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A New Bicyclic Dipeptide Isostere with Pyrrolizidinone Skeleton

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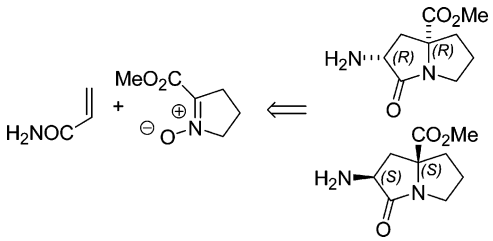

The synthesis of a new conformationally constrained Gly-(*s-cis*)Pro Turn Mimetic (GPTM) in both racemic and enantiomerically pure forms and their incorporation into peptides **18**, **21**, and **24** are reported. The synthetic strategy adopted to assemble the bicyclic pyrrolizidinone skeleton is based on the 1,3-dipolar cycloaddition of the cyclic nitrone **4a** derived from proline and acrylamide, followed by a reductive cleavage/cyclization domino process. The enantiomerically pure GPTMs are obtained by synthesis and separation of diastereomeric intermediates containing (1*R*)-1-phenylethylamine as chiral auxiliary. Analysis of pseudotripeptides **18**, **21**, and **22** by FT-IR and NMR shows that the amide proton of GPTM derivatives **21** is intramolecularly hydrogen bonded in $CDCl_3$, while DMSO was shown to disrupt this hydrogen bond.

Introduction

Conformationally constrained polypeptides are the object of substantial research activity, because turns are present in the active conformations of biologically important peptides.[1] Among the different strategies adopted to induce constraint in a peptide chain, of particular interest and success has been the replacement of a dipeptide substructure in a natural substrate with a constrained rigid analogue that is able to induce a folding of the peptide chain, such as a β-turn or a hairpin.[2,3] The design of a rigid constraint dipeptide leads consequentially to the construction of bicyclic aza-heterocycles that can provide the most useful features for the scope and embody a broad structural diversity easily accessible by numerous synthetic pathways. In fact, a large array of fused bicyclic (or polycyclic) lactams featuring all the ring combinations (4,5; 5,5; 5,6; 5,7; 5,8; 6,6; 6,7) have been synthesized and employed in peptidomimetics.[3,4] In search of a new member of this class that could combine the structural rigidity with the ease of access and the possible implementation of the structure to provide a full family of turn mimetics, we focused on pyrrolizinone **1** as our target dipeptide isostere (Scheme 1).[5]

Compound **1** (R=R′=H) and its enantiomer can be envisaged as Gly-Pro dipeptide mimetics rigidified by a

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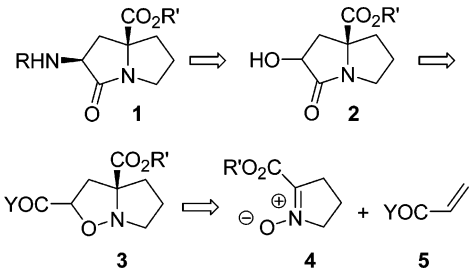
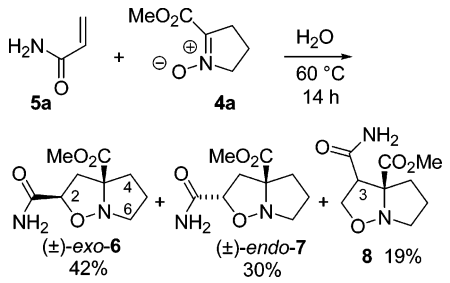
SCHEME 1



SCHEME 2



methylene bridge between the two amino acidic α carbons (GPTM, Gly-Pro Turn Mimetic), or as Ala-Pro dipeptide mimics with the Ala methyl linked to the Pro α carbon (APTM, Ala-Pro Turn Mimetic). The ability of **1** to induce a turn is conferred by the *cis* relationship of the amine and carboxylic groups on the convex face of the molecule. The high rigidity of the pyrrolizidinone ring, in fact, bestowed the compound with a β-turn mimetic ability similar to that of the best candidates of the class.[5,6]

Our retrosynthetic analysis for compound **1** suggested that it could straightforwardly derive from the hydroxylated analogue **2**, which in turn could be prepared through the well-known consecutive reductive cleavage/cyclization of an isoxazolidine **3** formed by 1,3-dipolar cycloaddition (1,3-DC) of nitrone **4** and acrylate or acrylamide dipolarophiles **5**. As nitrone **4** can be directly synthesized from proline esters and acrylates and acrylamides are widely available substrates, the general access to dipeptide isoster **1** is granted. Unfortunately, the formation of nitrone from proline leads to the loss of chirality of this starting material, and absolute configuration has to be introduced at a later stage of the synthesis. There are many ways to introduce absolute configuration in our compound, among these the resolution of racemic mixtures will be analyzed in this paper, whereas others are still object of studies in our laboratories. Actually, in the preliminary investigation of new peptidomimetic scaffolds both optical isomers are in principle necessary. Therefore, the separation of optically pure diastereomeric salts or derivatives leading to both enantiomers is sometimes more convenient than the asymmetric synthesis of one single optically pure diastereoisomer.

Herein we report the synthesis of racemic and enantiopure pyrrolizidinones **15**, the coupling of these bicyclic scaffolds with natural amino acids, and the conformational analysis of pseudotripeptides **18**, **21**, and **22** by FT-IR and ^{1}H NMR. This study indicates that both the enantiomeric GPTMs mimic the two central residues of VI β-turn types. In particular, the diastereomeric conjugates **21** adopt a VIa β-turn conformation in relatively nonpolar solvents such as CH_2Cl_2 and $CDCl_3$.

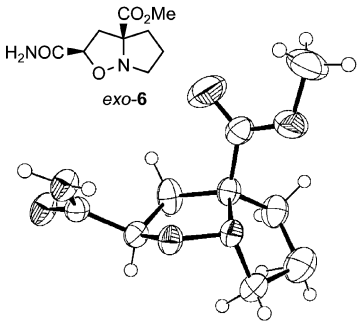


FIGURE 1. ORTEP drawing from the X-ray crystal structure of adduct *exo*-**6**.

Results and Discussion

The treatment of a concentrated (3.5 M) aqueous solution of the nitrone **4a**[7] with 2 equiv of acrylamide (**5a**) at 60 °C for 14 h afforded the 2-aminocarbonyl pyrrolo[1,2-*b*]isoxazolidines *exo*-**6** and *endo*-**7** and their 3-aminocarbonyl isomer **8** in 2.2:1.6:1 ratio and 91% overall yield (Scheme 2). The 1,3-DC under the reported reaction conditions shows a good regioselectivity (3.7:1) in favor of the desired 2-substituted adducts and a low *endo*/*exo* diastereoselectivity (1:1.4). Luckily, both the adducts *exo*-**6** and *endo*-**7** could be used in the synthesis of pyrrolizinone derivates **1** by selective conversion into a common intermediate (see below).

The ^{1}H NMR spectra of adducts *exo*-**6** and *endo*-**7** were differentiated mainly by the value of the coupling constants between 2-H and the two 3-H atoms [*exo*-**6**: $\delta_{2-H} = 4.59$ (dd, $J = 8.8$, 4.8 Hz); *endo*-**7**: $\delta_{2-H} = 4.55$ (dd, $J = 9.5$, 7.3 Hz)]. The NMR data were inconclusive to ascertain the relative configuration of *exo*-**6** and *endo*-**7**, but the structure of *exo*-**6** could be unequivocally assigned by X-ray crystallography of a single crystal (Figure 1).

The pyrrolizidine *trans*-**10** was directly obtained from *exo*-**6** by treatment with H_2 in the presence of a catalytic amount of $Pd(OH)_2$/C and 10 mol equiv of AcOH. The reaction goes through the N−O bond hydrogenolysis to the amino alcohol **9** followed by a spontaneous cyclization via intramolecular transamidation (Scheme 3). The domino process is very efficient and affords the analytically pure *trans*-**10** in 96% yield. As either the reductive cleavage and cyclization steps do not affect the configuration of the stereogenic centers (relative configuration: *exo*-**6**: 2*R**,3a*S**; *trans*-**10**: 2*R**,7a*S**), under the same hydrogenation conditions the diastereomer *endo*-**7** afforded the sole alcohol *cis*-**12** in 96% yield.

The alcohol *trans*-**10** features the pyrrolizin-3-one skeleton of the target GPTM bearing the hydroxyl group

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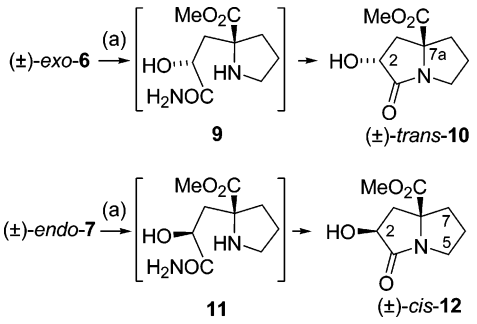
SCHEME 3[a]



[a] (a) H_2, $Pd(OH)_2$/C, AcOH, MeOH, 96%.

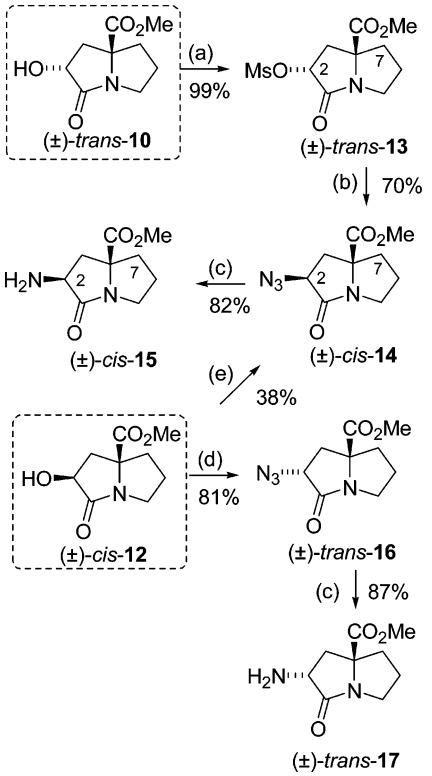
in the suitable position and orientation to introduce an amino moiety on the convex face of the bicyclic system through a S_N2 reaction. The base-induced epimerization of the *cis*-alcohol **12** to the thermodynamically more stable *trans* isomer **10** was attempted, but an inseparable diastereomeric mixture of **10** and **12** was always obtained. In particular, the hydroxy ester *cis*-**12** was hydrolyzed to the corresponding *cis*-hydroxy carboxylate by aqueous NaOH in MeOH in few minutes at room temperature. Under more vigorous reaction conditions [*t*BuOK (2 mol equiv), H_2O, sealed vial, 100 °C, 1 d], the *cis* derivative was in part isomerized, and the subsequent treatment with MeI in DMF at room temperature afforded a 1:1 mixture of *trans*-**10** and *cis*-**12** in 72% overall yield after chromatographic purification.

The alcohol *trans*-**10** was transformed into the corresponding amine *cis*-**15** through a three-step procedure consisting of mesylation, nucleophilic displacement with NaN_3, and reduction of the azido group with Raney-Ni in 57% overall yield (Scheme 4). The direct conversion of *trans*-**10** to the azide *cis*-**14** with diphenyl azidophosphate (DPPA) in the presence of a base[8] was also tested, but it was revealed to be less convenient because it afforded a mixture of the azide and the corresponding 2-[(diphenoxyphosphoryl)oxy]-pyrrolizidinone that did not undergo displacement by the azide ion even under strenuous conditions. On the contrary, the diastereomeric alcohol *cis*-**12** gave the sole azide *trans*-**16** in 81% yield by treatment with DPPA and 4-(dimethylamino)pyridine (DMAP)[9] at 65 °C for 3.5 h (Scheme 4). The reduction of *trans*-**16** in the presence of Raney-Ni provided the amine *trans*-**17** in 87% yield.

The introduction of the azide group proceeded with clean S_N2 inversion either from the mesilate *trans*-**13** with NaN_3 or from the alcohol *cis*-**12** with DPPA/DMAP. The alcohol *cis*-**12** could be also converted into the amino ester *cis*-**15** by a double inversion process. In fact, treatment of *cis*-**12** with $SOCl_2$ in the presence of pyridine, followed by NaN_3 in dimethylformamide, afforded the *cis*-**14** azide intermediate through two sequential S_N2 reactions (Scheme 4).

