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Regioselective Enzymatic Aminoacylation of Lobucavir to Give an Intermediate for Lobucavir Prodrug

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Abstract—Synthesis of lobucavir prodrug, L-valine, [(1*S*,2*R*,3*R*)-3-(2-amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)-2-(hydroxymethyl) cyclobutyl]methyl ester monohydrochloride (BMS 233866), requires regioselective coupling of one of the two hydroxyl groups of lobucavir (BMS 180194) with valine. Either hydroxyl group of lobucavir could be selectively aminoacylated with valine by using enzymatic reactions. *N*-[(Phenylmethoxy)carbonyl]-L-valine, [(1*R*,2*R*,4*S*)-2-(2-amino-6-oxo-1*H*-purin-9-yl)-4-(hydroxymethyl)cyclobutyl]methyl ester (**3**, 82.5% yield), was obtained by selective hydrolysis of *N*,*N′*-bis[(phenylmethoxy)carbonyl]bis[L-valine], *O*,*O′*-[(1*S*,2*R*,3*R*)-3-(2-amino-6-oxo-1*H*-purin-9-yl)cyclobuta-1,2-diyl]methyl ester (**1**) with lipase M, and L-valine, [(1*R*,2*R*,4*S*)-2-(2-amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)-4-(hydroxymethyl)cyclobutyl]methyl ester monohydrochloride (**4**, 87% yield) was obtained by hydrolysis of bis[L-valine], *O*,*O′*-[(1*S*,2*R*,3*R*)-3-(2-amino-6-oxo-1*H*-purin-9-yl)cyclobuta-1,2-diyl]methyl ester, dihydrochloride (**2**), with lipase from *Candida cylindracea*. The final intermediate for lobucavir prodrug, *N*-[(phenylmethoxy)carbonyl]-L-valine, [(1*S*,2*R*,4*R*)-3-(2-amino-6-oxo-1*H*-purin-9-yl)-2-(hydroxymethyl)cyclobutyl]methyl ester (**5**), could be obtained by transesterification of lobucavir using ChiroCLEC^{TM} BL (61% yield), or more selectively by using immobilized lipase from *Pseudomonas cepacia* (84% yield).

Introduction

Lobucavir (BMS 180194, Scheme 2) is a cyclobutyl guanine nucleoside analogue recently under development as an antiviral agent for treatment of herpes viruses and hepatitis B.[1] A prodrug form in which one of the two hydroxyls is coupled to valine, BMS 233866 (Scheme 3), has also been considered for development. Regioselective aminoacylation by valine is difficult to achieve by chemical procedures, but appeared to be suitable for an enzymatic approach. Riva et al. were the first to demonstrate enzymatic regioselective acylation of nucleosides, using trichloroethyl butyrate and subtilisin in *N*,*N*-dimethylformamide (DMF).[2] Singh et al. reported that using pyridine as a solvent instead of DMF gave higher yields and better regioselectivity for 5′-butyrylation using vinyl butyrate or trifluoroethyl butyrate with subtilisins.[3] Wong and co-workers developed mutant subtilisins with several amino acid substitutions that gave better stability in DMF and were used successfully for 5′-*O*-acetylation of nucleosides.[4,5] Lipases were also shown to carry out regioselective acylations of pyrimidine nucleosides in organic solvent using vinyl acetate or acid anhydrides.[6,7] Moris and Gotor reported that oxime esters were useful acyl donors for regioselective acylation of nucleosides by lipases[8,9] and extended this technique to regioselective aminoacylation using oxime aminoacyl esters prepared from *p*-nitrophenyl esters of Z-gly or Z-or Boc β-ala.[10]

Regioselective enzymatic hydrolysis of mutiply-acylated nucleosides has also been used as an approach to selective acylation. Selective 5′-*O*-deacetylation of tri-*O*-acetylribonucleosides by subtilisin or porcine pancreatic lipase was reported by Singh et al.[11] Katagiri et al. found that lipase MY deacylated the 5′-*O* and lipase xiii

