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COMMUNICATION

Unique biocatalytic resolution of racemic tetrahydroberberrubine *via* kinetic glycosylation and enantio-selective sulfation†

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In this communication, we document a facile kinetic glycosylation resolution of racemic tetrahydroberberrubine. We also demonstrate that the enantiomeric excess of the resolved products is increased *via* a second resolution of the minor product of the first glycosylation resolution. This provides a rare example of tandem kinetic resolution of racemates.

Glycosylations of both macromolecules and small molecules are critical for cellular functions.[1] Glycoconjugates play structural roles in the cell, are involved in cell-to-cell recognition[2] and communication,[3] and intra- and extra-cellular signalling.[4] Glycosylated small molecules, such as heparin, amikacin, and cytarabine, have been shown to be clinically useful for the treatment of a variety of diseases, including bacterial and fungal infections, cancer, and other human diseases.[5] Consequently there has been tremendous interest in the identification, synthesis or manipulation of glycosylated small molecules for potential biomedical applications. It has been demonstrated that for most of the alkaloidal glycosides found in plants, in particular benzyltetrahydroisoquinoline alkaloids, removal of the carbohydrate units lead to aglycone units that are marginally active or less specific,[6] underscoring the importance of the glycosyl units for biological activity. Consequently, methods (both chemical and enzymatic) that make possible the facile introduction of glycosyl units onto alkaloids in a regio-specific manner are needed. Thus far, only a handful of strategies have been reported for the regio-specific glycosylation of natural products.[7] Herein we describe, to the best of our knowledge, the first example of kinetic resolution *via* glycosylation of racemic substrates, using a whole cell approach (fermentation). *Gliocladium deliquescens* NRRL 1086 has been shown to facilely glycosylate ruscogenin.[8] We therefore hypothesized that *Gliocladium deliquescens* NRRL 1086 would be a good organism to selectively glycosylate alkaloids, *vide infra*.

Using a whole cell approach, a near-perfect resolution of tetrahydroberberrubine (±)−**1** was achieved *via* a tandem kinetic resolution (see Scheme 1). A tandem kinetic resolution is expected to give almost enantiomerically pure compounds if the first kinetic resolution step produces enantio-enriched product and the second resolution is selective for the minor enantiomer. This way, the second resolution depletes the minor enantiomer (obtained during the first resolution) and thereby increases the enantiomeric excess of the major product from the first resolution step.

Treating tetrahydroberberrubine (±)−**1** with *Gliocladium deliquescens* NRRL 1086 gave glycosylated products **M1** and **M2** as colorless crystals in a 15 : 1 ratio, after silica gel chromatographic separation. The identities of **M1** and **M2** were confirmed *via* HR-ESI-MS, ^{13}C-NMR, ^{1}H-NMR, HMBC, HSQC and DEPT. These analyses revealed that **M1** and **M2** were both glycosylated products of **1**. X-ray crystallographic analysis of **M1** (Fig. 1) provided further evidence for the assigned structure of **M1** and **M2**. The absolute configurations of **M1** and **M2** were assigned as 14*S* and 14*R* respectively. These absolute configurations were assigned by comparing the CD of **M1** and **M2** with those of 14*S* and 14*R* protoberberines.[9] **M1** exhibited negative Cotton effects around 210 nm (similar to 14*S* protoberberine) while **M2** showed positive Cotton effects around 210 nm (similar to that seen with 14*R* protoberberine).

With our aim to increase the enantiomeric excess of the major isomer, **M1**, we proceeded to monitor the reaction profile of the kinetic resolution of tetrahydroberberrubine (±)−**1** with *Gliocladium deliquescens* NRRL 1086 at different times, using HPLC monitoring. Our aim was to identify a time point whereby the ratio of **M1** to **M2** would be maximized. Interestingly, HPLC analysis of the progress of the reaction indicated that the *S*-isomer, **M1**, formed faster than the *R*-isomer, **M2**. Interestingly, we also observed that within the first 12 h of the reaction, the concentration of **M2** initially increased but then decreased. Curiously, the relative amount of another product, denoted **M3**, increased as the reaction time increased (see Fig. 2).

