121. Synthesis of D- and L-5-Oxaproline and of a New Captopril Analogue

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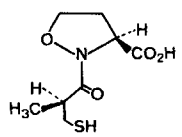
Summary

The 1,3-dipolar cycloaddition of the *C-t*-butyloxycarbonyl-N-mannosyl-nitrone **5**, formed *in situ* from the partially protected D-mannose-oxime **3** and the glyoxylate **4**, to ethylene gave preferentially the (3*S*)-*N*-glycosyl-isoxazolidine **6** which was transformed into the 3-isoxazolidine-carboxylate (L-5-oxaproline ester) **12** and into some derivatives thereof. The (*S*)-configuration of **12** was proved by chemical correlation with a derivative of L-asparagine. The D-5-oxaproline ester was obtained from the corresponding *N*-ribosyl-nitrone **24**. Two protected dipeptides containing either *C*-terminal- (**28**) or *N*-terminal-5-oxaproline (= Opro) (**30**) were synthesized. Starting from **12**, the analogue **1** of captopril® (**2**) was prepared and its activity as an inhibitor of the angiotensin-converting-enzyme (ACE) was examined.

We have reported briefly on the asymmetric synthesis of a new proline analogue of potential biochemical interest using sugar derivatives as reagents [1]. To determine if 5-oxaproline (3-isoxazolidinecarboxylic acid) can replace proline in biologically active compounds without loss of the biological activity, we intended to prepare the analogue **1** of captopril ((2*S*)-1-[(3-mercapto-2-methyl)propionyl]-L-proline, **2**)[2]) [2–4], where the proline moiety is replaced by 5-oxaproline. The present



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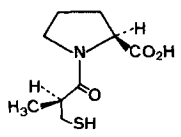

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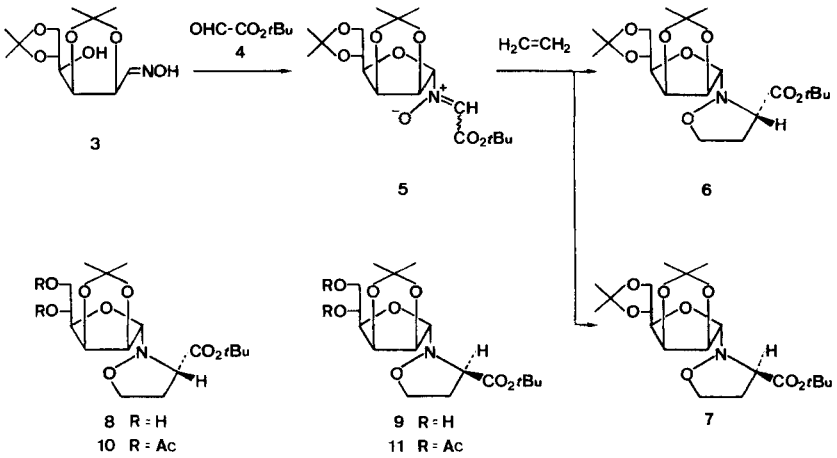
[2]) Captopril is a specific inhibitor of the *Angiotensin-Converting-Enzyme* (ACE), a carboxypeptidase responsible for the conversion of the decapeptide angiotensin I into the potent vasopressor octapeptide angiotensin II by cleavage of the *C*-terminal dipeptide histidyl-leucine [2] [3] [5] [6]. It has been shown to possess clinical usefulness as an orally active antihypertensive agent [3] [5] [7–9].

communication reports on the preparation of the analogue **1** and describes its inhibitory activity against ACE[2]). It also gives the experimental details of the synthesis of derivatives of both 5-oxaproline enantiomers.

The asymmetric synthesis of L-5-oxaproline is based upon a 1,3-dipolar cycloaddition of the *N*-glycosyl-*C*-alkoxycarbonyl-nitrones, obtained *in situ* from 2,3:5,6-di-*O*-isopropylidene-D-mannose oxime (**3**) and glyoxylate [1], to ethylene. Using the *t*-butyl glyoxylate (**4**)[3]), the *N*-glycosyl-isoxazolidinecarboxylates **6** and **7** *(Scheme 1)* were obtained in 91% yield and in a diastereoselectivity of 54% (see *Exper. Part*) favouring the spontaneously crystallizing (3*S*)-diastereoisomer **6**, which was pure after 3–4 recrystallizations in hexane (43%). The isomers **6** and **7** could not be separated but the diols **8** and **9** obtained from **6** and **7**, respectively, by partial deprotection (87.6%) were easily separated by chromatography on silica gel and

Scheme 1



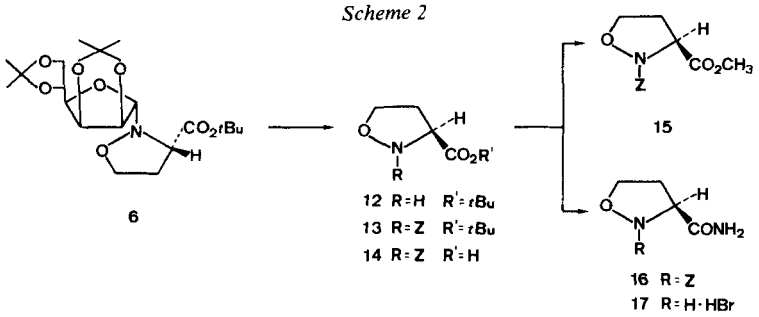
further characterized as the diacetates **10** and **11**. The *α*-D-configuration of the compounds **6–11** was deduced from the very small *J*(1′,2′)-values. Judging by the similarity of their coupling constants, compounds **6–11** possess very similar conformations. The ^{1}H-NMR. spectrum of the (3*R*)-diacetate **11** showed a deshielding for H–C(1′ and 2′) and a shielding for H–C(3 and 5′) relative to the chemical shifts of (3*S*)-diacetate **10**. Similarly, the ^{13}C-NMR. spectra of the minor isomers **7** and **11** showed a deshielding for C(1′,2′,4′,5′,4 and 5) and a shielding for C(3) relative to the chemical shifts of the major isomers **6** and **10**. These differences depend only weakly on the substituent at C(3) and may serve for the determination of the configuration at this center [16].

Glycoside cleavage of the *N*-glycosyl-isoxazolidine **6** gave the 5-oxaproline ester **12** (90%) *(Scheme 2)* which, upon benzyloxycarbonylation, afforded the fully

[3]) *t*-Butyl glyoxylate (**4**) was prepared by ozonolysis (MeOH/CH_2Cl_2 3:1, –70°, then $(CH_3)_2S$, –10° to 0°, 20 h and distillation, b.p. 55–60°/48 Torr [10–12]) of the corresponding maleate [13] or fumarate [14]. Acidic esterification of glyoxylic acid with isobutylene (isobutylene, H_2SO_4, *t*-BuOH, 3.5 h, r.t.) gave the (*R*,*S*)-2-hydroxy-4-methyl-4-pentanolide (39%, b.p. 60–63%, 0.001 Torr) and **4** (22%) (*cf.* [15]).

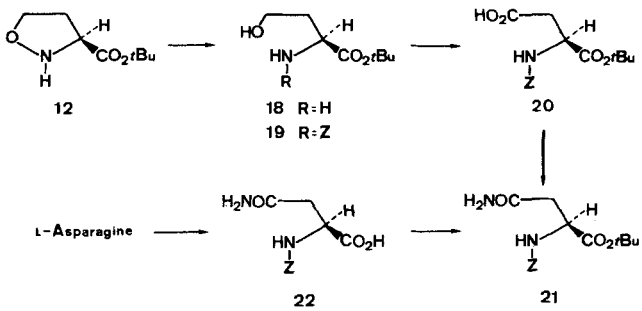
protected ester **13** (84%). Hydrolysis of the *t*-butyl-ester **13** with CF_3CO_2H yielded the *N*-benzyloxycarbonyl-5-oxaproline **14** (97%). This acid was, on one hand esterified to the methyl-ester **15**, and on the other hand, treated first with isobutyl chloroformate and then with NH_3, to give the protected amide **16** (88%). Deprotection of **16** with HBr yielded the hydrobromide of the amide **17**.

Scheme 2



The (3*S*)-configuration of the 5-oxaproline ester **12** was proved by correlation with the known L-asparagine derivative **21** [17] *(Scheme 3)*. Hydrogenolysis of the N,O-bond of **12** gave the L-homoserine ester **18** which was benzyloxycarbonylated to **19**. Oxidation of **19** with $KMnO_4$ afforded the partially protected aspartic-acid derivative **20**. Ammonolysis of the mixed anhydride formed from **20** and isobutyl chloroformate gave the asparagine-ester derivative **21**, which was also obtained from L-asparagine by benzyloxycarbonylation (→ **22**) [18] and esterification [17]. The samples prepared from **12** and from L-asparagine had the same melting point and the same specific rotation *(Table)*. Their mixed melting point showed no depression.

