Pergamon

Tetrahedron 57 (2001) 6353–6359

TETRAHEDRON

Synthesis and analysis of the sterically constrained L-glutamine analogues (3*S*,4*R*)-3,4-dimethyl-L-glutamine and (3*S*,4*R*)-3,4-dimethyl-L-pyroglutamic acid

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Received 23 January 2001; accepted 17 April 2001

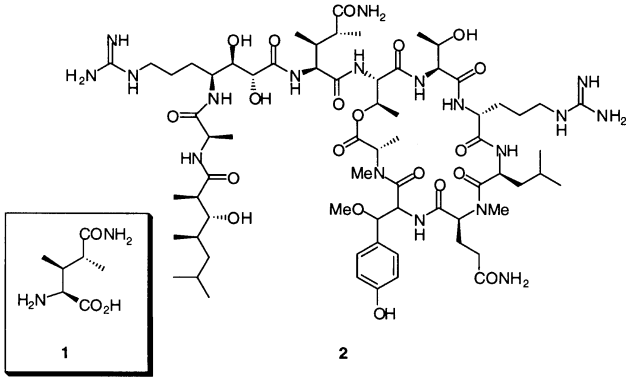
Abstract—The nonproteinogenic amino acids (3*S*,4*R*)-3,4-dimethyl-L-pyroglutamic acid and (3*S*,4*R*)-3,4-dimethyl-L-glutamine—found in the cyclic depsipeptides callipeltin B, callipeltin A, and papuamide A—were synthesized from a common intermediate derived from L-pyroglutamic acid. The diastereoselective introduction of the methyl groups was accomplished by cuprate addition and enolate alkylation, followed by a kinetic epimerization of the C-4 methyl substituent. (3*S*,4*R*)-3,4-Dimethyl-L-glutamine shows a conformational restriction of its side chain which may be related to its biological function in the natural products where it is found.

1. Introduction

The nonproteinogenic α-amino acid (3*S*,4*R*)-3,4-dimethyl-L-glutamine (**1**) was first encountered by Minale and co-workers during their characterization of callipeltin A (**2**), a cyclic depsipeptide with potent antiviral and antifungal properties isolated from the shallow water Lithistida sponge *Callipelta* sp.[1] In a companion publication, Minale and coworkers also isolated and characterized a related cyclic depsipeptide, callipeltin B (**3**), in which **1** had been cyclized to form (3*S*,4*R*)-3,4-dimethyl-L-pyroglutamic acid (**4**).[2] Both callipeltins A and B were shown to possess antitumor activity against several different tumor cell lines.[2] More recently, **1** has also been found in a family of related natural products, papuamides A–D (**5a**–**d**), isolated from *Theonella mirabilis* and *Theonella swinhoe*.[3] Papuamides A (**5a**) and B (**5b**) were shown, like callipeltin A, to inhibit the replication of HIV-1 in vitro; papuamide A was also found to be cytotoxic to a panel of tumor cell lines with a mean IC_{50} of 75 ng mL^{-1}.

It is noteworthy that in all of these cyclic depsipeptide natural products, each of which possesses antitumor, antiviral or antifungal activity, some form of **1** is present. While the modes of action of these molecules are not known, the conservation of **1** throughout the family suggests that it may play a role in one or more of the biological functions of **2**, **3** and **5a**. Additionally, the side chain methylation of **1** might be assumed to result in a conformational restriction of its side chain in comparison to that of L-glutamine.

As part of our efforts directed toward the synthesis and



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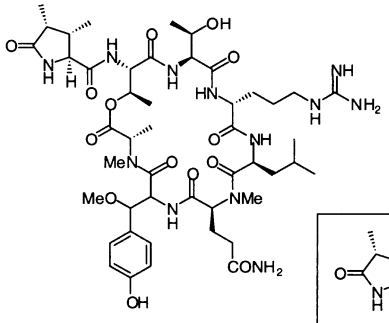

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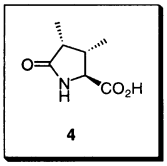

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Keywords: asymmetric synthesis; amino acids; conformational analysis; callipeltin A.

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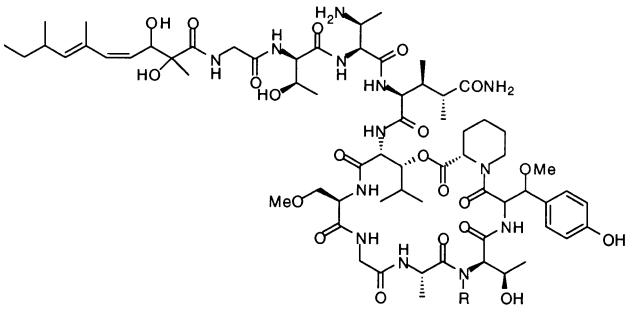
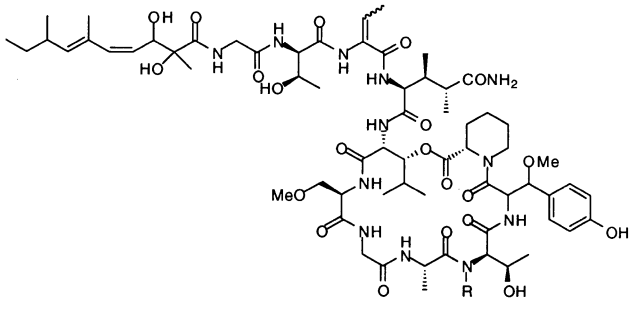
PII: S0040-4020(01)00501-4



