Enzymatic Synthesis of (−)- and (+)-Acetoxyhexamides and (−)- and (+)-Hydroxyhexamides

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The enantioselective hydrolysis of (±)-4-(1-acetoxyethyl)-*N*-(cyclohexylcarbamoyl)-benzenesulfonamides 3 with lipase Amano P from *Pseudomonas* sp. in a water-saturated solvent gave (*R*)-4-(1-hydroxyethyl)-*N*-(cyclohexylcarbamoyl)benzenesulfonamide 2 (39%, >99% ee) and unchanged (*S*)-3 (50%, 62% ee). On the other hand, enantioselective esterification of (±)-2 with lipase Amano P in the presence of vinyl acetate provided (*R*)-3 (41%, >99% ee) and unchanged (*S*)-2 (46%, 78% ee).

Key words acetohexamide; acetoxyhexamide; hydroxyhexamide; oral antidiabetic drug; lipase

Acetohexamide, 4-acetyl-*N*-(cyclohexylcarbamoyl)benzenesulfonamide, **1**, is widely used as an oral antidiabetic drug possessing a moderate duration of action.[1)] When this drug is administered in human and rabbits, acetohexamide undergoes conversion to an active metabolite (−)-4-(1-hydroxyethyl)-*N*-(cyclohexylcarbamoyl)benzenesulfonamide **2**, in the body. (−)-Hydroxyhexamide **2** is reported to be more pharmacologically active and has a longer elimination half-life than the parent drug.[2)] The stereoselective reduction of **1** using the enzyme[3a,b)] present in the cytosol of rabbit liver was also reported to give enantiomerically pure (−)-**2**.[3a)] In a preceeding paper,[4)] the absolute configuration of a metabolite (−)- hydroxyhexamide **2** from acetohexamide **1** was found to be *S* based on unequivocal chemical methods including X-ray analysis and the syntheses of (*R*-(+)- and (*S*)-(−)- hydroxyhexamides **2**, and (*R*)-(+)- and (*S*)-(−)-acetoxyhexamides, 4-(1-acetoxyethyl)-*N*-(cyclohexylcarbamoyl)benzenesulfonamides **3**. In a preliminary pharmacological study, both hydroxyhexamides, (*R*)-**2** and (*S*)-**2**, showed a potent hypoglycemic effect after oral administration in mice and rats. The efficacies of these two compounds were almost equivalent. The hypoglycemic effect of these two compounds was dose-dependent, onset early and was of significantly short duration. Acetates (*R*)-**3** and (*S*)-**3** were about 200 times more potent than acetohexamide in insulin secretion activities in cultured pancreatic β-cell. The two acetates (*R*)-**3** and (*S*)-**3** seemed to be absorbed quickly after oral administration in several species of experimental animals, and showed potent but short lasting hypoglycemic effects.[4)] We now describe the alternative syntheses of (*R*)-(+)- and (*S*)-(−)-hydroxyhexamides **2**, and (*R*)-(+)- and (*S*)-(−)-acetoxyhexamides **3** based on an enzymatic method in order to find a more pharmacologically active drug.

At first, in order to determine the enantiomeric excess (ee) of the enzymatic reaction products, authentic samples corresponding to the (*R*)-α-methoxy-α-trifluoromethylphenylacetates, (*R*)-**2**-(*R*)-MTPA and (*S*)-**2**-(*R*)-MTPA were synthesized from the known compounds (*R*)-(+)-4-(1-acetoxyethyl)benzenesulfonamide **5**[4)] and (*S*)-(−)-4-(1-hydroxyethyl)benzenesulfonamide **4**.[4)] Enantioselective acetylation of (±)-4-(1-hydroxyethyl)benzenesulfonamide **4** with Acylase I (No. A 2156) from *Aspergillus melleus* in the presence of vinyl acetate gave (*R*)-4-(1-acetoxyethyl)benzenesulfonamide **5** (98% ee) and (*S*)-**4** (98% ee).[4)] Alkaline hydrolysis of the acetoxy compound (*R*)-(+)-**5** with MeONa in MeOH gave the hydroxy compound (*R*)-(+)-**4** (95% $[\alpha]_D^{23}$ +31.9 (c=0.320, MeOH) corresponds to >98% ee), which was treated with (*S*)-(+)-α-methoxy-α-trifluoromethylphenylacetyl chloride ((*S*)-(+)-MTPACl)[5)] in pyridine to afford (*R*)-**4**-(*R*)- MTPA ester (76% yield). The unchanged (*S*)-(−)-**4** ($[\alpha]_D^{25}$ −30.6 (c=0.301, MeOH): corresponds to 98% ee) was also converted into the corresponding (*S*)-**4**-(*R*)-MTPA ester (89% yield). Thus obtained (*R*)-**4**-(*R*)-MTPA and (*S*)-**4**-(*R*)-MTPA were individually treated with cyclohexylisocyanate in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to provide (*R*)-**2**-(*R*)-MTPA (55%) and (*S*)-**2**-(*R*)-MTPA (54%), respectively. Chemical shifts due to the methoxyl group of both (*R*)-**2**-(*R*)-MTPA and (*S*)-**2**-(*R*)-MTPA are assigned as δ 3.47 and 3.57, respectively. Therefore, it is possible to determine an enantiomeric excess (ee) of the enzymatic reaction products by derivation into the corresponding (*R*)-MTPA ester.

