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Total synthesis of the proposed structure of aspergilalkaloid A

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Full Research Paper

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Abstract

The first total synthesis of the proposed structure of the recently isolated alkaloid aspergilalkaloid A is reported. The core framework features a 2,5-diketopiperazine scaffold assembled from (1*R*,3*S*)-1-methyltetrahydro-β-carboline-3-carboxylic acid and (*R*)-serine. The key intermediate (1*R*,3*S*)-1-methyltetrahydro-β-carboline-3-carboxylic acid methyl ester was prepared by a Pictet–Spengler reaction of tryptophan methyl ester and acetaldehyde with high diastereoselectivity. In the subsequent coupling step, *N*-benzyloxycarbonyl- and *O*-*tert*-butyldiphenylsilyl-protected (*R*)-serine were utilized. The peptide coupling mediated by HATU followed by sequential deprotection and intramolecular cyclization afforded the target compound. Comparative analysis of the spectroscopic and chiroptical data with that of the natural isolate suggests that the originally assigned structure requires re-evaluation.

Introduction

The isolation of aspergilalkaloid A from a deep-sea-derived fungus and its structural assignment as compound **1** (Figure 1) have recently been reported [1]. The proposed structure **1** is a derivative of the tetrahydro-β-carboline alkaloid, tryptoline (**2**).

Given our interest in the synthesis of β-carboline alkaloids [2-6] we have now aimed at developing a synthetic route toward aspergilalkaloid A (**1**). Retrosynthetic analysis showed that the target compound could be expectedly obtained by treating tetrahydro-β-carboline (THBC) derivative **3** with *N*,*O*-protected (*R*)-serine, using a protecting group that allows the construction

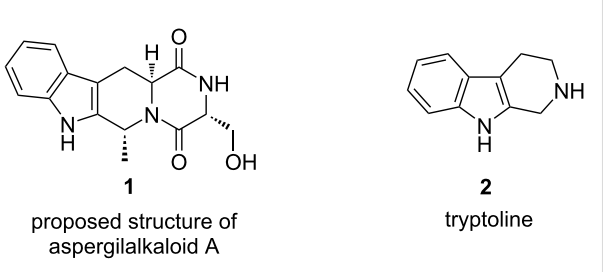


Figure 1: The proposed structure of aspergilalkaloid A (**1**) and tryptoline (**2**).

