Preprint Title	Deuterated reagents in multicomponent reactions to afford deuterium labeled products.
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Publication Date	10 Juni 2024
Article Type	Full Research Paper
Supporting Information File 1	Supporting info FinalV3.docx; 3.8 MB
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Deuterated reagents in multicomponent reactions to afford deuterium labeled products.

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ABSTRACT: The utility of bio-isosteres is broad in drug discovery and methodology herein enables the preparation of deuterium labeled products in the most fundamental of known bio-isosteric replacements. As such we report the use of both D1-aldehydes and D_2-isonitriles across 8 multi-component reactions (MCRs) to give diverse arrays of deuterated products. A highlight is the synthesis of several FDA approved calcium channel blockers, selectively deuterated at a T1/2 limiting metabolic soft-spot via use of D_1-aldehydes. Surrogate pharmacokinetic analyses of microsomal stability confirm prolongation of T1/2 of the new deuterated analogs. We also report the first preparation of D_2-isonitriles from D_3-formamides via a modified Leuckart-Wallach reaction and their use in an MCR to afford products with D_2-benzylic positions and likely significantly enhanced metabolic stability, a key parameter for property-based design efforts.

Multi-component reactions (MCRs) are one-pot reactions that utilize three or more readily available starting materials.[1] Typically, MCRs use reactive functional groups such as ketones or aldehydes, carboxylic acids, amines, and isocyanides where these simple building blocks can be utilized to form large libraries of drug-like compounds with synthetic ease.[2] In recent years use of deuterium in drug discovery has expanded beyond mechanistic and tracer studies to deuterium incorporation in small molecules in attempts to hijack the deuterium kinetic isotope effect to induce longer drug T1/2 and greater systemic exposure.[3] Herein, we describe applications of deuterium labeled reagents with MCRs through use of deuterated aldehydes and deuterated isocyanides, an area of study with sparingly few examples.
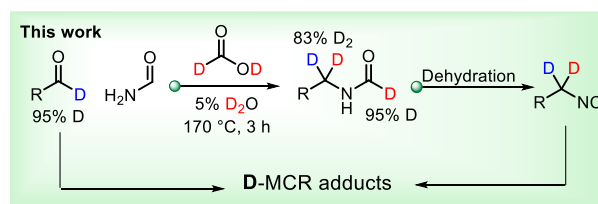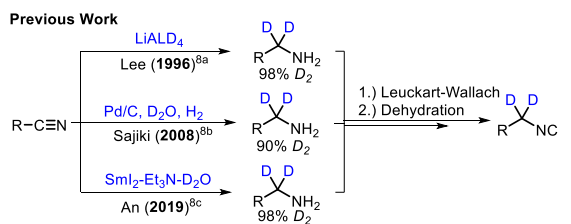
Scheme 1. Competitive examples of D_2-benzylamine formation via phenylnitriles.

One example by Srivastava obtained a 65% deuterated Passerini product starting from a 65% deuterated aldehyde.[4] Latterly, Yamamoto utilized a 90% deuterated D_2-isocyanide in a copper catalyzed [3+2] cycloaddition to afford a 60% deuterated D_2-pyrrole.[5] Use of such reagents in MCRs and determination of discrepancies in deuterium retention with MCRs has yet to be explored, although one would expect scrambling to be limited. Thus, we began by gathering highly deuterated aldehydes (>95% D) prepared via NHC catalysis[6] and developed a route to deuterated D_2-benzylic isocyanides with a goal to apply them to the field of isocyanide multi-component reaction (IMCR) chemistry which enables rapid access to a arrays of biologically relevant chemotypes or secondary reactions thereafter.[7] Many of these chemotypes have populated corporate collections through in-house production or external purchase and have progressed along the value chain to the clinic and full approval.[2] Literature inspection reveals the only established common method to prepare deuterated benzylic isonitriles is reduction of a nitrile in the presence of a deuterium source (**Scheme 1**).[8]

