

**Manchester
Metropolitan
University**

Taday, Freya and Ryan, James and Argent, Stephen P and Caprio, Vittorio and Maciá, Beatriz and O'Reilly, Elaine (2020) Asymmetric Construction of Alkaloids Employing a Key -Transaminase Cascade. *Chemistry - A European Journal*. ISSN 0947-6539

Downloaded from: <http://e-space.mmu.ac.uk/625055/>

Version: Accepted Version

Publisher: Wiley

DOI: <https://doi.org/10.1002/chem.202000067>

Please cite the published version

<https://e-space.mmu.ac.uk>

CHEMISTRY

A European Journal

A Journal of



Accepted Article

Title: Asymmetric Construction of Alkaloids Employing a Key ω -Transaminase Cascade

Authors: Freya Taday, James Ryan, Stephen P. Argent, Vittorio Caprio, Beatriz Maciá, and Elaine O'Reilly

This manuscript has been accepted after peer review and appears as an Accepted Article online prior to editing, proofing, and formal publication of the final Version of Record (VoR). This work is currently citable by using the Digital Object Identifier (DOI) given below. The VoR will be published online in Early View as soon as possible and may be different to this Accepted Article as a result of editing. Readers should obtain the VoR from the journal website shown below when it is published to ensure accuracy of information. The authors are responsible for the content of this Accepted Article.

To be cited as: *Chem. Eur. J.* 10.1002/chem.202000067

Link to VoR: <http://dx.doi.org/10.1002/chem.202000067>

Supported by
ACES

WILEY-VCH

COMMUNICATION

Asymmetric Construction of Alkaloids Employing a Key ω -Transaminase Cascade

Freya Taday,^[a] James Ryan,^{*[a,c]} Stephen P. Argent,^[a] Vittorio Caprio,^[b] Beatriz Maciá,^[b] Elaine O'Reilly^{*[a,c]}

Abstract: An ω -transaminase triggered intramolecular aza-Michael reaction has been employed for the preparation of cyclic β -enaminones in good yield and excellent enantio- and diastereoselectivity, starting from easily accessible prochiral ketonones and commercially available enzymes. The powerful thermodynamic driving force associated with the spontaneous aza-Michael reaction effectively displaces the transaminase reaction equilibrium towards product formation, using only two equivalents of isopropylamine. To demonstrate the potential of this methodology, we have combined this biocatalytic aza-Michael step with annulation chemistry, affording unique stereo-defined fused alkaloid architectures.

β -Enaminones are vinylogous amides with unique reactivity and represent important intermediates in the synthesis of a variety of *N*-heterocyclic compounds.^[1] Specifically, six-membered cyclic variants have been exploited for the preparation of a diverse array of bioactive piperidine alkaloids (Figure 1), including pinidinone and related 2,6-piperidine derivatives,^[2] apomitomycin,^[3] mesembrenone,^[4] desoxoprosophylline,^[5] cassine,^[6] deoxyfebrifugine,^[7] sedacryptine,^[8] selenopsine^[9] and the *Coccinellidae* defensive alkaloids, including hippodamine.^[10] However, despite their importance, there have been limited strategies reported for the enantioselective construction of chiral β -enaminones. Traditional approaches for their synthesis rely on the sulfide contraction methodology originally reported by Eschenmoser,^[11] modified Knoevenagel-type reactions on (alkylthio)alkylideniminium salts,^[12] lactam-derived iminium chlorides^[13] or related compounds.^[14] Alternatively, the direct condensation of chiral lactim ethers with keto esters followed by decarboxylation with boric acid represents a useful approach.^[15] More recently, a gold catalyzed cyclisation of *N*-Boc-6-alkynyl-3,4-dihydro-2*H*-pyridines was shown to provide a facile route.^[16]