5a R=CH_3
5b R=H



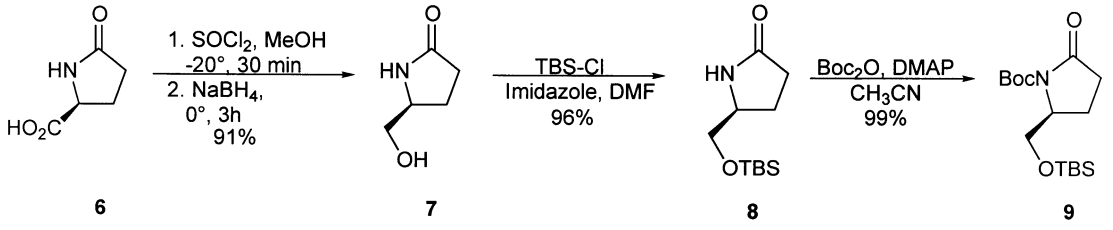
5c R=CH_3
5d R=H

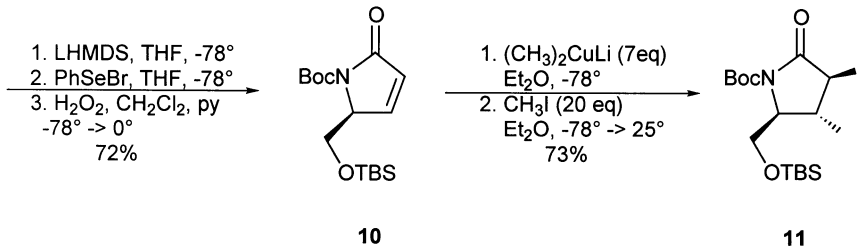
structure elucidation of callipeltins A and B, we wish to report herein the syntheses of (3*S*,4*R*)-3,4-dimethyl-L-glutamine (**1**) and (3*S*,4*R*)-3,4-dimethyl-L-pyroglutamic acid (**4**) from a common precursor derived initially from L-pyroglutamic acid.[4] We also have now investigated the conformational consequences of the side chain methylation of **1** by comparing the conformation preferences of a protected derivative of **1** to those of an analogously protected version of L-glutamine. The major concerns attending the syntheses of **1** and **4** from L-pyroglutamic acid were the acylation of the lactam nitrogen to facilitate nucleophilic ring opening of the lactam by a nitrogen nucleophile, avoiding epimerization of C_α by suitable protection of the carboxylate and producing the desired *cis* relationship of the two methyls in **4**. This latter concern was addressed through diastereoselective epimerization of the C-4 methyl.

2. Results and discussion

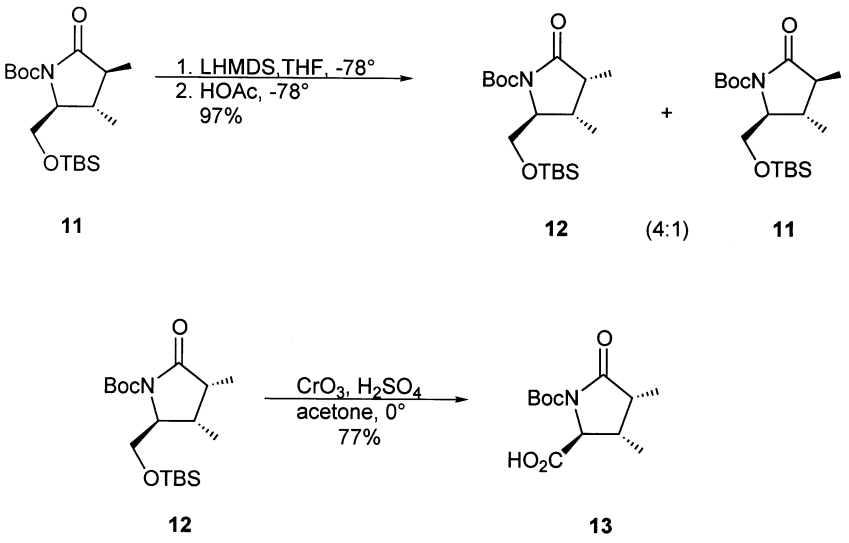
Reduction of L-pyroglutamic acid **6** to (5*S*)-5-hydroxymethylpyrrolidin-2-one **7** was required to reduce the acidity of C_α and thereby prevent racemization during later synthetic steps. Reduction of **6** to **7** was accomplished in 91% yield in one pot by conversion of the acid to the methyl ester followed by reduction with $NaBH_4$ to the alcohol (Scheme 1). Alcohol **7** was protected as the silyl ether **8** with *tert*-butyldimethylsilyl chloride and imidazole in DMF, followed by acylation using $(Boc)_2O$ and DMAP in acetonitrile to yield **9** in 95% overall yield. The α,β-unsaturation of intermediate **10** was installed in 72% yield from **9** by α-selenation of the lactam[5] and oxidative elimination in the presence of pyridine to avoid racemization of **10**. It was found in accord with literature precedent[6] that successful cuprate addition to **10** could be achieved using an excess of cuprate reagent. The resulting enolate was found to react with methyl iodide only when the reaction was allowed to warm to room temperature. Methylation of the enolate resulted in nearly exclusive formation of the *trans*, *trans* diastereomer **11**. The *cis* relationship of the C-4 methyl to the silyl ether and its *trans* relationship to the C-3 methyl were established by NOE spectroscopy.[7]

Enolization of compound **11** with LHMDS (Scheme 2) and quenching with acetic acid at −78°C gave a mixture of **11** and its C-4 epimer **12** in a 1 to 4 ratio. Epimers **11** and **12** were separated using flash chromatography and recovered **11** was resubmitted to the epimerization conditions. From





Scheme 1.



