New Methods towards the synthesis of beta-amino acids
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Chapter 3

Synthesis of α- and β-aryl-amino acids catalyzed by phenylalanine amino mutase

Phenylalanine amino mutase (PAM) from Taxus chinensis catalyzes the stereoselective isomerization of α-phenylalanine to β-phenyalanine. Mechanistic studies show that trans-cinnamic acid is an intermediate in this transformation. The synthetic strategy described here shows that the addition of ammonia to cinnamic acid derivates gives α- and β-amino acids with excellent enantioselectivities of >99%. The α/β distribution is determined, and parameters that are important for the observed selectivities are elucidated.

Part of this chapter will be published: Szymanski, W.; Wu, B.; Weiner, B.; Feringa, B. L.; Janssen, D. B. “Phenylalanine aminomutase catalyzed addition of ammonia to substituted cinnamic acids – a new route to enantiopure α- and β-amino acids”, manuscript in preparation.
2.1 Introduction

Phenylalanine aminomutase (PAM) from *Taxus chinensis*, a Pacific Yew tree, catalyzes the key step in the biosynthesis of the phenylisoserine side chain of the antitumor drug paclitaxel 3.01 (Taxol, figure 3.1). Current commercially available Taxol is made semi-synthetically; the side chain is synthesized by a chemical route and is subsequently attached to 10-deacetyl-baccatin III, which is a more abundant metabolite from *Taxus chinensis*. In the biosynthetic pathway, the phenylisoserine side chain is constructed in five steps: 1) conversion of (S)-α-phenylalanine to (R)-β-phenylalanine catalyzed by PAM, 2) ligase catalyzed activation to the corresponding CoA ester, 3) transfer of the activated β-phenylalanine to the C-13 hydroxy group of baccatin III catalyzed by CoA acyltransferase, 4) hydroxylation at the C-2 of the side chain catalyzed by cytochrome P450, and 5) N-benzoylation of the side chain catalyzed by N-benzoyltransferase.

![Figure 3.1. Structure of Taxol.](image)

One key step in the Taxol-biosynthesis is the isomerization of (S)-α-phenylalanine to (R)-β-phenylalanine first demonstrated in cell-free extracts from *Taxus brevifolia* (scheme 3.01). By deuterium labeling and kinetic isotope measurements, the mechanism of this hydrogen transfer has been elucidated. It turns out that the same hydrogen shifts from the C-3 to the C-2 position via elimination and addition (scheme 3.01). The shift of the amino group from C-3 of the substrate to C-2 of the product proceeds also with retention of configuration, and the pro-3S hydrogen shifts to C-2 of (R)-β-Phe with retention of configuration. A primary kinetic isotope effect calculated for the Cγ-H bond of the substrate indicates that C-H bond cleavage is rate limiting. From these data, Walker *et. al* have proposed that PAM binds the carboxylate and phenyl ring in a syn-periplanar orientation in the active site. The amino group and the pro-3S hydrogen of α-Phe are therefore positioned on the same side of the molecule, and exchange and reattachment can occur from this side with retention of configuration. This orientation should show (Z)-cinnamic acid as intermediate, however, (Z)-cinnamic acid was not identified as substrate or inhibitor for PAM (see paragraph 2.4). The stereochemistry observed in the reaction of PAM should be related to another
mechanism, which could include rotation or distortion of the intermediate cinnamate. Up to now, no crystal structure of PAM has been solved.

\[
\begin{align*}
\text{[PAM]} & \quad \text{(S)-}\alpha\text{-Phe} \quad \rightarrow \quad \text{[PAM]} & \quad \text{(R)-}\beta\text{-Phe}
\end{align*}
\]

Scheme 3.01. Isomerization of α-Phe to β-Phe catalyzed by PAM.

Walker has shown that various aromatic β-amino acids can be synthesized from their corresponding α-amino acids using PAM.\textsuperscript{10} Studies with fluorine substituents in \(\alpha\)-, \(m\)- and \(p\)-position of these amino acids reveal that electron withdrawing substituents enhance the activity.

PAM relies on the internal cofactor 4-methylideneimidazol-5-one (MIO) \textsuperscript{3.07} in its active site. MIO is formed by posttranslational modification from the internal tripeptide Ala-Ser-Gly \textsuperscript{3.04} by executing mechanical pressure during protein folding (scheme 3.02).\textsuperscript{11,12} PAM belongs to a family of enzymes, which also includes the recently characterized tyrosine aminomutase (TAM) from \textit{Streptomyces globiporus}\textsuperscript{13} and shows high sequence similarity to the family of phenylalanine ammonia lyase (PAL)\textsuperscript{14} and histidine ammonia lyase (HAL)\textsuperscript{15}. All members of that family contain the MIO cofactor.

MIO, which is a more electrophilic version of dehydroalanine, is formed by cyclization of glycine with alanine to give intermediate \textsuperscript{3.05}, from which then two molecules of water are subsequently eliminated to yield \textsuperscript{3.07} via \textsuperscript{3.06} (scheme 3.02).\textsuperscript{11}

\[
\begin{align*}
\text{3.04} & \quad \xrightarrow{\text{H}^+} \quad \text{3.05} \\
\text{3.05} & \quad \xrightarrow{\text{H}_2\text{O}} \quad \text{3.06} \\
\text{3.06} & \quad \xrightarrow{\text{H}_2\text{O}} \quad \text{3.07}
\end{align*}
\]

Scheme 3.02. Mechanism for the formation of MIO.
The catalytic mechanism of this enzyme family has been extensively investigated and debated.\textsuperscript{11} Even with crystal structures of PAL\textsuperscript{14} and HAL\textsuperscript{15} available no agreement has yet been achieved.

Two mechanisms were suggested for PAL/HAL and aminomutases (PAM and TAM), both of them are E1\textsubscript{cb}-like.\textsuperscript{9,11} In the suggested Friedel-Crafts mechanism the aromatic ring is involved in a nucleophilic attack from the o-position on the terminal carbon of MIO forming a MIO-phenylalanine adduct with a positive charge delocalized in the former phenyl ring (scheme 3.03).\textsuperscript{16} A base abstracts the $\beta$-proton and from the resulting intermediate ammonia and MIO are released, gaining back the aromaticity of the phenyl ring and leading to cinnamic acid. In the PAL catalyzed reaction cinnamic acid and ammonia are now released, while PAM catalyzes a hydroamination adding ammonia to C-3 and protonating at C-2 leading to the product, $\beta$-phenylalanine.

The alternative carbanion mechanism starts with conjugate addition of the amino group at C-2 to the terminal $\alpha,\beta$-unsaturated alkene of MIO giving a protonated ammonia-MIO adduct (scheme 3.04).\textsuperscript{14} The acidity of the C-3 proton is thus increased,\textsuperscript{17} and it is deprotonated to give a carbanion intermediate which is further stabilized through the inductive effect of the phenyl ring. Then, the ammonia-MIO adduct is eliminated and in a reverse conjugate amine addition added to C-3 while C-2 is protonated giving a MIO-$\beta$-phenylalanine adduct. Finally, MIO is released and $\beta$-Phe is formed.
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Poppe and Rétey have favoured the Friedel-Crafts mechanism because they argue that stabilization of the carbanion through the protonated amino group and the phenyl ring would not be sufficient because the C-3 proton has a pK_a of >40.11 On the other hand the energy to eliminate the aromaticity at the expense of 36 kcal/mol is also high.18 The enzyme shows 10-20% higher activity with m-tyrosine as substrate than with α-Phe, and with p-tyrosine lower activity is observed which the authors explain through enhanced nucleophilicity of the phenyl ring through resonance with an attack from the o-position if the Friedel-Crafts mechanism plays a role.19 However, x-ray analysis of cocrystals of α,α-difluoro-β-tyrosin with TAM revealed an electron density that fits with an amine-bound-MIO-adduct, therefore supporting the carbanion mechanism.20 Furthermore, the crystal structure of PAL supports the carbanion mechanism.14 MIO is not the only responsible factor for lowering the pK_a of the C-3 proton. The carbanion can be stabilized through the influence of dipole moments of six α-helices which point with their positive poles towards the environment of the active site. This observation would strongly disfavour a mechanism with a cationic intermediate. The basicity of the general histidine base that deprotonates the C-3 group is also enhanced by a nearby negative dipole of a helix. The observation by Walker and co-workers, that electron withdrawing substituents on the phenyl ring enhance the activity, also supports the carbanion mechanism through increased acidity on C-3.10

PAL (EC 4.3.1.5) from Rhodosporidium toruloides is a homotetramer with four active sites, which catalyzes the reversible deamination of (S)-α-phenylalanine to cinnamic acid (scheme 3.05).14 This enzyme plays a key role in the secondary phenylpropanoid metabolism in plants,21 and is important in plant stress responses.22 Trans-cinnamate is a precursor for compounds which are essential for mechanical support such as lignin.23
PAL from parsley\textsuperscript{24} has been used in the biocatalytic production of $\alpha$-amino acids in the reverse reaction, i.e. by the addition of high concentrations of ammonia to cinnamic acid derivatives. A variety of aryl substituted (2-fluoro, 3-fluoro, 4-fluoro, 2,5-difluoro, 3,5-difluoro, pentafluoro, 2-chloro, 3-chloro, 4-chloro, 2,5-dichloro, 3-bromo, 3-cyano, 4-cyano, 3-hydroxy, 4-hydroxy, 2-nitro, 3-nitro, 2-naphthyl phenylalanine)\textsuperscript{25}, 2-naphthyl phenylalanine and heteroaromatic (2-pyridyl, 3-pyridyl, 4-pyridyl, and 3-thienyl)\textsuperscript{26} $\alpha$-amino acids can be synthesized.

\begin{center}
\begin{tikzpicture}
\node at (-1cm,0cm) {\( \text{CO}_2\text{H} \)};
\node at (1cm,0cm) {\( \text{H} \)};
\node at (2cm,-1cm) {\( \text{NH}_3 \)};
\node at (-3cm,-1cm) {\( \text{H} \)};
\node at (-4cm,-2cm) {\( \text{CO}_2\text{H} \)};
\node at (0cm,-2cm) {\( \text{3.02} \)};
\node at (3cm,-2cm) {\( \text{3.00} \)};
\draw [->] (-2cm,-1cm) -- (-1cm,-1cm);
\draw [->] (1cm,-1cm) -- (2cm,-1cm);
\draw [->] (1cm,-1cm) -- (2cm,-1cm);
\draw [->] (2cm,-1cm) -- (3cm,-1cm);
\draw [->] (3cm,-1cm) -- (4cm,-1cm);
\end{tikzpicture}
\end{center}

\textit{Scheme 3.05. Deamination of $\alpha$-Phe catalyzed by PAL.}

In contrast to ammonia lyases, aminomutases do not release the $\alpha,\beta$-unsaturated carboxylic acid but catalyze the re-addition of ammonia to the $\beta$-position, thus preventing ammonia lyase activity (scheme 3.01). It is shown in this chapter that the addition of ammonia to cinnamic acids can be used to produce enantioenriched $\alpha$- and $\beta$-amino acids. For the investigation of the scope of the PAM catalyzed reaction, a series of cinnamic acids were synthesized as well as the corresponding $\alpha$- and $\beta$-substituted amino acids.
2.2 **Synthesis of cinnamic acid derivatives**

Cinnamic acid derivatives were synthesized via Knoevenagel condensation of benzaldehyde derivatives with malonic acid and catalytic amounts of piperidine (table 3.1). All compounds were obtained in very good yields.

<table>
<thead>
<tr>
<th>entry</th>
<th>R</th>
<th>compound</th>
<th>yield [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2-F</td>
<td>3.10</td>
<td>99</td>
</tr>
<tr>
<td>2</td>
<td>2-Cl</td>
<td>3.11</td>
<td>93</td>
</tr>
<tr>
<td>3</td>
<td>2-Br</td>
<td>3.12</td>
<td>73</td>
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<tr>
<td>4</td>
<td>3-F</td>
<td>3.13</td>
<td>99</td>
</tr>
<tr>
<td>5</td>
<td>3-Cl</td>
<td>3.14</td>
<td>96</td>
</tr>
<tr>
<td>6</td>
<td>3-Br</td>
<td>3.15</td>
<td>92</td>
</tr>
<tr>
<td>7</td>
<td>4-Br</td>
<td>3.16</td>
<td>88</td>
</tr>
<tr>
<td>8</td>
<td>4-Et</td>
<td>3.17</td>
<td>96</td>
</tr>
<tr>
<td>9</td>
<td>4-n-Pr</td>
<td>3.18</td>
<td>92</td>
</tr>
<tr>
<td>10</td>
<td>4-NO 2</td>
<td>3.19</td>
<td>95</td>
</tr>
<tr>
<td>11</td>
<td>4-tert-Bu</td>
<td>3.20</td>
<td>99</td>
</tr>
</tbody>
</table>

By hydrogenation of the corresponding alkyne with Lindlar’s catalyst and hydrogen gas, (Z)-ethyl cinnamate 3.21 was synthesized, and subsequently hydrolyzed with base to give (Z)-cinnamic acid 3.22 (scheme 3.06).

