Pergamon

Tetrahedron: *Asymmetry* 11 (2000) 4965–4973

TETRAHEDRON: *ASYMMETRY*

New access to the synthetic building block L-aspartic acid β-semialdehyde via Grignard reaction

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Received 14 November 2000; accepted 5 December 2000

Abstract

A new, efficient, and inexpensive synthesis of protected L-aspartic acid β-semialdehyde has been developed starting from L-glutamic acid via a substituted L-allylglycine derivative as intermediate. The key step of the reaction sequence was a strongly solvent-dependent Grignard reaction of an L-glutamic acid semiester. The desired regioselective addition to the C-5 ester group was achieved in 1,2-dimethoxyethane while reactions in diethyl ether gave products resulting from additional attack at the carboxylic acid functionality.

1. Introduction

L-Aspartic acid β-semialdehyde (**1**) is a natural non-proteinogenic amino acid and a key intermediate in the biosynthesis of the essential amino acids L-lysine, L-methionine, L-threonine, and L-isoleucine in higher plants and bacteria.[1] It also represents a relevant synthetic intermediate. The potential access to a variety of polyfunctional non-proteinogenic and unnatural amino acids using **1** has been demonstrated.[2] Further applications include the total syntheses of rhizobitoxin[3] and sinefungin,[4] and also of the phytotoxic agent D,L-γ-hydroxyphosphinothricine via protected, racemic **1**.[5]

An established route to **1** employs ozonolysis of L-allylglycine[6] or its derivatives,[7] which in turn is accessible by malonester syntheses[8] and via glycine-derived[9] or other stable imines[10] followed by enzyme-catalyzed racemate resolution. Other known methods start from the chiral pool and proceed through oxidation of L-homoserines, which can be prepared from L-methionine,[2] and by controlled reduction[11] or, preferably, by reduction–oxidation[12] of L-aspartic acid. However, *N*-alkoxycarbonyl derivatives of homoserines reportedly have the tendency of lactonization.[13] Furthermore, many reducing agents are not compatible with widely-used protecting

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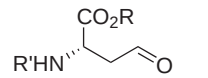
0957-4166/00/$ - see front matter
PII: S0957-4166(00)00487-0

groups.[14] Common oxidation methods for the synthesis of some aldehydes have been successfully utilized,[15] although they led to unsatisfactory results for other authors.[16] None of these methods is predominant in the literature.

The need for enantiomerically pure **1** for biochemical and organic-preparative studies demands an efficient, inexpensive synthetic route of general validity. We describe a method that combines the strengths of both known approaches using the chiral pool of the biogenic amino acids and the mild ozonolysis of an L-allylglycine analogue by an overall Barbier–Wieland degradation. To avoid stability problems and because of its intended further use in natural product synthesis, the doubly protected derivative of **1**, *N*-ethoxycarbonyl-L-aspartic acid α-methylester β-semialdehyde **2**, had been chosen as a target molecule (see Fig. 1).

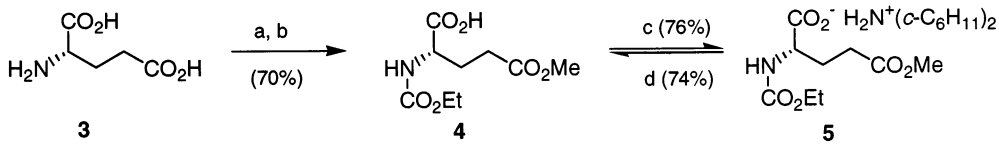


1 R=H R'=H
2 R=Me R'=CO_2Et

Figure 1.

2. Results and discussion

L-Glutamic acid **3** served as starting material. The two acid functions were differentiated by selective esterification of the γ-carboxyl group to secure chemoselectivity in later stages in the synthesis. Esterification and subsequent protection of the amino functionality were carried out analogous to our established method for L-aspartic acid.[17] The NMR-spectroscopically pure, yet oily semiester **4** was obtained in high yield. It was further characterized as its dicyclohexylammonium salt **5**, which served as a storage compound because of its stability (see Scheme 1).