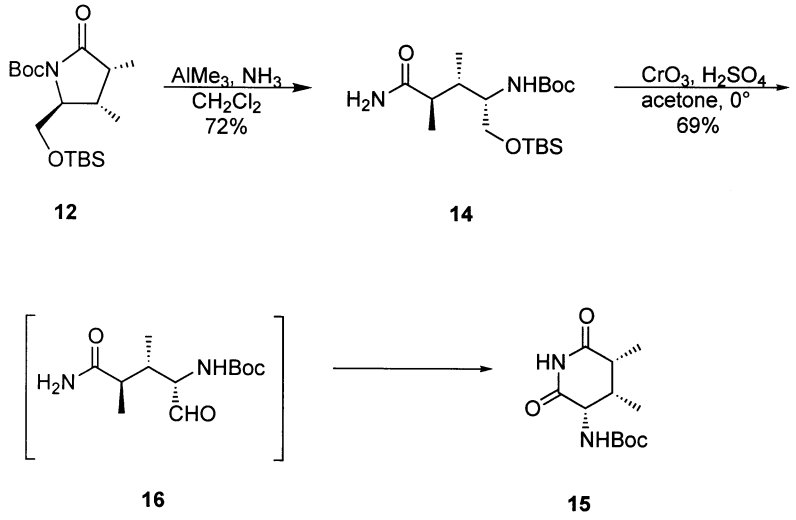
Scheme 2.

this intermediate (**12**), deprotection of the silyl ether and oxidation of the alcohol provides a Boc-protected version of **4**. In the event, both operations were accomplished in one step, in 77% yield, using a Jones oxidation. The product thus obtained (**13**) could be cleanly deprotected with TFA in methylene chloride to afford (3*S*,4*R*)-3,4-dimethyl-L-pyroglutamic acid **4**.

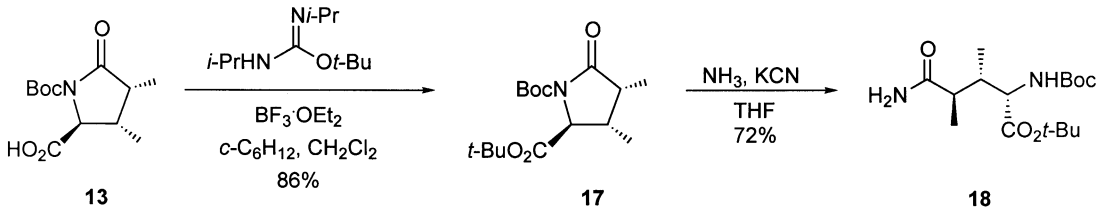
The advanced intermediate **12** was also employed as a synthetic precursor to (3*S*,4*R*)-3,4-dimethyl-L-glutamine. In our early efforts, the acylated lactam **12** was treated with ammonia in the presence of $AlMe_3$,[8] opening the ring to form the acyclic amide **14** (Scheme 3). However, when **14** was submitted to Jones oxidation, the desired carboxylic acid was produced in low (<40%) yield, and a relatively nonpolar compound (**15**) was obtained as the major product. Isolation and characterization of **15** showed it to be a cyclic imide, presumably formed by cyclization of the intermediate α-amino aldehyde **16** and oxidation of the resultant cyclic hemiaminal. Deprotection of the silyl group of **14** followed by treatment with several different oxidizing agents led to no decrease in the formation of **15**.

A second strategy was therefore explored in which *N*-(*tert*-butoxycarbonyl)-(3*S*,4*R*)-3,4-dimethyl-L-pyroglutamic acid (**13**) was used as the precursor of **1**. This strategy began with conversion of **13** to the *t*-butyl ester **17** (Scheme 4) using *N*,*N*′-diisopropyl-*O*-*tert*-butylisourea.[9] Ring opening of lactam **17** using ammonia in the presence of catalytic KCN resulted in the formation of *N*-(*tert*-butoxycarbonyl)-(3*S*,4*R*)-3,4-dimethyl-L-glutamine *tert*-butyl ester **18**.[10] Deprotected **1** can be obtained from **18** by treatment with TFA in methylene chloride.

Upon completion of the syntheses of protected forms of **1** and **4**, ^{1}H-NMR and computational studies were undertaken to elucidate the conformational consequences of the 3,4-dimethyl substituents on the L-glutamine side chain. To this end, vicinal coupling constants for the side chain protons of **18** and *N*-(*tert*-butoxycarbonyl)-L-glutamine

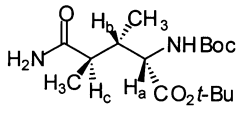


Scheme 3.



Scheme 4.

Table 1. Experimental and computed coupling constants found in **18** and **19**



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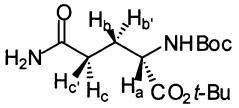


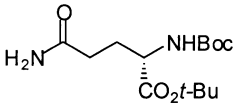
19

Compound	J_{ab} (Hz)	$J_{ab'}$ (Hz)	J_{bc}[a] (Hz)	$J_{b'c}$[a] (Hz)
18 (experimental)	4.8	–	8.4	–
18 (calculated)[b]	4.3 (4.6)		6.4 (5.2)	
19 (experimental)	3.8	7.6	7.4	7.8
19 (calculated)[b]	4.3 (3.5)	11.6 (11.1)	9.0 (7.1)	3.3 (5.0)

[a] Coupling constants to H_c and $H_{c'}$ are reported as a single value due to magnetic shift equivalence.

[b] The value outside of parentheses is the coupling constant calculated for the lowest energy conformer. The value inside the parentheses is a Boltzmann-weighted average obtained from all conformers within 3 kcal mol^{-1} of the global minimum.

tert-butyl ester (**19**) were obtained from homonuclear decoupling experiments performed in methanol-d_4. The results are shown in Table 1, along with vicinal coupling constants computed from molecular mechanics calculations.



