# Chemoenzymatic Enantioselective Formal Synthesis of (-)-Gephyrotoxin-223

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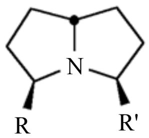
**ABSTRACT:** *(-)-Gephyrotoxin-223 was formally synthesized from chiral synthon 1 which has been chemoenzymatically synthesized in the presence of Candida Antartica lipase.*

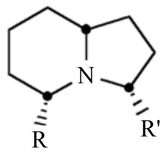
**KEY WORDS:** *Gephyrotoxin, Indolizidine, Chemoenzymatic, Candida Antartica Lipse, Alkaloids, Desymmetrization.*

## INTRODUCTION

For a wide range of simple bicyclic alkaloids that have been found in skin extracts of dendrobatid frogs and mantelline frogs, the term "izidine" alkaloids might be used [1]. The "izidine" alkaloids detected in amphibian skin include 3,5-disubstituted pyrrolizidines, 3,5-disubstituted and 5-monosubstituted indolizidines, 5,8-disubstituted indolizidines, 5,6,8-trisubstituted indolizidines. Many of them probably originate from dietary ants. In this paper, we will present our work on the synthesis of (-)-gephyrotoxin-223 as one of the 3,5-disubstituted indolizidines.



**3,5-disubstituted pyrrolizidines**



**3,5-disubstituted indolizidines**
**5-monosubstituted indolizidines (R′=H)**

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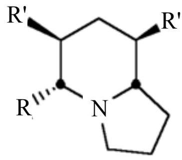
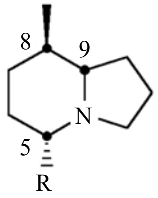
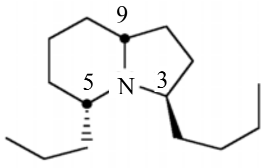


**5,6,8-trisubstituted indolizidines**

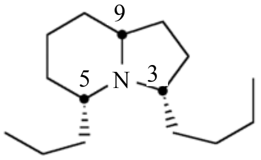


**5,8-disubstituted indolizidines**

In 1978, 3,5-disubstituted indolizidine structures were proposed [2] for three alkaloids found in the dendrobatid frog *Dendrobates histrionicus*. One of them was (-)-gephyrotoxin-223 (indolizidine 223AB) of which the 5E, 9E isomer has been found in a Colombian frog *Dendrobates histrionicus* and its 5Z,9Z isomer was the sole diastereomer found in Panamanian *Dendrobates specious*.



**5E,9E-223AB**



**5Z,9Z-223AB**

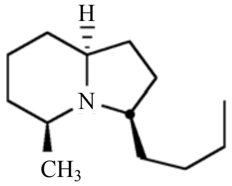
About 15 alkaloids detected in extracts of amphibian skin have been proven to be 3,5-disubstitued indolizidines and they represent another alkaloid class present as venom constituents in myrmicine ants. Recently, both monomorine I and the amphibian diastereomer 5E,9E–**195B** were found in a Puerto Rican myrmicine ant.

When Dendrobatid frogs (*Dendrobates auratus*), were fed Pharoah's ants (*Monomorium pharaonis*), they efficiently accumulated the ant alkaloid monomorine I and two minor 3,5-disubstitued indolizidines in their skin.

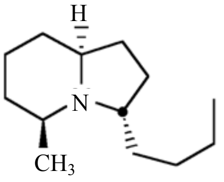
Indolizidine 5E,9E–**195B** was a major alkaloid in a dendrobatid frog (*Dendrobates auratus*) raised in outside cages in Hawaii.

It seems highly likely that the 3,5-disubstitued indolizidines found in amphibian skin are the result of sequestration from myrmicine ants.

Of the 5-monosubstituted indolizidines, we have reported [3,4] enantioselective synthesis of the enantiomeric pairs of indolizidine 167B and indolizidine 209D and of the 3,5-disubstituted indolizidines, we have performed the formal asymmetric synthesis of (-)-gephyrotoxin-223 (indolizidine 223AB) via enzymatic desymmetrization.



