Efficient Synthesis and Analysis of Chiral Cyanohydrins

Stina Lundgren

KTH Chemical Science and Engineering

Doctoral Thesis

Stockholm 2007

Akademisk avhandling som med tillstånd av Kungliga Tekniska högskolan i Stockholm framlägges till offentlig granskning för avläggande av teknologie doktorsexamen i kemi med inrikting mot organisk kemi, fredagen den 13 april 2007 kl 10.15 i sal F3, KTH, Lindstedsvägen 26, Stockholm. Avhandlingen försvaras på engelska. Opponent är Professor Ben Feringa, University of Groningen, Nederländerna.

ISBN 978-91-7178-599-2
ISSN 1654-1081
TRITA-CHE-Report 2007:11

Universitetsservice US AB, Stockholm

Abstract

This thesis deals with the development of new methods for efficient synthesis and analysis in asymmetric catalysis. It focuses on the preparation of chiral cyanohydrins by enantioselective addition of cyanide to prochiral aldehydes.

The initial part of the thesis describes the development of a dual Lewis acid–Lewis base activation system for efficient synthesis of chiral O-acylated and O-carbonylated cyanohydrins. This system was used for the preparation of a variety of cyanohydrins in high isolated yields and with up to 96% ee. Activation of the cyanide by nucleophilic attack of the Lewis base at the carbonyl carbon atom was supported experimentally.

Secondly, convenient procedures for the synthesis of polymer-bound chiral $YbCl_3$-pybox and Ti-salen complexes are described. The polymeric complexes were employed in cyanation of benzaldehyde.

A T-shaped microreactor was used for screening of reaction conditions for the enantioselective cyanation of benzaldehyde using trimethylsilyl cyanide and acetyl cyanide as cyanide sources. A microreactor charged with the polymeric Ti-salen complex was used for enantioselective cyanation of benzaldehyde.

Finally, an enzymatic method for high throughput analysis of ee and conversion of products from chiral Lewis acid–Lewis base-catalysed additions of α-ketonitriles to prochiral aldehydes was developed. The method could be used for the analysis of a variety of O-acylated cyanohydrins. Microreactor technology was successfully combined with high throughput analysis for efficient catalyst optimisation.

Keywords: asymmetric catalysis, cyanohydrins, microreactor, polymer-supported catalyst, ketonitriles, titanium, lanthanide, Lewis acid, Lewis base, high throughput analysis, enzyme.

List of Publications

This thesis is based on the following papers, referred to in the text by their Roman numerals:

I. **"Dual Lewis Acid–Lewis Base Activation in Enantioselective Cyanation of Aldehydes using Acetyl Cyanide and Cyanoformate as Cyanide Sources"** S. Lundgren, E. Wingstrand, M. Penhoat and C. Moberg, *J. Am. Chem. Soc*. **2005**, *127*, 11592.

II. **"Lewis Acid–Lewis Base-Catalysed Enantioselective Addition of α-Ketonitriles to Aldehydes"** S. Lundgren, E. Wingstrand and C. Moberg, *Adv. Synth. Cat.* **2007,** *349*, 364.

III. **"Polymer-Supported Pyridine-Bis(oxazoline). Application to Ytterbium Catalyzed Silylcyanation of Benzaldehyde"** S. Lundgren, S. Lutsenko, C. Jönsson and C. Moberg, *Org. Lett.* **2003**, *5*, 3663.

IV. **"Enantioselective Cyanation of Benzaldehyde: an Asymmetric Polymeric Catalyst in a Microreactor"** S. Lundgren and C. Moberg**,** *Preliminary manuscript*.

V. "**Asymmetric Catalysis in a Micro Reactor–Ce, Yb and Lu Catalysed Enantioselective Addition of Trimethylsilyl Cyanide to Benzaldehyde"** C. Jönsson, S. Lundgren, S. J. Haswell and C. Moberg, *Tetrahedron* **2004**, *60*, 10515.

VI. **"High-Throughput Enzymatic Method for Enantiomeric Excess Determination of O-Acetylated Cyanohydrins"** A. Hamberg, S. Lundgren, M. Penhoat, C. Moberg and K. Hult, *J. Am. Chem. Soc.* **2006**, *128*, 2234.

VII. **"High Throughput Synthesis and Analysis of Acylated Cyanohydrins"** A. Hamberg, S. Lundgren, E. Wingstrand, C. Moberg and K. Hult, *Chem. Eur. J*. **2007**, ASAP.

Abbreviations

Å	Ångström(s)
binol	1,1'-bi-2-naphthol
°C	degrees Celsius
CALB	*Candida antarctica* lipase B
DABCO	1,4-diazabicyclo[2.2.2]octane
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	N,N'-dicyclohexylcarbodiimide
DIEA	diisopropylethylamine
DMAP	N,N-dimethylaminopyridine
ee	enantiomeric excess
EMDc	enzymatic method for determination of conversion
EMDee	enzymatic method for determination of enantiomeric excess
ent	enantiomer
EOF	electroosmotic flow
equiv.	equivalent
GC	gas chromatography
HLADH	horse liver alcohol dehydrogenase
HPLC	high pressure liquid chromatography
IR	infrared
J	coupling constant (in NMR spectroscopy)
µ	micro
mol	mole(s)
MS	mass spectrometry
NAD^+	nicotineamide adenine dinucleotide
NADH	reduced NAD^+
NADPH	reduced nicotineamide adenine dinucleotide phosphate
NMR	nuclear magnetic resonance
PEG	polyethylene glycol
PLE	pig liver esterase
PS	polystyrene
pybox	pyridine-bis(oxazoline)
TG	tentagel
THF	tetrahydrofuran
TMSCN	trimethylsilyl cyanide
T	temperature
TsCl	tosyl chloride
UV	ultraviolet
V	volt

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1. Introduction

1.1 Asymmetric Catalysis

The concept of replacing a racemic mixture of biologically active substances with a single enantiomer – the chiral switch – has become important within the pharmaceutical industry.[1] Moreover, the demand for chiral small organic molecules for applications as pesticides, flavours and aromas is steadily increasing.[2] The reason for this development is that the enantiomers often exhibit different biological activities. Therefore, the performance of the single enantiomer usually is superior to that of the racemic mixture. Moreover, regulations often demand that both enantiomers are evaluated separately before approval of a biologically active compound. As a result, the need for new methods for synthesising, separating, and analysing chiral compounds has emerged.

In broad outlines, enantiomerically pure compounds are obtained in three different ways:

- *Resolution.* By this method a racemic mixture is resolved into pure enantiomers. This is the classical way of producing enantiomerically pure compounds and it is still the most commonly used approach within the pharmaceutical industry.[3]
- *The chiral pool approach.* This method uses a pure enantiomer of a natural product as starting material for the synthesis of an enantiomerically pure compound.
- *Asymmetric synthesis.* This method starts from an achiral compound or a racemate, from which one enantiomer is produced in excess.

Asymmetric synthesis can be divided into three groups according to the chiral inductions: auxiliary controlled, reagent controlled and catalyst controlled. Asymmetric catalysis, the concept of using a chiral catalyst, was awarded the Nobel Prize in Chemistry in 2001. This is an excellent strategy for the preparation of enantiomerically pure compounds since a catalytic amount of a chiral substance can be used for producing a large amount of chiral product.[4]

Although highly selective catalysts have been found for a large number of catalytic transformations, there are only a few general catalytic systems that are enantioselective for a wide range of reactions and substrates. Moreover, it is difficult to predict which catalyst that will be the best for a given target molecule.

Therefore, the structure of the catalyst as well as the reaction conditions usually need to be optimised for each particular transformation. Methods based on combinatorial chemistry, parallel synthesis, rapid screening, and efficient analytical tools have been developed in order to speed up the optimization process. It is advantageous to conduct optimization of reaction parameters in a microreactor since it enables fast screening with low consumption of reagents. Moreover, since screening of many reaction parameters typically consumes large amounts of reagents, downsizing of reactions is desirable. For this reason, various types of microreactors are gaining increased importance for catalyst screening and development.

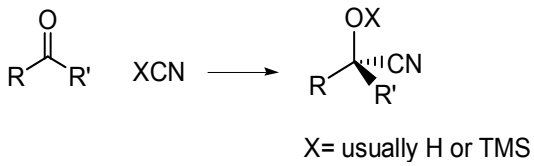
1.2 The Aim of This Thesis

The aim of this thesis is to develop new efficient methods in asymmetric metal catalysis. This involves the optimization of reactions, the development of new reaction technologies such as microfluidic based systems, and methods for high throughput screening and analysis. The work includes catalysts based on homogeneous as well as on heterogeneous systems.

2. Enantioselective Synthesis of Cyanohydrins

2.1 Background

Addition of cyanide to a carbonyl group generates a cyanohydrin (Scheme 1). Cyanohydrins are found in over 2500 plants, bacteria, fungi, and insects.[5] Many plants use them as a natural defence system against herbivores. Cyanohydrins also serve as a source of nitrogen for the biosynthesis of amino acids.[6] Efficient procedures for the synthesis of cyanohydrins have been known for more than 100 years.[7]

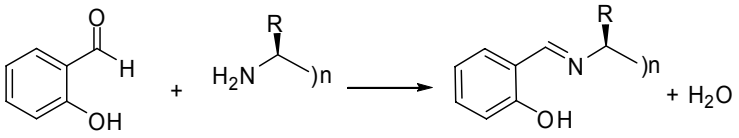


Scheme 1. Cyanide addition to carbonyl compounds.

Enantioselective addition of cyanide to prochiral ketones and aldehydes constitutes an important organic reaction, since the resulting chiral cyanohydrins are versatile building blocks.[8] Except for their use as synthetic intermediates, cyanohydrins also play an important role as pesticides in agriculture. The first asymmetric synthesis of cyanohydrins was achieved by Rosenthaler in 1908 using an enzyme for the chiral induction.[9] Since then, several enantioselective reactions have been developed based on the use of enzyme, Lewis acid, or Lewis base catalysis.[8] Today, the most important class of reactions is based on Lewis acid catalysis.[8b] Several metal complexes have been employed for the enantioselective addition of trimethylsilyl cyanide (TMSCN) to carbonyl compounds.

2.2 Schiff Bases Derived from Salicylaldehyde

Schiff bases derived from salicylaldehyde are among the most successful types of ligands used in enantioselective addition of cyanide to ketones and aldehydes. They are prepared through the condensation of an amine with salicylaldehyde (Scheme 2).

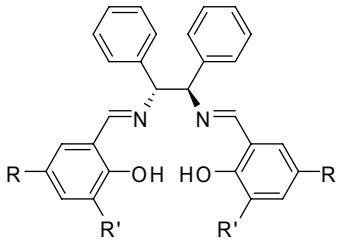


Scheme 2. Synthesis of Schiff base ligand derived from salicylaldehyde.

The first use of a Schiff base ligand for enantioselective cyanide addition to aldehydes was reported by the group of Inoue in 1991.[10] They employed Ti complexes of peptide-derived Schiff base ligands and titanium tetraetoxide ($Ti(OEt)_4$). The catalyst which induced the highest enantioselectivity gave the product with up to 97% ee at –60 °C. A few years after this work, Oguni and co-workers used Schiff base ligands prepared from β-amino alcohols for the addition of TMSCN to aldehydes.[11] A catalyst prepared from titanium tetraisopropoxide ($Ti(O^iPr)_4$) and a valinol based ligand gave the product with up to 96% ee at –80 °C.

The condensation of a diamine and two salicylaldehydes forms a tetradentate Schiff base, salen ligand. These types of compounds are versatile ligands and are enantioselective for a wide range of different reactions.[12]

Catalytic systems employing salen ligands for enantioselective cyanide addition to carbonyls were developed independently by two research groups. In 1996, Jiang and co-workers reported a salen ligand originating from 1,2-diphenyl-ethylenediamine (**1**).[13] The highest selectivity was achieved with a catalyst prepared from $Ti(O^iPr)_4$ and Schiff base ligands derived from non-substituted salicylaldehyde. This catalytic system gave silylated cyanohydrins with up to 87% ee at –78 °C.



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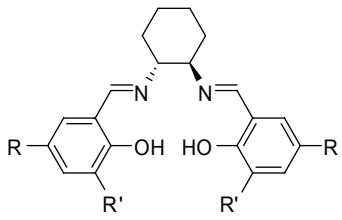
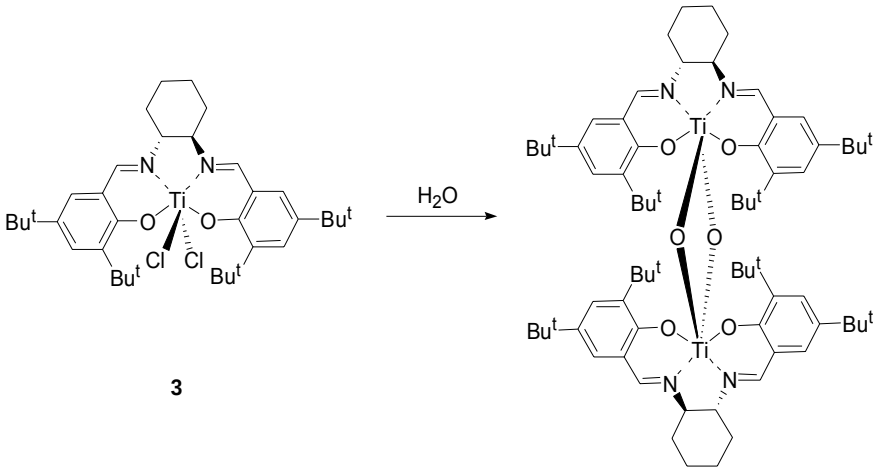


2

Simultaneously, the group of Belokon' and North used salen ligands derived from 1,2-diaminocyclohexane (**2**).[14] Ligands derived from substituted salicylaldehyde gave highest selectivity and TMS-protected cyanohydrins were synthesised with up to 77% ee at –80 °C. A more efficient catalyst was achieved when the Ti-salen complex **3** was treated with water (Scheme 3), a procedure that gave a Ti-salen dimer bridged by two oxygen atoms (**4**).[15] This catalyst proved to be extremely reactive and only 0.1 mol% of the catalyst was required for the reaction to be over in less than five minutes. Moreover, the use of the Ti-salen dimer **4** resulted in high enantioselectivity and cyanohydrins were formed with up to 86% ee at room temperature.



3 **4**

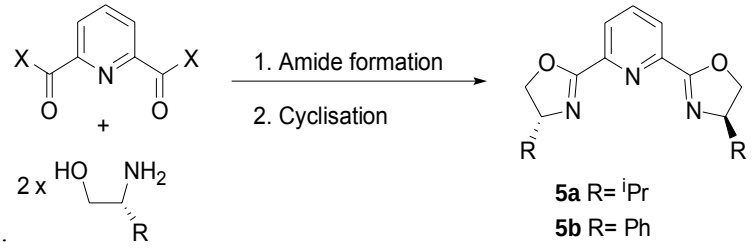
Scheme 3. Preparation of dimeric Ti-salen complex **4**.

2.3 Pyridine-2,6-bisoxazoline (pybox)

Pyridine-2,6-bisoxazoline (pybox) is another example of a versatile ligand that can be applied in a variety of different enantioselective reactions.[16] Pybox is a tridentate ligand developed by Nishiyama and co-workers in 1989.[17] It is usually prepared via amide formation by reaction of a pyridine-2,6-dicarboxylic acid derivatives and amino alcohols followed by cyclisation to afford the oxazoline rings (Scheme 4).

The first use of pybox in enantioselective cyanohydrin synthesis was reported by Iovel and co-workers in 1997.[18] They employed a complex of isopropyl-pybox (**5a**) and $AlCl_3$ for the addition of TMSCN to aldehydes. This catalyst provided

high yields of a variety of cyanohydrins, but the enantiomeric excess was only reported for the reaction with benzaldehyde (90% ee).



Scheme 4. Preparation of pybox ligands.

