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Synthesis and Evaluation of Aza HUN-7293

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Abstract—The aza analogue of the cyclic heptadepsipeptide HUN-7293 (**1**), which is a potent naturally occurring inhibitor of inducible cell adhesion molecule expression, and its C_2^3 (MLEU^3 C_2) epimer were prepared via solution-phase synthesis. Biological evaluations of these two compounds as inhibitors of cell adhesion molecules expression are detailed. © 2000 Elsevier Science Ltd. All rights reserved.

HUN-7293 (**1**), a naturally occurring cyclic heptadepsipeptide which was isolated from a fungal broth, is a potent inhibitor of inducible cell adhesion molecule expression and the prototypical lead for a new class of potential therapeutics for the treatment of chronic inflammatory disorders or autoimmune diseases.[1] We recently disclosed the first total synthesis of HUN-7293 enlisting as key steps the linear heptadepsipeptide construction by Mitsunobu esterification of the constituent tetrapeptide and tripeptide, and a final cyclization step conducted at the secondary amide site linking residues 3 and 4 (MLEU^3–LEU^4), Figure 1.[2] In order to gain a better understanding of the structure–activity relationships of this potent natural product, the synthesis of its analogues is underway in our lab.

Herein, we report two key analogues and the development of methodology suitable for the rapid, parallel synthesis of a library of HUN-7293 analogues. Thus, the approach to aza HUN-7293 (**2**, Fig. 1) was developed with two purposes in mind. First, by replacing the ester oxygen atom in **1** with the nitrogen atom in **2**, the significance of the ester bond in the natural product would be defined. Moreover, since the construction of each of the amide bonds could be achieved by solution-phase chemistry and pure products could be obtained by simple aqueous acid/base extractions which we have employed in preparing a number of libraries,[3–16] the approach could be developed in such a way as to provide the technology for the synthesis of a library of HUN-7293 analogues.

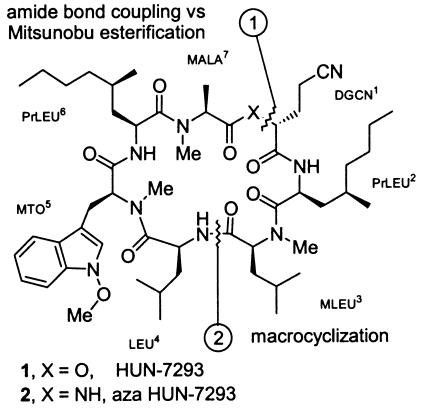

Figure 1.

The additional D-amino acid **3**, which is not present in natural products, was made adopting a literature procedure[17] starting from D-Gln (Scheme 1). *N*-BOC deprotection of dipeptide **4** (HCl–EtOAc) and coupling of **5** with **3** cleanly provided tripeptide **6**. All the peptide coupling reactions were conducted with product isolation and purification by sequential acid/base/brine washes, providing the products sufficiently pure by ^{1}H NMR and HPLC analysis for subsequent use.

The known tetrapeptide **10** was prepared by an improved coupling of deprotected dipeptides **8**[2] and **9**[2] using HATU–2,6-lutidine, providing a diastereomeric ratio of 32:1 (HPLC) in favor of the desired product **10** (Scheme 1).[2] This is a significant improvement over our original conditions (HATU, $NaHCO_3$) which provided a 13:1 ratio of diastereomers that are not separable by

