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Second-generation artificial hydrogenases based on the biotin–avidin technology: Improving selectivity and organic solvent tolerance by introduction of an (*R*)-proline spacer

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Abstract

We report on our efforts to create efficient artificial metalloenzymes for the enantioselective hydrogenation of *N*-protected dehydroamino acids using streptavidin as host protein. Introduction of an (*R*)-proline spacer between the biotin anchor and the diphosphine moiety affords a versatile ligand **Biot**-(***R***)-**Pro**-**1** which displays good (*S*)-selectivities in the presence of streptavidin (91% ee). The resulting artificial metalloenzyme [Rh(**Biot**-(***R***)-**Pro**-**1**)(COD)]$^+$ ⊂ WT-Sav displays increased stability against organic solvents. ***To cite this article: U.E. Rusbandi et al., C. R. Chimie 10 (2007).***

Résumé

Nous décrivons nos efforts de recherche dans la création de métalloenzymes artificielles efficaces dans l'hydrogénation énantiosélective de déhydroaminoacides *N*-protégés. L'introduction d'un espaceur (*R*)-proline entre le résidu biotinyle et la partie diphosphine fournit le ligand **Biot**-(***R***)-**Pro**-**1**, qui donne de bonnes sélectivités en faveur du produit d'hydrogénation (*S*) en présence de streptavidine (jusqu'à 91% ee). La métalloenzyme artificielle [Rh(**Biot**-(***R***)-**Pro**-**1**)(COD)]$^+$ ⊂ WT-Save ainsi obtenue montre une stabilité accrue vis-à-vis des solvants organiques. ***Pour citer cet article : U.E. Rusbandi et al., C. R. Chimie 10 (2007).***

Keywords: Artificial metalloenzymes; Biotin–avidin technology; Asymmetric catalysis; Enantioselective hydrogenation

Mots-clés : Métalloenzymes artificielles ; Technologie biotine–avidine ; Catalyse asymétrique ; Hydrogénation énantiosélective

1. Introduction

The field of artificial metalloenzymes, based on the incorporation of an active catalyst, in a macromolecular host has enjoyed a revival in recent years [1,2]. Both covalent and supramolecular anchoring strategies have

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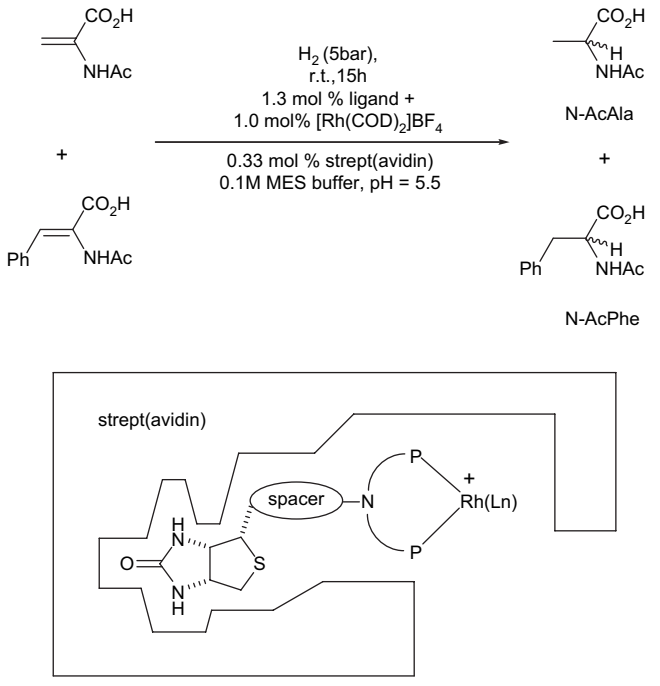
successfully been exploited to produce enantioselective hybrid catalysts for ester hydrolysis [3], dihydroxylation [4], epoxidation [5], sulfoxidation [6–11], hydrogenation [12–19], transfer hydrogenation [20,21] and Diels–Alder reactions [22–24].

