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TETRAHEDRON: *ASYMMETRY*

# Enzymatic resolution of 2-substituted tetrahydroquinolines. Convenient approaches to tricyclic quinoxalinediones as potent NMDA-glycine antagonists

Seiji Katayama, Nobuyuki Ae and Ryu Nagata *

*Sumitomo Pharmaceuticals Research Center, 1-98 Kasugade naka 3 chome, Konohanaku, Osaka 554, Japan*

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**Abstract**

Two approaches leading to the enantiomerically pure tricyclic quinoxalinedione class of NMDA-glycine antagonists using enzymatic resolutions are described. An intermediate, racemic methyl 1,2,3,4-tetrahydroquinoline-2-carboxylate **3**, was resolved to (*S*)-**3** in 97% ee and 47% yield (E=67) using α-chymotrypsin. In an improved method, hydrolysis of another intermediate, racemic methyl 1,2,3,4-tetrahydroquinoline-2-acetate **4**, with Novozym® 435 provided the desired (*S*)-**4** in high enantioselectivity and yield (93% ee, 50%, E=94). 

## 1. Introduction

There is increasing evidence that over-excitation of the NMDA receptor plays an important role in neuronal cell death during ischemic or hypoxic conditions such as stroke.[1] Several binding sites on the NMDA receptor, including glutamate, glycine, and channel blocker binding sites, have been identified and these sites offer a target for the drug treatment of not only stroke but other neurodegenerative disorders such as Alzheimer's and Huntington's diseases.[2,3] We have recently synthesized a series of tricyclic quinoxalinediones as potent antagonists of the glycine site of the NMDA receptor.[4–6] Among them, the six-membered ring fused tricyclic quinoxalinedione **1** ($K_i$=9.9 nM) and its anilide **2** ($K_i$=2.6 nM) showed extremely high affinity for the glycine site, as determined by radio ligand binding assay using [$^3$H] 5,7-dichlorokynurenic acid.

* Corresponding author. E-mail: rnagata@sumitomopharm.co.jp

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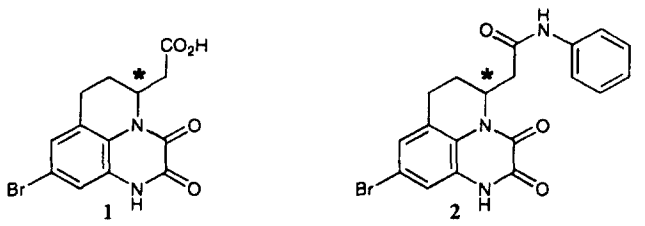
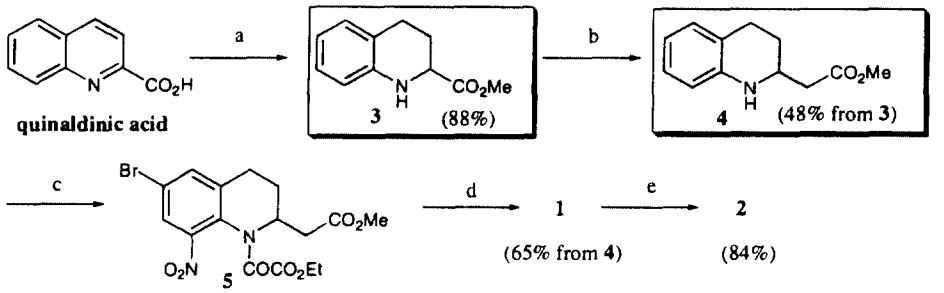


(1)

In particular, only the *S* isomer ($K_i$=0.96 nM) of **2** retained the affinity and the *R* isomer ($K_i$=82 nM) was 85-fold weaker than the *S* isomer. Consequently, we focused our attention on the synthesis of the enantiomerically pure *S* isomer, (*S*)-**2**, and its derivatives. Tricyclic quinoxalinediones **1** and **2** have been synthesized from methyl 1,2,3,4-tetrahydroquinoline-2-carboxylate (**3**) via methyl 1,2,3,4-tetrahydroquinoline-2-acetate (**4**) according to the route outlined in Scheme 1.[4]

