New Efficient Strategy for the Incorporation of (S)-Isoserine into Peptides[1,2]

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A new efficient synthesis of (*S*)-isoserine derivatives from (*S*)-malic acid using hexafluoroacetone as protecting and activating reagent is described. Via this route (*S*)-isoserine is obtained as mono- and diactivated species suitable for the incorporation of isoserine into the N- and C-terminal positions of peptides.

Introduction

The 1-amino-2-hydroxyethylene substructure in combination with a carboxyl group is present in a large number of naturally occurring biologically active compounds, which contain amino acids such as isoserine,[3] phenylisoserine,[4] GABOB (4-amino-3-hydroxybutanoic acid),[5] carnitine,[6] and statine [(3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid].[7] Several syntheses of the enantiopure (*S*)- and (*R*)-isoserines have been reported,[3,8] as well as the chemical[9] and enzymatic[10] resolution of the racemate.[3,11]

(*S*)-Isoserine is a constituent of antibiotics such as edeine[12] and tatumine[13] which are produced by the *Bacillus brevis* V_m[4] strains. The biological activity of other antibiotics, such as butirosin[14] and gentamycin,[15] has been enhanced by replacing naturally occurring amino acids by isoserine. Therefore, isoserine represents an interesting nonproteinogenic amino acid, which is excellently suited for peptide modification. Isosteres of natural di- and tripeptides are valuable building blocks for the design of proteolytically stable peptides[16] and mechanism-based protease inhibitors.[17] Furthermore, isoserine represents an interesting chiral building block.[18]

In this paper we describe a stereoconservative synthesis of (*S*)-isoserine derivatives[19] from (*S*)-malic acid using hexafluoroacetone as a protecting and activating agent.[20] The new strategy provides a general and preparatively simple access to isoserine-containing peptides.

Results and Discussion

(*S*)-Malic acid (**1**) is transformed into 2,2-bis(trifluoromethyl)-4-oxo-1,3-dioxolan-5-ylacetic acid (**2**) upon reaction with hexafluoroacetone in dimethyl sulfoxide.[21] The hydroxyl and the adjacent carboxyl group are protected simultaneously. Furthermore, the carboxyl group incorporated into the five-membered heterocycle is now highly activated toward nucleophiles. Under appropriate reaction conditions, the remaining carboxyl group of malic acid can be functionalized regioselectively.[1,2,22] Treatment of **2** with thionyl chloride yields the acid chloride **3** which on heating with trimethylsilyl azide in toluene gives the isocyanate **4**. When trimethylsilyl azide is added in excess, a [3 + 2] cycloadduct (**5**) is formed.

Compound **4** is a colorless, distillable liquid representing a double-activated isoserine derivative, in which the isocyanate function is the more reactive center. For example, the addition of equimolar amounts of alcohols results in the formation of urethanes in high yields. Consequently, addition of benzyl alcohol, 9-fluorenylmethanol, and *tert*-butyl alcohol provides a highly efficient access to the Z-, Fmoc-, and Boc-protected, carboxyl group-activated derivatives of (*S*)-isoserine (**4** → **6**), respectively.

Hexafluoroacetone as a protective group for α-amino, α-hydroxy, and α-mercapto acids is superior to other carbonyl compounds, e.g., formaldehyde,[8a] because of the very mild reaction conditions to be applied for protection and deprotection. Deprotection of the hydroxyl and the carboxyl group of **6** can be accomplished in one step on stirring with H_2O/2-propanol at room temperature to give the N-protected isoserine derivatives **7**. All reaction steps can be monitored by ^{19}F NMR spectroscopy. Upon

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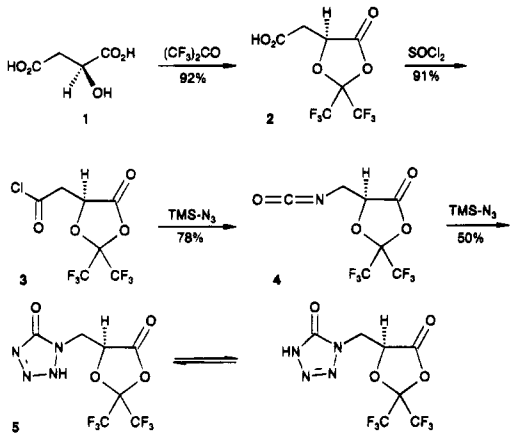
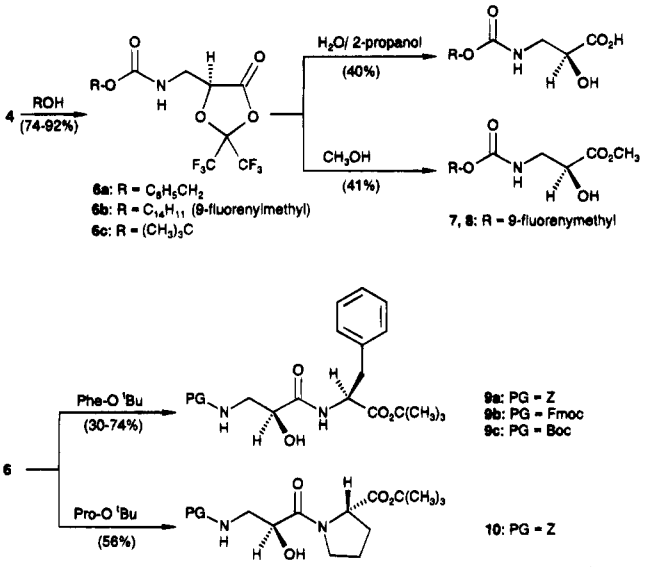
Scheme 1



Scheme 2



reaction of **6** with alcohols, the N-protected isoserine esters **8** become readily available.

