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SYNTHESIS OF DOLASTATIN 15 MIMETIC PEPTOIDS

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Abstract: Eight peptoids have been synthesized as peptidomimetics of the cytostatic Dolastatin 15, a depsipeptide isolated from the Indian sea hare *Dolabella auricularia*. The compounds have been tested against several human cancer cell lines and did not show any cytostatic properties.

Marine organisms are a rich source of secondary metabolites that have been shown to exhibit various biological activities and several marine natural products have emerged as promising candidates for drug development.[1] The sea hare *Dolabella auricularia*, a shell-less mollusk, has long been known to secrete complex mixtures of defensive substances.[2] Pettit and coworkers have isolated the potent cytostatic Dolastatins 10 and 15 from this organism.[3] Dolastatin 10 (lymphocytic leukemia P388, ED_{50} = 0.1 ng/mL) and also a derivative of the slightly less active Dolastatin 15 (P388, ED_{50} = 2.4 ng/mL) are currently in clinical development as chemotherapeutic agents for cancer therapy.[4] Dolastatin 15 is a linear depsipeptide consisting of seven building blocks that are amino acids or direct derivatives of amino acids, whereas Dolastatin 10 reveals a more complex structure (Fig. 1). The so-called peptide site (positions 1-3) with N,N-dimethyl-valine, valine and dolaisoleuine (Dolastatin 10) or N-methyl-valine (Dolastatin 15) is believed to bind to the β-subunit of microtubules[5] and there is considerable evidence that suppression of the dynamics of the spindle microtubules during mitosis is responsible for the cytostatic properties.[6]

Oligomers of N-substituted glycines, so-called peptoids, are a new class of non-natural polymers.[7] They can serve as peptidomimetics with similar binding properties, as was shown for parent peptide sequences and their peptoid analogues of three different receptor systems (inhibition of α-amylase by peptides derived from

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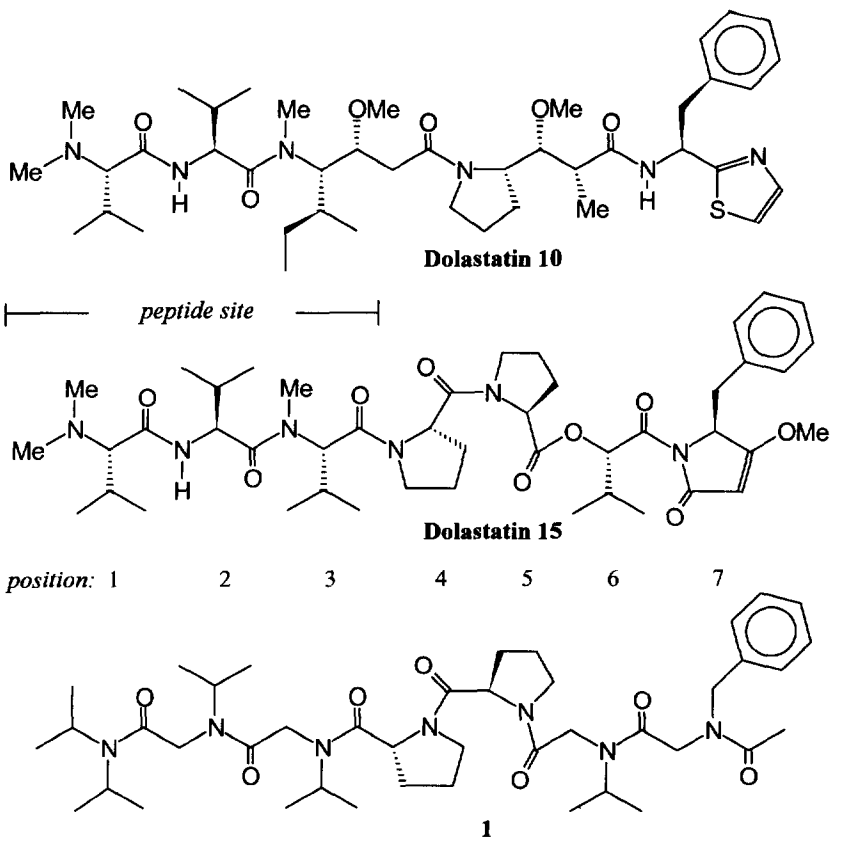