Next, the enantioselective hydrolysis of (±)-**3** and enantioselective esterification of (±)-**2** were carried out. From a screening experiment for the enantioselective hydrolysis of (±)-**3** using various kinds of lipase, the commercially available enzyme, lipase Amano P, from *Pseudomonas* sp. was found to be effective. When (±)-**3** was exposed to the lipase Amano P in a water-saturated mixed solvent (isopropyl ether-benzene (4 : 1)) at 33 °C, hydrolyzed product (+)-**2** (39%, $[\alpha]_D^{27}$ +21.64 (c=0.23, $CHCl_3$) corresponding to >99% ee) and unchanged (−)-**3** (50%, $[\alpha]_D^{26}$ −45.75 (c=0.33, MeOH) corresponding to 62% ee) were obtained (Table 1, entry 1). In the case of the enantioselective hydrolysis of (±)-**3** using the lipase Amano P, the reaction proceeded extremely slowly and required 10 d to complete (entry 1). On the other hand, the lipase Amano P was used for enantioselective esterification of (±)-**2** in the presence of vinyl acetate, an acetate (+)-**3** (41%, $[\alpha]_D^{27}$ +60.70 (c=0.36, MeOH) >99% ee) and unchanged (−)-**2** (46%, $[\alpha]_D^{27}$ −15.15 (c=0.29, $CHCl_3$), 78% ee). The absolute structure of enzymatic reaction products was determined by comparison with the sign of $[\alpha]_D$ of au-

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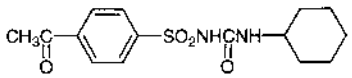
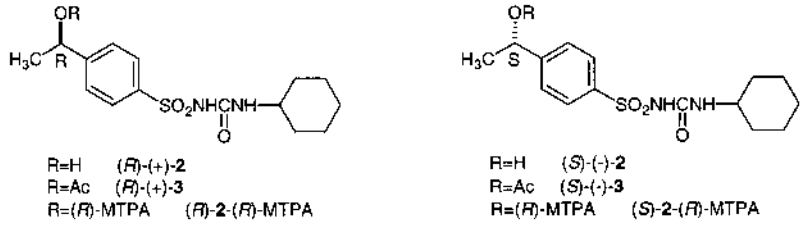


Acetohexamide **1**



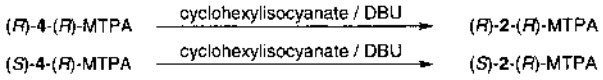
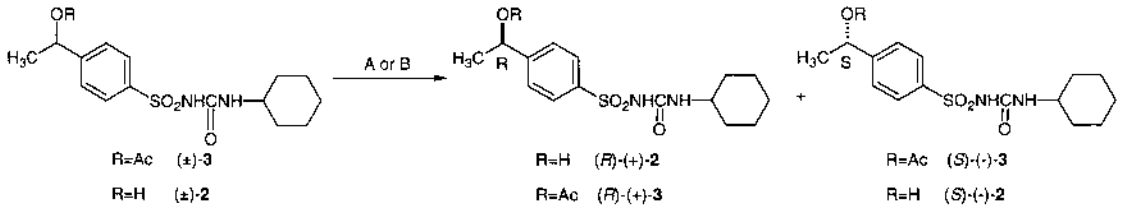


Chart 1

Table 1



Entry	Substrate (g)	Conditions	Time	Products (%, % ee)	
1	(±)-**3** (1.00)	A	10 d	(*R*)-(+)-**2** (39, >99)	(*S*)-(−)-**3** (50, 52)
2	(±)-**2** (1.00)	B	21 h	(*R*)-(+)-**3** (41, >99)	(*S*)-(−)-**2** (46, 78)

A, lipase Amano P in water-satureted mixed solvent (PhH: *iso*-Pr_2O (1 : 4)) at 40 °C; B, lipase Amono P in the presence of vinyl acetate at 40 °C.

thentic samples.

