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Orthogonally Protected, Carboxy-Activated *L*-Homoisoserine, 2-Methyl-*L*-homoisoserine, and Homoisocysteine Derivatives. New Building Blocks for Peptide and Depsipeptide Modification[#]

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Summary. Starting from *L*-malic, *L*-citramalic, and *rac*. thiomalic acids routes to *L*-homoisoserine, 2-methyl-*L*-homoisoserine and rac. homoisocysteine have been developed. The new orthogonally protected and carboxy-activated building blocks are *GABA* as well as α-hydroxy and α-mercapto acid derivatives, suitable for the construction of peptide and depsipeptide surrogates.

Keywords. Malic acid; Citramalic acid; Thiomalic acid; Hexafluoroacetone; γ-Amino acids; α-Hydroxy acids; α-Mercapto acids; *Wolff* rearrangement; *Curtius* rearrangement; *Arndt-Eistert* reaction; Peptide modification; Depsipeptide modification; Azapeptides; Hydroxamates.

Introduction

The number of reports dealing with peptidomimetics built from two or more different types of monomers is rapidly growing [1]. The application of ω-peptides (β-peptides and γ-peptides) as drug candidates and of ω-amino acids as building blocks for the incorporation into strategical positions of biologically active peptides is a newly emerging area of current research [2]. This is mainly due to the ability of these amino acids to modify the geometry of the peptide backbone [2d], providing proteolytically stable bioactive domains. Insertion of one or more extra carbon atoms into intramolecular hydrogen bound, folded structures leads to the

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[#] Dedicated to Prof. Dr. *Manfred Mühlstädt* on the occassion of his 75th birthday

creation of unusual turns and novel helical folds [3], which are often responsible for biological activity [4].

Helix stability increases on homologation of monomers. Incorporation of α-methylated ω-amino acids further stabilizes helical structures, provided that the spatial orientation of the α-placed group is compatible with the main backbone [5]. Structural diversity can be achieved by incorporation of additional functional groups, like –OH and –SH, into different positions of the backbone, suitable for further transformations.

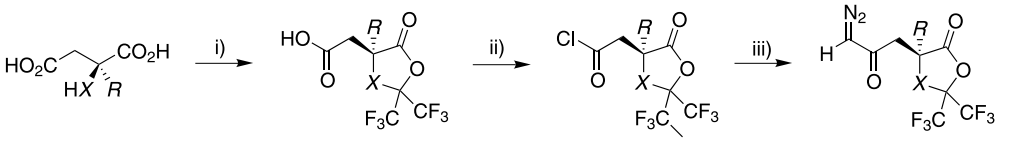
Prominent members of this class of amino acids are *GABA* (4-aminobutyrate) and homoisoserine (2-hydroxy-4-aminobutyrate). The latter was isolated from antibiotics [6] and phytosiderophores [7]. Homoisoserine is an inhibitor of *GABA* uptake and exhibits antitumor activities [8]. Because of these and other interesting properties, several routes to homoisoserine have been developed [9].

However, to the best of our knowledge, 2-methylhomoisoserine has not been found in nature. More surprisingly, this amino acid seems to be unknown. Likewise, homoisocysteine (4-amino-2-mercaptobutyrate) has not been found in nature so far. Although, homoisocysteine was first mentioned in a Japanese patent in 1961, there exists nearly no information about its chemistry [10].

In a series of papers we disclosed that a new protection/activation concept developed for the site-selective derivatization of multifunctional amino acids, like iminodiacetic acid [11], aspartic acid [12], and homologues [13] can also be applied to multifunctional α-hydroxy (malic and citramalic acid) [14] and α-mercapto acids (thiomalic acid) [15].

Results and Discussion

We now report on the syntheses of *L*-homoisoserine, 2-methyl-*L*-homoisoserine and *rac*. homoisocysteine and their incorporation into peptides using *HFA* (hexafluoroacetone) as protecting and activating reagent [16]. Malic (**1a**), citramalic (**1b**), and thiomalic acid (**2**) react readily with *HFA* in solvents like *DMSO* (dimethylsulphoxide) or *DMF* (dimethylformamide) at room temperature to give 2,2-bis(trifluoromethyl)-1,3-dioxolan-4-ones (**3**) [17] and 2,2-bis(trifluoromethyl)-1,3-oxathiolan-5-ones (**4**) [18] in excellent yields (86–87%). A second equivalent of *HFA* is necessary to trap the water which is eliminated during lactone formation. In a tandem process, protection of the α-placed group (–OH, –SH) and the adjacent carboxy group are achieved. Noteworthy, the α-carboxy group is protected and activated, while the ω-carboxy group remains unaffected and can be selectively derivatized after separate activation (Scheme 1). Compared to conventional strate-



1a *X*=O, *R*=H
1b *X*=O, *R*=CH_3
2 *X*=S, *R*=H (*rac.*)

3a *X*=O, *R*=H; 87%
3b *X*=O, *R*=CH_3; 87%
4 *X*=S, *R*=H (*rac.*); 86%

5a *X*=O, *R*=H; 89%
5b *X*=O, *R*=CH_3; 86%
6 *X*=S, *R*=H (*rac.*); 88%

7a *X*=O, *R*=H; 92%
7b *X*=O, *R*=CH_3; 90%
8 *X*=S, *R*=H (*rac.*); 93%

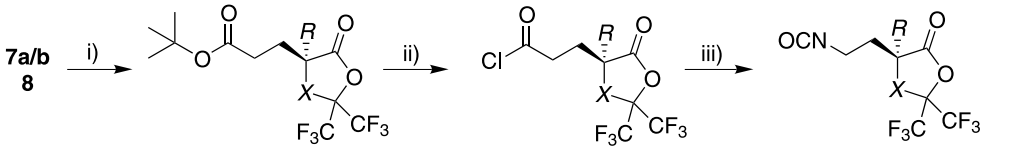
Scheme 1. i) *HFA*, *DMSO*, rt; ii) $SOCl_2$, reflux; iii) CH_2N_2, Et_2O, $-78\rightarrow0^\circ C$

gies, this concept saves steps, as demonstrated by a two step *Aspartame* synthesis with an overall yield of 72% [19].

On heating compounds **3**/**4** with an excess of thionyl chloride, acid chlorides **5**/**6** are formed (86–87%). Compounds **5**/**6** are preparatively useful members of a new class of dielectrophiles, because their two electrophilic centers are different in reactivity, which allows site-selective derivatizations. With diazomethane (>3 equivalents) the acid chloride moiety reacts to give the diazoketones **7**/**8**. Best yields (90–93%) were obtained when the acid chlorides were slowly dropped into a solution of diazomethane in ether with stirring at $-78 \rightarrow 0°C$. An excess of diazomethane is necessary to trap the HCl formed during the reaction to suppress the formation of chloroketones, which are difficult to separate from diazoketones **7**/**8**.

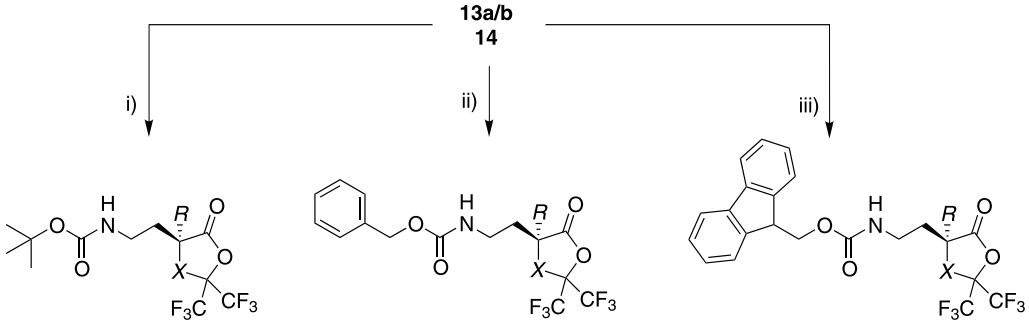
Arndt-Eistert homologation can be achieved *via* photo-*Wolff* rearrangement [20] of diazoketones **7**/**8** in aqueous dioxane. The corresponding α-hydroxy and α-mercapto glutaric acid derivatives can be readily transformed into acid chlorides on treatment with thionyl chloride.

