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Synthesis of ether analogues derived from HUN-7293 and evaluation as inhibitors of VCAM-1 expression

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Abstract—The cyclic depsipeptide HUN-7293 (**1**) and its D-lactate analogue **2** are highly potent inhibitors of inducible cell adhesion molecule expression. We report the synthesis of ether analogues varying in stereochemistry and side chain at the former hydroxyl acid position by employing a 'cut and paste chemistry' methodology starting from **1**. As an additional fruit of this synthetic effort, a cyclodepsipeptide featuring a tertiary amine instead of a tertiary amide between PrLEU and MALA was obtained. Results on the inhibitory profile of these compounds in assays of VCAM-1 and ICAM-1 protein expression are discussed.

Cell adhesion molecules such as ICAM-1, VCAM-1 and E-selectin play essential roles in the immune response, primarily by regulating leucocyte activation and trans-endothelial cell migration.[1] Natural products represent some of the most potent inhibitors of VCAM-1 expression, offering therapeutic potential for numerous inflammatory disorders and autoimmune diseases.[2] Using two independent screening assays, we identified cyclic heptadepsipeptide HUN-7293 (**1**) and the structurally related cyclic depsipeptide **2**, which contains a D-lactate as the hydroxy acid moiety.[3] Of note, **1** and **2** were isolated from different fungal broths. Both compounds were chosen as leads for a medicinal chemistry programme aiming at improved selectivity for VCAM-1.[2] The three-dimensional (3-D) structure of HUN-7293 was assessed by crystal structure analysis.[4] Total synthesis of **1** was achieved by Dale Boger's group in collaboration with Novartis scientists.[5] Preparation and testing of a cyclodepsipeptide pharmacophore library enabled the elucidation of some structure–activity relationships.[6,7]

In the 3-D structure of **1**, determined in solution as well as in the crystalline state,[4] the ester bond exists in the *trans*-configuration and, therefore, suggested replacement of the ester by a more stable amide. However, introduction of an amide bond via total as well as chemical point mutation using a 'cut and paste' chemistry approach yielded aza HUN-7293, which showed a 10- to 20-fold loss of inhibitory potency in the VCAM-1 expression assay.[8,9] We reasoned that this was due to the more hydrophilic nature and/or rigidity of the amide bond compared to the natural ester bond. Therefore, our next aim was the synthesis of the less polar, yet more flexible, ether analogues.

In this paper we report the preparation of ether analogues of **2** by a semi-synthetic approach starting from HUN-7293 (**1**), accessible in kilogram amounts by a fermentation process.[3] To assess also the biological impact of the D-stereochemistry of the hydroxyl acid, we envisaged preparing macrocyclic ether analogues derived from L- and D-lactic acids as well as glycolic acid featuring no side chain (Fig. 1).

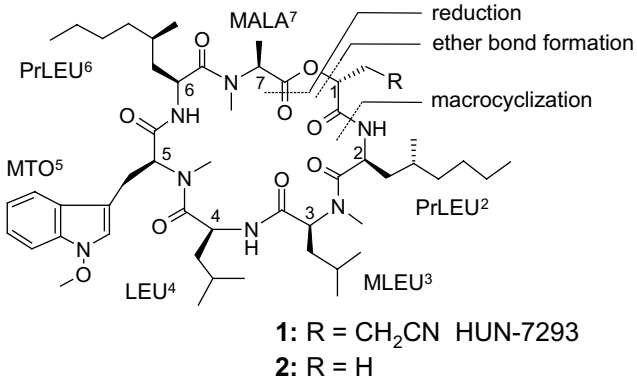

Figure 1.