Scheme 3



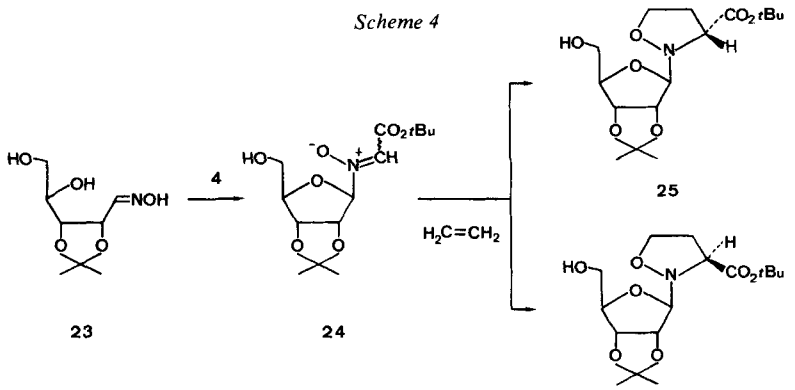
Using the ribose-derived oxime **23**, we expected [19] preferential formation of the *N*-glycosyl-D-5-oxaproline ester **25**. Reaction of the oxime **23** *(Scheme 4)* [19] with the glyoxylate **4** (→ **24**) in the presence of ethylene, gave the two *N*-glycosyl-isoxazolidines **25** (62%) and **26** (25.3%) (d.e. 42%)[4]). The D-configurated ester **12** (m.p. 53–53.5°; $[\alpha]_D^{25} = +26.5$ ($c = 1$, $CHCl_3$)) was obtained by glycoside cleavage of the major isomer **25**.

[4]) Utilizing 2,3-*O*-isopropylidene-5-*O*-trityl-D-ribose oxime [20] instead of **23** gave a greater diastereoselectivity (d.e. 72%) but a poorer yield (78%) [1].

Table. *Comparison of the specific rotations and melting points of the asparagine ester* **21** *prepared from H-Opro-OtBu* **12** *or from* L-*asparagine*

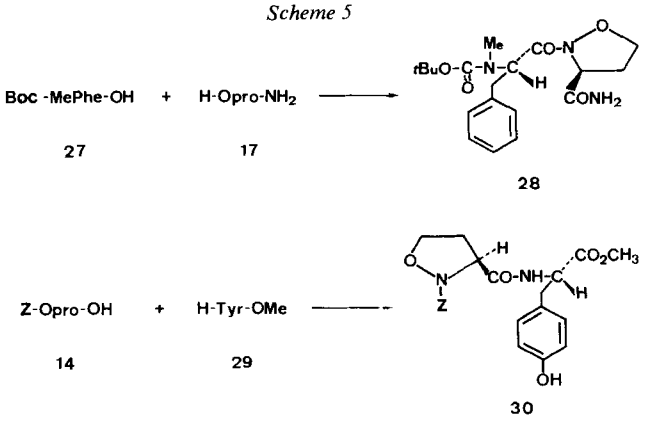
21 (from **12**)	m.p. 103.5–104°	$[\alpha]_D^{25} = -14.0°$ ($c = 1.99$, EtOH)
21 (from L-asparagine)	m.p. 104–104.5°	$[\alpha]_D^{25} = -14.8°$ ($c = 2$, EtOH)

Scheme 4



To check if 5-oxaproline can be incorporated into peptides, we synthesized the protected didpeptides **28** and **30** containing 5-oxaproline (Opro) either as the *C*- or *N*-terminal amino-acid *(Scheme 5)*. L-5-oxaprolinamide **17** was coupled with Boc-(*N*-methyl)-L-phenylalanine **27** [21] by the method of the mixed anhydride [22] yielding the dipeptide **28** (90%). By the same method, Z-Opro-OH **14** (Z = benzyloxycarbonyl) was coupled with L-tyrosine methyl ester (**29**) [23] to give Z-L-5-oxaprolyl-L-tyrosine methyl ester **30** (87%). Elemental analysis and spectroscopic data of these fully protected dipeptides **28** and **30** were in agreement with their structures.

Scheme 5

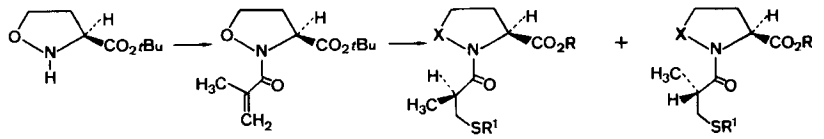


To prepare the captopril analogue **1**, the 5-oxaproline ester **12** was treated with methacryloyl chloride in pyridine to give the methacryloyl-5-oxaproline ester **31** (93%). The mercapto group was introduced by conjugate addition of thioacetic acid yielding a roughly 1:1-mixture of the diastereoisomers **32** (44% from **12**)

and **33** (40% from **12**) which were separated by chromatography and transformed into **34** and **35**, respectively by hydrolysis with CF_3CO_2H.

The (*S*)-configuration at C(2′) of **34** was inferred from the comparison of the pharmacological activity of the two diastereoisomers **34** and **35**. In the ACE-inhibitory-test (IC_{50}, HHL-assay, [24]), the acetyl derivative **34** was 3 times and its diastereoisomer **35** 300 times less active than the analogous (2′*S*)-configurated captopril derivative **36**[5]).

Scheme 6



12 **31** **32** X=O, R=*t*Bu, R¹=Ac; **33** X=O, R=*t*Bu, R¹=Ac; **34** X=O, R=H, R¹=Ac; **35** X=O, R=H, R¹=Ac; **1** X=O, R=R¹=H; **36** X=CH_2, R=H, R¹=Ac; **37** X=CH_2, R=H, R¹=Ac; **2** X=CH_2, R=R¹=H; **38** X=CH_2, R=R¹=H

Ammonolysis of the more active *S*-acetyl derivative **34** gave the captopril analogue **1**, which was 5 times less active than captopril (**2**) in the ACE-inhibitory-test, indicating that 5-oxaproline can replace proline in a pharmacologically active compound without substantial loss of biological activity.

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Experimental Part

General remarks: see [1] [16]. Mass spectra (MS.) were registered on *Du-Pont 21-491* (I), *Varian 112S* (II), *AEI MS 30* (III) and *CEC 21-110B* (IV) instruments. The following solvent mixtures were used for chromatography: A: CH_2Cl_2/MeOH 9:1; B: EtOAc/Et_2O/Hexane 1:2:4; C: EtOAc/Et_2O/hexane 2:4:3; D: EtOAc/Et_2O/hexane 2:4:5; E: EtOAc/Et_2O/hexane 2:3:5; F: EtOAc/hexane/MeOH 40:8:1.

t-*Butyl (3S)- and (3R)-2-(2,3:5,6-di-O-isopropylidene-α-D-mannofuranosyl)-3-isoxazolidinecarboxylate* (**6** and **7**). A solution of the oxime **3** ([19]; 27 g, 98.2 mmol), and *t*-butyl glyoxylate (**4**; 20.2 g, 90%, 140 mmol) in $CHCl_3$ (70 ml) was pressurized in an autoclave with ethylene (65 atm). The mixture was stirred for 1.5 h at r.t., for 17 h at 75–80° (bath temp.) and then cooled to r.t. After

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[5]) The relative specific rotations of the (2′*S*)- and (2′*R*)-configurated oxa-analogues of *S*-acetylcaptopril (**34** and **35**: $[\alpha]_D = -169°$ ($c = 0.7$, DMF) and $-54°$ ($c = 0.5$, DMF)) are similar to those of captopril (**2**) and of its (2′*R*)-diastereoisomer **38** ($[\alpha]_D = -131.0°$ ($c = 1.7$, EtOH) and $-41.1°$ ($c = 2.3$, EtOH)) [5]. The solvent dependency was found to be small in the case of the (2′*S*,3*S*)-captopril *t*-butylammonium salt ($[\alpha]_D = -67°$ ($c = 0.8$, EtOH) and $-57°$ ($c = 1.1$, DMF))and in the case of (2′*S*,3′*S*)-*S*-acetylcaptopril (**36**) ($[\alpha]_D = -164.4°$ ($c = 2.4$, EtOH) and $-137°$ ($c = 0.9$, DMF)). The values published for the corresponding *S*-acetylcaptopril derivatives **36** (2′*S*) and **37** (2′*R*) are: $[\alpha]_D = -164.4°$ ($c = 2.4$, EtOH) and $+17.8°$ ($c = 1.3$, EtOH) [5].

concentration, a sample was chromatographed on silica gel (20 g, B) to afford mixtures of the two diastereoisomers **6** and 7. By capillary GC. (capillary column *GROB OV-61*, $T_{inj} = 230°$, $T_{col} = 180°$, $P_{H2} = 0.4$ atm), this mixture contained 23% of **7** (retention time 8.47 min) and 77% of **6** (retention time 9.86 min). The crystalline, crude reaction product was recrystallized in hexane yielding fractions A–C; A: 9.8 g (m.p. 94–96°); B: 19 g (m.p. 96–97°); C: 8.3 g (m.p. 95–96°); ratio of the diastereoisomers (GC.): A 1.3: 98.7, B 25.2: 74.5 and C 54: 46. HPLC.-chromatography (*LICHROSORB Si-60* column, dioxane/hexane 1:4, 95 bar) confirmed these ratios. Repeated recrystallizations gave the major product **6** (17.4 g, 43%, purity > 99%).

