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Stereoselective Hydrogenation of Folic Acid Dimethyl Ester Benzenesulfonate: A New Access to Optically Pure L-Tetrahydrofolic Acid

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Abstract: Folic acid was converted to the corresponding dimethyl ester benzenesulfonate and stereoselectively hydrogenated in organic solvents. An extended screening with rhodium- and iridium-diphosphine catalysts was performed. Under optimized conditions the asymmetric hydrogenation provided a solution of tetrahydrofolic acid dimethyl ester with up to 44% de. The de could be increased to 98% by fractional crystallization of L-tetrahydrofolic acid dimethyl ester benzenesulfonate from the hydrogenation mixture. Hydrolysis of the ester afforded optically pure L-tetrahydrofolic acid, which can be isolated or converted into its 5- or 10- or 5,10-substituted derivatives.

Keywords: folic acid; homogeneous catalysis; iridium; P ligands; rhodium; stereoselective hydrogenation

Introduction

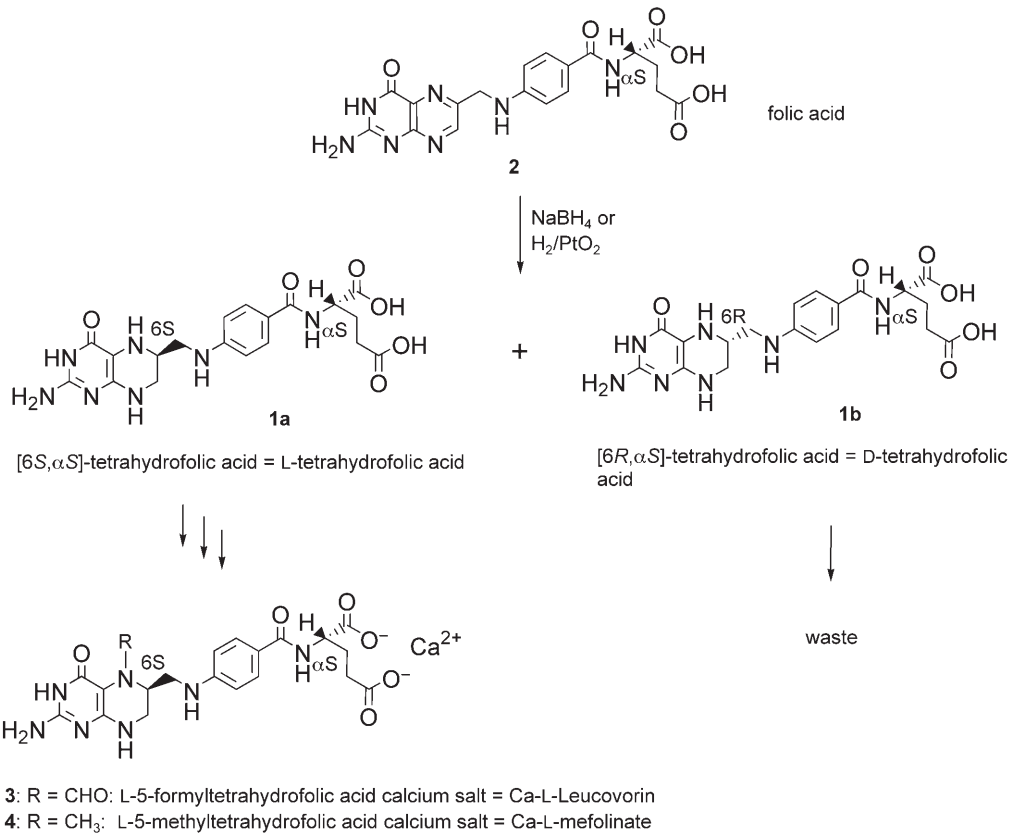
L-Tetrahydrofolic acid, which is [6S,αS]-tetrahydrofolic acid (**1a**), plays a central role in cell metabolism.[1] Some tetrahydrofolic acid derivatives are manufactured for pharmaceutical applications. For example, L-leucovorin (**3**; [6*S*,α*S*]-5-formyltetrahydrofolic acid, folinic acid) is utilized 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, may be effective in lowering elevated homocysteine levels[3] which are reported to increase the risk of cardiovascular diseases.[4]

As shown in Scheme 1, L-tetrahydrofolic acid (**1a**) is the key intermediate in the conventional industrial four-step synthesis of Ca-L-leucovorin (**3**) and Ca-L-mefolinate (**4**). It is formed by 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 chiral center in position 6. While the physiological enzymatic reduction by dihydrofolate reductase is stereoselective,[7] leading to the optically pure natural [6*S*,α*S*]-tetrahydrofolic acid, the conventional chemical reductions always afford an equimolar mixture of diastereoisomers. Optically pure [6*S*,α*S*]-tetrahydrofolic acid can then be isolated from the racemic mixture, for example, as a sulfonate salt by repeated fractional crystallization.[8] With this procedure at least half of the starting material is lost and the numerous crystallizations result in a low overall yield of 28% of L-tetrahydrofolic acid sulfonate salt with reference to folic acid.[8]

