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Article

Total Synthesis of Homo- and Heterodimeric Bispyrrolidinoindoline Dioxopiperazine Natural Products

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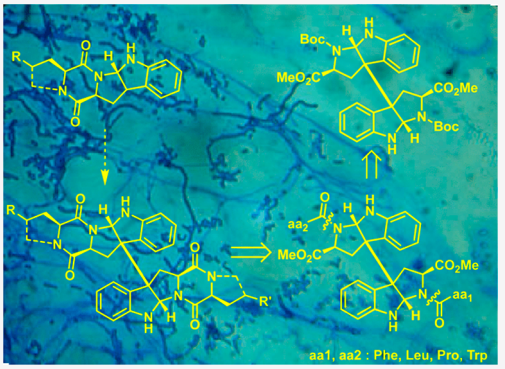
ABSTRACT: Total synthesis and structural confirmation of homo- and heterodimeric bispyrrolidinoindoline dioxopiperazine alkaloids isolated from fungi and bacteria, namely, ditryptoleucine A, ditryptoleucine B (**11**), the *N,N′*-bis-demethylated analogue (+)-**12**, (−)-dibrevianamide F (**13**), (−)-SF-5280-451 (**14**), tetratryptomycin A (**15**), (−)-tryprophenaline (**17**), and (−)-SF-5280-415 (**18**), has been carried out starting from the corresponding bispyrrolidinoindolines derived from tryptophan. Our efforts to synthesize all possible diastereomers of the natural ditryptoleucine isolates uncovered structural factors that determine the rate and efficiency of dioxopiperazine ring formation, leading in some cases to mixtures of diastereomers by concomitant epimerization, to the formation of their putative monomeric dioxopiperazine dipeptide biogenetic precursors, and to the alternative formation of a dimer with a fused 1,3,5-triazepan-6-one heterocycle.



2,5-Dioxopiperazines, commonly named 2,5-diketopiperazines (DKPs), are representative members of the cyclic dipeptide (CDP) family of natural products, which are mainly generated by intramolecular condensation of dipeptide precursors. Bioactive natural products with 2,5-dioxopiperazine scaffolds are known to be produced by marine and terrestrial fungi, bacteria, plants, and even animals.[1]

Within this family, the dimeric tryptophan-derived bispyrrolidinoindoline dioxopiperazine alkaloids, which have been mainly isolated from *Streptomyces* sp. actinobacteria as well as from *Aspergillus*/*Eurotium* fungal species,[2,3] contain 2,5-dioxopiperazines fused to hexahydropyrrolo[2,3-*b*]indole skeletons (Scheme 1 and Figure 1). The two subunits of these dimeric alkaloids are connected through the C-3 and C-3′ atoms, forming a characteristic arrangement of two contiguous quaternary stereogenic centers adjacent to two aminals (see **9**, Figure 1).

The biogenesis of symmetrical and nonsymmetrical C-3/C-3′ bispyrrolidinoindoline dioxopiperazine alkaloids, as indicated for (−)-ditryptophenaline **9** (Figure 1), involves as the key step the coupling reaction, catalyzed by P450-dependent oxidase enzymes, of indole-2,5-dioxopiperazine units **4** derived from tryptophan-tRNA **2** (Scheme 1).[2] The radical generated at the dioxopiperazine ring of **4** evolves to produce the pyrrolidinoindoline dioxopiperazine radical **5**. Radical recombination of dioxopiperazine units gives rise to C-3/C-3′ homodimer (−)-ditryptophenaline **9**.[3−6] Additional natural products, including **8** (Scheme 1), are alternatively generated through connection of the monomeric pyrrolidinoindoline dioxopiperazine radical at C-3 of **6** to the N-1′ position (C-6′/C-3 or C-7′/C-3 heterodimeric positional isomers have also been isolated)[3,6] of the radical species generated in **6** (and stabilized by resonance with the indole ring as depicted in **7**).[3−8]

The biosynthetic machineries of these microorganisms[3] further contribute to diversify the already densely functionalized alkaloid scaffold. Not only permutations on the amino acids acting as biogenetic building blocks (Ala, Leu, Val, Phe, Trp, Pro) that condense with the bispyrrolidinoindoline core but also covalent modifications at the indole and dioxopiperazine nitrogen substituents are commonly found in the structures of these dimeric natural products.[3,6]

Some representative members (**9**−**18**) of the family of alkaloids relevant to the work described herein are depicted in Figure 1. They are characterized by sharing homo- and heterodimeric structures derived from Leu, Phe, Pro, and Trp, the latter not only forming the bispyrrolidinoindoline core but also appearing as the distal amino acid of the terminal dioxopiperazine rings in tetratryptomycin A (**15**).[6,8] A small group of related homo- and heterodimeric bispyrrolidinoindoline dioxopiperazine natural products derived from Ala and Val as terminal amino acid components are collected in the Supporting Information (Figure S1).

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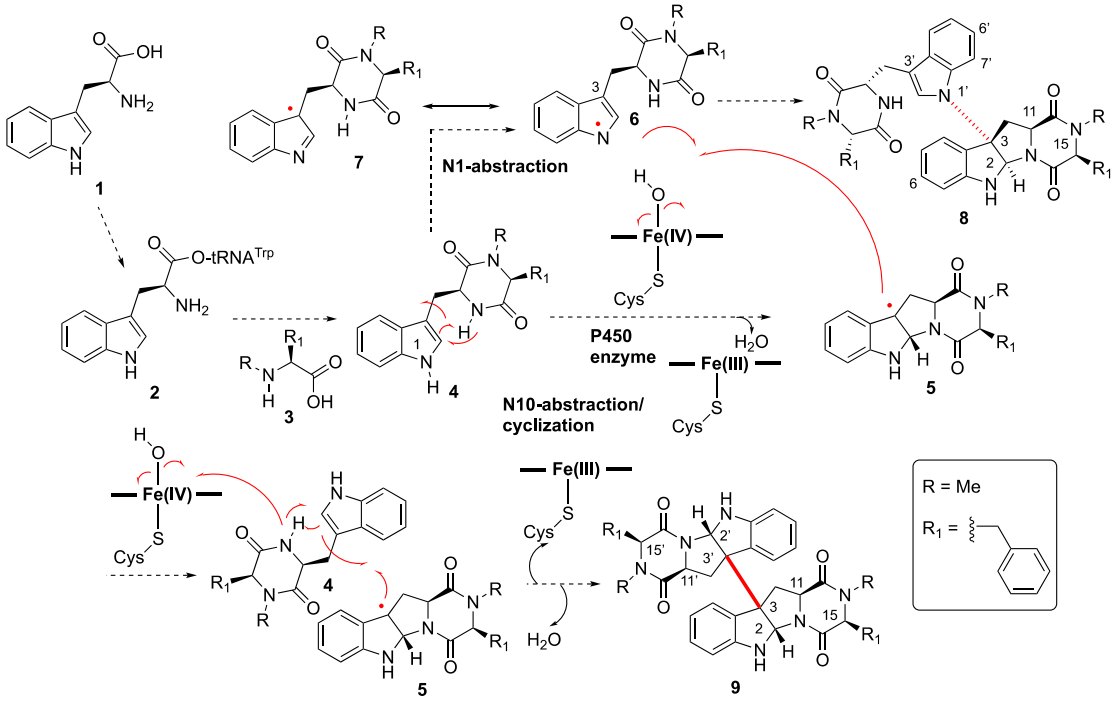
Scheme 1. General Biogenesis[a] of a Symmetrical C-3/C-3′ Bispyrrolidinoindoline Dioxopiperazine Alkaloid (9) and the C-3/N-1′ Dioxopiperazine Analogue (8)[3−6]



[a]Only one of the possible configurations is shown.

(−)-Ditryptophenaline (**9**) was the first alkaloid of this group isolated from the mycelium of several strains of *Aspergillus* obtained from contaminated food[9] (and more recently from other sources[10−12]), and the connectivity and three-dimensional arrangement of the proposed structure was confirmed by X-ray diffraction analysis[9] and total synthesis.[13,14] Additional symmetrical dioxopiperazine dimers include (+)-WIN 64821 (**10**), isolated from an *Aspergillus* sp. SC319 culture originally extracted from soil,[15] ditryptoleucines A and B (**11**), isolated from *Aspergillus oryzae* (RIB40),[16] (+)-**12** from the fungus *Aspergillus violaceofuscus* present in a *Reniochalina* sp. marine sponge,[17] (−)-SF5280-451 (**14**),[18,19] isolated from *Aspergillus sydowii* (MSX19583),[18] and (−)-tetratryptomycin A (**15**) obtained from a large-scale fermentation of *Streptomyces albus* J1074 expressing a gene cluster[8] from *Saccharopolyspora antimicrobica* (Supporting Information, Figure S2).[20]

Heterodimeric bispyrrolidinoindoline dioxopiperazine natural products in this series include (+)-WIN 64745 (**16**), which was also isolated from *Aspergillus* sp. SC319 culture,[15] and (−)-SF5280-415 (**18**), which was obtained, together with (−)-SF5280-451 (**14**), from the marine-derived *Aspergillus* sp. SF-5280.[19]

Finally, as a result of the relaxed substrate specificity exhibited by the P450 oxidase enzyme DtpC, homo- and heterodimeric analogues termed (−)-dibrevianamide F (**13**) and (−)-tryprophenaline (**17**), in addition to **9**, were isolated from *A. flavus* and shown to be biosynthetically generated through radical mechanisms, as indicated in Scheme 1 for parent **9**.[4,5]

A wide range of biological activities and pharmacological effects have been reported for these dimeric alkaloids, most notably the inhibition of foam cell formation in macrophages and the inhibition of ubiquitin-specific protease 7 (USP7) by **16**,[21] the inhibition of the LPS-induced expression of cytokine I6-10 by (+)-**12**,[17] and the inhibition of protein tyrosine phosphatase PTP1B (the activation of which in the heart has been associated with heart failure) by **18**.[19] Likewise, intermediates used as precursors for the synthesis of **16** and selected diastereomers have shown antifungal[22] and antitumor activities.[23] Most likely, the complex dimeric skeletons might play the role (in chemical space) of privileged structures[24] through multipoint interactions with their biological targets, which could explain the variety of biological activities of these compounds.

