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Total Synthesis and Biological Evaluation of Halipeptins A and D and Analogues

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Abstract: The marine-derived halipeptins A (**1a**) and D (**1d**) and their analogues **3a**, **3d** and **4a**, **4d** were synthesized starting from building blocks **10**, **13**, **14a** or **14d**, **15**, and **16**. The first strategy for assembling the building blocks, involving a macrolactamization reaction to form the 16-membered ring hydroxy thioamide **52d** as a precursor, furnished the epi-isoleucine analogue (**4d**) of halipeptin D, whereas a second approach involving thiazoline formation prior to macrolactamization led to a mixture of halipeptins A (**1a**) and D (**1d**) and their analogues **3a**, **3d** (epimers at the indicated site) and **4a**, **4d** (epimers at the indicated site). The same route starting with D-Ala resulted in the exclusive formation of the epimeric halipeptin D analogue **3d**. The synthesized halipeptins, together with the previously constructed oxazoline analogues **5d** and **6d**, were subjected to biological evaluation revealing anti-inflammatory properties for **1a**, **1d**, and **6d** while being noncytotoxic against human colon cancer cells (HCT-116).

Introduction

The halipeptins (A−D, Figure 1) are a group of marine-derived depsipeptides with interesting molecular architectures and biological properties. Their fascinating tale began with the isolation of halipeptins A and B by the Gomez-Paloma group in 2001 from the sponge *Haliclona sp.*, and the assignment to them of depsipeptide structures containing an intriguing 1,2-oxazetidine-type structural motif as shown in **2a** and **2b** (Figure 1).[1] Adding to the attractiveness of these molecules as targets for total synthesis was the potent anti-inflammatory activity attributed to the most abundant of the two, halipeptin A (**2a**). The latter compound exhibited 60% reduction of carrageenan-induced paw edema in mice at the intraperitoneal dose of 0.3 mg kg^{-1} body weight, thus rivaling in potency commercial anti-inflammatory drugs.[1]

The story of halipeptins took another turn in 2002 when the same group reported a third member of the family, halipeptin C (**1c**, Figure 1), and revised their originally proposed oxazetidine structures of halipeptins A and B to the thiazoline structures **1a** and **1b**, respectively (Figure 1).[2] Adding support to the new structures was yet another development in the field, that involving the isolation of halipeptin D (**1d**, Figure 1) from a different sponge, *Leiosella* cf. *arenifibrosa*, by Faulkner and Manam.[3] In 2003, these researchers communicated to us the structure of halipeptin D (**1d**) and their finding that their newly isolated natural product exhibited potent cytotoxic properties [IC_{50} = 7 nM against human colon cancer HCT-116 cell line and an average IC_{50} = 420 nM against a BMS ODCP (oncology diverse cell panel) of tumor cell lines].[4] These results were in sharp contrast to those of Gomez-Paloma for their halipeptins A−C (**1a**−**1c**), which apparently were devoid of any significant cytotoxicity properties.[1,2] It should also be mentioned that neither the Gomez-Paloma group nor the Faulkner−Manam team could assign with certainty the absolute stereochemistries at C-3 and C-4, although the former group had reached the conclusion, through chemical synthesis studies, that the absolute stereochemistry of the C-7 stereocenter was of the (*S*) configuration in halipeptin B (**1b**).[1]

Faced with these intriguing and still unresolved questions and because of the natural scarcity of these compounds, we embarked on a program directed toward their total synthesis, initially targeting halipeptins A (**1a**) and D (**1d**).[5] In this Article, we describe in detail our investigations in this area, including the chemical synthesis of these two natural products (**1a** and

† Current address: Dipartimento di Scienze Farmaceutiche, University degli Studi di Salerno, Italy.
‡ Current address: Dipartimento di Farmacologia Sperimentale, University degli Studi di Napoli, Italy.

(1) Randazzo, R.; Bifulco, G.; Giannini, C.; Bucci, M.; Debitus, C.; Cirino, G.; Gomez-Paloma, L. *J. Am. Chem. Soc.* **2001**, *123*, 10870−10876.
(2) Della Monica, C.; Randazzo, A.; Bifulco, G.; Cimino, P.; Aquino, M.; Izzo, I.; De Riccardis, F.; Gomez-Paloma, L. *Tetrahedron Lett.* **2002**, *43*, 5707−5710.
(3) Nicolaou, K. C.; Schlawe, D.; Kim, D.; Longbottom, D. A.; de Noronha, R. G.; Lizos, D. E.; Rao Manam, R.; Faulkner, D. J. *Chem.-Eur. J.* **2005**, *11*, 6197−6211.
(4) (a) Izzo, I.; Avallone, E.; Della Corte, L.; Maulucci, N.; De Riccardis, F. *Tetrahedron: Asymmetry* **2004**, *15*, 1181−1186 (for some biological data of halipeptin D, see ref 3 in this publication). (b) Data obtained by D. J. Faulkner and which appeared in a summary review, see: Fenical, W.; et al. *Pharm. Biol.* **2003**, *41*, 6−14.
(5) Nicolaou, K. C.; Kim, D.; Schlawe, D.; Lizos, D. E.; de Noronha, R. G.; Longbottom, D. *Angew. Chem., Int. Ed.* **2005**, *44*, 4925−4929.

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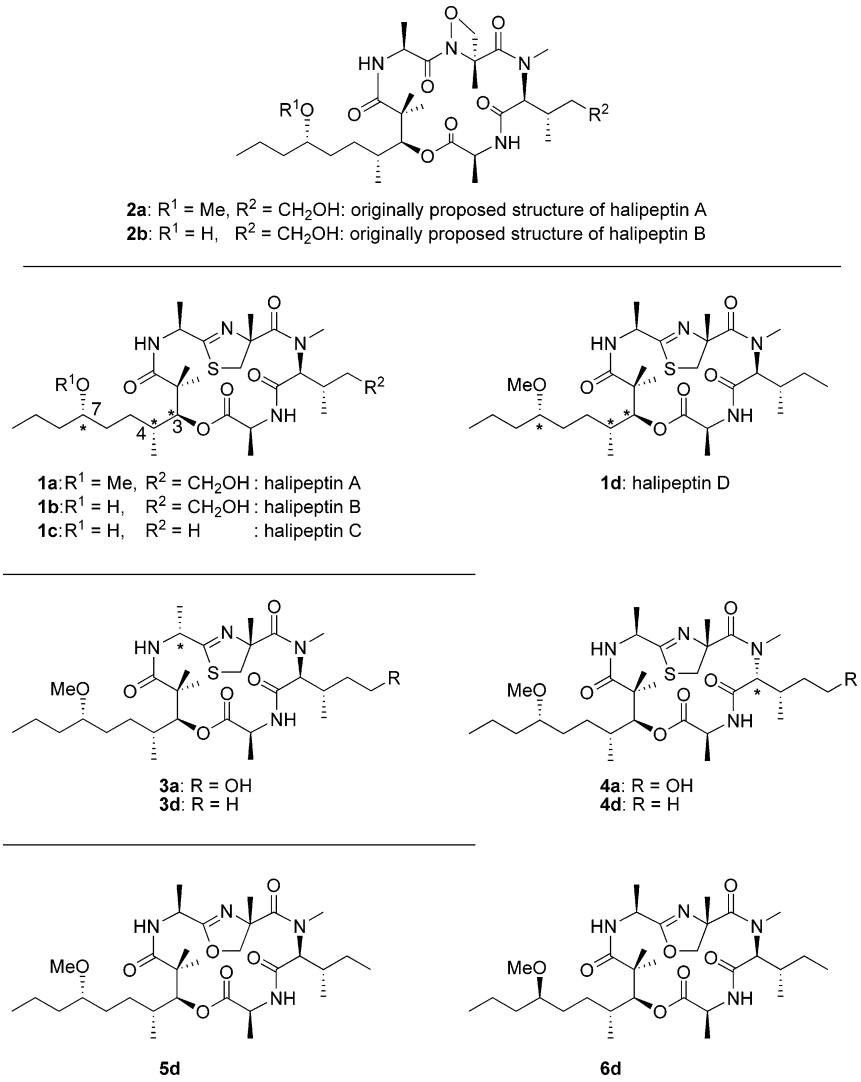


Figure 1. Structures of halipeptins A−D and analogues thereof.

1d), their epimers **3a**, **3d**, **4a**, and **4d** (Figure 1), and their biological properties, as well as those of a number of oxazoline analogues of halipeptin D (**5d**[3] and **6d**[3] Figure 1).

Results and Discussion

In contemplating a total synthesis of the halipeptins, a number of their structural features deserve special attention and dictate certain strategies and tactics. A detailed discussion of their structural motifs is, therefore, deemed important at this juncture. Thus, even a cursory inspection of the structures of halipeptins reveals a striking number of methyl groups situated on, or around, the periphery of their macrocyclic depsipeptide ring. These methyl groups translate into an unusually high degree of strain for a 16-membered ring that otherwise could have been relatively free of such energetic barriers, as far as its construction is concerned. Further constrains are imposed upon this macrocycle by the thiazoline ring and its adjacent cisoid amide bond locked in that configuration by the two methyl groups flanking its carbonyl group. The three stereocenters situated next to the carbonyl and thiazoline moieties should not be ignored, for they could epimerize at certain stages of the growth of the molecule or at the end of the synthesis. Also, the architectural uncertainties left unanswered by the structural elucidation studies, particularly

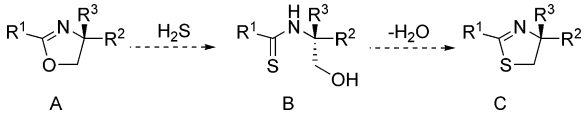


Figure 2. Oxazoline−thiazoline conversion.

at C-3, C-4, and C-7,[6] demanded flexible strategies for the construction of key intermediates, especially of the hydroxyl decanoic acid fragment carrying the remote methoxy group of halipeptins A (**1a**) and D (**1d**). It was with these considerations in mind that we entered into the endeavor we are about to describe, beginning with the retrosynthetic analysis of the halipeptin molecule.

