Acta Chemica Scandinavica, 1994: 48: 517–525
Printed in Belgium – all rights reserved

ACTA
CHEMICA SCANDINAVICA
ISSN 0904–213X

Synthesis of Amino Acids with Modified Principal Properties 3:* Sulfur-Containing Amino Acids

Ulf Larsson and Rolf Carlson†

Department of Organic Chemistry, Umeå University, S-901 87 Umeå, Sweden

Larsson, U. and Carlson, R., 1994. Synthesis of Amino Acids with Modified Principal Properties 3: Sulfur-Containing Amino Acids. – Acta Chem. Scand. 48: 517–525 © Acta Chemica Scandinavica 1994.

The synthesis and characterization of seven medium-sized, medium-polar, sulfur-containing amino acids are presented. The compounds were prepared with the objective of finding amino acids which possess physical and chemical properties such that their principal properties would be clearly different from those of previously known amino acids. The principal properties are determined as latent variables in principal component analysis of molecular property descriptors.

The following amino acids were prepared in homochiral form from L-cysteine: (2*R*)-2-amino-3-(2-hydroxyethylsulfanyl)propanoic acid, (2*R*)-2-amino-3-(2-hydroxyethylsulfonyl)propanoic acid, (3*R*)-perhydro-1,4-thiazine-3-carboxylic acid and (3*R*)-1,1-dioxoperhydro-1,4-thiazine-3-carboxylic acid. (3*R*)-1-Oxoperhydro-1,4-thiazine-3-carboxylic acid was prepared in *N,C* protected form, but all attempts to obtain the free amino acid failed. The principal properties of this amino acid were determined on the racemic diastereomers obtained by oxidation of the racemic perhydro-1,4-thiazine-3-carboxylic acid prepared from 2-aminoethanethiol and ethyl 3-bromo-2-oxopropanoate. With the exception of (3*R*)-perhydro-1,4-thiazine-3-carboxylic acid for which the synthesis has been previously described, the amino acids described are new compounds. An improved synthesis of (3*R*)-perhydro-1,4-thiazine-3-carboxylic acid via the cyclization of (2*R*)-benzyl 2-amino-3-(2-hydroxyethylsulfanyl)propanoate under Mitsunobu conditions is presented. Full experimental details for the syntheses are given.

The principal property scores of the synthesized sulfur-containing amino acids and of the structurally related 3-(2-aminoethyl)cysteine which was commercially available are given.

Multivariate characterization by principal component analysis of physical and chemical descriptors of amino acids to determine their principal properties has been shown to yield three significant principal components.[1,2] The first component accounts mainly for lipophilic–hydrophilic properties of the amino acid side chain. The second component is mainly related to the electronic distribution as measured by variation of NMR data. The third component is largely composed of molecular descriptors related to the 'size' of the side chain although there is also a slight contribution from descriptors related to variation of the electronic distribution. These results indicate that structural modifications which alter the lipophilic–hydrophilic properties, but leave the 'size' of the side chain relatively unperturbed or *vice versa*, might give new amino acids which could be projected in the principal component score plot (principal property map) with an expected displacement along those principal components related to size and to lipophilic properties. For discussion of principal properties of amino acids and quantitative structure–activity relationships of peptides, see Ref. 3. For a general discussion of the concept of principal properties, see Ref. 4.

In recent studies,[1,5] we have shown that it is possible to obtain amino acids with new principal properties by applying the principles suggested above. Ref. 1 describes the synthesis and characterization of some polyfluorinated analogs of norleucine and norvaline and it was shown that replacement of one or more methylene groups (CH_2) by difluoromethylene groups (CF_2) in the amino acid side chain had a large influence on the principal properties related to lipophilic–hydrophilic properties whereas this structural change had only a small influence on the principal properties related to the size of the side chain. In Ref. 5 is described how the principal properties of amino acids related to serine and lysine are altered by the incorporation of one or more ethyleneoxy moieties in the side chain.

The present work covers the synthesis and characterization of some sulfur-containing amino acids. Our intentions were to prepare modified amino acids with side

* For Part 2, see Ref. 5.
† To whom correspondence should be addressed.

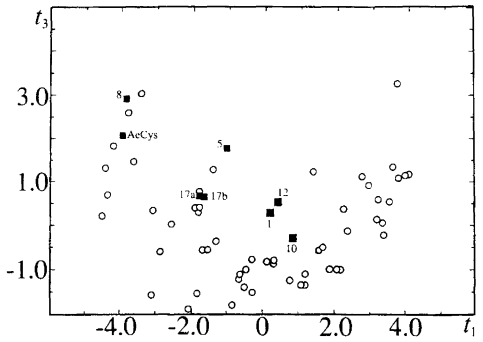


Fig. 1. Score plot obtained by projecting the descriptor data of the sulfur-containing amino acids (■) onto the principal component model obtained from the data of 55 amino acids (○) given in Ref. 2, but without including the ^{1}H NMR descriptor data. The variation in lipophilicity is displayed along the t_1 axis and the variation in size along the t_2 axis.

chains of medium to high polarity which could be foreseen to be projected into empty areas of the principal property plot, see Fig. 1. If this could be achieved, it would indicate that these amino acids possess unique principal properties.

Methods and results

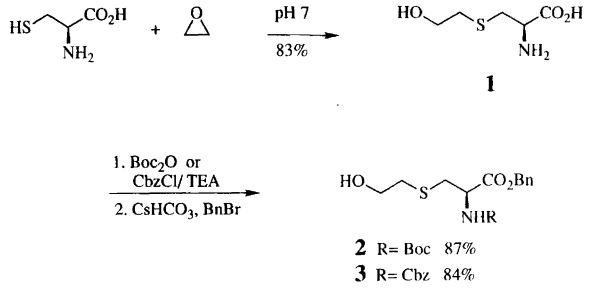
Synthesis of amino acids. The syntheses of the amino acids are summarized in Schemes 1–6. Full experimental details are given in the Experimental section.

(−)-(2*R*)-2-Amino-3-(2-hydroxyethylsulfanyl)propanoic acid (**1**) was prepared from L-cysteine and ethylene oxide[5,6] in 83% yield. It was then *N*-protected and esterified according to standard methods[7,8] to give compounds **2** and **3** in 87 and 84% yields, respectively (Scheme 1).

Oxidation of **2** with potassium hydrogenpersulfate,[9],* gave the sulfone **4** in 93% yield, which was first debenzylated by catalytic phase-transfer hydrogenation using palladium black and formic acid in methanol.[10] The removal of the *tert*-butyloxycarbonyl (BOC) group was achieved by trifluoroacetic acid mediated hydrolysis[11] to give **5** in 78% yield (Scheme 2).

