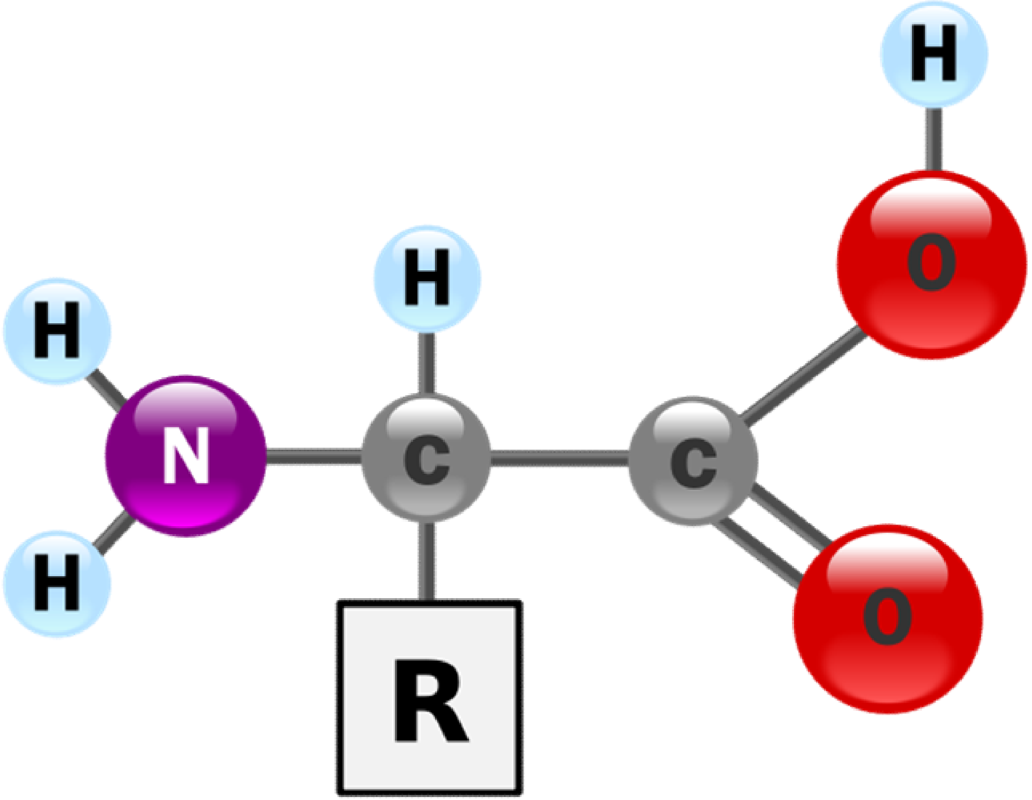
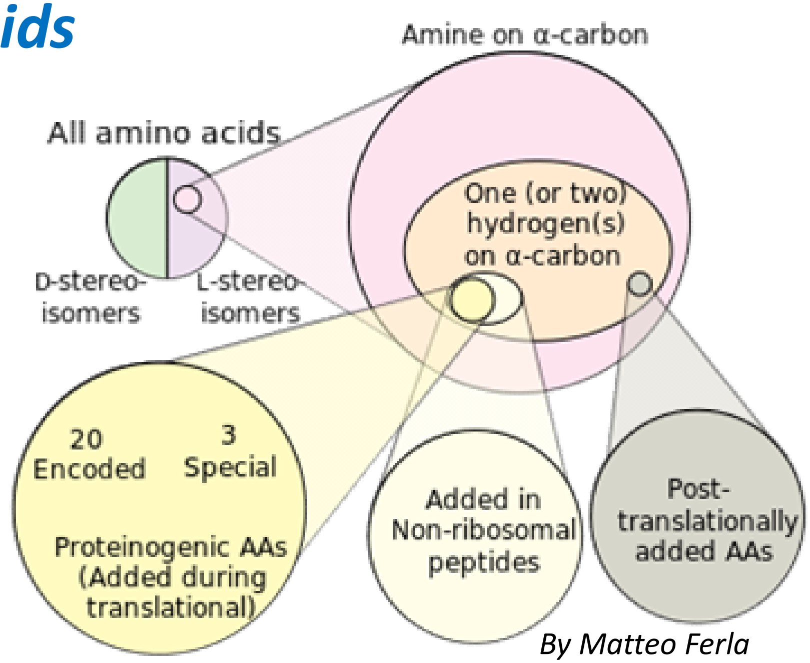
# *Aminoacids*

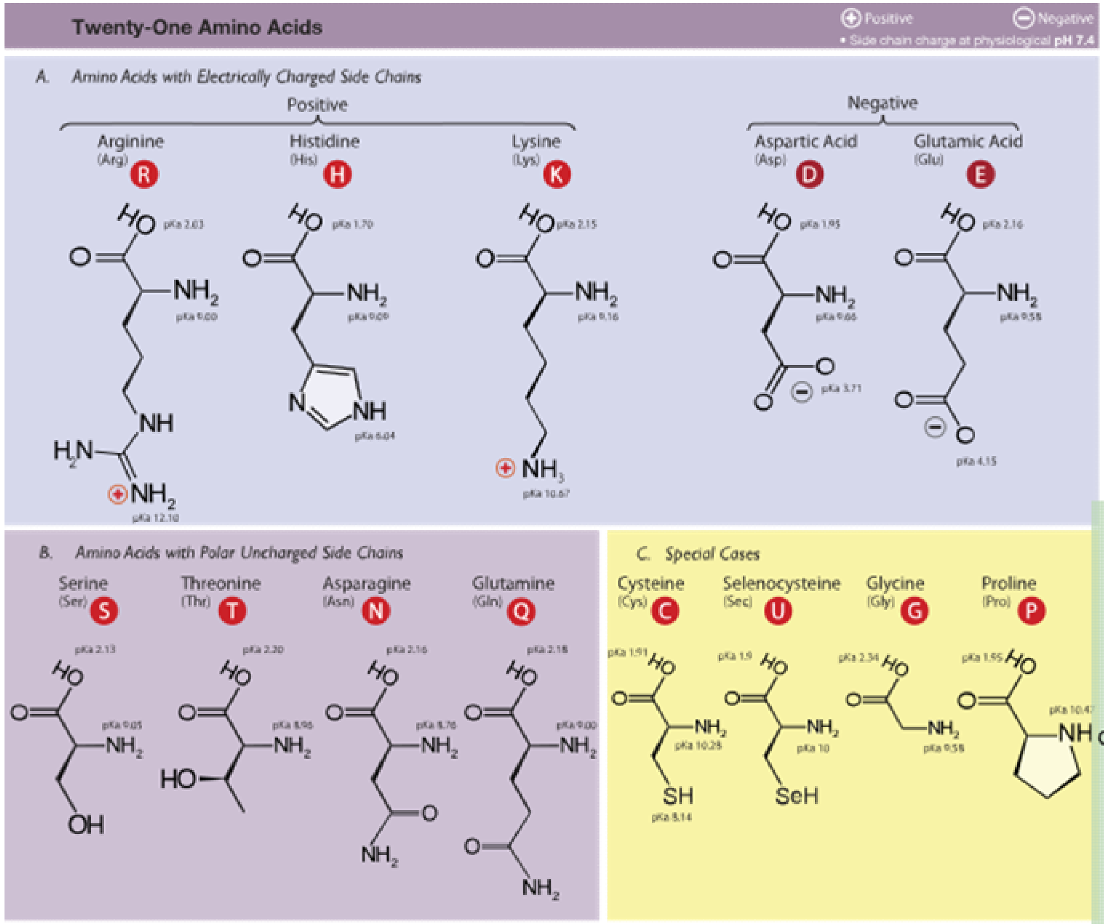


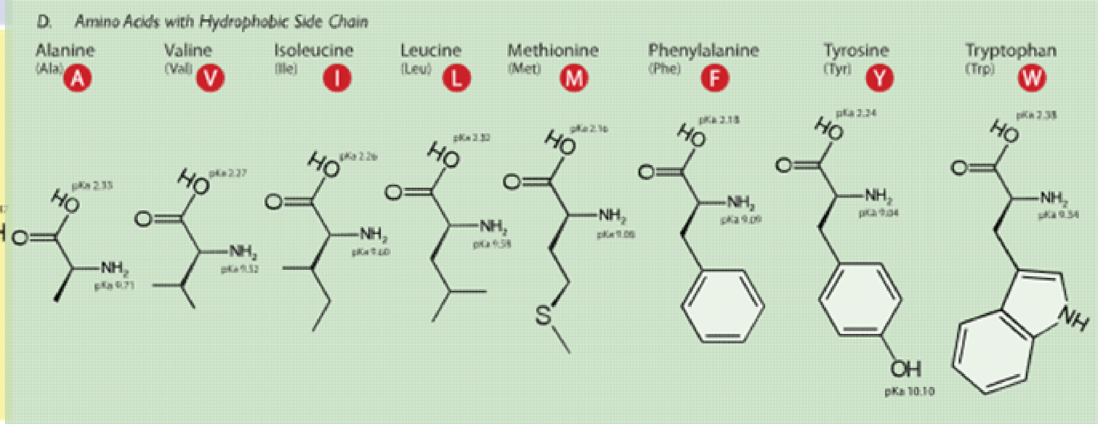
# *Proteinogenic aminoacids*

## *genetically encoded*

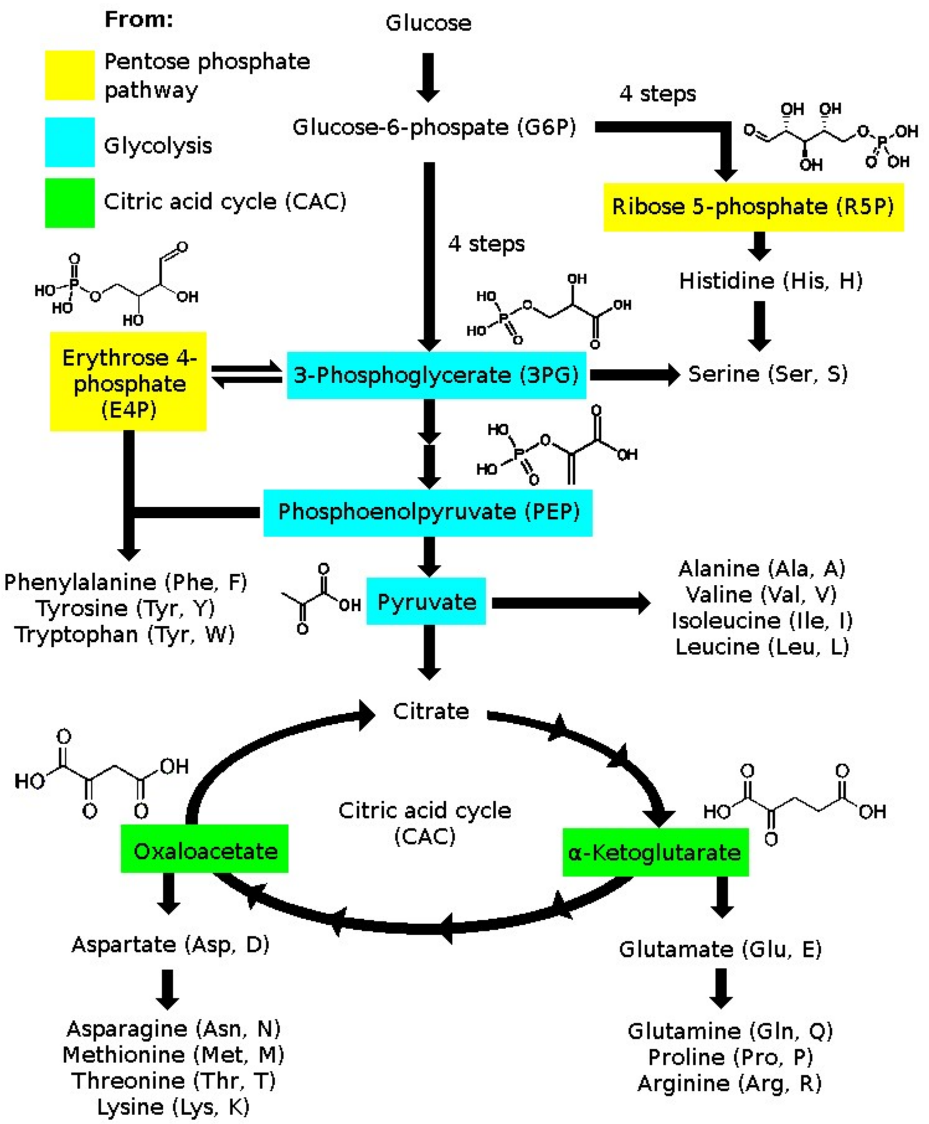


*By Matteo Ferla*





# *Biosynthesis of biogenic aminoacids*



**From:**

- 🟨 Pentose phosphate pathway
- 🟦 Glycolysis
- 🟩 Citric acid cycle (CAC)

Glucose

Glucose-6-phospate (G6P)

4 steps

Ribose 5-phosphate (R5P)

Histidine (His, H)

4 steps

Erythrose 4-phosphate (E4P)

3-Phosphoglycerate (3PG)

Serine (Ser, S)

Phosphoenolpyruvate (PEP)

Phenylalanine (Phe, F)
Tyrosine (Tyr, Y)
Tryptophan (Tyr, W)

Pyruvate

Alanine (Ala, A)
Valine (Val, V)
Isoleucine (Ile, I)
Leucine (Leu, L)

Citrate

Citric acid cycle
(CAC)

Oxaloacetate

α-Ketoglutarate

Aspartate (Asp, D)

Asparagine (Asn, N)
Methionine (Met, M)
Threonine (Thr, T)
Lysine (Lys, K)

Glutamate (Glu, E)

Glutamine (Gln, Q)
Proline (Pro, P)
Arginine (Arg, R)

