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PROGRESS TOWARDS THE TOTAL SYNTHESIS OF THE BIOACTIVE CALOTHRIXINS A AND B

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Dedicated to Dr. Keiichiro Fukumoto, HETEROCYCLES Editor, Emeritus Professor at Tohoku University on the occasion of his 75th birthday.

Abstract - During the past decade, the total synthesis of calothrixin A and B, bioactive metabolites from cyanobacteria *Calothrix* sp., has been independently reported by six groups. Here, we describe the development of these synthetic efforts, including two biomimetic routes *via* indolo[2,3-*a*]carbazole.

INTRODUCTION

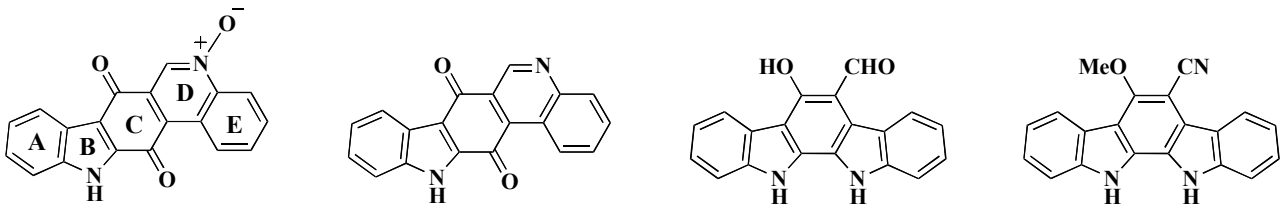


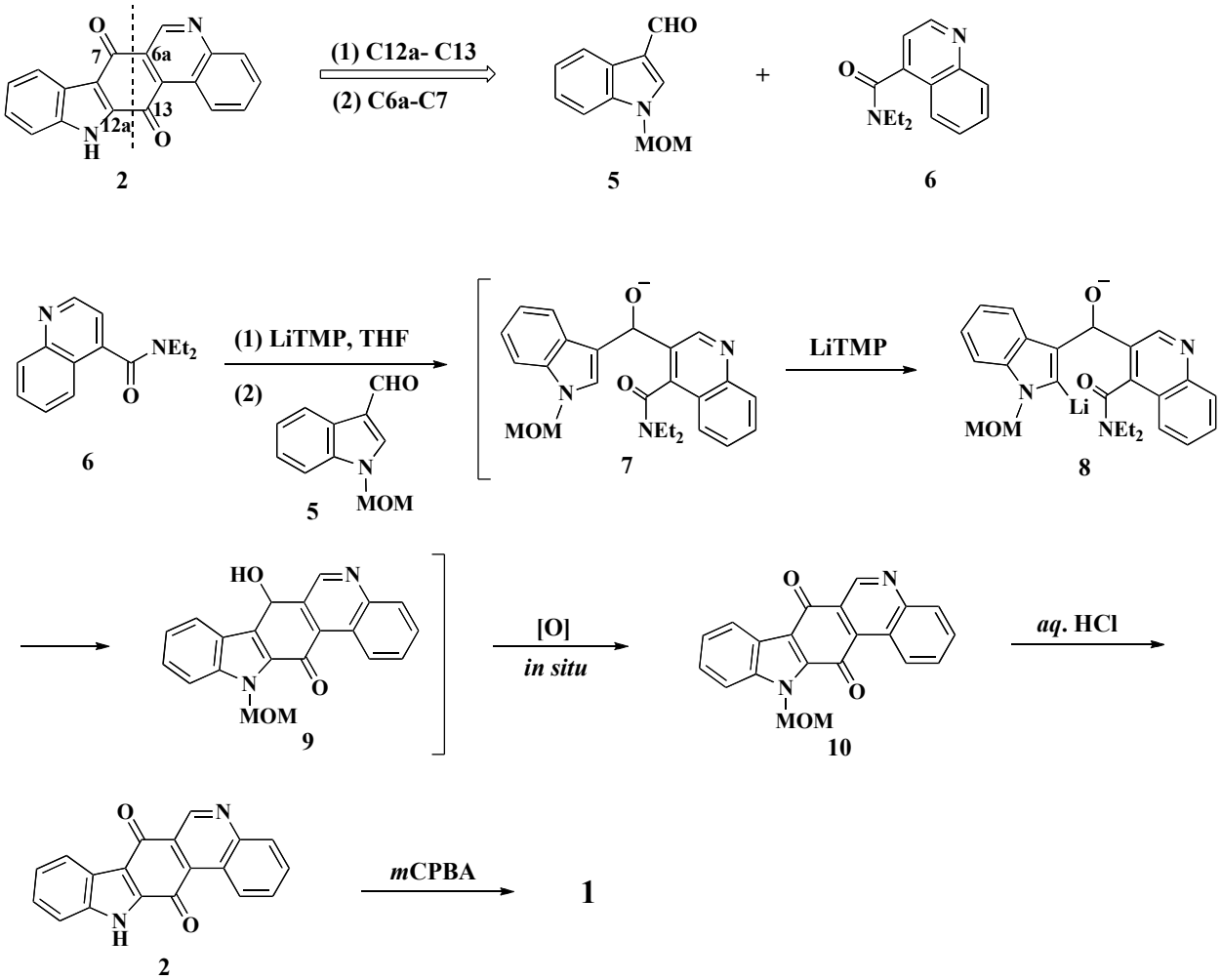
Figure 1

Calothrixins A (**1**) and B (**2**), which possess a novel indolo[3,2-*j*]phenanthridine pentacyclic ring system, were originally isolated from *Calothrix* cyanobacteria in 1999 by the group of Rickards (Figure 1).[1] These unique pentacyclic quinones exhibit remarkable biological activity, most notably their growth inhibitory effects at nanomolar concentrations on a chloroquine resistant strain of the malarial parasite *Plasmodium falciparum*, as well as activity against human Hela cancer cells, and inhibition of RNA polymerase activity.[1-6] Rickards *et al*. proposed that calothrixins A (**1**) and B (**2**) may be derived biosynthetically from a hypothetical metabolite **3** of the relatively common indolo[2,3-*a*]carbazole type,[1] which is closely related to the known 6-cyano-5-methoxyindolo[2,3-*a*]carbazole (**4**)[7,8] isolated from cyanobacterium *Nostoc sphaericum*. To date, eight synthetic schemes, including two biosynthetic routes,

to calothrixins (**1** and **2**) have been reported by six research groups. In this review, we will summarize each total synthesis in turn, including the two biosynthetic routes.

