An Efficient Strategy to Orthogonally Protected (*R*)- and (*S*)-α-Methyl(alkyl)serine-Containing Peptides via a Novel Azlactone/Oxazoline Interconversion Reaction†

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A novel strategy for the synthesis of (*R*)- and (*S*)-α-methyl(alkyl)serine-containing peptides is presented. Using (*S*)-phenylalanine cyclohexylamide **6** as chiral auxiliary, the optically pure azlactones (*R*)- and (*S*)-**2** were synthesized via a novel azlactone/oxazoline interconversion reaction (Figures 3 and 6). These azlactones constitute fully protected and activated synthetic equivalents of (*R*)- and (*S*)-α-methylserine and can be directly incorporated into peptides without further protective group manipulations. Like other α,α-dialkylated glycines, optically pure α-alkylserines can be used to stabilize *β*-turn and α-helical conformations in short peptides.

1. Introduction

In recent years, non-proteinogenic amino acids have been the focus of many investigations.[1−3] Among the nonstandard amino acids, the α,α-dialkylated glycines are especially interesting, because they can stabilize short peptides in rather well defined conformations, such as *β*-turns,[4−9] 3_{10}- and α-helical[10−14] and extended[9,15−18] conformations, depending on the nature of the α-substituents.

We have developed a general method for the preparation of a wide range of cyclic and open-chain α,α-disubstituted glycines, and we have studied the conformational properties of such building blocks when incorporated into small peptides.[6−10] These building blocks, together with other templates, were used in a program with the aim to mimic exposed epitopes of structurally known proteins with conformationally constrained peptides.[19] As part of this program we were interested in investigating the conformational properties of (*R*)- and (*S*)-α-methylserine **1** (Figure 1) and analogues since the conformational properties of α,α-disubstituted glycines bearing functionalized and polar side chains have been much less investigated.

Interestingly, calculated Ramachandran plots[20,21] of (*R*)- and (*S*)-*N*-acetyl-α-methylserine *N*-methylamide (Figure 2) revealed a strong preference for α-helical conformations, similarly to α-aminoisobutyric acid (AIB).[22]

In addition, it was known from screening the Protein Crystal Structure Data Base,[23] that (*S*)-serine is relatively often found in the last C-terminal turn of α-helices. In this position, the side chain hydroxyl group of serine can be engaged in a H-bond with an amide carbonyl group in the preceding turn.[24]

For these structural aspects and for reasons of increased chemical stability we were interested in studying the conformational properties of (*R*)- and (*S*)-α-methylserine **1** as a potential C-terminal α-helix stabilizing building block.

In this paper we present a novel synthetic strategy featuring the (*R*)- and (*S*)-4-(bromomethyl)-4-methyl-2-phenyloxazol-5(4*H*)-ones ("azlactones") **2** (Figure 3) as fully protected and activated analogues of (*R*)- and (*S*)-**1**, ready for incorporation into peptides.

2. Synthesis of the Optically Pure Azlactones (*R*)- and (*S*)-2

Several publications dealing with the asymmetric synthesis of fully orthogonally protected α-methylserine derivatives appeared in the literature.[25−35] Much less is

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‡ Part of the Ph.D. thesis of M. Altorfer, University of Zurich, 1996.
† Presented in part at the annual meeting of the New Swiss Chemical Society, Abstract published in *Chimia* **1994**, *48*, 276.
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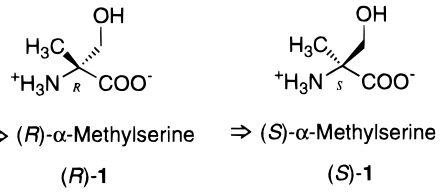


Figure 1.

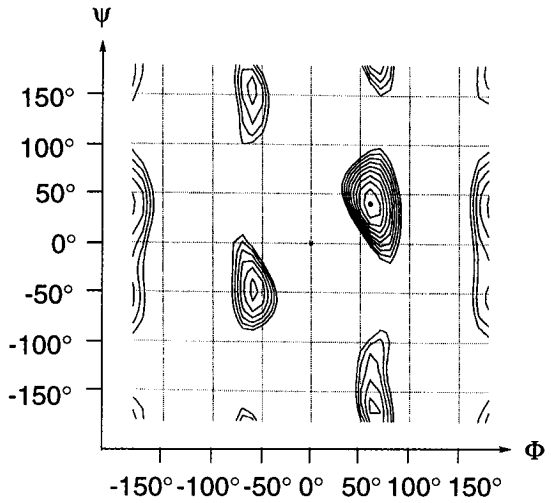


Figure 2. Ramachandran plot of N-Ac-(*S*)-α-Me-Ser(OH)-NHMe. Contour spacing: 0.25 kcal/mol. Contour lines (Φ/Ψ): min 7.57 kcal/mol (Φ = +61°/Ψ = +40°), max 9.82 kcal/mol (Φ = 0°/Ψ = 0°).

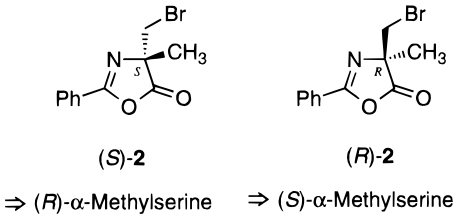


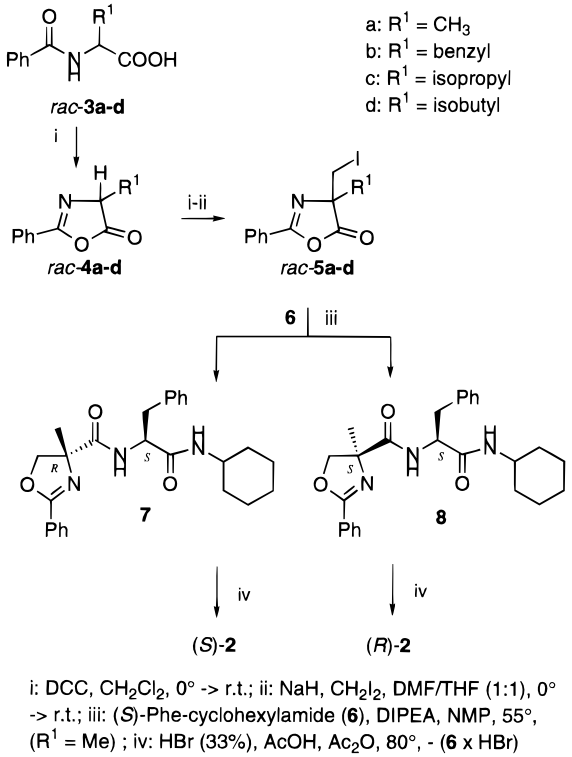
Figure 3. Fully protected and activated analogues of (*R*)- and (*S*)-α-methylserine.

known about the incorporation of these building blocks into peptides and the conformational behavior of (*R*)- and (*S*)-α-methylserine-containing peptides.[36,37]

Our synthetic strategy starts from the corresponding commercially available racemic N-benzoylated amino acids **3a**−**d** as outlined in Scheme 1.

