# Chemoenzymatic Synthesis of Chiral Isoxazole Derivatives

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The synthesis of the two enantiomers of 1-(3-bromo-5-isoxazolyl)-2-(*tert*-butylamino)ethanol (1), a potent and selective $\beta_2$-adrenergic stimulant, has been efficiently accomplished by enzyme-catalyzed transformations. The absolute configurations are attributed to (+)- and (−)-1 by correlation with (*S*)-3-butyn-2-ol. The *S* enantiomer was prepared in >98% enantiomeric excess by reducing $\alpha$-bromo ketone 4 in the presence of alcohol dehydrogenase from *Thermoanaerobium brockii* and the *R* enantiomer was obtained in 97% ee through a kinetic resolution of the racemic bromohydrin (±)-5, in organic solvents, catalyzed by lipase P from *Pseudomonas fluorescens*. The experimental conditions for the lipase-catalyzed asymmetric transesterifications were optimized in order to improve reaction rates and the enantiomeric excess of the products.

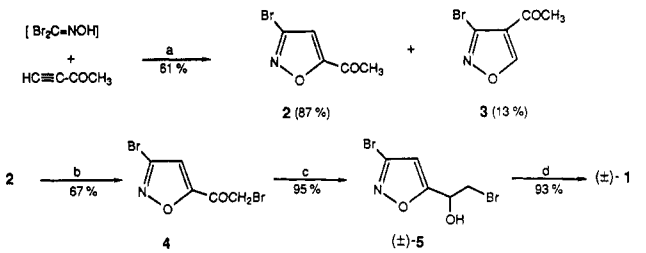
## Introduction

The use of bioconversion processes for the preparation of chiral derivatives has received more and more attention due to the increased interest in asymmetric synthesis.[2] This is particularly true in medicinal chemistry, since the potency and/or the selectivity of a chiral drug are usually dependent upon the configuration of its stereocenters.[3]

In pursuing our program devoted to the application of the 1,3-dipolar cycloaddition strategy to the synthesis of biologically active compounds,[4] we became interested in the asymmetric synthesis of both the enantiomers of 1-(3-bromo-5-isoxazolyl)-2-(*tert*-butylamino)ethanol (broxaterol) (1)[5] (Figure 1). This compound is a potent and selective $\beta_2$-adrenergic stimulant, and, for this reason, it has been proposed as a bronchodilator for treatment of asthma. Furthermore, resolution showed that the activity was mainly associated with the (−)-isomer which is, at least, 100 times more potent than its (+)-isomer.[5] Nevertheless, the configuration of the stereocenter remained unassigned and, as a consequence, it was difficult to compare its pharmacological profile with that of the other chiral $\beta$-adrenergic drugs.

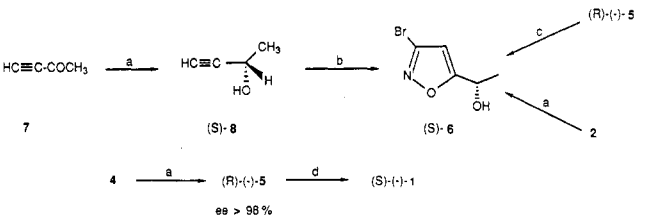
This paper reports a preparative route to (+)- and (−)-broxaterol (1) through the asymmetric reduction of an $\alpha$-bromo ketone catalyzed by alcohol dehydrogenase from *Thermoanaerobium brockii* (TBADH) and through kinetic resolution of a racemic bromohydrin by lipase P (from *Pseudomonas fluorescens* (LP)) catalysis. The absolute configurations of (+)- and (−)-Broxaterol are also assigned through chemical correlations. In addition, since isoxazoles are masked forms of different functionalities,[6] we extended our studies to other keto isoxazoles whose asymmetric reduction to carbinols can provide a valuable route for the synthesis of highly functionalized chiral building blocks. The reduction was carried out in the presence of the following catalysts: TBADH, 3$\alpha$,20$\beta$-hydroxysteroid dehydrogenase (20$\beta$-HSDH), and *Saccharomyces cerevisiae*. To our knowledge, in the sole report dealing with an enzyme-catalyzed reduction of keto isoxazoles, the reducing agent was actively fermenting yeast (*S. cerevisiae*), which yielded the corresponding chiral carbinols in high ee.[7]

**Scheme I**[a]




[a] (a) $NaHCO_3/CH_2Cl_2$; (b) $C_6H_5N^+Me_3Br_3^-$/THF; (c) $NaBH_4$/EtOH; (d) *t*-$BuNH_2$/MeOH.

**Scheme II**[a]




[a] (a) TBADH-catalyzed reduction; (b) $Br_2C$=NOH, $NaHCO_3/CH_2Cl_2$; (c) *n*-$Bu_3SnH$, AIBN/toluene; (d) *t*-$BuNH_2$/MeOH.