The ^1H NMR spectra of the 2,7a-disubstituted hexahydro-3*H*-pyrrolizin-3-ones **10** and **12**−**17** show two distinctive patterns characteristic for the *cis* and *trans* derivatives, respectively (Table 1). In particular, the coupling constants between 2-H and the methylene hydrogens on C-1 in the *trans*-substituted derivatives were both large (7.5−8.4 and 9.9−11.4 Hz), while the corresponding *cis*-diasteromers show a much smaller coupling constant (1.5−3.0 Hz) besides a large one (6.8−8.2 Hz). Moreover, the difference between the chemical shift ($\Delta\delta$) of the two hydrogens of the C-1 methylene moiety in the *trans* bicyclic lactams is significantly larger than in the *cis* compounds (Table 1). Analogues features were found in all the other synthesized derivatives belonging to the *trans* or *cis* series (see below and Table 1).

SCHEME 4[a]



[a] (a) MsCl, TEA, CH_2Cl_2; (b) NaN_3, DMF; (c) Raney-Ni, MeOH; (d) DPPA, DMAP, toluene; (e) i) $SOCl_2$, Py, 54%; ii) NaN_3, DMF, 71%.

The primary amine *cis*-**15** proved to be configurationally less stable than the corresponding alcohol *cis*-**12** and azide *cis*-**14** as it slowly isomerizes to the thermodynamically more stable *trans*-**17** even at room temperature in $CDCl_3$ solution. It is, therefore, advisable to use immediately the free amino ester in couplings with other amino acids or to protect the amino group.

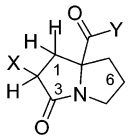
To test their potential utilization in the synthesis of peptides, the two racemic amines *cis*-**15** and *trans*-**17** were coupled with L-Phe either at the *N*-terminus or *C*-terminus under classic solution-phase peptide synthesis conditions. In all cases the reaction conditions were not optimized because the diastereomeric pseudotripeptides appeared to be hardly separable. Nevertheless, the product mixtures were useful to study the conformation in solution of both pairs of diastereomers at the same time (see below).

The reaction of *cis*-**15** and Boc-Phe-OH in the presence of bromo[tri(1-pyrrolidinyl)]phosphonium hexafluoro-

(8) Thompson, A. S.; Humphrey, G. R.; DeMarco, A. M.; Mathre, D. J.; Grbowski, J. J. *J. Org. Chem.* **1993**, *58*, 5886−5888.

(9) In this case the use of DMAP as base afforded better results than 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) or 1,4-diazabicyclo[2.2.2]-octane (DABCO).

TABLE 1. Selected ^{1}H NMR Data of 2,7a-Disubstituted Hexahydro-3*H*-pyrrolizin-3-ones (Values Referring to *trans*-2,7a Compounds Are Underlined to Facilitate Comparisons)



Y = OMe:
10,12 (X = OH); **13** (X = OMs);
trans-**Cl** (X = Cl); **14,16** (X = N_3);
15, 17 (X = NH_2); **18,19** (X = BocPhe);
23 (X = NHCH(Me)Ph);
24 [X = FmocAsp(*t*Bu)]

Y = OH: **25** [X = FmocAsp(*t*Bu)]

Y = PheOMe: **21** (X = NHBoc)

cmpd	δ_{2-H} (ppm)[a]	δ_{1-Ha} (ppm)[a]	δ_{1-Hb} (ppm)[a]	$J_{1a,2}$ (Hz)	$J_{1b,2}$ (Hz)
10	4.71	3.00	1.96	7.5	10.5
12	4.37	2.54	2.25	1.5	6.8
13	5.55	~3.2[b]	~2.1[b]	8.4	9.9
***trans*-Cl**	4.84	3.20	2.20	7.7	11.0
14	4.18	2.54	2.24	1.5	7.6
15	3.60	2.38[c]	2.24[d]	8.2	3.0
16	4.54	2.94	1.84	8.1	11.0
17	3.92	2.96	~1.7[b]	7.6	11.4
18a,b[e]	4.65 4.55	~2.4[b]	2.26 2.11	8.2	3.0
19[g]	~4.6[f]	3.19	1.86	7.7	10.9
21[g]	~4.2[f]	2.56	2.29	10.2	4.7
23a	3.31	2.16	2.03	2.2	7.9
23b	3.34	2.49	2.09	2.8	7.5
24a	4.68[h]	2.46	2.30[i]	9.0	2.5
25a	~4.5[b]	2.50	~2.3[f]	9.5	n.d.
24b	4.69[h]	2.41	2.30	8.5	2.2
25b	~4.4[b]	~2.4[b]	~2.4[b]	n.d.	n.d.

[a] All doublet doublet (dd) except where indicated. [b] Multiplet (m) (referred to the resonance of two or more hydrogen). [c] X part of an AXY system. [d] Y part of an AXY system. [e] Recorded at 55 °C. [f] m (referred to the resonance of only one hydrogen). [g] One diastereomer. [h] Doublet triplet (dt). [i] Broad doublet (br d).

phosphate (PyBroP) and *N*-ethyl-*N*,*N*-diisopropylamine (DIPEA) afforded a mixture of the two diasteromeric tripeptide isosteres (2*R*,7a*R*)-**18a** and (2*S*,7a*S*)-**18b** with a not optimized 55% yield (Scheme 5). Similarly, the amine *trans*-**17** provided (2*R*,7a*R*)-**19a** and (2*S*,7a*S*)-**19b** in 76% yield. In this case an accurate chromatographic separation allowed recovery of one of the two diastereomers **19** in low yield (12%) (Scheme 5).

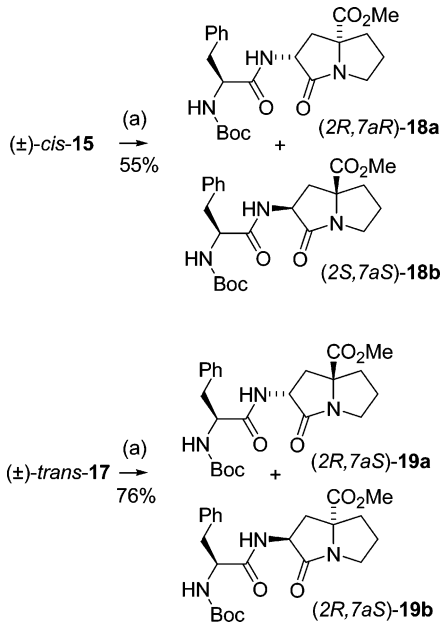
The diastereomeric mixtures of tripeptide isosteres **21** and **22** were prepared by sequential treatment of *cis*-**15** and *trans*-**17**, respectively, with $(Boc)_2O$ to protect the amino moiety, NaOH to hydrolyze the methyl ester, and finally, L-Phe-OMe in the presence of the usual coupling reagents (Scheme 6).

Besides demonstrating the good reactivity of the *N*- and *C*-termini of both the enantiomers of *cis*-**15** and *trans*-**17**, the syntheses of compounds **18**, **19**, **21**, and **22** confirmed the conservation of the relative configuration between C-2 and C-7a under the coupling reaction conditions and during the purification of the final products. The *cis* and *trans* orientation of the amino and carboxylic acid groups on the bicyclic system in **18**, **21** and **19**, **22**, respectively, could be easily recognized by analyzing the ^{1}H NMR spectra that showed the same typical patterns already observed in the other compounds belonging to the *cis* or *trans* series (Table 1).

Enantiopure bicyclic scaffolds were required for peptidomimetic synthesis because of the difficulties in separating diastereomeric pseudotripeptides. To make both enantiomers of amine *cis*-**15** available, the resolution of a racemate of an intermediate of the synthetic sequence was planned by using a chiral auxiliary to create separable diastereomeric compounds.

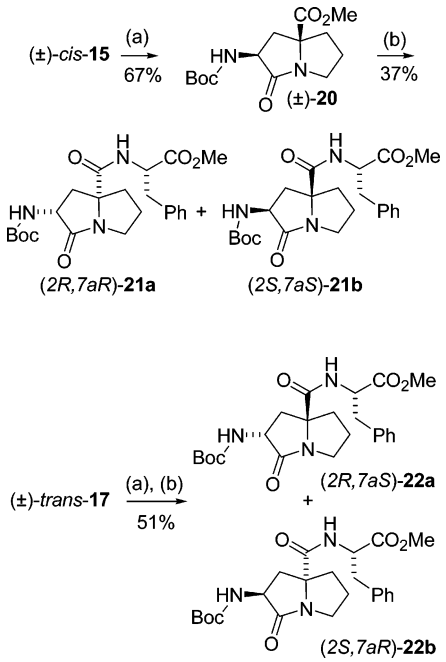
The commercial inexpensive (1*R*)-1-phenylethylamine did not react with mesylate *trans*-**13** even at high temperature. Therefore, the more reactive trifluoromethanesulfonyl (Tf) leaving group was introduced. The triflate of *trans*-**10** obtained by treatment of the alcohol with Tf_2O and pyridine[10] in CH_2Cl_2 at 0 °C reacted with the

SCHEME 5[a]



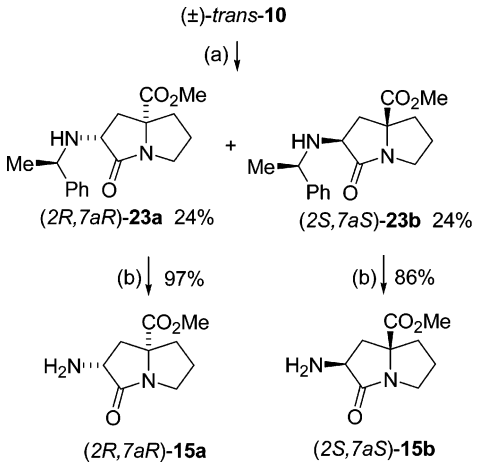
[a] (a) Boc-Phe-OH, PyBroP, DIPEA.

SCHEME 6[a]



[a] (a) $(Boc)_2O$, DIPEA; (b) i) NaOH, MeOH; ii) Phe-OMe, PyBroP, DIPEA.

SCHEME 7[a]



[a] (a) i) Tf_2O, Py, CH_2Cl_2, 0 °C; ii) (*R*)-PhMeCHNH$_2$, CH_2Cl_2, 0 °C; (b) H_2, $Pd(OH)_2$/C, MeOH, 35 atm, 20 h.

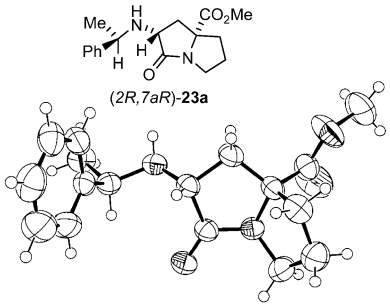


FIGURE 2. ORTEP drawing from the X-ray crystal structure of optically pure amine (2*R*,7a*R*)-**23a**.

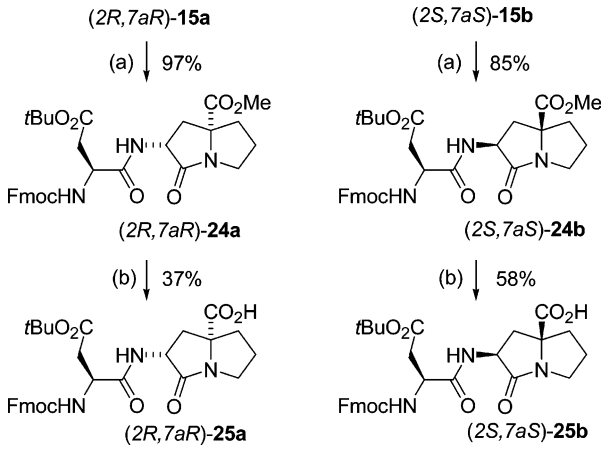
enantiomerically pure amine to afford equimolecular amounts of the diastereomeric (2*R*,7a*R*)-**23a** and (2*S*,7a*S*)-**23b** (48% overall yield) that were separated by chromatography on silica gel (Scheme 7). The ^{1}H NMR analysis confirmed the complete inversion at the C-2 (Table 1), leading to the formation of *cis*-disubstituted pyrrolizidinones.

The secondary amines (2*R*,7a*R*)-**23a** and (2*S*,7a*S*)-**23b** proved to be configurationally more stable than *cis*-**15** and could be safely purified and characterized. The absolute configuration of (2*R*,7a*R*)-**23a** could be ascertained by single-crystal X-ray crystallography (Figure 2).

