Interaction of Papain-like Cysteine Proteases with Dipeptide-Derived Nitriles†

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A series of 44 dipeptide nitriles with various amino acids at the P^2 position and glycine nitrile at position P^1 were prepared and evaluated as inhibitors of cysteine proteinases. With respect to the important contribution of the P^2-S^2 interaction to the formation of enzyme−inhibitor complexes, it was focused to introduce structural diversity into the P^2 side chain. Nonproteinogenic amino acids were introduced, and systematic fluorine, bromine, and phenyl scans for phenylalanine in the P^2 position were performed. Moreover, the *N*-terminal protection was varied. Kinetic investigations were carried out with cathepsin L, S, and K as well as papain. Changes in the backbone structure of the parent *N*-(*tert*-butoxycarbonyl)-phenylalanyl-glycinenitrile (**16**), such as the introduction of an *R*-configured amino acid or an azaamino acid into P^2 as well as methylation of the P^1 nitrogen, resulted in a drastic loss of affinity. Exemplarily, the cyano group of **16** was replaced by an aldehyde or methyl ketone function. Structure−activity relationships were discussed with respect to the substrate specificity of the target enzymes.

Introduction

The huge class of cysteine proteinases comprises enzymes participating in a multitude of biological processes and can be subdivided into several clans, including the clans CA (papain-like enzymes), CD (caspases and related proteases), and PA (picornaviral proteases). A more detailed classification can be found in the MEROPS database.[1−3] To the first clan belong proteases from parasites as well as from mammals, and papain-like cysteine proteases are promising targets for therapeutic invention in parasitic infections and systemic human diseases, respectively.[4,5] At present, 11 human papain-like cysteine proteases have been described: the cathepsins B, C, H, F, K, L, O, S, V, W, and X. They show a high degree of homology and are mainly located in lysosomes but can also be secreted as inactive precursors from various cells, such as macrophages, fibroblasts, osteoclasts, and malignant cells.[6] The mature enzymes share a common fold consisting of two domains, the *N*-terminal L (left) and the *C*-terminal R (right) domain. The V-shaped active site cleft is located between the two domains binding the substrate in an extended conformation (for review, see refs 7 and 8). The catalytic center is constituted of the residues Cys 25, His 159, and Asn 175 (papain numbering). The latter residue does not directly contribute to catalysis, but acts as hydrogen bond acceptor toward the imidazole entity of His 159 and thereby enables the formation of a permanent thiolate−imidazolium ion pair. Substrates are mainly recognized by the S^2, S^1, and $S^{1\prime}$ binding sites (nomenclature according to Schechter and Berger).[9] The S^2 pocket represents the best defined binding site and determines the primary substrate specificity.[10] Besides housekeeping tasks in the turnover of cellular proteins, thiol-dependent cathepsins also execute specific and individual functions in physiological processes.[11,12] Their activity is controlled by limited proteolysis of latent precursors as well as by endogenous inhibitors.[8,13] To an ever increasing extent it becomes obvious that cysteine proteases are implicated in pathological conditions. For example, the cathepsins B, C, L, and X have been shown to be key players in the proliferation, vascularization, and invasion of multiple pancreatic islet tumors and cervical cancer.[14]

Cathepsin L (EC 3.4.22.15) shows the highest proteolytic activity of all lysosomal proteases and is widely expressed in various tissues. Increased activities of cathepsin L were observed in several tumors. Very recently, this protease has been demonstrated to act as central mediator of the invasive capacity of *S*-adenosylmethionine decarboxylase-transformed malignant fibroblasts, probably by proteolytic degradation of extracellular matrix constituents.[15] Its involvement in the metastasis of human melanoma cells has also been reported.[16]

Cathepsin S (EC 3.4.22.27) can be found in lymphatic organs such as lymph nodes and spleen. It is crucial for MHC class II mediated antigen presentation in B-cells, dendritic cells, and nonprofessional antigen presenting cells such as intestinal epithelial cells. In detail, cathepsin S cleaves off the CLIP peptide from the invariant chain Ii, which is associated with MHC class II $\alpha\beta$ heterodimers in order to prevent premature antigen binding.[17,18] The role of this protease in the pathogenesis of the autoimmune disease myasthenia gravis has been elaborated recently.[19]

Cathepsin K (EC 3.4.22.38) is predominantly expressed in osteoclasts.[20] Its collagenolytic capability is even higher than that of the matrix metalloproteinases, because it is able to cleave native collagen at multiple

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† Dedicated to Prof. Dr. Bernd Wiederanders, Jena, on the occasion of his 65th birthday.

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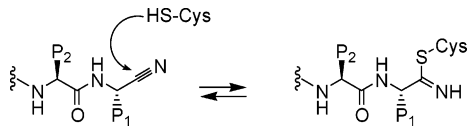
sites.[21] It has been shown that the collagenase activity of cathepsin K requires the formation of supramolecular complexes between the protease and chondroitin 4-sulfate.[22] Bone remodeling is an essential physiological process balanced between degradation and reconstruction. Bone resorption starts with acid-induced demineralization, followed by proteolytic degradation of the organic matrix—mainly consisting of type I collagen—preponderantly mediated by cathepsin K secreted from osteoclasts. Enhanced degradation causes diseases such as osteoporosis. Hence, cathepsin K is a promising drug target for the treatment of osteoporosis, and strong efforts have been focused on the development of inhibitors for this enzyme.

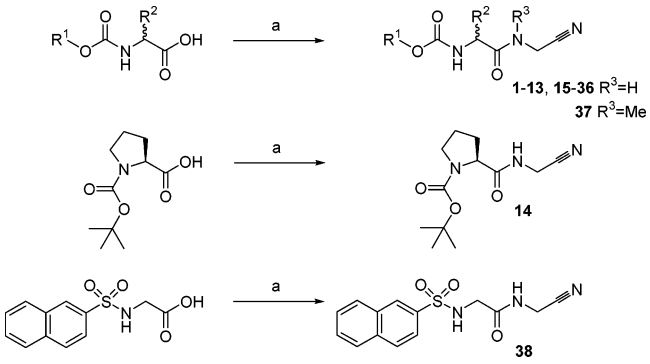
Due to the vital role of papain-like cysteine proteases in human physiology and pathology, the development of potent and selective inhibitors for these enzymes represents a great challenge. Several classes of compounds with electrophilic sites able to interact with the active thiol have been reported to inhibit cysteine cathepsins.[1,23,24] Most of them are peptide derived inhibitors such as peptidyl aldehydes, ketones, halomethyl ketones, diazoketones, *O*-acylhydroxamates, epoxides, vinylsulfones, and nitriles. The latter class has gained much attention in recent years.[25,26] Peptide nitriles bind to the active site thiol via reversible formation of a covalent thioimidate linkage (Chart 1).[27,28] Nitriles are class-selective protease inhibitors which strongly inhibit cysteine proteases while serine proteases are not or only weakly affected.[29] This selectivity can be explained from two different perspectives. According to the HSAB principle, the addition of the soft, polarizable sulfur nucleophile of the cysteine proteases to the soft carbon electrophile of the C−N triple bond is favored over that of the hard oxygen nucleophile of the serine proteases. From an enzymological point of view, there is a difference in stabilization of the tetrahedral transition state of amide bond hydrolysis. Cysteine protease-catalyzed reactions pass through a less constrained transition state as compared to serine proteases; the hydrogen bonding to the oxyanion hole is not as crucial as in serine proteases.[30,31] Hence, and in contrast to serine proteases, cysteine proteases should be more prone to accept the planar thioimidate formed from a nitrile inhibitor instead of the tetrahedral transition state formed upon the competing hydrolysis of a substrate molecule.

Chart 1. Reversible Formation of Thioimidates from Peptide Nitriles and Cysteine Proteases



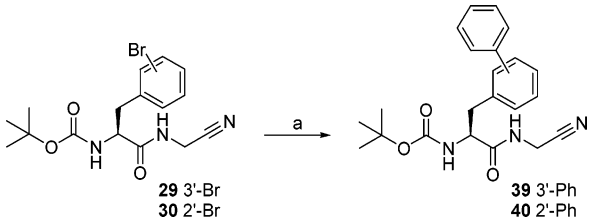
Herein we report a detailed study on the structure−activity relationships of dipeptide derived nitriles as inhibitors of cysteine proteases. A series of 48 compounds including 44 nitriles were prepared and evaluated toward the canonical papain and the therapeutically relevant cathepsins L, S, and K on the basis of the inhibition kinetics. With respect to the known importance of the P^2-S^2 interaction for specificity and affinity, the design was aimed to explore the requirements and limitations of the P^2 side chain of the peptidic inhibitors. Further variations were introduced at the *N*-terminal group and the backbone structure, whereas glycine was kept constant at P^1. In addition, the bioactivity of a selected nitrile was compared to that of the analogous aldehyde and methyl ketone inhibitors, as well as the corresponding propargyl- and allylamide.

Scheme 1[a]



[a] Reagents and conditions: (a) (1) *N*-methylmorpholine, $ClCO_2CH_2CH(CH_3)_2$, THF; (2) $H_2NCH_2CN{\cdot}0.5H_2SO_4$ or $CH_3NHCH_2CN{\cdot}HCl$, H_2O, 1 N NaOH, −25 °C to room temperature.

Scheme 2[a]

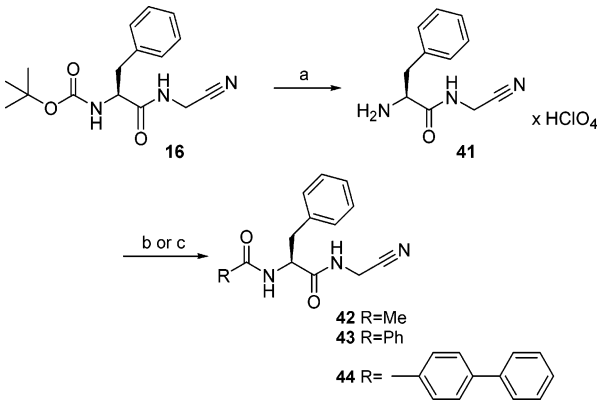


[a] Reagents and conditions: (a) phenyl boronic acid, Na_2CO_3, $Pd(PPh_3)_4$, DME/H_2O, microwave.

Results and Discussion

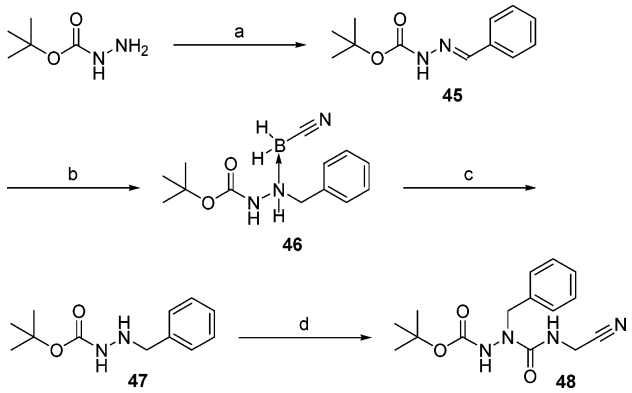
Synthesis. Wieland's mixed anhydride method using isobutyl chloroformate as activating agent, *N*-methylmorpholine as tertiary base, and THF as solvent provides a powerful and reliable synthetic tool for the formation of amide bonds in solution.[32] This method was used to couple proteinogenic and nonproteinogenic *N*-protected amino acids carrying different aliphatic and aromatic side chains, activated in situ, with aminoacetonitrile to obtain dipeptide nitriles **1**−**36** and **38** mostly in high yields and purity (Scheme 1). The *N*-methylated compound **37** was prepared by using *N*-methyl aminoacetonitrile instead of aminoacetonitrile. For the preparation of the biphenyl derivatives **39** and **40** a microwave-assisted, palladium-catalyzed Suzuki cross coupling of the phenyl bromides **29** and **30** with phenylboronic acid was applied (Scheme 2).[33−35] The acyl derivatives **42**-**44** were synthesized by deprotection of **16** using formic acid and subsequent acylation of the free dipeptide nitrile **41** under Schotten−Baumann conditions to yield **42** and **43** (Scheme 3). To prepare **44**, biphenyl-4-carbonic acid was activated as mixed anhydride and reacted with **41**. The synthetic route to the aza analogue **48** includes the multistep reductive alkylation of *tert*-butyl carbazate with benzaldehyde using sodium cyanoborohydride as reductant (Scheme 4). The intermediate cyanoborane complex **46** was isolated and subsequently destroyed with sodium hydroxide solution.[36] The resulting compound **47** was

Scheme 3[a]



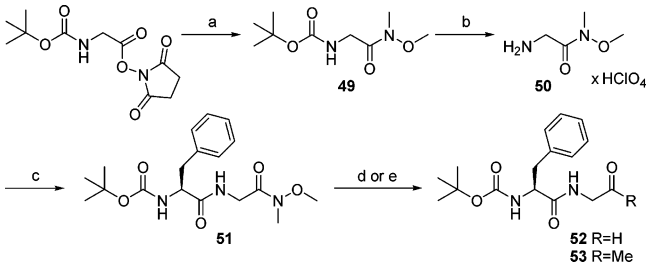
[a] Reagents and conditions: (a) (1) HCOOH, room temperature; (2) 70% $HClO_4$/*i*PrOH; (b) Ac_2O or BzCl, sat. $NaHCO_3$, 4 °C; (c) (1) biphenyl-4-carbonic acid, *N*-methylmorpholine, $ClCO_2CH_2$-$CH(CH_3)_2$ in THF; (2) **41** in H_2O/1 N NaOH, −25 °C to room temperature.

Scheme 4[a]



[a] Reagents and conditions: (a) benzaldehyde, THF, room temperature; (b) $NaBH_3CN$, AcOH, THF; (c) 1 N NaOH, MeOH; (d) CDI, triethylamine, $H_2NCH_2CN{\cdot}0.5H_2SO_4$, DMF, 4 °C to room temperature.

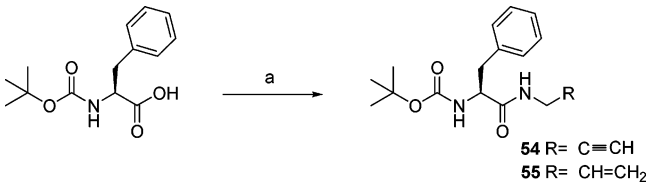
Scheme 5[a]



[a] Reagents and conditions: (a) $H_3CNHOCH_3{\cdot}HCl$, 1 N NaOH, dioxane, room temperature; (b) (1) 4 N HCl/dioxane, room temperature; (2) 70% $HClO_4$/*i*PrOH; (c) (1) Boc-Phe-OH, *N*-methylmorpholine, $ClCO_2CH_2CH(CH_3)_2$, THF; (2) **50** in H_2O/1 N NaOH, −25 °C to room temperature; (d) (1) $LiAlH_4$, Et_2O; (2) sat. NH_4Cl; (e) (1) Mg, CH_3I, Et_2O; (2) sat. NH_4Cl.

reacted with the azolide formed in situ from *N*,*N*′-carbonyldiimidazole and aminoacetonitrile sulfate,[37] to yield the azadipeptide nitrile **48**. Attempts to prepare the aldehyde **52** (Scheme 5) by oxidation of the corresponding primary alcohol with the hypervalent iodine reagent iodoxybenzoic acid (IBX) resulted in a complex mixture of products. Therefore, the Weinreb amide **51** was prepared in three steps and subsequently reduced with lithium aluminum hydride[38] to furnish **52**. The

Scheme 6[a]



[a] Reagents and conditions: (a) (1) *N*-methylmorpholine, $ClCO_2CH_2CH(CH_3)_2$, THF; (2) propargylamine or allylamine, −25 °C to room temperature.

preparation of the methyl ketone **53** was accomplished by reacting Weinreb amide **51** with methyl Grignard reagent.[39] The phenylalanine derived propargyl- and allylamides **54** and **55**, respectively, were prepared from the corresponding amines by the mixed anhydride method (Scheme 6).

Inhibition Kinetics. The dipeptide nitriles **1**−**40**, **42**−**44,** and **48**, the aldehyde **52**, the methyl ketone **53,** and the unsaturated amides **54** and **55** were investigated toward four cysteine proteases, the plant enzyme papain, and the mammalian cathepsins L, S, and K. Papain was assayed with the chromogenic substrate Z-Phe-Arg-NHNp (Np = 4-nitrophenyl). To reduce enzyme consumption, more sensitive fluorimetric assays were chosen to characterize the inhibitory activity of these compounds toward the cathepsins. The specific substrates Z-Phe-Arg-NHMec, Z-Val-Val-Arg-NHMec, and Z-Leu-Arg-NHMec (Mec = 4-methylcoumarin-7-yl), containing the fluorescent C-terminal leaving group 7-amino-4-methylcoumarin (AMC), were employed to monitor the activities of cathepsin L, cathepsin S, and cathepsin K, respectively. The K_m values of the substrates were required for the kinetic analyses and had to be determined under the same experimental conditions used in the inhibition experiments. The determination of the K_m value of the papain substrate Z-Phe-Arg-NHNp was difficult due to low affinity (K_m > 500 μM) and poor solubility even in the presence of 12% DMSO (<500 μM). As described in the Experimental Section, K_m was determined by monitoring the complete papain-catalyzed hydrolysis at a preferably high initial substrate concentration. The data were analyzed with differential equations 3 and 4 describing both the catalytic activity and the slow, but significant, inactivation of the enzyme over a comparably long period of time to obtain $K_m = 965 \pm 244$ μM. The K_m values of the cathepsins were determined as usual in separate measurements in the presence of different substrate concentrations and were calculated from the initial velocities.

All nitrile inhibitors showed time-independent inhibition. It could be concluded from the inhibitor structures and literature data that these dipeptide nitriles act as reversible, active site directed inhibitors and exhibit competitive behavior. Thus, the slopes of the progress curves could directly be used to determine K_i' values from which K_i values were calculated. A representative kinetic analysis is illustrated in Figure 1, showing the inhibition of cathepsin S by the naphthylalanine-derived compound **34**. The velocities of the enzyme-catalyzed reaction were influenced by the inhibitor concentration already at the beginning of the measurement and kept constant until its end. It could be deduced from the

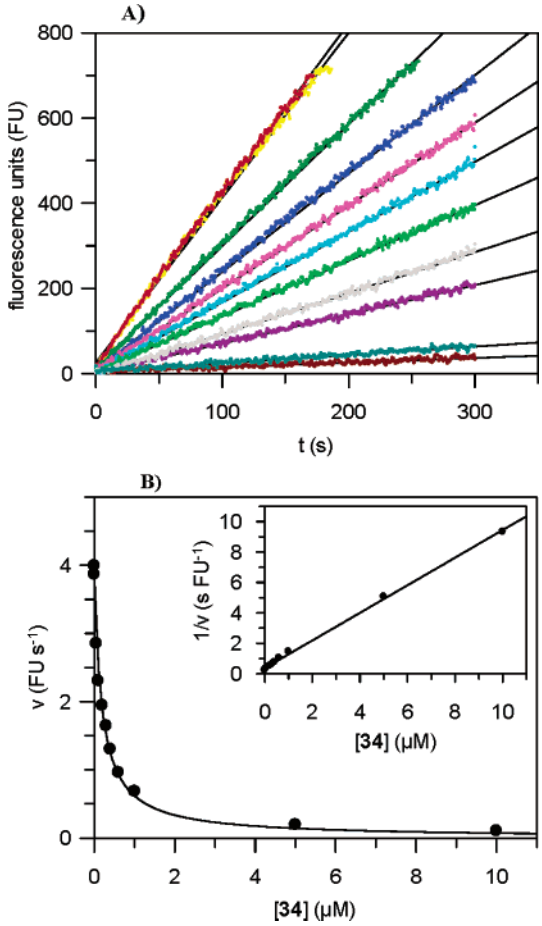


Figure 1. (A) Monitoring of the human cathepsin S-catalyzed hydrolysis of Z-Val-Val-Arg-NHMec (40 μM) in the presence of increasing concentrations of compound **34** (50 mM potassium phosphate pH 6.5, 50 mM NaCl, 2 mM EDTA, 0.01% Triton X-100, 25 μM DTT, and 1% DMSO, 37 °C). The reaction was initiated by addition of the enzyme. Data were obtained by measuring the fluorescence emission at 440 nm after excitation at 360 nm. Ordinate values were corrected for background fluorescence. Color scheme: red, [I] = 0; yellow, [I] = 0; dark green, [I] = 0.05 μM; dark blue, [I] = 0.1 μM; magenta, [I] = 0.2 μM; light blue, [I] = 0.3 μM; light green, [I] = 0.4 μM; gray, [I] = 0.6 μM; violet, [I] = 1 μM; turquoise, [I] = 5 μM; brown, [I] = 10 μM. (B) Plot of the rates of hydrolysis of Z-Val-Val-Arg-NHMec versus concentrations of **34**. The linear dependence shown in the Dixon plot (inset) indicates competitive inhibition. Nonlinear regression gave an apparent inhibition constant $K_i' = (1 + [S]/K_m)K_i = 0.25 \pm 0.02$ μM. A Michaelis constant $K_m = 19.2 \pm 0.8$ μM was determined separately.

instantaneously attained equilibrium that the enzyme–inhibitor interaction involves the rapid formation of the covalent thioimidate complex.