Based on Whitesides' early report [25], we have been exploiting the biotin–avidin technology to produce artificial hydrogenases for the enantioselective reduction of *N*-protected dehydroamino acids [13,14,18,19] as well as the reduction via transfer hydrogenation of aromatic ketones [20,21]. For optimization purposes, we rely both on chemical- and genetic-strategies (i.e. chemogenetic) to yield both (*R*)- and (*S*)-selective hydrogenases. For the proof-of-principle, we focused exclusively on *achiral* ligands and spacers, thus ensuring that (enantio)selectivity is provided by second coordination sphere between the catalyst–substrate and the host protein. Following this chemogenetic optimization, good enantioselectivities (>90% ee) for both enantiomers of *N*-protected amino acids (*N*-acetamidoalanine *N*-AcAla and *N*-acetamidophenylalanine *N*-AcPhe) could be obtained using streptavidin (WT-Sav hereafter) as host protein. Herein we report on our efforts to produce active and selective artificial hydrogenases based on the biotin–avidin technology, with an emphasis on the chemical optimization using enantiopure α-amino acids (Scheme 1). A preliminary account of this work has appeared recently [19].

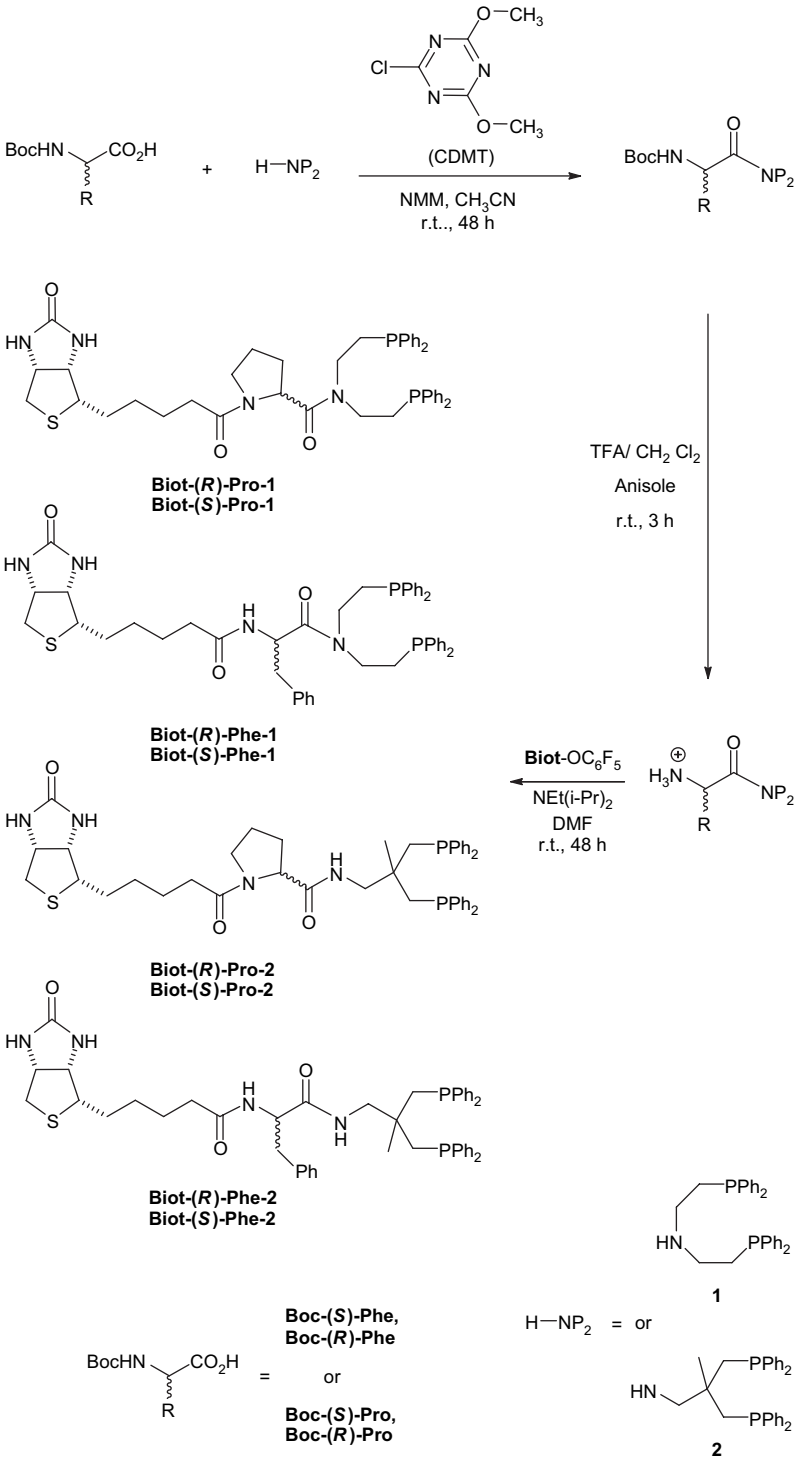
2. Results and discussion

2.1. Choice of ligand and spacer

In previous studies [13,14,18], we demonstrated that the nature of the aminodiphosphine moiety (H-**NP$_2$**) plays a critical role in both the activity and the selectivity of the resulting artificial metalloenzymes. Although the diphosphine **1** proved superior in most cases, the diphosphine **2** displayed interesting substrate- and enantioselectivities in a few cases [14]. We thus included both scaffolds **1** and **2** and combined them with enantiopure amino acid spacers (aa) to afford **Biot-aa-NP$_2$**. For these studies, we selected phenylalanine ((*R*)-Phe, (*S*)-Phe) and proline ((*R*)-Pro, (*S*)-Pro) as spacer to afford a total of eight biotinylated ligands, Scheme 2. The choice of spacer was influenced by the fact that the biotin-binding pockets in both Sav and Avi are hydrophobic; we thus speculated that the presence of an aromatic moiety on the spacer could favorably interact with the tryptophane (and phenylalanine in WT-Avi) residues present in the binding pocket. Proline clearly stands out among the natural amino acids; we reasoned that introduction of pyrrolidine ring as a spacer would restrict the degrees of freedom of the catalyst within streptavidin (catalyst ⊂ WT-Sav; the inclusion symbol "⊂" will be used throughout this section to describe artificial metalloenzymes: metal fragment ⊂ host protein).



Scheme 1. Artificial hydrogenases based on the biotin (strept)avidin technology for the reduction of *N*-protected dehydroamino acids.



Scheme 2. Synthesis of biotinylated diphosphine ligands incorporating chiral spacers.

2.2. Ligand synthesis

In the spirit of peptidic coupling with enantiopure amino acids and in order to avoid epimerization upon amide bond formation, we opted for an N → C coupling scheme, rather than the C → N approach used previously with achiral amino acid spacers, (Scheme 2) [13,18].

The aminodiphosphine scaffold H-**NP$_2$ 1** or **2** was coupled with the Boc-protected amino acids activated in situ with CDMT (chlorodimethoxytriazine) [26]. The yields of the purified product critically depend on the diphosphine moiety: 60–70% yields were obtained with the diphosphine **1**, whereas with the sterically congested, neopentylic amine **2**, they only reach 20–30% [19].

The deprotection of the amino group was achieved with trifluoroacetic acid in the presence of two equivalents of anisole to avoid attack of the *tert*-butyl cation on the aromatic groups at phosphorus [27].