2.3 **Synthesis of amino acids**

2.3.1 **Synthesis of α-amino acids**

A sequence involving Knoevenagel condensation with methyl isocyanocate and substituted benzaldehyde derivatives as electrophiles, followed by homogeneous or heterogeneous hydrogenation, and deprotection of both the amine and the ester moiety by hydrolysis were employed to synthesize racemic α-amino acids (scheme 3.07).
Methyl isocyanoacetate 3.24 reacts with catalytic amounts of copper(I) to form an organocopper-isocyanide adduct where the acidic α-hydrogen is replaced by Cu(I). This intermediate adds to the aldehyde to form a tetrahedral intermediate, which cyclizes to a 2-oxazoline releasing the copper catalyst. Upon addition of the base t-BuOK, ring opening yields the N-formyl-dehydroamino acid ester. All products were obtained as a mixture of (E)- and (Z)-isomers in moderate to good yields ranging from 35-74% (table 3.2). Some yields could be low due to the fact that t-BuOK was not purified prior to use.29

In order to avoid dehalogenation, halogen substituted N-formyl-dehydroamino esters were homogeneously hydrogenated employing 1 mol% of Rh(COD)_2BF_4 and 2 mol% of triphenylphosphine as catalyst with 20 bar of hydrogen gas in an autoclave (table 3.3). The N-formylamino esters were obtained in very good yields. Due to their low solubility in CH_2Cl_2, 2-bromo- and 3-bromo-substituted dehydroamino esters 3.38 and 3.41 were dissolved in CHCl_3 and the hydrogenation was performed at 60°C (table 3.3, entry 3, 6).
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leading in case of 2-bromo substrate 3.38 to lower yield. Alkyl substituted substrates 3.42, 3.45 and 3.46 could be hydrogenated using Pd on activated carbon as a heterogenous catalyst and 5 bar of hydrogen gas (table 3.3, entry 7, 10 and 11).28b

Table 3.3. Hydrogenation of dehydroamino esters.

<table>
<thead>
<tr>
<th>entry</th>
<th>R</th>
<th>compound</th>
<th>yield [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2-F</td>
<td>3.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>97</td>
</tr>
<tr>
<td>2</td>
<td>2-Cl</td>
<td>3.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86</td>
</tr>
<tr>
<td>3</td>
<td>2-Br</td>
<td>3.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58</td>
</tr>
<tr>
<td>4</td>
<td>3-F</td>
<td>3.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91</td>
</tr>
<tr>
<td>5</td>
<td>3-Cl</td>
<td>3.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>84</td>
</tr>
<tr>
<td>6</td>
<td>3-Br</td>
<td>3.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91</td>
</tr>
<tr>
<td>7</td>
<td>3-Me&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.42</td>
<td>85</td>
</tr>
<tr>
<td>8</td>
<td>4-Br</td>
<td>3.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>83</td>
</tr>
<tr>
<td>9</td>
<td>4-CF&lt;sub&gt;3&lt;/sub&gt;</td>
<td>3.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80</td>
</tr>
<tr>
<td>10</td>
<td>4-Et</td>
<td>3.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>94</td>
</tr>
<tr>
<td>11</td>
<td>4-n-Pr</td>
<td>3.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>83</td>
</tr>
</tbody>
</table>

<sup>a</sup> homogeneous hydrogenation using Rh(COD)<sub>2</sub>BF<sub>4</sub> and PPh<sub>3</sub>; <sup>b</sup> heterogeneous hydrogenation using Pd/C.

Deprotection of the amino group was achieved using 6N aq. HCl in methanol but the esters were only partly hydrolyzed. The crude amino acid esters were deprotected using aqueous LiOH, and the corresponding amino acids were crystallized as their hydrochloride salts from EtOH and Et<sub>2</sub>O in moderate to very good yields (table 3.4).
Table 3.4. Synthesis of α-amino acids.

<table>
<thead>
<tr>
<th>entry</th>
<th>R</th>
<th>compound</th>
<th>yield [%]</th>
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<td>2-Cl</td>
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<td>34</td>
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<td>2-Br</td>
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<td>3-F</td>
<td>3.50</td>
<td>91</td>
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<td>3.51</td>
<td>34</td>
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<td>3-Br</td>
<td>3.52</td>
<td>88</td>
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<td>3-Me</td>
<td>3.53</td>
<td>99</td>
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<tr>
<td>8</td>
<td>4-Br</td>
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<td>80</td>
</tr>
<tr>
<td>9</td>
<td>4-CF$_3$</td>
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<td>87</td>
</tr>
<tr>
<td>10</td>
<td>4-Et</td>
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</tr>
<tr>
<td>11</td>
<td>4-$n$-Pr</td>
<td>3.57</td>
<td>37</td>
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</tbody>
</table>

The α-amino acids were used for the chromatographic separation of α- and β-regioisomers and for the analysis, identification and characterization of the enantiomers of the α- and β-amino acids produced by PAM.
2.3.2 Synthesis of β-amino acids

The racemic β-amino acids were synthesized via the Rodionov reaction (table 3.5). Ammonia is produced in situ from ammonium acetate, and forms an imine with the aldehyde at which malonic acid is attacking. A subsequent decarboxylation provided the β-amino acids via this one-step procedure in low to moderate yields.

\[ \text{RCHO} + \text{HO}_2\text{C-CO}_2\text{H} \xrightarrow{\text{NH}_4\text{OAc, EtOH, } \Delta} \text{R-NH}_2\text{CO}_2\text{H} \]

<table>
<thead>
<tr>
<th>entry</th>
<th>R</th>
<th>compound</th>
<th>yield [%]</th>
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<td>3.60</td>
<td>13</td>
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<td>4</td>
<td>3-F</td>
<td>3.61</td>
<td>67</td>
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<td>3.62</td>
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<td>3.66</td>
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<td>4-i-Pr</td>
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<td>60</td>
</tr>
<tr>
<td>12</td>
<td>4-NO₂</td>
<td>3.69</td>
<td>21</td>
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</table>

The β-amino acids were used for separation of α- and β-regioisomers and for the analysis, identification and characterization of the enantiomers of the α- and β-amino acids produced by PAM.

2.4 Synthesis of α- and β-amino acids using PAM

PAM exhibits ammonia lyase activity as previously discussed (paragraph 3.1). The use of the reverse lyase reaction, i.e. the addition of ammonia to cinnamic acid derivatives, to synthesize enantiopure α- and β-amino acids was investigated (scheme 3.08). The gene for PAM from *Taxus chinensis* was cloned in the pBAD-His expression plasmid, and the recombinant enzyme expressed in *E. coli* as an N-terminal hexahistidine protein. The 95% pure protein was used in the following experiments.
Scheme 3.08. Synthesis of \( \alpha \) - and \( \beta \)-amino acids using PAM.

After having establishing that PAM catalyzes the addition of ammonia to cinnamic acid, a variety of cinnamic acid derivatives was studied as potential substrates. For every accepted substrate kinetic parameters were determined with UV-Vis spectroscopy employing the Michaelis-Menten equation to explore the influence on binding affinity and catalytic activity. Initial ratios for the formation of \( \alpha \)- and \( \beta \)-isomers were determined by HPLC to analyze the parameters influencing their formation.

Table 3.6. PAM catalyzed addition of ammonia to ortho-substituted cinnamic acids.

<table>
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<tr>
<th>entry</th>
<th>R</th>
<th>( K_m ) [mM]</th>
<th>( k_{cat} ) ([s^{-1}] \times 10^3)</th>
<th>( k_{cat}/K_m ) ([1 \text{ mol}^{-1} \text{s}^{-1}] \times 10^3)</th>
<th>initial ( \alpha/\beta ) ratio</th>
<th>( \alpha ) ee [%]</th>
<th>( \beta ) ee [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>24±1</td>
<td>1.8±0.1\times10^3</td>
<td>13</td>
<td>51:49</td>
<td>&gt;99 (S)</td>
<td>&gt;99 (R)</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>226±11</td>
<td>13±1</td>
<td>17</td>
<td>98:2</td>
<td>&gt;99 (S)</td>
<td>nd</td>
</tr>
<tr>
<td>3</td>
<td>Cl</td>
<td>359±20</td>
<td>8.6±1</td>
<td>42</td>
<td>&gt;99:1</td>
<td>&gt;99 (S)</td>
<td>nd</td>
</tr>
<tr>
<td>4</td>
<td>Br</td>
<td>145±11</td>
<td>6.9±1</td>
<td>21</td>
<td>99:1</td>
<td>&gt;99 (S)</td>
<td>nd</td>
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<tr>
<td>5</td>
<td>Me</td>
<td>110±8</td>
<td>9.3±1.4</td>
<td>12</td>
<td>&gt;99:1</td>
<td>&gt;99 (S)</td>
<td>nd</td>
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<tr>
<td>6</td>
<td>OMe</td>
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<td>-</td>
<td>&lt;0.1</td>
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</table>

nd = not determined.

\( \alpha \)- And \( \beta \)-phenylalanine are formed in a 1:1 ratio (table 3.6, entry 1), both with >99% ee. 2-Methoxy- and 2-hydroxy cinnamic acid did not show any conversion (table 3.6, entry 6-7). Substrates with fluoro, chloro, bromo and methyl substituents in ortho-position were accepted by PAM, and lead to almost exclusive formation of the \( \alpha \)-isomer (table 3.6, entry 2-5). This selectivity is likely due to steric hindrance resulting in effectively shielding of the \( \beta \)-position. One assumption is that the affinity of PAM (expressed as \( K_m \)) towards \( \alpha \)-substituted cinnamic acids depends on the hydrophobicity of the substituent. Considering the halogen substitution, the lowest \( K_m \)-value, so the highest affinity, is observed for the bromo substituted substrate, while the more hydrophilic fluoro substituted substrate has a 2-fold higher \( K_m \)-value (table 3.6, entry 2 and 4). This could suggest that a hydrophobic pocket exists around the ortho position. However, the chloro substituted substrate does not fit this pattern of reactivity. It seems that size does not matter for accepting a substrate in the active site of PAM, because the \( K_m \) value for the
bromo substituted substrate is smaller than the $K_m$ value for the smaller fluoro-substituted substrate. The catalytic activity $k_{cat}$ is increased for all substituted cinnamic acids, the highest for fluoro cinnamic acid, indicating that stronger electron withdrawing substituents might influence the acidity at C-3 (see paragraph 3.1). However, the electron donating methyl substituted phenyl ring does not match with this observation, because its activity is higher than for substrates bearing electron withdrawing bromo and chloro substituents (table 3.6, entry 5).

Addition of ammonia to meta-substituted cinnamic acids gives a mixture of $\alpha$- and $\beta$-amino acids (table 3.7). For halogen substituents the $\alpha$-isomer dominates (table 3.7, entry 2-4), but the substrate with the electron donating methyl substituent yields 80% of the $\beta$-isomer (table 3.7, entry 5). The affinity of PAM for 3-methyl cinnamic acid is higher than for its natural substrate $\alpha$-Phe. The catalytic activity is in all cases higher than for cinnamic acid. 3-Methoxy and 3-hydroxy cinnamic acid showed no detectable activity (table 3.7, entry 6-7).