In this work, we hypothesized that D_1-aldehydes could be converted to D_2-benzylic isocyanides using D_2-formic acid via Leuckart-Wallach reaction followed by dehydration. Surprisingly, the Leuckart-Wallach reaction gave D_3-formamides which are scarce in the primary literature. The common method to prepare D_1-formamides (D-C=O) is through a Leuckart-Wallach reaction with an amine and D_1-methyl/ethyl formate or D_1-dimethylformamide.[9] Stockmann and coworkers produced D_2-formamides (N-D, D-C=O) via acid catalyzed nitrile hydrolysis with HCl and D_2O.[10] A tentative mechanism to D_3-formamides is shown but is open to debate, **Scheme 2**. Thus, formamide adds to the D_1-aldehyde **A** to form hemiaminal **B** which eliminates D_2O to give imine **D**. Deprotonation of formamide **D** forms the resonance and zwitterrion stabilized isocyanate **E**.[11] We then hypothesize that zwitterion **E** rearranges with loss of CO_2 to form D_3-formamide.
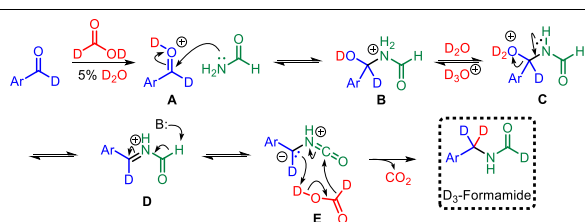
Scheme 2. Proposed tentative mechanism of D3-formamide formation via modified Leuckart-Wallach reaction with D2-formic acid.

Thus, using the methodology developed herein, deuterated aldehydes can be converted into D2-isocyanides. The optimized conditions for this reaction are summarized below (**Table 1**).

**Table 1**. Optimization of deuterated Leuckart-Wallach reaction.
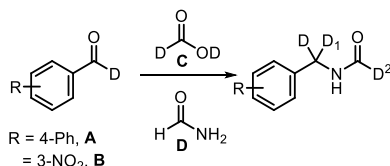
Entry	Aldehyde	C eq.	D eq.	Yield %	Deuteration % D^1, D^2
1	A	7.2	12	61	51, 42
2	A	6	1	24	70, 88
3	A	20	1	27	83, 94
4	A	12	10	80	27, 61
5[a]	B	20	2	83	78, 40

Standard conditions: 3 hours at 170 °C 3 hours. a. Reaction was heated to 180 °C for 5 minutes in a microwave reactor.

It is important to note that 1 equivalent of formamide and excess D2-formic acid leads to increased deuteration of formamide product. Excess reaction time increases side product formation and thermal degradation of the aldehyde starting material. To combat this, a microwave irradiation was employed which dramatically increased the overall yield of the reaction (entry 5[a]). In summary, optimal reaction conditions to give high yield and high deuteration % are 20 equivalents of D2-formic acid, 1 equivalent of formamide, and heating to 170 °C in a microwave reactor for 5 minutes.

The ability to deuterate at benzylic positions is particularly relevant as benzylic C-H bonds are common in biologically relevant chemotypes and moreover appear in approximately 25% of the top selling 200 pharmaceuticals.[12] Benzyl cation stability is a driver of metabolism at these sites which benzylic C-H bonds readily undergo metabolism driven by cytochrome P450 oxidases via single-electron oxidation.[13] This metabolic lability may be tempered by hydrogen replacement with deuterium, an almost perfect bio-isosteric replacement (C-H to C-D) which maintains 3D surface, shape and flexibility.[3b] Indeed, early incorporation of deuterium during hit generation may negate the need for late-stage C-H functionalization which often requires strong external oxidants or affords products with significantly lower biological activity.[14] Thus, eight MCRs were evaluated for D-reagent scope of reactivity and determination of deuterium retention using a combination of deuterated aldehydes, D_1-, and/or D_2-isocyanides.

