Yeast-Mediated Resolution of β-Keto Esters of Prochiral Alcohols

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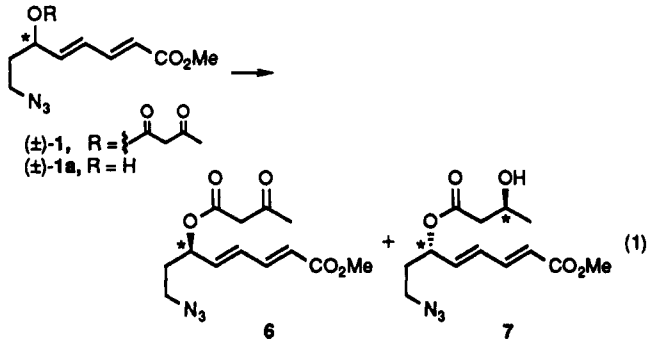
Several racemic alcohols were converted to their β-keto esters with diketene, and the resulting compounds were subjected to kinetic resolution by means of bakers' yeast. The unreacted keto esters were separated from the reduced hydroxy esters by chromatography, and the products were analyzed for levels of enantiomeric excess. Chiral shift reagents, Mosher esters, and optical rotation of the enantiomers of the alcohols were the criteria used to determine the optical integrity of the resolved alcohols after hydrolysis of the esters. Absolute stereochemistry was determined for the resolution products of all the substrates. Some rationale is advanced to account for the observed levels of enantiomeric excess and for the apparent diastereospecificity of the enzymatic resolution. The utility of this process as means of resolution of prochiral alcohols as well as an application of such resolution to the preparation of both enantiomers of a pyrrolizidine alkaloid synthon are indicated.

Introduction

During our approach to the enantiodivergent synthesis of pyrrolizidine diols, we required both enantiomers of azido diene 1. The availability of enantiomerically pure azides 1 would permit the preparation of any existing pyrrolizidinediol of the dihydroxyheliotridane series, as the stereochemistry of the oxygenated center controls the relative stereochemistry of the remaining stereocenters in the racemic series.[2] It occurred to us that the yeast-mediated reduction of β-keto esters could be employed for such a purpose provided that the enzymes recognized a secondary site for resolution elsewhere in the molecule, in particular the alkoxide center of the ester, as shown in eq 1. This kinetic resolution was proposed on the basis of



the belief that one enantiomer of a keto ester would be reduced faster than the other, thus leading to a separable mixture of keto ester 6 and hydroxy ester 7. A convenient method of resolution of secondary or tertiary alcohols based on this rationale could then be developed.

Of the asymmetric reductions via microbial methods currently known, the bakers' yeast (*Saccharomyces cerevisiae*) mediated reductions of β-keto esters[3-17] to the corresponding β-hydroxy esters have gained popularity for several reasons: availability and low cost of the reagents, operational simplicity, reasonable enantioselectivity of the process, and the use of the resulting chiral metabolites as building blocks in the synthesis of antibiotics and natural products.[4,7] Whereas some studies have shown the effect of the substrate concentration[12,18] and the size of the ester group[4,8] on the enantioselectivity of the formation of the hydroxy ester, others have reported adjustments in the reaction conditions[17] for better selectivity.

Although many examples exist where some resolution occurred at other prochiral centers on the keto ester portion, the enantiomeric excess at these secondary sites was usually only marginal. Its magnitude diminished as a function of the distance of the chiral center from the site of enzymatic reduction at the ketone carbonyl of the β-keto ester. To our knowledge, no examples are known where such a resolution took place at a chiral center contained within the alkoxide portion of the ester. The reason this data seems absent is probably because all of the resolutions reported in the literature used methyl, ethyl, or benzyl esters—none utilized esters of secondary alcohols. We reasoned that, as the distance of the ester–alkoxide center

