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Rational *De Novo* Design of NADH Mimic for Stereoselective Reduction Based on Molecular Orbital Calculation

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***Abstract**: The methodology of rational design of NADH mimics in stereoselective reduction of carbonyl and imino groups based on molecular orbital calculation was described. The designed NADH mimics **1a** and **1b** were subjected to the reduction of benzoylformate and acetyliminophenylacetate. As expected from the calculations of the transition-states, the reduction with **1a** proceeded with high stereoselectivity in both substrates, while **1b** showed much lower chirality transfer.*

Introduction

Nicotinamide adenine dinucleotide (NAD^+) and its phosphate ($NADP^+$) are major cofactors in the enzymatic oxido-reduction and have been studied extensively.[1] The reduced forms of these cofactor, NADH and NADPH, are particularly interesting because, generally in the dehydrogenase and reductase reactions, one hydrogen of the prochiral C-4 methylene group in the dihydropyridine ring is transferred to a trigonal center of the specific substrate giving rise to a new chiral tetragonal center stereospecifically, even in the stereochemically cryptic cases. The first seminal stereoselective reduction of an achiral substrate with NADH mimics was reported by Ohno *et al.* in 1975.[2] Since then, varieties of NADH mimics have been synthesized by imposing various chiral auxiliaries and functional groups on the side chain of the dihydronicotinamide and its analogs or a methyl group of the 1,4-dihydropyridine structure, to analyze the features of such stereospecific reduction and to explore applications of the potential of the dihydropyridine chemistry to organic synthesis.[3,4]

Because most of the achiral and chiral NADH mimics so far reported require the presence of a suitable bivalent metal ion such as magnesium (II) to achieve efficient reductions, specific coordination of the metal ion is believed to be involved in to the polar groups of the substrate and the NADH mimics.[3-5] Generally, by imposing a chiral auxiliary group, such coordination induces formation of chiral ternary complex so that one particular enantiotopic face of a trigonal substrate preferentially approaches to one particular face of the dihydropyridine ring thereby leading to the stereoselective reduction.

Recently, we proposed a general chirality transfer mechanism based on the calculation of the transition-state of the hydride transfer from dihydropyridine derivative to benzoylformate by semiempirical molecular orbital method.[6] Also proposed was importance of an additional intramolecular polar coordinating group to a bivalent metal ion for enhancement of the stereoselectivity in the reduction of benzoylformate with such NADH mimics. These results allowed us more precise and rational design of NADH model compounds with high reactivity and stereoselectivity based on these approach. In this paper, we describe the novel design of the NADH models possessing intramolecular polar coordinating group and their ability for the reduction of benzoylformate and acetyliminophenylacetate as standard substrates for NADH mimics.[3-5,7]

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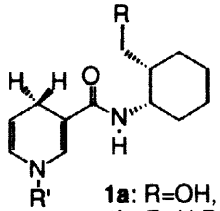
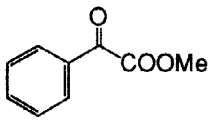
Results and Discussion

Design of chiral NADH mimics

The objective of this study is to develop the methodology of rational design of chiral NADH mimics for the stereoselective reduction of C=O bond in benzoylformate **2** and C=N bond in acetyliminophenylacetate **3**, which ultimately lead to chiral mandelate and phenylglycinate, respectively. The design was based on the transition-state of the hydride transfer from dihydropyridine derivative to benzoylformate previously deduced by semiempirical molecular orbital method.[6] Preliminary computational modeling by imposing a chiral auxiliary possessing a coordination group to a metal ion, especially a hydroxyl group, into the transition-state structure allowed us to figure out *de novo* a candidate molecule **1a**. The designed molecule **1a** contains a conformationaly rigid cyclohexane ring, which appears to be important in fixing the conformation of a reacting ternary complex. Role of such a hydroxy oxygen was previously noted by Bourguignon *et al.*[8] As a control to **1a**, a deoxygenated derivative **1b** was also synthesized and examined as well.



1a: R=OH, R'=Bn
1b: R=H,R'=Bn
1c: R=OH, R'=Me
1d: R=H, R'=Me



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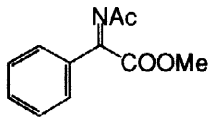

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Although several hydride transfer reactions mediated by NADH derivatives were theoretically calculated on the *ab initio* level,[9] the systems were too much simplified. We thus undertook an advantage of semi-empirical molecular orbital method, which is also capable of yielding relevant calculated structures of transition-state even for rather complicated systems with the saving of computational resources. At first, to test the ability for stereoselective reduction of the NADH mimics **1a** and **1b**, we undertook MNDO-PM3 calculation[10] for the transition-state of reduction of methyl benzoylformate as reported previously.[6] Because four reaction pathways were essentially conceivable, calculation must be carried out for these four possible pathways in the presence of magnesium (II) ion by using the more simplified molecules **1c** and **1d**, where the migrating hydrogen was either the *pro-R* or *pro-S* hydrogen atom at the C-4 position of dihydronicotinamide and the electrophilic trigonal center was either the *re*-face or the *si*-face of benzoylformate (or acetyliminophenylacetate) as shown in Figure 1.