The chiral auxiliary was easily removed by hydrogenolysis of (2*R*,7a*R*)-**23a** and (2*S*,7a*S*)-**23b** at 35 atm in the presence of a catalytic amount of $Pd(OH)_2$/C (Scheme 7) to afford the enantiomerically pure amines (2*R*,7a*R*)-**15a** and (2*S*,7a*S*)-**15b** in high yields (86−97%). Albeit the *cis*-amines have to be protected or utilized in the subsequent step to avoid epimerization, (2*S*,7a*S*)-**15b** could be purified by fast chromatography on silica to determine its optical rotation value (see Experimental Section).

The GPTM (2*R*,7a*R*)-**15a** and (2*S*,7a*S*)-**15b** were coupled with the orthogonally protected amino acid Fmoc-Asp(O*t*Bu)-OH in the presence of PyBroP and DIPEA and afforded the corresponding tripeptide isosteres (2*R*,7a*R*)-**24a** and (2*S*,7a*S*)-**24b** in excellent yields (97 and 85%, respectively) (Scheme 8). Only one diastereomer **24** from each enantiomer **15** was obtained, attesting that no racemization occurs at the aspartic acid stereocenter under the coupling reaction conditions.

SCHEME 8[a]



[a] (a) Fmoc-Asp(O*t*Bu)-OH, PyBroP, DIPEA; (b) NaOH, $CaCl_2$, *i*PrOH, H_2O.

Tripeptides (2*R*,7a*R*)-**24a** and (2*S*,7a*S*)-**24b** were useful model compounds to explore the possibility of hydrolyzing the methyl ester at the GPTM *C*-terminus in the presence of the Fmoc protecting group and without any C-2 epimerization. The alkaline hydrolysis in the presence of $CaCl_2$, following the procedure described by Pascal and Sola,[11] allowed obtaining of the Fmoc-Asp(O*t*Bu)-GPTM-OH (2*R*,7a*R*)-**25a** and (2*S*,7a*S*)-**25b** in 37 and 58% yield, respectively (Scheme 8). The relative *cis* configuration of **24a**, **24b**, **25a**, and **25b** could be confirmed following the same criteria previously illustrated (Table 1).

In a preliminary communication, we reported computational studies on model hexapeptides Ac-Ala-Ala-GPTM-Ala-Ala-NHMe that showed effective turn restraints induced by the insertion of the dipeptide isosteres.[5,6] In particular, a high percentage of conformers of both the diastereomeric Ac-Ala-Ala-GPTM-Ala-Ala-NHMe peptides possessed geometrical parameters characteristic of an open β-turn i.e., a β-turn not stabilized by a hydrogen bond between the termini of the turn region ($NH_{Ala5}-CO_{Ala2}$).

To validate the modeling predictions, we investigated some structural features of the protected pseudotripeptides **18**, **21**, and **22** by FT-IR and ^{1}H NMR spectroscopies.[12] The pseudotripeptide mixtures could be directly analyzed without isolation of the single components because the ^{1}H NMR signals of the two diastereomers were sufficiently differentiated, and in all cases, no significant difference was observed between diastereomers **a** and **b**. Compounds **18** and **21** were used as models because they possess the minimum structural

(10) Zhang, C.; Ludin, C.; Eberle, M. K.; Stoeckli-Evans, H.; Keese, R. *Helv. Chim. Acta* **1998**, *81*, 179−181.

(11) Pascal, R.; Sola, R. *Tetrahedron Lett.* **1988**, *39*, 5031−5034.

(12) (a) Dado, G. P.; Gellman, S. H. *J. Am. Chem Soc*. **1993**, *115*, 4228−4245. (b) Gellman, S. H.; Dado, G. P.; Liang, G.-B.; Adams, B. R. *J. Am. Chem. Soc*. **1991**, *113*, 1164−1173. (c) Boussard, G.; Marraud, M. *J. Am. Chem. Soc.* **1985**, *107*, 1825−1828. (d) Stevens, E. S.; Sugarawa, N.; Bonora, G. M.; Toniolo, C. *J. Am. Chem. Soc*. **1980**, *102*, 7048−7050.

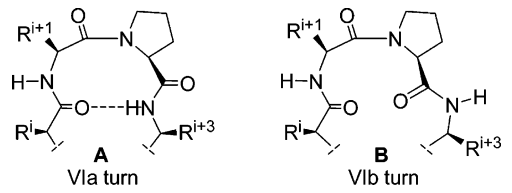


FIGURE 3. Natural VI β-turns contain a *s-cis*-Pro at the i + 2 position.

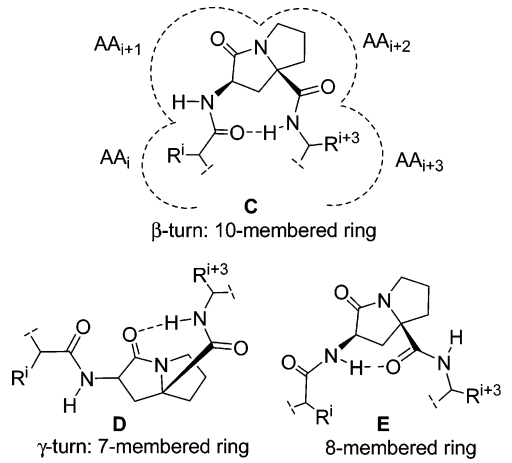
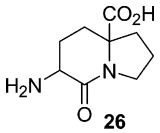


FIGURE 4. Hydrogen-bonded conformations available to peptides incorporating a GPTM.

elements to form the characteristic intramolecular hydrogen bond that is necessary to prove the formation of β-turns.



26

Analogously to the homologue indolizidinone **26** studied by Germanas et al.,[4h] GPTM could mimic the two central residues of natural VI β-turns that have a *s-cis*-proline in position i + 2 (Figure 3).[13] Structure **C** (Figure 4) represents a type VIa β-turn where the amino acids in i + 1 and i + 2 positions have been replaced with the GPTM moiety. Conjugated GPTM could also adopt other conformations stabilized by different intramolecular hydrogen bonds, including **D** and **E** (Figure 4). On the contrary, the *trans* isomers of GPTM preclude the formation of the aforementioned internal hydrogen-bonded conformations except for **D** (Figure 4).

The analysis and comparison of the N−H stretch region of pseudopeptides **21** and **22** recorded at different concentrations proved the presence of intramolecular hydrogen bonds in the GPTM derivatives **21**. In particular, the IR spectrum of a 30 mM solution of *trans* derivatives **22** in CH_2Cl_2 showed a relatively strong band at 3411 cm^{-1} attributable to a non-hydrogen-bonded NH stretch, and only a weak band at 3333 cm^{-1} for a hydrogen-bonded NH stretch (Figure 5).[14] As the concentration was reduced to 7 mM, the second band disappeared, showing that the hydrogen bonding was intermolecular rather than intramolecular.

(13) Richardson, J. S. *Adv. Protein Chem.* **1981**, *34*, 167−339.

At a concentration of 30 mM, the IR spectrum of the *cis* derivatives **21** exhibited two bands in the region of free NH stretch (3449 and 3408 cm^{-1}) and one attributable to a hydrogen-bonded NH stretch (3339 cm^{-1}) (Figure 5). The indication that these NH hydrogens were involved in intramolecular hydrogen bonds came from the lack of any significant concentration dependence (from 30 to 2 mM) of the IR spectrum of **21**.

The ^1H NMR analysis of compounds **21** and **22** in $CDCl_3$ solution supported the presence of internal hydrogen bonds in both diastereomers **21a** and **21b**. In general, chemical shift values (δ) of the carboxamide hydrogens higher than 7 ppm are strongly indicative of NH involved in hydrogen bonds.[14] In fact, the δ NH^3 (Figure 6) of *cis* isomers **21** measure 7.31 and 7.11 ppm in the absence of significant aggregation (~2 mM $CDCl_3$ solution).[15] As a confirmation, the corresponding NH^3 of *trans* isomers **22**, which can be considered as reference compounds with no hydrogen-bonded NH, resonate upfield, namely at 6.55 and 6.54 ppm.

The absence of intramolecular hydrogen bonding in the *trans* isomers **22** rules out the formation of 7-membered ring hydrogen bonding (γ-turn conformation **D**) also for *cis* isomers **21**. The formation of structure **E** (Figure 4) involving NH^2 hydrogen bond was dismissed by observing the NH stretch region of the IR spectrum of **18**. In particular, the 7 mM solution of compounds **18** in CH_2Cl_2 showed only a band at 3420 cm^{-1} that was attributable to free NH groups (Figure 5). The combination of these data suggests that 10-membered ring hydrogen bonding (β-turn conformation **C**, Figure 4) occurs in **21**.

In DMSO the NH^3 protons of both isomers **21** undergo a downfield shift (δ_{DMSO} = 8.16 and 8.11 ppm), which proves that they are both accessible to the solvent, i.e., NH^3 protons are no longer internally hydrogen bonded in the presence of a competitive solvent.

The NH^3 hydrogen bond strength in $CDCl_3$ was qualitatively evaluated by measuring the $\Delta\delta$ NH^3 upon small addition of a competitive solvent such as DMSO and the temperature dependence ($\Delta\delta/\Delta T$) in $CDCl_3$.[4h,14] Both the parameters were in accord with NH^3 in equilibrium between hydrogen-bonded and non-hydrogen-bonded states. In particular, δ NH^3 in each compound **18**, **21**, and **22** shifted downfield upon addition of a small quantity of DMSO-d_6 to the $CDCl_3$ solution ($\Delta\delta_{10\%\ DMSO}$: **21** +0.46 and +0.63; **22** +0.49 and +0.38, **18** +0.81 and +0.77 ppm; Figure 7).

The internal NH^3 hydrogen bonding in both tripeptide mimetics **21** showed a large temperature dependence (**21**: $\Delta\delta/\Delta T$ = −5.0 and −4.9 ppb/K; Figure 8) in accord with the presence of amide protons in equilibrium between hydrogen-bonded and non-hydrogen-bonded states.[14] As expected, the non-hydrogen-bonded NH^3 in the *trans* derivatives **22** showed a low-temperature coefficient (**22**: $\Delta\delta/\Delta T$ = −1.3 and −1.4 ppb/K). The $\Delta\delta/\Delta T$ values of NH^2 in **18** were too large in absolute value (−3.4 and −2.8 ppb/K) for a non-hydrogen-bonded state according to general classifications.[12,14] Anyway, in this case the lack of hydrogen bonds was unambiguously demonstrated by the IR analysis (see Figure 5).

(14) Belvisi, L.; Gennari, C.; Mielgo, A.; Potenza, D.; Scolastico, C. *Eur. J. Org. Chem.* **1999**, 389−400.

(15) For all model compounds **18**, **21**, and **22**, NH δ values were independent of concentration at or below 5.0 mM at 295 K.

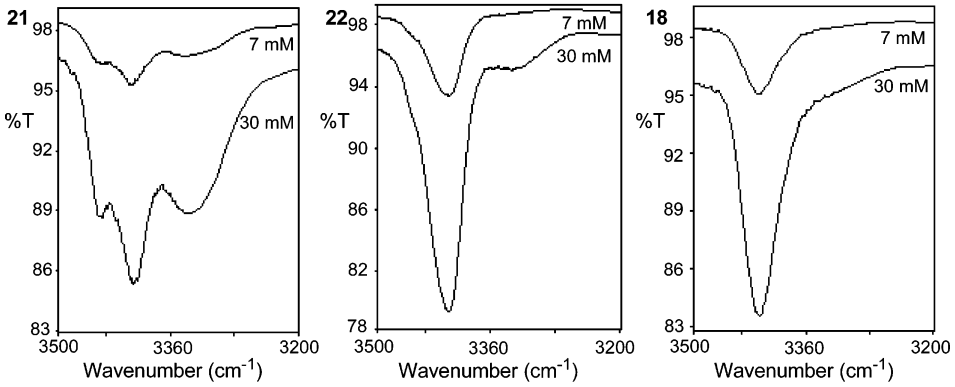


FIGURE 5. NH stretch region of FT-IR spectra for diastereomeric mixtures of pseudotripeptides **18**, **21**, and **22** (7 and 30 mM solutions in CH_2Cl_2 at room temperature). **18**: maximum at 3420 cm^{-1}; **21**: maxima at 3449, 3408, 3339 cm^{-1}; **22** (30 mM): maxima at 3411, 3333 cm^{-1}; **22** (7 mM): only maximum at 3411 cm^{-1}.