## Results and Discussion

Racemic broxaterol was prepared by the reaction sequence in Scheme I, which is a valid alternative to the previously reported synthesis.[5,8] We prepared 3-bromo-5-acetylisoxazole (2), contaminated by its regioisomer 3 (2/3 = 87/13), by reacting bromonitrile oxide, generated "in situ", with 3-butyn-2-one. Adduct 2, separated from isomer 3 by column chromatography, was efficiently transformed into $\alpha$-bromo ketone 4 by treatment with phenyltrimethylammonium tribromide.[9] The key intermediate 4 was then transformed into (±)-1 by conventional reactions (Scheme I).

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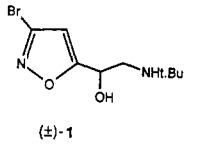
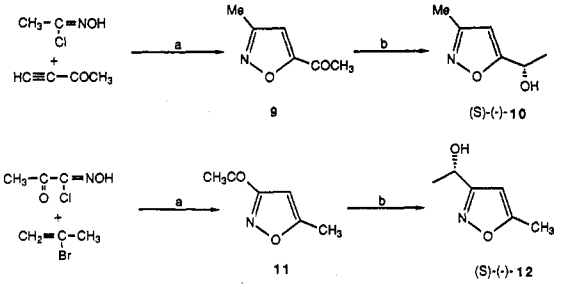



(±)-1

**Figure 1.**

**Scheme III**[a]




[a] (a) $NaHCO_3/CH_2Cl_2$; (b) TBADH-catalyzed reduction.

**Table I. Asymmetric Reduction of Ketones 2, 4, 7, 9, and 11 with TBADH, 20β-HSDH, and Bakers' Yeast**

| substrate | enzyme | rel rate | abs conf | ee,[a] % | $[\alpha]^{20}_D$, deg |
|---|---|---|---|---|---|
| 2 | TBADH | 88 | *S* | >98 | -17.45 |
| 2 | 20β-HSDH | | *R* | 69 | +12.07 |
| 2 | Bakers' yeast | | *S* | 75 | -12.33 |
| 4 | TBADH | 100 | *R* | >98[b] | -8.25 |
| 7 | TBADH | | *S* | 86 | -35.67 |
| 9 | TBADH | 13 | *S* | >98 | -24.82 |
| 11 | TBADH | 8 | *S* | >98 | -25.72 |

[a] Determined by $^1H$ NMR analysis as MTPA esters. [b] Determined by HPLC.

(*S*)-(-)-1 was synthesized through the asymmetric reduction of **4**, catalyzed by TBADH, followed by treatment with *tert*-butylamine (Scheme II). The TBADH-catalyzed reduction of bromo ketone **4** was remarkably enantioselective (>98% ee), making this a useful transformation for syntheses. The enantiomeric excess of (-)-**5** was ascertained by HPLC analysis; base-line separation of the two enantiomers was accomplished on a chiral stationary phase consisting of $\alpha_1$-acid glycoprotein (see the Experimental Section).

The *S* configuration was assigned to (-)-1 through correlation with (*S*)-(-)-3-butyn-2-ol (**8**) (Scheme II), whose configuration had previously been attributed.[10] It is worth pointing out that the TBADH-catalyzed reduction of 3-butyn-2-one yields (*S*)-3-butyn-2-ol, a reversal in stereoselectivity from that of 2-butanone.[11] A similar inversion of enzyme stereospecificity within a homogeneous series of aliphatic saturated ketones has previously been observed and rationalized.[11] As shown in Scheme II, the correlation of (*S*)-**8** with (-)-1 was obtained through the intermediate (*S*)-**6**, which was prepared both by reacting (*S*)-**8** with bromonitrile oxide and by reducing (-)-**5** with tributyltin hydride under conditions that form radicals.

The potential use of TBADH in organic synthesis is clearly illustrated by the generality and high enantioselectivity of its reduction of heterocyclic ketones **2**, **4**, **9**, and **11** (Schemes I and III), as shown in Table I. It is interesting to observe that the TBADH-catalyzed reductions of both 3-acetyl-5-methylisoxazole (**11**) and 3-methyl-5-acetylisoxazole (**9**) are equally effective in terms of enantiomeric excess and yield stereocenters with the same configuration.[12] Since the isoxazole nucleus can be considered the masked form of a 1,3-diketone, the same chiral (*S*)-α-hydroxy 1,3-diketone can be prepared by reducing either ketone **9** or ketone **11**.

**Table II. PPL- and LP-Catalyzed Transesterification of (±)-5**

| acylating ester | enzyme | degree of conversion,[a] % | yield, % | ee,[b] % | abs conf |
|---|---|---|---|---|---|
| TFE butyrate | PPL[c] | 29 | 25 | 60 | *S* |
| TFE butyrate | PPL | 32 | 28 | 80 | *S* |
| TFE butyrate | LP | 31 | 28 | 86 | *S* |
| TFE octanoate | LP | 30 | 27 | 97 | *S* |

[a] Determined by gas chromatography. [b] Determined by chiral HPLC on the bromohydrin generated by a PPL-catalyzed hydrolysis of the ester (see the Experimental Section). [c] The reaction was carried out in the absence of 4-Å molecular sieves.

