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A NOVEL HETEROSPIROCYCLIC 2H-AZIRIN-3-AMINE AS SYNTON FOR 3-AMINOTHIOLANE-3-CARBOXYLIC ACID

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Dedicated to Professor Ekkehard Winterfeldt on the occasion of his 75th birthday

Abstract – The heterospirocyclic 2H-azirin-3-amine (9), i.e., N-methyl-N-phenyl-5-thia-1-azaspiro[2.4]hept-1-en-2-amine, was prepared from the commercially available thiolane-3-one (10) via the corresponding 3-carbonitrile (11) and 3-thiocarboxamide (14). This azirine reacted with thiobenzoic acid to give 3-benzoylamino-N-methyl-N-phenylthiolane-3-thiocarboxamide (15). With N-protected valine, the protected dipeptides (16) and (17), which contain a heterocyclic amino acid, were obtained as mixtures of diastereoisomers.

INTRODUCTION

In recent years, we have shown that 2H-azirin-3-amines are useful synths for 2,2-disubstituted glycines, i.e., α,α-disubstituted α-amino acids in the synthesis of conformationally restricted peptides, cyclopeptides, and cyclodepsipeptides. A special group of α,α-disubstituted α-amino acids are carbo- and heterocyclic ones. Since the first isolation of the natural (−)-(S)-3-aminopyrrolidine-3-carboxylic acid (cucurbitin, (S)-1), it is of increasing interest because of its antihistaminic and antiallergic activities (for enantioselective syntheses, see 19,20). The analogous 3-aminotetrahydrofuran-21 and 3-aminothiolane-3-carboxylic acid (3)22 are also biologically active compounds (e.g., as enzyme inhibitors).

As carbocyclic 1-amino carboxylic acids (e.g., 1-aminocyclopentane carboxylic acid) in peptides behave similar to α-aminoisobutyric acid (Aib), it is of interest to prepare peptides, which contain heterocyclic amino acids of type (1–3), and to study the influence of these amino acids on the peptide conformations. Because the ‘azirine/oxazolone method’ proved to be a very convenient and efficient method for the coupling of sterically demanding α,α-disubstituted α-amino acids, we prepared the 2H-azirines (4–6) as
synthons for heterocyclic α-amino acids and the 1-(2H-azirin-3-yl)prolinates (7, 8) as dipeptide synthons.4,25–28 All these azirines react smoothly with amino acids or peptide acids to give the corresponding dipeptides or extended peptide chains.

In the present paper, we report the synthesis of racemic N-methyl-N-phenyl-5-thia-1-azaspiro[2.4]hept-1-en-2-amine (9) as a synthon for 3-aminothiolane-3-carboxylic acid (3, Atc), a homocysteine analogue.

RESULTS AND DISCUSSION
The commercially available thiolane-3-one (10) was chosen as the starting material for the synthesis of 9. We intended to transform 10 into the carbonitrile (11) by using tosylmethyl isocyanide (Tosmic) as the reagent according to ref.29 This method has previously been used in the synthesis of 5 (X = S).25 Unfortunately, the yield in the case of 10 → 11 was unsatisfactory (< 20%) and, therefore, the three-step procedure via reduction to thiolane-3-ol, tosylation, and substitution with sodium cyanide in DMSO was used, leading to 11 in 45% yield (Scheme 1).30 The basic hydrolysis of 11 gave thiolane-3-carboxylic acid (12) in 98% yield, and the amide (13) was obtained in 83% yield via DCC coupling with N-methylaniline in the presence of 4-(dimethylamino)pyridine (DMAP). As it was known that amides of type (13) often react only sluggishly with phosgene,2 13 was transformed into the corresponding thioamide (14, 96% yield) by treatment with Lawesson reagent in boiling toluene (cf. ref.26). Then, the desired azirine (9) was prepared
according to a modified protocol\textsuperscript{32} of the azirine synthesis of Rens and Ghosez.\textsuperscript{33} Subsequent treatment of a solution of 14 in dichloromethane with phosgene in toluene and catalytic amounts of DMF, change of the solvent to THF and addition of 1,4-diazabicyclo[2.2.2]octane (DABCO), and reaction of the intermediate $\alpha$-chloroenamine in a mixture of DMF and THF with sodium azide yielded 9 (Scheme 1). After chromatographic workup, the azirine (9) was obtained in 70\% yield, contaminated with small amounts (\leq 5\%) of the amide (13).\textsuperscript{34} As this amide does not interfere with the forthcoming reactions, we avoided the tedious and loss-making separation of 9 and 13.

\begin{center}
\textbf{Scheme 1}
\end{center}

\begin{picture}(200,200)
\put(50,100){\includegraphics[width=10cm]{scheme1.png}}
\end{picture}

\textit{Reagents and conditions:} a) NaBH\textsubscript{4}, NaOAc, MeOH, H\textsubscript{2}O, 0°C; b) DMAP, 4-toluenesulfonyl chloride, CH\textsubscript{2}Cl\textsubscript{2}; c) NaCN, DMSO, reflux; d) NaOH, EtOH/H\textsubscript{2}O 1:2; e) N-methylaniline, DCC, DMAP, CH\textsubscript{2}Cl\textsubscript{2}, rt; f) Lawesson reagent, toluene, reflux; g) COCl\textsubscript{2}, toluene, CH\textsubscript{2}Cl\textsubscript{2}, DMF, 0°C; h) DABCO, THF, rt; i) NaN\textsubscript{3}, DMF/THF 2:1, rt.

