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NADH Mimics on Diacetone-D-glucose: Stereoselective Biomimetic Reduction of Benzoylformate and Interpretation of Chirality Transfer Deduced by Molecular Orbital Approach[1]

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Key Words: NADH mimics; synthesis; diacetone-D-glucose template; asymmetric reduction; semi-empirical calculation

Abstract: *We have prepared novel NADH mimics, in which the 1,4-dihydronicotinamide structure is connected to the diacetone-D-glucose molecule via its C-1 nitrogen, e.g. compound* ***1a*** *and* ***1b****, and through the amide bond, e.g. compound* ***2-6****, and analyzed their ability to stereoselective reduction of methyl benzoylformate. Although NADH mimics* ***1-3*** *and* ***6*** *turned out to be less effective in chirality transfer toward methyl benzoylformate, much higher chirality transfer was observed in the reactions with the compounds (****4*** *and* ***5****) possessing free hydroxyl groups at 5',6'-position of furanose. Importance of an additional intramolecular coordinating substituent to bivalent metal ion has been demonstrated in enhancing the stereoselectivity in the reduction of benzoylformate with such NADH mimics. To materialize these observations, transition-states of the hydride transfer from 1-methyl-1,4-dihydronicotinamide to methyl benzoylformate in the presence of magnesium (II) ion were calculated by semi-empirical molecular orbital method, MNDO-PM3. Also discussed in this paper is a general chirality transfer mechanism deduced from the theoretical transition-state modeling.*

Introduction

Enzyme-mediated oxidation-reduction is highly specific in terms of chemo-, regio- as well as stereoselectivity. These specificities are among major concerns of current organic and bioorganic chemistry, for example, the use of certain alcohol dehydrogenases in the preparation of chiral carbinols is well documented.[2] Most of the oxido-reduction enzymes usually utilize a cofactor (or coenzyme) for the responsible reaction as an electron-donor or acceptor. Each cofactor is generally required as a co-substrate in a stoichiometric amount to the corresponding substrate. The enzyme proteins play an important role in providing a specific three-dimensional arrangement of each chemical species involved, by which the specificities are in fact materialized.

Nicotinamide adenine dinucleotide (NAD^+) and its phosphate ($NADP^+$) are major cofactors in the enzymatic oxido-reduction and have been studied extensively.[3] 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.[4] Since then, varieties of NADH mimics have been synthesized by imposing various chiral auxiliaries and functional groups to the dihydronicotinamide and its analogs, to analyze the features of such stereospecific reduction and to explore applications of the potential of the dihydropyridine chemistry to organic synthesis.[5]

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.[6] So far, while several models of chelation has been postulated for each mimics depending upon purely empirical consideration, a

generalized model which can explicate the majority of results has yet to be clarified.

Generally, by imposing a chiral auxiliary functional group, such chelation 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. Therefore, crucial requirement to clarify and to pursue the dehydrogenase-like stereoselective reductions is to develop suitable and efficient chiral NADH mimics.

Recently, we developed highly enantioselective synthetic methodology based upon the chirality transcription concept using a versatile chiral template, diacetone-D-glucose, which was successfully utilized in the syntheses of chirally deuterated glycines, chirally monodeuterated glycerol, chiral acetic acid, *etc.*[7] A key feature of this chirality transcription approach is ascribable to highly efficient diastereoface selection of allylic alcohol systems attached to the template. Similar efficient face selection was thus anticipated in the NADH mimics prepared by imposing the dihydronicotinamide structure into the diacetone-D-glucose template, which could be effective in the stereoselective reduction of carbonyl compounds. While sugar substituted NADH mimics were previously prepared,[8] the present approach to the design of mimics and their chemical features have been well-apart from those reported *vide infra*.

The present paper deals with the preparation of novel NADH models, in which the 1,4-dihydronicotinamide structure is connected to the diacetone-D-glucose molecule *via* its C-1 nitrogen, *e.g.* compound **1a** and **1b**, and through the amide bond, *e.g.* compound **2-6**. All the models contained an amide bond attached to dihydropyridine ring as the natural counterparts. We observed, in the reduction of benzoylformate with these NADH mimics in the presence of magnesium (II), that the presence of an additional intramolecularly coordinating substituent other than two carbonyl groups of the benzoylformate and the carbonyl group of dihydronicotinamide in NADH mimics significantly enhanced the stereoselectivity. To materialize these observations, we took advantage of recent advances in theoretical chemistry and computational resources to evaluate the ternary complex in the transition state of this reaction. In particular, it seemed that the MNDO-PM3 semi-empirical molecular orbital method is appropriate for yielding relevant calculated structures of this molecular complexity for the crucial ternary transition-state complexes. Discussed also in this paper is the chirality transfer mechanism deduced from the theoretical transition-state modeling.

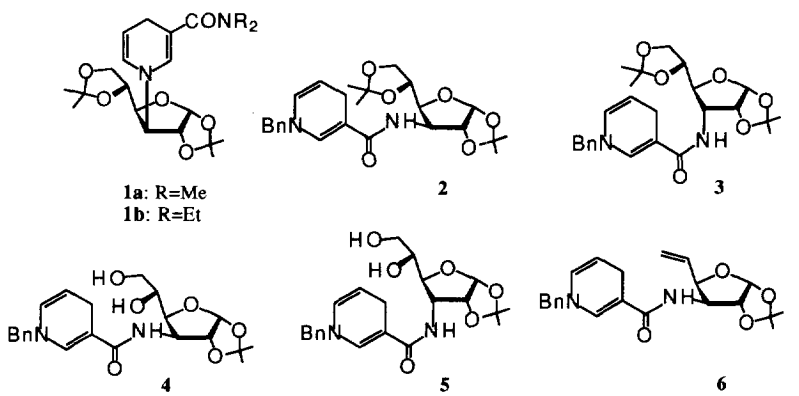


Figure 1

Results and Discussion

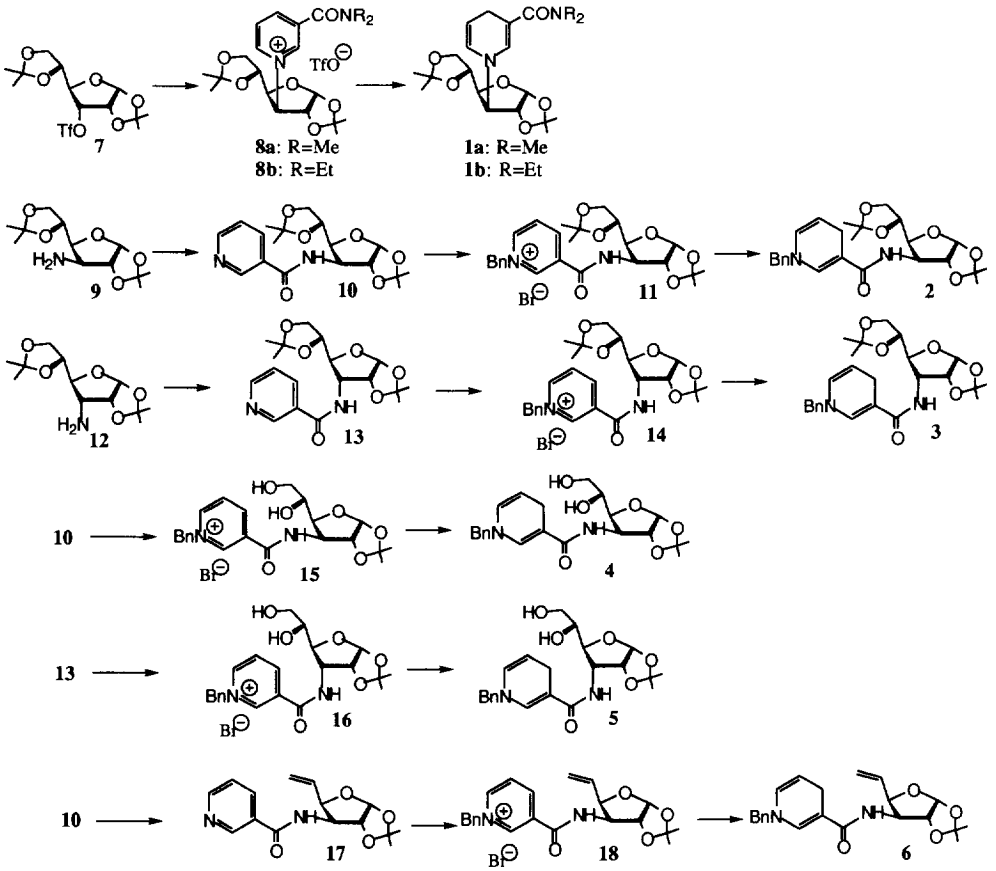
Chiral NADH mimics to be exploited first were those in which the reactive 1,4-dihydronicotinamide structure was bound to C-3' of diacetone-D-glucose at the N-1 position such as **1a** and **1b**. Primarily, these mimics were supposed to be stereo-cognates to the natural NAD(P)H in terms of its furanoside nature rather than pyranoside. Actually in the natural NAD(P)H, the dihydropyridine is attached rather to C-1' of ribofuranose than the C-3' in the mimics. Secondarily, a significant steric constraint between the bulky substituent on C-4' of the furanose ring and the dihydropyridine ring on C-3' could be expected as a key feature of the diacetone-D-glucose template. This could hopefully discriminate the faces of the dihydropyridine chemically. The target molecules **1a** and **1b** were synthesized as shown in Scheme 1. Heating of known 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose 3-trifluoromethanesulfonate **7**[8] with *N,N*-dimethyl or *N,N*-diethylnicotinamide without solvent gave pyridinium trifluoromethanesulfonate derivatives **8a** and **8b**, respectively, in fair yields. Reduction of these pyridinium salts **8a** and **8b** into the corresponding dihydronicotinamide derivatives was efficiently performed by treating with a large excess of $Na_2S_2O_4$ in 1 M $NaHCO_3$-CH_2Cl_2, as reported in the literature.[10]

