“Dial Up and Lock In”: Asymmetric Organo-Brønsted Acid Catalysis Incorporating Stable Isotopes

Bew and co-workers report an asymmetric organo-Brønsted-acid-catalyzed reaction that incorporates one or more stable isotopes and affords structurally and functionally diverse chiral non-racemic aziridines with excellent levels of isotope incorporation. The utility of the methodology is further substantiated by their straightforward transformation into high-value isotope-derived optically active natural and un-natural α-amino acids. The iso-organocatalysis approach advanced in this work is extendable to other reactions and should therefore prove a versatile approach to sought-after isotope-labeled compounds.

HIGHLIGHTS

- Stable-isotope incorporation mediated by organo-Brønsted acid
- Asymmetric synthesis of aziridines with stable isotopes
- Incorporation of single or multiple and similar or different isotopes
- Generation of optically active stable-isotope-derived amino acids

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“Dial Up and Lock In”: Asymmetric Organo-Brønsted Acid Catalysis Incorporating Stable Isotopes


SUMMARY
An operationally simple organo-Brønsted-acid-catalyzed asymmetric and regioselective “dial up and lock in” of one or more stable isotopes into organic compounds is unknown. Here, we describe a newly designed, chemically versatile protocol mediating single- or multiple-isotope incorporation into aziridines via a one-pot, three-component, two-step process. By exploiting easy-to-generate isotope-derived starting materials, it allows complete control of isotope positioning, affords >95 atom % isotope incorporation, and generates cis-aziridines with excellent optical activities and regioselectivities. Demonstrating a “low entry point,” and thus easy access to a broad range of researchers, it requires no specialist laboratory equipment and employs readily attainable reaction conditions. Demonstrating their utility, the aziridines are easily transformed into sought-after chiral non-racemic α-amino acids appended with one to three (or more) identical or different isotopes. The widespread use of these compounds ensures that our methodology will be of interest to biological, medicinal, pharmaceutical, agrochemical, biotechnology, materials, and process chemists alike.

INTRODUCTION
Stable isotopes such as deuterium ([2H]), carbon ([13C]), nitrogen ([15N]), and oxygen ([18O]) are widely employed in contemporary science. Generating an isotopologue by incorporating a single isotope can propel a compound to “front line” applications in medicinal chemistry, toxicology, biology, structural biology, and mechanism determination. Differences in physical properties are evident in comparisons of otherwise identical, natural-isotope-abundant compounds with isotope-enriched isotopologues. Thus, the seemingly trivial substitution of a hydrogen atom for a deuterium atom (2-fold greater mass) affords a compound with a shorter, stronger C-[2H] bond and modifies its polarity, molar volume, Van der Waal properties, dipole moment, pKa, and lipophilicity; all of these can, but not always will, afford detectable chemical-reactivity and/or physicochemical differences. Isotope-dependent technologies require not only state-of-the-art instrumentation but also, importantly, convenient and efficient synthetic routes to compounds with unambiguous, site-specific isotope incorporation. Taking these points into consideration, isotope chemistry goes beyond academic interest or research curiosity; it is instead at the forefront of a raft of technologies that exploit labeled compounds in a plethora of cutting-edge applications. For example, over 3,400 volatile organic compounds (VOCs) have been...
detected in deep alveolar breath. This biological medium is a treasure trove of information relating to dysfunctional metabolic processes. Uniting isotope chemistry and synthetic chemistry, non-invasive “breathomics” allows a personalized-medicine approach to identifying patients who would benefit from a particular targeted therapy. The CYP2D6 metabolism of mono-[13C]-labeled dextromethorphan (1) is a breathomics biomarker that generates [13C]O₂ and 2, as shown in Figure 1. If we compare the isotope ratios in the exhaled [13C]O₂/[12C]O₂ via infrared (IR) spectrometry, [13C]-1 is a point-of-care phenotypic screen for breast cancer that can identify patients who would benefit from tamoxifen therapy. Only by dovetailing the site-specific synthesis of [13C]H₃-1 with an understanding of tumor metabolism and IR spectrometry is it possible to use 1 as a non-invasive precision-medicine tool. Indeed, the direct relationship among advances in state-of-the-art equipment, development of contemporary analytical techniques, and software has allowed huge gains in the detection sensitivity of isotopes. The result? An upsurge of technologies and associated synthesis methodology exploiting single- or multi-isotope-derived compounds.

The growing demand for molecules that incorporate single or multiple different isotopes can be further highlighted by their key use in biophysical analytical techniques. These include ¹H-NMR for in situ metabolic analysis using [13C₂]-dopamine, in vivo screening of inhibitors using long-lifetime hyperpolarizable [13C]-[2]H-bioprobes, NMR-based stereo-array [13C]-[2]H-isotope labeling for determining protein structure, rate-constant analysis in enzyme-catalyzed reactions, stimulated Raman scattering for imaging proteome degradation, metabolic fingerprinting, in vivo labeling of proteins in living cells, coherent anti-Stokes Raman spectroscopy for determining the bacteriorhodopsin photocycle, [¹³C]=[¹⁸O]-labeled peptides and 2D IR spectroscopy for amylin-inhibitor complexation, stimulated emission depletion microscopy with [¹³C]-[¹⁵N]-α-amino acids in correlated optical and isotopic nanoscopy, multi-isotope imaging mass spectrometry for protein study, proteomics- and mass-spectrometry-based MARQUIS and iTRAQ using labeled peptides, triple-resonance isotope-edited NMR using [¹³C]-[¹⁵N]-pyrimidines for monitoring catabolism and drug activity, deuterated starting materials in physical organic chemistry as kinetic isotope probes of reaction mechanisms, and stable-isotope-labeled pharmaceuticals as internal standards for pharmaceutical bioanalysis.

Installing an isotope at a specific location within a desirable and tractable compound is an expensive, often deceptively difficult undertaking in comparison to generating its naturally abundant counterpart; the difficulty increases substantially when multiple and different isotopes are required at specific sites via a single asymmetric reaction. Catalysis underpins modern synthetic chemistry and is built on three pillars: (1) transition-metal catalysis, (2) biocatalysis, which also includes microalgae,

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and (3) organocatalysis; these three examples have been used exclusively to generate optically active isotope-labeled compounds. In stark contrast, the development and application of organo-Brønsted-acid-mediated protocols that transform isotope-derived starting materials into high-value products is non-existent. The third pillar must be strengthened!

Exploiting “better, milder, faster, cheaper” organocatalysis has matured to such an extent that it is now, more often than not, the first port of call in planning a synthetic route to a high-value product with a specific form and function.28–32 Surprisingly, given all the aforementioned examples of isotope applications, two aspects of important contemporary science, that is, organo-Brønsted acid catalysis and isotope chemistry, have yet to be dovetailed together.

From an organocatalysis point of view, developing an isotope-incorporating protocol that generates compounds with one isotope or, especially, multiple and different isotopes presents specific problems that necessitate special consideration. From a reaction-screening and product-optimization perspective, both enantiomeric forms of the desired organocatalyst should be commercially available and in sufficient quantity or synthesized from cheap, readily available starting materials. To ensure widespread utilization, the starting materials should have >90% isotope enrichment. It is, however, worth noting that there are far fewer commercial-isotope-enhanced starting materials than natural-isotope-abundant starting materials. These need to be readily generated from the corresponding natural-isotope-abundant precursors with the use of commercial, easy-to-handle sources of isotope-derived reagents, e.g., [2H] → [2H]2O (e.g., Aldrich catalog no. 151882), [15N] → [15N]H3 (e.g., Aldrich catalog no. 488011), [13C] → α-[13C]-benzoic acid (e.g., Aldrich catalog no. 277746), and [18O] → H2[18O] (e.g., Aldrich catalog no. 329878).