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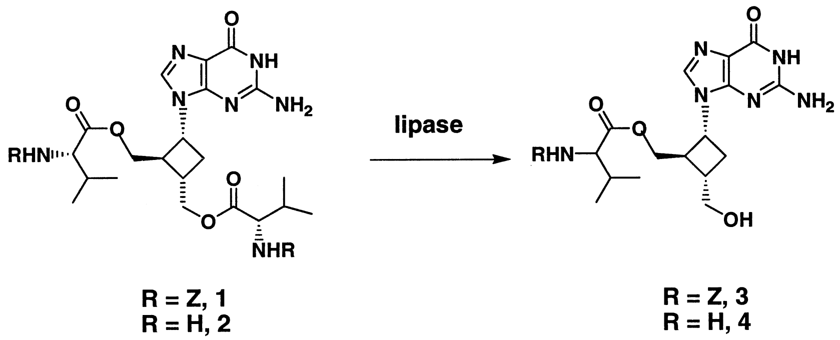
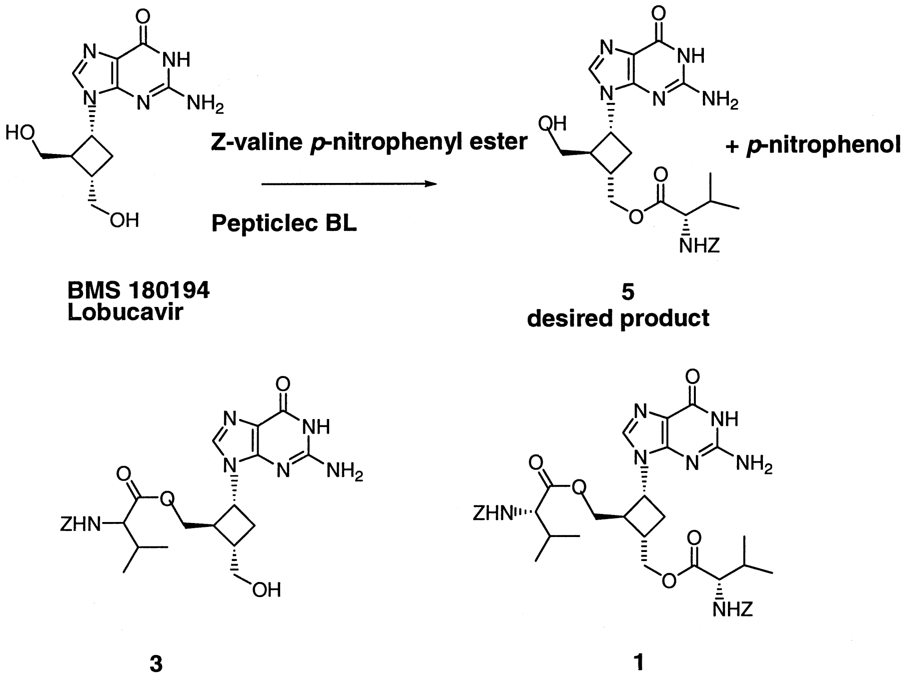


Scheme 1. Regioselective hydrolysis of diesters **1** and **2**.



Scheme 2. Transesterification of Z-valine *p*-nitrophenyl ester with lobucavir.

(*Pseudomonas* sp.) deacylated the 3′-*O* of carbocyclic 3′5′-di-*O*-acyloxetanocin.[12]

Regioselective acylation of only one of the 2 hydroxyls of lobucavir with *N*-protected L-valine is a problem that we have approached by chemical aminoacylation of both hydroxyl groups followed by selective enzymatic hydrolysis and also by selective enzymatic aminoacylation.

