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Synthesis and biological evaluation of unnatural derivatives of narciclasine: 7- aza-narciclasine and its *N*-oxide

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Abstract

Several unnatural derivatives of narciclasine were prepared in which the C-7 carbon was replaced with nitrogen. The 7-aza derivative and its *N*-oxide were prepared by the coupling of iodopicolinic acid with a conduramine unit derived chemoenzymatically from bromobenzene. Intramolecular Heck reaction was used to construct the carbostyryl ring system. The compounds were submitted to biological screening against cancer cell lines. Full experimental and spectra data are provided for all new compounds.

Pancratistatin (**1**) and narciclasine (**3**) represent some of the most potent cytotoxic components of the Amaryllidaceae constituents. These compounds, and their 7-deoxy derivatives, 7-deoxypancratistatin (**2**) and lycoricidine (**4**), have been the focus of many synthetic ventures in the last three decades.[1] Many unnatural or truncated derivatives of pancratistatin have been prepared and evaluated for biological activity with the intent to produce more bioavailable agents then the highly water-insoluble natural products.[2]

Most of the effort aimed at unnatural or truncated derivatives was focused on structural modifications of pancratistatin or 7-deoxypancratistatin. Modifications of narciclasine, one of the most potent of Amaryllidaceae constituents,[1d] are less common.[2b,3]

Initially, we chose to investigate a truncated derivative of narciclasine such as **5**, without the methylenedioxy bridge and with nitrogen instead of carbon at C-7, Figure 2. The rationale for this design was based on the assumption that the *N*-oxide **6** derived from **5** could potentially mimic the donor-acceptor functionality responsible for increased activity in the alkaloids containing the 7-hydroxy-moiety.[2g,l]

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The synthesis begins with the preparation of the conduramine **8**, which is generated from the diene diol **9**, by a similar strategy that was utilized in the in the total synthesis of lycoricidine.[4] Coupling of conduramine **8** with iodo picolinic acid **7** would be followed by the Heck reaction, in analogy with the strategy used in lycoricidine synthesis. Diol **9**, Scheme 1, is produced in the yields of 18-20 g/L by the whole cell fermentation of bromobenzene with *E. coli* JM109 (pDTG601A), a recombinant organism developed by Gibson and over-expressing toluene dioxygenase.[5]

Diol **9** was protected with acetonide group and used immediately for hetero Diels-Alder reaction with fragment generated by oxidation of *t*-butyloxycarbohydroxamic acid , Scheme 1. Reduction of bicyclic product **11** with aluminum amalgam led to alcohol **12**. This sequence was performed on a multigram scale and did not require the isolation of any intermediates.

Protection of alcohol **12** with *t*-butylsilyl group led to protected conduramine **13**.

Second coupling fragment, iodo acid **7**, was prepared by a simple direct *ortho*-metalation of picolinic acid with lithium tetramethylpiperidide (LTMP) and I_2 *via* a known literature procedure, Scheme 2.[6] The free acid **7** was not obtained in good yield and proved somewhat unstable upon prolonged storage. Therefore the lithium salt **15** was ultimately used as a coupling partner because of its increased stability.

Coupling of the protected conduramine **13** with 3-iodopicolinic acid **7** was initially attempted to produce **17** directly. Unfortunately, under a variety of coupling conditions: 1,1‘-carbonyldiimidazole (CDI), *N,N’*-dicyclohexylcarbodiimide/hydroxybenzotriazole (DCC/HOBt**), or** *N,N,N’,N’*-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU), no desired product was observed, Scheme 3. Therefore conduramine **13** was selectively deprotected with trifluoroacetic acid to afford the free amine **8**. Coupling of amine **8** and lithium salt **15** provided amide **16**, in a moderate yield that can probably be explained by steric hindrance of *ortho*-iodo group. Reprotection of the amide with di-*tert*-butyl dicarbonate in the presence of DMAP provided carbamate **17**. All of the currently existing Heck approaches to narciclasine skeleton[4,7] require tertiary amide or imide moiety for successful transformation; most of them also use toxic thallium (I) salts as a base. Nevertheless, both products **14** and **15** were submitted to a range of standard Heck coupling conditions. We decided to avoid the use of thallium salts and therefore silver phosphate was used as a substitute.[7c] The only conditions that led to the desired product **18**, involved the reaction of **17** in the presence of $Pd(OAc)_2$/1,2-bis(diphenylphosphino)ethane and silver phosphate. The summary of the various conditions attempted is shown in Table 1.