1. KELLY'S TOTAL SYNTHESIS

The first total synthesis of calothrixin A (**1**) and B (**2**) was completed in short steps in 2000 by the Kelly group (Scheme 1).[9] The known *N*-MOM-3-formylindole (**5**)[10a] and quinoline-4-carboxamide (**6**),[10b] derived from the disconnection at the C6a to C7 bond and the C12a to C13 bond in Scheme 1, were chosen for the construction of the C-ring on the basis of *o*-lithiation chemistry. Namely, treatment of **6** with 4 equivalents of lithium tetramethylpiperidide (LiTMP), followed by addition of the aldehyde **5** afforded the *N*-MOM calothrixin B (**10**) in a one pot operation (12% yield from **6**). Removal of the *N*-MOM protecting group of **10** gave the calothrixin B (**2**) (74%), which was oxidized with *m*CPBA yielding calothrixin A (**1**) (71%).

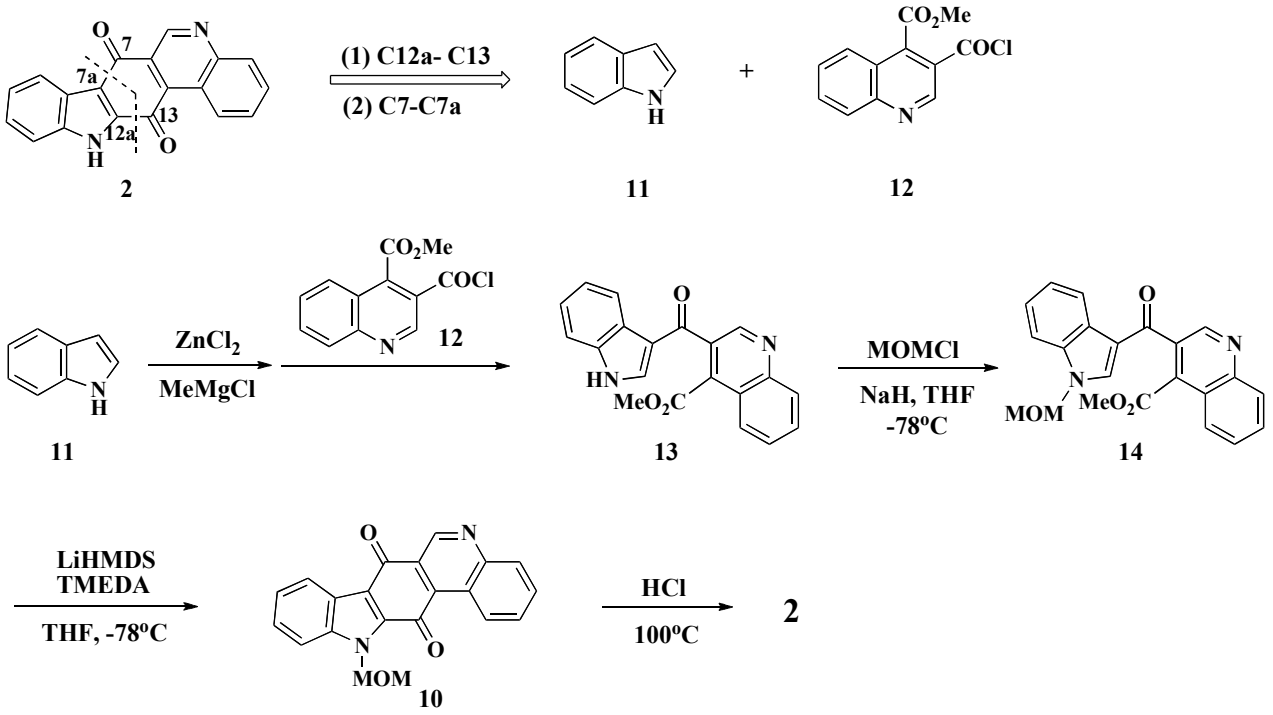


Scheme 1

2. CHAI'S TOTAL SYNTHESIS (1)

Chai's retro synthetic analysis[11,12] in Scheme 2 employs an initial disconnection at the C12a to C13 bond. A second disconnection is then made between C7a and C7 to give indole **11** and the quinoline-3-acyl