A comparison of the ee values for the TBADH-, 20β-HSDH-, and *S. cerevisiae*-catalyzed reductions of **2** (Table I) indicates that TBADH is the most specific catalyst; it is worth pointing out that 20β-HSDH has a reversed enantioselectivity. We also tested alcohol dehydrogenase from horse liver, but this enzyme did not reduce ketone **2** at an appreciable rate. This result is likely due to the presence of nitrogen in the heterocyclic portion of the substrate, which inactivates the enzyme by complexing the zinc ion of its active site.[2e]

The degree of the enantioselectivity of the TBADH-catalyzed reductions (i.e., **7** vs **2**) seems to be related to the difference in size between the two groups attached to the carbonyl moiety. This trend supports the hypothesis[11] of the presence in the enzyme's active site of two hydrophobic pockets of different sizes, which accommodate the small (i.e., the methyl group) and the large group (i.e., the acetylenic group or the heterocyclic ring). The enzyme directs hydride transfer at the *re* face of the carbonyl moiety, leading to the formation of alcohols with the *S* configuration. The extent of the enantioselectivity seems to parallel the increase in size of the large group attached to the carbonyl.

The relative rates of reduction of the structurally related substrates **2**, **9**, and **11** indicate that, whereas a shift of the reacting group from the 5- to the 3-position of the heterocyclic ring (i.e., **9** vs **11**) does not markedly affect the reaction rate, the replacement of the 3-methyl group with bromo (i.e., **9** vs **2**) provokes a very pronounced change (Table II). As a consequence, the accommodation of the aromatic moiety in the appropriate enzyme's hydrophobic pocket is primarily related to the dimension and lipophilic character of the group but is also affected by electronic factors.

In an effort to prepare the *R* enantiomer of broxaterol in high optical purity, we tested the enzyme-catalyzed asymmetric transesterification of (±)-**5** in organic media. This compound was a good substrate for pig liver esterase, *Candida cylindracea* lipase, chymotrypsin, and subtilisin, but, unfortunately, the selectivity was poor. On the contrary, porcine pancreatic lipase (PPL) and lipase P (LP) proved to be quite selective catalysts (Table II). With 30% conversion, the enantiomeric excesses of the formed esters were 60–97%, depending on the enzyme and conditions employed (Table II). Removal of water from the medium was essential for obtaining high reaction rates and high

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(12) The configuration to (*S*)-(-)-**10** and (*S*)-(-)-**12** was attributed by comparing their specific rotation with that of (*S*)-(-)-**6**. In addition, the configuration of (*S*)-**12** was confirmed by comparing its circular dichroic curve with that of (*S*)-**10** (see the Experimental Section).

**Table III. Effect of the Acylating Ester on the Enantioselectivity of the LP-Catalyzed Transesterification of (±)-5**

| acylating ester | degree of conversion,[a] % | ee,[b] % |
|---|---|---|
| TFE butyrate | 61 | 33 |
| | 75 | 40 |
| TFE hexanoate | 60 | 42 |
| | 77 | 30 |
| TFE octanoate | 62 | 44 |
| | 68 | 50 |
| | 75 | 53 |
| TFE dodecanoate | 58 | 43 |
| | 70 | 54 |
| TFE hexadecanoate | 61 | 46 |
| | 74 | 57 |

[a] Determined by gas chromatography. [b] The values refer to the unreacted bromohydrin and were determined by chiral HPLC directly in the reaction mixtures. The esters did not affect the resolution of the bromohydrin since they were eluted in the void volume of the column.

optical purity. This was particularly true for PPL, which is a cruder enzyme preparation and contains a larger amount of absorbed water per enzyme unit. The results confirm recent observations of the importance of enzyme dehydration in PPL-catalyzed transesterification reactions.[13] Activated esters, such as trifluoroethyl (TFE) esters, were used as acylating reagents because, as already shown by Klibanov and co-workers,[14] they significantly increased the reaction rates as compared to nonactivated esters.

Since chemical hydrolysis of the esters, under usual conditions, caused extensive degradation of the substrate, PPL was also used for the preparation of (*S*)-(+)-**5** by quantitative hydrolysis of the esters synthesized either with LP or PPL itself. Butyrate esters were also hydrolyzed with the less specific pig liver esterase, which, however, did not recognize long-chain esters. (*S*)-(+)-**5** was finally transformed into (*R*)-(+)-**1** by a conventional reaction with *tert*-butylamine.