After cyclization of *rac*-**3a-d**, using *N*,*N*-dicyclohexylcarbodiimide (DCC) in CH_2Cl_2, the intermediate racemic 4-monosubstituted-2-phenyl-oxazol-5(4*H*)-ones **4a**−**d** were

Scheme 1



i: DCC, CH_2Cl_2, 0° -> r.t.; ii: NaH, CH_2I_2, DMF/THF (1:1), 0° -> r.t.; iii: (*S*)-Phe-cyclohexylamide (**6**), DIPEA, NMP, 55°, (R^1 = Me) ; iv: HBr (33%), AcOH, Ac_2O, 80°, - (**6** x HBr)

iodomethylated with CH_2I_2, NaH as base in DMF/THF (1:1), giving rise to the key precursor (*rac*)-**5a**−**d** in medium to good overall yields (40−75%).[38]

The racemic 4-(iodomethyl)-4-methyl-2-phenyloxazol-5(4*H*)-one **5a** was further treated in analogy to our previously reported method[10] with the chiral auxiliary (*S*)-Phe-cyclohexylamide **6** in *N*-methylpyrrolidone (NMP) and diisopropylethylamine (DIPEA) at 55 °C to form the easily separable diastereomeric oxazolines **7** and **8** in 46% and 48% isolated yield, respectively, after separation by flash chromatography (FC).[39] The absolute configurations of dipeptides **7** and **8** were unambiguously determined by a crystal structure of **8** (Figure 4).[51]

Treatment of **7** and **8** with 33% HBr in acetic acid and acetic anhydride, to assure anhydrous conditions, gave after recovery of **6**·HBr (85% yield, >99% optical purity) the two optically pure azlactones (*R*)- and (*S*)-**2** in 70−80% isolated yield after FC.

In summary these azlactones (*R*)- and (*S*)-**2**, which constitute fully orthogonally protected and activated analogues of (*R*)- and (*S*)-α-methylserine **1**, were obtained in four steps (including the separation of the diastereomers **7** and **8**) in an overall yield of roughly 30% (Scheme 1).

3. Hydrolytic Oxazoline Ring Openings

In order to test the selective hydrolytic cleavage of the oxazoline moiety,[40] we treated the diastereomeric oxazolines **7** and **8** separately with 2 N aqueous HCl in dioxane at room temperature giving cleanly the intermediate free amines (*R*/*S*)-**9** and (*S*/*S*)-**10**, after neutralization of the

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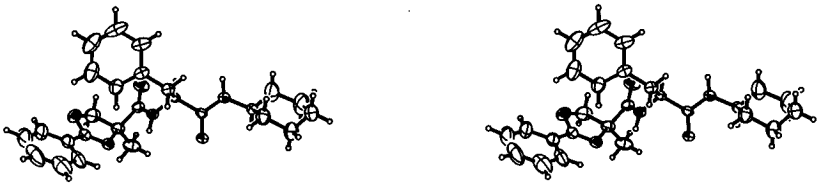


Figure 4. ORTEP diagram of **8**.

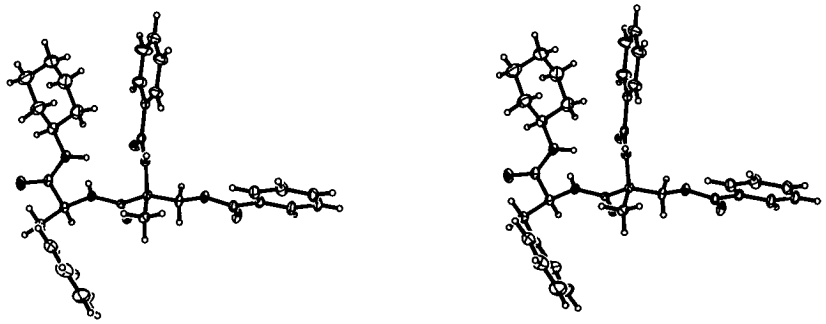
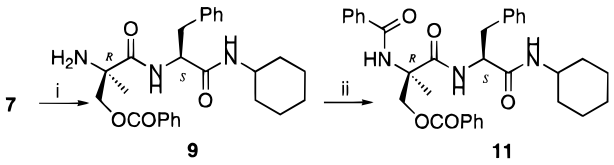


Figure 5. ORTEP diagram of **11**.

Scheme 2



acidic reaction mixture with aqueous $NaHCO_3$ and extraction with $CHCl_3$ or EtOAc (Scheme 2).

The amines (*R*/*S*)-**9** and (*S*/*S*)-**10** were immediately N-benzoylated with benzoyl chloride and DIPEA in CH_2Cl_2 at room temperature affording the N,O-dibenzoylated dipeptides (*R*/*S*)-**11** and (*S*/*S*)-**12** in high yield. The absolute configurations of **11** and **12** were reconfirmed by a crystal structure of **11** (Figure 5).[51]