Enzymes represent valuable catalysts for the synthesis of chiral compounds that are challenging to access using more

traditional synthetic chemistry. The growing biocatalyst toolbox means that enzymes can now be considered alongside other homogeneous catalysts, and this has facilitated the concept of biocatalytic retrosynthesis.^[17] The prevalence of chiral amines in bioactive natural products and pharmaceutical drugs has led to considerable interest in the development and application of transaminase (TA) enzymes for their synthesis.^[18] TAs belong to fold types I and IV of pyridoxal 5-phosphate (PLP)-dependent enzymes and catalyse the reversible transfer of an amino group from a suitable donor to a carbonyl acceptor. While α -TAs exclusively convert α -amino and α -keto acids, ω -TAs can accommodate substrates with a distal carboxylate group. Perhaps the most synthetically interesting subgroup of ω -TAs are the amine TAs (ATAs), which are capable of accepting substrates lacking a carboxylate group. These enzymes are of considerable interest due to their potential for the synthesis of chiral primary amines from the corresponding prochiral ketones.^[18]

There are a number of examples of the application of ATAs in cascade reactions for the synthesis of nitrogen heterocycles, starting from readily available or easily accessible dicarbonyl compounds.^[19] We previously reported a novel biocatalytic disconnection for the regio- and stereoselective synthesis of a range of 2,6-disubstituted piperidines, relying on a key ω -TA triggered intramolecular aza-Michael reaction and subsequent epimerization.^[20] To complement the existing literature, we now report the expansion of the ω -TA-triggered intramolecular aza-Michael reaction to include ynones as the aza-Michael acceptors (Scheme 1), affording cyclic β -enaminones in excellent regio- and stereoselectivity. The products contain a number of functional handles for further derivatization and represent useful synthetic building blocks. Additionally, we demonstrate that condensation

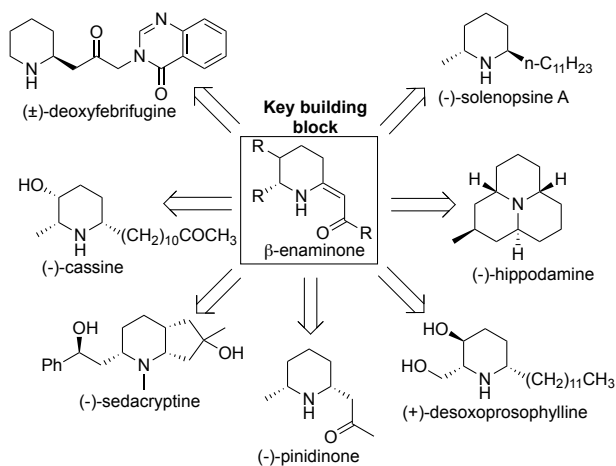


Figure 1. A selection of natural products that can be made from key β -enaminone building blocks.

[a] F. Taday, Dr J. Ryan, Dr E. O'Reilly
School of Chemistry, University of Nottingham, University Park,
Nottingham, NG7 2RD (UK)
E-mail: james.ryan2@ucd.ie

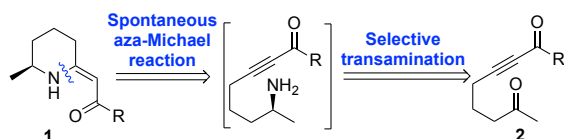
[b] Dr. B. Maciá, Dr V. Caprio
Faculty of Science & Engineering, Division of Chemistry &
Environmental Science, Manchester Metropolitan University,
Chester Street, Manchester M1 5GD (UK)

[c] Dr E. O'Reilly (current address)
School of Chemistry, University College Dublin, Belfield, Dublin 4,
(Ireland)
E-mail: elaine.oreilly@ucd.ie

Supporting information for this article is given *via* a link at the end of the document.

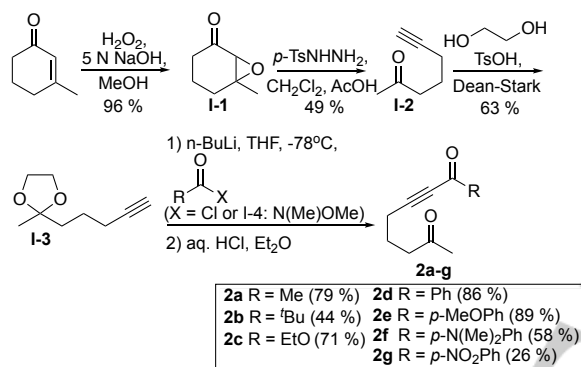
COMMUNICATION

of these enzyme-derived enaminones with various [3+3] acceptors enables the straightforward synthesis of hexahydroquinoline (HHQ) and tetrahydroquinoline (THQ) derivatives.



