Competent route to the unsymmetric dimer architectures: Total syntheses of (−)-lycodine, (−)-complanadines A and B, and evaluation of their neurite outgrowth activities

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Abstract: Valuable synthetic routes to the *Lycopodium* alkaloid lycodine (**1**), and its unsymmetric dimers, complanadines A (**4**) and B (**5**) were developed. Regioselective construction of the bicyclo[3.3.1]nonane core structure of lycodine was achieved by a remote functionality-controlled Diels-Alder reaction and the subsequent intramolecular Mizoroki-Heck reaction. A key coupling reaction of the lycodine units, pyridine *N*-oxide (**66**) and aryl bromide (**65**), through C-H arylation at the C1 position of **66** provided the unsymmetric dimer structure in the late stage of the synthesis. This strategy greatly simplified the construction of the dimeric architecture and functionalization. Complanadines A (**4**) and B (**5**) were synthesized by adjusting the oxidation level of the bipyridine mono-*N*-oxide (**67**). The diverse utility of this common intermediate (**67**) suggests a possible biosynthetic pathway of complanadines in nature. Both enantiomers of lycodine (**1**) and complanadines A (**4**) and B (**5**) were prepared in sufficient quantities for biological evaluation. The effect on neuron differentiation of PC-12 cells upon treatment with culture medium in which human astrocytoma cells has been cultured in the presence of **1**, **4**, or **5**, was evaluated.

Introduction

The *Lycopodium* alkaloids with a complex C_{16} tri- or tetracyclic skeleton were originally identified in the family of club moss *Lycopodium*[1] and more than 250 congeners have been reported. They often show intriguing biological activities.[2] Lycodine (**1**), one of the oldest member of the *Lycopodium* alkaloids, was isolated from *L. annotinum* by Anet and Eves (Figure 1).[3a] The structure of **1** was determined using spectroscopic methods (^{1}H NMR and IR) and derivatization from the related *Lycopodium* alkaloid β-obscurine (**2**).[3b] Lycodine contains a bicyclo[3.3.1]nonane skeleton attached by pyridine and piperidine rings. The absolute stereochemistry was assumed to be identical with that of the structurally related lycopodine (**3**), which was determined by X-ray crystallography.[4a,b] Complanadine A (**4**), isolated from *Lycopodium complanatum* by Kobayashi and co-workers in 2000 is the first dimeric alkaloid of the lycodine family.[6a] The compound (**4**) featuring an unsymmetric C1-C2‘ linkage[7] between the two lycodine units was reported to induce secretion of neurotropic factors from human astrocytoma (1321N1) cells, which promoted neuronal differentiation of PC-12 cells.[6b] These phenomena are promising for the treatment of Alzheimer‘s disease. Furthermore, **4** showed cytotoxicity against murine leukemia L1210 cells in vitro.[6a] While its congeners, complanadines B (**5**), D (**6**) and E (**7**) were also isolated,[6b,c,d] the scarcity of **5**–**7** has hampered extensive biological studies on their neurite outgrowth activity.[6b,c,d]

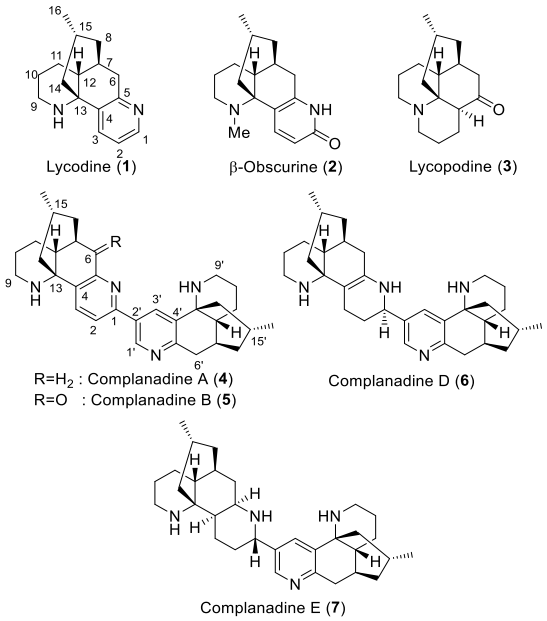

Figure 1. Lycodine, complanadines and structurally related *Lycopodium* alkaloids.

The Heathcock[5a] and Takayama groups[5b] reported the synthesis of racemic **1** and an enantioselective synthesis of **1**, respectively. Total synthesis of **4** was recently reported by the Sarpong[8a] and Siegel[9] groups. Sarpong and co-workers also reported a total synthesis of **5**,[8b] whereas the Lewis group reported a synthetic study toward complanadines.[10] We were also interested in the syntheses of **1** and its unsymmetric dimers **4** and **5**, because dimeric alkaloids often show distinct and/or improved biological activities when compared to those of the

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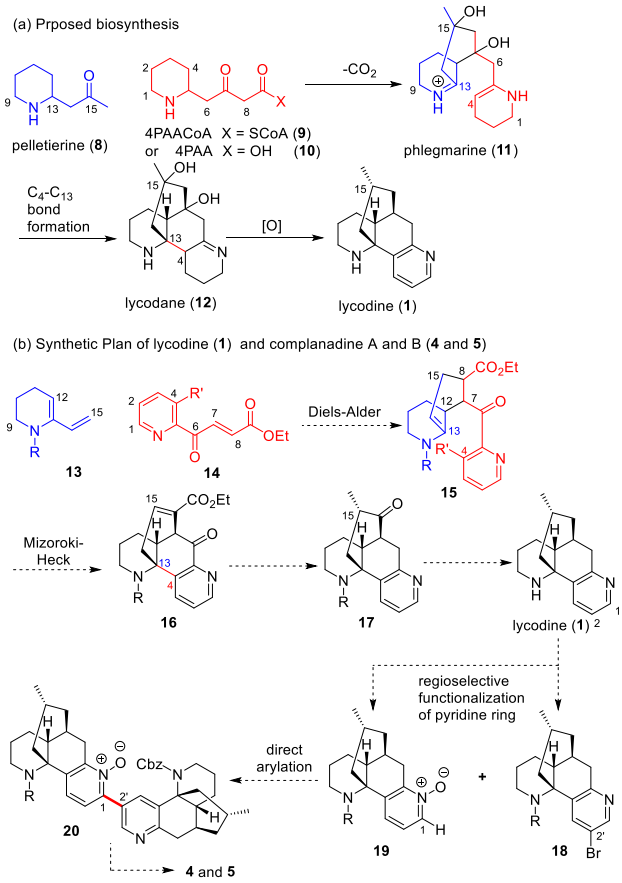
monomer.[11] Recently, we accomplished a total synthesis of **1**,[12a] and moreover, total syntheses of **4** and **5** based on a strategy inspired by the biosynthetic hypothesis.[12b] In this article, we describe the full details of our synthetic studies of these molecules and their enantiomers, which enabled evaluation of their neurite outgrowth activities for the first time.

