular diseases. Interestingly, PTX is recognized to

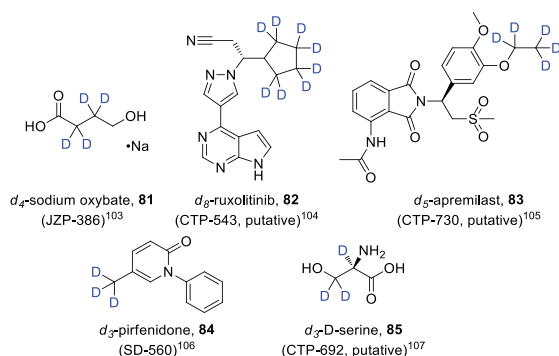


Figure 20. Structures of clinical candidates from Tables 3 and 4 not shown elsewhere.

have a number of favorable effects other than those on blood flow, including anti-inflammatory, antiproliferative, immunomodulatory, and antifibrotic properties, all of which have contributed to make PTX a good drug for treating chronic kidney disease (CKD). Such a renoprotective function is mainly attributed to one of PTX active metabolites, which is 1-[(S)-5-hydroxyhexyl]-3,7-dimethylxanthine (HDX **104**). In order to increase the stability of HDX, five hydrogen atoms at the 4- and 6-positions of the hexyl moiety of HDX were replaced by deuterium atoms (CTP-499 **105**). Preclinical evidence suggests that, similar to HDX, CTP-499 has antifibrogenic, antioxidative, and anti-inflammatory properties in rat models of CKD.¹¹⁸

A phase I study (NCT01328821) on CTP-499 was conducted to assess its PK, safety, and tolerability after single dose administration. CTP-499 exposure was herein compared with that of PTX in male healthy adults (phase Ia). Interestingly, the plasma concentrations of CTP-499 and related major metabolites (M1-M5) were shown to be higher compared to those of PTX.¹¹⁹ The tolerated dose and the effects of food on CTP-499 PK were also defined herein.¹²⁰ Hence, a subsequent phase Ib randomized, placebo-controlled study was performed to investigate safety and tolerability of CTP-499 in CKD patients. The deuterated compound was reported to be safe and to be rapidly absorbed, with a low interindividual variability.¹²¹ On this basis, a 24-week phase II study (NCT01487109) was initiated to evaluate the safety and efficacy of CTP-499 in patients affected by diabetic nephropathy and treated with an ACE inhibitor and/or an

angiotensin II receptor blocker. Even if the study is now completed, trial results are not yet publicly available.

Nomenclature of Deuterated Compounds. Most regulated countries require that drugs that enter the market are assigned an approved name. While a number of countries have their own naming agency (United States, Great Britain, Japan, China), most countries rely on the World Health Organization (WHO) to allocate an international non-proprietary name (INN), i.e., the global approved name. Importantly, also where national agencies exist, there is always the will to align their decisions to those of WHO, whenever possible, to reduce confusion which may lead to medication errors. Names follow conventions and deuterated compounds usually are flagged with the prefix deu- and take the identical INN of the parent nondeuterated compound, if allocated beforehand (for example, deutetabenazine). A list of all WHO approved names and structures is published by the WHO. The list includes deutolperisone, deutetabenazine, deudextromethorphan, and deutivacaftor. One of the deuterated compounds in clinical trials well illustrates why this system is in place. Indeed, clinical trials have been initiated in a number of cancer types with a compound referred to by the applicant and in the literature as donafenib (**106**, Figure 24). It is likely that this name was chosen by the company developing the compound and not by any naming agency as it represents a deuterated form of the known and approved tyrosine kinase inhibitor sorafenib. If clinical trials were to be successful and the drug were to be approved, it would therefore most likely receive the INN deusorafenib to show the relationship with sorafenib. It is also interesting that the naming conventions of WHO has changed through the years. Indeed, in the 1990s a deuterated derivative of ascorbic acid, possibly the first deuterated drug to receive an INN, was named zilascorb(2H) (**107**, Figure 24). Zilascorb(2H) was studied in phase I trials for cancer and was later abandoned.

FINAL REMARKS AND CONCLUSIONS

While the use of deuterium in drug design is steadily increasing, a number of issues have probably slowed down the uptake of this bioisosteric replacement in recent years, namely, (i) an erroneous perceived possible toxicity of deuterium, (ii) risks associated with intellectual property, (iii) uncertainties around the costs associated with the active principle, and (iv) from an industrial setting, the regulatory path that will be applied by regulatory agencies for deuterium greening.

Table 4. Deuterated Compounds Declared To Be in Clinical Development but Not Found with the Systematic Search Described in the Text^a

deuterated compd (alternative names)	parent compd	therapeutic indication	phase	status
CTP-354, 4 (d_9 -L838417; C-21191)	L-838417	Spasticity	Phase I	Completed
SD-1077, 5 (d_3 -L-DOPA)	L-DOPA	Parkinson's disease	Phase I	Unknown ^b
CTP-518, 29 (d_{15} -atazanavir)	atazanavir	HIV infections	Phase I	Unknown ^b
CTP-347, 53 (d_2 -paroxetine)	paroxetine	Menopause, hot flashes and vasomotor symptoms	Phase I	Completed
PXL065, 58 (d_1 -pioglitazone; DRX-065)	pioglitazone	Adrenomyeloneuropathy, nonalcoholic steatohepatitis	Phase I	Recruiting
BDD-10103, 71 (d_7 -tolperisone)	tolperisone	Spastic paralyzes	Phase I	Unknown ^b
SD-254, 72 (d_9 -venlafaxine)	venlafaxine	Major depressive disorder	Phase I	Completed
SD-560, 84 (d_3 -pirfenidone)	pirfenidone	Idiopathic pulmonary fibrosis	Phase I	Unknown ^b
CTP-692, 85 (d_3 -D-serine)	D-serine	Schizophrenia	Phase I	Recruiting

^aInformation was found on search engines and on company Web sites, and therefore the list might not be complete. ^bNo explicit information concerning the status of the trial is available on the company Web site.

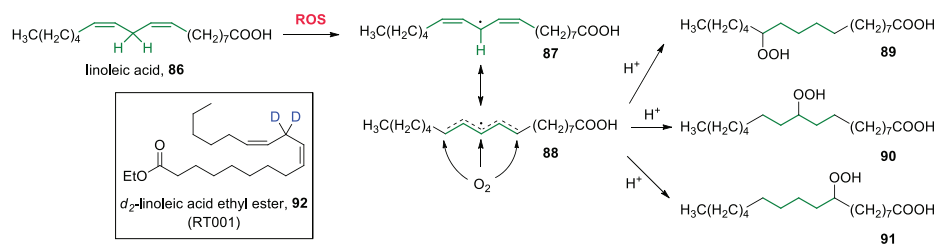


Figure 21. Lipoperoxidation process of linoleic acid and structure of RT001.

