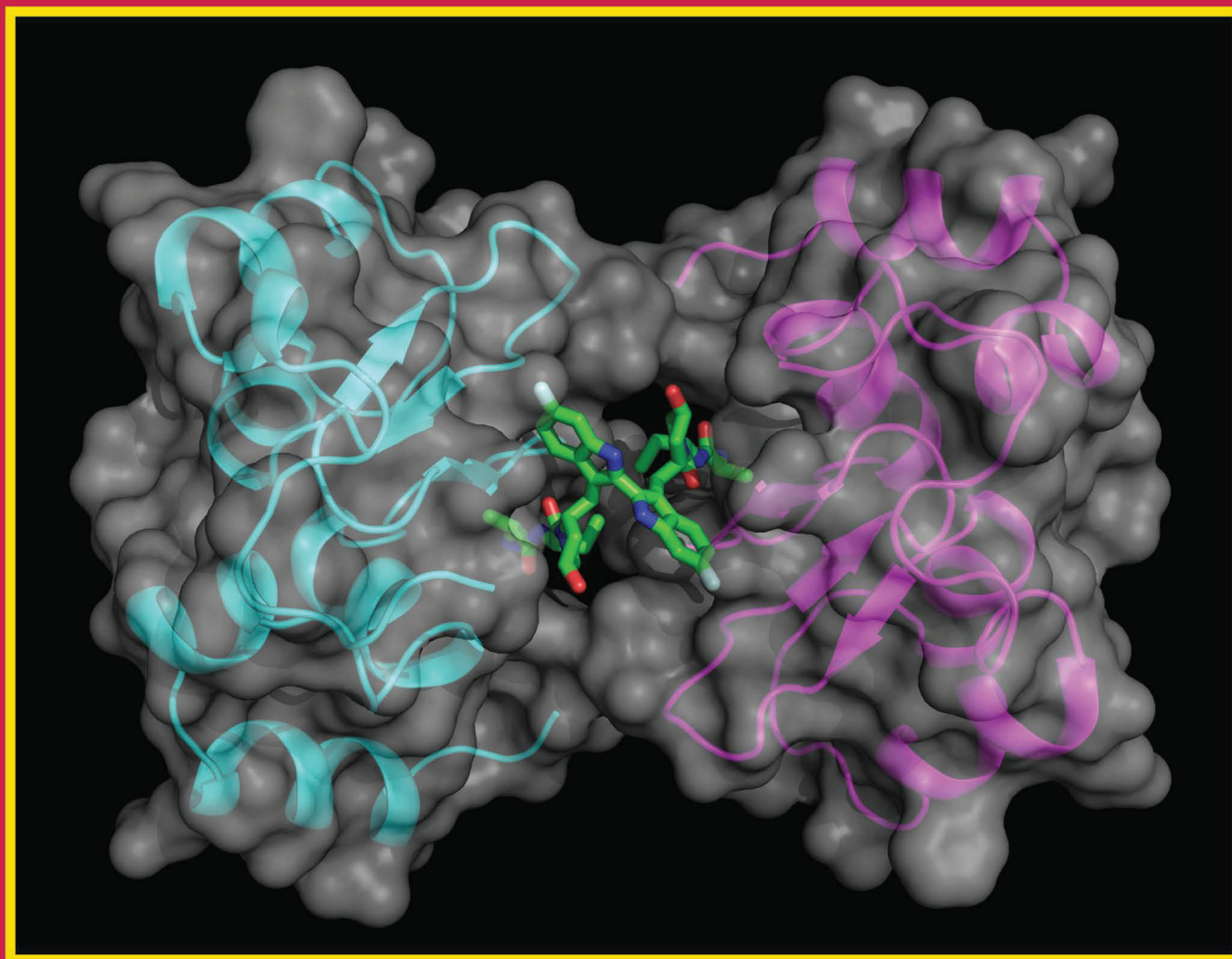


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ON THE COVER: The surface (gray) and cartoon (cyan and magenta) representations of two XIAP BIR3 domains are shown along with a stick representation of the bound birinapant (PDB ID: 4KMP). The atoms in birinapant are colored according to the following convention: C, green; N, blue; O, red; F, pale cyan. (Condon, S. M.; et al. *J. Med. Chem.* 2014, 57, 3666–3677)

Perspectives

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dx.doi.org/10.1021/jm4007998**Using Deuterium in Drug Discovery: Leaving the Label in the Drug**

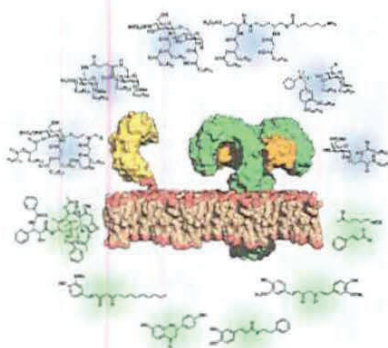
Thomas G. Gant*



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dx.doi.org/10.1021/jm401006s**Toll-like Receptor 4 (TLR4) Modulation by Synthetic and Natural Compounds: An Update**

Francesco Peri* and Valentina Calabrese



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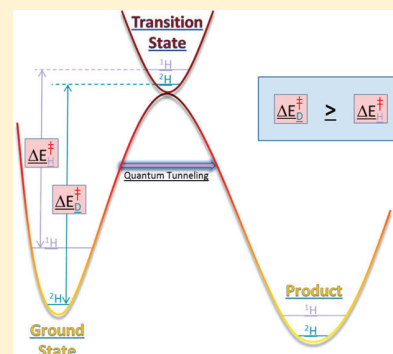


Using Deuterium in Drug Discovery: Leaving the Label in the Drug

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ABSTRACT: Deuterium, the stable isotope of hydrogen, is known to medicinal chemists for its utility in mechanistic, spectroscopic, and tracer studies. In fact, well-known applications utilizing deuterium exist within every subdiscipline in pharmaceutical discovery and development. Recent emphasis on incorporation of deuterium into the active pharmaceutical ingredient has come about as a result of inquiries into the potential for substantial benefits of the deuterium kinetic isotope effect on the safety and disposition of the drug substance. This Perspective traces the author's experience in reviving and expanding this potential utility, first suggested many decades prior by the discoverer of this, the simplest of all isotopes.



■ A REINTRODUCTION TO DEUTERIUM

Harold Urey, one of the most brilliant chemists of the 20th century, discovered deuterium about a year before the identification of the neutron. Even without a full picture of this isotope, he still understood the importance of what he'd done sufficiently well to predict much of what has happened to science, with respect to deuterium, as a result. This isotope has found its way into tens of thousands of scientific publications. And the pharmaceutical industry is probably second only to the nuclear industry in its appreciation of, and reliance on, deuterium. The next step in this growing relationship will be to actually incorporate the isotope into the final product. A slew of companies are now racing to do just that. But until that final approval by the FDA, the actual incorporation of deuterium into a medicine will continue to be a curiosity to most, something to poke with a stick, not quite admitting that its inevitable acceptance is right around the corner. Several good reviews have been written on the subject,¹ going back to a time before any companies existed for the sole purpose of exploiting deuterium as there are today. So this is not a review but a perspective. And the perspective that follows is based in part on the most common questions that I've gotten from many hundreds of chemists, biologists, clinicians, physicists, venture capitalists, patent lawyers (ex-chemists), and even consultants (retired chemists). Since some readers may find certain sections more to their interest than others, a bit of redundant language is included to allow each topic to stand alone.

■ DARING TO USE A NEW NUCLEUS

Introducing a new nucleus to the pharmaceutical world is never easy. It was not so long ago that medicinal chemists who suggested incorporating boron into a protease inhibitor were subjected to a derisive retort at a minimum. Then along came the lightning fast FDA approval of bortezomib, and it has become commonplace to find boron in advanced discovery projects, often even landing in the company pipeline. Bortezomib

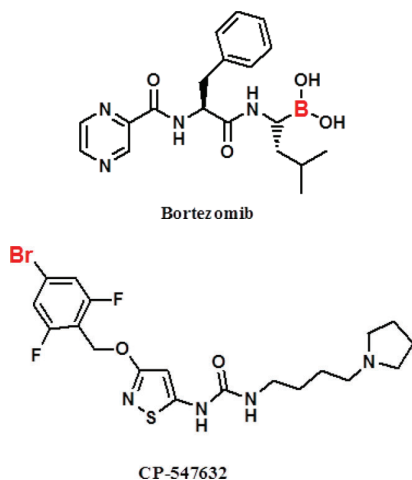
(Velcade) is one of the most effective new drugs to hit the oncology world in a generation and would not have happened if not for a fearless champion or two.² Boron is a superb "warhead" within certain parameters of application. The only clear negative to its use is the generally high level of synthetic skill needed to generate sufficient analogs for a vigorous drug discovery program.³ In a similar example, this author made the "bold" move in the 1990s of appending a bromide onto an aromatic group on a tyrosine kinase inhibitor (CP-547632).⁴ There were concerns about the overall stability of the active pharmaceutical ingredient (API), since there was only scant precedent to support its consideration. The highly supportive data for the drug itself were not sufficient to allay concerns. On the other hand, the industry-wide acceptance of bromide *ion*, present as salt form counterion in about 4% of all drugs,⁵ is largely unquestioned, even in light of this ion's known sedative and other bioactive properties (thus, the vernacular use of the word "bromide" referring to a relaxant) and its extraordinary half-life (~2 weeks), leading to legitimate concerns for the use of this counterion in chronically administered medications. It turns out that this use of a stable *covalent* bromide, as an aryl bromide specifically, is a terrific substitute, a bioisosteric replacement, for a methyl group in tolyl form, in so many regards. It has similar group electronegativity, similar van der Waals volume, and similar hydrophobic binding properties. But it is many-fold more stable to oxidation, both CYP-mediated and autoxidative, relative to an aromatic methyl group. In standard preclinical stability assays, all similar analogs proved to be remarkably stable to the battery of purposefully harsh *hν*, and other corrosive, conditions employed to represent the kind of environmental thrashing that commercial products must endure. This particular molecule is still the most stable drug this author has ever made. Consider the conundrum that the primary concern was stability, although

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stability was rapidly demonstrated to be one of the drugs many positive attributes. There are currently far more drugs of commercial value sporting heavy metals or radioactive elements than there are drugs with either covalent boron or an innocuous arylbromide. Sometimes data are no match for preconceptions. And then the author decided to use deuterium.