Compounds **6a–c** represent a new class of activated isoserine derivatives which is excellently suited for the N-terminal incorporation of isoserine into peptides (**6** → **9**; **6** → **10**). The peptide bond-forming process is coupled with the deprotection of the α-hydroxyl function, which can be functionalized subsequently.[22]

Carboxylic acids add to the isocyanates to give the mixed anhydrides, which eliminate carbon dioxide upon heating to give the corresponding amides.[23,24] This reaction sequence applied to **4** represents a method for selective N-acylation of isoserine (**4** → **11**). The addition of N-protected amino acids to isocyanate **4** provides a new method for the C-terminal introduction of isoserine into peptides (**4** → **12**).

Since the dioxolan-4-ones **12** are carboxyl-activated species, they can be directly used for selective functionalization of the carboxyl group. On reaction with alcohols, compounds **12** are readily transformed into N-protected dipeptide esters of type PG-Xaa-(H)-Ise-OR.[22]

Nucleophilic cleavage of the lactone ring of compounds **12** with amino acid esters offers a preparatively simple access to N-protected tripeptide derivatives (**12** → **13**; **12** → **14**) having the isoserine unit, which concomitant deprotection of the hydroxyl group for further transformations, e.g., with DAST.[22]

All steps of the described synthetic sequences proceed in a stereoconservative manner (^{1}H NMR analysis).[19] Further applications of the new method for the introduction of (*S*)- as well as (*R*)-isoserine into the N- and C-terminal positions of peptides and cyclic peptides with the aim of producing proteolytically stable biologically active peptide mimetics and to synthesize mechanism-based protease inhibitors will be described elsewhere.

Experimental Section

General. Melting points were determined on a Totolli apparatus and are uncorrected. Optical rotations were measured at 589 nm (Na D line). ^{1}H NMR spectra were recorded at 360 MHz. Splitting multiplicities are given as singlet (s), doublet (d), triplet (t), quartet (q), septet (sept), and multiplet (m). The chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS) in $CDCl_3$, acetone-d_6, or DMSO-d_6; J values are given in hertz (Hz). ^{13}C NMR spectroscopy was performed at 90 MHz. ^{19}F NMR spectra were recorded at 84, 235, or 340 MHz with trifluoroacetic acid (TFA) as external standard. For flash chromatography, silica gel 60 (30–60 μm) was used with the solvent system given in the text. Organic solvents were dried and distilled prior to use.

Hexafluoroacetone as Protecting Group for α-Functional Carboxylic Acids: (5*S*)-2,2-Bis(trifluoromethyl)-4-oxo-1,3-dioxolan-5-ylacetic Acid (2). (*S*)-Malic acid (**1**) (13.40 g, 100.0 mmol) was reacted with 34.9 g (210.0 mmol) of hexafluoroacetone in 30 mL of DMSO. After completion of the reaction, the solution was poured into a mixture of 600 mL of water/dichloromethane. The organic layer was separated, and the aqueous phase was extracted with dichloromethane (3 × 150 mL). The combined organic layer was washed with water (2 × 100 mL) and dried ($MgSO_4$). The solvent was removed in vacuo, and the crude product was recrystallized from chloroform/hexanes: yield 92%; mp 75 °C; $[\alpha]_D$ −12.7° (*c* 1.2, acetone); IR (KBr) 3300–2800, 1850, 1720 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.98 (dd, J = 7, 18 Hz, 1H), 3.12 (dd, J = 4, 18 Hz, 1H), 5.05 (dd, J = 4, 7 Hz, 1H), 11.43 (s, br, 1H); ^{13}C NMR ($CDCl_3$) δ 35.9, 71.5, 98.0 (sept, J = 36 Hz), 118.8 (q, J = 287 Hz), 119.8 (q, J = 288 Hz), 166.8, 174.1; ^{19}F NMR ($CDCl_3$) δ −2.11 (q, J = 7 Hz, 3F), −1.79 (q, J = 7 Hz, 3F).

(5*S*)-2,2-Bis(trifluoromethyl)-4-oxo-1,3-dioxolan-5-ylacetyl Chloride (3). **2** (28.20 g, 100.0 mmol) and 60 mL of thionyl chloride were kept under reflux for 6 h. After removal of the excess of thionyl chloride, the residue was distilled: yield 91%; bp 71 °C (15 Torr); $[\alpha]_D$ −15.6° (*c* 1.0, $CHCl_3$); IR (film) 1850, 1790 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.46 (dd, J = 8, 19 Hz, 1H), 3.63 (dd, J = 3, 19 Hz, 1H), 5.08 (dd, J = 3, 8 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 47.2, 70.7, 97.4 (sept, J = 36 Hz), 118.6 (q, J = 287 Hz), 119.4 (q, J = 288 Hz), 165.8, 169.0; ^{19}F NMR ($CDCl_3$) δ −2.36 (m, 6F).