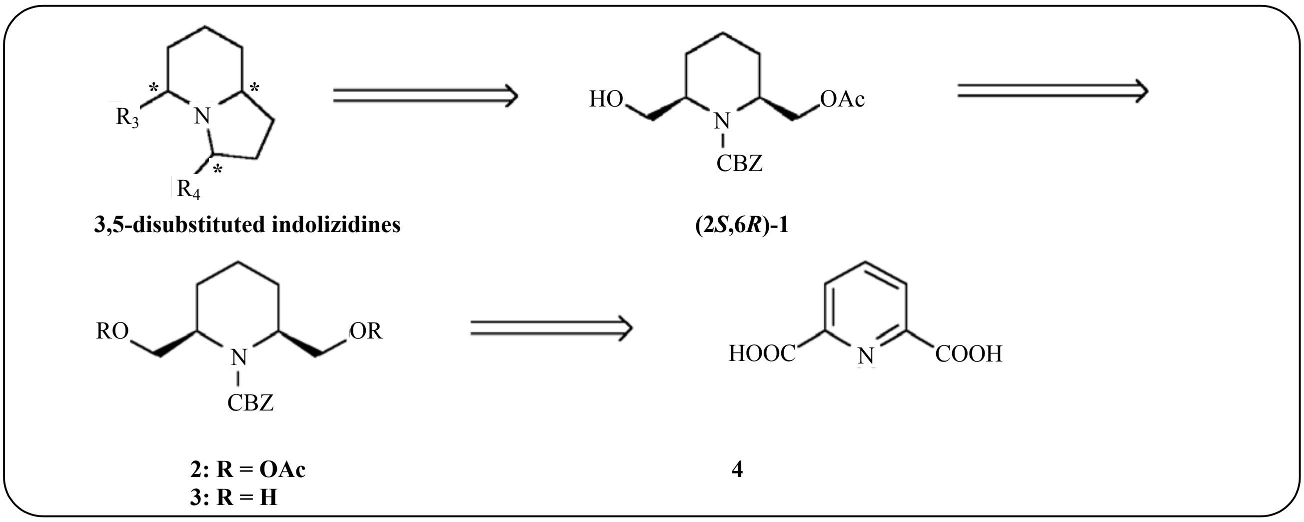
**Monomorine I**



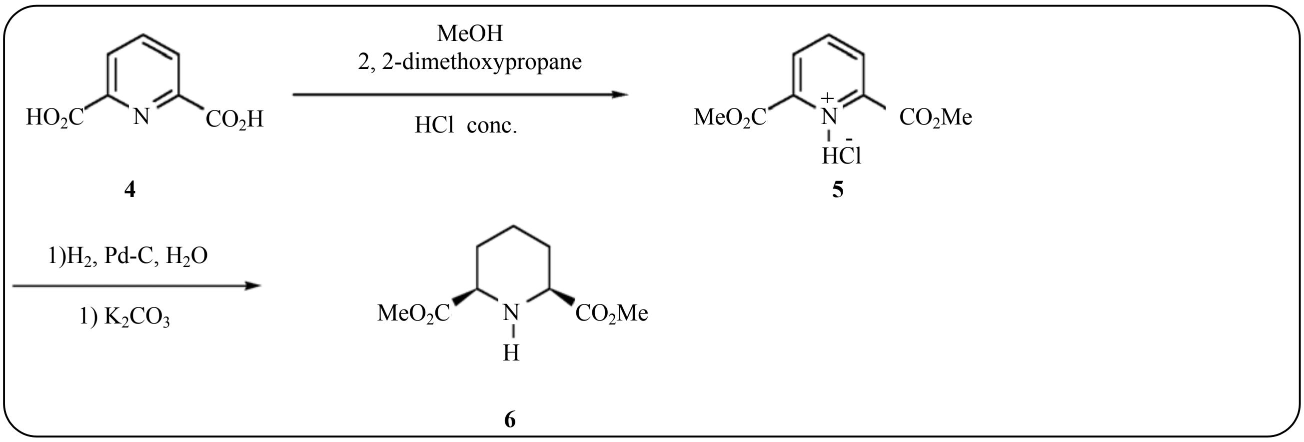
**5E,9E–195B**

***Retrosynthetic analysis***

Our construction strategy for 3,5-disubstituted indolizidines is based on the retrosynthetic analysis illustrated in scheme 1. This analysis introduces the chiral synthon 1 as the key intermediate which was prepared



