

Aldol condensations on a 3-alkylidene-2,5-diketopiperazine - synthesis of two marine natural products

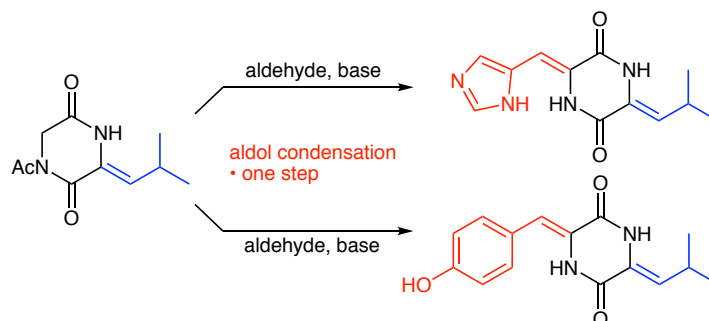
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Abstract The synthesis of two marine natural products containing a 3-alkylidene-6-arylidene-2,5-diketopiperazine scaffold by employing two consecutive aldol condensations starting with 1,4-diacetyl-2,5-diketopiperazine is reported. The target compounds contain a phenol or an imidazole group as aryl substituents, respectively, and suitable conditions for the aldol condensation of 1-acyl-3-alkylidene-2,5-diketopiperazine with the required functionalized aromatic aldehydes were developed. Provided the optimal base was used, introduction of the phenol group did not require use of a protecting group. Boc-protection was beneficial for introduction of the imidazole group, and conditions for carrying out the aldol condensation and Boc-deprotection in one step was identified. Additionally, the stereochemistry of the target compounds was confirmed by NMR analysis.

Key words 2,5-diketopiperazine; natural product; total synthesis; aldol condensation; alkylidene; arylidene.

2,5-Diketopiperazine (DKPs), which formally consists of a head-to-tail cyclized dipeptide, is a privileged scaffold found in numerous natural products displaying a wide range of bioactivities, e.g. antibacterial, antiviral, anticancer, anti-inflammatory, antioxidant and antibiofouling, as well as in several approved drugs, e.g. the PDE5 inhibitor tadalafil and the oxytocin receptor antagonists retosiban and epelsiban.² The DKP ring can be the main structural feature of smaller compounds e.g. (-)-phenylahistin³ (**1**) or part of a complex structure e.g. the spiro-annulated brevianamide A (**2**) (Figure 1). Unsaturated DKPs such as 3-dehydro- and 3,6-didehydro-2,5-DKPs formally derived from dehydroamino acids have attracted attention as anticancer agents e.g. the tubulin polymerisation inhibitor plinabulin⁴ (now in Phase 3 clinical trials for treatment of non-small cell lung cancer) and the plasmogen activator inhibitor XR5967.⁵

Due to the potential pharmaceutical interest of 3,6-bis-arylidene-DKPs, synthetic methods for their preparation are well established.^{2a, 2c, 6} However, few examples for the preparation of 3-alkylidene-6-arylidene-DKPs are known.⁷ Reported methods for the formation of 3-alkylidene-DKPs are based on the reaction of alkyl aldehydes with a preformed DKP via a condensation,^{7c-h, 8} a Wittig reaction^{8g, 9} or cyclisation of dipeptides containing a dehydroamino acid.^{7b, 7c} Among these methods, condensation reactions are most frequently utilized for the preparation of 3-alkylidene-6-arylidene-DKPs.^{7a,c-h} However with one exception,^{7a} condensation reactions towards 3-alkylidene-6-arylidene-DKPs are limited to the introduction of aryl substituents that are non-reactive under basic conditions or require several protection-deprotections of the DKP nitrogen atoms.

During our efforts to explore bioactive marine natural products we became interested in DKP-based compounds and the synthesis of the natural 3-alkylidene-6-arylidene-DKPs **3** and **4** (Figure 1).¹⁰ The compounds are related to the reported albonoursin.^{7b, 7d-g, 11} However, instead of having a non-reactive phenyl substituent, natural products **3** and **4** contain either a phenol or an imidazole group, respectively. Our synthetic investigations show that two subsequent condensation reactions can give access to compounds **3** and **4** from the same starting material. Base-reactive aromatic aldehydes can be employed, provided the appropriate conditions are used. In

addition, access to **4** allowed for confirmation of the reported stereochemistry by NMR analysis.