The structural complexity of these natural products with six stereocenters makes them challenging targets for organic synthesis. Following putative biomimetic oxidative dimerization reaction of dioxopiperazine biogenetic precursors (see **4**, Scheme 1), (−)-ditryptophenaline (**9**) (Figure 1) was first synthesized, although in very low yield (3%),[13] and more recently, the sequence was adapted to the syntheses of **9**−**11** and **16** (Figure 1).[21,25] Departing from the biomimetic oxidative dimerization, synthetic efforts have mainly focused on the construction of bispyrrolidinoindolines such as **9** (Figure 1) and *ent*-**10** from bisoxindole diamines[26] and of (−)-**9**, (+)-**10** (Figure 1), and a 1′-(2-phenylethylene)-ditryptophenaline derivative[27,28] by the Co(I)-mediated dimerization of 3a-bromocyclotryptophans (see **21**/**26** in Scheme 2).[27−32] On the basis of the bioinspired Co(I)-promoted dimerization we have also reported the synthesis of (+)-**10** (Figure 1) starting from C3-bromopyrrolidinoindoline **21** (Scheme 2) and extended the protocol to nonsymmetrical dimeric alkaloids including (+)-**16** (Figure 1).[33,34]

Given the structural similarities within this family of alkaloids (Figure 1) and our previous approaches to symmetrical and nonsymmetrical congeners displaying the congested dimeric scaffold,[33,34] we set out to address the

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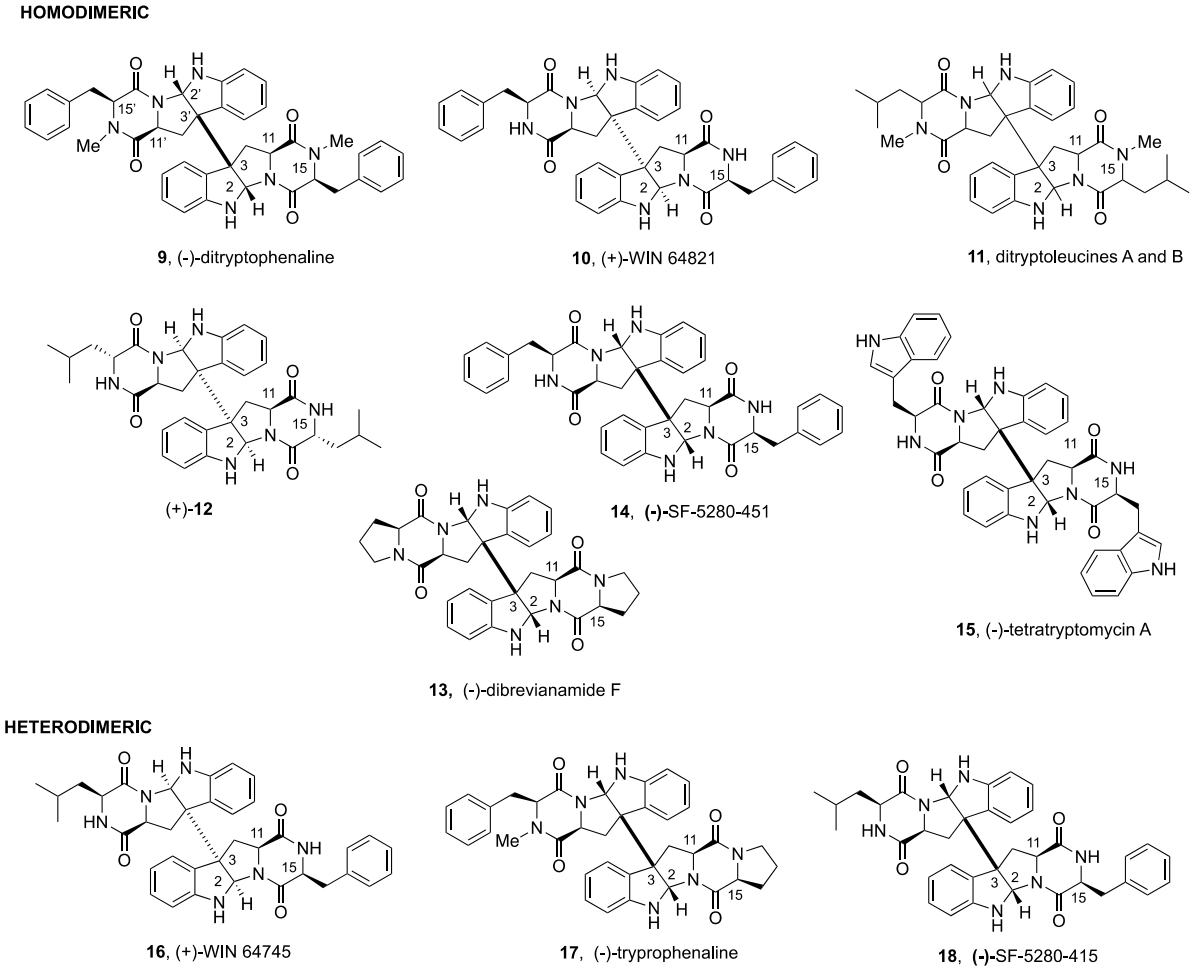

Figure 1. Homo- and heterodimeric bispyrrolidinoindoline dioxopiperazine alkaloids derived from Trp, Leu, Phe, and Pro.

total synthesis of the remaining members of the family of homo- and heterodimeric natural products shown in Figure 1, namely, **11**−**15**, **17**, and **18**.

RESULTS AND DISCUSSION

As illustrated in Scheme 2 A, our general retrosynthetic analysis to these alkaloids, as indicated for **11**, involves the late-stage formation of the dioxopiperazine rings from precursors such as **19**, which would be generated by an amide condensation reaction between the required amino acids and the N-deprotected methyl esters of the bispyrrolidinoindoline **20**. Two protecting groups (PGs) for the amine of the second amino acid component, namely, Boc and Cbz, were initially considered in synthetic planning, in particular with regard to optimizing the reaction conditions for deprotection and release of dipeptides **19** prior to or concomitant with dioxopiperazine ring formation.[21,25,33,34] Because the relative configuration of the ring fusion carbons of the hexahydropyrrolo[2,3-*b*]indole core is conserved during the dimerization process[27] and this can be traced back to the configuration of the protected tryptophan derivative (**23**) used in the bromocyclization step, an easy entry into the required diastereo- and enantiomeric series of homo- and heterobispyrrolidinoindoline dioxopiperazine alkaloids should be feasible by adequate choice of the synthetic partners (Scheme 2A).[33,34] Thus, the Co(I)-induced dimerization of bromopyrrolidinoindoline **21**, which could be generated by diastereoselective bromocyclization reaction[35] of protected D-Trp derivative (*R*)-**23** (Scheme 2), was selected for the connection of the two monomeric units.[27] The use of appropriately protected D- or L-amino acids, as well as the epimerization at C-2 of bispyrrolidinoindoline dioxopiperazines when required, would allow targeting of alkaloids shown in Figure 1.

Moreover, as a synthesis tactic, these natural products (Figure 1) were divided in two groups: A, homodimers that incorporate the diastereoisomers (**19**) of the bispyrrolidinoindoline core structure of protected D-Trp, namely, (*R*)-**23**, and each enantiomer of protected (*N*-Me-)L-Leu and (*N*-Me-)D-Leu, toward the synthesis of **11** and **12** (exemplified for **11** in Scheme 2A); and B, homo/heterodimers from L-Trp derivatives that connect the corresponding protected amino acids (L-Phe, L-Leu, L-Pro, and L-Trp) to bispyrrolidinoindoline **25** derived from protected L-Trp, namely, (*S*)-**23**, toward the synthesis of **13**−**15**, **17**, and **18** (exemplified for **14** in Scheme 2B).

The *exo*-bromopyrrolidinoindolines **21** and **26** (Scheme 2) required for the Co(I)-promoted dimerization[27,36] were independently synthesized in three-step sequences[33] starting from commercial D- or L-Trp (Supporting Information, Section S6.2), namely, formation of the methyl ester, amine protection as bis-Boc carbamate derivatives (*R*)-**23** and (*S*)-**23**, and a stereoselective bromocyclization reaction.[33−35] Treatment of (*R*)-**23** or (*S*)-**23** with *N*-bromosuccinimide (NBS) under the optimized conditions (1 equiv of PPTS, CH_2Cl_2, 25 °C)[34]

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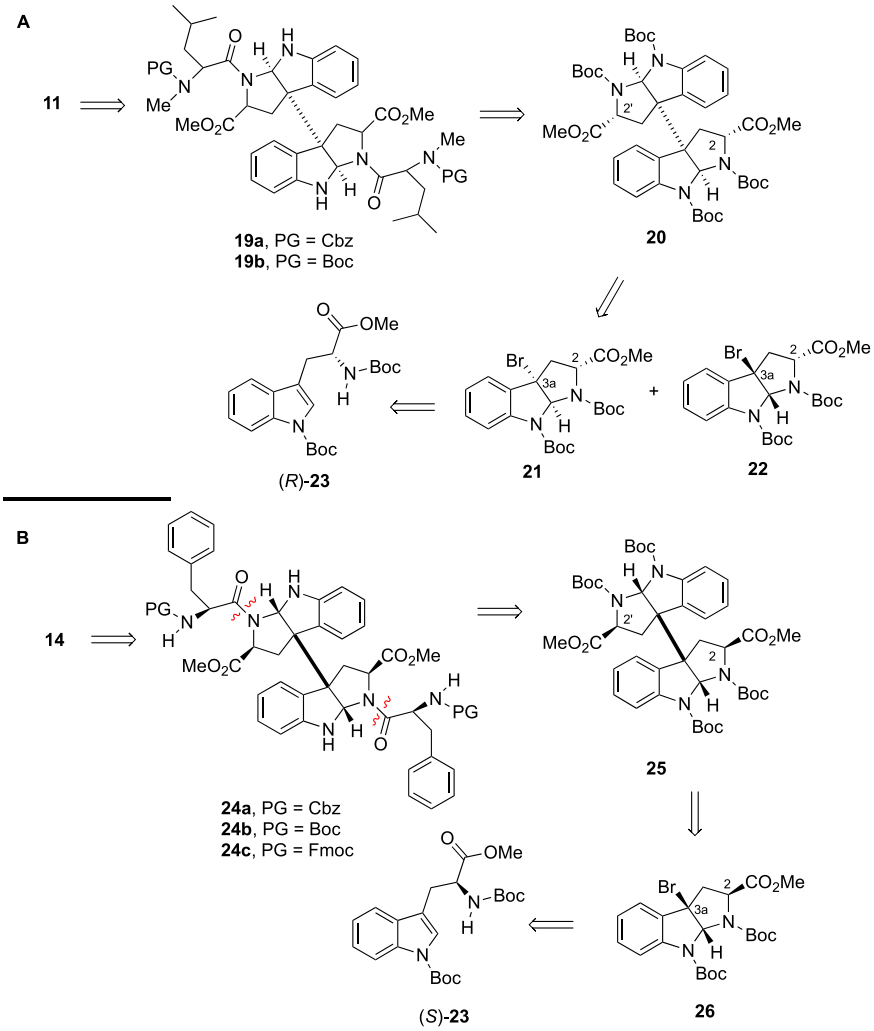
Scheme 2. Retrosynthetic Analysis of the Proposed Structures of Bispyrrolidinoindoline Dioxopiperazine Alkaloids (Figure 1) Adapted to 11 and 14, Respectively: (A) from Protected D-Trp, (*R*)-23, and Coupling to Protected (*N*-Me-)L-Leu and (*N*-Me-)D-Leu; (B) from Protected L-Trp, (*S*)-23, and Coupling to Protected L-Phe



afforded in 93% yield either **21** or **26** and their *anti*-diastereomers (only **22** is shown) in a 94:6 ratio (Section S6.2), a highly diastereoselective bromocyclization that was justified through DFT calculations.[35]

