Preparation of cyclohexene isotopologues and stereoisotopomers from benzene

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The hydrogen isotopes deuterium (D) and tritium (T) have become essential tools in chemistry, biology and medicine[1]. Beyond their widespread use in spectroscopy, mass spectrometry and mechanistic and pharmacokinetic studies, there has been considerable interest in incorporating deuterium into drug molecules[1]. Deutetrabenazine, a deuterated drug that is promising for the treatment of Huntington's disease[2], was recently approved by the United States' Food and Drug Administration. The deuterium kinetic isotope effect, which compares the rate of a chemical reaction for a compound with that for its deuterated counterpart, can be substantial[1,3,4]. The strategic replacement of hydrogen with deuterium can affect both the rate of metabolism and the distribution of metabolites for a compound[5], improving the efficacy and safety of a drug. The pharmacokinetics of a deuterated compound depends on the location(s) of deuterium. Although methods are available for deuterium incorporation at both early and late stages of the synthesis of a drug[6,7], these processes are often unselective and the stereoisotopic purity can be difficult to measure[7,8]. Here we describe the preparation of stereoselectively deuterated building blocks for pharmaceutical research. As a proof of concept, we demonstrate a four-step conversion of benzene to cyclohexene with varying degrees of deuterium incorporation, via binding to a tungsten complex. Using different combinations of deuterated and proteated acid and hydride reagents, the deuterated positions on the cyclohexene ring can be controlled precisely. In total, 52 unique stereoisotopomers of cyclohexene are available, in the form of ten different isotopologues. This concept can be extended to prepare discrete stereoisotopomers of functionalized cyclohexenes. Such systematic methods for the preparation of pharmacologically active compounds as discrete stereoisotopomers could improve the pharmacological and toxicological properties of drugs and provide mechanistic information related to their distribution and metabolism in the body.

Typically, hydrogenation of benzene using D_2 gas leads to isotopologue mixtures of cyclohexane[9–11]. However, Taube et al.[12] demonstrated that the complex $[Os(NH_3)_5(\eta^2\text{-benzene})]^{2+}$ could be deuterated to form a single stereoisotopomer of $[Os(NH_3)_5(\eta^2\text{-cyclohexene-}d_4)]^{2+}$ using D_2 and a Pd/C catalyst. We posited that benzene bound in this manner could also be converted to cyclohexene using four well defined additions of two protons and two hydrides, passing through an η^2-1,3-cyclohexadiene intermediate (Fig. 1). If these reactions could be performed regio- and stereoselectively, one could access a diverse set of isotopologues and even stereoisotopomers of cyclohexene using various combinations of proteated and deuterated reagents.

The dearomatization agent {WTp(NO)(PMe_3)} is considerably more activating than its osmium predecessor[13]. Strong π-backbonding renders arene and diene complexes of this system highly nucleophilic and resistant to substitution[13]. Furthermore, this system displays considerable electronic asymmetry, and the benzene complex WTp(NO)(PMe_3)(η^2-benzene) (**1**) can be prepared on a multi-gram scale[14] and in enantioenriched form[15]. Treatment of an acetone-d_6 solution of **1** with diphenylammonium triflate (DPhAT; $pK_a \approx 0$) at −30 °C affords its clean conversion to the η^2-benzenium complex [WTp(NO)(PMe_3)(η^2-C_6H_7)](OTf) (**2**; Fig. 2). Using chilled diethyl ether as a precipitating solvent, **2** can be isolated from dichloromethane in 86% yield (1.9 g). As an acetonitrile solution, the η^2-benzenium complex **2** is moderately stable at room temperature but soon decomposes (half-life, $t_{1/2} \approx 6$ min). At 0 °C, however, **2** exists in equilibrium with its diastereomer **3** in a 10:1 ratio (Fig. 2) and persists for three hours without substantial decomposition. The major isomer (**2**) is formed with the metal binding two internal carbons of the five-carbon π system, and with the newly formed

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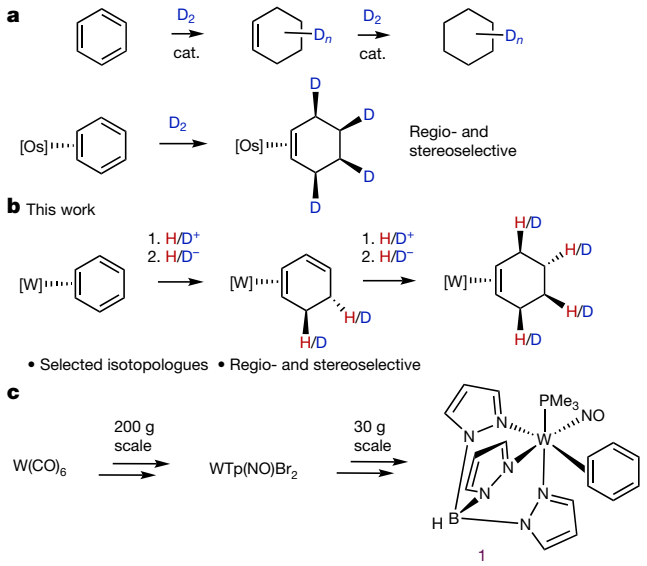