Retrosynthetic Analysis. Mindful of the known methods[7] of converting an oxazoline to a thiazoline through the corresponding hydroxymethyl thioamide (i.e., A→B→C, Figure 2), we initially considered the possibility of obtaining the final product (e.g., halipeptin D, **1d**) from its oxazoline counterpart

(6) (a) In their total synthesis of halipeptin A, Ma et al. have shown the decanoic acid fragment to be 3(*S*),4(*R*),7(*S*): Yu, S.; Pan, X.; Lin, X.; Ma, D. *Angew. Chem., Int. Ed.* **2005**, *44*, 135−138. (b) Hara, S.; Makino, K.; Hamada, Y. *Tetrahedron Lett.* **2006**, *47*, 1081−1085.

(7) (a) Wipf, P.; Miller, C. P.; Venkatraman, S.; Fritch, P. C. *Tetrahedron Lett.* **1995**, *36*, 6395−6398. (b) Wipf, P.; Fritch, P. C. *Tetrahedron Lett.* **1994**, *35*, 5397−5400. (c) Lafargue, P.; Guenot, P.; Lellouche, J.-P. *Heterocycles* **1995**, *41*, 947−958.

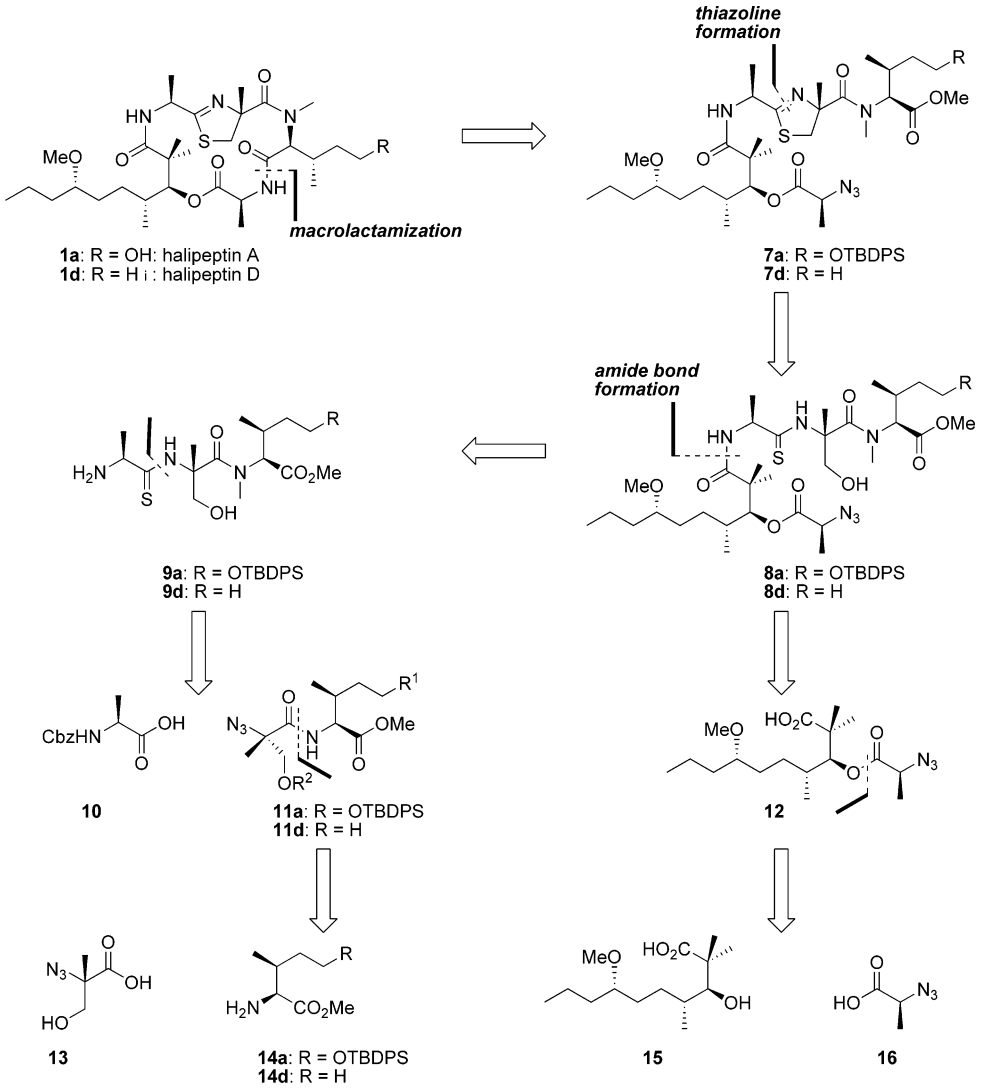


Figure 3. Second generation retrosynthetic analysis of halipeptins A and D (**1a**, **1d**).

as shown in Figure 2. Such an approach would not only allow easier entry into the larger (and therefore less strained) macrocycle of the required hydroxyl amide precursors, but would also give birth to a new series of designed molecules for testing, the oxazoline halipeptins. Our investigations along these lines have been reported elsewhere, and although they provided entry into a variety of oxazoline analogues of the halipeptins, they failed to deliver the natural products.[3]

A new strategy for the total synthesis of halipeptins A (**1a**) and D (**1d**) was, therefore, sought along the path depicted retrosynthetically in Figure 3.[5] Thus, retrolactamization of **1a** and **1d**, accompanied by functional group manipulations, led to azide methyl esters **7a** and **7d** as potential open-chain precursors (Figure 3). Subsequent dismantling of the thiazoline ring allowed the generation of hydroxy thioamides **8a** and **8d**, while disconnection at the indicated amide bond led to the hydroxy amino esters **9a** and **9d** and carboxy azido ester **12** as potential building blocks for these constructions (Figure 3). Further disconnection of **9a** and **9d** revealed L-alanine derivative **10** and dipeptide equivalents **11a** and **11d**, the latter two intermediates being traced to the simpler starting materials **13**, and **14a** and **14d**. A similar simplification of ester **12** as shown led to hydroxy acid **15** and D-alanine derivative **16** as the starting points for that construction (Figure 3). The strategy thus derived from this analysis had the advantages of convergence and late-stage installment of the thiazoline moiety thought to be necessary for avoiding early problems of epimerization of the center α to it.[8]

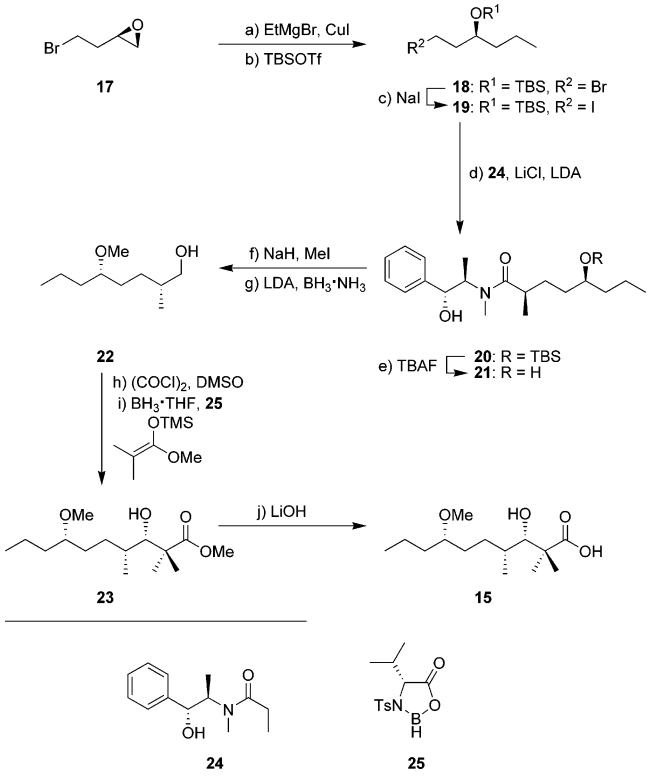
Construction of Building Blocks. Following our first synthesis[3] of the hydroxy decanoic acid fragment **15**,[6,9] we developed a second generation synthesis to this building block starting from commercially available bromo epoxide **17**[10] as shown in Scheme 1. Thus, regioselective opening of the epoxide ring within **17** with EtMgBr in the presence of CuI gave the corresponding bromo alcohol in 99% yield, whose exposure to TBSOTf and 2,6-lutidine furnished TBS-protected compound **18** (99% yield). Displacement of the bromide residue from the latter compound (**18**) with NaI resulted in the quantitative formation of the desired iodide **19**, which was used to alkylate the Myers auxiliary **24** (*n*BuLi-*i*Pr$_2$NH, LiCl, THF, −78 → 25 °C),[11]

(8) (a) Hirotsu, Y.; Shiba, T.; Kaneko, T. *Bull. Chem. Soc. Jpn.* **1970**, *43*, 1870−1873. (b) Wipf, P.; Fritch, P. C. *Tetrahedron Lett.* **1994**, *35*, 5397−5400.

(9) For alternative syntheses of this decanoic acid, see: Della Monica, C.; Maulucci, N.; De Riccardis, F.; Izzo, I. *Tetrahedron: Asymmetry* **2003**, *14*, 3371−3378 and ref 6.

(10) (*R*)-4-Bromo-1,2-epoxybutane (**17**) is commercially available from "Synthon Chiragenics Corporation", 7 Deer Park Drive, Monmouth Junction, NJ 08852.

