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A Facile Chemoenzymatic Approach: One-Step Syntheses of Monoterpenoid Indole Alkaloids

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Abstract: Facile chemoenzymatic syntheses of cytotoxic monoterpenoid indole alkaloids with novel skeletons and multiple chiral centers are described. Synthesis of these alkaloids was achieved by a simple one-step reaction using strictosidine and 12-aza-strictosidine as the key intermediates. Strictosidines were prepared by coupling of secologanin with tryptamine and 7-aza-tryptamine, respectively, using the immobilized recombinant *Rauvolfia* strictosidine synthase. A detailed stereochemical analysis is presented herein. The results provide an opportunity for a chemoenzymatic approach that leads to an increased diversification of complex alkaloids with improved structures and activities.

Keywords: alkaloids · chemoenzymatic approach · cytotoxicity · indoles · stereochemistry

Introduction

Monoterpenoid indole alkaloids (MIAs) are a diverse class of natural products that comprise about 2000 structurally complex and important members, including the anticancer agents camptothecin and vinblastine.[1] The biological properties associated with these alkaloids have been the driving force for chemists who are looking to develop synthetic methods to generate novel alkaloids.[2] Aza-indoles, isosteric to the indole moiety, are important scaffolds for improving water solubility and feature unique hydrogen-bonding properties compared to the indole scaffold.[3] Several natural products and synthetic analogues that contain a highly active aza-indole moiety have been reported. Case in point: PharmaMar's Variolin B is currently undergoing preclinical studies as an antitumor drug.[4] Owing to their complex makeup that contains multiple rings and chiral centers, alkaloids remain one of the most challenging targets for new synthetic strategies and methodologies. Enzymes that are capable of performing reactions in a stereoselective manner are increasingly recognized as useful catalysts for organic synthesis, particularly for molecules that possess chiral centers.[5]

Strictosidine synthase (STR1) derived from *Rauvolfia* (Figure 1), originally derived from *Catharanthus roseus*, is a key enzyme for biosynthesis of alkaloids that conducts the Pictet–Spengler condensation.[6] As a critical initiator of probably all monoterpenoid indole alkaloid biosynthetic pathways, research on this enzyme has continued over several decades and great developments have been made.[7] Recently, O'Connor et al. worked on the *C. roseus* strictosidine synthase,[8] primarily focusing on substrate specificity and in vivo transformations;[8c] and Loris and co-workers focused on the structure-based redesign of *Rauvolfia* STR1[7c] for the synthesis of strictosidines.[7c,8] Despite the achievements relating to STR1, the possibility of utilizing STR1 for systematic chemoenzymatic synthesis of MIAs remains underexploited and limited owing to the low expression levels of soluble STR1. Previously, we have reported an improved STR1-His_6 expression system that utilizes the co-expression of plasmid pQE-2-STR1 with the molecular chaperone pG-Tf2 in *Escherichia coli* M15.[9] The immobilized STR1-His_6 exhibits excellent stability (for the *C. roseus* enzyme, see Ref. [7a]), thus making it a good candidate to enzymatically produce strictosidine (**1**; Scheme 1) and 12-aza-strictosidine