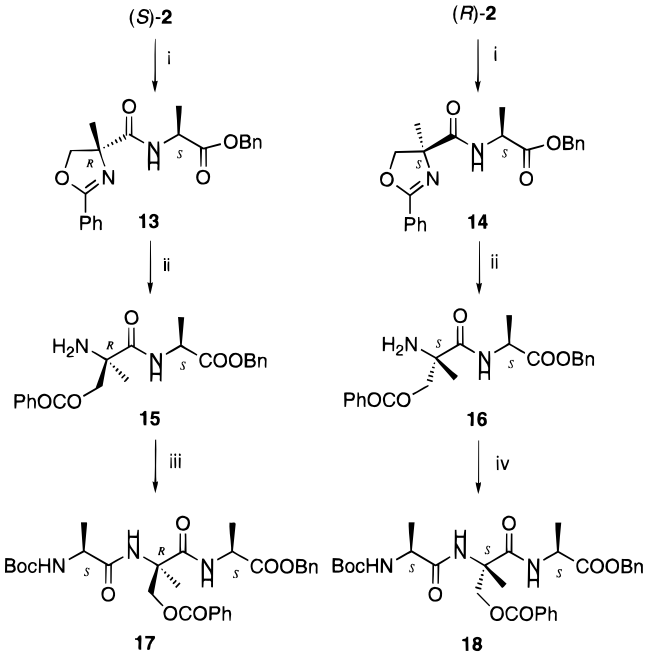
The interesting β-turn I conformation observed in the X-ray structure of **11** will be discussed in more detail in section 6. It is important to note that under the reaction conditions we did not observe an O → N-benzoyl-shift in the intermediate amines **9** and **10**, which is an important prerequisite for our strategy to incorporate the key azlactones (*R*)- and (*S*)-**2** into peptides without further protective group manipulations.

4. Incorporation of Azlactones (*R*)- and (*S*)-2 into Tripeptides

As outlined in Scheme 3 we have efficiently coupled the azlactones (*R*)- and (*S*)-**2** with (*S*)-alanine benzyl ester hydrochloride in the presence of DIPEA in NMP at 50 °C to yield the corresponding oxazoline derivatives (*R*/*S*)-**13** and (*S*/*S*)-**14** in 85% and 92% isolated yield, respectively.

After hydrolysis of **13** and **14** using 2 N aqueous HCl in dioxane at room temperature, neutralization of the reaction mixture with $NaHCO_3$ and extraction with $CHCl_3$ or EtOAc, the free amines (*R*/*S*)-**15** and (*S*/*S*)-**16** were immediately coupled to Boc-(*S*)-Ala-OH in DMF and DIPEA using *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TATU)[41] and 1-hydroxy-7-azabenzotriazole (HOAT) as coupling reagents to give the fully protected tripeptides **17** and **18** in 81% and 74% yield, respectively. The highly efficient coupling reagent TATU/HOAT developed recently by Carpino works especially well for the coupling of sterically hindered amines as noted also by Rich.[42]

Scheme 3



In peptides **17** and **18** the *O*-benzoyl group is stable towards the hydrogenation conditions (Pd black, EtOH, H_2) to liberate the free C-terminus or to the acidic conditions to remove the Boc-group (CF_3COOH, CH_2Cl_2) to liberate the N-terminus. In addition, treatment of **17** and **18** with NaCN in BnOH at 50 °C cleanly liberates

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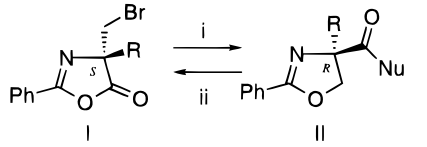
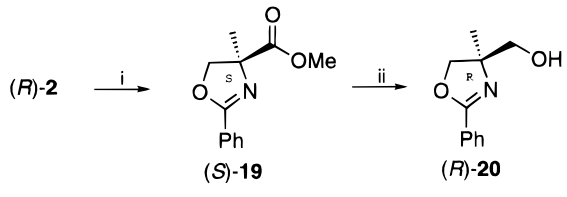
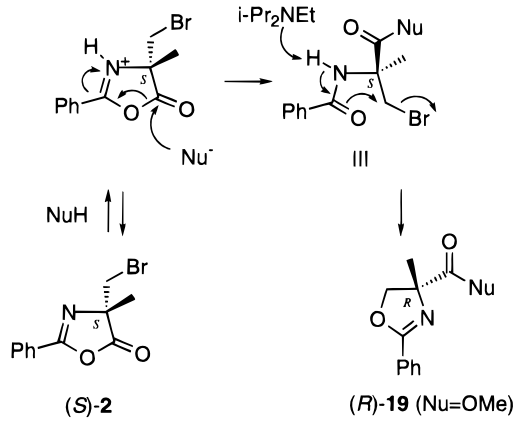


Figure 6. Azlactone/oxazoline interconversion.

Scheme 4



Scheme 5. Putative Mechanism for the Azlactone/ Oxazoline Interconversion



the free serine hydroxyl group by transesterification (compare reference[43]) without modification of the C- and N-terminal protective groups. These experiments demonstrate the orthogonality of the three protective groups in the peptides **17** and **18** and confirm our strategy to use building blocks (*R*)- and (*S*)-**2** as fully protected and activated α-methylserine analogues.

These building blocks were also converted successfully into the oxazoline methyl esters (*R*)- and (*S*)-**19** as shown in Scheme 4, which after reduction with $LiAlH_4$ in THF gave the corresponding oxazoline alcohols (*S*)- and (*R*)-**20** in high yields.

These alcohols constitute versatile optically pure trifunctional building blocks for synthesis of a range of (*R*)- and (*S*)-α-methylserine analogues.

5. Mechanistic Considerations

The synthesis of the key building blocks (*R*)- and (*S*)-**2** and their integration into the fully protected tripeptides **17** and **18** (Scheme 3) take advantage of a novel direct azlactone/oxazoline interconversion (**I** ↔ **II**, Figure 6).

In Scheme 5 we describe a putative mechanism for the azlactone/oxazoline interconversion (**I** → **II**, Figure 6) as shown for the conversion of (*S*)-**2** → (*R*)-**19**. In a first step we anticipate an attack of the nucleophile to the activated azlactone carboxyl group C(5) by protonation

