Beyond L-proline: Investigation of the properties of other natural amino acids in an organocatalytic warfarin synthesis

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Abstract

Proline is indisputably the model organocatalytic amino acid. However, other naturally occurring amino acids remain a potent and perhaps overlooked source of organocatalytic potential. In this work, we investigate the capacity of various natural amino acids to promote enantioselectivity in a synthesis of warfarin. We have identified L- and D-arginine as enantioselective catalysts for this reaction and have developed a recrystallization method to isolate the enantiomers of warfarin with high enantiopurity. In addition, we use methylated derivatives of arginine provide insight into the reaction mechanism.

Keywords

warfarin; organocatalysis; L-arginine; D-arginine; enantioselective

INTRODUCTION

Warfarin is a commonly prescribed anti-coagulant compound that is administered in racemic form. However, the biological activities of (R)- and (S)- warfarin are quite different (S enantiomer is ~5 fold more active than the R enantiomer), which can further complicate warfarin administration regimen for sensitive patients [1,2]. To circumvent these side effects, personalized medicine approaches to warfarin administration have been proposed [3]. Such strategies include the administration of enantiopure warfarins [4]. While the clinical relevance of these approaches is still a matter of debate [3], the enantioselective synthesis of warfarin from simple starting materials remains a useful proving ground for organocatalytic molecules.

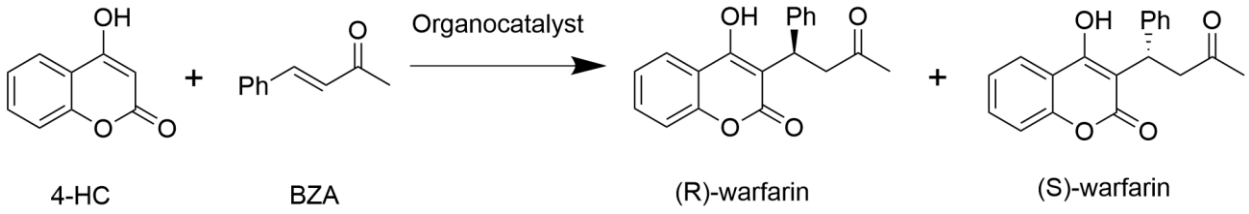
Organocatalytic approaches to the synthesis of warfarin have been known for quite some time [5,6]. Some of these approaches have both high yields and enantioselectivities (Diphenylethylenediamine (DPEN) catalysts, Cinchona alkaloids), while others have high yields but low enantioselectivities (L-proline) [7,8]. Organocatalytic applications of L-proline are widespread throughout the chemical literature, with particular utility in the catalysis of carbon-carbon bond forming reactions such as Aldol additions, Michael additions, Knoevenagel condensation, and Robinson Annulation [9]. The elevated pKa of the cyclic amine and unique cyclic structure of L-proline are typically important contributors to these enamine-based reaction mechanisms [10]. By contrast, while L-proline and L-proline-based molecules are often

an early avenue of exploration for catalysis of new carbon-carbon bond forming reactions [9], the organocatalytic properties of other natural amino acids are frequently found to be inferior to those of L-proline. Seminal work in the organocatalysis field demonstrates that this is often a correct assumption [11]. However, for many organocatalytic reactions the superiority of L-proline versus other natural amino acids is not explicitly demonstrated.

In this work, we investigate the ability of amino acids other than L-proline to catalyze an organocatalytic synthesis of warfarin. While we confirm that L-proline is an effective catalyst for this reaction, we find that it is not uniquely capable of catalyzing this transformation, as all amino acids investigated in this study produced at least moderate yields of warfarin. Furthermore, we discovered that L-arginine is also an effective catalyst of this transformation with an enantioselectivity surpassing that of other natural amino acids. We also find that L- and D- isomers of arginine have opposing enantioselectivities in the production of (*R*)- and (*S*) - warfarin. Although these enantioselectivities do not approach those previously reported for DPEN catalysts [8,12], we demonstrate that a modified recrystallization procedure results in highly enriched samples of (*R*)- and (*S*)- warfarin from L- and D-Arg catalyzed reactions.

RESULTS AND DISCUSSION

We first investigated the ability of various L-amino acids to catalyze the synthesis of warfarin from hydroxycoumarin and benzylidene acetone (Scheme 1) in a DMSO/water (90% DMSO/10% water) mixture. L-Pro has been previously characterized as an effective catalyst for this reaction [8] and was used as a positive control.



