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Total Synthesis and Structural Confirmation of Chlorodysinosin A

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Abstract: The first enantiocontrolled total synthesis of the marine sponge metabolite chlorodysinosin A is described. The structure and absolute configuration are identical to those of dysinosin A except for the presence of a novel 2*S*,3*R*-3-chloroleucine residue in the former. A concise stereocontrolled synthesis of the new chlorine-containing amino acid fragment was developed. An X-ray cocrystal structure of synthetic chlorodysinosin A with the enzyme thrombin confirms the structure and configuration assignment achieved through total synthesis. Within the aeruginosin family of natural products, chlorodysinosin A is the most potent inhibitor of the serine proteases thrombin, factor VIIa, and factor Xa, which are critical enzymes in the process leading to platelet aggregation and fibrin mesh formation in humans.

The aeruginosins are a family of peptide natural products of aquatic origin characterized structurally by the presence of a substituted 2-carboxyperhydroindole core residue.[1] Most of the aeruginosins exhibit inhibitory activity against the serine protease thrombin, which is the penultimate enzyme in the blood coagulation cascade that converts fibrinogen into soluble fibrin monomers and activates the process of platelet aggregation.[2] A number of aeruginosins also display inhibitory activity against other coagulation enzyme factors vital for hemostasis.[3] The medicinal implications of identifying novel, potent, and selective serine protease inhibitors have fostered the development of efficient methodologies for the synthesis of the aeruginosin subunits as well as analogues for biological evaluation in our laboratories[4] and elsewhere.[5] To date, 21 members of the aeruginosin family have been isolated from freshwater algae[6] and marine sponges.[7] The total syntheses of six aeruginosins have been completed,[8−12] four of these involving revisions of the originally proposed structures.[13]

Within the aeruginosin family, the dysinosins represent a subset of structures isolated from the *Dysideidae* family of

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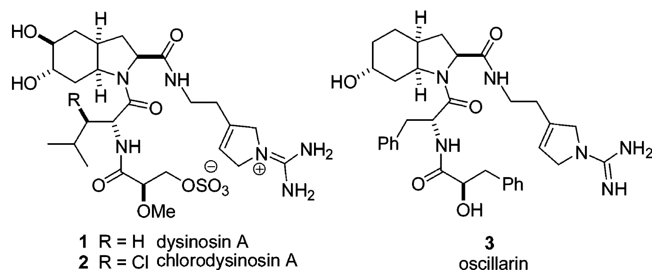

Figure 1.

marine sponges.[7] We recently reported the total synthesis and structural confirmation of dysinosin A (**1**) (Figure 1) which revealed a unique 5,6-dihydroxyoctahydroindole-2-carboxylic acid core structure and an unprecedented amidinopyrroline P_1 basic subunit.[12] Oscillarin (**3**), isolated from the freshwater algae *Oscillatoria aghardii*,[6e] is the third member of the aeruginosin family which was found to contain an amidinopyrroline subunit as a naturally occurring arginine mimetic residue. The originally misassigned structure for this unique heterocyclic entity was corrected in conjunction with the total synthesis of oscillarin, which was also found to be the most active aeruginosin against thrombin (IC_{50} = 28 nM).[11]

Soon after, researchers at Pharmacia Corp. isolated a chlorinated analogue of dysinosin A (**2**, here referred to as chlorodysinosin A) and reported its potent inhibitory activity against thrombin, trypsin, and factor VIIa.[7b] Structural studies of **2** revealed that it differed from **1** only in the presence of a 3-chloroleucine residue[14] instead of D-leucine. Although the stereochemistry of **2** was not assigned, its structural similarity to **1** suggested identical absolute configurations for the individual subunits. This assumption could not, however, be made to predict the configuration of the novel 3-chloroleucine residue, although we assumed the D-leucine backbone to be the same as that in **1**.