A particular concern with isotope-derived compounds or methodology incorporating isotopes is “scrambling.” Yet to be developed is an organo-Brønsted acid protocol that has excellent substrate scope and allows, at will, isotope “switching”—that is, the incorporation and transformation of starting materials with multiple different or identical isotope “patterns” into isotope-defined products without scrambling and with excellent levels of incorporation. The importance of being able to “dial up and lock in”—that is, insert an isotope into a predetermined position on a molecule and ensure that it remains in position—stems from the extremely difficult task of separating scrambled compounds. Therefore, it is critical, at the outset, that a reaction be sufficiently robust that it transforms structurally and functionally diverse derived starting materials consistently and reliably into products with >95 atom % site-specific “dialed up and locked in” isotopes.

Syntheses of isotope-labeled aziridines have focused mainly on incorporating [2H] and, to a significantly lesser extent, [13C]. In summary, they utilize one or more of the following: multi-step protocols; strongly basic reaction conditions; and/or formation of hazardous nitrene intermediates that require toxic metal salts that afford, in some cases, [2H]-aziridines in low yields and/or with reduced levels of [2H] incorporation.33–49

As outlined in Figure 2, we report an organocatalysis milestone: a one-pot, multi-component, asymmetric organo-Brønsted-acid-catalyzed aza-Darzens reaction that “dials up and locks in” one or more identical or different isotopes into optically active aziridines.
Our protocol uses an easy-to-synthesize catalyst that transforms cheap, readily synthesized starting materials into high-value aziridines with excellent levels of isotope incorporation, yield, and diastereo- and stereoselectivities. Confirming their status and utility, aziridines are readily transformed into a plethora of functionalized and optically active “secondary” products with ring opening, offering a straightforward entry point to isotope-derived natural or unnatural \( \alpha \)-amino acids, e.g., \( 3 \) (Figure 2).

Further substantiating their importance as secondary products is their transformation into sought-after tertiary products, e.g., alkaloids, antibiotics, heteroaromatic rings, and heterocyclic peptides with, again, chemoselectively “dialed in” isotopes.50 Because non-isotope-enhanced aziridines and \( \alpha \)-amino acids are important, it is not surprising that this extends to their isotopologue counterparts. By way of example, Figures 1 and 2 outlines select examples of cutting-edge applications for isotope-enhanced optically active \( \alpha \)-amino acids (e.g., \( 3 \)) in a wealth of biophysical technologies, e.g., Figures 2H,51-54 2I,55-57 2J,58-60 2K,61 2L,62,63 2M,64-66 2N,67-69 2O,70,71 and 2P.72

We report an organocatalytic approach to isotope incorporation that supplements and is complementary to transition-metal and biocatalysis approaches. A conceptually straightforward “mix and match” approach, outlined in Figure 3, includes a convenient entry point to optically active aziridines adorned with different types and numbers of identical or different isotopes and, importantly, generates them by using a handful of readily synthesized simple core starting materials. Developing an isotope-enhanced protocol should, in many respects, mirror the advantages...
associated with conventional, non-isotope-enhanced organocatalytic reactions. They should be straightforward to set up, require minimal maintenance, and have no requirements for specialist equipment, e.g., gloveboxes, (photo)bioreactors, fermenters, or pressurized facilities for handling gases. Preferably, there should be no requirement for highly inert, rigorously anhydrous atmospheres or ultra-dry solvents.

Our procedure employs low-cost, "out of the box" screw-capped vials or crimp-sealed microwave tubes, and importantly, the levels of laboratory expertise required for executing single- and multi-isotope organo-Brønsted-acid-catalyzed reactions are comparable. Indeed, the process of generating one, two, three, or four, etc., isotope-derived optically active aziridines is identical. In a comparison of organic and transition-metal catalysts, the differences are clear: many of the latter require ligand and metal pre-complexation before the starting materials are added (often, the metal salts and/or ligands are expensive and sensitive to air and/or moisture, which complicates handling). In contrast, shelf-stable organo-Brønsted acids are weighed with no special precautions to preclude air or water; the catalyst is added with the reactants and removed by a simple filtration through alumina or silica gel. Additionally, many structurally and functionally diverse asymmetric...
RESULTS AND DISCUSSION

Independently, both organo-Brønsted acid catalysis and multicomponent reactions have an impressive and established track record of synthetic transformations. Not surprisingly, when these are dovetailed, the resulting protocols are robust and powerful methods for the construction of molecular species augmented with complexity, but importantly, they are generated via fewer synthetic operations, isolations, and purification steps.

Organo-Brønsted acids, e.g., BINOL-phosphoric acid based on 4, are privileged catalysts. Symptomatic of their use is N-substituted imine protonation and activation, a widely employed strategy for lowering the lowest unoccupied molecular orbital energies of C=N bonds while increasing their susceptibility to nucleophilic attack. With this tactic, racemic or optically active non-isotope-enhanced aziridines can be generated from alkyl diazoacetates and in situ synthesized or preformed N-substituted imines. Given the widespread and operational simplicity of organo-Brønsted acid “imine activation,” it is somewhat surprising that an isotope-incorporating variant of the aza-Darzens reaction has not been reported.

Initiating our research, we screened organocatalysts 4–8 for their ability to “activate” imine 9 by allowing its reaction with α-[2H]-diazoeeter 10 or 11 and thus afford [2H]C₂-12 (R = Et or tBu; Figure 4). Incorporating the 2-pyridyl group within
(E)-1-phenyl-N-(pyridyl-2-ylmethylene)methanamine 9, we surmised that proton transfer from 4–8 would generate an intramolecular bifurcated hydrogen bond between the two sp² nitrogens and afford an optically active and electrophilic immonium ion [13]. Our rationale for this approach, and its potential benefits, originated from published density functional theory (DFT) calculations (BHandHLYP method) on an asymmetric Mannich reaction that indicated that a bifurcated hydrogen bond was able to activate and create a rigid optically active environment around an N-(2-hydroxyphenyl)-derived imine. Disappointingly, 4–8 were unable to activate 9, and no reaction was observed. Indeed, 9 was returned with good mass balance even when (1) the reaction was performed neat, i.e., [2H]-10 was the solvent; (2) the reaction occurred in the presence of 4 Å molecular sieves to remove any potentially detrimental trace amounts of water; (3) 4–8 were left for 120 hr at ambient temperature and subsequently at an elevated temperature, rescinding the possibility of a slow reaction; (4) 4–8 were increased to 20 mol %, negating the possibility that they had poor catalyst turnovers; and (5) a series of solvents with diverse dielectric properties were investigated in a search for a strong “solvent effect.” All to no avail.

