Accessing tetrahydrofuran-based natural products by microbial Baeyer–Villiger biooxidation†

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A heterobicyclic lactone obtained by stereoselective Baeyer–Villiger biooxidation with recombinant whole-cells expressing cyclopentanone monooxygenase from *Comamonas* sp. NCIMB 9872 was used for formal total syntheses of various natural products containing a tetrahydrofuran structural motif.

Stereoselective Baeyer–Villiger oxidations have received increasing attention in recent years.[1,2] In particular, enzyme-mediated oxygenation processes have successfully been introduced as a versatile strategy for the formation of chiral lactones.[3] Progress in genome sequencing and mining led to the discovery of an increasing number of novel flavin-containing Baeyer–Villiger monooxygenases (BVMOs).[4] Together with recent advances in molecular biology to modify BVMO biocatalysts by random and knowledge based approaches,[5] these developments led to the generation of a tool-box of oxygenation biocatalysts capable of solving several of the challenges associated with chiral Baeyer–Villiger oxidations.

In order to circumvent elaborate cofactor recycling strategies required for NADPH-dependent BVMOs together with troublesome isolation of enzymes of limited stability, we have successfully applied recombinant whole-cell biotransformations to satisfy the expectations and needs of synthetically oriented chemists. Usually, we utilize *Escherichia coli*-based overexpression systems for BVMOs of various microbial origin as benign and easily-cultivated organisms.[6,7]

Recently, we have identified sub-clustering of several BVMOs based on homology in protein sequence.[8] The two groups of biocatalysts are capable of oxidizing cyclic ketones to enantiocomplementary lactones in a significant number of substrates. In addition, cyclopentanone monooxygenase (CPMO; CE 1.14.13.16) from *Comamonas* sp. NCIMB 9872 (EC)[9] and a related protein from a *Brevibacterium* sp.[4f] display a certain tolerance for structurally demanding ketone precursors.[10] Within this contribution we present the first utilization of a chiral lactone obtained by recombinant whole-cell mediated biooxidation with CPMO in natural compound synthesis. The biooxidation product served as a platform for facile entry to several tetrahydrofuran-based natural products starting from achiral precursors.

Applying a recently outlined sonochemical protocol for a [4 + 3] cycloaddition[11] of furan **1** and tetrabromoacetone **2** followed by reductive dehalogenation, we were able to optimize the synthesis of oxabicycloketone **3** in a single chemical operation (Scheme 1). This prochiral compound is an excellent substrate for CPMO and the corresponding lactone (+)-**4** was obtained in good optical purity (95% ee/chiral phase GC, $[\alpha]_D^{20}$ = +85.2, *c* 0.2, $CHCl_3$) in a desymmetrization step[12] introducing two new stereogenic centers in a single biotransformation. Optimization of the fermentation conditions was performed taking advantage of the SFPR (substrate feeding and product removal) methodology recently introduced into Baeyer–Villiger biooxidations.[13] This strategy allows an increase in compound concentrations beyond levels for enzyme inhibition or toxicity to the living whole-cells by using a resin as a solid phase reservoir for both substrate and product. Compound (+)-**4** was obtained in 70% isolated yield from biotransformations using a precursor concentration of 5 g L^{-1} fermentation broth.

Having established facile access to intermediate (+)-**4** on the gram scale, we embarked on a study to utilize the functional diversity and structural rigidity of the compound within subsequent chemical transformations. Based on previous work by Noyori on a similar structural core,[14] we expected a high degree of diastereoselectivity for the dihydroxylation of (+)-**4** *via* osmylation. Applying standard Upjohn conditions using NMO as the oxidant to recycle OsO_4, we observed rapid conversion of the precursor to a new compound. However, the corresponding diol was very difficult to isolate in satisfactory yield due to its excellent water solubility. Consequently, we tried to circumvent aqueous reaction conditions for the dihydroxylation and rather preferred to perform the subsequent protection to acetonide (+)-**5** in a single operation. Best results were obtained using $AlCl_3$ in the protection step and compound **5** ($[\alpha]_D^{20}$ = +73.0, *c* 0.66, $CHCl_3$; lit.:[15] $[\alpha]_D^{22}$ = +82.6, *c* 0.6, $CHCl_3$) was obtained in acceptable yield (47%) as a pivotal precursor[14] for the total synthesis of (+)-showdomycin displaying biological activities as an antibiotic and cytostatic.[16]

With the completion of the formal total synthesis of (+)-showdomycin we were also able to establish the absolute configuration of biooxidation product (+)-**4** as (1*S*,6*S*).