Herein we report the first total synthesis and structural and stereochemical confirmation of **2**, including the 2*S*,3*R* configuration of the 3-chloroleucine subunit. In addition, it was our aim to compare the enzymatic inhibitory activity of **2** against representative serine proteases involved in the blood coagulation cascade to that of **1**, which lacks the chloro substituent. While many chlorinated peptide natural products have been isolated over the years, mostly from cyanobacteria,[15] β-chloro-α-amino acids have only been found in a few natural products which include the astins,[16] FR900148,[17] and FR225659.[18] To the best of our knowledge, the isolation of chlorodysinosin A marked the first occurrence of the 3-chloroleucine residue in a natural product.

Synthesis

Despite various reports describing the preparation of halogenated α-amino acids,[19] the synthetic utility of β-halogenated residues is limited by their susceptibility to base-promoted elimination across the α,β-carbons.[20] In our synthesis plan toward **2**, we made an intuitive assumption that the amino acid component was 3*R*-chloro-D-leucine. A number of challenges were clearly apparent, not the least of which was the propensity of the amino acid or its derivatives to undergo β-elimination. To circumvent this problem, we chose 3*R*-chloro-D-leucinol to be our initial subtarget for synthesis. Toward this objective, we considered a regioselective opening of an appropriately *N*-substituted aziridine precursor with a source of chloride anion.[21]

The synthesis of 3-chloroleucinol commenced with the known epoxy alcohol **4** (Scheme 1).[22] The *N*-substituted aziridines **7a**−**d** were obtained in good yield over four efficient steps by way of intermediates **5a,b** and **6**. Our initial attempts to open the *N*-Boc (**7a**) and *N*-Ts (**7b**) aziridines with $MgCl_2$[19e] were unsuccessful (Table 1, entries 1 and 3). A recent report on the use of $CeCl_3 \cdot 7H_2O$ to cleave unhindered *N*-Ts aziridines[23] prompted us to apply these conditions to subtrates **7a** and 7**b**. While the *N*-Boc-aziridine proved to be unreactive (Table 1, entry 2), a regioisomeric mixture of chlorosulfonamides was obtained in 69% yield from **7b** (Table 1, entry 4). The desired 3-chloro *N*-tosyl derivative could be isolated as the major

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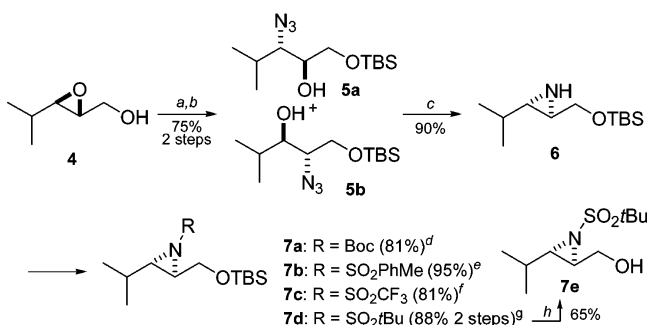
Table 1

entry	SM[a]	conditions	yield[b]	3-Cl:2-Cl[c]	R′
1	**7a**	10 equiv of $MgCl_2$, Et_2O, 72 h, rt	no rxn		TBS
2	**7a**	1.1 equiv of $CeCl_3 \cdot 7H_2O$, MeCN, 90 °C, 24 h	no rxn		TBS
3	**7b**	10 equiv of $MgCl_2$, Et_2O, 72 h, rt	no rxn		TBS
4	**7b**	1.1 equiv of $CeCl_3 \cdot 7H_2O$, MeCN, 90 °C, 24 h	69%	2:1	TBS
5	**7c**	1.1 equiv of $CeCl_3 \cdot 7H_2O$, MeCN, 90 °C, 24 h	57%	1:1	TBS
6	**7d**	2.0 equiv of $CeCl_3 \cdot 7H_2O$, MeCN, 90 °C, 48 h	56%	10:1	5:1 H:TBS
7	**7d**	4.0 equiv of $CeCl_3 \cdot 7H_2O$, MeCN, 90 °C, 72 h	80%	10:1	H
8	**7e**	4.0 equiv of $CeCl_3 \cdot 7H_2O$, MeCN, 90 °C, 48 h	74%	10:1	H

