β-Lactam Derivatives as Enzyme Inhibitors: 1-Peptidyl Derivatives of 4-Phenylazetidin-2-one as Inhibitors of Elastase and Papain

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***Key Words*:** *1,4-Disubstituted azetidin-2-ones; serine protease inhibitors; porcine pancreatic elastase; cysteine protease inhibitors; papain*

Summary

N-Peptidyl substituted azetidin-2-ones were synthesized and evaluated as inhibitors of the serine protease elastase, and the cysteine protease papain. All compounds were synthesized from 4-phenylazetidin-2-one, either from the racemate or from the pure enantiomers. The (*S*)-enantiomer was prepared by enantioselective synthesis from (*S*)-β-phenyl-β-alanine, while the (*R*)-enantiomer was obtained by enzymatic resolution with α-chymotrypsin. *N*-Alkylation with bromoacetates introduced a spacer group which, after hydrolysis to the free acid, was acylated with amino acid esters or di- or tripeptide esters. The enzymatic assays proved some derivatives to be effective inhibitors of PPE and/or papain. N-BOC protected amino acid derivatives without a spacer group inhibited PPE reversibly, while derivatives with spacer group showed either weak or no inhibitory properties. On the other hand, papain was inactivated irreversibly by ethyl (*RS*)-2-oxo-4-phenylazetidin-1-acetate. The highest inhibitory activity against papain was found for the diastereomers of *N*-(2-oxo-4-phenylazetidin-1-acetyl)-L-alanyl-L-valine benzyl ester, a compound with a spacer group.

Introduction

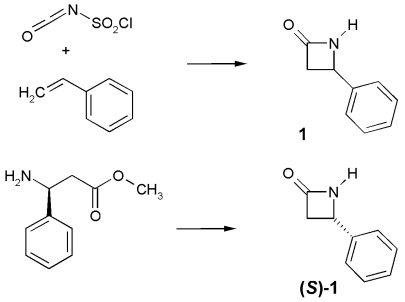
Proteases have been found to be involved in the pathogenesis of a large number of diseases. In particular, the serine protease elastase (HLE) is claimed to play a key role in the pathogenesis of lung diseases such as emphysema[1], ARDS[2], fibrosis[3], but also in arthritis[4] and arteriosclerosis[5]. Cysteine proteases[6], including calpains and most cathepsins, play a major role in processes with abnormal protein degradation, e.g. muscular dystrophy[7] or myocardial infarcts[8]. Increased levels of these proteases are also found in cancer[9], Alzheimer's disease[10], and cataracts[11]. It has been shown that inhibition of these proteases can have therapeutic effects on some of these diseases[12]. All thiol dependent cathepsins belong to the papain gene family[13]. Therefore, papain from the latex of the carica papaya fruit represents a simple and inexpensive model for the evaluation of cysteine protease inhibitors.

The development of protease inhibitors is based on the catalytic mechanisms and structures of the active sites of the proteases. All inhibitors developed up to now bear a chemically reactive group ("electrophilic trap"), which allows for the nucleophilic attack of the active site amino acids of the proteases. In addition, most inhibitors possess a peptide moiety responsible for recognition by the enzyme. Among the classes of cysteine protease inhibitors that appear most promising are epoxides[14] and aziridines[15]. Effective inhibitors of elastase include peptide aldehydes[16], trifluoromethyl ketones[17], as well as bicyclic[18] and monocyclic β-lactam derivatives[19,20]. In continuation of our interest in β-lactams as enzyme inhibitors[21] we reasoned that modification of our structures would even lead to potent or more potent inhibitors against proteases.

In this paper, we describe the syntheses of a number of aminoacyl and peptidyl substituted azetidin-2-ones and their activities as inhibitors of elastase and papain. In our concept we use the β-lactam ring as the electrophilic trap, and choose amino acids L-alanine, L-valine, and L-proline for the recognition part as they are reported to be effective in other inhibitors[16, 17]. Porcine pancreatic elastase (PPE) is similar to HLE. It is easily available, and therefore it is used in our experiments.

Results and Discussion

Racemic 4-phenylazetidin-2-one (**1**) was prepared in a two step synthesis from styrene and chlorosulfonylisocyanate according to Graf[22]. The (*S*)-enantiomer **(*S*)-1** was obtained in four steps from 3-amino-3-phenylpropionic acid. Esterification with methanol/$SOCl_2$ gave the methyl ester HCl,



Scheme 1

[1)] Part of the Ph. D. Thesis of K. A., University of Freiburg, 1997.

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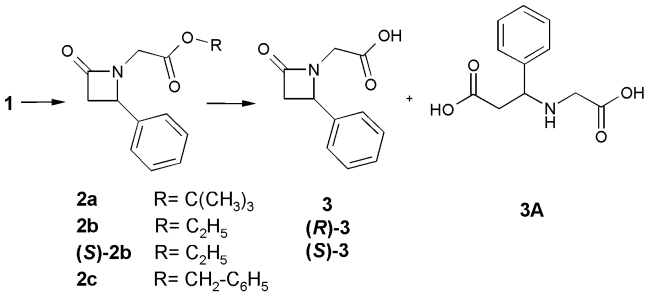
which was separated by kinetic resolution with (*R,R*)-tartaric acid via the diastereomeric salts according to Wassermann and Berger[23].

The formation of the β-lactam ring was directly achieved from methyl (*S*)-3-amino-3-phenylpropionate with *tert*-butylmagnesium chloride after *N*-silylation with chlorotrimethylsilane in the presence of triethylamine and 4-dimethylaminopyridine.

A very mild method for the *N*-alkylation of azetidin-2-ones is the use of phase-transfer catalysis (ptc)[24]. In agreement with this method described by Reuschling and Pietsch, *N*-alkylation of **1** and of **(*S*)-1** with alkyl bromoacetates was performed via solid/liquid phase transfer catalysis (Scheme 2)[25] yielding the corresponding compounds **2**.



Scheme 2

Hydrolysis of **2a** with trifluoroacetic acid gave the racemic β-lactam carboxylic acid **3** (83%). Alkaline hydrolysis of **2b** with refluxing 1 M NaOH gave the β-lactam carboxylic acid **3** and a ring opened by-product **3A**. This side reaction was avoided by reduced temperature (60 °C) during hydrolysis. The enzymatic hydrolysis of **2b** with α-chymotrypsin under pH-stat conditions[26] yielded the (*R*)-enantiomer **(*R*)-3** with 84% enantiomeric excess. An enzyme screening including porcine liver esterase (PLE), cholesterol esterase (ChE), porcine pancreatic lipase (PPL), lipase from *Penicillium roqueforti* (PrL), proteinase K (PrK), and α-chymotrypsin (CTR) selected α-chymotrypsin as the most appropriate enzyme for the enzymatic resolution of **2b** (Table 1).

Table 1. Enzyme screening for **2b**.

Enzyme (amount*)	Turnover (%) after 3 d	Opt. rotation acid / ester
without	0	0 / 0
PLE (200 µl)	100	0 / 0
ChE (50 mg)	19	n.d.** / –7°
PPL (500 mg)	0	0 / 0
PrL (50 mg)	0	0 / 0
PrK (50 mg)	39	56° / –91°
CTR (64 mg)	~50	80° / –113°

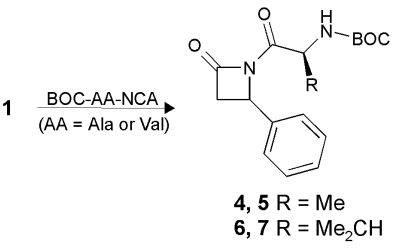
* Amount used for 150 mg **2b**. ** n.d. = not determined

The enantiomeric excess usually can be determined by ^{1}H NMR shift experiments using optical active shift reagents like TFMC-praseodymium(III) or TFEC-europium(III)[27]. However, this method failed, even when we used different concentrations of **3** and the reagents. Alternatively, we used HPLC with a Whelck-O1 column, showing that the product of enzymatic hydrolysis of **2b** consists of a 92:8 mixture of the enantiomers. The absolute configuration was established by CD spectroscopy. In accordance with the literature[28], we postulate for the compound with a positive Cotton effect at λ = 220 nm (Δε = +4.9), a negative effect at λ = 232 nm, and a positive effect at λ = 241 nm the (*S*)-configuration, **(*S*)-2b**. Vice versa, the CD spectrum of the acid **(*R*)-3** shows negative Cotton effects at λ = 217 nm (Δε = –10.7), and λ = 259 nm, and a positive effect at λ = 235 nm (Δε = +6.6). Furthermore, ***S*-2b** remaining from the enzymatic hydrolysis gave an equivalent CD spectrum and nearly identical $[\alpha]_D^{25}$ values as ***S*-2b** synthesized from ***S*-1**.

Hydrolysis of **(*S*)-2b** with NaOH or with PLE led to the (*S*)-enantiomer **(*S*)-3** with 51% or 59% yield. The catalytic hydrogenolysis of **2c** seemed to be not an appropriate route to **3**. Using an excess of hydrogen, the main product of this reaction was not **3**, but the ring opened N-(3-phenylpropionyl)glycine[29]. Reaction with equivalent amounts of H_2 showed preferred hydrogenolysis of the ester (83%), but the cyclic hetero benzyl bond was still attacked (17%).

The structures of compounds **2** and **3** were characterized by their spectroscopic data. The IR spectra show a strong carbonyl absorption between 1740 and 1765 cm^{-1} establishing the intact β-lactam ring. Furthermore, the ring is characterized by the ABX system of ring protons found in the ^{1}H NMR spectra with three signal groups around δ = 2.9, 3.45 and 4.85 ppm, and J_{AX} = 2.5, J_{AB} = 15.0 and J_{BX} = 5.2–5.5 Hz. The methylene group at nitrogen is detected by an AB system at δ = 3.3–3.5 and 4.25–4.40 ppm, $J_{AB} \approx 17.5$ Hz.

Due to the diminished reactivity of the nitrogen in **1** the acylation of the β-lactam ring was achieved only by deprotonation with BuLi followed by the reaction with *N*-protected amino acid *N*-carboxyanhydrides (NCA)[30]. In accordance with former results[31], under these conditions only acylation of the nitrogen occured. No C-3 acylated products were detected. The yields varied between 18 and 46%. From **1** and L-alanine we obtained **4**. Compound **5** was prepared from **(*S*)-1** and L-alanine, **6** was obtained from **1** and L-valine, and **7** from L-valine and **(*S*)-1**. The reaction failed when TEA was used for the deprotonation. Furthermore, experiments using anhydrides or chloroformates for activation of the amino acid were unsuccessful. During the syntheses no racemization was observed, as could be demonstrated by ^{1}H NMR-spectroscopy.



4, 5 R = Me
6, 7 R = Me_2CH

The peptides needed for the reactions with **3** were synthesized by coupling the respective *C*-protected amino acids or peptides with DCC/*N*-hydroxysuccinimide. Sterically hindered peptides could be obtained by coupling with diphenyl phosphorazidate (DPPA) (0 °C, DMF). The coupling of **3, (*S*)-3**, and **(*R*)-3** with peptides was achieved with DPPA or *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide · HCl (EDCl), alternatively to give the compounds **8–34** (Table 2). To obtain the unprotected analogs **35–42**, either the phenyl esters (**8**, **9**) were hydrolyzed (pH 10) with hydrogen peroxide[32] to yield **35** and **36**, or the trichloroethyl-protecting group[33] in the derivatives **23–25** and **32–34** was deprotected with zinc in glacial acetic acid to give **37–42** in quantitative yields[34].

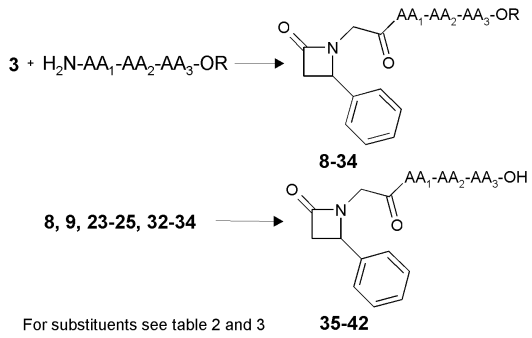


Table 2. Peptidyl β-lactams prepared from **3**.