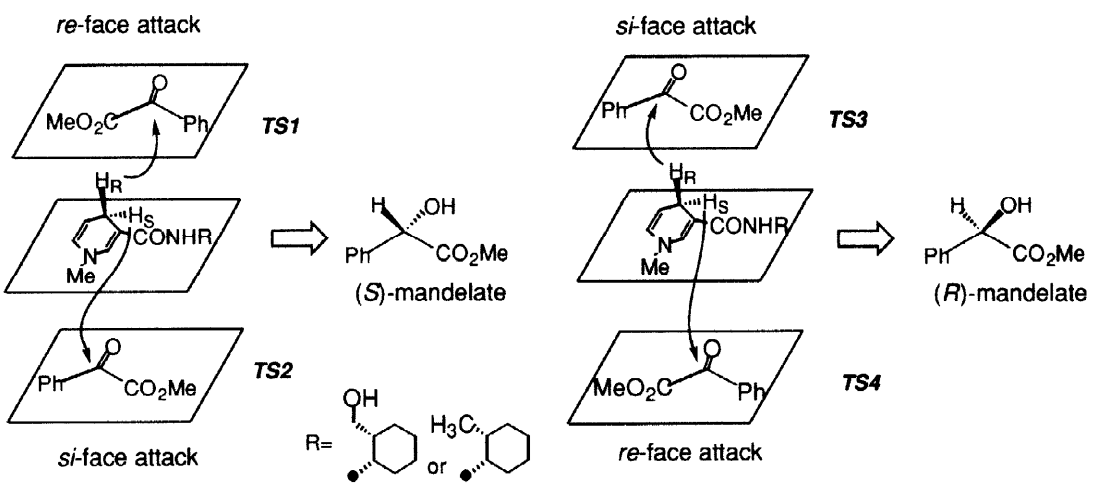


Figure 1

Prior to the actual calculation of the transition-states, the conformation of the cyclohexane moiety of the NADH mimics of **1c** and **1d** was deduced by molecular mechanics MM2 method.[11] Then, by imposing the most stable conformation, calculations of the transition-states (***TS1-TS4***) in the presence of a magnesium (II) ion were performed with MNDO-PM3 semi-empirical molecular orbital method.[10] The plausible transition-state structures for each reaction mode were successfully located on the restricted Hartree-Fock (RHF) energy hypersurface. The resulted transition-state structures for the four possible pathways of the hydride transfer of **1c** to benzoylformate (***TS1-TS4***) are shown in Figure 2. The calculated values of the heat of formation (ΔH_f) for each transition-state are also included in Figure 2. These results strongly suggested that the reduction of benzoylformate with NADH mimics **1a** would yield (*R*)-mandelate with high stereoselectivity depending upon the difference of ΔH_fs of ***TS1-TS4***.

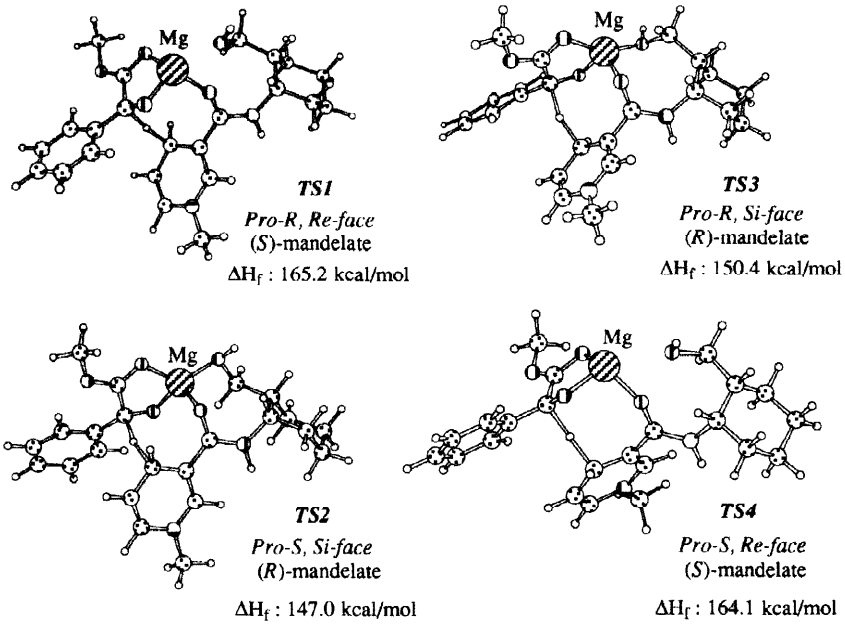


Figure 2

Subsequently, all possible transition-states in the reduction of benzoylformate **2** with **1d** and in the reduction of acetyliminophenylacetate **3** with **1c** and **1d** were similarly calculated. The most stable transition-state in the reaction of **3** with **1c** is shown in Figure 3. The predicted stereochemical courses of the reduction from these calculations are summarized in Table 1. These results strongly suggested that the NADH mimics **1c** (**1a**) possessing a hydroxyl group may significantly enhance the stereoselectivity in the reduction of benzoylformate and acetyliminophenylacetate compared with **1d** (**1b**). Furthermore, reversal of stereoselectivity may also be anticipated in the reduction depending on the presence or absence of the free hydroxyl group. With these calculation results in hand, we tested the NADH mimics **1a** and **1b** in the experimental reduction of benzoylformate and acetyliminophenylacetate.