Scheme 1. A retrosynthetic approach for the synthesis of chiral cyclic β -enaminones starting from prochiral ketonyones.

Ketonyone substrates **2a-g** were prepared *via* epoxidation of commercially available 3-methyl-2-cyclohexanone, followed by Eschenmoser fragmentation (Scheme 2). Subsequent acetal protection and acylation of the alkyne with the appropriate reagent provided the substrates after acidic work-up (see ESI for details).



Scheme 2. Synthesis of **2a-g** starting from 3-methyl-2-cyclohexanone.

Initially, four commercially available ATAs were screened for activity against **2a-d**, by employing previously optimized conditions.^[20] To allow access to both enantiomers of **1**, two (*R*)-selective (ATA117 and ATA025) and two (*S*)-selective variants (ATA113 and ATA256) were screened. These enzymes have been developed by Codexis and represent some of the most widely utilized commercially available ATAs.^[21] This initial screen showed that ATA025, ATA256 and ATA113 showed the most promising activity (data not shown) and these enzymes were therefore selected for preparative-scale reactions (Table 1).

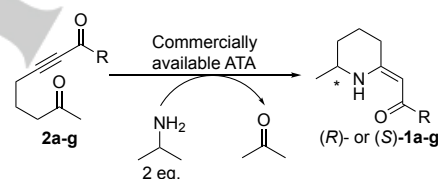
ATA025 achieved excellent conversions with all ketonyones (88-99%, entries 1, 3, 5, 7, 9 and 13), with the exception of the dimethylamine derivative **2f** (entry 11), where 46% conversion to the β -enaminone was observed. (*S*)-selective ATA256 provided the highest conversions (95-97%) for non-aromatic substrates **2a-c** and the electron deficient aromatic derivative **2g** at 40 mM substrate concentration (entries 2, 4, 6 and 14). The highest conversions with ketonyone substrates **2d**, **2e**, **2f** were achieved with (*S*)-ATA113 (53-92%, entries 8, 10 and 12). In all cases, the reactions proceeded in good to excellent enantiomeric excess (83->99%).

The challenging equilibrium typically associated with transaminase-mediated reactions means that in order to achieve high conversions to the desired amine product, it is often necessary to use extremely high equivalents of the amine donor

and/or remove the co-product *in-situ*.^[18,19,20] However, the strong thermodynamic driving force associated with the intramolecular aza-Michael reaction effectively displaces the reaction equilibrium towards product formation, necessitating the use of only two equivalents of isopropylamine. β -Enaminones **1a-g** were isolated in the *cis*-configuration, presumably due to the added stability of the intramolecular hydrogen bond between the amine and carbonyl formation, consistent with previous reports.^[2,6,16]

During the purification of (*S*)-**1d**, a minor product was isolated, characterized and determined to be the carbo-[3+3] annulation product **3**, arising from the reaction of the β -enaminone **1d** with unreacted ketonyone **2d** (Scheme 3). Although this compound was only isolated as a minor component (<10% yield), we postulated that the reaction could be biased towards the formation of the annulation product, if the enzymatic transformation could be slowed down sufficiently to allow the annulation reaction to occur. Adjusting the amine donor equivalents from 2 to 1 was enough to allow the condensation reaction to predominate, enabling the isolation of **3** in 78% yield (Scheme 3). Trace amounts of annulation product was also observed in a number of the biotransformations to produce **2a-g**, but these compounds were not isolated and characterised. It is likely that the formation of these side products could also be enhanced by lowering the equivalents of amine donor.

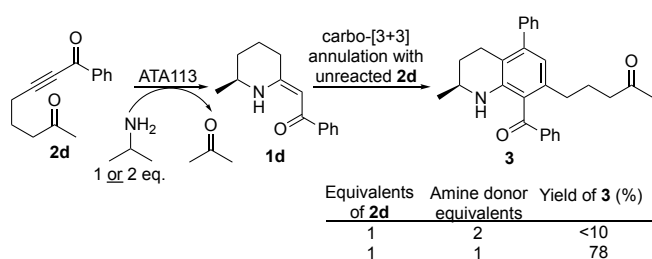
Table 1. Conversion of ketonyones **2a-g** to the corresponding cyclic β -enaminones using commercially available transaminases.



