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Synthesis of a Novel Heterospirocyclic 3-(*N*-Methyl-*N*-phenylamino)-2*H*-azirine and its Use as an Amino Acid Equivalent in the Preparation of a Model Tripeptide

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Abstract: The synthesis of a novel heterospirocyclic 3-amino-2H-azirine based on the reaction between amide enolates and diphenylphosphorochloridate is described. This compound has been shown to be a useful amino acid equivalent and the synthesis of a model tripeptide was achieved in good overall yields.

INTRODUCTION

3-Amino-2*H*-azirines are known to be valuable synthetic intermediates suited for a wide variety of uses[1]. Indeed, they undergo reactions with different substrates containing acidic protons. For instance, reactions with activated phenols[2] and heterocycles with acidic NH-groups[3,4] lead to different types of heterocycles. Of special importance, is the reaction with carboxylic acids and amino acids affording α,α-disubstituted α-amino acid derivatives. This latter reaction is the starting point for the development of the so called "azirine/oxazolone method" in peptide synthesis[1,5]. This strategy has been widely employed in the synthesis of linear oligopeptides[6,7], cyclic peptides[8] and depsipeptides[5,9] containing α,α-disubstituted α-amino acids as well as in the synthesis of peptaibols[10].

On the other hand, the non-protein amino acids[11] are of growing importance because of their intrinsic biological activity as well as the unique properties they impart when incorporated into peptide chains. In this context, cyclic amino acids are of particular interest due to their diverse physiological properties and their use in the synthesis of conformationally restricted peptides[12].

RESULTS

As a part of our ongoing program dealing with the synthesis of novel 3-amino-2*H*-azirines[13] and their use as amino acid equivalents in peptide synthesis, we were interested in the preparation of compounds of type **1** *via* the novel method recently described by us, which is based on the reaction between amide enolates and diphenylphosphorochloridate followed by reaction with NaN_3[14,15].

§ Taken in part from the PhD thesis of *JMV*, University of Zürich, 1992.
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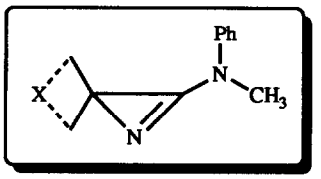
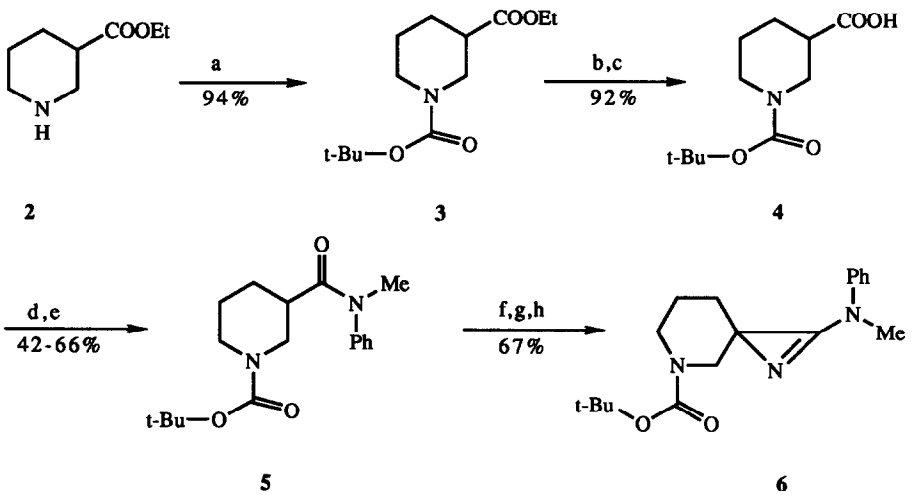


1, X = HN, O, S

In this context, as a first example, the readily available β-aminoester **2** was selected as the starting material and the following synthetic sequence was performed, as shown in *Scheme 1*.