[(5*S*)-2,2-Bis(trifluoromethyl)-4-oxo-1,3-dioxolan-5-yl]-methyl Isocyanate (4). A solution of compound **3** (3.01 g, 10.0 mmol) in 25 mL of toluene was added dropwise to trimethylsilyl azide (1.27 g, 11.0 mmol) in 25 mL of the same solvent and stirred at 80 °C for several hours until N_2 evolution ceased. After removal of the solvent, the residue was distilled: yield 78%; bp 50 °C (0.05 Torr); $[\alpha]_D$ −29.2° (*c* 1.3, $CHCl_3$); IR (film) 2290, 1845 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.83 (dd, J = 4, 14.5 Hz, 1H), 3.97 (dd, J = 3.5, 14.5 Hz, 1H), 4.76 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 42.9, 74.5, 97.9 (sept, J = 36 Hz), 118.8 (q, J = 285 Hz), 119.8 (q, J = 288 Hz), 125.1, 165.3; ^{19}F NMR ($CDCl_3$) δ −3.35 (q, J = 8 Hz, 3F), −2.68 (q, J = 8 Hz, 3F).

1-[[(5*S*)-2,2-Bis(trifluoromethyl)-4-oxo-1,3-dioxolan-5-yl]methyl]-2*H*-tetrazol-5-one (5). A solution of compound **3** (1.50 g, 5.0 mmol) in 25 mL of ethyl acetate was added dropwise to trimethylsilyl azide (1.27 g, 11.0 mmol) in the same solvent and stirred at 80 °C for several hours until N_2 evolution

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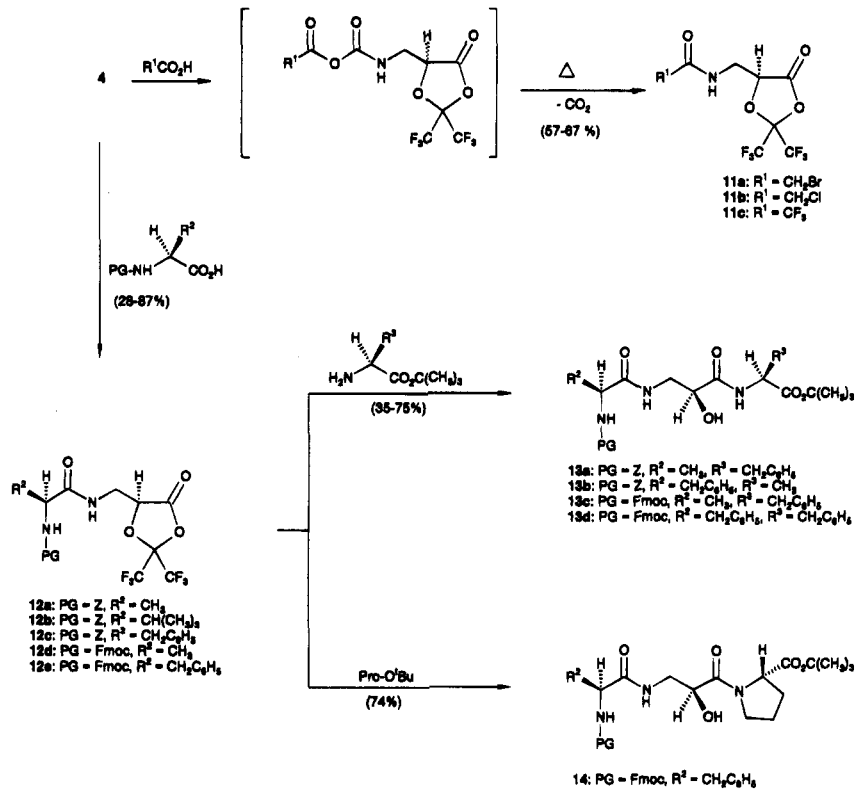
Scheme 3



ceased. After removal of the solvent, the residue was distilled: yield 50%; mp 141 °C; bp 130 °C (0.4 Torr); IR (KBr) 1855, 1735, 1710 cm^{-1}; ^1H NMR (acetone-d_6) δ 4.55 (dd, J = 4, 16 Hz, 1H), 4.65 (dd, J = 4, 16 Hz, 1H), 5.57 (m, 1H), 13.25 (s, br, 1H); ^{13}C NMR (acetone-d_6) δ 43.5, 73.8, 97.9 (sept, J = 36 Hz), 119.4 (q, J = 287 Hz), 120.4 (q, J = 289 Hz), 152.3, 165.7; ^{19}F NMR (acetone-d_6) δ = −3.02 (m, 6F).

Reaction of 4 with Alcohols. General Procedure: A solution of equimolar amounts of **4** and the corresponding alcohol (10 mmol) in 20 mL of chloroform was stirred at 70 °C for 40 h. Removal of the solvent and the unreacted starting materials in vacuo afforded a white solid, which was purified by recrystallization from chloroform/hexanes.

