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[Lys^3]Didemnins as Potential Affinity Ligands

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Abstract—The synthesis and biological activity of N^{ε}-Z-[Lys^3]didemnin B are reported. This novel analogue retains antiproliferative, cytotoxic, and protein biosynthesis inhibition activities, but at reduced levels. This result suggests the use of [Lys^3]didemnin derivatives as potential affinity probes for studying the molecular target(s) of the didemnin class of natural products.

Introduction

The didemnin family of cyclic depsipeptide natural products (Fig. 1) exhibits antitumor, antiviral, and immunosuppressive activities,[1–3] as well as the ability to induce very rapid apoptosis[4] and to inhibit eukaryotic protein biosynthesis.[5] Aplidine, a congener having a pyruvate residue at position 9 and enhanced antitumor activity, is undergoing clinical evaluation against cancer.[6] The mechanistic basis of these biological activities is incompletely understood. Two didemnin binding proteins, eukaryotic elongation factor 1α (EF-1α) and palmitoyl protein thioesterase 1 (PPT1), have been identified using an affinity chromatography ligand.[7,8] A mechanistic model for the EF-1α-mediated inhibition of protein biosynthesis in vitro by didemnins has been proposed[9] and recently refined[10] in which didemnins are believed to inhibit the EF-2-catalyzed translocation step of the elongation cycle. Additional work demonstrated that inhibition of protein biosynthesis is not sufficient to induce apoptosis in MCF7 cells,[11] indicating that other antiproliferative or cytotoxic mechanisms may be operating. Recent studies with PPT1, the other didemnin binding protein, showed that inhibition of PPT1 activity by a series of didemnin congeners is independent of both antiproliferative potency and side-chain structure.[12] These results imply that an unidentified molecular target may play a role in mediating the biochemical effects of this group of natural products.

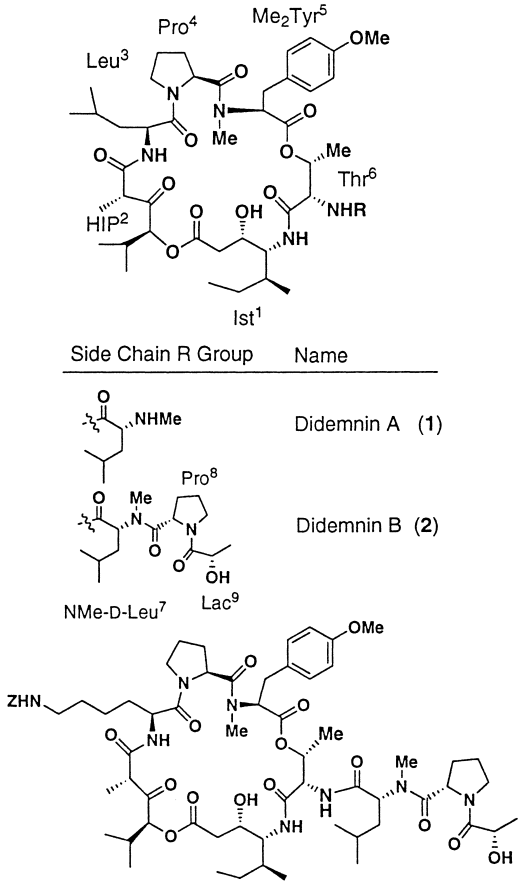

Figure 1. Structures of didemnins A, B, and analogue **3**.