19

The calculated vicinal coupling constants shown in Table 1 were obtained from conformational searches of both **18** and **19** using the AMBER* force field,[11] the GB/SA solvation model[12] for water and the torsional Monte Carlo conformational search algorithm[13] as implemented in the MacroModel molecular modeling package.[14] Vicinal coupling constants of individual conformations and Boltzmann-weighted averages of all conformations were computed using the Altona equation.[15]

A comparison of the experimentally derived and calculated coupling constants shows that the observed coupling constants of **19** are more consistent with those of an average of conformations than with any single conformation examined. This was in accord with the observation that the diastereotopic C_γ protons of **19** appear in the 300 MHz NMR spectra as a single resonance and behave as magnetically shift equivalent protons. In contrast, the observed coupling constants of **18** are marginally more consistent with those computed for the lowest energy conformer obtained from molecular mechanics calculations than with those of an ensemble average. Additionally, it was found that the number of available conformations of **19** (41) within 3 kcal mol^{-1} of the ground state was substantially greater than the comparable number of conformations of **18** (30). Even more striking was a comparison of the number of conformers within 1 kcal mol^{-1} of the ground state: **19** had seven minima in that range, whereas **18** had only two.

Further information was obtained from an examination of the global minima in the two conformational searches. The molecular mechanics global minimum of **19** (Fig. 1) has side chain torsion angles $\chi_1 = -170°$ and $\chi_2 = -53°$, whereas that of **18** (Fig. 2) has $\chi_1 = -48°$ and $\chi_2 = -41°$. This difference represents a fundamental change in

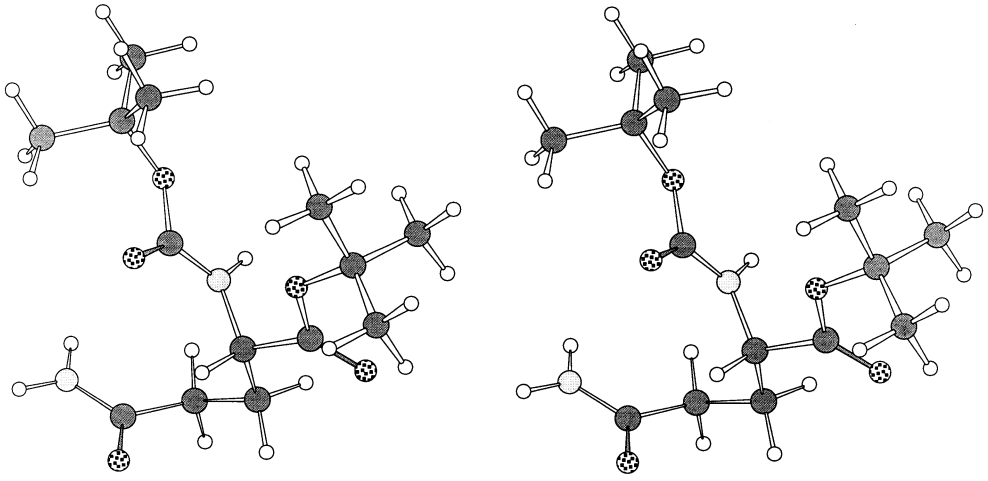


Figure 1. Stereo pair view of the lowest energy conformation of **19** obtained from molecular mechanics calculations.

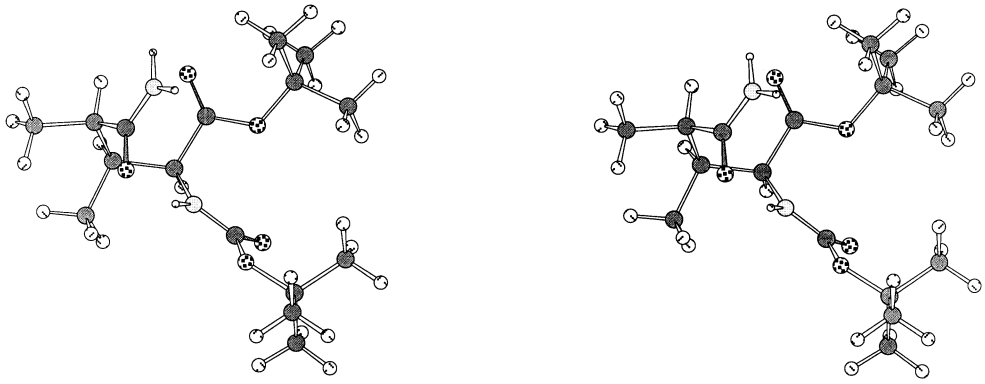


Figure 2. Stereo pair view of the lowest energy conformation of **18** obtained from molecular mechanics calculations.

side chain conformation, as that of **19** adopts an *anti*, *-gauche* conformation where that of **18** exists in a slightly distorted *-gauche*, *-gauche* arrangement. Examination of the other low energy conformer of **18** reveals that it too exists in a *-gauche*, *-gauche* conformation that differs only subtly from the global minimum. Thus, it appears that the methyl substituents on the side chain of **18** may bias its conformation to *-gauche*, *-gauche*. The only analogous conformer found for the parent system **19** was the highest energy conformer in the search, nearly 3 kcal mol^{-1} above the ground state. Therefore, the methyl substituents appear not only to restrict the rotation of the side chain, but also to selectively stabilize a high energy conformer of the glutamine side chain.

3. Conclusion

Protected derivatives of the nonproteinogenic amino acids (3*S*,4*R*)-3,4-dimethyl-L-pyroglutamic acid (**4**) and (3*S*,4*R*)-3,4-dimethyl-L-glutamine (**1**) were synthesized in good yields from readily available L-pyroglutamic acid in 8 and 10 steps, respectively. It was also shown that (3*S*,4*R*)-3,4-dimethyl-L-glutamine adopts a different conformation than L-glutamine in methanol and that it appears to rigidify the side chain into a conformation unavailable to that of L-glutamine. This observation may provide an insight into the role of the methyl groups of (3*S*,4*R*)-3,4-dimethyl-L-glutamine in the biological activity exhibited by the natural products that contain it.