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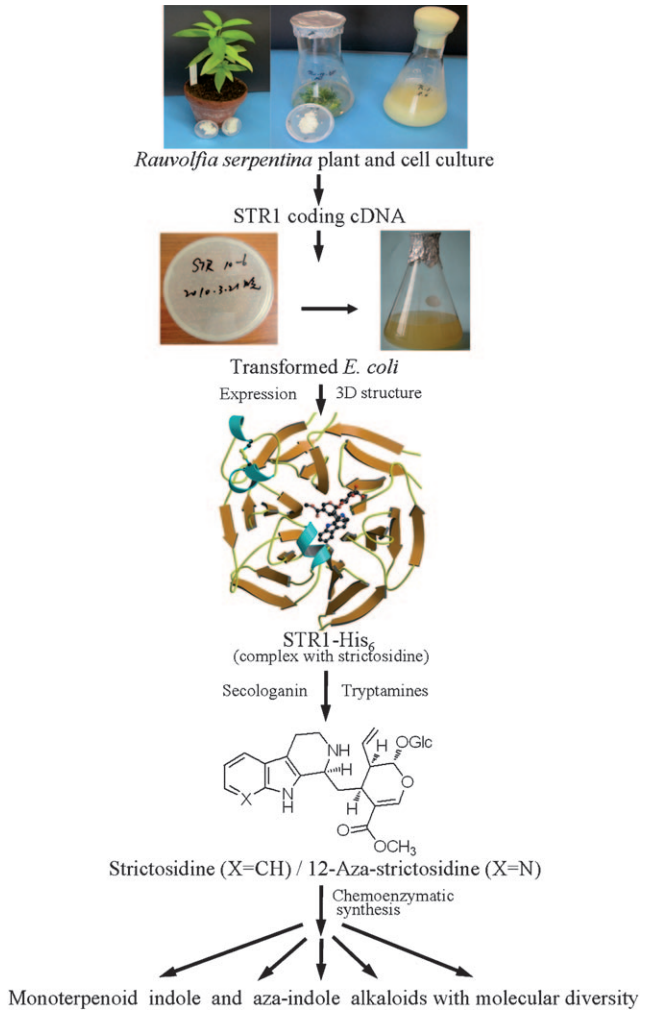


Figure 1. Strategy for STR1-based chemoenzymatic approaches that lead to complex indole alkaloids (figure modified from Ref. [9]).

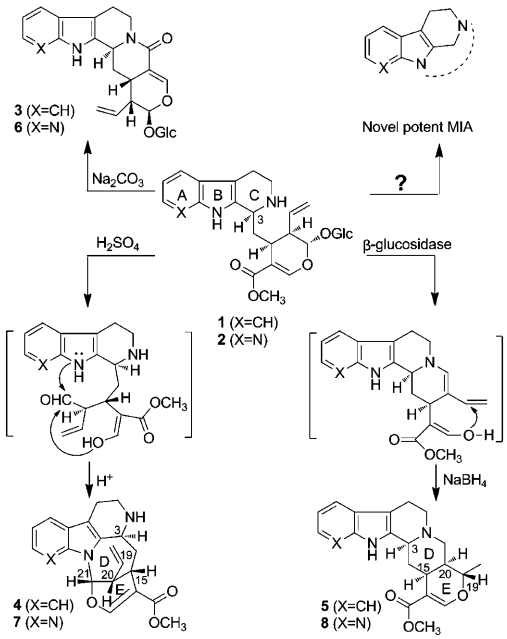
(**2**). Owing to the potent active intermediate formed after deglucosylation, strictosidine and 12-aza-strictosidine were used to explore a possible simple chemoenzymatic synthesis of MIAs, especially the novel alkaloids **6**–**8** with molecular diversity and improved structures (Scheme 1). The strategy is summarized in Figure 1.

Results and Discussion

The prepurified recombinant STR1-His_6 was immobilized on nickel nitrilotriacetate (Ni-NTA) to get the key intermediates **1** and **2** by enzymatic coupling of secologanin with

Abstract in Chinese:

吲哚类生物碱具有广泛的生物活性，但其多环多手性中心的结构特点对传统化学合成提出了很大挑战。我们基于酶-化学方法，以重组异胡豆苷合成酶催化所得的异胡豆苷和12-氮杂异胡豆苷为原料，实现了一步反应快速、原子经济性合成了具有多个手性中心和新结构母核的吲哚类生物碱，并对其结构进行了确证，同时发现新结构母环中氮原子的引入可能有利于其细胞毒活性。



Scheme 1. Chemoenzymatic synthesis of MIAs.