Data of **6**. M.p. 96–97°, Rf (D) = 0.48, $[\alpha]_D^{25} = -38.8°$ ($c = 1$, $CHCl_3$). – IR. ($CHCl_3$): 2980*s*, 2930*m*, 2880*w*, 1730*s*, 1470*w*, 1450*m*, 1380*s*, 1370*s*, 1150*s*, 1115*m*, 1065*s*, 1040*m*, 980*w*, 885*w*, 860*w*. – ^{1}H-NMR. (200 MHz, $CDCl_3$): 1.33 (*s*, 3 H); 1.35 (*s*, 3 H); 1.43 (*s*, 3 H); 1.47 (*s*, 3 H); 1.48 (*s*, 9 H); 2.40 (*d*×*d*×*d*×*d*, $J = 11$, 8, 8 and 8, 1 H, H–C(4)); 2.51 (*d*×*d*×*d*×*d*, $J = 11$, 8, 8 and 4.2, 1 H, H–C(4)); 3.93 (*d*×*d*, $J = 8$ and 4.3, 1 H, H–C(6′)); 3.99 (*d*×*d*, $J = 8$ and 8, 2 H, H–C(5)); 4.01 (*d*×*d*, $J = 8$ and 4.2, 1 H, H–C(3)); 4.09 (*d*×*d*, $J = 8$ and 6.3, 1 H, H–C(6′)); 4.18 (*d*×*d*, $J = 8$ and 3.6, 1 H, H–C(4′)); 4.34 (*s*, 1 H, H–C(1′)); 4.38 (*d*×*d*×*d*, $J = 8$, 6.3 and 4.3, 1 H, H–C(5′)); 4.87 (*d*×*d*, $J = 6$ and 3.6, 1 H, H–C(3′)); 5.01 (*d*, $J = 6$, 1 H, H–C(2′)). – ^{13}C-NMR. ($CDCl_3$): 170.32 (*s*); 112.28 (*s*); 109.23 (*s*); 96.95 (*d*); 84.11 (*d*); 83.36 (*d*); 81.47 (*s*); 80.22 (*d*); 73.00 (*d*); 66.80 (*t*); 66.69 (*t*); 63.29 (*d*); 31.54 (*t*); 28.01 (*qa*); 26.82 (*qa*); 25.96 (*qa*); 25.21 (*qa*); 24.54 (*qa*). – MS. (I): 415 (2, M^+), 400 (2), 344 (5), 315 (33), 249 (10), *186* (100), 168 (10), 146 (10), 128 (17), 102 (17), 99 (11), 85 (12), 57 (11), 43 (18).

$C_{20}H_{33}NO_8$ (415.48) Calc. C 57.83 H 7.95 N 3.37% Found C 57.58 H 7.99 N 3.49%

Data of **7**. Rf (D) = 0.48. – ^{13}C-NMR. ($CDCl_3$): 169.55 (*s*); 112.21 (*s*); 108.80 (*s*); 98.37 (*d*); 84.57 (*d*); 84.47 (*d*); 81.64 (*s*); 80.42 (*d*); 73.81 (*d*); 67.19 (*t*); 66.55 (*t*); 61.24 (*d*); 33.56 (*t*); 27.96 (*qa*); 26.82 (*qa*); 26.01 (*qa*); 25.13 (*qa*); 24.34 (*qa*).

t-*Butyl (3S)- and (3R)-2-(2,3-*O*-isopropylidene-α-*D*-mannofuranosyl)-3-isoxazolidinecarboxylate* (**8** and **9**). A solution of a 1:1-mixture of **6** and 7 (16 g, 38.5 mmol) in aq. HCOOH (80%, 200 ml) was stirred for 4 min at r.t. and then poured into ice cold 1M Na_2CO_3 (2 l). The products were extracted with EtOAc (3 × 1.2 l). The org. phases were washed with 0.5M Na_2CO_3 (2 × 300 ml) dried and evaporated i.v. Chromatography (LSC.) of the residue on silica gel (1.1 kg, F) gave **8** (6.7 g, 46.4%, Rf (F) = 0.40) and **9** (5.95 g, 41.2%, Rf (F) = 0.33). For analysis, a solution of **8** (500 mg, 1.3 mmol) in a mixture of pyridine (3.5 ml) and Ac_2O (2 ml) was kept for 2 h at r.t., concentrated i.v. and the residue purified by chromatography on silica gel (30 g, C) giving **10** (595 mg, 97%). In a similar way, **9** (500 mg, 1.3 mmol) gave **11** (592 mg, 97%). Analytical samples were prepared by distillation i.v.: b.p. = 95–100°/0.001 Torr.

Data of t-*butyl (3S)-2-(5,6-di-*O*-acetyl-2,3-*O*-isopropylidene-α-*D*-mannofuranosyl)-3-isoxazolidine-carboxylate* (**10**). Rf (C) = 0.44. $[\alpha]_D^{25} = -19.3°$ ($c = 1$, $CHCl_3$). – IR. ($CHCl_3$): 2990*m*, 2940*m*, 2900*w*, 1740*s*, 1480*w*, 1465*m*, 1395*m*, 1385*m*, 1372*s*, 1160*s*, 1100*s*, 1080*s*, 1050*s*, 1020*m*, 980*w*, 950*w*, 900*w*, 885*w*, 860*m*. – ^{1}H-NMR. (90 MHz, $CDCl_3$): 1.30 (*s*, 3 H); 1.44 (*s*, 3 H); 1.47 (*s*, 9 H); 2.04 (*s*, 6 H); 2.43 (*d*×*d*×*d*, $J = 7.2$, 7.2 and 7.2, 1 H, H–C(4)); 2.44 (*d*×*d*×*d*, $J = 7.2$, 7.2 and 5.4, 1 H, H–C(4)); 3.91 (*d*×*d*, $J = 7.2$ and 5.4, 1 H, H–C(3)); 3.97 (*d*×*d*, $J = 7.2$ and 7.2, 2 H, H–C(5)); 4.14 (*d*×*d*, $J = 12$ and 6, 1 H, couples with H–C(5′), H–C(6)); 4.34 (*d*×*d*, $J = 8.4$ and 4, 1 H, couples with H–C(5′), H–C(4′)); 4.37 (*s*, 1 H, H–C(1′)); 4.51 (*d*×*d*, $J = 12$ and 2.4, 1 H, couples with H–C(5′), H–C(6′)); 4.77 (*d*×*d*, $J = 6$ and 4, 1 H, H–C(3′)); 4.97 (*d*, $J = 6$, 1 H, H–C(2′)); 5.29 (*d*×*d*×*d*, $J = 8.4$, 6 and 2.4, 1 H, H–C(5′)). – ^{13}C-NMR. ($CDCl_3$): 170.50 (*s*); 169.94 (*s*); 169.32 (*s*); 112.50 (*s*); 69.53 (*d*); 83.63 (*d*); 81.35 (*s*); 80.10 (*d*); 79.91 (*d*); 69.10 (*d*); 66.63 (*t*); 63.43 (*t*); 63.14 (*d*); 31.39 (*t*); 28.01 (*qa*); 25.98 (*qa*); 24.78 (*qa*); 20.93 (*qa*); 20.78 (*qa*). – MS. (II): 459 (< 1, M^+), 358 (4), 287 (17), 128 (10), 127 (17), 109 (13), 99 (13), 98 (17), 85 (31), 84 (12), 81 (15), 71 (18), 59 (23), 57 (43), *43* (100).

$C_{21}H_{33}NO_{10}$ (459.49) Calc. C 54.89 H 7.24 N 3.05% Found C 54.65 H 7.28 N 2.97%

Data of t-*butyl (3R)-2-(5,6-di-*O*-acetyl-2,3-*O*-isopropylidene-α-*D*-mannofuranosyl)-3-isoxazolidine-carboxylate* (**11**). Rf (C) = 0.44, $[\alpha]_D^{25} = +102.9°$ ($c = 1$, $CHCl_3$). – IR. ($CHCl_3$): 2995*m*, 2950*w*, 2900*w*, 1745*s*, 1460*w*, 1400*w*, 1390*m*, 1380*s*, 1162*s*, 1122*w*, 1058*s*, 1052*m*, 1040*m*, 1020*w*, 985*w*, 945*w*, 885*w*, 850*w*. – ^{1}H-NMR. (90 MHz, $CDCl_3$): 1.29 (*s*, 3 H); 1.45 (*s*, 3 H); 1.47 (*s*, 9 H); 2.03 (*s*, 6 H); 2.40 (*d*×*d*×*d*, $J = 7.2$, 7.2 and 7.2, 1 H, H–C(4)); 2.41 (*d*×*d*×*d*, $J = 7.2$, 7.2 and 6, 1 H, H–C(4)); 3.83 (*d*×*d*, $J = 7.2$ and 6, 1 H, H–C(3)); 3.87 (*d*×*d*, $J = 7.2$ and 7.2, 1 H, H–C(5)); 3.92 (*d*×*d*, $J = 7.2$ and 7.2, 1 H, H–C(5)); 4.19 (*d*×*d*, $J = 12$ and 4.8, 1 H, couples with H–C(5′), H–C(6′)); 4.31 (*d*×*d*, $J = 8.4$ and 4.2,

1 H, couples with H–C(5'), H–C(4')); 4.55 ($d \times d$, $J = 12$ and 2.4, 1 H, couples with H–C(5'), H–C(6')); 4.75 ($d \times d$, $J = 6.5$ and 4.2, 1 H, H–C(3')); 4.88 (*s*, 1 H, H–C(1')); 5.01 (*d*, $J = 6.5$, 1 H, H–C(2')); 5.16 ($d \times d \times d$, $J = 8.6$, 4.8 and 2.4, 1 H, H–C(5')). – ^{13}C-NMR. ($CDCl_3$): 170.35 (*s*); 169.50 (*s*); 169.30 (*s*); 112.40 (*s*); 98.51 (*d*); 84.22 (*d*); 81.78 (*s*); 81.55 (*d*); 80.31 (*d*); 69.54 (*d*); 67.26 (*t*); 63.42 (*t*); 61.52 (*d*); 33.50 (*t*); 27.97 (*qa*); 26.05 (*qa*); 24.67 (*qa*); 20.94 (*qa*); 20.75 (*qa*). – MS. (II): 459 (< 1, M^+), 388 (1), 287 (25), 229 (7), 127 (15), 109 (10), 98 (10), 85 (22), 57 (19), *43* (100), 41 (10).