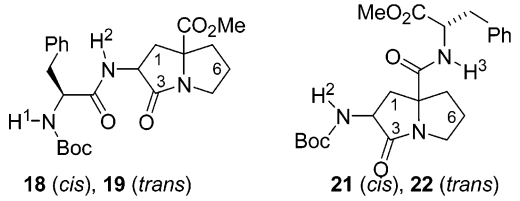


FIGURE 6. C and NH numbering in tripeptides **18**, **19**, **21**, and **22**.

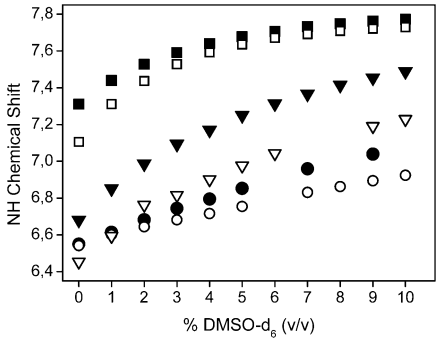


FIGURE 7. ^1H NMR chemical shift of NH^3 proton of pseudotripeptides **21** (■, □), **22** (●, ○), and NH^2 proton of **18** (▼, ▽) at different proportions of DMSO-d_6 in $CDCl_3$ (25 °C).[16]

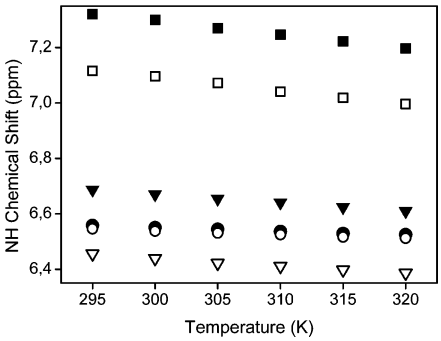


FIGURE 8. ^1H NMR chemical shift of NH^3 proton of pseudotripeptides **21** (■, □), **22** (●, ○), and of NH^2 proton of **18** (▼, ▽) as a function of temperature (~2 mM solution in $CDCl_3$).[16]

Comparison of the data sets from diastereomers **21a** and **21b** suggests that the tendency to form intramolecular hydrogen bonding of the pseudopeptide BocGPTM-PheOMe and its "mirror image"[17] is qualitatively similar. The same resemblance between the conformational characteristics of conjugates of the enantiomeric *cis*-indolizidinones **26** have been previously described.[4i]

In conclusion, all spectroscopic data demonstrated that the amide NH^3 hydrogen in both compounds **21** experiences a weak internal hydrogen bond, forming a 10-membered ring as in structure **C** (Figure 4). The reported results suggest that GPTMs are potential β-turn inducers, and encourage us to further explore the real synthetic applications and conformational behavior of these compounds.

Conclusion

The synthesis of the new conformationally constrained Gly-(*s-cis*)Pro turn mimetic **15** in both racemic and enantiomerically pure forms was accomplished. Preliminary results concerning the coupling of the GPTMs with other amino acids [Boc-Phe-OH, Phe-OMe, and Fmoc-Asp(O*t*Bu)-OH], and the hydrolysis of the methyl ester moiety at the GPTM *C*-terminus, either in the presence of Boc- or Fmoc protected amino groups, proved that these compounds can be used in peptide synthesis without epimerization at the C-2 stereocenter. The study of different protecting groups of the carboxylic moiety, more suitable for the preparation of the Fmoc-GPTM-OH building blocks for solid-phase peptide synthesis is currently carried out in our group.

The racemic *trans* isomer **17** of the GPTM was also coupled with Boc-Phe-OH and Phe-OMe. Compound *trans*-**17** itself can be regarded as a Gly-(*s-cis*)Pro mimetic which, in contrast to *cis*-**15** when incorporated in a peptide, prevents the bending of the peptide chain.

Experimental conformation studies by FT-IR and ^1H NMR performed on model compounds **18**, **21**, and **22** demonstrated that GPTM are effective VI β-turn mimetics and can induce either hydrogen bonded stabilized, or opened β-turn conformations. Accordingly, the bicyclic system **1** (R=R′=H) and its enantiomer represent two

(16) The δ NH obscured by the phenyl proton resonances were determined from the gCOSY spectrum when a coupling constant existed between the amide hydrogen and the corresponding $C_\alpha H$.

(17) Sibanda, B. L.; Thornton, J. M. *Nature* **1985**, *316*, 170−174.

(18) The X-ray CIF files have been deposited at the Cambridge Crystallographic Data Centre and allocated with the deposition numbers **CCDC 231007** for compound *exo*-**6** and **CCDC 231008** for compound (2*R*,7a*R*)-**23a**.

new dipeptide scaffolds for the synthesis of VI β-turn mimetics. Incorporation of GPTMs and their *trans* isomers in selected peptide chains is now in progress in our laboratories.

Experimental Section

Methyl (2*R,3a*S**)-2-(Aminocarbonyl)tetrahydropyrrolo[1,2-*b*]isoxazole-3a(4*H*)-carboxylate (*exo*-6), Methyl (2*S**,3a*S**)-2-(Aminocarbonyl)tetrahydropyrrolo[1,2-*b*]-isoxazole-3a(4*H*)-carboxylate (*endo*-7), and Methyl 3-(Aminocarbonyl)tetrahydropyrrolo[1,2-*b*]isoxazole-3a(4*H*)-carboxylate (8).** A solution of nitrone **4a** (400 mg, 2.8 mmol) and acrylamide (**5a**, 400 mg, 5.6 mmol) in bidistilled H_2O (0.8 mL) was stirred at 60 °C for 14 h. The mixture was diluted with AcOEt and concentrated. The crude product was crystallized from AcOEt, yielding adduct *exo*-**6** as a white solid (188 mg). The mother liquor was concentrated and purified by column chromatography on silica gel (eluent: $CHCl_3$/MeOH + 1% concd NH_4OH, 20:1) to obtain **8** as a white solid (116 mg, 19%), *exo*-**6** (62 mg; total yield: 250 mg, 42%), and a mixture of *endo*-**7** and **5a**. The pure adduct *endo*-**7** (179 mg, 30%) was obtained as a pale-yellow viscous oil by chromatographic separation on silica gel (eluent: AcOEt).

exo-**6**:[18] R_f = 0.30; mp = 163−164 °C; ^{1}H NMR (200 MHz) δ 6.87 (br s, NH), 5.37 (br s, NH), 4.59 (dd, J = 8.8, 4.8 Hz, 1H), 3.76 (s, 3H), 3.48−3.20 (m, 2H), 3.18 (dd, J = 13.2, 4.8 Hz, 1H), 2.63 (dd, J = 13.2, 8.8 Hz, 1H), 2.42−2.26 (m, 1H), 2.22−1.84 (m, 3H); ^{13}C NMR (75 MHz) δ 174.0 (s), 172.9 (s), 77.4 (t), 77.0 (s), 57.4 (t), 52.8 (q), 44.1 (t), 35.8 (t), 24.2 (t); MS (EI) *m/z* (%) 214 (3), 155 (100), 138 (50), 110 (70), 82 (26), 54 (22); IR ($CDCl_3$) 3519, 3402, 2958, 1737, 1689, 1571, 1202 cm^{-1}. Anal. calcd for $C_9H_{14}N_2O_4$: C, 50.46; H, 6.59; N, 13.08. Found: C, 50.57; H, 6.52; N, 12.89.

endo-**7**: R_f = 0.23 ($CHCl_3$/MeOH + 1% concd NH_4OH 20:1); R_f = 0.06 (AcOEt); ^{1}H NMR (200 MHz) δ 6.28 (br s, 1H), 5.79 (br s, 1H), 4.55 (dd, J = 9.5, 7.3 Hz, 1H), 3.78 (s, 3H), 3.44−3.11 (m, 2H), 3.26 (dd, J = 12.8, 7.3 Hz, 1H), 2.42−2.24 (m, 1H), 2.29 (dd, J = 12.8, 9.5 Hz, 1H), 2.20−1.86 (m, 3H); ^{13}C NMR (75 MHz) δ 172.9 (s), 172.6 (s), 77.7 (s), 77.7 (d), 57.2 (t), 52.8 (q), 43.6 (t), 34.6 (t), 23.9 (t); MS (EI) *m/z* (%) 214 (2), 155 (100), 138 (53), 110 (67), 82 (20), 54 (19); IR ($CDCl_3$) 3522, 3405, 2960, 1737, 1693, 1198 cm^{-1}. Anal. calcd for $C_9H_{14}N_2O_4$: C, 50.46; H, 6.59; N, 13.08. Found: C, 50.20; H, 6.68; N, 12.80.

8: R_f = 0.32; mp = 103−104 °C; ^{1}H NMR (200 MHz) δ 6.75 (br s, 1H), 5.91 (br s, 1H), 4.29 (dd, J = 9.9, 9.1 Hz, 1H), 4.10 (dd, J = 9.1, 7.3 Hz, 1H), 3.80 (s, 3H), 3.77 (dd, J = 9.9, 7.3 Hz, 1H), 3.47 (ddd, J = 11.4, 7.0, 4.4 Hz, 1H), 3.15 (ddd, J = 11.4, 8.8, 6.6 Hz, 1H), 2.20−1.86 (m, 4H); ^{13}C NMR (75 MHz) δ 175.9 (s), 170.7 (s), 77.6 (s), 67.2 (t), 56.4 (t), 54.5 (d), 53.4 (q), 32.6 (t), 24.4 (t); MS (EI) *m/z* (%) 214 (5), 155 (100), 108 (47), 85 (17), 59 (13); IR ($CDCl_3$) 3500, 3379, 2959, 1724, 1689, 1590, 1253 cm^{-1}. Anal. calcd for $C_9H_{14}N_2O_4$: C, 50.46; H, 6.59; N, 13.08. Found: C, 50.24; H, 6.48; N, 13.06.

Methyl (2*R,7a*S**)-2-Hydroxy-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate (*trans*-10).** A mixture of *exo*-**6** (680 mg, 3.17 mmol) and AcOH (1.8 mL, 31.7 mmol) in MeOH (26 mL) was hydrogenated over 20% $Pd(OH)_2$/C (84 mg) at room temperature and atmospheric pressure for 12 h. The catalyst was removed by filtration on a short pad of Celite. The filtrate was concentrated, diluted with CH_2Cl_2 (50 mL), and treated with K_2CO_3 and Na_2SO_4 for 30 min. The mixture was filtered and concentrated to give analytically pure *trans*-**10** (606 mg, 96%) as a white solid.

trans-**10**: R_f = 0.22 (AcOEt); mp = 105−106 °C; ^{1}H NMR (500 MHz) δ 4.71 (dd, J = 10.5, 7.5 Hz, 1H), 4.36 (br s, OH), 3.74 (s, 3H), 3.68 (dt, J = 11.4, 7.8 Hz, 1H), 3.16 (m, 1H), 3.00 (dd, J = 12.7, 7.5 Hz, 1H), 2.46 (ddd, J = 12.7, 7.5, 3.2 Hz, 1H), 2.12−1.99 (m, 2H), 1.96 (dd, J = 12.7, 10.5 Hz, 1H), 1.73 (dt, J = 12.7, 9.7 Hz, 1H); ^{13}C NMR (75 MHz) δ 175.1 (s), 173.4 (s), 72.4 (d), 69.4 (s), 52.8 (q), 42.0 (t), 41.8 (t), 36.1 (t), 25.0 (t); MS (EI) *m/z* (%) 199 (0.5), 140 (100), 112 (82), 84 (64), 56 (18); IR ($CDCl_3$) 3352, 2957, 1736, 1686, 1321, 1205, 1173 cm^{-1}. Anal. calcd for $C_9H_{13}NO_4$: C, 54.26; H, 6.58; N, 7.03. Found: C, 53.88; H, 6.64; N, 6.77.