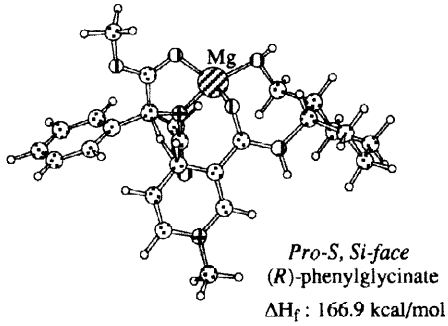


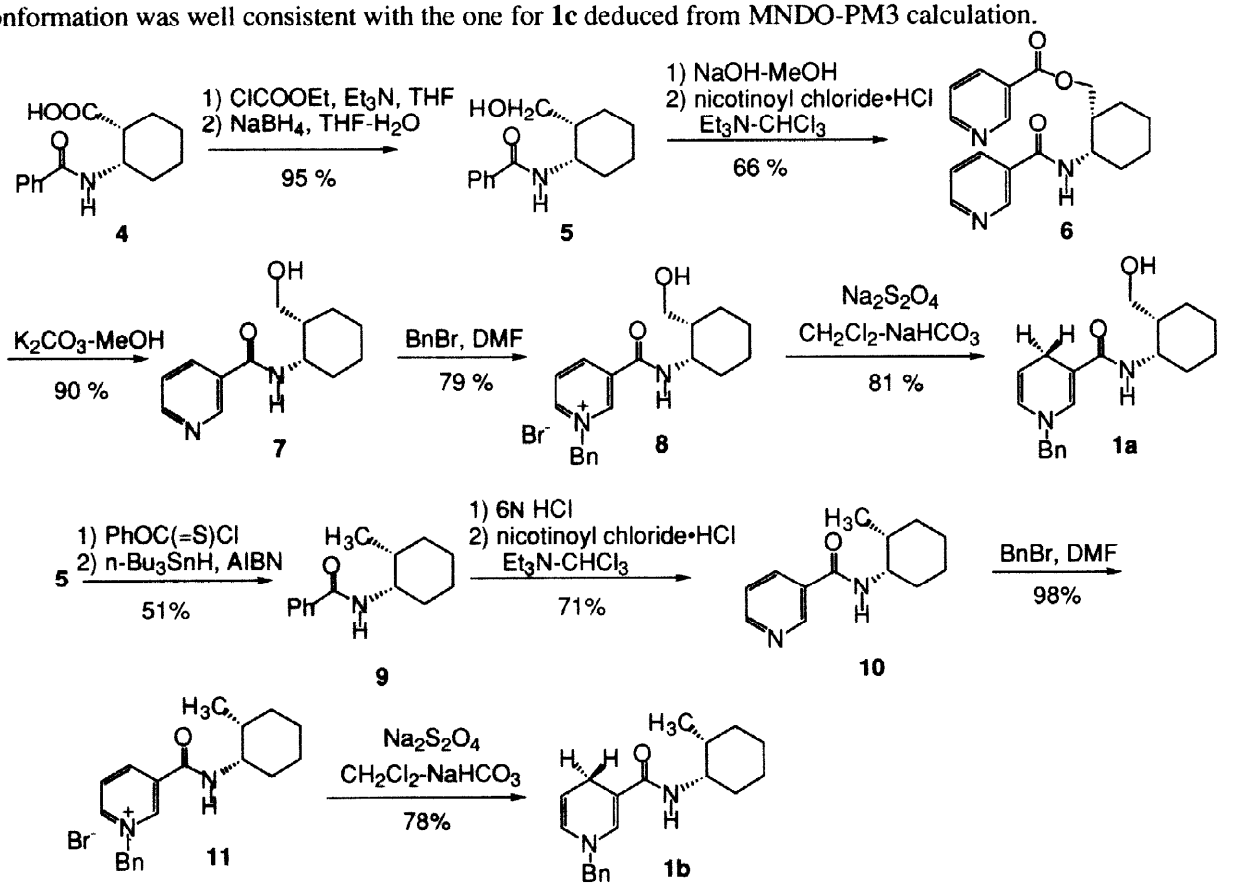
Figure 3

Table 1. The predicted stereochemical course of NADH mimics from the calculated transition-states.

NADH mimics	substrate	expected major product	expected enatiomeric excess (%)
1c (1a)	**2**	(*R*)-mandelate	>99
1d (1b)	**2**	(*S*)-mandelate	38
1c (1a)	**3**	(*R*)-phenylglycinate	>99
1d (1b)	**3**	(*S*)-phenylglycinate	4

Synthesis of NADH mimics 1a and 1b, and stereoselectivity in the reduction of benzoylformate and acetyliminophenylacetate with 1a and 1b.

The syntheses of chiral NADH mimics **1a** and **1b** are summarized in Scheme 1. The commercially available (-)-*cis*-2-benzamidocyclohexanecarboxylic acid **4** was treated with ethyl chloroformate and triethylamine in THF to give a mixed anhydride, which was reduced with sodium borohydride in THF-water to furnish alcohol **5** in 95 % yield. The amide moiety of **5** was hydrolyzed with 30% NaOH in methanol, and the resulting aminocyclohexcylmethanol was then treated with an excess amount of nicotinoyl chloride hydrochloride in the presence of triethylamine to give **6** in 66 % yield. Selective hydrolysis of the ester moiety of **4** was performed with 1 equivalent of K_2CO_3 in methanol to give **7** in 90 % yield. Conversion of **7** into NADH mimic **1a** was accomplished by the following. Thus, quaternarization of **7** with benzyl bromide in DMF gave **8** in 79 % yield. Transformation of these pyridinium salts **8** into the dihydronicotinamide form was performed by treating with $Na_2S_2O_4$ in 1M $NaHCO_3$-CH_2Cl_2,[12] and the desired NADH mimic **1a** was obtained in 81 % yield. The conformation of the cyclohexane moiety of **1a** was analyzed by ^{1}H-NMR. The observed coupling constants as depicted in Figure 3 clearly indicated that the hydroxymethyl group was located in an equatorial orientation and the amide group in an axial position in the chair cyclohexane structure. This conformation was well consistent with the one for **1c** deduced from MNDO-PM3 calculation.