5-[(5*S*)-[(Benzyloxycarbonyl)amino]methyl]-2,2-bis(trifluoromethyl)-1,3-dioxolan-4-one (6a): yield 92%; mp 61 °C; $[\alpha]_D$ −10.8° (*c* 1.5, $CHCl_3$); IR (KBr) 3305, 1855, 1730, 1705, 1555 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.61 (m, 1H), 3.78 (m, 1H), 4.74 (m,), 5.10 (s, br, 2H), 5.34 (t, br, 1H), 7.33 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 41.3, 67.6, 74.4, 97.7 (sept, J = 36 Hz), 118.8 (q, J = 287 Hz), 119.6 (q, J = 289 Hz), 128.3, 128.5, 128.7, 136.0, 156.3, 166.2; ^{19}F NMR ($CDCl_3$) δ −3.12 (q, J = 8 Hz, 3F), −2.84 (q, J = 8 Hz, 3F).

5-[(5*S*)-[(9-Fluorenylmethoxycarbonyl)amino]methyl]-2,2-bis(trifluoromethyl)-1,3-dioxolan-4-one (6b): yield 83%; mp 117 °C; IR (KBr) 3340, 1840, 1700, 1535 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.67 (m, 1H), 3.78 (m, 1H), 4.21 (m, 1H), 4.48 (m, 2H), 4.77 (m, 1H), 5.08 (t, br, 1H), 7.29−7.33 (m, 2H), 7.37−7.43 (m, 2H), 7.54−7.58 (m, 2H), 7.74−7.78 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 41.2, 47.0, 67.2, 74.1, 97.6 (sept, J = 36 Hz), 118.7 (q, J = 288 Hz), 119.5 (q, J = 288 Hz), 120.0, 124.8, 127.0, 127.7, 141.3, 143.5, 156.1, 166.0; ^{19}F NMR ($CDCl_3$) δ −3.06 (q, J = 8 Hz, 3F), −2.80 (q, J = 8 Hz, 3F).

5-[(5*S*)-[(*tert*-Butyloxycarbonyl)amino]methyl]-2,2-bis(trifluoromethyl)-1,3-dioxolan-4-one (6c): yield 74%; mp 81 °C; $[\alpha]_D$ −15.0° (*c* 1.0, $CHCl_3$); IR (KBr) 3450, 1845, 1715, 1505 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.45 (s, 9H), 3.58 (m, 1H), 3.80 (m, 1H), 4.78 (m, 1H), 5.01 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 28.2, 40.9, 74.6, 80.7, 97.7 (sept, J = 36 Hz), 118.8 (q, J = 287 Hz), 119.6 (q, J = 288 Hz), 155.5, 166.4; ^{19}F NMR ($CDCl_3$) δ −3.17 (q, J = 8 Hz, 3F), −2.68 (q, J = 8 Hz, 3F).

***N*-(9-Fluorenylmethoxycarbonyl)isoserine (Fmoc-Ise, 7).** Compound **6b** (0.19 g, 0.4 mmol) was stirred in 10 mL of 2-propanol/H_2O (ratio 1:1) for 2 days at room temperature. The solvent was removed in vacuo, and the remaining solid was washed carefully with ether: yield 40%; mp 168 °C; IR (KBr) 3400, 3260, 1755, 1675, 1550 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.14 (dd, J = 5, 14 Hz, 1H), 3.29 (dd, J = 5, 14 Hz, 1H), 4.00−4.30 (m, 4H), 5.50 (m, 1H), 7.26−7.38 (m, 4H), 7.65−7.67 (m, 2H), 7.82−7.84 (m, 2H), 12.5 (br, 1H); ^{13}C NMR (DMSO-d_6) δ 44.0, 46.4, 65.3, 69.0, 119.8, 125.0, 126.8, 127.3, 140.4, 143.6, 155.9, 173.7.

***N*-(9-Fluorenylmethoxycarbonyl)isoserine Methyl Ester [H-Ise(Fmoc)-OMe, 8].** Compound **6b** (0.36 g, 0.75 mmol) was stirred in 20 mL of methanol for 2 days at room temperature. The excess of methanol was removed in vacuo; the remaining oil was dissolved in dichloromethane and washed with H_2O. The dried solution ($MgSO_4$) was evaporated and the residue recrystallized from dichloromethane/hexanes: yield 41%; mp 126 °C; $[\alpha]_D$ +18.0° (*c* 1.0, $CHCl_3$); IR (KBr) 3660−3140, 1740, 1695, 1545 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.09 (m, 1H), 3.19 (m, 1H), 3.51 (s, 3H), 4.02−4.19 (m, 4H), 5.51 (t, br, 1H), 7.15−7.32 (m, 4H), 7.55−7.60 (m, 2H), 7.75−7.80 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 44.1, 46.7, 51.5, 65.5, 69.3, 120.0, 125.2, 127.0, 127.6, 140.7, 143.8, 156.2, 172.9.

General Procedure for the Preparation of N-Protected Dipeptides. The N-protected dipeptides were prepared by reaction of **6** (5 mmol) with amino acid *tert*-butyl esters (5 mmol) in 10 mL of ether at room temperature. After the reaction was complete (monitored by ^{19} F NMR), the solvent was removed in vacuo. The residue was taken up in dichloromethane and washed with water. When the dried ($MgSO_4$) solution was evaporated and the residue purified by recrystallization from chloroform/hexanes, the products **9** were obtained as white powders.