3 **4** **5**

Scheme 1. (a) MeOH, HCl, 20 h, rt; then $NaHCO_3$; (b) $NaHCO_3$, $ClCO_2Et$, 16 h, rt; (c) $NH(c\text{-}C_6H_{11})_2$, Et_2O; (d) $NaHCO_3$, Et_2O; then H_2SO_4, Et_2O

Grignard reagents are known to add to ester groups but usually not to free acid or carbamate functionalities, even though we recently observed side products arising from attack at the acid functionality of *N*-alkoxycarbonyl-L-aspartic acid β-methyl ester.[18] Reaction with semiester **4** was expected to mainly afford a tertiary alcohol that would dehydrate to a substituted L-allylglycine derivative under mild conditions. Phenylmagnesium bromide was chosen as the Grignard reagent to favor subsequent acid-catalyzed dehydration because of the stabilizing effect of the phenyl groups. Four equivalents of Grignard reagent are needed for the reaction: two equivalents to abstract the acidic protons of the carbamate nitrogen and carboxylic acid functions, and the other two for the nucleophilic addition onto the ester functionality.

Initially, phenylmagnesium bromide was prepared in diethyl ether where quantitative formation of phenylmagnesium bromide was assumed.[19] The Grignard reaction was carried out in the same solvent by addition of the organometallic compound to semiester **4**. Unexpectedly, hydroxy ketone **6** and diol **7** were the major products while much of the starting material **4** remained unreacted. Only minute quantities of the hydroxy acid **8** could be found (see Fig. 2).[20]

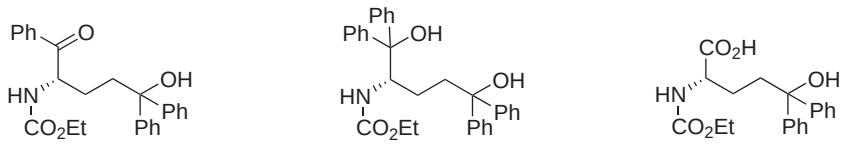


Figure 2.

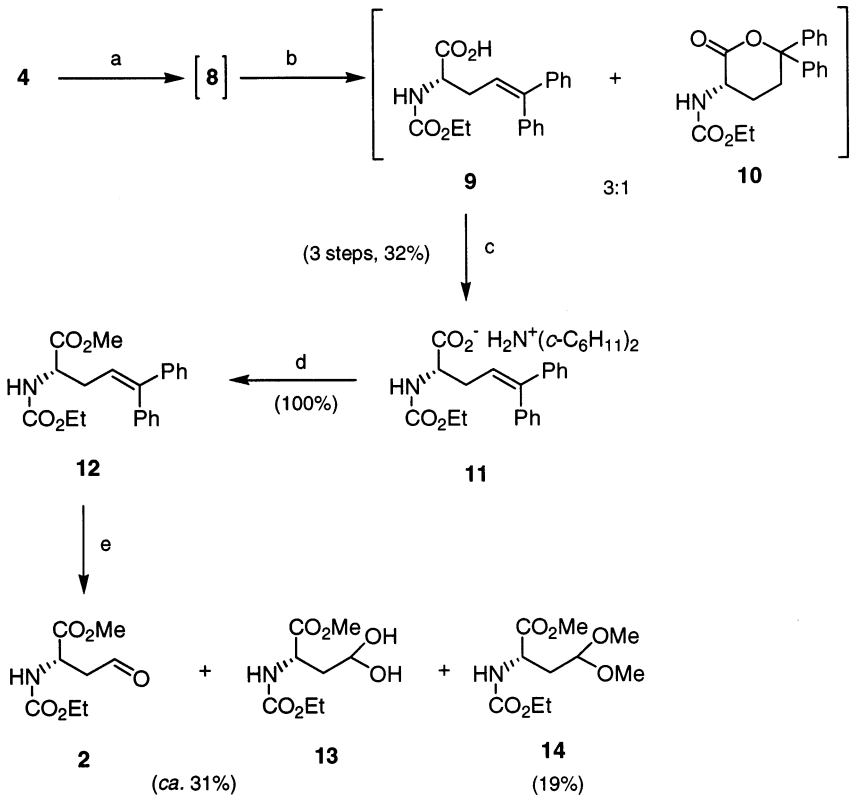
It was then attempted to increase the selectivity of the Grignard reaction by choosing a bis-donative solvent such as 1,2-dimethoxyethane (DME) which can slow down the reaction rate by chelating the Grignard reagent.[19] Furthermore, chelation of the magnesium cations by the doubly deprotonated semiester **4** could sterically hinder nucleophilic attack at C-1. Similar complexes with 2,3-dimethoxybutane have been postulated in the literature.[21] Under these conditions, hydroxy acid **8** was indeed the major product, while hydroxy ketone **6** and diol **7** were not found to have formed. The optimal ratio of bromobenzene to semiester **4** was found to be 10:1, indicating a low yield in the preparation of phenylmagnesium bromide in DME presumably due to coupling reactions.[21] Stronger acidification during the reaction work-up resulted in the desired dehydration and at 0°C formation of alkenoic acid **9** was favored over lactonization to compound **10** (3:1) (see Scheme 2). Compound **9** could be purified by precipitation as its dicyclohexylammonium salt **11** directly from the crude reaction mixture followed by careful washing with aqueous $NaHCO_3$ to remove leftover traces of the more polar starting material **4**. The 32% overall yield for the last three steps (Grignard reaction, dehydration, salt precipitation) can still be potentially improved if lactone **10** could be converted into **9**.