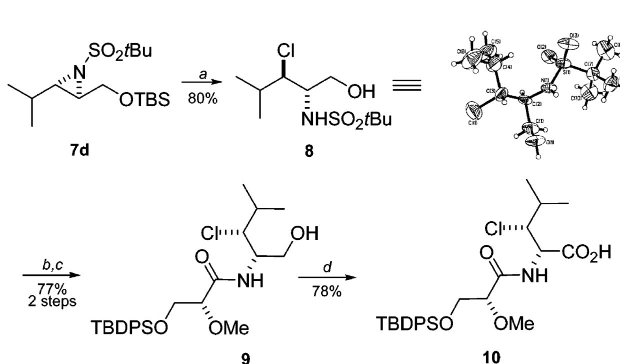
[a] For the structure of **7e** and R groups, see Scheme 1. [b] Combined isolated yield. [c] Determined by ^{1}H NMR.

Scheme 1 [a]



[a] Reagents and conditions: (a) NaN_3, NH_4Cl, MeOEtOH/H_2O (9:1), reflux; (b) TBSCl, Et_3N, DMAP, DCM, 0 °C; (c) PPh_3, MeCN, 50 °C; (d) Boc_2O, Et_3N, DCM; (e) TsCl, Et_3N, DCM; (f) Tf_2O, pyr, DCM, 0 °C; (g) *t*-BuSOCl, Et_3N, DCM, 0 °C then *m*CPBA, DCM, 0 °C; (h) 0.5 M TBAF/THF, 2 h.

Scheme 2 [a]



[a] Reagents and conditions: (a) 4.0 equiv of $CeCl_3 \cdot 7H_2O$, MeCN, 90 °C, 72 h; (b) 0.1 M TfOH/DCM, anisole; (c) PyBOP, (*R*)-3-*tert*-butyldiphenylsilyloxy-2-methoxypropionic acid, 2,6-lutidine, DCM; (d) 0.1 M H_5IO_6/wet MeCN, cat. CrO_3, 0 °C.

product in over 40% yield, but the modest regioselectivity and harsh conditions eventually required for the deprotection of the *N*-tosyl group led us to investigate alternative activating groups.

Treatment of the trifluoromethylsulfonamide **7c** with $CeCl_3 \cdot 7H_2O$ in refluxing MeCN led to a 1:1 mixture of 2-chloro and 3-chloro regioisomers (Table 1, entry 5). We were pleased to find that the *tert*-butylsulfonamide **7d** gave significantly improved regioselectivity in favor of the desired 3-chloro isomer (Table 1, entry 6). By employing 4.0 equiv of $CeCl_3 \cdot 7H_2O$ in refluxing MeCN for 72 h, it was possible to achieve efficient, regioselective aziridine opening and cleavage of the TBS group affording (2*S*,3*R*)-*N*-Bus-3-chloroleucinol (**8**) as a crystalline solid in 80% yield (Table, entry 7). A single-crystal X-ray structure of **8** confirmed the stereo- and regiochemistry of the product.

The *tert*-butylsulfonyl (Bus) protecting group, originally introduced by Weinreb and co-workers,[24] and later utilized in the opening of aziridines with secondary amines by Sharpless and co-workers,[25] has received scant attention as an *N*-protecting group. The high regioselectivity of $CeCl_3$-mediated opening of the Bus-protected **7d** may be due to a steric clash between the *tert*-butyl and isopropyl groups, thus providing an open path for S_N2 attack at C3. The less sterically demanding *N*-tosyl and *N*-trifluoromethylsulfonyl groups of **7b** and **7c** may allow for a competitive reaction at C2. This hypothesis is supported by the fact that cleavage of the aziridine bearing the smallest *N*-sulfonyl group studied (**7c**) resulted in the lowest regioselectivity at C3.