Figure 1. Comparison of the Dolastatins 10 and 15 and mimetic peptoid **1**

tendamistat, inhibition of the hepatitis A virus 3C protease and binding to the TAT-RNA of the HIV virus). This led us to propose Dolastatin 15 mimetic peptoids as illustrated in Fig. 1. To maintain the same relative orientation of the carbonyl functional groups and the side chains the mimetic peptoid has to be designed as the corresponding retrosequence of the parent peptide. In consequence, N,N-dimethyl-valine is mimicked by C-terminal N,N-diisopropyl amide and (S)-proline is represented by its enantiomer, according to the retro-inverso-concept that has been applied to peptides.[8] As for the positions 6-7, it has been shown that the thiazole ring in Dolastatin 10 can be omitted [9] as well as the (S)-2-hydroxy-isovaleric acid (pos. 6) and the (S)-dolapyrrolidone (pos. 7) in Dolastatin 15 can be replaced by the C-terminal proline benzyl amide without appreciable loss of activity.[6] All possible combinations of stereoisomers for the dipeptide unit -Pro-Pro- have been synthesized (compounds **1**, **4**, **5**, and **6**, Table 1). Except for products **2**, **3** and **8** N-terminal Nphe was capped as the acetamide. Compound **8** is designed analogous to the above mentioned Dolastatin 15 derivative in which positions 6 and 7 are replaced by the C-terminal proline benzyl amide.

Table 1.

Entry	sequence	M_r	FAB-MS $(M+Na)^+$	yield[a] [%]
1	Ac-Nphe-Nval-D-Pro-D-Pro-Nval-Nval-N(iPr)$_2$	782.04	804	33
2	H-Nphe-Nval-D-Pro-D-Pro-Nval-Nval-OH	656.82	679	75
3	Ac-Nphe-Nval-D-Pro-D-Pro-Nval-Nval-OH	698.86	721	76
4	Ac-Nphe-Nval-Pro-Pro-Nval-Nval-N(iPr)$_2$	782.04	804	27
5	Ac-Nphe-Nval-D-Pro-Pro-Nval-Nval-N(iPr)$_2$	782.04	804	29
6	Ac-Nphe-Nval-Pro-D-Pro-Nval-Nval-N(iPr)$_2$	782.04	804	15
7	Ac-Nphe-Nval-D-Pro-D-Pro-Nphe-Nval-N(iPr)$_2$	830.06	852	10
8	Z-D-Pro-D-Pro-Nval-Nval- N(iPr)$_2$	627.83	650	61

[a] yield after RP-HPLC purification, respectively.

The synthesis of the compounds **1 - 8** combined the advantages of submonomeric solid phase synthesis (SPS) of peptoids[10] and standard Fmoc-monomer SPS of peptides. For example, the dipeptoid H-Nval-Nval- was synthesized on the 2-chloro-trityl chloride resin according to a submonomer SPS protocol.[11] The sterically hindered coupling of Fmoc-Pro to the secondary amine with a branched alkyl chain was carried out in good yield with PyBroP[12] in dichloromethane. After capping with acetic anhydride the second proline was coupled with PyBroP followed by submonomer synthesis of -Nval- and -Nphe- and final acetylation. The hexapeptoid was cleaved from the resin and reacted directly with propane phosphonic acid anhydride and diisopropylamine. After purification by HPLC compound **1** was obtained in 33% overall yield. All products were characterized by FAB-MS (Table 1). Compounds **1 - 8** were tested for inhibition of colony formation in liquid culture of LNCAP lung carcinoma cells, SK-OV-3 ovarian cancer cells, KB human epidermoid carcinoma cells and L-1210 leukemia cells. Up to concentrations of 31.6 μg/mL no significant inhibition of cell growth was found.

In conclusion, eight Dolastatin 15 mimetic peptoids have been synthesized. Because of the modification of stereochemical and structural properties of the peptoids compared to the parent structure we expected reduced activity. On the other hand, a new peptoid based lead structure would have been accessible to high synthetic variability for further optimization. Complete loss of activity proved the concept of peptoids in retro-sequence as peptidomimetics not to be applicable for the cytostatic Dolastatin 15.