Improved yields are obtained when a silver benzoate catalyzed decomposition [21] of the diazoketones **7**/**8** is performed in *tert*-butanol to trap the ketenes as *tert*-butylesters **9**/**10**. Deprotection of the *tert*-butylester and transformation into the acid chlorides **11**/**12** are achieved in excellent yields on heating with an excess of thionyl chloride. The acid chlorides **11**/**12** can be readily purified by distillation under reduced pressure (Scheme 2).



9a *X*=O, *R*=H; 66% **11a** *X*=O, *R*=H; 80% **13a** *X*=O, *R*=H; 88%
9b *X*=O, *R*=CH_3; 77% **11b** *X*=O, *R*=CH_3; 78% **13b** *X*=O, *R*=CH_3; 70%
10 *X*=S, *R*=H (*rac.*); 55% **12** *X*=S, *R*=H (*rac.*); 84% **14** *X*=S, *R*=H (*rac.*); 82%

Scheme 2. i) ^tBuOH, $Ph$$CO_2$Ag, reflux; ii) $SOCl_2$, reflux; iii) $(CH_3)_3SiN_3$, toluene, 80–90°C



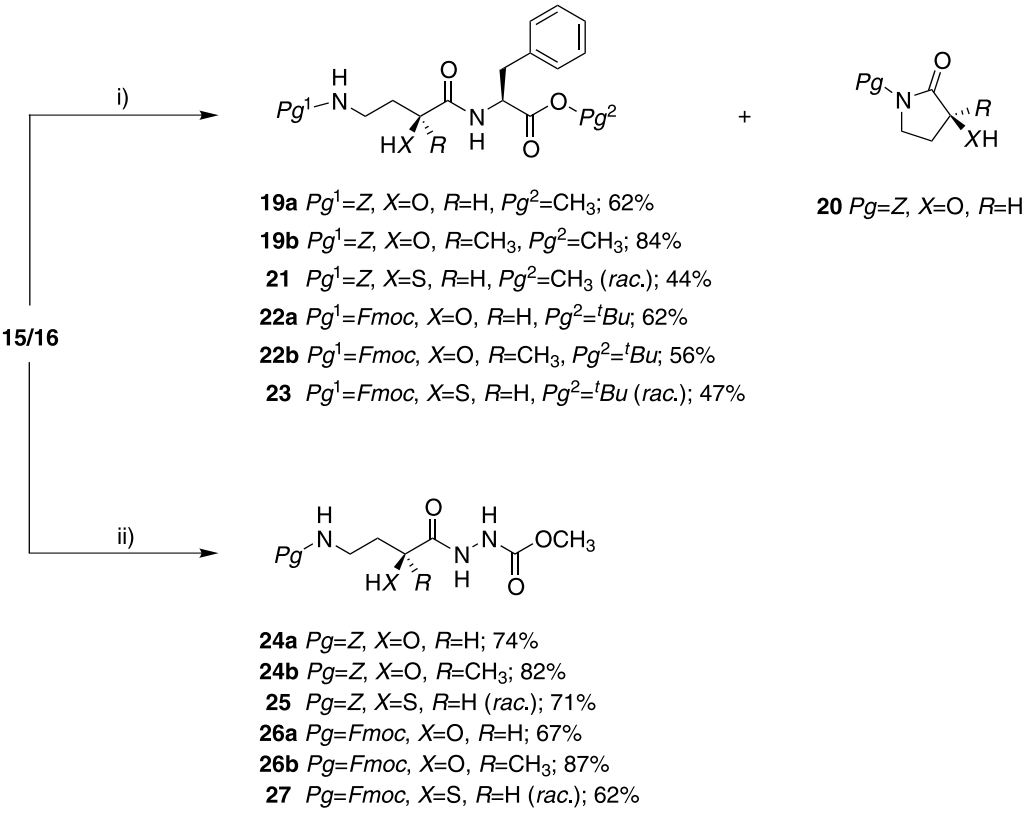
15a/1 *X*=O, *R*=H; 61% **15a/2** *X*=O, *R*=H; 51% **15a/3** *X*=O, *R*=H; 66%
15b/1 *X*=O, *R*=CH_3; 70% **15b/2** *X*=O, *R*=CH_3; 74% **15b/3** *X*=O, *R*=CH_3; 76%
16/1 *X*=S, *R*=H (*rac.*); 64% **16/2** *X*=S, *R*=H (*rac.*); 76% **16/3** *X*=S, *R*=H (*rac.*); 76%

Scheme 3. i) ^tBuOH, $CHCl_3$, reflux; ii) $Ph$$CH_2OH$, $CHCl_3$, reflux; iii) 9-fluorenylmethanol, $CHCl_3$, reflux

Introduction of the isocyanato group into the ω-position requires two steps, namely *Curtius* rearrangement of the azides [22] obtained from **11**/**12** and trimethylsilyl azide. Compounds **13**/**14** are members of a class of dielectrophiles with high synthetic potential. Urethane protection of the γ-amino group (*e.g. Boc*-NH, *Z*-NH, *Fmoc*-NH) can be achieved in high yields on heating the isocyanates **13**/**14** with 1 equivalent of the corresponding alcohol to give the carboxy-activated orthogonally protected derivatives of homoisoserine (**15a**), 2-methylhomoisoserine (**15b**), and homoisocysteine (**16**), respectively (Scheme 3). Further highly useful synthetical applications of compounds **13**/**14** are *i.a. N*-glycosylation [23], *N*-lipidation [24], and isopeptide formation [25]. Compounds **13**/**14** can be stored in a fridge on exclusion of moisture for several weeks without decomposition.

Likewise, urethane-protected carboxy-activated species **15**/**16** are valuable starting materials for the generation of libraries of homoisoserine, 2-methylhomo-

Scheme 4. i) CH_3OH, reflux; ii) $HCl{\cdot}NH_2OH$, propene oxide, *DMF*, rt



Scheme 5. i) $HCl \cdot$ H-*Phe*-OPg^2, *DIPEA*, *DMF*, rt; ii) $H_2NNHCO_2CH_3$, *DMF*, rt

isoserine and homoisocysteine derivatives. Since they represent *GABA* derivatives they are interesting *per se* or as building blocks for the synthesis of drug candidates. Esters, *e.g.* **17**, are readily obtainable on heating with an excess of corresponding alcohols, hydroxamates, *e.g.* **18**, are obtainable on cleavage of the lactone moiety with the reagent pair hydroxylamine hydrochloride/propene oxide (Scheme 4). Hydroxamates exhibit strong inhibitory effects on metal-containing enzymes (metallozymes) [26] and meet growing interest since a hydroxamate subunit is the pharmacophor of several HDAC inhibitors [27].

Small peptides [28] and peptide surrogates (depsipeptides **19**, **21–23** and azapeptides **24–27**) become readily available using **15** and **16** as acyl transfer reagents. Nucleophilic cleavage of the lactone ring is always coupled with a deprotection of the α-placed functional group (Scheme 5). Consequently, chain elongation can be continued without the need of any extra deprotection step. Depending on the reaction conditions applied, up to 10% of *N*-protected 3-hydroxypyrrolidin-2-ones represented by structure **20** are formed *via* an intramolecular cyclocondensation process [29]. *N*-Unprotected 3-hydroxypyrrolidin-2-ones were obtained from compounds **15** in good yields on selective cleavage of the urethane protecting group and intramolecular ring closure [20c, 30].

For *L*-malic acid we proved that all reaction steps studied so far proceed stereoconservatively (NMR analysis). In the case of thiomalic acid only the racemate was commercially available, therefore all reactions described are starting from *rac* thiomalic acid. On further aspects of the synthetic potential of the *HFA* protection/activation concept we report elsewhere.

Experimental

General

Solvents were purified and dried prior to use. Reagents were used as purchased. TLC was performed on Silica Gel 60 F_{254} (Merck) with detection by UV light or phosphomolybdic acid/ceric sulphate in 5% aqueous sulfuric acid followed by heating. Melting points (uncorrected) were determined on a Boetius heating table. Optical rotation indices were measured using a Schmidt & Haensch Polartronic-D polarimeter in a 5 cm cell. Mass spectra were recorded on a VG 12-250 and a MAT 212 (Masslab) electron ionization spectrometer (EI-MS, EI = 70 eV), on a Bruker Daltonics APEX II ESI-FT-ICR spectrometer or on a Finnigan ZAB-HSQ spectrometer (FAB-matrix: 3-*NBA*). IR spectra were obtained with a FTIR spectrometer (Genesis ATI Mattson/Unicam). ^{1}H (400 MHz, 600 MHz, 700 MHz), ^{13}C (101 MHz, 151 MHz), and ^{19}F (376 MHz) NMR were recorded on Bruker DRX-AVANCE spectrometers. TMS was used for ^{1}H and ^{13}C NMR spectra, and CCl_3F for ^{19}F NMR spectra as internal references. Flash chromatography was performed using silica gel (32–63 μm) with solvent systems given in the text. Elemental analyses were performed with a CHNO-S Rapid apparatus (Fa. Heraeus); their results agreed with calculated values.