Scheme 1. Second Generation Synthesis of Hydroxydecanoic Acid Building Block (**15**)[a]

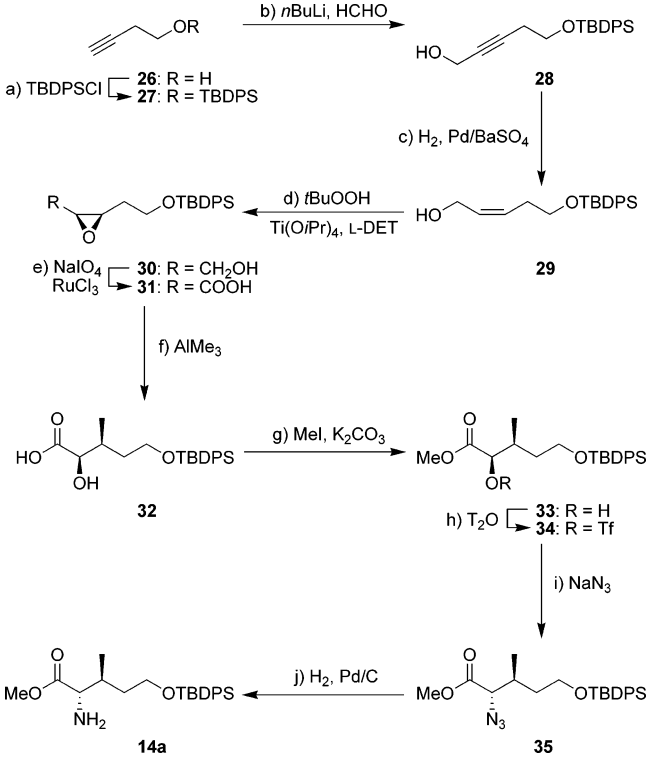


[a] (a) EtMgBr (1.0 M in THF, 2.0 equiv), CuI (1.1 equiv), THF, 0 °C, 30 min, 99%; (b) TBSOTf (1.5 equiv), 2,6-lutidine (2.0 equiv), ether, 0 °C, 15 min, 99%; (c) NaI (2.0 equiv), acetone, 25 °C, 12 h, 99%; (d) (i) *n*BuLi (2.5 M in hexanes, 4.0 equiv), LiCl (10.0 equiv), *i*Pr_2NH (4.2 equiv), THF, −78 °C, 15 min; (ii) **24** (2.0 equiv) −78 → 25 °C, 19 h, 66% (12:1); (e) TBAF (1.0 M in THF, 4.0 equiv), THF, 25 °C, 4 h, 99%; (f) NaH (60% suspension in oil, 3.0 equiv), MeI (4.0 equiv), THF, 25 °C, 12 h, 95%; (g) *n*BuLi (2.5 M in hexanes, 4.0 equiv), *i*Pr_2NH (4.0 equiv), $BH_3{\cdot}NH_3$ (4.0 equiv), THF, −78 → −25 °C, 16 h, 82%; (h) $(COCl)_2$ (2.0 equiv), DMSO (3.0 equiv), CH_2Cl_2, −78 °C; then Et_3N (4.0 equiv), −78 °C, 3 h; (i) **25** (1.0 equiv), $Me_2C{=}C(OMe)OTMS$ (1.2 equiv), CH_2Cl_2, −78 °C, 1.5 h, 89%; (j) $LiOH{\cdot}H_2O$ (6.0 equiv), $MeOH/H_2O$ (4:1), 25 °C, 20 h, 99%. TBAF = tetra-*n*-butylammonium fluoride; TBS = *tert*-butylsilyl; TMS = trimethylsilyl.; Tf = trifluoromethanesulfonyl.

leading to hydroxy amide **20** in 66% yield and 12:1 diastereoselectivity (determined by ^1H NMR spectroscopic analysis). Treatment of **20** with TBAF, followed by methylation (NaH, MeI) of the free hydroxyl groups within the resulting diol (**21**, 99% yield), afforded the corresponding bis-methoxy compound, which was cleaved to afford alcohol **22** (*n*BuLi-*i*Pr_2NH, $BH_3{\cdot}NH_3$, 82% yield). The latter compound (**22**) was oxidized under Swern conditions ($(COCl)_2$−DMSO, Et_3N), leading to the corresponding aldehyde in good yield. Mukaiyama aldol reaction of the resulting aldehyde in the presence of the boron compound **25** yielded hydroxy ester **23** in 89% yield and ca. 95:5 diastereoselectivity.[12] Finally, LiOH-induced ester hydrolysis of **23** led to the targeted decanoic hydroxy acid derivative **15** in quantitative yield. This 10-step synthesis of **15** (44% overall yield) is our preferred route to this intermediate.

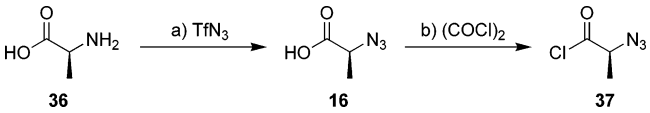
The next intermediate to be targeted was the hydroxyisoleucine derivative **14a** (Scheme 2).[6,13] Its construction began with

Scheme 2. Construction of TBDPS-Protected δ-Hydroxy-L-isoleucine Methyl Ester (**14a**)[a]



[a] (a) TBDPSCl (1.1 equiv), imidazole (2.2 equiv), CH_2Cl_2, 25 °C, 16 h, 99%; (b) *n*BuLi (2.5 M in hexanes, 1.05 equiv), *p*-formaldehyde (1.1 equiv), ether, −78 → 25 °C, 14 h, 72%; (c) H_2 (1 atm), 5% $Pd/BaSO_4$ (7 wt %), quinoline (5 wt %), MeOH, 25 °C, 20 min, 98%; (d) $Ti(OiPr)_4$ (0.3 equiv), L-DET (0.35 equiv), *t*BuOOH (2.0 equiv), MS (4 Å), CH_2Cl_2, 20 °C, 17 h, 99%, 92% ee (Mosher ester); (e) $NaIO_4$ (4.1 equiv), $RuCl_3{\cdot}H_2O$ (2.2 mol %), $CCl_4/CH_3CN/H_2O$ (1:1:1.5), 25 °C, 2 h, 80%; (f) $AlMe_3$ (3.0 equiv), hexanes, 25 °C, 30 h, 80%; (g) MeI (1.3 equiv), K_2CO_3 (1.1 equiv), acetone, 25 °C, 18 h, 90%; (h) Tf_2O (1.5 equiv), pyridine (10.0 equiv), CH_2Cl_2, 0 °C, 20 min; (i) NaN_3 (1.5 equiv), DMF, 25 °C, 40 min, 75% (two steps); (j) H_2 (1 atm), 10% Pd/C (30 wt %), EtOH, 25 °C, 2.5 h, 99%. DET = diethyl tartrate; DMF = *N*,*N*-dimethylformamide; MS = molecular sieves; TBDPS = *tert*-butyldiphenylsilyl; Tf = trifluoromethanesulfonyl.

Scheme 3. Preparation of D-Alanine Equivalent **37**[a]



[a] (a) TfN_3 (3.0 equiv), $CuSO_4{\cdot}5H_2O$ (0.5 equiv), Et_3N (4.0 equiv), MeOH, H_2O, CH_2Cl_2, 25 °C, 16 h, 80%; (b) $(COCl)_2$ (1.0 equiv), DMF, 25 °C, 30 min. DMF = *N*,*N*-dimethylformamide; Tf = trifluoromethanesulfonyl.

homopropargylic alcohol **26**, which was protected as a TBDPS ether (TBDPSCl, imid., 99% yield) and then converted to propargyl alcohol derivative **28** by reaction with *n*BuLi and *p*-formaldehyde (72% yield). The acetylenic bond within compound **28** was then selectively reduced to afford cis olefin **29** through the action of hydrogen in the presence of 5% $Pd/BaSO_4$ and quinoline (98% yield).[14] Sharpless asymmetric epoxidation [$Ti(iPrO)_4$, L-DET, *t*BuOOH] of the latter compound (**29**) gave hydroxy epoxide **30** in 99% yield and 92% ee.[14] (This enantioriched compound was curried through until the coupling with