In the IR spectrum (neat), the new azirine (9) shows a characteristic C=N absorption at 1760 cm\textsuperscript{-1}, and indicative signals for C(2) and C(3) appear in the $^{13}$C-NMR spectrum at 161.1 and 44.7 ppm, respectively. The Cl-MS of 9 shows the base peak at $m/z$ 219 ([M$+1$]$^+$).

A characteristic reaction of 2\textit{H}-azirin-3-amins is that with thiobenzoic acid.\textsuperscript{25,26} In the present case, addition of 1.2 equiv. of thiobenzoic acid to a solution of 9 in dichloromethane at 0°C and stirring of the mixture at room temperature for 18 h, followed by chromatographic workup, gave the racemic
monothiodiamide (15) in 90% yield (Scheme 2). This reaction is a chemical proof for the azirine structure, i.e., it shows that 9 is a synthon for 3-aminothiolane-3-carboxylic acid (3, Atc).

Scheme 2

To examine the use of this new 2H-azirin-3-amine as a building block in peptide synthesis, reactions of 9 were carried out with Z-Val-OH and Fmoc-Val-OH. For this purpose, a mixture of 9 and 1.1 equiv. of the N-protected valine in dichloromethane was stirred at room temperature for 21 h. Chromatographic separation of the mixture obtained with Z-Val-OH (SiO₂, ethyl acetate/hexane) gave two diastereoisomeric dipeptides Z-Val-Atc-N(Me)Ph (16a and 16b, Scheme 3) in 42 and 41% yield and a mixed fraction of the diastereoisomers (13%). The faster moving diastereoisomer (16a) with an R_f-value of 0.29 showed a mp of 175–177°C, whereas the mp of the second isomer (16b) with R_f = 0.23 was 76–79°C.35

Scheme 3

A similar result was obtained in the reaction of 9 with Fmoc-Val-OH: the two diastereoisomers 17a and 17b were isolated in 45 and 22% yield, respectively, along with a mixed fraction in 32% yield. Again, the faster
moving isomer (17a) showed the higher mp (194–196°C) in comparison with a broad ‘melting interval’ 100–110°C for 17b.

With the aim of demonstrating the usefulness of the reaction described above for peptide synthesis, the dipeptide 17a was selectively deprotected at the N- as well as the C-terminus. Heating of 17a in a 1:1 mixture of aqueous 6N HCl and acetonitrile under reflux for 3 h gave the corresponding acid 18a in 89% yield (Scheme 4). On the other hand, the amino group of 17a was deprotected by treatment with diethylamine, leading to 19a in 78% yield.

Scheme 4

CONCLUSIONS
We have shown that the novel 2H-azirin-3-amine (9) can be prepared in a multi-step synthesis via thioamide 14. The crucial formation of the three-membered ring was achieved via a modified protocol of the Rens and Ghosez procedure by successive treatment of 14 with phosgene, DABCO, and sodium azide. The model reaction of 9 with a N-protected valine derivative, e.g., Fmoc-Val-OH, successful separation the diastereoisomeric protected dipeptides, and the selective deprotection of the N- as well as the C-terminus demonstrate convincingly that the heterospirocyclic azirine (9) is a useful building block for peptide synthesis.

EXPERIMENTAL

General remarks. TLC: silica gel 60 F_{254} plates (0.25 mm, Merck). Prep. TLC: silica gel 60 F_{254} plates (2 mm, Merck). Column chromatography (CC): silica gel 60 (0.043–0.063 mm, Merck). Melting points: Büchi B-510 apparatus, uncorrected. IR spectra: Perkin-Elmer-Spektrum ONE FT-IR, in KBr or as film; in cm⁻¹. ¹H-NMR (300 MHz) and ¹³C-NMR (75 MHz) spectra: Bruker AVX-300 and ARX-300 instrument, in CDCl₃; chemical shifts in ppm, coupling constants J in Hz. CI-MS: Finnigan MAT-95 instrument; NH₃ as carrier gas. ESI-MS: Finnigan TSQ-700 (API).
Synthesis of N-Methyl-N-phenyl-5-thia-1-azaspiro[2.4]hept-1-en-2-amine (9). 3-Hydroxythiolane. To a solution of 3-oxothiolane (10) (11.6 mL, 14.0 g, 0.137 mol) in methanol (140 mL) in an ice bath, NaBH₄ (11.1 g, 0.293 mol) and AcONa (23.5 g, 0.286 mol) in water (85 mL) were slowly added (70 min). After stirring for another 45 min at 0°C, the mixture was neutralized with conc. H₂SO₄ (8 mL) and then extracted with Et₂O (5 × 1). The combined organic phases were dried with MgSO₄, filtered, the solvent was evaporated, and the white precipitate was filtered. Addition of CH₂Cl₂ led to the formation of additional precipitate. Filtration and bulb-to-bulb distillation (90°C, 8 mbar) gave 12.86 g (90%) of 3-hydroxythiolane as colorless oil. Rf (SiO₂, AcOEt/hexane 1:1) = 0.34 (UV254 and Ce(SO₄)₂). IR (neat): 3375v, 2936v, 1709m, 1427s, 1332s, 1263s, 1196s, 1140m, 1064s, 1026s, 954vs, 886w, 831m, 687s, 645s. 1H-NMR (CDCl₃): 4.64–4.60 (m, H-C(3)); 3.03–2.80 (m, 4H, CH₂(2), CH₂(5)); 2.20–2.10 (m, 1H of CH₂(4)); 1.93–1.81 (m, 1H of CH₂(4)). 13C-NMR (CDCl₃): 74.4 (d, C(3)); 39.6, 37.9 (2t, C(2), C(5)); 28.1 (t, C(4)). CI-MS: 122 (100, [M+NH₄]⁺), 105 (13, [M+H]⁺), 104 (5, [M⁺]), 86 (25, [M–H₂O]+).