General Trends in Cysteine Protease Inhibition. The inhibition constants of 37 dipeptide nitriles toward papain and the cathepsins L, S, and K are summarized in Table 1. An aliphatic R^2 residue attached at the P^2 position required at least two carbon atoms to achieve inhibition. Introduction of a further methyl group at the R^2 chain mostly enhanced the inhibitory potency (**5** versus **4**; **7** versus **6**). Rigidization of the geminal methyl groups in **7** led to the cyclopropyl derivative **10** and resulted in a loss of inhibition, particularly toward cathepsins L and K. This might be due to the reduced sterical demand of a cyclopropyl group in comparison to an isopropyl moiety. The carba-analogue **13** was accepted by the four proteases to a similar extent as the parent methionine derivative **12**. These data demonstrate that hydrophobic interactions between the P^2 aliphatic residues and the S^2 binding sites account for the affinity of the dipeptide nitriles toward the target enzymes.

The proline derived compound **14** did not exhibit inhibitory activity. A successive extension of the spacer between the α-carbon and the phenyl ring was realized to provide inhibitors **15**, **16**, **21**, and **22**. The phenylglycine derivative **15** showed weak activities with a K_i value toward papain being 3 orders of magnitude higher than that of the phenylalanine inhibitor **16**. Nitrile **16** was a potent inhibitor of papain, and both cathepsins L and S. Further extension diminished enzyme inhibition, however, even the introduction of a three-atom-linker in the *O*-benzylserine derivate **22** resulted in K_i values of 11 and 2.2 μM toward papain and cathepsin S, respectively.

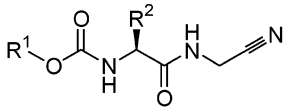
Further structural alterations were made on the basis of inhibitor **16**. Bioisosteric replacement of the benzene ring by five-membered hetarenes yielded **18**–**20**. These structures were well tolerated by the target proteases. The thiophene derivatives were significantly more potent that the 2-furyl compound **18**. The attachment of the thiophene ring at position 2′, compared to position 3′, was somewhat preferred by the four enzymes (**19** versus **20**).

For the benzene ring of **16**, systematic fluorine and bromine scans were performed. The fluorine scan was done to evaluate the fluorophilic/fluorophobic potential of the S^2 pockets. Monofluorination (**24**–**26**) was well tolerated, but no enhancement of the affinities was observed. Perfluorination, as realized in **27**, significantly decreased the affinity, when compared to inhibitor **16**. This could be explained by electrostatic repulsions due to the directly opposed quadrupole moments of perfluorobenzenes[40] rather than by a raised sterical demand of the pentafluorophenyl group. From these results, it can be concluded that the S^2 pockets of the four proteases are slightly fluorophobic. Studies in which the systematic fluorination of aromatic residues in thrombin inhibitors led to improved affinities were recently published.[41,42] The systematic bromine substitution of the aromatic ring in **16** was also accepted by the proteases and resulted in mostly equipotent inhibitors (**28**, **29**, **30** versus **16**).

The usefulness of bicyclic aromatic cores was investigated with the inhibitors **31**–**34**. The tryptophan and 3′-thionaphthenylalanine derived compounds **31** and **32** behaved almost equally toward each of the four enzymes and inhibited cathepsin L and cathepsin S nearly to the same extent. The replacement of the indole and thionaphthene moieties by the bioisosteric naphthalene resulted in the case of the α-substitution (**33**) in a loss of inhibitory activity. In contrast, introduction of β-naphthalene (**34**) furnished very potent inhibitors of cathepsin S, cathepsin L, and papain.

To investigate substituents, in which the two aromatic rings are connected by a single bond, the three possible biphenyl derivatives **35**, **39**, and **40** were synthesized and evaluated. While the *para* and *meta* biphenylalanine-derived compounds showed remarkable inhibitory

Table 1. Inhibition of Papain, Cathepsin L, Cathepsin S, and Cathepsin K by Dipeptide Nitriles **1**−**35**, **39**, and **40**



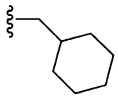
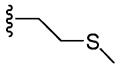
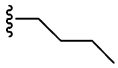
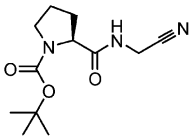
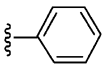
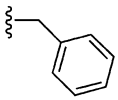
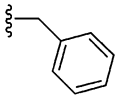
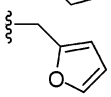
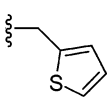
compd.	R^1	R^2	K_i (μM)			
			papain	bovine cathepsin L	human cathepsin S	human cathepsin K
1	*t*Bu	H	> 3000	> 1000	> 1000	> 1000
2	Bn	H	1599 ± 109	> 1000	> 1000	> 1000
3	*t*Bu	Me	22 ± 2	> 100	292 ± 30	136 ± 12
4	*t*Bu		5.4 ± 0.2	> 100	28 ± 2	5.0 ± 0.2
5	*t*Bu		2.0 ± 0.1	2.8 ± 0.1	6.2 ± 0.4	6.5 ± 0.4
6	*t*Bu		0.75 ± 0.02	22 ± 2	0.90 ± 0.07	1.1 ± 0.1
7	*t*Bu		2.0 ± 0.1	1.2 ± 0.1	0.13 ± 0.01	0.12 ± 0.01
8	Bn		3.1 ± 0.2	0.75 ± 0.02	0.13 ± 0.01	0.035 ± 0.001
9	*t*Bu		26 ± 1	4.9 ± 0.5	4.1 ± 0.1	5.8 ± 0.3
10	*t*Bu		13 ± 1	47 ± 10	0.65 ± 0.04	3.3 ± 0.2
11	*t*Bu		2.2 ± 0.1	1.1 ± 0.1	0.044 ± 0.001	5.3 ± 0.2
12	*t*Bu		0.65 ± 0.01	19 ± 1	0.66 ± 0.03	0.73 ± 0.03
13	*t*Bu		1.57 ± 0.05	19 ± 1	0.55 ± 0.02	1.27 ± 0.14
14			537 ± 13	> 100	> 100	> 100
15	*t*Bu		83 ± 2	29 ± 3	1.7 ± 0.08	90 ± 7
16	*t*Bu		0.26 ± 0.01	0.52 ± 0.02	0.62 ± 0.05	4.1 ± 0.2
17	Bn		0.36 ± 0.01	0.18 ± 0.02	0.51 ± 0.05	0.26 ± 0.01
18	*t*Bu		1.2 ± 0.1	14 ± 1	2.8 ± 0.2	4.1 ± 0.2
19	*t*Bu		0.19 ± 0.01	1.6 ± 0.1	0.27 ± 0.01	1.2 ± 0.1

Table 1 (Continued)

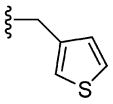
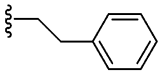
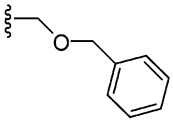
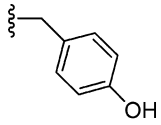
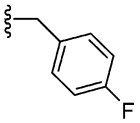
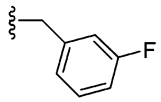
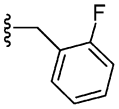
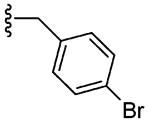
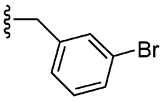
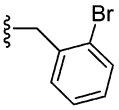
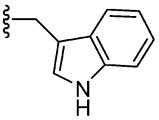
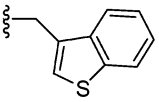
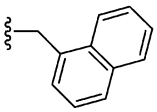
compd.	R^1	R^2	K_i (μM)			
			papain	bovine cathepsin L	human cathepsin S	human cathepsin K
20	*t*Bu		0.57 ± 0.01	4.5 ± 0.4	0.76 ± 0.03	2.1 ± 0.1
21	*t*Bu		12 ± 0.2	9.4 ± 0.9	1.9 ± 0.1	8.5 ± 0.3
22	*t*Bu		11 ± 1	33 ± 4	2.2 ± 0.2	> 100
23	*t*Bu		0.19 ± 0.01	0.40 ± 0.02	0.97 ± 0.09	30 ± 2
24	*t*Bu		0.27 ± 0.01	0.64 ± 0.07	0.59 ± 0.03	13 ± 1
25	*t*Bu		0.50 ± 0.01	0.70 ± 0.04	0.86 ± 0.06	6.3 ± 0.3
26	*t*Bu		0.40 ± 0.02	0.47 ± 0.03	0.77 ± 0.07	3.3 ± 0.3
27	*t*Bu		14 ± 1	2.5 ± 0.3	15 ± 1	> 100
28	*t*Bu		0.029 ± 0.001	0.63 ± 0.08	0.19 ± 0.04	28 ± 4
29	*t*Bu		0.33 ± 0.02	0.27 ± 0.02	0.34 ± 0.02	6.5 ± 0.5
30	*t*Bu		0.46 ± 0.02	1.1 ± 0.1	0.61 ± 0.03	4.2 ± 0.2
31	*t*Bu		7.6 ± 0.2	0.35 ± 0.01	0.24 ± 0.02	29 ± 6
32	*t*Bu		7.4 ± 0.2	0.32 ± 0.02	0.30 ± 0.01	34 ± 3
33	*t*Bu		28 ± 0.2	1.7 ± 0.2	1.3 ± 0.1	66 ± 9

Table 1 (Continued)

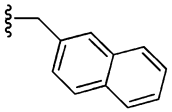
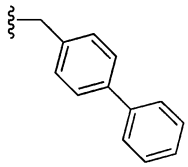
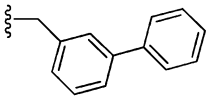
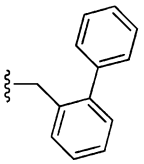
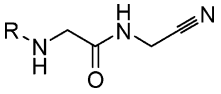
compd.	R^1	R^2	K_i (μM) papain	bovine cathepsin L	human cathepsin S	human cathepsin K
34	*t*Bu		0.021 ± 0.002	0.12 ± 0.01	0.083 ± 0.005	83 ± 14
35	*t*Bu		0.016 ± 0.001	0.34 ± 0.03	0.44 ± 0.07	16 ± 4
39	*t*Bu		1.2 ± 0.01	0.43 ± 0.03	0.27 ± 0.03	26 ± 3
40	*t*Bu		299 ± 9	> 100	> 100	> 100

Table 2. Inhibition of Papain, Cathepsin L, Cathepsin S, and Cathepsin K by Glycine Derivatives **1**, **2**, and **38**



compd	R	K_i (μM) papain	bovine cathepsin L	human cathepsin S	human cathepsin K
1[a]	Boc	>3000	>1000	>1000	>1000
2[a]	Z	1599 ± 109	>1000	>1000	>1000
38	2-naphthyl-sulfonyl	10 ± 0.2	25 ± 5	2.5 ± 0.2	>100

[a] Compounds are also included in Table 1.

potency toward papain, cathepsin L, and cathepsin S, the *ortho* linkage led to a dramatic decrease in inhibitory activity.

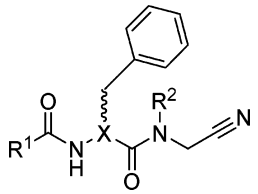
The data given in Table 1 underscore the importance of the P^2 residue. Accordingly, the glycylglycine derivatives **1** and **2** are not active. Nevertheless, one compound lacking a P^2 residue showed significant inhibitory properties obviously mediated by the 2-naphthylsulfonyl group of **38** (Table 2).

Further structural variations, outlined in Table 3, were carried out starting from inhibitor **16** and maintaining its phenylalanine and cyanomethyl substructures. Compounds **16**, **17**, and **42**−**44** (Table 3) differ in the nature of *N*-terminal protecting group and share the remaining molecular structure. Without exception, these phenylalanine derivatives exhibited affinities in the lower micromolar or nanomolar range. However, the small acetyl moiety was less favorable and all bulkier substituents including carbamate and acyl protecting groups enhanced the interaction (**42** versus **16**, **17**, and **43**, **44**, respectively).

The intervention in the peptidic backbone had a dramatic influence on the bioactivity. *N*-Methylation (**37**) decreased the affinity by 3 orders of magnitude. The calculated changes in the free enthalpies of binding are in the range $\Delta\Delta G_{obs} > 2$ kcal/mol, suggesting the amide N−H to be a strong H-bond donor. The exchange of the phenylalanine of **16** by azaphenylalanine as realized in **48** was not tolerated. The change of the configuration from *S* to *R* (**36**) also led to a drastic reduction in the binding affinity. Our observations with the azadipeptide nitrile are in accordance with those reported by Calabretta et al. on azapeptidyl methylketones as substrate analogue inhibitors of papain and cathepsin B.[36] The poor affinity resulting from the C/N-replacement was explained by the geometry changed from tetrahedral to nearly trigonal-planar. Furthermore, the capacity of the amide N−H bond to act as an H-bond donor is reduced. Quantum chemical calculations have shown that azapeptides cannot adopt β-strand conformations, which is crucial for protease−peptide interactions, because the dihedral angle ϕ is restricted.[43,44] However, cystatin derived azapeptides with azaglycine in the P^1 position have been shown to be very potent inhibitors of papain-like cysteine proteases.[45] Although the P^2 benzyl group of the *R*-configured compound **36** could be expected to fit worse than that of **48**, the latter compound appeared to be even less potent than **36**. Therefore, the inhibitor geometry might be more distorted in the aza analogue **48** than in the *R*-configured **36**.

Furthermore, the nitrile warhead was exchanged by the electrophilic carbonyl group as realized in the aldehyde **52** and the methyl ketone **53** (Table 4). The dipeptidyl aldehyde **52** showed significantly increased affinity to all enzymes. This can be understood by the stronger electrophilicity of the aldehyde carbonyl carbon when compared to the cyano carbon. The strong elec-

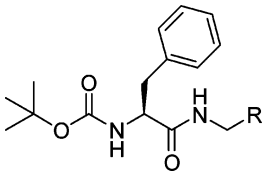
Table 3. Inhibition of Papain, Cathepsin L, Cathepsin S, and Cathepsin K by Phenylalanine Derivatives



					K_i (μM)			
compd	R^1	R^2	X	conf	papain	bovine cathepsin L	human cathepsin S	human cathepsin K
16[a]	*t*BuO	H	CH	S	0.26 ± 0.01	0.52 ± 0.02	0.62 ± 0.05	4.1 ± 0.2
17[a]	BnO	H	CH	S	0.36 ± 0.01	0.18 ± 0.02	0.51 ± 0.05	0.26 ± 0.01
42	Me	H	CH	S	0.38 ± 0.02	5.8 ± 0.5	1.4 ± 0.1	7.5 ± 0.5
43	Ph	H	CH	S	0.062 ± 0.002	0.23 ± 0.02	0.077 ± 0.004	1.5 ± 0.1
44	4-biphenyl	H	CH	S	0.016 ± 0.001	0.55 ± 0.07	0.24 ± 0.03	0.30 ± 0.03
37	*t*BuO	Me	CH	S	>100	>100	>100	>100
36	*t*BuO	H	CH	R	41 ± 2	>100	46 ± 13	>100
48	*t*BuO	H	N		725 ± 21	>100	>50	>100

[a] Compounds are also included in Table 1.

Table 4. Inhibition of Papain, Cathepsin L, Cathepsin S, and Cathepsin K by Dipeptide Analogues **16**, **52**, and **53**



		papain	bovine cathepsin L			human cathepsin S			human cathepsin K		
compd	R	K_i (μM)	K_i (μM)	k_{on} ($M^{-1}\,s^{-1}$)	k_{off} ($10^{-3}\,s^{-1}$)	K_i (μM)	k_{on} ($M^{-1}\,s^{-1}$)	k_{off} ($10^{-4}\,s^{-1}$)	K_i (μM)	k_{on} ($M^{-1}\,s^{-1}$)	k_{off} ($10^{-4}\,s^{-1}$)
16[a]	CN	0.32 ± 0.01	0.52 ± 0.02			0.62 ± 0.05			4.1 ± 0.2		
52	CHO	0.015 ± 0.0005	0.022 ± 0.001			0.025 ± 0.001			0.074 ± 0.004		
53	COMe	187 ± 24	2.3 ± 0.3	515 ± 32	1.2 ± 0.25	2.1 ± 0.1	336 ± 19	7.1 ± 0.7	2.1 ± 0.3	228 ± 21	4.8 ± 1.2

[a] Compound is also included in Table 1.

trophilic aldehyde is thought to be the determining factor for the affinity. The thiohemiacetal formed by the nucleophilic attack of the cysteine sulfur also accounts for the pronounced activity of **52**. The thiohemiacetal has a tetrahedral geometry different from the planar thioimidate that results from the corresponding nitrile inhibitors. A crystal structure of cathepsin S complexed with a dipeptidyl aldehyde revealed that the formation of the tetrahedral adduct is not stereospecific, because complexes with *S*- and *R*-configurations could be observed.[46] Thus, the aldehyde carbonyl can be attacked at both the *si* and the *re* face, forming adducts in which either the hydrogen atom or the hydroxy group points toward the oxyanion hole, respectively, which may further account for the enhanced affinity. It was found for inhibitor **52** that the relative impact of the specificity-mediating P^2-benzyl group was reduced resulting in lower differences in inhibitory potency toward the four enzymes. For comparison, the nitrile **16** revealed a discriminating affinity for cathepsins L and S over cathepsin K. In contrast, the dipeptidyl methyl ketone **53** exhibited K_i values which were 1 order of magnitude higher toward cathepsins L and S than those of compound **16**, while inhibition of cathepsin K was not affected by this structural replacement. Interestingly, inhibitor **53** showed time-dependent inhibition against the cathepsins (Figure 2). The reactions were analyzed by the formalism of slow-binding kinetics, i.e., eq 5 and the corresponding data analysis as noted in the Experimental Section. A one-step mechanism could be concluded from the linear dependence of the pseudo-first-order rate constant, k_{obs}, on the inhibitor concentration, [I], as well as from the initial velocities, v_i, being independent of the inhibitor concentration.[47] The drop in the activity caused by the attachment of the methyl group (**53** versus **52**) might be due to the enhanced steric hindrance in the ketone-derived tetrahedral complex, rather than the reduced electrophilicity of the ketone carbonyl group. The methyl group might not fit into the oxyanion hole and thus the carbonyl would only be attacked at the *re* face. The methyl ketone **53** completely lacked selectivity for the cathepsins with K_i values between 2.1 and 2.3 μM. The second-order rate constants for the formation of the enzyme−inhibitor complex, k_{on}, were very similar.