Our attention was next turned to dihydronicotinamide analogs containing the diacetone glucose moiety through an amide bond. Since the rotational energy barrier of an amide bond was known to be relatively large,[11] the conformation in the transition state was expected to be significantly restricted. To this end, known 3-amino-3-deoxy-*allo*- and *gluco*-furanose derivatives **9** and **12**[11] were converted in high yield to the nicotinamide derivatives **10** and **13**, respectively, by the reaction with nicotinic acid in the presence of *N,N'*-dicyclohexylcarbodimide as shown in Scheme 1. Quaternarization of **10** or **13** with benzyl bromide in absolute ethanol gave **11** and **14**, respectively . Prolonged reaction caused a break-down of the protecting group at the 5',6'-position of the furanose probably due to an acidic product derived from benzyl bromide. Thus, compounds **10** and **13** were separately treated with benzyl bromide for longer reaction time to yield the hydrolyzed products **15** and **16**, respectively. Transformation of these pyridinium salts **11**, **14**, **15** and **16** into the dihydronicotinamide form was performed by treating with $Na_2S_2O_4$ in 1M $NaHCO_3$-CH_2Cl_2 to provide the desired NADH mimics (**2-5**).

To evaluate the effects of free hydroxyl groups of **4** and **5** for the stereoselective reduction, 5',6'-dideoxygenated derivative **6** was also prepared. Thus, the above mentioned nicotinamide derivative **10** was selectively hydrolyzed in 90% acetic acid to afford a dihydroxyl intermediate, which in turn was mesylated and was further subjected to the Tipson-Cohen conditions with zinc powder in the presence of sodium iodide[13] to give a 5',6'-dideoxy-5',6'-didehydro derivative **17**. After quaternarization, final reduction of **18** with $Na_2S_2O_4$ in a same manner described above afforded a simplified vinyl compound **6**.

The potential of **1-6** to enantioselective reduction was examined by a standard methodology. Methyl benzoylformate was treated with the synthesized NADH mimics **1-6** in the presence of an equimolar amount of magnesium perchlorate in acetonitrile under inert atmosphere to afford methyl mandelate smoothly. Chirality of the resulting mandelate was analyzed by ^{1}H-NMR spectroscopy after derivatizing it to (+)-(*R*)-MTPA ester. The results are summarized in Table 1.

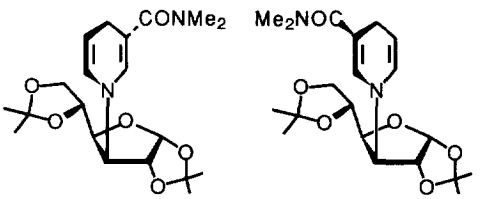
NADH mimics **1a** and **1b** turned out to be rather ineffective in chirality transfer toward methyl benzoylformate (21 and 14 % ee, respectively). This may probably be due to free rotation of the C-3'-N-1 bond and a flexible conformation as a whole. In fact, NOE effect was observed between the hydrogen at the 3'-position of the carbohydrate template and both hydrogens at the 2- and 6-positions of the dihydropyridine moiety in **1a** in ^{1}H-NMR. Conceivably of course, the mimic **1a** was possibly in two major conformations, *i.e. syn-a* and *anti-b* (Figure 2), and such an easy conformational interconversion might have allowed an approach of the substrate *via* a stereochemically unconstrained path and caused in scrambling of stereochemistry. Thus, it seemed important to restrict the pertinent C-3'-N-1 bond as suggested generally in the enzyme reactions. However, this was not the case. The mimic **1b** was as active as or even less efficient than **1a** in chirality transfer. Apparently, even the bulkiness of *N,N*-diethyl group introduced into the carboxamide moiety was not demanding enough to control conformational flexibility. In addition, these results could also ruled out major involvement of the N-1 nitrogen in the formation of a ternary complex in the transition state, because the close proximity of N-1 to the chiral center in **1a** and **1b** had almost nothing to do with chirality transfer.



Scheme 1

Table 1. Stereoselectivity in the reduction of methyl benzoylformate with NADH mimics (**1-6**)

NADH Mimics	Reaction Time (h)	Yield (%)	ee (%)	Configuration of mandelate
1a	48	58	21	*R*
1b	48	43	14	*R*
2	29	18	23	*S*
3	26	33	19	*R*
4	168	68	80	*S*
5	48	33	50	*R*
6	144	25	23	*R*



syn-a *anti-b*

Figure 2

Reduction of methyl benzoylformate with the *gluco*-type NADH mimic **2** afforded (*S*)-mandelate stereoselectively, while the *allo*-isomer **3** gave (*R*)-mandelate predominantly. Reversal of stereoselectivity was observed depending on the configuration at the C-3' position of the furanoside. However, enantioselectivities observed in the reduction with **2** and **3** were still rather low (23 and 19 % ee, respectively). Ineffectiveness in chirality transfer seemed to be because the reaction center (C-4) of dihydropyridine is 4-bonds away from the chiral center in all cases. In the reduction of benzoylformate with the compounds **4** and **5** without a 5',6'-isopropylidene protecting group, the tendencies of chirality transfer within *gluco*- and *allo*-series was same as in the corresponding protected cases. It should be pointed out, however, that much higher chirality transfer was observed in the reactions with **4** and **5** compared to the corresponding **2** and **3** in both *gluco*- and *allo*-series. In the reduction of methyl benzoylformate with the *gluco* series **4**, (*S*)-mandelate was obtained in 80 % enantiomeric excess (ee), whereas the *allo* derivative **5** afforded (*R*)-mandelate in 50 % enantiomeric excess. Althrough the observed ee were not necessarily superior to those reported previously,[5] the improvement of stereoselectivity from the protected cases to the unprotected appeared to be significant, because the isopropylidene protecting group was much sterically demanding than the hydroxyl groups, yet yielding less selectivity. We reasoned these higher chirality transfer that the 5',6'-hydroxyl groups in compounds **4** and **5** can coordinate more effectively to a magnesium (II) ion than the ethereal oxygens at the 5'- and 6'-positions of **2** and **3**, thereby fixing a transient ternary complex. Steric bulkiness of the C-4' substituent was not necessarily important for control of the transition state structure. As also indicated in Table 1, the enantioselectivity in the reduction of benzoylformate with **6** was as low as with **1a**, **1b**, **2**, or **3** (23 % ee). Again, the absence of free hydroxyl groups in the NADH mimics apparently reduced the stereoselectivity under these conditions.

The crucial interactions between the relevant hydroxyl groups and magnesium ion were confirmed by ^{1}H-NMR spectroscopic analysis, where the spectra of compounds **4** and **5** in deuterated acetonitrile were altered by gradual addition of magnesium (II) ion. Especially, the signals of H-5' and H-6' of the furanose were significantly shifted to higher frequency region (up to about 1 ppm). These results strongly suggested that the polar hydroxyl group(s) can coordinate to magnesium (II) ion, in addition to the previously suggested complexation between the substrate's carbonyl group and the magnesium ion. This multiple chelation could firmly fix the conformation of a reacting ternary complex so that both the reactivity and stereoselectivity of the hydride transfer were efficiently enhanced.

It has been well recognized that the most NADH mimics require a suitable bivalent metal ion such as magnesium (II) or zinc (II) for highly efficient and stereoselective reduction.[5] Apparently, the metal ion is involved in coordination to the polar substituents of both the substrate and the NADH mimics and the resulting formation of a specific ternary complex induces selective transfer of one particular hydride from the reducing dihydropyridine group to a preferred face of the enantiotopic trigonal carbon of the substrate leading to the stereoselective reduction. However, close features and precise nature of such important ternary complex are still not clear.