Wolff Rearrangement of HFA-Protected Diazoketones (General Procedure)

A stirred solution of diazoketones **7**/**8** (20 mmol) in dry *tert*-butanol (50 cm^3) was heated (oil bath temperature 85°C). Then silver benzoate (200 mg) was added in two portions. When no gas evolution was observed even on further addition of silver benzoate the solvent was evaporated *in vacuo* and the residue purified by column chromatography (eluent: dichloromethane (*DCM*)).

tert-Butyl 3-[(5S)-2,2-bis(trifluoromethyl)-4-oxo-1,3-dioxolan-5-yl]propionate
(**9a**, $C_{12}H_{14}F_6O_5$)

Yield 66%; colorless oil; $[\alpha]_D = -17.4°$ $cm^3\,g^{-1}\,dm^{-1}$ ($c = 1.0$, *DCM*); IR (film): $\bar{\nu} = 2981$, 2937, 1851, 1730 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): $\delta = 1.43$ (s, 9H), 2.03–2.09 (m, 1H), 2.21–2.27 (m, 1H), 2.45 (m, 2H), 4.76 (dd, $J = 8.3$, 5.1 Hz, 1H) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): $\delta = 26.9$, 27.9, 29.7, 74.1, 81.3, 97.6 (sept, $J = 36.0$ Hz), 118.8 (q, $J = 287.2$ Hz), 119.6 (q, $J = 289.8$ Hz), 167.5, 170.7 ppm; ^{19}F NMR (376 MHz, $CDCl_3$): $\delta = -81.5$ (m, 6F) ppm; MS (EI): m/z (%) = 352 $[M]^+$ (1), 336 (29), 295 $[M - C_4H_9]^+$ (14), 278 (100), 85 (18), 59 (90), 42 $[C_2H_2O]^+$ (24).

tert-Butyl 3-[(5S)-2,2-bis(trifluoromethyl)-5-methyl-4-oxo-1,3-dioxolan-5-yl]propionate
(**9b**, $C_{13}H_{16}F_6O_5$)

Yield 77%; colorless oil; $[\alpha]_D = -6.6°$ $cm^3\,g^{-1}\,dm^{-1}$ ($c = 1.21$, *DCM*); IR (film): $\bar{\nu} = 1844$, 1732 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 1.45$ (s, 9H), 1.59 (s, 3H), 2.18 (m, 2H), 2.43 (m, 2H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 22.0$, 28.1, 29.2, 32.8, 81.3, 97.0 (sept, $J = 35.7$ Hz), 119.2 (q, $J = 288.4$ Hz), 119.3 (q, $J = 289.0$ Hz), 170.1, 170.9 ppm; ^{19}F NMR (376 MHz, $CDCl_3$): $\delta = -81.1$ (q, $J = 8.3$ Hz, 3F), -81.3 (q, $J = 8.3$ Hz, 3F) ppm; MS (EI): m/z (%) = 366 $[M]^+$ (1), 351 (1), 293 $[M - C_4H_9O]^+$ (25), 147 (8), 99 (100), 69 (55), 57 (98).

rac. tert-Butyl 3-[2,2-bis(trifluoromethyl)-5-oxo-1,3-oxathiolan-4-yl]propionate
(**10**, $C_{12}H_{14}F_6O_4S$)

Yield 55%; slightly yellow oil; IR (film): $\bar{\nu} = 2980$, 2933, 1817, 1726 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): $\delta = 1.42$ (s, 9 H), 2.09–2.17 (m, 1H), 2.35–2.45 (m, 3H), 4.34 (dd, $J = 7.8$, 3.1 Hz, 1H) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): $\delta = 27.9$, 28.2, 32.0, 45.1, 81.4, 82.9 (sept, $J = 35.0$ Hz), 120.8 (q, $J = 283.5$ Hz), 121.3 (q, $J = 284.5$ Hz), 170.5, 170.8 ppm; ^{19}F NMR (376 MHz, $CDCl_3$): $\delta = -76.4$ (q, $J = 9.2$ Hz, 3F), -77.5 (q, $J = 9.2$ Hz, 3F) ppm; MS (EI): m/z (%) = 368 $[M]^+$ (1), 352 (29), 311 (39), 294 (49), 101 (42), 57 (100), 41 (34).

Synthesis of Propionyl Chlorides **11**, **12** *(General Procedure)*

Thionyl chloride (30 cm^3) was added to compounds **9**/**10** (20 mmol) with stirring. Then the reaction mixture was heated under reflux for 12 h. After removal of the excess of thionyl chloride, the residue was distilled *in vacuo*.

3-[(5S)-2,2-Bis(trifluoromethyl)-4-oxo-1,3-dioxolan-5-yl]propionyl chloride
(**11a**, $C_8H_5ClF_6O_4$)

Yield 80%; colorless oil; bp 47–48°C/106.7 Pa; $[\alpha]_D = -17.1°$ $cm^3\,g^{-1}\,dm^{-1}$ ($c = 1.05$, *DCM*); IR (film): $\bar{\nu} = 1851$, 1793 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 2.20$ (ddt, $J = 14.9$, 8.4, 7.0 Hz, 1H), 2.39 (ddt, $J = 14.9$, 7.5, 5.0 Hz, 1H), 3.18 (t, $J = 7.0$ Hz, 2H), 4.75 (dd, $J = 8.4$, 5.0 Hz, 1H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 26.9$, 41.5, 73.3, 97.8 (sept, $J = 36.4$ Hz), 118.9 (q, $J = 286.8$ Hz), 119.6 (q, $J = 288.8$ Hz), 167.0, 172.7 ppm; ^{19}F NMR (376 MHz, $CDCl_3$): $\delta = -81.4$ (m, 6F) ppm; MS (EI): m/z (%) = 316/314 $[M]^+$ (1), 293 (33), 279 $[M - Cl]^+$ (20), 247/245 $[M - CF_3]^+$ (6), 217 (19), 203 (11), 97 (17), 85 (76), 57 (44), 42 $[C_2H_2O]^+$ (100).

3-[(5S)-2,2-Bis(trifluoromethyl)-5-methyl-4-oxo-1,3-dioxolan-5-yl]propionyl chloride
(**11b**, $C_9H_7ClF_6O_4$)

Yield 78%; yellow oil; bp 56–60°C/53.3 Pa; $[\alpha]_D = -14.3°$ $cm^3\,g^{-1}\,dm^{-1}$ ($c = 1.26$, *DCM*); IR (film): $\bar{\nu} = 1842$, 1797 cm^{-1}; ^1H NMR (700 MHz, $CDCl_3$): $\delta = 1.62$ (s, 3H), 2.28 (m, 2H), 3.08 (m, 1H), 3.17

(m, 1H) ppm; ^{13}C NMR (176 MHz, $CDCl_3$): $\delta = 22.0$, 32.5, 40.7, 80.4, 97.1 (sept, $J = 35.9$ Hz), 119.0 (q, $J = 288.5$ Hz), 119.1 (q, $J = 289.0$ Hz), 169.5, 172.4 ppm; ^{19}F NMR (376 MHz, $CDCl_3$): $\delta = -81.2$ (m, 6F) ppm; MS (EI): m/z (%) = 328 $[M]^+$ (2), 293 $[M - Cl]^+$ (37), 259 (3), 231 (6), 195 (4), 167 (5), 117 (8), 99 (51), 89 (15), 69 (20).

rac. 3-[2,2-Bis(trifluoromethyl)-5-oxo-1,3-oxathiolan-4-yl]propionyl chloride
(**12**, $C_8H_5ClF_6O_3S$)

Yield 84%; yellow oil; bp 52°C/240 Pa; IR (film): $\bar{\nu} = 1813$, 1735 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 2.29$–2.35 (m, 1H), 2.47–2.53 (m, 1H), 3.17 (m, 2H), 4.35 (t, $J = 6.6$ Hz, 1H) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): $\delta = 27.9$, 43.2, 44.1, 83.0 (sept, $J = 35.0$ Hz), 120.6 (q, $J = 284.0$ Hz), 121.2 (q, $J = 284.5$ Hz), 169.9, 172.7 ppm; ^{19}F NMR (376 MHz, $CDCl_3$): $\delta = -76.4$ (q, $J = 9.2$ Hz, 3F), -77.4 (q, $J = 9.2$ Hz, 3F) ppm; MS (EI): m/z (%) = 330 $[M]^+$ (1), 294 $[M - Cl]^+$ (100), 266 (31), 252 (26), 101 (69), 73 (29).