tryptamine and 7-aza-tryptamine, respectively.[9] The alkaloids (**3**–**8**) were simply prepared by a one-step reaction. The lactams **3**[10] and **6** were obtained by conversion of **1** and **2** under mild basic conditions (Scheme 1). Nacycline (**4**)[11] and 12-aza-nacycline (**7**) with a seven-membered ring were synthesized by simple acidic catalyzation by using 2 M H_2SO_4 (involving Schiff base formation and asymmetric electrophilic addition) to result in D and E ring closure (Scheme 1). A one-pot chemoenzymatic approach was carried out to obtain tetrahydroalstonine (**5**)[12] and its aza analogue (**8**). The reaction was initiated by β-glucosidase-catalyzed deglucosylation of **1** and **2** to form the active intermediate with the D ring closure. Regiospecific electrophilic addition and reduction of the intermediate afforded **5** and **8** according to the Markovnikov rule. All the reactions were monitored by analytical HPLC (Figure 2) and the synthe-

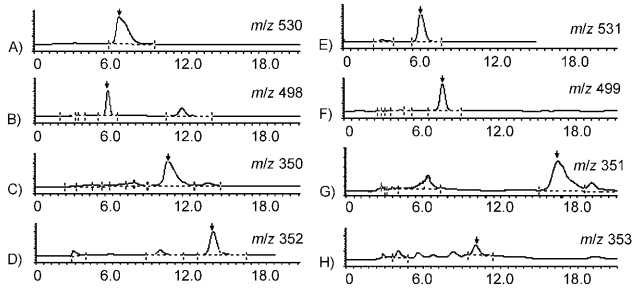


Figure 2. Reaction monitored by HPLC in the synthesis of MIAs. A) Strictosidine afforded B) strictosidine lactam, C) nacycline, and D) tetrahydroalstonine. E) 12-Aza-strictosidine afforded F) 12-aza-strictosidine lactam, G) 12-aza-nacycline, and H) 12-aza-tetrahydroalstonine. The *m*/*z* data were obtained by ESI-MS using purified alkaloids.

sized MIAs were successfully purified by semipreparative HPLC.

The synthesized MIAs were characterized by MS, one-dimensional NMR spectroscopy (^{1}H and ^{13}C), and two-dimensional NOESY spectroscopic data. The 12-aza-strictosidine was found to have the same 3*S* configuration as strictosidine.[9,10b] To determine the configuration of the newly formed chiral center C-21 of nacyclines (**4**, **7**) and C-19 and C-20 of tetrahydroalstonines (**5**, **8**), NOESY spectroscopic data and virtual structural models were compared and analyzed (Figure 3). The synthesized MIAs with specific configurations (*R*/*S*) and preferred energy-minimized structures were constructed by SYBYL 6.91.[13] The constructed structural model of (21*S*)-12-aza-nacycline (Figure 3A) shows that H-21 is close to H-20 and H-19, whereas the (21*R*)-12-aza-nacycline (Figure 3B) indicates that there are no correlations of H-21 with either H-20 or H-19. Although H-20 and H-15 appear in one multiple peak in the ^{1}H NMR spectra, expansion of the NOESY spectroscopic data clearly identifies H-20 with a chemical shift at $\delta = 3.34$ ppm since H-15 can not have any correlation with H-21 in both configurations (*R*/*S*; Figure 3A and B). The correlation between H-21 with H-19 and especially with H-20 matches the *S* configuration of C-21 quite well, which supports the idea that (21*S*)-12-aza-nacycline (**7**) was prepared. Figure 3C and D represent the structural models of (19*S*,20*S*)-12-aza-tetrahydroalstonine and its isomer 19*R*,20*S*. Comparison of the structural models with the NOESY spectroscopic data indicates that C-20 has the same *S* configuration as C-15 since they have very strong correlation to each other. The very weak correlation between H-19 and H-20 indicates the *S* configuration of C-19, which is further supported by the observed correlation of H-19 and H-21β. The stereochemistry of **4** and **5** was also determined to be (3*S*,15*S*,20*R*,21*S*)-nacycline and (3*S*,15*S*,19*S*,20*S*)-tetrahydroalstonine, respectively, with the detailed stereochemical description in the Supporting Information. The stereochemistry of strictosidine lactam and 12-aza-strictosidine lactam remains unchanged.