Scheme 1



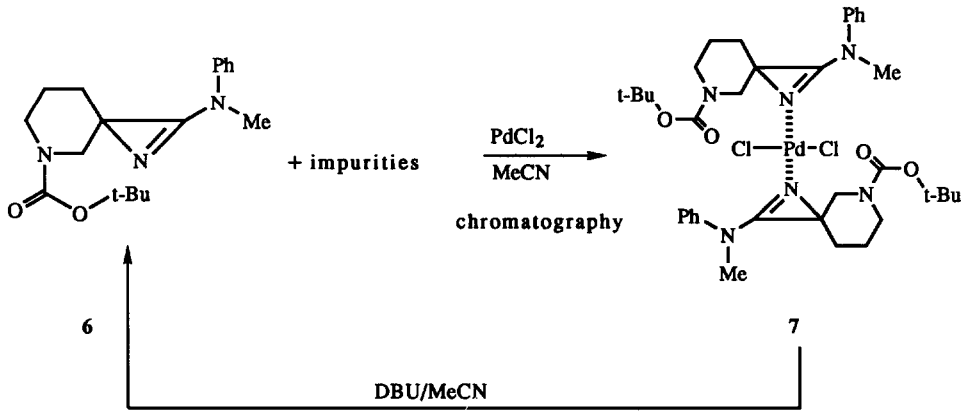
a: *$(BOC)_2CO$, CH_2Cl_2, Hünig base, 0°-r.t. over night;* b: *1N NaOH, THF, r.t. 24 h;* c: *2N HCl;* d: *DCC (60%), CME-CDI (66%), CDI (42%);* e: *N-Methylaniline;* f-h: *LDA/THF, 0°C; DPPCl, 0°C-r.t.; NaN_3/DMF, r.t. 4-5 d, Ar.*

Protection of **2** with the tert-butyloxycarbonyl group (BOC) led to the protected ester **3** in 94% yield. Saponification and acidification afforded the BOC-protected β-amino acid **4** in 92 % yield, which upon treatment with a peptide coupling reagent and *N*-methyl aniline led to the amide **5**. In this particular case, the use of the water-soluble carbodiimide *N*-cyclohexyl-*N'*-(2-morpholinoethyl) methyl p-toluenesulfonate (CME-CDI) gave the best yields (66%) compared with other coupling reagents like dicyclohexyl carbodiimide (DCC) and carbonyl diimidazole (CDI). Amide **5**, was allowed to react under the usual conditions already described[15] by the sequential treatment *f-h* which finally furnished the corresponding 3-amino-2*H*-azirine **6** in 67% yield.

Compound **6**, isolated as a slightly yellow oil after chromatography on silica gel (SiO_2), was further purified by complexation with $PdCl_2$[16], forming **7** (*Scheme 2*). Filtration over a small column of SiO_2 and decomplexation with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in MeCN at 0° for 30 min. led to the

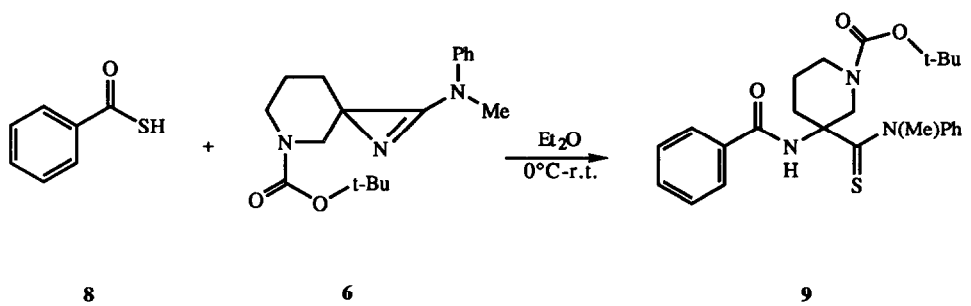
removal of the accompanying impurities mainly in the form of amide **5**, from which it is very difficult to separate completely **6**, even by HPLC means.

Scheme 2



Aminoazirine **6** was fully characterized by the usual spectroscopic methods and its reactivity tested by making use of the excellent reaction of benzothiocarboxylic acid **8** with 2,2-disubstituted 3-amino-2*H*-azirines to give the corresponding crystalline thioamides[17]. In this case, compound **9** was isolated in 95% yield as a colourless powder (*Scheme 3*).