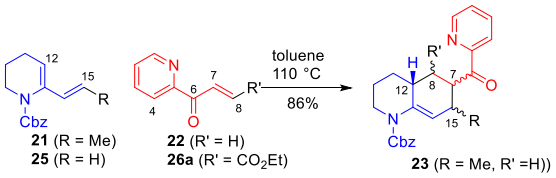
Key features of the biosynthetic hypothesis of **1** are i) dimerization of a pelletierine unit and ii) bond formation between C4 and C13 of the phlegmarine intermediate **11** (Scheme 1a).[13] Furthermore, complanadines (**4** and **5**) are derived from two units of **1**.[6b,14] Therefore, we envisaged that the Diels-Alder reaction of diene **13** and dienophile **14** would provide phlegmarine skeleton **15** (Scheme 1b). The intramolecular Mizoroki-Heck reaction[15] of **15** should give a bicyclo[3.3.1]nonane **16**.[16] Installation of the C15 methyl group would lead to **17** and then to **1**. Coupling of *m*-bromopyridine **18** and pyridine *N*-oxide **19** via C1 C–H activation[17] should afford bipyridyl mono-*N*-oxide **20** designed as a common intermediate for the syntheses of complanadines.



Scheme 1. Proposed biosynthesis of lycodine, and synthetic plan of lycodine and complanadine A and B.

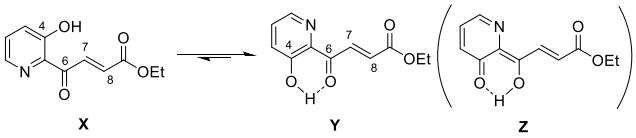
Diels-Alder reaction for construction of phlegmarine skeleton

Since the Diels-Alder reaction of diene **21**[18] with α,β–unsaturated ketone **22** only led to undesired regioisomer **23** as anticipated (Scheme 2), an electron withdrawing ester group was added at C8 as dienophile **26a** to reverse the regioselectivity.[19] Additionally, to facilitate NMR analysis of the Diels-Alder adducts, diene **25** was employed for the reaction instead of **21**.



Scheme 2. Diels-Alder reaction of **21** and **22**.

The Diels-Alder reaction of **25**[18] and **26a**[12a] proceeded smoothly in toluene at 110 °C, while thermodynamically stable tetrasubstituted olefins **27aE** and **27aF** were obtained through double bond isomerization (Table, entry 1). For avoiding this undesired double bond isomerization, several dienophiles were screened. While introduction of a TfO group at the C4 position was not effective, the presence of methoxy (at C1) or hydroxyl (at C4) groups suppressed this isomerization (entries 2–5). For the reaction of **26c**, no regioselectivity was observed, while a dienophile with a bromine atom at C4 further favored the undesired regioisomer (entries 3, 4). These results indicate that the electron density of the pyridine ring did not perturb the olefin of the dienophile. On the other hand, introduction of a hydroxyl group improved the selectivity (entry 5). To discern the origins of this selectivity, we conducted a DFT calculation for the most stable conformation of **26e**.[20] It was found that conformer **Y**, which was stabilized by a hydrogen bond, was the most stable (Scheme 3). Considering the resonance of conformer **Y** (i.e., **Z**), introduction of the hydroxyl group at C4 reduces the electron withdrawing property of the C6 carbonyl carbon and improves selectivity. The obtained four diastereomers **27eA–D** (R^1 = OH, R^2 = H) were carefully separated by silica gel column chromatography and their relative stereochemistries were determined by extensive NMR analysis, including NOESY experiments.



Scheme 3. Resonance conformers of **26e**.