No.	AA_1	AA_2	AA_3	Educt	Yield * mg/%	Optical rotation
8	Ala(OPh)	–	–	**3**	98/19%	–34.7
9	Ala(OPh)	–	–	**(*S*)-3**	100/19%	–111.4
10	Ala(OBn)	–	–	**3**	229/51%	–35.4
11	Phe(OMe)	–	–	**3**	250/36%	–17.3
12	Phe(OMe)	–	–	**(*S*)-3**	270/50%	–33.3
13	Phe(OMe)	–	–	**(*R*)-3**	200/56%	–20.3
14	Val(OBn)	–	–	**3**	213/44%	–22.2
15	Ala	Ala(OBn)	–	**3**	114/22%	–45.3
16	Ala	Ala(OBn)	–	**(*S*)-3**	248/39%	–114.6
17	Ala	Val(OBn)	–	**3**	322/29%	–28.6
18	Ala	Val(OBn)	–	**(*S*)-3**	456/41%	–110.9
19	Ala	Val(OBn)	–	**(*R*)-3**	275/48%	–17.0
20	Val	Ala(OBn)	–	**3**	453/40%	–52.1
21	Val	Ala(OBn)	–	**(*S*)-3**	264/39%	–111.4
22	Val	Ala(OBn)	–	**(*R*)-3**	130/23%	–7.4
23	Val	Ala(OTCE)	–	**3**	302/62%	–45.2
24	Val	Ala(OTCE)	–	**(*S*)-3**	230/47%	–101.1
25	Val	Ala(OTCE)	–	**(*R*)-3**	155/42%	–42.3
26	Val	Pro(OBn)	–	**3**	170/28%	–77.4
27	Val	Pro(OBn)	–	**(*S*)-3**	181/30%	–128.9
28	Val	Pro(OBn)	–	**(*R*)-3**	85/14%	–45.9
29	Val	Pro	Ala(OBn)	**3**	126/18%	–65.2
30	Val	Pro	Ala(OBn)	**(*S*)-3**	165/24%	–150.7
31	Val	Pro	Ala(OBn)	**(*R*)-3**	165/24%	–55.4
32	Val	Pro	Ala(OTCE)	**3**	300/41%	–76.9
33	Val	Pro	Ala(OTCE)	**(*S*)-3**	325/44%	–134.4
34	Val	Pro	Ala(OTCE)	**(*R*)-3**	152/43%	–31.8

* The yields refer to the last step of the synthesis.

Table 3. Peptidyl β-lactams (free acids).

No.	AA_1	AA_2	AA_3	Educt	Yield * mg/%	Optical rotation
35	Ala(OH)	–	–	**8**	115/46%	–22.9
36	Ala(OH)	–	–	**9**	39/47%	–37.8
37	Val	Ala(OH)	–	**23**	75/98%	–30.7
38	Val	Ala(OH)	–	**24**	129/98%	–12.5
39	Val	Ala(OH)	–	**25**	73/97%	–7.1
40	Val	Pro	Ala(OH)	**32**	78/99%	–61.8
41	Val	Pro	Ala(OH)	**33**	139/98%	–90.4
42	Val	Pro	Ala(OH)	**34**	91/97%	–30.5

***** All compounds are obtained as viscous materials.

Enzyme Kinetics

In order to evaluate whether the inhibition is reversible or irreversible, the time-dependent decrease of enzyme activity was monitored by the increase of UV absorption at 405 nm due to the release of *p*-nitroaniline from the respective substrates: Ac-Ala-Ala-Ala-pNA for elastase, and N^{α}-benzoyl-arginine-*p*-nitroanilide-HCl (L-BAPA) for papain.

In cases where no time-dependent inhibition was observed, the inhibitor constants K_I were calculated from Dixon plots. Lineweaver-Burke and Eadie-Hofstee plots indicated that the mode of inhibition of PPE is competitive for the substrate.

Alternatively, inactivation rates for time-dependent inhibition were determined by dilution assays according to Kitz and Wilson[35] or continuously as described by Tian und Tsou[36]. K_I values characterizing the inhibition of PPE and inactivation rates characterizing the inhibition of papain are presented in Table 4.

Discussion

The highest inhibitory activity against PPE was found for the *N*-acylated compounds **4**, **5**, and **6**. This is in good accordance with the results from Doherty et al. who found high inhibition constants for *N*-acylated β-lactams bearing a leaving group in position 4[37]. Compounds **10**, **11**, **14**, **15**, **17**, **18**, **20**, **26**, and **29** showed no or only weak inhibitory activity against PPE. Compared to the *C*-protected compound **20**, the deprotected compound **37** was a less efficient inhibitor. Compounds **2b** and **3** did not inhibit PPE. This does not seem to be surprising since **2b** serves as a substrate for the serine protease α-chymotrypsin, and the product of this enzymatic hydrolysis was **(*R*)-3**. Interestingly, the pure (*S*,*R*)-diastereomer **5** was less effective than the diastereomeric mixture **4**. This might suggest that the (*R*,*S*)-diastereomer of **5**, which was not available, is the better inhibitor than the (*S*,*R*)-diastereomer **5**. All compounds inhibited PPE in a competitive, reversible manner.

Table 4. Results of the enzymatic tests.

No.	Elastase K_I [mM]	Papain k_{2nd} [$M^{-1}min^{-1}$]	
		Continuous Assay	Dilution Assay
2a	–	–	8
2b	n.i.	–	57
(*S*)-2b	–	–	n.i.
3	n.i.	9	–
(*S*)-3	–	7	–
(*R*)-3	–	–	19
4	0.76	–	24
5	2.08	–	–
6	1.58	–	–
10	n.i.	n.i.	–
11	n.i.	11	–
14	1.6	78	–
15	n.i.	n.i.	–
17	1.4	773	–
18	n.i.	563	–
19	–	761	–
20	2.4	n.i.	–
26	n.i.	n.i.	–
29	n.i.	n.i.	–
37	2.1	n.i.	–
40	n.i.	n.i.	–

–: not determined; n.i.: no inhibition

The mixture of diastereomers **17** and the diastereomers **18** and **19** were the most potent inhibitors of papain. The diastereomeric compounds **18** and **19** did not show a stereodifferentiating profile. Furthermore, the *N*-alkylated compound **2b**, the *N*-acylated compounds **4**, and the β-lactam peptides **11** and **14** were weak inhibitors of papain. All compounds were irreversible inhibitors.

The different mechanisms of inhibition for PPE and papain may be explained by the higher nucleophilic properties of the thiolate residue of the catalytic active cysteine residue compared to the hydroxy group of the active site serine residue. Therefore, a nucleophilic attack with formation of a covalent bond to the carbonyl carbon is facilitated. Furthermore, the suitability of **2b** as a substrate for the enzymatic hydrolysis with a serine protease also indicates that the phenyl-substituted β-lactam ring-system can act only as a reversible inhibitor of elastase. Thus, the inhibitors either compete with the substrate for the active site or bind at a site near the active site, which also hinders the interaction of the substrate with the catalytic site.

Acknowledgment

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Experimental

General: Mp: not corrected; *Linström* apparatus. IR spectra (cm^{-1}): *Perkin-Elmer IR 841*; in KBr, if not noted otherwise. NMR spectra: *Varian U-300* (300 MHz) for ^1H; *Varian U-300* (75.4 MHz) for ^{13}C; δ in ppm; ^1H-values and ^{13}C-values from spectra in $CDCl_3$, if not noted otherwise. Standard ref. for all spectra in $CDCl_3$ or D_4-MeOH was the signal of the undeuterated solvent obtained in D_2O. UV spectra: *Pharmacia Ultrospec III*. Polarimeter: *Perkin-Elmer 241*. Mass Spectra: *Finnigan MAT 312*. pH-stat: *Metrohm 702 SM Titrino*. Elementary analyses: Pharmazeutisches Institut or Chemisches Laboratorium der Universität Freiburg. All compounds gave satisfactory elemental analyses or were proven by high resolution MS. R_f values from cc (silica gel 60 Merck 7734). Tlc (Merck Alufolien, silica gel 60 F_{254}, No. 5549).

Tetrahydrofuran (THF) was stored with KOH, then refluxed with Na and benzophenone, and distilled prior to use. Other solvents were dried/ purified according to literature procedures. Unless noted otherwise, reactions were conducted under an atmosphere of dry nitrogen.

PPE was purchased from Serva (Nr. 20929), α-chymotrypsin, and L-BAPA from Merck Darmstadt, proteinase K from Sigma, cholesterol esterase from Boehringer Mannheim, and papain from carica papya, PLE, PPL, and lipase from penicillium roqueforti from Fluka. All enzymes were used without further purification. All substrates were purchased from Bachem.

Abbreviations: L-BAPA = N^{α}-Benzoylarginine-*p*-nitroanilide HCl; BuLi = *n*-Butyllithium, 1.6 M solution in *n*-hexane; EtOAc = Ethyl acetate; TEA = Triethylamine; CTMS = Chlorotrimethylsilane; DMAP = 4-Dimethylaminopyridine; TMEDA = Tetramethylethylenediamine; DPPA = Diphenyl phosphorazidate; EDCl = *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide HCl; DCC = Dicyclohexylcarbodiimide; *ar* = aromatic.

*(RS)-4-Phenylazetidin-2-one (**1**)*

Overall yield: 60%.– Mp 105 °C (ref.[22] 107 °C).

(S)-4-Phenylazetidin-2-one (**(S)-1**)

To a solution of methyl (*S*)-3-amino-3-phenylpropionate (6.0 g, 33 mmol) in diethyl ether (150 ml) at 0 °C were added TEA (4.6 ml, 33 mmol), a catalytic amount of DMAP (≈ 50 mg) and CTMS (4.2 ml, 33 mmol). After stirring for 3 h, the mixture was maintained at room temp. for 12 h. The precipitate was filtered off, diethyl ether (600 ml) was added, and the solution was cooled to –5 °C. *tert*-Butylmagnesium chloride (18.2 ml, 37 mmol, as 2 M solution in diethyl ether) was added dropwise. The mixture was stirred vigorously for 30 min at –5 °C, and then 12 h at room temp. With cooling, the solution was acidified with HCl-satd. diethyl ether. The precipitated salt was dissolved in water (100 ml), the phases were separated and the aqueous layer was extracted with diethyl ether (100 ml). The combined organic layers were dried (Na_2SO_4) and concentrated to dryness. The residue was dissolved in methanol (50 ml), warmed to 50 °C and the solvent was removed *in vacuo*. The product was crystallized with cyclohexane. Recrystallization from cyclohexane/chloroform (1:1). Yield: 1.5 g (31%). Light yellow crystals.– Mp 108 °C (ref.[22] 107 °C).– $[\alpha]_D^{25}$ = –130.2° (*c* = 1.12, EtOH).

General Procedure for the N-Alkylation of **1**

The appropriate bromoacetate (10 mmol), and powdered KOH (0.62 g, 11 mmol) were added to a solution of **1** or **(*S*)-1** (1.47 g, 10 mmol), and tetrabutylammonium bromide (0.36 g, 1 mmol) in THF (50 ml). The mixture was stirred for 8–10 h (tlc control), filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by cc with EtOAc or recrystallized from ethanol/water (1:1).

tert-Butyl (RS)-2-(2-Oxo-4-phenylazetidin-1-yl) Acetate (**2a**)

Yield: 2.51 g (96%). Colourless crystals.– Mp 64 °C.– IR: ν = 3006, 2977, 2931 (CH), 1753 (C=O).– ^{1}H NMR: δ = 1.44 [s, 9H, $C(CH_3)_3$], 2.89, 3.45, 4.88 (ABX, J_{AX} = 2.5 Hz, J_{BX} = 5.5 Hz, J_{AB} = 15.0 Hz, 3H, 3-H, 3-H′, 4-H), 3.33, 4.27 (AB, *J* = 17.7 Hz, 2H, CH_2), 7.31 (m, 5H, *ar*-H).– Anal. ($C_{15}H_{19}NO_3$).

Ethyl (RS)-2-(2-Oxo-4-phenylazetidin-1-yl)acetate (**2b**)

Yield: After cc 1.65 g (71%). Colourless liquid [24].– R_f = 0.59.

