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Synthesis and cytotoxicity of desmethoxycallipeltin B: Lack of a quinone methide for the cytotoxicity of callipeltin B

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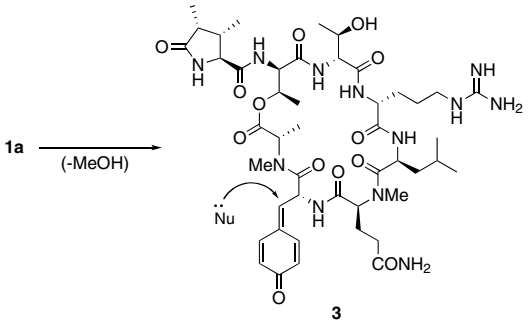
Abstract—The desmethoxy analogue of the cytotoxic, cyclic depsipeptide callipeltin B was synthesized to evaluate the role of its β-MeOTyr residue. The IC_{50} of desmethoxycallipeltin B, in which the β-MeOTyr residue was replaced by D-Tyr, against HeLa cells was found to be 128 ± 10 μM in an MTT assay, compared to 98 ± 5 μM for the natural product itself. The roughly comparable cytotoxicities suggest that the cytotoxicity of callipeltin B does not arise through the formation of a quinone methide intermediate.

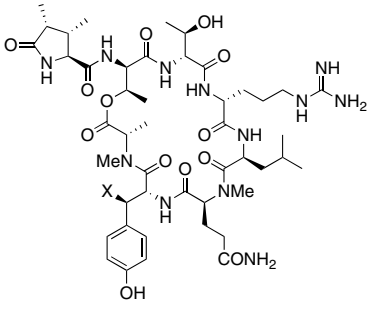
Callipeltin B (**1a**) and its congener callipeltin A (**2**) are cyclic depsipeptides isolated from the lithistid marine sponge *callipelta* sp. by Minale and co-workers. Both molecules were reported to exhibit broad-spectrum cytotoxicity against various tumor cell lines.[1,2] Additionally, the related cyclic depsipeptides papuamides A–D[3] and neamphamide A[4] were isolated from other marine sponges and also shown to exhibit broad-spectrum cytotoxicity against tumor cell lines. Because all of these cyclic depsipeptides contained the novel amino acid β-methoxytyrosine (β-MeOTyr), speculation arose as to whether the cytotoxicity of **1a** resulted from elimination of methanol from β-MeOTyr to form a reactive quinone methide intermediate (**3**, Scheme 1) that subsequently reacted with a biological nucleophile (Fig. 2).

Such an idea received indirect support during the recent synthesis of **1a**, when acid deprotection of **1a** resulted in the formation of a side-product that MALDI-MS showed had lost methanol.[5] To test such a hypothesis, desmethoxycallipeltin (**1b**), in which the β-MeOTyr residue of **1a** was replaced by a D-Tyr, was synthesized and assayed for cytotoxicity (Fig. 1).

Herein we report the synthesis of **1b** using the same strategy used for the synthesis of **1a** (Scheme 2).[5]



Scheme 1. Formation and reaction of a putative quinone methide intermediate **3**.



1a, X=OCH_3
1b, X=H

Figure 1. Structures of callipeltin B (**1a**) and desmethoxycallipeltin B (**1b**).

Keywords: Cyclic depsipeptide; Cytotoxicity; Quinone methide; Solid phase synthesis; MTT assay.
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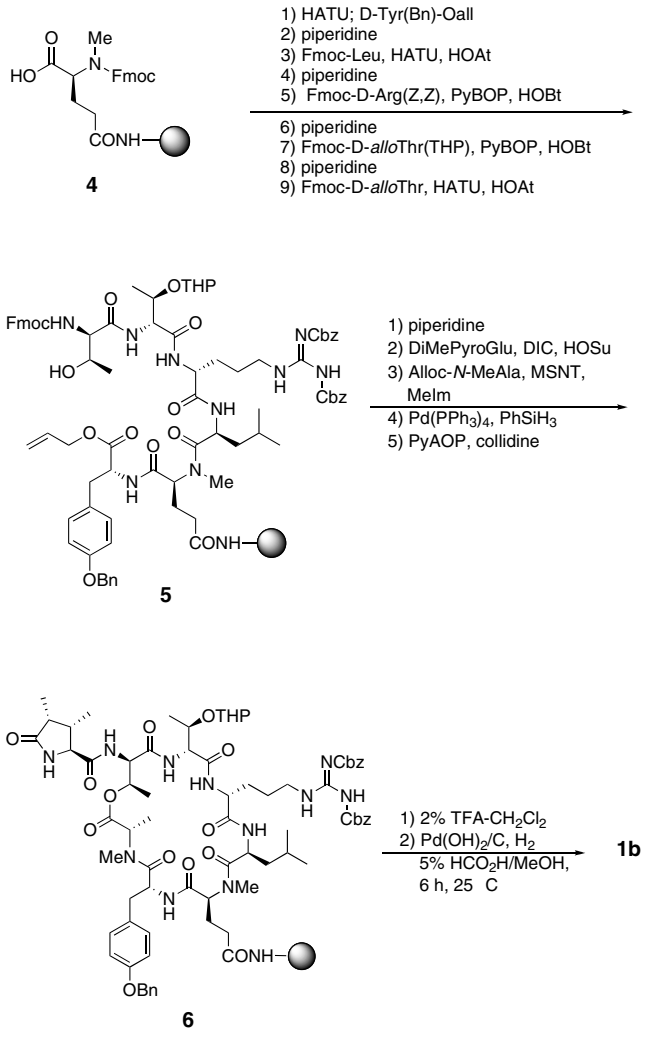