Methyl (2*S,7a*S**)-2-Hydroxy-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate (*cis*-12).** The adduct *endo*-**7** (2.30 g, 10.7 mmol) was converted into analytically pure *cis*-**12** (2.05 g, 96%) by the same procedure as described for the synthesis of *trans*-**10** starting from *exo*-**6**.

cis-**12**: white solid; R_f = 0.24 (AcOEt); mp = 130−131 °C; ^{1}H NMR (500 MHz) δ 4.49 (br s, 1H), 4.37 (dd, J = 6.8, 1.5 Hz, 1H), 3.76 (s, 3H), 3.64 (dt, J = 11.7, 7.8 Hz, 1H), 3.28 (ddd, J = 11.7, 9.3, 4.4 Hz, 1H), 2.54 (dd, J = 14.2, 1.5 Hz, 1H), 2.35 (ddd, J = 12.7, 7.3, 2.4 Hz, 1H), 2.25 (dd, J = 14.2, 6.8 Hz, 1H), 2.16−2.00 (m, 2H), 1.62 (m, 1H); ^{13}C NMR (50 MHz) δ 174.3 (s), 173.9 (s), 74.0 (d), 72.2 (s), 52.7 (d), 41.4 (t), 39.7 (t), 36.0 (t), 25.5 (t); MS (EI) *m/z* (%) 199 (0.3), 140 (100), 112 (80), 84 (60); IR ($CDCl_3$) 3379, 2957, 1738, 1692, 1210 cm^{-1}. Anal. calcd for $C_9H_{13}NO_4$: C, 54.26; H, 6.58; N, 7.03. Found: C, 53.91; H, 6.51; N, 6.84.

Partial Isomerization of *cis*-12 to *trans*-10. A suspension of *cis*-**12** (100 mg, 0.5 mmol) in H_2O (1 mL) was treated with *t*BuOK (111 mg, 1 mmol) and then heated in a sealed flask at 100 °C in an oven for 1 d. The solvent was removed under reduced pressure, and the residue containing the hydroxy carboxylates was treated with DMF and MeI (156 μL, 2.5 mmol). The heterogeneous mixture was stirred at room temperature overnight and then concentrated. The crude product was purified by chromatography on silica gel (eluent: CH_2Cl_2/MeOH, 20:1) to afford a 1:1 mixture of *trans*-**10** and *cis*-**12** (72 mg, 72% yield).

Methyl (2*R,7a*S**)-2-[(Methylsulfonyl)oxy]-3-oxotetrahydro-*1H*-pyrrolizine-7a(*5H*)-carboxylate (*trans*-13).** Methanesulfonyl chloride (MsCl, 0.325 mL, 4.2 mmol) was added dropwise to a solution of hydroxy ester *trans*-**10** (422 mg, 2.1 mmol) and triethylamine (TEA, 1.463 mL, 10.5 mmol) in CH_2Cl_2 (3 mL) at 0 °C. The mixture was stirred in dry atmosphere at room temperature overnight, and the resulting suspension was diluted with CH_2Cl_2, washed sequentially with H_2O and brine, dried over Na_2SO_4, and concentrated. The crude mesylate *trans*-**13** (578 mg, 99%) was sufficiently pure to be used in the next step without further purification. A sample purified by column chromatography on silica gel (eluent: AcOEt/petroleum ether, 1:1) afforded analytically pure *trans*-**13** as a white solid.

trans-**13**: white solid; R_f = 0.25; mp = 88−90 °C (Et_2O); ^{1}H NMR (200 MHz) δ 5.55 (dd, J = 9.9, 8.4 Hz, 1H), 3.77 (s, 3H), 3.70 (dt, J = 11.7, 7.7 Hz, 1H), 3.29 (s, 3H), 3.30−3.05 (m, 2H), 2.55 (ddd, J = 12.4, 6.6, 3.6 Hz, 1H), 2.28−2.00 (m, 3H), 1.72 (dt, J = 12.4, 9.6 Hz, 1H); ^{13}C NMR (50 MHz) δ 172.4 (s), 168.1 (s), 79.1 (d), 69.0 (s), 53.0 (q), 41.8 (t), 39.7 (t), 39.6 (q), 35.6 (t), 24.8 (t); MS (EI) *m/z* (%) 277 (M^+, 35), 201 (14), 183 (10), 158 (100), 130 (33), 94 (31), 67 (61); IR ($CDCl_3$), 2957, 1714, 1413, 1358, 1281 cm^{-1}. Anal. calcd for $C_{10}H_{15}NO_6S$: C, 43.32; H, 5.45; N, 5.05. Found: C, 42.93; H, 5.09; N, 5.08.

Methyl (2*S,7a*S**)-2-Azido-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate (*cis*-14). Synthesis from Mesylate *trans*-13.** A mixture of the crude mesylate *trans*-**13** (578 mg, ~2.08 mmol) and NaN_3 (270 mg, 4.15 mmol) in DMF (5 mL) was heated to 40 °C overnight. The reaction mixture was diluted with CH_2Cl_2, treated dropwise with 10% aqueous HCl solution (10 mL), and vigorously stirred for 15 min. The separated organic phase was washed sequentially with 5% $NaHCO_3$, H_2O, and brine and was dried over Na_2SO_4 and concentrated. The crude product was purified by column chromatography on silica gel (eluent: AcOEt/petroleum ether, 1:1) to afford azide *cis*-**14** (325 mg, 70%) as a white solid.

Synthesis from Alcohol *cis*-12. Alcohol *cis*-**12** (205 mg, 1.03 mmol) was dissolved in $SOCl_2$ (2 mL), to which pyridine (104 μL, 1.03 mmol) was added dropwise at room temperature. The mixture was refluxed during 1 h and concentrated under reduced pressure, and the crude product was purified by chromatography on silica gel (petroleum ether/AcOEt, 1:1). The

pure methyl (2*S**,7a*R**)-2-chloro-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate (*trans*-**chloride**) derivative was obtained as a yellow oil (122 mg, 54%).

trans-**chloride**: yellow oil; R_f = 0.20 (petroleum ether/AcOEt, 1:1); ^{1}H NMR (200 MHz) δ 4.84 (dd, J = 11.0, 7.7 Hz, 1H), 3.76 (s, 3H), 3.85−3.64 (m, 1H), 3.29−3.11 (m, 1H), 3.20 (dd, J = 13.2, 7.7 Hz, 1H), 2.49 (ddd, J = 12.4, 6.4, 3.8 Hz, 1H), 2.20 (dd, J = 13.2, 11.0 Hz, 1H), 2.16−2.00 (m, 2H), 1.73 (dt, J = 12.4, 9.3 Hz, 1H); ^{13}C NMR (50 MHz) δ 172.7 (s), 169.0 (s), 70.1 (s), 57.2 (d), 53.0 (q), 43.6 (t), 42.5 (t), 35.9 (t), 25.4 (t); MS (EI) *m/z* (%) 160 (34), 158 (100), 130 (14), 84 (59), 69 (27), 67 (36); IR ($CDCl_3$) 2956, 1738, 1712, 1405, 1210 cm^{-1}. Anal. calcd for $C_9H_{12}ClNO_3$: C, 49.67; H, 5.56; N, 6.44. Found: C, 49.21; H, 5.32; N, 6.70.

A mixture of the *trans*-**chloride** (122 mg, 0.56 mmol) and NaN_3 (73 mg, 1.12 mmol) in DMF (3 mL) was heated to 40 °C overnight. The reaction mixture was diluted with CH_2Cl_2, treated dropwise with 10% aqueous HCl solution (10 mL), and vigorously stirred for 15 min. The separated organic phase was washed sequentially with 5% $NaHCO_3$, H_2O, and brine and was dried over Na_2SO_4 and concentrated. The crude product was purified by column chromatography on silica gel (eluent: AcOEt/petroleum ether, 1:1) to afford azide *cis*-**14** (90 mg, 71%) as a white solid.

cis-**14**: R_f = 0.26; mp = 84−87 °C (Et_2O); ^{1}H NMR (200 MHz) δ 4.18 (dd, J = 7.6, 1.5 Hz, 1H), 3.78 (s, 3H), 3.66 (dt, J = 11.7, 7.8 Hz, 1H), 3.31 (ddd, J = 11.7, 8.8, 4.5 Hz, 1H), 2.54 (dd, J = 14.4, 1.5 Hz, 1H), 2.32 (ddd, J = 12.4, 6.6, 3.2 Hz, 1H), 2.24 (dd, J = 14.4, 7.6 Hz, 1H), 2.19−2.03 (m, 2H), 2.66 (dt, J = 12.4, 9.6 Hz, 1H); ^{13}C NMR (75 MHz) δ 173.1 (s), 169.2 (s), 71.9 (s), 63.5 (d), 52.5 (q), 41.4 (t), 37.1 (t), 36.0 (t), 25.3 (t); MS (EI) *m/z* (%) 165 (75), 137 (100), 110 (35), 81 (35); IR ($CDCl_3$) 2957, 2116, 1735, 1703, 1412, 1208 cm^{-1}. Anal. calcd for $C_9H_{12}N_4O_3$: C, 48.21; H, 5.39; N, 24.99. Found: C, 48.08; H, 5.34; N, 24.74.

Methyl (2*S,7a*S**)-2-Amino-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate (*cis*-15).** To a solution of *cis*-**14** (56 mg, 0.25 mmol) in MeOH (2.5 mL) was added dropwise a water suspension (∼1 mL) of Raney-Ni. The mixture was stirred at room temperature for 30 min, filtered through a short pad of Celite, and concentrated under reduced pressure to give analytically pure *cis*-**15** (41 mg, 82%) as a colorless oil.

cis-**15**: R_f = 0.06 (AcOEt/petroleum ether, 6:1); ^{1}H NMR (200 MHz) δ 3.76 (s, 3H), 3.68−3.52 (m, 1H), 3.60 (dd, J = 8.2, 3.0 Hz, 1H), 3.19 (ddd, J = 11.6, 9.1, 4.2 Hz, 1H) 2.41 (ddd, J = 12.4, 7.0, 2.6 Hz, 1H), 2.38 (X part of an AXY system, J = 14.0, 8.2 Hz, 1H) 2.24 (Y part of an AXY system, J = 14.0, 3.0 Hz, 1H) 2.15−1.88 (m, 2H), 1.64−1.44 (m, 1H); ^{13}C NMR (50 MHz) δ 176.3 (s), 174.4 (s), 71.9 (s), 57.2 (d), 52.8 (q), 41.6 (t), 39.5 (t), 35.9 (t), 25.6 (t). Anal. calcd for $C_9H_{12}N_4O_3$: C, 54.53; H, 7.12; N, 14.13. Found: C, 54.57; H, 7.29; N, 13.92.

Methyl (2*R,7a*S**)-2-Azido-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate (*trans*-16).** To a solution of compound *cis*-**12** (61 mg, 0.3 mmol) and DMAP (50 mg, 0.4 mmol) in toluene (0.6 mL) was added DPPA (86 μL, 0.4 mmol). The mixture was heated at 65 °C for 3.5 h, then purified by column chromatography on silica gel (eluent: initially petroleum ether, then AcOEt/petroleum ether, 1:1) to afford analytically pure *trans*-**16** (56 mg, 81%) as a colorless oil.

trans-**16**: R_f = 0.44 (AcOEt/petroleum ether, 1:1); ^{1}H NMR (200 MHz) δ 4.54 (dd, J = 11.0, 8.1 Hz, 1H), 3.76 (s, 3H), 3.71 (dt, J = 11.5, 7.5 Hz, 1H), 3.26−3.10 (m, 1H), 2.94 (dd, J = 13.2, 8.1 Hz, 1H), 2.48 (ddd, J = 12.4, 6.2, 4.4 Hz, 1H), 2.18−2.00 (m, 2H), 1.84 (dd, J = 13.2, 11.0 Hz, 1H), 1.69 (dt, J = 12.5, 9.2 Hz, 1H); ^{13}C NMR (50 MHz) δ 173.0 (s), 170.4 (s), 69.6 (s), 62.2 (d), 52.9 (q), 42.0 (t), 39.3 (t), 35.9 (t), 25.3 (t); MS (EI) *m/z* (%) 225 (MH^+, 43), 165 (77), 137 (92), 110 (19), 81 (28), 59 (100); IR ($CDCl_3$) 2959, 2113, 1707, 1407, 1328, 1207 cm^{-1}. Anal. calcd for $C_9H_{12}N_4O_3$: C, 48.21; H, 5.39; N, 24.99. Found: C, 48.23; H, 5.47; N, 25.39.