The prevalence of deuterium appears to be fairly consistent throughout the universe,⁶ the majority of the universe extending far beyond the influence of the pharmaceutical industry. This consistency of deuterium's occurrence tells us a lot about the formation of galaxies, and other systems large and small from the earliest stages of the big bang onward to nearby solar chemistry and further to the water vapor that continues to enter Earth via cometary origins.⁷ Deuterium is produced in vast quantities in stars, including the one nearby, but this 2 amu nucleus is in such high demand in various downstream processes, themselves so kinetically superior to the production of deuterium, that negligible ^2H (^2D) ever leaves the sun in any form; ionic, atomic, or molecular (Figure 1). These processes that occur in the sun

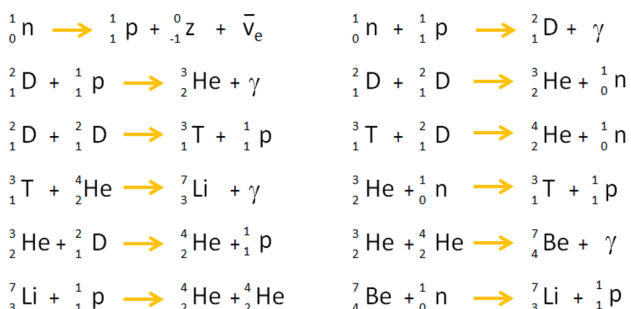


Figure 1. A large proportion of the fusion reactions occurring in stars involve deuterium. And as a result, in spite of a robust process for the production of this isotope, little deuterium leaves unincorporated.

give a different meaning and heft to the phrase “kinetic isotope effect” (KIE). But in addition to what we have learned about the universe and our solar system from the *relative* constancy and scarcity of deuterium's presence around the cosmos (about 1% of 1% with respect to ^1H), we also learn at least as much from the *slight* variations in its abundance from place to place on Earth. Variation in ^2H representation, relative to its more popular isotopic counterpart, ^1H , in bodies of water can imply the presence of evaporative, condensative, and/or reactive processes, all of which will have an intrinsic isotope effect taking place on a

breathhtaking scale. Applications finding success span the gap from paleoreconstructive climatic analysis⁸ to sleuthing for present day sources of organic chemicals used in the drug trade.⁹ So with the deuterium kinetic isotope effect (DKIE) taking place all around us, in stars, in oceans, in plants,¹⁰ and in ourselves, it is not surprising that many have sought to actively harness this phenomenon, not merely look after it.

■ WHY DOES THE DKIE WORK?

The deuterium kinetic isotope effect is more than a mathematical construct with physical implications; it is a picturesque event that fascinates any chemist who encounters it.¹¹ Imagine first a carbon–hydrogen bond exercising its freedom to a degree, vibrating back and forth as these pairs do. In a reaction with activated oxygen, for example, the C–H vibration is surreptitiously interrupted and replaced by the translational motion of the transition state. It is no surprise that *either* of these modes of movement may be influenced greatly by changing the mass of the moving parts; that substituting a deuterium for the hydrogen alters the rate of change from one mode of movement to another, as well as alters the frequency of the individual modes themselves. It may be a greater surprise to some that for the majority of studied cases in biological media, this observed change in rate, the kinetic isotope effect, is very small, often so small as to be difficult to detect. Thus, one discovers that a kinetic isotope effect can be smothered by other phenomena. It can fail to take the mantle of the rate-limiting step. It is very common to discuss the kinetic isotope effect by presenting first the anharmonicity of bond oscillations, referring to the Morse potential of C–H vs C–D bonds. Ultimately the short-range repulsion of the nuclei and the long-range attraction produced by the stabilizing orbital system define the energy diagram. But systems on the scale of a small molecule drug can be envisioned to be so much more complex than the oscillator initially examined by Morse. The necessity of accounting for the allowed sinusoidal rhythms of the full molecular system will produce energy states that one cannot predict by simple inspection. Furthermore, the typical explanation of kinetic isotope effect by reference to differing zero point energies (Figure 2) is “oversimplified to the point of

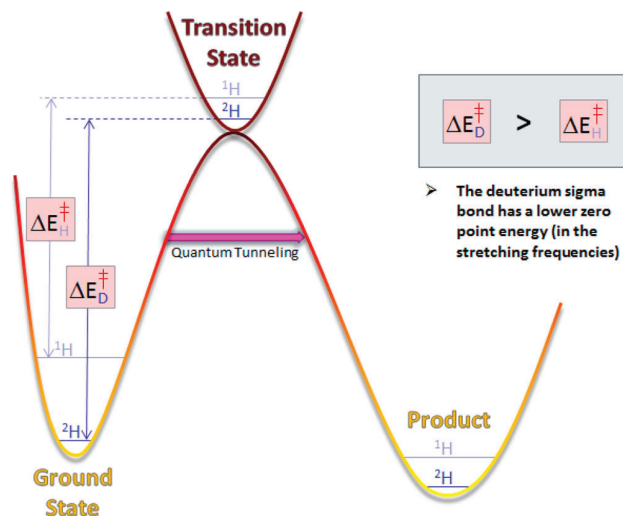


Figure 2. The standard explanation for the KIE begins with differing zero point energies of the isotopic nuclei. Tunneling is a physically allowed bypass of the transition state energy maximum.

absurdity".¹² And so out of respect for the complexity of the true origin of the isotope effects and owing to the high quality of many existing reviews,¹³ the origins of the DKIE are largely left out of this Perspective. However, certain types of substitutions just work, and other types just do not work. No computational chemist or other theoretical scientist has yet come up with a method for predicting which cases will work a priori. They are still playing "Jeopardy"; give them the answer, and they will provide a great question. It can, however, be easily explained why so many expected DKIEs never materialize in a biological setting; the rate-limiting step must involve bond-breaking/bond-making with respect to the C–H or C–D bonds under study. And the complexity of the catalytic cycle of a typical P_{450} affords a great many other steps that may become the slow step, thus rendering deuterium substitution meaningless to the final rate.¹⁴ Of similar difficulty for theoreticians is the prediction of large DKIEs, i.e., the potential for tunneling, the reason for the occasional outlier with KIE > 10, even occasionally as high as 70,¹⁵ as in the example in Figure 3. It must be realized that to achieve stabilization in a

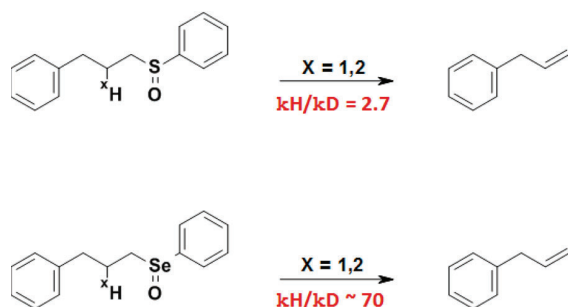


Figure 3. The magnitude of kinetic isotope effects cannot be predicted with any certainty. Tunneling is less foreseeable.

biological setting, the C–^{*}H bond-breaking step must be a rate-limiting process among many other potentially rate-limiting processes¹⁶ including, for example, the rate of initial binding of the drug to the offending oxidizing enzyme. Furthermore, a bound drug may tumble in the oxidase binding pocket, leading to exposure of alternative sites of the drug to oxidation. This may be referred to as "metabolic switching".¹⁷ Thus, a judicious and intuitive, yet scientifically rigorous process for selecting drugs that might be amenable to this approach is a necessity for increasing the chances of success. Many drugs simply will not change in any measurable way when substituted with deuterium in place of common hydrogen atoms, regardless of the site-specific placement or the number of such substitutions.

■ OXIDATION OF CARBON–HYDROGEN BONDS IS CENTRAL TO LIFE

Splitting carbon–hydrogen bonds and borrowing the energy of their electrons is fundamental to life, occurring in myriad ways in respiring species, so it is no surprise that this general mechanism is reused for the destruction of xenogenic materials.¹⁸ In the oxidation of drugs, it is most common for a cytochrome to act as an electron acceptor¹⁹ rather than, say, oxygen in a typical oxidase or NAD⁺ in a typical dehydrogenase. These cytochromes perform a service by converting the overly hydrophobic into hydrophilics and by adding handles to substances needing such handles for phase II conjugation. So in the case of oxidation of a C–H bond in a drug, the resulting alcohol is likely to be excreted more readily, although this is not always so. The resulting

oxidized species may go on to additional oxidations. It is a simple transformation, simpler even than it is usually portrayed. For example, some CYP oxidations are described as "dealkylations" even though these are actually just C–H oxidations with a twist; the hydroxylated product breaks down to reveal a *net* dealkylation, as with the collapse of a hemiketal or hemiaminal.

■ DEUTERIUM IN THE PHARMACEUTICAL INDUSTRY

It is quite difficult to name a scientific discipline not actively using deuterium in some fashion, particularly with special attention to the kinetic isotope effect that is possibly its most distinguishing characteristic. The pharmaceutical industry has found almost endless utility for deuterium. ²H is incorporated into drug analogs for the sake of creating mass spectrometry standards. It finds multiple uses in protein crystallography, NMR,²⁰ MS,²¹ and medical imaging²² and also has widespread utility in the elucidation of mechanisms,²³ both organic²⁴ and bio-organic.^{25,26} Much of this utility was quickly and accurately predicted by the discoverer of deuterium, physical chemist Harold C. Urey.²⁷ But until recently, the culture of drug discovery has steadfastly resisted the purposeful incorporation of deuterium into an API despite its extraordinary safety profile, proven capacity to improve drugs, low cost, and the unparalleled predictability of its effects. Perhaps it is the initially frustrating complexity of deuterium chemistry that foils broader use. Perhaps half of all envisioned incorporations are chemically trivial while the other half are surprisingly tough to accomplish in an efficient manner, particularly if it is important to leave other sites in the protio form. Having personally run hundreds of deuterium reactions, much of the chemistry has become second nature. Having supervised the deuterium incorporation chemistry for slightly more than 200 chemotypes, it is clear that no chemist gets to that second nature quickly. Too much of the deuterium substitution chemistry provides results that run counter to the falsely acquired intuition about the relative stability of a hydrogen atom. If a hydrogen atom, attached to a carbon atom, is not in an environment that would stabilize its carbanion should the proton meet a base, then it should not be touched by basic media. Similar statements can be made about hydride anions, hydrogen radicals, tautomerizations, bifurcated hydrogen bonds, or any other situation involving hydrogen. But in practice it is surprisingly fickle, this hydrogen mimic, deuterium. Consider a typical reaction, one that you have run ad nauseum. What is there, in all your arsenal of analytical equipment, that shouts out to tell you when two hydrogen atoms have exchanged places? Nothing. Not high field NMR, not triple quad MS, not RP-HPLC, not microanalysis. The hydrogen atoms could be exchanging repeatedly, but they will leave no trace of their utter disregard for your understanding of the atomic world. Deuterium chemistry upsets that ignorance-is-bliss feeling. Very strange things happen, strange to a chemist with a common understanding of the facility and requisite conditions of hydrogen exchange. When deuterium is exchanged for hydrogen, all of the aforementioned analytical techniques will announce the event in no uncertain terms. But they will do so to the degree to which their design specifications will allow. Those of us who have worked in the field of organometallic chemistry, especially those of us who did so when the 300 MHz NMR was the workhorse high field instrument, a deuterium quench was often used as evidence for a particular intermediate derived from the protio compound. Certainly full deuterium incorporation was a gold standard of having achieved the organometallic reagent cleanly in the first place. Failure to achieve full deuteration upon quench

was evidence of the failure to cleanly prepare the organometallic reagent in the first place. Or was it? The evidence for scrambling upon deuterium quench was difficult to prove unless it occurred on a large scale. A scrambling of 5% or less would look more like poor technique than scrambling, since the incorporation of a percent of so of deuterium at an odd sight or two would be tough to prove in short order. The advent of higher field instruments and their widespread availability has resulted in an easier path to the proof of such events. Further, the now ready availability of tens of thousands of commercially available deuterium-containing reagents has spurred greater experimentation with deuterium in general.²⁸ Though virtually all such deuterated reagents come ultimately from heavy water, the constant availability of this inexpensive reagent has finally created catalogs containing a deuterated version of virtually every potentially useful reagent that a chemist may request. In spite of this availability, many of the most useful new reactions in the field of deuterium continue to utilize heavy water. The work illustrated in Figure 4 is one of the more useful developments to occur in

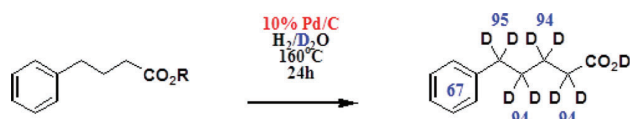


Figure 4. Deuterium substitution of seemingly unactivated C–H bonds was an extraordinary achievement and was accomplished under relatively mild conditions.

advancement of this field. And the reactions are certainly an eye-opener for what is possible in the realm of exchange reactions under relatively mild conditions. The figure shown is not a cherry-picked example. This chemistry is widely applicable (Figure 5). Generally speaking, the palladium chemistry can be tweaked to substitute deuterium at all, or many, of the aliphatic positions that have a σ bond trail to a π system, often leaving the aromatic positions undisturbed.²⁹ If platinum is used, the reverse

is observed, high substitution rates at aromatic positions and little change at adjacent aliphatic positions.³⁰ The creativity in this subdiscipline of deuterium incorporation extends to enzymatic reactions as well,³¹ although in all regards, anyone working in this area, synthetic or biosynthetic, should expect the unexpected.

