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Chiral polyamines from reduction of polypeptides: asymmetric pyridoxamine-mediated transaminations

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Abstract—BH_3·THF can reduce polypeptides to polyamines with retention of chirality. The resulting polyamines are intriguing general platforms for asymmetric catalysis, given the diverse structures available and their relative ease of synthesis. We have constructed a number of chiral pyridoxamine catalysts based on reduced peptides. These compounds transaminate α-ketoacids with moderate to good enantioselectivity, while their peptidyl counterparts show almost no chiral induction.

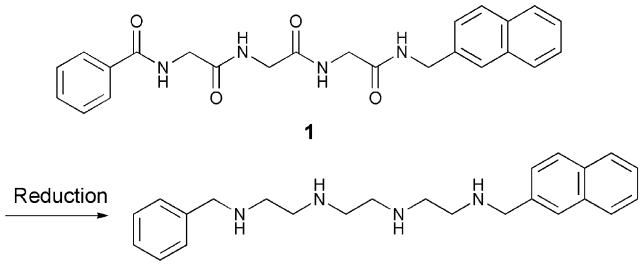
We have reported the use of polyethylenimines as catalysts in transaminations by pyridoxamine derivatives.[1] The ethylenediamine units have attractive properties as acid/base catalysts, but the polymers are of course achiral. If polypeptides could be reduced to polyamines without chain cleavage and with retention of the amino acid chirality, this would be an attractive approach to chiral catalysts.

In particular, the amine and ammonium catalytic components of the polymer would be in a chiral environment. They could promote asymmetric proton transfers, and also serve as metal ion ligands and as nucleophiles. In contrast with the polymers made by polymerization of ethylenimine, they should also have well defined structures and lengths, reflecting those of their parent polypeptides. Furthermore, when made from polypeptides, the polyamines should be easy to modify and optimize.

Discovery of low molecular weight chiral catalysts has been a focus of research during the past several decades.[2] This search spawned the development of many interesting compounds that function as asymmetric catalysts.[3] Among these compounds, the peptide-based chiral catalysts are of special interest because (1) they offer intriguing analogies to enzymatic systems where the chiral induction relies heavily on the conformational determinants and functional group arrays, (2) they are easy to synthesize and modify, and (3) their optimization can be accomplished via library screening.[4]

We tried $LiAlH_4$, Red-Al, BH_3·Me_2S, $BH_3/TiCl_4$, and BH_3·THF in the reduction[5] of the tripeptide **1** (Scheme 1), and found that use of BH_3·THF provides the best yield in the reduction.[6] The workup of the BH_3·THF reduction is also the least tedious; methanol completely removes boron as its volatile ester, $B(OCH_3)_3$. The ^1H NMR[7,8] of **2** and **2′** (derived from the dimethylamino amides of L- and D-alanine via the BH_3·THF reduction and Mosher derivatization,[9] Scheme 2) confirmed that stereochemistry was retained with BH_3·THF.

Next, we applied the BH_3·THF reduction method to peptides of various lengths. We found that the yields of the polyamines are 80% ($n = 3$), 75% ($n = 6$), 60% ($n = 9$), and 41% ($n = 12$), respectively (Fig. 1). Combining these observations, it is conceivable that a large



Scheme 1.

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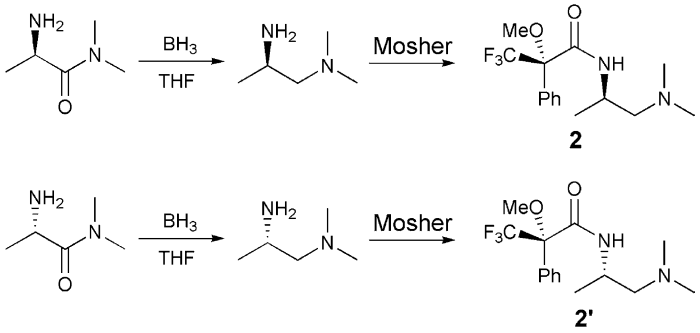


Scheme 2.

