Total synthesis of cyclotripetidic natural products anacine, aurantiomide C, polonimides A and C, and verrucine F

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Abstract

The total synthesis of cyclotripeptidic natural products possessing a central piperazino[2,1-b]quinazolin-3,6-dione core is described, through an original strategy involving the pivotal cyclocondensation of an electrophilic homoserine lactone intermediate. The alkylidene group was spontaneously installed by autooxidation during the cyclocondensation process, while the propionamide side-chain was introduced through the nickel-catalyzed aminocarbonylation of a bromoethyl intermediate. This last reaction is unprecedented on such highly functionalized intermediates. Finally, we explored structural modifications and interconversions of the natural products. Overall, this work led to anacine, aurantiomide C, polonimide A and C, and verrucine F.

Numerous studies have documented the synthesis of the 4-quinazolinone framework, with the Niementowski reaction standing out as a leading approach. This reaction proceeds under heating through the acid-catalyzed condensation of anthranilic acid with an amide. As a privileged structure, the 4-quinazolinone template holds an important medicinal value. Synthetic studies have thus been reported towards more functionalized and complex structures. In natural products, the fused piperazino[2,1-b]quinazolin-3,6-dione core is found in tripeptide derivatives predominantly found in marine fungi. They exhibit a diverse array of biological activities, encompassing antibacterial, anticancer, antifungal, anti-HIV, anti-inflammatory, and antimalarial properties. In particular, anacine (1), aurantiomide C (4) and derivatives (Figure 1), isolated from a marine sponge-associated fungus, Penicillium aurantiogriseum, have demonstrated a significant inhibitory potential against chitinase enzymes and cytotoxicity towards various cell lines. Biosynthetic studies demonstrated the involvement of a non-ribosomal peptide synthetase in the assembly of fumiquinazolines from anthranilic acid and amino acids. Notably, this route undergoes an important oxidative metabolism leading to structure functionalization, and more specifically to oxepin derivatives. Incidentally, we recently reported the total synthesis of some of those oxepin natural products.

Figure 1. Examples of cyclopetidic natural products featuring a glutamate-derived piperazino[2,1-b]quinazolin-3,6-dione.

In 1994, Danishefsky reported the first total synthesis on N-acetyldeedemin, a compound that reverses the multidrug resistance of cancer cells. It featured a pivotal aza-Wittig reaction for the closure of the central ring, forging the C=N bond of the piperazino[2,1-b]quinazolin-3,6-dione core (Scheme 1a). This reaction was also adopted by Snider and co-workers for the total synthesis of fumiquinazolines. Alternatively, the Niementowski condensation approach (Scheme 1b) was used by Wang...
and Ganesan during the liquid and solid phase syntheses of fumiquinazolines and fiscalin B from tripeptide precursors.\textsuperscript{28–30} The solid-phase method was also applied by Wang and Sim to the total synthesis of anacine (1), and verrucines A (8) and B (9).\textsuperscript{31} Finally, Tseng and Chu devised a microwave-assisted synthesis in presence of Zn(OTf)$_2$ to facilitate a condensation process.\textsuperscript{32}


Herein, we report an alternative cyclocondensation strategy towards piperazino[2,1-b]quinazolin-3,6-diones I (Scheme 2), and its application to the total synthesis of anacine (1), aurantiomide C (4), polonimides A and C (7), and verrucine F (10). During the cyclocondensation step, we took benefit of the electrophilicity of an L-homoserine lactone residue on tripeptide intermediate II (made from isatoic anhydride 11, homoserine lactone 12 and Boc-protected L-leucine 13 or L-phenylalanine 14), allowing the release of a 2-hydroxyethyl substituent in position 4 (I, X = OH). From here, after bromination (I, X = Br), a rare nickel-catalyzed aminocarbonylation installed the propionamide side-chain of the natural products.


The choice of L-homoserine lactone (12) as a starting material in this study came from our initial difficulties encountered during coupling attempts between 11 and dimethyl L-glutamate 15, only resulting in a poor 7% yield of dipeptide 16 (Scheme 3, route a). Conversely, the reaction of 11 with L-homoserine lactone hydrochloride (12), which is easily accessible from L-methionine (see the supporting information), was successfully performed in presence of 4-dimethylaminopyridine (DMAP) to provide anthranyclic amide 17 in a 80% yield (route b). Then, tripeptide intermediates 18 and 19 were obtained through the reaction of 17 with N-Boc- L-leucine (13) or N-Boc- L-phenylalanine (14), respectively, in presence of the coupling reagent 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ).\textsuperscript{33}