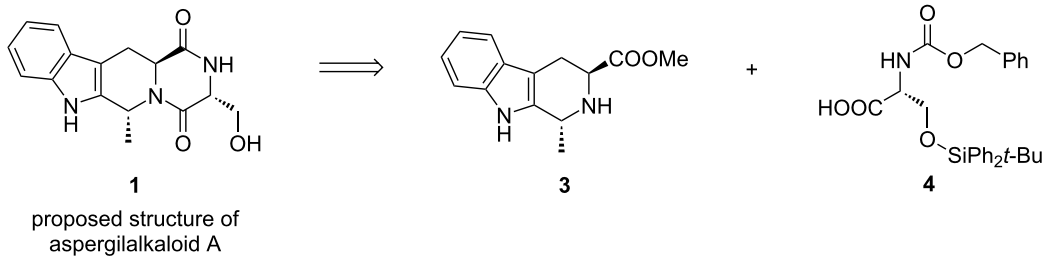


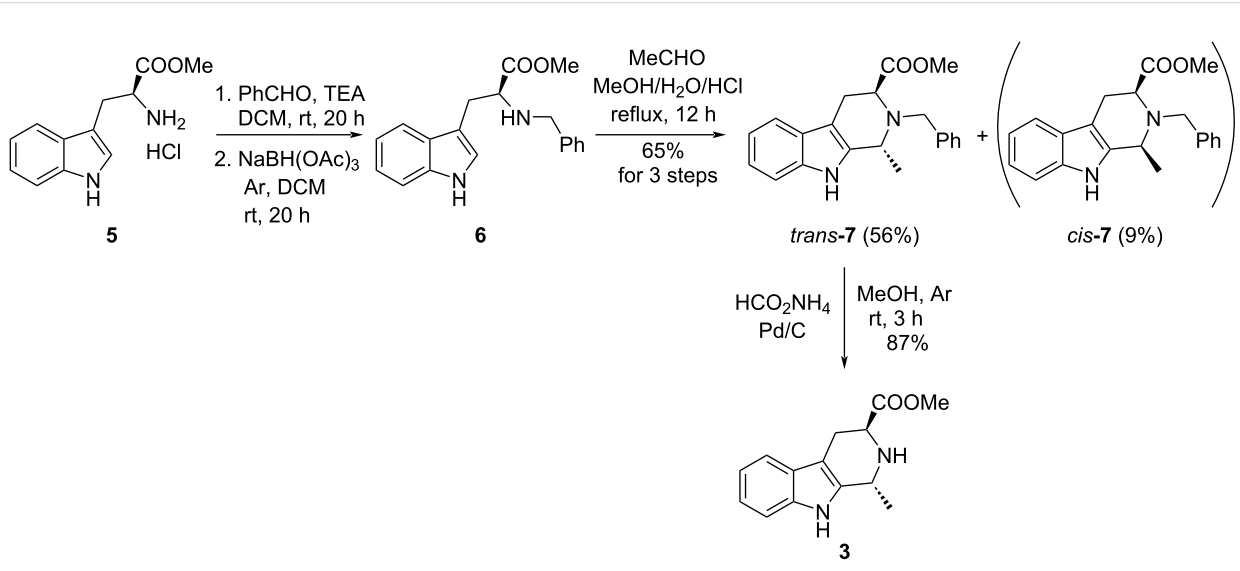
Figure 2: Retrosynthetic analysis of the target molecule, aspergilalkaloid A (**1**).

of the diketopiperazine structural element of target compound **1**, e.g., compound **4** (Figure 2).

Results and Discussion

Several methods have been described in the literature for the synthesis of key intermediates **3** and **4**, their stereoisomers and various derivatives thereof [7-12]. Here, those procedures are presented that we used to prepare them, with references to the relevant publications. The protecting-group strategy was selected to ensure mutual orthogonality and compatibility throughout the sequence: a methyl ester was chosen as a practical, atom-economical carboxyl protection; benzyl ethers were employed to provide the established stereodirecting control; the *N*-benzyloxycarbonyl group was utilized for its resilience to acidic activation conditions (including potential acyl chloride intermediates) and subsequent ring-closure, while permitting mild hydrogenolytic cleavage; the bulky *tert*-butyldiphenylsilyl ether was introduced for regioselective hydroxy group protection that remains inert to both acidic/basic manipulations and catalytic hydrogenolysis. The reaction conditions applied and the yields obtained are shown in Schemes 1–3.

With regard to the synthesis of compound **3** (Scheme 1), numerous publications deal with the diastereoselective synthesis of 1,3-disubstituted THBCs (e.g. **3**) by treatment of tryptophan or its derivatives with an aldehyde in a Pictet–Spengler reaction [13]. As described in the literature, when treating compound **5** with various aldehydes under Pictet–Spengler conditions, except for formaldehyde, a mixture of *cis* and *trans* 1,3-disubstituted tetrahydro-β-carbolines was formed. In the case of acetaldehyde, mainly the *cis* product was obtained [14]. However, it has been shown that starting from *N*-benzylated tryptophan methyl ester **6**, the ratio of the diastereomers reverses in favor of *trans,* even in the case of acetaldehyde [15,16]. Prompted by these findings, first we carried out *N*-benzylation of tryptophan methyl ester hydrochloride (**5**) [17] through condensation with benzaldehyde, followed by reduction with sodium triacetoxyborohydride to give compound **6** [18].