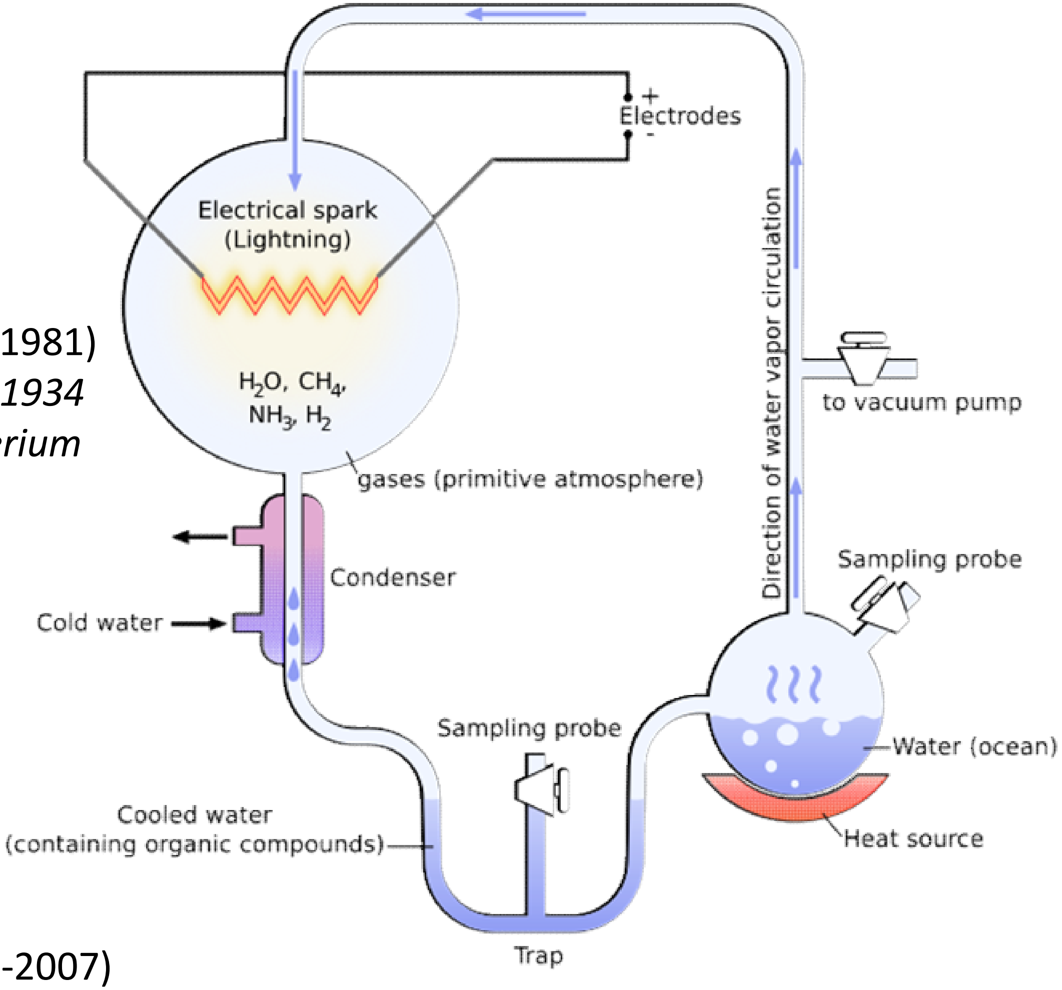
# *Atmosphere composition for young terrestrial planets*

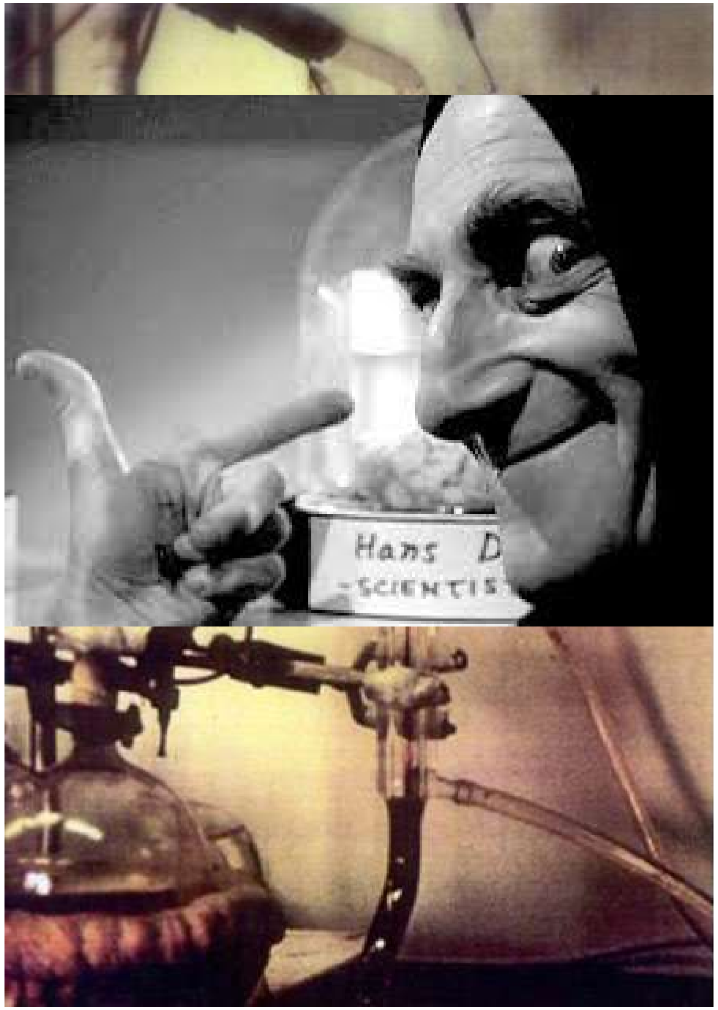
| | **Reduced** | **Neutral** | **Oxic** |
|---|---|---|---|
| Carbon (C) | $CH_4$ | $CO$, $CO_2$ | $CO_2$ |
| Nitrogen (N) | $NH_3$ | $N_2$ | $N_2$ |
| Oxygen (O) | $H_2O$ | $H_2O$, $CO$, $CO_2$ | $O_2$ |
| Hydrogen (H) | $H_2$, $CH_4$, $NH_3$, $H_2O$ | $H_2O$ | $H_2O$ |

# *Miller-Urey experiment - 1952*

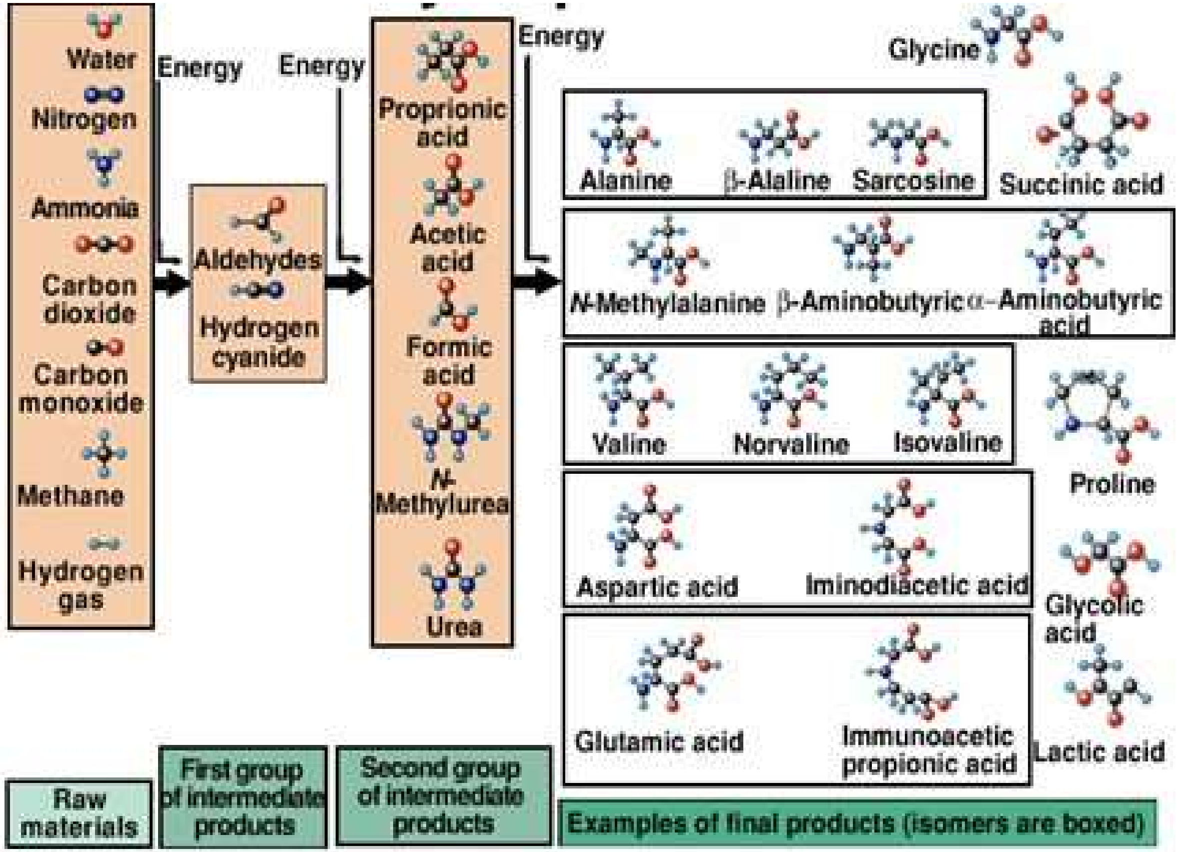
Harold Urey (1893-1981)
*UCSD, Nobel prize 1934*
*Discovery of deuterium*

Stanley Miller (1930-2007)
*UCSD San Diego, CA, USA*





# *Products of the Miller-Urey experiment*



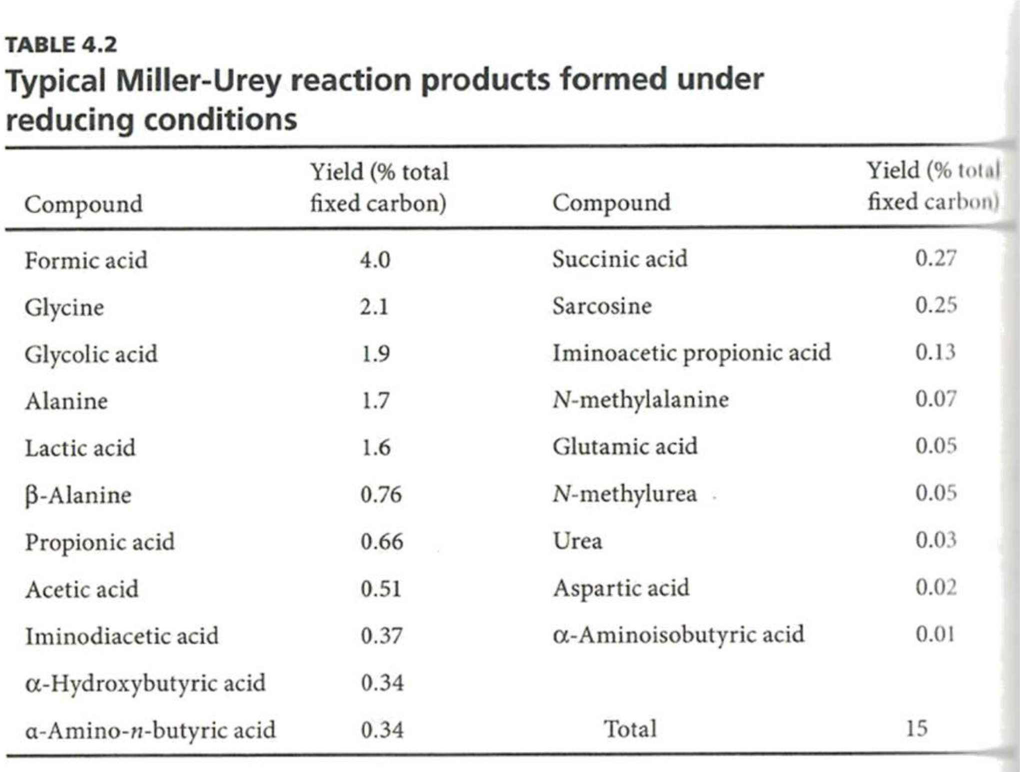


**TABLE 4.2**

**Typical Miller-Urey reaction products formed under reducing conditions**

| Compound | Yield (% total fixed carbon) | Compound | Yield (% total fixed carbon) |
|---|---|---|---|
| Formic acid | 4.0 | Succinic acid | 0.27 |
| Glycine | 2.1 | Sarcosine | 0.25 |
| Glycolic acid | 1.9 | Iminoacetic propionic acid | 0.13 |
| Alanine | 1.7 | *N*-methylalanine | 0.07 |
| Lactic acid | 1.6 | Glutamic acid | 0.05 |
| β-Alanine | 0.76 | *N*-methylurea | 0.05 |
| Propionic acid | 0.66 | Urea | 0.03 |
| Acetic acid | 0.51 | Aspartic acid | 0.02 |
| Iminodiacetic acid | 0.37 | α-Aminoisobutyric acid | 0.01 |
| α-Hydroxybutyric acid | 0.34 | | |
| α-Amino-*n*-butyric acid | 0.34 | Total | 15 |

Cysteine and methionine also present, when $H_2S$ is added to the reaction mixture