Table II shows that in the LP-catalyzed transesterifications, TFE octanoate yielded higher ee than TFE butyrate. This evidence prompted us to investigate systematically the effect of the chain length of the acyl donor on the enantioselectivity (Table III). The esterification of (±)-**5** was extended beyond 50%, and the optical purity of the remaining substrate was determined. This approach was chosen for convenience, since the remaining bromohydrin could be directly analyzed by chiral HPLC without any workup of the reaction mixture. The trend emerging from the data collected in Table III indicates that enantioselectivity increases with the increase in chain length of the acyl donor. The reaction rates were similar for all the esters tested except hexadecanoate, which had a lower rate (about 60%) (data not reported). In addition, the data in Table III show that, as previously observed for related systems,[15] high ee of the bromohydrin (*S*)-**5** cannot be attained by extending the conversion beyond 50%. This can be ascribed to the fact that, unlike ester hydrolysis carried out in water,[16] transesterification in organic media is reversible.[15] Therefore, high enantiomeric excesses can be obtained only for the ester product and not for the unreacted substrate. We do not know at present if the

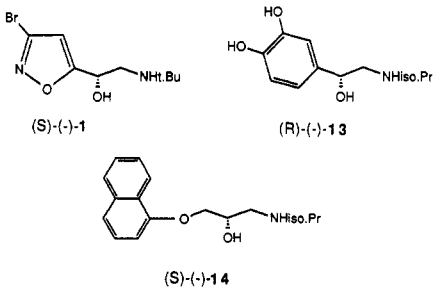


**Figure 2.**

increase in enantioselectivity as a function of the chain length of the acyl donor is due to an influence on the equilibrium constant of the reaction[15] or to steric effects at the enzyme's active site. In any case, the combined effect of anhydrous conditions plus the use of a long-chain acylating donor made it possible to synthesize the desired bromohydrin (*S*)-(+)-**5** in 97% ee, which was then transformed into the final derivative (*R*)-(+)-**1**.

## Conclusion

This work provides a further example of the utility and versatility of enzyme-catalyzed transformations for the asymmetric synthesis of chiral building blocks. The synthesis of the two enantiomers of broxatherol and, consequently, the assignment of structure to its most active enantiomer, made it possible to delineate a qualitative structure–activity relationship with the most common β-adrenergic drugs. Figure 2 shows the active enantiomers of the β-agonists broxaterol and isoproterenol (**13**)[17,18] and of the β-blocker propranolol (**14**).[17,18] In spite of the different notations for the chiral center, the spatial arrangement of the substituents around the stereocenter is the same for the three drugs, making it reasonable to hypothesize that the isoxazole moiety of broxaterol fits the same receptor subsite as the catechol nucleus of isoproterenol or the aromatic group of propranolol.

## Experimental Section

**Materials and Methods.** Alcohol dehydrogenase from *T. brockii* (TBADH) (purified powder), porcine pancreatic lipase (PPL), pig liver esterase, NAD, and NADP were obtained from Sigma. 3α,20β-Hydroxysteroid dehydrogenase (20β-HSDH) was purchased from Boehringer, and lipase P (LP) was purchased from Amano. Organic solvents were reagent grade. $^1$H NMR spectra were recorded in $CDCl_3$ solution at 80 or 200 MHz. GLC analyses were carried out on a 5-m HP1 capillary silica gel column coated with methylsilicone gum. $N_2$ was used as the carrier gas at 30 mL/min. Rotatory power determinations were carried out with a Perkin-Elmer 241 polarimeter, coupled with a Haake N3-B thermostat. Circular dichroic spectra of (*S*)-(−)-**10** and (*S*)-(−)-**12** were recorded at a concentration of 0.2 mg/mL with a JASCO 500 A spectropolarimeter in acetonitrile with an optical path of 0.1 cm, at 25 °C. Merck silica gel 60 $F_{254}$ analytical thin-layer chromatography plates were used throughout this work. Microanalyses (C, H, and N) agree with theoretical value ±0.3%. (*R*)-(+)-MTPA esters were prepared according to the procedure described previously.[19] Liquids were characterized by the oven temperature for Kugelrohr distillations.

**3-Bromo-5-acetylisoxazole (2).** A suspension of dibromoformaldoxime[20] (6.1 g, 30 mmol), 3-butyn-2-one (2.5 g, 37 mmol),

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and sodium bicarbonate (12.6 g, 0.15 mol) in dichloromethane (70 mL) was stirred at room temperature until gas evolution ceased. The slurry was then treated with diluted HCl (30 mL) and extracted with dichloromethane. The organic layer was dried over sodium sulfate, and the solvent was removed under vacuum. A $^1$H NMR spectrum of the residue (3.5 g, 61% yield) showed the presence of both the regioisomers **2** (87%) and **3** (13%). Regioisomer **2** was purified from **3** by silica gel column chromatography (eluent: cyclohexane/ethyl acetate, 4:1). Compound **2** crystallizes from ligroin/ethyl acetate as colorless needles: mp 61–62 °C (lit.[5] mp 56–58 °C); $^1$H NMR for **2** $\delta$ 2.58 (s, 3 H, Me), 6.90 (s, 1 H, H-4); for **3** $\delta$ 2.58 (s, 3 H, Me), 8.87 (s, 1 H, H-4).