To investigate the contributions of the covalent and noncovalent interactions to enzyme−ligand recognition, the cyano group of **16** was replaced by nonelectrophilic entities such as the ethynyl group in **54** and the vinyl group in **55** (Table 5). The propargylamide **54** resembles **16** in the unbound state, and the allylamide **55** mimics the geometry of the enzyme-bound thioimidate. The poor affinities of **54** and **55** demonstrate the importance of the covalent interaction. This result, combined with the fact that the glycine derivative **1** is by 4 orders of magnitude weaker than **16**, indicates the high cooperativity between the covalent and noncovalent interaction. The finding that the non-nitriles **54** and **55** are somewhat active against papain might result from ion−

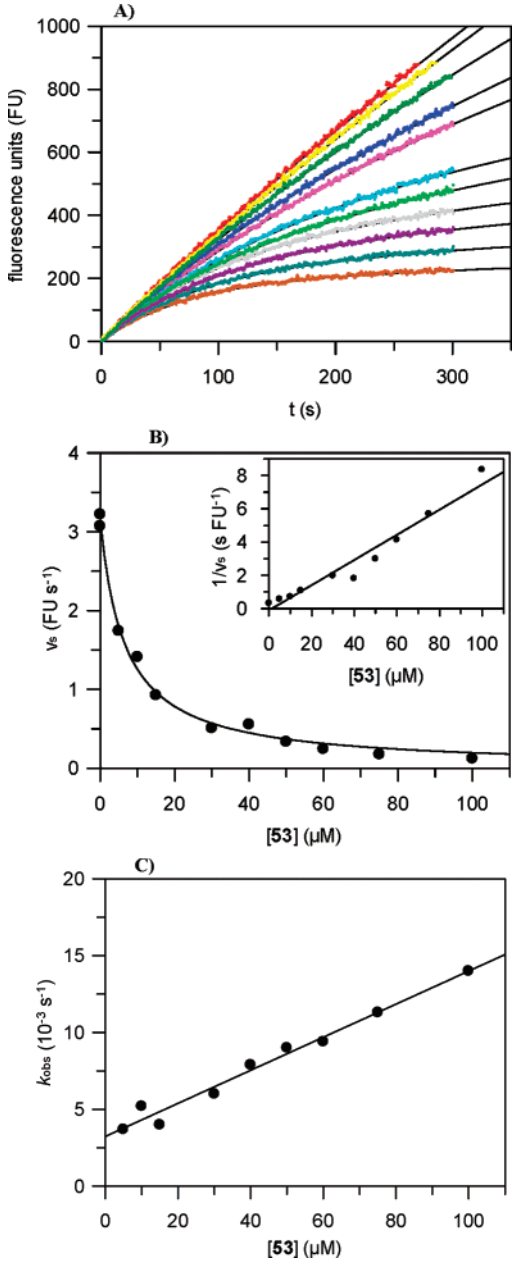
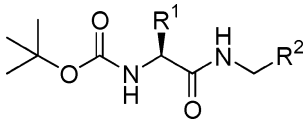


Figure 2. (A) Monitoring of the human cathepsin S-catalyzed hydrolysis of Z-Val-Val-Arg-NHMec (40 μM) in the presence of increasing concentrations of compound **53** (50 mM potassium phosphate pH 6.5, 50 mM NaCl, 2 mM EDTA, 0.01% Triton X-100, 25 μM DTT, and 1% DMSO, 37 °C). The reaction was initiated by addition of the enzyme. Data were obtained by measuring the fluorescence emission at 440 nm after excitation at 360 nm. Ordinate values were corrected for background fluorescence. Color scheme: red, [I] = 0; yellow, [I] = 0; dark green, [I] = 5 μM; dark blue [I] = 10 μM; magenta, [I] = 15 μM; light blue [I] = 30 μM; light green, [I] = 40 μM; gray, [I] = 50 μM; violet, [I] = 60 μM; turquoise, [I] = 75 μM; brown, [I] = 100 μM. (B) Plot of the steady state rates of the hydrolysis of Z-Val-Val-Arg-NHMec versus concentrations of **53**. The linear dependence shown in the Dixon plot (inset) indicates competitive inhibition. Nonlinear regression gave an apparent inhibition constant $K_i' = (1 + [S]/K_m)K_i = 6.6 \pm 0.5$ μM. A Michaelis constant $K_m = 19.2 \pm 0.8$ μM was determined separately. (C) Plot of the k_{obs} values versus concentrations of **54**. The linear dependence indicates a one-step mechanism for the enzyme−inhibitor interaction. Linear regression gave an apparent second order rate constant $k_{on}' = k_{on}/(1 + [S]/K_m) = 109 \pm 6$ M^{-1} s^{-1}. From the corresponding k_{on} value, a first order rate constant $k_{off} = k_{on}K_i = 7.1 \times 10^{-4} \pm 0.7 \times 10^{-4}$ s^{-1} was calculated.

Table 5. Inhibition of Papain, Cathepsin L, Cathepsin S, and Cathepsin K by Dipeptide Analogues **1**, **16**, **54**, and **55**



			K_i (μM)			
compd	R^1	R^2	papain	bovine cathepsin L	human cathepsin S	human cathepsin K
1[a]	H	C≡N	>3000	>1000	>1000	>1000
16[a]	Bn	C≡N	0.26 ± 0.01	0.52 ± 0.02	0.62 ± 0.05	4.1 ± 0.2
54	Bn	C≡CH	497 ± 14	>1000	>100	>1000
55	Bn	C=CH_2	497 ± 52	>1000	>100	>1000

[a] Compounds are also included in Table 1.

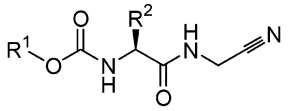
induced dipole interaction between the active site thiolate and the C−C multiple bonds.

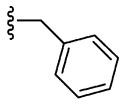
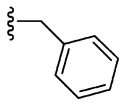
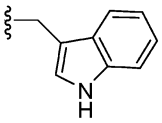
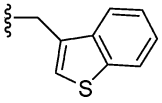
Inhibition of Papain. Papain was scarcely affected by compounds with aliphatic residues in P^2 (Table 1). Strong inhibition could be achieved by introduction of various aromatic structures into P^2. As most potent nitriles, **35** (Table 1) and **44** (Table 3) were identified, both bearing a 4-biphenyl moiety, either in P^2 (**35**), or as the *N*-terminal acyl residue (**44**), respectively. The biphenyl structural element is considered as a "privileged structure" in drug discovery, since the two aromatic units are combined in a well-balanced degree of flexibility and rigidity.[48] However, the bromine scan showed that a bromine atom attached in the *para* position improved the affinity to nearly the same extent (**28** versus **35**, Table 1). Thus, the enhancement of the activity of the parent compound **16** by *para*-phenyl substitution could be attributed to the hydrophobic character of the *para*-substituent (phenyl or bromo) rather than to a specific aromatic interaction within the S^2 pocket. An impact of a *para*-substituent to lower K_m has been found within a series of substrates containing phenylalanine analogues.[49] The most dramatic structural impact was observed upon switching the linkage of the naphthalene ring to the dipeptide scaffold from α to β position, lowering K_i by 3 orders of magnitude (**33** versus **34**).

With regard to the substrate specificity, it is known that papain mainly accepts substrates containing a bulky nonpolar amino acid in P^2, e.g. phenylalanine. The S^2 binding pocket is defined by the hydrophobic, nonaromatic side chains of Pro 68, Val 133, Val 157, Asp 158, and Ala 166. Crystal structures of the enzyme complexed with corresponding chloromethyl ketones revealed van der Waals contacts of the phenyl group with the valine side chains[50] as well as with Asp 158 and Pro 168.[51] However, the structural basis for the preference of aromatic over aliphatic residues in P^2 is not completely understood. The present study confirmed the strong preference of papain for aromatic side chains in the P^2 position. However, phenylalanine was not the best accepted amino acid in P^2. Certain, well-defined modifications of the benzene ring enhanced the activity of the resulting structures, others drastically reduced it. Noteworthy, the differences in the binding affinity within the group of dipeptide nitriles bearing an aromatic structure in P^2 were more obvious for papain than for the cathepsins.

Inhibition of Cathepsin L. The trend observed with papain, that less efficient inhibition was achieved by

Table 6. Comparison of the Inhibition of Bovine and Human Cathepsin L by Selected Compounds



compd.	R^1	R^2	K_i (μM)	
			bovine cathepsin L	human cathepsin L
16	*t*Bu		0.52 ± 0.02	0.70 ± 0.03
17	Bn		0.18 ± 0.02	0.23 ± 0.02
31	*t*Bu		0.35 ± 0.01	0.43 ± 0.02
32	*t*Bu		0.32 ± 0.02	0.29 ± 0.01

compounds with aliphatic residues in P^2 (Table 1), is also valid for cathepsin L. Monocyclic hetarenes in P^2 side chains were less well tolerated by cathepsin L than by the other enzymes. Bicyclic aromatic structures (**31**, **32**, **34**, and to a lower extent **33**) were explicitly accepted in the S^2 pocket. The β-naphthylalanine derivative **34** is the best inhibitor against cathepsin L ($K_i = 0.12$ μM) of all compounds investigated in this study. With respect to the binding of bicyclic aromatic structures, the S^2 pocket of cathepsin L is more flexible than in the case of papain. When the *tert*-butoxycarbonyl group was replaced by benzyloxycarbonyl, inhibition became more pronounced (**7** versus **8**; **16** versus **17**). The Z-protected compound **17** was the second-best inhibitor of the dipeptide nitrile series and exhibited a slight preference toward cathepsin L over cathepsin S. In addition, the differences in the inhibitory behavior toward the bovine and the human cathepsin L were investigated by evaluating selected compounds at the human enzyme. The results are shown in Table 6. The, at best, marginal differences indicated that the human enzyme can be substituted by bovine cathepsin L, at least for inhibition studies with derivatives addressing the nonprimed binding sites.

The P^2 substrate specificity of cathepsin L resembles that of papain. The S^2 site is constituted by the side chains of Asp 158, Gly 160, Met 157, Ala 205, Met 68, and Leu 67 (papain numbering).[52] The pronounced preference for aromatic binding partners, as confirmed in this study, might be explained by sulfur−arene interactions mediated by one of the two methionine side chains, as it has been observed in several protein−ligand complexes.[40]

Inhibition of Cathepsin S. The S^2 pocket of cathepsin S is formed by the side chains of Phe 67, Met 68, Gly 133, Val 157, Gly 160, and Phe 205 (papain numbering).[53] This protease prefers substrates containing either valine or leucine in P^2; the substrate specificity is less restricted.[54] In accordance, potent inhibitors of the present series bear aliphatic as well as aromatic residues in the P^2 position, a feature which distinguished cathepsin S from the other examined enzymes (Table 1). This observation reflects the plasticity of the S^2 site, which can be attributed to a conformational switch of Phe 205, a phenomenon discovered upon comparison of enzyme−inhibitor complexes containing either P^2-Leu or P^2-Phe derived inhibitors.[46] The leucine-derived inhibitors **7** and **8** possessed high potency. Prolongation and cyclization of the leucine side chain by three methylene groups led to the cyclohexylalanine derivative **11**, which was the most potent inhibitor ($K_i = 0.044$ μM) among all considered nitriles. It inhibited cathepsin S with high selectivity over the cathepsins K and L. This observation accords with that made by Ward et al.[26] A further particularly active compound was the β-naphthylalanine derivative **34**, which also inhibited cathepsin L in a comparable manner. Replacement of the phenyl group in **16** by a 2-thienyl moiety in **19** intensified the inhibition of cathepsin S. The thienylalanine derivative **19** showed a weak preference for cathepsin S, while the biphenyl compounds **35** and **39** did not differentiate well between cathepsin L and S. With respect to Z or Boc as the *N*-protecting group, cathepsin S behaved similarly. Surprisingly, when the Z-derivative **17** was shortened by the CH_2O unit, as realized in the benzoyl-protected **43**, the potency increased by nearly 1 order of magnitude (Table 3).

Inhibition of Cathepsin K. The substrate specificity of cathepsin K is similar to that of cathepsin S; leucine is favored over phenylalanine in P^2.[20] Its S^2 subsite is defined by Tyr 67, Met 68, Ala 133, Leu 157, Ala 160, and Leu 205 (papain numbering).[55] Cathepsin K showed the highest affinities toward dipeptide nitriles with a leucine side chain (**7**, **8**, Table 1). Every structural

change of this moiety increased the inhibition constant, which then mostly escaped from the nanomolar range. Thus, cathepsin K was unique in that it possessed minor promiscuity with respect to the acceptance of a variety of P^2 residues, in particular those from nonproteinogenic amino acids. Especially certain space-filling aromatic substituents at P^2 were not at all tolerated, e.g. the *O*-benzylserine (**22**), tyrosine (**23**), *para*-bromophenylalanine (**28**), and *β*-naphthylalanine (**34**) derivatives, all of which were found to be substantially less potent, compared to the other proteases. Enhanced inhibitory activity was observed when the *N*-terminal group was changed from *tert*-butoxycarbonyl to benzyloxycarbonyl (**7** versus **8**; **16** versus **17**). The replacement of the *tert*-butoxycarbonyl group by benzoyl (**16** versus **43**, Table 3) had no remarkable effect, whereas a 4-phenylbenzoyl group shifted the inhibition constant into the nanomolar range (**44** versus **43**). These observations implicated that an aromatic entity needs to have an exact distance to the P^2-nitrogen for favorable interactions.

Conclusions

Potent inhibitors of papain-like cysteine proteases were designed due to the introduction of structural diversity into the P^2 side chain of dipeptidyl nitriles with an aminoacetonitrile portion at the P^1 position. This approach is based on the major contribution of the P^2-S^2 interaction to facilitate strong binding of the inhibitors to the enzyme. Although the formation of a covalent thioimidate complex is well established for the reaction of cysteine proteinases with nitrile inhibitors, the cooperative effect of both the covalent attachment and the P^2-S^2 interaction are crucial factors for inhibition. Among the four enzymes used in this study, cathepsin K showed a rather distinct profile with respect to inhibitor structures. Papain, cathepsin L, and cathepsin S share the P^2 substrate specificity and tended to exhibit similar affinities to a number of inhibitors. As papain represents the best characterized and cheapest cysteine protease, it might be used as model enzyme in the preliminary search for inhibitors of the cathepsins L and S. It can be concluded from the data of this study that selectivity for one of the target enzymes over the other closely related proteases is difficult to achieve with dipeptide-derived compounds. The cysteine proteases did not show promiscuity with respect to changes within the backbone structure or methylation at the P^1 nitrogen as found by systematic structural changes of a parent compound **16**. All enzymes behaved sensitively to subtle changes in the P^2 entity. It was demonstrated that various nonproteinogenic amino acids could be introduced into the P^2 position to result in an enhanced affinity. The present series was mainly focused on variations within the P^2 side chain. However, the *N*-protecting group was found to be a suitable unit to accentuate the enzyme−inhibitor interaction. Thus, a combination of the P^2 side chain, as established in this study, with diverse groups at the P^2 nitrogen might be a promising strategy for future investigations.

Experimental Section

General Methods and Materials. Melting points were determined on a Büchi 510 oil bath apparatus and are not corrected. Thin-layer chromatography was performed on Merck aluminum sheets. Preparative column chromatography was performed on silica gel 60 (Fluka) 70−230 mesh. Preparative HPLC was performed on a Eurospher 100 C_{18} column (250 × 20 mm) with a mixture of MeOH and H_2O at a flow rate of 20 mL/min. Microwave assisted syntheses were done in a CEM discover system. ^{1}H NMR spectra (500 MHz) and ^{13}C NMR spectra (125 MHz) were recorded on a Bruker Avance 500. Mass spectra were obtained on a API 2000 spectrometer from Applied Biosystems (ESI, sprayed from a 10^{-5} M solution in 2 mM NH_4OAc/MeOH 1:1; volumetric flow rate 10 μL/min). Optical rotations were determined on a Perkin-Elmer 241 Polarimeter. Enzymatic activity of papain and cathepsins was measured with chromogenic substrates spectrophotometrically at a Varian Cary Bio 50 UV/vis spectrophotometer or fluorometrically at a Perkin-Elmer luminescence spectrometer LS 50 B, respectively. Papain was purchased from Sigma, Germany. Bovine liver cathepsin L and human cathepsin L, His Tag were purchased from Calbiochem, Germany. Recombinant human cathepsin S was either expressed in insect cells[56] or purchased from Calbiochem; the two preparations showed a similar K_m toward Z-Val-Val-Arg-NHMec. Recombinant human Cathepsin K (expressed in yeast) was a gift of D. Brömme.[57] Human cathepsin L, His Tag was purchased from Calbiochem.

The substrates, Z-Phe-Arg-NHNp, Z-Phe-Arg-NHMec, Z-Val-Val-Arg-NHMec, Z-Leu-Arg-NHMec, were from Bachem, Bubendorf, Switzerland. The amino acid derivatives were purchased from Novabiochem, Läufelfingen, Switzerland, Bachem, Bubendorf, Switzerland, Acros, Geel, Belgium, Aldrich, Steinheim, Germany, and Synthetech Inc., Albany, NY. *N*-(2-Naphthylsulfonyl)-glycine was prepared as described in the literature.[58] DTT (= (±)-*threo*-2,3-dihydroxy-1,4-butanedithiol), Brij 35 P, and Triton X-100 were obtained from Fluka; CHAPS (= 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate) was from Sigma. Mathematical data analyses were done with the programs Grafit 4 (Erithacus Software), GraphPad Prism 4 (GraphPad Software) and EASY-FIT.[59]

***N*-(*tert*-Butoxycarbonyl)-glycyl-glycine-nitrile (1). General Procedure for Dipeptide Nitriles 1−38.** Boc-Gly-OH (1 g, 5.7 mmol) was dissolved in THF (15 mL) and cooled to −25 °C. Subsequently, *N*-methylmorpholine (1.73 mL, 5.7 mmol) and isobutyl chloroformate (0.75 mL, 5.7 mmol) were sequentially added under vigorous stirring. Immediately after the formation of a white precipitate (*N*-methylmorpholine hydrochloride), a solution of aminoacetonitrile sulfate (0.66 g, 6.3 mmol) in H_2O (0.5 mL), precooled on ice, and 1 N NaOH (6.3 mL) were added. The resulting mixture was allowed to warm to room temperature. After 2 h, THF was removed under reduced pressure and the residual aqueous mixture was diluted with a small volume of H_2O, adjusted to pH 1 (10% $NaHSO_4$) and extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with H_2O (10 mL), sat. $NaHCO_3$ (2 × 10 mL), and brine (10 mL), dried over Na_2SO_4, and evaporated to dryness. The solid residue was recrystallized from cyclohexane/ethyl acetate to obtain **1** (0.83 g, 68%): mp 110−112 °C (lit.[60] mp 108−110 °C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39 (s, 9H), 3.78 (d, J = 5.6 Hz, 2H), 4.11 (d, J = 5.8 Hz, 2H), 5.41 (br s, 1H), 7.27 (t, J = 5.2 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.41, 28.31, 44.18, 80.83, 116.06, 156.44, 170.29; MS (ESI) *m/z* (rel intensity) (pos.) 236 (35, $[M + Na]^+$), 231 (100, $[M + NH_4]^+$), 214 (66, $[M + H]^+$), 158 (62, $[M - (CH_3)_2CCH_2 + H]^+$), 114 (5, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$) (neg.) 272 (57, $[M + CH_3COO]^-$), 212 (86, $[M - H]^-$), 138 (100, $[M - t\text{BuOH} - H]^-$). Anal. ($C_9H_{15}N_3O_3$) C, H, N.

***N*-(Benzyloxycarbonyl)-glycyl-glycine-nitrile (2).** The crude product was recrystallized from cyclohexane/ethyl acetate, yield 90%: mp 144−146 °C (lit.[61] mp 148−150 °C); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.65 (d, J = 6.3 Hz, 2H), 4.12 (d, J = 5.7 Hz, 2H), 5.03 (s, 2H), 7.25−7.40 (m, 5H), 7.52 (t, J = 6.2 Hz, 1H), 8.58 (t, J = 5.4 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.14, 43.49, 65.74, 117.68, 127.87, 127.95, 128.48, 137.07, 156.63, 170.11; MS (ESI) *m/z* (rel intensity) (pos.) 270 (32, $[M + Na]^+$), 265 (100, $[M + NH_4]^+$), 248 (52, $[M + H]^+$), 204

(38, $[M - CO_2 + H]^+$) (neg.) 306 (32, $[M + CH_3COO]^-$), 246 (45, $[M - H]^-$), 138 (100, $[M - BnOH - H]^-$). Anal. ($C_{12}H_{13}N_3O_3$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-alanyl-glycine-nitrile (3).** The oily residue obtained after removal of ethyl acetate solidified in the refrigerator, and the solid was suspended in petrol ether and collected by filtration, yield 72%: mp 92−95 °C (lit.[60] 90−92 °C); $[\alpha]^{20}_D = -26.0$ ($c = 1.13$, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.16 (d, $J = 7.3$ Hz, 3H), 1.37 (s, 9H), 3.95 (m, 1H), 4.10 (d, $J = 5.7$ Hz, 2H), 7.00 (d, $J = 7.0$ Hz, 1H), 8.46 (t, $J = 5.4$ Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.87, 27.23, 28.32, 49.68, 78.27, 117.71, 155.24, 173.58; MS (ESI) *m/z* (rel intensity) (pos.) 266 (5, $[M + K]^+$), 250 (9, $[M + Na]^+$), 245 (36, $[M + NH_4]^+$), 228 (35, $[M + H]^+$), 172 (100, $[M - (CH_3)_2CCH_2 + H]^+$), 128 (68, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$), (neg.) 286 (20, $[M + CH_3COO]^-$), 226 (100, $[M - H]^-$), 152 (79, $[M - t$BuOH $- H]^-$). Anal. ($C_{10}H_{17}N_3O_3$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-2-aminobutanoyl-glycine-nitrile (4).** The oily residue obtained after removal of the solvent crystallized in the refrigerator, and the solid was suspended in petrol ether and collected by filtration, yield 80%: mp 80−82 °C; $[\alpha]^{20}_D = -27.8$ ($c = 2.79$, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.94 (t, $J = 7.4$ Hz, 3H), 1.43 (s, 9H), 1.59−1.70 (m, 1H), 1.80−1.90 (m, 1H), 4.01−4.09 (m, 1H), 4.14 (d, $J = 5.4$ Hz, 2H), 5.14 (d, $J = 7.9$ Hz, 1H), 7.31 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 9.99, 25.33, 27.29, 28.29, 55.51, 80.67, 115.88, 156.09, 172.58; MS (ESI) *m/z* (rel intensity) (pos.) 280 (4, $[M + K]^+$), 264 (54, $[M + Na]^+$), 259 (100, $[M + NH_4]^+$), 242 (97, $[M + H]^+$), 186 (99, $[M - (CH_3)_2CCH_2 + H]^+$), 142 (16, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$) (neg.) 300 (57, $[M + CH_3COO]^-$), 240 (99, $[M - H]^-$), 166 (100, $[M - t$BuOH $- H]^-$). Anal. ($C_{11}H_{19}N_3O_3$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-valyl-glycine-nitrile (5).** The crude product was recrystallized from petrol ether/ethyl acetate, yield 54%: mp 110−111 °C; $[\alpha]^{20}_D = -28.4$ ($c = 1.20$, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.93 (d, $J = 7.0$ Hz, 3H), 0.95 (d, $J = 6.9$ Hz), 1.43 (s, 9H), 2.03−2.16 (m, 1H), 3.92−3.99 (m, 1H), 4.09 (dd, $J = 17.3$, 5.7 Hz, 1H), 4.18 (dd, $J = 17.5$, 5.8 Hz, 1H), 5.23 (d, $J = 7.9$ Hz, 1H), 7.36 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.08, 19.18, 27.23, 28.30, 30.74, 59.82, 80.50, 115.82, 155.21, 172.36; MS (ESI) *m/z* (rel intensity) (pos.) 294 (4, $[M + K]^+$), 278 (8, $[M + Na]^+$), 273 (39, $[M + NH_4]^+$), 256 (30, $[M + H]^+$), 200 (100, $[M - (CH_3)_2CCH_2 + H]^+$), 156 (47, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$), (neg.) 314 (25, $[M + CH_3COO]^-$), 254 (100, $[M - H]^-$), 180 (67, $[M - t$BuOH $- H]^-$). Anal. ($C_{12}H_{21}N_3O_3$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-norvalyl-glycine-nitrile (6).** The crude product was recrystallized from petrol ether/ethyl acetate, yield 44%: mp 120−123 °C; $[\alpha]^{20}_D = -23.1$ ($c = 1.87$, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.92 (t, $J = 7.4$ Hz, 3H), 1.29−1.42 (m, 2H), 1.43 (s, 9H), 1.53−1.62 (m, 1H), 1.74−1.82 (m, 1H), 4.05−4.19 (m, 3H), 5.10 (d, $J = 7.3$ Hz, 1H), 7.28 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.64, 18.86, 27.31, 28.28, 33.99, 54.10, 80.69, 115.88, 156.09, 172.77; MS (ESI) *m/z* (rel intensity) (pos.) 294 (4, $[M + K]^+$), 278 (61, $[M + Na]^+$), 273 (93, $[M + NH_4]^+$), 256 (100, $[M + H]^+$), 200 (94, $[M - (CH_3)_2CCH_2 + H]^+$), 156 (25, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$) (neg.) 314 (62, $[M + CH_3COO]^-$), 254 (96, $[M - H]^-$), 180 (100, $[M - t$BuOH $- H]^-$). Anal.($C_{12}H_{21}N_3O_3$) C, H; N: calcd 16.46, found 15.94.

