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Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 17 (2006) 544–549

# Aqueous diastereoselective hydrogenation of folic acid to tetrahydrofolic acid in the presence of water-soluble Rh and Ir diphosphine complexes

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Received 16 November 2005; accepted 26 December 2005
Available online 21 February 2006

Dedicated to Jack Halpern on the occasion of his 80th birthday

**Abstract**—Rhodium and iridium catalysts with chiral, water soluble diphosphine ligands, were used for the diastereoselective hydrogenation of folic acid disodium salt in water. Using a modified Rh/Josiphos type at 30 °C, L-tetrahydrofolic acid, a relevant pharmaceutical intermediate, was obtained with a selectivity of up to 49% de; at 70 °C turnover numbers of up to 2800 were achieved, albeit with lower selectivity. These results define the state of the art for this reaction.


## 1. Introduction

L-Tetrahydrofolic acid **1a** ([6*S*,α*S*]-tetrahydrofolic acid) plays a central role in cell metabolism as a carrier of one carbon units of different oxidation state.[1] Tetrahydrofolates serve as cofactors in a variety of enzymatic reactions involved in nucleotide and amino acid biosynthesis and several tetrahydrofolic acid derivatives are manufactured for pharmaceutical applications. For example, L-leucovorin **3** ([6*S*,α*S*]-5-formyltetrahydrofolic acid, folinic acid) is utilized in cancer chemotherapy as it enhances the antitumor activity of 5-fluorouracil.[2] L-Mefolinate **4** ([6*S*,α*S*]-5-methyltetrahydrofolic acid), which is an active metabolite of folic acid, is effective in lowering elevated homocysteine levels[3] reported to increase the risk of cardiovascular diseases.[4]

As shown in Scheme 1, L-tetrahydrofolic acid **1a** is a key intermediate in the conventional industrial four-step synthesis of Ca-L-leucovorin **3** and Ca-L-mefolinate **4**. It was produced by the reduction of folic acid **2** with $NaBH_4$[5] or by catalytic hydrogenation with $H_2/PtO_2$[6] in aqueous solutions. The reduction of the pyrazine ring produces a new stereogenic center in position 6. While the physiological enzymatic reduction by dihydrofolate reductase is stereoselective,[7] leading to the natural, enantiomerically pure [6*S*,α*S*]-tetrahydrofolic acid **1a**, conventional chemical reductions always afford an equimolar mixture of diastereomers. [6*S*,α*S*]-Tetrahydrofolic acid **1a** can then be isolated, for example, as a sulfonate salt by repeated fractional crystallization. With this procedure at least half of the starting material is lost while the numerous crystallizations result in a low overall yield of 28% of L-tetrahydrofolic acid sulfonate salt.[8]

A stereoselective hydrogenation, which preferentially affords the desired [6*S*,α*S*]-diastereomer **1a** would, therefore, be a significant improvement of the synthesis. The development of such a stereoselective process is an ambitious task for two major reasons. First, most effective enantioselective hydrogenation catalysts are Rh, Ru, and Ir diphosphine complexes, which are not soluble in water and, second, only very few stereoselective reductions of heteroaromatic rings are known and in most cases, selectivities and/or catalytic activity are low.[9] In principle, there are two approaches for solving these problems. One can either adapt the substrate to existing catalysts or modify existing catalysts to be able to work in water. In fact, both approaches have been described in the literature. An early attempt by Boyle and Keating[10] to hydrogenate a silylated folic acid derivative in benzene using a Rh–DIOP complex failed, because no