Failure to generate a sufficiently activated form of 9 could have been associated with the relatively low pKₐs of 4–8, i.e., ~13 in MeCN. On the contrary, (R)- and (S)-BINOL N-triflylphosphoramides (14) are, as outlined in Figure 4, considerably more reactive and acidic (pKₐs of ~6.5 in MeCN). As a consequence, these have widespread application in the organo-Brønsted acid catalysis world. Independently reacting [2H]-10 or [2H]-11 ([2H] > 95 atom %) with (E)-1-phenyl-N-(pyridyl-2-ylmethylene)methanamine 9 in the presence of (S)-14 (Ar = Ph, 10 mol %) for 12 hr at ambient temperature consumed both reactants. ¹H-NMR analysis of the two unpurified reactions indicated that 12 (R = Et or tBu) had been generated cleanly and efficiently (no enamine). The unoptimized yields were good (78% for R = Et and 71% for R = tBu), and the absence of the characteristic doublet in both ¹H-NMR spectra at ~2.6 ppm (associated with the aziridine proton, i.e., HC-CO₂Et or tBu) confirmed excellent levels ([2H] > 95 atom %) of regiospecific [2H]-incorporation on C₂ of 12; no evidence for isotope scrambling was observed. The excellent levels of [2H]-incorporation on C₂ verified several important points: (1) during the reaction, the deuterium on [2H]-10 and [2H]-11 did not undergo [1H] ↔ [2H] exchange; (2) once “installed” on 12, the deuterium on C₂ did not scramble onto, for example, the [2H]C₃ position; and (3) (S)-14 did not promote [1H] ↔ [2H] exchange in aziridine 12 or alkyl α-[2H]diazoacetate [2H]-10 or [2H]-11.

Disappointingly, chiral column high-performance liquid chromatography (HPLC) analysis established that aziridines 12 (R = Et or tBu) were racemic. So, although we were delighted that (S)-14 (Ar = Ph) catalyzed the synthesis of 12, its formation as a racemic mixture was frustrating. Completing a comprehensive reaction condition, a substrate, solvent, and catalyst screening program established that at ~80°C (E)-2-(tert-butoxy-N-pyridin-2-ylmethylene)aniline 15 reacted with [2H]-11. Although the reaction can be successfully conducted in various solvents, for catalyst-optimization studies we chose a combination of [2H]chloroform/dichloromethane (8:2 v/v) as a convenient solvent mixture because it allowed ¹H-NMR monitoring of the reactions. Screening a wide array of catalysts, we eventually settled on “sterically tuned” 3,3’-anthracenyl-(S)-17 (10 mol %), which afforded [2H]C₂-16 (Figure 4) in an 82% yield and, importantly, an excellent 99% enantiomeric excess (ee). ¹H-NMR analysis and careful integration of the signals associated with tert-butyl 1-(2-tert-butoxyphenyl)-3-(pyridin-2-yl)-[2H]C₂-aziridine-2-carboxylate (16) confirmed regiospecific [2H] > 95 atom % at C₂ (see Figures S1 and S2).
We next undertook a substrate study incorporating an array of preformed structurally and functionally diverse N-(2-tert-butoxyphenyl)imines based on 18 (Table 1), sterically demanding [2H]11, and catalyst (S)-17. Employing preformed imines rather than generating them in situ assured us that any lack of reaction or low yield was not associated with slow or incomplete N-(2-tert-butoxyphenyl)imine formation at −80°C. We confirmed the utility of (S)-17 to mediate the efficient synthesis of [2H]C2-labeled aziridines by incorporating a diverse array of unfunctionalized monocyclic aromatic (e.g., benzaldehyde), multicyclic aromatic (e.g., 2-naphthaldehyde), and substituted aromatic (e.g., 4-chloro, 4-bromo, 4-fluoro, pentafluoro, 4-nitro, 4-cyano, 4-(O-Fmoc)phenyl) carboxaldehydes, as well as heterocyclic 2-pyridinecarboxaldehyde. All were successfully synthesized in good (55%) to excellent (97%) yields and, in the majority of examples, excellent ee (90%–99%); see, for example, 16 and 22–29 in Table 1 (and Figures S5–S19). Furthermore, incorporating the sterically less demanding isopropyl ([2H]-19) or allyl ([2H]diazooacetate [2H]-20) afforded [2H]C2-30–[2H]C2-32 in good yields and excellent (90%–96%) ee. Importantly, universally excellent regiospecific [2H] > 95 atom % incorporation at the C2-atom, as determined by 1H-NMR (see Figures S20–S24) and mass spectrometry molecular and fragment ion analysis, was observed for [2H]C2-21–[2H]C2-32 and was directly related to the isotopic enrichment employed in starting materials 11, 19, and 20. Substantiating this, the 1H-NMR spectrum of [2H]C2-21 (see Figures S3 and S4) confirmed that the characteristic doublet associated with the proton on C2 at 3.1 ppm had disappeared with concomitant formation of a broad singlet at 3.4 ppm associated with the proton on C3. This observation was general and consistent for all 1H-NMR spectra of [2H]C2-21–[2H]C2-32, albeit the chemical shifts of individual C3-protons varied slightly. cis-Diastereoselectivity had been tentatively assigned to the

<table>
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<tr>
<th>Number</th>
<th>Aryl Group (Ar)</th>
<th>R Group</th>
<th>Yield (%)</th>
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For synthesis, see Figures S4–S15.

Table 1. Organo-Brønsted-Acid-Catalyzed Synthesis of Optically Active [2H]C2-21–[2H]C2-32

We next undertook a substrate study incorporating an array of preformed structurally and functionally diverse N-(2-tert-butoxyphenyl)imines based on 18 (Table 1), sterically demanding [2H]-11, and catalyst (S)-17. Employing preformed imines rather than generating them in situ assured us that any lack of reaction or low yield was not associated with slow or incomplete N-(2-tert-butoxyphenyl)imine formation at −80°C. We confirmed the utility of (S)-17 to mediate the efficient synthesis of [2H]C2-labeled aziridines by incorporating a diverse array of unfunctionalized monocyclic aromatic (e.g., benzaldehyde), multicyclic aromatic (e.g., 2-naphthaldehyde), and substituted aromatic (e.g., 4-chloro, 4-bromo, 4-fluoro, pentafluoro, 4-nitro, 4-cyano, 4-(O-Fmoc)phenyl) carboxaldehydes, as well as heterocyclic 2-pyridinecarboxaldehyde. All were successfully synthesized in good (55%) to excellent (97%) yields and, in the majority of examples, excellent ee (90%–99%); see, for example, 16 and 22–29 in Table 1 (and Figures S5–S19). Furthermore, incorporating the sterically less demanding isopropyl ([2H]-19) or allyl ([2H]diazooacetate [2H]-20) afforded [2H]C2-30–[2H]C2-32 in good yields and excellent (90%–96%) ee. Importantly, universally excellent regiospecific [2H] > 95 atom % incorporation at the C2-atom, as determined by 1H-NMR (see Figures S20–S24) and mass spectrometry molecular and fragment ion analysis, was observed for [2H]C2-21–[2H]C2-32 and was directly related to the isotopic enrichment employed in starting materials 11, 19, and 20. Substantiating this, the 1H-NMR spectrum of [2H]C2-21 (see Figures S3 and S4) confirmed that the characteristic doublet associated with the proton on C2 at 3.1 ppm had disappeared with concomitant formation of a broad singlet at 3.4 ppm associated with the proton on C3. This observation was general and consistent for all 1H-NMR spectra of [2H]C2-21–[2H]C2-32, albeit the chemical shifts of individual C3-protons varied slightly. cis-Diastereoselectivity had been tentatively assigned to the
C2,3-substituents on [2H]C2-21–[2H]C2-32. Confirmation of this assignment was essential. Subjecting a crystal of [2H]C2-27 to X-ray analysis (Figure 5) clearly showed the cis-diastereoselective relationship between the tert-butoxycarbonyl and 4-nitrophenyl substituent, as well as their trans-relationship with the N-(2-tert-butoxyphenyl) group attached to the nitrogen of the aziridine (see Accession Numbers).