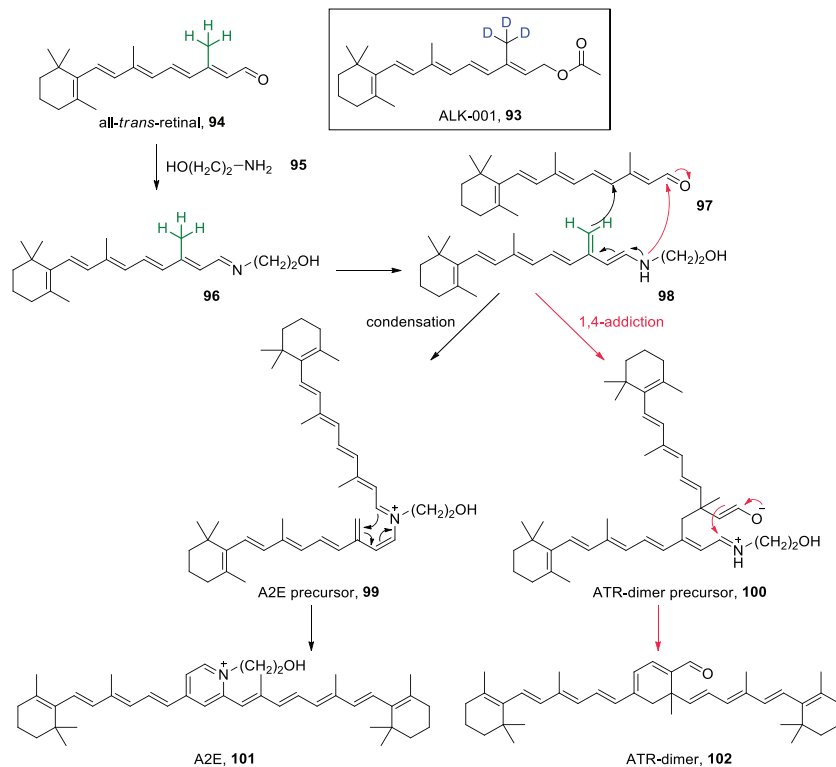


Figure 22. Biosynthesis of vitamin A polyene dimers and structure of ALK-001.

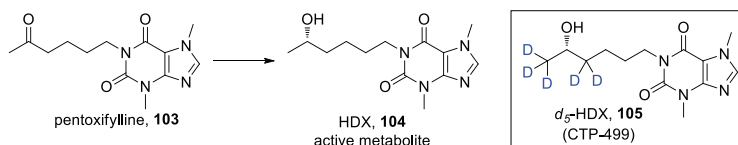


Figure 23. Pentoxifylline, HDX, and d₅-HDX.

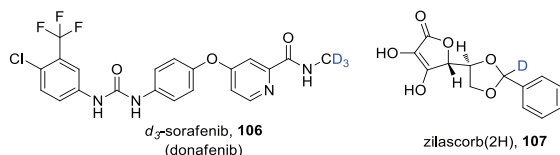


Figure 24. d₃-Sorafenib and zilascorb(2H).

Most studies relating to the toxicity of deuterium are relatively old, yet data are reassuring. While it could be expected that deuterium, at high enough concentrations, may be toxic, it is estimated that the threshold for clinically relevant side effects is between 200 and 400 mg/kg of pure D₂O (i.e., 14 g for a 70 kg person), well above what can be envisaged for a medical use.¹²² Indeed, the amount of deuterium in bioactive molecules would be lower by 2–3 orders of magnitude. Safety of deuterium per se is not therefore a real concern. Another issue that limited deuterated drug R&D in the past was

sensitive analytical techniques (e.g., LC/MS/MS) to determine the percentage of deuterium content of a compound with a single deuterium.

Patentability of deuterated compounds is a subtler issue. De novo deuterated compounds obviously do not present any issues of obviousness, but deuterium switches are more complex from an intellectual point of view. Patent applications for deuterated compounds in this latter category have been allowed, rejected, or allowed after restriction.^{27b,c} Presumably, the different outcome depends on the effect that the deuterated compound has compared to the ancestor, on the data that support the claims, and on the prior art of the nondeuterated compound. Therefore, it is possible to protect the intellectual property in this instance if well supported by data. Indeed, the present review highlights that while the rationale to deuterate a compound may in many instances be strong, the outcome of deuteration is instead unpredictable and in no way obvious. It should be noted that postapproval litigations over deuterated drugs may be possible. Also, it must be considered that for many years deuteration was not an obvious practice and therefore should not be considered obvious in the prior art when considering older patents. It is therefore not surprising that the prevalence of patents that protect both deuterated and nondeuterated drugs is significantly increasing (independently of whether examples are present in the patent), an indication of the fact that the pharmaceutical industry is becoming sensitive to this issue, both scientifically (when examples are given) and legally (when examples are not given).

A further concern relating to a new deuterated compound is usually given by manufacturing costs. For everyday academic researchers who work on small scales, this is, in our experience, not an issue, as reagents are only moderately more expensive than their nondeuterated counterparts. Yet in a previous review in this journal, Gant suggested that this is not a concern also in industrial scale-up settings, and the cost of reagents should not represent a barrier, and bulk purchases will further reduce the price.^{6a} Indeed, the cost of enriched D₂O as a manufacturing deuterium source is low enough (and will probably decrease in the future) to generally enable acceptable costs for manufacturing heavy drugs. Furthermore, a deuterium switch can potentially lead to a lower dose, which ultimately may cost less to produce. While this is generally true, it should be noted that also in this case it will actually depend on the single compound. Most drugs currently in development relate to rare diseases or are for indications in which manufacturing costs will not affect at all the sale price. It is at present unclear whether costs might become a limiting factor for drugs in primary care or for compounds that might need to compete in the generic arena. The news that an abbreviated, hybrid pathway was employed (505(b)(2) by the FDA for deutetrabenazine approval increases the chance that deuterium-greening may turn to an opportunity, reducing risks and lowering the costs of preclinical and clinical development.

In summary, a critical appraisal of the literature suggests that deuterium is entering the medicinal chemist's toolbox, and it is foreseeable that drugs will be increasingly endowed with deuterium atoms in the future. Deuterium incorporation is particularly appropriate for those soft spots that suffer from strict and rigid structure–activity relationships, where no change in steric hindrance or electronegativity is tolerated. While a pure deuterium switch has become more difficult due to patentability concerns, we are already witnessing an

increasing use of deuterium incorporation during de novo drug discovery and development. Nevertheless, medicinal chemists should be aware that designing and developing a successful deuterated drug are far from straightforward. First of all, both intuition and rigor are necessary for selecting compounds and positions that might be amenable to precision deuteration and this cannot go beyond the understandings of metabolic fates, an evaluation of the nature of the oxidation reactions, their DKIEs and the synthetic feasibility of the deuterated compound. A beneficial effect of deuterium in vitro does not guarantee a success in biological systems. Indeed, substantial differences among species are often found and many different factors competing with DKIE might mask or even reverse the desired effects of deuteration. While finding an effect of deuteration may be left to a serendipitous approach, the literature is now providing examples of extremely rigorous and elegant approaches to obtaining the most from deuterium. This is nicely exemplified by the thorough work of Stringer et al.⁵¹ when developing the CRF₁ antagonist d₃-NVS-CRF38.

In conclusion, the examples provided by the literature would suggest that deuterium isosteric replacement will come of age and will be a great opportunity for medical chemists. Indeed, we share the vision of Sheila DeWitt, CEO of a pharmaceutical company that focuses its efforts on deuterated drugs, that in a recent interview stated “Fluorine was in less than 2% of drugs in the 1970s but is now in as many as 25% of approved drugs. You see fluorine in places you never thought possible. The same will happen to deuterium.”¹²³ On this note, it is interesting to appreciate that nowadays deuterium has also been used for bioisosteric replacements when fluorine replacement is unfeasible,⁴⁴ further strengthening the parallel between the two.

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Notes

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■ ABBREVIATIONS USED

AADC, aromatic L-amino acid decarboxylase; ABCA4, ATP-binding cassette, subfamily A, member 4; AO, aldehyde oxidase; CCR5, C–C chemokine receptor type 5; CdSe, cadmium selenide; CEO, Chief Executive Officer; CKD, chronic kidney disease; CRF1, corticotropin-releasing hormone receptor 1; CYP, cytochrome P₄₅₀; DBH, dopamine β-hydroxylase; DKIE, deuterium kinetic isotope effect; DNA-PK, DNA-dependent protein kinase; DP, deuterated pool; FA, Friedreich's ataxia; FXN, frataxin; H, protium; HDX, 1-[(S)-5-hydroxyhexyl]-3,7-dimethylxanthine; HLM, human liver microsome; HK, (2S,6S;2R,6R)-hydroxyketamine; HNK, (2S,6S;2R,6R)-hydroxynorketamine; INN, international nonproprietary name; KET, ketamine; KIE, kinetic isotope effect; L-DOPA, levodopa; MAO, monoamine oxidase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PBR, peripheral benzodiazepine receptor; PK, pharmacokinetics; PTX, pentoxifylline; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species; RPE, retinal pigment epithelium; SAR, structure–activity relationship; SGLT2, sodium–glucose cotransporter-2; TSPO, translocator protein; Tyk2, tyrosine kinase 2.