***N*-(*tert*-Butoxycarbonyl)-leucyl-glycine-nitrile (7).** The oily residue solidified in the refrigerator. It was suspended in petrol ether and collected by filtration, yield 92%: mp 114−116 °C; $[\alpha]^{20}_D = -23.4$ ($c = 2.11$, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.90 (d, $J = 6.3$ Hz, 3H), 0.93 (d, $J = 6.6$ Hz, 3H), 1.43 (s, 9H), 1.46−1.56 (m, 1H), 1.59−1.71 (m, 2H), 4.13 (d, $J = 5.7$ Hz, 2H), 4.13−4.21 (m, 1H), 5.08 (d, $J = 4.1$ Hz, 1H), 7.41 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.77, 22.87, 24.67, 27.33, 28.28, 40.59, 52.73, 80.70, 115.91, 156.17, 173.17; MS (ESI) *m/z* (rel intensity) (pos.) 292 (6, $[M + Na]^+$), 287 (100, $[M + NH_4]^+$), 270 (60, $[M + H]^+$), 214 (100, $[M - (CH_3)_2CCH_2 + H]^+$), 170 (21, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$) (neg.) 328 (48, $[M + CH_3COO]^-$), 268 (100, $[M - H]^-$), 194 (73, $[M - t$BuOH $- H]^-$). Anal. ($C_{13}H_{23}N_3O_3$) C, H, N.

***N*-(Benzyloxycarbonyl)-leucyl-glycine-nitrile (8).** In addition to the General Procedure, the free carboxylic acid Z-Leu-OH was released from its commercially available dicyclohexylammonium salt by the following method: Z-Leu-OH × dicyclohexylamine (1 g, 2.2 mmol) was suspended in ethyl acetate (20 mL) and shaken with ice-cold 2 N H_2SO_4 (6 mL). The organic phase was separated, the aqueous phase was diluted with H_2O (10 mL) and extracted with ethyl acetate (2 × 20 mL). The combined organic layers were washed with H_2O (10 mL) and brine (10 mL), dried over Na_2SO_4, and concentrated to dryness under reduced pressure. The oily residue obtained thereby was subjected to the standard mixed anhydride coupling to obtain a pure crude product, yield 94%: mp 112−113 °C; $[\alpha]^{20}_D = -22.3$ ($c = 2.15$, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.89 (d, $J = 6.0$ Hz, 3H), 0.91 (d, $J = 6.3$ Hz, 3H), 1.49−1.56 (m, 1H), 1.60−1.68 (m, 2H), 4.06 (d, $J = 5.7$ Hz, 2H), 4.17−4.27 (m, 1H), 5.06 (d, $J = 12.3$ Hz, 1H), 5.10 (d, $J = 12.3$ Hz, 1H), 5.38 (d, $J = 8.2$ Hz, 2H), 7.21 (br s, 1H), 7.29−7.35 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.79, 22.82, 24.62, 27.38, 40.68, 53.20, 67.43, 115.90, 127.96, 128.37, 128.62, 135.83, 156.61, 172.70; MS (ESI) *m/z* (rel intensity) (pos.) 326 (6, $[M + Na]^+$), 321 (100, $[M + NH_4]^+$), 304 (66, $[M + H]^+$), 260 (34, $[M - CO_2 + H]^+$) (neg.) 362 (67, $[M + CH_3COO]^-$), 302 (100, $[M - H]^-$), 194 (71, $[M - BnOH - H]^-$). Anal. ($C_{16}H_{21}N_3O_3$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-isoleucyl-glycine-nitrile (9).** The crude product was recrystallized from petrol ether/ethyl acetate, yield 48%: mp 130−132 °C; $[\alpha]^{20}_D = 22.5$ ($c = 1.23$, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.88 (t, $J = 7.4$ Hz, 3H), 0.92 (d, $J = 6.6$ Hz, 3H), 1.06−1.18 (m, 1H), 1.42 (s, 9H), 1.47−1.57 (m, 1H), 1.79−1.92 (m, 1H), 3.97 (dd, $J = 8.5$, 7.3 Hz, 1H), 4.10 (dd, $J = 17.8$, 6.0 Hz, 1H), 4.17 (dd, $J = 17.5$, 5.9 Hz, 1H), 5.20 (d, $J = 7.9$ Hz), 7.33 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.09, 15.48, 24.81, 27.23, 28.30, 36.92, 58.99, 80.52, 115.80, 156.16, 172.44; MS (ESI) *m/z* (rel intensity) (pos.) 308 (4, $[M + K]^+$), 292 (9, $[M + Na]^+$), 287 (57, $[M + NH_4]^+$), 270 (42, $[M + H]^+$), 214 (100, $[M - (CH_3)_2CCH_2 + H]^+$), 170 (50, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$), (neg.) 328 (26, $[M + CH_3COO]^-$), 268 (100, $[M - H]^-$), 194 (87, $[M - t$BuOH $- H]^-$). Anal. ($C_{13}H_{23}N_3O_3$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-cyclopropylalanyl-glycine-nitrile (10).** The corresponding free carboxylic acid was released from its dicyclohexylammonium salt as described in the preparation of **8**. The crude product was recrystallized from *n*-hexane/ethyl acetate, yield 72%: mp 124−125 °C (*n*-hexane/ethyl acetate); $[\alpha]^{20}_D = -17.3$ ($c = 2.28$, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ −0.02−0.04 (m, 1H), 0.05−0.12 (m, 1H), 0.30−0.43 (m, 2H), 0.64−0.77 (m, 1H), 1.31−1.39 (m, 1H), 1.37 (s, 9H), 1.48−1.60 (m, 1H), 3.91−4.00 (m, 1H), 4.05−4.16 (m, 2H), 6.94 (d, $J = 7.9$ Hz, 1H), 8.52 (t, $J = 5.7$ Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 4.05, 4.60, 7.87, 27.16, 28.37, 36.58, 54.85, 78.21, 117.62, 155.46, 172.94; MS (ESI) *m/z* (rel intensity) (pos.) 306 (3, $[M + K]^+$), 290 (10, $[M + Na]^+$), 285 (41, $[M + NH_4]^+$), 268 (38, $[M + H]^+$), 212 (100, $[M - (CH_3)_2CCH_2 + H]^+$), 168 (32, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$) (neg.) 326 (19, $[M + CH_3COO]^-$), 266 (100, $[M - H]^-$), 192 (89, $[M - t$BuOH $- H]^-$). Anal. ($C_{13}H_{21}N_3O_3$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-cyclohexylalanyl-glycine-nitrile. (11)** The corresponding free carboxylic acid was released from its dicyclohexylammonium salt as described in the preparation of **8**. Recrystallization was done from petrol ether/ethyl acetate, yield 53%: mp 81−83 °C; $[\alpha]^{20}_D = -12.8$ ($c = 1.08$, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.80−1.01 (m, 3H), 1.06−1.27 (m, 3H), 1.43 (s, 9H), 1.53−1.77 (m, 7H), 4.13 (d, $J = 5.4$ Hz, 2H), 4.15−4.23 (m, 1H), 5.01 (d, $J = 7.9$ Hz, 1H), 7.31 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 25.97, 26.15, 26.31, 27.32, 28.28, 32.47, 33.58, 33.98, 39.18, 52.08, 80.75, 115.88, 156.17, 173.16; MS (ESI) *m/z* (rel intensity) (pos.) 348 (6, $[M + K]^+$), 332 (8, $[M + Na]^+$), 327 (46, $[M + NH_4]^+$), 310 (42, $[M + H]^+$) 254 (100, $[M - (CH_3)_2CCH_2 + H]^+$), 210 (65, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$) (neg.) 368 (29, $[M + CH_3COO]^-$), 308 (100, $[M - H]^-$), 234 (50, $[M - t$BuOH $- H]^-$). Anal. ($C_{16}H_{27}N_3O_3$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-methionyl-glycine-nitrile (12).** This compound was obtained as a pure crude product, yield 91%: mp 98−99 °C; $[\alpha]^{20}_D = -22.2$ ($c = 1.50$, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.37 (s, 9H), 1.72−1.88 (m, 2H), 2.02 (s, 3H), 2.36−2.48 (m, 2H), 3.96−4.06 (m, 1H), 4.10 (d, J = 5.7 Hz, 2H), 7.06 (d, J = 7.9 Hz, 1H), 8.52 (t, J = 5.5 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.74, 27.28, 28.30, 29.31, 31.39, 53.41, 78.39, 117.66, 155.55, 172.65; MS (ESI) *m/z* (rel intensity) (pos.) 326 (6, $[M + K]^+$), 310 (13, $[M + Na]^+$), 305 (50, $[M + NH_4]^+$), 288 (57, $[M + H]^+$), 232 (100, $[M - (CH_3)_2CCH_2 + H]^+$), 188 (57, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$) (neg.) 346 (14, $[M + CH_3COO]^-$), 286 (100, $[M - H]^-$), 212 (82, $[M - t\text{BuOH} - H]^-$). Anal. ($C_{12}H_{21}N_3O_3S$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-norleucyl-glycine-nitrile (13).** The corresponding free carboxylic acid was released from its dicyclohexylammonium salt as described in the preparation of **8**. The product was obtained as colorless oil, which solidified in the refrigerator; it was suspended in petrol ether and collected by filtration, yield 82%: mp 67−75 °C; $[\alpha]^{20}_D = -17.1$ (c = 2.11, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85−0.90 (m, 3H), 1.25−1.36 (m, 4H), 1.42 (s, 9H), 1.53−1.64 (m, 1H), 1.74−1.85 (m, 1H), 4.05−4.20 (m, 3H), 5.17 (d, J = 7.9 Hz, 1H), 7.39 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.81, 22.28, 27.30, 27.67, 28.28, 31.78, 54.29, 80.63, 115.90, 156.09, 172.87; MS (ESI) *m/z* (rel intensity) (pos.) 308 (4, $[M + K]^+$), 292 (10, $[M + Na]^+$), 287 (43, $[M + NH_4]^+$), 270 (35, $[M + H]^+$), 214 (100, $[M - (CH_3)_2CCH_2 + H]^+$), 170 (52, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$) (neg.) 328 (24, $[M + CH_3COO]^-$), 268 (100, $[M - H]^-$), 194 (56, $[M - t\text{BuOH} - H]^-$). Anal. ($C_{13}H_{23}N_3O_3$) C, H, N: calcd 15.60; found 15.06.

***N*-(*tert*-Butoxycarbonyl)-prolyl-glycine-nitrile (14).** The crude product was recrystallized from petrol ether/ethyl acetate, yield 39%: mp 139−140 °C; $[\alpha]^{20}_D = -49.2$ (c = 1.39, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.33 & 1.39 (s, 9H), 1.68−1.85 (m, 3H), 2.02−2.19 (m, 1H), 3.23−3.33 (m, 1H), 3.35−3.41 (m, 1H), 4.00−4.05 (m, 1H), 4.05−4.16 (m, 2H), 8.49−8.54 & 8.54−8.61 (m, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 23.35 (i) & 24.03 (w), 27.13, 28.06 (i) & 28.26 (w), 30.79 (i) & 30.98 (w), 46.54 (i) & 46.78 (w), 59.52 (w) & 59.77 (i), 78.86 (i) & 78.90 (w), 117.61 (i) & 117.67 (w), 153.27 (i) & 153.80 (w), 172.98 (w) & 173.39 (i); MS (ESI) *m/z* (rel intensity) (pos.) 292 (6, $[M + K]^+$), 276 (8, $[M + Na]^+$), 271 (29, $[M + NH_4]^+$), 254 (48, $[M + H]^+$), 198 (100, $[M - (CH_3)_2CCH_2 + H]^+$), 154 (87, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$) (neg.) 312 (28, $[M + CH_3COO]^-$), 252 (100, $[M - H]^-$), 178 (47, $[M - t\text{BuOH} - H]^-$). Anal. ($C_{12}H_{19}N_3O_3$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-phenylglycyl-glycine-nitrile (15).** Recrystallization of the crude product was done from petrol ether/ethyl acetate, yield 66%: mp 176−177 °C; $[\alpha]^{20}_D = 96.5$ (c = 2.01, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.37 (s, 9H), 4.13 (d, J = 5.7 Hz, 2H), 5.17 (d, J = 8.2 Hz, 1H), 7.26−7.30 (m, 1H), 7.31−7.35 (m, 2H), 7.35−7.39 (m, 2H), 7.42 (d, J = 6.6 Hz, 1H), 8.80 (t, J = 5.5 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 27.30, 28.27, 57.81, 78.65, 117.48, 127.53, 127.89, 128.46, 138.09, 155.11, 171.02; MS (ESI) *m/z* (rel intensity) (pos.) 312 (30, $[M + Na]^+$), 307 (99, $[M + NH_4]^+$), 290 (100, $[M + H]^+$), 234 (93, $[M - (CH_3)_2CCH_2 + H]^+$), 190 (70, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$) (neg.) 348 (22, $[M + CH_3COO]^-$), 288 (99, $[M - H]^-$), 214 (100, $[M - t\text{BuOH} - H]^-$). Anal. ($C_{15}H_{19}N_3O_3$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-phenylalanyl-glycine-nitrile (16).** The crude product was recrystallized from cyclohexane/ethyl acetate, yield 59%: mp 136−138 °C (lit.[60] mp 136.5−137.5 °C); $[\alpha]^{20}_D = 1.2$ (c = 1.10, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.37 (s, 9H), 2.98−3.08 (m, 2H), 4.05 (d, J = 5.8 Hz, 2H), 4.30−4.45 (m, 1H), 5.16 (d, J = 7.3 Hz, 1H), 6.94 (br s, 1H) 7.13−7.18 (m, 2H), 7.20−7.25 (m, 1H), 7.26−7.31 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.27, 28.22, 38.20, 55.63, 80.74, 115.57, 127.16, 128.80, 129.21, 136.04, 155.73, 171.92; MS (ESI) *m/z* (rel intensity) (pos.) 342 (3, $[M + K]^+$), 326 (21, $[M + Na]^+$), 321 (82, $[M + NH_4]^+$), 304 (59, $[M + H]^+$), 248 (100, $[M - (CH_3)_2CCH_2 + H]^+$), 204 (68, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$) (neg.) 362 (33, $[M + CH_3COO]^-$), 302 (100, $[M - H]^-$), 228 (83, $[M - t\text{BuOH} - H]^-$). Anal. ($C_{16}H_{21}N_3O_3$) C, H, N.

***N*-(Benzyloxycarbonyl)-phenylalanyl-glycine-nitrile (17).** The crude product was recrystallized from petrol ether/ethyl acetate, yield 72%: mp 139−141 °C; $[\alpha]^{20}_D = -12.9$ (c = 2.07, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.01−3.09 (m, 2H), 3.99 (d, J = 5.7 Hz, 2H), 4.46 (m, 1H), 5.04 (s, 2H), 5.61 (d, J = 6.7 Hz, 1H) 7.12−7.18 (m, 3H), 7.21−7.37 (m, 8H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.24, 38.29, 55.96, 67.21, 115.64, 127.17, 127.91, 128.26, 128.55, 128.77, 129.22, 135.85, 135.88, 156.13, 171.57; MS (ESI) *m/z* (rel intensity) (pos.) 360 (12, $[M + Na]^+$), 355 (100, $[M + NH_4]^+$), 338 (50, $[M + H]^+$), 294 (62, $[M - CO_2 + H]^+$), (neg.) 396 (25, $[M + CH_3COO]^-$), 336 (100, $[M - H]^-$), 228 (51, $[M - BnOH - H]^-$). Anal. ($C_{19}H_{19}N_3O_3$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-2′-furylalanyl-glycine-nitrile (18).** The commercially available *tert*-butylammonium salt of the corresponding protected amino acid was employed directly in the mixed anhydride procedure. Therefore, this reaction was performed without *N*-methylmorpholine. Fractionation of the crude material on silica gel using petrol ether/ethyl acetate (2:1) as eluent led to a colorless oil, which crystallized in the refrigerator overnight, yield 31%: mp 112−113 °C; $[\alpha]^{20}_D = -10.3$ (c = 1.07, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.41 (s, 9H), 3.05−3.17 (m, 2H), 4.07 (dd, J = 17.2, 5.2 Hz, 1H), 4.14 (dd, J = 17.4, 6.0 Hz, 1H), 4.42 (br s, 1H), 5.17 (d, J = 7.6 Hz, 1H), 6.12 (d, J = 3.2 Hz, 1H), 6.28 (dd, J = 3.0, 2.1 Hz, 1H), 6.93 (br s, 1H), 7.31−7.34 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.36, 28.24, 30.44, 53.62, 80.98, 108.21, 110.57, 115.57, 142.23, 150.18, 155.68, 171.43; MS (ESI) *m/z* (rel intensity) (pos.) 332 (6, $[M + K]^+$), 316 (10, $[M + Na]^+$), 311 (36, $[M + NH_4]^+$), 294 (42, $[M + H]^+$), 238 (100, $[M - (CH_3)_2CCH_2 + H]^+$), 194 (90, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$) (neg.) 352 (13, $[M + CH_3COO]^-$), 292 (100, $[M - H]^-$), 218 (76, $[M - t\text{BuOH} - H]^-$). Anal. ($C_{14}H_{19}N_3O_4$) C: calcd 57.33, found 58.18; H: calcd 6.53, found 6.94; N: calcd 14.33, found 13.14.