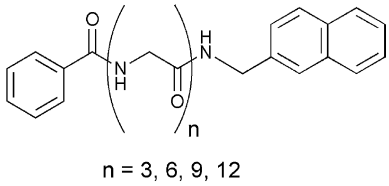
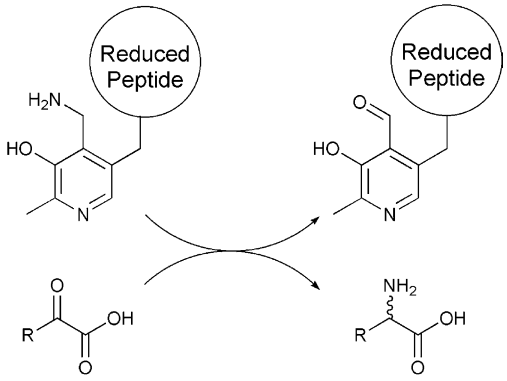


Figure 1. Polyglycine pre-cursors for BH_3·THF reduction.

library of potential asymmetric catalysts can be constructed from chiral polyamines with diverse lengths utilizing the BH_3·THF reduction method. The longest reduced peptide reported previously comprised only two amino acid units.[10]

With a successful synthetic method for reduced peptides in hand, our next target was to construct reduced peptide based catalysts. In our initial efforts, we synthesized the first pyridoxamine catalysts linked to reduced peptides. Imperiali's group had recently studied some peptide-based pyridoxamine catalysts.[11] Thus, we were very interested to compare the peptide based pyridoxamine catalysts with our reduced counterparts (Scheme 3).

Our synthesis (Scheme 4) starts with commercially available pyridoxamine. After Boc protection of the amino group, the benzylic OH group was selectively protected by TBDMS. The phenolic OH group was protected by the MOM group. The benzylic silyl ether was then



Scheme 3. Proposed pyridoxamine–mediated transamination of α-ketoacids to α-amino acids.

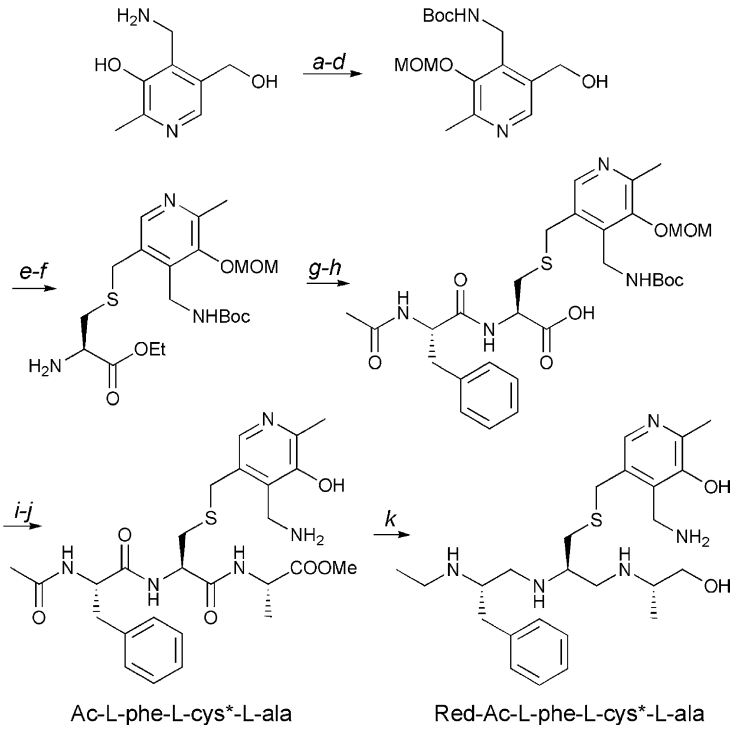
deprotected with TBAF and activated with mesyl chloride. The resulting mesylate was displaced by the thiol group of L-cysteine methyl ester to afford a fully protected pyridoxamine-ligated cysteine (Cys*) in 48% overall yield from pyridoxamine. Then this pyridoxamine-ligated cysteine unit was coupled with an N-protected amino acid (e.g., Ac-L-Phe) at the *N*-terminus, followed by ester hydrolysis and amidation with a C-protected amino acid (e.g., L-Ala-OMe) at the *C*-terminus. Using this procedure we can synthesize various peptides that incorporate one pyridoxamine unit.