The crude ammonium salts were reacted with activated biotin, namely (+)-biotin-pentafluorophenylester [28], under basic conditions. After chromatography on silica gel under inert atmosphere, the desired ligands were isolated as white to pale yellow oils in 5–41% overall yield (three steps). Next, we proceeded to test these eight ligands with WT-Sav in their performance as artificial hydrogenases. All experimental details concerning the synthesis and characterization of all intermediates and ligands can be found in the supporting information of our preliminary communication [19].

2.3. Screening results

As the biotinylated ligands bear an enantiopure amino acid moiety relatively close to the chelating diphosphine moiety, we checked their performance, both in terms of activity and of selectivity in the hydrogenation of α-acetamidoacrylic acid in the absence of protein (Table 1).

Catalysis reactions were run in degassed (nitrogen bubbling) MES buffer (0.1 M, pH = 5.5, MES = 2-(*N*-morpholino)ethanesulfonic acid). To ensure solubility, the rhodium complexes were prepared in situ in DMSO, which, upon mixing with the protein and the substrate, accounts for 9% of the total volume. Experimental details, including catalytic setup and GC operating conditions can be found in Ref. [14].

All ligands afford high conversion rates and, not unexpectedly, low enantioselectivity: a maximum of 8% ee in favor of the (*R*)-*N*-AcAla, entry 7, and (*S*))-*N*-AcAla, entry 9. We next tested these ligands in the hydrogenation of a cocktail containing both α-acetamidoacrylic and α-acetamidocinnamic acid in the presence of WT-Sav.

The results displayed in Table 2 suggest that the ligands carrying the diphosphine moiety **1**, **Biot-aa-1**, perform better than the corresponding **Biot-aa-2**, both in terms of activity and of selectivity. This general feature emphasizes once again the critical role of the ligand structure on the performance of the artificial metalloenzyme. Hydrogenations of α-acetamidocinnamic acid with hybrid catalysts derived from **Biot-aa-2** yield modest enantioselectivities (up to 60% ee) and modest conversions. In contrast, with ligands based on diphosphine **1**, **Biot-aa-1**, good conversions and good selectivities (up to 91% ee) are found. The most efficient ligand is derived from unnatural (*R*)-Proline, **Biot-(*R*)-Pro-1**, which affords full conversion in each case and up to 91% ee (*S*) for *N*-AcPhe and 86% ee (*S*) for *N*-AcPhe in the presence of WT-Sav.

Depending on the absolute configuration of the Phe spacer, nearly identical, but opposite enantioselectivities (>60% ee) are obtained for [Rh(**Biot-Phe-1**)(COD)]$^+$ ⊂ WT Sav, Table 2, entries 3 and 4.

It appears that the amino acid spacer interacts very significantly with the protein environment, thus

Table 1
Results for the hydrogenation of α-acetamidoacrylic acid in the absence of protein

Entry	Ligand	ee (%) *N*-AcAla	Conversion (%)
1	**Biot-1**	0	81
2	**Biot-(*S*)-Phe-1**	7 (*S*)	100
3	**Biot-(*R*)-Phe-1**	3 (*R*)	91
4	**Biot-(*S*)-Phe-2**	7 (*S*)	92
5	**Biot-(*R*)-Phe-2**	2 (*R*)	100
6	**Biot-(*S*)-Pro-1**	8 (*R*)	100
7	**Biot-(*R*)-Pro-1**	3 (*R*)	100
8	**Biot-(*S*)-Pro-2**	8 (*S*)	100
9	**Biot-(*R*)-Pro-2**	0	100

Reaction conditions: The substrate (100 equiv α-acetamidoacrylic acid vs. Rh) was dissolved in 0.1 M MES ([substrate] = 24 mM), added to a solution containing [Rh(Ligand)(COD)]BF_4 (0.62 mM in DMSO, 1 equiv) in a 3-mL Pyrex tube and hydrogenated at 5 bar H_2 for 15 h.