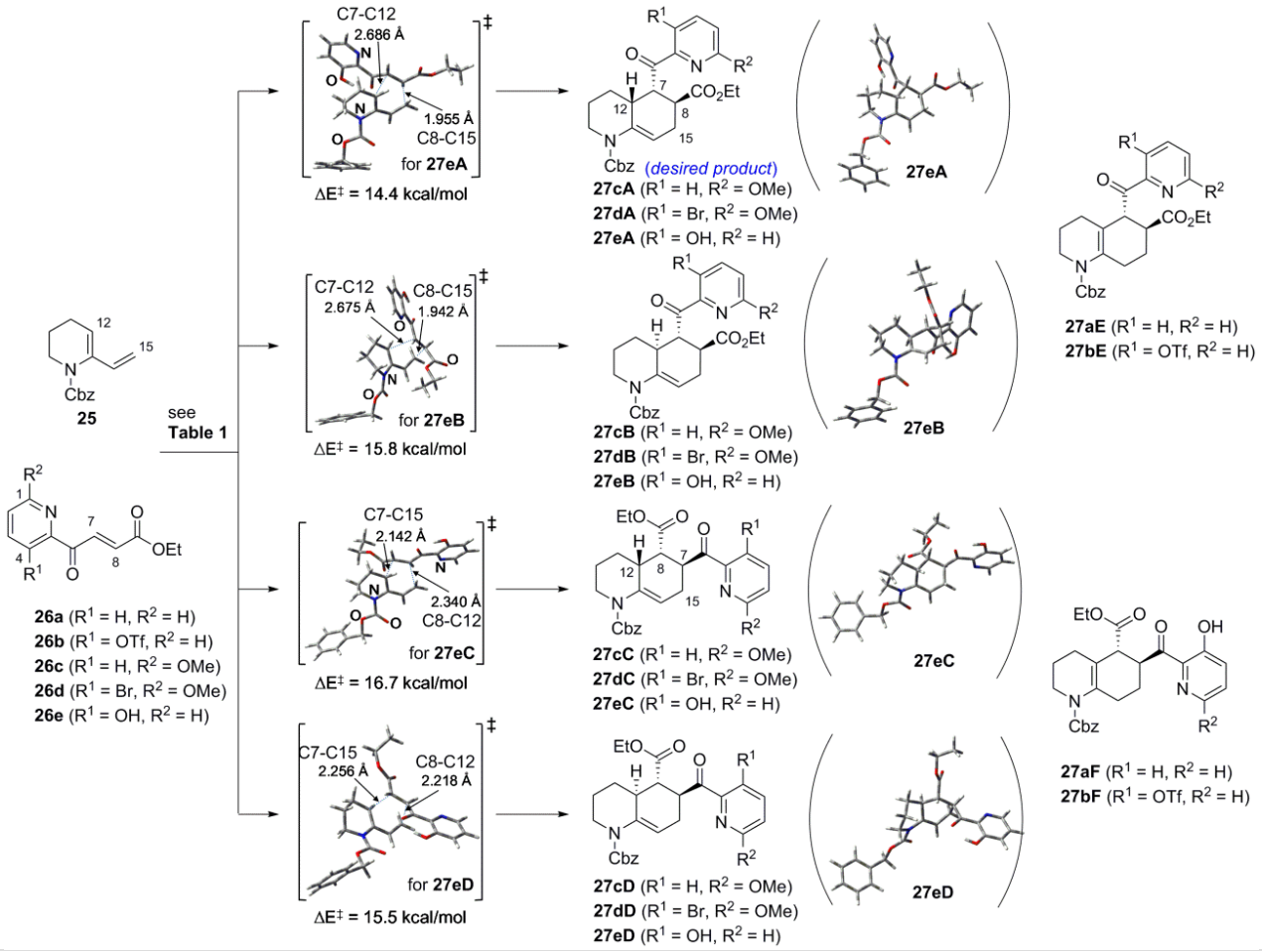


Table 1. Diels-Alder reaction of **25** and **26a-e** and optimized transition states for **25** and **26e** at the B3LYP/6-31G(d) level.

Entry	Dienophile	R^1	R^2	Conditions	Yield (%)				Regioselectivity (**27xA**+**27xB**):(**27xC**+**27xD**)
					27xA	**27xB**	**27xC**	**27xD**	
1	**26a**	H	H	Toluene, 110 °C	**27aE**: 40		**27aF**: 31		1.3:1
2	**26b**	OTf	H	Toluene, 110 °C	**27bE**: 34		**27bF**: 33		1.0:1
3	**26c**	H	OMe	Toluene, 110 °C	22	12	13	19	1.1:1
4	**26d**	Br	OMe	Toluene, 110 °C	14	19	19	29	0.7:1
5	**26e**	OH	H	Toluene, 110 °C	45	15	6	21	2.2:1
6	**26e**	OH	H	Pyridine, 80 °C	32	15	8	13	2.2:1
7	**26e**	OH	H	Ethanol, 80 °C	44	11	10	17	2.0:1
8	**26e**	OH	H	CH_3CN, 80 °C	51	11	10	19	2.1:1
9	**26e**	OH	H	CH_3CN, RT	52	7	9	14	2.6:1
10	**26e**	OH	H	$BF_3 \cdot OEt_2$[b], CH_2Cl_2, -78 °C	7	1	4	12	0.5:1
11	**26e**	OH	H	K_2CO_3[b], CH_3CN, RT	12	1	2	3	2.6:1
12	**26e**	OH	H	Et_3N[c], CH_3CN, 0 °C	22	4	5	7	2.2:1
13	**26e**	OH	H	$NaH_2PO_4 \cdot 2H_2O$[b], CH_3CN, RT	56	6	9	17	2.4:1

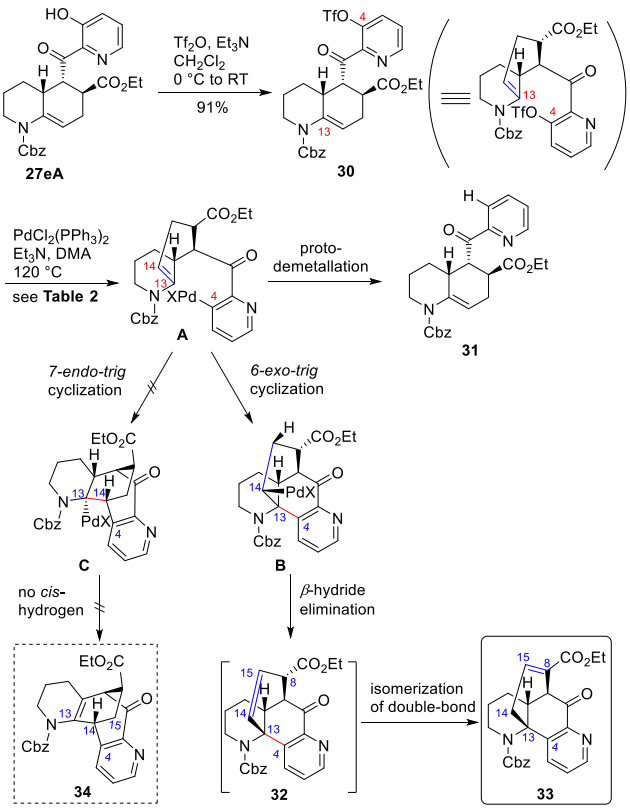
[a] Isolated yield. [b] 1 equiv. [c] 0.05 equiv.

We then investigated solvents, temperatures and additives using diene **25** and dienophile **26e**. Using pyridine, ethanol and acetonitrile at 80 °C resulted in similar selectivity (entries 6–8). These results were consistent with DFT calculations, which indicated that conformer **Y** is the most stable species in these solvents. This Diels-Alder reaction in acetonitrile proceeded at room temperature with better selectivity (entry 9). Addition of a Lewis acid, such as $BF_3 \cdot OEt_2$, resulted in low yield and selectivity, probably because the carbopyridyl moiety is a better Lewis acid acceptor than the ester (entry 10). Furthermore, decomposition of **26e** was also observed under these conditions. Various bases and inorganic salts were also examined.[21] Use of K_2CO_3 or Et_3N did not improve the yield or selectivity due to the low stability of dienophile **26e** under basic conditions (entries 11, 12). Addition of a weak Brønsted acid, $NaH_2PO_4 \cdot 2H_2O$, was found to suppress effectively the decomposition of **26e** and resulted in an improved yield of the desired product **27eA** (entry 13). The optimized conditions ($NaH_2PO_4 \cdot 2H_2O$, CH_3CN, rt) were applied successfully to a large scale synthesis of the desired adduct **27eA**.