**3-Bromo-5-(bromoacetyl)isoxazole (4).** A solution of **2** (1.35 g, 7.1 mmol) and phenyltrimethylammonium tribromide (2.66 g, 7.1 mmol) in anhydrous THF (15 mL) was stirred at 25 °C until the orange color disappeared, and then a second aliquot (0.266 g) of brominating agent was added with stirring. The mixture was poured into water (20 mL) and extracted with ether (3 × 20 mL). The extracts were dried over anhydrous sodium sulfate, and the solvents were removed under vacuum. The residue (1.27 g, 66.6% yield) was distilled (147–152 °C (0.5 mmHg)) and then crystallized from isopropyl ether: mp 53–54 °C; $^1$H NMR $\delta$ 4.35 (s, 2 H, $CH_2Br$), 7.08 (s, 1 H, H-4).

**1-(3-Bromo-5-isoxazolyl)-2-bromoethanol (5).** Derivative **5** was obtained in 95% yield by adding, portionwise, an excess of $NaBH_4$ (0.45 g, 11.9 mmol) to a 30-mL ethanol solution of **4** (0.7 g, 2.58 mmol) cooled at 0 °C. The reaction was stirred at 25 °C until TLC showed the disappearance of the starting material (about 1 h). The solvent was removed under vacuum, and the residue was treated with dilute HCl and extracted with dichloromethane (3 × 15 mL). The organic extracts were dried over anhydrous sodium sulfate, the solvent was removed at reduced pressure, and the residue was Kugelrohr distilled at 150–155 °C (0.5 mmHg): $^1$H NMR $\delta$ 3.20 (d, 1 H, OH), 3.80 (m, 2 H, $CH_2Br$, $J_{A,B}$ = 10.8 Hz, $J_{A,X}$ = 6.0 Hz, $J_{B,X}$ = 4.3 Hz), 5.18 (m, 1 H, C*H*OH), 6.52 (s, 1 H, H-4).

**1-(3-Bromo-5-isoxazolyl)-2-(*tert*-butylamino)ethanol (1).** A stirred solution of **5** (0.5 g, 1.78 mmol) and *tert*-butylamine (0.65 g, 8.9 mmol) in methanol (20 mL) was heated under reflux for 3 h. The solvent and the excess *tert*-butylamine were removed under vacuum. The residue, column chromatographed on silica gel (eluent: chloroform/methanol, 95:5), yielded 0.42 g (93.5%) of (±)-**1**; **1** crystallizes from diisopropyl ether as colorless needles: mp 83.5–84.5 °C; $^1$H NMR $\delta$ 1.30 (s, 9 H, 3 Me), 3.15 (m, 2 H, $CH_2N$), 5.00–5.50 (m, 3 H, C*H*OH, OH, NH), 6.40 (s, 1 H, H-4).

**3-Methyl-5-acetylisoxazole (9).** Following the methodology previously reported for **2**, 1.75 g (18.7 mmol) of acetohydroxymoyl chloride was reacted with 3-butyn-2-one (0.6 g, 8.82 mmol) in the presence of excess sodium bicarbonate and yielded 0.623 g (56.5%) of adduct **9**. The $^1$H NMR spectrum of the crude mixture did not show the presence of the 4-substituted regioisomer. Compound **9** crystallizes from isopropyl ether: mp 71–72 °C; $^1$H NMR $\delta$ 2.42 (s, 3 H, Me-3), 2.64 (s, 3 H, COMe), 6.80 (s, 1 H, H-4).

**3-Acetyl-5-methylisoxazole (11).** In analogy to the protocol described for the synthesis of **2**, 0.81 g (6.67 mmol) of pyruvohydroximoyl chloride was reacted with 2-bromopropene (1.78 mL, 20 mmol) in the presence of sodium bicarbonate. Adduct **11** (0.21 g, 25% yield) was purified by Kugelrohr distillation: 110–115 °C (22 mmHg): $^1$H NMR $\delta$ 2.51 (s, 3 H, Me-5), 2.67 (s, 3 H, COMe), 6.40 (s, 1 H, H-4).

**TBADH Reduction of 2, 4, 7, 9, and 11.** The following procedure is representative. A 1-L Erlenmeyer flask was loaded with 640 mL of solution containing the following: acylisoxazole **2** (1 g, 5.26 mmol), 2-propanol (40 mL), TBADH (100 units), NADP (120 mg, 0.15 mmol), and 0.05 M potassium phosphate buffer, pH 6.5 (600 mL).[21] The flask was placed in a water bath at 30 °C and gently stirred. The reduction was followed by GLC and interrupted at the disappearance of the starting material (about 2 h). The reaction mixture was extracted with ethyl acetate (3 × 100 mL), and the organic layer was dried over sodium sulfate and evaporated to dryness. The residue, flash chromatographed on silica gel (eluent: cyclohexane/ethyl acetate, 4:1), yielded 0.829 g (82%) of (*S*)-(−)-**6**: bp 110–120 °C (1 mmHg): $[\alpha]^{20}_D$ −17.45° (*c* 1.1, $CHCl_3$).