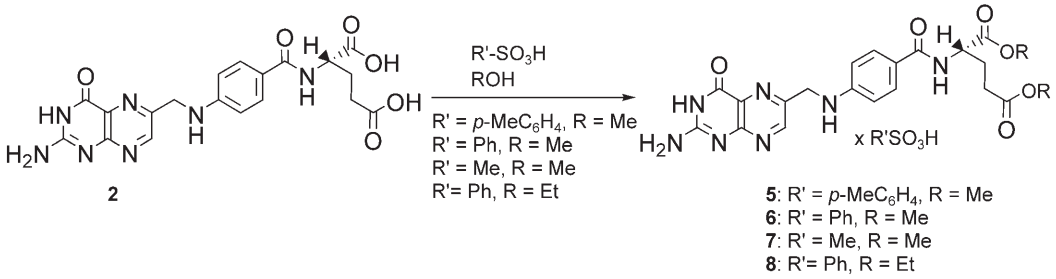
Stereoselective hydrogenation which preferentially affords the desired [6*S*,α*S*]-diastereomer would therefore significantly improve the efficiency of this synthesis. Several attempts in this direction have been described in the literature.

Boyle et al.[9] solubilized folic acid by silylation and performed hydrogenation experiments in benzene using Rh/Diop [*O*-isopropylidene-2,3-dihydroxy-1,4-bis(diphenylamino)butane], but failed to detect any conversion of starting material. Brunner et al.[10] were able to hydrogenate folic acid in aqueous buffer solutions with up to 42% de but low activity using 2.5 mol % of chiral rhodium diphosphine catalysts adsorbed onto silica gel.

In this contribution we describe a new, alternative route to optically pure L-tetrahydrofolic acid which is based on the stereoselective hydrogenation of folic acid dimethyl ester benzenesulfonate in organic solvents that works with less than 0.2 mol % unmodified chiral rhodium diphosphine catalysts.[11]



Scheme 1. Conventional synthesis of reduced tetrahydrofolic acid derivatives Ca-L-mefolinate and Ca-L-leucovorin.

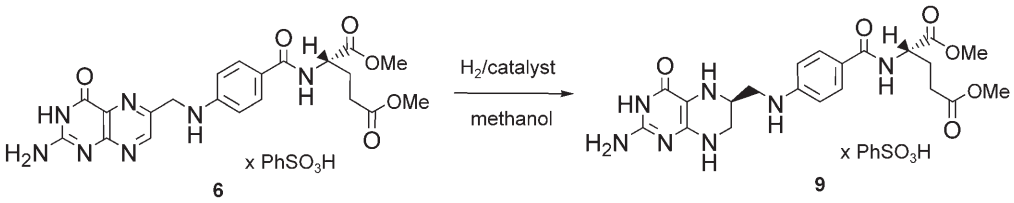


Scheme 2. Esterification of folic acid with different sulfonic acids in alcohols.

Results and Discussion

Esterification of Folic Acid

Folic acid is practically insoluble in the organic solvents that are commonly used in catalytic enantioselective hydrogenations. We therefore first searched for easily accessible folic acid derivatives which are soluble in organic solvents and after reduction can easily be converted to tetrahydrofolic acid. The introduction of the *N,N*-dimethylaminomethylene group at the N-2 position which is often used in pteridine chemistry to increase solubility[12] or N-2,N-10-acetylation of folic acid was not successful. Folic acid dimethyl ester hydrochloride salt which was prepared according to Pfiffner et al.[13] by treatment of folic acid with methanolic HCl was found to be sparingly soluble in methanol. Since chloride is an anion that can coordinate with our catalysts and thereby influence their properties and HCl corrodes steel autoclaves we replaced HCl by benzenesulfonic acid. Addition of 1–2 equivalents of benzenesulfonic acid to a suspension of folic acid in methanol gave folic acid dimethyl ester benzenesulfonate that crystallizes with about 1.2 equivalents of benzenesulfonic acid. The esterification was complete after 1 hour at 90 °C or alternatively after overnight reaction at room temperature. The conversion also worked with toluenesulfonic acid and methanesulfonic acid leading to the corresponding salts. Folic acid diethyl ester was obtained by using ethanol (Scheme 2).