Methyl (2*S,7a*R**)-2-Amino-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate (*trans*-17).** The azide *trans*-**16** (119 mg, 0.53 mmol) was converted into the amine *trans*-**17** (91 mg, 87%) by the same procedure as described for the synthesis of *cis*-**15** starting from *cis*-**14**.

trans-**17**: R_f = 0.06 (AcOEt/petroleum ether, 6:1); ^{1}H NMR (200 MHz) δ 3.92 (dd, J = 11.4, 7.6 Hz, 1H), 3.73 (s, 3H), 3.65 (dt, J = 11.6, 7.8 Hz, 1H), 3.24−3.08 (m, 1H), 2.96 (dd, J = 12.6, 7.6 Hz, 1H), 2.43 (ddd, J = 12.6, 6.2, 4.4 Hz, 1H), 2.15−1.93 (m, 2H), 1.80−1.58 (m, 2H); ^{13}C NMR (50 MHz) δ 175.6 (s), 173.8 (s), 69.4 (s), 55.9 (d), 52.7 (q), 43.0 (t), 41.8 (t), 36.2 (t), 25.2 (t); MS (EI) *m/z* (%) 198 (M^+, 25), 139 (37), 111 (100), 84 (33), 70 (10).

Methyl (2*R*,7a*R*)- and (2*S*,7a*S*)-2-({(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-phenylpropanoyl}amino)-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate [(2*R*,7a*R*)-18a and (2*S*,7a*S*)-18b]. DIPEA (72 μL, 0.41 mmol) was added to a solution of crude *cis*-**15** (40 mg, ∼0.20 mmol), Boc-L-Phe-OH (54 mg, 0.20 mmol), and PyBroP (54 mg, 0.20 mmol) in CH_2Cl_2 (2 mL) with cooling in an ice/water bath. The mixture was allowed to stand at room temperature overnight and then concentrated. The crude compound was diluted with AcOEt, then filtered, and washed sequentially with 5% $KHSO_4$, 5% $NaHCO_3$, and brine. The organic solution was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (eluent: AcOEt/petroleum ether, 1:1) to afford a 1:1 inseparable mixture of the two diastereoisomers (2*R*,7a*R*)-**18a** and (2*S*,7a*S*)-**18b** (49 mg, 55%).

18 (1:1 mixture of two diastereomers): R_f = 0.16; ^{1}H NMR (300 MHz) δ 7.31−7.17 (m, 5H + 5H), 6.96−6.88 (m, NH), 6.83−6.72 (m, NH), 5.13−4.98 (m, NH + NH), 4.67 (dt, J = 2.8, 8.6 Hz, 1H) 4.54 (m, 1H) 4.44−4.24 (m, 1H + 1H), 3.71 (s, 3H + 3H), 3.64 (dt, J = 11.7, 7.8 Hz, 1H), 3.61 (dt, J = 11.7, 7.5 Hz, 1H), 3.28−2.92 (m, 3H + 3H), 2.50−2.35 (m, 2H + 2H), 2.25 (dd, J = 14.4, 3.0 Hz, 1H), 2.18−1.88 (m, 3H + 2H), 1.64−1.48 (m, 1H + 1H), 1.36 (s, 9H), 1.35 (s, 9H); ^{1}H NMR (300 MHz, 55 °C) δ 7.32−7.14 (m, 5H + 5H), 6.79−6.71 (m, NH), 6.66−6.57 (m, NH), 5.02−4.88 (m, NH + NH), 4.65 (dt, J = 3.0, 8.2 Hz, 1H) 4.55 (dt, J = 3.0, 8.2 Hz, 1H) 4.42−4.24 (m, 1H + 1H), 3.72 (s, 3H + 3H), 3.72−3.58 (m, 1 H + 1H), 3.26−2.94 (m, 3 H + 3H), 2.50−2.36 (m, 2H + 2H), 2.26 (dd, J = 14.1, 3.0 Hz, 1H), 2.17−1.96 (m, 2H + 2H), 2.11 (dd, J = 14.1, 3.0 Hz, 1H), 1.64−1.49 (m, 1 H + 1H), 1.39 (s, 9 H), 1.38 (s, 9 H); ^{13}C NMR (75 MHz) δ 174.0 and 173.9 (s), 171.9 and 173.8 (s), 171.3 and 171.2 (s), 155.3 and 155.2 (s), 136.8 and 136.62 (s), 129.5 and 129.4 (d, 2C), 128.6 (d, 2C + 2C), 127.0 and 126.8 (d), 80.2 (s, 1C + 1C), 71.8 (s, 1C + 1C), 56.0 (d, 1C + 1C), 54.3 and 54.1 (d), 52.8 (q, 1C + 1C), 42.2 and 42.1 (t), 38.6 (t, 1C + 1C), 37.4 and 37.3 (t), 35.4 and 35.2 (t), 28.3 (q, 3C + 3C), 25.7 and 25.6 (t); MS (EI) *m/z* (%) 445 (0.4), 389 (6), 269 (29), 254 (30), 183 (39), 164 (51), 120 (85), 91 (27), 57 (100); IR ($CDCl_3$) 3422, 3031, 2982, 1704, 1488, 1211, 1160 cm^{-1}. Anal. calcd for $C_{23}H_{31}N_3O_6$: C, 62.01; H, 7.01; N, 9.43. Found: C, 61.72; H, 7.04; N, 9.63.

Methyl (2*R*,7a*S*)- and (2*S*,7a*R*)-2-({(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-phenylpropanoyl}amino)-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate [(2*R*,7a*S*)-19a and (2*S*,7a*R*)-19b]. DIPEA (161 μL, 0.92 mmol) was added to a solution of amine *trans*-**17** (91 mg, 0.46 mmol), Boc-L-Phe-OH (122 mg, 0.46 mmol), and PyBroP (215 mg, 0.46 mmol) in CH_2Cl_2 (4.5 mL) with cooling in an ice/water bath. The reaction mixture was treated by following the same procedure described in the previous synthesis. The crude product was purified by column chromatography on silica gel (eluent: AcOEt/petroleum ether, 6:1) to afford a mixture of the two diastereomers [(2*R*,7a*S*)-**19a** and (2*S*,7a*R*)-**19b** (156 mg, 76%)]. The diastereomeric mixture was partially separated by column chromatography (eluent: AcOEt/petroleum ether, 2:1) to afford a mixture of **19a** and **19b** (117 mg, 57%) and one of the two diastereomers **19** (24 mg, 12%) as a white solid.

19 (one diastereomer): R_f = 0.18 (AcOEt/petroleum ether, 1:1); mp = 141−144 °C; $[\alpha]^{20}_D$ = −21.8 (c = 0.11, $CHCl_3$); ^{1}H

NMR (300 MHz) δ 7.35−7.10 (m, 5H), 6.52−6.41 (m, 1H), 5.04−4.90 (m, 1H), 4.68−4.52 (m, 1H), 4.42−4.30 (m, 1H), 3.78 (s, 3H), 3.67 (dt, J = 11.2, 7.8, Hz, 1H), 3.24−3.13 (m, 1H), 3.19 (dd, J = 12.8, 7.7 Hz, 1H), 3.07 (d, J = 6.3 Hz, 2H), 2.48 (ddd, J = 12.5, 6.9, 3.7 Hz, 1H), 2.16−2.00 (m, 2H), 1.86 (dd, J = 12.8, 10.9 Hz, 1H), 1.69 (dt, J = 12.9, 9.5, Hz, 1H), 1.41 (s, 9H); ^{13}C NMR (75 MHz) δ 173.3 (s), 171.6 (s), 171.5 (s), 155.2 (s), 136.5 (s), 129.4 (d, 2C), 128.7 (d, 2C), 127.0 (d), 80.4 (s), 70.3 (s), 55.9 (d), 54.5 (d), 52.8 (q), 42.2 (t), 41.1 (t), 38.5 (t), 36.2 (t), 28.3 (q, 3C), 25.4 (t); MS (EI) *m/z* (%) 445 (0.2), 389 (2), 269 (15), 254 (23), 183 (16), 164 (25), 120 (56), 91 (40), 57 (100); IR ($CDCl_3$) 3693, 3417, 2936, 1705, 1483, 1164 cm^{-1}. Anal. calcd for $C_{23}H_{31}N_3O_6$: C, 62.01; H, 7.01; N, 9.43. Found: C, 61.88; H, 6.62; N, 9.33.

Methyl (2*R,7a*R**)-2-[(*tert*-Butoxycarbonyl)amino]-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate (20).** DIPEA (163 mL, 0.95 mmol) and $(Boc)_2O$ (207 mg, 0.95 mmol) were added to a solution of amine *cis*-**15** (188 mg, 0.95 mmol) in a 4:1 mixture of CH_2Cl_2 (7.6 mL) and EtOH (1.9 mL) at room temperature. The reaction mixture was stirred overnight at room temperature, and then the solvents were removed under reduced pressure. The residue was dissolved in a 2:1 mixture of CH_2Cl_2 and Et_2O and treated with a 10% solution of $KHSO_4$. The two phases were separated, the aqueous layer was extracted with a 2:1 mixture of CH_2Cl_2 and Et_2O, and the combined organic extracts were dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (eluent: AcOEt/petroleum ether, 2:1) to afford racemic Boc-GPTM-OMe (170 mg, 67%) as a white solid.

20: R_f = 0.22 (AcOEt/diethyl ether, 1:2); mp = 161−163 °C; ^{1}H NMR (400 MHz) δ 5.18 (br s, NH), 4.44 (m, 1H), 3.82 (s, 3H), 3.70 (dt, J = 11.8, 8.0 Hz, 1H), 3.28 (ddd, J = 11.8, 9.5, 3.9 Hz, 1H), 2.54−2.45 (m, 2H), 2.39 (dd, J = 14.3, 3.2 Hz, 1H), 2.19−2.12 (m, 1H), 2.12−2.02 (m, 1H), 1.61 (ddd, J = 12.4, 10.9, 9.0 Hz, 1H), 1.46 (s, 9H); ^{13}C NMR (50 MHz) δ 174.1 (s), 172.4 (s), 155.2 (s), 80.0 (s), 71.7 (s), 55.4 (d), 52.8 (q), 42.0 (t), 37.6 (t),35.2 (t), 28.2 (q, 3C), 25.5 (t); MS *m/z* (%) 241 (0.3) [$M^+ - CMe_3$], 239 (14), 225 (8), 183 (100), 165 (16), 137 (12), 122 (90), 111 (25), 57 (94); IR ($CDCl_3$) 3433, 2982, 2956, 1733, 1710, 1502, 1165 cm^{-1}. Anal. calcd for $C_{14}H_{22}N_2O_5$: C, 56.36; H, 7.43; N, 9.39. Found: C, 56.55; H, 7.34; N, 9.15.

Methyl (2*S*)-2-{[((2*R*,7a*R*)-2-[(*tert*-Butoxycarbonyl)amino]-3-oxotetrahydro-1*H*-pyrrolizin-7a(5*H*)-yl)carbonyl]amino}-3-phenylpropanoate and Methyl (2*S*)-2-{[((2*S*,7a*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-oxotetrahydro-1*H*-pyrrolizin-7a(5*H*)-yl)carbonyl]amino}-3-phenylpropanoate [(2*R*,7a*R*)-21a and (2*S*,7a*S*)-21b]. A 1 M solution of NaOH (170 mL, 0.17 mmol) was added dropwise to a mixture of **20** (51 mg, 0.17 mmol) in MeOH (0.24 mL) at 0 °C. The reaction mixture was stirred during 1 h at room temperature, and then the solvent was removed under reduced pressure. DIPEA (30 mL, 0.17 mmol) and PyBroP (79 mg, 0.17 mmol) were added to the mixture of the crude residue and L-Phe-OMe (30 mg, 0.17 mmol) in CH_2Cl_2 (1.7 mL) at 0 °C. The reaction mixture was stirred overnight at room temperature, then the solvent was removed under reduced pressure, and the residue was dissolved in AcOEt and filtered. The solution was washed twice with a 5% aqueous solution of $KHSO_4$, twice with a 5% aqueous solution of $NaHCO_3$, and once with brine and then dried over Na_2SO_4, filtered, and concentrated. The product was purified by column chromatography (eluent: AcOEt/Et_2O, 1:1) to afford a 1:1 mixture of diastereomers **21a** and **21b** (28 mg, 37%) as a white solid.