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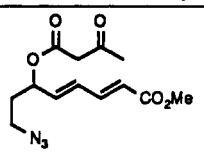
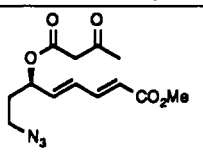
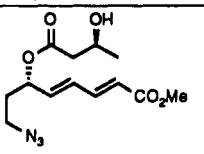
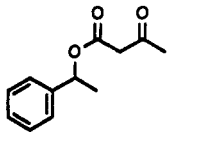
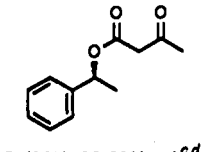
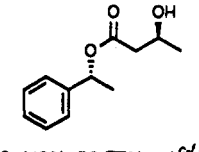
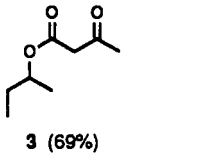
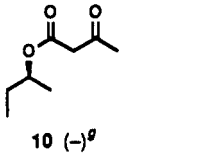
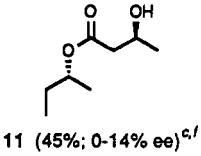
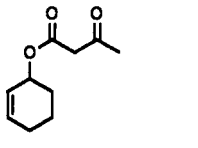
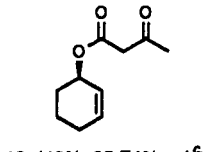
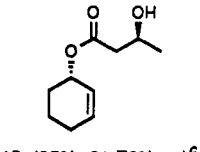
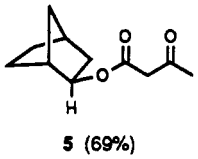
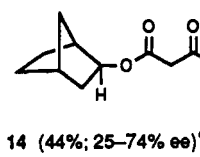
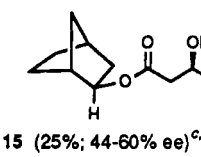
Table I. Yeast Reduction of β-Keto Esters

racemic keto ester[a] (yield)	keto ester[b] (yield)	hydroxy ester[b] (yield)
 1 (83%)	 6 (19%, 70% ee)[c]	 7 (49%; 70% ee)[c]
 2 (85%)	 8 (36%; 38-63% ee)[c,d,f]	 9 (49%; 59-77% ee)[c-f,i]
 3 (69%)	 10 (–)[g]	 11 (45%; 0-14% ee)[c,f]
 4 (81%)	 12 (42%; 25-74% ee)[c,f,h]	 13 (35%; 61-76% ee)[c,f]
 5 (69%)	 14 (44%; 25–74% ee)[c,f,h]	 15 (25%; 44-60% ee)[c,e,f,h,i]

[a] Isolated yield. [b] Isolated yield of diastereomer. [c] By NMR of (*R*)-Mosher ester of free alcohol obtained from hydrolysis of ester. [d] By NMR of Eu(hfc)$_3$. [e] By comparison of $[\alpha]_D$ of authentic 3-hydroxybutanoic acid with that of the same obtained by hydrolysis. [f] Determined by comparison of $[\alpha]_D$ with known standards. [g] Could not be isolated. [h] By NMR of (*S*)-Mosher ester of the free alcohol obtained from hydrolysis. [i] By NMR of Mosher esters derived from hydroxy esters.

from the primary reduction site would always remain the same, regardless of the structural variations of the alcohol, the secondary resolution at this center may therefore be more successful than that of additional randomly chosen stereogenic centers on the keto ester moiety.

We report herein several examples of the kinetic resolution of a prochiral center located on the alkoxide portion of the β-keto ester and the assignment of absolute stereochemistry at the resolved secondary chiral site for the alcohols. The utility of this kinetic resolution was exploited in an attempt at the enantiocontrolled total synthesis of pyrrolizidine alkaloids that has been reported elsewhere.[19]

Results and Discussion

The requisite β-keto esters were prepared in excellent yields from the corresponding alcohols by their reaction with diketene. Microbial reduction of each of substrates **1–5**, shown in Table I, was carried out with dry bakers' yeast under "fermenting conditions"[9] for 16 h to give the unreacted β-keto ester and the β-hydroxy ester (eq 1). These compounds were isolated from the reaction mixtures by extractions with ethyl acetate and were easily separated by silica gel chromatography. The yields were enhanced by an improved extraction technique (see Experimental Section). Spectral evidence suggested the presence of a single diastereomer for each of the β-hydroxy esters. The hydrolysis of the hydroxy esters furnished (3*S*)-3-hydroxybutanoic acid, identical with an authentic sample.[20] These observations suggested that resolution was occurring at the secondary alkoxide site and that the process was diastereoselective. It remained to determine the level of induction at the secondary chiral center.

Determination of the Extent of Resolution. Mosher Ester Method. Following the chromatographic separation of the unreacted β-keto ester and the reduced β-hydroxy ester, each compound was hydrolyzed and the free alcohols converted to the Mosher esters of (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid. The Mosher esters derived from 1-phenylethanols and cyclohex-2-enols showed the highest enantiomeric excess (75 and 76% ee, respectively), followed by azido dienols **6** and **7** (70%) (Table I). Curiously, the secondary alkoxide centers of the hydroxy esters showed, in most cases, a higher degree of resolution than those of their enantiomers, obtained from the hydrolysis of the unreacted keto esters. Both (*R*)-(+)- and (*S*)-(–)-Mosher esters were also prepared from hydroxy esters **9** and **15**. The results of their ^{1}H NMR analysis were compared to those of the Mosher esters of the free alcohols and were found in excellent agreement (Table I).

