



Cite this: *Green Chem.*, 2015, **17**, 852

Received 25th November 2014,
Accepted 12th December 2014

DOI: 10.1039/c4gc02325k

www.rsc.org/greenchem

One-pot triangular chemoenzymatic cascades for the syntheses of chiral alkaloids from dopamine†

B. R. Lichman,^a E. D. Lamming,^b T. Pesnot,^b J. M. Smith,^a H. C. Hailes*^b and J. M. Ward*^a

We describe novel chemoenzymatic routes to (*S*)-benzylisoquinoline and (*S*)-tetrahydroprotoberberine alkaloids using the enzymes transaminase (TAM) and norcoclaurine synthase (NCS) in a one-pot, one-substrate 'triangular' cascade. Employment of up to two C–C bond forming steps allows for the rapid generation of molecular complexity under mild conditions.

Introduction

In order to minimise waste products and reduce the usage of finite resources, chemistry must find 'green' alternatives to traditional synthetic methods.¹ The employment of enzymes as catalysts offers significant potential in this regard as enzymes often demonstrate exquisite chemo- and enantio-selectivity, and operate in mild conditions.² Furthermore, using enzymes in one-pot cascades can enable the formation of highly complex compounds from cheap starting materials, frequently without the requirement of intermediate isolation or functional group protection strategies.³

Benzylisoquinoline alkaloids (BIAs) are a large, diverse family of natural products found in both plants⁴ and animals.⁵ Many BIAs have pharmacological activities—these include analgesic,⁶ antimicrobial⁷ and antitumor⁸ effects—and consequently are common synthetic targets.⁹ A key step in the synthesis of many of these compounds is the formation of the tetrahydroisoquinoline (THIQ) moiety *via* a Pictet–Spengler condensation.¹⁰ Recently, a mild one-pot biomimetic approach to the formation of THIQs was developed, using phosphate buffer as a catalyst.¹¹ There has also been recent progress made in the enzymatic synthesis of diverse chiral THIQs using the plant enzyme norcoclaurine synthase (NCS).¹²

Norlaudanosoline (tetrahydropapaveroline) **1a** is a BIA found in mammals: it is the precursor to 'endogenous' morphine,⁵ and also has a number of neuronal effects including roles in Parkinson's disease¹³ and drug addiction.¹⁴ Synthetically, **1a** is a versatile BIA precursor, and consequently it has been employed in synthetic biology/metabolic engineering routes to various BIAs.¹⁵ BIA **1a** can be accessed by a Pictet–Spengler condensation between dopamine **2a** and 3,4-dihydroxyphenylacetaldehyde **3a**.¹⁶

Tetrahydroprotoberberine alkaloids (THPBs, berbines) are a subgroup of the BIAs found in both plants and animals, and include compounds such as canadine¹⁷ and spinosine.^{18a} The THPB moiety can be accessed from **1a** *via* a Pictet–Spengler reaction with formaldehyde.¹⁸ The two compounds directly formed by this reaction (**4** and **5**) have been shown to have potential in cancer chemotherapeutics.¹⁹ The regioselectivity of this chemical Pictet–Spengler reaction (major product: 10,11-dihydroxy **4**) contrasts with the regioselectivity of the plant berberine-bridge enzyme (BBE), which typically forms 9,10-dihydroxy-THPBs from a *N*-methylated BIA precursor.²⁰ Thus chemical approaches to THPBs provide a complementary method to the reported BBE approach.

Triangular cascade design

In this work, we present one-pot two-enzyme one-substrate syntheses of (*S*)-**1a** and (*S*)-**1b** using the enzymes transaminase (TAM) and NCS. The reaction involves *in situ* generation and utilisation of reactive aldehyde species. We also demonstrate a one-pot, two-enzyme, three-step chemoenzymatic synthesis of the THPB (*S*)-**4**. This synthesis uses the same cascade route as for (*S*)-**1a**, but involves the sequential addition of formaldehyde to trigger a second Pictet–Spengler cyclisation (Scheme 1).