Keywords: HUN-7293; VCAM-1 expression; Ether analogues.
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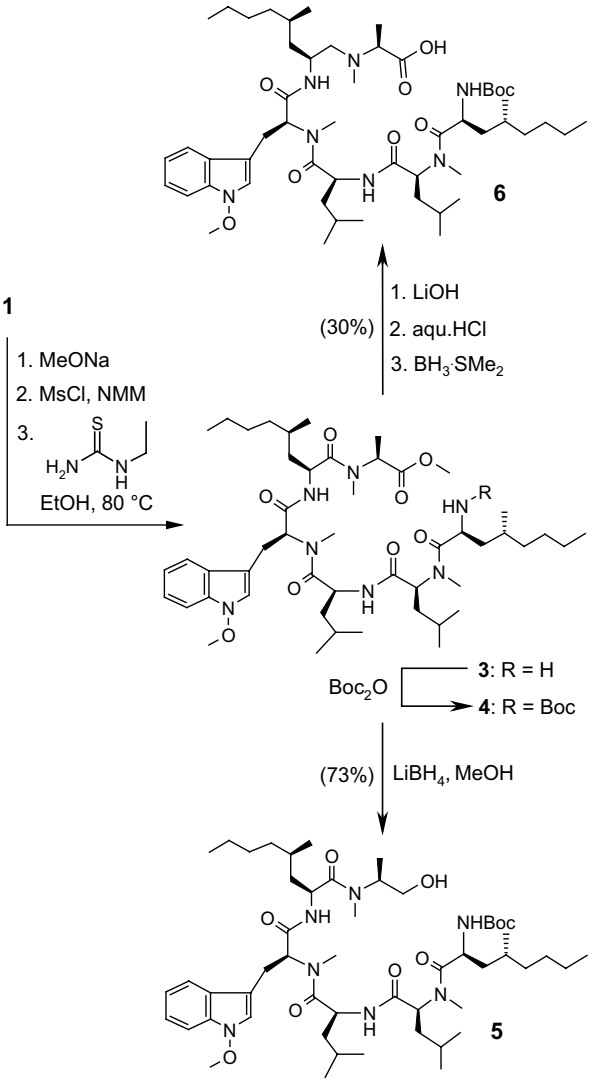
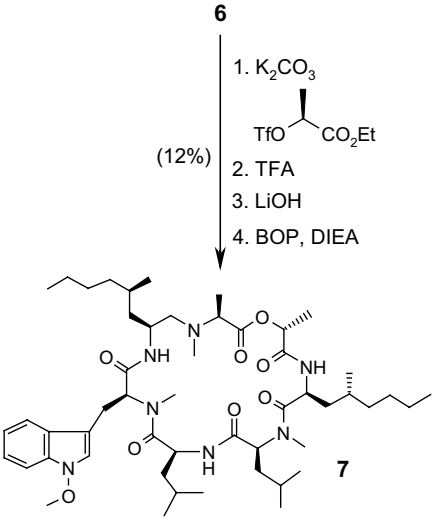
First, we transformed **1** into the hexapeptide ester **3** by a three-step sequence, as recently published.[8] Briefly, alkaline ester cleavage in MeOH (MeOH, NaH, rt) liberating the hydroxy group of the hydroxy acid moiety followed by a two-step protocol consisting of activation of the hydroxyl group via mesylation (Mes-Cl, NMM, in CH_2Cl_2, 0 °C to rt, 3 h) and treatment with *N*-ethylthiourea (EtOH, 80 °C, 24 h) gave **3** in 70–75% yield. *N*-BOC protection of **3** (BOC_2O, CH_2Cl_2, rt, 18 h) delivered **4** in quantitative yield. Reduction of **4** by portionwise addition of an excess of $LiBH_4$ to a solution of the ester in MeOH provided the alcohol **5** (73%) as well as unreacted starting material **4** (25%).

In the course of an alternative route towards the primary alcohol **5** we cleaved the methylester of **3** (LiOH, THF/H_2O (5:1), rt, 18 h). Acidic work-up gave the intermediate carboxylic acid, which was treated with borane-dimethylsulfide complex in THF. The major product isolated (30%), however, turned out to be not identical with **5** but according to mass spectroscopy a compound of the same molecular weight (970), which was later identified as the tertiary amine **6** resulting from reduction of the secondary amide ligating 6-PrLEU and 7-MALA (Scheme 1).