Scheme 3. Reduction of folic acid dimethyl ester benzenesulfonate to tetrahydrofolic acid dimethyl ester benzenesulfonate.

Stereoselective Hydrogenation of Folic Acid Dimethyl Ester Benzenesulfonate[11]

The solubility of folic acid dimethyl ester benzenesulfonate in methanol is 0.5%. However, it was found that suspensions of folic acid dimethyl ester benzenesulfonate in methanol of up to a maximum of 10% content can be hydrogenated very well with homogeneous catalysts leading to methanolic solutions of tetrahydrofolic acid dimethyl ester benzenesulfonate. These findings encouraged us to perform a broad screening of unmodified rhodium and iridium catalysts with a large variety of chiral diphosphine ligands (Table 1) in order to identify effective catalytic systems for the stereoselective hydrogenation of folic acid dimethyl ester benzenesulfonate (Scheme 3).

The ligands in Table 1 are divided in 4 classes: (a) biaryl ligands, (b) ligands with a ferrocene backbone which include Josiphos-type ligands (IV) as well as Hayashi's BPPFA type ligands (V), (c) Duphos type ligands (VI–VII) and (d) miscellaneous commercially available ligands. The hydrogenations that gave > 70% conversion and de > 35% are highlighted. The results in Table 1 clearly show that biaryl-type ligands are the ligands of choice. With this class of ligands a de of up to 44% and conversions up to 80% are obtained. From all other ligands, only BPPM can match these results. The fact that (*R*)- and (*S*)-Binap exhibit the same activity and lead to products with the same de but with just different configurations (entries 1 and 2) proves that the existing chiral center in the folic acid dimethyl ester has no influence on the outcome of the hydrogenation.

Iridium diphosphine catalysts which are known to be highly active in the hydrogenation of imines[14] were also included in our catalyst screening. As expected, the metal has an important and unpredictable effect on activity and de. Based on the results obtained so far, rhodium is clearly superior to iridium in the present reaction. It remains to be seen whether ligands that gave poor results with rhodium will in some cases give better results with iridium. The use of ruthenium was also envisaged. Ru-[*R*-(+)-BINAP]-Br_2 showed no activity, therefore we refrained from using ruthenium for further screening experiments.

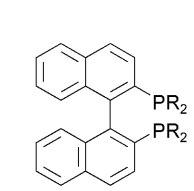
There are plenty of examples in the literature showing that factors such as anions or other additives, solvent, or reaction conditions can strongly affect the performance of catalysts. With the objective to optimize selectivity and activity we studied the influence of such factors on rhodium and iridium catalysts with BINAP and BPPM ligands (Table 2).

In Table 2 we show the influence of the anion on catalyst performance. While the performance of the Rh(+)-BINAP catalyst was not much influenced by the addition of additives, the activity and stereoselectivity of the Rh(+)-BPPM, Ir-BPPM and Ir-BINAP catalysts were strongly affected. In all these cases, addition of coordinating anions can lead to catalysts that produce the product with inverse configuration. However, none of the modified catalyst systems delivered more than 44% de.

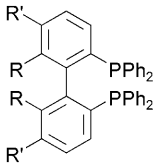
Due to its good activity, high de results and commercial availability the Rh(+)/*R*-(+)-BINAP catalyst was chosen for further optimization. In a series of experiments the influence of the solvent was studied. Due to the limited solubility of folic acid dimethyl ester benzenesulfonate, only a small number of solvents, in particular alcohols and mixtures thereof, were tested (Table 3). The hydrogenation of folic acid diethyl ester in ethanol led to a slight increase in de from 44% to 51% and a conversion of 70% (entry 2). Ethanol may therefore be a viable alternative to methanol. A significant increase in de from 44% to 61% and 55% was observed with 2-propanol and ethylene glycol, respectively, as solvent. Unfortunately, presumably due to the low solubility of **6** in these alcohols, the conversions were quite low (10% and 57%, entries 4 and 6).

Using methanol as solvent, the influence of the hydrogen pressure was studied (Figure 1). While the diastereomeric excess of the product is not significantly influenced, the hydrogen pressure has a clear effect on the chemoselectivity of the reaction. At low pressures the undesired *p*-aminobenzoylglutamic acid dimethyl ester (ABGA ester) is formed as the main product. Since we found that the product tetrahydrofolic acid dimethyl ester benzenesulfonate is stable under hydrogenation conditions, we assume that dihydrofolic acid dimethyl ester, which is supposed to be an intermediate, decomposes under hydrogenation conditions. The cleavage of the ABGA ester seems to be enhanced if the hydrogenation reaction is slowed down, for example, at low pressure. Reduction of the reaction temperature from 70 °C to 50 °C or 30 °C had no influence on diastereose-

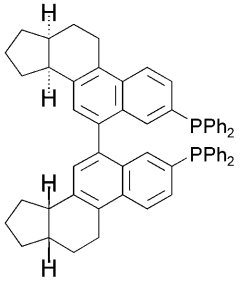
Table 1. Asymmetric hydrogenation of **6** with Rh and Ir catalysts with various diphosphine ligands.



