Synthesis of Bis-Aminoazirines and their Application in Peptide Synthesis

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Dedicated to Professor Branko Stanovnik on the occasion of his 70th birthday

Abstract

The ‘cyclohexane-bridged’ bis-(3-amino-2H-azirines) cis- and trans-N,N’-dimethyl-N,N’-diphenyl-1,7-diazadispiro[2.2.2.2]deca-1,7-diene-2,8-diamine (cis-21 and trans-21) were synthesized from the corresponding bis-thioamide 20 by consecutive treatment with COCl₂, 1,4-diazabicyclo[2.2.2]octane (DABCO) and NaN₃. The reaction of these bis-azirines with different natural α-amino acids gave peptide amides 23. In addition, hydrolysis of the C-terminal amide groups of 23c and subsequent coupling with the Aib synthon 2, i.e., 2,2,N-trimethyl-N-phenyl-2H-azirin-3-amine, showed the applicability of building blocks 21 for peptide synthesis and peptide chain ligation.

Keywords: 2H-azirin-3-amines, α,α-disubstituted α-amino acids, aminoisobutyric acid (Aib), peptide synthesis

1. Introduction

Tools for the reduction of the conformational freedom of a protein and, therefore, for controlling its three-dimensional structure are of considerable interest in organic, medicinal and biochemistry. Conformational constraints can be achieved by incorporation of non-protein amino acids into the polypeptide chain. Substitution of the H-atom at C(α) of a natural amino acid is one of the widest spread strategies for backbone modification leading to an enhanced tendency to form secondary structures such as β-turns or helices. The most well-known α,α-disubstituted α-amino acid (2,2-disubstituted glycine) is aminoisobutyric acid (Aib), which is ubiquitous in natural peptaibols and responsible for the helical conformation of these oligopeptides with antibiotic properties.
The preferred formation of the $3_{10}$-helical conformation has been demonstrated for several Aib-containing oligopeptides.\textsuperscript{14–20}

A useful strategy for the introduction of these disubstituted amino acids into peptides is the ‘azirine/oxazolone method’,\textsuperscript{18–21} in which 2$H$-azirin-3-amines 1 are used as amino acid synthons. The reaction of the latter, e.g., the Aib synthon 2, with amino or peptide acids leads to peptide amides, the terminal amide groups of which can be hydrolyzed selectively.

Based on the first synthesis of Rens and Ghosez,\textsuperscript{24} numerous 2$H$-azirin-3-amines have been prepared,\textsuperscript{22,25–27} including enantiomerically pure chiral compounds,\textsuperscript{28,29} spirocyclic\textsuperscript{30} and heterospirocyclic compounds (e.g., 3),\textsuperscript{31,32} as well as dipeptide synthons of type 4 and 5\textsuperscript{18,33–35} (Figure 1). These molecules have been applied successfully in the synthesis of peptaibols,\textsuperscript{18–20,23,36} endo-thiopeptides,\textsuperscript{37–40} cyclic peptides,\textsuperscript{41–43} and cyclic depsipeptides.\textsuperscript{44–47} Furthermore, the crystal structure of the 1,4-bis(2,2-dimethyl-2$H$-azirin-3-yl)piperazine (6) has been published,\textsuperscript{48} without disclosing its synthesis and reactions.

A very general and effective way of introducing a global constraint into a peptide chain is the formation of a covalent bond between distant parts in the sequence, e.g., by formation of disulfide bridges between cysteine residues.\textsuperscript{49} Because this might possibly also be achieved between two carboxylic acid functions by using a bis-(3-amin-2$H$-azirine) of type 7 (Scheme 1), it was of interest to develop a synthesis of such molecules by using similar methods as described for the monomeric analogues.\textsuperscript{24,50–52}

In the present paper we report the synthesis of the bis-(3-amino-2$H$-azirines) cis-21 and trans-21, representing the first examples of this new class of molecules. Reactions with several N-protected α-amino acids confirmed that these new building blocks are suitable for peptide synthesis.