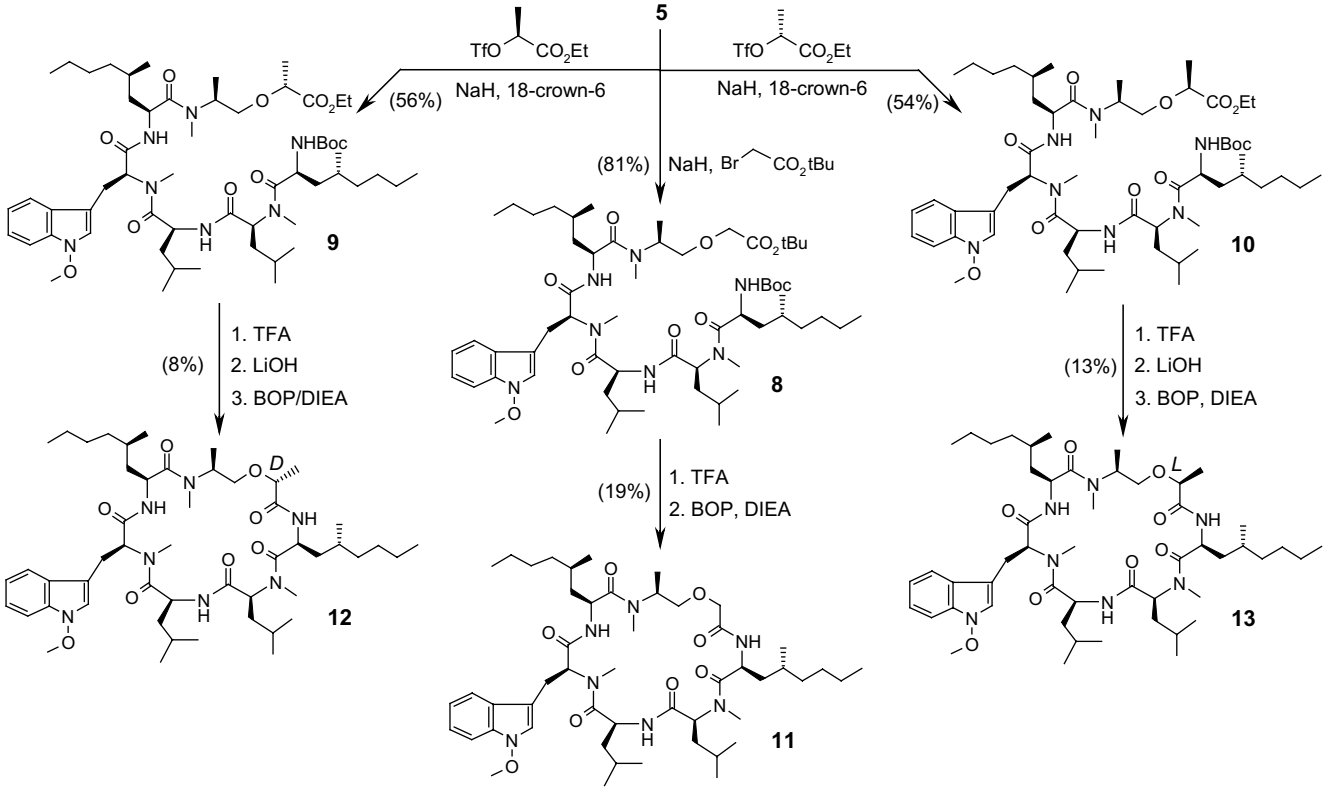
Scheme 1.



Scheme 2.

The fact, that application of an acidic protocol for HPLC analysis, our standard method (RP18/H_2O–MeCN gradient, 0.1% H_3PO_4) for this class of compounds, did not work in case of **6** immediately indicated that it should be an amine. To unambiguously identify the site affected in the borane reaction we transformed **6** into a cyclic molecule. This was accomplished by reaction of the amino acid **6** with ethyl L-*O*-trifluoromethylsulfonyllactate (L-TfO-Lac-OEt) in presence of K_2CO_3 in a mixture of CH_2Cl_2 and pentane[10] at rt overnight forming the ester with the natural D-stereochemistry in 88% yield. Sequential acidic removal of the BOC group (TFA, CH_2Cl_2, rt, 3 h) and alkaline cleavage of the ethylester (LiOH, THF/H_2O (5:1), rt, 18 h) gave the unprotected peptide. Applying the procedure found to be effective in the semi-synthesis of aza HUN-7293,[8] we achieved macrocyclization by forming the amide bond between the newly introduced lactate moiety and 2-PrLEU (0.34 mM in CH_3CN, BOP, DIEA, rt, 14 h). Purification by chromatography on SiO_2 (EtOAc/*c*-Hex/MeOH = 3:6:1) and size exclusion chromatography (Sephadex® LH_{20}, MeOH) provided the cyclic amine **7**, in 13% isolated overall yield from **6** (Scheme 2).