Ethyl (S)-2-(2-Oxo-4-phenylazetidin-1-yl)acetate (**(S)-2b**)

Yield: After cc 1.51 g (65%). Colourless liquid.– R_f = 0.59.– $[\alpha]_D^{25}$ = –117.3° (0.99, $CHCl_3$).– IR (film): ν = 2983 (CH), 1765 (C=O).– ^{1}H NMR: δ = 1.25 (t, *J* = 7.0 Hz, 3H, CH_3), 2.92, 3.48, 4.88 (ABX, J_{AX} = 2.4 Hz, J_{BX} = 5.2 Hz, J_{AB} = 15.0 Hz, 3H, 3-H, 3-H′, 4-H), 3.44, 4.35 (AB, *J* = 17.4 Hz, 2H, CH_2), 4.16 (m, 2H, CH_2CH_3), 7.37 (m, 5H, *ar*-H).– Anal. ($C_{13}H_{15}NO_3$).

Benzyl (RS)-2-(2-Oxo-4-phenylazetidin-1-yl)acetate (**2c**)

Yield: After cc 2.48 g (84%). Colourless liquid. – R_f = 0.58.– IR (film): ν = 3033, 2958 (CH), 1759 (C=O).– ^{1}H NMR: δ = 2.87, 3.42, 4.84 (ABX, J_{AX} = 2.6 Hz, J_{BX} = 5.4 Hz, J_{AB} = 14.9 Hz, 3H, 3-H, 3-H′, 4-H), 3.42, 4.40 (AB, *J* = 18.1 Hz, 2H, CH_2), 5.13 [AB, *J* = 12.2 Hz, 2H, CH_2(benzyl)], 7.30 (m, 10H, *ar*-H).– Anal. ($C_{18}H_{17}NO_3$).

(RS)-2-(2-Oxo-4-phenylazetidin-1-yl)acetic Acid (**3**)

Method a: Trifluoroacetic acid (5 ml) was added at 0 °C to a solution of **2a** (4.0 g, 15.3 mmol) in CH_2Cl_2 (5 ml). After stirring for 1 h at 0 °C, the mixture was hydrolyzed with 0.1 M NaOH. The layers were separated, the aqueous layer was adjusted to pH 2 with 2 M HCl and extracted with CH_2Cl_2 (2 × 25 ml). The combined organic layers were dried (Na_2SO_4) and evaporated. Yield: 2.6 g (83%). Colourless liquid.

Method b: A solution of **2b** (2.0 g, 8.6 mmol) in 1 M NaOH solution (20 ml) was warmed to 60 °C for 20 min. After cooling, the mixture was extracted with EtOAc, and the organic phase was discharged. The aqueous layer was adjusted to pH 2 with 2 M HCl and extracted with EtOAc (3 × 50 ml). The combined organic layers were dried (Na_2SO_4) and evaporated. Yield: 1.5 g (87%). Colourless liquid.

IR (film): ν = 3440 (OH), 2928 (CH), 1737 (C=O).– ^{1}H NMR: δ = 2.94, 3.48, 4.88 (ABX, J_{AX} = 2.3 Hz, J_{BX} = 5.2 Hz, J_{AB} = 15.0 Hz, 3H, 3-H, 3-H′, 4-H), 3.47, 4.27 (AB, *J* = 18.3 Hz, 2H, CH_2), 7.35 (m, 5H, *ar*-H), 9.07 [s(br), 1H, CO_2H].– MS (70 eV): *m/z* (%) = 205 (2.3) [M^+], 177 (19), 160 (2.9) [M^+-CO_2H], 146 (1.5) [M^+-CH_2CO_2H], 104 (100).– $C_{11}H_{11}NO_3$ (205.21).– HRMS: Calcd. 205.0738; found 205.0739.

(S)-2-(2-Oxo-4-phenylazetidin-1-yl)acetic Acid (**(S)-3**)

From **(*S*)-2b** (2.0 g, 8.6 mmol) as described for **3** (method b). Yield: 0.9 g (51%). Colourless liquid.– $[\alpha]_D^{25}$ = –98.2° (*c* = 0.97, $CHCl_3$).

(R)-2-(2-Oxo-4-phenylazetidin-1-yl)acetic Acid (**(R)-3**)

Phosphate buffer (350 ml, pH 8, 0.05 M), and α-chymotrypsin (350 mg) were added to a solution of **2b** (3.5 g, 15 mmol) in acetone (35 ml). As soon as the pH started decreasing, the solution was adjusted to the initial pH with 0.1 M NaOH. When 0.4 eq. of sodium hydroxide was used, the reaction was stopped by adding EtOAc (200 ml). The phases were separated, and the aqueous layer was extracted with EtOAc (3 x 200 ml). The combined organic layers were dried (Na_2SO_4) and evaporated (phase I). The aqueous phase was adjusted to pH 2 with 2 M HCl, and extracted with EtOAc (3 x 200 ml). The combined organic layers were dried and evaporated (phase II). Phase I: Yield: 1.0 g (57%) **(*S*)-2b**. Colourless liquid. Phase II: Yield: 1.1 g (65%) **(*R*)-3**. Colourless liquid.– $[\alpha]_D^{25}$ = +80.3° (*c* = 1.09, $CHCl_3$).

(RS)-3-Aza-4-phenylhexane-1,6-diacid (**3A**)

By-product from the synthesis of **3**, method b. Yield: ca. 10%.– Mp. 201 °C.– IR: ν = 3426 (OH, NH), 2929 (CH), 1716, 1656 (C=O).– ^{1}H NMR ([D_4]MeOH): 3.07, 3.23, 4.77 (ABX, J_{AX} = 7.8 Hz, J_{BX} = 6.7 Hz, J_{AB} = 16.8 Hz, 3H, 3-H, 3-H′, 4-H), 3.61, 3.75 (AB, *J* = 16.8 Hz, 2H, CH_2), 7.29 (m, 5H, *ar*-H).– MS (70eV): *m/z* (%) = 223 (2), 177 (57) [M^+-CH_2CO_2H], 151 (44) [M^+-NH-CH_2-CO_2H]. HREIMS: Calcd. 223.0842; found 223.0845.– Anal. ($C_{11}H_{13}NO_4$).

General Procedure for the N-Acylation of **1, (*S*)-1**

A solution of **1** or **(*S*)-1** (6.8 mmol) in THF (50 ml) was cooled to –78 °C, BuLi (4.7 ml, 7.5 mmol) was added, and the mixture was stirred for 10 min. A solution of the appropriate BOC-amino-acid-NCA (6.8 mmol) in THF (10 ml) was added, and the reaction was completed by stirring at –78 °C for 1 h. The mixture was warmed to room temp., and subsequently quenched with a satd. solution of NH_4Cl (100 ml). The layers were separated, and the aqueous layer was extracted with EtOAc (2 × 50 ml). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. The product was purified by cc (silica gel/EtOAc), if not otherwise noted.

(S,R)-1-[(S)-N-(tert-Butoxycarbonyl)alanyl]-4-phenylazetidin-2-one (**4**)

From **1** and BOC-ala-NCA. Yield: 1.00 g (46%). Colourless solid.– Mp 140 °C.– R_f = 0.64.– α_D^{25} = –68.9° (*c* = 0.92, EtOH).– ^{1}H NMR: δ = 1.40 [2s, 9H, $C(CH_3)_3$], 1.42 (2AB, 3H, CH_3), 2.98, 3.53, 5.08 (2ABX, 3H, 3-H, 3-H′, 4-H), 4.92 [m, 1H, α-H(ala)], 5.08 (m, 1H, N-H), 7.31 (m, 5H, *ar*-H).– $C_{17}H_{22}N_2O_4$ (318.38).

(S)-1-[(S)-N-(tert-Butoxycarbonyl)alanyl]-4-phenylazetidin-2-one (**5**)

From **(*S*)-1** and BOC-ala-NCA. Yield: 0.48 g (22%). Light yellow solid.– Mp 137 °C.– R_f = 0.64.– $[\alpha]_D^{25}$ = –147.7° (*c* = 0.89, EtOH).– IR: ν = 3411 (NH), 1777, 1705 (C=O).– ^{1}H NMR: δ = 1.44 [d, 3H, CH_3; s, 9H, $C(CH_3)_3$], 2.98, 3.53, 5.07 (ABX, J_{AX} = 3.4 Hz, J_{BX} = 6.6 Hz, J_{AB} = 16.5 Hz, 3H, 3-H, 3-H′, 4-H), 4.93 [m, 1H, α-H(ala)], 5.10 (m, 1H, NH), 7.31 (m, 5H, *ar*-H).– Anal. ($C_{17}H_{22}N_2O_4$).

(S,R)-1-[(S)-N-(tert-Butoxycarbonyl)valyl]-4-phenylazetidin-2-one (**6**)

From **1** and BOC-val-NCA. Yield: 0.42 g (18%). Colourless solid.– Mp 40 °C.– R_f = 0.52.– α_D^{25} = -34.8° (*c* = 0.99, EtOH).– ^{1}H NMR: δ = 0.81, 1.07 (2d, *J* = 6.8 Hz, 6H, 2 CH_3), 1.43 [s, 9H, $C(CH_3)_3$], 2.25 [m, 1H, β-H(val)], 3.00. 3.53, 5.05 (ABX, J_{AX} = 3.7 Hz, J_{BX} = 6.7 Hz, J_{AB} = 16.6 Hz, 3H, 3-H, 3-H′, 4-H), 4.85 [m, 1H, α-H(val)], 5.0 (m, 1H,

N-H), 7.30 (m, 5H, *ar*-H).– MS (70 eV): *m/z* (%) = 346 (6) [M^+], 290 (12) [M^+-*t*Bu], 132 (64), 116 (97).– $C_{19}H_{26}N_2O_4$ (346.42).– HRMS: Calcd. 346.1889; found 346.1893.

*(S)-1-[(S)-N-(tert-Butoxycarbonyl)valyl]-4-phenylazetidin-2-one (**7**)*

From **(*S*)-1** and BOC-val-NCA. Yield: 0.14 g (6%). Colourless solid.– Mp 38 °C.– R_f = 0.52.– $[\alpha]_D^{25}$ = –129.7° (*c* = 0.78, EtOH).– IR: ν = 3377 (NH), 2971, 2933 (CH), 1793, 1707 (C=O).– ^{1}H NMR: δ = 0.81, 1.07 (2d, *J* = 6.8 Hz, 6H, 2 CH_3), 1.43 [s, 9H, $C(CH_3)_3$], 2.25 [m, 1H, β-H(val)], 3.00. 3.53, 5.05 (ABX, J_{AX} = 3.7 Hz, J_{BX} = 6.7 Hz, J_{AB} = 16.6 Hz, 3H, 3-H, 3-H′, 4-H), 4.85 [m, 1H, α-H(val)], 5.0 (m, 1H, N-H), 7.30 (m, 5H, *ar*-H).– MS (70 eV): *m/z* (%) = 346 (6) [M^+], 290 (12) [M^+-*t*Bu], 132 (64), 116 (97).– $C_{19}H_{26}N_2O_4$ (346.42).– HRMS: Calcd. 346.1889; found 346.1893.

General Procedure for the Coupling of **3, *S*-3** *or* ***R*-3** *with Amino Acid or Peptide Esters*

Method a: DPPA (1.1 eq) and TEA (2.1 eq) were added at 0 °C to a solution of **3, (*S*)-3** or **(*R*)-3** (1 eq) and the *N*-deprotected peptide or amino acid ester (1.04 eq) in DMF (8 ml). The mixture was stirred at 0 °C for 10 h. EtOAc was added, and the mixture was washed with water (3 × 50 ml), then with a satd. $NaHCO_3$ solution (50 ml), and then with brine (50 ml). The organic layer was dried (Na_2SO_4), the solvent was removed *in vacuo*, and the residue was purified by cc (silica gel, EtOAc).

Method b: EDCl (1.1 eq) and TEA (1 eq) were added to a solution of **3, (*S*)-3**, or **(*R*)-3** (1 eq) and the *N*-deprotected peptide or amino acid ester (1 eq) in DMF (20 ml). The mixture was stirred for 2 h at room temp., EtOAc was added, and the organic phase was separated, washed with water (50 ml), 1 M HCl (50 ml), water (50 ml), a satd. $NaHCO_3$ solution (50 ml), and finally with water (50 ml). The organic layer was dried (Na_2SO_4), and the solvent was removed *in vacuo*. The residue was purified by cc (silica gel/EtOAc).