Ketones **4** (1 g, 3.71 mmol), **7** (0.5 g, 7.3 mmol), **9** (0.1 g, 0.8 mmol), and **11** (0.1 g, 0.8 mmol) were treated similarly to give (*R*)-(−)-**5** (0.573 g), (*S*)-(−)-**8** (0.1 g), (*S*)-(−)-**10** (89 mg), and (*S*)-(−)-**12** (60 mg).

(*R*)-(−)-**5**: bp 150–5 °C (0.5 mmHg); $[\alpha]^{20}_D$ −8.25° (*c* 2.0, chloroform).

(*S*)-(−)-**8**: bp 60–65 °C (150 mmHg); $[\alpha]^{25}_D$ −35.67° (*c* 2.2, dioxane).

(*S*)-(−)-**10**: bp 100–5 °C (0.5 mmHg); $[\alpha]^{20}_D$ −24.82° (*c* 1.1, chloroform); UV ($CH_3CN$) $\lambda_{max}$ 210, $\epsilon$ = 5105; CD ($CH_3CN$) $[\theta]_{210}$ = −254 deg cm$^2$ dmol$^{-1}$.

(*S*)-(−)-**12**: bp 75–80 °C (0.8 mmHg); $[\alpha]^{20}_D$ −25.72° (*c* 0.7, chloroform); UV ($CH_3CN$) $\lambda_{max}$ 215, $\epsilon$ = 5982; CD ($CH_3CN$) $[\theta]_{215}$ = −1524 deg cm$^2$ dmol$^{-1}$.

**20$\beta$-HSDH Reduction of 2.** A 500-mL Erlenmeyer flask was loaded with 210 mL of solution containing the following: **2** (0.35 g, 1.84 mmol), ethanol (20 mL), NAD (60 mg, 0.09 mmol), 0.05 M potassium phosphate buffer, pH 6.9 (190 mL), 0.1 M sodium formate, 3$\alpha$,20$\beta$-HSDH (140 units), and formate dehydrogenase (80 units).[22] The reaction was incubated at 25 °C until disappearance (about 2 h) of the starting material (GLC control). The reaction mixture was extracted with ethyl acetate (3 × 100 mL), and the organic layer was dried over sodium sulfate and evaporated to dryness. The crude product was purified by column chromatography and yielded 276 mg (78%) of (*R*)-(+)-**6**: $[\alpha]^{20}_D$ +12.07° (*c* 1.0, chloroform).

***S. cerevisiae* Reduction of 2.** Derivative **2** (0.4 g) was reduced by using the procedure reported by Seebach and co-workers[23] and yielded 0.28 g of (*S*)-(−)-**6**: $[\alpha]^{20}_D$ −12.33° (*c* 0.6, chloroform).

**LP-Catalyzed Production of (*S*)-(+)-5.** (*S*)-(+)-**5** was prepared through the enantioselective enzymatic transesterification of (±)-**5** followed by an aspecific enzymatic hydrolysis of the ester.

**A.** Trifluoroethyl octanoate (45 mmol) was incubated with lipase P (6.0 g), (±)-**5** (2.4 g, 9 mmol), and molecular sieves (15 g, 4-Å) in 300 mL of *n*-hexane/benzene (9:1) at 45 °C with shaking (265 rpm). The reaction, monitored by GLC, was interrupted after reaching 30% conversion (about 4 h) by filtering the organic medium to remove the enzyme. The organic solution was then concentrated to dryness, and the residue was flash chromatographed over silica gel (eluent: *n*-hexane/ethyl acetate, 4:1) to give 0.95 g of the octanoate ester, which was not characterized but directly hydrolyzed by the following procedure.

**B.** The ester was dissolved in acetone (30 mL) and added to 300 mL phosphate buffer, pH 7.5, containing PPL (3 g). The mixture was shaken at 25 °C until GLC analysis showed the disappearance of the starting material (about 1 h). The mixture was filtered to remove the enzyme, and the solution was extracted with ethyl acetate (4 × 100 mL). The extracts were dried over anhydrous sodium sulfate, and the solvent was removed under vacuum. The residue, column chromatographed on silica gel (eluent: cyclohexane/ethyl acetate, 3:2), gave 0.58 g of (*S*)-(+)-**5**: $[\alpha]^{20}_D$ +8.00° (*c* 1.8, chloroform). (*S*)-(+)-**5** was also prepared, under similar conditions, with trifluoroethyl butyrate as the acylating agent.

**Preparation of (*S*)-(−)-1.** Bromohydrin (*R*)-(−)-**5** (240 mg, 0.89 mmol) was transformed into amino alcohol (*S*)-(−)-**1** (218 mg, 93.5% yield) by the procedure previously reported for the racemic form. (*S*)-(−)-**1** crystallizes from ligroin/ethyl acetate as light pink needles: mp 139–140 °C dec; $[\alpha]^{20}_D$ −25.00° (*c* 0.7, methanol).

The hydrochloride of (*S*)-(−)-**1** was obtained as colorless prism from 2-propanol/ligroin: mp 130–165 °C dec; $[\alpha]^{20}_D$ −25.24° (*c* 0.7, water) (lit.[5] mp 185 °C); $[\alpha]^{20}_D$ −25.4° (*c* 5, water).

