Tetrahedron Letters 52 (2011) 6142–6144

Contents lists available at SciVerse ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

A novel approach for the synthesis of lophocladines A, B and C1 analogues

Wannaporn Disadee [a,b,*], Poonsakdi Ploypradith [a,b], Thammarat Aree [c], Narongsak Chaichit [d], Somsak Ruchirawat [a,b,e]

[a] Laboratory of Medicinal Chemistry, Chulabhorn Research Institute, Lak Si, Bangkok 10210, Thailand
[b] Program on Chemical Biology, Chulabhorn Graduate Institute, Lak Si, Bangkok 10210, Thailand
[c] Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand
[d] Department of Physics, Faculty of Science and Technology, Thammasat University, Pathum Thani 12121, Thailand
[e] The Center of Excellence on Environmental Health, Toxicology and Management of Chemicals, Lak Si, Bangkok 10210, Thailand

ARTICLE INFO

Article history:
Received 19 July 2011
Revised 28 August 2011
Accepted 7 September 2011
Available online 10 September 2011

Keywords:
Nitrogen heterocycles
Nucleophilic substitution
2,7-Naphthyridine
Lophocladine

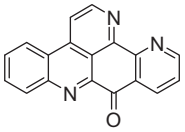
ABSTRACT

A novel approach for the syntheses of lophocladines A and B has been developed. These compounds were prepared in 4–6 steps with moderate to excellent overall yields. The key step involved the nucleophilic substitution of 4-chloronicotinic acid with the carbanion generated from phenylacetonitrile. Subsequent reduction of the cyano group, lactamization and oxidation furnished lophocladine A in 50% yield over 4 steps. Further amination with various amines led to lophocladine B and its C1 analogues in good yields. In addition, the synthesized compounds were evaluated for their cytotoxicity against leukaemia cells.

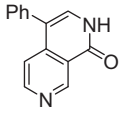
Naphthyridine derivatives have prompted considerable scientific interest because of their broad spectrum of application in the pharmaceutical sciences, agriculture and chemical industry.[1] In a number of studies, the 2,7-naphthyridine structural fragment was found to be a component of various polycyclic alkaloids such as the marine alkaloid ascididemine (**1**) (Fig. 1) which exhibits a wide range of pharmacological activity.[2a] Recently, Gross et al. isolated the marine algal alkaloids, lophocladines A (**2**) and B (**3a**), from a red alga, *Lophocladia* sp.[3] These 2,7-naphthyridines exhibited different modes of biological activity as observed from their preliminary screening. Lophocladine A showed affinity for the NMDA (*N*-methyl-D-aspartic acid) receptor while also exhibiting δ-opioid receptor antagonist activities. On the other hand, lophocladine B exhibited cytotoxicity towards human lung tumour cells and breast cancer cells.[3] Although these compounds were found to be of biological interest,[2b,2c] synthetic accessibility has been limited.[1,4] To date, only two groups have reported the total synthesis of lophocladines A and B via a similar key step reaction, namely cyclisation of enamines generated from 4-benzyl- or 4-methyl-3-cyanopyridine[5] and Bredereck's reagent.[6] Thus, despite the earlier approaches, a new and more efficient synthetic route is desirable.

Addition of nucleophiles to the α- and γ-position of π-electron deficient *N*-heterocyclic compounds[7] is an important method for the synthesis of functionalized compounds and has been used in a number of syntheses.[8] An intermolecular version of this reaction could provide a valuable route to further transformations such as second ring fusion to the napthyridine ring system. Herein, we report the application of this methodology for the synthesis of lophocladines A and B.