2. Results and Discussion

In preliminary studies we elucidated the appropriateness of bis-(N-methyl-N-phenylamides) for azirine synthesis and attempted the preparation of compounds with two (3-amino-2$H$-azirine) structures linked together by an alkyl chain. The preparation of 2,4-dimethylpentaenoic acid bis-(methylphenylamide) (9) and 2,7-dimethyloctanedioic acid bis-(methylphenylamide) (10) was achieved via α-alkylation of two equivalents of N-methyl-N-phenylpropanamide (8) with dibromomethane or 1,4-dibromobutane, respectively (Scheme 2). Since the conversion of these compounds into the corresponding bis-azirines according to the procedure of Villalgordo and Heimgartner\textsuperscript{52} was not successful, 9 and 10 were converted into the corresponding thioamides 11 and 12 by reaction with P$_2$S$_5$ and hexamethyldisiloxane (HMDO).\textsuperscript{53} These are the required starting materials for a synthesis analogous to the procedure described earlier.\textsuperscript{50,51} In the case of 11, consecutive treatment with COCl$_2$ in CH$_2$Cl$_2$, evaporation of the solvent, dissolution of the residue in THF, addition of 1,4-diazabicyclo[2.2.2]octane (DABCO), filtration, and reaction of the filtrate with NaN$_3$ gave 4$H$-
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Scheme 2

\[ \text{thiopyran-2,6-diamine 13 in quantitative yield, instead of the desired bis-azirine.} \]

A plausible mechanism for the formation of 13 is shown in Scheme 3. The key step is the cyclization of the mono-chloriminium salt 16 via nucleophilic attack of the neighboring thioamide group to give the bis-iminium salt 17, which stabilizes by twofold deprotonation. It is likely that this side reaction is favored in the case of the formation of a six-membered ring.

After the treatment of 12 under the same conditions, no product could be isolated due to decomposition of the crude material during the purification process. However, analysis of the crude product by mass spectrometry showed the absence of a nine-membered cyclization product analogous to 13, but indicated the presence of a mixture of the corresponding mono-azirine 14 and the bis-azirine 15. In addition, an IR-absorption at 1750 cm\(^{-1}\), which is characteristic for 2H-azirin-3-amines, was observed. Although the desired compound could not be isolated, it seemed possible that bis-(3-amino-2H-azirines) could be synthesized by this method using phosgene.
Since in the case of 15 the formation of different stereoisomers is possible, which probably would be difficult to separate because of the flexibility of the molecule, we decided to use a cyclohexane ring as a symmetric and less flexible connection between the two three-membered heterocycles. The preparation of the required starting material 20 for the azirine synthesis was performed starting with the commercially available cis/trans-1,4-cyclohexanedicarboxylic acid 18. Conversion to the bis-(N-methyl-N-phenylamide) 19 (cis/trans mixture) via the bis-acyl chloride and subsequent thionation with P2S5/HMDO gave cyclohexane-1,4-dicarboxylic acid bis-(N-methyl-N-phenylthioamide) 20. Treatment of the latter with COCl2, DABCO and NaN3 as described above yielded a mixture of cis-21 and trans-21. These isomers were separated by means of column chromatography and were obtained as solid products in 31% and 39% yield, respectively (Scheme 4).

Recrystallization of cis-21 from a mixture of CH2Cl2, acetone, hexane, and Et2O by slow evaporation of the solvent gave crystals that were sufficiently adequate for a crude X-ray crystal structure determination, which allowed the essential conformation of the molecule to be established. The molecular structure of cis-21 is shown in Figure 2.

The cyclohexane ring shows the chair conformation with the unsubstituted N(9) and N(16) atoms of the aziri-

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* NMR spectroscopy did not give any indication for the presence of two diastereoisomers. As the reaction pathway for the synthesis of 3-amino-2H-azirines involves intermediates with a sp2-C(α) atom of the thioamide,22 the stereochemical properties of the starting material are irrelevant.
ne rings cis to one another and in pseudo-equatorial and pseudo-axial orientations, respectively.