We began with the venerable Ugi 4-component reaction (Ugi-4CR), first reported by Ivar Ugi in 1959.[15] The Ugi-4CR utilizes an amine, carbonyl, carboxylic acid, and isocyanide component to afford α-aminoacyl amide derivatives **1a** – **1g** in good isolated yield (**Scheme 3**). A D_1-aldehyde and D_1-isocyanide were independently used in conjunction with supporting reagents to afford **1b** – **1d** and **1e** and **1f** resepectively with no observation of deuterium scrambling. A post-condensation modification of **1d**, representative of the large swath of chemical space accessible by Ugi-Deprotect-Cyclize (UDC) methodology, gave the dihydroquinoxaline **1g** in good yield with high deuterium retention.[16]
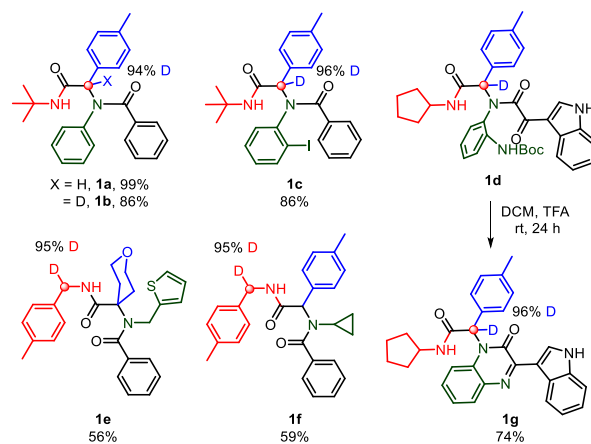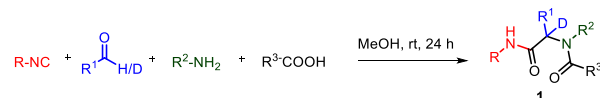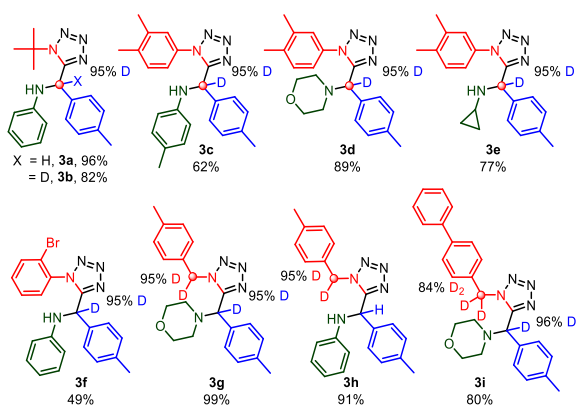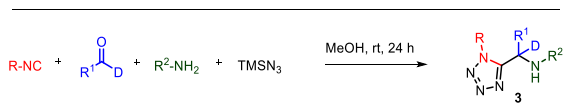

Scheme 3. Ugi-4CR products: no deuterium scrambling observed.

The catalytic three-component Ugi reaction was first reported by List in 2008[17] and is comprised of reaction of an isonitrile, amine, and aldehyde/ketone, in the presence of phenylphosphinic acid (PPA), to give α-amino amides. Examples of deuterated Ugi-3CR products are represented in **Scheme 3**. Like the Ugi 4-CR reaction, there was no deuterium scrambling in the Ugi 3-CR. Using a >95% deuterated aldehyde gave a >95% deuterated product. The low yield in **2c** is explained by the difficulty to produce aliphatic deuterated aldehydes which led us to believe that the starting material was partially decomposed. Nonetheless, both aliphatic and aromatic deuterated aldehydes have been demonstrated to work without loss of deuterium in the Ugi-3CR.
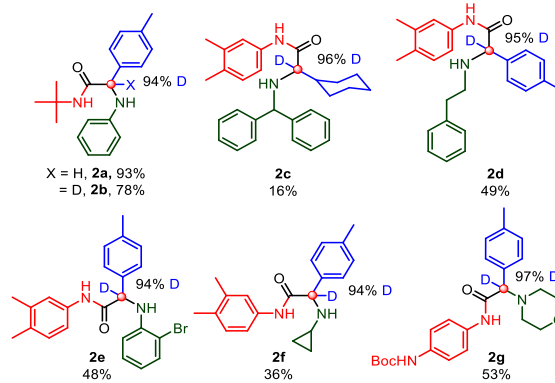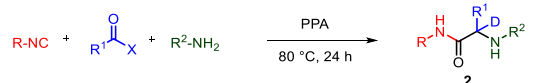
Scheme 4. Ugi-3CR products. No deuterium scrambling observed.