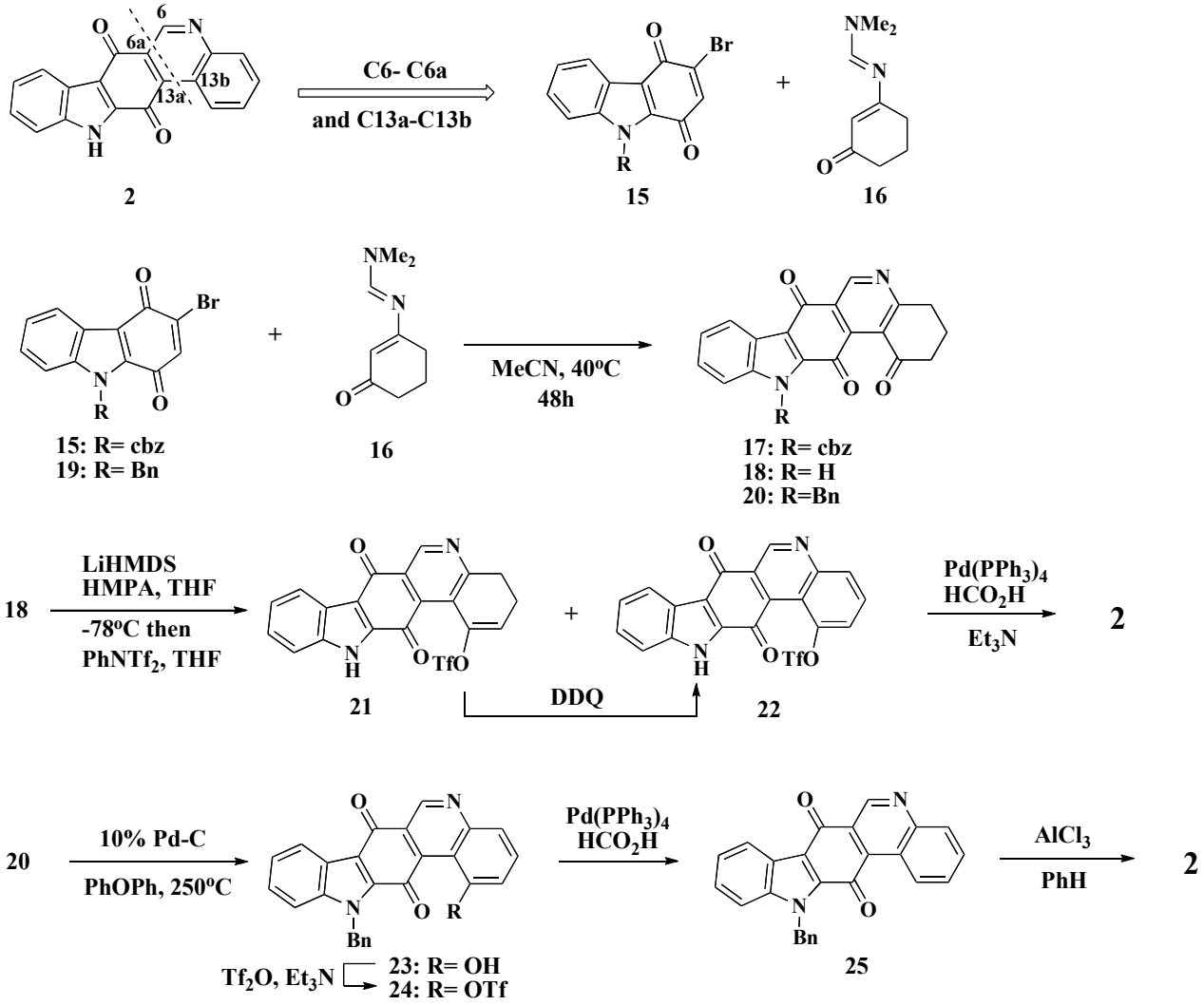
chloride **12** (Scheme 2). Coupling of indole **11** to 3-chlorocarbonyl-4-methoxycarbonylquinoline (**12**)[13a,b] was carried out as follows. Treatment of indole **11** with $ZnCl_2$ and MeMgCl followed by the addition of quinoline **12** under Friedel-Crafts conditions without $AlCl_3$ reproducibly afforded the desired 3-acylindole **13** (80%). Subsequent treatment of the 3-acylindole **13** with NaH in THF followed by addition of MOMCl yielded *N*-MOM-3-acylindole **14** (99%). Lithiation of **14** with two equivalents of LDA (or LHMDS) in the presence of TMEDA gave the *N*-MOM calothrixin B (**10**) (54%). Cleavage of the *N*-MOM group was achieved by heating in conc. HCl to give calothrixin B (**2**) (83%).



Scheme 2

3. GUINGANT'S TOTAL SYNTHESIS

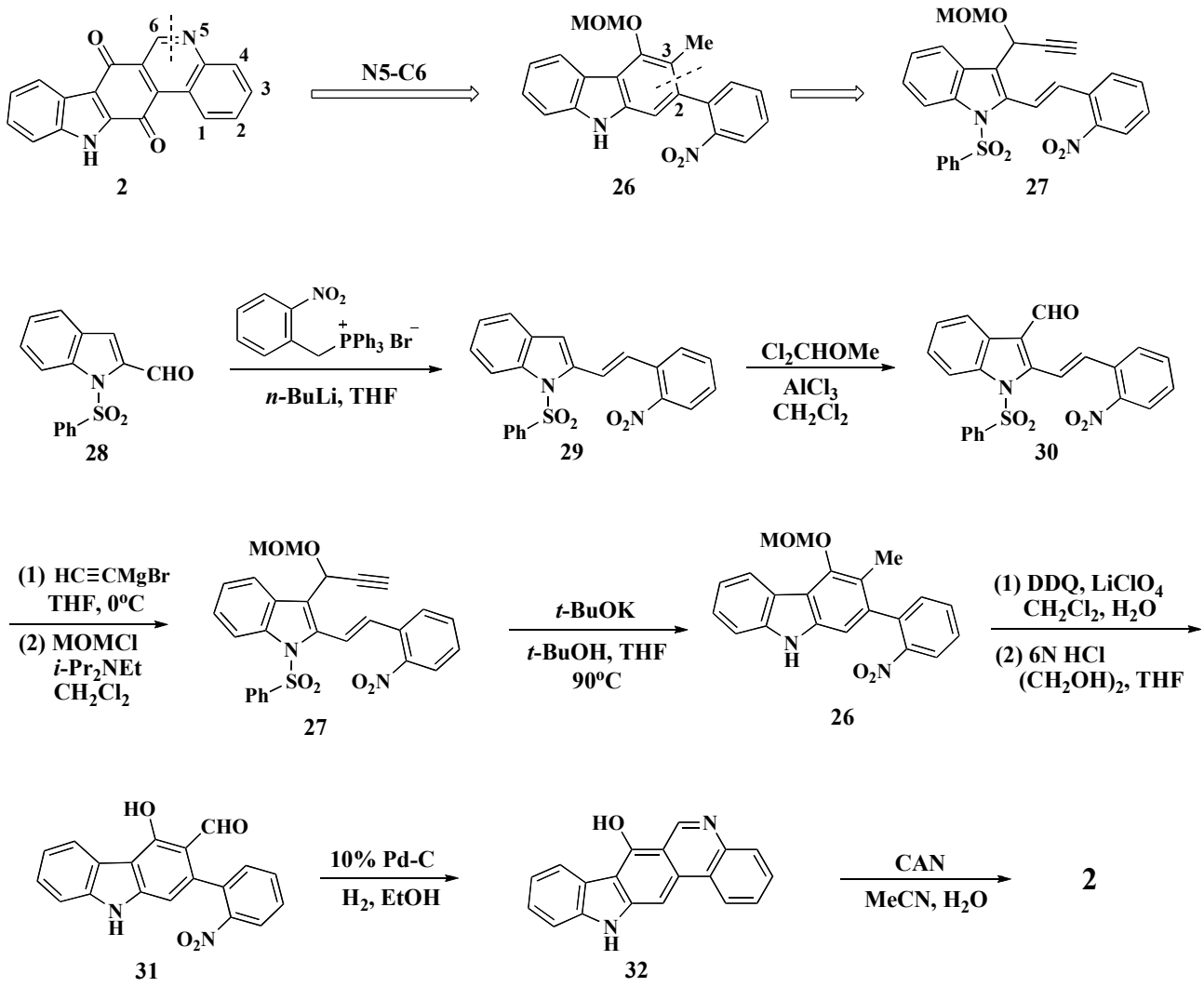
Simultaneous creation of the C6-C6a and C13a-C13b bonds based on an intermolecular hetero Diels-Alder reaction has been carried out by the Gingant group.[14,15] The key cycloaddition of the dienophile **15**[16a] with the diene **16**[16b,c] afforded the deprotected pentacyclic product **18** (80%) as a major component after 48 h. Similarly, dienophile **19** reacted with diene **16** to give the desired adduct **20** (80%). Deprotonation of **18** followed by triflation afforded a mixture of enol triflate **21** and aryl triflate **22**. The mixture was subsequently heated in the presence of DDQ in dioxane to yield the triflate **22** (51% from two steps). Reductive cleavage of the *O*-triflate group with $Pd(PPh_3)_4$ and HCOOH provided calothrixin B (**2**) (75%). In addition, calothrixin B (**2**) can also be generated from the *N*-benzyl protected adduct **20**. Oxidation of **20** with 10% Pd-C afforded phenol **23** (73%), which was then treated with Tf_2O and Et_3N to give the triflate **24** (94%). Reductive cleavage of the *O*-triflate group of **24** gave the *N*-benzyl calothrixin B **25** (95%). Finally, treatment with $AlCl_3$ in benzene to remove the *N*-benzyl group produced calothrixin B (**2**) (57%).



