

A Short Chemoenzymatic Synthesis of (+)-Narciclasine

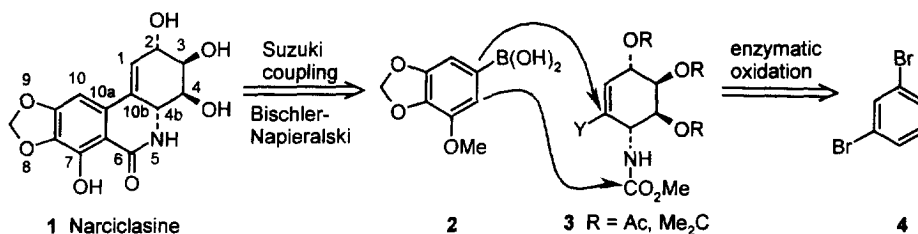
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Abstract: The title alkaloid has been synthesized in eight operations from dibromobenzene and *o*-vanillin, via enzymatic oxidation of the former compound, Suzuki coupling and a Bischler-Napieralski type cyclization as the key transformations. © 1999 Elsevier Science Ltd. All rights reserved.

Narciclasine (**1**), the last of the antitumor Amaryllidaceae alkaloids to yield to total synthesis,¹ is found in the extract of *Lycoris radiata*,² *Pancratium litorale*,³ and *Pancratium maritimum*,⁴ as well as in several *Narcissus* species.⁵ Of the four well-known compounds (pancratistatin, 7-deoxypancratistatin, lycoricidine, and narciclasine) that have attracted attention as potential antineoplastic agents,⁶ narciclasine is the only one whose mode of activity has been briefly investigated.^{9c}



Scheme 1. Design of narciclasine synthesis.

The closely related and more abundant lycoricidine lacks the 7-hydroxyl group believed to be important in the biological activity of narciclasine.² Pancratistatin (1*R*-hydroxy-10*β*H derivative of **1**), and 7-deoxypancratistatin are related in the same way, but the latter substance is about 10 times less active than the former in identical cell line screens.⁷ An attempt to convert the more abundant congeners to pancratistatin by hydration of the C1-C10b olefin was unsuccessful.⁸ To date a truly practical synthesis of **1** or its congeners has not materialized. Thus, the supply-and-demand issue of antitumor screening of these alkaloids has not been alleviated. Several syntheses of these alkaloids have been reported,^{1,9-11} and the various synthetic strategies were recently summarized in two excellent reviews.¹² In 1995, a chemoenzymatic strategy from this laboratory led to the first (and, at 13 steps, still the shortest) enantioselective synthesis of pancratistatin.^{9b-c} In subsequent studies, we addressed several major improvements to shorten further the synthetic sequence and to provide a fully general and highly efficient route to all four alkaloids. In this report, we describe a brief route (eight operations) to the title compound via an enzymatic dihydroxylation of *m*-dibromobenzene.

m-Dibromobenzene was subjected to the whole-cell fermentation with *E. coli* JM109(pDTG601A), an organism developed by Gibson¹³⁻¹⁵ for the overexpression of toluene dioxygenase (TDO), whose structure has recently been solved.¹⁶ Biooxidation yielded the new metabolite **5** (4 g/L, >99% ee) that presents unique symmetry and two chemically different vinylic bromine atoms (C3 bromine is hydrogen bonded to C2 OH), Scheme 2. The

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rotation of the symmetry axis that takes place during the transformation of **4** into **5**, positions the bromine atoms in different planes.⁸ This symmetry rotation will later allow the selective incorporation of the aromatic fragment of narciclasine. Diol **5** was transformed in a one-pot operation to bicyclic oxazine **6** in 70% yield, as shown in Scheme 2. Reduction of this material under Keck's conditions^{17,18} to yield the conduramine oxidation state as previously reported,^{10c-d,19} gave predominantly the fully dehalogenated conduramine derivative **7b**.