The method described demonstrates for the first time that alkenoic acid **9** is easily accessible in enantiomerically pure form. Until now, only a far more complex synthesis of a racemic mixture of the *N*-acylated 2-amino-5,5-diphenyl-4-pentenoic acid was known.[22]

Esterification of **11** using an H_2SO_4/MeOH mixture led to the desired methyl ester **12**, but also gave lactone **10** as a side product. However, when MeOH and dimethyl sulfate were used, **12** was obtained quantitatively (see Scheme 2). Ozonolysis of **12** in CH_2Cl_2/MeOH at −60°C followed by reductive work-up with dimethyl sulfide afforded a mixture of semialdehyde **2**, its hydrate **13**, and dimethyl acetal **14**, in a combined yield of 50% (see Scheme 2), all of which can potentially be used as building blocks in subsequent synthetic efforts. Reaction of semialdehyde **2** and its hydrate **13** with 2,4-dinitrophenylhydrazine afforded a crystalline derivative that has been identified unambiguously as hydrazone **15** (see Fig. 3).

3. Conclusion

A new synthesis of protected L-aspartic acid β-semialdehyde **1** was developed in overall yield of ca. 12%. The synthesis is inexpensive since it makes use of the chiral pool, does not require



Scheme 2. (a) PhMgBr, DME, 2 h, reflux; then H_2O; (b) H_2SO_4, pH 1.0, 0°C; (c) $NH(c\text{-}C_6H_{11})_2$, Et_2O; (d) Me_2SO_4, MeOH, 2 h, steam bath; (e) O_3, CH_2Cl_2/MeOH, −60°C; then Me_2S, 14 h, rt

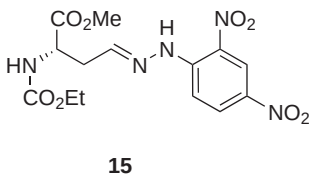


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Figure 3.

costly reagents or possibly harsh oxidizing/reducing conditions, and consists of only a few steps. It therefore represents a superior alternative to the established methods in the literature. However, the relatively low pH that is necessary for the dehydration excludes acid-sensitive *N*-protecting groups. The key to the success of the synthesis was the control of the regioselectivity in the reaction of the *N*-protected semiester **4** with phenylmagnesium bromide, where using DME as the solvent allowed the nucleophilic addition to be directed successfully towards the ester group. Exploiting this selective reactivity may enhance the applicability of Grignard reactions in natural product synthesis.