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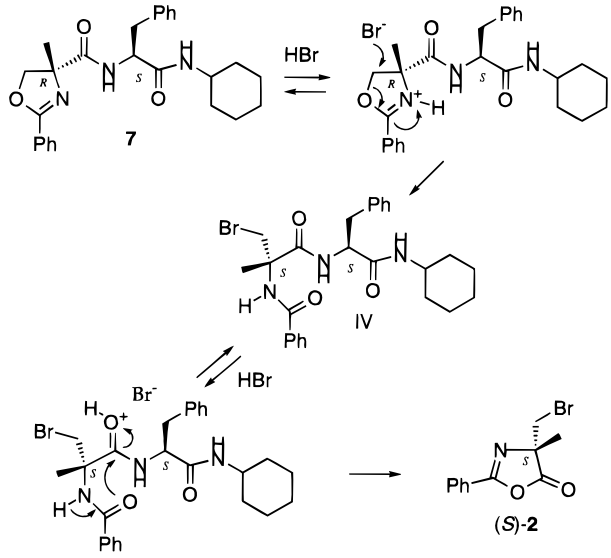
Scheme 6. Putative Mechanism for the Oxazoline/ Azlactone Interconversion



of N(3). After ring opening and formation of **III**, the *N*-benzoyl group displaces, in an intramolecular base-assisted S_N2-reaction, the side chain bromo group to form the stable oxazoline ring moiety, similar to the formation of oxazolines starting from β-halo serine amides.[44]

The oxazoline/azlactone interconversion (**II** → **I**, Figure 6) is depicted in Scheme 6 for the transformation of **7** into (*S*)-**2**.

Under the strongly acidic reaction conditions the oxazoline ring is protonated at N(3) and presumably opened by bromine attack at C(5) forming the linear dipeptide intermediate **IV** (in analogy to reference[45]). As previously shown,[6,10] dipeptides of type **IV** are selectively cleaved forming **6**·HBr, which can be recovered, and (*S*)-**2**. The driving force for this selective amide cleavage and for the preferred formation of the 4,4-disubstituted azlactone (*S*)-**2**, can be attributed to a strong "*gem*-dialkyl effect" as shown earlier.[46]

6. Discussion of the Crystal Structures

The peptide backbone conformations of the compounds **8** and **11** in the crystalline state (cf. stereo plots in Figures 4 and 5) exhibit some interesting aspects of peptide folding.

Whereas the oxazoline derivative **8**, due to the blocked N-terminal amide group, is not capable of forming a β-turn, the open chain derivative **11** exhibits a nearly perfect β-turn type I[47,48] conformation as outlined in Figure 7. The former extended structure **8** is found in a conformational space region populated by 1% of turn types in the aforementioned representative protein database extract. Furthermore both structures have their phenylalanine side chain in a (−) synclinal orientation, allowing the central amide group to be engaged in intermolecular hydrogen bonding as observed in the crystal packing of the structures. The torsionally unrestricted *O*-benzoyl hydroxymethyl group in **11** is oriented in a (+) synclinal conformation. This is a nice example

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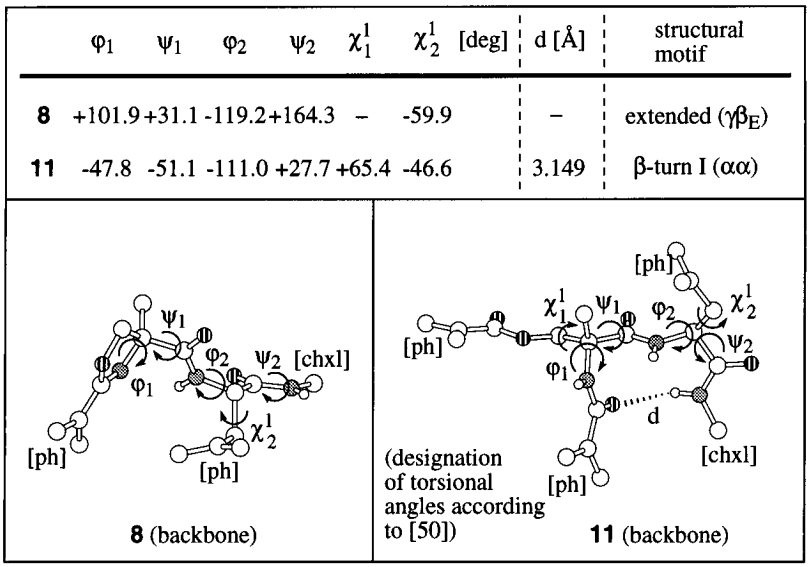

Figure 7.

of the *gauche* effect[49] showing a preferentially staggered orientation of the more electronegative carbon heteroatom bonds.

7. Summary and Outlook

Starting from commercial *rac*-*N*-benzoyl-Ala-OH the key (*R*)- and (*S*)-4-(bromomethyl)-4-methyl-2-phenyloxazol-5(4*H*)-ones **2** were efficiently prepared in optically pure form (Scheme 1).

Using a series of azlactone/oxazoline interconversions (Figure 6), these building blocks were incorporated without additional protective group manipulations into the tripeptides **17** and **18**. It is noteworthy, that after the hydrolytic cleavage of the oxazolines **7** and **8** (Scheme 2), **13** and **14** (Scheme 3) no *O* → *N*-benzoyl-shift was observed under the reaction conditions employed for the subsequent acylation of the intermediate amines **9**, **10**, **15**, and **16**. This prerequisite in combination with the novel direct azlactone/oxazoline interconversion forms the cornerstones of our strategy to synthesize and incorporate the fully protected and activated α-methylserine analogues (*R*)- and (*S*)-**2** into tripeptides **17** and **18** (Scheme 3).

As indicated in Scheme 2, our strategy should not be limited to the (*R*)- and (*S*)-α-methylserine **1**, since the α-iodomethylation of the 4-monosubstituted-2-phenyloxazol-5(4*H*)-ones **4b**−**d**[38] proceeded smoothly to give the racemic 4-iodomethylated azlactones **5b**−**d** in high yields. These azlactones **5b**−**d** correspond to the precursors of the "α-chimeras"[6] (α,α-disubstituted glycines combining two side chains of proteinogenic amino acids at the α-center) of Ser-Phe **5b**, Ser-Val **5c**, and Ser-Leu **5d**. Work to synthesize the corresponding optically pure azlactones (*R*)- and (*S*)-**2b**−**d** is in progress and will be reported in due course.

Experimental Section

General. See ref 6. Optical rotation measurements were made at 20 °C, and the values are expressed as specific rotation $[\alpha]_D$ in angular degrees. Melting points are given in °C.

