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Functionalized platforms based on marine cyclopeptides: different pathways to the hexapeptide

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Abstract

The synthesis of macrocyclic, roughly planar, exclusively *syn*-functionalized hexapeptides, related to dolastatin I and bistratamide C from enantiomerically pure oxazole precursors is reported. The platforms can either be synthesized in a stepwise procedure by final cyclization of the linear oxazole trimers or in a direct 'one-pot' reaction. The investigation on the coupling of these building blocks into linear dimers and trimers with modern peptide coupling reagents is reported. © 2000 Published by Elsevier Science Ltd.

Keywords: coupling reagents; cyclisation; peptides; oxazoles; thiazoles.

Introduction. In recent years numerous biologically active cyclopeptides[1] incorporating oxazole and thiazole units[2] have been identified as metabolic products of algae, fungi and primitive marine organisms and their structures have been elucidated. Compounds like dolastatin I (**1**)[3] or bistratamide C (**2**)[4] (Scheme 1) containing three substituted five-membered heterocycles often exhibit biological activities, such as cytotoxicity.[2b]

In these compounds the three heterocycles are linked by *trans* amide bonds. When incorporating only aromatic heterocycles, the cyclotrimers are roughly planar due to conjugation of the amide bonds with the aromatic rings.[5] The orientation of the three substituents depends on the absolute configuration of the α-carbon atoms. All *syn*-substituted structural analogues with functionalized side chains are rare in the large family of marine cyclopeptides.[2b,6] They may not only exhibit different biological activity than their unfunctionalized counterparts, but are also of potential interest as template molecules in supramolecular chemistry, because of the limited conformational

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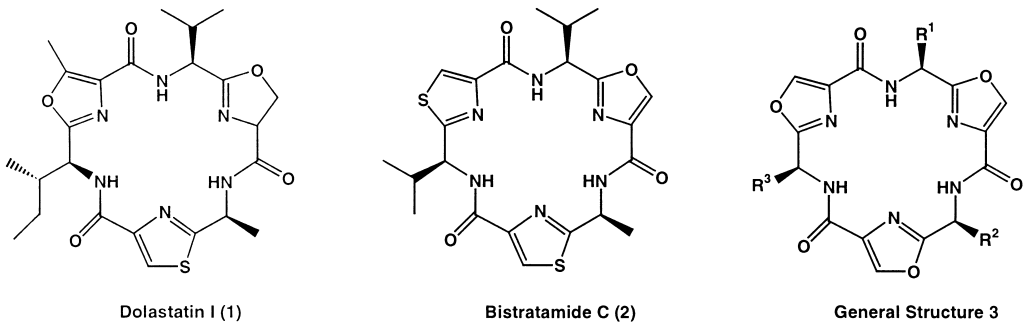
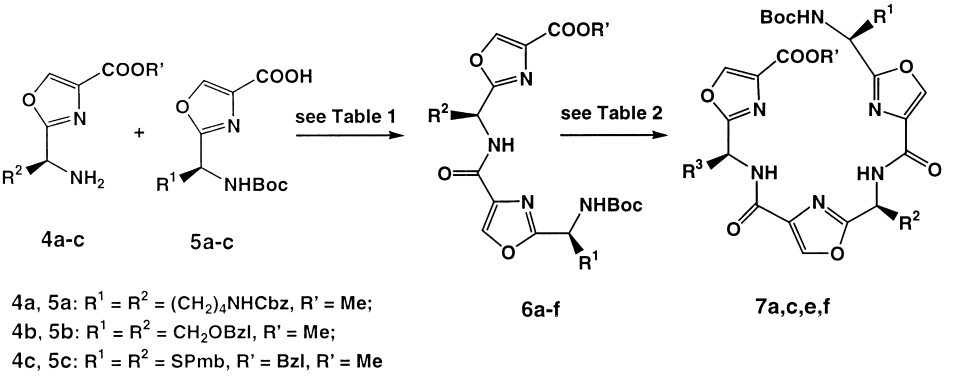


Scheme 1. Examples of molecules with a rigid platform-shaped skeleton

flexibility within the macrocyclic backbone. A large variety of molecules have been designed that incorporate these features; notable representatives are calixarenes, resorcinarenes and tetraarylporphyrines.[7a–c] In this context, a general synthetic route to **3** tolerating a variety of functionalized side chains R^1 to R^3 was desirable. Furthermore, the projected synthesis should allow the introduction of three different side chains in order to provide addressability.