Several interpretative attempts for the ternary complex formation have been proposed so far on the purely empirical bases to mostly explain the observed experimental outcome in the enantioselective carbonyl reduction with chiral NADH mimics. For example, Ohno *et al.* rationalized their observation of chirality transfer by

adopting a ternary complex, in which a crucial magnesium ion was placed between the dihydronicotinamide ring and the substrate, but was not specifically located (Figure 3A).[4,6] Vekemans *et al.* suggested an intuitive ternary complex as shown in Figure 3B.[14] Meyers *et al.* rationalized their observation of stereoselectivity of the reduction by assuming the formation of a rigid ternary complex, in which the ketonic carbonyl group of the substrate was assumed to lie over the dihydropyridine ring with the oxygen facing to the ring nitrogen (Figure 3C).[15] Even though all the mechanisms and complexes proposed for the stereoselective reduction with the NADH mimics seems to be able to accommodate each experimental evidence, all remains to essentially be substantiated by closer examination. In addition, it seems important to clarify whether or not a general characteristics for the reduction with those NADH mimics can be extracted. Further, while the structural mechanism of hydrogen transfer in these reactions has been discussed as mentioned above, it is yet to be clarified whether the reaction proceeds *via* a concerted mechanism or a three-step mechanism involving two single electron transfers.[6,16]

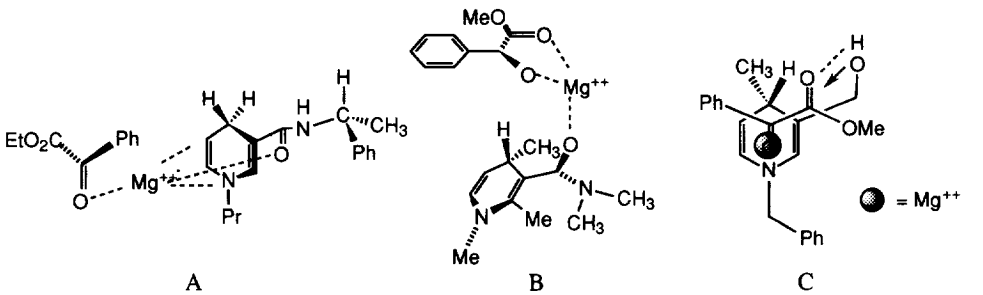


Figure 3. Ternary complexes previously proposed in the literatures.

As described above, the presence of an additional intramolecularly coordinating substituent other than two carbonyl groups of the benzoylformate and the carbonyl group of dihydronicotinamide in the NADH mimics significantly enhanced the stereoselectivity in the reduction of the benzoylformate with the furanose-based NADH mimics in the presence of magnesium (II). To get more insight into these observations, we took advantage of recent advances in theoretical chemistry and computational resources to evaluate the ternary complex in the transition state of this reaction. In particular, it seemed that the MNDO-PM3 semi-empirical molecular orbital method[17] is capable of yielding relevant calculated structures of the crucial and rather complicated ternary complexes in the transition states. So far, only the reaction between formaldehyde and simple dihydronicotinamide in the absence of a metal ion was calculated on the *ab initio* level.[18]

The model reaction selected for our computational study was the hydride transfer from 1-methyl-1,4-dihydronicotinamide to methyl benzoylformate in the presence of magnesium (II) ion. Although four cannomical reaction pathways were essentially conceivable in terms of prochirality, two reaction modes were equivalent *a priori* because individual species involved in the reaction are not chiral. Therefore, calculation was carried out for two possible pathways of the reaction, where the migrating hydrogen was arbitrarily defined to the *pro-R* 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 as depicted in Figure 4.

The possible transition state structures for each reaction mode were successfully located on the restricted Hartree-Fock (RHF) energy hypersurface by the use of MNDO-PM3 calculation. The results strongly suggest that a concerted hydrogen transfer mechanism is favored under these conditions. The transferring hydrogen may attack either face of the trigonal center of benzoylformate, *i. e.* the *pro-R* hydrogen atom at the C-4 position of dihydronicotinamide can be transferred to the *re*-face of the ketone of benzoylformate (TS-1) or to the *si*-face (TS-2). The general structures of these transition states are quite similar. Thus, in both transition states, a magnesium ion is located in the position where it can coordinate to two carbonyl oxygens of methyl benzoylformate as well as the oxygen atom of carboxamide group of dihydronicotinamide (distance of oxygen-magnesium, 1.82-1.84 Å), and the distances of the breaking and forming carbon-hydrogen bonds in the

transition states are approximately 1.41 Å and 1.51 Å, respectively.

It should be noted that the carbonyl group of carboxamide is *syn*-oriented to the migrating hydrogen atom in both cases. The *syn*-orientation of carbonyl group was previously suggested by Donkersloot *et al.* in the *ab initio* calculation of the transition state of the reaction between 1,4-dihydronicotinamide and formaldehyde in the absence of magnesium ion.[18] Further, the dihydropyridine ring is almost plannar and the transferring hydrogen atom nearly locates vertically to the dihydropyridine plane. Between the two transition-states obtained, the transition state involving the *pro-R* hydrogen atom at the C-4 position of dihydronicotinamide transferring to the *re*-face of benzoylformate (TS-1) turned out to be energetically more favorable by 0.37 kcal/mol than to the *si*-face attack (TS-2). This may be attributable to the more proper orientation of each coordinating groups, *i. e.*, magnesium ion, the chelating three carbonyl groups and the transferring hydrogen atom, in the crucial ternary complex of the transition state.

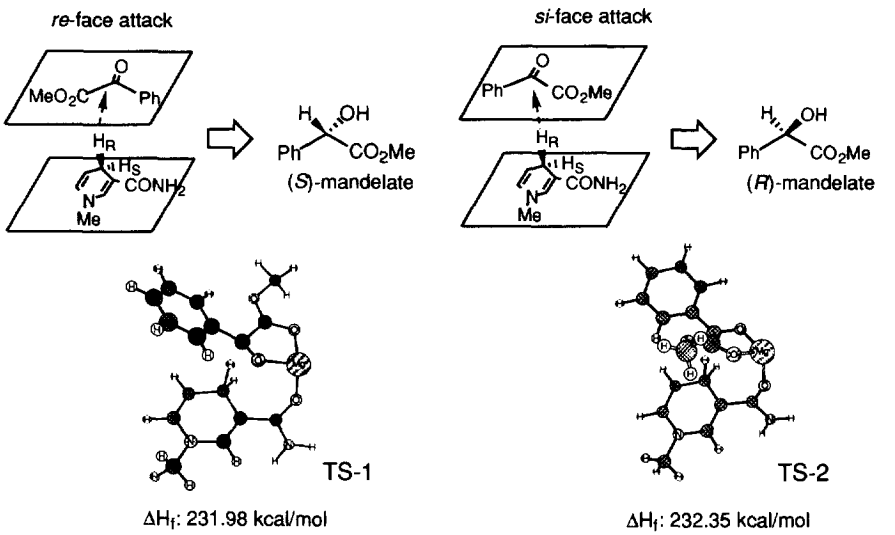


Figure 4. Calculated transition-states of the hydride transfer from 1-methyl-1,4-dihydronicotinamide to methyl benzoylformate in the presence of magnesium (II) ion.

To the best of our knowledge, this is the first transition-state structure in the presence of metal ion to be elucidated at the semi-empirical level of orbital approach. A working model structure proposed recently by Vekemans *et al.* based on the purely empirical bases turned out to be quite similar to the present results.[14,19] It seems that, in this reaction, the stereoselectivity is dictated mainly by the *syn*-directed carbonyl group of a carboxamide at the C-3 position of dihydropyridine ring. Therefore, fixation of the orientation of the polar substituent at the C-3 position in NADH mimics is of particular importance in controlling the stereoselectivity in the reduction of benzoylformate.

Another important feature in the calculated transition state is that magnesium ion is tri-coordinated. This could mean that an additional polar functionality may be coordinate to the vacant locus of the tri-coordinated magnesium ion. An additional intramolecular coordination thus seems entropically feasible and favorable to control and stabilize the structure of the ternary complex for better reactivity and stereoselectivity.

As described above, the present experimental results indicated that the presence of free hydroxyl groups in the NADH mimics apparently enhanced the stereoselectivity as shown in the cases of the compounds **4** and **5**. The ternary complexes in the reduction of methyl benzoylformate of NADH mimics **4** and **5** based on the calculated transition-state structure are outlined in Figure 5. In these postulated transition-states, the amide bond is positioned in the energetically favored *s-trans* conformations and the magnesium ion is located in as tetra-coordinated to the suitably positioned 5'-hydroxyl group in addition to the three carbonyl groups. These ternary

structures with the tetra-coordinated magnesium ion can rationalize the direction or trajectory of the hydride transfer and the stereoselectivity as well.