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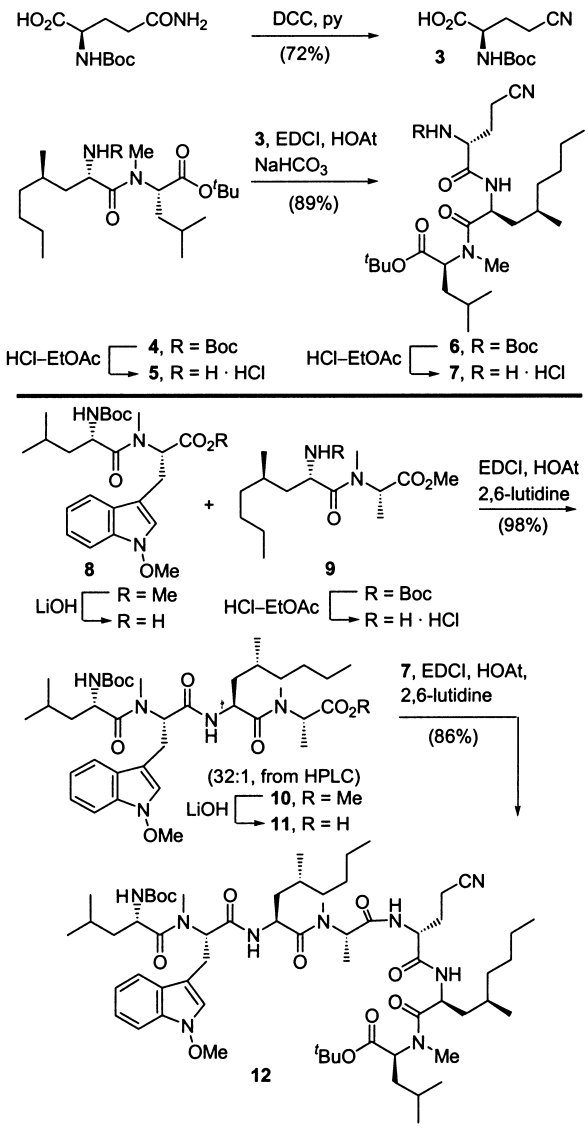
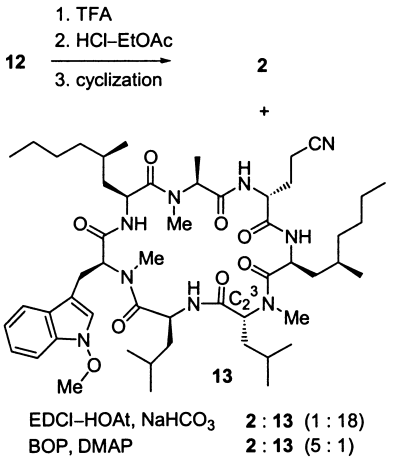
normal phase SiO_2 chromatography, but may be separated by reverse phase HPLC. Similar observations were made with EDCI–HOAt where the coupling of **8** and **9** (90–98%) proceeded with less epimerization in the presence of 2,6-lutidine (>30:1) versus $NaHCO_3$ (14:1). Unlike the sample of tetrapeptide **10** utilized in the total synthesis of **1**,[2] each of the constituent dipeptide precursors **8** and **9**, as well as **10** were isolated and purified only by aqueous acid/base extractions. Linear heptapeptide **12** was prepared by coupling **7** and **11** and under the reaction conditions studied (HOAt, EDCI, 2,6-lutidine in CH_2Cl_2–DMF 5:1, −30 °C, 3 h, 86%), no significant epimerization was detected.

The final macrocyclization reaction proved more problematic (Scheme 2). In the efforts on the natural product, the macrolactamization reaction of the depsipeptide conducted at the MLEU[3]–LEU[4] site proceeded in superb conversion (72%) under a range of conditions. Although C_2[3] epimerization was observed under some conditions, racemization of the sensitive activated MLEU[3] *N*-methyl carboxylate could be suppressed such that it was not problematic. Adapting the reaction conditions successful in the total synthesis of HUN-7293 (EDCI–HOAt, $NaHCO_3$ in 5 mM DMF, 0 °C, 6 h),[2] one major product **13**, the C_2[3] epimer of **2**, was obtained in 38% yield and only a trace of **2** was detected (18:1, **13**:**2**). The best result obtained enlisted a cyclization effected by treatment with BOP (DMAP in 5 mM CH_3CN, 25 °C, 1 h), and a reversed diastereomeric ratio was observed (**13:2** = 1:5) in 22% combined yield. Clearly, the substitution of an amide for the depsipeptide ester slows the rate of macrocyclization such that C_2[3] epimerization and preferential closure to **13** containing a L-amine/D-carboxylate cyclization site is observed.[18] The final products **2** and **13** were purified by HPLC (reverse phase *Prep*LC™, 86–100% MeOH–H_2O gradient, 10 mL/min, R_t = 17 min for **2** and 19 min for **13**) to provide samples suitable for biological assay.