Generation of radicals

$$H_2 \xrightarrow{\text{High-energy electrons or UV light}} 2\ H^\bullet$$

$$H_2O \longrightarrow H^\bullet + HO^\bullet$$

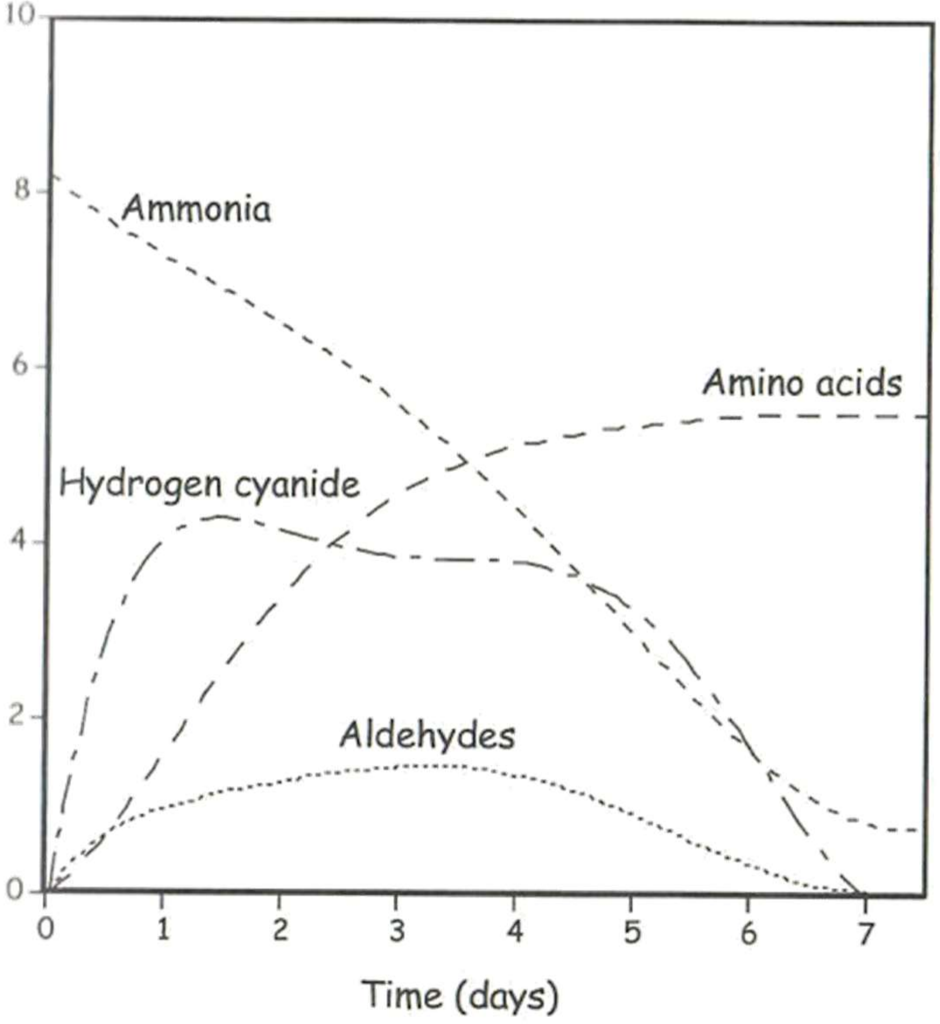
$$CH_4 \longrightarrow {}^\bullet CH_3 + {}^\bullet H$$

Radical reactions

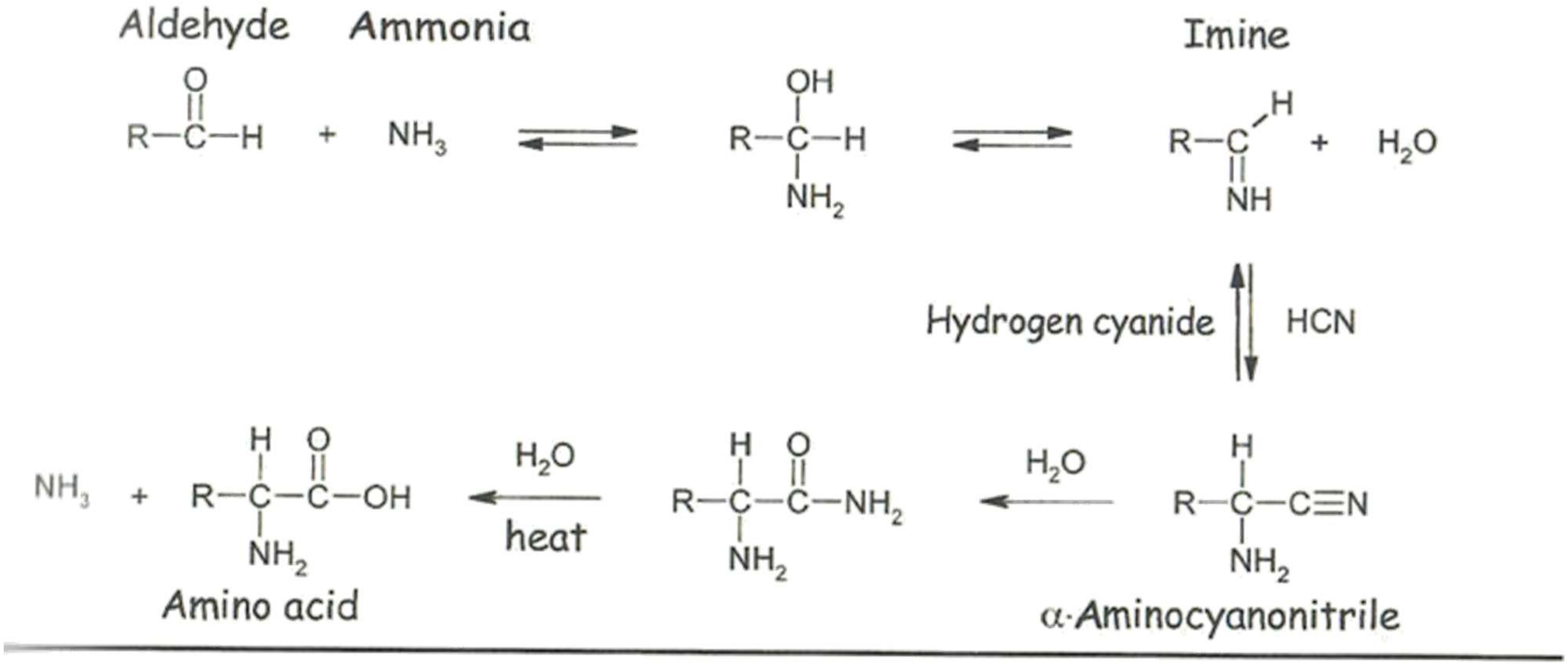
$${}^\bullet CH_3 + H_2O \longrightarrow H_3C{-}O^\bullet + H_2$$

$$H_3C{-}O^\bullet + H^\bullet \longrightarrow \underset{\text{Formaldehyde}}{H_2C{=}O} + H_2$$

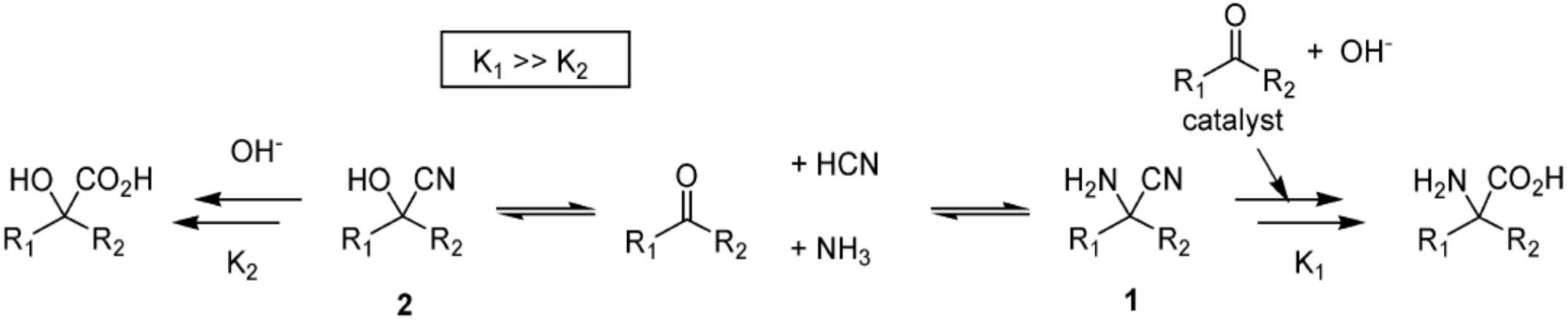
$${}^\bullet CH_3 + {}^\bullet CH_3 \longrightarrow \underset{\text{Ethane}}{H_3C{-}CH_3}$$



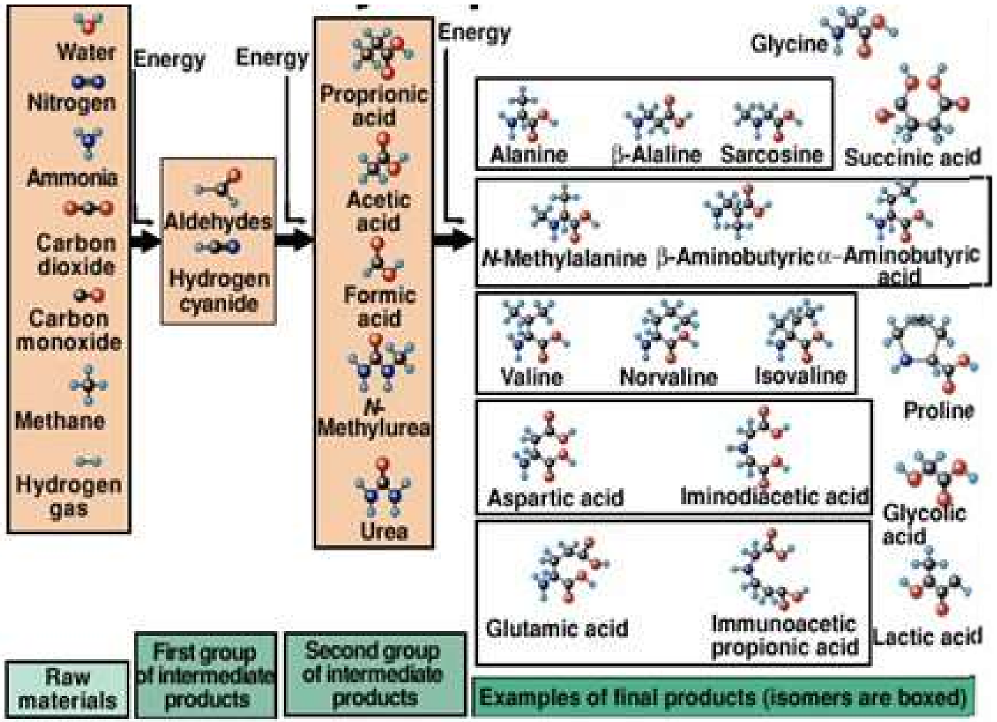
# *Strecker reaction*



### Scheme 1. Synthesis of *α*-Amino Acids through the Strecker Reaction



# *α-Aminoacid production in the Miller-Urey experiment*



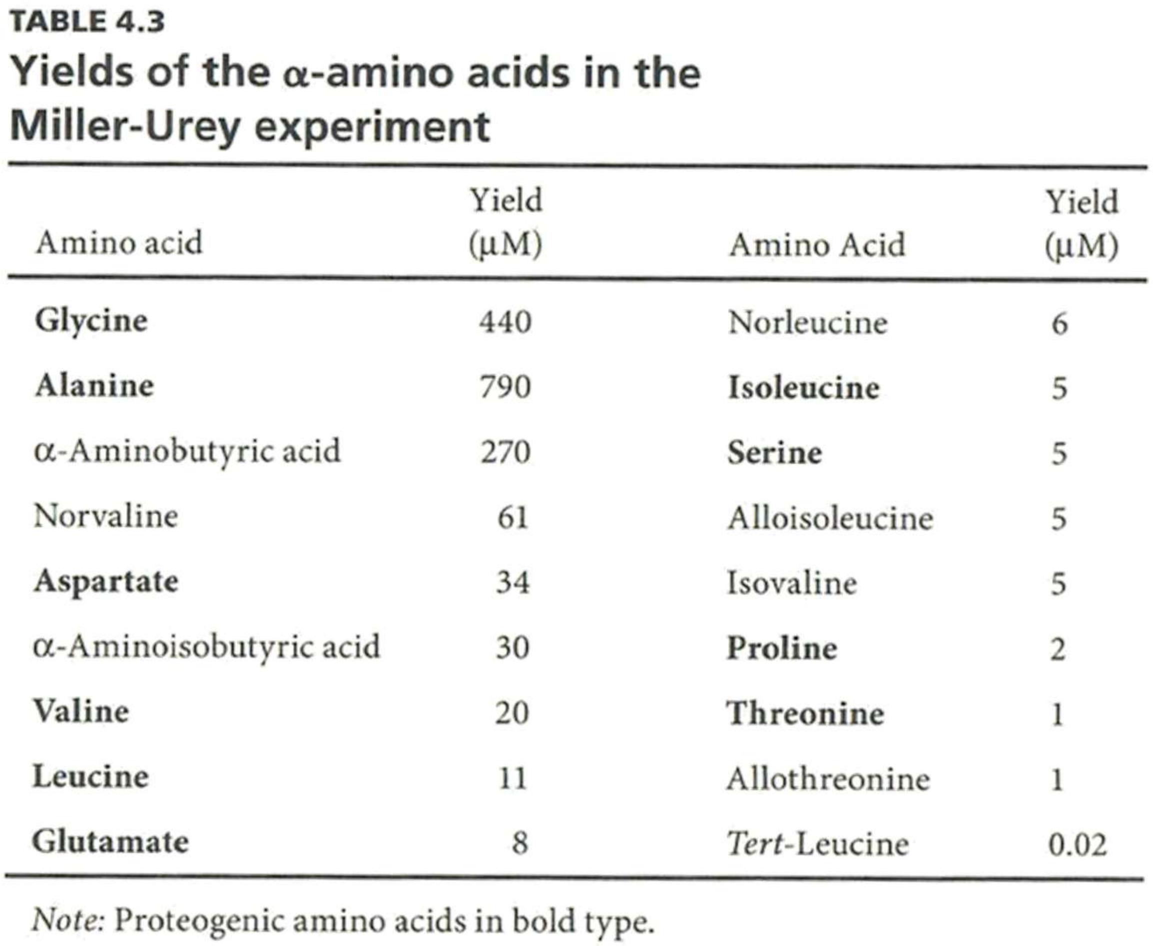


**TABLE 4.3**

**Yields of the α-amino acids in the Miller-Urey experiment**

| Amino acid | Yield (μM) | Amino Acid | Yield (μM) |
|---|---|---|---|
| **Glycine** | 440 | Norleucine | 6 |
| **Alanine** | 790 | **Isoleucine** | 5 |
| α-Aminobutyric acid | 270 | **Serine** | 5 |
| Norvaline | 61 | Alloisoleucine | 5 |
| **Aspartate** | 34 | Isovaline | 5 |
| α-Aminoisobutyric acid | 30 | **Proline** | 2 |
| **Valine** | 20 | **Threonine** | 1 |
| **Leucine** | 11 | Allothreonine | 1 |
| **Glutamate** | 8 | *Tert*-Leucine | 0.02 |

*Note:* Proteogenic amino acids in bold type.