Method a was used, if not noted otherwise.

*{2-[(S,R)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-alanine Phenyl Ester (**8**)*

Method a: From **3** (300 mg, 1.46 mmol), and L-alanine phenyl ester HCl (307 mg, 1.52 mmol). Yield: 98 mg (19%).

Method b: From **3** (450 mg, 2.2 mmol), and L-alanine phenyl ester HCl (442 mg, 2.2 mmol). Yield: 93 mg (12%). Colourless liquid.– R_f = 0.63.– α_D^{25} = –34.7° (*c* = 1.13, EtOH).– ^{1}H NMR: δ = 1.48, 1.53 (d, *J* = 7.3 Hz, 3H, CH_3), 2.89 (2.91), 3.46, 4.78 (ABX, J_{AX} = 2.7 Hz, J_{AB} = 15.0 Hz, 3H, 3-H, 3-H′, 4-H), 3.46, 4.15 (AB, 2H, CH_2), 4.70 [m, 1H, α-H(ala)], 6.74 [m, 1H, N-H(ala)], 7.17 (m, 10H, *ar*-H).– $C_{20}H_{20}N_2O_4$ (352.39). Ratio of diastereomers (^{13}C NMR) 1:1.

*{2-[(S)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-alanine Phenyl Ester (**9**)*

From (***S*)-3** (300 mg, 1.46 mmol), and L-alanine phenyl ester HCl (307 mg, 1.52 mmol). Yield: 100 mg (19%). Hard, glasslike liquid.– R_f = 0.63.– α_D^{25} = 111.4° (c = 0.90, EtOH).– IR (film): ν = 3291 (NH), 3067, 2936 (CH), 1746, 1674 (C=O).– ^{1}H NMR: δ = 1.53 (d, *J* = 7.6 Hz, 3H, CH_3), 2.98, 3.49, 4.77 (ABX, J_{AX} = 2.6 Hz, J_{BX} = 5.1 Hz, J_{AB} = 14.9 Hz, 3H, 3-H, 3-H′, 4-H), 3.49, 4.20 (AB, *J* = 16.6 Hz, 2H, CH_2), 4.77 [dq, *J* = 7.3 Hz, 1H, α-H(ala)], 6.74 (d, *J* = 7.3 Hz, 1H, N-H), 7.26 (m, 10H, *ar*-H).– ^{13}C NMR: δ = 17.76 [CH_3(ala)], 44.41 (CH_2), 46.85 (C-3), 48.21 [α-C(ala)], 55.32 (C-4), 121.15, 126.12, 126.53, 128.73, 128.85, 129.04, 129.44, 137.04, 150.32 (*ar*-C), 167.02, 168.42, 171.31 (C=O).– MS (CI): *m/z* (%) = 353 (100) [M^++1].– MS (70 eV): *m/z* (%) = 259 (74), 231 (50) [M^+-COOPh], 189 (100) [M^+-ala(OPh)], 160 (36) [M^+-CH_2COala(OPh)], 146 (41), 127 (76), 118 (52), 104 (34), 91 (48).– $C_{20}H_{20}N_2O_4$ (352.39).

*{2-[(S,R)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-alanine Benzyl Ester (**10**)*

From **3** (250 mg, 1.22 mmol), and L-alanine benzyl ester tosylate (445 mg, 1.26 mmol). Yield: 229 mg (51%). Colourless liquid.– R_f = 0.52.– α_D^{25} = –35.4° (*c* = 1.21, EtOH).– IR (film): ν = 3306 (NH), 1745, 1675 (C=O).– ^{1}H NMR: δ = 1.33, 1.40 (2d, *J* = 7.2 Hz, 3H, CH_3), 2.90, 2.95, 3.45, 4.70, 4.75 (2 ABX, 3H, 3-H, 3-H′, 4-H), 3.45, 4.10 (AB, 2H, CH_2), 4.55 [m, 1H, α-H(ala)], 5.16 [2AB, 2H, CH_2(benzyl)], 6.60 (m, 1H, N-H), 7.31 (m, 10H, *ar*-H).– ^{13}C NMR: δ = 17.59 [2 CH_3(ala)], 43.65, 43.90 (CH_2), 46.61, 46.67 (C-3), 47.87, 48.00 [C-α (ala)], 55.00, 55.06 (C-4), 66.76, 66.85 [CH_2(benzyl)], 126.29, 126.35, 127.77, 127.85, 128.09, 128.15, 128.30, 128.35, 128.44, 128.76, 128.79, 135.09, 135.12, 137.02, 137.16 (*ar*-C), 166.67, 166.78, 168.02, 168.08, 172.11 (C=O).– MS (70 eV): *m/z* (%) = 366 (8.0) [M^+], 338 (46), 275 (98), 189 (26), 118 (76), 91 (100).– $C_{21}H_{22}N_2O_4$ (366.42).– HRMS: Calcd. 366.1579; found 366.1579. Ratio of diastereomers (^{13}C NMR) 1:1.

*{2-[(S,R)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-phenylalanine Methyl Ester (**11**)*

From **3** (400 mg, 1.9 mmol), and L-phenylalanine methyl ester HCl (463 mg, 2.0 mmol). Yield: 250 mg (36%). Colourless liquid.– R_f = 0.52.– α_D^{25} = –17.3° (*c* = 0.97, EtOH).

*{2-[(S)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-phenylalanine Methyl Ester (**12**)*

From **(*S*)-3** (300 mg, 1.46 mmol), and L-phenylalanine methyl ester HCl (328 mg, 1.52 mmol). Yield: 270 mg (50%). Colourless liquid.– R_f = 0.42.– $[\alpha]_D^{25}$ = –33.3° (*c* = 0.70, EtOH).– IR (film): ν = 3299 (NH), 1749, 1687 (C=O).– ^{1}H NMR: δ = 2.90, 3.43, 4.68 (ABX, J_{AX} = 2.4 Hz, J_{BX} = 5.1 Hz, J_{AB} = 14.9 Hz, 3H, 3-H, 3-H′, 4-H), 3.04, 3.12, 4.81 [ABX, J_{AX} = 6.4 Hz, J_{BX} = 5.6 Hz, J_{AB} = 13.9 Hz, 3H, β-H(phe), α-H(phe)], 3.37, 4.12 (AB, *J* = 16.9 Hz, 2H, CH_2), 3.74 (s, 3H, OCH_3), 6.46 (d, *J* = 8.1 Hz, 1H, NH), 7.25 (m_c, 10H, *ar*-H).– ^{13}C NMR: δ = 37.27 [β-C(phe)], 44.44 (CH_2), 47.08 (C-3), 52.41 (OCH_3), 53.19 [α-C(phe)], 55.24 (C-4), 126.50, 127.22, 128.64, 128.80, 129.12, 129.19, 135.56, 137.10 (*ar*-C), 166.84, 168.09, 171.58 (C=O).– MS (70 eV): *m/z* (%) = 366 (39) [M^+], 205 (75), 175 (55), 162 (96), 149 (67) 131 (70), 118 (100).– $C_{21}H_{22}N_2O_4$ (366.42).– HRMS: Calcd. 366.1580; found 366.1580.

*{2-[(R)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-phenylalanine Methyl Ester (**13**)*

From **(*R*)-3** (200 mg, 0.97 mmol), and L-phenylalanine methyl ester HCl (218 mg, 1.01 mmol). Yield: 200 mg (56%). Colourless liquid.– R_f = 0.42.– $[\alpha]_D^{25}$ = –20.3° (*c* = 1.53, EtOH).– IR (film): ν = 3304 (NH), 1742, 1680 (C=O).– ^{1}H NMR: δ = 2.87, 3.36, 4.49 (ABX, J_{AX} = 2.6 Hz, J_{BX} = 5.2 Hz, J_{AB} = 14.7 Hz, 3H, 3-H, 3-H′, 4-H), 3.04, 3.18, 4.80 [ABX, J_{AX} = 5.8 Hz, J_{BX} = 5.8 Hz, J_{AB} = 14.0 Hz, 3H, β-H(phe), α-H(phe)], 3.36, 4.08 (AB, *J* = 16.9 Hz, 2H, CH_2), 3.70 (s, 3H, OCH_3), 6.40 (d(br), 1H, N-H), 7.25 (m, 10H, *ar*-H).– ^{13}C NMR: δ = 37.60 [β-C(phe)], 44.41 (CH_2), 47.06 (C-3), 52.40 (OCH_3), 53.10 [α-C(phe)], 55.24 (C-4), 126.47, 127.23, 128.65, 128.75, 129.07, 129.24, 135.73, 137.10 (*ar*-C), 166.89, 168.06, 171.49 (C=O).– MS (70 eV): *m/z* (%) = 366 (1.9) [M^+], 162 (44), 131 (32), 118 (99), 91 (100).– $C_{21}H_{22}N_2O_4$ (366.42).– HRMS: Calcd. 366.1580; found 366.1583.

*{2-[(S,R)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-valine Benzyl Ester (**14**)*

From **3** (250 mg, 1.22 mmol), and L-valine benzyl ester tosylate (509 mg, 1.27 mmol). Yield: 213 mg (44%). Colourless liquid.– R_f = 0.42.– α_D^{25} = –22.2° (*c* = 1.22, EtOH).– IR (film): ν = 3302 (NH), 1742, 1683 (C=O).– ^{1}H NMR: δ = 0.88 [m, 6H, CH_3(val)], 2.19 [m, 1H, β-H(val)], 2.94, 3.45, 4.71, 4.78 (2 ABX, J_{AX} = 2.4 Hz, J_{BX} = 5.4 Hz, 3H, 3-H, 3-H′, 4-H), 3.43, 3.53, 4.12, 4.20 (2 AB, *J* = 16.6 Hz, 2H, CH_2), 4.55 [m, 1H, α-H(val)], 5.18 [m, 2H, CH_2(benzyl)], 6.65 (m, 1H, N-H), 7.35 (m, 10H, *ar*-H).– ^{13}C NMR: δ = 17.62, 17.67, 18.92 [CH_3(val)], 31.03, 31.07 [β-C(val)], 44.28, 44.50 (CH_2), 46.89, 46.92 (C-3), 55.24, 55.40 (C-4), 57.08, 57.33 [α-C(val)], 66.97, 67.07 [CH_2(benzyl)], 126.49, 128.28, 128.36, 128.39, 128.46, 128.54, 128.59, 128.64, 128.71, 129.02, 129.06, 129.16, 135.26, 135.28, 137.29, 137.37 (*ar*-C), 167.42, 168.23, 168.34, 171.39, 171.44 (C=O).– MS (70 eV): *m/z* (%) = 394 (16) [M^+], 366 (40), 303 (83), 259 (41), 216 (74), 118 (92), 104 (75), 91 (100).– $C_{23}H_{26}N_2O_4$ (394.47).– HRMS: Calcd. 394.1893; found 394.1892. Ratio of diastereomers (^{13}C NMR) 1:1.

*{2-[(S,R)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-alanyl-L-alanine Benzyl Ester (**15**)*

From **3** (250 mg, 1.22 mmol), and L-alanyl-L-alanine benzyl ester HCl (361 mg, 1.26 mmol). Yield: 114 mg (22%). Colourless liquid.– R_f = 0.29.– α_D^{25} = –45.3° (*c* = 1.30, EtOH). Ratio of diastereomers (^{13}C NMR) 1:1.

