Enantioselective Synthesis of Alkyl Fluorides via Biocatalytic Reduction

Helen Allan[†],[1] Yu Wang[†],[1] Bethan Winterson,[1] Alexandra King,[1] Abil E. Aliev,[1] Rachel Szpara,[1] Victor Laserna,[1] Charlotte E. Coomber,[1] John M. Ward,[2] Jack W. E. Jeffries,[2] Helen C. Hailes*,[1] Tom D. Sheppard*[1]

[1]Department of Chemistry, University College London, 20 Gordon Street, London, WC1H 0AJ, UK.

[2]Department of Biochemical Engineering, University College London, London, WC1E 6BT, UK.

[†]These authors contributed equally

Abstract Here we report the first biocatalytic asymmetric synthesis of alkyl fluorides via reduction of α-fluoroenones and α-fluoroenoates using ene reductase enzymes. The reduction of a wide range of (*Z*) or (*E*)-α-fluoroenones was shown to proceed in high yield and selectivity using ene reductases, with the different alkene geometries leading to opposite enantiomers of the chiral fluoroalkane. The reaction could also be successfully extended to α-fluoroenoates to access enantioenriched α-fluoroesters with only the *E*-alkene isomers under-going reduction, enabling mixtures of alkene geometries to be employed. The selectivity and substrate scope were rationalized using in silico substrate-enzyme molecular docking studies.

Fluorinated molecules make up ~20% of marketed drugs and an ever increasing number of pharmaceutical candidates;[1] an even larger proportion of agrochemicals in development contain fluorine.[2] This reflects the significant utility of fluorine in biologically active molecules, and there is considerable interest in novel methods for the synthesis of fluorinated organic compounds. Importantly, the fluorine atom provides a largely inert functional group that can increase metabolic stability and compound permeability. Moreover, it can perturb lipophilicities and pKas – impacting on binding affinities, and C(sp^3)-F centers can provide control over the molecular shape through conformational interactions of the polar C-F bond.[1c,3] Compounds bearing a fluorine atom at a chiral center have been underexplored due to the synthetic challenges of preparing these compounds in high enantiopurity in a scalable fashion. However, examples describing the stereoselective replacement of an sp^3 C-H with C-F and the resulting impact on biological activities highlight the importance of such compounds.[3a,4]

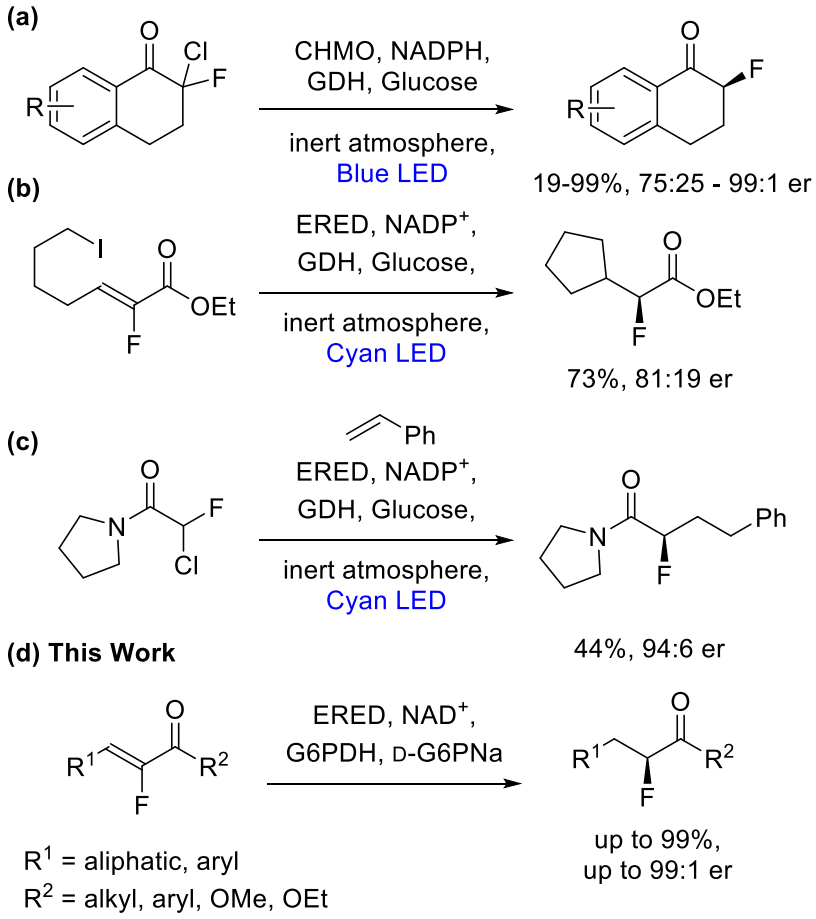
Approaches reported for the synthesis of chiral centers containing a C-F bond proceeding with high levels of enantioselectivity include electrophilic fluorination of carbonyl compounds[5] or addition of amines to fluorovinylpyiridines[6] using organocatalysis, the use of hydrogen-bonding phase transfer

catalysts for catalytic asymmetric S_N2 reactions of fluoride,[7] the use of a chiral fluorinating reagent,[8] and the asymmetric hydrogenation of fluoroalkenes using homogeneous catalysts containing rare transition metals (Rh, Ir).[9] Direct fluorination of enantioenriched alcohols with reagents such as DAST is perhaps the most accessible approach, but it is potentially hazardous to scale-up and promotes competing elimination processes often resulting in low product yields.[10] The development of readily scalable and accessible methods for the enantioselective preparation of alkyl fluorides remains a challenge.