Applying another chemical oxygenation strategy, epoxidation of lactone (+)-**4** gave epoxide (+)-**6**§ also with excellent diastereoselectivity, as already expected from the above series. Methanolysis of the lactone was anticipated to proceed chemoselectively based on previous reports on related structures,[17] and afforded ester (−)-**7** in a smooth transformation. Subsequent Lewis acid-mediated intramolecular attack[18] of the epoxide ring gave rise to fused bicyclolactone (−)-**8** ($[\alpha]_D^{22}$ = −30.1, *c* 0.86, MeOH; lit.:[19] $[\alpha]_D^{24}$ = −29.2, *c* 0.25, MeOH) in excellent yield. This

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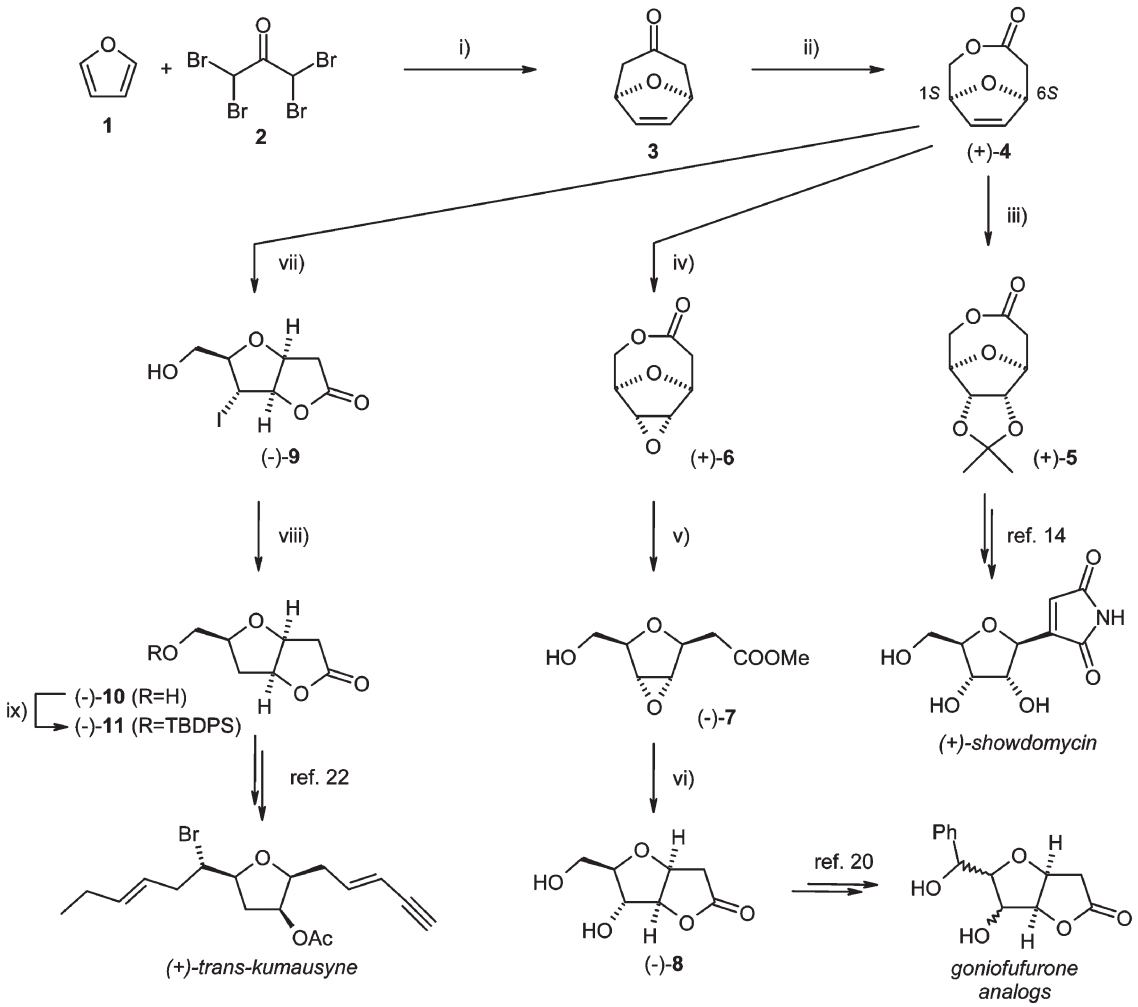