4. Experimental

4.1. General methods

Organic solvents were purified before use. Diethyl ether (Et_2O) and 1,2-dimethoxyethane (DME) were freed of peroxides by storage over KOH. After subsequent drying over sodium until positive benzophenone reaction, both ethers were distilled under an inert argon atmosphere. Preparations of phenylmagnesium bromide were also carried out under argon. TLC analysis was performed using Polygram Sil G/UV_{254} plates from Macherey–Nagel. The R_f values of salts given are equivalent to those of the corresponding acids since acidic mobile phases have been used. Silica gel S (0.032–0.063 mm, 230–400 mesh) from Riedel–de Haën was used for flash chromatography. Melting points were determined on a Büchi 510 apparatus and are uncorrected. Optical rotations were measured with the automatic polarimeter AA 1000 from Technolab. IR spectra were recorded on a Perkin–Elmer FTIR 1750 instrument. ^1H and ^{13}C NMR experiments were carried out on a Bruker AC 200 spectrometer operating at 200 and 50 MHz, respectively, using tetramethylsilane as the internal standard. Mass spectra were obtained on an HP5988 instrument from Hewlett–Packard, operated in the EI mode at 70 eV. Combustion analyses were performed by Beller Mikroanalytisches Labor, Göttingen (Germany).

4.2. N-*Ethoxycarbonyl-L-glutamic acid γ-methylester* **4**

L-Glutamic acid **3** (22.1 g, 150 mmol) was suspended in MeOH (400 mL). Addition of 37% aqueous HCl (45 mL, 540 mmol) resulted in a colorless solution that was stirred at 25°C for 20 h. H_2O (120 mL) and $NaHCO_3$ (46.0 g, 548 mmol) were added, and MeOH was evaporated in vacuo. H_2O was added to give a final volume of 250 mL followed by further $NaHCO_3$ (33.6 g, 400 mmol). Ethyl chloroformate (32.6 g, 300 mmol) was added dropwise to the solution that was then stirred for 16 h at 25°C. The solution was washed with Et_2O (3×50 mL) and the organic phases were discarded. The aqueous solution was cooled using an ice bath and acidified to pH 2 with cold 50% H_2SO_4. The product was extracted with Et_2O (4×150 mL). The combined ethereal phases were washed with H_2O (50 mL), dried over $MgSO_4$, and the solvent was evaporated to give semiester **4** as a colorless to slightly yellowish oil (24.6 g, 70%): R_f 0.23 [EtOAc/*n*-hexane/AcOH (30:60:1)]; ^1H NMR ($CDCl_3$) δ 1.18 (t, $J=7.1$ Hz, 3H, OCH_2CH_3), 1.86–2.08 (m, 1H, $CHH'CHN$), 2.08–2.30 (m, 1H, $CHH'CHN$), 2.43 (td, $J=7.7$, 2.8 Hz, 2H, CH_2CO_2Me), 4.07 (q, $J=7.1$ Hz, 2H, OCH_2), 4.20–4.42 (m, 1H, CH), 5.61 (br d, $J=8.1$ Hz, 0.75H, NH), 6.54 (br d, $J=7.0$ Hz, 0.25H, NH), 10.12 (br s, 1H, CO_2H); ^{13}C NMR ($CDCl_3$) δ 14.3 (q, OCH_2CH_3), 27.2 (t, CH_2CHN), 30.0 (t, CH_2CO_2Me), 51.8 (q, OCH_3), 52.9 (d, CH), 61.4 (t, OCH_2), 156.5 (s, $NHCO_2Et$), 173.5 (s, CO_2Me), 175.3 (s, CO_2H).