After screening several cyclocondensation conditions, including temperature, reaction time, and solvent selection, we found that microwave heating at 140 °C in water in presence of Zn(OTf)$_2$ (1.0 equiv), as inspired by Tseng and Chu, afforded cis-cycloripeptide 20 in a 55% yield. The whole process was accompanied by the Boc deprotection. Unfortunately, an epimerization at position 1 could not be avoided and gave a substantial amount of trans isomer 21 in 35% yield (this amount could vary depending on the reaction conditions used). From the literature, the epimerization was known to occur regioselectively at position 1 under these acidic conditions, or at position 4 under basic conditions. Most importantly for the following discussion, isopropylidene byproduct 22 was also formed in minute amount (2%), presumably resulting from an autooxidation process in presence of oxygen and favored by the rigorous condition used. The formation of this product (22) was magnified by prolonged heating, or minimized at higher concentration of reactants or by using DMF as a co-solvent.

To achieve the synthesis of anacine 1, the primary alcohol of tricyclic compound 20 was planned to be converted into an amide group, with subsequent homologation (Scheme 4). Firstly, a modified Mitsunobu protocol in presence of PPh$_3$, 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) and tetra-n-butylammonium cyanide, was attempted to directly substitute the hydroxyl with a cyanide group toward intermediate 26. However, under these conditions at 80 °C, an intramolecular substitution of the activated hydroxyl group by the adjacent ketopiperazine amide occurred, leading to cyclic imidate 23 and precluding the cyanation. A two-step route was thus used from 20, involving a bromination to follow by the substitution of the bromide by a cyanide in presence of KCN and 18-crown-6 ether. This step inevitably yielded a separable mixture of cis isomer 26 (47%) and of trans isomer 27 (26%). Finally, the hydrolysis of the nitrile group of isomer 26 under aqueous HCl condition yielded anacine 1 in a 63% yield. The same sequence was applied to alcohol intermediate 21, giving polonimide C (7) in comparable yields through isomer 27. Fortunately, crystals of 1 were obtained (CCDC 2309741) and allowed confirmation of the relative configuration (Scheme 4). However, the compounds turned out to crystallize as a racemate. Quasi complete racemization of 1 and 7 was confirmed by measuring their respective optical rotations: for 1, $[\alpha]_D^{25} = +5.0$ (c 0.26, MeOH) [lit.16: $[\alpha]_D^{25} = +233.3$ (c 0.21, MeOH)], resulting in an optical purity of 2%; for 7, $[\alpha]_D^{25} = +53.9$ (c 0.33, MeOH) [lit.16: $[\alpha]_D^{25} = +171.0$ (c 0.3, MeOH)], resulting in an optical purity of 32%. This short sequence of reaction highlights the difficulties of this stepwise approach to anacine 1, and the need to explore alternative strategies not only for the introduction of the amide group, but also the synthesis of other natural product congeners.

Scheme 4. First synthetic route to anacine and polonimide, showing an X-ray crystallographic structure of anacine.
As mentioned before, prolonged reaction times for the cyclocondensation of tripeptide 18 enhanced the production of autooxidation product 22. Considering this side-product could be a good entry to the synthesis of aurantiomide C, further optimization was performed by conducting the reaction under microwave, in a solution saturated with oxygen. We observed that the presence of Zn(OTf)₂ catalyst significantly reduced the yields of 22. Ultimately, the optimal yield for this reaction was achieved at a concentration of 0.1M of tripeptide 18 in water and in the absence of Zn(OTf)₂ at 130 °C (microwave) during 4 hours, resulting in a 55% yield of 22 (Scheme 5). Subsequently, following an acylation reaction with the Mosher reagents (Figure S1 in the supporting information), NMR analysis allowed to determine an enantiomeric ratio (e.r.) of 78:22 for 22 (\([\alpha]_D^{25} = -23.4, c 0.72, \text{MeOH}\)). Compound 22, as all those of this series, crystallized as a racemate (CCDC 2309742).

After successful bromination of the primary alcohol 22 (see compound S5 in the supporting information), the formation of cyclic imidate by-product S6 similar to 23 (see supporting information) could not be avoided upon a cyanation attempt. The amide function was thus protected as an ethyl imidate (29), in presence of Meerwein’s reagent (80% yield). Furthermore, inspired by a recent report of Zhou and co-workers, the alkyl bromide on 29 was straightforwardly converted into homologated amide 31 through an aminocarbonylation. The reaction was catalyzed by NiCl₂(dppp) (20 mol%) in presence of t-butylisonitrile (1.0 equiv) and Cs₂CO₃ (1.0 equiv) in a mixture of acetonitrile and water at 150 °C (sealed tube). This step generated t-butylic amide 31 in 62% yield. Notably, this isonitrile coupling is unique with such a functionalized substrate, since the related literature only reported poorly functionalized alkyl bromide substrates. Finally, convergent deprotection of the two protecting groups (ethyl imidate and t-butylic amide) by acidolysis in trifluoroacetic acid (TFA) at the needed temperature of 120 °C was performed in 81% yield, thus achieving the total synthesis of aurantiomide C (4) with a global yield of 15%. This compound, crystallizing as a racemate, showed a marked reduction of the optical rotation compared to the literature (\([\alpha]_D^{25} = +5.2, c 0.25, \text{CHCl}_3\); Lit.\([\alpha]_D^{24} = +25.8, c 0.1, \text{CHCl}_3\)) corresponding to an optical purity of 20%.