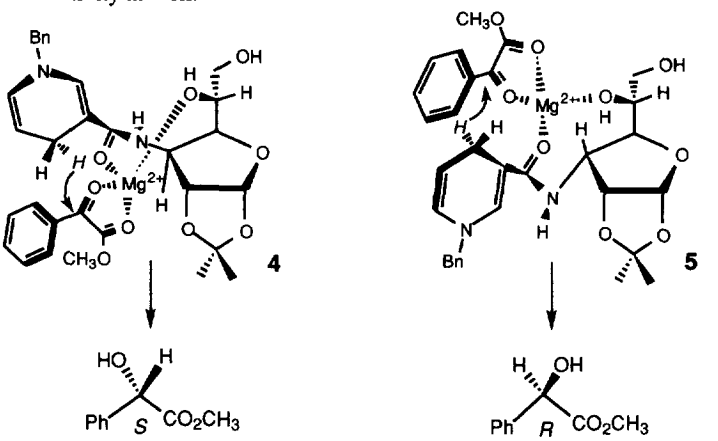
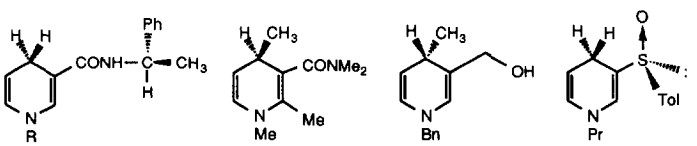


Figure 5. Proposed transition-states for the reduction of methyl benzoylformate with **4** and **5**

The present transition-state model can be extended further to generalize the previously reported results of benzoylformate reduction with the NADH mimics and the stereoselectivities thereof. More specifically, we tested compatibility of our theoretical transition state model with the results of the stereoselective reduction with the NADH mimics reported by Ohno *et al.*,[4] Vekemans *et al.*,[14] Meyers *et al.*,[15] and Iwata *et al.*[20] Ohno *et al.* obtained (*R*)-mandelate in the stereoselective reduction of ethyl benzoylformate with **19a** in an enantiomeric excess of 20 %.[4] Vekemans *et al.* reported that the reduction of methyl benzoylformate with **20** afforded (*R*)-mandelate in a 95 % optical yield.[14] Meyers *et al.* utilized **21** in the stereoselective reduction of methyl benzoylformate and (*S*)-mandelate was obtained in an optical yield of 94 %.[15] In the asymmetric reduction of methyl benzoylformate with the chiral sulfoxide derivative **22**, (*R*)-mandelate was obtained by Iwata *et al.* in 96 % enantiomeric excess.[20]



19a: R = ^{n}Pr
19b: R = CH_2Ph
19c: R = 2,6-dichlorobenzyl

20 **21** **22**

Figure 6

As shown below in Figure 7, the results reported by Vekemans *et al.* and Meyers *et al.* seem to be explained by simply applying their NADH mimics **20** and **21**, which yielded (*R*)- and (*S*)-mandelate, respectively, to the present calculated favored transition-state model (TS-1) (Figure 7B and 7C), respectively. To analyze the results by Ohno *et al.* and Iwata *et al.*, favorable conformations of their NADH mimics were first deduced in our hands by molecular mechanics calculation, especially of the conformation of the chiral auxiliary. Thus, the dihydropyridine moiety, the benzoylformate and the oxygen atom of the C-3 substituent in these cases were firstly superimposed into the calculated favored transition state (TS-1) and its enantiomeric form as well to construct a pair of diastereoisomeric complex structures. Then rest of the structures were next incorporated to derive the whole structure of each NADH mimics. The most favorable conformation was then estimated by

MM2 calculation,[21] during which the reactive site comprising from the dihydropyridine moiety, the benzoylformate and the oxygen atom of the C-3 substituents were fixed throughout. The resulting final steric energies were compared between each pair of the diastereoisomeric transition state structures. The most favored transition-state structures, in both cases of Ohno *et al.* and Iwata *et al.*, were again those incorporating the favored transition-state (TS-1) as shown in Figure 7A and 7D, respectively.

These transition-state structures were therefore in good accordance with the experimental results in terms of stereochemical outcomes. Thus, the chelated ternary complexes (7A and 7D) constructed on our transition-state model (TS-1) appeared to be appropriate to rationalize the reported stereoselectivities in all cases. The present transition-state model for the reduction of methyl benzoylformate with NADH mimics now seems to be general and is useful to understand the origin of selectivity over varieties of reactions with the NADH mimics.

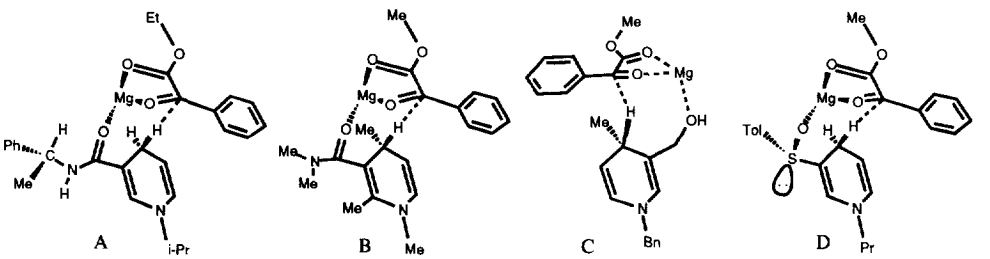


Figure 7. Plausible transition-states for the stereoselective reduction of benzoylformate with various NADH mimics reported by Ohno *et al.* (A), Vekemans *et al.* (B), Meyers *et al.* (C), and Iwata *et al.* (D), based on our calculated preferred transition-state.

In summary, with newly developed NADH mimics possessing a furanose moiety as a chiral auxiliary in an isosteric manner to natural NADH and NADPH, importance of an additional intramolecular polar coordinating group to a bivalent metal ion has been demonstrated with enhancement of the stereoselectivity in the reduction of benzoylformate with such NADH mimics. We suggest the general transition-state model for the stereoselective reduction of benzoylformate with the NADH mimics based on the theoretical calculation by the PM3 semi-empirical molecular orbital method. These results apparently facilitate more precise and rational design of NADH model compounds with high reactivity and stereoselectivity. 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. It is well established that varieties of enzymes utilizing NAD(P)/NAD(P)H as coenzyme require a bivalent metal ion such as zinc or magnesium.2 Thus, the present study may provide a clue to the ternary complexes of the enzyme reactions, in which the amide carbonyl group of the dihydronicotinamide cofactor is chelated to the enzyme-supported metal ion and is fixed in *syn*-direction to the migrating hydrogen during the hydride transfer, thereby controlling the stereospecificity in the enzyme reactions. The present approach seems useful and significant in understanding the otherwise unobservable transition-states of the enzyme reactions.

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 FX-200 and/or a JEOL GSX-270 spectrometer. Deuteriochloroform (99.8 % atom enriched, Aldrich) was used for the NMR solvent throughout, unless otherwise stated. ^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 for reduction of methyl benzoylformate without further purification.

1-(3-Deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranos-3-yl)-3-*N*,*N*-dimethylcarbamoylpyridinium trifluoromethanesulfonate 8a

A mixture of **7** (1.03 g, 2.63 mmol) and *N*,*N*-dimethylnicotinamide (1.16 g, 3 eq) was stirred for 24 h at 120°C. After cooled to room temperature, the mixture was applied to column chromatography over silica gel (100 g) with $CHCl_3$-methanol (50:1-2:1) to give **8a** as an oil (0.94 g, 66 %). UV λ_{max}/nm (ε): 207.5 (6800), 270.0 (2800); IR ν_{max}/cm^{-1} (nujol): 2850, 1660, 1460, 1380, 1280, 1170, 1080, 1040; ^{1}H NMR (200 MHz): δ 1.12 (s, 3H, isopropylidene-Me), 1.37 (s, 3H, isopropylidene-Me), 1.45 (s, 3H, isopropylidene-Me), 1.59 (s, 3H, isopropylidene-Me), 3.03 (s, 3H, N-Me), 3.13 (s, 3H, N-Me), 3.26 (dt, *J*=7.4, 3.8 Hz, 1H, 5'-H), 4.00 (m, 2H, 6'-H), 4.46 (dd, *J*=7.4, 3.2 Hz, 1H, 4'-H), 5.28 (d, *J*=3.2 Hz, 1H, 3'-H), 5.39 (dd, *J*=3.2, 2.6 Hz, 1H, 2'-H), 6.40 (d, *J*=2.6 Hz, 1H, 1'-H), 8.24 (t, *J*=7.2, Hz, 1H, 5-H), 8.64 (d, *J*=7.2 Hz, 1H, 4-H), 8.82 (s, 1H, 2-H); ^{13}C NMR (67.9 MHz): δ 24.5, 25.6, 26.1, 26.7, 35.8, 39.3, 67.4, 71.9, 77.2, 79.6, 84.1,105.5, 110.5, 113.0, 117.8, 122.5, 129.0, 136.0, 145.7, 164.0. High resolution FAB-MS: *m*/*z*: 393.2036. Calcd. for $C_{20}H_{29}N_2O_6$: 393.2027.