To rationalize this selectivity, the activation energy of this reaction was estimated by DFT calculations at the B3LYP/6-31G(d) level. The activation energies for **27eA**–**D** were $\Delta E^{\ddagger}$ = 14.4, 15.8, 16.7, 15.5 kcal/mol, respectively (Table 1). These results are consist with the ratio of the products (i.e., entry 5) and indicated that the transition state for the desired product **27eA** was the most favorable among the isomers. Additionally, it was found that the distance between C7 and C12, C8 and C15 were 2.686 and 1.955 Å, respectively. These results suggested that this Diels-Alder reaction proceeds through a stepwise process rather than a concerted mechanism. It is noted that *endo*/*exo* selectivity was explained by secondary interactions of the π-orbital of the pyridyl ketone with the diene, which was larger than that of an ester's LUMO, according to DFT calculations of the transition state for **27eA** (Table 1). Therefore, the transition state, in which the pyridyl ketone and ester are in the *endo* and *exo* positions, respectively, was favored to produce the desired adduct **27eA** as the major product.

Construction of the Tetracyclic Core and Total Synthesis of Lycodine

After triflation of **27eA**, we investigated the Mizoroki-Heck reaction of compound **30** for construction of the tetracyclic core (Scheme 4). In our initial attempts, the reaction using a catalytic amount of tetrakis(triphenylphosphine)palladium ($Pd(PPh_3)_4$) and triethylamine in dimethylformamide (DMF) did not proceed at 100 °C. We assumed that the oxidative addition of compound **30** would require higher temperature, thus **30** was treated with $Pd(PPh_3)_4$ in DMF at 120 °C. Predictably, the desired *6-exo-trig* cyclization proceeded to produce tetracyclic compound **32**, which was immediately isomerized to the conjugated unsaturated ester **33** in 32% yield with a significant amount of reduced compound **31** (30%). The regioselectivity was exclusive, and the *7-endo-trig* product **34** was never observed under these conditions. It is rationalized that the *6-exo-trig* cyclization via **B** would be preferable than the *7-endo-trig* cyclization via **C** due to electronic factors in the hetero-substituted olefin and difficulty of β-hydride elimination from **C**.[22] To suppress the production of by-product **31**, a few catalysts and solvents were screened. The use of $PdCl_2(PPh_3)_2$ in dimethylacetamide (DMA) were found to be effective. Additionally, use of $PdCl_2(PPh_3)_2$ reduced an amount of phosphine oxide as by-product, thus facilitating purification. However, these optimized conditions were not reproducible over the concentration of 0.1 M. For example, the 0.1 M reaction in DMA resulted in quick precipitation of Pd black and recovery of a significant amount of starting material (Table 2, entry 1). These results indicated that the palladium catalyst was deactivated before oxidative addition of triflate **30** because two pyridyl ketone moieties can coordinate to the palladium and accelerate precipitation of the palladium black. Thus, the reaction was carried out under high dilution conditions (entries 2–4). Consequently, the reaction at 0.005 M of DMA provided **33** in 73% yield with good reproducibility.



Scheme 4. Construction of tetracyclic core through intramolecular Mizoroki-Heck reaction of **30**.

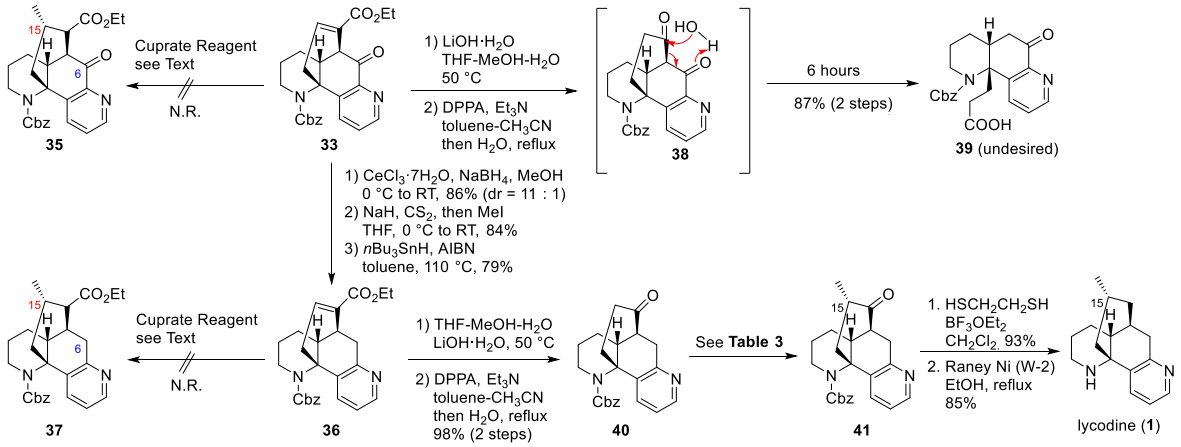
Table 2. Concentration effect of intramolecular Mizoroki-Heck reaction of **30**.

entry	Reaction time (h)	Concentration of **30** (M)	Yield of **33**[a] (%)
1	48	0.1	18
2	22	0.05	42
3	24	0.01	61
4	24	0.005	73

[a] Isolated yield.

With the tetracyclic core **33** in hand, we focused on (i) introduction of the C15 methyl group, (ii) reduction of the ketone to a methylene, and (iii) removal of the ethyl ester moiety for the total synthesis of racemic lycodine. We initially attempted 1,4-addition of cuprate reagents into the unsaturated ester of **33** to introduce the C15 methyl group. However, the reaction with various organocopper reagents (MeCu+TMSI, MeCu+TMSCl, Me_2CuLi+TMSCl, Me(Th)CuLi+TMSCl)[23] did not furnish the 1,4-addition product **35**, and the starting material **33** was recovered (Scheme 5).[24] The pyridyl ketone moiety might be problematic due to coordination with the cuprate reagent. Therefore, the ketone of **33** was reduced by a three-step transformation, including Luche reduction[25] and Barton-McCombie deoxygenation.[26] **36** was treated with methyl cuprate reagents again to access compound **37**; however, these attempts only resulted in recovery of the starting material. We concluded that this is presumably due to the low reactivity of the α,β-unsaturated ester rather than coordination of pyridine to the cuprate reagent.[27] Thus, we switched tasks to the removal of the ethyl ester moiety by hydrolysis and decarboxylation by Curtius rearrangement.[28] Compound **33** was hydrolyzed under basic conditions to give a carboxylic acid, which was treated with diphenylphosphoryl azide (DPPA) and then water for 30 min. While **38** was obtained in a 30% yield, a prolonged reaction time (6 h) resulted in retro-Claisen[29] fragmentation of the 1,3-diketone moiety of **38** to give thermally stable **39** in 87% yield. To prevent this undesired side-reaction, the deoxygenated compound **36** was used and converted to ketone **40** in 98% yield over two steps using the same sequence.