***rac*-4-Methyl-2-phenyl-4*H*-oxazol-5-one (4a).** To a stirred suspension of *rac*-*N*-benzoyl-Ala-OH **3** (10.0 g, 51.7 mmol) in CH_2Cl_2 (150 mL) at 0 °C and under Ar was added DCC (11.1 g, 53.8 mmol) in small portions, so that the reaction temperature did not exceed 5 °C. The reaction mixture was stirred at 0 °C for 30 min and at rt for 40 min and filtered, and the solvent was evaporated. The residue was triturated in Et_2O (200 mL) and filtered. Evaporation of the solvent yielded 9.1 g (quantitative) of **4a** as a colorless oil that was used in the next step without further purification.

***rac*-4-(Iodomethyl)-4-methyl-2-phenyl-4*H*-oxazol-5-one (5a).** To a stirred solution of **4a** (9.1 g, 52 mmol) and CH_2I_2 (20.9 mL, 0.26 mol) in dry THF/DMF (1:1, 300 mL) at 0 °C and under Ar, was added NaH dispersion (60%, 2.48 g, 62 mmol) in small portions so that the reaction temperature did not exceed 15 °C. The reaction mixture was stirred at 0 °C for 30 min and poured onto ice, 5% aqueous NaH_2PO_4 (200 mL), and CH_2Cl_2 (200 mL). The organic layer was extracted with H_2O (200 mL) and saturated brine (200 mL) and dried (Na_2SO_4), and the solvents were evaporated. The residue was chromatographed on SiO_2 (500 g) with hexane/EtOAc (10:1−7:1) to yield 5.5 g (34%) of **5a** as white crystals. Mp 98−100 °C. IR (KBr): 3435w, 3064w, 3028w, 2969w 1814s, 1649s, 1577w, 1450m, 1317m, 1293m, 1229m, 1006s, 929m, 884m, 694s. ^{1}H-NMR ($CDCl_3$, 250 MHz): 8.1−8.0 (m, 2H); 7.65−7.45 (m, 3H); 3.57, 3.50 (2d, J = 10, 2H); 1.70 (s, 3H). MS (EI): 315 (3, (M)), 188 (35), 174 (12), 105 (100), 77 (44), 51 (16).

(*R*)-4-Methyl-2-phenyl-4,5-dihydrooxazole-4-carboxylic Acid (*S*)-[1-(cyclohexylcarbamoyl)-2-phenylethyl]-amide (7) and (*S*)-4-Methyl-2-phenyl-4,5-dihydrooxazole-4-carboxylic Acid (*S*)-[1-(cyclohexylcarbamoyl)-2-phenylethyl]amide (8). A mixture of azlactone **5a** (1.65 g, 5.24 mmol), **6** (1.99 g, 8.1 mmol) and DIPEA (1.85 mL, 10.48 mmol) in NMP (15 mL) was stirred for 18 h at 55 °C, cooled to rt, and poured onto H_2O (50 mL) and EtOAc (80 mL). The organic fraction was extracted with H_2O (2 × 30 mL) and dried ($MgSO_4$), and the solvent was evaporated. The residue was chromatographed on SiO_2 (300 g) with EtOAc/hexane (1:2−2:3) to give first, after crystallisation from Et_2O/hexane, 1.05 g (46%) of **7** as a white solid. Mp 137−138 °C. $[\alpha]_D$ = +47 (c = 0.2, $CHCl_3$). IR (KBr): 3376w, 3302m, 3062w, 3029w, 2930m, 2853w, 1645s, 1521s, 1495m, 1451m, 1352m, 1301w, 1063m, 696s. ^{1}H-NMR (250 MHz, $CDCl_3$): 8.0−7.9 (m, 2H); 7.6−7.35 (m, 4H); 7.35−7.2 (m, 5H); 5.38 (d (br), 1H); 4.61, 4.23 (2d, J = 8.5, 2H); 4.55−4.45 (m, 1H); 3.7−3.55 (m, 1H); 3.2−3.0 (m, 2H); 1.75−1.4 (m, 5H); 1.49 (s, 3H); 1.35−0.75 (m, 5H). MS (EI): 433 ((M),1), 307 (16), 273 (8), 160 (100), 132 (32), 105 (35), 104 (44), 91 (18), 77 (32), 55(28), 41 (28).

Further elution yielded, after recrystallisation from Et_2O/hexane, 1.10 g (48.%) of **8** as a white solid. Mp 121.5−123.0

(49) Wolfe, S. *Acc. Chem. Res.* **1972**, *5*, 102.

(50) IUPAC-IUB Commission on Biochemical Nomenclature. *Biochemistry* **1970**, *9*, 3471.

(51) The author has deposited atomic coordinates for this structure with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

°C. $[\alpha]_D = -55$ ($c = 0.2$, $CHCl_3$). IR (KBr): 3381w, 3292m, 3060w, 2932m, 2855w, 1676s, 1644s, 1533s, 1450w, 1353w, 1301w, 1062m, 697s. ^{1}H-NMR (250 MHz, $CDCl_3$): 8.0−7.9 (m, 2H); 7.6−7.45 (m, 3H); 7.25 (d (br), 1H); 7.09 (s, 5H); 5.69 (d (br), 1H); 4.6−4.45 (m, 1H); 4.48, 4.19 (2d, $J = 8.5$, 2H); 3.8−3.65 (m, 1H); 3.15-2.95 (m, 2H); 1.95−1.5 (m, 5H); 1.55 (s, 3H); 1.45−0.9 (m, 5H). MS (EI): 433 ((M),1), 307 (17), 273 (7), 160 (100), 132 (27), 105 (34), 104 (41), 91 (16), 77 (39), 55(26), 41 (23).

(*S*)-4-(Bromomethyl)-4-methyl-2-phenyl-4*H*-oxazol-5-one [(*S*)-2]. A solution of **7** (400 mg, 0.92 mmol) in HBr/AcOH (33%, 2 mL) and Ac_2O (0.8 mL) was stirred in a sealed tube at 70 °C for 4.5 h. The solvents were removed under high vacuum, and the residue was chromatographed on SiO_2 (15 g) with hexane/EtOAc (3:1) to yield 175 mg (71%) of (*S*)-**2** as colorless oil. $[\alpha]_D = -16$ ($CHCl_3$, $c = 0.1$). IR (film) 3065w, 3035w, 2984w, 2934w, 1824s, 1655s, 1580w, 1493w, 1451m, 1321m, 1293s, 1269w, 1244w, 1131w, 1094m, 1007s, 934m, 887m, 781m, 696s. ^{1}H-NMR ($CDCl_3$, 250 MHz): 8.1−8.0 (m, 2H); 7.65−7.45 (m, 3H); 3.75, 3.69 (2d, $J = 10$, 2H); 1.66 (s, 3H). MS (EI): 269, 267 (8, (M)), 174 (25), 144 (10), 105 (100), 104 (30), 77 (35), 51 (10).