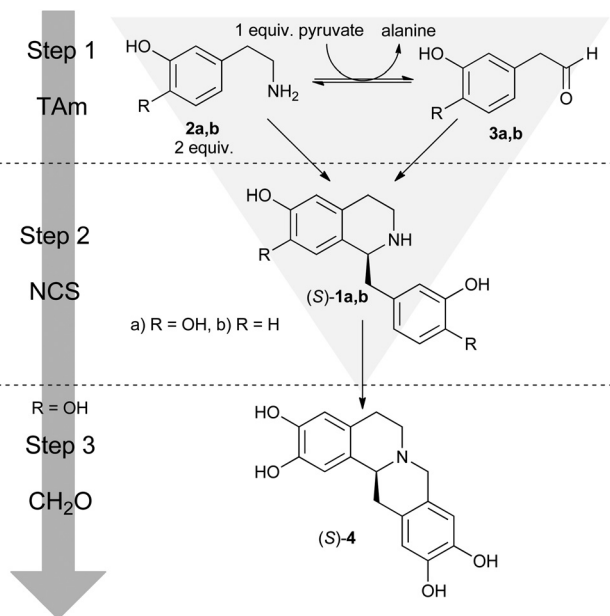
Previous attempts to achieve this type of cascade *in vitro* and *in vivo* have utilised a monoamine oxidase (MAO) as the catalyst in the first step, converting **2a** to **3a**. Several MAO approaches have focused on forming *rac*-**1a** through a spontaneous Pictet–Spengler reaction (typically phosphate catalysis).¹⁶ A number of *in vivo* metabolic cascades forming

^aDepartment of Biochemical Engineering, University College London, Bernard Katz Building, Gordon Street, London, WC1H 0AH, UK. E-mail: j.ward@ucl.ac.uk

^bDepartment of Chemistry, University College London, Christopher-Ingold Building, 20 Gordon Street, London, WC1H 0AJ, UK. E-mail: h.c.hailes@ucl.ac.uk

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c4gc02325k





Scheme 1 Overview of the biocatalytic and non-enzymatic cascades presented in this work, including the 'triangular' cascade.

(*S*)-reticuline report the use of recombinant NCS as the Pictet–Spengler catalyst. However, it is not clear that NCS is active in these systems: any chirality reported seems to be the result of (*S*)-selective enzymes downstream of NCS.^{15a–d}

Here, we employ a TAM for the conversion of **2a** to **3a** (step 1), which then combine to form **1a** (step 2). TAMs have an advantage over MAOs in this system: the conversion of **2a** to **3a** with TAMs can be controlled by stoichiometrically limiting the quantity of co-substrate (in this instance, pyruvate) available. Furthermore, we avoid the problems associated with driving TAM reactions to completion, as the Pictet–Spengler reaction

removes equal quantities of molecules from both sides of the TAM equilibrium. This three-sided 'triangular' cascade design results in a system with high atom economy.³

Results and discussion

The first step towards establishing the one-pot cascade system was the identification of a TAM with good activity towards **2a**. The screening reaction was conducted in phosphate buffer, which enabled the *in situ* formation of *rac*-**1a** from **2a** and **3a** (Table 1). The screen identified two TAMs with activity towards **2a** at pH 7.5: CV2025 (*Chromobacterium violaceum*)²¹ and PP_3718 (*Pseudomonas putida*), providing 21% and 15% conversion of **1a** from 50 mM **2a** respectively. Trace activities were found in PP_0596 (*P. putida*), SaV_2612 (*Streptomyces avermitilis*) and VF_JS17 (*Vibrio fluvialis*)²² (Table 1, ESI† Fig. S1). We selected the best performing TAM, CV2025, for use in further studies.

In order to form (*S*)-**1a** from **2a** two aspects of the previous TAM screening reaction conditions were modified. Changing the buffer from phosphate to HEPES removed most of the background chemical reaction¹¹ and adding purified Δ29TfNCS enabled catalytic formation of the chiral product (*S*)-**1a** from **2a**. Optimum reaction conditions were determined by varying the concentrations of **2a**, NCS and TAM present. A balance between reaction components was crucial to ensure the rates of the two steps were matched; a build-up of **3a** would cause an increase in undesirable side-reactions including the non-enzymatic Pictet–Spengler condensation, which would reduce the final enantiomeric excess (ee) of the product.