No product of the Heck reaction was ever observed under similar conditions when the unprotected amide **16** was used. Deprotection of the silyl group with TBAF led to an alcohol **19**.

The synthesis was completed by acid-catalyzed deprotection of the Boc carbamate **19** to furnish the HCl salt of **5**, which upon chromatography with basic eluent was isolated as a free base **5**. Oxidation of **5** to its respective *N*-oxide **6** was performed, as shown in Scheme

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3, and subjected to screening in two cancer cell lines to compare the cytotoxicity results to those of the recently tested pancratistatin C-1 homologues.[2j]

In our previous study it has been shown[2r,t] that C-1 analogues **20** and **21** also displayed pronounced activity against pancreatic (BxPC-3), prostate (DU-145), and lung (NCI-H460) cancer cell lines. The respective IC_{50} values for these three lines for C-1 acetate **20** were 0.07, 0.06 and 0.07 μM and for C-1 benzoate **21**: 0.01, 0.01. 0.03 μM. For the latter compound, IC_{50} values exceeded those of the natural product narciclasine (**3**): 0.05, 0.03, 0.05 μM respectively.

The activity of pancratistatin and narciclasine against cancer cell lines is known to be ~100 times higher than that of the 7-deoxy-derivatives. It appears that the 7-hydroxy group is crucial for maintaining effective inhibition of cancer cell growth. The notion that the 7-*N*-oxide functionality would somehow mimic this requirement was shown not to be correct and the aza-derivatives were found to be inactive. The compounds tested also lacked the methylene dioxy group, which is a known contributor to the pharmacophore of these compounds. Our next goal will be the preparation of aza-derivatives of narciclasine that retain the 7-hydroxy moiety as well as the methylene dioxy unit. The results of these endeavors will be reported in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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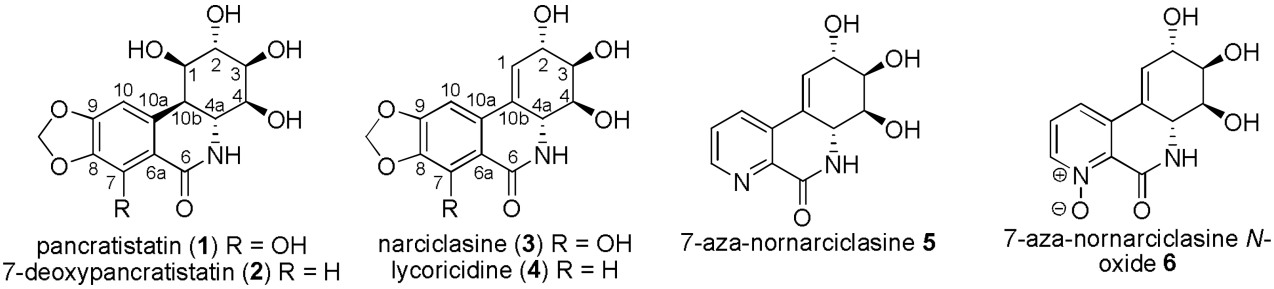

Figure 1.
Amaryllidaceae alkaloids and aza-narciclasine.

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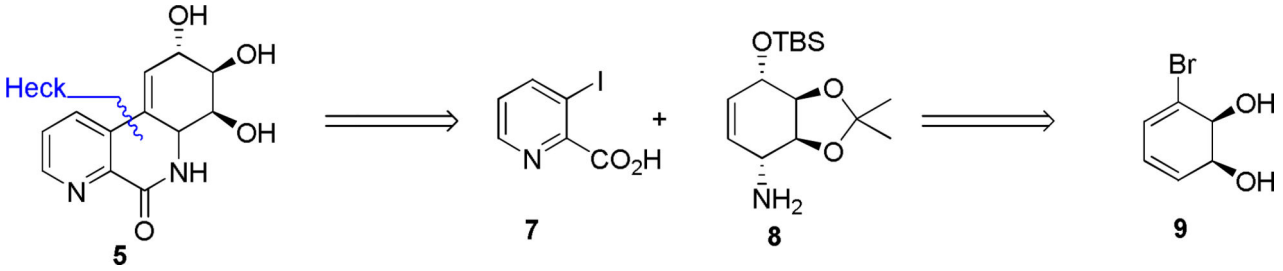

Figure 2.
Retrosynthetic plan for aza-narciclasine analogues.