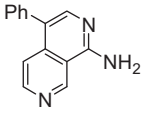
At the outset, unprotected 4-chloronicotinic acid (**4**)[9] was treated with a solution of 2-phenylacetonitrile under various conditions. After some experimentation, we found that this reaction required 1.5 equiv of 2-phenylacetonitrile and 2 equiv of potassium *tert*-butoxide (KO^tBu) in *N*-methylpyrrolidone (NMP) at 130 °C for 30 min and gave cleanly the desired product **5** in good yield (65%). We also examined the use of microwave irradiation (MW) for this reaction and found that the best result (85% yield) was obtained using conditions at 110 °C and 30 W for 4 min. As a consequence of this successful procedure, we turned our attention to the preparation of nicotinate **6**, as shown in Scheme 1. The most effective conditions for the methylation of **5** were found to involve



ascididemine (**1**)



lophocladine A (**2**)

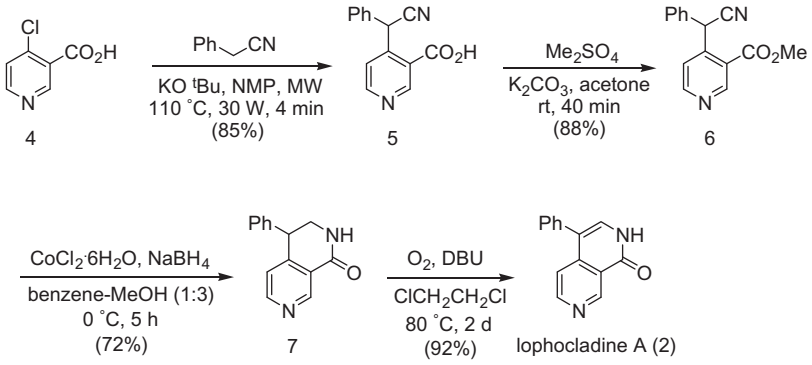


lophocladine B (**3a**)

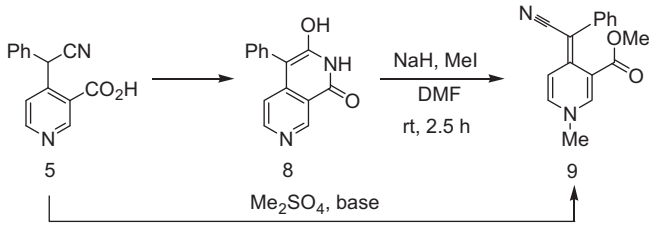
Figure 1. Examples of naturally occurring bioactive compounds containing the 2,7-naphthyridine ring system.

* Corresponding author. Tel.: +66 2 574 0622; fax: +66 2 574 0622x1513
E-mail address: wannapor@cri.or.th (W. Disadee).

0040-4039/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2011.09.032



Scheme 1. Synthesis of lophocladine A (**2**).



Scheme 2. Base-mediated dearomatization of naphthyridone **8**.

the reaction in acetone with a stoichiometric amount of K_2CO_3 and dimethyl sulfate (Me_2SO_4)[10] at room temperature for 40 min. The yields decreased slightly with the weaker bases $NaHCO_3$ and Na_2CO_3, due to incomplete methylation. For the next step, we investigated the lactamization and subsequent oxidation of **7** to lophocladine A. We first explored the reduction of **6** using catalytic hydrogenation (Pd/C or PtO_2), however, these methods failed to reduce the cyanide group, even under high pressures of H_2 (75 psi), and products of pyridine ring hydrogenation were observed. After some experimentation, we found that **6** could be converted into **7** by using $NaBH_4$ and $CoCl_2$ in a solvent mixture of benzene and MeOH.[11] The cyclised product **7** was obtained in 72% yield. With the naphthyridine framework in hand, we further oxidized compound **7** into laphocladine A (**2**) using various reagents: 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ), KO^{t}Bu/O_2, and 1,8-diazabicycloundec-7-ene (DBU)/O_2. In general, the best results were obtained using strong bases in the presence of O_2 (KO^{t}Bu in DMF at rt for 1 h, 87%; or DBU in dichloroethane at 80 °C for 2 days, 92%).

It should be noted that compound **5** was unstable and slowly cyclised into **8** in a polar solvent such as dimethylsulfoxide (DMSO) (Scheme 2).[12] In general, nitrile **5** could be prepared in good yields, despite being contaminated with the cyclised product **8** in <5% yield. Transformation of **8** into lophocladine A (**2**) was possible via palladium-catalyzed deoxygenation, however, preparation of the *O*-triflate proved to be problematic. The reaction of **8** in the presence of NaH in DMF at room temperature gave complete conversion into an intermediate that was trapped with iodomethane (MeI) to give *N*-methyl-1,4-dihydropyridine **9** in good yield (91%).[13] The structure of this dearomatized product was confirmed by X-ray crystallographic analysis (Figure 2).[14] Thus, the preparation of **6** was carefully carried out under controlled conditions to avoid the formation of **9**.