First reported in 1961, the Ugi-azide reaction differs from the classical Ugi 4-CR in that an azide anion traps out the intermediate nitrilium ion, leading to formation of α-aminotetrazoles.[18] Thus, it comprises reaction of an isocyanide, carbonyl, amine and TMSN3 to give tetrazole containing products. Isolated yields for eight analogs are reported with excellent retention of the deuterium label (**Scheme 5**). Of note, **3i** was prepared from combination of a D2-isocyanide and a D1-aldehyde.

Scheme 5. Ugi-azide reaction products, no deuterium scrambling observed.

Another versatile IMCR is the Passerini reaction[19] discovered 60 years prior to the Ugi reaction. It uses the reactivity of isocyanides, aldehydes, and carboxylic acids to yield α-acyloxy amides (**Scheme 6**). Six deuterated analogs are reported in good yield with no observation of deuterium scrambling. For the preparation of **4d**, a deuterated isocyanide was solely employed and for **4a-b** and **4e-4f,** water was used as solvent. For the latter, product precipitates from the aqueous solution which deters undesirable side-reactions whilst also aiding rate of the reaction.
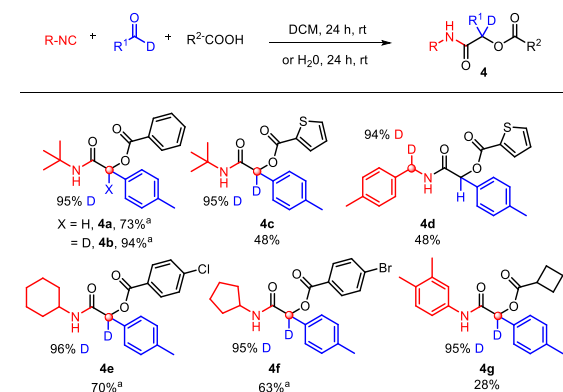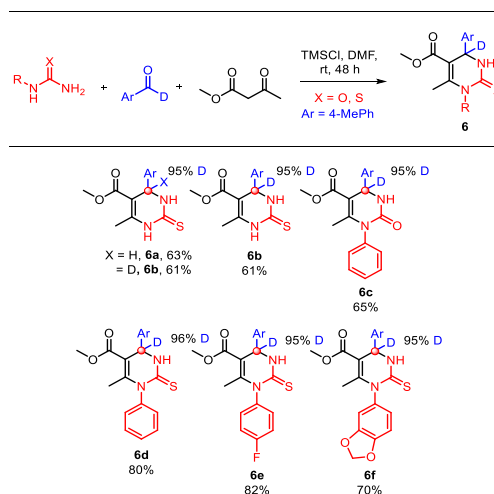

Scheme 6. Passerini products, no deuterium scrambling observed.
[a]Water was used as solvent.

The fifth MCR employed herein is the ubiquitous Strecker reaction[20] where cyanide, aldehyde, and carboxylic acid react to afford α-aminonitriles. **5c** was converted to the deuterated amino acid **5d** under acidic conditions. This finding opens up the possibility of scale-production of deuterium-labled α-amino acids. Deuterated Strecker products are presented in **Scheme 7** in good yield with no observed deuterium scrambling.
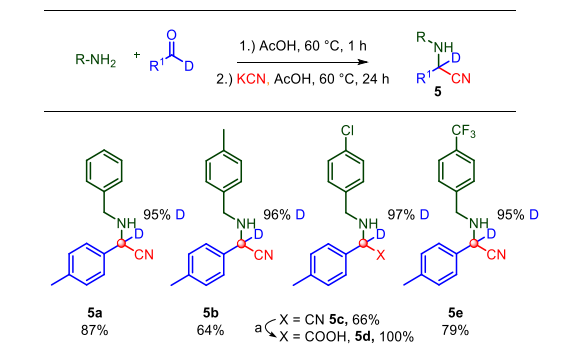
Scheme 7. Strecker reaction products (precursors to D_1-α-amino acids), no deuterium scrambling was observed. a.) The cyano group was converted to a carboxylic acid via typical saponification conditions without loss of deuterium (See SI for conditions).