■ ESTABLISHING CHEMICAL MECHANISMS

Isotopic labeling studies have been used to quickly elucidate chemical mechanistic pathways that would be otherwise quite challenging to derive (Figure 6). Studies of the stunning beauty of the ene–diyne antibiotic/antimitotic mechanisms-of-action were confidently exposed as “Bergman cyclizations” (figure) producing distant but predictable radical actors, juxtaposed for efficient reaction with the DNA minor groove, leading in turn to a strand scission. Far more challenging than this elegant but purposeful incorporation of deuterium is the deduction of mechanism through analysis of natural abundance. The sequential fractionation and frequent use of convergent synthesis in natural systems afford a complex but telltale patterning of isotopic ratios that can be quite revealing of nature’s methods of construction.³² Most mechanistic studies, so often employing deuterium incorporation, are more workman-like, not so striking, but critical for the everyday progression of scientific progress (no graphic examples can be given after that near-slandorous description). But sometimes a mechanistic study exemplifies the workings of deuterium. It must always be considered that a mechanistic study relying on deuterium may inadvertently produce the same product by a different mechanism because of the DKIE. Any mechanism exemplified by a deuterium quenching study, as above, is best accompanied by supporting experimentation.

■ DEUTERIUM: THE BEST OF THE BIOISOSTERES

There are differences between a C–H bond and a C–D bond: the C–D bond is a bit shorter,³³ has reduced electronic polarizability, lesser hyperconjugative stabilization of adjacent bonds (including the developing antibonding orbital of a newly

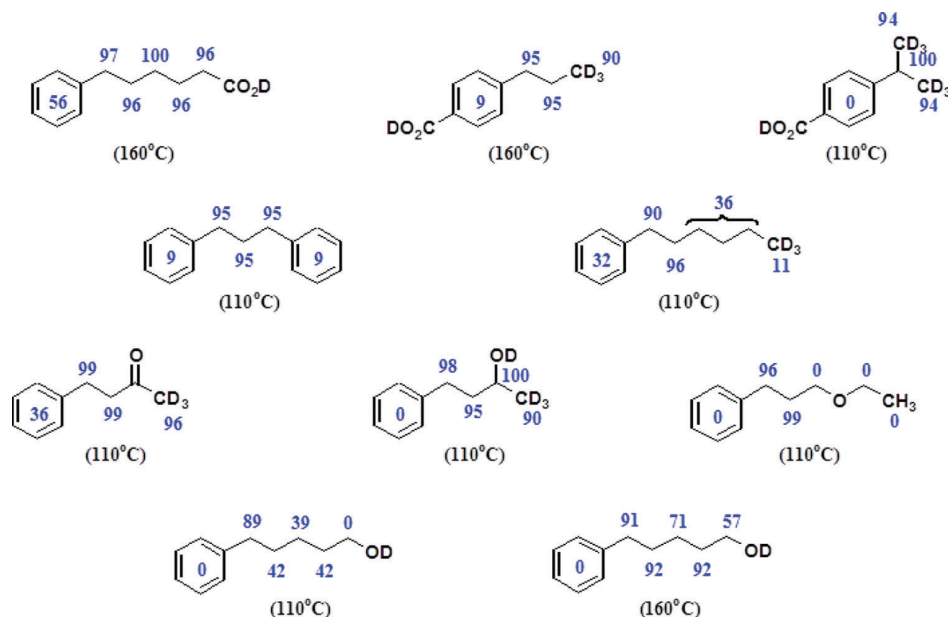


Figure 5. The applicability is broad, and the apparent limitations are intriguing. This discovery should inspire many more advancements in deuterium incorporation.

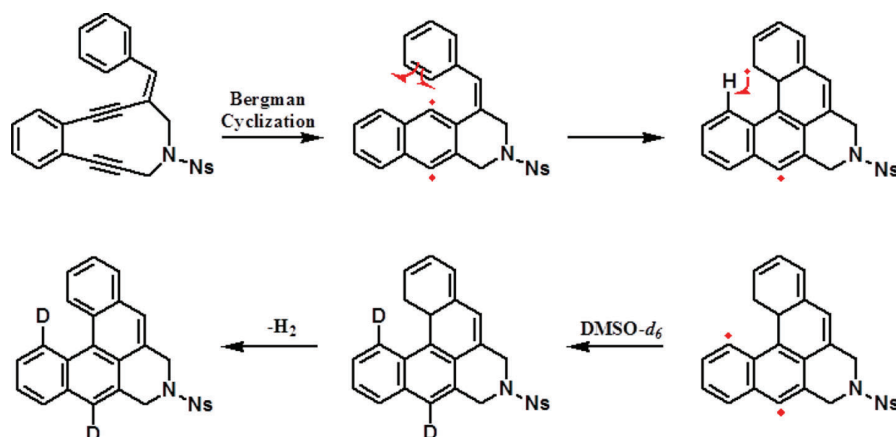


Figure 6. This logical schematic reveals the fate of the deuterium-substituted quenching agent, information unavailable with the use of protio reagents.

forming bond), potentially weaker van der Waals stabilization, and changes that are difficult to predict including changes in intramolecular volume and transition state volume. And the differences do go on and on. But in an R&D setting, what are the variances that may translate to a clinically relevant difference? Not many. The differences are so small that most eluded detection for decades after deuterium's discovery. Most of these differences are still only detectable in a few highly contrived circumstances. And the physicochemical differences that do exist (Figure 7), readily apparent in the hands of the world's finest

| Symbol | Name | Natural Abundance | Stability | Physical Properties Of $^1\text{H}_2\text{O}$ |
|--------------------------------|-------------------------------|-------------------|--|---|
| $^1_1\text{H} \equiv \text{H}$ | Hydrogen | 99.98% | Stable | MW = 18.0153 g/mol mp = 0°C bp = 100°C d = 1.000 g/cm ³ @ 4°C |
| $^2_1\text{H} \equiv \text{D}$ | Deuterium "Heavy Hydrogen" | 0.0156% | Stable | MW = 20.0276 g/mol mp = 3.79°C bp = 101.4°C d = 1.105 g/cm ³ @ 11°C |
| $^3_1\text{H} \equiv \text{T}$ | Tritium | Trace | Radioactive $T_{1/2} = 12.3$ years β decay | MW = 22.0315 g/mol mp = 4.49°C bp = 101.5°C d = 1.215 g/cm ³ @ 13°C |

Figure 7. Deuterium, a rare and stable isotope, gives rise to altered physicochemical characteristics in compounds where bonding is critical, such as in highly hydrogen bonded compounds.

physicists and physical chemists, are generally undetectable in the clinical setting. The C–D diatomic pair is the one true nonradioactive bioisosteric, bioisotopic, bioisoelectronic replacement for the ubiquitous C–H bond. For contrast, compare the C–D alternative to the most common C–H replacement in medicinal chemistry applications, the C–F switch. One of the most common questions I hear in regard to the D-switch approach is "why wouldn't you just throw a fluorine on that site?" And so the C–F group remains perhaps the most common replacement for an oxidatively labile C–H group in drug discovery. Fluorine substitution, although certainly a proven way to avert oxidation at the specific site of replacement, is massively different in so many properties relative to the hydrogen it is replacing. Fluorine has a van der Waals volume almost 100% larger than hydrogen, is substantially more electronegative (3.98

vs 2.20 on the Pauling scale, respectively), and is capable of acting as a hydrogen bond *acceptor*, to name just a few stark differences. As such, the resulting fluorinated drug is different in every conceivable manner along the entirety of the ~10-year drug discovery and development timeline. In stark contrast, a deuterated drug is virtually indistinguishable from its medicinal protio predecessor in so many ways, including in vitro pharmacodynamics (PD) and physicochemical properties. In fact it is virtually indistinguishable in *all* of its biological properties with the useful exception of those processes involving bond-breaking at the specific C–H site(s) that are oxidatively labile. The C–D bonds may be substantially more stable to oxidative processes because of the kinetic isotope effect (KIE), more specifically the deuterium kinetic isotope effect (DKIE), separated out from any other isotope effects because of its distinctively higher potential magnitude. As a consequence, ADMET can be altered, in some cases to an astonishing extent.³⁴ The following is a highly abbreviated list of parameters that may change quite substantially when switching from a C–H bond to a C–F bond, but *will not generally exhibit a clinically measurable change when switching from a C–H bond to a C–D bond*.³⁵

- Mechanism of action (in the case of multiple subtype binding)
- Target binding affinity
- Subtype binding
- Cellular activity
- Pharmacology model results
- PK/PD relationship
- Permeability
- Crystallinity
- Polymorph selection
- Salt form selection
- Solubility in all vehicles
- Maximum absorbable dose
- Minimum efficacious plasma concentration
- Efflux susceptibility
- Mechanisms saturate at same concentration
- Protein binding/serum binding
- Immunogenicity understood
- GI tolerability
- CNS tolerability
- BBB penetration
- Volume of distribution
- -S9 Ames assay
- -S9 micronucleus assay

- Tox species selection
- Off-target profile
- Pass three species tox
- Pass hERG, QTc assays
- Long-term carc studies
- No reactive metabolites
- Formulation issues
- Delivery options realistic
- Dose-proportionate exposure
- Exposure profile responsible for efficacy (e.g., AUC vs C_{max} effect)
- Phase I clinical trial is devoid of SAEs.

