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Enantiocomplementary access to carba-analogs of C-nucleoside derivatives by recombinant Baeyer–Villiger monooxygenases

Dario A. Bianchi [b], Roberto Moran-Ramallal [a], Naseem Iqbal [a], Florian Rudroff [a], Marko D. Mihovilovic [a,*]

[a] Vienna University of Technology, Institute of Applied Synthetic Chemistry, Getreidemarkt 9/163-OC, A-1060 Vienna, Austria
[b] Instituto de Química Rosario (IQUIR, CONICET-UNR) and Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, S2002LRK Rosario, Argentina

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ABSTRACT

A novel and stereoselective synthetic route towards carba-C-nucleosides was investigated applying an enantiodivergent biooxidation strategy by two different Baeyer–Villiger monooxygenases. Within only three chemo-enzymatic steps it was possible to introduce four chiral centers starting from commercially available non-chiral starting material.

C-Nucleosides are an important class of compounds that display increased hydrolytic and enzymatic stability and have been identified as important probes in the pursuit of novel antiviral and antiproliferative drugs.[1] There are several known ribose-based C-nucleosides that are potent antiproliferatives like formycin,[2] tiazofurin[3,4] and showdomycin.[5] Carba-C-nucleosides are C-nucleoside analogs in which the furanose core is substituted by a cyclopentane ring. Syntheses of classical C-nucleosides are often based on derivatizations of natural pentoses or other sugars as starting materials. Consequently, such strategies are of limited use to access corresponding carba analogs. Hence, the development of stereoselective routes to this compound class has attracted the interest of medicinal chemists and pharmaceutical industry.

The enzyme mediated Baeyer–Villiger reaction represents a distinct approach in the synthesis of chiral esters and lactones from carbonyl precursors.[6] Generation of chirality by transformation of prochiral cyclic ketones provides a very atom efficient and industrially valuable synthetic approach towards the preparation of biological active compounds. Baeyer–Villiger monooxygeanses (BVMOs) have attained explicit attention in recent years due to their excellent chemo-, regio- and stereoselectivity and their attribute to substitute potentially dangerous peroxide reagents with cheap and environmentally friendly atmospheric oxygen.[7] Recent developments led to the generation of a tool-box of oxygenation biocatalysts capable of applying chiral Baeyer–Villiger oxidations for the synthesis of potential new pharmaceuticals.[8]

In this study we present the synthesis and utilization of both antipodal chiral lactones **2** obtained by enzyme mediated Baeyer–Villiger oxidation of readily accessible prochiral intermediate **1**. Both enantiomeric biooxidation products can serve as precursor for the synthesis of various unnatural carba-C-nucleosides like carba-showdomycin (Scheme 1).

Compound **1** was synthesized according to a previously described sonochemical protocol by a [4+3] cycloaddition of cyclopentadiene and tetrabromoacetone followed by reductive dehalogenation, in a single chemical operation.[9,10]

By applying our recently published decision guidance for quantitative and comparative evaluation of chiral catalysts[11] we were able to select the two most promising enzyme candidates from the vast BVMO family to produce chiral lactones **2**. Consequently, enzymes of choice turned out to be cyclopentanone monooxygenase ($CPMO_{Coma}$; EC 1.14.13.16)[12,13] from *Comamonas* sp. NCIMB 9872 and a more recently characterized cyclohexanone monooxygenase from *Xanthobacter* sp. ZL5 ($CHMO_{Xantho}$).[14] Both enzymes were overexpressed in *Escherichia coli* and, 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.[15,16]

We had recently discovered that ketone **1** can indeed undergo stereoselective oxygenation by BVMOs.[17] As expected from our decision guidance algorithm,[11] desymmetrization of prochiral

* Corresponding author. Tel.: +43 1 58801 15420; fax: +43 1 58801 15499.
E-mail address: mmihovil@pop.tuwien.ac.at (M.D. Mihovilovic).