The azido compound **6** was prepared in 85% yield from tosylated **2** and tetramethylguanidinium azide. Oxidation of **6** with potassium hydrogenpersulfate by the same method as for **4** afforded the sulfone **7** in 95% yield. Debenzylation and reduction of the azido moiety by catalytic phase-transfer hydrogenation (palladium black and formic acid) and subsequent hydrolysis of the *tert*-butyloxycarbonyl group by hydrochloric acid gave **8** in 94% yield, see Scheme 2.

* A commercially available solid persulfate reagent, Oxone®, was used. This reagent is composed of potassium hydrogenpersulfate, potassium sulfate and potassium hydrogensulfate in a 2:1:1 mole ratio.



Scheme 1.

Compound **9** was synthesized from **3** under Mitsunobu conditions (triphenylphosphine and diethyl azodicarboxylate)[12,13] in 55% yield as two diastereomers (slow *N* inversion). However, all attempts to deprotect **9** by catalytic phase-transfer catalysis were unsuccessful. Deprotection could be achieved by a more drastic method, *viz.*, treatment of **9** with aluminium trichloride in anisole.[14] After purification by ion exchange chromatography the free amino acid **10** was obtained in 57% yield (Scheme 3).

Oxidation of **9** with potassium hydrogenpersulfate gave the sulfone **11** in 83% yield. The sulfone was also deprotected with aluminium chloride in anisole to give **12** in 49% yield. Treatment of **9** with bromine in aqueous 10% potassium hydrogencarbonate and dichloromethane[15] gave the sulfoxide **13** in 96% yield as a mixture of four diastereomers (Scheme 3). The sulfoxide is not stable under strong Lewis acid conditions and we did not attempt to remove the protective groups.

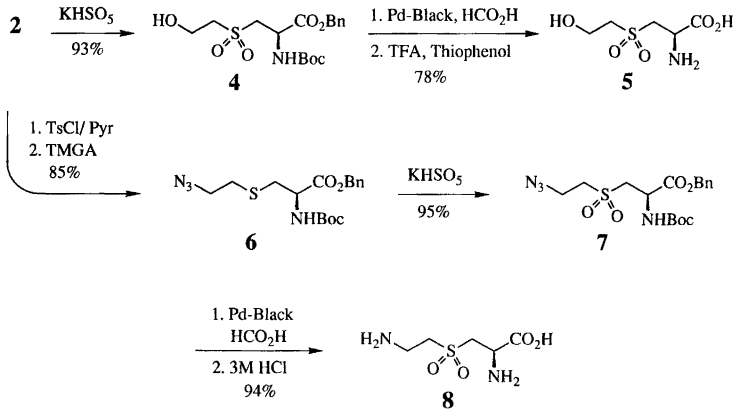
Treatment of **2** under Mitsunobu conditions (as for **3** gave the cyclized product **14** in 28% yield (Scheme 4).

However, all our attempts to cyclize the sulfone derived from **3** failed. The only product that could be isolated was the vinylic sulfone **15**, see Scheme 5.

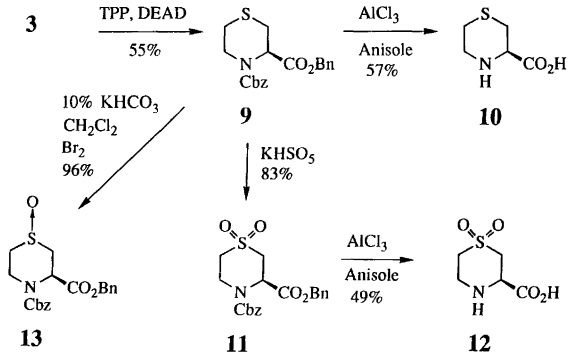
As our attempts to obtain the chiral sulfoxide amino acid analogs of **10** were unsuccessful, the principal properties of these amino acids were determined from the racemic compounds which were prepared by different routes. Treatment of ethyl bromopyruvate with 2-aminoethanethiol afforded a cyclic imine which upon treatment with sodium cyanoborohydride gave racemic thiopipecolic acid ethyl ester **16** in 81% yield (isolated as the hydrochloride), see Scheme 6.

Hydrolysis of **16** with barium hydroxide and purification on Amberlite IR-120 gave racemic perhydro-1,4-thiazine-3-carboxylic acid.

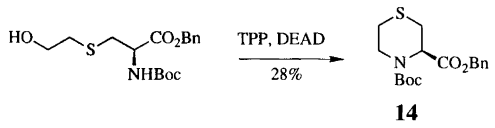
Oxidation of **16** with sodium periodate to yield the corresponding sulfoxide esters gave a mixture of four stereoisomers which was partially separated by column chromatography into two diastereomeric fractions each containing mixtures of the enantiomeric pairs. The two diastereomeric fractions were hydrolyzed using barium hydroxide and purified by ion exchange chromatography to yield two diastereomeric batches of the racemic sulfoxide amino acids (**17a,b**). However, we were unable to assign the relative configuration of the sulfoxide groups.



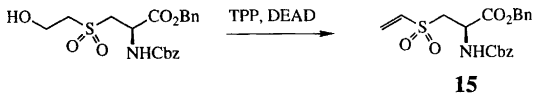
Scheme 2.



Scheme 3.



Scheme 4.

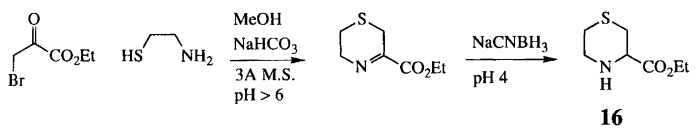


Scheme 5.

The racemic sulfone amino acid **18** was prepared from **16** by oxidation with potassium permanganate[16] followed by hydrolysis.

Characterization and principal properties of the amino acids. To characterize the amino acids described above by their principal properties, the descriptors summarized in Table 1 were used. These descriptors are the same as were used in Refs. 1 and 5. One commercially available modified amino acid, (2*R*)-2-amino-3-(2-aminoethylsulfanyl)propanoic acid [(2-aminoethyl)cysteine] (AeCys) was also characterized and these data are included in Table 1. This amino acid is structurally related to the modified amino acids synthesized in the present work. To permit a comparison with previously described principal properties of known amino acids, the principal property scores, t_1–t_3, of the new amino acids were determined as follows. The descriptors of the new amino acids were projected onto the principal model of the previously characterized amino acids. By this procedure, it is possible to see how the modifications of the amino acid structures have altered the principal properties related to size and lipophilicity in comparisons with previously characterized amino acids. An alternative to this procedure would have been to append the descriptor data of the new amino acids to the data of old amino acids and recompute a principal component model. However, such a procedure would have altered the previously determined principal component scores and would have made a comparison as to the change of the principal properties of the new amino acids more difficult.

