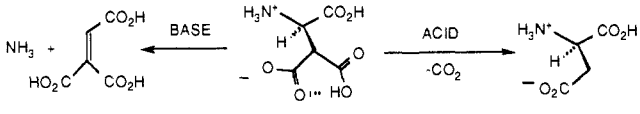
Asymmetric Synthesis of β-Carboxyaspartic Acid

Robert M. Williams,*† Peter J. Sinclair, and Weixu Zhai

Contribution from the Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523. *Received April 24, 1987*

Abstract: Bromoglycinate **4** was coupled with the (trimethylsilyl)ketene acetal of dibenzyl malonate to furnish predominantly the *syn*-lactone **6**. Hydrogenolysis of **6** directly afforded β-carboxyaspartic acid (Asa) in 30% overall yield in >98% ee. The optical purity of the synthetic Asa was determined by decarboxylation and esterification to aspartic acid diethyl ester.

β-Carboxyaspartic acid (**1**, Asa) was detected in the ribosomal protein hydrolysates of *Escherichia coli* by Koch and associates in 1982.[1] Asa is a homologue of γ-carboxyglutamic acid (Gla), a biologically important amino acid formed by a posttranslational vitamin K dependent γ-carboxylation of glutamyl residues in polypeptide precursors of prothrombin, bone, and kidney proteins; Asa may be formed by a related sequence. Asa has also recently been detected[1b] in the base hydrolysate of human atherosclerotic plaque, but the exact biological significance of Asa remains unclear at present. This is due, in part, to the lability of Asa. Asa is a notoriously unstable amino acid that is sensitive to decarboxylation, forming aspartic acid (**2**, Asp) under acidic conditions, and is sensitive to elimination of the amine function, forming carboxymaleic acid (**3**) under basic conditions. Asa is also the most acidic natural amino acid known (first pK_a ~0.8) and exists as the β-carboxy zwitterion.[2a] The inherent lability of Asa is sufficiently problematical that the harsh conditions employed in conventional protein-sequencing techniques have limited the detection of Asa in natural systems. Indeed, the sample obtained by Koch[1] from *E. coli* was not gathered in sufficient quantity to obtain complete spectroscopic characterization[2] (including an optical rotation) on the natural Asa but was instead correlated to a synthetic, racemic sample.[1]

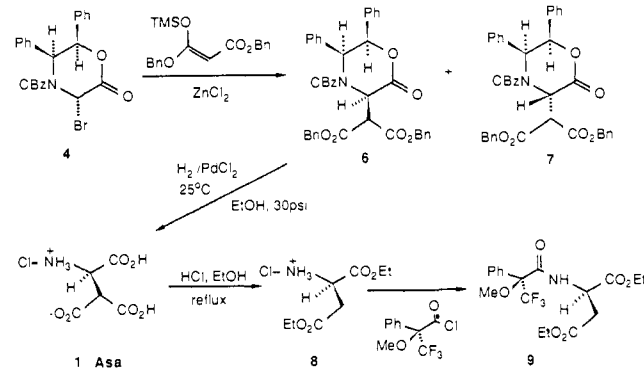


3 1 Asa 2 Asp

Several syntheses of Asa in *racemic* form have now been recorded;[1,3] successful resolution of *dl*-Asa, however, has not been reported. In a unique approach, Sargeson and Dixon[4] synthesized and resolved an optically active cobalt complex of β-carboxyaspartic acid β-diethyl ester. However, under the conditions employed to reduce the cobalt complex[4] (Zn, pH 6, 25 °C) and hydrolyze the esters (2 equiv, 1 N NaOH, 80 °C, 1 h), complete racemization ensued, resulting in the isolation of *dl*-Asa. Recently, Schöllkopf[5] reported the synthesis of several optically active triesters of Asa utilizing their bis(lactim) ether amino acid synthesis; however, the free amino acid was not reported.[5]

The development of numerous approaches to the asymmetric synthesis of α amino acids is mandated by the diversity of functionality and attendant stability of the α-"R" groups. Recent reports in this area have examined chiral glycine enolate alkylation,[6] chiral enolate amination,[7] and enolate halogenation[8]/azide substitutions to prepare amino acids in optically active form. Indeed, impressive advances in this field have been achieved with respect to the asymmetric induction attained. Nonetheless, the experimental conditions required to obtain the final free, zwitterionic α-amino acid in pure form may, in some instances, preclude the utilization of a particular method should the functionality in the α-R residue be labile to the conditions employed. As discussed above, Asa is a deceptively simple amino acid that, in addition to its intrinsic biological and chemical significance, poses an especially challenging synthetic test for new and existing

Scheme I



4 6 7 1 Asa 8 9

methodology. Due to the ease with which Asa suffers decarboxylation, elimination, and racemization, it seems unlikely that the optically pure, free amino acid will be readily accessed by existing technology.