Synthesis of Isocyanates **13**/**14** *(General Procedure)*

Trimethylsilyl azide (21 mmol) was added to a stirred solution of propionyl chloride **11**/**12** (20 mmol) in dry toluene (30 cm^3) at room temperature. After 12 h the reaction mixture was heated to 80–90°C (bath temperature). When the reaction was complete (^{19}F NMR analysis, *ca.* 12 h) the solvent was evaporated under reduced pressure and the residue was purified by distillation *in vacuo* or by column chromatography (eluent: *DCM*).

(5S)-5-(2-Isocyanatoethyl)-2,2-bis(trifluoromethyl)-1,3-dioxolan-4-one (**13a**, $C_8H_5F_6NO_4$)

Yield 88%; yellow oil; bp 65°C/70.7 Pa; $[\alpha]_D = -15.3°$ cm^3 g^{-1} dm^{-1} ($c = 1.1$, $CHCl_3$); IR (film): $\bar{\nu} = 2281$, 1853 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.07$ (dddd, $J = 14.6$, 9.0, 5.9, 5.4 Hz, 1H), 2.22 (dddd, $J = 14.6$, 8.4, 5.8, 4.2 Hz, 1H), 3.61 (ddd, $J = 13.4$, 8.4, 5.4 Hz, 1H), 3.67 (ddd, $J = 13.4$, 5.9, 5.8 Hz, 1H), 4.80 (dd, $J = 9.0$, 4.2 Hz, 1H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 33.0$, 38.5, 72.5, 98.0 (sept, $J = 36.0$ Hz), 119.0 (q, $J = 287.2$ Hz), 119.8 (q, $J = 289.1$ Hz), 123.16, 167.56 ppm; ^{19}F NMR (376 MHz, $CDCl_3$): $\delta = -81.5$ (m, 6F) ppm; MS (EI): m/z (%) = 293 $[M]^+$ (2), 224 $[M - CF_3]^+$ (10), 250 (10), 196 (11), 155 (15), 99 (23), 69 $[CF_3]^+$ (50), 56 $[CH_2NO]^+$ (100).

(5S)-5-(2-Isocyanatoethyl)-5-methyl-2,2-bis(trifluoromethyl)-1,3-dioxolan-4-one
(**13b**, $C_9H_7F_6NO_4$)

Yield 70%; yellow oil; bp 34–38°C/45.3 Pa; $[\alpha]_D = -6.8°$ cm^3 g^{-1} dm^{-1} ($c = 1.33$, *DCM*); IR (film): $\bar{\nu} = 2276$, 1844 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.66$ (s, 3H), 2.17 (t br, $J = 7.0$ Hz, 2H), 3.59 (m, 2H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 22.3$, 37.4, 38.5, 80.6, 97.4 (sept, $J = 35.9$ Hz), 119.3 (q, $J = 288.0$ Hz), 122.6, 169.9 ppm; ^{19}F NMR (376 MHz, $CDCl_3$): $\delta = -81.1$ (m, 6F) ppm; MS (EI): m/z (%) = 307 $[M]^+$ (3), 238 (28), 169 (14), 72 (66), 56 $[CH_2NCO]^+$ (100).

rac. 4-(2-Isocyanatoethyl)-2,2-bis(trifluoromethyl)-1,3-oxathiolan-5-one (**14**, $C_8H_5F_6NO_3S$)

Yield 82%; light yellow oil; bp 36–38°C/24.0 Pa; IR (film): $\bar{\nu} = 2279$, 1815 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 2.11$ (m, 1H), 2.48 (m, 1H), 3.58–3.62 (m, 1H), 3.66–3.71 (m, 1H), 4.32 (m, 1H) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): $\delta = 34.3$, 40.7, 43.4, 83.3 (sept, $J = 35.0$ Hz), 120.7 (q, $J = 284.0$ Hz), 121.3 (q, $J = 284.5$ Hz), 123.1, 170.4 ppm; ^{19}F NMR (376 MHz, $CDCl_3$): $\delta = -76.4$ (q, $J = 9.2$ Hz, 3F), -77.4 (q, $J = 9.2$ Hz, 3F) ppm; MS (EI): m/z (%) = 309 $[M]^+$ (2), 281 $[M - CO]^+$

(35), 266 (50), 253 $[M-NCO]^+$ (18), 236 (20), 171 (23), 113 (29), 82 (39), 72 (63), 69 $[CF_3]^+$ (30), 56 $[CH_2NCO]^+$ (28).

Introduction of Boc-, Z-, and Fmoc Protection (General Procedure)

A solution of equimolar amounts (10 mmol) of the isocyanate (**13**, **14**) and the corresponding alcohol was heated in dry $CHCl_3$ (30 cm^3) for 12 h under reflux (TLC control). After evaporation of the solvent, the residue was purified by column chromatography (eluent: *DCM*) or crystallization.

(5S)-5-[2-(tert-Butyloxycarbonylamino)ethyl]-2,2-bis(trifluoromethyl)-1,3-dioxolan-4-one (**15a**/**1**, $C_{12}H_{15}F_6NO_5$)

Yield 61%; mp 43°C; $[\alpha]_D = -8.0°\ cm^3\ g^{-1}\ dm^{-1}$ ($c = 0.75$, *DCM*); IR (KBr): $\bar{\nu} = 3620–3035$, 1854, 1782, 1700 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): $\delta = 1.40$ (s, 9H), 1.91–2.21 (m, 2H), 3.26–3.35 (m, 2H), 4.45 (m, 1H, NH), 4.69 (dd, $J = 8.0$, 4.0 Hz, 1H) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): $\delta = 27.8$, 31.7, 33.9, 72.9, 79.4, 97.2 (sept, $J = 36.0$ Hz), 118.4 (q, $J = 286.0$ Hz), 119.1 (q, $J = 286.0$ Hz), 155.4, 167.4 ppm; ^{19}F NMR (376 MHz, $CDCl_3$): $\delta = -81.5$ (m, 6F) ppm; MS (EI): m/e (%) = 352 $[M-CH_3]$ (1.5), 69 $[CF_3]^+$ (41), 56 $[C_4H_9]^+$ (100).

(5S)-5-[2-(Benzyloxycarbonylamino)ethyl]-2,2-bis(trifluoromethyl)-1,3-dioxolan-4-one (**15a**/**2**, $C_{15}H_{13}F_6NO_5$)

Yield 51%; colorless crystals; mp 55°C ($CHCl_3$/hexanes); $[\alpha]_D = -13.4°\ cm^3\ g^{-1}\ dm^{-1}$ ($c = 1.05$, *DCM*); IR (KBr): $\bar{\nu} = 3500–3300$, 1830, 1689, 1539 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): $\delta = 1.92–2.20$ (m, 2H), 3.32–3.41 (m, 2H), 4.69 (dd, $J = 8.0$, 4.0 Hz, 1H), 5.06 (s, 2H), 5.23 (m, 1H, NH), 7.32 (m, 5H) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): $\delta = 31.2$, 36.1, 66.3, 72.5, 97.0 (sept, $J = 35.0$ Hz), 118.1 (q, $J = 288.0$ Hz), 118.8 (q, $J = 289.0$ Hz), 127.5, 127.6, 127.9, 135.5, 155.8, 167.1 ppm; ^{19}F NMR (376 MHz, $CDCl_3$): $\delta = -81.5$ (m, 6F) ppm; MS (EI): m/z (%) = 401 $[M]^+$ (22), 108 $[C_6H_5CH_2OH]^+$ (100), 92 $[C_7H_8]^+$ (31).