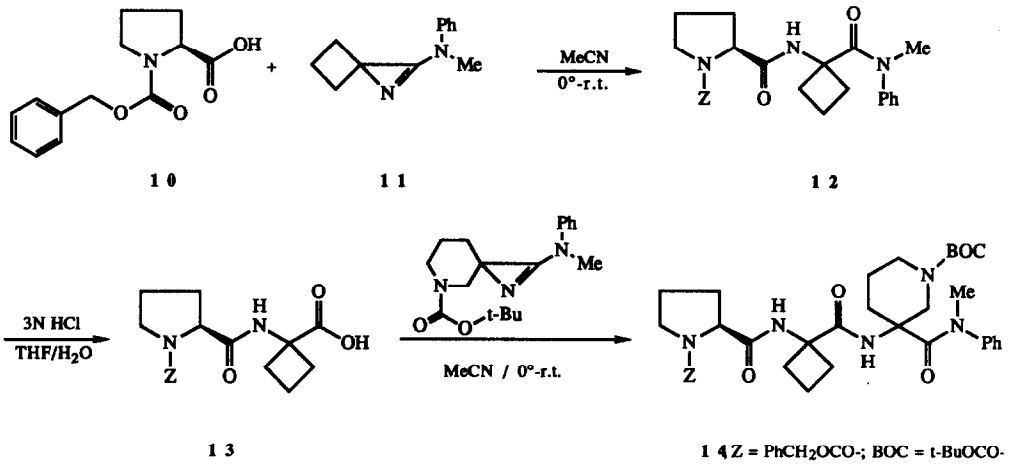
Scheme 3



Once the pure heterospirocyclic azirine **6** had been obtained, and it had ben shown that its reactivity toward thiocarboxylic acids was analogous to that of the other 2,2-disubstituted 3-amino-2*H*-azirines, the next step was to study the application of this type of azirine to the synthesis of oligopeptides containing heterocyclic amino acids.

As mentioned above, the "azirine/oxazolone method" has been shown to be the method of choice for the synthesis of the peptaibols, a class of polipeptidic antibiotics and ionophores[5]. In an analogous way, we were interested to know if this strategy is also valid when heterospirocyclic azirines were used instead. For that purpose, a model tripeptide was selected and the synthesis is depicted in *Scheme 4*.

Scheme 4



10 11 12

13

14 Z = $PhCH_2OCO$-; BOC = t-BuOCO-

Reaction of Z-L-proline (**10**) with aminoazirine **11**[13] in dry MeCN at 0°C led after 5 hours to the corresponding dipeptide **12** in 88% yield, isolated as a colourless foam. Selective hydrolysis of **12** under the standard conditions of 3N HCl (THF/H_2O 1:1) led to the formation of the protected acid dipeptide **13** in quantitative yields which was further reacted with aminoazirine **6** in dry MeCN to give the fully protected model tripeptide **14** in 80% yield, isolated as a colourless oil.

In conclusion, we have presented a very straightforward synthesis of a hitherto unknown type of 3-amino-2*H*-azirines. The complexation/decomplexation procedure with $PdCl_2$ has been shown to be a very effective method for the purification of **6**. The reactivity of **6** toward thiocarboxilic acids is the same as that displayed by other azirines. Furthermore, the incorporation of **6** in peptidic chains as heterocyclic amino acid equivalents has been studied by the synthesis of **14**. The synthesis of **14** has enabled us to optimise procedures and further investigations using other aza-heterocycles with different ring sizes as well as the corresponding oxa- and thio-analogues are in progress. The synthesis of optically active **6** which could be used for the preparation of enantiomerically pure peptides is being developed and will be published elsewhere.

EXPERIMENTAL SECTION

General: CH_2Cl_2 was distilled over $CaCl_2$ and dried over molecular sieves (4Å). THF was distilled over Na/benzophenone and MeCN and DMF were purchased from *Fluka* and dried over molecular sieves prior to use. Analytical Thin Layer Chromatography (TLC) were carried out on silica gel (SiO_2) plates 60F 254 (*Merck*); preparative column chromatography (flash-chromatography)[18] on *Merck* 60 silica gel, 0.040-0.063 mesh. The eluent mixture is given in volume ratio (v/v). Unless otherwise stated, IR spectra were recorded in $CHCl_3$ solutions and NMR spectra were recorded in $CDCl_3$ (^1H at 300 or 400 MHz; ^{13}C at 50.4 MHz). MS at 70eV, CI-MS with 2-methylpropane or NH_3 as carriers. Melting points (m.p.) were determined on a hot plate Mettler-FP-5 apparatus and are uncorrected.