4. Experimental

4.1. Data for compounds

4.1.1. (*S*)-5-Hydroxymethyl-2-pyrrolidinone (7). To a solution of (*S*)-pyroglutamic acid (**6**) (2.00 g, 15.5 mmol) in methanol (50 mL) at −15°C was added thionyl chloride ($SOCl_2$) (2.04 g, 17.1 mmol) dropwise with stirring. After stirring for 30 min at −15°C the reaction was allowed to warm to room temperature over an hour and stirred for an additional hour at room temperature. The solvent was then removed under reduced pressure producing a thick, clear oil. This oil was then redissolved in ethanol (50 mL) and cooled to 0°C. To the solution was slowly added sodium borohydride ($NaBH_4$) (1.18 g, 31.0 mmol) which would rapidly evolve gas. The reaction was then allowed to warm to room temperature overnight producing a milky white solution. The reaction was quenched by addition of a 5% solution of citric acid (100 mL) until the solution became clear with a solid gray precipitate. The solution was then decanted from the precipitate and the solvent removed under reduced pressure leaving a thick oil. To the oil was added a 25% methanol/ethyl acetate solution (200 mL) and the resulting solution was filtered. The solvent was removed under reduced pressure from the supernatant and the solid was dissolved in CH_2Cl_2 (200 mL) and filtered. The CH_2Cl_2 was removed under reduced pressure to yield **7** as a white solid (1.63 g, 14.2 mmol, 91%): ^{1}H NMR ($CDCl_3$) δ 7.54 (s, 1H), 4.89 (br s, 1H), 3.73 (m, 1H), 3.64 (dd, J=11.1, 3.6 Hz, 1H), 3.47 (m, 1H), 2.29 (m, 2H), 2.10 (m, 1H), 1.73 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 179.7, 65.7, 56.7, 30.4, 22.7; FABHRMS calculated: 116.0712, found: 116.0714.

4.1.2. (5*S*)-5-{[(*tert*-Butyl)dimethylsilyloxy]methyl}pyrrolidin-2-one (8). To a solution of **7** (1.63 g, 14.2 mmol) in dimethylformamide (DMF) (100 mL) was added imidazole (2.41 g, 35.4 mmol) and *tert*-butyldimethylsilyl chloride (TBSCl) (2.57 g, 17.0 mmol). After 24 h the solution was diluted with Et_2O (300 mL) and washed with water (3×100 mL) and brine (3×100 mL) and dried over Na_2SO_4. The solvent was removed to give **8** as a colorless oil (3.14 g, 13.6 mmol, 96%): ^{1}H NMR ($CDCl_3$) δ 5.99 (br s, 1H), 3.74 (m, 1H), 3.61 (dd, J=10.1, 4.1 Hz, 1H), 3.44 (dd, J=10.0, 7.6 Hz, 1H), 2.33 (m, 2H), 2.16 (m, 1H), 1.73 (m, 1H), 0.87 (s, 9H), 0.05 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 178.6, 66.6, 55.9, 30.0, 25.9, 25.8, 22.9, 11.2, −3.5, −5.4, −5.5.

4.1.3. (5*S*)-*N*-(*tert*-Butoxycarbonyl)-5-{[(*tert*-butyl)dimethylsilyloxy]methyl}-pyrrolidin-2-one (9). To a solution of **8** (3.14 g, 13.61 mmol) in acetonitrile (CH_3CN) (100 mL) at 0°C was added DMAP (0.17 g, 1.361 mmol) and di-*tert*-butyl dicarbonate (5.94 g, 27.22 mmol) with stirring. The reaction was allowed to slowly warm to room temperature overnight. The solvent was removed under reduced pressure to yield an dark red oil which was purified by flash chromatography using 10% ethyl acetate/CH_2Cl_2 to yield **9** as an amber oil (4.49 g, 13.59 mmol, 99%): $[\alpha]^{24}_D$=−59.7° (*c* 0.10, $CHCl_3$) {lit. $[\alpha]^{23}_D$=−62.2° (*c* 1.23, $CHCl_3$)}; ^{1}H NMR ($CDCl_3$) δ 4.41 (m, 1H), 3.89 (dd, J=10.4, 4.1 Hz, 1H), 3.66 (dd, J=10.4, 2.3 Hz, 1H), 2.67 (m, 1H), 2.34 (m, 1H), 2.04 (m, 1H), 1.50 (s, 9H), 0.85

(s, 9H), 0.02 (s, 3H), 0.00 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 175.1, 150.2, 82.8, 64.5, 59.0, 32.5, 28.2, 26.0, 21.3, 18.3, −5.4, −5.5; FABHRMS calculated: 330.2102, found: 330.2100.

