these enzymes cleave at the hydrophilic head-group region (phosphate ester) of DMPC. The significance of the detection system described is that it both mimics the natural membrane interface, and also provides a visual reporting component (the conjugated polymer) for the rapid detection of biocatalysis.

The simple, one-step detection method for enzymatic catalysis and inhibition allows convenient adaptation to the high-throughput screening of catalytic inhibitors. In addition, this method may be applied to detect deadly neurotoxins that have enzyme-like activities (for example, $\beta$-bungarotoxin). Future efforts are geared towards the study of factors that affect enzyme recognition and activity, parameters that influence reorganization of the conjugated polymer membrane, and adaptation of the colorimetric method to other enzyme systems.

### *Experimental Section*

Figure 2a: The polymerized vesicles composed of 40% DMPC/60% PDA, 1mM total lipid, were diluted 1:10 in 50mM Tris buffer (pH 7.0) to a final volume of 0.5 mL in a standard cuvette, and the spectra were recorded with a Hewlett Packard Spectrophotometer (model 9153C). Bee venom phospholipase $A_2$ (Sigma) was dissolved in a buffer (pH 8.9) of 10mM Tris, 150mM NaCl, and 5mM $CaCl_2$ to yield a final concentration of 1.4 mg mL$^{-1}$. 50 μL of this solution was added to the cuvette and the spectrum recorded after 60 min.

Figure 2b: 5 μL of the 1.4 mg mL$^{-1}$ solution of $PLA_2$ was added to 50 μL of DMPC/DPA vesicles (0.1mM final total lipid concentration). The experiment was carried out in a standard 96-well plate with a $UV_{max}$ kinetic microplate reader (Molecular Devices). The absorption of the vesicle solution was monitored as a function of time at 620 and 490 nm. The data was then plotted as colorimetric response (CR) versus time to yield the color response curves. Colorimetric response is defined here as the percentage change in the absorption at 620 nm relative to the total absorption maxima.[6]

Figure 2c: 5 μL of 40% DTPC/PDA vesicles diluted with 45 μL of 50mM Tris pH 7.0 and 5 μL of 6mM DTNB were incubated with 10 μL of 1.4 mg mL$^{-1}$ $PLA_2$. The absorbance at 412 nm was monitored over time.

Figure 4b: MJ33 was added to 5 μL of unpolymerized 40% DMPC/PDA vesicles (0.015 mol ratio of MJ33 in the substrate interface) in 40 μL of 50mM Tris (pH 7.0) and 5 μL of a solution of 50mM Tris, 150mM NaCl, and 5mM $CaCl_2$ (pH 8.9). The mixture was incubated at room temperature for 20 min and polymerized prior to measuring the absorption at 490 and 620 nm. 5 μL of a 1.4 mg mL$^{-1}$ solution of $PLA_2$ was added and the colorimetric response recorded as above. For $Zn^{2+}$ inhibition the enzyme was dissolved in 10mM Tris, 150mM NaCl, and 0.1mM $ZnCl_2$ at pH 8.9.

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## Practically Perfect Asymmetric Autocatalysis with (2-Alkynyl-5-pyrimidyl)alkanols

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Organic synthesis plays a central role in natural and technical sciences, and the development of organic reactions that proceed with perfect chemo- and stereoselectivity is an important goal for organic chemists.[1] Reactions that are catalyzed by enzymes in living organisms proceed with extremely high chemo- and stereoselectivities. Enzymes are, however, macromolecules that consist of thousands of amino

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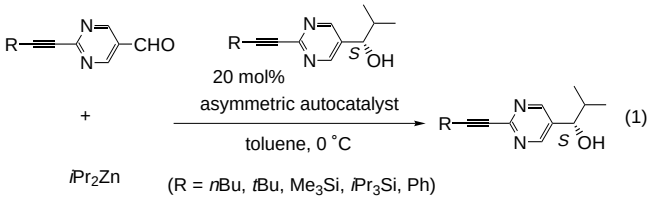
acids. It is a challenging problem in asymmetric synthesis to design a chiral catalyst that exhibits extremely high enantioselectivity.[2] Although highly enantioselective reactions (>90% *ee*) are known these days, it is much more difficult to develop an enantioselective reaction that gives greater than 99.5% *ee*, even in non-autocatalytic asymmetric synthesis. In general, it is difficult to achieve a reaction with an extremely high *ee* value (>99.5%) because the difference in Gibbs free energy ($\Delta\Delta G^{\ddagger}$) between the transition states that give the *R* and *S* isomers kinetically increases exponentially with the increase in enantioselectivity.[3] We have studied asymmetric autocatalysis,[4, 5] where the catalyst and product have the same structure and configuration.