*Scheme 1: Retrosynthesis of the 3,5-disubstituted indolizidines.*



*Scheme 2: Synthesis of cis-2,6-bis(methoxycarbonyl)piperidine 6.*

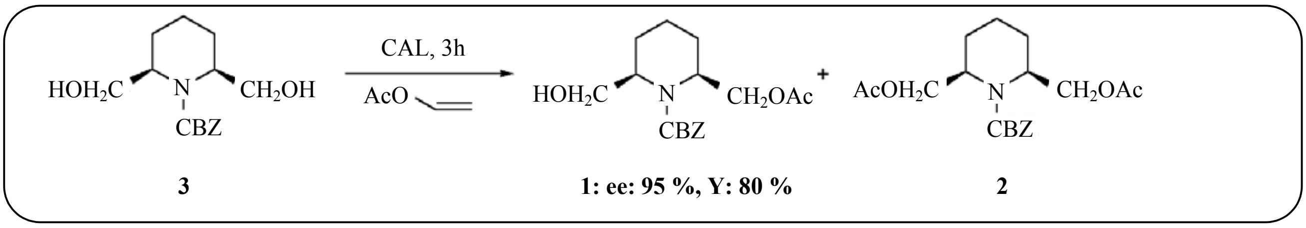
from 2,6-dicarboxylic acid 4 in several steps. As we know, the enzymatic hydrolysis of *meso*-diester 2 in the presence of *Aspergillus niger* lipase (ANL) [5,6] provided the corresponding (+)-(2*R*,6*S*)-monoacetate 1, while the enzymatic acetylation of *meso*-diol [3,4] 3 gave the (-)-(2*S*,6*R*)-monoacetate 1. These two enantiomeric pairs were used as chiral building blocks in the enantioselective synthesis of both enantiomers of 3,5-disubstituted indolizidines.

## DISCUSSION

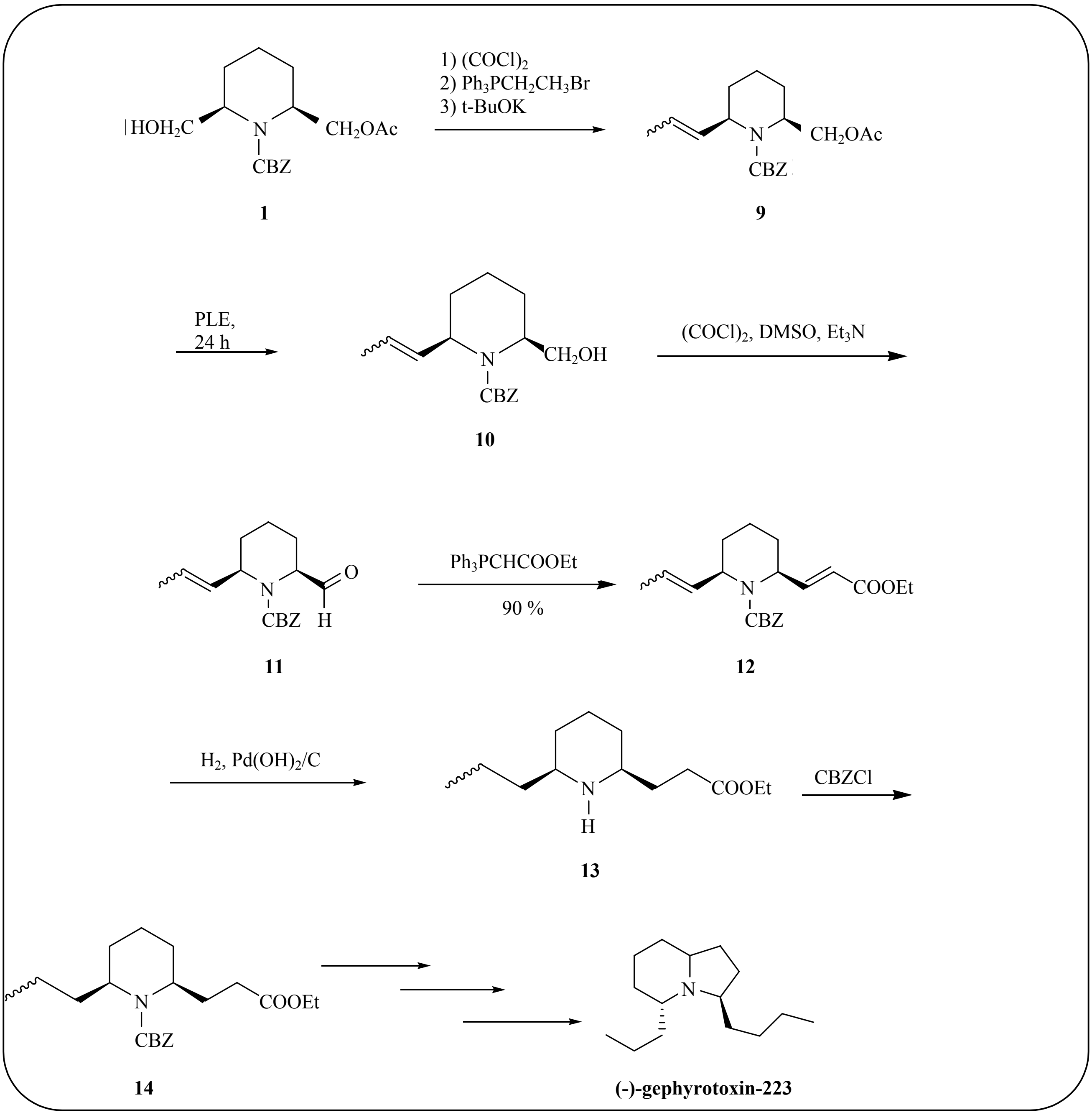
We report here the chemoenzymatic enantioselective formal synthesis of both enantiomers of (-)-gephyrotoxin-223 (indolizidine **223AB)** from the unstable aldehyde **11**, readily prepared [3] from the (2*R*,6*S*)-monoacetate **1** as chiral synthon which was prepared as shown in scheme 2, 3, 4. The aldehyde **11** was added to Wittig ylide, ethyl (triphenylphosphoranylidene)acetate, to provide the olefin **12**, which was hydrogenated over Pearlman's Catalyst to yield aminoester **13**. The protection of the amino group of **13** was effected using benzylchloroformate giving the desired intermediate **14** $\{[\alpha]_D^{25}$ + 5.2° (c 0.745, $CHCl_3$); lit. [7], $[\alpha]_D^{26}$ +5.1° (c 3.51, $CHCl_3$)$\}$, identical with that already prepared by Momose. The preparation of carbamate **14** constitute [8] a formal synthesis of (-)-gephyrotoxin-223 [(-)-indolizidine **223AB**] (scheme 5).

