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Chemo-enzymatic synthesis of chiral 2-substituted succinic acid derivatives

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Abstract

Prochiral discrimination by the biocatalyst Alcalase®, an enzyme preparation of subtilisin Carlsberg, was used to effect enantio- and regioselective monohydrolysis of a variety of (*RS*)-2-substituted succinate diesters to afford the corresponding half esters in modest to excellent enantiomeric excesses (>99%). Exploitation of malonate chemistry, as well as recycling of the unhydrolyzed isomer from the enantioselective hydrolysis, has resulted in a process which is both practical and economical. © 1999 Published by Elsevier Science Ltd. All rights reserved.

1. Introduction

The use of hydrolytic enzymes in organic synthesis, for the preparation of enantiomerically pure carboxylic acids, has received increased attention over the last 10 years.[1] This is, in part, due to the ability of these enzymes to catalyze the hydrolysis of both natural and unnatural substrates. Many groups have realized the potential of using enantiomeric hydrolysis of esters as a practical and cost-effective means of producing important synthetic building blocks in enantiomerically pure form.[2–4] Attractive features of this approach include the readily available racemic materials and the adaptability of the enzymatic hydrolysis step to multi-gram scales.

The use of substituted succinic acid derivatives as peptidomimetics has found a number of applications in medicinal chemistry.[5–9] We have successfully utilized these fragments for the introduction of a succinamide unit in the development of BILA 2157 BS, a highly bioavailable and potent renin inhibitor[10] shown in Fig. 1. In particular, we needed an efficient method to prepare the required 2(*R*)-(2-amino-4-thiazolyl)methyl succinyl core of our lead compound. A number of approaches to chiral succinic acid derivatives have been reported in the literature including catalytic asymmetric reduction,[8] the use of chiral auxiliaries,[5,6,9,10] or the chemical resolution by recrystallization.[7,11] While some of these approaches work well, they suffer from disadvantages of costly auxiliaries, lengthy preparations of chiral ligands,

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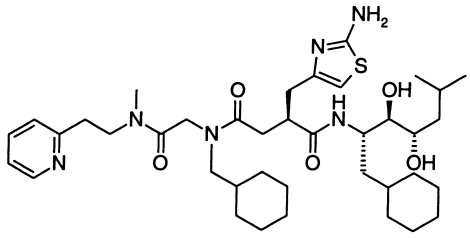


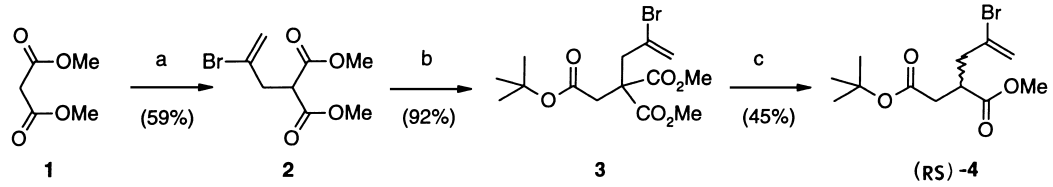
Figure 1. Renin inhibitor BILA 2157 BS

or the lack of generality. In addition, a number of groups have made use of hydrolytic enzymes such as lipases[12] and esterases[13,14] to prepare enantiomerically enriched materials. We have explored this latter approach to find a cost-effective alternative to the use of chiral auxiliaries in the preparation of enantiomerically pure 2-substituted succinic acid derivatives by enantioselective and regioselective hydrolysis of (*RS*)-2-substituted succinate diesters.[15] An initial screen of various enzymes led us to select a serine protease, subtilisin Carlsberg, as the enzyme of choice. Recently, the use of subtilisin Carlsberg has been reported for the enantioselective hydrolysis of diethyl 2-isobutyl succinate.[16] We wish to report our findings that subtilisin Carlsberg efficiently resolves a variety of 2-substituted succinate diesters with varied functionalities in high enantiomeric excesses. In particular, we have focused on the preparation and enantioselective hydrolysis of 2-(*RS*)-(2-bromo-2-propenyl) succinate diester **4**, which is a key and versatile intermediate for the preparation of a number of interesting functional groups. For example, this vinyl bromide side chain is readily transformed in a 'one-pot' process into the desired 2-amino-4-thiazolylmethyl moiety[17] required in our renin inhibitors.