Scheme 1. An organocatalytic warfarin synthesis from 4-hydroxycoumarin (4-HC) and benzylideneacetone (BZA). Organocatalysts (0.17 mmol) were added to a reaction of 81 mg (0.5 mmol) of 4-hydroxycoumarin and 88 mg (0.6 mmol) of benzylideneacetone (BZA) with 900 µL DMSO AND 100 µL distilled water. All reactions were incubated in an end-over-end rotator at room temperature for 7 days.

Among the twelve amino acids tested (Table 1), three (L-Trp, L-Arg, L-Pro) produced good yields of warfarin, ranging from 75 – 85%. By contrast, Gly, L-Ala, L-Lys, L-Met, and L-His produced moderate yields of warfarin (35 – 52%), while low yields were achieved with L-Tyr, L-Glu, L-Leu, and L-Ile (10 – 20%). Generally, these yields fall within expectations for an enamine-catalyzed mechanism in which an amine-bearing sidechain with an elevated pKa promotes catalysis (L-Lys, L-His, L-Trp, L-Arg, L-Pro); however, it is somewhat surprising that Gly, which lacks a sidechain, is a modestly competent catalyst for this reaction. Furthermore, L-Met, which contains a sulfur bearing sidechain, also produced moderate yields of warfarin, on par with that of L-Lys and L-His.

Table 1: Yields of L-amino acid catalyzed warfarin reactions[a].

Catalyst	Yield (%)
Uncatalyzed	4
Tyrosine (L-Tyr)	14
Glutamic Acid (L-Glu)	14
Leucine (L-Leu)	18
Isoleucine (L-Ile)	20
Glycine (Gly)	34
Phenylalanine (L-Phe)	35
Lysine (L-Lys)	44
Methionine (L-Met)	47
Histidine (L-His)	52
Tryptophan (L-Trp)	77
Arginine (L-Arg)	78
Proline (L-Pro)	86

[a]Determined by HPLC

We subsequently characterized the enantioselectivities of a subset of these amino acids (Table 2). These amino acid catalysts were selected based on their high warfarin yields or, in the case of Gly, the absence of a sidechain. We also compared these enantioselectivities to R,R- and S,S- DPEN, catalysts previously shown to promote enantioselective synthesis of warfarin [12,13]. All of the L-amino acids favored the production of (*R*)-warfarin, with the exception of Gly, which demonstrated no selectivity. Surprisingly, L-Arg had the highest enantioselectivity among the amino acids investigated, on par with R,R-DPEN. As anticipated, R,R and S,S- DPEN produced opposing selectivities. We note that while higher enantioselectivities are observed with DPEN catalysts under THF/AcOH solvent conditions, solubility limitations of our natural amino acid catalysts prevented a direct comparison under such conditions.

Table 2: Enantiomeric excess values for selected catalysts [a].

Catalyst	ee (%R)
Glycine (Gly)	0
Proline (L-Pro)	+11
Tryptophan (L-Trp)	+23
Lysine (L-Lys)	+30
Histidine (L-His)	+37
Arginine (L-Arg)	+48
R,R-DPEN	+48
S,S-DPEN	-44

[a]Determined by chiral HPLC

Having identified L-Arg as a potent and moderately enantioselective catalyst for warfarin synthesis, we next investigated whether a D-Arg catalyst might impose the opposing stereoselectivity (Figure 1). For this experiment, six teams of investigators set up independent trials of either D- or L-Arg catalyzed warfarin reactions (one reaction per team). The reactions from the L-Arg trials favored the R-enantiomer (37 (+/-9)% ee), while the D-Arg trials favored synthesis of the S-enantiomer of warfarin (-41 (+/-5)%). Interestingly, this trend did not hold true for D- versus L-proline, which both had a slight excess of the R-enantiomer of warfarin in this reaction (L-Pro: 11%; D-Pro: 11%).