Scheme 3

4. HIBINO'S TOTAL SYNTHESIS

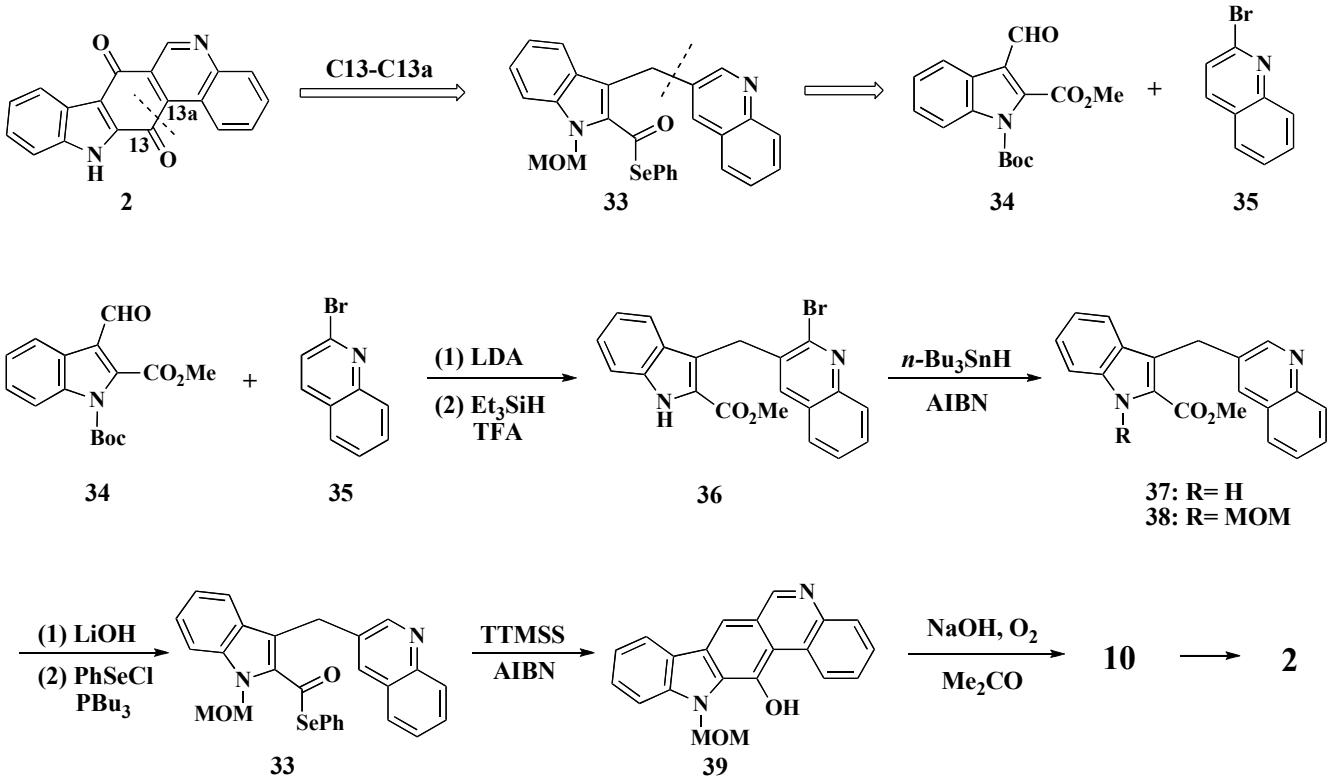
It was presumed that an 4-oxygenated 2,3,4-trisubstituted carbazole ring **26**, derived from a disconnection at the N5-C6, might be obtained from **27** by an allene-mediated electrocyclic reaction of the 6π-electron system including the indole 2,3-bond (Scheme 4).[17] The Wittig reaction of 2-formylindole **28**[18] with 2-nitrobenzyltriphenylphosphorane gave the *trans*-2-(2-styryl)indole **29** (96%). Subsequent treatment of **29** with Cl_2CHOMe in the presence of $AlCl_3$ afforded the 3-formylindole **30** (96%). The Grignard reaction of **30** with ethynylmagnesium bromide yielded the propargyl alcohol, which was protected with MOMCl and *i*-Pr_2NEt to produce the *O*-MOM ether **27** (86% from **30**). The desired 4-oxygenated 2,3,4-trisubstituted carbazole **26** was obtained by heating **27** in the presence of *t*-BuOK at 90°C (29%), along with an elimination of the phenylsulfonyl group. Sequential oxidation of **26** with DDQ followed by deprotection with 6*N* HCl gave the 3-formylcarbazole **32** (70%). Reduction of the nitro group of **31** with 10% Pd-C and H_2 followed by the condensation afforded the indolo[3,2-*j*]phenanthridine **32**, which was oxidized with CAN to provide calothrixin B (**2**) (67% from **31**).