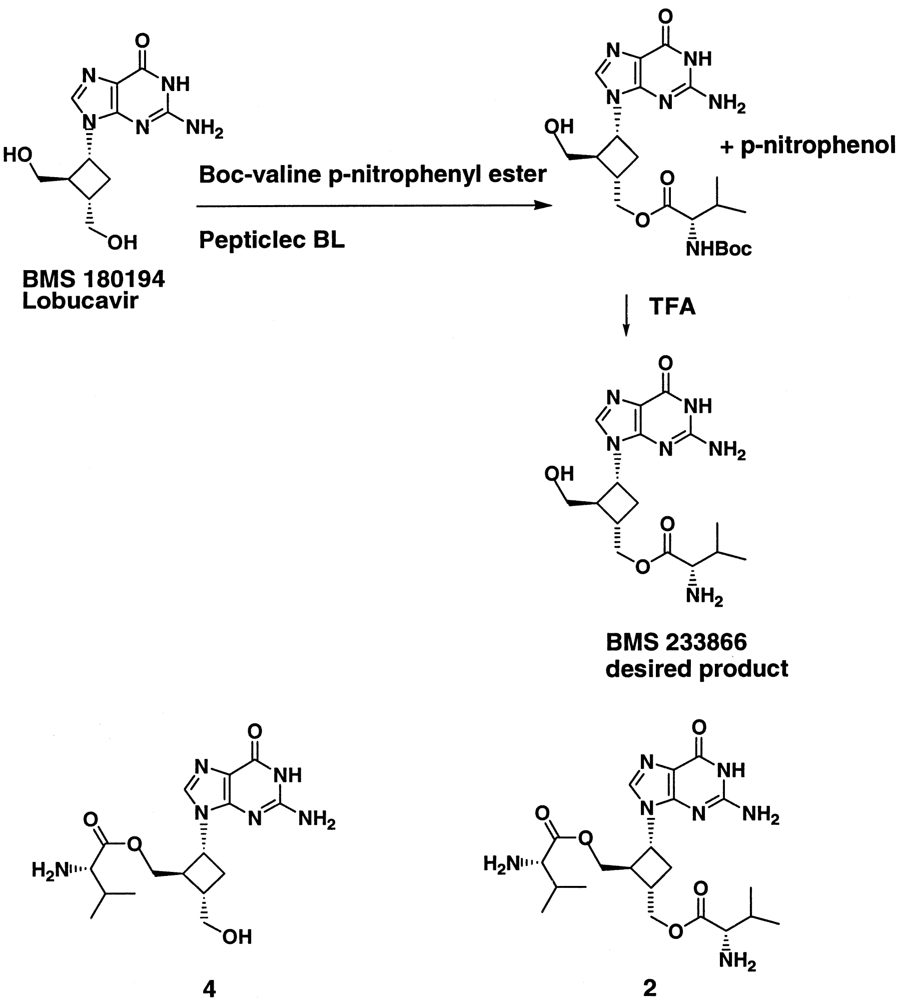
Results

Hydrolysis

Screening lipases for regioselective hydrolysis of diester **1** revealed several that gave **3**, but none that gave the desired product **5**. On a 50 g scale, lipase M in 90% toluene/10% buffer as described in Experimental gave **3** in 75.2% yield. Lipases were also screened for hydrolysis of **2** to BMS 233866. Again, selectivity was for the undesired isomer **4**. The most selective enzyme was lipase from *Candida cylindracea*, which gave 87 M% yield of **3** on a 2 g scale in phosphate buffer as described in Experimental.

Coupling

Enzyme and solvent screening. Initial efforts to couple lobucavir to Z-valine by transesterification focused on using lipases that converted **1** to **3**, or **2** to **4** in the hydrolysis direction (lipases M, *C. cylindracea*, and others). If these enzymes selected the same hydroxyl site in the coupling reaction, lobucavir would be converted to the desired product (**5**, Scheme 2). These lipases did not give any coupled products, but ChiroCLEC^{TM} BL (cross-linked crystals of subtilisin) gave **5**. Undesired side products were also produced in smaller amounts (**3** and **1**). The rate and yield for the coupling reaction were very dependent on the choice of solvent. The best yield for the reaction was obtained using 70% acetone/30% DMF as solvent (Fig. 1). Addition of 0.01 or 0.1% water slightly and 1.0% water strongly decreased the rate and yield of the coupling reaction. Other solvents



Scheme 3. Transesterification of Boc-valine *p*-nitrophenyl ester with lobucavir and subsequent deprotection.

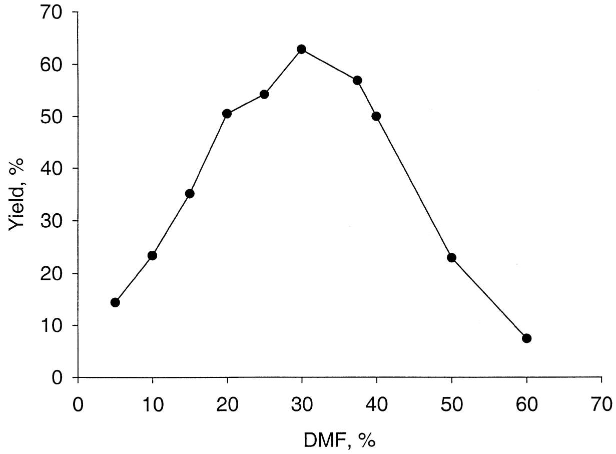


Figure 1. Effect of DMF concentration on yield after 2 days. The reaction contained 10 mg/mL lobucavir, 50 mg/mL (3.56 equiv) Z-valine *p*-nitrophenyl ester, and 15 mg/mL ChiroCLEC^{TM} BL.

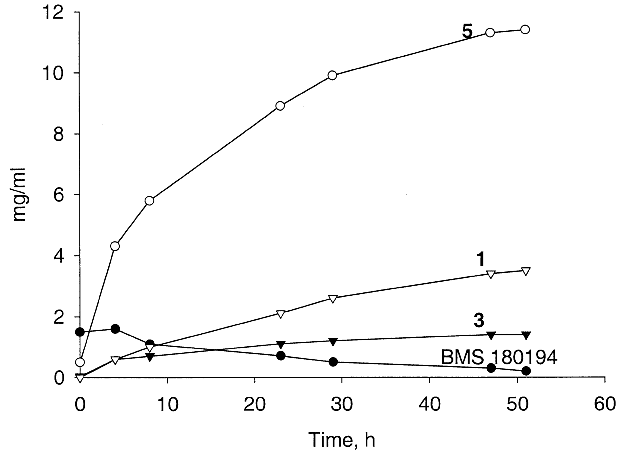


Figure 2. Kinetics of tranesterification of Z-valine *p*-nitrophenyl ester with lobucavir using ChiroCLEC^{TM} BL. The reaction contained 10 mg/mL lobucavir, 50 mg/mL Z-valine *p*-nitrophenyl ester, and 15 mg/mL ChiroCLEC^{TM} BL in 70% acetone/30% DMF. Initial lobucavir concentrations are difficult to assay because of the low solubility in the reaction solvent.

giving lower yields and/or lower regioselectivity were tetrahydrofuran, DMF, acetone, acetonitrile, methylene chloride, methyl *t*-butyl ether, methyl ethyl ketone, methylisobutyl ketone, ethyl acetate, toluene, isooctane, pyridine, dimethyl sulfoxide, dioxane, *t*-butanol, and *t*-amyl alcohol.

The time course of a 7 g batch is shown in Figure 2. The yield for this reaction was 8.30 g, 63.1 M% **5** (desired product). Undesired products were **3** (1.02 g, 7.8 M%), **1** (2.55 g, 13.2 M%), and unreacted BMS 180194

(2.1 M%). The reaction was scaled up to give 607 g of **5** (53.8 M%) from a 600 g input of lobucavir and 4.5 kg of **5** (54.8 M%) from a 4.4 kg input of lobucavir (Table 2).