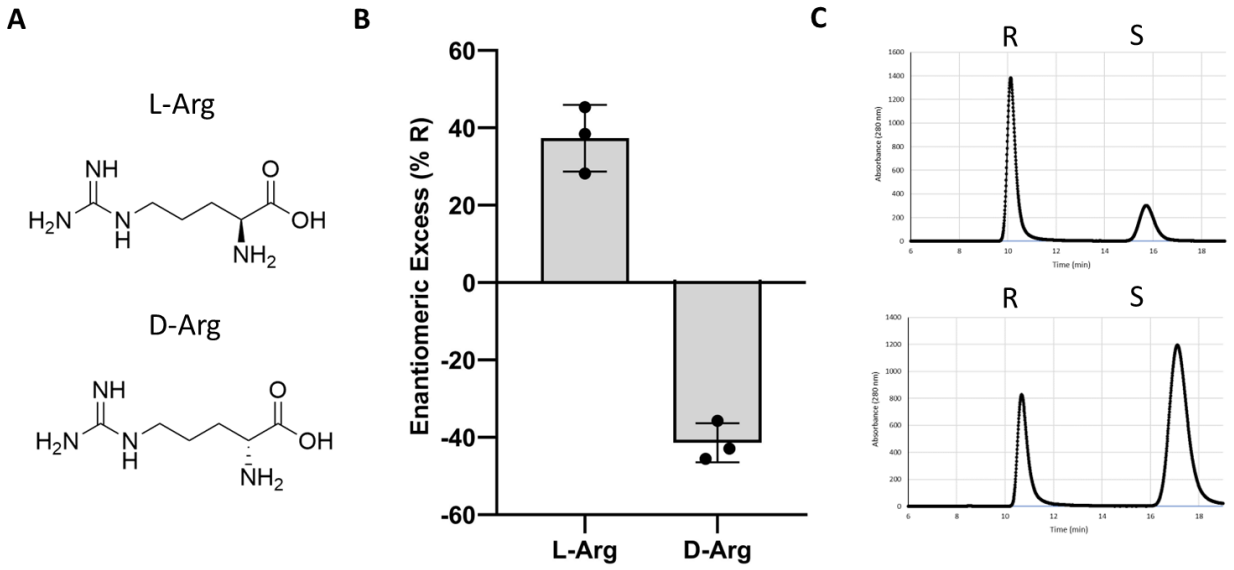


Figure 1. Enantioselectivity of L-arginine versus D-arginine. A. Structures of L- and D-arginine. B. Results of trials of L- and D-arginine catalysts. C. Representative chiral HPLC traces from L-arg (top) and D-arginine (bottom) warfarin reactions.

To provide additional insight into the mechanism of the arginine catalyst, we subsequently investigated the ability of methylated derivatives of L-Arg to catalyze the

warfarin transformation (Table 4 and Figure 2). In comparison to L-Arg, a single-methylated arginine (N^G-Monomethyl-L-arginine; NGMA) produced a good yield of warfarin and demonstrated a slightly elevated preference for the R-enantiomer. By contrast, a double-methylated variant (N^G, N^G-Dimethylarginine**;** ADMA) produced a diminished yield of warfarin yet retained a similar induction of stereoselectivity. This result demonstrates two important features of the L-arginine catalyst: it tolerates sidechain functionalization, which implies that further derivation beyond the methyl group is possible. Such derivatives could significantly enhance stereoselectivity, whereas dual methylation demonstrates that additional functionalization of the guanidine group hampers catalytic efficiency. Interestingly, a methoxy-ester arginine (Methoxy Arginine) derivative had near-background activity. This result indicates that the carboxy portion of the amino acid plays a critical role in catalysis, and strongly implicates Bronsted acid-type catalysis as a component the reaction mechanism [14]. Bronsted acid catalysis would explain the general catalytic effect observed with all amino acids investigated in this study (Table 1).

Table 4: Yields and ee values for arginine derivatives.

Catalyst	Yield (%)	ee (%R)
Uncatalyzed	4	n.d.
L-Arginine	78	+48
NGMA	68	+52
ADMA	36	+41
Methoxy Arginine	7	-2

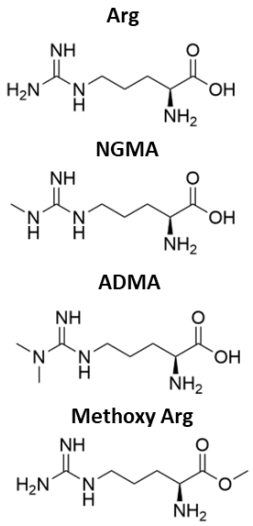


Figure 2. Structures of arginine and methylated derivatives (Arg = L-arginine; NGMA = N^G-Monomethyl-L-arginine; ADMA = N^G, N^G-Dimethylarginine; Methoxy Arg = L-Arg, methoxy ester).