## EXPERIMENTAL

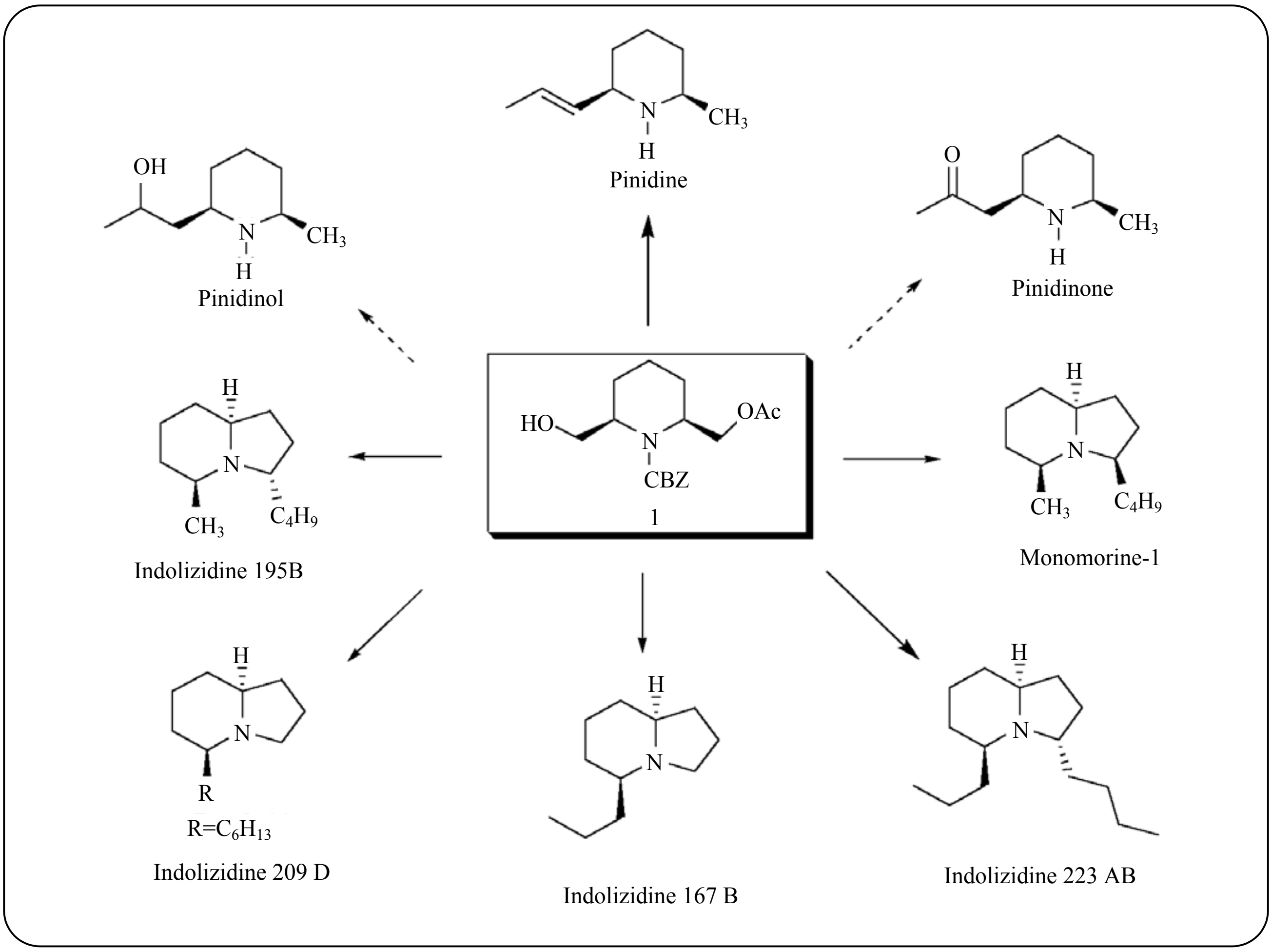
IR spectra were ecorded using a Bomen MB-100 spectrophotometer. NMR spectra were recorded in $CDCl_3$ solutions at 300 MHZ ($^1H$), 282 MHZ ($^{19}F$), 75 MHZ ($^{13}C$) on a Bruker AC – 300 instrument. Optical rotation



*Scheme 3: Enzyme-catalyzed acetylation of cis-diol 3 with CAL.*



*Scheme 4: Chemoenzymatic enantioselective formal synthesis of (-)-gephyrotoxin-223.*



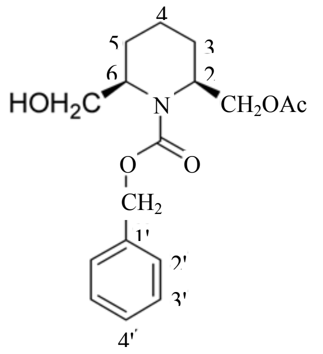
*Scheme 5: Synthesis of piperidine alkaloids from chiral monoacetate 1.*

values were obtained from a JASCO DIP-300 polarimeter (c as g of compound per 100 mL). Elemental analyses performed on a Carlo Ebra 1106 instrument. Column purifications were conducted by flash chromatography on silica gel 60 (230-400 mesh).

### *Enzyme-catalyzed acylation of N-carbobenzoxy-cis-2,6-dihydroxymethyl-piperidine 3: preparation of N-carbobenzoxy- cis- 2S- acetoxymethyl- 6R- hydroxymethyl-piperidine 1*

