An Improved Stereoselective Synthesis of L-Alanosine

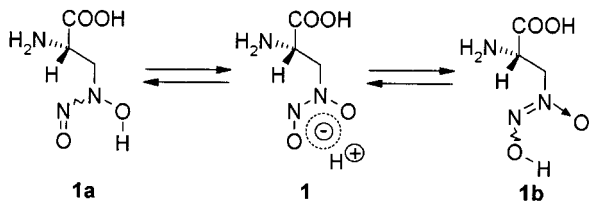
Paolo Strazzolini,*[a] Maria Grazia Dall'Arche,[a] Maura Zossi,[a] and Andrea Pavsler[a]

Keywords: Amino acids / Antitumour agents / Asymmetric synthesis / Chiral pool / Structure elucidation

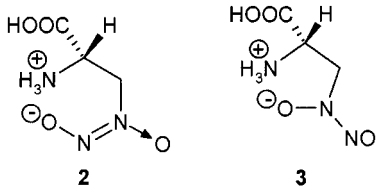
An improved, stereoselective synthesis of the natural, non-proteogenic amino acid L-alanosine has been developed, starting from the readily available and cheap substrate L-serine, in six steps and 49% overall yield. The process is very efficient, is suitable for large-scale production, and affords L-alanosine with properties comparable with those of the natural substance. In addition, the structural assignment concerning some previously reported synthetic alkylated derivatives of the natural amino acid has been definitively confirmed.

Introduction

L-Alanosine [(*S*)-3-(hydroxynitrosoamino)alanine (**1**); Scheme 1],[1] which is an antitumour antibiotic produced by fermentation of *Streptomyces alanosinicus* n. sp. ATCC 15710,[2] has the structure of an α-amino acid with an L configuration, and was the first naturally occurring substance found to contain an *N*-nitroso-hydroxylamino group.[3] Like many other amino acids, **1** exists as a zwitterion, where the role of the more acidic function ($pK_a < 1$) is taken by the *N*-nitroso-hydroxylamino group,[3] which is known to exist as an equilibrium of two tautomers,[4] namely the hydroxynitrosoamino (**1a**, Scheme 1) and the diimide *N*-oxide (**1b**) forms.[5] In the solid state, **1** has been shown to be present as the diimide oxide tautomer **1b**,[6] and this configuration has been considered likely to be favoured also in solution, taking into account the reported structural assignment concerning a number of alkylation products derived from **1** (see below).[7] This circumstance might explain the preferential formation of a six-membered



Scheme 1



Scheme 2

cyclic zwitterion (**2**, Scheme 2), which is more stable than the corresponding five-membered one (**3**), and could also be responsible for the difficulties encountered in *N*-acylation of **1**,[7] as well as for the anomalous behaviour observed, compared with that shown by other α-amino acids.[8]

L-Alanosine exhibits a number of interesting biological and pharmacological properties,[1] including antiviral and antimicrobial activity, immunosuppressive and anti-arthritis action, as well as insect growth regulatory function,[9] but its anti-cancer activity, which is associated with its ability to interfere with the metabolism of aspartic acid,[10] has appeared since the beginning to be by far the most attractive one. In view of the renewed interest concerning the applications of **1** in cancer therapy,[11] and in the course of a study aimed at obtaining new L-alanosine derivatives with potential anti-cancer activity, we needed to be able to produce large amounts of **1** by chemical synthesis as we were unavailable to obtain it by a fermentation process.[3]

A number of syntheses of **1** have been proposed in the literature to date; however, the majority either only produce the amino acid in its racemic form,[12] or require tedious enantiomeric separations in order to afford the active isomer.[13] Recently, a stereoselective process taking the starting material from the chiral pool[14] appeared as a Japanese patent,[15] but it does not seem promising in view of a large-scale application. We therefore decided to develop a method for producing **1** which could offer acceptable safety and

[a] Department of Chemical Sciences and Technologies, University of Udine,
Via del Cotonificio 108, 33100 Udine, Italy
Fax: (internat.) + 39-0432-558-803
E-mail: strazzolini@dstc.uniud.it

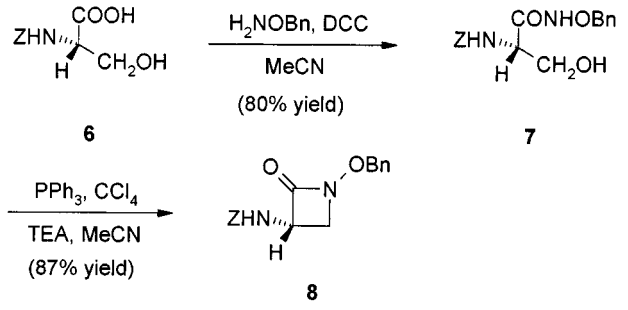
  DOI: 10.1002/ejoc.200400508

economical standards, as well as being suitable for potential scale-up.

Results and Discussion

Among the variety of methods available for the synthesis of pure stereoisomers of chiral amino acids, the strategy based on the elaboration of α-amino acid precursors appears, when applicable, the most convenient in terms of feasibility, simplicity and low costs.[16] In this context, the naturally occurring chiral amino acid serine represents, for the organic chemist, an attractive synthetic template as both isomers are readily available and cheap starting materials.[17] Nevertheless, many of the possible synthetic approaches starting from L-serine (**4**) that were potentially able to afford the important intermediate (*S*)-2-amino-3-(hydroxyamino)propanoic acid (**5**) proved to be inadequate,[18] due to the risk of incurring competitive eliminations,[19] as well as unwanted epimerisations and intramolecular cyclisations;[18] moreover, even the use of reactive derivatives of **4**, like activated aziridines[20] or β-lactones,[21] often resulted in the formation of isomeric mixtures. For these reasons, the more straightforward strategy suggested in a recent patent,[15] based on the intermediacy of an azetidinone derivative of **4**, even if not satisfying as such (vide infra), seemed to prove the most promising synthetic approach (Scheme 3).