### Table 3.7. PAM catalyzed addition of ammonia to meta-substituted cinnamic acids.

<table>
<thead>
<tr>
<th>entry</th>
<th>R</th>
<th>$K_m$ [mM]</th>
<th>$k_{cat}$ [s$^{-1}$] $\times 10^3$</th>
<th>$k_{cat}/K_m$ [l mol$^{-1}$ s$^{-1}$] $\times 10^3$</th>
<th>initial $\alpha$:$\beta$ ratio</th>
<th>$\alpha$ ee [%]</th>
<th>$\beta$ ee [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>24±1</td>
<td>1.8±0.1 $\times 10^3$</td>
<td>13</td>
<td>51:49</td>
<td>&gt;99 (S)</td>
<td>&gt;99 (R)</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>68±2</td>
<td>5.6±0.5 $\times 10^3$</td>
<td>10</td>
<td>86:14</td>
<td>92</td>
<td>nd</td>
</tr>
<tr>
<td>3</td>
<td>Cl</td>
<td>111±6</td>
<td>9.4±1.1 $\times 10^3$</td>
<td>12</td>
<td>94:6</td>
<td>&gt;99 (S)</td>
<td>nd</td>
</tr>
<tr>
<td>4</td>
<td>Br</td>
<td>nd$^a$</td>
<td>nd$^a$</td>
<td>nd$^a$</td>
<td>94:6</td>
<td>&gt;99 (S)</td>
<td>nd</td>
</tr>
<tr>
<td>5</td>
<td>Me</td>
<td>10±2</td>
<td>7.6±2.9 $\times 10^3$</td>
<td>1.3</td>
<td>20:80</td>
<td>&gt;99 (S)</td>
<td>&gt;99 (R)</td>
</tr>
<tr>
<td>6</td>
<td>OMe</td>
<td>-</td>
<td>-</td>
<td>&lt;0.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>OH</td>
<td>-</td>
<td>-</td>
<td>&lt;0.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

$^a$ Kinetic parameters were not determined due to insufficient solubility. nd = not determined.
Table 3.8. PAM-catalyzed addition of ammonia to para-substituted cinnamic acids.

\[
\begin{array}{cccccccc}
\text{entry} & R & K_m & k_{cat} & k_{cat}/K_m & \alpha/\beta \text{ ratio} & \alpha \text{ ee} & \beta \text{ ee} \\
& & [\text{mM}] & [\text{s}^{-1}] & [\text{M}^{-1}\text{s}^{-1}] & \% & \% \\
1 & H & 24 \pm 1 & 1.8 \pm 0.1 & 13 & 51:49 & >99 (S) & >99 (R) \\
2 & F & 37 \pm 1 & 2.5 \pm 0.2 & 15 & 35:65 & >99 (S) & >99 (R) \\
3 & Cl & 46 \pm 1 & 0.40 \pm 0.01 & 115 & 41:59 & >99 (S) & >99 (R) \\
4 & Br & 29 \pm 1 & 0.20 \pm 0.01 & 161 & 52:48 & 85 (S) & >99 (R) \\
5 & Me & 35 \pm 1 & 0.89 \pm 0.06 & 39 & 4.96 & >99 (S) & >99 (R) \\
6 & OMe & 27 \pm 1 & 0.79 \pm 0.04 & 34 & 14:86 & >99 (S) & >99 (R) \\
7 & OH & - & - & <0.1 & - & - & - \\
8 & Et & 38 \pm 3 & 0.44 \pm 0.10 & 86 & 12.88 & nd & >99^a \\
9 & n-Pr & 18 \pm 1 & 0.11 \pm 0.01 & 164 & 9:91 & nd & >99^a \\
10 & i-Pr & 46 \pm 3 & 2.1 \pm 0.6 & 22 & 9:91 & nd & >99^a \\
11 & NO₂ & 133 \pm 1 & 1.0 \pm 0.10 & 12 & 98:2 & >99 (S) & nd \\
12 & CF₃ & 81 \pm 4 & - & 81 & 83:17 & 43 (S) & nd \\
13 & t-Bu & - & - & <0.1 & - & - & - \\
\end{array}
\]

\(^a\) The absolute configuration was not determined. nd = not determined.

The catalytic efficiency is generally lower for cinnamic acids substituted in the para-position compared to those in the meta- or ortho-position (table 3.8, entry 2-6 and 8-11). As \(K_m\) values are lower, the affinity of PAM for these substrates is higher, they seem to fit better into the active site. 4-Methoxycinnamic acid is accepted by PAM in contrast to \(m\)- and \(o\)-methoxy substituted substrates (table 3.8, entry 6). Therefore, a larger range of \(p\)-substituted cinnamic acids was studied. The affinity of PAM seems to be influenced by three parameters: 1) hydrophobicity, 2) steric effects, and 3) electronic effects of the substituents. Lower \(K_m\) values are observed for lipophilic groups, as they decrease in the order methyl > propyl (table 3.8, entry 5+9), while they increase in the order bromo < fluoro < trifluoromethyl < nitro (table 3.8, entry 2-3 and 11-12). Steric effects disturb this trend, because the affinity in the series methyl, \(n\)-propyl, \(iso\)-propyl and \(tert\)-butyl initially rises until \(n\)-propyl, and then decreases for the bulkier branched substituent \(iso\)-propyl, while the sterically more demanding substrate \(tert\)-butylcinnamic acid is not accepted by PAM (table 3.8, entry 5, 8-10, 13). The ratio for \(\alpha\)- and \(\beta\)-amino acids is dominated by electronic properties of the respective substituents: cinnamic acids with electron donating substituents, such as methyl, ethyl, propyl, \(iso\)-propyl and methoxy (table 3.8, entry 5-6 and 8-10), are predominantly converted to their \(\beta\)-amino acids while
cinnamic acids with strongly electron withdrawing trifluoromethyl- and nitro-
substituents give mostly their α-amino acids (table 3.8, entry 11-12). Bromo-substituted
cinnamic acid gives an approximate ratio of 1:1, chloro-cinnamic acid a ratio of 40:60 of
α- to β-amino acid, and fluoro-cinnamic acid displays a 2:1 ratio in favour of the β-
isomer (table 3.8, entry 2-4).
Both the aromatic ring and the carboxylate group are activating the double bond for a
conjugate addition, so the tendency for α- or β-amino acid formation should be
influenced by the electronic properties of the substituents on the aromatic ring. Cinnamic
acids with electron donating groups in the para-position are activated towards conjugate
addition to the α,β-unsaturated carboxylic acid because the electronrich aromatic ring
cannot stabilize a negative charge (scheme 3.09). This could be the same reason for the
preferred formation of α-amino acids with electron donating substituents in the meta-
position (table 3.7). The strongly electron withdrawing substituted para-nitrocinnamic
acid gave exclusively α-amino acids, which could suggest that a conjugate addition to C-
2 is occurring and the produced negative charge can be delocalized to the nitro group
(scheme 3.09). Deviations from this trend could result from steric effects that might
influence the enzyme-substrate complex and thus the α/β ratio, as for example, observed
for ortho-substituents.

![Scheme 3.09. Electronic effects on the regioselectivity of PAM catalyzed addition reactions.](image)

A Hammett plot of the initial percentage of β-isomer formation for meta-substituted
cinnamic acids vs. the Hammett constants for the respective substituents shows a
linear behaviour, and therefore, a good correlation (figure 3.2). However, it should be
emphasized that four data points are not sufficient to draw unequivocal conclusions.
Chapter 3

Figure 3.2. Correlation between the Hammett constant $\sigma_p$ and the initial percentage of $\beta$-isomer formation for meta-substituted substrates of table 3.7.

For para-$\beta$-amino acids more data points were available to display the initial percentage of $\beta$-isomer formation for meta-substituents vs. the Hammett constants $\sigma_p$ in a Hammett plot. The graph shows a linear behaviour, and therefore, a good correlation of $\beta$-isomer formation and electronic effects of the substituents in para-position (figure 3.3). These observations show that the initial $\alpha/\beta$ ratio is dominated by electronic properties of $m$- and $p$-substituted cinnamic acids.

Figure 3.3. Correlation between the Hammett constant $\sigma_p$ and the initial percentage of $\beta$-isomer formation for para-substituted substrates of table 3.8.
Although kinetic efficiencies ($k_{cat}$) were collected for 18 substrates, no definitive trend in the catalytic rate was observed with strong electron withdrawing or donating substituents.34

Additional cinnamic acids were investigated in the addition of ammonia-catalyzed by PAM (figure 3.4). The β-Dopa35 precursor 3.70 was not accepted by PAM, and neither substrates with trisubstituted double bonds with methyl groups in C-2 position (3.71 and 3.73) or at C-3 (3.72). (Z)-Cinnamic acid is also not a substrate for PAM. PAM did not accept substrates where the phenyl ring was replaced with a furyl- (3.74), cyclohexyl-residue (3.75), an olefin (3.76) or a phenyl-substituted olefin (3.77).

### 2.5 Conclusion

Enantiopure α- and β-aryl-amino acids can be synthesized by PAM-catalyzed addition of ammonia to cinnamic acid derivatives. The substrate scope of PAM is rather broad which makes it an interesting enzyme for the biocatalytic synthesis of β-aryl-amino acids. However, the catalytic activity ($k_{cat}$) of 0.0001-0.0076 s$^{-1}$ is yet too low to use this enzyme in industrial applications. Studies towards the affinity of the enzyme for its substrates ($K_m$) indicate that a small hydrophobic pocket exists around the ortho-position of the substrate, and a larger hydrophobic groove around the para-position which tolerates non-branched aliphatic chains. Substrates with substituents in ortho-position lead to the selective formation of α-amino acids which is attributed to steric hindrance at C-3. Synthetic applications of this biocatalyst are depending on enantioselectivities and regioselectivities of α/β-formation. β-Amino acids are formed in excess (>90%) from cinnamic acids with electron donating groups in the para-position and to a smaller extent with these substituents in meta-position, all of them with excellent enantioselectivities. For substrates with electron withdrawing substituents the formation of α-amino acids is preferred. In most cases the enantioselectivities exceed 99%.
Concerning the debate about the mechanism involving PAM and PAL (see paragraph 3.1), the observed rates for the amination of the substituted cinnamic acids favor the carbanion mechanism. Substrates with electron withdrawing substituents in meta-position show a rate acceleration compared to cinnamic acid. This would strongly disfavor the Friedel-Crafts mechanism because the intermediate positive charge would partially be located in meta-position. Also, electron withdrawing substituents in ortho- and para position enhance the catalytic activity, which is in agreement with a stabilization of a carbanion-intermediate. This catalytic system represents a new addition to the biocatalytic synthesis of enantiopure β-amino acids.

2.6 Experimental

This project was performed in collaboration with Wiktor Szymanski and Bian Wu from the Department of Biochemistry. The PAM gene (T. chinensis) was ligated into a pBAD-His vector, expressed in E. coli TOP10 cells, and purified by metal-based affinity column chromatography by Wu Bian as described in reference 9.

General methods. see chapter 2.

Determination of kinetic parameters for the amination activity of PAM. Kinetic parameters of the PAM-catalyzed ammonia addition reaction were measured with UV-Vis spectroscopy. A 6M aq. ammonia solution was prepared and the pH was adjusted to pH=10 by bubbling CO₂ into the solution. In a typical assay, (E)-cinnamic acid or a derivative was incubated at various concentrations with purified PAM (0.06 mg, 0.76 μmol) in aqueous ammonia solution (300 μl). The reaction mixture was incubated at 30°C. The ammonia addition activity was monitored by UV-Vis spectroscopy. The initial rates were plotted against the substrate concentration and these data were fitted to the Michaelis-Menten equation to obtain the kinetic constants.

Stereochemical analysis of the phenylalanine products by chiral HPLC. Purified PAM (0.02 mg, 0.25 μmol, 0.03 mol%) was added to 5 mM of (E)-cinnamic acid (1 mmol) or a derivative in aqueous ammonia solution (6M, pH 10, 200 μl, 1.2 mmol). The reaction mixture was incubated for 24 h at 30°C. Subsequently, a 20-μl portion was taken and it was quenched by heating for 5 min at 99°C. A 40-μl portion of 2M aq. NaOH was added, and the sample was then frozen in liquid nitrogen. Subsequently, the sample was lyophilized and dissolved in 55 μl of 2M aq. HClO₄. Analysis were carried out with reversed phase HPLC on a Crownpak column with UV detection at 210 nm.