To further enhance the enantiopurity of the mixtures of (*R*)- and (*S*)-warfarin obtained from D- and L-Arg catalyzed reactions, we sought to enrich these enantiomers by recrystallization. However, we found that simple acetone-water recrystallization was an unreliable method for enantiomeric enrichment. Instead, following flash column isolation

of warfarin (hexanes/ethyl acetate) and removal of solvent via rotary evaporation, the resulting yellowish oil was placed under hexanes and allowed to sit for 48 h under reduced pressure. During this interval, the hexane layer evaporated and warfarin began to crystallize. Under these conditions, warfarin crystallizes in a roughly 1:1 ratio of R:S warfarin with the enantiomerically enriched product remaining as a yellow liquid. This enriched yellow liquid was then separated from the crystals by pipette (crystals can also be removed by centrifugation and pipetting away the yellow liquid) and recrystallized using 80% acetone/ 20% water, yielding highly enriched warfarin (Figure 3). For example, from scaled up (7X) L- or D-Arg catalyzed reactions, we obtained 132 mg of (*R*)-Warfarin (12% overall yield) and 173 mg of (*S*)-Warfarin (16% overall yield), both in >99% ee. These overall yields are in line with those obtained from resolution of warfarin enantiomers with quinidine-diastereomer approaches, with fewer recrystallization steps [5].

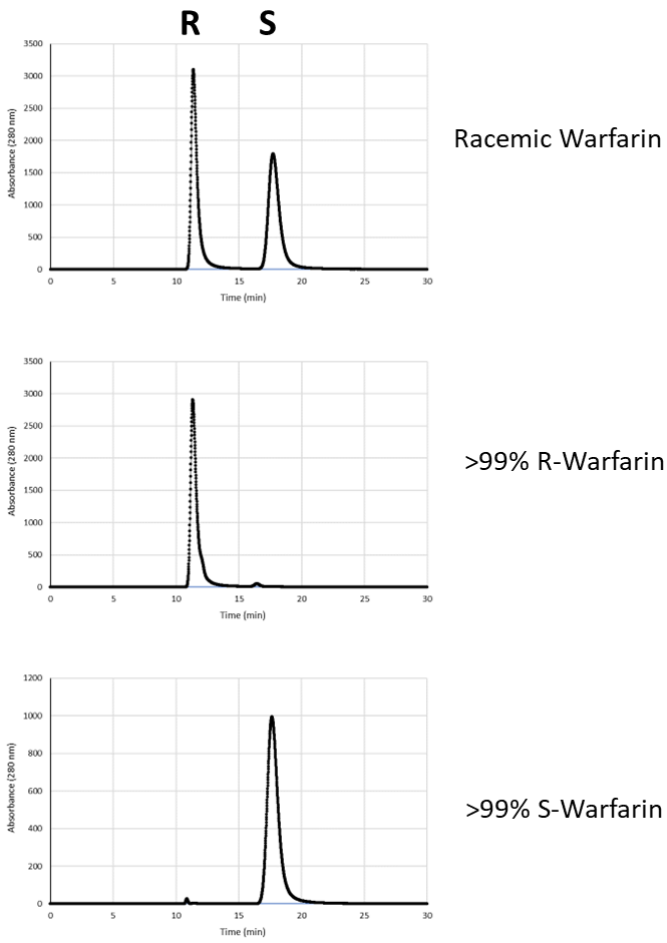


Figure 3. Comparison of chiral HPLC traces of recrystallized products from L- and D-Arg catalyzed reactions (middle and bottom) and commercially available racemic warfarin (top).

CONCLUSION

This work demonstrates the range of yields and enantioselectivities accessible with commercially available amino acids in a mixed solvent-based synthesis of warfarin. Surprisingly, L- and D- arginine have opposing enantioselectivities in this synthesis. While these enantioselectivities may be considered modest by current standards, recrystallization approaches can be used to obtain both (*R*)- and (*S*)- warfarin

enantiomers in excess of 99% purity. Furthermore, analysis of sidechain methylated arginine derivatives suggests that synthetic arginine-based organocatalysts with enhanced enantioselectivities are within reach.

EXPERIMENTAL

General procedure for warfarin synthesis

In 2 mL screw top reaction vials, combine the following: 0.081 g (81 mg) of 4 - hydroxycoumarin, 0.088 g (88 mg) of benzylideneacetone, 0.020 g (20 mg) of L-proline (or other amino acid catalyst), 900 µL DMSO, 100 µL distilled, deionized H2O. After addition of all reactants, secure screw top lids on vials and place reactions on end-over-end rotator for seven days at room temperature.

SUPPORTING INFORMATION

High resolution mass spectrometry, 1H NMR, and FTIR of R- and S-warfarins, experimental and instrumental details, commercial sources of catalysts.