In this paper, we record the first asymmetric synthesis of Asa utilizing the electrophilic glycinate template we have recently developed,[9] which exploits the mild conditions of heterogeneous hydrogenation to obtain the final, free amino acid.

Results and Discussion

Bromoglycinate **4**[9] was condensed with the (trimethylsilyl)-ketene acetal of dibenzyl malonate (**5**) in the presence of $ZnCl_2$ to furnish the lactones **6** and **7** (5.6:1 ratio) in 53% yield (Scheme I). These compounds were found to be configurationally stable

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and were readily separable by HPLC to afford each optically pure diastereoisomer. The major product was shown to possess the syn relative configuration as described below. Koch[1] had previously demonstrated that *dl*-Asa is stable to the conditions of catalytic hydrogenolysis. Thus, hydrogenation of **6** in EtOH at 40 psi in the presence of catalytic $PdCl_2$ followed by filtration of the catalyst, evaporation of the solvent, and trituration of the residue with Et_2O to extract the bibenzyl left a solid residue that consisted of Asa contaminated with Asp (ca. 4:1). The unfortunate presence of Asp is likely due to the mildly acidic medium[10] and relatively long reaction time (24 h at 25 °C). However, the extremely low pK_a of Asa enabled clean separation of Asp by stirring an aqueous solution of the mixture with an acidic ion-exchange resin (Dowex 50W-X8, H^+ form), filtration, and evaporation, leaving pure Asa as its hydrochloride salt in 30% overall yield. The Asa so obtained displayed a specific optical rotation, $[\alpha]^{25}_D$ −13.2° (*c* 0.8, H_2O).

The optical purity of the synthetic Asa was determined by decarboxylation and esterification (HCl, EtOH, reflux) to D-diethyl aspartate hydrochloride (**8**), which was converted into the corresponding (−)-MTPA (Mosher) amide[6a] **9** and analyzed by ^{19}F NMR. Comparison with authentic Mosher amides prepared from both racemic and optically pure aspartic acid diethyl ester indicated the synthetic material to be ≥98% ee. The fact that D-aspartic acid diethyl ester is obtained from **6** clearly establishes that the coupling of the *anti*-bromide[9c] **4** with **5** proceeds primarily via an S_N2 pathway, giving net *inversion* of stereochemistry.

These data are significant in that it is established that racemization does not accompany the decarboxylation of Asa to Asp nor does any racemization attend the other manipulations described in isolating Asa. This work also records the first specific rotation for Asa and can provide optically active Asa for studies that, due to the difficulty in obtaining sufficient quantities of Asa from natural sources, were not previously possible. It must also be pointed out that we have successfully coupled other malonate esters to **4**, and by analogy to our synthesis of β-ethyl aspartate,[9a] it should also be possible to synthesize optically active β-diesters of Asa for incorporation into peptides.

Experimental Section

(3*R*,5*S*,6*R*)-4-[(Benzyloxy)carbonyl]-3-(*O*,*O*'-dibenzylmalonyl)-5,6-diphenyl-2,3,5,6-tetrahydro-2*H*-1,4-oxazin-2-one (6) and (3*S*,5*S*,6*R*)-4-[(Benzyloxy)carbonyl]-3-(*O*,*O*'-dibenzylmalonyl)-5,6-diphenyl-2,3,5,6-tetrahydro-2*H*-1,4-oxazin-2-2-one (7). To a stirred solution of dibenzyl malonate (410 μL, 1.85 mmol, 2.8 equiv) in dry THF (6 mL) at 0 °C was added NaH (88 mg, 1.85 mmol, 2.8 equiv, 50% dispersion in oil). After 25 min, chlorotrimethylsilane (245 μL, 1.94 mmol, 3 equiv) was added. The mixture was stirred 20 min at 0 °C and 25 min at 25 °C and transferred to a solution of bromide **4** (300 mg, 0.65 mmol, 1.0 equiv) in dry THF (8 mL) via syringe. To this solution was added a 1.95 M solution of $ZnCl_2$ in THF (400 μL, 0.77 mmol, 1.2 equiv). The resulting orange solution was allowed to stir for 4 h, poured into H_2O, and thoroughly extracted with CH_2Cl_2. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, passed through a small plug of silica gel, concentrated, and separated by HPLC on Whatman Partisil 10 silica gel column (50 cm) (eluted with 1:5 EtOAc/hexanes at a flow rate of 5 mL/min), affording 36.6 mg (8%) of **7** and 207 mg (46%) of **6**.

