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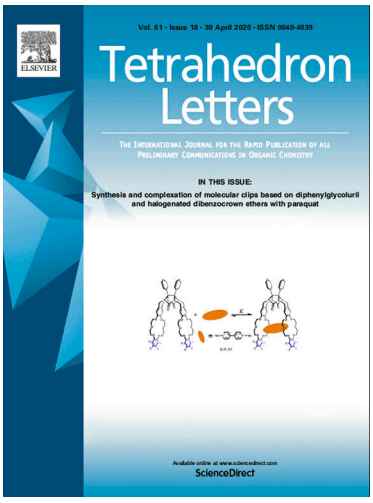

Total Synthesis of Inubosin B

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Total Synthesis of Inubosin B

Mohamed Farouk Hamissa[a,b,*], Petr Niederhafner[a,b], Martin Šafařík[a], Marta Telus[a], Lenka Kolářová [a], Leah Koutná[a], Hana Šestáková[a], Radko Souček[a], Jaroslav Šebestík[a,*]

[a] Institute of Organic Chemistry and Biochemistry, Academy of Sciences, Flemingovo náměstí 2, 16610 Prague 6, Czech Republic.

[b] Department of Chemistry of Natural Compounds, Faculty of Food and Biochemical Technology, University of Chemistry and Technology, Prague, Technická 5, 166 28 Prague 6, Czech Republic.

KEYWORDS: Neuroregeneration, Neurodegeneration, Acridine, Alkaloid, Total synthesis

Corresponding Authors

*mfahamissa@hotmail.com and sebestik@uochb.cas.cz

Abstract: Inubosin B is the most active member of the acridine alkaloids isolated from *Streptomyces sp.* IFM 11440 culture. The inubosins initiate neuroregeneration via a neurogenine 2 pathway. In this work, we have described the total synthesis of inubosin B *via* two synthetic routes. The effects of various coupling, cyclization and reduction reactions are discussed including common pitfalls and side reactions. Reverse phase chromatography with TFA was crucial for the isolation of the product from aluminum ions present in the reduction media.

Introduction

Neurodegenerative diseases are characterized by the progressive loss of neurons leading to neuron death.[1-3] Currently, no efficient strategies for curing neurodegenerative diseases are available, and all marketed drugs and treatment protocols instead focus on relieving symptoms and slowing the rate of decline in patients.[4-6] On the other hand, adult neurogenesis is the differentiation of neural stem cells to neurons in order to compensate for the neuronal loss.[7-9] Accordingly, the induction of neurogenesis can be a therapeutic strategy for neurodegenerative diseases.[2, 10, 11]

Natural products are important for drug discovery and activity optimization.[12-17] The inubosins are a group of naturally occurring acridine alkaloids recently isolated from the extract of a culture of *Streptomyces sp.* IFM 11440.[18] They induce neurogenesis by promoting the activity of neurogenin2 (Ngn2).[18] In comparison to the reference drug baicalin and other members of the inubosin family, inubosin B had superior activity in increasing mRNA expression of Ngn2.[18] The superior activity of inubosin B compared to inubosins A and C is due to the absence of the hydroxyl group at position 5 of the acridine ring, as well as the presence of the hydroxyl group at position 4 (Fig. 1).[18]