Scheme 4

5. BENNASAR'S TOTAL SYNTHESIS

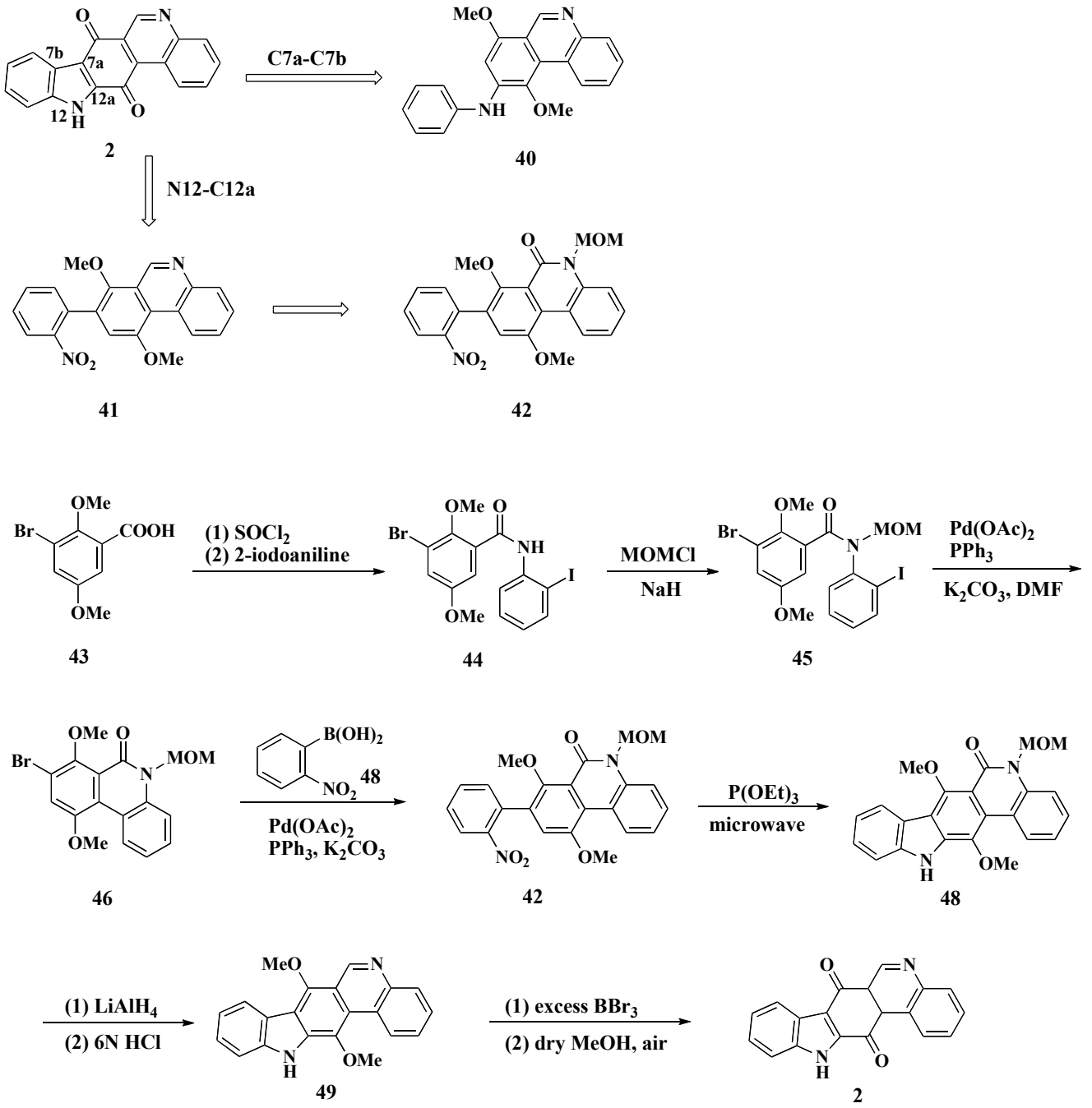
A new radical-based route at the C13 and C13a of calothrixin B (**2**) has been developed by the Bennasar group (Scheme 5).[19] A radical precursor, selenoester **33** is synthesized from *N*-Boc-3-formylindole **34** as shown in Scheme 5. The reaction of **34** with 3-lithio-2-bromoquinoline, prepared from 2-bromoquinoline **35** with LDA, followed by Et_3SiH reduction of the resulting alcohol provided the 3-alkylated 2-bromoquinoline **36** (55%). The 2-bromoquinoline **36** was converted into the deprotected quinoline **37** by treatment with *n*-Bu_3SnH (90%). After *N*-protection of **37** with MOMCl and NaH (90%), hydrolysis of **38** with LiOH, followed by phenylselenation of the resulting carboxylic acid, gave the target compound **33** (80%) as a radical precursor. The connection of the C13 and C13a bond with the *N*-MOM-substituted 2-indolylacyl radical, generated from selenoester **33** under reductive conditions (TTMSS, AIBN), yields the pentacyclic phenol **39** (90%). The phenol **39** was converted to the known *N*-MOM calothrixin B **10** by oxidation with molecular oxygen in NaOH medium (98%).