Having successfully developed a route for the synthesis of lophocladine A (**2**), we subsequently synthesized lophocladine B (**3a**) and its derivatives using the known chlorination/amination method shown in Table 1. Chlorination of **2** was performed using

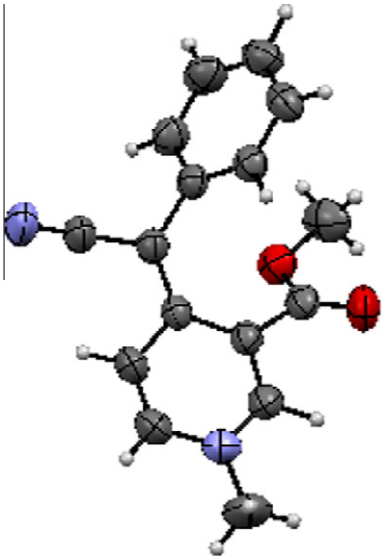
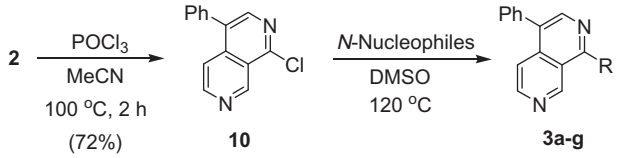


Figure 2. ORTEP structure of compound **9**.

Table 1
Synthesis of lophocladine B (**3a**) and its C1 analogues



Entry	R	Product	Yield[a] (%)
1	NH_2	**3a**	48[b]
2	HN Ph	**3b**	95
3	HN	**3c**	96
4	HN	**3d**	98
5	N O	**3e**	98
6	N_3	**3f**	97
7	MeONMe	**3g**	89

[a] Isolated yield.
[b] Two-step yield (see Supplementary data).

phosphorus oxychloride ($POCl_3$) and the desired product **10** was obtained in good yield (72%).[15] In general, lophocladine derivatives **3b-g** were prepared in excellent yields.

Table 2
Cytotoxic activities

Entry	Compound	IC_{50} (μM)[a]	
		MOLT-3	HL-60
1	**2**	> 150	> 150
2	**3a**	32	45
3[b]	**3b**	24	51
4[b]	**3c**	59	> 150
5	**3d**	27	43
6[b]	**3e**	> 150	> 150
7	**3f**	> 150	> 150
8	**3g**	80	> 150
9[b]	**10**	31	17
10	**VP-16**	0.04	1.3

[a] XTT assay. The IC_{50} values are given as the mean of three duplicate experiments.
[b] Compounds did not completely dissolve in the test medium.

Lophocladines A, B and C1 analogues were evaluated for in vitro cytotoxic activity against human acute promyelocytic leukaemia (HL-60)[16] and mouse acute lymphoblastic leukaemia (MOLT-3) cell lines using an XTT [2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide] assay. As shown in Table 2, although all the compounds were less potent than the reference compound (etoposide, **VP-16**), the order of potency as a function of the naphthyridine C1 position was halogen, primary- and secondary-amine > *tert*-amine, suggesting that this position is also important for enhanced cytotoxic activity.

In conclusion, we have developed a novel method for the preparation of lophocladine A and B, as well as its C1 analogues and have evaluated their cytotoxicity. Our strategy can be applied for the synthesis of other C4 analogues using commercially available heteroaromatic aldehydes to generate various cyano derivatives.