ACKNOWLEDGEMENTS

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Supporting Information

GENERAL EXPERIMENTAL DATA

Warfarin Reactions: Amino acids (0.17 mmol) were added to a reaction of 81 mg (0.5 mmol) of 4-hydroxycoumarin and 88 mg (0.6 mmol) of benzylideneacetone (BZA) with 900 µL DMSO AND 100 µL distilled water. All reactions were incubated in an end-over-end rotator at room temperature for 7 days (Xie, et. al. 2012).

Thin Layer Chromatography (TLC): TLC analysis was performed on each reaction using silica gel plates with 2:1 Hexanes:Ethyl Acetate as the mobile phase. Plates were visualized with UV illumination followed by anisaldehyde staining with heating until purple warfarin spots were visible.

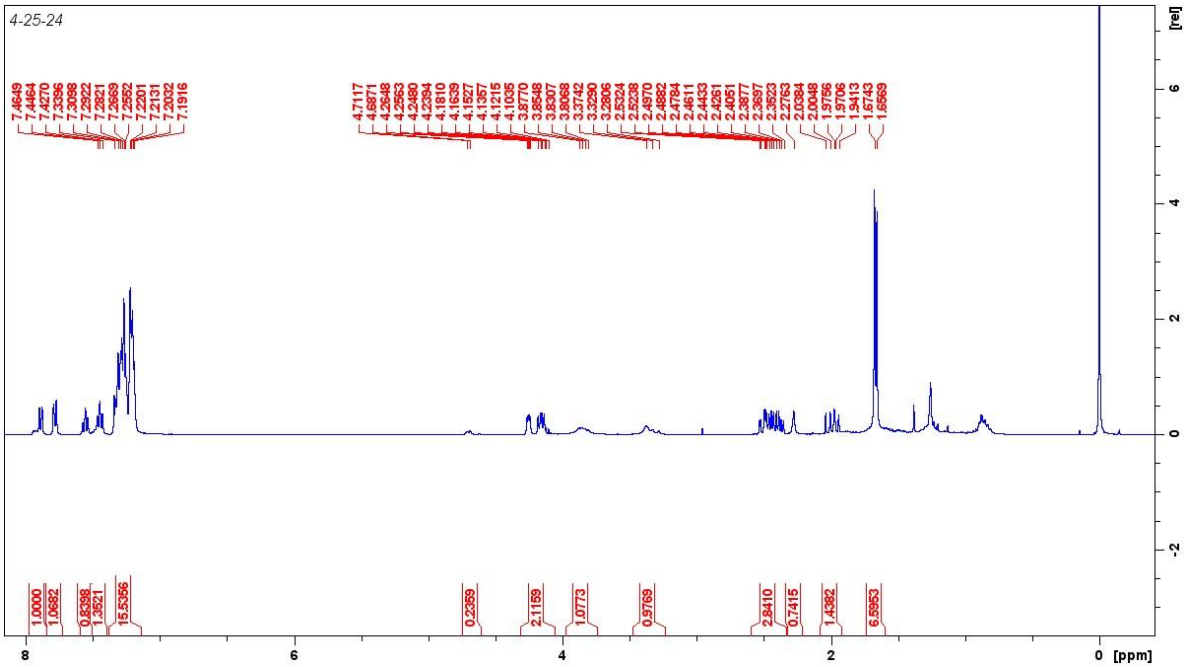
High Performance Liquid Chromatography (HPLC) for reaction yields: Diluted samples (1:200 dilution of crude reaction in ethanol) were analyzed using a Hitachi HPLC equipped with a Zorbax SB-C18, 3.5 µm, 4.6 x 150 mm column; 40% 0.1% Formic acid & 60% methanol mobile phase; 1 mL/min flow rate; 280 nm wavelength UV detector; 20 min run time; and PC/Chrom software. Yields were determined using a standard curve.

Flash Chromatography: Warfarin was isolated using silica gel flash column chromatography with Hexanes:Ethyl acetate mobile phase (4:1, 2:1, 0:1).