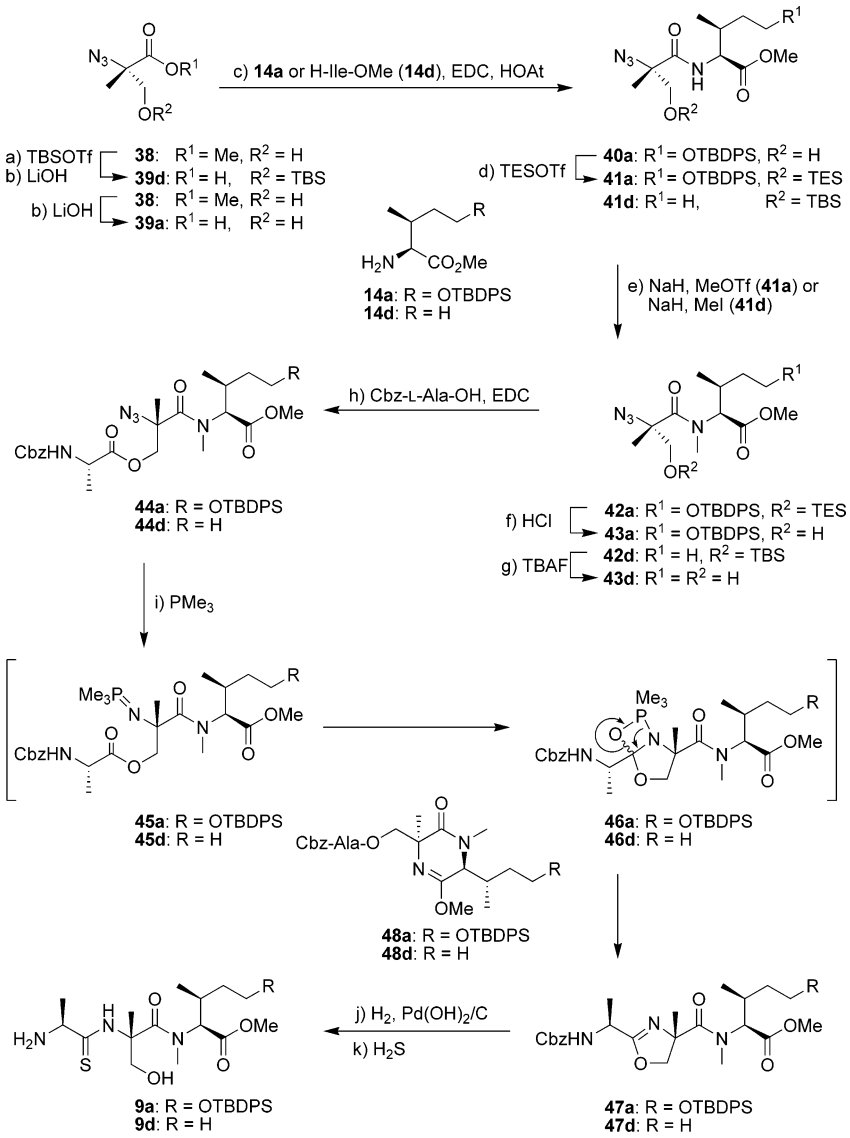
(11) (a) Myers, A. G.; Yang, B. H.; Chen, H.; Gleason, J. L. *J. Am. Chem. Soc.* **1994**, *116*, 9361−9362. (b) Myers, A. G.; Yang, B. H.; Chen, H.; McKinstry, L.; Kopecky, D. J.; Gleason, J. L. *J. Am. Chem. Soc.* **1997**, *119*, 6496−6511.

(12) Kiyooka, S.-I.; Kaneko, Y.; Komura, M.; Matsuo, H.; Nakano, M. *J. Org. Chem.* **1991**, *56*, 2276−2278.

(13) For other syntheses of this building block, see: (a) Izzo, I.; Avallone, E.; Della Corte, L.; Maulucci, N.; De Riccardis, F. *Tetrahedron: Asymmetry* **2004**, *15*, 1181−1186. (b) Hara, S.; Makino, K.; Hamada, Y. *Tetrahedron* **2004**, *60*, 8031−8035.

(14) For these steps, see: Still, W. C.; Ohmizu, H. *J. Org. Chem.* **1981**, *46*, 5242−5244.

Scheme 4. Synthesis of Thioamides **9a** and **9d**[a]



[a] (a) TBSOTf (2.0 equiv), 2,6-lutidine (4.0 equiv), CH_2Cl_2, 0 °C, 1.5 h, 98%; (b) $LiOH·H_2O$ (3.0 equiv), MeOH/H_2O (4:1), 0 → 25 °C, 2 h, 100%; (c) **14a** (1.0 equiv), EDC (1.2 equiv), HOAt (1.2 equiv), iPr$_2$NEt (3.0 equiv), CH_2Cl_2, 25 °C, 17 h, 55% (**38a**); **14d** (2.0 equiv), EDC (2.0 equiv), HOAt (2.0 equiv), iPr$_2$NEt (3.0 equiv), CH_2Cl_2, 25 °C, 17 h, 91% (**40d**); (d) TESOTf (1.3 equiv), 2,6-lutidine (2.0 equiv), CH_2Cl_2, 0 °C, 20 min, 85%; (e) NaH (4.0 equiv), MeOTf (2.5 equiv), THF, 0 °C, 1 h, 83% (**41a**); NaH (3.0 equiv), MeI (4.0 equiv), DMF, 0 °C, 1 h, 96% (**41d**); (f) aqueous HCl (1 N; 20 equiv), THF, 25 °C, 1 h, 100%; (g) TBAF (3.0 equiv), THF, 25 °C, 2 h, 98%; (h) EDC (4.0 equiv), Cbz-L-Ala-OH (4.0 equiv), CH_2Cl_2, 25 °C, 0.5 h; then **43a** or **43d**, 18 h, **44a**, 88%; **44d**, 96%; (i) PMe_3 (1.8 equiv), toluene, 25 °C, 2 h, **47a**, 79%; **47d**, 83%; (j) H_2 (1 atm), 20% $Pd(OH)_2$/C (30 wt %), EtOH, 25 °C, 1 h, 100%; (k) H_2S (excess), MeOH/Et_3N (2:1), 25 °C, 48−72 h, complete conversion, product not isolated. Cbz = benzyloxycarbonyl; CDI = carbonyldiimidazole; EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; HOAt = 1-hydroxy-7-azabenzotriazole; TBAF = tetra-*n*-butylammonium fluoride; TBS = *tert*-butyldimethylsilyl; TES = triethylsilyl.

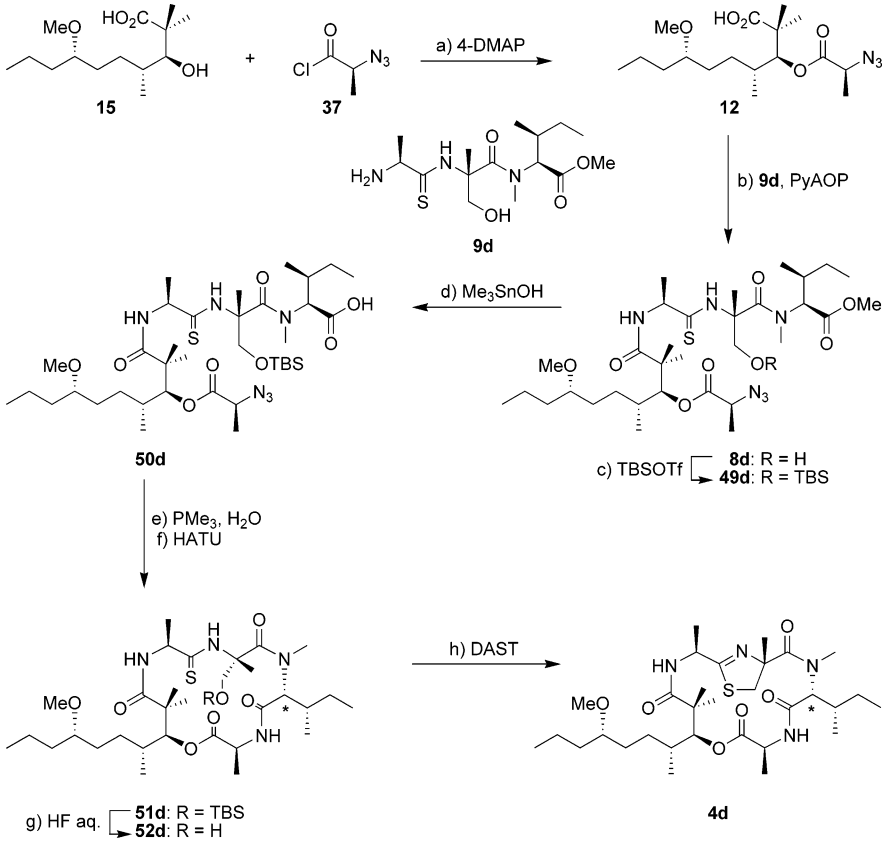
enantiomerically pure **39a** at which point the pure diastereomer **40a** was separated by chromatography, see below). Oxidation of hydroxy epoxide **30** with $NaIO_4$ in the presence of catalytic amounts of $RuCl_3$ furnished carboxylic acid **31** whose reaction with $AlMe_3$ proved both regioselective and efficient, leading to hydroxy acid **32** (80% yield) into which the required methyl group had been incorporated in a stereoselective manner.[14] Treatment of hydroxy acid **32** with near stoichiometric amounts of K_2CO_3 and MeI resulted in the formation of hydroxy methyl ester **33** in 90% yield. Triflate formation (Tf_2O, py) followed by reaction of the resulting triflate (**34**) with NaN_3 led to azide **35** in 75% overall yield for the two steps. With its inverted stereochemistry, azide ester **35** served well as a precursor to the desired amino ester **14a** upon reduction with hydrogen in the presence of 10% Pd/C (99% yield).

Acid chloride **37**, an L-alanine equivalent, was prepared by the method of Wong et al.[15] as shown in Scheme 3. Thus, reaction of L-alanine (**36**) with TfN_3 in the presence of $CuSO_4·6H_2O$ and Et_3N resulted in the formation of azide **16** (with retention of stereochemistry) in 80% yield. Treatment of the latter compound (**16**) with $(COCl)_2$ in DMF furnished acid chloride **37** in high yield, an acylating agent used directly and without purification in the subsequent coupling (vide infra).

Assembly of Building Blocks into Halipeptins A and D. We begin the description of the building block assembly with the construction of alanine-α-methylserine-*N*-methylisoleucine

(15) Formed in situ by reaction of oxalyl chloride with the corresponding acid: (a) Alper, P. B.; Hung, S.-C.; Wong, C.-H. *Tetrahedron Lett.* **1996**, *37*, 6029−6032. (b) Lundquist, J. T.; Pelletier, J. C. *Org. Lett.* **2001**, *3*, 781−783.