To examine the reactivity of the new bis-(3-amino-2H-azirines) cis-21 and trans-21, they were reacted with Z-protected L-alanine (22a)** in CHCl₃, which lead to the corresponding peptides 23a (Scheme 5). Compound trans-21 was chosen for reactions with other natural α-amino acids, which bear different protecting groups, i.e., Z-Phe-OH (22b), Fmoc-Leu-OH (22c), and Z-Lys(Boc)-OH (22d), leading to peptides 23b–d. In an analogous manner, the reaction of trans-21 with acetic acid gave the corresponding trans-1,4-bis(acetylamino)cyclohexane-1,4-dicarboxamide 24 (not shown in Scheme 5). All reactions proceeded smoothly at room temperature to give the products in high to very high yields (81–97%) without the formation of any side products.

The terminal amide groups of peptide 23c were selectively hydrolyzed using the standard procedure (3N HCl in water/THF) to give the peptide 25c with unprotected C-termini. The latter was subsequently reacted with the Aib synthon 2 to yield the extended peptide 26c, showing that bis-azirine trans-21 (as a representative of both diastereoisomers) is a convenient building block for the ‘azirine/oxazolone method’ (Scheme 6).

3. Conclusions

The first examples of bis-(2H-azirin-3-amines) connected via the C(2)-atoms, i.e., cis- and trans-21, were prepared in good yields from the corresponding bis-(thioamide) by using the ‘phosgene methodology’. Furthermore, it has been shown that the reactivity of these bis-azirines is similar to that of known 2H-azirin-3-amines and, therefore, they can be used as synthons of bis-(amino acids) in the preparation of ligated peptide chains.

** Abbreviations: Z = benzyloxycarbonyl, Boc = tert-butyloxycarbonyl, Fmoc = (9H-fluoren-9-yl)methoxycarbonyl
4. Experimental

4.1. General Procedures

Melting points were determined on a Büchi 540 apparatus; they are uncorrected. IR spectra were recorded on a Perkin-Elmer spectrum one spectrophotometer; absorption bands in cm⁻¹. ¹H NMR (300 MHz) and ¹³C NMR (75.5 MHz) spectra were obtained on a Bruker ARX-300 instrument at room temperature (r.t.); TMS was used as internal standard, δ in ppm. Mass spectra (MS) were recorded on a Finnigan SSQ-700 spectrometer for chemical ionization (CI, with NH₃) and electrospray ionization (ESI, in MeOH + NaI), and on a Finnigan MAT95 spectrometer for HR-MS (CI). Thin-layer chromatography (TLC) was performed on Merck TLC aluminium sheets, silica gel 60 F₂₅₄, and flash chromatography (CC) on Uetikon-Chemie Chromatographiegel C-560. The Aib synthon 2 was prepared according to ref. 52, and amide 8 was prepared according to ref. 30. All other products used were commercially available.

4.1.1. General Procedure 1 (GP1)

To a solution of diisopropylamine (2.16 ml, 15.3 mmol) in THF (30 ml) at –80 °C was added butyllithium (8.8 ml, 1.6 M in hexane, 14.1 mmol), and the mixture was stirred for 30 min. Then, amide 8 (2.1 g, 12.8 mmol) in THF (5 ml) was added, and after 30 min stirring at –80 °C, the corresponding dibromoalkane (6.4 mmol) was added. The reaction mixture was allowed to warm up to r.t. slowly. A saturated aqueous NH₄Cl-solution (40 ml) was added and the aqueous layer extracted with Et₂O (2 x 30 ml). The combined organic fractions were washed with water (40 ml) and brine (20 ml). After drying over MgSO₄, filtration, and evaporation of the solvent, the residue was purified by CC.

4.1.2. General Procedure 2 (GP2)

A mixture of the corresponding amide, P₂S₅, and hexamethyldisiloxane (HMDO) in CHCl₃, was heated under reflux until complete conversion of the amide was observed by TLC. After cooling to r.t., the solvent was evaporated and the residue purified by CC.

4.1.3. General Procedure 3 (GP3)

To a solution of the corresponding thioamide (3.81 mmol) and 3 drops of DMF in CH₂Cl₂ (20 ml) at 0 °C was added phosgene (20% solution in toluene, 9 ml, 18.1 mmol). The mixture was stirred at r.t. for 60 min and was then concentrated under reduced pressure. THF (40 ml) and DABCO (852 mg, 7.62 mmol) were added and the mixture was stirred for 30 min at r.t. The formed precipitate was filtered under nitrogen, and after the addition of

*** Warning: Phosgene is a highly toxic gas (b.p. 8 °C), which has to be handled with extreme caution. Its solution in toluene is more safe and was used in a closed system.
DMF (30 ml) and NaNO₂ (2.0 g, 30.8 mmol), the suspension was stirred for 60 h at ambient temperature. Filtration and evaporation of the solvent gave the crude product that was purified as indicated.