[*N*-(Benzyloxycarbonyl)isoseryl]phenylalanine *tert*-butyl ester [H-Ise(Z)-Phe-O^{t}Bu, 9a]: yield 30%; mp 87 °C; IR (KBr) 3700−3100, 1735, 1725, 1660, 1535 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40 (s, 9H), 2.99 (dd, J = 7, 14 Hz, 1H), 3.08 (dd, J = 6, 14 Hz, 1H), 3.41 (m, 1H), 3.56 (ddd, J = 3, 6, 14 Hz, 1H), 4.15 (dd, J = 3, 5 Hz, 1H), 4.74 (m, 1H), 5.08 (d, J = 12 Hz, 1H), 5.12 (d, J = 12 Hz, 1H), 5.36 (br, 1H), 7.12−7.29 (m, 5H), 7.33−7.41 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 27.8, 38.3, 44.8, 53.2, 67.3, 72.8, 82.4, 126.9, 128.1, 128.2, 128.3, 128.5, 129.4, 135.9, 136.0, 158.7, 170.2, 171.6.

[*N*-(9-Fluorenylmethoxycarbonyl)isoseryl]phenylalanine *tert*-butyl ester [H-Ise(Fmoc)-Phe-O^{t}Bu, 9b]: yield 74%; mp 153 °C; $[\alpha]_D$ −13.0° (*c* 1.0, $CHCl_3$); IR (KBr) 3620−3100, 1730, 1720, 1660, 1535, 1515 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.39 (s, 9H), 2.98 (dd, *J* = 7, 14 Hz, 1H), 3.09 (dd, *J* = 6, 14 Hz, 1H), 3.40 (m, 1H), 3.54 (ddd, *J* = 3.5, 6, 14.5 Hz, 1H), 4.16 (m, 2H), 4.37 (dd, *J* = 7, 11 Hz, 1H), 4.42 (dd, *J* = 7, 11 Hz, 1H), 4.74 (m, 1H), 5.49 (t, br, 1H), 7.10−7.20 (m, 6H), 7.24−7.29 (m, 2H), 7.34−7.42 (m, 2H), 7.53−7.55 (m, 2H), 7.72−7.74 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 27.9, 38.5, 44.9, 47.1, 53.3, 67.3, 72.9, 82.5, 120.0, 125.0, 127.0, 127.1, 127.8, 128.4, 129.4, 136.1, 141.30, 143.7, 158.8, 170.3, 171.6.

[*N*-(*tert*-Butyloxycarbonyl)isoseryl]phenylalanine *tert*-butyl ester [H-Ise(Boc)-Phe-O^{t}Bu, 9c]: yield 58%; mp 83 °C; IR (KBr) 3600−2900, 1738, 1715, 1660, 1540, 1510 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.39 (s, 9H), 1.44 (s, 9H), 3.02 (dd, *J* = 7, 14 Hz, 1H), 3.09 (dd, *J* = 7, 14 Hz, 1H), 3.35 (m, 1H), 3.49 (m, 1H), 4.14 (m, 1H), 4.74 (m, 1H), 5.33 (t, br, 1H), 7.18−7.31 (m, 5H), 7.51 (d, *J* = 8 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 27.9, 28.3, 38.6, 44.6, 53.3, 73.2, 80.4, 82.4, 127.0, 128.4, 129.5, 136.2, 158.6, 170.3, 171.9.

[*N*-(Benzyloxycarbonyl)isoseryl]proline *tert*-butyl ester [H-Ise(Z)-Pro-O^{t}Bu, 10]: yield 56%; mp 80 °C; IR (KBr) 3500−3200, 1735, 1705, 1645, 1530 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.44 (s, 9H), 1.88−2.00 (m, 3H), 2.17 (m, 1H), 3.16 (m, 1H), 3.47 (m, 1H), 3.57 (m, 1H), 3.67 (m, 1H), 4.35−4.50 (m, 2H), 5.07 (d, *J* = 12 Hz, 1H), 5.15 (d, *J* = 12 Hz, 1H), 5.75 (t, br, 1H), 7.28−7.34 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 24.9, 27.9, 28.8, 45.0, 46.8, 59.9, 66.7, 68.9, 81.8, 127.9, 128.0, 128.5, 136.5, 156.8, 170.8, 170.9.

Reaction of 4 with Carboxylic Acids or N-Protected Amino Acids. General Procedure: Equimolar amounts of **4** and the corresponding carboxylic acid or N-protected amino acid (2 mmol) in 20 mL of toluene were stirred for 18 h at 100 °C. The toluene was removed in vacuo to give a residue, which was dissolved in 30 mL of dichloromethane. The organic layer was washed with saturated $NaHCO_3$ solution and water. Removal of the solvent afforded a crude product. Purification was achieved by flash chromatography (solvent: ethyl acetate/hexanes) or recrystallization from chloroform/hexanes.