Therefore, it appears that the enantiomeric excess of the major enantiomer, **M1**, could be increased to over 99% by leaving the reaction to go on for longer, contradicting the recommended

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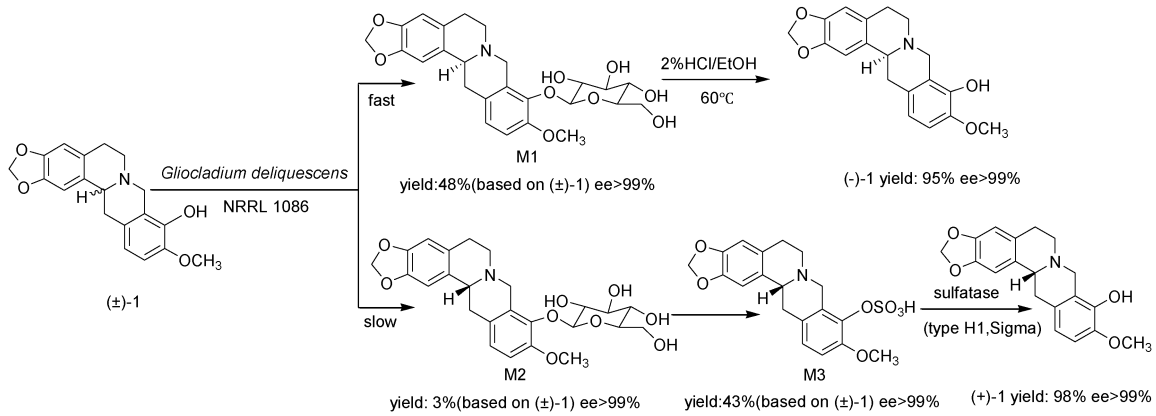


Scheme 1 Biotransformation and hydrolytic pathway of tetrahydroberberrubine (±)–**1**.

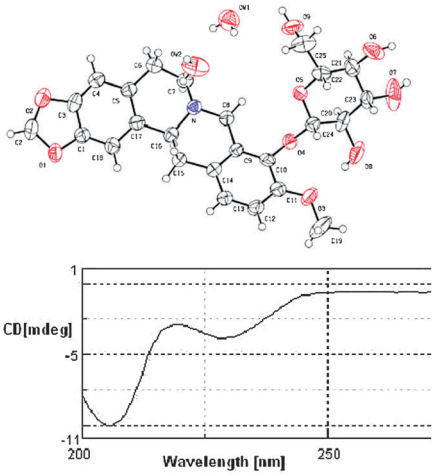


Fig. 1 X-ray crystal structure of **M1** and the CD spectra.

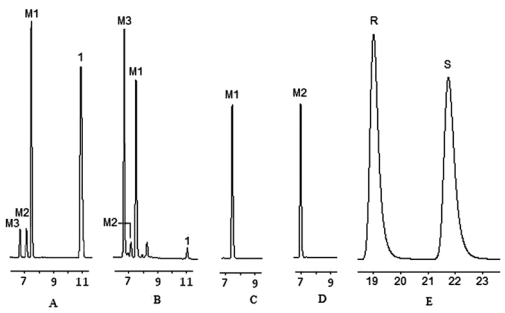


Fig. 2 HPLC chromatograms of the time-course analysis of microbial glycosylation of (±)–**1** to **M1** and **M2**. A. 12 h; B. 120 h; C. chromatogram of pure **M1**; D. chromatogram of pure **M2**; E. chiral chromatogram of the substrate **1**.

procedure for traditional kinetic resolution, which posits that it is not advisable to leave a kinetic resolution reaction going for longer periods because this would lead to the transformation of the other enantiomer into the product. Nonetheless, leaving our whole cell glycosylation resolution reaction on for about 36 h led to the isolation of the *S*-(–)-enantiomer, **M1** in 41% yield (close to the theoretical yield) and in greater than 99% ee.