Synthesis of ethyl-*N*-(t-butyloxycarbonyl)piperidine-3-carboxylate (3). In a 250 ml round bottom flask, 7 g (44.6 mmol = 6.86 ml) of ethyl piperidine-3-carboxylate (**2**) and 1.2 equiv. of *Hünig* base were dissolved in

100 ml of dry CH_2Cl_2. To this solution at 0°C with good stirring a solution of 9.81 g (44.6 mmol) of Boc-anhydride in 30 ml of dry CH_2Cl_2 was added. The reaction mixture was stirred overnight, while increasing the temperature from 0°C to r.t. After evaporation of the solvent under reduced pressure, the residue was distilled at 96°/2.10^{-2} Torr over a *vigreux* column: 10.6 g (93%) of **3** as a colourless oil. IR: 2990*m*, 2940*m*, 2860*m*, 1725*s*, 1680*s*, 1475*m*, 1470*m*, 1430*s*, 1395*m*, 1385*m*, 1370*s*, 1305*m*, 1270*s*, 1245*s*, 1150*s*, 1100*w*, 1080*w*, 1030*m*, 1000*w*, 950*w*, 905*w*, 880*w*, 860*m*, 815*w*, 710*w*, 660*w*. ^{1}H-NMR: 4.13 (*q*, *J* =7.1, CH_3CH_2); 3.91 (*br. s*, HC(3)); 2.96 (*br. s*, 1 H); 2.85-2.75 (*m*, 1 H); 2.45-2.4 (*m*, 1 H); 2.05-2.0 (*m*, 1 H); 1.8-1.5 (*m*, 4 H); 1.45 (*s*, $(CH_3)_3$); 1.25 (*t*, *J* = 7.1, CH_3CH_2). ^{13}C-NMR: 172.8 (*s*, C=O); 154.2 (*s*, C=O); 79.1 (*s*, $(CH_3)_3C$); 60.0 (*t*, CH_3CH_2); 45.3, 43.6 (2*t*, C(2), C(6)); 41.0 (*d*, C(3)); 28.0 (*q*, $(CH_3)_3C$); 26.9, 23.9 (2*t*, C(4), C(5)); 13.8 (*q*, CH_3CH_2). EI-MS: 257 (<5%, $M^{+\bullet}$), 202 (8), 200 (6), 184 (10), 156 (28), 128 (25), 84 (30), 57 (100), 56 (23), 55 (11).

Synthesis of *N*-(t-butyloxycarbonyl)piperidine-3-carboxylic acid (4). In a 250 ml round bottom flask, 6.67 g (25.94 mmol) of **3** were dissolved in 75 ml of THF. To this solution, 8 ml of 4N NaOH were added at r.t. and the reaction mixture was stirred for 48 h. After this time, the solvent was removed under reduced pressure, 2N HCl was added until pH ~2 was reached and the solution was extracted twice with CH_2Cl_2. The combined organic layers were dried over anhydrous $MgSO_4$, the solvent evaporated and the resulting colourless powder dried i. HV, yielding 5.88 g (~100%) of **4**. M.p.: 159-160°C. IR (KBr): 3180*s*, 3000*m*, 2970*m*, 2870*m*, 1730*s*, 1660*s*, 1480*s*, 1440*s*, 1390*m*, 1370*s*, 1345*m*, 1310*s*, 1270*s*, 1240*s*, 1215*s*, 1180*s*, 1150*s*, 1085*m*, 1045*w*, 1000*w*, 955*w*, 940*w*, 905*w*, 850*s*, 810*w*, 770*m*, 725*w*, 665*w*, 640*m*. ^{1}H-NMR: 10.35 (*br. s*, COOH); 4.10 (*br. s*, 1 H); 3.04 (*br. s*, 1 H); 2.9-2.8 (*m*, 1 H); 2.55-2.45 (*m*, 1 H); 2.1-2.05 (*m*, 1 H); 1.75-1.5 (*m*, 3 H); 1.45 (*s*, $(CH_3)_3C$). ^{13}C-NMR: 178.8 (*s*, C=O); 154.7 (*s*, C=O); 79.9 (*s*, $(CH_3)_3C$); 45.4, 44.0 (2*t*, C(2), C(6)); 41.0 (*d*, C(3)); 28.3 (*q*, $(CH_3)_3C$); 27.1, 24.1 (2*t*, C(4), C(5)). CI-MS: 230 (12, $[M+1]^+$), 174 (100).