The use of biocatalysts is continuing to grow and play an important role in the fine chemical and pharmaceutical industries, providing a sustainable and green synthetic strategy for the preparation of high-value chemicals. They have significant potential compared to traditional organic chemistry strategies, avoiding the use of expensive chiral ligands, toxic or rare transition metals, toxic solvents or extreme temperatures and pressures. Biocatalysts are also typically employed under mild reaction conditions in aqueous media, giving excellent chemo- and stereoselectivities. To date, the use of enzymes to generate sp^3 fluorine centers from non-chiral starting materials is limited, with early examples using aldolases to produce fluoroalcohols.[11] A more recent approach used a cyclohexanone monooxygenase (CHMO) under anerobic conditions and light activation, to achieve a photoinduced reductive dehalogenation via an electron transfer mechanism (Scheme 1a). While cyclic substrates gave products in high enantiopurity (enantiomeric ratio, er) poor enantiocontrol was observed with an acyclic substrate.[12] A non-haem iron enzyme has also been reported for enantioselective C(sp^3)–F bond formation via radical fluorine transfer. However, fluorination was limited to the benzylic positions of *ortho*-arylamides.[13]

Ene reductases (EREDs) are flavin-containing enzymes that reduce alkenes activated with an electron-withdrawing group, via a *trans*-hydrogenation.[14] The predominant family of EREDs is the old yellow enzyme (OYE) nicotinamide NAD(P)H dependent oxidoreductases which catalyze the reduction of α,β-unsaturated compounds including ketones, aldehydes, and nitro compounds; esters and acids are less readily accepted.[14] In recent years many EREDs have been described, including some that can accept sterically challenging enones which were recently discovered in our laboratory via a sequence-based functional metagenomics strategy.[14,15] EREDs have been applied in the synthesis of industrially useful compounds such as pregabalin precursors, flavor precursor molecules and other high value chiral building blocks on an industrially relevant scale.[16] Interestingly, Hyster et al. have recently reported a single example of a fluoroalkene photoenzymatic radical cyclisation using an

ERED (Scheme 1b)[17] and a single example of the ERED photocatalyzed coupling of an α,α-chloro-fluoroamide with styrene (Scheme 1c).[18] However, no routes have been described to single isomer C(sp^3)-F centers using bioreduction strategies of fluoroenones/enoates.[19] EREDs have been shown to reduce α-chloro/bromoenoates and α-chloro/bromoenones, with generally high selectivity but modest yields, particularly for aromatic-α,β-unsaturated compounds.[20] Here, a highly flexible novel biocatalytic approach to enantioenriched sp^3 fluorides, via the reduction of α-fluoroenones and α-fluoroenoates, is described to provide access to a diverse array of functionalized chiral fluorinated compounds in good to excellent yields and stereoselectivities (Scheme 1d).



Scheme 1 Biocatalytic routes to stereogenic sp^3 fluorine centers (a) A photoinduced reductive dehalogenation via an electron transfer mechanism.[12] (b) A fluoroalkene photoenzymatic radical cyclisation using an ERED.[17] (c) A photoenzymatic hydroalkylation of styrene using an ERED.[18] (d) Our approach via a bioreductive ERED reaction.

Initially we focused our attention on the reduction of α-fluoroenones, which could be readily prepared via the Horner-Wadsworth-Emmons olefination of aldehydes using a fluorinated phosphonate under aqueous conditions.[21] Authentic samples of the racemic products were synthesized via the Pd-catalyzed decarboxylation of fluorinated β-ketoesters.[22] Several EREDs, heterologously expressed in *E. coli* BL21 (DE3) were selected, including NCR from the bacterium *Zymomonas mobilis*

which has been used successfully with a range of linear substrates, and our previously reported EREDs pQR1445 and pQR1907 from a drain metagenome which exhibited good organic solvent tolerance.[15] The EREDs were co-expressed with glucose-6-phosphate dehydrogenase (G6PDH) to recycle the NADH co-factor *in situ*, utilizing D-glucose-6-phosphate sodium salt (D-G6PNa) as co-substrate. Enzyme lysates were employed in all reactions as these are typically used in industry, negating the need for costly enzyme purification.