An alternative and more efficient route toward the pyridoxamine-ligated peptides is outlined in Scheme 5. The disulfide-linked peptide scaffold is first constructed from either methyl cystinate·2HCl for dipeptides or *N*-Boc protected cystine for tripeptides. Reduction of the disulfide bond with $NaBH_4$, followed by alkylation of the resulting free thiol with 5-(bromomethyl)pyridoxamine[12] afforded the desired pyridoxamine-ligated di- and tripeptides in 55–88% yield over two steps.

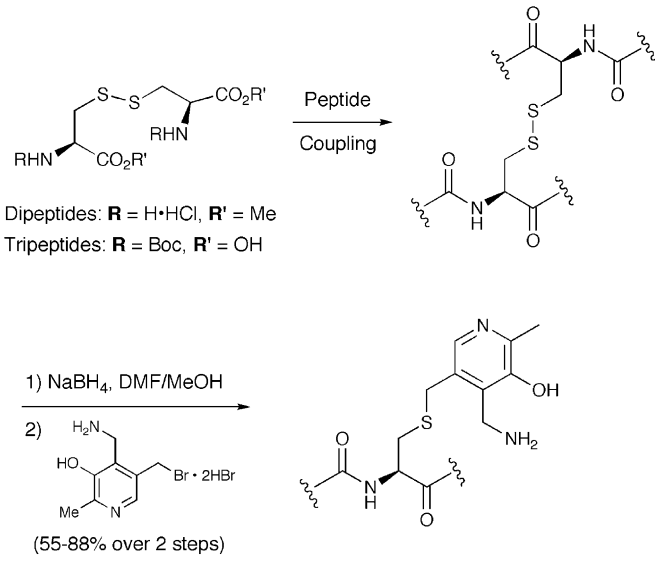
The pyridoxamine-containing peptides were purified by preparative reverse-phase HPLC, and then reduced by the aforementioned BH_3·THF method (Scheme 4). MS and NMR studies of the reduction products revealed that the pyridoxamine moiety was not damaged by the reduction. After another round of reverse-phase HPLC purification, we successfully obtained reduced peptides incorporating pyridoxamine.

While rather large enantiopure oligoamines are attainable via our newly developed procedure, our initial studies focused on learning the properties of relatively small systems. We examined a tripeptide framework, Ac-Phe-Cys*-Ala, and its reduced form, Red-Ac-Phe-Cys*-Ala. The central Cys* indicates the location of the pyridoxamine unit; 'Red' means reduced. In total, five different combinations of stereochemistry were considered: LLL, LLD, DLL, DLD, and DDL.[13,14]

We found that before reduction, all the peptide-based pyridoxamines exhibited very low enantioselectivity (ee < 5%) in the transamination reactions with either phenylpyruvic or pyruvic acid as substrates in water (Table 1). On the other hand, the reduced peptide-based pyridoxamines showed moderate ee values ranging from 5% to 54% in aqueous media. The validity of the



Scheme 4. Reagents and conditions: (a) Boc_2O, Et_3N, 90%; (b) TBDMSCl, Im, 95%; (c) MOMCl, NaH, 75%; (d) TBAF, 86%; (e) MsCl, Et_3N, quantitative; (f) L-Cys-OEt, NaH, 87%; (g) Ac-L-Phe, HATU, *i*-Pr_2NEt, 95%; (h) LiOH, THF, 70%; (i) L-Ala-OMe, HATU, *i*-Pr_2NEt, 82%; (j) TFA, 95%; (k) BH_3·THF, 80%.



Scheme 5. Alternative route toward pyridoxamine-ligated cysteine containing di- and tripeptides.

observed enantioselectivity was confirmed by comparing Red-Ac-Phe-Cys*-D-Ala with the corresponding mirror image, Red-Ac-D-Phe-D-Cys*-Ala. The LLD catalyst afforded L-phenylalanine and L-alanine in 39% and 54% ee, respectively. The enantiomeric DDL catalyst generated the corresponding D-enantiomers of phenylalanine and alanine in 38% and 50% ee, respectively. We have shown that such transaminations can occur with many catalytic turnovers, using sacrificial amino acids,[1b] but in this work we use the pyridoxamine as a reagent. As in other such transaminations, we see generally high chemical yields of the product amino acids.