■ REFERENCES

- (1) Established by the internationally accepted Vienna Standard Mean Ocean Water (VSMOW). Rosman, K. J. R.; Taylor, P. D. P. Isotopic compositions of the elements 1997 (Technical Report). *Pure Appl. Chem.* **1998**, *70*, 217–235.
- (2) Urey, H. C.; Brickwedde, F. G.; Murphy, G. M. A hydrogen isotope of mass 2 and its concentration. *Phys. Rev.* **1932**, *40*, 1–15.
- (3) Miller, S. L. A production of amino acids under possible primitive earth conditions. *Science* **1953**, *117*, 528–529.
- (4) Cooper, G. J. T.; Surman, A. J.; McIver, J.; Colón-Santos, S. M.; Gromski, P. S.; Buchwald, S.; Suárez Marina, I.; Cronin, L. Miller-Urey spark-discharge experiments in the deuterium world. *Angew. Chem., Int. Ed.* **2017**, *56*, 8079–8082.
- (5) (a) Baillie, T. A. The use of stable isotopes in pharmacological research. *Pharmacol. Rev.* **1981**, *33*, 81–132. (b) Atzrodt, J.; Derdau, V.; Kerr, W. J.; Reid, M. Deuterium- and tritium-labelled compounds: applications in the life sciences. *Angew. Chem., Int. Ed.* **2018**, *57*, 1758–1784.
- (6) (a) Gant, T. G. Using deuterium in drug discovery: leaving the label in the drug. *J. Med. Chem.* **2014**, *57*, 3595–3611. (b) Tung, R. D. Deuterium medicinal chemistry comes of age. *Future Med. Chem.* **2016**, *8*, 491–494.
- (7) (a) Harbeson, S. L.; Tung, R. D. Deuterium in drug discovery and development. *Annu. Rep. Med. Chem.* **2011**, *46*, 403–471. (b) Liu, J. F.; Harbeson, S. L.; Brummel, C. L.; Tung, R.; Silverman, R.; Doller, D. A decade of deuterium in medicinal chemistry. *Annu. Rep. Med. Chem.* **2017**, *50*, 519–542.
- (8) Meanwell, N. A. Synopsis of some recent tactical application of bioisosteres in drug design. *J. Med. Chem.* **2011**, *54*, 2529–2591.
- (9) O' Ferrall, R. A. M. A pictorial representation of zero-point energy and tunnelling contributions to primary hydrogen isotope effects. *J. Phys. Org. Chem.* **2010**, *23*, 572–579.
- (10) Bell, R. P. Liversidge Lecture. Recent advances in the study of kinetic hydrogen isotope effects. *Chem. Soc. Rev.* **1974**, *3*, 513–544.
- (11) Ling, K. H.; Hanzlik, R. P. Deuterium isotope effects on toluene metabolism. Product release as a rate-limiting step in cytochrome P-450 catalysis. *Biochem. Biophys. Res. Commun.* **1989**, *160*, 844–849.
- (12) Westheimer, F. H. The magnitude of the primary kinetic isotope effect for compounds of hydrogen and deuterium. *Chem. Rev.* **1961**, *61*, 265–273.
- (13) Klinman, J. P.; Kohen, A. Hydrogen tunneling links protein dynamics to enzyme catalysis. *Annu. Rev. Biochem.* **2013**, *82*, 471–496.
- (14) Slaughter, L. M.; Wolczanski, P. T.; Klinckman, T. R.; Cundari, T. R. Inter- and intramolecular experimental and calculated equilibrium isotope effects for (silo₂)₂(tBu₃SiND)TiR + RH (silo = tBu₃SiO): inferred kinetic isotope effects for RH/D addition to transient (silo₂)₂Ti=NSitBu₃. *J. Am. Chem. Soc.* **2000**, *122*, 7953–7975.
- (15) Schneider, F.; Mattern-Dogru, E.; Hillgenberg, M.; Alken, R. G. Changed phosphodiesterase selectivity and enhanced in vitro efficacy by selective deuteration of sildenafil. *Arzneim. Forsch.* **2007**, *57*, 293–298.
- (16) Belleau, B.; Burba, J.; Pindell, M.; Reiffenstein, J. Effect of deuterium substitution in sympathomimetic amines on adrenergic responses. *Science* **1961**, *133*, 102–104.
- (17) Elison, C.; Rapoport, H.; Laursen, R.; Elliott, H. W. Effect of deuteration of N-CH₃ group on potency and enzymatic N-demethylation of morphine. *Science* **1961**, *134*, 1078–1079.
- (18) Sipes, I. G.; Gandolfi, A. J.; Pohl, L. R.; Krishna, G.; Brown, B. R. Comparison of the biotransformation and hepatotoxicity of halothane and deuterated halothane. *J. Pharmacol. Exp. Ther.* **1980**, *214*, 716–720.
- (19) Darland, G. K.; Hajdu, R.; Kropp, H.; Kahan, F. M.; Walker, R. W.; Vandenheuvel, W. J. Oxidative and defluorinative metabolism of fludalanine, 2-2H-3-fluoro-D-alanine. *Drug Metab. Dispos.* **1986**, *14*, 668–673.
- (20) Higashi, T.; Ogawa, S. Isotope-coded ESI-enhancing derivatization reagents for differential analysis, quantification and profiling of metabolites in biological samples by LC/MS: A review. *J. Pharm. Biomed. Anal.* **2016**, *130*, 181–193.
- (21) (a) Halford, B. Deuterium switcheroo breathes life into old drugs. *Chem. Eng. News* **2016**, *94*, 32–36. (b) Timmins, G. S. Deuterated drugs: where are we now? *Expert Opin. Ther. Pat.* **2014**, *24*, 1067–1075. (c) Timmins, G. S. Deuterated drugs: updates and obviousness analysis. *Expert Opin. Ther. Pat.* **2017**, *27*, 1353–1361.
- (22) Schmidt, C. First deuterated drug approved. *Nat. Biotechnol.* **2017**, *35*, 493–494.
- (23) Claassen, D. O.; Carroll, B.; De Boer, L. M.; Wu, E.; Ayyagari, R.; Gandhi, S.; Stamler, D. Indirect tolerability comparison of deutetrabenazine and tetrabenazine for Huntington disease. *J. Clin. Mov. Disord.* **2017**, *4*, 3–13.