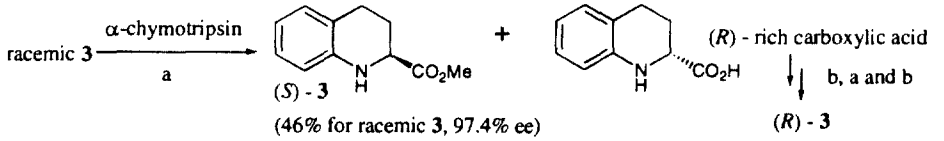



Conditions: (a) (1) $H_2/PtO_2$, MeOH, (2) MeOH/$SOCl_2$; (b) (1) $LiAlH_4$, THF, (2) $I_2/PPh_3$/imidazole, toluene/acetonitrile, (3) NaCN, DMF, (4) 12 N HCl, (5) MeOH/$SOCl_2$; (c) (1) NBS, DMF, (2) $ClCOCO_2Et/NEt_3$, $CH_2Cl_2$, (3) $NO_2^+BF_4^-$, $CH_2Cl_2$; (d) (1) $TiCl_3$, $H_2O$/acetone, (2) 1 N NaOH, MeOH-THF; (e) $PhNH_2$, WSC-HOBt, DMF.

Scheme 1.

We report here two approaches for the preparation of the key intermediate (*S*)-**4**, leading to enantiomerically pure (*S*)-**2**, as well as its derivatives.

Synthesis of enantiomerically pure **3** has been reported in the literature,[7] which describes the conversion of racemic **3** into diastereomers by coupling with an enantiomerically pure benzoyl-protected amino acid derivative followed by a fractional recrystallization and hydrolysis. In our hands, it turned out that this approach was too tedious for multi-gram synthesis. We then attempted to resolve racemic **3** using an enantioselective enzymatic hydrolysis. As a result, we found that treatment of racemic **3** with α-chymotrypsin in 0.1 M phosphate buffer (pH 7.5) afforded (*S*)-**3** in high enantiomeric excess and chemical yield (97% ee, 46%), and with a high enantiomeric ratio[19] (E=67). Enantiomerically pure (*R*)-**3** could also be obtained as shown in Scheme 2. In this way, we were able to supply a number of enantiomerically pure derivatives of **2**, so that we could identify in vivo active glycine antagonists including SM-18400.[8–10]




Conditions: (a) 0.1 M phosphate buffer (pH 7.5) ; (b) $SOCl_2$ / MeOH

Scheme 2.

Although this approach was useful, we required a more straightforward and practical route leading to the key intermediate (*S*)-**1** for extensive pharmacological and toxicological studies of SM-18400

Table 1

| Entry | Biocatalyst [b] (mg / 1 mg of substrate) | Time (h) | Conversion (%) | Residual ester [c] (% ee) | Absolute configuration [d] | E [e] |
|---|---|---|---|---|---|---|
| 1 | Lipase MY, *Candida rugosa* (2.5) | 135 | 61 | 29 | *S* | 1.9 |
| 2 | Lipase FAP, *Rhizopus oryzae* (2.5) | 73 | 67 | 53 | *S* | 2.7 |
| 3 | Lipase OF360, *Candida cylindracea* (0.5) | 48 | 58 | 34 | *S* | 2.2 |
| 4 | *Rhizopus chinensis* lipase (2.5) | 183 | 48 | 26 | *S* | 2.3 |
| 5 | *Mucor Miehei* lipase (0.25) | 58 | 55 | 22 | *S* | 1.7 |
| 6 | SP-525, *Candida antarctica* (0.25) | 0.2 | 67 | 95 | *S* | 9.3 |
| 7 | α-chymotrypsin (0.25) | 56 | 60 | 74 | *R* | 6.2 |
| 8 | Lipase PU15 (0.25) | 21 | 75 | 99 | *R* | 8.6 |
| 9 | SP-523, *Fumicola* (0.25) | 48 | 54 | 27 | *R* | 2.0 |
| 10 | Subtilisin A, *Bacillus licheniformis* (0.25) | 11 | 66 | 45 | *R* | 2.4 |

[a] All reactions were carried out by shaking a mixture of substrate (50 mg), catalyst (12.5–125 mg) in 0.1M phosphate buffer (5.0 ml) at 30°C. [b] Suppliers of enzymes: entry 1, 3, Meitoh Ltd.; entry 2, 8, Amano Ltd.; entry 4, Daikin Ltd.; entry 5, Biocatalysts Ltd.; entry 6, 9, 10, Novo Nordisk Ltd.; entry 7, Sigma. [c] Enantiomeric excess (ee) was determined by HPLC with chiral stationary phase.[14] [d] Absolute configuration of **4** was determined by comparison with that derived from enantiomerically active **3**. [e] E value (enantiomeric ratio), see ref. 19.