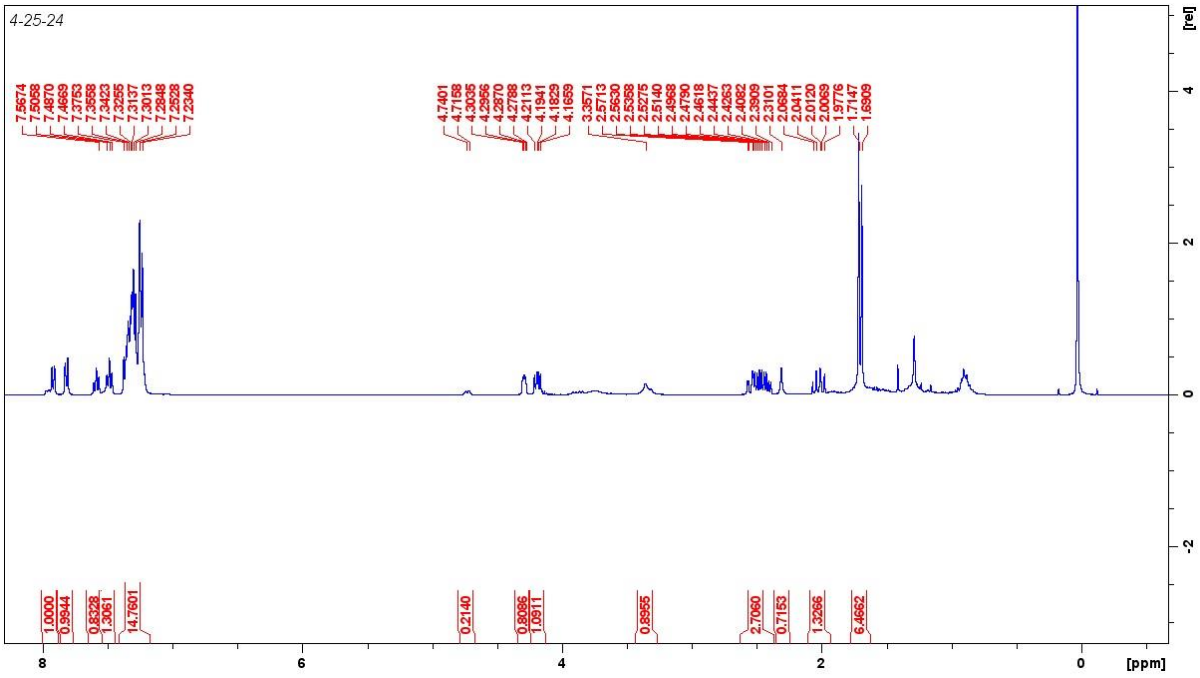
Chiral Chromatography for % enantiomeric excess: Purified sample was analyzed using a Shimadzu HPLC equipped with Chiralcel OD-H chiral column; 50% Heptane, 50% IPA, 0.1% Acetic Acid mobile phase. Samples were monitored by UV/VIS detection at 220 and 280 nm.

ANALYTICAL DATA

R-warfarin (>99% ee, L-Arg catalyzed reaction) Bruker 400 MHz Proton NMR, CDCl3 (TMS)

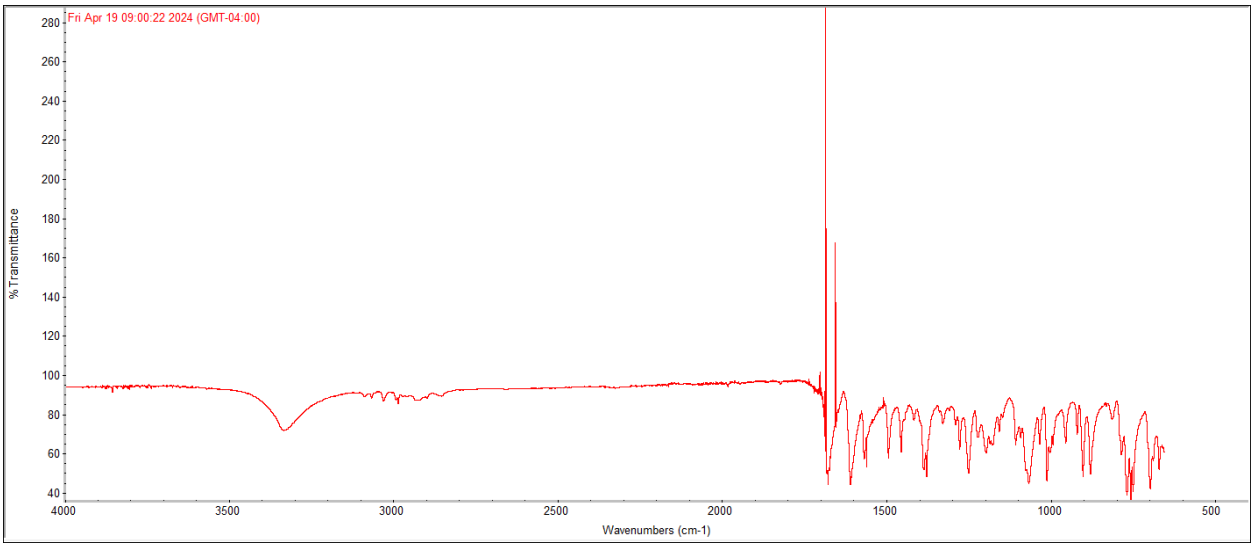


S-warfarin (>99% ee, D-Arg catalyzed reaction) Bruker 400 MHz Proton NMR, CDCl3 (TMS)

