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P_1,P_3 Truncated Analogs of Oscillarin and their Inhibitory Activity against Blood Coagulation Factors

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Abstract: Based on modeling and available X-ray co-crystal structure data of oscillarin with the enzyme thrombin, a series of P_1,P_3 truncated analogs were prepared using the azabicyclic octahydroindole carboxamide core as a scaffold. The P_1 subunit of the original natural product was replaced by a 4-amidinobenzamide group, and the P_3 subunit was simulated by N-benzylsulfonyl glycine amide or an N-acetyl D-phenylalanine amide. Single digit micromolar inhibition was found against trypsin, thrombin, Factor Xa, and Factor XIa for some analogs.

Keywords: Aeruginosin mimics · Sulfonamide · Thrombin inhibition

1. Introduction

Natural products obtained from diverse sources and possessing fascinating architectures are invariably endowed with some level of pharmacological activities.[1] In the best of cases, these are manifested by beneficial, even life-saving properties as exemplified by potent antitumor agents. Indeed, a large number of such metabolites have cytotoxic activities, which is why they have received particular attention as potential chemotherapeutic agents.[2] However, within the existing and constantly growing harvest of new natural products potent *in vitro* activity is found also in other medically important areas.[3] Synthetic chemistry has played a pivotal role in providing additional quantities of rare natural products through total synthesis, as well as in their chemical modification with the objective of studying structure–activity relationships (SARs) toward a safer and more effective drug.[4]

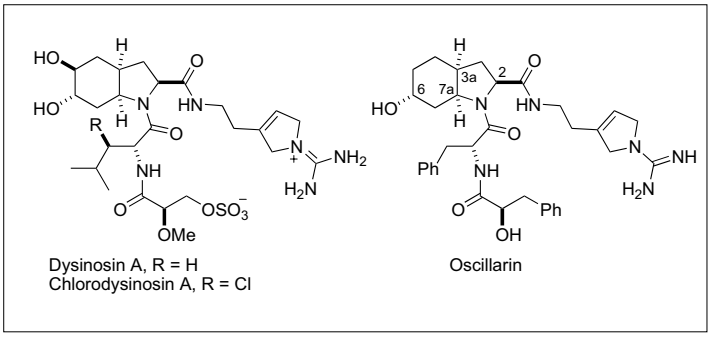

Fig. 1. Structures of the natural dysinosin A, chlorodysinosin A, and oscillarin

The aeruginosins are a relatively new class of linear peptides with a characteristic central 2-carboxyoctahydroindole core unit.[5] Over 20 aeruginosins have been isolated from blue-green algae or marine sponges over the last two decades, and their structures determined by chemical, spectroscopic, and X-ray crystallographic methods. Of the seven total syntheses, four have resulted in structural revision.[6] The aeruginosins are potent *in vitro* inhibitors of serine proteases such as thrombin, Factor VIIa, and Factor Xa which are implicated in intrinsic and extrinsic pathways leading to blood clots in humans.[7–10] Chlorodysinosin A[11] and oscillarin,[6e] depicted in Fig. 1, are among the most potent inhibitors of thrombin (5.7 nM and 28 nM, respectively). In spite of this, the aeruginosins are not likely candidates for further development as antithrombotic agents because of their highly polar nature. Fortunately, their exquisitely deployed pharmacophores and overall three-dimensional shape are also mimicked by non-natural, often achiral synthetic molecules with potent *in vitro* thrombin inhibition.[12] Very limited efforts have been reported to prepare analogs of aeruginosin 298A.[13,14]

Our continued interest in the chemistry and biology of the aeruginosins[5] led us to consider the synthesis of truncated analogs containing the central perhydroindole carboxamide core subunit to which we appended the well known *p*-amidinobenzyl subunit as a mimic of the P_1 basic extremity. We were particularly interested in replacing the P_3 amino acid with sulfonamide appendages bearing a hydrophobic benzyl group.[15] As a prototype, we chose 6*R*-hydroxyl and 6*S*-hydroxyl core octahydroindole 2-carboxamides maintaining the 'natural' configuration of the aeruginosins.

2. Results and Discussion

2.1. *Chemistry*

We adopted the Bonjoch protocol[6c] to prepare the (2*S*,3a*S*,6*R*,7a*S*)-configured oc-

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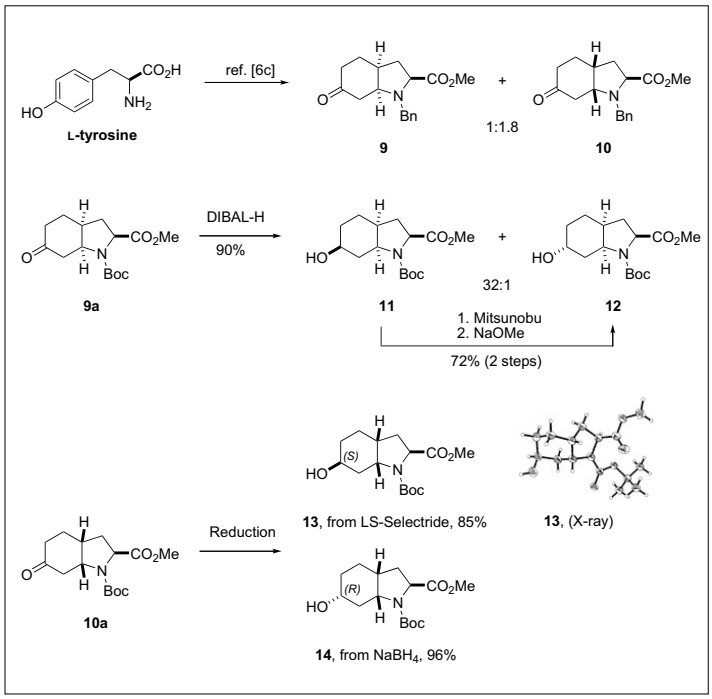