### 4. 1. 4. General Procedure 4 (GP4)

A solution of azirine cis-21 or trans-21 and the corresponding carboxylic acid in CHCl₃ was stirred at r.t. until complete conversion of the starting materials was observed (TLC). The mixture was then washed three times with saturated aqueous NaHCO₃ solution and dried over MgSO₄. Evaporation of the solvent gave the crude product that was purified as indicated.

### 4. 2. Attempted Synthesis of Bis-azirines of Type 15

**2,4, N,N'-Tetramethyl-N,N'-diphenylpentanedioic Acid Diamide (9).** Prepared according to GP1; with dibromomethane (1.11 g, 0.45 ml, 6.4 mmol), CC (SiO₂, hexane/AcOEt 1:1, then 2:3). Yield: 950 mg (44%) of amide 10. White powder; mp 55–56 °C. IR (KBr): ν max 3041w, 2930m, 2857w, 1694m, 1492s, 1461s, 1443s, 1383s, 1272m, 1101m, 773m, 700s cm⁻¹. 1H NMR (CDCl₃): δ 7.19–7.47 (10H, m, 2 Ph). 13C NMR (CDCl₃): δ 37.4 (q, 2 MeN); 37.8 (t, CH₂); 127.3, 127.6, 129.6 (3d, 10 arom. CH); 143.9 (s, 2 arom. CN); 212.1 (s, 2 CO). ESI-MS (m/z): 361 (100, [M¹+Na⁺]).

**2,7,N,N'-Tetramethyl-N,N'-diphenylbutane-bis-thioic Acid Diamide (10).** Prepared according to GP1; with 1,4-dibromobutane (1.38 g, 0.5 ml, 6.4 mmol), CC (SiO₂, hexane/AcOEt 1:1, then 2:3). Yield: 930 mg (84%) of thioamide 11. Yellowish powder; mp 109 °C. IR (KBr): ν max 3398m, 2933m, 2857w, 2222w, 2155m, 2029w, 1750s, 1654m, 1599s, 1501s, 1460w, 1113m, 755cm⁻¹. ESI-MS of the crude mixture (m/z): 337 (100, [M¹+H⁺]), 91 (5%).

**2,4, N,N'-Tetramethyl-N,N'-diphenylpentane-bis-thioic Acid Diamide (11).** Prepared according to GP2; with amide 9 (1.01 g, 3.0 mmol), P₅S₅ (1.28 g, 5.7 mmol) and HMDMO (3.97g, 5.2 ml, 24.4 mmol) in CHCl₃ (20 ml), 14 h, CC (SiO₂, hexane/AcOEt 6:1). Yield: 930 mg (84%) of thioamide 11. Yellowish powder; mp 110–112 °C. IR (KBr): ν max 3043w, 2973m, 2925m, 286w, 1592m, 1492s, 1465s, 1379s, 1343m, 1268m, 1106s, 1069m, 992s, 777m, 771m, 699s cm⁻¹. 1H NMR (CDCl₃): δ 0.74 (6H, d, J = 6.4 Hz, 2 MeCH); 1.83 (2H, t, J = 6.9 Hz, CH₂); 2.73–2.80 (2H, m, 2 MeCH); 3.69 (6H, s, 2 MeN); 7.34–7.49 (10H, m, 2 Ph). 13C NMR (CDCl₃): δ 20.2 (q, 2 MeCH); 40.5 (t, CH₂); 44.9 (d, 2 MeCH); 45.4 (q, 2 Me-N); 125.5, 128.4, 129.9 (3d, 10 arom. CH); 145.3 (s, 2 arom. CN); 211.1 (s, 2 CS). ESI-MS (m/z): 393 (100, [M¹ + Na⁺]).