So the C–D switch will give results that are clinically or preclinically identical to the protio predecessor in the aforementioned assays, whereas the C–F analog will give different results, potentially positive differences, but also potentially program-ending results if in a clinical rather than a preclinical setting. And yet the switch to a C–F bond remains standard practice in the face of unwanted C–H oxidation of a drug in a biological system, whether the assay be in vitro (e.g., aldehyde oxidases, cytochrome P_{450} , monoamine oxidases, alcohol dehydrogenases), in cellulo (e.g., hepatocytes), or in vivo (e.g., rodent, dog, or primate, including human). The change to C–F changes potentially everything. The switch to C–D changes almost nothing except the chemical route, the rate of oxidation, and perhaps the ratio of metabolites. Quite often, with some creativity, the chemical route has far fewer changes in the case of the deuterium switch, relative to the fluorine switch. The changes to the route for the deuterium switch tend to be inexpensive, especially relative to the fluorine switch. And with deuterium, the rate and profile of drug oxidation will change in a wholly predictable manner without any need for allometry. In fact, it is a simple, logical, and still stunning statement that in vitro oxidative changes measured with human reagents should typically accurately foretell in vivo changes in human without any use for the highly inaccurate practice of allometry. This holds particularly true in the case wherein the clearance mechanisms are understood and are reliant on oxidative mechanisms as the primary contributor to clearance.

■ EMPLOYING DEUTERIUM IN THE DESIGN AND STUDY OF NEW MEDICINES

My history includes experience with several hundred classes of deuterated drugs with sufficient metabolic data to build a picture of what does and does not work in this field. But I have only put one such drug into the clinic, so I rely on a scant few historical examples of clinically tested deuterated drugs to build a picture that I am about to convey here. These trials have not been run against the protio version of the drug, with rare exception, so absolutist statements are avoided. Since this is not a review, only a few examples are included to illustrate some principles.

■ D₁-HALOTHANE

Several biotechnology companies have sought to specialize in deuterium incorporation in the past decade or so. This followed approximately a half dozen high quality individual attempts to use deuterium to improve a particular medicine. Generally, the weakness of many of these approaches has been the weakness one sees in the industry as a whole: a reticence to tackle the most challenging problems and a disproportionate reliance on the familiar. Consider the case of halothane. This volatile anesthetic is known to cause hepatotoxicity, and there is support for the

theory that an acyl chloride is the reactive intermediate responsible for forming DNA adducts (Figure 8), and likely

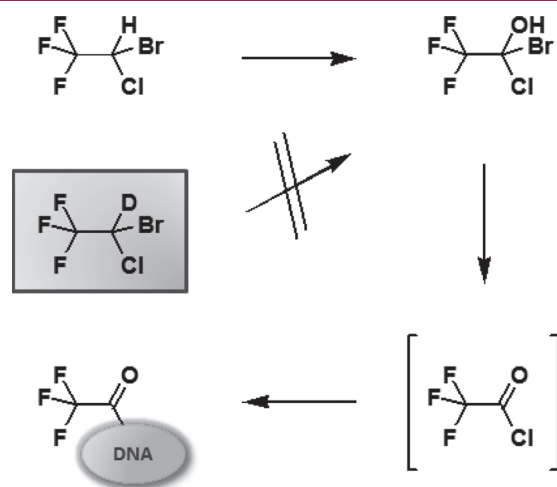


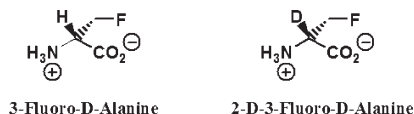
Figure 8. Halothane, when oxidized, breaks down to a reactive intermediate. Deuterium substitution slows that process.

other adducts, in the liver. A very nice synthesis of D₁-halothane was developed, and a dramatic reduction in DNA adducts was observed.³⁶ The problem here was certainly not that this work did not represent a significant improvement to the agent at hand. The problem was that this work was published in 1976, a time when safer alternatives to halothane were already known, such as sevoflurane, now itself being replaced to an extent by desflurane. So this beautifully executed deuteration solved a problem that had already been solved elsewhere. If any medicine was to be subjected to the powerful influence of deuterium substitution, why would one choose a drug that, in essence, no longer needed improvement? Well, the very accomplished scientist who carried out this work had been working on halothane for many years; this was his area of expertise. This is representative of what has largely held up a great many innovations: a focus on problems benefitting from accessibility rather than application to problems of greatest need and import. In defense of the very insightful person who carried out what is clearly a beautiful piece of work on halothane, the corporate environment rarely allows even this level of true inquiry and experimentation.

■ 2-²H-3-FLUORO-D-ALANINE

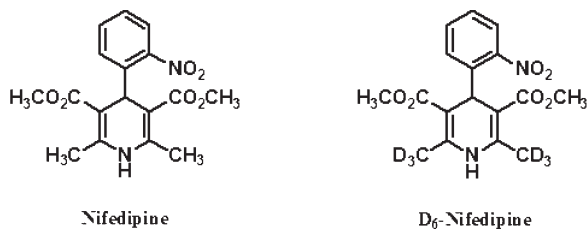
The antibiotic 2-²H-3-fluoro-D-alanine, also known as fludalanine is a different manifestation of the same trouble that deuteration approaches have had elsewhere. The example represents a highly successful application of deuteration in the limited sphere of deuteration chemistry, but the example was carried out in the service of a drug that was not to be successful for other reasons. This particular drug failed as an antibiotic because of rapid resistance to the drug, not a trait that deuteration was ever meant to address. The singly deuterated fludalanine did in fact lower oxidative clearance in vivo, as demonstrated in a primate model, by a factor of about 2.5-fold,³⁷ the same ratio observed for the kinetic isotope effect in the in vitro assay with the molecular nemesis of the protio drug, namely, D-amino oxidase. The oxidase converted the amino acid to 3-fluoropyruvate, and this was followed by reduction to 3-fluorolactate, the metabolite that was to be monitored in patients. This was a very intelligently designed and executed program and may have changed the

acceptability of deuterium as a tool of medicinal chemistry if not for those other failings of this drug. Again though, this was not an example of deuteration looking for its best application but rather an expert in a specific field, antibiotics in this instance, looking for patch kit for a metabolic problem. The patch seems to have worked beautifully, but the drug failed as drugs so often do. There is no published evidence that the protio drug was compared in human, in a head-to-head trial with the deuterated drug, so we will perhaps never know for certain if the deuterium played a role in improving the drug in the clinical setting or not. Improvements in animal models do not necessarily translate to improvements in humans given the frequent lack of correlation between oxidase (or other) orthologs.



■ D₆-NIFEDIPINE

With the majority of the great many deuterated drugs, the stated goal was an increase in half-life. This is a pretty mundane goal given that the possibilities for deuteration include reduction of reactive or off-target-active metabolites, i.e., safety end points. But improvement of half-life can do more than simply make a drug last longer. Increased half-life, if it translates to less frequent dosing, improves patient compliance and hence both efficacy and safety indirectly. Longer half-life should lead to decreased discontinuation effects³⁸ (read, “withdrawal”) in many agents, again a safety improvement. And in the case of drugs used for treating a condition with the capacity for rebound effects, say, hypertension, one can envision increased half-life leading to decreased mortality as the patient spends less time in the trough. And the trough itself may be less of a plunge with a deuterated drug.³⁹ So nifedipine was deuterated and the protected analog gave rise to a 34% increase in efficacy in rodents, but the true benefit may have been much more profound had this agent seen clinical trials. It did not.



■ A TIME AND PLACE FOR DEUTERIUM INCORPORATION

The quality of a drug may be gauged as a ratio of desirable medicinal traits such as efficacy, percentage of patients responding to treatment, and improvement in quality of life measures versus those undesirable traits such as harmful side effects and failures to respond to treatment. Often the titration of proper dose to balance these traits is a challenge to physicians. Patient compliance with dosing regimens is predictably poor with medicines having extensive side effects or poor efficacy. And since ascending doses often produce greater compliance-damaging side effects, as well as greater efficacy, a conundrum arises in the optimization of treatment per patient. And the side effects are not necessarily related to the same biological

mechanism/s responsible for efficacy. As medicines become ever more targeted to specific biological mechanisms and the individual proteins that we suspect may be awry in some patients, we still find ourselves largely at the mercy of chance in avoiding “off-target” activity, whether by parent drug or by undesirable metabolite activities. This off-target activity can occur most acutely through overexposure to the medicine during the postdose time of maximum exposure. Simply reducing the dose will reduce overexposure but can also dramatically reduce the time a patient is sufficiently exposed to allow the desired mechanism of action to work. There are tens of thousands of potential off-target protein binding sites with which our medicines and respective metabolites can interfere, if one includes splice variants, differing post-translational modifications, and the like each with potentially multiple allosteric sites wherein undesired modulation (agonism, inverse agonism, antagonism, etc.) may occur. This modulation can be fleeting and reversible or, potentially worse, covalent and irreversible. Despite the animal toxicology studies required for all new chemical entities (NCEs), it is not possible to rigorously predict human ills originating from NCEs until many humans are treated in the clinic. So what are we to do with an existing medicine that has previously proven to be clinically effective but may have a therapeutic index that hinders performance and induces adverse events (AEs) in humans that are (a) not so severe to eliminate the drug from the physicians armament but (b) not so benign that the utility of the drug may ever reach the heights of benefit once hoped for?

Furthermore, it is common that medicines will not have the pharmacokinetic profile desired for a given biological target or therapeutic application. ADME processes can often also be attributed as the genesis of toxicity through the metabolic conversion of the drug to an undesired metabolite⁴⁰ or alternatively by the necessity of massive peak exposure concentrations in order to provide therapeutic coverage over the desired time frame (often 24 h per dose); thus, the broader acronym ADMET is fit for this discussion, as toxicity is so often linked to ADME properties.^{41,42} Unfortunately, traditional new drug discovery techniques, although so often able to address specific ADMET issues uncovered in human studies of a prior drug, will most times result in a new drug that possesses *different* ADMET properties rather than *better* ADMET properties. Finally, there is no guarantee that the new drug will have *any* efficacy, much less *better* efficacy.

And so the new crop of deuteration biotechnologies have been testing an old concept and have taken it to human trials to see if one could avoid or diminish the conundrums listed above. That is, they have sought to *improve* ADMET while leaving *untouched* the known desirable traits of a value-proven medicine. The technique is ideally suited for application to a drug with many positive attributes but with liabilities suited for repair with this method. This is not an early discovery technique. It is simply not worth the effort for a discovery level analog that has perhaps passed through a couple dozen discovery gates with many hundreds of other preclinical and clinical gates guarding the road ahead. It is an approach for more mature projects. But here is the unmet need in this area: those highly promising projects that had a devastating fall in middle stage clinical trials. The failing of the drug candidate may not necessarily be understood; one of the strengths of deuterated analogs is in their capacity to reveal the root of a protio drugs’ failure. The Achilles heel may be revealed in this way and repaired in the same effort. And there are so many such targets of opportunity; thousands of previously clinically

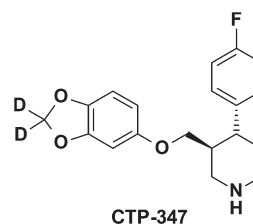
exposed drugs fit this description and are now lying on the shelf. Thousands of drugs for which millions of dollars' worth of data exist to light the path for a straightforward D-switch project with a higher probability of success and a higher net present value (NPV)⁴³ than the original project ever possessed. It includes projects that never made it to phase III, maybe never made it to phase II, but these are projects that do have *some* human data. These are actually the ideal targets for this approach, and yet they are seldom considered. People become fatigued with projects that have let them down. And it is unusual for a successor to pick up a predecessor's project and try to revive it; this act simply goes against human nature, regardless of its merits.