The result of the enantioselective hydrolysis of (±)-**3** and enantioselective esterification of (±)-**2** with the lipase Amano P from *Pseudomonas* sp. was explained by applying the reported empirical rules[6] to predict which enantiomer possessing a secondary hydroxy group reacts faster in lipase-catalyzed reactions by comparing the relative sizes of substituents at the stereocenter. A methyl group seems to be a relatively small sized substituent (M) and the benzenesulfonamide moiety appears to be a relatively large sized substituent (L).

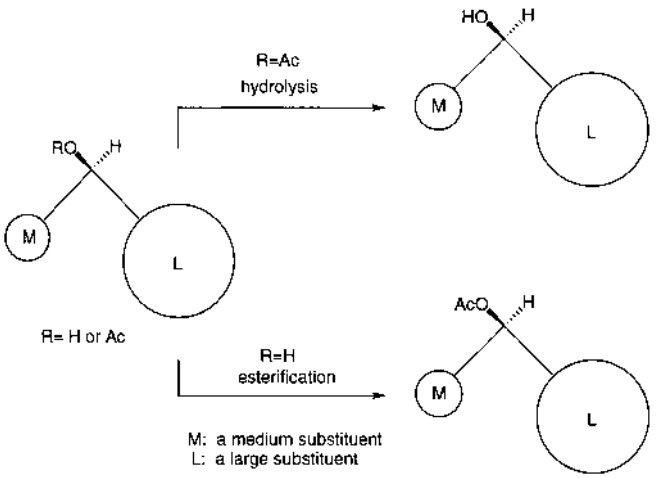


Chart 2

Experimental

All melting points were measured on a Yanaco MP-3S micro melting point apparautus and are uncorrected. ^{1}H-NMR spectra were recorded on a JEOL EX 400 spectrometer. Spectra were taken with 5—10% (w/v) solution in $(CD_3)_2SO$ or $CDCl_3$ with Me_4Si as an internal reference. The mass spectra (FAB-MS) were obtained with a JEOL JMS-AX 500 spectrometer. IR spectra were recorded on a JASCO FT/IR-300 spectrometer. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. All evaporations were performed under reduced pressure. For column chromatography, silica gel (Kieselgel 60) was employed.

(*R*)-2-(*R*)-MTPA Ester A mixture of DBU (27 mg, 0.18 mmol) and (*R*)-**4**-(*R*)-MTPA (46 mg, 0.11 mmol) in benzene (1 ml) was stirred for 30 min at room temperature, then cyclohexylisocyanate (0.04 g, 0.32 mmol) was added, and the whole mixture was stirred for 1 h at room temperature. This was diluted with 6 M aqueous HCl, then extracted with AcOEt. The organic layer was dried over $MgSO_4$ and evaporated to give an oily product, which was chromatographed on silica gel (10 g, *n*-hexane–AcOEt=3 : 1) to afford the (*R*)-**2**-(*R*)-MTPA (34 mg, 55%). ^{1}H-NMR ($CDCl_3$) δ:1.14—1.38 (5H, m), 1.58 (3H, d, J=6.6 Hz), 1.65—1.84 (5H, m), 3.47 (3H, s), 3.57—3.65 (1H, m), 6.15 (1H, q, J=6.6 Hz), 6.46 (1H, d, J=7.8 Hz), 7.35—7.44 (5H, m), 7.49, 7.89 (each 2H, d, J=8.6 Hz), 8.84 (1H, br s). FAB-MS (negative) *m*/*z*: 541 $[M-H]^-$.

(*S*)-2-(*R*)-MTPA Ester A mixture of DBU (32 mg, 0.21 mmol) and (*S*)-**4**-(*R*)-MTPA (36 mg, 0.085 mmol) in benzene (1 ml) was stirred for 30 min at room temperature, then cyclohexylisocyanate (0.04 g, 0.32 mmol) was added and the whole mixture was stirred for 1 h at room temperature. This was diluted with 6 M aqueous HCl and extracted with AcOEt. The organic layer was dried over $MgSO_4$ and evaporated to give an oily product, which was chromatographed on silica gel (10 g, *n*-hexane–AcOEt=3 : 1) to afford (*S*)-**2**-(*R*)-MTPA (25 mg, 54%). ^{1}H-NMR ($CDCl_3$) δ:1.09—1.39 (5H, m), 1.63 (3H, d, J=6.7 Hz), 1.52—1.94 (5H, m), 3.57 (3H, s), 3.60—3.65 (1H, m), 6.13 (1H, q, J=6.7 Hz), 6.44 (1H, d, J=7.8 Hz), 7.33—7.42 (5H, m), 7.37, 7.83 (each 2H, d, J=8.5 Hz), 8.09 (1H, br s). FAB-MS (negative) *m*/*z*: 541 $[M-H]^-$.