3-(4-Toluenesulfonyloxy)thiolane. To a solution of 3-hydroxythiolane (8.89 g, 85.34 mmol) in abs. CH₂Cl₂ (150 mL) at 0°C were added 4-(dimethylamino)pyridine (DMAP) (20.85 g, 170.6 mmol) and p-toluenesulfonyl chloride (16.27 g, 85.32 mmol), the mixture was stirred for 20 h at rt, and washed with 1N HCl (30 mL). The aqueous phase was extracted with CH₂Cl₂ (40 mL, 3 ×). The combined organic phase was washed with sat. aqueous NaCl solution, dried with MgSO₄, and the solvent evaporated to give 26 g of crude product. Purification by column chromatography (SiO₂, hexane/AcOEt 10:1 → 1:1, 2 ×) yielded 19.87 g (90 %) of 3-(4-toluenesulfonyloxy)thiolane as colorless crystals; mp 58–60°C. Rf (SiO₂, hexane/AcOEt 5:1) = 0.19 (UV₂₅₄ and Ce(SO₄)₂). IR (KBr): 3052v, 2965w, 1913w, 1642w, 1595m, 1492w, 1431m, 1361vs, 1337vs, 1316s, 1304m, 1292m, 1237m, 1197s, 1172vs, 1092s, 1048w, 1018w, 1007m, 971m, 926vs, 891vs, 857s, 833m, 808s, 871vs, 667vs, 629vs. ¹H-NMR (CDCl₃): 7.82–7.78 (m, 2 arom. H); 7.37–7.34 (m, 2 arom. H); 5.23–5.18 (m, H-C(3)); 3.03–2.81 (m, 4H, CH₂(2), CH₂(5)); 2.45 (s, 3H, Me); 2.35–2.25 (m, 1H of CH₂(4)); 2.04–1.89 (m, 1H of CH₂(4)). ¹³C-NMR (CDCl₃): 144.8, 133.9 (2s, 2 arom. C); 129.8, 127.6 (2d, 4 arom. C); 83.6 (d, C(3)); 36.42, 36.38 (2t, C(2), C(5)); 27.9 (t, C(4)); 21.5 (q, Me). CI-MS: 276 (100, [M+NH₄]⁺), 104 (4, [M–Ts+1]⁺), 86 (10, [M–TsOH]⁺). Anal. Calcd for C₁₁H₁₁O₃S₂: C, 51.14; H, 5.46; S, 24.82. Found: C, 50.88; H, 5.24; S, 24.70.

Thiolane-3-carbonitrile (11). To a solution of 3-(4-toluenesulfonyloxy)thiolane (10.34 g, 40.0 mmol) in DMSO (200 mL) was added NaCN (9.8 g, 200 mmol), the mixture was heated to reflux for 8 h, and stirred for another 20 h at rt. Then, the mixture was poured into water (700 mL), extracted with Et₂O (300 mL, 4 ×), and the combined Et₂O phase was extracted with sat. aqueous NaCl solution, dried with MgSO₄, and the solvent evaporated. Yield: 3.461 g of crude 11. Chromatographic purification (SiO₂,
hexane/AcOEt 10:1 $\rightarrow$ 5:1) gave 2.48 g (55%) 11 as colorless oil; bp 105°C/8 mbar. 

Rf (SiO$_2$, hexane/AcOEt 5:1) = 0.23 (UV$_{254}$ and Ce(SO$_4$)$_2$). IR (neat): 3635 w, 2941 s, 2866 m, 2240 s, 1621 w, 1439 s, 1333 w, 1303 m, 1252 m, 1226 m, 1184 w, 1126 w, 1080 w, 1041 w, 1013 m, 955 m, 930 w, 889 m, 813 w, 743 w, 711 w, 686 w. 

$^1$H-NMR (CDCl$_3$): 3.21–2.91 (m, 5H, CH$_2$(2), CH(3), CH$_2$(5)); 2.40–2.22 (m, 2H, CH$_2$(4)).

$^{13}$C-NMR (CDCl$_3$): 119.9 (s, CN); 34.9, 34.3 (2t, C(2), C(5)); 32.7 (d, C(3)); 29.9 (t, C(4)).


Thiolane-3-carboxylic acid (12). Thiolane-3-carbonitrile (11) (2.499 g, 22.08 mmol) was dissolved in EtOH and added to a solution of NaOH (9.189 g, 229.72 mmol) in water (85 mL) and EtOH (43 mL). The mixture was heated to reflux for 2.75 h, then cooled to 0°C, and acidified with conc. HCl (28 mL). Ethanol was removed by distillation, and the remaining solution was extracted with CH$_2$Cl$_2$ (5×), dried with MgSO$_4$, and evaporated to dryness to give 2.858 g (98%) of 12 as pale pink crystals; mp 61–62°C. 

Rf (SiO$_2$, acetone/5 drops of AcOH) = 0.52 (Ce(SO$_4$)$_2$). IR (KBr): 2937 s, 1699 v, 1422 v, 1350 m, 1281 s, 1245 v, 1197 s, 1091 m, 1061 w, 1013 w, 940 s, 893 m, 879 m, 824 w, 736 w, 687 w, 652 w. 