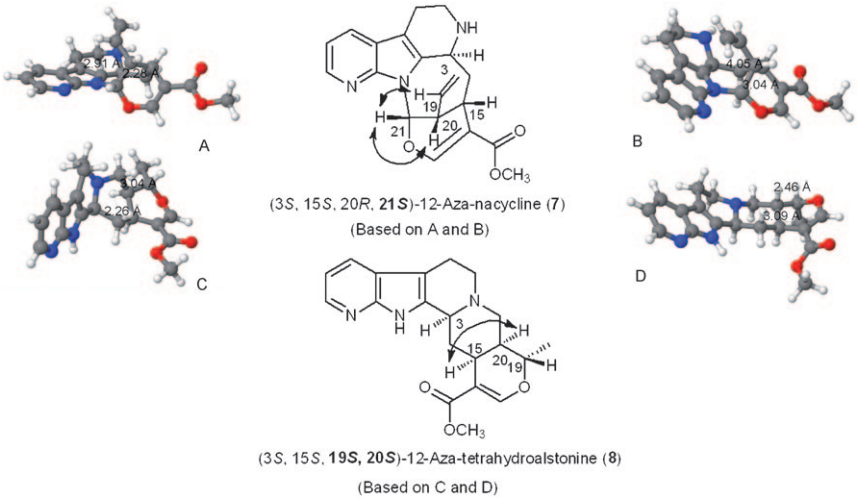


Figure 3. Energy-minimized structural models with a certain configuration showing the distances between the protons of the newly formed chiral center of **7** (C-21), **8** (C-19, 20), and their surroundings. A) (3*S*,15*S*,20*R*,21*S*)-12-aza-nacycline; B) (3*S*,15*S*,20*R*,21*R*)-12-aza-nacycline; C) (3*S*,15*S*,19*S*,20*S*)-12-aza-tetrahydroalstonine; and D) (3*S*,15*S*,19*R*,20*S*)-12-aza-tetrahydroalstonine.

All the synthesized MIAs were tested in vitro against three human cancer cell lines such as KB, A549, and K562 with the positive control Camptothecin (CPT). The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay[14] results suggest that these MIAs are selectively cytotoxic toward the A549 cell line, and the incorporated nitrogen of the indole moiety might be superior to the carbon atom for the cytotoxicities of the synthesized alkaloids (Table 1). The (21*S*)-12-aza-nacycline (**7**) exhibits the most potent cytotoxicity of these MIAs with an IC_{50} value of 6.2 μM.

Table 1. Cytotoxicity of synthesized MIAs toward the A549 cell line.

MIA	IC_{50} [μM]	MIA	IC_{50} [μM]
3	–[a]	**6**	11.3
4	–[a]	**7**	6.2
5	8.1	**8**	16.6
CPT[b]	0.11		

[a] $IC_{50} > 50$ μM is considered to be inactive and is omitted here. [b] Camptothecin.

Conclusion

In conclusion, a convenient chemoenzymatic synthesis of novel MIAs with multiple chiral centers was described. The nacycline, strictosidine lactam, tetrahydroalstonine, and their aza analogues were successfully achieved according to this strategy using immobilized strictosidine synthase catalyzation followed by a one-step reaction. The stereochemistry of these alkaloids was also determined by comparison of the virtual structural model and spectral data. Preliminary in vitro biological evaluation indicates that these alkaloids are selectively cytotoxic toward the A549 cell line and the newly incorporated nitrogen might be preferred for cytotoxity. These results open an opportunity to chemoenzymatic approaches that lead to diversification of complex monoterpenoid indole alkaloids with improved novel structures and activities.

Experimental Section

General Methods

Strictosidine synthase was prepared by expression of the plasmid pQE-2-STR1-pG-Tf2-His_6 in *E. coli* M15 cells. Secologanin was isolated from *Lonicera tatarica*. The 7-aza-tryptamine was synthesized from 7-aza-indole. The 12-aza-strictosidine was obtained by conversion of 7-aza-tryptamine and secologanin using the immobilized prepurified STR1-His_6 enzymatic catalytic system. Strictosidine was also synthesized by the same method. The detailed description of these experimental procedures is given in the Supporting Information. The MS, NMR, and NOESY spectral data and the stereochemistry determination of **4** and **5** are also provided in the Supporting Information. The β-glucosidase was purchased from Fluka. All the other chemical reagents and biological material were obtained from standard commercial sources and were of analytical reagent grade. ESI-MS data were recorded with a Bruker Esquire 3000+ spectrometer. NMR spectra and NOESY spectroscopic data were recorded with Bruker AVIII (500 MHz) or Bruker DRX 700 instruments with TMS as an internal standard, and CD_3OD and $CDCl_3$ were used as solvent. TLC was performed on silica gel (GF_{254}). Column chromatography was performed on silica gel 60 (70–230 mesh, E. Merck). All the synthesized MIAs were purified by semipreparative HPLC with a LiChrospher RP-18 EC column (250×10 mm, 10 μm, Merck).