In 1999 the group of Aspinall developed a more efficient catalyst based on a lanthanide-pybox complex.[19] The highest ee reported for the lanthanide-catalysed addition of TMSCN to benzaldehyde was achieved with a catalyst prepared from phenyl-pybox (**5b**) and $YbCl_3$. A more reactive and only slightly less enantioselective catalyst was formed when the phenyl-pybox **5b** was replaced with isopropyl-pybox **5a**.

2.4 Dual Activation

A new approach to achieve efficient asymmetric catalysis is to use dual activation of the nucleophile and the electrophile.[20] Dual activation can be achieved by employing a Lewis acid for activation of the electrophile and a Lewis base for activation of the nucleophile. This can result in a very efficient catalytic system resembling the catalytic machinery of an enzyme.[21]

There are mainly two types of dual activation systems (Figure 1).

- Systems based on the use of a bifunctional catalyst with two separate catalytic moieties incorporated into the same molecule (System A).
- Systems based on the use of two separate catalysts combined into one dual activation system (System B).

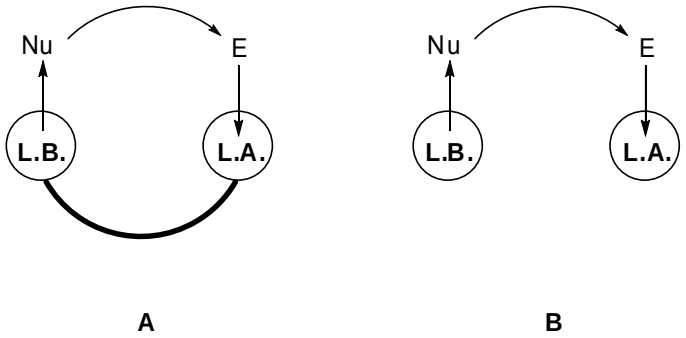
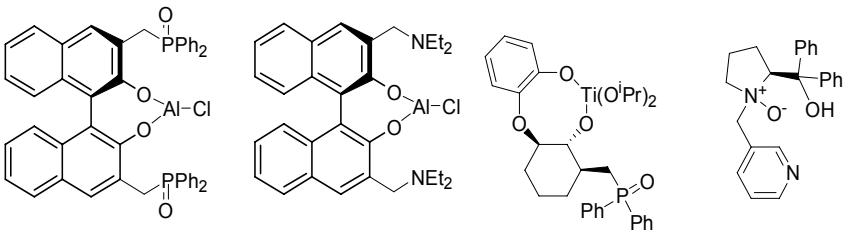


Figure 1. A bifunctional catalyst (system A) and two separate catalysts (system B).

The bifunctional catalyst has the advantage of keeping the Lewis acid and Lewis base moieties apart, thereby avoiding undesired complex formation between the Lewis base and the Lewis acid. Moreover, coordination of both the electrophile and the nucleophile in a defined chiral setting might lead to higher selectivity. However, bifunctional catalysts often demand advanced design and elaborate synthesis. The use of separate Lewis acid and Lewis base catalysts has the advantage of allowing the two catalysts to be easily varied which enables efficient screening of the resulting catalytic properties.

The first dual activation system for enantioselective cyanide addition to aldehydes was reported in 1993 by Corey and co-workers.[22] They used a Mg-bisoxazoline complex for Lewis acid activation of the aldehyde and an uncomplexed bisoxazoline for Lewis base activation of the cyanide. The system was very enantioselective towards aliphatic nonconjugated aldehydes and gave the product with up to 95% ee at –78 °C.

A few years later Shibasaki and co-workers published a very efficient bifunctional catalyst based on Al-binol (**6**).[23] The Al complex afforded the Lewis acid moiety while the phosphine oxide arms constituted the Lewis base moiety. The presence of additional phosphine oxide as well as slow addition of the TMSCN were essential ingredients in order to achieve high selectivity. This catalyst afforded cyanohydrins derived from aliphatic and aromatic aldehydes with up to 98% ee at –78 °C.



6 **7** **8** **9**

The group of Saá and Najera prepared a similar bifunctional catalyst with diethylamino groups instead of the phosphine oxide groups (**7**).[24] Neither additional phosphine oxide nor slow addition of the TMSCN was necessary. This catalytic system provided cyanohydrins with up to 98% ee on addition of TMSCN to aliphatic and aromatic aldehydes at room temperature. Another advantage was that the ligand could be recovered after the reaction and later reused.

A carbohydrate-based Ti catalyst (**8**) was later developed by Shibasaki and co-workers.[25] This catalyst was more reactive than **6**, thus, lower catalyst loading was possible. Moreover, neither slow addition of the TMSCN nor the use of extra phosphine oxide was required.

The group of Feng employed a bifunctional catalyst (**9**) containing an *N*-oxide for cyanosilylation of ketones.[26] This catalyst gave the product with up to 69% ee. Later, Feng and co-workers published a catalytic system based on a Ti-salen complex combined with an *N*-oxide for the addition of TMSCN to ketones.[27] This catalyst afforded cyanohydrins with up to 81% ee at –20 °C.

2.5 Cyanide Sources

Although TMSCN is associated with several disadvantages, it is the most commonly used cyanide source in enantioselective cyanations of carbonyl compounds. The reason for this is that it directly provides the TMS-protected cyanohydrin. The protecting group prevents the reverse reaction to occur, a reaction that could cause racemerization. However, TMSCN is expensive and would contribute significantly to the cost if it were used in large scale production. Therefore, inexpensive hydrogen cyanide (HCN) is used for multi-ton scale production of enantioenriched mandelonitrile derivatives using enzyme catalysis.[28] However, the drawback of both TMSCN and HCN is their extreme toxicity. Consequently, there is a need to find cyanation reactions using cheap, easily handled and less toxic cyanide sources for production of protected

cyanohydrins of synthetic interest. Asymmetric reactions that provide cyanohydrins from alternative cyanide sources have only recently been described. Examples of cyanide sources that have been used are presented in Figure 2.

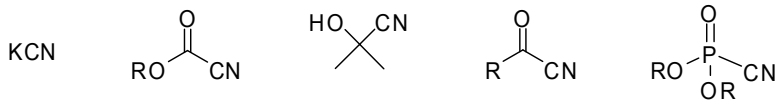


Figure 2. Examples of alternative cyanide sources.

2.6 Synthesis of Chiral O-Carbonylated Cyanohydrins[I]

Alkyl cyanoformates, which directly give the alkyl carbonyl-protected cyanohydrins, are attractive to use in cyanation reactions because they are cheaper and less toxic than TMSCN. Moreover, alkyl carbonylated cyanohydrins are stable and not easily hydrolysed by moisture in air. They are useful synthetic intermediates and can be applied in the synthesis of, for example, β-amino alcohols[29] and γ-substituted unsaturated nitriles from O-carbonylated allylic cyanohydrins.[30]

In 2001, Deng and co-workers reported the first use of alkyl cyanoformate in enantioselective cyanide addition to carbonyls.[29] They employed dimeric chincona alkaloid derivatives as Lewis base catalysts for the addition of ethyl cyanoformate to ketones. Ethyl carbonylated cyanohydrins were formed with up to 97% ee. High catalyst loading was necessary and the reaction required up to seven days reaction time.

In 2002, Shibasaki and co-workers used a binol-based heterobimetallic complex in combination with phosphine oxides for the addition of ethyl cyanoformate to aldehydes.[31] Using 10 mol% of catalyst at –78 °C, cyanohydrins were formed with up to 98% ee.

The group of Najera and Saá utilised their bifunctional catalyst **7** for addition of methyl cyanoformate to aldehydes.[32] By employing 10 mol% of catalyst in the presence of 4 Å molecular sieves, cyanohydrins were obtained with up to 80% ee at room temperature.

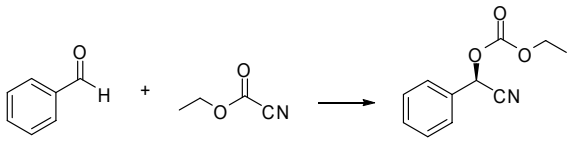
Belokon' and North showed that the dimeric Ti-salen complex **4** could be used for the addition of ethyl cyanoformate to aldehydes.[33] High enantioselectivities

were accomplished at –40 °C, but the reaction was sluggish and required two equivalents of ethyl cyanoformate.

Inspired by the previously reported results, we decided to investigate whether dual Lewis acid–Lewis base activation could be employed in order to improve the reaction. Tertiary amines were selected for activation of the nucleophile and the dimeric Ti-salen complex **4** was selected for activation of the carbonyl function. We were pleased to find that in the presence of DMAP at –40 °C, cyanation of benzaldehyde was completed within four hours in contrast to 18 hours without DMAP (entries 1 and 2, Table 1). The use of DMAP resulted in only a minor decrease of the enantioselectivity (from 95 to 93% ee). Lowering the catalyst loading gave a slightly higher enantioselectivity but a longer reaction time (entry 3).

By decreasing the amount of ethyl cyanoformate, the selectivity increased and a slightly lower reactivity was observed (entry 4). Replacing DMAP by DABCO gave a catalytic system with essentially the same reactivity, although the selectivity was somewhat lower (entry 5). Employing Et_3N and DIEA as Lewis base gave essentially quantitative conversion of starting material into product with high enantioselectivity within three hours (entries 6 and 7). Using chiral tertiary amines as Lewis base did not improve the reaction to any major extent (entries 8-10). Combining the chiral Lewis bases with *ent*-**4** instead of **4** had little effect on the selectivity (entries 11-13), but resulted in slower reactions in the case of cinchonidine and quinine (entries 12 and 13).

Table 1. Cyanation of benzaldehyde by ethyl cyanoformate catalysed by **4** at –40 °C.



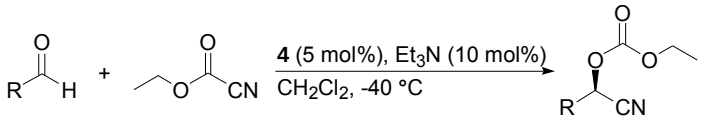
10a **11a** **12a**

entry	%**4**	Lewis base (%)	equiv. **2**	time (h)	% conv.[a]	% ee[b]	abs. conf.[c]
1[33]	5	-	2	18	100	95	*S*
2	5	DMAP (10)	2	4	99	93	*S*
3	1	DMAP (2)	2	8	78	95	*S*
4	5	DMAP (10)	1.2	8	95	94	*S*
5	5	DABCO (10)	1.2	7	90	90	*S*
6	5	Et_3N (10)	1.2	3	97	92	*S*
7	5	DIEA (10)	1.2	3	96	89	*S*
8	5	Sparteine (10)	1.2	3	98	78	*S*
9	5	Cinchonidine (10)	1.2	4	98	94	*S*
10	5	Quinine (10)	1.2	4	93	93	*S*
11	5[d]	Sparteine (10)	1.2	3	98	79	*R*
12	5[d]	Cinchonidine (10)	1.2	7	93	94	*R*
13	5[d]	Quinine (10)	1.2	7	97	94	*R*

[a]Determined by GC-MS. [b]Determined by chiral GC. [c]Assigned by comparing the sign of optical rotation with literature data, see references in paper I. [d]*ent*-**4** was used.

The dual activation system using Ti-salen complex **4** and Et_3N was further explored by adding ethyl cyanoformate to a variety of different aromatic and aliphatic aldehydes (Table 2). High isolated yields and enantioselectivities were obtained for aromatic aldehydes (entries 1-4) as well as for unsaturated and aliphatic aldehydes (entries 5-7).

Table 2. Cyanation of aldehydes by ethyl cyanoformate catalysed by **4** at –40 °C.[a]



10a-k **11a** **12a-g**

a R = C_6H_5 e R = (*E*)-CH=CHPh
b R = 4-CH_3-C_6H_4 f R = $C(CH_3)_3$
c R = 4-CH_3O-C_6H_4 g R = $(CH_2)_4CH_3$
d R = 4-Cl-C_6H_4

entry	aldehyde (R)	product	time (h)	% yield[b]	% ee[c]	abs. conf.[d]
1	**10a** (C_6H_5)	**12a**	4	95	92	*S*
2	**10b** (4-CH_3-C_6H_4)	**12b**	6	88	94	*S*
3	**10c** (4-CH_3O-C_6H_4)	**12c**	6	79	94	*S*
4	**10d** (4-Cl-C_6H_4)	**12d**	4	90	93	*S*
5	**10e** ((*E*)-CH=CHPh)	**12e**	7	97	93	*S*
6	**10j** ($C(CH_3)_3$)	**12f**	5	81	73	*S*
7	**10g** ($(CH_2)_4CH_3$)	**12g**	6	89	90	*S*

[a]Reaction conditions: 5 mol% **4**, 10 mol% Et_3N, 2 equiv. ethyl cyanoformate, –40 °C. [b]Isolated yield. [c]Determined by chiral GC. [d]Assigned by comparing the sign of optical rotation with literature data, see references in paper I.

2.7 Synthesis of Chiral O-Acylated Cyanohydrins[I,II]

Chiral acylated cyanohydrins can be used for the synthesis of a variety of important compounds, e.g. the synthesis of α-acetoxy amides,[34] γ-cyanoallylic alcohols from O-acetylated allylic cyanohydrins[35] and *N*-acyl β-amino alcohols.[36] Furthermore, acylated cyanohydrins are important insecticides used in agriculture.[37]

In 2002, the group of Belokon' and North developed a system based on the use of potassium cyanide (KCN) as cyanide source for the synthesis of acylated cyanohydrins.[38] The dimeric salen-Ti complex **4** could be used for the preparation of enantiomerically enriched acetylated cyanohydrins, with up to 95% ee at –40 °C, in the presence of acetic anhydride as a trapping agent. The advantages of cyanide salts are that they are easily handled, cheap and non-

volatile. However, a drawback of this method is that it requires four equivalents of acetic anhydride and KCN. Moreover, several by-products are observed, e.g. one equivalent of potassium acetate. In addition, the low solubility of alkali salts in organic solvents demands for the use of highly diluted systems.

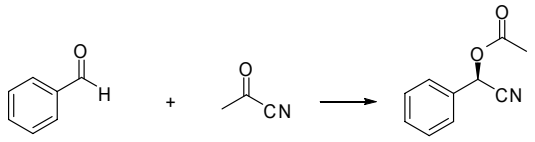
Hanefeldt and co-workers have developed a system based on dynamic kinetic resolution of *in situ* obtained racemic cyanohydrins.[39] They combined a base-catalysed equilibrium between an aldehyde and the corresponding unprotected cyanohydrin with enantiospecific esterfication of the (*S*)-cyanohydrin by *Candida antarctica* lipase B (CALB). It was possible to achieve a quantitative yield since the remaining cyanohydrin was continuously racemized by the base. Acylated cyanohydrins were obtained with up to 98% ee.

Recently, Belokon' and North presented a two step procedure that combined the Ti-salen-catalysed cyanation with enantiospecific hydrolysis by an enzyme.[40] In the first step, non-racemic acetylated cyanohydrins were synthesised by reacting KCN, acetic anhydride, and aldehyde. This was followed by selective hydrolysis of the unwanted minor (*S*)-enantiomer by CALB. Through this procedure (*R*)-cyanohydrins could be synthesised with up to 99% ee. However, this method is time-consuming and only affords the (*R*)-enantiomer with high enantiomeric purity.

α-Ketonitriles, also called acyl cyanides, are attractive to use in cyanation reactions. They are easy to prepare, cheap and usually have lower toxicity than most other cyanide sources. Moreover, cyanation of carbonyl compounds by addition of ketonitriles directly gives acylated cyanohydrins. Although many unselective processes for the synthesis of acylated cyanohydrins by addition of ketonitriles to carbonyl compounds are known, no asymmetric process was described in the literature. Therefore we wanted to investigate if dual activation could be used for the addition of ketonitriles to aldehydes. Earlier attempts by the research group of Belokon' and North to apply acetyl cyanide in the Ti-salen-catalysed system had failed.[38] Accordingly, our initial results were not very promising since no reaction occurred between acetyl cyanide and benzaldehyde at –40 °C when only **4** was used as catalyst (entry 1, Table 3). A slow unselective reaction took place at room temperature (entry 2). To our delight, the addition of DMAP enabled the reaction to occur readily at –40 °C (entry 3). The use of DABCO, Et_3N, DIEA and DBU as Lewis bases was also effective (entries 4-7). However, activation by DIEA and DBU resulted in decreased enantioselectivity. The reaction also proceeded using a secondary amine, but lower selectivity was

obtained (entry 8). In contrast to the results using DMAP, no reaction was observed when pyridine was used (entry 9). The use of chiral bases did not offer any major advantages and replacement of **4** with *ent*-**4** had a minor effect on the reaction outcome (entries 10-15).