(5S)-5-[2-(9-Fluorenylmethoxycarbonylamino)ethyl]-2,2-bis(trifluoromethyl)-1,3-dioxolan-4-one (**15a**/**3**, $C_{22}H_{17}F_6NO_5$)

Yield 66%; mp 119°C ($CHCl_3$/hexanes); $[\alpha]_D = -7.9°\ cm^3\ g^{-1}\ dm^{-1}$ ($c = 1.14$, *DCM*); IR (KBr): $\bar{\nu} = 3600–3300$, 1834, 1697, 1535 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): $\delta = 1.98$ (m, 1H), 2.18 (m, 1H), 3.32–3.41 (m, 2H), 4.16 (m, 1H), 4.44 (m, 2H), 4.63 (dd, $J = 8.0$, 4.0 Hz, 1H), 4.95 (m, 1H, NH), 7.26–7.30 (m, 2H), 7.35–7.39 (m, 2H), 7.53–7.55 (m, 2H), 7.72–7.74 (m, 2H) ppm; ^{13}C NMR (151 MHz, $CHCl_3$): $\delta = 32.3$, 37.1, 47.7, 67.1, 73.6, 98.1 (sept, $J = 36.0$ Hz), 119.3 (q, $J = 288.0$ Hz), 120.1 (q, $J = 289.0$ Hz), 120.5, 125.4, 127.6, 128.3, 141.9, 144.3, 157.0, 168.3 ppm; ^{19}F NMR (376 MHz, $CDCl_3$): $\delta = -81.5$ (m, 6F) ppm; MS (EI): m/z (%) = 489 $[M]^+$ (14), 323 $[M-C_3F_6O]^+$ $^+$ (14), 179 $[C_{14}H_{11}]^+$ (100), 166 (54), 69 (41), 55 (21).

(5S)-5-[2-(tert-Butyloxycarbonylamino)ethyl]-2,2-bis(trifluoromethyl)-5-methyl-1,3-dioxolan-4-one (**15b**/**1**, $C_{13}H_{17}F_6NO_5$)

Yield 70%; colorless crystals; $R_f = 0.22$ (eluent: *DCM*); mp 49–51°C; $[\alpha]_D = -4.0°\ cm^3\ g^{-1}\ dm^{-1}$ ($c = 1.0$, *DCM*); IR (KBr): $\bar{\nu} = 3340$, 1842, 1687, 1543 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): $\delta = 1.43$ (s, 9H), 1.65 (s, 3H), 2.07 (m, 2H), 3.29 (m, 1H), 3.44 (m, 1H), 4.70 (s br, 1H, NH) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): $\delta = 22.0$, 28.4, 35.1, 37.5, 79.8, 81.1, 97.1 (sept, $J = 35.7$ Hz), 119.2 (q, $J = 288.5$ Hz), 155.7, 170.2 ppm; ^{19}F NMR (376 MHz, $CDCl_3$): $\delta = -81.2$ (m, 6F) ppm; MS (EI): m/z (%) = 381 $[M]^+$ (2), 367 (5), 328 (16), 282 (14), 59 (93), 57 (100).

(5S)-5-[2-(Benzyloxycarbonylamino)ethyl]-2,2-bis(trifluoromethyl)-5-methyl-1,3-dioxolan-4-one (**15b**/**2**, $C_{16}H_{15}F_6NO_5$)

Yield 74%; colorless oil; $R_f = 0.32$ (eluent: *DCM*); $[\alpha]_D = -4.0°\ cm^3\ g^{-1}\ dm^{-1}$ ($c = 1.0$, *DCM*); IR (film): $\bar{\nu} = 3500–3200$, 1842, 1720, 1699, 1527 cm^{-1}; ^1H NMR (700 MHz, $CDCl_3$): $\delta = 1.63$ (s br, 3H); 2.09 (m, 2H); 3.35 (m, 1H), 3.48 (m, 1H), 5.01 (t br, 1H, NH), 5.09 (s br, 2H), 7.33 (m, 5H) ppm; ^{13}C NMR (176 MHz, $CDCl_3$): $\delta = 22.0$, 35.6, 37.3, 67.0, 81.0, 97.1 (sept, $J = 35.7$ Hz), 119.2 (q, $J = 288.6$ Hz), 128.2, 128.3, 128.6, 136.4, 156.3, 170.1 ppm; ^{19}F NMR (376 MHz, $CDCl_3$): $\delta = -81.2$ (m, 6F) ppm; MS (FAB, 3-*NBA*): $m/z = 416$ $[M + H]^+$.

(5S)-5-[2-(9-Fluorenymethyloxycarbonylamino)ethyl]-2,2-bis(trifluoromethyl)-5-methyl-1,3-dioxolan-4-one (**15b**/**3**, $C_{23}H_{19}F_6NO_5$)

Yield 76%; mp 75–78°C; $R_f = 0.30$ (eluent: *DCM*); $[\alpha]_D = -5.5°\ cm^3\ g^{-1}\ dm^{-1}$ ($c = 1.09$, *DCM*); IR (KBr): $\bar{\nu} = 3500–3300$, 1838, 1720, 1693, 1541 cm^{-1}; ^1H NMR (700 MHz, $CDCl_3$): $\delta = 1.64$ (s, 3H), 2.09 (t br, $J = 7.0$ Hz, 2H), 3.36 (m, 1H), 3.49 (m, 1H), 4.20 (t br, $J = 7.0$ Hz, 1H), 4.42 (d br, $J = 7.0$ Hz, 2H), 4.90 (t br, 1H, NH), 7.30 (m, 2H), 7.40 (m, 2H), 7.57 (m, 2H), 7.76 (m, 2H) ppm; ^{13}C NMR (176 MHz, $CDCl_3$): $\delta = 22.0$, 35.5, 37.3, 47.3, 66.8, 81.0, 97.3 (sept, $J = 35.0$ Hz), 119.1 (q, $J = 288.3$ Hz), 120.0, 125.0, 127.1, 127.8, 141.2, 143.8, 156.2, 170.1 ppm; ^{19}F NMR (376 MHz, $CDCl_3$): $\delta = -81.2$ (m, 6F) ppm; MS (EI): m/z (%) = 503 $[M]^+$ (2), 178 (100), 165 (13).

rac. 4-[2-(tert-Butyloxycarbonylamino)ethyl]-2,2-bis(trifluoromethyl)-1,3-oxathiolan-5-one (**16**/**1**, $C_{12}H_{15}F_6NO_4S$)

Yield 64%; colorless oil; IR (film): $\bar{\nu} = 3600–3200$, 1815, 1685, 1525 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 1.45$ (s, 9H), 2.06 (m, 1H), 2.41 (m, 1H), 3.33 (m, 2H), 4.25 (dd, $J = 9.8$, 4.1 Hz, 1H), 4.73 (t br, 1H, NH) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 28.3$, 34.6, 38.4, 44.0, 80.2, 83.3 (sept, $J = 34.9$ Hz), 120.9 (q, $J = 283.5$ Hz), 121.4 (q, $J = 284.3$ Hz), 156.1, 171.1 ppm; ^{19}F NMR (376 MHz, $CDCl_3$): $\delta = -76.5$ (q, $J = 9.1$ Hz, 3F), -77.4 (q, $J = 9.1$ Hz, 3F) ppm; MS (EI): m/z (%) = 383 $[M]^+$ (1), 327 (69), 282 (100), 243 (17), 178 (12), 117 (71); MS (FAB, 3-*NBA*): $m/z = 384$ $[M + H]^+$.

rac. 4-[2-(Benzyloxycarbonylamino)ethyl]-2,2-bis(trifluoromethyl)-1,3-oxathiolan-5-one (**16**/**2**, $C_{15}H_{13}F_6NO_4S$)

Yield 76%; light yellow oil; IR (film): $\bar{\nu} = 3500–3200$, 1815, 1699 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 2.06$ (m, 1H), 2.38 (m, 1H), 3.23–3.50 (m, 2H), 4.22 (m, 1H), 5.06 (t br, $J = 6.0$ Hz, 1H, NH), 5.10 (s br, 2H), 7.29–7.39 (m, 5H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 34.4$, 38.8, 43.9, 67.2, 83.3 (sept, $J = 34.8$ Hz), 120.9 (q, $J = 283.3$ Hz), 121.3 (q, $J = 284.3$ Hz), 128.2, 128.4, 128.6, 136.2, 156.8, 171.0 ppm; ^{19}F NMR (376 MHz, $CDCl_3$): $\delta = -76.5$ (q, $J = 9.2$ Hz, 3F), -77.3 (q, $J = 9.2$ Hz, 3F) ppm; MS (EI): m/z (%) = 417 $[M]^+$ (34), 147 (35) 100 (88), 92 (100), 69 $[CF_3]^+$ (84).