Entry	Substrate	R-substituent	ATA	Conv %	yield (%) ^d	<i>e.e.</i>
1	2a	Me	025	91 ^b	78	>99 ^e (<i>R</i>)
2	2a	Me	256	95 ^b	43	>99 ^e (<i>S</i>)
3	2b	^t Bu	025	>99 ^b	54	94 ^e (<i>R</i>)
4	2b	^t Bu	256	97 ^b	95	98 ^e (<i>S</i>)
5	2c	OEt	025	92 ^b	73	83 ^e (<i>R</i>)
6	2c	OEt	256	96 ^b	60	>99 ^e (<i>S</i>)
7	2d	Ph	025	88 ^b	72	97 ^e (<i>R</i>)
8	2d	Ph	113	91 ^b	85	>99 ^e (<i>S</i>)
9	2e	<i>p</i> -MeOPh	025	88 ^c	79	94 ^f (<i>R</i>)
10	2e	<i>p</i> -MeOPh	113	92 ^c	52	98 ^f (<i>S</i>)
11	2f	<i>p</i> -N(Me) ₂ Ph	025	46 ^c	43	>99 ^f (<i>R</i>)
12	2f	<i>p</i> -N(Me) ₂ Ph	113	53 ^c	29	98 ^f (<i>S</i>)
13	2g	<i>p</i> -NO ₂ Ph	025	98 ^c	58	>99 ^f (<i>R</i>)
14	2g	<i>p</i> -NO ₂ Ph	256	94 ^c	53	97 ^f (<i>S</i>)

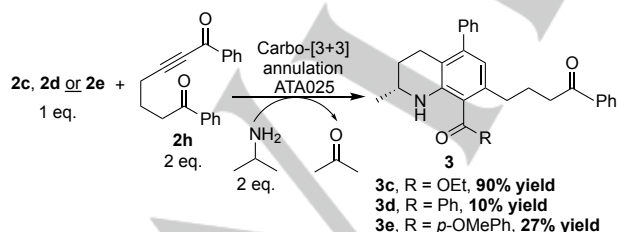
(a) Reaction conditions: ω -TA (5 mg/mL), substrate (40 mM), isopropyl amine (80 mM), DMSO (10%), pyridoxyl-5'-phosphate (PLP, 1 mM), HEPES buffer (5 mL, 100 mM, pH 7.5), 30 °C, 200 rpm, 24 h. (b) Conversion determined by GC-FID. (c) Conversion determined by ¹H NMR. (d) Isolated yield after flash chromatography. (e) *e.e.* determined by GC-FID using a chiral column. (f) *e.e.* determined by HPLC using a chiral column.

COMMUNICATION



Scheme 3. Carbo-[3+3] annulation product **3**, observed as a side product (<10%) during the reaction of **2d** with the commercial ATAs. Adjusting the equivalents of amine donor from 2 to 1 allowed the isolation of **3** in 78% yield.