Scheme 1. Synthesis of the alcohol intermediates **11–14**

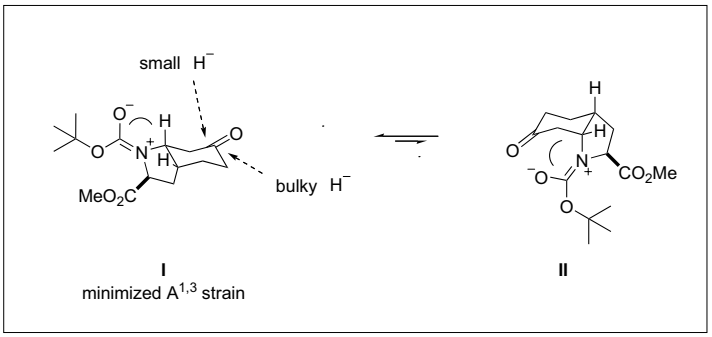
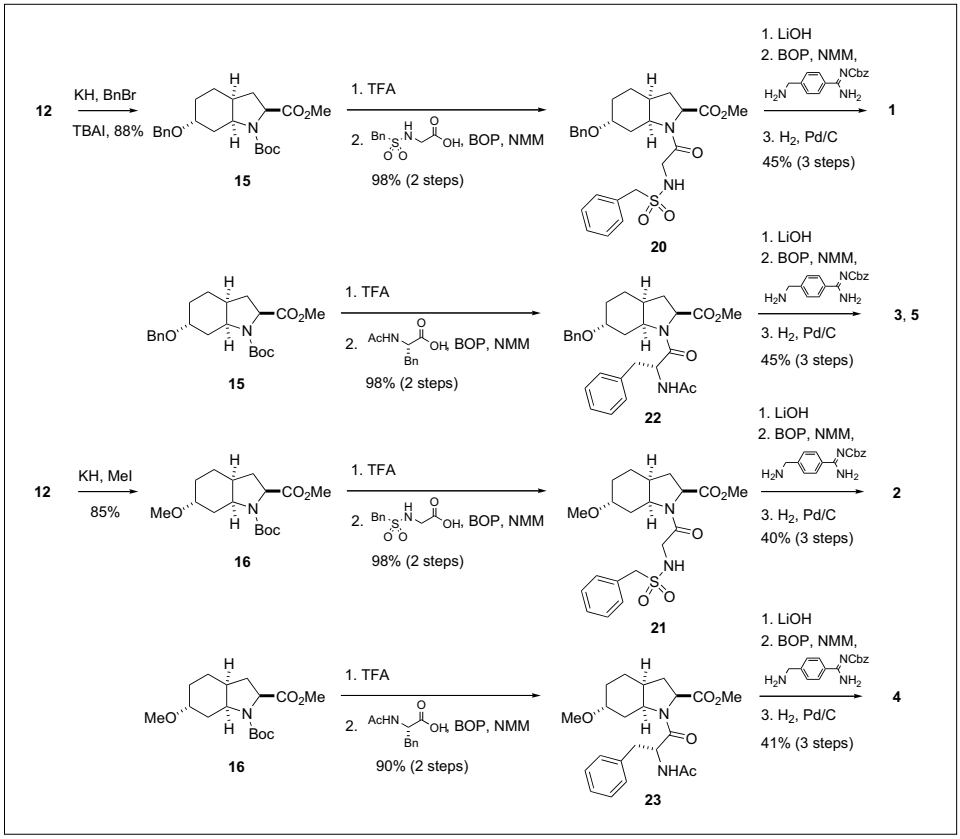


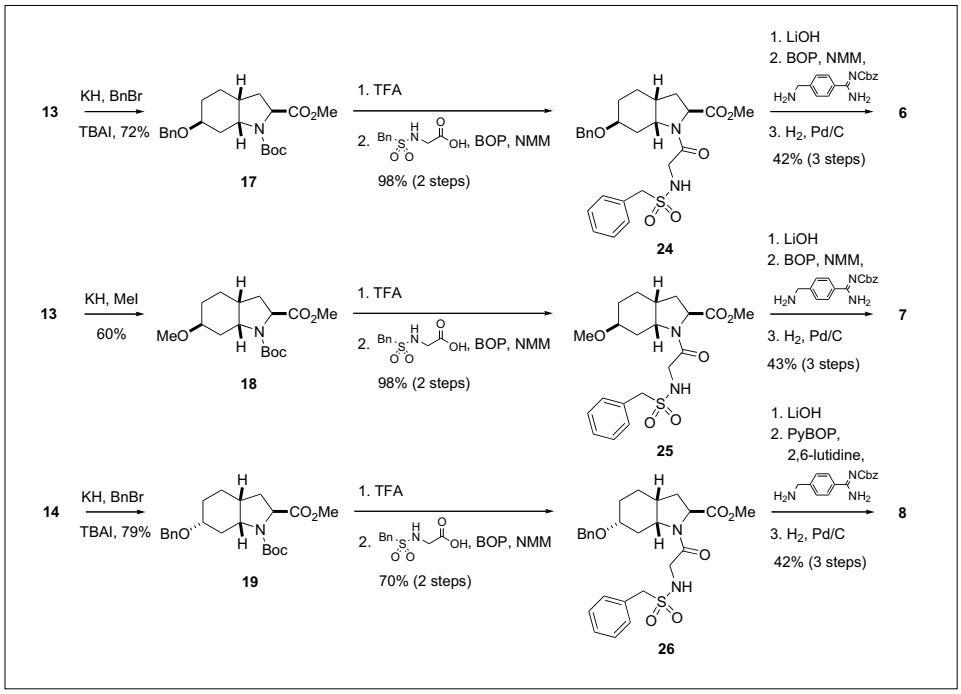
Fig. 2. Proposed preferred conformation **I** of intermediate **10a**

tahydroindole **12** as a versatile intermediate (Scheme 1). The synthetic route also led to the (2*S*,3a*R*,6*S*,7a*R*)- and (2*S*,3a*R*,6*R*,7a*R*)-configured diastereomers **13** and **14**. In this protocol L-tyrosine undergoes a reductive dearomatization followed by a remarkable intramolecular cyclization to two diastereomeric 6-oxo-octahydroindole-2-carboxylic acid methyl esters, *endo*-**9** and *exo*-**10**, that are further manipulated to the intended intermediates. Variation of the N-substituent (Bn, Ac, Boc) as well as the size of the reducing agent afforded the intended 6*R*- or 6*S*-alcohols in good overall yields and high diastereoselectivity.[6c] We found it optimal to reduce the N-Boc *endo*-ketone **9a** with DIBAL-H, which led in high yield to the 6*S*-alcohol **11** and the 6*R*-alcohol **12** in a 32:1 ratio, as outlined in Scheme 1. Inversion of C(6) in derivative **11** by the Mitsunobu procedure over the *p*-nitrobenzoate intermediate followed by hydrolysis gave **12** in a very good yield over two steps. A similar set of reactions furnished the diastereomers **13** and **14** from the N-Boc *exo*-ketone **10a** (Scheme 1). Reduction of **10a** using LS-Selectride furnished stereoselectively the 6*S*-alcohol **13**, while $NaBH_4$ gave the inverse 6*R*-alcohol **14**. An X-ray analysis confirmed the proposed structure of **13** (Scheme 1). The stereoselectivity of the reduction can be explained by the preferred conformation of the N-Boc *exo*-ketone **10a**, which adopts the chair conformation **I** with minimized $A^{1,3}$ strain between the N-Boc C–O and the C(7a) methine group (Fig. 2). An axial hydride attack is favored according to the Cieplak effect,[16] which states that vicinal antiperiplanar σ C–H orbitals are better stabilizers than σ C–C orbitals of the incipient σ* orbital formed as the hydride approaches the carbonyl group. The torsional strain in the transition state is also less with an axial attack compared to an equatorial attack.[17] However, the 1,3-diaxial hydrogens block an axial attack from bulkier hydride reagents resulting in an equatorial attack when LS-Selectride is used.

Although coupling with benzylsulfonylglycine or N-acetyl-L-phenylalanine could be performed on the free hydroxyl intermediates, we opted to protect the alcohols as the benzyl and methyl ethers (Schemes 2 and 3). The latter was done in order to probe the importance of a free hydroxyl group on thrombin inhibition. Hydrolysis of the N-Boc group with TFA, followed by coupling of the resulting amine with the P_3 substituent in the presence of BOP and N-methyl morpholine in DMF afforded the corresponding sulfonamides and amides **20–26** in near quantitative yields (Schemes 2 and 3). The methyl esters were cleaved with aq. LiOH and the resulting carboxylic acids were coupled with N-Cbz *p*-amidino-



Scheme 2. Synthesis of the *endo*-series analogs **1–5**



Scheme 3. Synthesis of the *exo*-series analogs **6**–**8**

benzylamine in modest yields. Final deprotection gave the analogs **1**–**8**. In an effort to investigate whether a large 6-O-benzyl could fit in the S_2 or S_3 pocket, we prepared analog **5** by exposing the reaction to a shorter hydrogenation time in order to cleave the Cbz-protecting group selectively leaving the benzyl protecting group intact.

