First Total Syntheses of Aeruginosin 298-A and Aeruginosin 298-B, Based on a Stereocontrolled Route to the New Amino Acid 6-Hydroxyoctahydroindole-2-carboxylic Acid

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Abstract: The first total syntheses of aeruginosin 298-A (**1**) and aeruginosin 298-B (**3**) are described. The syntheses of the alternative putative structures **2** and **4** were also accomplished. The key common strategic element is the stereocontrolled synthesis of (2*S*,3a*S*,6*R*,7a*S*)-6-hydroxyoctahydroindole-2-carboxylic acid (L-Choi, **5**) from L-tyrosine. The synthesis of this new bicyclic α-amino acid, which is the core of aeruginosins, involves Birch reduction of *O*-methyl-L-tyrosine (**6**) and aminocyclization of the resulting dihydroanisole **7** in acid medium, followed by *N*-benzylation to give the diastereoisomers **12** and **13**. Upon acid treatment with HCl-MeOH, the last two produce an equilibrium mixture in which the *endo* isomer **13** significantly predominates. Hydrogenation of **13** in the presence of $(Boc)_2O$ gives **16**, which on reduction with LS-Selectride furnishes the alcohol **22**, a protected L-Choi. Successive couplings of **22** with D-leucine, protected (*R*)-(4-hydroxyphenyl)lactic acid, and L-arginine fragments, followed by reduction to the argininol level and a deprotection end step complete the synthetic sequence to produce aeruginosin 298-A (**1**). Spectral comparison showed that peptide **2**, with the structure previously proposed for aeruginosin 298-A, was different from the natural product. However, synthetic **1** was found to be identical to the isolated natural sample of aeruginosin 298-A. These results unequivocally establish that the absolute stereochemistry of aeruginosin 298-A, formerly assigned incorrectly, is D-Hpla-D-Leu-L-Choi-L-Argol, as shown by structure **1**. Aeruginosin 298-B was also synthesized and shown to be a mixture of rotamers of D-Hpla-D-Leu-L-ChoiNH_2 (**3**), rather than an epimeric mixture of **3** and the L-Leu-incorporating **4**.

Keywords: aeruginosins · amino acids · peptides · total synthesis · thrombin inhibitor

Introduction

Cyanobacteria (blue-green algae) are prokaryotic, photosynthetic microorganisms that have been shown to be a rich source of a variety of structurally novel, bioactive nitrogen compounds.[1] Among the wide range of serine protease inhibitors found in cyanobacteria, aeruginosins are a group of linear peptides that have in common a new, nonproteinogenic α-amino acid that contains a *cis*-perhydroindole ring. The aeruginosins isolated from *Microcystis* species contain the new bicyclic amino acid 2-carboxy-6-hydroxyoctahydroindole (Choi),[2] while those found in *Oscillatoria agardhii* incorporate a 6-chloro analog (Ccoi).[3] Other common structural trends in aeruginosins are: i) a D-amino acid unit linked to the Choi nitrogen atom,[4] ii) an (*R*)-(4-hydroxyphenyl)lactic acid (D-Hpla) moiety or derivative at the N-terminus, and iii) a guanidine-containing unit at the C-terminus (except for aeruginosin 298-B), in which the arginine is present in a reduced (Argol or Argal) or decarboxylated (Agmatin) form. In addition to the thirteen aeruginosins isolated by Murakami since 1994[5] (see Scheme 1), Carmelli has reported the isolation of microcin SF608, a closely related peptide, from *Microcystis sp.* waterbloom.[6]

Aeruginosin 298-A was the first member of this family to be reported and our initial synthetic target. It was isolated from the toxic strain of *M. aeruginosa* NIES-298 and found to inhibit thrombin and trypsin.[2a] The absolute stereochemistry of aeruginosin 298-A was determined in 1998 by single-crystal X-ray diffraction analysis of the ternary complex of aeruginosin 298-A bound to hirugen-thrombin,[7] but the putative structure **2** assigned then should be revised in the light of the data presented in this work.[8]

In this paper, we describe the first synthesis of the amino acid (2*S*,3a*S*,6*R*,7a*S*)-6-hydroxy-octahydroindole-2-carboxylic acid (**5**, L-Choi), which is the common structural motif shared by almost all aeruginosins, and we report the use of Choi derivatives for the total syntheses of aeruginosin 298-A (**1**) and the peptide **2**, the structure of which is the same as that

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previously proposed for aeruginosin 298-A.[2a, 7] Our results have led to a revision of the structure originally assigned to the natural product, which we have concluded to be **1** rather than **2**. We also describe the syntheses of aeruginosin 298-B (**3**) and its epimer **4** (Scheme 2), thus clarifying that this natural product exists as a mixture of conformational isomers and not a mixture of Leu epimers as has been reported.[2e]

The construction of the whole carbon skeletons for these peptides involves the synthesis of an appropriately functionalized and stereochemically pure octahydroindole (Choi core), and its stepwise elongation with the other fragments, the unit linked at the C-terminus being incorporated in the last step.

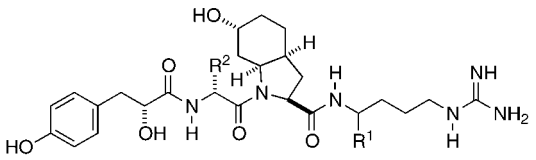
Results and Discussion

The first stage: synthesis of azabicyclic ketone 13: Our approach to the synthesis of the amino acid L-Choi (**5**) involved the stereoselective formation of ketone **13**,[9, 10] which could be obtained by Birch reduction of tyrosine derivative **6**, followed by an aminocyclization onto the masked enone present in the Birch reduction compound (Scheme 3). This synthetic route to octahydroindol-6-ones takes advantage of the presence of the β-amino ketone moiety for the formation of the last bond, which involves an

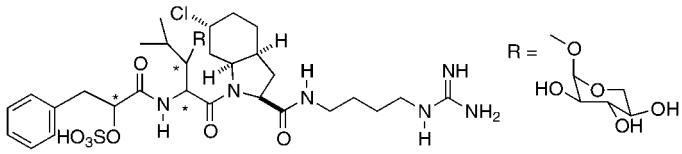
Abstract in Spanish: *Se describen las síntesis totales de las aeruginosinas 298-A (**1**) y 298-B (**3**). También se han llevado a cabo las síntesis de las estructuras putativas **2** y **4**. El elemento estratégico común es la síntesis estereocontrolada del ácido (2S,3aS,6R,7aS)-6-hidroxioctahidroindol-2-carboxílico (L-Choi, **5**) a partir de la L-tirosina. La síntesis de este nuevo aminoácido bicíclico, núcleo central de las aeruginosinas, implica la reducción de Birch de la O-metil-L-tirosina (**6**), seguida de aminociclación en medio ácido del dihidroanisol **7** resultante y subsiguiente N-bencilación, proporcionando los azabiciclos **12** y **13** que posteriormente se equilibran bajo tratamiento ácido (HCl-MeOH). La hidrogenación del mayoritario isómero endo **13** en presencia de $(Boc)_2O$ proporciona **16**, que por reducción con LS-Selectide rinde el alcohol **22**, una forma protegida de L-Choi. Sucesivos acoplamientos a partir de **22** con fragmentos de D-Leucina y formas protegidas del ácido (R)-hidroxifenilláctico y L-arginina, seguidos de una reducción al grado de argininol y una etapa final de desprotección, completan la secuencia sintética para la obtención de la aeruginosina 298-A.*

*La comparación de los datos espectroscópicos mostró que el péptido **2**, con la estructura propuesta para la aeruginosina 298-A, era diferente al producto natural. Sin embargo, el compuesto sintético **1** mostró ser idéntico a la aeruginosina 298-A natural. Estos resultados establecen de forma inambigua que la configuración absoluta de la aeruginosina 298-A, incorrectamente asignada anteriormente, es D-Hpla-D-Leu-L-Choi-L-Argol.*

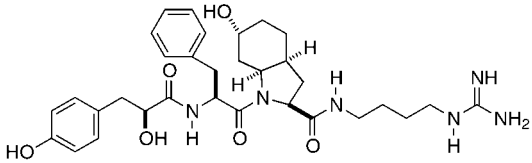
*La aeruginosina 298-B también fue sintetizada y su estructura es una mezcla de rotámeros de D-Hpla-D-Leu-L-ChoiNH_2 (**3**) en vez de la anteriormente propuesta de una mezcla epimérica de **3** y **4**, que incorpora una L-Leu.*



	R^1	R^2	ref
Aeruginosin 89-A[a]	CHO	D-Leu	2e
Aeruginosin 98-B[b]	H	D-alloLeu	2b
Aeruginosin 102-A[c]	CHO	D-Tyr	2c
Aeruginosin 103-A[d]	CHO	D-Tyr	2d
Aeruginosin 298-A[e]	CH2OH	D-Leu	2a/4

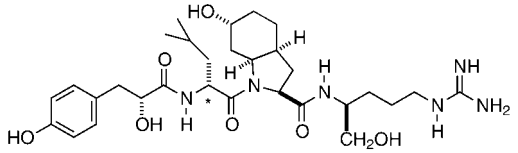


Aeruginosin 205-A L-Plas, (2*R*,3*S*)-HLeu
Aeruginosin 205-B D-Plas, (2*S*,3*R*)-HLeu
ref 3

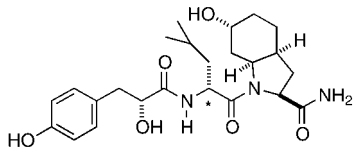


Microcin SF608 ref 6

Scheme 1. Structure of aeruginosins. [a] Phenol ring sulfated and *o*-substituted by a chloro atom. L-Argal unit has hemiaminal form. Aeruginosin 89-B has a D-Argal unit. [b] Aeruginosins 98-A and 98-C have a chloro and a bromo atom, respectively, as *o*-substituents in the phenol ring. Aeruginosin 101 is the *o*,*o*-dichloro derivative. The Choi 6-hydroxyl is sulfated in all cases. [c] Sulfated phenol ring. L-Argal unit has hemiaminal form. Aeruginosin 102-B has a D-Argal unit. [d] Hemiaminal cyclic form (*O*-ethyl). [e] Aeruginosin 298-B has a carboxamido group at Choi C(2).



Revised structure of aeruginosin 298-A (**1**)
Original structure of aeruginosin 298-A (**2**, L-Leu epimer at C*)



Revised structure of aeruginosin 298-B (**3**)
Original structure of aeruginosin 298-B
(3:1 mixture of **3** and its L-Leu epimer at C* **4**)

Scheme 2. Previously proposed and revised structures for aeruginosins 298-A and 298-B.

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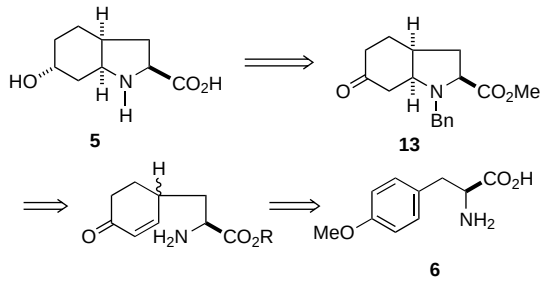
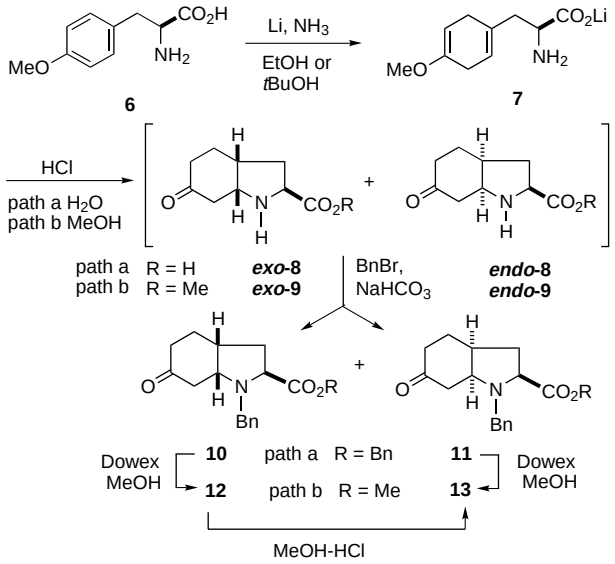


Scheme 3. Retrosynthesic analysis of L-Choi.

intramolecular 1,4-addition of an amino group across a cyclohexenone intermediate.[11]

Our studies began with the Birch reduction of *O*-methyltyrosine (**6**), by using lithium in ammonia and ethanol.[12] In our initial studies,[13] the resulting dihydroanisole **7** was treated in aqueous acid medium (3 N HCl) to give a mixture of *cis* compounds **8** (*exo* and *endo* isomers),[14] which was submitted to a benzylation process (BnBr, EtOH, $NaHCO_3$) to give the two octahydroindoles **10** and **11** in a 1:1 ratio and 44% overall yield from **6** (Scheme 4). Both **10** and **11** were



Scheme 4. Synthesis of *cis*-6-oxo-octahydroindole-2-carboxylic acid derivatives.

converted by a transesterification process into the corresponding methyl esters **12** and **13**. Although the configurations of **10**–**13** were unequivocally assigned by NMR experiments (see below), both **12** and **13** were transformed into the corresponding *N*-acetyl derivatives **14** and **15** for analytical purposes, by hydrogenation in an acetic anhydride medium, in order to check their NMR data against those corresponding to the *endo* derivative (*rac*-**15**), the X-ray structure of which had been reported previously.[9b]

Taking into account that only the ketone **13** (*endo* isomer) can be considered to be a precursor of the target Choi, we were pleased to find that treatment of *exo* isomer **12** with methanolic HCl produced the desired *endo* isomer **13** in 87% yield, accompanied by about 11% of starting material, which could be removed by chromatographic separation and then reused. The equilibration of these β-amino ketones[15] may occur through acid-mediated β-elimination, vinylogous enolization, and intramolecular Michael readdition.

At that point, we thought we might improve the synthesis of azabicyclo **13** by carrying out the cyclization of dihydroanisole **7** under the same reaction conditions as used for the equilibration (**12** → **13**) so as at least to avoid the transesterification process (Scheme 4, path b). Thus, dihydroanisole **7** was treated with a methanolic solution of hydrogen chloride to give a mixture of octahydroindoles **9** (*exo*/*endo*), which was benzylated to furnish a diastereomeric mixture of *exo* and *endo* isomers **12** and **13** in a 1.8:1 ratio, in 52% overall yield. The probable explanation for this improvement (milder reaction conditions and better yields) is that cyclization takes place much more readily when α-amino esters are used instead of α-amino acids for the intramolecular 1,4-addition.[9c] After the acid-mediated equilibration of the mixture of diastereoisomers **12** and **13**, enantiopure octahydroindolone **13** could be obtained in multigram amounts, the overall yield for these four initial steps being 44%. The stereochemical integrity of **12** and **13** was confirmed by ^{1}H NMR by using the Pirkle alcohol procedure.[16]

The above results indicate that the secondary amine *exo*-**9** is slightly more stable than the isomer *endo*-**9**, but that, after *N*-benzylation, **13** (the *endo* isomer) is thermodynamically more stable than **12** (the *exo* isomer). This stability change agrees with molecular mechanics calculations,[17] which showed the hydrochloride of **13** to be at least 2.65 kcal mol^{-1} more stable than the hydrochloride of **12**, probably because only the *endo* isomer **13** can incorporate an intramolecular hydrogen bond between the carbonyl of the ester group at C(2) and the proton of the amine salt.

NMR structural analysis of octahydroindolones: Two NMR features clearly differentiate the *exo* and *endo* series of *cis*-6-oxo-octahydroindole-2-carboxylic acid alkyl esters: i) the ^{1}H NMR coupling constant pattern of H-2 [which is a doublet in the *exo* series (**10**, **12**, and **14**) but a doublet of doublets, in fact a pseudo triplet, in the *endo* series (**11**, **13**, **15**, and **16**][18, 19] and the zero values of the ^3J coupling between H-2 and H-3β in *exo* compounds—indicating that the pyrrolidine ring adopts a conformation in which C(2) is puckered in toward the *exo* surface of the bicyclic system, and ii) the chemical shifts of H-3a and H-7a, which are more deshielded in the *exo* derivatives than in the *endo* compounds due to the *syn* relationship of the alkoxycarbonyl group with both protons (Figure 1).