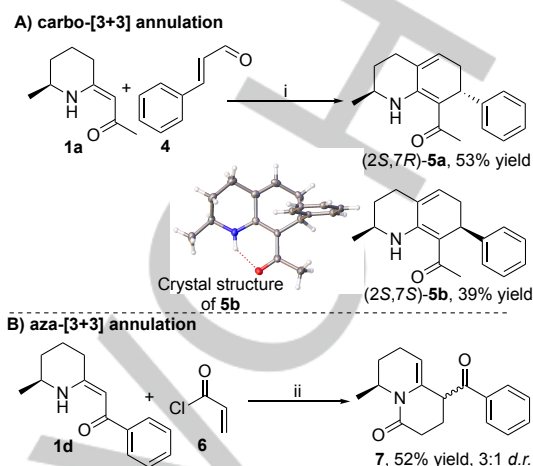
To demonstrate the potential scope of this novel, one-pot ATA-annulation cascade, we explored its feasibility for the synthesis of HHQ derivatives arising from the condensation of β -enaminones **1** with an alternative ketoynone. Partner **2h**, bearing a phenyl substituent on both carbonyls, was selected to investigate this crossed condensation (Scheme 4). Wild-type ATAs typically accept ketone substrates where at least one of the substituents is 'small' (e.g. methyl or ethyl), due to the nature of their active site pocket. While the properties of the commercial enzymes (ATA113, ATA256, ATA025) have been optimised, these catalysts have not been engineered to accept substrates with two bulky substituents. This means that diphenyl ynone **2h** will not readily undergo transamination in the presence of the commercial enzymes, which simplifies a one-pot ATA-annulation cascade, ensuring only a single annulation product is isolated. As **2h** is not a substrate for the enzyme, there was no requirement to limit the equivalents of amine donor and therefore 2 equivalents were employed. The one-pot transamination of **2c** followed by carbo-[3+3] annulation with **2h** proceeded extremely efficiently, affording the target product **3c** in 90% yield after 24 hours (Scheme 4). Selective transamination of **2d** in the presence of **2h** afforded **3d** in only 10% yield after 24 hours. This is somewhat surprising, given that **2d** is efficiently converted to **1d** in the absence of **2h** (Table 1, entry 7). The one-pot cascade with **2e** also proceeded quite poorly, allowing the isolation of **3e** in 27% yield. It is likely that these reactions can be improved with further optimisation, but this was not carried out in this study.



Scheme 4. ATA/Carbo-[3+3] annulation cascade of **2c**, **2d**, or **2e** with **2h** in the presence of 2 equivalents of isopropylamine, affording **3**.

Additionally, we explored two annulation reactions involving cyclic β -enaminones **1a** and **1d**.^[22] The methyl-methyl derivative **1a** reacted with cinnamaldehyde **4** in the presence of piperidinium acetate, to provide the separable THQ diastereomers **5a** (53%

yield) and **5b** (39% yield) (Scheme 5A), via a carbo-[3+3] annulation. Subsequently, **5b** was crystallised to determine the overall conformation, as 2D-NMR experiments were inconclusive.



Scheme 5. Annulation reactions of **1a** and **1d**. A) Carbo-[3+3] annulation: i) piperidinium acetate, sodium sulfate, EtOAc:Tol (2:3), 100 °C, 16 h. Crystal structure of **5b** (CCDC 1945711). B) Aza-[3+3] annulation: ii) acryloyl chloride, THF, Δ , 16 h.

The rather low crude *d.r.* (57:43) observed for **5** can be explained by the formation of a relatively flat hexadiene with a subsequent lack of axial/equatorial bias affecting stereofacial selectivity. The aza-[3+3] annulation of **1d** with acryloyl chloride **6** provided lactam **7** in a 52% yield and a 3:1 *d.r.* (Scheme 5B).

In conclusion, we have expanded the scope of the ω -TA triggered intramolecular aza-Michael reaction strategy to include ynone substrates, providing an expedient and general chemoenzymatic route to valuable chiral cyclic β -enaminone scaffolds in good yield and enantiomeric excess. This methodology relies on the exceptional levels of regio- and stereoselectivity associated with the biocatalytic transamination of prochiral ketoynone substrates. To highlight the utility of these functionalisable compounds (**1a-e**) *en route* to stereodefined scaffolds of potential synthetic and medicinal interest, a novel, one pot tandem TA/aza-Michael/condensation cascade was developed, allowing access to polyfunctionalised hexahydroquinolines **3**. Two variations of annulation reactions were also successfully conducted, providing hexahydroquinolines **5** and quinolizidines **7**.

Acknowledgements

Research leading to these results has received funding from the (EPSRC). The UK Catalysis Hub is kindly thanked for resources and support provided via our membership of the UK Catalysis Hub Consortium and funded by (EPSRC) (grants EP/K014706/2, EP/K014668/1, EP/K014854/1, EP/K014714/1 and EP/M013219/1). We acknowledge the School of Chemistry UoN for supporting FT.

