# Enzyme-Catalyzed Regioselective Hydrolysis of Aspartate Diesters

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The application of enzymes to organic synthesis promises to provide improved conversion efficiency, significant increases in reaction rates, and higher selectivity than may be achieved in the corresponding uncatalyzed processes.[1] While each of these advantages are important, most attention has focused on achieving improved selectivity, for example, in the resolution of racemic mixtures or the hydrolysis of *meso* diesters.[2–4] Enzymes have proven especially useful for the kinetic resolution of racemic amino acids, either by hydrolyzing one enantiomer of the *N*-acetamide derivative[5] or by enantioselective saponification of an amino acid ester.[6] Typically, these methods result in preferential hydrolysis of the L-(*S*)-amino acid. However, interest in obtaining differentially protected (*R*)-aspartate for incorporation into synthetic targets led us to examine the saponification of (*R*)-aspartate dimethyl ester (**1a**). Under nonenzymic conditions, base-catalyzed hydrolysis of this compound proceeds rapidly to provide the diacid within 30 min. Acid-catalyzed hydrolysis of the same diester affords some selectivity due to protonation of the α-amino group which disfavors hydrolysis of the α-ester. However, our best result under these conditions yielded only a 1:4 mixture of compounds **2a** and **3a**, although Rapoport has reported better results using a mixture of $CuCO_3$·Cu(OH)$_2$ in EtOH at 70 °C.[7] Consequently, we decided to examine the utility of enzymes as selective catalysts of diester hydrolysis. Here, we report the use of porcine liver esterase to differentiate the aspartate carboxylate functions by regioselective hydrolysis of the α-ester.

## Results and Discussion

A variety of enzymes catalyze selective hydrolysis of the α-ester of aspartate dimethyl ester as shown in Table 1. Best results were obtained using porcine liver esterase (PLE; E.C. 3.1.1.1) which provides 98:2 selectivity for hydrolysis of the α-ester to give monoester **2a** within 30 min (entry 6 in Table 1). The regioselectivity of this hydrolysis reaction is partially reversed when the aspartate amino group is protected as its formamide,[8] giving a 55:45 ratio of α-ester hydrolysis to β-ester hydrolysis. We anticipated that an increased bias for β-ester hydrolysis might be achieved by increasing the size of the amino substituent. However, *N*-acetyl-(*R*)-aspartate dimethyl ester is not a substrate for PLE.[8] Interestingly, hydrolysis of (*S*)-aspartate dimethyl ester by PLE also results in selective hydrolysis of the α-ester to give the enantiomer of monoester **2a** (**2'a**) within 1.5 h. The 3-fold rate difference for hydrolysis of these two enantiomers is probably insufficient for achieving a clean kinetic resolution of racemic aspartate diesters.

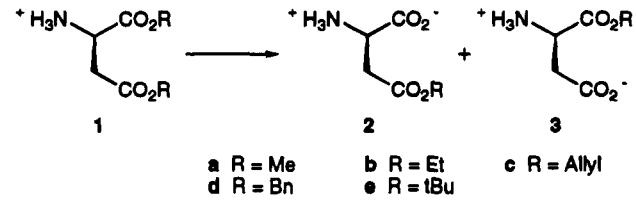


**Figure 1.**

**Table 1. Hydrolysis of (*R*)-Aspartate Dimethyl Ester (1a) to Monoesters 2a and 3a[a]**

| entry | enzyme | product ratio **2a**:**3a** | time (h) |
|---|---|---|---|
| 1 | subtilisin Carlsberg | 81:19 | 12 |
| 2 | papain | 84:16 | 24 |
| 3 | proteinase K | 95:5 | 5 |
| 4 | elastase | 92:8 | 24 |
| 5 | thermolysin | 90:10 | 24 |
| 6 | PLE | 98:2 | 0.5 |
| 7 | carboxypeptidase Y | 87:13 | >24 |
| 8 | butyrylcholinesterase | 85:15 | >24 |
| 9 | chymotrypsin | 82:18 | >24 |

[a] Saponifications were performed following the general procedure described in the Experimental Section.