***N*-[(5*S*)-2,2-Bis(trifluoromethyl)-4-oxo-1,3-dioxolan-5-yl]methyl bromoacetamide (11a):** yield 57%; mp 74 °C; bp 125 °C (0.1 Torr); $[\alpha]_D$ −7.0° (*c* 1.1, $CHCl_3$); IR (film) 3300, 1850, 1670, 1655 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.86 (m, 2H), 3.92 (s, 2H), 4.89 (m, 1H), 7.37 (t, br, 1H); ^{13}C NMR ($CDCl_3$) δ 28.2, 40.1, 73.8, 97.7 (sept, *J* = 36 Hz), 118.8 (q, *J* = 287 Hz), 119.5 (q, *J* = 288 Hz), 166.1, 167.2; ^{19}F NMR ($CDCl_3$) δ −3.16 (q, *J* = 8 Hz, 3F), −2.86 (q, *J* = 8 Hz, 3F).

***N*-[(5*S*)-2,2-Bis(trifluoromethyl)-4-oxo-1,3-dioxolan-5-yl]methyl chloroacetamide (11b):** yield 65%; mp 47 °C; $[\alpha]_D$ −11.1° (*c* 1.6, DMSO); IR (film) 3700−3160, 1840, 1670, 1535 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.85 (ddd, *J* = 5.5, 6.5, 14.5 Hz, 1H), 3.91 (ddd, *J* = 5, 6, 14.5 Hz, 1H), 4.09 (s, 2H), 4.89 (dd, *J* = 5, 5.5 Hz, 1H), 7.18 (t, br, 1H); ^{13}C NMR ($CDCl_3$) δ 39.4, 41.9, 73.4, 97.4 (sept, *J* = 36 Hz), 118.4 (q, *J* = 287 Hz), 118.8 (q, *J* = 289 Hz), 165.7, 166.8; ^{19}F NMR ($CDCl_3$) δ −3.22 (q, *J* = 8 Hz, 3F), −2.92 (q, *J* = 8 Hz, 3F).

***N*-[(5*S*)-2,2-Bis(trifluoromethyl)-4-oxo-1,3-dioxolan-5-yl]methyl trifluoroacetamide (11c):** yield 67%; mp 47 °C; $[\alpha]_D$ −13.6° (*c* 1.1, $CHCl_3$); IR (KBr) 3320, 1825, 1705, 1550 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.88 (m, 1H), 4.00 (ddd, *J* = 5, 6, 15 Hz, 1H), 4.89 (m, 1H), 7.09 (s, br, 1H); ^{13}C NMR ($CDCl_3$) δ 39.4, 73.3, 97.8 (sept, *J* = 36 Hz), 115.4 (q, *J* = 288 Hz), 118.6 (q, *J* = 288 Hz), 119.4 (q, *J* = 290 Hz), 158.1 (q, *J* = 39 Hz), 165.5; ^{19}F NMR ($CDCl_3$) δ −3.08 (q, *J* = 7 Hz, 3F), −2.93 (q, *J* = 7 Hz, 3F), 1.73 (s, 3F).

***N*-(Benzyloxycarbonyl)alanine-*N'*-[[(5*S*)-2,2-bis(trifluoromethyl)-4-oxo-1,3-dioxolan-5-yl]methyl]amide (12a):** yield 87%; mp 123 °C; $[\alpha]_D$ −32.7° (*c* 0.5, $CHCl_3$); IR (KBr) 3330, 1850, 1695, 1665, 1540 cm^{-1}; ^1H NMR (acetone-d_6) δ 1.32 (d, *J* = 7 Hz, 3H), 3.66 (m, 1H), 3.92 (m, 1H), 4.24 (m, 1H), 5.05 (d, *J* = 12.5 Hz, 1H), 5.10 (d, *J* = 12.5 Hz, 1H), 5.25 (m, 1H), 6.60 (d, *J* = 8 Hz, 1H), 7.28−7.38 (m, 5H), 7.80 (t, br, 1H); ^{13}C NMR (acetone-d_6) δ 18.5, 40.2, 51.2, 66.5, 74.8, 97.8 (sept, *J* = 36 Hz), 119.6 (q, *J* = 287 Hz), 120.4 (q, *J* = 287 Hz), 128.4, 128.5, 129.0, 137.9, 156.6, 166.8, 174.1; ^{19}F NMR (acetone-d_6) δ −3.05 (q, *J* = 8 Hz, 3F), −2.65 (q, *J* = 8 Hz, 3F).

***N*-(Benzyloxycarbonyl)valine-*N'*-[[(5*S*)-2,2-bis(trifluoromethyl)-4-oxo-1,3-dioxolan-5-yl]methyl]amide (12b):** yield 56%; mp 132 °C; $[\alpha]_D$ −4.0° (*c* 1.0, acetone); IR (KBr) 3330, 1850, 1690, 1660, 1545 cm^{-1}; ^1H NMR (acetone-d_6) δ 0.93 (d, *J* = 7 Hz, 3H), 0.96 (d, *J* = 7 Hz, 3H), 2.15 (m, 1H), 3.74 (m, 1H), 3.89 (m, 1H), 4.08 (m, 1H), 5.04 (d, *J* = 13 Hz, 1H), 5.10 (d, *J* = 13 Hz, 1H), 5.26 (dd, *J* = 5, 7 Hz, 1H), 6.39 (d, *J* = 8 Hz, 1H), 7.38 (m, 5H), 7.87 (t, br, 1H); ^{13}C NMR (acetone-d_6) δ 18.0, 19.6, 31.7, 40.7, 61.2, 66.9, 74.8, 97.6 (sept, *J* = 35 Hz), 119.9 (q, *J* = 287 Hz), 120.6 (q, *J* = 289 Hz), 128.6, 128.7, 129.2, 138.2, 157.2, 166.8, 173.0; ^{19}F NMR (acetone-d_6) δ −3.08 (q, *J* = 8 Hz, 3F), −2.75 (q, *J* = 8 Hz, 3F).