Furthermore, $CeCl_3$-mediated opening of hydroxymethyl aziridine **7e** (Table 1, entry 8) provided the corresponding 3-chlorosulfonamide with the same regioselectivity and yield as those of **7d**, despite diminished steric bulk at C2.

With a concise and selective synthesis of (2*S*,3*R*)-*N*-*tert*-butylsulfonyl-3-chloroleucinol (**8**), we proceeded with the elaboration of amide **10**. Treatment of **8** with 0.1 M TfOH smoothly cleaved the *N*-Bus group, and the resulting amine salt was coupled with (*R*)-3-*tert*-butyldiphenylsilyloxy-2-methoxypropionic acid[12] in the presence of PyBOP (Scheme 2). Oxidation of **9** under a variety of well-known conditions resulted in complex mixtures of products. Gratifyingly, oxidation with H_5IO_6 and catalytic CrO_3, developed by Reider, Grabowski, and co-workers,[26] provided the desired *N*-terminal acid **10** in 78% yield.

We have recently described an efficient intramolecular *N*-acyliminium ion halocarbocyclization route to the 2-carboxy-5-hydroxyoctahydroindole (Choi) core found in oscillarin and various other members of the aeruginosin family.[4b,11] As shown in Scheme 3, treatment of the mixture of hemiaminal acetates **12** with $SnBr_4$ in CH_2Cl_2 at −78 °C afforded, within a few minutes, the brominated intermediate **13** in good yield. This method was exploited toward a new synthesis of the dihydroxyhexahydroindole core previously obtained via tin-mediated *C*-allylation and subsequent ring closing metathesis of a diene intermediate.[12] Thus, dehydrohalogenation of **13** by heating in neat DBU gave the previously obtained RCM product **14** in

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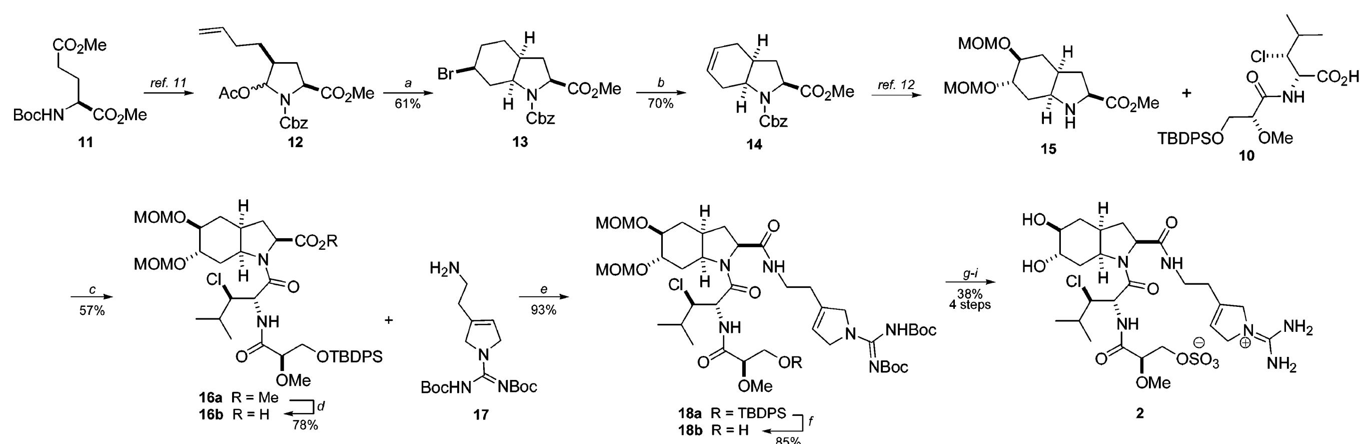
Scheme 3 [a]



[a] Reagents and conditions: (a) $SnBr_4$, DCM, −78 °C, 5 min; (b) neat DBU, 80 °C, 4 h; (c) DEPBT, 2,6-lutidine, DCM, 0 °C, 4 h; (d) 20 equiv of MeSnOH, 1,2-DCE, 75 °C, 48 h; (e) PyBOP, 2,6-lutidine, 2.0 equiv, DCM, 0 °C, 4 h; (f) 0.3 M TBAF/THF, 15 min, 0 °C; (g) SO_3•pyr, cat. Bu_2SnO, DCM, 18 h, then repeat; (h) 10% TFA/DCM, rt, 6 h; (i) preparative RP-HPLC.