**Preparation of (*R*)-(+)-1.** This compound was prepared in 90% yield by reacting (*S*)-(+)-**5** with excess *tert*-butylamine via the procedure reported for the racemic form: $[\alpha]^{20}_D$ +24.22° (*c* 0.5, methanol).

**(*S*)-(−)-1-(3-Bromo-5-isoxazolyl)ethanol (*S*)-(−)-6. A.** Bromohydrin (*R*)-(−)-**5** (70 mg, 0.258 mmol), AIBN (42 mg), and

(21) NADPH, the hydrogen donor for the TBADH-catalyzed reduction of **2**, was continuously "in situ" regenerated from NADP at the expense of 2-propanol in a reaction catalyzed by TBADH itself.[11]

(22) NADH, the hydrogen donor for the 20$\beta$-HSDH-catalyzed reduction of **2**, was continuously "in situ" regenerated from NAD at the expense of formate in a reaction catalyzed by formate dehydrogenase.[1f]

(23) Seebach, D.; Sutter, M. A.; Weber, R. H.; Zuger, R. H.; Zuger, M. F. *Org. Synth.* **1985**, *63*, 1.

tributyltin hydride (70 μL) in benzene (5 mL) were stirred and refluxed under nitrogen for 4 h. Methyl iodide (0.5 mL) was added to the reaction mixture, and reflux was continued overnight. Chromatography on a silica gel column gave (*S*)-(−)-**6**: yield 38 mg (76.7%); $[\alpha]^{20}_D$ −13.57° (*c* 1.0, chloroform).

**B.** A suspension of dibromoformaldoxime (1.2 g, 6 mmol), (*S*)-(−)-3-butyn-2-ol (0.2 g, 2.8 mmol), and sodium bicarbonate (2.4 g) in dichloromethane (30 mL) were stirred at room temperature until evolution of gas ceased. The slurry was treated with diluted HCl (20 mL) and extracted with dichloromethane (2 × 30 mL). The organic extracts were dried over anhydrous sodium sulfate, and the solvent was removed under vacuum. Column chromatography of the residue gave 0.503 g (89% yield) of (*S*)-(−)-**6**: $[\alpha]^{20}_D$ −14.95° (*c* 1.1, chloroform).

**Determination of Enantiomeric Excess of 5, 6, 10, 12. A.** The enantiomeric excess of **5** was directly determined by HPLC with a commercially available chiral column (4 mm × 100 mm) packed with $\alpha_1$-acid glycoprotein (LKB Enantiopac); eluent: sodium phosphate buffer 8 mM, pH = 5, containing NaCl (0.05 M) and 2-propanol (0.5%); flow rate 0.3 mL/min. Retention times (min): 14.8 (*S*) and 19.4 (*R*); peak resolution $R_S$ = 1.73.

**B.** Chiral alcohols **6**, **10**, and **12** and the corresponding racemic forms were converted into the (*R*)-(+)-MTPA esters,[19] and the ee were determined by integration of the doublets of the methyl group α to the hydroxyl group in the 200-MHz $^1$H NMR spectrum: **6** *S* form δ 1.74, *R* form δ 1.67; **10** *S* form δ 1.69, *R* form δ 1.61; **12** *S* form δ 1.68, *R* form δ 1.60. $^1$H NMR spectral data of the (*R*)-(+)-MTPA esters of **6**(*S*) and **6**(*R*), **10**(*S*) and **10**(*R*), **12**(*S*), and **12**(*R*) are reported below.

**6**(*S*): δ 1.74 (d, 3 H, Me, *J* = 6.1 Hz), 3.60 (b s, 3 H, OMe), 6.14 (s, 1 H, H-4), 6.23 (q, 1 H, CH), 7.40–7.55 (m, 5 H, Ar).

**6**(*R*): δ 1.67 (d, 3 H, Me, *J* = 6.1 Hz), 3.60 (b s, 3 H, OMe), 6.33 (s, 1 H, H-4), 6.23 (q, 1 H, CH), 7.40–7.55 (m, 5 H, Ar).

**10**(*S*): δ 1.69 (d, 3 H, Me, *J* = 6.7 Hz), 2.21 (s, 3 H, Me-3), 3.50 (q, 3 H, OMe, $J_{C,F}$ = 1.3 Hz), 5.87 (s, 1 H, H-4), 6.17 (q, 1 H, CH), 7.40–7.55 (m, 5 H, Ar).

**10**(*R*): δ 1.61 (d, 3 H, Me, *J* = 6.7 Hz), 2.26 (s, 3 H, Me-3), 3.50 (q, 3 H, OMe, $J_{C,F}$ = 1.3 Hz), 6.05 (s, 1 H, H-4), 6.17 (q, 1 H, CH), 7.40–7.55 (m, 5 H, Ar).