The regioselective hydrolysis of aspartate dimethyl esters by PLE can be performed on a preparative scale to obtain gram quantities of enantiomerically pure aspartate β-methyl ester. Both enantiomers are available since both (*R*)- and (*S*)- dimethyl aspartate are substrates for the enzyme. Moreover, the product aspartate β-methyl ester is readily converted to synthetically useful, differentially protected aspartates as shown in Scheme 1.

Aspartate β-methyl ester has been prepared previously by several groups, either by resolution,[9] by selective hydrolysis of the dimethyl ester,[7] or by selective esterification.[7,10] The use of PLE compares favorably with these methods in its expediency and simplicity. Moreover, the hydrolysis of (*R*)-aspartate dimethyl ester by PLE is complementary to previously published methods for differentiating aspartate esters using enzymes, since none of the published methods provide access to (*R*)-aspartate β-methyl ester. Esters of the (*S*)-*N*-Boc-aspartate α-carboxylic acid have been prepared using papain,

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(1) (a) Whitesides, G. M.; Wong, C. H. *Angew. Chem., Int. Ed. Engl.* **1985**, *57*, 617–638. (b) Jones, J. B. *Tetrahedron* **1986**, *42*, 3351–3403. (c) Turner, N. J. *Nat. Prod. Rep.* **1994**, *11*, 1–15 and references therein.

(2) Boland, W.; Frössl, C.; Lorenz, M. *Synthesis* **1991**, *12*, 1049–1072.

(3) Adachi, K.; Kobayashi, S.; Ohno, M. *Chimia* **1986**, *40*, 311–314.

(4) Ohno, M.; Kobayashi, S.; Iimori, T.; Wang, Y.-F.; Izawa, T. *J. Am. Chem. Soc.* **1981**, *103*, 2405–2406.

(5) Mori, K.; Iwasawa, H. *Tetrahedron* **1980**, *36*, 2209–2213.

(6) (a) Miyazawa, T.; Takitani, T.; Ueji, S.; Yamada, T.; Kuwata, S. *J. Chem. Soc., Chem. Commun.* **1988**, 1214–1215. (b) Cohen, S. G.; Crossley, J.; Khedouri, E. *Biochemistry* **1963**, *2*, 820–823. (c) Cohen, S. G.; Crossley, J. *J. Am. Chem. Soc.* **1964**, *20*, 4999–5003. (d) Chen, S.-T.; Wang, K.-T.; Wong, C. H. *J. Chem. Soc., Chem. Commun.* **1986**, 1514–1516. (e) Chen, S.-T.; Wang, K.-T. *Synthesis* **1987**, 581–582. (f) Pugniere, M.; Castro, B.; Domergue, N.; Previero, A. *Tetrahedron: Asymmetry* **1992**, *3*, 1015–1018.

(7) Gmeiner, P.; Feldman, P. L.; Chu-Moyer, M. Y.; Rapoport, H. *J. Org. Chem.* **1990**, *55*, 3068–3074.

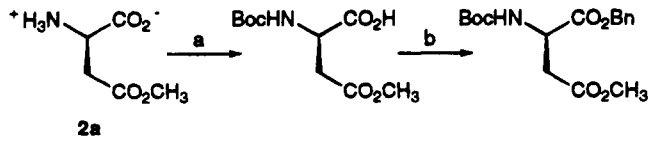
(8) (*R*)-*N*-Formyl aspartate dimethyl ester was prepared in 83% yield from (*R*)-aspartate dimethyl ester by treatment with 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide, formic acid, and *N*-methylmorpholine in $CH_2Cl_2$. (*R*)-*N*-Acetylaspartate dimethyl ester was prepared in 59% yield from (*R*)-aspartate dimethyl ester by treatment with acetic anhydride and triethylamine in $CH_2Cl_2$ and purified by column chromatography. Both products were characterized by $^1$H NMR, $^{13}$C NMR, and MS.

(9) Yoshioka, R.; Ohtsuki, O.; Senuma, M.; Tosa, T. *Chem. Pharm. Bull.* **1989**, *37*, 883–886.