Table 3. Cyanation of benzaldehyde with acetyl cyanide catalysed by **4**.[a]



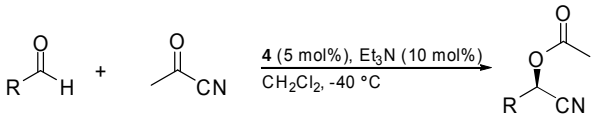
10a **13a** **14a**

entry	Lewis base (%)	T (°C)	time (h)	% conv.[b]	% ee[c]	abs. conf.[d]
1	-	–40	24	0	-	-
2	-	25	24	30	53	*S*
3	DMAP (10)	–40	6	57	94	*S*
4	DABCO (10)	–40	9	67	92	*S*
5	Et_3N (10)	–40	8	96	94	*S*
6	DIEA (10)	–40	8	97	81	*S*
7	DBU (10)	–40	2	99	20	*S*
8	Diethylamine (10)	–40	8	41	81	*S*
9	Pyridine (10)	–40	24	0	-	-
10	Sparteine (10)	–40	8	93	65	*S*
11	Cinchonidine (10)	–40	9	78	96	*S*
12	Quinine (10)	–40	9	80	92	*S*
13[e]	Spartein (10)	–40	8	96	67	*R*
14[e]	Cinchonidine (10)	–40	9	75	92	*R*
15[e]	Quinine (10)	–40	9	73	95	*R*

[a] Reaction conditions: 5 mol% **4**, 2 equiv. acetyl cyanide. [b] Determined by GC-MS.[c] Determined by chiral GC. [d] Assigned by comparing the sign of optical rotation with literature data see references in papers I and II. [e] *ent*-**4** was used.

A number of aliphatic and aromatic aldehydes were subjected to enantioselective addition of acetyl cyanide catalysed by Ti-salen complex **4** and Et_3N (Table 4). The reaction with aromatic aldehydes resulted in high isolated yields and with high enantioselectivities in all cases, except with 2-pyridine carboxaldehyde (entries 1-4, 8-11). Unsaturated and aliphatic acetylated cyanohydrins were synthesised in high yields and with good enantioselectivities (entries 5-7).

Table 4. Cyanation of aldehydes by acetyl cyanide.[a]



10a-k **13a** **14a-k**

a R = C_6H_5 e R = (*E*)-CH=CHPh i R = 3-(PhO)C_6H_4
b R = 4-CH_3-C_6H_4 f R = $C(CH_3)_3$ j R = 3-Pyridyl
c R = 4-CH_3O-C_6H_4 g R = $(CH_2)_4CH_3$ k R = 2-Pyridyl
d R = 4-Cl-C_6H_4 h R = 2-Furyl

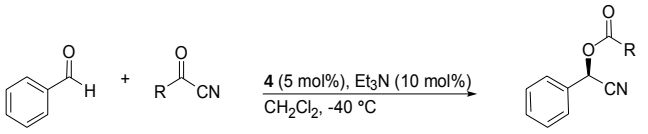
entry	aldehyde (R)	product	time (h)	% yield[b]	% ee[c]	abs. conf.[d]
1	**10a** (C_6H_5)	**14a**	10	89	94	*S*
2	**10b** (4-CH_3-C_6H_4)	**14b**	10	90	96	*S*
3	**10c** (4-CH_3O-C_6H_4)	**14c**	12	72	94	*S*
4	**10d** (4-Cl-C_6H_4)	**14d**	8	89	95	*S*
5	**10e** ((*E*)-CH=CHPh)	**14e**	12	64	93	*S*
6	**10f** ($C(CH_3)_3$)	**14f**	6	84	76	*S*
7	**10g** ($(CH_2)_4CH_3$)	**14g**	6	89	90	*S*
8	**10h** (2-Furyl)	**14h**	12	93	89	*R*
9	**10i** (3-PhO-C_6H_4)	**14i**	48	84	85	*S*
10	**10j** (3-Pyridyl)	**14j**	12	91	86	n.d
11	**10k** (2-Pyridyl)	**14k**	12	87	20	n.d

[a]Reaction conditions: 5 mol% **4**, 10 mol% Et_3N, 2 equiv. of acetyl cyanide, –40 °C. [b]Isolated yield. [c]Determined by chiral GC. [d]Assigned by comparing the sign of optical rotation with literature data, see references in papers I and II.

Six different ketonitriles were synthesised by reacting the corresponding acid bromide or chloride with copper cyanide. The obtained ketonitriles were applied in cyanations of benzaldehyde catalysed by salen-Ti **4** and Et_3N (Table 5). Linear aliphatic ketonitriles afforded cyanohydrins in high yields and with good enantioselectivities (entries 1-4). The use of 3-methyl-2-oxobutanenitrile **13e** also resulted in high yield of the product with high enantioselectivity (entry 5). In contrast, the more bulky 3,3-dimethyl-2-oxobutanenitrile **13f** was unreactive at –40 °C. Increasing the temperature to room temperature gave the product in high yield with good enantioselectivity (entry 6). Cinnamoyl cyanide was the most reactive ketonitrile and afforded the product in high yield with excellent

enantioselectivity (entry 7). Commercially available benzoyl cyanide was also included in the study and gave the product with 75% ee (entry 8).

Table 5. Cyanation of benzaldehyde by α-ketonitriles.[a]



10a **13 a-h**

14a R = CH_3
14l R = CH_2CH_3
14m R= $(CH_2)_2CH_3$
14n R = $(CH_2)_3CH_3$
14o R = $CH(CH_3)_2$
14p R = $C(CH_3)_3$
14q R = (*E*)-CH=CHPh
14r R = C_6H_5

entry	α-Ketonitrile (R_1)	product	time (h)	% yield[b]	% ee[c]	abs. conf.[d]
1	**13a** (CH_3)	**14a**	10	89	94	*S*
2	**13b** (CH_2CH_3)	**14l**	10	89	93	*S*
3	**13c** ($(CH_2)_2CH_3$)	**14m**	10	90	92	*S*
4	**13d** ($(CH_2)_3CH_3$)	**14n**	10	85	93	*S*
5	**13e** ($CH(CH_3)_2$)	**14o**	10	86	92	*S*
6[e]	**13f** ($C(CH_3)_3$)	**14p**	10	81	79	*S*
7	**13g** ((*E*)-CH=CHPh)	**14q**	4	89	94	*S*
8	**13h** (C_6H_6)	**14r**	26	76	75	*S*

[a]Reaction conditions: 5 mol% **4**, 10 mol% Et_3N, 2 equiv of acetyl cyanide, –40 °C. [b]Isolated yield. [c]Determined by chiral GC or chiral HPLC. [d]Assigned by comparing the sign of optical rotation with literature data, see references in paper II. [e]Reaction run at room temperature.

2.8 Mechanism[II]

The group of Belokon' and North has previously reported an increase in enantioselectivity with time in cyanation reactions using **4** as catalyst.[15,40] We observed the same effect when combining **4** with a Lewis base (a, Figure 3). A change in ee with time was also observed when the Ti-salen catalyst was kept at room temperature for three hours before cooling followed by addition of reactants (b, Figure 3). However, when the reaction mixture was kept at –40 °C for three hours before the addition of the Lewis base, a constant ee was observed (c, Figure 3). A constant ee was also obtained when only the Ti-salen catalyst was kept at –40 °C before addition of the reactants and the Lewis base. Keeping the reaction mixture at –40 °C for three hours before addition of the Lewis base did not only result in a constant ee, but also in a slightly higher ee than that

observed when the normal procedure was used. This phenomenon was particularly noticeable when DBU was employed as the Lewis base. Cooling prior to the addition of DBU resulted in an ee of 69%, as compared to 20% under the normal conditions.

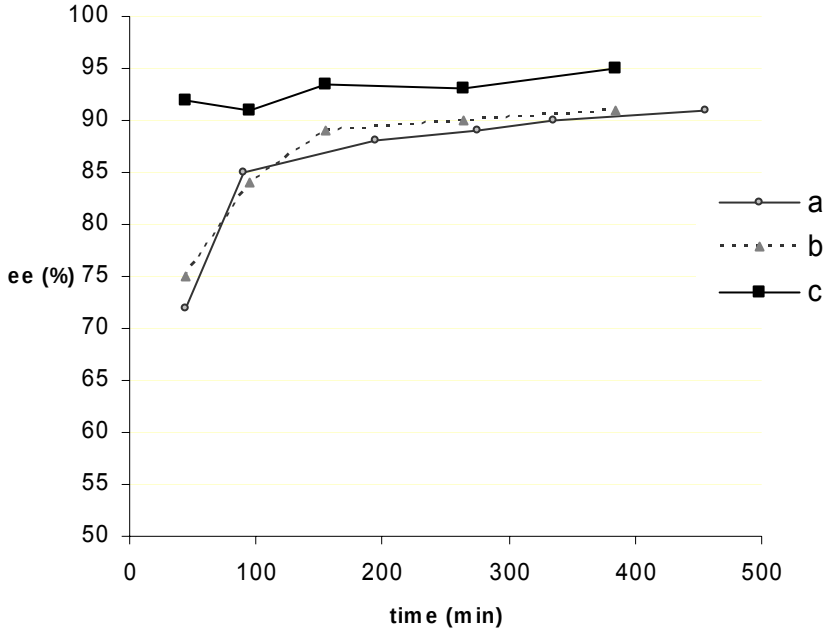
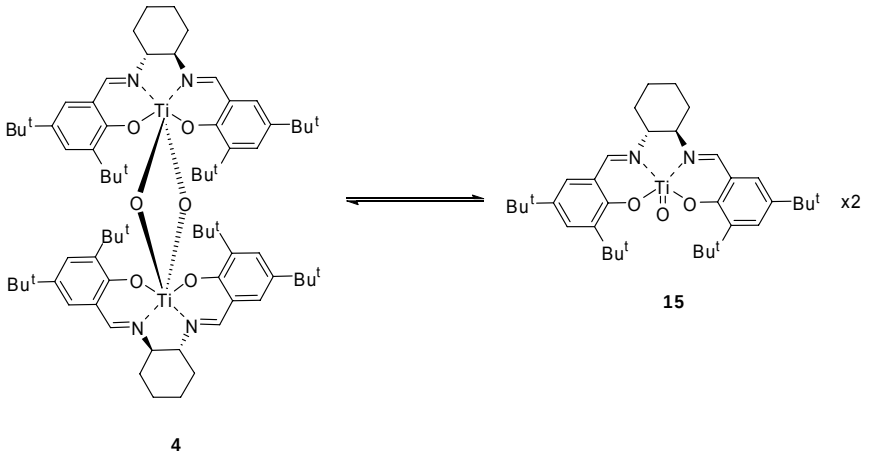


Figure 3. a) normal conditions; b) Ti-salen complex kept at room temperature for three hours before cooling to –40 °C and addition of aldehyde, ketonitrile, and Lewis base; c) Ti-salen complex kept at –40 °C for three hours before the addition of aldehyde, ketonitrile, and Lewis base.

It has previously been reported that the dimeric Ti-salen complex **4** is in equilibrium with its monomer **15** (Scheme 5) and that the monomer to dimer ratio varies with the temperature, solvent and concentration.[40]



4 **15**

Scheme 5. Equilibrium between Ti-salen dimer **4** and monomer **15**.

To further study this effect, we recorded ^{1}H NMR spectra of the Ti complex at ambient and low temperature. The spectrum taken at room temperature showed a dimer to monomer ratio of 4:1. This ratio increased to 9:1 on cooling the sample to –40 °C. The ratio increased with time at –40 °C and after 90 minutes a constant ratio of 19:1 was observed (Figure 4).

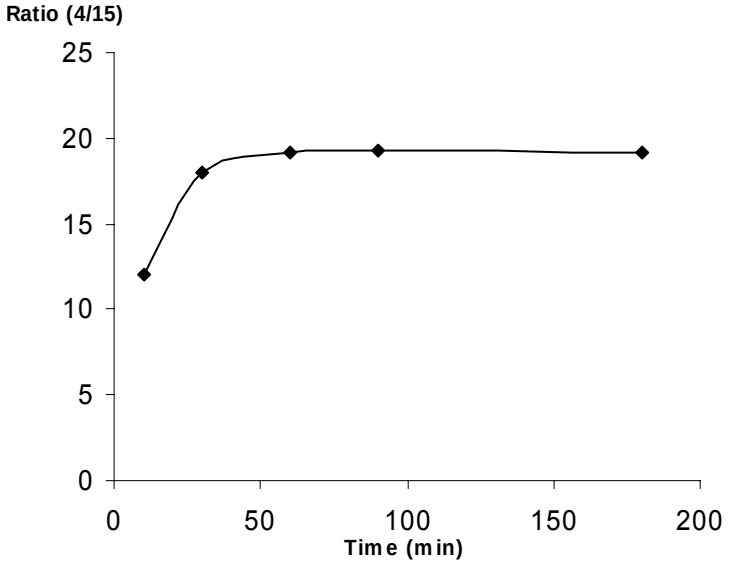
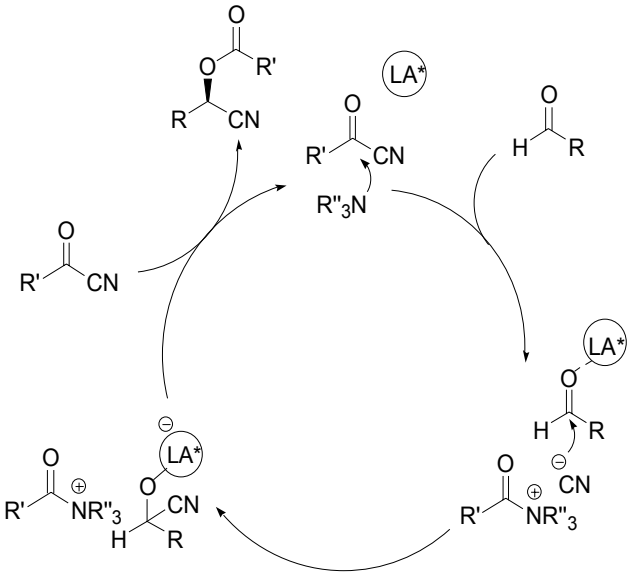


Figure 4. Ratio of dimer **4**/monomer **15** increased with time at –40 °C. The ratio was obtained by integration of the aromatic protons at δ = 7.55 (s, 1H, ArH) for the monomer and at δ = 7.41 (s, 1H, ArH) for the dimer.

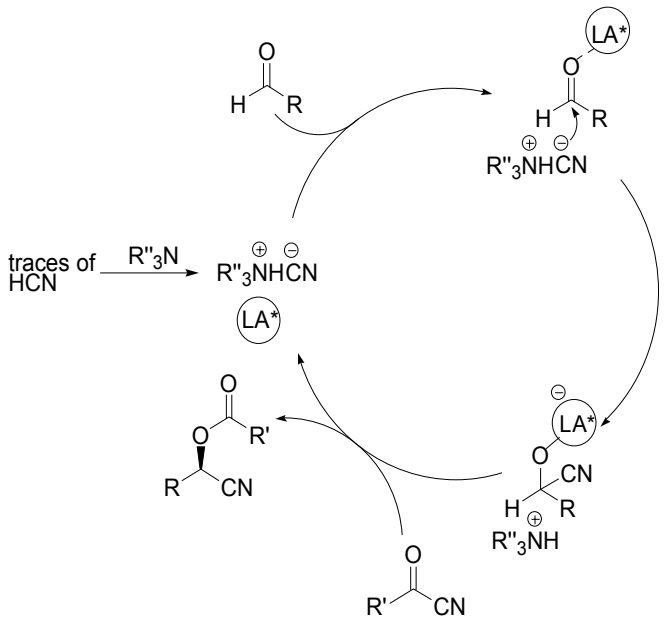
Considering the increase in both ee and dimer to monomer ratio with time at –40 °C, it is reasonable to assume that the dimeric species results in higher enantioselectivity. The lower ee in the initial part of the reaction may be caused by a less selective reaction catalysed by the monomer.