Total Synthesis of Bispyrrolidinoindoline Dioxopiperazine Alkaloids Derived from L-Trp and Enantiomers of Leu. As only ^{1}H NMR and ^{13}C NMR spectroscopic data, but not relative and absolute configurations, were provided for ditryptoleucine **11**,[16] in order to address the preparation of the series of diastereomers and extend the protocol to the synthesis of (+)-**12** (Figure 1), the configuration at C-2/C-2′ in homodimer **20** was inverted to efficiently generate **27** (Scheme 3). The selectivity is considered to be due to the thermodynamic preference of *exo*-2-acylhexahydropyrrolo-[2,3-*b*]indoles to place the acyl group at the *endo* position under base-promoted equilibration conditions[37] and thus reduce torsional interactions around the formal diazabicyclo[3.3.0]octane core.[38] Thus, treatment of the *exo* dimer **20** with 4 equiv of lithium hexamethyldisilazide (LiHMDS) at −15 °C in THF and quenching of the corresponding lithium ester enolates with MeOH at −78 °C[37] afforded the *endo* diastereomer **27** in 93% yield (Scheme 3). The ^{1}H NMR chemical shifts for the methyl esters of both diastereoisomers allowed the structural assignment of their relative configurations, because the *endo* bispyrrolidinoindoline shows a shielded signal (δ_H ∼3.1) relative to the *exo* diastereomer (δ_H ∼3.7).[37]

Subsequent cleavage of the four *N*-Boc protecting groups in **20**, **25**, and **27** took place upon treatment with TMSI in CH_3CN at 0 °C (Scheme 3).[39] Given the water solubility of the resulting bispyrrolidinoindolines **28**−**30**, a resin bearing diisopropylamino groups was added to the reaction mixture[40] followed by wet MeOH, with the purpose of quenching the acidic media, which allowed isolation of these dimeric compounds in high yields (93−95%).

The twofold coupling of *endo*-**28** and *exo*-**29** with *N*-Cbz-D-Leu (**31a**) and *N*-Boc-D-Leu (**31b**) and with their enantiomers derived from L-Leu was carried out in the presence of HATU and Et_3N in DMF to provide the corresponding dimers, **33a,b** and **34a,b** and **35a,b** and **37a,b**, in variable yields (Schemes 4−6), which were purified but not fully characterized due to the typical line broadening observed in their NMR spectra.

Nitrogen deprotection and dioxopiperazine formation[2] were then performed under alternative reaction conditions, namely, catalytic hydrogenation for *N*-Cbz-protected precursors and acidic treatment for *N*-Boc-protected analogues. Only the

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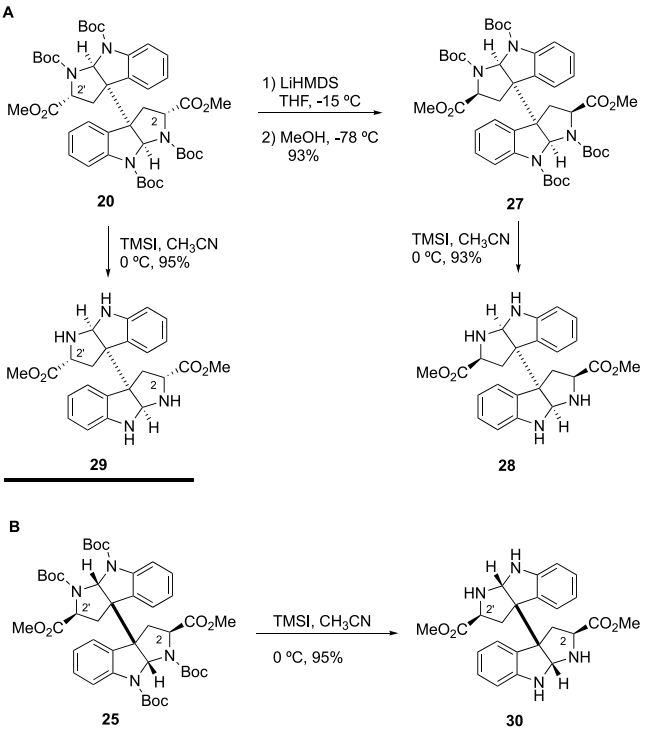
Scheme 3. (A) Synthesis of Diastereomeric Bispyrrolidinoindolines (28, 29) Derived from D-Trp; (B) Synthesis of Enantiomeric Bispyrrolidinoindoline (30) Derived from L-Trp



procedures that provided the highest yields on the optimized reaction conditions are shown in Schemes 4−6 (Supporting Information, Section S6, contains a full description of the experimental work), which differed slightly depending upon the relative configuration of the bispyrrolidinoindoline precursors.

Formation of the dioxopiperazines by condensation of the amino and the methyl ester groups of **34** to afford *endo*-L-ditryptoleucine **11a** (Scheme 4A) was found to be the most challenging of the diastereomeric series, as anticipated by prior experience on the synthesis of **16**[33,34] and related dioxopiperazines.[21] Hydrogenolysis of **34a** (PG = Cbz) promoted by Pd on C in MeOH as catalyst for 16 h led to *cyclo*-(L-Trp-L-Me-Leu) **32**,[41,42] a putative *N*-Me derivative of natural *cyclo*-(L-Trp-L-Leu), in quantitative yield (Supporting Information, Section S2.9),[41] in what could be formally considered as a retrobiogenetic process.[1] The formation of a related dioxopiperazine, namely, *cyclo*-(L-Trp-L-Phe), was also reported during the early efforts on the structure elucidation of (+)-**10** (Figure 1).[15] The deprotection/cyclization of *N*-Boc-protected dipeptide **34b** required heating in toluene at 140 °C with SiO_2 in a microwave reactor (MW, 300 W power) for 1 h,[43] which afforded homodimer **11a** in 70% yield (Scheme 4) together with additional byproducts (Supporting Information, Scheme S1). The presence of impurities required further purification by HPLC (Luna 5 μm PFP, 3 mL/min, 0.1% HCO_2H in 30:70 to 30:70 v/v, CH_3CN/Milli-Q H_2O gradient; t_R = 11.3 min) and afforded *endo*-L-ditryptoleucine **11a** in 47% yield. Moreover, upon increasing the reaction temperature from 140 °C to 180 °C, *cyclo*-(L-Trp-L-Me-Leu) (**32**)[41,42] was instead obtained in 65% yield (Supporting Information, Section S6.2.9).

In striking contrast, hydrogenolysis of the *N*-Cbz protecting group of **33a** (PG = Cbz) using Pearlman's catalyst [$Pd(OH)_2$ on C (20% wt loading)][44] was followed by dioxopiperazine ring formation[21] upon treatment with excess 28−30% aqueous NH_4OH in MeOH at rt for 2 h and afforded uneventfully the *endo*-D-ditryptoleucine diastereomer **11b** in 99% yield (Scheme 4B; see also Supporting Information, Scheme S2).

Similarly, the synthesis of the *exo*-L-ditryptoleucine diastereomer **11d** (Scheme 5) by *N*-Cbz deprotection[21] and ensuing dioxopiperazine ring formation was carried out in an overall 94% yield upon stirring solutions of bispyrrolidinoindoline **36a** in MeOH (0.01 M) with Pd on C (10% wt loading) at rt for 15h.[21]

Lastly, hydrogenation of **35a** (PG = Cbz) using Pd on C (10% wt loading) but with excess aqueous ammonia (28% NH_4OH) in MeOH at rt for 4 h afforded *exo*-D-ditryptoleucine **11c** in 67% yield accompanied not only by precursors resulting from deprotection but also by diastereomer **11d** (Scheme 5), which confirmed the facile epimerization of diketopiperazine rings under those reaction conditions (Supporting Information, Scheme S4).

Interestingly, when either **35a** (PG = Cbz) or the deprotected derivative was treated with Pearlman's catalyst [$Pd(OH)_2$ on C (20% wt loading)][44] in MeOH at rt under a hydrogen atmosphere for 6 h, an unexpected compound was obtained in high yield (88%). Interpretation of the spectroscopic data and MS analysis suggested the formation of a product with the dimeric structure **37** (Scheme 6). Instead of the expected fused diketopiperazine, homodimer **37** contains a methyl octahydro-2a,5,6a-triazabenzo[*a*]cyclopenta-[*cd*]azulene-2-carboxylate skeleton resulting from the generation of a fused 1,3,5-triazepan-6-one heterocycle with the three nitrogen atoms, including those of the pyrrolidinoindoline fragment, as part of the new ring.[45]

Without discarding effects related to the undefined composition of Pearlman's catalyst,[46] pyrrolidinone fragments have been previously obtained upon deprotection of *N*- and *O*-benzyl indole/phenol-containing pyrrolidine structures under similar reaction conditions. An intriguing mechanistic proposal (Supporting Information, Scheme S5) was further advanced in order to justify the appearance of oxidation products under formal hydrogenation reaction conditions.[47] In accordance with the proposed mechanism (Scheme S5), efforts to promote the formation of the dioxopiperazine ring in **11c** starting from the deprotected precursor under the same conditions but in the absence of $Pd(OH)_2$ on C proved to be fruitless after 15 h of stirring.

The contrasting behavior of the diastereomeric precursors on dioxopiperazine ring formation has already been noted.[21,33,34] We have carried out computational studies aimed to justify the structural requirement for cyclization of the series of diastereomers **33−36** on route to the ditryptoleucine family of natural products, and the results can be found in the Supporting Information, Figure S6.