***N*-(Benzyloxycarbonyl)phenylalanine-*N'*-[[(5*S*)-2,2-bis(trifluoromethyl)-4-oxo-1,3-dioxolan-5-yl]methyl]amide (12c):** yield 46%; mp 143 °C; $[\alpha]_D$ −9.4° (*c* 1.0, DMSO); IR (KBr) 3310, 1830, 1680, 1660, 1530 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.02−3.12 (m, 2H), 3.56−3.70 (m, 2H), 4.43 (m, 1H), 4.61 (m, 1H), 5.07 (m, 2H), 5.36 (d, *J* = 8 Hz, 1H), 6.36 (br, 1H), 7.15−7.38 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 38.2, 39.8, 56.1, 67.3, 73.4, 97.1 (sept, *J* = 36 Hz), 118.6 (q, *J* = 287 Hz), 119.4 (q, *J* = 289 Hz), 127.2, 128.0, 128.3, 128.5, 128.8, 129.1, 135.6, 135.9, 156.1, 165.7, 171.7; ^{19}F NMR ($CDCl_3$) δ −3.00 (m, 6F).

***N*-(9-Fluorenylmethoxycarbonyl)alanine-*N'*-[[(5*S*)-2,2-bis(trifluoromethyl)-4-oxo-1,3-dioxolan-5-yl]methyl]amide (12d):** yield 28%; mp 164 °C; $[\alpha]_D$ −10.0° (*c* 1.0, $CHCl_3$); IR (KBr) 3325, 1850, 1680, 1530 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.37 (d, *J* = 6.5 Hz, 3H), 3.75 (m, 2H), 4.18−4.22 (m, 2H), 4.45 (m, 2H), 4.79 (m, 1H), 5.17 (m, 1H), 6.62 (br, 1H), 7.29−7.33 (m, 2H), 7.38−7.43 (m, 2H), 7.56−7.58 (m, 2H), 7.76−7.78 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 18.1, 39.5, 47.0, 50.5, 67.2, 73.2, 97.5 (sept, *J* = 36 Hz), 118.7 (q, *J* = 288 Hz), 119.4 (q, *J* = 287 Hz), 119.9, 124.9, 127.1, 127.7, 141.3, 143.6, 156.6, 166.0, 173.9; ^{19}F NMR ($CDCl_3$) δ −3.09 (q, *J* = 8 Hz, 3F), −2.83 (q, *J* = 8 Hz, 3F).

***N*-(9-Fluorenylmethoxycarbonyl)phenylalanine-*N'*-[[(5*S*)-2,2-bis(trifluoromethyl)-4-oxo-1,3-dioxolan-5-yl]methyl]amide (12e):** yield 52%; mp 184 °C; IR (KBr) 3310, 1850, 1780−1600, 1535 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.02 (m, 1H), 3.12 (m, 1H), 3.65 (m, 2H), 4.17 (m, 1H), 4.40−4.50 (m, 3H), 4.61 (m, 1H), 5.28 (m, 1H), 6.25 (br, 1H), 7.15−7.32 (m, 7H), 7.38−7.42 (m, 2H), 7.50−7.53 (m, 2H), 7.75−7.77 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 38.3, 39.9, 47.1, 56.2, 67.2, 73.5, 97.5 (sept, *J* = 36 Hz), 118.7 (q, *J* = 288 Hz), 119.4 (q, *J* = 288 Hz), 120.0, 125.0, 127.1, 127.3, 127.8, 128.8, 129.2, 136.0, 141.3, 143.6, 156.1, 165.8, 171.8; ^{19}F NMR ($CDCl_3$) δ −3.01 (m, 6F).

General Procedure for the Preparation of Tripeptides. A solution of equimolar amounts of **12** and the corresponding amino acid *tert*-butyl ester (5 mmol) in 10 mL of ether was stirred at room temperature. After completion of the reaction (monitored by ^{19}F NMR), the solvent was removed in vacuo. The residue was dissolved in dichloromethane and the organic layer washed with water. The dried ($MgSO_4$) solution was evaporated, and the crude product was purified by flash chromatography (solvent: ethyl acetate/hexanes) or recrystallization from chloroform/hexanes).

[[*N*-(Benzyloxycarbonyl)alanyl]isoseryl]phenylalanine *tert*-butyl ester [H-Ise(Z-Ala)-Phe-O^{t}Bu, 13a]: yield 35%; mp 47 °C; IR ($CHCl_3$) 3500−3140, 1715, 1665, 1505 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.29 (d, *J* = 7 Hz, 3H), 1.37 (s, 9H), 3.03 (dd, *J* = 7, 14 Hz, 1H), 3.08 (dd, *J* = 7, 14 Hz, 1H), 3.44 (m, 1H), 3.53 (m, 1H), 4.10−4.25 (m, 2H), 4.70 (m, 1H), 5.00 (d, *J* = 12 Hz, 1H), 5.09 (d, *J* = 12 Hz, 1H), 5.91 (d, *J* = 7 Hz, 1H), 7.15−7.33 (m, 10 H), 7.44 (t, br, 1H), 7.53 (d, *J* = 8 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 18.4, 27.9, 38.0, 44.0, 50.7, 53.6, 67.0, 71.9, 82.5, 127.0, 128.0, 128.2, 128.4, 128.5, 129.3, 136.1, 136.2, 156.1, 170.6, 172.1, 175.1.