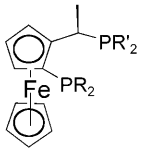
Ia: R = Ph
Ib: R = *p*-Tolyl



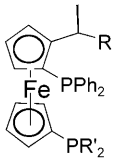
IIa: R = Me, R'= H
IIb: R = OMe, R'= H
IIc: R = OMe, R'=Cl



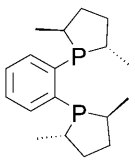
III



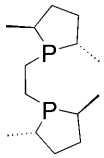
IVa: R = Ph, R' = Ph
IVb: R = Ph, R' = 4-$CF_3C_6H_4$
IVc: R = Ph, R' = 3,5-Xylyl
IVd: R = Ph, R' = *c*-C_6H_{11}
IVe: R = *c*-C_6H_{11}, R' = Ph
IVf: R = 4-$CF_3C_6H_4$, R' = Ph



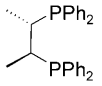
Va: R = NMe_2, R' = Ph
Vb: R = NMe_2, R' = *c*-C_6H_{11}
Vc: R = OH, R' = Ph



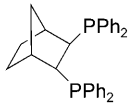
VI: Me-Duphos



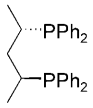
VII: Me-BPE



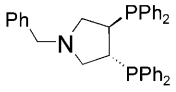
VIII: Chiraphos



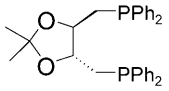
IX: Norphos



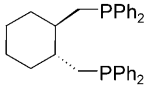
X: BDPP



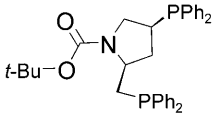
XI: Pyrphos HCl



XII: Diop



XIII: C6-Diop



XIV: BPPM

Entry	Formula	Name/Abbreviation	% de	Rh Conversion [%]	% de	Ir Conversion [%]
Biaryl-Type Ligands						
1	Ia	*R*-(+)-BINAP	**44**	**78**	30	**80**
2	Ia	*S*-(−)-BINAP	−**44**	**76**		
3	Ib	Tol-*R*-(+)-BINAP	**42**	**76**		
4	IIa	*R*-(+)-BIPHEMP	**42**	**72**		
5	IIb	*R*-(+)-MeO-BIPHEP	**38**	**81**	−6.0	68
6	IIc	*R*-(+)-Cl-MeO-BIPHEP	**42**	**79**	−11	**73**
7	III		**42**	**80**	15.9	**88**
Ferrocene Ligands						
8	IVa	Josiphos; R = Ph, R′ = Ph	12	60		
9	IVb	Josiphos; R = Ph, R′ = 4-$CF_3C_6H_4$	−11	9		
10	IVc	Josiphos; R = Ph, R′ = 3,5-Xylyl	14	58	7	6
11	IVd	Josiphos; R = Ph, R′ = *c*-C_6H_{11}	22	69	−8	53
12	IVe	Josiphos; R = *c*-C_6H_{11}, R′ = Ph	23	20		
13	IVf	Josiphos; R = 4-$CF_3C_6H_4$, R′ = Ph	−23	12		
14	Va	*S,R*-BPPFA	−17	64	**35**	59
15	Vb	*S,R*-Ph_2P, cy_2PFA	9	2		
16	Vc	*S,R*-BPPFOH	14	81		
Phospholane-Type Ligands						
17	VI	(*R,R*)-Me-Duphos	0.4	2		
18	VII	(*R,R*)-BPE	4	23		
Miscellaneous Ligands						
19	VIII	(*R,R*)-Chiraphos	0.4	15		
20	IX	(*S,S*)-Norphos	7	27		
21	X	(*S,S*)-BDPP	−2	40	4	39
22	XI	Pyrphos x HCl	9	46		
23	XII	(−)-Diop	14	2	−9	46
24	XIII	(−)-C6-Diop	2	65		
25	XIV	(−)-(2*S*,4*S*)-BPPM	**38**	**82**	−32	**76**

Reaction conditions: substrate/catalyst molar ratio (S/C) = 100, ligand/metal molar ratio = 1.25, MeOH (c = 5.3%), 70 °C, 80 bar, 17 h, for preparation of catalyst precursors $[Rh(COD)_2]BF_4$ or $[Ir(COD)Cl]_2$, (COD = cyclooctadiene) were used, conversion and de were directly measured by HPLC.