21: [1:1 mixture of two diastereomers (the absolute configuration of **21a** and **21b** was not established, but most of the hydrogen resonances could be attributed to one diastereomer (I) or to the other (II) by analyzing gCOSY and NOESY 1D spectra of the mixture)]: R_f = 0.29 (AcOEt/diethyl ether, 1:2); ^{1}H NMR (400 MHz) δ 7.38−7.20 [m, 4H + (4H + NH); I and II], 7.20−7.14 (m, 1H + 1H; I and II), 7.11 (br s, NH; II), 5.48 (br d, J = 7.2 Hz, NH; I), 5.05 (br s, NH; II), 4.83−4.73 (m, 1H + 1H; I and II), 4.24−4.11 (m, 1H; I), 4.09−3.98 (m, 1H; II), 3.75 (s, 3H), 3.71 (s, 3H), 3.66 (dt, J = 11.9, 8.3 Hz, 1H; II), 3.53 (dt, J = 11.8, 8.2 Hz, 1H; I), 3.29 (dd, J = 14.1, 5.1 Hz, 1H; I), 3.21 (dd, J = 13.9, 5.8 Hz, 1H; II), 3.11 (ddd, J = 11.9, 9.9, 3.6 Hz, 1H; II), 3.08 (dd, J = 13.9, 7.8 Hz, 1H; II), 2.95 (dd, J = 14.1, 9.8 Hz, 1H; I), 2.70 (m, 1H; I), 2.65−2.47 (m, 1H + 2H; I and II), 2.56 (dd, J = 14.4, 10.2 Hz, 1H; I), 2.29 (dd, J = 14.4, 4.7 Hz, 1H; I), 2.04−1.81 (m, 2H + 3H; I and II), 1.46 (s, 9H), 1.44 (s, 9H), 1.40−1.20 (m, 1H + 1H; I and II); ^{13}C NMR (50 MHz) δ 175.6 and 174.9 (s), 173.4 and 173.0 (s), 172.1 and 171.7 (s), 155.3 and 155.2 (s), 136.4 and 136.3 (s), 129.1 and 128.9 (d, 2C), 128.6 and 128.5 (d, 2C), 127.1 (d, 1C + 1C), 80.4 and 80.3 (s), 72.2 and 71.7 (s), 54.7 and 54.0 (d), 53.4 and 53.3 (d), 52.5 and 52.3 (q), 43.2 and 42.6 (t), 37.5 and 37.1 (t), 37.1 and 35.5 (t), 34.3 and 34.0 (t), 28.4 and 28.3 (q, 3C), 24.8 and 24.6 (t); IR (CH_2Cl_2, 30 mM) 3449, 3408, 3339, 2979, 2955, 1741, 1709, 1679, 1507, 1367, 1164 cm^{-1}. Anal. calcd for $C_{23}H_{31}N_3O_6$: C, 62.01; H, 7.01; N, 9.43. Found: C, 61.87; H, 6.97; N, 9.44.

Methyl (2*S*)-2-{[((2*R*,7a*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-oxotetrahydro-1H-pyrrolizin-7a(5*H*)-yl)carbonyl]amino}-3-phenylpropanoate and Methyl (2*S*)-2-{[((2*S*,7a*R*)-2-[(*tert*-Butoxycarbonyl)amino]-3-oxotetrahydro-1H-pyrrolizin-7a(5*H*)-yl)carbonyl]amino}-3-phenylpropanoate [(2*R*,7a*S*)-22a and (2*S*,7a*R*)-22b]. Tripeptides **22a,b** were synthesized starting from amine *trans*-**17** by the same procedure as described for the synthesis of **21a,b** starting from *cis*-**15**.

22 (1:1 mixture of two diastereomers): yield 51%; R_f = 0.25 (AcOEt/diethyl ether, 1:2); ^{1}H NMR (400 MHz) δ 7.32−7.12 (m, 4H + 4H), 7.14−7.09 (m, 1H + 1H), 6.85 (br s, NH), 6.83 (br s, NH), 5.12 (br s, NH), 5.06 (br d, J = 3.7 Hz, NH), 4.89−4.81 (m, 1H + 1H), 4.45 (m, 1H), 4.21 (m, 1H), 3.77 (s, 3H), 3.74 (s, 3H), 3.64−3.51 (m, 1H + 1H), 3.26 (dd, J = 14.1, 5.3 Hz, 1H), 3.22 (dd, J = 15.0, 5.3 Hz, 1H), 3.10−2.96 (m, 4H), 2.88 (m, 1H), 2.78 (dd, J = 12.8, 8.1 Hz, 1H), 2.47 (ddd, J = 12.5, 6.7, 3.3 Hz, 1H), 2.31 (ddd, J = 12.4, 6.9, 3.2 Hz, 1H), 2.01−1.80 (m, 5H), 1.67−1.58 (m, 2H), 1.50−1.38 (m, 1H), 1.43 (s, 9H), 1.41 (s, 9H); ^{13}C NMR (50 MHz) δ 174.7 and 174.5 (s), 172.8 and 172.7 (s), 171.7 and 171.5 (s), 155.1 (s, 1C + 1C), 136.0 and 135.6 (s), 129.0 and 128.9 (d, 2C), 128.8 and 128.6 (d, 2C), 127.3 and 127.2 (d),80.0 (s, 1C + 1C), 71.0 (s, 1C + 1C), 54.4 (d, 1C + 1C), 52.9 and 52.8 (d), 52.5 and 52.4 (q), 43.1 and 43.0 (t), 41.7 and 41.3 (t), 37.4 and 37.3 (t), 36.8 and 36.6 (t), 28.2 (q, 3C + 3C), 25.0 and 24.8 (t); IR (CH_2Cl_2, 30 mM) 3411, 3333, 3057, 2956, 1740, 1711, 1680, 1503, 1368, 1165 cm^{-1}. Anal. calcd for $C_{23}H_{31}N_3O_6$: C, 62.01; H, 7.01; N, 9.43. Found: C, 61.74; H, 6.87; N, 9.71.

Methyl (2*R*,7a*R*)- and (2*S*,7a*S*)-3-Oxo-2-{[(1*R*)-1-phenylethyl]amino}tetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate [(2*R*,7a*R*)-23a and (2*S*,7a*S*)-23b]. A solution of the alcohol *trans*-**10** (395 mg, 1.98 mmol) and pyridine (0.177 mL, 2.19 mmol) in CH_2Cl_2 (4.5 mL) was added to a solution of trifluoromethane sulfonic anhydride (Tf_2O, 0.367 mL, 2.18 mmol) in CH_2Cl_2 (4.5 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min and then filtered through a short pad of silica gel (eluent CH_2Cl_2). After evaporation of the solvent, the crude triflate was dissolved in CH_2Cl_2 (4.5 mL) and added to a solution of (1*R*)-1-phenylethanamine (0.765 mL, 5.94 mmol) in CH_2Cl_2 (4 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, and then the solvent was evaporated. The crude product was purified by chromatography on silica gel (eluent: Et_2O/iPr_2O, 5:1 then 2:1) to afford the two pure diastereomers (2*R*,7a*R*)-**23a** (144 mg, 24%) and (2*S*,7a*S*)-**23b** (144 mg, 24%) as a white crystalline solid and a colorless oil, respectively.

(2*R*,7a*R*)-**23a**:[18] R_f = 0.3 (Et_2O); mp = 63 °C (iPr_2O); $[\alpha]^{26}_D$ = +5.89 (c = 0.475, $CHCl_3$); ^{1}H NMR (500 MHz) δ 7.36−7.33 (m, 2H), 7.32−7.27 (m, 2H), 7.24−7.19 (m, 1H), 4.13 (q, J = 6.6 Hz, 1H), 3.74 (s, 3H), 3.62 (td, J = 11.6, 8.0 Hz, 1H), 3.31 (dd, J = 7.9, 2.2 Hz, 1H), 3.20 (ddd, J = 11.6, 9.2, 4.1 Hz, 1H),

2.31 (ddd, J = 12.7, 7.3, 2.6 Hz, 1H), 2.16 (dd, J = 13.9, 2.2 Hz, 1H), 2.10−1.96 (m, 2H), 2.03 (dd, 13.9, 7.9 Hz, 1H), 1.85 (br s, 1H), 1.49 (ddd, J = 12.7, 10.6, 8.8 Hz, 1H), 1.31 (d, J = 6.6 Hz, 3H); ^{13}C NMR (50 MHz) δ 175.2 (s), 174.6 (s), 145.0 (s), 128.3 (d, 2C), 127.0 (d, 2C), 126.9 (d), 71.9 (s), 60.9 (d), 56.4 (d), 52.6 (q), 41.6 (t), 39.0 (t), 36.0 (t), 25.6 (t), 24.2 (q); MS (EI) *m/z* (%) 303 (MH^+, 1), 287 (10), 124 (49), 120 (100), 105 (87), 77 (11); IR ($CDCl_3$) 3331, 2955, 1735, 1688, 1451, 1209 cm^{-1}. Anal. calcd for $C_{17}H_{22}N_2O_3$: C, 67.53; H, 7.33; N, 9.26. Found: C, 67.64; H, 7.28; N, 9.70.

(2*S*,7a*S*)-**23b**: R_f = 0.25 (Et_2O); $[\alpha]^{26}_D$ = −72.9 (c = 0.48, $CHCl_3$); ^{1}H NMR (500 MHz) δ 7.35−7.28 (m, 2H), 7.27−7.25 (m, 2H), 7.24−7.20 (m, 1H), 3.85 (q, J = 6.6 Hz, 1H), 3.74 (s, 3H), 3.65 (dt, J = 11.6, 7.8 Hz, 1H), 3.34 (dd, J = 7.5, 2.8 Hz, 1H), 3.24 (ddd, J = 11.6, 8.6, 5.0 Hz, 1H), 2.49 (dd, J = 13.6, 2.8 Hz, 1H), 2.26 (ddd, J = 12.7, 6.8, 3.3 Hz, 1H), 2.09 (dd, J = 13.6, 7.5 Hz, 1H), 2.07−1.98 (m, 2H), 1.82 (br s, 1H), 1.56 (dt, J = 12.7, 9.7 Hz, 1H), 1.37 (d, J = 6.6 Hz, 3H); ^{13}C NMR (50 MHz) δ 175.0 (s), 174.6 (s), 144.4 (s), 128.6 (d, 2C), 127.1 (d), 126.5 (d, 2C), 72.0 (s), 60.0 (d), 55.67 (d), 52.6 (q), 42.1 (t), 37.2 (t), 35.7 (t), 25.6 (t), 24.0 (q); MS (EI) *m/z* (%) 303 (MH^+, 1), 287 (8), 124 (44), 120 (100), 105 (67), 77 (7); IR ($CDCl_3$) 3330; 2955; 1734; 1690; 1450; 1214 cm^{-1}. Anal. calcd for $C_{17}H_{22}N_2O_3$: C, 67.53; H, 7.33; N, 9.26. Found: C, 67.51; H, 7.37; N, 9.48.

Methyl (2*R*,7a*R*)-2-Amino-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate ((2*R*,7a*R*)-15a). A solution of (2*R*,7a*R*)-**23a** (181 mg, 060 mmol) in MeOH (5 mL) was hydrogenated in an autoclave in the presence of 20% $Pd(OH)_2$/C (50 mg) at room temperature and a pressure of 35 atm for 20 h. The catalyst was removed by filtration through a short pad of Celite, and then the solution was concentrated to give the crude amine (2*R,*7a*R*)-**15a** (116 mg, 97%), sufficiently pure to be used in the next step without further purification.

(2*R,*7a*R*)-**15a**: spectral properties are identical with those of *cis*-**15**.

Methyl (2*S*,7a*S*)-2-Amino-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate [(2*S*,7a*S*)-15b)]. The amine (2*S*,7a*S*)-**23b** (295 mg, 0.98 mmol) was hydrogenated under the same conditions as its diastereomers (2*R*,7a*R*)-**23a**. The purification of the crude amine (200 mg) by column chromatography on silica gel (eluent: CH_2Cl_2/MeOH 50:1 then 25:1 and 20:1) afforded the pure compound (2*S,*7a*S*)-**15b** (166 mg, 86%).

(2*S,*7a*S*)-**15b**: $[\alpha]^{25}_D$ = +28.1 (c = 0.66, $CHCl_3$); spectral properties are identical with those of *cis*-**15**.