NMR studies also allowed us to assign the conformational preference of *cis*-6-oxo-octahydroindole-2-carboxylic acid alkyl ester derivatives, including the positions of the nitrogen lone-pair electrons, when present. As shown in Figure 2, c_1 and c_2 refer to the *N*-outside and *N*-inside conformers of the conformationally mobile *cis*-octahydroindole system,[20] respectively (H-7a axial and H-7a equatorial with respect to the carbocyclic ring). *N*-Benzylated compounds (**10**–**13**) have deformed c_2 conformations (half-boat in the cyclohexanone ring), rather than the flip form c_2 (chair). The H7–H7a coupling constants (both 5 Hz) are consistent with gauche

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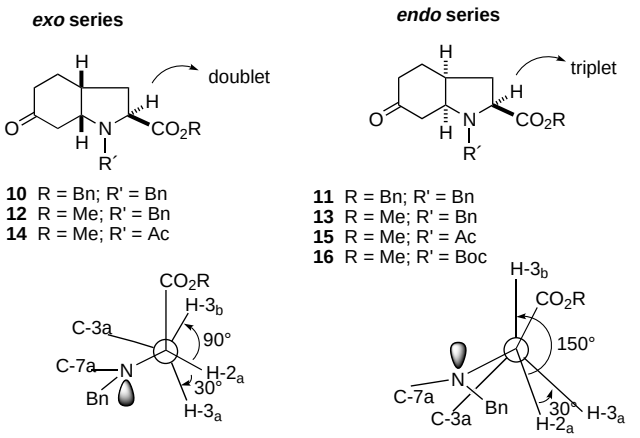


Figure 1. NMR behavior of *exo* and *endo cis*-octahydroindole-2-carboxylic acid derivatives.

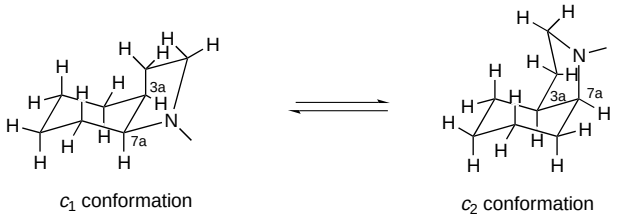


Figure 2. Conformational equilibria in octahydroindoles.

couplings; this indicates that the H-7a proton is equatorially situated with respect to the carbocyclic ring. NMR NOESY data and molecular mechanics calculations[18] agree with the twisted conformation depicted in Figure 3 for azabicyclic compound **13**.

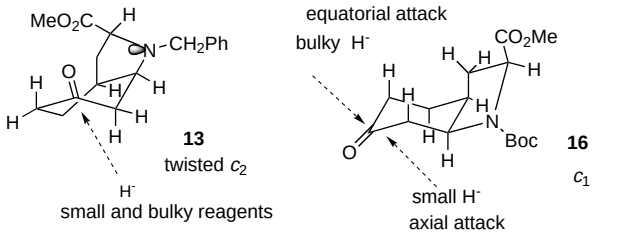
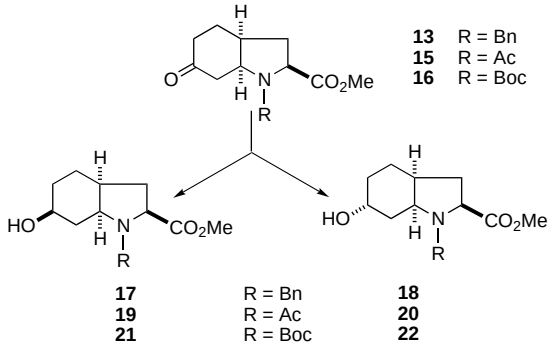


Figure 3. Different behavior in reduction processes involving ketones **13** and **16**.

In contrast, *N*-acetyl derivatives **14** and **15**, together with *N*-Boc compound **16**, had c_1 as the preferred conformation (compare, for example, the H7ax–H7a coupling of 11 Hz). These derivatives have a strong preference for a c_1 conformation to avoid the $A^{1,3}$ strain between the *N*-substituent and the C(7) methylene group.[21] Whereas the 500 MHz ^{1}H NMR spectrum of the *N*-Boc derivative **16** at 20 °C is difficult to interpret, owing to the hindered rotation around the carbon–nitrogen bond of the carbamate moiety, all protons are well resolved in the spectrum at 80 °C, and the proton H-2 appears as a broadened triplet ($J = 8.5$ Hz) at $\delta = 4.26$.

Synthesis of L-Choi (5): To reach the target **5** from ketone **13**, which is available in multigram quantities following path b shown in Scheme 4, we needed to produce the alcohol with the 6*R* configuration. All attempts to satisfactorily obtain the alcohol **18** from ketone **13** were fruitless, because under all the conditions used ($NaBH_4$, L-Selectride, LS-Selectride, and so on) the 6*S* alcohol **17** was the major product (Scheme 5,



Scheme 5. Reduction of octahydroindolones (cf. Table 1).

Table 1). However, we decided to optimize the synthesis of alcohol **17** in order to subject it to the Mitsunobu inversion and so achieve the 6*R* configuration. When the reduction of **13** was carried out with $NaBH_4$ in MeOH at -23 °C with addition

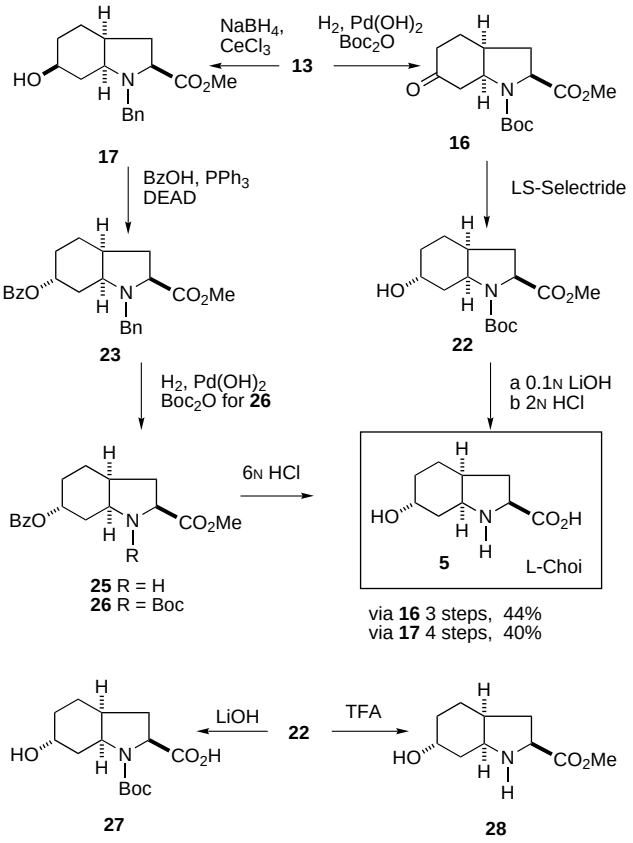
Table 1. Yields and diastereoselectivities obtained in reductions of azabicyclic ketones **13**, **15**, and **16** as shown in Scheme 5.

Substrate	R	Reagent	Compounds (yield)	
1	**13**	Bn	$NaBH_4$, $CeCl_3$[a]	**17** (75%)	**18** (10%)
2	**13**	Bn	LS-Selectride[b]	**17** (51%)	**18** (14%)
3	**15**	Ac	$NaBH_4$[a]	**19** (88%)	**20** (5%)
4	**15**	Ac	LS-Selectride[b]	**19** (9%)	**20** (56%)
5	**16**	Boc	$NaBH_4$, $CeCl_3$[a]	**21** (91%)	**22** (3%)
6	**16**	Boc	LS-Selectride[b]	**21** (7%)	**22** (56%)

[a] at -23 °C in MeOH. [b] at -78 °C in THF.

of $CeCl_3$, the process occurred in 85% yield, the ratio of alcohols **17** and **18** being 7.5:1. By submitting pure alcohol **17** to Mitsunobu conditions (BzOH 1.1 equiv, PPh_3 1.5 equiv, diethyl-azodicarboxylate (DEAD) 1.1 equiv, THF, RT, 17 h) we isolated the benzoate **23** in 59% yield;[22] this represents a triprotected derivative of target **5** (Scheme 6). It is noteworthy that benzoylation of the alcohol **18**, with the correct configuration at its four stereocenters, gave **23** in poor yield; this indicates the low reactivity of its hydroxyl group.

At the same time, we investigated ketone reduction in the *N*-acetyl derivative **15**. The most interesting finding was that the reduction took place with a different stereoselectivity to that observed in the *endo* derivative **13**, which incorporates an *N*-benzyl substituent. Thus, when ketone **13** was reduced with L-Selectride, it gave alcohol **20** as the major product, with the correct configuration at C(6). When $NaBH_4$ was used, the epimeric alcohol **19** was formed almost exclusively, as expected.

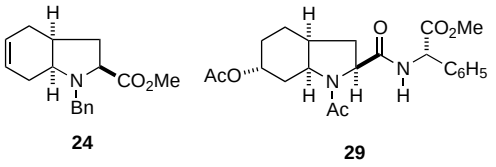


Scheme 6. Synthesis of L-Choi and protected derivatives.

With this information, and taking into account the same preferred conformation of *N*-acetyl compound **15** and the *N*-Boc derivative **16** (vide supra), we decided to study the reduction of this latter ketone to avoid the extra step of inversion at C(6) required when reducing the *N*-benzyl ketone **13**. Ketone **16**, which is available from **13** (H_2, Pd–C, Boc_2O) (Scheme 6), was reduced with LS-Selectride to afford a chromatographically separable 8:1 mixture of alcohols **22** and **21**. The different reaction course taken by the basic nitrogen azabicyclo **13** and the *N*-acyl and *N*-Boc derivatives **15** and **16** can be explained by their different preferred conformations, as shown in Figure 3.[23]

Although the isolated yield of the desired 6*R* alcohol **22** could not be improved to more than 56%, the simplicity of the procedure enabled us to prepare this valuable protected α-amino acid in amounts sufficient to achieve the target Choi and also to carry out the peptide synthesis in the aeruginosin field. The new amino acid Choi was prepared from both **22** and **23**. Debenzylation of **23** was sluggish, and the secondary amine **25** was accompanied by *N*-alkylation products from the alcohol used as solvent.[24] However, **23** was easily converted to the *N*-Boc compound **26** in 95% yield by catalytic, hydrogenolytic debenzylation in the presence of $(Boc)_2O$. The three protecting groups of **26** underwent solvolysis in 6N HCl to give Choi in its hydrochloride form. The target Choi, as well as its monoprotected derivatives **27** and **28**, was also obtained from **22**, as outlined in Scheme 6.

The identity of our synthetic L-Choi **5** and the natural compound isolated by Murakami from aeruginosins by acid



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hydrolysis was confirmed by the conversion of **5** into **29**, and comparison of its ^1H NMR data (500 MHz) with those described for the same compound prepared from the natural source by bisacetylation and coupling with (*S*)-methoxyphenylglycine.[2e]

NMR structural analysis of L-Choi derivatives: The 6-hydroxyoctahydroindoles **5** and **17**–**29** show different conformational behavior depending on the substituent on the nitrogen atom, as occurs in the ketone derivative series. Compounds with a benzyl substituent at the nitrogen atom adopt the inside c_2 conformation, whereas *N*-acetyl and *N*-Boc compounds prefer the outside c_1 conformation (Figure 4). As a consequence, in compound **23**, with the correct 6*R* configuration, the benzoyloxy substituent at C(6) is equatorial ($\delta = 5.37$, tt, $J = 10.5$ Hz and 4 Hz for H-6), whereas in compound **22**, with the same configuration, the hydroxyl group is located axially ($\delta = 4.11$, brs for H-6). NMR data show that the secondary amine **28** exists as a mixture of conformers c_1 and c_2 and the preferred conformation of the hydrochloride of Choi (**5**) is c_1. The disposition of the hydroxyl group can also be diagnosed by IR spectra, since the C–O bond appears at longer wavelengths for alcohols with an axial hydroxyl group than for those with an equatorial one. For instance, absorptions were observed at 1017 cm^{-1} in **22** and 1066 cm^{-1} in **21**.

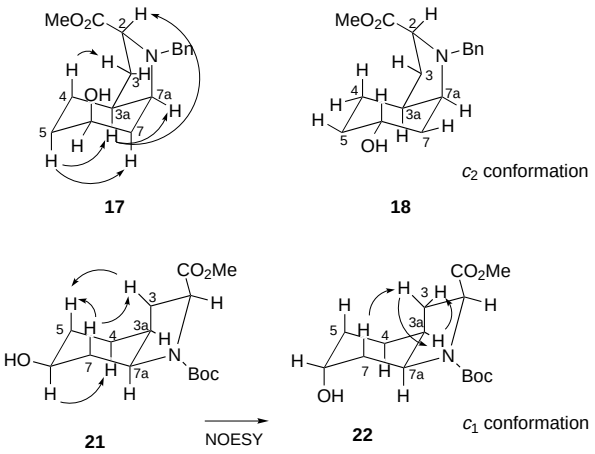

Figure 4. Conformations of 6-hydroxy-octahydroindole-2-carboxylic acid derivatives.

The synthesis of the putative aeruginosin 298-A (2): Among several possible scenarios for the use of the Choi derivatives in the preparation of aeruginosins, the strategy used to produce D-Hpla-L-Leu-L-Choi-L-Argol (**2**), thought at the time to be natural aeruginosin 298-A, was centered around an initial coupling with L-Leu, followed by incorporation of D-Hpla

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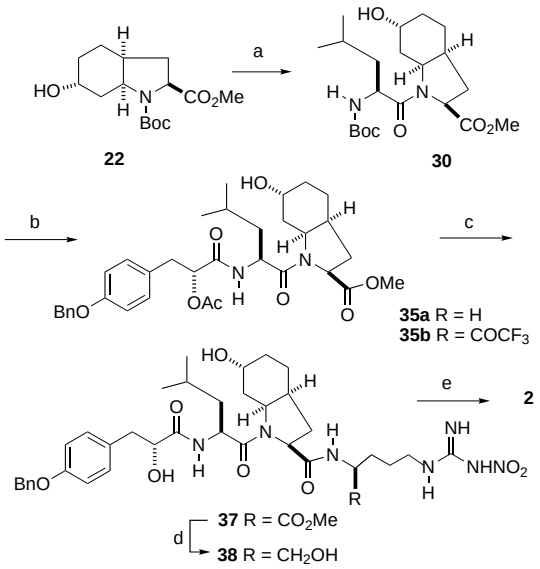
and, finally, a unit of L-Arg (Scheme 7). Later on, the same reaction sequence was successfully used for the synthesis of the real aeruginosin 298-A (**1**), by incorporating D-Leu.

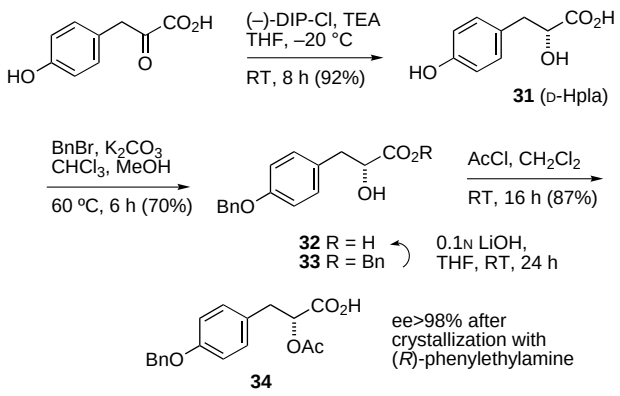
Having successfully achieved the preparation of Choi, we had at our disposal two derivatives, **22** and **26**, suitable for the



Scheme 7. Synthesis of the putative aeruginosin 298-A (**2**): a) TFA, CH_2Cl_2, 0 °C, 45 min; Boc-L-Leu, BOP, NMM, CH_2Cl_2, 0 °C, 30 min, then 22 h, 43 % from **22**; b) TFA, CH_2Cl_2, 0 °C, 45 min; **34**, BOP, NMM, CH_2Cl_2, 0 °C, 30 min, then 18 h, 60 %; c) 0.1N LiOH, THF, 20 h, 92 %; L-Arg-(NO_2)OMe·HCl, BOP, NMM, DMF, 0 °C, 30 min, then 24 h 46 %; d) $LiBH_4$ (2M in THF), THF, 3 h, 50 %; e) H_2, Pd-C 10 %, EtOAc-MeOH, 6N HCl, 1 atm, 8 h, 70 %.

aeruginosin synthesis. Preliminary studies showed that the hydroxyl group at C(6) did not require protection against unwanted coupling reactions, and consequently we carried out the synthetic sequence using the *N*-Boc derivative **22**, which has a free hydroxyl group. Removal of the Boc group from Choi derivative **22**, followed by coupling to Boc-L-Leu, by using benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP), gave dipeptide **30**.[25]

As summarized in Scheme 8, the protected D-Hpla **34** was synthesized in a straightforward sequence. Enantioselective reduction of (4-hydroxyphenyl)pyruvic acid by using the



Scheme 8. Synthesis of D-Hpla and protected derivatives.

methodology recently described by Wang [(−)-DIP-Cl, THF, Et_3N],[26] resulted in a 92 % yield of the α-hydroxy acid **31** (D-Hpla) in 86 % ee.[27] After sequential protection of both hydroxyl groups and recrystallization with (+)-1-phenylethylamine, we obtained the required **34** in > 98 % ee.