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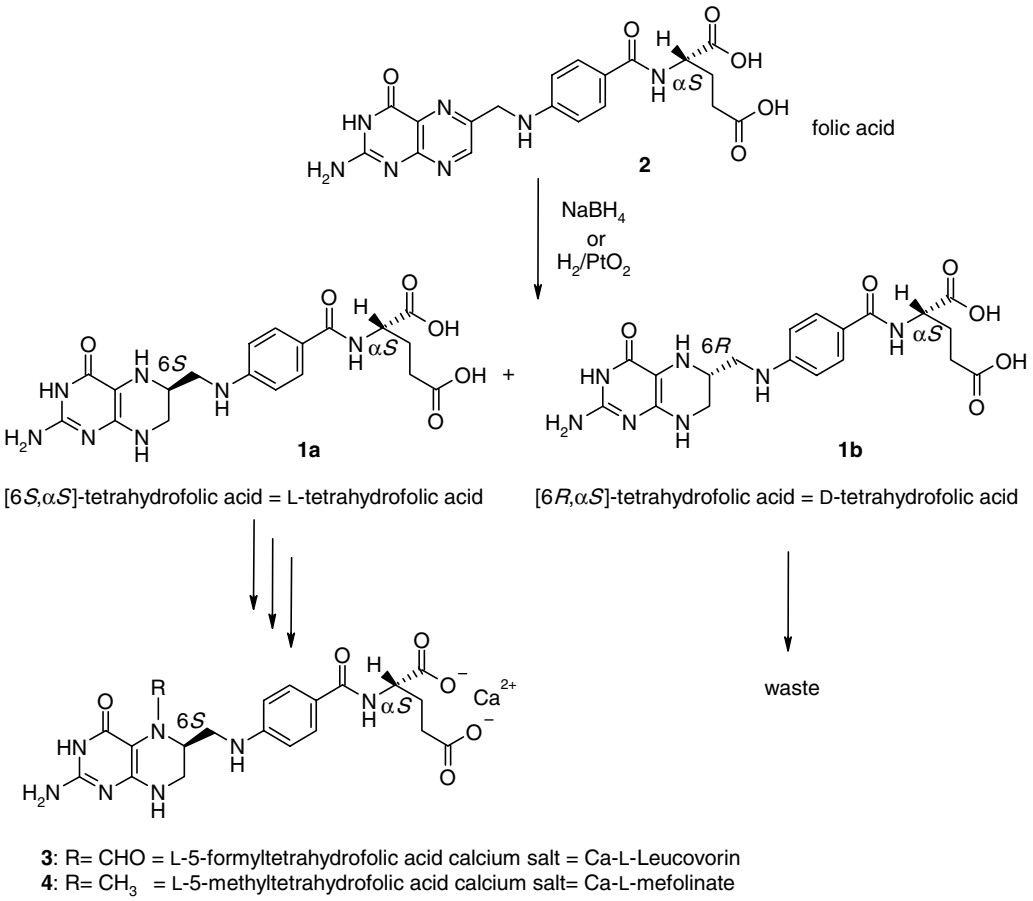
doi:10.1016/j.tetasy.2005.12.034