We previously reported the first highly enantioselective autocatalytic reaction,[5b] but even this reaction did not achieve perfect enantioselectivity: when a pyrimidylalkanol with a high *ee* value was used as an asymmetric autocatalyst, the *ee* value of the resulting pyrimidylalkanol remained at up to 98.2% and the chemical yield was no greater that 80%, as a result of the formation of by-products and the recovery of unreacted aldehyde. We report here an unprecedented practically perfect asymmetric autocatalytic reaction that gives extremely high enantioselectivity (>99.5% *ee*) and almost quantitative chemical yield (>99%).

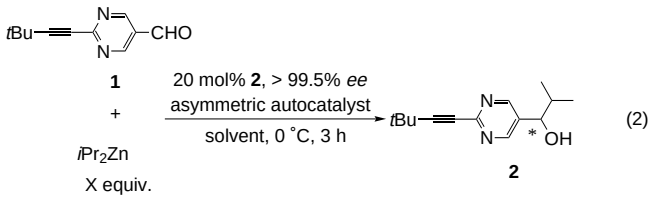
First, to find a better asymmetric autocatalyst, several 5-pyrimidylalkanols that possessed an alkynyl group at their 2-positions were used as asymmetric autocatalysts in the enantioselective alkylation with diisopropylzinc (2.2 equiv) [Eq. (1)]. Differences in selectivity were clarified by the use




(1)

of (2-alkynylpyrimidyl)alkanols with a low *ee* value. An alkanol, including the catalyst, with 21.2% *ee* was obtained from the use of a pyrimidylalkanol with an *n*-butyl group on the alkyne with 5.8% *ee*. The introduction of a *tert*-butyl or trimethylsilyl group was much more effective (5.5 →69.6% *ee* for a *tert*-butyl and 8.4 →74.2% *ee* for a trimethylsilyl group). On the other hand the introduction of a more bulky triisopropylsilyl group reduced the catalytic activity (8.6 → 8.8% *ee*). A phenyl group was also effective (5.9 → 47.3% *ee*), but less than a *tert*-butyl group. These results imply that a moderate electron-withdrawing effect that arises from the alkynyl group and the appropriate bulkiness of the alkyne are indispensable for a practically perfect asymmetric autocatalyst. Thus, 1-(2-*tert*-butylethynyl-5-pyrimidyl)-2-methyl-1-propanol was found to be a very efficient asymmetric autocatalyst.

The enantioselective isopropylation of 2-(*tert*-butylethynyl)pyrimidine-5-carbaldehyde (**1**) with diisopropylzinc (in toluene) and the enantiomerically pure (>99.5% *ee*) (*S*)-pyrimidylalkanol (*S*)-**2** as an asymmetric autocatalyst was performed in toluene [Eq. (2)]. This reaction resulted in the formation of (*S*)-pyrimidylalkanol (*S*)-**2** in 98% yield and




(2)

with 99.1% *ee* (Table 1, entry 1). When cumene was used as the solvent instead of toluene the *ee* value increased to 99.3% (entry 2). The *ee* value reached in excess of 99.5% when a cumene solution of diisopropylzinc was used (entry 4). The use of 1.7 equivalents of diisopropylzinc gave (*S*)-**2** with a

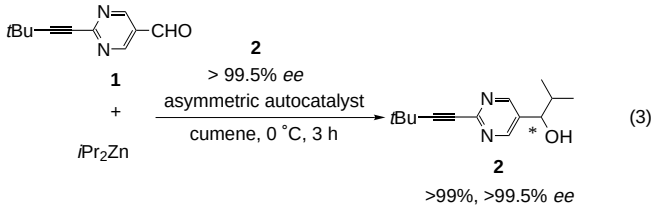
Table 1. Asymmetric autocatalytic reaction as shown in Equation (2) with (*S*)- and (*R*)-**2** with >99.5% *ee*.