**2,7,N,N'-Tetramethyl-N,N'-diphenylbutane-bis-thioic Acid Diamide (12).** Prepared according to GP2; with amide 10 (1.14 g, 3.0 mmol), P₅S₅ (1.28 g, 5.7 mmol) and HMDMO (3.97g, 5.2 ml, 24.4 mmol) in CHCl₃ (20 ml), 14 h, CC (SiO₂, hexane/AcOEt 10:1). Yield: 408 mg (33%) of thioamide 12. Yellowish powder; mp 109 ºC. IR (KBr): ν max 3427w, 2957m, 2858m, 1694m, 1492s, 1461s, 1443s, 1383s, 1272m, 1101m, 773m, 700s cm⁻¹. 1H NMR (CDCl₃): δ 1.08 (6H, d, J = 6.6 Hz, 2 MeCH): 0.89–1.09, 1.24–1.39, 1.60–1.82 (4H + 2H + 2H, 3m, 4 CH₃); 2.56–2.70 (2H, m, 2 MeCH); 3.72 (6H, s, 2 MeN); 7.07–7.14, 7.36–7.48 (4H + 6H, 2m, 2 Ph). 13C NMR (CDCl₃): δ 22.1 (q, 2 MeCH); 27.6, 38.1 (2t, 4 CH₂); 43.9 (d, 2 MeCH); 45.4 (q, 2 MeN); 125.4, 128.3, 129.9 (3d, 10 arom. CH); 145.62 (s, 2 arom. CN); 212.1 (2 s, 2 CO). ESI-MS (m/z): 435 (100%, [M¹+Na⁺]).

**3,5,N,N'-Tetramethyl-N,N'-diphenyl-4H-thiopyran-2,6-diamine (13).** Prepared according to GP3; with thioamide 11 (1.41 g), CC (SiO₂, hexane/AcOEt 4:1). Yield: 1.23 g (96%) of 13. Orange oil. IR (film): ν max 3026w, 2985w, 2913w, 2903m, 2810w, 1598s, 1577m, 1499s, 1451m, 1363s, 1297m, 1236m, 1108w, 1034m, 1001m, 909m, 748s cm⁻¹. 1H NMR (CDCl₃): δ 1.69 (6H, s, 2 Me); 3.05 (6H, s, 2 MeN); 6.72–6.80, 7.15–7.22 (6H + 4H, 2m, 2 Ph). 13C NMR (CDCl₃): δ 18.7 (q, 2 Me); 37.4 (q, 2 MeN); 40.0 (t, CH₂); 113.0, 117.6, 128.9 (3d, 10 arom. CH); 125.1, 133.8, 147.0 (3s, 2 C=C + 2 arom. CN). CI-MS (m/z): 337 (100, [M¹+H⁺]), 91 (5%).

**4. 3. Synthesis of cis- and trans-N,N'-Dimethyl-N,N'-diphenylcyclohexane-1,7-diazadispiro[2.2.2.2]deca-1,7-diene-2,8-diamine (cis-21 and trans-21)**

**N,N'-Dimethyl-N,N'-diphenylcyclohexane-1,4-dicarboxamide (19).** A solution of 1,4-cyclohexanedicarboxylic acid (cis/trans mixture, 10.0 g, 58 mmol) in SOCl₂ (100 ml)
was heated to reflux for 6 h. The mixture was concentrated under reduced pressure and the residue dried in high vacuum. Ethyl acetate (300 ml) and, after cooling to 0 °C, triethylamine (13.1 g, 18.2 ml, 130 mmol) and N-methyl-l alanilin (13.9 g, 14 ml, 130 mmol) were added slowly. The mixture was stirred at r.t. for 16 h; then water (200 ml) was added. The organic layer was extracted with 1N aqueous HCl-solution (3 × 100 ml), 1N aqueous NaOH-solution (3 × 100 ml), and brine (50 ml). Evaporation of the solvent and crystallization from toluene gave bis-amide (19) (mixture of 2 diastereoisomers, ratio ca. 1:1). White powder; mp 156–158 °C. IR (KBr): ν max 3433, w 2950m, 2929m, 2905m, 2827m, 1744s, 1596s, 1503s, 1320m, 1235m, 1189m, 1101s, 1034s, 752s, 690m cm–1. 1H NMR (CDCl3): δ 1.31–1.39, 2.54–2.63 (4H + 4H, 2m, 2 CH2); 3.49 (6H, s, 2 MeN); 7.11–7.21, 7.39–7.48 (4H + 6H, 2m, 2 Ph). 13C NMR (CDCl3): δ 13.32 (t, 4 CH, 151.8, 151.7, 151.6, 151.5 (4d, 10 arom. CH); 176.8 (s, 2 C=N); Me-N and spiro-C could not be detected. CI-MS (m/z): 345 (100, [M + H]+), 238 (19), 108 (41). HR-CIMS (m/z): 345.2082 ([M + H]+). Anal. Calcd. for C22H25N4: 345.2079 (δ = 0.8 ppm).