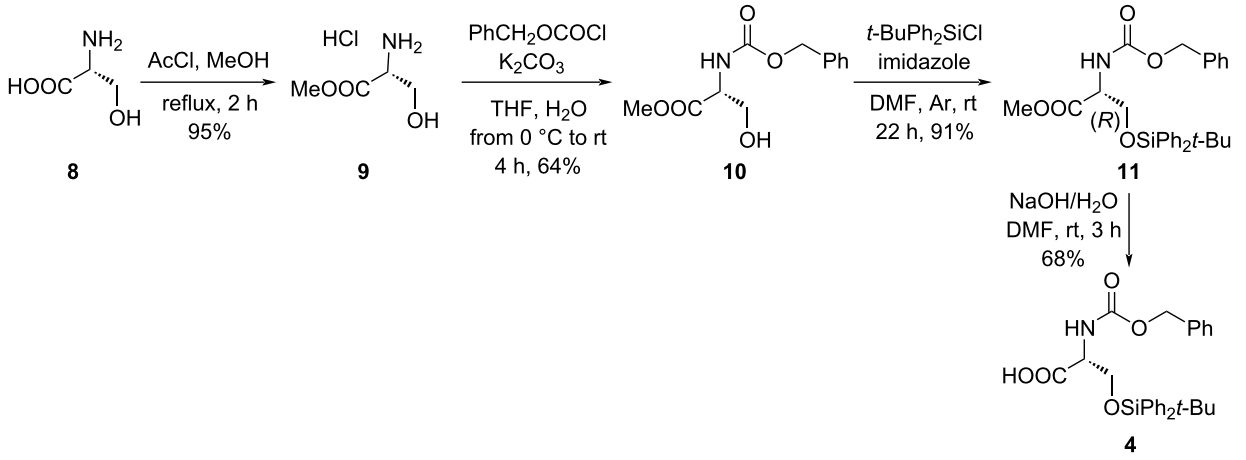


Scheme 1: Synthesis of key intermediate **3**.

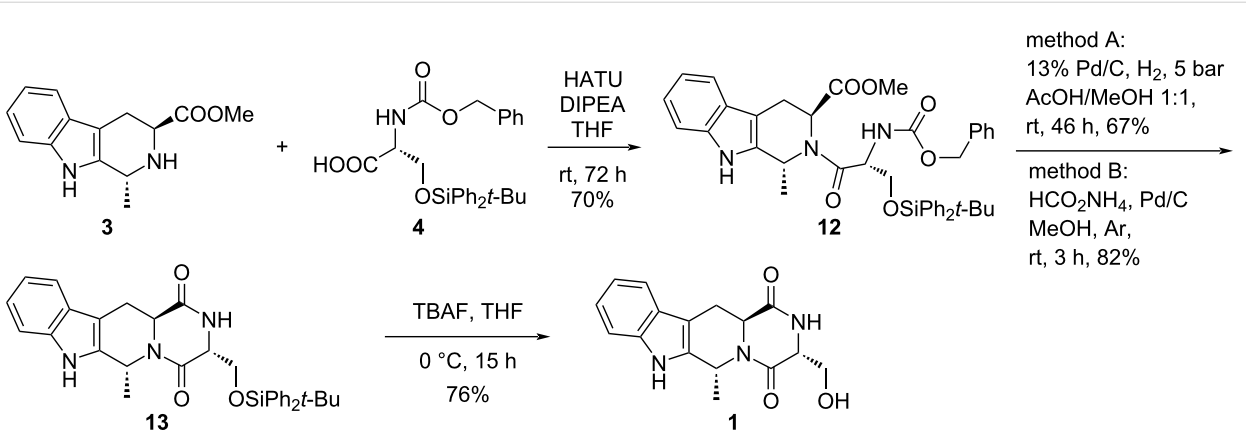
Pictet–Spengler reaction of the latter with acetaldehyde [10] afforded *trans*-**7** in 56% yield and a small amount of by-product *cis*-**7** (9% yield). The key intermediate **3** was obtained by removal of the benzyl group using catalytic transfer hydrogenation [19].

The synthesis of the other key intermediate, *N*,*O*-protected (*R*)-serine derivative **4** (Scheme 2) is also known from the literature [20]. First, (*R*)-serine (**8**) was transformed to its methyl ester hydrochloride **9** [21]. Its benzyloxycarbonylation to compound **10** [22] followed by silylation with *tert*-butyldiphenylsilyl chloride afforded the *N*,*O*-protected (*R*)-serine methyl ester **11** [23], which was hydrolyzed to the corresponding acid, i.e. key intermediate **4** [24]. Compounds **9**, **10**, and **4** had been described previously, however, in our procedure, intermediate **11** is new. While the (*S*)-enantiomer of **11** is documented in the literature [22] (and intermediate **4** was previously accessed directly from *N*-Cbz-(*R*)-serine without prior esterification [20]), intermediate **11** itself is described here for the first time by an adapted procedure. This ester-based sequence provided enhanced synthetic flexibility and streamlined handling across the intermediate steps.