Reusability

ChiroCLEC^{TM} BL was used three times without a noticeable loss of activity, but yields dropped on the fourth and fifth uses. Unreacted Z-valine *p*-nitrophenyl ester, recovered after isolation of **5**, was used in a second coupling reaction to give **5** in 58.5% yield.

Other esters with ChiroCLEC^{TM} BL

Z-valine methyl ester gave only about 15% of the yield obtained with the *p*-nitrophenyl ester, and the reaction was much slower. In 20 mg scale experiments, Z-valine trifluoroethyl ester gave a somewhat better yield than the *p*-nitrophenyl ester. Four equivalents Z-valine *N*-hydroxysuccinimide ester gave a 50% yield after 4 days, and reaction was a little slower than with the *p*-nitrophenyl ester. *N*-Boc-valine *p*-nitrophenyl ester gave a significant improvement in yield to 76 M% (Table 1) and reaction rate. The amount of enzyme required to complete the reaction in about 52 h was decreased by half when the Boc-ester was substituted for the Z-ester.

Transesterification by *Pseudomonas cepacia* lipase

In a 20 mg scale reaction, ChiroCLEC-PC using 7 equiv Z-valine trifluoroethyl ester gave an 84.6% yield of **5** with no **3** and only 1.8% **1** (Fig. 3). Under the same conditions, Amano lipase PS30 immobilized on Accurel polypropylene gave an 81.4% yield of **5** with no **3** and only 0.7% **1**.

Discussion

Either hydroxyl group of lobucavir could be selectively aminoacylated with valine by using enzymatic reactions. Monoester **3** (82.5% yield) was obtained by selective hydrolysis of **1** with lipase M, and **4** (87% yield) was obtained by hydrolysis of **2** with lipase from *C. cylindracea*. However, these two products were not the desired intermediates for lobucavir prodrug. ChiroCLEC^{TM} BL (cross-linked crystals of subtilisin) catalyzed a regioselective aminoacylation of lobucavir using Z-L-valine *p*-nitrophenyl ester as acyl donor to give **5** in 62% yield. Undesired side products were **3** (7.8%) and **1** (13%). Rate, yield, and regioselectivity of the transesterification of Z-valine *p*-nitrophenyl ester with lobucavir using ChiroCLEC^{TM} BL were strongly affected by solvent. 70% Acetone/30% DMF gave the highest rate and yield of **5** of any of the solvents tested.

Table 2. Scaleup of transesterification of Z-valine *p*-nitrophenyl ester with lobucavir

Lobucavir input, g	Desired product, **5**		Other	
	g	Molar% yield	Molar% yield	
7	8.07*	61.4	BMS 180194	2.4
			3	7.6
			1	12.6
600	607	53.8	BMS 180194	0.4
			3	7.2
			1	23.0
4400	4531	54.8	BMS 180194	2.6
			3	7.2
			1	11.7

*Mean of 3 reactions.

Rate and yield in the aminoacylation of lobucavir by ChiroCLEC^{TM} BL were also strongly affected by the leaving group and *N*-protecting groups on the valine ester. When several Z-L-valine esters were compared, *p*-nitrophenyl = trifluoroethyl > *N*-hydroxysuccinimide > > methyl ester in rate and yield for the reaction. When Boc-L-valine *p*-nitrophenyl ester was used, followed by deprotection of analytical samples, a 76% yield of BMS 233866 was achieved using 1/2 the amount of ChiroCLEC^{TM} BL required for the reaction with the Z-valine *p*-nitrophenyl ester. Undesired side products were **4** (8.3%) and **2** (11.5%).

Wong and co-workers developed mutant subtilisins with several amino acid substitutions that gave better stability in DMF and were used successfully for 5′-*O*-acetylation of nucleosides.[4,5] The cross-linked enzyme crystals of subtilisin are an alternative for increased stability in organic solvents and were reused for four more reactions in acetone/DMF.

ChiroCLEC^{TM} PC (cross-linked crystals of *P. cepacia* lipase) or Amano lipase PS30 (also *P. cepacia* lipase) immobilized on Accurel polypropylene gave the highest yield and regioselectivity. Z-valine trifluoroethyl ester was transesterified with lobucavir to give **5** in 84% yield, with no **3** and only 0.4% **1**. With this lipase Z-valine *p*-nitrophenyl ester was not an effective substrate. Moris

Table 1. Yields in transesterification reactions with lobucavir

Enzyme	Ester	Desired product	Molar% yield	Other	Molar% yield
CLEC^{TM} BL	Z-valine *p*-nitrophenyl ester	**5**	63.1	BMS 180194	2.1
				3	7.8
				1	13.2
CLEC^{TM} BL	Boc-valine *p*-nitrophenyl ester	BMS 233866	76.0	BMS 180194	7.1
				4	8.3
				2	11.5
CLEC^{TM} PC	Z-valine trifluoroethyl ester	**5**	84.6	BMS 180194	0.3
				3	0.0
				1	1.9