Scheme 5

6. CHAI'S TOTAL SYNTHESIS (2)

Two synthetic strategies for construction of the B-ring have been attempted by intramolecular aryl-aryl coupling reaction of 9-anilinophenanthridine **40** derived from a disconnection at the C7a and C7b, and a nitrene insertion reaction of 8-(2-nitrophenyl)phenanthridine **41** derived from a disconnection at the N12 and C12a (Scheme 6).[20] However, attempts to cyclize the anilinophenanthridine **40** using catalytic amounts of $Pd(OAc)_2$ in refluxing glacial AcOH in the presence of molecular oxygen failed. A second strategy involved using a nitrene intermediate generated from the 8-(2-nitropheny)phenanthridine **41** and its equivalent **42**. The nitrophenylphenanthridine **42** was synthesized by using Suzuki-Miyaura coupling reaction of the 8-bromophenanthridine **46** (prepared from 3-bromo-2,5-dimethoxybenzoic acid (**43**) in three steps in 82% yield, according to the Harayama's phenanthridine route[21]) with 2-nitrophenyl boronic acid (**47**) in the presence of $Pd(OAc)_2$, PPh_3, K_2CO_3 in DMF at 150° C (96%). The nitro compound **42** was then cyclized in the presence of triethylphosphite in a sealed tube at 174° C under microwave irradiation to the indolophenanthridinone **48** (89% yield). Subsequent reduction of the amide group of **48** with $LiAlH_4$ gave the 7,10-dimethoxyindolo[3,2-*j*]phenanthridine **49**, which was treated with excess BBr_3 followed by quenching with dry MeOH and molecular oxygen to form calothrixin B (**2**) (97% from **48**).

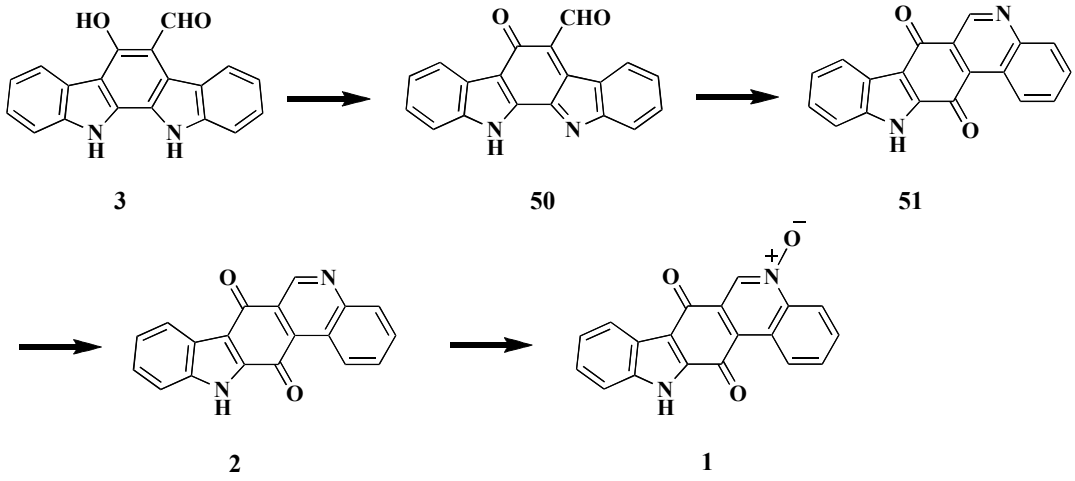


Scheme 6

7. HYPOTHETICAL BIOSYNTHETIC ROUTE OF CALOTHRIXINS A (1) AND B (2)

A biosynthetic route to generate calothrixins A (**1**) and B (**2**) has been proposed by Rickards group as follows. The pentacyclic indolo[3,2-*j*]phenanthridine ring system may be derived biosynthetically from a hypothetical metabolite, 6-formyl-5-hydroxyindolo[2,3-*a*]carbazole (**3**). Oxidation of a phenol **3** to quinone-imine **50**, followed by hydrolytic cleavage of the resulting imino group affords an *o*-aminophenylcarbazole-1,4-quinone **51**. Rotation around the biaryl bond and condensation of an amine with a formyl group provides the pentacyclic calothrixin B (**2**). *N*-Oxidation generates the pentacyclic calothrixin A (**1**) (Scheme 7).[1] This ring system of **1** is unique amongst natural products and was