We found that LLL provided ee values similar to those given by LLD. Meanwhile, DLL provided ee values close to those given by DLD, so the *C*-terminal amino acid unit in the tripeptide does not have much influence on the enantioselectivity of the whole system. Thus we synthesized Red-Ac-Phe-Cys*, a substrate that did not have the *C*-terminal amino acid unit. The ee values for

Table 1. The % ee values in the transamination reactions between the peptide or reduced peptide based pyridoxamine and phenylpyruvic or pyruvic acid in aqueous media[a,b,c]

Catalyst	Phenylpyruvic acid (%)	Pyruvic acid (%)
Red-Me_2-Cys*	9 (L)	6 (D)
Red-Ac-Phe-Cys*-Ala	33 (L)	52 (L)
Red-Ac-Phe-Cys*-D-Ala	39 (L)	54 (L)
Red-Ac-D-Phe-Cys*-Ala	14 (L)	19 (L)
Red-Ac-D-Phe-Cys*-D-Ala	5 (L)	14 (L)
Red-Ac-D-Phe-D-Cys*-Ala	38 (D)	50 (D)
Red-Ac-Phe-Cys*	**37** (L)	**64** (L)
Red-Ac-Ala-Cys*	31 (L)	18 (L)
Red-Ac-Trp-Cys*	40 (L)	52 (L)
Red-Ac-Tyr-Cys*	24 (L)	37 (L)
Red-Bz-Phe-Cys*	37 (L)	39 (L)
Red-*i*-PrCO-Phe-Cys*	31 (L)	45 (L)
Red-*t*-BuCO-Phe-Cys*	15 (L)	35 (L)
Red-$HOCH_2CO$-Phe-Cys*	48 (L)	52 (L)
Ac-Phe-Cys*-Ala	<5	<5
Ac-Phe-Cys*-Ala	<5	<5
Ac-Phe-Cys*-D-Ala	<5	<5
Ac-D-Phe-Cys*-Ala	<5	<5
Ac-D-Phe-Cys*-D-Ala	<5	<5
Ac-D-Phe-D-Cys*-Ala	<5	<5
Ac-Phe-Cys*	<5	<5

[a] Values are means of two or three experiments. Standard deviation is about 1–2%.

[b] Transamination reaction condition: catalyst (ca. 2.5×10^{-3} M), ketoacid (3.8×10^{-2} M), EDTA (7.7×10^{-3} M). pH = 7.5, t = 20 °C.

[c] The amino acid product was derivatized with *o*-phthalaldehyde and N-Boc-cysteine before analysis by reverse-phase HPLC. For more details see Ref. 1e.

this LL catalyst are indeed very close to those provided by LLL and LLD. The dipeptidyl catalyst that was not reduced, that is Ac-Phe-Cys*, showed nearly zero enantioselectivity in the transamination.

By contrast, the *N*-terminal amino acid unit has a crucial role in chiral induction. Change of LLL or LLD to DLL or DLD in the Ac-Phe-Cys*-Ala series decreases the ee values from 39% to 5% for phenylalanine and from 54% to 14% for alanine. Removal of both the *N*- and *C*-terminal residues (i.e., Red-Me_2-Cys*) led to very low and poorly defined enantioselectivity.[15] We therefore synthesized a selection of reduced dipeptidyl catalysts that differ in the *N*-terminal amino acid *N*- and α-C-substituents. Red-Ac-Phe-Cys* achieves better enantiomeric excesses than Red-Ac-Ala-Cys*, Red-Ac-Trp-Cys*, or Red-Ac-Tyr-Cys*. Red-Ac-Phe-Cys* is also better than Red-*t*-BuCO-Phe-Cys*, Red-*i*-PrCO-Phe-Cys*, or Red-$HOCH_2CO$-Phe-Cys*. The highest ee value (64%) in water came from Red-Ac-Phe-Cys* for the transamination of pyruvic acid to L-alanine.

We next studied the transamination between Red-Ac-L-Phe-L-Cys* with other α-ketoacids and in different solvents. Under aqueous conditions, the highest ee values were obtained from pyruvic acid, providing L-alanine in 64% ee. Larger α-ketoacid substituents, especially those containing aromatic systems (e.g., phenylpyruvic acid) negatively influenced the enantioselectivity of the transamination reaction, possibly by disrupting structurally-stabilizing intra-molecular hydrophobic interactions in the oligoamine reagent. Similarly, when the transamination reactions were conducted in methanol, the observed ee values of the corresponding amino acids were significantly lower than those obtained under aqueous conditions. An exception to this phenomenon was observed with β-branched-α-ketoacids; L-valine was obtained in 85% ee from transamination of 3-methyl-2-oxobutanoic acid with Red-Ac-Phe-Cys* in methanol, representing an increase of 35% ee over the aqueous conditions. Similar results were obtained from 3-methyl-2-oxopentanoic acid (data not shown) (Table 2).