Initial screens explored the reduction of (*Z*)-3-fluoro-4-phenyl-3-buten-2-one (**1a**) and reactions were monitored by HPLC analysis. The highest activity was seen with NCR, which gave the complete conversion of **1a** to the desired fluoroalkane ((*S*)-**2a**) with excellent stereocontrol (>95:5 er, chiral HPLC analysis, Figure 1a). A range of substrates were then screened against NCR, pQR1445 and pQR1907. The highest activity was seen with NCR in all but one case. The NCR ERED was found to accept a wide range of α-fluoroenones and reductions occurred with excellent yields and stereoselectivities with both (*Z*) and (*E*)-α-fluoroenones being accepted by the enzyme (Figure 1a, 1b). Interestingly the opposite stereochemistry was observed starting from the (*Z*)-alkene compared to when the (*E*)-alkene was used. Substrates with a range of β-substituents were accepted, yielding fluorides containing electron poor (**2b**, **2d**) and electron rich (**2c**) aryl groups, a range of aryl substitution patterns (**2e**, **2f**), a heteroaromatic group (**2g**) and alkyl groups (**2h**, **2i**). While most products were obtained in high yield and selectivity, the substrates for obtaining the (*R*)-o-tolyl product ((*R*)-**2e**) and pyridyl products (**2g**) were less well accepted. For the preparation of (*R*)-**2e** a higher enzyme concentration was needed but the reaction could not be pushed to completion and low stereoselectivity was observed. Low conversions were seen for both (*Z*)- and (*E*)- pyridyl substrates (**1g**) using NCR (<50% at 80% v/v enzyme lysate concentration). Changing the enzyme to pQR1445 (20% enzyme lysate concentration) gave excellent yields and moderate to good selectivities (94%, (*S*)-**2g**: 9:91 er; (*R*)-**2g**: 7:1 er). When the ethyl ketone (**1j**) was subjected to the reaction conditions the (*E*)-fluoroenone gave the corresponding fluoroalkane (*R*)-**2j** in 98% yield and 99:1 er, however, the (*Z*)-fluoroenone was poorly accepted and we saw <5% conversion to (*S*)-**2j**. A phenyl ketone was well accepted, however, giving fluoride (*S*)-**2k** in excellent yield and enantiopurity from the *Z*-enone, though the corresponding *E*-enone could not be purified effectively.