- (24) Rodrigues, F. B.; Duarte, G. S.; Costa, J.; Ferreira, J. J.; Wild, E. J. Tetrabenazine versus deutetabenazine for Huntington's disease: twins or distant cousins? *Mov. Disord. Clin. Pract.* **2017**, *4*, 582–585.
- (25) Garay, R. P.; Grossberg, G. T. AVP-786 for the treatment of agitation in dementia of the Alzheimer's type. *Expert Opin. Invest. Drugs* **2017**, *26*, 121–132.
- (26) (a) Xie, J. H.; Gillooly, K.; Zhang, Y.; Yang, X.; Zupa-Fernandez, A.; Cheng, L.; Strnad, J.; Heimrich, E.; Zhou, X.; Chen, J.; Chaudhry, C.; Li, S.; Moslin, R.; Wroblewski, S.; Weinstein, D.; Burke, J. 349 - BMS-986165 is a highly potent and selective allosteric inhibitor of TYK2, blocks IL-12, IL-23 and type I interferon signaling and provides for robust efficacy in preclinical models of systemic lupus erythematosus and inflammatory bowel disease. *Gastroenterology* **2018**, *154*, S-1357. (b) Weinstein, D.; Wroblewski, S.; Moslin, R.; Lin, S.; Zhang, Y.; Spergel, S.; Mertzman, M.; Tokarski, J.; Sun, H.; Chiney, M.; Elzinga, P.; Aranibar, N.; Chimalakonda, A.; Strnad, J.; Zupa-Fernandez, A.; Cheng, L.; Gillooly, K.; McIntyre, K.; Carter, P.; Lombardo, L.; Burke, J.; Macor, J. Discovery of a Pseudokinase Domain Ligand as an Allosteric Inhibitor of TYK2 for the Treatment of Autoimmune Diseases. *Abstracts of Papers*, 252nd National Meeting and Exposition of the American Chemical Society, Philadelphia, PA, August 21–25, 2016; American Chemical Society: Washington, DC, 2016.
- (27) (a) Papp, K.; Gordon, K.; Thaci, D.; Morita, A.; Gooderham, M.; Foley, P.; Girgis, I. G.; Kundu, S.; Banerjee, S. Phase 2 trial of selective tyrosine kinase 2 inhibition in psoriasis. *N. Engl. J. Med.* **2018**, *379*, 1313–1321. (b) An Investigational Study To Evaluate Experimental Medication BMS-986165 Compared to Placebo and a Currently Available Treatment in Participants with Moderate to Severe Plaque Psoriasis (POETYK-PSO-1). ClinicalTrials.gov identifier: NCT03624127. Last update: October 10, 2018. Accessed on October 22, 2018. (c) An Investigational Study To Evaluate Experimental Medication BMS-986165 Compared to Placebo and a Currently Available Treatment in Participants with Moderate-to-Severe Plaque Psoriasis (POETYK-PSO-2). ClinicalTrials.gov identifier: NCT03611751. Last update: October 10, 2018. Accessed on October 22, 2018.
- (28) (a) Khan, A. J.; Misenko, S. M.; Thandoni, A.; Schiff, D.; Jhawar, S. R.; Bunting, S. F.; Haffty, B. G. VX-984 is a selective inhibitor of non-homologous end joining, with possible preferential activity in transformed cells. *Oncotarget* **2018**, *9*, 25833–25841. (b) Boucher, D.; Newsome, D.; Takemoto, D.; Hillier, S.; Wang, Y.; Arimoto, R.; Maxwell, J.; Charifson, P.; Fields, S. Z.; Tanner, K.; Penney, M. S. Preclinical Characterization of VX-984, a Selective DNA-Dependent Protein Kinase (DNA-PK) Inhibitor in Combination with Doxorubicin in Breast and Ovarian Cancers. *Abstracts of Papers*, 2016 San Antonio Breast Cancer Symposium, San Antonio, TX, December 6–10, 2016.
- (29) First-in-Human Study of the Safety, Tolerability, and Pharmacokinetic/Pharmacodynamic Profile of VX-984 in Combination with Chemotherapy. ClinicalTrials.gov identifier: NCT02644278. Last update: June 5, 2018. Accessed on November 15, 2018.
- (30) Scott-Stevens, P.; Attack, J. R.; Sohal, B.; Worboys, P. Rodent pharmacokinetics and receptor occupancy of the GABA_A receptor subtype selective benzodiazepine site ligand L-838417. *Biopharm. Drug Dispos.* **2005**, *26*, 13–20.
- (31) (a) Liu, J. F.; Scott Harbeson, S.; Vinita Uttamsingh, V.; Morales, A. J.; Nguyen, S.; Bridson, G.; Cheng, C.; Aslanian, A.; Wu, L. CTP-354: A Novel Deuterated Subtype-Selective GABA(A) Modulator for Treatment of Neuropathic Pain, Spasticity and Anxiety Disorders. Presented at the American College of Neuropsychopharmacology (ACNP) 51st Annual Meeting, Hollywood, FL, December 2–6, 2012. (b) Braman, V.; Liu, J. F.; Harbeson, S.; Uttamsingh, V.; Bridson, G.; Wu, L.; Shipley, J. E. Preliminary Clinical Outcomes for CTP-354, a Novel Subtype-Selective GABA(A) Modulator. Presented at the American Neurological Association (ANA) 2014 Annual Meeting, Baltimore, MD, October 12–14, 2014.
- (32) Alex, A. A.; Harris, C. J.; Smith, D. A. *Attrition in the Pharmaceutical Industry: Reasons, Implications, and Pathways Forward*; Wiley: Hoboken, NJ, 2016.
- (33) (a) Nelson, S. D.; Trager, W. F. The use of deuterium isotope effects to probe the active site properties, mechanism of cytochrome P450-catalyzed reactions, and mechanisms of metabolically dependent toxicity. *Drug Metab. Dispos.* **2003**, *31*, 1481–1498. (b) Guengerich, F. P. Kinetic deuterium isotope effects in cytochrome P450 reactions. *Methods Enzymol.* **2017**, *596*, 217–238. (c) Guengerich, F. P. Kinetic deuterium isotope effects in cytochrome P450 oxidation reactions. *J. Labelled Compd. Radiopharm.* **2013**, *56*, 428–431.
- (34) Sharma, R.; Strelevitz, T. J.; Gao, H.; Clark, A. J.; Schildknecht, K.; Obach, R. S.; Ripp, S. L.; Spracklin, D. K.; Tremaine, L. M.; Vaz, A. D. N. Deuterium isotope effects on drug pharmacokinetics. I. System-dependent effects of specific deuteration with aldehyde oxidase cleared drugs. *Drug Metab. Dispos.* **2012**, *40*, 625–634.
- (35) Malmöf, T.; Rylander, D.; Alken, R. G.; Schneider, F.; Svensson, T. H.; Cenci, M. A.; Schilström, B. Deuterium substitutions in the L-DOPA molecule improve its anti-kinetic potency without increasing dyskinesias. *Exp. Neurol.* **2010**, *225*, 408–415.
- (36) Schneider, F.; Erisson, L.; Beygi, H.; Bradbury, M.; Cohen-Barak, O.; Grachev, I. D.; Guzy, S.; Loupe, P. S.; Levi, M.; McDonald, M.; Savola, J. M.; Papapetropoulos, S.; Tracewell, W. G.; Velinova, M.; Spiegelstein, O. Pharmacokinetics, metabolism and safety of deuterated L-DOPA (SD-1077)/carbidopa compared to L-DOPA/carbidopa following single oral dose administration in healthy subjects. *Br. J. Clin. Pharmacol.* **2018**, *84*, 2422–2432.
- (37) Schofield, J.; Derda, V.; Atzrodt, J.; Zane, P.; Guo, Z.; van Horn, R.; Czepczor, V.; Stoltz, A.; Pardon, M. Effect of deuteration on metabolism and clearance of Nerispiridine (HP184) and AVE5638. *Bioorg. Med. Chem.* **2015**, *23*, 3831–3842.
- (38) Pestov, N. B.; Okkelman, I. A.; Shmanai, V. V.; Hurski, A. L.; Giaccia, A. J.; Shchepinov, M. S. Control of lysyl oxidase activity through site-specific deuteration of lysine. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 255–258.
- (39) Schneider, F.; Hillgenberg, M.; Koytchev, R.; Alken, R. G. Enhanced plasma concentration by selective deuteration of rofecoxib in rats. *Arzneim. Forsch.* **2006**, *56*, 295–300.
- (40) Knutson, D. E.; Kodali, R.; Divović, B.; Treven, M.; Stephen, M. R.; Zahn, N. M.; Dobričić, V.; Huber, A. T.; Meirelles, M. A.; Verma, R. S.; Wimmer, L.; Witzigmann, C.; Arnold, L. A.; Chiou, L. C.; Ernst, M.; Mihovilovic, M. D.; Savić, M. M.; Sieghart, W.; Cook, J. M. Design and synthesis of novel deuterated ligands functionally selective for the γ -aminobutyric acid type A receptor (GABA_AR) $\alpha 6$ subtype with improved metabolic stability and enhanced bioavailability. *J. Med. Chem.* **2018**, *61*, 2422–2446.
- (41) Manley, P. W.; Blasco, F.; Mestan, J.; Aichholz, R. The kinetic deuterium isotope effect as applied to metabolic deactivation of imatinib to the des-methyl metabolite, CGP74588. *Bioorg. Med. Chem.* **2013**, *21*, 3231–3239.
- (42) Kerekes, A. D.; Esposito, S. J.; Doll, R. J.; Tagat, J. R.; Yu, T.; Xiao, Y.; Zhang, Y.; Prelusky, D. B.; Tevar, S.; Gray, K.; Terracina, G. A.; Lee, S.; Jones, J.; Liu, M.; Basso, A. D.; Smith, E. B. Aurora kinase inhibitors based on the imidazo[1,2-a]pyrazine core: fluorine and deuterium incorporation improve oral absorption and exposure. *J. Med. Chem.* **2011**, *54*, 201–210.
- (43) Xu, G.; Lv, B.; Roberge, J. Y.; Xu, B.; Du, J.; Dong, J.; Chen, Y.; Peng, K.; Zhang, L.; Tang, X.; Feng, Y.; Xu, M.; Fu, W.; Zhang, W.; Zhu, L.; Deng, Z.; Sheng, Z.; Welihinda, A.; Sun, X. Design, synthesis, and biological evaluation of deuterated C-aryl glycoside as a potent and long-acting renal sodium-dependent glucose cotransporter 2 inhibitor for the treatment of type 2 diabetes. *J. Med. Chem.* **2014**, *57*, 1236–1251.
- (44) Zhan, Z.; Peng, X.; Sun, Y.; Ai, J.; Duan, W. Evaluation of deuterium-labeled JNJ38877605: pharmacokinetic, metabolic, and *in vivo* antitumor profiles. *Chem. Res. Toxicol.* **2018**, *31*, 1213–1218.
- (45) Dunsæd, C. B.; Dornish, J. M.; Pettersen, E. O. The bioavailability and dose dependency of the deuterated anti-tumour