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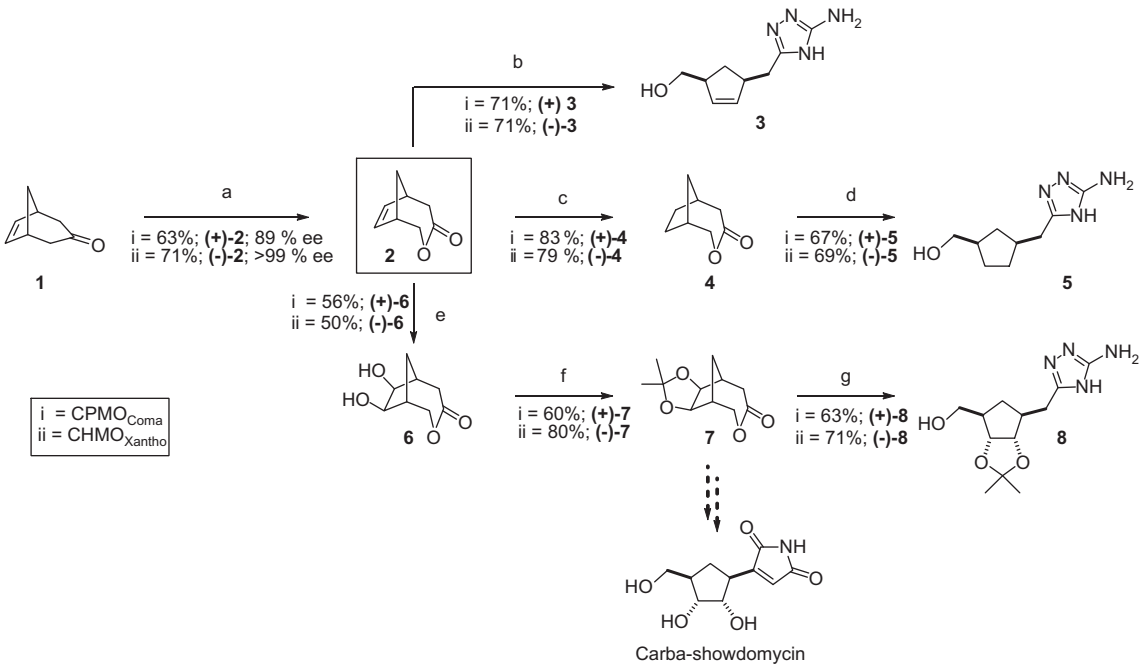

Scheme 1. Reagents and conditions: (a) *E. coli* expressing (i) $CPMO_{Coma}$ or (ii) $CHMO_{Xantho}$, shake flask, TB-medium, 24 °C; (b) aminoguanidine bicarbonate, pyridine, N_2-atmosphere, reflux, 1 h; (c) H_2, 1 bar, Pd/C (10% w/w), EtOAc, rt, overnight; (d) aminoguanidine bicarbonate, pyridine, N_2-atmosphere, reflux, 30 min; (e) cat. OsO_4, NMO, CH_2Cl_2/*tert.* BuOH, N_2-atmosphere, rt, 24 h; (f) 2,2-dimethoxypropane, cat. *p*-toluenesulfonic acid, dry acetone, N_2-atmosphere, rt, 26 h; (g) aminoguanidine bicarbonate, pyridine, N_2-atmosphere, reflux, 1 h.[21]

ketone **1** produced two new stereogenic centers in a single biotransformation step. The biooxygenation was catalyzed either by CPMO from *Comamonas* sp. or $CHMO_{Xantho}$ in enantiocomplementary manner, as also predicted from our previously reported enzyme clustering analysis based on sequence homology of enzymes:[18] $CPMO_{Coma}$ gave (+)-**2** in very good optical purity (89% ee/chiral phase GC, $[\alpha]_D^{20} = +87.6$ (*c* 0.41, $CHCl_3$)), whereas $CHMO_{Xantho}$ yielded in (−)-**2** in excellent stereoselectivity (99% ee/chiral phase GC, $[\alpha]_D^{30} = -83.7$ (*c* 1.0, $CHCl_3$)). Biotransformations were performed in classical shake flask experiments under non-growing conditions up to a concentration of 4 mM and a reaction time of 17–19 h. Thereby cells were grown in TB media at 30 °C until they reach an OD_{590} of 15. Subsequently the temperature was decreased to 24 °C, IPTG and after 1 h ketone **1**, dissolved in dioxane were added. After full conversion, extractive work up with dichloromethane and subsequent column chromatography compound **2** was isolated in good yields (63% of (+)-**2** and 71% of (−)-**2**).

Access to novel non-natural carba-C-nucleoside **3** via the biooxidative strategy was achieved by the reaction of enantiopure lactone **2** with aminoguanidine bicarbonate under reflux in pyridine.[19] Both stereoisomers were obtained in good yields ((+)-**3**, 71%, (−)-**3**, 71%). For the elaboration of saturated lactone **4** catalytic hydrogenation of enantiopure lactone **2** was performed in the presence of H_2 at 1 bar and rt. The desired saturated product **4** was obtained in very good yields ((+)-**4**, 83%, (−)-**4**, 79%). Hence, transformation of compound **4** into the carba-C-nucleoside **5** was performed according to the procedure described above. Aminoguanidine bicarbonate and saturated lactone **4** was refluxed in pyridine until full conversion was observed. Column chromatography of product **5** yielded in 63% of (+)-**5**, and 69%, of (−)-**5**, respectively. Access to the carba analog of showdomycin was achieved via a diastereoselective dihydroxylation of lactone **2**. As reported previously by our group,[10] high degree of diastereoselectivity was expected for the dihydroxylation of the oxa bicyclic analog employing osmylation. Standard chemical oxygenation strategy, using catalytic amounts of OsO_4 and NMO as oxidant to recycle the Os (VI) species, lead to a rapid conversion of the precursor **2** to the desired diol. The transformation proceeds with total diastereoselectivity, forming only one diol. In accordance to the oxa bicyclic series we expect the dihydroxylation at the exo face of the bicyclic lactone. The corresponding diol was obtained after isolation and purification via column chromatography in moderate yields (56% of (+)-**6**; 50% of (−)-**6**). Subsequent protection to ketal **7** was carried out in the presence of acetone and *p*-TsOH. After column purification **7** was obtained in good to very good yields (60% of (+)-**7**; 80% of (−)-**7**) as a pivotal precursor for the total synthesis of carba showdomycin.[20] The triazole-containing C-nucleoside precursor **8** was obtained after the reaction of amino guanidine bicarbonate in good yield ((+)-**8**, 63%, (−)-**8**, 71%) according to the procedure described above, exemplifying access to target compounds containing a non-natural nucleobase.