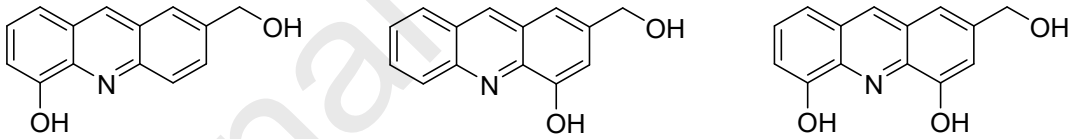


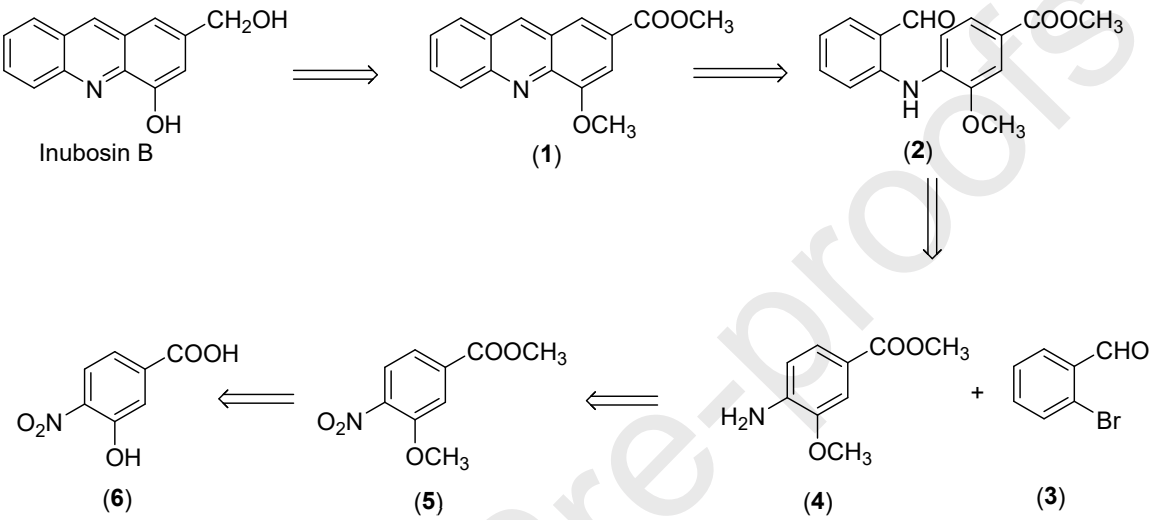
Figure 1. Structures of inubosins A, B, and C.

However, to the best of our knowledge, there are no synthetic routes towards inubosin B; thus, we carried out a total synthesis of inubosin B *via* two methods. Furthermore, our synthetic route could potentially be used for the development of inubosin-like compounds, which can be tested for various biological activities.[19, 20]

Results and Discussion

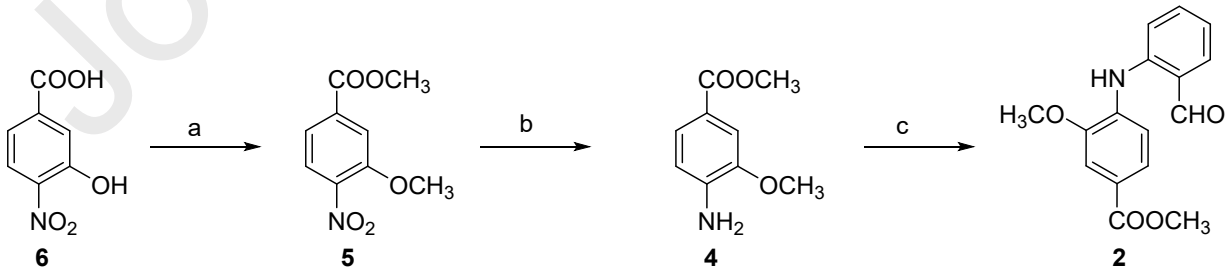
For the synthesis of inubosin B, we proposed a retrosynthetic scheme based on the typical reactivities of acridines.[21] The alcoholic and phenolic groups can be transformed into the

corresponding ester and ether groups (**1**), respectively. The acridine ring is then opened to *N*-phenyl-benzaldehyde (**2**). After N-C bond disconnection, commercially available 2-bromobenzaldehyde (**3**) and ester (**4**) are obtained as the precursors. Finally, the amino group of the ester is transformed to a nitro group, and the phenol and ester are deprotected to give commercially available 3-hydroxy-4-nitrobenzoic acid (**6**).



Scheme 1. Retrosynthesis of inubosin B.