Optical Rotation Method. The optical rotation of the hydroxybutanoate fragments isolated following hydrolysis of hydroxy esters **7**, **9**, **11**, **13**, and **15** was compared to that of an authentic sample (Aldrich).[20] The stereochemistry of the hydroxybutanoate fragments was found to be *S* in all cases. This result is in agreement with the known

(19) Hudlicky, T.; Seoane, G.; Price, J. D.; Gadamasetti, K. G. (in part) *Synlett* **1990**, 433.

(20) Purchased from the Aldrich Chemical Co.

tendency of this particular strain of yeast to furnish *S* configuration at the hydroxy center.[4,6,9,17] In each case, this primary resolution site showed levels of enantiomeric excess at 70–74%, a result consistent with expectations for bakers' yeast.

The keto esters and hydroxy esters were hydrolyzed and the optical rotation of the alcohols determined. In the cases of known compounds, a direct measure of the enantiomeric excess was available by comparison of $[\alpha]_D$ of the alcohols with that of available standards for the assignment of absolute stereochemistry. The magnitudes of enantiomeric excess of the free alcohols were in the range of 31–75% (see Experimental Section).

Shift Reagent Study. A comparative determination of the percent enantiomeric excess for compounds **8** and **9**, derived from the resolution of **2**, was obtained from chiral shift studies with $Eu(hfc)_3$, and the results were found to be in close agreement to those obtained from NMR studies of the Mosher esters. The values of enantiomeric excess for hydroxy ester **9**, as determined by four different methods, were found in excellent agreement (within 1%).

Absolute Stereochemistry. Comparison of the ^1H NMR of the (*R*)-(+)-Mosher esters prepared from the free alcohols derived from hydrolysis of **8** and **9** with the (*R*)-(+)-Mosher esters prepared from the commercially available (*R*)-(+)-*sec*-phenethyl alcohol[18] showed that the kinetically resolved (i.e., reduced) β-hydroxy ester **9** possessed the *R* configuration at the alkoxide center; therefore, the unreacted β-keto ester **8** from the microbial reduction would bear *S* absolute stereochemistry. The optical rotations of 1-phenylethanol, 2-butanol, and cyclohex-1-enol obtained from hydrolyses of **9**, **11**, and **13**, respectively, were compared to those of standards (see Experimental Section and refs 27 and 28). The determination of absolute stereochemistry for the alkoxide center in **7** was made by its conversion to dihydroxyheliotridane,[19] although the conversion of **7** to the natural product was accompanied by extensive racemization.[21]

Noyori Asymmetric Hydrogenation. A comparison of enzymatic and chemical resolution for two compounds (**2** and **5**) was made by hydrogenation of these keto esters under Noyori's conditions.[22] In both cases, the hydroxybutanoate fragment was found to have 94% enantiomeric excess. Although this method was found to be superior to the yeast reduction at the primary site of resolution in the two cases studied, it proved of little practical value as the two diastereomers could not be separated by either column, flash, thin-layer, or high-pressure chromatography. Unlike the mixtures from the yeast reductions where the two products differ greatly in adsorptive properties, the diastereomeric hydroxy esters from Noyori's hydrogenation are too similar to be separated easily. The two mechanisms also differ: it appears that the enzymes recognize and adjust to the environment of the chiral center at the alkoxide site and perform a kinetic resolution, whereas the Noyori catalyst recognizes only the *re* or the *si* face of the keto ester via an enantiospecific process. Thus, even if substrates are found whose diastereomeric hydroxy esters can be separated, the Noyori process cannot be used for those compounds that contain unsaturation or functionalities that would be reduced during the high-pressure hydrogenation. The yeast-mediated resolution provides a good alternative, although much work must be invested in the proper choice of organism to improve the optical yields.[23]

Diastereoselectivity of the Reduction Process. The assignment of absolute stereochemistry for **8** and **9** (as well as the other alcohols) allows some conclusions to be drawn about the diastereoselectivity of the enzymatic process. Additional diastereomers were not detected in **9**, yet the optical purity of the alcohol obtained by hydrolysis was only ~70%. We therefore investigated the yeast reduction of both antipodes of keto ester **2** prepared from commercially available enantiomers of 1-phenylethanol in detail. The *R* enantiomer was completely reduced in 12 h to its (*S*)-hydroxybutenoate. Both (*S*)-Mosher ester NMR and comparison of $[\alpha]_D$ of methyl 3(*S*)-*tert*-butyldimethylsiloxy)butenoate derived from the hydrolysis showed ~70% ee at the hydroxybutenoate site. The *S* enantiomer of **2** was reduced in 24 h and by the same criteria was also found to contain the *S* configuration at the hydroxybutenoate fragment.