Summarizing, we have developed stereoselective routes to several novel carba-C-nucleosides that are potential bioactive products. The biooxidative desymmetrization strategy and subsequent chemical oxidation via OsO_4 displayed excellent enantio- and diastereoselective control for the synthetic elaboration of key intermediate **2** 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 chemo-enzymatic operations. Hence, we consider the outlined synthetic routes to crucial precursors for the above carba-C-nucleosides as a novel approach taking advantage of a sustainable biooxygenation technique. Major synthetic strategies involved diastereoselective dihydroxylation, hydrogenation, and heterocyclic ring-closure reactions. We anticipate that this general approach will provide a flexible general synthesis of novel carba-C-nucleosides and related biologically important compounds. Currently, we are investigating the absolute configuration of compound **7** and the further chemical elaboration of lactone **2**, in particular to extend the methodology to related cyclopentan-based compounds.

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21. Physical and spectral properties of selected compounds: (**2**) ^{1}H NMR: (200 MHz, $CDCl_3$): 1.75 (d, J = 16 Hz, 1H), 2.00–2.20 (m, 1H), 2.50–2.90 (m, 4H), 4.00–4.20 (m, 2H), 5.80–5.90 (m, 1H), 6.05–6.15 (m, 1H). ^{13}C NMR: (50 MHz, $CDCl_3$): 36.7 (d), 42.6 (d), 44.4 (t), 44.7 (t), 70.7 (t) 131.0 (d), 137.0 (d), 174.1 (s); Elemental Anal. Calcd for $C_8H_{10}O_2$: C, 69.55; H, 7.30. Found: C, 69.29; H, 7.25. (**3**) Colorless oil; ^{1}H NMR (200 MHz, CD_3OD): 1.19–2.16 (m, 2H), 2.35–2.56 (m, 1H), 2.59–3.06 (m, 1H), 2.65–2.98 (m, 3H), 3.19–3.22 (m, 1H), 3.25 (br s, OH), 4.82 (br s, N–H), 5.58 (dd, J = 7.8 Hz, J = 1.4 Hz, 1H), 5.65 (dd, J = 7.8 Hz, J = 1.4 Hz, 1H). ^{13}C NMR (50 MHz, CD_3OD): 34.0 (t), 35.3 (t), 45.8 (d), 49.1 (d), 67.5 (t), 133.6 (d), 136.2 (d). (**5**) ^{1}H NMR (200 MHz, methanol-d_4): 1.13–1.31 (m, 2H), 1.57–1.67 (m, 3H), 1.78–2.17 (m, 2H), 2.35–2.55 (m, 1H), 3.20–3.34 (m, 1H). ^{13}C NMR (50 MHz, CD_3OD): 29.1 (t), 32.5 (t), 34.4 (t), 37.5 (d), 40.5 (t), 43.2 (d), 67.6 (t). Elemental Anal. Calcd for $C_9H_{16}N_4O$: C, 55.08; H, 8.22; N, 28.55. Found: C, 54.70; H, 8.18; N, 27.96. (**7**) Colorless solid; mp: 115–119 °C. ^{1}H NMR (200 MHz, acetone-d_6): 1.26 (d, J = 0.5 Hz, 3H), 1.36 (d, J = 0.5 Hz, 3H), 1.59 (d, J = 11.3 Hz, 1H), 2.22–2.30 (m, 2H), 2.48 (m, 1H), 2.76 (m, 2H), 4.17–4.36 (m, 3H), 4.69 (dd, J = 5.3 Hz, J = 1.4 Hz, 1H). ^{13}C NMR (50 MHz, acetone-d_6): 24.6 (q), 27.2 (q), 37.8 (t), 40.4 (d), 40.9 (t), 45.2 (d), 72.0 (t), 83.4 (d), 85.5 (d), 110.8 (s), 175.1 (s); Elemental analysis: Anal. Calcd for $C_{11}H_{16}O_4$: C, 62.25; H, 7.60. Found: C, 62.40; H, 7.47. (**8**) Colorless oil; ^{1}H NMR (200 MHz, methanol-d_4): 1.28 (s, 3H), 1.2 (s, 3H), 2.05–2.17 (m, 3H), 2.31–2.57 (m, 2H), 3.29–3.37 (m, 2H), 4.19–4.41 (m, 1H). ^{13}C NMR (50 MHz, methanol-d_4): 25.4 (q), 27.3 (q), 34.9 (t), 38.5 (t), 43.5 (d), 52.1 (d), 64.5 (t), 84.2 (d), 86.6 (d), 113.8 (s), 174.0 (s).