When the present study was initiated, a two-component principal component model of amino acid data was available.[17] The data set upon which this model was based contained the present chromatographic descriptors (lipophilicity) and descriptors related to the size of the side chain but did not include the α proton NMR shift descriptors. When the NMR data are omitted from the set of the descriptors of the new amino acids and the remaining descriptors were projected on the two-component principal component model determined from the truncated data set the score plot shown in Fig. 1 was



Scheme 6.

Table 1. Descriptors and principal property scores of the amino acids.

	Descriptor[a]												Principal property scores		
Amino acid	1	2	3	4	5	6	7	8	9	10	11	12	t_1	t_2	t_3
1	72	29	42	22	49	31	62	49.5	165.2	4.15	3.93	3.41	0.28	0.56	0.03
5	55	22	28	21	26	25	33	59.0	197.2	4.49	4.25	3.79	−0.39	4.23	−0.04
8	1	5	2	1	6	19	0	61.5	196.2	4.40	4.20	–[b]	−3.33	4.47	1.23
10	67	35	51	24	54	43	74	41.5	160.2	3.94	3.86	3.36	0.70	−0.78	0.05
12	56	23	52	25	48	44	55	51.0	178.2	4.30	4.00	3.71	0.64	1.41	−0.41
17a[c]	48	14	40	5	39	21	30	46.2	163.2	4.33	4.01	3.84	−1.13	2.75	−0.63
17b[c]	55	12	38	5	36	19	29	46.2	163.2	4.34	3.98	3.83	−1.24	1.41	−0.41
(AeCys)[d]	2	7	6	1	14	14	0	53.0	164.2	4.10	3.97	–[b]	−3.90	1.80	1.58

[a]Descriptors: 1–7 are R_f values determined from a test battery of seven eluents,[1] 8 is the van der Waal's volume ($cm^3\ mol^{-1}$) of the side chain, 9 is the molar mass ($g\ mol^{-1}$), 10–12 are the ^1H NMR shifts for the α-proton recorded in deuterium oxide at different pD, 10 (pD 2), 12 (pD 7), 12 (pD 12.5). [b]The amino acid was unstable at this pD. [c]Diastereomerically enriched fractions. [d](2-Aminoethyl)cysteine was purchased from Sigma.

obtained. The positions of the projected new amino acids in this score plot show a more distinct displacement along the axes related to variations in lipophilic–hydrophilic properties and variations in size.

Discussion

Seven sulfur-containing amino acids were synthesized with the intention of obtaining compounds with different principal properties from those previously characterized.[1,2] The new amino acids were prepared through a series of simple modifications of (−)-(2*R*)-2-amino-3-(2-hydroxyethylsulfanyl)propanoic acid (**1**). One of the amino acids, (−)-(3*R*)-perhydro-1,4-thiazine-3-carboxylic acid (**10**) had previously been prepared through a synthetic sequence involving a nucleophilic ring opening of a protected chiral aziridinecarboxylic acid by 2-chloroethanethiol as a critical step.[18] The thus obtained ω chloro sulfide product was deprotected and cyclized to yield the free amino acid **10**. However, the synthesis of the aziridine[19,20] requires many steps and for this reason, the overall yield is moderate. We found it more convenient to prepare **10** by a direct cyclization of **1** under Mitsunobu conditions.[12] Although the yield in this cyclization is moderate the overall yield is better owing to a shorter synthetic path.

Fig. 1 is a score plot based upon a principal component analysis of molecular descriptors related to lipophilic–hydrophilic properties and size of the side chains of known amino acids. It is seen that the projections of some of the new amino acids described in this paper are found in previously empty areas of the score plot. For these new amino acids it can therefore be concluded that they have acquired new principal properties with respect to lipophilicity and size.

A different picture emerges if the NMR descriptors are included. The presence of the strong electron-withdrawing sulfoxide and sulfone groups in the amino acid side chain has an influence on the chemical shift of the amino acid α proton. Although the variation of the NMR descriptors is largely described by the second principal component, the NMR descriptors also give slight contributions to the other components. The differences between the projections shown in Fig. 1 and Fig. 2 are attributed to this.

A principal component model computed after appending the property descriptors of the new amino acids described in this and previous papers in this series to the descriptors of the amino acids in Ref. 2, afforded the score plot shown in Fig. 3. Inclusion of the new amino acids in the data set changes the directions of the principal component vectors and the score plots are altered, cf. Figs. 1 and 2. Most probably, this would call for a future revision of the principal property *z* scales.

Experimental

General methods. ^1H and ^{13}C NMR were recorded on a Bruker AC80, ACP250 or AC500 instrument, using deuteriochloroform as the solvent and tetramethylsilane, TMS, as an internal reference. NMR spectra of the amino acids were recorded in deuterium oxide and with sodium 2,2-dimethyl-2-silapentane-5-sulfonate, DSS, as an internal reference. Electron impact (EI) mass spectra

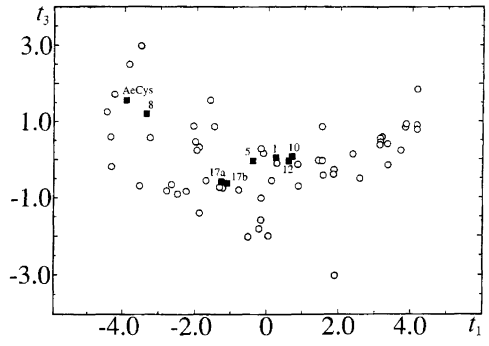


Fig. 2. Principal property plot obtained by projecting the data in Table 1 onto a principal component model including the ^1H NMR data in the set of descriptors.

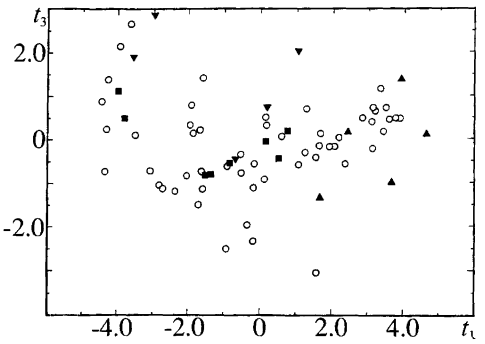


Fig. 3. Score plot obtained in principal component analysis of a data set of property descriptors obtained after appending the data of the amino acids described in this and preceding papers in this series to the data of 55 amino acids given in Ref. 2. Markers in the plot: ○, amino acids given in Ref. 2; ■, the present work; ▼, the polar amino acids described in Ref. 5; ▲, the fluorinated amino acids described in Ref. 1.

were obtained on an HP GC/MSD 5830/5970 system. Chemical ionization (CI) mass spectra were recorded on a Finnigan INCOS 500 instrument. Mass spectra were reported as follows: *m/z* (% relative abundance) [assignment]. IR spectra were recorded on a Perkin-Elmer 681 spectrometer and are reported in cm^{-1}. Optical rotations were measured on a Perkin-Elmer 141 or 241 polarimeter using chloroform as the solvent unless otherwise stated. All reactions were monitored by thin layer chromatography, TLC (silica gel 60) and, when possible, by capillary gas chromatography, GLC. For GLC analyses a Carlo-Erba Fractovap 4130 instrument equipped with a flame ionization detector was used with a Supelco SPB-5 15 m × 0.23 mm capillary column. Principal component analysis for the characterization of the principal properties was carried out using the SIMCA package (SIMCA-R 4.4 version). This software is available from UMETRI AB, P.O. Box 1456, S-901 24 Umeå, Sweden and from MDS, Inc., 371 Highland Ave., Winchester, MA 01890, USA.