Introduction of the C15 methyl group was then investigated using enolate chemistry. Treatment of **40** with LiHMDS, then followed by iodomethane gave a trace amount of the desired product with recovery of **40** (Scheme 5, Table 3, entry 1). Warming the reaction temperature did not improve the yield of methylated product **41**. This was presumably due to the formation of the undesired aggregate of the lithium enolate, which was not reactive. Thus, we examined a lithium-free enolate derived from a silyl enol ether, prepared from ketone **40** under standard conditions (LiHMDS, Et_3N, TMSCl, THF, –78 °C). Following Noyori's report,[30a] the silyl enol ether was treated with iodomethane, TASF and 4 Å molecular sieves, the reaction proceeded to give the desired methyl ketone **41** in 37% yield along with **40** (47%) and a small amount of dimethylated product (entry 2). After several screenings of fluoride sources, we found that benzyltrimethylammonium fluoride (BTAF) was the best fluoride source for this transformation (entries 3, 4).[30b] In our technique, removing water from BTAF would be easier than from the other fluoride sources. The stereochemistry of **41** was confirmed by extensive NMR spectroscopic analysis, including NOESY experiments (Figure 2). In the course of this reaction, no epimers at C15 were observed, because iodomethane approaches from the desired face to avoid the steric hindrance of the pyridine ring.



Scheme 5. Total synthesis of racemic lycodine (**1**).

Table 3. Introduction of the C15 methyl group.

entry	Conditions	Yield[a] (%)	
		41	**40**
1	LiHMDS, MeI, THF, –78 °C	5	52
2	1. LiHMDS, Et_3N, TMSCl, THF, –78 °C 2. MeI, TASF, MS 4 Å, THF, RT	37	47
3	1. LiHMDS, Et_3N, TMSCl, THF, –78 °C 2. MeI, TBAF, MS 4 Å, THF, RT	47	18
4	1. LiHMDS, Et_3N, TMSCl, THF, –78 °C 2. MeI, BTAF, MS 4 Å, THF, RT	64	14

[a] Isolated yield. TASF = Tris(dimethylamino)sulfonium difluorotrimethylsilicate, BTAF = benzyltrimethylammonium fluoride

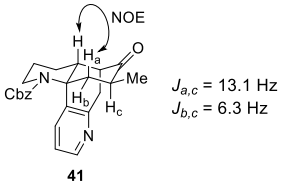
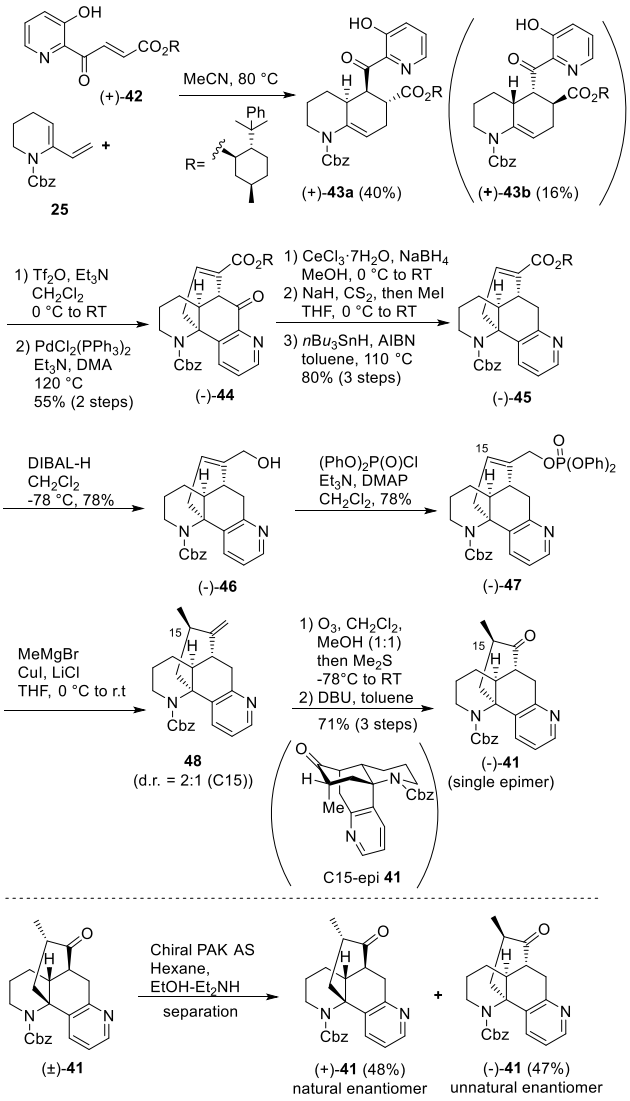


Figure 2. Stereochemistry of the C15 methyl group of **41**.

Finally, thioacetalization of methyl ketone **41** and subsequent one-pot reduction and deprotection of the Cbz group using Raney Ni (W-2) gave racemic lycodine (**1**). Spectroscopic (^{1}H and ^{13}C NMR, UV, IR)[31] and high-resolution mass spectrometric data of the synthetic sample were identical to those of the natural product. The established synthetic route, which is 15 steps from methyl 3-hydroxypicolinate, is robust and convenient for accessing the racemic lycodine and a protected monomer for synthesis of the complanadines.

Synthesis of Optically Pure Lycodine and Determination of Absolute Stereochemistry

Next, we turned our attention to the synthesis of the enantiomerically pure form of **41**, which is essential for the total synthesis of complanadines via dimerization. We employed a chiral auxiliary, which was installed to the ester moiety, because the hydroxyl group on pyridine is important for regioselectivity. The Diels-Alder reaction of diene **25** and dienophile (+)-**42** bearing the chiral auxiliary prepared from the natural (+)-pulegone,[32] proceeded smoothly in acetonitrile to provide (+)-**43a** as the major product in moderate yield (40%), which was separated from the other diastereomers by silica gel column chromatography (Scheme 6). The major adduct had the desired relative and unnatural absolute stereochemistry, which was determined later. The second major diastereomer was (+)-**43b** (16%), and the other diastereomers were obtained in less than 10% yields. Although the diastereoselectivity was moderate, enough material was obtained to continue the synthesis. Thus, (+)-**43a** was converted to tetracyclic (−)-**45** using the same sequence developed in the racemic synthesis. When (−)-**45** was treated under basic conditions such as LiOH in THF-MeOH-H_2O, and NaOEt in EtOH, it resisted solvolysis presumably due to steric hindrance. Thus, the chiral auxiliary of (−)-**45** was removed by reduction of the unsaturated ester using DIBAL-H. The absolute stereochemistry was determined at this stage. After oxidation of the obtained allyl alcohol (−)-**46**, Curtius rearrangement and diastereoselective reduction, the Kusumi-Mosher's method[33] was applied to the resultant secondary alcohol (not shown, see Supporting Information). The results indicated that the absolute stereochemistry derived from the Diels-Alder reaction was the unnatural type.