Synthesis of *N*-(t-butyloxycarbonyl)piperidine-3-carboxylic acid *N*-methylanilide (5). To a solution of 4 g (12.57 mmol) of **4**, in 60 ml of dry MeCN, 1 equiv. of CME-CDI (5.484 g) was added, followed by 1.2 equiv. of *N*-methylaniline (1.598 g). The reaction mixture was heated up to 50°C under an Ar atmosphere overnight. The by-product urea was filtered off, washed with AcOEt, the solvent removed under reduced pressure and AcOEt added to the residue. The solution was washed successively with water, citric acid 5%, water, $NaHCO_3$ 10% and again water, the organic layer dried over $MgSO_4$, the solvent evaporated and the residue purified by flash-chromatography on SiO_2 (Hex/AcOEt 9:1). The amide **5** was isolated as a colourless powder (2.641 g, 66%) and dried i. HV. Other condensing reagents like DCC or CDI, under the same conditions, gave less sactisfactory results (60% and 42% respectively). M.p.: 100.5-101 °C. IR (KBr): 3000*w*, 2950*m*, 2860*m*, 1680*s*, 1655*s*, 1595*m*, 1490*s*, 1465*m*, 1450*m*, 1430*s*, 1415*s*, 1390*s*, 1365*s*, 1305*m*, 1270*m*, 1250*s*, 1175*m*, 1155*s*, 1120*m*, 1080*w*, 1070*m*, 1035*m*, 1020*w*, 995*m*, 950*w*, 940*w*, 920*w*, 885*m*, 860*w*, 790*w*, 770*m*, 700*m*, 675*w*, 645*w*. ^{1}H-NMR: 7.45-7.3 (*m*, 3 arom. H); 7.2-7.15 (*m*, 2 arom. H); 4.08 (*br. s*, 2 H); 3.23 (*s*, CH_3N); 2.9-2.85 (*m*, 1 H); 2.65-2.55 (*m*, 1 H); 2.35-2.25 (*m*, 1 H); 1.75-1.65 (*m*, 2 H); 1.6-1.5 (*m*, 1 H); 1.37 (*s*, $(CH_3)_3C$); 1.25-1.2 (*m*, 1 H). ^{13}C-NMR: 173.1 (*s*, C=O); 154.1 (*s*, C=O); 143.4 (*s*, 1 arom. C); 129.6, 127.7, 126.8 (3*d*, 5 arom. CH); 79.0 (*s*, $(CH_3)_3C$); 46.6, 46.3 (2*t*, C(2), C(4)); 43.5 (*d*, C(3)); 43.4 (*q*, CH_3N); 28.1 (*q*, $(CH_3)_3C$); 27.6, 23.9 (2*t*, C(4), C(5)). CI-MS: 319 (100, $[M+1]^+$). Anal. calc. for $C_{18}H_{26}N_2O_3$ (318.41): C 67.89, H 8.23, N 8.79; found: C 68.07, H 8.45, N 8.61.

Synthesis of 5-(t-butyloxycarbonyl)-2-(*N*-methyl-*N*-phenylamino)-1,5-diazaspiro[2.5]oct-1-en (6). To a solution of 2.5 g (7.86 mmol) of **5** in 40 ml of dry THF at 0° and under an Ar atmosphere 1.1 equiv. (5.76 ml) of LDA (1.5 M in cyclohexane, Aldrich or Fluka) were added. The solution was stirred at 0°C for 60 min., then 1.03 equiv. diphenylphosphorochloridate (DPPCl) were added via a syringe at 0°C. After 20-30 min., the ice-bath was removed and the mixture was stirred for 24 h. The solid which precipitated in the reaction mixture was filtered off under Ar and the THF solution dropped into 20 ml. of a dry DMF suspension containing 1.556 g (3 equiv.) of NaN_3. The mixture was then stirred for 3 days. After this time, Et_2O was added, the mixture filtered through a celite pad and the solvent was removed under reduced pressure. The resulting residue was dissolved in Et_2O, washed twice with $NaHCO_3$ 5%, and the aqueous layer washed with Et_2O. The combined organic layers were dried over $MgSO_4$. Removal of the solvent under reduced pressure and flash-chromatography of the resulting residue(Hex/AcOEt 19:1) led to 1.65 g (67%) of **6**, isolated as a slightly yellow solid. M.p.: 83.6-84.5 °C. IR : 2980*m*, 2940*m*, 2860*w*, 1760*s*, 1680*s*, 1600*m*, 1500*s*, 1480*m*, 1425*s*, 1370*m*, 1320*m*, 1300*m*, 1275*s*, 1250*s*, 1156*s*, 1130*m*, 1095*m*, 1030*w*, 1005*m*, 970*w*, 930*w*, 900*w*, 870*w*, 690*m*, 660*w*. ^{1}H-NMR: 7.58 (*br. s*, 1 arom. H); 7.37 (*br. s*, 2 arom. H); 7.15-7.1 (*m*, 2 arom. H); 3.9-3.55 (*m*, 2 H); 3.47 (*s*, CH_3N); 1.85-1.65 (*m*, 6 H); 1.44 (*s*, $(CH_3)_3C$). ^{13}C-NMR: 166.2 (*s*, C=N); 142.2 (*s*, 1 arom.C); 129.3, 123.7, 117.2 (3*d*, 5 arom. CH); 79.5 (*s*, $(CH_3)_3C$); 52.0, 43.8, 36.2 (3*t*, 3 CH_2); 33.5 (*q*, CH_3N); 28.3 (*q*, $(CH_3)_3C$); 24.4 (*br. t*, CH_2). At r.t. C(2) could not be detected. CI-MS: 316 (53, $[M+1]^+$), 260 (100), 218 (80).