1-(3-Deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranos-3-yl)-3-*N*,*N*-dimethylcarbamoyl-1,4-dihydropyridine 1a

A mixture of **8a** (452 mg, 0.83 mmol) and $Na_2S_2O_4$ (2.85 g, 16.4 mmol) in CH_2Cl_2 (50 ml) and aqueous 1M $NaHCO_3$ (40 ml) was stirred for 5 h in the dark. Two layers were separated and the aqueous layer was extracted twice with $CHCl_3$. The combined organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated to dryness to give **1a** as amorphous (314 mg, 96 %). UV λ_{max}/nm (ε): 209.0 (8000), 336.5 (3400); IR ν_{max}/cm^{-1} ($CHCl_3$): 3000, 2700, 1640, 1600, 1490, 1430, 1250, 1160, 1080, 1020; ^{1}H NMR (200 MHz): δ 1.25 (s, 3H, isopropylidene-Me), 1.32 (s, 3H, isopropylidene-Me), 1.41 (s, 3H, isopropylidene-Me), 1.52 (s, 3H, isopropylidene-Me), 3.01 (s, 6H, N-Me x 2), 3.10 (m, 2H, 4-H), 3.63 (d, *J*=4.0 Hz, 1H, 3'-H), 4.00-4.23 (m, 4H, 4'-, 5'- and 6'-H), 4.60 (dt, *J*=8.0, 3.0 Hz, 1H, 5-H), 4.70 (d, *J*=4.0 Hz, 1H, 2'-H), 5.64 (dd, *J*=8.0, 1.6 Hz, 1H, 6-H), 5.88 (d, *J*=4.0 Hz, 1H, 1'-H), 6.06 (d, *J*=1.6 Hz, 1H, 2-H); ^{13}C NMR (67.9 MHz): δ 23.4, 25.3, 25.8, 26.4, 26.9, 37.5, 67.5, 68.0, 72.3, 77.2, 84.3, 101.6, 102.6, 105.1, 109.5, 111.6, 128.1, 135.0, 172.1.

3-*N*,*N*-Diethylcarbamoyl-1-(3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranos-3-yl)pyridinium trifluoromethanesulfonate 8b

A mixture of **7** (1.04g, 2.66 mmol) and *N*,*N*-diethylnicotinamide (1.5g, 3 eq) was stirred for 16 h at 120°C. After cooled to room temperature, the mixture was applied to column chromatography over silica gel (125 g) with $CHCl_3$-methanol (50:1-2:1) to give **8b** as an oil (1.03 g, 67 %). UV λ_{max}/nm (ε): 206.0 (11800), 273.5 (3400); IR ν_{max}/cm^{-1} ($CHCl_3$): 3000, 1640, 1490, 1460, 1380, 1260, 1210, 1180, 1140; ^{1}H NMR (200 MHz): δ 1.14 (s, 3H, isopropylidene-Me), 1.22 (t, *J*=7.2 Hz, 3H, CH_2CH_3), 1.30 (t, *J*=7.2 Hz, 3H, CH_2CH_3), 1.38 (s, 3H, isopropylidene-Me), 1.45 (s, 3H, isopropylidene-Me), 1.60 (s, 3H, isopropylidene-Me), 3.25 (dt, *J*=9.6, 4.6 Hz, 1H, 5'-H), 3.35 (q, *J*=7.2 Hz, 2H, CH_2CH_3), 3.58 (q, *J*=7.2 Hz, 2H, CH_2CH_3), 4.01 (m, 2H, 6'-H), 4.44 (dd, *J*=9.6, 4.0 Hz, 1H, 4'-H), 5.22 (d, *J*=4.0 Hz, 1H, 3'-H), 5.47 (d, *J*=4.0 Hz, 1H, 2'-H), 6.38 (d, *J*=4.0 Hz, 1H, 1'-H), 8.25 (dd, *J*=6.0, 8.0 Hz, 1H, 5-H), 8.52 (d, *J*=8.0 Hz, 1H, 4-H), 8.60 (s, 1H, 2-H), 8.71 (d, *J*=6.0 Hz, 1H, 6-H); ^{13}C NMR (67.9 MHz): δ 12.5, 14.1, 24.6, 25.7, 26.1, 26.8, 40.3, 43.3, 67.4, 71.8, 77.2, 79.6, 84.0,105.6, 110.5, 113.0, 122.8, 129.1, 137.0, 143.2, 144.2, 163.3. High resolution FAB-MS: *m*/*z*: 421.2339. Calcd. for $C_{22}H_{33}N_2O_6$: 421.2340.

3-*N*,*N*-Diethylcarbamoyl-1-(3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranos-3-yl)-1,4-dihydropyridine 1b

Compound **8b** (637 mg, 1.12 mmol) was treated in the same manner as described for the preparation of

8a to give **1b** as amorphous (444 mg, 94 %). UV λ_{max}/nm (ε): 206 (8700), 333.5 (2600); IR ν_{max}/cm^{-1} ($CHCl_3$): 3000, 1720, 1600, 1420, 1270, 1250, 1170, 1080, 1030; ^{1}H NMR (200 MHz): δ 1.11 (t, *J*=7.1 Hz, 6H, CH_2CH_3 x 2) 1.31 (s, 3H, isopropylidene-Me), 1.34 (s, 3H, isopropylidene-Me), 1.40 (s, 3H, isopropylidene-Me), 1.51 (s, 3H, isopropylidene-Me), 3.10 (m, 2H, 4-H), 3.40 (q, *J*=7.1 Hz, 4H, CH_2CH_3), 3.62 (d, *J*=4.0 Hz, 1H, 3'-H), 3.95-4.22 (m, 4H, 4'-, 5'- and 6'-H), 4.56 (dt, *J*=8.0, 3.8 Hz, 1H, 5-H),4.69 (d, *J*=4.0 Hz, 1H, 2'-H), 5.62 (dd, *J*=8.0, 1.6 Hz, 1H, 6-H), 5.86 (d, *J*=4.0 Hz, 1H, 1'-H), 5.99 (s, 1H, 2-H); ^{13}C NMR (67.9 MHz): δ 13.7, 23.9, 25.3, 25.8, 26.3, 27.0, 40.8, 41.2, 67.5, 68.0, 72.3, 77.2, 81.0, 84.3, 101.0, 103.9, 105.0, 109.5, 111.5, 128.4, 132.5, 171.6.

3-*N*-(3-Deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranos-3-yl)carbamoylpyridine 10

A mixture of 3-amino-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose **9** (3.17 g, 12.2 mmol), nicotinic acid (3.08 g, 25.1 mmol), and *N,N'*-dicyclohexylcarbodiimide (5.08 g, 24.7 mmol) in *N,N*-dimethylformamide (50 ml) was stirred overnight at room temperature. The mixture was diluted with ethyl acetate and the insoluble materials were filtered off. The filtrate was washed with brine, dried over Na_2SO_4, filtered and concentrated to dryness. The residue was chromatographed over silica gel (250 g) with hexane-ethyl acetate (1:1-0:1) to give **10** as colorless crystals (4.41 g, 99 %). m.p. 135.5-136.5°C (from hexane-ethyl acetate); IR ν_{max}/cm^{-1} ($CHCl_3$): 3370, 2990, 1665, 1510, 1375; ^{1}H NMR (270 MHz): δ 1.30 (s, 3H, isopropylidene-Me), 1.33 (s, 3H, isopropylidene-Me), 1.34 (s, 3H, isopropylidene-Me), 1.55 (s, 3H, isopropylidene-Me), 3.85 (dd, *J*=8.3, 6.8 Hz, 1H, 6'-H), 4.16 (dd, *J*=8.3, 6.3 Hz, 1H, 6'-H), 4.30 (dd, *J*=5.9, 3.7 Hz, 1H, 3'-H), 4.48 (br. q, *J*=6.3 Hz, 1H, 5'-H), 4.57 (dd, *J*=6.3, 3.7 Hz, 1H, 4'-H), 4.80 (d, *J*=3.9 Hz, 1H, 2'-H), 5.95 (d, *J*=3.9 Hz, 1H, 1'-H), 7.21 (br. d, *J*=5.9 Hz, 1H, NH), 7.40 (ddd, *J*=7.8, 4.9, 1.0 Hz, 1H, 5-H), 8.13 (dt, *J*=7.8, 2.0 Hz, 1H, 4-H), 8.75 (dd, *J*=4.9, 1.5 Hz, 1H, 6-H), 8.98 (d, *J*=2.0 Hz, 1H, 2-H); ^{13}C NMR (67.9 MHz): δ 24.9, 26.0, 26.5, 56.8, 66.9, 73.4, 77.4, 84.1, 104.4, 109.9, 112.0, 123.4, 129.6, 135.1, 148.0, 152.4, 165.5. *Anal.* Calcd. for $C_{18}H_{24}N_2O_6$: C, 59.33; H, 6.64; N, 7.69. Found: C, 59.08; H, 6.80; N, 7.58.