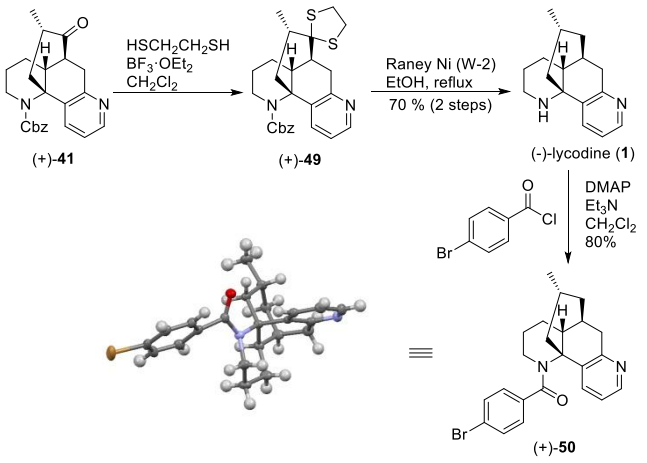
Scheme 6. Diels-Alder reaction of **25** and (+)-**42** with the chiral auxiliary and alternative routes to access optically active (−)-**41**.

With the enantiopure tetracyclic core (−)-**46** in hand, we synthesized the monomer **41** (unnatural). To avoid a lengthy route through re-oxidation of allyl alcohol (−)-**46** and decarboxylation, the C15 methyl group was introduced via γ–selective allylic alkylation of the allyl phosphate. Allyl alcohol (−)-**46** was converted to the corresponding allyl phosphate (−)-**47** in 78% yield. Treatment of (−)-**47** with low order methyl cuprate gave compound **48** as a 2:1 epimer at C15, which was inconsequential, while the regioselectivity is about γ:α = 3:1.[34] After ozonolysis of the mixture, epimerization using DBU proceeded smoothly to give the thermally stable methyl ketone (−)-**41** in 71% yield over three steps, because of the disfavored 1,3-diaxal interaction in C15-epi **41**.

As an alternative method, racemic **41** could be separated into its optically active forms using chiral high-performance liquid chromatography. Use of an amylose chiral column (Daicel Chiral-Pak AS) with a mixture of ethanol/hexane/diethylamine (5.8%/94%/0.2%) as the eluent provided (+)- and (−)-**41** in 48 and 47% yields, respectively. This also provided sufficient quantities of material for further studies.

Thioacetalization, reduction and deprotection of the Cbz group as before resulted in completion of the total synthesis of both natural and unnatural lycodine ((−)-**1**, (+)-**1**) (Scheme 7). Spectroscopic (^{1}H NMR, ^{13}C NMR, UV, IR, optical rotation) and high-resolution mass spectrometric data of the synthetic sample were identical to those of the natural product.

Although the absolute stereochemistry had already been determined using the modified Kusumi-Mosher's method, the stereochemistry of synthetic natural type lycodine was further confirmed by X-ray crystallographic analysis. The synthetic (−)-lycodine **1** was treated with *p*-bromobenzoyl chloride and recrystallized from THF-hexane to give its *p*-bromobenzoate derived as a single crystal (+)-**50**. X-ray crystallographic analysis indicated that the synthetic sample was the natural type enantiomer. [4, 35]



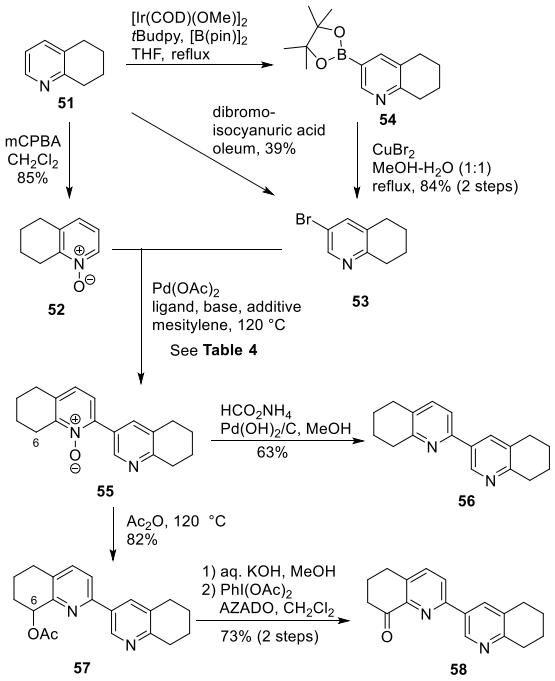
Scheme 7. Synthesis of (−)-lycodine (**1**) and confirmation of its absolute stereochemistry by X-ray crystallography. Thermal ellipsoids are shown at the 50% probability level.