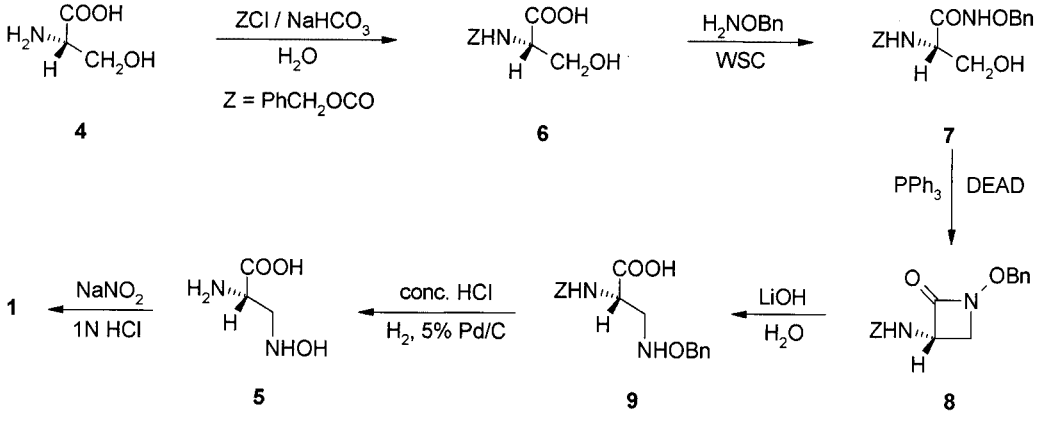
Therefore, L-serine (**4**) was converted, in nearly quantitative yield and according to a previously described procedure,[22] into the corresponding *N*-(phenylmethyloxy)carbonyl derivative **6**, which was, in turn, transformed into the corresponding *O*-protected hydroxylamide **7**. The conditions reported in the literature for this reaction (Scheme 3)[15] involve the use of expensive water-soluble carbodiimides (WSCs), which is impractical for large-scale processes. Even though the employment of the much more convenient dicyclohexylcarbodiimide (DCC) had been discouraged for this application,[23] it was possible to set up suitable and very simple conditions to perform the reaction between **6** and *O*-(phenylmethyl)hydroxylamine, using DCC in MeCN (Scheme 4) to obtain **7** in very good isolated yield (80%), essentially comparable with those reported employing WSCs.[23]



Scheme 4

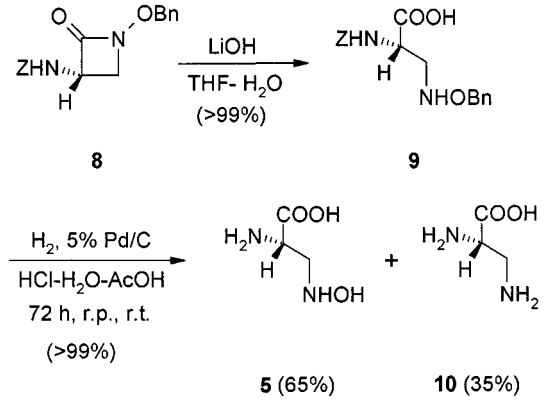
When we tried to repeat the cyclisation reaction in order to prepare the azetidinone intermediate **8** (Scheme 3),[15] using classical Mitsunobu conditions,[24] in preliminary experiments we succeeded in obtaining the desired compound, but in quite unsatisfactory yield due to difficulties encountered in separating it from the reduced counterpart ($DEADH_2$) of diethyl azodicarboxylate (DEAD). This separation, which requires column chromatography,[25] is not suitable for large-scale production; moreover, it must be pointed out that DEAD is a rather expensive, potentially unstable chemical whose use in large batches is best avoided for safety reasons. Therefore, we looked for alternative conditions to perform the cyclisation and decided to apply the elegant reaction proposed by Miller,[23] which consists of treating **7** with PPh_3, CCl_4 and TEA in MeCN (Scheme 4), and affords the azetidinone **8** in very high isolated yield after a simple workup requiring just a fast passage through a SiO_2 column in order to separate the formed $OPPh_3$. Alternate conditions that do not require any purification by SiO_2 treatment have also been reported.[26]

Subsequent hydrolytic ring-opening to give quantitatively the protected hydroxyamino derivative **9** (Scheme 5), performed with LiOH in THF/H_2O,[27] proceeded smoothly in accordance with the literature,[15] whereas the following de-



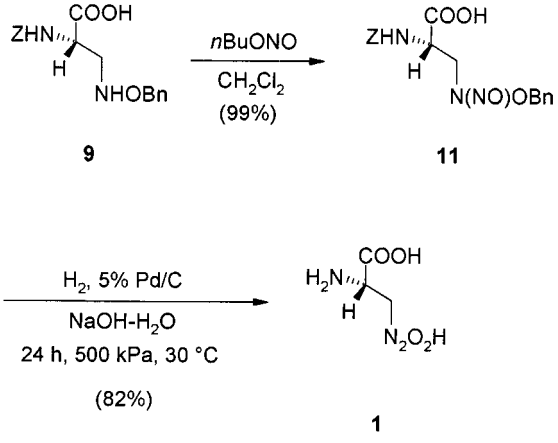
Scheme 3

protection step was somehow disappointing. In fact, when an aqueous HCl/AcOH solution of **9** was hydrogenolysed in the presence of 5% Pd/C, at room temperature and atmospheric pressure, careful monitoring of the composition of the reaction mixture (^{1}H NMR) showed that the complete conversion of substrate **9** gave the expected fully deprotected intermediate **5** (Scheme 5), accompanied by a consistent amount (35%) of the over-reduction product (*S*)-2,3-diaminopropanoic acid (**10**). The hydrogenolytic susceptibility of the N−O bond in hydroxylamines has been well documented.[19,27,28]