Fig. 1 | Methods for the deuteration of benzene. a, Existing methods for the selective deuteration of benzene can lead to over-reduction and a mixture of isotopologues. **b**, The current approach provides access to cyclohexene isotopologues and stereoisotopomers. **c**, The dearomatized benzene complex WTp(NO)(PMe_3)(η^2-benzene).

sp^3 carbon distal to the PMe_3 ligand. The minor isomer (**3**) is bound at a terminus of the π system with the sp^3 carbon proximal to the phosphine. Proton nuclear magnetic resonance (NMR) data and density functional theory (DFT) calculations (Supplementary Information; Supplementary Figs. 1–3) of these η^2-benzenium complexes (**2**, **3**) suggest that they are similar in structure to complexes of the form [WTp(NO)(PMe_3)(η^2-allyl)]$^+$ (ref. [16]), where the allyl ligand is tightly bound to the metal through only two carbons. A third carbon, weakly associated to the metal, resembles a carbocation, and is indicated as such in the figures (Fig. 2). Combining cold solutions of **2** and tetrabutylammonium borohydride generates WTp(NO)(PMe_3)(η^2-1,3-cyclohexadiene) exclusively (**4**). Despite the coexistence of the allyl conformer **3** in solution, the WTp(NO)(PMe_3)(η^2-1,4-cyclohexadiene) complex (**8**) is undetected (Fig. 2) in the reaction mixture[16]. The η^2-diene complex **4** is then treated with either DPhAT or HOTf/MeOH acids to generate the η^2-allyl complex (**6**)[16]. When **6** is subjected to base, it deprotonates to form **5**, a stereoisomer of **4** (ref. [16]), in which the uncoordinated double bond is now distal to the PMe_3 (ref. [16]). Combining the allyl complex **6** with a hydride source produces the desired η^2-cyclohexene complex **7** (67%). Crystals suitable for X-ray structure determinations are grown for complexes of cyclohexadiene **4**, allyl complex **6**, and cyclohexene **7**, and a rendering of these structures, along with key nuclear Overhauser effect (NOE) interactions are provided in Supplementary Information (Supplementary Fig. 4). Overlapping signals in the ^{1}H NMR spectrum of cyclohexene complex **7** precludes unambiguous stereochemical assignments of some of the ring proton signals.

By methylating the nitrosyl ligand of **7** (CH_3OTf) to generate [WTp(NOMe)(PMe_3)(η^2-C_6H_{10})]OTf (**9**)[17], the chemical shifts of the cyclohexene ring separate to the point that each proton can be assigned with high confidence (Supplementary Information sections G and H). An X-ray structure determination of **9** provides conclusive evidence for methylation of the nitrosyl oxygen (Fig. 2), analogous to earlier literature reports[18]. Strong NOE interactions between the ring *endo* protons and the methylated nitrosyl ligand further facilitate these assignments, and quantitative NOE experiments support the stereochemical assignments of all diastereotopic protons on the cyclohexene ring (Supplementary Information section H).

Deuterium studies

With all hydrogen resonances for the methylated η^2-cyclohexene complex **9** fully assigned, we investigated the regio- and stereochemical fidelity of the reaction sequence (Fig. 3). When the η^2-benzenium complex **11** was prepared from **1** using [$MeOD_2^+$]OTf, a loss of signal intensity was observed, corresponding to the methylene *endo* proton. This indicates that protonation of the η^2-benzene occurs *syn* to the metal (Fig. 3). A complementary experiment was next performed, starting with the fully deuterated benzene complex, **17**, in which $MeOH_2^+$ was used as the acid source. In this case, protonation led to a single broad proton resonance for the deuterated η^2-benzenium complex **18**. This proton signal is ~0.03 ppm upfield from its proteo counterpart, consistent with a primary H/D isotopic shift[19]. The *endo*-selective protonation of the benzene ligand in **1** is in stark contrast to the addition of carbon and heteroatom electrophiles, which have been observed to add *anti* to η^2-arene and η^2-diene ligands of tungsten complexes[13]. When η^2-benzenium complexes **11** and **18** were treated with $NaBD_4$ or $NaBH_4$, respectively, the complementary cyclohexadiene complexes **12** and **19** were formed (Fig. 3). A comparison of NOESY data for all three isotopologues of the cyclohexadiene complex (**4**, **12**, **19**) confirmed that the proton delivered from the borohydride reagent was *anti* to the metal (Fig. 3). The cyclohexadiene complexes **12** and **19** were then taken forward to their π-allyl analogues **13**, **15** and **20** (Fig. 3). In contrast to protonation of the η^2-benzene ligand of **1**, the acidic hydrogen was delivered predominantly *anti* to the metal (Fig. 3).