6: oil; ^1H NMR (200 MHz, 393 K, DMSO-d_6)[11] δ 4.19 (1 H, d, *J* = 5.5 Hz), 4.89 (2 H, s), 4.99 (2 H, d, *J* = 12.7 Hz), 5.17 (2 H, d, *J* = 12.5 Hz), 5.74 (1 H, d, *J* = 3.2 Hz), 5.75 (1 H, d, *J* = 5.5 Hz), 6.37 (1 H, d, *J* = 3.2 Hz), 7.0–7.4 (25 H, m); IR (NaCl, neat) 3050, 2980, 1740, 1700, 1495, 1445, 1415, 1395, 1205, 1140 cm^{-1}; $[\alpha]^{25}_D$ +60.6° (*c* 1.51, CH_2Cl_2). Anal. ($C_{41}H_{35}NO_8$) C, H, N.

7: mp 168–169 °C, recrystallized from EtOAc/hexanes; ^1H NMR (200 MHz, 393 K, DMSO-d_6)[11] δ 4.83 (1 H, d, *J* = 5.0 Hz), 4.95 (2 H, s), 5.16 (1 H, d, *J* = 3 Hz), 5.19 (2 H, s), 5.22 (2 H, s), 5.68 (1 H, d, *J* = 5 Hz), 6.14 (1 H, d, *J* = 3 Hz), 6.57 (1 H, s), 6.61 (1 H, s), 6.8–6.9 (4 H, m), 7.1–7.4 (19 H, m); IR (KBr, disk) 3060, 3030, 2940, 1750, 1735, 1700, 1495, 1450, 1390, 1345, 1315, 1275, 1215, 1175, 1140, 1105 cm^{-1}; mass spectrum (NH_3/CI) *m/z* 687.9 (M^+ + 18, 0.7), 670.3 (M^+, 1.4) 579 (M^+ − 91, 1.1); $[\alpha]^{25}_D$ +23.8°(*c* 1.14, CH_2Cl_2). Anal. ($C_{41}H_{35}NO_8$) C, H, N.

D-β-Carboxyaspartic Acid (Asa). To a solution of **6** (77.6 mg, 0.12 mmol, 1.0 equiv) in THF (1.5 mL) and 0.03 N HCl/EtOH (1.5 mL) was added $PdCl_2$ (11.8 mg, 0.03 mmol, 0.3 equiv). The mixture was stirred under an atmosphere of H_2 at 40 psi for 27 h, purged with N_2, filtered through Celite, and concentrated. The residue was dissolved in $CHCl_3$, concentrated, twice, to remove excess HCl, and triturated with Et_2O several times, leaving 32 mg (white solid) of the crude Asa contaminated with a small amount of Asp (by ^1H NMR analysis). The residue was dissolved in ultrapure H_2O (4 mL) and stirred with ion-exchange resin (600 mg wet weight, Dowex 50W-X8, H^+ form, that was washed with 1 N NaOH, H_2O, 10% H_2SO_4, H_2O to pH 6, THF, and H_2O) for 15 min. The resin was filtered and the filtrate passed through a C-18 cartridge (Millipore C-18 Seppak, wet first with MeCN and then with H_2O) and concentrated to yield 8 mg of Asa HCl as a white solid, $[\alpha]^{25}_D$ −13.25° (*c* 0.8, H_2O, pH 2.56).

This material was identical with an authentic, racemic sample purchased from Sigma Chemical Co. (with the exception of optical rotation). The ^1H NMR (200 MHz) displayed a singlet at δ 4.57 in D_2O.

The optical purity of the synthetic Asa was determined by refluxing the sample in HCl/EtOH (2.5 mL, 1 N) and evaporation. The resulting aspartic acid diethyl ester hydrochloride (8 mg) was acylated in CCl_4 (0.2 mL) with (−)-MTPA-Cl (α-methoxy-α-(trifluoromethyl)phenylacetyl chloride; 10 μL, 0.07 mmol, 2 equiv) in the presence of pyridine (0.2 mL) for 8 h. The mixture was diluted with H_2O (1 mL), taken up in Et_2O, and washed with 1 N HCl, saturated $NaHCO_3$ solution, and H_2O. The ether layer was separated, dried over anhydrous $MgSO_4$, filtered, and evaporated to furnish the amide **9**. Analysis of the crude amide so obtained by ^{19}F NMR against an authentic diastereomeric mixture of the amides resulting from *dl*-aspartic acid diethyl ester hydrochloride revealed an enantiomeric excess ≥98%.

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(10) The hydrogenolysis requires 0.3 equiv of $PdCl_2$; Thus, 0.6 equiv of HCl can be generated in the reaction medium.

(11) ^1H NMR spectra for **6** and **7** are recorded at 393 K. This is done to preclude the line broadening caused by conformational exchange of the urethane moiety at room temperature.