4.3. *Dicyclohexylammonium 5-methyl-(S)-N-ethoxycarbonylglutamate* **5**

Compound **5** was prepared for characterization and storage purposes. Dicyclohexylamine (5.80 g, 32.0 mmol) was added to a solution of semiester **4** (7.46 g, 32.0 mmol) in Et_2O (100 mL). Ammonium salt **5** crystallized overnight in the refrigerator. The colorless crystals were filtered, washed with cold Et_2O and dried in vacuo (10.1 g, 76%): R_f 0.23 [EtOAc/*n*-hexane/AcOH (30:60:1)]; mp 127–128°C; $[\alpha]_D^{20}$ +11.2 (*c* 1, MeOH); IR (KBr) ν_{max} 3227, 3000–2400, 1737, 1719, 1631, 1547, 1448, 1406, 1374, 1266, 1169, 1075 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.03–2.52 (m, 24H, 10

ring-CH_2, $CH_2CH_2CO_2Me$), 1.17 (t, $J=7.1$ Hz, 3H, OCH_2CH_3), 2.81–3.02 (m, 2H, 2 ring-CH), 3.58 (s, 3H, OCH_3), 3.91–4.13 (m, 3H, OCH_2, $NCHCO_2^-$), 5.60 (br d, $J=6.1$ Hz, 1H, NH), 8.20–9.70 (br, 2H, N^+H_2); ^{13}C NMR ($CDCl_3$) δ 14.6 (q, OCH_2CH_3), 4×24.7 (t, ring-CH_2), 2×25.0 (t, ring-CH_2), 28.9 (t, CH_2CHN), 4×29.0 (t, ring-CH_2), 30.3 (t, CH_2CO_2Me), 51.4 (q, OCH_3), 2×52.5 (d, ring-CH), 55.2 (d, $NCHCO_2^-$), 60.4 (t, OCH_2), 156.1 (s, $NHCO_2Et$), 174.0 (s, CO_2Me), 175.4 (s, CO_2^-). Anal. calcd for $C_{21}H_{38}N_2O_6$: C 60.85; H 9.24. Found: C, 60.81; H, 9.21.

To liberate semiester **4**, the ammonium salt **5** (7.50 g, 18.1 mmol) was mixed in a separatory funnel with an aqueous solution of $NaHCO_3$ (6.50 g, 77.4 mmol) in H_2O (70 mL) and Et_2O (35 mL). NaOH was added until the aqueous phase reached pH 9.5. The ethereal phase was separated, and the aqueous phase extracted with Et_2O (2×35 mL) to remove dicyclohexylamine. The aqueous phase was acidified to pH 2 with 50% H_2SO_4 and extracted with Et_2O (4×60 mL). The organic phases were dried over $MgSO_4$ and the solvent was evaporated to give semiester **4** (3.13 g, 74%).

4.4. Dicyclohexylammonium (S)-2-(ethoxycarbonylamino)-5,5-diphenyl-4-pentenoate ***11***

Grignard reactions were carried out in Et_2O and DME at various stoichiometric ratios and using different reaction work-ups. Reactions in DME using a molar ratio of bromobenzene/semiester **4** of 10:1 gave the best results. The following procedure proved to be the most efficient.