1-Benzyl-3-*N*-(3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranos-3-yl)carbamoylpyridinium bromide 11

Benzyl bromide (0.75 ml, 6.3 mmol) was added to a solution of **10** (1.47 g, 4.05 mmol) in ethanol (15 ml). The resulting mixture was stirred for 23 h at room temperature. After removal of the solvent, the residue was chromatographed over silica gel (170 g) with $CHCl_3$-methanol (50:1-2:1) to give **11** as colorless amorphous (1.27 g, 59 %). UV λ_{max}/nm (ε): 204 (2.77 x 10^4); IR ν_{max}/cm^{-1} ($CHCl_3$): 3190, 2950, 1670, 1375, 1065, 1015; ^{1}H NMR (270 MHz): δ 1.20 (s, 3H, isopropylidene-Me), 1.32 (s, 3H, isopropylidene-Me), 1.38 (s, 3H, isopropylidene-Me), 1.54 (s, 3H, isopropylidene-Me), 3.96 (dd, *J*=8.8, 6.1 Hz, 1H, 6'-H), 4.05 (dd, *J*=8.8, 4.9 Hz, 1H, 6'-H), 4.33 (dd, *J*=7.8, 3.9 Hz, 1H, 4'-H), 4.39 (m, 1H, 5'-H), 4.76 (d, *J*=3.4 Hz, 1H, 2'-H), 4.84 (dd, *J*=8.8, 3.9 Hz, 1H, 3'-H), 6.11 (s, 2H, PhCH_2), 6.30 (d, *J*=3.4 Hz, 1H, 1'-H), 7.43 (m, 3H, aromatic), 7.63 (m, 2H, aromatic), 8.04 (dd, *J*=8.3, 6.4 Hz, 1H, 5-H), 9.00 (d, *J*=8.3 Hz, 1H, 4-H), 9.16 (d, *J*=6.4 Hz, 1H, 6-H), 9.27 (d, *J*=8.8 Hz, 1H, NH), 10.34 (s, 1H, 2-H); ^{13}C NMR (67.9 MHz): δ 25.2, 26.3, 26.7, 26.8, 56.7, 64.6, 66.9, 73.1, 80.1, 84.5, 105.4, 109.1, 111.9, 127.8, 129.5, 129.8, 130.4, 131.9, 134.4, 144.4, 145.3, 145.5, 161.1. *Anal.* Calcd. for $C_{25}H_{31}N_2O_6Br$: C, 56.08; H, 5.84; N, 5.23. Found: C, 56.32; H, 6.05; N, 4.95.

1-Benzyl-3-*N*-(3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranos-3-yl)carbamoyl-1,4-dihydropyridine 2

A mixture of **11** (260 mg, 0.486 mmol) and $Na_2S_2O_4$ (910 mg, 5.23 mmol) in CH_2Cl_2 (30 ml) and aqueous 1M $NaHCO_3$ (25 ml) was stirred for 3 h in the dark. Two layers were separated and the aqueous layer was extracted twice with $CHCl_3$. The combined organic phase was washed with brine, dried over Na_2SO_4, filtered and concentrated to dryness to give **2** as amorphous (221 mg, 99 %). UV λ_{max}/nm (ε): 208.5 (2.0 x 10^4), 349.5 (3900); IR ν_{max}/cm^{-1} ($CHCl_3$): 3400, 2990, 1675, 1575, 1500, 1380, 1165, 1075, 1015; ^{1}H NMR

(270 MHz): δ 1.29 (s, 3H, isopropylidene-Me), 1.34 (s, 3H, isopropylidene-Me), 1.41 (s, 3H, isopropylidene-Me), 1.52 (s, 3H, isopropylidene-Me), 3.15 (m, 2H, 4-H), 3.89 (dd, *J*=8.3, 5.1 Hz, 1H, 6'-H), 4.10 (dd, *J*=8.3, 5.6 Hz, 1H, 6'-H), 4.22 (m, 2H, 4'- and 5'-H), 4.28 (s, 2H, PhCH_2), 4.46 (dd, *J*=6.8, 3.2 Hz, 1H, 3'-H), 4.68 (d, *J*=3.7 Hz, 2'-H), 4.73 (dt, *J*=7.8, 3.4 Hz, 1H, 5-H), 5.61 (d, *J*=6.8 Hz, 1H, NH), 5.75 (dq, *J*=7.8, 1.7 Hz, 1H, 6-H), 5.87 (d, *J*=3.7 Hz, 1H, 1'-H), 7.15 -7.46 (m, 6H, aromatic and 2-H); ^{13}C NMR (67.9 MHz): δ 22.5, 25.2, 26.0, 26.6, 26.7, 56.2, 57.4, 67.1, 73.3, 78.2, 84.5, 98.8, 102.6, 104.6, 109.7, 111.9, 127.2, 127.8, 128.8, 129.2, 137.2, 139.6, 167.8.

3-*N*-(3-Deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranos-3-yl)carbamoylpyridine 13

3-Amino-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose **12** (2.29 g, 8.84 mmol) was treated in the same manner as described for the preparation of **10** to give **13** as colorless crystals (3.02 g, 94 %). m.p. 109-110 °C; IR ν_{max}/cm^{-1} ($CHCl_3$): 3430, 2970, 1660, 1505, 1370; ^{1}H NMR (270 MHz): δ 1.34 (s, 3H, isopropylidene-Me), 1.37 (s, 3H, isopropylidene-Me), 1.44 (s, 3H, isopropylidene-Me), 1.59 (s, 3H, isopropylidene-Me), 3.99 (dd, *J*=9.3, 5.4 Hz, 1H, 4'-H), 4.01 (dd, *J*=8.3, 5.9 Hz, 1H, 6'-H), 4.16 (dd, *J*=8.3, 6.4 Hz, 1H, 6'-H), 4.28 (br. q, *J*=5.9 Hz, 1H, 5'-H), 4.42 (ddd, *J*=9.3, 8.1, 4.9 Hz, 3'-H), 4.77 (dd, *J*=4.9, 3.4 Hz, 1H, 2'-H), 5.89 (d, *J*=3.4 Hz, 1H, 1'-H), 6.54 (br. d, *J*=8.1 Hz, 1H, NH), 7.42 (dd, *J*=8.3, 4.9 Hz, 1H, 5-H), 8.12 (dt, *J*=8.3, 1.7 Hz, 1H, 4-H), 8.76 (dd, *J*=4.9, 1.7 Hz, 1H, 6-H), 8.98 (d, *J*=1.7 Hz, 1H, 2-H); ^{13}C NMR (67.9 MHz): δ 25.1, 26.3, 26.4, 26.6, 54.2, 65.7, 75.9, 78.9, 79.0, 104.3, 109.7, 112.8, 123.5, 129.7, 135.1, 147.9, 152.5, 165.3. *Anal.* Calcd. for $C_{18}H_{24}N_2O_6$: C, 59.33; H, 6.64; N, 7.69. Found: C, 59.04; H, 6.60; N, 7.70.

3-*N*-(3-Deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranos-3-yl)carbamoyl-1-benzylpyridinium bromide 14

Compound **13** (1.73 g, 4.75 mmol) was treated in the same manner as described for the preparation of **11** to give **14** as colorless amorphous (1.63 g, 64 %). UV λ_{max}/nm (ε): 204.5 (2.56 x 10^4); IR ν_{max}/cm^{-1} ($CHCl_3$): 3190, 2950, 1670, 1375, 1020; ^{1}H NMR (270 MHz): δ 1.31 (s, 6H, isopropylidene-Me$_2$), 1.41 (s, 3H, isopropylidene-Me), 1.69 (s, 3H, isopropylidene-Me), 4.12 (dd, *J*=8.8, 7.3 Hz, 1H, 6'-H), 4.30 (dd, *J*=8.8, 5.4 Hz, 1H, 6'-H), 4.47 (m, 1H, 5'-H), 4.63 (ddd, *J*=9.8, 7.8, 4.9 Hz, 1H, 3'-H), 4.76 (dd, *J*=9.8, 2.9 Hz, 1H, 4'-H), 4.81 (dd, *J*=4.9, 3.9 Hz, 1H, 2'-H), 5.90 (d, *J*=3.9 Hz, 1H, 1'-H), 6.13 (d, *J*=13.7 Hz, 1H, PhCH_2), 6.14 (d, *J*=13.7 Hz, 1H, PhCH_2), 7.43 (m, 3H, aromatic), 7.67 (m, 2H, aromatic), 8.01 (dd, *J*=8.3, 5.9 Hz, 1H, 5-H), 8.94 (d, *J*=8.3 Hz, 1H, 4-H), 9.21 (d, *J*=5.9 Hz, 1H, 6-H), 9.28 (d, *J*=7.8 Hz, 1H, NH), 10.39 (s, 1H, 2-H); ^{13}C NMR (67.9 MHz): δ 24.7, 26.0, 26.5, 27.0, 53.4, 64.3, 65.3, 74.7, 78.7, 104.1, 109.7, 112.7, 127.7, 129.6, 129.7, 130.3, 132.0, 134.2, 144.4, 145.2,145.5,161.2. *Anal.* Calcd. for $C_{25}H_{31}N_2O_6Br$: C, 56.08; H, 5.84; N, 5.23. Found: C, 56.12; H, 5.94; N, 5.00.