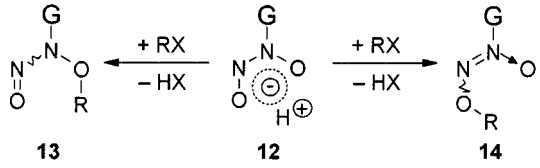
Scheme 5

In order to overcome this drawback, and also taking into account the observed,[7] unexpected resistance exhibited by the *N*-nitroso-hydroxylamine function in the course of hydrogenolytic dealkylation, we decided to anticipate the nitrosation step, which was smoothly accomplished under aprotic conditions[29] by addition of a moderate excess of *n*-butyl nitrite (*n*BuONO) in CH_2Cl_2, affording the intermediate **11** in quantitative yield (Scheme 6). Surprisingly enough, compound **11** (as its sodium salt) could be eventually deprotected under hydrogenolytic conditions (5% Pd/C, H_2O, 24 h, at moderate pressure and 30 °C) without suffering any over-reduction to give L-alanosine (**1**) in fairly good yield (Scheme 6) and with properties comparable with those of the natural amino acid and an undetectable ash content. The optical purity of **1** (*S* configuration) was confirmed by measuring its specific rotation {$[\alpha]_D^{20} = -45.0$ (c = 0.5, 0.1 M NaOH)}, which was found to be equal to the values previously reported.[3,13c]

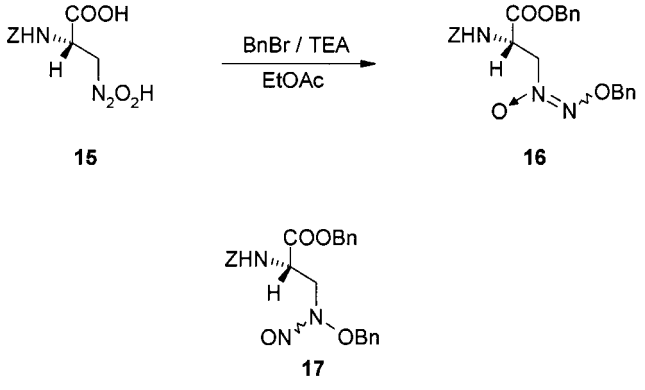


Scheme 6

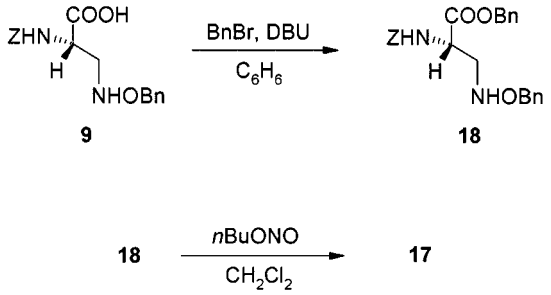
The availability of the protected intermediate **9** offered the possibility to unequivocally verify the correct structural assignment previously attributed to some products obtained by alkylation of a masked derivative of **1**.[7] It is well-known[4,5] that alkylation products of *N*-nitroso-hydroxylamines (**12**, Scheme 7) may exist in two isomeric arrangements, namely **13** and **14**. In the course of a study directed towards the synthesis of some derivatives of **1**,[7] (*S*)-3-(hydroxy-nitrosoamino)-*N*-[(phenylmethyloxy)-carbonyl]alanine (*N*-Z-L-alanosine, **15**) was protected at the acidic functions by dialkylation with alkyl halides in the presence of TEA. In one case, when the alkylating agent was phenylmethyl bromide, the structure assigned to the predominant, if not exclusive, isomer formed was, on the basis of chemical and physico-chemical properties, the diimide *N*-oxide one (**16**, Scheme 8), rather than the *N*-nitroso form **17**. In order to definitively confirm that attribution, compound **9** was esterified with phenylmethyl bromide in benzene and in the presence of DBU (Scheme 9),[30] and the obtained dibenzylated derivative **18** was subsequently *N*-nitrosated under the aprotic conditions reported above, to afford the previously unknown *N*-nitroso derivative **17**, whose properties proved very different to those of compound **16**, thus unequivocally confirming the structural assignment given in the literature.[7]



Scheme 7



Scheme 8



Scheme 9

Conclusion

In view of the renewed interest concerning L-alanosine and its applications in cancer therapy, an improved, stereoselective chemical synthesis has been developed, allowing the isolation of the pure amino acid starting from cheap and readily available L-serine in six steps and 49% overall yield. This process is characterised by its simplicity and low cost, and is safe, eco-friendly and potentially suitable for large-scale applications. Moreover, the present work has firmly established the correctness of the structural assignment concerning some previously reported alkyl derivatives of L-alanosine, thus casting more light on the chemistry of *N*-nitroso-hydroxylamines.

Experimental Section

General Remarks: Unless otherwise specified, reagents and solvents are commercially available (Aldrich Italia, Milano, Italy) and were used as received. Commercial PPh_3 was carefully dried prior to use by keeping it over P_2O_5, in vacuo at 70 °C for 1 h. Anhydrous CCl_4 and CH_3CN were obtained by heating under reflux over P_2O_5, followed by distillation under an inert atmosphere. Anhydrous NEt_3 (TEA) was obtained by distillation after prolonged treatment with CaH_2. *O*-(Phenylmethyl)hydroxylamine was prepared as described elsewhere.[31] Compounds **5**, **7**, **8** and **9** have been already described in the literature (see below). TLC analyses and column chromatography were performed on silica gel 60 from Merck (Darmstadt, Germany) under suitable conditions. The course of all the described reactions was monitored by TLC and by a parallel accurate ^1H NMR quantitative evaluation. Melting points (uncorrected) were previously determined in open-ended capillary tubes by using a Mettler FP 61 automatic system, and subsequently visually confirmed by a Büchi 512 apparatus. Elemental analyses were obtained by a Carlo−Erba CHN/OS 1106 analyzer for all isolated compounds and found to be satisfactory. The ash content in **1** was determined by weighing the residue left by a suitable sample after mineralisation (1 h at 800 °C). Optical activities were measured at 20 °C on a Atago Polax-D polarimeter at 589 nm in a 1.0-dm tube. IR spectra were recorded on a Nicolet FTIR Magna 550 spectrophotometer using the KBr technique. Unless otherwise indicated, ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker AC-200 spectrometer at 200 and 50 MHz, respectively. The proton chemical shifts are reported in ppm on the δ scale relative to TMS as an internal reference (δ = 0.00 ppm); the carbon chemical shifts are reported in ppm relative to the central line of the $CDCl_3$ triplet (δ = 77.00 ppm). When D_2O was the solvent, spectral references were used and are indicated where appropriate. Coupling constants are given in Hz. Some ^1H NMR multiplets are characterised by the term app (apparent): this refers only to their appearance and may be an oversimplification. MS measurements were carried out with a Fisons TRIO-2000 apparatus, working in the positive-ion electron impact mode (70 eV), by direct introduction of the sample into the ion source and heating from 50 up to 300 °C. The five most intense peaks and the molecular peak (when detectable) for each individual compound with bracketed intensity values are reported.