- agent 4,6-benzylidene-*d*₁-D-glucose in mice and rats. *Cancer Chemother. Pharmacol.* **1995**, *35*, 464–470.
- (46) Guo, S.; Pang, X.; Peng, L.; Zhan, M.; Fan, L.; Gong, Y.; Kang, F.; Chen, Y. Design, synthesis and biological evaluation of deuterated tivozanib for improving pharmacokinetic properties. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 2425–2428.
- (47) Pang, X.; Wang, Y.; Chen, Y. Design, synthesis, and biological evaluation of deuterated apalutamide with improved pharmacokinetic profiles. *Bioorg. Med. Chem. Lett.* **2017**, *27*, 2803–2806.
- (48) Velthuisen, E. J.; Baughman, T. M.; Johns, B. A.; Temelkoff, D. P.; Weatherhead, J. G. Synthesis and pharmacokinetic profile of highly deuterated breacanvir analogs. *Eur. J. Med. Chem.* **2013**, *63*, 202–212.
- (49) Pang, X.; Peng, L.; Chen, Y. Effect of *N*-methyl deuteration on pharmacokinetics and pharmacodynamics of enzalutamide. *J. Labelled Compd. Radiopharm.* **2017**, *60*, 401–409.
- (50) Jiang, J.; Pang, X.; Li, L.; Dai, X.; Diao, X.; Chen, X.; Zhong, D.; Wang, Y.; Chen, Y. Effect of *N*-methyl deuteration on metabolism and pharmacokinetics of enzalutamide. *Drug Des., Dev. Ther.* **2016**, *10*, 2181–2191.
- (51) Stringer, R. A.; Williams, G.; Picard, F.; Sohal, B.; Kretz, O.; McKenna, J.; Krauser, J. A. Application of a deuterium replacement strategy to modulate the pharmacokinetics of 7-(3,5-dimethyl-1*H*-1,2,4-triazol-1-yl)-3-(4-methoxy-2-methylphenyl)-2,6-dimethylpyrazolo[5,1-*b*]oxazole, a novel CRF1 antagonist. *Drug Metab. Dispos.* **2014**, *42*, 954–962.
- (52) Harbeson, S. L.; Morgan, A. J.; Liu, J. F.; Aslanian, A. M.; Nguyen, S.; Bridson, G. W.; Brummel, C. L.; Wu, L.; Tung, R. D.; Pilja, L.; Braman, V.; Uttamsingh, V. Altering metabolic profiles of drugs by precision deuteration 2: discovery of a deuterated analog of ivacaftor with differentiated pharmacokinetics for clinical development. *J. Pharmacol. Exp. Ther.* **2017**, *362*, 359–367.
- (53) Helfenbein, J.; Lartigue, C.; Noirault, E.; Azim, E.; Legaillard, J.; Galmier, M. J.; Madelmont, J. C. Isotopic effect study of propofol deuteration on the metabolism, activity, and toxicity of the anesthetic. *J. Med. Chem.* **2002**, *45*, S806–S808.
- (54) Czarnik, A. W. Deuterium-Enriched Maraviroc. U.S. Patent 0,062,331, March 05, 2009.
- (55) Barratt, M. J.; Frail, D. E. *Drug Repositioning: Bringing New Life to Shelves Assets and Existing Drugs*; Wiley: Hoboken, NJ, 2012.
- (56) Hearn, B. R.; Fontaine, S. D.; Pfaff, S. J.; Schneider, E. L.; Henise, J.; Ashley, G. W.; Santi, D. V. Primary deuterium kinetic isotope effects prolong drug release and polymer biodegradation in a drug delivery system. *J. Controlled Release* **2018**, *278*, 74–79.
- (57) Chen, J.; Mannargudi, B. M.; Xu, L.; Uetrecht, J. Demonstration of the metabolic pathway responsible for nevirapine-induced skin rash. *Chem. Res. Toxicol.* **2008**, *21*, 1862–1870.
- (58) Mutlib, A. E.; Gerson, R. J.; Meunier, P. C.; Haley, P. J.; Chen, H.; Gan, L. S.; Davies, M. H.; Gemzik, B.; Christ, D. D.; Krahn, D. F.; Markwalder, J. A.; Seitz, S. P.; Robertson, R. T.; Miwa, G. T. The species-dependent metabolism of efavirenz produces a nephrotoxic glutathione conjugate in rats. *Toxicol. Appl. Pharmacol.* **2000**, *169*, 102–113.
- (59) Phillips, D. H.; Potter, G. A.; Horton, M. N.; Hewker, A.; Crofton-Sleigh, C.; Jarman, M.; Venitt, S. Reduced genotoxicity of [D5-ethyl]-tamoxifen implicates alpha-hydroxylation of the ethyl group as a major pathway of tamoxifen activation to a liver carcinogen. *Carcinogenesis* **1994**, *15*, 1487–1492.
- (60) Calinski, D. M.; Zhang, H.; Ludeman, S.; Dolan, M. E.; Hollenberg, P. F. Hydroxylation and *N*-dechloroethylation of ifosfamide and deuterated ifosfamide by the human cytochrome p450s and their commonly occurring polymorphisms. *Drug Metab. Dispos.* **2015**, *43*, 1084–1090.
- (61) Jia, H.; Dai, G.; Weng, J.; Zhang, Z.; Wang, Q.; Zhou, F.; Jiao, L.; Cui, Y.; Ren, Y.; Fan, S.; Zhou, J.; Qing, W.; Gu, Y.; Wang, J.; Sai, Y.; Su, W. Discovery of (S)-1-(1-(imidazo[1,2-*a*]pyridin-6-yl)ethyl)-6-(1-methyl-1*H*-pyrazol-4-yl)-1*H*-[1,2,3]triazolo[4,5-*b*]pyrazine (volitinib) as a highly potent and selective mesenchymal-epithelial transition factor (c-Met) inhibitor in clinical development for treatment of cancer. *J. Med. Chem.* **2014**, *57*, 7577–7589.