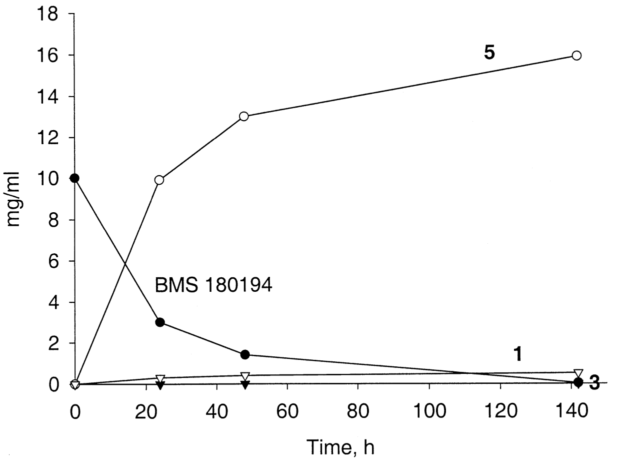


Figure 3. Kinetics of transesterification of Z-valine trifluoroethyl ester with lobucavir using ChiroCLEC^{TM} PC. The reaction contained 10 mg/mL lobucavir, 89.4 mg/mL (7.12 equiv) Z-valine trifluoroethyl ester, and 15 mg/mL ChiroCLEC^{TM} PC in 80% *t*-butyl-methyl ether/20% DMF. The first sample was taken after 24 h.

and Gotor also noted that *p*-nitrophenyl esters of *N*-protected amino acids were not effective aminoacylating agents when using *P. cepacia* lipase and other lipases.[10]

Conclusion

The final intermediate (**5**) for lobucavir prodrug could be obtained by transesterification of lobucavir using ChiroCLEC^{TM} BL (61% yield), or more selectively by using immobilized lipase from *P. cepacia* (84% yield). Coupling of Z-valine or valine to the other hydroxyl was achieved by chemical aminoacylation of both hydroxyls, followed by regioselective enzymatic hydrolysis using lipase M from *Mucor javanicus* or lipase from *C. cylindracea*, respectively.

Experimental

Hydrolysis of 1

Diester **1** (50.0 g, 68.32 mmol) was dissolved in 2.5 L toluene by stirring at 50–55 °C, then added to a 15-L jacketed reactor equipped with an air-driven stirrer and a condenser kept at 4–8 °C. The reactor was heated to 40 °C with a circulator. Another 7.5 L toluene was added to the reactor. The stirrer was turned to full power and 250 g lipase MAP-10 was added to give a fine suspension of the enzyme. One litre of 0.1 M potassium phosphate buffer, pH 7.0, was added, and the reaction was continued until HPLC analysis indicated that the concentration of **1** had decreased to 0.12 g/L. Reactions were stopped after 71 h. HPLC analysis showed **3** (28.38 g, 83.3 M%), **1** (1.32 g, 2.6 M%), **5** (0.06 g, 0.2 M%), and BMS 180194 (0.13 g, 0.7 M%). After completion of the reaction, the whole solution was passed through a coalescing filter to break the emulsion with the resulting toluene layer separated. The remaining small amount of emulsion was further extracted with toluene followed by separation of the resulting organic phase. The combined toluene extracts were pooled and concentrated at 40 °C (~23 mmHg) to give 40.4 g oily solid containing 20.8 g **3**.

Isolation and purification of 3

A warm solution of crude **3** (40.2 g containing 19.96 g **3**) in ethyl acetate (700 mL) was washed with water (1×150 mL and 1×50 mL) by stirring at 45 °C (bath). The separated organic phase was then treated with additional water (50 mL) at 45 °C (bath). The mixture was slowly cooled to room temperature with stirring and stirred at room temperature for 4 h. A white precipitate formed as the organic phase was cooled to room temperature, the solid was collected by filtration, washed with ethyl acetate:hexane (10:90), air-dried and dried in vacuo (18 h) to provide the desired **3** as a white solid (18.85 g, 98.4 HPLC area%, 94% yield): mp 99.5 °C, $[\alpha]_D$ −0.19.3° (*c* 1.01, MeOH). ^{1}H NMR (270 MHz, DMSO-d_6) δ 7.83 (s, 1H), 7.62 (d, 1H, $J = 7.6$ Hz), 7.22–7.43 (m, 5H), 6.35 (s, 2H), 5.03 (s, 2H), 4.67 (t, 1H, $J = 5.4$ Hz), 4.38–4.53 (m, 1H), 4.17 (d, 2H, $J = 5.3$ Hz), 3.88–3.83 (t, 1H, $J = 7.0$ Hz), 3.53 (m, 2H), 2.92 (m, 1H), 2.36 (m, 1H), 2.00–2.23 (m, 2H), 1.87 (m, 1H), 0.78 (d, 6H, $J = 6.4$ Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.5, 156.6, 156.2, 153.0, 150.8, 136.7, 135.5, 128.1 (2 C), 127.6 (2 C), 127.5, 116.6, 65.3, 65.1, 62.9, 59.4, 47.0, 43.8, 33.1, 29.3 (2 C), 18.7, 17.7. IR (KBr) 3423, 3337, 2691, 1700, 1628, 1535, 1095 cm^{-1}. Anal. calcd for $C_{24}H_{30}N_6O_6 \cdot 1.0\ H_2O$: C, 55.78; H, 6.25; N, 16.27. Found: C, 55.47; H, 6.10; N, 16.01.