(*S*)-*N*-[(Phenylmethyloxy)carbonyl]serine (6): Compound **6** was prepared in 87% yield, according to a previously described procedure.[22]

(*S*)-{2-[(Phenylmethyloxy)amino]-1-(hydroxymethyl)-2-oxoethyl}carbamic Acid Phenylmethyl Ester (7):[25] A solution of *N*,*N*-dicyclohexylcarbodiimide (DCC, 4.28 g, 21.0 mmol) in CH_3CN (80 mL) was slowly added dropwise, at 0 °C, into a well-stirred solution of **6** (4.78 g, 20.0 mmol) and *O*-(phenylmethyl)hydroxylamine (2.46 g, 20.0 mmol) in CH_3CN (80 mL), and the reaction mixture was stirred for 4 h at 0 °C and overnight at room temperature. After completion of the reaction, the obtained white precipitate (*N*,*N*-dicyclohexylurea; DCU) was separated by filtration and the resulting clear reaction mixture concentrated to a small volume under reduced pressure. After the addition of Et_2O, a crystalline product formed, which was collected by filtration, washed with Et_2O and dried for 30 min at 40 °C under vacuum. White solid (80% yield): m.p. (from CH_3CN/Et_2O) 127−129 °C. $[\alpha]_D^{20}$ = −27.5 (c = 2.0, CH_3OH). IR (pellet): $\tilde{\nu}$ = 3425 br. m, 3230 m, 1670 s, 1539 s, 1456 w, 1294 m, 1247 m, 1100 w, 1073 w, 1044 w, 914 w, 737 s, 696 m, 637 w cm^{-1}. ^1H NMR: δ = 9.66 (s, 1 H, O−N*H*−C=O), 7.38−7.25 (m, 10 H, C_6H_5), 5.96 (d, J = 7.8 Hz, 1 H, C−N*H*−C=O), 5.01 (s, 2 H, Ph−CH_2), 4.83 (s, 2 H, Ph−CH_2), 4.11 (m, 1 H, CH_2−C*H*), 3.86 (m, 1 H, O*H*), 3.61 (m, 2 H, CH−CH_2) ppm. ^{13}C NMR: δ = 168.5 (O−N−C=O), 156.5 (N−C=O), 135.7, 134.8, 129.3, 128.9, 128.6 (2 overlapped signals), 128.3, 128.0, 78.4, 67.4, 62.3, 53.5 ppm. MS (50 °C): m/z = 91 (100), 79 (56), 108 (55), 77 (43), 107 (41). $C_{18}H_{20}N_2O_5$ (344.37): calcd. C 62.78, H 5.85, N 8.14; found C 62.65, H 5.86, N 8.12.

(*S*)-[1-(Phenylmethyloxy)-2-oxo-3-azetidinyl]carbamic Acid Phenylmethyl Ester (8):[23,25] A solution of PPh_3 (4.51 g, 17.2 mmol) in dry CH_3CN (33 mL) was added dropwise, at room temperature and under an inert atmosphere, to a stirred solution of **7** (5.64 g, 16.4 mmol) in dry CH_3CN (50 mL), containing CCl_4 (1.70 mL, 17.6 mmol) and anhydrous triethylamine (TEA, 3.50 mL, 25.1 mmol). The resultant clear reaction mixture was stirred for 24 h at room temperature. After completion of the reaction, the obtained white precipitate (TEA·HCl and Ph_3PO) was separated by filtration and the resulting clear reaction mixture concentrated under reduced pressure. The residue was redissolved in EtOAc (350 mL), washed with 10% aqueous Na_2SO_4 (3 × 170 mL), dried over anhydrous Na_2SO_4, filtered, concentrated to a small volume at reduced pressure and passed through a short SiO_2 column by elution with hexane/EtOAc (1:1, v/v). The fractions containing the product of interest were combined and the solvent was evaporated off, yielding a solid residue which was collected and dried for 30 min at 40 °C under vacuum. White solid (87% yield): m.p. (from hexane/EtOAc) 91−92 °C. $[\alpha]_D^{20}$ = −12.5 (c = 1.0, CH_3OH). IR (pellet): $\tilde{\nu}$ = 3309 br. m, 3065 w, 2967 w, 1802 s, 1703 s, 1543 s, 1455 w, 1337 w, 1272 s, 1227 w, 1132 w, 1077 m, 970 s, 913 w, 854 w, 755 m, 699 w, 578 w cm^{-1}. ^1H NMR: δ = 7.47−7.27 (m, 10 H, C_6H_5), 5.57 (d, J = 6.8 Hz, 1 H, N*H*), 5.08 (s, 2 H, Ph−CH_2), 4.95

(s, 2 H, Ph−CH_2), 4.55 (sym m, J = 6.8, J_{BX} = 4.5, J_{AX} = 2.0 Hz, 1 H, CH_2−CH), 3.51 (app t, J_{AB} = 4.8, J_{BX} = 4.5 Hz, 1 H, CH−CH_2), 3.24 (dd, J_{AB} = 4.8, J_{AX} = 2.0 Hz, 1 H, CH−CH_2) ppm. ^{13}C NMR: δ = 162.4 (C=O), 155.6 (N−C=O), 135.8, 134.6, 129.2, 129.1, 128.7, 128.5, 128.3, 128.1, 77.9, 67.3, 54.1, 53.6 ppm. MS (50 °C): m/z = 91 (100), 92 (9), 77 (7), 108 (7), 79 (6). $C_{18}H_{18}N_2O_4$ (326.35): calcd. C 66.25, H 5.56, N 8.58; found C 66.14, H 5.58, N 8.57.

