Pergamon

Tetrahedron Letters, Vol. 37, No. 3, pp. 311-314, 1996

0040-4039/96 $15.00 + 0.00

0040-4039(95)02160-4

Analogs of the β-Turn of the Cyclodepsipeptide Didemnin B

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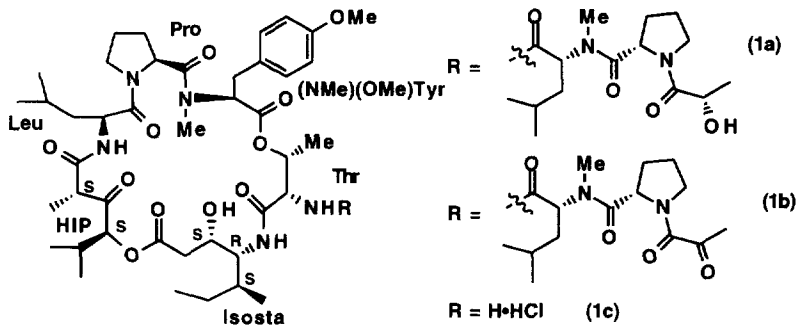
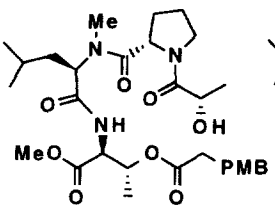
Abstract: *Three side chain analogs of didemnin B were synthesized. All three analogs of the only conventional type II β-turn present in didemnin B differed in the functionality incorporated in the threonine residue. The cytotoxic activities of these analogs were evaluated, and one of them exhibited weak activity.*



The didemnins are a class of cyclodepsipeptides isolated in 1981 from a Caribbean tunicate of the family *Didemnidae*. Most didemnins contain a common macrocycle and differ only in the side chains attached to the backbone through the amino group of threonine.[1] These natural products display a wide spectrum of biological activity including antiviral, antitumor, and immunomodulatory properties. Their biological activities are dependent on the structures of the side chains. Didemnin B (**1a**) was tested extensively and was thought to be the most active didemnin.[2-5] Recently, *N*-pyruvoyl-*N*-prolyl didemnin A (**1b**) has shown some exceptional preliminary results.[6] This compound differs from didemnin B by having the lactyl portion in its oxidized form.

Recently, we have reported the synthesis and biological activities of various side chain analogs of didemnin B.[7] The macrocycle without any side chains was essentially devoid of activity. These results confirmed previous assumptions that the side chain is an important feature for retention of biological activity. Analysis of the X-ray crystal structure of didemnin B[8] and NMR investigations confirmed the presence of a conventional type II β-turn present in the side chain.[9, 10] This turn contains the lactoyl moiety of the side chain, L-proline, *N*-Me-D-leucine and the threonine residue of the tetrapeptide at the *i*, *i*+1, *i*+2 and *i*+3 positions respectively.

β-Turns play a significant role in receptor binding and antibody recognition. As part of a research program aimed at investigating the structure/activity relationships of didemnin B, we designed three β-turn analogs. Two of these compounds (**2**, **3**) contain 4-methoxydihydrocinnamic acid, as a substitute for the tyrosine side chain, one of the proposed elements of the pharmacophore. Analogs **2** and **3** differ only in the protection of the carboxylic acid residue of threonine. The third analog (**4**) differs from **3** only at the alcohol functionality of the threonine residue.



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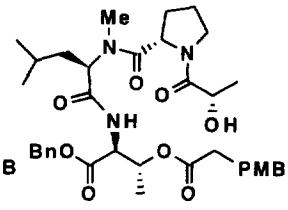

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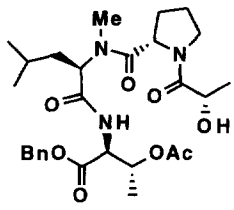

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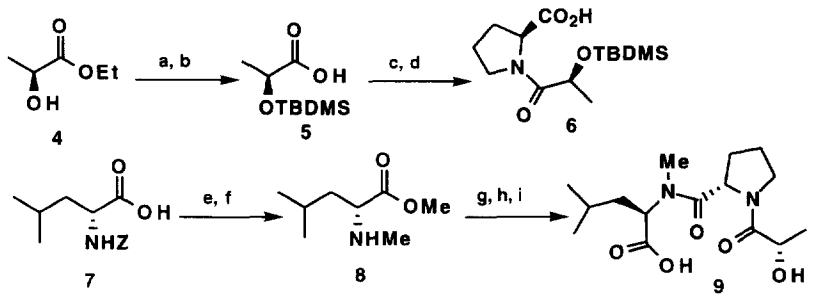
PMB = *p*-methoxybenzyl

The three analogs consist of two major fragments: the didemnin B side chain and the functionalized threonine residue. The improved synthesis of the didemnin B side chain (**Scheme 1**) began with coupling of proline methyl ester and the protected lactic acid **5**. Hydrolysis of the proline methyl ester gave **6**, which was immediately coupled to *N*-Me-D-leucine methyl ester gave the fully protected side chain. Hydrolysis of the resulting methyl ester, followed by the removal of *tert*-butyldimethylsilyl group under acidic conditions gave the didemnin B side chain (**9**).