Crystals for an X-ray crystal-structure determination were grown from a solution of cis-21 in a mixture of CH2Cl2, acetone, hexane, and Et2O by slow evaporation of the solvent.

**Data of trans-21:** Yellowish powder; mp 98–99 °C. IR (KBr): ν max 3433, w 2950m, 2929m, 2905m, 2827m, 1744s, 1596s, 1503s, 1320m, 1235m, 1189m, 1101s, 1034s, 752s, 690m cm–1. 1H NMR (CDCl3): δ 1.31–1.39, 2.54–2.63 (4H + 4H, 2m, 2 CH2); 3.49 (6H, s, 2 MeN); 7.11–7.21, 7.39–7.48 (4H + 6H, 2m, 2 Ph). 13C NMR (CDCl3): δ 13.32 (t, 4 CH, 151.8, 151.7, 151.6, 151.5 (4d, 10 arom. CH); 176.8 (s, 2 C=N); Me-N and spiro-C could not be detected. CI-MS (m/z): 345 (100, [M + H]+). Anal. Calcd. for C22H25N4: 345.2088 ([M + H]+). calcd. for C22H25N4: 345.2079 (δ = 2.6 ppm).

### 4. Reactions of cis-21 and trans-21 with Amino Acids

Benzyl cis-[[S]-1-4-[[[Benzyloxy carbonyl]amino]propanoylamino]-1,4-bis(N-methyl-N-phenylcarbamoyl)cyclohexylcarbamoyl]ethyl carbamate (cis-23a). Prepared according to GP4 with cis-21 (30 mg, 0.087 mmol) and Z-Ala-OH (43 mg, 0.19 mmol) in CHCl3 (5 ml), 2 h, CC (SiO2, hexane/AcOEt 1:3). Yield: 63 mg (91%) of cis-23a. Yellowish powder; mp 177–178 °C. IR (KBr): ν max 3433, w 2950m, 2929m, 2905m, 2827m, 1744s, 1596s, 1503s, 1320m, 1235m, 1189m, 1101s, 1034s, 752s, 690m cm–1. 1H NMR (CDCl3): δ 1.31–1.39, 2.54–2.63 (4H + 4H, 2m, 2 CH2); 3.49 (6H, s, 2 MeN); 7.11–7.21, 7.39–7.48 (4H + 6H, 2m, 2 Ph). 13C NMR (CDCl3): δ 13.32 (t, 4 CH, 151.8, 151.7, 151.6, 151.5 (4d, 10 arom. CH); 176.8 (s, 2 C=N); Me-N and spiro-C could not be detected. CI-MS (m/z): 345 (100, [M + H]+). Anal. Calcd. for C22H25N4: 345.2079 (δ = 0.8 ppm).

Prepared according to GP4; with *trans*-21 (100 mg, 0.29 mmol) and Z-Phe-Oh (190 mg, 0.64 mmol) in CHCl3 (10 ml), 2 h, CC (SiO2, CH2Cl2/MeOH 25:1). Yield: 220 mg (81%) of *trans*-23b.