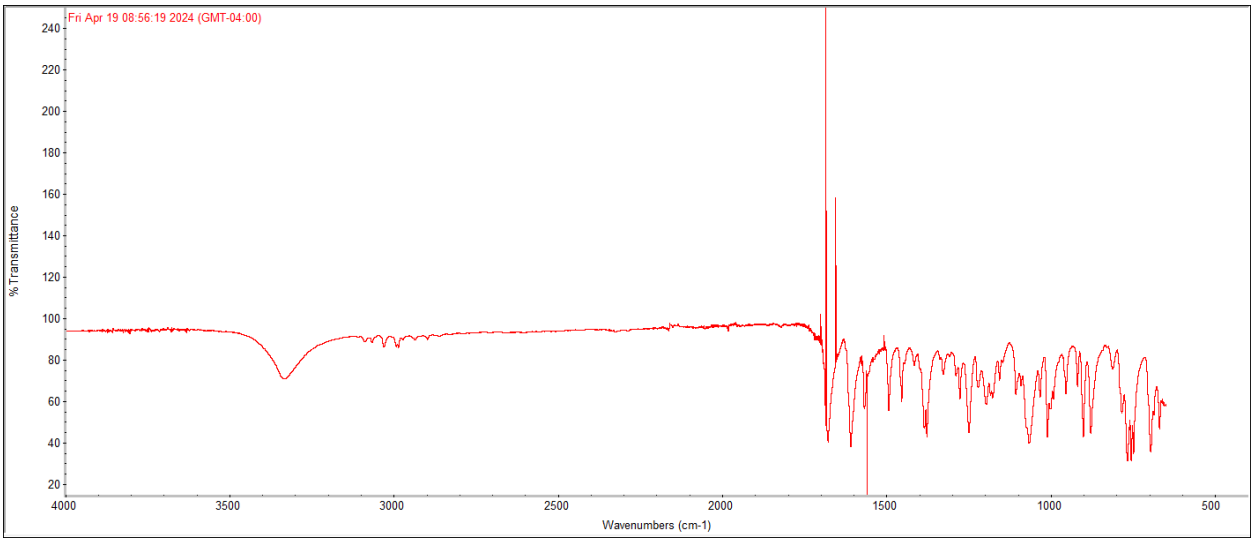


FTIR of crystalline solids (Thermo Nicolet iS10 with Golden Gate ATR)