The 19th centrury Biginelli reaction utilizes an aryl aldehyde, urea, and acetoacetate component to give 3,4-dihydropyrimidin-2(1H)-ones.[21] Such molecules are widely used as calcium channel blockers and antihypertensive agents.[22]

**Scheme 8.** Biginelli reaction products, no deuterium scrambling was observed.
Six site-specific deuterated Biginelli products are revealed in good yield with no deuterium scrambling, **Scheme 8**.

The Groebke-Blackburn-Bienaymé (GBB) reaction is an intramolecular variant of the Ugi reaction where the intermediate nitrilium ion is intercepted by heteroatoms from the amino-heterocyclic input. Discovered in 1998, and reported independently by three different research groups, it is a three component reaction of α-amino-heterocycles, aldehydes, and isocyanides which affords various aza-bicyclic molecules.[23] The methodology has been widely used for file enhancement purposes. Three deuterated GBB products are presented, **Scheme 9**. Unlike the previous MCRs, labeling via a deuterated aldehyde is not feasible as deuterium is removed in favor of the aromatic bicyclic product. Both D_2- and D_1- isocyanides are exemplified in **7a**-**7c**.
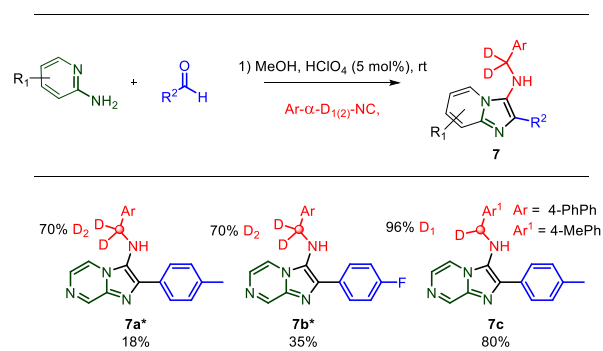
Scheme 9. GBB reaction products, no deuterium scrambling was observed. *A 70% D_2-isocyanide was used in **7a** and **7b**.

Finally, we studied the compatibility of the Hantzsch dihydropyridine synthesis with D_1-aldehydes. The reaction is a condensation of ethyl acetoacetate with acetaldehyde and ammonia to give 1,4-dihydropyridine.[24] Such scaffolds are seen in several FDA approved calcium channel blockers including nifedipine, nicardipine and nimodipine and three site-specific deuterated analogs of approved 1,4-dihydropyridines (DHPs) are presented, **Scheme 10**. In line with all prior MCRs, good yields were observed with no deuterium scrambling.

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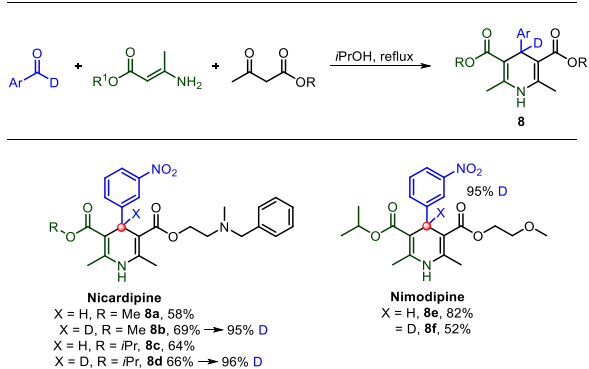
Scheme 10. Modified Hantzsch pyridine synthesis to afford 1,4-dihydropyridines. No deuterium scrambling was observed.

Calcium channel blockers derived from this methodology are heavily metabolized by CYP3A4 via dehydrogenation to afford inactive pyrdines, **Scheme 11**.[25] As such, bio-isosteric deuterium-hydrogen exchange at this position was thought a reasonable approach to extend drug T1/2 through exploitation of the kinetic isotope effect underpinned by the C-D bond being slightly shorter and stronger than a C-H bond. Such site specific labeling was hypothesized to slow CYP3A4 metabolism.
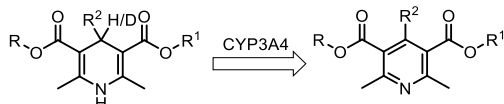
Scheme 11. CYP3A4 mediated dehydrogenation of dihydropyridines. To evaluate the hypothesis, surrogate studies were conducted in mouse liver microsomes to compare deuterated DHPs with their non-deuterated counterparts (**Table 1**).