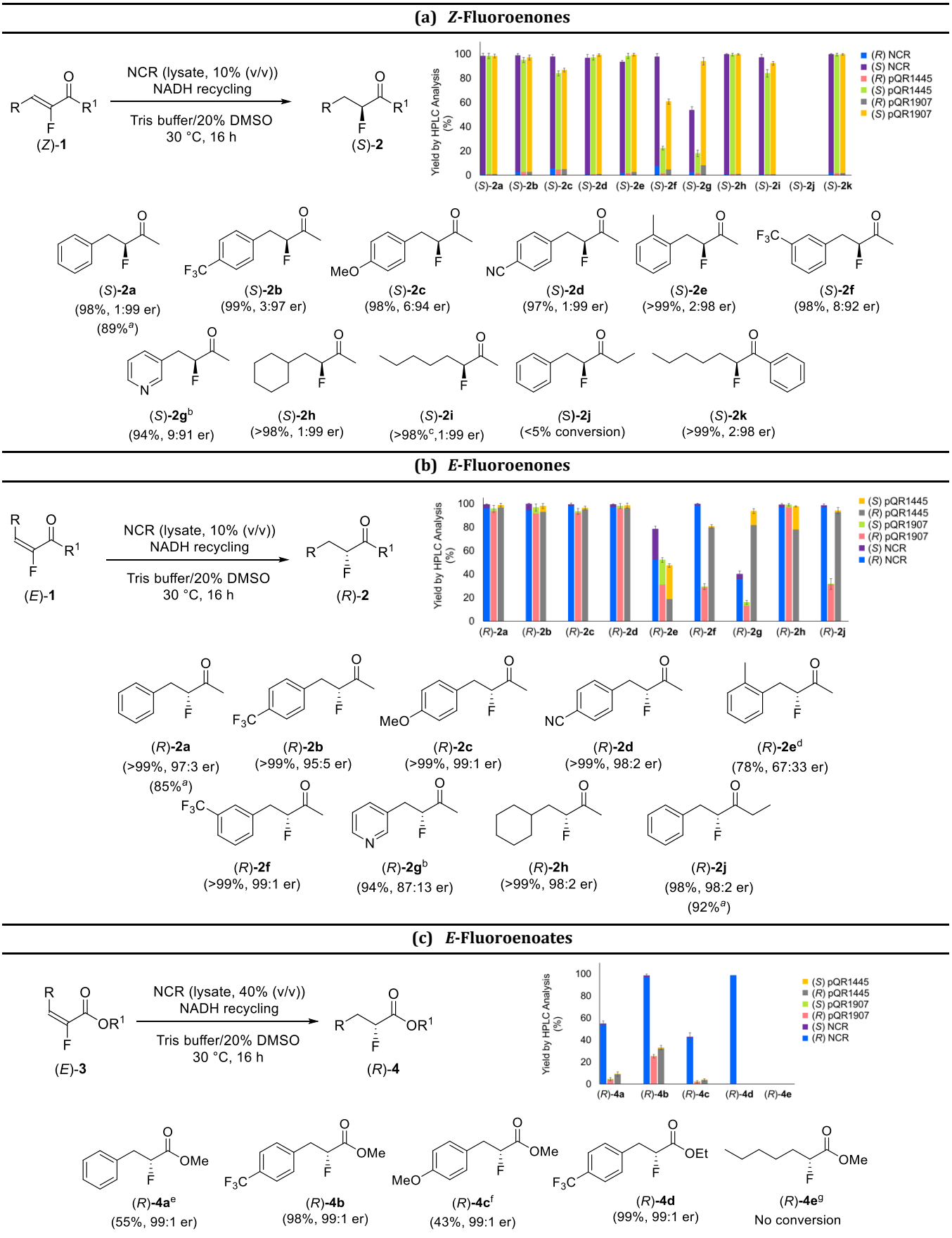


Figure 1. (a,b) Substrate Scope of Fluoroenones. Substrate (10 mM), ERED and G6PDH lysates (10% (v/v), co-expression, total protein in the lysates 1 mg mL^{-1}), NAD+ (1 mM), G6PNa (50 mM), in Tris–HCl (100 mM) and DMSO (20%) at pH 7.5, 30 °C, 16 h, 700 rpm. Reactions were performed in triplicate. Yields and enantiomeric ratios were determined by HPLC

or GC analysis. [a]Isolated yield from preparative scale reaction. [b]pQR1445 used, 20% (v/v) enzyme lysate concentration. [c]Conversion; no starting material remaining by GC but both product and starting material are volatile. [d]80% (v/v) enzyme lysate concentration. (c) Substrate Scope of Fluoroenoates - 40% (v/v) enzyme lystate concentration; [e]from 8:1 (*E*:*Z*); [f]from 5:1 (*E*:*Z*); [g]from 13:1 (*E*:*Z*); where the SM was a mixture of isomers, yield is based upon the conversion of the *E* isomer to the product.

Less activated alkenes such as enoates are considered as borderline substrates for ene reductases. We reasoned that the electronegative fluorine atom may activate these substrates sufficiently to enable reduction to occur. A range of (*E*)- and (*Z*)-fluoroenoates were synthesized via a Horner-Wadsworth-Emmons reaction-(*E*) or $TiCl_4$ mediated aldol condensation-(*Z*) and subjected to the reaction (Figure 1c).[23] Authentic samples of reaction products were synthesized via a one-pot substitution/Krapcho decarboxylation.[24] Whilst aryl (*E*)-enoates were accepted by the enzyme, the conversion was lower than with (*E*)-enones. The enzymes were most active towards electron poor enoates (**4b**, 98% yield) compared to electron rich enoates (**4c**, 55% yield). Both methyl (**4a**-**c**) and ethyl (**4d**) esters were accepted. In all cases the stereocontrol was excellent. However, (*E*)-alkylenoate (**3e**) and all tested (*Z*)-fluoroenoates showed no conversions.[25] This was in line with the ketone results above, where a more sterically demanding ketone substituent (Et), comparable in size to OMe/OEt, led to low reactivity of the (*Z*)-isomer. This enzyme selectivity is especially useful when preparing the (*E*)-enoates via a Horner-Wadsworth-Emmons reaction. The resultant mixture of (*E*)- and (*Z*)- enoates can be used in the ERED reaction without the need for prior separation with no decrease in the enantiopurity of the product. The reaction was amenable to biocatalytic preparative scale reactions (20-130 mg) giving an isolated yield of 89% of (*S*)-**2a** from (*Z*)-**1a**; 86% of (*R*)-**2a** from (*E*)-**1a** and 92% of (*R*)-**2j** from (*E*)-**1j**. Authentic reference standards of (*R*)-**4a** and (*R*)-**2j** were synthesized in order to accurately assign the absolute stereochemistry of the products (correlated by chiral HPLC) from the biocatalytic reactions. Experimental procedures and discussion of the assignments can be found in the Supporting Information.

Docking studies were conducted to rationalize the high reactivities and excellent stereoselectivities observed with NCR. Using the reported X-ray crystallographic data for NCR (PBD database (4A3U)26 and substrates (*E*)-**1a** and (*E*)-**3a** and also (*Z*)-**1a** and (*Z*)-**3a** as ligands, in silico molecular docking was carried out using AutoDock Vina (v.1.2.0).[27] As indicated (Figure 2) the ligands were orientated in the catalytic pocket of NCR with the carbonyl group complexed to His172 (3.69-4.41 Å, Van der Waal interaction) and Asn175 (2.91-3.17 Å, H-bond). Hydride transfer occurs from the reduced flavin to the β-C of the ligands while the α-C is reported to be protonated by Tyr177. The EREDs readily ac-

cepted (*E*)-**1a**, (*E*)-**3a**, and (*Z*)-**1a** as substrates, while (*Z*)-**3a** was not accepted at all. This can be attributed to the different orientations of the substrates in the catalytic pocket (Figure 2). Substrates (*E*/*Z*)-**1a** and (*E*)-**3a** can adopt productive conformations with hydride addition and then protonation in a *trans*-fashion to the alkene (Figure 2a-c). However, for substrate (*Z*)-**3a**, docked conformations are not productive as they would require hydride delivery and subsequent protonation to occur from the same face of the alkene (Figure 2d).