This means that the lack of diastereomers in the reductions of *racemic* **2** indicated a much slower rate of reduction and a lag time for the *S* enantiomer of **2** in reactions under 8–12 h of reduction time. Prolonged reaction time would therefore lead to an identical 1:1 mixture of diastereomers of hydroxy esters as obtained, for example, from the Noyori hydrogenation. The precise rates of reduction for both enantiomers of several compounds will be determined in a separate study.

Conclusion

From the initial investigations reported in this manuscript, it appears that the enantiomeric excess in products resulting from the resolving capacity of the bakers' yeast reduction seems to be somewhat dependent on the bulk of the ester substituent. Phenyl and cyclohexyl groups have afforded better resolution than the [2.2.1] bicyclo system or the *sec*-butyl group. Strains of yeast are known that produce *R* or *S* configurations selectively and in high enantiomeric excess; this process may therefore be used to achieve improved resolutions of prochiral alcohols.[24] Recently, a procedure that utilizes immobilized cells for the reduction of keto esters with high enantiomeric excess was reported.[25] The application of this secondary kinetic resolution to the preparation of chiral alcohols is self-evident, and the degree of resolution appears to depend on the choice of organism and/or the steric influence of the ester group. Studies designed to provide some insight into the enzymatic processes that are responsible for this unique resolution are in order.

(21) Interesting results were obtained in the resolution of β-keto ester **1**. The optical rotation of the hydroxy ester was consistent from one experiment to the next, but the rotation of the unreacted enantiomer of **1** varied widely. The reason for this anomaly was due to the epimerization of the chiral center that is conjugated with the unsaturated ester functionality. In one experiment, the keto ester **6** was allowed to stir in MeOH with complete racemization observed after 100 h. On the other hand, the optical rotation of **7** remained constant under the same conditions. Subjecting the racemic keto ester **1** to longer reaction times with additions of fresh yeast resulted in an increased percentage of **7** by continuous resolution–racemization–resolution cycle of the unresolved enantiomers of **1**. This latter observation indeed would show promise in acquiring either enantiomer of those racemates that contain an acidic proton adjacent to the secondary chiral site.

(22) Noyori, R.; Okhuma, T.; Kitamura, M.; Takaya, H.; Sayo, N.; Kumobayashi, H.; Akutagawa, S. *J. Am. Chem. Soc.* **1987**, *109*, 5856.

(23) Confirmation of these observations involved the preparation of (*S*)-Mosher esters of **9**, which originated from three different sources: (a) the yeast reduction of racemic **2**, (b) Noyori hydrogenation of racemic **2**, and (c) the yeast reduction of optically pure **2** prepared from the commercially available (*R*)-*sec*-phenethyl alcohol. The expanded methoxy regions of the ^1H NMR spectra (see supplemental material) clearly indicate approximately 75% ee in the first case, a 1:1 mixture of diastereomers in the second, and no change in ee in the third at the secondary chiral centers.

(24) Sugai, T.; Fugita, M.; Mori, K. *Nippon Kagaku Kaishi* **1983**, 1315.

(25) Naoshima, Y.; Akakabe, Y. *Y. Org. Chem.* **1989**, *54*, 4237.

Experimental Section[26]

General Procedure for the Preparation of β-Keto Esters. (This example provides weights for **1a**; other compounds were prepared on a scale of approximately 1 mmol unless otherwise noted.) To a solution of the alcohol (250 mg, 1.18 mmol), Et_3N (0.2 mL, 1.42 mmol), and DMAP (20 mg) in CH_2Cl_2 (5 mL) at 0 °C was added diketene (0.11 mL, 1.42 mmol) over 5 min. After being stirred for 30 min, the reaction mixture was worked up with aqueous KOH (0.1 M, 10 mL) and H_2O (5 mL). The organic layer was separated, and the aqueous phase was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic phase was dried and concentrated, and the crude reaction product was purified by flash column chromatography (5% deactivated SiO_2, 1 in. × 3.5 in., ether–hexane (1:9)), yielding analytically pure β-keto ester.

Methyl 8-azido-6-acetoacetoxy-2,4-octadienoate (1): 83%; R_f = 0.32 (ether–hexane (3:2)); IR (neat) 2940, 2080, 1705, 1640, 1615, 1430, 1305, 1265, 1140, 1000, 800, 725 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.93 (m, 2 H), 2.24 (s, 3 H), 3.36 (t, 2 H), 3.48 (s, 2 H), 3.72 (s, 3 H), 5.49 (q, 1 H), 5.92 (d, J = 15.7 Hz, 1 H), 5.95 (dd, J = 15.2, 6.8 Hz, 1 H), 6.38 (dd, J = 15.3, 10.5 Hz, 1 H), 7.2 (dd, J = 15.4, 10.5 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 29.9 (CH_3), 33.5 (CH_2), 47.4 (CH_2), 50.0 (CH_3), 51.4 (CH_2), 72.1 (CH), 123.1 (CH), 130.6 (CH), 137.5 (CH), 142.7 (CH), 166.0 (C, double intensity), 199.4 (C); MS m/z (relative intensity) 296 (0.5), 295 (0.5), 279 (1.0), 267 (2), 252 (1), 236 (4), 224 (2), 183 (15), 166 (75), 106 (100), 85 (53), 79 (40); high-resolution EIMS calcd for $C_{13}H_{17}N_3O_5$ (protonated) 296.2944, found 296.1372.