2. Results and discussion

*2.1. Preparation of (*RS*)-2-substituted succinate diesters*

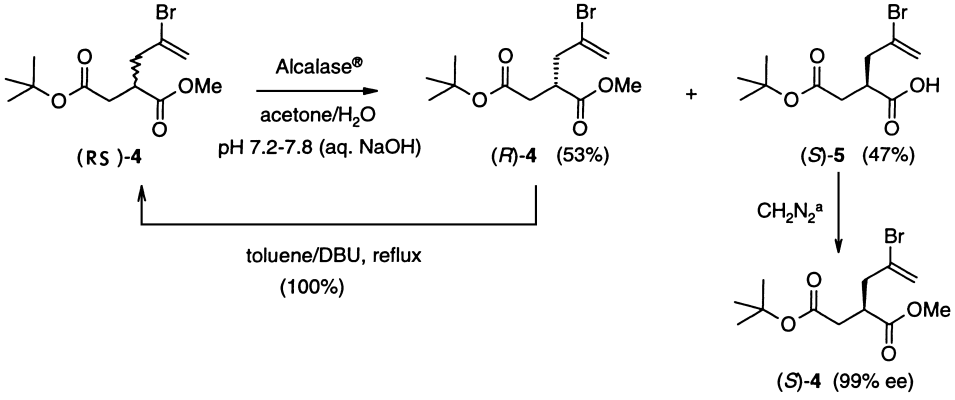
The required racemate (*RS*)-**4** was readily prepared by a protocol involving sequential alkylation of dimethyl malonate and demethoxycarbonylation as shown in Scheme 1. The first alkylation was carried out with 2,3-dibromopropene utilizing an excess of dimethylmalonate **1** (1.5 equiv.) to decrease dialkylation. The monoalkylated malonate **2** was readily isolated by distillation under reduced pressure in 59% yield. The preparation of dialkylated product **3** was easily accomplished by, first, deprotonation of **2** with sodium bis(trimethylsilyl)amide, followed by treatment with *tert*-butyl bromoacetate. For convenience, sodium bis(trimethylsilyl)amide was used as the base to facilitate slow addition; however, a number of bases worked equally well including potassium *tert*-butoxide. Purification by distillation under reduced pressure afforded **3** in 92% yield, although the crude product was sufficiently clean to be used directly in the next step without purification. Demethoxycarbonylation with lithium bromide in aqueous DMF was carried out on triester **3**, involving mono-saponification and decarboxylation in a 'one-pot' protocol. This gave directly racemate **4** in 45% yield after purification, along with a minor contaminant arising from transesterification of the *tert*-butyl ester to the corresponding methyl ester (<6%). The yields for this operation can be improved by a two-step process involving mono-saponification (aqueous potassium hydroxide) and decarboxylation.[18] This straightforward approach to (*RS*)-**4** makes use of relatively inexpensive reagents, no cryogenic conditions, and requires minimal purification. Racemate **4** was prepared in a practical fashion on a multi-gram scale (270 g). Further optimizations of this sequence for adaptation to multi-kilogram quantities have been carried out and will be reported elsewhere.[18]



Scheme 1. (a) NaOMe in MeOH, 2,3-dibromopropene, 0°C to rt (1.5 h); (b) NaHMDS in THF, *t*-butyl bromoacetate, 0°C to rt (15 min); (c) LiBr and H_2O in DMF, 135°C (8 h)

*2.2. Enantio- and regioselective hydrolysis of (*RS*)-2-substituted succinate diesters*

A number of potential hydrolytic enzymes were screened for their suitability as catalysts for the enantioselective hydrolysis of racemic vinyl bromide **4** (Scheme 2), including α-chymotrypsin, papain and pig liver esterase. While no conversion was observed with papain, α-chymotrypsin produced a low conversion (30%) and poor enantioselectivity (70% ee). The enzyme which produced the desired enantioselective hydrolysis was found to be subtilisin Carlsberg, a serine protease. We also determined that a crude and inexpensive preparation of this enzyme, Alcalase® 2.4L,[19] performed equally well at a fraction of the cost.



Scheme 2. [a]The extent of enantioselective hydrolysis was determined by esterification of the half ester with diazomethane and subsequent analysis by HPLC using a Chiralpak® AS column, 0.25% ethanol in hexane

Treatment of a suspension of (*RS*)-**4** in acetone:water (1:7) with a catalytic amount of Alcalase® under controlled NaOH conditions using a pH autotitrator resulted in rapid hydrolysis to give the desired carboxylic acid (*S*)-**5** in 47% yield and the unhydrolyzed ester (*R*)-**4** in 53% yield as illustrated in Scheme 2. The progress of the enzymatic reaction was readily monitored by following the amount of NaOH solution consumed with time. The reaction initially progressed rapidly and then slowed considerably as the concentration of the substrate was depleted. The hydrolysis was complete after consumption of 0.5 equivalents of the NaOH (0.4 M) solution. Stabilization of the pH at this point was usually diagnostic that enantioselective hydrolysis had occurred. Once completed, the components of the reaction were separated using a simple acid–base extraction protocol to afford the diester (*R*)-**4** and the half ester (*S*)-**5**. An aliquot of half ester (*S*)-**5** was derivatized to the methyl ester (*S*)-**4** by treatment with diazomethane and the enantiomeric purity determined to be >99% ee by HPLC analysis on a chiral support (ChiralPak® AS, 4.6×250 mm, eluents: 0.25% ethanol in hexane).

The enzymatic reaction, in addition to being enantioselective, was completely regioselective since the *tert*-butyl ester was unaffected during the hydrolysis. The absolute configuration of the resolved material was confirmed by independent synthesis[20] of the vinyl bromide (*S*)-**4** using Evans' alkylation method.[21]

The authentic material was identical (optical rotation and HPLC analysis on a chiral support) to the resolved succinate (*S*)-**4**, confirming the natural amino acid configuration at the α-center as expected. In this approach, the unhydrolyzed ester antipode was also available in high optical purity if the reaction was allowed to go to theoretical completion. Hence, (*R*)-**4** was determined to be 97% ee by HPLC analysis (chiral support).