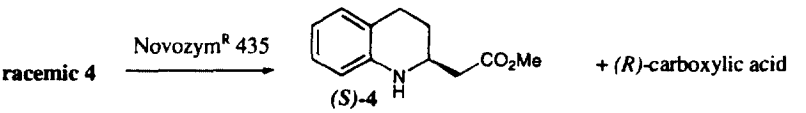
and related compounds. We therefore attempted to resolve racemic **4**, since **4** was readily available by a simple two-step sequence from quinoline-*N*-oxide.[11–13] More than 30 lipases and proteases were screened to determine whether enantioselective hydrolysis of racemic **4** could be achieved. In all cases, the hydrolyses were carried out at a substrate concentration of 0.05 M with a given enzyme (25–250 wt%) in 0.1 M phosphate buffer (pH 7.5) at 30°C and the enantiomeric excess of the residual substrate was analyzed by HPLC with a chiral stationary phase.[15] Selected examples are listed in Table 1. Among the enzymes screened, SP-525 (entry 6, available from Novo Nordisk Ltd) afforded the desired (*S*)-**4** with high enantioselectivity (95% ee at 67% conversion, E=9.3) and showed remarkable reactivity. An immobilized form of SP-525 is available as Novozym® 435, which provided a somewhat lower enantioselectivity compared with SP-525 (58% ee at 51% conversion, E=6.2), but was still acceptable. Considering the ease of handling and the commercial availability, we decided to optimize the reaction conditions further, using Novozym® 435.

We changed the reaction medium from phosphate buffer to an organic solvent containing a constant amount of water, since such solvent systems have been successfully employed in enzymatic hydrolysis to improve the enantioselectivity.[15–18] The results are summarized in Table 2.

Fortunately, some of the solvents increased the enantioselectivity remarkably (E>37) as well as the chemical yield. In particular, polar solvents such as THF (entry 4), acetone (entries 5 and 6) and dioxane (entry 3) gave favorable results. The amount of water in the organic solvent dramatically affected the enantioselectivity. For example, an increase in the water content in acetone from 5% to 10% improved the enantioselectivity (entry 5: 89% ee, E=78; entry 6: 94% ee, E=115), but an increase to 30% remarkably decreased it (entry 7: 76% ee, E=14).

The typical procedure used for this enzymatic hydrolysis is as follows: to a solution of racemic **4** (9.00 g, 43.9 mmol) in THF (400 ml) containing 5% water (20 ml) was added Novozym® 435 (1.60 g). The mixture was gently stirred at 30°C for 72 h, when the progress of the reaction stopped (usually

Table 2




| Entry | Solvent | Time (h) | Conversion (%) | Residual ester[c] (% ee) | E[d] |
|---|---|---|---|---|---|
| 1 | diisopropyl ether (saturated with $H_2O$ and 20% acetone)[b] | 4 | 39 | 58 | 37 |
| 2 | acetonitrile (containing 5% $H_2O$) | 4 | 45 | 78 | 100 |
| 3 | dioxane (containing 5% $H_2O$) | 50 | 49 | 89 | 78 |
| 4 | THF (containing 5% $H_2O$) | 96 | 50 | 95 | 146 |
| 5 | acetone (containing 5% $H_2O$) | 32 | 49 | 89 | 78 |
| 6 | acetone (containing 10% $H_2O$) | 20 | 50 | 94 | 115 |
| 7 | acetone (containing 30% $H_2O$) | 6 | 51 | 76 | 14 |

[a] All reactions were carried out by shaking a mixture of substrate (80 mg) and catalyst beads (16 mg) in a given organic solvent (4.0 ml) at 30°C. [b]The solvent system was prepared according to the procedure described in ref. 17. [c]Enantiomeric excess (ee) was determined by HPLC with chiral stationally phase.[14] [d] E value (enantiomeric ratio), see ref. 19.

ca. 50% conversion). The reaction mixture was filtered to remove the catalyst beads. After evaporation of the filtrate, the residue was partitioned between ethyl acetate and aqueous $NaHCO_3$ solution, from which the (*R*)-carboxylic acid could also be recovered. The organic layer was washed with water and brine, dried, and evaporated. The residue was purified by flash chromatography to give (*S*)-**4** (4.53 g, 93% ee, 50% yield, E=94) as a colorless oil. Compound (*S*)-**4** formed a salt with *d*-camphorsulfonic acid. Recrystallization of the salt from ethyl acetate, followed by desalination enhanced the enantiomeric purity (97.5% ee, $[\alpha]_D^{20}$ +104.7 (c=1.0, $CHCl_3$)).

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