Total Synthesis of Complanadines A and B

After completion of the total synthesis of lycodine (**1**), we investigated the challenging dimerization process. Biosynthetically, there are two possibilities: the intermediate is (i) the natural product itself, and (ii) a compound that is not isolated. Considering the unsymmetric structure of the bipyridyl linkage and the oxidation level state of the pyridine ring, we assumed that complanadines are biosynthetically produced through the latter possibility. Inspired by this idea, we chose bipyridine mono-*N*-oxide **20** as the more reasonable compound, which could readily be accessed from bromopyridine **18** and its pyridine *N*-oxide **19** using C-H activation chemistry (Scheme 1). Due to the unprecedented complexity of both the coupling partners **18** and **19**, tetrahydroquinoline **51** was employed as a model compound to establish feasible conditions for the functionalization of the pyridine ring and direct arylation via C-H activation of pyridine *N*-oxide. Pyridine *N*-oxide **52** was readily prepared by *m*CPBA oxidation of **51** (Scheme 8). Treatment of **51** with dibromoisocyanuric acid in oleum gave bromopyridine **53** in 39% yield as a single product.[36] Because the Cbz protecting group in monomer **41** would not be tolerated under these harsh conditions, we also employed Miyaura-Hartwig borylation[37] of **51** by using the procedure of Sarpong and co-workers.[8a] Subsequent bromination of **54** with $CuBr_2$ to give **53** in 84% improved the yield over two steps.[38]

With both coupling partners in hand, we examined the key C-H arylation reaction. Based on Fagnou's report,[17] **52** and **53** were treated with a catalytic amount of $Pd(OAc)_2$, tri-*tert*-butylphosphine tetrafluoroborate (tBu$_3$P·HBF$_4$) as ligand and potassium carbonate in mesitylene at 120 °C to give bipyridine mono-*N*-oxide **55** in 33% yield. After screening electron rich phosphine ligands, such as tricyclohexylphosphine tetrafluoroborate (Cy$_3$P·HBF$_4$) and 2-di-*tert*-butyl-phosphino-2'-(*N*,*N*-dimethylamino)biphenyl (*t*BuDavePhos), the latter gave a better yield. Addition of pivalic acid (PivOH) was effective.[39] Finally, employment of cesium carbonate improved the product yield significantly.

The coupling product **55** was reduced with palladium hydroxide on carbon and ammonium formate as a homogeneous hydrogen source to give the complanadine A model (**56**). By heating **55** in acetic anhydride, a sigmatropic rearrangement occurred to introduce an oxygen functionality at the C6 picolinic position.[40] The obtained **57** was further converted to the complanadine B model **58** by hydrolysis and oxidation.[41]



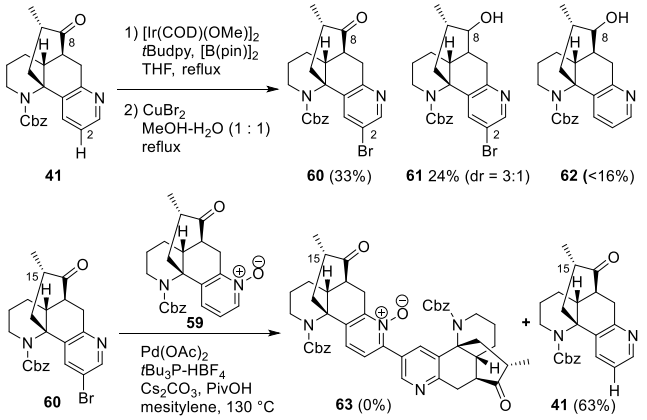
Scheme 8. Bipyridine mono-*N*-oxide framework construction using model compounds.

Table 4. Optimization of C-H arylation of pyridine *N*-oxide **52**.

Entry	Ligand	Additive	Base	Yield[a] (%)
1	tBu$_3$P· HBF$_4$	None	K_2CO_3	33
2	Cy$_3$P· HBF$_4$	None	K_2CO_3	37
3	*t*BuDavePhos	None	K_2CO_3	41
4	*t*BuDavePhos	PivOH	K_2CO_3	46
5	*t*BuDavePhos	PivOH	Cs_2CO_3	56

[a] Isolated yield.

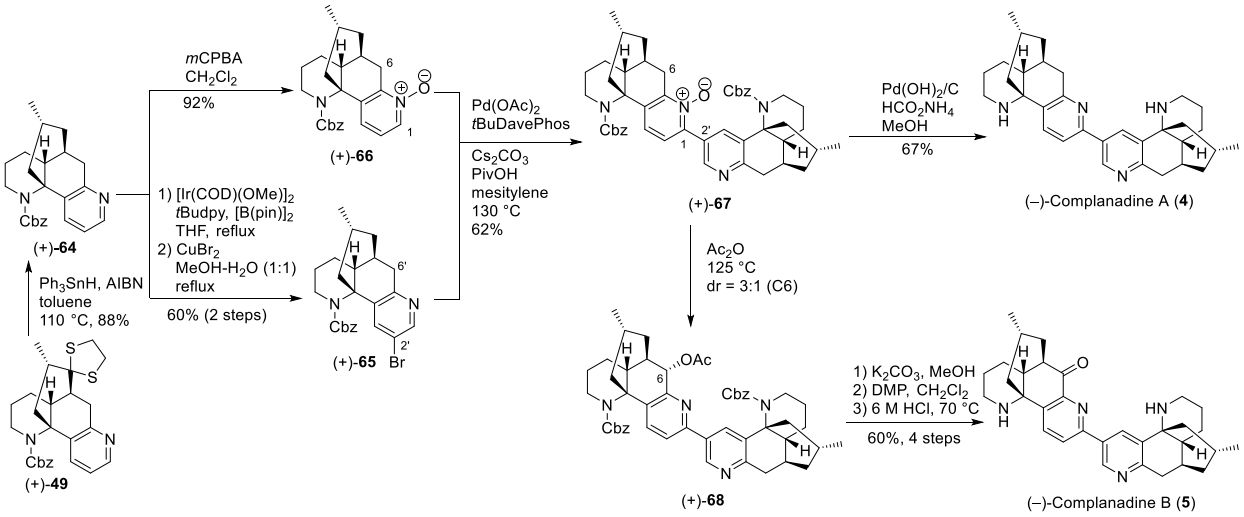
We initially applied this sequence to methyl ketone **41**. *m*CPBA oxidation gave pyridine *N*-oxide **59** in good yield, but the Miyaura-Hartwig borylation was problematic. Two-step bromination afforded *m*-bromopyridine **60** in only 33% yield along with alcohols **61, 62** and a small amount of by-product derived from bromination of the Cbz group (Scheme 9).[42] Coupling between **59** and **60** gave no desired product **63** under the conditions using tBu$_3$P· HBF$_4$; although, it was not the optimized ligand. The de-brominated compound **41** and unaffected pyridine *N*-oxide **59** were obtained. We further considered the mechanism of these problematic transformations. In the Miyaura-Hartwig borylation reaction, bis(pinacolato)diboron is converted to pinacolborane after C-H activation of pyridine, then the pinacolborane reduces the ketone to an alcohol.[37] In the case of palladium-catalyzed direct arylation, the reason why the reaction did not proceed is unclear; although, we suspect the presence of the C15 enolizable proton.



Scheme 9. Unsuccessful attempt of the bipyridyl mono-*N*-oxide framework construction using methyl ketone **41**.