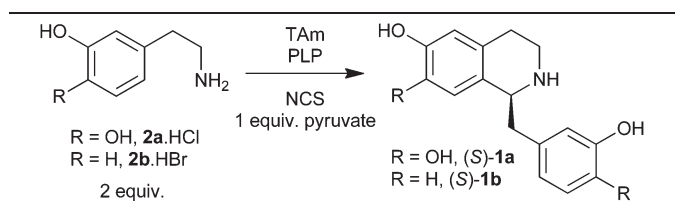
BIA (*S*)-**1a** was produced from **2a** with very good conversions and excellent enantioselectivity. In all cases, an increase in TAM concentration resulted in a greater consumption of **2a**,

Table 1 TAM mediated synthesis of *rac*-**1a** from **2a**^a

Entry	TAM	Organism	Uniprot entry name	Gene name	Conv. ^b (%)
1	Empty vector				n.d. ^c
2	BSU_09260	<i>Bacillus subtilis</i>	YHXA_BACSU	yhxA	n.d.
3	CV_2025 ²¹	<i>Chromobacterium violaceum</i>	Q7NWX4_CHRVO	CV_2025	21
4	Dgeo_1416	<i>Deinococcus geothermalis</i>	Q11YH3_DEIGD	argD/lysJ	n.d.
5	KPN_00255	<i>Klebsiella pneumoniae</i>	A6T537_KLEP7	gabT	n.d.
6	PP_0596	<i>Pseudomonas putida</i>	Q88Q98_PSEPK	PP_0596	Trace
7	PP_3718	<i>Pseudomonas putida</i>	Q88GK3_PSEPK	PP_3718	14
8	SaV_2612	<i>Streptomyces avermitilis</i>	Q82JZ2_STRAW	SAV_2612	Trace
9	SaV_4551	<i>Streptomyces avermitilis</i>	Q82ER2_STRAW	SaV_4551	n.d.
10	VF_JS17 ²²	<i>Vibrio fluvialis</i>	F2XBU9_VIBFL	JS17	Trace

^a 50 mM **2a**, 25 mM pyruvate, 1 mM PLP, 10% v.v⁻¹ TAM, 37 °C, 4 h. ^b Concentration of *rac*-**1a**, determined by analytical HPLC. ^c n.d. = not detected. See ESI Fig. S1 for reaction time course.



Table 2 One pot synthesis of (S)-BIAs^a

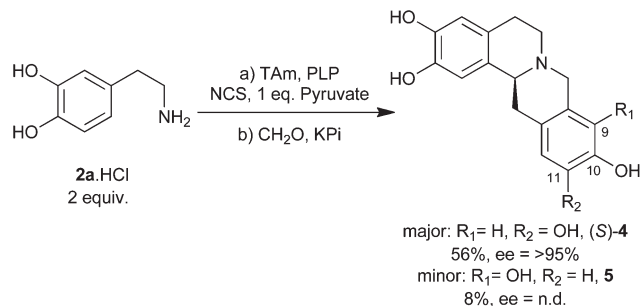
Amine [mM]	CV2025 (v.v ⁻¹) [%]	NCS [$\mu\text{g mL}^{-1}$]	Conv. ^b [%]	ee ^c [%]	
2a	20	100	74 (74)	99	
2a	20	20	86 (89)	99	
2a	20	30	82 (93)	99	
2a	20	10	500	77 (82)	99
2a	20	20	500	87 (92)	99
2a	20	30	500	84 (95)	98
2a	50	10	100	36 (42)	98
2a	50	20	100	55 (66)	97
2a	50	30	100	70 (78)	99
2a	50	10	500	42 (48)	98
2a	50	20	500	65 (73)	98
2a	50	30	500	72 (85)	96
2b	20	20	500	56 (57)	90

^a General reaction conditions: 2 equivalents **2a** or **2b**, 1 equivalent pyruvate, purified $\Delta 29\text{TfNCS}$ and CV2025 lysate, 50 mM HEPES pH 7.5, 37 °C, 3 h. ^b Determined by analytical HPLC. Conversions in brackets refer to depletion of primary amine. ^c Determined by chiral HPLC of the crude product.

generally leading to an increase in product formation (Table 2). However, at lower concentrations of **2a** (20 mM) the use of 20% v.v⁻¹ CV2025 lysate, rather than 30%, was optimal. The enantioselectivity was excellent under all conditions. In terms of conversion, the effect of NCS concentration was more apparent with higher concentrations of dopamine. The best conditions identified were those with 20 mM **2a**, 500 $\mu\text{g mL}^{-1}$ NCS and 20% v.v⁻¹ CV2025 lysate, which provided an 87% conversion of (S)-**1a** from **2a** with an ee of 99%.