So by converting an existing failed, or failing, medicine to its selectively deuterated counterpart, one might confer stability to specifically targeted C–H bonds.^{44,45} Given the high likelihood of “surprises” when doing these kinds of investigations, it is usually worthwhile to make a limited number of additional analogs with deuterium incorporated at positions not believed to contribute to the problem. For analogs that are easy to prepare, it is best not to be too clever by half when so much is at stake. Thankfully, we can usually purchase synthetic precursors containing the necessary deuterium, even if the ultimate process route will be slightly different, just to get our answers quickly. In the instance that deuterium must be incorporated by novel methods, the analysis may require some sophistication to characterize incorporation rates and sometimes even to determine the incorporation sites themselves (perhaps an order of magnitude more difficult than a typical project). But it is all readily accomplished with a thoughtful approach. Thus, C–H bonds, commonly destroyed stereoselectively by oxidase systems *in vivo* such as the cytochrome P₄₅₀'s (CYPs) and monoamine oxidases (MAOs) and less frequently by flavins, Fenton species, and the like and which fare much better as an isotopically substituted C–D bond, can usually be installed in less time than it might take to synthesize any of the top 10 traditional analogs that may have been otherwise prepared in its wake.

In the past, this approach has been too often used merely to improve the half-life of a commonplace drug or a drug that had already been made obsolete. The greatest benefit will come to those who can focus on the more difficult but higher value applications. This includes increasing safety by reducing propensity for reactive metabolites. Screening for such improvements is extremely facile, using glutathione-capture assays and DNA–adduct measurements, just to name two of many rapid, reliable methods. A great many proprietary methods exist to facilitate this characterization which has proven to be very interesting in producing “cleaner” drugs following deuterium incorporation. Other high value applications to be investigated include reduction of CYP inhibition, improvement of distribution patterns, and selective alteration of active metabolite ratios. And the likeliest success will be had by those who can judiciously resurrect programs that failed by an easily cured malady. This approach is waiting for a curious pharmacological archeologist willing to dig up the bones of old projects, reanimating their DNA. The value of any new drug is a measure of cost versus benefit. And though I hope for companies to focus on projects with greater safety benefits than those illustrated here, the other part of the equation is just as important, the reduction of production costs. Will any company focus on the clear potential for this approach to reduce costs?

■ CLINICAL TRIALS OF DEUTERATED DRUGS

Eighty years after the discovery of deuterium we may be on the verge of the first therapeutic agent with an enhanced incorporation of this stable, naturally occurring isotope. But with only a few companies having put a deuterated drug into the clinic, it is too early to project the rate of success of this approach. Merck struck out with fludalanine but deserves great credit for the high quality of the approach from the beautiful process chemistry to the proof-of-concept in primates. And Merck deserves appreciation from any organization working in this area for its courage to make the attempt, to make a new path. BiRDS, Auspex, and Concert also have agents in the clinic at this writing. CTP-347 has been shown to lessen the effect on CYP2D6 relative to the expectation from the protio counterpart, although it is not clear that this improvement is clinically meaningful, thus justifying taking the agent forward.⁴⁶ CTP-499, a pentoxifylline metabolite deuterated at multiple sites, is apparently going forward at this moment, although it is not clear that deuterium substitution has made a clinically meaningful impact.⁴⁷ For example, is it worth the effort to deuterate a drug for the sake of half-life extension if one ends up with (by all appearances⁴⁸) a b.i.d. dosing schedule for an extended release formulation?⁴⁹ Yet still there are not many data points, although Concert has done very well in generating partnerships, so it appears that there is potentially something of great value that will be, at minimum, illuminating when it is fully revealed.



These companies are creating multiple clinically advancing candidates in therapeutic categories including oncology, cardiovascular disease, and inflammation. Traditional drug discovery is of course still utilized, but it is combined with the deuterium kinetic isotope effect (DKIE) allowing creation of novel patentable drugs with predictable pharmacodynamics and tailored pharmacokinetics and toxicology. The concept has been around for decades, but now the efforts are sufficiently dedicated to see it through. Auspex was first to put a deuterated drug head-to-head with its protio counterpart in humans, and the results were compelling although not well communicated to the scientific community.⁵⁰ The drug is reported to be on track for a phase II clinical trial in neuropathic pain, so hopefully the company's silence represents the stealth of progress.

■ DECREASING RACIAL AND GENDER DIFFERENCES IN DRUG EFFICACY AND TOXICITY

Many oxidizing enzymes are known to exist in substantially distinct polymorphic forms, one allelic product giving rise to fast metabolizers, the other to slow metabolizers. These differences can be amplified in one racial group, giving rise to conundrums of clinical trial design because it is impossible to include sufficient numbers of every conceivable genetically distinctive group. Whether the drug is administered to Caucasian or African-descent patients, for example, can alone determine response, whether measuring efficacy or toxicity per cohort, if not per patient. One of the most popular commercially available drugs,

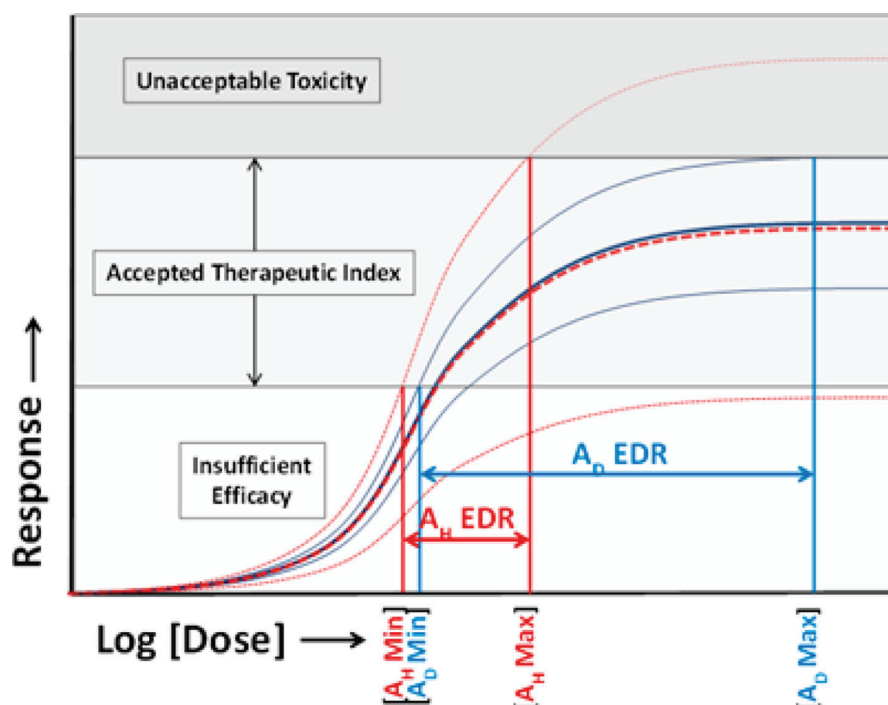


Figure 9. Decreasing interpatient variability offers an array of benefits that can be cashed in by (a) increasing dose and efficacy while retaining mean AEs or (b) decreasing dose and AEs while retaining mean efficacy. But then, there's always (c), splitting the difference. EDR = Effective Dosing Range.

nicotine, is metabolized in part by CYP2A6 and is therefore more slowly metabolized by the African-descent consumer,⁵¹ although in this instance not necessarily a patient just yet. This may affect the efficacy of this highly addictive substance. In the case of a drug that is produced to *treat* patients rather than *make* patients, deuteration has the potential to decrease oxidation through genetically variable mechanisms, thus decreasing inter-racial variability by diverting the clearance of drugs through more universal pathways. This means particularly the avoidance of CYPs such as CYP2D6, CYP2C9, and CYP2C19 in addition to the aforementioned CYP2A6. In general, by shunting of metabolism through a more universal clearance mechanism, the racial component of medication applicability is reduced.

■ REDUCING INTERPATIENT VARIABILITY

Improving the safety profile of a drug through the introduction of follow-on analogs while perfectly maintaining efficacy has proven to be difficult to achieve, perhaps well-nigh impossible, in practice. Generally, a new drug within a class may solve a long-standing problem only to bring along its own adverse events. For example, paroxetine is more potent than fluoxetine, so it may be expected to outperform fluoxetine in refractory depression. Unfortunately the shorter half-life leads to a greater likelihood of discontinuation effects in SSRIs.³⁸ This leads to poor adherence to dosing schedules which likely diminishes efficacy in the real world. More famously, rofecoxib has greater selectivity (for COX2/COX1) than celecoxib,⁵² so it may be expected to induce fewer peptic ulcerations. Unfortunately, it is more likely to cause more cardiac arrests perhaps because of this same increased selectivity.⁵³ Thus, the question about improvement in reduction of peptic ulceration potential was never really answered to anyone's satisfaction. If only there was a sure-fire way to improve efficacy while leaving toxicity unchanged or similarly to decrease toxicity while holding efficacy constant. I will posit that there is

such an option available. The simple act of deuteration stands to offer a graceful solution to this dilemma if properly applied. The act of shunting clearance through universal mechanisms, those pathways for which there are lesser interindividual variances, as in the CYP2A6 example above, stands to offer a unique opportunity to improve *most* drugs in this manner. In a further application of this idea, one could expect lesser *inpatient* variability as well. Consider a drug that is normally metabolized by CYP3A4. Suppose that we utilize the deuteration approach to decrease this metabolism, resulting in more of the parent drug being eliminated “as is” in our imaginary case, through simple passive diffusion mechanism so that only the physicochemical properties control the clearance. A standard response to this suggestion is that CYP3A4 is not one of the polymorphically expressed CYPs, so there is no problem here provided we do not have a drug–drug interaction to worry about. Well, first off, there are, in point of fact, a variety of CYP3A4 alleles, and I would not be so certain that there are no differences, just none that have yet been sufficiently elucidated with respect to their effect on drug disposition. But leaving that possibility aside, we are certain that expression levels of CYP3A4 can vary tremendously, within even one individual over time and not merely between individuals. This way of viewing the world of metabolism causes all oxidative clearance mechanisms to become opportunities for reducing variability of response. The graph (Figure 9) illustrates the benefit of reducing variability. Consider that in any clinical trial design, there is always that uncomfortable discussion wherein two camps develop on either side of a battle line. The more conservative side wants to err on the side of safety with a low dose, risking utter failure to meet efficacy goals (a leftward shift along the dose axis in Figure 9), risking the trap of the “insufficient efficacy” domain. The more risk-tolerant folks want to go with the higher dose, ensuring efficacy (a rightward shift along the dose axis in Figure 9) and risking entry into the