Removal of the Boc group from **30**, followed by coupling with the D-Hpla derivative **34** by using BOP, gave compound **35**.[25] After saponification of the methyl ester in **35**, which simultaneously induced deprotection of the acetate group in the Hpla fragment, coupling to the desired tetramer unit system was achieved by treatment of acid **36** with arginine (N^{ω}-NO_2) methyl ester in DMF medium, by using BOP.

At this point, only two operations were now required to complete the synthesis: reduction of the ester moiety of the arginine unit in compound **37** and removal of phenol and guanidino protecting groups. $LiBH_4$ reduction[28] of **37** (1.75 mmol for each mmol of ester)[29] in THF brought about the transformation of the arginine moiety methyl ester to the hydroxymethyl group. Finally, treatment of the resulting compound **38** with palladium on charcoal in acid medium effected the cleavage of the benzyl group and the deprotection of the guanidine moiety to give the target **2**. Purification by reversed-phase HPLC provided pure **2** (positive Sakaguchi test) in 63 % yield. However, the ^1H and ^{13}C NMR spectra of our synthetic aeruginosin 298-A (**2**) unexpectedly showed some chemical shifts different from those reported in the literature for the natural product, although the patterns of the peaks were quite similar. The most significant differences in the NMR data of **2** in comparison with those reported for the natural compound correspond to the signals attributable (COSY, HSQC) to H-7a ($\delta = 4.4$) and H-7eq ($\delta = 1.83$) of Choi and the methyl groups of Leu ($\delta = 0.84$ and 0.85), as well as to C(7) of Choi ($\delta = 34.4$) and C(1) of Leu ($\delta = 170.5$). For detailed NMR data of **2**, see Tables 2 and 3.

At this stage, we concluded that the original structure elucidation[2a, 7] of aeruginosin 298-A was incorrect and should be revised. After careful analyses of NMR data of **2** and several aeruginosins, as well as microcin SF608, which has an L-amino acid linked to the Choi nucleus, we suspected that the correct configuration for aeruginosin 298-A should include a D-Leu unit. Working from this perspective, stereoselective synthesis of **1**, an aeruginosin incorporating a D-Leu instead of an L-Leu, was carried out.

The synthesis and structural reassignment of aeruginosin 298-A (1): Working as described for the synthesis of **2**, but coupling the Choi derivative **22** with Boc-D-Leu, we prepared the new peptide D-Leu-L-Choi **39**. In this series, the Choi hydroxyl group was more reactive in the coupling processes than it had been in the above series, undergoing trifluoroacetylation more easily. Thus, peptides **39** and **40** were isolated as a mixture of compounds with both free and trifluoroacetylated Choi hydroxyl groups. Although this lengthened the analytical process, it did not constitute a synthetic problem, since the trifluoroacetate was removed in the saponification step to provide **41**. More importantly, the higher solubility of the trifluoroacetylated intermediates enhanced the yields of the coupling steps. Thus, following the sequence depicted in

Table 2. ^{1}H NMR data of natural, putative, and synthetic aeruginosins 298-A and 298-B[a]

		298-A	**1** (D-Leu)		**2** (L-Leu)		**298-B**[b]		**3** (D-Leu)		**4** (L-Leu)	
[c]			*trans*	*cis*	*trans*	*cis*	*major*	*minor*	*trans*	*cis*	*trans*	*cis*
Hpla	2	4.04 m	4.04 m		4.01 ddd (8, 4, 5)		4.01 m	4.00 m	4.01 m	4.00 m	4.01 m	3.95 m
	3	2.64 dd (14, 7.5)	2.64 dd (14, 7.5)	2.55 dd (14, 7.5)	2.55 dd (14, 8)		2.63 dd (13.3, 7.7)	2.61 dd (14.4, 7.3)	2.63 dd (14, 7.5)	2.61 dd (14, 7)	2.53 dd (13.8, 4.8)	2.45 dd (13.8, 7.8)
		2.85 dd (14, 3.6)	2.85 dd (14, 3.6)	2.80 dd (14, 3.6)	2.78 d (14, 4.5)		2.84 dd (14.1, 3.9)	2.81 dd (13.7, 4.3)	2.85 dd (14, 3.5)	2.81 d (14, 4)	2.78 dd (13.8, 4.8)	2.87 dd (13.8, 4.8)
	5, 9	6.98 d (8.5)	6.98 d (8.5)	6.94 d (8.5)	6.94 d (8.5)		6.98 dd (8.6, 1.7)	6.95 dd (8.6, 1.7)	6.98 d (8.5)	6.95 d (8.5)	6.95 d (8.4)	6.98 d (8.4)
	6, 8	6.60 (8.5)	6.62 d (8.5)	6.61 d (8.5)	6.60 d (8.5)		6.62 dd (8.6, 1.7)	6.60 dd (8.6, 1.7)	6.62 d (8.5)	6.60 d (8.5)	6.60 d (8.4)	6.64 d (6.0)
	2OH		5.72 d (5.5)	5.46 d (6.0)	5.40 d (6.5)				5.76 br	5.5 br	5.32 d (6.0)	5.53 d (6.0)
	7OH		9.12 brd		9.16 s				9.14 s		9.10 s	
Leu	2	4.51 ddd (10.1, 8.3, 3.5)	4.51 m		4.51 ddd (11, 9.3, 3.5)		4.53 t (9, 0)	4.19 ddd (10, 9, 3)	4.52 t (9.0)	4.19 ddd (13.5, 12.5, 3.5)	4.50 br	
	3	1.20 t (3.5)	1.20 t (3)	1.40	1.2 – 1.3 m		1.20 ddd (13.3, 9, 4.7)	1.24 m	1.20 m	1.24 m	1.25 m	
		1.40 m	1.40 m	1.60	1.4 – 1.6 m		1.33 m	1.41 m	1.34 m	1.41 m	1.49 m	
	4	1.35 m	1.35 m		1.4 – 1.6 m		1.32 m	1.28 m	1.32 m	1.27 m	1.49 m	
	5	0.82 d (6.4)	0.81d (6.0)	0.68 d (6.0)	0.84 d (6.5)	0.80 d (7.0)	0.80 d (6.4)	0.76 d (6.0)	0.80 d (6.4)	0.71 d (6.0)	0.84 d (6.6)	0.82 d (6.6)
		0.88 d (6.5)	0.87 d (6.5)	0.77 d (6.0)	0.85 d (6.5)	0.88 d (7.0)	0.87 d (6.4)	0.87 d (6.0)	0.87 d (6.0)	0.76 d (6.0)	0.85 d (6.6)	0.91 d (6.6)
	NH	7.45 d (8.3)	7.46 d (6.0)	7.50 d (6.0)	7.69 d (9.0)		7.40 d (8.5)	7.33 d (9.0)	7.40 d (9.0)	7.35 d (9.0)	7.62 d (8.4)	
Choi	2	4.16 t (8.9)	4.17 t (8.7)	4.63	4.23 dd (9.5, 8.5)	4.29 dd (9.5, 8.5)	4.12 t (8.0)	4.64 t (8.6)	4.12 t (9.0)	4.64 t (9.0)	4.19 dd (10.2, 1.8)	4.24 m
	3β	1.80 td (12.7, 10.1)	1.80 td (13, 9)		1.83 m		1.80 ddd (14.2, 13.3, 8)	1.85 m	1.80 m	1.85 m	1.80 m	
	3α	2.0 m	2.0 m		1.96 ddd (12, 7, 7)		1.98 m	2.22 m	1.99 m	2.22 m	1.99 m	
	3a	2.27 m	2.27 m		2.2 ddddd (13, 7, 7, 7, 0.5)		2.25 m	2.22 m	2.25 m	2.22 m	2.18 ddddd (13, 7, 6.5, 6.5, 1)	
	4	1.43 m	1.43 m		1.4 – 1.6 m		1.41 m	1.45 m	1.42 m	1.45 m	1.43 m	
		2.02 m	2.00 m		2.04 m		2.02 m	2.02 m	2.02 m	2.20 m	1.99 m	
	5	1.43 m	1.43 m		1.2 – 1.3 m		1.42 m	1.39 m	1.42 m	1.39 m	1.43 m	
								1.98 m		1.98 m		
	6	3.92 s	3.91 s	3.81 s	3.91 s	3.84 s	3.92 br	3.84 br	3.91 brs	3.84 brs	3.90 brs	3.85 brs
	7ax	1.65 t (11.8)	1.65 t (11)		1.72 t (12)		1.65ddd (14, 11.6, 2)	1.43 m	1.65 m	1.43 m	1.70 t (12)	
	7eq	2.02 m	2.00 m		1.83 m		2.00 m	1.85 m	2.00 m	1.85 m	1.80 m	
	7a	4.04 m	4.04 m	4.28 ddd (11, 6, 6)	4.40 ddd (11, 6, 6)	4.05 ddd (11, 6, 6)	4.02 m	4.27 ddd (11.5, 6, 6)	4.02 m	4.27 ddd (11.5, 6, 6)	4.39 ddd (11, 6, 6)	4.24 m
	NH_2						6.83 br	7.10 br	6.84 br	7.10 br	6.80 br	
							7.22 br	7.60 br	7.23 br	7.60 br	7.25 br	
Arg	1	3.21dd (10.7, 6.2)	3.21 dd (10.5, 6)		3.24 dd (11.5, 5)							
		3.31 dd (10.7, 5.1)	3.31 dd (10.5, 4)		3.31 dd (10.5, 5)							
	2	3.64 m	3.60 m		3.67 m	3.42 m						
	3	1.30 m	1.30 m		1.2 – 1.3 m							
		1.60 m	1.60 m		1.4 – 1.6 m							
	4	1.50 m	1.50 m		1.4 – 1.6 m							
	5	3.07 m	3.07 m		3.06 m							
	1OH		4.63 t (6.1)	4.70 t (6.1)	4.70 t (5.5)	4.74 t (5.5)						
	2NH	7.54 s	7.58 d (8.5)	7.80 d (8.0)								
	5NH	7.52 s	7.55 t (5.5)	7.60 t (5.5)	7.42 brs							

[a] All spectra were recorded in [D_6]DMSO at 500 MHz (**1** – **3**) or 600 MHz (**4**) and the peak assignments are derived from COSY and NOESY experiments. [b] Described as a D-Leu and L-Leu mixture of epimers. [c] *trans*/*cis* rotamer ratio 6:1 for **1**, 5:1 for **2**; 3:1 for **3**, and 7:1 for **4**.

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Table 3. ^{13}C NMR Chemical shifts of Natural, Putative, and Synthetic Aeruginosins 298-A and 298-B[a]

		298-A	**1**	**2**	**298-B**		**3**		**4**
			trans	*trans*	*major*[b]	*minor*	*trans*[c]	*cis*[c]	*trans*
Hpla	1	172.8	172.7	173.1	172.3	172.3	172.5	172.5	173.3
	2	72.1	72.0	72.4	71.8	72.0	72.1	72.1	72.4
	3	39.3	39.2	39.4	39.0	39.0	39.2	39.2	40.0
	4	128.1	128.0	126.2	127.9	125.2	128.1	125.0	129.0
	5, 9	130.4	130.4	130.3	130.0	129.9	130.4	130.3	130.6
	6, 8	114.7	114.6	114.8	114.4	114.4	114.6	114.6	115.0
	7	155.7	155.7	155.7	155.4	155.4	155.6	155.6	156.0
Leu	1	169.8	169.6	170.5	169.4	169.8	169.5	169.7	170.6
	2	48.1	48.0	47.9	47.4	46.9	47.8	47.8	48.1
	3	41.8	42.0	41.6	41.8	41.7	42.2	42.1	42.1
	4	23.9	23.9	24.3	23.6	23.7	23.8	23.8	24.5
	5	23.3	23.4	23.6	22.9	23.0	23.3	23.4	23.9
	5′	21.4	21.4	21.7	21.4	21.3	21.5	21.0	22.0
Choi	1	171.3	171.2	171.4	173.0	173.3	173.3	173.4	173.6
	2	59.9	59.9	60.4	59.4	59.1	59.5	59.4	59.5
	3	30.7	30.5	30.4	30.1	30.1	30.4	30.4	30.7
	3a	36.0	36.0	36.6	35.8	35.9	36.0	36.1	37.0
	4	19.0	19.0	19.2	18.8	18.7	19.0	19.0	19.4
	5	26.0	26.0	26.1	25.9	25.9	26.0	25.9	26.3
	6	63.9	63.8	64.1	63.7	63.7	63.8	63.8	64.3
	7	33.4	33.4	34.3	33.2	33.4	33.5	33.5	34.6
	7a	54.0	54.0	54.5	53.6	53.9	53.9	54.0	54.7
Argol	1	63.3	63.2	63.5					
	2	50.2	50.2	50.1					
	3	28.0	27.8	28.3					
	4	25.0	25.1	25.1					
	5	40.8	40.7	40.9					
	C=N	156.7	157.0	156.8					

[a] All spectra were recorded in [D_6]DMSO at 75 MHz and the assignments were aided by HSQC experiments. [b] These data correspond to the major and minor components described for the natural product. [c] *trans-cis* rotamers of the D-Leu derivative.

Scheme 9, we synthesized **1**, which had NMR spectral data matching those reported by Murakami when he isolated aeruginosin 298-A.[2a] Thus, we concluded that the stereostructure of the natural product corresponds to that of **1**.[30]

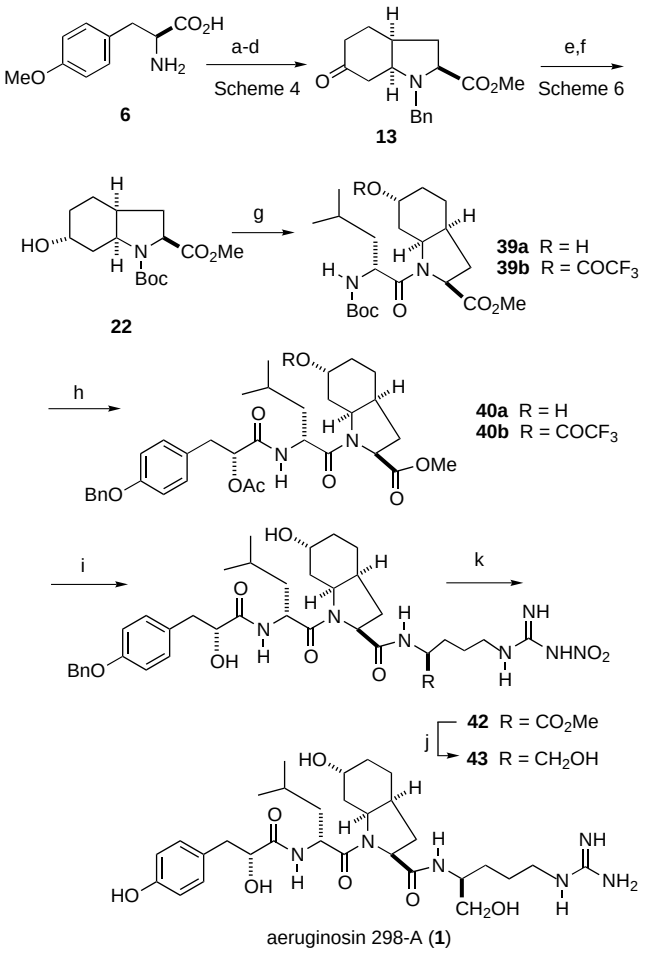
In fact, aeruginosin 298-A (**1**) in DMSO solution appears as a 6:1 mixture of *trans* and *cis* rotamers about the Choi–Leu peptide bond. The relative populations of the amide *cis* and *trans* isomers were measured in the ^1H NMR spectra by integration of the isomeric methyl proton signals of Leu. The assigned isomer geometry was based on NOESY experiments and the chemical shift values for the signals of H-2 and H-7a of Choi. In the ^1H NMR spectra of **1** and its precursors, which also exhibit this phenomenon,[31] the H-2 and H-7a protons appear more shielded in the *trans* isomer than in the *cis* isomer (see Table 2). The NMR of the L-Leu epimer peptide **2** and its precursors (Scheme 8) also show this rotational isomerism, although the change in the chemical shifts of H-2 and H-7a follows a different rule: in the L-Leu compounds, the H-2 signal of the *trans* isomer appears upfield to that of the *cis* isomer, but the H-7a signal of the *trans* isomer appears downfield from that of the *cis* isomer[32] (see Table 2).