Table 1. Microsomal stability package of deuterated and non-deuterated dihydropyridines.

Sample ID	MLM $t_{1/2}$	MLM Cl[a]
H-Nicardipine	2.8 min	1968.2
D-Nicardipine	3.0 min	1860.8
H-Nimodipine	2.1 min	2572.3
D-Nimodipine	2.6 min	2098.5
H-*i*Pr-Nicardipine	8.0 min	687.0
D-*i*Pr-Nicardipine	14.2 min	386.6

[a]Mouse liver microsomes clearance (MLM Cl), units:mL/min/kg

D-Nicardipine saw a marginal increase in stability when compared to its non-deuterated counterpart H-Nicardipine (3.0 vs 2.8 mins) with both molecules being rapidly metabolized. D-nimodipine witnessed a 23% improvement in T1/2 over its non-deuterated counterpart although both molecules were metabolized quickly. The most significant improvement was seen with D-*i*Pr-nicardipine - a 77% increase in MLM stability compared to H-iPr-nicardipine. Note that baseline stability of H-iPr-Nicardipine was higher (8 mins) related to enhanced ester stabilty with an iso-propyl group versus a methyl ester. Collectively these results point to high potential for translation in vivo where novel deuterated analogs exhibit longer T1/2 and by extension oral bioavailability.

In summary we have presented the first method to prepare highly deuterated D₃-formamides and D₂-isocyanides from deuterated aldehydes. Furthermore, we have demonstrated that large libraries of deuterated drug-like molecules can be produced rapidly with MCR technology, with particular value for site selective deuteration of often metabolically soft benzylic C-H sites. Lastly and most importantly, preliminary surrogate metabolic stability studies on site selective D₁-DHPs suggest these novel deuterated analogs may afford increased exposure in an *in vivo* setting. The methodology is likely to have wide utility for the drug-hunting community at large.

References

(1) (a) Domling, A.; Kang, K.; Wang, W.; *Chem. Rev.*, **2012**, *112*, 3083-3135. (b) Zhu, J.; Wang, Q.; Wang, M.; Multicomponent Reactions in Organic Synthesis, **2014**, John Wiley & Sons. (b) Rotstein, B. H.; Zaretsky, S.; Rai, V.; Yudin, A. K. *Chem. Rev.*, **2014**, *114*, 8323-8359. (c) Brauch, S.; van Berkel, S.S.; Westermann, B.; *Chem. Soc. Rev.*, **2013**, *42*, 4948-4962.

(2) (a) Hulme, C.; Ayaz, M.; Martinez-Ariza, G.; Medda, F.; Shaw, A.; **2015**, Small Molecule Medicinal Chemistry: Strategies and Technologies, ed. W. Czechtizky, P. Hamley, Wiley, Weinheim, ch. 6, 145-187. (b) Hulme, C.; Bienaymé, H.; Nixey, T.; Chenera, B.; Jones, W.; Tempest, P.; Smith A. L.; *Methods in Enzymology*, **2003**, *369*, 469-496.

(3) (a) Gant, T.G.; *J. Med. Chem.*, **2014**, *57*, 3595-3611. (b) Pirali, T.; Serafini, M.; Cargnin, S.; Genazzani, A. A.; *J. Med. Chem.*, **2019**, *62*, 5276-5297. (c) Meanwell, N.A.; *J. Med. Chem.*, **2011**, *54*, 2529-2591.

(4) Ghoshal, A.; Ambule, M. D.; Sravanthi, R.; Taneja, M.; Srivastava, A. K. *New J. Chem.*, **2019**, *43*, 14459–14474.

(5) Kamijo, S.; Kanazawa, C.; Yamamoto, Y.; *J. Am. Chem. Soc.*, **2005**, *127*, 9260–9266.