***N*-(*tert*-Butoxycarbonyl)-2′-thienylalanyl-glycine-nitrile (19).** Pure product was obtained by recrystallization from *n*-hexane/ethyl acetate, yield 61%: mp 117−119 °C; $[\alpha]^{20}_D = -5.0$ (c = 1.21, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.42 (s, 9H), 3.27 (dd, J = 15.0, 6.5 Hz, 1H), 3.32 (dd, J = 15.0, 6.5 Hz, 1H), 4.07 (dd, J = 17.5, 5.2 Hz, 1H), 4.15 (dd, J = 17.4, 6.0 Hz, 1H), 4.37 (m, 1H), 5.05 (d, J = 5.4 Hz, 1H), 6.82 (br s, 1H), 6.85 (dd, J = 3.5, 0.95 Hz, 1H), 6.93 (dd, J = 5.1, 3.5 Hz, 1H), 7.17 (dd, J = 5.2, 1.1 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.39, 28.25, 31.91, 55.38, 81.11, 115.49, 125.08, 127.02, 127.25, 137.60, 155.68, 171.33; MS (ESI) *m/z* (rel intensity) (pos.) 348 (6, $[M + K]^+$), 332 (15, $[M + Na]^+$), 327 (52, $[M + NH_4]^+$), 310 (40, $[M + H]^+$), 254 (100, $[M - (CH_3)_2CCH_2 + H]^+$), 210 (82, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$) (neg.) 368 (17, $[M + CH_3COO]^-$), 308 (100, $[M - H]^-$), 234 (70, $[M - t\text{BuOH} - H]^-$). Anal. ($C_{14}H_{19}N_3O_3S$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-3′-thienylalanyl-glycine-nitrile (20).** Pure product was obtained by recrystallization from *n*-hexane/ethyl acetate, yield 42%: mp 137−139 °C; $[\alpha]^{20}_D = -1.3$ (c = 1.06, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.40 (s, 9H), 3.04−3.15 (m, 2H), 4.06 (dd, J = 17.4, 5.3 Hz, 1H), 4.12 (dd, J = 17.2, 5.9 Hz, 1H), 4.28−4.42 (m, 1H), 5.02 (d, J = 6.6 Hz, 1H), 6.78 (br s, 1H), 6.92 (dd, J = 4.9, 1.2 Hz, 1H), 7.02−7.05 (m, 1H), 7.28 (dd, J = 5.0, 2.9 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.36, 28.25, 32.37, 54.89, 80.98, 115.54, 123.02, 126.50, 128.22, 136.12, 155.72, 171.78; MS (ESI) *m/z* (rel intensity) (pos.) 348 (6, $[M + K]^+$), 332 (13, $[M + Na]^+$), 327 (47, $[M + NH_4]^+$), 310 (41, $[M + H]^+$), 254 (100, $[M - (CH_3)_2CCH_2 + H]^+$), 210 (84, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$) (neg.) 368 (14, $[M + CH_3COO]^-$), 308 (100, $[M - H]^-$), 234 (87, $[M - t\text{BuOH} - H]^-$). Anal. ($C_{14}H_{19}N_3O_3S$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-homophenylalanyl-glycine-nitrile (21).** The oily crude product crystallized in the refrigerator; it was suspended in Et_2O and collected by filtration, yield 47%: mp 95−98 °C; $[\alpha]^{20}_D = -21.0$ (c = 1.12, dioxane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.44 (s, 9H), 1.87−1.97 (m, 1H), 2.09−2.19 (m, 1H), 2.61−2.74 (m, 2H), 4.04−4.16 (m, 3H), 5.13 (br s, 1H), 7.12−7.21 (m, 4H), 7.24−7.28

(m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.33, 28.29, 31.76, 33.23, 53.70, 80.81, 115.80, 126.30, 128.36, 128.58, 140.34, 156.06, 172.42; MS (ESI) *m/z* (rel intensity) (pos.) 356 (7, $[M + K]^+$), 340 (37, $[M + Na]^+$), 335 (100, $[M + NH_4]^+$), 318 (75, $[M + H]^+$), 262 (88, $[M - (CH_3)_2CCH_2 + H]^+$), 218 (53, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$) (neg.) 376 (41, $[M + CH_3COO]^-$), 316 (96, $[M - H]^-$), 242 (100, $[M - t$BuOH $- H]^-$); Anal. ($C_{17}H_{23}N_3O_3$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-*O*-benzylseryl-glycine-nitrile (22).** The oily crude product crystallized in the refrigerator; it was suspended in petrol ether and collected by filtration, yield 83%: mp 81−85 °C; $[\alpha]^{20}_D = 3.2$ ($c = 1.11$, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.43 (s, 9H), 3.57 (dd, $J = 9.5$, 6.3 Hz, 1H), 3.86−3.91 (m, 1H), 4.06−4.20 (m, 2H), 4.30 (br s, 1H), 4.49 (d, $J = 11.7$ Hz, 1H), 4.55 (d, $J = 12.0$ Hz, 1H), 5.34 (br s, 1H), 7.02 (t, $J = 5.7$ Hz, 1H), 7.26−7.31 (m, 3H), 7.32−7.37 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.43, 28.24, 53.92, 69.28, 73.60, 80.78, 115.64, 127.88, 128.14, 128.61, 137.06, 155.45, 170.82; MS (ESI) *m/z* (rel intensity) (pos.) 372 (6, $[M + K]^+$), 356 (13, $[M + Na]^+$), 351 (43, $[M + NH_4]^+$), 334 (35, $[M + H]^+$), 278 (100, $[M - (CH_3)_2CCH_2 + H]^+$), 234 (36, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$), (neg.) 392 (14, $[M + CH_3COO]^-$) 332 (100, $[M - H]^-$), 258 (90, $[M - t$BuOH $- H]^-$). Anal. ($C_{17}H_{23}N_3O_4$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-tyrosyl-glycine-nitrile (23).** The crude product was recrystallized from cyclohexane/ethyl acetate, yield 80%: mp 166−168 °C; $[\alpha]^{20}_D = 6.8$ ($c = 1.16$, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.30 (s, 9H), 2.63 (dd, $J = 13.7$, 9.6 Hz, 1H), 2.82 (dd, $J = 13.7$, 4.9 Hz, 1H), 4.03−4.07 (m, 1H), 4.10 (d, $J = 5.7$ Hz, 2H), 6.63 (d, $J = 8.2$ Hz, 2H), 6.90 (d, $J = 8.5$ Hz, 1H), 7.00 (d, $J = 8.2$ Hz, 2H), 8.55 (t, $J = 5.35$ Hz, 1H), 9.12 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 27.21, 28.28, 36.58, 56.03, 78.24, 115.02, 117.59, 127.94, 130.17, 155.37, 155.94, 172.63; MS (ESI) *m/z* (rel intensity) (pos.) 358 (7, $[M + K]^+$), 342 (13, $[M + Na]^+$), 337 (68, $[M + NH_4]^+$), 320 (48, $[M + H]^+$), 264 (100, $[M - (CH_3)_2CCH_2 + H]^+$), 220 (34, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$) (neg.) 378 (50, $[M + CH_3COO]^-$), 318 (100, $[M - H]^-$), 244 (51, $[M - t$BuOH $- H]^-$). Anal. ($C_{16}H_{21}N_3O_4$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-4′-fluorophenylalanyl-glycine-nitrile (24).** Recrystallization of the crude product was done from *n*-hexane/ethyl acetate, yield 38%: mp 140 °C; $[\alpha]^{20}_D = 0.1$ ($c = 1.15$, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.38 (s, 9H), 2.97 (dd, $J = 14.0$, 7.1 Hz, 1H), 3.06 (dd, $J = 14.0$, 6.9, 1H), 4.01−4.13 (m, 2H), 4.27−4.38 (m, 1H), 5.08 (d, $J = 8.2$ Hz, 1H), 6.89 (br s, 1H), 6.93−7.01 (m, 2H), 7.09−7.16 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.32, 28.21, 37.23, 55.61, 80.97, 115.51, 115.67 (d, $J = 21.1$ Hz), 130.78 (d, $J = 8.2$ Hz), 131.72 (d, $J = 3.5$ Hz), 155.70, 162.01 (d, $J = 244.4$ Hz), 171.67; MS (ESI) *m/z* (rel intensity) (pos.) 360 (7, $[M + K]^+$), 344 (14, $[M + Na]^+$), 339 (45, $[M + NH_4]^+$), 322 (31, $[M + H]^+$), 266 (100, $[M - (CH_3)_2CCH_2 + H]^+$), 222 (63, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$) (neg.) 380 (10, $[M + CH_3COO]^-$), 320 (100, $[M - H]^-$), 246 (88, $[M - t$BuOH $- H]^-$). Anal. ($C_{16}H_{20}N_3O_3F$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-3′-fluorophenylalanyl-glycine-nitrile (25).** Recrystallization of the crude product was done from *n*-hexane/ethyl acetate, yield 64%: mp 150 °C; $[\alpha]^{20}_D = -3.0$ ($c = 1.25$, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.38 (s, 9H), 2.99 (dd, $J = 13.9$, 7.6 Hz, 1H), 3.09 (dd, $J = 13.7$, 6.5 Hz, 1H), 4.02−4.15 (m, 2H), 4.31−4.42 (m, 1H), 5.09 (d, $J = 8.2$ Hz, 1H), 6.86−6.99 (m, 4H), 7.22−7.29 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.33, 28.20, 37.65, 55.39, 81.07, 114.16 (d, $J = 20.8$ Hz), 115.51, 116.14 (d, $J = 21.1$ Hz), 124.92 (d, $J = 2.7$ Hz), 130.32 (d, $J = 8.2$ Hz), 138.61 (d, $J = 7.4$ Hz), 155.75, 162.89 (d, $J = 245.1$ Hz), 171.58; MS (ESI) *m/z* (rel intensity) (pos.) 360 (6, $[M + K]^+$), 344 (13, $[M + Na]^+$), 339 (48, $[M + NH_4]^+$), 322 (30, $[M + H]^+$), 266 (100, $[M - (CH_3)_2CCH_2 + H]^+$), 222 (64, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$), (neg.) 380 (10, $[M + CH_3COO]^-$), 320 (100, $[M - H]^-$), 246 (84, $[M - t$BuOH $- H]^-$). Anal. ($C_{16}H_{20}N_3O_3F$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-2′-fluorophenylalanyl-glycine-nitrile (26).** Recrystallization of the crude product was done from *n*-hexane/ethyl acetate, yield 42%: mp 137−139 °C (*n*-hexane/ethyl acetate); $[\alpha]^{20}_D = -4.8$ ($c = 1.30$, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.35 (s, 9H), 2.97−3.08 (m, 1H), 3.16 (dd, $J = 13.0$, 5.1 Hz, 1H), 4.04−4.15 (m, 2H), 4.33−4.45 (m, 1H), 5.12 (d, $J = 7.9$ Hz, 1H), 6.97 (br s, 1H), 6.99−7.09 (m, 2H), 7.16−7.25 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.32, 28.17, 31.35, 54.70, 80.83, 115.42 (d, $J = 22.1$ Hz), 115.54, 123.20 (d, $J = 15.4$ Hz), 124.47 (d, $J = 3.5$ Hz), 129.06 (d, $J = 8.2$ Hz), 131.69 (d, $J = 4.5$ Hz), 155.75, 161.27 (d, $J = 243.4$ Hz), 171.57; MS (ESI) *m/z* (rel intensity) (pos.) 360 (5, $[M + K]^+$), 344 (10, $[M + Na]^+$), 339 (40, $[M + NH_4]^+$), 322 (35, $[M + H]^+$), 266 (100, $[M - (CH_3)_2CCH_2 + H]^+$), 222 (73, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$) (neg.) 380 (15, $[M + CH_3COO]^-$), 320 (100, $[M - H]^-$), 246 (70, $[M - t$BuOH $- H]^-$). Anal. ($C_{16}H_{20}N_3O_3F$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-pentafluorophenylalanyl-glycine-nitrile (27).** The obtained semisolid crude material was purified by fractionation on silica gel using petrol ether/ethyl acetate (2:1) as eluent to yield a solid residue, which was recrystallized from *n*-hexane/ethyl acetate, yield 12%: mp 139−140 °C (*n*-hexane/ethyl acetate); $[\alpha]^{20}_D = -12.7$ ($c = 1.05$, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.36 (s, 9H), 3.03 (dd, $J = 14.5$, 9.5 Hz, 1H), 3.27 (dd, $J = 14.4$, 4.3 Hz, 1H), 4.11 (dd, $J = 17.5$, 5.5 Hz, 1H), 4.20 (dd, $J = 17.4$, 6.0 Hz, 1H), 4.36−4.48 (m, 1H), 5.16 (d, $J = 8.8$ Hz, 1H), 7.15 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.47, 28.05, 53.32, 81.35, 109.80−110.40 (m), 115.48, 137.46 (dm, $J = 249.8$ Hz), 140.44 (dm, $J = 251.5$ Hz), 145.46 (dm, $J = 244.1$ Hz), 155.76, 170.59; MS (ESI) *m/z* (rel intensity) (pos.) 432 (7, $[M + K]^+$), 416 (19, $[M + Na]^+$), 411 (82, $[M + NH_4]^+$), 394 (24, $[M + H]^+$), 338 (100, $[M - (CH_3)_2CCH_2 + H]^+$), 294 (64, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$) (neg.) 392 (100, $[M - H]^-$), 372 (22, $[M - HF - H]^-$), 318 (25, $[M - t$BuOH $- H]^-$), 298 (32, $[M - t$BuOH $- HF - H]^-$). Anal. ($C_{16}H_{16}N_3O_3F_5$) C, H; N: calcd 10.68, found 10.26.

***N*-(*tert*-Butoxycarbonyl)-4′-bromophenylalanyl-glycine-nitrile (28).** This compound was obtained as a pure crude product, yield 99%: mp 139−140 °C; $[\alpha]^{20}_D = 3.3$ ($c = 2.17$, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.38 (s, 9H), 2.95 (dd, $J = 14.1$, 7.1 Hz, 1H), 3.04 (dd, $J = 13.9$, 6.6 Hz, 1H), 4.05 (dd, $J = 17.5$, 5.8 Hz, 1H), 4.10 (dd, $J = 17.7$, 5.7 Hz, 1H), 4.29−4.37 (m, 1H), 5.06 (d, $J = 8.5$ Hz, 1H), 6.89 (br s, 1H), 7.04 (d, $J = 8.2$ Hz, 2H), 7.41 (d, $J = 8.5$ Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.35, 28.21, 37.39, 55.36, 81.07, 115.48, 121.21, 130.98, 131.90, 135.04, 155.69, 171.57; MS (ESI) *m/z* (rel intensity) (pos.) 406 (45, $[M + Na]^+$, ^{81}Br), 404 (45, $[M + Na]^+$, ^{79}Br), 401 (99, $[M + NH_4]^+$, ^{81}Br), 399 (100, $[M + NH_4]^+$, ^{79}Br), 384 (34, $[M + H]^+$, ^{81}Br), 382 (34, $[M + H]^+$, ^{79}Br), 328 (34, $[M - (CH_3)_2CCH_2 + H]^+$, ^{81}Br), 326 (35, $[M - (CH_3)_2CCH_2 + H]^+$, ^{79}Br), 284 (6, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$, ^{81}Br), 282 (6, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$, ^{79}Br) (neg.) 382 (95, $[M - H]^-$, ^{81}Br), 380 (100, $[M - H]^-$, ^{79}Br), 308 (43, $[M - t$BuOH $- H]^-$, ^{81}Br), 306 (43, $[M - t$BuOH $- H]^-$, ^{79}Br). Anal. ($C_{16}H_{20}N_3O_3Br$) C, H; N: calcd 10.99, found 10.36.

***N*-(*tert*-Butoxycarbonyl)-3′-bromophenylalanyl-glycine-nitrile (29).** This compound was obtained as a pure crude product, yield 99%: mp 139 °C; $[\alpha]^{20}_D = 4.7$ ($c = 1.71$, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.39 (s, 9H), 2.95 (dd, $J = 13.8$, 7.5 Hz, 1H), 3.08 (dd, $J = 13.7$, 6.5 Hz, 1H), 4.07 (dd, $J = 17.8$, 6.5 Hz, 1H), 4.12 (dd, $J = 17.5$, 5.9 Hz, 1H), 4.30−4.40 (m, 1H), 5.10 (d, $J = 8.2$ Hz, 1H), 6.97 (br s, 1H), 7.10 (d, $J = 7.6$ Hz, 1H), 7.14−7.19 (m, 1H), 7.33 (s, 1H), 7.37 (d, $J = 7.9$ Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.36, 28.23, 37.59, 55.43, 81.08, 115.50, 122.72, 127.93, 130.32, 130.35, 132.23, 138.53, 155.73, 171.51; MS (ESI) *m/z* (rel intensity) (pos.) 406 (38, $[M + Na]^+$, ^{81}Br), 404 (38, $[M + Na]^+$, ^{79}Br), 401 (99, $[M + NH_4]^+$, ^{81}Br), 399 (100, $[M + NH_4]^+$, ^{79}Br), 384 (28, $[M + H]^+$, ^{81}Br), 382 (29, $[M + H]^+$, ^{79}Br), 328 (26, $[M - (CH_3)_2CCH_2 + H]^+$, ^{81}Br), 326 (27, $[M - (CH_3)_2CCH_2 + H]^+$, ^{79}Br), 284 (4, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$, ^{81}Br), 282 (4, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$, ^{79}Br) (neg.) 382 (95, $[M - H]^-$, ^{81}Br), 380 (100, $[M - H]^-$, ^{79}Br), 308 (44, $[M - t$BuOH $- H]^-$, ^{81}Br), 306 (45, $[M - t$BuOH $- H]^-$, ^{79}Br). Anal. ($C_{16}H_{20}N_3O_3Br$) C, H; N: calcd 10.99, found 10.47.

***N*-(*tert*-Butoxycarbonyl)-2′-bromophenylalanyl-glycine-nitrile (30).** The crude product was recrystallized from petrol ether/ethyl acetate, yield 50%: mp 141−143 °C (petrol ether/

ethyl acetate); $[\alpha]^{20}_D = -8.8$ ($c = 1.17$, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.34 (s, 9H), 3.03−3.18 (m, 1H), 3.22−3.36 (m, 1H), 4.07 (dd, $J = 17.7$, 4.8 Hz, 1H), 4.13 (dd, $J = 17.3$, 6.0 Hz, 1H), 4.43−4.50 (m, 1H), 5.13 (d, $J = 8.5$ Hz, 1H), 6.83 (br s, 1H), 7.08−7.13 (m, 1H), 7.21−7.26 (m, 2H), 7.53 (d, $J = 8.2$ Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.38, 28.19, 37.90, 54.51, 80.84, 115.50, 124.79, 127.86, 128.92, 131.64, 133.02, 135.89, 155.69, 171.51; MS (ESI) *m/z* (rel intensity) (pos.) 406 (31, $[M + Na]^+$, ^{81}Br), 404 (31, $[M + Na]^+$, ^{79}Br), 401 (99, $[M + NH_4]^+$, ^{81}Br), 399 (100, $[M + NH_4]^+$, ^{79}Br), 384 (52, $[M + H]^+$, ^{81}Br), 382 (53, $[M + H]^+$, ^{79}Br), 328 (39, $[M - (CH_3)_2CCH_2 + H]^+$, ^{81}Br), 326 (40, $[M - (CH_3)_2CCH_2 + H]^+$, ^{79}Br), 284 (8, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$, ^{81}Br), 282 (8, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$, ^{79}Br) (neg.) 382 (96, $[M - H]^-$, ^{81}Br), 380 (100, $[M - H]^-$, ^{79}Br), 308 (42, $[M - t$BuOH $- H]^-$, ^{81}Br), 306 (42, $[M - t$BuOH $- H]^-$, ^{79}Br). Anal. ($C_{16}H_{20}N_3O_3Br$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-tryptophanyl-glycine-nitrile (31).** The foamy crude product crystallized upon dissolving in cyclohexane und subsequent addition of ethyl acetate, yield 90%: mp 135−138 °C; $[\alpha]^{20}_D = 0.7$ ($c = 1.20$, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.40 (s, 9H), 3.15 (dd, $J = 13.1$, 7.4 Hz, 1H), 3.22−3.35 (m, 1H), 3.88 (dd, $J = 17.3$, 5.7 Hz, 1H), 3.95 (dd, $J = 17.3$, 4.7 Hz, 1H), 4.44 (br s, 1H), 5.16 (br s, 1H), 6.40 (t, $J = 5.8$ Hz, 1H), 7.02 (d, $J = 2.2$ Hz, 1H), 7.08−7.13 (m, 1H), 7.16−7.21 (m, 1H), 7.34 (d, $J = 7.9$ Hz, 1H), 7.58 (d, $J = 7.9$ Hz, 1H), 8.29 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.15, 28.18, 28.25, 55.08, 80.64, 109.76, 111.41, 115.60, 118.53, 119.86, 122.38, 123.57, 127.19, 136.23, 155.59, 172.27; MS (ESI) *m/z* (rel intensity) (pos.) 381 (8, $[M + K]^+$), 365 (26, $[M + Na]^+$), 360 (100, $[M + NH_4]^+$), 343 (71, $[M + H]^+$), 287 (96, $[M - (CH_3)_2CCH_2 + H]^+$), 243 (15, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$) (neg.) 401 (49, $[M + CH_3COO]^-$), 341 (100, $[M - H]^-$), 267 (37, $[M - t$BuOH $- H]^-$). Anal. ($C_{18}H_{22}N_4O_3$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-3′-thionaphthenylalanyl-glycine-nitrile (32).** Recrystallization of the crude product was done from *n*-hexane/ethyl acetate, yield 68%, 144−146 °C; $[\alpha]^{20}_D = -8.8$ ($c = 1.15$, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.37 (s, 9H), 3.10−3.47 (m, 2H), 3.94−4.08 (m, 2H), 4.38−4.60 (m, 1H), 5.15 (d, $J = 8.2$ Hz, 1H), 6.70 (br s, 1H), 7.19 (s, 1H), 7.31−7.40 (m, 2H), 7.76 (dd, $J = 7.1$, 1.7 Hz, 1H), 7.84 (dd, $J = 6.9$, 1.6 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.34, 28.21, 31.01, 54.20, 80.94, 115.44, 121.45, 123.03, 124.36, 124.52, 124.60, 130.59, 138.39, 140.48, 155.66, 171.72; MS (ESI) *m/z* (rel intensity) (pos.) 398 (6, $[M + K]^+$), 382 (15, $[M + Na]^+$), 377 (81, $[M + NH_4]^+$), 360 (41, $[M + H]^+$), 304 (100, $[M - (CH_3)_2CCH_2 + H]^+$), 260 (68, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$) (neg.) 418 (9, $[M + CH_3COO]^-$), 358 (100, $[M - H]^-$), 284 (50, $[M - t$BuOH $- H]^-$). Anal. ($C_{18}H_{21}N_3O_3S$) C, H; N: calcd 11.69, found 11.18.