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References and Notes

1. König, G. M.; Wright, A. D. *Planta Medica* **1996**, *62*, 193-211; Flam, F. *Science* **1994**, *266*, 1324-1325.
2. Pettit, G. R.; Singh, S. B.; Hogan, F.; Burkett, D. D. *J. Med. Chem.* **1990**, *33*, 3133-3138.
3. Pettit, G. R.; Kamano, Y.; Herald, C. L.; Fujii, Y.; Kizu, H.; Boyd, M. R. Boettner, F. E.; Doubek, D. L.; Schmidt, J. M.; Chapuis, J.-C.; Michel, C. *Tetrahedron* **1993**, *49*, 9151-9170.
4. Hauthal, H. G. *Nachr. Chem. Tech. Lab.* **1995**, *43*, 1206-1208.
5. Bai, R.; Pettit, G. R.; Hamel, E. *J. Biol. Chem.* **1990**, *265*, 17141-17149; Bai, R.; Friedman, S. J.; G. R. Pettit, Hamel, E. *Biochem. Pharmacol.* **1992**, *43*, 2637-2645.
6. de Arruda, M.; Cocchiaro, C. A.; Nelson, C. M.; Grinnell, C. M.; Janssen, B.; Haupt, A.; Barlozzari, T. *Cancer Res.* **1995**, *55*, 3085-3092.
7. Simon, R. J., Kania, R. S., Zuckermann, R. N., Huebner, V. D., Jewell, D. A., Banville, S., Ng, S., Wang, L., Rosenberg, S., Marlowe, C. K., Spellmeyer, D. C., Tan R., Frankel, A. D., Santi, D. V., Cohen, F. E., Bartlett, P. A. *Proc. Natl. Acad. Sci.* **1992** *89*, 9367-9371; Kessler, H., *Angew. Chem.* **1993,** *105*, 572-573; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 543-544.
8. Chorev, M.; Goodman, M. *TIBTECH* **1995**, *13*, 438-445 and references cited therein.
9. Miyazaki, K.; Kobayashi, M.; Natsume, T.; Gondo, M.; Mikami, T.; Sakakibara, K.; Tsukagoshi, S. *Chem. Pharm. Bull.* **1995**, *43*, 1706-1718; Pettit, G. R.; Srirangam, J. K.; Barkoczy, J.; Williams, M. D.; Durkin, K. P. M.; Boyd, M. R.; Bai, R.; Hamel, E.; Schmidt, J. M.; Chapuis, J.-C. *Anti-Cancer Drug Design* **1995**, *10*, 529-544.
10. Zuckermann, R. N., Kerr, J. M., Kent, S. B. H., Moos, W. H. *J. Am. Chem. Soc.* **1992**, *114*, 10646-10647.
11. Experimental procedures: CH_2Cl_2 and DIPEA were dried over CaH_2.
    Loading of the resin: To 1.0 g 2-chloro-trityl chloride resin were added 5 mmol (0.70 g) bromoacetic acid and 7 mmol (1.2 mL) DIPEA in 15 mL CH_2Cl_2. The mixture was shaken for 1 h at RT and the resin was washed with CH_2Cl_2 and ether. In general a loading of 0.9 - 1.2 mmol/g was achieved.
    Coupling of bromoacetic acid: To a stirred solution of 10 eq. bromoacetic acid in CH_2Cl_2 (maximum concentration 0.3 M) are added 8 eq. bromoacetic acid bromide and 20 eq. DIPEA. The mixture is shaken for 10 min with the resin followed by washing of the resin with CH_2Cl_2.
    Substitution with primary amine: The resin is shaken for 4 h with a solution of 30 eq. isopropylamine or benzylamine in NMP (ca. 2 M) followed by washing of the resin with NMP and CH_2Cl_2.
    Capping: The resin is shaken for 10 min with a solution of 10 eq. acetic acid anhydride and 5 eq. DIPEA in CH_2Cl_2 followed by washing of the resin with CH_2Cl_2.
    Coupling of proline: A solution of 2.0 eq. Fmoc-Pro-OH, 2.0 eq. PyBrOP and 3 eq. DIPEA in CH_2Cl_2 is added to the resin and shaken for 1 h followed by washing of the resin with CH_2Cl_2. During the reaction the pH is adjusted to 8 by addition of DIPEA. Subsequently Fmoc is cleaved off by reacting the resin with 20% piperidine in DMF followed by washing of the resin with CH_2Cl_2.
    Cleaving of the resin: 1 g of the resin is shaken for 30 min with 20 mL of a mixture of acetic acid, trifluoroethanol and CH_2Cl_2 (1:1:3) and the resulting solution is filtered from the resin. The procedure is repeated once and the combined filtrates are evaporated in vacuo after addition of 20 mL of toluene.
    Formation of the C-terminal amide: To a ice-cooled solution of 0.5 mmol of the crude peptoid in 5 mL of CH_2Cl_2 1.5 mmol propane phosphonic acid anhydride (0.88 mL of a 50% solution in ethyl acetate) and 7.5 mmol diisopropylamine are added. After incubation for 2 h at 0°C and another 12 h at RT the solution is extracted with 5 mL saturated $NaHCO_3$, 5% $KHPO_4$ and water, respectively. The organic layer is evaporated in vacuo and the crude product is purified by HPLC.
12. Frérot, E.; Coste, J.; Pantaloni, A.; Dufour, M.-N.; Jouin, P. Tetrahedron 1991, 47, 259-270.