1-Phenylethyl acetoacetate (2): 24-mmol scale, 84.4%; R_f = 0.34 (ether–hexane (1:2)); IR (neat) 2460, 1710, 1640, 1440, 1400, 1350, 1140, 1050, 750, 690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.33 (m, 5 H), 5.92 (q, 1 H), 3.44 (s, 2 H), 2.2 (s, 3 H), 1.55 (d, 3 H); ^{13}C NMR ($CDCl_3$) δ 21.8, 29.9, 50.2, 73.4, 126.1, 128.0, 128.5, 140.9, 166.2, 200.1; MS m/z (relative intensity) 206 (21), 193 (3), 178 (46), 162 (3), 121 (72), 105 (100), 104 (60), 103 (21), 85 (14), 77 (30); high-resolution EIMS calcd for $C_{12}H_{14}O_3$ 206.2408, found 206.1154. Anal. Calcd for $C_{12}H_{14}O_3$: C, 69.89; H, 6.84; O, 23.27. Found: C, 69.97; H, 6.72, O, 23.09.

2-Butyl acetoacetate (3): 40-mmol scale, 68.8%; R_f = 0.4 (ether–hexane (1:2)); IR (neat) 2965, 2930, 2870, 1730, 1710, 1640, 1410, 1360, 1310, 1240, 1150, 1110, 1025, 990 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.9 (q, 1 H), 3.4 (s, 2 H), 2.2 (s, 3 H), 1.55 (m, 2 H), 1.21 (d, 3 H), 0.88 (t, 3 H); ^{13}C NMR ($CDCl_3$) δ 9.4, 19.2, 28.6, 29.8, 50.3, 73.4, 166.6, 200.3. Anal. Calcd for $C_8H_{14}O_3$: C, 60.74; H, 8.92. Found: C, 60.09; H, 9.01.

Cyclohex-2-enyl acetoacetate (4): 20 mmol, 81.1%; R_f = 0.37 (ether–hexane (1:2)); IR (neat) 2400, 1700, 1610, 1400, 1130, 990, 900, 710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.96 (dt, J = 10 Hz, 1 H), 5.7 (d, J = 10.5 Hz, 1 H), 5.31 (broad m, 1 H), 3.42 (s, 2 H), 2.25 (s, 3 H), 2.01 (m, 2 H), 1.81 (m, 1 H), 1.6–1.8 (m, 3 H); ^{13}C NMR ($CDCl_3$) δ 18.7, 24.8, 28.2, 30.0, 50.4, 69.3, 125.1, 133.2, 166.8, 200.4. Anal. Calcd for $C_{10}H_{14}O_3$: C, 65.92; H, 7.74. Found: C, 66.14; H, 8.00.

Bicyclo[2.2.1]heptanyl acetoacetate (5): 26 mmol, 68.8%; R_f = 0.38 (ether–hexane (1:2)); IR (neat) 2940, 2850, 1730, 1710, 1635, 1615, 1400, 1350, 1310, 1230, 1140, 1055 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.64 (d, 1 H), 3.4 (s, 2 H), 2.28 (dd, J = 5, 12 Hz), 2.24 (s, 3 H), 1.72 (m, 1 H), 1.56–1.39 (m, 4 H), 1.18–1.07 (m, 3 H); ^{13}C NMR ($CDCl_3$) δ 24.0, 27.9, 29.8, 35.1, 35.7, 39.2, 41.2, 50.1, 78.4, 166.5, 200.2. Anal. Calcd for $C_{11}H_{16}O_3$: C, 67.32; H, 8.22. Found: C, 67.76; H, 8.44.