“unacceptable toxicity” domain, suffering a few more adverse events along the way, and perhaps risking an AE profile that unjustifiably ends the program. Some version of this scenario actually happens every time a clinical trial is put on the table. In the case of the deuteration of a protio drug that has already been through clinical trials, the benefit of capturing and reusing the roughly one to two billion dollars’ worth of readily available clinical information allows the deuteration approach to design a trial where the D_X -drug can be pushed to higher doses with no increase in AEs or alternatively pulled back to lower doses with equivalent efficacy and concomitant decrease in AEs. This is all made possible by the decrease in interpatient variability. With reduced interpatient variability, there will be fewer “outliers” who overdose because of poor metabolism. And there will be fewer failure-to-treat patients due to excessive metabolism. Figure 9 demonstrates this principle for a common drug A_H (red lines) and a specific deuterated drug having reduced interpatient variability, A_D (blue lines). As illustrated in this idealized example, the safe and effective dosing range can be increased quite dramatically if indeed the clearance mechanisms for drug A_H are variable in some regard whether through polymorphic expression or through fluctuating expression levels of the primary clearance proteins or cofactors. And though Figure 9 is idealized and theoretical, Table 1 is not. Table 1 represents the real results

Table 1. Reduction in Interpatient Variability As Observed in the Clinical Trial of Deuterated Venlafaxine vs Protio Venlafaxine

| measured parameter | reduction in interpatient variability, % |
|--|--|
| plasma half-life ($T_{1/2}$) | ≥ 20 |
| time at maximum plasma concentration (T_{max}) | ≥ 15 |
| maximum plasma concentration (C_{max}) | ≥ 40 |
| area under the curve (AUC) | ≥ 20 |

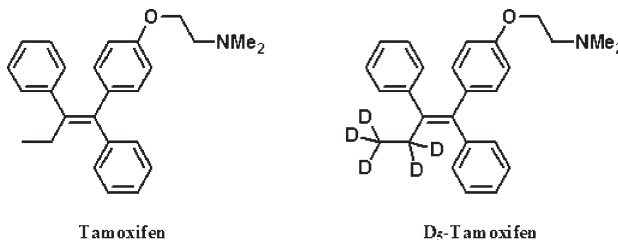
obtained in this author’s clinical trial of D_9 -venlafaxine (SD-254) vs the commercially available SNRI, venlafaxine, both formulated equivalently. Venlafaxine is one of the most prescribed treatments for neuropathic pain and/or depression. Interpatient variability dropped in every parameter recapped here and to no small degree. The observed reduction in the C_{max} variability of $\geq 40\%$ could be expected to have a substantial effect on the rate of adverse events for many drugs.

■ REDUCTION OF UNWANTED METABOLITES MAY LEAD TO DIRECT REDUCTION OF ADVERSE EVENTS

The simplistic dose–response curve in Figure 9 reveals the concept that at equivalent doses and with a drug that dramatically reduces interpatient variability, particularly with respect to C_{max} , far fewer patients should suffer adverse events from off-target mechanisms requiring a higher exposure than the target mechanism. Another manner in which a deuterated drug may reduce side effects is through reduction of undesired metabolites. All of this was observed in the author’s study of the SNRI D_9 -venlafaxine vs protio venlafaxine.⁵⁰ The total net incidence of reduction for all adverse events monitored was a staggering 35%, although the study was not powered sufficiently to break out individual AEs.

■ DEUTERIUM SUBSTITUTION FOR THE PURPOSE OF REDUCING GENOTOXICITY

Reducing reactivity can have benefits that extend far beyond the typical deuteration application, simply converting a t.i.d. drug to a q.d. drug, for example. Consider the opportunities to reduce reactive metabolites. An otherwise simple hydroxylation may convert a stable, highly target-specific drug into an indiscriminate electrophile. Extreme reactivity may produce an electrophile that bonds essentially irreversibly to the CYP itself. Impart a bit of stability to the electrophile and instead of one-hit kinetics, we produce a species that may diffuse away from the endoplasmic reticulum or mitochondrial structures to other cellular targets including even the nucleus. This thinking certainly helps in understanding a path to genotoxicity for tamoxifen. And the insightful deuteration of this drug led to a heavy version of tamoxifen with reduced genotoxicity.⁵⁴ This D_5 version of the drug was used quite brilliantly to shed light on the mechanism of the observed genetic toxicity of this targeted breast cancer treatment. Evidence was recently given for decay of the hydroxy metabolite to a quinone methide⁵⁵ that then forms protein conjugates. There may be multiple mechanisms by which such a metabolite does indeed go on to covalently modify DNA, but with one such degradation product so elegantly identified, we can decisively proclaim that deuteration has in this instance prevented a specific type of oncogenic event, not prevention in its entirety but by about 2- to 3-fold in magnitude, depending on the assay. It is important to consider the context under which these dangerous adducts form. In a living system, glutathione and perhaps cysteine may intercept a reactive metabolite of any given xenogen. So kinetically, the extent to which deuteration may attenuate oncogenic DNA adducts will be derived from the concentrations of reactive species produced, including the rate of production, the relative reactivity of the metabolite, and the locale of the metabolite. A metabolite may be deactivated more proficiently by glutathione if it is concentrated within the cell, whereas cysteine will more likely perform this function if the metabolite is actively expelled from a cell. If either cysteine or glutathione is required for removal of a troublesome metabolite, then the tamoxifen-treated patient is reliant on a sufficiently cysteine-rich (or cystine-rich) diet to limit the rate of this metabolite’s DNA–adduct formation. Recall that glutathione is itself a tripeptidyl product of cysteine, so sulfur is the nucleophilic atom responsible for outcompeting nitrogenous nucleophiles in sensitive regions of DNA, RNA, or proteins.



Considering the extraordinary power of simple deuteration to limit a carcinogenic process, why has this application of the deuterium approach failed to find widespread application even in companies devoted solely to the discovery of deuterium containing medicines? Perhaps the answer lies in the complexity of the process at hand. Reactive metabolites do not overtly announce their presence. Such a species will react at multiple sites so that the purification of discrete substances is going to be a rare occurrence. To go after a reactive metabolite is to chase an

idea. This exercise would require diligence, courage, great insight, and faith in one's possession of the aforementioned traits.

How often are such reactive species likely produced from the medicines that we rely on most? In my experience, it is rare to find a commercially available drug that, when assayed in humans and analyzed for parent as well as all metabolites, results in a satisfying mass balance, a recovery approaching anything close to 100%. Often the mass balance is actually omitted from such metabolite analyses, reporting only relative quantities. This is often the case even in the event that the drug is radioactively labeled, allowing quite reliable tracking of the drug, since a definitive identification of the metabolite structure(s) is unnecessary. In general, *most drugs leave behind at least 10% of their mass permanently, or at least semipermanently, embedded in the patient.* A review of the subject⁵⁶ found that of ~170 drugs examined for mass balance, more than 15% of these drugs left behind $\geq 20\%$ of their mass in the patient. That is a lot of molecular detritus building up day after day with no understanding of the long-term effects. Now this does not guarantee that the drug is covalently attached to anything, much less covalently attached to DNA. It is possible that a certain proportion of the unaccounted-for mass has simply found its way to a deep tissue depot, a completely different troubling thought. It does, however, leave behind a well-justified concern about the fate of these drug or drug fragments perhaps forever embedded in these patients and the likelihood that drug fragments have attached themselves via reactive metabolites to nucleophilic biomolecules. What is the cumulative effect of this apparent continuous modification of biological molecules (protein residues, nucleobases, etc.) by drug products? Does it interest anyone that deuteration offers a potential solution to a problem of entirely unknown magnitude? Leaving behind 20% or more of a drug in a patient's body forever every time that patient takes that medicine strikes me as a problem worth solving even if the clinical implications are not, as-yet, even mildly understood. More to the point, the answer to the question, "Why is this potentially powerful solution to a shockingly common problem not pursued?" is that this is a complex, ill-defined problem and our industry investigates easily stated problems that will likely fall to easily stated answers. We like to keep our complexity limited to the technology, not the problem we are solving. Deuteration is a relatively simple technology but with great capacity to address complex problems.

■ SAFETY MARGIN FOR DEUTERATED DRUGS

Those questions surrounding the use of deuterium in medicines that are spoken in the most hesitant voice are certainly those asking about the underlying toxicity. The natural abundance of deuterium is ~0.0156%, give or take a bit, based on where you live. This means that a human body of typical size, such as the author's, will contain approximately 1 g of deuterium all of the time. This deuterium will be distributed throughout one's body water as DHO, and it will be present in differing ratios in every conceivable position on every biomolecule that normally possesses hydrogen atoms. So considering that this approach has the purposeful end point of diverting metabolism *away* from the deuterated site, the increase in deuterium load is not surprisingly estimated at $\ll 1 \mu\text{g}$ per day for typical medication doses of 100 mg per day. This is an increase in the deuterium load of $\ll 0.1\%$ even allowing for the accumulation at steady state. Studies with direct treatment with deuterium oxide treated mammals have found that doses of $>25\%$ total replacement of body water are required to observe frank toxicity, and even this

toxicity is reversible.⁵⁷ The safety of deuterium is sufficiently well documented to the point that humans are frequently purposefully exposed to this isotope in singly and doubly labeled water experiments, as a matter of course, in various metabolic studies.⁵⁸

■ DEUTERIUM IS PRESENT IN VIRTUALLY ALL MEDICINES

It is helpful to keep in mind that every existing drug that contains hydrogen also contains deuterium at approximately its natural abundance of 0.0156%. Starting with a known incorporation rate of the isotope at each position, occupied nominally by hydrogen, a natural enrichment or depletion of certain sites is virtually certain to occur at various sites over time as the drug circulates repeatedly through various defensive tissues. Tissues most notable for their defensive strategies for the removal of xenogens include lung, liver, and gut wall. It is useful to remember too that gut flora contribute substantially to the oxidation of drugs. Gut flora exist in such abundance that there are 10-fold more such organisms than there are cells that compose the very human supporting these flora. Since these defensive tissues frequently use oxidative means to prepare xenogens for removal, a kinetic isotope effect stands as a mechanism for increasing the proportion of the more deuterium-rich isotopomers over time. A great many C–H bonds are labile to these processes, far more than typically portrayed in package inserts. The typical drug metabolism study might have you believing that there are but a few CYPs involved in the clearance of a particular drug and that each CYP attacks a specific site at a specific rate. The real picture is far more complex and less tidy and with great interpatient variability. Consider that ibuprofen, one of the simplest of all medicines, has more than 100 metabolites, most of which have not been definitively described in terms of structure, although the metabolites were certainly analytically discrete. So if one were to follow the fate of the wonderfully complex isotopomeric mixture of a given drug, an initially complex mixture would be expected to change in a complex manner in response to a myriad of isotopic-ratio altering events, and the velocity of change could be measured over time, with the acceleration converging on zero for each process at a different time of day for every site at the point of steady state and no sooner. Interestingly, in following a typical drug on its journey through the human body, one could in theory use this information therapeutically, such as in (a) therapeutic drug monitoring, (b) patient compliance studies, and (c) patient genotyping. Initial drug characterization would be a key element of such an exercise because the abundance of deuterium at each position is likely to be different from the global natural abundance because of differences in natural levels in raw materials used in the synthesis. Another critically important, if mundane, alteration in the natural abundance at a given site in the drug may arise from isotopic enrichment or depletion caused by the synthetic and/or purification methods used to produce the API.