We started our investigations into the synthesis of natural products **3** and **4** by evaluating in which order to introduce the alkylidene and arylidene side chains using the 1,4-diacetyl DKP

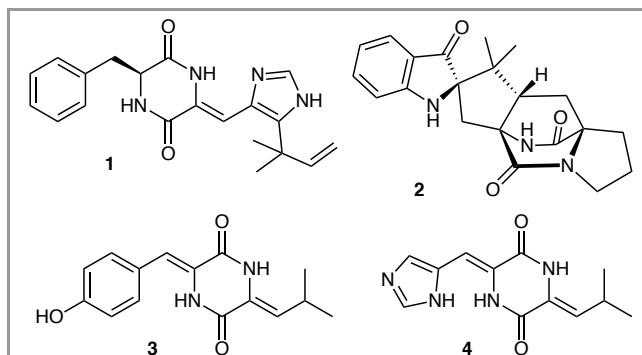
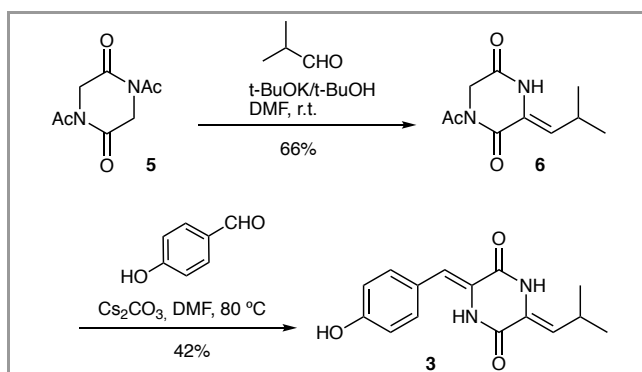


Figure 1. Biologically active 2,5-diketopiperazines

5 as starting material (Scheme 1). In accordance with previous reports,^{7d} successful introduction of an alkylidene side chain was not efficient once an arylidene side chain had been installed. However, by first reacting **5** with isobutyraldehyde (Scheme 1) and then treating the resulting 1-acetyl-3-alkylidene-DKP **6** with either 4-hydroxybenzaldehyde (Scheme 1) or protected imidazole-5-carbaldehyde (Scheme 2) the natural products **3** and **4** were successfully obtained.

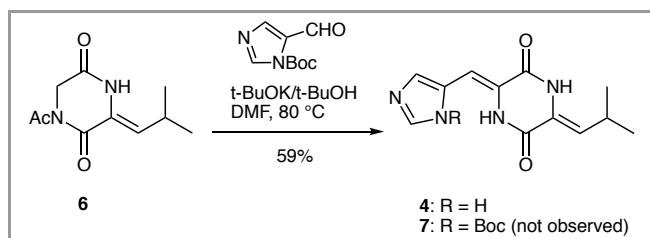


Scheme 1. Successful route to natural product **3**.

The condensation of **5** with isobutyraldehyde was first attempted using the conditions reported by Folkes and co-workers for condensations with aromatic aldehydes.¹² Using Cs_2CO_3 in degassed DMF at room temperature gave **6** in 21% yield after 18 h. Increasing the reaction temperature to 80 °C improved the yield to 42%. We initially used a 1:1:1 ratio between DKP **5**, Cs_2CO_3 and aldehyde, and the work-up consisted of adding crushed ice to the reaction mixture and then isolating the precipitated product in pure form by filtration. Further attempts to improve the reaction by using an excess of base or aldehyde necessitated chromatographic purification due to the more complex crude products this gave, and did not result in improved yields.

We then turned to the conditions described by Gallina^{7d} employing *t*-BuOK as base at room temperature (Scheme 1). Reaction of DKP **5** with 1.2 equiv. of isobutyraldehyde in the presence of 1.05-1.1 equiv. of *t*-BuOK/*t*-BuOH in DMF as solvent

gave mono-substituted DKP **6**¹³ in up to 66% yield (Scheme 2). A larger excess of isobutyraldehyde gave the product in lower purity and failed to provide **6** in higher yield.



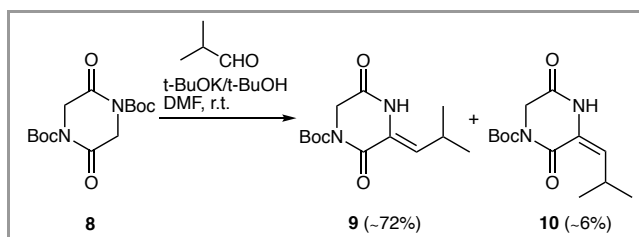
Scheme 2. Synthesis of natural product **4**.