(*S*)-3-[(Phenylmethyloxy)amino]-*N*-[(phenylmethyloxy)carbonyl]-alanine (9):[15] Compound **9** was obtained according to a described procedure.[27] Solid LiOH·H_2O (0.90 g, 21.4 mmol) was added in one portion to a stirred solution of **8** (4.52 g, 13.9 mmol) in THF (70 mL) and H_2O (35 mL) and the clear reaction mixture was kept at room temperature overnight under an inert atmosphere. After completion of the reaction, the mixture was acidified by addition of 2 M HCl (33 mL), and subsequently extracted with EtOAc (3 × 65 mL). The combined organic phase was washed with H_2O (100 mL), 10% aqueous Na_2SO_4 (2 × 100 mL), dried over anhydrous Na_2SO_4, filtered and concentrated to dryness, yielding a solid residue which was collected and dried for 30 min at 60 °C under vacuum. Yellowish solid (>99% yield): m.p. (from EtOAc) 73−75 °C. $[\alpha]_D^{20}$ = −20.0 (c = 1.0, CH_3OH). IR (pellet): $\tilde{\nu}$ = 3309 br. m, 3039 w, 2940 w, 1730 s, 1524 s, 1455 m, 1397 m, 1334 w, 1245 s, 1132 w, 1061 m, 915 w, 786 w, 740 s, 699 m, 578 w cm^{-1}. ^{1}H NMR: δ = 8.18 (br. s, 2 H, COOH + O−NH), 7.45−7.14 (m, 10 H, C_6H_5), 5.83 (d, J = 7.6 Hz, 1 H, O=C−NH), 5.09 (s, 2 H, Ph−CH_2), 4.63 (s, 2 H, Ph−CH_2), 4.50 (sym m, J = 7.6, J_{AX} = 5.1, J_{BX} = 4.1 Hz, 1 H, CH_2−CH), 3.43 (dd, J_{AB} = 14.0, J_{BX} = 4.1 Hz, 1 H, CH−CH_2), 3.21 (dd, J_{AB} = 14.0, J_{AX} = 5.1 Hz, 1 H, CH−CH_2) ppm. ^{13}C NMR: δ = 175.0 (C=O), 156.2 (N−C=O), 137.3, 135.9, 128.5, 128.4, 128.4, 128.2, 128.1, 128.1, 75.9, 67.2, 52.3, 52.1 ppm. MS (80 °C): m/z = 91 (100), 108 (42), 79 (37), 77 (35), 107 (32), 344 (<1) [M^+]. $C_{18}H_{20}N_2O_5$ (344.37): calcd. C 62.78, H 5.85, N 8.14; found C 62.69, H 5.87, N 8.13.

Hydrogenolysis of (*S*)-3-[(Phenylmethyloxy)amino]-*N*-[(phenylmethyloxy)carbonyl]alanine (9):[15] 5% Pd/C (35 mg) was added to a solution of **9** (350 mg, 1.00 mmol) in AcOH (2.5 mL) and 2.0 M HCl (3.0 mL) and the mixture was vigorously stirred under a H_2 atmosphere, at room temperature and pressure, until complete conversion of the starting material was evidenced by TLC and ^{1}H NMR analyses (72 h). The reaction mixture was then filtered in order to eliminate the catalyst, diluted with 2.0 M HCl (1.0 mL) and concentrated to dryness, yielding a solid residue which was collected and dried for 60 min over KOH pellets at 60 °C under vacuum. A suitable sample was then dissolved in D_2O and analysed by ^{1}H and ^{13}C NMR spectroscopy, which showed the presence of a mixture of two substances, in the ratio 65:35 (mol/mol). In fact, besides the expected deprotected hydroxyamino derivative **5** (65%), obtained as the corresponding dihydrochloride and identified on the basis of its NMR properties (vide infra), the over-reduction product (*S*)-2,3-diaminopropanoic acid (**10**, 35%) was also present, also in its dihydrochloride form, which was identified by comparison with a commercial authentic sample.

(*S*)-2-Amino-3-(hydroxyamino)propanoic Acid (5) Dihydrochloride:[13c,15] ^{1}H NMR (D_2O, spectral reference = 2853.00 Hz): δ = 4.42 (dd, J_{AX} = 6.4, J_{BX} = 5.3 Hz, 1 H, CH_2−CH), 3.38 (dd, J_{AB} = 14.6, J_{BX} = 5.3 Hz, 1 H, CH−CH_2), 3.73 (dd, J_{AB} = 14.6, J_{AX} = 6.4 Hz, 1 H, CH−CH_2) ppm. ^{13}C NMR (D_2O, spectral reference = 3313.00 Hz): δ = 171.7 (C=O), 51.8 (CH_2), 51.5 (CH) ppm.

(*S*)-2,3-Diaminopropanoic Acid (10) Dihydrochloride: ^{1}H NMR (D_2O, spectral reference = 2853.00 Hz): δ = 4.29 (dd, J_{AX} = 5.9, J_{BX} = 7.8 Hz, 1 H, CH_2−CH), 3.48 (dd, J_{AB} = 13.6, J_{BX} = 7.8 Hz, 1 H, CH−CH_2), 3.42 (dd, J_{AB} = 13.6, J_{AX} = 5.9 Hz, 1 H, CH−CH_2) ppm. ^{13}C NMR (D_2O, spectral reference = 3313.00 Hz): δ = 171.4 (C=O), 52.1 (CH_2), 40.7 (CH) ppm.