Methyl (2*R*,7a*R*)-2-[((2*S*)-4-*tert*-Butoxy-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-4-oxobutanoyl)amino]-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate [(2*R*,7a*R*)-24a]. DIPEA (200 μL, 1.15 mmol) was added to a solution of amine (2*R*,7a*R*)-**15a** (116 mg, ∼0.58 mmol), Fmoc-L-Asp(O*t*Bu)-OH (238 mg, 0.58 mmol), and PyBroP (270 mg, 0.58 mmol) in $CHCl_3$ (6 mL) with cooling in an ice/water bath. The mixture was allowed to stand at room temperature overnight and then concentrated. The crude compound was diluted with AcOEt, then filtered and washed sequentially with 5% $KHSO_4$, 5% $NaHCO_3$, and brine. The organic solution was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (eluent: AcOEt/Et_2O, 1:1) to afford (2*R*,7a*R*)-**24a** (334 mg, 97%) as a white solid.

(2*R*,7a*R*)-**24a**: R_f = 0.21; mp = 85−86 °C; $[\alpha]^{27}_D$ = −3.26 (c = 0.49, $CHCl_3$); ^{1}H NMR (500 MHz) δ 7.78−7.75 (m, 2H), 7.63−7.58 (m, 2H), 7.42−7.38 (m, 2H), 7.35−7.30 (m, 2H), 7.29−7.25 (m, 1H), 5.99−5.94 (m, 1H), 4.68 (dt, J = 2.5, 8.4 Hz, 1H), 4.58−4.51 (m, 1H), 4.41 (d, J = 7.1 Hz, 2H), 4.24 (t, J = 7.1 Hz, 1H), 3.72 (s, 3H), 3.65 (dt, J = 11.6, 8.1 Hz, 1H), 3.23 (ddd, J = 11.6, 9.7, 3.7 Hz, 1H), 2.86 (dm, J = 16.5 Hz, 1H), 2.69 (dd, J = 16.5, 5.6 Hz, 1H), 2.50−2.42 (m, 1H), 2.46 (dd, J = 14.3, 9.0 Hz, 1H), 2.30 (br d, J = 14.3 Hz, 1H), 2.18−2.00 (m, 2H), 1.59 (ddd, J = 12.5, 10.9, 8.8 Hz, 1H), 1.45 (s, 9H); ^{13}C NMR (50 MHz) δ 173.9 (s), 172.1 (s), 170.7 (s), 170.4 (s), 156.1 (s), 143.7 (s, 2C), 141.2 (s, 2C), 127.6 (d, 2C), 127.0 (d, 2C), 125.1 (d, 2C), 119.9 (d, 2C), 81.7 (s), 72.0 (s), 67.4 (t), 54.4 (d), 53.0 (q), 51.5 (d), 47.1 (d), 42.1 (t), 37.7 (t), 36.9 (t), 35.1 (t), 28.0 (q, 3C), 25.7 (t); MS (EI) *m/z* (%) 458 (<1), 333 (1), 236 (55), 178 (100), 176 (48), 165 (11), 152 (23), 122 (60), 88 (14); IR ($CDCl_3$) 3417, 3068, 2982, 1721, 1498, 1247 cm^{-1}. Anal. calcd for $C_{32}H_{37}N_3O_8$: C, 64.96; H, 6.30; N, 7.10. Found: C, 64.61; H, 5.96; N, 7.11.

(2*R*,7a*R*)-2-[((2*S*)-4-*tert*-Butoxy-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-4-oxobutanoyl)amino]-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylic Acid [(2*R*,7a*R*)-25a]. The tripeptide (2*R*,7a*R*)-**24a** (296 mg, 0.50 mmol) and NaOH (24, mg, 0.6 mmol) were added to a 0.8 M solution of $CaCl_2$ in *i*PrOH/H_2O 7:3 (11.2 mL). The reaction mixture was stirred for 6 h at room temperature and then was neutralized with 1 M AcOH and concentrated. The residue was dissolved in CH_2Cl_2, and the solution was dried over Na_2SO_4, filtered, and concentrated. The crude compound was purified by column chromatography on silica gel (eluent: $CHCl_3$/MeOH/AcOH, 200:10:0.1) to afford the compound (2*R*,7a*R*)-**25a** (108 mg, 37%) as a white solid.

(2*R*,7a*R*)-**25a**: R_f = 0.22 ($CHCl_3$/MeOH/AcOH = 20:1:0.1); mp = 108−110 °C; $[\alpha]^{27}_D$ = −0.56 (c = 1.25, $CHCl_3$); ^{1}H NMR (400 MHz) δ 7.79−7.72 (m, 2H), 7.61−7.66 (m, 2H), 7.44−7.29 (m, 4H + NH), 5.96−5.88 (m, 1H), 4.60−4.30 (m, 2H), 4.46 (d, J = 6.8 Hz, 2H), 4.23 (t, J = 6.8 Hz, 1H), 3.69 (dt, J = 11.8, 8.0 Hz, 1H), 3.26−3.17 (m, 1H) 2.90 (br d, J = 17.1 Hz, 1H), 2.69 (dt, J = 11.9, 4.4 Hz, 1H), 2.59 (br dd, J = 17.1, 3.8 Hz, 1H), 2.50 (dd, J = 14.6, 9.5 Hz, 1H), 2.37−2.26 (m, 1H), 2.13−2.03 (m, 2H), 1.58−1.42 (m, 1H), 1.44 (s, 9H); ^{13}C NMR (50 MHz, CD_3OD) δ 176.8 (s), 174.7 (s), 173.0 (s), 171.3 (s), 159.1 (s), 145.2 (s, 2C), 142.6 (s, 2C), 128.8 (d, 2C), 128.2 (d, 2C), 126.3 (d, 2C), 120.9 (d, 2C), 82.4 (s), 73.3 (s), 68.2 (t), 55.4 (d), 53.1 (d), 48.3 (d), 44.5 (t), 38.7 (t), 36.8 (t), 35.9 (t), 28.3 (q, 3C), 26.3 (t); MS (EI) *m/z* (%) 178 (100), 176 (24), 165 (4), 152 (17), 150 (10), 111 (2), 89 (5), 86 (9), 84 (10), 76 (11); IR ($CDCl_3$) 3688, 3519, 3404, 2928, 1705, 1602, 1506, 1369, 1216 cm^{-1}. Anal. calcd for $C_{31}H_{35}N_3O_8$: C, 64.46; H, 6.11; N, 7.27. Found: C, 64.96; H, 6.20; N, 6.99.

Methyl (2*S*,7a*S*)-2-[((2*S*)-4-*tert*-Butoxy-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-4-oxobutanoyl)amino]-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylate [(2*S*,7a*S*)-24b]. The amine (2*S*,7a*S*)-**15b** (166 mg, 0.84 mmol) was coupled with Fmoc-L-Asp(O*t*Bu)-OH (345 mg, 0.84 mmol) to afford (2*S*,7a*S*)-**24b** (422 mg, 85%) by the same procedure as described for the synthesis of (2*R*,7a*R*)-**24a**.

(2S,7aS)-**24b**: white solid, R_f = 0.38 (AcOEt/Et_2O, 1:1); mp = 82−83 °C; $[\alpha]^{27}_D$ = +43.30 (c = 1.03, $CHCl_3$); ^{1}H NMR (500 MHz) δ 7.78−7.76 (m, 2H), 7.63−7.60 (m, 2H), 7.43−7.39 (m, 2H), 7.37.34−7.30 (m, 2H), 7.27−7.24 (m, 1H), 5.94−5.91 (m, 1H), 4.69 (dt, J = 2.2, 8.3 Hz, 1H), 4.56−4.50 (m, 1H), 4.46−4.40 (m, 2H), 4.25 (t, J = 7.0 Hz, 1H), 3.78 (s, 3H), 3.66 (dt, J = 11.7, 8.1 Hz, 1H), 3.24 (ddd, J = 11.7, 9.6, 3.7 Hz, 1H), 2.97 (dd, J = 17.1, 4.0 Hz, 1H), 2.59 (dd, J = 17.1, 5.3 Hz, 1H), 2.53 (dd, J = 12.2, 7.0 Hz, 1H), 2.41 (dd, J = 14.3, 8.5 Hz, 1H), 2.30 (dd, J = 14.3, 2.2 Hz, 1H), 2.20−2.12 (m, 1H), 2.11−2.01 (m, 1H), 1.60 (ddd, J = 12.5, 11.1, 8.8 Hz, 1H), 1.45 (s, 9H); ^{13}C NMR (50 MHz) δ 174.0 (s), 171.6 (s), 171.2 (s), 170.5 (s), 155.8 (s), 143.6 (s, 2C), 141.2 (s, 2C), 127.7 (d, 2C), 127.0 (d, 2C), 125.0 (d, 2C), 119.9 (d, 2C), 81.8 (s), 72.1 (s), 67.2 (t), 54.8 (d), 53.0 (q), 51.1 (d), 47.0 (d), 41.8 (t), 37.7 (t), 37.4 (t), 35.2 (t), 27.9 (q, 3C), 25.7 (t); MS (EI) *m/z* (%) 333 (1), 236 (55), 178 (100), 176 (26), 165 (6), 152 (26), 122 (36), 88 (82), 76 (50); IR ($CDCl_3$) 3417, 3068, 2976, 1712, 1496, 1215 cm^{-1}. Anal. calcd for $C_{32}H_{37}N_3O_8$: C, 64.96; H, 6.30; N, 7.10. Found: C, 64.63; H, 6.29; N, 7.14

(2*S*,7a*S*)-2-[((2*S*)-4-*tert*-Butoxy-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-4-oxobutanoyl)amino]-3-oxotetrahydro-1*H*-pyrrolizine-7a(5*H*)-carboxylic acid [(2*S*,7a*S*)-25b]. The tripeptide (2*S*,7a*S*)-**24b** (170 mg, 0.29 mmol) was hydrolyzed to (2*S*,7a*S*)-**25b** by the same pro-

cedure as described for the synthesis of (2*R*,7a*R*)-**25a**. The crude compound was purified by column chromatography on silica gel (eluent: CH_2Cl_2/MeOH/TFA, 40:1:0.2) to afford (2*S*,7a*S*)-**25b** (96 mg, 58%) as a white solid.

(2*S*,7a*S*)-**25b**: R_f = 0.24 (CH_2Cl_2/MeOH/TFA, 20:1:0.2); mp = 113−114 °C; $[\alpha]^{25}_D$ = +28.97 (*c* = 1.07, $CHCl_3$); ^{1}H NMR (400 MHz)) δ 7.77−7.72 (m, 2H), 7.65−7.59 (m, NH), 7.59−7.54 (m, 2H), 7.41−7.36 (m, 2H), 7.31−7.27 (m, 2H), 6.07−6.01 (m, NH), 4.66−4.58 (m, 1H), 4.52−4.32 (m, 3H), 4.24−4.16 (m, 1H), 3.64 (dt, *J* = 11.7, 8.1 Hz, 1H), 3.33−3.20 (m, 1H), 2.81−2.72 (m, 1H), 2.72−2.62 (m, 1H), 2.59−2.52 (m, 1H), 2.45−2.37 (m, 2H), 2.14−2.03 (m, 2H), 1.58−1.45 (m, 1H), 1.42 (s, 9H); ^{13}C NMR (50 MHz) δ 175.2 (s), 172.2 (s), 171.3 (s), 170.6 (s), 156.2 (s), 143.3 (s, 2C), 141.1 (s, 2C), 127.7 (d, 2C), 127.0 (d, 2C), 125.1 (d, 2C), 119.9 (d, 2C), 82.0 (s), 72.0 (s), 67.4 (t), 54.7 (d), 51.4 (d), 46.9 (d), 42.1 (t), 37.9 (t), 36.6 (t), 35.2 (t), 28.0 (q, 3C), 25.5 (t); MS (EI) *m/z* (%) 236 (1), 178 (100), 176 (22), 165 (12), 152 (11), 122 (8), 111 (7), 88 (9), 76 (12); IR ($CDCl_3$) 3688, 3419, 2982, 1710, 1516, 1368, 1224, 1156 cm^{-1}. Anal. calcd for $C_{31}H_{35}N_3O_8$: C, 64.46; H, 6.11; N, 7.27. Found: C, 64.11; H, 6.40; N, 6.83.

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Supporting Information Available: General experimental methods; conformational analysis, NMR, and IR methods; X-ray crystallographic data for compounds *exo*-**6** and (2*R*,7a*R*)-**23a** in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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