***N*-(*tert*-Butoxycarbonyl)-1′-naphthylalanyl-glycine-nitrile (33).** The crude product was recrystallized from petrol ether/ethyl acetate, yield 42%: mp 167 °C; $[\alpha]^{20}_D = -5.5$ ($c = 1.07$, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.35 (br s, 9H), 3.48 (br s, 2H), 3.94 (d, $J = 5.1$ Hz, 2H), 4.45−4.52 (m, 1H), 5.16 (br s, 1H), 6.36 (br s, 1H), 7.28 (d, $J = 7.0$ Hz, 1H), 7.39 (dd, $J = 8.2$, 6.9 Hz, 1H), 7.45−7.54 (m, 2H), 7.76 (d, $J = 8.2$ Hz, 1H), 7.85 (d, $J = 8.4$ Hz, 1H), 8.06 (d, $J = 8.2$ Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.20, 28.19, 35.65, 55.17, 80.69, 115.31, 123.27, 125.59, 125.94, 126.63, 127.93, 128.10, 128.99, 131.71, 132.18, 133.92, 155.57, 171.75; MS (ESI) *m/z* (rel intensity) (pos.) 392 (4, $[M + K]^+$), 376 (49, $[M + Na]^+$), 371 (100, $[M + NH_4]^+$), 354 (36, $[M + H]^+$), 298 (44, $[M - (CH_3)_2CCH_2 + H]^+$), 254 (4, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$) (neg.) 412 (6, $[M + CH_3COO]^-$), 352 (100, $[M - H]^-$), 278 (64, $[M - t$BuOH $- H]^-$). Anal. ($C_{20}H_{23}N_3O_3$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-2′-naphthylalanyl-glycine-nitrile (34).** The crude product was recrystallized from petrol ether/ethyl acetate, yield 39%: mp 147 °C; $[\alpha]^{20}_D = 18.7$ ($c = 1.27$, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.35 (s, 9H), 3.21 (d, $J = 6.9$ Hz, 2H), 4.00 (dd, $J = 17.3$, 5.7 Hz, 1H), 4.06 (dd, $J = 17.3$, 6.0 Hz, 1H), 4.39−4.49 (m, 1H), 5.06 (br s, 1H), 6.67 (br s, 1H), 7.51 (dd, $J = 8.2$, 1.6 Hz, 1H), 7.41−7.48 (m, 2H), 7.61 (s, 1H), 7.74−7.81 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.30, 28.19, 38.19, 55.70, 80.95, 115.43, 125.93, 126.34, 127.16, 127.64, 127.66, 128.04, 128.66, 132.50, 133.46, 133.50, 155.69, 171.78; MS (ESI) *m/z* (rel intensity) 392 (3, $[M + K]^+$), 376 (47, $[M + Na]^+$), 371 (100, $[M + NH_4]^+$), 354 (34, $[M + H]^+$), 298 (47, $[M - (CH_3)_2CCH_2 + H]^+$), 254 (5, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$) (neg.) 412 (5, $[M + CH_3COO]^-$), 352 (100, $[M - H]^-$), 278 (64, $[M - t$BuOH $- H]^-$). Anal. ($C_{20}H_{23}N_3O_3$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-4′-biphenylalanyl-glycine-nitrile (35).** The crude product was recrystallized from petrol ether/ethyl acetate, yield 68%: mp 164−165 °C; $[\alpha]^{20}_D = 7.3$ ($c = 1.12$, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.39 (s, 9H), 3.03−3.16 (m, 2H), 4.02−4.15 (m, 2H), 4.33−4.45 (m, 1H), 5.06 (d, $J = 5.4$ Hz), 6.73 (br s, 1H), 7.24 (d, $J = 7.9$ Hz, 2H), 7.29−7.34 (m, 1H), 7.38−7.43 (m, 2H), 7.51−7.56 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.31, 28.23, 37.64, 55.59, 80.95, 115.57, 126.97, 127.36, 127.54, 128.80, 129.68, 134.98, 140.14, 140.50, 155.70, 171.76; MS (ESI) *m/z* (rel intensity) (pos.) 418 (4, $[M + K]^+$), 402 (12, $[M + Na]^+$), 397 (100, $[M + NH_4]^+$), 380 (43, $[M + H]^+$), 324 (42, $[M - (CH_3)_2CCH_2 + H]^+$), 280 (7, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$), (neg.) 378 (100, $[M - H]^-$), 304 (48, $[M - t$BuOH $- H]^-$). Anal. ($C_{22}H_{25}N_3O_3$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-D-phenylalanyl-glycine-nitrile (36).** The crude product was recrystallized from petrol ether/ethyl acetate, yield 56%: mp 136−138 °C (petrol ether/ethyl acetate); $[\alpha]^{20}_D = -0.6$ ($c = 1.86$, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.38 (s, 9H), 3.00−3.10 (m, 2H), 4.04 (dd, $J = 17.4$, 5.7 Hz, 1H), 4.09 (dd, $J = 17.4$, 6.0 Hz, 1H), 4.30−4.40 (m, 1H), 5.01 (br s, 1H), 6.66 (br s, 1H), 7.14−7.19 (m, 2H), 7.21−7.26 (m, 1H), 7.27−7.33 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.29, 28.23, 38.01, 55.66, 80.88, 115.46, 127.24, 128.88, 129.22, 136.00, 155.66, 171.75; MS (ESI) *m/z* (rel intensity) (pos.) 342 (3, $[M + K]^+$), 326 (42, $[M + Na]^+$), 321 (100, $[M + NH_4]^+$), 304 (79, $[M + H]^+$), 248 (90, $[M - (CH_3)_2CCH_2 + H]^+$), 204 (57, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$) (neg.) 362 (43, $[M + CH_3COO]^-$), 302 (99, $[M - H]^-$), 228 (100, $[M - t$BuOH $- H]^-$). Anal. ($C_{16}H_{21}N_3O_3$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-phenylalanyl-sarcosine-nitrile (37).** This compound was prepared from Boc-Phe-OH (0.64 g, 2.40 mmol) and *N*-methyl-aminoacetonitrile hydrochloride (0.28 g, 2.64 mmol) according to the General Procedure. The obtained oily crude material was purified by fractionation on silica gel using petrol ether/ethyl acetate (2:1) as eluent to yield a solid residue, which was recrystallized from *n*-hexane/ethyl acetate, yield 62%: mp 96−98 °C; $[\alpha]^{20}_D = 0.4$ ($c = 1.28$, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.38 (w) & 1.40 (i) (s, 9H), 2.68 (s, 3H), 2.93 (dd, $J = 12.9$, 8.9 Hz, 1H), 3.00 (dd, $J = 13.0$, 5.7 Hz, 1H), 3.98 (d, $J = 17.1$ Hz, 1H), 4.44 (d, $J = 17.1$ Hz, 1H), 4.75−4.83 (m, 1H), 5.29 (d, $J = 8.7$ Hz, 1H), 7.14−7.20 (m, 2H), 7.21−7.26 (m, 1H), 7.26−7.31 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.89 (w) & 28.24 (i), 35.17, 35.85, 37.03, 51.85, 78.34, 116.55, 126.55, 128.26, 129.44, 137.44, 155.31, 172.47; MS (ESI) *m/z* (rel intensity) (pos.) 356 (8, $[M + K]^+$), 340 (10, $[M + Na]^+$), 335 (29, $[M + NH_4]^+$) 318 (59, $[M + H]^+$), 262 (100, $[M - (CH_3)_2CCH_2 + H]^+$), 218 (64, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$) (neg.) 376 (41, $[M + CH_3COO]^-$), 316 (79, $[M - H]^-$), 242 (100, $[M - t$BuOH $- H]^-$). Anal. ($C_{17}H_{23}N_3O_3$) C, H, N.

***N*-(2-Naphthylsulfonyl)-glycyl-glycine-nitrile (38).** This compound was prepared according to the General Procedure for dipeptide nitriles to give a pure crude product, yield 19%: mp 165 °C (dec); ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.51 (s, 2H), 4.06 (d, $J = 5.4$ Hz, 2H), 7.64−7.72 (m, 2H), 7.82 (dd, $J = 9.3$, 1.9 Hz, 1H), 8.01−8.05 (m, 1H), 8.11 (d, $J = 8.8$ Hz, 1H), 8.13−8.16 (m, 1H), 8.20 (br s, 1H), 8.43 (d, $J = 1.6$ Hz, 1H), 8.64 (t, $J = 5.5$ Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 27.21, 45.15, 117.46, 122.42, 127.61, 127.68, 127.96, 128.87, 129.32, 129.47, 131.81, 134.36, 137.27, 168.72; MS (ESI) *m/z* (rel intensity) (pos.) 326 (14, $[M + Na]^+$), 321 (100, $[M + NH_4]^+$), 304 (42, $[M + H]^+$) (neg.) 362 (5, $[M + CH_3COO]^-$), 302 (100, $[M - H]^-$), 275 (25, $[M - HCN - H]^-$). Anal. ($C_{14}H_{13}N_3O_3S$) C, H; N: calcd 13.85, found 12.62.

***N*-(*tert*-Butoxycarbonyl)-3′-biphenylalanyl-glycine-nitrile (39). General Procedure for Biphenyl Derivatives**

39−**40.** Compound **29** (0.20 g, 0.52 mmol), phenylboronic acid (0.1 g, 0.78 mmol), and Na_2CO_3 (0.12 g, 1.14 mmol) were dissolved in dimethoxyethane (8 mL) and H_2O (4 mL). After degassing with Ar, $Pd(PPh_3)_4$ (0.057 g, 0.05 mmol) was added under an inert atmosphere. The resulting mixture was placed into a microwave reactor (sealed tube) and irradiated for 30 min at 30 W and 70 °C. The reaction mixture was transferred into a round-bottom flask and evaporated. The residue was suspended in H_2O (5 mL) and extracted with ethyl acetate (320 mL). The combined organic layers were washed with sat. $NaHCO_3$ (215 mL) and brine (15 mL), treated with silica gel, and evaporated. The obtained brownish residue was dissolved in a mixture of MeOH (24 mL) and H_2O (6 mL) and subjected to solid-phase extraction with a C_{18} cartridge (J. T. Baker), and the cartridge was washed with the same amount of MeOH/H_2O. The combined eluates were partly evaporated, and the remaining aqueous suspension was diluted with the required amount of MeOH (18 mL) to obtain a solution, which was fractionated by preparative HPLC (isocratic elution, MeOH/H_2O 60:40). The combined product fractions were concentrated and lyophilized to obtain **39** (43 mg, 22%): mp 154 °C; $[\alpha]^{20}_D$ = 11.3 (*c* = 1.02, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.38 (s, 9H), 3.10 (dd, *J* = 13.6, 7.6 Hz, 1H), 3.16 (dd, *J* = 13.9, 6.6 Hz, 1H), 4.05 (dd, *J* = 17.4, 4.1 Hz, 1H), 4.12 (dd, *J* = 17.5, 6.1 Hz, 1H), 4.35−4.43 (m, 1H), 4.96 (br s, 1H), 6.61 (br s, 1H), 7.15 (d, *J* = 7.6 Hz, 1H), 7.31−7.35 (m, 1H), 7.37 (s, 1H), 7.38−7.44 (m, 3H), 7.46−7.49 (m, 1H), 7.53−7.57 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.33, 28.20, 37.83, 55.70, 80.99, 115.46, 126.05, 127.12, 127.50, 127.97, 128.11, 128.80, 129.33, 136.58, 140.63, 141.79, 155.70, 171.65; MS (ESI) *m/z* (rel intensity) (pos.) 418 (3, $[M + K]^+$), 402 (35, $[M + Na]^+$), 397 (100, $[M + NH_4]^+$), 380 (38, $[M + H]^+$), 324 (39, $[M - (CH_3)_2CCH_2 + H]^+$), 280 (6, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$) (neg.) 378 (100, $[M - H]^-$), 304 (62, $[M - t\text{BuOH} - H]^-$). Anal. ($C_{22}H_{25}N_3O_3$) C, N; H: calcd 6.64, found 7.19.

***N*-(*tert*-Butoxycarbonyl)-2′-biphenylalanyl-glycine-nitrile (40):** yield 21%; mp 79−83 °C; $[\alpha]^{20}_D$ = 4.2 (*c* = 1.04, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.32 (s, 9H), 2.82−2.98 (m, 1H), 3.22 (dd, *J* = 14.2, 5.1 Hz, 1H), 3.97 (dd, *J* = 17.7, 5.0 Hz, 1H), 4.02 (dd, *J* = 17.8, 6.1 Hz, 1H), 4.03−4.12 (m, 1H), 4.64 (d, *J* = 7.9 Hz, 1H), 6.40 (br s, 1H), 7.21−7.24 (m, 1H), 7.26−7.33 (m, 5H), 7.35−7.39 (m, 1H), 7.41−7.46 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.25, 28.14, 34.65, 55.42, 80.62, 115.52, 127.09, 127.38, 127.88, 128.61, 129.34, 129.98, 130.35, 133.87, 141.13, 142.45, 155.64, 171.76; MS (ESI) *m/z* (rel intensity) (pos.) 418 (48, $[M + K]^+$), 402 (35, $[M + Na]^+$), 397 (100, $[M + NH_4]^+$), 380 (85, $[M + H]^+$), 324 (52, $[M - (CH_3)_2CCH_2 + H]^+$), 280 (13, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$), (neg.) 438 (7, $[M + CH_3COO]^-$), 378 (100, $[M - H]^-$), 304 (76, $[M - t\text{BuOH} - H]^-$). Anal. ($C_{22}H_{25}N_3O_3 \cdot 0.5H_2O$) C, H, N.

Phenylalanyl-glycine-nitrile Hydroperchlorate (41). Compound **16** (8.4 g, 27.7 mmol) was dissolved in formic acid (50 mL) and stirred for 6 h at room temperature. The solvent was thoroughly removed under reduced pressure and the residue was dissolved in *i*PrOH (30 mL). The resulting solution was treated with 70% $HClO_4$ (2.4 mL, 27.7 mmol) under ice cooling. Since precipitation did not occur, the solution was evaporated (***CAUTION: Perchloric acid is explosive!***) and the remaining residue dissolved in H_2O (70 mL). The resulting mixture was extracted with Et_2O (2 × 20 mL). The aqueous phase was lyophilized to obtain **41** as a semisolid, yellow residue (6.64 g, 79%), which is pure enough for further steps: ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.98 (dd, *J* = 13.9 Hz, *J* = 7.3 Hz, 1H), 3.05 (dd, *J* = 13.9 Hz, *J* = 6.6 Hz, 1H), 4.00 (br s, 1H), 4.20 (d, *J* = 5.7 Hz, 2H), 7.19−7.23 (m, 2H), 7.25−7.30 (m, 1H), 7.31−7.36 (m, 2H), 8.21 (s, 3H), 9.06 (t, ^3J = 5.5 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 27.26, 36.97, 53.58, 117.00, 127.47, 128.79, 129.57, 134.62, 168.87.

***N*-Acetyl-phenylalanyl-glycine-nitrile (42).** To a solution of **41** (0.9 g, 3.0 mmol) in H_2O (10 mL), $NaHCO_3$ was added as solid until saturation (pH 8−9). Acetic anhydride (0.42 mL, 4.5 mmol) was added dropwise to this mixture under ice cooling. After stirring for 30 min, H_2O (30 mL) was added and the remaining precipitate was filtered off, washed with H_2O (10 mL), dried over P_4O_{10}, and recrystallized from petrol ether/ethyl acetate to obtain **42** (0.15 g, 20%): mp 180−182 °C (lit.[62] mp 190−192 °C); $[\alpha]^{20}_D$ = 7.4 (*c* = 1.43, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.97 (s, 3H), 3.07 (dd, *J* = 7.3, 2.5 Hz, 2H), 4.04 (dd, *J* = 5.7, 1.9 Hz, 2H), 4.60−4.66 (m, 1H), 6.03 (d, *J* = 7.6 Hz, 1H), 6.68 (br s, 1H), 7.16−7.20 (m, 2H), 7.23−7.27 (m, 1H), 7.28−7.33 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 23.13, 27.34, 37.63, 54.38, 115.34, 127.37, 128.96, 129.14, 135.90, 170.69, 171.20; MS (ESI) *m/z* (rel intensity) (pos.) 268 (32, $[M + Na]^+$), 263 (93, $[M + NH_4]^+$), 246 (100, $[M + H]^+$), 190 (47, $[M - H_2NCH_2CN + H]^+$) (neg.) 304 (36, $[M + CH_3COO]^-$), 244 (100, $[M - H]^-$), 217 (50, $[M - HCN - H]^-$). Anal. ($C_{13}H_{15}N_3O_2$) C, H, N.

***N*-Benzoyl-phenylalanyl-glycine-nitrile (43).** To a solution of **41** (0.9 g, 3.0 mmol) in H_2O (10 mL), $NaHCO_3$ was added as solid until saturation (pH 8−9). Benzoyl chloride (0.31 mL, 2.7 mmol) was added dropwise to this mixture under ice cooling. After stirring for 30 min, H_2O (30 mL) was added and the remaining precipitate was filtered off, washed with H_2O (10 mL), dried over P_4O_{10}, and recrystallized from H_2O/EtOH to obtain **43** (0.22 g, 27%): mp 199−200 °C; $[\alpha]^{20}_D$ = −38.5 (*c* = 1.10, dioxane); ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.01 (dd, *J* = 13.7, 10.6 Hz, 1H), 3.13 (dd, *J* = 13.9, 4.4 Hz, 1H), 4.15 (dd, *J* = 5.5, 1.2 Hz, 2H), 4.66−4.71 (m, 1H), 7.13−7.18 (m, 1H), 7.22−7.27 (m, 2H), 7.30−7.34 (m, 2H), 7.41−7.46 (m, 2H), 7.48−7.53 (m, 1H), 7.78−7.82 (m, 2H), 8.65 (d, *J* = 8.5 Hz, 1H), 8.75 (t, *J* = 5.5 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 27.33, 36.94, 54.83, 117.63, 126.43, 127.59, 128.24, 128.27, 129.22, 131.47, 133.98, 138.25, 166.47, 172.17; MS (ESI) *m/z* (rel intensity) (pos.) 330 (33, $[M + Na]^+$), 325 (21, $[M + NH_4]^+$), 308 (100, $[M + H]^+$), 252 (23, $[M - H_2NCH_2CN + H]^+$) (neg.) 366 (6, $[M + CH_3COO]^-$), 306 (100, $[M - H]^-$), 279 (29, $[M - HCN - H]^-$). Anal. ($C_{18}H_{17}N_3O_2 \cdot 0.25H_2O$) C, H; N: calcd 13.47, found 12.94.

***N*-(4-Phenylbenzoyl)-phenylalanyl-glycine-nitrile (44).** This compound was prepared according to the General Procedure for dipeptide nitriles using biphenyl-4-carbonic acid (0.5 g, 2.50 mmol) instead of the protected amino acid and **41** (0.84 g, 2.76 mmol) instead of aminoacetonitrile sulfate. The crude product was recrystallized from petrol ether/ethyl acetate, yield 17%: mp 214−215 °C; $[\alpha]^{20}_D$ = −44.2 (*c* = 1.24, dioxane); ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.03 (dd, *J* = 13.9, 10.8 Hz, 1H), 3.15 (dd, *J* = 13.9, 4.4 Hz, 1H), 4.14 (dd, *J* = 5.7, 1.0 Hz, 2H), 4.67−4.75 (m, 1H), 7.14−7.18 (m, 1H), 7.23−7.27 (m, 2H), 7.32−7.35 (m, 2H), 7.37−7.42 (m, 1H), 7.46−7.50 (m, 2H), 7.70−7.76 (m, 4H), 7.87−7.92 (m, 2H), 8.72 (d, *J* = 8.5 Hz, 1H), 8.77 (t, *J* = 5.5 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 27.35, 36.95, 54.85, 117.64, 126.44, 126.50, 126.99, 128.19, 128.26, 128.29, 129.14, 129.23, 132.77, 138.28, 139.28, 143.04, 166.11, 172.18; MS (ESI) *m/z* (rel intensity) (pos.) 406 (24, $[M + Na]^+$), 401 (11, $[M + NH_4]^+$), 384 (100, $[M + H]^+$), 328 (25, $[M - H_2NCH_2CN + H]^+$) (neg.) 442 (5, $[M + CH_3COO]^-$), 400 (6, $[M + H_2O - H]^-$), 382 (100, $[M - H]^-$), 355 (18, $[M - HCN - H]^-$). Anal. ($C_{24}H_{21}N_3O_2 \cdot 0.25H_2O$) C, H, N.