(*S*)-3-[Nitroso(phenylmethyloxy)amino]-*N*-[(phenylmethyloxy)-carbonyl]alanine (11): Butyl nitrite (3.10 g, 30.0 mmol) was added dropwise to a solution of **9** (3.44 g, 10.0 mmol) in CH_2Cl_2 (6.5 mL) and the resultant clear, yellow mixture was stirred for 1 h at room temperature in the dark. After completion of the reaction, the solvent was evaporated off under reduced pressure and the residue was treated with Et_2O until crystallisation occurred. The obtained product was collected by filtration, washed with Et_2O and dried for 30 min at 40 °C under vacuum. Yellowish solid (99% yield): m.p. (from Et_2O) 79−80 °C. $[\alpha]_D^{20}$ = −15.0 (c = 1.0, $CHCl_3$). IR (pellet): $\tilde{\nu}$ = 3420 m, 3039 br. s, 1757 m, 1743 m, 1670 s, 1531 s, 1448 m, 1425 m, 1408 w, 1311 w, 1278 m, 1199 s, 1117 w, 1071 m, 1015 w, 909 w, 876 w, 753 m, 744 w, 697 s, 611 w cm^{-1}. ^{1}H NMR: δ = 10.39 (br. s, 1 H, COOH), 7.36−7.27 (m, 10 H, C_6H_5), 5.59 (d, J = 7.5 Hz, 1 H, NH), 5.07 (s, 2 H, Ph−CH_2), 4.94 (s, 2 H, Ph−CH_2), 4.64 (sym m, J = 7.5, J_{AX} = 4.3, J_{BX} = 3.9 Hz, 1 H, CH_2−CH), 4.53 (dd, J_{AB} = 13.6, J_{BX} = 3.9 Hz, 1 H, CH−CH_2), 4.39 (dd, J_{AB} = 13.6, J_{AX} = 4.3 Hz, 1 H, CH−CH_2) ppm. ^{13}C NMR: δ = 173.0 (C=O), 155.8 (N−C=O), 135.6, 133.3, 129.6, 129.4, 128.7, 128.5, 128.3, 128.0, 77.4, 67.5, 54.1, 51.8 ppm. MS (50 °C): m/z = 91 (100), 108 (86), 107 (67), 79 (46), 77 (26), 373(<1) [M^+]. $C_{18}H_{19}N_3O_6$ (373.37): calcd. C 57.91, H 5.13, N 11.26; found C 57.79, H 5.14, N 11.25.

(*S*)-3-(Hydroxynitrosoamino)alanine (1, L-alanosine):[3,13c] 5% Pd/C (444 mg) was added to a solution of **11** (1.87 g, 5.00 mmol) in 0.5 M NaOH (10.0 mL) and the mixture was vigorously stirred under a H_2 atmosphere, at 30 °C and 500 kPa, until complete conversion of the starting material was evidenced by TLC and ^{1}H NMR analyses (24 h). The reaction mixture was then filtered in order to eliminate the catalyst, concentrated to ca. 5 mL, filtered again and the pH (7.6) corrected to 5.4 by careful addition of 1 M HCl. Absolute EtOH (10 mL) was added to the solution and the resulting cloudy mixture was left standing at 0 °C for 12 h, producing a precipitate. The gummy residue was separated from the mother liquor, treated with additional absolute EtOH (5 mL) and triturated until a crystalline solid was obtained, which was filtered, washed with Et_2O, collected and dried over P_2O_5 for 30 min at 60 °C under vacuum. Brownish solid (82% yield): m.p. (from EtOH) 184−187 °C (decomp.). $[\alpha]_D^{20}$ = −45.0 (c = 0.5, 0.1 M NaOH). IR (pellet): $\tilde{\nu}$ = 3437 w, br., 3073 br. m, 1642 s, 1517 m, 1476 w, 1393 m, 1343 w, 1285 w, 1257 m, 1163 w, 1092 w, 1077 m, 952 s, 920 w, 864 m, 814 w, 664 m, 625 w, 549 w cm^{-1}. ^{1}H NMR (2 M NaOD in D_2O, spectral reference: 2853.00 Hz): δ = 3.49 (dd, J_{AX} = 5.0, J_{BX} = 8.0 Hz, 1 H, CH_2−CH), 3.80 (dd, J_{AB} = 13.0, J_{AX} = 5.0 Hz, 1 H, CH−CH_2), 3.87 (dd, J_{AB} = 13.0, J_{BX} = 8.0 Hz, 1 H, CH−CH_2) ppm. ^{13}C NMR (2 M NaOD in D_2O, spectral reference = 3313.00 Hz): δ = 181.7 (C=O), 65.4 (CH_2), 56.2 (CH) ppm. MS: not determined. $C_3H_7N_3O_4$ (149.11): calcd. C 24.17, H 4.73, N 28.18; found C 24.12, H 4.75, N 28.10.

(*S*)-3-[(Phenylmethyloxy)amino]-*N*-[(phenylmethyloxy)carbonyl]-alanine Phenylmethyl Ester (18): Compound **18** was obtained essentially according to a described procedure.[30] A solution of phenylmethyl bromide (1.11 g, 6.50 mmol) in benzene (15 mL) was added dropwise to a solution consisting of **9** and 1,8-diazabicy-

clo[5.4.0]undec-7-ene (DBU; 0.83 g, 5.40 mmol) in benzene (35 mL) and the obtained mixture was stirred for 24 h at room temperature. After this time, the resulting suspension was diluted with Et_2O (100 mL), freed from formed DBU·HBr by filtration, and the clear filtrate was washed with H_2O (100 mL), 1 M HCl (100 mL), 5% aqueous $NaHCO_3$ (100 mL), and 10% aqueous Na_2SO_4 (100 mL). It was then dried over anhydrous Na_2SO_4, filtered and the solvent evaporated off. The obtained oily residue was submitted to column chromatography (SiO_2, hexane/EtOAc), affording pure **18**. Colourless liquid (57% yield). $[\alpha]_D^{20}$ = not determined. IR (film): $\tilde{\nu}$ = 3427 br. m, 3348 br. m, 3039 w, 2927 m, 1723 s, 1497 m, 1454 w, 1386 m, 1346 w, 1200 s, 1168 w, 1055 s, 1027 w, 914 w, 824 w, 741 m, 700 m, 597 w cm^{-1}. ^1H NMR: δ = 7.42−7.16 (m, 15 H, C_6H_5), 5.79 (d, J = 7.8 Hz, 1 H, O=C−N*H*), 5.74 (br. s, 1 H, O−N*H*), 5.12 (s, 2 H, Ph−CH_2), 5.08 (s, 2 H, Ph−CH_2), 4.61−4.46 (m, 3 H, CH_2−C*H* + Ph−CH_2−O−N), 3.47 (dd, J_{AB} = 14.0, J_{BX} = 3.6 Hz, 1 H, CH−CH_2), 3.22 (dd, J_{AB} = 14.0, J_{AX} = 4.4 Hz, 1 H, CH−CH_2) ppm. ^{13}C NMR: δ = 171.1 (C=O), 155.9 (N−C=O), 137.1, 136.1, 135.3, 128.5 (3 overlapped signals), 128.4, 128.31, 128.28, 128.2, 128.1, 127.9, 76.0, 67.2, 67.0, 52.9, 52.7 ppm. MS (100 °C): *m*/*z* = 91 (100), 181 (29), 92 (28), 148 (25), 108 (16), 435 (<1) [M^+]. $C_{25}H_{26}N_2O_5$ (434.49): calcd. C 69.11, H 6.03, N 6.45; found C 69.08, H 6.04, N 6.43.