■ WHEN DOES DEUTERATION FAIL TO ACCOMPLISH THAT WHICH SEEMS OBVIOUS?

It is often falsely assumed that one simply replaces a C–H pair that is subject to oxidation with a C–D pair so that stability ensues. This naive view is surprisingly pernicious and not one by which practitioners of this approach are burdened. And to make matters more difficult, there are no hard and fast rules for predicting what examples will work, although experience

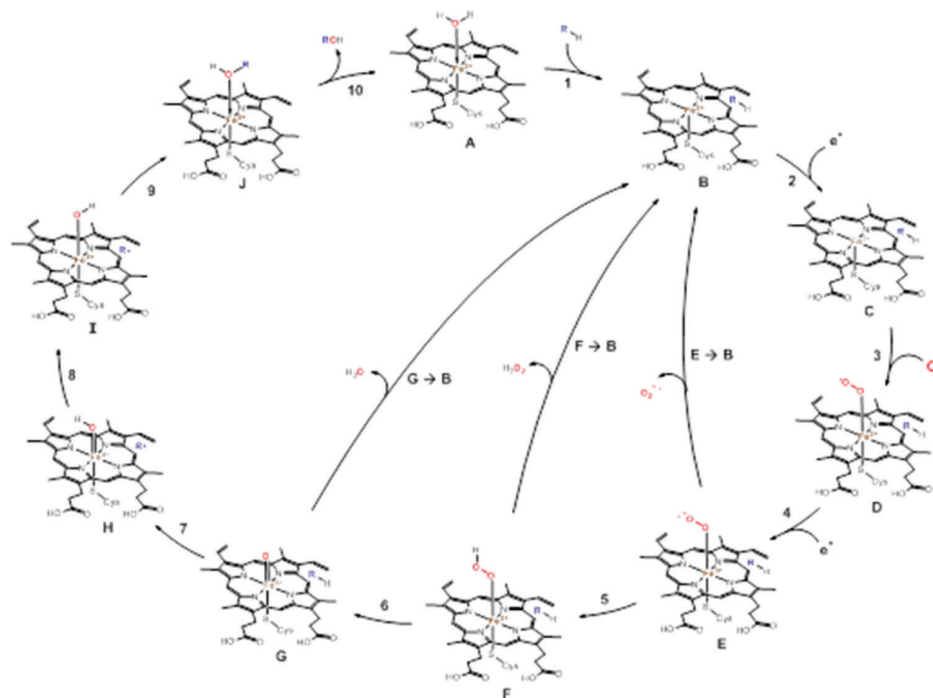


Figure 10. Step 1 is substrate binding. Step 2 is an electron transfer from cytochrome P450 reductase (CPR). Step 3 is binding of the iron center to molecular oxygen. Step 4 is another electron transfer although the recipient is the dioxygen radical. Step 5 is a quench to produce the hydroperoxide. Alternatively, in a process referred to as a autoxidation shunt, species E may revert back to species B with concomitant loss of peroxyanion radical. Step 6 is the formation of the reactive iron oxide. Alternatively, in a process referred to as a peroxide shunt, species F may revert back to species B with concomitant loss of hydrogen peroxide. Step 7 is a hypothetical hydrogen atom abstraction from the substrate. The reactive iron oxide can alternatively produce an oxirane with π systems. Alternatively, in a process referred to as an oxidase shunt, species G may revert back to species B with concomitant loss of water. Step 8 is simply an electron transfer thus reducing iron back to Fe(III). It is also hypothesized that the G converts directly to species I. Step 9 is the recombination of the R radical with oxygen. This step is likely very fast or concerted, since rearranged products are not common. Step 10 is the release of the oxidized product and coordination of water to Fe(III). The figure is adapted from ref 14.

certainly imbues one with empirical rules. And most such replacements would work quite well if under thermodynamic control. However, as with much of nature's way, it is kinetic control that determines the outcome of most oxidations. As previously noted, the oxidation of the C– x H bond must reside in a rate-limiting step for a kinetic isotope effect to be observed in a biological system. A DKIE will likely take place regardless; it just might otherwise occupy a portion of the long series of steps that occur rapidly. In some instances, the mechanism of oxidation allows solely for secondary isotope effects, as in the case of aromatic oxidation. The oxidase-induced reaction generally produces first an oxirane from the aromatic ring rather than a hydrogen atom abstraction (as in step 7, Figure 10). Thus, deuterium can provide at best a ~20% enhancement (DKIE \approx 1.2) for oxirane production where we are typically looking for at least a 300% enhancement (DKIE \geq 3) for most therapeutic improvements. In fact, deuterium substitution in the case of aromatic oxidation may actually lead to *more* facile oxidation; DKIE < 1 and maybe DKIE < 0.9. Further, it is possible to create a deuterated analog that induces the creation of an altogether new active, or worse yet, reactive, metabolite. This is primarily envisioned to occur in the instance that metabolic switching is induced by the stabilized C–D bond. That is, the stabilized bond may allow the drug to tumble in the CYP binding pocket where previously it simply reacted at the active site. The tumbling drug may now have added opportunities to find an alternative local minima for binding that produces a new binding mode and thus a new metabolite. This is sufficiently rare that I have not seen this

occur after approximately 200 chemotypes investigated. It can happen, although it will be a rare event.

PATENTABILITY

The patentability of this approach is well established; more than 100 USPTO-granted patents directly covering deuterium-substituted versions of approved or, at a minimum, well-known biologically active substances attest to this fact. However, there is a fair degree of confusion in the general pharmaceutical industry populace as to what constitutes a patentable claim in this space. When I first officially began work in this area in 1995, my organizations' lawyers made the straightforward observation that simply adding language to a patent covering deuterated analogs for the purpose of improving some aspect of ADMET was not sufficient to provide real protection. That is to say, unsupported language had no value whatsoever with the USPTO unless it was followed up with demonstrable art. This is an obvious conclusion in most contexts, but for some reason, it had seemed less obvious for deuterium for many chemists who were quite practiced in the art of prophetic patents. A novel, nonobvious prophetic statement would be ultimately unenforceable in the practice of preventing others from operating in the deuterium-substituted space of the composition of matter unless it was supported by real science in a subsequent filing on the same matter. The omission of methods to produce the deuterated analogs constituted a failure to enable the prophetic invention. Further, a lack of biological data to demonstrate an improvement is a failure to demonstrate the best mode, among other failings.

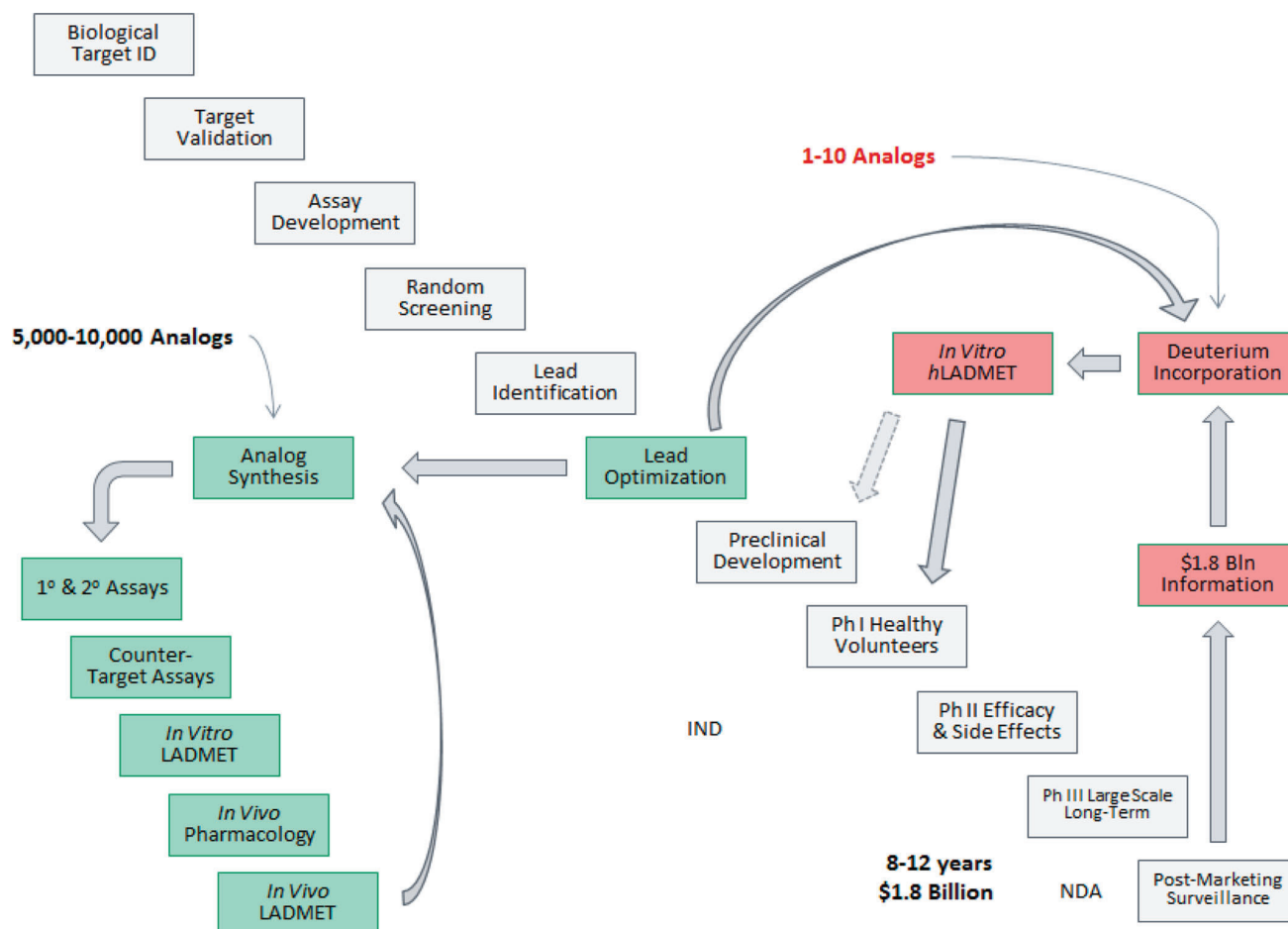


Figure 11. In the event that an approved drug acts as the lead molecule, the deuterium substitution approach may utilize roughly to 2 billion dollars' worth of information with only the minimal analoging, straightforward human metabolism projections, and metabolite analyses constituting truly new science. The remainder of the pathway ranges from highly predictable to entirely predictable, if it is necessary at all (LADMET = liberation, absorption, distribution, metabolism, excretion, and toxicity).

Nevertheless, language "protecting" deuterated analogs made its way into the boilerplate. Why would this be done? Why make statements that you are claiming the right to deuterated analogs if you have made no attempt whatsoever to enable that claim? The obvious answer is simply that those *not* skilled in the art of reading patents will be shooed away from deuterium-substituted versions of the properly claimed protio composition-of-matter. It is useful to realize that only a select few companies' employees, as a consequence of the corporate culture, have no fear of patents. The corollary is that most companies' employees live in eternal fear of patents and are easily scared away from a valuable, though wholly unprotected, volume of unprotected space. So a few well-placed words can easily protect what is, in reality, entirely unprotected.