Acknowledgments

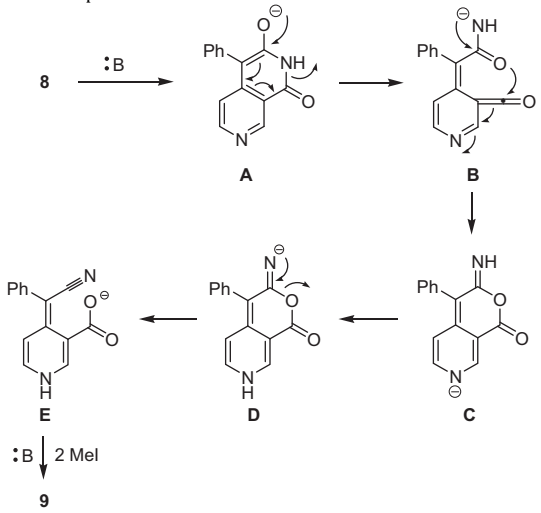
The authors gratefully acknowledge all researchers from the Central Facilities, the Chulabhorn Research Institute for the evaluation of cytotoxic activity. The authors would like to thank Dr. M. Paul Gleeson, the Kasetsart University, for careful reading of the final manuscript. This work was partially supported by the Thailand Research Fund (Grant No. RTA 4880008 to N.C. and T.A.; TRG 5280001 to W.D.), Chulabhorn Research Institute and the Center of Excellence on Environmental Health, Toxicology and Management of Chemicals, Thailand.

Supplementary data

Supplementary data (General methods, experimental procedures, spectroscopic data, copies of ^1H and ^{13}C NMR of all new compounds and crystallographic information (CIF) for compound **9**.) associated with this paper can be found, in the online version, at doi:10.1016/j.tetlet.2011.09.032.