Chemicals. Starting materials for synthesis, reagents and solvents were *pro analysi* grade and were supplied by Aldrich, Merck, Sigma or Jansen. Dichloromethane was distilled from diphosphorus pentoxide and stored over 3 Å molecular sieves. *N,N*-Dimethylformamide (DMF) was dried over 4 Å molecular sieves followed by distillation under reduced pressure. Tetrahydrofuran (THF) was distilled from sodium–potassium amalgam. Benzyl chloroformate was distilled at 1 mmHg.

*(−)-(2R)-2-Amino-3-(2-hydroxyethylsulfanyl)propanoic acid (**1**).* L-Cysteine, (2.42 g, 20.0 mmol) was dissolved in 20 ml of water and the pH of the solution was adjusted to pH 7 with 1 M aqueous sodium hydroxide.[6] The solution was cooled to 0 °C and ethylene oxide (1.97 ml, 40.0 mmol) (condensed at −10 °C) was added. The reaction mixture was allowed to stand at room temperature for 1.5 h and was then extracted with ether to remove unchanged ethylene oxide. The ether layer was discarded. The aqueous layer was evaporated to dryness and the residual white crystalline mass recrystallized from abs. ethanol to afford 2.74 g (83%) of pure **1**. ^{1}H NMR (80.13 MHz, pH 2): δ 4.15 (dd, 1 H, *J* 6.8 and 5.0 Hz), 3.78 (t, 2 H), 3.17 (d, *J* 4.9 Hz), 3.15 (d, *J* 6.9 Hz). ^{13}C NMR (20.15 MHz): δ 176.1; 63.5, 57.1, 37.2, 35.6. IR (KBr): 3350, 3200–2800, 2600, 2100, 1600, 1485, 1410, 1070, 1000, 850. $[\alpha]_D^{25} = -28.3°$ (*c* 0.053, H_2O).

*(2R)-(−)-Benzyl 2-(tert-butoxycarbonylamino)-3-(2-hydroxyethylsulfanyl)propanoate (**2**).* To a solution of **2** (2.00 g, 12.1 mmol) and 0.53 g (1.1 equiv.) sodium hydroxide in 25 ml of water were added 10 ml *tert*-butyl alcohol followed by 2.77 g (1.05 equiv.) di-*tert*-butyl dicarbonate.[7] The reaction mixture was allowed to stand at room temperature overnight and then extracted with pentane. The pentane layer was then extracted with saturated aqueous sodium hydrogencarbonate. The pH of the combined aqueous layers was adjusted to pH 1–2 by the addition of potassium hydrogensulfate solution and then extracted several times with ethyl acetate. The organic layers were combined and the ethyl acetate was removed by evaporation. The viscous residue was dissolved in 50 ml of methanol and titrated with cesium hydrogencarbonate solution to pH 7.[8] The resulting mixture was evaporated to dryness and then further dried under vacuum. The dry cesium salt thus obtained was dissolved in 30 ml of DMF and benzyl bromide (2.17 g, 1.05 equiv.) was added. The reaction mixture was allowed to stand at room temperature for 3 h and then diluted with water and extracted several times with ethyl acetate. The combined organic layers were washed in sequence with aqueous sodium hydrogen carbonate, water and saturated aqueous sodium chloride and then dried ($MgSO_4$). The crude product was obtained after evaporation of the solvent. Purification by flash chromatography (Silica gel 60; heptane–ethyl acetate 5:4) yielded 3.74 g (87%) of **2** as a viscous oil. ^{1}H NMR (250.13 MHz): δ 7.36 (m, 5 H), 5.57 (d, 1 H, *J* 8.2 Hz), 5.18 (s, 2 H), 4.58 (m, 1 H), 3.67 (t, 2 H), 2.97 (m, 2 H), 2.90 (s, 1 H), 2.67 (t, 2 H), 1.44 (s, 9 H). ^{13}C NMR (62.89 MHz): δ 171.6, 156.1, 135.8, 129.3, 129.3, 129.2, 81.0, 68.2, 61.5, 54.4, 36.7, 35.4, 29.0. IR (neat film): 3400, 3100–3000, 2950–2850, 1740, 1710, 1500, 1165, 1055, 1020, 750, 695. $[\alpha]_D^{25} = -1.4°$ (*c* 0.25).

*(2R)-(−)-Benzyl-2-(benzyloxycarbonylamino)-3-(2-hydroxyethylsulfanyl)propanoate (**3**).* To a solution of **1** (0.80 g, 4.84 mmol) and potassium hydrogencarbonate 1.02 g (2.1 equiv.) in 15 ml of dioxane and 15 ml of water was added dropwise benzyl chloroformate (0.91 g, 1.1 equiv.) and the resulting reaction mixture was allowed to stand at room temperature overnight. The solvents were evaporated off and the residual salt was dissolved in 15 ml of DMF. Benzyl bromide (0.91 g, 1.1 equiv.) was added and the reaction mixture was allowed to stand at room temperature overnight. The DMF was removed by evapora-

tion under reduced pressure and the residue was dissolved in ca. 10 ml of ethyl acetate. The solution was washed with water and brine and dried ($MgSO_4$). After filtration, evaporation of the solvent and purification by flash chromatography (Silica Gel; heptane–ethyl acetate 1:1) 1.59 g (84%) of **3** was obtained as a viscous oil. ^{1}H NMR (250.13 MHz): δ 7.30 (m, 10 H), 6.00 (d, 1 H, *J* 8.1 Hz), 5.15 (d, 2 H, *J* 2.8 Hz), 5.09 (s, 2 H), 4.62 (m, 1 H), 3.59 (q, 2 H), 2.96 (m, 2 H), 2.85 (t, 1 H), 2.59 (t, 2 H). ^{13}C NMR (62.89 MHz): δ 170.6, 156.0, 136.1, 135.0, 128.6, 128.5, 128.4, 128.2, 128.1, 67.5, 67.1, 60.9, 54.1, 35.8, 34.5. IR (neat film): 3350, 3100–3000, 2900–2820, 1740, 1720, 1520, 1500, 1340, 1260, 1215, 1055, 740, 700. $[\alpha]_D^{25} = -3.2°$ (*c* 0.15).