*General Procedure for the Synthesis of Strictosidine Lactams (**3**, **6**)*

12-Aza-strictosidine (20 mg, 0.038 mmol) was dissolved in a water solution of Na_2CO_3 (5%, 2 mL). The mixture was stirred at 75°C for 30 min under nitrogen. After the reaction was completed, the mixture was extracted by EtOAc (10 mL×3). The combined extract was washed with water (10 mL×3), brine (10 mL×1), and dried over anhydrous Na_2SO_4. The solvent was then removed in vacuo and the residue was dissolved in methanol (2.0 mL). After centrifugation, the supernatant was subjected to semipreparative HPLC to afford 12-aza-strictosidine lactam (**6**, 18 mg, yield: 95.0%) using MeOH/H_2O as the eluent (gradient: 0–10 min: 55:45; 10–20 min: 75:25; 20–35 min: 80:20; UV: 254 nm). ^{1}H NMR (700 MHz, CD_3OD): δ=8.12 (brs, 1H), 7.83 (dd, J=7.7, 1.4 Hz, 1H), 7.36 (s, 1H), 7.11 (dd, J=7.7, 4.9 Hz, 1H), 5.64 (m, 1H), 5.39 (d, J=2.1 Hz, 1H), 5.36 (dd, J=16.8, 1.4 Hz, 1H), 5.30 (dd, J=10.5, 2.1 Hz, 1H), 5.09 (m, 1H), 4.93 (dd, J=12.6, 5.6 Hz, 1H), 4.55 (d, J=8.4 Hz, 1H), 3.84 (dd, J=11.9, 2.8 Hz, 1H), 3.61 (dd, J=11.9, 5.6 Hz, 1H), 3.29–3.10 (m, 4H), 2.96–2.90 (m, 2H), 2.78 (m, 1H), 2.69–2.66 (m, 1H), 2.54 (ddd, J=13.3, 4.2, 2.1 Hz, 1H), 2.05 ppm (dt, J=14.0, 6.3 Hz, 1H); ^{13}C NMR (175 MHz,): δ=167.1, 149.6, 149.3, 143.0, 136.2, 134.3, 127.8, 121.9, 120.6, 116.7, 109.7, 109.1, 100.4, 98.0, 78.3, 78.0, 74.3, 71.4, 62.9, 54.9, 44.7, 44.5, 27.2, 25.0, 21.7 ppm; MS (ESI): m/z: 500 $[M+H]^+$.

The synthetic scheme of strictosidine lactam (**3**) is identical to that of 12-aza-strictosidine lactam, except the starting material was substituted with strictosidine. The final yield was 76.5%.