Thus, the ketone was reduced to methylene through radical reduction[43] of thioacetal (+)-**49** and then the established sequence was applied to Cbz-protected lycodine (+)-**64** (Scheme 10). Miyaura-Hartwig borylation and bromination afforded bromopyridine (+)-**65** in 60% yield, with a small amount of by-product derived from bromination of the Cbz group. Pyridine *N*-oxide (+)-**66** was obtained in 92% yield by *m*CPBA oxidation of (+)-**64**. Treatment of (+)-**65** and (+)-**66** with 10 mol % of Pd(OAc)$_2$, 20 mol % of *t*BuDavePhos, cesium carbonate (3 equiv.) and PivOH (0.3 equiv.) in mesitylene at 130 °C furnished bipyridine mono-*N*-oxide (+)-**67** in 62% yield. This was one of the most difficult examples of the C-H coupling of a pyridine *N*-oxide in terms of molecular complexity. These results indicate that the C15 enolizable proton of **59** or **60** would have hampered the C(sp^2)-H activation process, and the benzylic proton at C6 did not affect the coupling of (+)-**65** and (+)-**66**.[17c] Following our model studies, coupling product (+)-**67** was reduced with palladium hydroxide on carbon and ammonium formate to give (−)-complanadine A (**4**). Rearrangement, basic hydrolysis, oxidation and deprotection completed the total synthesis of (−)-complanadine B (**5**). The ^{1}H, ^{13}C NMR data of the synthetic samples were identical to those of the natural products. For biological studies, unnatural enantiomers (+)-**4** and (+)-**5** were also synthesized from the common intermediate (−)-**41** in the same manner as their natural enantiomer.

It is noteworthy that our synthesis requires only one pair of monomers for the synthesis of two unsymmetric dimers. By designing an appropriate intermediate based on the C-H arylation reaction, we no longer need to prepare several monomers for each unsymmetric natural product. This fact suggests an intermediacy of a mono-*N*-oxide, such as **67**, in the biosynthesis of dimeric alkaloids.



Scheme 10. Total synthesis of complanadines A (**4**) and B (**5**).

Evaluation of Biological Activity

Because several compounds including antipodes of natural products were obtained, we examined the effect of these compounds on the neuronal differentiation of rat pheochromocytoma (PC12) cells. Human astrocytoma (1321N1) cells were incubated for 48 h with lycodine, complanadines A and B and their enantiomers ((−)-**1**, (−)-**4**, (−)-**5**, (+)-**1**, (+)-**4**, (+)-**5**), and then PC-12 cells were cultivated for 48 h in the above 1321N1 culture medium, respectively. In the case of (−)-**4** (5 μM), neurite extension on PC-12 cells was observed as described by the isolation group (Figure 3), while cytotoxicity of (−)-**4** hampered evaluation over a concentration of 10 μM. In sharp contrast, the other synthetic compounds ((−)-**1**, (−)-**5**, (+)-**1**, (+)-**4**, (+)-**5**) did not show any change at a concentration of 10 μM. These results suggest that not only the dimeric structure but chirality and oxidation levels were important for biological activity of (−)-complanadine A (**4**).

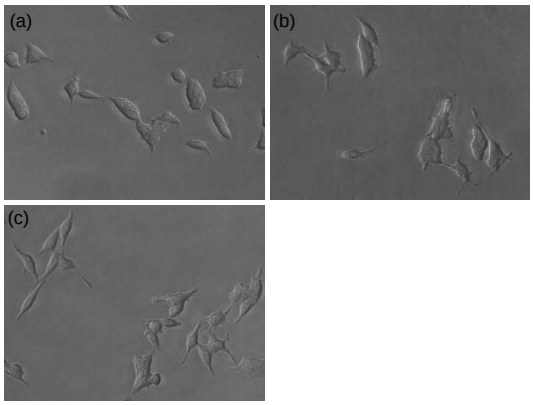


Figure 3. Glial cell-mediated morphological changes to PC-12 cells by (−)-complanadine A (**4**). (a) Control (48 h + 48 h), (b) complanadine A (5 μM, 48 h + 48 h), (c) ionomycin (1 μM; 48 h + 48 h, positive control).

Conclusions

We accomplished the total syntheses of both enantiomers of lycodine ((−)-**1,** (+)-**1**) and complanadines A and B ((−)-**4,** (+)-**4**, (−)-**5,** (+)-**5**). Key transformations included (i) the remote functional group controlled diastereoselective Diels-Alder reaction, (ii) the *6-exo-trig* intramolecular Mizoroki-Heck reaction to construct the tetracyclic lycodine core, and (iii) the direct arylation to pyridine *N*-oxide to yield the unsymmetric dimer at the final stage. The bipyridyl mono-*N*-oxide functionality of **67** provided successful access to the inherently different oxidation levels of the left domain in both **4** and **5**, and enabled their biological evaluation. Preliminary biological studies indicated that not only the dimeric structure but also chirality and oxidation levels are important for the biological activity of complanadine A (−)-**4**.

Acknowledgments

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Keywords: total synthesis • complanadine • lycodine • C-H arylation • palladium

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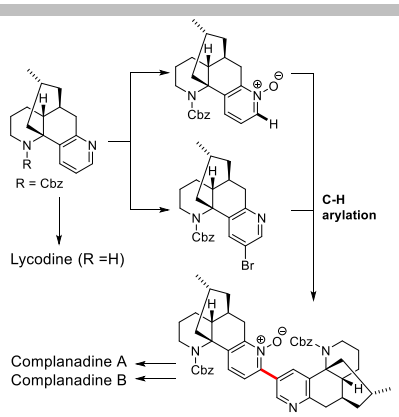
Entry for the Table of Contents

Layout 1:

FULL PAPER

Unsymmetric dimers from the protected monomer: Total syntheses of the *Lycopodium* alkaloid lycodine, and its unsymmetric dimers, complanadines A and B are described. Unsymmetric dimerization was achieved through regioselective functionalization of the protected monomer and direct C–H arylation. Examination of neuronal differentiation revealed the importance of the dimeric structure, chirality and oxidation levels for the biological activity of natural complanadine A.



L. Zhao, C. Tsukano, E. Kwon, H. Shirakawa, S. Kaneko, Y. Takemoto, and M. Hirama*

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Competent route to the unsymmetric dimer architectures: Total syntheses of (−)-lycodine, (−)-complanadines A and B, and evaluation of their neurite outgrowth activities