2.2. *Enzyme Activity*

Analogs **1**–**8** were tested for their inhibitory *in vitro* activities against thrombin and Factors VIIa, IXa, Xa, and XIa, and trypsin. The results are presented as IC_{50} values in the Table.[18] Only the sulfonamide analog **1** and the amide analog **3**, both with a free 6*R*-hydroxyl on the azabicycle demonstrated significant activities in the thrombin and trypsin assays (thrombin IC_{50} = 2.39 μM and 13.1 μM and trypsin IC_{50} = 0.182 μM and 2.05 μM, respectively, Table). Furthermore, modest Factor XIa and Factor VIIa activities were noted with the sulfonamide **1**. The amide analog **3** bearing a benzyl P_3 side chain was less potent against thrombin and trypsin compared to the P_3 truncated sulfonamide **1**. Apparently, the combination of an N-terminal benzyl and a truncated P_3 sulfonamide was more favorable than a P_3 benzyl and a truncated N-terminal acetamide for efficient inhibition. Surprisingly, the 'synthetic' 6*S*-*exo* O-methyl analog **7** showed thrombin, trypsin, and Factor Xa activity, while the unmethylated analog **6** was inactive. One compound, analog 6*R*-*exo* **8**, was prepared possessing the same stereochemistry of the core azabicycle as that found in natural aeruginosin EI461.[6d] This is the only natural aeruginosin identified to date with (2*S*,3a*R*,6*R*,7a*R*)-configuration of the nitrogen-containing bicycle. A comparison of **8** to its diastereomer **1** shows the 'naturally' more common (2*S*,3a*S*,6*R*,7a*S*)-core configuration of **1** to be superior against thrombin (thrombin IC_{50} = 18.5 μM and 2.39 μM, respectively, Table). In general the compounds in this truncated series of aeruginosin analogs were more active against trypsin than thrombin (**1**, **3**, and **7**, Table).

Clearly, the stereochemistry of the 2-carboxy-6-hydroxy-octahydroindole core azabicycle, often referred to as Choi, has a significant impact on the SARs of this class of compounds. In the absence of representative X-ray co-crystal structures complexed with thrombin the different effects of a free hydroxyl compared to a methoxy or benzyloxy in the 6*R*-*endo* and 6*S*-*exo* series are difficult to rationalize. However, it is likely that a steric clash with the S_2 pocket of the bulkier ether-protected 6*R*-*endo*-analogs favors smaller 6*R*-substituents. In fact, preliminary results show that endo-azabicycles without any substituent in the 6-position are even slightly more active against thrombin than the corresponding 6*R*-hydroxyl-substituted endo-analogs.[19] According to preliminary modeling of analogs **1**–**4** and **6**, **7** both the 6*R*-*endo*- and 6*S*-*exo*-series with either a hydroxyl or methoxy substituent could be favorably accommodated in the thrombin active site.

It has previously been noted that the shape and orientation of the P_3 side chain is of pivotal importance for high inhibitory activity against the coagulation factors.[11] A 3*R*-chloro substituent on the D-leucine side chain in chlorodysinosin A compared to the unsubstituted D-leucine in dysinosin A gave a remarkable enhancing effect on the activity against thrombin and Factor VIIa (chlorodysinosin A, IC_{50} thrombin, 5.7 nM, FVIIa, 39 nM; dysinosin A, IC_{50} thrombin, 46 nM, FVIIa, 326 nM).[11] The two most active inhibitors **1** and **7** in this series of truncated analogs contained non-traditional P_3 groups, exemplified by the benzyl sulfonamide appendage.

3. Conclusion

We have prepared a series of truncated aeruginosins focusing on the core Choi subunit as a central scaffold and varying the P_3 substituent, as well as the stereochemistry of the 6-hydroxyl group. A glycine sulfonamide P_3 substituent with a 4-amidinobenzamide P_1 surrogate **1** exhibited promising *in vitro* activity against trypsin, thrombin, Factor VIIa, and FXIa. A diastereomeric *exo*-analog with a 6-O-methyl substituent maintained activity against trypsin, thrombin, FVIIa, and FXa, but was inactive against FXIa. Many other totally synthetic N-terminal sulfonamides[12,15] and amides[12] bearing no relation to the aeruginosins have shown high potency against the coagulation factors including thrombin. The fact that these truncated aeruginosin analogs exhibit noticeable inhibitory activity is encouraging and argues well for more potent variants by fine-tuning the P_3 appendage.

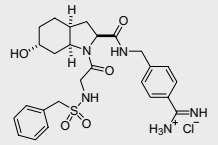
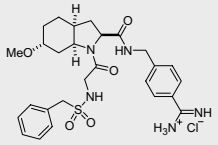
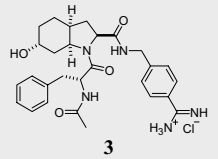
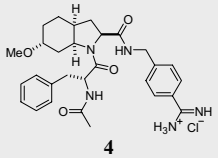
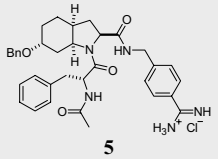
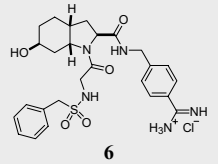
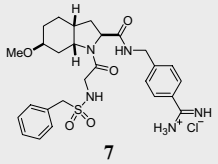
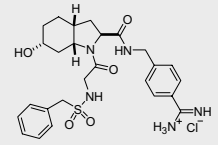
4. Experimental Section

4.1. *General Information*

(2*S*,3a*S*,6*S*,7a*S*)-6-Hydroxyoctahydroindole-1,2-dicarboxylic acid 1-*tert*-butyl ester 2-methyl ester (11). To a solution of **9a** (1.5 g, 5.2 mmol) in THF (30 ml) at –78 °C was added DIBAL-H (6 ml of a 1 M solution in THF, 6.0 mmol) and the reaction mixture was stirred for 5 h. The reaction was quenched by addition of 2 M aq. NH_4Cl (5 ml) and NaCl (300 mg) and the mixture was stirred overnight. The THF was removed under reduced pressure and the aqueous mixture extracted with CH_2Cl_2. The combined organic phases were dried (Na_2SO_4), concentrated under reduced pressure and the crude residue was purified by flash chromatography over silica gel (EtOAc/hexane, 2:3) to give the known **11** (1.3 g, 87%) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ (rotamers) 4.21 (m, 1H), 3.94 (m, 1H), 3.72 (s, 3H), 3.54 (m, 1H), 2.40 (m, 1H), 2.24 (m, 1H), 2.11 (m, 1H), 2.03–1.97 (m, 3H), 1.77–1.68 (m, 3H), 1.51–1.33 (m, 10H).[6c]

(2*S*,3a*S*,6*R*,7a*S*)-6-Hydroxyoctahydroindole-1,2-dicarboxylic acid 1-*tert*-butyl ester

Table. *In vitro* enzyme inhibitory activities

compound	Enzyme IC_{50} (μM)[a]					
	trypsin	thrombin	FVIIa	FIXa	FXa	FXIa
 **1**	0.182	2.39	25.5	>44.4	nd	5.34
 **2**	nd	>44.4	nd	nd	nd	nd
 **3**	2.05	13.1	>44.4	>44.4	>44.4	>44.4
 **4**	nd	>44.4	nd	nd	nd	nd
 **5**	nd	>44.4	nd	nd	nd	nd
 **6**	nd	>44.4	nd	nd	nd	nd
 **7**	0.192	3.25	22.7	>44.4	5.34	>44.4
 **8**	nd	18.5	nd	nd	nd	nd

[a] nd, not determined.