The practicality and efficiency of the enzymatic resolution route was further improved by converting the unhydrolyzed enantiomer (*R*)-**4** back to the racemate through base catalyzed racemization (DBU in refluxing toluene, 18 h) and could subsequently be retreated under the enzyme reaction conditions. In order to demonstrate the improvement in overall efficiency of the resolution sequence, recycling of the unhydrolyzed enantiomer (*R*)-**4** in two additional cycles resulted in a combined yield of 82% of (*S*)-**4** in high purity (98% ee). The feasibility of doing this reaction on large scale was also investigated. Utilizing the same protocol as outlined above (molarity of the NaOH solution increased to 1 M), the enantioselective resolution was carried out on a preparative scale (100 g of (*RS*)-**4**) which gave the desired (*S*)-**4** after 48 h in 46% yield and 98% ee after one cycle. The use of an autotitrator for the controlled addition of the sodium hydroxide solution in the hydrolysis step, particularly on large scale, is practical and provided excellent reproducibility between different runs. On small scales, however, the autotitrator was not critical in performing the reaction. For example, Alcalase® treatment of a suspension of the versatile vinyl bromide intermediate (*RS*)-**4** in phosphate buffer solution (pH 7.2) produced similarly high enantiomeric excesses and yields.

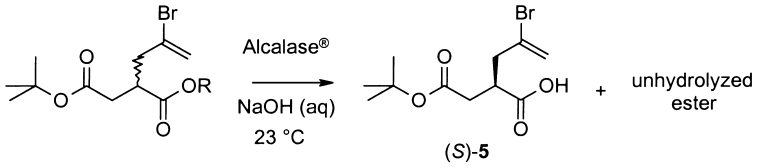
2.3. Effect of ester size and temperature on the enzymatic hydrolysis

The rate of the enzymatic hydrolysis was found to be dependent on the size of the ester group to be hydrolyzed. While all esters studied were hydrolyzed with good enantioselectivities to the half esters, the optimal group under our reaction conditions was the methyl ester analog with relative conversion rates of two to five times faster than for other esters (Me>Et>*n*-Pr>Bn) (Table 1). The effect of temperature was also examined. As shown in Table 2, the selectivity was not affected between 23°C and 37°C. The enantioselectivity of the hydrolysis, however, decreased at 55°C. It is not clear whether this observed decrease is due to increased competition with the uncatalyzed process (direct NaOH hydrolysis) or a loss in specificity of the enzyme.

2.4. Effect of the 2-substitutent on the enzymatic hydrolysis

The generality of the enzymatic hydrolysis was evaluated with a variety of 2-substituted succinate diesters (Table 3). These analogs were prepared in analogous fashion to the vinyl bromide derivative **4**, and were subjected to the identical enzymatic hydrolysis conditions. The hydrolyzed materials were derivatized to the methyl ester (diazomethane treatment) and analyzed by HPLC using a chiral support as before. As can be seen from Table 3, the nature of the C-2 side chain had a significant impact on the degree of selectivity that was obtained. When the group was small (R=Me), a 4.6:1 ratio of enantiomers was obtained. Changing the nature of the group as in entries 2 and 3 resulted in improved ratios of 9:1 and 15:1, respectively. Interestingly, this enzyme also tolerated fairly large heterocyclic groups with different functionality (entries 4 and 5). Hence utilizing this approach, the 2(*R*)-(2-amino-4-thiazolyl)methyl succinic acid moiety needed for BILA 2157 BS can be accessed by either enantioselective hydrolysis of the heterocycles (*RS*)-**12** and (*RS*)-**13**), or the (*RS*)-**4** vinyl bromide precursor. Finally, introduction of a benzyl group (entry 6) was also very well tolerated in the specificity pocket of the enzyme and

Table 1
Effect of various ester groups on the enantioselective hydrolysis reaction



(*S*)-**5**

Entry	Compound	R	t ½[a] min	% yield acid[b]	% ee[c]
1	(*RS*)-**4**	Me	45	48	99
2	(*RS*)-**6**	Et	100	46	95
3	(*RS*)-**7**	n-Pr	250	43	93
4	(*RS*)-**8**	Bn	380	30[d]	97
5	(*RS*)-**8**	Bn	235	37[d,e]	95

[a] Time at which 50% of the theoretical amount of NaOH solution had been consumed.
[b] Maximum yield is 50%.
[c] Determined by HPLC analysis of the derivatized methyl ester using a chiral support.
[d] Reaction not carried out to completion.
[e] Reaction performed at 37°C.

Table 2
Temperature effect on enzymatic hydrolysis of (*R*,*S*)-**4**

T (°C)	% Yield[a]	% ee[b]
23	48	99
37	48	99
55	50	85

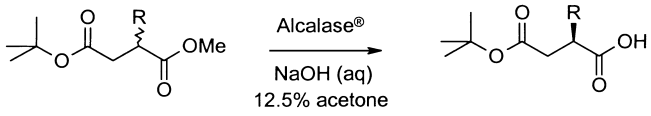
[a] Maximum yield 50%
[b] Determined by HPLC analysis of the derivatized methyl esters using a chiral support.

