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CHEMOENZYMATIC SYNTHESIS OF A C_5-CHIRAL BUILDING BLOCK: A SUBSTRATE MODIFICATION APPROACH.

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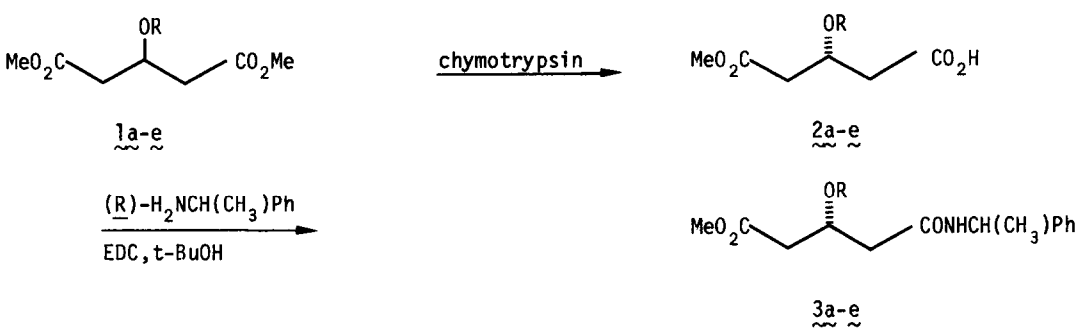
Abstract: The enantioselectivity of α-chymotrypsin hydrolysis of prochiral dimethyl-3-hydroxyglutarates was controlled by the proper choice of the hydroxyl protecting groups. This strategy allows the synthesis of a versatile C_5-chiral building block.

In an effort to establish a reiterative strategy for the synthesis of both the syn-[1] and anti-1,3-diol[2] and 1,3-polyol units found in some polyene macrolides and in numerous natural products, we were faced with the elaboration of chiral β-hydroxyketones. It was envisaged that a C_5-chiral building fragment composed of an unsymmetrically disposed 1,5-functionality would be a suitable general precursor. Our interests in enzyme-catalyzed asymmetric synthesis[3] led us towards the α-chymotrypsin enzymatic hydrolysis of the prochiral dimethyl 3-hydroxyglutarate (1a)[4]. This reaction had been previously reported to be totally enantioselective[4]. However, as reported by Rosen et al.[5] and more recently by Brooks et al.[6], the ee was very dependent upon the reaction conditions and was never high enough to be of value for synthetic purposes. It was therefore decided to reinvestigate the reaction conditions involved in the enzymatyic hydrolysis in order to optimize the ee. Such studies have already led to striking improvements in similar situations[7].

Unfortunately, as shown in Table 1, the enantiomeric excesses, obtained from the hydrolysis of 1a, were disappointingly constant and low (~ 61%) even after exhaustive variations in the reaction conditions. The ee's were determined by capillary GC or by ^{1}H-NMR on the diastereomeric (R)-α-methylbenzylamide 3a (Scheme 1). The R absolute configuration for 2a agreed with literature information and corresponded to an enantioselective pro-S ester hydrolysis[4].

Scheme 1



1a-e 2a-e 3a-e

a, R=H; b,R=Bn;
c, R=Bz; d,R=MOM, e, R=TBDMS

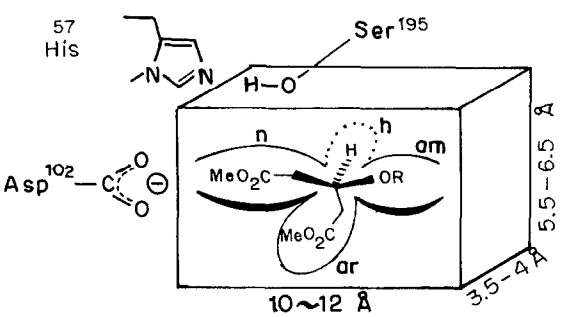
TABLE 1. Chymotrypsin-catalyzed Hydrolysis of 1a.

Enz. Subst. (w/w)	pH	Solvent[a]	Yield[b] (%)	ee[c]	Conf.
1:2	6.7	A	100	64	R
1:5	7.0	A	92	62	R
1:2	7.0	A	86	58	R
1:1	7.0	A	95	65	R
1:5	7.8	A	83	57	R
1:2	7.8	A	99	63	R
1:1	7.8	A	61	63	R
1:2	7.8	B	100	55	R
2:1	7.0	C	67	62	R
70[d]	7.0	A	100	15	S

[a] A=0,01M Na_2HPO_4 buffer[4]; B=A + 20% MeOH; C = PBS. Reactions performed at room temperature.
[b] Yields of isolated 2a based on recuperated 1a.
[c] Determined by GC and ^{1}H-NMR on 3e.
[d] PLE units.