2-methyl ester (12). To a solution of PPh_3 (0.029 g, 0.11 mmol) and 4-nitrobenzoic acid (0.019 g, 0.11 mmol) in THF (4 ml) at –30 °C was added **11** (0.028 g, 0.092 mmol) in THF (3 ml). DIAD (22 μl, 0.11 mmol) was added carefully over 10 min. and the reaction mixture was stirred at RT for 2 h. The reaction was neutralized by addition of aq. $NaHCO_3$ (sat.), concentrated under reduced pressure, dissolved in H_2O, and extracted with CH_2Cl_2. The combined organic phases were dried (Na_2SO_4), concentrated under reduced pressure, and the crude residue was purified by flash chromatography over silica gel (EtOAc/hexane, 5:15–7:13). The purified product was dissolved in MeOH (3 ml) and NaOMe (0,05 ml of a 1 M solution in MeOH) was added at 0 °C. The reaction mixture was stirred for 1 h, neutralized with 2 M aq. NH_4Cl and concentrated under reduced pressure. The residue was dissolved in H_2O and extracted with CH_2Cl_2. The combined organic phases were dried (Na_2SO_4), concentrated under reduced pressure, and purified by flash chromatography over silica gel (EtOAc/hexane, 7:13) to give the known **12** (0.020 g, 72% over two steps) as a colorless oil whose spectroscopic data matched those reported previously.[6c]

(2*S*,3a*R*,6*S*,7a*R*)-6-Hydroxyoctahydroindole-1,2-dicarboxylic acid 1-*tert*-butyl ester 2-methyl ester (13). To a solution of **10a** (0.098 g, 0.33 mmol) in THF (4 ml) at –78 °C was added LS-Selectride (0.43 ml as a 1 M solution in THF, 0.43 mmol) over 15 min. and the reaction mixture was stirred for 6 h. The reaction was quenched by addition of 3 ml aq. $NaHSO_4$ (sat.) and the mixture was stirred overnight. After the addition of aq. $NaHCO_3$ (3 ml) the mixture was concentrated under reduced pressure and the resulting residue was dissolved in sat. aq. NaCl (5 ml) and extracted with CH_2Cl_2 and $CHCl_3$/*i*-propanol (3:1). The combined organic phases were dried (Na_2SO_4), concentrated under reduced pressure, and purified by flash chromatography over silica gel (EtOAc/hexane, 2:3) to give **13** (0.084 g, 85%) as a white solid. The product was recrystallized by slow evaporation from EtOAc. ^1H NMR (400 MHz, $CDCl_3$) δ (rotamers) 4.48–4.21 (m, 2H), 4.15–4.03 (m, 1H), 3.74 (s, 3H), 2.62 (m, 1H), 2.21–2.12 (m, 3H), 1.89–1.76 (m, 3H), 1.62–1.57 (m, 2H), 1.45–1.40 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (rotamers) 173.1, 153.4, 79.4, 65.1, 57.8, 53.6, 51.5, 34.7, 34.1, 32.0, 30.8, 29.0, 27.5, 20.2, 19.5; mp 111–113 °C.

(2*S*,3a*R*,6*R*,7a*R*)-6-Hydroxyoctahydroindole-1,2-dicarboxylic acid 1-*tert*-butyl ester 2-methyl ester (14). To a solution of **10a** (0.042 g, 0.14 mmol) in anhydrous MeOH (2 ml) at –15 °C was added $NaBH_4$ (0.0054 mg, 0.14 mmol) and the reaction mixture was stirred for 20 min. The reaction was quenched by addition of 2 M aq. NH_4Cl (1 ml) and the MeOH was removed under reduced pressure. The residue was diluted with H_2O (3 ml) and extracted with CH_2Cl_2. The combined organic phases were dried (Na_2SO_4), concentrated under reduced pressure, and purified by flash chromatography over silica gel (EtOAc/hexane, 3:7) to give the known **14** (0.041 g, 96%) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ (rotamers) 4.31 (dd, 1H, J = 2.3, 7.5 Hz), 4.14–4.04 (dm, 1H, J = 8.1 Hz), 3.73 (s, 3H), 3.57 (m, 1H), 2.49–2.42 (m, 2H), 2.25 (m, 1H), 2.05–1.71 (m, 5H), 1.48–1.40 (m, 11H).[6d]

(2*S*,3a*S*,6*R*,7a*S*)-6-Benzyloxyoctahydroindole-1,2-dicarboxylic acid 1-*tert*-butyl ester 2-methyl ester (15). To a solution of **12** (0.035 g, 0.12 mmol) in DMF (2 ml) was added BnBr (0.5 ml, 4.2 mmol) and a catalytic amount of TBAI (20 mol%). KH (0.010 g, 0.25 mmol) was added at 0 °C and the reaction mixture was stirred at RT for 2 h. The reaction was quenched by addition of MeOH (2 ml) and 2 M aq. NH_4Cl (2 ml) and the mixture was concentrated under reduced pressure. The residue was diluted with H_2O (8 ml) and extracted with EtOAc. The combined organic phases were dried (Na_2SO_4), concentrated under reduced pres-

sure, and purified by flash chromatography over silica gel (EtOAc/hexane, 5:95–3:7) to give **15** (0.040 g, 88%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (rotamers) 7.34–7.26 (m, 5H), 4.60 (t, 1H, *J* = 12 Hz), 4.74 (d, 1H, *J* = 12 Hz), 4.28–4.09 (m, 2H), 3.74 (s, 3H), 2.62–2.34 (m, 2H), 2.14–1.97 (m, 3H), 1.77–1.67 (m, 1H), 1.58–1.41 (m, 13H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (rotamers) 173.9, 153.8, 138.7, 128.1, 127.9, 127.8, 127.1, 126.9, 79.4, 72.2, 69.2, 64.8, 59.0, 58.4, 53.7, 51.6, 36.0, 31.5, 29.8, 29.5, 24.0, 19.6; ESI/MS for $C_{22}H_{31}NO_5$ calculated ($M+H^+$) 390.2, found 390.3.

(2*S*,3a*S*,6*R*,7a*S*)-6-Methoxyoctahydroindole-1,2-dicarboxylic acid 1-*tert*-butyl ester 2-methyl ester (16). To a solution of **12** (0.298 g, 1.0 mmol) in THF (10 ml) at 0 °C was added MeI (2 ml, 32.0 mmol) and KH (0.060 g, 1.5 mmol) and the reaction mixture was stirred at RT for 40 min. The reaction was quenched by the addition of MeOH (2 ml) and 2 M aq. NH_4Cl (5 ml) and the mixture was concentrated under reduced pressure. The residue was diluted with H_2O (5 ml) and extracted with EtOAc. The combined organic phases were dried (Na_2SO_4), concentrated under reduced pressure, and purified by flash chromatography over silica gel (EtOAc/hexane, 3:7) to give **16** (0.264 g, 85%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (rotamers) 4.29–4.26 (m, 1H), 4.09–3.93 (m, 1H), 3.73 (s, 3H), 3.53 (s, 1H), 3.33 (s, 3H), 2.47–2.28 (m, 2H), 2.19–2.13 (m, 1H), 2.04–1.95 (m, 2H), 1.73–1.61 (m, 2H), 1.50–1.40 (m, 11H); ESI/MS for $C_{16}H_{27}NO_5$ calculated ($M+H^+$) 314.2, found 314.1.

(2*S*,3a*R*,6*S*,7a*R*)-6-Benzyloxyoctahydroindole-1,2-dicarboxylic acid 1-*tert*-butyl ester 2-methyl ester (17). Same procedure as for compound **15**, starting from **13**, gave **17** (72%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (rotamers) 7.41–7.28 (m, 5H), 4.71 (s, 1H), 4.65–4.57 (m, 1H), 4.49–4.46 (m, 1H), 4.35–4.26 (m, 2H), 3.74 (s, 3H), 2.66–2.47 (m, 2H), 2.26–2.23 (m, 1H), 2.10–2.06 (m, 2H), 1.85–1.73 (m, 2H), 1.54–1.42 (m, 11H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (rotamers) 173.5, 153.8, 140.6, 138.6, 138.5, 128.2, 127.2, 126.9, 79.4, 69.2, 64.9, 58.3, 57.7, 53.8, 51.8, 34.5, 32.1, 31.0, 30.0, 25.1, 24.6, 20.3; ESI/MS for $C_{22}H_{31}NO_5$ calculated ($M+H^+$) 390.2, found 390.3.