Comparison of the spectroscopic data with those reported[16] for ditryptoleucines A and B (**11**) (Supporting Information, Section 6.2.9; Tables S1−S4) suggested the assignment of the relative configuration of ditryptoleucine A to diastereomer *exo*-L **11d**. Although the same conclusion was reached by Ishikawa and co-workers after their synthesis of ditryptoleucine A,[21] the absolute configuration of *exo*-L **11d** was assigned by structural analogy with those of known analogues shown in Figure 1 and the assumption that all these homo- and heterodimeric

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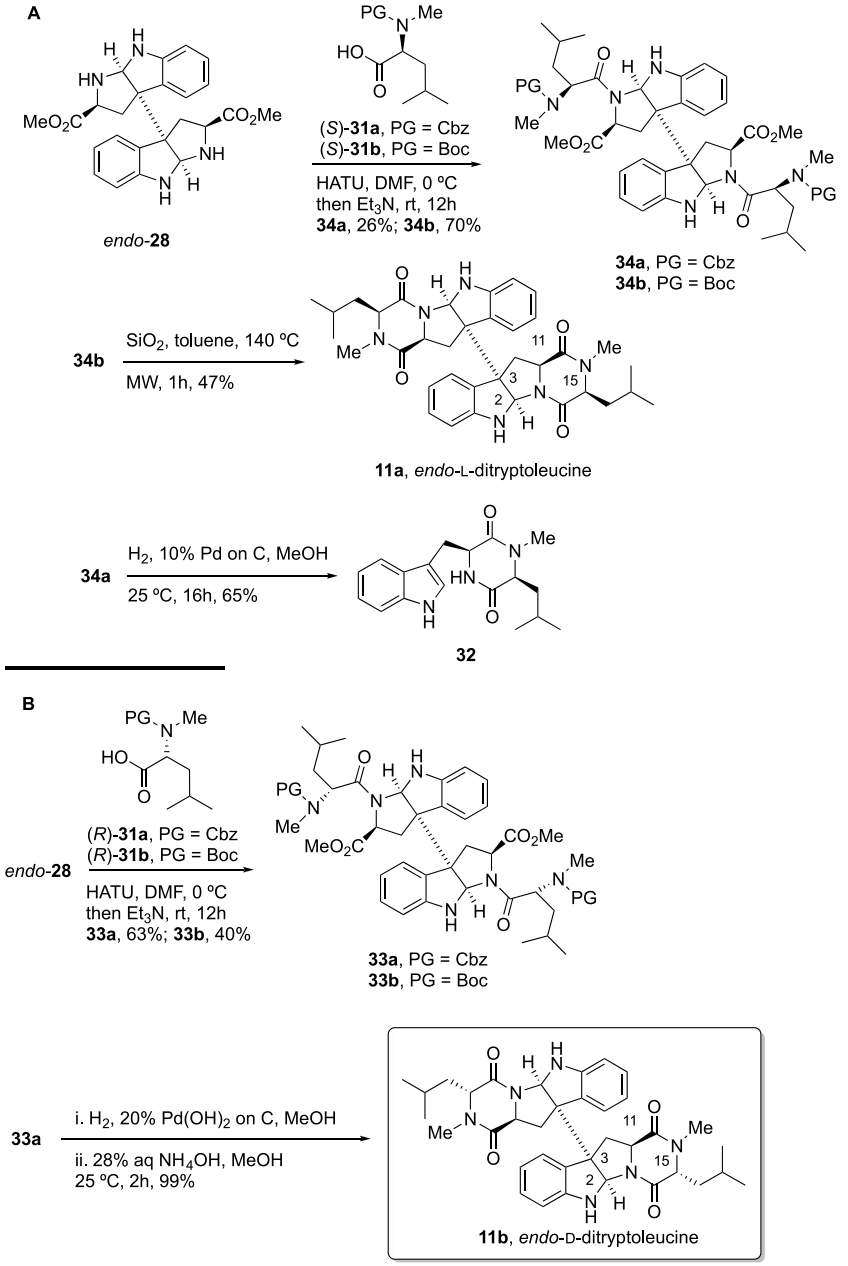
Scheme 4. (A) Synthesis of *endo*-L-Ditryptoleucine 11a;[a] (B) Synthesis of *endo*-D-Ditryptoleucine 11b



[a]MW: microwave.

alkaloids in Nature were derived from L-Trp. However, the recent isolation from *Aspergillus versicolor* 16F-11 (KM605199) obtained from the marine sponge *Phakellia fusca*,[48] and structural assignment of (+)-asperflocin, the formal C-11′-epimer of (+)-WIN 64821 (**10**) (Figure 1), suggested that it could be biosynthesized by the fungus through the random selection of L-Trp and D-Trp.[48]

Our synthetic sample of *endo*-D **11b** showed also ^{1}H NMR data rather similar to those reported for natural ditryptoleucine B[16] and was assigned this structure despite some discrepancies of the ^{13}C NMR chemical shifts with those reported for the natural product (Section 6.2.9; Tables S1−S4). As indicated for *exo*-L **11d**, neither specific rotation nor ECD spectra were reported for these natural products, and therefore the absolute configuration remains uncertain.[16]

Bispyrrolidinoindoline dioxopiperazine (+)-**12**,[18] being formally the *N*-demethylated analogue of *endo*-D-ditryptoleucine **11d**, was made following the same general synthetic sequence (Scheme 7), namely, the condensation promoted by HATU and Et_3N of *endo*-**28** with *N*-Cbz-D-Leu (*R*)-**38a**[49] to afford the bis-amide intermediate **39** in 48% yield and final amine deprotection−dioxopiperazine ring formation upon hydrogenation with Pearlman's $Pd(OH)_2$ on C catalyst (20% wt loading)[44] in MeOH, followed by treatment with 28−30% aqueous NH_4OH in MeOH (Scheme 7). In addition to (+)-**12** (62% yield), the deprotected uncyclized tetrapeptide was also obtained (Supporting Information, Section S6.2.11), thus suggesting the more favorable DKP formation of the *N*-methyl derivatives as shown for the synthesis of **11d**. The absolute configuration of natural product (+)-**12** had been proposed,[17] after confirmation of the presence of D-Leu by application of

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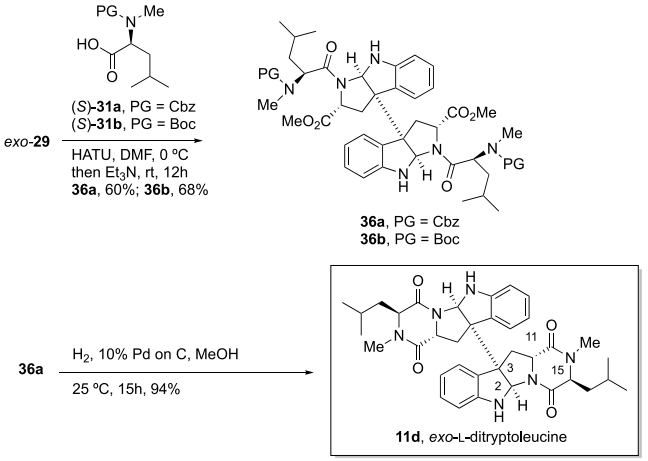
Scheme 5. Synthesis of *exo*-L-Ditryptoleucine 11d



Marfey's method.[50,51] Comparison of NMR data (Supporting Information, Section S6.2.11; Table S5) and specific rotation values of the synthetic compound ($[\alpha]^{24}_{D}$ +300 (*c* 0.34, $CHCl_3$)) with those of the natural product ($[\alpha]^{25}_{D}$ +530 (*c* 0.3, MeOH))[18] confirmed the stereostructure of (+)-**12**.

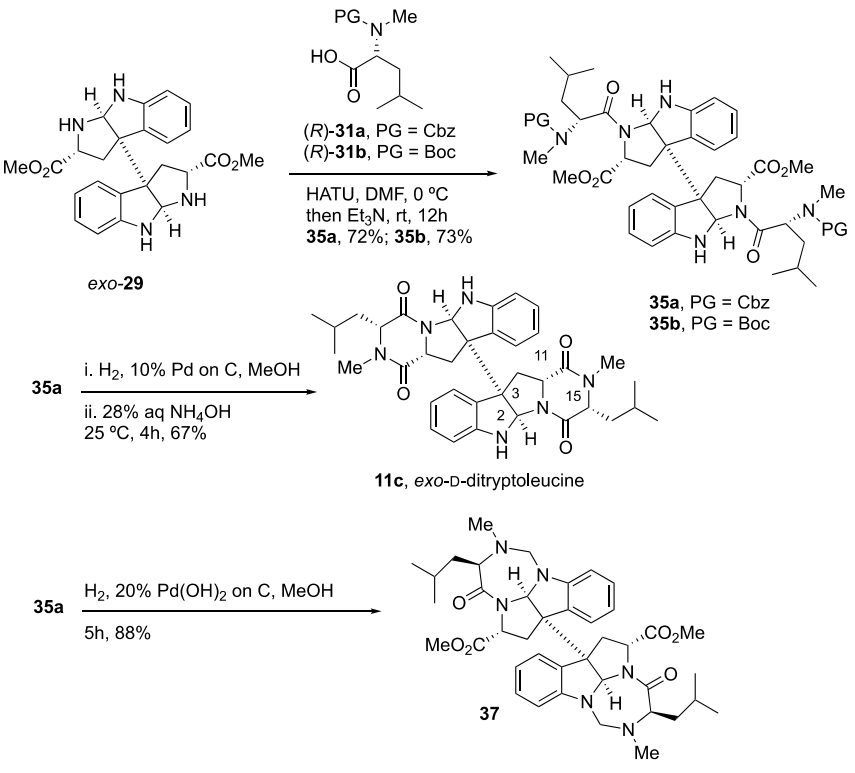
Total Synthesis of Bispyrrolidinoindoline Dioxopiperazine Alkaloids Derived From L-Trp, L-Phe, and L-Pro. Similarly, by choosing the enantiomer of the bispyrrolidinoindoline core, namely, *exo*-**30**, itself prepared from **25** (derived from L-Trp; Supporting Information, Section S6.2) by deprotection of the *N*-Boc groups (Scheme 3), another series of natural products with the same configurations at C-11 and C-15, but differing in the nature of the first and second terminal amino acids, was prepared.

Homodimeric Bispyrrolidinoindoline Dioxopiperazines. For the synthesis of the homodimeric alkaloids, dimers **41**, **43**, and **46** (Scheme 8) were instead required. The former was obtained in 70% yield upon coupling **30** with *N*-Cbz-L-Pro **40** (2.4 molar equiv) promoted by COMU and DIPEA in DMF. Bis-amide **41** was deprotected by hydrogenolysis to the bispyrrolidinoindoline derivative, which spontaneously underwent cyclization to generate (−)-dibrevianamide F (**13**) in almost quantitative yield (Scheme 8), in accordance with the results of the *exo* diastereomers discussed before (Scheme 6). The ^{1}H NMR and ^{13}C NMR spectra (Supporting Information, Section 6.2.12; Table S6) and the specific rotation value of synthetic (−)-**13** ($[\alpha]^{24}_{D}$ −369 (*c* 0.5, $CHCl_3$)) were similar to those reported for the natural product ($[\alpha]^{24}_{D}$ −483.91 (*c* 0.32, $CHCl_3$)).[4] The X-ray crystal structures of both (−)-**13** and (−)-**17** (Figure 1) already confirmed their absolute configuration after isolation from natural sources.[4]

Similarly, (−)-ditryptophenaline (**9**) was also synthesized (Section S6.2.13) in almost quantitative yield (94%) from **30** using protected *N*-Cbz-*N*-Me-L-Phe (2.4 molar equiv) as reactant in the presence of COMU and DIPEA in DMF, and the intermediate (73% yield) was deprotected by hydrogenolysis catalyzed by $Pd(OH)_2$ on C (20%) in MeOH followed by 28% aqueous NH_4OH at rt (Supporting Information, Section S6.2.13, Table S7).