Scheme 1 (i) Cu/Zn-couple, ultrasound, MeCN, 10 °C, then Cu/Zn-couple, NH_4Cl, EtOH–MeCN, −78 °C to rt, 72%; (ii) *E. coli* expressing CPMO, isopropyl-β-D-thiogalactopyranoside (IPTG), lab-bench fermenter, 25 °C, 70%, 95% ee; (iii) OsO_4–NMO, CH_2Cl_2, rt, then acetone–$AlCl_3$, 0 °C to 40 °C, 47%; (iv) mCPBA, CH_2Cl_2, reflux, 98%; (v) MeOH–H_2O, K_2CO_3, rt, 98%; (vi) $SnCl_4$, CH_2Cl_2, −78 °C, 98%; (vii) MeCN–H_2O, KOH, rt, then I_2–KI, 40 °C, dark, 75%; (viii) Bu_3SnH, PhMe, 60 °C, 99%; (ix) TBDPSCl, imidazole, DMF, rt, 92%.

compound has been described as a potential precursor for structural analogs of the cytotoxic metabolite goniofufurone in the recent literature.[20]

Finally, we hydrolyzed lactone (+)-**4** to the corresponding hydroxy-acid, which was directly submitted to a halolactonization reaction in a single operation. Using KI–I_2 we obtained iodolactone (−)-**9** with excellent diastereoselectivity. Dehalogenation under radical conditions enabled access to product (−)-**10** ($[\alpha]_D^{25}$ = −53.3, *c* 0.42, MeOH), which is a pivotal precursor for the synthesis of *trans*-kumausyne.[21] However, based on the absolute configuration of biooxygenation product (+)-**4**, optical rotation of this compound as an intermediate for (+)-*trans*-kumausyne disagreed with literature reports.[22] Hence, we transformed (−)-**10** into the TBDPS-protected advanced precursor (−)-**11** ($[\alpha]_D^{26}$ = −23.8, *c* 0.75, $CHCl_3$). Opposed to one publication,[22] we did not observe a change of sign for specific rotation upon this conversion. Our findings are in agreement with reports for precursors of the (−)-*trans*-kumausyne series, where positive rotations are reported for both enantiomeric compounds **10** and **11**.[23] Correspondingly, our synthetic sequence allows rapid access to the non-natural configuration of this natural product.

Summarizing, we have developed routes to several pivotal precursors for natural products containing a tetrahydrofuran structural core utilizing biooxidative desymmetrization. The combined chemo-enzymatic oxidation strategies displayed excellent enantio- and diastereoselective control for the synthetic elaboration of key intermediate (+)-**4** involving diverse subsequent transformations. Our approach allows the generation of up to four chiral centers from achiral and commercially available precursors in as few as three chemical operations. Hence, we consider the outlined synthetic routes to crucial precursors for the above natural products as efficient shortcuts to previous sequences taking advantage of a sustainable biooxygenation technique.

Currently, we are investigating the further chemical elaboration of lactone (+)-**4**, in particular to extend the methodology to related carbo- and heterobicyclic compounds.