$^1$H-NMR (CDCl$_3$): 10.43 (br. s, OH); 3.75–2.87 (m, 5H, CH$_2$(2), CH(3), CH$_2$(5)); 2.33–2.23 (m, 2H, CH$_2$(4)). 

$^{13}$C-NMR (CDCl$_3$): 179.3 (s, COOH); 47.9 (d, C(3)); 33.6, 33.1 (2t, C(2), C(5)); 30.5 (t, C(4)). CI-MS: 133 (100, [M+H$^+$]+), 132 (46, [M–H$^-$$]+), 131 (10), 116 (3, [M–CO$_2$H$^-$$]+), 87 (12, [M–OH$^-$$]+), 86 (9), 85 (7). Anal. Calcd for C$_5$H$_8$O$_2$S: C, 45.43; H, 6.10; S, 24.26. Found: C, 45.44; H, 5.84; S, 24.09.

N-Methyl-N-phenylthiolane-3-carboxamide (13). To a solution of the carboxylic acid (12) (2.84 g, 21.49 mmol) in CH$_2$Cl$_2$ (45 mL) was added DCC (4.88 g, 23.65 mmol). Immediately, a white precipitate formed, and the mixture got warm. Then, N-methylaniline (4.61 g, 42.99 mmol) and DMAP (0.26 g, 2.13 mmol) were added and the mixture stirred for 17.5 h at rt. The precipitate was filtered, the filtrate concentrated, and the residue dissolved in AcOEt (60 mL). The solution was extracted successively with 1N HCl (3×), with sat. aqueous NaHCO$_3$ solution (1×), and with sat. aqueous NaCl solution (1×), dried with MgSO$_4$, and evaporated. Purification by column chromatography (2×, SiO$_2$, hexane/AcOEt 5:1) gave 3.96 g (83%) of amide (13). Colorless crystals; mp 63–64°C. 

Rf (hexane/AcOEt 1:1) = 0.43 (UV$_{254}$). IR (KBr): 2934 m, 2860 w, 1654 vs, 1592 s, 1494 s, 1426 s, 1387 s, 1331 m, 1295 s, 1260 m, 1204 w, 1122 s, 1071 w, 1024 w, 997 w, 892 w, 845 w, 777 m, 750 w, 701 s, 659 w, 571 m. 

$^1$H-NMR (CDCl$_3$): 7.47–7.35 (m, 3 arom. H); 7.22–7.18 (m, 2 arom. H); 3.28 (s, 3H, Me); 3.07–2.59 (m, 5H, CH$_2$(2), CH(3), CH$_2$(5)); 2.19–2.10 (m, 2H, CH$_2$(4)). $^{13}$C-NMR (CDCl$_3$): 172.6 (s, CO); 143.6 (s, 1 arom. C); 129.9, 128.0, 127.1 (3d, 5 arom. CH); 46.2 (d, C(3)); 37.6 (q, Me); 35.1, 34.6 (2t, C(2), C(5)); 31.0 (t, C(4)). ESI-MS: 260 (4, [M+K$^+$]+), 244 (100, [M+Na$^+$]+). Anal. Calcd for C$_{13}$H$_{15}$NOS: C, 65.12; H, 6.83; N, 6.33; S, 14.49. Found:
N-Methyl-N-phenylthiolane-3-thiocarboxamide (14). To a solution of amide (13) (6.12 g, 27.65 mmol) in toluene (28 mL) was added Lawesson reagent (6.71 g, 16.59 mmol) and the mixture heated to 130°C. After 1 h, the mixture was cooled to rt, filtered, washed with Et₂O, and evaporated. Purification by column chromatography (hexane/AcOEt 10:1) yielded 6.28 g (96%) of thioamide (14). Pale yellow crystals; mp 74–76°C. Rf (hexane/AcOEt 5:1) = 0.32 (UV 254 and Schlittler). IR (KBr): 2927 m, 2857 w, 1590 m, 1582 m, 1489 v, 1450 v, 1381 v, 1330 s, 1281 s, 1215 m, 1191 s, 1100 s, 1068 s, 1020 m, 993 m, 971 m, 886 w, 801 w, 767 s, 700 s, 615 w, 558 s. ¹H-NMR (CDCl₃): 7.52–7.40 (m, 3 arom. H); 7.19–7.16 (m, 2 arom. H); 3.73 (s, 3H, Me); 3.31 (t, J = 10.1 Hz, 1H); 3.11–3.02 (m, 1H); 2.96–2.90 (m, 1H); 2.79–2.73 (m, 1H); 2.66–2.57 (m, 1H); 2.47–2.36 (m, 1H); 2.17–2.12 (m, 1H). ¹³C-NMR (CDCl₃): 205.8 (s, CS); 145.3 (s, 1 arom. C); 130.2, 128.7, 125.3 (3 d, 5 arom. CH); 53.2 (d, C(3)); 45.8 (q, Me); 39.1, 38.2 (2 t, C(2), C(5)); 30.8 (t, C(4)). CI -MS: 240 (9), 239 (15), 238 (100, [M+H]+), 237 (4, M⁺). Anal. Calcd for C₁₂H₁₅NS₂: C, 60.72; H, 6.37; N, 5.90; S, 27.01. Found: C, 60.50; H, 6.12; N, 5.80; S, 27.06.