R-warfarin (>99% ee) from L-Arg catalyzed reaction

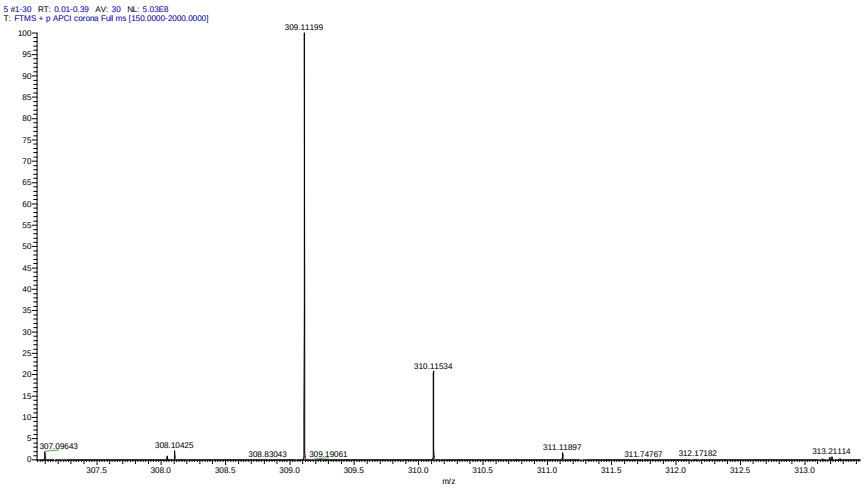


S-warfarin (>99% ee) from D-Arg catalyzed reaction



High Resolution Mass Spectrometry

R-warfarin from L-Arg catalyzed reaction



Zoom Inset

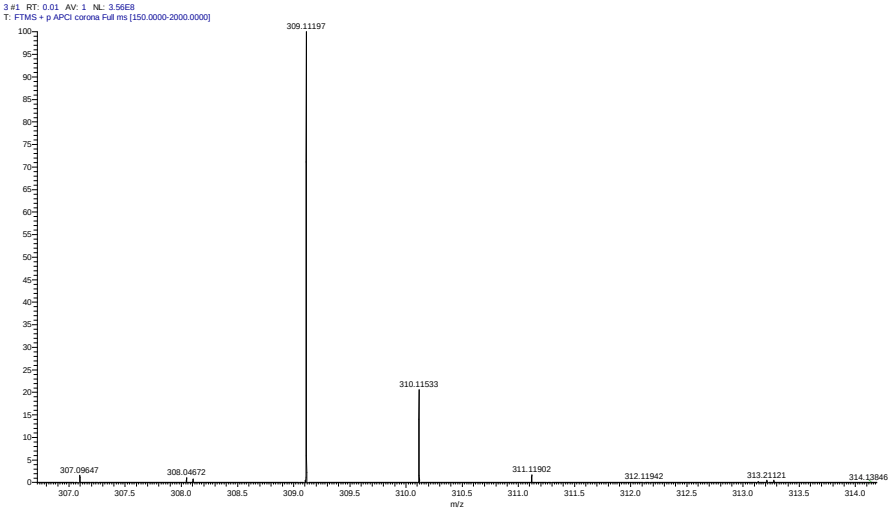
m/z = 309.11199

mass error = -0.47 ppm

Assigned Chemical Formula: $C_{19}H_{17}O_4$ $[M+H]^+$

Assessment: The observed mass and isotope pattern are consistent with the proposed chemical formula.

S-warfarin from D-Arg catalyzed reaction



Zoom Inset

m/z = 309.11197

mass error = -0.53 ppm

Assigned Chemical Formula: $C_{19}H_{17}O_4$ $[M+H]^+$

Assessment: The observed mass and isotope pattern are consistent with the proposed chemical formula.

ESI

Samples were analyzed with a Q Exactive HF-X (ThermoFisher, Bremen, Germany) mass spectrometer. Samples were introduced via a heated electrospray source (HESI) at a flow rate of 10 µL/min. HESI source conditions were set as: nebulizer temperature 400 deg C, sheath gas (nitrogen) 20 arb, auxillary gas (nitrogen) 0 arb, sweep gas (nitrogen) 0 arb, capillary temperature 320 degrees C, RF voltage 45 V. The mass range was set to 100-1000 m/z. All measurements were recorded at a resolution setting of 120,000. Solutions were analyzed at 0.1 mg/mL or less based on responsiveness to the ESI mechanism. Xcalibur (ThermoFisher, Breman, Germany) was used to analyze the data. Molecular formula assignments were determined with Molecular Formula Calculator (v 1.3.0). All observed species were singly charged, as verified by unit *m/z* separation between mass spectral peaks corresponding to the ^{12}C and $^{13}C^{12}C_{c-1}$ isotope for each elemental composition.

Q Exactive HF-X system acknowledgement:

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Organocatalyst Sources and Product Numbers

Catalyst	Source	Catalog No.
Tyrosine (L-Tyr)	Thermo Scientific	A11141.18
Glutamic Acid (L-Glu)	Fisher Scientific	A125
Leucine (L-Leu)	Fisher Scientific	BP385
Isoleucine (L-Ile)	Fisher Scientific	BP384
Glycine (Gly)	Fisher Scientific	BP381
Phenylalanine (L-Phe)	Thermo Scientific	A13238.14
Lysine (L-Lys)	Fisher Scientific	BP386
Methionine (L-Met)	Fisher Scientific	BP388
Histidine (L-His)	Fisher Scientific	BP382
Tryptophan (L-Trp)	Thermo Scientific	140590250
Arginine (L-Arg)	Thermo Scientific	A15738.22
Proline (L-Pro)	Fisher Scientific	BP392
Proline (D-Pro)	Millipore Sigma	858919
R,R-DPEN	Millipore Sigma	364010
S,S-DPEN	Millipore Sigma	364002
Arginine (D-Arg)	Millipore Sigma	A2646
NGMA	Sigma Aldrich	M7033
ADMA	Millipore Sigma	SMB00938

L-Arg, Methoxy ester	Millipore Sigma	11030