To demonstrate the versatility of this system, it was used to synthesise (S)-**1b** from 2-(3-hydroxyphenyl)ethylamine **2b**. The *para*-hydroxyl group of **2a** is not required for the Pictet–Spengler condensation, and thus **2b** is a suitable substrate for NCS.^{12b} Using the optimum conditions determined previously for **2a**, (S)-**1b** was formed from **2b** with a fair conversion of 56% and a high ee of 90%. The lower conversion and ee compared to (S)-**1a** is possibly reflective of a poorer affinity of TfNCS towards for **2b** compared to the natural substrate **2a**.

In order to form THPBs (S)-**4** and (S)-**5**, a one-pot synthesis of (S)-**1a** was conducted (as described above) and formaldehyde was added to the reaction after 3 hours. This triggered the second Pictet–Spengler condensation and resulted in the formation of (S)-**4** and (S)-**5** in a ratio of approximately 7 : 1 (Scheme 2). This cascade features 2 enzymes, 3 steps and the formation of 4 bonds, including 2 C–C bonds. The overall reaction occurred with good conversion: the second Pictet–Spengler step alone (from (S)-**1a**) provided 74% conversion (64% and 9% for (S)-**4** and (S)-**5** respectively), which translates as an overall 64% conversion (56% and 8%) from **2a**. As the chirality



Scheme 2 One-pot chemoenzymatic synthesis of (S)-**4** and (S)-**5**. Reaction conditions: (a) 20 mM **2**, 10 mM sodium pyruvate, 500 $\mu\text{g mL}^{-1}$ NCS and 20% v.v⁻¹ CV2025 lysate, 50 mM HEPES pH 7.5, 37 °C, 3 h. (b) 40 mM formaldehyde, 1 M sodium phosphate, pH 6, 30 min, 37 °C.

of the system is established by NCS, the subsequent addition of formaldehyde does not affect the ee: only the (S)-enantiomer of the major product **4** was observed by chiral HPLC.

The major regioisomer **4** formed by this method has 10,11-dihydroxy regiochemistry. BBE, a plant enzyme which catalyses the formation of (S)-THPBs *en route* to berberine, typically forms products with 9,10-dihydroxy regioselectivity.²⁰ This enzyme has recently been used in a novel *in vitro* deracemisation cascade to produce (S)-THPBs.^{20b} The synthesis presented here and the BBE catalysed cascade can be seen as complementary methods, providing efficient chemoenzymatic routes to 10,11-dihydroxy-(S)-THPBs and 9,10-dihydroxy-(S)-THPBs respectively.

Finally, to demonstrate the preparative potential of our cascades, we performed syntheses of (S)-**1a** and (S)-**4** on a 0.5 mmol scale. The concentrations of components in these cascades were the same as those used in the micro-scale cascades (Scheme 2). The synthesis scaled with no complications: conversion of **2a** to (S)-**1a** was achieved in 2 hours with 86% conversion and 62% isolated yield (>95% ee). (S)-**4** was formed from **2a** in 2.5 hours with 47% conversion and 42% isolated yield (>95% ee). The identities of these products were verified by NMR.

Conclusions

In summary, we have developed one-pot syntheses of chiral alkaloids, using the enzymes TAM and NCS in a ‘triangular’ cascade. BIA (S)-**1a** was formed from **2a** with very good conversion and excellent enantioselectivity in only 2 hours. (S)-**1b** was formed in a similar manner with fair conversion and a high ee. A chemical extension to the cascade enabled the formation of (S)-**4** in a one-pot three-step reaction from the low-cost starting material **2a**, again with excellent enantioselectivity and an overall reaction time of less than 3 hours.