The resulting η^2-allyl complexes (**13**, **15**, **20**) underwent a conformational change ('allyl shift') such that the second proton added became H^{6exo} (conversion of **4** to **6**; Fig. 2), while the first proton added became H^{5endo}. For allyl complexes **13** and **20**, full stereoselective protonation was achieved. However, with the preparation of **15** or **26** we experienced difficulties in achieving full deuterium incorporation, owing to an unusually large deuterium kinetic isotope effect (DKIE) ($k_H/k_D \approx 37$ at −30 °C for the deuteration of **12** or **4**, where k_H and k_D are specific rate constants for protonation and deuteration, respectively). This DKIE was determined for **4** as the average value from three separate experiments in which **26** was generated from acidic solutions with different H/D ratios (Supplementary Information section K). This DKIE could be decreased by raising the temperature to 22 °C; however, this compromised the stereofidelity of the resulting deuterated product (**15**), with *endo* deuteration of the η^2-diene **12**, which competed with *exo* deuteration. Consequently, stereoselective deuterium incorporation at the H^{6exo} position of cyclohexene (that is, **16**, **33**–**35**, **41**, **44**, **49**, **51**; Fig. 3) could not be achieved above ~75–80%. A similar outcome was observed when we tried to convert the d_6-isotopologue diene **19** to allyl **30**. Finally, treatment of **13**, **15** or **20** with a hydride or deuteride source again confirmed that the corresponding η^2-cyclohexene products (**14**, **16**, **21**) are formed by nucleophilic addition *anti* to the metal (Fig. 3). Similarly to the 1,3-diene complex **4**, its isomer **5** undergoes *exo* protonation to form the allyl complex **24**. Remarkably, treatment of the 1,4-cyclohexadiene complex (**8**) with D^+ ($D_2NPh_2^+$ in MeOD) also undergoes direct *exo* protonation (Fig. 3), this time providing allyl **25**. The direct exogenous protonation of the unconjugated C=C bond in **8** appears to result in a carbocation that can be stabilized by the participation of the nitrosyl ligand, as revealed by DFT calculations. A subsequent [1,2]-hydride shift results in the formation of the allyl complex **25** (Supplementary Fig. 5). Unambiguous assignment of the deuterated hydrogen atom in **25** comes from its conversion to **9**-d_1 (via **39**; Fig. 3). To demonstrate regio- and stereocontrol of deuterium incorporation, additional deuterated isotopomers of the allyl complex were prepared from the monodeuterated dienes **22** and **23** and from the benzene-d_6-derived allyls **30** and **31** (Fig. 3). The allyl complexes **24**–**31** were then combined with deuteride or hydride to form 18 additional cyclohexene complexes, **32**–**46**, **49**–**51**. In principle, one can selectively make ten different isotopologues of the cyclohexene complex

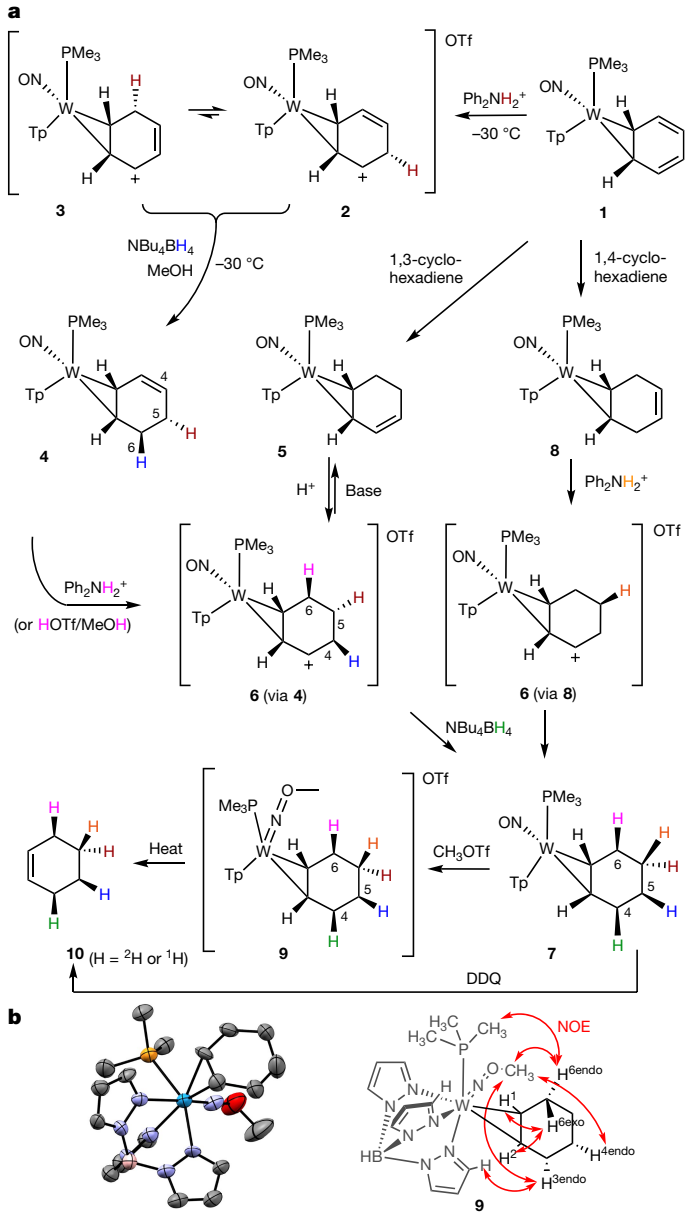

Fig. 2 | Formation of tungsten-bound cyclohexene from benzene. **a**, Sequential reduction of benzene to cyclohexene bound to tungsten (by addition of ^{1}H or ^{2}H). **10** was confirmed by ^{13}C-NMR and MRR spectroscopy and **9** was confirmed by quantitative NOE. **b**, Solid-state molecular structure from a single-crystal X-ray diffraction study and relevant NOE interactions (red arrows) for the methylated cyclohexene complex **9** ($Ph_2NH_2^+$ as OTf salt).

using the procedures outlined above (d_0–d_4; d_6–d_{10}), eight of which (**7**, **16**, **32**–**38**) are reported herein.