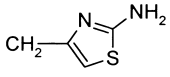
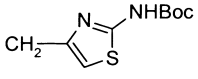
produced essentially optically pure material (129:1). Based on reaction scales of 1 mmol, these reactions were complete between a few hours (entry 1) to 24 h for the most demanding substrate (entry 5).

3. Conclusions

Subtilisin Carlsberg is known to be somewhat of a permissive enzyme which tolerates a variety of different functionalized substrates to bind in the specificity pocket (S1 binding pocket).[22] Taking advantage of this feature of the enzyme, a variety of (*RS*)-2-substituted succinate diesters can be hydrolyzed with modest to excellent enantiospecificity, depending on the nature of the substituent.

Table 3
Enantioselective hydrolysis reaction with various succinate diesters



Entry	Compound	R	t ½ h[a]	% yield acid[b]	Ratio
1	(*RS*)-**9**	CH_3	1	46.5	4.6:1[c]
2	(*RS*)-**10**	CH_2CONMe_2	2	50	9:1
3	(*RS*)-**11**	CH_2CH_2SMe	3	41	15:1
4	(*RS*)-**12**		1.5	43[d]	34:1[e]
5	(*RS*)-**13**		1.5	46	46:1[e]
6	(*RS*)-**14**	CH_2Ph	1.5	40	129:1[c]

[a] Time at which 50% of the theoretical amount of NaOH solution had been consumed. Completed reactions take approximately 18 h.
[b] The maximum theoretical yield is 50%.
[c] Optical rotation consistent with literature values for the (*R*) isomer.
[d] The acid was converted to the methyl ester before isolation using Cs_2CO_3 and MeI in DMF.
[e] Verified by independent synthesis of the (*R*) isomer.

Furthermore, an inexpensive source of this enzyme (Alcalase® 2.4L) was shown to be equally effective. The enzymatic hydrolysis can be carried out on scales ranging from 0.5 g to 100 g without loss of enantioselectivity or regioselectivity as demonstrated in the case of the vinyl bromide derivative. Further improvements on this approach were realized by racemization of the 'unhydrolyzed' enantiomer and recycling. The enzymatic hydrolysis was shown to be an efficient and practical method for the preparation of a variety of different homochiral succinic acid derivatives. In particular, the vinyl bromide analog (*RS*)-**4** was effectively resolved in 82% yield after two additional enzymatic cycles. The rate of the enzymatic reaction was shown to be influenced by the nature of the ester group to be hydrolyzed (Me>Et>*n*-Pr>Bn) with no loss of selectivity between 23°C and 37°C. The simplicity and cost effectiveness of this strategy for the preparation of large amounts of these fragments will be reported shortly.

4. Experimental

All reagents, solvents and starting materials were obtained from commercial sources and used without further purification. Column chromatography was performed on silica gel, Merck grade 60 (230–400 mesh). Optical rotations were measured on a Perkin–Elmer 241 polarimeter. NMR spectra were obtained at 400 MHz for ^1H and 100 MHz for ^{13}C, on a Bruker AMX 400 spectrometer. All chemical shifts are reported in ppm in deuterochloroform with TMS as an internal standard. IR spectra were recorded on

a Perkin–Elmer 781 spectrometer. FAB mass spectra were recorded on an Autospec, VG spectrometer and CI mass spectra were obtained on a Kratos MS50 spectrometer. The pig liver esterase (EC 3.1.1.1), α-chymotrypsin (EC 3.4.21.1) and papain (EC 3.4.22.2) were purchased from Sigma. Alcalase® 2.4L (food grade) was obtained from Novo Nordisk Biochem., Inc. Enzymatic reactions were conducted using a pH autotitrator. The enantiomeric purities were determined using an analytical HPLC chiral column, Chiralpak® AS column (4.6×250 mm); mobile phase, 0.25% ethanol in hexane, 0.5 mL/min, isocratic (condition A).

*4.1. 2-(2-Bromo-2-propenyl)-1,3-propanedioic acid dimethyl ester **2***

To a 5 L three-necked flask equipped with a mechanical stirrer was added anhydrous methanol (1.8 L) followed by freshly cut metallic sodium (31 g, 1.35 g-atom) portionwise over 1.5 h. To the clear solution was added dimethyl malonate (267 g, 2.0 mol, 1.5 equiv.) in anhydrous methanol (250 mL) over 1 h. The solution was cooled to 0°C before 2,3-dibromopropene (300 g, ~90% technical grade, 1.3 mol) was added in anhydrous methanol (200 mL) over 3 h. The resulting yellow solution was stirred at room temperature for 14 h until neutral to litmus paper. The methanol was removed under reduced pressure and the residue was taken up in ether (800 mL) and washed with distilled water (800 mL). The aqueous phase was re-extracted with ether (300 mL) and the combined organic phases washed with saturated brine, and dried over anhydrous $MgSO_4$. The solution was filtered and concentrated to give a yellow oil which was purified by vacuum distillation. After the initial forerun, the mono-alkylated product **2** distilled at 101–106°C (1 mm Hg) as a clear colorless oil (201 g, 59%). R_f=0.54 (4:1 hexane:EtOAc); ^{1}H NMR δ 5.69 (m, 1H, olefinic), 5.48 (d, *J*=2.0 Hz, 1H, olefinic), 3.81 (t, *J*=7.5 Hz, 1H), 3.75 (s, 6H), 3.02 (dd, *J*=1.0, 7.5, 2H); ^{13}C NMR δ 168.3, 129.2, 119.7, 52.6, 50.3, 40.4; IR (neat, cm^{-1}) ν 3000 (w), 2955 (m), 2840 (w), 1740 (s), 1625 (m); MS (FAB) *m/z*: 253 $(MH+2)^+$, 251 $(MH)^+$. Anal. calcd for $C_8H_{11}BrO_4$: C, 38.27; H, 4.42; found: C, 38.21; H, 4.44.