The cascades presented here exhibit high atom economy, the *in situ* generation of reactive intermediates, and the rapid accumulation of molecular complexity through C–C bond forming steps. Overall, these syntheses demonstrate the



remarkable potential of *in vitro* biocatalysis for the formation of complex chiral compounds.

We gratefully acknowledge the Wellcome Trust for student-ship funding to B. R. L., EPSRC and GlaxoSmithKline for student-ship funding to E. D. L., and the Biotechnology and Biological Sciences Research Council (BBSRC) (BB/G014426/1) for funding T. P.

Notes and references

- (a) J. H. Clark, *Green Chem.*, 1999, **1**, 1–8; (b) R. A. Sheldon, *Pure Appl. Chem.*, 2000, **72**, 1233–1246.
- B. M. Nestl, S. C. Hammer, B. A. Nebel and B. Hauer, *Angew. Chem., Int. Ed.*, 2014, **53**, 3070–3095.
- For reviews see: (a) E. Ricca, B. Brucher and J. H. Schrittwieser, *Adv. Synth. Catal.*, 2011, **353**, 2239–2262; (b) R. C. Simon, N. Richter, E. Busto and W. Kroutil, *ACS Catal.*, 2014, **4**, 129–143.
- J. M. Hagel and P. J. Facchini, *Plant Cell Physiol.*, 2013, **54**, 647–672.
- (a) C. Poeaknapo, J. Schmidt, M. Brandsch, B. Dräger and M. H. Zenk, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 14091–14096; (b) C. Boettcher, M. Fellermeier, C. Boettcher, B. Dräger and M. H. Zenk, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 8495–8500; (c) N. Grobe, M. Lamshöft, R. G. Orth, B. Dräger, T. M. Kutchan, M. H. Zenk and M. Spiteller, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 8147–8152.
- A. J. Goodman, B. Le Bourdonnec and R. E. Dolle, *Chem-MedChem*, 2007, **2**, 1552–1570.
- K. Iwasa, M. Moriyasu, Y. Tachibana, H. Kim, Y. Wataya, W. Wiegrebe, K. F. Bastow, L. M. Cosentino, M. Kozuka and K.-H. Lee, *Bioorg. Med. Chem.*, 2001, **9**, 2871–2884.
- P. Jabbarzadeh Kaboli, A. Rahmat, P. Ismail and K.-H. Ling, *Eur. J. Pharmacol.*, 2014, **740**, 584–595.
- M. Chrzanowska and M. D. Rozwadowska, *Chem. Rev.*, 2004, **104**, 3341–3370.
- J. Stöckigt, A. P. Antonchick, F. Wu and H. Waldmann, *Angew. Chem., Int. Ed.*, 2011, **50**, 8538–8564.
- T. Pesnot, M. C. Gershtater, J. M. Ward and H. C. Hailes, *Chem. Commun.*, 2011, **47**, 3242–3244.
- (a) A. Bonamore, I. Rovardi, F. Gasparrini, P. Baiocco, M. Barba, C. Molinaro, B. Botta, A. Boffi and A. Macone, *Green Chem.*, 2010, **12**, 1623–1627; (b) T. Pesnot, M. C. Gershtater, J. M. Ward and H. C. Hailes, *Adv. Synth. Catal.*, 2012, **354**, 2997–3008; (c) B. M. Ruff, S. Bräse and S. E. O'Connor, *Tetrahedron Lett.*, 2012, **53**, 1071–1074; (d) M. Nishihachijo, Y. Hirai, S. Kawano, A. Nishiyama, H. Minami, T. Katayama, Y. Yasohara, F. Sato and H. Kumagai, *Biosci. Biotechnol. Biochem.*, 2014, **78**, 701–707; (e) J. J. Maresh, S. O. Crowe, A. a. Ralko, M. D. Aparece, C. M. Murphy, M. Krzeszowiec and M. W. Mullooney, *Tetrahedron Lett.*, 2014, **55**, 5047–5051.