With hindsight, the D-configuration of the Leu subunit is consistent with the absolute configuration of related members of the aeruginosin family that are also potent inhibitors of thrombin. Assuming a related biosynthetic pathway and function for other aeruginosins, it would have been surprising to find that 298-A is an exception. Furthermore, from the X-ray co-crystal structure of the aeruginosin 98-B (a relative of 298-A)/thrombin complex reported by Clardy,[33] the configuration of the allo-Ile unit corresponds to D on the basis of its van der Waals interactions with Trp215 and Leu99 and neither an L-Ile nor L-allo-Ile side chain could be accommodated by thrombin. In addition, the D-configuration of the P3 substituent is consistent with the structure–activity relationship of D-Phe-L-Pro-arginine inhibitors of thrombin[34] and recently described peptidomimetics.[35]

The synthesis of aeruginosin 298-B (3). Our next concern was to synthesize aeruginosin 298-B (**3**) by utilizing the same advanced intermediate **41** as in the above synthesis of aeruginosin 298-A (**1**). At the same time, we also decided to synthesize the epimer **4**, which incorporates an L-Leu, since the natural product isolated from *microcystis aeruginosa* in 1999[2e] had been reported to be a 3:1 mixture of **3** and **4** (see Scheme 2).

From the D-Hpla-D-Leu-L-Choi derivative **41**, a two-step sequence consisting of coupling with ammonium hydroxide by using PyBOP to give the amide **44** and a deprotecting debenzylation gave aeruginosin 298-B **3** (Scheme 10).

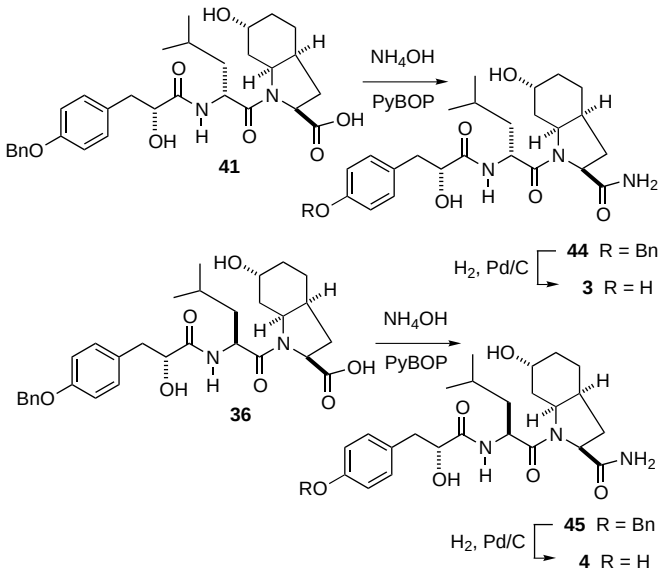
Aeruginosin 298-B (**3**) showed minor NMR signals with approximately 20% of the intensity of the major signals. Virtually all signals were doubled. In fact, the ^1H and ^{13}C NMR data of **3** matched the duplicate signals reported by Murakami for the natural product. It was therefore concluded that the dichotomy of the signals observed in **3**, and hence in the natural aeruginosin, was due to geometrical



Scheme 9. Synthesis of aeruginosin 298-A (**1**): a) Li, NH_3, THF-*t*BuOH (2.5:1), −78 °C; b) MeOH, 8 N HCl, 35 °C, 48 h; c) BnBr, $NaHCO_3$, EtOH, 70 °C, 6 h; d) MeOH, 9 N HCl, 65 °C, 17 h (44% from **6**); e) H_2, $Pd(OH)_2$ 20%, Boc_2O, EtOAc, 48 h, 79%, f) *LS*-Selectride (1 M in THF, 1.3 equiv), THF, −78 °C, 53% for isomer **22**; g) TFA, CH_2Cl_2, 0 °C, 45 min; Boc-D-Leu, BOP, NMM, CH_2Cl_2, 0 °C, 30 min, then 22 h, 73% from **22**; h) TFA, CH_2Cl_2, 0 °C, 45 min; **34**, BOP, NMM, CH_2Cl_2, 0 °C, 30 min, then 18 h, 60%; i) 0.1 N LiOH, THF, 20 h, 85%; L-Arg(NO_2)OMe·HCl, PyBOP, NMM, DMF, 0 °C, 30 min, then 22 h, 90%; j) $LiBH_4$ (2 M in THF), THF, 3 h, 53%; k) H_2, Pd-C 10%, EtOAc/MeOH (1:1), 6 N HCl (2 drops), 1 atm, 8 h, 95%.

isomerism in the Choi–Leu peptide bond and not to a mixture of D- and L-Leu residues. The ^1H chemical shifts of the major peaks, based on NOESY studies, corresponded to attributable signals for the *trans* isomer (Choi, H-2 $\delta = 4.12$; H-7a $\delta = 4.02$; Leu, H-2 $\delta = 4.52$), while the minor peaks (Choi, H-2 $\delta = 4.64$; H-7a $\delta = 4.27$; Leu, H-2 $\delta = 4.19$) corresponded to those of the *cis* isomer. Thus, as had been observed in the aeruginosin 298-A series, both the H-2 and H-7a protons of Choi appeared further upfield in the *trans* rotamer than in the *cis* rotamer.

As expected, the NMR data of **4** (Table 2)—the L-Leu epimer of aeruginosin 298-B (**3**), prepared by starting from acid **36** and following the same procedure as used for the synthesis of **3** (Scheme 10)—are clearly different from the minor signals described for the natural product and erroneously attributed to **4** when aeruginosin 298-B was isolated. In fact, duplicate signals again appear in NMR spectra of compound **4**, due to *cis-trans* amide isomerism, but they do not correspond to those reported for the natural aeruginosin.



Scheme 10. Syntheses of aeruginosin 298-B (**3**) and its epimer **4**.

Conclusion

In conclusion, we have accomplished the first total syntheses of aeruginosins 298-A (11 steps, 3.1% yield) and 298-B (10 steps, 2.6% yield); this allows the unequivocal reassignment of the absolute configuration of both natural aquatic peptides. Interestingly, this fact shows that all aeruginosins[2, 3] have the D (*R*) configuration in the α-amino acid attached to the perhydroindole nitrogen.

Since our previous communication[9] and the writing of this manuscript, another synthesis of aeruginosin 298-A has been published.[36] The synthesis, reported by Wipf, is conceptually different from that reported here, the main differences being: i) L-Choi was prepared by an oxidative process rather than a reductive one, ii) D-Hpla was synthesized from (*R*)-benzylglycidol instead of from a phenylpyruvic acid derivative, iii) orthogonal protecting groups requiring two deprotection steps were used instead of one-step removal protecting groups, and iv) a different coupling sequence was used.

Experimental Section

General: All reactions were carried out under argon atmosphere with dry, freshly distilled solvents under anhydrous conditions. Unless otherwise noted, analytical thin layer chromatography was performed on SiO_2 (silica gel 60 F_{254}, Merck), and spots were located with iodoplatinate reagent (compounds **5**–**30**), 1% aqueous $KMnO_4$ (compounds **3**, **4**, **31**–**44**) or the Sakaguchi reagent (compounds **1** and **2**). Chromatography refers to flash chromatography and was carried out on SiO_2 (silica gel 60, SDS, 230–240 mesh ASTM). Drying of organic extracts during workup of reactions was performed over anhydrous Na_2SO_4. Evaporation of solvent was accomplished with a rotary evaporator. Optical rotations were recorded with a Perkin—Elmer 241 polarimeter. ^1H and ^{13}C NMR spectra were recorded with a Varian 300 or a Varian VXR-500 instrument. Chemical shifts are reported in ppm downfield (δ) from Me_4Si. IR spectra were recorded on a Nicolet 205 FT-IR spectrophotometer, and only noteworthy absorptions are listed. Melting points were determined in a capillary tube. Microanalyses and HRMS were performed by the Centro de Investigación y Desarrollo (CSIC), Barcelona. Abbreviations: BOP, benzotriazol-1-yloxy-

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tris(dimethylamino)phosphonium hexafluorophosphate; NMM, (*N*-methylmorpholine); PyBOP (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate

***O*-Methyl-L-tyrosine hydrochloride (6)**: This compound was prepared following the previously reported procedure,[37] either from *N*-Boc-L-tyrosine (Fluka) or *N*-acetyl-L-tyrosine (Fluka) on 82 mmol and 112 mmol scales (90% and 77% overall yield, respectively) in two steps (i, Me_2SO_4, aq NaOH; ii, 4N HCl).

Methyl (2*S*,3a*R*,7a*R*)- and (2*S*,3a*S*,7a*S*)-1-benzyl-6-oxo-octahydroindole-2-carboxylate (12 and 13): Ammonia (200 mL) was added to a cooled (−78 °C) solution of **6**·HCl (13.85 g, 60 mmol) in a mixture of THF/*t*BuOH (2.5:1) (250 mL). Small chips of lithium (2.35 g, 0.34 mmol) were added with vigorous stirring until the solution was a persistent deep blue, and stirring was maintained for 90 min. The cooling bath was removed, the ammonia was allowed to evaporate overnight, and the reaction mixture was concentrated to give lithium (2*S*)-2-amino-3-(2,5-dihydro-4-methoxyphenyl)propanonate **(7)** as a white solid: ^{1}H NMR (200 MHz, D_2O): $\delta = 1.8-2.2$ (m, 2H), 2.45 (br s, 4H), 3.03–3.07 (m, 1H), 3.29 (s, 3H), 4.56 (br s, 1H), 5.24 (br s, 1H); ^{13}C NMR (50 MHz, D_2O): $\delta = 31.0$ (t), 31.2 (t), 45.1 (t), 56.6 (d and q), 94.8 (d), 123.5 (d), 134.8 (s), 154.9 (s), 185.6 (s). The lithium salt **7** was dissolved in a freshly prepared 8.5N solution of HCl in MeOH (70 mL), and the solution was stirred at 35 °C for 48 h. The mixture was concentrated to dryness, and the residue[38] was dissolved in EtOH (100 mL). $NaHCO_3$ (35 g, 0.42 mol) and benzyl bromide (25 mL, 0.21 mmol) were added, and the mixture was stirred at 70 °C until the disappearance of the starting material **9** was observed by TLC (7 h). If necessary, additional $NaHCO_3$ (1 equiv) and benzyl bromide (1 equiv) were added. The reaction mixture was filtered, the solvent removed, and the residue taken up with CH_2Cl_2 (25 mL) and extracted with 2N HCl (4 × 10 mL). The aqueous extracts were basified with Na_2CO_3 to pH 10–11 and extracted with CH_2Cl_2 (6 × 50 mL). Purification of the latter organic extracts by chromatography (CH_2Cl_2) provided a partially separated 1.8:1 mixture of compounds **12** and **13**. The first purification gave 3.6 g of **12**, 2.52 g of an *exo-endo* isomer mixture, and 2.57 g of **13**.[39, 40] Further careful chromatographic treatment allowed the separation of the fractions containing mixtures of *exo* and *endo* isomers. After the overall process, 5.47 g (32%) of **12** and 3.10 g (18%) of **13** were isolated as colorless oils (combined yield 50% from **6**). On standing, **13** solidified as colorless needles

Exo isomer **12**: $R_f = 0.64$ (hexane/CH_2Cl_2/EtOAc 6:9:2); $[\alpha]_D^{20} = -43.4$ ($c = 3.6$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, COSY, NOESY): $\delta = 1.72$ (dq, $J = 14$, 5 Hz, 1H; H-4$_{eq}$), 1.85 (dt, $J = 14$, 7.5 Hz, 1H; H-3$_\alpha$), 1.98–2.09 (m, 2H; H-3$_\beta$ and H-4$_{ax}$), 2.21 (dt, $J = 14$, 5.7 Hz, 1H; H-5$_{eq}$), 2.41 (ddd, $J = 14$, 10.4, 5 Hz, 1H; H-5$_{ax}$), 2.54 (d, $J = 4.7$ Hz, 2H; H-7), 2.80 (m, 1H; H-3a), 3.54 (d, $J = 7.5$ Hz, 1H; H-2), 3.66 (s, 3H; OCH_3), 3.72 (dt, $J = 9$, 4.5 Hz, 1H; H-7a), 3.55 and 3.90 (2d, $J = 13.5$ Hz, 1H each; CH_2Ph), 7.20–7.27 (m, 5H; Ph); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 25.4$ (C(4)), 33.3 (C(3)), 34.2 (C(3a)), 35.3 (C(5)), 42.1 (C(7)), 51.0 (OCH_3), 52.2 (CH_2Ph), 59.2 (C(7a)), 61.8 (C(2)), 127.0, 128.2, 128.6 and 138.6 (Ph), 174.2 (COOMe), 212.3 (CO); IR (film): $\tilde{\nu} = 1732$, 1717 cm^{-1}; elemental analysis calcd (%) for $C_{17}H_{21}NO_3$ (287.4): C 71.08, H 7.32, N 4.88; found C 71.03, H 7.34, N 4.82.

Endo isomer **13**: $R_f = 0.54$ (hexane/CH_2Cl_2/EtOAc 6:9:2); m.p. 68 °C; $[\alpha]_D^{20} = -50.5$ ($c = 3.3$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, COSY, NOESY): $\delta = 1.71$ (ddd, $J = 12.5$, 9, 6.5 Hz, 1H; H-3$_\alpha$), 1.83–1.96 (m, 2H; H-4), 2.17 (ddd, $J = 17.5$, 8.5, 4.5 Hz, 1H; H-5), 2.31 (ddd, $J = 12.5$, 8.5, 8 Hz, 1H; H-3$_\beta$), 2.43 (m, 1H; H-3a), 2.44 (dd, $J = 15.5$, 4.8 Hz, 1H; H-7$_{ax}$), 2.54 (ddd, $J = 17.5$, 8, 5 Hz, 1H; H-5), 2.58 (dd, $J = 15.5$, 5.5 Hz, 1H; H-7$_{eq}$), 3.05 (dt, $J = 9$, 5 Hz, 1H; H-7a), 3.26 (t, $J = 8.5$ Hz, 1H; H-2), 3.41 (s, 3H; OCH_3), 3.57 and 3.83 (2d, $J = 14$ Hz, 1H each; CH_2Ar), 7.21–7.24 (m, 5H; Ar); ^{13}C NMR (50 MHz, $CDCl_3$, HMQC): $\delta = 26.6$ (C(4)), 34.6 (C(3a)), 34.8 (C(3)), 36.6 (C(5)), 42.1 (C(7)), 51.6 (OCH_3), 56.2 (CH_2Ar), 61.5 (C(7a)), 65.6 (C(2)), 127.1, 127.5, 127.9 and 136.6 (Ar), 173.8 (COOMe), 211.7 (CO); IR (film): $\tilde{\nu} = 1746$, 1716 cm^{-1}; elemental analysis calcd (%) for $C_{17}H_{21}NO_3$ (287.4): C 71.08, H 7.32, N 4.88; found C 71.01, H 7.38, N 4.85.

Epimerization of *exo* isomer 12 to *endo* isomer 13: A solution of ketone **12** (5 g) in MeOH/HCl (9N, 500 mL) was heated at 65 °C overnight. MeOH was evaporated, the residue was taken up with HCl (2N, 50 mL) and THF (6 mL), and the mixture was stirred at room temperature for 20 min. Solid Na_2CO_3 was added, and the basic solution was extracted with EtOAc and $CHCl_3$. The dried extracts were concentrated to give a 1:7 mixture of **12** and **13**, respectively. Careful separation by chromatography (CH_2Cl_2) afforded 4.2 g (84%) of ketone **13**, with 350 mg of the starting material being recovered.

This process can obviously be applied to mixtures of *exo-endo* derivatives (**12** and **13**), thus avoiding some of the chromatographic process in the aforementioned synthetic procedure.