The synthesis of inubosin B began with the bis-methylation of 3-hydroxy-4-nitrobenzoic acid (**6**) using methyl iodide.[22] Then, the nitro group was reduced to the amine group (**4**) with Zn and AcOH.[23] The coupling of amine (**4**) and 2-bromobenzaldehyde (**3**) was carried out *via* Buchwald-Hartwig amination using a suitable palladium catalyst (Scheme 2). We have found that the combination of $Pd_2(dba)_3$ with DPEPhos provided the highest yield of the desired aldehyde **2** (Table 1).[24, 25]

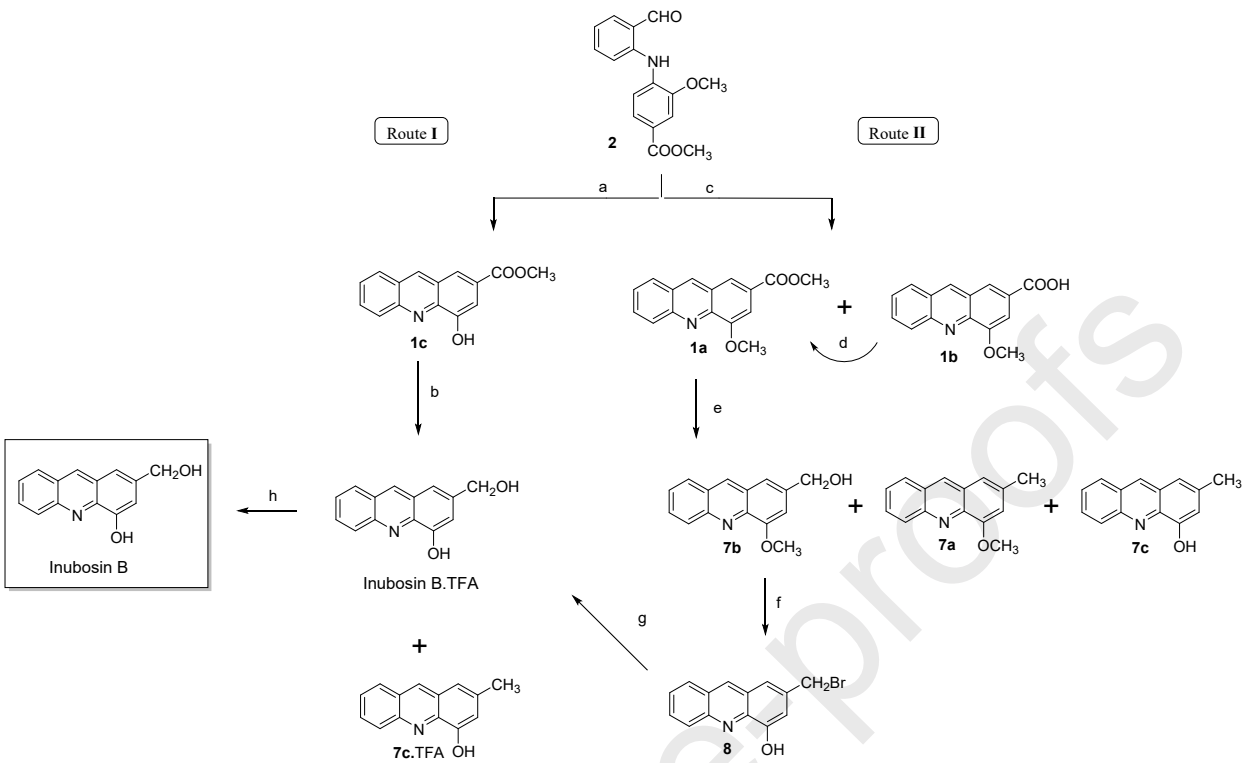


Scheme 2. Synthesis of precursor **2**. Reagents and conditions: (a) MeI, K_2CO_3, DMF, 40-45 °C, 86%; (b) Zn, AcOH, MeOH, RT, 88%; (c) 2-bromobenzaldehyde, $Pd_2(dba)_3$, DPEPhos (see Table 1), K_2CO_3, toluene, 90 °C, 55%.

Table 1. Optimization of the Buchwald-Hartwig amination reaction to give **2**.