- M. A. Collins, *Neurotoxicology*, 2004, **25**, 117–120.
- L. Yao, P. Fan, M. Arolfo, Z. Jiang, M. F. Olive, J. Zablocki, H.-L. Sun, N. Chu, J. Lee, H.-Y. Kim, K. Leung, J. Shryock, B. Blackburn and I. Diamond, *Nat. Med.*, 2010, **16**, 1024–1028.
- For examples which synthesise norlaudanoline *in situ*: (a) H. Minami, J.-S. Kim, N. Ikezawa, T. Takemura, T. Katayama, H. Kumagai and F. Sato, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 7393–7398; (b) A. Nakagawa, H. Minami, J.-S. Kim, T. Koyanagi, T. Katayama, F. Sato and H. Kumagai, *Nat. Commun.*, 2011, **2**, 326, DOI: 10.1038/ncomms1327; (c) A. Nakagawa, H. Minami, J.-S. Kim, T. Koyanagi, T. Katayama, F. Sato and H. Kumagai, *Bioeng. Bugs*, 2012, **3**, 49–53; (d) J.-S. Kim, A. Nakagawa, Y. Yamazaki, E. Matsumura, T. Koyanagi, H. Minami, T. Katayama, F. Sato and H. Kumagai, *Biosci. Biotechnol. Biochem.*, 2013, **77**, 2166–2168; examples which use racemic norlaudanoline as a feedstock: (e) K. M. Hawkins and C. D. Smolke, *Nat. Chem. Biol.*, 2008, **4**, 564–573; (f) E. Fossati, A. Ekins, L. Narcross, Y. Zhu, J.-P. Falguyret, G. A. W. Beaudoin, P. J. Facchini and V. J. J. Martin, *Nat. Commun.*, 2014, **5**, 3283, DOI: 10.1038/ncomms4283.
- (a) P. Holtz, K. Stock and E. Westermann, *Nature*, 1964, **203**, 656–658; (b) L. K. Hoover, M. Moo-Young and R. L. Legge, *Biotechnol. Bioeng.*, 1991, **38**, 1029–1033; (c) A. Nakagawa, C. Matsuzaki, E. Matsumura, T. Koyanagi, T. Katayama, K. Yamamoto, F. Sato, H. Kumagai and H. Minami, *Sci. Rep.*, 2014, **4**, 6695, DOI: 10.1038/srep06695.
- E. F. Queiroz, F. Roblot, A. Cavé, M. e Q. Paulo and A. Fournet, *J. Nat. Prod.*, 1996, **7**, 438–440.
- (a) K. Iwasa, W. Cui, T. Takahashi, Y. Nishiyama, M. Kamigauchi, J. Koyama, A. Takeuchi, M. Moriyasu and K. Takeda, *J. Nat. Prod.*, 2010, **73**, 115–122; (b) K. D. McMurtrey, L. R. Meyerson, J. L. Cashaw and V. E. Davis, *J. Org. Chem.*, 1984, **49**, 947–948.
- W. Cui, K. Iwasa, H. Tokuda, A. Kashihara, Y. Mitani, T. Hasegawa, Y. Nishiyama, M. Moriyasu, H. Nishino, M. Hanaoka, C. Mukai and K. Takeda, *Phytochemistry*, 2006, **67**, 70–79.
- (a) J. H. Schrittwieser, V. Resch, S. Wallner, W.-D. Lienhart, J. H. Sattler, J. Resch, P. Macheroux and W. Kroutil, *J. Org. Chem.*, 2011, **76**, 6703–6714; (b) J. H. Schrittwieser, B. Groenendaal, V. Resch, D. Ghislieri, S. Wallner, E.-M. Fischereeder, E. Fuchs, B. Grischek, J. H. Sattler, P. Macheroux, N. J. Turner and W. Kroutil, *Angew. Chem., Int. Ed.*, 2014, **53**, 3731–3734.
- U. Kaulmann, K. Smithies, M. E. B. Smith, H. C. Hailes and J. M. Ward, *Enzyme Microb. Technol.*, 2007, **41**, 628–637.
- J.-S. Shin, H. Yun, J.-W. Jang, I. Park and B.-G. Kim, *Appl. Microbiol. Biotechnol.*, 2003, **61**, 463–471.