Scheme 1

The deoxy-analog **1b** was also prepared from **5** by deoxygenation of the hydroxyl group according to Barton's procedure.[13] Treatment of **5** with phenyl chlorothionoformate in the presence of 4-dimethylamino-pyridine, and the reduction of the resulting thionocarbonate with tributyltin hydride in the presence of AIBN in refluxing benzene afforded **9** in 51 % yield. Acid hydrolysis, followed by condensation with nicotinoyl group gave **10** in 71 % yield. The conformation of the cyclohexane moiety of **10** was also verified by ^{1}H-NMR analysis at this stage as shown in Figure 4. The most stable conformation of this moiety was again consistent with the calculation. Transformation of **10** into dihydronicotinamide form was carried out similarly as described above to give NADH mimic **1b** in 76 % yield in two steps.

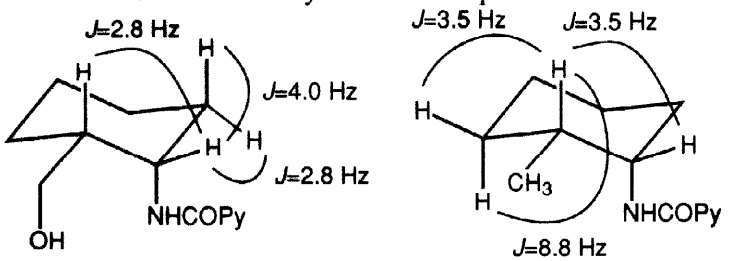


Figure 4

Having had the desired NADH mimics, the selective potential of **1a** and **1b** in reduction was examined by standard methodology. Methyl benzoylformate and methyl acetyliminophenylacetate[14] were separately treated with the synthesized NADH mimics **1a** or **1b** in the presence of an equimolar amount of magnesium perchlorate in acetonitrile under inert atmosphere. The chiral purity and absolute configuration of the resulting mandelate and *N*-acetylphenylglycinate were analyzed by ^{1}H-NMR spectroscopy after derivatization into (+)-(*R*)-MTPA ester for mandelate or in the presence of chiral shift reagent europium tris[3-(heptafluoropropyl-hydroxymethylene)-(+)-camphorate] [(+)-Eu(hfc)$_3$] for *N*-acetylphenylglycinate. The results are summarized in Table 2.

Table 2. The stereoselectivity of NADH mimics **1a** and **1b**.

NADH mimics	substrate	product	chemical yield (%)	observed enantiomeric excess (%)
1a	**2**	methyl (*R*)-mandelate	81	80
1b	**2**	methyl (*S*)-mandelate	40	20
1a	**3**	methyl (*R*)-phenylglycinate	38	70
1b	**3**	n.d.	< 10	n.d.

n.d.; not determined.

As anticipated from the calculation, the NADH mimic **1a** turned out to be more effective in chirality transfer toward both of methyl benzoylformate and acetyliminophenylacetate (80 and 70 % ee, respectively) than its counterpart **1b**. Significantly low enantioselectivity (20 % ee) was observed in the reduction of benzoylformate with **1b** as predicted by the calculation. Furthermore, reversal of stereoselectivity was also observed as anticipated due to the absence of a free hydroxyl group in the reduction of the benzoylformate. The reduction of acetyliminophenylacetate with **1b** was sluggish, probably because its intrinsic lower electrophilicity compared with a carbonyl group. As suggested from the transition-state structures as shown in Figure 2, the multiple chelation appeared to firmly fix the conformation of a reacting ternary complex **1a** so that both the reactivity and stereoselectivity of the hydride transfer were efficiently enhanced. However, the observed enantioselectivities were slightly lower than the calculated values. This may be due to the limitation that the calculations were carried out without considerations of effects of the solvent, the counter ion, and thermal vibration. Although the observed enantioselectivities were not necessarily superior to those reported previously,[4] the present transition-state modeling studies appear to address a new way in precise and rational design of NADH model compounds for the reduction of carbonyl and imino groups.

In summary, the methodology of rational design of NADH mimics for stereoselective reduction of

carbonyl and imino groups based on molecular orbital method was successfully developed. As expected from the calculations of the transition-states, the designed NADH mimics **1a** was able to reduce both substrates in the predictable fashion with high stereoselectivity, while its deoxy-analog **1b** showed much lower chirality transfer. The transition state model is not only useful in interpreting the stereoselectivity of the reduction with artificial NADH mimics, but plausibly shows a general reaction surface of the oxidation-reduction of NAD(P)/NAD(P)H in the enzymes as well.

Experimental

Melting points were measured with a Yanagimoto BY-1 melting point apparatus and are uncorrected. UV spectra were measured on a Shimadzu UV-160A spectrometer in ethanol solution. IR spectra were taken on a Hitachi 285 infrared spectrometer. ^1H and ^{13}C NMR spectra was recorded on a JEOL GSX-270 and/or a JEOL LA-300 spectrometer. Deuteriochloroform (99.8 % atom enriched, Aldrich) was used for the NMR solvent throughout. ^1H and ^{13}C NMR chemical shift were reported in δ value based on internal reference, tetramethylsilane (0 ppm) and $CDCl_3$ (77.0 ppm), respectively. Column chromatography was carried out with a Kieselgel 60 (70-230 mesh, Merck). All reactions were carried out in an inert (Ar or N_2) atmosphere. Because of their instability to air and light, synthetic NADH mimics were immediately used after preparation without further purification.