One caveat of this approach was the necessity of a chromatographic step in the work-up protocol. The preparation of **6** has been carried out several times using *t*-BuOK as base, and in some cases, evaporation of the solvent following extraction afforded pure **6** after washing with distilled water, however, on other instances flash chromatographic purification of the crude product was necessary in order to obtain **6** in satisfactory purity. Irrespectively of the base used (Cs_2CO_3 or *t*-BuOK) only one stereoisomer of **6** was observed, which correlates well with the high (*Z*)-stereoselectivity reported for aldol condensation of *N*-acetyl DKPs with aldehydes.^{8h}

For the second condensation step, use of either Cs_2CO_3 or *t*-BuOK as base with 4-hydroxybenzaldehyde (Scheme 1) or imidazole-5-carbaldehyde with or without *N*-Boc protection (Scheme 2) failed to give any product at room temperature. At 80 °C, however, **3**¹⁴ could be obtained in 42% yield when Cs_2CO_3 was used as base (Scheme 1). Reaction with *t*-BuOK as base (1.0 or 2.5 equiv) resulted in a complex crude product mixture, in which the desired compound was only present in minor amounts. Shin and co-workers reported similar problems when employing 2-hydroxybenzaldehyde under the same reaction conditions,^{7a} which were solved by re-acetylating the free N-H of **6**.

Reactions of **6** with unprotected imidazole-5-carbaldehyde using either either Cs_2CO_3 or *t*-BuOK at 80 °C resulted in the formation of a complex mixture of products, **4** being one of them albeit in modest amounts. The flash chromatographic purification proved to be inefficient with several unidentified products co-eluting with the desired product. We then switched to using the *N*-Boc-protected imidazole-5-carbaldehyde. Using 1.2 equiv. of *t*-BuOK as base, a much cleaner crude product, which also was amenable to flash chromatographic purification was obtained (Scheme 2). We found that the *N*-Boc-protected aldehyde decomposed with loss of the Boc-group during the reaction and the best results were obtained when an excess of the Boc-protected aldehyde (2.5 equiv.) was used. Further, the Boc-group of compound **7** (Scheme 2) was also found to be labile under the reaction conditions and as a result the natural product **4**¹⁵ (instead of **7**) was isolated in 59% yield, thus avoiding an additional deprotection step. Use of a larger excess of *t*-BuOK did not improve the outcome of the reaction. Instead significant deacetylation of **6** was observed.¹⁶ Unfortunately, use of Cs_2CO_3 in the condensation of **5** with *N*-Boc-protected imidazole-5-carbaldehyde was largely unsuccessful and gave **7** in a mere 5% yield (data not shown).

In an effort to increase the yield of the natural product **4**, we investigated the use of bis-Boc-protected DKP **8** (Scheme 3) in the condensation reactions. Application of the same conditions as for condensation with the bis-acetyl protected **5** gave a much cleaner crude product, however unlike for reactions with **5**, the aldol condensation of **8** resulted in the formation of both (*E*)- and (*Z*)-double bond isomers in an estimated 9:1 ratio between the (*Z*)-isomer **9** and the (*E*)-isomer **10**, respectively. The isomers **9** and **10** were isolated by careful flash chromatography in 72% and 6% yield, respectively.



Scheme 3. Attempted synthesis using *N*-Boc protected DKPs.

The second condensation step was attempted with 4-hydroxybenzaldehyde (using either 1.2 or 2.5 equiv. *t*-BuOK as base) but only traces of product were seen in either case. Reaction with *N*-Boc-imidazole-5-carbaldehyde (using 1.2 equiv. *t*-BuOK) did not give any product, and only minor amounts of **4** could be observed when reacting the unprotected aldehyde both when 1.05 equiv. Cs_2CO_3 or 1.2 equiv. *t*-BuOK were used as base.

In the original work by Huang, Zhu and coworkers both double bonds in **4** were suggested to be of (*Z*)-configuration.⁹ It is reasonable to anticipate that our synthetic product is the (3*Z*,6*Z*)-isomer of **4** based on the high (*Z*)-stereoselectivity reported for aldol condensation of *N*-acetyl DKPs with aldehydes.^{8h} In any case, a NOESY analysis of **4** was performed to confirm the stereochemistry (see SI). NOE contacts of similar intensity were observed from 4-H to 11-H, 13-H and 15-H. The corresponding distances through space for the (3*Z*)- and the (3*E*)-isomer were compared based on 3D models from molecular mechanic simulations (Figure 2). For the (3*Z*)-rotamers, the distances were estimated to 3.7, 2.4/5.0 and 5.4/4.0 Å, respectively (see SI), which was in agreement with the observed NOE contacts. In contrast, for the (3*E*)-rotamers, the corresponding distances were 2.3, 5.1/5.4 and 7.2/7.0 Å, respectively, which should lead to rather different NOE contacts for the three interactions. An NOE contact from 1-H to 8-H was observed, while no NOE contact was found to 7-H, which is in agreement with previous analysis¹⁰ for similar systems and indicates a (6*Z*)-configuration.