Scheme 2. Synthesis of desmethoxycallipeltin B (**1b**).

D-Tyr(Bn) allyl ester was synthesized in two steps from commercially available Boc-D-Tyr(Bn). All the other residues were either commercially available or had been previously synthesized in our group during the synthesis of **1a**.[5,6] As before, the Sieber amide resin[7] was acylated with the MeGln residue of **1b** to afford the intermediate **4**. All deprotection and coupling reactions were monitored by cleavage of a 1–2 mg sample of resin using 2% TFA-CH_2Cl_2 followed by analysis of the crude cleavage mixture using reverse phase HPLC and MALDI-MS.

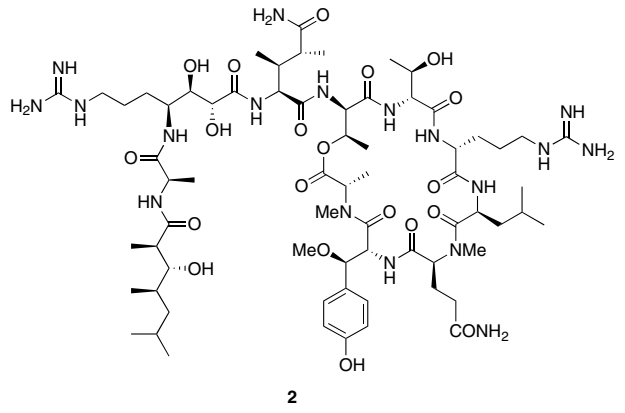

Figure 2. Structure of callipeltin A (**2**).

The macrocyclized product **6** was cleaved from the resin with 2% TFA-CH_2Cl_2 and the crude cleavage product was purified by RP-HPLC to afford 2.6 mg of purified **2** in 11% overall yield. The identity of **1b** was confirmed by MALDI-MS and ^{1}H NMR. The homogeneity of the synthetic **1b** was confirmed by RP-HPLC and ^{1}H NMR.

With synthetic **1b** in hand, we assayed **1b** for cytotoxicity alongside synthetic **1a**, which served as the control. The MTT fluorogenic assay[8,9] was chosen as the method for quantifying cytotoxicity, and it was performed with DMEM-supplemented HeLa cells. Analysis of the dose–response data obtained from the assay afforded IC_{50} values for both **1a** and **1b**. The mean IC_{50} values obtained from duplicate assays were 98 ± 5 μM for **1a** and 128 ± 10 μM for **1b**.

Although **1b** is slightly less cytotoxic than **1a**, the small difference in IC_{50} values of **1a** and **1b** is not what one would expect if the β-MeOTyr residue were essential for cytotoxicity. This result strongly suggests that a quinone methide intermediate is not responsible for the cytotoxicity of **1a**. In light of the recent report that the closely related cyclic depsipeptide callipeltin A is a sodium ionophore,[10] a reasonable alternative source of cytotoxicity could be the ability of both **1a** and **1b** to act as sodium ionophores. This hypothesis is now being actively investigated in our laboratory. The small difference in activity between **1a** and **1b** that is observed is possibly attributable to a conformational change that results from removal of the methoxy substituent of β-MeOTyr. Indirect support for such an explanation is provided by a comparison of the ROESY spectra of **1a** and **1b**, which show noticeable differences.[11]

In summary, an analogue of callipeltin B that lacks a β-MeOTyr residue has been synthesized and its cytotoxic effect on HeLa cells quantified using an MTT assay. Substitution of D-Tyr for β-MeOTyr does not substantially affect the cytotoxicity of callipeltin B, leading to the conclusion that a quinone methide intermediate is unlikely to be the principal source of the cytotoxicity of callipeltin B. Studies are ongoing in our laboratories to further elucidate the roles played by the various non-proteinogenic amino acids in **1a** and to further explore the mode of action of **1a** and related natural products.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.07.003.

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