Hydrolysis of diester 2

Diester **2** (2.0 g) and 20 g lipase from *C. cylindracea* were stirred in 800 mL 0.1 M phosphate buffer, pH 7.0, at 28 °C for 18 h. Reaction product concentrations determined by HPLC were 1.62 mg/mL **4** (87M%), 0.006 mg/mL BMS 233866, 0.2 mg/mL BMS 180194, and 0.025 mg/mL remaining diester **2**.

Coupling reactions

Analytical scale coupling reactions were carried out on a heated block in 2 mL capped vials mixed with a magnetic stirring bar. For a preparative batch, lobucavir (7.00 g, 26.39 mmol), *N*-CBZ-L-valine *p*-nitrophenyl ester (35 g, 93.98 mmol, 3.56 equiv), and 10.5 g ChiroCLEC BL were added to a jacketed 1-L three-necked round-bottom flask equipped with an air-driven stirrer and a condenser maintained at 5 °C by a circulating water bath. 490 mL acetone followed by 210 mL DMF were added, a stopper was put in the third neck of the flask and the mixture was stirred fast enough to keep the enzyme and lobucavir suspended. The jacket temperature was raised to 45 °C with a second circulating water bath, and the reaction was monitored by taking 0.1 mL samples for analysis by HPLC. Other reaction conditions are described in the figure legends. The immobilized enzyme was removed by filtration at the end of the reaction, and acetone was removed with a rotary evaporator at 40 °C. The recovered immobilized enzyme was stored at 4 °C for reuse. A 600 g batch was carried out in a 100 L reactor under nitrogen, and a 4.4 kg batch was carried out in a pilot plant reactor.

Isolation and purification of 5

A concentrated reaction mixture (678.0 g) from the selective enzymatic acylation of BMS 180194 (20.0 g) with *p*-nitrophenyl ester of CBZ-L-valine (200 g) in DMF–acetone was concentrated to remove most of the DMF at 40 °C (~1 mm Hg) to provide a crude product (318 g, containing ~100 g DMF). One portion of this crude (130.0 g) was treated with ethyl acetate (200 mL) and water (80 mL). Crystalline **5** seeds were added to the mixture, which was then stirred at 25 °C for 0.5 h and then at 65 °C for 4 h. The mixture was cooled to 25 °C for 3 h and then cooled to 0 °C (ice bath) for 20 min. The crystals were collected by filtration, washed with water (20 mL) and ethyl acetate (15 mL), and dried under vacuum to give 8.1 g (46% yield, 98 HPLC area%) of **5** with 98 AP. The crude **5** was further purified by stirring in ethyl acetate (100 mL) and water (30 mL) at 65 °C (bath) for 3 h and then cooling to ambient temperature. Filtration and washing with ethyl acetate (15 mL) followed by drying under vacuum provided 7.6 g (43%) of pure **5** as a white solid: mp 205.0–206.0 °C. $[\alpha]_D$ −0.6° (*c* 1.02, MeOH). ^{1}H NMR (270 MHz, DMSO-d_6) δ 7.91 (s, 1H), 7.72 (d, 1H, *J* = 7.6 Hz), 7.20–7.45 (m, 5H), 6.43 (s, 2H), 5.05 (s, 2H), 4.70 (t, 1H, *J* = 4.7 Hz), 4.50 (m, 1H), 4.12–4.31 (m, 2H), 3.96 (t, 1H, *J* = 7.4 Hz), 3.49 (m, 2H), 3.37 (s, 1H, OH), 2.77 (m, 1H), 2.40 (m, 1H), 2.26 (m, 1H), 1.95–2.20 (m, 2H), 0.91 (d, 6H, *J* = 6.5 Hz); ^{13}C NMR (68 MHz, DMSO-d_6) δ 173.1, 158.1, 157.7, 154.5, 152.3, 138.1, 137.3, 129.6 (2 C), 129.1 (3 C), 118.0, 68.0, 66.8, 62.0, 61.1, 49.2, 47.5, 31.3, 30.9, 30.6, 20.3, 19.5. IR (KBr) 3600–3000, 1740, 1694, 1624, 1601, 1534, 1362, 1028 cm^{-1}. Anal. calcd for $C_{24}H_{30}N_6O_6$: C, 57.76; H, 6.07; N, 16.84. Found: C, 57.67; H, 5.79; N, 16.62.