70% yield. Following the four-step procedure described for dysinosin A,[12] **14** was converted to **15** in preparation for coupling to the chloro amino acid fragment **10**.

Although we had achieved our first objectives with the synthesis of fragments **10** and **15**, we were cognizant that the assembly of **2** by previously employed methods for dysinosin A[12] had to be extensively modified due to the sensitive nature of the 3-chloroleucine moiety. The first of the anticipated challenges was encountered during attempted amide bond formation. Use of triethylamine or diisopropylethylamine in combination with EDC/HOBt, HBTU/HOBt, or PyBOP gave only low (<15%) yields of **16a** (Scheme 3). By employing 2,6-lutidine as a base in combination with various coupling reagents,[27] yields improved slightly, although mixtures of eliminated products still predominated. After extensive optimization, we found that **16a** could be obtained reproducibly in 55−60% yield when coupling was mediated by 1.5 equiv of *recrystallized DEPBT*.[28]

The next major hurdle to overcome was the hydrolysis of the methyl ester in **16a**. As expected, the various hydroxide bases effected the desired ester cleavage, but at the expense of unwanted elimination. Unsuccessful attempts with both sodium hydroperoxide[29] and enzymatic methods such as pig liver esterase[30] led us to explore the $MeTeAlMe_2$ complex, recently reported by Corey and co-workers.[31] Although we observed efficient ester cleavage without elimination of HCl under the described conditions, the reaction was attended by rapid deprotection of the MOM ethers. However, we were gratified to find that **16b** could be obtained *without any detectable elimination* by employing an excess of Me_3SnOH with heating in 78% yield.[32] Coupling of carboxylic acid intermediate **16b** to amine **17** in the presence of PyBOP and 2,6-lutidine proceeded in high yield to give **18a**.

The installation of the sulfate ester and final deprotection was the last challenge to face en route to the intact intended target **2**. This was achieved by treatment of **18a** with TBAF for 15 min to cleave the TBDPS ether. Longer reaction times resulted in the formation of byproducts, including elimination. Reaction of **18b** with SO_3•pyr and catalytic Bu_2SnO (2 times),[33] followed by global deprotection with 10% TFA/DCM and purification of the crude peptide by RP-HPLC, gave **2** in 38% overall yield. The spectral data obtained from synthetic **2** matched those reported for the natural product[7b] in every respect, thus confirming the structure of chlorodysinosin A as well as the configuration of the (2*S*,3*R*)-3-chloroleucine residue.

Biological and Structural Data

Although our "hunch" in choosing the (2*S*,3*R*)-3-chloroleucine diastereomer (rather than the equally possible 3*S*) proved correct, we had not yet appreciated the subtleties of replacing a pro-*R* methine hydrogen in **1** by a chlorine atom in **2**, until their enzyme inhibitory activities were compared. Chlorodysinosin A proved to be the most potent inhibitor to date of thrombin and factor VIIa among the known aeruginosins (IC_{50} values for **2**: thrombin = 5.7 nM, trypsin = 37 nM, and factor VIIa = 39 nM; for **1**: thrombin = 46 nM, factor VIIa = 326 nM).[34]

A cocrystal structure of **2** with thrombin (at 2 Å resolution) confirms the structure and configurational assignments through synthesis and provides insights into the enhanced binding.[35] While the overall conformation of **2** bound to the thrombin active site is very similar to the binding mode of **1**, there are some interesting differences (Figure 2, left). For instance, both Glu192 and Arg173 differ in the orientation of their respective side chains in the cocrystal structures of **1** and **2** bound to