Methyl (2*S*,3a*S*,7a*S*) 1-*tert*-butoxycarbonyl-6-oxo-octahydroindole-2-carboxylate (16): A suspension of **13** (712 mg, 2.48 mmol), Boc_2O (704 mg, 3.2 mmol), and $Pd(OH)_2$/C (20%, 142 mg) in EtOAc (5 mL) was stirred under hydrogen (atmospheric pressure) at room temperature for 48 h. The catalyst was then removed by filtration through a short pad of Celite, which was washed with $CHCl_3$ (30 mL), EtOAc (30 mL), and MeOH (30 mL). The organic filtrates were concentrated and chromatographed (Al_2O_3, hexane/EtOAc 1:1) to furnish carbamate **16** as a yellow oil. Yield: 658 mg, 89%; $R_f = 0.54$ (hexane/EtOAc); $[\alpha]_D^{20} = +30.2$ ($c = 3.6$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, COSY, NOESY) (1:1 mixture of *trans* and *cis* rotamers): $\delta = 1.37$ and 1.42 (2 × s, 9H; CH_3), 1.88 (br s, 1H; H-4$_{ax}$), 1.96–2.03 (m, 2H; H-3, H-4$_{eq}$), 2.12–2.18 (m, 1H; H-5$_{ax}$), 2.34–2.44 (m, 2H; H-3, H-5$_{eq}$), 2.50–2.60 (m, 1H; H-3a), 2.64–2.70 (br s, 1H; H-7$_{ax}$), 2.84 and 2.96 (2 × br s, 1H; H-7$_{eq}$), 3.72 (s, 3H; OCH_3), 4.11 and 4.22 (2 × br s, 1H; H-7a), 4.28 and 4.37 (2 × br s, 1H; H-2); (300 MHz, [D$_6$]DMSO, 80 °C): $\delta = 1.37$ (s, 9H; CH_3), 1.80 (dddd, $J = 13.5$, 9, 5.5, 5.5 Hz, 1H; H-4$_{ax}$), 1.89–2.02 (m, 2H; H-3$_\alpha$, H-4$_{eq}$), 2.11 (dddd, $J = 15.9$, 7.8, 5.4, 0.9 Hz, 1H; H-5$_{eq}$), 2.36 (ddd, $J = 15.9$, 9, 5.4 Hz, 1H; H-5$_{ax}$), 2.40 (dt, $J = 13$, 8 Hz, H-3$_\beta$), 2.53 (masked, H-3a), 2.54 (dd, $J = 15.3$, 9.9 Hz, 1H; H-7$_{ax}$), 2.63 (ddd, $J = 15.3$, 6.6, 0.9 Hz, 1H; H-7$_{eq}$), 3.67 (s, 3H; OCH_3), 4.11 (ddd, $J = 9.6$, 7.5, 6.6 Hz, 1H; H-7a), 4.26 (t, $J = 8.5$ Hz, 1H; H-2); ^{13}C NMR (75 MHz, $CDCl_3$, HMQC): $\delta = $ (*cis rotamer*) 24.5 (C(4)), 28.3 (CH_3), 33.1 (C(3)), 35.5 (C(3a)), 36.5 (C(5)), 44.0 (C(7)), 52.2 (OCH_3), 57.4 (C(7a)), 59.3 (C(2)), 80.6 ($OCMe_3$), 153.0 (NCO), 173.6 (COOMe), 210.1 (CO); (*trans rotamer*) 24.3 (C(4)), 28.3 (CH_3), 34.1 (C(3)), 35.5 (C(3a)), 36.5 (C(5)), 43.0 (C(7)), 52.2 (OCH_3), 57.2 (C(7a)), 59.7 (C(2)), 80.5 (CMe_3), 153.5 (NCO), 173.6 (COOMe), 209.7 (CO); ^{13}C NMR (75 MHz, [D$_6$]DMSO, 80 °C): $\delta = 23.7$ (C(4)), 27.8 (C(CH_3)$_3$), 33.1 (C(3)), 35.2 (C(3a)), 36.0 (C(5)), 42.9 (C(7)), 51.5 (OCH_3), 56.9 (C(7a)), 59.2 (C(2)), 79.2 (C(CH_3)$_3$), 152.5 (NCO_2), 172.8 (CO_2Me), 209.0 (C(6)); IR (film): $\tilde{\nu} = 1749$, 1716, 1698 cm^{-1}; MS (EI) *m/z*: 297, 238, 196, 182, 138 (100), 80, 57; elemental analysis calcd (%) for $C_{15}H_{23}NO_5$ (297.33): C 60.59, H 7.80, N, 4.71; found C, 60.39, H 8.05, N 4.55.

Alternatively, the chromatography can be avoided if the reaction mixture is treated with a pH 3 buffer of AcOH/NaOAc (20 mL) at room temperature for 48 h. The aqueous solution was basified with Na_2CO_3 and extracted with CH_2Cl_2 and $CHCl_3$. The organic extracts were concentrated to give pure carbamate **16** with the same yield as above, operating on a 6.6 mmol scale (1.67 g of **16**).

Methyl (2*S*,3a*S*,6*S*,7a*S*) 1-benzyl-6-hydroxy-octahydroindole-2-carboxylate (17): $CeCl_3$ (296 mg, 1.2 mmol) was added to a solution of **13** (2.28 g, 7.94 mmol) in MeOH (60 mL). The reaction was stirred at room temperature for 20 min, then $NaBH_4$ (1 g, 27.8 mmol) was added. The mixture was stirred at −23 °C for 6 h. After removal of MeOH, water (100 mL) was added, and the solution was extracted with EtOAc (2 × 100 mL) and $CHCl_3$ (3 × 100 mL). The dried organic extracts were concentrated to give an 8:1 mixture of alcohols **17** and **18**, which was purified by chromatography (Al_2O_3, hexane/EtOAc 1:2). The first eluate gave **17** (1.72 g, 75%): $R_f = 0.77$ (Al_2O_3, hexane/EtOAc, 1:2); m.p. 49–51 °C; $[\alpha]_D^{20} = +11.8$ ($c = 1$ in CH_3OH); ^{1}H NMR (500 MHz, $CDCl_3$, COSY, NOESY): $\delta = 1.40-1.46$ (m, 2H; H-4$_{eq}$, H-5$_{ax}$), 1.54 (dd, $J = 13$, 3.5 Hz, 1H; H-3$_\beta$), 1.60 (dt, $J = 15$, 3.5 Hz, 1H; H-7$_{ax}$), 1.85 (m, 1H; H-5$_{eq}$), 1.90 (m, 1H; H-4$_{ax}$), 2.04 (ddddd, $J = 12.5$, 6, 6, 5.5, 5.5 Hz, 1H; H-3a), 2.20 (td, $J = 13$, 6.5 Hz, 1H; H-3$_\alpha$), 2.39 (dd, $J = 15$, 2.5 Hz, 1H; H-7$_{eq}$), 2.93 (s, $\nu_{1/2} = 3$ Hz, 1H; H-7a), 3.31 (s, 3H; OCH_3), 3.32 (masked, 1H; H-2), 3.33 and 4.24 (2 × d, $J = 13$ Hz, 1H each; NCH_2Ph), 4.04 (s, $\nu_{1/2} = 2$ Hz, 1H; H-6$_{eq}$), 5.40 (br, 1H; OH), 7.24 (m, 5H; PhH); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 27.5$ (C(4)), 33.3 (C(3)), 34.8 (C(5)), 35.5 (C(7)), 37.0 (C(3a)), 51.5 (OCH_3), 57.0 (CH_2Ar), 64.5 (C(7a)), 65.1 (C(2)), 66.3 (C(6)), 127.0 (*p*-C), 127.9 (*o*-C), 129.3 (*m*-C), 137.8 (*ipso*-C), 175.2 (CO_2Me); IR (film): $\tilde{\nu} = 3550$, 1748 cm^{-1}; MS (EI) *m/z*: 289, 230 (100), 212, 170, 122, 91; elemental analysis calcd (%) for $C_{17}H_{23}NO_3$ (289.35): C 70.59, H 7.96, N 4.84; found C 70.78, H 8.03, N 4.84. The second eluate gave 229 mg (10%) of 6*R* isomer **18**.[40]

Methyl (2*S*,3a*S*,6*R*,7a*S*) 1-*tert*-butoxycarbonyl-6-hydroxy-octahydroindole-2-carboxylate (22): A solution of carbamate **16** (219 mg, 0.73 mmol) in THF (5 mL) was added to a cooled (−78 °C) solution of LS-Selectride®

(1M in THF, 0.95 mL) in THF (5 mL). The mixture was stirred at −78 °C for 2 h and then allowed to warm to room temperature. After addition of 5% $NaHSO_4$ (7 mL) and continued stirring for 2 h, the mixture was basified with solid Na_2CO_3 to pH 8 and extracted with CH_2Cl_2 (3 × 5 mL) and $CHCl_3$/*i*PrOH (4:1, 4 × 15 mL). The dried organic extracts gave a mixture of alcohols **21** and **22** in 1:8 ratio. Purification by chromatography (Al_2O_3, hexane/EtOAc 1:1 to EtOAc) furnished the alcohol **22** (97 mg, 44%) and then a mixture of **21**[40] and **22** (40 mg).

Alcohol 22: Overall yield: 56%; $R_f = 0.45$ (Al_2O_3, EtOAc); m.p. 160–162 °C; $[\alpha]_D^{20} = -31.1$ ($c = 2.4$ in CH_3OH); ^{1}H NMR (500 MHz, $CDCl_3$, COSY, NOESY) (mixture of conformers, 60% of *trans* and 40% of *cis*): $\delta = 1.37$ and 1.40 (2 × s, 9H; CH_3), 1.47 (masked, 1H; H-4), 1.56 (br, 2H; H-5), 1.67 (br t, $J = 12.5$ Hz, 1H; H-7_{ax}), 1.92 (br, 1H; H-3_α), 2.08–2.14 (m, 2H; H-3_β, H-4), 2.23 (br, 1H; H-7_{eq}), 2.32 (br, 1H; H-3a), 3.70 (s, 3H; OCH_3), 4.11 (br s $\nu_{1/2} = 10$ Hz, 1H; H-6_{eq}), 4.18 (br, 1H; H-7a), 4.25 (br, 1H; H-2); ^{13}C NMR, (75 MHz, $CDCl_3$, HMQC): $\delta = 19.4$ (C(4)), 26.3 (C(5)), 28.2 (C(*CH*$_3$*)*$_3$), 31.6 and 32.4 (C(3)), 33.6 (C(7)), 35.8 and 36.4 (C(3a)), 51.9 (OCH_3), 53.4 (C(7a)), 58.8 and 59.3 (C(2)), 65.8 and 66.1 (C(6)), 79.8 ($C(CH_3)_3$), 153.2 (NCO_2), 174.0 (CO_2Me); MS (EI) *m/z*: 371, 312, 270, 256, 212(100), 196, 182, 166, 122, 80, 57; IR (KBr): $\tilde{\nu} = 3487$, 1754, 1673, 1017 (OH_{ax}) cm^{-1}; elemental analysis calcd (%) for $C_{15}H_{25}NO_5 \cdot \frac{1}{4}H_2O$ (303.85): C 59.29, H 8.46, N. 4.61; found C 59.29, H 8.20, N 4.23.

Methyl (2*S*,3a*S*,6*R*,7a*S*)-6-benzoyloxy-1-benzyl-octahydroindole-2-carboxylate (23): Benzoic acid (250 mg, 2 mmol) and triphenylphosphine (710 mg, 2.7 mmol) were added to a cooled (0 °C) solution of alcohol **17** (515 mg, 1.8 mmol) in THF (16 mL). Diethyl azodicarboxylate (0.43 mL, 2.7 mmol) was added slowly, and the mixture was stirred at 0 °C for 1 h and then overnight at room temperature. The solvent was evaporated, and the residue was partitioned between saturated Na_2CO_3 (150 mL) and CH_2Cl_2 (100 mL). The dried organic extracts were concentrated and chromatographed (SiO_2, hexane/EtOAc 3:1) to furnish 392 mg (56%) of **23** and 29 mg (6%) of alkene **24**.[40] After further careful chromatography of slightly impure **23** (CH_2Cl_2), and trituration of the resulting solid with hexane, **23** was obtained as white crystals. $R_f = 0.68$ (hexane/EtOAc 3:1); m.p. 79–80 °C; $[\alpha]_D^{20} = -41.7$ ($c = 1.5$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, COSY): $\delta = 1.49$–1.62 (m, 2H; H-3 and H-5), 1.64 (m, 1H; H-4), 1.69 (ddd, 13.8, 10.5, 8 Hz, 1H; H-7_{ax}), 1.95 (m, 1H; H-4), 2.05 (m, 1H; H-3a), 2.10 (m, 1H; H-3), 2.15 (m, 1H; H-5), 2.38 (dm, $J = 13.8$ Hz, 1H; H-7_{eq}), 2.95 (dd, $J = 8$, 3.5 Hz, 1H; H-7a), 3.28 (dd, $J = 11$, 4.8 Hz, 1H; H-2), 3.53 and 4.07 (2 × d, $J = 13.5$ Hz, 1H each; NCH_2Ph), 5.37 (tt, $J = 10.5$, 4 Hz, 1H; H-6_{ax}), 7.20–7.35 (m, 5H; PhH), 7.43 (tt, $J = 7.5$, 1.5 Hz, 2H; *m*-Ar), 7.54 (tt, $J = 7.5$, 1.5 Hz, 1H; *p*-Ar), 8.02 (dd, $J = 8$, 1.5 Hz, 2H; *o*-Ar); ^{13}C NMR, (50 MHz, $CDCl_3$, HMQC): $\delta = 26.9$ (C(4)), 30.8 (C(5)), 31.8 (C(7)), 33.8 (C(3)), 36.8 (C(3a)), 51.5 (OCH_3), 56.5 (CH_2Ar), 63.3 (C(7a)), 64.8 (C(2)), 70.4 (C(6)), 127.0 (C *p*-Bn), 127.9 (C *m*-Bz), 128.2 (C *m*-Bn), 129.4 (C *o*-Bz), 129.6 (C *o*-Bn), 130.9 (C *ipso*-Bz), 132.6 (C *p*-Bz), 137.5 (C *ipso*-Bn), 165.9 (OCOPh), 175.1 (CO_2Me); IR (film): $\tilde{\nu} = 1747$, 1715 cm^{-1}; elemental analysis calcd (%) for $C_{24}H_{27}NO_4$ (393.5): C 73.26, H. 6.91, N. 3.56; found C 72.90, H 6.94, N 3.55.

Methyl (2*S*,3a*S*,6*R*,7a*S*)-6-benzoyloxy-1-*tert*-butoxycarbonyl-octahydroindole-2-carboxylate (26): $Pd(OH)_2$/C (20%, 20 mg) and Boc_2O (72 mg, 0.33 mmol) were added to a solution of **23** (100 mg, 0.25 mmol) in EtOAc (4 mL), and the mixture was stirred under atmospheric hydrogen pressure for 15 h. The catalyst was removed by filtration through a short pad of Celite, which was washed with EtOAc and $CHCl_3$ (100 mL). The filtrate and washings were combined and treated with a buffer solution AcOH/NaAcO (pH 3) for 24 h. The mixture was basified with solid Na_2CO_3 to pH 9 and extracted with CH_2Cl_2 (3 × 15 mL) and $CHCl_3$ (3 × 15 mL). The dried organic extracts were concentrated to give pure **26**. Yield: 101 mg, 99%; $R_f = 0.85$ (hexane/EtOAc 1:2); $[\alpha]_D^{20} = -99.5$ ($c = 0.5$ in $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.40$ (s, 9H), 1.6–2.3 (m, 7H), 2.4–2.6 (m, 2H), 3.76 (s, 3H), 4.28 (m, 2H), 5.35 (br s, $\nu_{1/2} = 14$ Hz, 1H), 7.44 (m, 3H), 8.04 (dd, $J = 8$, 1.2, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 20.0$ (C(4)), 23.6 (C(5)), 28.2 (C(*CH*$_3$*)*$_3$), 30.8 (C(3)), 32.3 (C(7)), 35.6 (C(3a)), 51.0 (C(7a)), 53.6 (OCH_3), 59.3 (C(2)), 69.8 (C(6)), 79.9 (*O*CCH_3), 128.2 (*m*-C), 129.3 (*o*-C), 130.4 (*ipso*-C), 132.8 (*p*-C), 153.8 (NCO_2), 165.6 (OCOPh), 174.0 (CO_2Me); IR (KBr): $\tilde{\nu} = 1750$, 1705, 1700 cm^{-1}.