Recovery of excess *p*-nitrophenyl ester of CBZ-L-valine

The mother liquor from the first crystallization was concentrated and treated with ethyl acetate:hexane (2:5, 500 mL) at 50 °C for 0.5 h. The mixture was then cooled to 0 °C (ice bath, 1 h). A small amount of a brown oil was present. The supernatant was decanted to a 1 L flask. Ethyl acetate (100 mL) was added to the supernatant, which was then washed with aqueous K_3PO_4 solution (10%, 3×150 mL) and NaH_2PO_4 (std. 100 mL) and dried (Na_2SO_4). The solid (Na_2SO_4) was removed by filtration. Concentration of the filtrate provided an oil which was dissolved in ethyl acetate (70 mL)/hexane (300 mL) at 40 °C (oil bath). The solution was seeded and additional hexane (500 mL) was added while stirring and slowly cooling to 0 °C. The crystalline solid was collected by filtration and washed with hexane (50 mL) to give 49.0 g of *p*-nitrophenyl ester of CBZ-L-valine (>99.5 AP) as a white solid: mp 65.0–66.0 °C (identical to an authentic sample). The mother liquor was concentrated and an additional 4.5 g of *p*-nitrophenyl ester of CBZ-L-valine (>99.5 AP) was obtained in the second crop by crystallization from ethyl acetate (20 mL) and hexane (280 mL).

HPLC analysis

For analysis of hydrolysis reactions, samples were evaporated to dryness under a nitrogen stream, then dissolved in 75% acetonitrile/25% potassium dihydrogen phosphate (0.68%) and 1-heptanesulfonic acid, sodium salt (0.1%), in water. For coupling reactions with Z-valine esters, 0.1 mL samples were evaporated to dryness under a nitrogen stream at 45 °C. The residue was taken up in 2 mL 50% acetonitrile/50% water containing 0.4% trifluoroacetic acid. To this sample was added 1 mL 50% acetonitrile, 50% water containing 0.05 M KH_2PO_4 and 0.01% heptanesulfonic acid, sodium salt. After mixing and sonicating, 1 mL acetonitrile was added and the sample was mixed and sonicated again. For coupling reactions with Boc-valine *p*-nitrophenyl ester, samples were dried, treated for 30 min at room temperature with trifluoroacetic acid, dried again, then dissolved as outlined for the Z-valine ester samples.

Samples were analyzed with a YMC, Inc., ODS-A, C-18, 5 μ, 4.6×150 mm column. The mobile phase was a gradient of A: potassium dihydrogen phosphate (0.68%) and 1-heptanesulfonic acid, sodium salt (0.1%), in water and B: acetonitrile (0 min 5%, 30 min 14%, 50 min 14%, 70 min 50%, 100 min 50%, 105 min 5%, 110 min 5%). Flow rate was 1 mL/min, detection was at 254 nm, temperature was ambient, and injection volume was 20 μL. Retention times (min) were: BMS 180194, 7.2; **4**, 25.5; BMS 233866, 31.8; **2**, 55.5; Z-valine, 58.5; **3**, 63.4; **5**, 64.9; Z-valine methyl ester, 72.9; **1**, 76.2; Z-valine-*p*-nitrophenyl ester, 88.2.

Materials

Commercial materials were obtained from the following sources: Z-L-valine and Z-valine trifluoroethyl esters, Chem-Impex International, Boc-L-valine *p*-nitrophenyl ester, Bachem; Z-valine *N*-hydroxysuccinimide ester, Nova; ChiroCLEC^{TM} BL and ChiroCLEC^{TM} PC, Altus Biologics; M-10 (*Mucor javanicus*) lipase, Amano; *C. cylindracea* lipase, Biocatalysts; lipase PS30 (*P. cepacia*) from Amano was immobilized by adsorption on Accurel polypropylene as described previously.[13]

Preparation of diester 1

Cbz-L-valine (3.02 g, 5.0 mmol) and EDC (3.26 g, 17.0 mmol) were added to a suspension of BMS 180194 (monohydrate, 1.417 g, 5.0 mmol) in MeCN (20 mL) under an argon atmosphere. After 2.5 h, the reaction was quenched with AcOH (0.3 g, 5.0 mmol) and the solvent was removed under vacuum. The residue was treated with ethyl acetate (60 mL) and water (20 mL). The organic phase was washed with water (3×20 mL), 1 M aq H_3PO_4 (3×15 mL), brine (10 mL), aq $NaHCO_3$ (3×15 mL) and brine. During these washings, 2 mL of brine was added each time after shaking to facilitate the separation of the layers. The organic solution was dried ($MgSO_4$), filtered, and concentrated, and the residue was dried under vacuum to provide 4.07 g (98.3 AP) of crude product. Crystallization of the crude product from EtOAc:TBME:heptane (2:2:1, 25 mL) gave 2.99 g

(82%, 99.4 AP) of 1 as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.71 (s, 1H), 7.91 (s, 1H), 7.74 (d, 1H, J = 8.1 Hz), 7.63 (d, 1H, J = 8.1 Hz), 7.23–7.45 (m, 10H), 6.44 (s, 2H), 4.97–5.10 (m, 4H), 4.52 (m, 1H), 4.26 (d, 1H, J = 5.1 Hz), 4.20 (d, 1H, J = 5.1 Hz), 4.08–4.25 (m, 2H), 3.98 (dd, 1H, J = 6.8 Hz, 7.7 Hz), 3.89 (dd, 1H, J = 6.0 Hz, 8.0 Hz), 3.00 (m, 1H), 2.42 (m, 1H), 2.30 (m, 1H), 2.23 (m, 1H), 2.06 (m, 1H), 1.89 (m, 1H), 0.91 (d, 6H, J = 6.4 Hz), 0.78 (d, 6H, J = 5.0 Hz); ^{13}C NMR (68 MHz, DMSO-d_6) δ 172.1, 172.0, 157.2, 156.7 (2 C), 153.7, 151.4, 137.2 (2 C), 136.2, 128.6 (4 C), 128.1 (6 C), 117.1, 66.7, 65.9, 65.1, 60.2, 60.0, 59.9, 47.3, 45.0, 30.3, 30.1, 29.9, 29.8, 19.3, 19.2, 18.5, 18.2. IR (KBr) 3600–3000, 1701, 1626, 1595, 1532, 1028 cm^{-1}. Anal. calcd for $C_{37}H_{45}N_7O_9 \cdot 0.35\ H_2O$: C, 60.20; H, 6.24; N, 13.29. Found: C, 59.89; H, 6.17; N, 13.10.