(E)-Cinnamic acid (3.08), (E)-4-fluoro-cinnamic acid, (E)-4-chloro-cinnamic acid, (E)-4-methyl-cinnamic acid, (E)-4-hydroxy-cinnamic acid were purchased from Acros organics. (E)-2-Methyl-cinnamic acid, (E)-2-methoxy-cinnamic acid, (E)-2-hydroxy-cinnamic acid, (E)-3-methyl-cinnamic acid, (E)-3-methoxy-cinnamic acid, (E)-3-
hydroxy-cinnamic acid, (E)-4-iso-propyl-cinnamic acid, (E)-4-methoxy-cinnamic acid were obtained from Sigma-Aldrich-Fluka. (R)-β-Phenylalanine, (S)-β-phenylalanine, (R)-3-amino-3-(2-fluoro-phenyl)-propionic acid, (R)-3-amino-3-(2-chloro-phenyl)-propionic acid, (R)-3-amino-3-(2-bromo-phenyl)-propionic acid, (R)-3-amino-3-(2-methyl-phenyl)-propionic acid, (S)-3-amino-3-(2-methyl-phenyl)-propionic acid, (R)-3-amino-3-(3-fluoro-phenyl)-propionic acid, (R)-3-amino-3-(3-chloro-phenyl)-propionic acid, (R)-3-amino-3-(3-bromo-phenyl)-propionic acid, (R)-3-amino-3-(3-methyl-phenyl)-propionic acid, (R)-3-amino-3-(4-fluoro-phenyl)-propionic acid, (R)-3-amino-3-(4-chloro-phenyl)-propionic acid, (S)-3-amino-3-(2-fluoro-phenyl)-propionic acid, (R)-3-amino-3-(4-bromo-phenyl)-propionic acid, (R)-3-amino-3-(4-nitro-phenyl)-propionic acid were synthesized by Peptech Corp. (±)-α-Phenylalanine, (±)-β-phenylalanine, (R)-α-phenylalanine, (S)-α-phenylalanine, (S)-4-nitro-α-phenylalanine and (±)-4-nitro-α-phenylalanine were obtained from Sigma-Aldrich-Fluka. (R)-β-Phenylalanine, (S)-β-phenylalanine, (S)-3-amino-3-(4-fluoro-phenyl)-propionic acid, (R)-3-amino-3-(4-fluoro-phenyl)-propionic acid, (S)-3-amino-3-(4-chloro-phenyl)-propionic acid, (R)-3-amino-3-(4-chloro-phenyl)-propionic acid, (S)-3-amino-3-(4-methyl-phenyl)-propionic acid, (R)-3-amino-3-(4-methyl-phenyl)-propionic acid, (S)-3-amino-3-(4-methoxy-phenyl)-propionic acid, (R)-3-amino-3-(4-methoxy-phenyl)-propionic acid, (S)-4-fluoro-α-phenylalanine, (R)-4-fluoro-α-phenylalanine, (S)-4-chloro-α-phenylalanine, (R)-4-chloro-α-phenylalanine, (S)-4-methyl-α-phenylalanine, (R)-4-methyl-α-phenylalanine, (S)-4-methoxy-α-phenylalanine and (R)-4-methoxy-α-phenylalanine were purchased from Peptech Corp.

**General procedure for the synthesis of cinnamic acid derivatives.**\(^{30b}\) A mixture of substituted benzaldehyde (4.00 mmol), malonic acid (8.80 mmol) and piperidine (70 μL) in pyridine (1.80 mL) was stirred under reflux for 80-180 min. The reaction mixture was cooled and slowly poured into ice-cold aqueous HCl (2N, 35 mL). The precipitate was filtered off and dried in vacuum.

**General procedure for the synthesis of N-formyl dehydroamino acid esters.**\(^{28b}\) To a solution of aldehyde (13.2 mmol, 1.2 eq.) and methyl isocyanoacetate (1.0 mL, 11.0 mmol) in dry Et\(_2\)O (10 mL) Cu\(_2\)O (79 mg, 0.55 mmol, 5 mol%) was added. After stirring for 3h at room temperature the mixture was cooled to 0°C and t-BuOK (1.28 g, 11.0 mmol) in dry THF (10 mL) was added. The mixture was stirred for 30 min at 0°C, acetic acid (0.65 mL, 11.0 mmol) in CH\(_2\)Cl\(_2\) (27 mL) was added, and the solution slowly warmed to room temperature. The organic layer was washed with H\(_2\)O (20 mL), dried over MgSO\(_4\) and concentrated in vacuum. The crude mixture was purified by flash column chromatography (n-pentane/EtOAc) or recrystallized from EtOAc/CH\(_2\)Cl\(_2\).\(^{28b}\)

**General procedure for hydrogenation of nonhalogen-substituted dehydroamino acids.**\(^{28b}\) In a pressure secure vial one spatula tip of Pd/C was added to the N-formyl
dehydroamino acid ester (2.0 mmol) in MeOH (5.0 mL). The vial was placed in an autoclave and 5 bar H₂ was applied. After stirring over night, the mixture was filtered over celite, and concentrated in vacuum. The crude mixture was purified by flash column chromatography (pentane/EtOAc).

**General procedure for hydrogenation of halogen-substituted dehydroamino acid esters.** In a pressure secure vial Rh(COD)₂BF₄ (8.1 mg, 0.02 mmol, 1 mol%) and PPh₃ (10.5 mg, 0.04 mmol, 2 mol%) were added to the N-formyl dehydroamino acid ester (2.0 mmol) in CH₂Cl₂ (5.0 mL). The vial was placed in an autoclave and 20 bar H₂ was applied. After stirring over night, the mixture was filtered over celite, and concentrated in vacuum. The crude mixture was purified by flash column chromatography (pentane/EtOAc).

**General procedure for the synthesis of α-amino acids.** To the N-formyl amino ester (0.5 mmol) in MeOH (2.0 mL), 5M aq. HCl (2 mL) was added. After heating to 40°C overnight, the solvent was evaporated in vacuum. The residue was dissolved in EtOH (2 mL) and Et₂O (5 mL) was added. The precipitated amino acid esters were filtered, and the crystals dried in vacuum. LiOH (0.15 g, 6.26 mmol) was added to the amino acid ester in MeCN (2 mL) and H₂O (2 mL), and the mixture stirred for 3 d. The sample was concentrated in vacuum, acidified with 5M aq. HCl (5 mL), and concentrated in vacuum. The precipitate was redissolved in EtOH and crystallized from Et₂O. The crystals were filtered and dried in vacuum.

3-Amino-3-(2-methyl-phenyl)-propanoic acid. HPLC (Crownpack CR(+), 4 mm x 150 mm, HClO₄ in 15% MeOH, pH 2.3, flow 0.3 mL/min, −7°C) 86.6 (R-α), 100.5 (S-α) min.

4-Fluoro phenylalanine. HPLC (Crownpack CR(+), 4 mm x 150 mm, HClO₄ in 15% MeOH, pH 2.5, flow 0.3 mL/min, −5°C) 22.0 (R-α), 35.6 (S-α), 53.8 (R-β), 68.3 (S-β) min.

4-Chloro phenylalanine. HPLC (Crownpack CR(+), 4 mm x 150 mm, HClO₄ in 15% MeOH, pH 2.7, flow 0.3 mL/min, −6°C) 45.8 (R-α), 73.9 (S-α), 136.6 (R-β), 155.1 (S-β) min.

4-Methyl phenylalanine. HPLC (Crownpack CR(+), 4 mm x 150 mm, HClO₄ in 15% MeOH, pH 2.6, flow 0.3 mL/min, −5°C) 34.7 (R-α), 71.5 (S-α), 109.8 (R-β), 126.3 (S-β) min.
4-Methoxy phenylalanine. HPLC (Crownpack CR(+), 4 mm x 150 mm, HClO₄ in 15% MeOH, pH 2.7, flow 0.3 mL/min, −6°C) 24.7 (R-α), 41.5 (S-α), 74.4 (R-β), 106.2 (S-β) min.

4-Nitro phenylalanine. HPLC (Crownpack CR(+), 4 mm x 150 mm, HClO₄ in 10% MeOH, pH 2.2, flow 0.3 mL/min, −6°C) 58.6 (R-α), 72.1 (S-α) min.

4-iso-propyl phenylalanine. HPLC (Crownpack CR(+), 4 mm x 150 mm, HClO₄ in 15% MeOH, pH 1.8, flow 0.5 mL/min, −7°C) 280 (R-α), 294 (S-α) min.

(E)-2-Fluoro-cinnamic acid 3.10. Light yellow solid; 99%; >99% (E) isomer; mp. 173-174°C (lit. 175°C)⁴⁶; ¹H NMR (400 MHz, CDCl₃): δ=6.56 (d, 3J=16.0 Hz, 1H; CH), 7.10-7.59 (m, 4H; CH), 7.93 (d, 3J=16.0 Hz, 1H; CH). ¹H NMR data consistent with literature.⁴⁷

(E)-2-Chloro-cinnamic acid 3.11. White solid, 93%; >99% (E) isomer; mp. 211°C (lit. 208-210°C)⁴⁸; ¹H NMR (400 MHz, CDCl₃): δ=6.59 (d, 3J=16.0 Hz, 1H; CH), 7.86 (d, 3J=16.4 Hz, 1H; CH), 7.36-7.92 (m, 4H; CH). ¹H NMR data were consistent with the literature.⁴⁸

(E)-2-Bromo-cinnamic acid 3.12. White solid, 73%; >99% (E) isomer; mp. 220°C (lit. 218-219°C)⁴⁹; ¹H NMR (400 MHz, CDCl₃): δ=6.54 (d, 3J=16.0 Hz, 1H; CH), 7.82 (d, 3J=15.6 Hz, 1H; CH), 7.32-7.90 (m, 4H; CH). ¹H NMR data were consistent with the literature.⁵⁰

(E)-3-Fluoro-cinnamic acid 3.13. White solid, 99%; >99% (E) isomer; mp. 168-169 ºC (lit. 166-167)⁵¹; ¹H NMR (400 MHz, DMSO-d₆): δ=6.60 (d, 3J=16.0 Hz, 1H; CH), 7.21-7.61 (m, 5H; CH). ¹H NMR data were consistent with the literature.⁵²

(E)-3-Chloro-cinnamic acid 3.14. White solid, 96%; >99% (E) isomer; mp. 161-162°C (lit. 162-163°C)⁵⁴; ¹H NMR (400 MHz, CDCl₃): δ=6.60 (d, 3J=16.0 Hz, 1H; CH), 7.55 (d, 3J=15.6 Hz, 1H; CH), 7.40-7.80 (m, 4H; CH). ¹H NMR data were consistent with the literature.⁵⁵
\((E)-3\)-Bromo-cinnamic acid 3.15. White solid, 92%; >99% (\(E\)) isomer; mp. 175-176°C (lit. 176-178°C)\(^{44}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta=6.61\) (d, \(^3J=15.6\) Hz, 1H; CH), 7.65 (d, \(^3J=16.0\) Hz, 1H; CH), 7.39-7.90 (m, 4H; CH). Spectral data were consistent with the literature.

\((E)-4\)-bromo-cinnamic acid 3.16. White solid, 88%; >99% (\(E\)) isomer; mp. 264-265°C (lit. 264-266°C)\(^{45}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta=6.55\) (d, \(^3J=16.0\) Hz, 1H; CH), 7.55 (d, \(^3J=15.6\) Hz, 1H; CH), 7.58-7.65 (m, 4H; CH). Spectral data were consistent with the literature.

\((E)-4\)-Ethyl-cinnamic acid 3.17. White solid, 96%; >99% (\(E\)) isomer; mp. 142-144°C (lit. 143°C)\(^{46}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta=1.25\) (t, \(^3J=7.6\) Hz, 3H; CH\(_3\)), 2.68 (q, \(^3J=7.6\) Hz, 2H; CH\(_2\)), 6.42 (d, \(^3J=15.6\) Hz, 1H; CH), 7.22-7.49 (m, 4H; CH). 7.78 (d, \(^3J=16.0\) Hz, 1H; CH); 13C NMR (50 MHz, CDCl\(_3\)): \(\delta=24.0, 24.5, 38.2, 116.3, 128.6, 129.3, 131.8, 146.3, 147.3, 184.8\). MS (EI) \(m/z\) 190 (M\(^+\), 40), 161 (100), 115 (75); HRMS calcd. for C\(_{12}\)H\(_{14}\)O\(_2\) 190.0994, found 190.0996.

\((E)-4\)-n-Propyl-cinnamic acid 3.18. White solid, 92%; >99% (\(E\)) isomer; mp. 176-177°C; \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta=0.95\) (t, \(^3J=7.2\) Hz, 3H; CH\(_3\)), 1.61-1.70 (m, 2H; CH\(_2\)), 2.62 (t, \(^3J=8.0\) Hz, 2H; CH\(_2\)), 6.42 (d, \(^3J=15.6\) Hz, 1H; CH), 7.21-8.49 (m, 4H; CH), 7.78 (d, \(^3J=16.0\) Hz, 1H; CH); 13C NMR (50 MHz, CDCl\(_3\)): \(\delta=24.0, 24.5, 38.2, 116.3, 128.6, 129.3, 131.8, 146.3, 147.3, 184.8\). MS (EI) \(m/z\) 204 (M\(^+\), 24), 189 (100); HRMS calcd. for C\(_{13}\)H\(_{16}\)O\(_2\) 204.1150, found 204.1159.