# *Aminoacid production under hydrothermal conditions*

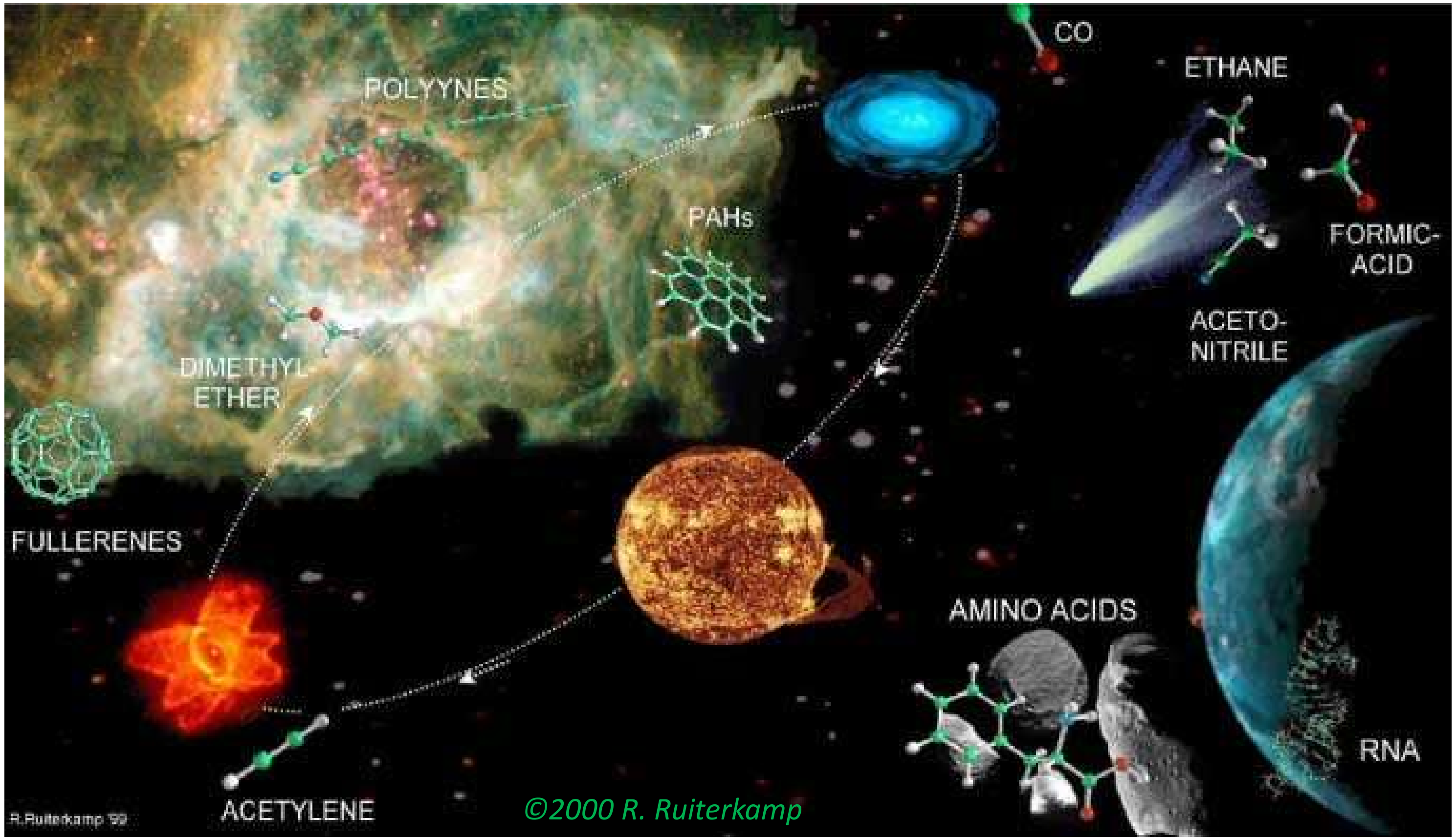
$Ni(OH)_2/KCN/CO$ in alkaline aqueous conditions (80-120$^0$C) → α-amino and α-hydroxyacids

Huber, C.; Wächtershäuser, G. *Science* **2006**, *314*, 630–632

$Ca(OH)_2/NiSO_4/KCN/CO$ in alkaline (pH 9.1-12.9) aqueous conditions (145-280$^0$C) → α-amino and α-hydroxyacids (higher yields): glycine, alanine, serine, glycolate, lactate, glycerate

Huber, C.; Eisenreich, H.; Wächtershäuser, G. *Tetrahedron Lett.* **2010**, *51*, 1069-1071

# *Extraterrestrial origin of biomolecules*



©2000 R. Ruiterkamp

# *Extraterrestrial origin of biomolecules*

Murchison meteorite
*chondrite*

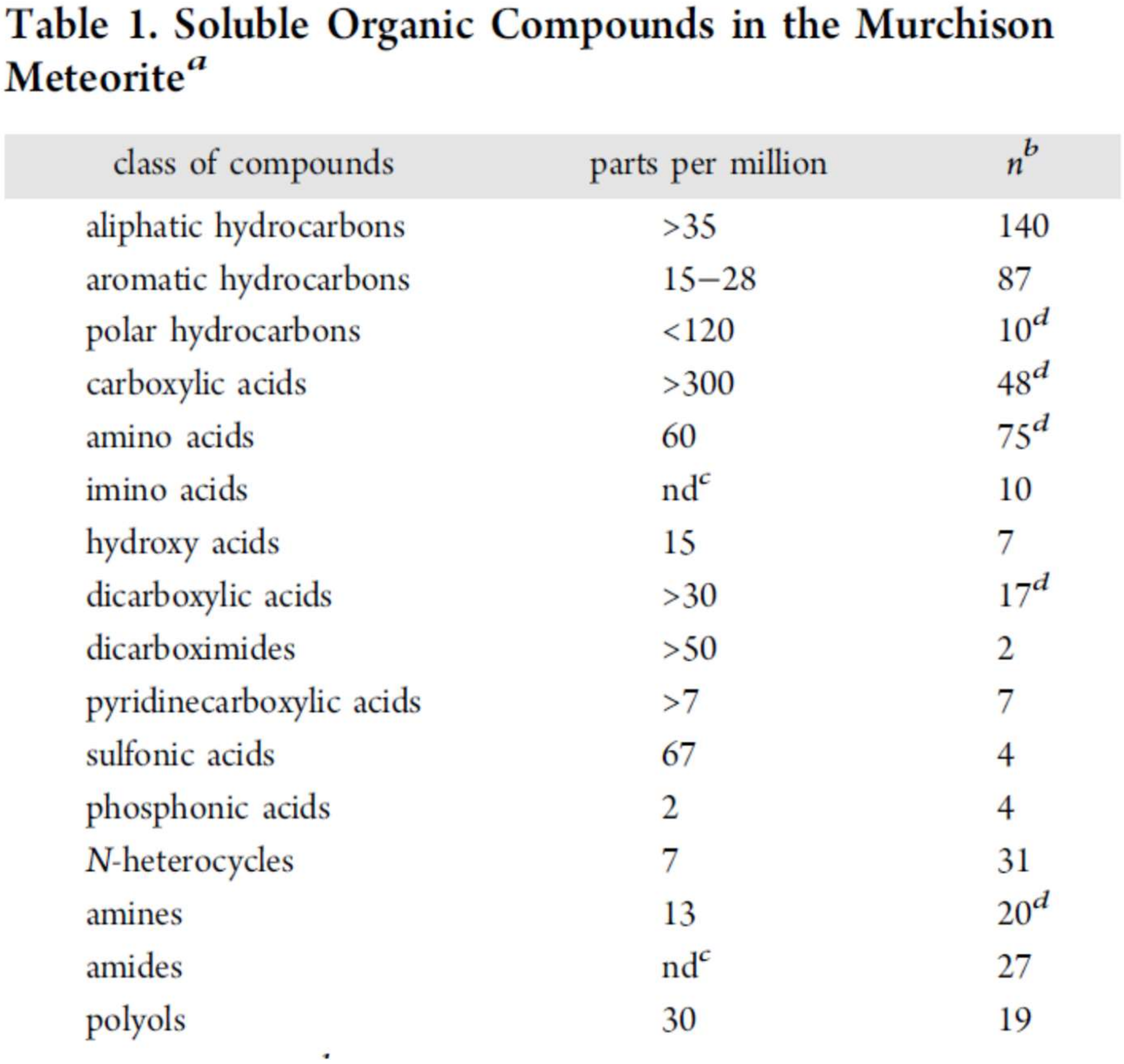
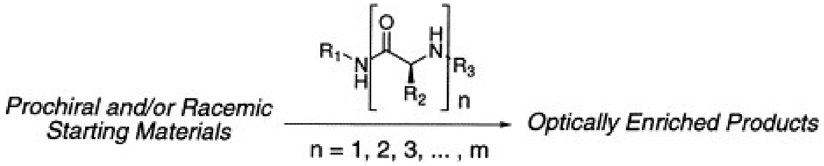


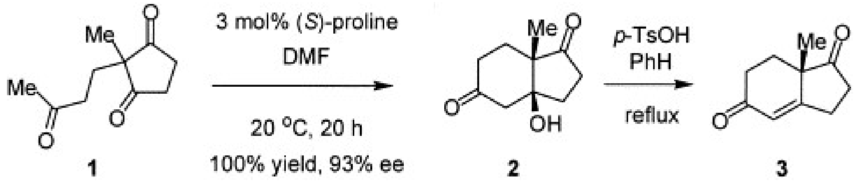
**Table 1. Soluble Organic Compounds in the Murchison Meteorite[a]**

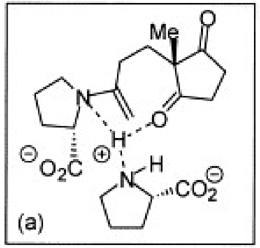
| class of compounds | parts per million | *n*[b] |
|---|---|---|
| aliphatic hydrocarbons | >35 | 140 |
| aromatic hydrocarbons | 15–28 | 87 |
| polar hydrocarbons | <120 | 10[d] |
| carboxylic acids | >300 | 48[d] |
| amino acids | 60 | 75[d] |
| imino acids | nd[c] | 10 |
| hydroxy acids | 15 | 7 |
| dicarboxylic acids | >30 | 17[d] |
| dicarboximides | >50 | 2 |
| pyridinecarboxylic acids | >7 | 7 |
| sulfonic acids | 67 | 4 |
| phosphonic acids | 2 | 4 |
| *N*-heterocycles | 7 | 31 |
| amines | 13 | 20[d] |
| amides | nd[c] | 27 |
| polyols | 30 | 19 |

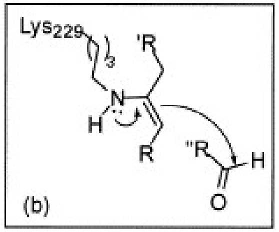
# *Catalytic properties of aminoacids - organocatalysis*