Because (−)-SF-5280−451 (**14**) is formally the bis-*N*,*N*′-demethylated analogue of (−)-**9**, exchange of the amino acid component was required for the coupling to **30**. Whereas *N*-Cbz-L-Phe **42a**[40] as coupling partner of **30** in the presence of HATU and 2,6-lutidine in DMF provided a mixture of homodimer **43a** in 47% yield and monocoupled product **44a** in 23% yield (Scheme 8 B), *N*-Fmoc-L-Phe **42c** under the same conditions afforded **43c** in 74% yield. Deprotection of the dimeric structure **43c** upon treatment with Et_2NH in MeOH

Scheme 6. Synthesis of *exo*-D-Ditryptoleucine 11c and Bispyrrolidinoindoline 1,3,5-Triazepan-6-one 37



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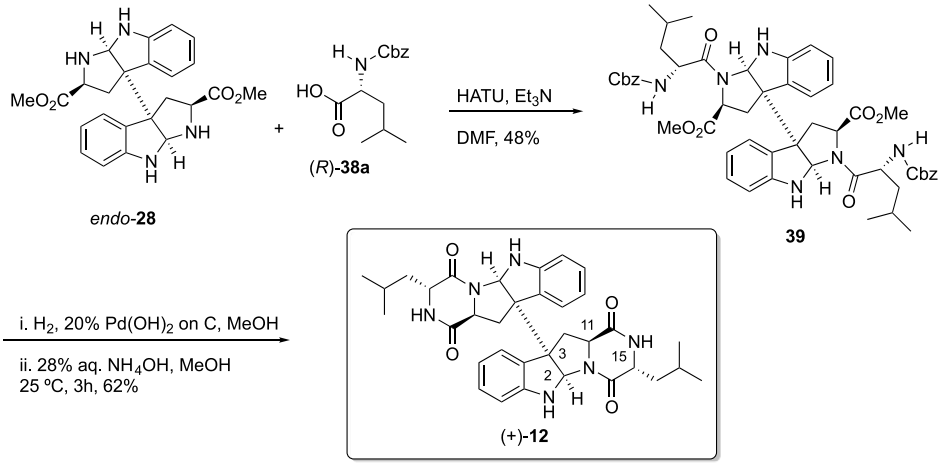
Scheme 7. Synthesis of Homodimeric Bispyrrolidinoindoline Dioxopiperazine (+)-12



for 15 h gave rise to (−)-**14** in 78% yield. The ^{1}H NMR and ^{13}C NMR spectra (Section S6.2.13; Table S7) and the specific rotation value ($[\alpha]^{24}_{D}$ −393 (*c* 0.13, $CHCl_3$); cf. $[\alpha]^{24}_{D}$ −343 (*c* 0.04, CH_2Cl_2))[18] were similar to those reported for the natural product. The configuration of (−)-**14** had previously been proposed based on the similarity of the ECD spectrum to that of (−)-**9**.[18] (−)-SF-5280−451 (**14**) has also been recently prepared,[23] together with several diastereomers, using the same strategy based on the epimerization of the bispyrrolidinoindolines and peptide formation followed by DKP generation, but its natural occurrence[18] was not mentioned.

Similarly, the recently discovered (−)-tetratryptomycin A (**15**)[6] was more conveniently synthesized in 38% combined yield by 2-fold condensation of **30** and *N*-Fmoc-L-Trp **45c** in the presence of DMT-MM in EtOH at room temperature and, without further purification (Scheme 8C), deprotection of the resulting intermediate **46c** upon treatment with morpholine. The ^{1}H NMR and ^{13}C NMR spectra (Supporting Information, Section S6.2.15; Table S9) were similar to those reported for the natural product. Although no absolute configuration was reported for (−)-**15**,[6] it has been assumed as indicated based on the similarity of the ECD spectrum[6] with that described for analogues.[52] When *N*-Cbz-L-Trp **45a** was instead used for coupling with **30**, not only did the condensation reaction promoted by BOPCl and Et_3N in THF turn out to be less efficient (22−36% yield), but the acyclic tetrapeptide **46a** underwent cleavage of the pyrrolidinoindoline fragment upon treatment with H_2 and Pd on C followed by aqueous ammonia as described for **34a** (Scheme 4A) and provided rather complex reaction mixtures, from which *cyclo*-[L-Trp-L-Trp] (**47**)[53] (Scheme 8C) was the only product that could be obtained (in 16% yield) and fully characterized (Supporting Information, Section S6.2.16). Natural **47** has been previously isolated during biogenetic studies aimed to discover P450 dimerization enzymes after heterologous expression of candidate genes and gene clusters in *Streptomyces albus* J1074, which led to the discovery of cWW synthases termed TtpA1 and TtpA2.[6] Additional cyclodipeptides leading biogenetically to the same product[42,54] and further involvement of **47** in the biogenesis of related natural congeners have recently been demonstrated.[55−57]

Heterodimeric Bispyrrolidinoindoline Dioxopiperazines. We have reported the selective one-pot formation of bispyrrolidinoindoline dioxopiperazines as condensation products resulting from the monocoupling of protected amino acid peptide components when the reaction temperature was maintained at −15 °C.[33,34] However, in addition to the heterodimeric bis-coupled reaction product, the homodimeric analogues were also isolated in lower to comparable yields depending upon the nature of the amino acid (cf. 10−15% for L-Phe, 19% for D-Val, and 41% for D-Ala).[58] Similar observations were made by Ishikawa et al. during the preparation of related alkaloids using an analogous strategy.[21]

In order to overcome some of these limitations, for the nonsymmetrical bispyrrolidinoindoline dioxopiperazine natural products (−)-tryprophenaline (**17**) and (−)-SF-5280−415 (**18**), the choice of sequential monocoupling reactions allowed further improvement of the selectivity (Schemes 9 and 10). Thus, Cbz-protected (*S*)-**31a** (1.3 molar equiv) was added to a solution of bispyrrolidinoindoline **30** in DMF at 0 °C, followed by COMU and DIPEA to provide, after 26 h, monocoupled derivative **48** in 93% yield. Removal of the Cbz protecting group using $Pd(OH)_2$ on C as catalyst followed by treatment with NH_4OH gave rise to dioxopiperazine **49**. A second condensation of **49** with an excess of protected L-Pro **40** (2.4 equiv) afforded **50**, and final hydrogenation of the latter component with 10% Pd on C in MeOH provided (−)-**17** in 77% yield (Scheme 9). Comparison of the NMR data (Supporting Information, Section S6.2.17; Table S10) and specific rotation values ($[\alpha]^{25}_{D}$ −419 (*c* 0.13, $CHCl_3$); cf. $[\alpha]^{27}_{D}$ −378.95 (*c* 0.43, $CHCl_3$))[4] allowed confirmation of the structure of the natural product.[4]

A similar ordering of events was used for the synthesis of (−)-**18** (Scheme 10). First, condensation of **30** with *N*-Cbz-Phe (*S*)-**42a** (1.3 mol equiv) in the presence of HATU and anhydrous 2,6-lutidine (5 molar equiv) for 20 h at rt led selectively to **44a**. HPLC monitoring of the reaction progress confirmed the selectivity of the monocoupling reaction. Deprotection by hydrogenolysis afforded monoprotected derivative **51** in 51% yield. Condensation of intermediate **51** with excess (2.6 molar equiv) *N*-Cbz-Leu (*S*)-**38a** (2.6 equiv) promoted by COMU and DIPEA as indicated above, followed by deprotection of **52** under catalytic hydrogenation conditions, also resulted in DKP ring formation and afforded (−)-**18** in 74% yield (Scheme 10).

Comparison of the NMR data (Supporting Information, Section S6.2.18; Table S11) and specific rotation value ($[\alpha]^{25}_{D}$ −135 (*c* 0.055, MeOH); cf. $[\alpha]^{27}_{D}$ −261 (*c* 0.5, MeOH))[19]

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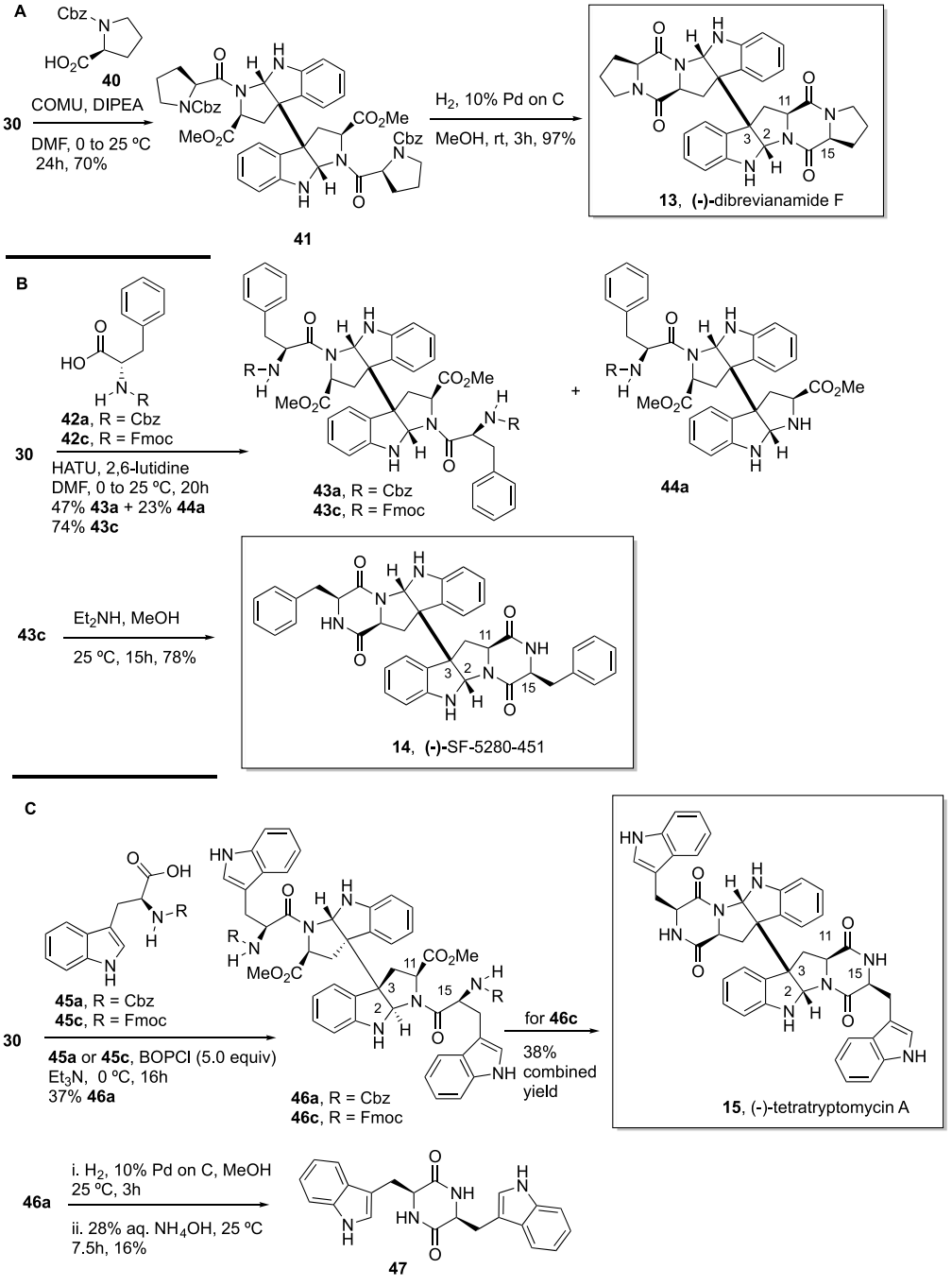
Scheme 8. (A) Synthesis of (−)-Dibrevianamide F (13); (B) Synthesis of (−)-SF-5280-451 (14); (C) Synthesis of (−)-Tetratryptomycin A (15)