\((E)-4\)-Nitro-cinnamic acid 3.19. Yellow solid, 95%; >99% (\(E\)) isomer; mp. 292-293°C (lit. 293°C)\(^{44}\); \(^1\)H NMR (400 MHz, DMSO-d\(_6\)): \(\delta=6.74\) (d, \(^3J=16.4\) Hz, 1H; CH), 7.68 (d, \(^3J=16.4\) Hz, 1H; CH), 7.96-8.24 (m, 4H; CH). Spectral data were consistent with the literature.\(^{48}\)

\((E)-4\)-tert-Butyl-cinnamic acid 3.20. White solid, 99%; >99% (\(E\)) isomer; mp. 202-204°C (lit. 201-203°C)\(^{11}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta=1.35\) (s, 9H; (CH\(_3\)))\(_3\)), 6.43 (d, \(^3J=15.6\) Hz, 1H; CH), 7.42-8.51 (m, 4H; CH), 7.78 (d, \(^3J=16.0\) Hz, 1H; CH); 13C NMR (50 MHz, CDCl\(_3\)): \(\delta=31.4, 35.2, 116.4, 126.2, 128.5, 131.5, 147.1, 172.0\). MS (EI) \(m/z\) 204 (M\(^+\), 24), 189 (100); HRMS calcd. for C\(_{13}\)H\(_{16}\)O\(_2\) 204.1150, found 204.1159.
Ethyl (Z)-cinnamate 3.21. Ethyl phenylpropionate (2.87 mmol), quinoline (0.47 mL, 4.00 mmol) and 1-octene (3.0 mL) were dissolved in hexane (12.0 mL). Lindlar’s catalyst (0.15 g) was added, and the suspension was stirred under H₂ pressure (balloon) for 140 min. The solvent was evaporated, and the product was purified by flash column chromatography (pentane:Et₂O = 99:1) to give the product as a yellow oil (0.24 g, 1.37 mmol, 48%). ¹H NMR (400 MHz, DMSO-d₆): δ=1.25 (t, 3J=7.2 Hz, 4H; CH₃), 4.18 (q, 3J=7.2 Hz, 1H; CH₂), 5.95 (d, 3J=12.8 Hz, 1H; CH), 6.95 (d, 3J=12.8 Hz, 1H; CH), 7.32-7.59 (m, 5H; CH). Spectral data were consistent with the literature.

(Z)-Cinnamic acid 3.22. To a solution of ethyl (Z)-cinnamate 3.21 (0.23 g, 1.30 mmol) in ethanol (5.0 mL) was added aqueous 2 N NaOH (11 mL). The mixture was stirred for 120 min, acidified with aqueous 4 N HCl (6 mL) and extracted with Et₂O (3 x 20 mL). The combined organic layers were dried (MgSO₄) and the solvent was evaporated in vacuum. The residue was suspended in hexane (8 mL) and the solid product was filtered off, to give white crystals (0.19 g 1.25 mmol, 97%). Mp. 67°C (lit. 67-68°C); ¹H NMR (400 MHz, DMSO-d₆): δ=5.98 (d, 3J=12.8 Hz, 1H; CH), 7.07 (d, 3J=12.8 Hz, 1H; CH), 7.07 (d, 3J=12.8 Hz, 1H; CH), 7.35-7.62 (m, 5H; CH). Spectral data were consistent with the literature.

β-Cyclohexyl-acrylic acid 3.75. The product was obtained as white solid (75 %), mp. 56°C (lit. 56-57°C); ¹H NMR (400 MHz, DMSO-d₆): δ=1.14-2.18 (m, 11H; CH₂ + CH), 5.75 (d, 3J=16.0 Hz, 1H; CH), 7.02 (dd, 3J=16.4 Hz 3J= 6.8 Hz, 1H; CH). Spectral data were consistent with the literature.

Methyl 3-(2-fluorophenyl)-2-formamidoacrylate 3.25. Column chromatography (n-pentane/EtOAc 1:1) yielded the product as a white solid (1.09 g, 4.67 mmol, 42%) as a trans:cis (60:40) mixture. mp. 91°C; ¹H NMR (300 MHz, CDCl₃): δ=3.84 (s, 3H; CH₃), 3.88 (s, 3H; CH₃), 7.02-7.18 (m, 4H; CH), 7.25-7.60 (m, 6H; CH), 7.66 (s, 1H; CH), 8.19 (bs, 2H; CHO). ¹³C NMR (75 MHz, CDCl₃): δ=53.2 (CH₃), 53.4 (CH₃), 115.7 (C), 116.0 (C), 116.5 (CH), 116.7 (CH), 120.4 (CH), 124.2 (CH), 125.0 (CH), 125.0 (CH), 126.1 (C), 129.8 (CH), 130.1 (CH), 131.3 (CH), 131.3 (CH), 131.5 (CH), 131.6 (CH), 135.0 (CO), 163.7 (CO), 164.7 (CO), 165.3 (CO). HR-ESI-MS: m/z calcd for C₁₁H₁₁FNO₃ [M+H]+ 224.0717, found 224.0717.
Methyl 3-(2-chlorophenyl)-2-formamidoacrylate 3.26. Column chromatography (n-pentane/EtOAc 1:1) yielded the product as a white solid (1.40 g, 5.84 mmol, 53%) as a trans:cis (60:40) mixture. \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta=3.83\) (s, 3H; CH\(_3\)), 3.87 (s, 3H; CH\(_3\)), 7.20-7.52 (m, 9H; CH), 7.71 (s, 1H; CH), 8.14-8.24 (m, 2H; CHO). \(^1\)C NMR (100 MHz, CDCl\(_3\)): \(\delta=52.9\) (CH\(_3\)), 53.1 (CH\(_3\)), 123.0 (C), 125.1 (C), 127.0 (CH), 127.2 (CH), 127.8 (CH), 129.3 (CH), 129.5 (CH), 129.6 (CH), 130.4 (C), 130.7 (CH), 134.3 (C), 134.4 (C), 135.0 (C), 135.3 (C), 159.2 (CO), 163.8 (CO), 164.5 (CO), 165.0 (CO). HR-ESI-MS: m/z calcd for C\(_{11}\)H\(_{11}\)ClNO\(_3\) [M+H]\(^+\) 240.0422, found 240.0421.

Methyl 3-(2-bromophenyl)-2-formamidoacrylate 3.27. Recrystallization from EtOAc/CH\(_2\)Cl\(_2\) yielded the product as a white solid (1.10 g, 3.87 mmol, 39%) as a trans:cis (60:40) mixture. mp. 137°C; \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta=3.80\) (s, 3H; CH\(_3\)), 3.86 (s, 3H; CH\(_3\)), 7.07-7.49 (m, 8H; CH), 7.50-7.60 (m, 2H; CH), 7.66 (s, 1H; CH), 7.90 (s, 1H; CH), 8.00-8.06 (m, 2H; CHO). \(^1\)C NMR (100 MHz, CDCl\(_3\)): \(\delta=52.8\) (CH\(_3\)), 53.0 (CH\(_3\)), 124.1 (C), 124.3 (C), 124.4 (C), 125.7 (C), 125.9 (C), 126.9 (CH), 127.8 (CH), 129.4 (CH), 129.6 (CH), 130.0 (CH), 130.1 (CH), 130.3 (CH), 132.5 (CH), 133.1 (CH), 133.4 (C), 134.2 (C), 159.1 (CO), 163.4 (CO), 164.2 (CO), 164.8 (CO). HR-ESI-MS: m/z calcd for C\(_{11}\)H\(_{11}\)BrNO\(_3\) [M+H]\(^+\) 283.9917, found 283.9916.

Methyl 3-(3-fluorophenyl)-2-formamidoacrylate 3.28. Column chromatography (n-pentane/EtOAc 1:1) yielded the product as a white solid (1.73 g, 7.42 mmol, 67%) as a trans:cis (60:40) mixture. mp. 105°C; \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta=3.78\) (s, 3H; CH\(_3\)), 3.84 (s, 3H; CH\(_3\)), 6.81-7.02 (m, 3H; CH), 7.09-7.35 (m, 6H; CH), 7.65-7.85 (m, 1H; CH), 7.95-8.26 (m, 3H; CH). \(^1\)C NMR (75 MHz, CDCl\(_3\)): \(\delta=52.7\) (CH\(_3\)), 53.1 (CH\(_3\)), 114.8 (C), 115.8 (C), 116.6 (CH), 123.7 (CH), 124.7 (CH), 125.4 (CH), 127.6 (CH), 126.0 (CH), 128.1 (CH), 129.6 (CH), 130.2 (CH), 131.0 (CH), 131.6 (CH), 133.1 (C), 135.9 (C), 137.5 (C), 160.1 (CO), 164.5 (CO), 165.4 (CO), 170.8 (CO). HR-ESI-MS: m/z calcd for C\(_{11}\)H\(_{11}\)FNO\(_3\) [M+H]\(^+\) 224.0718, found 224.0718.

Methyl 3-(3-chlorophenyl)-2-formamidoacrylate 3.29. Column chromatography (n-pentane/EtOAc 1:1) yielded the product as a white solid (1.26 g, 5.75 mmol, 48%) as a trans:cis (60:40) mixture. mp. 117°C; \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta=3.83\) (s, 3H; CH\(_3\)), 3.88 (s, 3H; CH\(_3\)), 7.14-7.29 (m, 4H; CH), 7.33-7.60 (m, 6H; CH), 7.76 (s, 1H; CH), 8.05-8.11 (m, 2H; CHO). \(^1\)C NMR (100 MHz, CDCl\(_3\)): \(\delta=52.8\) (CH\(_3\)), 53.1 (CH\(_3\)), 123.6 (CH), 124.2 (C), 125.9 (C), 126.4 (CH), 127.2 (CH), 127.9 (CH), 129. (CH), 129.4 (CH), 130.0 (CH), 130.2 (CH), 131.5 (C), 132.4 (C), 133.9 (C), 134.3 (C), 135.1 (C), 135.9 (C), 137.5 (C), 160.1 (CO), 164.5 (CO), 165.4 (CO), 164.5 (CO), 164 (CO), 165.4 (CO), 170.8 (CO). HR-ESI-MS: m/z calcd for C\(_{11}\)H\(_{11}\)ClNO\(_3\) [M+H]\(^+\) 240.0422, found 240.0421.
158.9 (CO), 163.4 (CO), 164.2 (CO), 164.9 (CO). HR-ESI-MS: m/z calced for C_{11}H_{11}ClNO_{3} [M+H]^+ 240.0422, found 240.0422.

Methyl 3-(3-bromophenyl)-2-formamidoacrylate 3.30. Recrystallization from EtOAc/CH_{2}Cl_{2} yielded the product as a white solid (1.06 g, 3.81 mmol, 35%) as a trans: cis (60:40) mixture. \(^{1}H\) NMR (400 MHz, CDCl_{3}): \(\delta=3.84\) (s, 3H; CH_{3}), 3.88 (s, 3H; CH_{3}), 7.17-7.29 (m, 2H; CH), 7.29-7.50 (m, 6H; CH), 7.5-7.65 (m, 2H; CH), 8.14-8.26 (m, 2H; CH). \(^{13}C\) NMR (100 MHz, CDCl_{3}): \(\delta=52.9\) (CH_{3}), 53.1 (CH_{3}), 122.4 (C), 123.0 (C), 123.2 (C), 125.1 (C), 126.7 (CH), 127.6 (CH), 128.1 (CH), 129.9 (CH), 130.5 (CH), 130.6 (CH), 132.3 (CH), 132.4 (CH), 134.8 (C), 135.6 (C), 159.1 (CO), 163.7 (CO), 164.5 (CO), 165.0 (CO). HR-ESI-MS: m/z calced for C_{11}H_{11}BrNO_{3} [M+H]^+ 283.9917, found 283.9915.

Methyl 2-formamide-3-(4-(trifluoromethyl)phenyl)acrylate 3.33. Column chromatography (n-pentane/EtOAc 1:1) yielded the product as a white solid (2.11 g, 7.73 mmol, 70%) as a trans: cis (60:40) mixture. \(^{1}H\) NMR (400 MHz, CDCl_{3}): \(\delta=3.81\) (s, 3H; CH_{3}), 3.86 (s, 3H; CH_{3}), 7.27-7.34 (m, 1H; CH), 7.40 (s,
1H; CH), 7.48-7.68 (m, 6H; CH), 7.83 (s, 1H; CH), 7.96-8.08 (m, 1H; CH), 8.10-8.22 (m, 2H; CH). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta=52.9\) (CH\(_3\)), 53.0 (CH\(_3\)), 123.2 (C), 123.7 (C), 124.6 (CH), 124.8 (CH), 125.1 (CH), 125.2 (CH), 126.8 (CH), 127.0 (C), 128.8 (CH), 129.7 (CH), 130.2 (CH), 136.3 (C), 137.2 (CH), 138.9 (CH), 159.4 (CO), 159.7 (CO), 164.0 (CO), 165.0 (CO). HR-ESI-MS: \(m/z\) calcd for C\(_{12}\)H\(_{11}\)F\(_3\)NO\(_3\) [M+H]\(^+\) 274.0686, found 274.0685.