$C_{21}H_{33}NO_{10}$ (459.49) Calc. C 54.89 H 7.24 N 3.05% Found C 54.92 H 7.26 N 3.01%

t-*Butyl* (S)-*3-isoxazolidinecarboxylate* (**12**). A solution of **6** (22 g, 52.9 mmol) in MeOH (300 ml) containing aq. HCl (30 ml, 36%) was stirred for 6 h at r.t. After neutralization with ice cold 1M Na_2CO_3, the product was extracted with Et_2O (3 × 700 ml). The org. phases were washed with 0.5M Na_2CO_3, dried and evaporated i.v. Chromatography of the residue on silica gel (60 g, D) gave **12** (8.3 g, 90%) recrystallized from hexane, m.p. 52–53°, Rf (D) = 0.27, $[\alpha]_D^{25} = -25.5°$ ($c = 1$, $CHCl_3$). – IR. ($CHCl_3$): 3220*w*, 2960*s*, 2933*s*, 2877*m*, 1727*s*, 1483*m*, 1465*m*, 1400*m*, 1375*s*, 1355*s*, 1155*s*, 1050*m*, 982*w*, 945*w*, 915*w*, 872*m*, 850*m*. – ^{1}H-NMR. (200 MHz, $CDCl_3$): 1.48 (*s*, 9 H); 2.24 ($d \times d \times d \times d$, $J = 13$, 8, 5.6 and 4.5, 1 H, H–C(4)); 2.51 ($d \times d \times d \times d$, $J = 13$, 9.4, 8 and 8, 1 H, H–C(4)); 3.72 ($d \times d \times d$, $J = 8$, 8 and 8, 1 H, H–C(5)); 3.86 ($d \times d$, $J = 9.4$ and 5.6, 1 H, H–C(3)); 3.99 ($d \times d \times d$, $J = 8$, 8 and 4.5, 1 H, H–C(5)); 5.34 (*s*; 1 H, exchangeable with D_2O, NH). – ^{13}C-NMR. ($CDCl_3$): 171.07 (*s*); 82.09 (*s*); 69.36 (*t*); 61.11 (*d*); 34.87 (*t*); 27.92 (*qa*).

For analysis **12** was transformed in the hydrochloride by treatment with HCl (1 equiv.) in Et_2O; m.p. 96–97°, $[\alpha]_D^{25} = -35.2°$ ($c = 0.94$, EtOH).

$C_8H_{16}ClNO_3$ (209.67) Calc. C 45.83 H 7.69 N 6.68% Found C 45.98 H 7.60 N 6.52%

t-*Butyl* (S)-*2-benzyloxycarbonyl-3-isoxazolidinecarboxylate* (**13**). To a solution of **12** (4 g, 23.1 mmol) in $CHCl_3$ (50 ml) were added 1M $NaHCO_3$ (50 ml), 1M Na_2CO_3 (50 ml) and benzyl chloroformate (6 ml, 90%, 38.1 mmol). The mixture was vigorously stirred for 8.5 h at r.t. The product was extracted with EtOAc (3 × 300 ml). The org. phases were washed with 1M HCl (2 × 120 ml), 0.5M Na_2CO_3 (120 ml), dried and evaporated i.v. Chromatography of the residue on silica gel (400 g, B) gave (5.97 g, 84%) recrystallized from Et_2O/petroleum ether. M.p. 48.5–49°, Rf(B) = 0.20, $[\alpha]_D^{25} = -85.3°$ ($c = 1.1$, $CHCl_3$). – IR. (KBr): 3090*w*, 3060*w*, 3038*w*, 3018*w*, 3000*w*, 2973*m*, 2960*w*, 2935*w*, 2880*w*, 1735*s*, 1585*w*, 1500*m*, 1465*w*, 1453*m*, 1445*m*, 1390*s*, 1370*s*, 1365*m*, 1350*m*, 1335*s*, 1328*w*, 1308*s*, 1280*s*, 1260*w*, 1248*w*, 1220*s*, 1205*m*, 1160*s*, 1120*s*, 1095*w*, 1075*w*, 1030*w*, 1015*w*, 1003*w*, 1000*w*, 975*w*, 950*s*, 930*w*, 900*m*, 852*m*, 768*m*, 760*s*, 730*s*, 710*w*, 695*m*, 630*w*, 620*w*, 580*w*. – ^{1}H-NMR. (90 MHz, $CDCl_3$): 1.46 (*s*, 9 H); 2.34 ($d \times d \times d \times d$, $J = 10$, 7.6, 7.6 and 5.3, 1 H, H–C(4)); 2.57 ($d \times d \times d \times d$, $J = 10$, 8.8, 7.6 and 5.3, 1 H, H–C(4)); 3.81 ($d \times d \times d$, $J = 7.6$, 7.6 and 7.6, 1 H, H–C(5)); 4.11 ($d \times d \times d$, $J = 7.6$, 7.6 and 5.3, 1 H, H–C(5)); 4.59 ($d \times d$, $J = 8.8$ and 5.3, 1 H, H–C(3)); 5.20 (*s*, 2 H); 7.33 (*s*, 5 H). – ^{13}C-NMR. ($CDCl_3$): 169.05 (*s*); 156.00 (*s*); 135.42 (*s*); 128.28 (*d*); 128.07 (*d*); 127.89 (*d*); 82.11 (*s*); 68.45 (*t*); 67.86 (*t*); 59.81 (*d*); 33.09 (*t*); 27.78 (*qa*). – MS. (I): 263 (3), 219 (4), 130 (8), 93 (76), 78 (13), 68 (39), 65 (42), 64 (26), 55 (23), 40 (30), 38 (32), 31 (22), *27* (100).

$C_{16}H_{21}NO_5$ (307.35) Calc. C 62.53 H 6.89 N 4.55% Found C 62.44 H 6.92 N 4.51%

(S)-*2-Benzyloxycarbonyl-3-isoxazolidinecarboxylic acid* (**14**). A solution of **13** (8.2 g, 26.7 mmol) in CF_3COOH (40 ml) and 1-chlorobutane (40 ml) was stirred for 2 h under Ar and then evaporated i.v. The resulting residue was dissolved in EtOAc (120 ml), the solution washed with H_2O (50 ml), dried and evaporated i.v. to yield **14** (6.5 g, 97%). Rf (C_6H_6/MeOH/EtOAc/HOAc 22:2:4:1) = 0.19, $[\alpha]_D^{25} = -9.3°$ ($c = 1$, AcOH 95%). – IR. ($CHCl_3$): 3660*w*, 3495*w*, 3035*m*, 3015*m*, 2960*w*, 2890*w*, 1728*s*, 1497*w*, 1452*m*, 1395*m*, 1340*m*, 1303*m*, 1195*w*, 1075*m*, 1030*w*, 960*w*, 906*w*. – ^{1}H-NMR. (90 MHz, $CDCl_3$): 2.53 (*m*, 2 H, H–C(4)); 3.78 ($d \times d \times d$, $J = 7.4$, 7.4 and 7.4, 1 H, H–C(5)); 4.06 ($d \times d \times d$, $J = 7.4$, 7.4 and 5.6, 1 H, H–C(5)); 4.71 ($d \times d$, $J = 8.4$ and 5.2, 1 H, H–C(3)); 5.17 (*s*, 2 H); 7.30 (*m*, 5 H); 10.02 (*s*, 1 H). – ^{13}C-NMR. ($CDCl_3$): 177.41, 176.35 (*s*); 155.35, 154.33 (*s*); 136.23 (*s*); 128.25 (*d*); 127.69 (*d*); 127.45 (*d*); 67.27, 67.07 (*t*); 46.86, 46.53 (*t*); 30.84, 29.56 (*t*).

Methyl (S)-*2-benzyloxycarbonyl-3-isoxazolidinecarboxylate* (**15**). To a solution of **14** (290 mg, 1.15 mmol; crude product obtained by treating **13** with TFA, workup with H_2O, dissolving of the residue in DMF, evaporation and drying i.v.) in Et_2O (60 ml) was added diazomethane. Evaporation of the solvent yielded **15** (308 mg). An analytical sample was prepared by distillation i.v.: b.p. 75–80°/0.005 Torr. Rf(C) = 0.25, $[\alpha]_D^{25} = -97.8°$ ($c = 1$, $CHCl_3$). – IR. ($CHCl_3$): 3030*m*, 3020*m*, 2960*m*, 1730*s*,

1500w, 1455*w*, 1440*m*, 1395*m*, 1310*s*, 1080*s*, 1030*w*, 992*w*, 942*w*, 910*w*, 842*w*, 695*m*. – ^{1}H-NMR. (90 MHz, $CDCl_3$): 2.43 ($d\times d\times d\times d$, J = 13.5, 8.1, 8.1 and 5.7, 1 H, H–C(4)); 2.52 ($d\times d\times d\times d$, J = 13.5, 8.7, 8.1 and 5, 1 H, H–C(4)); 3.69 (*s*, 3 H); 3.76 ($d\times d\times d$, J = 8.1, 8.1 and 8.1, 1 H, H–C(5)); 4.11 ($d\times d\times d$, J = 8.1, 8.1 and 5, 1 H, H–C(5)); 4.75 ($d\times d$, J = 8.7 and 5.7, 1 H, H–C(3)); 5.18 (*s*, 2 H); 7.31 (*s*, 5 H). – ^{13}C-NMR. ($CDCl_3$): 170.47 (*s*); 156.40 (*s*); 135.32 (*s*); 128.32 (*d*); 128.13 (*d*); 127.98 (*d*); 68.75 (*t*); 68.06 (*t*); 59.33 (*d*); 52.53 (*qa*); 32.79 (*t*). – MS. (I): 265 (< 1, M^+), 163 (4), 162 (32), 108 (6), 105 (4), 92 (8), *91* (100).