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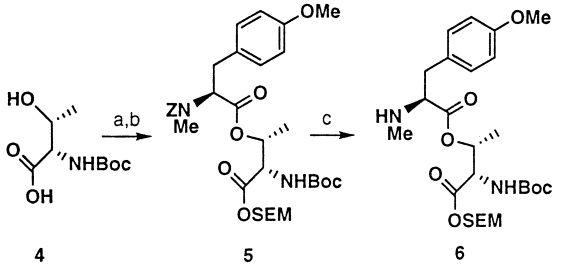
Rationale and Chemical Synthesis

In order to clarify the mechanistic basis for the effects of didemnins on cells, we have focused on synthetic didemnin analogues designed for use as probe molecules. Biological activity is highly sensitive to the structure of the side-chain peptide anchored to Thr[6] of the didemnin macrocycle.[13–16] For example, didemnin A (**1**) shows consistently lower levels of activity than didemnin B (**2**).[2,15] The affinity ligand used to identify EF-1α and PPT1 was a didemnin A derivative in which the *N*-Me-D-Leu[7] residue was acylated with a biotinylated linker.[7] Since the two binding proteins identified using this ligand do not seem sufficient to explain the antiproliferative effects of didemnins, we believed it would be fruitful to synthesize additional affinity ligand candidates. Our first target is compound **3**, in which the macrocyclic Leu[3] residue is replaced by a protected Lys[3] residue. This analogue provides a protected nucleophilic amino group for eventual derivatization to yield an affinity probe that leaves intact the side-chain peptide of didemnin B, an important structural determinant of biological activity.

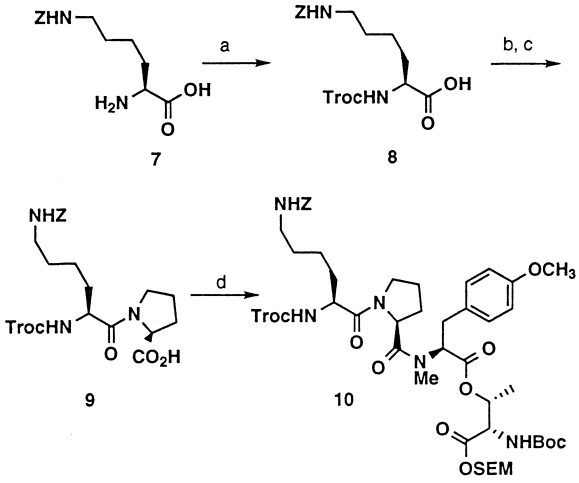
The synthesis of **3** follows the overall approach used in our laboratory to obtain several congeners and analogues of the didemnins.[14,17] *N*-Boc-threonine (**4**) was sequentially alkylated with SEM-Cl (Scheme 1) and then acylated with a derivative of *N*-Z-tyrosine preactivated with isopropenyl chloroformate to yield depsipeptide **5** in one operation. Catalytic hydrogenolysis afforded secondary amine **6**.



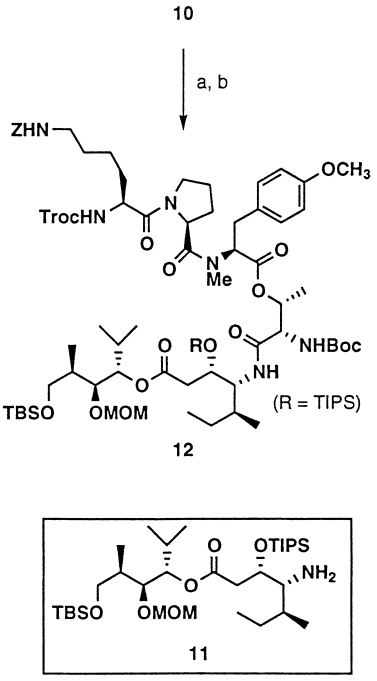
Scheme 1. (a) Et_3N, SEM-Cl, THF, then (b) Z-Me_2TyrOH, IPCF, Et_3N, DMAP (cat), CH_2Cl_2, 85% (2 steps); (c) 10% Pd/C, H_2, EtOAc/MeOH (1:1), quant.

The tetrapeptide fragment was elaborated as shown in Scheme 2. Monoprotected lysine derivative **7** was converted to its Troc carbamate and then coupled to proline methyl ester using the PyBroP reagent, followed by saponification, to afford dipeptide **9**. Preactivation of **9** with BOP-Cl, followed by reaction with secondary amine **6** (Scheme 1) furnished the tetrapeptide SEM ester **10**.