(2*S*,3a*R*,6*S*,7a*R*)-6-Methoxyoctahydroindole-1,2-dicarboxylic acid 1-*tert*-butyl ester 2-methyl ester (18). Same procedure as for compound **16**, starting from **13**, gave **18** (60%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (rotamers) 4.33–4.24 (m, 1H), 4.19–4.02 (m, 1H), 3.72 (s, 3H), 3.51 (m, 1H), 3.34 (s, 3H), 2.51–2.42 (m, 2H), 2.29–2.21 (m, 1H), 2.05–1.96 (m, 1H), 1.83–1.79 (m, 1H), 1.75–1.62 (m, 1H), 1.52–1.40 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (rotamers) 173.4, 153.8, 79.4, 73.9, 58.3, 55.4, 53.5, 51.7, 34.5, 32.1, 31.0, 30.0, 29.2, 25.2, 24.3, 20.2; ESI/MS for $C_{16}H_{27}NO_5$ calculated ($M+H^+$) 314.2, found 314.2.

(2*S*,3a*R*,6*R*,7a*R*)-6-Benzyloxyoctahydroindole-1,2-dicarboxylic acid 1-*tert*-butyl ester 2-methyl ester (19). Same procedure as for compound **15**, starting from **14**, gave **19** (79%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (rotamers) 7.42–7.20 (m, 5H), 4.66–4.42 (m, 2H), 4.32 (m, 0.3H), 4.25 (m, 0.7H), 4.02 (m, 0.7H), 3.89 (m, 0.3H), 3.71 (s, 0.9H), 3.71 (s, 2.1H), 3.55 (m, 0.3H), 3.28 (m, 0.7H), 2.67 (m, 0.7H), 2.52–2.38 (m, 1.3H), 2.36–2.18 (m, 1H), 1.94–1.56 (m, 4H), 1.51–1.08 (m, 11H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (rotamers) 173.5, 173.1, 153.7, 153.0, 138.6, 128.6, 128.3, 128.2, 128.1, 127.40, 127.37, 127.3, 127.2, 126.7, 79.8, 75.3, 74.9, 69.9, 69.8, 64.9, 58.3, 57.9, 56.4, 56.2, 52.0, 51.8, 34.6, 33.8, 33.6, 31.9, 30.8, 28.3, 28.1, 26.8, 26.5, 22.75, 22.68; ESI/MS for $C_{22}H_{31}NO_5$ calculated ($M+H^+$) 390.2, found 390.2.

(2*S*,3a*S*,6*R*,7a*S*)-6-Benzyloxy-1-(2-(phenylmethanesulfonylaminoacetyl)octahydroindole-2-carboxylic acid methyl ester (20). To a solution of **15** (0.034 g, 0.087 mmol) in CH_2Cl_2 (2 ml) was added TFA (0.4 ml) and the reaction mixture was stirred at RT for 30 min. The reaction mixture was concentrated under reduced pressure and co-evaporated with toluene. The crude residue was dissolved in DMF (3 ml) and the sulfonamide subunit (0.052 g, 0.22 mmol) was added. The mixture was cooled to 0 °C and BOP (0.065 g, 0.15 mmol) and NMM (62 µl, 0.56 mmol) were added. After stirring for 24 h the reaction was quenched by addition of 2 M aq. NH_4Cl (3 dr.) and the mixture was partitioned between EtOAc and H_2O. The combined organic phases were dried (Na_2SO_4), concentrated under reduced pressure, and purified by flash chromatography over silica gel (EtOAc/hexane, 95:5) to give **20** (0.043 g, 98% over two steps) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (rotamers) 7.46–7.28 (m, 10H), 5.30 (s, 1H), 4.72 (d, 1H, *J* = 12 Hz), 4.44–4.41 (m, 2H), 4.32–4.30 (m, 2H), 3.84–3.79 (m, 1H), 3.77 (s, 3H), 3.58–3.54 (m, 1H), 2.39–2.37 (m, 1H), 2.23–2.20 (m, 1H), 2.10–1.94 (m, 5H), 1.87–1.84 (m, 1H), 1.62–1.55 (m, 2H), 1.44–1.41 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (rotamers) 172.4, 165.5, 138.1, 130.5, 128.8 (2C), 128.4, 128.2, 128.1, 127.4, 127.2 (2C), 127.1, 71.8, 69.8, 59.5, 58.8, 53.7, 52.0, 44.3, 36.6, 30.8, 29.9, 22.4, 19.0, 13.9; ESI/MS for $C_{26}H_{32}N_2O_6S$ calculated ($M+H^+$) 501.2, found 501.4.

(2*S*,3a*S*,6*R*,7a*S*)-6-Methoxy-1-(2-(phenylmethanesulfonylaminoacetyl)octahydroindole-2-carboxylic acid methyl ester (21). Same procedure as for compound **20**, starting from **16**, gave **21** (98% over two steps) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46–7.36 (m, 5H), 5.44–5.39 (m, 1H), 4.41 (m, 1H), 4.31–4.29 (m, 2H), 3.79–3.73 (m, 5H), 3.64–3.60 (dd, 2H, *J* = 4.5, 16.0 Hz), 3.27 (s, 3H), 2.21–2.18 (m, 1H), 2.05–2.02 (m, 1H), 1.99–1.87 (m, 3H), 1.85–1.77 (m, 1H), 1.66–1.49 (m, 2H), 1.45–1.32 (m, 1H); ESI/MS for $C_{20}H_{28}N_2O_6S$ calculated ($M+H^+$) 425.2, found 425.3.

(2*S*,3a*S*,6*R*,7a*S*)-1-((*R*)-2-Acetylamino-3-phenylpropionyl)-6-benzyloxyoctahydroindole-2-carboxylic acid methyl ester (22). Same procedure as for compound **20** gave **22** (98% over two steps) as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (rotamers) 8.02 (s, 1H), 7.43–7.13 (m, 10H), 6.77–6.71 (m, 1H), 4.97–4.94 (m, 1H), 4.62–4.42 (m, 3H), 4.19–4.09 (m, 1H), 3.76 (m, 3H), 2.47–2.38 (m, 1H), 2.15–1.93 (m, 6H), 1.87–1.63 (m, 3H), 1.52–1.49 (m, 1H), 1.39–1.36 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (rotamers) 172.4, 169.5, 162.3, 138.4, 135.8, 129.2, 129.0, 128.0 (2C), 127.1 (2C), 126.9, 126.6, 126.5, 71.6, 69.3, 58.8, 54.7, 51.6, 51.5, 38.7, 36.9, 36.5, 31.1, 30.0, 22.9, 22.6, 19.2; ESI/MS for $C_{28}H_{34}N_2O_5$ calculated ($M+H^+$) 479.2, found 479.4.