Levels of isotopic purity for the cyclohexene ligand isotopologues were determined by recording high-resolution mass spectrometry (HRMS) data for the corresponding complexes as their methylated adducts (Fig. 2.; **9**-d_n) to create a suitable cation for electrospray ionization mass analysis. Using the isotope envelope of **9**-d_0 as a reference (Supplementary Fig. 6), the isotopic purity of **7**, **16** and **32**–**38** (as converted to **9**-d_n) was estimated to be >90%, with the exception of **16** (79%), for which the high DKIE of the second protonation prevented complete deuteration at the H^{6exo} position (see above). Finally, as a demonstration of how the {WTp(NO)(PMe_3)} system precisely governs both the stereochemistry and the regiochemistry of protonation and hydride addition, a series of five monodeuterated (**32**, **39**–**42**), seven dideuterated (**14**, **33**, **35**, **43**–**46**), and four trideuterated (**34**, **49**–**51**) isotopomers of the cyclohexene complex were prepared using these methods (Fig. 3).

Oxidation of the tungsten complex **7** with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) releases the free cyclohexene (Fig. 2; **10**). Such action on **32**, **42**, **45** and **46** confirmed the expected regiochemistry of these d_1 and d_2 isotopomers of cyclohexene via ^{13}C NMR. Introduction of a single deuterium in 3-deuterocyclohexene or 4-deuterocyclohexene allows one to distinguish all six of the carbons in the ^{13}C NMR spectrum, owing to isotopic shifting of the now asymmetric cyclohexene carbons (Supplementary Fig. 7). Alternatively, solvent-free heating of various isotopologues of the methylated complex **9** induce the release of the cyclohexene ligand for analysis by molecular rotation resonance (MRR) spectroscopy (Supplementary Information section L)[20]. These experiments determined that: (1) over-deuteration is exceedingly low (<2%). (2) The stereoselectivity is excellent when assessed by observation of undesired *cis*/*trans* isomers, which in the worst case is 22:1 and in other cases it is 40:1 or higher. (3) The dominant stereoisotopomers in all cases are those predicted by the ^{1}H NMR data. As a final check of the stereochemical assignments, the locations of the deuterium atoms were confirmed for complex **45** using neutron diffraction measurements (Supplementary Information section I; TOPAZ at Oak Ridge National Laboratory).

Mechanistic considerations

The reaction of **1** with D^+ to form **11** results in deuterium incorporation exclusively *endo* to the metal, but this does not conclusively show which carbon is initially protonated (Supplementary Fig. 8). Given that the *endo* proton of the benzene ligand in **1** completely preempts protonation from an exogenous acid (*exo*), we propose that the protonation must be concerted—that C–H bond formation is intramolecular and simultaneous with electronic changes at the metal—which could lower the activation barrier for this process relative to protonation by an external acid. Such a mechanism could occur via a hydride intermediate, but this seems sterically untenable. Instead, we propose a mechanism (Supplementary Information section M) in which the nitrosyl ligand is first protonated to form a hydroxylimido ligand analogous to that reported by Legzdins et al.[21]. This action is followed by a concerted proton transfer in which a gamma carbon of the benzene is protonated simultaneously with the release of electron density back into the tungsten through the NO group. The role of nitrosyl ligands in intramolecular proton transfer has been previously documented[22]. By contrast, the stereochemistry and kinetics of η^2-diene protonation (for example, **4**; Fig. 2) indicates that the hydrogen is delivered exogenously, *anti* to the tungsten (Fig. 1). We speculate that whereas *endo* protonation may still be accessible for these 1,3-cyclohexadiene complexes, the less-delocalized diene ligand is probably more basic than its η^2-benzene predecessor, and its direct *exo* protonation apparently preempts the purported *endo* mechanism at −30 °C.

Transition-metal-promoted *endo* deuteration of benzene was observed in the η^4-benzene complexes $Cr(CO)_3(\eta^4\text{-benzene})^{2-}$ and $Mn(CO)_3(\eta^4\text{-benzene})^-$ by Cooper et al.[23,24], and was proposed to occur via hydride intermediates[23,24]. More recently, Chirik et al. explored the molybdenum-catalysed reduction of benzene and cyclohexadiene, with D_2 (g), which resulted in mixtures of isotopologues of cyclohexane[11]. However, reduction of cyclohexene with D_2 produced a single *cis* isotopomer of 1,2-dideuterocyclohexane using the molybdenum catalyst.