Based on previous pathways used for the total synthesis of natural products[4,8] we investigated the synthesis of linear precursors (Scheme 2) containing differently functionalized side chains allowing for the synthesis of 'addressable' platforms.



Scheme 2. Coupling of the building blocks to open-chain dimers and trimers

Results and discussion. The retrosynthetic disconnection of the three amide bonds of **3** leads to three heterocyclic building blocks each containing an amino- and an acid-terminus. The building blocks were synthesized according to standard procedures.[5] The coupling of the mono deprotected subunits to linear dimers and trimers was extensively studied as outlined in Tables 1 and 2. It was first attempted with *i*-butylchloroformate and NMM.[4] The reaction led to a large amount of side-products. The desired dimers were obtained in yields of 18–26% almost independent of the different side chains R^1 to R^2. Thus, in order to improve the coupling procedure, several modern peptide coupling reagents were investigated and a series of homo- and heterodimers was generated. The best results were obtained with PyBOP, followed by BOP and HBTU.[9] The *N*-termini of the dimers **6a**,**c**,**e**,**f** were deprotected in almost quantitative yields with TFA followed

by the coupling with an acid monomer activated by PyBOP to give the 'open-chain' trimers **7a**,**c**,**e**,**f** in reasonable to good yields (Table 2).

Table 1
Coupling experiments to the dimers **6a–f**

Amino block	Acid block	Coupling agent	Yield %	Dimer
4a	**5a**	ClCOO*i*Bu, NMM	23	**6a**, $R^1 = R^2 = (CH_2)_4NHZ$, R' = Me
4a	**5b**	ClCOO*i*Bu, NMM	18	**6b**, $R^1 = CH_2OBzl$, $R^1 = (CH_2)_4NHZ$, R' = Me
4b	**5a**	ClCOO*i*Bu, NMM	26	**6c**, $R^1 = (CH_2)_4NHZ$, $R^2 = CH_2OBzl$, R' = Me
4c	**5a**	ClCOO*i*Bu, NMM	20	**6d**, $R^1 = CH_2OBzl$, $R^2 = SPMB$, R' = Bzl
4b	**5b**	BOP, *Hünigs base*	32	**6e**, $R^1 = R^2 = CH_2OBzl$, R' = Me
4c	**5c**	BOP, *Hünigs base*	45	**6f**, $R^1 = R^2 = SPMB$, R' = Bzl
4b	**5a**	EDC, DMAP	17	**6c**, $R^1 = (CH_2)_4NHZ$, $R^2 = CH_2OBzl$, R' = Me
4b	**5a**	HBTU, NMM	49	**6c**, $R^1 = (CH_2)_4NHZ$, $R^2 = CH_2OBzl$, R' = Me
4b	**5a**	PyBOP, *Hünigs base*	61	**6c**, $R^1 = (CH_2)_4NHZ$, $R^2 = CH_2OBzl$, R' = Me

NMM: *N*-Methyl morpholine; BOP: Benzotriazolyl-*N*-oxy-tris(dimethylamino)phosphonium hexafluorophosphate; PyBOP: Benzotriazol-1-yl-oxytripyrrolidinephosphonium hexafluorophosphate; HBTU: *O*-Benzotriazol-1-yl-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate: EDC: 1-Ethyl-3(3-dimethylaminopropyl)carbodiimde.