To a solution of diol **3** (53 mg, 0.19 mmol) in vinylacetate (3 mL) was added 60 mg of *Candida antarctica* lipase (CAL), and the mixture was stirred for 3 h at rt. The progress of the reaction was monitored by TLC. As the reaction proceeded, the amount of diacetate present in the reaction mixture increased before the complete disappearance of starting material was observed. When the spot of diacetate became as visible as the spot of the starting diol, the reaction was stopped. The mixture was filtered for removing of enzyme and then concentrated. Purification of the crude product was done by flash chromatography using 20 % ethyl acetate / 80 % petroleum ether to pure ethyl acetate to give (2*S*,6*R*)-monoacetate **1** as a colourless oil in 80 % yield (48.81 mg, 0.151 mmol) with an ee ≥ 95 % as measured by $^{19}F$ NMR of *R*-Mosher's ester made from **1**. $[\alpha]_D^{25}$ - 4.98 (c 2.08, $CHCl_3$); IR (neat) vmax: 3450 (OH), 2930, 1745 (C=O, Ac), 1690 (C=O, $NCO_2$) $cm^{-1}$ ; $^1H$ NMR ($CDCl_3$) δ: 7.35 - 7.25 (m, 5H, Ph), 5.12 (AB system, 2H, J = 12.4 Hz, $CH_2Ph$), 4.48 (m, 1H, $C^2H$), 4.31 (m, 1H, $C^6H$), 4.13 (dd, 1H, $J_1$ = 8.0 Hz, $J_2$ = 10. 9 Hz, $C\underline{H}HOAc$), 3.95 (dd, 1H, $J_1$ = 6.9 Hz, $J_2$ = 10. 9 Hz, $CH\underline{H}OAc$), 3.56 (d, 2H, J = 7.6 Hz,$C\underline{H_2}OH$), 2.85 (br s, 1H, OH), 1.92 (s, 3H, $CH_3CO$), 1.81-1.45 (m, 6H, $C^3H_2$, $C^4H_2$ and $C^5H_2$); $^{13}C$

NMR ($CDCl_3$) δ: 170.60 (C=O, Ac), 156.79 (C=O, $NCO_2$), 136.43 ($C^{1'}$), 128.36 ($C^{3'}$, $C^{5'}$), 127.89 ($C^{4'}$), 127.72 ($C^{2'}$, $C^{6'}$), 67.28 ($\underline{C}H_2OAc$), 64.38 ($CH_2Ph$), 64.02 ($\underline{C}H_2OH$), 51.71 ($C^6$), 48.45 ($C^2$), 24.84 ($C^3$), 24.32 ($C^5$), 20.5 ($C^4$), 14.47 ($CH_3$).



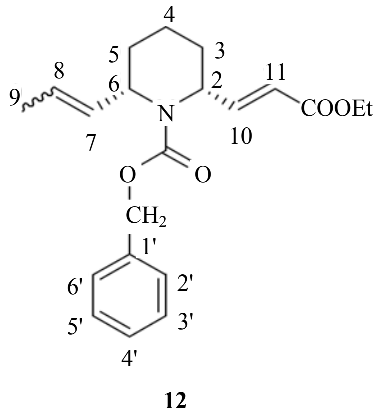
**(2S,6R)-1**

### *Preparation of N-carbobenzoxy- cis- 2R- (trans-carboethoxyethylene)-6S-(1-propenyl)-piperidine 12*

The synthesis of alcohol **10** was reported from chiral synthon **1** in our previous aricle [3] and then it will transformed to the aldehyde 11 as following experiment. Oxalyl chloride (2 eq, 4 mmol, 0.34 mL) was dissolved in 6 mL anhydrous $CH_2Cl_2$, cooled to -78°C and stirred under $N_2$. To this solution, anhydrous DMSO (3 eq, 6 mmol, 0.43 mL) in 2 mL anhydrous $CH_2Cl_2$ was added dropwise during 10 min and stirred to react for 5 min at – 78 °C. The alcohol **10** (2 mmol, 438 mg) in 2 mL anhydrous $CH_2Cl_2$ was added dropwise to the reaction mixture, which was stirred for 1 hr at -78°C. The reaction was completed by addition of anhydrous $Et_3N$ (4 eq, 1.12 mL, 8 mmol). After 5 min, the dry ice/acetone bath was removed and the reaction temperature was left to rise to rt. The reaction was diluted with 10 mL of $CH_2Cl_2$ and then poured into 30 mL $CH_2Cl_2$ / 10 mL 10 % $NH_4OH$ solution. The aqueous phase was extracted 3 times with $CH_2Cl_2$, and the combined $CH_2Cl_2$ fractions were washed with brine and dried with $MgSO_4$. Evaporation of the solvent gave a mixture of a light yellow oil and a white solid. After dissolving the oily product in anhydrous ether, the mixture was filtered through a $MgSO_4$ pad and then the ether evaporated to give the highly unstable aldehyde **11**, which was used immediately in the Wittig reaction. The purification and analysis of the resulting aldehyde **11** was not possible because it decomposes very fast.