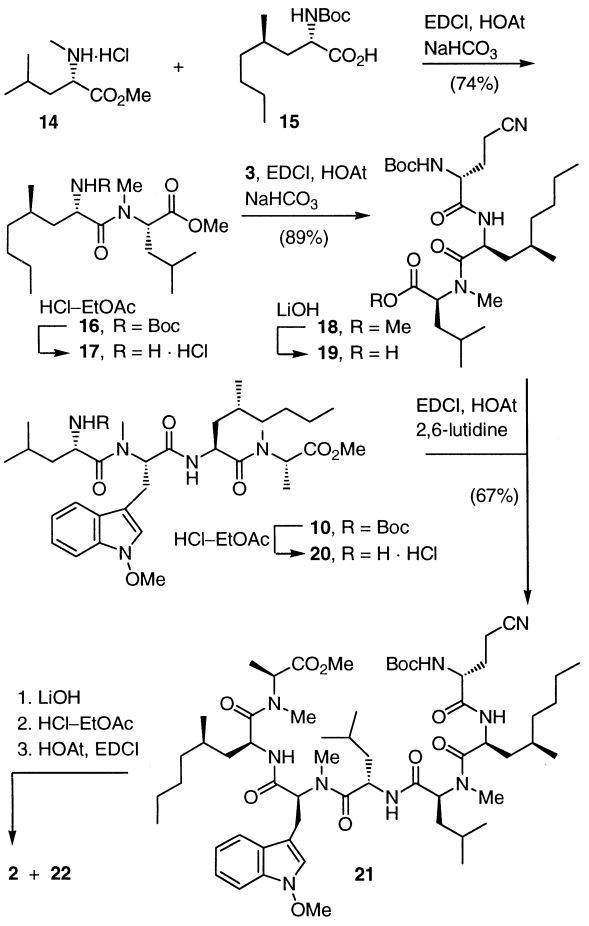
The approach in which the coupling and macrocyclization steps were reversed was also explored and represented an especially attractive alternative. Macrocyclization at the MALA[7]–DGCN[1] site represents closure at a D-amine terminus which has been shown to provide superb conversions relative to an L-amine terminus.[18] The disadvantage of this closure site, which is also embodied in the LEU[4]–MLEU[3] site, is that it is conducted with activation of a racemization prone the *C*-terminus *N*-methyl carboxylate. Thus, coupling of NMe–Leu–OCH_3 with **15**,[2] *N*-BOC deprotection of **16**, and coupling of **17** with **3** provided the key tripeptide **18** bearing a *C*-terminus protecting group suitable for subsequent hydrolysis upon treatment with LiOH to provide **19** (Scheme 3). *N*-BOC deprotection of **10**, coupling of **19** and **20**, and sequential *N*-BOC deprotection and methyl ester hydrolysis of **21** provided the precursor for the macrocyclization reaction. Closure was best effected by treatment with EDCI–HOAt (2,6-lutidine, CH_2Cl_2–DMF 5:1, 0 °C, 5 h) providing **2** and its C_2[7] epimer **22** (2.1:1, 30%). Like the closure of **12** (Scheme 2), the use of $NaHCO_3$ in place of 2,6-lutidine led to greater racemization (1:1 **2**:**22**, 12%). The use of DPPA (*i*Pr_2NEt, DMF, 0 °C, 43 h; **2**:**22** 2.1:1, 18%) or BOP (DMAP, CH_3CN, 25 °C, 1 h, **2**:**22**, 1.8:1, 17%) provided comparable, but less



Scheme 1.



Scheme 2.



Scheme 3.

satisfactory results. Thus, closure at the D-amine terminus of the MALA[7]–DGCN[1] site offered no advantage.

Aza HUN-7293 (**2**) and its $C_2{}^3$ epimer were compared with **1** in their ability to inhibit inducible cell adhesion molecule expression (VCAM-1, ICAM-1, E-selectin) in ELISA assays previously disclosed (Table 1).[1,20] In each case, **2** proved to be roughly 10–20× (10–60×) less potent than **1** at inhibiting VCAM-1, ICAM-1, or E-selectin expression and the sample of **2** prepared by synthesis herein and by degradation of **1** and semisynthesis[19] were indistinguishable within the error limits of the assays (IC_{50} ±30–40%, $n = 1$–3). In addition, the $C_2{}^3$ epimer **13** exhibited a further 10-fold loss in potency relative to **2** indicating that the $C_2{}^3$ stereochemistry is important to the properties of **1**.

Having established that the depsipeptide ester is important to the biological properties of **1** and facilitates macrocyclization, these studies have been extended to the ongoing preparation of a library of analogues incorporating the depsipeptide ester enlisting the solution-phase isolation and purification of intermediates by sequential aqueous acid/base extractions. The results of these studies will be disclosed in due course.