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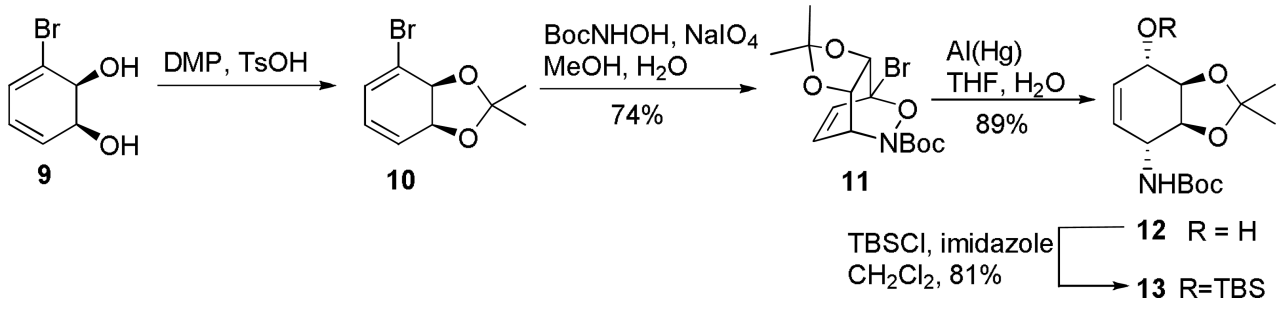

Scheme 1.

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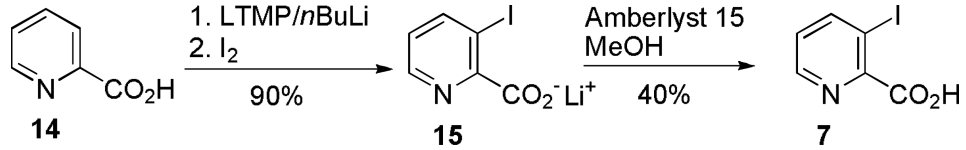

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Scheme 2.

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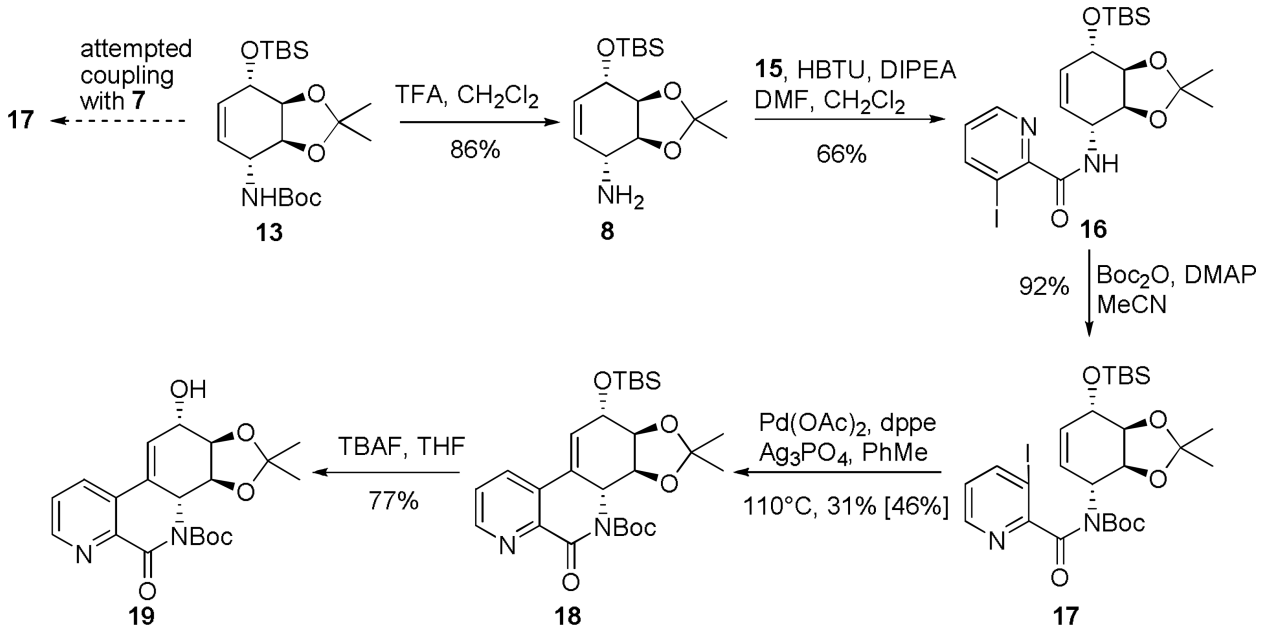