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Notes and references

§ *Physical and spectral properties of selected compounds*. **6**: colorless crystals, mp: 78–80 °C; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.92 (dd, 2H, J = 3.8 and 16.5 Hz), 3.05 (dd, 1H, J = 2.5 and 16.5 Hz), 3.76 (s, 2H), 4.19 (dd, 1H, J = 2.7 and 3.6 Hz), 4.20–4.50 (m, 2H); ^{13}C-NMR (50 MHz, $CDCl_3$) δ 41.9 (t), 52.6 (d), 54.2 (d), 69.3 (d), 71.3 (t), 74.2 (d), 171.5 (s). **7**: colorless oil; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.43 (br s, 1H), 2.57 (d, 2H, J = 7.5 Hz), 3.67 (s, 3H), 3.50–3.75 (m, 4H), 4.11 (t, 1H, J = 4.3 Hz), 4.43 (t, 1H, J = 7.5 Hz); ^{13}C-NMR (50 MHz, $CDCl_3$) δ 38.2 (t), 52.1 (d), 58.6 (d), 59.9 (d), 62.7 (t), 74.8 (q), 79.7 (d), 171.2 (s). **9**: beige oil, which slowly solidified, mp 84–86 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.79 (br s, 1H), 2.72 (d, 2H, J = 3.3 Hz), 3.62 (dd, 1H, J = 4.6 and 12.4 Hz), 3.80 (dd, 1H, J = 3.0 and 12.4 Hz), 4.15 (dd, 1H, J = 2.1 and 7.5 Hz), 4.27 (ddd, 1H, J = 3.0, 4.6 and 7.5 Hz), 4.75–4.82 (m, 1H), 5.15 (dd, 1H, J = 2.1 and 4.5 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 20.0 (d), 36.2 (t), 61.2 (t), 78.2 (d), 90.7 (d), 92.8 (d), 173.8 (s).

1 For general reviews on Baeyer–Villiger reactions see: (*a*) G. R. Krow, *Org. React.*, 1993, **43**, 251; (*b*) M. Renz and B. Meunier, *Eur. J. Org. Chem.*, 1999, 737.

2 For reviews on stereoselective Baeyer–Villiger oxidations see: (*a*) M. D. Mihovilovic, F. Rudroff and B. Grötzl, *Curr. Org. Chem.*, 2004, **8**, 1057; (*b*) C. Bolm, in *Peroxide Chemistry*, ed. W. Adam, Wiley-VCH: Weinheim, 2000, p. 494; (*c*) G. Strukul, *Angew. Chem., Int. Ed.*, 1998, **37**, 1198.

3 For recent reviews see: (*a*) N. M. Kamerbeek, D. B. Janssen, W. J. H. van Berkel and M. W. Fraaije, *Adv. Synth. Catal.*, 2003, **345**, 667; (*b*) M. D. Mihovilovic, B. Müller and P. Stanetty, *Eur. J. Org. Chem.*, 2002, 3711.

4 (*a*) D. Bonsor, S. F. Butz, J. Solomons, S. Grant, I. J. S. Fairlamb, M. J. Fogg and G. Grogan, *Org. Biomol. Chem.*, 2006, **4**, 1252; (*b*) M. W. Fraaije, N. M. Kamerbeek, A. J. Heidekamp, R. Fortin and D. B. Janssen, *J. Biol. Chem.*, 2004, **279**, 3354; (*c*) J. B. van Beilen, F. Mourlane, M. A. Seeger, J. Kovac, Z. Li, T. H. M. Smits, U. Fritsche and B. Witholt, *Environ. Microbiol.*, 2003, **5**, 174; (*d*) P. Brzostowicz, D. M. Walters, S. M. Thomas, V. Nagarajan and P. E. Rouviere, *Appl. Environ. Microbiol.*, 2003, **69**, 334; (*e*) M. W. Fraaije, N. M. Kamerbeek, W. J. H. van Berkel and D. B. Janssen, *FEBS Lett.*, 2002, **518**, 43; (*f*) P. C. Brzostowicz, K. L. Gibson, S. M. Thomas, M. S. Blasko and P. E. Rouviere, *J. Bacteriol.*, 2000, **182**, 4241.

5 (*a*) M. D. Mihovilovic, F. Rudroff, A. Winninger, T. Schneider, F. Schulz and M. T. Reetz, *Org. Lett.*, 2006, **8**, 1221; (*b*) M. Bocola, F. Schulz, F. Leca, A. Vogel, M. W. Fraaije and M. T. Reetz, *Adv. Synth. Catal.*, 2005, **347**, 979; (*c*) M. T. Reetz, B. Brunner, T. Schneider, F. Schulz, C. M. Clouthier and M. M. Kayser, *Angew. Chem., Int. Ed.*, 2004, **43**, 4078.

6 For the first *E. coli* based expression systems for BVMOs utilized in whole-cell biocatalysis see: (*a*) G. Chen, M. M. Kayser, M. D. Mihovilovic, M. E. Mrstik, C. A. Martinez and J. D. Stewart, *New J. Chem.*, 1999, **23**, 827; (*b*) S. D. Doig, L. M. O'Sullivan, S. Patel, J. M. Ward and J. M. Woodley, *Enzyme Microb. Technol.*, 2001, **28**, 265.