(27) For a review of peptide coupling methods as applied to the synthesis of complex natural products, see: Humphrey, J. M.; Chamberlin, A. R. *Chem. Rev.* **1997**, *97*, 2243.
(28) (a) Li, H.-T.; Jiang, X.-H.; Ye, Y.-H.; Fan, C.-X.; Romoff, T.; Goodman, M. *Org. Lett.* **1999**, *1*, 91. (b) Fan, C.-X.; Hao, X.-L.; Ye, Y.-H. *Synth. Commun.* **1996**, *26*, 1455. For discussions on the effectiveness of DEPBT in difficult coupling reactions en route to complex natural products, see: (c) Jiang, W.; Wanner, J.; Lee, R. J.; Bounaud, P.-Y.; Boger, D. L. *J. Am. Chem. Soc.* **2003**, *125*, 1877. (d) Ye, Y.-H.; Li, H.-T.; Jiang, X.-H. *Biopolymers* **2005**, *80*, 172.
(29) See: Hanessian, S.; Kagotani, M. *Carbohydr. Res.* **1990**, *202*, 67 and referenced cited therein.
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(32) For a recent application, see: (a) Nicolaou, K. C.; Estrada, A. A.; Zak, M.; Lee, S. H.; Safina, B. S. *Angew. Chem., Int. Ed.* **2005**, *44*, 1378. See also: (b) David, S.; Hanessian, S. *Tetrahedron* **1985**, *4*, 643.
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(34) Nilsson, T.; Sjoling-Ericksson, A.; Deinum, J. *J. Enzyme Inhibition* **1998**, *13*, 11.
(35) Data for cocrystal structure of **2** bound to thrombin has been registered with the protein data bank under PDB ID: 2GDE.

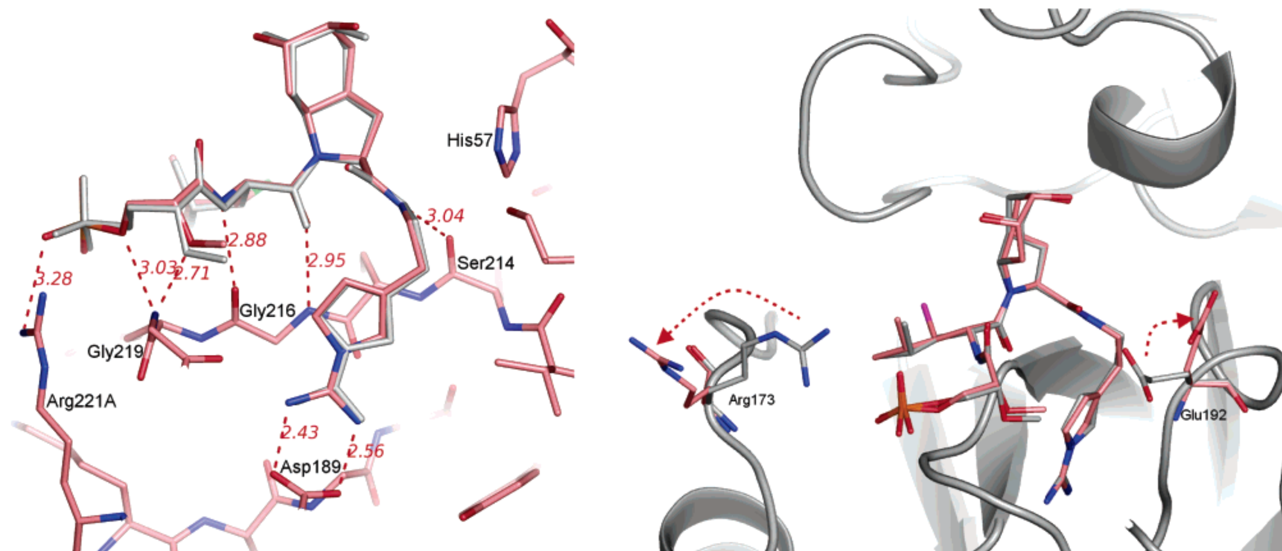

Figure 2. (Left) Overlay of the cocrystal structures of **1** (grey) and **2** (pink) with thrombin. (Right) Overlay of the cocrystal structures of **1** (grey) and **2** (pink) showing changes in the thrombin residue side chain orientations in the case of **2**.