**12**(*S*): δ 1.68 (d, 3 H, Me, *J* = 6.1 Hz), 2.35 (d, 3 H, Me-5, *J* = 1.0 Hz), 3.55 (q, 3 H, OMe, $J_{C,F}$ = 1.1 Hz), 5.76 (q, 1 H, H-4, *J* = 1.0 Hz), 6.17 (q, 1 H, CH), 7.30–7.70 (m, 5 H, Ar).

**12**(*R*): δ 1.60 (d, 3 H, Me, *J* = 6.1 Hz), 2.38 (d, 3 H, Me-5, *J* = 1.0 Hz), 3.55 (q, 3 H, OMe, $J_{C,F}$ = 1.1 Hz), 5.93 (q, 1 H, H-4, *J* = 1.0 Hz), 6.17 (q, 1 H, CH), 7.30–7.70 (m, 5 H, Ar).

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**Registry No.** (±)-**1**, 119596-01-9; (*R*)-(+)-**1**, 104164-30-9; (*S*)-(−)-**1**, 119717-13-4; (*S*)-(−)-**1**·HCl, 104182-21-0; **2**, 76596-53-7; **3**, 119619-10-2; **4**, 76596-54-8; (±)-**5**, 119596-02-0; (±)-**5** butyrate ester, 119596-15-5; (±)-**5** hexanoate ester, 119596-16-6; (±)-**5** octanoate ester, 119596-13-3; (±)-**5** dodecanoate ester, 119596-17-7; (±)-**5** hexadecanoate ester, 119596-18-8; (*R*)-(−)-**5**, 119677-65-5; (*S*)-(+)-**5**, 119677-66-6; (*R*)-(+)-**6**, 119596-05-3; (*R*)-**6** (*R*)-(+)-MTPA ester, 119596-08-6; (*S*)-(−)-**6**, 119596-03-1; (*S*)-**6** (*R*)-(+)-MTPA ester, 119596-07-5; **7**, 1423-60-5; (*S*)-(−)-**8**, 2914-69-4; **9**, 55086-61-8; (*S*)-(−)-**10**, 119596-04-2; (*R*)-**10** (*R*)-(+)-MTPA ester, 119596-10-0; (*S*)-**10** (*R*)-(+)-MTPA, 119596-09-7; **11**, 24068-54-0; (*S*)-(−)-**12**, 119596-06-4; (*S*)-**12** (*R*)-(+)-MTPA ester, 119596-11-1; (*R*)-**12** (*R*)-(+)-MTPA ester, 119596-12-2; TBADA, 9031-72-5; 3α,20β-HSDH, 72855-18-6; dibromoformaldoxime, 74213-24-4; *tert*-butylamine, 75-64-9; acetohydroximoyl chloride, 683-58-9; pyruvohydroximoyl chloride, 5471-68-1; 2-bromopropene, 557-93-7; 2,2,2-trifluoroethyl octanoate, 2264-29-1; 2,2,2-trifluoroethyl butyrate, 371-27-7; formate dehydrogenase, 9028-85-7; 2,2,2-trifluoroethyl hexanoate, 2822-57-3; 2,2,2-trifluoroethyl dodecanoate, 70253-78-0; 2,2,2-trifluoroethyl hexadecanoate, 119596-14-4; lipase, 9001-62-1.

# Oligosaccharides Corresponding to Biological Repeating Units of *Shigella flexneri* Variant Y Polysaccharide. 2. Synthesis and Two-Dimensional NMR Analysis of a Hexasaccharide Hapten[1]

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The block synthesis of a hexasaccharide portion of the biological repeating unit, [2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-β-D-Glc*p*NAc-(1-], of the *Shigella flexneri* variant Y polysaccharide is described. The synthetic strategy relies on the use of the key trisaccharide intermediate, α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*, as a glycosyl donor. Thus, the trisaccharide bromide in conjunction with the β-D-Glc*p*NPhth-(1→2)-α-L-Rha*p*-(1→2)-α-L-Rha*p* unit under Helferich conditions yielded the blocked hexasaccharide in 85% yield. Attempts at coupling the tetrasaccharide donor, α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-β-D-Glc*p*NPhth, with the disaccharide acceptor, α-L-Rha*p*-(1→2)-α-L-Rha*p*, to give the hexasaccharide under a variety of conditions were unsuccessful. The blocked derivatives were synthesized as their allyl glycosides. Removal of the blocking groups, hydrogenation of the allyl group, and N-acetylation yielded the hexasaccharide hapten, α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-β-D-Glc*p*NAc-(1→2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*, as its propyl glycoside, for use in inhibition studies with complementary monoclonal antibodies, and in NMR and X-ray studies. The detailed NMR analysis of the protected and deprotected hexasaccharides by use of two-dimensional NMR techniques is also described.

## Introduction

A program of research designed to probe the interaction of antigens with antibodies at a molecular level is in progress.[1] The approach consists of the synthesis of well-defined, complex carbohydrate antigens and their testing in inhibition reactions with complementary monoclonal antibodies. The objective is to define the molecular specificity of these antibodies in terms of the

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