Initial experiments scheduled to achieve ether bond formation between **5** and L-TfO-Lac-OEt, again using K_2CO_3 as the base, did not produce the desired ether but the corresponding carbonate arising from trapping carbon dioxide by the alkoxy anion and subsequent reaction of the intermediate monoalkylcarbonate with the electrophile. However, we then succeeded by using the combination NaH/18-crown-6 (4 equiv) in THF. Alkylation of the alcohol **5** with *tert*-butyl bromoacetate cleanly delivered the open-chained ether **8** (NaH, THF, rt, 4 h, 81%). Similarly, reaction with L- and D-TfO-Lac-OEt gave the ether analogue with the natural D-configuration **9** and its corresponding L-isomer **10** in



Scheme 3.

56% and 54% yield, respectively. *N*-Boc deprotection of **8–10** was again pursued by treatment with TFA in CH_2Cl_2. In the case of **8** simultaneously the *tert*-butylester was cleaved liberating the free amino acid ready for cyclization in a single step (64%). The ethylesters of **9** and **10** had to be hydrolyzed in a separate step under alkaline conditions (LiOH, THF/H_2O, rt, 18 h) providing the crude deprotected peptides in 94% and 90% yield, respectively. Macrocyclization at a concentration of 0.22–0.3 mM (BOP, DIEA, CH_3CN, rt, 24 h) gave the title compounds **11–13** in 29%, 8% and 14% yield over three steps, respectively. Yields in the macrocyclization step for the amine **7** and the α-substituted ether derivatives **12** and **13** were clearly lower than that observed for cyclic peptides. We think this reflects the less effective pre-organization of the more flexible open-chained precursors. In accordance with this hypothesis the NMR spectrum (d_3-MeOD) indicates that only 30% of the ether **12** exists in that for those compound class typical basket–like conformation,[4] which, carrying out the experiment in $CDCl_3$, even disappeared totally (Scheme 3).

Inhibition of the induced expression of the cell adhesion molecule VCAM-1 and ICAM-1 by compounds **2**, **7**, **12–14** was assessed by ELISA assays disclosed previously (Table 1).[11]

Comparing the biological activities of the ether analogue **12** with natural D-configuration with the cyclic peptide **14** featuring a D-alanine as a replacement for the hydroxyl acid (IC_{50} = 92 nM),[12] **12** was found to be seven times more potent, however, still clearly less potent than the natural product **2**. Switching the configuration of the alkoxy acid from D-(**12**) to L-stereochemistry (**13**) caused an additional 40-fold reduction of activity emphasizing the importance of the appropriate chirality at this position for biological activity. An IC_{50} value somewhere in between for the analogue **11**, lacking a methyl group and, therefore, chirality, fits the picture. The amine analogue **7** appeared to be almost equipotent as **12**, but features at least 10-times higher selectivity (>150) for inhibition of VCAM-1 versus ICAM-1 (Fig. 2).

Table 1. Activity (nM, IC_{50}) in cell ELISA for TNFα-induced VCAM-1 and ICAM-1 expression in the human microvascular endothelial cell line HMEC-1, using previously described method[11]

Compound	VCAM-1	ICAM-1
	HMEC-1	HMEC-1
1	1	25
2	2	178
7	18	>30,000
11	200	nt[a]
12	15	1662
13	630	>30,000
14	92	6925

[a] Not tested.

In conclusion, using a 'cut and paste' chemistry approach, we have synthesized three ether analogues, **11–13**, of the HUN-7293 analogue **2**. Compound **12** with natural D-stereochemistry was found to be significantly

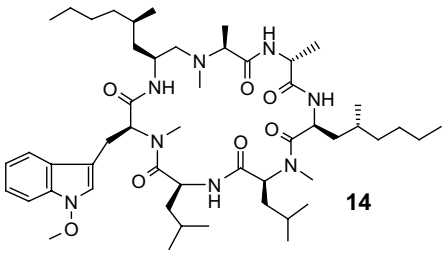


Figure 2.

more potent than the corresponding cyclic peptide, however, it was not as effective as the natural lactone **2**. Inversion of the stereocentre resulted in a 40-fold decrease in potency reflecting the importance of the natural D-stereochemistry at this position. The lactone derivative **7**, structurally featuring a tertiary amine instead of one amide bond, showed a significantly higher selectivity for inhibition of VCAM-1 versus ICAM-1 at a comparable level of potency, a profile at which we were aiming.

Acknowledgements

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12. Obtained by the methodology described in Ref. 9.