### *Wittig reaction of aldehyde 11*

To a solution of crude aldehyde **11** (2.0 mmol, assuming 100 % yield from Swern oxidation of alcohol **10**) in anhydrous benzene (40 mL), ethyl (triphenylphosphoranylidene) acetate (1.5 eq, 3.0 mmol) was added. The reaction mixture was refluxed for 4 hr, and then poured into the solution of 50 mL ethyl acetate / 25 mL 10 % $Na_2S_2O_3$. The aqueous phase was extracted 3 times with ethyl acetate. The organic fractions were dried with $MgSO_4$ and evaporated. The residue was purified by flash chromatography using 5% ethyl acetate / 95 % petroleum ether to 15 % ethyl acetate / 85 % petroleum ether to give **12** as a white solid in 80 % yield (437.4 mg, 1.6 mmol) from alcohol **10**. $[\alpha]_D^{25}$ -105.9 (c 0.375, $CHCl_3$) (from CAL); IR (neat) νmax : 3050 - 2944, 1717 (CO, $CO_2Et$), 1693 (CO, $NCO_2$), 1655 (C=C) $cm^{-1}$ ; $^1H$ NMR ($CDCl_3$) δ: 7.95 - 7.23 (m, 5H, Ph), 6.97 – 6.90 (dd, 1H, $J_1$ = 5.1 Hz, $J_2$ = 16 Hz, $C^{10}H$), 5.89 (dd, 1H, $J_1$ = 2 Hz, $J_2$ = 16 Hz, $C^{11}H$); 5.57 – 5.35 (m, 2H, $C^7H$ and $C^8H$), 5.11 – 5.02 (m, 3H, $CH_2Ph$ and $C^2H$), 4.90 (m, 1H, $C^6H$), 4.16 – 4.09 (q, 2H, J = 7.1 Hz, $CH_2$, Et), 1.89 – 1.47 (m, 6H, $C^3H_2$, $C^4H_2$ and $C^5H_2$), 1.55 (d, 3H, J= 6.42 Hz, $C^9H_3$), 1.25 – 1.20 (t, 3H, J= 7.23 Hz, $CH_3$, Et). $^{13}C$ NMR ($CDCl_3$) δ: 166.29 (C=O, $CO_2Et$), 155.59 (C=O, $NCO_2$), 149.04 ($C^{10}$), 136.45 ($C^{1'}$), 130.16 ($C^7$), 128.31 ($C^{3'}$, $C^{5'}$), 127.92 ($C^{4'}$), 127.87 ($C^{2'}$, $C^{6'}$), 125.70 ($C^8$), 121.43 ($C^{11}$), 67.29 ($CH_2Ph$), 60.28 ($O\underline{C}H_2CH_3$), 50.77 ($C^2$), 47.59 ($C^6$), 30.04 ($C^5$), 27.58 ($C^3$), 14.96 ($C^4$), 14.12 ($CH_3$), 12.71 ($C^9H_3$); HRMS (CI, $NH_3$) calcd. ($MH^+$) : 358.2018, Found ($MH^+$): 358.2025 ±0.0011.

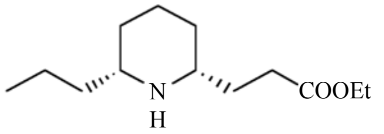


**12**

### *Preparation of (2R,6R)- N-carbobenzoxy-cis-2-(carboethoxyethyl)-6-propylpiperidine 14*

### *Hydrogenation of diene 12*

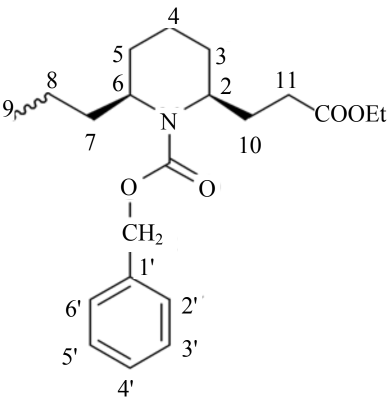
A suspension of diene **12** (50 mg, 0.14 mmol) and palladium hydroxide (10 mg) in EtOH (5 mL) was stirred under a hydrogen atmosphere overnight at 40 psi. After filtration of the reaction mixture through a bed of Celite, the filtrate was evaporated to give crude amino ester **13**. It was used without further purification in the next step.