(10) Albert, R.; Danklmaier, J.; Hönig, H.; Kandolf, H. *Synthesis* **1987**, 635–637.

0022-3263/95/1960-8110$09.00/0 

**Scheme 1**[a]




**2a**

[a] Reaction conditions: (a) $(tBuO)_2CO$, $Et_3N$; (b) BnBr, $Cs_2CO_3$.

**Table 2. Results of Preparative Experiments Using PLE**

| substrate | cosolvent[a] | reaction temp | reaction time (h) | yield (%) |
|---|---|---|---|---|
| **1a** | none | rt | 5 | 92 |
| **1'a** | none | rt | 5 | 91 |
| **1b** | none | rt | 5 | 96 |
| **1'c** | none | rt | 18 | 87 |
| **1'd** | 23% MeOH | 35 °C | 18 | 61 |
| **1e** | 5% MeOH | rt | 18 | 86 |
| **1'e** | 5% MeOH | rt | 18 | 82 |

[a] Reactions were performed in 0.1 M potassium phosphate buffer (pH 7.5) containing the indicated proportions of organic cosolvent to assist dissolution of the substrate.

but this enzyme does not accept (*R*)-*N*-Boc-aspartate as a substrate.[11] Hydrolysis of (*R,S*)-*N*-(benzyloxycarbonyl)-aspartate dimethyl ester by porcine pancreatic lipase yields (*S*)-*N*-(benzyloxycarbonyl)aspartate α-methyl ester and (*R*)-*N*-(benzyloxycarbonyl)aspartate dimethyl ester with excellent enantioselectivity, but unprotected aspartate dimethyl ester is hydrolyzed unselectively.[12] Similarly, α-chymotrypsin catalyzes the hydrolysis of (*R,S*)-*N*-acetylaspartate diethyl ester to give (*S*)-*N*-acetylaspartate β-ethyl ester and (*R*)-*N*-acetylaspartate diethyl ester as products.[6]

While the differential hydrolysis of aspartate dimethyl ester fulfilled our original aim, we were stimulated to investigate the possibility of obtaining other aspartate monoesters by the same method. In particular, we wished to extend our work to the preparation of differentially protected aspartates that could be used in solid-phase peptide synthesis. To test the generality of our method, we examined the ability of PLE to catalyze the hydrolysis of four other aspartate diesters (Table 2). Both (*R*)-aspartate diethyl ester (**1b**) and (*S*)-aspartate diallyl ester (**1'c**) are excellent substrates for PLE giving the respective β-monoester with complete regioselectivity. (*S*)-Aspartate dibenzyl ester (**1'd**) is hydrolyzed slowly (possibly due to the poor solubility of this compound in water); however, it also provides only the β-ester product. Remarkably, both enantiomers of aspartate di-*tert*-butyl ester (**1e**) are hydrolyzed regioslectively by PLE to provide the corresponding aspartate β-*tert*-butyl ester in >80% yield. However, comparison of the isolated enantiomeric products with each other, and with literature data, indicates that some racemization may occur in this case.

In summary, we have described an application of the enzyme PLE to the regioselective hydrolysis of aspartate diesters to provide differentially protected amino acids. This method provides rapid and convenient access to both L- and D- aspartate β-monoesters including compounds that are useful for solution and solid-phase peptide synthesis.

(11) Cantacuzène, D.; Pascal, F.; Guerreiro, C. *Tetrahedron* **1987**, *43*, 1823–1826.
(12) Guibé-Jampel, E.; Rousseau, G.; Salaün, J. *J. Chem. Soc., Chem. Commun.* **1987**, 1080–1081.

## Experimental Section

**General.** All enzymes and aspartate derivatives were purchased from Sigma (St. Louis, MO), except butyrylcholinesterase which was obtained from Worthington (Freehold, NJ). D-Aspartic acid was obtained from the Aldrich Chemical Co. (Milwaukee, WI). $^1H$ and $^{13}C$ NMR spectra were recorded using AM-300 and AM-360 spectrometers. Mass spectra were obtained using a VG 7070E mass spectrometer. Melting points are uncorrected.