Methyl (2*S*,3a*S*,6*R*,7a*S*) 6-hydroxy-octahydroindole-2-carboxylate (28): Trifluoroacetic acid (TFA; 1.4 mL, 18.2 mmol) was added to a solution of **22** (110 mg, 0.37 mmol) in CH_2Cl_2 (3 mL). The reaction mixture was stirred at 0 °C for 45 min. After evaporation of the solvent and excess reagent, the trifluoroacetate salt of **28** was obtained quantitatively (115 mg). An analytical sample was obtained by trituration with $CHCl_3$: $R_f = 0.57$ (EtOAc/EtOH 1:1); m.p. 126–128 °C ($CHCl_3$); $[\alpha]_D^{20} = -25.7$ ($c = 0.7$ in CH_3OH); ^{1}H NMR (500 MHz, $CDCl_3 + CD_3OD$, COSY): $\delta = 1.30$ (br, 1H; H-4_{ax}), 1.39 (m, 1H; H-5_{eq}), 1.53 (tm, $J = 10$ Hz, 1H; H-5_{ax}), 1.66 (br, 1H; H-3), 1.89 (br m, 3H; H-3, H-4_{eq} and H-7), 2.35 (br s, 2H; H-3a, H-7), 3.74 (s, 3H; OCH_3), 3.91 (br s, 2H; H-2, H-6), 4.37 (br s, $\nu_{1/2} = 24$ Hz, 1H; H-7a); ^{13}C NMR (75 MHz, $CDCl_3 + CD_3OD$): $\delta = 20.4$ (C(4)), 27.5 (C(5)), 31.1 (C(3)), 31.5 (C(7)), 36.2 (C(3a)), 53.5 (OCH_3), 57.3 (C(7a)), 57.6 (C(2)), 64.1 (C(6)), 170.4 (COOMe); IR (KBr): $\tilde{\nu} = 3403$, 2937, 1748, 1677, 1019; elemental analysis calcd (%) for $C_{12}H_{18}F_3NO_5 \cdot \frac{1}{2}H_2O$ (322.26): C 44.72, H 5.90, N 4.35; found C 44.81, H 5.98, N, 4.43. A sample of **28**·TFA was dissolved in a saturated aqueous Na_2CO_3 solution (2 mL) and extracted with a mixture of $CHCl_3$/*i*PrOH (4:1, 5 × 2 mL). The concentrated dried organic extracts gave **28**: $R_f = 0.18$ (EtOAc/EtOH 3:1); m.p. 83–85 °C; $[\alpha]_D^{20} = -26.2$ ($c = 0.3$ in CH_3OH); ^{1}H NMR (500 MHz, $CDCl_3$, COSY, NOESY): $\delta = 1.20$–1.30 (m, 2H; H-4, H-5_{ax}), 1.55–1.61 (m, 2H; H-4, H-7_{ax}), 1.67 (ddd, $J = 13$, 4.5, 2.5 Hz, 1H; H-3), 1.81 (m, 1H; H-5_{eq}), 1.92 (br, 2H; H-3a, NH), 2.12 (br d, $J = 13$ Hz, 1H; H-7_{eq}), 2.23 (ddd, $J = 13$, 11, 7 Hz, 1H; H-3), 3.32 (br s $\nu_{1/2} = 20$ Hz, 1H; H-7a), 3.72 (s, 3H; OCH_3), 3.77 (br, 1H; H-2), 3.92 (dddd, $J = 8$, 8, 4, 4 Hz, 1H; H-6_{eq}); ^{13}C NMR, 75 MHz, $CDCl_3$): $\delta = 25.8$ (C(4)), 33.7 (C(5)), 36.0 (C(3)), 36.2 (C(7)), 37.1 (C(3a)), 52.2 (OCH_3), 58.2 (C(7a)), 59.0 (C(2)), 66.3 (C(6)), 176.0 (CO_2Me); IR (KBr): $\tilde{\nu} = 3420$, 2931, 1724, 1079 cm^{-1}.

(2*S*,3a*S*,6*R*,7a*S*)-6-Hydroxy-octahydroindole-2-carboxylic acid (5): **From 22**: LiOH (0.1N, 3.2 mL) was added to a cooled (0 °C) solution of **22** (35 mg, 0.12 mmol) in THF (3 mL), and the mixture was stirred at room temperature for 20 h. The mixture was extracted with Et_2O (1 × 5 mL), and the aqueous phase was acidified with 5% aqueous $NaHSO_4$ to pH 4. Extraction of aqueous layer with CH_2Cl_2 (3 × 10 mL) and $CHCl_3$/*i*PrOH (4:1, 3 × 10 mL) gave an organic extract which provided (2*S*,3a*S*,6*R*,7a*S*) 1-*tert*-butoxycarbonyl-6-hydroxy-octahydroindole-2-carboxylic acid (**27**) as a white solid: Yield: 31 mg, 91%; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.43$ (s, 9H), 1.50–1.80 (br, 4H), 1.98–2.20 (br, 3H), 2.35 (br, 2H), 4.20 (br s, 1H), 4.15–4.32 (br, 2H), 5.60 (br, 2H); ^{13}C NMR, (75 MHz, $CDCl_3$): 19.5 (C(4)), 26.4 (C(5)), 28.3 (C(*CH*$_3$*)*$_3$), 29.6 (C(3)), 33.6 (C(7)), 35.9 (C(3a)), 53.6 (C(7a)), 59.2 (C(2)), 65.9 (C(6)), 80.6 (*C*$(CH_3)_3$); IR (KBr): $\tilde{\nu} = 3446$, 2932, 1733, 1681, 1011 (OH_{ax}) cm^{-1}. A solution of **27** (31 mg, 0.12 mmol) in HCl (1N, 10 mL) was stirred at room temperature for 2 h. After concentration and drying, the hydrochloride salt of **5** (26.5 mg, 100%) was isolated as an hygroscopic solid: $R_f = 0.57$ (BuOH/H_2O/AcOH 4:1:1); $[\alpha]_D^{20} = -20.9$ ($c = 0.9$ in CH_3OH); ^{1}H NMR (300 MHz, CD_3OD): $\delta = 1.40$–1.49 (m, 2H; H-3, H-4), 1.65–1.85 (m 3H; H-3, 2H-5), 1.90–2.10 (m, 2H. H-4, H-7), 2.49 (br s, 2H; H-3a, H7), 3.40–4.10 (m, 2H; H-2, H-6), 4.45 (br s, 1H; H-7a); ^{13}C NMR, (75 MHz, CD_3OD): $\delta = 21.8$ (C(4)), 29.0 (C(3)), 32.1 (C(5)), 32.5 (C(7)), 38.0 (C(3a)), 59.3 (C(7a)), 59.5 (C(2)), 65.7 (C(6)), 172.4 (CO_2H); IR (KBr): $\tilde{\nu} = 3409$, 1734 cm^{-1}; elemental analysis calcd (%) for $C_9H_{16}ClNO_3 \cdot \frac{1}{4}CHCl_3$ (251.6): C 44.15, H 6.52, N 5.56; found C 43.84, H 6.64, N 5.48.

From 25: A solution of **25**·HCl (47 mg, 0.15 mmol) in HCl (6N, 4.2 mL) was heated at 85 °C for 8 h. The reaction mixture was cooled to room temperature and washed with CH_2Cl_2 (7 × 5 mL). The aqueous solution was concentrated to give **5**·HCl as a hygroscopic solid (31 mg, 100%).

***(R)*-*β*-(4-Hydroxyphenyl)lactic acid [D-Hpla] (31)**: This compound was obtained by following Wang's method.[26] A solution of *β*-(4-hydroxyphenyl)pyruvic acid (Aldrich, 1.5 g, 8.3 mmol) in THF (30 mL) was treated with triethylamine (1.15 mL, 8.3 mmol) at −20 °C. After 5 min at this temperature, a solution of (−)-DIP-Cl (3.2 g, 9.96 mmol) in THF (10 mL) was added, and the resulting solution was stirred at room temperature for 8 h. The reaction mixture was quenched with 2N aqueous NaOH (10 mL) and H_2O (5 mL). The aqueous layer was washed with *tert*-butylmethylether (2 × 20 mL), and the organic layer with H_2O (2 × 30 mL). The combined aqueous phases were acidified with 2N HCl to pH 1 and extracted with EtOAc (4 × 70 mL). The dried and concentrated organic extracts gave D-Hpla (**31**) as a white solid. Yield: 1.4 g, 92%; $R_f = 0.42$ (EtOAc/MeOH 1:1); m.p. 164–166 °C; $[\alpha]_D^{20} = +10.8$ ($c = 0.52$ in MeOH);[41] ^{1}H NMR (200 MHz, $CDCl_3 + CD_3OD$): $\delta = 2.90$ (dd, $J = 14$, 8 Hz, 1H; H-3), 3.10 (dd, $J = 14.4$, 4.4 Hz, 1H; H-3), 3.4 (br s, 1H; OH), 4.34 (dd, $J = 7.8$, 4.4 Hz, 1H; H-2), 6.75 (d, $J = 8.4$ Hz, 2H; H-6, H-8), 7.10 (d, $J = 8.8$ Hz, 2H; H-5, H-9); ^{13}C NMR (50 MHz, $CDCl_3 + CD_3OD$): $\delta = 40.7$ (C(3)), 73.0 (C(2)), 116.0 (C(6), C(8)), 129.5 (C(4)), 131.5 (C(5), C(9)), 157.0 (C(7)), 177.5

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(C(1)); IR (KBr): $\tilde{\nu}=3251$, 1737 cm^{-1}; elemental analysis calcd (%) for $C_9H_{10}O_4\cdot\frac{1}{2}H_2O$ (190.95): C 56.53, H 5.76; found C 56.56, H 6.02.

(*R*)-β-[4-(Benzyloxy)phenyl]lactic acid [HO-D-Hpla(Bn)-OH] (32): K_2CO_3 (516 mg, 3.3 mmol) was added to a solution of hydroxy acid **31** (152 mg, 0.83 mmol) in $CHCl_3$/MeOH (2:1, 6 mL). After the mixture had been stirred for 15 min at 60 °C, benzyl bromide (0.25 mL, 2.10 mmol) was added dropwise, and the mixture was stirred at this temperature for 6 h. The solvent was removed, H_2O (20 mL) was added, and the mixture was extracted with EtOAc (3 × 20 mL). The dried organic extracts were concentrated to give the dibenzylated compound **33** and benzyl alcohol. The basic aqueous phase was acidified with 2N HCl to pH 1 and extracted with $CHCl_3$/*i*PrOH (4:1, 4 × 30 mL). After being washed with brine, dried, and concentrated, the organic extract yielded 95 mg (42%) of **32** as a white solid.

HO-D-Hpla(Bn)-OBn (**33**): $R_f=0.69$; (EtOAc/MeOH 1:1); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta=2.60$ (br s, 1H; OH), 2.89 (dd, $J=14.0$, 6.2 Hz, 1H; H-3), 3.10 (dd, $J=14.0$, 4.4 Hz, 1H; H-3), 3.72 (s, 2H; CO_2CH_2Ar), 4.38 (dd, $J=6.2$, 4.4 Hz, 1H; H-2), 5.00 (s, 2H; OCH_2Ar), 6.89 (d, $J=$ 8.4 Hz, 2H; H-6, H-8), 7.10 (d, $J=8.4$ Hz, 2H; H-5, H-9), 7.33 (m, 10H; ArH).

LiOH (0.1N, 9.6 mL, 0.96 mmol) was added to a cooled (0 °C) solution of the above mixture of **33** and benzyl alcohol (228 mg) in THF (2 mL), and the comnbined mixture was stirred at room temperature for 24 h. The solution was extracted with EtOAc (2 × 8 mL) and acidified with 2N HCl to pH 1. This aqueous phase was extracted with $CHCl_3$/*i*PrOH (4:1, 4 × 20 mL). The organic extracts were washed with brine, dried, and concentrated to give an additional 63 mg of acid **32** (70% overall yield from **31**): $R_f=0.42$ (EtOAc/MeOH 1:1); m.p. 140–142 °C; $[\alpha]_D^{20}$: +14 ($c=$ 0.61 in MeOH);[41] ^{1}H NMR (200 MHz, $CDCl_3+CD_3OD$) $\delta=2.87$ (dd, $J=$ 13.8, 7.0 Hz, 1H; H-3), 3.08 (dd, $J=14.2$, 4.0 Hz, 1H; H-3), 4.36 (dd, $J=7.0$, 4.4 Hz, 1H; H-2), 5.04 (s, 2H; OCH_2Ar), 6.91 (d, $J=8.8$ Hz, 2H; H-6, H-8), 7.18 (d, $J=8.8$ Hz, 2H; H-5, H-9), 7.41 (m, 5H; ArH); ^{13}C NMR (50 MHz, $CDCl_3+CD_3OD$) $\delta=40.7$ (C(3), 70.8 (OCH_2Ar), 72.8 (C(2)), 115.6 (C(6), C(8)), 128.5 (*p*-C), 128.7 (*m*-C), 129.4 (*o*-C), 138.8 (*ipso*-C), 131.0 (C(4)), 131.5 (C(5), C(9)), 158.9 (C(7)), 177.2 (C(1)); IR (KBr): $\tilde{\nu}=3448-2928$, 1727 cm^{-1}; elemental analysis calcd (%) for $C_{16}H_{16}O_4\cdot\frac{1}{2}H_2O$ (280.95): C 68.30, H 6.05; found C 68.22, H 6.24.

(*R*)-2-Acetyloxy-3-(4-benzyloxyphenyl)propanoic acid [AcO-D-Hpla(Bn)-OH] (34): Acetyl chloride (4 mL, 56 mmol) was added dropwise to a cooled (0 °C) solution of hydroxy acid **32** (1 g, 3.67 mmol) in CH_2Cl_2 (7 mL). The solution was stirred at room temperature for 16 h and concentrated. Purification by chromatography (SiO_2, CH_2Cl_2/AcOH 1%) gave **34** as a white solid. Yield: 1 g, 87%; $R_f=0.50$ (hexane/CH_2Cl_2/AcOH 3:6:1); m.p. 109—110 °C; $[\alpha]_D$: +7.4 ($c=0.7$, $CHCl_3$); ee ≥98%, after crystallization (see Supporting Information); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta=2.10$ (s, 3H; CH_3CO), 3.06 (dd, $J=14.6$, 8.4 Hz, 1H; H-3), 3.20 (dd, $J=14.4$, 4 Hz, 1H; H-3), 5.04 (s, 2H; OCH_2Ar), 5.20 (dd, $J=8.6$, 4.2 Hz, 1H; H-2), 6.92 (d, $J=8.4$ Hz, 2H; H-6, H-8), 7.16 (d, $J=8.8$ Hz, 2H; H-5, H-9), 7.34 (m, 5H; ArH), 10.0 (br s, 1H; COOH); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta=20.5$ (CH_3CO), 36.2 (C(3)), 70.0 (OCH_2Ar), 79.0 (C(2)), 114.8 (C(6), C(8)), 127.5 (C *p*-Ar), 128.0 (C *m*-Ar), 128.5 (C *o*-Ar), 137.0 (C *i*-Ar), 128.0 (C(4)), 130.3 (C(5), C(9)), 158.0 (C(7)), 170.6 (C(1)), 174.8 (CH_3CO_2); IR (KBr): $\tilde{\nu}=3213-2926$, 1760, 1706 cm^{-1}; elemental analysis calcd (%) for $C_{18}H_{18}O_5$ (314.32): C 68.77, H 5.71; found C 68.38, H 5.81.

Boc-D-Leu-L-Choi-OMe (39): BOP (644 mg, 1.46 mmol) and NMM (0.5 mL, 3.92 mmol) were added to a cooled (0 °C) solution of **28**·TFA (350 mg, 1.12 mmol) and Boc-D-Leu (314 mg, 1.36 mmol) in CH_2Cl_2 (23 mL). After the mixture had been stirred at 0 °C for 30 min, the temperature was allowed to rise to room temperature, and stirring was continued for 22 h. The solution was diluted with CH_2Cl_2(53 mL) and washed successively with aqueous $NaHSO_4$ (5%, 3 × 60 mL), saturated aqueous $NaHCO_3$ (2 × 60 mL), and brine (1 × 50 mL). The organic portion was dried and concentrated, and the crude product was purified by chromatography (hexane/EtOAc 1:1) to give alcohol **39a** (114 mg) and trifluoroacetate **39b** (335 mg) in a 1:2 ratio and 73% combined yield.