The Grignard reagent was prepared by dropwise addition of a solution of bromobenzene (35.3 g, 225 mmol) in DME (40 mL) to a slurry of magnesium (5.83 g, 240 mmol) in DME (60 mL). The reaction started after heating the mixture. To complete the reaction, the mixture was heated under reflux for an additional 2 h. The Grignard reagent was transferred into a dropping funnel under an argon atmosphere, while the remaining precipitate and excess magnesium were removed by this procedure. The Grignard reagent was then added to a solution of semiester **4** (5.00 g, 21.4 mmol) in DME (75 mL) under vigorous stirring and sonication. The white suspension was heated under reflux for 2 h and it finally turned yellow. The mixture was hydrolyzed with H_2O (100 mL), acidified until pH 1.0 with H_2SO_4 (50%) under cooling of the flask with ice. The precipitate dissolved, and the red organic phase was separated. The aqueous, mixed phase was extracted with Et_2O (4×100 mL). The extracts were combined with the previously separated organic phase and washed with aqueous $NaHCO_3$ solution of pH 8.0 (2×25 mL) and then further washed with H_2O (25 mL). The ethereal phase was concentrated to a final volume of 30 mL and dicyclohexylamine (3.88 g, 21.4 mmol) was added. The mixture was allowed to crystallize overnight in the refrigerator, the precipitated product was then collected by filtration and recrystallized from $CHCl_3$/*n*-hexane to afford pure **11** (3.54 g, 32% from **4**): R_f 0.47 [EtOAc/*n*-hexane/AcOH (30:60:1)]; mp 140–141°C; $[\alpha]_D^{20}$ +36.5 (*c* 1, MeOH); IR (KBr) ν_{max} 3422, 3054, 3025, 3000–2400, 1708, 1631, 1595, 1557, 1539, 1495, 1444, 1420, 1397, 1242, 1062, 758, 701 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.00–2.03 (m, 23H, 10 ring-CH_2, CH_3), 2.48–3.06 [m, 4H, CH_2CHN, $N^+(CH)_2$], 3.97–4.28 (m, 3H, OCH_2, $NCHCO_2^-$), 5.62 (br d, $J=7.1$ Hz, 1H, NH), 6.21 [t, $J=7.0$ Hz, 1H, $CHC(Ph)_2$], 7.15–7.43 (m, 10H, $CH_{ar.}$), 7.90–8.90 (br, 2H, N^+H_2); ^{13}C NMR ($CDCl_3$) δ 14.7 (q, CH_3), 4×24.7 (t, ring-CH_2), 2×25.0 (t, ring-CH_2), 4×29.1 (t, ring-CH_2), 34.0 (t, CH_2CHN), 52.4 [d, $N^+(CH)_2$], 55.6 (d, $NCHCO_2^-$), 60.3 (t, OCH_2), 126.0 [d, $CHC(Ph)_2$], 126.7, 127.2, 127.9, 128.0, 129.9 (d, 10×$CH_{ar.}$), 140.0, 142.8, 143.1 [s, 2×$C_{ar.}^{ipso}$, $C(Ph)_2$], 155.9 (s, $NHCO_2Et$), 175.6 (s, CO_2^-). Anal. calcd for $C_{32}H_{44}N_2O_4$: C, 73.81; H, 8.52. Found: C, 73.87; H, 8.42.

4.5. Methyl (S)-2-(ethoxycarbonylamino)-5,5-diphenyl-4-pentenoate **12**

A solution of ammonium salt **11** (2.09 g, 4.02 mmol) and dimethyl sulfate (506 mg, 4.02 mmol) in MeOH (20 mL) was heated in a steam bath for 2 h. H_2O (50 mL) was added and the solution extracted with Et_2O (4×50 mL). The combined ethereal phases were washed with saturated aqueous $NaHCO_3$ (50 mL) and then with H_2O (50 mL). The solvent was evaporated to give **12** as an oily, yellowish residue (1.42 g, 100%): R_f 0.48 [Et_2O/*n*-pentane (1:1)]; IR (film) ν_{max} 3336, 3056, 3025, 2981, 2953, 1745, 1723, 1599, 1525, 1497, 1444, 1372, 1213, 1178, 1059, 762, 702 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.14 (t, $J=7.1$ Hz, 3H, OCH_2CH_3), 2.37–2.72 (m, 2H, CH_2CHN), 3.61 (s, 3H, OCH_3), 4.02 (q, $J=7.1$ Hz, 2H, OCH_2), 4.32–4.47 (m, 1H, CHN), 5.16 (br d, $J=8.4$ Hz, 1H, NH), 5.91 [t, $J=7.4$ Hz, 1H, $CHC(Ph)_2$], 6.95–7.39 (m, 10H, $CH_{ar.}$); ^{13}CNMR ($CDCl_3$) δ 14.5 (q, OCH_2CH_3), 32.7 (t, CH_2CHN), 52.3 (q, OCH_3), 53.5 (d, CHN), 61.1 (t, OCH_2), 122.6 [d, $CHC(Ph)_2$], 127.2, 127.3, 127.7, 128.1, 128.3, 129.6 (d, 10×$CH_{ar.}$), 139.3, 142.0, 145.4 [2×$C_{ar.}^{ipso}$, $C(Ph)_2$], 155.9 (s, $NHCO_2Et$), 172.3 (s, CO_2Me); MS (EI) *m*/*z* (%) 353 (2) [M^+], 294 (52) [$M^+-CO_2CH_3$], 205 (88), 178 (17), 165 (30), 144 (16), 115 (23), 90 (84), 77 (9), 59 (6) [$CO_2CH_3^+$], 29 (56), 28 (100) [$C_2H_4^{\bullet+}$].