We envisioned that the reaction was initiated by nucleophilic attack of the Lewis base on the carbonyl group of the ketonitrile or ethyl cyanoformate (Scheme 6). This forms a good acylating agent and cyanide ions that can react with the aldehyde to form the cyanohydrin. A similar role of the Lewis base was proposed by Deng and co-workers for the addition of ethyl cyanoformate to ketones.[29] In addition, similar mechanisms were proposed for the addition of ketonitriles to carbonyl groups catalysed by DABCO[41] and DBU.[42]



Scheme 6. Proposed dual Lewis base–Lewis acid reaction mechanism.

Najera and Saá have proposed an alternative mechanism for the addition of benzoyl cyanide and methyl cyanoformate to aldehydes.[43] They suggest a Brønsted base mechanism where the first step is the deprotonation of HCN (trace amounts present in the reaction mixture) by the tertiary amine. Through this process cyanide ions are obtained that can attack the aldehyde (Scheme 7).



Scheme 7. Proposed Brønsted base–Lewis acid reaction mechanism.

In order to study the mechanism we set up a number of experiments:

- A ^{1}H NMR spectrum of a mixture of HCN and Et_3N in CD_2Cl_2 was recorded. This showed no formation of trietylammonium ions, which indicates that deprotonation is unfavoured in dichloromethane. This result is not enough to distinguish between the two proposed mechanisms, since undetectable amounts of cyanide may be enough to initiate the reaction.
- $H^{13}CN$ was bubbled through a mixture of **4** and Et_3N prior to cooling to –40 °C and addition of the reactants. A sample was taken after 10 minutes (5% conversion) and analysed by GC-MS. The mass spectra showed no ^{13}C incorporation in the product. Therefore, it seems unlikely that HCN is a source of cyanide ions. Moreover, this result also implies that no free cyanide ions are present in the solution, since that would give fast isotope scrambling between $H^{13}CN$ and CN^-.
- A ^{13}C NMR spectrum of a mixture of **4** and acetyl cyanide in CD_2Cl_2 was recorded. The ^{13}C NMR signal from the carbonyl carbon atom of acetyl cyanide was shifted in the presence of the Ti complex. This indicates coordination of the acetyl cyanide to the Ti atom.

Analysing the results, it seems reasonable to believe that both the aldehyde and the cyanide are coordinated to the two Ti atoms of the dimeric Ti-salen complex

(Figure 5). Attack of the cyanide on the aldehyde within the dimeric Ti complex explains the lack of ^{13}C scrambling between $H^{13}CN$ and CN^-.

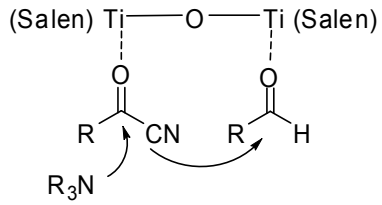


Figure 5. Proposed mechanism for cyanation of aldehydes.

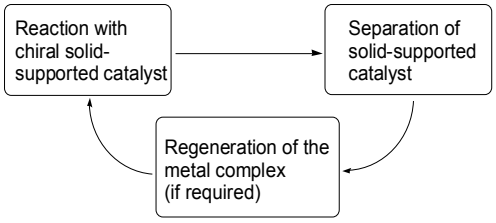
2.9 Conclusion

A dual activation system for enantioselective addition of cyanide to aldehydes has been described. This system provided highly enantioenriched and synthetically important O-carbonylated and O-acylated cyanohydrins in high yield with 100% atom efficiency. Moreover, the catalytic system, with the Lewis acidic and Lewis basic moieties residing in different molecules is suitable for high throughput screening of the catalytic properties. Experimental data support a mechanism involving attack of the cyanide on the aldehyde within the dimeric Ti-salen complex.

3. Polymer-Supported Catalysts

3.1 Background

There is a current trend in asymmetric metal catalysis to develop reusable catalytic systems. During the past ten years, numerous solid-supported ligands have been prepared and applied in a variety of asymmetric transformations.[44] Several types of solid supports have been used e.g. inorganic, polymeric and hybrid organic-inorganic materials. The major advantages of the heterogeneous systems are the possibilities to separate the chiral catalyst from the reaction mixture as well as to reuse the catalyst (Scheme 8). Moreover, the use of solid-supported metal complexes might minimise the leakage of metal into the reaction mixture, an aspect particularly important in pharmaceutical applications. Another benefit with the heterogeneous systems is the possibility to use a continuous flow with the chiral catalyst in a fixed bed.



Scheme 8. Separation and recycling of heterogeneous catalytic systems.

There is also a growing interest to use heterogeneous catalysis in combinatorial applications.[45] However, more complicated synthetic routes are usually required in order to obtain the heterogeneous systems. Moreover, lower conversion and selectivity have been observed with the heterogeneous catalysts compared to the corresponding homogeneous catalysts, probably due to inflexibility of the solid-supported catalysts.

It is attractive to combine the advantages of heterogeneous catalysis with the flexibility of homogeneous catalysis. This can partly be achieved by the use of grafted co-polymers. They consist of a non-soluble core, commonly cross-linked polystyrene (PS), and soluble arms, often polyethylene glycol (PEG). Tentagel® (TG) and Argogel® are two examples of commercially available polymers of this kind. These polymers have higher swelling capacity compared to traditionally

cross-linked PS-polymers. The solubility of the PEG-chain allows the use of ^{13}C NMR for analysis of the polymer-bound ligands and the polymer functionality.

3.2 Synthesis of Polymer-Supported Pybox Ligands[III]

Before we started our research, there was only one report in the literature of a polymer-supported pybox ligand.[46] Today, there are a few published examples of immobilised pybox ligands.[48-51,III] In all ligands, the pybox is attached to the solid-support via introduction of a spacer at the 4-position of the pyridine ring (Figure 6). The spacer serves as a handle and should ideally reduce the interactions between the solid support and the attached catalyst.

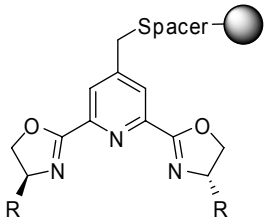
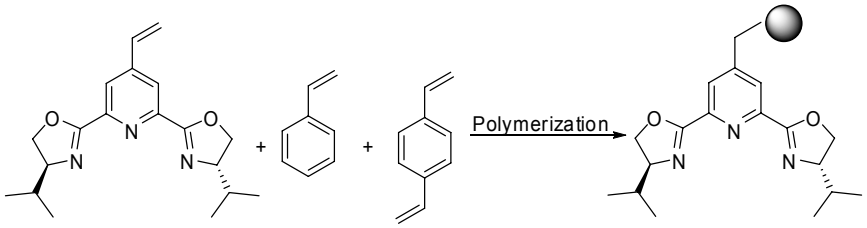


Figure 6. Solid-supported pybox ligand.

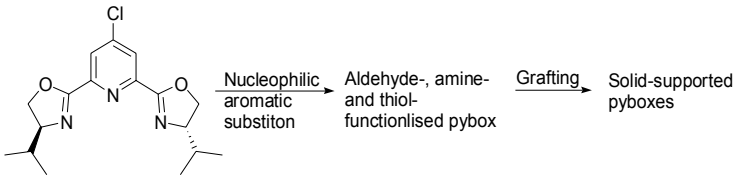
The first polymer-supported pybox ligand was developed by Mayoral and co-workers in 2003.[46] They synthesised a vinyl-functionalised pybox and subjected it to co-polymerisation with styrene and divinylbenzene (Scheme 9). The resulting polymer-bound pybox was employed in enantioselective Ru-catalysed cyclopropanation of styrene and ethyl diazoacetate. The products were formed in high yields with up to 85% ee. The catalytic complex could be reused up to three times, but the reactivity was reduced after the second run.



Scheme 9. Synthesis of polymer-supported pybox ligands via co-polymerisation.

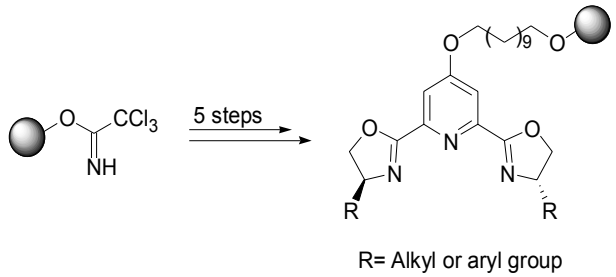
Later Mayoral and co-workers synthesised amine-, aldehyde-, and thiol-functionalised pybox ligands via nucleophilic aromatic substitution of 4-chloro pybox (Scheme 10).[47,48] These pybox derivatives were immobilised onto solid

supports such as silica,[47,49] Merrifield resin[48] and modified starch.[50] The resulting polymer-bound catalysts were employed in Ru-catalysed cyclopropanation.



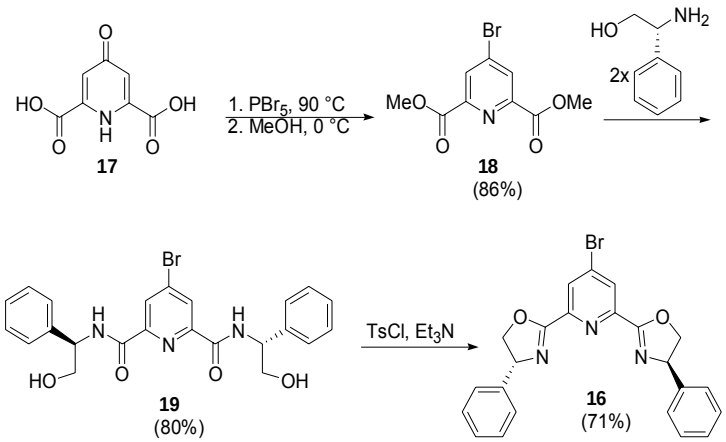
Scheme 10. Synthesis of solid-supported pybox ligands via nucleophilic aromatic substitution.

In 2005, Portnoy and co-workers prepared polymer-supported pybox ligands by stepwise solid phase synthesis (Scheme 11).[51] The resulting polymer-supported pybox ligands were applied in Cu(I)-catalysed enantioselective addition of terminal alkynes to imines. Chiral propargylamines were formed with up to 83% ee. The polymer-bound pybox-Cu(I) complexes could be reused after the reaction, although the recovered catalysts exhibited lower reactivity. The drawback of the stepwise solid phase synthesis is that it is time-consuming due to the lower reactivity of the solid-supported substrates. Moreover, high conversion in each step of the solid-supported starting material into the desired product is necessary for the method to be useful.



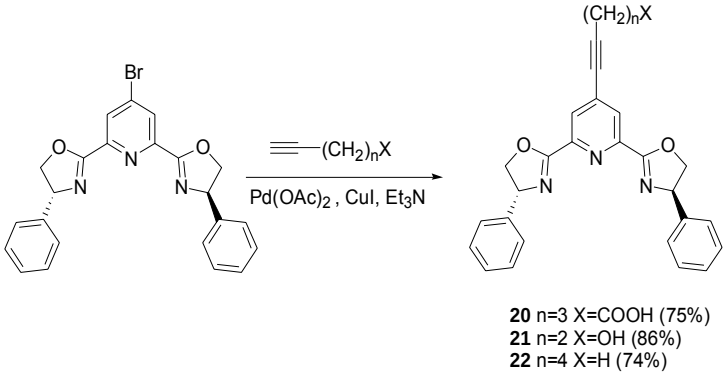
Scheme 11. Synthesis of polymer supported pybox ligands via solid phase synthesis.

Our strategy was to use 4-bromo-phenyl pybox **16** as a key intermediate for the introduction of a spacer. 4-Bromo-phenyl pybox **16** was synthesised starting from chelidamic acid (**17**). Initially, **17** was transformed to 4-bromopyridine-2,6-dicarboxylic acid dimethyl ester (**18**), which was further reacted with (*R*)-phenyl glycinol generating the diamide **19** (Scheme 12). This was followed by ring closure to afford **16**.



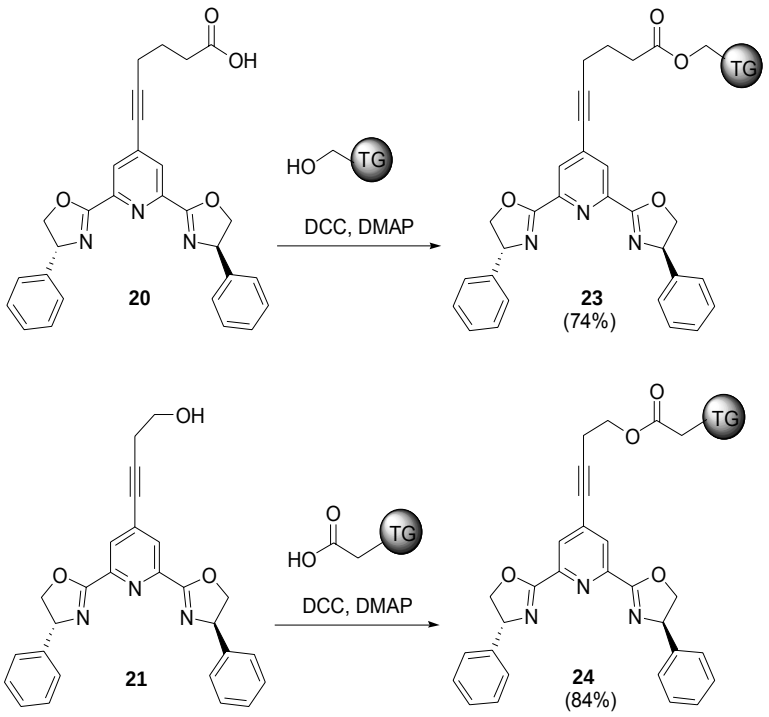
Scheme 12. Synthesis of 4-bromo-phenyl pybox.

For the attachment of the spacers we decided to employ Sonogashira coupling, which is a Pd-catalysed cross-coupling reaction that tolerates a large variety of functional groups.[52] In this manner, terminal alkynes were conveniently connected to the 4-position of the pyridine ring in the pybox (Scheme 13). Carboxyl- and hydroxyl-functionalised pybox ligands **20** and **21** were synthesised in 75% and 86% isolated yield, respectively. In order to study the effect of an unfunctionalised alkyne pybox, we also prepared **22** in 74% isolated yield via reaction of 1-hexyne with **16**.



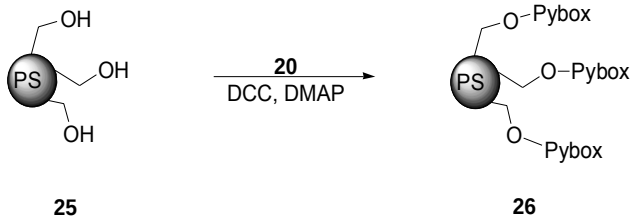
Scheme 13. Synthesis of 4-alkynyl-substituted phenyl pybox ligands.

Pybox derivatives **20** and **21** were immobilised onto hydroxyl- and carboxyl-functionalised TG-polymers via ester bond formation in the presence of the coupling reagent DCC (Scheme 14). The polymer-supported pybox ligands were obtained in high yields according to elemental analysis.



Scheme 14. Immobilisation of pybox ligands onto TG-polymers.