*4.2. 2-(2-Bromo-2-propenyl)-2-(methoxycarbonyl)-1,4-butanedioic acid 4-(1,1-dimethyl-ethyl) 1-methyl ester **3***

The mono-alkylated product **2** (201 g, 0.8 mol) was dissolved in anhydrous THF (800 mL) and cooled to 0°C. Sodium bis(trimethylsilyl)amide (881 mL, 1 M solution in THF) was cannulated into the reaction mixture over 45 min. After a further 30 min, *tert*-butyl bromoacetate (172 g, 130 mL, 0.9 mol) was added in anhydrous THF (200 mL) over 1 h. Stirring was continued for 3.5 h (room temperature) before the reaction mixture was diluted with hexane (500 mL) and filtered through Celite. Concentration in vacuo gave a brownish oil which was purified by vacuum distillation at 140–145°C (1 mm Hg) to give 271 g (92%) of a colorless oil. R_f=0.60 (4:1 hexane:EtOAc); ^{1}H NMR δ 5.64 (bs, 1H), 5.59 (bs, 1H), 3.75 (s, 6H), 3.35 (s, 2H), 3.07 (s, 2H), 1.43 (s, 9H); ^{13}C NMR δ 169.4, 168.4, 127.2, 122.3, 81.3, 54.6, 52.9, 43.5, 37.2, 27.9; IR (neat, cm^{-1}) ν 2980 (s), 2950 (s), 1760 (vs), 1640 (m); MS (FAB) *m/z*: 367 $(M+Na)^+$, 365 $(MH)^+$. Anal. calcd for $C_{14}H_{21}BrO_6$: C, 46.04; H, 5.80; found: C, 45.65; H, 5.77.

*4.3. 2-(*RS*)-(2-Bromo-2-propenyl)-1,4-butanedioic acid 4-(1,1-dimethylethyl) 1-methyl ester **4***

The dialkylated malonate derivative **3** (52.6 g, 0.14 mol) was dissolved in DMF (20 mL) and added to a solution of lithium bromide (12.51 g, 0.14 mol, 1 equiv.) in H_2O (5.2 mL, 0.29 mol, 2 equiv.) and DMF (20 mL). The clear solution was heated to 135°C for 8 h before being concentrated under high vacuum. The residue was taken up in EtOAc (120 mL) and washed with water (1×60 mL). The extraction was

repeated twice more with EtOAc (2×60 mL). The combined organic phase was washed with saturated brine (100 mL) and dried ($MgSO_4$), filtered and concentrated to give 27.6 g (63%) of a pale yellow oil. A sample of this material (9.55 g) was further purified by flash chromatography eluting with hexane:ethyl acetate (20:1) to provide 6.8 g of pure 2-(*RS*)-**4** (45%); ^{1}H NMR δ 5.64 (s, 1H), 5.48 (d, *J*=1.5 Hz, 1H), 3.71 (s, 3H), 3.14 (m, 1H), 2.85 (dd, *J*=14.5, 6.5 Hz, 1H), 2.61 (dd, *J*=14.5, 8 Hz, 1H), 2.57 (dd, *J*=16.5, 8 Hz, 1H), 2.48 (dd, *J*=16.5, 6.5 Hz, 1H), 1.44 (s, 9H); ^{13}C NMR δ 173.90, 170.38, 130.46, 119.55, 80.91, 51.87, 42.75, 39.75, 35.82, 27.96; IR (neat, cm^{-1}) ν 2980 (s), 1730 (s), 1630 (m); MS (EI) *m/z*: 309 $(MH+2)^+$, 307 $(MH)^+$; HRMS (FAB) calcd for $C_{12}H_{20}BrO_4$ $(MH)^+$, 307.0545. Found, 307.0538. Anal. calcd for $C_{12}H_{19}BrO_4$: C, 47.05; H, 6.26; found: C, 46.84; H, 6.36.

*4.4. 2-(*RS*)-Methyl-1,4-butanedioic acid 4-(1,1-dimethylethyl) 1-methyl ester (*RS*)-**9***

Using the same protocol as above and methyl iodide as the alkylating reagent, (*RS*)-**9** was obtained in 33% overall yield. ^{1}H NMR δ 3.69 (s, 3H), 2.92–2.4 (m, 1H), 2.64 (dd, *J*=16.2, 8.26 Hz, 1H), 2.33 (dd, *J*=16, 6.0 Hz, 1H), 1.44 (s, 9H), 1.20 (d, *J*=7.3 Hz, 3H); MS (EI) *m/z*: 203 $(MH)^+$. Optical rotation for acid of (*R*)-**9**: $[\alpha]_D^{25}$ +1.6 (c=2.5, MeOH), lit. value[23] $[\alpha]_D^{25}$ +3.0 (c=0.64, MeOH).