Preparation of 2

A solution of **1** (45.54 g, 62.3 mmol) in MeOH (500 mL) and 10% Pd/C (4.55 g) were added to a 2-L Parr hydrogenation bottle under an Ar atmosphere. Concentrated HCl (10.3 mL, 125 mmol) was added and the mixture was hydrogenated at 25 psi for 4 h (reaction was complete by TLC and HPLC). The reaction mixture was sparged with N_2 for 1 h, filtered through Celite, and washed with MeOH. Concentration of the filtrate provided the crude product as a foam. The foam was triturated with acetone (500 mL) and then stirred for 2 h. The product was collected by filtration, washed with acetone, and dried under vacuum to give 32.7 g (97.1%, ~100 AP) of **2** as a white solid: mp 192.5 °C. $[\alpha]_D$ +15.6° (c 1.0, MeOH). ^{1}H NMR (270 MHz, DMSO-d_6) δ 8.66 (br-s, 7H), 8.25 (s, 1H), 6.77 (s, 2H), 4.63 (m, 1H), 4.20–4.46 (m, 4H), 3.93 (br-s, 1H), 3.79 (br-s, 1H), 3.10 (m, 1H), 2.28–2.53 (m, 3H), 2.22 (m, 1H), 2.06 (m, 1H), 1.00 (d, 3H, J = 7.0 Hz), 0.96 (d, 3H, J = 7.0 Hz), 0.86 (d, 3H, J = 7.0 Hz), 0.84 (d, 3H, J = 7.0 Hz); ^{13}C NMR (68 MHz, DMSO-d_6) δ 168.7 (2 C), 156.2, 154.1, 150.8, 136.3, 115.0, 67.4, 65.6, 57.4 (2 C), 47.2, 44.2, 29.5, 29.3 (2 C), 29.2, 18.7, 18.3, 17.6 (2 C). IR (KBr) 3430, 1744, 1686, 1630, 1595 cm^{-1}. Anal. calcd for $C_{21}H_{33}N_7O_5 \cdot 2\ HCl \cdot 1.0\ H_2O$: C, 44.55; H, 6.75; N, 17.09; Cl, 14.05. Found: C, 44.48; H, 6.72; N, 16.95; Cl, 14.13.

References and Notes

1. Ireland, C.; Leeson, P. A.; Castaner, J. *Drugs Future* **1997**, *22*, 359.
2. Riva, S.; Chopineau, J.; Kieboom, A. P. G.; Klibanov, A. M. *J. Am. Chem. Soc.* **1988**, *110*, 584.
3. Singh, H. K.; Cote, G. L.; Hadfield, T. M. *Tetrahedron Lett.* **1994**, *35*, 1353.
4. Wong, C.-H.; Chen, S.-T.; Hennen, W. J.; Bibbs, J. A.; Wang, Y.-F.; Liu, J. L.-C.; Pantoliano, M. W.; Whitlow, M.; Bryan, P. N. *J. Am. Chem. Soc.* **1990**, *112*, 945.
5. Zhong, Z.; Liu, J. L.-C.; Dinterman, L. M.; Finkelman, M. A. J.; Mueller, W. T.; Rollence, M. L.; Whitlow, M.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 683.
6. Uemura, A.; Nozaki, K.; Yamashita, J.; Yasumoto, M. *Tetrahedron Lett.* **1989**, *30*, 3817.
7. Nozaki, K.; Uemura, A.; Yamashita, J.; Yasumoto, M. *Tetrahedron Lett.* **1990**, *31*, 7327.
8. Moris, M.; Gotor, V. *J. Org. Chem.* **1993**, *58*, 653.
9. Moris, M.; Gotor, V. *Tetrahedron* **1993**, *44*, 10089.
10. Moris, M.; Gotor, V. *Tetrahedron* **1994**, *50*, 6927.
11. Singh, H. K.; Cote, G. L.; Sikorski, R. S. *Tetrahedron Lett.* **1993**, *34*, 5201.
12. Katagiri, N.; Morishita, Y.; Yamaguchi, M. *Tetrahedron Lett.* **1998**, *39*, 2613.
13. Patel, R. N.; Banerjee, A.; Ko, R. Y.; Howell, J. M.; Li, W.-S.; Comezoglu, F. T.; Partyka, R. A.; Szarka, L. *Biotechnol. Appl. Biochem.* **1994**, *20*, 23.