Table 2. Rhodium and iridium diphosphine-catalyzed asymmetric hydrogenations of **6** in the presence of additives.

Entry	Catalyst precursor	Additive	% de	Conversion [%]
1	$[Rh(COD)_2]BF_4$/*R*-(+)-BINAP	–	44	78
2	$[Rh(COD)Cl]_2$/*R*-(+)-BINAP	–	44	n.d.
3	$[Rh(COD)_2]BF_4$/*R*-(+)-BINAP	10 equivs. LiCl/Rh	42	n.d.
4	$[Rh(COD)_2]BF_4$/*R*-(+)-BINAP	10 equivs. NaI/Rh	35	32
5	$[Ir(COD)_2]BF_4$/*R*-(+)-BINAP	–	18	73
6	$[Ir(COD)Cl]_2$/*R*-(+)-BINAP	–	30	80
7	$[Ir(COD)Cl]_2$/*R*-(+)-BINAP	10 equivs. LiCl/Ir	−22	**57**
8	$[Ir(COD)Cl]_2$/*R*-(+)-BINAP	10 equivs. LiBr/Ir	−7	58
9	$[Ir(COD)Cl]_2$/*R*-(+)-BINAP	10 equivs. Bu_4NI/Ir	−10	25
10	$[Rh(COD)_2]BF_4$/(−)-(2*S*,4*S*)-BPPM	–	38	82
11	$[Rh(COD)Cl]_2$/(−)-(2*S*,4*S*)-BPPM	–	16	65
12	$[Rh(COD)2]BF_4$/(−)-(2*S*,4*S*)-BPPM	1 equiv. Bu_4NI/Rh	−1.2	67
13	$[Rh(COD)2]BF_4$/(−)-(2S,4S)-BPPM	10 equivs. NaI/Rh	−38	13
14	$[Ir(COD)_2]BF_4$/(−)-(2*S*,4*S*)-BPPM	–	−11	38
15	$[Ir(COD)Cl]_2$/(−)-(2*S*,4*S*)-BPPM	–	−32	76
16	$[Ir(COD)Cl]_2$/(−)-(2*S*,4*S*)-BPPM	10 equivs. LiCl/Ir	−40	77
17	$[Ir(COD)Cl]_2$/(−)-(2*S*,4*S*)-BPPM	10 equivs. LiI/Ir	14	76

Reaction conditions: substrate/catalyst molar ratio (S/C) = 100, MeOH (c = 5.3%), ligand/metal molar ratio = 1.25, 70 °C, 80 bar, 17 h, conversion and de were directly measured by HPLC.

Table 3. Asymmetric hydrogenation of **6** in different solvents catalyzed by rhodium-(+)-BINAP complex.

Entry	Solvent	% de	Conversion [%]
1	MeOH	44	78
2	EtOH	51	70
3	MeOH/THF, 1:1	44	70
4	2-propanol	61	10
5	2-propanol/MeOH, 1:1	48	32
6	ethylene glycol	55	57
7	1,2-propanediol	51	62

Reaction conditions: $[Rh(COD)_2]BF_4$/R-(+)-BINAP as catalyst, substrate/catalyst molar ratio (S/C = 100), *R*-(+)-BINAP/Rh molar ratio = 1.25, concentration = 1.25 g of **6**/30 mL solvent, 70 °C, 80 bar, 17 h, conversion and de were directly measured by HPLC.

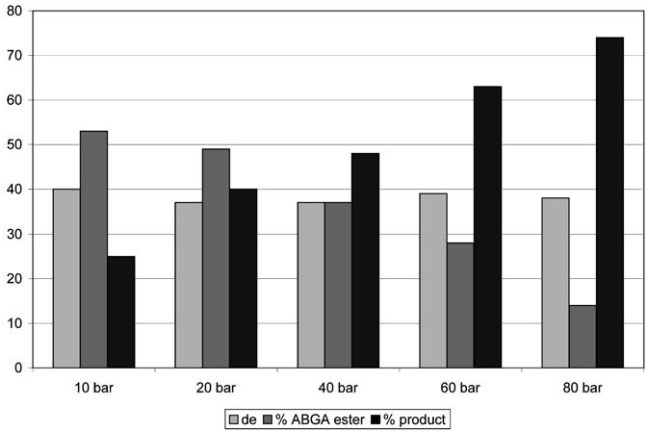


Figure 1. Asymmetric hydrogenation of **6** at different reaction pressures catalyzed by rhodium-(+)-BINAP complex. *Conditions:* $[Rh(COD)_2]BF_4$/*R*-(+)-BINAP as catalyst, substrate/catalyst molar ratio (S/C = 500), *R*-(+)-BINAP/Rh molar ratio = 1.25, MeOH (c = 13.4%), 70 °C, 17 h; conversion and de were directly measured by HPLC.