Table 2. The % ee values in the transamination reactions between Red-Ac-Phe-Cys* and various α-ketoacids in water and in methanol[a,b]

Ketoacid	H_2O (%)	CH_3OH (%)
Pyruvic acid	64 (L)	18 (L)
Phenylpyruvic acid	38 (L)	24 (L)
3-Methyl-2-oxobutanoic acid	50 (L)	85 (L)
4-Methyl-2-oxopentanoic acid	43 (L)	39 (L)
2-Oxopentanoic acid	45 (L)	24 (L)
α-Ketoglutaric acid	47 (L)	10 (L)
Oxolacetic acid	53 (L)	22 (L)

[a] Values are means of two or three experiments. Standard deviation is about 1–2%.

[b] Transamination reaction condition: catalyst (ca. 2.5×10^{-3} M), ketoacid (3.8×10^{-2} M), EDTA (7.7×10^{-3} M). pH = 7.5, t = 20 °C.

In summary, we have found that BH_3·THF can be utilized to reduce polypeptides to polyamines with retention of chirality. We propose that the resulting polyamines are highly interesting platforms for asymmetric catalysis, since a large diversity of chiral structures can be inexpensively synthesized in parallel.

In our first study, we successfully utilized the reduced polypeptides to construct a number of chiral pyridoxamine derivatives. These transaminate α-ketoacids to the corresponding α-amino acids with moderate enantioselectivity, whereas their peptidyl counterparts show almost no chiral induction.

In subsequent publications we will report other applications of these unique polyamines, with their well defined structures and chiralities. Not only are they chirally defined, they also can carry many useful side-chains related to those of proteins. Thus they have wide potential applications in synthesis and catalysis, and in biology.

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6. Typical procedure: 0.30 g of **1** was dissolved in 10 mL of THF, and 20 mL of BH_3 solution in THF (1 M) was added. The mixture was heated under reflux for 2 days. The solvent was removed and then 30 mL of MeOH was added. The mixture was heated under reflux again for 1 day. After the removal of solvent, the residue was dissolved in 2 mL of CH_2Cl_2. After 2 mL of HBr (33% in AcOH) was added dropwise, the product tetrahydrobromide (0.39 g, yield = 80%) precipitated as a white solid. ^{1}H NMR (D_2O, 300 MHz): 8.10, m, 3H; 7.60, m, 9H; 4.90, s, 2H; 4.35, s, 2H; 3.61, m, 12H. CI MS: 377.4 (tetramine M+1).
7. ^{1}H NMR (D_2O, 300 MHz) for **2**: 8.05, d, 1H, J = 8.1 Hz; 7.60, m, 5H; 4.12, m, 1H; 3.50, s, 3H; 2.39, m, 2H; 2.19, s, 6H; 1.19, d, 3H, J = 6.6 Hz.
8. ^{1}H NMR (D_2O, 300 MHz) for **2′**: 8.10, d, 1H, J = 8.1 Hz; 7.60, m, 5H; 4.12, m, 1H; 3.60, s, 3H; 2.51, m, 2H; 2.28, s, 6H; 1.10, d, 3H, J = 6.6 Hz.
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14. Red-Ac-Phe-Cys*-Ala: ^{1}H NMR (D_2O, 300 MHz): 7.99, s, 1H; 7.25, m, 5H; 4.31, s, 2H; 3.80, s, 3H; 3.65–2.60, 15H; 2.59, s, 3H; 1.31, m, 6H. CI MS: 476.3 (M+1).
15. When Red-Me_2N-Cys* was reacted with 2-oxopentanoic acid in MeOH with $Zn(OAc)_2$, D-norvaline was obtained in 39% ee: Zimmerman, S. C.; Czarnik, A. W.; Breslow, R. *J. Am. Chem. Soc.* **1983**, *105*, 1694–1695.