General Procedure for the Bakers' Yeast Reduction of the β-Keto Esters (e.g., Cyclohex-2-enyl Acetoacetate (4)). To a solution of D-glucose (12.6 g) and yeast extract (0.42 g) in distilled water (84 mL) at 37–38 °C with stirring was added dry bakers' yeast (8.4 g, Fleischmann's Yeast, Inc., Oakland, CA 94603). The mixture was stirred open to air for 30 min, maintaining the temperature at 33–34 °C. The β-keto ester (1 g, neat or dissolved in DMSO) was added, and the mixture was stirred open to air for 0.5 h at 24–25 °C. It was stirred at room temperature for an additional 16 h with air bubbling through the solution. Brine (100 mL) was added, and the mixture was extracted with EtOAc (3 × 500 mL). The combined organic phase was dried and concentrated to give 810 mg of crude oil. Purification by flash chromatography (SiO_2, 2.5 in. × 3.5 in., ether–hexane (1:4)) gave the unreacted β-keto ester **12** (171 mg, 19%, $[\alpha]^{25}_D$ = 9.34) and the hydroxy ester **13** (302 mg, 33%, $[\alpha]^{25}_D$ = −0.078). Modification of the extraction technique (adding excess NaCl to the mixture, extracting with EtOAc, centrifugation, and further extraction with EtOAc) improved the yields of the β-keto ester **12** (42%) and the β-hydroxy ester **13** (35%).

Methyl 8-azido-6-((3-hydroxy-1-oxobutanyl)oxy)-2,4-octadienoate (7): $[\alpha]^{25}_D$ = +5.35; R_f = 0.19 (SiO_2, ether–hexane (3:2)); ^1H NMR ($CDCl_3$) δ 1.19 (d, J = 6.3 Hz, 3 H), 1.89 (m, 2 H), 2.42 (m, 2 H), 2.92 (b s, 1 H), 3.31 (t, 2 H), 3.69 (s, 3 H), 4.15 (m, 1 H), 5.44 (q, 1 H), 5.88 (d, J = 15.5 Hz, 1 H), 5.94 (dd, J = 15.8, 7.9 Hz, 1 H), 6.33 (dd, J = 15.3, 10.9 Hz, 1 H), 7.18 (dd, J = 15.3, 11 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 22.6 (CH_3), 33.4 (CH_2), 43.2 (CH_2), 47.4 (CH_2), 51.6 (CH_3), 64.4 (CH), 71.3 (CH), 122.9 (CH), 130.4 (CH), 138.0 (CH), 142.8 (CH), 166.9 (C), 171.6 (C); IR (neat) 3440, 2940, 2070, 1710, 1645, 1610, 1430, 990, 715 cm^{-1}; CIMS m/z (relative intensity) 298 (5), 270 (18), 194 (13), 166 (100), 151 (7), 134 (19), 106 (33); high-resolution EIMS calcd for $C_{13}H_{19}N_3O_5$ (protonated) 298.3181, found 298.1502.

1-Phenylethyl 3(*S*)-hydroxybutanoate (9): $[\alpha]^{25}_D$ = +60.38; ^1H NMR ($CDCl_3$) δ 7.32 (m, 5 H), 5.9 (q, 1 H), 4.17 (m, 1 H), 2.46 (m, 2 H), 1.55 (d, 3 H), 1.2 (d, 3 H); ^{13}C NMR ($CDCl_3$) δ 21.9, 22.2, 43.0, 64.0, 72.4, 125.8, 127.7, 128.2, 141.1, 171.6; IR (neat) 3420, 3010, 2960, 2910, 1715, 1440, 1365, 1280, 1165, 1050, 750, 690 cm^{-1}; MS m/z (relative intensity) 208 (4), 122 (61), 121 (25), 107 (20), 105 (100), 104 (71), 77 (28); high-resolution MS calcd for $C_{12}H_{16}O_3$ 208.2566, found 208.1311. Anal. Calcd for $C_{12}H_{16}O_3$: C, 69.21; H, 7.75. Found: C, 69.18; H, 7.78.

2-Butyl 3(*S*)-hydroxybutanoate (11): $[\alpha]^{25}_D$ = +13.7; ^1H NMR ($CDCl_3$) δ 4.86 (q, 1 H), 4.16 (m, 1 H), 3.06 (d, 1 H), 2.55–2.31 (m, 2 H), 1.68–1.45 (m, 2 H), 1.19 (d, 4 H), 0.87 (t, 3 H); ^{13}C NMR ($CDCl_3$) δ 9.0, 18.9, 22.2, 28.3, 43.1, 63.8, 71.9, 171.7. Anal. Calcd for $C_8H_{16}O_3$: C, 59.97; H, 10.07. Found: C, 59.82; H, 10.08.