**Methyl 3-(4-ethylphenyl)-2-formamide acrylate 3.34.** Column chromatography (n-pentane/EtOAc 1:1) yielded the product as a white solid (1.41 g, 6.05 mmol, 55%) as a trans:cis (60:40) mixture. mp. 72-74°C; \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta=1.23\) (t, \(\text{j}=7.5\) Hz, 6H; CH\(_3\)), 2.64 (q, \(\text{j}=7.5\) Hz, 4H; CH\(_2\)), 3.83 (s, 3H; CH\(_3\)), 3.87 (s, 3H; CH\(_3\)), 7.11-7.27 (m, 6H; CH), 7.33-7.49 (m, 4H; CH), 8.12-8.35 (m, 2H; CO). \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta=15.1\) (CH\(_3\)), 28.5 (CH\(_2\)), 28.6 (CH\(_2\)), 52.6 (CH\(_3\)), 52.7 (CH\(_3\)), 121.5 (C), 123.1 (CH), 124.1 (C), 127.2 (CH), 127.7 (CH), 128.0 (CH), 128.6 (CH), 129.8 (CH), 129.4 (C), 129.8 (CH), 130.0 (CH), 130.6 (C), 144.0 (C), 146.3 (CH), 159.4 (CO), 159.6 (CO), 164.2 (CO), 165.1 (CO). HR-ESI-MS: \(m/z\) calcd for C\(_{13}\)H\(_{16}\)NO\(_3\) [M+H]\(^+\) 234.1125, found 234.1123.

**Methyl 3-(4-propylphenyl)-2-formamide acrylate 3.35.** Column chromatography (n-pentane/EtOAc 1:1) yielded the product as a white solid (0.48 g, 1.92 mmol, 58%) as a trans:cis (60:40) mixture. \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta=0.87\) (t, \(\text{j}=6.2\) Hz, 6H; CH\(_3\)), 1.56 (quind, \(\text{j}=7.0\) Hz, 4H; CH\(_2\)), 2.50 (t, \(\text{j}=7.3\) Hz, 4H; CH\(_2\)), 3.71 (s, 3H; CH\(_3\)), 3.77 (s, 3H; CH\(_3\)), 6.98-7.23 (m, 6H; CH), 7.32-7.45 (m, 4H; CH), 8.06-8.32 (m, 2H; CO). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta=13.4\) (CH\(_3\)), 23.8 (CH\(_3\)), 23.9 (CH\(_3\)), 37.3 (CH\(_3\)), 37.4 (CH\(_2\)), 52.2 (CH\(_2\)), 52.4 (CH\(_2\)), 121.7 (C), 122.8 (C), 125.2 (CH), 127.6 (CH), 128.2 (CH), 128.3 (CH), 128.8 (CH), 129.6 (CH), 130.3 (CH), 129.8 (CH), 130.7 (CH), 131.6 (C), 133.5 (CH), 142.1 (C), 144.4 (C), 144.6 (CH), 159.6 (CO), 160.3 (CO), 164.5 (CO), 165.1 (CO). HR-ESI-MS: \(m/z\) calcd for C\(_{14}\)H\(_{18}\)NO\(_3\) [M+H]\(^+\) 248.1281, found 248.1282.

**Methyl 3-(2-fluorophenyl)-2-formamidopropanoate 3.36.** Column chromatography (n-pentane/EtOAc 1:1) yielded the product as a white solid (0.94 g, 1.94 mmol, 97%). mp. 58-60°C; \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta=3.04-3.14\) (m, 1H; CH\(_2\)), 3.14-3.21 (m, 1H; CH\(_2\)), 3.69 (s, 3H; CH\(_3\)), 4.89 (m, 1H; CH), 6.40-6.70 (m, 1H; NH), 6.93-7.06 (m, 2H; CH), 7.17-7.26 (m, 1H; CH), 7.08-7.14 (m, 1H; CH), 7.15-7.22 (m, 1H; CH), 8.06 (s, 1H; CHO). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta=31.6\) (CH\(_3\)), 51.3 (CH\(_3\)), 52.8 (CH), 115.5 (C), 115.7 (C), 124.4 (CH), 129.3 (CH), 129.4 (CH), 131.9 (CH), 161.1.
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(CO), 171.8 (CO). HR-ESI-MS: m/z calcd for C_{14}H_{12}FNO_{3} [M+H]^+ 226.0874, found 226.0875.

**Methyl 3-(2-chlorophenyl)-2-formamidopropanoate 3.37.** Column chromatography (n-pentane/EtOAc 1:1) yielded the product as a white solid (0.42 g, 1.73 mmol, 86%). \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) = 2.97 (dd, \(^J=14.0\) Hz, \(^3J=14.0\) Hz, 1H; CH\(_2\)), 3.07 (dd, \(^2J=13.6\) Hz, \(^J=5.2\) Hz, 1H; CH\(_2\)), 3.68 (s, 3H; CH\(_3\)), 4.82-4.89 (m, 1H; CH), 6.81 (bs, 1H; NH), 6.93-7.01 (m, 1H; CH), 7.05-7.09 (m, 1H; CH), 7.11-7.20 (m, 2H; CH), 8.05 (s, 1H; CHO). \(^1\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) = 37.1 (CH\(_3\)), 51.5 (CH\(_2\)), 52.3 (CH), 127.1 (CH), 127.2 (CH), 129.2 (CH), 129.6 (CH), 134.0 (C), 137.6 (C), 160.8 (CO), 171.2 (CO). HR-ESI-MS: m/z calcd for C\(_{14}H_{13}ClNO_{3} [M+H]^+ 242.0579, found 242.0577.

**Methyl 3-(2-bromophenyl)-2-formamidopropanoate 3.38.** The dehydroamino acid was dissolved in CHCl\(_3\), and the hydrogenation performed at 60°C and 20 bar H\(_2\). Column chromatography (n-pentane/EtOAc 1:1) yielded the product as a white solid (0.48 g, 1.92 mmol, 58%). mp. 101°C; \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) = 3.16 (dd, \(^2J=14.0\) Hz, \(^J=8.0\) Hz, 1H; CH\(_2\)), 3.32 (dd, \(^2J=13.8\) Hz, \(^J=8.0\) Hz, 1H; CH\(_2\)), 3.70 (s, 3H; CH\(_3\)), 4.98 (q, \(^3J=8.0\) Hz, 1H; CH), 6.45 (d, \(^J=6.4\) Hz, 1H; NH), 7.06-7.12 (m, 3H; CH), 7.17-7.26 (m, 1H; CH), 7.52 (d, \(^J=8.0\) Hz, 1H; CH), 8.09 (s, 1H; CHO). \(^1\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) = 37.8 (CH\(_3\)), 50.9 (CH\(_2\)), 52.2 (CH), 124.8 (C), 127.5 (CH), 128.8 (CH), 131.1 (CH), 132.9 (CH), 135.5 (C), 160.6 (CO), 171.5 (CO).

**Methyl 3-(3-fluorophenyl)-2-formamidopropanoate 3.39.** Column chromatography (n-pentane/EtOAc 1:1) yielded the product as a white solid (0.41 g, 1.81 mmol, 91%). \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) = 3.01 (dd, \(^2J=13.8\) Hz, \(^J=7.8\) Hz, 1H; CH\(_2\)), 3.30 (dd, \(^2J=14.0\) Hz, \(^J=5.4\) Hz, 1H; CH\(_2\)), 3.68 (s, 3H; CH\(_3\)), 4.85-4.92 (m, 1H; CH), 6.50-6.95 (m, 4H; NHCH), 7.26-7.25 (m, 1H; CH), 8.07 (s, 1H; CHO). \(^1\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) = 37.2 (CH\(_3\)), 51.5 (CH\(_2\)), 52.3 (CH), 114.0 (CH), 116.1 (CH), 124.7 (CH), 129.9 (CH), 138.1 (C), 160.8 (CO), 163.7 (C), 171.2 (CO). HR-ESI-MS m/z calcd for C\(_{14}H_{13}FNO_{3} [M+H]^+ 226.0874, found 226.0874.

**Methyl 3-(3-chlorophenyl)-2-formamidopropanoate 3.40.** Column chromatography (n-pentane/EtOAc 1:1) yielded the product as a white solid (0.40 g, 1.67 mmol, 84%). mp. 88-90°C; \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) = 3.14 (dd, \(^2J=13.8\) Hz, \(^J=7.8\) Hz, 1H; CH\(_2\)), 3.30 (dd, \(^2J=14.0\) Hz, \(^J=6.0\) Hz, 1H; CH\(_2\)), 3.69 (s, 3H; CH\(_3\)), 4.96 (q, \(^3J=7.2\) Hz, 1H; CH), 6.53 (d, \(^2J=7.2\) Hz, 1H; NH), 7.12-7.20 (m, 3H; CH), 7.29-7.35
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(m, 1H; CH), 8.08 (s, 1H; CHO). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$=35.3 (CH$_3$), 50.7 (CH$_2$), 52.5 (CH), 128.8 (CH), 128.5 (CH), 129.5 (CH), 131.2 (CH), 133.7 (C), 134.2 (C), 160.7 (CO), 171.6 (CO). HR-ESI-MS: m/z calcd for C$_{11}$H$_{13}$ClNO$_3$ [M+H]$^+$ 242.0579, found 242.0579.

Methyl 3-(3-bromophenyl)-2-formamidopropanoate 3.41. The dehydroamino acid was dissolved in CHCl$_3$, and the hydrogenation performed at 60°C and 20 bar H$_2$. Column chromatography (n-pentane/EtOAc 1:1) yielded the product as a white solid (0.41 g, 1.81 mmol, 91%). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$=3.01 (dd, $^2J$=13.8 Hz, $^3J$=6.2 Hz, 1H; CH$_2$), 3.09 (dd, $^2J$=14.0 Hz, $^3J$=5.6 Hz, 1H; CH$_2$), 3.70 (s, 3H; CH$_3$), 4.89 (q, $^3J$=6.5 Hz, 1H; CH), 6.58 (d, $^3J$=6.8 Hz, 1H; NH), 7.02 (d, $^3J$=7.6 Hz, 1H; CH), 7.12 (t, $^3J$=7.8 Hz, 1H; CH), 7.24 (s, 1H; CH), 7.33 (d, $^3J$=8.0 Hz, 1H; CH), 8.10 (s, 1H; CHO). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$=37.2 (CH$_3$), 51.6 (CH$_2$), 52.4 (CH), 122.3 (CH), 127.7 (CH), 130.0 (CH), 130.1 (CH), 132.1 (C), 137.9 (C), 160.6 (CO), 171.2 (CO).

Methyl 2-formamido-3-m-tolylpropanoate 3.42. Column chromatography (n-pentane/EtOAc 1:1) yielded the product as a white solid (0.38 g, 1.71 mmol, 85%). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$=2.24 (s, 3H; CH$_3$), 2.90-3.10 (m, 2H; CH$_2$), 3.63 (s, 3H; CH$_3$), 4.79-4.89 (m, 1H; CH), 6.84-6.94 (m, 2H; CH), 6.95-7.03 (m, 1H; CH), 7.06-7.15 (m, 1H; CH), 7.99 (s, 1H; CHO). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$=20.9 (CH$_3$), 37.2 (CH$_3$), 51.6 (CH$_2$), 51.9 (CH), 125.8 (CH), 127.4 (CH), 128.0 (CH), 129.6 (CH), 135.3 (C), 137.7 (C), 160.8 (CO), 171.4 (CO). HR-ESI-MS m/z calcd for C$_{12}$H$_{16}$NO$_3$ [M+H]$^+$ 222.1125, found 222.1126.

Methyl 3-(4-bromophenyl)-2-formamidopropanoate 3.43. Column chromatography (n-pentane/EtOAc 1:1) yielded the product as a white solid (0.48 g, 1.67 mmol, 83%). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$=2.97 (dd, $^2J$=14.0 Hz, $^3J$=6.4 Hz, 1H; CH$_2$), 3.07 (dd, $^2J$=14.0 Hz, $^3J$=5.6 Hz, 1H; CH$_2$), 3.67 (s, 3H; CH$_3$), 4.85 (q, $^3J$=6.5 Hz, 1H; CH), 6.60-6.70 (m, 1H; NH), 6.95 (d, $^2J$=8.4 Hz, 1H; CH), 7.34 (d, $^2J$=8.0 Hz, 1H; CH), 8.06 (s, 1H; CHO). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$=37.3 (CH$_3$), 51.9 (CH$_2$), 52.8 (CH), 121.4 (C), 131.2 (CH), 131.8 (CH), 134.9 (C), 161.1 (CO), 171.6 (CO). HR-ESI-MS: m/z calcd for C$_{11}$H$_{13}$BrNO$_3$ [M+H]$^+$ 286.0073, found 286.0071.