**Scheme 1.**

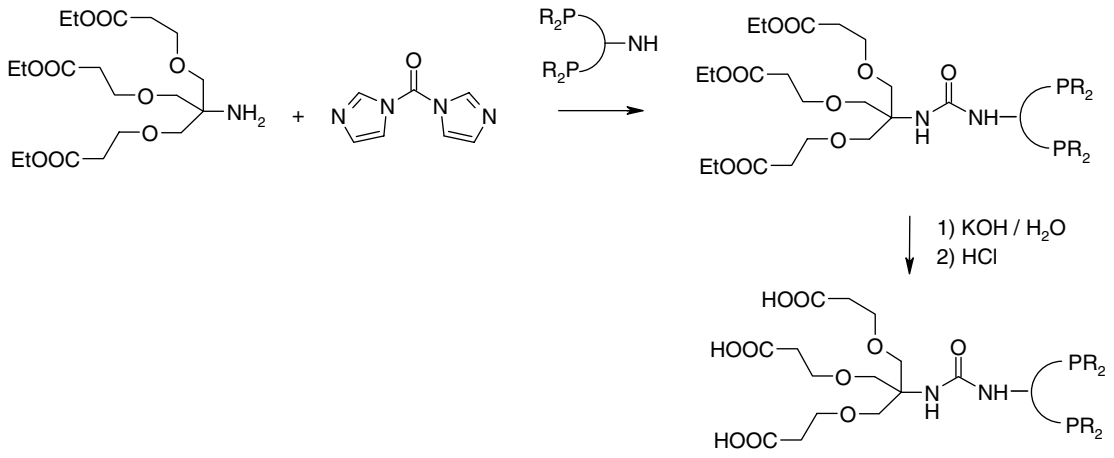
conversion was obtained. Recently we demonstrated that such an approach is actually feasible and that folic acid dimethylester benzenesulfonate can be hydrogenated in organic solvents with up to 60% de and turnover numbers up to 400.[11] Using a Rh/BPPM complex adsorbed on silica gel Brunner et al.[12] were able to hydrogenate folic acid in aqueous buffer solutions with up to 42% de but low activity and catalyst productivity. This case also demonstrates a third difficulty, namely, analysis of the reaction products. In the first paper,[12a] up to 90% des were claimed, which later turned out to be an artifact due to the enrichment of one diastereomer during the preparation of the analytical sample, which required product derivatization.[12c]

Brunner's approach to adsorb conventional catalysts on an inorganic support is very elegant since it requires no synthetic modification of the chiral ligand. On the other hand, the use of porous solids may, among other things, lead to poor mass transport. In fact, turnover numbers (ton) as well as turnover frequencies (tof) as published by Brunner were very low and far from practical interest. Herein we report the use of water soluble Rh and Ir complexes for the stereoselective hydrogenation of folic acid disodium salt in water with up to 49% de and turnover numbers up to 2800.[11a] We also report a direct HPLC method to determine the diastereomeric excess, which does not require any product derivatization or work-up before analysis.

## 2. Results and discussion

The disodium salt of folic acid was obtained by suspending folic acid in water and adjusting the pH to 7.5 with 30% sodium hydroxide solution followed by the addition of ethanol. The isolated folic acid disodium salt was used as a substrate for all the hydrogenation experiments described herein.

Although chiral water soluble diphosphine ligands have been known for many years,[13] their diversity is still very small compared to that of classical chiral diphosphine ligands and, to the best of our knowledge, no such ligand is commercially available. For catalyst screening, especially for such a 'difficult' substrate, a major challenge was to gain access to as many water soluble ligands as possible. In recent years we have developed a modular toolbox that allows tethering functionalized diphosphine ligands to various solid supports.[14] The same functionalized ligands were made water soluble by attaching them to water soluble vehicles, using a concept originally developed by Malmström and Andersson.[15] They tethered chiral diphosphine ligands containing an N–H group to non-cross linked polyacrylic acid via amide bonds. While in many cases this concept worked well, it was also shown that the attachment of several complexes to one polymer string can give rise to a high local catalyst concentration. Depending on the immobilized complex, the resulting interactions between active




**Scheme 2.**

centers can lead to negative effects on their catalytic performance. To avoid this problem, we also used a well defined dendrimer fragment with carboxylic acid groups as water soluble vehicles, which can be attached to functionalized ligands (see Scheme 2).[16]

The water soluble ligands used herein are depicted in Figure 1. PPM and functionalized Josiphos ligands[17] were attached to polyacrylic acid,[15] as well as to the dendritic vehicle (ligands **5**–**7**).[16] The BIPHEMP[18] and Pyrphos moieties were only attached to the dendrimer (ligands **8** and **10**). Additionally, a sulfonated biaryl ligand **9** was obtained from Roche.