Table 2
Results for the hydrogenation of α-acetamidoacrylic acid and α-acetamidocinnamic acid (as a mixture) in the presence of [Rh(**Biot-aa-NP$_2$**)(COD)]$^+$ ⊂ WT-Sav

Entry	Ligand	*N*-AcAla		*N*-AcPhe	
		ee (%)	Conv. (%)	ee (%)	Conv. (%)
1	**Biot-(*R*)-Pro-1**	−86	100	−91	100
2	**Biot-(*S*)-Pro-1**	23	100	23	100
3	**Biot-(*R*)-Phe-1**	66	100	64	100
4	**Biot-(*S*)-Phe-1**	−73	100	−64	87
5	**Biot-1**	94	100	93	84
6	**Biot-(*R*)-Phe-2**	5	100	6	68
7	**Biot-(*S*)-Phe-2**	−56	100	−16	37
8	**Biot-(*R*)-Pro-2**	9	100	−14	54
9	**Biot-(*S*)-Pro-2**	−40	100	−32	73
10	**Biot-2**	−6	38	0	2

Positive and negative ee values correspond to (*R*)- and (*S*)-product, respectively. Reaction conditions: both substrates (50 equiv each vs. Rh) were dissolved in 0.1 M MES ([substrates] = 24 mM), added to a solution containing [Rh(**Biot-aa-NP$_2$**)(COD)]BF_4 (0.62 mM in DMSO, 1 equiv) and streptavidin (tetrameric, 100 μl, 0.33 equiv vs. Rh, 0.21 mM in H_2O) in a 3-mL Pyrex tube and hydrogenated at 5 bar H_2 for 15 h.

Table 3
Catalytic runs with **Biot-1** and **Biot-(*R*)-Pro-1** in the presence of increasing amounts of DMSO

Entry	% DMSO	Ligand	*N*-AcAla		*N*-AcPhe	
			ee (%)	Conv. (%)	ee (%)	Conv. (%)
1	9	**Biot-1**	94	100	94	85
2	9	**Biot-(*R*)-Pro-1**	−87	100	−91	100
3	18	**Biot-1**	90	100	88	90
4	18	**Biot-(*R*)-Pro-1**	−87	100	−91	100
5	27	**Biot-1**	81	100	80	80
6	27	**Biot-(*R*)-Pro-1**	−87	100	−91	100
7	36	**Biot-1**	52	80	60	58
8	36	**Biot-(*R*)-Pro-1**	−87	100	−87	100
9	45	**Biot-1**	15	72	23	25
10	45	**Biot-(*R*)-Pro-1**	−87	100	−87	96

Reaction conditions: cf Table 2, volumes adapted with the desired amount of DMSO.

dramatically influencing the position of the Rh moiety within the chiral environment provided by the host protein.

Having created (*S*)-selective artificial hydrogenases with WT-Sav as host protein, we proceeded to evaluate the solvent tolerance of [Rh(**Biot-(*R*)-Pro-1**)(COD)]$^+$ ⊂ WT-Sav. For this purpose, catalytic runs were performed in the presence of increasing amounts of different organic solvents. For comparison, we use [Rh(**Biot-1**)(COD)]$^+$ ⊂ WT-Sav, which was previously shown to be a versatile (*R*)-selective hybrid catalyst [13,14,18]. In order to ensure solubilisation of the catalyst precursor, typical catalytic runs are performed with a buffer:DMSO ratio of 91:9, which was used as a starting point.

The results of the catalytic runs with [Rh(**Biot-1**)(COD)]$^+$ ⊂ WT-Sav and [Rh(**Biot-(*R*)-Pro-1**)(COD)]$^+$ ⊂ WT-Sav in the presence of 9 to 45% dimethylsulfoxide are collected in Table 3.

With **Biot-1** ⊂ WT-Sav, we observe a significant erosion of enantioselectivity (and to a lesser extent conversion), which drops to 15% ee *N*-AcAla and to 23% ee for *N*-AcPhe in the presence of 45% DMSO. From these data, it is clear that the **Biot-1**-based catalytic system does not tolerate large amounts of DMSO.