Pd-source	**Pd loading [mol%]**	**Ligand**	**Ligand loading [mol%]**	**Yield [%]**
$Pd(OAc)_2$	0.047	DPEPhos	0.1	25
$Pd_2(dba)_3$	0.025	DPEPhos	0.05	55
$Pd_2(dba)_3$	0.025	dppf	0.05	30

Deviations from the retrosynthetic scheme began to appear during acridine ring closure (Scheme 3). Using the $AlCl_3$-promoted method for acridine closure,[24] we were able to cyclize the acridine ring and to cleave the 4-methoxy group in one step to obtain methyl 4-hydroxyacridine-2-carboxylate (**1c**) containing 3.46% of aluminum according to elemental analysis which corresponds to a 1:3 complex (3.44% is the theoretical calculation for $Al(C_{15}H_{10}NO_3)_3$). This metallo-complex is similar to the aluminum 8-hydroxyquinoline complex tris(8-hydroxyquinolinate)aluminum.[26-28] Alternatively, the ring was cyclized using *p*-toluene sulfonic acid (*p*-TSA), which partially cleaved the methyl ester group leading to a mixture of the minor **1a** and the major **1b** products. Presumably due to its zwitterionic nature, carboxylic acid **1b** remains in the aqueous phase during washing and is sparingly soluble in MeOH and insoluble in $CHCl_3$ and THF. In order to increase the yield of ester **1a**, acid **1b** was converted to **1a** using $SOCl_2$/MeOH (Scheme 3).



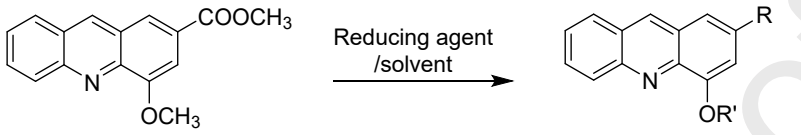
Scheme 3. Total synthesis of inubosin B. Reagents and conditions: (a) $AlCl_3$, toluene, 90 °C, 60%; (b) DIBAL-H, THF, 25 °C, inubosin B.TFA 15% and **7c.**TFA 4%; (c) *p*-TSA, toluene , 90 °C, **1a** 32%, **1b** 39 %; (d) $SOCl_2$/DMF, 50 °C, then MeOH , 0-25 °C, 78%; (e) DIBAL-H or $LiAlH_4$, THF (see Table 2); (f) conc. HBr, reflux, 44%; (g) DMSO, H_2O, 50 °C, inubosin B.TFA 18%; (h) pH 5.5 using aqueous NaOH, 93%.

The reduction of methyl 4-methoxyacridine-2-carboxylate (**1a**) to 2-hydroxymethyl-4-methoxy-acridine (**7b**) was very challenging (Table 2). The reducing agent and the solvent played an important role in the reaction: e.g. the use of $LiAlH_4$ or DIBAL-H led to side reactions, such as reduction of the alcohol to a methyl group and deprotection of the phenolic methoxy group.

Typically, unsubstituted acridines or acridine-9-carboxylates are reduced to 9,10-dihydroacridne using $LiAlH_4$.[29, 30] However, this was not observed in a detectable amount during our synthesis, even after careful LC-MS analysis (for more details the ESI: LC-MS reduction of **1a** at -15 and 25 °C). This difference in reactivity can be explained by calculation of the natural bond orbitals. The inubosin B precursor **1a** has LUMO containing some electron density at the carbonyl group, which is susceptible to reduction, whereas the 9-substituted acridine esters has

the LUMO localized only on the ring due to steric distortion and the breakage of conjugation. Thus, the hydride may initially react with the acridine ring in the case of alkyl acridine-9-carboxylates, whereas compound **1a** can react with hydride at the carboxyl position (Fig. S3), which also has the highest positive natural charge.

Table 2. Reduction of ester **1a** to alcohol **7b** accompanied by over-reduction



1a

7a: R: CH_3, R': CH_3.
7b: R: CH_2OH, R': CH_3.
7c: R: CH_3, R': H.

Reducing agent/solvent	Molar ratio	Temp.	Time [h]	Average yield* [%] 7a	7b	7c
$LiAlH_4$/THF	1	25 °C	4		10	
$LiAlH_4$/THF	1	66 °C	4		15	
$LiAlH_4$/THF	1.5	25 °C	1		15	
$LiAlH_4$/THF	1.5	25 °C	4	65		
$LiAlH_4$/THF	2	66 °C	1	55		20
$LiAlH_4$/THF	1.5	Microwave	0.25	55		20
DIBAL-H/hexane	3	25 °C	4		20	
DIBAL-H/hexane	4	25 °C	1	60		
DIBAL-H/toluene	3	25 °C	4		20	
DIBAL-H/toluene	4	25 °C	1	60		
DIBAL-H/THF	3	25 °C	4		70	

* the average yields were calculated from 4 runs.