1-Benzyl-3-*N*-(3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranos-3-yl)carbamoyl-1,4-dihydropyridine 3

Compound **14** (1.11 g, 2.07 mmol) was treated in the same manner as described for the preparation of **2** to give **3** as amorphous (894 mg, 95 %). UV λ_{max}/nm (ε): 208.0 (1.8 x 10^4), 355.0 (3900); IR ν_{max}/cm^{-1} ($CHCl_3$): 3440, 2990, 1670, 1640, 1580, 1500, 1375, 1160, 1060, 1015; ^{1}H NMR (270 MHz): δ 1.34 (s, 6H, 2 x isopropylidene-Me), 1.45 (s, 3H, isopropylidene-Me), 1.55 (s, 3H, isopropylidene-Me), 3.18 (m, 2H, 4-H), 3.94 (dd, *J*=9.3, 3.4 Hz, 1H, 4'-H), 3.99 (dd, *J*=8.3, 6.4 Hz, 1H, 6'-H), 4.12 (dd, *J*=8.3, 6.8 Hz, 1H, 6'-H), 4.25 (m, 2H, 3'- and 5'-H), 4.29 (s, 2H, PhCH_2), 4.63 (dd, *J*=4.9, 3.9 Hz, 1H, 2'-H), 4.75 (dt, *J*=7.8, 3.4 Hz, 1H, 5-H), 5.60 (d, *J*=8.3 Hz, 1H, NH), 5.75 (dq, *J*=7.8, 1.5 Hz, 1H, 6-H), 5.85 (d, *J*=3.9 Hz, 1H, 1'-H), 7.15-7.46 (m, 6H, aromatic and 2-H); ^{13}C NMR (67.9 MHz): δ 22.3, 25.3, 26.4, 26.4, 26.6, 53.1, 57.4, 64.8, 75.6, 79.1, 79.1, 98.7, 102.7, 104.4, 109.5, 112.5, 127.8, 128.8, 129.1, 137.2, 139.7, 167.7.

1-Benzyl-3-*N*-(3-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranos-3-yl)carbamoylpyridinium bromide 15

A mixture of **10** (3.02 g, 8.28 mmol) and benzyl bromide (1.5 ml, 13 mmol) in ethanol (22 ml) was stirred for 68 h at room temperature. After removal of the solvent, the residue was chromatographed over silica gel (200 g) with $CHCl_3$-methanol (50:1-3:1) to give **15** as amorphous (2.22 g, 54 %). UV λ_{max}/nm (ε): 203.0 (1.81 x 10^4); IR ν_{max}/cm^{-1} (KBr): 3370, 1665, 1550, 1495, 1375, 1315, 1210, 1065, 1015 cm^{-1}; ^1H NMR (270 MHz, CD_3OD): δ 1.31 (s, 3H, isopropylidene-Me), 1.50 (s, 3H, isopropylidene-Me), 3.62 (dd, *J*=11.7, 5.4 Hz, 1H, 6'-H), 3.74 (dd, *J*=11.7, 3.4 Hz, 1H, 6'-H), 3.87 (ddd, *J*=7.8, 5.4, 3.4 Hz, 1H, 5'-H), 4.27 (dd, *J*=7.8, 3.9 Hz, 1H, 4'-H), 4.63 (d, *J*=3.9 Hz, 1H, 3'-H), 4.73 (d, *J*=3.4 Hz, 1H, 2'-H), 5.97 (s, 2H, PhCH_2), 5.99 (d, *J*=3,4 Hz, 1H, 1'-H), 7.48 (m, 3H, aromatic), 7.59 (m, 2H, aromatic), 8.22 (dd, *J*=8.1, 6.4 Hz, 1H, 5-H), 8.95 (dt, *J*=8.1, 1.5 Hz, 1H, 4-H), 9.18 (br. d, *J*=6.4 Hz, 1H, 6-H), 9.57 (s, 1H, 2-H); ^{13}C NMR (67.9 MHz, CD_3OD): δ 27.2, 27.6, 59.2, 65.5, 66.9, 71.5, 80.1, 86.3, 106.9, 113.8, 130.4, 131.2, 131.5, 132.0, 135.0, 136.9, 146.4, 146.8, 148.3, 164.7. High resolution FAB-MS: *m/z*: 415.1892. Calcd. for $C_{22}H_{27}N_2O_6$: 415.1870.

1-Benzyl-3-*N*-(3-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranos-3-yl)carbamoyl-1,4-dihydropyridine 4

Compound **15** (993 mg, 2.01 mmol) was treated in the same manner as described for the preparation of **2** to give **4** as amorphous (723 mg, 87 %). UV λ_{max}/nm (ε): 211.5 (2.3 x 10^4), 355.5 (7000); IR ν_{max}/cm^{-1} ($CHCl_3$): 3330, 2995, 1680, 1565, 1505, 1165, 1070, 1020; ^1H NMR (270 MHz): δ 1.30 (s, 3H, isopropylidene-Me), 1.52 (s, 3H, isopropylidene-Me), 2.60 (br. s, 1H, OH), 3.13 (m, 2H, 4-H), 3.6 -3.8 (m, 3H, 5'- and 6'-H), 4.12 (dd, *J*=7.3, 2.9 Hz, 1H, 4'-H), 4.29 (s, 2H, PhCH_2), 4.47 (dd, *J*=7.3, 2.9 Hz, 1H, 3'-H), 4.55 (d, *J*=3.7 Hz, 1H, 2'-H), 4.76 (dt, *J*=8.3, 3.4 Hz, 1H, 5-H), 4,79 (br. s, 1H, OH), 5.72 (m, 2H, 6-H, NH), 5.88 (d, *J*=3.7 Hz, 1H, 1'-H), 7.2 -7.4 (m, 6H, aromatic and 2-H); ^{13}C NMR (67.9 MHz): δ 22.4, 26.1, 26.4, 29.6, 56.7, 57.5, 64.0, 69.3, 79.4, 84.3, 97.5, 103.3, 104.3, 112.0, 127.2, 127.9, 128.8, 136.9, 140.7, 169.4.

1-Benzyl-3-*N*-(3-deoxy-1,2-*O*-isopropylidene-α-D-allofuranos-3-yl)carbamoyl-pyridinium bromide 16

Compound **13** (3.34 g, 9.3 mmol) was treated in the same manner as described for the preparation of **15** to give **16** as amorphous (2.16 g, 56 %). UV λ_{max}/nm (ε): 203.5 (1.76 x 10^4); IR ν_{max}/cm^{-1} (KBr): 3360, 1660, 1540, 1495, 1375, 1210, 1015; ^1H NMR (270 MHz, CD_3OD): δ 1.32 (s, 3H, isopropylidene-Me), 1.54 (s, 3H, isopropylidene-Me), 3.59 (dd, *J*=13.4, 6.3 Hz, 1H, 6'-H), 3.63 (dd, *J*=13.4, 5.0 Hz, 1H, 6'-H), 3.85 (dt, *J*=6.3, 5.0 Hz, 1H, 5'-H), 4.25 (dd, *J*=9.8, 4.9 Hz, 1H, 4'-H), 4.56 (dd, *J*=9.8, 4.9 Hz, 1H, 3'-H), 4.80 (1H, 2'-H), 5.85 (d, *J*=3.4 Hz, 1H, 1'-H), 5.94 (s, 2H, PhCH_2), 7.46 (m, 3H, aromatic), 7.56 (m, 2H, aromatic), 8.22 (dd, *J*=8.3, 6.4 Hz, 1H, 5-H), 8.96 (dt, *J*=8.3, 1.4 Hz, 1H, 4-H), 9.17 (br. d, *J*=6.4 Hz, 1H, 6-H), 9.50 (br. s, 1H, 2-H); ^{13}C NMR (67.9 MHz, CD_3OD): δ 27.4, 27.9, 55.8, 64.7, 66.9, 74.1, 80.3, 81.4, 106.6, 114.6, 130.3, 131.2, 131.6, 132.0, 135.0, 137.0, 146.4, 146.8, 148.3, 164.4. High resolution FAB-MS: *m/z*: 415.1892. Calcd. for $C_{22}H_{27}N_2O_6$: 415.1870.

1-Benzyl-3-*N*-(3-deoxy-1,2-*O*-isopropylidene-α-D-allofuranos-3-yl)carbamoyl-1,4-dihydropyridine 5

Compound **16** (982 mg, 1.98 mmol) was treated in the same manner as described for the preparation of **2** to give **5** as amorphous (691 mg, 84 %). UV λ_{max}/nm (ε): 212.0 (2.3 x 10^4), 355.0 (7300); IR ν_{max}/ cm^{-1} ($CHCl_3$): 3440, 3250, 2990, 1680, 1560, 1510, 1170, 1020, 875 cm^{-1}; ^1H NMR (270 MHz): δ 1.36 (s, 3H, isopropylidene-Me), 1.54 (s, 1H, isopropylidene-Me), 2.48 (br. s, 1H, OH), 3.17 (m, 2H, 4-H), 3.6-3.8 (m, 4H, 4'-, 5'- and 6'-H), 4.19 (m, 1H, 3'-H), 4.30 (s, 2H, PhCH_2), 4.62 (dd, *J*=5.4, 3.9 Hz, 1H, 2'-H), 4.79 (dt, *J*=7.8, 3.4 Hz, 1H, 5-H), 5.23 (br. s, 1H, OH), 5.75 (dd, *J*=7.8, 1.7 Hz, 1H, 6-H), 5.85 (d, *J*=3.9 Hz, 1H, 1'-H), 6.05 (br. d, *J*=7.3 Hz, 1H, NH), 7.2-7.4 (m, 6H, aromatic and 2-H); ^{13}C NMR (67.9 MHz): δ 22.1, 26.6, 54.9, 57.5, 64.2, 72.5, 79.7, 81.5, 97.8, 103.3, 104.0, 112.6, 127.1, 127.8, 128.8, 128.9, 136.9, 140.8, 169.4.