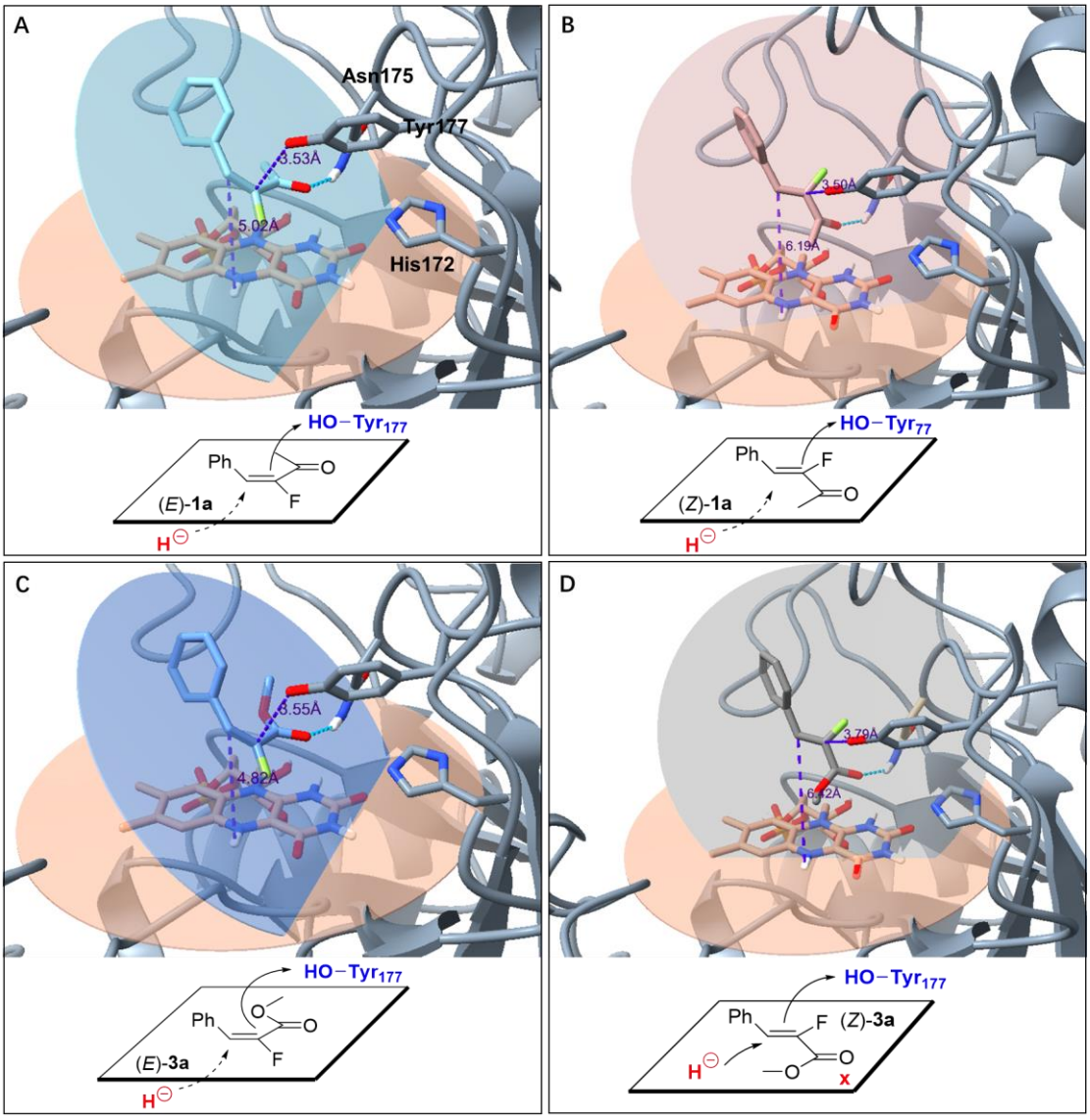


Figure 2. Docking studies of substrates (*E*/*Z*)-**1a** and **3a** with NCR. (a) (*E*)-**1a** with NCR, giving product (*R*)-**2a**. (b) (*Z*)-**1a** with NCR, giving product (*S*)-**2a**. (c) (*E*)-**3a** with NCR, giving product (*R*)-**4a**. (d) (*Z*)-**3a** with NCR. No product was generated with this substrate as the hydride delivery and the protonation from Tyr177 cannot take place from opposite faces.

In conclusion, a novel approach to enantioenriched sp^3 fluorides has been developed, via the bioreduction of α-fluoroenones and α-fluoroenoates. Notably, this provides access to a diverse array of functionalized chiral fluorinated compounds in good to excellent yields and stereoselectivities.

Acknowledgements We are grateful for financial support from the BBSRC LIDo PhD programme (BB/M009513/1) for funding A.K, from the EPSRC for funding Y.W. (EP/W019132/1) and R.S. (EP/R513143/1), and the Leverhulme Trust for funding B.W (RPG-2023-173) and V.L./C.E.C. (RPG-2017-221). We acknowledge 700 MHz NMR equipment support by EPSRC (EP/P020410/1). We thank K. Karu (UCL Mass Spectrometry Facility) in the Department of Chemistry at UCL.