(*R*)-4-(Bromomethyl)-4-methyl-2-phenyl-4*H*-oxazol-5-one [(*R*)-2]. To a stirred mixture of **8** (500 mg, 1.15 mmol) in 33% HBr/AcOH (2 mL) in a pyrolysis tube at 0 °C under Ar was added acetic anhydride (0.8 mL). The reaction mixture was stirred for 5 h at 80 °C and cooled to rt, and the solvents were removed under reduced pressure. The residue was dried over K_2CO_3 in a desiccator and chromatographed on SiO_2 (40 g) with EtOAc/hexane (1:4) to yield, after drying under reduced pressure, 231 mg (75%) of (*R*)-**2** as a colorless oil. $[\alpha]_D = +17$ ($c = 0.15$, $CHCl_3$). IR, MS, and NMR spectra in close agreement to (*S*)-**2**.

(*R*)-2-(Benzoylamino)-2-[[(*S*)-1-(cyclohexylcarbamoyl)-2-phenylethyl]carbamoyl]propyl Benzoate (11). To a stirred solution of **7** (910 mg, 2.10 mmol) in dioxane (3 mL) at 0 °C was added 2 N aqueous HCl (3 mL). The reaction mixture was stirred for 2h at rt and poured onto ice, saturated aqueous $NaHCO_3$ (15 mL), and $CHCl_3$ (15 mL). The organic layer was dried ($MgSO_4$) and the solvent evaporated. After drying under high vacuum overnight, the residue was dissolved in NMP (4 mL), and DIPEA (0.72 mL, 4.2 mmol) and benzoyl chloride (0.37 mL, 3.15 mmol) were added at 0 °C. The reaction mixture was stirred at rt for 4 h and poured onto ice, 2 N aqueous HCl (10 mL), and EtOAc (20 mL). The aqueous layer was extracted with EtOAc (2 × 10 mL), the combined organic fractions were dried ($MgSO_4$), and the solvents were evaporated. The residue was recrystallized from EtOAc/hexane to yield 1.06 g (91%) of **11** as a white solid. Mp 202.5−204.0 °C. $[\alpha]_D = +10$ ($c = 0.2$, $CHCl_3$). IR (KBr): 3359m, 3324m, 3065w, 2935w, 2854w, 1704s, 1659s, 1641s, 1581w, 1539m, 1366w, 1280s, 1258m, 1119w, 722m, 695w. ^{1}H-NMR (250 MHz, $CDCl_3$): 8.0−7.9 (m, 2H); 7.75−7.65 (m, 2H); 7.6−7.5 (m, 2H); 7.5−7.35 (m, 4H); 7.25−7.1 (m, 5H); 7.10 (s (br), 1H); 6.64 (d (br), $J = 8$, 1H); 6.25 (d (br), $J = 8$, 1H); 4.91, 4.67 (2d, $J = 12$, 2H); 4.65−4.55 (m, 1H); 3.8−3.6 (m, 1H); 3.3−3.0 (m, 2H); 1.85−1.5 (m, 5H); 1.62 (s, 3H); 1.4−1.0 (m, 5H). MS (ISP): 578.6 ($(M + Na)^+$, 50), 556.6 ($(M + H)^+$, 100).

(*S*)-2-(Benzoylamino)-2-[(*S*)-1-(cyclohexylcarbamoyl)-2-phenylethyl]carbamoyl]propyl Benzoate (12). A mixture of oxazoline **8** (910 mg, 2.10 mmol) in dioxane (3 mL) was treated according to oxazoline **7** to yield, after recrystallization from EtOAc/hexane, 1.05 g (90%) of **12** as a white solid. Mp 166−167 °C. $[\alpha]_D = +15.0$ ($c = 0.2$, $CHCl_3$). IR (KBr): 3418w (br), 3328w (br), 3063w, 2932w, 2854w, 1726m, 1645s, 1601w, 1534m (br), 1451m, 1315w, 1272s, 1112w, 713m, 695w. ^{1}H-NMR (250 MHz, $CDCl_3$): 8.2−8.1 (m, 2H); 7.93 (s (br), 1H); 7.75−7.45 (m, 8H); 6.9−6.7 (m, 5H); 6.32 (d (br), 1H); 4.74, 4.49 (2d, $J = 12$, 2H); 4.75−4.6 (m, 1H); 3.85−3.65 (m, 1H); 3.4−2.65 (m, 2H); 1.95−1.55 (m, 5H); 1.71 (s, 3H); 1.45−1.05 (m, 5H). MS (ISP): 578.65 ($(M + Na)^+$, 40), 556.5 ($(M + H)^+$, 100).

Benzyl (*S*)-2-[(*R*)-4-Methyl-2-phenyl-4,5-dihydrooxazol-4-yl]carbonyl]amino]propionate (13). To a stirred solution of (*S*)-**2** (175 mg, 0.65 mmol) and H-(*S*)-Ala-OBn·HCl (280 mg, 1.30 mmol) in *N*,*N*-dimethylacetamide (DMA) (2.5 mL) at 0 °C under Ar was added DIPEA (0.56 mL, 3.25 mmol). The reaction mixture was stirred at 50 °C overnight and poured onto ice, H_2O (30 mL), and EtOAc (30 mL). The organic layer was extracted with saturated brine (30 mL) and dried ($MgSO_4$), and the solvent was evaporated. The residue was chromatographed on SiO_2 (15 g) with hexane/EtOAc (2:1) to yield 203 mg (85%) of **13** as a colorless oil. $[\alpha]_D = +65$ ($CHCl_3$, $c = 0.08$). IR (KBr): 3385w, 2979w, 2930w, 1742s, 1677s, 1644m, 1512m, 1451m, 1354w, 1298w, 1258w, 1199m, 1171m, 1139w, 1064m, 1026w, 967w, 750w, 697s. ^{1}H-NMR ($CDCl_3$, 250 MHz): 8.0−7.9 (m, 2H); 7.55−7.35 (m, 3H); 7.3−7.2 (m, 6H); 5.17, 5.09 (2d, $J = 12$, 2H); 4.61, 4.25 (2d, $J = 9$, 2H); 4.7−4.55 (m, 1H); 1.58 (s, CH_3); 1.47 (d, $J = 7$, 3H). MS (ISP): 367 (100, $[M + H]^+$).