The first series of hydrogenations was performed with catalysts formed in situ from $[Rh(NBD)_2]BF_4$ and the ligands depicted in Figure 1. The results are listed in Table 1, entries 1–10. Whereas the diastereoselectivities ranged from 18% (Pyrphos **10**) to 47% for PPM-de **5b** and 46% de for BIPHEMP **8** (entries 2 and 6), the catalytic activity varied much more. It is interesting to note that the dendrimer vehicle gave catalysts with significantly better activities and selectivities than the polyacrylic acid vehicle with PPM (entries 1 and 2). Similarly, the dendritic Josiphos **7b** reached a very remarkable tof of 184 $h^{-1}$ (entry 5), almost twice as high as that of the corresponding Josiphos-pa **7a** (entry 4).

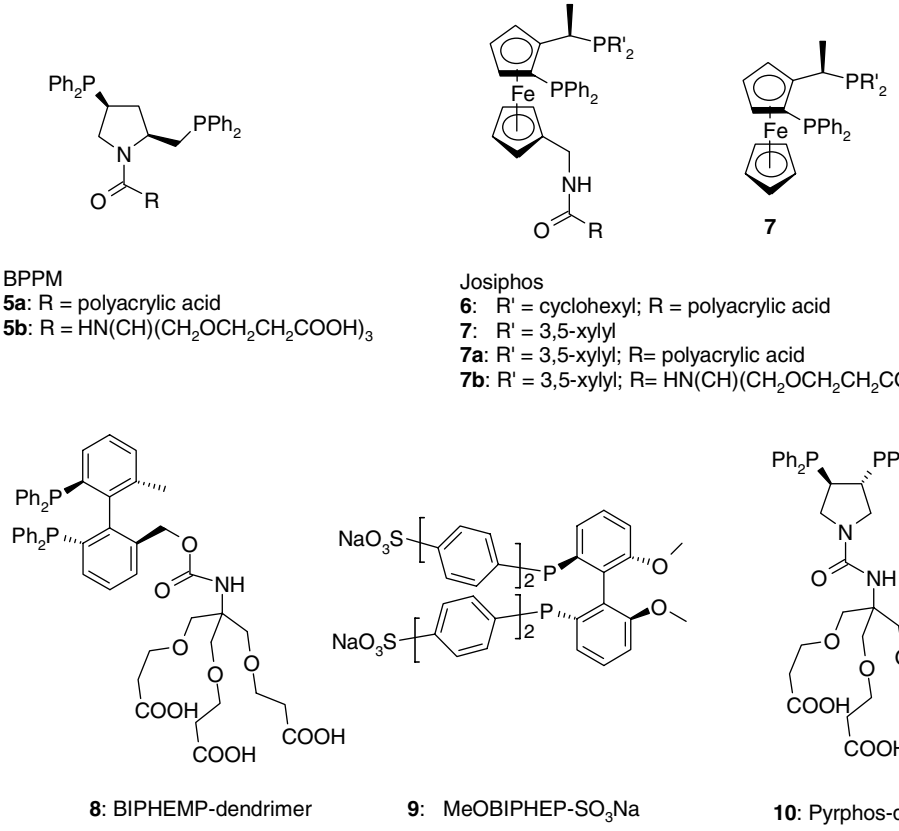



**Figure 1.** Water soluble diphosphine ligands.

**Table 1.** Asymmetric hydrogenation of folic acid disodium salt in water

| Entry | Catalyst[a] | $t$ (h) | Conv. (%) | de (%) | tof ($h^{-1}$) | Comments |
|---|---|---|---|---|---|---|
| 1 | Rh/PPM-pa **5a** | 18.5 | 79 | 28 | 4 | |
| 2 | Rh/PPM-de **5b** | 4 | 87 | 47 | 22 | |
| 3 | Rh/Josiphos-pa **6** | 17.5 | 61 | 36 | 3 | |
| 4 | Rh/Josiphos-pa **7a** | 0.9 | 90 | 30 | 100 | |
| 5 | Rh/Josiphos-de **7b** | 0.5 | 92 | 31 | 184 | |
| 6 | Rh/BIPHEMP-de **8** | 3.2 | 89 | 46 | 27 | |
| 7 | Rh/MeOBIPHEP-$SO_3Na$ **9** | 14 | 87 | 33 | 6 | |
| 8 | Rh/Pyrphos-de **10** | 2 | 82 | 18 | 41 | |
| 9 | Ir/Josiphos-de **7b** | 23 | 2 | 9 | 0.1 | 13.4 equiv NaI/Ir |
| 10 | Ir/Josiphos-pa **7a** | 20 | 6 | 1 | 0.3 | 1320 equiv NaI/Ir |