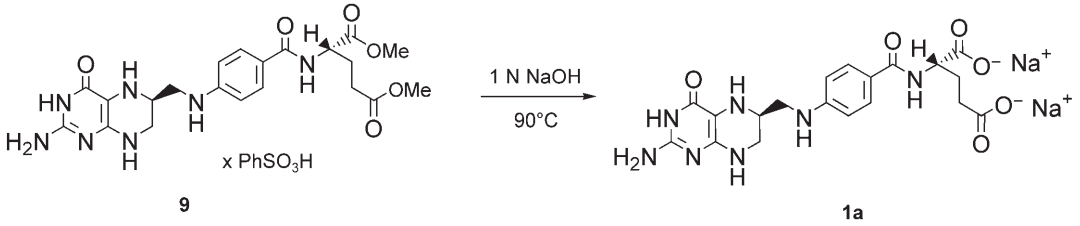
lectivity but led to an incomplete conversion due to an impaired solubility of the substrate.

The Overall Process from Folic Acid to L-Tetrahydrofolic Acid

In a final phase of our work efforts were made to integrate this novel diastereoselective hydrogenation of folic acid esters into a process that yields optically pure L-tetrahydrofolic acid as final product. The *in situ* formation of the folic acid dimethyl ester benezenesulfonate in the hydrogenation vessel and subsequent hydrogenation of the resulting suspension proved to be possible. Also, by chance it was observed that colorless crystals separated from the hydrogenation solutions when left at room temperature. This material was identified as L-tetrahydrofolic acid dimethyl ester benzenesulfonate with an optical purity of 99%. The crystallization procedure was thoroughly optimized to obtain the material from the hydrogenation mixture in a high chemical yield and with at least 94% de. Using this procedure 2.5 kg of folic acid were converted to L-tetrahydrofolic acid dimethyl ester benzenesulfonate with an optical purity of 98% de and a chemical yield of 45%. This reaction was performed with S/C = 500 at 60 bar and 70 °C. The use of lower catalyst loadings must be further investigated.

Table 4. Comparison of the conventional route with the stereoselective route.

	Conventional route	Stereoselective route (one-pot process)
starting material	folic acid	folic acid
chemical step	no	*in situ* preparation of ester
reduction/hydrogenation	$NaBH_4$[5,8] or H_2/PtO_2[6]	Rh(I)/chiral diphosphine
purification/optical enrichment	2 crystallization steps required to obtain > 98% de	1 crystallization step required to obtain > 98% de
isolated yield	28% L-tetrahydrofolic acid toluene-sulfonate with respect to folic acid	45% L-tetrahydrofolic acid dimethyl ester benzenesulfonate with respect to folic acid
chemical step before conversion to derivatives	no	quantitative *in situ* hydrolysis of ester



Scheme 4. Hydrolysis of L-tetrahydrofolic acid dimethyl ester benzenesulfonate to L-tetrahydrofolic acid disodium salt.

L-Tetrahydrofolic acid dimethyl ester benzenesulfonate was hydrolyzed quantitatively with 1 N NaOH to give a solution of L-tetrahydrofolic acid disodium salt (Scheme 4). L-Tetrahydrofolic acid may be isolated as the free acid,[16] calcium salt[17] or sulfonate[8] or it may be converted *in situ* to different substituted derivatives of L-tetrahydrofolic acid[15] (Scheme 1).

We converted *in situ* formed **1a** to 5-methyltetrahydrofolic acid calcium salt[18] (Ca-L-mefolinate) which was of identical quality as that obtained in the traditional process. The biological activity measured by growth of *L. casei* was found to be identical with that one of traditionally made Ca-L-mefolinate which means that the assignment of the natural 6*S*-diastereoisomer was correct and no racemization in the glutamic acid moiety has occurred during ester hydrolysis. The rhodium content was below 2 ppm which is acceptable.

Conclusion

We have investigated the stereoselective hydrogenation of folic acid diesters with homogeneous catalysts in an alcoholic medium and found a new, efficient route for the synthesis of optically pure L-tetrahydrofolic acid. The simple conversion of folic acid to folic acid dimethyl ester benzenesulfonate with subsequent stereoselective hydrogenation in combination with fractional crystallization of L-tetrahydrofolic acid dimethyl ester benzenesulfonate with an optical purity of 99% allows the conversion of folic acid to optically pure L-tetrahydrofolic acid dimethyl ester benzenesulfonate in an economic one-pot process with a chemical yield of 45%.