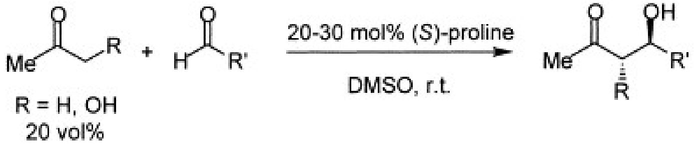
## *Robinson annulation*

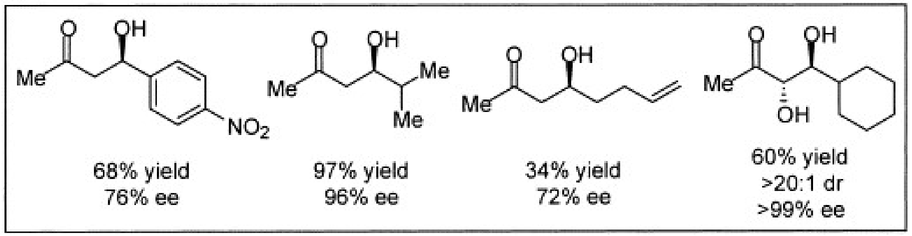


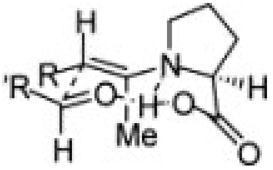




## *aldol reaction*



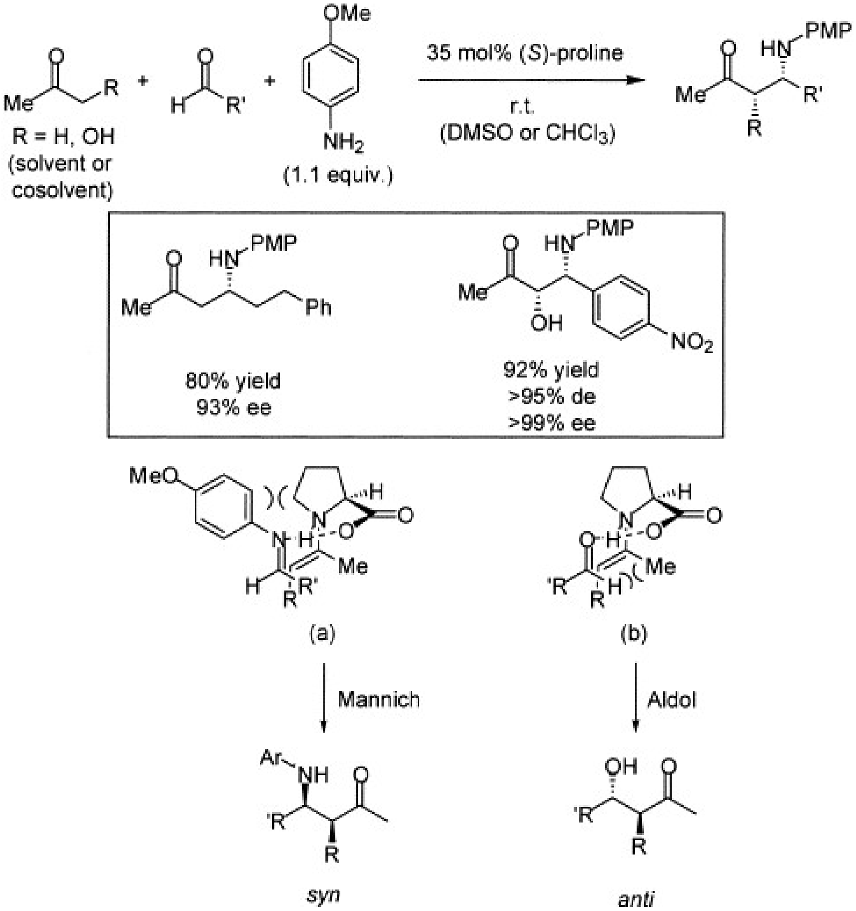




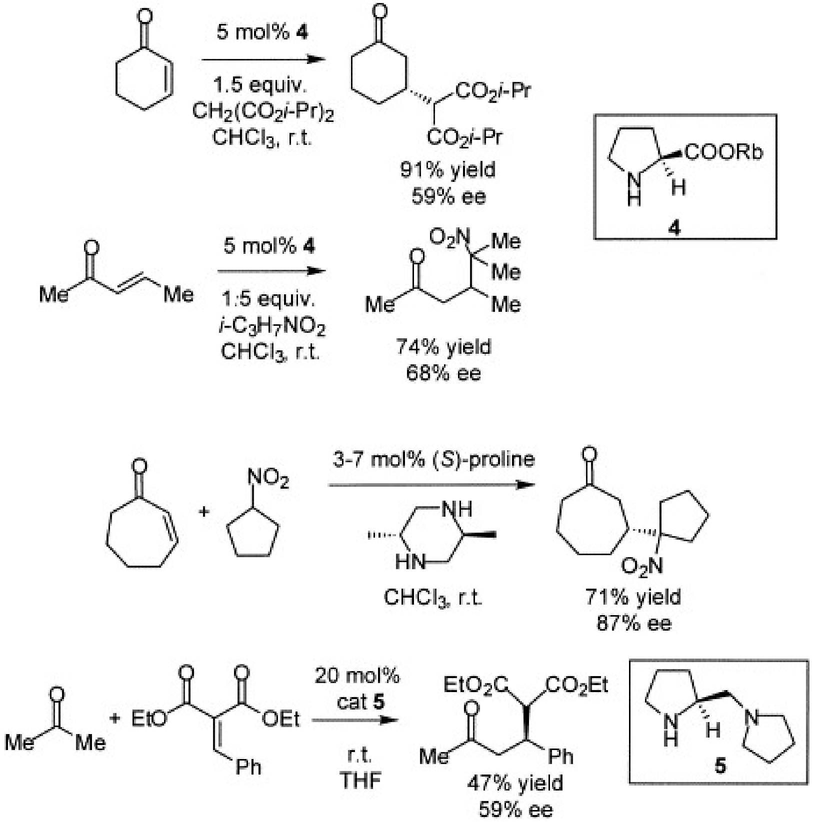
Jarvo, E. R., Miller, S. J. *Tetrahedron* **2002**, *58(13)*, 2481-2495.

# *Catalytic properties of aminoacids - organocatalysis*

## *Mannich reaction*



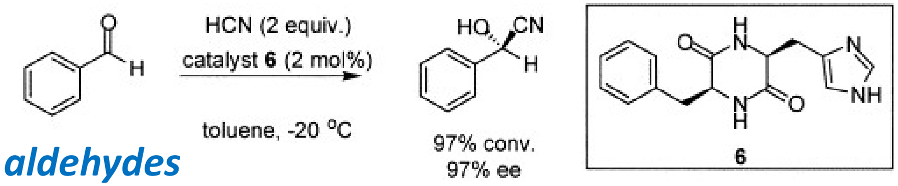
## *Michael addition*



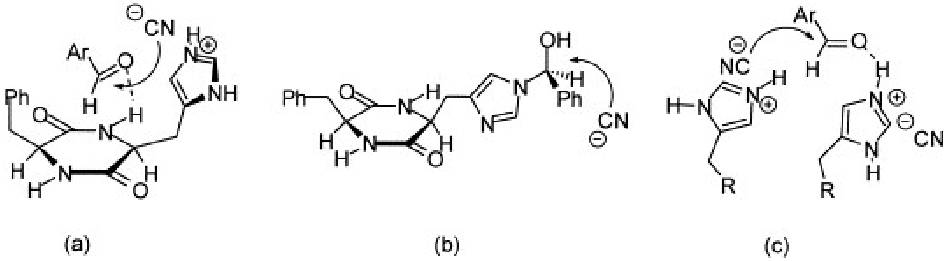
Jarvo, E. R., Miller, S. J. *Tetrahedron* **2002**, *58(13)*, 2481-2495.

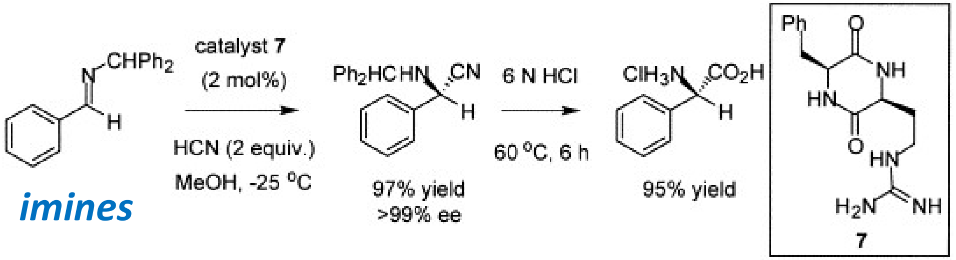
# *Catalytic properties of aminoacids - organocatalysis*

## *Hydrocyanation*



***aldehydes***



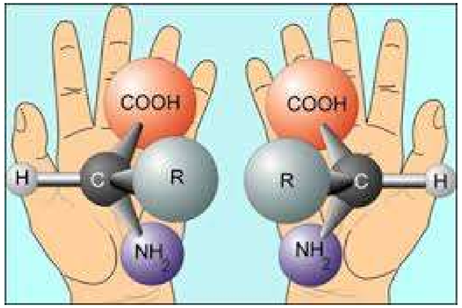


***imines***

***assymetric Strecker reaction!!!***

Jarvo, E. R., Miller, S. J. *Tetrahedron* **2002**, *58(13)*, 2481-2495.

# *The origins of homochirality*



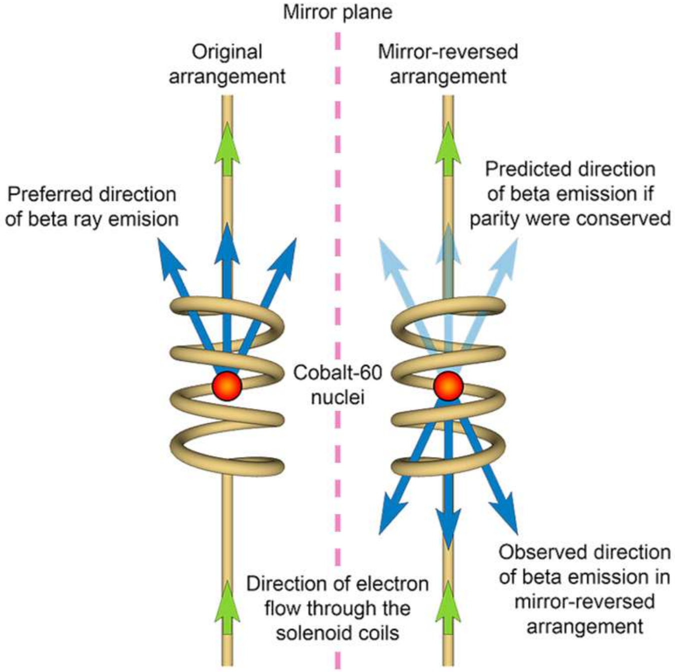
Currently known biopolymers are homochiral

Structural propensity and catalytic activity strongly depends on the enantopurity
→ Homochirality must have been involved early in the process of life formation
→ Chiral monomers could be only partially enantioenriched

Chien-Shiung Wu (1956) – experiment on $^{60}$Co decay

General cause of homochirality:
the initial symmetry breaking + subsequent assymetry amplification:

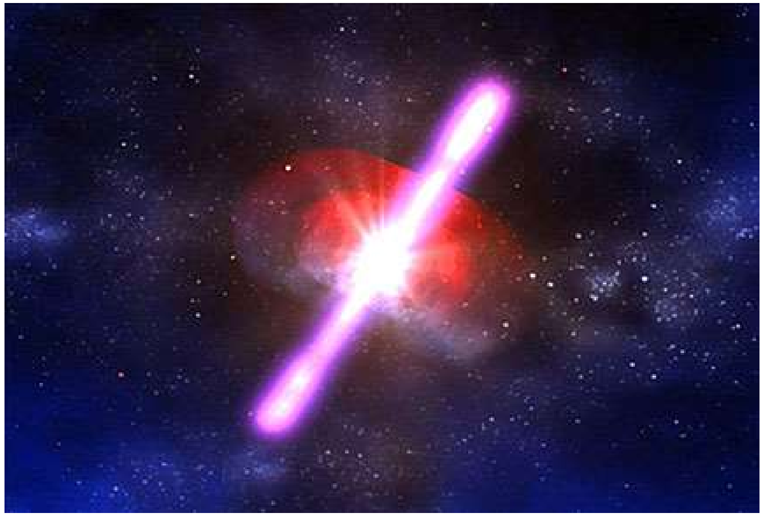
- ***The pairity violation***
- ***Stochastic symmetry disturbances***



Electroweak interactions and the pairity violation principle cause *L*-aminoacids and *D*-sugars to be SLIGHTLY MORE STABLE than their enantiomers

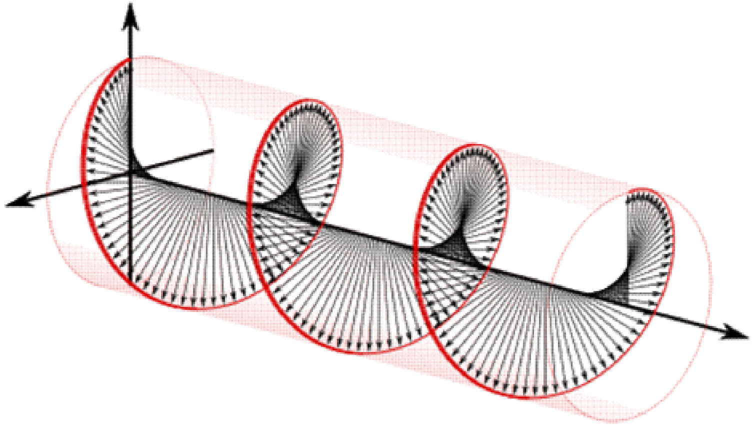
Differentiation in left and right handedness is inherent property of weak interactions

# *The origins of homochirality*

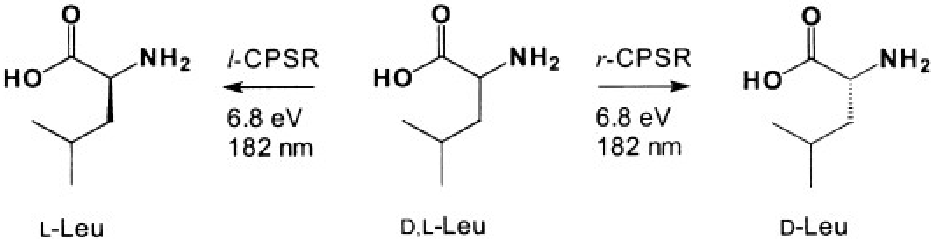


K. Wiersema et al., *Nature* **509** 201, 2014

Circularly polarized light (CPL) from gamma ray bursts



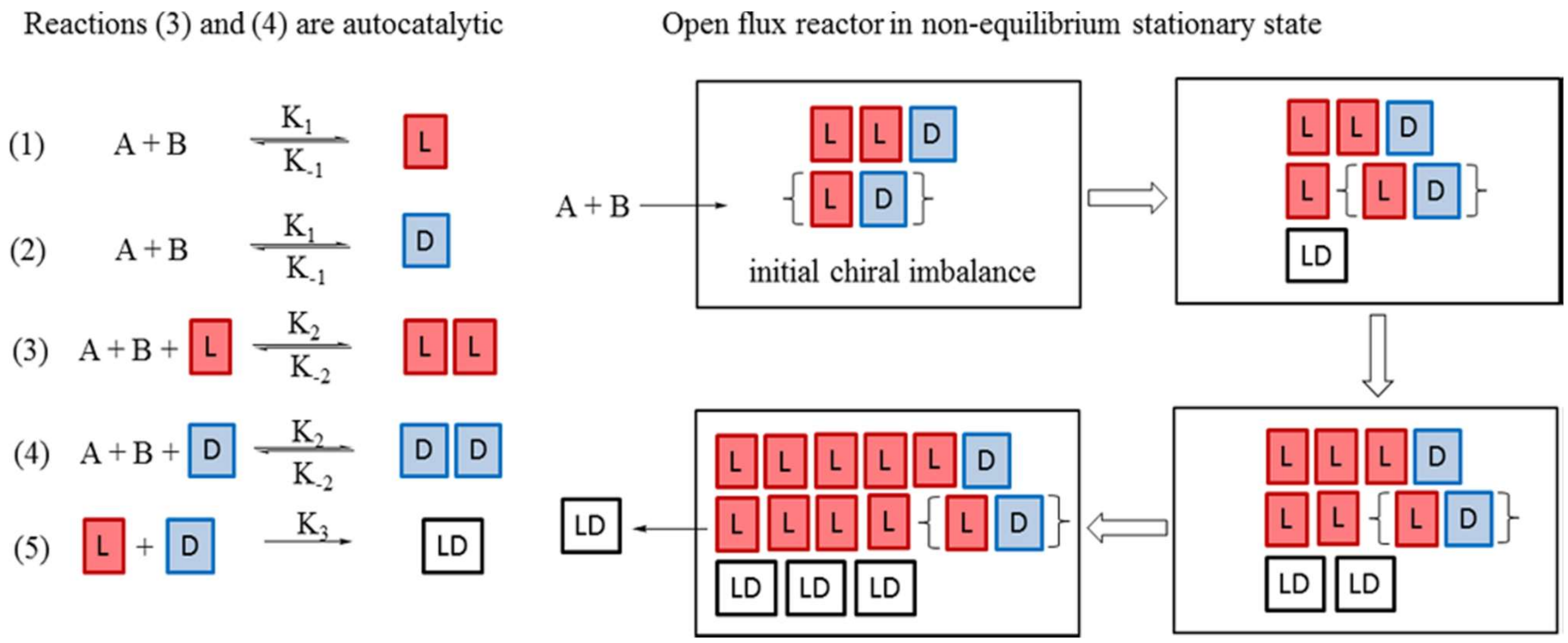
Small enantiomeric excess can be obtained by enantioselective degradation of aminoacids with CPL