***tert*-Butoxycarbonylbenzylidenehydrazine (45).** Benzaldehyde (0.77 mL, 7.6 mmol) was added dropwise to a solution of *tert*-butyl carbazate (1 g, 7.6 mmol) in THF (10 mL). After stirring for 4 h at room temperature, the solvent was removed under reduced pressure to obtain **45** (1.47 g, 88%) as white solid, which was used without further purification in the next step: mp 203 °C (lit.[63] mp 186 °C); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.52 (s, 9H), 7.30−7.36 (m, 3H), 7.62−7.68 (m, 2H), 7.82 (br s, 1H), 8.03 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.26, 81.46, 127.16, 128.54, 129.80, 133.89, 143.68, 152.46.

1-*tert*-Butoxycarbonyl-2-benzylhydrazine × BH_2CN (46). To a solution of **45** (1.3 g, 5.9 mmol) in THF (15 mL) and AcOH (9 mL) $NaBH_3CN$ (0.93 g, 14.8 mmol) was added as solid under ice cooling. The resulting mixture was stirred for 18 h at room temperature. Subsequently, ethyl acetate (50 mL) and sat. $NaHCO_3$ (50 mL) were added and the two phases were separated. The aqueous phase was alkalized with solid $NaHCO_3$ and extracted with ethyl acetate (2 × 50 mL). The combined organic phases were washed with sat. $NaHCO_3$ (1 × 25 mL) and brine (25 mL), dried over Na_2SO_4, and

evaporated under reduced pressure to obtain **46** (1.54 g, 100%) as a white residue: mp 143−144 °C (dec) (lit.[36] mp 152 °C) (petrol ether/ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.39 (s, 9H) 4.11 (dd, J = 12.8, 5.8 Hz, 1H), 4.30 (dd, J = 12.8, 4.9 Hz, 1H), 6.40 (br s, 1H), 6.86 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.91, 62.70, 84.89, 128.92, 129.75, 130.40, 130.81, 154.41; MS (ESI) *m/z* (rel intensity) (pos.) 279 (74, $[C_{12}H_{18}N_2O_2 + {}^{11}BH_2CN + NH_4]^+$), 278 (20, $[C_{12}H_{18}N_2O_2 + {}^{10}BH_2CN + NH_4]^+$), 262 (97, $[C_{12}H_{18}N_2O_2 + {}^{11}BH_2CN + H]^+$), 261 (27, $[C_{12}H_{18}N_2O_2 + {}^{10}BH_2CN + H]^+$), 206 (100, $[C_{12}H_{18}N_2O_2 + {}^{11}BH_2CN - (CH_3)_2CCH_2 + H]^+$), 205 (30, $[C_{12}H_{18}N_2O_2 + {}^{10}BH_2CN - (CH_3)_2CCH_2 + H]^+$) (neg.) 260 (98, $[C_{12}H_{18}N_2O_2 + {}^{11}BH_2CN - H]^-$), 259 (29, $[C_{12}H_{18}N_2O_2 + {}^{10}BH_2CN - H]^-$), 233 (100, $[C_{12}H_{18}N_2O_2 + {}^{11}BH_2CN - HCN - H]^-$), 232 (31, $[C_{12}H_{18}N_2O_2 + {}^{11}BH_2CN - HCN - H]^-$.

1-*tert*-Butoxycarbonyl-2-benzylhydrazine (47). Compound **46** (1.51 g, 5.8 mmol) was dissolved in MeOH (10 mL) and treated with 1 N NaOH (10 mL) for 2 h at room temperature. After removal of the organic solvent, the aqueous residue was extracted with ethyl acetate (3 × 20 mL). The combined organic phases were washed with brine (20 mL), dried over Na_2SO_4, and concentrated to obtain **47** as a colorless oil (1.24 g, 96%), which was pure enough for further conversion: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.44 (s, 9H), 3.96 (s, 2H), 4.15 (br s, 1H), 6.01 (br s, 1H), 7.25−7.35 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.32, 55.87, 80.72, 127.58, 128.55, 128.94, 137.41, 156.79.

***N*-(*tert*-Butoxycarbonyl)-azaphenylalanyl-glycine-nitrile (48).** To a solution of *N*,*N*′-carbonyldiimidazole (0.36 g, 2.25 mmol) in DMF (10 mL) solid aminoacetonitrile sulfate (0.24 g, 2.25 mmol) and diisopropylethylamine (0.38 mL, 2.25 mmol) were added under ice cooling. After 1 h, a solution of **47** (0.5 g, 2.25 mmol) in DMF (7 mL) was added. After stirring for 13 h at room temperature, the solvent was removed under reduced pressure. The residue was suspended in H_2O (5 mL), acidified to pH 1 (10% $NaHSO_4$), and extracted with ethyl acetate (3 × 15 mL). The combined organic layers were washed with H_2O (10 mL), sat. $NaHCO_3$ (2 × 10 mL) and brine (10 mL), dried over Na_2SO_4, and evaporated to obtain an oily residue, which was fractionated on silica gel (petrol ether/ethyl acetate 1:1). The fractions containing the main product were combined and delivered from solvent to obtain **48** (0.35 g, 51%): mp 131−133 °C; ^{1}H NMR (500 MHz, DMSO-d_6, 70 C) δ 1.35 (s, 9H), 4.04 (d, J = 6.0 Hz, 2H), 4.55 (br s, 2H), 7.16−7.22 (m, 1H), 7.23−7.33 (m, 5H), 8.68 (br s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6, 70 C) δ 27.78, 28.77, 51.58, 79.77, 117.93, 126.87, 127.89, 128.38, 136.98, 154.01, 157.40; MS (ESI) *m/z* (rel intensity) (pos.) 327 (22, $[M + Na]^+$), 322 (100, $[M + NH_4]^+$), 305 (51, $[M + H]^+$), 249 (74, $[M - (CH_3)_2CCH_2 + H]^+$), 205 (16, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$) (neg.) 363 (36, $[M + CH_3COO]^-$), 303 (99, $[M - H]^-$), 229 (100, $[M - t\text{BuOH} - H]^-$). Anal. ($C_{15}H_{20}N_4O_3$) C, H; N: calcd 18.41, found 17.47.

***N*-(*tert*-Butoxycarbonyl)-glycine-*N*′-methoxy-*N*′-methylamide (49).** To a solution of *N*,*O*-dimethylhydroxylamine hydrochloride (3.59 g, 36.8 mmol) in H_2O (5 mL), 1 N NaOH (36.8 mL) was added under ice cooling. The resulting solution was added to a suspension of *N*-(*tert*-butoxycarbonyl)-glycine *N*′-hydroxysuccinimide ester (2.50 g, 9.2 mmol) in dioxane (20 mL) and stirred for 6 h at room temperature. After evaporation of the solvent, the remaining aqueous residue was adjusted to pH 1−2 with 10% $NaHSO_4$ and extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with sat. $NaHCO_3$, H_2O, and brine (each 20 mL), dried over Na_2SO_4, and evaporated to obtain **49** (1.24 g, 62%): mp 100 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.42 (s, 9H), 3.17 (s, 3H), 3.68 (s, 3H), 4.05 (d, J = 3.8 Hz, 2H), 5.24 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.33, 32.36, 41.70, 61.43, 79.62, 155.87, 170.21.

Glycine *N*′-methoxy-*N*′-methylamide Hydroperchlorate (50). Compound **49** (1.19 g, 5.45 mmol) was dissolved in 4 N HCl/dioxane (10 mL) and stirred for 2 h at room temperature. After removal of the solvent, the residual oil was dissolved in *i*PrOH (15 mL) and treated with 70% $HClO_4$ (0.46 mL, 5.45 mmol) under ice cooling (***CAUTION: Perchloric acid is explosive!***). The precipitate was collected by filtration to obtain **50** (0.68 g, 72%): mp 175−178 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.16 (s, 3H), 3.71 (s, 3H), 3.88 (s, 2H), 7.98 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 32.04, 39.32, 61.60, 166.99.

***N*-(*tert*-Butoxycarbonyl)-phenylalanyl-glycine-*N*′-methoxy-*N*′-methylamide (51).** Boc-Phe-OH (0.8 g, 3.0 mmol) was dissolved in THF (15 mL) and cooled to −25 °C. Subsequently, *N*-methylmorpholine (0.33 mL, 3.0 mmol) and isobutyl chloroformate (0.39 mL, 3.0 mmol) were added under vigorous stirring. Immediately, a solution of **50** (0.72 g, 3.3 mmol) in H_2O (0.5 mL) and 1 N NaOH (3.3 mL) was added. The resulting mixture was allowed to warm to room temperature. After 2 h, THF was removed under reduced pressure and the aqueous residue was diluted with a small volume of H_2O, adjusted to pH 1−2 (10% $NaHSO_4$), and extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with H_2O (10 mL), sat. $NaHCO_3$ (2 × 10 mL), and brine (10 mL), dried over Na_2SO_4, and evaporated to dryness to obtain **51** (1.07 g, 98%): mp 65 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.20 (w) & 1.28 (i) (s, 9H), 2.72 (dd, J = 13.9, 10.7 Hz, 1H), 3.03 (dd, J = 13.9, 3.8 Hz, 1H), 3.11 (s, 3H), 3.70 (s, 3H), 3.99 (dd, J = 17.5, 4.8 Hz, 1H), 4.07 (dd, J = 17.6, 5.4 Hz, 1H), 4.19−4.26 (m, 1H), 6.90 (d, J = 8.8 Hz, 1H), 7.13−7.21 (m, 1H), 7.22−7.32 (m, 4H), 8.00 (t, J = 5.2 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 28.26, 32.23, 37.67, 39.83, 55.47, 61.30, 78.13, 126.23, 128.09, 129.30, 138.44, 155.34, 169.63, 172.19.

***N*-(*tert*-Butoxycarbonyl)-phenylalanyl-glycine-aldehyde (52).** A solution of **51** (0.56 g, 1.53 mmol) in dry Et_2O (20 mL) was added all at once to a suspension of $LiAlH_4$ (0.32 g, 8.42 mmol) in dry Et_2O (80 mL) under Ar at −35 °C. The resulting mixture was allowed to warm to room temperature over 2 h. Subsequently, it was cooled to −35 °C again and treated with 25% $KHSO_4$ (5 mL). The mixture was filtered over Celite, and the residue was washed with Et_2O (2 × 20 mL). The combined filtrates were washed with 10% $NaHSO_4$ (25 mL), sat. $NaHCO_3$ (2 × 25 mL), and brine (25 mL), dried over Na_2SO_4, and evaporated to dryness to obtain **52** as white foam (0.33 g, 70%): mp 88−90 °C; $[\alpha]^{20}_D$ = 6.9 (c = 1.06, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.39 (s, 9H), 3.03−3.09 (m, 2H), 4.05 (dd, J = 20.4, 4.6 Hz, 1H), 4.12 (dd, J = 20.2, 5.1 Hz, 1H), 4.36−4.45 (m, 1H), 4.99 (br s, 1H), 6.60 (br s, 1H), 7.09−7.34 (m, 5H), 9.54 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.23, 38.32, 50.03, 55.70, 80.46, 127.06, 128.72, 129.23, 136.41, 155.43, 171.74, 195.94; MS (ESI) *m/z* (rel intensity) (pos.) 339 (22, $[M + CH_3OH + H]^+$), 324 (7, $[M + NH_4]^+$), 307 (100, $[M + H]^+$), 251 (36, $[M - (CH_3)_2CCH_2 + H]^+$), 207 (5, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$), (neg.) 397 (33, $[M + CH_3OH + CH_3COO]^-$), 383 (51, $[M + H_2O + CH_3COO]^-$), 365 (5, $[M + CH_3COO]^-$), 305 (100, $[M - H]^-$), 231 (93, $[M - t\text{BuOH} - H]^-$). Anal. ($C_{16}H_{22}N_2O_4$) H, N; C: calcd 62.73, found 61.05.

***N*-(*tert*-Butoxycarbonyl)-phenylalanyl-glycine-methylketone (53).** A small portion of a solution of CH_3I (0.95 mL, 15.3 mmol) in dry Et_2O (10 mL) was added dropwise to a suspension of Mg sparks (0.37 g, 15.3 mmol) in dry Et_2O (5 mL). A drop of Br_2 was added to initiate the reaction. The residual amount of CH_3I was added dropwise over a period of 30 min. After stirring for an additional 1 h almost all of the metal dissolved. Subsequently, a solution of **51** (0.56 g, 1.53 mmol) in dry Et_2O (10 mL) was added dropwise under ice cooling. After stirring the mixture for an additional 1 h at room temperature, sat. NH_4Cl (20 mL) was added and the aqueous phase was extracted with Et_2O (2 × 20 mL). The combined organic phases were washed with brine (10 mL), dried over Na_2SO_4, and evaporated. The obtained oily-crystalline residue was fractionated on silica gel (petrol ether/ethyl acetate 1:1) to obtain **53** (245 mg, 55%): mp 110−111 °C; $[\alpha]^{20}_D$ = −6.0 (c = 1.14, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.38 (s, 9H), 2.14 (s, 3H), 2.98−3.06 (m, 1H), 3.08 (dd, J = 13.6, 6.6 Hz, 1H), 4.01 (dd, J = 20.0, 4.1 Hz, 1H), 4.10 (dd, J = 19.7, 4.9 Hz, 1H), 4.34−4.44 (m, 1H), 4.97 (br s, 1H), 6.51−6.62 (m, 1H), 7.15−7.18 (m, 2H), 7.19−7.23 (m, 1H), 7.25−7.29 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.23, 28.22, 38.35, 49.65, 55.65, 80.27, 126.97, 128.66, 129.21, 136.49, 155.35, 171.28, 202.18;

MS (ESI) *m/z* (rel intensity) (pos.) 359 (3, $[M + K]^+$), 343 (6, $[M + Na]^+$), 338 (38, $[M + NH_4]^+$) 321 (100, $[M + H]^+$), 265 (56, $[M - (CH_3)_2CCH_2 + H]^+$), 221 $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$), (neg.) 379 (11, $[M + CH_3COO]^-$); 319 (28, $[M - H]^-$), 245 (100, $[M - t\text{BuOH} - H]^-$). Anal. ($C_{17}H_{24}N_2O_4$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-phenylalanyl-propargylamide (54). General Procedure for Unsaturated Amides 54 and 55.** Boc-Phe-OH (1 g, 3.8 mmol) was dissolved in THF (20 mL) and cooled to −25 °C. Subsequently, *N*-methylmorpholine (0.41 mL, 3.8 mmol) and isobutyl chloroformate (0.51 mL, 3.8 mmol) were added under vigorous stirring. Immediately, propargylamine (0.29 mL, 4.2 mmol) was added. The suspension was allowed to warm to room temperature. After 2 h, THF was removed under reduced pressure and the solid residue was dissolved in H_2O (10 mL), adjusted to pH 1−2 (10% $NaHSO_4$), and extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with H_2O (10 mL), sat. $NaHCO_3$ (2 × 10 mL), and brine (10 mL), dried over Na_2SO_4, and evaporated to dryness to obtain a solid residue, which was recrystallized from cyclohexane to obtain **54** (0.75 g, 65%): mp 102−104 °C (lit.[64] mp 98−100 °C); $[\alpha]^{20}_D = +3.6$ (c = 1.32, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.22 (w) & 1.28 (i) (s, 9H), 2.71 (dd, J = 12.2, 10.8 Hz, 1H), 2.92 (dd, J = 13.7, 4.5 Hz, 1H), 3.09 (t, J = 2.5, 1H), 3.83−3.88 (m, 2H), 4.10−4.17 (m, 1H), 6.85 (d, J = 8.4 Hz, 1H), 7.15−7.20 (m, 1H), 7.21−7.30 (m, 4H), 8.33 (t, J = 5.1 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.15, 28.27, 37.65, 55.69, 73.17, 78.11, 81.13, 126.29, 128.11, 129.32, 138.1, 155.31, 171.53; MS (ESI) *m/z* (rel intensity) (pos.) 341 (3, $[M + K]^+$), 325 (6, $[M + Na]^+$), 303 (54, $[M + H]^+$), 247 (72, $[M - (CH_3)_2CCH_2 + H]^+$), 203 (100, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$), (neg.) 361 (47, $[M + CH_3COO]^-$), 301 (66, $[M - H]^-$), 227 (100, $[M - t\text{BuOH} - H]^-$). Anal. ($C_{17}H_{22}N_2O_3$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-phenylalanyl-allylamide (55).** The compound was obtained as pure crude product, yield 100%: mp 105−107 °C (lit.[65] mp 88−90 °C); $[\alpha]^{20}_D = +1.0$ (c = 2.11, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.22 (w) & 1.29 (i) (s, 9H), 2.74 (dd, J = 13.7, 10.0 Hz, 1H), 2.93 (dd, J = 13.8, 4.9 Hz, 1H), 3.63−3.74 (m, 2H), 4.12−4.19 (m, 1H), 5.02 (dd, J = 10.3, 1.3 Hz, 1H), 5.09 (dd, J = 17.2, 1.7 Hz, 1H), 5.69−5.80 (m, 1H), 6.85 (d, J = 8.7 Hz, 1H), 7.15−7.20 (m, 1H), 7.21−7.30 (m, 4H), 7.99 (t, J = 5.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.27, 37.80, 40.9, 55.93, 78.08, 115.06, 126.27, 128.11, 129.31, 135.27, 138.31, 155.32, 171.53; MS (ESI) *m/z* (rel intensity) (pos.) 343 (2, $[M + K]^+$), 327 (6, $[M + Na]^+$), 305 (45, $[M + H]^+$), 249 (69, $[M - (CH_3)_2CCH_2 + H]^+$), 205 (100, $[M - (CH_3)_2CCH_2 - CO_2 + H]^+$), (neg.) 363 (60, $[M + CH_3COO]^-$), 303 (31, $[M - H]^-$), 229 (100, $[M - t\text{BuOH} - H]^-$). Anal. ($C_{17}H_{24}N_2O_3$) C, H, N.

Inhibition Experiments. Papain. Enzyme activities were calculated from kinetic measurements performed by spectrophotometric detection of the product *p*-nitroaniline (pNA) at 25 °C in a multicell holder (parallel measurements for each single inhibitor determination; final volume 1 mL) at a wavelength of 405 nm. A 4 mM stock solution of the chromogenic substrate Z-Phe-Arg-NHNp was prepared in DMSO; the final concentration was 200 μM. The assay medium was 0.1 M sodium phosphate pH 6.5, 2.5 mM EDTA (ethylenediaminetetraacetic acid), 300 μM DTT, and 12% DMSO. Stock solutions of the inhibitors were prepared in DMSO. In the absence of inhibitor, 70 μL of DMSO was added to the cuvette. A papain stock solution was prepared in 1 mM HCl. For daily activation, the papain stock solution was diluted 1:100 in 15 mM DTT, 0.1 M sodium phosphate pH 6.5, 2.5 mM EDTA and incubated at 25 °C for 1 h. The activated enzyme was kept on ice. After thermal equilibration, the reaction was initiated by addition of the enzyme (20 μL); its final concentration catalyzed the conversion of the substrate with a rate of 1−2 μM/min. Progress curves were monitored over 10 min. Rates were determined for 7 different inhibitor concentrations in duplicate. The apparent inhibition constants K_i' were determined by fitting

$$v = v_0/(1 + [I]/K_i') \quad (1)$$

to the experimental data, where v is the rate, v_0 is the rate in the absence of inhibitor, [I] is the inhibitor concentration and K_i' is the apparent inhibition constant. The true inhibition constants K_i were calculated by correction of K_i' according to

$$K_i = K_i'/(1 + [S]/K_m) \quad (2)$$

where [S] is the substrate concentration and K_m is the Michaelis constant.

To determine the K_m value, the total hydrolysis of Z-Phe-Arg-NHNp (480 μM) was followed in the presence of a papain concentration which was 20-fold higher than in the inhibition assays; other conditions were as noted above. Hydrolysis went to completion in 30 min. Progress curves of six independent experiments were analyzed by fitting the following set of differential equations to the experimental data using the program EASY-FIT,

$$\frac{d[P]}{dt} = k_{cat}[E_t]\frac{([S_0] + [P_0] - [P])}{K_m + ([S_0] + [P_0] - [P])} \quad (3)$$

$$\frac{d[E_t]}{dt} = -k_{inac}[E_t]\frac{K_m}{K_m + ([S_0] + [P_0] - [P])} \quad (4)$$

where [P] is the product concentration (dependent experimental variable) and t is the time (independent experimental variable). For the calculation of the pNA concentrations, an extinction coefficient ϵ_{405} = 9.96 cm^2/μmol was used. The following parameters were obtained by nonlinear regression: $[E_t]$ (total concentration of active enzyme), K_m (Michaelis constant), $[S_0]$ (initial substrate concentration), $[P_0]$ (initial product concentration), and k_{inac} (rate constant for enzyme inactivation). As the determination of k_{cat} (catalytic constant) was beyond the scope of this investigation, a reasonable value was taken from the literature (k_{cat} = 1980 min^{-1}).[66] The following results were obtained: K_m = 965 ± 244 μM, $[S_0]$ = 437 ± 2 μM, $[P_0]$ = 40 ± 0.8 μM.