Synthesis of bis[5-(t-butyloxycarbonyl)-2-(*N*-methyl-*N*-phenylamino)-1,5-diazaspiro[2.5]-oct-1-en] palladium dichloride complex (7). To a suspension of 380 mg (1.58 mmol) of $PdCl_2$ (40% Pd) in 5 ml of dry MeCN, a solution of 1 g (3.17 mmol) of azirine **6** in 5 ml of MeCN was added under Ar at 0°C. The reaction mixture was stirred for 12 h. After this time, the solvent was removed under reduced pressure and the residue filtered through a short column of SiO_2 (Hex/AcOEt 4:1): 2.250 g (89%) **7**. Dark-orange foam. IR: 2985*s*, 2950*m*, 2860*w*, 1790*s*, 1685*s*, 1600*s*, 1500*s*, 1450*m*, 1430*s*, 1395*m*, 1370*s*, 1325*w*, 1275*s*, 1250*s*, 1165*s*, 1135*s*, 1090*m*, 1055*m*, 1030*w*, 1000*w*, 970*w*, 940*w*, 900*w*, 870*w*, 855*w*, 835*w*, 690*m*, 660*w*. ^{1}H-NMR ((D6) DMSO/400MHz): 7.7-7.0 (*m*, 10 arom. H); 3.72 (*s*, 2 H); 3.6-3.35 (*m*, 9 H); 3.29 (*s*, 3 H); 1.85-1.3 (*m*, 26 H). ^{13}C-NMR ((D6) DMSO): 164.8, 164.2 (2*s*, C=N); 153.6, 153.2 (2*s*, 2 C=O); 141.9, 141.1 (2*s*, 2 arom. C); 129.3, 129.1, 126.1, 123.1, 120.1, 116.7 (6*d*, 10 arom. CH); 78.8 (*s*, $(CH_3)_3C$); 78.5, 59.5, 50.1, 48.1, 42.0 (5*t*, 5 CH_2); 36.2 (*br. q*, CH_3N); 31.9, 30.1 (2*t*, 2 CH_2); 27.8 (*q*, $(CH_3)_3C$); 22.2 (*t*, CH_2). ESI-MS: 653 (10, $[M\text{-}PdCl_2+Na]^+$), 338 (100, $([M\text{-}PdCl_2]/2+Na)^+$).

Decomplexation of palladium complex 7. To a well stirred solution of 2 g (2.49 mmol) of complex **7** in dry MeCN, 1.25 equiv. of (DBU) were added at 0°C. Total decomplexation was achieved in 30 min. (monitored by TLC). The solvent was removed and the residue filtered through a short column of SiO_2 (Hex/AcOEt 4:1): 745 mg (95%) azirine **6**, colourless solid.

Synthesis of *N*-[*N*-(t-butyloxycarbonyl)-1-(*N*-methyl-*N*-phenyl-thio-carbamoyl)piperidin-3-yl]-benzamide (9). To a well stirred solution of 300 mg (0.95 mmol) **6** in 3 ml of Et_2O, 1.1 equiv. (144 mg) of thiobenzoic acid (**8**) were added at 0°C. The reaction mixture was stirred overnight while raising the temperature from 0°C to r.t. The product **9**, was filtered, washed with hexane and dried i. HV: 408 mg (95%)