*{2-[(S)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-alanyl-L-alanine Benzyl Ester (**16**)*

From **(*S*)-3** (300 mg, 1.46 mmol), and L-alanyl-L-alanine benzyl ester HCl (436 mg, 1.52 mmol). Yield: 248 mg (39%). Colourless solid.– Mp 108 °C.– R_f = 0.29.– $[\alpha]_D^{25}$ = 114.6° (*c* = 0.96, EtOH).– IR: ν = 3305 (NH), 1741, 1656 (C=O).– ^{1}H NMR: δ = 1.30, 1.43 (2d, each *J* = 6.8 Hz, 3H, CH_3), 2.97, 3.51, 4.78 (ABX, J_{AX} = 2.4 Hz, J_{BX} = 5.1 Hz, J_{AB} = 14.9 Hz, 3H, 3-H, 3-H′, 4-H), 3.46, 4.12 (AB, *J* = 16.9 Hz, 2H, CH_2), 4.42, 4.59 [2dq, each *J* = 7.3 Hz, 1H, α-H(ala)], 5.18 [AB, *J* = 12.2 Hz, 2H, CH_2(benzyl)], 6.47 (d, *J* = 7.1 Hz, 1H, N-H), 6.69 (d, *J* = 7.3 Hz, 1H, N-H), 7.38 (m, 10H, *ar*-H).– ^{13}C NMR: δ = 17.88, 18.30 [CH_3(ala)], 44.24 (CH_2), 46.99 (C-3), 48.23, 48.73 [α-C(ala)], 55.38 (C-4), 67.11 [CH_2(benzyl)], 126.55, 128.08, 128.39, 128.56, 128.74, 129.07, 135.28, 137.26 (*ar*-C), 166.96, 168.33, 171.57, 172.39 (C=O).– MS (70 eV): *m/z* (%) = 437 (3.7) [M^+], 259 (31), 189 (40), 91 (100).– $C_{24}H_{27}N_3O_5$ (437.50).– HRMS: Calcd. 437.1950; found 437.1949.

*{2-[(S,R)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-alanyl-L-valine Benzyl Ester (**17**)*

From **3** (500 mg, 2.44 mmol), and L-alanyl-L-valine benzyl ester HCl (798 mg, 2.53 mmol). Yield: 322 mg (29%). Colourless liquid.– R_f = 0.52.– α_D^{25} = –28.6° (*c* = 1.26, EtOH). Ratio of diastereomers (^{13}C NMR) 2 : 1.

*{2-[(S)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-alanyl-L-valine Benzyl Ester (**18**)*

From **(*S*)-3** (500 mg, 2.44 mmol), and L-alanyl-L-valine benzyl ester HCl (798 mg, 2.53 mmol). Yield: 456 mg (41%). Colourless solid.– Mp 95 °C.– R_f = 0.43.– $[\alpha]_D^{25}$ = 110.9° (*c* = 0.97, EtOH).– IR: ν = 3309 (NH), 1739, 1655 (C=O), 1534.– ^{1}H NMR: δ = 0.85, 0.89 [2d, each *J* = 6.9 Hz, 3H, CH_3(val)], 1.28 [d, *J* = 7.0 Hz, 3H, CH_3(ala)], 2.17 [m, 1H, β-H(val)], 2.92, 3.46, 4.79 (ABX, J_{AX} = 2.5 Hz, J_{BX} = 5.3 Hz, J_{AB} = 14.8 Hz, 3H, 3-H, 3-H′, 4-H), 3.40, 4.15 (AB, *J* = 16.8 Hz, 2H, CH_2), 4.44 [dq, *J* = 7.3 Hz, 1H, α-H(ala)], 4.52 [dd, *J* = 4.9 Hz, *J* = 8.5 Hz, 1H, α-H(val), 5.14 (AB, *J* = 12.2 Hz, 2H, CH_2), 6.76 [d, *J* = 8.4 Hz, 1H, N-H(val)], 7.04 [d, *J* = 7.3 Hz, 1H, N-H(ala)], 7.33 (m, 10H, *ar*-H).– ^{13}C NMR: δ = 17.55 [CH_3(ala)], 18.08, 18.94 [CH_3(val)], 31.12 [β-C(val)], 44.49 (CH_2), 47.02 (C-3), 48.90 [α-C(ala)], 55.47 (C-4), 57.27 [α-C(val)], 67.10 [CH_2(benzyl)], 126.58, 128.36, 128.47, 128.59, 128.84, 129.14, 129.29, 135.24, 137.19 (*ar*-C), 167.00, 168.31, 171.45, 171.73 (C=O).– MS (70 eV): *m/z* (%) = 465 (19) [M^+], 330 (50), 258 (54), 191 (75), 127 (75), 118 (78), 94 (98), 73 (100).– $C_{26}H_{31}N_3O_5$ (465.55).– HRMS: Calcd. 465.2264; found 465.2264.

*{2-[(R)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-alanyl-L-valine Benzyl Ester (**19**)*

From **(*R*)-3** (250 mg, 1.22 mmol), and L-alanyl-L-valine benzyl ester HCl (399 mg, 1.27 mmol). Yield: 275 mg (48%). Colourless liquid.– R_f = 0.43.– $[\alpha]_D^{25}$ = –17.0° (*c* = 0.64, EtOH).– IR: ν = 3306 (NH), 1747, 1658 (C=O).– ^{1}H NMR: δ = 0.82, 0.84 [2d, each *J* = 7.1 Hz, 3H, CH_3(val)], 1.28 [d, *J* = 6.8 Hz, 3H, CH_3(ala)], 2.14 [m, 1H, β-H(val)], 2.85, 3.38, 4.81 (ABX, J_{AX} = 2.4 Hz, J_{BX} = 5.1 Hz, J_{AB} = 14.9 Hz, 3H, 3-H, 3-H′, 4-H), 3.43, 4.21 (AB, *J* = 16.9 Hz, 2H, CH_2), 4.48 [dd, *J* = 5.1 Hz, *J* = 8.2 Hz, 1H, α-H(val)], 4.53 [dq, *J* = 7.1 Hz, 1H, α-H(ala)], 5.07, 5.17 (AB, *J* = 12.2 Hz, 2H, CH_2), 7.10 [d, *J* = 8.2 Hz, 1H, N-H(val)], 7.33 [m, 10H, *ar*-H; 1H, N-H(ala)].– ^{13}C NMR: δ = 17.46 [CH_3(ala)], 17.94, 18.76 [CH_3(val)], 30.72 [β-C(val)], 43.53 (CH_2), 46.87 (C-3), 48.68 [α-C(ala)], 55.17 (C-4), 57.20 [α-C(val)], 66.78 [CH_2(benzyl)], 126.35, 128.12, 128.21, 128.36, 128.46, 128.84, 135.14, 137.21 (*ar*-C), 167.05, 168.10, 171.31, 172.04 (C=O). Mixture of diastereomers 5 : 1; only the signals of the main product are listed.– MS (FAB): *m/z* (%) = 466 (100) [M^++1].– $C_{26}H_{31}N_3O_5$ (465.55).

*{2-[(S,R)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-valyl-L-alanine Benzyl Ester (**20**)*

From **3** (500 mg, 2.44 mmol), and L-valyl-L-alanine benzyl ester HCl (767 mg, 1.22 mmol), method b. Yield: 453 mg (40%). Colourless liquid.– R_f = 0.43.– α_D^{25} = –52.1° (*c* = 0.58, EtOH).– Ratio of diastereomers (^{13}C NMR) 1:1.– $C_{26}H_{31}N_3O_5$ (465.55).

*{2-[(S)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-valyl-L-alanine Benzyl Ester (**21**)*

From **(*S*)-3** (300 mg (1.46 mmol), and L-valyl-L-alanine benzyl ester HCl (503 mg, 1.60 mmol), method b. Yield: 264 mg (39%). Colourless solid.– Mp 133 °C.– R_f = 0.40.– $[\alpha]_D^{25}$ = –111.4° (*c* = 0.90, EtOH).– IR: ν = 3293 (NH), 3067, 2963 (CH), 1748, 1642 (C=O).– ^{1}H NMR: δ = 0.86, 0.91 [2d, each *J* = 6.6 Hz, 3H, CH_3(val)], 1.43 [d, *J* = 7.3 Hz, 3H, CH_3(ala)], 2.06 [m, 1H, β-H(val)], 2.95, 3.51, 4.76 (ABX, J_{AX} = 2.4 Hz, J_{BX} = 5.4 Hz, J_{AB} = 15.0 Hz, 3H, 3-H, 3-H′, 4-H), 3.44, 4.15 (AB, *J* = 16.9 Hz, 2H, CH_2), 4.22 [dd, *J* = 6.1 Hz, *J* = 8.8 Hz, 1H, α-H(val)], 4.60 [dq, *J* = 7.3 Hz, 1H, α-H(ala)], 5.15 [AB, *J* = 12.2 Hz, 2H, CH_2(benzyl)], 6.36 [d, *J* = 7.3 Hz, 1H, N-H(ala)], 6.67 [d, *J* = 8.8 Hz, 1H, N-H(val)], 7.37 (m, 10H, *ar*-H).– ^{13}C NMR: δ = 17.95 [CH_3(ala), CH_3(val)], 18.99 [CH_3(val)], 31.24 [β-C(val)], 44.47 (CH_2), 47.04 (C-3), 48.15 [α-C(ala)], 55.30 (C-4), 58.33 [α-C(val)], 67.13 [CH_2(benzyl)], 126.49, 128.13, 128.40, 128.55, 128.74, 129.09, 135.24, 137.28 (*ar*-C), 167.18, 168.34, 170.35, 172.38 (C=O).– MS (70 eV): *m/z* (%) = 465 (3.5) [M^+], 258 (75), 217 (79), 156 (73), 118 (81), 94 (100).– $C_{26}H_{31}N_3O_5$ (465.55).– HRMS: Calcd. 465.2264; found 465.2264.

*{2-[(R)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-valyl-L-alanine Benzyl Ester (**22**)*

From **(*R*)-3** (250 mg, 1.22 mmol), and L-valyl-L-alanine benzyl ester HCl (384 mg, 1.22 mmol), method b. Yield: 130 mg (23%). Colourless solid.– Mp 142 °C.– R_f = 0.40.– $[\alpha]_D^{25}$ = –7.4° (*c* = 1.07, EtOH).– IR: ν = 3295 (NH), 1763, 1643 (C=O).– ^{1}H NMR: δ = 0.89, 0.91 [2d, each *J* = 6.6 Hz, 3H, CH_3(val)], 1.36 [d, *J* = 7.3 Hz, 3H, CH_3(ala)], 2.05 [m, 1H, β-H(val)], 2.91, 3.44, 4.78 (ABX, J_{AX} = 2.4 Hz, J_{BX} = 5.1 Hz, J_{AB} = 14.9 Hz, 3H, 3-H, 3-H′, 4-H), 3.44, 4.16 (AB, *J* = 16.6 Hz, 2H, CH_2), 4.25 [dd, *J* = 6.6 Hz, *J* = 8.5 Hz, 1H, α-H(val)], 4.56 [dq, *J* = 7.3 Hz, 1H, α-H(ala)], 5.11, 5.18 (d, AB, *J* = 12.4 Hz, CH_2), 6.67 [d, *J* = 7.3 Hz, 1H, N-H(ala)], 6.67 [d, *J* = 8.5 Hz, 1H, N-H(val)], 7.33 (m, 10H, *ar*-H).– ^{13}C NMR: δ = 17.99 [CH_3(ala)], 18.07 [CH_3(val)], 19.00 [CH_3(val)], 31.21 [β-C(val)], 44.41 (CH_2), 47.10 (C-3), 48.16 [α-C(ala)], 55.52 (C-4), 58.45 [α-C(val)], 67.21 [CH_2(benzyl)], 126.51, 128.19, 128.46, 128.60, 128.75, 129.10, 135.20, 137.25 (*ar*-C), 167.27, 168.18, 170.11, 172.31 (C=O).– MS (70 eV): *m/z* (%) = 465 (0.9) [M^+], 259 (27), 217 (79), 188 (12), 155 (30), 118 (45), 91 (100).– $C_{26}H_{31}N_3O_5$ (465.55).– HRMS: Calcd. 465.2264; found 465.2274.

*{2-[(S,R)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-valyl-L-alanine 2,2,2-Trichloroethyl Ester (**23**)*

From **3** (250 mg, 1.22 mmol), and L-valyl-L-alanine 2,2,2-trichloroethyl ester HCl (452 mg, 1.27 mmol). Yield: 302 mg (62%). Colourless solid.– Mp 71 °C.– R_f = 0.63.– α_D^{25} = –45.2° (*c* = 1.06, EtOH).– $C_{21}H_{26}Cl_3N_3O_5$ (508.83).