*4.5. 2-(*RS*)-[2-(Dimethylamino)-2-oxoethyl]-1,4-butanedioic acid 4-(1,1-dimethylethyl) 1-methyl ester (*RS*)-**10***

Using the same protocol as above and 2-chloro-*N*,*N*-dimethylacetamide as the alkylating reagent, (*RS*)-**10** was obtained in 28% yield overall. ^{1}H NMR δ 3.70 (s, 3H), 3.32–3.25 (m, 1H), 3.01 (s, 3H), 2.94 (s, 3H), 2.85–2.76 (m, 1H), 2.67–2.50 (m, 3H), 1.44 (s, 9H); MS (FAB) *m/z*: 274 $(MH)^+$. Optical rotation for the acid of (*R*)-**10**: $[\alpha]_D^{25}$ +6.14 (c=3.76, MeOH), $[\alpha]_{365}^{25}$ +23.14 (c=3.76, MeOH).

*4.6. 2-(*RS*)-[2-(Methylthio)ethyl]-1,4-butanedioic acid 4-(1,1-dimethylethyl) 1-methyl ester (*RS*)-**11***

Using the same protocol as above and 2-chloroethyl methyl sulfide as the alkylating reagent, (*RS*)-**11** was obtained in 30% yield overall. ^{1}H NMR δ 3.70 (s, 3H), 2.98–2.88 (m, 1H), 2.78–2.60 (m, 1H), 2.55–2.47 (m, 2H), 2.45–2.37 (m, 1H), 2.10 (s, 3H), 2.02–1.92 (m, 1H), 1.84–1.73 (m, 1H), 1.44 (s, 9H); MS (FAB) *m/z*: es$^-$: 247 $(M-H)^-$. Optical rotation for the acid of (*R*)-**11**: $[\alpha]_D^{25}$ +13.11 (c=3.18, MeOH), $[\alpha]_{365}^{25}$ +46.2 (c=3.18, MeOH).

*4.7. 2-(*RS*)-[(2-Amino-4-thiazolyl)methyl]-1,4-butanedioic acid 4-(1,1-dimethylethyl) 1-methyl ester (*RS*)-**1***

The vinyl bromide (*RS*)-**4** (5.00 g, 16.3 mmol) was dissolved in acetonitrile (20 mL) and H_2O (7 mL) before being treated with *N*-bromosuccinimide (NBS) (4.06 g, 23.0 mmol) in one portion. The reaction mixture was stirred 35 min before the excess NBS was quenched with 2-methoxypropene (0.63 mL, 6.5 mmol) with the disappearance of color. Thiourea (1.50 g, 19.6 mmol) was added all at once and the reaction stirred 1 h before the acetonitrile was removed in vacuo. The residue was partitioned between saturated aqueous $NaHCO_3$ and EtOAc. The organic phase was washed with saturated brine and then dried ($MgSO_4$), filtered and concentrated in vacuo. The product was purified by flash chromatography (5% MeOH/$CHCl_3$) to give 2.7 g (55%). ^{1}H NMR δ 6.17 (s, 1H), 4.89 (bs, 2H), 3.68 (s, 3H), 3.2 (m, 1H), 2.95 (m, 1H), 2.55–2.8 (m, 2H), 2.45 (m, 1H), 1.43 (s, 9H); MS (FAB) *m/z*: 301 $(MH)^+$; HRMS

(FAB) calcd for $C_{13}H_{21}N_2O_4S$ $(MH)^+$, 301.1222. Found, 301.1232. Optical rotation for the methyl ester (*R*)-**12** (derivatized by diazomethane addition to the resolved acid): $[\alpha]_D^{25}$ +2.75 (c=0.8, MeOH).

*4.8. 2-(*RS*)-[2-[[(1,1-Dimethylethoxy)carbonyl]amino]-4-thiazolylmethyl]-1,4-butanedioic acid 4-(1,1-dimethylethyl) 1-methyl ester (*RS*)-**13***

To (*RS*)-**12** (0.76 g, 2.5 mmol) in anhydrous THF (20 mL) was added NaH (70 mg, 2.8 mmol). This mixture was stirred for 30 min before Boc anhydride (0.72 g, 3.3 mmol) was added. The reaction was stirred 48 h before careful addition of wet THF. The mixture was partitioned between EtOAc (100 mL) and H_2O (50 mL). The organic phase was washed with saturated brine, dried ($MgSO_4$), filtered and concentrated in vacuo. Purification by chromatography gave the mono-Boc derivative (*R,S*)-**13** (0.215 g, 21%). ^{1}H NMR δ 8.85 (bs, 1H), 6.55 (s, 1H), 3.66 (s, 3H), 3.17 (m, 1H), 3.05 (m, 1H), 2.84 (m, 1H), 2.57 (m, 1H), 2.41 (m, 1H), 1.6 (s, 9H), 1.43 (s, 9H); MS (FAB) *m/z*: 401 $(MH)^+$; HRMS (FAB) calcd for $C_{18}H_{29}N_2O_6S$ $(MH)^+$, 401.1746. Found, 401.1757. Specific rotation for the acid of (*R*)-**13**: $[\alpha]_D^{25}$ +5.16 (c=0.31, MeOH).