A grafted cross-linked PS-polymer (**25**), was also included in the study. This polymer has soluble arms and consists of beads with a diameter of only 15 μm. Pybox derivative **20** was bound to this polymer by ester bond formation in the presence of DCC (Scheme 15).



25 **26**

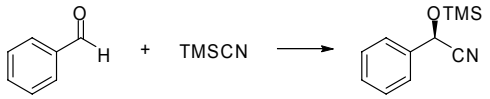
Scheme 15. Immobilisation of pybox ligands onto polymer **25**.

3.3 Cyanation of Benzaldehyde Catalysed by Polymeric $YbCl_3$-$Pybox^{III}$

The polymer-supported pybox ligands were applied in Yb-catalysed additions of TMSCN to benzaldehyde (Table 6). The results were compared to those obtained

with the corresponding homogenous catalyst **5b**-$YbCl_3$ (page 6). In our hands, the catalytic system based on **5b**-$YbCl_3$ gave the product with 84% ee (entry 1), in contrast to the ee value of 89% reported by Aspinall and co-workers.[19] The alkyne substituent had a minor influence on the reaction outcome. Thus, the Yb complex of ligand **22** afforded the product in 84% ee (entry 2). The catalysts based on the TG bound ligands **23** and **24** provided the product in high yields and with good enantioselectivity (entries 3-6). The polymeric pybox complex could be reused up to four times with only a slight decrease in reactivity after each run. The catalytic system obtained with the polymer-supported ligand **26** was less reactive and required a longer reaction time (entries 7 and 8). However, the polymer bound pybox ligands could be reused up to 30 times by simple filtration and washing of the polymer after the reaction.

Table 6. Cyanation of benzaldehyde catalysed by various $YbCl_3$-pybox derivatives.[a]



10a **27**

entry	catalyst	number of runs	time (h)	% conv.[b]	% ee[c]	abs. conf.[d]
1	**5b-$YbCl_3$**		0.5	90	84	*R*
2	**22-$YbCl_3$**		0.5	92	84	*R*
3	**23-$YbCl_3$**	1	0.5	89	81	*R*
4	**23-$YbCl_3$**	4	0.5	66	81	*R*
5	**24-$YbCl_3$**	1	0.5	88	80	*R*
6	**24-$YbCl_3$**	4	0.5	72	81	*R*
7	**26-$YbCl_3$**	1	0.5	30	77	*R*
8	**26-$YbCl_3$**	1	25	89	78	*R*

[a]Reaction conditions: 10 mol% $YbCl_3$, 20 mol% ligand, 1.2 equiv. TMSCN, 0-25 °C. [b]Determined by GC/MS. [c]Determined by chiral GC. [d]Assigned by comparing the sign of optical rotation with literature data see references in paper III.

3.4 Synthesis of Polymer-Supported Ti-salen Complexes[IV]

There are several reports on immobilisation of salen ligands onto solid support.[53] The attachment has mostly been achieved by covalent binding. In general, three different approaches have been used for the synthesis of solid-supported salen ligands (Figure 7).

- Connection at one of the salicylaldehyde moieties (A).
- Connection at the diamine moiety (B).
- Polymerisation of a difunctionalised salen ligand (C).

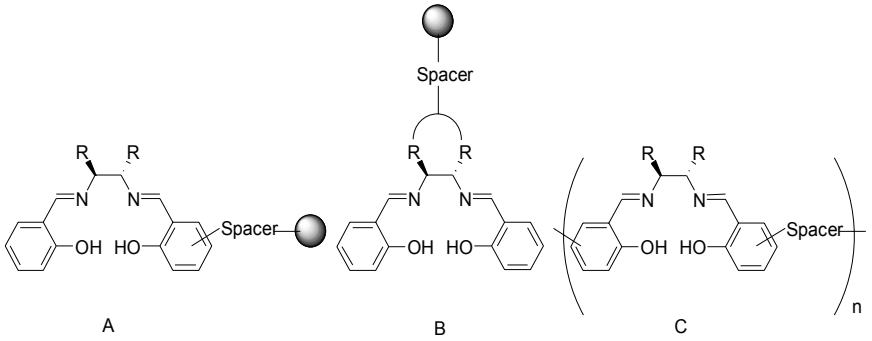
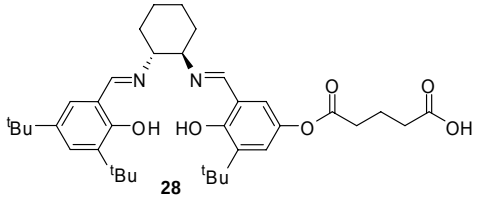


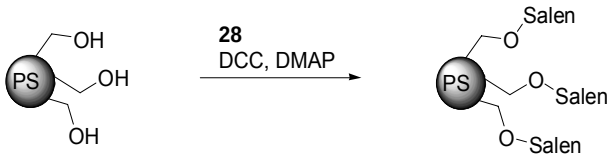
Figure 7. Different approaches for the synthesis of solid-supported salen ligands.

For the synthesis of a polymer-supported salen ligand we chose **28** as a suitable precursor. This salen ligand derivative could be obtained via an unsymmetrical phenolic salen ligand[54] following a published procedure.[55]



28

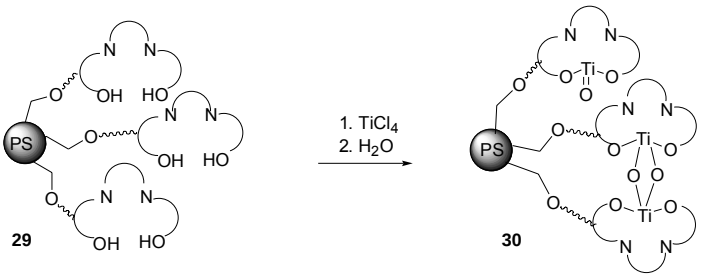
Salen ligand **28** could conveniently be attached to the hydroxyl-functionalised polymer **25** affording **29** by ester bond formation (Scheme 16).



25 **29**

Scheme 16. Immobilisation of salen ligand **28** onto polymer **25**.

The polymeric Ti-salen complex was prepared by mixing the polymer-bound ligand **29** with $TiCl_4$ in dichloromethane before addition of a phosphate buffer (pH 7) (Scheme 17). After removal of solvents and washing the polymer with dichloromethane, **30** was obtained as a bright yellow solid.



Scheme 17. Preparation of polymeric Ti-salen complex.

3.5 Cyanation of Benzaldehyde Catalysed by Polymeric Ti-Salen[IV]

The polymeric Ti-salen complex **30** was applied in enantioselective addition of TMSCN to benzaldehyde. This afforded the TMS-protected cyanohydrin with 73% ee at room temperature. Catalyst **30** was also combined with Et_3N in dual Lewis acid–Lewis base-catalysed addition of acetyl cyanide to benzaldehyde. This gave the acetylated cyanohydrin in 68% ee. The polymeric Ti-salen complex could be reused up to five times without any decrease of neither reactivity nor selectivity in the catalytic system.

3.6 Conclusion

Polymer-supported pybox ligands have been synthesised and applied in addition of TMSCN to benzaldehyde. The use of the polymer-supported pybox ligands resulted in formation of the products with up to 81% ee. The polymer-supported $YbCl_3$ complexes could be recycled and reused up to four times with only a minor decrease in catalytic activity. We have also synthesised a polymer-supported Ti-salen catalyst. This complex has been used in the enantioselective addition of TMSCN and acetyl cyanide to benzaldehyde. The polymeric Ti-salen complex could be reused up to five times without change in reactivity or selectivity.

4. Microreactors

4.1 Background

Microfluid technologies have received a considerable amount of attention in recent years.[56] The applications of microstructured devices are mainly found in analytical chemistry and biochemistry. However, there is also an increasing interest in the use of microreactors for organic synthesis.[57] Microreactor technology offers a number of advantages such as low consumption of reagents, increased safety and efficient optimization of reaction conditions. They are particularly useful to employ in connection to high throughput methods.[58] The main disadvantages of microreactors are the limited reaction time and the low tolerance to product precipitation. However, in some cases higher yield and selectivity have been observed in comparison to conventional batch systems.

A microreactor is commonly defined as a network of channels formed in a planar surface. The channels have dimensions in the range of 10-500 μm and the total volume of the microreactors varies between 0.1 μL-10 mL. The flow is laminar and the mixing occurs only by diffusion. Diffusion is fast in channels of small dimensions and therefore the mixing is very rapid. The increased surface to volume ratio of microreactors makes the heat transport more efficient than in traditional round-bottom flasks. This results in very efficient heat and mass transport. There are several commercially available microfluidic-based devices that are fabricated in polymers, metals, quartz, silicon, and glass. Chemically inert metal or glass-fabricated microreactors are usually used for synthetic applications.

There are basically two ways to achieve flow for transportation of fluids within a microfluidic-based reactor; electroosmotic flow (EOF) and pressure driven flow. The former flow technique, EOF, is based on the use of channels with a negatively charged surface and a fluid containing charged or polarized molecules. A positive fluid layer is formed next to the charged surface (Figure 8). By applying a voltage, a flow towards the negative electrode is generated. This flow profile is constant across the channel. The main advantages of EOF are that the technique is uncomplicated and easily miniaturised. The disadvantage of EOF is that it is limited to the use of polar solvents such as THF, acetonitrile, and methanol. Furthermore, the flow generated by electroosmosis is dependent on the channel material as well as on the composition of the fluid. In other words,

any changes in the reaction fluid or any surface contamination will affect the flow. Other drawbacks of EOF are the separation of the reactants due to difference in polarity and the unwanted electrochemical reactions that can take place.

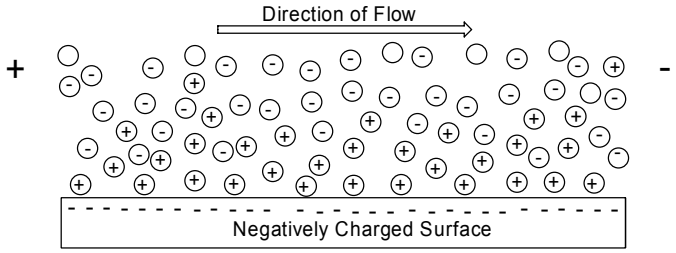


Figure 8. The principle of EOF.

Pressure driven flow is the most straightforward pumping technique and a broad range of flow rates can be obtained by the use of a syringe or an HPLC pump. The advantage of this technique is the broad spectrum of different solvents that can be used. This approach generates a flow that is constant over the channel system and that is stable towards variations of the liquid content. One of the main drawbacks of this system is the parabolic flow profile obtained.

Although there are many articles concerning organic synthesis in a microreactor,[57] there are only a few examples in the literature regarding the use of microreactors in asymmetric synthesis.

In 2004, Watts and co-workers published a procedure for diastereoselective alkylations of enolates in a pressure driven microreactor.[59] The chirality transfer was controlled by the use of Evans' auxiliary and the product was formed with up to 94:6 diastereomeric ratio.

The research group of de Bellefon developed a liquid–gas phase microreactor for hydrogenation of prochiral methyl acetamidocinnamate.[60] They employed a microreactor with a volume of 15 μL for the screening of chiral Rh catalysts and substrates. Products were formed in high yields with up to 63% ee.

Recently, Kitamori and co-workers reported the use of a super-cooled microreactor for phase transfer-catalysed alkylations of Schiff bases.[61] Although products with up to 99% ee have been synthesised under normal batch reactions

at 0 °C,[62] the enantiomeric excess of the product formed in the microreactor was only 50% at –20 °C.

4.2 The Use of Microreactor for $LnCl_3$ Pybox-Catalysed Cyanation of Benzaldehyde[V]

We employed a T-shaped microreactor with two inlets (A and B) and one outlet (C; Figure 9). The microreactor, with approximate channel dimensions of 100×50 μm, was fabricated in borosilicate glass at Hull University, England.[63] In our first project, the reaction fluids were mobilised by the use of EOF. A continuous flow was generated by applying voltages ranging from 80-750 V to electrodes in A and B with C set to ground. We used this system to study the lanthanide pybox-catalysed addition of TMSCN to benzaldehyde. Two standard solutions were prepared and added to inlets A and B, respectively. One solution contained the pybox-lanthanide catalyst and benzaldehyde and the other TMSCN.

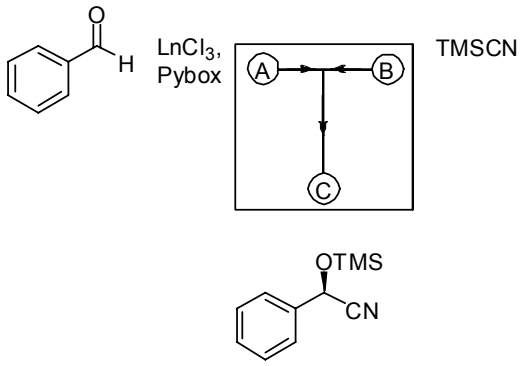


Figure 9. A schematic view of the microreactor consisting of two inlets (A and B) and one outlet (C).

Initially we investigated the effect of the applied voltage on the conversion and selectivity (Figure 10). In the Lu-catalysed reaction the highest conversion (97%) was achieved when the applied voltages were 140 V on inlet A and 220 V on inlet B. Higher voltages resulted in lower conversion. The conversion achieved in a batch reaction using the same stock solutions was 87% after 30 minutes.

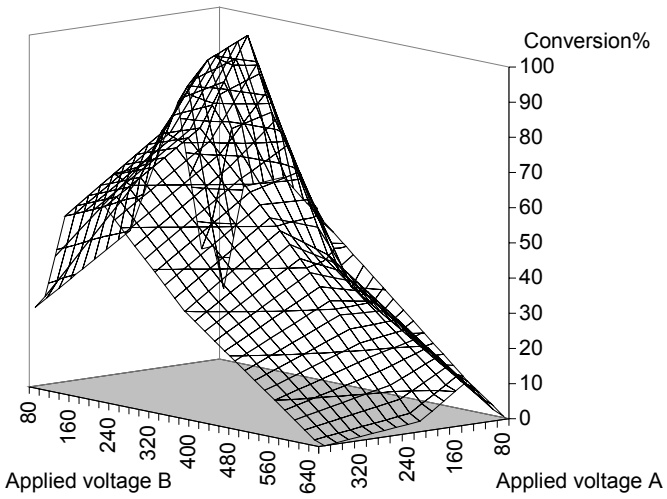


Figure 10. The influence of the applied voltages on the conversion of the $LuCl_3$ pybox-catalysed reaction. All reactions were carried out in acetonitrile at room temperature using 1.2 equiv. TMSCN, 8 mol% pybox ligand and 4 mol% $LuCl_3$.

To explore the effectiveness of the microreactor for optimization of reaction conditions, we decided to study the influence of a variety of metal sources and additives on the reaction outcome. A negative aspect of our system was that when we exchanged $LuCl_3$ for other lanthanide chlorides, re-optimization of the applied voltages was required. We discovered, however, that each additive affected the selectivity similarly, whether the reactions were run in the microreactor or under conventional conditions. In general, the selectivity was always lower when the reaction was conducted in the microreactor compared to batch conditions (Figure 11). On the other hand, the reactivity achieved using optimal microreactor operating conditions was higher than that observed in analogous batch reactions.

The lower selectivity observed for the reactions performed in the microreactor can be caused by several factors, for instance an unselective background reaction catalysed by the reactor walls in combination with slow diffusion of the lanthanide complex and/or decomposition of the chiral catalyst.