rac. 4-[2-(9-Fluorenylmethyloxycarbonylamino)ethyl]-2,2-bis(trifluoromethyl)-1,3-oxathiolan-5-one (**16**/**3**, $C_{22}H_{17}F_6NO_4S$)

Yield 76%; viscous oil; mp 88°C; IR (film): $\bar{\nu} = 3600–3300$, 1817, 1695 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 2.02$ (m, 1H), 2.32 (m, 1H), 3.29 (m, 1H), 3.38 (m, 1H), 4.11 (m, 1H), 4.18 (dd, $J = 10.0$, 4.0 Hz, 1H), 4.48 (m, 2H), 4.88 (m, 1H, NH), 7.30 (m, 2H), 7.39 (m, 2H), 7.56 (m, 2H), 7.75 (m, 2H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 34.4$, 38.7, 43.9, 47.3, 66.7, 83.3 (sept, $J = 34.8$ Hz), 120.1, 120.8 (q, $J = 283.9$ Hz), 121.3 (q, $J = 284.6$ Hz), 124.9, 127.1, 127.8, 141.4, 143.7, 156.7, 171.0 ppm; ^{19}F NMR (376 MHz, $CDCl_3$): $\delta = -76.6$ (q, $J = 9.0$ Hz, 3F), -77.2 (q, $J = 9.0$ Hz, 3F) ppm; MS (EI): m/z (%) = 505 $[M]^+$ (19), 178 (100), 165 (34).

Methyl (2S)-4-tert-butyloxycarbonylamino-2-hydroxy-2-methylbutyrate (**17**, $C_{11}H_{21}NO_5$)

A solution of **15b**/**1** (3.81 g, 10 mmol) in dry methanol (30 cm^3) was heated under reflux for 72 h (TLC control). Then the solvent was evaporated under reduced pressure and the residue was purified by column chromatography (eluent: $CHCl_3$/methanol, 20/1, $R_f = 0.32$). Yield 49%; colorless oil; $[\alpha]_D = -4.2°\ cm^3\ g^{-1}\ dm^{-1}$ ($c = 0.48$, methanol); IR (film): $\bar{\nu} = 3600–3200$, 2978, 2933, 1782, 1712, 1696 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 1.35$ (s, 3H), 1.36 (s, 9H), 1.74 (m, 1H), 2.01 (m, 1H), 2.97 (s, 1H, OH), 3.10 (m, 1H), 3.20 (m, 1H), 3.72 (s, 3H), 4.83 (s br, 1H, NH) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 26.6$, 28.4, 36.2, 39.2, 52.9, 74.8, 83.4, 156.0, 177.5 ppm; MS (ESI): $m/z = [M + Na]^+$ calcd 270.13119, found 270.13144.

tert-Butyl (S)-3-hydroxycarbamoyl-3-hydroxybutylcarbamate (**18**, $C_{10}H_{20}N_2O_5$)

To a stirred solution of **15b**/**1** (3.81 g, 10 mmol) in *DMF* (30 cm^3) hydroxylamine hydrochloride (0.70 g, 10.0 mmol) and propylene oxide (0.60 g, 10 mmol) were added. The reaction mixture was stirred for 24 h at room temperature (TLC control). Then the solvent was evaporated under reduced pressure and the residue was purified by column chromatography (eluent: $CHCl_3/CH_3OH$), 10/1, $R_f = 0.11$). **18** was isolated as a hygroscopic white solid after lyophilization. Yield 67%; $[\alpha]_D = -3.0°\ cm^3\ g^{-1}\ dm^{-1}$ ($c = 0.50$, CH_3OH); IR (KBr): $\bar{\nu} = 3600–3100$, 2976, 2929, 1689 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 1.22$ (s, 3H), 1.35 (s, 9H), 1.56 (m, 1H), 1.75 (m, 1H), 2.83 (m, 1H), 3.01 (m, 1H), 5.14 (s, 1H, OH), 6.59 (t br, 1H, NH), 8.59 (s br, 1H), 10.29 (s br, 1H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 26.4$, 28.2, 35.7, 39.9, 73.2, 77.3, 155.5, 171.7 ppm; MS (ESI): $m/z = [M + Na]^+$ calcd 271.12644, found 271.12670, $[2M + Na]^+$ calcd 519.26366, found 519.26389.

*Aminolysis of **15** and **16** (General procedure)*

To a stirred solution of equimolar amounts of *DIPEA* (1.29 g, 10.0 mmol) in *DMF* (20 cm^3) a solution of *L-Phe-OMe* × HCl (2.15 g, 10.0 mmol) in *DMF* was added. After 5 min **15**/**16** (10.0 mmol) was added. When the reaction was complete (TLC control) *DMF* was evaporated *in vacuo* and the residue was purified by column chromatography.

Methyl [(2S)-4-(benzyloxycarbonylamino)-2-hydroxybutyryl]phenylalaninate
(**19a**, $C_{22}H_{26}N_2O_6$)

Yield 62%; yellow oil; crystallizing on standing, mp 69–71°C; $R_f = 0.38$ (eluent: ethyl acetate/petroleum ether, 1/3); $[\alpha]_D = -5.6°\ cm^3\ g^{-1}\ dm^{-1}$ ($c = 1.0$, CH_3OH); IR (KBr): $\bar{\nu} = 3700–3200$, 1725, 1711, 1660 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): $\delta = 1.56$ (m, 1H), 1.91 (m, 1H), 3.03 (dd, $J = 14.0$, 7.2 Hz, 1H), 3.14 (dd, $J = 14.0$, 5.7 Hz, 2H), 3.37 (m, 1H), 3.68 (s, 3H), 4.06 (m, 1H), 4.54 (s br, 1H, OH), 4.84 (dt br, $J = 7.8$, 5.9 Hz, 1H), 5.04 (d, $J = 12.3$ Hz, 1H), 5.07 (d, $J = 12.3$ Hz, Hz, 1H), 5.38 (t, $J = 6.2$ Hz, 1H, NH), 7.10–7.33 (m, 10H), 7.40 (d, $J = 8.1$ Hz, 1H; NH) ppm; ^{13}C NMR (151 MHz, $CDCl_3$): $\delta = 34.9$, 37.0, 38.1, 52.3, 52.8, 67.0, 69.1, 127.1, 128.1, 128.2, 128.5, 128.6, 129.2, 135.9, 136.3, 157.8, 171.9, 173.5 ppm; MS (ESI): $m/z = [M + Na]^+$ calcd 399.10015, found 399.10077, $[M + H]^+$ calcd 377.11820, found 377.11871.

Methyl [(2S)-4-(benzyloxycarbonylamino)-2-hydroxy-2-methylbutyryl]phenylalaninate
(**19b**, $C_{23}H_{28}N_2O_6$)

Yield 84%; colorless oil; $R_f = 0.48$ (eluent: $CHCl_3/CH_3OH$, 10/1); $[\alpha]_D = -5.9°\ cm^3\ g^{-1}\ dm^{-1}$ ($c = 1.0$, CH_3OH); IR (film): $\bar{\nu} = 3600–3150$, 3100–2850, 1736, 1662 cm^{-1}; ^1H NMR (400 MHz, *DMSO*-d_6): $\delta = 1.19$ (s, 3H), 1.54 (m, 1H), 1.75 (m, 1H), 2.71 (m, 1H), 3.00 (m, 1H), 3.06 (d br, $J = 7.0$ Hz, 2H), 3.62 (s, 3H), 4.52 (dt, $J = 8.1$, 7.1 Hz, 1H), 4.99 (s br, 2H), 5.44 (s, 1H, OH), 6.97 (t,

$J = 5.2$ Hz, 1H, NH), 7.12–7.38 (m, 10H), 7.77 (d, $J = 8.1$ Hz, 1H, NH) ppm; ^{13}C NMR (101 MHz, *DMSO*-d_6): $\delta = 26.3$, 35.9, 36.3, 40.6, 51.8, 52.7, 65.0, 73.2, 126.4–137.2, 155.7, 171.6, 175.0 ppm; MS (ESI): $m/z = [M + H]^+$ calcd 429.20201, found 429.20238, $[M + Na]^+$ calcd 451.18396, found 451.18448.