Preliminary results indicate that (S)-17 is an excellent activator of preformed N-(2-tert-butoxyphenyl)imines, given that it induced very good levels of diastereo- and stereoselectivity within a diverse array of N-(2-tert-butoxyphenyl)-C2,3-disubstituted [2H]C2-aziridines. Confident that our methodology was robust, we focused our attention on the cis-diastereoe- and stereoselective synthesis of [2H]C2-aziridinyl alkyl esters via an operationally simplified one-pot, three-component, two-step protocol employing 2-tert-butoxaniline (34), aryl [2H]aldehydes (e.g., 33, [2H] > 95 atom %), and natural-isotope-abundant ([2H] = 0.0156%) alkyl diazoesters. Probing this, we reacted commercially available α-[2H]-benzaldehyde (2H > 98 atom %), 2-tert-butoxaniline (34; Table 2), tert-butyl α-diazoacetate (35), and organo-Bønsted acid (R)-17 (10 mol %). Gratifyingly, [2H]C3-36 was afforded in an unoptimized 65% yield and 88% ee; these values compare favorably with those derived from (S)-17 for the synthesis of [2H]C2-21 (65% yield and 81% ee; Table 1). No isotope scrambling was observed, and [2H]C3-36 was afforded with >95 atom % deuterium incorporation at C3. A series of functionalized aryl α-[2H]aldehydes ([2H] > 95 atom %) based on 33 were readily synthesized by a slightly modified procedure originally reported by Curley et al.121 Reacting these with 2-tert-butoxaniline 34, tert-butyl α-diazoacetate 35, and (R)-17 (10 mol %) in our standard one-pot, multi-component, two-step protocol afforded [2H]C3-aziridines 37–43; chiral column HPLC analysis confirmed that they had been generated with high to excellent ee. Furthermore, Table 2 demonstrates that universally excellent regiospecific [2H] > 95 atom % incorporation at the C3-atom was observed for [2H]C3-37–[2H]C3-43. These preliminary results have established two viable synthetic routes to [2H]C2- and [2H]C3-aziridines with either preformed N-(2-tert-butoxyphenyl)imines or the operationally more straightforward one-pot, multi-component, two-step protocol. Both afford, within experimental error, identical results. Furthermore, switching between the enantiomeric forms of catalyst 17 and translocating the deuterium atom from the formyl group of the aldehyde to the α-diazoester had little effect on
the yield, enantioselectivity, or percentage of deuterium incorporation. The reaction
times for producing [2H]C3-36–[2H]C3-45 (see Figures S25–S44) via the one-pot,
three-component, two-step protocol were longer than those for generating [2H]
C2-21–[2H]C2-32 via non-isotope-enhanced preformed aryl N-(2-tert-butoxy-
phenyl)imines. It seemed unlikely that the slower reaction rate affording [2H]C 3-
36–[2H]C3-45 could be attributed to a significant secondary kinetic isotope effect. 122
Instead, a more likely explanation was associated with the slow rate of aryl
N-(2-tert-butoxyphenyl)-[2H]-imine formation at /C080/C14 with the sterically encumbered
2-tert-butoxyaniline. A mixture of a-diazoester 35 and pre-synthesized (E)-N-(4-
nitro-[2H]-benzylidene)-2-tert-butoxyaniline was cooled to /C080/C14, and (R)-
17(10 mol %) was subsequently added. Consistent with previous chemistry outlined in Table 1 (affording (stereo)isotopomer
[2H]C2-27 from the corresponding non-isotopically-labeled imine), aziridine [2H]C3-
43 was afforded in 48 hr with a similar yield and ee to [2H]C2-27. In a demonstration of ee reproducibility between different isotopologue syntheses (Tables 2 and 3), it is noteworthy that 21 and 36 (both Ph), 23 and 39 (both 4-chlorophenyl), 25 and 38 (both 4-fluorophenyl), 27 and 43 (both 4-nitrophenyl), and 28 and 42 (both 4-cyano-
phenyl) afforded, within experimental error, very similar ee values. To further exemplify the utility of our methodology, particularly the ease with which multiple deuterium atoms can be established into optically active aziridines, we wanted to
generate the first example of an organo-Bronsted-acid-catalyzed stereoselective reaction that installed two deuterium atoms.

\[ \text{Table 2. Organo-Bronsted-Acid-Catalyzed Synthesis of Optically Active [2H]C3-36–[2H]C3-45} \]

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<td>65%</td>
<td>88%</td>
</tr>
<tr>
<td>37</td>
<td>4-bromophenyl</td>
<td>tert-butyl</td>
<td>67%</td>
<td>83%</td>
</tr>
<tr>
<td>38</td>
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<td>tert-butyl</td>
<td>72%</td>
<td>86%</td>
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<tr>
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<td>4-chlorophenyl</td>
<td>tert-butyl</td>
<td>67%</td>
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</tr>
<tr>
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<td>67%</td>
<td>69%</td>
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<tr>
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<td>tert-butyl</td>
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<td>65%</td>
<td>84%</td>
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<td>45</td>
<td>4-bromophenyl</td>
<td>ethyl</td>
<td>52%</td>
<td>74%</td>
</tr>
</tbody>
</table>

For synthesis, see Figures S16–S24.

Comparing the ee values associated with C3-(3-chlorophenyl) [2H]C3-40 (69% ee; Table 2) and [2H]C2-[2H]C3-52 (76% ee; Table 3) with those of [2H]C2-21–[2H]C2-22 (81% and 99% ee, respectively), [2H]C2-24–[2H]C2-32 (80%–99% ee; Table 1), [2H]C3-37–[2H]C3-38 (83% and 86% ee, respectively), and [2H]C3-42–[2H]C3-45 (74%–93% ee; Table 2) provides evidence that the 3-chlorophenyl group generates aziridines with reduced ee. Similarly, incorporating a C3-(2-chlorophenyl) group afforded [2H]C2-[2H]C3-53 and [2H]C2-41 with lower ee (i.e., 52% and 64%, respectively) than, for example, C3-(4-chlorophenyl)-, (4-bromophenyl), or (4-fluorophenyl)-derived [2H]C2-[2H]C3-49–[2H]C2-[2H]C3-52 (77%–97% ee; Table 3). Currently, it is unclear why the 2-chlorophenyl- and 3-chlorophenyl-afford lower ee than the halide-derived examples above or pentafluoro-[2H]C2-26 (97% ee); it does, however, seem specifically related to the inclusion of a 2- or 3-chlorophenyl and is not solely a consequence of the electronegativity of chlorine.123