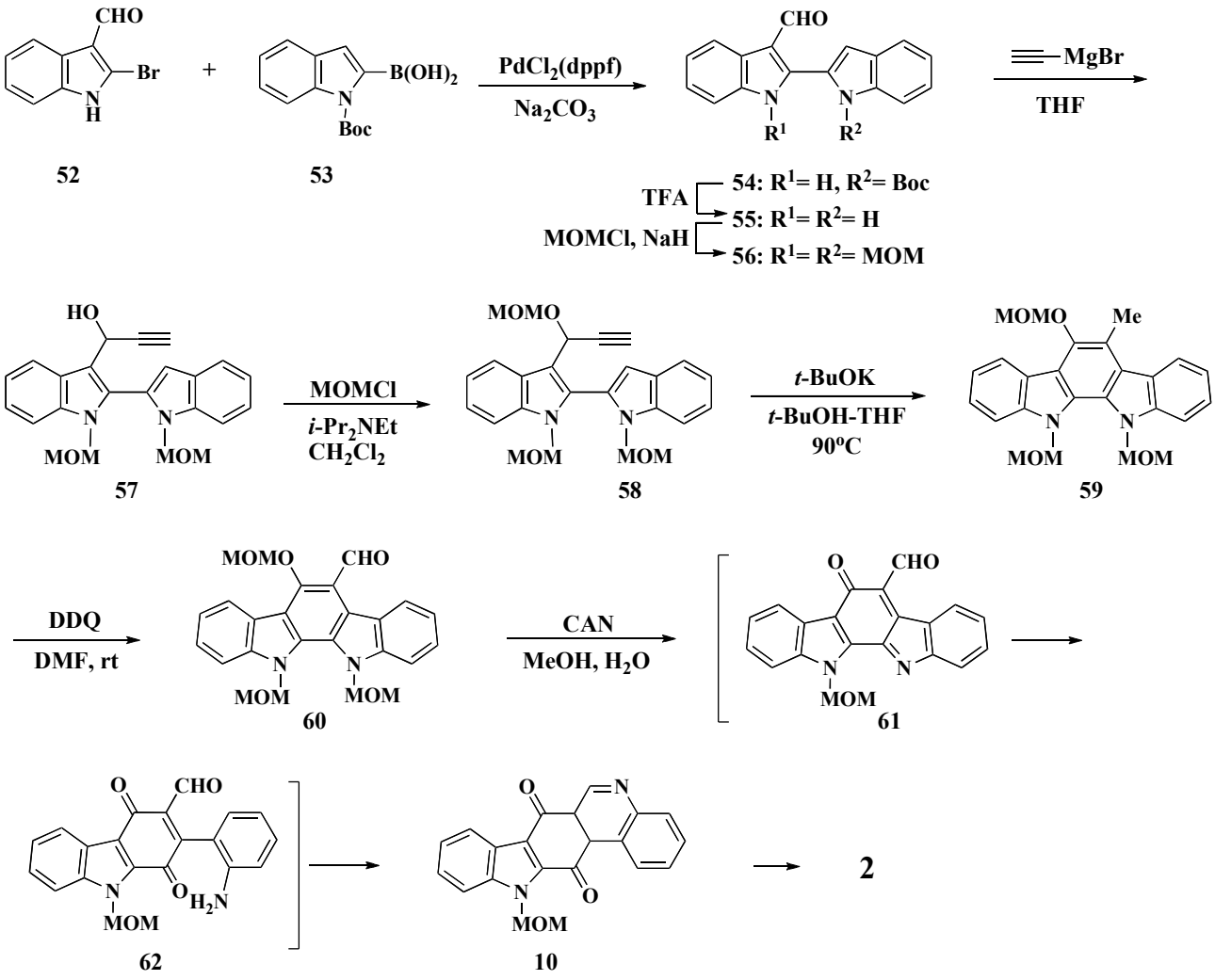
proposed to arise in nature from the more common tryptophan derived indolo[2,3-*a*]carbazole framework.[8] Recently, two biomimetic total syntheses of **1** and **2** have appeared in the literature.[22,23,24]



Scheme 7

8. HIBINO'S BIOMIMETIC TOTAL SYNTHESIS

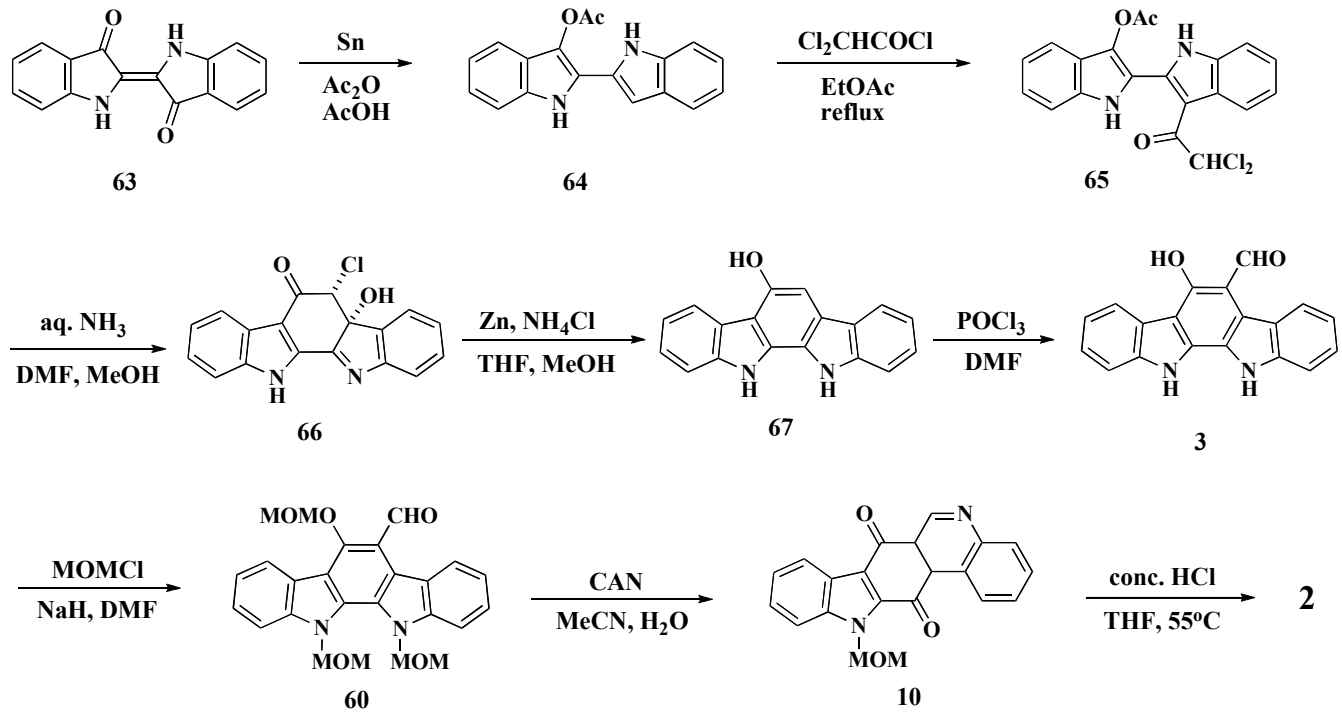
It was envisaged that a 6-hydroxy-5-formylindolo[2,3-*a*]carbazole (**3**) or its derivative **60** might be derived through an allene-mediated electrocyclic reaction of the 6π-electron system involving two [*b*]-bond of indoles, generated from a propargyl ether **58** (Scheme 8).[22] The Suzuki-Miyaura coupling reaction of 2-bromo-3-formylindole (**52**)[25] with indole-2-boronic acid **53** gave the bisindole **54** (96%). Cleavage of the *N*-Boc group in **54** with TFA (82%), followed by protection of the nitrogen atom of bisindole **55** with MOMCl and NaH afforded *N*,*N*'-bis(methoxymethyl)bisindole **56** (71%). The Grignard reaction of **56** with ethynylmagnesium bromide yielded the propargyl alcohol **57** (86%), which was protected with MOMCl to produce the MOM ether **58** (78%). The propargyl ether **58** was treated with *t*-BuOK in *t*-BuOH amd THF at 90°C to yield the desired indolo[2,3-*a*]carbazole **59** (93%). Oxidation of 5-methylindolocarbazole **59** to 5-formylindolocarbazole **60** was attempted with DDQ in DMF to generate the expected indolocarbazole **60** (55%) as a protected metabolite **3**. Further oxidation of **60** in order to convert to a quinone-imine like compound was examined with cerium ammonium nitrate (CAN) to give the *N*-MOM calothrixin **10** directly (40%). Treatment of **10** with conc. HCl afforded calothrixin B (**2**) (65%).