Reaction conditions: $p = 80$ bar, $T = 70$ °C, substrate/catalyst ratio (s/c) 100, substrate concentration 0.13 mol/l, ligand/metal molar ratio = 1.25, pH = 7 (phosphate buffer).
[a] $[Rh(NBD)_2]BF_4$ or $[Ir(COD)Cl]_2$ (NBD = norbornadiene, COD 1,5-cyclooctadiene).

**Table 2.** Asymmetric hydrogenation of folic acid disodium salt in water catalyzed by $[Rh(NBD)_2]BF_4$/Josiphos-de **7b**

| Entry | $p$ | $T$ | s/c | $t$ (h) | Conv. (%) | de (%) | tof ($h^{-1}$) | Comments |
|---|---|---|---|---|---|---|---|---|
| 1 | 80 | 70 | 100 | 0.5 | 92 | 31 | 184 | Standard |
| 2 | 80 | 70 | 100 | 23.5 | 23 | 21 | 1 | Josiphos **7** |
| 3 | 80 | 70 | 100 | 8 | 90 | 26 | 11 | $[Rh(COD)Cl]_2$/Josiphos **7**/$SiO_2$ |
| 4 | 20 | 70 | 100 | 2 | 94 | 26 | 47 | |
| 5 | 80 | 30 | 100 | 12 | 97 | 49 | 8 | |
| 6 | 80 | 30 | 100 | 16.5 | 26 | 38 | 2 | pH = 6 |
| 7 | 80 | 70 | 100 | 0.3 | 92 | 26 | 307 | 100 equiv NaBr/Rh |
| 8 | 80 | 70 | 100 | 1 | 100 | 23 | 100 | 20 equiv KI/Rh |
| 9 | 80 | 70 | 1000 | 4 | 89 | 30 | 223 | Substrate = 0.28 M |
| 10 | 80 | 70 | 3000[a] | 8 | 91 | 26 | 341 | Substrate = 0.24 M |

Reaction conditions: pH 7, phosphate buffer, substrate concentration 0.13 mol/l, ligand/metal molar ratio = 1.25.
[a] Substrate was purified by stirring over Rh/C before use.

Since iridium diphosphine complexes in the presence of iodide are known to be highly efficient catalysts for the hydrogenation of imines,[19] a few experiments were carried out with selected Ir complexes (entries 9 and 10). However, the diastereoselectivities as well as activities were very low.

The dendritic Rh/Josiphos-de **7b** catalyst was chosen for further investigations. The reason for this choice were the very high activity observed and the fact that Josiphos ligands are available on large scale, which is not the case for the PPM and the functionalized BIPHEMP ligands used in this investigation. In order to check whether it was really necessary to have water soluble catalysts, we carried out experiments with the unfunctionalized Josiphos **7**, as well as with Josiphos adsorbed on a silica gel and we investigated the effect of various reaction parameters. The results of these studies are compiled in Table 2. A comparison of entries 2 and 3 with the standard experiment (entry 1) clearly shows that the unfunctionalized or adsorbed Rh complexes have very low activity, even though the stereoselectivity is not affected so much. This clearly demonstrates that it is essential to have a catalyst, which is soluble in the reaction medium. The stereoselectivity was not much affected by the reaction pressure (entry 4) but depended strongly on the reaction temperature. Decreasing the reaction temperature from 70 to 30 °C resulted in an increase of the de from 31% to 49% (entry 5), but as expected the reaction times became longer and the tof decreased strongly. At 0 °C no conversion was observed any more. Changing the pH from 7 to 6 decreased the de somewhat but slowed the reaction even further, probably because under these conditions, the starting material was not completely dissolved (entry 6). The addition of additives, such as NaBr or KI, went along with a slight decrease of diastereoselectivity but the presence of NaBr was beneficial for the catalytic activity (entries 7 and 8). While it was possible to run the reaction at an s/c ratio of 1000 within a reasonable reaction time (entry 9), a further increase to 3000 was only possible with an additional purification of the substrate. When the folic acid disodium salt was stirred over Rh/C before use, a conversion of >90% was reached after 8 h (entry 10). Without this pre-treatment there was no conversion, which indicates that the substrate contains a catalyst poison that is adsorbed on Rh/C.