$C_{13}H_{15}NO_5$ (265.26) Calc. C 58.86 H 5.70 N 5.28% Found C 58.99 H 5.80 N 5.41%

(S)-*2-Benzyloxycarbonyl-3-isoxazolidinecarboxamide* (**16**). To a solution of **14** (7.6 g, 30.1 mmol, crude product; obtained by treating **13** with TFA and usual workup) in THF (90 ml), at –20°, were added *N*-methylmorpholine (5 ml, 45 mmol) and isobutyl chloroformate (4.5 ml, 95%, 34 mmol). A white precipitate formed, and this mixture was stirred for 8 min at –20° and then treated with aq. NH_3 (6.8 ml, 25%, 90 mmol). After dissolution of the precipitate, the solution was again stirred between –10° and +15° for 2 h and then concentrated i.v. The residue was diluted with 120 ml of 0.5M Na_2CO_3 and the product extracted with EtOAc (3×300 ml). The org. phases were washed with 0.5M Na_2CO_3 (2×120 ml), 1M HCl (3×120 ml), 0.5M Na_2CO_3 (120 ml), dried and concentrated i.v. Chromatography of the residue on silica gel (250 g, A) gave **16** (6.6 g, 88%) which was recrystallized in EtOAc/petroleum ether. M.p. 232–234°, Rf(A) = 0.37, $[\alpha]_D^{25} = -126.5°$ (c = 1, $CHCl_3$). – IR. (KBr): 3370*s*, 3180*m*, 3020*w*, 2935*w*, 2875*m*, 1715*s*, 1640*s*, 1500*m*, 1450*m*, 1400*s*, 1350*s*, 1335*s*, 1320*s*, 1250*w*, 1220*m*, 1200*w*, 1148*m*, 1118*m*, 1070*m*, 1030*w*, 992*m*, 980*m*, 950*w*, 905*w*, 897*m*, 815*w*, 770*m*, 732*m*, 700*m*, 690*w*. – ^{1}H-NMR. (90 MHz, $CDCl_3$): 2.59 ($d\times d\times d$, J = 8, 8 and 6, 2 H, 2 H–C(4)); 3.78 ($d\times d\times d$, J = 8, 8 and 8, 1 H, H–C(5)); 4.14 ($d\times d\times d$, J = 8, 6 and 6, 1 H, H–C(5)); 4.74 ($d\times d$, J = 8 and 8, 1 H, H–C(3)); 5.25 (*s*, 2 H); 5.82 (*s*, 1 H); 6.55 (*s*, 1 H); 7.38 (*s*, 5 H). – ^{13}C-NMR. ($CDCl_3$): 172.67 (*s*); 158.09 (*s*); 134.88 (*s*); 128.48 (*d*); 128.14 (*d*); 69.56 (*t*); 68.74 (*t*); 61.60 (*d*); 32.49 (*t*). – MS. (I): 250 (1, M^+), 207 (4), 162 (32), 108 (19), 107 (12), 92 (9), *91* (100), 79 (10).

$C_{12}H_{14}N_2O_4$	Calc.	C 57.60	H 5.64	N 11.19	O 25.57%
(250.26)	Found	„ 57.8	„ 5.7	„ 11.1	„ 25.4%

(S)-*Homoserine* t-*butyl ester* **18**. The 5-oxaproline ester **12** (1.7 g, 9.8 mmol) in EtOH (35 ml) was hydrogenated at normal pressure and for 20 h at r.t. in the presence of Rh/C (5%, 100 mg). The mixture was filtered and concentrated i.v. yielding **18** (1.75 g). Rf ($MeOH/CH_2Cl_2$ 3%) = 0.11. $[\alpha]_D^{25} = +4.9°$ (c = 2, EtOH). – IR. ($CHCl_3$): 3620*w*, 3390*m*, 3310*m*, 3000*m*, 2980*s*, 2995*m*, 2870*w*, 1725*s*, 1592*m*, 1480*m*, 1458*m*, 1435*w*, 1395*m*, 1370*s*, 1300*m*, 1158*s*, 1122*w*, 1070*s*, 987*m*, 965*m*, 875*w*, 847*w*. – ^{1}H-NMR. (90 MHz, $CDCl_3$): 1.45 (*s*, 9 H); 1.75 ($d\times d\times d$, J = 10, 10 and 5, 1 H, H–C(3)); 2.01 ($d\times d\times d$, J = 10, 5 and 5, 1 H, H–C(3)); 2.63 (*s*, 3 H, exchangeable with D_2O; NH_2 and OH); 3.51 ($d\times d$, J = 10 and 5, 1 H, H–C(2)); 3.83 ($d\times d$, J = 5 and 5, 2 H, H–C(4)). – ^{13}C-NMR. ($CDCl_3$): 174.41 (*s*); 81.25 (*s*); 61.41 (*t*); 54.73 (*d*); 35.56 (*t*); 27.98 (*qa*).

(S)-N-*Benzyloxycarbonylhomoserine* t-*butyl ester* (**19**). To a solution of **18** (1.3 g, 7.42 mmol) in $CHCl_3$ (10 ml) were added 1M $NaHCO_3$ (10 ml), 1M Na_2CO_3 (10 ml) and benzyl chloroformate (1.6 ml, 90%, 10 mmol). The mixture was vigorously stirred for 5 h at r.t. The product was extracted with Et_2O (3×100 ml). The org. phases were washed with 1M HCl (2×40 ml), 0.5M Na_2CO_3 (40 ml), dried and concentrated i.v. Chromatography of the residue on silica gel (250 g, E) gave **19** (1.8 g, 79%). An analytical sample was obtained by distillation i.v.: b.p. 88–92°/0.003 Torr. Rf(C) = 0.24, $[\alpha]_D^{25} = -32.5°$ (c = 2, EtOH). – IR. ($CHCl_3$): 3630*w*, 3430*m*, 3100*w*, 3080*w*, 3025*s*, 2995*m*, 2970*m*, 2940*m*, 2900*w*, 1723*s*, 1510*s*, 1482*w*, 1470*w*, 1460*m*, 1435*m*, 1400*m*, 1375*s*, 1350*s*, 1315*s*, 1160*s*, 1090*w*, 1055*s*, 1032*w*, 972*w*, 902*w*, 848*w*. – ^{1}*H*-NMR. (90 MHz, $CDCl_3$): 1.43 (*s*, 9 H); 1.70 ($d\times d\times d\times d$, J = 13.5, 7.5, 4.5 and 4.5, 1 H, H–C(3)); 2.11 ($d\times d\times d\times d$, J = 13.5, 9.5, 6.8 and 4.5, 1 H, H–C(3)); 3.10 (*s*, 1 H, exchangeable with D_2O, OH); 3.64 ($d\times d$, J = 7.5 and 4.5, 1 H, H–C(4)); 3.65 ($d\times d$, J = 6.8 and 4.5, 1 H, H–C(4)); 4.37 ($d\times d\times d$, J = 9.5, 9.5 and 4.5, 1 H, H–C(2)); 5.10 (*s*, 2 H); 5.76 (*d*, J = 9.5, 1 H, NH); 7.32 (*s*, 5 H). – ^{13}C-NMR. ($CDCl_3$): 171.38 (*s*); 156.69 (*s*); 135.95 (*s*); 128.36 (*d*); 128.06 (*d*); 127.97 (*d*); 82.33 (*s*); 67.11 (*t*); 58.32 (*t*); 51.60 (*d*); 37.98 (*t*); 27.92 (*qa*). – MS. (I): 275 (22), 208 (11), 108 (67), 107 (37), 101 (16), 97 (11), *91* (100), 79 (26), 59 (27), 57 (33).