N-Methyl-N-phenyl-5-thia-1-azaspiro[2.4]hept-1-en-2-amine (9). To a solution of thioamide (14) (2.011 g, 8.47 mmol) in abs. CH₂Cl₂ (14 mL) were added 5 drops of DMF. Under an argon atmosphere at 0°C, a 2M solution of phosgene in toluene (5.5 mL, 11.01 mmol) was added dropwise within 5 min, and the mixture was stirred for 30 min. Then, the evolution of CO₂ ceased, the solvent was evaporated, and the residue was dissolved in abs. THF (10 mL). Still at 0°C, DABCO (0.965 g, 8.60 mmol) was added and the mixture stirred for 20 min. In a N₂ atmosphere, the precipitated salt was removed by filtration, washed with DMF (10 mL), and to the filtrate was added NaN₃ (1.159 g, 17.83 mmol). The mixture was stirred at rt for 6 d, filtered via Celite, and washed with Et₂O. Column chromatography (hexane/AcOEt 5:1) gave 9, contaminated with 5% (¹H-NMR) of 13, as brownish, viscose oil: 1.379 g (i.e. 1.29 g of 9, 70% yield; corrected on the basis of the ¹H-NMR spectrum). Rf (hexane/AcOEt 2:1) = 0.28 (UV₂₅₄ and Ce(SO₄)₂). IR (neat): 3460 w, 3062 m, 3045 m, 2918 s, 1760 vs, 1675 s, 1653 s, 1598 vs, 1501 vs, 1457 s, 1425 s, 1409 s, 1387 m, 1321 vs, 1303 s, 1281 s, 1259 s, 1224 s, 1207 s, 1188 m, 1159 m, 1110 vs, 1096 vs, 1033 s, 1003 s, 980 m, 965 m, 894 m, 842 w, 755 vs, 692 vs, 662 s, 620 w. ¹H-NMR (CDCl₃): 7.44–7.37 (m, 3 arom. H); 7.21–7.10 (m, 2 arom. H); 3.48 (s, 3H, Me); 3.21–3.12 (m, 1 H of CH₂(6)); 3.02 (d, 2J = 12.1 Hz, 1H of CH₂(4)); 2.96–2.87 (m, 1H of CH₂(6)); 2.71 (d, 2J = 12.1 Hz, 1H of CH₂(4)); 2.27 – 2.17 (m, 1 H of CH₂(7)); 2.00–1.92 (m, 1 H of CH₂(7)). ¹³C-NMR (75.5 MHz, CDCl₃): 161.0 (s, C(2)); 142.0 (s, 1 arom. C); 129.4, 123.5, 116.1 (3d, 5 arom. CH); ca. 53 (br., C(3)); 38.2, 37.3 (2t, 2 CH₂S); ca. 34 (br., Me); 29.5 (t, CH₂(7)). CI-MS: 219 (100, [M+H]+), 218 (4, M⁺), 217 (5).
Reaction of N-methyl-N-phenyl-5-thia-1-azaspiro[2.4]hept-1-en-2-amine (9) with thiobenzoic acid. N-f-3{-[N-Methyl-N-phenylamino]thioxomethyl[thiolan-3-yl]benzamide (15). A solution of azirine (9) (68 mg, 0.31 mmol) in abs. CH₂Cl₂ (3 mL) was cooled to 0°C. Then, thiobenzoic acid (58 mg, 0.42 mmol) was added dropwise and the mixture was stirred at rt. After 18 h, the solvent was evaporated and the product purified by prep. TLC (CH₂Cl₂/MeOH 50:1): 99.8 mg (90%) of 15 as colorless solid; mp 170–172°C. Rf (hexane/AcOEt 1:1) = 0.42 (UV254 and Ce(SO₄)₂). IR (KBr): 3401m, 3059vw, 3034vw, 2941w, 2869vw, 1667vs, 1580w, 1509vs, 1483vs, 1465s, 1452s, 1443s, 1377vs, 1280s, 1257m, 1234m, 1222m, 1198w, 1176w, 1111s, 1073m, 1037m, 1001w, 988w, 924w, 845vw, 772m, 705vs, 619w. ¹H-NMR (CDCl₃): 7.51–6.97 (9, 10 arom. H); 5.79 (br. s, NH); 3.73 (s, 3H, MeN); 3.49 (d, ²J = 11.4 Hz, 1H of CH₂(2)(Atc)); 3.40–3.29 (m, 1H); 3.12–3.05 (m, 1H); 2.98 (d, ²J = 11.4 Hz, 1H of CH₂(2)(Atc)); 2.90–2.84 (m, 1H); 2.61–2.51 (m, 1H). ¹³C-NMR (CDCl₃): 202.2 (s, CS); 165.8 (s, CONH); 147.1 (s, 1 arom. CN); 133.5 (s, 1 arom. C); 131.8, 129.8, 129.1, 128.4, 128.0, 126.8, 125.6, 125.2 (8d, 10 arom. CH); 73.6 (s, C(3)(Atc)); 50.5 (q, Me); 43.1 (t, 2 CH₂S); 28.0 (t, CH₄(4)(Atc)). ESI-MS: 395 (7, [M+K]⁺), 379 (8, [M+Na]⁺), 357 (100, [M+H]⁺), 250 (22). Anal. Calcd for C₁₉H₂₀N₂O₂S₂: C, 64.06; H, 5.50; N, 7.66; S, 17.75.