References and notes

1. For a review on naphthyridines, see: Litvinov, V. P.; Roman, S. V.; Dyachenko, V. D. *Russ. Chem. Rev.* **2001**, *70*, 299–320.
2. For reviews on marine alkaloids, see: (a) Molinski, T. F. *Chem. Rev.* **1993**, *93*, 1825–1838; (b) Sithranga Boopathy, N.; Kathiresan, K. *J. Oncol.* **2010**, *2010*, 214186; (c) Güven, K. C.; Percot, A.; Sezik, E. *Mar. Drugs* **2010**, *8*, 269–284.
3. Gross, H.; Goeger, D. E.; Hills, P.; Mooberry, S. L.; Ballantine, D. L.; Murray, T. F.; Valeriote, F. A.; Gerwick, W. H. *J. Nat. Prod.* **2006**, *69*, 640–644.
4. For a recent synthesis of 2,7-naphthyridine-containing analogues, see: Dai, X.; Cheng, C.; Ding, C.; Yao, Q.; Zhang, A. *Synlett* **2008**, 2989–2992.
5. 4-Benzyl-3-cyanopyridine and 4-methyl-3-cyanopyridine were prepared in low to good overall yields, see: (a) Pfleger, K.; Fuchs, W.; Pailer, M. *Monatsh. Chem.* **1978**, *109*, 597–602; (b) Cameron, D. W.; Deutscher, K. R.; Feutrill, G. I.; Hunt, D. E. *Aust. J. Chem.* **1982**, *35*, 1451–1498; (c) Shing, T.-L.; Chia, W.-L.; Shiao, M.-J.; Chau, T.-Y. *Synthesis* **1991**, 849–850; (d) Reimann, E.; Wichmann, P.; Höfner, G. *Sci. Pharm.* **1996**, *64*, 637–646.
6. (a) Lotter, M.; Schilling, J.; Reimann, E.; Bracher, F. *Arch. Pharm. Chem. Life Sci.* **2006**, *339*, 677–679; (b) Zhang, A.; Ding, C.; Cheng, C.; Yao, Q. *J. Comb. Chem.* **2007**, *9*, 916–919.
7. For previous studies on nucleophilic substitution of *N*-heteroaromatic compounds, see: (a) Yin, Z.; Zhang, Z.; Kadow, J. F.; Meanwell, N. A.; Wang, T. *J. Org. Chem.* **2004**, *69*, 1364–1367; (b) Cherng, Y.-J. *Tetrahedron* **2002**, *58*, 4931–4935; (c) Sommer, M. B.; Begtrup, M.; Boegesoe, K. P. *J. Org. Chem.* **1990**, *55*, 4817–4821; (d) Yamanaka, H.; Ohba, S. *Heterocycles* **1990**, *31*, 895–909.
8. For selected publications, see: (a) Seto, M.; Aramaki, Y.; Imoto, H.; Aikawa, K.; Oda, T.; Kanzaki, N.; Iizawa, Y.; Baba, M.; Shiraishi, M. *Chem. Pharm. Bull.* **2004**, *52*, 818–829; (b) Adam, S. *Tetrahedron* **1991**, *47*, 7609–7614; (c) Sakamoto, T.; Kondo, Y.; Yamanaka, H. *Chem. Pharm. Bull.* **1985**, *33*, 626–633.
9. Due to its stability and ease of preparation, 4-chloronicotinic acid (**4**) was used instead of other halo compounds. 4-Iodonicotinic acid and 4-bromopyridine were found to be unstable and easily underwent polymerization, see: (a) Lazaar, J.; Rebstock, A.-S.; Mongin, F.; Godard, A.; Trécourt, F.; Marsais, F.; Quéguiner, G. *Tetrahedron* **2002**, *58*, 6723–6728; (b) Feast, W. J.; Tsibouklis, J. *Polym. Int.* **1994**, *35*, 67–74.
10. Dimethyl sulfate was used instead of iodomethane due to ease of handling.
11. For selected publications on $NaBH_4/CoCl_2$ reduction of nitriles, see: (a) Bhat, A. S.; Gervay-Hague, J. *Org. Lett.* **2001**, *3*, 2081–2084; (b) Gaucher, A.; Zuliani, Y.; Cabaret, D.; Wakselman, M.; Mazaleyrat, J.-P. *Tetrahedron: Asymmetry* **2001**, *12*, 2571–2580; (c) Zhang, F.-Y.; Corey, E. J. *Org. Lett.* **2000**, *2*, 1097–1100; (d) Osby, J. O.; Heinzman, S. W.; Ganem, B. *J. Am. Chem. Soc.* **1986**, *108*, 67–72.
12. This type of reaction is not well known and only one example was found in the literature, see: Huffman, J. *J. Heterocycl. Chem.* **1987**, *24*, 549–553.
13. It is interesting to note that compound **9** was found in $CDCl_3$ solution as an equilibrating mixture of two diastereomers (ratio = 1:4 at 23 °C, and 1:3 at 50 °C). The assignment of the major isomer was strongly supported by the shielded OMe resonance (δ 2.99), due to the anisotropic effect, with respect to that of the minor isomer (δ 3.92). A proposed mechanism for the base-mediated dearomatization of naphthyridone **8** to dihydropyridine **9** is shown below. The reaction is probably based on the formation of a ketene intermediate **B**, which undergoes cyclisation via an intramolecular reaction into **C**. Subsequent lactone ring-opening and trapping of the intermediate **E** with the electrophile leads to **9**.



14. Complete crystallographic data for compound **9** have been deposited with the Cambridge Crystallographic Data Centre, CCDC No. 728653.
15. For selected publications on $POCl_3$ chlorination of *N*-heteroaromatic compounds, see: (a) England, D. B.; Padwa, A. *Org. Lett.* **2008**, *10*, 3631–3634; (b) Cappelli, A.; Giuliani, G.; Anzini, M.; Riitano, D.; Giorgi, G.; Vomero, S. *Bioorg. Med. Chem.* **2008**, *16*, 6850–6859; (c) Vanejevs, M.; Jatzke, C.; Renner, S.; Mueller, S.; Hechenberger, M.; Bauer, T.; Klochkova, A.; Pyatkin, I.; Kazyulkin, D.; Aksenova, E.; Shulepin, S.; Timonina, O.; Haasis, A.; Gutcaits, A.; Parsons, C. G.; Kauss, V.; Weil, T. *J. Med. Chem.* **2008**, *51*, 634–647.
16. Lotter previously reported that lophocladine B showed cytotoxic activity against HL-60 in an MTT assay with IC_{50} = 1 μM, see: Ref. 6a.