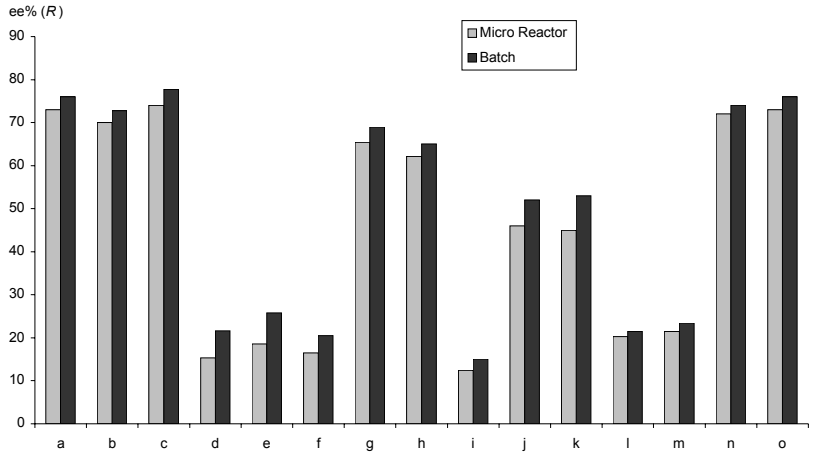


Figure 11. The influence of additives on the enantioselectivity of the $LuCl_3$-pybox-catalysed reaction. All reactions were carried out in acetonitrile at room temperature using 1.2 equiv. TMSCN, 8 mol% pybox ligand **5b**, 4 mol% $LuCl_3$ and 4 mol% of the additive. (a) no additive; (a) L-menthol; (c) D-menthol; (d) *N*,*N*-dimethylaniline *N*-oxide; (e) pyridine *N*-oxide; (f) tritylamine; (g) neomenthol; (h) *R*-(-)-2-butanol; (i) (-)-sparteine; (j) *R*-(+)-1-ethylphenylamine; (k) *S*-(-)-1-ethylphenylamine; (l) triphenylphosphine oxide; (m) dimethylphenylphosphine oxide; (n) THF; (o) diethyl ether. The ee values were determined by chiral GC.

4.3 The Use of Microreactor for Ti-Salen-Catalysed Cyanation of Benzaldehyde[VII]

The addition of acetyl cyanide to benzaldehyde catalysed by Ti-salen in combination with various Lewis bases was studied using the T-shaped microreactor (Figure 12). The Ti-salen complex and benzaldehyde were introduced at inlet A and the acetyl cyanide and Lewis base at inlet B. The products were collected after 20 or 40 minutes at outlet C. In order to avoid the problems associated with EOF, a pressure driven flow was used for achieving the mobility of the liquids. A flow of 1 μL/min was applied by employing a syringe pump.

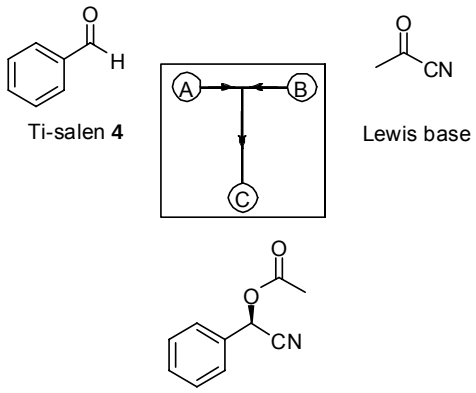


Figure 12. A schematic view of the microreactor used for the addition of acetyl cyanide to benzaldehyde catalysed by Ti-salen complex **4** and various Lewis bases.

The results obtained using a number of different Lewis bases combined with two different reaction times are presented in Figures 13.

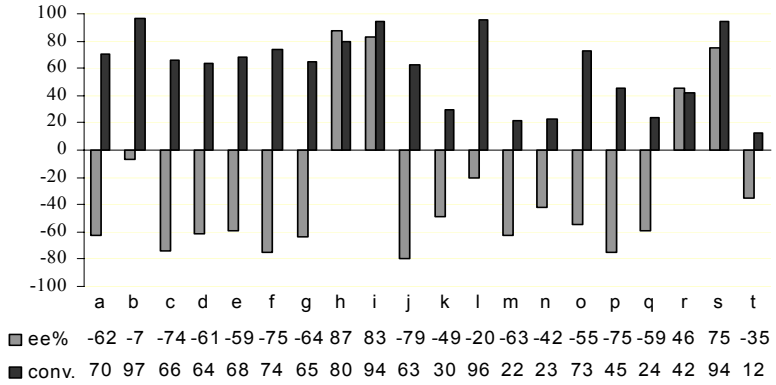


Figure 13. The influence of different Lewis bases and reaction times on the enantioselectivity and conversion. All reactions were carried out in dichloromethane at room temperature for 40 min (a-j) or 20 min (k-t) using 2 equiv. acetyl cyanide, 5 mol% **4** (a-g, j, k-q, t) or *ent*-**4** (h, i, r, s) and 10 mol% Lewis base, (a) Et3N; (b) DBU; (c) cinchonidine; (d) quinine; (e) DIPEA; (f) DMAP; (g) DEA; (h) cinchonidine; (i) quinine; (j) DABCO; (k) Et_3N; (l) DBU; (m) cinchonidine; (n) quinine; (o) DIPEA; (p) DMAP; (q) DEA; (r) cinchonidine; (s) quinine; (t) DABCO. Conversions and ee values were determined by GC. Positive ee values denote an excess of the (*R*)-enantiomer whereas negative ee values denote an excess of the (*S*)-enantiomer.

4.4 The Use of Microreactor for Polymeric Ti-salen-Catalysed Cyanations of Benzaldehyde[IV]

As discussed in Chapter 3, the use of a solid-supported catalyst offers several advantages. Therefore, heterogeneous catalysts have been used in combination with microreactor technologies. The microreactors used for this purpose usually consist of a filter chamber that keeps the solid-supported catalyst in place. There are also examples where the catalyst has been immobilised in a membrane. The reactants are then passed through the catalyst for the reaction to take place. In contrast to batch reactions, the catalyst is always in excess over the reactants.

We employed a microreactor for the cyanation of benzaldehyde catalysed by the polymeric Ti-salen catalyst **30** contained in the filter chamber (Figure 14). The microreactor had a total volume of 70 μL and the reaction solution was transported through the catalyst by applying a pressure driven flow.

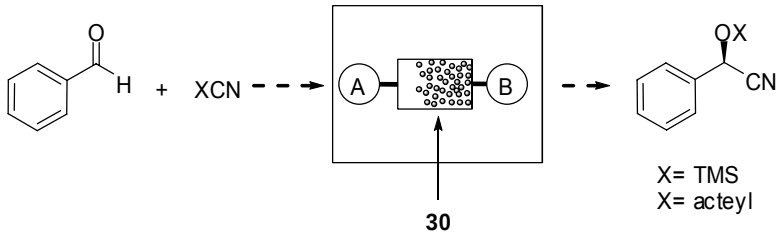


Figure 14. A schematic view of the microreactor with a filter containing the polymeric Ti-salen catalyst **30**. The system catalyses cyanation of benzaldehyde.

Initially, we studied the addition of TMSCN to benzaldehyde (Table 7). We discovered that the conversion increased with decreased flow rate. This can be explained by the longer contact time between the catalyst and the reactants at lower flow rates. At a flow of 0.8 μL/min, a conversion of 92% was obtained (entry 4). The selectivity was essentially constant to variations in flow rate and the product was formed with 70-72% ee. The reproducibility was studied by comparing the reaction outcome of two runs using the same flow rate (entry 2 and 3).

Table 7. Cyanation of benzaldehyde by TMSCN catalysed by **30** using the microreactor.[a]

entry	flow (μL/min)	% conv.[b]	% ee[c]	abs. conf.[d]
1	2	32	70	*S*
2	1	79	72	*S*
3	1	82	71	*S*
4	0.8	92	72	*S*

[a]Reaction conditions: 1.2 equiv. TMSCN, 20-25 °C. Determined by GC-MS.[c]Determined by chiral GC. [d]Assigned by comparing the sign of optical rotation with literature data, see references in paper IV.

We also studied the addition of acetyl cyanide to benzaldehyde catalysed by the polymeric Ti-salen complex and Et_3N (Table 8). A reaction solution of acetyl cyanide, benzaldehyde and Et_3N in dichloromethane was prepared and flowed through the microreactor. In this case, the enantioselectivity varied with the flow rate. Higher ee values were obtained at lower flow rates. This is probably due to the unselective background reaction catalysed by Et_3N that occurs in the absence of the chiral Lewis acid.

Table 8. Cyanation of benzaldehyde by acetyl cyanide catalysed by **30** and Et_3N using the microreactor.[a]

entry	flow (μL/min)	% conv.[b]	% ee[c]	abs. conf.[d]
1	1	35	32	*S*
2	0.8	27	40	*S*
3	0.8	29	39	*S*
4	0.6	25	69	*S*

[a]Reaction conditions: 2 mol% Et_3N, 2 equiv. acetyl cyanide, 20-25 °C. [b]Determined by GC-MS. [c]Determined by chiral GC. [d]Assigned by comparing the sign of optical rotation with literature data, see references in paper IV.

4.5 Conclusion

In this chapter we have shown that it is possible to use flow-through microreactors for enantioselective addition of cyanide to aldehydes, employing homogenous and heterogonous metal complexes. Pressure driven flow as well as electroosmotic flow can be used to transport relatively large metal complexes within a microfluidic device. Finally, we have demonstrated that microreactors can be used for optimization studies in asymmetric catalysis.

5. High Throughput Analysis

5.1 Background

The development of high throughput optimization techniques in asymmetric catalysis allows for many reactions to be carried out in a short time. As a result, it becomes important to be able to analyse large numbers of samples both fast and accurately. Today, the most commonly used techniques are chiral chromographic methods, such as GC and HPLC, for analysis of the reaction outcome. These methods are time-consuming, since they require serial analysis and pre-treatment of the reaction mixtures. Consequently, only a restricted number of samples can be analysed per day. Therefore, several high throughput methods for analysis of the outcome of asymmetric reactions have been developed.[64] Unfortunately, none of them is general for all types of substrates. Methods for parallel analysis, which only require a small amount of substrate, are particularly useful. Many of the methods described are based on the use of microtiter plates, which allows for parallel analysis of 96 or more samples.

In general, mass spectrometry is not an appropriate method for analysis of ee since the two enantiomers have identical masses. However, by employing mass-tagged or isotopically labelled pseudo-enantiomeric mixtures or pseudo-prochiral compounds, it is possible to study asymmetric transformations with mass spectrometry.[65] The use of an automated sampler for microtiter plates combined with an MS system allows for analysis of up to 1000 samples per day.

Mioskowski and co-workers proved that an immunoassay could be used for analysis of the reaction outcome.[66] An enantiospecific antibody was used for ee determination of chiral α-ketoesters. Moreover, the total yield could be determined using an antibody, which binds to both enantiomers.

In 2001, Feringa and co-workers used doped liquid crystals as colour indicators.[67] The ee could be determined both visually, by inspection of the colour, or spectrometrically by measuring the maximum reflected wavelength. An advantage of this method is that microgram quantities of a substrate can be accurately analysed.

Techniques utilizing fluorescent sensors capable of chiral recognition have proven to offer several advantages.[68] The high sensitivity enables analysis of small amounts of substrates. By employing optical fibers, fluorescent sensors can be used for on-line monitoring of reactions.

5.2 Enzymatic Method for Determination of ee

Enzymatic methods for determination of ee (EMDee) have been used in some cases. Such methods use selective enzymes in order to differentiate between the two enantiomers. The IR thermographic method, developed by Reetz and co-workers, belongs to this category of high throughput analytical methods.[69] They determined the selectivity of kinetic resolution of epoxides by using a selective lipase for the acetylation of one of the product enantiomers. The amount of the resulting ester was determined by IR thermography.

The term EMDee was used for the first time in 2001 by Seto and co-workers.[70] They employed an (*S*)-selective alcohol dehydrogenase for oxidation of chiral secondary alcohols. The ee was determined by measuring the rate of the formation of NADPH. An enzyme with opposite selectivity was used for determination of the amount of the other enantiomer. In this way it was possible to distinguish between reactions with low enantioselectivity but high conversion and those with high enantioselectivity but low conversion.

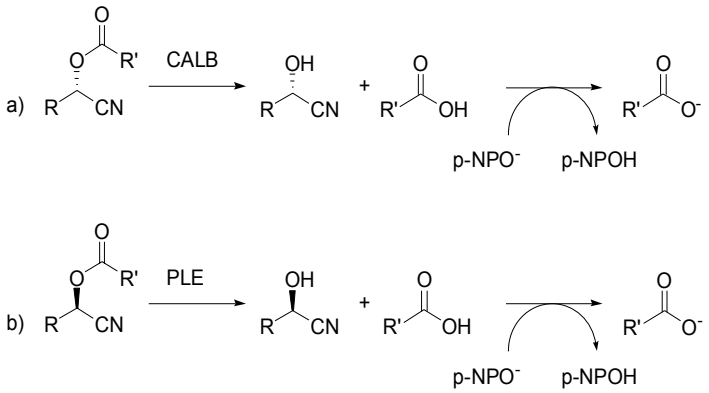
Later Seto and co-workers used a pH indicator for ee determination of chiral esters.[71] One of the enantiomeric esters was hydrolysed by a selective enzyme. The produced acid was titrated with yellow *p*-nitrophenolate to generate uncoloured *p*-nitrophenol. By studying the rate of hydrolysis, recorded spectrometrically, the enantiomeric excess was accessible.

Li and co-workers employed two different alcohol dehydrogenases with opposite specificity for the oxidation of chiral alcohols.[72] This method accessed both ee and conversion. In a similar manner, Berkowitz and co-workers used two dehydrogenases for *in situ* determination of enantioselectivity and relative rates in hydrolytic kinetic resolutions of racemic propylene oxide.[73]

Recently, Seto and co-workers developed a method for ee determination of chiral sulfoxides.[74] The ee was obtained by measuring the inhibition of alcohol dehydrogenase-catalysed oxidation of ethanol.

5.3 Analysis Using a pH Indicator[VII]

The aim of our research was to develop a method for high throughput analysis of the reaction outcome in enantioselective additions of acyl cyanides to aldehydes. We decided to use one selective enzyme, CALB, for the hydrolysis of the (*S*)-acylated cyanohydrin followed by hydrolysis of the remaining (*R*)-enantiomer with pig liver esterase (PLE). Initially, we used a pH-indicator, *p*-nitrophenolate, for the analysis of the amount of acid produced in the two separate hydrolysis steps (Scheme 18). The ee was obtained by recording the colour change spectrometrically in the two steps.



Scheme 18. pH indicator for analysis of a) (*S*)-O-acylated cyanohydrin, and b) (*R*)-O-acylated cyanohydrin.

This method worked satisfactorily, when using cuvettes, for the analysis of the cyanohydrin originating from benzaldehyde and acetyl cyanide. It provided a linear relationship of the ee obtained from GC analysis and the ee obtained by the enzymatic method (Figure 15). However, attempts to conduct the enzymatic analysis on a microtiter plate using a UV plate reader resulted in low reproducibility. A possible explanation could be that the weak buffer, needed to give measurable changes in absorbance, made the system sensitive towards contaminations.