Reaction of N-methyl-N-phenyl-5-thia-1-azaspiro[2.4]hept-1-en-2-amine (9) with Z-Val-OH. Benzyl N-f-3{-Methyl-(S)-2-[(R,S)-3-{{[N-methyl-N-phenylamino]carbonyl[thiolan-3-yl]amino]-1-oxobutyl}carbamate (Z-Val-Atc-N(Me)Ph (16)). To a solution of azirine (9) (377 mg, 1.73 mmol) in abs. CH₂Cl₂ (10 mL) at 0°C, a solution of Z-Val-OH (514 mg, 2.05 mmol) in abs. CH₂Cl₂ (8 mL) was added slowly. The mixture was stirred at rt for 21 h and the solvent was evaporated. Column chromatography of the residue (SiO₂, hexane/AcOEt 5:1 → 2:1) gave 772 mg (95%) of 16 in three fractions: 339 mg (42%) of the faster moving diastereoisomer 16a (Rf (hexane/AcOEt 1:1) = 0.29 (UV254 and Ce(SO₄)₂), 101 mg (13%) of a mixture of the two diastereoisomers, and 332 mg (41%) of the slower moving diastereoisomer 16b (Rf (hexane/AcOEt 1:1) = 0.23 (UV254 and Ce(SO₄)₂).

Diastereoisomer 16a: Colorless crystals; mp 175–177°C. IR (KBr): 3364s, 3267vs, 3061w, 3026w, 2958m, 1715vs, 1652vs, 1595m, 1538vs, 1507vs, 1436s, 1389s, 1310w, 1264s, 1237vs, 1161w, 1129w, 1100s, 1024m, 1006m, 971w, 859w, 778w, 769w, 742m, 701s, 653m. ¹H-NMR (CDCl₃): 7.43–7.29 (m, 8 arom. H); 7.08–7.05 (m, 2 arom. H); 5.52 (d, ²J = 6.7 Hz, NH(Val)); 5.29 – 5.10 (m, NH(Atc)); 5.21, 5.13 (AB, J = 12.3 Hz, 2H, CH₂O); 3.64–3.60 (m, H-C(2)(Val)); 3.22 (s, 3H, MeN); 3.18 (d, ²J = 11.6 Hz, 1H of CH₂(2)(Atc)); 2.89–2.63 (m, 4H, 2 CH₂); 2.56–2.49 (m, 1H of CH₂(2)(Atc)); 1.90–1.84 (m, H-C(3)(Val)); 0.89 (d, ³J = 6.8 Hz, 3H, Me); 0.80 (d, ³J = 6.8 Hz, 3H, Me). ¹³C-NMR (CDCl₃): 170.2, 168.4 (2s, 2 CO); 156.1 (s, OCONH); 143.6 (s, 1 arom. CN); 136.4 (s, 1 arom. C); 129.6, 128.4, 128.1, 128.0, 127.3 (5d, 10 arom. CH); 69.5 (s, C(3)(Atc)); 66.9 (t, CH₂O); 59.2 (d, C(2)(Val)); 40.6 (q, MeN);
Diastereoisomer 16b: Colorless solid; mp 76–79°C. $^1$H-NMR (CDCl$_3$): 7.36–7.27 (m, 8 arom. H); 7.15–7.21 (m, 2 arom. H); 5.92 (br. s, NH(Atc)); 5.48 (d, $^3$J = 8.6 Hz, NH(Val)); 5.16, 5.09 (AB, $^1$J = 12.3 Hz, CH$_2$O); 3.78–3.74 (m, H-C(2)(Val)); 3.44 (d, $^3$J = 11.8 Hz, 1H of CH$_2$O); 3.21 (s, 3H, Me); 2.94–2.80 (m, 2H); 2.65–2.56 (m, 1H); 2.50–2.31 (m, 2H); 2.19–2.08 (m, 1H); 0.93 (d, $^3$J = 6.8 Hz, 3H, Me); 0.85 (d, $^3$J = 6.8 Hz, 3H, Me). $^{13}$C-NMR (CDCl$_3$): 170.6, 169.2 (2s, 2 CO); 156.2 (s, OCONH); 143.9 (s, 1 arom. CN); 136.2 (s, 1 arom. C); 129.4, 128.4, 128.2, 128.0, 127.9, 127.3 (6d, 10 arom. CH); 69.5 (s, C(3)(Atc)); 67.0 (t, CH$_2$O); 59.7 (d, C(2)(Val)); 40.6 (q, MeN); 39.9, 39.4, 28.1 (3t, 3 CH$_2$); 30.8 (d, C(3)(Val)); 19.2, 17.1 (2q, 2 Me). ESI-MS: 292 (100, [M+Na$^+$]). Anal. Calcd for C$_{25}$H$_{31}$N$_3$O$_4$S: C, 63.94; H, 6.65; N, 8.95; S, 6.83. Found: C, 63.87; H, 6.57; N, 8.81; S, 6.88.

Reaction of N-methyl-N-phenyl-5-thia-1-azaspiro[2.4]hept-1-en-2-amine (9) with FMOC-Val-OH. [9H-Fluoren-9-yl)methyl] N-[3-methyl-(S)-2-(((R,S)-3-(([(N-methyl-N-phenylamino)carbonyl]thiolan-3-yl)amino)-1-oxobutyl)carbamate (Fmoc-Val-Atc-N(Me)Ph (17)). To a solution of 9 (467 mg, 2.14 mmol) in abs. CH$_2$Cl$_2$ (15 mL) at 0°C, FMOC-Val-OH (855 mg, 2.52 mmol) was added and the mixture stirred for 21 h at rt. Then, the solvent was evaporated, and the residue separated by column chromatography (SiO$_2$, hexane/AcOEt 5:1 → 2:1) to give 1.18 g (99%) 17 in three fractions: 537 mg (45%) of the faster moving diastereoisomer 17a ($R_f$ (hexane/AcOEt 1:1) = 0.28 (UV 254 and Ce(SO$_4$)$_2$), 378 mg (32%) of a mixture of the two diastereoisomers, and 265 mg (22%) of the slower moving diastereoisomer 17b ($R_f$ (hexane/AcOEt 1:1) = 0.22 (UV254 and Ce(SO$_4$)$_2$).