N-[(1*S*, 2*R*)-2-hydroxymethylcyclohexyl]benzamide (5)

Ethyl chloroformate (0.9 ml, 9.45 mmol) was added dropwise to a mixture of (-)-*cis*-2-benzamidocyclohexanecarboxylic acid **4** (2.01 g, 8.13 mmol) and triethylamine (1.3 ml, 9.38 mmol) in THF (20 ml) at -5°C. After 90 min, the resulted precipitates were filtered and washed with THF. A suspension of $NaBH_4$ (0.8 g, 21.1 mmol) in water (5 ml) was added to the filtrate at 0°C and the mixture was stirred for 1 h. Saturated aqueous NH_4Cl solution was added and the mixture was extracted with ether. The extract was washed with brine, dried over Na_2SO_4 and concentrated to dryness. The residue was chromatographed over silica gel with hexane-ethyl acetate (5:1-2:1) to afford **5** (1.79 g, 95 %) as white solid. m.p. 132.5-133.5 °C. IR ν_{max}/cm^{-1} ($CHCl_3$): 3650, 3450, 3010, 2960, 1650. ^1H NMR δ 1.12 (m, 1H, cyclohexyl), 1.40 (m, 3H, cyclohexyl), 1.72-1.92 (m, 5H, cyclohexyl), 3.28 (dt, 1H, *J*=2.0, 5.0 Hz, C*H*$_2$OH), 3.42 (ddd, 1H, *J*=5.0, 4.0, 2.0 Hz, C*H*$_2$OH), 4.44 (dd, 1H, *J*=5.0, 2.0 Hz, OH), 4.52 (dd, 1H, *J*=4.0, 2.0 Hz, 1'-H), 6.58 (s, 1H, NH), 7.43-7.54 (m, 3H, aromatic), 7.77 (m, 2H, aromatic). ^{13}C-NMR δ 21.5, 23.9, 24.7, 30.0, 43.0, 45.6, 63.9, 126.9, 128.7, 131.7, 134.1, 168.5. *Anal.* Calcd for $C_{14}H_{19}NO_2$: C, 72.07; H, 8.21; N, 6.00. Found: C, 72.36; H, 8.33; N, 5.90 .

3-*N*-[(1*S*, 2*R*)-2-(3-nicotinoyloxymethyl)cyclohexyl]carbamoylpyridine (6)

A solution of 30 % aqueous sodium hydroxide (4 ml) was added to a solution of **5** (1.28 g, 5.48 mmol) in methanol (6 ml) and the mixture was refluxed for 36 h. The reaction mixture was acidified with 6N HCl (pH=1) and was extracted with $CHCl_3$. The aqueous phase was neutralized with $NaHCO_3$ and evaporated. $CHCl_3$ was added to the residue and the insoluble materials were filtered off. The filtrate was dried over Na_2SO_4 and evaporated to give a crude 1-(*R*)-*cis*-2-aminocyclohexanemethanol (596 mg, 84 %), which was used without further purification. To a solution of 1-(*R*)-*cis*-2-aminocyclohexanemethanol (596 mg, 4.62 mmol) in CH_2Cl_2 (10 ml) were added nicotinoyl chloride hydrochloride (1.76 g, 9.88 mmol) and triethylamine (5.72 ml, 41.1 mmol) at 0°C. The mixture was stirred at room temperature for 10 h. Water was added and the mixture was extracted with $CHCl_3$. The extract was washed with saturated aqueous $NaHCO_3$ solution and brine, dried over Na_2SO_4 and concentrated to dryness The residue was chromatographed over silica gel with chloroform-methanol (10:1) to give **6** (1.03 g, 66 %) as yellow solid. m.p. 140.0-140.5 °C. IR ν_{max}/cm^{-1} ($CHCl_3$): 3450, 3330, 1720, 1660. ^1H-NMR δ 1.35-1.80 (m, 8H, cyclohexyl), 2.28 (s, 1H, 2'-H), 4.23 (dd, 1H, *J*=10.5, 6.5 Hz, C*H*$_2$OH), 4.33 (dd, 1H, *J*=10.5, 7.0 Hz, C*H*$_2$OH), 4.50 (m, 1H, 1'-H), 6.60 (s, 1H, NH), 7.30 (m, 2H, 5- and 5''-H), 7.98 (dt, 1H, *J*=8.0, 1.5 Hz, 4-H), 8.18 (dt, 1H, *J*=8.0, 1.5 Hz, 4''-H), 8.59 (dd, 1H, *J*=4.8, 1.5 Hz, 6-H), 8.66 (dd, 1H, *J*=5.0, 1.5 Hz, 6''-H), 8.88 (d, 1H, *J*=1.5 Hz, 2-H), 9.20 (d, 1H, *J*=1.5

Hz, 2"-H). ^{13}C-NMR δ 22.0, 23.6, 25.2, 29.9, 38.8, 46.9, 66.0, 123.2, 123.4, 125.9, 130.4, 135.0, 137.0, 147.7, 150.8, 152.1, 153.4, 165.1, 165.3. *Anal.* Calcd for $C_{19}H_{21}N_3O_3$: C, 67.24; H, 6.24; N, 12.38. Found: C, 67.07; H, 6.20; N,12.17 .