We studied the possibility of reducing the oxazine to unsaturated ketone **8**. Tributyltin hydride or *tris*-trimethylsilylsilane (TTMSS) are suited for this transformation but cannot be applied to oxazine **6** since overreduction of the vinylic bromine is unavoidable under such conditions. Conversely, Mo(CO)₆²⁰ cleanly reduced dibrominated oxazine **6** to the corresponding bromo ketone **8** with concomitant (and interesting) cleavage of the acetonide protecting group. With this result in hand, we explored the possibility of directed hydride reduction by means of Zn(BH₄)₂²¹ or Na(AcO)₃BH₄.²² To date this reaction has provided triol **9** in only a disappointing 10% diastomeric excess (HPLC). To circumvent the problem of overreduction of the bromine atom, we decided to couple the aromatic portion of the alkaloid directly to oxazine **6** and postpone the bridge opening to a later stage in the synthesis. Surprisingly, oxazine **10** was resistant to aluminum amalgam reduction, while stronger reducing agents led to fully saturated products. This result closed the direct reduction pathway to the α -hydroxy compound; therefore, we chose to transform **10** into unsaturated ketone **12** with TTMSS.

Interestingly, a considerable amount of **11** (10-15%) was formed during Suzuki coupling of bromide **6** with borate **2** (prepared from *o*-vanillin in four steps and 20% overall yield²³) probably through a Pd insertion-type mechanism. Guided by this observation we decided to add acetonitrile and Mo(CO)₆ directly to the Suzuki reaction mixture after the coupling was finished. Heating of this mixture for 10 hours afforded ketone **11** in 45% yield, reaching the advanced intermediate **11** in only three steps from *m*-dibromobenzene.

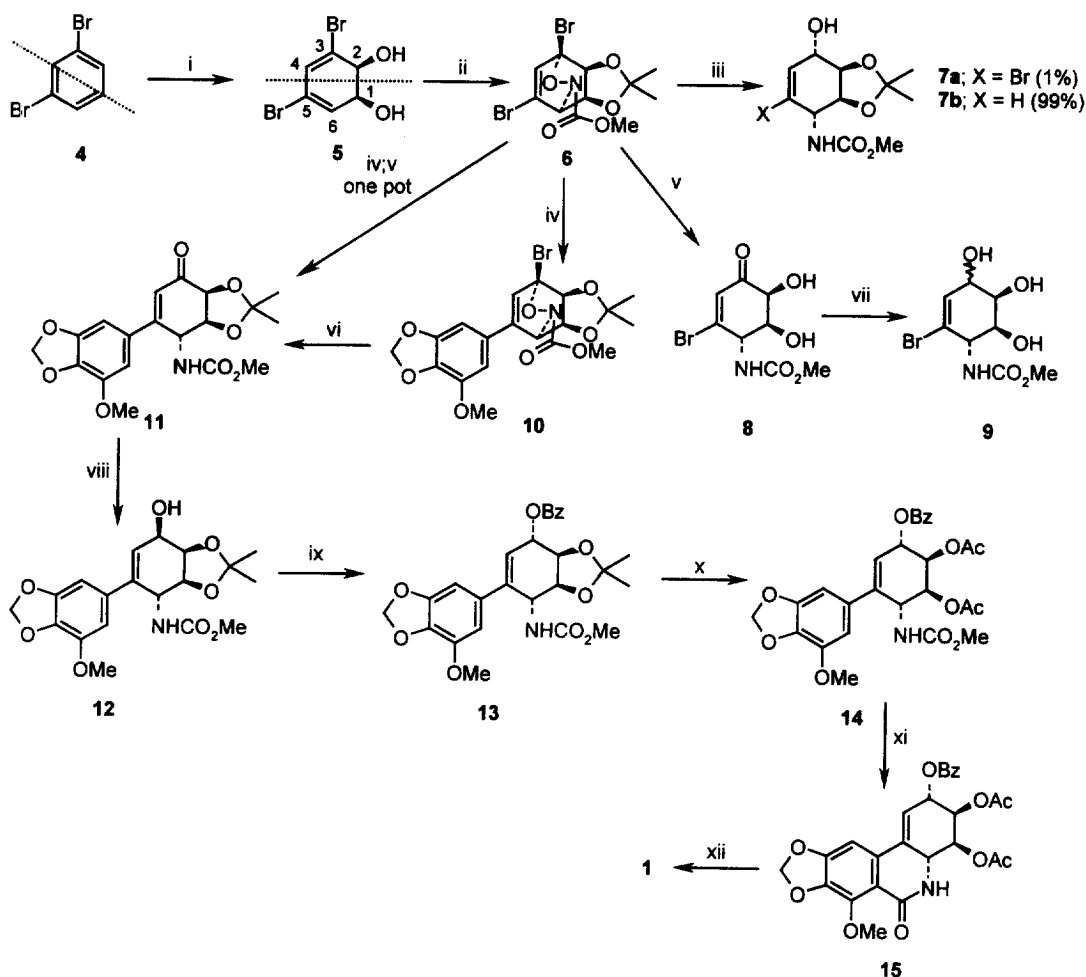
In order to set the stereochemistry at C2 (narciclasine numbering), we applied the known NaBH₄ reduction followed by Mitsunobu inversion sequence as reported by Chida in his lycoricidine preparation.^{10b,c} This procedure gave cleanly the desired α -benzoate **14** in 50% (from **12**).