Diastereoisomer 17a: Colorless solid; mp 194–196°C. IR (KBr): 3351 m, 3273 s, 2961 w, 2935 w, 1720v s, 1661v s, 1633v s, 1515v s, 1495w, 1450s, 1389m, 1257s, 1227s, 1170w, 1134w, 1103w, 1030m, 973vw, 758m, 738s, 702m, 649w, 621w. $^1$H-NMR (CDCl$_3$): 7.77 (d, $^3$J = 7.5 Hz, 2 arom. H); 7.64 (t, $^3$J = 6.7 Hz, 2 arom. H); 7.58–7.29 (m, 7 arom. H); 7.10–7.08 (m, 2 arom. H); 5.55 (d, $^3$J = 8.8 Hz, NH(Val)); 5.22 (br. s, NH(Atc)); 4.53–4.42 (m, 2H, CH$_2$O); 4.27 (t, $^3$J = 6.9 Hz, H-C(9) of fluorene); 3.66–3.62 (m, H-C(2)(Val)); 3.25 (s, 3H, MeN); 3.18 (d, $^3$J = 11.6 Hz, 1 H (Atc)); 2.91–2.48 (m, 5H (Atc)); 1.90–1.84 (m, H-C(3)(Val)); 0.89 (d, $^3$J = 6.7 Hz, 3H, Me); 0.81 (d, $^3$J = 6.7 Hz, 3H, Me). $^{13}$C-NMR (CDCl$_3$): 170.2, 168.4 (2s, 2 CO); 156.0 (s, OCONH); 143.7, 141.3, 129.6 (3s, 5 arom. C); 128.2, 127.7, 127.3, 127.0, 125.0, 124.9, 120.0 (7d, 13 arom. CH); 69.6 (s, C(3)(Atc)); 66.9 (t, CH$_2$O); 59.2 (d, C(2)(Val)); 47.6 (d, C(9) of fluorene); 40.6 (q, MeN); 40.4, 38.5, 27.4 (3t, 3 CH$_2$(Atc)); 32.2 (d, C(3)(Val)); 19.1, 16.9 (2q, 2 Me). ESI-MS: 596 (3, [M+K$^+$]), 580 (100, [M+Na$^+$]). Anal. Calcd for C$_{32}$H$_{35}$O$_4$N$_3$S: C, 68.91; H, 6.33; N, 7.53; S, 5.75. Found: C, 68.68; H, 6.16; N, 7.39; S, 5.51.
Diastereoisomer 17b: Colorless solid; mp ca. 100–110°C. 1H-NMR (CDCl₃): 7.77 (d, 3J = 7.5 Hz, 2 arom. H); 7.62–7.56 (m, 2 arom. H); 7.40 (t, 3J = 7.1 Hz, 2 arom. H); 7.33–7.21 (m, 5 arom. H); 7.14 (d, 3J = 7.1 Hz, 2 arom. H); 5.70 (br. s, NH(Atc)); 5.32 (d, 3J = 9.2 Hz, NH(Val)); 4.44 (d, 3J = 7.0, CH₂O); 4.23 (t, 3J = 6.8 Hz, H-C(9) of fluorene); 3.73 (br. s, H-C(2)(Val)); 3.47 (d, 2J = 11.8 Hz, 1H(Atc)); 3.24 (s, 3H, MeN); 2.98–2.82 (m, 2H(Atc)); 2.64–2.55 (m, 1H(Atc)); 2.48–2.31 (m, 2H(Atc)); 2.15–2.05 (m, H-C(3)(Val)); 0.91 (d, 3J = 6.3 Hz, Me); 0.83 (d, 3J = 6.5 Hz, 3H, Me). 13C-NMR (CDCl₃): 170.5, 169.1 (2s, 2 CO); 156.2 (s, OCONH); 143.9, 143.6, 141.3 (3s, 5 arom. C); 129.5, 128.0, 127.7, 127.3, 127.0, 124.9, 124.8, 120.0 (8d, 13 arom. CH); 69.6 (s, C(3)(Atc)); 66.9 (t, CH₂O); 59.6 (d, C(2)(Val)); 47.1 (d, C(9) of fluorene); 40.7 (q, MeN); 40.0, 39.4, 28.1 (3t, 3 CH₂(Atc)); 30.7 (d, C(3)(Val)); 19.1, 17.1 (2q, 2 Me). ESI-MS: 596(4, [M+K]+), 580 (100, [M+Na]+), 538 (8), 522 (3), 179 (10). Anal. Calcd for C₃₂H₃₅O₄N₃S: C, 68.91; H, 6.33; N, 7.53; S, 5.75. Found: C, 68.69; H, 6.33; N, 7.24; S, 5.53.