3-*N*-[(1*S*, 2*R*)-2-hydroxymethylcyclohexyl]carbamoylpyridine (7)

A mixture of **6** (946 mg, 2.79 mmol) and potassium carbonate (200 mg, 1.45 mmol) in methanol (8 ml) was stirred for 10 min at room temperature. The reaction was quenched by addition of water and the mixture was extracted with $CHCl_3$. The extract was washed with brine, dried over Na_2SO_4 and concentrated to dryness. The residue was chromatographed over silica gel with $CHCl_3$-methanol (20:1) to give **7** (590 mg, 90 %) as white solid. m.p. 104-104.5 °C (hexane-ethyl acetate). IR ν_{max}/cm^{-1} ($CHCl_3$): 3450, 3390, 3000, 2940, 2850, 1660. ^{1}H-NMR δ 1.66-2.00 (m, 9H, cyclohexyl), 3.48 (m, 2H, C*H*$_2$OH), 4.37 (s, 1H), 4.48 (m, 1H, 1'-H), 7.00 (s, 1H, NH), 7.40 (dd, 1H, *J*=8.0, 4.8 Hz, 5-H), 8.13 (dt, 1H, *J*=8.0, 1.5 Hz, 4-H), 8.72 (dd, 1H, *J*=4.8, 1.6 Hz, 6-H), 8.97 (d, 1H, *J*=1.6 Hz, 2-H). ^{13}C-NMR δ 21.9, 24.2, 24.5, 29.5, 42.2, 47.0, 63.9, 123.6, 130.0, 135.3, 147.6, 152.2, 166.2. *Anal.* Calcd for $C_{13}H_{18}N_2O_2$: C, 66.64; H, 7.74; N, 11.96. Found: C, 66.86; H, 7.75; N,11.92 .

1-Benzyl-3-*N*-[(1*S*, 2*R*)-2-hydroxymethylcyclohexyl]carbamoylpyridinium bromide (8)

Benzyl bromide (0.33 ml, 2.77 mmol) was added to a solution of **7** (441 mg, 1.88 mmol) in DMF (5 ml). The resulting mixture was stirred for 24 h at room temperature. After removal of the solvent, the residue was chromatographed over silica gel with $CHCl_3$-methanol (6:1) to give **8** (603 mg, 79 %) as yellow oil. UV λmax (ε): 207.5 nm (18000). IR ν_{max}/cm^{-1} ($CHCl_3$): 3660, 3330, 2950, 2860, 1660, 1630. ^{1}H-NMR δ 1.25-2.10 (m, 9H, cyclohexyl), 2.80 (s, 1H, 2'-H), 3.55 (m, 1H, C*H*$_2$OH), 3.70 (m, 1H, C*H*$_2$OH), 4.32 (s, 1H, OH), 4.38 (m, 1H, 1'-H), 6.20 (s, 2H, PhC*H*$_2$), 7.40 (m, 3H, aromatic), 7.63 (m, 2H, aromatic), 8.04 (dd, 1H, *J*=8.0, 5.5 Hz, 5-H), 8.80 (d, 1H, *J*=8.0 Hz, 4-H), 8.92 (d, 1H, *J*=8.0 Hz, 6-H), 9.29 (d, 1H, *J*=5.5 Hz, 2-H). ^{13}C-NMR δ 22.8, 23.3, 25.7, 28.9, 41.0, 49.9, 63.3, 64.3, 76.5, 127.8, 129.5, 1129.7, 130.2, 132.2, 134.8, 144.0, 145.0, 145.4, 161.4. High resolution FAB-MS: Found: m/z: 325.1930. Calcd for $C_{20}H_{25}N_2O_2$: 325.1918.

1-Benzyl-3-*N*-[(1*S*, 2*R*)-2-hydroxymethylcyclohexyl]carbamoyl-1,4-dihydropyridine (1a)

A mixture of **8** (166 mg, 0.41 mmol) and $Na_2S_2O_4$ (0.79 g, 4.55 mmol) in CH_2Cl_2 (20 ml) and aqueous 1M $NaHCO_3$ (18 ml) was stirred for 2 h in the dark. Layers were separated and the aqueous layer was extracted with $CHCl_3$. The combined organic phase was washed with brine, dried (Na_2SO_4), filtered and concentrated to dryness to give **1a** as amorphous (109 mg, 81 %). UV λmax(ε): 211.5 nm (21000), 349.5nm (6100). IR ν_{max}/cm^{-1} ($CHCl_3$): 3470, 3360, 3000, 2940, 2860, 1690, 1630. ^{1}H-NMR δ 0.85 (m, 1H, cyclohexyl), 1.18-1.36 (m, 4H, cyclohexyl), 1.62-1.81 (m, 4H, cyclohexyl), 3.10 (dt, *J*=3.0, 12.0 Hz, 1H, C*H*$_2$OH), 3.20 (d, 2H, *J*=3.0, 12.0 Hz, 4-H), 3.32 (dt, *J*=3.0, 12.0 Hz, 1H, C*H*$_2$OH), 4.30 (s, 2H, PhC*H*$_2$), 4.42 (dt, *J*=4.0, 2.8 Hz, 1'-H), 4.78 (dt, 1H, *J*=8.0, 3.0 Hz, 5-H), 5.04 (dd, 1H, *J*=10.0, 3.0 Hz, OH), 5.38 (dd, 1H, *J*=8.0, 2.0 Hz, 6-H), 5.78 (d, 1H, *J*= 2.0 Hz, 2-H), 7.20-7.40 (m, 6H, aromatic and NH). ^{13}C-NMR δ 21.4, 22.5, 23.4, 25.0, 30.4, 43.2, 44.0, 57.5, 63.7, 98.1, 102.4, 127.2, 127.8, 128.3, 128.8, 129.2, 137.1, 139.8, 168.8.