**General Procedure for Enzyme-Catalyzed Hydrolysis of (*R*)-Aspartate Dimethyl Ester.** (*R*)-Aspartate dimethyl ester (16 mg, 8 μmol) was dissolved in 0.1 M potassium phosphate buffer (pH 7.5, 1.2 mL), 30 units of enzyme were added, and the mixture was incubated at rt (Table 1, entries 2, 6, 7, 9) or 37 °C until no starting material could be detected by TLC (4:1 2-propanol:$H_2O$). The solution was lyophilized to dryness, and the residue was redissolved in $D_2O$. Product ratios were determined by 360 MHz $^1H$ NMR spectroscopy.

**(*R*)-Aspartate β-Methyl Ester (2a).** PLE (285 units) was added to a solution of (*R*)-aspartate dimethyl ester (1.53 g, 7.78 mmol) in 0.1 M potassium phosphate (pH 7.5, 120 mL) and stirred at rt for 5 h. Following evaporation of the solvent *in vacuo*, the residue was resuspended in methanol and filtered. The product monoester was crystallized from methanol–$CH_2Cl_2$ to give a white solid (1.05 g, 7.14 mmol, 92%): mp 192–193 °C (lit.[9] mp 188–190 °C); $[\alpha]^{25}_D = -9.1°$ ($c = 0.027$, $H_2O$) [lit.[9] $[\alpha]^{25}_D = -21.7°$ ($c = 1.0$, MeOH)]; $^1H$ NMR (300 MHz, $D_2O$) δ 3.0 (2H, d, $J = 5.6$ Hz), 3.7 (3H, s), 4.0 (1H, t, $J = 5.6$ Hz); $^{13}C$ NMR (75 MHz, $D_2O$) δ [relative to 3-(trimethylsilyl)-1-propanesulfonic acid] 37.2, 53.5, 55.4, 175.3, 175.7; HRMS (FAB) *m/z* calcd for $C_5H_9NO_4$ 148.0610, found 148.0613.

**(*S*)-Aspartate β-methyl ester (2'a):** 91% yield; mp 192–193 °C (lit.[10] mp 193–194 °C); $[\alpha]^{25}_D = +8.8°$ ($c = 0.024$, $H_2O$) [lit.[10] $[\alpha]^{25}_D = -2.5°$ ($c = 1$, EtOH:$H_2O$, 1:3), $[\alpha]^{25}_D = +20.6°$ ($c = 2$, 1 N HCl)].

**(*R*)-Aspartate β-Ethyl Ester (2b).** PLE (55 units) was added to a solution of (*R*)-aspartate diethyl ester hydrochloride (0.27 g, 1.21 mmol) in 0.1 M potassium phosphate (pH 7.5, 26 mL) and stirred at rt for 5 h. Following evaporation of the solvent *in vacuo*, the residue was resuspended in methanol and filtered. Evaporation of the methanol provided the product as a white solid (0.19 g, 1.17 mmol, 96%): mp 192–194 °C (lit.[10] mp 206–208 °C); $[\alpha]^{25}_D = -3.6°$ ($c = 0.03$, $H_2O$) [lit.[10] for *S*-enantiomer $[\alpha]^{25}_D = +3.8°$ ($c = 1.0$, $H_2O$)]; $^1H$ NMR (300 MHz, $D_2O$) δ 1.2 (3H, t, $J = 7.1$ Hz), 2.9–3.0 (2H, m), 3.9 (1H, dd, $J = 6.1$, 5.5 Hz), 4.2 (2H, q, $J = 7.1$ Hz); $^{13}C$ NMR (75 MHz, $D_2O$) δ [relative to 3-(trimethylsilyl)-1-propane sulfonic acid] 17.6, 38.1, 53.7, 64.9, 174.9, 176.6; HRMS (FAB) *m/z* calcd for $C_6H_{11}NO_4$ 162.0766 [M + H], found 162.0773.