Acidic deprotection of the methoxy group using HBr[31] led to brominated analogue **8**, which was unstable during chromatographic analysis and slowly reacted with the solvent. This

brominated analogue (**8**) was hydrolyzed using a dimethyl sulfoxide (DMSO) and water mixture,[32] followed by HPLC purification to obtain inubosin B as the trifluoroacetate salt.

Due to the additional steps involved in route **II**, we returned to the cyclization using $AlCl_3$ (route **I**) leading to **1c**, followed by reduction using DIBAL-H in THF, which can lead directly to inubosin B. However, monitoring of the reduction of **1c** using a TLC system was misleading and the ester (**1c**) was easily over reduced to the corresponding methyl acridine (**7c**).

The use of HPLC was determined to be optimal for both monitoring of the reaction progress and for compound separation. Moreover, the purification using HPLC with reverse phase chromatography (MeCN/H_2O mobile phase containing 0.5% TFA) led to the removal of aluminum from the sample (0.03% according to elemental analysis), and inubosin B was obtained as the trifluoroacetate salt in 15% yield (see Scheme 3). The HPLC-purified inubosin B.TFA had distinct Rf values (hexane-acetone-AcOH, 6:3:0.1): 0.4 and (toluene-EtOH-TFA, 7:2:0.2): 0.35 using 2D-TLC (ESI, Fig. S2). The last step was conversion of the salt to the free base by adjusting the pH to 5.5 with 0.02 N aqueous NaOH to obtain inubosin B in 93% yield. There was good agreement between the NMR spectra of naturally occurring and synthesized inubosin B (see ESI for details).

In conclusion, we obtained inubosin B *via* two synthetic routes and observed that the usage of reverse phase chromatography with the MeCN/H_2O mobile phase containing TFA led to removal of the aluminum ions from the acridine samples. In the future, this total synthesis may open the way towards inubosin analogues which are unavailable from natural resources.

Acknowledgments

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Chimera is developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIGMS P41-GM103311). We thank Dr. Martin Dračinský, Ph. D for NMR measurement of the inubosin B free base, and Dr. Jan Ježek, Ph. D for corrections of the English language.

The authors declare no conflict of interests.

Supplementary Data

Experimental procedures, ESI-HRMS, ^{1}H and ^{13}C-NMR spectra of all synthesized compounds, HPLC traces of all synthesized acridines, 2D TLC for purified inubosin B.TFA, NMR comparison for inubosin B, comparison of the LUMO orbital, and an excel sheet for the reduction of **1a**.

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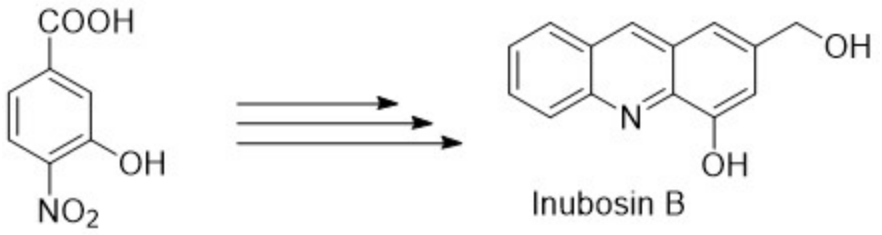
[a]*Institute of Organic Chemistry and Biochemistry, Academy of Sciences, Flemingovo náměstí 2, 16610 Prague 6, Czech Republic.*

[b]*Department of Chemistry of Natural Compounds, Faculty of Food and Biochemical Technology, University of Chemistry and Technology, Prague, Technická 5, 166 28 Prague 6, Czech Republic.*

Graphical Abstract



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[b]*Department of Chemistry of Natural Compounds, Faculty of Food and Biochemical Technology, University of Chemistry and Technology, Prague, Technická 5, 166 28 Prague 6, Czech Republic.*

Highlights

- Total synthesis of Inubosin B.
- Over reduction reactions.
- Reverse phase removal of aluminum from oxine like chelators.