The protected linear precursor was synthesized as outlined in Scheme 3. Treatment of **10** with $MgBr_2$ etherate[18] caused smooth and selective cleavage of the SEM ester, in the presence of three carbamates and an alkyl ester.[19] Coupling to the previously-reported[17] HIP-isostatine amine fragment (**11**) using HATU afforded linear silyl ether **12**.



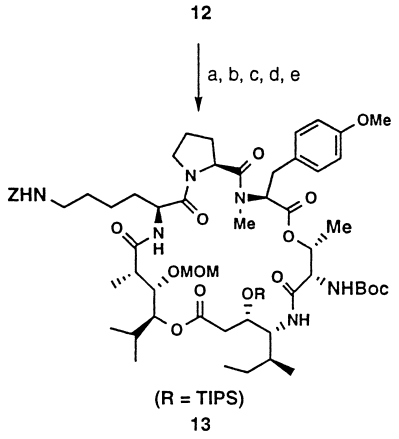
Scheme 2. (a) 2,2,2-Trichloroethyl chloroformate, Na_2CO_3, H_2O, dioxane, 87%; (b) proline methyl ester hydrochloride, PyBroP, DIEA, CH_2Cl_2, 77%; (c) LiOH, H_2O, THF, 74%; (d) BOP-Cl, NMM, CH_2Cl_2, then amine **6** (Scheme 1).



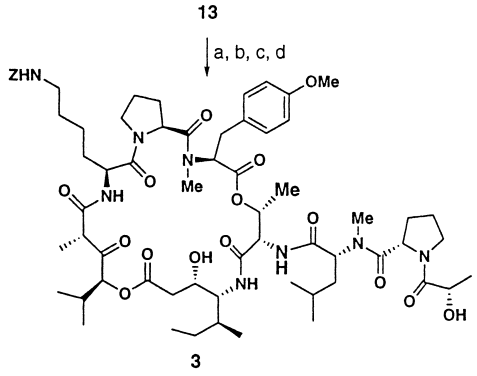
Scheme 3. (a) $MgBr_2{\cdot}Et_2O$, CH_2Cl_2, 96%; (b) HATU, DIEA, CH_2Cl_2, amine **11**, 61%.

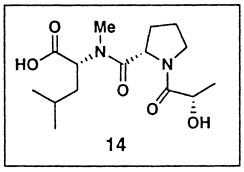
Hydrolysis of the silyl ether (Scheme 4) followed by stepwise oxidation using Dess–Martin[20] and Masamune[21] conditions delivered the linear carboxylic acid. Reductive fragmentation with acid-washed zinc dust followed by treatment with HATU induced macrocyclization in 55% yield.

The cyclic MOM ether (**13**) was cleaved using dimethylboron bromide (Scheme 5) and the resulting alcohol was oxidized under Dess–Martin conditions. The Boc and TIPS protecting groups were removed selectively using anhydrous HCl to obtain a macrocyclic amine salt. BOP-mediated coupling of this salt with the previously reported[17] side-chain acid (**14**) afforded analogue **3**.[22]



Scheme 4. (a) HOAc, H_2O, THF, 84%; (b) Dess–Martin periodinane, 96%; (c) $KMnO_4$, *t*BuOH, NaH_2PO_4, quant.; (d) Zn, HOAc, H_2O, quant.; (e) HATU, DIEA, CH_2Cl_2, 55%.





Scheme 5. (a) Me_2BBr, CH_2Cl_2, −30°C, quant.; (b) Dess–Martin periodinane, 95%; (c) HCl, EtOAc, −30 to 0°C, quant.; (d) BOP, NMM, CH_2Cl_2, side-chain acid **14**, 81%.