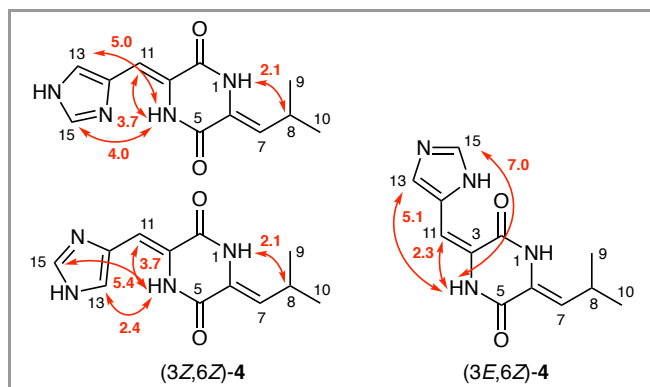


Figure 2. NOE contacts and distances (Å) for stereoisomers of **4**.

In conclusion, the syntheses of the marine natural products **3** and **4** were conducted in two steps with an overall yield of 28% and 39%, respectively. We have shown that a double condensation strategy is applicable to the synthesis of 3-alkylidene-6-arylidene DKPs containing functionalized aryl substituents. Efficient introduction of the imidazole group required Boc-protection, however, we have identified conditions that allows for aldol condensation and Boc-deprotection to be carried out in one step. Our results indicate that the outcome of the second condensation is more sensitive to the choice of base than the first condensation reaction. In addition, NMR analysis supported that the natural product **4** has a (3*Z*,6*Z*)-configuration.

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Supporting Information

YES

Primary Data

NO

References and Notes

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- (13) Experimental procedure and characterization data for **6** are in accordance with reference 6d.
- (14) Experimental procedure for the condensation of **6** with 4-hydroxybenzaldehyde: **6** (100 mg, 0.5 mmol), 4-hydroxybenzaldehyde (58 mg, 0.5 mmol) and Cs₂CO₃ (163 mg, 0.5 mmol) were dissolved in anhydrous degassed DMF (3 mL) and the reaction mixture was stirred at 80 °C for 6 h. After cooling to room temperature, crushed ice was added and the resulting white precipitate was isolated by filtration and washed with water. The solid material was dried in vacuo to give the title compound (57 mg, 42%) as a white solid. Characterization data for **3** are in accordance with reference 9. See SI for comparison. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.24 (s, 1H), 9.80 (s, 1H), 9.78 (s, 1H), 7.36 (d, *J* = 8.6 Hz, 2H), 6.79 (d, *J* = 8.6 Hz, 2H), 6.67 (s, 1H), 5.67 (d, *J* = 10.4 Hz, 1H), 2.94 (dhept, *J* = 10.4, 6.5 Hz, 1H), 0.97 (d, *J* = 6.5 Hz, 7H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 158.1, 157.6, 157.6, 131.0, 125.4, 125.1, 124.01, 123.96, 115.6, 115.1, 23.9, 22.3. HRMS (ESI): *m/z* [M+Na]⁺ calcd for C₁₅H₁₆N₂O₃Na: 295.1053; found: 295.1057.
- (15) Experimental procedure for the condensation of **6** with *N*-Boc-protected imidazole-5-carbaldehyde: **6** (168 mg, 0.8 mmol), *N*-Boc imidazole-5-carbaldehyde (392 mg, 2.0 mmol), *t*-BuOK (185 mg, 1.65 mmol) and *t*-BuOH (1.7 mL) were added to a dry round bottomed flask. The flask evacuated, back-filled with argon and sealed with a septum before anhydrous DMF (6 mL) was added. The mixture was stirred at 80 °C for 8 h and then cooled to room temperature before the reaction mixture was partitioned between ethyl acetate (15 mL) and a saturated NH₄Cl-solution (15 mL). The aqueous layer was extracted further with ethyl acetate (2 x 15 mL) before the combined organic layers were

dried over anhydrous Na_2SO_4 and evaporated in vacuo until approx ~1 mL of an oily residue remained (contains residual DMF). The oily residue was purified by flash column chromatography (EtOAc) to give the title compound as a pale yellow amorphous solid (116 mg, 59%). $R_f = 0.58$ (EtOAc). Characterization data for **4** are in accordance with reference 9. See SI for comparison. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 12.60 (s, 1H), 11.73 (s, 1H), 10.18 (s, 1H), 7.95 (s, 1H), 7.52 (s, 1H), 6.60 (s, 1H), 5.69 (d, $J = 10.4$ Hz, 1H), 2.96 (dhept, $J = 10.4, 6.5$ Hz, 1H), 0.97 (d, $J = 6.5$ Hz, 6H); ^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 157.3, 156.2, 136.5, 136.5, 125.4, 124.9, 124.7, 119.2, 104.4, 23.8, 22.2. HRMS (ESI): m/z [M-H] $^-$ calcd for $\text{C}_{12}\text{H}_{13}\text{N}_4\text{O}_2$: 245.1044; found 245.1041.

- (16) Use of 1.8 equiv. of *t*-BuOK gave an estimated 1:0.6 ratio of **4** and deacetylated **6** in the crude product.