Benzyl (*S*)-2-[(*S*)-4-Methyl-2-phenyl-4,5-dihydrooxazol-4-yl]carbonyl]amino]propionate (14). To a stirred solution of (*R*)-**2** (220 mg, 0.82 mmol) and H-(*S*)-Ala-OBn·HCl (354 mg, 1.64 mmol) in DMA (3 mL) was added under Ar at 0 °C DIPEA (0.70 mL, 4.1 mmol). The reaction mixture was stirred for 17 h at 50 °C, cooled to rt, and poured onto ice, H_2O (40 mL), and EtOAc (40 mL). The organic layer was extracted with saturated brine (40 mL) and dried ($MgSO_4$), and the solvents were evaporated. The residue was chromatographed on SiO_2 (25g) with EtOAc/hexane (1:2) to yield 275 mg (92%) of **14** as a white foam. $[\alpha]_D = -26.0$ ($c = 0.2$, $CHCl_3$). IR (KBr): 3380w , 3060w, 3025w, 2975w, 1742s, 1676s, 1644m, 1513m, 1353w, 1298w, 1200w, 1171w, 1065m, 697s. ^{1}H-NMR (250 MHz, $CDCl_3$): 8.05−7.95 (m, 2H); 7.6−7.3 (m, 8H); 7.23 (br d, $J = 7.5$, 1H); 5.23, 5.16 (2d, $J = 12.0$, 2H); 4.65, 4.25 (2d, $J = 8.0$, 2H); 4.7−4.55 (m, 1H); 1.56 (s, 3H); 1.41 (d, $J = 7.5$, 3H). MS (ISP): 367.4 ($(M + H)^+$, 100).

(*R*)-2-[[(*S*)-1-(Benzyloxycarbonyl)ethyl]carbamoyl]-2-[[(*S*)-2-(*tert*-butoxycarbonylamino)propionyl]amino]-propyl Benzoate (17). To a stirred solution of **13** (200 mg, 0.55 mmol) in dioxane (3 mL) at 5 °C under Ar was added 0.5 N aqueous HCl (2.2 mL). The reaction mixture was stirred at rt for 2 h and poured onto ice, saturated aqueous $NaHCO_3$ (40 mL), and CH_2Cl_2 (40 mL). The organic layer was dried ($MgSO_4$), and the solvent was evaporated to yield crude **15**.

To a stirred solution of Boc-(*S*)-Ala-OH (155 mg, 0.82 mmol), TATU (263 mg, 0.82 mmol), and HOAT (149 mg, 1.09 mmol) in DMF (5 mL) at 0 °C under Ar were added DIPEA (0.19 mL, 1.10 mmol) and crude **15**. The reaction mixture was stirred at rt for 1 h and poured onto H_2O (40 mL) and EtOAc (40 mL). The organic layer was extracted with saturated aqueous $NaHCO_3$ (40 mL) and 1 N aqueous HCl (40 mL) and dried ($MgSO_4$), and the solvents were evaporated. The residue was chromatographed on SiO_2 (15 g) with hexane/EtOAc (1:1) to give 246 mg (81%) of **17** as a white powder.

Mp 167.5−168.5 °C. $[\alpha]_D = -2$ (MeOH, $c = 0.1$). IR (KBr): 3377w, 3300m, 3068w, 2982w, 2938w, 1741m, 1726s, 1683s, 1654s, 1532m, 1454w, 1389w, 1365w, 1271s, 1174m, 1109m, 1071w, 1024w, 715w. ^{1}H-NMR ($CDCl_3$, 250 MHz): 8.1−8.0 (m, 2H); 7.6−7.35 (m, 3H); 7.35−7.25 (m, 5H); 7.16 (m, 2H); 5.18, 5.11 (2d, $J = 12$, 2H); 4.95 (s (br), 1H); 4.77, 4.65 (2d, $J = 12$, 2H); 4.7−4.5 (m, 1H); 4.15−3.95 (m, 1H); 1.68 (s, 3H); 1.39 (s, 9H); 1.39 (d, $J = 7$, 3H); 1.34 (d, $J = 7$, 3H). MS (ISP): 578 (100, $(M+Na)^+$), 556 (92, $(M + H)^+$), 500 (25), 328 (20), 327 (22). Anal. Calcd for $C_{29}H_{37}N_3O_8$ (555.63): C 62.69, H 6.71, N 7.56. Found: C 62.38, H 6.82, N 7.39.

(*S*)-2-[[(*S*)-1-(Benzyloxycarbonyl)ethyl]carbamoyl]-2-[[(*S*)-2-(*tert*-butoxycarbonylamino)propionyl]amino]-propyl Benzoate (18). To a stirred solution of **14** (250 mg, 0.68 mmol) in dioxane (3 mL) at 0 °C was added 0.5 N aqueous HCl (1 mL). The reaction mixture was stirred for 3 h at rt and poured onto ice, saturated aqueous $NaHCO_3$, and CH_2Cl_2. The organic layer was dried ($MgSO_4$), the solvents were evaporated, and the residue was dried under reduced pressure to give crude **16**, which was not further purified. The residue was dissolved in CH_2Cl_2 (1 mL), followed by addition of a prestirred mixture of Boc-(*S*)-Ala-OH (257 mg, 1.36 mmol), 1-hydroxybenzotriazole (HOBT) (230 mg, 1.70 mmol), *N*-[3-(dimethylamino)propyl]-*N*-ethylcarbodiimide hydrochloride (EDCI) (274 mg, 1.43 mmol) and DIPEA (0.23 mL) in DMF (5 mL) at 0 °C. The reaction mixture was stirred for 18 h at rt and poured onto H_2O and EtOAc, the organic layer was

extracted with H_2O, saturated aqueous $NaHCO_3$ solution, and 1 N aqueous HCl and dried ($MgSO_4$), and the solvents were evaporated. The residue was crystallized from EtOAc/hexane to yield, after drying under reduced pressure, 280 mg (74%) of **18** as a white amorphous solid.