4.1.4. (5*S*)-*N*-(*tert*-Butoxycarbonyl)-5-{[(*tert*-butyl)dimethylsiloxy]methyl}-3-pyrrolin-2-one (10). To a solution of **9** (7.93 g, 24.0 mmol) in THF (200 mL) at −78°C was slowly added LiHMDS in hexanes (26.4 mL, 26.4 mmol) and left stirring for 15 min. A solution of phenylselenenyl bromide (9.89 g, 41.9 mmol) in THF (50 mL) was transferred via cannula to the reaction and allowed to stir for 1 h at −78°C. The reaction was quenched by addition of saturated NH_4Cl (50 mL) and Et_2O (100 mL) and allowed to warm to room temperature. The solution was washed with sat. NH_4Cl (3×100 mL) followed by brine (3×100 mL) and the organic layer dried over Na_2SO_4. The solvent was removed under reduced pressure leaving behind a red oil that was then dissolved in CH_2Cl_2 (200 mL) and cooled to −78°C. Pyridine (5.81 mL) was added, followed by slow addition of 30% H_2O_2 (8.13 mL) and the temperature was allowed to warm to 0°C over 1 h. The solution was diluted with Et_2O (500 mL) and washed with H_2O (3×100 mL) followed by brine (3×100 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The resulting oil was purified using flash column chromatography with 10% EtOAc/petroleum ether giving the desired product as a colorless solid (6.64 g, 19.4 mmol, 81% yield): $[\alpha]^{24}_D$=−169° (*c* 1.0, $CDCl_3$){lit. $[\alpha]^{23}_D$=−175.6° (*c* 0.9, $CHCl_3$)}; ^{1}H NMR ($CDCl_3$) δ 7.26 (m, 1H), 6.12 (dd, *J*=6.1, 1.7 Hz, 1H), 4.60 (m, 1H), 4.15 (dd, *J*=9.6, 3.7 Hz, 1H), 3.71 (dd, *J*=9.6, 6.8 Hz, 1H), 1.55 (s, 9H), 0.86 (s, 9H), 0.04 (d, *J*=3.9 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 169.7, 150.0, 149.7, 127.3, 83.2, 63.8, 62.7, 28.4, 25.9, 18.4, −5.2, −5.3; mp 57–59°C; IR (KBr) 1781.5, 1701.3, 1460.8 cm^{-1}; Anal. Calcd for $C_{18}H_{35}NO_4Si$: C, 60.46; H, 9.87; N, 3.92. Found: C, 60.58; H, 9.86; N, 3.85.

4.1.5. (3*S*,4*R*,5*S*)-*N*-(*tert*-Butoxycarbonyl)-5-{[(*tert*-butyl)dimethylsiloxy]methyl}-3,4-dimethylpyrrolidin-2-one (11). To a yellow–orange mixture of copper iodide (6.04 g, 31.8 mmol) in Et_2O at 0°C methyl lithium in Et_2O (46.38 mL, 63.5 mmol) was added turning it into a clear solution. The lithium dimethyl cuprate solution was cooled to −78°C and a solution of **10** (1.49 g, 4.54 mmol) in Et_2O was added via cannula forming a cloudy yellow solution. The reaction was allowed to stir at −78°C for 1 h and then methyl iodide (12.90 g, 90.8 mmol) was added. Formation of a white precipitate is observed at this point. The reaction was left stirring for 30 min and then the temperature was allowed to slowly rise to room temperature over a period of 3 h. The reaction was quenched by addition of sat. NH_4Cl (3 mL) and Et_2O (10 mL). The solution was washed with NH_4Cl (3×30 mL), H_2O (3×30 mL), and brine (3×30 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. Column chromatography purification eluted with 10% EtOAc/petroleum ether gave compound **11** (1.19 g, 3.33 mmol, 73% yield): $[\alpha]^{24}_D$=−55° (*c* 0.5, $CDCl_3$); ^{1}H NMR ($CDCl_3$) δ 3.84 (dd, *J*=10.3, 5.2 Hz, 1H), 3.74 (dd, *J*=10.3, 2.7 Hz, 1H), 3.66 (m, 1H), 2.96 (p, *J*=7.5 Hz, 1H), 2.43 (p, *J*=7.5 Hz, 1H), 1.53 (s, 9H), 1.07 (d, *J*=7.3 Hz, 3H), 0.98 (d, *J*=7.1 Hz, 3H), 0.86 (t *J*=2.9 Hz, 9H), 0.03 (d, *J*=4.6 Hz, 6H); ^{13}C NMR δ 176.6, 150.5, 82.5, 64.7, 63.1, 40.6, 32.9, 28.0, 25.8, 18.1, 15.8, 10.1, −5.5, −5.6; IR (neat) 2957.4, 2861.4, 1787.6, 1754.1, 1711.7, 1466.2 cm^{-1}; FABHRMS calculated: 358.2414, found: 358.2412; Anal. Calcd for $C_{18}H_{35}NO_4Si$: C, 60.46; H, 9.87; N, 3.92. Found: C, 60.56; H, 9.97; N, 3.95.

4.1.6. (3*R*,4*R*,5*S*)-*N*-(*tert*-Butoxycarbonyl)-5-{[(*tert*-butyl)dimethylsiloxy]methyl}-3,4-dimethylpyrrolidin-2-one (12). To a solution of **11** (0.20 g, 0.57 mmol) in THF (4.5 mL) at −78°C was added LiHMDS in hexanes (0.85 mL, 1 M, 0.85 mmol). The reaction was allowed to stir at −78°C for 1 h, the temperature was lowered to −98°C and glacial HOAc (49 μL, 0.85 mmol) was added quickly. The reaction was quenched after 5 min by the addition of sat. NH_4Cl (2 mL) and sat. $NaHCO_3$ (2 mL) and diluted with Et_2O (5 mL) and H_2O (5 mL). The mixture was allowed to warm to room temperature and the layers separated. The organic layer was washed with H_2O (3×10 mL) and brine (3×10 mL). After drying the organic layer over Na_2SO_4 and removing the solvent under reduced pressure, the isomers were separated via flash column chromatography with 10% EtOAc/petroleum ether giving the desired product **12** in a 4:1 ratio (0.20 g, 97%) with **11**: $[\alpha]^{24}_D$=−43.9° (*c* 1.1, $CDCl_3$); ^{1}H NMR ($CDCl_3$) δ 4.03 (dd, *J*=10.3, 4.3 Hz, 1H), 3.69 (dd, *J*=10.3, 2.2 Hz, 1H), 3.53 (m, 1H), 2.09 (m, 1H), 1.96 (m, 1H), 1.53 (s, 9H), 1.22 (d, *J*=7.1 Hz, 3H), 1.15 (d, *J*=6.6 Hz, 3H), 0.87 (s, 9H), 0.03 (d, *J*=3.2 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 176.8, 150.7, 82.9, 64.6, 61.5, 45.6, 35.2, 28.3, 26.0, 18.8, 18.5, 15.0, −5.2, −5.3; mp 57–59°C; IR (KBr) 1781.5, 1701.3, 1460.8 cm^{-1}; Anal. Calcd. for $C_{18}H_{35}NO_4Si$: C, 60.46; H, 9.87; N, 3.92. Found C, 60.58; H, 9.86; N, 3.85.