The high stereoselectivity enabled by the tungsten system provides unprecedented control over the preparation of specific isotopologues and isotopomers of cyclohexene, starting from either benzene complex **1** or its deuterated analogue **17**, and using either proteated or deuterated sources of acids and hydrides (Supplementary Table 1). As an illustration, consider the d_2 isotopologue of the cyclohexene complex, **7**-d_2. Given that the {WTp(NO)(PMe_3)} system is available in

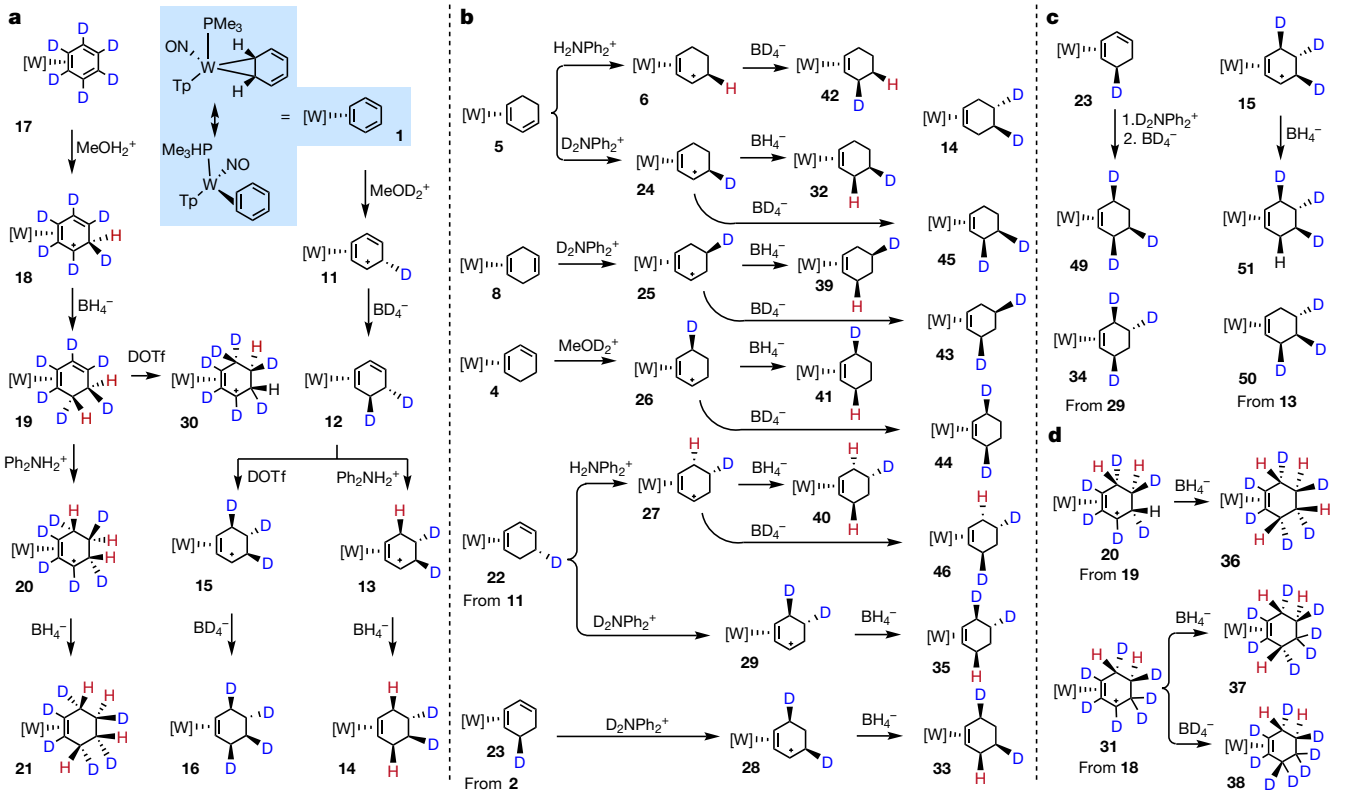

Fig. 3 | Synthesis of isotopologues and stereoisotopomers of the cyclohexene complex 7. a, Detailed syntheses of d_2, d_4 and d_6 isotopologues. **b**, Synthesis of d_1 and d_2 isotopologues. **c**, Synthesis of d_3 isotopologues. **d**, Synthesis of d_6, d_7 and d_8 isotopologues.

enantioenriched form[15], one has access to 14 different isotopomers (individual enantiomers of **14**, **33**, **35**, **43**–**46**; Supplementary Table 2). The cyclohexene-d_2 ligand of these complexes, once removed from the metal by oxidative decomplexation, would be available as 11 individual isotopomers: both enantiomers of *cis*-3,4-, *trans*-3,4-, *cis*-3,5-, *trans*-3,5-, *trans*-4,5- and the meso compound *cis*-3,6-dideuterocycloohexene. Similarly, 11 distinct isotopomers of cyclohexene-d_8 should be available using this methodology starting from benzene-d_6. Regarding cyclohexene complexes **7**-d_3 and **7**-d_7, eight isotopomers of each would be available, and all 16 of these complexes would yield a unique, chiral cyclohexene (eight cyclohexene-d_3 and eight cyclohexene-d_7). In total (Supplementary Table 2), the methodology outlined herein could provide access to 52 unique isotopomers of cyclohexene derived from benzene and benzene-d_6. For reference, the total number of isotopomers for cyclohexene is 528.