L-Tetrahydrofolic acid dimethyl ester benzenesulfonate can be quantitatively hydrolyzed to L-tetrahydrofolic acid which can be used as an *in situ* formed intermediate for the synthesis of various substituted, pharmaceutically active L-tetrahydrofolic acid derivatives.[15] In comparison, the conventional process which is based on a non-stereoselective hydrogenation yields only 28% L-tetrahydrofolic acid toluenesulfonate.[8] Table 4 compares the conventional process with our new stereoselective route.

We estimate that our new process in its actual state can compete with the conventional process which requires two crystallization steps to obtain a low isolated yield of optically pure L-tetrahydrofolic acid. Certainly, a critical factor of the stereoselective process is the cost of the chiral Rh catalyst. On the other hand, it is known that the reduction with $NaBH_4$ requires a large excess of the reagent which results in cost-intensive work-up and waste disposal. Also, to obtain acceptable results by heterogeneous hydrogenation a high loading of the expensive PtO_2 catalyst is required. Further work to improve the diastereoselectivity and productivity of the catalyst and the competitiveness of the process is in progress. First experiments indicate that the recycling of the Rh metal is possible.

Experimental Section

General Data

All manipulations of oxygen- and moisture-sensitive materials were conducted 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. Nuclear magnetic resonance (NMR) spectra were measured on a Bruker NMR-spectrometer operating at 200 MHz using TMS as internal standard. Optical rotations were measured on a Perkin Elmer PE 341 spectrometer.

The hydrogenations were run in steel autoclaves, equipped with a gas entrainment impeller and a sensor for temperature and pressure. Reaction pressure was kept constant by continuous supply of hydrogen from a reservoir.

Folic Acid Dimethyl Ester *p*-Toluenesulfonate (5)

To a solution of 4 g (21 mmol) *p*-toluenesulfonic acid monohydrate in 250 mL methanol 2 g (4.19 mmol) folic acid dihydrate (**2**) were added. The mixture was stirred overnight, evaporated to dryness and treated with water to give a yellow precipitate which was sucked off, washed with water and dried at 40 °C and 20 mbar vacuum to give **5** as a bright yellow powder; yield: 1.7 g (3.6 mmol, 86%). ^{1}H NMR (DMSO-d_6, 200 MHz): δ = 8.74 (1H, s), 8.31 (1H, d), 7,97 (1H, bs), 7,66 (2H,d), 7.53 (2H,d), 7.15 (2H, d), 6.66 (2H, d), 4.57 (2H, s), 4.39 (1H, m), 3.62 (3H, s), 3.57 (3H, s), 2.42 (2H, m), 2.30 (3H, s), 2.03 (2H, m).

Folic Acid Dimethyl Ester Benzenesulfonate (6)

To a solution of 530 g benzenesulfonic acid in 20 L of methanol 800 g folic acid dihydrate (**2**; 1.68 mmol) were added at 40 °C under a nitrogen atmosphere. The mixture was refluxed for 1 hour, cooled to room temperature and concentrated to a volume of 5 L. The precipitated product was sucked off, washed with 1 L of methanol and dried at 40 °C and 20 mbar vacuum to give **6** as an intense yellow powder; yield: 966 g (1.45 mol, 86%). ^{1}H NMR (DMSO-d_6, 200 MHz): δ = 8.78 (1H, s), 8.46 (2H, bs), 8.32 (1H, d), 7.64–7.68 (m), 7.35–7.40 (m), 6.66 (2H, d), 0.8 (2H, s), 4.39 (1H, m), 3.62 (3H, s), 3.57 (3H, s), 2.42 (2H, m), 1.98–2.11 (2H, m).

Folic Acid Dimethyl Ester Methanesulfonate (7)

To a solution of 8.05 g (83.8 mmol) methanesulfonic acid in 500 mL methanol 4 g (8.38 mmol) folic acid monohydrate (**2**) were added. The mixture was stirred at room temperature overnight, the precipitate was sucked off, washed with methanol and dried at 40 °C and 20 mbar vacuum to give **7** as a bright yellow powder; yield: 4.14 g (7.32 mmol, 87%). ^{1}H NMR (DMSO-d_6, 200 MHz): δ = 8.77 (1H, s), 8.40 (2H, bs), 8.31 (1H, d), 7.66 (2H, d), 6.66 (2H, d), 4.60 (2H, s), 4.36 (1H, m), 3.61 (3H, s), 3.57 (3H, s), 2.38–2.52 (5H + DMSO, m), 2.01 (2H, m).