(*S*)-3-[(Phenylmethyloxy)nitrosoamino]-*N*-[(phenylmethyloxy)-carbonyl]alanine Phenylmethyl Ester (17): Butyl nitrite (0.89 g, 5.70 mmol) was added dropwise to a solution of **18** (0.81 g, 1.90 mmol) in CH_2Cl_2 (1.5 mL) and the resultant clear, yellow mixture was stirred for 1 h at room temperature in the dark. After completion of the reaction, the solvent was evaporated off at reduced pressure and the residue was submitted to column chromatography (SiO_2, hexane/Et_2O), affording pure solid **17**, which was dried for 30 min at 40 °C under vacuum. Yellow solid (89% yield): m.p. (from hexane/Et_2O) 63−65 °C. $[\alpha]_D^{20}$ = +15.0 (*c* = 1.0, CH_3OH). IR (film): $\tilde{\nu}$ = 3348 br. m, 3039 w, 2954 w, 1736 s, 1715 s, 1531 s, 1442 m, 1374 w, 1310 w, 1283 m, 1235 w, 1109 w, 1067 m, 1029 w, 960 w, 910 m, 875 w, 741 m, 697 m, 602 w cm^{-1}. ^1H NMR: δ = 7.38−7.21 (m, 15 H, C_6H_5), 5.51 (d, J = 7.4 Hz, 1 H, N*H*), 5.08 (s, 2 H, Ph−CH_2), 5.08 (d, J_{AB} = 12.1 Hz, 1 H, Ph−CH_2), 5.01 (d, J_{AB} = 12.1 Hz, 1 H, Ph−CH_2), 4.90 (s, 2 H, Ph−CH_2), 4.81−4.74 (m, 3 H, CH−CH_2 + CH_2−C*H*) ppm. ^{13}C NMR: δ = 168.9 (C=O), 155.5 (N−C=O), 135.8, 134.5, 133.5, 129.6, 129.3, 128.63 (2 overlapped signals), 128.61, 128.5 (2 overlapped signals), 128.2, 128.0, 77.3, 68.0, 67.3, 54.3, 52.2 ppm. MS (100 °C): *m*/*z* = 91 (100), 108 (78), 107 (70), 106 (64), 105 (64), 463 (<1) [M^+]. $C_{25}H_{25}N_3O_6$ (463.49): calcd. C 64.79, H 5.44, N 9.07; found C 64.58, H 5.45, N 9.04.

(*S*)-*N*-[(Phenylmethyloxy)carbonyl]-3-[(phenylmethyloxy)-*NNO*-azoxy]alanine Phenylmethyl Ester (16): Compound **16** was prepared as previously reported.[7] Brownish solid (48% yield): m.p. (from hexane/Et_2O) 78−80 °C. $[\alpha]_D^{20}$ = not determined. IR (film): $\tilde{\nu}$ = 3330 br. m, 3035 w, 2934 w, 1741 s, 1689 s, 1521 s, 1456 w, 1413 w, 1392 w, 1348 m, 1290 w, 1221 s, 1053 m, 1021 w, 955 w, 913 w, 782 w, 740 m, 697 m, 588 w cm^{-1}. ^1H NMR: δ = 7.42−7.23 (m, 15 H, C_6H_5), 5.77 (d, J = 7.5 Hz, 1 H, N*H*), 5.23−5.08 [m (2 overlapped signals of AB systems), 4 H, Ph−CH_2], 5.09 (s, 2 H, Ph−CH_2), 4.74 (sym m, J = 7.5, J_{AX} = 4.1, J_{BX} = 3.7 Hz,1 H, CH_2−C*H*), 4.61 (dd, J_{AB} = 13.3, J_{BX} = 3.7 Hz, 2 H, CH−CH_2), 4.49 (dd, J_{AB} = 13.3, J_{AX} = 4.1 Hz, 1 H, CH−CH_2) ppm. ^{13}C NMR: δ = 168.3 (C=O), 155.7 (N−C=O), 135.8, 135.1, 134.7, 128.8−128.0 (complex), 76.0, 68.0, 67.2, 63.1, 51.6 ppm. MS (100 °C): *m*/*z* = 91 (100), 159 (21), 107 (17), 92 (16), 187 (15). $C_{25}H_{25}N_3O_6$ (463.49): calcd. C 64.79, H 5.44, N 9.07; found C 64.70, H 5.44, N 9.06.

Acknowledgments

We are indebted to Prof. G. Verardo for MS data collection and to Dr. P. Martinuzzi for recording the NMR spectra. Thanks are also due to the University of Udine (F. U. R. D.) for financial support.