Scheme 5. Synthesis of epi-isoleucine Halipeptin D Stereoisomer **4d**[a]



[a] (a) **37** (10.0 equiv), 4-DMAP (0.5 equiv), Et_3N (12 equiv), DMF, 50 °C, 2 h, 94%; (b) **9d** (2.0 equiv), PyAOP (2.5 equiv), iPr$_2$NEt (2.5 equiv), DMF, 25 °C, 17 h, 92%; (c) TBSOTf (1.2 equiv), 2,6-lutidine (1.5 equiv), CH_2Cl_2, 0 °C, 0.5 h, 99%; (d) Me_3SnOH (20 equiv), 1,2-dichloroethane, 80 °C, 48 h, 52% at 79% conversion; (e) PMe_3 (3.0 equiv), THF/H_2O (9:1), 25 °C, 48 h; (f) HATU (2.0 equiv), HOAt (2.0 equiv), iPr$_2$NEt (2.0 equiv), CH_2Cl_2, 25 °C, 18 h, 38% (two steps); (g) 50% aqueous HF/CH_3CN (1:99 by volume), 25 °C, 24 h, 100%; (h) DAST (2.0 equiv), CH_2Cl_2, −78 → 20 °C, 2 h, 80%. DAST = diethylaminosulfurtrifluoride; 4-DMAP = 4-(dimethylamino)pyridine; DMF = *N*,*N*-dimethylformamide; HATU = *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HOAt = 1-hydroxy-7-azabenzotriazole; PyAOP = (7-azabenzotriazol-1-yloxy) tripyrrolidinophosphonium hexafluorophosphate; TBS = *tert*-butyldimethylsilyl.

tripeptides **44a** and **44d** (Scheme 4). Our initial forays to these tripeptide fragments were met with failure due to the propensity of the intermediate dipeptides to undergo intramolecular bridging to diketopiperazines. We, therefore, adopted an alternative approach to these tripeptide units that did not involve a free amino group as part of the growing molecule as depicted in Scheme 4. Thus, the previously known α-hydroxy-α-methyl-serine equivalent **38**[16] was silylated (TBSOTf, 2,6 lut., 98% yield) and the resulting TBS ether methyl ester was hydrolyzed (LiOH, 100% yield) to afford carboxylic acid **39a**. This α-methyl-serine equivalent was then coupled to the δ-hydroxy isoleucine methyl ester derivative **14a**[6,13] (EDC, HOAt, 55% yield) to furnish dipeptide **40a**. TES protection (TESOTf, 2,6 lut., 85% yield) of the hydroxyl group of the latter compound (**40a**) allowed *N*-methylation (NaH, MeOTf, 83% yield) of its amide moiety through the intermediacy of the bis-silyl ether **41a** to afford compound **42a**. Exposure of the latter substance to 1 N aqueous HCl in THF then led to selective mono-desilylation of the latter compound (**42a**) to give the desired hydroxy azido fragment **43a** required for the total synthesis of halipeptin A (**1a**). The hydroxy azido fragment **43d** required for the construction of halipeptin D (**1d**) was prepared by TBAF-induced desilylation (98% yield) of the TBS ether **42d**, whose synthesis has been described elsewhere.[3]

Each of the hydroxy azido dipeptides thus obtained (**43a** and **43d**) was converted to the corresponding ester by coupling with Cbz-L-Ala-OH (EDC, **44a**, 88% yield; **44d**, 96% yield) as a prelude to the tripeptide construction. It was anticipated that reaction of the azide group with a phosphine would result in azaylid formation (i.e., **45a** and **45d**) and subsequent addition to the nearest grouping, an occurrence that was expected to lead to the desired oxazolines (**47a** and **47d**) by expulsion of phosphine oxide from the corresponding four-membered ring phosphine derivatives (i.e., **46a** and **46d**) as shown in Scheme 4.[17]

Much to our delight, this cascade sequence was realized in the laboratory by treating **44a** or **44d** with PMe_3 in benzene or toluene solution at room temperature. The coveted oxazolines **47a** and **47d** were obtained in 79% and 83% yield, respectively, accompanied only by small amounts of the undesired dihydropyrazinones **48a** (17%) and **48d** (16%), respectively. The latter compounds were presumably formed upon hydrolysis

(16) (a) Avenoza, A.; Cativiela, C.; Corzana, F.; Peregrina, J. M.; Sucunza, D.; Zurbano, M. M. *Tetrahedron: Asymmetry* **2001**, *12*, 949−958. (b) Smith, N. D.; Goodman, M. *Org. Lett.* **2003**, *5*, 1035−1038.

(17) For previous examples of oxazoline construction through aza-Wittig cyclization, see: (a) Gololobov, Yu. G.; Gusar, N. I.; Chaus, M. P. *Tetrahedron* **1985**, *41*, 793−799. (b) Mulzer, J.; Meier, A.; Buschmann, J.; Luger, P. *Synthesis* **1996**, 123−132. (c) Kato, H.; Ohmori, K.; Suzuki, K. *Synlett* **2001**, 1003−1005. For thiazoline formations with triphenylphosphine, see: (d) Kok, G. B.; Campbell, M.; Mackey, B.; von Itzstein, M. *J. Chem. Soc., Perkin Trans. 1* **1996**, *23*, 2811−2815. (e) Chen, J.; Forsyth, C. J. *Org. Lett.* **2003**, *5*, 1281−1284. (f) Chen, J.; Forsyth, C. J. *J. Am. Chem. Soc.* **2003**, *125*, 8734−8735.

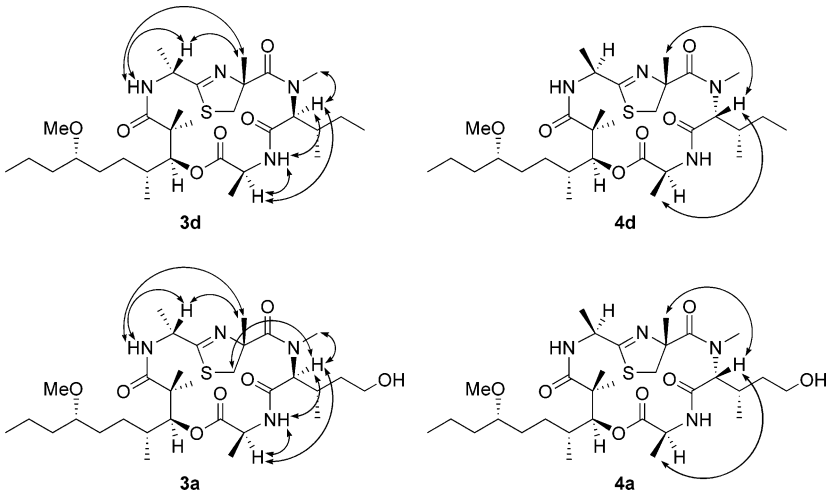
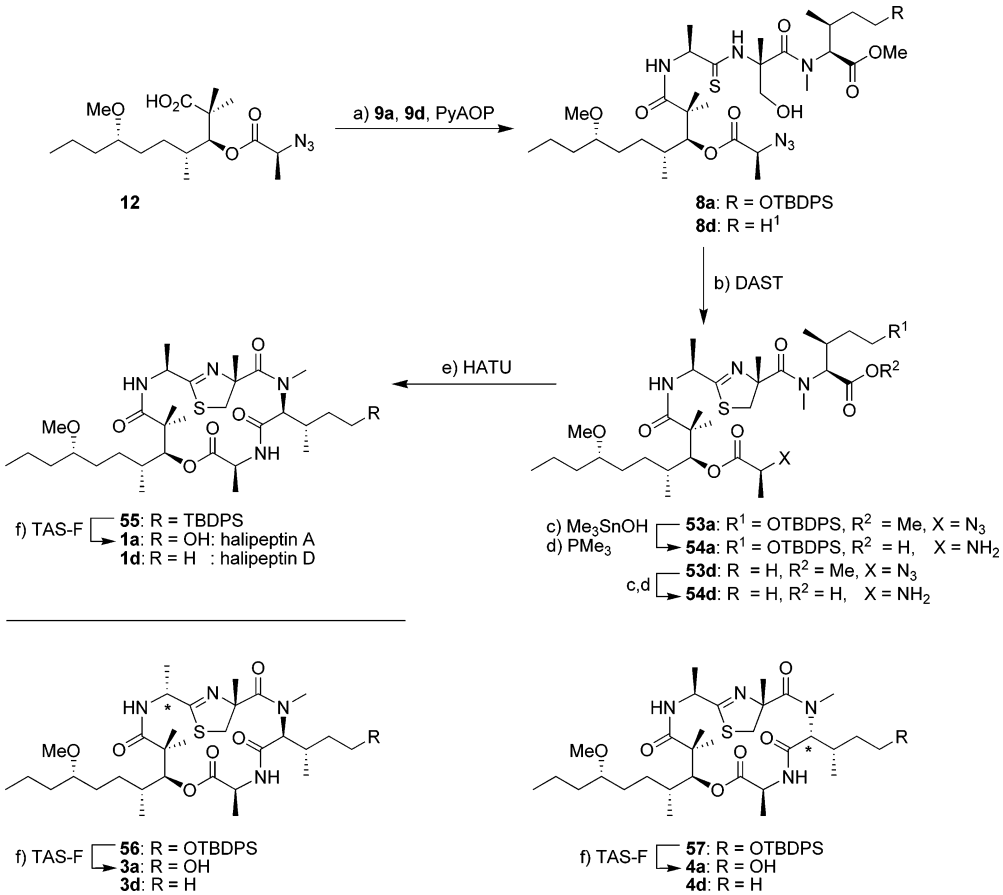


Figure 4. Selected nOe correlations for halipeptin A and D analogues **3a**, **3d**, and **4a**, **4d**.