*{2-[(S)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-valyl-L-alanine 2,2,2-Trichloroethyl Ester (**24**)*

From **(*S*)-3** (250 mg, 1.22 mmol), and L-valyl-L-alanine 2,2,2-trichloroethyl ester HCl (452 mg, 1.27 mmol). Yield: 230 mg (47%). Colourless solid.– Mp 123 °C.– $[\alpha]_D^{25}$ = –101.1° (*c* = 1.05, EtOH).– IR: ν = 3301 (NH), 1765, 1650 (C=O).– ^{1}H NMR: δ = 0.89, 0.95 [2d, each *J* = 6.8 Hz, 3H, CH_3(val)], 1.52 [d, *J* = 7.3 Hz, 3H, CH_3(ala)], 2.10 [m, 1H, β-H(val)], 2.97, 3.52, 4.78 (ABX, J_{AX} = 2.4 Hz, J_{BX} = 5.1 Hz, J_{AB} = 14.9 Hz, 3H, 3-H, 3-H′, 4-H), 3.48, 4.16 (AB, *J* = 16.9 Hz, 2H, CH_2), 4.27 [dd, *J* = 6.3 Hz, *J* = 8.8 Hz, 1H, α-H(val)], 4.66, 4.93 (AB, *J* = 11.9 Hz, 2H, CH_2), 4.71 [dq, *J* = 7.3 Hz, 1H, α-H(ala)], 6.44 [d, *J* = 7.3 Hz, 1H, N-H(ala)], 6.69 [d, *J* = 8.8 Hz, 1H, N-H(val)], 7.34 (m, 5H, *ar*-H).– ^{13}C NMR: δ = 17.77 [CH_3(ala)], 17.89, 19.03 [CH_3(val)], 31.06 [β-C(val)], 44.78 (CH_2), 47.01

(C-3), 48.06 [α-C(ala)], 55.41 (C-4), 58.37 [α-C(val)], 74.20 (CH_2), 94.48 (CCl_3), 126.47, 128.83, 129.14, 137.14 (*ar*-C), 167.22, 168.43, 170.45, 171.04 (C=O).– MS (70 eV): *m/z* (%) = 507 (2) [M^+], 287 (17), 259 (82), 217 (100), 188 (47), 155 (73).– $C_{21}H_{26}Cl_3N_3O_2$ (506.82).

*{2-[(R)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-valyl-L-alanine 2,2,2-Trichloroethyl Ester (***25***)*

From **(*R*)-3** (150 mg, 0.73 mmol), and L-valyl-L-alanine 2,2,2-trichloroethyl ester HCl (270 mg, 0.76 mmol). Yield: 155 mg (42%). Colourless liquid.– $[\alpha]_D^{25}$ = –42.3° (*c* = 0.55, EtOH).– IR: ν = 3281 (NH), 1758, 1650 (C=O).– ^{1}H NMR: δ = 0.95, 0.97 [2d, each *J* = 6.8 Hz, 3H, CH_3(val)], 1.49 [d, *J* = 7.3 Hz, 3H, CH_3(ala)], 2.12 [m, 1H, β-H(val)], 2.95, 3.49, 4.77 (ABX, J_{AX} = 2.4 Hz, J_{BX} = 5.1 Hz, J_{AB} = 14.9 Hz, 3H, 3-H, 3-H′, 4-H), 3.49, 4.14 (AB, *J* = 16.6 Hz, 2H, CH_2), 4.24 [dd, *J* = 6.6 Hz, *J* = 8.5 Hz, 1H, α-H(val)], 4.64, 4.92 (AB, *J* = 12.0 Hz, 2H, CH_2), 4.67 [dq, *J* = 7.3 Hz, 1H, α-H(ala)], 6.44 [d, *J* = 7.3 Hz, 1H, N-H(ala)], 6.72 [d, *J* = 8.5 Hz, 1H, N-H(val)], 7.35 (m, 5H, *ar*-H).– ^{13}C NMR: δ = 17.38 [CH_3(ala)], 18.12, 18.98 [CH_3(val)], 31.12 [β-C(val)], 44.01 (CH_2), 47.09 (C-3), 48.00 [α-C(ala)], 55.43 (C-4), 58.27 [α-C(val)], 74.05 (CH_2), 94.53 (CCl_3), 126.42, 128.63, 129.00, 137.29 (*ar*-C), 167.49, 168.04, 170.78, 170.97 (C=O). Ratio of diastereomers 3 : 1; only the signals of the main product are listed.– MS (70 eV): *m/z* (%) = 507 (3) [M^+], 259 (100), 217 (95), 155 (55).– $C_{21}H_{26}Cl_3N_3O_2$ (506.82).

*{2-[(S,R)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-valyl-L-proline Benzyl Ester (***26***)*

From **3** (250 mg, 1.22 mmol), and L-valyl-L-proline benzyl ester HCl (443 mg, 1.27 mmol). Yield: 170 mg (28%). Colourless liquid.– Ratio of diastereomers (^{13}C NMR) 3 : 2.– α_D^{25} = –77.4° (*c* = 0.78, EtOH).

*{2-[(S)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-valyl-L-proline Benzyl Ester (***27***)*

From **(*S*)-3** (250 mg, 1.22 mmol), and L-valyl-L-proline benzyl ester HCl (443 mg, 1.27 mmol). Yield: 181 mg (30%). Colourless liquid.– $[\alpha]_D^{25}$ = –128.9° (*c* = 1.65, EtOH).– IR (film): ν = 3297 (NH), 1753, 1680, 1629 (C=O).– ^{1}H NMR: δ = 0.81, 0.94 [2d, each *J* = 6.7 Hz, 3H, CH_3(val)], 1.99 [m, 3H, 3-H(pro), 4-H(pro), 4-H′(pro); m, 1H, β-H(val)], 2.23 [m, 1H, 3-H′(pro)], 2.93, 3.51, 4.78 (ABX, J_{AX} = 2.4 Hz, J_{BX} = 5.4 Hz, J_{AB} = 14.9 Hz, 3H, 3-H, 3-H′, 4-H), 3.39, 4.21 (AB, *J* = 16.9 Hz, 2H, CH_2), 3.64, 3.75 [2m, each 1H, 5-H(pro), 5-H′(pro)], 4.37 [m, 1H, α-H(pro); 1H, α-H(val)], 5.11, 5.18 [AB, *J* = 12.2 Hz, 2H, CH_2(benzyl)], 6.69 [d, *J* = 9.3 Hz, 1H, N-H(val)], 7.35 (m, 10H, *ar*-H).– ^{13}C NMR: δ = 17.49, 19.15 [CH_3(val)], 24.81 [(4′-C(pro)], 28.94 [3′-C(pro)], 31.31 [β-C(val)], 44.06 (CH_2), 47.17 [5′-C(pro)], 47.20 (C-3), 55.16 (C-4), 55.47 [α-C(pro)], 58.91 [α-C(val)], 66.83 [CH_2(benzyl)], 126.49, 128.12, 128.25, 128.47, 128.65, 129.03, 135.46, 137.39 (*ar*-C), 166.94, 168.14, 170.27, 171.54 (C=O).– MS (70 eV): *m/z* (%) = 491 (1.4) [M^+], 259 (16), 217 (23), 91 (85).– HRMS: Calcd. 491.2420, found 491.2420.– $C_{28}H_{33}N_3O_5$ (491.59).

*{2-[(R)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-valyl-L-proline Benzyl Ester (***28***)*

From **(*R*)-3** (250 mg, 1.22 mmol), and L-valyl-L-proline benzyl ester HCl (443 mg, 1.27 mmol). Yield: 85 mg (14%). Colourless liquid.– $[\alpha]_D^{25}$ = –45.9° (*c* = 0.85, EtOH).– IR (film): ν = 3252 (NH), 1748, 1632 (C=O).– ^{1}H NMR: δ = 0.88, 0.95 [2d, each *J* = 6.8 Hz, 3H, CH_3(val)], 2.00 [m, 3H, 3-H(pro), 4-H(pro), 4-H′(pro); m, 1H, β-H(val)], 2.18 [m, 1H, 3-H′(pro)], 2.88, 3.46, 4.79 (ABX, J_{AX} = 2.4 Hz, J_{BX} = 5.4 Hz, J_{AB} = 14.7 Hz, 3H, 3-H, 3-H′, 4-H), 3.41, 4.19 (AB, *J* = 16.6 Hz, 2H, CH_2), 3.63, 3.75 [2m, each 1H, 5-H, 5-H′(pro)], 4.53 [m, 1H, α-H(pro); 1H, α-H(val)], 5.09, 5.17 [AB, *J* = 12.2 Hz, 2H, CH_2(benzyl)], 6.84 [d, *J* = 8.8 Hz, 1H, N-H(val)], 7.35 (m, 10H, *ar*-H).– ^{13}C NMR: δ = 17.63, 19.22 [CH_3(val)], 24.84 [4′-C(pro)], 28.97 [3′-C(pro)], 31.38 [β-C(val)], 43.77 (CH_2), 47.23 [5′-C(pro); C-3], 55.33 (C-4), 55.54 [α-C(pro)], 58.94 [α-C(val)], 66.88 [CH_2(benzyl)], 126.50, 128.16, 128.28, 128.50, 128.58, 129.00, 135.49, 137.47 (*ar*-C), 167.13, 167.94, 170.27, 171.53 (C=O).– Ratio of diastereomers 4 : 1; only the signals of the main product are listed.– MS (70 eV): *m/z* (%) = 491 (11) [M^+], 287 (29), 259 (71), 217 (79), 155 (52), 91 (100).– HRMS: Calcd. 491.2420, found 491.2430.– $C_{28}H_{33}N_3O_5$ (491.59).

*{2-[(S,R)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-valyl-L-prolyl-L-alanine Benzyl Ester (***29***)*

From **3** (250 mg, 1.22 mmol), and L-valyl-L-prolyl-L-alanine benzyl ester HCl (523 mg, 1.27 mmol). Yield: 126 mg (18%). Colourless liquid.– α_D^{25} = –65.2° (*c* = 0.73, EtOH).– Ratio of diastereomers 4 : 1.– $C_{31}H_{38}N_4O_6$ (562.67).

*{2-[(S)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-valyl-L-prolyl-L-alanine Benzyl Ester (***30***)*

From **(*S*)-3** (250 mg, 1.22 mmol), and L-valyl-L-prolyl-L-alanine benzyl ester HCl (523 mg, 1.27 mmol). Yield: 165 mg (24%). Colourless solid.– Mp 112 °C.– $[\alpha]_D^{25}$ = –150.7° (*c* = 0.89, EtOH).– IR: ν = 3309 (NH), 1742, 1675, 1629 (C=O).– ^{1}H NMR: δ = 0.83, 0.95 [2d, each *J* = 6.8 Hz, 3H, CH_3(val)], 1.38 [d, *J* = 7.1 Hz, 3H, CH_3(ala)], 1.98 [m, 3H, 3-H(pro), 4-H(pro), 4′-H(pro); m, 1H, β-H(val)], 2.31 [m, 1H, 3-H′(pro)], 2.96, 3.53, 4.79 (ABX, J_{AX} = 2.5 Hz, J_{BX} = 5.4 Hz, J_{AB} = 14.9 Hz, 3H, 3-H, 3-H′, 4-H), 3.44, 4.21 (AB, *J* = 16.8 Hz, 2H, CH_2), 3.60, 3.70 [2m, each 1H, 5-H(pro), 5-H′(pro)], 4.56 [m, 1H, α-H(pro); 1H, α-H(val); 1H, α-H(ala)], 5.14, 5.20 [AB, *J* = 12.5 Hz, 2H, CH_2(benzyl)], 6.66 [d, *J* = 8.8 Hz, 1H, N-H(val)], 7.17 [d, *J* = 7.3 Hz, 1H, NH(ala)], 7.35 (m, 10H, *ar*-H).– ^{13}C NMR: δ = 17.58, 19.26 [CH_3(val)), 18.05 [CH_3(ala)), 25.00 [4′-C(pro)], 27.45 [3′-C(pro)], 31.44 [β-C(val)], 44.14 (CH_2), 47.14 (C-3), 47.71 [5′-C(pro)], 48.22 [α-C(ala)], 55.30 (C-4), 55.55 [α-C(pro)], 59.78 [α-C(val)], 67.04 [CH_2 (benzyl)], 126.54, 128.12, 128.37, 128.56, 128.73, 129.09, 135.38, 137.45 (*ar*-C), 167.10, 168.30, 170.59, 171.36, 172.57 (C=O).– MS (70 eV): *m/z* (%) = 562 (8.0) [M^+], 287 (39), 259 (27), 217 (23).– HRMS: calcd. 562.2791, found 562.2810.– $C_{31}H_{38}N_4O_6$ (562.67).