■ A CRASS BUT NECESSARY TOPIC

A surprising number of questions come in about the cost-of-goods when using this approach. The short answer is that deuterium substitution actually saves a great deal of money at almost every step of the drug discovery and development process. But to convince the reader, let us start with raw material, "the raws". The ultimate source of most reagents for these transformations is the inexpensive, safe, and readily available heavy water (D_2O). A recent price for the common reagent D_2O

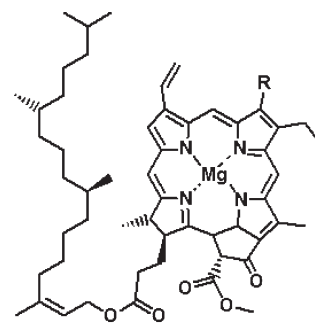
was \$88.70 per 100 g. This price drops considerably with a bulk purchase, but considering that our reagent has a molecular weight of just 18 g/mol, this 100 g purchase may just constitute a bulk purchase, since it may well get us through phase I, depending of course on our specific needs. Consider also that the price quoted is for 99.9% deuterium incorporation, and we can save substantially by opting for 99.8% incorporation, usually creating no clinically relevant difference in the performance of the final product. Just as germane is the observation that this approach is frequently applied to a drug whose protio synthesis has been well worked out, so the cost of the deuterio synthesis often benefits from the reduction in overall process development costs. The primary benefit that produces the reduced production costs, however, is clearly in the reduction of drug clearance. Even in most instances where the primary goal is something other than half-life extension, the deuterated analog still improves stability enough to dictate a lower effective dose. Thus, the usual 1–5% increase in cost of materials is more than offset by the ~10–70% reductions in dose. In assessing the cost-of-goods (COGs), it is instructive to consider the great number of discovery and development activities that may be references in full to the protio version with no alteration and possibly no need for repeating. Even the activities that must be repeated are often guided so effectively by the protio precedent that a single run-through is

sufficient. A D-switch drug discovery pathway consists most conspicuously of three activities: (1) an analoging effort, (2) metabolism studies using human reagents, and (3) extensive metabolite analyses. Every other typical activity is so utterly predictable that deuteration companies are understandably asking the FDA for exceptions to many of these activities. Consider just one activity that *must* be repeated when transferring from the protio effort to a deuterium-substituted drug: the analoging effort. The original protio effort may easily have required 5–10k analogs to traverse iteratively from hit to lead to the invention of the specific structure found in the approved medicine. A deuterium-substitution follow-on effort (Figure 11) will typically require only 1–10 analogs (a) to prove the viability of the approach (or lack thereof) and (b) to find the ideal substitution pattern.⁵⁹ In addition to the analoging effort, the human metabolism studies and the assays for toxic, or otherwise undesirable, metabolites are the primary activities required for discovery of the improved drug substance. The analoging effort may, in the ideal scenario, use the protio synthetic route to such an extent that merely purchasing a deuterated reagent allows for no other significant changes to synthesis. In the event that the entire synthesis must be reworked, this still constitutes a minor effort when one considers that frequently the 5–10k analog efforts of typical drug discovery efforts require the de novo syntheses of dozens or even hundreds of unique core structures. A nonchemist may not appreciate the virtually guaranteed weeks-long headaches inherent in synthesizing even one “probe analog” with that extra nitrogen in the aromatic ring, for example. Those efforts do not exist in this synthetically simplified world of the D-switch. Ultimately, when it becomes clear which breaks, if any, are routinely granted by the FDA, one can produce a scalar to estimate full discovery and development costs of a deuterated drug. However, even in the complete absence of any real breaks from the FDA on typical development requirements, the cost of R&D in this space will be far less than half of a typical program because of the near complete absence of the range-finding, wandering-in-the dark trials that must accompany the successful NDA of any other kind of drug.

POTENTIAL FOR BIOENGINEERING WITH DEUTERIUM

Bioengineering is not just for the production of biologics, despite what your corporate structure may suggest. Techniques for producing small molecules in heavy water culture have been around for decades and are surprisingly amenable to techniques available to most drug discovery teams. Thus, one can envision analog production by way of semisynthesis *from* perdeuterated starting materials rather than the more typical deuterium incorporation *into* protio materials. Some of the more interesting and useful perdeuterated materials produced in this manner include chlorophyll and perdeuteroglucose.⁶⁰ All of the species most frequently tapped for biologics production have contributed to this cause, including bacteria, yeast, mammalian culture, and even algae.⁶¹ *E. coli* has been cultured stably in >99% D₂O for more than 1 year, the primary difference being slower growth. Yeast acclimates surprisingly rapidly to >99% D₂O; several species have been used with ready success. Most mammalian cultures are limited to moderate deuterium levels; CHO and other mammalian cells typically hit a wall when D₂O concentration exceeds ~50%,⁶² although one variant was produced in >99% D₂O, albeit with up-regulation of heat shock proteins. Algae production⁶³ may be less familiar to the

typical discovery team, but this species has been maintained stably in >99% D₂O for more than 1 year in the production of chlorophyll.⁶⁴ In general, cultures grow more slowly in deuterium oxide, although many contravening results have been found including the stimulation of growth in *Mycobacterium tuberculosis* at D₂O levels up to ~80%.⁶⁵ The opportunities for deuterium incorporation into semisynthetic schemes are limited only by the chemists' imagination (and perhaps their knowledge of the biosynthesis and the relevant substitution chemistry, in reverse).



Perdeuteriochlorophyll
C₅₅D₇₄MgN₄O₅

LURE OF TRITIUM

Deuterium can occasionally net a more stable drug with few clinically relevant changes, so what is the effect of adding yet another neutron to a site anticipated for oxidative attack? Well, the increase in kinetic stability is quite substantial. Unfortunately, stable deuterium has now given way to radioactive tritium. Few medicinal chemists have the training or the facilities to directly work with tritium, but fortunately, it is a suitable approach to simply model tritium with deuterium. Using the simple relationship illustrated in eq 1 (the Swain equation⁶⁶),

$$\frac{k_H}{k_T} = \left(\frac{k_H}{k_D} \right)^{1.442} \quad (1)$$

one can run the necessary in vitro or in vivo experiments with deuterium and have a reasonable expectation as to the outcome of the hot experiment. As an example, a DKIE of 3 is predicted to translate to a tritium KIE (TKIE) of ~4.9. The most spectacular gains may be in the unpredictable instance where tunneling occurs for the hydrogen substituted species, a phenomenon far less likely with a tritiated analog. This is not a method for the faint of heart but could be envisioned to have applications in oncology applied to particularly lethal suicide inhibitors.

CONCLUSION

Deuterium incorporation is an easy technique to initiate. And with thoughtful and well-informed decisions along the way, with a properly designed assay strategy, reaching a decision point is characteristically quick and painless. But in the absence of assays for unexpected benefits, it is also very easy to miss important improvements that are so often obtained with this technology. It certainly does not always work the way we might hope, but it pays to keep an open mind and a diligent analytical strategy. The technology is mature in many regards but nascent in others, allowing the solution of a host of otherwise intractable problems while opening up opportunities for novel research. Whether this

is what happens really will depend on the emergence of that one person who is in possession of both the unique abilities and the opportunity. There are many folks out there with the former quality; it is the latter possession, opportunity, that can be elusive. Harold Urey predicted most of what has happened with deuterium back in the 1930s.⁶⁷

*"The chemical properties of two isotopes are in general very similar... The differences in physical and chemical properties of ... compounds of hydrogen and deuterium are much greater than in the case of any other two isotopes which have been investigated thus far... The velocities of chemical reactions in which deuterium replaces hydrogen differ more markedly than do the equilibrium properties. Hydrogen reacts with chlorine 13.4 times more rapidly at 0°C than deuterium, and similar differences are observed in the case of other chemical reactions... perhaps more interesting than the gross effects of life and death of living organisms in deuterium oxide can be secured by using deuterium as an indicator in the study of metabolic processes within living things. It often is of interest to trace a variety of atoms or compounds through living organisms. Deuterium makes possible such studies for if given to an animal in its food the particular compounds of the food can again be identified in the excretory products, in the blood, in the fat deposits of the body, or other tissues, and hence the course of the foods through the animal body can be traced. Studies of this kind will probably prove to be among the most interesting applications of deuterium."*⁶⁷

As to the purposeful production of a deuterium-enriched compound for human consumption, the difficult question to answer is, "when?"

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Notes

The author declares no competing financial interest.

Biography

Thomas G. Gant completed undergraduate studies at Wayne State University, MI, and doctoral studies at Colorado State University. Postdoctoral studies were carried out at Harvard University, MA, under the mentorship of Nobel laureate Professor E. J. Corey. His professional career in drug discovery and DKIE applications to medicines began at Pfizer (1995), moving then to Millennium Pharmaceuticals, leading the chemistry efforts for the Velcade backup. He then became the Scientific Founder (2004) and CSO of Auspex Pharmaceuticals, completing the first clinical trial to directly demonstrate a substantial kinetic isotope effect of a deuterated drug (SD-254) in humans. He is currently Associate Professor and Director of Pharmacy Post-Baccalaureate studies at Lake Erie College of Osteopathic Medicine, PA, and is President of the pharmaceutical consulting firm Recondite Falls Discovery, LLC.

ABBREVIATIONS USED

Standard Abbreviations

ADME, absorption, distribution, metabolism, and excretion; ADMET, absorption, distribution, metabolism, excretion, and toxicity; AE, adverse event; API, active pharmaceutical ingredient; AUC, area under the curve; BBB, blood–brain barrier; b.i.d., twice a day; CNS, central nervous system; CYP, cytochrome P; DNA, deoxyribonucleic acid; FDA, Food and Drug Administration; GI, gastrointestinal; hERG, human ether-

a-go-go-related gene; HPLC, high-performance liquid chromatography; MHz, megahertz; MS, mass spectrometry; NAD⁺, nicotinamide adenine dinucleotide; NDA, new drug application; NMR, nuclear magnetic resonance; PD, pharmacodynamics; PK, pharmacokinetics; q.d., once daily ("quaque die"); RNA, ribonucleic acid; t.i.d., three times daily ("ter in die")

Nonstandard Abbreviations

AMU, atomic mass unit; C_{max}, maximal concentration; CPR, cytochrome P₄₅₀ reductase; COGs, cost-of-goods; DKIE, deuterium kinetic isotope effect; EDR, effective dosing range; ID, identification; KIE, kinetic isotope effect; LADMET, liberation, absorption, distribution, metabolism, and toxicology; MAO, monoamine oxidase; NPV, net present value; PhII, phase II; PhIII, phase III; QTc, corrected QT interval, where QT is an interval between the start of the Q-wave and the end of the T-wave in an electrocardiogram; R&D, research and development; RP, reverse phase; S9, a microsomal component containing cytochrome P₄₅₀ enzymes; SAE, serious adverse event; TKIE, tritium kinetic isotope effect; USPTO, United States Patent and Trademark Office

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