Biological Activity and Conclusions

Analogue **3** was tested in both the NCI-60 human tumor cell screen[23] and also a cell-free assay for protein biosynthesis inhibition activity,[9] as shown in the Table 1. Since the ε-amino group of lysine would be acylated by a linker during affinity chromatography, the benzyl carbamate protecting group at this position was left intact for the purpose of obtaining a benchmark for biological activity.

Table 1. Comparison of biological activities of **2** and **3**

Biological activity	2	3
Growth inhibition (GI_{50})	13 nM	6.4 μM
Total growth inhibition (TGI)	66 nM	22 μM
Cytotoxicity (LC_{50})	3.8 μM	25 μM
Cell-free protein biosynthesis inhibition (IC_{50})	3 μM	154 μM

The biological activities of the protected analogue (**3**) were less than those of didemnin B. The antiproliferative activity, as measured by the 50% growth inhibition parameter (GI_{50}) was substantially reduced. Interestingly, cytotoxic activity, as measured by the LC_{50}, was only modestly reduced. This result is in agreement with reports of intact cytotoxic activity in natural congeners having modifications at HIP[2] [24,25] or inversion of configuration at Pro[4],[26] indicating a tolerance for modification in this region. The reduction in protein biosynthesis inhibition potency was of an intermediate magnitude, as measured in this cell-free translation assay. The observation that the leucine-to-lysine modification exerts such disparate effects on these types of activity underscores the complex and multifaceted nature of the biological actions of didemnins.

In spite of the reduction in potency, the residual antiproliferative and cytotoxic activities of **3** suggest its potential use as a competent but unoptimized affinity chromatography ligand for studying the molecular target(s) of the didemnins. Previous studies have indicated that the potency of didemnins can be attenuated, in some cases by several orders of magnitude, by modification of the side-chain peptide structure. This suggests that second-generation affinity probes based on the [Lys[3]]didemnin scaffold with modified side chains might be feasible. Studies with **3** and similar analogues are currently underway.

Acknowledgements

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22. Compound **3**: R_f 0.26 (5% MeOH/$CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.71–1.05 (m, 18H), 1.16–1.42 (m, 13H), 1.43–1.80 (m, 6H), 1.82–2.20 (m, 10H), 2.26–2.66 (m, 6H), 2.85–2.99 (m, 2H), 3.12–3.74 (m, 11H), 3.79 (s, 3H), 3.99–4.13 (m, 2H), 4.24–4.39 (m, 2H), 4.50 (brs, 1H), 4.59–5.31 (m, 7H), 5.43–5.45 (m, 1H), 6.15 (m, 1H), 6.82 (d, J = 8.3 Hz, 2H), 7.03 (d, J = 8.2 Hz, 2H), 7.20 (d, J = 10.2 Hz, 1H), 7.28–7.39 (m, 6H), 7.66–7.67 (m, 1H), 7.72 (d, J = 9.3 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.6, 14.1, 14.5, 15.0, 15.4, 16.3, 16.9, 17.7, 18.6, 20.2, 21.2, 22.7, 23.2, 23.3, 24.5, 25.1, 25.9, 27.1, 27.8, 28.4, 29.3, 29.7, 29.9, 30.3, 31.3, 31.4, 31.9, 32.5, 33.8, 33.9, 36.2, 38.8, 41.0, 45.3, 46.8, 47.2, 49.1, 50.8, 55.2, 55.3, 56.4, 57.3, 57.8, 66.1, 66.3, 66.4, 67.8, 70.2, 75.6, 81.5, 114.1, 128.0, 128.4, 130.0, 130.3, 137.0, 156.5, 158.6, 168.6, 169.3, 170.4, 170.7, 171.7, 172.7, 173.4, 174.2, 204.6; IR (neat) 3330 (m), 2957 (m), 2931 (m), 2359 (w), 1730 (m), 1635 (s), 1539 (m), 1514 (m), 1456 (m), 1247 (m), 1168 (m), 731 (w) cm^{-1}; LRMS m/z calcd for $C_{65}H_{96}N_8O_{17}Na$ (M + Na): 1283.7, found 1283.6.
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