*Up to 2.6% ee*

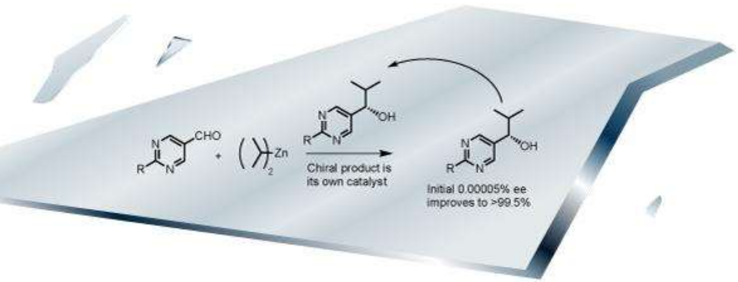
*Meierhenrich, U. J.; Nahon, L.; Alcaraz, C.; Bredehoft, J. H.; Hoffmann, S. V.; Barbier, B.; Brack, A. Angew. Chem., Int. Ed. 2005, 44, 5630*

# *Stochastic induction of assymetry – Frank model*



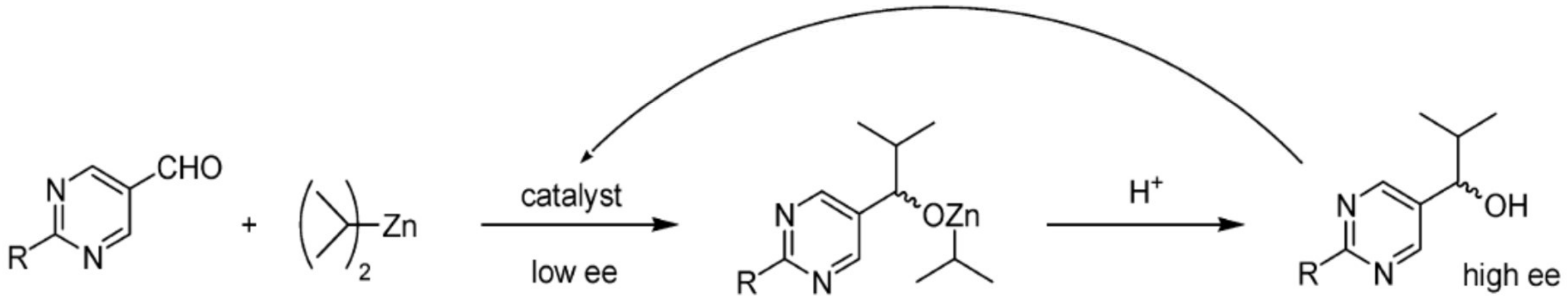
If a chiral dissipative structure catalyzes its own formation and inhibits formation of the opposite enantiomer, any stochastic symmetry breaking in the system will be amplified

# *autocatalytic Soai reaction – extreme chirality amplification*



*Organometallic reaction*
***- NOT prebiotic***

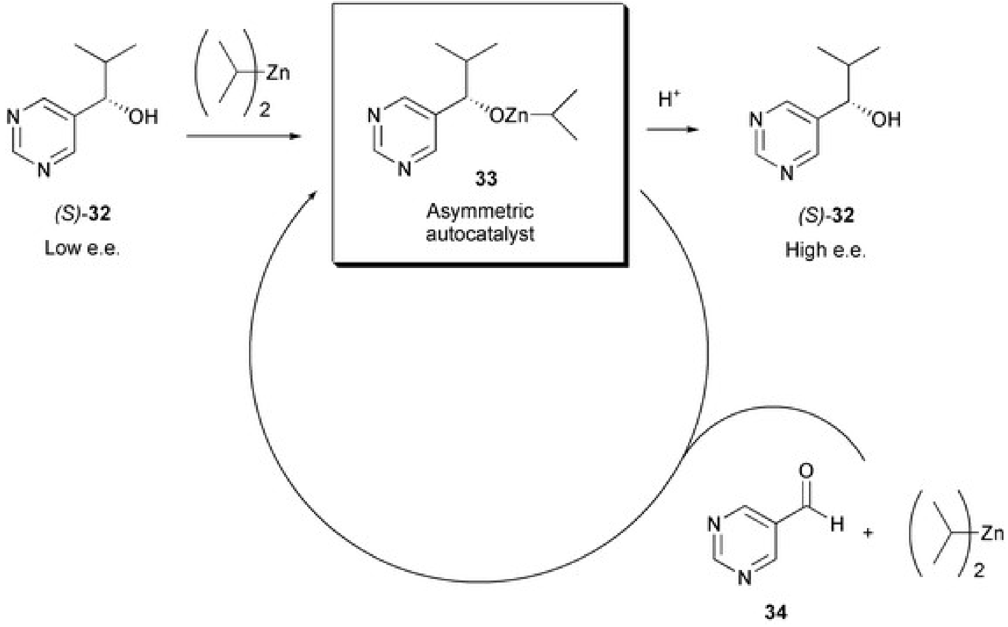
Scheme 9. Soai Autocatalytic Reaction

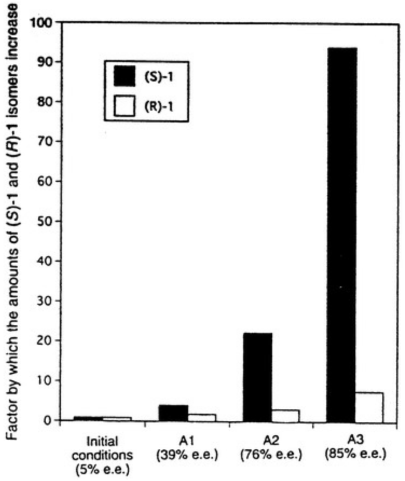


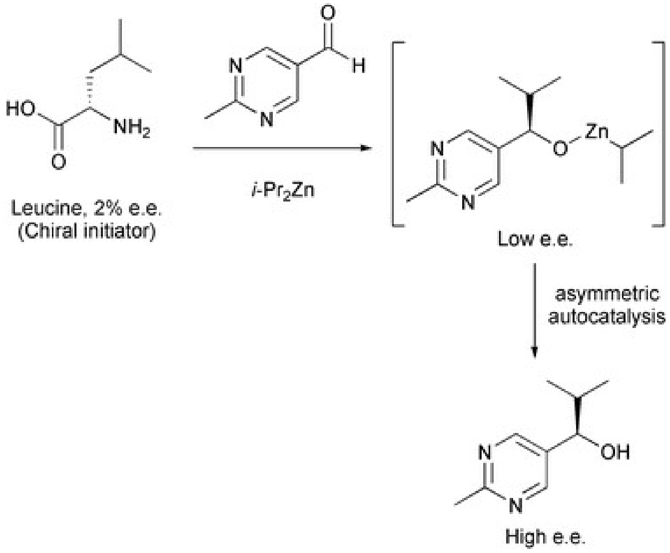
***CPL***
***Aminoacids***
***$^{12}C/^{13}C$-enantiomers!***

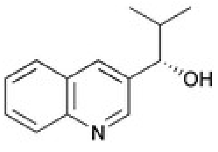
*Extremelly sensitive*
*chirality detector*

# *autocatalytic Soai reaction – extreme chirality amplification*





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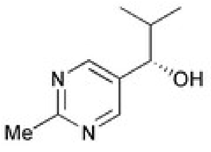
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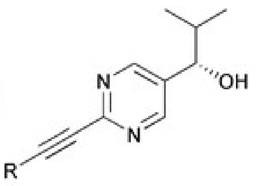
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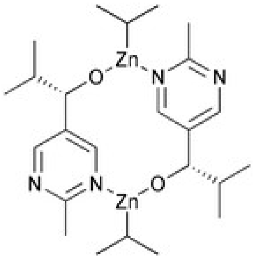
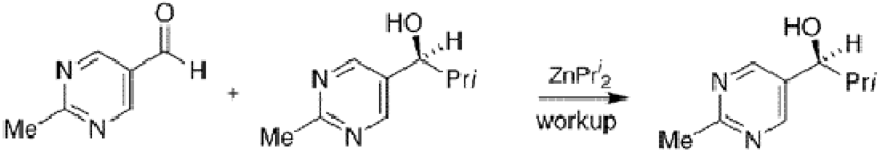

**38**

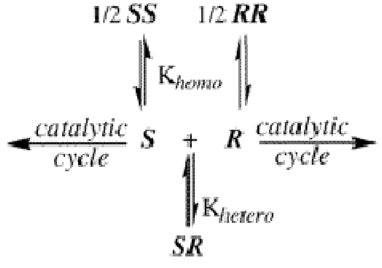
# *autocatalytic Soai reaction – extreme chirality amplification*



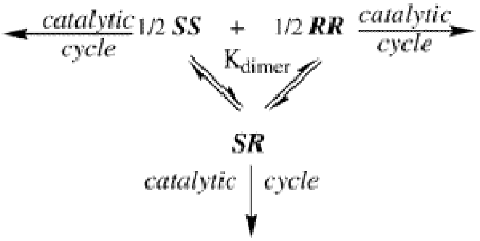
**1**

**2**
catalytic, low ee

**2**
higher ee



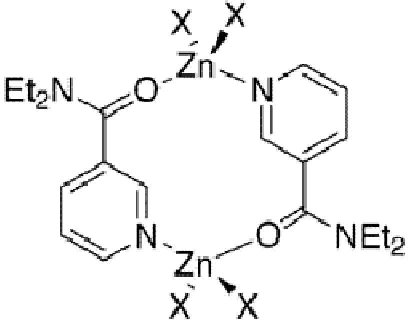
**a**



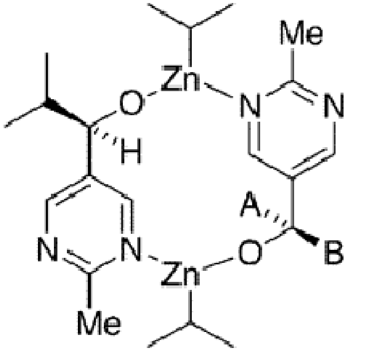
**b**

$$g = \frac{\text{activity of } [SR]}{\text{activity of } [RR]} \quad \beta = \frac{[SR]}{[RR] + [SS]} \quad K = \frac{([SR])^2}{([RR])([SS])}$$

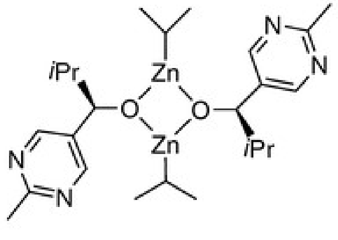
$$ee_{dx} = \frac{[RR] - [SS]}{[RR] + [SS] + g[SR]} \quad ee_{x+dx} = \frac{ee_x[\mathbf{2}] + ee_{dx}\frac{dx}{2}[\mathbf{1}]_0}{[\mathbf{2}] + \frac{dx}{2}[\mathbf{1}]_0} \tag{2}$$



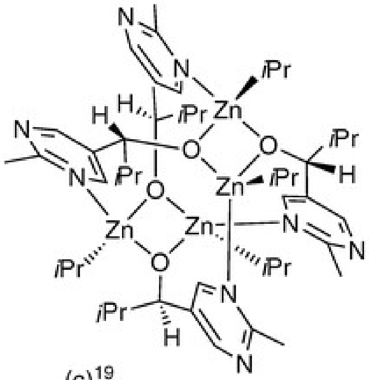
**7a**, X = Cl,
**7b**, X = SCN



***(S,S)*-8** A = H, B = Pr$^i$
***(R,S)*-8** A = Pr$^i$, B = H



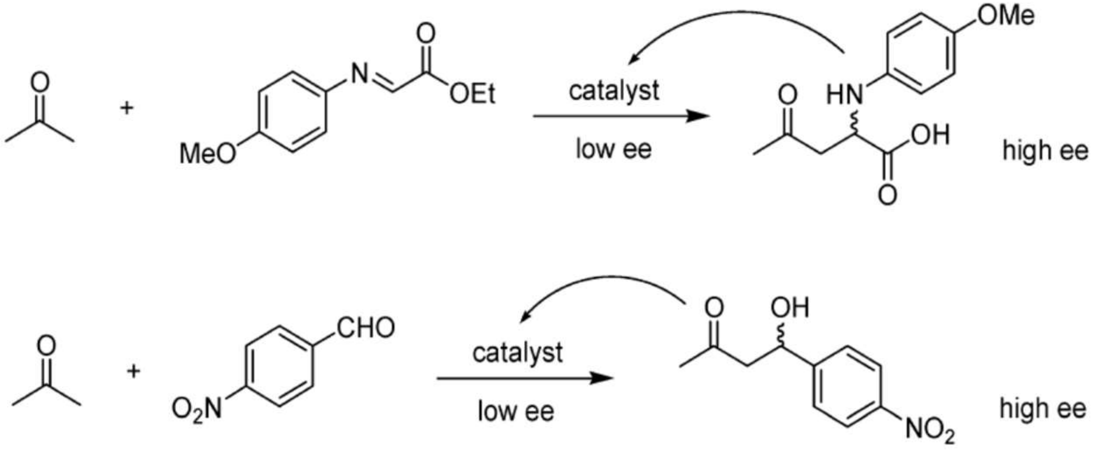
(b)[19]