Scheme 6. Completion of the Total Synthesis of Halipeptins A (**1a**) and D (**1d**) and Their Analogues **3a**, **3d**, **4a**, and **4d**[a]

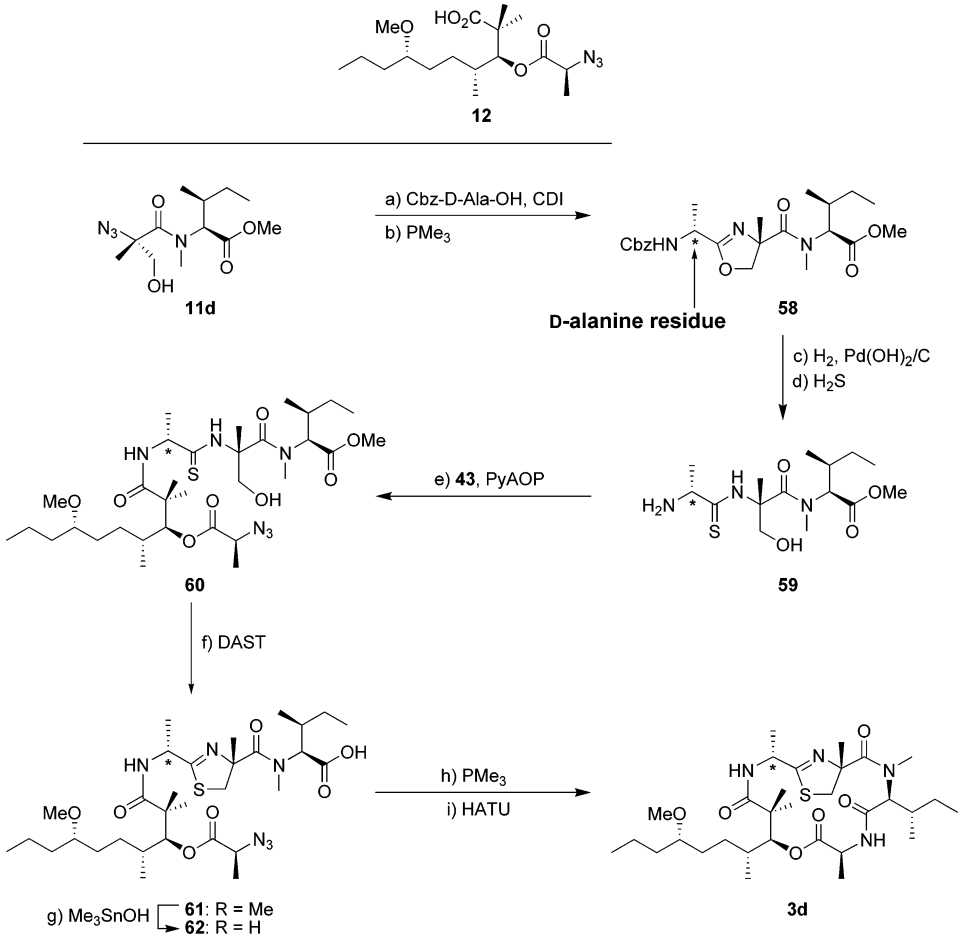


[a] (a) **9a** (1.0 equiv), PyAOP (1.5 equiv), *i*Pr_2NEt (2.0 equiv), DMF, 25 °C, 17 h, 71%; (b) DAST (1.5 equiv), CH_2Cl_2, −78 → 20 °C, 1 h, **53a**, 85%; **53d**, 84%; (c) Me_3SnOH (20 equiv), 1,2-dichloroethane, 90 °C, 48−72 h, 82% from **53a**; 85% from **53d**; (d) PMe_3 (2.5 equiv), THF/H_2O (9:1), 25 °C, 2 h; (e) HATU (2.0 equiv), HOAt (2.0 equiv), K_2CO_3 (10.0 equiv), CH_2Cl_2, 25 °C, 24 h, **55**, 31%; **56**, 10%; **57**, 14%; **1d**, 25%; **3d**, 5%; **4d**, 18%; (f) TAS-F (5.0 equiv), DMF, 25 °C, 24 h, **1a**, 85%; **3a**, 81%; **4a**, 85%. DAST = diethylaminosulfurtrifluoride; DMF = *N*,*N*-dimethylformamide; HATU = *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HOAt = 1-hydroxy-7-azabenzotriazole; PyAOP = (7-azabenzotriazol-1-yloxy) tripyrrolidinophosphonium hexafluorophosphate; TBDPS = *tert*-butyldiphenylsilyl; TAS-F = tris-(dimethylamino)sulfur-(trimethylsilyl)difluoride; Tf = trifluoromethanesulfonyl.

of the aza-ylids by traces of water leading to the corresponding amines, which then engaged the proximal methyl ester moieties in ring formation. Chromatographic purification of **47a** and **47d** then allowed their efficient conversion to thioamides **9a** and **9d**, respectively, by a two-step sequence involving hydrogenolysis of the Cbz group [H_2, 10% $Pd(OH)_2$/C, 100% yield] and subsequent thiolysis of their oxazoline rings (H_2S, Et_3N)[7a] as shown in Scheme 4. The obtained crude products **9a** and **9d** were employed in the subsequent step without further purification.

Our first attempt to synthesize halipeptin D (**1a**) led, rather unexpectedly, to epi-isoleucine halipeptin D (**4d**) as shown in Scheme 5.

Scheme 7. Incorporation of D-Alanine into the Total Synthesis of Halipeptin **3d**[a]



[a] (a) CDI (4.0 equiv), Cbz-D-Ala-OH (4.0 equiv), CH_2Cl_2, 25 °C, 18 h, 69%; (b) PMe_3 (1.8 equiv), toluene, 25 °C, 2 h, 80%; (c) H_2 (1 atm), 10% $Pd(OH)_2$/C (30 wt %), EtOH, 25 °C, 1 h, 99%; (d) H_2S (excess), MeOH/Et_3N (2:1), 25 °C, 72 h; (e) **43** (1.0 equiv), PyAOP (1.5 equiv), *i*Pr_2NEt (2.0 equiv), DMF, 25 °C, 18 h, 63%; (f) DAST (1.5 equiv), CH_2Cl_2, −78 → 20 °C, 1 h, 72%; (g) Me_3SnOH (20 equiv), 1,2-dichloroethane, 90 °C, 72 h; (h) PMe_3 (2.5 equiv), THF/H_2O (9:1), 25 °C, 2 h; (i) HATU (2.0 equiv), HOAt (2.0 equiv), *i*Pr_2NEt (6.0 equiv), CH_2Cl_2, 25 °C, 24 h, 59%. Cbz = benzyloxycarbonyl; CDI = carbonyldiimidazole; DAST = diethylaminosulfurtrifluoride; DMF = *N*,*N*-dimethylformamide; EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; HATU = *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HOAt = 1-hydroxy-7-azabenzotriazole; PyAOP = (7-azabenzotriazol-1-yloxy) tripyrrolidinophosphonium hexafluorophosphate.

Table 1. Cytotoxicity (IC_{50}, μM)[a] of Synthetic Halipeptins A (**1a**) and D (**1d**) and Their Analogues (**3a**, **3d**, **4a**, **4d**, **5d**, and **6d**)

	1a	1d	3a	3d	4a	4d	5d	6d
HCT-116[b]	>200	32.5	>200	105.4	>200	111.4	>200	58.0

[a] The concentration at which the compound inhibits 50% of the cell growth. [b] Human colon carcinoma cell line.

Fearing epimerization of the stereocenter adjacent to the thiazoline moiety, we followed a strategy that called for late stage construction of the thiazoline ring.[8] Thus, treatment of hydroxy acid fragment **15** with excess azido chloride **37** (prepared in situ from the corresponding acid and oxalyl chloride)[15] in the presence of Et_3N and 4-DMAP yielded the sterically crowded ester **12** in 94% yield. That no epimerization had occurred during this coupling reaction at the azide-bearing site, despite the basic conditions employed, was confirmed by synthesizing the corresponding epimeric material through a similar sequence utilizing **15** and ***ent*-37**.

With both fragments **12** and **9d** in hand, we were in a position to assemble the entire chain needed for the macrocycle construction. The coupling of these two fragments was best achieved through the action of PyAOP in the presence of *i*Pr_2NEt, leading to advanced intermediate **8d** in 92% yield. Protection of the hydroxyl group (TBSOTf, 2,6 lut., 99% yield) within the latter intermediate then produced TBS ether **49d**, whose Me_3SnOH-induced methyl ester cleavage led to carboxylic acid azide **50d** in 52% yield (79% conversion).[18] The following two steps were carried out without purification of the intermediate amino acid. Thus, the Staudinger reduction of carboxylic acid azide **50d** with PMe_3 in THF:H_2O (9:1) was followed by macrolactamization employing HATU, HOAt, and *i*Pr_2NEt in CH_2Cl_2 (ambient temperature) to afford macrocycle **51d** in 38% overall yield for the two steps. Removal of the TBS group from the latter compound (**51d**) was smoothly and quantitatively achieved by exposure to 50% aqueous HF in CH_3CN, leading to hydroxy thioamide macrocycle **52d**. However,

(18) (a) Nicolaou, K. C.; Estrada, A. A.; Zak, M.; Lee, S. H.; Safina, B. S. *Angew. Chem., Int. Ed.* **2005**, *44*, 1378−1382. (b) Furlan, R. L. E.; Mata, E. G.; Mascaretti, O. A. *J. Chem. Soc., Perkin Trans. 1* **1998**, 355−358. (c) Furlan, R. L. E.; Mata, E. G.; Mascaretti, O. A.; Pena, C.; Coba, M. P. *Tetrahedron* **1998**, *54*, 13023−13034. (d) Furlan, R. L. E.; Mata, E. G.; Mascaretti, O. A. *Tetrahedron Lett.* **1996**, *37*, 5229−5232.