In strong contrast, hybrid catalysts derived from **Biot-(*R*)-Pro-1** tolerate large amounts of DMSO. Indeed, only a very modest erosion of enantioselectivity is observed.

Other organic co-solvents (9%) were tested in the presence of **Biot-(*R*)-Pro-1** ⊂ WT-Sav. Ethanol, methanol, acetone, tetrahydofuran and dimethylformamide were selected, and the results are summarized in Table 4.

Except for THF, which is recognized as a strongly denaturing solvent, both the activity and the enantioselectivity is by and large maintained with the other water-miscible solvents tested. Interestingly, dimethylformamide, which is seldom used for enantioselective hydrogenation of enamides with cationic rhodium due to its coordination ability, is compatible with artificial hydrogenases. These results consolidate our findings that the catalytic system based on the **Biot-(*R*)-Pro-1** ligand is indeed robust. This prompted us to evaluate further its performance under biphasic reaction conditions (Table 5). For substrate solubility reasons, ethylacetate was selected as non-miscible solvent and used as a 1:1 biphasic mixture combined with MES buffer.

Under biphasic conditions, [Rh(**Biot-(*R*)-Pro-1**)(COD)]$^+$ ⊂ WT-Sav, performs much better than its [Rh(**Biot-1**)(COD)]$^+$ analog.

Table 4
Catalytic runs with **Biot-(*R*)-Pro-1** in the presence of 9% (in volume) of various organic co-solvents

Solvent (9% in volume)	*N*-AcAla		*N*-AcPhe	
	ee (%)	Conv. (%)	ee (%)	Conv. (%)
DMSO	−87	100	−91	100
EtOH	−86	100	−86	100
MeOH	−81	100	−83	100
Acetone	−81	100	−82	100
DMF	−87	100	−88	100
THF	−38	38	−44	35

Reaction conditions: cf Table 2, adapted with the desired solvent.

Table 5
Biphasic catalytic runs with [Rh(**Biot-(*R*)-Pro-1**)(COD)]$^+$ ⊂ WT-Sav in the presence of 50% ethylacetate

Catalyst	*N*-AcAla		*N*-AcPhe	
	ee (%)	Conv. (%)	ee (%)	Conv. (%)
[Rh(**Biot-(*R*)-Pro-1**)(COD)]$^+$ ⊂ WT-Sav	−83	96	−88	90
[Rh(**Biot-1**)(COD)]$^+$ ⊂ WT-Sav	30	56	31	5

Reaction conditions: cf Table 2, adapted with the desired amount of ethylacetate.

3. Conclusion

The straightforward incorporation of enantiopure amino acid spacers into biotinylated diphosphine ligands allowed us to identify an efficient artificial hydrogenase based on the biotin–avidin technology. The catalytic system [Rh(**Biot-(*R*)-Pro-1**)(COD)]$^+$ ⊂ WT-Sav displays unprecedented (*S*) selectivity (up to 91% ee in streptavidin) and enhanced stability towards organic co-solvents. It therefore broadens the scope of

artificial metalloenzymes based on the biotin–avidin technology. Its efficiency in biphasic medium opens the way to new applications in the asymmetric hydrogenation of water insoluble prochiral alkenes with artificial metalloenzymes and recycling of the water soluble catalyst.