Scheme 8

9. MOODY'S BIOMIMETIC TOTAL SYNTHESIS

A synthetic route to the key indolo[2,3-*a*]carbazole **3** is based on the synthesis of 5-cyano-6-methoxy-12-methylindolo[2,3-*a*]carbazole starting from indigo **63** (Scheme 9)[23,24] according to the reaction scheme devised by Somei.[26] Namely, a mixture of indigo **63** and tin powder in a solution of Ac_2O in AcOH was heated at 64-66°C to give the monoacylated bisindole **64** (85%). Acylation of **65** with Cl_2CH_2COCl in EtOAc gave the dichloroacetylated bisindole **65** (79%), which was treated with aqueous NH_3 to cause ring closure, thus producing *cis*-chlorohydrin **66** (75%). The reduction of **66** with Zn and NH_4Cl afforded the known 5-hydroxyindolo[2,3-*a*]carbazole **67** (55%) and subsequent Vilsmeier reaction provided the desired 5-formyl-6-hydroxyindolocarbazole **3** (82%). In an attempt to effect the proposed biomimetic transformation, the indolocatbazole **3** was subjected to many different oxidation conditions. Unfortunately, however, all attempts to oxidize **3** have been unsuccessful. As a result, it was found that the fully protected indolocarbazole **60**[22] with MOM-group (44%) could be oxidized by CAN to produce the *N*-MOM calothrixin B **10** (31%). Finally, deprotection of *N*-MOM group with conc. HCl afforded calothrixin B (**2**) (100%).



Scheme 9

CONCLUSIONS

The first total synthesis of calothrixin A and B was achieved by the Kelly group, which involved two key C-C bond forming reactions at C6a and C7, followed by C12a and C13, using an *o*-lithiation strategy.[9] The structures **1** and **2** have been confirmed. Two further total syntheses have been reported by the Chai group. One is a short and concise route to calothrixin B (**2**) using Friedel-Crafts and lithiation reactions for the formation at the C7 to C7a, and the C12a to C13.[11,12] The alternative synthesis is based on N-C bond formation at the N12 to C12a atom, utilizing a nitrene intermediate.[20] This route, which involves seven steps, gave the highest overall yield (68%) among the eight reported synthetic schemes. Next, the Guingant group used a regioselective hetero Diels-Alder strategy as a key synthetic step in order to construct the D-ring.[14,15] The cycloaddition reaction between the catbazole-1,4-quinone **15** and 2-azadiene **16** proceeds in 80% yield based on simultaneous C-C-bond formation at the C6 to C7a and C13a to C13b atoms. We chose an allene-mediated electrocyclic reaction involving the indole 2,3-bond for the construction of an appropriate 4-oxygenated 2,3,4-trisubstituted carbazole ring **26**, which was converted to calothrixin B (**2**) through condensation at N5 to C6.[17] The Bennasar group[19] used a regioselective intramolecular homolytic acylation at C13 to C13a. The 2-indolylacyl radical, generated from the phenylselenoester **33**, underwent reaction to give the indolo[3,2-*j*]phenanthridine **39** in 90% yield. In 1995, a synthetic approach to the pentacyclic system of calothrixins using an electrocyclic reaction of 2,3-dialkenylindole, including the indole [*b*]-bond,[27] was reported by the Srinivasan group.[28] Recently, synthesis of a calothrixin B isomer with a novel 7*H*-indolo[2,3-*j*]phenanthridine-7,13(8*H*)-dione structure has been completed by the Gingant group employing a similar reaction.[29]

The first biomimetic total synthesis of calothrixin A and B was reported by our research group. Construction of a hypothetical metabolite, 5-formylindolo[2,3-*a*]carbazole ring **60** was carried out using an allene-mediated electrocyclic reaction involving two [*b*]-bonds of indoles.[22] Oxidation of the fully MOM-protected 5-formylindolo[2,3-*a*]carbazole **60** with CAN provided *N*-MOM calothrixin B (**2**) in 40% yield. Moreover, the Moody group[23,24] generated 5-formylindolo[2,3-*a*]carbazole **3** from indigo **6**. Unfortunately, despite many attempts, oxidation of the non-protected carbazole **3** to give the pentacyclic calothrixin B (**2**) could not be achieved. Therefore, they employed our fully protected indolo[2,3-*a*]carbazole **60** as an intermediate for the synthesis of calothrixin A and B. Efficient oxidation of **60** was achieved using CAN, and subsequent condensation provided *N*-MOM calothrixin B (**2**). The biosynthetic route to calothrixins A (**1**) and B (**2**), proposed by Rickards group,[1] has been proved by two independent research groups.

ACKNOWLEDGEMENTS

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Dr. Satoshi Hibino was born in 1945. He graduated from Osaka Pharmaceutical University followed by Graduate School of Pharmaceutical Sciences, Tohoku University where he obtained his Ph. D. degree under the supervision of Prof. Tetsuji Kametani. After two years of Post-Doc (1975-1977) with Professor Steven M. Weinreb (PSU) at Chem. Dept. of Fordham University, he worked at Tokyo Pharmaceutical University during five years. He moved to Fukuyama University as an Associate Prof. and then he became a Professor in 1986. His research interests are in synthetic organic chemistry including natural products.