Keywords: Biocatalytic cascade • Transaminase • Aza-Michael • Ketoynone • Annulation

COMMUNICATION

- [1] a) J. V. Greenhill, *Chem. Soc. Rev.* **1977**, *6*, 277-294; b) A.-Z. A. Elassar, A. A. El-Khair, *Tetrahedron* **2003**, *59*, 8463-8480; c) A. Z. A. Hassanien, S. A. S. Ghozlan, M. H. Elnagdi, *J. Heterocycl. Chem.* **2003**, *40*, 225-228; d) A. K. Chattopadhyay, S. Hanessian, *Chem Commun* **2015**, *51*, 16437-16449.
- [2] S. Fréville, P. Delbecq, V. M. Thuy, H. Petit, J. P. Célérier, G. Lhommet, *Tetrahedron Lett.* **2001**, *42*, 4609-4611.
- [3] T. Kametani, Y. Kigawa, H. Nemoto, M. Ihara, K. Fukumoto, *J. Chem. Soc., Perkin Trans. 1* **1980**, 1607-1613.
- [4] A. S. Howard, R. B. Katz, J. P. Michael, *Tetrahedron Lett.* **1983**, *24*, 829-830.
- [5] C. Herdeis, J. Telsner, *Eur. J. Org. Chem.* **1999**, *1999*, 1407-1414.
- [6] C. Herdeis, P. Kupper, S. Ple, *Org. Biomol. Chem.* **2006**, *4*, 524-529.
- [7] J. Michael, C. de Koning, D. Pienaar, *Synlett* **2006**, *2006*, 383-386.
- [8] A. G. Wee, G. J. Fan, *Org. Lett.* **2008**, *10*, 3869-3872.
- [9] D. Bacos, J. P. Célérier, E. Marx, S. Rosset, G. Lhommet, *J. Heterocycl. Chem.* **1990**, *27*, 1387-1392.
- [10] A. I. Gerasyuto, R. P. Hsung, *Org. Lett.* **2006**, *8*, 21, 4899-4902.
- [11] a) M. Roth, P. Dubs, E. Götschi, A. Eschenmoser, *Helv. Chim. Acta* **1971**, *54*, 710-734; b) B. A. D. b) Neto, A. A. M. Lapis, A. B. Bernd, D. Russowsky, *Tetrahedron* **2009**, *65*, 2484-2496.
- [12] M. M. Gugelchuk, D. J. Hart, Y. M. Tsai, *J. Org. Chem.* **1981**, *46*, 3671-3675.
- [13] J. P. Célérier, E. Deloisy, G. Lhommet, P. Maitte, *J. Org. Chem.* **1979**, *44*, 3089.
- [14] a) J. P. Célérier, E. Deloisy, P. Kapron, G. Lhommet, P. Maitte, *Synthesis* **1981**, *2*, 130-133. b) J. P. Célérier, M. G. Richaud, G. Lhommet, *Synthesis* **1983**, *3*, 195-197.
- [15] a) P. Delbecq, J.-P. Celerier, G. Lhommet, *Tetrahedron Lett.* **1990**, *31*, 4873-4874; b) P. Delbecq, D. Bacos, J. P. Celerier, G. Lhommet, *Can. J. Chem.* **1991**, *69*, 1201-1206.
- [16] A. Oppedisano, C. Prandi, P. Venturello, A. Deagostino, G. Goti, D. Scarpì, E. G. Occhiato, *J. Org. Chem.* **2013**, *78*, 11007-11016.
- [17] a) N. J. Turner, E. O'Reilly, *Nat. Chem. Biol.* **2013**, *9*, 285; b) A. Wells, H.-P. Meyer, *ChemCatChem* **2014**, *6*, 918-920; c) A. P. Green, N. J. Turner, *Perspectives in Science* **2016**, *9*, 42-48; d) M. Honig, P. Sondermann, N. J. Turner, E. M. Carreira, *Angew. Chem. Int. Ed.* **2017**, *56*, 8942-8973; e) R. de Souza, L. S. M. Miranda, U. T. Bornscheuer, *Chem. Eur. J.* **2017**, *23*, 12040-12063.
- [18] a) M. Fuchs, J. E. Farnberger, W. Kroutil, *Eur. J. Org. Chem.* **2015**, *2015*, 6965-6982; b) F. Guo, P. Berglund, *Green Chem.* **2017**, *19*, 333-360; c) I. Slabu, J. L. Galman, R. C. Lloyd, N. J. Turner, *ACS Catal.* **2017**, *7*, 8263-8284; d) A. Gomm, E. O'Reilly, *Curr. Opin. Chem. Biol.* **2018**, *43*, 106-112; e) S. A. Kelly, S. Pohle, S. Wharry, S. Mix, C. C. R. Allen, T. S. Moody, B. F. Gilmore, *Chem. Rev.* **2018**, *118*, 349-367; f) M. D. Patil, G. Grogan, A. Bommaris, H. Yun, *Catalysts* **2018**, *8*, 254; g) C. K. Savile, J. M. Janey, E. C. Mundorff, J. C. Moore, S. Tam, W. R. Jarvis, J. C. Colbeck, A. Krebber, F. J. Fleitz, J. Brands, P. N. Devine, G. W. Huisman, G. J. Hughes, *Science* **2010**, *329*, 305.
- [19] a) R. C. Simon, B. Grischek, F. Zepeck, A. Steinreiber, F. Belaj, W. Kroutil, *Angew. Chem. Int. Ed.* **2012**, *51*, 6713-6716; b) E. O'Reilly, C. Iglesias, D. Ghislieri, J. Hopwood, J. L. Galman, R. C. Lloyd, N. J. Turner, *Angew. Chem. Int. Ed.* **2014**, *53*, 2447-2450; c) S. P. France, S. Hussain, A. M. Hill, L. J. Hepworth, R. M. Howard, K. R. Mulholland, S. L. Flitsch, N. J. Turner, *ACS Catal.* **2016**, *6*, 3753-3759; d) S. E. Payer, J. H. Schrittwieser, B. Grischek, R. C. Simon, W. Kroutil, *Adv. Synth. Catal.* **2016**, *358*, 444-451; e) B. Z. Costa, J. L. Galman, I. Slabu, S. P. France, A. J. Marsaioli, N. J. Turner, *ChemCatChem* **2018**, *10*, 4733-4738.
- [20] J. Ryan, M. Siaucilius, A. Gomm, B. Macia, E. O'Reilly, V. Caprio, *J. Am. Chem. Soc.* **2016**, *138*, 15798-15800.
- [21] The sequence data for these commercially available enzymes is not freely available. However, the sequence for ATA256 can be found in the following patent: M. Crowe, M. Foulkes, G. Francese, D. Grimler, E. Kuesters, K. Laumen, Y. Li, C. Lin, J. Nator, T. Ruch, D. Smith, S. Song, S. Teng, Chemical Processes for Preparing Spiroindolones and Intermediates Thereof. US Patent US 2015/0045562 A1, filed 22 March 2013 and issued 12 February 2015.
- [22] S. K. Ghosh, G. S. Buchanan, Q. A. Long, Y. Wei, Z. F. Al-Rashid, H. M. Sklenicka, R. P. Hsung, *Tetrahedron* **2008**, *64*, 883-893.