(c)[19]

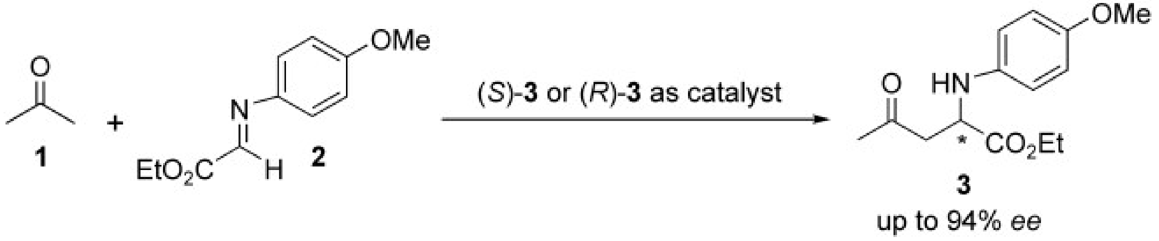
# *autocatalytic organic reactions*

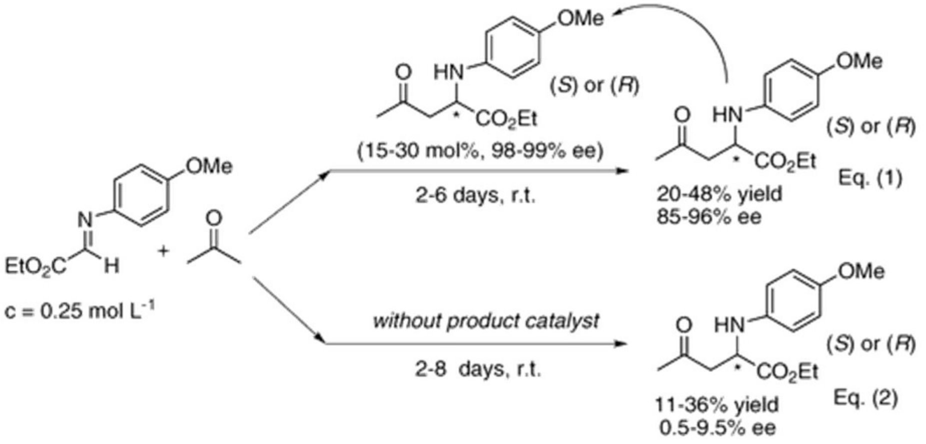
Scheme 10. Mannich and Aldol Autocatalytic Reactions[a]

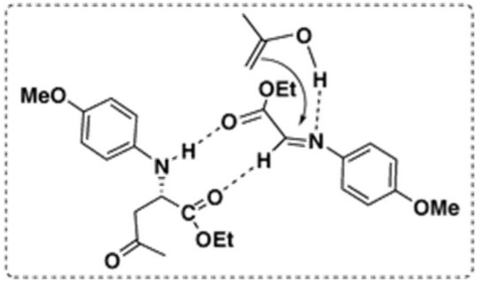


*Meaningful transformations for the prebiotic syntheses of aminoacids and sugars*

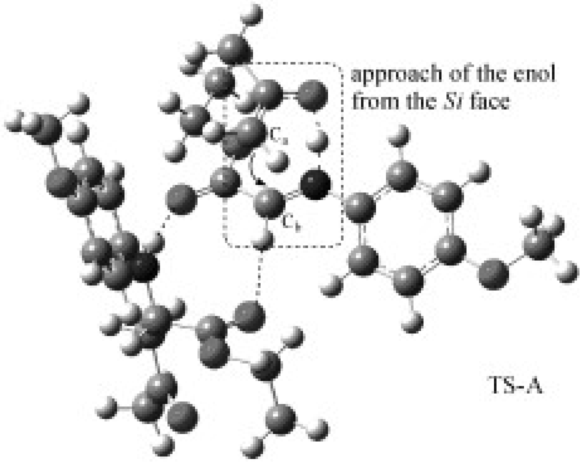
# *autocatalytic organic reactions*



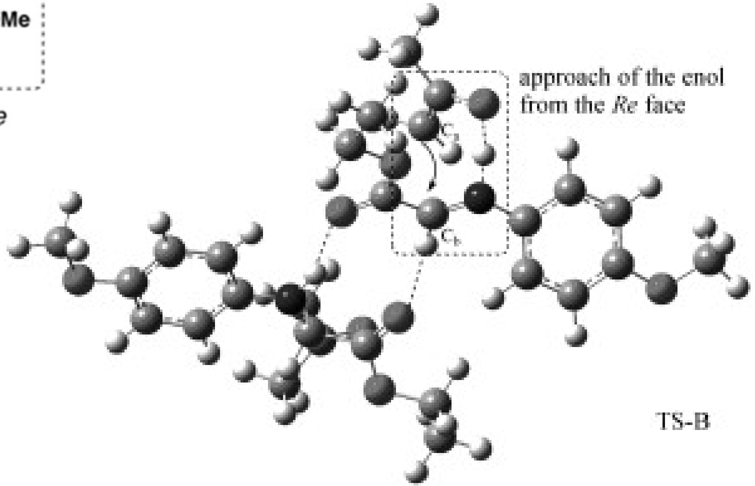




*proposed transition-state structure*



TS-A



TS-B

# *Organocatalysis – the origin of homochirality*

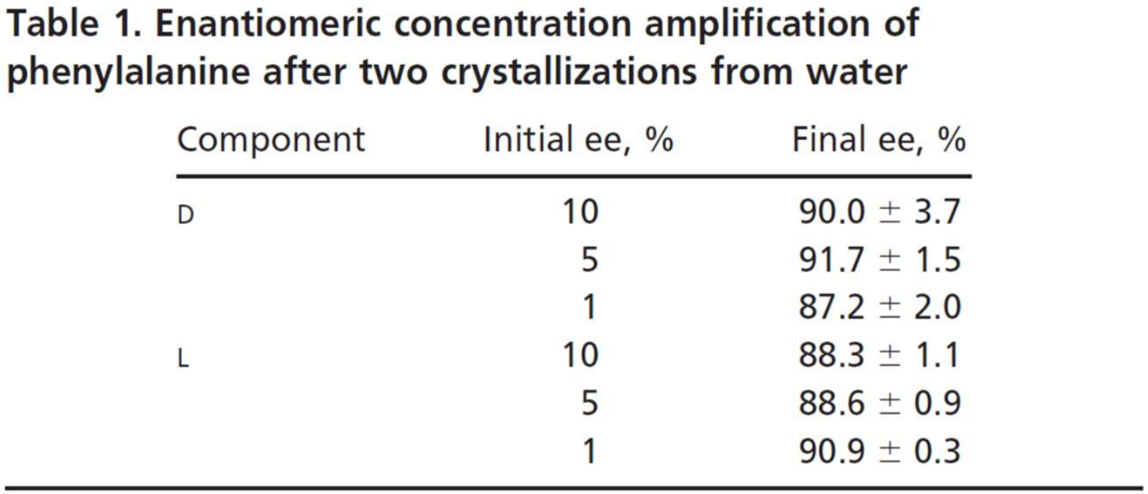
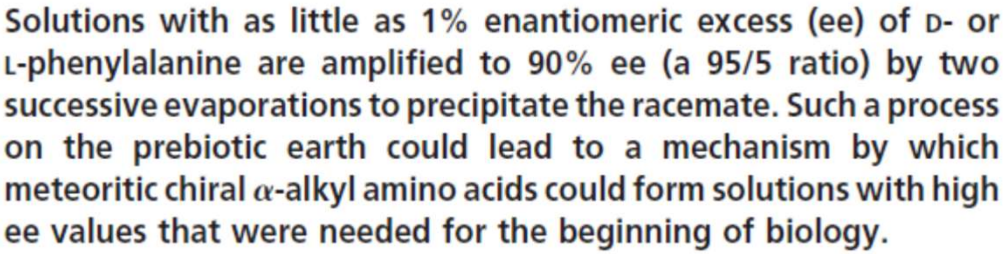


**Table 1. Enantiomeric concentration amplification of phenylalanine after two crystallizations from water**

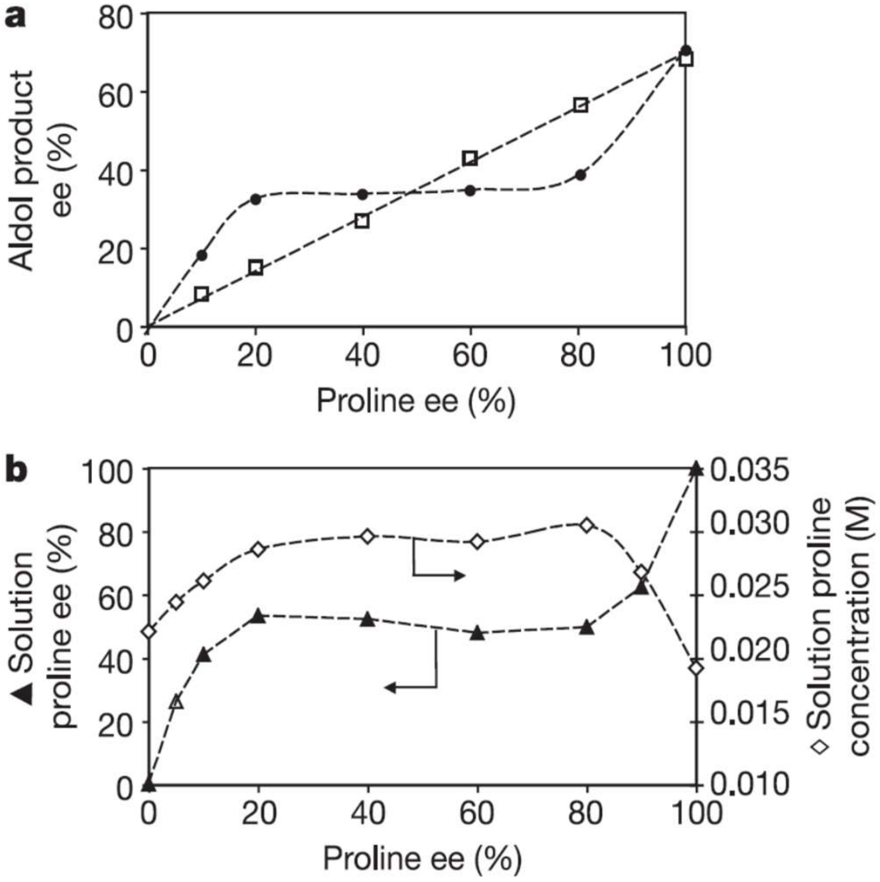
| Component | Initial ee, % | Final ee, % |
|---|---|---|
| D | 10 | 90.0 ± 3.7 |
| | 5 | 91.7 ± 1.5 |
| | 1 | 87.2 ± 2.0 |
| L | 10 | 88.3 ± 1.1 |
| | 5 | 88.6 ± 0.9 |
| | 1 | 90.9 ± 0.3 |

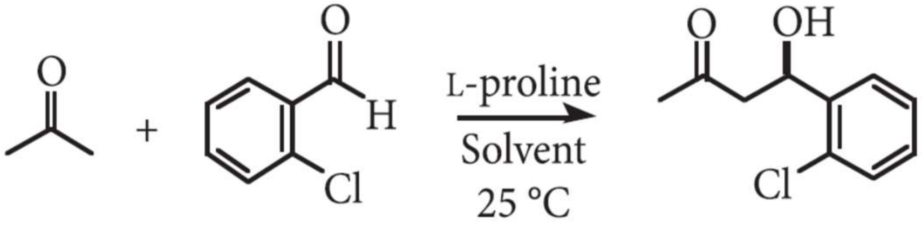


Solutions with as little as 1% enantiomeric excess (ee) of D- or L-phenylalanine are amplified to 90% ee (a 95/5 ratio) by two successive evaporations to precipitate the racemate. Such a process on the prebiotic earth could lead to a mechanism by which meteoritic chiral α-alkyl amino acids could form solutions with high ee values that were needed for the beginning of biology.