9; Colourless powder. M.p.: 192-193°C. IR (KBr): 3400*s*, 3060*w*, 3030*w*, 3000*w*, 2980*w*, 2930*w*, 2870*w*, 1670*s*, 1600*m*, 1580*w*, 1520*s*, 1490*s*, 1480*s*, 1470*s*, 1450*s*, 1430*s*, 1375*s*, 1295*s*, 1270*s*, 1240*s*, 1170*s*, 1140*s*, 1105*s*, 1075*m*, 1030*m*, 1010*w*, 995*m*, 955*w*, 905*m*, 870*m*, 800*w*, 770*w*, 760*m*, 735*w*, 710*s*, 700*s*, 670*w*, 640*w*. ^{1}H-NMR: 7.4-7.05 (*m*, 10 arom. H); 6.38 (*br. s*, NH); 4.7-4.6 (*m*, 1 H); 4.2-3.9 (*m*, 1 H); 3.71 (*s*, CH_3N); 3.5-3.1 (*m*, 2 H); 2.9-2.55 (*m*, 2 H); 1.40 (*br. s*, $(CH_3)_3C$); 1.25-1.2 (*m*, 2 H). ^{13}C-NMR: 204.6 (*s*, C=S); 164.8 (*s*, C=O); 147.2 (*s*, C=O); 133.3, 133.1 (2*s*, 2 arom. C); 131.3, 129.4, 127.8, 127.3, 126.5, 124.4 (6*d*, 10 arom. CH); 80.1 (*s*, $(CH_3)_3C$); 66.4 (*s*, C(3')); 53.2 (*t*, CH_2); 50.8 (*q*, CH_3N); 44.3, 33.0, (2*t*, 2 CH_2); 28.0 (*q*, $(CH_3)_3C$); 21.9 (*t*, CH_2). CI-MS: 454 (80, $[M+1]^+$), 398 (100), 354 (61). Anal. calc. for $C_{25}H_{31}N_3O_3S$ (453.59): C 66.19, H 6.88, N 9.26, S 7.06; found: C 66.33, H 6.60, N 9.15, S 7.33.

Synthesis of *N*-benzyloxycarbonyl-prolyl-1-aminocyclobutane carboxylic acid-*N*-methyl-anilide (Z-Pro-Acb-N(CH_3)Ph) (12). To a solution of 747 mg (3mmmol) of Z-L-proline (**10**) in 8 ml of dry MeCN, another solution containing 558 mg (3 mmol) of aminoazirine **11** in 2 ml of dry MeCN was added at 0°. The reaction mixture was stirred for 5 hours while raising the temperature from 0° to r.t. The solvent was removed under reduced pressure and the residue filtered through a short columm of SiO_2 (/Hex/AcOEt 1:1) to give 1.148 g (88%) of **12** isolated as a colourless amorphous solid. M.p.: 55-57°C. IR: 3420*w*, 3310*w*, 1690*s*, 1680*s*, 1650*s*, 1600*s*, 1500*s*, 1455*s*, 1415*s*, 1375*s*, 1360*s*, 1335*s*, 1310*m*, 1255*s*, 1190*m*, 1120*s*, 1090*s*, 1045*w*, 1030*w*, 990*w*, 920*w*, 875*w*, 840*w*, 700*s*, 660*w*, 610*w*. ^{1}H-NMR: 7.35-7.25 (*m*, 8 arom. H); 7.06 (*d*, J = 7, 2 arom. H); 5.08 (*d*, J = 4.3, $PhCH_2O$); 3.85 (*br., s*, 1H); 3.4-3.35 (*m*, 2H); 3.23 (*s*, MeN); 3.0 (*br., s*, 1H); 2.83 (*br., s*, 1H); 2.39 (*br., s*, 1H); 2.05-1.85 (*m*, 8H). ^{13}C-NMR: 171.1, 169.0, 144.4 (3 s, 3 C=O); 136.0, 128.9 (2 s, 2 arom. C); 128.9, 128.3, 128.0, 127.5, 126.9, 126.6 (6 d, 10 arom. CH); 67.1 (t, PhCH2O); 59.9 (d, CH); 59.7 (s, C(2) from Acb); 46.8 (t, CH2)); 39.5 (q, MeN); 33.5, 32.8, 26.8, 24.4, 15.4 (5 t, 5 CH2). CI-MS: 436 (100, $[M+1]^+$).