(2*S*,3a*S*,6*R*,7a*S*)-1-((*R*)-2-Acetylamino-3-phenylpropionyl)-6-methoxyoctahydroindole-2-carboxylic acid methyl ester (23). Same procedure as for compound **20**, starting from **16**, gave **23** (90% over two steps) as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (rotamers) 7.32–7.21 (m, 5H), 6.40–6.38 (m, 1H), 4.45–4.40 (m, 1H), 3.75–3.73 (d, 3H, *J* = 12 Hz), 3.47–3.39 (m, 1H), 3.34–3.26 (m, 3H), 3.22–3.14 (m, 0.5H), 3.11–3.03 (m, 0.5H), 2.96–2.88 (m, 2H), 2.49–2.32 (m, 1H), 2.20–2.06 (m, 1H), 2.04–1.99 (m, 2H), 1.93–1.90 (m, 2H), 1.88–1.79 (m, 1H), 1.75–1.57 (m, 2H), 1.54–1.40 (m, 1H), 1.34–1.15 (m, 3H); ESI/MS for $C_{22}H_{30}N_2Oy$ calculated ($M+H^+$) 403.2, found 403.2.

(2*S*,3a*R*,6*S*,7a*R*)-6-Benzyloxy-1-(2-(phenylmethanesulfonylaminoacetyl)octahydroindole-2-carboxylic acid methyl ester (24). Same procedure as for compound **20**, starting from **17**, gave **24** (98% over two steps) as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (rotamers) 7.44–7.36 (m, 10H), 5.34 (s, 1H), 4.54–4.43 (m, 3H), 4.30 (s, 2H), 4.12 (m, 0.5H), 3.95 (m, 0.5H), 3.73–3.71 (m, 5H), 3.64–3.60 (m, 1H), 2.63–2.61 (m, 1H), 2.30–2.27 (m, 2H), 2.13–2.01 (m, 4H), 1.83–1.81 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (rotamers) 171.8, 162.2, 138.1, 130.5, 128.8 (2C), 128.3, 128.2, 128.1 (2C), 127.3 (2C), 127.2, 127.0, 71.6, 69.8, 59.3, 58.0, 53.3, 44.4, 36.2, 34.9, 31.7, 29.5, 22.5, 18.9; ESI/MS for $C_{26}H_{32}N_2O_6S$ calculated ($M+H^+$) 501.2, found 501.2.

(2*S*,3a*R*,6*S*,7a*R*)-6-Methoxy-1-(2-(phenylmethanesulfonylaminoacetyl)octahydroindole-2-carboxylic acid methyl ester (25). Same procedure as for compound **20**, starting from **18**, gave **25** (98% over two steps) as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (rotamers) 7.42–7.29 (m, 5H), 5.52

(s, 1H), 4.44 (d, 1H, *J* = 9.5 Hz), 4.30–4.29 (m, 2H), 3.82–3.78 (m, 1H), 3.73–3.63 (m, 5H), 3.49 (s, 1H), 3.25 (s, 3H), 2.63–2.56 (m, 1H), 2.30–2.19 (m, 2H), 2.03–1.91 (m, 4H), 1.79–1.77 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (rotamers) 171.8, 162.3, 130.5, 130.0, 129.0, 128.7, 128.3, 128.2, 73.6, 60.0, 59.1, 58.0, 55.4, 52.0, 53.3, 44.4, 36.1, 31.0, 21.9, 20.7; ESI/MS for $C_{20}H_{28}N_2O_6S$ calculated ($M+H^+$) 425.2, found 425.3.

(2*S*,3a*R*,6*R*,7a*R*)-6-Benzyloxy-1-(2-(phenylmethanesulfonylaminoacetyl)octahydroindole-2-carboxylic acid methyl ester (26). Same procedure as for compound **20**, starting from **19**, gave **26** (70% over two steps) as colorless oil. ^{1}H NMR, (400 MHz, $CDCl_3$) δ (major rotamer) 7.48–7.27 (m, 10H), 5.30 (m, 1H), 4.62–4.40 (m, 3H), 4.48–4.25 (m, 2H), 3.84 (dd, 1H, *J* = 4.8, 16.5 Hz), 3.73–3.69 (m, 4H), 3.62 (dd, 1H, *J* = 4.4, 16.5 Hz), 3.25 (m, 1H), 2.84 (m, 1H), 2.31 (m, 1H), 2.10–1.66 (m, 5H), 1.46–1.15 (m, 2H); ^{13}C NMR, (100 MHz, $CDCl_3$) δ (rotamers) 172.0, 165.9, 138.2, 130.9, 130.8, 129.1, 128.8, 128.7, 128.5, 128.4, 127.7, 127.5, 74.8, 70.3, 59.7, 58.3, 56.6, 52.4, 44.9, 35.2, 34.9, 29.8, 26.0, 22.4; ESI/MS for $C_{26}H_{32}N_2O_6S$ calculated ($M+H^+$) 501.2, found 501.2.

(2*S*,3a*S*,6*R*,7a*S*)-1-((*R*)-2-Acetylamino-3-phenylpropionyl)-6-methoxyoctahydroindole-2-carboxylic acid 4-carbamimidoylbenzylamide (4). To a solution of **23** (0.019 g, 0.047 mmol) in THF (2 ml) was added 1 dr. H_2O and LiOH (0.008 g, 0.19 mmol). After stirring at RT for 19 h the THF was removed under reduced pressure and the residue acidified with 2 M aq. HCl. The mixture was extracted with EtOAc and the combined organic phases were dried (Na_2SO_4) and concentrated under reduced pressure. The crude carboxylic acid was dissolved in DMF (2 ml) and bis N-Cbz *p*-amidinobenzylamine (0.028 g, 0.097 mmol) was added. The mixture was cooled to 0 °C and BOP (0.028 g, 0.064 mmol) and NMM (30 µl, 0.27 mmol) were added. The reaction mixture was stirred for 3 h and the reaction was quenched by addition of 2 M aq. NH_4Cl (0.8 ml). The mixture was extracted with EtOAc and the combined organic phases were dried (Na_2SO_4), concentrated under reduced pressure, and purified by flash chromatography over silica gel (EtOAc/hexane, 4:1–1:0, EtOAc/MeOH, 97:3–95:5) to give the fully protected target molecule (0.013 g, 41%). The benzyl- and Cbz-protected intermediate (0.010 g, 0.015 mmol) was dissolved in MeOH (2 ml) and 1 dr. aq. HCl (conc.) and Pd/C 10 wt. % (20 mol%) were added and the mixture was stirred under H_2 (1 atm) at RT for 24 h. The Pd/C catalyst was removed by filtration though celite and the filtrate was concentrated under reduced pressure to give **4** (0.0085 g, 100 %) as the hydrochloride salt. ^{1}H NMR (400 MHz, CD_3OD) δ (rotamers) 9.30 (s, 1H), 8.78 (s, 1H), 7.89–7.80 (m, 2H), 7.70–7.61 (m, 2H), 7.36–7.22 (m, 5H), 4.76–4.40 (m, 5H), 3.38–3.24 (m, 3H), 3.19–3.09 (m, 1H), 3.03–2.95 (m, 1H), 2.93–2.82 (m, 1H), 2.47–2.30 (m, 1H), 2.19–1.88 (m, 3H), 1.80–1.21 (m, 8H); ^{13}C NMR (100 MHz, CD_3OD) δ (rotamers) 176.7, 175.1, 170.6, 163.4, 149.6, 140.8, 132.9, 132.8 (2C), 132.2, 132.0 (2C), 131.6, 131.4, 130.5, 130.3, 78.4, 64.6, 58.9, 58.5, 41.4, 40.4, 34.6, 34.3, 33.1, 32.8, 26.3, 24.5, 22.8; $[\alpha]_D$ –3.17° (*c* 0.82, MeOH); HRMS for $C_{29}H_{37}N_5O_4$ calculated ($M+H^+$) 520.29249, found 520.29183.