(3S)-1-Benzyloxycarbonylamino-3-hydroxypyrrolidin-2-one (**20**, $C_{12}H_{13}NO_4$)

A solution of **15a**/**2** (5 mmol) in *DMF* (25 cm^3) was heated in a microwave oven under reflux for 30–60 min (TLC control). Then the solvent was evaporated *in vacuo* and the residue was purified by column chromatography (eluent: *DCM*). Yield 64%; colorless oil; $R_f = 0.21$ (eluent: *DCM*); $[\alpha]_D = -17.8°\ cm^3\ g^{-1}\ dm^{-1}$ ($c = 0.45$, *DCM*); IR (film): $\bar{\nu} = 3600$–3000, 1721, 1702, 1691 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.95$ (m, 1H), 2.40 (m, 1H), 3.54 (dt br, $J = 10.0$, 7.0 Hz, 1H), 3.86 (t br, $J = 10.0$ Hz, 1H), 4.37 (t, $J = 10.0$ Hz, 1H), 4.68 (s br, 1H, OH), 5.24 (s br, 2H), 7.26–7.41 (m, 5H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 26.8$, 42.1, 68.2, 70.3, 128.1, 128.4, 128.5, 134.9, 151.1, 174.8 ppm; MS (EI): m/z (%) = 235 $[M]^+$ (9), 209 (5), 197 (5), 167 (8), 147 (62), 115 (24), 18 (91), 95 (28), 91 (100), 79 (19), 69 (46).

Methyl [4-(benzyloxycarbonylamino)-2-mercaptobutyryl]phenylalaninate (**21**, $C_{22}H_{26}N_2O_5S$)

Yield 44%; yellow oil; $R_f = 0.55$ (eluent: $CHCl_3/CH_3OH$, 10/1, mixture of 2 diastereomers); IR (film): $\bar{\nu} = 3450$–3300, 3064, 3032, 2951, 1745, 1651 cm^{-1}; ^{1}H NMR (400 MHz, *DMSO*-d_6): $\delta = 1.62$ (m, 1H), 1.84 (m, 1H), 2.66/2.71 (2d, $J = 9.2$, 8.5 Hz, 1H, SH), 2.90 (m, 2H), 3.04 (m, 2H), 3.37 (m, 1H), 3.59/3.61 (s, 3H), 4.47 (m, 1H), 5.01 (s br, 2H), 7.10–7.40 (m, 10H arom, 1NH), 8.48/8.50 (2d, $J = 5.9$, 6.3 Hz, 1H, NH) ppm; ^{13}C NMR (101 MHz, *DMSO*-d_6): $\delta = 35.8$, 36.5/36.7, 38.0/38.1, 38.5/38.7, 51.7/51.8, 53.5/53.6, 65.1/65.2, 126.4/126.5, 128.1/128.2, 128.3, 129.0/129.0, 136.9/137.0, 137.0/137.1, 155.9/156.0, 171.5/171.6, 171.7/171.9 ppm; MS (ESI): $m/z = [M + H]^+$ calcd 431.16352, found 431.16364, $[M + Na]^+$ calcd 453.14546, found 453.14598, $[2M + Na]^+$ calcd 883.30171, found 883.30230.

tert-Butyl [(2S)-4-(9-fluorenylmethyloxycarbonylamino)-2-hydroxybutyryl]phenylalaninate (**22a**, $C_{32}H_{36}N_2O_6$)

Yield 62%; mp 126°C; $[\alpha]_D = 6.7°\ cm^3\ g^{-1}\ dm^{-1}$ ($c = 1.00$, *DCM*); IR (KBr): $\bar{\nu} = 3360$–3290, 1720, 1685, 1630, 1530 cm^{-1}; ^{1}H NMR (101 MHz, $CDCl_3$): $\delta = 1.37$ (s, 9H), 1.57 (m, 1H), 1.91 (m, 1H), 3.01 (dd, $J = 14.0$, 7.0 Hz, 1H), 3.08 (dd, $J = 14.0$, 7.0 Hz, 1H), 3.15 (m, 1H), 3.38 (m, 1H), 3.96 (m, 1H), 4.12 (m, 1H), 4.36 (m, 2H), 4.63 (s br, 1H, OH), 4.74 (m, 1H), 5.51 (m, 1H, NH), 7.13–7.17 (m, 2H), 7.18–7.27 (m, 5H), 7.32–7.36 (m, 2H), 7.44 (d, $J = 7.0$ Hz, 1H, NH), 7.51–7.54 (m, 2H), 7.69–7.71 (m, 2H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 27.4$, 34.4, 36.4, 37.8, 46.8, 52.7, 66.3, 68.3, 81.8, 119.5, 124.5, 126.3, 126.6, 128.0, 129.0, 140.8, 140.8, 143.2, 143.3, 157.4, 170.1, 172.9 ppm; MS (EI): m/z (%) = 500 $[M - CO_2]^+$ (0.7), 178 (100).

tert-Butyl [(2S)-4-(9-fluorenylmethyloxycarbonylamino)-2-hydroxy-2-methylbutyryl]phenylalaninate (**22b**, $C_{33}H_{38}N_2O_6$)

Yield 56%; mp 129°C; $R_f = 0.36$ (eluent: $CHCl_3/CH_3OH$, 10/1); $[\alpha]_D = -4.0°\ cm^3\ g^{-1}\ dm^{-1}$ ($c = 0.75$, CH_3OH); IR (KBr): $\bar{\nu} = 3600$–3200, 2975, 2929, 1725, 1654 cm^{-1}; ^{1}H NMR (400 MHz, *DMSO*-d_6): $\delta = 1.30$ (s, 3H), 1.34 (s, 9H), 1.58 (m, 1H), 1.79 (m, 1H), 2.77 (m, 1H), 3.02 (m, 3H), 4.20 (t, $J = 6.8$ Hz, 1H), 4.26 (d, $J = 6.8$ Hz, 2H), 4.41 (dt, $J = 7.2$, 7.1 Hz, 1H), 5.46 (s br, 1H, OH), 7.07 (t br, $J = 5.0$ Hz, 1H, NH), 7.14–7.29 (m, 5H), 7.64 (d, $J = 9.0$ Hz, 1H, NH), 7.32 (m, 2H), 7.40 (m, 2H), 7.67 (m, 2H), 7.88 (m, 2H) ppm; ^{13}C NMR (101 MHz, *DMSO*-d_6): $\delta = 27.4$, 27.5, 35.9, 36.7, 39.7,

46.6, 53.2, 65.1, 79.7, 80.8, 120.0, 125.0, 126.4, 126.9, 127.5, 128.0, 129.1, 136.9, 140.6, 143.8, 155.8, 170.1, 174.8 ppm; MS (ESI): $m/z = [M + H]^+$ calcd 559.28026, found 559.28079, $[M + Na]^+$ calcd 581.26221, found 581.26252.

tert-Butyl [4-(9-fluorenylmethyloxycarbonylamino)-2-mercaptobutyryl]phenylalaninate (**23**, $C_{32}H_{36}N_2O_5S$)

Yield 47%; yellow oil; $R_f = 0.45$ (eluent: $CHCl_3/CH_3OH$, 10/1, mixture of diastereomers); IR (film): $\bar{\nu} = 3600–3200$, 2992, 2948, 1740, 1655 cm^{-1}; ^1H NMR (400 MHz, *DMSO*-d_6): $\delta = 1.28/1.30$ (s, 9H), 1.81 (m, 2H), 2.79–3.11 (m, 5H), 3.54 (m, 1H), 4.19 (t br, 1H), 4.28 (d br, 2H), 4.41 (m, 1H), 7.12–7.91 (m, 14H), 8.37/8.46 (d br, 1H, NH) ppm; ^{13}C NMR (101 MHz, *DMSO*-d_6): $\delta = 27.4$, 31.4, 36.9, 37.9, 46.7, 50.6, 54.6, 65.3, 80.8, 120.0, 125.0, 126.4, 127.0, 127.5, 128.1, 129.1, 136.8, 140.7, 143.8, 155.9, 156.0, 170.1 ppm; MS (ESI): $m/z = [M + H]^+$ calcd 561.24187, found 561.24177.

Incorporation of Azaglycine (General Procedure)

15 (10.0 mol) was added to a stirred solution of NH_2NHCO_2Me (0.90 g, 10.0 mol) in *DMF* (15 cm^3). When the reaction was complete (TLC control) *DMF* was evaporated and the residue was purified by column chromatography (eluent: $CHCl_3/CH_3OH$, 10/1). Reactions with **16** were performed under argon.