(6) Geng, H.; Chen, X.; Gui, J.; Zhang, Y.; Shen, Z.; Qian, P.; Chen, J.; Zhang, S.; Wang, W.; *Nat. Catal.*, **2019**, *2*, 1071–1077.

(7) (a) Ayaz, M.; De Moliner, F.; Dietrich, J.; Hulme, C.; Isocyanide Chemistry: Applications in Synthesis and Material Science, **2012**, 335-384. (b) Bedard, N.; Fistrovich, A.; Schofield, K.; Shaw, A.; Hulme, C.; Multicomponent Reactions towards Heterocycles: Concepts and Applications. **2022**, Wiley‐VCH GmbH, 339-409.

(8) (a) Kim, S. S.; Yang, K. W.; Lee, C. S.; *J. Org. Chem.*, **1996**, *61*, 4827–4829. (b) Kurita, T.; Aoki, F.; Mizumoto, T.; Maejima, T.; Esaki, H.; Maegawa, T.; Monguchi, Y.; Sajiki, H.; *Chem. Eur. J.*, **2008**, *14*, 3371–3379. (c) Ding, Y.; Luo, S.; Weng, C.; An, J.; *J. Org. Chem.*, **2019**, *84*, 15098–15105.

(9) a) Kesharwani, T.; Verma, A. K.; Emrich, D.; Ward, J. A.; Larock, R. C.; *Org. Lett.*, **2009**, *11*, 2591–2593; b) Suchý, M.; Elmehriki, A. A. H.; Hudson, R. H. E.; *Org. Lett.*, **2011**, *13*, 3952–3955.

(10) Stockmann, V.; Bakke, J. M.; Bruheim, P.; Fiksdahl. A.; *Tetrahedron*, **2009**, *65*, 3668–3672.

(11) Kende, A. S.; Brands, K. M. J.; Blass, B.; *Tetrahedron Lett.*, **1993**, *34*, 579–582.

(12) McGrath, N. A.; Brichacek, M.; Njardarson, J. T.; *J. Chem. Educ.*, **2010**, *87*, 1348–1349.

(13) Engst, W.; Landsiedel, R.; Hermersdörfer, H.; Doehmer, J.; Glatt, H.; *Carcinogenesis*, **1999**, *20*, 1777-1785.

(14) (a) Xu, W.; Wang, W.; Liu, T.; Xie, J.; Zhu, C. *Nature Comm.*, **2019**, 10, 4867. https://doi.org/10.1038/s41467-019-12844-9. (b) Guo, S.; Abu Salim, D. I.; Cook, S. P.; *J. Am. Chem. Soc.*, **2018**, *140*, 12378–12382. (c) Qin, Y.; Zhu, L.; Luo, S.; *Chem. Rev.*, **2017**, *117*, 9433–9520.

(15) Ugi, I.; *Angew. Chem.*, **1959**, *71*, 373–388.

(16) (a) Tempest, P.; Ma, V.; Thomas, S.; Hua, Z.; Kelly, M. G.; Hulme, C.; *Tetrahedron Lett.*, **2001**, *42*, 4959–4962. (b) Dietrich, J.; Kaiser, C.; Meurice, N.; Hulme, C.; *Tetrahedron Lett.*, **2010**, *51*, 3951-3955 (c) Hulme, C.; Gore, V.; *Curr. Med. Chem.*, **2003**, *10*, 51-80. (d) Xu, Z.; De Moliner, F.; Cappelli, A.P.; Ayaz, M.; Hulme, C.; *Synlett.*, **2014**, 25, 225-228

(17) (a) Pan, S. C.; List, B.; *Angew. Chem. Int. Ed Engl.*, **2008**, *47*, 3622–3625. (b) Ayaz, M.; Martinez-Ariza, G.; Hulme, C.; *Synlett.*, **2014** 25, 1680-1684.