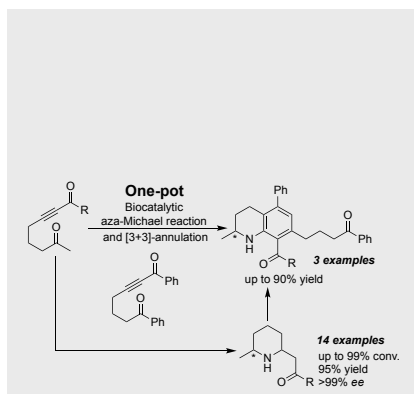
COMMUNICATION

Entry for the Table of Contents (Please choose one layout)

Layout 1:

COMMUNICATION

A chemoenzymatic cascade has been developed for the synthesis of hexahydroquinoline and tetrahydroquinoline derivatives, relying on a key transaminase-mediated aza-Michael reaction followed by [3+3] annulation.



Freya Taday, James Ryan,* Stephen P. Argent, Vittorio Caprio, Beatriz Maciá, Elaine O'Reilly*

Page No. – Page No.

Asymmetric Construction of Alkaloids Employing a Key ω -Transaminase Cascade

Layout 2:

COMMUNICATION

*Author(s), Corresponding Author(s)**

Page No. – Page No.

Title

Text for Table of Contents