White powder; mp 177–178 °C. IR (KBr): νmax 3304s, 3063m, 3039m, 2955s, 2926w, 1648s, 1592m, 1549s, 1524s, 1493w, 1433s, 1403s, 1368s, 1346m, 1303s, 1275m, 1187s, 1101m, 1034m, 750m, 699s cm–1. 1H NMR (d6-DMSO): δ 1.37 (18H, s, 2 Me3C); 1.24–1.70, 1.94–2.10 (12H + 8H, 2m, 2 CH2); 3.30 (6H, s, 2 MeN); 7.40–7.72 (10H, 2 CH). Trans-1,4-Bis(acetylamino)hexanoylamino-5-(tert-butilino)pentylcarbamate (*trans*-23d).

Prepared according to GP4; with *trans*-21 (100 mg, 0.29 mmol) and Z-Lys(Boc)-OH (243 mg, 0.64 mmol) in CHCl3 (10 ml), 2 h, CC (SiO2, CH2Cl2/MeOH 15:1). Yield: 295 mg (92%) of *trans*-23d. White powder; mp 190–191 °C. IR (KBr): νmax 3372s, 3034w, 2959m, 2932w, 2865w, 1717s, 1659s, 1626s, 1593m, 1525s, 1454s, 1365s, 1250s, 1170s, 1101m, 1034m, 750m, 699s cm–1. 1H NMR (d6-DMSO): δ 1.37 (18H, s, 2 MeC); 1.24–1.70, 1.94–2.10 (12H + 2H, 2 CH2); 3.30 (6H, s, 2 CH2); 7.40–7.80 (10H, 2 CH).
23c (80 mg, 0.076 mmol) in THF (15 ml) was slowly added an aqueous HCl-solution (32%, 6.4 ml), and the mixture was stirred at r.t. for 24 h. Then, aqueous HCl (2M solution, 10 ml) was added and the mixture was extracted with Et₂O (3 × 10 ml). After drying over MgSO₄ and evaporation of the solvent, the residue was recrystallized from acetone/hexane, yielding trans-25c (64 mg, 96%). White powder; mp 171–173 °C. IR (KBr): νmax 3325s, 3065m, 2956s, 1719s, 1662s, 1525s, 1540m, 1383m, 1262s, 1108m, 1050m, 785m, 739m cm⁻¹. ¹H NMR (CD₂OD): δ 6.09, 6.5 Hz, 2 Me₂CH; 4.15–1.78, 1.91–2.20 (6H + 8H, 2m, 2 Me₂C); 1.45–1.5, 2.6–2.7 (8H, m + d, J = 6.6 Hz, 2 CH₂O); 6.4 Hz, 2 CH₃; 176.9 (2s, 2 CON + 2 CO₂). ESI-MS (arom. C + 2 arom. CN); 158.0 (s, 2 N-CO-O); 175.2, 128.8, 130.2 (8d, 26 arom. CH); 142.5, 145.1, 145.2 (3s, 8 arom. C); 25.8 (d, 2 Me₂CH₂); 41.1 (q, 2 MeN); 41.4 (t, 2 MeCH₂); 27.9, 29.7 (2, 2 Me₂CH₂); 41.1 (q, 2 MeN); 41.4 (t, 2 MeCH₂); 27.9, 29.7 (2, 2 CH₂O); 43.8 (d, 2 CH₂O); 55.7 (d, 2 CHN); 68.0 (t, 2 CH₂O); 73.9 (8d, 26 arom. CH); 142.5, 145.1, 145.2 (3s, 8 arom. C + 2 arom. CN). ¹³C NMR (CD₃OD): δ 25.9 (d, 2 Me₂CH₂); 67.8 (t, 2 CH₂O); 120.8, 126.1, 126.2, 128.1, 128.6 (5d, 16 arom. CH); 142.5, 145.1, 145.4 (3s, 8 arom. C); 158.3 (s, 2 O-CO-N); 175.4, 176.9 (2s, 2 CON + 2 CO₂), ESI-MS (m/z); 985 (100, [M + Na⁺]). [α]D 25o = −16.4 (c 10, acetone).

(9H-Fluoren-9-yl)methyl trans-[(S)-1-[4-(S)-2-[[9H-Fluoren-9-yl]methoxy]carbonylamino]-4-methylpentanoylamino]-1,4-bis[1-methyl-1-(N-methyl-N-phenylcarbamoyl)ethylcarboxyl]-3-methylbutylcarbamate (trans-26c). A solution of trans-25c (150 mg, 0.17 mmol) and aminourine 2 (63 mg, 0.36 mmol) in THF (10 ml) was stirred at r.t. for 48 h. Then, the solvent was evaporated and the residue was purified by CC (SiO₂, CH₂Cl₂/MeOH 20:1), yielding 64 mg (96%) of 25c.