Scheme 3.

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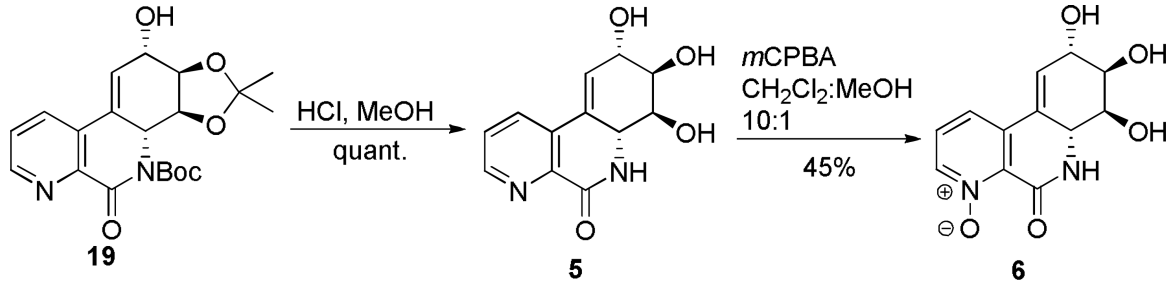

Scheme 4.

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Table 1

Conditions attempted for the Heck cyclization.

Conditions	Outcome
$Pd(dppf)_2Cl_2$, dppf, Ag_3PO_4 dioxane, 60°C, 24 h	No reaction
$Pd(OAc)_2$, dppe, Ag_3PO_4, Et_3N toluene, 80°C, 18 h	Traces of **18**
$Pd(OAc)_2$, PPh_3, Et_3N, $AgNO_3$, MeCN, 24 h, r.t to 80°C	No reaction
$Pd(OAc)_2$, dppe, $AgNO_3$, Cs_2CO_3, toluene, 24 h, 110°C	**18**, 35% (45 % brsm)
$Pd(OAc)_2$, BINAP, Ag_3PO_4, Et_3N toluene, 80°C, 18 h	No reaction

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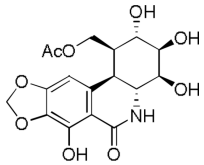
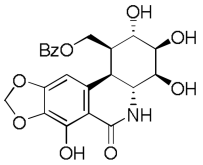
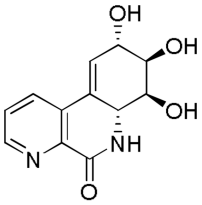
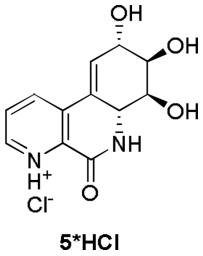
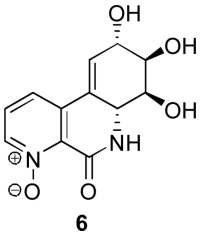
Table 2

Activity of aza-analogues with C-1 homologues as standards [IC_{50} (μM)]

Cell line	 **20**	 **21**	 **5**	 **5*HCl**	 **6**
HeLa (cervical cancer)	**<0.4 μM**	**<0.4 μM**	**Non-inhibitory**	**>100 μM**	**>100 μM**
MCF7 (breast cancer)	**<0.4 μM**	**<0.4 μM**	**Non-inhibitory**	**>100 μM**	**>100 μM**