4.1.7. (2*S*)-*N*-(*tert*-Butoxycarbonyl)-(3*S*,4*R*)-3,4-dimethylpyroglutamic acid (13). To a solution of **12** (0.41 g, 1.2 mmol) in acetone at 0°C was added Jones reagent (0.86 mL, 2.32 mmol), which had been cooled to 0°C. The solution instantly became bright orange and after 10 min a precipitate had formed and the solution had turned amber in color. The reaction stirred for 30 min at 0°C and 1 h at room temperature. The reaction was quenched by addition of isopropanol (25 mL) and slow addition of sat. $NaHCO_3$ (10 mL) at 0°C. The volatiles were removed under reduced pressure and the resultant mixture was diluted with H_2O (15 mL) and ethyl acetate (25 mL). The solution was then extracted with Et_2O (3×20 mL) and the aqueous layers were combined and acidified with 0.1N HCl to pH 3 at 0°C. The acidified solution was extracted with ethyl acetate (3×20 mL). The combined organic fractions were dried over Na_2SO_4. Removal of the solvent gave **13** as a glassy solid (0.23 g, 77% yield): $[\alpha]^{24}_D$=−10.3° (*c* 0.8, $CDCl_3$); ^{1}H NMR ($CDCl_3$) δ 10.66 (br s, 1H), 4.20 (d, *J*=1.7 Hz, 1H), 2.79 (m, 1H), 2.54 (m, 1H), 1.46 (s, 9H), 1.08 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 176.0, 175.6, 150.0, 84.0, 64.4, 40.8, 34.4, 28.0, 25.8, 15.4, 10.0; mp 107–110 C; IR (KBr) 3190.1, 2986.9, 1788.4, 1741.6 cm^{-1}.

4.1.8. (4*S*)-*N*-(*tert*-Butoxycarbonyl)-4-{[(*tert*-butyl)dimethylsiloxy]methyl}-(2*R*,3*S*)-dimethylglutamine (14). To a solution of **12** (75 mg, 0.21 mmol) in CH_2Cl_2 (4.2 mL) at room temperature was bubbled $NH_{3(g)}$ for

3 min. Trimethylaluminum (0.16 mL, 0.32 mmol) was added rapidly dropwise, and left stirring for 6 h. The reaction was quenched by addition of 0.1N HCl (3 mL) and the resulting suspension was stirred for 5 min and filtered. The solution was extracted with Et_2O (3×10 mL), the organic layer dried over Na_2SO_4 and concentrated under reduced pressure. Purification using column chromatography with 30% EtOAc/petroleum ether gave the desired product **14** as a white solid (57 mg, 0.16 mmol, 72% yield): $[\alpha]^{24}_D=-54.8°$ (*c* 1.01, $CDCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.65 (br s, 1H), 5.44 (br s, 1H), 5.08 (d, *J*=9.8 Hz, 1H), 3.76 (dd, *J*=10.1, 2.5 Hz, 1H), 3.60 (m, 2H), 2.53 (m, 1H), 1.60 (m, 1H), 1.45 (s, 9H), 1.09(d, *J*=7.08 Hz, 3H), 0.88 (s, 9H), 0.86 (s, 6H), 0.11 (s, 3H), 0.10 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 176.0, 157.4, 80.1, 63.2, 54.6, 39.5, 38.8, 28.4, 25.9, 18.3, 16.6, 11.7, −5.5; mp 105–106°C; FABHRMS calculated: 375.2679, found 375.2662; IR (KBr) 3438.0, 3206.1, 2957.6, 1669.0, 1626.3 cm^{-1}.

4.1.9. [(4*S*,5*R*)-Dimethyl-2,6-dioxo-piperidin-3*S*-yl]-carbamic acid *tert*-butyl ester (15). To a solution of **14** (20 mg, 0.077 mmol) in acetone at 0°C was added Jones reagent (0.17 mL, 1.67 mmol) which had been cooled to 0°C. The solution instantly became bright orange and after 10 min a precipitate had formed and the solution had turned amber in color. The reaction stirred for 30 min at 0°C and 1 h at room temperature. The reaction was quenched by addition of isopropanol (1 mL) and slow addition of sat. $NaHCO_3$ (0.5 mL) at 0°C. The volatiles were removed under reduced pressure and the resultant mixture was diluted with H_2O (5 mL) and diethyl ether (2 mL). The mixture was extracted with Et_2O (3×5mL) and the combined organic fractions were dried over Na_2SO_4. Removal of the solvent yielded a white solid (13 mg, 62% yield): ^{1}H NMR ($CDCl_3$) δ 7.83 (br s, 1H), 5.44 (br s, 1H), 4.54 (m, 1H), 2.87 (m, 1H), 2.69 (m, 1H), 1.47 (s, 9H), 1.28 (d, *J*=7.2 Hz, 3H), 0.85 (d, *J*=7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 173.7, 171.1, 80.5, 57.6, 41.2, 35.7, 29.7, 28.3, 28.2, 12.4, 8.0, 1.0; HRMS calculated: 257.1501, found 257.1499; IR (film) 2979.7, 2931.8, 1712.7, 1494.9 cm^{-1}.