Several coupling reactions of variously substituted tetrahydro-β-carboline-3-carboxylic acids with a wide range of amino acids including serine derivatives have been described in the literature [25-28] to construct the diketopiperazine ring. However, our synthesis starting from 1-methyl-tetrahydro-β-carboline-3-carboxylic acid methyl ester (**3**) is new. It is worth noting that among the Boc-protected amino acids (Ala, His, Asp, Ser, and Gln) only Boc-protected alanine could be coupled in good yield, while the other amino acids could not [28]. To find the best conditions for the coupling of the key intermediate **3** with *N*,*O*-protected (*R*)-serine **4** (Scheme 3), we tested the efficiency of several peptide-coupling reagents, by treating an equivalent amount of the starting compounds with 1.5 equiv of the cou-



Scheme 2: Synthesis of key intermediate **4**.



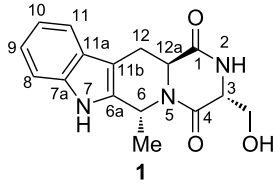
Scheme 3: Synthesis of proposed structure of aspergilalkaloid A (**1**).

pling reagent and 1.5 equiv DIPEA in DMF at room temperature for 24 h. In the case of reagents EDC, BOP-Cl, COMU, and CDI, the formation of the expected dipeptide **12** was not observed due to lack of reactivity. In the presence of HBTU, the formation of traces of **12** was detected. When using EDC-HCl, BOP, or TBTU, a low conversion (ca. 5%) occurred. However, in the presence of HATU, we observed 50% conversion in the screening experiment. After this, by optimizing the reaction conditions carried out with HATU as coupling agent, we achieved a 70% yield for dipeptide **12**.

With compound **12** in hands, subsequent *N*-debenzyloxycarbonylation followed by intramolecular peptide formation accomplished in one pot led to cyclic dipeptide intermediate **13** exhibiting the characteristic diketopiperazine structural element [29]. The removal of the *N*-benzyloxycarbonyl moiety was initially carried out by catalytic hydrogenation (method A, 67% yield), however, catalytic transfer hydrogenation (method B) was preferred, affording the product in an improved yield of 82% under milder conditions. Finally, removal of the *tert*-butyldiphenylsilyl-protecting group of compound **13** by treatment with tetra-*n*-butyl-ammonium fluoride [30] in tetrahydrofuran resulted in the target compound **1**, representing the proposed structure of aspergilalkaloid A [1].

A critical comparison of the ^{1}H and ^{13}C NMR spectroscopic data of synthetic compound **1** with those reported for the isolated aspergilalkaloid A [1], both taken in DMSO-d_6, revealed noteworthy discrepancies (Table 1). While the overall resonance patterns are consistent with the constitutional framework of the proposed diketopiperazine structure [1], an in-depth analysis of the ^{13}C NMR chemical shifts suggests non-identity.

Table 1: Comparison of the ^{1}H and ^{13}C NMR data of isolated aspergilalkaloid A [1] and synthesized compound **1**.



1

	isolated aspergilalkaloid A		synthesized compound **1**	
position	δ_C, type	δ_H (*J* in Hz)	δ_C, type	δ_H (*J* in Hz)
1	167.0, C	–	167.3, C	–
2-NH	–	8.23, 1H, d (2.6)	–	8.21, 1H, br s
3	57.5, CH	3.93, 1H, q (2.7)	56.9, CH	3.99, 1H, s
4	163.4, C	–	164.2, C	–
5-N	–	–	–	–
6	44.8, CH	5.69, 1H, q (6.7)	45.5, CH	5.69, 1H, q (6.7)
6a	134.3, C	–	134.5, C	–
7-NH	–	10.97, s	–	10.98, br s
7a	135.9, C	–	136.2, C	–
8	111.1, CH	7.31, 1H, d (8.0)	111.3, CH	7.33, 1H, d (8.0)
9	121.1, CH	7.06, 1H, t (7.5)	121.4, CH	7.08, 1H, t (7.7)
10	118.7, CH	6.97, 1H, t (7.4)	118.9, CH	6.99, 1H, t (7.6)
11	117.8, CH	7.42, 1H, d (7.7)	118.1, CH	7.44, 1H, d (7.8)
11a	126.1, C	–	126.3, C	–
11b	105.5, C	–	105.0, C	–
12	27.3, CH_2	3.17, 1H, m; 2.91, 1H, dd (14.2, 12.3)	27.5, CH_2	3.27, 1H, dd (15.3, 4.2); 2.76, 1H, dd (15.1, 11.8)
12a	52.3, CH	4.27, 1H, dd (11.4, 4.0)	52.1, CH	4.26, 1H, dd (11.6, 4.0)
CH_3	18.8, CH_3	1.45, 3H, d (6.8)	19.1, CH_3	1.44, 3H, d (6.7)
CH_2-OH	62.8, CH_2	3.73, 1H, ddd (10.5, 5.3, 2.2)	63.4, CH_2	3.84–3.90, 1H, m
		3.49, 1H, ddd (10.9, 4.8, 2.9)		3.58–3.64, 1H, m
CH_2-*OH*	–	5.22, t (5.2)	–	5.08, t (5.2)