$C_{16}H_{23}NO_5$ (309.37) Calc. C 62.12 H 7.49 N 4.53% Found C 62.22 H 7.40 N 4.49%

α-(t-Butyl) (S)-N-*benzyloxycarbonylaspartate* (**20**). To a stirred solution of **19** (700 mg, 2.26 mmol) in acetone (18 ml) were added, $KMnO_4$ (1.25 g, 7.91 mmol) and acetic acid (9 ml) during 30 min. The mixture was stirred for further 3.5 h at r.t. The major part of the solvents was removed i.v., the residue was poured into ice-cold 1M H_2SO_4 (90 ml) containing $NaHSO_3$ (3 g) and the product was extracted with EtOAc (3×150 ml). The org. phases were extracted with 0.5M Na_2CO_3 (3×150 ml). These extracts were neutralized with ice-cold 2M H_2SO_4 and then reextracted with EtOAc (3×200 ml). The org. phases were dried and concentrated i.v., yielding **20** (614 mg, 84%) as a sirup which was homogeneous on TLC. Rf(A)=0.45, $[\alpha]_D^{25}=-7.6°$ ($c=0.8$, $CHCl_3$). – IR. ($CHCl_3$). – IR. ($CHCl_3$): 3510*w*, 3440*m*, 2985*s*, 2835*m*, 1720*s*, 1505*s*, 1455*m*, 1398*m*, 1340*s*, 1160*s*, 1070*m*, 1050*m*, 1030*m*, 990*w*, 930*w*, 965*w*, 845*w*. – ^{1}H-NMR. (90 MHz, $CDCl_3$): 1.42 (*s*, 9 H); 4.85 (*m*, 2 H, 2 H–C(3)); 4.52 (*m*, 1 H, H–C(2)); 5.10 (*s*, 2 H); 5.77 (*s*, 1 H, exchangeable with D_2O, NH); 7.20 (*s*, 5 H); 9.23 (*s*, 1 H, exchangeable with D_2O, CO_2H). – ^{13}C-NMR. ($CDCl_3$): 175.83 (*s*); 169.20 (*s*); 155.85 (*s*); 135.89 (*s*); 128.82 (*d*); 127.93 (*d*); 127.59 (*d*); 82.71 (*s*); 67.03 (*t*); 50.69 (*d*); 36.69 (*t*); 27.73 (*qa*).

(S)-N-*Benzyloxycarbonylaspargine* t-*butyl ester* (**21**). To a solution of **20** (180 mg, 0.56 mmol) in THF (3 ml), at −20°, were added *N*-methylmorpholine (92 µl, 0.84 mmol) and isobutyl chloroformate (76 µl, 0.58 mmol). The mixture was stirred for 7 min at −20° and then treated with aq. NH_3 (25%, 125 µl). The stirring was continued for 2 h between −15° and +10° after dissolution of the precipitate. Analogous workup as indicated for the preparation of **16**, evaporation of the EtOAc and recrystallization of the residue in EtOAc/petroleum ether gave **21** (162 mg, 90%), m.p. 103.5–104°. ([17]: m.p. 105–106°). $[\alpha]_D^{25}=-14.02°$ ($c=1.99$, EtOH). ([17]: $[\alpha]_D^{25}=-14.9°$ ($c=2$, EtOH)). – IR. (KBr): 3400*s*, 3340*s*, 3205*m*, 2990*m*, 2940*w*, 1745*s*, 1700*s*, 1665*s*, 1540*s*, 1455*w*, 1450*w*, 1425*m*, 1395*s*, 1370*m*, 1360*w*, 1290*s*, 1280*m*, 1230*m*, 1160*s*, 1055*s*, 1030*w*, 1010*w*, 915*w*, 895*w*, 852*w*, 785*w*, 750*w*, 740*w*, 700*m*, 665*w*, 645*w*, 600*w*, 585*w*. – ^{1}H-NMR. ($CDCl_3$): 1.44 (*s*, 9 H); 2.65 (*d*×*d*, $J=16$ and 4.5, 1 H, H–C(3)); 2.88 (*d*×*d*, $J=16$ and 4.5, 1 H, H–C(3)); 4.44 (*d*×*d*×*d*, $J=8.5$, 4.5 and 4.5, 1 H, H–C(2)); 5.11 (*s*, 2 H); 5.76 (*s*, 2 H, exchangeable with D_2O, NH_2); 6.00 (*d*, $J=8.5$, 1 H, NH); 7.33 (*s*, 5 H).

$C_{10}H_{22}N_2O_5$ (322.36) Calc. C 59.62 H 6.88 N 8.69% Found C 59.80 H 6.85 N 8.77%

t-*Butyl (3*R*)- and (3*S*)-2-(2,3-*O*-isopropylidene-β-*D*-ribofuranosyl)-3-isoxazolidinecarboxylate* (**25** and **26**). An autoclave containing the oxime **23** ([19] 300 mg, 1.45 mmol), *t*-butyl glyoxylate (**4**; 365 mg, 90%, 2.53 mmol) and $CHCl_3$ (5 ml) was pressurized with ethylene (65 atm). The mixture was stirred for 17 h at 75–80° (bath temp.) and then cooled to r.t. After evaporation of the solvent, the residue was chromatographed on silica gel (30 g, C) yielding **25** (313 mg, 62%, crystallized spontaneously) and **26** (128 mg, 25.3%).

Data of **25**. Rf (D)=0.28; m.p. 108–109° (recrystallized in Et_2O/hexane) and 84.5–85° (sublimed at 90°/0.0001 Torr and recrystallized in Et_2O/hexane). $[\alpha]_D^{25}=+20.3°$ ($c=1$, $CHCl_3$). – IR. (KBr): 3337*s*, 2985*m*, 2950*w*, 2945*w*, 2920*m*, 2905*w*, 1735*s*, 1460*w*, 1397*w*, 1385*w*, 1377*m*, 1368*w*, 1358*m*, 1292*m*, 1275*w*, 1252*m*, 1240*w*, 1225*w*, 1215*m*, 1207*m*, 1163*s*, 1112*s*, 1105*s*, 1095*m*, 1065*w*, 1055*s*, 1015*w*, 990*w*, 985*w*, 970*w*, 938*w*, 915*w*, 890*w*, 870*w*, 845*w*, 808*w*, 778*w*, 685*w*, 655*w*, 645*w*, 570*w*, 530*w*. – ^{1}H-NMR. (90 MHz, $CDCl_3$): 1.33 (*s*, 3 H); 1.50 (*s*, 12 H); 2.30–2.80 (*m*, 2 H, 2 H–C(4)); 3.58 (*d*×*d*×*d*, $J=13$, 10 and 2.4, 1 H, tranformed with D_2O to *d*×*d*, $J=13$ and 2.4, H–C(5′)); 3.70 (*d*×*d*×*d*, $J=13$, 4 and 2, 1 H, transformed with D_2O to *d*×*d*, $J=13$ and 2, H–C(5′)); 3.90–4.20 (*m*, 3 H, H–C(3) and 2 H–C(5)); 4.33 (*s*, 1 H, H–C(1′)); 4.43 (*d*×*d*, $J=2.4$ and 2, 1 H, H–C(4′)); 4.64 (*d*×*d*, $J=10$ and 4, 1 H, exchangeable with D_2O, OH); 4.93 (*d*, $J=6$, 1 H); 4.96 (*d*, $J=6$, 1 H). – ^{13}C-NMR. (Cl_2CD–$CDCl_2$, 81°): 170.07 (*s*); 111.93 (*s*); 99.64 (*d*); 89.22 (*d*); 84.65 (*d*); 82.36 (*d*+*s*); 66.91 (*t*); 64.52 (*d*); 63.58 (*t*); 31.06 (*t*); 27.87 (*qa*); 26.64 (*qa*); 25.00 (*qa*). – MS. (I): 345 (<1, M^+), 258 (6), 244 (6), 214 (4), 182 (5), 173 (45), 157 (6), 146 (6), *117* (100), 115 (15), 73 (8), 72 (24), 71 (15), 59 (19).

$C_{16}H_{27}NO_7$ (345.40) Calc. C 55.64 H 7.88 N 4.05% Found C 55.70 H 7.90 N 4.04%

Data of **26**. M.p. 69–70° (Et_2O/hexane). Rf(C)=0.25. $[\alpha]_D^{25}=-100.9°$ ($c=1$, $CHCl_3$). – IR. (KBr): 3160*s*, 2980*m*, 2960*m*, 2940*m*, 2880*m*, 1740*s*, 1480*w*, 1460*w*, 1450*w*, 1382*m*, 1370*m*, 1343*w*, 1290*w*, 1245*m*, 1220*s*, 1160*s*, 1125*w*, 1088*m*, 1072*s*, 1050*s*, 1015*m*, 995*w*, 952*w*, 927*w*, 905*w*, 870*m*, 850*m*, 817*w*, 765*m*, 703*w*. – ^{1}H-NMR. (200 MHz, $CDCl_3$): 1.33 (*s*, 3 H); 1.47 (*s*, 9 H); 1.50 (*s*, 3 H); 2.41 (*d*×*d*×*d*×*d*, $J=11.5$, 9, 7 and 7, 1 H, H–C(4)); 2.53 (*d*×*d*×*d*×*d*, $J=11.5$, 7, 7 and 6, 1 H, H–C(4)); 3.59 (*d*×*d*, $J=14$ and 3.5, 1 H, H–C(5′)); 3.81 (*d*×*d*, $J=14$ and 2, 1 H, H–C(5′)); 3.84 (*s*, 1 H, exchangeable with D_2O, OH); 3.85 (*d*×*d*, $J=9$ and 6, 1 H, H–C(3)); 3.96 (*d*×*d*, $J=7$ and 7, 2 H, 2 H–C(5)); 4.36 (*d*×*d*×*d*, $J=3.5$, 2 and 2, 1 H, H–C(4′)); 4.69 (*s*, 1 H, H–C(1′)); 4.70 (*d*×*d*, $J=6$ and 2, 1 H, H–C(3′));

4.81 (*d*, J = 6, 1 H, H–C(2')). – ^{13}C-NMR. ($CDCl_3$): 169.16 (*s*); 112.51 (*s*); 98.81 (*d*); 87.70 (*d*); 83.66 (*d*); 82.36 (*s*); 81.91 (*d*); 66.33 (*t*); 63.91 (*t*); 62.15 (*d*); 32.50 (*t*); 27.86 (*qa*); 26.73 (*qa*); 24.95 (*qa*). – MS. (I): 345 (2, M^+), 330 (2), 315 (6), 314 (10), 258 (17), 244 (11), 173 (61), 158 (14), *117* (100), 115 (16), 73 (13), 59 (38), 57 (25).