Cathepsins. Enzyme activities were calculated from kinetic measurements performed by fluorimetric detection of the product AMC at 37 °C in a stirred cell. The wavelengths for excitation and emission were 360 and 440 nm, respectively. The reaction volume of the assay was 2 mL. To assay cathepsin L, Z-Phe-Arg-NHMec was used as substrate at a concentration of 10 μM in 100 mM sodium phosphate pH 6.0, 100 mM NaCl, 5 mM EDTA, 0.01% Brij 35, 25 μM DTT, and 1% DMSO. For cathepsin S, Z-Val-Val-Arg-NHMec was chosen as substrate at a concentration of 40 μM. The assay medium consisted of 50 mM potassium phosphate pH 6.5, 50 mM NaCl, 2 mM EDTA, 0.01% Triton X-100, 25 μM DTT, and 1% DMSO. In the cathepsin K assay, Z-Leu-Arg-NHMec was used as substrate at a concentration of 20 μM in 100 mM sodium citrate pH 5.0, 100 mM NaCl, 1 mM EDTA, 0.01% CHAPS, 25 μM DTT, and 1% DMSO. Stock solutions of the substrates were prepared in DMSO in a 1000-fold higher concentration than the final concentrations. Dilutions were done in the corresponding assay medium without DTT and DMSO. Stock solutions of the inhibitors were prepared in DMSO. The enzyme dilutions were daily prepared from a stock with the corresponding assay medium without DMSO, containing 5 mM DTT (the complete amount of DTT required for the final concentration noted above), and kept on ice.

After thermal equilibration, 10 μL of the enzyme solution was added and product formation was monitored over 5 min. The inhibition constants were computed from the initial velocities determined at 10 to 12 different inhibitor concentrations and duplicate or triplicate measurements in the absence of the inhibitor. The K_i' values were obtained from nonlinear regressions according to eq 1, and K_i was calculated from eq 2. Michaelis constants were determined at 10 to 12 different substrate concentrations by the standard method to obtain the following values: cathepsin L, K_m = 5.3 ± 0.3 μM; cathepsin S, K_m = 19.2 ± 0.8 μM; cathepsin K, K_m = 6.2 ± 1.2 μM. Progress curves of the reactions of cathepsins L, S, and K in

the presence of inhibitor **53** were biphasic and were analyzed by nonlinear regression using eq 5,

$$[P] = v_s t + \{(v_0 - v_s)(1 - \exp(-k_{obs} t))\}/k_{obs} + d \quad (5)$$

where v_s is the steady state rate, v_0 is the initial rate, k_{obs} is the observed pseudo-first-order rate constant, and d is the offset. The second-order rate constant k_{on} was obtained by linear regression according to eq 6.

$$k_{obs} = k_{on}[I] + k_{off} \quad (6)$$

The first-order rate constant k_{off} for the dissociation of the enzyme−inhibitor complex was calculated according to eq 7.

$$k_{off} = k_{on} K_i \quad (7)$$

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Supporting Information Available: Elemental analytical data for synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Leung-Tong, R.; Li, W.; Tam, T. F.; Karimian, K. Thiol-Dependent Enzymes and Their Inhibitors: A Review. *Curr. Med. Chem.* **2002**, *9*, 979−1002.
(2) Rawlings, N. D.; Tolle, D. P.; Barrett, A. J. *MEROPS*: the peptidase database. *Nucleic Acids Res.* **2004**, *32*, D160−D164; http://merops.sanger.ac.uk.
(3) Barrett, A. J.; Rawlings, N. D. Evolutionary Lines of Cysteine Proteinases. *Biol. Chem.* **2001**, *382*, 727−733.
(4) Lecaille, F.; Kaleta, J.; Brömme, D. Human and Parasitic Papain-Like Cysteine Proteases: Their Role in Physiology and Pathology and Recent Developments in Inhibitor Design. *Chem. Rev.* **2002**, *102*, 4459−4488.
(5) Turk, D.; Turk, B.; Turk, V. Papain-like lysosomal cysteine proteases and their inhibitors: drug discovery targets? *Biochem. Soc. Symp.* **2003**, *70*, 15−30.
(6) Kirschke, H.; Barrett, A. J.; Rawlings, N. D. *Lysosomal Cysteine Proteases*; Oxford University Press: Oxford, 1998; p 57.
(7) Turk, V.; Turk, B.; Turk, D. Lysosomal cysteine proteases: facts and opportunities. *EMBO J.* **2001**, *20*, 4629−4633.
(8) Grzonka, Z.; Jankowska, E.; Kasprzykowski, F.; Lankiewicz, L.; Wiczk, W.; Wieczerzak, E.; Ciarkowski, J.; Drabik, P.; Janowski, R.; Kozak, M.; Jaskolski, M.; Grubb, A. Structural studies of cysteine proteases and their inhibitors. *Acta Biochim. Pol.* **2001**, *48*, 1−20.
(9) Schechter, I.; Berger, A. On the Size of the Active Site in Proteases. I. Papain. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157−162.
(10) Turk, D.; Guncar, G.; Podobnik, M.; Turk, B. Revised Definition of Substrate Binding Sites of Papain-Like Cysteine Proteases. *Biol. Chem.* **1998**, *379*, 137−147.
(11) Turk, B.; Turk, D.; Turk, V. Lysosomal cysteine proteases: more than scavengers. *Biochim. Biophys. Acta* **2000**, *1477*, 98−111.
(12) Nägler, D. K.; Menard, R. Family C1 Cysteine Proteases: Biological Diversity or Redundancy? *Biol. Chem.* **2003**, *384*, 837−843.
(13) Wiederanders, B.; Kaulmann, G.; Schilling, K. Functions of Propeptide Parts in Cysteine Proteases. *Curr. Protein Pept. Sci.* **2003**, *4*, 309−326.
(14) Joyce, J. A.; Baruch, A.; Chehade, K.; Meyer-Morse, N.; Giraudo, E.; Tsai, F.-Y.; Greenbaum, D. C.; Hager, J. H.; Bogyo, M.; Hanahan, D. Cathepsin cysteine proteases are effectors of invasive growth and angiogenesis during multistage tumorigenesis. *Cancer Cell* **2004**, *5*, 443−453.
(15) Ravanko, K.; Järvinen, K.; Helin, J.; Kalkkinen, N.; Hölttä, E. Cysteine Cathepsins Are Central Contributors of Invasion by Cultured Adenosylmethionine Decarboxylase-Transformed Rodent Fibroblasts. *Cancer Res.* **2004**, *64*, 8831−8838.
(16) Rousselet, N.; Mills, L.; Jean, D.; Tellez, C.; Bar-Eli, M.; Frade; R. Inhibition of Tumorigenicity and Metastasis of Human Melanoma Cells by Anti-Cathepsin L Single Chain Variable Fragment. *Cancer Res.* **2004**, *64*, 146−151.
(17) Honey, K.; Rudensky, A. Y. Lysosomal Cysteine Proteases Regulate Antigen Presentation. *Nat. Rev. Immunol.* **2003**, *3*, 472−482.
(18) Beers, C.; Burich, A.; Kleijmeer, M. J.; Griffith, J. M.; Wong, P.; Rudensky, A. Y. Cathepsin S Controls MHC Class II-Mediated Antigen Presentaion of Epithelial Cells In Vivo. *J. Immunol.* **2005**, *175*, 1205−1212.
(19) Yang, H.; Kala, M.; Scott, B. G.; Goluszko, E.; Chapman, H. A.; Christadoss, P. Cathepsin S Is Required for Murine Autoimmune Myasthenia gravis Pathogenesis. *J. Immunol.* **2005**, *174*, 1729−1737.
(20) Brömme, D.; Okamoto, K.; Wang, B. B.; Biroc, S. Human Cathepsin O2, a Matrix Protein-degrading Cysteine Protease Expressed in Osteoclasts. *J. Biol. Chem.* **1998**, *271*, 2126−2132.
(21) Garnero, P.; Borel, O.; Byrjalsen, I.; Ferreras, M.; Drake, F. H.; McQueney, M. S.; Foged, N. T.; Delmas, P. D.; Delaissse, J.-M. The Collagenolytic Activity of Cathepsin K Is Unique among Mammalian Proteinases. *J. Biol. Chem.* **1998**, *273*, 32347−32352.
(22) Li, Z.; Hou, W.-S.; Escalente-Torres, C. R.; Gelb, B. D.; Brömme, D. Collagenase Activity of Cathepsin K Depends on Complex Formation with Chondroitin Sulfate. *J. Biol. Chem.* **2002**, *277*, 28669−28676.
(23) Shaw, E. Cysteinyl Proteinases and their Selective Inactivation. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1990**, *63*, 271−347.
(24) Otto, H.-H.; Schirmeister, T. Cysteine Proteases and Their Inhibitors. *Chem. Rev.* **1997**, *97*, 133−171.
(25) Greenspan, P. D.; Clark, K. L.; Tommasi, R. A.; Cowen, S. D.; McQuire, L. W.; Farley, D. L.; van Duzer, J. H.; Goldberg, R. L.; Zhou, H.; Du, Z.; Fitt, J. J.; Coppa, D. E.; Fang, Z.; Macchia, W.; Zhu, L.; Capparelli, M. P.; Goldstein, R.; Wigg, A. M. Identification of Dipeptidyl Nitriles as Potent and Selective Inhibitors of Cathepsin B through Structure-Based Drug Design. *J. Med. Chem.* **2001**, *44*, 4524−4534.
(26) Ward, Y. D.; Thomson, D. S.; Frye, L. L.; Cywin, C. L.; Morwick, T.; Emmanuel, M. J.; Zindell, R.; McNeil, D.; Bekkali, Y.; Girardot, M.; Hrapchak, M.; DeTuri, M.; Crane, K.; White, D.; Pav, S.; Wang, Y.; Hao, M.-H.; Grygon, C. A.; Labadia, M. E.; Freeman, D. M.; Davidson, W.; Hopkins, J. L.; Brown, M. L.; Spero D. M. Design and Synthesis of Dipeptide Nitriles as Reversible and Potent Cathepsin S Inhibitors. *J. Med. Chem.* **2002**, *45*, 5471−5482.
(27) Moon, J. B.; Coleman, R. S.; Hanzlik, R.-P. Reversible Covalent Inhibition of Papain by a Peptide Nitrile. ^{13}C NMR Evidence for a Thioimidate Ester Adduct. *J. Am. Chem. Soc.* **1986**, *108*, 1350−1351.
(28) Brisson, J.-R.; Carey, P. R.; Storer, A. C. Benzoylamidoacetonitrile Is Bound as a Thioimidate in the Active Site of Papain. *J. Biol. Chem.* **1986**, *261*, 9087−9089.
(29) Liang, T.-C.; Abeles, R. H. Inhibition of Papain by Nitriles: Mechanistic Studies Using NMR and Kinetic Measurements. *Arch. Biochem. Biophys.* **1987**, *252*, 626−634.
(30) Asboth, B.; Polgar, L. Transition-State Stabilization at the Oxyanion Binding Sites of Serine and Thiol Proteinases: Hydrolyses of Thiono and Oxygen Esters. *Biochemistry* **1983**, *22*, 117−122.
(31) Asboth, B.; Stokum, E.; Khan, I. U.; Polgar, L. Mechanism of Action of Cysteine Proteinases: Oxyanion Binding Site Is Not Essential in the Hydrolysis of Specific Substrates. *Biochemistry* **1985**, *24*, 606−609.
(32) Wieland, T.; Bernhard, H. Über Peptidsynthesen. 3. Die Verwendung von Anhydriden aus *N*-acylierten Aminosäuren und Derivaten anorganischer Säuren. *Liebigs Ann. Chem.* **1951**, *572*, 190−194.
(33) Miyaura, N.; Suzuki, A. Palladium-Catalyzed Cross-Coupling Reactions of Organoboron Compounds. *Chem. Rev.* **1995**, *95*, 2547−2483.
(34) Larhed, M.; Moberg, C.; Hallberg, A. Microwave-Accelerated Homogeneous Catalysis in Organic Chemistry. *Acc. Chem. Res.* **2002**, *35*, 717−727.
(35) Gong, Y.; He, W. Direct Synthesis of Unprotected 4-Aryl Phenylalanines via the Suzuki Reaction under Microwave Irradiation. *Org. Lett.* **2002**, *4*, 3803−3805.
(36) Calabretta, R.; Gallina, C.; Giordano, C. Sodium cyanoborohydride reduction of (benzyloxycarbonyl)- and (*tert*-butoxycarbonyl)hydrazones. *Synthesis* **1991**, 536−539.
(37) Gante, J.; Kessler, H.; Gibson, C. Synthesis of Azapeptides. In *Houben-Weyl Methods of Organic Chemistry*, Vol. E22c *Synthesis of Peptidomet*ics; Goodman, M., Felix, A., Moroder, L., Toniolo, C., Eds.; Georg Thieme Verlag: Stuttgart, 2002; pp 311−323.
(38) Krauser, J. A.; Powers, J. C. Peptide Aldehydes. In *Hoben-Weyl Methods of Organic Chemistry*, Vol. E22c *Synthesis of Peptidomet*ics; Goodman, M., Felix, A., Moroder, L., Toniolo, C., Eds.; Georg Thieme Verlag: Stuttgart, 2002; pp 199−217.
(39) Nahm, S.; Weinreb, S. M. N-Methoxy-N-methylamides as Effective Acylating Agents. *Tetrahedron Lett.* **1981**, *22*, 3815−3818.
(40) Meyer, E. A.; Castellano, R. K.; Diederich, F. Interaction with Aromatic Rings in Chemical and Biological Recognition. *Angew. Chem., Int. Ed.* **2003**, *42*, 1210−1240.

(41) Olsen, J. A.; Banner, D. W.; Seiler, P.; Obst-Sander, U.; D'Arcy, A.; Stihle, M.; Müller, K.; Diederich, F. A Fluorine Scan of Thrombin Inhibitors to Map the Fluorophilicity/Fluorophobicity of an Enzyme Active Site: Evidence for C−F···C═O Interactions. *Angew. Chem., Int. Ed.* **2003**, *42*, 2507−2511.
(42) Olsen, J. A.; Banner, D. W.; Seiler, P.; Wagner, B.; Tschopp, T.; Obst-Sander, U.; Kansy, M.; Müller, K.; Diederich, F. Fluorine Interactions at the Thrombin Active Site: Protein Backbone Fragments H−C−C═O Comprise a Favorable C−F Environment and Interactions of C−F with Electrophiles. *ChemBioChem* **2004**, *5*, 666−675.
(43) Thormann, M.; Hofmann, H.-J. Conformational properties of azapeptides. *J. Mol. Struct. (THEOCHEM)* **1999**, *469*, 63−76.
(44) Tyndall, J.; Nall, T.; Fairlie, D. P. Proteases Universally Recognize Beta Strands In Their Active Sites. *Chem. Rev.* **2005**, *105*, 973−999.
(45) Wieczerzak, E.; Drabik, P.; Lankiewicz, L.; Oldziej, S.; Grzonka, Z.; Abrahamson, M.; Grubb, A.; Brömme, D. Azapeptides Structurally Based upon Inhibitory Sites of Cystatins as Potent and Selective Inhibitors of Cysteine Proteases. *J. Med. Chem.* **2002**, *45*, 4202−4211.
(46) Pauly, T. A.; Sulea, T.; Ammirati, M.; Sivaraman, J.; Danley, D. E.; Griffor, M. C.; Kamath, A. V.; Wang, I.-K.; Laird, E. R.; Seddon, A. P.; Menard, R.; Cygler, M.; Rath, V. L. Specificity Determinants of Human Cathepsin S Revealed by Crystal Structures of Complexes. *Biochemistry* **2003**, *42*, 3203−3213.
(47) Morrison, J. F.; Walsh, C. T. The Behavior and Significance of Slow-Binding Enzyme Inhibitors. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1988**, *61*, 201−301.
(48) Hajduk, P. J.; Bures, M.; Praestgard, J.; Fesik, S. W. Privileged Molecules for Protein Binding Identified from NMR-Based Screening. *J. Med. Chem.* **2000**, *43*, 3443−3447.
(49) Lecaille, F.; Serveau, C.; Gauthier, F.; Lalmanach, G. Revisiting the S2 specificity of papain by structural analogs of Phe. *FEBS Lett.* **1999**, *445*, 311−314.
(50) Storer, A. C.; Menard, R. Recent insights into cysteine protease specificity: Lessons for drug design. *Perspect. Drug Discovery Des.* **1996**, *6*, 33−46.
(51) Janowski, R.; Kozak, M.; Jankowska, E.; Grzonka, Z. Two polymorphs of a covalent complex between papain and a diazomethylketone inhibitor. *J. Pept. Res.* **2004**, *64*, 141−150.
(52) Fujishima, A.; Imai, Y.; Nomura, T.; Fujisawa, Y.; Yamamoto, Y.; Sugawara, T. The crystal structure of human cathepsin L complexed with E-64. *FEBS Lett.* **1997**, *407*, 47−50.
(53) McGrath, M. E.; Palmer, J. T.; Brömme, D.; Somoza, J. R. Crystal structure of human cathepsin S. *Protein Sci.* **1998**, *7*, 1294−1302.
(54) Brömme, D.; Steinert, A.; Friebe, S.; Fittkau, S.; Wiederanders, B.; Kirschke, H. The specificity of bovine spleen cathepsin S. A comparison with rat liver cathepsins L and B. *Biochem. J.* **1989**, *264*, 475−481.
(55) McGrath, M. E.; Klaus, J. E.; Barnes, M. G.; Brömme, D. Crystal structure of human cathepsin K complexed with a potent inhibitor. *Nat. Struct. Biol.* **1997**, *4*, 105−108.
(56) Maubach, G.; Schilling, K.; Rommerskirch, W.; Wenz, I.; Schultz, J. E.; Weber, E.; Wiederanders, B. The inhibition of cathepsin S by its propeptide. Specificity and mechanism of action. *Eur. J. Biochem.* **1997**, *250*, 745−750.
(57) Linnevers, C. J.; McGrath, M. E.; Armstrong, R.; Mistry, F. R.; Barnes, M. G.; Klaus, J. L.; Palmer, J. T.; Katz, B. A.; Brömme, D. Expression of human cathepsin K in Pichia pastoris and preliminary crystallographic studies of an inhibitor complex. *Protein Sci.* **1997**, *6*, 919−921.
(58) Nikolenko, L. M. Preparation of arylsulfonyl derivatives of amino acids. *Zh. Obshch. Khim.* **1956**, *26*, 806−808.
(59) Schittkowski, K. EASY-FIT: A Software System for Data Fitting in Dynamic Systems. *Struct. Multidiscip. Optim.* **2002**, *23*, 153−169.
(60) Moser, H.; Fliri, A.; Steiger, A.; Costello, G.; Schreiber, J.; Eschenmoser, A. 130. Poly(dipeptamidinium)-Salze: Definition und Methoden zur präparativen Herstellung. *Helv. Chim. Acta* **1986**, *69*, 1224−1262.
(61) Grzonka, Z.; Rekowska, E.; Liberek, B. Tetrazole Analogues of Amino Acides and Peptides-V. Syntheses of Peptide Derivatives Containing Tetrazole Analogues of Amino Acids in C-terminal Position. *Tetrahedron* **1971**, *27*, 2317−2322.
(62) Reid, J. D.; Hussain, S.; Sreedharan, S. K.; Bailey, T. S. F.; Surapong, P.; Thomas, E. W.; Verma, C. S.; Brocklehurst, K. Variation in aspects of cysteine proteinase catalytic mechanism deduced by spectroscopic observation of dithioester intermediates, kinetic analysis and molecular dynamic simulations. *Biochem. J.* **2001**, *357*, 343−352.
(63) Wieczerzak, E.; Kozlowska, J.; Lankiewicz, L.; Grzonka, Z. The efficient synthesis of azaamino acids. *Pol. J. Chem.* **2002**, *76*, 1693−1697.
(64) Curran, T. P.; Grant, A. L.; Lucht, R. L.; Carter, J. C.; Affonso, J. π-Ligands for Generating Transition Metal-Peptide Complexes: Coordination of Amino Acid Derivatives to Tungsten Utilizing Alkyne Ligands. *Org. Lett.* **2002**, *4*, 2917−2920.
(65) Suzuki, K.; Hiroki, F.; Sasaki, Y.; Shiratori, M.; Sakadura, S.; Kisara, K. Studies on analgesic oligopeptides. V. Structure-activity relationship of tripeptide alkylamides, Tyr-D-Arg-Phe-X. *Chem. Pharm. Bull.* **1988**, *36*, 4834−4840.
(66) Tchoupe, J. R.; Moreau, T.; Gauthier, F.; Bieth, J. G. Photometric or fluorometric assay of cathepsin B, L and H and papain using substrates with an aminotrifluoromethylcoumarin leaving group. *Biochim. Biophys. Acta* **1991**, *1076*, 149−151.

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