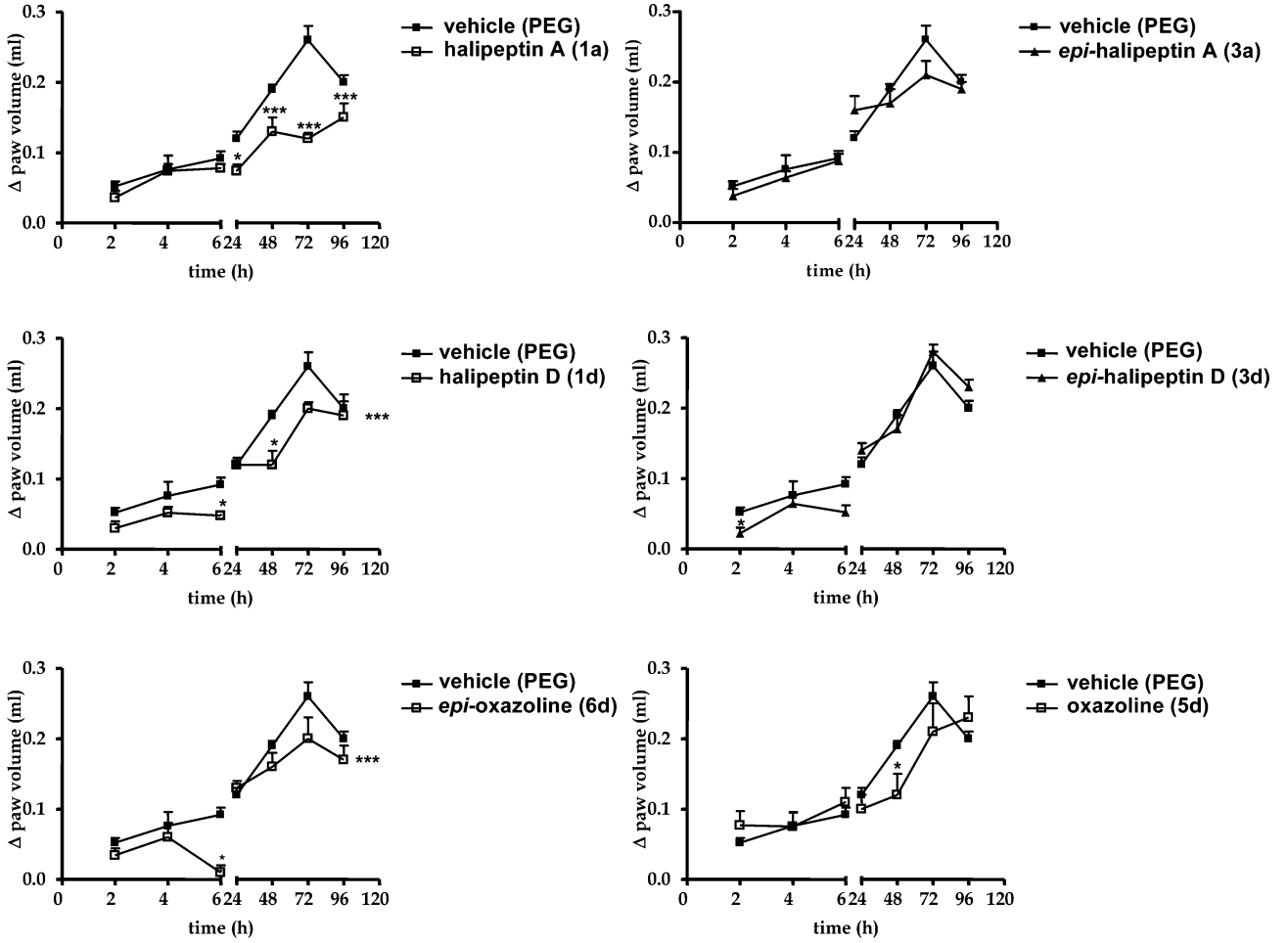


Figure 5. Dose-dependent inhibition on the first (0−6 h) and second phases (24−96 h) of carrageenan-induced paw edema by synthetic halipeptins (**1a**, **1d**, **3a**, **3d**, **5d**, and **6d**). Data are expressed as mean ± s.e. mean and analyzed by using ANOVA for multiple comparisons followed by Dunnett's test. Statistical significance is set at $p < 0.05$.

although the performance of DAST in the final step to prepare the targeted halipeptin D (**1d**) was admirable in terms of furnishing (80% yield) the desired thiazoline moiety,[19] this route failed in reaching its goal, yielding instead epi-isoleucine halipeptin D (**4d**) as indicated in Scheme 5. Apparently, somewhere along the line, most likely at the macrocyclization step, the conditions employed caused epimerization at the indicated (*) site, a fact supported by NMR spectroscopic studies of **4d** (^{1}H, ^{13}C, ROESY, see Figure 4). This outcome, a consequence of the sensitivity of these molecules toward epimerization, forced upon us a change in strategy and tactics toward halipeptins D (**1d**) and A (**1a**), as will be described below.

Our revised strategy for the total synthesis of halipeptins A (**1a**) and D (**1d**) and their analogues improvising for thiazoline construction prior to macrocyclization is depicted in Scheme 6. Thus, coupling of the sterically congested ester **12** with amino fragment **9a** or **9d** (see Scheme 4 for their preparation) under the influence of PyAOP and *i*Pr$_2$NEt led to thiamides **8a** (71% yield) and **8d** (92% yield), respectively. Reaction of these intermediates with DAST in CH_2Cl_2 at −78 °C resulted in smooth thiazoline formation[19] (**53a**, 82% yield; **53d**, 85% yield). The methyl ester group of the resulting compounds (**53a**, **53d**) was then cleaved by treatment with Me_3SnOH, leading to the corresponding carboxylic acid azides (82%, 85%, respectively),[18] which were reduced under Staudinger conditions [PMe_3, THF: H_2O (9:1)] to obtain the crude amino acids **54a** and **54d**, ready for macrocyclization. Our first attempts to cyclize the amino acids under the same conditions as before (i.e., HATU, HOAt, *i*Pr$_2$NEt) led to the formation of the same epimerized halipeptin D (**4d**) in 17% overall yield from the corresponding azido carboxylic acid. This time, however, **4d** was accompanied by halipeptin D (**1d**) and analogue **3d** (epimer at the indicated (*) site adjacent to the thiazoline ring, ROESY analysis, see Figure 4), which were formed in 13% and 5% yield, respectively, from the corresponding azido carboxylic acid. Switching the base from *i*Pr$_2$NEt to K_2CO_3 in the macrolactamization step resulted in an increase of both the yield and the preference for halipeptin D (**1d**) to 25%, with **3d** and **4d** being formed in 5% and 18% yield, respectively. These isomeric halipeptins D could easily be separated by preparative thin-layer chromatography (PTLC, silica gel) due to the rather large differences in their R_f values.

In a similar way, the HATU/HOAt/K_2CO_3 macrolactamization protocol was employed to produce the TBDPS derivative of halipeptin A (**55**) from amino acid **54a** in 31% yield, together with its epimers **56** (10% yield) and **57** (14% yield) after separation with PTLC (Scheme 6). Treatment of these derivatives with TAS-F generated halipeptin D (**1d**, 86% yield) and

(19) Lafargue, P.; Guenot, P.; Lellouche, J.-P. *Heterocycles* **1995**, *41*, 947−958.

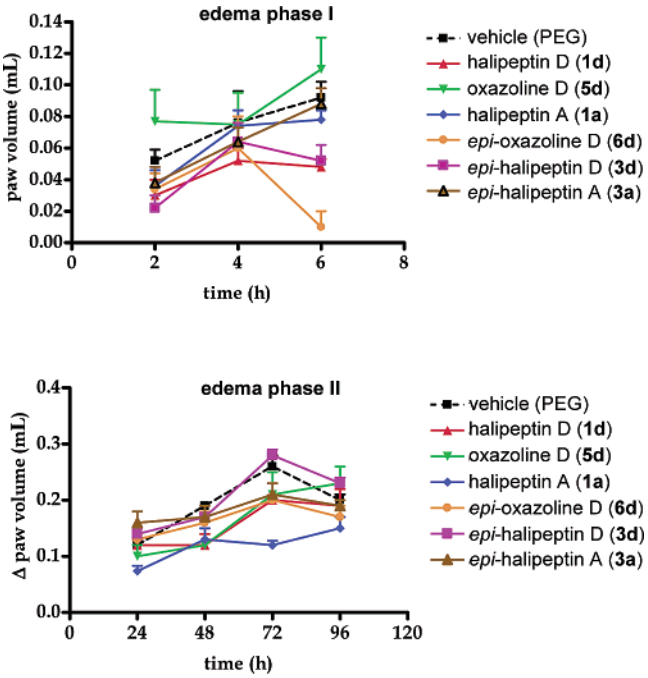


Figure 6. Dose-dependent inhibition of phase I (0−6 h) and phase II (24−96 h) of carrageenan-induced paw edema by synthetic halipeptins (**1a**, **1d**, **3a**, **3d**, **5d**, and **6d**). Data are expressed as mean ± s.e. mean and analyzed by using ANOVA for multiple comparisons followed by Dunnett's test. Statistical significance is set at $p < 0.05$.