Having established a preference for catalyst 17 to afford cis-aziridines, we sought a tentative mechanistic rational to help explain their formation. Protonation, (S)-17, of the N-arylimine nitrogen attached to the bulky ortho-tert-butoxyaryl substituent (54) affords electrophilic immonium ion pair 55 and a five-membered intramolecular hydrogen-bonded immonium complex. Minimizing steric interactions (see highlighted red structures on 57) of the bulky tert-butyl α-[2H]diazoacetate toward ion pair 55 preferentially affords 56. Alternative approaches of the tert-butyl α-[2H]
diazoacetate proceed via intermediates with increased steric congestion (58, a clash between tert-butyl ester and bulky N-aryl group) or require 120° rotation around the newly formed central C–C bond of 59 so that the –N₂ leaving group is in a suitable orientation for S_N2 displacement and ring closure (57) via nucleophilic attack of the aryl nitrogen atom (Scheme 1). The preference for generating cis-aziridines instead of trans-aziridines is interesting and worthy of comment. Similar to cis-21, Newman projections 60–62 (Scheme 1) present a clearer understanding of why this is the case. Generating trans-21 requires that the diazo leaving group be anti-periplanar to the incoming nucleophilic aryl nitrogen; however, as outlined in 62, this conformation results in a severe clash between the bulky tert-butyl ester and the N-bound ortho-tert-butoxyaryl group. This results in a transition state with increased energy, and consequently trans-21 is not generated. In contrast, although 60 and 61 (Scheme 1) have reduced steric interactions, both conformations have the diazo leaving group in the wrong conformation (not anti-periplanar to the incoming arylamine nucleophile). Indeed, the only way that 60 and 61 can afford trans-21 is via a 120° clockwise (60) or anti-clockwise (61) rotation around the central C–C bond.

However, doing this only serves to re-establish the severe steric interactions observed in 62; consequently, 60 and 61 are unlikely to be efficient pathways to trans-21. A comprehensive mechanistic and computational study that fully explains the stereoselectivity observed with triflimide (S)-17 or (R)-17 has yet to be undertaken. However, using high-level quantitative ONIOM (Our Own N-layered Integrated Molecular Orbital and Molecular Mechanics) calculations, Goodman et al. developed a BINOL-derived phosphoric acid (based on 4; Figure 4) model that helps explain the observed stereoselectivities when nucleophiles add to imines.125 Extrapolating the Goodman bifunctional phosphoric acid imine activation model by substituting a triflimide group for the hydroxyl group of the phosphoric acid affords a quadrant model that projects the catalyst with the BINOL oxygens in the plane of the paper (blue bonds and atoms, 63; Scheme 2), the triflimide above (pink atoms and bonds), and the P=O group below (red bonds and atoms), each with the bulky 3- and 3'-aryl groups on either side. E-imines (e.g., 54; Scheme 1) are more stable than Z-imines; consequently, aldimines have a larger energy difference between the E- and Z-forms.
Although a type I Z-transition state (data not shown) is more compact, the energy required to rotate the ortho-tert-butoxyphenyl is greater than the energy of the steric interactions with the large 3,3'-substituents. With this in mind, we propose that, similarly to 63, 54 generates an activated type I E-hydrogen-bonded transition-state complex (Scheme 2) with the bulky ortho-tert-butoxyaryl group projecting into the left-hand empty quadrant. Binding the tert-butyl α-[2H]diazoacetate to the P=O within the second vacant quadrant aligns the nucleophile directly below the activated imine double bond of 63, affording a complex similar to 64. Stereoselective attack of the α-[2H]diazoacetate generates a new optically active C–C bond with the diazo leaving group anti-periplanar to the arylamine group; subsequent cyclisation affords the desired optically enhanced aziridine cis-21.

By exploiting the quadrant model (66, Figure 6) and incorporating new catalysts, it could be possible to increase the stereoselectivity and/or rate of the aza-Darzens reaction. As noted, activating the imine of 9 with phosphoric acid 4 (pKa = 3.15 in DMSO) was not possible. Replacing it, however, with the more acidic (S)-17 (pKa = –3.36 in DMSO) activated the imine and afforded the cis-aziridines. Thus, testing second-generation, more acidic catalysts such as 67 (pKa –3.83 in DMSO) and 68 (pKa –4.58 in DMSO; Figure 6) will allow the important aspect of pKa to be probed. Similarly, incorporating TRIP-derived N-triflimide 69 and sulfur- and selenium-substituted N-triflimides 70 and 71 (anticipated to have similar a pKa to 67 and 68) will allow investigation of the effect that larger 3- and 3'-substituents have on the stereoselectivity and reaction rate. Furthermore, Yamamoto and Sai126 recently reported an asymmetric Hosomi-Sakurai reaction catalyzed by 72; improved diastereo- and enantioselectivity was attributed to the incorporation of the perfluoroalkyl tether and the bulky tert-butylidiphenylsilyl groups, which increased the steric bias within the filled quadrants I and IV (66; Figure 6). Exploiting this, synthesizing aziridines with increased optical activities via enhanced reaction rates by using lower catalyst loadings seems viable.

Optically active α- and/or β-deuterated natural and unnatural α-amino acids are important (bio)chemical motifs (see Figures 2H–2P) mainly generated via multi-step, enolate-based chemistry using Schöllkopf bis-lactim ethers, Evans oxazolidinones, Williams’ oxazoline, Oppolzer’s sultam, or Seebach imidazolidinone chiral auxiliaries.127–141 The development of an operationally simple, catalysis-based protocol that transforms easily accessible starting materials into cis-aziridines “en route” to natural or unnatural, optically active, isotope-enhanced α-amino acids holds considerable merit. Isotope-enhanced α-amino acids are valuable bioprobes that have been used for investigating protein-protein interactions,142 identifying unknown compounds from orphan gene clusters (the so-called genomisotopic approach),143 and determining the metabolic
and conformational stabilities\(^{144}\) of proteins and peptides. Preliminary hydrogenation studies using catalytic quantities (20 mol %) of palladium hydroxide on carbon (20 mol % Pd on C) focused on [2H]C2-21 (Table 1) and [2H]C2-[2H]C3-46 (Table 3). Optimally active N-aryl phenylalanine tert-butyl ester \(\alpha\)-[2H]-73 (Figures S61 and S62) and \(\alpha\)-[2H]- and \(\beta\)-[2H]-74 (Figures S63 and S64) were afforded in excellent 93% and 90% yields, respectively (Figure 7). It is worth noting that there was with little evidence (\(^{1}H\)-NMR) for loss of the deuterium or erosion of the optical activity incorporated via the asymmetric aza-Darzens reaction. Analytical chiral column HPLC analysis confirmed that \(\alpha\)-[2H]-73 and \(\alpha\)-[2H]-[2H]-74 had 80% and 70% ee, respectively. These values match, within experimental error, those of the starting-material aziridines (e.g., [2H] C2-21 had 81% ee, and [2H]C2-[2H]C3-46 had 67% ee). Thus, under the mild, closely monitored hydrogenation conditions, these substrates did not undergo “deuterium washout.” Encouraged by this, we sought to expand our repertoire such that it included multicyclic, heteroaryl, and haloaryl washout.”