Table 2
Coupling experiments to linear trimers **7**

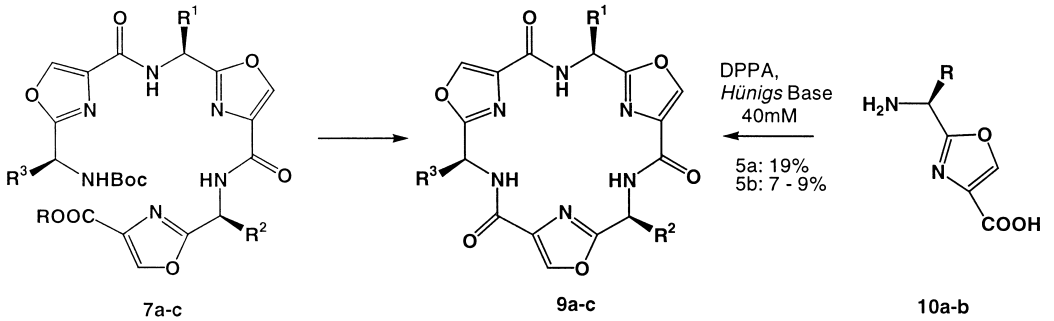
Amino dimer	Acid block	Yield %	Trimer
6a	**5a**	47	**7a**, $R^1 = R^2 = R^3 = (CH_2)_4NHZ$, R' = Me
6c	**5b**	75	**7c**, $R^1 = (CH_2)_4NHZ$, $R^2 = R^3 = CH_2OBzl$, R' = Me
6e	**5c**	64	**7e**, $R^1 = R^2 = CH_2OBzl$, $R^3 = SPMB$, R' = Me
6f	**5c**	58	**7f**, $R^1 = R^2 = R^3 = SPMB$, R' = Bzl

Deprotection of the acid terminus of the linear trimers **7a–c** by saponification of the methyl ester followed by cleavage of the Boc-group with TFA or HCl gave the cyclization precursors **8a–c** in 71 to 75%. The macrocyclization was tried under various conditions. High dilution and activation by PyBOP, BOP or HBTU gave only low yields of the cyclopeptides **9a–c** (12–15%).

However, there are several methods available for peptide cyclizations.[10] In many examples the ring closure via the pentafluorophenyl ester method[11] shows exceptional results. The methyl ester **7a** was transformed into a pentafluorophenyl ester,[12,13] the Boc-group was removed by HCl in dioxane, and the cyclization was subsequently performed in a two-phase chloroform-aqueous base system. This procedure gave **9a** in 58% yield. Removal of the protective groups on the side chains was achieved under standard conditions.

For the synthesis of platforms with three identically functionalized substituents a 'one-pot'-procedure seems to be suitable and has been investigated. Starting from the appropriate deprotected oxazole building blocks (Scheme 3) **10a–b**, the cyclic trimers **9a–b** could be synthesized under high dilution conditions by using DPPA as the coupling agent in moderate yields.[14] The overall yields of both pathways are in the same range (about 10–20%) starting from the

monomeric building block. Therefore, the 'one-pot'-procedure, with respect to yields, has no advantage over the stepwise synthesis, but gives much faster access to molecular platforms such as **9a**–**b**, bearing three identical side-chain substituents. The cyclization of the open-chain fragments **8a**–**c** with the pentafluorophenyl ester method highly improves the yields of cyclic products **9a**–**c** compared to the formerly used cyclization protocol.[15]



a) R = Me, $R^1 = R^2 = R^3 = (CH_2)_4NHCbz$; **b)** R = Me, $R^1 = R^2 = R^3 = CH_2NHCbz$;
c) R = Me, $R^1 = R^2 = CH_2OBzl$, $R^3 = (CH_2)_4NHCbz$