*4.9. 2-(*RS*)-Benzyl-1,4-butanedioic acid 4-(1,1-dimethylethyl) 1-methyl ester (*RS*)-**14***

Using the above procedure and benzyl bromide as the alkylating reagent, (*RS*)-**14** was obtained in 46% yield overall. ^{1}H NMR δ 7.32–7.25 (m, 2H), 7.24–7.19 (m, 1H), 7.18–7.14 (m, 2H), 3.66 (s, 3H), 3.11–2.99 (m, 2H), 2.74 (dd, *J*=13, 8 Hz, 1H), 2.58 (dd, *J*=16.5, 9 Hz, 1H), 2.33 (dd, *J*=16.5, 5 Hz, 1H), 1.41 (s, 9H); MS (FAB) *m/z*: 279 $(MH)^+$. Specific rotation for the acid of (*R*)-**14**: $[\alpha]_D^{25}$ +8.3 (c=5.2, $CHCl_3$), lit. value[24] $[\alpha]_D^{25}$ +6.4 (c=0.11, $CHCl_3$).

*4.10. Typical procedure for the kinetic resolution of succinate derivatives using subtilisin Carlsberg. Kinetic resolution of 2-(*RS*)-(2-bromo-2-propenyl)-1,4-butanedioic acid 4-(1,1-dimethylethyl) 1-methyl ester **4***

Racemate **4** (5.01 g, 16.3 mmol) was dissolved in acetone (5 mL) and diluted with deionized H_2O (30 mL). The system was equipped with an automatic pH titrator and a peristaltic pump connected to a 0.4 M aqueous NaOH solution. The pH of the rapidly stirred suspension was adjusted to 7.5 before a crude preparation of subtilisin Carlsberg (0.2 g) (Alcalase® 2.4L, 'food grade' enzyme preparation) was added. The automatic titrator setting was adjusted to between pH 7.2 and pH 7.6 and later readjusted after 12 h to pH 7.5 and pH 7.8 until the pH stabilized. The acetone was removed under reduced pressure and saturated aqueous $NaHCO_3$ (10 mL) was added. The unreacted (*R*)-ester was extracted with EtOAc (2×80 mL), washed serially with H_2O and brine, dried ($MgSO_4$) and concentrated under reduced pressure to give a colorless oil (2.67 g). This material was analyzed by HPLC using a chiral support (condition A) and was determined to be the (*R*)-**4** isomer predominating in a ratio of 68:1 (97% ee); $[\alpha]_D^{25}$ −1.24 (c=1.37, $CHCl_3$), $[\alpha]_{365}^{25}$ −1.61 (c=1.37, $CHCl_3$); MS (CI) *m/z*: 309 $(M+2)^+$, 307 $(M)^+$. Anal. calcd for $C_{12}H_{19}BrO_4$: C, 46.92; H, 6.23; found: C, 47.20, H, 6.21.

The basic aqueous phase from above was rendered acidic by addition of 10% HCl (ca. 20 mL) and then extracted with (1:1) EtOAc:Et_2O (2×80 mL). The combined extracts were serially washed with brine and dried ($MgSO_4$). Concentration under reduced pressure followed by drying the residue under high vacuum gave (*S*)-**5** as a colorless oil (2.25 g, 47% yield). $[\alpha]_D^{25}$ −4.58 (c=1.0, MeOH), $[\alpha]_D^{25}$ −1.08 (c=2.69, $CHCl_3$), $[\alpha]_{365}^{25}$ −2.38 (c=2.69, $CHCl_3$); R_f=0.28 (30% EtOAc:hexane); ^{1}H NMR δ 10.98 (bs, 1H), 5.66 (s, 1H), 5.51 (d, *J*=1.5 Hz, 1H), 3.16 (m, 1H), 2.89 (dd, *J*=14.5, 6.0 Hz, 1H), 2.65 (dd, *J*=14.5, 8.5 Hz,

1H), 2.57 (dd, *J*=17, 7.5 Hz, 1H), 2.52 (dd, *J*=17, 5.5 Hz, 1H), 1.44 (s, 9H); ^{13}C NMR δ 179.7, 170.4, 130.2, 119.9, 81.3, 42.3, 39.7, 35.4, 28.0; IR (neat, cm^{-1}) ν 3660–2400 (s), 1730–1700 (s), 1630 (m); MS (CI) *m/z*: 295 $(M+2)^+$, 293 $(M)^+$. Anal. calcd for $C_{11}H_{17}BrO_4$: C, 45.20; H, 5.87; found: C, 45.43, H, 5.87. An aliquot of this material was reacted with diazomethane to give the corresponding methyl ester (*S*)-**4** which was then analyzed by HPLC using a chiral column (condition A) which determined a ratio of 214:1 of (*S*)- to (*R*)-enantiomers (>99% ee). $[\alpha]_D^{25}$ +1.7 (c=0.98, $CHCl_3$); MS (CI) *m/z*: 309.3 $(M+2)^+$, 307.3 $(M)^+$. Anal. calcd for $C_{12}H_{19}BrO_4$: C, 47.05; H, 6.26; found: C, 47.17; H, 6.39.