**13**

### *N-Protection of amino-ester 13*

To a solution of crude amino-ester **13** in anhydrous THF (2 mL) under dry $N_2$ at 0°C was added di*iso*propylethylamine (DiPEA) (1.3 eq, 38.2 μL) followed by benzylchloroformate (1.2 eq, 30 μL). The ice bath was immediately removed and the reaction was stirred for 4 hr at room temperature. The mixture was diluted by addition $CH_2Cl_2$ (10 mL). To this solution was added 1N HCl (2 mL) and then extraction was effected 3 times with $CH_2Cl_2$. The combined organic fractions were dried with $MgSO_4$ and evaporated. The crude product of **14** was purified by flash chromatography using 5 % ethyl acetate / 95 % hexane to 30 % ethyl acetate / 70 % hexane to provide **14** (48.07 mg, 0.133 mmol) as an oil in 95 % yield. $[\alpha]_D^{25}$: -5.2 (c 0.745, $CHCl_3$) (from CAL), lit.[7] $[\propto]_D^{26}$ 5.1° (c 3.51, $CHCl_3$); IR (neat) νmax : 2944, 1718 (CO, $CO_2Et$), 1692 (CO, $NCO_2$) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ: 7.35 - 7.27 (m, 5H, Ph), 5.17 – 5.04 (AB system, 2H, J = 12.42 Hz, $CH_2Ph$), 4.21 (m, 2H, $C^2H$, $C^6H$), 4.11 – 4.07 (m, 2H, $CH_2$, Et), 2.29 (m, 2H, $C^{11}H_2$), 1.95 – 1.79 (m, 2H, $C^{10}H_2$), 1.60 – 1.40 (m, 8H, $C^3H_2$, $C^4H_2$, $C^5H_2$, and $C^7H_2$), 1.24 - 1.13 (m, 2H, $C^8H_2$), 1.24 – 1.20 (t, 3H, J = 6.4 Hz, $C^{13}H_3$, Et), 0.90 – 0.86 (t, 3H, J = 7 Hz, $C^9H_3$). $^{13}C$ NMR ($CDCl_3$) δ: 173.2 (C=O, $CO_2$), 155.98 (C=O, $NCO_2$), 137.04 ($C^{1'}$), 128.29 ($C^{3'}$, $C^{5'}$), 127.70 ($C^{2'}$, $C^{4'}$, $C^{6'}$), 66.86 ($CH_2Ph$), 60.19 ($C^{12}$), 50.46 ($C^6$), 49.94 ($C^2$), 36.79 ($C^7$), 32.03 ($C^5$), 29.70 ($C^3$), 29.56 ($C^{10}$), 28.05 ($C^{11}$), 27.36 ($C^4$), 14.19 ($C^8$), 14.07 ($C^{13}H_3$), 13.86 ($C^9H_3$)



## CONCLUSION

In this work, we wished to show the effectiveness of enzymes in the enantioselective synthesis of (-)-gephyrotoxin-223.

At first, *meso* and *cis*-2,6-piperidines **2**, **3**, were prepared from commercially available pyridine 2,6-dicarboxylic acid **4**. The enzymatic hydrolysis of N-carbobenzoxy-*cis*-2,6-diacetoxymethyl-piperidine **2** with *Aspergillus niger* lipase gave (2*R*,6*S*)-monoacetate **1**, N-carbobenzoxy-cis-2*R*-acetoxy-methyl-6*S*-hydroxymethyl-piperidine, in 5 days with good yield (83 %) and excellent enantiomeric excess (ee ≥ 98 %).The enzymatic acetylation of *meso*-diol **3**, N-carbobenzoxy-*cis*-2,6-dihydroxy-methylpiperidine employing vinyl acetate as solvent and acyl donor in the presence of *Candida antarctica* lipase gave (2*S*,6*R*)-monoacetate **1**, N-carbobenzoxy-*cis*-2*S*-acetoxymethyl-6*R*-hydroxymethyl-piperidine, in only 3 h in good yield (80 %) and high enantiomeric purity (ee ≥ 95 %). In this manner, the time of desymmetrization has been decreased from 5 days to just 3 hours, which was an excellent improvement. By exploiting these two desymmetrization methods, we have synthesized the two enantiomeric pairs of chiral synthon **1**, which were used in the synthesis of both enantiomeric pairs of several piperidines alkaloids (scheme 5). This is an eloquent demonstration of the beauty of our work.

Thus, in this work, the both enantiopure (-)-gephyrotoxin-223 were formally synthesized from pyridine-2,6-dicarboxylic acid **4** .

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