- (62) Bhadra, P. B.; Hassanzadeh, A.; Arsic, B.; Allison, D. G.; Morris, G. A.; Barber, J. Enhancement of the properties of a drug by mono-deuteration: reduction of acid-catalysed formation of a gut-motilide enol ether from 8-deuterio-erythromycin B. *Org. Biomol. Chem.* **2016**, *14*, 6289–6296.
- (63) Shao, L.; Abolin, C.; Hewitt, M. C.; Koch, P.; Varney, M. Derivatives of tramadol for increased duration of effect. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 691–694.
- (64) Uttamsingh, V.; Gallegos, R.; Liu, J. F.; Harbeson, S. L.; Bridson, G. W.; Cheng, C.; Wells, D. S.; Graham, P. B.; Zelle, R.; Tung, R. Altering metabolic profiles of drugs by precision deuteration: reducing mechanism-based inhibition of CYP2D6 by paroxetine. *J. Pharmacol. Exp. Ther.* **2015**, *354*, 43–54.
- (65) Zhu, Y.; Zhou, J.; Jiao, B. Deuterated clopidogrel analogues as a new generation of antiplatelet agents. *ACS Med. Chem. Lett.* **2013**, *4*, 349–352.
- (66) (a) Smith, S. W. Chiral toxicology: It's the same thing...only different. *Toxicol. Sci.* **2009**, *110*, 4–30. (b) Csuk, R. *Biocatalysis in the Pharma and Biotech Industries*; Patel, R. N., Ed.; CRC Press: Boca Raton, FL, 2007; pp 699–716. (c) Hutt, A. J.; Valentova, J. The chiral switch: The development of single enantiomer drugs from racemates. *Acta Fac. Pharm. Univ. Comenianae* **2003**, *50*, 7–23. (d) Ali, I. Homochiral drug design and development by racemization. *Comb. Chem. High Throughput Screening* **2007**, *10*, 326–335. (e) Somogyi, A.; Bochner, F.; Foster, D. Inside the isomers: The tale of chiral switches. *Aust. Prescr.* **2004**, *27*, 47–49.
- (67) Maltais, F.; Jung, Y. C.; Chen, M.; Tanoury, J.; Perni, R. B.; Mani, N.; Laitinen, L.; Huang, H.; Liao, S.; Gao, H.; Tsao, H.; Block, E.; Ma, C.; Shawgo, R. S.; Town, C.; Brummel, C. L.; Howe, D.; Pazhanisamy, S.; Raybuck, S.; Namchuk, M.; Bennani, Y. L. In vitro and in vivo isotope effects with hepatitis C protease inhibitors: enhanced plasma exposure of deuterated telaprevir versus telaprevir in rats. *J. Med. Chem.* **2009**, *52*, 7993–8001.
- (68) Mori, T.; Ito, T.; Liu, S.; Ando, H.; Sakamoto, S.; Yamaguchi, Y.; Tokunaga, E.; Shibata, N.; Handa, H.; Hakoshima, T. Structural basis of thalidomide enantiomer binding to cereblon. *Sci. Rep.* **2018**, *8*, 1294–1307.
- (69) Yamamoto, T.; Tokunaga, E.; Nakamura, S.; Shibata, N.; Toru, T. Synthesis and configurational stability of (S)- and (R)-deuteriothalidomides. *Chem. Pharm. Bull.* **2010**, *58*, 110–112.
- (70) Jacques, V.; Czarnik, A. W.; Judge, T. M.; Van der Ploeg, L. H.; DeWitt, S. H. Differentiation of antiinflammatory and antitumorogenic properties of stabilized enantiomers of thalidomide analogs. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112*, E1471–E1479.
- (71) Jacques, V.; Van der Ploeg, L.; Dewitt, S. H. DRX- 065, the Deuterated (R)-Enantiomer of Pioglitazone, as a Nonalcoholic Steatohepatitis (NASH) Drug Candidate: Preclinical and Phase 1 Results. *Abstracts of Papers*, 256th National Meeting and Exposition of the American Chemical Society, Boston, MA, August 19–23, 2018; American Chemical Society: Washington, DC, 2018.
- (72) Abdallah, C. G.; Adams, T. G.; Kelmendi, B.; Esterlis, I.; Sanacora, G.; Krystal, J. H. Ketamine's mechanism of action: a path to rapid-acting antidepressants. *Depression Anxiety* **2016**, *33*, 689–697.
- (73) Zanos, P.; Moaddel, R.; Morris, P. J.; Georgiou, P.; Fischell, J.; Elmer, G. I.; Alkondon, M.; Yuan, P.; Pribut, H. J.; Singh, N. S.; Dossou, K. S.; Fang, Y.; Huang, X. P.; Mayo, C. L.; Wainer, I. W.; Albuquerque, E. X.; Thompson, S. M.; Thomas, C. J.; Zarate, C. A.; Gould, T. D. NMDAR inhibition-independent antidepressant actions of ketamine metabolites. *Nature* **2016**, *533*, 481–486.
- (74) Zhang, M.-R.; Maeda, J.; Ito, T.; Okauchi, T.; Ogawa, M.; Noguchi, J.; Suhara, T.; Halldin, C.; Suzuki, K. Synthesis and evaluation of *N*-(5-fluoro-2-phenoxyphenyl)-*N*-(2-[¹⁸F]-fluoromethoxy-5-methoxybenzyl)acetamide: a deuterium-substituted radioligand for peripheral benzodiazepine receptor. *Bioorg. Med. Chem.* **2005**, *13*, 1811–1818.
- (75) Moon, B. S.; Kim, B. S.; Park, C.; Jung, J. H.; Lee, Y. W.; Lee, H.-Y.; Chi, D. Y.; Lee, B. C.; Kim, S. E. [¹⁸F]Fluoromethyl-PBR28 as a potential radiotracer for TSPO: preclinical comparison with