[[*N*-(Benzyloxycarbonyl)phenylalanyl]isoseryl]alanine *tert*-butyl ester [H-Ise(Z-Phe)-Ala-O^{t}Bu, 13b]: yield 75%; mp 109 °C; $[\alpha]_D$ +6.0° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.34 (d, *J* = 7 Hz, 3H), 1.46 (s, 9H), 3.03 (m, 1H), 3.07 (dd, *J* = 6.5, 14 Hz, 1H), 3.36 (m, 1H), 3.63 (m, 1H), 4.07 (m, 1H), 4.36−4.48 (m, 2H), 4.98 (d, *J* = 12 Hz), 5.05 (d, *J* = 12 Hz, 1H), 5.57 (d, *J* = 7.5 Hz, 1H), 7.16−7.37 (m, 11H), 7.43 (d, *J* = 7.5 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 18.0, 27.9, 38.2, 44.0,

48.5, 56.4, 67.1, 71.7, 82.2, 127.1, 128.0, 128.2, 128.5, 128.7, 129.2, 135.9, 136.1, 156.1, 171.9, 172.2, 173.6.

[[*N*-(9-Fluorenylmethoxycarbonyl)alanyl]isoseryl]-phenylalanine *tert*-butyl ester [H-Ise(Fmoc-Ala)-Phe-O^{t}Bu, 13c]: yield 46%; mp 148 °C; IR (KBr) 3660–3140, 1720, 1660, 1525 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.32 (d, J = 6 Hz, 3H), 1.38 (s, 9H), 3.01 (dd, J = 7, 14 Hz, 1H), 3.06 (dd, J = 7, 14 Hz, 1H), 3.41 (m, 1H), 3.58 (m, 1H), 4.10–4.20 (m, 3H), 4.34 (m, 2H), 4.71 (m, 1H), 5.70 (d, J = 6 Hz, 1H), 7.13–7.29 (m, 8H), 7.35–7.39 (m, 2H), 7.48 (d, J = 8 Hz, 1H), 7.52–7.55 (m, 2H), 7.72–7.74 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 18.3, 27.9, 37.9, 44.1, 47.0, 50.6, 53.6, 67.1, 71.8, 82.5, 119.9, 124.95, 125.02, 126.9, 127.0, 127.7, 128.4, 129.2, 136.2, 141.2, 143.5, 143.7, 156.1, 170.7, 172.1, 175.0.

[[*N*-(9-Fluorenylmethoxycarbonyl)phenylalanyl]-isoseryl]phenylalanine *tert*-butyl ester [H-Ise(Fmoc-Phe)-Phe-O^{t}Bu, 13d]: yield 45%; mp 143 °C; IR (KBr) 3620–3120, 1720, 1650, 1530 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 9H), 2.95–3.10 (m, 4H), 3.30 (m, 1H), 3.52 (m, 1H), 4.00–4.15 (m, 2H), 4.30–4.44 (m, 3H), 4.70 (m, 1H), 5.50 (d, J = 8 Hz, 1H), 6.97 (br, 1H), 7.12–7.40 (m, 15H), 7.46–7.49 (m, 2H), 7.72–7.75 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 27.9, 38.0, 38.3, 44.2, 47.0, 53.7, 56.4, 67.2, 71.8, 82.6, 120.0, 125.0, 127.0, 127.1, 127.2, 127.8, 128.4, 128.7, 129.3, 136.2, 141.3, 143.6, 143.7, 156.1, 170.8, 171.8, 173.5.

[[*N*-(9-Fluorenylmethoxycarbonyl)phenylalanyl]-isoseryl]proline *tert*-butyl ester [H-Ise(Fmoc-Phe)-Pro-O^{t}Bu, 14]: yield 74%; mp 83 °C; IR (KBr) 3600–3120, 1720, 1640, 1525 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H), 1.86–1.98 (m, 3H), 2.18 (m, 1H), 3.00 (m, 1H), 3.15 (m, 1H), 3.35–3.70 (m, 4H), 4.10–4.25 (m, 2H), 4.30–4.40 (m, 4H), 5.29 (s, br, 1H), 5.73 (d, J = 8 Hz, 1H), 6.99 (t, br, 1H), 7.15–7.30 (m, 7H), 7.30–7.40 (m, 2H), 7.45–7.53 (m, 2H), 7.72–7.74 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 24.9, 27.9, 28.8, 38.7, 43.1, 46.9, 47.1, 56.1, 59.9, 67.1, 68.4, 82.2, 119.9, 125.1, 125.2, 126.9, 127.1, 127.7, 128.5, 129.4, 136.6, 141.2, 143.8, 155.9, 170.8, 171.2, 171.7.

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