Selective deprotection of dipeptide (17). (R,S)-3-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3-methyl-1-oxobutyl)amino)thiolan-3-carboxylic acid (18a). To a mixture of MeCN (5 mL) and 6N HCl (5 mL), 17a (0.369 g, 0.662 mmol) was added and the mixture stirred for 4 d at rt. After this time, no change could be detected. Then, the mixture was heated to reflux for 3 h, extracted with CH₂Cl₂ (20 mL, 3×), the organic phase was dried with MgSO₄, and the solvent evaporated. Yield of 17a: 0.309 g (89%). Colorless solid; mp ca. 95–105°C. R₇ (acetone + 3 drops of AcOH) = 0.59 (UV 254 and Ce(SO₄)₂). IR (KBr): 3310 s, 3066 m, 2963 s, 2612 w, 1716 v s, 1660 v s, 1538 v s, 1477 m, 1449 s, 1392 m, 1372 m, 1320 m, 1246 v s, 1186 m, 1139 w, 1106 w, 1031 m, 759 s, 740 s, 694 v w, 665 w, 621 w. 1H-NMR (CDCl₃): 7.72 (d, 3J = 7.4 Hz, 2 arom. H); 7.55 (d, 3J = 7 Hz, 2 arom. H); 7.36 (m, 2 arom. H); 7.24 (m, 2 arom. H); 6.44 (br. s, NH); 5.94 (br. s, NH); 4.41–4.32 (m, 2H, CH₂O); 4.20–4.15 (m, H-C(9) of fluorene); 3.21–3.18 (m, H-C(2)(Val)); 2.92–2.64 (m, 4H, 2 CH₂(Atc)); 2.40–2.15 (m, H-C(9) of fluorene); 3.21–3.18 (m, H-C(2)(Val)); 2.92–2.64 (m, 4H, 2 CH₂(Atc)); 2.40–2.15 (m, H-C(3)(Val)); 2.14–2.04 (m, 2H, CH₂(Atc)); 0.95–0.90 (m, 6H, 2 Me). 13C-NMR (CDCl₃): 175.8, 172.4 (2s, 2 CO); 156.9 (s, OCONH); 143.7, 143.5, 141.2 (3s, 4 arom. C); 127.6, 127.0, 125.0, 119.9 (4d, 8 arom. CH); 68.3 (s, C(3)(Atc)); 67.3 (t, CH₂O); 60.0 (d, C(2)(Val)); 47.0 (d, C(9) of fluorene); 39.3, 37.5, 28.7 (3t, 3 CH₂(Atc)); 31.1 (d, C(3)(Val)); 20.6, 17.5 (2q, 2 Me). ESI-MS: 507 (10, [M+K]+), 491 (100, [M+Na]+), 362 (4), 179 (8). Anal. Calcd for C₂₅H₂₈N₂O₅S·H₂O: C, 61.71; H, 6.21; N, 5.76; S, 6.59. Found: C, 61.59; H, 5.96; N, 5.36; S, 5.48.

{3-(((S)-2-Amino-3-methyl-1-oxobutyl)amino)thiolan-3-yl)-N-methyl-N-phenylcarboxamide (19). 240 mg (0.430 mmol) of 17b were dissolved in Et₂NH (5 mL), and the mixture was stirred at rt for 100 min. Then, the solvent was evaporated, the residue dissolved in CH₂Cl₂ and purified by prep. TLC. Yield of 17b: 112 mg (78%). Colorless solid; mp ca. 50–55°C. R₇ (hexane/AcOEt 1:4) = 0.22 (UV₂₅₄ and Ce(SO₄)₂). IR (KBr): 3314s, 3058m, 2959vs, 2870s, 1651vs, 1592vs, 1494vs, 1453s, 1369vs, 1291s, 1247m, 1222m,
1172w, 1127m, 1073m, 1025w, 997v, 961w, 871w, 846w, 775m, 734m, 703s, 666m, 615w. \(^1\)H-NMR (CDCl\(_3\)): 7.42–7.30 (m, 3 arom. H); 7.28–7.21 (m, 2 arom. H); 3.28 (s, 3H, MeN); 3.25–3.21 (m, 2H); 2.95–2.70 (m, 6H); 2.62–2.56 (m, 1H); 2.23–2.04 (m, 1H); 1.43 (br. s, 1 H); 0.92 (d, \(^3\)J = 6.9 Hz, 3H, Me); 0.74 (d, \(^3\)J = 6.9 Hz, 3H, Me). \(^1^3\)C-NMR (CDCl\(_3\)): 173.2, 169.1 (2s, 2 CO); 144.6 (s, 1 arom. C); 129.2, 127.6, 127.0 (3d, 5 arom. CH); 68.9 (s, C(3)(Atc)); 59.7 (d, C(2)(Val)); 40.9 (q, MeN); 40.7, 38.9, 27.7 (3t, 3 CH\(_2\)(Atc)); 30.5 (d, C(3)(Val)); 19.5, 16.1 (2q, 2 Me). ESI-MS: 693 (10, [2M+Na]\(^+\)), 671 (7, [2M+H]\(^+\)), 358 (27, [M+Na]\(^+\)), 336 (100, [M+H]\(^+\)), 229 (32), 201 (13).

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REFERENCES AND NOTES


30. As a third alternative, the recently published Mitsunobu strategy was used: thiolan-3-ol in Et₂O at 0°C was treated with PPh₃ and diethyl azodicarboxylate (DEAD), and α-hydroxyisobutyronitrile was added. The nitrile (11) was obtained in low yield because of a difficult separation of some side products.


34. The amide (13) is formed by hydrolysis of the intermediate α-chloroenamine, which is present after incomplete reaction with sodium azide, during the workup procedure.

35. As in the reaction of the O-analogue (6) with Z-Ala-OH the dipeptide Z-Ala-Thp-N(Me)Ph with the bigger $R_f$-value has been shown to be the $(S,R)$-13 somer, we tentatively assign the $(S,R)$-configuration to the faster moving dipeptide (16a).