*(2R)-(−)-Benzyl-2-(*tert*-butoxycarbonylamino)-3-(2-hydroxyethylsulfonyl)propanoate (**4**).* To a solution of **2** (1.50 g, 4.22 mmol) in 20 ml of methanol at 0°C was slowly added (3.85 g, 3 equiv.) of Oxone[9] dissolved in 20 ml of water. The ice-bath was removed and the reaction mixture was allowed to stand at room temperature for 4 h. The methanol was removed from the reaction mixture by evaporation under reduced pressure and the remaining aqueous solution was extracted with several portions of ethyl acetate. The combined organic layers were washed with water. After drying ($MgSO_4$), evaporation of the solvent, and purification by flash chromatography (Silica gel 60; hexane–ethyl acetate 1:2) 1.52 g (93%) of **4** was obtained as a white solid, m.p. 96–97°C. ^{1}H NMR (250.13 MHz): δ 7.35 (m, 5 H), 5.87 (d, 1 H, *J* 7.2 Hz), 5.19 (s, 2 H), 4.72 (m, 1 H), 4.02 (q, 2 H), 3.76 (m, 2 H), 3.22 (m, 2 H), 3.13 (s, 1 H), 1.41 (s, 9 H). ^{13}C NMR (62.89 MHz): δ 169.6, 155.4, 134.8, 128.6, 128.5, 80.9, 68.1, 57.1, 56.2, 55.4, 49.9, 28.2. IR (KBr): 3525, 3375, 3100–3000, 2990–2880, 1750, 1700, 1690, 1525, 1195, 1047, 950, 730, 700. $[\alpha]_D^{25} = -3.9°$ (*c* 0.13).

*(−)-(2R)-2-Amino-3-(2-hydroxyethylsulfonyl)propanoic acid (**5**).* A solution of **4** (1.52 g, 3.92 mmol) in 60 ml of 10% formic acid in methanol was slowly passed (flow rate ca. 2 ml min^{-1}) through a (1.5 × 5 cm) column loosely packed with palladium-black.[10] The eluate was collected and the operation was repeated. Two passages were enough for complete debenzylation. Methanol and remaining formic acid was evaporated off to yield the debenzylated product. To remove the *N*-Boc group the crude debenzylated product was dissolved in 10 ml of dichloromethane and thiophenol (6.0 ml, 15 equiv.) was added. The mixture was cooled at 0°C and 100 ml of trifluoroacetic acid was added dropwise over a period of 45 min.[11] When the addition was complete, the reaction mixture was allowed to stand at 0°C for 1 h after which time the deprotection was complete. The course of the reaction was monitored by TLC (butanol–acetic acid–water 4:1:1; R_f 0.21). The reaction mixture was evaporated to dryness and the residue was dissolved in water. The aqueous solution was extracted with ethyl acetate to remove traces of reagents and unchanged starting material. The organic layer was discarded. The aqueous layer was evaporated to dryness and the crude **5** was recrystallized from methanol to yield 0.60 g (78%) of **5** as a white solid. ^{1}H NMR (80.13 MHz): δ 4.33 (dd, 1 H, *J* 9.0 and 3.3 Hz), 4.09 (t, 2 H), 3.94 (d, 1 H, *J* 3.3 Hz), 3.83 (d, 1 H, *J* 9.2 Hz), 3.56 (t, 2 H). ^{13}C NMR (20.15 MHz): δ 173.0, 58.4, 57.6, 56.7, 51.3. IR (KBr): 3480, 3390, 3240, 3100–2800, 2600, 1620, 1520, 1280, 1120, 1050, 840, 810. $[\alpha]_D^{25} = -6.9°$ (*c* 0.02, H_2O).

*(2R)-(−)-Benzyl 3-(2-azidoethylsulfanyl)-2-(*tert*-butoxycarbonylamino)propanoate (**6**).* To a solution of **2** (2.64 g, 7.43 mmol) in 15 ml of pyridine under argon at 0°C was added *p*-toluenesulfonyl chloride (2.83 g, 2 equiv.). The mixture was cooled to −25°C and kept at this temperature for 36 h after which time all **2** had been consumed. The mixture was warmed to 0°C and a cold (0°C) aqueous solution of potassium hydrogensulfate was added. The mixture was kept at 0°C and was extracted with cold diethyl ether. The ethereal layer was dried ($MgSO_4$) and filtered, and the ether was removed by evaporation at ice-bath temperature. The crude tosylate thus obtained was dissolved in 40 ml of dichloromethane and under a protective atmosphere of argon and tetramethylguanidinium azide (3.52 g, 3 equiv.) was added. The reaction mixture was allowed to stand at room temperature. The course of the reaction was monitored by TLC and after 40 h the tosylate had been consumed. The reaction mixture was washed with aqueous potassium hydrogensulfate and dried ($MgSO_4$). After filtration and evaporation of the solvent, the crude product was purified by flash chromatography (Silica gel 60; heptane–ethyl acetate 7:2) to yield 2.41 g (85%) of pure **6** as a viscous oil. ^{1}H NMR (250.13 MHz): δ 7.35 (m, 5 H), 5.55 (d, 1 H, *J* 7.8 Hz), 5.17 (d, 6.6 Hz), 4.57 (m, 1 H), 3.34 (t, 2 H), 2.98 (m, 2 H), 2.62 (t, 2 H), 1.43 (s, 9 H). ^{13}C NMR (62.89 MHz): δ 170.7, 155.1, 135.1, 128.6, 128.4, 80.1, 67.4, 53.6, 50.9, 34.6, 31.6, 28.2. IR (neat film): 3400, 3100–3000, 2990–2850, 2100, 1745, 1715, 1500, 1165, 750, 700. $[\alpha]_D^{25} = -1.6°$ (*c* 0.25).