*General Procedure for the Synthesis of (21S)-Nacyclines (**4**, **7**)*

12-Aza-strictosidine (20 mg, 0.038 mmol) was dissolved in a solution of H_2SO_4 (2 M, 0.4 mL). The mixture was stirred at 85–90°C for 30 min under nitrogen. After the reaction was completed, saturated sodium carbonate was added (under cooling with an ice bath) into the solution to adjust the pH to 8–9. The aqueous layer was extracted by EtOAc (10 mL×3). The combined extract was washed with water (10 mL×3), brine (10 mL×1), and dried over anhydrous Na_2SO_4. The solvent was then removed in vacuo and the residue was purified by semipreparative HPLC using MeOH/H_2O (80:20, UV: 230 nm) to afford (21*S*)-12-aza-nacycline (**7**, 8.8 mg, yield: 65.7%). ^{1}H NMR (500 MHz, $CDCl_3$): δ=10.21 (s, 1H), 8.33 (d, J=4.5 Hz, 1H), 7.79 (d, J=7.5 Hz, 1H), 7.54 (s, 1H), 7.07 (dd, J=7.5, 4.5 Hz, 1H), 6.18 (s, 1H), 4.64 (q, J=6.5 Hz, 1H), 4.34 (d, J=11.5 Hz, 1H), 3.76 (m, 1H), 3.71 (d, J=9.0 Hz, 1H), 3.68 (s, 1H), 3.58 (dd, J=10.5, 4.5 Hz, 1H), 3.34 (m, 1H), 3.30 (m, 1H), 3.24 (m, 1H), 2.89 (m, 1H), 2.71 (d, J=14.5 Hz, 1H), 1.75 (m, 1H), 1.52 ppm (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=167.9, 154.5, 148.9, 142.2, 133.8, 126.2, 119.8, 115.5, 107.3, 106.8, 76.4, 52.3, 50.9, 49.0, 33.6, 27.5, 21.9, 20.3, 18.7 ppm; MS (ESI): m/z: 352 $[M+H]^+$.

The synthesis of (21*S*)-nacycline (**4**) is identical to that of 12-aza-nacycline, except strictosidine was used as the starting material. The final yield was 82.3%.

*General Procedure for the Synthesis of (19S,20S)-Tetrahydroalstonines (**5**, **8**)*

12-Aza-strictosidine (20 mg, 0.038 mmol) and β-glucosidase (5 mg) were dissolved in a solution of acetate buffer (50 μM, pH 5.0, 2 mL). The mixture was stirred at 37°C for 24 h under nitrogen. After the reaction was completed, the mixture was freeze-dried in vacuo. Then anhydrous methanol (5 mL) was added to the residue and the inactivated enzyme was removed by centrifugation. The supernatant was directly used for reaction by addition of AcOH (32 μL, 0.57 mmol) under N_2 and the mixture was stirred at RT for 15 min. $NaBH_4$ (21 mg, 0.19 mmol) was added to the reaction mixture and stirred overnight. The methanol was removed in vacuo and the reaction was quenched by addition of water (5 mL). The aqueous layer was extracted with EtOAc (10 mL×3). The combined extract was washed with water (10 mL×3), brine (10 mL×1), and dried over anhydrous Na_2SO_4. The solvent was then removed in vacuo and the residue was purified by semipreparative HPLC using MeOH/H_2O (80:20, UV: 230 nm) to afford (19*S*,20*S*)-12-aza-tetrahydroalstonine (**8**, 4.7 mg, 35.1%). ^{1}H NMR (500 MHz, $CDCl_3$): δ=8.28 (d, J=5.0 Hz, 1H), 7.81 (s, 1H), 7.76 (d, J=8.0 Hz, 1H), 7.56 (s, 1H), 7.04 (t, J=8.0 Hz, 1H), 4.52 (dt, J=10.5, 6.5 Hz, 1H), 3.76 (s, 1H), 3.43 (dd, J=11.5, 2.0 Hz, 1H), 3.12 (dd, J=12.5, 1.5 Hz, 1H), 2.99–2.90 (m, 2H), 2.83 (dt, J=12.0, 4.5 Hz, 1H), 2.76 (dd, J=12.5, 3.5 Hz, 1H), 2.70–2.56 (m, 3H), 1.72 (m, 1H), 1.53 (q, J=12.5 Hz, 1H), 1.41 ppm (d, J=6.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=168.0, 155.8, 149.2, 142.3, 135.5, 126.2, 120.1, 115.7, 109.7, 106.6, 72.5, 59.9, 56.6, 53.5, 51.3, 38.6, 34.3, 31.5, 21.8, 18.7 ppm; MS (ESI): m/z: 354 $[M+H]^+$.

The synthesis of (19*S*,20*S*)-tetrahydroalstonine (**5**) is identical to that of 12-aza-tetrahydroalstonine, except the starting material was substituted with strictosidine. The final yield was 80.6%.

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