(2*S*,3a*S*,6*R*,7a*S*)-6-Hydroxy-1-(2-(phenylmethanesulfonylaminoacetyl)octahydroindole-2-carboxylic acid 4-carbamimidoylbenzylamide (1). Same procedure as for compound **4**, starting from **20**, gave **1** (45% over three steps) as the hydrochloride salt. ^{1}H NMR (400 MHz, CD_3OD) δ (rotamers) 7.81–7.31 (m, 11H), 4.66–4.32 (m, 5H), 4.18–3.59 (m, 3H), 2.53–1.97 (m, 3H), 1.87–1.74 (m, 2H), 1.68–1.46 (m, 2H), 1.38–1.21 (m, 4H); ^{13}C NMR (100 MHz, CD_3OD) δ (rotamers) 174.7, 169.2, 168.1, 147.1, 132.1 (2C), 131.2, 129.5 (2C), 129.4 (2C), 129.1, 128.9, 128.1, 127.9, 66.6, 62.2, 59.9, 55.6, 45.6, 43.6, 38.2, 34.2, 31.8, 26.9, 20.1; $[\alpha]_D$ +0.97° (*c* 1.23, MeOH); HRMS for $C_{26}H_{33}N_5O_5S$ calculated ($M+H^+$) 528.22888, found 528.22752.

(2*S*,3a*S*,6*R*,7a*S*)-6-Methoxy-1-(2-(phenylmethanesulfonylaminoacetyl)octahydroindole-2-carboxylic acid 4-carbamimidoylbenzylamide (2). Same procedure as for compound **4**, starting from **21**, gave **2** (40% over three steps) as the hydrochloride salt. ^{1}H NMR (400 MHz, CD_3OD) δ (rotamers) 7.86–7.36 (m, 9H), 4.67–4.33 (m, 5H), 4.14–3.72 (m, 2H), 3.68–3.51 (m, 1H), 3.43–3.25 (s, 3H), 2.55–1.97 (m, 3H), 1.90–1.55 (m, 5H), 1.50–1.19 (m, 4H); ^{13}C NMR (100 MHz, CD_3OD) δ (rotamers) 172.9, 171.4, 167.3, 166.3, 145.3, 130.4, 129.5, 127.8, 127.7, 127.4, 127.3, 127.2, 126.1, 74.3, 60.4, 58.4, 54.4, 54.0, 43.9, 41.8, 38.5, 37.3, 30.2, 26.7, 22.1, 21.5, 18.5; $[\alpha]_D$ –0.63° (*c* 1.23, MeOH); HRMS for $C_{27}H_{35}N_5O_5S$ calculated ($M+H^+$) 542.24383, found 542.24317.

(2*S*,3a*S*,6*R*,7a*S*)-1-((*R*)-2-Acetylamino-3-phenylpropionyl)-6-hydroxyoctahydroindole-2-carboxylic acid 4-carbamimidoylbenzylamide (3). Same procedure as for compound **4**, starting from **22**, gave **3** (45% over three steps) as the hydrochloride salt. ^{1}H NMR (400 MHz, CD_3OD) δ (rotamers) 9.28 (s, 1H), 8.75 (s, 1H), 7.89–7.77 (m, 2H), 7.75–7.62 (m, 2H), 7.36–7.20 (m, 5H), 4.81–4.41 (m, 5H), 3.18–3.09 (m, 1H), 3.05–2.97 (m, 1H), 2.95–2.86 (m, 1H), 2.47–2.38 (m, 1H), 2.22–2.09 (m, 3H), 2.07–1.77 (m, 4H), 1.72–1.24 (m, 5H); ^{13}C NMR (100 MHz, CD_3OD) δ (rotamers) 172.6, 171.3, 170.9, 166.4, 145.4, 136.3, 128.9, 128.8, 128.6, 128.1, 127.8, 127.4, 127.3, 126.6, 126.3, 126.1, 64.9, 60.4, 54.7, 52.3, 41.8, 37.2, 36.4, 33.2, 30.2, 25.0, 20.2, 18.3; $[\alpha]_D$ –5.98° (*c* 0.92, MeOH); HRMS for $C_{28}H_{35}N_5O_4$ calculated ($M+H^+$) 506.27684, found 506.27618.

(2*S*,3a*S*,6*R*,7a*S*)-1-((*R*)-2-Acetylamino-3-phenylpropionyl)-6-benzyloxyoctahydroindole-2-carboxylic acid 4-carbamimidoylbenzylamide (5). Essentially the same procedure as for compound **4**, apart from a shorter hydrogenation time (maximum 16 h), starting from **22**, gave **5** (45% over three steps) as the hydrochloride salt. ^{1}H NMR (400 MHz, CD_3OD) δ (rotamers) 7.85–7.72 (m, 2H), 7.62–7.50 (m, 2H), 7.37–7.22 (m, 10H), 4.74–4.62 (m, 2H), 4.57–4.38 (m, 5H), 3.19–3.09 (m, 1H), 3.06–2.97 (m, 1H), 2.95–2.83 (m, 1H), 2.47–2.38 (m, 1H), 2.20–2.01 (m, 3H), 1.97–1.26 (m, 10H); ^{13}C NMR (100 MHz, CD_3OD) δ (rotamers) 172.4, 171.3, 170.9, 166.1, 143.2, 138.5, 136.7, 130.3, 128.8, 128.7, 128.6, 128.0, 127.9, 127.6, 127.5, 127.4, 127.0, 126.8 (2C), 126.7, 126.5, 126.2, 72.3, 69.2, 60.4, 55.0, 52.5, 37.6, 36.3, 34.4, 30.5, 30.2, 22.6 20.3, 18.9; $[\alpha]_D$ –21.85° (*c* 1.3, MeOH); HRMS for $C_{33}H_{41}N_5O_4$ calculated ($M+H^+$) 596.32379, found 596.32313.

(2*S*,3a*R*,6*S*,7a*R*)-6-Hydroxy-1-(2-(phenylmethanesulfonylaminoacetyl)octahydroindole-2-carboxylic acid 4-carbamimidoylbenzylamide (6). Same procedure as for compound **4**, starting from **24**, gave **6** (42% over three steps) as the hydrochloride salt. ^{1}H NMR (400 MHz, CD_3OD) δ (rotamers) 9.18 (s, 1H), 8.72 (s, 1H), 7.81–7.27 (m, 9H), 4.64–4.30 (m, 5H), 4.19–3.62 (m, 3H), 2.57–2.31 (m, 3H), 2.28–1.92 (m, 3H), 1.89–1.26 (m, 7H); ^{13}C NMR (100 MHz, CD_3OD) δ (rotamers) 175.1, 169.4, 168.1, 147.1, 132.1, 131.2, 131.1, 129.5 (2C), 129.4 (2C), 129.0, 128.3, 127.8, 66.6, 61.2, 59.9, 55.3, 45.7, 38.2, 37.1, 35.4, 34.5, 31.7, 26.8, 20.0; $[\alpha]_D$ –0.82° (*c* 1.58, MeOH); HRMS for $C_{26}H_{33}N_5O_5S$ calculated ($M+H^+$) 528.22818, found 528.22752.