*(2R)-(+)-Benzyl 3-(azidoethylsulfonyl)-2-(*tert*-butoxycarbonylamino)propanoate (**7**).* To a solution of **6** (2.24 g, 5.89 mmol) in 25 ml of methanol at 0°C was added 5.38 g (3 equiv.) of Oxone[9] dissolved in 25 ml of water. The reaction mixture was stirred at room temperature for 4 h. The methanol was evaporated off under reduced pressure and the remaining aqueous solution was extracted with ethyl acetate. The organic phase was dried ($MgSO_4$), filtered, and the solvent removed by evaporation. After purification of the crude product by flash chromatography (Silica gel 60; hexane–ethyl acetate 2:3) 2.50 g (95%) of **7** were obtained as a white solid. ^{1}H NMR (250.13 MHz): δ 7.35 (m, 5 H), 5.71 (d, 1 H, *J* 6.8 Hz), 5.21 (s, 2 H), 4.70 (m, 1 H), 3.77 (m, 4 H), 3.20 (t, 2 H), 1.43 (s, 9 H). ^{13}C NMR (62.89 MHz): δ 169.2, 155.2, 134.7, 128.6, 128.5, 80.9, 68.2, 55.4, 53.9, 50.0, 44.6, 28.1. IR (KBr): 3350, 3100–3000, 2950–2850, 2100,

1745, 1700, 1520, 1310, 1275, 1135, 1063, 750, 700. $[\alpha]_D^{25} = 4.6°$ (*c* 0.25).

*(−)-(2R)-2-Amino-3-(2-aminoethylsulfonyl)propanoic acid dihydrochloride (**8**).* A solution of **7** (1.88 g, 4.56 mmol) in 200 ml of 5% formic acid in methanol was slowly passed (flow rate ca. 2 ml min^{-1}) through a (1.5 × 5 cm) column of loosely packed palladium black.[10] The reaction mixture was allowed to pass twice through the column to achieve complete debenzylation of the ester and reduction of the azide group. After two passages through the column, the reaction mixture was evaporated to dryness and the residue was dissolved in 200 ml of 3 M hydrochloric acid and the mixture was allowed to stand at ambient temperature overnight. After evaporation to dryness, the residual crude **8** was dissolved in the minimum amount of methanol. The hydrochloride was precipitated by trituration with diethyl ether. The white crystalline precipitate was collected by filtration and washed with diethyl ether. The yield of **8** was 1.02 g (94%). No impurities could be detected by TLC (Silica gel 60; butanol–acetic acid–water, 4:1:1, R_f 0.01). ^{1}H NMR (250.13 MHz): δ 4.81 (dd, 1 H, *J* 7.4 and 3.8 Hz), 4.24 (dd, 1 H, *J* 15.4 and 3.7 Hz), 4.06 (dd, 1 H, *J* 15.4 and 7.5 Hz), 3.91 (t, 2 H), 3.64 (t, 2 H). ^{13}C NMR (62.89 MHz): δ 171.0, 54.2, 53.0, 49.8, 35.2. IR (KBr): 3400, 3100–2800, 2600, 2000, 1650, 1605, 1580, 1505, 1300, 1140, 850, 745. $[\alpha]_D^{25} = -2.0°$ (*c* 0.02, H_2O).

*(−)-(3R)-4-(Benzyloxycarbonyl)-perhydro-1,4-thiazinecarboxylic acid benzyl ester (**9**).* To a solution of **3** (2.40 g, 6.16 mmol) in 15 ml of dry tetrahydrofuran was added diethyl azodicarboxylate (1.50 g, 1.4 equiv.) under an atmosphere of argon. To this solution was added dropwise over a period of 10 min a solution of triphenylphosphine (2.10 g, 1.3 equiv.) in 5 ml of tetrahydrofuran.[12,13] The reaction mixture was allowed to stand at room temperature for 5 h. The solvent was removed by evaporation and the residue was treated with ether to precipitate triphenylphosphine oxide. The precipitate was filtered off and the ether solution evaporated. The residue was purified by flash chromatography (Silica gel 60; heptane–ethyl acetate 4:1) to yield 1.25 g (55%) of **9**. ^{1}H NMR (250.13 MHz): δ 7.21 (m, 10 H), 5.15 (d, 1 H), 5.07 (m, 4 H), 4.34 (dd, 1 H, *J* 25.0 and 13.9 Hz), 3.23 (q, 1 H, *J* 24.5 and 12.8 Hz), 2.98 (t, 1 H), 2.79 (m, 1 H), 2.52 (m, 1 H), 2.28 (t, 1 H). ^{13}C NMR (62.89 MHz): δ 169.1, 158.0, 155.5, 135.9, 135.2, 128.3, 128.1, 127.9, 127.8, 127.6, 67.5, 67.0, 54.8, 54.3, 42.7,

42.3, 28.3, 26.8. IR (neat film): 3100–3000, 2990–2800, 1745, 1705, 1420, 1305, 1180, 1005, 750, 700. MS (EI): 371 (2) $[M^+]$, 280 (1), 236 (10), 192 (13), 91 (100), 65 (11). $[\alpha]_D^{25} = -75.6°$ (*c* 0.104).

*(−)-(3R)-Perhydro-1,4-thiazine-3-carboxylic acid (**10**).* To a solution of 200 mg (0.538 mmol) of **9** in 7.5 ml of anisole under an atmosphere of argon at 0°C was added in one portion anhydrous aluminium chloride (0.43 g, 6 equiv.).[14] The reaction mixture was allowed to stand at room temperature for 2.5 h and was then quenched by the addition of water. The aqueous phase was extracted with ethyl acetate to remove any unchanged starting material and was then neutralized with aqueous sodium hydroxide. The precipitated aluminium hydroxide was filtered off and the filtrate was passed through an Amberlite CG-120 column. The amino acid **10** was eluted with 0.2 M aqueous ammonia. The eluate was evaporated to dryness to yield a slightly yellow solid. After recrystallisation from ethanol–water and drying of the crystals at 0.1 mmHg over diphosphorus pentoxide, 45 mg (57%) of **10** was obtained as a white solid. ^{13}C NMR (20.15 MHz): δ 174.5, 61.5, 47.6, 29.7, 26.1. IR (KBr): 3036, 2852, 2352, 1596, 1394, 1346, 1308, 1193, 1159, 977, 942. $[\alpha]_D^{25} = -50.7°$ (*c* 0.013, H_2O) {lit.[15] $[\alpha]_D^{25} = -51.7°$ (*c* 0.01, H_2O); $[\alpha]_D^{13} = -52.94°$ (*c* 0.02, H^2O)}.