Scheme 3. Macrocyclization to the cyclopeptides **9a**–**c**

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9. **Procedure for the PyBOP coupling:** The amine building block (1 equiv.), the acid building block (1 equiv.) and PyBOP (1equiv.) were dissolved in anhydrous DMF (15ml/1mmol PyBOP) at rt, followed by slow addition of Hünigs Base (2.3 equiv.). Stirring was continued for 12 h. The reaction mixture was diluted with EtOAc, washed with 10% HCl, sat. $NaHCO_3$, brine and dried over $MgSO_4$. The solvent was evaporated and the products isolated by chromatography on silica with EtOAc:hexanes = 2:1. **Procedure for the BOP coupling**: The amine building

block (1 equiv.), the acid building block (1 equiv.) and BOP (1 equiv.) were dissolved in anhydrous CH_3CN or DMF (10 ml/1 mmol BOP) at rt, followed by slow addition of Hünigs Base (2.5 equiv.). Stirring was continued for 12 h. The reaction mixture was diluted with EtOAc and the organic layer was washed with 3 M HCl, sat. $NaHCO_3$ and brine, dried over $MgSO_4$ and the solvent was evaporated. The products were isolated by chromatography on silica with EtOAc:hexanes = 2:1. All compounds were characterized by ^1H and ^{13}C NMR and MS/HR-MS.

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13. **General procedure for the macrocyclization (pentafluorophenyl ester method)**: EDC×HCl (1.2 equiv.) was added to a stirred solution of compounds **7a**–**c** (1 equiv.) in CH_2Cl_2, followed by the addition of pentafluorophenol (1.3 equiv.) at –20°C. The mixture was slowly warmed to rt and stirred for 16 h, evaporated, dissolved in EtOAc, washed with brine, dried over Na_2SO_4 and evaporated. The crude pentafluorophenylester was dissolved in dioxane and HCl in dioxane was added at 0°C. Stirring was continued for 30 min at 0°C then 2 h at rt followed by evaporation of the solvent. The crude product was dissolved in $CHCl_3$ followed by the addition of a 1 M solution of $NaHCO_3$. The mixture was vigorously shaken for 10 min, followed by separation of the layers. The organic layer was dried and evaporated. The products were isolated by flash chromatography (silica gel, EtOAc).
14. **General procedure for the 'one–pot' macrocyclization**: To a 40 mM solution of **10a**–**b** in DMF, DPPA (2 equiv.) was slowly added at 0°C, followed by slow addition of Hünigs Base. The mixture was stirred at 0°C for 10 h then at rt for 48 h. The solvent was evaporated and the cyclic products were isolated by chromatography (silica gel, EtOAc:hexanes = 3:1). All compounds were characterized by ^1H and ^{13}C NMR and MS/HR-MS.
15. Selected analytical data: compound **9a**: ^1H NMR (600 MHz, acetone-d_6) δ (ppm) 1.15–1.25(m, 6H), 1.45–1.60 (m, 6H), 1.90–2.05 (m, 3H), 2.05–2.20 (m, 3H), 3.05–3.20 (m, 6H), 5.02 (s, 6H), 5.21 (q, ^3J = 5.9 Hz, 3H), 6.34 (t, ^3J = 5.3 Hz, 3H), 7.20–7.35 (m, 15H), 8.42 (d, ^3J = 6.6 Hz, 3H), 8.48 (s, 3H); ^{13}C NMR (150 MHz, acetone-d_6) δ (ppm) 22.65, 30.30, 35.00, 41.29, 49.07, 66.46, 128.72, 128.84, 129.40, 136.46, 138.72, 143.18, 157.45, 160.11, 165.24; FAB-MS: $[M+Cs]^+$ expected: 1120.3181; observed: 1120.3129. Compound **9b**: After cleavage of the Cbz-moiety in the side-chain amino functionalities by transfer-hydrogenation with Pd-black and 1,4-cyclohexadiene the corresponding tris-amine resulted (66%) which was identical in all means of spectroscopic data with the compound described in Ref. 5.