Figure 6. Alternative, More Reactive Organo-Brønsted Acids for the Synthesis of Isotope-Enhanced cis-Aziridines

The synthesis of unnatural \(\beta\)-(aminoaryl) and \(\beta\)-(haloaryl) \(\alpha\)-amino acids, e.g., 77 and 78, respectively, (Figure 7), is an important endeavor worthy of investigation.\(^{150–160}\) Only three isotope-enhanced 4-aminophenylalanines\(^{161,162}\) have been reported. Two of these detail the deuterium incorporation within the side chain groups—not, as would be preferable, at one or both of the \(\alpha\)- or \(\beta\)-carbons on the \(\alpha\)-amino propanoic acid chain (see \(\alpha\)-[2H]-[2H]-93–[2H]-95 in Scheme 3).\(^{163}\) To increase atom efficiency, we envisaged executing a one-pot, double-reduction process whereby the Baeyer strain in [2H]C2-27 is released while the 4-nitrophenyl is reduced to a 4-aminophenyl group. In a reaction employing previously successful conditions and palladium catalysts, 4-nitrophenyl derived [2H]C2-27, [2H]C3-43, and [2H]C2-[2H]C3-50 underwent “global” hydrogenation, affording the desired 4-aminophenyl-derived \(\alpha\)-amino esters \(\alpha\)-[2H]-77, \(\beta\)-[2H]-78, and \(\alpha\)-[2H]-[2H]-79 in 85%, 97%, and 82% yields and 86%, 89%, and 94% ee, respectively (Figures S69–S74).

The ee values are, within experimental error, identical to those of the starting materials. Demonstrating the utility of multi-isotope-derived unnatural 4-aminophenyl \(\alpha\)-amino acids based on \(\alpha\)-[2H]-77–[2H]-79, Herbert and Knaggs exploited rac-90 as a biosynthetic probe for the \(\beta\)-lactam antibiotic obafluorin;\(^{161}\) rac-90 helped to establish that the biosynthetic route did not proceed via a pyridoxal-phosphate-mediated decarboxylation.

Halophenyl- and especially fluorophenyl-derived\(^{164–171}\) \(\alpha\)-amino acids are important motifs widely employed in medical chemistry to generate “Teflon” proteins or help
stabilize tertiary and quaternary protein structures. Hydrogenating (4-fluorophenyl)-
[2H]C2-25 afforded the corresponding ring-opened tert-butyl ester α-amino acid (data not shown) in an excellent 95% yield and >95 atom % deuterium at the C2-site. Determining its optical purity was not possible by chiral column HPLC analysis; the corresponding racemic sample was not separable. However, chemoselective hydrolysis of the tert-butyl ester with fomic acid afforded carboxylic acid α-[2H]80.

β-Hydroxy-α-amino acids are key components of important bioactive natural products, e.g., vancomycin (antibacterial), bouvarдин (anticancer), orentcin (antibacterial), phomopsins (mycotoxin), ristocetin (antibiotic), and actaplanin (antibiotic). Furthermore, they are also essential building blocks for the synthesis of β-lactams, β-fluoro-α-amino acids, and carbohydrates. Consequently, many examples of asymmetric synthesis strategies afford non-isotope-enhanced β-hydroxy-β-aryl-α-amino acids. A non-exhaustive list includes lithium amide conjugate addition, 172 transition-metal-mediated hydroborations, 173 the use of chiral auxiliaries (i.e., oxazolidinones, 174 oxazolines, 175 and bis-lactim ethers 176), and substrate-specific biocatalysis. 177–180 And yet, despite this intensive research effort, only two reports have detailed the synthesis of α-[2H]-, β-[2H]-, and α-[2H]-β-[2H]-β-hydroxy-β-aryl-α-amino acids (Scheme 3). Thus, Hamada et al. reported a deuterium-incorporating mechanistic study that employed a dynamic kinetic resolution (DKR) reaction mediated by a cationic Ir-catalyzed complex and pre-deuterated β-keto-α-[2H]-α-amino carboxylate ester 92 (Scheme 3). The DKRs were stopped at low conversions to product to minimize complications caused by hydrogen-solvent deuterium exchange. Be that as it may, hydrogenation afforded a, presumably inseparable, 1:1.1 mixture of anti-α-[2H]-β-[2H]-β-93 and anti-α-[2H]-β-hydroxy-α-amino acid 94. Interestingly, because hydrogenation of the ketone on α-[2H]-92 proceeds via enol tautomerization, when deuterium gas was substituted for hydrogen and non-isotope-enhanced acetic acid was used, mono-deuterated isotopologue β-[2H]-95 was afforded together with naturally abundant anti-β-hydroxy-α-amino acid 96 in a 1:1.4 mixture (the article does not say whether they were separable).
In early mechanistic studies on asymmetric hydrogenation, Noyori et al. generated syn-\(\alpha\)-[2H]-\(\beta\)-hydroxy-\(\beta\)-aryl-\(\alpha\)-amino acid 98 by using RuBr\(_2\)-(R)-BINAP and, again, a pre-deuterated \(\beta\)-keto-\(\alpha\)-[2H]-\(\beta\)-aryl-\(\alpha\)-amino acid, 97. Worthy of note, Noyori reported that "2-deuterio 97 easily loses deuterium via enolization" and that conducting the hydrogenation at a very low 1.3% conversion afforded \(\alpha\)-[2H]-hydroxy-\(\beta\)-aryl-\(\alpha\)-amino acid 98 with only 80% [2H]-incorporation (ee not reported). Furthermore, and of particular concern, recovered 97 had significantly reduced (70%) deuterium incorporation. In summary, neither of these transition-metal-based methods is particularly suited to bespoke isotope incorporation; instead, they afford presumably inseparable isotopologues of \(\beta\)-hydroxy-\(\beta\)-aryl-\(\alpha\)-amino acids with relatively poor levels of isotope incorporation.\(^{181,182}\)

The potential for exploiting an organo-Brønsted-acid-promoted hydrolytic ring opening of isotope-labeled aziridines offered an alternative (cf. Scheme 3), efficient, operationally straightforward entry point to these high-value entities. Employing cheap "off the shelf" 4-toluenesulfonic acid (PTSA), aqueous acetonitrile (1:1), and mild reaction conditions readily transformed [2H]C\(_2\)-37 and [2H]C\(_3\)-38 (Table 2) into \(\beta\)-[2H]-84 and \(\beta\)-[2H]-85, respectively (Figure 7), in unoptimized 86% and 75% yields, respectively (Figures S85–S88). Importantly, there was no evidence of deuterium scrambling or reduced incorporation ([2H] > 95 atom %). Furthermore, chiral column HPLC analysis confirmed no erosion of their optical purities. Encouragingly, a conceptually important next step was to reinforce the utility of our methodology by generating unusual, highly functionalized, isotope-labeled, optically active \(\alpha\)- or \(\beta\)-[2H]-\(\beta\)-iodo-\(\alpha\)-amino esters 86 and 87 (Figure 7). In a slightly modified procedure,\(^{183}\) [2H]C\(_2\)-27 and [2H]C\(_3\)-43 reacted in 20 min with iodine- and polystyrene-immobilized thiophenol. Gratifyingly, \(\beta\)-iodo-\(\alpha\)-amino esters \(\beta\)-[2H]-86 and \(\alpha\)-[2H]-87 were afforded with >95 atom % deuterium incorporation in unoptimized 81% and 86% yields and 79% and 84% ee, respectively (Figures S89–S92). Further transformation of these benzyl-activated \(\alpha\)-amino acids, affording highly functionalized, desirable isotope-labeled building blocks, seems feasible.