7 For a *Saccharomyces cerevisiae* expression system see: J. D. Stewart, K. W. Reed and M. M. Kayser, *J. Chem. Soc., Perkin Trans. 1*, 1996, 755.

8 M. D. Mihovilovic, F. Rudroff, B. Grötzl, P. Kapitan, R. Snajdrova, J. Rydz and R. Mach, *Angew. Chem., Int. Ed.*, 2005, **44**, 3609.

9 (*a*) H. Iwaki, Y. Hasegawa, P. C. K. Lau, S. Wang and M. M. Kayser, *Appl. Environ. Microbiol.*, 2002, **68**, 5681; (*b*) M. Griffin and P. W. Trudgill, *Eur. J. Biochem.*, 1976, **63**, 199.

10 M. D. Mihovilovic, R. Snajdrova, A. Winninger and F. Rudroff, *Synlett*, 2005, 2751.

11 (*a*) M. D. Mihovilovic, B. Grötzl, W. Kandiller, R. Snajdrova, A. Muskotal, D. A. Bianchi and P. Stanetty, *Adv. Synth. Catal.*, 2006, **348**, 463; (*b*) A. M. Montana and P. M. Grima, *Tetrahedron Lett.*, 2001, **42**, 7809.

12 For an excellent review on biocatalytic desymmetrizations see: E. Garcia-Urdiales, I. Alfonso and V. Gotor, *Chem. Rev.*, 2005, **105**, 313.

13 (*a*) F. Rudroff, V. Alphand, R. Furstoss and M. D. Mihovilovic, *Org. Process Res. Dev.*, 2006, **10**, 599; (*b*) M.-C. Gutièrrez, R. Furstoss and V. Alphand, *Adv. Synth. Catal.*, 2005, **347**, 1051; (*c*) I. Hilker, M. C. Gutierrez, V. Alphand, R. Wohlgemuth and R. Furstoss, *Org. Lett.*, 2004, **6**, 1955.

14 (*a*) T. Sato, Y. Hayakawa and R. Noyori, *Bull. Chem. Soc. Jpn.*, 1984, **57**, 2515; (*b*) R. Noyori, T. Sato and Y. Hayakawa, *J. Am. Chem. Soc.*, 1978, **100**, 2561; (*c*) T. Sato, R. Ito, Y. Hayakawa and R. Noyori, *Tetrahedron Lett.*, 1978, **21**, 1829.

15 K. A. Cruickshank and C. B. Reese, *Synthesis*, 1983, **3**, 199.

16 (*a*) K. R. Darnall, L. B. Townsend and R. K. Robins, *Proc. Natl. Acad. Sci. U. S. A.*, 1967, **57**, 548; (*b*) H. Nishimura, M. Mayama, Y. Komatsu, H. Sato, N. Shimaoka and Y. Tanaka, *J. Antibiot., Ser. A.*, 1964, **17**, 148.

17 E. A. Anderson, A. B. Holmes and I. Collins, *Tetrahedron Lett.*, 2000, **41**, 117.

18 S. Lochynski, B. Frackowiak, T. Olejniczak, Z. Ciunik and C. Warwrzenczyk, *Tetrahedron: Asymmetry*, 2002, **13**, 1761.

19 C. Cagnolini, M. Ferri, P. R. Jones and P. J. Murphy, *Tetrahedron*, 1997, **53**, 4815.

20 (*a*) P. J. Murphy and S. T. Dennison, *Tetrahedron*, 1993, **49**, 6695; (*b*) P. J. Murphy, *J. Chem. Soc., Chem. Commun.*, 1992, 1096.

21 (*a*) D. J. Faulkner, *Nat. Prod. Rep.*, 1984, **1**, 251; (*b*) T. Suzuki, K. Koizumi, H. Suzuki and E. Kurosawa, *Chem. Lett.*, 1983, 1643.

22 R. R. Gadikota, C. S. Callam and T. L. Lowary, *J. Org. Chem.*, 2001, **66**, 9046.

23 H. B. Mereyala and R. R. Gadikota, *Tetrahedron: Asymmetry*, 2000, **11**, 743.