*{2-[(R)-2- Oxo-4-phenylazetidin-1-yl]acetyl}-L-valyl-L-prolyl-L-alanine Benzyl Ester (***31***)*

From **(*R*)-3** (250 mg, 1.22 mmol), and L-valyl-L-prolyl-L-alanine benzyl ester HCl (523 mg, 1.27 mmol). Yield: 165 mg (24%). Colourless solid.– Mp 125 °C.– $[\alpha]_D^{25}$ = –55.4° (*c* = 0.52, EtOH).– IR: ν = 3308 (NH), 1741, 1674, 1627 (C=O).– ^{1}H NMR: δ = 0.90, 0.97 [2d, each *J* = 6.6 Hz, 3H, CH_3(val)], 1.37 (d, *J* = 7.3 Hz, 3H, CH_3), 2.06 [m, 3H, 3-H(pro), 4-H(pro), 4-H′(pro); m, 1H, β-H(val)], 2.27 [m, 1H, 3-H′(pro)], 2.92, 3.48, 4.84 (ABX, J_{AX} = 2.4 Hz, J_{BX} = 5.1 Hz, J_{AB} = 14.7 Hz, 3H, 3-H, 3-H′, 4-H), 3.39, 4.24 (AB, *J* = 16.6 Hz, 2H, CH_2), 3.64 [m, 2H, 5-H(pro), 5′-H(pro)], 4.48 [dd, *J* = 4.4 Hz, *J* = 8.5 Hz, 1H, α-H(pro)], 4.54 [dq, *J* = 7.3 Hz, 1H, α-H(ala)], 4.58 [dd, *J* = 6.1 Hz, *J* = 8.9 Hz, 1H, α-H(val)], 5.13, 5.19 [AB, *J* = 12.3 Hz, 2H, CH_2(benzyl)], 6.79 [d, *J* = 8.8 Hz, 1H, N-H(val)], 7.22 [d, *J* = 7.6 Hz, 1H, N-H(ala)], 7.33 (m, 10H, *ar*-H).– ^{13}C NMR: δ = 17.49, 19.34 [CH_3(val)], 17.65 [CH_3(ala)], 25.09 [4′-C(pro)], 28.30 [3′-C(pro)], 31.30 [β-C(val)], 42.78 (CH_2), 46.92 (C-3), 47.71 [5′-C(pro)], 48.06 [α-C(ala)], 55.42 [C-4; α-C(pro)], 59.96 [α-C(val)], 66.83 [CH_2(benzyl)], 126.53, 128.04, 128.22, 128.46, 128.52, 128.95, 135.49, 137.41 (*ar*-C), 167.50, 167.80, 170.92, 171.05, 172.53 (C=O).– MS (70 eV): *m/z* (%) = 562 (0.4) [M^+], 287 (5.7), 259 (6.0), 217 (6.4), 155 (5.9), 118 (9.8), 91 (28).– HRMS: Calcd. 562.2791, found 562.2805.– $C_{31}H_{38}N_4O_6$ (562.67).

*{2-[(S,R)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-valyl-L-prolyl-L-alanine 2,2,2-Trichloroethyl Ester (***32***)*

From **3** (250 mg, 1.22 mmol), and L-valyl-L-prolyl-L-alanine 2,2,2-trichloroethyl ester HCl (576 mg, 1.27 mmol). Yield: 300 mg (41%). Colourless liquid.– α_D^{25} = –76.9° (*c* = 0.93, EtOH).– $C_{26}H_{33}Cl_3N_4O_6$ (603.93).

*{2-[(S)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-valyl-L-prolyl-L-alanine 2,2,2-Trichloroethyl Ester (***33***)*

From **(*S*)-3** (250 mg, 1.22 mmol), and L-valyl-L-prolyl-L-alanine 2,2,2-trichloroethyl ester HCl (576 mg, 1.27 mmol). Yield: 325 mg (44%). Colourless liquid.– $[\alpha]_D^{25}$ = –134.4° (*c* = 0.85, EtOH).– IR: ν = 3433 (NH), 1758, 1686, 1630 (C=O).– ^{1}H NMR: δ = 0.84, 0.95 [2d, each *J* = 6.8 Hz, 3H, CH_3(val)], 1.47 [d, *J* = 7.3 Hz, 3H, CH_3(ala)], 2.05 [m, 3H, 3-H(pro), 4-

H(pro), 4-H′(pro); m, 1H, β-H(val)], 2.37 [m, 1H, 3-H′(pro)], 2.96, 3.53, 4.78 (ABX, J_{AX} = 2.4 Hz, J_{BX} = 5.4 Hz, J_{AB} = 14.9 Hz, 3H, 3-H, 3-H′, 4-H), 3.44, 4.19 (AB, J = 16.8 Hz, 2H, CH_2), 3.60, 3.71 [2m, each 1H, 5-H(pro), 5′-H(pro)], 4.61 [m, 1H, α-H(pro); 1H, α-H(val); 1H, α-H(ala)], 4.64, 4.92 (AB, J = 12.0 Hz, 2H, CH_2), 6.63 [d, J = 8.5 Hz, 1H, N-H(val)], 7.23 [d, J = 7.3 Hz, 1H, N-H(ala)], 7.35 (m, 5H, *ar*-H).– ^{13}C NMR: δ = 17.67 [CH_3(ala), CH_3(val)], 19.17 (CH_3(val)], 24.93 (4′-C(pro)], 27.50 (3′-C(pro)], 31.35 (β-C(val)], 43.97 (CH_2), 47.00 (C-3), 47.74 [5′-C(pro), 48.00 [α-C(ala)], 55.27 (C-4), 55.59 [α-C(pro)], 59.66 [α-C(val)], 74.14 (CH_2), 94.55 (CCl_3), 126.47, 128.66, 129.03, 137.46 (*ar*-C), 167.10, 168.24, 170.85, 171.19, 171.42 (C=O).– MS (70 eV): *m/z* (%) = 604 (2.3) [M^+], 287 (46), 259 (37), 217 (37), 118 (23), 70 (100).– $C_{26}H_{33}Cl_3N_4O_6$ (603.93).

*{2-[(R)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-valyl-L-prolyl-L-alanine 2,2,2-Trichloroethyl Ester (**34**)*

From ***(R)*-3** (120 mg, 0.58 mmol), and L-valyl-L-prolyl-L-alanine 2,2,2-trichloroethyl ester HCl (273 mg, 0.61 mmol). Yield: 152 mg (43%). Colourless liquid.– $[\alpha]_D^{25}$ = –31.8° (*c* = 0.66, EtOH).– IR: ν = 3417 (NH), 1757, 1685, 1628 (C=O).– ^{1}H NMR: δ = 0.83, 0.94 [2d, each J = 6.8 Hz, 3H, CH_3(val)], 1.38 [d, J = 7.3 Hz, 3H, CH_3(ala)], 1.99 [m, 4H, 3-H(pro), 3-H′(pro), 4-H(pro), 4-H′(pro); m, 1H, β-H(val)], 2.83, 3.41, 5.00 (ABX, J_{AX} = 2.2 Hz, J_{BX} = 5.1 Hz, J_{AB} = 14.9 Hz, 3H, 3-H, 3-H′, 4-H), 3.13 (AB, J = 16.6 Hz, 1H, CH_2), 3.62, 3.69 [2m, each 1H, 5-H(pro), 5-H′(pro)], 4.53 [m, 1H, α-H(pro); 1H, α-H(val); 1H, α-H(ala); 1H, CH_2; 1H, CH_2], 4.88 (AB, J = 12.0 Hz, 1H, CH_2), 7.29 (m, 5H, *ar*-H), 7.77 [d, J = 7.1 Hz, 1H, N-H(ala)], 8.00 [d, J = 8.3 Hz, 1H, N-H(val)].– ^{13}C NMR: δ = 17.19 [CH_3(ala)], 17.45, 19.32 [CH_3(val)], 25.08 [4′-C(pro)], 28.48 [3′-C(pro)], 31.21 [β-C(val)], 42.53 (CH_2), 46.77 (C-3), 47.75 [5′-C(pro), 47.88 [α-C(ala)], 55.38 (C-4), 55.44 [α-C(pro)], 59.96 [α-C(val)], 74.06 (CH_2), 94.60 (CCl_3), 126.51, 128.51, 128.92, 137.21 (*ar*-C), 167.54, 167.87, 170.99, 171.21, 171.28 (C=O).– MS (70 eV): *m/z* (%) = 604 (2.3) [M^+], 455 (2.5), 287 (46), 259 (37), 217 (37), 70 (100).– $C_{26}H_{33}Cl_3N_4O_6$ (603.93).

*{2-[(S,R)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-alanine (**35**)*

A solution of H_2O_2 (30%, 74 µl) was added to a solution of **8** (250 mg, 0.90 mmol) in a mixture of acetone (8 ml) and water (3.3 ml), which was adjusted with NaOH (1M) to pH = 10.5. The mixture was kept with this pH for 5 min, then it was diluted with a few ml of water, and twice extracted with EtOAc. The aqueous layer was adjusted with dil. HCl to pH = 2, and twice extracted with EtOAc. These combined organic layers were dried (Na_2SO_4), and the solvent was removed in vacuo. Yield: 115 mg (46%). Yellowish viscous liquid.– α_D^{25} = –22.9° (*c* = 0.14, EtOH).– ^{1}H NMR ([D4]MeOH): δ = 1.34 (d, J = 7.6 Hz, 3H, CH_3), 2.88, 3.46, 4.84 (ABX, 3H, 3-H, 3-H′, 4-H), 3.46, 4.22 (2AB, each 2H, CH_2), 4.36 [m, 1H, α-H(ala)], 7.36 (m, 5H, ar-H).– $C_{14}H_{16}N_20_4$ (276.29).

*{2-[(S)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-alanine (**36**)*

From **9** (110 mg (0.29 mmol), acetone (4 ml), water (1.7 ml), and H_2O_2 (30%, 26 µl) as described for **35**. Yield: 39 mg (47%). Yellowish viscous liquid.– $[\alpha]_D^{25}$ = –37.8° (c = 0.45, EtOH).– IR: ν = 3404 (OH), 1744, 1665 (C=O).– ^{1}H NMR ([D4]MeOH): δ = 1.34 (d, J = 7.6 Hz, 3H, CH_3), 2.88, 3.46, 4.84 (ABX, 3H, 3-H, 3-H′, 4-H), 3.46, 4.22 (2AB, each 2H, CH_2), 4.36 [m, 1H, α-H(ala)], 7.36 (m, 5H, *ar*-H).– ^{13}C NMR ([D4]MeOH): δ = 17.57 [CH_3(ala)], 44.11 (CH_2), 47.40 (C-3), 56.40 (C-4), 127.73, 129.72, 130.11, 138.82 (*ar*-C), 169.11, 170.52, 175.53 (C=O).– MS (CI): *m/z* (%) = 277 (100) [M^++1].– MS (70 eV): *m/z* (%) = 259 (48), 248 (41), 231 (37) [M^+-COOH], 189 (78) [M^+-ala(OH)], 160 (39) [M^+-CH_2CO-ala(OH)], 146 (68), 132 (100), 118 (71), 104 (49), 91 (60).– $C_{14}H_{16}N_20_4$ (276.29).

General Procedure for the Cleavage of the 2,2,2-Trichloroethyl Group

The protected compound (100–200 mg) was dissolved in glacial acetic acid. Zinc powder (400 mg) was added. After stirring for 1 h at room temp., EtOAc was added and the suspension filtered. The filtrate was concentrated *in vacuo*, and the residue was dried over KOH *in vacuo*.

*{2-[(S,R)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-valyl-L-alanine (**37**)*

From **23** (100 mg, 0.20 mmol). Yield: 75 mg (98%). Colourless foam.– α_D^{25} = 30.7° (*c* = 0.38, EtOH).– $C_{19}H_{25}N_3O_5$ (375.43).