4.6. Ozonolysis of methyl (S)-2-(ethoxycarbonylamino)-5,5-diphenyl-4-pentenoate **12**

Ester **12** (1.41 g, 3.99 mmol) was dissolved in a mixture of CH_2Cl_2/MeOH (2:1) (300 mL) and ozonized at −60°C. Dimethyl sulfide (1 mL) was added and the solution stirred at room temperature overnight. The solvent was removed in vacuo. Flash chromatography of the residue gave semialdehyde **2**, partially hydrated to **13**, and its dimethyl acetal **14**.

4.7. Mixture of N-ethoxycarbonyl-L-aspartic acid α-methyl ester β-semialdehyde **2** and N-ethoxycarbonyl-L-aspartic acid α-methyl ester β-semialdehyde hydrate **13**[†]

Slightly yellowish oil (250 mg, ca. 31%); R_f 0.27 [Et_2O/*n*-pentane (2:1)]; IR (film) ν_{max} 3362, 2982, 2940, 2720, 1740, 1723, 1700, 1531, 1440, 1374, 1221, 1180, 1066 cm^{-1}; ^1H NMR ($CDCl_3$) for **2**: δ 1.17 (t, $J=7.1$ Hz, 3H, OCH_2CH_3), 3.02 (t, $J=5.0$ Hz, 2H, CH_2CHN), 3.68 (s, 3H, OCH_3), 4.05 (q, $J=7.1$ Hz, 2H, OCH_2), 4.58 (dt, $J=8.4$, 5.0 Hz, 1H, CHN), 5.76 (br d, $J=8.4$ Hz, 1H, NH), 9.66 (s, 1H, CHO); ^1H NMR ($CDCl_3$) for **13**: δ 1.17 (t, $J=7.1$ Hz, 3H, OCH_2CH_3), 1.98–2.10 (m, 2H, CH_2CHN), 3.68 (s, 3H, OCH_3), 4.05 (q, $J=7.1$ Hz, 2H, OCH_2), 4.37–4.48 (m, 1H, CHN), 5.20–5.32 [m, 1H, $CH(OH)_2$], 5.65 (br d, $J=7.9$ Hz, 1H, NH); ^{13}C NMR ($CDCl_3$) for **2**: δ 14.4 (q, OCH_2CH_3), 45.8 (t, CH_2CHN), 48.8 (d, CHN), 52.8 (q, OCH_3), 61.4 (t, OCH_2), 156.1 (s, $NHCO_2Et$), 171.2 (s, CO_2Me), 199.3 (d, CHO); ^{13}C NMR ($CDCl_3$) for **13**: δ 14.4 (q, OCH_2CH_3), 35.1 (t, CH_2CHN), 50.5 (d, CHN), 52.5 (q, OCH_3), 61.4 (t, OCH_2), 98.5 [d, $CH(OH)_2$], 156.1 (s, $NHCO_2Et$), 172.5 (s, CO_2Me); MS (EI) *m*/*z* (%) 203 (0.1) [M^+ for **2**], 175 (2), 171 (8), 144 (100) [($M^+-CO_2CH_3$) for **2**], 130 (10), 115 (11), 102 (6), 88 (6), 72 (17), 59 (6), 44 (52) [CH_2=CH-$OH^{\bullet+}$], 29 (25).

[†] NMR signal assignments were made with aid of 2D NMR correlation experiments and published NMR data for similar compounds.