The synthesis of the second fragment began with Boc-L-threonine which was esterified with benzyl bromide under basic conditions. The secondary alcohol was either acetylated to give **10** or treated with 4-methoxydihydrocinnamic acid in the presence of isopropenyl chloroformate to afford **14**. The Boc group was then removed, and the resulting amine was treated with the didemnin B side chain (**9**) to afford analogs **3**[11] and **4**. Analog **2** was synthesized in a similar fashion as

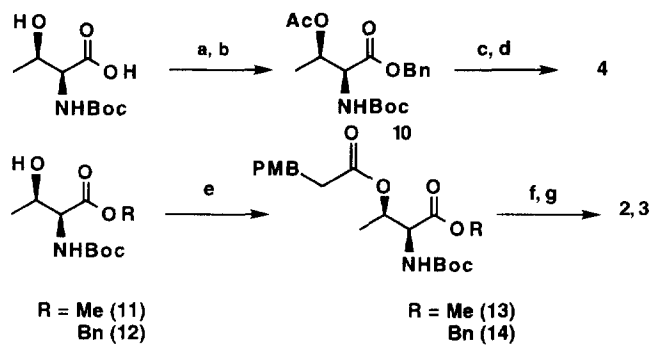
shown in **Scheme 2**.

Scheme 1



a) TBDMSCl, DMF, imidazole, 92%; b) LiOH, H_2O:THF (1:1); c) BOP, CH_2Cl_2, NMM, Pro-OMe, 59%; d) LiOH, H_2O:THF (1:1); e) KOH, $Bu_4N^+HSO_4^-$, THF, $(CH_3)_2SO_4$, 86%; f) 10% Pd/C, H_2 (40 psi), (1:1) EtOAc:MeOH, 95%; g) **6**, BOP-Cl, NMM, CH_2Cl_2, 62%; h) LiOH, H_2O:THF (1:1), 84%; i) AcOH:H_2O (3:1), THF, 75%

Scheme 2



a) BnBr, Li_2CO_3, DMF, 60%; b) Ac_2O, DMAP, Et_3N, 85%; c) TFA, CH_2Cl_2 d) **9**, BOP, NMM, CH_2Cl_2, 55-67%; e) isopropenyl chloroformate, DMAP, Et_3N, CH_2Cl_2, *p*-$MeOC_6H_4CH_2CH_2CO_2H$; f) TFA, CH_2Cl_2 ; g) **9**, BOP, NMM, CH_2Cl_2, 55-67%

All analogs were screened at the National Cancer Institute against various cell lines (**Table**). Analog **3** was weakly active and analogs **2** and **4** were devoid of activity. It is significant to note that analog **3** completely inhibits growth in human melanoma cell lines at 0.1 mM concentrations. Although **3** is much less active than **1a** (note the difference in units), this observation demonstrates that to achieve optimal activity and selectivity for the didemnin B receptor, the presence of the backbone (**1c**) and the side chains are necessary. Compound **3** is very weak inhibitor of protein synthesis *in vitro* (IC_{50} =

500 ± 150 μM).[12] However, we do not know whether this represents its mechanism of action in cells.

Cytotoxic Activity of Analogs Panel/Cell Line--LC_{50} (mM)					
Compound	Melanoma	CNS Cancer	Renal Cancer	Colon Cancer	Breast Cancer
1a	<0.0100	0.432 (μM)	0.494 (μM)	—	—
3	0.0537	0.0866	0.0905	0.0780	0.0600
1c	>100	>100	>100	—	>100

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11. *Spectroscopic data for* **3**: R_f 0.49 (5% MeOH/$CHCl_3$); $[\alpha]_D^{25}$ +47.2° (c=0.13, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 0.90 (d, J=6.63 Hz, 3H), 0.94 (d, J=6.63 Hz, 3H), 1.22 (d, J=6.44 Hz, 3H), 1.32 (d, J=6.66 Hz, 3H), 1.34-1.41 (m, 2H), 1.59-1.63 (m, 1H), 1.88-1.94 (m, 3H), 2.16-2.19 (m, 2H), 2.44-2.54 (m, 2H), 2.74-2.80 (m, 2H), 2.95 (s, 3H), 3.53-3.74 (m, 2H), 3.75 (s, 3H), 4.30-4.32 (m, 1H), 4.96 (t, J=6.82 Hz, 1H), 4.79 (dd, J=4.11, 4.31 Hz, 1H), 5.07 (AB, J=12.01 Hz, 2H), 5.50 (dd, J=4.39, 4.93 Hz, 1H), 5.49-5.51 (m, 1H), 6.80 & 7.07 (AB, J=8.68 Hz, 4H), 7.26-7.34 (m, 5H), 7.41 (d, J=8.4 Hz, 1H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 16.71, 20.03, 21.47, 23.37, 24.93, 25.82, 28.29, 29.73, 30.89, 35.71, 46.89, 54.92, 55.19, 56.39, 56.92, 65.61, 67.25, 68.00, 69.53, 113.77, 113.92, 128.14, 128.36, 128.48, 128.58 (2 overlapped carbons), 128.61, 129.21, 129.25, 132.49, 135.24, 169.40, 171.43, 171.93, 172.68, 173.49; IR ($CHCl_3$) 3460-3310 (br), 2980 (s), 1740 (s), 1660-1635 (br & s), 1510 (m), 1390 (m), 1260-1230 (br), 910 (s) cm-1; HRMS (M+Na) Calcd. for $C_{36}H_{49}N_3O_9$: 668.3568. Found 668.3568.
12. We thank Ms. Deepika Tandon and Professor Peter Toogood at the University of Michigan for measuring the inhibition of *in vitro* protein synthesis by this compound.

(*Received in USA* 12 *September* 1995; *revised* 8 *November* 1995; *accepted* 10 *November* 1995)