The ability of {WTp(NO)(PMe_3)} to be optically resolved on a practical scale and to retain its stereochemical configuration, even when undergoing ligand displacement[15], also makes it a valuable tool for determining the isotopic pattern of cyclohexene H/D isotopomers produced by other methods[8]. Consider, for example, a scenario in which an unknown isotopomer of cyclohexene-d_1 is combined with the resolved form of benzene complex (R)-**1** in solution and allowed to undergo ligand exchange. Even though the two faces of the cyclohexene ring will bind to tungsten with equal probability, the ^{1}H NMR spectrum will be unique for each of the five possible isotopomers (Supplementary Information section C; Supplementary Fig. 11). A similar approach could be taken for any cyclic alkene (for example, dehydropiperidines, pyrrolines, cyclopentenes) for which a ^{1}H NMR spectrum of a fully proteated species can be fully assigned (see above).

Deuterated building blocks for the MedChem database

The development of deutetrabenazine, is considered by many as a prelude to a new generation of medicines and therapies that incorporate deuterium into the active pharmaceutical ingredient[2]. Given that each stereoisotopomer of a biologically active substance will have its own unique pharmacokinetic profile, the ability to stereoselectively deuterate cyclohexene or other MedChem building blocks could enable the development of new probes, fragment libraries and leads for medicinal chemists, as well as provide a new tool for organic and organometallic mechanistic studies. Cyclohexene can be readily converted into perhydroindoles[25], perhydroisoquinolines[26] and azepines[27]. However, the inability to chemically differentiate the two alkene carbons or the enantioface of the deuterated cyclohexene limits its potential. Nevertheless, by replacing the benzene ligand in Fig. 2 with a substituted benzene, or by using a non-hydrogenic nucleophile in the conversion of **6** to **7** (Fig. 2), one can envision a series of 3-substituted cyclohexenes with highly defined isotopic patterns. As proof of concept, we prepared the α,α,α-trifluorotoluene complex WTp(NO)(PMe_3)(η^2-CF_3Ph) (ref. [28]), which can be elaborated into a 3-(trifluoromethyl)cyclohexene complex (**47**) analogous to the cyclohexene complex **7** (Supplementary Fig. 14). Liberation of the cyclohexene from {WTp(NO)(PMe_3)} can be accomplished by a one-electron oxidant such as DDQ, Fe(III) or $NOPF_6$ in yields of 70–75% (ref. [28]). Oxidation of **47** would generate a cyclohexene that has been previously shown to undergo diastereoselective epoxidation, and would therefore be an attractive building block for medicinal chemistry[29]. Repeating the synthesis of **47** with deuteride in the final step yields the *cis*-6-deutero-3-(trifluoromethyl)cyclohexene complex **52** in 95% yield. Various other isotopologues of **47** and **52** were also prepared (**47**, **52**, **53**, **54**), and the reaction pattern was found to be similar to that observed for benzene. The prepared compounds are summarized in Fig. 4, with synthetic details provided in Supplementary Information section B. Notably, in the syntheses of **47**, **52**, **53** and **54**, protonation at the carbon bearing the CF_3 group ultimately occurs *endo* to the metal, allowing the CF_3 group to assume an *exo* stereochemistry. However, if the purported diene intermediate is protonated under kinetic control, *exo* protonation forces the CF_3 group *endo*, and the result after a second hydride reduction is the cyclohexene complex