Methyl 2-formamido-3-(4-(trifluoromethyl)phenyl)propanoate 3.44. Column chromatography (n-pentane/EtOAc 1:1) yielded the product as a white solid (0.47 g, 1.72 mmol, 80%). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$=3.07 (dd, $^2J$=14.0 Hz, $^3J$=6.4 Hz, 1H; CH$_2$), 3.67 (s, 3H; CH$_3$), 4.85 (q, $^3J$=6.5 Hz, 1H; CH), 6.60-6.70 (m, 1H; NH), 6.95 (d, $^2J$=8.4 Hz, 1H; CH), 7.34 (d, $^2J$=8.0 Hz, 1H; CH), 8.06 (s, 1H; CHO). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$=37.3 (CH$_3$), 51.9 (CH$_2$), 52.8 (CH), 121.4 (C), 131.2 (CH), 131.8 (CH), 134.9 (C), 161.1 (CO), 171.6 (CO). HR-ESI-MS: m/z calcd for C$_{11}$H$_{13}$BrNO$_3$ [M+H]$^+$ 286.0073, found 286.0071.
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CDCl$_3$): $\delta=3.12$ (dd, $^2J=14.0$ Hz, $^3J=6.0$ Hz, 1H; CH$_2$), 3.22 (dd, $^2J=14.0$ Hz, $^3J=5.6$ Hz, 1H; CH$_2$), 3.72 (s, 3H; CH$_3$), 4.95 (q, $^2J=6.5$ Hz, 1H; CH), 6.45 (d, $^2J=6.4$ Hz, 1H; NH), 7.21 (d, $^2J=8.0$ Hz, 1H; CH), 7.51 (d, $^2J=8.0$ Hz, 1H; CH), 8.12 (s, 1H; CHO). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta=37.4$ (CH$_3$), 51.6 (CH$_3$), 52.5 (CH), 125.3 (C), 125.4 (CH), 129.6 (CH), 129.7 (C), 139.7 (C), 160.6 (CO), 171.2 (CO). HR-ESI-MS: m/z calcd for C$_{12}$H$_{13}$F$_3$NO$_3$ [M+H]$^+$ 276.0842, found 276.0844.

Methyl 3-(4-ethylphenyl)-2-formamidopropanoate 3.45. Column chromatography ($n$-pentane/EtOAc 1:1) yielded the product as a white solid (0.44 g, 1.87 mmol, 94%). $^1$H NMR (400 MHz, CDCl$_3$): $\delta=1.14$ (t, 3H, $^3J=7.6$ Hz; CH$_3$), 2.53 (q, 2H, $^2J=7.5$ Hz, 2H; CH$_2$), 2.95 (dd, $^2J=14.0$ Hz, $^3J=6.4$ Hz, 1H; CH$_2$), 3.05 (dd, $^2J=13.6$ Hz, $^3J=5.3$ Hz, 1H; CH$_2$), 3.62 (s, 3H; CH$_3$), 4.80-4.88 (m, 1H; CH), 6.97-7.08 (m, 4H; CH), 8.00 (s, 1H; CHO). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta=15.0$ (CH$_3$), 27.9 (CH$_2$), 36.8 (CH$_2$), 51.6 (CH$_2$), 51.8 (CH), 127.5 (CH), 128.7 (CH), 132.5 (C), 142.5 (C), 160.8 (CO), 171.4 (CO). HR-ESI-MS m/z calcd for C$_{13}$H$_{18}$NO$_3$ [M+H]$^+$ 236.1281, found 236.1281.

Methyl 3-(4-propylphenyl)-2-formamidopropanoate 3.46. Column chromatography ($n$-pentane/EtOAc 1:1) yielded the product as a white solid (0.34 g, 1.35 mmol, 83%). mp. 59-60°C; $^1$H NMR (400 MHz, CDCl$_3$): $\delta=0.86$ (t, 3H, $^2J=7.4$ Hz; CH$_3$), 1.55 (sextet, 2H, $^3J=7.6$ Hz; CH$_3$), 2.48 (q, 2H, $^3J=6.9$ Hz, 2H; CH$_2$), 2.96 (dd, $^2J=14.0$ Hz, $^3J=6.4$ Hz, 1H; CH$_2$), 3.05 (dd, $^2J=13.6$ Hz, $^3J=5.6$ Hz, 1H; CH$_2$), 3.62 (s, 3H; CH$_3$), 4.80-4.88 (m, 1H; CH), 6.88-7.09 (m, 5H; NH+CH), 8.00 (s, 1H; CHO). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta=13.4$ (CH$_3$), 24.0 (CH$_2$), 36.8 (CH$_2$), 37.1 (CH$_2$), 51.6 (CH$_2$), 51.8 (CH), 128.2 (CH), 128.6 (CH), 132.5 (C), 141.0 (C), 160.7 (CO), 171.4 (CO). HR-ESI-MS m/z calcd for C$_{14}$H$_{20}$NO$_3$ [M+H]$^+$ 250.1438, found 250.1437.

2-Fluorophenylalanine hydrochloride 3.47. Precipitation yielded the product as a white solid (0.06 g, 0.27 mmol, 54%). $^1$H NMR (300 MHz, CDCl$_3$): $\delta=3.09$ (dd, $^2J=14.7$ Hz, $^3J=7.5$ Hz, 1H; CH$_2$), 3.26 (dd, $^2J=14.6$ Hz, $^3J=5.9$ Hz, 1H; CH$_2$), 4.19 (d, $^2J=6.6$ Hz, 1H; CH), 6.97-7.10 (m, 2H; NH+CH), 7.15-7.29 (m, 1H; CH). $^{13}$C NMR (50 MHz, CDCl$_3$): $\delta=22.4$ (CH$_3$), 46.3 (CH), 108.4 (CH), 113.8 (C), 114.1 (C), 117.9 (CH), 123.1 (CH), 124.8 (CH), 164.1 (CO). HR-ESI-MS: m/z calcd for C$_{9}$H$_{11}$FNO$_2$ [M+H]$^+$ 184.0768, found 184.0767. HPLC (Crownpack CR(+), 4 mm x 150 mm, HClO$_4$ in 15% MeOH, pH 2.4, flow 0.3 mL/min, −7°C) 29.8 (R-α), 40.6 (S-α) min.
2-Chlorophenylalanine hydrochloride 3.48. Precipitation yielded the product as a white solid (0.03 g, 0.14 mmol, 34%). ¹H NMR (400 MHz, CDCl₃):

\[
\begin{align*}
\delta &= 3.04 \text{ (dd, } J=14.8 \text{ Hz, } 1H; CH₂), \\
&= 3.17 \text{ (dd, } J=14.4 \text{ Hz, } 3J=5.6 \text{ Hz, } 1H; CH₂), \\
&= 4.12-4.19 \text{ (m, } 1H; CH), \\
&= 7.06-7.11 \text{ (m, } 1H; CH), \\
&= 7.17-7.26 \text{ (m, } 3H; CH). \\
\end{align*}
\]

¹C NMR (50 MHz, CDCl₃): δ=28.3 (CH₂), 47.1 (CH), 120.8 (CH), 120.9 (CH), 122.2 (CH), 123.6 (CH), 127.0 (C), 129.3 (C), 164.2 (CO). HPLC (Crownpack CR(+), 4 mm x 150 mm, HClO₄ in 15% MeOH, pH 2.4, flow 0.3 mL/min, −7°C) 103.8 (S-), 165.6 (R-)

2-Bromophenylalanine hydrochloride 3.49. Precipitation yielded the product as a white solid (0.12 g, 0.42 mmol, 84%). ¹H NMR (300 MHz, CDCl₃):

\[
\begin{align*}
\delta &= 2.90-3.20 \text{ (m, } 2H; CH₂), \\
&= 4.10-4.52 \text{ (m, } 1H; CH), \\
&= 7.04-7.18 \text{ (m, } 2H; CH), \\
&= 7.23-7.37 \text{ (m, } 2H; CH). \\
\end{align*}
\]

¹C NMR (75 MHz, CDCl₃): δ=35.1 (CH₂), 53.8 (CH), 122.3 (C), 128.3 (CH), 130.9 (CH), 132.1 (CH), 136.2 (C), 170.9 (CO). HPLC (Crownpack CR(+), 4 mm x 150 mm, HClO₄ in 15% MeOH, pH 2.6, flow 0.3 mL/min, −7°C) 77.6 (R-), 94.3 (S-)

3-Fluorophenylalanine hydrochloride 3.50. Precipitation yielded the product as a white solid (0.09 g, 0.38 mmol, 91%). ¹H NMR (300 MHz, CDCl₃):

\[
\begin{align*}
\delta &= 3.08 \text{ (dd, } J=14.5 \text{ Hz, } 1J=7.7 \text{ Hz, } 1H; CH₂), \\
&= 3.22 \text{ (dd, } J=14.7 \text{ Hz, } J=5.7 \text{ Hz, } 1H; CH₂), \\
&= 4.96 \text{ (dd, } J=7.5 \text{ Hz, } J=5.9 \text{ Hz, } 1H; CH), \\
&= 6.89-7.02 \text{ (m, } 3H; CH), \\
&= 7.22-7.31 \text{ (m, } 1H; CH). \\
\end{align*}
\]

¹C NMR (75 MHz, CDCl₃): δ=35.4 (CH₂), 54.2 (CH), 114.8 (C), 115.1 (CH), 116.1 (CH), 125.4 (CH), 131.1 (CH), 136.7 (C), 171.6 (CO). HR-ESI-MS m/z calcd for C₉H₁₁FNO₂ [M+H]⁺ 184.0768, found 184.0768. HPLC (Crownpack CR(+), 4 mm x 150 mm, HClO₄ in 15% MeOH, pH 2.0, flow 0.3 mL/min, −7°C) 57.1 (R-), 74 (S-)

3-Chlorophenylalanine hydrochloride 3.51. Precipitation yielded the product as a white solid (0.03 g, 0.14 mmol, 34%). ¹H NMR (400 MHz, CDCl₃):

\[
\begin{align*}
\delta &= 3.04 \text{ (dd, } J=14.8 \text{ Hz, } 1J=7.6 \text{ Hz, } 1H; CH₂), \\
&= 3.17 \text{ (dd, } J=14.4 \text{ Hz, } J=5.6 \text{ Hz, } 1H; CH₂), \\
&= 4.12-4.19 \text{ (m, } 1H; CH), \\
&= 7.06-7.11 \text{ (m, } 1H; CH), \\
&= 7.17-7.26 \text{ (m, } 3H; CH). \\
\end{align*}
\]

¹C NMR (50 MHz, CDCl₃): δ=28.3 (CH₂), 47.1 (CH), 120.8 (CH), 120.9 (CH), 122.2 (CH), 123.6 (CH), 127.0 (C), 129.3 (C), 164.2 (CO). HPLC (Crownpack CR(+), 4 mm x 150 mm, HClO₄ in 15% MeOH, pH 2.0, flow 0.3 mL/min, −7°C) 118.6 (R-), 160.8 (S-).
3-Bromophenylalanine hydrochloride 3.52. Precipitation yielded the product as a white solid (0.023 g, 0.82 mmol, 88%). $^1$H NMR (300 MHz, CDCl$_3$): $\delta$=3.15-3.65 (m, 2H; CH$_2$), 4.28-4.52 (m, 1H; CH), 7.15-7.52 (m, 3H; CH), 7.55-7.85 (m, 1H; CH). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$=36.4 (CH$_2$), 52.9 (CH), 124.4 (C), 128.4 (CH), 130.0 (CH), 132.0 (CH), 133.4 (CH), 133.7 (C), 171.3 (CO). HPLC (Crownpack CR(+), 4 mm x 150 mm, HClO$_4$ in 15% MeOH pH 2.6, flow 0.3 mL/min, $-7^\circ$C) 114.4 ($R$-$\alpha$), 197.8 ($S$-$\alpha$) min.

3-methylphenylalanine hydrochloride 3.53. Precipitation yielded the product as a white solid (0.09 g, 0.43 mmol, 99%). $^1$H NMR (300 MHz, CDCl$_3$): $\delta$=2.18 (s, 3H; CH$_3$), 2.96-3.08 (m, 1H; CH$_2$), 3.09-3.21 (m, 1H; CH$_2$), 4.12-4.21 (m, 1H; CH), 6.92-7.03 (m, 2H; CH), 7.04-7.20 (m, 1H; CH), 7.12-7.21 (m, 1H; CH). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$=13.4 (CH$_3$), 28.5 (CH$_2$), 47.2 (CH), 119.3 (CH), 121.5 (CH), 122.1 (CH), 123.0 (CH), 127.0 (C), 132.4 (C), 154.3 (CO). HR-ESI-MS m/z calcd for C$_{10}$H$_{14}$NO$_2$ [M+H]$^+$ 180.1019, found 180.1020. HPLC (Crownpack CR(+), 4 mm x 150 mm, HClO$_4$ in 15% MeOH pH 2.3, flow 0.3 mL/min, $-7^\circ$C) 111.1 ($S$-$\beta$), 123.1 ($S$-$\alpha$), 150.8 ($R$-$\beta$), 155.8 ($R$-$\alpha$) min.