4.1.10. *tert*-Butyl (2*S*)-*N*-(*tert*-Butoxycarbonyl)-(3*S*, 4*R*)-3,4-dimethylpyroglutamate (17). To a solution of **13** (60 mg, 0.23 mmol) in CH_2Cl_2 (2 mL) freshly distilled *N*,*N*′-diisopropyl-*O*-*tert*-butylisourea (47 mg, 2.3 mmol) was added and allowed to stir at room temperature for 16 h. The solvent was removed under reduced pressure and the resultant solid was purified using flash column chromatography with 30% Et_2O/petroleum ether yielding a white solid (60 mg, 83%): $[\alpha]^{24}_D=-12.9°$ (*c* 1.2, $CDCl_3$); ^{1}H NMR ($CDCl_3$) δ 4.10 (d, *J*=5 Hz,1H), 2.65 (m, 1H), 2.30 (m, 1H), 1.44 (s, 9H), 1.42 (s, 9H), 1.10 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 175.3, 169.8, 149.7, 83.0, 82.1, 65.0, 40.4, 34.2, 27.9, 15.4, 9.8; mp 62–64°C; IR (film) 2979.2, 2928.3, 2880.5, 1792.7, 1735.0, 1717.2, 1457.7, 1370.1 cm^{-1}; Anal Calcd for $C_{16}H_{27}NO_5$: C, 61.32; H, 8.68; N, 4.47. Found: C, 61.59; H, 8.59; N, 4.44.

4.1.11. (2*S*)-*N*-(*tert*-Butoxycarbonyl)-(3*S*,4*R*)-3,4-dimethylglutamine-*tert*-butyl ester (18). To a solution of **17** (30 mg, 0.96 mmol) in THF (0.38 mL) at −35°C catalytic amounts of KCN were added. Ammonia (~1 mL) was condensed and the reaction was left to reach room temperature in a sealed, high pressure vessel. After 24 h of stirring, crude **18** was submitted to flash column chromatography with 20% EtOAc/petroleum ether, yielding an oil (27 mg, 86%): $[\alpha]^{24}_D=-9.4°$ (*c* 0.5, $CDCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.07 (bs,1H), 5.53 (bs, 1H), 5.31 (d, *J*=9 Hz, 1H), 4.15 (dd, *J*=9, 9 Hz,1H), 2.47 (m, 2H), 1.47 (s, 9H) 1.45 (s, 9H), 1.18 (d, *J*=7 Hz, 3H), 0.92 (d, *J*=7 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 175.8, 171.1, 156.3, 82.6, 80.5, 57.2, 42.1, 39.9, 28.3, 28.0, 16.2, 12.2; HRMS calculated: 331.2233, found 331.2221; IR (film) 3348.6, 1703.5, 1680.5, 1650.2, 1368.0, 1155.2 cm^{-1}.

References

1. Zampella, A.; D'Auria, M. V.; Paloma, L. G.; Casapullo, A.; Minale, L.; Debitus, C.; Henin, Y. *J. Am. Chem. Soc.* **1996**, *118*, 6202.
2. D'Auria, M. V.; Zampella, A.; Paloma, L. G.; Minale, L.; Debitus, C.; Rousakis, C.; Le Bert, V. *Tetrahedron* **1996**, *52*, 9859.
3. Ford, P. W.; Gustafson, K. R.; McKee, T. C.; Shigematsu, N.; Maurizi, L. K.; Pannell, L. K.; Williams, D. E.; deSilva, E. D.; Lassota, P.; Allen, T. M.; Soest, R. V.; Andersen, R. J.; Boyd, M. R. *J. Am. Chem. Soc.* **1999**, *121*, 5899.
4. During preparation of this manuscript, an alternate synthesis of **1** by a diastereoselective Michael addition appeared in print: Liang, B.; Carroll, P. J.; Joullié, M. M. *Org. Lett.* **2000**, *26*, 4157.
5. (a) Ohfune, Y.; Tomita, M. *J. Am. Chem. Soc.* **1982**, *104*, 3511. (b) Ackermann, J.; Matthes, M.; Tamm, C. *Helv. Chim. Acta.* **1990**, *73*, 122. (c) Shimamoto, K.; Ishida, M.; Shinozaki, H.; Ohfune, Y. *J. Org. Chem.* **1991**, *56*, 4167. (d) Ikota, N. *Chem. Pharm. Bull.* **1992**, *40*, 1925.
6. Herdeis, C.; Hubmann, H. P. *Tetrahedron: Asymmetry* **1992**, *3*, 1213.
7. An NOE in the *cis* epimer **12** was observed between the two methyl groups, whereas there was no corresponding NOE seen in the *trans* epimer **11**.
8. Armstrong, R. W.; De Mattei, J. A. *Tetrahedron Lett.* **1991**, 5749.
9. Santini, C.; Ball, R. G.; Berger, D. *J. Org. Chem.* **1994**, *59*, 2261.
10. Schoenfelder, A.; Mann, A. *Synth. Commun.* **1990**, *20*, 2585.
11. McDonald, D. Q.; Still, W. C. *Tetrahedron Lett.* **1992**, *33*, 7743.
12. Still, W. C.; Tempczyk, A.; Hawley, R. C.; Hendrickson, T. *J. Am. Chem. Soc.* **1990**, *112*, 6127.
13. Chang, G.; Guida, W. C.; Still, W. C. *J. Am. Chem. Soc.* **1989**, *111*, 4379.
14. Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. MacroModel ver. 5.0. *J. Comput. Chem.* **1990**, *11*, 440–467.
15. Haasnoot, C. A. G.; DeLeeuw, F. A. A. M.; Altona, C. *Tetrahedron* **1980**, *36*, 2783.