*(−)-(3R)-1,1-Dioxo-1,4-(benzyloxycarbonyl)perhydro-1,4-thiazine-3-carboxylic acid benzyl ester (**11**).* To a solution of **9** (0.50 g, 1.35 mmol) in 6 ml of methanol at 0°C was added a solution of Oxone (1.24 g, 3 equiv.) in 6 ml of water. The reaction mixture was allowed to stand at room temperature overnight. The methanol was removed by evaporation and the remaining aqueous solution was extracted with several portions of dichloromethane. The combined organic layers were dried ($MgSO_4$) and the solvent was removed by evaporation. Purification of the crude product by flash chromatography (Silica gel 60; heptane–ethyl acetate 1:1), afforded 0.45 g (83%) of **11** as a white solid, m.p. 125°C. ^{1}H NMR (250.13 MHz): δ 7.34 (m, 10 H), 5.44 (d, 1 H, *J* 43.3), 5.18 (m, 4 H), 4.57 (dd, 1 H, *J* 32.2 and 15.1 Hz), 3.89 (m, 1 H), 3.68 (m, 1 H), 3.26 (dd, 1 H, *J* 14.4 and 5.8 MHz), 2.99 (m, 2 H). ^{13}C NMR (62.89 MHz): δ 167.4, 155.4, 135.5, 134.8, 128.7, 128.6, 128.3, 68.8, 68.3, 54.3, 51.3, 50.8, 40.5. IR (KBr): 3100–3000, 2900–2850, 1750, 1420, 1320, 1180, 1130, 1000, 750, 700. MS (CI, direct inlet): 404 $[(M+H)^+]$, 432 $[(M+C_2H_5)^+]$. $[\alpha]_D^{25} = -15.7°$ (*c* 0.10).

*(−)-(3R)-1,1-Dioxoperhydro-1,4-thiazine-3-carboxylic acid (**12**).* The procedure for deprotection with aluminium trichloride, work-up, purification and drying of the final product was the same as for the synthesis of **10**. A solution of 170 mg (0.421 mmol) of **11** in 6.5 ml of anisole under argon was treated with 0.34 g (6 equiv.) aluminium trichloride. Deprotection was complete within 2 h. After work-up, purification and drying, 37 mg (49%) of **12** were obtained as white crystals. IR (KBr): 3030, 2966, 2420, 1591, 1391, 1371, 1276, 1121, 852. $[\alpha]_D^{40} = -3.4°$ (*c* 0.005).

*(−)-(3R)-1-Oxo-4-(benzyloxycarbonyl)perhydro-1,4-thiazine-3-carboxylic acid benzyl ester (**13**).* A small Erlenmeyer flask was charged with a solution of **9** 0.70 g (1.88 mmol) dissolved in 5 ml of dichloromethane and 5 ml aqueous 10% potassium hydrogencarbonate. The mixture was

vigorously stirred and bromine (0.11 ml, 1.1 equiv.) was added dropwise.[15] The reaction mixture was vigorously stirred at room temperature for 20 min after which time unchanged bromine was destroyed by the addition of solid sodium thiosulfate. The organic layer was separated, and the aqueous layer was extracted with several portions of ethyl acetate. The combined organic layers were dried ($MgSO_4$). After filtration, evaporation of the solvent, and purification of the crude product by flash chromatography (Silica gel 60; ethyl acetate), pure **13** 0.70 g (96%) was obtained as a viscous oil. ^{1}H NMR (250.13 MHz): δ 7.32 (m, 10 H), 5.25 (m, 1 H), 5.20 (m, 4 H), 4.20 (m, 1 H), 3.68 (m, 1 H), 3.20 (d, 1 H), 2.83 (m, 1 H), 2.58 (m, 2 H). ^{13}C NMR (250.13 MHz): δ 168.7, 168.2, 155.8, 155.3, 135.9, 135.6, 135.3, 134.7, 128.7, 128.6, 128.5, 128.4, 128.3, 128.1, 128.0, 68.3, 68.1, 67.9, 67.8, 67.5, 53.4, 49.8, 49.5, 48.4, 47.9, 44.5, 44.4, 44.0, 38.1, 30.7, 30.5. IR (neat film): 3100–3000, 2950–2820, 1745, 1705, 1415, 1300, 1175, 1045, 750, 700. $[\alpha]_D^{25} = -52.0°$ (*c* 0.10).

*(−)-(3*R*)-4-(*tert*-butoxycarbonyl)perhydro-1,4-thiazine-3-carboxylic acid benzyl ester (***14***).* To a solution of **2** (1.77 g, 4.98 mmol) and diethyl azodicarboxylate (1.13 g, 1.3 equiv.) in 20 ml of tetrahydrofuran under an argon atmosphere was added dropwise a solution of triphenylphospine (1.57 g, 1.2 equiv.) in 5 ml tetrahydrofuran. The resulting mixture was allowed to stand at room temperature for 4 h. The solvent was removed by evaporation and the residue was treated with diethyl ether to precipitate triphenylphosphine oxide. After filtration, evaporation of the ether, and purification of the crude product by flash chromatography (Silica gel 60; heptane–ethyl acetate 5:1) 0.48 g (28%) of **14** was obtained as a mixture of two diastereomers, m.p. 73–75°C. ^{1}H NMR (250.13 MHz): δ 7.35 (m, 5 H), 5.19 (m, 3 H), 4.38 (m, 1 H), 3.15 (m, 2 H), 2.89 (dd, 1 H, *J* 13.8 and 3.9 Hz), 2.69 (t, 1 H), 2.43 (t, 1 H), 1.41 (d, 9 H). ^{13}C NMR (62.89 MHz): δ 169.7, 169.6, 155.5, 154.9, 135.5, 128.5, 128.3, 128.2, 128.1, 128.0, 80.8, 67.1, 55.3, 53.7, 43.2, 41.8, 28.6, 28.3, 27.2. IR (KBr): 3100–3000, 2990–2850, 1743, 1700, 1405, 1310, 1150, 1000, 755, 700. $[\alpha]_D^{25} = -75.6°$ (*c* 0.104).

*(+)-(2*R*)-2-(Benzyloxycarbonylamino)-3-(vinylsulfonyl)propanoic acid benzyl ester (***15***).* ^{1}H NMR (80.13 MHz): δ 7.33 (m, 10 H), 6.61 (m, 2 H), 6.09 (m, 1 H), 5.76 (d, 1 H), 5.17 (s, 4 H), 4.65 (m, 1 H), 3.64 (d, 2 H). IR (KBr): 3350, 3100–3000, 2980–2830, 1745, 1700, 1530, 1300, 1125, 975, 750, 700. $[\alpha]_D^{25} = 2.6°$ (*c* 0.035).