***N*-[(1*S*,2*R*)-2-methylcyclohexyl]benzamide (9)**

A mixture of **5** (0.69 g, 2.97 mmol), 4-dimethylaminopyridine (1.1 g, 8.93 mmol), and phenyl chlorothionoformate (1.0 ml, 7.38 mmol) in CH_2Cl_2 (10 ml) was stirred at 0°C for 6h. Water was added and the mixture was extracted with ether. The extract was washed successively with 1N HCl, saturated aqueous $NaHCO_3$ solution and brine, dried over Na_2SO_4, and concentrated to dryness. A mixture of the obtained residue, n-Bu_3SnH (0.61 ml, 2.28 mmol), and AIBN (80 mg) in benzene (25 ml) was heated at reflux for 4.5 h. After removal of the solvent, the residue was chromatographed over silica gel with hexane-ethyl acetate

(10:1) to give **9** (379 mg, 51 %) as colorless powder. m.p. 108-108.5 °C (hexane-ethyl acetate). IR ν_{max}/cm^{-1} ($CHCl_3$): 3480, 3010, 2940, 2870, 1660. ^{1}H-NMR δ 0.95 (d, 3H, *J*=7.0 Hz, CH_3), 1.22-1.68 (m, 7H, cyclohexyl), 1.78 (m, 1H, cyclohexyl), 1.97 (m, 1H, cyclohexyl), 4.28 (m, 1H, cyclohexyl), 6.15 (d, 1H, *J*=7.8 Hz, NH), 7.40-7.54 (m, 3H, aromatic), 7.76 (m, 2H, aromatic). ^{13}C-NMR δ 16.50, 22.27, 23.51, 29.65, 30.26, 33.64, 49.76, 126.74, 128.55, 131.23, 135.27, 166.99. *Anal.* Calcd for $C_{14}H_{19}NO$: C, 77.38; H, 8.81; N, 6.45. Found: C, 77.23; H, 9.03; N, 6.75.

3-*N*-[(1*S*,2*R*)-2-methylcyclohexyl]carbamoylpyridine (10)

A mixture of **9** (1.34 g, 6.17 mmol) and 6N HCl (30 ml) was refluxed for 96 h. After being cooled to room temperature, the mixture was extracted three times with ether. The aqueous layer was concentrated to dryness. A mixture of the crystalline residue, nicotinoyl chloride hydrochloride (1.37 g, 7.68 mmol), and Et_3N (3.0 ml, 21.6 mmol) was stirred first at 0°C for 10 min, and then at room temperature for 5 h. Saturated aqueous NH_4Cl solution was added and the mixture was extracted three times with $CHCl_3$. The extract was washed with brine, dried, and concentrated to dryness. The residue was chromatographed over silica gel with hexane-ethyl acetate (1:1) to give **10** (954 mg, 71 %) as colorless powder. m.p. 88-89 °C (hexane-ethyl acetate). IR ν_{max}/cm^{-1} ($CHCl_3$): 3740, 3020, 2900, 2870, 1660· ^{1}H-NMR δ 0.95 (d, 1H, *J*=7.1 Hz, CH_3), 1.25-1.70 (m, 7H, cyclohexyl), 1.79 (m, 1H, cyclohexyl), 1.98 (m, 1H, cyclohexyl), 4.29 (m, 1H, cyclohexyl), 6.19 (d, 1H, *J*=8.0 Hz, NH), 7.40 (ddd, 1H, *J*=1.0, 5.0, 8.0 Hz, 4-H), 8.11 (ddd, 1H, *J*=1.8, 2.2, 8.0 Hz, 6-H), 8.72 (dd, *J*=1.8, 5.0 Hz, 5-H), 8.96 (dd, 1H, *J*=1.0, 2.2 Hz, 2-H). ^{13}C-NMR δ 16.43, 22.28, 23.37, 29.53, 30.23, 33.52, 50.09, 123.53, 130.87, 135.07, 147.58, 152.08, 165.06. *Anal.* Calcd for $C_{13}H_{18}N_2O$: C, 71.52; H, 8.31; N, 12.83. Found: C, 71.48; H, 8.43; N, 13.03.

1-Benzyl-3-*N*-[(1*S*,2*R*)-2-methylcyclohexyl]carbamoylpyridinium bromide (11)

A mixture of **10** (138 mg, 0.63 mmol) and benzyl bromide (0.15 ml, 1.26 mmol) in DMF (3 ml) was stirred at room temperature for 21 h. After removal of the solvent, the obtained residue was chromatographed over silica gel with $CHCl_3$-methanol (10:1) to give **11** (241 mg, 98 %) as amorphous. UV λmax(ε): 209.8 nm (19000). IR ν_{max}/cm^{-1} ($CHCl_3$): 3670, 2940, 1670. ^{1}H-NMR δ 1.02 (d, 3H, *J*=6.8 Hz, CH_3), 1.25-2.10 (m, 9H, cyclohexyl), 4.26 (m, 1H, cyclohexyl), 6.14 (s, 2H, $PhCH_2$), 7.45 (m, 3H, aromatic), 7.59 (m, 2H, aromatic), 7.94 (dd, 1H, *J*=6.2, 8.0 Hz, 5-H), 8.68 (d, 1H, *J*=1.2, 8.0 Hz, 4-H), 8.90 (dd, 1H, *J*=1.2, 6.2 Hz, 6-H), 10.28 (s, 1H, 2-H). ^{13}C-NMR δ 15.84, 22.63, 23.01, 28.70, 30.26, 33.24, 52.34, 64.37, 127.37, 129.52, 129.79, 130.33, 132.07, 135.27, 144.26, 144.80, 145.37, 161.14. High resolution FAB-MS: Found: *m/z*: 309.1967. Calcd for $C_{20}H_{25}N_2O$: 309.1988.