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References

[1] D. Qi, C.-M. Tann, D. Haring, M.D. Distefano, Chem. Rev. 101 (2001) 3081.
[2] (a) C.M. Thomas, T.R. Ward, Chem. Soc. Rev. 34 (2005) 337; (b) C. Letondor, T.R. Ward, ChemBioChem 7 (2006) 1845.
[3] R.R. Davies, M.D. Distefano, J. Am. Chem. Soc. 119 (1997) 11643.
[4] T. Kokubo, T. Sugimoto, T. Uchida, S. Tanimoto, M. Okano, J. Chem. Soc., Chem. Commun. (1983) 769.
[5] K. Okrasa, R.J. Kazlauskas, Chem. Eur. J. 12 (2006) 1587.
[6] F. van de Velde, L. Könemann, F. van Rantwijk, R.A. Sheldon, Chem. Commun. (1998) 1891.
[7] M. Ohashi, T. Koshiyama, T. Ueno, M. Yanase, H. Fujii, Y. Watanabe, Angew. Chem., Int. Ed. 42 (2003) 1005.
[8] T. Ueno, T. Koshiyama, M. Ohashi, K. Kondo, M. Kono, A. Suzuki, T. Yamane, Y. Watanabe, J. Am. Chem. Soc. 127 (2005) 6556.
[9] J.R. Carey, S.K. Ma, T.D. Pfister, D.K. Garner, H.K. Kim, J.A. Abramite, Z. Wang, Z. Guo, Y. Lu, J. Am. Chem. Soc. 126 (2004) 10812.
[10] A. Mahammed, Z. Gross, J. Am. Chem. Soc. 127 (2005) 2883.
[11] A.C.A. Fernandez-Gacio, J. Fastrez, O. Riant, P. Soumillion, ChemBioChem 7 (2006) 1013.
[12] T.R. Ward, Chem. Eur. J. 11 (2005) 3798.
[13] J. Collot, J. Gradinaru, N. Humbert, M. Skander, A. Zocchi, T.R. Ward, J. Am. Chem. Soc. 125 (2003) 9030.
[14] G. Klein, N. Humbert, J. Gradinaru, A. Ivanova, F. Gilardoni, U.E. Rusbandi, T.R. Ward, Angew. Chem., Int. Ed. 44 (2005) 7764.
[15] C.-C. Lin, C.-W. Lin, A.S.C. Chan, Tetrahedron: Asymmetry 10 (1999) 1887.
[16] L. Panella, J. Broos, J. Jin, M.W. Fraaije, D.B. Janssen, M. Jeronimus-Stratingh, B.L. Feringa, A.J. Minnaard, J.G. De Vries, Chem. Commun. (2005) 5656.
[17] M.T. Reetz, M. Rentzsch, A. Pletsch, M. Maywald, Chimia 56 (2002) 721.
[18] M. Skander, N. Humbert, J. Collot, J. Gradinaru, G. Klein, A. Loosli, J. Sauser, A. Zocchi, F. Gilardoni, T.R. Ward, J. Am. Chem. Soc. 126 (2004) 14411.
[19] M. Skander, C. Malan, A. Ivanova, T.R. Ward, Chem. Commun. (2005) 4815.
[20] C. Letondor, N. Humbert, T.R. Ward, Proc. Natl Acad. Sci. USA 102 (2005) 4683.
[21] C. Letondor, A. Pordea, N. Humbert, A. Ivanova, S. Mazurek, M. Novic, T.R. Ward, J. Am. Chem. Soc. 128 (2006) 8320.
[22] M.T. Reetz, N. Jiao, Angew. Chem., Int. Ed. 45 (2006) 2416.
[23] G. Roelfes, B.L. Feringa, Angew. Chem., Int. Ed. 44 (2005) 3230.
[24] G. Roelfes, A.J. Boersma, B.L. Feringa, Chem. Commun. (2006) 635.
[25] M.E. Wilson, G.M. Whitesides, J. Am. Chem. Soc. 100 (1978) 306.
[26] C.E. Garrett, G.C. Fu, J. Am. Chem. Soc. 120 (1998) 7479.
[27] Y. Okada, Curr. Org. Chem. 5 (2001) 1.
[28] C. Somlai, M. Berenyi, P. Maroy, Z. Naturforsch., B: Chem. Sci. 48 (1993) 511.