Methyl [(2S)-4-benzyloxycarbonylamino-2-hydroxybutyryl]azaglycinate (**24a**, $C_{14}H_{19}N_3O_6$)

Yield 74%; hygroscopic brownish crystals; mp 103–105°C; $[\alpha]_D = -21.6°$ $cm^3\,g^{-1}\,dm^{-1}$ ($c = 0.51$, CH_3OH); IR (KBr): $\bar{\nu} = 3600–3200$, 1732, 1697, 1653 cm^{-1}; ^1H NMR (600 MHz, *DMSO*-d_6): $\delta = 1.63$ (m, 1H), 1.80 (m, 1H), 3.12 (s br, 2H), 3.58 (s, 3H), 3.97 (s br, 1H), 5.01 (s br, 2H), 5.70 (s br, 1H, OH), 7.20 (s br, 1H, NH), 7.31–7.36 (m, 5H), 8.98 (s br, 1H, NH), 9.57 (s br, 1H, NH) ppm; ^{13}C NMR (151 MHz, *DMSO*-d_6): $\delta = 34.6$, 36.8, 51.7, 65.1, 68.4, 127.6, 128.2, 137.2, 156.0, 156.4, 173.1 ppm; MS (ESI): $m/z = [M + H]^+$ calcd 326.13466, found 326.13420, $[M + Na]^+$ calcd 348.11661, found 348.11595.

Methyl [(2S)-4-benzyloxycarbonylamino-2-hydroxy-2-methylbutyryl)azaglycinate (**24b**, $C_{15}H_{21}N_3O_6$)

Yield 82%; oil; $R_f = 0.21$ (eluent: $CHCl_3/CH_3OH$, 10/1); $[\alpha]_D = -2.7°$ $cm^3\,g^{-1}\,dm^{-1}$ ($c = 0.75$, CH_3OH); IR (film): $\bar{\nu} = 3700–3100$, 3100–2800, 1698 br cm^{-1}; ^1H NMR (400 MHz, *DMSO*-d_6): $\delta = 1.25$ (s, 3H), 1.61 (m, 1H), 1.82 (m, 1H), 2.98 (m, 1H), 3.18 (m, 1H), 3.56 (s, 3H), 4.99 (s br, 2H), 5.38 (s br, 1H, OH), 7.07 (t br, $J = 5.0$ Hz, 1H, NH), 7.31 (m, 5H), 8.94 (s br, 1H, NH), 9.44 (s br, 1H, NH) ppm; ^{13}C NMR (101 MHz, *DMSO*-d_6): $\delta = 26.4$, 35.9, 40.4, 51.4, 65.0, 73.4, 127.6, 128.2, 137.2, 155.8, 156.4, 174.8 ppm; MS (ESI): $m/z = [M + H]^+$ calcd 340.15031, found 340.15065, $[M + Na]^+$ calcd 362.13226, found 362.13268.

Methyl [4-benzyloxycarbonylamino-2-mercaptobutyryl]azaglycinate (**25**, $C_{14}H_{19}N_3O_5S$)

Yield 71%; hygroscopic white solid; $R_f = 0.27$ (eluent: $CHCl_3/CH_3OH$, 10/1); IR (KBr): $\bar{\nu} = 3500–3200$, 3032, 1745, 1716, 1670 cm^{-1}; ^1H NMR (600 MHz, *DMSO*-d_6): $\delta = 1.71$ (m, 1H), 1.92 (m, 1H), 2.86 (d, $J = 8.4$ Hz, 1H, SH), 3.09 (m, 2H), 3.33 (m, 1H), 3.59 (s, 3H), 5.01 (s br, 2H), 7.26 (t br, $J = 5.0$ Hz, 1H, NH), 7.29–7.39 (m, 5H), 9.18 (s br, 1H, NH), 9.86 (s br 1H, NH) ppm; ^{13}C NMR (101 MHz, *DMSO*-d_6): $\delta = 35.9$, 37.0, 38.0, 52.0, 65.3, 127.7, 128.3, 137.1, 156.1, 156.5, 171.7 ppm; MS (ESI): $m/z = [M + H]^+$ calcd 342.11182, found 342.11135.

Methyl [(2S)-4-(9-fluorenylmethyloxycarbonylamino)-2-hydroxybutyryl]azaglycinate (**26a**, $C_{21}H_{23}N_3O_6$)

Yield 67%; white solid; mp 144–145°C; $[\alpha]_D = -13.3°\ cm^3\ g^{-1}\ dm^{-1}$ ($c = 0.60$, CH_3OH), IR (KBr): $\bar{\nu} = 3500–3200, 1695\ cm^{-1}$; ^1H NMR (600 MHz, *DMSO*-d_6): $\delta = 1.63$ (m, 1H), 1.81 (m, 1H), 3.11 (m, 2H), 3.58 (s, 3H), 3.97 (m, 1H), 4.21 (t, $J = 7.0$ Hz, 1H), 4.27 (d, $J = 7.0$ Hz, 2H), 5.54 (s br, 1H, OH), 7.26 (t br, $J = 5.0$ Hz, 1H, NH), 7.33 (m, 2H), 7.41 (m, 2H), 7.69 (m, 2H), 7.89 (m, 2H), 8.99 (s br, 1H, NH), 9.57 (s br, 1H, NH) ppm; ^{13}C NMR (151 MHz, *DMSO*-d_6): $\delta = 34.5$, 36.7, 46.6, 51.7, 65.2, 68.4, 120.0, 125.1, 127.0, 127.5, 140.6, 143.8, 156.0, 156.4, 173.1 ppm; MS (ESI): $m/z = [M + H]^+$ calcd 414.16596, found 414.16553.

Methyl [(2S)-4-(9-fluorenylmethyloxycarbonylamino)-2-hydroxy-2-methylbutyryl]azaglycinate (**26b**, $C_{22}H_{25}N_3O_6$)

Yield 87%; white solid; mp 75–77°C; $R_f = 0.38$ (eluent: $CHCl_3/CH_3OH$, 10/1); $[\alpha]_D = -3°\ cm^3\ g^{-1}\ dm^{-1}$ ($c = 1.0$, CH_3OH); IR (KBr): $\bar{\nu} = 3600–3100, 3100–2900, 1811, 1737, 1697\ cm^{-1}$; ^1H NMR (400 MHz, *DMSO*-d_6): $\delta = 1.26$ (s, 3H), 1.63 (m, 1H), 1.84 (m, 1H), 2.99 (m, 1H), 3.19 (m, 1H), 3.57 (s, 3H), 4.21 (m, 3H), 5.48 (s br, 1H, NH), 7.16 (s br, 1H, NH), 7.32 (m, 2H), 7.40 (m, 2H), 7.67 (m, 2H), 7.87 (m, 2H), 8.95 (s br, 1H, NH), 9.46 (s br, 1H, NH) ppm; ^{13}C NMR (101 MHz, *DMSO*-d_6): $\delta = 26.4$, 35.8, 40.1, 46.6, 51.7, 65.2, 73.3, 119.9, 125.1, 126.9, 127.5, 140.6, 143.8, 155.9, 156.5, 174.8 ppm; MS (ESI): $m/z = [M + Na]^+$ calcd 450.16356, found 450.16395.

Methyl [4-(9-fluorenylmethyloxycarbonylamino)-2-mercaptobutyryl]azaglycinate (**27**, $C_{21}H_{23}N_3O_5S$)

Yield 62%; hygroscopic solid; $R_f = 0.21$ (eluent: $CHCl_3/CH_3OH$, 10/1); IR (KBr): $\bar{\nu} = 3500–3300, 2920, 2852, 1736, 1658\ cm^{-1}$; ^1H NMR (400 MHz, *DMSO*-d_6): $\delta = 1.72$ (m, 1H), 1.94 (m, 1H), 2.85 (d, $J = 9.7$ Hz, 1H, SH), 3.10 (m, 2H), 3.32 (m, 1H), 3.59 (s, 3H), 4.21 (t, $J = 6.7$ Hz, 1H), 4.31 (d, $J = 6.7$ Hz, 2H), 7.33 (m, 3H), 7.41 (m, 2H), 7.69 (m, 2H), 7.89 (m, 2H), 9.18 (s br, 1H, NH), 9.87 (s br, 1H, NH) ppm; ^{13}C NMR (101 MHz, *DMSO*-d_6): $\delta = 35.9$, 37.1, 38.0, 46.7, 51.9, 65.3, 120.1, 125.1, 127.0, 127.6, 140.7, 143.9, 156.1, 156.6, 171.7 ppm; MS (ESI): $m/z = [2M + Na]^+$ calcd 881.26090, found 881.26006.

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