## 3. Conclusion

We have demonstrated that a modular approach based on attaching different functionalized ligands to various water soluble groups is feasible for screening a broad range of water soluble homogeneous catalysts for a commercially relevant application. We also found that folic acid disodium salt can be hydrogenated stereoselectively in aqueous solution with water soluble Rh diphosphine complexes. Diastereoselectivities of up to 47–49% were obtained with Rh/BIPHEMP **8**, Rh/PPM-de **5b**,

and with Rh/Josiphos-de **7b** (at 30 °C), which is a slight improvement in comparison with Brunner's[12c] best de of 40%. However, good catalytic activity could only be achieved with Rh/Josiphos-de **7b** with tons up to 334 $h^{-1}$ at 70 °C. However, under these conditions, stereoselectivity varies between 26% and 31% de. Turnover numbers of >2700 were obtained (with substrate stirred over Rh/C before use), which is a huge improvement compared to Brunner's[12] best tons of 40. While catalyst activity and productivity are very close to being technically viable, the diastereoselectivities are still too low to make this an alternative to the classical process. Further efforts are needed to identify a catalytic system with similar activities and improved diastereoselectivities in order to make this new method an alternative to the classical process.

## 4. Experimental

### 4.1. General

All manipulations with oxygen and moisture sensitive materials were performed under an argon atmosphere. All solvents, which were used for preparing catalysts and for the hydrogenation reactions, were degassed at a vacuum line and flushed with argon. The buffer solution of pH 7, which was added to the reaction mixtures was prepared by dissolving 0.041 mol $Na_2HPO_4$ and 0.028 mmol $KH_2PO_4$ in 1 l of water.

The hydrogenations were run in steel autoclaves, equipped with sensors for temperature and pressure. The reaction pressure was kept constant by continuous supply of hydrogen from a reservoir. Hydrogen consumption was monitored indirectly by measurement of the pressure decrease in the reservoir. Hydrogenation solutions were stirred with a magnetic stirring bar. Folic acid dihydrate was purchased from Fluka (47620). Folic acid disodium salt had a purity of 95% and contained 5% water.

### 4.2. Preparation of the water soluble ligands

**4.2.1. Functionalized ligands.** (−)-(2*S*,4*S*)-2-Diphenylphosphinomethyl-4-diphenylphosphino-pyrrolidine was purchased from Aldrich (32,677-1). (3*R*,4*R*)-Bis(diphenylphosphino)pyrrolidine was prepared as described by Nagel et al.,[20] the Josiphos- and Xyliphos-ligands with an aminomethyl function at the bottom cyclopentadienyl ring were prepared as described in Ref. 17 and the BIPHEMP ligand with the OH function was synthesized as described in Ref. 18.

**4.2.2. Water soluble ligands.** The polyacrylic acid attached ligand **5a** was prepared as described in Ref. 14 while ligands **6a** and **7a** were prepared in an analogous way, as described in Ref. 17. Polyacrylic acid with an average MW of 2000 was used and was purchased as a 60% aqueous solution from Aldrich (19,202-3). The polymer loadings were 0.67 mmol ligand/g for ligand **5a**, 0.54 mmol ligand/g for ligand **6a**, and 0.22 mmol/g for ligand **7a**. The preparation of the dendrimer bound ligands **5b**, **7b**, **8**, and **10** is described in Ref. 16.