3-*N*-(5,6-Didehydro-1,2-*O*-isopropylidene-3,5,6-trideoxy-α-D-glucofuranos-3-yl)carbamoylpyridine 17

A solution of **10** (1.59 g, 4.11 mmol) in 90 % acetic acid (25 ml) was stirred overnight at room temperature, and then at 70°C for 4 h. The mixture was diluted with water and concentrated to dryness. A mixture of the resulting residue and methanesulfonyl chloride (1.1 ml) in pyridine (10 ml) was stirred overnight at room temperature. Water was added and the mixture was extracted with ethyl acetate. The extract was successively washed with 2N HCl, sat. $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated to dryness. The residue was dissolved in *N*,*N*-dimethylformamide (20 ml), to which NaI (3.78 g, 25.2 mmol) and zinc powder (1.98 g, 30.2 mmol) were added. The resulting mixture was stirred for 5 h under reflux. After cooled to room temperature, the mixture was diluted with ethyl acetate and the insoluble materials were removed by filtration. The filtrate was washed with brine, dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was chromatographed over silica gel (75 g) with hexane-ethyl acetate (5:1-1:1) to give **17** as colorless powder (427 mg, 50 %). m.p. 126.5-127.5°C (from hexane-ethyl acetate). IR ν_{max}/cm^{-1} ($CHCl_3$): 3440, 2990, 1665, 1510, 1070, 1010; ^1H NMR (270 MHz): δ 1.33 (s, 3H, isopropylidene-Me), 1.56 (s, 3H, isopropylidene-Me), 4.54 (dd, *J*=7.1, 3.9 Hz, 1H, 3'-H), 4.74 (d, *J*=3.9 Hz, 1H, 2'-H), 4.97 (m, 1H, 4'-H), 5.42 (dt, *J*=10.8, 1.7 Hz, 1H, 6'*E*-H), 5.61 (dt, *J*=17.6, 1.7 Hz, 1H, 6'Z-H), 5.88 (ddd, *J*=17.6, 10.8, 3.9 Hz, 1H, 5'-H), 5.90 (d, *J*=3.9 Hz, 1H, 1'-H), 6.36 (d, *J*=7.1 Hz, 1H, NH), 7.38 (dd, *J*=7.8, 4.9 Hz, 1H, 5-H), 8.06 (ddd, *J*=7.8, 2.4, 1.5 Hz, 1H, 4-H), 8.72 (dd, *J*=4.9, 1.5 Hz, 1H, 6-H), 8.91 (d, *J*=2.4 Hz, 1H, 2-H); ^{13}C NMR (67.9 MHz): δ 26.1, 26.4, 57.0, 77.8, 84.1, 104.0, 112.1, 118.5, 123.5, 129.6, 131.2, 135.0, 147.8, 152.5, 165.4. *Anal.* Calcd. for $C_{15}H_{18}N_2O_4$: C, 62.05; H, 6.25; N, 9.65. Found: C, 61.94; H, 6.32; N, 9.52.

1-Benzyl-3-*N*-(5,6-didehydro-1,2-*O*-isopropylidene-3,5,6-trideoxy-α-D-glucofuranos-3-yl)carbamoylpyridinium bromide 18

Compound **17** (129 mg, 0.445 mmol) was treated in the same manner as described for the preparation of **11** to give **18** as amorphous (164 mg, 80 %). UV λ_{max}/nm (ε): 204.0 (2.07 x 10^4); IR ν_{max}/cm^{-1} ($CHCl_3$): 3180, 2940, 1665, 1005; ^1H NMR (270 MHz): δ 1.33 (s, 3H, isopropylidene-Me), 1.54 (s, 3H, isopropylidene-Me), 4.70 (dd, *J*=8.8, 3.9 Hz, 1H, 2'-H), 4.82 (dd, *J*=5.9, 3.9 Hz, 1H, 4'-H), 4.92 (d, *J*=3.9 Hz, 1H, 2'-H), 5.09 (d, J=10.8 Hz, 1H, 6'*E*-H), 5.43 (d, *J*=17.1 Hz, 1H, 6'Z-H), 5.81 (ddd, *J*=17.1, 10.8, 5.9 Hz, 1H, 5'-H), 6.13 (s, 2H, PhCH_2), 6.41 (d, *J*=3.9 Hz, 1H, 1'-H), 7.41 (m, 3H, aromatic), 7.66 (m, 2H, aromatic), 8.09 (dd, *J*=8.3, 5.9 Hz, 1H, 5-H), 8.99 (d, *J*=8.3 Hz, 1H, 4-H), 9.15 (d, *J*=8.8 Hz, 1H, NH), 9.37 (d, *J*=5.9 Hz, 1H, 6-H), 10.32 (s, 1H, 2-H); ^{13}C NMR (67.9 MHz): δ 26.2, 26.6, 58.4, 64.5, 80.1, 84.0, 104.9, 111.6, 118.5, 127.9, 129.5, 129.7, 130.3, 131.8, 132.1, 134.2, 144.2, 145.3, 145.7, 161.0. *Anal.* Calcd. for $C_{22}H_{25}N_2O_4Br$: C, 57.27; H, 5.46; N, 6.07. Found: C, 57.08; H, 5.42; N, 6.11.

1-Benzyl-3-*N*-(5,6-didehydro-1,2-*O*-isopropylidene-3,5,6-trideoxy-α-D-glucofuranos-3-yl)carbamoyl-1,4-dihydropyridine 6

Compound **18** (178 mg, 0.387 mmol) was treated in the same manner as described for the preparation of **2** to give **6** as amorphous (121 mg, 80 %). UV λ_{max}/nm (ε): 207.5 (1.8 x 10^4), 347.0 (2500); IR ν_{max}/cm^{-1} ($CHCl_3$): 2990, 1670, 1570, 1500, 1160, 1070, 1010; ^1H NMR (270 MHz): δ 1.30 (s, 3H, isopropylidene-Me), 1.53 (s, 3H, isopropylidene-Me), 3.06 (m, 2H, 4-H), 4.28 (s, 2H, PhCH_2), 4.39 (dd, *J*=7.1, 3.7 Hz, 1H, 3'-H), 4.66 (d, *J*=3.7 Hz, 1H, 2'-H), 4.70 (dt, *J*=8.3, 3.4 Hz, 1H, 5-H), 4.89 (m, 1H, 4'-H), 5.35 (dt, *J*=10.7, 2.0 Hz, 1H, 6'*E*-H), 5.15 (d, *J*=7.1 Hz, 1H, NH), 5.51 (dt, *J*=17.1, 2.0 Hz, 1H, 6'Z-H), 5.74 (m, 1H, 6-H), 5.83 (d, *J*=3.7 Hz, 1H, 1'-H), 5.85 (m, 1H, 5'-H), 7.13-7.56 (m, 6H, aromatic and 2-H); ^{13}C NMR (67.9 MHz): δ 22.2, 26.0, 26.5, 56.4, 57.3, 77.8, 84.2, 98.7, 102.6, 104.0, 111.8, 117.9, 127.1, 127.7, 128.7, 129.8, 131.6, 137.1, 139.4, 167.7.

General method of reduction of benzoylformate with NADH mimics

To a solution of $Mg(ClO_4)_2$ (0.1 mmol) and NADH mimic (0.1 mmol) in dry acetonitrile (2.5 ml) was added methyl benzoylformate (14 μl, 0.1 mmol) *via* syringe. The mixture was stirred at room temperature and the reaction was monitored by TLC. After completion of the reaction, $CHCl_3$ 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. Yield of methyl mandelate was estimated at this stage by ^1H NMR without further purification. The absolute configuration of the resulting methyl mandelate were determined in ^1H NMR by comparison the corresponding (*R*)-(+)-α-methoxy-α-trifluoromethyl-phenylacetyl (MTPA) derivative with the authentic specimen. The optical purity was calculated from integrations of methine signals of (*R*)-MTPA derivative in ^1H NMR [(*S*)- and (*R*)-mandelate; 6.12 and 6.09 ppm, respectively]. MTPA ester of 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.

Computation

The MOPAC (version 6.0) molecular orbital package[22] utilizing the MNDO-PM3 Hamiltonian[17] was used for the semi-empirical MO calculations. The transition states were located using the SADDLE routine[23] implemented in MOPAC. No arbitrary assumption was imposed on to find the most likely geometry for the transition state. Further refinements for each transition state were carried out by the Baker's eigenvector following method[24] with the use of the keyword TS implemented in the MOPAC. 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.[25] MM2 force field was used for molecular mechanics calculations.[21] 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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