**Scheme 3. Synthesis of Deuterium Incorporating \(\beta\)-Hydroxy-\(\beta\)-aryl-\(\alpha\)-amino Acids 93–95 and 98**

![Scheme 3](image-url)
Protocols that afford optically active α-amino acid building blocks that are also appended with multiple and identical or differentiated isotopes, such as [2H] and [13C], [2H] and [18O], [2H] and [15N] or [2H], [2H] and [15N] or [2H] and [13C], are highly sought after for their physicochemical properties (Figure 1 and Figures 2H–2P). To facilitate uptake of this methodology, it is important to use readily available, easy-to-handle isotope-derived reagents (e.g., [2H]/deuterium oxide and [15N]/[14N][15N] ammonium hydroxide). With this in mind and the straightforward synthesis of β-[2H]-84 and β-[2H]-85 (Figure 7), we expanded our isotope “repertoire” by installing a high-value [18O]H group that generated α-[2H]-β-[18O]-hydroxy-α-amino esters. Brønsted-acid-mediated (PTSA) ring opening of [2H]C2-24 and [2H]C2-25 with readily available H2[18O] ([18O] > 95 atom %) afforded dual-isotope-differentiated α-[2H]-β-[18O]-hydroxy-derived 88 and 89 in unoptimized 72% and 43% yields (Figures S93–S96) and 95% and 85% ee, respectively (Figure 7). Using NMR to confirm [18O] installation is neither straightforward nor convenient. However, the high-resolution mass spectral analysis of 88 and 89 afforded intense signals at m/z 467.1524 and 407.2333, respectively (associated with [M+H]+), thereby confirming that both had [2H]- and [18O]-labeled oxygen (both >95 atom %).

To address the shortfall of aziridines appended with multiple and differentiated isotopes, we considered our isotope “mix and match” approach (Figure 3) to be a simple, effective solution that installs different combinations of [2H], [13C], and [15N]. However, to generate [15N]-labeled cis-aziridines with enhanced stereochemical purities, our catalyst-development studies had already established the importance of the 2-tert-butoxy group on 34 (Table 2). Yet, our desire to incorporate [15N] with >95% incorporation was frustrated by the lack of a commercial source of [15N]-34. For this reason, we opted to expedite our preliminary [15N]-incorporation studies by employing commercially available [15N]aniline (>95 atom %). Using this, we generated four “model” [15N]-aryl substrates: (E)-[15N]-(4-nitrobenzylidene) aniline, (E)-[15N]-(4-nitro-[2H]-benzylidene)aniline, (E)-[15N]-(4-cyanobenzylidene) aniline, and (E)-[15N]-(pyridin-2-ylmethylene)aniline (data not shown). Reacting these with [2H]-11 or 35 in an aza-Darzen reaction mediated by (S)-17 afforded double-differentially labeled [15N]-[2H]C2-99, [15N]-[2H]C2-100, [15N]-[2H]C2-101, and [15N]-[2H]C3-102 (Figures S97–S105) with enhanced optical purities and excellent >95 atom % [2H]- and [15N]-incorporation (Figure 8).

A notable isotope milestone we wanted to add to our expanding repertoire of isotopes was [13C] (cf. Figure 1). α-[13C]-Benzoic acid (99 atom% [13C]-incorporation) was transformed into ethyl α-[13C]-3-nitrobenzoate, the ester reduced, and the resulting [13C]aldehyde reacted with [15N]aniline, affording (E)-[15N]-(3-nitro-[13C]-benzylidene)aniline (data not shown). Supporting the ease with which additional, different isotopes can be incorporated, tert-butyl diazoacetate 35 was switched for [2H]-11; the former afforded [15N]-[13]C2-103, and the latter, illustrating the ease with which three isotopes differentially positioned can be “dialed up and locked in,” afforded [15N]-[2H]C2-[13]C3-104 (Figures S106–S111). Both were generated via identical protocols, and the products were afforded in essentially equal yields (Figure 8) and >95 atom % [13C]- and [15N]-incorporation. Anticipating that [15N]phenyl-derived [15N]-[13]C2-99–[15N]-[2H]C2-[13]C3-104 might have slightly lower ee values (cf. ortho-tert-butoxyphenyl-derived 16 [99% ee], 27 [90% ee], 42 [90% ee], and 43 [93% ee]), we were delighted that chiral column HPLC analysis confirmed that our unoptimized [15N]phenyl studies had afforded site-selective, multiple- and differentiated-isotope-derived cis-aziridines with 75%–87% ee.
Bioactive, optically active N-aryl α-amino acids equipped with isotopes have been used as PPARγ metabolism probes and for positron emission tomography studies.\textsuperscript{185,186} They are generally synthesized by transition-metal-mediated N-arylation of α-amino acids or via rhodium carbenoid N-H insertion chemistry. Having established an efficient route to a series of optically active deuterated N-aryl α-amino acids,\textsuperscript{73–89} (Figure 7), we sought to diversify our approach by generating a straightforward route to isotope-differentiated [15N]aryl α-[2H]-α-amino acids. Establishing that [15N]-inclusion was not detrimental, the efficient hydrogenation of [15N]-[2H]C\textsubscript{2}-100 (85% ee) afforded the unnatural [15N]aryl α-amino acid [15N]phenyl-2-pyridyl-α-[2H]-alanine tert-butyl ester\textsuperscript{105} with an 84% ee and >95 atom % for both isotopes. These percentages are, within experimental error, identical to those of the starting material (Figures S112 and S113).

Removing the N-aryl substituent, thereby generating optically active multi-isotope-derived NH-aziridines, was also important. Exploiting the ease with which electron-rich N-2,4-dimethoxyphenyl (2,4-DMP) groups are cleaved under mild oxidative conditions convinced us of the merits of incorporating [15N]-2,4-dimethoxyaniline. Employing a combination of copper(I) iodide (20 mol %), L-proline (40 mol %), and potassium carbonate in aqueous DMSO transformed gram quantities of 1-iodo-2,4-dimethoxybenzene and [15N]ammonium hydroxide (a cheap, commercial source of 98 atom % [15N]) into 2,4-dimethoxy[15N]aniline. Incorporating this and a selection of aryl α-[2H]aldehydes ([2H] > 95 atom %) into our standard protocol afforded isotope-differentiated [15N]-[2H]C\textsubscript{2}-[2H]C\textsubscript{3}-\textsuperscript{106}, [15N]-[2H]C\textsubscript{2}-[13]C\textsubscript{3}-\textsuperscript{107}, and [15N]-[2H]C\textsubscript{2}-[13]C\textsubscript{3}-\textsuperscript{108} in 80%, 83%, and 82% ee, respectively, and >95 atom % for all three isotopes (Figures S114–S123). The 2,4-dimethoxyphenyl group had several beneficial effects: (1) the methoxy groups helped generate the

![Figure 8. Organocatalytic Synthesis of Optically Active, Labeled [15N]- and [2H]C\textsubscript{2}- or [2H]C\textsubscript{3}-99–108 and [2H]C\textsubscript{2}-[2H]C\textsubscript{3}-[15N]H-109](image-url)

For synthesis, see Figures S52–S62.
pre-requisite electron-rich and oxidatively cleavable aryl ring attached to the [15N], and (2) our stereochemical optimization studies (Figure 4) had already identified the importance of increasing the steric bias at the ortho-position of the N-aryl group (cf. ortho-tert-butyloxy ether in 15 → 16; Figure 4). Further confirmation of the positive "ortho-effect" was identified in [15N]-[2H]C₂-[13]C₃-104 and [15N]-[2H]C₂-[13]C₃-107; thus, although it is evident that the yields (63% and 58%, respectively) are very similar, the inclusion of the ortho-methoxy substituent on 107 results in an increase in ee from 75% to 83%. The 2,4-dimethoxy groups were oxidatively cleaved off [15N]-[2H]C₂-[2H]C₃-106 with cerium(IV) ammonium nitrate in aqueous acetonitrile. [15N]H-[2H]C₂-[2H]C₃-109 was afforded in an unoptimized 58% yield. Furthermore, there was no reduction in optical purity, isotope "washout," or scrambling of the deuterium and all isotopes in >95 atom % (Figures S124–S126).