### 4.3. Procedures for the hydrogenation of folic acid disodium salt

**4.3.1. Hydrogenations with Rh catalysts.** To 25 μmol water soluble diphosphine ligand were added 5 ml water and 0.5 ml buffer solution of pH 7. By the addition of 0.1 M NaOH (about 1 ml), the carboxyl groups of the ligand were deprotonated. The resulting clear solution was added to 20 μmol $[Rh(NBD)_2]BF_4$ and stirring continued until a clear solution was obtained. This catalyst-solution was added to a solution of 2 mmol folic acid disodium salt in 11 ml water and 1.5 ml buffer solution of pH 7. At this point additives were added to the reaction mixture if required or the pH was adjusted to pH 6 by the addition of 4 ml of 1 M $KH_2PO_4$ solution. The mixed solution was added via a capillary into a 50 ml autoclave flushed with nitrogen. The autoclave was sealed and the hydrogenation started by stirring at 70 °C with constant hydrogen pressure of 80 bar unless otherwise is stated. The reactions were run until the hydrogen uptake stopped. After hydrogenation, the reaction mixtures were set under argon and samples for HPLC analysis of tetrahydrofolic acid taken. The hydrogenations with high s/c were performed in a 5–10 mmol substrate scale.

**4.3.2. Hydrogenations with Ir catalysts.** To 25 μmol diphosphine ligand and 12.5 μmol $[Ir(COD)Cl]_2$, 5 ml methanol was added. After stirring for 10 min, the methanol was evaporated and a solution of 1.25 g (2 mmol) folic acid disodium salt in 25 ml of water and 2 ml of buffer solution added. By addition of 0.1 M NaOH, a clear solution was obtained. At this point, additives were added if required. The solution was transferred via a narrow tube into a 50 ml autoclave flushed with nitrogen. The hydrogenations were then performed as described above.

**4.3.3. Pre-treatment of folic acid disodium salt.** Rh/C (600 mg) (Engelhard 4806) was washed three times with distilled water and added to a solution of 6 mmol folic acid disodium salt in 20 ml of water and 3.5 ml of buffer solution. After stirring under an argon atmosphere for 1 h, the Rh/C was filtered off and washed with 1 ml of water. The aqueous substrate solution was then used for the hydrogenation.

### 4.4. HPLC methods for the analysis of tetrahydrofolic acid

The content of tetrahydrofolic acid was determined by using the following HPLC method.[8] $Na_2HPO_4$ (0.03 mol) and $KH_2PO_4$ (0.03 mol) were dissolved in 1 l of water to give solvent A. Solvent B was composed of 800 ml solvent A and 200 ml methanol. By addition of phosphoric acid the pH was adjusted to pH 7.8. A solution of tetrahydrofolic acid in solvent A (1 mg/ml) was analyzed on an ODS column (Macherey and Nagel, Shandon-Hypersil, 250 × 4 mm) by using a linear gradi-

ent (flow rate 1 ml/min) from 0% to 85% solvent B within 28 min and UV detection at 280 nm. To protect tetrahydrofolic acid from oxidative degradation, all eluents were continuously flushed with argon.

The diastereoisomers of tetrahydrofolic acid were determined by using the following HPLC method.[8] The eluent was prepared by mixing 960 ml water, 40 ml acetonitrile, 8 g β-cyclodextrine, and 10 ml triethylamine. By adding of acetic acid, the pH was adjusted to pH 7.0 at which point 274 µl 37% formaldehyde was added. A solution of tetrahydrofolic acid in the eluent (0.5 mg/ml) was analyzed (flow rate 1 ml/min) on an ODS column (Macherey and Nagel Nucleosil/CART, 120-5, 250 × 4 mm) with UV-detection at 280 nm.

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