(2*S*,3a*R*,6*S*,7a*R*)-6-Methoxy-1-(2-(phenylmethanesulfonylaminoacetyl)octahydroindole-2-carboxylic acid 4-carbamimidoylbenzylamide (7). Same procedure as for compound **4**, starting from **25**, gave **7** (43% over three steps) as the hydrochloride salt. ^{1}H NMR (400 MHz, CD_3OD) δ (rotamers) 9.36 (s, 1H), 8.91 (s, 1H), 7.96–7.31 (m, 9H), 4.58–4.20 (m, 5H), 4.11–3.49 (m, 3H), 3.38–3.19 (m, 3H), 2.71–2.33 (m, 3H), 2.27–1.99 (m, 3H), 1.80–1.69 (m, 2H), 1.63–1.21 (m, 4H); ^{13}C NMR (100 MHz, CD_3OD) δ (rotamers) 175.1, 169.4, 168.1, 167.8, 147.1, 140.7, 132.2, 131.2, 131.0, 130.1, 129.8, 129.6, 129.5, 129.3, 129.1, 127.8, 75.9, 61.2, 60.2, 60.1, 56.2, 45.9, 43.5, 36.9, 32.2, 31.8, 23.1; $[\alpha]_D$ +4.51° (*c* 2.55, MeOH); HRMS for $C_{27}H_{35}N_5O_5S$ calculated ($M+H^+$) 542.24383, found 542.24317.

(2*S*,3a*R*,6*R*,7a*R*)-6-Hydroxy-1-(2-(phenylmethanesulfonylaminoacetyl)octahydroindole-2-carboxylic acid 4-carbamimidoylbenzylamide (8). Essentially the same procedure as for compound **4**, apart from using PyBOP and 2,6-lutidine instead of BOP and NMM and starting from **26**, gave **8** (42% over three steps) as the hydrochloride salt. ^{1}H NMR, (400 MHz, CD_3OD) δ (rotamers) 7.81–7.76 (m, 0.4H), 7.74–7.67 (m, 1.6H), 7.60–7.54 (m, 2H), 7.50–7.41 (m, 2H), 7.40–7.34 (m, 3H), 4.63–4.35 (m, 5H), 3.99–3.83 (m, 1.7H), 3.73 (m, 1.3H), 3.54–3.45 (m, 1H), 2.62–2.50 (m, 1H), 2.46–2.27 (m, 1H), 2.23–1.95 (m, 2H), 1.90–1.54 (m, 3H), 1.37–1.15 (m, 2H); ^{13}C NMR, (100 MHz, CD_3OD) δ (rotamers) 174.9, 174.6, 174.2, 169.4, 169.2, 168.1, 147.12, 147.09, 132.2, 131.3, 129.6, 129.5, 129.4, 129.1, 129.01, 128.97, 127.9, 68.9, 68.7, 62.2, 61.2, 60.3, 60.2, 58.8, 58.4, 45.9, 43.5, 38.2, 37.8, 36.6, 31.7, 30.1, 23.7; $[\alpha]_D$ –4.8° (*c* 0.73, MeOH); HRMS for $C_{26}H_{33}N_5O_5S$ calculated ($M+H^+$) 528.22752, found 528.22885.

4.2. *Enzyme Assays*

The enzyme assays were performed as described earlier.[18]

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[1] See for example: a) F. E. Koehn, G. T. Center, *Nat. Rev. Drug Discovery* **2005**, *4*, 206; b) D. J. Newman, G. M. Cragg, *Curr. Med. Chem.* **2004**, *11*, 1693; c) J. Clardy, C. Walsh, *Nature* **2004**, *432*, 829; d) P. Vuorela, M. Leinonen, P. Saikku, P. Tammela, J.-P. Rauha, T. Wennberg, H. Vuorela, *Curr. Med. Chem*. **2004**, *11*, 1375.

[2] D. J. Newman, G. M. Cragg, *Nat. Prod.* **2005**, 129.

[3] See for example: F. von Nussbaum, M. Brands, B. Hinzen, S. Weigand, D. Häbich, *Angew. Chem., Int. Ed*. **2006**, *45*, 5072.

[4] See for example: S. Hanessian, *ChemMedChem* **2006**, *1*, 1300, and references cited therein.

[5] Review on aeruginosins: K. Ersmark, J. R. Del Valle, S. Hanessian, *Angew. Chem., Int. Ed.* **2007**, in press. See also: M. Murakami, Y. Okita, H. Matsuda, T. Okino, K. Yamaguchi, *Tetrahedron Lett.* **1994**, *35*, 3129.

[6] Aeruginosin 298A: a) N. Valls, M. López-Canet, M. Vallribera, J. Bonjoch, *J. Am. Chem. Soc.* **2000**, *122*, 11248; b) P. Wipf, J. L. Methot, *Org. Lett.* **2000**, *2*, 4213. Aeruginosin 298B: c) N. Valls, M. López-Canet, M. Vallribera, J. Bonjoch, *Chem. Eur. J.* **2001**, *7*, 3446. Aeruginosin EI461: d) N. Valls, M. Vallribera, S. Carmeli, J. Bonjoch, *Org. Lett.* **2003**, *5*, 447. Oscillarin: e) S. Hanessian, M. Tremblay, J. F. W. Petersen, *J. Am. Chem. Soc*. **2004**, *126*, 6064.

[7] 'Hemostasis and Thrombosis. Basic Principles and Clinical Practice', Eds. R. W. Colman, J. L. Hirsh, J. V. Marder, A. W. Clowes, J. N. George, Lippincott Williams & Wilkins, Philadelphia, **2001**, p. 3.

[8] J. D. Kulman, E. W. Davie, *Encyclopedia of Biological Chemistry* **2004**, *3*, 457.

[9] E. W. Davie, *J. Biol. Chem.* **2003**, *278*, 50819.

[10] B. Dahlbäck, *Lancet* **2000**, *355*, 1627.

[11] S. Hanessian, J. R. Del Valle, Y. Xue, N. Blomberg, *J. Am. Chem. Soc*. **2006**, *128*, 10491. See also: G. H. Goetz, G. G. Harrigan, J. J. Likos, T. P. Kasten, PCT WO03/051831, **2003**.

[12] See for example: a) S. Srivastava, L. N. Goswami, D. K. Dikshit, *Med. Res. Rev.* **2005**, *25*, 66; b) R. M. Scarborough, A. Pandey, X. Zhang, *Annu. Rep. Med. Chem*. **2005**, *40*, 85; c) R. Pfau, *Curr. Opin. Drug Discov. Develop*. **2003**, *6*, 437; d) P. Betschmann, C. Lerner, S. Sahli, U. Obst, F. Diederich, *Chimia* **2000**, *54*, 633; e) D. Leung, G. Abbenante, D. P. Fairlie, *J. Med. Chem*. **2000**, *43*, 305; f) R. E. Babine, S. L. Bender, *Chem. Rev.* **1997**, *97*, 1359.

[13] Y. Fukuta, T. Ohshima, V. Gnanadesikan, T. Shibuguchi, T. Nemoto, T. Kisugi, T. Okino, M. Shibasaki, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 5433.

[14] T. Doi, Y. Hoshina, H. Mogi, Y. Yamada, T. Takahashi, *J. Comb. Chem.* **2006**, *8*, 571.

[15] S. Hanessian, E. Therrien, W. A. L. van Otterlo, M. Bayrakdarian, I. Nilsson, O. Fjellström, Y. Xue, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1032.

[16] A. S. Cieplak, *J. Am. Chem. Soc.* **1981**, *103*, 4540.

[17] M. Chérest, H. Felkin, *Tetrahedron Lett.* **1968**, *9*, 2205.

[18] D. Gustafsson, T. Antonsson, R. Bylund, U. Eriksson, E. Gyzander, I. Nilsson, M. Elg, C. Mattsson, J. Deinum, S. Pehrsson, O. Karlsson, A. Nilsson, H. Sörensen, *Thromb. Haemost*. **1998**, *79*, 110.

[19] S. Hanessian, K. Ersmark, X. Wang, J. R. Del Valle, N. Blomberg, Y. Xue, O. Fjellström, *Bioorg. Med. Chem.* **2007**, in press.