2-Cyclohexenyl 3(*S*)-hydroxybutanoate (13): $[\alpha]^{25}_D$ = −0.078; ^1H NMR ($CDCl_3$) δ 5.94 (dt, J = 10 Hz, 1 H), 5.67 (d, J = 10.1 Hz, 1 H), 5.28 (b s, 1 H), 4.16 (m, 1 H), 3.04 (b s, 1 H), 2.53–2.3 (m, 2 H), 2.03 (m, 2 H), 1.8 (m, 1 H), 1.77–1.5 (m, 3 H), 1.19 (d, 3 H); ^{13}C NMR ($CDCl_3$) δ 18.7, 22.4, 24.7, 28.2, 43.2, 64.3, 68.4, 125.3, 132.9, 172.3; IR (neat) 3400, 3000, 2910, 1700, 1365, 1275, 1155, 1050, 995, 900, 710 cm^{-1}. Anal. Calcd for $C_{10}H_{16}O_3$: C, 65.19; H, 8.75. Found: C, 65.03; H, 8.77.

Bicyclo[2.2.1]heptanyl 3(*S*)-hydroxybutanoate (15): $[\alpha]^{25}_D$ = +8.38; ^1H NMR ($CDCl_3$) δ 4.62 (b d, J = 7.1 Hz, 1 H), 4.15 (m, 1 H), 3.03 (d, J = 3.7 Hz, 1 H), 2.4 (d, J = 4 Hz, 1 H), 2.27 (m, 2 H), 1.75 (dd, J = 2.4, 7 Hz, 1 H), 1.68 (dd, J = 2.4, 7 Hz, 1 H), 1.55–1.3 (m, 5 H), 1.19 (d, J = 6.3 Hz, 3 H), 1.1 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 22.4, 24.2, 28.0, 35.2, 39.6, 41.4, 43.1, 64.3, 77.9, 172.4; IR (neat) 3380, 2930, 2840, 1700, 1620, 1360, 1300, 1160, 1050, 980, 930, 830, 755 cm^{-1}. Anal. Calcd for $C_{11}H_{18}O_3$: C, 66.64; H, 9.15. Found: C, 66.44; H, 9.19.

General Procedure for the Hydrolysis of β-Keto Esters or β-Hydroxy Esters. To the β-keto ester of β-hydroxy ester in MeOH was added KOMe (2.1 equiv) in one portion, and the solution was slowly warmed to 65 °C and was heated at reflux for 1.5–2 h. The reaction was acidified under dry conditions (addition of the pretitrated solution of dry HCl gas in dry MeOH). The solvent was removed under reduced pressure. Methylene chloride was added, and the CH_2Cl_2 solubles (alcohol) were either directly used in the next reaction or purified by a fast filtration

(26) All nonhydrolytic reactions were carried out under a nitrogen or argon atmosphere with standard techniques for the exclusion of air and moisture. Glassware used for moisture-sensitive reactions was flame-dried with an inert gas sweep. THF, ether, and DME were distilled from benzophenone ketyl, dichloromethane, and hexane from calcium hydride. Analytical TLC was performed on silica gel 60F-254 plates. Flash chromatography was performed on Keiselgel 60 (230–400 mesh) with EM Science solvents. Mass spectra were recorded on a DuPont 20-491 or a Varian MAT-112 instrument (low resolution) or on a double-focusing DuPont 21-110C or VGT instrument (exact mass). Infrared spectra were recorded on neat samples (NaCl plates) on a Perkin-Elmer 257 spectrometer. Proton NMR spectra were obtained on Bruker WP-270 instrument. Proton chemical shifts were reported in parts per million (δ) downfield from tetramethylsilane (TMS) as an internal reference (δ 0.0). Carbon chemical shifts are reported in parts per million relative to TMS, the spectra were calibrated to TMS or to the center line of the $CDCl_3$ triplet (δ 77.02). Microanalyses were performed by Galbraith Laboratory, Knoxville, TN. Optical rotations were carried out on a Perkin-Elmer 241 polarimeter.

through silica gel. The determination of % ee was performed by comparison of the $[\alpha]_D$ of the isolated alcohol with the $[\alpha]_D$ of a solution made of the known standard: **8**, 38% ee for (*S*)-phenylethanol (standard from Aldrich);[20] **9**, 59% ee for (*R*)-phenylethanol (standard from Aldrich);[20] **11**, 0% ee for (*R*)-2-butanol (standard from Aldrich);[20] **12**, 31% ee for (*R*)-cyclohexenol (literature standard);[27] **13**, 61% ee for (*S*)-cyclohexenol (literature standard);[27] **14**, 74% ee for (1*S*,2*S*,4*R*)-*exo*-2-norbornanol (literature standard);[28] **15**, 60% ee for (1*R*,2*R*,4*S*)-*exo*-2-norbornanol (literature standard).[28]

In those experiments where the isolation of hydroxybutanoic acid was desired, the hydrolysis mixture was extracted with ether prior to acidification and extraction of the carboxylic acid. The determination of % ee was performed by comparison of $[\alpha]_D$ of the isolated hydroxybutenoic acid with the $[\alpha]_D$ of a solution made of the known standard:[20] **9**, 77% ee; **15** (from Noyori hydrogenation), 94% ee.