likewise confirmed the structure proposed for the natural product. As indicated for symmetrical (−)-SF5280-451 (**6**), the nonsymmetrical (−)-SF5280-415 (**18**) showed the same absolute configuration as (−)-ditryptophenaline (**9**)[19] but different configurations on some of the stereocenters when compared to (+)-WIN 64745 (**16**).[15]

CONCLUSIONS

In summary, following the generation of the bispyrrolidinoindoline scaffold by using as a key step the Co(I)-induced dimerization of the C3a-bromo-hexahydropyrrolo[2,3-*b*]indole core to construct the C-3/C-3′ central bond, the subsequent amide formation with Leu, Phe, Pro, and Trp and final dioxopiperazine ring formation allowed completion of the synthesis of homodimeric (C_2-symmetric) bispyrrolidinoindoline dioxopiperazine natural products. Control of peptide bond formation by stepwise addition of the different amino acids before diketopiperazine ring construction led instead to the heterodimeric C_1-nonsymmetric alkaloids. The base-induced epimerization of the *exo*- to the *endo*-C2-acyl-hexahydropyrrolo[2,3-*b*]indole further expanded the stereochemical diversification of the family of dimeric alkaloids with the synthesis of the C-11/C-11′ epimers. Having the more congested dimeric scaffold, the exo-D-diastereomer under hydrogenation conditions using Pearlman's catalyst in MeOH

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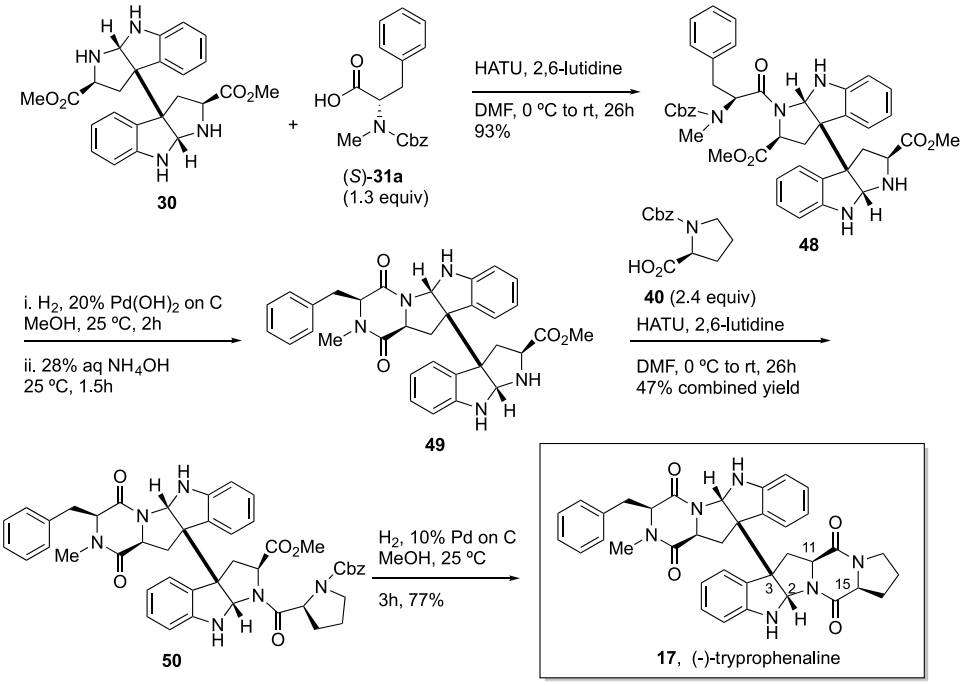
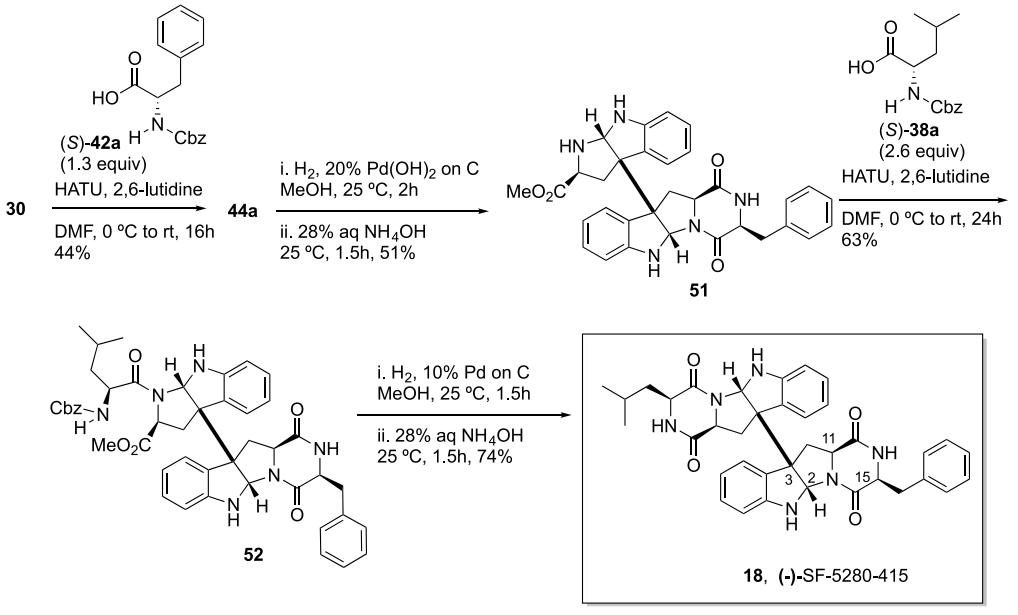
Scheme 9. Synthesis of (−)-Tryprophenaline (17)



Scheme 10. Synthesis of (−)-SF-5280-415 18



led instead to the quantitative formation of a novel bispyrrolidinoindoline-1,3,5-triazepan-6-one skeleton.

The sign of the specific rotation $[\alpha]_D$ data for this family of dimeric alkaloids is highly consistent with the configuration of the hexahydropyrroloindole monomer. A positive $[\alpha]_D$ value would indicate the *R* configuration at C-2/C-2′ and C-3/C-3′ as in (+)-WIN 64821 (**10**), (+)-**12**, and (+)-WIN 64745 (**16**), whereas a negative $[\alpha]_D$ value would confirm the *S* configuration for the same stereogenic centers in the series of (−)-ditryptophenaline (**9**) and analogues, namely, (−)-dibrevianamide F (**13**), (−)-SF-5280-451 (**14**), (−)-tetratryptomycin A (**15**), (−)-tryprophenaline (**17**), and (−)-SF-5280-415 (**18**). Although the absolute configuration of **11a** and **11b** remains to be corroborated by comparison with the values of natural ditryptoleucines,[16] total synthesis continues to be the ultimate proof for the confirmation and/or determination of the three-dimensional structures of natural products.[59]

EXPERIMENTAL SECTION

General Experimental Procedures. Specific rotations were obtained on a JASCO P-1020 polarimeter. IR spectra were obtained on a JASCO IR 4200 spectrophotometer from a thin film deposited onto NaCl glass. ^{1}H NMR spectra were recorded in $CDCl_3$, CD_3CN, or DMSO-d_6 at ambient temperature (or the indicated temperature) on a Bruker AMX-400 spectrometer at 400 (or 600 MHz) with residual protic solvent as the internal reference ($CDCl_3$, δ_H 7.26; CD_3CN, δ_H 1.94; DMSO-d_6, δ_H 2.50). ^{13}C NMR spectra were

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recorded in $CDCl_3$, CD_3CN, or DMSO-d_6 at ambient temperature unless otherwise indicated on the same spectrometer at 100 MHz, with the central peak of $CDCl_3$ (δ_C 77.16), CD_3CN (δ_C 118.26), or DMSO-d_6 (δ_C 39.52) as the internal reference. DEPT135 and bidimensional (COSY, HSQCed, HMBC, and NOESY) sequences were used where appropriate to aid in the assignment of signals. Mass spectra and HRMS (ESI^+) were taken on an Apex III FT ICR MS (Bruker Daltonics) apparatus.

Solvents were dried according to published methods and distilled before use. All reagents were commercial compounds of the highest purity available. Reactions were carried out under an argon atmosphere, unless indicated otherwise. Analytical TLC was performed on aluminum plates with Merck Kieselgel $60F_{254}$ and visualized by UV irradiation (254 nm) or by staining with an ethanolic solution of phosphomolibdic acid. Flash-column chromatography was carried out using Merck Kieselgel 60 (230−400 mesh) under pressure.

General Procedure for Boc-Deprotection of Homodimeric Bispyrrolidinoindolines. To a cooled (0 °C) solution of the protected homodimer **27** (0.4 g, 0.48 mmol, 1.0 equiv) in CH_3CN (11 mL, 0.05 M) was added TMSI (300 μL, 4.4 equiv). The resulting mixture was stirred for 90 min until the reaction was completed, which was followed by TLC. Diisopropylamine resin (400 mg) and MeOH (16 mL) were added to the mixture at 0 °C, the temperature was raised to 25 °C, and it was stirred for another 20 min. The suspension was filtered, the resin was washed with MeOH and CH_2Cl_2, and the solvent was eliminated under reduced pressure to afford the deprotected tetraamine. When the product was used in homocoupling reactions, the residue could be used without further purification assuming a quantitative yield. If the product was used in monocoupling reactions, the residue was purified by flash-column chromatography (silica gel, from 100:0 to 90:10 v/v CH_2Cl_2/MeOH) to afford 190 mg (93% yield) of deprotected bispyrrolidinoindoline **28**.