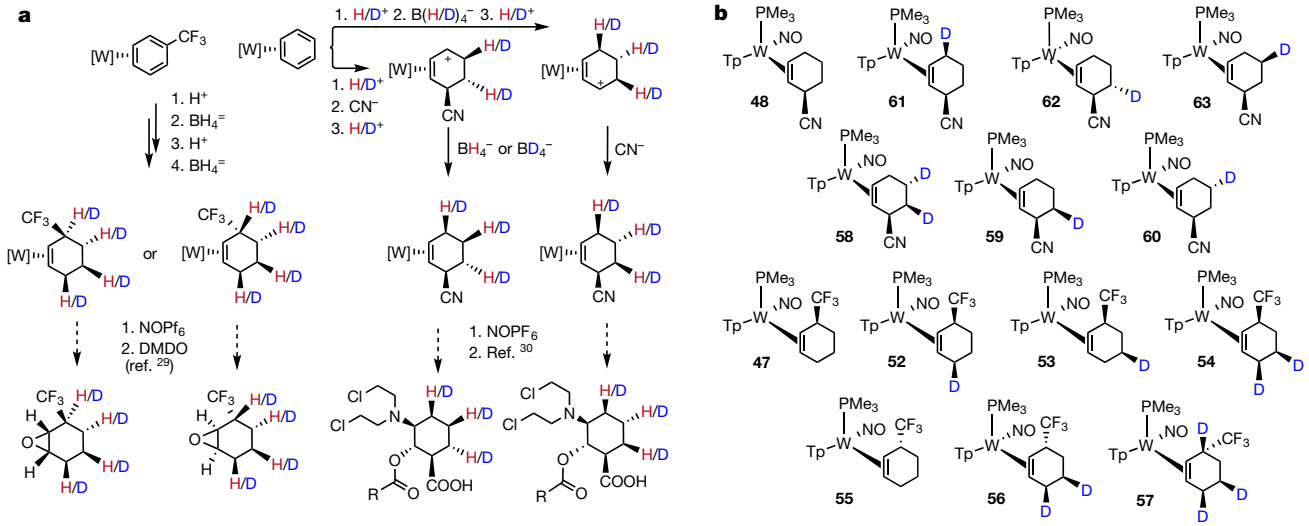

Fig. 4 | Examples of functionalized cyclohexene isotopomer complexes. **a**, Synthesis (speculated) of functionalized cyclohexenes; ref. [29] describes a single-step reaction with DMDO (70%); ref. [30] describes a nine-step synthesis that includes: (i) HCl/H_2O, (ii) isobutylene, (iii) mCPBA, (iv) NaN_3, (v) acetyl chloride, (vi) H_2/C, (vii) ethylene oxide, (viii) TsCl and (ix) TFA. **b**, Examples of chemo- and stereoselectively deuterated cyclohexene complexes with CF_3 and CN groups on the cyclohexene. See Supplementary Information section B for the full synthetic details of **47**, **48**, **52**–**63**.

55. By exploiting this reactivity feature, we were able to prepare other isotopologues of **55** with inversion of the stereocentre bearing the -CF_3 substituent (Fig. 4, **56**, **57**; Supplementary Fig. 14).

As further demonstration of the ability of this methodology to selectively prepare isotopomers of functionalized cyclohexenes, we prepared the tungsten complex of *cis*,*trans*-3-cyano-4,5-dideuterocyclohexene (**58**) by the addition of cyanide to the allyl intermediate **13** (57%; diastereometric ratio >98%; Fig. 4.). Other d_1-isotopolouges were also prepared (Supplementary Fig. 15), and their stereochemistry could again be controlled with the sequence of nucleophiles. For example, **58**, **59** and **60** could be prepared by generating the appropriate isotopologue of the tungsten-allyl complex and then treating with NaCN (Supplementary Fig. 16). Conversely, treating the benzenium **2** with NaCN leads to a cyano-substituted cyclohexadiene that can be subsequently combined with acid and a hydride source to generate other cyclohexene isotopomers (**61**–**63**; Supplementary Information section B). 3-cyanocyclohexene (proteo form) has been previously used as a precursor to cytotoxic mustards that are of interest in cancer research[30]. Allyl-substituted cyclohexenes theoretically exist as 1,024 different H/D isotopomers (512 for each enantiomer). Using the tungsten dearomatization methodology, the CF_3- and CN-substituted cyclohexenes are accessible as 64 and 60 unique isotopomers, respectively. We further note that a full range of both carbon and nitrogen nucleophiles has now been demonstrated to add to tungsten benzenium and allyl tungsten complexes[31], which demonstrates the broad scope of compounds that can now be prepared as various deuteroisotopomers.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-020-2268-y.

1. Gant, T. G. Using deuterium in drug discovery: leaving the label in the drug. *J. Med. Chem.* **57**, 3595–3611 (2014).
2. Dean, M. & Sung, V. W. Review of deutetrabenazine: a novel treatment for chorea associated with Huntington's disease. *Drug Des. Devel. Ther.* **12**, 313–319 (2018).
3. Thibblin, A. & Ahlberg, P. Reaction branching and extreme kinetic isotope effects in the study of reaction mechanisms. *Chem. Soc. Rev.* **18**, 209–224 (1989).
4. Thibblin, A. Unusually large kinetic deuterium isotope effects on oxidation reactions. 1. the mechanism of hydroxide-catalysed permanganate oxidation of PhCD(CF_3)OH and PhCD(CH_3)OH in water. *J. Phys. Org. Chem.* **8**, 186–190 (1995).
5. Nelson, S. D. & Trager, W. F. The use of deuterium isotope effects to probe the active site properties, mechanism, of cyctochrome P450-catalyzed reactions, and mechanisms of metabolically dependent toxicity. *Drug Metab. Dispos.* **31**, 1481–1497 (2003).
6. Loh, Y. Y. et al. Photoredox-catalyzed deuteration and tritiation of pharmaceutical compounds. *Science* **358**, 1182–1187 (2017).
7. Pony Yu, R., Hesk, D., Rivera, N., Pelczer, I. & Chirik, P. J. Iron-catalysed tritiation of pharmaceuticals. *Nature* **529**, 195–199 (2016).
8. Baldwin, J. E., Kiemle, D. J. & Kostikov, A. P. Quantitative analyses of stereoisomeric 3,4-d_2-cyclohexenes in the presence of 3,6-d_2-cyclohexenes. *J. Org. Chem.* **74**, 3866–3874 (2009).
9. Eisen, M. S. & Marks, T. J. Supported organoactinide complexes as heterogeneous catalysts. A kinetic and mechanistic study of facile arene hydrogenation. *J. Am. Chem. Soc.* **114**, 10358–10368 (1992).
10. Jones, R. A. & Seeberger, M. H. Synthesis of polymer-supported transition metal catalysts via phosphido linkages: heterogeneous catalysts for the hydrogenation of aromatic compounds under mild conditions. *J. Chem. Soc. Chem. Commun*. 373–374 (1985).
11. Joannou, M. V., Bezdek, M. J. & Chirik, P. J. Pyridine(diimine) molybdenum-catalyzed hydrogenation of arenes and hindered olefins: insights into precatalyst activation and deactivation pathways. *ACS Catal.* **8**, 5276–5285 (2018).
12. Harman, W. D. & Taube, H. The selective hydrogenation of benzene to cyclohexene on pentaammineosmium(II). *J. Am. Chem. Soc.* **110**, 7906–7907 (1988).
13. Liebov, B. K. & Harman, W. D. Group 6 dihapto-coordinate dearomatization agents for organic synthesis. *Chem. Rev.* **117**, 13721–13755 (2017).
14. Welch, K. D. et al. Large-scale syntheses of several synthons to the dearomatization agent {TpW(NO)(PMe_3)} and convenient spectroscopic tools for product analysis. *Organometallics* **26**, 2791–2794 (2007).
15. Lankenau, A. W. et al. Enantioenrichment of a tungsten dearomatization agent utilizing chiral acids. *J. Am. Chem. Soc.* **137**, 3649–3655 (2015).
16. Harrison, D. P. et al. Hyperdistorted tungsten allyl complexes and their stereoselective deprotonation to form dihapto-coordinated dienes. *Organometallics* **30**, 2587–2597 (2011).
17. Lis, E. C. et al. The uncommon reactivity of dihapto-coordinated nitrile, ketone, and alkene ligands when bound to a powerful π-base. *Organometallics* **25**, 5051–5058 (2006).
18. Arashiba, K. et al. Electrophilic O-methylation of a terminal nitrosyl ligand attained by an early–late heterobimetallic effect. *Organometallics* **25**, 560–562 (2006).
19. Jamison, C. J. Isotope effects on chemical shifts and coupling constants. *eMagRes* https://doi.org/10.1002/9780470034590.emrstm0251 (2007).
20. Pérez, C. et al. Broadband Fourier transform rotational spectroscopy for structure determination: the water heptamer. *Chem. Phys. Lett.* **571**, 1–15 (2013).
21. Sharp, W. B., Legzdins, P. & Patrick, B. O. O-Protonation of a terminal nitrosyl group to form an η1-hydroxylimido ligand. *J. Am. Chem. Soc.* **123**, 8143–8144 (2001).
22. Llamazares, A., Schmalle, H. W. & Berke, H. Ligand-assisted heterolytic activation of hydrogen and silanes mediated by nitrosyl rhenium complexes. *Organometallics* **20**, 5277–5288 (2001).