Mp 169.5−171 °C. $[\alpha]_D = -69$ ($c = 0.15$, EtOH). IR (KBr): 3390w , 3301m, 3060w, 2875w, 1731s, 1681s, 1651s, 1532 m, 1454m, 1365w, 1272s, 1164s, 1110w, 713w. ^{1}H-NMR (250 MHz, DMSO-d_6): 8.19 (s (br), 1H); 8.05−7.95 (m, 2H); 7.81 (d (br), $J = 7$, 1H); 7.7−7.45 (m, 3H); 7.40−7.25 (m, 5H); 7.05 (br d, $J = 6$, 1H); 5.08 (s, 2H); 4.84, 4.51 (2d, $J = 10$, 2H); 4.45−4.3 (m, 1H); 3.95−3.8 (m, 1H); 1.43 (s, 3H); 1.34 (s, 9H); 1.28 (d, $J = 7.5$, 3H); 1.05 (d, $J = 7.0$, 3H). MS (FAB): 578.2 ((M + Na)$^+$, 10), 556.2 ((M + H)$^+$,70), 217 (80), 149.1 (25), 109.1 (30), 91.2 (100).

Methyl (*S*)-4-Methyl-2-phenyl-4,5-dihydrooxazole-4-carboxylate [(*S*)-19]. A solution of **8** (5.0 g, 11.5 mmol) in HBr/AcOH (33%, 25 mL) and Ac_2O (10 mL) was stirred in a sealed tube at 70 °C for 4.5 h. The solvents were removed under high vacuum, the residue was dissolved in MeOH (10 mL), DIPEA (10 mL) was added, and the reaction mixture was stirred at 50 °C overnight and poured onto ice, H_2O (100 mL), and EtOAc (100 mL). The organic layer was dried (Na_2SO_4), and the solvents were evaporated. The residue was chromatographed on SiO_2 (100 g) with hexane/EtOAc (9:1−4:1) to give 1.61 g (63%) of (*S*)-**19** as white crystals.

Mp 73−74 °C. $[\alpha]_D = +60$ ($CHCl_3$, $c = 0.1$). IR (KBr): 3433s (br), 2991w, 2952m, 2899w, 1743s, 1635s, 1601w, 1578w, 1407m, 1449m, 1379m, 1363s, 1322m, 1304s, 1283s, 1215s, 1185m, 1155s, 1132m, 1083m, 1065s, 1020w, 1000w, 982w, 965s, 935w, 881w, 832w, 791m, 769w, 726m, 708s, 702s, 686w, 572w. ^{1}H-NMR ($CDCl_3$, 250 MHz): 8.05−7.95 (m, 2H); 7.55−7.35 (m, 3H); 4.83, 4.19 (2d, $J = 9$, 2H); 3.80 (s, 3H); 1.63 (s, 3H). MS (EI): 220 (0.5, (M + H)$^+$), 161 (12), 160 (100, [M − $COOCH_3$]), 132 (20), 105 (17), 104 (24), 77 (13).

Methyl (*R*)-4-Methyl-2-phenyl-4,5-dihydrooxazole-4-carboxylate [(*R*)-19]. A solution of **7** (100 mg, 0.23 mmol) in HBr/AcOH (33%, 0.5 mL) and Ac_2O (0.2 mL) was treated according to (*S*)-**19** to yield 32 mg (63%) of (*R*)-**19** as white crystals. Mp 72.5−74 °C. $[\alpha]_D = -58$ ($CHCl_3$, $c = 0.1$). IR, MS, and NMR spectra are in close agreement to (*S*)-**19**.

(*R*)-(4-Methyl-2-phenyl-4,5-dihydrooxazol-4-yl)methanol [(*R*)-20]. To a stirred solution of (*S*)-**19** (720 mg, 3.30 mmol) in THF (12 mL) at 0 °C under Ar was added $LiAlH_4$ (3.30 mL of a 1 M solution in THF, 3.30 mmol). The reaction mixture was stirred at rt for 30 min and cooled to 0 °C. 2 N NaOH (5 mL), saturated aqueous $NaHCO_3$ (20 mL), and Et_2O (100 mL) were added, and the mixture was stirred for 10 min at rt. The organic layer was decanted, the aqueous layer was extracted with Et_2O (3 × 30 mL), the combined organic phases were dried (Na_2SO_4), and the solvents were evaporated. The residue was chromatographed on SiO_2 (50 g) with hexane/EtOAc (4:1−1:2) to give 590 mg (93%) of (*R*)-**20** as white crystals.

Mp 120.5−122 °C. $[\alpha]_D = +17$ ($CHCl_3$, $c = 0.1$). IR (KBr): 3173s (br), 2973m, 2894m, 2862m, 1641s, 1602w, 1578m, 1487m, 1451m, 1424w, 1379w, 1355s, 1326s, 1304m, 1263m, 1204m, 1149w, 1075, 1061s, 1038m, 973s, 929w, 870w, 844w, 794m, 742w, 697s, 638w. ^{1}H-NMR ($CDCl_3$, 250 MHz): 7.95−7.85 (m, 2H); 7.5−7.3 (m, 3H); 4.47, 4.09 (2d, $J = 8$, 2H); 3.85−3.75 (m, 1H); 3.55−3.45 (m, 1H); 2.6−2.5 (m, 1H); 1.33 (s, 3H). MS (EI): 161 (12), 160 (100, [M − CH_2OH]), 132 (24), 105 (17), 104 (50), 77 (19).

(*S*)-(4-Methyl-2-phenyl-4,5-dihydrooxazol-4-yl)methanol [(*S*)-20]. A solution of (*R*)-**19** (120 mg, 0.55 mmol) in THF (2 mL) was treated according to (*R*)-**20** to yield 89 mg (85%) of (*S*)-**20** as white crystals. Mp 119−120.5 °C. $[\alpha]_D = -18$ ($CHCl_3$, $c = 0.1$). IR, MS, and NMR spectra are in close agreement to (*R*)-**20**.

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Supporting Information Available: Copies of ^{1}H-NMR spectra for compounds (*S*)-**2**, (*R*)-**2**, **5**, **7**, **8**, **11**−**14**, **17**, **18**, (*S*)-**19**, (*R*)-**19**, (*S*)-**20**, and (*R*)-**20** (15 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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