4.8. Methyl (S)-2-(ethoxycarbonylamino)-4,4-dimethoxybutanoate **14**

Slightly yellowish oil (187 mg, 19%); R_f 0.49 [Et_2O/*n*-pentane (2:1)]; IR (film) ν_{max} 3400, 2980, 2954, 2820, 1745, 1713, 1542, 1440, 1373, 1224, 1185, 1130–1000 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.17 (t, $J=7.1$ Hz, 3H, OCH_2CH_3), 1.90–2.18 (m, 2H, CH_2CHN), 3.24 (s, 3H, $CH_3OCHOCH'_3$), 3.27 (s, 3H, $CH_3OCHOCH'_3$), 3.67 (s, 3H, CO_2CH_3), 4.05 (q, $J=7.1$ Hz, 2H, OCH_2), 4.28–4.46 (m, 2H, $CHCH_2CH$), 5.57 (br d, $J=7.9$ Hz, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 14.4 (q, OCH_2CH_3), 34.8 (t, CH_2CHN), 50.7 (d, CHN), 52.2, 53.2, 53.6 [q, CO_2CH_3, $CH(OCH_3)_2$], 61.0 (t, OCH_2), 102.0 [d, $CH(OMe)_2$], 156.3 (s, $NHCO_2Et$), 172.8 (s, CO_2Me); MS (EI) *m*/*z* (%) 249 (0.01) [M^+], 218 (2), 190 (5) [$M^+-CO_2CH_3$], 161 (4), 158 (9), 129 (33), 88 (6), 75 (100) [$CH(OCH_3)_2^+$], 59 (5), 29 (6).

4.9. N-*Ethoxycarbonyl-L-aspartic acid α-methyl ester β-semialdehyde-(2,4-dinitro)phenylhydrazone* **15**

A freshly prepared solution of the mixture of **2** and **13** (100 mg, ca. 479 μmol) in EtOH (1 mL) was added to a solution of 2,4-dinitrophenylhydrazine (400 mg, 2.02 mmol) in concentrated H_2SO_4 (2 mL) and H_2O (25 mL). The resultant precipitate was filtered, washed with H_2O, recrystallized from EtOAc/*n*-hexane, and dried in vacuo. Yellow crystals of **15** were obtained (20.5 mg, ca. 11%): mp 167–168°C; $[\alpha]_D^{20}$ +40.2 (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.18 (t, $J=7.1$ Hz, 3H, OCH_2CH_3), 2.80–3.06 (m, 2H, CH_2CHN), 3.73 (s, 3H, OCH_3), 4.07 (q, $J=7.1$ Hz, 2H, OCH_2), 4.56–4.73 (m, 1H, $NCHCO_2Me$), 5.41 (br d, $J=8.1$ Hz, 1H, $NHCO_2Et$), 7.44 (t, $J=4.4$ Hz, 1H, CHNNH), 7.77 (d, $J=9.6$ Hz, 1H, $CH_{ar.}^{(6)}$), 8.28 (dd, $J=9.6$, 2.6 Hz, 1H, $CH_{ar.}^{(5)}$), 9.05 (d, $J=2.6$ Hz, 1H, $CH_{ar.}^{(3)}$), 11.0 (s, 1H, NNH); ^{13}C NMR ($CDCl_3$) δ 14.5 (q, OCH_2CH_3), 35.4 (t, CH_2CHN), 51.2 (d, $NCHCO_2Me$), 52.9 (q, OCH_3), 61.5 (t, OCH_2), 116.4 (d, $CH_{ar.}$), 123.4 (d, $CH_{ar.}$), 129.3 (s, $C_{ar.}^{quart.}$), 130.2 (d, $CH_{ar.}$), 138.4 (s, $C_{ar.}^{quart.}$), 144.9 (s, $C_{ar.}^{quart.}$), 146.5 (d, CHNNH), 156.0 (s, $NHCO_2Et$), 171.7 (s, CO_2Me). Anal. calcd for $C_{14}H_{17}N_5O_8$: C, 43.87; H, 4.47. Found: C, 44.65; H, 4.77.

Acknowledgements

The authors thank the Deutsche Forschungsgemeinschaft and the Fond der Chemischen Industrie for financial support.

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