Enantioselective Hydrolysis of (±)-3 with Lipase Amano P A suspension of (±)-**3** (1.00 g) and lipase (1.01 g) in water-saturated mixed solvent ((PhH(40 ml)–*iso*-Pr_2O (160 ml)) was incubated at 40 °C for 10 d. After the reaction mixture was filtered, the precipitate was washed with AcOEt. The combined organic layer was dried over $MgSO_4$ and evaporated. The residue was chromatographed on silica gel (50 g) to give acetate (−)-**3** (504 mg, 50%) from *n*-hexane–AcOEt (2 : 1) eluent and alcohol (+)-**2** (351 mg, 39%) from *n*-hexane–AcOEt (1 : 2) eluent, respectively. (−)-**3**: $[\alpha]_D^{26}$ −45.75 (c=0.33, MeOH) corresponds to 62% ee. FAB-MS (negative) *m*/*z*: 367 $[M-H]^-$. The spectral data (IR and NMR) of (−)-**3** were identical with those of the reported (±)-**3**.[4] A part of (+)-**2** was recrystallized from AcOEt-Et_2O to give colorless needles (+)-**2**. (+)-**2**: mp 141—143 °C, $[\alpha]_D^{27}$ +21.64 (c=0.23, $CHCl_3$) corresponds to >99% ee. FAB-MS (negative) *m*/*z*: 325 $[M-H]^-$. Spectral data (IR and NMR) of (+)-**2** were identical with those of the reported (±)-**2**.[4]

Enantioselective Acetylation of (±)-2 with Lipase Amano P A suspension of of (±)-**2** (1.00 g) and lipase (1.01 g) in the presence of vinyl acetate (100 ml) was incubated at 40 °C for 21 h. After the reaction mixture was filtered, the precipitate was washed with AcOEt. The combined organic layer was dried over $MgSO_4$ and evaporated. The residue was chromatographed on silica gel (50 g) to give acetate (+)-**3** (476 mg, 41%) from *n*-hexane–AcOEt (2 : 1) eluent and alcohol (−)-**2** (470 mg, 46%) from *n*-hexane–AcOEt (1 : 2) eluent, respectively. A part of (+)-**3** was recrystallized from AcOEt-Et_2O to give colorless needles (+)-**3**. (+)-**3**: mp 115—116 °C, $[\alpha]_D^{27}$ +60.70 (c=0.36, MeOH) corresponds to >99% ee. FAB-MS (negative) *m*/*z*: 367 $[M-H]^-$. (−)-**2**: $[\alpha]_D^{27}$ −15.15 (c=0.29, $CHCl_3$) corresponds to 78% ee.

General Procedure of (*R*)-MTPA Ester Formation from Enzymatic Product (Acetate) To a solution of acetate (*ca*. 60 mg, 0.16 mmol) in MeOH (1 ml) was added K_2CO_3 (*ca*. 50 mg, 0.36 mmol), and the whole mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with 6 M aqueous HCl and extracted with AcOEt. The organic layer was dried over $MgSO_4$ and evaporated to give a residue (*ca*. 40 mg). To a stirred solution of residue (alcohol: *ca*. 17 mg, 0.05 mmol) in pyridine (1 ml) was added (*S*)-MTPACl (*ca*. 40 mg, 0.16 mmol) and the whole mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with H_2O at 0 °C and extracted with AcOEt. The organic layer was washed with 6 M aqueous HCl and sat. aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$ and evaporated to give a residue, which was chromatographed on silica gel (5 g, *n*-hexane–AcOEt=4 : 1) to afford (*R*)-MTPA ester (*ca*. 18 mg, 63% yield). An enantiomeric excess of the resulting (*R*)-MTPA ester was determined by NMR analysis.

General Procedure of (*R*)-MTPA Ester Formation from Enzymatic Product (Alcohol) To a stirred solution of alcohol (*ca*. 40 mg, 0.12 mmol) in pyridine (1 ml) was added (*S*)-MTPACl (*ca*. 60 mg, 0.24 mmol), and the whole mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with H_2O at 0 °C, and extracted with AcOEt. The organic layer was washed with 6 M aqueous HCl and sat. aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$ and evaporated to give a residue, which was chromatographed on silica gel (5 g, *n*-hexane–AcOEt=4 : 1) to afford (*R*)-MTPA ester (*ca*. 40 mg, 60% yield). An enantiomeric excess of the resulting (*R*)-MTPA ester was determined by NMR analysis.

References and Notes

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