$C_{16}H_{27}NO_7$ (345.40) Calc. C 55.64 H 7.88 N 4.05% Found C 55.47 H 7.84 N 3.90%

L-N-*(*N-t-*Butoxycarbonyl*-N-*methyl*-L-*phenylalanyl)-3-isoxazolidinecarboxamide* (**28**). A solution of **16** (2.58 g, 10.3 mmol) in a 33% solution of HBr in HOAc (70 ml) was stirred for 2 h at r.t. After evaporation of the solvents, the crude residue (**17**) was dried i.v. To a solution of Boc-MePhe-OH **27** ([21]; 2.87 g, 10.3 mmol, dissolved in DMF, evaporated and dried i.v.) in THF (70 ml), at – 15°, were added *N*-methylmorpholine (4.55 ml, 40.8 mmol) and isobutyl chloroformate (1.6 ml, 90%, 11.5 mmol). A white precipitate formed. The mixture was stirred for 8 min at – 15° and then treated with a solution of the crude H-Opro-NH_2 · HBr **17** in DMF (5 ml). When the precipitate had dissolved, the solution was again stirred for 1.5 h between 0° and + 10° and concentrated i.v. Workup as indicated for the preparation of **16**, evaporation of the EtOAc and chromatography of the residue on silica gel (250 g, A) gave **28** (3.46 g, 90%) recrystallized in EtOAc/petroleum ether, m.p. > 250°, Rf(A) = 0.32, $[\alpha]_D^{25} = -159.6°$ (c = 1, $CHCl_3$). – IR. (KBr): 3430*s*, 3340*m*, 3220*m*, 3030*w*, 2980*m*, 2940*m*, 2890*w*, 1710*s*, 1670*s*, 1640*s*, 1632*s*, 1480*m*, 1460*s*, 1390*s*, 1372*s*, 1345*m*, 1331*m*, 1320*m*, 1300*w*, 1260*m*, 1220*w*, 1180*s*, 1160*s*, 1130*w*, 1035*m*, 1026*w*, 987*m*, 962*w*, 950*m*, 905*w*, 868*w*, 835*w*, 780*w*, 755*w*, 740*w*, 702*w*. – ^{1}H-NMR. (90 MHz, $CDCl_3$): 1.37 (*s*, 9 H); 2.30–3.20 (*m*, 4 H); 2.84 (*s*, 3 H); 3.65–4.60 (*m*, 2 H); 4.79 (*d* × *d*, J = 9 and 6, 1 H); 5.40 (*m*, 1 H); 5.80 (*s*, 1 H, amide); 6.50 (*s*, 1 H, amide); 7.23 (*s*, 5 H). – ^{13}C-NMR. (Cl_2CD–$CDCl_2$, 82.5°): 171.91 (*s*); 170.92 (*s*); 154.97 (*s*); 137.19 (*s*); 128.94 (*d*); 128.18 (*d*); 126.40 (*d*); 79.86 (*s*); 69.94 (*t*); 58.23 (*d*); 56.99 (*d*); 35.12 (*t*); 30.93 (*t* + *qa*); 28.18 (*qa*). – MS. (III): 378 (< 1), 347 (< 1). 322 (4), 321 (18). 304 (27), 286 (9), 277 (22), 262 (32), 234 (46), 230 (15), 207 (38), 206 (97), 187 (14), 186 (98), 179 (67), 178 (95), 172 (42), 160 (57), 139 (72), *134* (100), 133 (25), 132 (29), 131 (37), 128 (66), 117 (68), 116 (75), 92 (62), 91 (94), 77 (27), 72 (95), 71 (18), 65 (39), 57 (94).

N-*(*N-*Benzyloxycarbonyl*-L-*3-isoxazolidinecarbonyl)*-L-*tyrosine methyl ester* (**30**). To a solution of **14** (186 mg, 0.74 mmol, dissolved in DMF, evaporated and dried i.v.) in THF (3 ml), at 20° were added *N*-methylmorpholine (0.1 ml, 0.89 mmol) and isobutyl chloroformate (0.11 ml, 0.89 mmol). A white precipitate formed. The mixture was stirred for 6 min at – 20° and then treated with a solution of H-Tyr-OMe ([23]; 145 mg, 0.74 mmol) in THF (3 ml). When the precipitate had dissolved, the solution was stirred for 2.5 h between – 15° and + 20° and concentrated i.v. Workup as indicated for the preparation of **16**, evaporation of the EtOAc and chromatography of the residue on silica gel (40 g, CH_2Cl_2/MeOH 13:1) gave **30** (278 mg, 87%) as a white foam. Rf(A) = 0.41, $[\alpha]_D^{25} = -19.8°$ (c = 2, EtOH). – IR. ($CHCl_3$): 3600*w*, 3420*m*, 3340*m*, 3100*w*, 3070*w*, 3030*w*, 3010*m*, 2990*w*, 2960*m*, 2900*w*, 2540*w*, 2480*w*, 1955*w*, 1885*w*, 1745*s*, 1680*s*, 1617*m*, 1600*w*, 1515*s*, 1500*s*, 1500*w*, 1435*w*, 1360*s*, 1120*s*, 1090*m*, 1032*w*, 990*w*, 922*w*, 880*w*, 845*w*, 830*w*, 700*w*. – ^{1}H-NMR. (90 MHz, $CDCl_3$): 1.90–2.10 (*m*, 2 H, 2 H–C(4-Opro)); 2.90 (*d* × *d*, J = 14 and 6, 1 H, H–C(3-Tyr)); 3.00 (*d* × *d*, J = 14 and 5.5, 1 H, H–C(3-Tyr)); 3.41 (*d* × *d*, J = 6.3 and 6.3, 2 H, 2 H–C(5-Opro)); 3.67 (*s*, 3 H); 4.31 (*d* × *d*, J = 6 and 5.5, 1 H, H–C(2-Tyr)); 4.79 (*d* × *d*, J = 12 and 6, 1 H, H–C(3-Opro)); 5.11 (*s*, 2 H); 6.68 (*d*, J = 10, 2 H); 6.70 (*m*, 1 H); 6.86 (*d*, J = 10, 2 H); 7.50 (*m*, 1 H); 7.31 (*s*, 5 H). – ^{13}C-NMR. ($CDCl_3$): 171.62 (2*s*); 155.44 (*s*); 135.99 (*s*); 130.03 (*d*); 128.33 (*d*); 127.94 (*d*); 127.74 (*d*); 126.62 (*s*); 115.03 (*d*); 67.41 (*t*); 60.38 (*d*); 53.17 (*d*); 52.28 (*qa*); 47.04 (*t*); 37.05 (*t*); 29.63 (*t*). – MS. (IV): 428 (< 1, M^+), 321 (7), 292 (11), 276 (5), 250 (8), 249 (51), 236 (4), 222 (8), 205 (20), 194 (5), 180 (6), 179 (13), 178 (86), 162 (9), 161 (6), 160 (42), 174 (14), 108 (20), 107 (76), 92 (16), *91* (100), 88 (16), 85 (17), 83 (27), 77 (17), 70 (28), 65 (12), 57 (23).

$C_{22}H_{24}N_2O_7$ (428.44) Calc. C 61.67 H 5.65 N 6.54% Found C 61.95 H 5.93 N 6.40%

t-*Butyl (*3S*)-2-(2-methyl-2-propenoyl)-3-isoxazolidinecarboxylate* (**31**). To a stirred solution of **12** (300 mg, 1.73 mmol) in pyridine (4 ml), kept between – 15° and – 10°, was added over 20 min methacryloylchloride (0.2 ml, 2.03 mmol). Stirring was continued for 2 h between – 10° and – 5°. The residue obtained by evaporating the pyridine i.v. was diluted with 1M HCl (20 ml) and the product extracted with EtOAc (3 × 50 ml). The org. phases were washed with 1M HCl (2 × 20 ml), 0.5M Na_2CO_3 (12 ml), dried and concentrated i.v. Chromatography on silica gel (30 g, E) afforded **31** (387 mg, 93%). An analytical sample was distilled i.v.: b.p. 58–60°/0.01 Torr, Rf(D) = 0.38, $[\alpha]_D^{25} = -116.9°$ (c = 1,

$CHCl_3$). – IR. ($CHCl_3$): 3015*s*, 2995*s*, 2950*m*, 2900*m*, 1745*s*, 1660*s*, 1630*s*, 1460*s*, 1402*s*, 1377*s*, 1340*m*, 1300*m*, 1163*s*, 1042*m*, 1020*m*, 995*m*, 895*w*, 835*m*. – ^{1}H-NMR. (90 MHz, $CDCl_3$): 1.50 (*s*, 9 H); 1.99 (*s*, 3 H); 2.15–2.85 (*m*, 2 H, 2 H–C(4)); 3.88 (*d*×*d*×*d*, J=7.6, 7.6 and 7.6, 1 H, H–C(5)); 4.17 (*d*×*d*×*d*, J=7.6, 7.6 and 5.5, 1 H, H–C(5)); 5.75 (*d*×*d*, J=9.1 and 4.8, 1 H, H–C(3)); 5.40 (*m*, 1 H); 5.61 (*m*, 1 H). – ^{13}C-NMR. ($CDCl_3$): 169.31 (*s*); 168.92 (*s*); 138.46 (*s*); 120.89 (*t*); 82.21 (*s*); 68.92 (*t*); 58.20 (*d*); 32.61 (*t*); 27.84 (*qa*); 19.51 (*qa*). – MS. (I): 241 (1, M^+), 208 (5), 69 (20), 59 (28), 57 (13), 43 (15), 41 (23), 39 (8), 32 (23), *28* (100).