4-Bromophenylalanine hydrochloride 3.54. Precipitation yielded the product as a white solid (0.10 g, 0.35 mmol, 80%). $^1$H NMR (300 MHz, CDCl$_3$): $\delta$=3.05-3.38 (m, 2H; CH$_2$), 4.20-4.42 (m, 1H; CH), 7.07-7.32 (m, 2H; CH), 7.40-7.63 (m, 2H; CH). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$=35.2 (CH$_2$), 54.0 (CH), 118.8 (C), 118.9 (CH), 122.9 (CH), 131.5 (C), 164.0 (CO).

4-Trifluoromethylphenylalanine hydrochloride 3.55. Precipitation yielded the product as white a solid (0.10 g, 0.37 mmol, 87%). $^1$H NMR (300 MHz, CDCl$_3$): $\delta$=3.13 (dd, $^2$J=14.6 Hz, $^3$J=7.3 Hz, 1H; CH$_2$), 3.24 (dd, $^2$J=14.6 Hz, $^3$J=5.9 Hz, 1H; CH$_2$), 4.22 (d, $^3$J=6.8 Hz, 1H; CH), 7.31 (d, $^3$J=7.8 Hz, 2H; CH), 7.55 (d, $^3$J=7.8 Hz, 2H; CH). $^{13}$C NMR (50 MHz, CDCl$_3$): $\delta$=28.4 (CH$_2$), 46.9 (CH), 118.8 (C), 118.9 (CH), 122.9 (CH), 131.5 (C), 164.0 (CO).

4-Ethylphenylalanine hydrochloride 3.56. Precipitation yielded the product as a white solid (0.29 g, 1.27 mmol, 99%). $^1$H NMR (300 MHz, CD$_2$OD): $\delta$=2.21 (t, $^3$J=7.6 Hz, 3H; CH$_3$), 2.62 (q, $^3$J=6.7 Hz, 2H; CH$_2$), 3.15 (dd, $^3$J=14.7 Hz, $^4$J=7.4 Hz, 1H; CH$_2$), 3.28 (dd, $^4$J=14.8 Hz, $^3$J=5.8 Hz, 1H; CH$_2$), 4.23 (dd, $^3$J=7.2 Hz, $^4$J=6.0 Hz, 1H; CH$_2$), 7.18-7.24 (m, 4H; CH). $^{13}$C NMR (100 MHz, CD$_2$OD): $\delta$=15.0 (CH$_3$), 28.3 (CH$_2$), 35.7
(CH₂) 15.1 (CH), 128.4 (CH), 129.4 (CH), 131.5 (C), 143.9 (C), 170.8 (CO). HR-ESI-MS: m/z calcd for C₁₁H₁₆NO₂ [M+H]^+ 194.1176, found 194.1174.

4-Ethylphenylalanine hydrochloride 3.57. Precipitation yielded the product as a white solid (0.04 g, 0.15 mmol, 37%). ¹H NMR (300 MHz, CDCl₃): δ=0.84-1.06 (m, 3H; CH₃), 1.55-1.80 (m, 2H; CH₂), 2.55-2.78 (m, 2H; CH₂), 3.18-3.50 (m, 2H; CH₂), 4.30-4.50 (m, 1H; CH), 7.21-7.45 (m, 4H; CH). ¹³C NMR (75 MHz, CDCl₃): δ=13.1 (CH₃), 24.2 (CH₂), 35.3 (CH₂), 37.0 (CH₃), 54.2 (CH), 116.1 (CH), 118.8 (C), 124.3 (CH), 126.5 (CH), 131.2 (CH), 143.2 (C), 171.5 (CO). HR-ESI-MS: m/z calcd for C₁₂H₁₈NO₂ [M+H]^+ 208.1332, found 208.1334.

3-Amino-3-(2-fluoro-phenyl)-propanoic acid 3.58. White solid, 41%, mp. 219-220°C (lit. 234-236°C)⁵³; ¹H NMR (400 MHz, D₂O + K₂CO₃): δ=2.46-2.53 (m, 2H; CH₂), 4.36 (t, ²J=7.2 Hz, 1H; CH), 6.96-7.31 (m, 4H; CH). Spectral data consistent with literature.⁵³

3-Amino-3-(2-chloro-phenyl)-propanoic acid 3.59. White solid, 31%; mp. 230-232°C (lit. 219°C)⁵⁴; ¹H NMR (400 MHz, D₂O + K₂CO₃): δ=2.41 (dd, ²J=15.2 Hz, ³J=8.0 Hz, 1H; CH₂), 2.54 (dd, ²J=15.2 Hz, ³J=6.0 Hz, 1H; CH₂), 4.54-4.60 (m, 1H; CH), 7.13-7.36 (m, 4H; CH). Spectral data consistent with literature.⁵⁴

3-Amino-3-(2-bromo-phenyl)-propanoic acid 3.60. White solid, 13%; mp. 229-230°C; ¹H NMR (400 MHz, D₂O + K₂CO₃): δ=2.38 (dd, ²J=14.8 Hz, ³J=8.0 Hz, 1H; CH₂), 2.54 (dd, ²J=14.8 Hz, ³J=6.0 Hz, 1H; CH₂), 4.51-4.54 (m, 1H; CH), 7.05-7.52 (m, 4H; CH); ¹³C NMR (75 MHz, CDCl₃): δ=46.9, 52.8, 124.9, 126.5, 127.4, 130.3, 133.8, 146.2, 167.3. HR-ESI-MS calcd. for C₈H₁₁O₂BrNBr 243.9968, found 243.9968.

3-Amino-3-(3-chloro-phenyl)-propanoic acid 3.62. White solid, 56%; mp. 221-222°C; ¹H NMR (400 MHz, D₂O + K₂CO₃): δ=2.41-2.45 (m, 2H; CH₂), 4.10 (t, ²J=7.2 Hz, 1H; CH), 7.16-7.28 (m, 4H; CH); ¹³C NMR (75 MHz, CDCl₃): δ=46.9, 52.8, 124.9, 126.5, 127.4, 120.3, 133.8, 146.2, 167.3; HR-ESI-MS calcd. for C₈H₁₁O₂ClNCl 200.0473, found 200.0473.
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3-Amino-3-(3-bromo-phenyl)-propanoic acid 3.63. White solid, 55%; mp. 225-226°C (lit. 243-245°C);\(^\text{19b}\) \(^1\)H NMR (400 MHz, D\(_2\)O + K\(_2\)CO\(_3\)): \(\delta=2.40-2.44\) (m, 2H; CH\(_2\)), 4.03-4.10 (m, 1H; CH), 7.13-7.43 (m, 4H; CH). Spectral data consistent with literature.

3-Amino-3-(3-methyl-phenyl)-propanoic acid. 3.64. White solid, 56%; mp. 219°C (lit. 221-222°C);\(^\text{55}\) \(^1\)H NMR (400 MHz, D\(_2\)O + K\(_2\)CO\(_3\)): \(\delta=2.20\) (s, 3H; CH\(_3\)), 2.41-2.44 (m, 2H; CH\(_2\)), 4.09 (t, \(\text{J}=7.2\) Hz, 1H; CH), 7.02-7.19 (m, 4H; CH); Spectral data consistent with literature.\(^\text{55}\) HPLC (Crownpack CR(+), 4 mm x 150 mm, HClO\(_4\) in 15% MeOH, pH 2.6, flow 0.3 mL/min, \(-7^\circ\)C) 111.1 (S-α), 150.8 (R-β), 155.8 (R-α), 123.1 (S-β) min.

3-Amino-3-(4-bromo-phenyl)-propanoic acid 3.65. White solid, 29%; mp. 228-229°C (lit. 234°C);\(^\text{54}\) \(^1\)H NMR (400 MHz, D\(_2\)O + K\(_2\)CO\(_3\)): \(\delta=2.37-2.50\) (m, 2H; CH\(_2\)), 4.08 (t, \(\text{J}=6.8\) Hz, 1H; CH), 7.14-7.17 (m, 2H; CH), 7.39-7.42 (m, 2H; CH); Spectral data consistent with literature.\(^\text{54}\) HPLC (Crownpack CR(+), 4 mm x 150 mm, HClO\(_4\) in 15% MeOH, pH 2.6, flow 0.3 mL/min, \(-7^\circ\)C) 122.5 (R-α), 163.3 (S-α), 198.5 (R-β), 219.7 (S-β) min.

3-Amino-3-(4-ethyl-phenyl)-propanoic acid 3.66. White solid, 60%; mp. 220-221°C; \(^1\)H NMR (400 MHz, D\(_2\)O + K\(_2\)CO\(_3\)): \(\delta=1.04\) (t, \(\text{J}=7.6\) Hz, 3H; CH\(_3\)), 2.40-2.43 (m, 2H; CH\(_2\)), 2.48 (q, \(\text{J}=8.0\) Hz, 2H; CH\(_2\)), 4.05-4.10 (m, 1H, CH), 7.13-7.19 (m, 4H; CH); \(^13\)C NMR (75 MHz, CDCl\(_3\)): \(\delta=15.2, 28.0, 46.9, 52.8, 126.1, 128.3, 144.1, 167.7\); MS (EI) \(m/z\) 193 (M\(^+\), 16), 134 (100); Anal. calcd. for C\(_{11}\)H\(_{15}\)NO\(_2\) C 68.37, H 7.82, N 7.25, found C 68.20, H 7.88, N 7.19. HPLC (Crownpack CR(+), 4 mm x 150 mm, HClO\(_4\) in 15% MeOH, pH 2.6, flow 0.3 mL/min, \(-7^\circ\)C) 175.0 (R-β), 231.6 (S-β) min.

3-Amino-3-(4-propyl-phenyl)-propanoic acid 3.67. White solid, 31%, mp. 218-219°C; \(^1\)H NMR (400 MHz, D\(_2\)O + K\(_2\)CO\(_3\)): \(\delta=0.75\) (t, \(\text{J}=7.2\) Hz, 3H; CH\(_3\)), 1.42-1.50 (m, 2H; CH\(_2\)), 2.41-2.47 (m, 4H; CH\(_2\)), 4.10 (t, \(\text{J}=7.2\) Hz, 1H; CH), 7.12-7.20 (m, 4H; CH); \(^13\)C NMR (75 MHz, CDCl\(_3\)): \(\delta=13.1, 24.2, 37.0, 47.0, 52.8, 118.8, 121.5, 126.5, 128.9, 167.5\); HRMS calcd. for C\(_{12}\)H\(_{16}\)O\(_2\)N 208.1332,
found 208.1333; Anal. calcd. for C_{12}H_{17}NO_{2} C 69.54, H 8.27, N 6.76, found C 69.46, H 8.27, N 6.72. HPLC (Crownpack CR(+), 4 mm x 150 mm, HClO_{4} in 15% MeOH, pH 3.0, flow 0.3 mL/min, −7°C) 250.1 (R-) min, 387.3 (S-) min.

3-Amino-3-(4-iso-propyl-phenyl)-propanoic acid 3.68. White solid, 60%, mp. 242-243°C; 1H NMR (400 MHz, D_{2}O + K_{2}CO_{3}): 6=1.05 (d, J=6.8 Hz, 6H; CH_{3}), 2.39 (d, J=7.2 Hz, 2H; CH_{2}), 2.75 (sept, J=6.6 Hz, 1H; CH), 4.07 (t, J=7.2 Hz, 1H; CH), 7.15-7.20 (m, 4H; CH); 13C NMR (75 MHz, CDCl_{3}): 6=23.3, 36.7, 47.0, 51.2, 121.5, 124.2, 126.6, 126.8, 168.1; MS (EI) m/z 207 (M+, 16), 148 (100); Anal. calcd. for C_{12}H_{17}NO_{2} C 69.54, H 8.27, N 6.76, found C 69.40, H 8.25, N 6.74.

3-Amino-3-(4-nitrophenyl)-propanoic acid 3.69. A suspension of 4-nitrobenzaldehyde (1.00 g, 6.65 mmol), malonic acid (0.70 g, 6.70 mmol) and ammonium acetate (1.09 g, 14.2 mmol) in 2-propanol was heated under reflux for 22 h. The solid was filtered off, redissolved in aqueous 1 N HCl (10 mL) and washed with Et_{2}O (3 x 10 mL). The aqueous phase was evaporated in vacuum to give the product as yellow solid (0.30 g, 1.42 mmol, 21 %). 5

2.7 References

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31 The Rodionov reaction is known for giving low yields, see ref. 30.
34 This was similar to the results of kinetic studies for the isomerization of different α-amino acids to β-amino acids using PAM observed by Walker, see ref. 10.
51 Zweifel, G.; Lynd, R. A. Synthesis 1976, 9, 625.
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