References

(1) (a) Ali, S.; Zhou, J. Highlights on U.S. FDA-approved fluorinated drugs over the past five years (2018–2022). *Eur. J. Med. Chem.* **2023**, 115476. (b) Shabir, G.; Saeed, A.; Zahid, W.; Naseer, F.; Riaz, Z.; Khalil, N.; Muneeba; Albericio, F. Chemistry and Pharmacology of Fluorinated Drugs Approved by the FDA (2016–2022). *Pharmaceuticals* **2023**, *16*, 1162. (c) Inoue, I,; Sumii, Y.; Shibata, N. Contribution of Organofluorine Compounds to Pharmaceuticals. *ACS Omega*, **2020**, *5*, 10633-10640.

(2) (a) Jeschke, P. Recent developments in fluorine-containing pesticides. *Pest Manag. Sci.* **2024**, doi.org/10.1002/ps.7921. (b) Ogawa, Y.; Tokunaga, E.; Kobayashi, O.; Hirai, K.; Shibata, N. Current Contributions of Organofluorine Compounds to the Agrochemical Industry. *iScience* **2020**, *23*, 101467.

(3) (a) Purser, S.; Moore, P. R.; Swallow, S.; Gouverneur, V. Fluorine in medicinal chemistry. *Chem. Soc. Rev.* **2008**, *37*, 320-330; (b) Gillis, E. P.; Eastman, K. J.; Hill, M. D.; Donnelly, D. J.; Meanwell, N. A. Applications of fluorine in medicinal chemistry. *J. Med. Chem.* **2015**, *58*, 8315–8359; (c) Teschers, C. S.; Daniliuc, C. G.; Kehr, G.; Gilmour, R. Conformational control enabled by the fluorine gauche effect in a model of the β2-AR agonist salbutamol (Ventolin™). *J. Fluor. Chem.* **2018**, *210*, 1-5; (d) Bentler, P.; Erdeljac, N.; Bussmann, K.; Ahlqvist, M.; Knerr, L.; Bergander, K.; Daniliuc, C. G.; Gilmour, R. Stereocontrolled synthesis of tetrafluoropentanols: Multivicinal fluorinated alkane units for drug discovery. *Org. Lett.* **2019**, *21*, 7741-7745.

(4) Myers, A. G.; Barbay, J. K.; Zhong, B. Asymmetric Synthesis of Chiral Organofluorine Compounds: Use of Nonracemic Fluoroiodoacetic Acid as a Practical Electrophile and Its Application to the Synthesis of Monofluoro Hydroxyethylene Dipeptide Isosteres within a Novel Series of HIV Protease Inhibitors. *J. Am. Chem. Soc.* **2001**, *123*, 7207-7219.

(5) (a) Beeson, T. D.; MacMillan, D. W. C. Enantioselective Organocatalytic α-Fluorination of Aldehydes, *J. Am. Chem. Soc.* **2005**, *127*, 8826-8828; (b) Marigo, M.; Fielenbach, D.; Braunton, A.; Kjærsgaard, A.; Jørgensen K. A. Enantioselective Formation of Stereogenic Carbon–Fluorine Centers by a Simple Catalytic Method, *Angew. Chem. Int. Ed.* **2005**, *44*, 3703-3706; (c) Steiner, D. D.; Mase, N.; Barbas, C. F. Direct Asymmetric α-Fluorination of Aldehydes *Angew. Chem. Int. Ed.* **2005**, *44*, 3706-3710; (d) Li, F.; Wu, Z.; Wang, J. Oxidative Enantioselective α-Fluorination of Aliphatic Aldehydes Enabled by *N*-Heterocyclic Carbene Catalysis, *Angew. Chem. Int. Ed.* **2015**, *54*, 656-659; (e) Dong, X.; Yang, W.; Hu, W.; Sun J.; *N*-Heterocyclic Carbene Catalyzed Enantioselective α-Fluorination of Aliphatic Aldehydes and α-Chloro Aldehydes: Synthesis of α-Fluoro Esters, Amides, and Thioesters, *Angew. Chem. Int. Ed.* **2015**, *54*, 660-663; (f) Wheeler, P.; Voraa, H. U.; Rovis, T. Asymmetric NHC-catalyzed synthesis of α-fluoroamides from readily accessible α-fluoroenals, *Chem. Sci.* **2013**, *4*, 1674-1679; (g) Wang, L.; Jiang, X.; Chen, J.; Huang, Y. Enantio- and Diastereoselective Hydrofluorination of Enals by *N*-Heterocyclic Carbene Catalysis, *Angew. Chem. Int. Ed.* **2019**, *58*, 7410-7414; (h)

Kwiatkowski, P.; Beeson, T. D.; Conrad, J. C.; MacMillan, D. W. C. Enantioselective Organocatalytic α-Fluorination of Cyclic Ketones. *J. Am. Chem. Soc.* **2011**, *133*, 1738–1741.

(6) Ashford, M. W.; Xu, C.; Molloy, J. J.; Carpenter-Warren, C.; Slawin, A. M. Z.; Leach, A. G.; Watson, A. J. B. Catalytic Enantioselective Synthesis of Heterocyclic Vicinal Fluoroamines by Using Asymmetric Protonation: Method Development and Mechanistic Study. *Chem. Eur. J.* **2020**, *26*, 12249-12255.