1-Benzyl-3-*N*-[(1*S*,2*R*)-2-methylcyclohexyl]carbamoyl-1,4,dihydropiridine (1b)

A mixture of **11** (157 mg, 0.40 mmol), $Na_2S_2O_4$ (1.5 g, 8.6 mmol) in CH_2Cl_2 (9 ml), and aqueous 1M $NaHCO_3$ (8 ml) was stirred for 3 h in the dark. Layers were separated and the aqueous layer was extracted with $CHCl_3$. The combined organic phase was washed with brine, dried (Na_2SO_4), filtered and concentrated to dryness to give **1a** as amorphous (97 mg, 78 %) as amorphous. UV λmax(ε): 349.6 nm (7300). IR ν_{max}/cm^{-1} ($CHCl_3$): 3760, 3030, 2330, 1690, 1630. ^{1}H-NMR δ 0.85 (d, 3H, *J*=6.8 Hz, CH_3), 1.10-1.70 (m, 8H, cyclohexyl), 1.85 (m, 1H, cyclohexyl), 3.20 (br, 2H, 4-H), 4.17 (m, 1H, cyclohexyl), 4.29 (s, 2H, $PhCH_2$), 4.72 (dt, 1H, *J*=3.2, 7.9 Hz, 5-H), 5.22 (d, 1H, *J*=9.0 Hz, NH), 5.76 (ddt, 1H, *J*=1.3, 3.2, 7.9 Hz, 6-H), 7.16 (d, 1H, *J*=1.3 Hz, 2-H), 7.22-7.46 (m, 5H, aromatic). ^{13}C-NMR δ 22.19, 22.67, 23.67, 29.90, 30.29, 33.68, 48.81, 57.33, 99.63, 101.95, 127.20, 127.66, 128.73, 129.29, 129.69, 137.44, 138.73, 167.15.

Reduction of methyl benzoylformate

To a solution of $Mg(ClO_4)_2$ (0.1 mmol) and an NADH mimic (0.1 mmol) in dry acetonitrile (2.5 ml) was added methyl benzoylformate (14 μl, 0.1 mmol) *via* a syringe. The mixture was stirred at room temperature

and the reaction was monitored by TLC. After the reaction was completed, $CHCl_3$ and water were added. The layers were separated and the aqueous layer was extracted with $CHCl_3$. The combined organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated to dryness. The yield of methyl mandelate was estimated at this stage by ^1H NMR without further purification. The absolute configuration of the resulting methyl mandelate was determined with ^1H NMR by comparison the corresponding (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetyl (MTPA) derivative with an authentic specimen. The chiral purity was calculated from the integrations of methine signals of an (*R*)-MTPA derivative with ^1H NMR [6.12 ppm for (*S*)-mandelate and 6.09 ppm for (*R*)-mandelate]. MTPA ester of the resulted methyl mandelate was prepared as follows: each crude methyl mandelate product (15 mg) was dissolved in pyridine (0.2 ml), to which (*S*)-(-)-MTPA chloride (70 μl) (prepared from (*R*)-(+)-MTPA acid and $SOCl_2$) was added. The mixture was stirred for 30 min at room temperature. Water was added and the mixture was extracted with ethyl acetate. The extract was successively washed with 2N HCl, sat $NaHCO_3$, brine, dried over Na_2SO_4, filtered and concentrated to dryness. The residue was directly analyzed by ^1H NMR without further purification.

Reduction of methyl acetyliminophenylacetate

To a solution of $Mg(ClO_4)_2$ (0.2 mmol) and an NADH mimic (0.2 mmol) in dry acetonitrile (3 ml) was added methyl acetyliminophenylacetate (0.2 mmol) in acetonitrile (1 ml) *via* a syringe. The mixture was stirred at room temperature for 6.5 h, and then $CHCl_3$ and water were added. The layers were separated and the aqueous layer was extracted three times with $CHCl_3$. The combined organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated to dryness. The yield of methyl *N*-acetylphenylglycinate was estimated at this stage by ^1H NMR without further purification. The chiral purity of the resulted methyl *N*-acetylphenylglycinate was determined by ^1H NMR with the use of (+)-Eu(hfc)$_3$. After being chromatographed over silica gel (hexane-ethyl acetate, 1/1), the solution of the purified methyl *N*-acetylphenylglycinate (2.5 mg, 0.012 mmol) in $CDCl_3$ (0.6 ml) was mixed with (+)-Eu(hfc)$_3$ (28 mg, 0.024 mmol) and the optical purity was calculated from integrations of methyl signals of *N*-acetyl group in ^1H NMR [10.13 ppm for (*R*)-phenylglycinate and 10.28 ppm for (*S*)-phenylglycinate].

Computation

The MOPAC (version 6.0) molecular orbital package[15] utilizing the MNDO-PM3 Hamiltonian[10] was used for the semi-empirical MO calculations. Refinements for each transition state were carried out by the Baker's eigenvector following method[16] with the use of the keyword TS implemented in the MOPAC. No arbitrary assumption was imposed on to find the most likely geometry for the transition state. All the transition states were characterized by the presence of one and only one negative force constant in the Hessian matrix of a force calculation.[17] All starting geometries were generated using the graphics interface of Chem 3D™ (Cambridge Scientific Computing, Inc.). All calculations were performed on a IBM RS-6000 computer.

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