thrombin (Figure 2, right). The flexibility of Glu192 has been previously observed in substrate-bound thrombin structures.[36] Although Glu192 in thrombin adopts a different side chain orientation when bound to **2** rather than **1**, there is no evidence of a direct interaction between Glu192 and the bound ligand in either X-ray structure. The Arg173 residue in thrombin points toward the sulfate group of the bound ligand **1**, an orientation not frequently observed in other thrombin complexes. The presence of the chlorine seems to cause the side chain of this Arg173 to return to its "normal" orientation (Figure 2, right). Other differences in the orientation of the residues in the binding pocket include the backbone atoms of Glu217 (0.36 Å for C_α) and Gly219 (0.47 Å). Shifts of a similar scale are also observed for some of the ligand atoms.

Perhaps most significant, the addition of the chlorine does not seem to change the conformation of either the side chain of the leucine or the protein binding site. We rationalize the enhanced inhibitory activity of **2** by favorable changes in charge distribution, better accommodation of the chlorine in the S3 subsite, and stabilization of the χ^1 dihedral angle of the (2*S*,3*R*)-chloroleucine residue. The presence of the chlorine atom also enhances lipophilicity (log *P* of −1.8 for **2** vs −3.8 for **1**) contributing to increased hydrophobic interactions via a decreased desolvation penalty. In addition, the presence of a chlorine atom in the S3 subsite of thrombin leads to a release of water from the protein, which provides an entropic gain.

To test to what extent the chlorine atom stabilizes the biologically active conformation of the dysinosins, we ran a short molecular dynamics simulations starting from the bound conformations of **1** and **2**. Dysinosin A was found to sample two major conformations of the χ^1 dihedral angle (approximately trans and gauche), whereas the sampling of χ^1 in chlorodysinosin A (**2**) is clearly more restricted.[37] These results suggest that conformational stabilization of the leucine side chain in fact plays an important role in the enhanced potency of **2**. A detailed calorimetric study of the relative binding thermodynamics of the dysinosins would provide insight into the relative importance of the different terms and provide a basis for future refinements.[38]

Conclusion

We have described a concise total synthesis of chlorodysinosin A (**2**), a highly potent inhibitor of the serine proteases thrombin, trypsin, and factor VIIa. The synthesis also established the absolute configuration of the natural product. A novel 3*R*-chloro-D-leucine amide residue was synthesized in a stereocontrolled manner en route to the target compound. An X-ray cocrystal structure of **2** bound to thrombin reveals important differences with the des-chloro analogue, dysinosin A (**1**). The importance of chlorinated amino acids in natural products is highlighted by enhanced potency and functional novelty in these metabolites. The incorporation of a chlorine in related aeruginosins as hybrid analogues and the replacement of the 3-chloroleucine with other hydrophobic *β*-branched residues are currently being investigated.

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Note Added after ASAP and Print Publication: Due to a production error, the references following ref 21 were misnumbered and structure **3** in Figure 1 was incorrect in the version of this paper published on the Web July 24, 2006 (reflecting a change to Figure 1 after initial ASAP publication on July 21, 2006), and in the August 16, 2006 print issue. This electronic version of the paper, with the correct Figure 1 and Scheme 1 and the references correctly numbered, was published on August 22, 2006; an Addition and Correction appears in the September 6, 2006 issue (Vol. 128, No. 35, pp 11727−11728).

Supporting Information Available: Experimental procedures, ^{1}H, ^{13}C NMR spectra for all new compounds. Molecular modeling data and a table of comparative biological activities are provided for **1** and **2**. These materials are available free of charge via the Internet at http://pubs.acs.org.

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(37) See Supporting Information for experimental details on molecular modeling and for a comparison of χ^1 angles and energy minimized conformations of **1** and **2**.

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