- [¹¹C]PBR28 in a rat model of neuroinflammation. *Bioconjugate Chem.* **2014**, *25*, 442–450.
- (76) Moon, B. S.; Jung, J. H.; Park, H. S.; Contino, M.; Denora, N.; Lee, B. C.; Kim, S. E. Preclinical comparison study between [¹⁸F]fluoromethyl-PBR28 and its deuterated analog in a rat model of neuroinflammation. *Bioorg. Med. Chem. Lett.* **2018**, *28*, 2925–2929.
- (77) Nag, S.; Fazio, P.; Lehmann, L.; Kettschau, G.; Heinrich, T.; Thiele, A.; Svedberg, M.; Amini, N.; Leesch, S.; Catafau, A. M.; Hannestad, J.; Varrone, A.; Halldin, C. In vivo and in vitro characterization of a novel MAO-B inhibitor radioligand, [¹⁸F]-labeled deuterated fluorodeprenyl. *J. Nucl. Med.* **2016**, *57*, 315–320.
- (78) Nag, S.; Varrone, A.; Tóth, M.; Thiele, A.; Kettschau, G.; Heinrich, T.; Lehmann, L.; Halldin, C. In vivo evaluation in cynomolgus monkey brain and metabolism of [¹⁸F]fluorodeprenyl: a new MAO-B pet radioligand. *Synapse* **2012**, *66*, 323–330.
- (79) Ottoboni, S.; Caldera, P.; Trevor, A.; Castagnoli, N. Deuterium isotope effect measurements on the interactions of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine with monoamine oxidase B. *J. Biol. Chem.* **1989**, *264*, 13684–13688.
- (80) Drake, L. R.; Brooks, A. F.; Mufarreh, A. J.; Pham, J. M.; Koeppe, R. A.; Shao, X.; Scott, P. J. H.; Kilbourn, M. R. Deuterium kinetic isotope effect studies of a potential in vivo metabolic trapping agent for monoamine oxidase B. *ACS Chem. Neurosci.* **2018**, *9*, 3024–3027.
- (81) (a) Al-Qahtani, M. H.; Cleator, N.; Danks, T. N.; Garman, R. N.; Jones, J. R.; Stefaniak, S.; Morgan, A. D.; Simmonds, A. J. Microwave enhanced hydrogenation reactions using solid hydrogen, deuterium and tritium donors. *J. Chem. Res., Synop.* **1998**, 400–401. (b) Erb, T. W.; Jones, J. R.; Lu, S. Y. Microwave enhanced deuterations in the solid state using alumina doped sodium borodeuteride. *J. Chem. Res., Synop.* **1999**, 728–729. (c) Guaragna, A.; Pedatella, S.; Pinto, V.; Palumbo, G. Synthesis of C-protected 2,2-deutero β-amino acids. *Synthesis* **2006**, 2006, 4013–4016.
- (82) (a) Chapelle, M. R.; Kent, B. B.; Jones, J. R.; Lu, S. Y.; Morgan, A. D. Development of combined microwave-enhanced labelling procedures for maximising deuterium incorporation. *Tetrahedron Lett.* **2002**, *43*, S117–S118. (b) Derdau, V. Deuterated ammonium formate as deuterium source in a mild catalytic deuterium transfer reaction of pyridines, pyrazines and isoquinolines. *Tetrahedron Lett.* **2004**, *45*, 8889–8893.
- (83) Atzrodt, J.; Derdau, V.; Fey, T.; Zimmermann, J. The renaissance of H/D exchange. *Angew. Chem., Int. Ed.* **2007**, *46*, 7744–7765.
- (84) (a) Liu, C.; Chen, Z.; Su, C.; Zhao, X.; Gao, Q.; Ning, G. H.; Zhu, H.; Tang, W.; Leng, K.; Fu, W.; Tian, B.; Peng, X.; Li, J.; Xu, Q. H.; Zhou, W.; Loh, K. P. Controllable deuteration of halogenated compounds by photocatalytic D₂O splitting. *Nat. Commun.* **2018**, *9*, 80–88. (b) Loh, Y. Y.; Nagao, K.; Hoover, A. J.; Hesk, D.; Rivera, N. R.; Colletti, S. L.; Davies, I. W.; MacMillan, D. W. C. Photoredox-catalyzed deuteration and tritiation of pharmaceutical compounds. *Science* **2017**, *358*, 1182–1187.
- (85) Thulasiram, H. V.; Phan, R. M.; Rivera, S. B.; Poulter, C. D. Synthesis of deuterium-labeled derivatives of dimethylallyl diphosphate. *J. Org. Chem.* **2006**, *71*, 1739–1741.
- (86) Sattler, A. Hydrogen/Deuterium (H/D) exchange catalysis in alkanes. *ACS Catal.* **2018**, *8*, 2296–2312.
- (87) Atzrodt, J.; Derdau, V. Pd- and Pt-catalyzed H/D exchange methods and their application for internal MS standard preparation from a Sanofi-Aventis perspective. *J. Labelled Compd. Radiopharm.* **2010**, *53*, 674–685.
- (88) (a) Yung, C. M.; Skaddan, M. B.; Bergman, R. G. Stoichiometric and catalytic H/D incorporation by cationic iridium complexes: a common monohydro-iridium intermediate. *J. Am. Chem. Soc.* **2004**, *126*, 13033–13043. (b) Zhou, J.; Hartwig, J. F. Iridium-catalyzed H/D exchange at vinyl groups without olefin isomerization. *Angew. Chem., Int. Ed.* **2008**, *47*, S783–S787.
- (89) (a) Takahashi, M.; Oshima, K.; Matsubara, S. Ruthenium catalyzed deuterium labelling of α-carbon in primary alcohol and primary/secondary amine in D₂O. *Chem. Lett.* **2005**, *34*, 192.
- (b) Neubert, L.; Michalik, D.; Bahn, S.; Imm, S.; Neumann, H.; Atzrodt, J.; Derdau, V.; Holla, W.; Beller, M. Ruthenium-catalyzed selective α,β-deuteration of bioactive amines. *J. Am. Chem. Soc.* **2012**, *134*, 12239–12244. (c) Maegawa, T.; Fujiwara, Y.; Inagaki, Y.; Monguchi, Y.; Sajiki, H. A convenient and effective method for the regioselective deuteration of alcohols. *Adv. Synth. Catal.* **2008**, *350*, 2215–2218.
- (90) (a) Ma, S.; Villa, G.; Thuy-Boun, P. S.; Homs, A.; Yu, J.-Q. Palladium-catalyzed ortho-selective C-H deuteration of arenes: evidence for superior reactivity of weakly coordinated palladacycles. *Angew. Chem., Int. Ed.* **2014**, *53*, 734–737. (b) Sajiki, H.; Aoki, F.; Esaki, H.; Maegawa, T.; Hirota, K. Efficient C–H/C–D exchange reaction on the alkyl side chain of aromatic compounds using heterogeneous Pd/C in D₂O. *Org. Lett.* **2004**, *6*, 1485–1487.
- (91) Sajiki, H.; Ito, N.; Esaki, H.; Maesawa, T.; Maegawa, T.; Hirota, K. Aromatic ring favorable and efficient H–D exchange reaction catalyzed by Pt/C. *Tetrahedron Lett.* **2005**, *46*, 6995–6998.
- (92) Charifson, P. S.; Cottrell, K. M.; Deng, H.; Duffy, J. P.; Gao, H.; Giroux, S.; Green, J.; Jackson, K. L.; Kennedy, J. M.; Lauffer, D. J.; Ledebor, M. W.; Li, P.; Maxwell, J. P.; Morris, M. A.; Pierce, A. C.; Waal, N. D.; Xu, J. DNA-PK Inhibitors. U.S. Patent 9,925,188, March 27, 2018.
- (93) Alken, R.-G.; Stabingis, T. Deuterated 3-Piperidino-propiofenone and Medicaments Containing Said Compounds. Eur. Pat. Appl. 1383752, 2005.
- (94) Gant, T. G.; Sarshar, S. Methods of Reduction of Interpatient Variability. U.S. Patent 0,076,087, March 25, 2010.
- (95) <http://ir.concertpharma.com/news-releases/news-release-details/concert-pharmaceuticals-initiates-phase-1-clinical-trial-ctp-354> (accessed on Oct 16, 2018).
- (96) https://www.tevapharm.com/files/pipeline/Public_pipeline_Feb2017.pdf (accessed on Dec 27, 2018).
- (97) <http://ir.concertpharma.com/news-releases/news-release-details/concert-pharmaceuticals-initiates-phase-1b-clinical-trial-ctp> (accessed on Dec 27, 2018).
- (98) <http://ir.concertpharma.com/static-files/9dddc59a-d13d-4c64-86e5-d343562fda1b> (accessed on Oct 16, 2018).
- (99) <https://adisinsight.springer.com/trials/700276018> (accessed on Dec 27, 2018).
- (100) <https://www.businesswire.com/news/home/20081023005201/en/Auspex-Pharmaceuticals-Announces-Positive-Results-Clinical-Study> (accessed on Oct 16, 2018).
- (101) https://www.sec.gov/Archives/edgar/data/1454189/000156459015001726/aspx-ex991_2015031628.htm (accessed on Oct 16, 2018).
- (102) <https://www.concertpharma.com/product-pipeline/> (accessed on Dec 27, 2018).
- (103) Tung, R. D. 4-Hydroxybutyric Acid Analogs. U.S. Patent 9,815,763, November 14, 2017.
- (104) Wagner, A. T.; Cassella, J. V.; Graham, P. B.; Braman, V.; Uttamsingh, V.; Von Hehn, J.; Hamilton, C. E. Treatment of Hair Loss Disorders with Deuterated JAK Inhibitors. WO Patent 192,905, September 11, 2017.
- (105) Liu, J. F. Deuterated Isoindoline-1,3-Dione Derivatives as PDE4 and TNF-Alpha Inhibitors. Eur. Pat. Appl. 2690092, 2016.
- (106) Julie, F.; Liu, J. F.; Dong, Y. Deuterated Pirfenidone. U.S. Patent 0,131,485, May 21, 2009.
- (107) Masse, C. E. Deuterated 2-Amino-3-hydroxypropanoic Acid Derivatives. U.S. Patent 8,704,001, April 22, 2014.
- (108) Spittler, G. Linoleic acid peroxidation—the dominant lipid peroxidation process in low density lipoprotein—and its relationship to chronic diseases. *Chem. Phys. Lipids* **1998**, *95*, 105–162.
- (109) Yin, H.; Xu, L.; Porter, N. A. Free radical lipid peroxidation: mechanisms and analysis. *Chem. Rev.* **2011**, *111*, S944–S972.
- (110) (a) Shchepinov, M. S.; Chou, V. P.; Pollock, E.; Langston, J. W.; Cantor, C. R.; Molinari, R. J.; Manning-Bog, A. B. Isotopic reinforcement of essential polyunsaturated fatty acids diminishes nigrostriatal degeneration in a mouse model of Parkinson's disease. *Toxicol. Lett.* **2011**, *207*, 97–103. (b) Elharram, A.; Czegledy, N. M.;