[1] A. K. Tyagi, D. A. Cooney, *Adv. Pharmacol. Chemother.* **1984**, *20*, 69−121.
[2] [2a] J. E. Thiemann, G. Beretta, *J. Antibiot., Ser. A* **1966**, *19*, 155−160. [2b] Y. K. S. Murthy, J. E. Thiemann, C. Coronelli, P. Sensi, *Nature* **1966**, *211*, 1198−1199.
[3] C. Coronelli, C. R. Pasqualucci, G. Tamoni, G. G. Gallo, *Farmaco, Ed. Sci.* **1966**, *21*, 269−277; *Chem. Abstr.* **1966**, *65*, 2344e−g.
[4] B. Zeeh, H. Metzger, in *Methoden der Organischen Chemie, Houben-Weyl* (Ed.: E. Müller), G. Thieme Verlag, Stuttgart, **1971**; Vol. X/1 (Stickstoff-Verbindungen, I), pp. 1273−1279.
[5] T. E. Stevens, *J. Org. Chem.* **1964**, *29*, 311−315.
[6] M. A. F. Jalal, M. B. Hossain, D. van der Helm, *Acta Crystallogr., Sect. C* **1986**, *42*, 733−738.
[7] P. Strazzolini, A. Malabarba, P. Ferrari, M. Grandi, B. Cavalleri, *J. Med. Chem.* **1984**, *27*, 1295−1299.
[8] G. Powis, M. M. Ames, *J. Chromatogr.* **1979**, *170*, 195−201.
[9] [9a] R. G. Kratsas, *J. Econ. Entomol.* **1975**, *68*, 581−582. [9b] S. Matsumoto, S. Sakuda, A. Isogai, A. Suzuki, *Agric. Biol. Chem.* **1984**, *48*, 827−828.
[10] [10a] S. J. Anandaraj, H. N. Jayaram, D. A. Cooney, A. K. Tyagi, N. Han, J. H. Thomas, M. Chitnis, J. A. Montgomery, *Biochem. Pharmacol.* **1980**, *29*, 227−245. [10b] A. K. Tyagi, D. A. Cooney, *Cancer Res.* **1980**, *40*, 4390−4397. [10c] A. K. Tyagi, D. A. Cooney, H. N. Jayaram, J. K. Swiniarski, R. K. Johnson, *Biochem. Pharmacol.* **1981**, *30*, 915−924. [10d] P. J. Casey, J. M. Lowenstein, *Biochem. Pharmacol.* **1987**, *36*, 705−709.
[11] [11a] H. Hori, P. Tran, C. J. Carrera, Y. Hori, M. D. Rosenbach, D. A. Carson, T. Nobori, *Cancer Res.* **1996**, *56*, 5653−5658. [11b] A. Batova, M. B. Dicianni, M. Omura-Minamisawa, J. Yu, C. J. Carrera, L. J. Bridgeman, F. H. Kung, J. Pullen, M. D. Amylon, A. L. Yu, *Cancer Res.* **1999**, *59*, 1492−1497. [11c] H. Harasawa, Y. Yamada, M. Kudoh, K. Sugahara, H. Soda, Y. Hirakata, H. Sasaki, S. Ikeda, T. Matsuo, M. Tomonaga, T. Nobori, S. Kamihira, *Leukemia* **2002**, *16*, 1799−1807. [11d] P. B. Illei, V. W. Rusch, M. F. Zakowski, M. Ladanyi, *Clin. Cancer Res.* **2003**, *9*, 2108−2113. [11e] T. Efferth, E. Gebhart, D. D. Ross, A. Sauerbrey, *Biochem. Pharmacol.* **2003**, *66*, 613−621.
[12] C. N. Eaton, G. H. Denny Jr., M. A. Ryder, M. G. Ly, R. D. Babson, *J. Med. Chem.* **1973**, *16*, 289−290.
[13] [13a] G. C. Lancini, A. Diena, E. Lazzari, *Tetrahedron Lett.* **1966**, *16*, 1769−1772. [13b] G. C. Lancini, E. Lazzari, A. Diena, *Farmaco, Ed. Sci.* **1969**, *24*, 169−178; *Chem. Abstr.* **1969**, *70*, 97146h. [13c] Y. Isowa, H. Kurita, M. Ohmori, M. Sato, K. Mori, *Bull. Chem. Soc. Jpn.* **1973**, *46*, 1847−1850.
[14] J. E. Baldwin, A. C. Spivey, C. J. Schofield, *Tetrahedron: Asymmetry* **1990**, *1*, 881−884.
[15] S. Sachio, T. Kazuo, K. Yasushi, *Jpn. Kokai Tokkyo Koho* JP 122839 (2001); *Chem. Abstr.* **2001**, *134*, 326764.
[16] R. F. W. Jackson, N. Wishart, A. Wood, K. James, M. J. Wythes, *J. Org. Chem.* **1992**, *57*, 3397−3404.
[17] Y. S. Kulkarni, *Aldrichimica Acta* **1999**, *32*, 18−27.
[18] C. Dugave, A. Ménez, *Tetrahedron: Asymmetry* **1997**, *8*, 1453−1465.
[19] J. C. Spetzler, T. Hoeg-Jensen, *J. Pept. Sci.* **1999**, *5*, 582−592.
[20] M. E. Solomon, C. L. Lynch, D. H. Rich, *Tetrahedron Lett.* **1995**, *36*, 4955−4958.
[21] E. S. Ratemi, J. C. Vederas, *Tetrahedron Lett.* **1994**, *35*, 7605−7608.
[22] P. Strazzolini, M. Scuccato, A. G. Giumanini, *Tetrahedron* **2000**, *56*, 3625−3633.
[23] M. J. Miller, P. G. Mattingly, M. A. Morrison, J. F. Kerwin Jr., *J. Am. Chem. Soc.* **1980**, *102*, 7026−7032.

[24] [24a] M. Wada, O. Mitsunobu, *Tetrahedron Lett.* **1972**, *13*, 1279−1282. [24b] D. L. Hughes, *Org. React.* **1992**, *42*, 335−656.
[25] Y. Nagao, T. Kumagai, S. Yamada, E. Fujita, Y. Inoue, Y. Nagase, S. Aoyagi, T. Abe, *J. Chem. Soc., Perkin Trans. 1* **1985**, 2361−2367.
[26] D. M. Floyd, A. W. Fritz, J. Pluscec, E. R. Weaver, C. M. Cimarusti, *J. Org. Chem.* **1982**, *47*, 5160−5167.
[27] Y. Xu, M. J. Miller, *J. Org. Chem.* **1998**, *63*, 4314−4322.
[28] P. G. Mattingly, J. F. Kerwin, M. J. Miller, *J. Am. Chem. Soc.* **1979**, *101*, 3983−3985.
[29] M. Shiino, Y. Watanabe, K. Umezawa, *Bioorg. Chem.* **2003**, *31*, 129−135.
[30] N. Ono, T. Yamada, T. Saito, K. Tanaka, A. Kaji, *Bull. Chem. Soc. Jpn.* **1978**, *51*, 2401−2404.
[31] P. Strazzolini, A. Pavsler, *Ind. Eng. Chem. Res.*, manuscript submitted for publication.

Received July 20, 2004