Folic Acid Diethyl Ester Benzenesulfonate (8)

To a solution of 3.18 g (20.11 mmol) benzenesulfonic acid in 1.5 L ethanol 8 g folic acid dihydrate (16.76 mmol) were added at room temperature. The mixture was refluxed for 5 hours, cooled to room temperature and stirred for 12 hours. The separated product was sucked off and dried at 40 °C and 20 mbar vacuum to give **8** as a bright yellow powder; yield: 10.1 g (15.29 mmol, 92%). ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.77 (1H, s), 8.27 (3H, d, bs), 7.66 (m), 7.35 (m), 6.66 (2H, d), 4.59 (2H, s), 4.37 (1H, m), 3.98–4.13 (4H, m), 2.40 (2H, m) 1.97–2.06 (2H, m) 1.06–1.21 (6H, m).

General Hydrogenation Procedure

8.12 mg $[Rh(COD)_2]BF_4$ (20 μmol) or 4.93 mg $[Rh(COD)Cl]_2$ (10 μmol) or 6.72 mg $[Ir(COD)Cl]_2$ (10 μmol) (see Tables) and diphosphine ligand (25 μmol) were degassed in a Schlenk tube. For hydrogenations in MeOH 5 mL methanol were added to give a suspension. For hydrogenations which were performed in another solvent like methanol 7 mL THF and 3 mL methanol were added to give a solution. After evaporation of solvents 5 mL of the solvent used for the hydrogenation were added. 1.25 g (2 mmol) of **6** were suspended in 25 mL methanol and added *via* a narrow tube to the catalyst. The suspension was added to a 100 mL autoclave flushed with nitrogen. The autoclave was sealed and the hydrogenation run at 70 °C at a constant pressure of 80 bar. After 17 hours a sample for HPLC analysis was taken. The $[Ir(COD)_2]BF_4$ used in some hydrogenations was prepared by adding 3.97 mg (20 μmol) $AgBF_4$ to a solution of 6.72 mg $[Ir(COD)Cl]_2$ (10 μmol) in 3 mL degassed THF. After 10 min, the precipitated AgCl was filtered off under an argon atmosphere and the filtrate was transferred to a solution of diphosphine ligand (25 μmol) in 3 mL of degassed THF. After evaporation of the THF, 5 mL methanol were added and the hydrogenations performed as described above.

For the hydrogenations in the presence of various halides (Table 2) the additives were added to the metal complex/diphosphine reaction mixture.

Isolation of L-Tetrahydrofolic Acid Dimethyl Ester Benzenesulfonate (9) from a Hydrogenation Mixture Prepared According to Entry 1, Table 1

After hydrogenation the product solution which is susceptible to oxidative degradation was transferred into a Schlenk tube which was flushed with argon. The solution was concentrated to 1/6 of its original volume and stored at 4 °C for 2 h. The crystalline product was sucked off, washed with a small amount of ice-cold methanol and dried at 40 °C and 20 mbar in vacuum to afford **9**; yield: 0.57 g (0.9 mmol, 45%). The ratio of [6S,αS]/[6R,αS]-diastereoisomers was 99:1; $[\alpha]_{589}^{20}$: −69.8° (c 1, DMSO). ^{1}H NMR (DMSO-d_6): δ = 10.61 (1H, bs), 8.35 (1H, d), 7.6–7.74 (m), 7.51 (1H, bs), 7.30–7.37 (m), 6.70 (2H, d, 2H, bs), 4.42 (2H, m), 3.63 (3H, s), 3.58 (3H, s), 3.50 (1H, m), 3.38 (1H, m), 3.28 (1H, m), 2.44 (2H, m), 2.01–2.13 (2H, m).

HPLC Method for the Separation of Diastereoisomers of Tetrahydrofolic Acid Dimethyl Ester

The content and diastereomeric excess of tetrahydrofolic acid dimethyl ester was determined by using the following HPLC method: 6.8 g of β-cyclodextrin and 270 mL of 37% formaldehyde were dissolved in 1 L of water to give the sample dilution solution. 1 mL of this sample dilution solution was mixed with 15 mg of the hydrogenation mixture or 1 mg of isolated prod-

uct was dissolved in 100 mL of the sample dilution solution and analyzed (flow rate 1 mL/min.) on an OS column (Macherey and Nagel, Nucleosil, 240 × 4 mm) with UV detection at 300 nm. The eluent was prepared by mixing 6.8 g of β-cyclodextrin, 8.5 mL of triethylamine, 850 mL of water and 150 mL of acetonitrile. By addition of acetic acid the pH was adjusted to pH 7.5 and then 270 μL of 37% formaldehyde were added. Under these conditions the natural 6*S*-diastereoisomer is eluted about 4 minutes before the unnatural 6*R*-diastereoisomer.

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