General Procedure for Peptide Coupling of Leucine to Homodimeric Bispyrrolidinoindolines. For the coupling reactions, the two enantiomers of *N*-Boc- or *N*-Cbz-*N*-Me-Leu previously synthesized (Section S6.4) were used. The protected L- or D-Leu (0.33 mmol, 3.0 equiv) was added to a solution of the tetraamine (0.05 g, 0.11 mmol, 1.0 equiv) in DMF (2 mL, 0.06 M) at 0 °C, followed by HATU (0.13 g, 0.33 mmol, 3.0 equiv). After purging the resulting mixture for 5−10 min, the inert gas was removed and anhydrous Et_3N (71 μL, 0.52 mmol, 4.5 equiv) was added. The reaction mixture was stirred overnight at rt. H_2O and EtOAc were added, the aqueous layer was extracted with EtOAc (3×), the combined organic layers were washed with H_2O (4×), dried over anhydrous Na_2SO_4, and filtered, and the solvent was evaporated under reduced pressure. The residue was purified by flash-column chromatography (silica gel, from 80:20 to 50:50 v/v hexane/EtOAc) to afford the bis-coupled reaction products in yields ranging from 40% to 70%. The compounds **32**−**36** could not be fully characterized because of the complexity of the spectra, in most of the cases due to the presence of rotamers.

Synthesis of the Four Stereoisomers of Ditryptoleucine 11. *Synthesis of endo-L-Ditryptoleucine **11a** (Scheme 4).* To a solution of the dipeptide **34b** (40 mg, 0.045 mmol, 1.0 equiv) in toluene (2.2 mL, 0.02 M) was added silica gel (585 mg, 13 g/mmol), and the resulting suspension was heated in a microwave reactor at 140 °C for 1 h. The silica gel was washed with a 90:10 v/v CH_2Cl_2/MeOH solution, and the solvent was evaporated. Purification by flash-column chromatography (silica gel, 95:5 to 90:10 v/v CH_2Cl_2/MeOH) afforded 18 mg of impure ditryptoleucine. The product was further purified by HPLC-UV (Luna 5 μm PFP, 254 and 280 nm, 3 mL/min, 0.1% HCO_2H in 30:70 to 30:70 v/v CH_3CN/Milli-Q H_2O gradient; t_R = 11.3 min) to obtain 7.6 mg (47%) of a white solid, which was characterized as *endo*-L-ditryptoleucine **11a**. The purification by HPLC was required because the reaction conditions led to some epimerization of the substrate. $[\alpha]^{23}_D$ +110 (*c* 0.6, $CHCl_3$); UV (MeOH) λ_{max} (nm) 240, 299; IR (NaCl) ν_{max} (cm^{-1}) 3600−3100 (br, N−H), 2956 (m, C−H), 2869 (w, C−H), 1658 (s, C═O), 1460 (s); ^{1}H NMR(400 MHz, $CDCl_3$) δ_H 7.38 (d, *J* = 7.6 Hz, 2H, H_5 + $H_{5'}$), 7.11 (t, *J* = 8.0 Hz, 2H, H_7 + $H_{7'}$), 6.79 (t, *J* = 8.0 Hz, 2H, H_6 + $H_{6'}$), 6.60 (d, *J* = 7.9 Hz, 2H, H_8 + $H_{8'}$), 5.78 (s, 2H, 2×NH), 4.81 (s, 2H, H_2 + $H_{2'}$), 3.90 (app t, *J* = 10.0 Hz, 2H, H_{11} + $H_{11'}$), 3.8−3.7 (br, 2H, H_{15} + $H_{15'}$), 3.32 (dd, *J* = 13.5, 8.0 Hz, 2H, H_{12a} + $H_{12a'}$), 2.83 (s, 6H, 2×N$\underline{CH_3}$), 2.70 (dd, *J* = 13.5, 11.0 Hz, 2H, H_{12b} + $H_{12b'}$), 1.86 (ddd, *J* = 14.8, 7.9, 3.0 Hz, 2H, H_{17a} + $H_{17a'}$), 1.73 (dt, *J* = 14.8, 5.7 Hz, 2H, H_{17b} + $H_{17b'}$), 1.4−1.3 (m, 2H, H_{18} + $H_{18'}$), 0.66 (d, *J* = 6.6 Hz, 6H, 2×$\underline{CH_3}$), 0.52 (d, *J* = 6.5 Hz, 6H, 2×$\underline{CH_3}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 166.8 (C), 166.6 (C), 149.3 (C), 130.8 (C), 129.9 (CH), 125.1 (CH), 120.5 (CH), 110.4 (CH), 80.5 (CH), 61.7 (CH), 59.9 (C), 56.8 (CH), 39.8 (CH_2), 38.8 (CH_2), 31.9 (CH_3), 24.5 (CH), 23.5 (CH_3), 22.7 (CH_3); ESIMS *m*/*z* 625 $[M + H]^+$; HRESIMS (ESI^+) *m*/*z* 625.3496 $[M + H]^+$ (calcd for $C_{36}H_{45}N_6O_4$, 625.3493).

*Synthesis of endo-D-Ditryptoleucine **11b**.* To a solution of the dipeptide **33a** (93 mg, 0.1 mmol, 1.0 equiv) in MeOH (0.7 mL, 0.01 M) was added activated Pd on C (69 mg, 10% wt loading, 1.05 g/mmol). The argon atmosphere was replaced by hydrogen, which was allowed to bubble into the solution for 5−10 min. The resulting suspension was stirred at rt under hydrogen for 4 h. The reaction mixture was filtered through Celite washing with EtOAc, and the solvent was evaporated to afford 61.8 mg of the deprotected product, which was used in the next step without further purification.

Procedure 1. The deprotected dimer (10 mg, 0.014 mmol, 1.0 equiv) was treated with a 28−30% v/v aqueous solution of ammonia (52 μL, 3.68 mL/mmol) in MeOH (2.3 mL, 0.006 M). The resulting mixture was stirred at rt for 72 h. The solvent was eliminated under reduced pressure, and the residue was purified by flash-column chromatography (silica gel, from 100:0 to 95:5 v/v CH_2Cl_2/MeOH) to afford 8.1 mg (90% yield) of *endo*-D-ditryptoleucine **11b**.

Procedure 2. To a solution of the deprotected dipeptide (10 mg, 0.014 mmol, 1.0 equiv) in MeOH (1.4 mL, 0.01 M) were added Pearlman's catalyst (6 mg, 20% wt. loading, 0.41 g/mmol) and a 28−30% v/v aqueous solution of ammonia (52 μL, 3.68 mL/mmol). The resulting suspension was stirred at rt under hydrogen for 2 h, then filtered through Celite washing with MeOH, and the solvent was evaporated. The resulting residue was purified by flash-column chromatography (silica gel, 98:2 v/v CH_2Cl_2/MeOH) to afford 8.7 mg (99% yield for the two steps) of *endo*-D-ditryptoleucine **11b**. $[\alpha]^{23}_D$ +120 (*c* 0.17, $CHCl_3$); UV (MeOH) λ_{max} (nm) 243, 300; IR (NaCl) ν_{max} (cm^{-1}) 3600−3100 (br, N−H), 2960 (m, C−H), 2873 (w, C−H), 1663 (s, C═O), 1598 (w, C═O), 1465 (m); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.34 (d, *J* = 7.5 Hz, 2H, H_5 + $H_{5'}$), 7.14 (td, *J* = 7.7, 1.1 Hz, 2H, H_7 + $H_{7'}$), 6.79 (td, *J* = 7.5, 1.0 Hz, 2H, H_6 + $H_{6'}$), 6.63 (dd, *J* = 7.9, 0.7 Hz, 2H, H_8 + $H_{8'}$), 5.63 (s, 2H, 2×NH), 4.96 (s, 2H, H_2 + $H_{2'}$), 4.02 (app t, *J* = 8.9 Hz, 2H, H_{11} + $H_{11'}$), 3.67 (dd, *J* = 7.9, 5.8 Hz, 2H, H_{15} + $H_{15'}$), 3.22 (dd, *J* = 14.0, 9.0 Hz, 2H, H_{12a} + $H_{12a'}$), 2.83 (s, 6H, 2×N$\underline{CH_3}$), 2.9−2.8 (m, 2H, H_{12b} + $H_{12b'}$), 1.6−1.5 (m, 2H, H_{18} + $H_{18'}$), 1.5−1.4 (m, 4H, H_{17a} + $H_{17a'}$ + H_{17b} + $H_{17b'}$), 0.88 (d, *J* = 6.6 Hz, 6H, 2×$\underline{CH_3}$), 0.82 (d, *J* = 6.5 Hz, 6H, 2×$\underline{CH_3}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 168.3 (C), 167.2 (C), 149.1 (C), 130.5 (C), 129.9 (CH), 125.2 (CH), 120.0 (CH), 110.4 (CH), 80.7 (CH), 63.5 (CH), 60.6 (C), 56.6 (CH), 40.1 (CH_2), 38.3 (CH_2), 33.2 (CH_3), 24.8 (CH), 23.3 (CH_3), 22.5 (CH_3); MS ESIMS *m*/*z* 625 $[M + H]^+$; HRESIMS *m*/*z* 625.3497 $[M + H]^+$ (calcd for $C_{36}H_{45}N_6O_4$, 625.3493).