(7) (a) Pupo, G.; Vicini, A. C.; Ascough, D. M. H.; Ibba, F.; Christensen, K. E.; Thompson, A. L.; Brown, J. M.; Paton, R. S.; Gouverneur, V. Hydrogen Bonding Phase-Transfer Catalysis with Potassium Fluoride: Enantioselective Synthesis of β-Fluoroamines. *J. Am. Chem. Soc.* **2019**, *141*, 2878-2883. (b) Pupo, G.; Ibba, F.; Ascough, D. M. H.; Vicini, A. C.; Ricci, P.; Christensen, K. E.; Pfeifer, Morphy, J. R.; Brown, J. M.; Paton, R. S.; Gouverneur, V. Asymmetric nucleophilic fluorination under hydrogen bonding phase-transfer catalysis. *Science*, **2018**, *360*, 638-642.

(8) Cahard, D.; Audouard, C.; Plaquevent, J.-C.; Roques, N. Design, Synthesis, and Evaluation of a Novel Class of Enantioselective Electrophilic Fluorinating Agents: *N*-Fluoro Ammonium Salts of cinchona Alkaloids (F-CA-BF4). *Org. Lett.* **2000**, *2*, 3699–3701.

(9) e.g. (a) Ponra, S.; Yang, J.; Kerdphon, S.; Andersson, P. G. Asymmetric Synthesis of Alkyl Fluorides: Hydrogenation of Fluorinated Olefins. *Angew. Chem. Int. Ed.* **2019**, *58*, 9282-9287. (b) Lee, C.; Kim, M.; Han, S.; Kim, D.; Hoong, S. Nickel-Catalyzed Hydrofluorination in Unactivated Alkenes: Regio- and Enantioselective C–F Bond Formation. *J. Am. Chem. Soc.* **2024**, *146*, 9375–9384.

(10) Hu, W. L.; Hu, X. G.; Hunter, L. Recent Developments in the Deoxyfluorination of Alcohols and Phenols: New Reagents, Mechanistic Insights, and Applications. *Synthesis*, **2017**, 4917-4930.

(11) Fischer, M.; Kahlig, H.; Schmid, W. Gram scale synthesis of 3-fluoro-1-hydroxyacetone phosphate: a novel donor substrate in rabbit muscle aldolase-catalyzed aldol reactions. *Chem. Commun.* **2011**, *47*, 6647-6649.

(12) Peng, Y.; Wang, Z.; Chen, Y.; Xu, W.; Hu, Y.; Chen, Z.; Xu, J.; Wu, Q. Photoinduced Promiscuity of Cyclohexanone Monooxygenase for the Enantioselective Synthesis of α-Fluoroketones. *Angew. Chem. Int. Ed.* **2022**, *61*, e202211199.

(13) Zhao, Q.; Chen, Z.; Soler, J.; Chen, X.; Rui, J.; Tianlin Ji, N.; Yu, Q.E.; Yang, Q.; Garcia-Borràs, M.; Huang, X. Engineering non-haem iron enzymes for enantioselective C(sp^3)–F bond formation via radical fluorine transfer. *Nature Synthesis*, **2024**, https://doi.org/10.1038/s44160-024-00507-7.

(14) (a) Toogood, H. S.; Gardiner, J. M.; Scrutton, N. S. Biocatalytic Reductions and Chemical Versatility of the Old Yellow Enzyme Family of Flavoprotein Oxidoreductases. *ChemCatChem* **2010**, *2*, 892-914. (b) Toogood, H. S.; Scrutton, N. S. Discovery, Characterization, Engineering, and Applications of Ene-Reductases for Industrial Bio-catalysis. *ACS Catal.* **2018**, *8*, 3532-3549; (c) Roy, K. T.; Sreedharan, R.; Ghosh, P.; Gandhi, T.; Maiti, D. Ene-Reductase: A Multifaceted Biocatalyst in Organic Synthesis. *Chem. Eur. J.* **2022**, *28*, e202103949.

(15) Dobrijevic, D.; Benhamou, L.; Aliev, A. E.; Méndez-Sánchez, D.; Dawson, D.; Baud, D.; Tappertzhofen, N.; Moody, T. S.; Orengo, C. A.; Hailes, H. C.; Ward, J. M. Metagenomic ene-reductases for the bioreduction of sterically challenging enones. *RSC Adv.* **2019**, *9*, 36608-36614.

(16) (a) Winkler, C. K.; Clay, D.; Davies, S.; O'Neill, P.; McDaid, P.; De-barge, S.; Steflik, J.; Karmilowicz, M.; Wong, J. W.; Faber, K. Chemoenzymatic Asymmetric Synthesis of Pregabalin Precursors via Asymmetric Bioreduction of β-Cyanoacrylate Esters Using Ene-Reductases. *J. Org. Chem.*

2013, *78*, 1525-1533; (b) Mangan, D.; Miskelly, I.; Moody, T. S. A Three-Enzyme System Involving an Ene-Reductase for Generating Valuable Chiral Building Blocks. *Adv. Syn. Catal.* **2012**, *354*, 2185-2190; (c) Papadopoulou, A.; Peters, C.; Borchert, S.; Steiner, K.; Buller, R. Development of an Ene Reductase-Based Biocatalytic Process for the Production of Flavor Compounds. *Org. Process Res. Dev.* **2022**, *26*, 2102-2110.