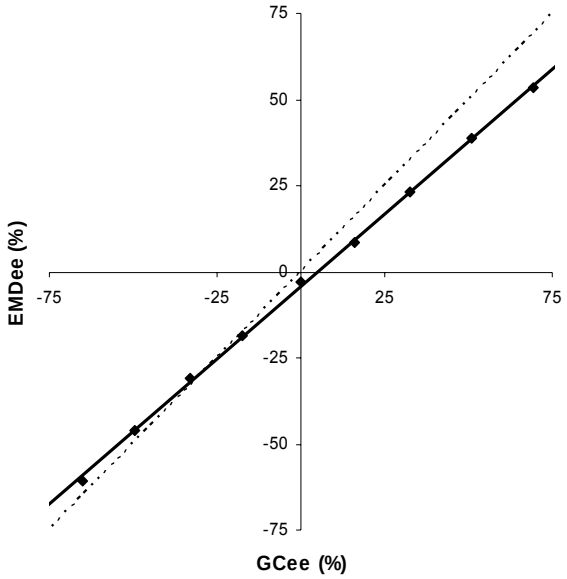
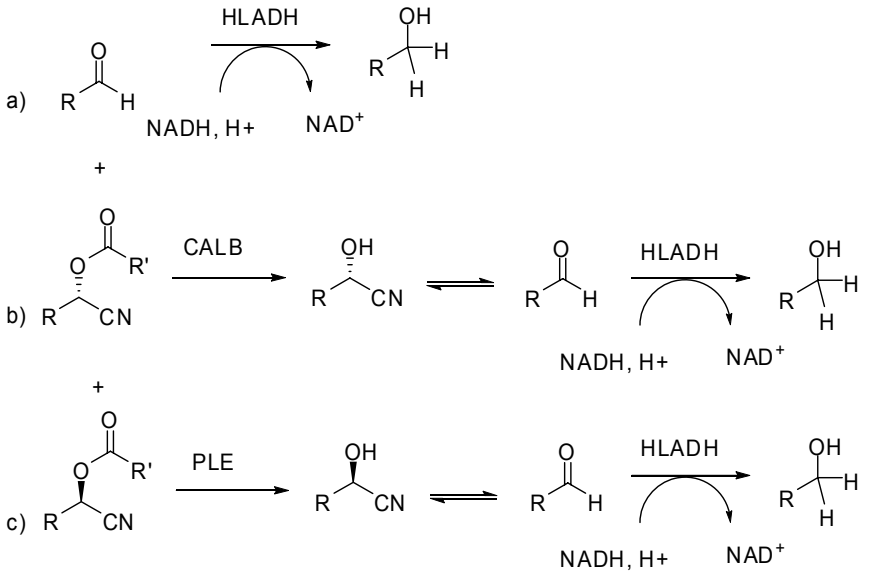


Figure 15. Example of enzymatic determination of enantiomeric excess plotted as a function of the corresponding values determined by GC. Positive ee values correspond to an excess of the (*R*)-enantiomer whereas negative values denote an excess of the (*S*)-enantiomer. The dotted line corresponds to the ideal case when EMDee equals GCee.

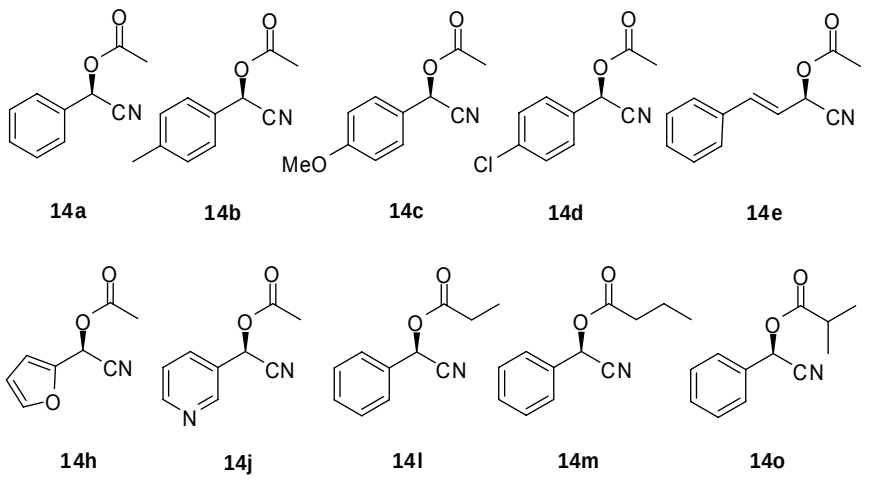
5.4 Analysis Using NADH[VI,VII]

In order to overcome the problems associated with the ee determination using a pH-indicator, we developed a three-step screening method based on the quantification of aldehyde using NADH and horse liver alcohol dehydrogenase (HLADH) (Scheme 19). NADH, which absorbs at 340 nm, was consumed in the reduction of aldehyde, producing non-absorbing NAD^+. The amount of unreacted aldehyde was analysed by reducing it to the corresponding alcohol and thereby measuring the consumed amount of NADH. Hydrolysis of the (*S*)-acylated cyanohydrin using CALB afforded the free cyanohydrin, which was in equilibrium with the corresponding aldehyde. Consequently the cyanohydrin could be analysed in the same way as the unreacted aldehyde. Finally, hydrolysis using PLE gave the unprotected cyanohydrin of the (*R*)-enantiomer, which was analysed in the same way as the (*S*)-enantiomer. Since each step gave a drop in absorbance, both enantiomeric excess and conversion could be determined.



Scheme 19. The reaction mixture for step-by-step analysis of a) unreacted aldehyde, b) (*S*)-O-acylated cyanohydrin, and c) (*R*)-O-acylated cyanohydrin.

Totally eighteen different substrates were evaluated. For each of the substrates **14a-14r,** the racemic sample as well as the (*R*)- and (*S*)-enantiomer were used for testing the enzymatic method. For ten of the substrates (**14a-14e**, **14h**, **14j**, **14l-14m** and **14o**), both ee values and conversions could be accurately determined by the enzymatic method. In other words, good correlations of the values determined by GC and the enzymatic method were obtained for these compounds. This is illustrated in Figure 16, where the relationships between the enzymatic and the GC determinations of ee and conversion for compounds **14b, 14c** and **14d** are shown.



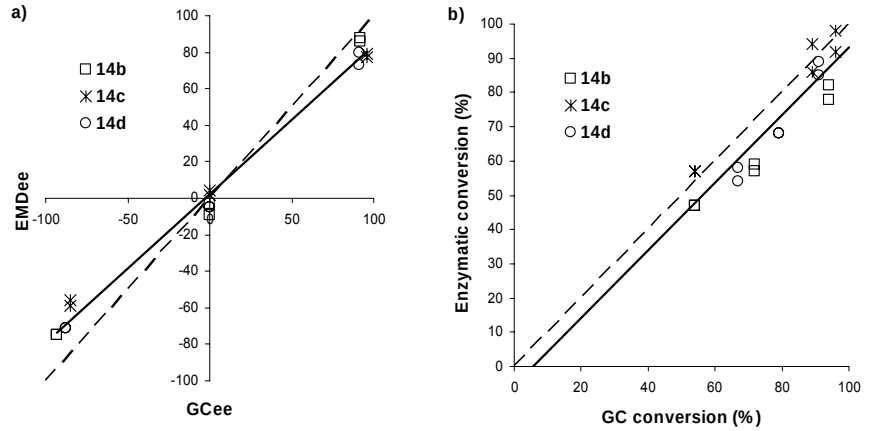
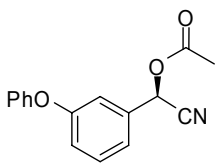
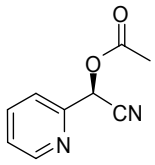


Figure 16. Enzymatic determination of ee (a) and conversion (b) plotted as a function of the corresponding values determined by GC for **14b** (□), **14c** (*) and **14d** (○). Positive ee values correspond to an excess of the (*R*)-enantiomer, negative to an excess of the (*S*)-enantiomer. The continuous lines are linear regressions of the results and the dashed lines correspond to the ideal case when the EMD value equals the GC value.

For substrates **14i** and **14k,** only the conversion could be correctly analysed. These substrates always showed high excess of the (*S*)-enantiomer, probably due to racemization during the analysis.

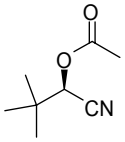


14i

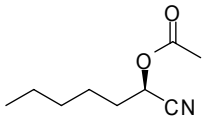


14k

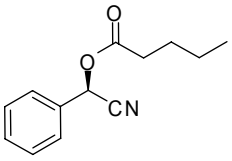
The low rate of the hydrolysis of **14f**, **14g** and **14n** using both CALB and PLE made the analysis of these substrates troublesome. The reduced rate could be due to inhibition of the hydrolysed product or slow formation of the aldehyde due to increased stability of the unprotected cyanohydrin. For substrates **14p**, **14q** and **14r,** no activity of CALB was observed. This was probably caused by the large acyl groups, which do not fit into the active site of the enzyme.



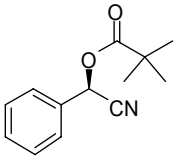
14f



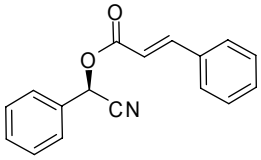
14g



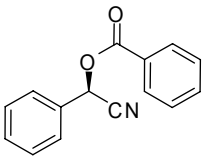
14n



14p



14q



14r

5.5 High Throughput Analysis[VII]

We wanted to study if it was possible to combine the enzymatic method with a microreactor for reaction optimization. Therefore, samples synthesised by employing the microreactor system presented in Figure 12 were subjected to the enzymatic analysis. To demonstrate that this method could be used for high throughput screening, crude reaction samples were analysed enzymatically on microtiter plates. Good correlations between the GC analysis and the enzymatic method were obtained (Figure 17). The ee values could be determined more

accurately for samples with a conversion higher than 50% (standard deviation 5 EMDee % units), compared to samples with a conversion lower than 50% (standard deviation 11 EMDee % units). It was, however, possible to use the enzymatic method for initial screening of enantioselectivity and conversion.

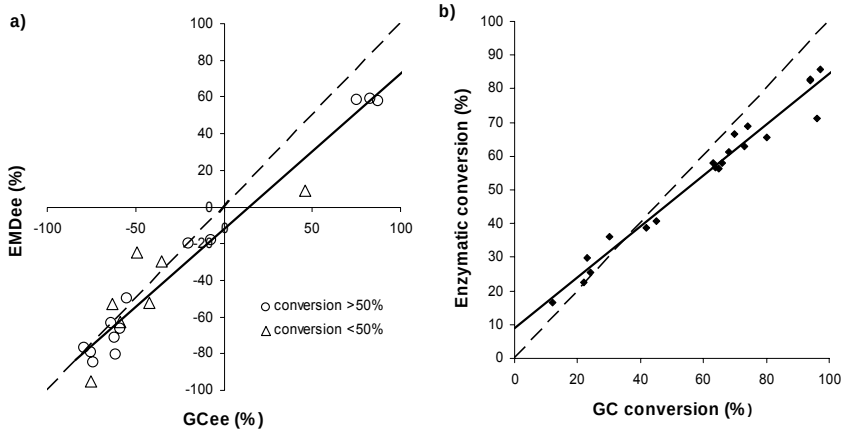


Figure 17. a) Average EMDee plotted as a function of ee determined by GC. Positive ee values correspond to an excess of the (*R*)-enantiomer whereas negative values denote an excess of the (*S*)-enantiomer. b) Average conversions obtained by the enzymatic method plotted as a function of ee determined by GC. The continuous lines are linear regressions of the results and the dashed lines correspond to the ideal case when EMD value equals GC value.

5.6 Conclusion

This chapter described the development of a high throughput method for determination of the conversions and the enantiomeric excesses of O-acylated cyanohydrins. Since the method used the relative end-points measured from one single sample, the method is insensitive to variations in sample concentration and volume. Moreover, the method proved to be stable towards alterations of the synthesis parameters such as different Lewis bases and catalysts used. To sum up, the enzymatic method in combination with a microreactor can be used for high throughput screening of reaction parameters.

6. Concluding Remarks and Outlook

This work concerns efficient synthesis and reaction optimization in asymmetric catalysis, in general, and enantioselective cyanations of aldehydes, in particular. A dual activation system for efficient synthesis of highly enriched cyanohydrins has been described. By employing this catalytic system, both cyanoformate and ketonitriles could be enantioselectively added to a variety of aliphatic and aromatic aldehydes. A relevant extension of this project would be to use other electrophiles, such as imines, epoxides, and ketones. It would also be interesting to take advantage of this system for the syntheses of pesticides, pharmaceuticals, and their lead compounds.

Two types of recyclable polymer-supported catalysts were prepared and employed in enantioselective additions of cyanide to benzaldehyde. There is still a need to find more general methods for the preparation of heterogeneous enantioselective catalysts, which are applicable to a broad range of transformations.

In this thesis microreactors have been employed for high throughput optimization of reaction parameters in enantioselective cyanide addition to aldehydes. Heterogeneous as well as homogeneous asymmetric catalysis have been successfully conducted in microreactors. A future challenge is to combine several different transformations in order to achieve a multi-step synthesis within a microfluidic device.

A high throughput enzymatic method for determination of both conversion and ee, applied to the additions of ketonitriles to aldehydes, has been described. Our method could only be used for analysis of acylated cyanohydrins, and in common with other high throughput analyses, it is not applicable to all types of substrates. In a general perspective, additional developments in this area could therefore be expected.

We demonstrated that a microreactor can be combined with the high throughput enzymatic analysis. A long term goal is to design a fully integrated microfluidic device for synthesis, separation, analysis, and screening of biological activity.

7. Acknowledgements

Writing a thesis is hard, finishing it is even harder. There are several people who have, in one way or another, made this thesis possible.

First of all, I would like to express gratitude to my supervisor, Christina Moberg. Your enthusiasm and energy have made my doctoral studies inspiring and stimulating. Thank you for helping me find my paths and giving me directions on the way.

Secondly, I am grateful for the financial support from the Swedish Foundation for Strategic Reasearch (SSF). The Aulin-Erdtman Foundation, the Lars Gunnar Silléns Foundation, the Knut and Alice Wallenberg Foundation, and KTH are acknowledged for making it possible for me to present this work at several conferences.

Ulla Jacobsson, Peter Somfai, Daniel Linder, Erica Wingstrand, and Anders Frölander are gratefully acknowledged for proof reading and giving valuable comments on the thesis. I am also grateful to Henry Challis, Lena Skowron and Ann Ekqvist for help with practical matters.

I also would like thank all the former and present colleagues at Organic Chemistry. I am particularly grateful to the members of the Ki group for fruitful discussions and invaluable help. Special thanks to Oscar Belda and Fredrik Rahm for guidance in the early days. I also would like to thank Erica Wingstrand for a great collaboration and help with research projects and Anders Frölander for being an excellent travelling companion and friend.

I also want to thank my co-authors: Christina Moberg, S. J. Haswell, Karl Hult, Sergey Lutsenko, Christina Jönsson, Anders Hamberg, Erica Wingstrand, and Maël Penhoat for productive collaborations.

On a private side, let me thank my family, Daniel's family and my friends. Their encouragements have been important for my personal and academic development. Finally, I would like to thank Daniel for all the support and love you always give me.

8. References

[1] a) A. M. Rouhi, *Chem. Eng. News* **2003**, *81*, 56-61; b) I. Agranat, H. Caner, J. Caldwell, *Nat. Rev. Drug Discov*. **2002**, *1*, 753-767; c) G. T. Tucker, *Lancet* **2000**, *355*, 1085-1087.

[2] A. M. Rouhi, *Chem. Eng. News* **2004**, *82*, 47-62.

[3] A. M. Thayer, *Chem. Eng. News* **2006**, *84*, 29-31.

[4] G.-Q. Lin, Y. –M. Li, A. S. C. Chan, Principles and Applications of Asymmetric Synthesis, Wiley, New York, 2001. 49-53.

[5] a) F. F. Fleming, *Nat. Prod. Rep.* **1999**, *16*, 597-606; b) C. J. Peterson, R. Tsao, J. R. Coats, *Pest Manag. Sci.* **2000**, *56*, 615-617; c) D.-S. Park, J. R. Coats, *J. Pestic. Sci.* **2005**, *30*, 99-102.

[6] J. E. Poulton, *Plant Physiol.* **1990**, *94*, 401-405.

[7] D.T. Mowry, *Chem. Rev.* **1948**, *42*, 189-283.

[8] a) J. M. Brunel, I. P. Holmes, *Angew. Chem. Int. Ed.* **2004**, *43*, 2752-2778; b) M. North, *Tetrahedron: Asymmetry* **2003**, *14*, 147-176; c) R. J. H. Gregory, *Chem. Rev.* **1999**, *99*, 3649-3682; d) Jacobsen, E. N.; Pfaltz, A.; Yamamoto, H. *Comprehensive Asymmetric Catalysis*; Springer: Berlin, 1999; e) F. Effenberger, *Angew. Chem. Int. Ed. Engl*. **1994**, *33*, 1555-1564.

[9] L. Rosenthaler, *Biochem. Z*. **1908**, *14*, 238-253.