*{2-[(S)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-valyl-L-alanine (**38**)*

From **24** (180 mg, 0.35 mmol). Yield: 129 mg (98%). Colourless foam.– $[\alpha]_D^{25}$ = –12.5° (*c* = 0.40, EtOH).– IR: ν = 3307 (OH), 2967 (CH), 1731, 1653 (C=O).– ^{1}H NMR ([D4]MeOH): δ = 0.86, 0.94 [2d, each J = 6.8 Hz, 3H, CH_3(val)], 1.39 [d, J = 7.3 Hz, 3H, CH_3(ala)], 2.20 [m, 1H, β-H(val)], 2.88, 3.48, 4.80 (ABX, J_{AX} = 2.4 Hz, J_{BX} = 5.1 Hz, J_{AB} = 14.9 Hz, 3H, 3-H, 3-H′, 4-H), 3.50, 4.26 (AB, J = 16.9 Hz, 2H, CH_2), 4.21 [d, J = 7.1 Hz, 1H, α-H(val)], 4.34 [q, J = 7.3 Hz, 1H, α-H(ala)], 7.37 (m, 5H, *ar*-H).– ^{13}C NMR ([D4]MeOH): δ = 17.99 [CH_3(ala)], 18.50, 19.65 [CH_3(val)], 32.07 [β-C(val)], 44.30 (CH_2), 47.42 (C-3), 56.45 (C-4), 60.01 [α-C(val)], 127.70, 129.74, 130.14, 138.86 (*ar*-C), 169.33, 170.61, 172.81, 187.16 (C=O).– MS (ESI): *m/z* (%) = 470 (64) $[M+2Na+K]^{3+}$, 438 (100) $[M+1H+Na+K]^{3+}$.– $C_{19}H_{25}N_3O_5$ (375.43).

*{2-[(R)-2- Oxo-4-phenylazetidin-1-yl]acetyl}-L-valyl-L-alanine (**39**)*

From **25** (100 mg, 0.20 mmol). Yield: 73 mg (97%). Colourless foam.– $[\alpha]_D^{25}$ = –7.1° (*c* = 0.35, EtOH).– IR: ν = 3309 (OH), 2970 (CH), 1752, 1643 (C=O).– ^{1}H NMR ([D4]MeOH): δ = 0.91, 0.96 [2d, each J = 6.8 Hz, 3H, CH_3(val)], 1.39 [d, J = 7.3 Hz, 3H, CH_3(ala)], 2.05 [m, 1H, β-H(val)], 2.87, 3.46, 4.81 (ABX, J_{AX} = 2.4 Hz, J_{BX} = 5.1 Hz, J_{AB} = 14.9 Hz, 3H, 3-H, 3-H′, 4-H), 3.53, 4.22 (AB, J = 16.7 Hz, 2H, CH_2), 4.18 [d, J = 6.8 Hz, 1H, α-H(val)], 4.67 [q, J = 7.3 Hz, 1H, α-H(ala)], 7.36 (m, 5H, *ar*-H).– ^{13}C NMR ([D4]MeOH): α = 18.11 [CH_3(ala)], 18.49, 19.67 [CH_3(val)], 32.01 [β-C(val)], 44.23 (CH_2), 47.45 (C-3), 56.62 (C-4), 60.11 [α-C(val)], 127.70, 129.67, 130.08, 138.97 (*ar*-C), 169.35, 169.55, 170.66, 172.89 (C=O).– Ratio of diastereomers 3 : 1; only the signals of the main product are listed.– MS (ESI): *m/z* (%) = 470 (100) $[M+2Na+K]^{3+}$, 438 (77) $[M+1H+Na+K]^{3+}$.– $C_{19}H_{25}N_3O_5$ (375.43).

*{2-[(S,R)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-valyl-L-prolyl-L-alanine (**40**)*

From **32** (100 mg, 0.17 mmol). Yield: 78 mg (100%). Colourless foam.– α_D^{25} = –61.8° (*c* = 0.38, EtOH).– $C_{24}H_{32}N_4O_6$ (472.54).

*{2-[(S)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-valyl-L-prolyl-L-alanine (**41**)*

From **33** (180 mg, 0.30 mmol). Yield: 139 mg (98%). Colourless foam.– $[\alpha]_D^{25}$ = –90.4° (*c* = 0.45, EtOH).– IR: ν = 3323 (OH), 2974 (CH), 1740, 1628 (C=O).– ^{1}H NMR ([D4]MeOH): δ = 0.88, 0.99 [2d, each J = 6.6 Hz, 3H, CH_3(val)], 1.39 [d, J = 7.3 Hz, 3H, CH_3(ala)], 2.07 [m, 4H, 3-H(pro), 3-H′(pro), 4-H(pro), 4-H′(pro); m, 1H, β-H(val)], 2.88, 3.47, 4.79 (ABX, J_{AX} = 2.4 Hz, J_{BX} = 5.1 Hz, J_{AB} = 14.7 Hz, 3H, 3-H, 3-H′, 4-H), 3.50, 4.21 (AB, J = 16.9 Hz, 2H, CH_2), 3.67, 3.85 [2m, each 1H, 5-H(pro), 5-H′(pro)], 4.30 [q, J = 7.3 Hz, 1H, α-H(ala)], 4.43 [m, 2H, α-H(pro), α-H(val)], 7.35 (m, 5H, *ar*-H).– ^{13}C NMR ([D4]MeOH): δ = 18.31 [CH_3(ala)], 18.70, 19.64 [CH_3(val)], 25.90 [4′-C(pro)], 30.30 [3′-C(pro)], 31.74 [β-C(val)], 44.08 (CH_2), 47.45 (C-3), 56.46 (C-4), 58.00 [α-C(pro)], 61.49 [α-C(val)], 127.71, 129.74, 130.12, 138.89 (*ar*-C), 169.32, 170.54, 172.28, 173.54, 178.05 (C=O). 5′-C(pro) α-C(ala) are not detectable.– MS (ESI): *m/z* (%) = 535 (100) $[M+1H+Na+K]^{3+}$.– $C_{24}H_{32}N_4O_6$ (472.54).

*{2-[(R)-2-Oxo-4-phenylazetidin-1-yl]acetyl}-L-valyl-L-prolyl-L-alanine (**42**)*

From **34** (120 mg, 0.20 mmol). Yield: 91 mg (97%). Colourless foam.– $[\alpha]_D^{25}$ = –30.5° (*c* = 0.30, EtOH).– IR: ν = 3429 (OH), 2975 (CH), 1741, 1632 (C=O).– ^{1}H NMR ([D4]MeOH): δ = 0.93, 1.00 [2d, each J = 6.8 Hz, 3H, CH_3(val)], 1.38 [d, J = 7.1 Hz, 3H, CH_3(ala)], 2.07 [m, 5H, 3-H(pro), 3-H′(pro), 4-H(pro), 4-H′(pro), β-H(val)], 2.86, 3.47 (ABX, J_{AX} = 2.4 Hz, J_{BX} = 5.1 Hz, J_{AB} = 14.9 Hz, 3H, 3-H, 3-H′, 4-H), 3.50, 4.21 (AB, J = 16.8 Hz, 2H, CH_2), 3.67, 3.86 [2m, each 1H, 5-H(pro), 5-H′(pro)], 4.30 [q, J = 7.1 Hz, 1H, α-H(ala)], 4.39 [d, J = 6.8 Hz, 1H, α-H(val)], 4.44 [dd,

J = 4.5 Hz, *J* = 6.1 Hz, 1H, α-H(pro)], 7.38 (m, 5H, *ar*-H). 4-H not visible.– ^{13}C NMR ([D4]MeOH): α = 18.23 [CH3(ala)], 18.65, 19.66 [CH3(val)], 25.93 [4′-C(pro)], 30.29 [3′-C(pro)], 31.64 [β-C(val)], 44.03 (CH_2), 47.49 (C-3), 56.64 (C-4), 58.09 [α-C(pro)], 61.49 [α-C(val)], 127.67, 129.68, 130.08, 138.97 (*ar*-C), 169.52, 170.58, 172.38, 173.55, 177.08 (C=O). 5′-C(pro) and α-C(ala) are not detectable.– MS (ESI): *m/z* (%) = 535 (100) $[M+1H+Na+K]^{3+}$.– $C_{24}H_{32}N_4O_6$ (472.54).

Enzyme Kinetics

Elastase

The assays were performed at PPE concentrations of 7.5 μg/ml and substrate concentrations between 0.46–0.52 mM Ac-Ala-Ala-Ala-pNA in 0.1 M tris-buffer, pH 8.0. Stock solutions of PPE were prepared by dissolving the enzyme in 1 mM acetic acid. The rate of enzymatic hydrolysis was monitored at 405 nm continuously for 20 min via the release of *p*-nitroaniline at 25 °C at 405 nm (ε = 9960 l mol^{-1} cm^{-1}). The K_m-value was calculated to be 0.69 mM. Both substrate and inhibitor were dissolved in DMSO, and all assays were performed with the same final concentration of DMSO (10–15%). Enzyme, inhibitor and substrate were mixed and the time-dependent increase in the UV absorption was monitored over 20 min (final concentrations [I]: **2b**: 2.06 mM; **3**: 0.46 mM; **4**: 0.81 mM; **5**: 0.75 mM; **6**: 0.63 mM; **10**: 1.10 mM; **11**: 0.86 mM; **14**: 0.69 mM; **15**: 0.70 M; **17**: 0.82 mM; **18**: 0.83 mM; **20**: 0.35-0.69 mM; **26**: 0.60 mM; **29**: 0.54 mM; **37**: 0.83 mM; **40**: 0.54 mM). Without inhibitor no significant decrease in enzyme activity occured during the time of the assay (steady-state conditions). For each inhibitor, two independent experiments were performed, each with at least three different inhibitor concentrations and two substrate concentrations, respectively.

Kinetic constants were obtained by non-linear or linear regression analysis with the program GraFit®[38].

Papain

The assays were performed at papain concentrations 1.3–3.5 μM and L-BAPA as substrate at concentrations of 1.37–1.80 mM. Solutions of papain were prepared freshly by incubating the enzyme (about 100 mg/10 ml = 30–35 μM) in 0.05 M sodium phophate buffer (pH 6.5), which contained 5 mM EDTA and 5 mM cysteine for 30 min at 25°C. The K_m value was determined as 2.5 mM from five independent experiments. The substrate was dissolved in 1 ml DMSO and diluted with buffer, the inhibitor was dissolved in DMSO. All assays were performed with the same final concentration of DMSO. The rate of enzymatic hydrolysis was monitored by the release of *p*-nitroaniline at 25 °C at 405 nm (ε = 9960 l mol^{-1} cm^{-1}).

Dilution Assay: For the inhibition experiments the enzyme was incubated with the inhibitor at various inhibitor concentrations (final concentrations [I]: **2a**: 2.49–4.97 mM; **2b**: 1.88–9.4 mM; ***(S)*-2b**: 1.7–9.3 mM; ***(R)*-3**: 0.32–1.93 mM; **4**: 0.33–3.28 mM) for varying periods of time (5–40 min, 5–7 values). Following this preincubation with inhibitor, the substrate was added and the remaining enzyme activity was monitored as described above. Without inhibitor, no significant decrease in enzyme activity was observed (steady-state conditions). The experiments were repeated with 3–7 different inhibitor concentrations. Two independent assays were carried out for each inhibitor.

Continuous Assay: Enzyme, inhibitor and substrate were mixed and the time-dependent increase in absorption was monitored at 405 nm over 20 min (final concentrations [I]: ***(S)*-3**: 1.8 mM; **3**: 2.8 mM; **10**: 1.1 mM; **11**: 1.1 mM; **14**: 0.70 mM; **15**: 0.71 mM; **17**: 0.17–0.83 mM; **18**: 0.17–0.84 mM; **19**: 0.82 mM; **20**: 0.70 mM; **26**: 0.61 mM; **29**: 0.55 mM; **37**: 0.84 mM; **40**: 0.63 mM). Without inhibitor, no significant decrease in enzyme activity occured (steady-state conditions). Non-linear or linear regression analysis with the program GraFit® gave the kinetic constants.

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