**(*S*)-Aspartate β-Allyl Ester *p*-Toluenesulfonate Salt (2'c·pTSA salt).** PLE (80 units) was added to a solution of (*S*)-aspartate diallyl ester *p*-toluenesulfonate salt (0.508 g, 1.32 mmol) in 0.1 M potassium phosphate (pH 7.5, 19 mL) and incubated at rt overnight. Following evaporation of the solvent *in vacuo*, the residue was resuspended in methanol and filtered. Evaporation of the methanol and recrystallization from $CH_3OH$–$Et_2O$ provided the title compound as a white solid (0.398 g, 1.15 mmol, 87%): mp 195–196 °C dec; $[\alpha]^{25}_D = -4.7°$ ($c = 0.048$, $H_2O$); $^1H$ NMR (360 MHz, $D_2O$) δ 2.4 (3H, s), 3.0 (2H, d, $J = 5.6$ Hz), 4.1 (1H, dd, $J = 5.7$, 5.6 Hz), 4.6 (2H, d, $J = 5.7$ Hz), 5.2–5.4 (2H, m), 5.8–6.0 (1H, m), 7.3 (2H, d, $J = 8.1$ Hz), 7.7 (2H, d, $J = 8.1$ Hz); $^{13}C$ NMR (75 MHz, $D_2O$) δ [relative to 3-(trimethylsilyl)-1-propanesulfonic acid] 23.2, 37.3, 53.4, 69.1, 121.5, 128.0, 132.1, 134.2, 142.1, 145.1, 174.4, 175.5; HRMS (FAB) *m/z* calcd for $C_7H_{11}NO_4$ 174.0766 [M + H], found 174.0771.

**(*S*)-Aspartate β-Benzyl Ester *p*-Toluenesulfonate Salt (2'd·pTSA salt).** PLE (150 units) was added to a solution of (*S*)-aspartate dibenzyl ester *p*-toluenesulfonate salt (0.327 g, 0.67 mmol) in 0.1 M potassium phosphate (pH 7.5, 8.5 mL) containing methanol (2.5 mL) and warmed to 35 °C to dissolve the aspartate. The reaction mixture was incubated at 35 °C overnight. After the aqueous mixture was washed twice with diethyl ether, the solvent was evaporated *in vacuo* and the resulting residue was resuspended in methanol and filtered. Evaporation of the methanol and recrystallization of the residue from methanol–diethyl ether provided the title compound as a white solid (0.155 g, 0.39 mmol, 61%): mp 205–207 °C dec; $[\alpha]^{25}_D$

= + 10.4° (c = 0.009, 1 N HCl) [lit.[10] for free amine $[\alpha]^{25}_D$ = + 27.9° (c = 2, 1 N HCl)]; $^1$H NMR (360 MHz, $D_2O$) δ 2.4 (3H, s), 3.0–3.1 (2H, t, J = 5.4 Hz), 4.0–4.1 (1H, m), 5.2 (2H, s), 7.3 (2H, d, J = 8.1 Hz), 7.3–7.5 (5H, m), 7.7 (2H, d, J = 8.1 Hz); $^{13}$C NMR (75 MHz, $D_2O$) δ [relative to 3-(trimethylsilyl)-1-propanesulfonic acid] 23.1, 37.3, 53.4, 70.2, 128.0, 131.0, 131.4, 131.5, 132.1, 137.8, 142.0, 145.1, 174.5, 175.4; HRMS (FAB) m/z calcd for $C_{11}H_{13}NO_4$ 224.0923 [M + H], found 224.0928.