General Procedure for the Preparation of the Mosher Esters. To the alcohol (obtained from the β-keto ester or the β-hydroxy ester by base hydrolysis) in CH_2Cl_2 were added DCC, DMAP, and the (*R*)- or the (*S*)-α-methoxy-α-(trifluoromethyl)-phenylacetic acid. The reaction mixture was stirred for 12 h at room temperature and worked up with H_2O, 1 N HCl, aqueous $NaHCO_3$. The organic phase was separated, dried, and concentrated. The determination of the enantiomeric excess was determined by ^1H NMR: **8**, 60% ee; **9**, 75% ee; **11**, 14% ee; **12**, 21% ee; **13**, 76% ee; **14**, 25–30% ee; **15**, 44–55% ee.

Procedure for the Determination of the % ee or % de with Eu(hfc)$_3$ as a Chiral Shift Reagent. Each NMR sample was prepared by mixing 100 μL of Eu(hfc)$_3$ (100 mg/mL $CDCl_3$), 10 mg of the β-keto ester or β-hydroxy ester, and 0.4 mL of $CDCl_3$. The % ee or % de was determined by the difference in the integration of those signals in the ^1H NMR spectrum that had been resolved in the NMR experiment done on the racemic sample and optimized for the relative concentration of the shift reagent (**8**, 73% ee; **9**, 74% ee).

Procedure for Hydrogenation (Noyori's Conditions). $RuCL_2$–COD (38 mg, 0.13 mM) and (*S*)-BINAP (100 mg, 0.16 mM) were transferred to a Schlenk vessel in an inert atmosphere (glovebox). A solution of triethylamine in toluene (0.135 mL, 1.87 mM, 5.5 mL, freshly distilled and degassed in three freeze–thaw cycles) was added by means of a cannula, and the mixture was refluxed under N_2 for 8 h. The solvent and triethylamine were removed by high vacuum, leaving behind a solid, red-brown residue. A solution of the requisite keto ester (for **2**, 200 mg, 1.03 mM; for **5**, 200 mg, 1.02 mM) in triply degassed 2-propanol freshly distilled from CaH_2 was added via a cannula to the Schlenk vessel and refluxed until it appeared that all catalyst had dissolved and the solution had acquired a bright orange color. The solution was added directly to a Parr pressure bomb, which was flushed with argon and then pressurized to 100 atm H_2. The reaction was stirred at 25–30 °C for 58 h, then the pressure was released and the solvents removed in vacuo. Kugelrohr distillation afforded a yellowish oil, which appeared homogeneous by TLC and NMR, and corresponded to the diastereomers of **9** (196 mg, 97%) and **15** (198 mg, 98%). This material was analyzed for % ee by spectral methods. Attempts at the separation of diastereomers proved unsuccessful (see Discussion Section).

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Supplementary Material Available: ^1H and ^{13}C NMR spectra for compounds **6**–**15** and ^1H NMR spectra of *S*-Mosher ester of **9** (23 pages). Ordering information is given on any current masthead page.

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Interconversion and Hydrolysis of Monomethyl and Monoisopropyl Esters of Adenosine 2′- and 3′-Monophosphates: Kinetics and Mechanisms

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First-order rate constants for mutual isomerization and hydrolytic cleavage of the monomethyl and monoisopropyl esters of adenosine 2′- and 3′-monophosphates (2′- and 3′-AMP) have been determined by HPLC over a wide pH range. Both reactions proceed at comparable rates under acidic conditions, exhibiting a second-order dependence of rate on hydronium ion concentration at $1 < pH < 2$, and a first-order dependence in more acidic solutions. Moreover, hydrolytic depurination takes place at $pH < 3$. In the pH range 4 to 9 a pH-independent phosphate migration prevails. By contrast, in alkaline solutions the methyl esters are hydrolyzed to a mixture of 2′- and 3′-AMP, the reaction rate being proportional to the hydroxide ion concentration at $[OH^-] < 0.1$ mol dm^{-3}. No sign of mutual isomerization was detected under these conditions. With the isopropyl esters alkaline degradation of the adenine moiety is considerably faster than the phosphodiester hydrolysis. Mechanisms of phosphate migration and phosphodiester hydrolysis under various conditions have been discussed.

Introduction

Phosphodiester bonding plays a vital role in biological chemistry by linking nucleoside units to nucleic acids. Numerous kinetic and mechanistic works on hydrolysis of simple mono-, di-, or triesters of phosphoric acid refer, in their introduction, to the importance of detailed understanding of the chemical behavior of this bond.[1] However, surprisingly few attempts have been made to apply the

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