Compound 39a: $R_f=0.80$ (EtOAc); $[\alpha]_D^{20}=+26.0$ ($c=3.3$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, COSY, NOESY) mixture of 85% of *trans* and 15% of *cis* conformers; *trans* rotamer: $\delta=0.89$ and 0.96 (2 × d, $J=6.5$ Hz, 6H; H-5 and H-5′ Leu), 1.32 (ddd, $J=13.5$, 9, 4 Hz, 1H; H-3 Leu), 1.40 (s, 9H; $C(CH_3)_3$), 1.50 (m, 3H; H-4 Choi, H-5 Choi, H-3 Leu), 1.63 (m, 2H; H-5 Choi, H-4 Leu), 1.78 (ddd, $J=13$, 13, 1.5 Hz, 1H; H-7 Choi) 1.93 (ddd, $J=12.5$, 12.5, 10 Hz, 1H; H-3 Choi), 2.16 (m, 2H; H-3 Choi, H-4 Choi), 2.23 (m, 1H; H-7 Choi), 2.40 (ddddd, $J=12$, 6, 6, 6, 6 Hz, 1H; H-3a Choi); 3.73 (s, 3H; OCH_3), 4.14 (br s, 1H; H-6 Choi), 4.20 (ddd, $J=12.6$, 6 Hz, 1H; H-7a Choi), 4.31 (dd, $J=9$, 2 Hz, 1H; H-2 Choi), 4.47 (ddd, $J=9.5$, 9.5, 4.5 Hz, 1H; H-2 Leu), 5.2 (d, $J=9$ Hz, 1H; NH); *cis* rotamer: 0.85 and 0.90 (6H; H-5, H-5′ Leu), 4.01 (1H; H-2 Leu), 4.07 (1H; H-6 Choi), 4.56 (1H; H-7a Choi), 4.92 (1H; H-2 Choi), 5.04 (1H; NH Leu); ^{13}C NMR (75 MHz, $CDCl_3$, HSQC): $\delta=19.2$(C(4) Choi), 21.9 and 24.6 (C(5), C(5′) Leu), 23.5 (C(4) Leu), 25.7 (C(5) Choi), 28.3 ($C(CH_3)_3$), 30.4 (C(3) Choi), 34.1 (C(7) Choi), 37.0 (C(3a) Choi), 43.5 (C(3) Leu), 49.2 (C(2) Leu), 52.2 (OCH_3), 54.3 (C(7a) Choi), 59.0 (C(2) Choi), 65.4 (C(6) Choi), 79.5 ($C(CH_3)_3$), 155.2 (NCO_2), 170.7 (C(1) Leu), 173.0 (CO_2Me); IR (KBr): $\tilde{\nu}=3425$, 1748, 1702, 1638, 1173, 1017 cm^{-1}; elemental analysis calcd (%) for $C_{21}H_{36}N_2O_6$(412.5): C 61.14, H 8.80, N 6.79; found C 60.89, H 8.97, N 6.64.

Compound 39b: $R_f=0.60$ (EtOAc); $[\alpha]_D$: −36.5 ($c=1.3$ in $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) *trans-cis* rotamer 5:1 ratio, *trans*-rotamer: $\delta=$ 0.94 and 0.99 (2 × d, $J=6.6$ Hz, 6H; H-5, H-5′ Leu), 1.42 (s, 9H; $C(CH_3)_3$), 1.42 (masked, 1H; H-3 Leu), 1.60–2.16 (m, 5H; 2H-5 Choi, H-4 Choi, H-3 L, H-4 Leu); 2.20–2.65 (m, 6H; 2H-3 Choi, H-4 Choi, 2H-7 Choi, H-3a Choi); 3.75 (s, 3H; OCH_3), 4.15 (m, 1H; H-7a Choi); 4.21 (2H; H-2 Choi, H-2 Leu), 5.16 (d, $J=8.7$ Hz, 1H; NH), 5.39 (br s, 1H; H-6 Choi); ^{13}C NMR (200 MHz, $CDCl_3$) *trans* rotamer: $\delta=19.3$ (C(4) Choi), 21.0 and 24.4 (C(5) L, C(5′) Leu), 22.8 (C(5) Choi), 23.4 (C(4) Leu), 28.2 ($C(CH_3)_3$), 30.2 (C(3) Choi), 30.8 (C(7) Choi), 36.6 (C(3a) Choi), 43.5 (C(3) Leu), 50.0 (C(2) Leu), 52.3 (CO_2Me), 53.5 (C(7a)), 59.1 (C(2) Choi), 74.4 (C(6) Choi), 80 ($C(CH_3)_3$), 155.6 (NCO_2), 172 (C(1) Leu), 173 (CO_2Me).

AcO-D-Hpla(Bn)-D-Leu-L-Choi-OMe (40): TFA (1.42 mL, 18.5 mmol) was added to a cooled (0 °C) solution of **39b** (257 mg, 0.51 mmol) in CH_2Cl_2 (7 mL), and the mixture was stirred for 45 min and concentrated. A solution of the resulting material in CH_2Cl_2 (2.8 mL) with NMM (86 μL, 0.77 mmol) was added to a cooled (0 °C) solution of the D-Hpla derivative **34** (157.2 mg, 0.51 mmol) in CH_2Cl_2 (2.8 mL), which had been previously stirred at this temperature with BOP (226 mg, 0.51 mmol) and NMM (120 μL, 1.02 mmol) for 30 min. The mixture was stirred at room temperature for 16 h. CH_2Cl_2 (14 mL) was added, and the organic layer was successively washed with HCl (1N, 3 × 15 mL), saturated $NaHCO_3$ solution (3 × 15 mL), and brine (1 × 10 mL). The concentrated dried extract was chromatographed (hexane/EtOAc 2:1 to EtOAc) to give **40b** (199 mg, 0.28 mmol) and later **40a** (16 mg, 0.026 mmol), the combined yield being 61%.

Compound 40a: $R_f=0.40$ (EtOAc); $[\alpha]_D$: −10.7 ($c=1.2$ in $CDCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, COSY, NOESY) *trans-cis* rotamer 4:1 ratio, *trans*-rotamer: $\delta=0.83$ and 0.91 (2 × d, $J=$6Hz, 6H; H-5, H-5′ Leu), 1.26–1.32 (m, 2H; H-3 Leu, H-4 Leu), 1.39 (t, $J=9$ Hz, 1H; H-3 Leu), 1.47–1.55 (m, 2H; H-4 Choi, H-5 Choi), 1.63 (m, 1H; H-5 Choi), 1.71 (ddd, $J=14$, 13, 5 Hz, 1H; H-7 Choi), 1.94 (ddd, $J=12$, 12, 8 Hz, 2H; H-3 Choi), 2.10 (s, 3H; CH_3O), 2.16 (m, 3H; H-3, H-4, H-7 Choi), 2.39 (ddddd, $J=13$, 6.5, 6.5, 6.5, 6 Hz, 1H; H-3a Choi), 3.08 (m, 2H; H-3 Hpla), 3.70 (s, 3H; OCH_3), 4.14 (s, 1H; H-6 Choi), 4.19 (ddd, $J=12$, 6, 6 Hz, 1H; H-7a Choi), 4.30 (dd, $J=10$, 8 Hz, 1H; H-2 Choi), 4.80 (td, $J=9.5$, 4.5 Hz, 1H; H-2 Leu), 5.00 (s, 2H; OCH_2Ar), 5.31 (dd, $J=6.5$, 5 Hz, 1H; H-2 Hpla), 6.60 (d, $J=9.5$ Hz, 1H; NH), 6.85 (d, $J=8.5$ Hz, 2H; H-6, H-8 Hpla); 7.06 (d, $J=85$ Hz, 2H; H-5, H-9 Hpla), 7.30 (t, $J=7.5$ Hz, 1H; H *p*-Ar), 7.36 (dd, $J=7.5$, 2H; H *m*-Ar), 7.40 (d, $J=7.5$ Hz, 2H; H *o*-Ar); *cis* rotamer: $\delta=2.01$ (OAc), 2.95 (H-3 Hpla), 4.06 (H-6 Choi), 4.52 (H-7a Choi), 4.76 (H-2 Leu), 4.90 (H-2 Choi), 5.28 (H-2 Hpla), 6.63 (NH Leu); ^{13}C NMR (50 MHz, $CDCl_3$, HSQC) *trans* rotamer: $\delta=19.2$ (C(4) Choi), 20.8 and 24.3 (C(5), C(5′) Leu), 22.0 (CH_3CO), 22.1 (C(4) Leu), 25.6 (C(5) Choi), 30.4 (C(3) Choi), 34.0 (C(7) Choi), 36.6 (C(3) Hpla), 37.0 (C(3a) Hpla), 42.7 (C(3) Leu), 48.3 (C(2) Leu), 52.3 (OCH_3), 54.4 (C(7a) Choi), 59.0 (C(2) Choi), 65.5 (C(6) Choi), 70 (OCH_2Ar), 74.2 (C(2) Hpla), 114.6 (C(6), C(8) Hpla), 127.5 (*o*-C), 128.0 (*p*-C), 128.5 (*m*-C), 130.6 (C(5), C(9) Hpla), 137.0 (*ipso*-C), 157.7 (C(7) Hpla), 168.5 (C(1) Hpla), 169.3 (C(1) Leu), 170.0 (CH_3CO_2), 172.4 (CO_2Me); IR (NaCl): $\tilde{\nu}=3425-3350$, 1747, 1636, 1250, 1017, 801, 752 cm^{-1}.

Compound 40b: $R_f=0.50$ (EtOAc); $[\alpha]_D$: −20 ($c=2$ in $CDCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, COSY, NOESY) *trans-cis* rotamer 4:1 ratio, *trans* rotamer: $\delta=0.83$ and 0.91 (2 × d, $J=6.5$ Hz, 6H; H-5, H-5′ Leu), 1.14–1.32 (m, 2H; H-3 Leu, H-4 Leu), 1.43 (t, $J=9$ Hz, 1H; H-3 Leu), 1.60–1.71 (m, 2H; H-4 Choi, H-5 Choi), 1.90–1.98 (m, 4H; H-4 Choi, H-5 Choi, H-7 Choi, H-3 Choi), 2.24 (ddd, $J=13$, 7.5, 6.5 Hz, 1H; H-3 Choi), 2.44–2.54

(m, 2H; H-3a Choi, H-7 Choi), 3.10 (m, 2H; H-3 Hpla), 3.71 (s, 3H; OCH_3), 4.07 (ddd, $J = 12$, 6, 6 Hz, 1H; H-7a Choi), 4.34 (dd, $J = 9.5$, 8 Hz, 1H; H-2 Choi), 4.73 (ddd, $J = 10$, 9.5, 3.5 Hz, 1H; H-2 Leu), 5.00 (s, 2H; OCH_2Ar), 5.30 – 5.34 (m, 2H; H-2 Hpla, H-6 Choi), 6.51 (d, $J = 9.5$ Hz, 1H; NH), 6.85 (d, $J = 8.5$ Hz, 2H; H-6, H-8 Hpla), 7.06 (d, $J = 8.5$ Hz, 2H; H-5, H-9 Hpla), 7.30 (t, $J = 7.5$ Hz, 1H; H *p*-Ar), 7.36 (dd, $J = 7.5$ Hz, 2H; H *m*-Ar), 7.40 (d, $J = 7.5$ Hz, 2H; H *o*-Ar); ^{13}C NMR (300 MHz, $CDCl_3$, HSQC) *trans* rotamer: $\delta = 19.3$ (C(4) Choi), 21.0 (CH_3CO), 21.0 and 23.2 (C(5), C(5′) Leu), 22.7 (C(5) Choi), 24.0 (C(4) Leu), 30.2 (C(3) Choi), 30.6 (C(7) Choi), 36.3 (C(3) Hpla), 36.4 (H-3a Choi), 42.8 (C(3) Leu), 48.1 (C(2) Leu), 52.4 (CO_2CH_3), 53.7 (C(7a)), 59.0 (C(2) Choi), 69.7 (OCH_2Ar), 74.1 (C(2) Hpla), 74.3 (C(6) Choi), 114.5 (C(6), C(8)), 127.5 (*o*-C), 128.0 (C(4) Hpla), 128.0 (*m*-C), 128.5 (*p*-C), 130.6 (C(5) , C(9 Hpla), 137.0 (*ipso*-C), 157.7 (C(7) Hpla), 168.5 (C(1) Hpla), 169 (C(1) Leu), 170.3 (CH_3CO_2), 172.4 (CO_2Me).

HO-D-Hpla(Bn)-D-Leu-L-Choi-L-Arg(NO_2)-OMe (42): A cooled solution of LiOH (0.1N, 10 mL, 1 mmol) was added dropwise to a cooled (0 °C) solution of peptide **40** (197 mg, 0.28 mmol) in THF (10 mL). After having been stirred at room temperature for 20 h, the solution was washed with Et_2O (2 × 15 mL) and acidified to pH 1 with 1N HCl. The aqueous solution was extracted with EtOAc (4 × 30 mL) and $CHCl_3$/*i*PrOH (4:1, 5 × 30 mL). The combined extracts were washed with brine, dried, and concentrated to give acid **41**. Yield: 120 mg, 80%; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 0.82$ (t, $J = 6$ Hz, 6H; H-5, H-5′ Leu), 1.25 – 1.80 (br, 6H), 1.80 – 2.40 (br, 5H), 2.80 (br, 1H; H-3 Hpla), 3.11 (br, 1H; H-3 Hpla), 4.1 – 4.3 (m, 4H; H-2, H-6, H-7a Choi, H-2 Hpla), 4.82 (br t, 1H; H-2 Leu), 4.97 (s, 2H; OCH_2Ar), 6.84 (d, $J = 8.4$ Hz, 2H; H-6, H-8 Hpla), 7.0 (br s, 4H; NH, OH, COOH), 7.2 (d, $J = 8.4$ Hz, 2H; H-5, H-9 Hpla), 7.29 – 7.38 (m, 5H; Ar); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 19.1$ (C(4) Choi), 21.5 and 23.3 (C(5), C(5′) Leu), 24.2 (C(4) Leu), 25.0 (C(5) Choi), 30.0 (C(3) Choi), 33.3 (C(7) Choi), 37.0 (C(3a) Choi), 39.0 (C(3) Hpla), 41.7 (C(3) Leu), 48.3 (C(2) Leu), 59.2 (C(2) Choi), 65.5 (C(6) Choi), 70.0 (OCH_2Ar), 72.3 (C(2) Hpla), 114.3 (C(6) and C(8) Hpla), 127 (*o*-C), 128.0 (*m*-C), 128.4 (*p*-C), 130.7 (C(5) and C(9) Hpla), 137.0 (*ipso*-C), 157.5 (C(7) Hpla), 171.0 (CO_2H), 174.0 and 174.4 (C(1) Hpla and C(1) Leu).

PyBOP (109.3 mg, 0.21 mmol) and NMM (82 μL, 0.73 mmol) were added to a cooled (0 °C) solution of acid **41** (111 mg, 0.21 mmol) and the methyl ester of N^{ω}-nitro-arginine·HCl (Fluka, 56.64 mg, 0.21 mmol) in DMF (2.3 mL). After the solution had been stirred for 30 min at 0 °C and 22 h at room temperature, EtOAc (80 mL) was added, and the solution was washed with saturated aqueous $NaHCO_3$ (1 × 60 mL) and H_2O (5 × 60 mL). The organic layer was dried and concentrated, and the residue was purified by chromatography (EtOAc) to give **42** (147 mg, 91%) as a white foam. $R_f = 0.36$ (EtOAc/MeOH 1:1); $[\alpha]_D$: +20.8 ($c = 6.5$ in $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 0.87$ (t, 6.6 Hz, 6H; H-5, H-5′ Leu), 1.25 (br, 2H; H-3 Leu, H-3 Arg), 1.60 (br, 6H; H-4, H-5 Choi, H-3, H-4 Leu, 2H-4 Arg), 1.80 – 2.00 (br, 2H; H-3 Choi, H-7 Choi), 3.73 (s, 3H; OCH_3), 4.13 (br s, 2H; H-7a Choi, H-6 Choi), 4.23 (br, 2H; H-2 Hpla, H-2 Choi), 4.55 (br, 1H; H-2 Arg), 4.86 (br, 1H; H-2 Leu), 4.99 (s, 2H; OCH_2Ar), 6.88 (d, $J = 8.8$ Hz, 2H; H-6, H-8 Hpla), 7.17 (d, $J = 8.8$ Hz, 2H; H-5, H-9 Hpla), 7.27 (t, $J = 7$ Hz, 1H; H *p*-Ar), 7.35 (t, $J = 7$ Hz, 2H; H *m*-Ar), 7.41 (t, $J =$ 7Hz, 2H; H *o*-Ar); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 19.2$ (C(4) Choi), 21.6 and 23.4 (C(5), C(5′) Leu), 24.5 (C(4) Leu), 25.7 (C(4) Arg), 29.7 (C(5) Choi, C(3) Choi, C(3) Arg), 33.5 (C(7) Choi), 36.8 (C(3a) Choi), 39.6 (C(3) Hpla), 40.7 and 41.7 (C(5) Arg, C(3) Leu), 48.9 (C(2) Arg), 51.5 (C(2) Leu), 52.7 (OCH_3), 55.1 (C(7a) Choi), 60.4 (C(2) Choi), 65.5 (C(6) Choi), 69.9 (OCH_2Ar), 72.6 (C(2) Hpla), 114.5 and 114.6 (C(6), C(8) Hpla), 127.4 (*o*-C), 127.8 (*p*-C), 128.5 (*m*-C), 129.1 (C(4) Hpla), 130.7 (C(5), C(9) Hpla), 137.0 (*ipso*-C), 157.7 (C(7) Hpla), 159.1 (C=NH), 171.5 (C(1) Leu), 172.2 (CON), 172.3 (C(1) Arg), 174.0 (C(1) Hpla); IR (NaCl): $\tilde{\nu} = 3360 – 3317$, 1741, 1670, 1625, 1261, 1014, 757, 688 cm^{-1}.