- Golod, M.; Milne, G. L.; Pollock, E.; Bennett, B. M.; Shchepinov, M. S. Deuterium-reinforced polyunsaturated fatty acids improve cognition in a mouse model of sporadic Alzheimer's disease. *FEBS J.* **2017**, *284*, 4083–4095. (c) Raefsky, S. M.; Furman, R.; Milne, G.; Pollock, E.; Axelsen, P.; Mattson, M. P.; Shchepinov, M. S. Deuterated polyunsaturated fatty acids reduce brain lipid peroxidation and hippocampal amyloid beta-peptide levels, without discernible behavioural effects in an APP/PS1 mutant transgenic mouse model of Alzheimer's disease. *Neurobiol. Aging* **2018**, *66*, 165–176. (d) Berbée, J. F. P.; Mol, I. M.; Milne, G. L.; Pollock, E.; Hoeke, G.; Lütjohann, D.; Monaco, C.; Rensen, P. C. N.; van der Ploeg, L. H. T.; Shchepinov, M. S. Deuterium-reinforced polyunsaturated fatty acids protect against atherosclerosis by lowering lipid peroxidation and hypercholesterolemia. *Atherosclerosis* **2017**, *264*, 100–107. (e) Abeti, R.; Uzun, E.; Renganathan, I.; Honda, T.; Pook, M. A.; Giunti, P. Targeting lipid peroxidation and mitochondrial imbalance in Friedreich's ataxia. *Pharmacol. Res.* **2015**, *99*, 344–350.
- (111) Zesiewicz, T.; Heerinckx, F.; De Jager, R.; Omidvar, O.; Kilpatrick, M.; Shaw, J.; Shchepinov, M. S. Randomized, clinical trial of RT001: early signals of efficacy in Friedreich's ataxia. *Mov. Disord.* **2018**, *33*, 1000–1005.
- (112) Abeti, R.; Parkinson, M. H.; Hargreaves, I. P.; Angelova, P. R.; Sandi, C.; Pook, M. A.; Giunti, P.; Abramov, A. Y. Mitochondrial energy imbalance and lipid peroxidation cause cell death in Friedreich's ataxia. *Cell Death Dis.* **2016**, *7*, No. e2237.
- (113) Selvadurai, L. P.; Harding, I. H.; Corben, L. A.; Georgiou-Karistianis, N. Cerebral abnormalities in Friedreich ataxia: A review. *Neurosci. Biobehav. Rev.* **2018**, *84*, 394–406.
- (114) Tanna, P.; Strauss, R. W.; Fujinami, K.; Michaelides, M. Stargardt disease: clinical features, molecular genetics, animal models and therapeutic options. *Br. J. Ophthalmol.* **2017**, *101*, 25–30.
- (115) Weng, J.; Mata, N. L.; Azarian, S. M.; Tzekov, R. T.; Birch, D. G.; Travis, G. H. Insights into the function of Rim protein in photoreceptors and etiology of Stargardt's disease from the phenotype in abcr knockout mice. *Cell* **1999**, *98*, 13–23.
- (116) Kaufman, Y.; Ma, L.; Washington, I. Deuterium enrichment of vitamin A at the C20 position slows the formation of detrimental vitamin A dimers in wild-type rodents. *J. Biol. Chem.* **2011**, *286*, 7958–7965.
- (117) (a) Ma, L.; Kaufman, Y.; Zhang, J.; Washington, I. C20-D3-vitamin A slows lipofuscin accumulation and electrophysiological retinal degeneration in a mouse model of Stargardt disease. *J. Biol. Chem.* **2011**, *286*, 7966–7974. (b) Charbel Issa, P.; Barnard, A. R.; Herrmann, P.; Washington, I.; MacLaren, R. E. Rescue of the Stargardt phenotype in Abca4 knockout mice through inhibition of vitamin A dimerization. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112*, 8415–8420.
- (118) (a) Aslanian, A.; Hogan, K.; Qin, S.; Liu, J. F.; Cheng, C.; Bridson, G. W.; Graham, P.; Tung, R.; Wu, L. CTP-499, a Novel Drug for the Potential Treatment of Chronic Kidney Disease, Has Anti-Fibrotic, Anti-Inflammatory, and Anti-Oxidative Activities with In Vivo Efficacy. Presented at the American Society of Nephrology (ASN) Kidney Week Meeting 2011, Philadelphia, PA, November 8–13, 2011. (b) Aslanian, A.; Hogan, K.; West, K.; Bridson, G.; Wu, L. CTP-499, a Novel Drug for the Treatment of Chronic Kidney Disease, Ameliorates Renal Fibrosis and Inflammation In Vivo. Presented at the American Society of Nephrology (ASN) Kidney Week 2012, San Diego, CA, October 30–November 4, 2012.
- (119) Parasrampur, D.; Braman, V.; Cheng, C.; Grotbeck, B.; Turnquist, D.; Hogan, K.; Aslanian, A.; Wu, L.; Liu, J.; Zelle, R.; Graham, P.; Shipley, J.; Tung, R. First Human Exposure Suggests a Unique Metabolic Profile with Multiple Active Species for CTP-499, a Novel Agent for Treatment of Chronic Kidney Disease. Presented at the American Society of Nephrology (ASN) Kidney Week Meeting, Philadelphia, PA, November 8–13, 2011.
- (120) Braman, V.; Graham, P.; Cheng, C.; Turnquist, D.; Harnett, M.; Sabounjian, L.; Shipley, J. A randomized phase I evaluation of CTP-499, a novel deuterium-containing drug candidate for diabetic nephropathy. *Clin. Pharmacol. Drug Dev.* **2013**, *2*, 53–66.
- (121) Sabounjian, L.; Graham, P.; Wu, L.; Braman, V.; Cheng, C.; Liu, J.; Shipley, J.; Neutel, J.; Dao, M. A first-in-patient, multicenter, double-blind, 2-arm, placebo-controlled, randomized safety and tolerability study of a novel oral drug candidate, CTP-499, in chronic kidney disease. *Clin. Pharmacol. Drug Dev.* **2016**, *5*, 314–325.
- (122) Jones, P. J.; Leatherdale, S. T. Stable isotopes in clinical research: safety reaffirmed. *Clin. Sci.* **1991**, *80*, 277–280. (b) Bila, W. C.; Lamounier, J. A.; Everton de Freitas, A.; Silva, V. R.; Turani, S. D.; Dutra de Oliveira, J. E. Stable isotopes and body composition in children: History, fundamentals, and clinical applications. *Health* **2013**, *5*, 61–68.
- (123) Mullard, A. Deuterated drugs draw heavier backing. *Nat. Rev. Drug Discovery* **2016**, *15*, 219–221.