A modification of the Bischler-Napieralski reaction reported by Banwell^{24a} and applied with success by the same author in simplified models of phenanthridone alkaloids,^{24b} was chosen to close ring B of the target after the required manipulation of acetonide **14** into diacetate **15** (30% over the three steps). Finally, removal of the ester and methyl ether protective groups in **16** (Amberlyst basic, MeOH and LiCl, DMF respectively)²⁵ rendered synthetic narciclasine (**1**) whose ¹H NMR and optical rotation matched the reported data for the alkaloid.²⁶

Overall, we have completed a total synthesis of narciclasine in 12 steps carried out as only 8 individual chemical operations from *m*-dibromobenzene (14 from *o*-vanillin). A complete description of the routes depicted in Scheme 2, with focus on the results obtained in the reduction of the oxazine system under different conditions as well as the Zn(BH₄)₂ reduction of dihydroxy ketones, will appear in an upcoming full account.

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i) *E. coli* JM109 (pDTG601A), 4g/L; ii) DMP, acetone, TsOH, rt; then NHCO_2Me , NaIO_4 , rt, 70%; iii) $\text{Al}(\text{Hg})$, THF, 80%; iv) borate **2**, $\text{Pd}(\text{PPh}_3)_4$, aq. Na_2CO_3 , PhH, reflux, 30%; v) $\text{Mo}(\text{CO})_6$, $\text{MeCN-H}_2\text{O}$, reflux, 75%; vi) TTMSS, AIBN, PhH, reflux, 80%; vii) $\text{Zn}(\text{BH}_4)_2$, DME, -10°C , 70%; viii) NaBH_4 , CeCl_3 , MeOH, 0°C , 80%; ix) BzOH , Bu_3P , DEAD, THF, rt, 65%; x) Dowex 50X8-100, MeOH, rt; then Ac_2O , py, DMAP, rt, 70%; xi) TiF_2O , DMAP, CH_2Cl_2 , 0°C , 40%; xii) Amberlyst A21, MeOH, rt; then LiCl , DMF, 120°C , 20%.

Scheme 2. Summary of the narciclasine synthesis.

References and notes

- § We can designate these planes as "prochemotopic", in addition to "proenantiotopic", since the vinylic bromine atoms will become chemically different after the next operation. The Diels-Alder cycloaddition transformed the bromine at C3 into an allylic one.
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