In conclusion, we have developed an exciting approach that allows the installation of single or multiple and identical or different isotopes into a sought-after class of heterocycles. The exceptional ease of our process offers further prospects for isotope incorporation; it uses mild conditions, is operationally straightforward, and is cost effective. Utilizing readily available starting materials and an organo-Brønsted acid catalyst, it transforms, with equal ease, combinations of isotope-enhanced or naturally abundant starting materials into optically active aziridines with site-specific isotope inclusion. Demonstrating the broad utility of our process, the resulting aziridines are readily transformed into isotope-derived, secondary "high-value feedstocks" based on natural or unnatural α- or β-substituted optically active α-amino acids. The future requirements of isotope-derived compounds are increasing; therefore, the design and synthesis of reactions and catalysts that facilitate the incorporation of single or multiple isotopes is almost certainly going to intensify. As the first example of an isotope-incorporating organo-Brønsted acid protocol, we anticipate that our work will open the door to the development of a raft of alternative isoorganocatalytic chemistry. Ultimately, easier access to isotope-labeled compounds will assist chemists and biologists in gaining a better understanding of the fundamental processes associated with the health and well-being research-intensive sectors of the biotech, medtech, agritech, pharmaceutical, and academic communities alike.

EXPERIMENTAL PROCEDURES

Synthesis of 2-(2H)-tert-Butyl-1-(2-tert-butoxyphenyl)-3-(perfluorophenyl) aziridine-2-carboxylate: 26

Pentafluorobenzaldehyde (40 mg, 0.26 mmol), O-tert-butoxyaniline (43 mg, 0.26 mmol), and catalyst (S)-17 (22 mg, 0.027 mmol, 10%) were added to a flame-dried Biotage 2 mL microwave vial under nitrogen. 800 μL of [2H]chloroform was added (pre-dried over 4 Å molecular sieves), and the vial was sealed with a polytetrafluoroethylene crimp cap. 200 μL of anhydrous dichloromethane (DCM) was added via syringe through the septum, and the reaction mixture was cooled to −80°C. After 30 min, >95% deuterated tert-butyl α[2H]diazooacetate (40 μL, 0.29 mmol) was added via syringe, and the reaction mixture was stirred at −80°C. It was monitored by 1H-NMR until the reaction was deemed complete. At this point, the reaction mixture was passed through a short plug of silica and eluted with diethyl ether. The solvents were removed under reduced pressure, and the residue was purified by flash chromatography (14% diethyl ether in 40°C–60°C petroleum ether). A sample was submitted to chiral column analytical HPLC analysis (iso-hexane/iso-propanol = 95/5, 1 mL/min, 3.97 min (first peak), 5.14 min (second peak), 97% ee; Chiralpak AD). Optically active
2-[2H]-tert-butyl-1-(2-tert-butoxyphenyl)-3-(pentafluorophenyl)aziridine-2-carboxylate (26) was afforded as a yellow oil in an 82% yield.

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 7.09–6.83 (m, 4H, ArH), 3.30 (s, 1H, HC$_3$), 1.40 (s, 9H, C(CH$_3$)$_3$), 1.38 (s, 9H, C(CH$_3$)$_3$); $^{13}$C NMR (CDCl$_3$, 100 MHz): 167.2, 148.2, 147.7, 147.7, 145.3, 144.5, 144.3, 139.3, 139.1, 139.0, 136.0, 135.8, 135.8, 123.7, 123.1, 122.8, 120.7, 110.5, 110.2, 110.2, 82.1, 80.5, 43.55, 36.8, 28.6, 27.6 ppm; $\left[\gamma\right]_D^{23} + 121$ (c 1.1 CHCl$_3$); FT-IR (thin film cm$^{-1}$): 2979, 2933, 1743, 1738, 1594, 1524, 1502, 1451, 1393, 1369, 1331 cm$^{-1}$; MS (ES): 459.2 [M+H]$^+$, 481.1 [M+Na]$^+$; HRMS (EI): exact mass calculated for [C$_{23}$H$_{24}$F$_5$NO$_3$]+ requires m/z 459.1812, found m/z 459.1809.

**Representative Synthesis of α-Deuterated α-Amino acid: 76**

Pd(OH)$_2$/C (20% Pd by weight [9.6 mg, 0.014 mmol, 20%]) was added to a solution of optically active [2H]C$_2$-16 (25 mg, 0.06 mmol) in 4 mL of ethyl acetate. The reaction mixture was stirred at 30 °C under 43 psi of H$_2$ for 8 hr in a Biogte Endeavour catalyst screening system. The reaction was monitored by the uptake of hydrogen gas. When deemed complete, the reaction mixture was filtered through Celite and eluted with DCM. The washings were combined, and the solvent was removed under reduced pressure. The resulting material was purified by flash chromatography (15% diethyl ether in 40%–60% petroleum ether), allowing subsequent analysis to confirm that 76 had formed in a 64% yield. A sample was submitted to chiral column analytical HPLC analysis (iso-hexane/iso-propanol = 95/5, 1 mL/min, 4.19 min [first peak], 8.01 min [second peak], 97% ee; Chiralpak AD).

$^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 8.73 (d, 1H, J 4.67 Hz, Ar-H), 8.02–7.60 (m, 1H, Ar-H), 7.57–7.32 (m, 2H, Ar-H), 6.77–6.49 (m, 2H, Ar-H), 3.40 (s, 2H, $\beta$-CH$_2$), 1.37 (s, 9H, C(CH$_3$)$_3$), 1.34 (s, 9H, C(CH$_3$)$_3$); $^{13}$C NMR (CDCl$_3$, 75 MHz): 171.6, 143.1, 141.1, 139.6, 125.7, 123.9, 123.0, 122.3, 117.2, 111.2, 82.3, 80.0, 39.1, 29.1, 28.0 ppm; $\left[\gamma\right]_D^{23} + 22$ (c 0.5 CHCl$_3$); FT-IR (thin film): 2978, 2931, 1735, 1598, 1511, 1507, 1368, 1253, 1157 cm$^{-1}$; MS (EI)$^+$: m/z 372.3 [M+H]$^+$, 394.2 [M+Na]$^+$; HRMS (EI)$^+$: exact mass calculated for [C$_{22}$H$_{30}$DN$_2$O$_3$]+ requires m/z 372.2392, found m/z 372.2396.

**ACCESSION NUMBERS**

cis-27 has been deposited in the Cambridge Crystallographic Data Centre under accession number CCDC: 1510574.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Supplemental Experimental Procedures and 126 figures and can be found with this article online at http://dx.doi.org/10.1016/j.chempr.2016.11.008.

**AUTHOR CONTRIBUTIONS**

S.P.B. designed the experiments and wrote the paper. D.U.B., G.D.H.-G., P.P., Z.D., and S.M.T. designed and conducted the experiments. M.P. and S.J.C collected and interpreted the X-ray crystal diffraction data.

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REFERENCES AND NOTES


179. The lower yield for 89 was associated with a problematic purification.