Synthesis of *N*-Benzyloxycarbonyl-prolyl-1-aminocyclobutane carboxylic acid (Z-Pro-Acb) (13). 800 mg (1.83 mmmol) of dipeptide **12** were dissolved at 0° in 9 ml of 3N HCl (THF/H_2O 1:1). The mixture was stirred overnight while raising the temperature from 0° to r.t. The solvent was removed under reduced pressure and the residue partitioned between H_2O and Et_2O. The layers were separated and the aqueous fraction washed twice with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, and the solvent removed to give 630 mg (≈100%) of **13** which was isolated as a colourless oil, pure enough to be used in the next step without further purification. IR: 3420*w*, 3300*w*, 3000*m*, 2960*m*, 2880*w*, 1710*s*, 1690*s*, 1680*s*, 1515*s*, 1500*s*, 1455*s*, 1420*s*, 1360*s*, 1310*m*, 1260*m*, 1240*m*, 1185*m*, 1120*s*, 1090*m*, 1040*w*, 985*w*, 920*w*, 870*w*, 700*m*, 660*w*, 610*w*. ^{1}H-NMR: 7.56 (*s*, 1 arom. H); 7.45-7.3 (*m*, 4 arom. H); 6.70 (*br. s*, NH); 5.11 (*br., s*, $PhCH_2O$); 4.30 (*br., s*, 1H); 3.5-3.45 (*m*, 2H); 2.85-2.6 (*m*, 2H); 2.25-1.8 (*m*, 8H). ^{13}C-NMR: 175.8, 172.8, 136.1 (3 *s*, 3 C=O); 128.4 (*s*, arom. C); 128.4, 128.1, 127.8 (3 *d*, 5 arom. CH); 67.4 (t, $PhCH_2O$); 60.3 (*s*, C(2) from Acb); 58.3 (*d*, CH); 47.2, 47.1, 31.1, 27.0, 24.2, 15.1 (6 *t*, 6 CH_2). CI-MS: 347 (100, $[M+1]^+$).

Synthesis of *N*-Benzyloxycarbonyl-prolyl-1-aminocyclobutanecarbonyl-4-(*N*-t-butyloxycarbonyl)-piperidine carboxylic acid-*N*-methylanilide (Z-Pro-Acb-BOC-piperidinyl-N(Me)Ph) (14). A solution of 456 mg (1.44 mmol) of aminoazirine **6** in 2 ml of dry MeCN was added at r.t. to another solution containing 500 mg (1.44 mmol) of acid dipeptide **13** in 2 ml of dry MeCN. The reaction mixture was stirred overnight. The solvent was removed under reduced presure and the resulting residue filtered over an small columm of

SiO_2 (Hex/AcOEt 1:1) to give 764 mg (80%) of tripeptide **14** isolated as a colourless oil. IR: 3420*w*, 3280*w*, 3000*m*, 2980*m*, 1680*s*, 1600*w*, 1500*m*, 1450*m*, 1425*s*, 1370*m*, 1360*m*, 1300*w*, 1275*m*, 1250*m*, 1160*s*, 1120*m*, 1090*m*, 1040*w*, 1030*w*, 1000*w*, 960*w*, 910*w*, 865*w*, 700*m*, 660*w*. ^{1}H-NMR: 7.45-7.3 (*m*, 8 arom. H); 7.2-7.15 (*m*, 2 arom. H); 6.97 (*br., s*, NH); 5.15 (*s*, $PhCH_2O$); 4.55-4.5 (*m*, 1H); 4.2-4.15 (*m*, 1H); 4.09 (*br., s*, 1H); 3.5-3.45 (*m*, 2H); 3.27, 3.24 (2 *s*, 4 H); 2.65 (*br., s*, 1H); 2.4-1.7 (*m*, 12H); 1.6-1.55 (*m*, 2H); 1.43 (*s*, 9H)* . ^{13}C-NMR** : 171.8, 169.2, 155.7, 155.1, 154.9 (5 s, 5 C=O); 145.0, 136.1 (2 s, 2 arom. C); 129.2, 129.1, 128.4, 128.0, 127.0, 126.9 (6 d, 10 arom. CH); 79.7 (s, $(CH_3)_3C$); 67.2 (t, $PhCH_2O$); 60.6 (d, CH); 59.5, 58.5 (2 s, C(2) from Acb and C(4) from piperidine); 49.1, 48.5, 46.9, 42.8 (4 t, 4 CH_2); 40.5 (q, MeN); 31.2, 30.9, 30.1 (3 t, 3 CH_2); 28.3 (q, $(CH_3)_3C$); 24.4, 20.5, 15.3 (3 t, 3 CH_2). CI-MS: 555 [100 (M-$PhCH_2O$+1)]$^+$.

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* Doubling of the signal observed
** Doubling of some signals observed