rac-*Perhydro-1,4-thiazine-3-carboxylic acid ethyl ester hydrochloride (***16***).* **Hydrogen cyanide is evolved during the reaction and the experiment was carried out in an efficient hood.** A 500 ml Erlenmeyer flask was purged with argon and charged with 2-aminoethanethiol hydrochloride (2.62 g, 23.1 mmol), sodium hydrogencarbonate (4.85 g, 2.5 equiv.), 2.0 g of 3 Å molecular sieves (activated at 150°C, 1 mmHg) and 115 ml of dry methanol. To the flask were added 10 mg of bromocresol purple (pH indicator). To this solution was added ethyl 3-bromo-2-oxopropanoate (5.00 g, 23.1 mmol) at such a rate that the pH was maintained above pH 6. The addition took about 2 h. When the addition was complete, the reaction mixture was allowed to stand for an additional 30 min where upon sodium cyanoborohydride (2.90 g, 2 equiv.) was added. The reaction mixture was acidified to pH 4 and was kept at this pH by the careful addition of 6 M hydrochloric acid. When all of the imine had been reduced (ca. 3 h) the excess of hydride reagent was destroyed by the addition of hydrochloric acid to maintain a pH in the range 1–2. When the gas evolution has ceased, the reaction mixture was filtered through a pad of Celite to remove the molecular sieves. Methanol was evaporated and the remaining aqueous phase was extracted with diethyl ether to remove any unchanged bromo ester. The ethereal layer was discarded. The aqueous layer was made alkaline by the addition of sodium hydroxide solution and then extracted with several portions of diethyl ether. The combined organic layers were dried over magnesium sulfate and filtered. Gaseous hydrogen chloride was passed through the solution to precipitate the amino acid ester hydrochloride. After evaporation of the ether, 3.94 g (81%) of pure **16** were obtained as white crystals. ^{1}H NMR (250.13 MHz, D_2O): δ 4.39 (m, 1 H), 4.34 (q, 2 H), 3.76 (dt, 1 H, *J* 13.2, 4.6 and 3.9 Hz), 3.42 (dt, 1 H, *J* 9.7, 3.5 and 3.5 Hz), 3.09 (m, 2 H), 2.98 (m, 2 H), 1.30 (t, 3 H). ^{13}C NMR (62.89 MHz, D_2O): δ 170.1, 66.6, 59.1, 47.5, 28.7, 26.0, 15.8. MS (EI, free base): 175 (4) [M^+], 146 (1), 102 (100), 74 (41), 56 (32). IR (KBr): 2980, 2920, 2720, 2640, 2450, 2100, 1745, 1375, 1270, 1220, 1090, 1030.

rac-*Perhydro-1,4-thiazine-3-carboxylic acid.* Hydrolysis of **16** was achieved by stirring the ester in 2 M aqueous barium hydroxide overnight followed by neutralization of reaction mixture by the addition of 6 M sulfuric acid. The precipitated barium sulfate was removed by filtration and the clear solution was passed through an Amberlite IR-120 column. The amino acid was eluted with 0.2 M aqueous ammonia. The eluate was evaporated to dryness to yield the free amino acid as a white solid.

rac-*1-Oxoperhydro-1,4-thiazine-3-carboxylic (***17a,b***).* Oxidation of **16** to the corresponding sulfoxide was achieved by adding an aqueous solution of sodium metaperiodate (1.15 equiv.) to a cold (0°C) solution of **16** in water–methanol (1:1). When the addition was complete the reaction mixture was left at room temperature overnight. Methanol was removed by evaporation under reduced pressure and the residual aqueous solution was extracted with several portions of dichloromethane. After drying ($MgSO_4$) and evaporation the crude sulfoxide ester was obtained as a mixture of two diastereomeric enantiomeric pairs. Flash chromatography (Silica gel 60; hexane–dichloromethane gradient elution) afforded par-

tial separation of the diastereomers which were collected in two fractions. Evaporation of the solvent afforded two batches of different diastereomeric composition of the sulfoxide. However, we were unable to assign the relative configuration of the sulfoxide group in these fractions. Hydrolysis of the crude sulfoxide ester fractions with barium hydroxide and work-up as given above afforded two batches of diastereomerically enriched sulfoxide amino acids as white solids. These samples were used to determine the principal properties of these amino acids.

rac-*1,1-Dioxoperhydro-1,4-thiazine-3-carboxylic acid* (**18**). The crude sulfoxide ester from the above procedure was dissolved in 0.5 M aqueous sulfuric acid and an aqueous solution of potassium permanganate was added with stirring over a period of 2 h. The excess of permanganate was destroyed by the addition of formic acid.[16] The reaction mixture was neutralized with aqueous barium hydroxide, filtered and passed through an Amberlite IR-120 column. Elution with aqueous ammonia and evaporation of the eluate to dryness afforded **18** as a white solid.

References

1. Larsson, U., Carlson, R. and Leroy, J. *Acta Chem. Scand. 47* (1993) 380.
2. Jonsson, J., Eriksson, L., Hellberg, S., Sjöström, M. and Wold, S. *Quant. Struct.-Act. Relat. 8* (1989) 204.
3. Hellberg, S., Sjöström, M. and Wold, S. *Acta Chem. Scand., Ser. B 40* (1986) 135.
4. Carlson, R. *Design and Optimization in Organic Synthesis*, Elsevier, Amsterdam 1992, pp. 337–428.
5. Larson, U. and Carlson, R. *Acta Chem. Scand. 48* (1994) preceding paper.
6. Black, D.K. *J. Chem. Soc. C* (1966) 1123.
7. Keller, O., Keller, W.E., van Look, G. and Wersin, G. *Org. Synth. Coll. Vol. VII* (1990) 73.
8. Wang, S., Gisin, B.F., Winter, D.P., Makofske, R., Kulesha, I.D., Tzougraki, C. and Meienhofer, J. *J. Org. Chem. 42* (1977) 1286.
9. Trost, B.M. and Curran, D.P. *Tetrahedron Lett. 22* (1981) 1287.
10. ElAmin, B., Anantharamaiah, G.M., Royer, G.P. and Means, G.E. *J. Org. Chem. 44* (1979) 3442.
11. Lundt, B.F., Johansen, N.L., Volund, A. and Markussen, J. *Int. J. Protein Res. 12* (1978) 258.
12. Mitsunobu, O. *Synthesis* (1981) 1.
13. Mattingly, P.G., Kervin, J.F. and Miller, M.J. *J. Am. Chem. Soc. 101* (1979) 3983.
14. Tsuji, T., Kataoka, T., Yoshioka, M., Sendo, Y., Nishitami, Y., Hirai, S., Meada, T. and Nagata, W. *Tetrahedron Lett. 30* (1979) 2793.
15. Drabowicz, J., Midura, W. and Mikolajczyk, M. *Synthesis* (1979) 39.
16. Carson, J.F., Boggs, L.E. and Lundin, R. *J. Org. Chem. 33* (1968) 3739.
17. Eriksson, L., Jonsson, J., Sjöström, M. and Wold, S. *Quant Struct.-Act. Relat. 7* (1988) 144.
18. Kogami, Y. and Okawa, K. *Bull. Chem. Soc. Jpn. 60* (1987) 2963.
19. Najima, K., Takai, F., Tanaka, T. and Okawa, K. *Bull. Chem. Soc. Jpn. 51* (1978) 1577.
20. Nakajima, K., Masahiro, N., Yamada, S. and Okawa, K. *Bull. Chem. Soc. Jpn. 55* (1982) 3049.

Received January 4, 1994.