**(S)-Aspartate β-*tert*-Butyl Ester (2'e).** PLE (60 units) was added to a solution of (S)-aspartate di-*tert*-butyl ester hydrochloride (0.18 g, 0.64 mmol) in 0.1 M potassium phosphate (pH 7.5, 10 mL) containing methanol (0.5 mL), and this mixture was stirred at rt overnight. Following evaporation of the solvent *in vacuo*, the residue was resuspended in methanol and filtered. The product was recrystallized from $CH_3OH$–$Et_2O$ to provide a white solid (0.104 g, 0.55 mmol, 86%): mp 177–178 °C (lit.[13] mp 198–199 °C dec); $[\alpha]^{25}_D$ = +4.8° (c = 0.024, $H_2O$) [lit.[13] $[\alpha]^{25}_D$ = +8.5° (c = 1.3, $H_2O$)]; $^1$H NMR (300 MHz, $D_2O$) δ 1.5 (9H, s), 3.0 (2H, d, J = 5.5 Hz), 4.0 (1H, dd, J = 5.7, 5.7 Hz); $^{13}$C NMR (75 MHz, $D_2O$) δ [relative to 3-(trimethylsilyl)-1-propanesulfonic acid] 29.9, 38.3, 53.5, 86.6, 173.8, 175.6; HRMS (FAB) m/z calcd for $C_8H_{15}NO_4$ 190.1079 [M + H], found 190.1083.

**(R)-Aspartate β-*tert*-butyl ester (2e):** 82% yield; mp 179–180 °C; $[\alpha]^{25}_D$ = −6.0° (c = 0.047, $H_2O$).

**(R)-*N*-Boc-aspartate β-Methyl Ester.** (R)-Aspartate β-methyl ester (53 mg, 0.36 mmol) was dissolved in MeOH (1.8 mL), and triethylamine (0.2 mL) and di-*tert*-butyl dicarbonate (153 mg, 0.72 mmol) were added successively. The mixture was stirred under nitrogen at rt for 5 h. Following removal of the solvent *in vacuo*, cold 1 M $HCl_{aq}$ was added and the product extracted with ethyl acetate. The combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and evaporated. Column chromatography of the residue on silica gel (10% MeOH in $CH_2Cl_2$) gave the title compound as a white solid (65 mg, 75%): $^1$H NMR (300 MHz, $CDCl_3$) δ 1.5 (9H, s), 2.8 (1H, dd, J = 17.28, 4.8 Hz), 3.1 (1H, dd, J = 17.27, 4.28 Hz), 3.7 (3H, s), 4.5–4.6 (1H, m), 5.6 (1H, d, J = 8.41 Hz); $^{13}$C NMR (75 MHz, $CDCl_3$) δ 29.2, 36.4, 49.8, 52.0, 80.4, 155.6, 171.4, 175.3; HRMS (CI, $NH_3$) m/z calcd for $C_{10}H_{17}NO_6$ 248.1134, found 248.1137.

**(R)-*N*-Boc-aspartate α-Benzyl β-Methyl Ester.** (R)-*N*-Boc-aspartate β-methyl ester (76 mg, 0.3 mmol) was dissolved in dry DMF (4 mL), and cesium carbonate (203 mg, 0.62 mmol) added followed by benzyl bromide (0.11 mL, 0.92 mmol). This mixture was stirred overnight under nitrogen at rt and then partitioned between ethyl acetate and water. The aqueous layer was extracted three times with ethyl acetate, and the combined extracts were washed with brine, dried over anhydrous magnesium sulfate, filtered, and evaporated. The product was purified by chromatography on silica gel (20% ethyl acetate in hexanes) to give a white solid (53 mg, 80%): $^1$H NMR (300 MHz, $CDCl_3$) δ 1.4 (9H, s), 2.8 (1H, dd, J = 17.27, 4.8 Hz), 3.0 (1H, dd, J = 17.27, 4.28 Hz), 3.6 (3H, s), 4.5–4.6 (1H, m), 5.1 (2H, d, J = 7 Hz), 7.2–7.4 (5H, m); $^{13}$C NMR (75 MHz, $CDCl_3$) δ 28.2, 36.6, 50.0, 51.9, 67.4, 80.1, 128.2, 128.4, 128.5, 135.2, 155.4, 170.9, 171.3; IR ($CDCl_3$) 2281, 1750, 1722, 1504, 1370, 1166 $cm^{-1}$; HRMS (CI, $NH_3$) m/z calcd for $C_{17}H_{23}NO_6$ 338.1604, found 338.1601.

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(13) Roeske, R. *J. Org. Chem.* **1963**, *28*, 1251–1253.