$C_{12}H_{19}NO_4$ (241.29) Calc. C 59.73 H 7.94 N 5.81% Found C 59.68 H 8.00 N 5.80%

t-*Butyl (3*S,*2'*S*)- and (3*S,*2'*R*)-2-(3-acetylthio-2-methylpropanoyl)-3-isoxazolidinecarboxylate* (**32** and **33**). To a stirred solution of **12** (10.3 g, 59.4 mmol) in pyridine (80 ml), kept between −10° and −5°, was added, over 50 min, methacryloyl chloride (6.5 ml, 67.1 mmol). The resulting solution was stirred for 2 h at −8° and worked up as indicated above. The solution of the crude residue of the extraction in thioacetic acid (22 ml) was stirred at 0° for 15 h and then poured in ice-cold 1M Na_2CO_3 (260 ml). The product was extracted with EtOAc (3×500 ml). The org. phases were washed with 0.5M Na_2CO_3 (2×300 ml), stirred and concentrated i.v. Chromatography of the red residue on silica gel (470 g, D) gave **32** (8.34 g, 44%) as white solid recrystallized from Et_2O/petroleum ether and **33** (7.56 g, 40%) as colourless sirup.

Data of **32**. M.p. 68–69°, Rf(D)=0.36, $[\alpha]_D^{25}=-154.1°$ (c=1, $CHCl_3$). – IR. ($CHCl_3$): 2980*s*, 2930*m*, 2880*w*, 1740*s*, 1690*s*, 1650*s*, 1460*s*, 1400*m*, 1370*s*, 1360*w*, 1300*m*, 1160*s*, 1025*w*, 1020*w*, 990*m*, 960*m*, 850*m*, 630*m*. – ^{1}H-NMR. (90 MHz, $CDCl_3$): 1.25 (*m*, 3 H); 1.45 (*s*, 9 H); 2.28 (*s*, 3 H); 2.30–2.85 (*m*, 2 H, 2 H–C(4)); 3.00–3.20 (*m*, 3 H); 2.92 (*d*×*d*×*d*, J=7.5, 7.5 and 7.5, 1 H, H–C(5)); 4.17 (*d*×*d*×*d*, J=7.5, 7.5 and 6, 1 H, H–C(5)); 4.67 (*d*×*d*, J=9.3 and 5.3, 1 H, H–C(3)). – ^{13}C-NMR. ($CDCl_3$): 195.34 (*s*); 172.33 (*s*); 168.55 (*s*); 82.12 (*s*); 68.89 (*t*); 56.77 (*d*); 37.18 (*d*); 32.74 (*t*); 31.08 (*t*); 30.46 (*qa*); 27.84 (*qa*); 16.96 (*qa*). – MS. (III): 317 (7, M^+), 289 (3), 261 (3), 243 (5), 228 (6), 186 (14), 173 (20), 162 (10), 147 (16), 145 (80), 137 (27), 118 (18), *117* (100), 114 (14), 109 (15), 103 (44), 75 (22), 72 (68), 69 (70), 57 (81).

$C_{14}H_{23}NO_5S$ (317.40) Calc. C 52.98 H 7.30 N 4.41% Found C 52.70 H 7.36 N 4.38%

Data of **33**. Rf(D)=0.29, $[\alpha]_D^{25}=-34.7°$ (c=1, $CHCl_3$). – IR. ($CHCl_3$): 2980*s*, 2930*m*, 2880*w*, 1740*s*, 1690*s*, 1650*s*, 1460*s*, 1400*m*, 1380*w*, 1375*s*, 1300*w*, 1160*s*, 1035*w*, 1020*w*, 990*m*, 965*m*, 855*m*, 630*m*. – ^{1}H-NMR. (90 MHz, $CDCl_3$): 1.18 (*m*, 3 H); 1.48 (*s*, 9 H); 2.31 (*s*, 3 H); 2.30–2.85 (*m*, 2 H, 2 H–C(4)); 2.90–3.30 (*m*, 3 H); 3.93 (*d*×*d*×*d*, J=7.5, 7.5 and 7.5, 1 H, H–C(5)); 4.20 (*d*×*d*×*d*, J=7.5, 7.5 and 6, 1 H, H–C(5)); 4.62 (*d*×*d*, J=9.3 and 3.5, 1 H, H–C(3)). – ^{13}C-NMR. ($CDCl_3$): 195.24 (*s*); 171.64 (*s*); 168.41 (*s*); 82.14 (*s*); 58.75 (*t*); 57.04 (*d*); 37.10 (*d*); 32.77 (*t*); 31.52 (*t*); 30.45 (*qa*); 27.79 (*qa*); 16.07 (*qa*). – MS. (III): 317 (16, M^+), 261 (4), 243 (8), 241 (11), 218 (13), 215 (10), 186 (22), 173 (32), 147 (15), 146 (22), 145 (84), 128 (10), 118 (20), *117* (100), 114 (20), 103 (53), 75 (33), 72 (69), 69 (61), 57 (63), 43 (71).

$C_{14}H_{23}NO_5S$ (317.40) Calc. C 52.98 H 7.30 N 4.41% Found C 52.92 H 7.28 N 4.39%

*(3*S,*2'*S*)-2-(3-Acetylthio-2-methylpropanoyl)-3-isoxazolidinecarboxylic acid* (**34**). A solution of **32** (1 g, 3.15 mmol) in CF_3COOH (5 ml) and 1-chlorobutane (5 ml) was stirred for 2 h at r.t. and then concentrated i.v. The residue was dissolved in a mixture of dioxane/H_2O 4:1 and this solution was put on a column of weakly basic resin (*BioRad AG3*×*4*, 20 ml). The column was eluted with the same mixture of solvents (120 ml) yielding **34** (700 mg, 85%). $[\alpha]_D^{20}=-169°$ (c=0.7, DMF). – ^{1}H-NMR. (90 MHz, (D_6)DMSO): 1.00–1.20 (*m*, 3 H); 2.26 (*s*, 3 H); 2.35–3.05 (*m*, 5 H); 3.87 (*d*×*d*×*d*, J=7.5, 7.5 and 7.5, 1 H, H–C(5)); 4.20 (*d*×*d*×*d*, J=7.5, 7.5 and 5, 1 H, H–C(5)); 4.64 (*d*×*d*, J=8 and 5, 1 H, H–C(3)). – MS. (Field desorption): 261 (M^+).

*(3*S,*2'*R*)-2-(3-Acetylthio-2-methylpropanoyl)-3-isoxazolidinecarboxylic acid* (**35**). In a similar way as described for the preparation of **34**, **33** (880 mg, 2.77 mmol) gave **35** (500 mg, 69%). $[\alpha]_D^{20}=-54°$ (c=0.5, DMF). – ^{1}H-NMR. (90 MHz, (D_6)DMSO): 0.95–1.25 (*m*, 3 H); 2.35 (*s*, 3 H); 2.35–3.10 (*m*, 5 H); 3.86 (*d*×*d*×*d*, J=7.8, 7.8 and 7.8, 1 H, H–C(5)); 4.21 (*d*×*d*×*d*, J=7.8, 7.8 and 4.5, 1 H, H–C(5)); 4.64 (*d*×*d*, J=9.3 and 5.3, 1 H, H–C(3)). – MS. (Field desorption): 261 (M^+).

*(3*S,*2'*S*)-2-(3-Mercapto-2-methylpropanoyl)-3-isoxazolidinecarboxylic acid* (**1**). A solution of **34** (420 mg, 1.6 mmol) in MeOH (2.5 ml) and conc. aq. NH_3 (2.5 ml) was stirred for 2 h at r.t. under Ar. N_2 was bubbled through the mixture for 2 h. The solution was then adjusted to pH 4 with 4M *HOAc*,

dioxane (8 ml) was added, and the solvents were evaporated i.v. The residue was triturated with Et_2O to afford **1** (330 mg, 94%) as viscous solid. $[\alpha]_D^{20} = -87°$ ($c = 0.8$, DMF). – ^{1}H-NMR. (90 MHz, (D_6)DMSO): 0.95–1.25 (*m*, 3 H); 2.15–2.85 (*m*, 5 H); 3.79 (*d*×*d*×*d*, $J = 7$, 7 and 7, 1 H, H–C(5)); 4.08 (*d*×*d*×*d*, $J = 7$, 7 and 5, 1 H, H–C(5)); 4.42 (*d*×*d*, $J = 8.5$ and 5.7, 1 H, H–C(3)). – MS. (Field ionisation after treatment with diazomethane): 247 (17, S–CH_3 and CO_2CH_3), *233* (100, SH and CO_2CH_3).

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