Since none of the variations in the reaction conditions with 1a had a dramatic influence on the enantioselectivity, we varied the size of the hydroxyl protecting group in order to take better advantage of the dimension of the enzyme's active site[8,9]. It was hypothesized that two different orientations of the substrate in the binding site could have been responsible for the lack of enantioselectivity. Thus, it seems likely that the h (hydrogen) domain is not sterically congested enough to prevent the binding of the unprotected 3-hydroxy group in competition for the binding to the am (amide) domain (Scheme 2). This undesired orientation would allow the pro-R ester to be hydrolyzed and would explain the formation of the S monoacid of 2a. Therefore, to prevent this mode of binding, a suitable derivatization of the hydroxyl function could constrain the substrate to bind to the am domain provided that the pro-R ester group would still have a higher affinity for the ar (aromatic) domain.

Scheme 2



A new strategy was therefore undertaken. Various bulky hydroxyl protecting groups were found to successfully raise the ee of the products. Table 2 shows a number of results using this substrate modification approach. For example, the water soluble MOM derivative 1d gave an ee well above the unprotected substrate (93%). Interestingly, the configuration of

TABLE 2. Chymotrypsin-Catalyzed Hydrolysis of 1b-d.

Enz. Subst. (w/w)	pH	Solvent[a]	Yield[b] (%)	ee[c]	Conf.
1:1(1b)	7.8	C	68	84	R
368[d](1b)	7.0	C	78	12	S
1:2(1c)	7.8	C[e]	42	84	R
1:1(1c)	7.8	C	86	92	R
1:2(1c)	7.8	C[f]	68	91	R
160[d](1c)	7.0	D	79	33	S
180[d](1c)	7.0	C	77	59	S
1:2(1d)	7.8	A[f]	100	88	R
1:2(1d)	7.0	A	95	93	R
1:1(1d)	7.8	A	100	93	R
2:1(1d)	7.8	A[g]	92	93	R
330[d](1d)	7.0	A	100	14	S

[a]A=0,0.1M Na_2HPO_4 buffer; B=A+20% CH_3CN; C=A+20% dioxane; D=A+20% MeOH. Reactions performed at room temperature[11].

[b]Yields of isolated 2b-d based on recuperated 1b-d. Yields may vary depending on the extent of completion which was followed by the equivalent of base consumed.

[c]Determined by GC and/or ^{1}H-NMR on 3b-d[13].

[d]PLE units; [e]At 36°C; [f]Membrane-Enclosed Enzymatic Catalysis (MEEC)[14].

[g]Same results at 0°C and 36°C (complete in 2 hrs).

the major enantiomer remained R, suggesting that the focalized orientation of the substrate within the active site was as predicted and corresponded to the pro-S ester group binding to the n(active) site. However, the results of Table 2 demonstrate that the strategy appears not to be general since it does not apply to the other enzymatic system studied. Hence, the data with pig liver esterase (PLE) are included and remain comparable with previous results on 1a[10].

In conclusion, the present report establishes another facet for the utilization of enzymes in asymmetric synthesis and that a closer look at the enzyme's active site should be undertaken before abandoning their usage. The above strategy should therefore be considered complementary to the existing ones[7].

Typical Experiment:

α-Chymotrypsin (EC 3.4.21.1, Sigma Type II from bovine pancreas, 1.00 g, 15 μmol) dissolved in 16 mL of 0.01M Na_2HPO_4 buffer was equally distributed in four dialysis bags (cellulose acetate, M.W. cut off 12-14 kDa) which were added to a solution of 1d (1.00 g, 4.55 mmol) in the same buffer. The pH was adjusted to 7.8 with 0.4N NaOH and kept constant throughout the reaction with a Radiometer automatic titrator. The extent of the reaction was estimated by the volume of base consumed during the reaction performed at 23°C. The reaction was complete after overnight contact. The solution was extracted with ether to remove the remaining starting material (40 mg) after which the reaction mixture was

acifified to 2 with 2.5N HCl. This solution was extracted with EtOAc and processed as usual. The products remaining in the dialysis bags were isolated by repeating the above steps after dialyzing the contents with a fresh solution of the buffer. The R monoacid 2d was thus isolated in a yield of 91%. The enantioselectivity of the reaction was determined on the derivative 3d obtained by the coupling of (R)-α-methylbenzylamine with a carbodiimide (EDC).

A reactor such as the one just described is repeatedly utilized for a scale-up of the product and compensates for the high enzyme-substrate ratio used. Without a decrease in the enantioselectivity the time of the reaction could be decreased 3-4 fold when the reactions are performed at 36°C.

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