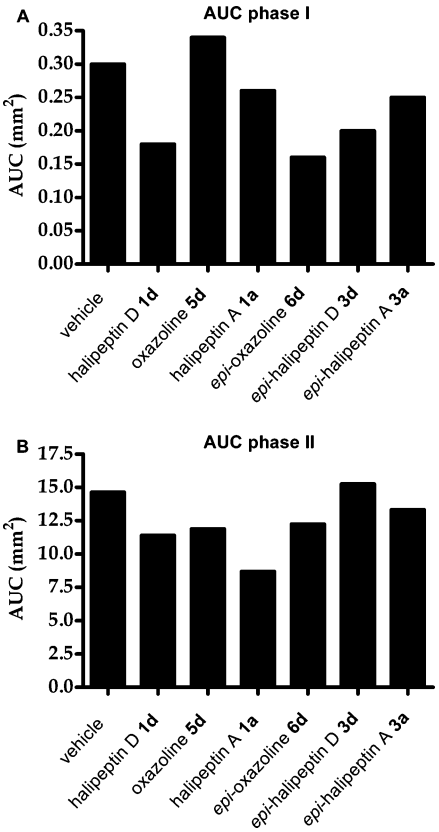


Figure 7. Inhibition of carrageenan-induced paw edema by synthetic halipeptins (**1a**, **1d**, **3a**, **3d**, **5d**, and **6d**). Data are expressed as area under the curve (AUC) for phase I (A) and phase II (B) edema.

its epimers **3a** (81% yield, ROESY analysis, see Figure 4) and **4a** (85% yield, ROESY analysis, see Figure 4).

At this juncture, we became curious whether the unnatural halipeptin D **3d** containing the thiazoline-bound D-alanine residue was more stable than the naturally occurring substance halipeptin D (**1d**) and, therefore, set out to synthesize it. Scheme 7 summarizes this endeavor, which proceeded along the same lines as before and in similar yields, except for the macrolactamization step that yielded only the unnatural halipeptin D (**3d**), and in good yield (59% yield, two steps). The absence of any epimerized products at the end of this synthesis reflects the higher stability of this molecule (**3d**) relative to its natural isomer (**1d**) and ensures a relatively easy access to this unnatural analogue (**3d**).

Biological Evaluations of Synthetic Halipeptins A and D and Analogues

Cytotoxic Properties. As mentioned above, Faulkner and Manam reported that halipeptin D (**1d**) exhibited potent cytotoxic properties [$IC_{50} = 7$ nM against human colon cancer HCT-116 cell line and an average IC_{50} value of 420 nM against a BMS ODCP (oncology diverse cell panel) of tumor cell lines].[4] These results were in sharp contrast to those of Gomez-Paloma et al. for their halipeptins A−C (**1a**−**1c**), which apparently were devoid of any significant cytotoxicity properties.[1,2] We have also reported elsewhere that the oxazoline analogues of halipeptin D, compounds **5d** and **6d** (Figure 1), were found to have only weak cytotoxicity.[3] Intrigued by the previous findings and by the discrepancy between the results of the Gomez-Paloma group and Faulkner and Manam, we proceeded to test synthetic halipeptins A (**1a**) and D (**1d**), and their unnatural analogues, **3a**, **3d**, **4a**, and **4d**, for cytotoxic activity. As shown in Table 1, all of these synthetic halipeptins exhibited only weak cytotoxicity against tumor cells despite the previous reports regarding naturally derived halipeptin D (**1d**) by Faulkner and Manam.[3,4] These observations may be explained if we assume that naturally derived halipeptin D (**1d**) was contaminated by one or more potent contaminants, presumably coexisting within the marine species. Thus, we believe that the halipeptins are devoid of significant cytotoxicity, a fact that makes their anti-inflammatory properties even more meaningful and potentially useful.

Anti-inflammatory Properties. It was previously demonstrated that natural halipeptin A (**1a**) exhibited 60% reduction of carrageenan-induced paw edema in mice at the intraperitoneal dose of 0.3 mg kg^{-1} body weight.[1,2] The significance of this activity can be better appreciated when compared to those of the commercial agents, indomethacin and naproxen, which exhibit 40-fold and 130-fold lower potencies in the same test, respectively, than halipeptin A (**1a**) (ED_{50} 12 mg kg^{-1} for indomethacin, and 40 mg kg^{-1} for naproxen).[20] These findings prompted us to investigate the anti-inflammatory properties of our synthetic halipeptins (**1a**, **1d**, **3a**, **3d**, **5d**,[3] and **6d**,[3] Figure 1) using the same biological assay. As shown in Figure 5, administration of halipeptin A (**1a**, 1 mg kg^{-1}) resulted in reduction of the volume in the second phase of the mouse paw edema. More specifically, a significant inhibition occurred at 24 h ($*p < 0.05$), 48 h, and 72 h ($***p < 0.001$) as compared to the corresponding control time points. These results confirmed the original findings of the Gomez-Paloma group[1,2] regarding the anti-inflammatory activity of natural halipeptin A (**1a**). Synthetic halipeptin D (**1d**) exhibited an anti-inflammatory

(20) Calhoun, W.; Chang, J.; Carlson, R. P. *Agents Actions* **1987**, *21*, 306−309.

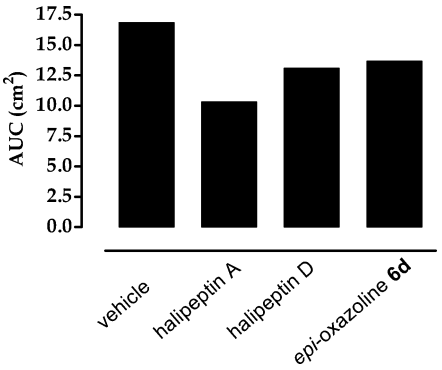


Figure 8. Inhibition of carrageenan-induced paw edema by halipeptin A (**1a**), halipeptin D (**1d**), and oxazoline analogue (**6d**). Data are expressed as area under the curve (AUC) for combined phase I and phase II edema.

action similar to that observed for halipeptin A (**1a**) on the second phase of the edema. In addition, halipeptin D (**1d**) significantly reduced the edema at the 6 h time point of the first phase (*$p < 0.05$), suggesting that its anti-inflammatory action was faster on onset as compared to that of halipeptin A (**1a**, Figure 5). The oxazoline analogue **6d** displayed an anti-inflammatory activity similar to that of halipeptin D (**1d**) in the second phase of the mouse paw edema. However, oxazoline **6d** displayed pronounced reduction of the mouse paw edema in the first phase at the 6 h time point (Figure 5). Most impressively, swelling was almost undetectable in the acute phase (phase I) of the edema (within the first 6 h). This was considerably more pronounced than those displayed by the natural halipeptins A (**1a**) and D (**1d**).

Figure 6 depicts, in color, the graphs for phase I and phase II of carrageenan-induced paw edema by the various synthetic halipeptins for easy comparison purposes. As shown in that figure, halipeptins **3a**, **3d**, and **5d** did not exhibit significant anti-inflammatory activity as compared to **1a**, **1d**, and **6d**.

Figure 7 shows the total reduction of edema for phase I (graph A) and phase II (graph B) as measured by the under the curve area (AUC) for synthetic halipeptins **1d**, **5d**, **1a**, **6d**, **3d**, and **3a**, demonstrating the potent early (phase I) effects of halipeptin D (**1d**) and its oxazoline counterpart (**6d**; the AUC for both compounds was significantly reduced as compared to vehicle ### $p < 0.001$) and the late effect (phase II) of halipeptin A (**1a**) (### $p < 0.001$).

The potent overall effect of halipeptins A (**1a**), D (**1d**), and epi-oxazoline halipeptin D (**6d**), as measured by the area under the curve (AUC), is demonstrated in Figure 8.

Conclusion

The described chemistry provides entries into the halipeptin family of compounds including the naturally occurring halipeptins A (**1a**) and D (**1d**), and their epimers **3a**, **3d** and **4a**, **4d**. By rendering these materials available, as well as their oxazoline analogues **5d**[3] and **6d**,[3] chemical synthesis allowed their biological evaluation in cytotoxicity and inflammation assays. The former investigations revealed no significant cytotoxicity for any of the tested synthetic halipeptins, including the naturally occurring halipeptins A (in line with previous reports)[1,2] and D (in contrast to previous reports).[4] To explain the discrepancy between synthetic and naturally derived halipeptin D (**1d**), we assumed contamination of the natural sample by potent cytotoxic impurity(ies) that may have been present in the original host of this natural product.

In contrast to the cytotoxicity assays, our anti-inflammatory tests involving carrageenan-derived mouse edema detected strong anti-inflammatory activity for both synthetic halipeptins A (**1a**, confirming the observation of Gomez-Paloma et al. with a natural sample)[1,2] and D (**1d**), as well as of oxazoline analogue **6d**.[3] The latter compound, in particular, exhibited remarkable edema reduction in both the first and the second phases of the condition, proving it to be a highly potent anti-inflammatory agent. In view of these promising results, further studies in the field may be warranted. These studies would include investigations directed toward the elucidation of the mechanism of action of the halipeptins and the design and synthesis of analogues that may prove useful as anti-inflammatory agents.

Acknowledgment. We thank Dr. D. H. Huang and Dr. G. Siuzdak for NMR spectroscopic and mass spectrometric assistance, respectively. Financial support for this work was provided by the National Institutes of Health, the CaPCURE Foundation, the Skaggs Institute for Chemical Biology, postdoctoral fellowships from the Ernst Schering Research Foundation (to D.S.) and the Royal Society-Fulbright Commission (to D.A.L.), and a graduate fellowship from the Portuguese Foundation for Science & Technology (to R.G.N.). R.G.N. is an exchange graduate student to TSRI from REQUIMTECQFB, Chemistry Department, FCT, Universidade Nova de Lisboa, Caparica, Portugal.

Supporting Information Available: Experimental procedures and compound characterization, and full citation for ref 4b. This material is available free of charge via the Internet at http://pubs.acs.org.

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