[10] a) A. Mori, H. Nitta, M. Kudo, S. Inoue, *Tetrahedon Lett.* **1991**, *32*, 4333-4336; b) H. Nitta, D. Yu, M. Kudo, A. Mori, S. Inoue, *J. Am. Chem. Soc*. **1992**, *114*, 7969-7975.

[11] M. Hayashi, T. Inoue, Y. Miyamoto, N. Oguni, *Tetrahedron* **1994**, *50*, 4385-4398.

[12] a) P. G. Cozzi, *Chem. Soc. Rev.* **2004**, *33*, 410-421; b) T. Katsuki, *Chem. Soc. Rev.* **2004**, *33*, 437-444; c) T. P. Yoon, E. N. Jacobsen, *Science* **2003**, *299*, 1691-1693.

[13] a) W. Pan, X. Feng, L. Gong, W. Hu, Z. Li, A. Mi, Y. Jiang, *Synlett* **1996**, 337-338; b) Y. Jiang, L. Gong, X. Feng, W. Hu, W. Pan, Z. Li, A. Mi, *Tetrahedron* **1997**, *53*, 14327-14338.

[14] Y. Belokon', N. Ikonnikov, M. Moscalenko, M. North, S. Orlova, V. Taravov, L. Yashkina, *Tetrahedron: Asymmetry* **1996**, *7*, 851-855.

[15] Y. N. Belokon', S. Caveda-Cepas, B. Green, N. S. Ikonnikov, V. N. Khrustalev, V. S. Larichev, M. A. Moscalenko, M. North, C. Orizu, V. I. Tararov, M. Tasinazzo, G. I. Timofeeva, L. V. Yashkina. *J. Am. Chem. Soc*. **1999**, *121*, 3968-3973.

[16] G. Desimoni, G. Faita, P. Quadrelli, *Chem. Rev.* **2003**, *103*, 3119-3154.

[17] H. Nishiyama, H. Sakaguchi; T. Nakamura, M. Horihata, M. Kondo, K. Itoh, *Organometallics* **1989**, *8*, 846-848.

[18] I. Iovel, Y, Popelis, M. Fleisher, E. Lukevics, E. *Tetrahedron: Asymmetry* **1997**, *8*, 1279-1285.

[19] a) H. C. Aspinall, N. Greeves, P. M. Smith, *Tetrahedron Lett.* **1999**, *40*, 1763-1766; b) H. C. Aspinall, J. F. Bickley, N. Greeves, R. V. Kelly, P. M. Smith, *Organometallics.* **2005**, *24*, 3458-3467.
[20] a) M. Kanai, N. Kato, E. Ichikawa, M. Shibasaki, *Synlett* **2005**, 1491-1508; b) J.-A. Ma, D. Cahard, *Angew. Chem. Int. Ed.* **2004**, *34*, 4566-4583; c) H. Gröger, *Chem Eur. J.* **2001**, *7*, 5246-5251.
[21] H. Steinhagen, G. Helmchen, *Angew. Chem. Int. Ed. Engl*. **1996**, *35*, 2339-2342.
[22] E. J. Corey, Z. Wang, *Tetrahedron Lett.* **1993**, *34*, 4001-4004.
[23] Y. Hamashima, D. Sawada, M. Kanai, M. Shibasaki, *J. Am. Chem. Soc*. **1999** *121*, 2641-2642.
[24] J. Casas, C. Nájera, J. M. Sansano, J. M. Saá, *Org. Lett*. **2002**, *4*, 2589-2592.
[25] a) Hamashima, Y.; Kanai, M.; Shibasaki, M. *J. Am. Chem. Soc*. **2000**, *122*, 7412- 7413; b) Yabu, K.; Masumoto, S.; Yamasaki, S.; Hamashima, Y.; Kanai, M.; Du, W.; Curran, D. P.; Shibasaki, M. *J. Am. Chem. Soc*. **2001**, *123*, 9908-9909.
[26] Y. Shen, X. Feng, G. Zhang, Y. Jiang, *Synlett* **2002**, 1353-1355.
[27] F. Chen, X. Feng, B. Qin, G. Zhang, Y. Jiang, *Org. Lett.* **2003**, *5*, 949-952.
[28] M. Breuer, K. Ditrich, T. Habicher, B. Hauer, M. Kesseler, R. Stuermer, T. Zelinski, *Angew. Chem. Int. Ed.* **2004,** 43, 788-824.
[29] S.-K. Tian, L. Deng, *J. Am. Chem. Soc.* **2001**, *123*, 6195-6196.
[30] A. Baeza, J. Casas, C. Najéra, J. M. Sansano, *J. Org. Chem*. **2006**, *71*, 3837-3848.
[31] a) J. Tian, N. Yamagiwa, S. Matsunaga, M. Shibasaki, *Angew. Chem. Int Ed.* **2002**, *41*, 3636-3638; b) J. Tian, N. Yamagiwa, S. Matsunaga, M. Shibasaki, *Org. Lett.* **2003**, *5*, 3021-3024; c) N. Yamagiwa, J. Tian, S. Matsunaga, M. Shibasaki, *J. Am. Chem. Soc*. **2005**, *127*, 3413-3422.
[32] J. Casas, A. Baeza, J. M. Sansano, C. Nájera, J. M. Saá, *Tetrahedron: Asymmetry* **2003**, *14*, 197-200.
[33] Y. N. Belokon', A. J. Blacker, L. A. Clutterbuck, M. North, *Org. Lett*. **2003**, *5*, 4505-4507.
[34] M. North, A. W. Parkins, A. N. Shariff, *Tetrahedron Lett*. **2004**, *45*, 7625-7627.
[35] H. Abe, H. Nitta, A. Mori, S. Inoue, *Chem. Lett.* **1992**, 2443-2446
[36] L. Veum, S. R. M. Pereira, J. C. van der Waal, U. Hanefeld, *Eur. J. Org. Chem.* **2006**, 1664-1671.
[37] a) H. Huang, J. E. Stok, D. W. Stoutamire, S. J. Gee, B. D. Hammock, *Chem. Res. Toxicol*. **2005**, *18*, 516-527; b) G. Shan, B. D. Hammock, *Anal. Biochem*. **2001**, *299*, 54-62; c) C. E. Wheelock, Å. M. Wheelock, R. Zhang, J. E. Stok, C. Morisseau, S. E. Le Valley, C. E. Green, B. D. Hammock, *Anal. Biochem*. **2003**, *315*, 208-222; d) C. J. Peterson, R. Tsao, A. L. Eggler, J. R. Coats, *Molecules* **2000**, *5*, 648-654; e) M. Beckmann, K.-J. Haack, *Chem. Unsere Zeit* **2003**, *37*, 88-97.
[38] a) Y. N. Belokon, A. V. Gutnov, M. A. Moskalenko, L. V. Yashkina, D. E. Lesovoy, N. S. Ikonnikov, V. S. Larichev, M. North, *Chem. Commun*. **2002**, 244-245; b) Y. N. Belokon, P. Carta, A. V. Gutnov, V. Maleev, M. A. Moskalenko, L.

V. Yashkina, N. S. Ikonnikov, N. V. Voskoboev, V. N. Khrustalev, M. North, *Helv. Chim. Acta* **2002**, *85*, 3301-3312.
[39] a) L. Veum, L. T. Kanerva, P. J. Halling, T. Maschmeyer, U. Hanefeld, *Adv. Synth. Catal.* **2005**, *347*, 1015-1021; b) L. Veum, U. Hanefeld, *Synlett* **2005**, 2382-2384.
[40] Y. N. Belokon', A. J. Blacker, P. Carta, L. A. Clutterbuck, M. North, *Tetrahedron* **2004**, *60*, 10433-10447.
[41] H. M. R. Hoffmann, Z. M. Ismail, R. Hollweg, A. R. Zein, *Bull. Chem. Soc. Jpn*. **1990**, *63*, 1807-1810.
[42] W. Zhang, M. Shi, *Org. Biomol. Chem*. **2006**, *4*, 1671-1674.
[43] a) A. Baeza, C. Nájera, J. M. Sansano, J. M. Saá, *Tetrahedron: Asymmetry* **2005**, *16*, 2385-2389; b) A. Baeza, J. Casas, C. Nájera, J. M. Sansano, J. M. Saá, *Eur. J. Org. Chem* **2006**, 1949-1958.
[44] a) M. Heitbaum, F. Glorius, I. Escher, *Angew. Chem. Int. Ed.* **2006**, *45*, 4732-4762; b) S. Bräse, F. Lauterwasser, R. E. Ziegert, *Adv. Synth. Catal.* **2003**, *345*, 869-929; c) Q.-H. Fan, Y.-M. Li, A. S. C. Chan, *Chem. Rev.* **2002**, *102*, 3385-3466.
[45] a) C. A. Christensen, M. Meldal, *Chem. Eur. J.* **2005**, *11*, 4121-4131; b) C. R. Landis, T. P. Clark, *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 5428-5432.
[46] A. Cornejo, J. M. Fraile, J. I. García, E. García-Verdugo, M. J. Gil, G. Legaretta, S. V. Luis, V. Martínez-Merino, J. A. Mayoral, *Org. Lett.* **2002**, *4*, 3927-3930.
[47] A. Cornejo, J. M. Fraile, J. I. García, M. J. Gil, V. Martínez-Merino, J. A. Mayoral, *Molecular Diversity* **2003**, *6*, 93-105.
[48] A. Cornejo, J. M. Fraile, J. I. García, M. J. Gil, S. V. Luis, V. Martinez-Merino, J. A. Mayoral, *J. Org. Chem.* **2005**, *70*, 5536-5544.
[49] A. Cornejo, J. M. Fraile, J. I. García, M. J. Gil, S. V. Luis, V. Martínez-Merino, J. A. Mayoral, *C. R. Chimie* **2004**, *7*, 161-167.
[50] A. Cornejo, V. Martinez-Merino, M. J. Gil, C. Valerio, C. Pinel, *Chem. Lett.* **2006**, *35*, 44-45.
[51] A. Weissberg, B. Halak, M. Portnoy, *J. Org. Chem.* **2005**, *70*, 4556-4559.
[52] E. Negishi, L. Anastasia. *Chem. Rev.* **2003**, *103*, 1979-2017.
[53] C. Baleizão, H. Garcia, *Chem. Rev.* **2006**, *106*, 3987-4043.
[54] D. A. Annis, E. N. Jacobsen, *J. Am. Chem. Soc*. **1999**, *121*, 4147-4154.
[55] T. S. Reger, K. D. Janda, *J. Am. Chem. Soc*. **2000**, *122*, 6929-6934.
[56] a) C. Haber, *Lab Chip* **2006**, *6*, 1118-1121; b) A. M. Thayer, *Chem. Eng. News* **2005**, *83*, 43-52.
[57] a) P. Watts, C. Wiles, *Org. Biomol. Chem.* **2007**, *5*, 727-732; b) B. Ahmed-Omer, J. C. Brandt T. Wirth, *Org. Biomol. Chem.* **2007**, *5*, 733-740; c) P. Watts, C. Wiles, *Chem. Commun.* **2007**, 443-467; d) M. Brivio, W. Verboom, D. N. Reinhoudt, *Lab Chip* **2006**, *6*, 329-344; e) P. Watts, S. J. Haswell, *Chem. Soc. Rev.* **2005**, *34*, 235-246; f) F. J. Keil, *Chem. Eng. Sci*. **2004**, *59*, 5473-5478; g) P. D. I. Fletcher, S. J. Haswell, E. Pombo-Villar, B. H. Warrington, P. Watts, S. Y. F. Wong, X. Zhang, *Tetrahedron* **2002**, *58*, 4735-4757; h) S. H. Dewitt, *Curr. Opin. Chem. Biol*. **1999**, 350-356.

[58] a) P. Watts, *QSAR Comb. Sci*. **2005**, 701-711; b) K. Geyer, J. D. C. Codée, P. H. Seeberger, *Chem. Eur. J.* **2006**, 12, 8434-8442.
[59] C. Wiles, P. Watts, S. J. Haswell, E. Pombo-Villar, *Lab Chip* **2004**, *4*, 171-173.
[60] D. de Bellefon, T. Lamouille, N. Pestre, F. Bornette, H. Pennemann, F. Neumann, V. Hessel, *Catalysis Today* **2005**, *110*, 179-187.
[61] S. Matsuoka, A. Hibara, M. Ueno, T. Kitamori, *Lab Chip* **2006**, *6*, 1236-1238.
[62] T. Ooi, M. Kameda, K. Maruoka, *J. Am. Chem. Soc.* **2003**, *125*, 5139-5151.
[63] a) G. M.Greenway, S. J. Haswell, D. O. Morgan, V. Skelton. P. Styring, P. *Sens. Actuators B* **2000**, *63*, 153-158; b) P. D. Christensen, S. W. P. Johnson, T. McCreedy, V. Skelton, N. G. Wilson, *Anal. Commun.* **1998**, *35*, 341-343.
[64] a) T. J. Edkins, D. R. Bobbitt, *Anal. Chem.* **2001**, *73*, 488A-496A; b) M.G. Finn, *Chirality* **2002**, *14*, 534-X; c) M. Reetz, *Angew. Chem. Int. Ed.* **2002**, *41*, 1335-1338; d) M. Tsukamoto, H. B. Kagan, *Adv. Synth. Catal*. **2002**, *344*, 453-463.
[65] a) M. T. Reetz, M. H. Becker, H.-W. Klein, D. Stöckigt, *Angew. Chem. Int. Ed.* **1999**, *38*, 1758-1761; b) J. Guo, J. Wu, G. Siuzdak, M. G. Finn, *Angew. Chem. Int. Ed.* **1999**, *38*, 1755-1758.
[66] F. Taran, C. Gauchet, B. Mohar, S. Meunier, A. Valleix, P. Y. Renard, C. Créminon, J. Grassi, A. Wagner, C. Mioskowski, *Angew. Chem. Int. Ed.* **2002**, *41*, 124-127.
[67] a) R. A. van Delden, B. L. Feringa, *Angew. Chem. Int. Ed.* **2001**, *40*, 3198-3200; b) R. Eelkema, R. A. van Delden, B. L. Feringa, *Angew. Chem. Int. Ed.* **2004**, *43*, 5013-5016.
[68] a) G. T. Copeland, S. J. Miller, *J. Am. Chem. Soc.* **1999**, *121*, 4306-4307; b) G. Klein, J.-L. Reymond, *Helv. Chim. Acta* **1999**, *82*, 400-407; c) G. A. Korbel, G. Lalic, M. D. Shair, *J. Am. Chem. Soc.* **2001**, *123*, 361-362; d) M. Matsushita, K. Yoshida, N. Yamamoto, P. Wirsching, R. A. Lerner, K. D. Janda, *Angew. Chem. Int. Ed.* **2003**, *42*, 5984-5987; d) L. Pu, *Chem Rev.* **2004**, *104*, 1687-1716 ; e) R. Corradini, C. Paganuzzi, R. Marchelli, S. Pagliari, S. Sforza, A. Dossena, G. Galaverna, A. Duchateau, *J. Mater. Chem.* **2005**, *15*, 2741-2746; f) C. D. Tran, D. Oliveira, *Anal. Biochem.* **2006**, *356*, 51-58.
[69] M. T. Reetz, A. Zonta, K. Schimossek, K.-E. Jaeger, K. Liebeton, *Angew. Chem. Int. Ed*. **1997**, *36*, 2830-2832.
[70] P. Abato, C. T. Seto, *J. Am. Chem. Soc*. **2001**, *123*, 9206-9207.
[71] M. B. Onaran, C. T. Seto, *J. Org. Chem*. **2003**, *68*, 8136-8141.
[72] Z. Li, L. Bütikofer, B. Witholt, *Angew. Chem. Int. Ed*. **2004**, *43*, 1698-1702.
[73] S. Dey, K. R. Karukurichi, W. Shen, D. B. Berkowitz, *J. Am. Chem. Soc*. **2005**, *127*, 8610-8611.
[74] C. M. Sprout, C. T. Seto, *Org. Lett.* **2005**, *7*, 5099-5102.