Scheme 5. Total synthesis of aurantiomide C and verrucine F.
Notes: a Ratio obtained by NMR analysis of the (R)-Mosher esters (S1 and S2, see Figure S1); b Since the NMR analysis could not distinguish the diastereomeric Mosher esters of 28 (S3 and S4, see Figure S2), the enantiomeric ratio was obtained by HPLC analysis (see the supporting information).

In a very similar sequence from tripeptide 19, verrucine F (10) was obtained in a global yield of 15% (Scheme 5). Notably, the autooxidation step occurring during the tripeptide condensation was more efficient, supposedly due to the stabilizing conjugation with the phenyl group. It gave cyclotripeptide 28 in 72% yield ([\(\alpha\)]\(_{D}^{25}\) = -139.1, c 0.34, MeOH; also crystallizing as a racemate, CCDC 2309746), with an e.r. of 60:40 measured by HPLC analysis of Mosher esters S3 and S4 (Figure S2). Furthermore, the final deprotection step necessitated two separated steps under acidic conditions to remove the t-butyl group (aqueous HCl) and the ethyl imidate (TFA), to afford natural product 10 in 75% yield ([\(\alpha\)]\(_{D}^{25}\) = -29, c 0.1, MeOH). The optical purity could not be evaluated in that case, due to the absence of data in the literature.

To complete this work, the reactivity of aurantiomide C (4) was investigated (Scheme 6), as an attempt to reach other natural products (1-3, 5, 6). Compound 4 turned out to be particularly stable. Although aurantiomides A (2) and B (3) were unreachable by the acid-catalyzed methoxylation and hydration of 4, respectively, the reduction of the isopropylidene double bond could be efficiently performed in presence of triethylsilane and TFA in dichloromethane, to give diastereomerically pure anacine (1) in 80% yield. The optical rotation of this new sample of 1 ([\(\alpha\)]\(_{D}^{25}\) = +20.3, c 0.23, MeOH; lit.\(^{15}\): [\(\alpha\)]\(_{D}^{25}\) = +233.3, c 0.21, MeOH) suggested an optical purity of 8.7%. Furthermore, during our methoxylation attempts, polonimide A (5) was inadvertently obtained in 96% yield in presence of para-toluenesulfonic acid (PTSA) and acetic acid ([\(\alpha\)]\(_{D}^{20}\) = -1.4, c 0.3, MeOH; lit.\(^{16}\): [\(\alpha\)]\(_{D}^{20}\) = +14, c 0.3, MeOH). Finally, while an attempt of oxygen-mediated autooxidation of anacine (1) was inefficient, its oxidation in presence of DDQ in a methanol/water mixture successfully led to aurantiomide C (4) in 91% yield, with no formation of 2 or 3.

Scheme 6. Conversion of aurantiomide C into anacine and polonimide A.

In summary, we successfully achieved the total synthesis of several cyclotripeptide natural products possessing a central piperazino[2,1-b]quinazolin-3,6-dione structure: anacine (1), aurantiomide C (4), and verrucine F (10). The tricyclic core was
constructed by a Niementowski-type cyclocondensation method involving a key homoserine lactone residue. Furthermore, relying on a nickel-catalyzed isonitrile coupling, it was possible to perform the aminocarbonylation of a bromoethyl side-chain to install the amide function of these natural products. Some of these steps had to be performed under drastic conditions that led to the epimerization or partial racemization of products, mostly resulting in a scalemic mixture of the final natural products. During this work, we took advantage of this epimerization to synthesize polonimide C (7), diastereoisomer of anacine (1), while the ester polonimide A (5) could be generated by the acid-catalyzed methanolysis of amide 4. Finally, it is interesting to mention that both 1 and 4 can be interconverted under specific redox conditions.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI:
Detailed experimental procedures, spectroscopic and crystallographic data, and 1H and 13C NMR spectra (PDF)

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Notes
The authors declare no competing financial interest.

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