D-Hpla-D-Leu-L-Choi-L-Argol [Aeruginosin 298-A] (1): $LiBH_4$ (0.34 mmol, 170 μL of 2M solution in THF) was added dropwise to a cooled (0 °C) solution of **42** (147 mg, 0.19 mmol) in THF (15 mL). The resulting solution was stirred at room temperature for 3 h, and cooled again to 0 °C. A saturated $NaHCO_3$ solution (74 mL) was added, and the resulting mixture stirred for 10 min. The aqueous layer was extracted with Et_2O (1 × 60 mL) and EtOAc (5 × 60 mL). The dried organic extracts were concentrated to give a solid, which was crystallized (EtOAc/MeOH 95:5) to give 75 mg (53%) of **43**: $R_f = 0.57$ (EtOAc/MeOH 1:1); $[\alpha]_D$: = +5.2 ($c = 0.45$ in MeOH); ^{1}H NMR (300 MHz, CD_3OD, 40 °C): $\delta = 0.86$ and 0.89 (2 × d, $J =$ 6.3 Hz, 6H; H-5, H-5′ Leu), 1.10 – 1.60 (m, 8H; H-4 Argol, 2H-3 Leu, H-4 Leu, H-5 Choi, 2H-3 Argol, H-4 Choi), 1.70 (br s, 2H; H-5 Choi, H-4 Argol), 1.82 (br t, $J = 12$ Hz, 1H; H-7_{ax} Choi), 2.00 – 2.20 (m, 3H; H-7_{eq} Choi, 2H-3 Choi), 2.22 – 2.42 (m, 2H; H-4 Choi, H-3a Choi), 2.85 (dd, $J =$ 14, 6.5 Hz, 1H; H-3 Hpla), 3.00 (dd, $J = 14$, 4 Hz, 1H; H-3 Hpla), 3.27 (m, 2H; 2H-5 Argol), 3.48 (d, $J = 5.5$ Hz, 2H; 2H-1 Argol), 3.87 (br d, 1H; H-6 Choi), 4.24 (m, 1H; H-7a Choi), 4.26 – 4.34 (m, 2H; H-2 Hpla, H-2 Choi), 4.63 (dd, $J = 10$, 4 Hz, 1H; H-2 Leu), 5.02 (s, 2H; OCH_2Ar), 6.88 (d, $J =$ 8.5 Hz, 2H; H-6, H-8 Hpla), 7.16 (d, $J = 8.5$ Hz, 2H; H-5, H-9 Hpla), 7.34 (m, 5H; 1H *p*-Ar, 2H *m*-Ar, 2H *o*-Ar); ^{13}C NMR (75 MHz, CD_3OD, 40 °C): $\delta = 20.2$ (C(4) Choi), 22.1 and 23.8 (C(5), C(5′) Leu), 25.6 (C(4) Leu), 26.8 (C(3) Argol), 29.4 (C(5) Choi, C(4) Argol), 31.8 (C(3) Choi), 34.7 (C(7) Choi), 38.1 (C(3a) Choi), 40.6 (C(3) Hpla), 42.1 (C(3) Leu), 42.6 (C(5) Argol), 50.3 (C(2) Leu), 52.1 (C(2) Argol), 56.3 (C(7a) Choi), 62.2 (C(2) Choi), 65.1 (C(1) Argol), 66.5 (C(6) Choi), 71.0 (OCH_2Ar), 73.7 (C(2) Hpla), 115.6 (C(6), C(8) Hpla), 128.4 (*o*-C), 128.7 (*p*-C), 129.4 (*m*-C), 131.0 (C(4) Hpla), 131.8 (C(5), C(9) Hpla), 138.8 (*ipso*-C), 158.9 (C(7) Hpla), 160.9 (C=NH), 174.2 (CON), 175.7 (C(1) Hpla).

Pd/C (10%, 69 mg) was added to a solution of **43** (69 mg, 0.093 mmol) in EtOAc/MeOH (1:1, 4.5 mL) and HCl (two drops, 6N), and the mixture was stirred under hydrogen at atmospheric pressure for 8 h, the course of the reaction being monitored by TLC. Upon complete conversion, the catalyst was removed by filtration with Celite, which was washed with EtOAc/MeOH (1:1, 50 mL) and MeOH (50 mL). The organic solution was concentrated to give 59 mg (95%) of the hydrochloride of aeruginosin 298-A (**1**) as a white powder; HPLC: Waters Nova-Park C-18 analytical; A: H_2O/TFA 0.05%, B: CH_3CN/TFA 0.05%; isocratic elution with A/B 68:32 over 45 min; $t_r = 1.40$ min; $R_f = 0.60$ (BuOH/AcOH/H_2O 4:1:1); $[\alpha]_D$: – 72.7 ($c = 0.23$ in H_2O); ^{1}H NMR: see Table 2; ^{13}C NMR: see Table 3; HRFABMS: calcd for $C_{30}H_{49}N_6O_7$, $[M+H]^+$ *m/z*: 605.3665; found 605.3663.

D-Hpla-D-Leu-L-Choi-NH_2 [Aeruginosin 298-B] (3): NH_4OH (33% NH_3, 20 μL, 1 mmol), PyBOP (51.5 mg, 0.1 mmol), and NMM (30 μL, 0.26 mmol) were added sequentially to a cooled (0 °C) solution of acid **41** (42 mg, 0.076 mmol) in CH_2Cl_2 (1.5 mL). The mixture was stirred for 30 min at this temperature and at room temperature for 22 h. $CHCl_3$ was added, and the organic solution was washed successively with $NaHSO_4$ (5%, 3 × 10 mL), saturated $NaHCO_3$ solution (3 × 10 mL), and brine (1 × 10 mL). The organic layer was dried and concentrated. Chromatography (EtOAc/MeOH 1 – 2%) of the residue gave HO-D-Hpla(Bn)-D-Leu-L-Choi-NH_2 (**44**). Yield: 16 mg, 39%; $R_f = 0.60$; (EtOAc/MeOH 1:1); ^{1}H NMR major rotamer (200 MHz, $CDCl_3$): $\delta = 0.89$ (m, 9H; H-5, H-5′ Leu), 1.2 – 2.4 (m), 2.8 (dd, $J = 14$, 6.5 Hz, 1H; H-3 Hpla), 3.1 (dd, $J = 14$, 4 Hz, 1H; H-3′ Hpla), 4.1 (br, 1H; H-6 Choi), 4.2 (br, 1H; H-2 Hpla), 4.4 (m, 2H; H-2 Choi, H-7a Choi), 4.6 (m, 1H; H-2 Leu), 5.0 (s, 2H; OCH_2Ar), 6.2 (br, NH Leu), 6.7 (br, NH_2 Choi), 6.9 (d, $J = 8.4$ Hz, 2H; H-6, H-8 Hpla), 7.2 (d, $J = 8.4$ Hz, 2H; H-5, H-9 Hpla), 7.4 (m, 5H; Ar); IR (NaCl): $\tilde{\nu} = 3392 – 3210$, 1682, 1639, 1014, 753, 582 cm^{-1}.

Pd/C (10%, 4.8 mg) was added to a solution of peptide **44** (16 mg, 0.028 mmol) in EtOAc/MeOH (2%, 1 mL), and the mixture was stirred under hydrogen at atmospheric pressure for 20 h. The catalyst was removed by filtration through Celite and washed with MeOH (45 mL). Evaporation of the solvent gave aeruginosin 298-B (**3**). Yield: 13 mg, 97%; $R_f = 0.56$ (EtOAc/MeOH 1:1); ^{1}H NMR: see Table 2; ^{13}C NMR: see Table 3; HRMS: calcd for $C_{24}H_{35}N_3O_6$ 461.2526; found 461.2533.

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[13] For the first generation synthesis of **13**, see:J. Bonjoch, J. Catena, E. Isábal, M. López-Canet, N. Valls, *Tetrahedron*: *Asymmetry* **1996**, *7*, 1899–1902.

[14] Compounds **8** are mixtures of keto and hydrate forms, as the ^{13}C NMR data of the reaction mixture shows. The tendency of secondary β-amino ketones toward hydration is well known: a) W. Wysocka, *Heterocycles* **1982**, *19*, 1–5; b) C. Herdeis, W. Engel, *Arch. Pharm*. **1992**, *325*, 419–423.

[15] For equilibration of β-amino ketones based on Mannich or retro-Michael processes, see inter alia: a) P. Slosse, C. Hootelé, *Tetrahedron* **1981**, *37*, 4287–4292; b) J. Bonjoch, N. Casamitjana, J. Gràcia, J. Bosch, *Tetrahedron Lett*. **1989**, *30*, 5655–5658; c) W. Deng, L. E. Overman, *J. Am. Chem. Soc*. **1994**, *116*, 11241–11250; d) R. W. Scott, J. Epperson, C. H. Heathcock, *J. Org. Chem*. **1998**, *63*, 5001–5012; e) D. Compère, C. Marazano, B. C. Das, *J. Org. Chem*. **1999**, *64*, 4528–4532.

[16] The spectra of these compounds and of the corresponding racemic derivatives were recorded on fairly concentrated samples (ca. 0.15 M in $CDCl_3$) in the presence of 15 mg of (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol. For this analytical procedure, see: a) W. H. Pirkle, D. L. Sikkenga, *J. Org. Chem*. **1977**, *42*, 1370–1374; b) M. Majewski, R. Lazny, *J. Org. Chem*. **1995**, *60*, 5825–5830

[17] Structures were set up with standard bond lengths and angles by using the Chem-X molecular modeling package, version Jan97. All structures were initially optimized by using the steepest descents and conjugate gradient methods. After semiempirical optimization with the MOPAC program (version 6.0), charge distributions were calculated with the aid of the AM1 method. The PULAY keyword and the eigenvector following the (EF) routine for minimum search with GNORM = 0.1 were used in the optimization step. Conformation analysis was performed with full AM1 optimization. All calculations were performed on a Digital Alpha Station 300.

[18] This diagnostic rule fits with the NMR data of all *N*-substituted derivatives synthesized in this work. *N*-Tosylated *cis*-2-carboxyoctahydroindoles follow the same pattern: A. Nuhrich, J. Moulines, *Tetrahedron* **1991**, *47*, 3075–3088.

[19] *N*-unsubstituted *cis*-2-carboxyoctahydroindoles follow an inverse rule, showing the *exo* derivatives as triplets and *endo* compounds as doublets of doublets ($\approx$10, 4 Hz) for H-2: a) R. Henning, H. Urbach, *Tetrahedron Lett*. **1983**, *24*, 5339–5342; b) M. Vincent, B. Marchand, G. Rémond, S. Jaguelin-Guinamant, G. Damien, B. Portevin, J.-Y. Baumal, J.-P. Volland, J.-P. Bouchet, J.-H. Lambert, B. Serkiz, W. Luitjen, M. Laubie, P. Schiaci, *Drug Des. and Discovery* **1992**, *9*, 11–28; c) S. G. Pyne, A. Javidan, B. W. Skelton, A. H. White, *Tetrahedron* **1995**, *51*, 5157–5168.

[20] M. Mokotoff, S. T. Hill, *J. Heterocycl. Chem*. **1988**, *25*, 65–71.

[21] a) The same conformational change has been observed in decahydroquinolines,[21b] and octahydropyrrolo[3,2-*c*]carbazoles;[11a]b) See inter alia:H. Booth, D. V. Griffiths, *J. Chem. Soc. Perkin Trans. 2* **1975**, 111–118; A. I. Meyers, G. Milot, *J. Am. Chem. Soc*. **1993**, *115*, 6652–6660; M. Naruse, S. Aoyagi, C. Kibayashi, *J. Chem. Soc. Perkin Trans. 1* **1996**, 1113–1124.

[22] Alkene **24** is formed as a by-product.

[23] For some recent contributions about facial diastereoselection in cyclohexanones, see: a) S. Tomoda, T. Senju, *Tetrahedron* **1999**, *55*, 3871–3882; b) T. Laube, *J. Org. Chem*. **1999**, *64*, 8177–8182; c) A. S. Cieplak, *Chem. Rev*. **1999**, *99*, 1265–1336.

[24] For a detailed mechanism for this type of alkylation processes, see: X. Fu, J. M. Cook, *J. Org. Chem*. **1993**, *58*, 661–672.

[25] In some runs, this compound appears to be partially trifluoroacetylated at the Choi oxygen, but this is not a synthetic problem since the trifluoroacetate is cleaved before the last coupling, in the saponification step.

[26] Z. Wang, B. La, J. M. Fortunak, X.-J Meng, G. W. Kabalka, *Tetrahedron Lett*. **1998**, *39*, 5501–5504.

[27] An enantiopure sample of (*R*)-(4-hydroxyphenyl)lactic (D-Hpla) (see Supporting Information) has $[\alpha]_D^{20} = +10.8$ ($c = 0.52$ in MeOH). This compound was prepared by Murakami[2e] by diazotation of 4-amino-D-phenylalanine in 31% yield.

[28] Y. Hamada, T. Shiori, *Chem. Pharm. Bull*. **1982**, *30*, 1921—1924. Using this reagent in aprotic solvent, Murakami has synthesized the L-Argol.[2e]

[29] A slight excess of the reducing agent induces the cleavage of the Choi–Leu amide bond. See: K. Soai, A. Ookawa, *J. Org. Chem*. **1986**, *51*, 4000–4005.

[30] Due to a dearth of natural product, we were unable to obtain an authentic sample of aeruginosin 298-A. However, comparisons were performed with the ^1H NMR and ^{13}C NMR spectra of natural aeruginosin 298-A. The optical rotation of synthetic **1**, $[\alpha]_D^{25} = -73$, is very different from the reported value for the natural **1**, $[\alpha]_D^{25} = +22.3$. We are unable to comment on the significance of this discrepancy, except to note that our sample was a hydrochloride salt while the natural product had been isolated as a trifluoroacetate salt.

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[34] a) S. Bajusz, E. Szell, D. Bagdy, E. Barabas, G. Horvath, M. Dioszegi, Z. Fittler, G. Szabo, A. Juhasz, E. Tomori, G. Szilagyi, *J. Med. Chem*. **1990**, *33*, 1729–1735; b) M. R. Wiley, N. Y. Chirgadze, D. K. Clawson, T. J. Craft, D. S. Gifford-Moore, N. D. Jones, J. L. Olkowski, A. L. Schacht, L. C. Weir, G. F. Smith, *Bioorg. Med. Chem. Lett*. **1995**, *5*, 2835–2840.

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[36] P. Wipf, J.-L. Methot, *Org. Lett*. **2000**, *2*, 4213–4216.

[37] a) B. Wünsch, M. Zott, *Liebigs Ann. Chem*. **1992**, 39–45; b) W. Siedel, K. Sturm, R. Geiger, *Chem. Ber*. **1963**, *96*, 1636–1440.

[38] Mixtures of *exo*- and *endo*-**9** and their corresponding dimethyl acetals were observed by NMR during this stage.
[39] In some runs, on elution with CH_2Cl_2/EtOAc (9:1), small quantities (<3% yield) of a *trans* isomer were isolated.
[40] For analytical data see Supporting Information.
[41] The optical rotation value corresponds to that of a sample obtained from the recrystallyzed Hpla derivative **34** (ee >98%).

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