*Synthesis of exo-D-Ditryptoleucine **11c**.* To a solution of the dipeptide **35a** (15 mg, 0.016 mmol, 1.0 equiv) in MeOH (1.6 mL, 0.01 M) was added activated Pd on C (16 mg, 10% wt loading, 1.05 g/mmol). The argon atmosphere was replaced by hydrogen, which was bubbled into the solution for 5 min. The resulting suspension was stirred at rt under hydrogen for 2 h, and the hydrogen was then replaced by argon and a 28−30% v/v aqueous solution of ammonia (59 μL, 3.68 mL/mmol). After 2.5 h of stirring, the reaction mixture was filtered through Celite washing with EtOAc and the solvent was evaporated. The residue was purified by flash-column chromatography (silica gel, from 100:0 to 90:10 v/v CH_2Cl_2/MeOH) to afford 6.7 mg (67% yield for the two steps) of *exo*-D-ditryptoleucine **11c**. $[\alpha]^{25}_D$ +271 (*c* 0.34, $CHCl_3$); UV (MeOH) λ_{max} (nm) 244, 303; IR

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(NaCl) ν_{max} (cm^{-1}) 3600−3100 (br, N−H), 2957 (m, C−H), 2870 (w, C−H), 1658 (s, C═O), 1605 (w, C═O), 1457 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.24 (d, J = 7.7 Hz, 2H, H_5 + $H_{5'}$), 7.09 (td, J = 7.6, 1.2 Hz, 2H, H_7 + $H_{7'}$), 6.73 (td, J = 7.5, 1.0 Hz, 2H, H_6 + $H_{6'}$), 6.55 (d, J = 7.8 Hz, 2H, H_8 + $H_{8'}$), 5.45 (s, 2H, 2×NH), 5.16 (s, 2H, H_2 + $H_{2'}$), 3.95 (app ddd, J = 11.4, 5.7, 1.9 Hz, 2H, H_{11} + $H_{11'}$), 3.86 (dd, J = 5.3, 2.5 Hz, 2H, H_{15} + $H_{15'}$), 2.86 (s, 6H, 2×N$\underline{CH_3}$), 2.8−2.7 (m, 4H, H_{12a} + $H_{12a'}$ + H_{12b} + $H_{12b'}$), 1.7−1.6 (m, 2H, H_{18} + $H_{18'}$), 1.4−1.2 (m, 4H, H_{17a} + $H_{17a'}$ + H_{17b} + $H_{17b'}$), 0.83 (d, J = 6.6 Hz, 6H, 2×$\underline{CH_3}$), 0.54 (d, J = 6.6 Hz, 6H, 2×$\underline{CH_3}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 166.4 (C), 165.2 (C), 151.3 (C), 130.3 (CH), 126.4 (C), 125.9 (CH), 119.5 (CH), 110.0 (CH), 79.1 (CH), 61.2 (CH), 59.5 (C), 58.7 (CH), 38.5 (CH_2), 37.7 (CH_2), 32.3 (CH_3), 24.6 (CH), 23.8 (CH_3), 22.8 (CH_3); ESIMS m/z 625 $[M + H]^+$; HRESIMS m/z 625.3497 $[M + H]^+$ (calcd for $C_{36}H_{45}N_6O_4$, 625.3493).

Synthesis of exo-L-Ditryptoleucine ***11d****. Procedure 1.* To a solution of the dipeptide **36a** (93 mg, 0.09 mmol, 1.0 equiv) in MeOH (9 mL, 0.01 M) was added activated Pd on C (112 mg, 10% wt loading, 1.05 g/mmol). The argon atmosphere was replaced by hydrogen, which was allowed to bubble into the solution for 3−5 min. The resulting suspension was stirred at rt under hydrogen overnight. The reaction was filtered through Celite washing with EtOAc, and the solvent was evaporated under vacuum to afford 62.4 mg of *exo*-L-ditryptoleucine **11d** (94% yield for the two transformations). Further purification was not required.

Procedure 2. To a solution of the dipeptide **36a** (70 mg, 0.07 mmol, 1.0 equiv) in MeOH (7 mL, 0.01 M) was added Pearlman's catalyst (28.7 mg, 20% wt loading, 0.41 g/mmol). The argon atmosphere was replaced by hydrogen, which was allowed to bubble into the solution for 10 min. The resulting suspension was stirred at room temperature under hydrogen for 5 h. The reaction was filtered through Celite washing with MeOH, and the solvent was evaporated. The residue was purified by flash-column chromatography (silica gel, from 100:0 to 90:10 v/v CH_2Cl_2/MeOH) to afford 36.5 mg (85% yield for the two steps) of *exo*-L-ditryptoleucine **11d**: $[\alpha]^{25}_D$ +418 (c 0.38, $CHCl_3$); UV (MeOH) λ_{max} (nm) 244, 301; IR (NaCl) ν_{max} (cm^{-1}) 3600−3100 (br, N−H), 2957 (m, C−H), 2870 (w, C−H), 1608 (s, C═O), 1464 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.23 (d, J = 7.6 Hz, 2H, H_5 + $H_{5'}$), 7.15 (t, J = 7.5 Hz, 2H, H_7 + $H_{7'}$), 6.80 (t, J = 7.5 Hz, 2H, H_6 + $H_{6'}$), 6.63 (t, J = 7.8 Hz, 2H, H_8 + $H_{8'}$), 5.25 (s, 2H, H_2 + $H_{2'}$), 5.00 (s, 2H, 2×NH), 3.82 (dd, J = 10.4, 6.7 Hz, 2H, H_{11} + $H_{11'}$), 3.74 (t, J = 6.9 Hz, 2H, H_{15} + $H_{15'}$), 2.92 (s, 6H, 2×N$\underline{CH_3}$), 2.7−2.6 (m, 4H, H_{12a} + $H_{12a'}$ + H_{12b} + $H_{12b'}$), 1.7−1.6 (m, 2H, H_{18} + $H_{18'}$), 1.48 (t, J = 7.0 Hz, 4H, H_{17a} + $H_{17a'}$ + H_{17b} + $H_{17b'}$), 0.87 (d, J = 6.5 Hz, 12H, 4×$\underline{CH_3}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 167.4 (C), 167.0 (C), 150.4 (C), 130.3 (CH), 127.0 (C), 125.9 (CH), 119.7 (CH), 110.5 (CH), 79.0 (CH), 63.5 (CH), 59.3 (C), 58.3 (CH), 41.1 (CH_2), 36.5 (CH_2), 33.5 (CH_3), 24.9 (CH), 23.2 (CH_3), 22.8 (CH_3); ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 7.23 (d, J = 7.4 Hz, 2H, H_5 + $H_{5'}$), 7.05 (td, J = 7.6, 1.2 Hz, 2H, H_7 + $H_{7'}$), 6.71 (s, 2H, 2×NH), 6.66 (td, J = 7.9, 1.0 Hz, 2H, H_6 + $H_{6'}$), 6.61 (dd, J = 7.9, 1.0 Hz, 2H, H_8 + $H_{8'}$), 5.09 (s, 2H, H_2 + $H_{2'}$), 3.9−3.8 (m, 2H, H_{15} + $H_{15'}$), 3.8−3.7 (m, 2H, H_{11} + $H_{11'}$), 2.81 (s, 6H, 2×N$\underline{CH_3}$), 2.6−2.4 (m, 4H, H_{12a} + $H_{12a'}$ + H_{12b} + $H_{12b'}$), 1.6−1.5 (m, 2H, H_{18} + $H_{18'}$), 1.5−1.4 (m, 4H, H_{17a} + $H_{17a'}$ + H_{17b} + $H_{17b'}$), 0.84 (d, J = 6.5 Hz, 6H, 2×$\underline{CH_3}$), 0.81 (d, J = 6.4 Hz, 6H, 2×$\underline{CH_3}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C 166.7 (C), 165.5 (C), 151.5 (C), 129.7 (CH), 127.2 (C), 125.3 (CH), 118.0 (CH), 109.4 (CH), 77.7 (CH), 62.2 (CH), 58.8 (C), 57.5 (CH), 40.0 (CH_2), 37.0 (CH_2), 32.5 (CH_3), 24.4 (CH), 23.3 (CH_3), 22.4 (CH_3); ESIMS m/z 625 $[M + H]^+$; HRESIMS m/z 625.3497 $[M + H]^+$ (calcd for $C_{36}H_{45}N_6O_4$, 625.3493).

Synthesis of Bispyrrolidinoindoline 1,3,5-Triazepan-6-one ***37****.* To a solution of the bis-coupled product **35a** (10 mg, 0.01 mmol, 1.0 equiv) in MeOH (1 mL, 0.01 M) was added Pearlman's catalyst (4 mg, 20% wt loading, 0.41 g/mmol). The argon atmosphere was replaced by hydrogen, which was allowed to bubble into the solution for 5 min. The resulting suspension was stirred at rt under hydrogen for 5 h. The reaction mixture was filtered through Celite washing with $CHCl_3$ and MeOH, and the solvent was evaporated under reduced pressure to afford 7.5 mg (88% yield) of compound **37** as a white solid: $[\alpha]^{25}_D$ +131 (c 0.16, $CHCl_3$); UV (MeOH) λ_{max} (nm) 252, 306; IR (NaCl) ν_{max} (cm^{-1}) 2953 (m, C−H), 2867 (w, C−H), 1745 (s, C═O), 1651 (s, C═O), 1601 (w, C═O), 1464 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.20 (td, J = 7.8, 1.2 Hz, 2H, H_7 + $H_{7'}$), 7.09 (d, J = 7.4 Hz, 2H, H_5 + $H_{5'}$), 6.77 (t, J = 7.4 Hz, 2H, H_6 + $H_{6'}$), 6.70 (d, J = 8.0 Hz, 2H, H_8 + $H_{8'}$), 5.28 (s, 2H, H_2 + $H_{2'}$), 4.79 (d, J = 14.8 Hz, 2H, H_{16a} + $H_{16a'}$), 4.40 (d, J = 14.8 Hz, 2H, H_{16b} + $H_{16b'}$), 3.99 (dd, J = 10.0, 6.9 Hz, 2H, H_{11} + $H_{11'}$), 3.7−3.6 (m, 2H, H_{15} + $H_{15'}$), 3.69 (s, 6H, 2×$CO_2\underline{Me}$), 2.50 (dd, J = 12.7, 10.1 Hz, 2H, H_{12a} + $H_{12a'}$), 2.40 (dd, J = 12.7, 7.0 Hz, 2H, H_{12b} + $H_{12b'}$), 2.15 (s, 6H, H_{21} + $H_{21'}$), 1.7−1.5 (m, 4H, H_{17a} + $H_{17a'}$ + H_{18} + $H_{18'}$), 1.4−1.3 (m, 2H, H_{17b} + $H_{17b'}$), 0.92 (d, J = 6.5 Hz, 6H, 2×$\underline{CH_3}$), 0.89 (d, J = 6.4 Hz, 6H, 2×$\underline{CH_3}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 173.1 (C), 173.0 (C), 150.1 (C), 130.6 (CH), 126.9 (C), 126.0 (CH), 118.9 (CH), 107.3 (CH), 82.6 (CH), 67.9 (CH_2), 62.7 (CH), 59.8 (C), 59.1 (CH), 52.8 (CH_3), 37.0 (CH_2), 35.4 (CH_3), 34.0 (CH_2), 24.6 (CH), 24.0 (CH_3), 22.3 (CH_3); ESIMS m/z 713 $[M + H]^+$; HRESIMS m/z 713.4028 $[M + H]^+$ (calcd for $C_{40}H_{53}N_6O_6$, 713.4021).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.0c01273.

Structures of additional homodimeric and heterodimeric bispyrrolidinoindoline dioxopiperazine natural products, further efforts on the synthesis of ditryptoleucines, mechanistic proposal for formation of **37**, experimental procedures, spectroscopic characterization, and copies of NMR spectra for synthetic intermediates and final products, and computational studies on bispyrrolidinoindoline dioxopiperazine ring formation (PDF)

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Notes

The authors declare no competing financial interest.

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