(17) Clayman, P. D.; Hyster, T. K. Photoenzymatic Generation of Unstabilized Alkyl Radicals: An Asymmetric Reductive Cyclization. *J. Am. Chem. Soc.* **2020**, *142*, 15673-15677.

(18) Liu, Y.; Bender, S. G.; Sorigue, D.; Diaz, D. J.; Ellington, A. D.; Mann, G.; Allmendinger, S.; Hyster, T. K. Asymmetric Synthesis of α-Chloroamides via Photoenzymatic Hydroalkylation of Olefins. *J. Am. Chem. Soc.* **2024**, *146*, 7191–7197.

(19) Luo, F.; Wang, P.; Gong, Y. Highly enantioselective bioreduction of 2-fluorocinnamyl alchohols mediated by Saccharomyces cerevisiae. *Tetrahedron Lett.* **2010**, *51*, 1693-1695.

(20) (a) Brenna, E.; Gatti, F. G.; Manfredi, A.; Monti, D.; Parmeggiani, F. Biocatalyzed Enantioselective Reduction of Activated C=C Bonds: Synthesis of Enantiomerically Enriched α-Halo-β-arylpropionic Acids. *Eur. J. Org. Chem.* **2011**, 4015-4022; (b) Tasnádi, G.; Winkler, C. K.; Clay, D.; Sultana, N.; Fabian, W. M. F.; Hall, M.; Ditrich, K.; Faber, K. A Substrate-Driven Approach to Determine Reactivities of α,β-Unsaturated Carboxylic Esters Towards Asymmetric Bioreduction. *Chem. Eur. J.* **2012**, *18*, 10362-10367; (c) Parmeggiani, F.; Casamajo, A. R.; Colombo, D.; Ghezzi, M. C.; Galman, J. L.; Chica, R. A.; Brenna, E.; Turner, N. J. Biocatalytic retrosynthesis approaches to D-(2,4,5-trifluorophenyl)alanine, key precursor of the antidiabetic sitagliptin. *Green Chem.* **2019**, *21*, 4368-4379; (d) Parmeggiani, F.; Brenna, E.; Colombo, D.; Gatti, F. G.; Tentori, F.; Tessaro, D. "A Study in Yellow": Investigations in the Stereoselectivity of Ene-Reductases. *ChemBioChem* **2022**, *23*, e202100445; (e) Ferreira, I. M.; Jimenez, D. E. Q.; Lopes, S. Q.; Oliveira, A. de N. de.; Matos, M. J. S.; Porto, A. L. M. Ene-reductases: Contribution from Brazilian fungi in organic synthesis. *Results in Chem.* **2023**, *5*, 100965.

(21) (a) Coutrot, P.; Grison, C.; Lachgar, M.; Ghribi, A. A general and efficient synthesis of β-ketophosphonates. *Bull. Soc. Chim. Fr.* **1995**, *132*, 925-942; (b) Coutrot, P.; Grison, C. Synthese Generale de β-Cetophosphonates α-Fluorés Precurseurs d'Enones α-Fluorées. Application en Serie Pyrethrenique. *Tetrahedron Lett.* **1988**, *29*, 2655-2658.

(22) Shimizu, I.; Ishii, H. Synthesis of α-fluoroketones based on palladium-catalyzed Decarboxylation reactions of allyl β-keto carboxylates. *Tetrahedron*, **1994**, *50*, 487-495.

(23) Augustine, J. K.; Bombrun, A.; Venkatachaliah, S.; Jothib, A. Titanium mediated olefination of aldehydes with α-haloacetates: an exceptionally stereoselective and general approach to (Z)-α-haloacrylates. *Org. Biomol. Chem.* **2013**, *11*, 8065-8072.

(24) Zhu, F., Xu, P., Zhou, F., Wang, C., Zhou, J. Recycle Waste Salt as Reagent: A One-Pot Substitution/Krapcho Reaction Sequence to α-Fluorinated Esters and Sulfones. *Org. Lett.* **2015**, *17*, 962-975.

(25) The lack of reactivity of a single (*Z*)-fluoroenoate has been observed previously by Faber et al.. (See reference 20b).

(26) Reich, S.; Wolfgang Hoeffken, H.; Rosche, B.; Nestl, B. M.; Hauer, B. Crystal Structure Determination and Mutagenesis Analysis of the Ene Reductase NCR. *ChemBioChem* **2012**, *13*, 2400.

(27) (a) Eberhardt, J.; Santos-Martins, D.; Tillack, A. F.; Forli. S. Auto-Dock Vina 1.2.0: New Docking Methods, Expanded Force Field, and Python Bindings. *J. Chem. Inf. Model.* **2021**, *61*, 3891-3898; (b)

Trott, O.; Olson, A. J. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. *J. Comp. Chem.* **2010**, *31*, 455-461.