| Entry | X | Solvent | Asym. autocat. and product *ee* [%] | Newly formed product yield [%] | Newly formed product *ee* [%] |
|---|---|---|---|---|---|
| 1 | 2.2 | toluene[a] | 99.3 (*S*) | 98 | 99.1 (*S*) |
| 2 | 2.2 | cumene[a] | 99.4 (*S*) | 98 | 99.3 (*S*) |
| 3 | 2.2 | *tert*-butylbenzene[a] | 99.3 (*S*) | 99 | 99.1 (*S*) |
| 4 | 2.2 | cumene[b] | >99.5 (*S*) | 99 | >99.5 (*S*) |
| 5 | 1.7 | cumene[b] | >99.5 (*S*) | >99 | >99.5 (*S*) |
| 6 | 1.7 | cumene[b] | >99.5 (*R*) | >99 | >99.5 (*R*) |

[a] With 1M $iPr_2Zn$ in toluene. [b] With 1M $iPr_2Zn$ in cumene.

greater than 99.5% *ee* and induced a further increase in yield (>99%; entry 5). (*R*)-Pyrimidylalkanol ((*R*)-**2**), with the opposite configuration, is also an extremely efficient asymmetric autocatalyst (entry 6). We utltimately achieved a practically perfect asymmetric autocatalytic reaction in terms of either configurations (>99.5% *ee*, >99% yield).

Under the best reaction conditions (Table 1, entry 5), the reaction was performed successively, with the products of one round serving as the reactants for the next entry (Table 2). Even after ten rounds, all the asymmetric autocatalytic reactions proceeded perfectly (>99%, >99.5% *ee*). In our reaction, the factor by which the amount of (*S*)-**2** multiplied relative to the amount of (*S*)-**2** initially used as an asymmetric




(3)

autocatalyst (entry 1) was approximately $10^3$ in five rounds (entry 5) and approximately $10^7$ in ten rounds (entry 10), with no deterioration of the catalyst. Thus, in the present asymmetric autocatalytic reaction, the factor by which a chiral molecule multiplies is practically unlimited.

In this asymmetric autocatalytic reaction, only one product (catalyst) is obtained. Therefore, if this product can be converted into important chiral synthetic intermediates, the significance of this scheme is surely enhanced. In fact, we previously reported that chiral 5-pyrimidylalkanols can be transformed into $\alpha$-hydroxycarboxylic acid derivatives without racemization.[6]

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Table 2. Consecutive asymmetric autocatalytic reaction[a] shown in Equation (3). The compounds **2a**–**k** only distinguish in which round the catalyst is used.

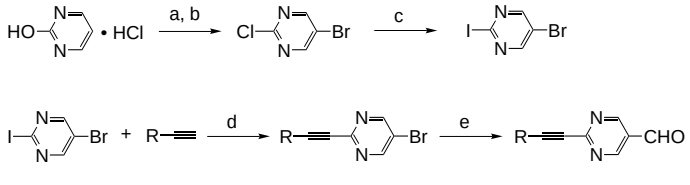
| Entry | Asym. Autocat. *ee* [%] | Product yield [%] | Product *ee* [%] | Amplified factor[b] |
|---|---|---|---|---|
| 1 | > 99.5 (**2a**) | > 99 | > 99.5 (**2b**) | 6 |
| 2 | > 99.5 (**2b**) | > 99 | > 99.5 (**2c**) | $6^2$ |
| 3 | > 99.5 (**2c**) | > 99 | > 99.5 (**2d**) | $6^3$ |
| 4 | > 99.5 (**2d**) | > 99 | > 99.5 (**2e**) | $6^4$ |
| 5 | > 99.5 (**2e**) | > 99 | > 99.5 (**2f**) | $6^5 \approx 8 \times 10^3$ |
| 6 | > 99.5 (**2f**) | > 99 | > 99.5 (**2g**) | $6^6$ |
| 7 | > 99.5 (**2g**) | > 99 | > 99.5 (**2h**) | $6^7$ |
| 8 | > 99.5 (**2h**) | > 99 | > 99.5 (**2i**) | $6^8$ |
| 9 | > 99.5 (**2j**) | > 99 | > 99.5 (**2k**) | $6^9$ |
| 10 | > 99.5 (**2k**) | > 99 | > 99.5 (**2l**) | $6^{10} \approx 6 \times 10^7$ |