23. Leong, V. S. & Cooper, N. J. Electrophilic activation of benzene in $[Cr(\eta^4\text{–}C_6H_6)(CO)_3]^{2-}$. *J. Am. Chem. Soc.* **110**, 2644–2646 (1988).
24. Thompson, R. L., Lee, S., Rheingold, A. L. & Cooper, N. J. Reductive activation of the coordinated benzene in manganese complex $[Mn(\eta^6\text{-}C_6H_6)(CO)_3]^+$: synthesis and characterization of the η^4-naphthalene complex $PPN[Mn(\eta^4\text{–}C_{10}H_8)(CO)_3]$. *Organometallics* **10**, 1657–1659 (1991).
25. Ungureanu, I.; Klotz, P.; Mann, A. Phenylaziridine as a masked 1,3 dipole in reactions with nonactivated alkenes. *Angew. Chem.* **39**, 4615–4617 (1991).
26. Sarkar, N., Banerjee, A. & Nelson, S. G. [4 + 2] Cycloadditions of *N*-alkenyl iminium ions: structurally complex heterocycles from a three-component Diels–Alder reaction sequence. *J. Am. Chem. Soc.* **130**, 9222–9223 (2008).
27. Shim, S. C., Doh, C. H., Kim, T. J., Lee, H. K. & Kim, K. D. A new and convenient synthesis of N-substituted perhydroazepines from adipaldehyde and primary amines with tetracarbonylhydridoferrate, $HFe(CO)_4^-$, as a selective reducing agent. *J. Heterocycl. Chem.* **25**, 1383–1385 (1988).
28. Wilson, K. B. et al. Sequential tandem addition to a tungsten–trifluorotoluene complex: a versatile method for the preparation of highly functionalized trifluoromethylated cyclohexenes. *J. Am. Chem. Soc.* **139**, 11401–11412 (2017).
29. Murray, R. W., Singh, M., Williams, B. L. & Moncrieff, H. M. Diastereoselectivity in the epoxidation of substituted cyclohexenes by dimethyldioxirane. *J. Org. Chem.* **61**, 1830–1841 (1996).
30. Leiris, S., Lucas, M., Dupuy d'Angeac, A. & Morère, A. Synthesis and biological evaluation of cyclic nitrogen mustards based on carnitine framework. *Eur. J. Med. Chem.* **45**, 4140–4148 (2010).
31. Wilson, K. B. et al. Highly functionalized cyclohexenes derived from benzene: sequential tandem addition reactions promoted by tungsten. *J. Org. Chem.* **84**, 6094–6116 (2019).

Data availability

All data are available in the main text and Supplementary Information, including NMR spectra, experimental details, crystallographic information, DFT calculations, rotational spectroscopy and HRMS data. Supplementary crystallographic data for this paper (**4**, **7**, **9** (X-ray) and **45** (neutron)) can be obtained from the Cambridge Crystallographic Data Centre at www.ccdc.cam.ac.uk/structures (CCDC 1885723-1885725 and 1972890).

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Competing interests The authors declare no competing interests.

Additional information

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