(18) (a) Ugi, I.; Steinbrückner, C.; *Angew. Chem.*, **1960**, *72* (7–8), 267–268; (b) Ugi, I.; Steinbrückner, C.; *Chem. Ber.*, **1961**, *94*, 734–742. (c) Gunawan, S.; Petit, J.; Hulme, C.; *ACS Comb. Sci.*, **2012**, *14*, 160-163. (d) Gunawan, S.; Hulme, C.; *Org. Biomol. Chem.*, **2013**, *11*, 6036-6046.

(19)   (a) Passerini, M.; Gazz, S.; *Chim. Ital.,* **1921**, *51*, 126–129. (b) Banfi, L.;
        Basso, A.; Lambruschini, C.; Moni, L.; Riva, R.; *Chem. Sci.*, **2021**, *12*,
        15445-15472. (c) Banfi, L.; Basso, A.; Guanti, G.; Riva, R.; *Mol. Divers.*,
        2003, *6*, 227-235. (d) Shaw, A.Y.; Medda, F.; Hulme, C. *Tetrahedron
        Lett.*, **2012**, *53*, 1313-1315

(20)   (a) Strecker, A.; *Ann. Chem. Pharm.*, **1850**, *75*, 27. (b) Ayaz, M.; De
        Moliner, F.; Morales, G.A.; Hulme, C.; Science of Synthesis, **2014**, 99-
        122.

(21)   Biginelli, P.; *Chem. Ges.,* **1891**, *24*, 1317–1319.

(22)   Rovnyak, G. C.; Atwal, K. S.; Hedberg, A.; Kimball, S. D.; Moreland, S.;
        Gougoutas, J. Z.; O'Reilly, B. C.; Schwartz, J.; Malley, M. F.; *J. Med.
        Chem.,* **1992**, *35*, 3254–3263.

(23)   (a) Groebke, K.; Weber, L.; Mehlin, F. *Synlett,* **1998**, *6*, 661–663; (b)
        Blackburn, C.; Guan, B.; Fleming, P.; Shiosaki, K.; Tsai, S.; *Tetrahedron
        Lett.,* **1998**, *39*, 3635–3638; (c) Bienaymé, H.; Bouzid, K.; *Angew. Chem.
        Int. Ed Engl.,* **1998**, *37*, 2234–2237. (d) Hulme, C.; Lee, YS. *Mol. Divers.*,
        2008 *12*, 1-15. (e) Umkehrer, M.; Ross, G.; Jäger, N.; Burdack, C.; Kolb,
        J.; Hu, H.; Alvim-Gaston, M.; Hulme, C.;
        Tetrahedron Lett., **2007**, *48*, 2213-2216.

(24)   Hantzsch, A. *Berichte der deutschen chemischen Gesellschaft* **1881**, *14*,
        1637–1638.

(25)   Zhu, Y.; Wang, F.; Li, Q.; Zhu, M.; Du, A.; Tang, W.; Chen, W.; *Drug
        Metab. Dispos.,* **2014**, *42*, 245–249.

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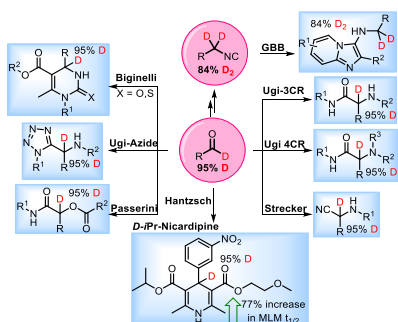
Deuterium is of high utility as a bio-isosteric replacement for hydrogen, often used to negate metabolic liabilities in small molecules through the kinetic isotope effect. Herein we report the use of both D1-aldehydes and D1/D2-isonitriles across 8 multi-component reactions (MCRs) to afford arrays of deuterated products. Highly significant is the D-congener synthesis of several FDA approved calcium channel blockers, deuterated at a T1/2 limiting metabolic soft-spot via use of D1-aldehydes. Surrogate pharmacokinetic analyses of microsomal stability confirm prolongation of T1/2 of these new deuterated analogs. We also report the first preparation and use of D2-isonitriles from D3-formamides via a modified Leuckart-Wallach reaction which afford products with D2-benzylic positions of likely enhanced metabolic stability. Conceptually, the compatibility of this chemistry with the Strecker reaction will also enable the synthesis of α-deuterated amino acids.