[a] Molar ratio **1**:*i*$Pr_2$Zn (in cumene):catalyst **2** = 1.0:1.7:0.2. [b] The factor by which the amount of **2** has multiplied based on the amount of **2** used as an asymmetric autocatalyst in entry 1.

### *Experimental Section*

Entry 5, Table 1: A solution of **1** (94.2 mg, 0.50 mmol) in cumene (5.0 mL) was added at 0 °C to a mixture of (*S*)-**2** (23.3 mg, 0.10 mmol, > 99.5 % *ee*) in cumene (12.0 mL) and *i*$Pr_2$Zn (0.85 mL of a 1 M solution in cumene, 0.85 mmol) that had been stirred for 15 min at 0 °C. The reaction mixture was stirred for 3 h at 0 °C, then quenched by the addition of 1 M hydrochloric acid (3 mL) and saturated aqueous $NaHCO_3$ (9 mL) at 0 °C. The mixture was filtered through celite and the filtrate was extracted with ethyl acetate (4 × 15 mL). The extract was dried over anhydrous sodium sulfate and evaporated under reduced pressure. Cumene was removed by flash column chromatography ($SiO_2$, hexane, then hexane/ethyl acetate, 3/1) to give pure **2** (138.8 mg). HPLC analysis of the obtained **2** on a column with a chiral stationary phase (Daicel Chiralcel OD, eluent 3 % 2-propanol in hexane, flow rate 1.0 mL min$^{-1}$, 254 nm UV detector, retention time 18.1 min for (*S*)-**2**, 26.9 min for (*R*)-**2**) showed that it had an enantiomeric purity of > 99.5 % *ee*. The newly formed (*S*)-alcohol (138.8 − 23.3 = 115.5 mg, 99.2 % yield) had an enantiomeric purity of > 99.5 % *ee*.

Preparation of 2-alkynylpyrimidine-5-carbaldehydes (Scheme 1): Commercially available 2-hydroxypyrimidine hydrochloride was halogenated to form 5-bromo-2-chloropyrimidine by the improved procedure.[7] Halogen exchange occurred at the 2-position by the reaction with hydroiodic acid to give 5-bromo-2-iodopyrimidine, which was then coupled with alkynes to give 2-alkynyl-5-bromopyrimidines in 80–99 %.[8] Lithiation of the bromides by *n*- or *tert*-butyllithium and the subsequent formylation[9] by ethyl formate gave the 2-alkynylpyrimidine-5-carbaldehydes in 25–60 %.




Scheme 1. Synthesis of 2-alkynylpyrimidine-5-carbaldehydes. a) $Br_2$, $H_2O$; b) $POCl_3$, $PhNMe_2$, 55 % over two steps; c) 57 % HI, $CH_2Cl_2$, 93 %; d) 1–2 mol % [$Pd(PPh_3)_4$], 2–4 mol % CuI, *i*$Pr_2$NH, 80–99 %; e) *n*BuLi or *t*BuLi then $HCO_2Et$, THF or $Et_2O$, 25–60 %.

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## A Functionalized Heterocubane with Extensive Intermolecular Hydrogen Bonds**

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Hans-Georg Schmidt, and Mathias Noltemeyer

*Dedicated to Professor Alan H. Cowley on the occasion of his 65th birthday*

The use of inorganic cage compounds as molecular building blocks for the rational design of materials is an attractive and challenging avenue for the materials chemist. One example is the silicate cage compounds $(RSiO_{1.5})_n$ (R = organic or inorganic group), which are potentially a very useful class of compounds.[1–6] They have been used as three-dimensional building block units for the synthesis of new materials, such as precursors for ceramics and models in various fields.[1, 3, 4] In addition, an exciting structural organization of the cubic silicate species $[Si_8O_{20}]^{8-}$ was achieved recently with various

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