*4.11. Racemization of 2-(*R*)-(2-bromo-2-propenyl)-1,4-butanedioic acid 4-(1,1-dimethyl-ethyl) 1-methyl ester (*R*)-**4***

To the unreacted ester (*R*)-**4** (1.64 g, 5.3 mmol), which is a mixture of the (*R*)- and (*S*)-enantiomers in a ratio of 68:1, was added toluene (15 mL) and DBU (0.85 mL, 5.5 mmol). This mixture was heated at reflux for 18 h and then diluted with H_2O and extracted with EtOAc. The organic phase was washed serially with 5% aqueous HCl and saturated brine, dried ($MgSO_4$) and concentrated under reduced pressure to give material identical to the racemate **4** (1.57 g, 96%). This material was shown by HPLC using a chiral support (condition A) to be a mixture of the (*R*)- and (*S*)-enantiomers in a ratio of 1:1.

*4.12. Preparation of (*RS*)-acid **5***

To racemate **4** (10.4 g, 33.9 mmol) in THF (30 mL) was added H_2O (15 mL) and aqueous 2 M NaOH (19 ml, 38 mmol) at room temperature. After 5 h, the aqueous phase was acidified with aqueous 1N HCl to pH 2.5 and the mixture diluted with EtOAc (50 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated to give the corresponding acid (*R,S*)-**5** (8.81 g, 89%) as a white solid. ^{1}H NMR δ 5.66 (bs, 1H), 5.51 (bs, 1H), 3.22–3.13 (m, 1H), 2.89 (dd, *J*=14.5, 6.0 Hz, 1H), 2.67–2.61 (dd, *J*=14.5, 9 Hz, 1H), 2.58–2.54 (m, 2H), 1.44 (s, 9H).

*4.13. Preparation of ester derivatives: preparation of (*R,S*)-2-(2-bromo-2-propenyl)-1,4-butanedioic acid 4-(1,1-dimethylethyl) 1-benzyl ester (*RS*)-**8***

Acid (*RS*)-**5** (4.00 g, 13.6 mmol) was dissolved in anhydrous DMF (60 mL) under a nitrogen atmosphere and treated with solid K_2CO_3 (4.15 g, 30.0 mmol). To this slurry was added benzyl bromide (2.44 mL, 20.5 mmol) over 5 min. After stirring at rt (16 h), the DMF was removed under reduced pressure and the residue partitioned between EtOAc (100 mL) and H_2O (125 mL). The aqueous phase was extracted with EtOAc (2×50 mL) and the combined extracts washed with saturated brine, dried ($MgSO_4$), filtered and concentrated. The crude oil was purified by flash chromatography to give the desired benzyl ester (*RS*)-**8** (4.55 g, 87%). ^{1}H NMR δ 7.38–7.31 (m, 5H), 5.58 (bs, 1H), 5.44 (d, *J*=1.5 Hz, 1H), 5.16 (d, *J*=12.5 Hz, 1H), 5.12 (d, *J*=12.5 Hz, 1H), 3.23–3.15 (m, 1H), 2.86 (ddd, *J*=14.5, 6.5, 1 Hz, 1H), 2.66–2.56 (m, 2H), 2.50 (dd, *J*=16.5, 5 Hz, 1H), 1.41 (s, 9H); MS (FAB) *m/z*: 385 $(MH+2)^+$, 383 $(MH)^+$.

*4.14. 2-(*R,S*)-(2-Bromo-2-propenyl)-1,4-butanedioic acid 4-(1,1-dimethylethyl) 1-ethyl ester (*RS*)-**6***

Using the above procedure and EtI as the alkylating reagent, (*RS*)-**6** was obtained in quantitative yield. ^{1}H NMR δ 5.63 (bs, 1H), 5.49 (d, *J*=1.5 Hz, 1H), 4.16 (dq, *J*=7.0, 1.5 Hz, 2H), 3.16–3.08 (m, 1H), 2.85 (ddd, *J*=14.5, 6.5, 1 Hz, 1H), 1.44 (s, 9H), 1.26 (t, *J*=7 Hz, 3H); MS (FAB) *m/z*: 323 $(MH+2)^+$, 320.8 $(MH)^+$.

4.15. 2-(RS)-(2-Bromo-2-propenyl)-1,4-butanedioic acid 4-(1,1-dimethylethyl) 1-propyl ester (RS)-7

Using the same procedure and propyl iodide as the alkylating reagent, (*RS*)-**7** was obtained in 83% yield. ^{1}H NMR δ 5.62 (bs, 1H), 5.47 (d, *J*=1.65 Hz, 1H), 4.05 (t, *J*=7.0 Hz, 2H), 3.21–3.05 (m, 1H), 2.90–2.77 (m, 1H), 2.67–2.48 (m, 3H), 1.75–1.55 (m, 1H), 1.42 (s, 9H), 0.93 (t, *J*=7.5 Hz, 3H); MS (FAB) *m/z*: 337 $(MH+2)^+$, 335 $(MH)^+$.

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