**4.5. X-ray Crystal-Structure Determination of cis-21 (see Figure 2).****

All measurements were made on a Nonius Kappa-CCD area detector diffractometer using graphite-monochromated MoKα radiation (λ 0.71073 Å) and an Oxford Cryosystems Cryostream 700 cooler. Data reduction was performed with HKL Denzo and Scalepack. The intensities were corrected for Lorentz and polarization effects, but not for absorption. Equivalent reflections were merged. Data collection and refinement parameters are given below, and a view of the molecule is shown in Figure 2. The structure of cis-21 was solved by direct methods using SIR92, which revealed the positions of all non-hydrogen atoms. The non-hydrogen atoms were refined anisotropically. All of the H-atoms were placed in geometrically calculated positions and refined using a riding model where each H-atom was assigned a fixed isotropic displacement parameter with a value equal to 1.2Ueq of its parent C-atom (1.5Ueq for the Me groups). Refinement of the structure was carried out on F² using full-matrix least-squares procedures, which minimized the function Σ(Fo-Fc)². A correction for secondary extinction was applied. Although the conformation of the molecule is clearly defined, the refinement results are poor. This appears to be because of the nature of the crystals. Either they are twinned or are intergrown, as the diffraction images show evidence of interleaving lattices with many reflections overlapping. Data recorded from several crystals yielded similar results. An attempt to extract twinned data was unsuccessful. The imprecise nature of the geometric parameters means that it is inadvisable to attempt to draw any far-reaching conclusions from a detailed analysis of the geometrical parameters. Neutral atom scattering factors for non-H-atoms were taken from ref. 58a and the scattering factors for H-atoms were taken from ref. 59. Anomalous dispersion effects were included in Fc 60; the values for f' and f'' were those of ref. 58b. The values of the mass attenuation coefficients are those of ref. 58c. All calculations were performed using the SHELXL97 program.

Crystal data for cis-21: C₃2H₄₂N₄O₄, M = 344.46, colorless, prism, crystal dimensions 0.20 × 0.30 × 0.35 mm, monoclinic, space group P21/c, Z = 4, a = 19.4051(6) Å, b = 9.7739(3) Å, c = 10.1001(2) Å, β = 98.747(2)°, V = 1893.34(3) Å³, T = 160 K, Dc = 1.208 g cm⁻³, μ(MoKα) = 0.0732 mm⁻¹, scan type φ, o. 2θmax = 60°, total reflections measured 48208, symmetry independent reflections 5539, reflections with I > 2σ(I) 4123, reflections used in refinement 5539, parameters refined 239, R(F) [I > 2σ(I) reflections] = 0.1315, wR(F²) all data] = 0.4719 (w = [σ²(Fo)² + (0.2P)²]⁻¹, where P = (Fo - 2Fc)²/3), goodness of fit 1.923, secondary extinction coefficient 0.17(4), final Δmax/σ 0.001, Δσ(max/min) = 0.50/−0.45 e Å⁻³.

**** CCDC-704899 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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Povzetek

Opisana je sinteza ’ciklheksan-premostenih’ bis-(3-amino-2H-azirinov), cis- in trans-N,N’-dimetil-N,N’-difenil-1,7-
diazadispiro[2.2.2.2]deka-1,7-dien-2,8-diaminov iz ustreznih bis-thioamidov 20 z zaporednimi pretvorbami s COCl2, 1,4-diazabiciklo[2.2.2]oktanom in NaN3. Reakcije teh bis-azirinov z različnimi naravnimi α-amino kislinami so vodile do peptid amidov 23. Hidroliza C-terminalnih amidnih skupin spojina 23c s sledečim pripajanjem na Aib sintona 2, 2,2,N-trimetil-N-fenil-2H-aziran-3-amin, je pokazala uporabnost gradnikov 21 v sintezi peptidov.