We were intrigued by the transformation of **M2** into a third product, **M3**, so we proceeded to isolate **M3**, using Sephedex LH-20 chromatography and characterized this product. **M3** exhibited a quasi-molecular ion at $m : z$ 406.0974 ($[M + H]^+$) in its high-resolution ESI mass spectrum (HR-ESI-MS), indicating its molecular formula to be $C_{19}H_{20}NO_7S$ (calc. 406.0960). Interestingly, **M3** did not contain any sugar moiety but rather contained an SO_3 group. The ^{1}HNMR and ^{13}CNMR, HSQC, HMBC, DEPT spectra of **M3** were similar to those of **1** but the carbon signal of C-9 shifted upfield by 3.1 ppm to δ 140.9 ppm. We speculated that **M3** might be the sulphate of analogue of **1**. To confirm this **M3** was incubated with sulfatase,[10] which hydrolyzed **M3** into (+)–**1**. The NMR (both ^{1}H and ^{13}C) of (+)–**1**, which was obtained *via* the action of sulfatase on **M3** was identical to that of (–)–**1**, which was obtained *via* the hydrolysis of **M1** (see Scheme 1). Additionally, the sign of the CD spectra of (+)–**1** at 210 nm was opposite to that of (–)–**1** (see Fig. 3), providing compelling evidence that (–)–**1** and (+)–**1** are indeed enantiomers. The resolution method described in this communication therefore highlights a tandem kinetic resolution using an enzymatic method whereby the second kinetic resolution selects the minor product of the first kinetic resolution.

To provide more concrete evidence for this tandem kinetic resolution hypothesis, we subjected pure **M1** to *Gliocladium deliquescens* NRRL 1086 but recovered **M1** after 120 h, whereas subjecting pure **M2** to *Gliocladium deliquescens* NRRL 1086 afforded **M3** after 120 h. Importantly, it appears that the sulfation reaction is more selective than the glycosylation reaction and hence allows for a remarkable complete resolution of both (–)–**1** and (+)–**1** into 48% and 43% yield, respectively, and in greater than 99% ee for each enantiomer.

We next examined if the resolution protocol discussed above could be used to resolve other closely related benzylisoquinoline alkaloids (compounds **2–6**; position of the phenolic hydroxyl is different, see Fig. 4). We discovered that only compound **2**, which contained a free hydroxyl group on C-9, could be transformed into glycosylated products (ESI†) using *Gliocladium deliquescens* NRRL 1086.

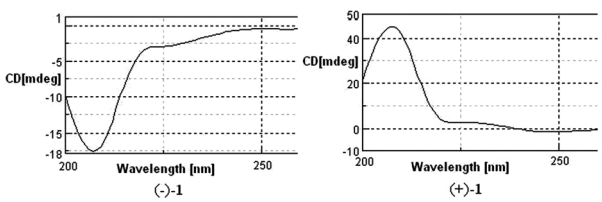
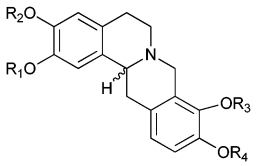


Fig. 3 CD spectra of (–)–**1** and (+)–**1**.



2 R_1,R_2,R_4=CH_3,R_3=H
3 R_1,R_2,R_3=CH_3,R_4=H
4 R_1,R_3,R_4=CH_3,R_2=H
5 R_2,R_3=CH_3,R_1,R_4=H
6 R_3,R_4=CH_3,R_1,R_2=H

Fig. 4 Structures of compounds **2–6**.

In the biosynthetic pathway of natural benzylisoquinoline alkaloids, it is assumed that the stereochemistry of the chiral center C-14 is formed *via* stereospecific enzymatic reactions.[11] In this communication, we demonstrate that alternatively the formation of the C-14 could be non-stereospecific but then subsequent stereospecific diversification, *via* differential reactions, of the 9-hydroxyl group,[12] could also account for stereospecific production of these metabolites.

In conclusion, we have documented an interesting case of enantio-selective glycosylation and sulphation of tetrahydroberberrubine, which not only led to the successful preparation of enantiomerically pure glycosidic benzylisoquinoline alkaloid but also provided a unique paradigm for resolution of racemic compounds. This work provides an interesting starting point to develop other tandem kinetic resolution strategies using enzymes or even organic catalysts. Ongoing work in our laboratory is further exploring the substrate scope of glycosylation systems (whole cell) and enzymes with the ultimate goal of expanding the types of substrates that could be amenable to stereoselective glycosylations and resolution.

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