Table 1. Activity (nM, IC_{50}) in cell ELISA for VCAM-1, ICAM-1, and E-selectin expression using primary human endothelial cells (HUVEC) and a human microvascular cell line (HMEC-1)

Compound	VCAM-1		ICAM-1		E-Selectin
	HUVEC	HMEC-1	HUVEC	HMEC-1	HUVEC
1	3	1	26	24	44
2[a]	33	22	540	540	540
2[b]	64	58	950	nt[c]	1000
13	560	550	>3000	>3000	>10,000

[a]Derived from natural HUN-7293, ref 19.
[b]Synthetic **2** prepared herein.
[c]Not tested.

Acknowledgements

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19. Compound **2** proved identical to a sample of **2** prepared from **1** by degradation and semisynthesis. We thank Dr. E. Schreiner of Novartis for this compound and for making this comparison.

For **2**: ^{1}H NMR (500 MHz, CD_3OD, major rotamer) δ 7.65 (d, J = 8.1 Hz, 1H), 7.59 (d, J = 7.7 Hz, 1H), 7.22–7.25 (m, 2H), 7.09–7.12 (m, 1H), 5.05 (dd, J = 9.2, 5.1 Hz, 1H), 4.94 (dd, J = 10.6, 4.0 Hz, 1H), 4.82 (m, overlapped with water peak, 1H), 4.46 (m, 2H), 4.36–4.40 (m, 2H), 4.07 (s, 3H), 3.22–3.32 (m, overlapped with solvent peak, 2H), 3.27 (s, partially overlapped, 3H), 2.93 (s, 3H), 2.59 (s, 3H), 2.16–2.21 (m, 1H), 1.94–1.99 (m, 1H), 1.82–1.84 (m, 1H), 1.10–1.75 (m, 27H), 0.88–1.05 (m, 18H), 0.46 (d, J = 6.6 Hz, 3H), 0.00 (d, J = 6.6 Hz, 3H), −0.59 (ddd, J = 12.8, 11.0, 1.8 Hz, 1H); IR (neat) ν_{max} 3296, 2923, 2246, 1732, 1682, 1661, 1634, 1615, 1538, 1463, 1385, 1204 cm^{-1}; MALDI–FTMS m/z 998.6455 ($[M+Na]^+$, $C_{53}H_{85}N_9O_8Na$ requires 998.6413).

For **13**: ^{1}H NMR (500 MHz, CD_3OD, major rotamer) δ 8.24 (br d, J = 8.1 Hz, 1H), 7.63 (br d, J = 8.1 Hz, 1H), 7.57 (br d, J = 7.6 Hz, 1H), 7.21–7.24 (m, 2H), 7.10 (dd, J = 7.6, 7.6 Hz, 1H), 5.19 (dd, J = 12.3, 3.9 Hz, 1H), 5.05 (dd, J = 7.2, 7.2 Hz, 1H), 4.96 (dd, J = 9.5, 4.4 Hz, 1H), ∼4.84 (overlapped with water peak, 1H), 4.52–4.54 (m, 2H), 4.45 (dd, J = 8.3, 5.3 Hz, 1H), 4.06 (s, 3H), 3.20–3.21 (m, 2H), 3.12 (br s, 3H), 2.94 (s, 3H), 2.78 (s, 3H), 2.52 (br dd, J = 7.0, 7.0 Hz, 2H), 2.17–2.21 (m, 1H), 1.98–2.03 (m, 1H), 1.81 (br dd, J = 13.2, 11.7 Hz, 1H), 1.69–1.74 (m, 2H), 1.59–1.63 (m, 1H), 1.20–1.41 (m, 28H), 0.85–0.96 (m, 12H), 0.52 (d, J = 6.6 Hz, 3H), 0.05 (d, J = 6.6 Hz, 3H), −0.38 (br dd, J = 13.2, 11.7 Hz, 1H); IR (neat) ν_{max} 3290, 2956, 2927, 2871, 2246, 1745, 1721, 1698, 1678, 1651, 1634, 1531, 1463, 1410, 1385, 1248, 1173, 1098 cm^{-1}; MALDI–FTMS m/z 998.6422 ($[M+Na]^+$, $C_{53}H_{85}N_9O_8Na$ requires 998.6413).

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