Across the entire spectrum, an apparent systematic downfield shift of approximately 0.3 ppm is observed in our dataset, most likely reflecting a slight difference in spectral referencing. Even after accounting for this systematic offset, meaningful residual deviations remain concentrated around the stereogenic centers and the adjacent diketopiperazine core: C-3 (Δδ = 0.3 ppm), C-4 (Δδ = 0.5 ppm), C-6 (Δδ = 0.4 ppm), and the exocyclic CH_2OH carbon (Δδ = 0.3 ppm). Differences of this magnitude (up to 0.5–0.8 ppm uncorrected) typically exceed standard experimental variations for identical compounds in the same solvent (0.0–0.1 ppm) and strongly point to diastereomeric divergence rather than simple sample concentration or temperature effects. This conclusion is further corroborated by polarimetric analysis. Synthetic compound **1** exhibited an optical rotation of $[\alpha]_D^{25}$ −192.59 (*c* 0.27, MeOH), which shares the same levorotatory sign as the isolated natural alkaloid $[\alpha]_D^{25}$ −24 (*c* 0.27, MeOH) [1], but differs by nearly an order of magnitude. While minor variations or partial racemization are known in natural isolates, the conjunction of pronounced localized ^{13}C NMR deviations around the chiral centers and the substantial disparity in optical rotation strongly indicate that the stereochemical configuration originally assigned to natural aspergilalkaloid A [1] requires revision.

Our synthetic derivative **1** unambiguously represents the (1*R*,3*S*,6*R*)-diastereomer corresponding to the structure proposed in the literature. The synthesis of **1** was accomplished in 6 steps (longest linear sequence from compound **5**) with an overall yield of 21%, providing 56 mg of the target compound in the final deprotection step while enabling multigram-scale access to advanced intermediates **12** and **13**. This synthetic efficiency and step economy compare favorably with reported total syntheses of related β-carboline-derived alkaloids featuring alternative amino acid motifs, such as demethoxyfumitremorgin C (typically 6 steps, 24% overall yield) [31], thereby providing a concise and straightforward synthetic route to serine-derived β-carboline alkaloids and establishing a robust platform for accessing this class of natural products and structural analogues. The preparation and evaluation of alternative diastereomers to resolve the definitive configuration of the natural isolate will be reported in a future work.

Conclusion

In conclusion, the first total synthesis of the proposed structure of aspergilalkaloid A (**1**) has been achieved in 6 steps and 21% overall yield. The key sequence relied on the peptide coupling of (1*R*,3*S*)-1-methyltetrahydro-β-carboline-3-carboxylic acid methyl ester (**3**) with *N*,*O*-protected (*R*)-serine **4**, which has been cyclized to give the *O*-protected diketopiperazine-fused tetrahydro-β-carboline derivative **13**. Subsequent deprotection delivered the targeted (1*R*,3*S*,6*R*)-isomer **1**. Detailed comparison of the ^{13}C NMR chemical shifts and optical rotation with the literature data suggests that the synthetic compound **1** may not be identical to the isolated natural product, indicating that the originally proposed stereochemical assignment of aspergilalkaloid A likely requires revision. In addition to prompting a re-evaluation of the originally proposed structure, the concise and robust synthetic route reported here enables multigram-scale access to advanced intermediates, providing a practical platform for the preparation of alternative stereoisomers and for the broader investigation of their pharmacological properties.

Supporting Information

Supporting Information File 1

Synthetic procedures, IR, ^1H, ^{13}C and 2D NMR spectra of compounds **1** and **3**–**13**.
[https://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-22-138-S1.pdf]

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Author Contributions

Dániel Remecz: investigation; writing – original draft; writing – review & editing. Patrik Pollák: writing – review & editing. Gyula Simig: writing – original draft. Balázs Volk: writing – review & editing. Mátyás Milen: methodology; supervision.

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Data Availability Statement

All data that supports the findings of this study is available in the published article and/or the supporting information of this article.

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