*Breslow, R., Levine, M. Proc. Natl. Acad. Sci. USA* **2006,** *103(35),* 12979-12980

# *Chirality amplification in biphasic systems*





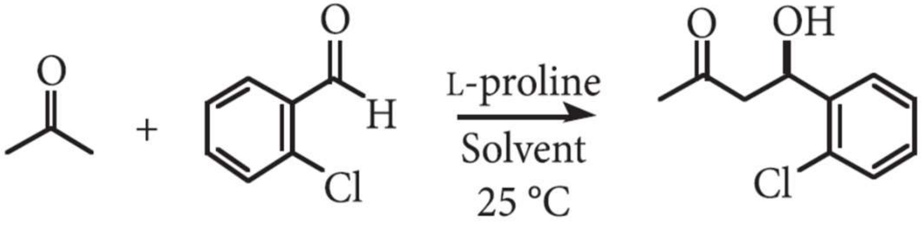
Reaction and solution behaviour as a function of the overall proline enantiomeric excess.

a, Product enantiomeric excess versus proline enantiomeric excess for the aldol reaction of equation

b, Solution proline enantiomeric excess (left axis, triangles) and solution proline concentration (right axis, diamonds) as a function of the overall enantiomeric excess for proline at 0.1 M

*Klussmann, M., et al. Nature* **2006,** *441,* 621-623

# *Chirality amplification in biphasic systems*



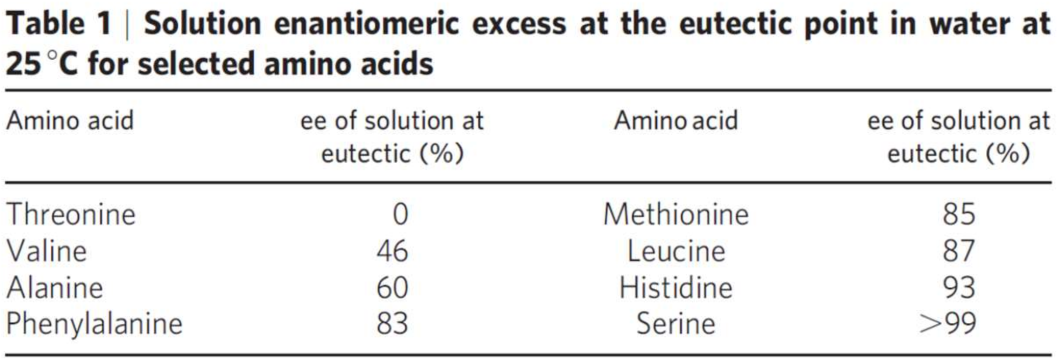
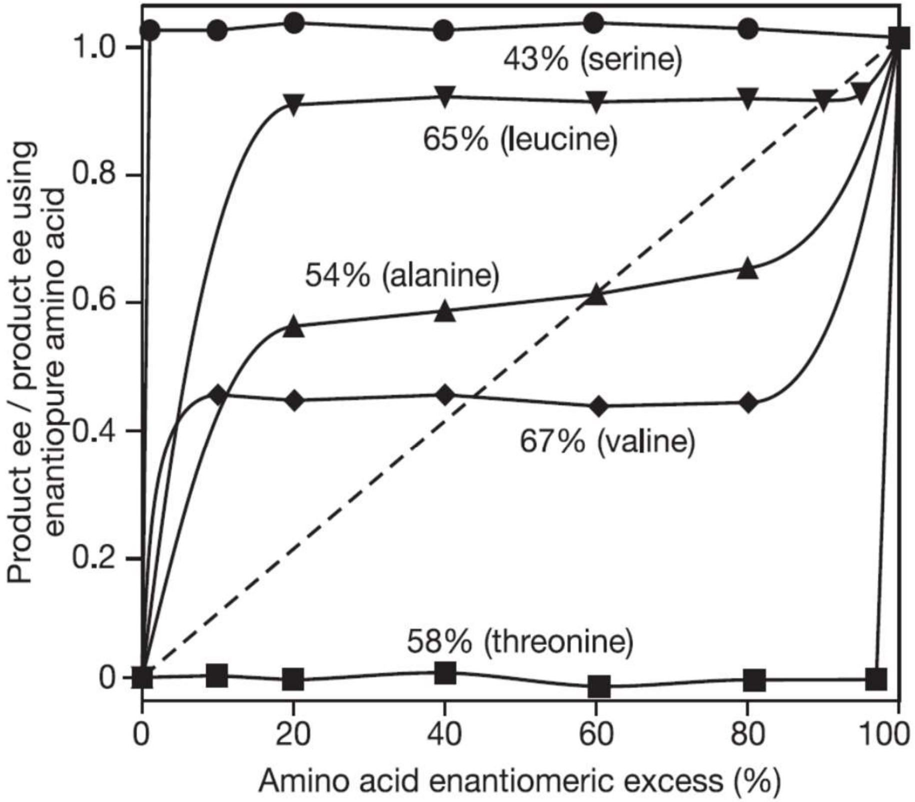


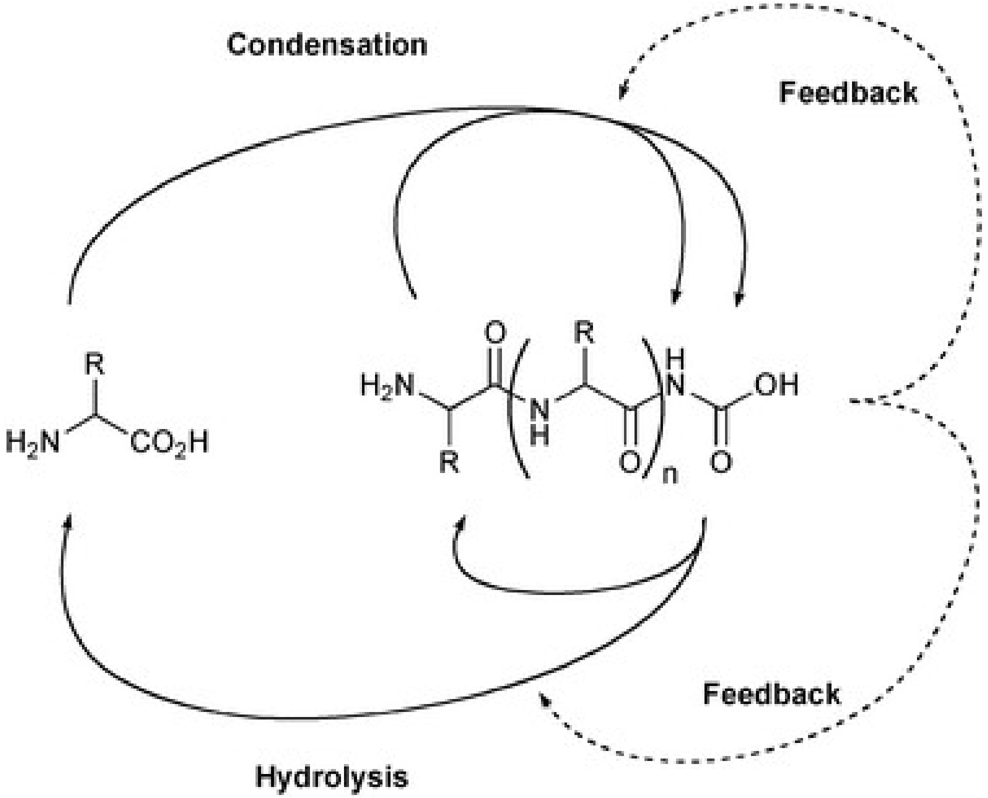
**Table 1** | **Solution enantiomeric excess at the eutectic point in water at 25 °C for selected amino acids**

| Amino acid | ee of solution at eutectic (%) | Amino acid | ee of solution at eutectic (%) |
|---|---|---|---|
| Threonine | 0 | Methionine | 85 |
| Valine | 46 | Leucine | 87 |
| Alanine | 60 | Histidine | 93 |
| Phenylalanine | 83 | Serine | >99 |

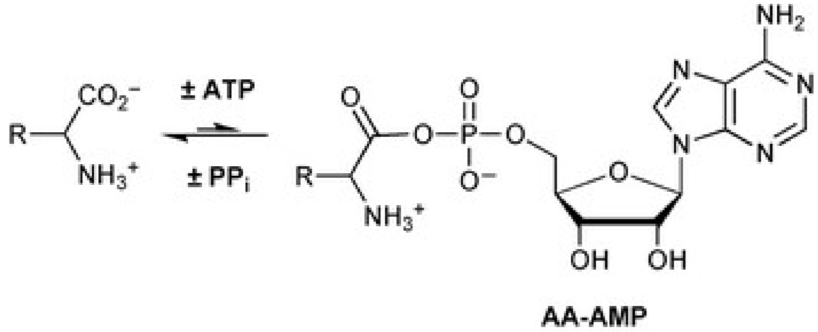


*Klussmann, M., et al. Nature* **2006,** *441*, 621-623

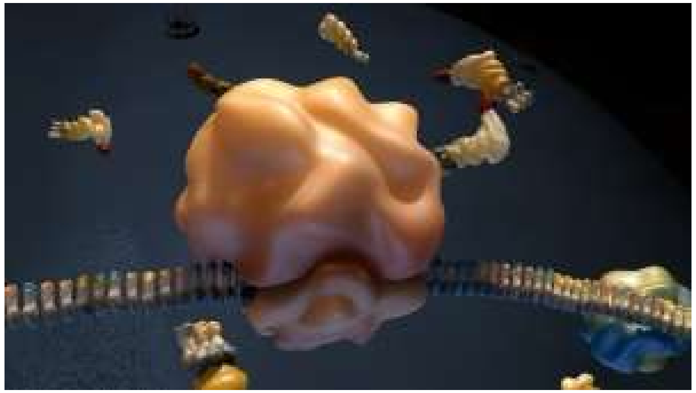
# *Condensation of aminoacids into peptides*



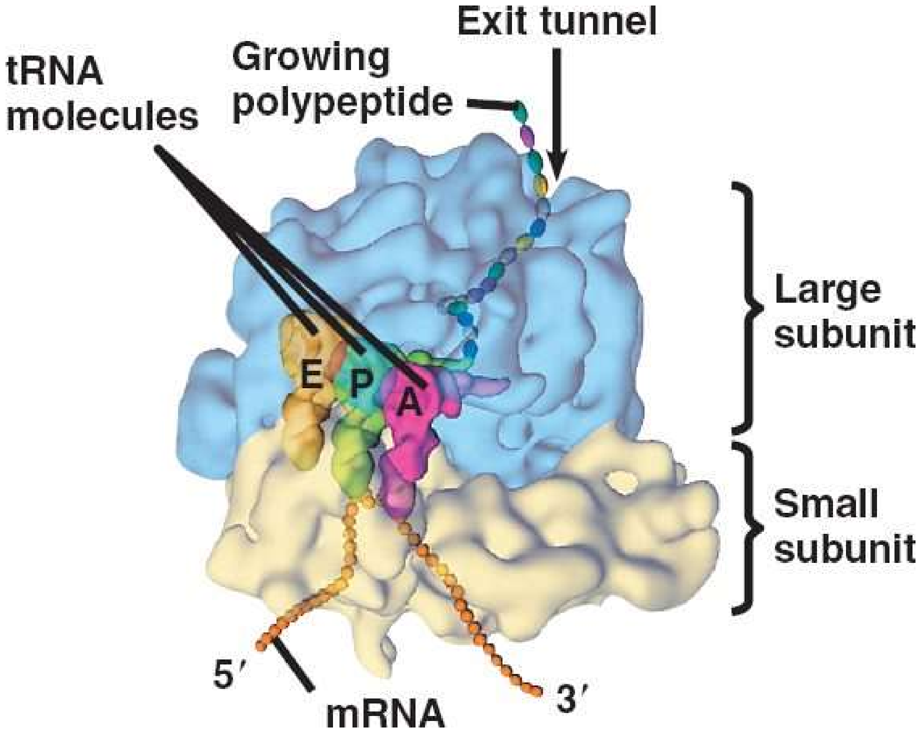
# *Biochemical condensation of aminoacids into peptides*



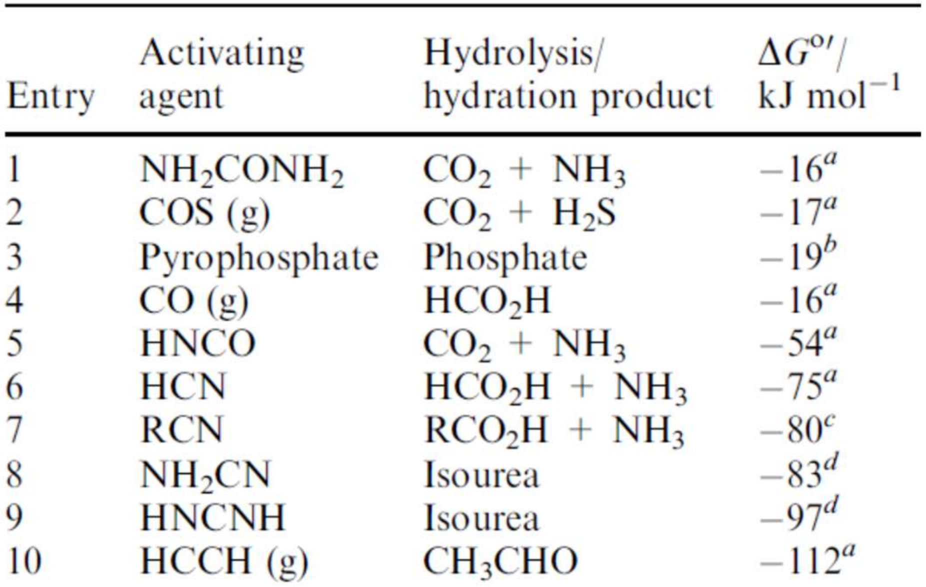
$$\text{AA-AMP} \xrightarrow{+\,\text{tRNA}} \text{AA-tRNA} \xrightarrow{\text{Ribosome}} \text{Peptides}$$



*Nature Publishing Group, www.nature.com/nrg/multimedia*

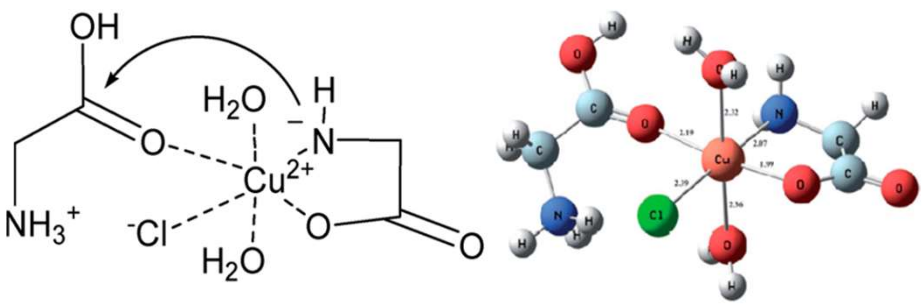


# *Prebiotically relevant peptide condensation agents*



| Entry | Activating agent | Hydrolysis/ hydration product | $\Delta G^{o\prime}$/ kJ $mol^{-1}$ |
|---|---|---|---|
| 1 | $NH_2CONH_2$ | $CO_2 + NH_3$ | −16[a] |
| 2 | COS (g) | $CO_2 + H_2S$ | −17[a] |
| 3 | Pyrophosphate | Phosphate | −19[b] |
| 4 | CO (g) | $HCO_2H$ | −16[a] |
| 5 | HNCO | $CO_2 + NH_3$ | −54[a] |
| 6 | HCN | $HCO_2H + NH_3$ | −75[a] |
| 7 | RCN | $RCO_2H + NH_3$ | −80[c] |
| 8 | $NH_2CN$ | Isourea | −83[d] |
| 9 | HNCNH | Isourea | −97[d] |
| 10 | HCCH (g) | $CH_3CHO$ | −112[a] |

Danger, G.; Plasson, R.; Pascal, R. *Chem. Soc. Rev.* **2012**, *41*, 5416.

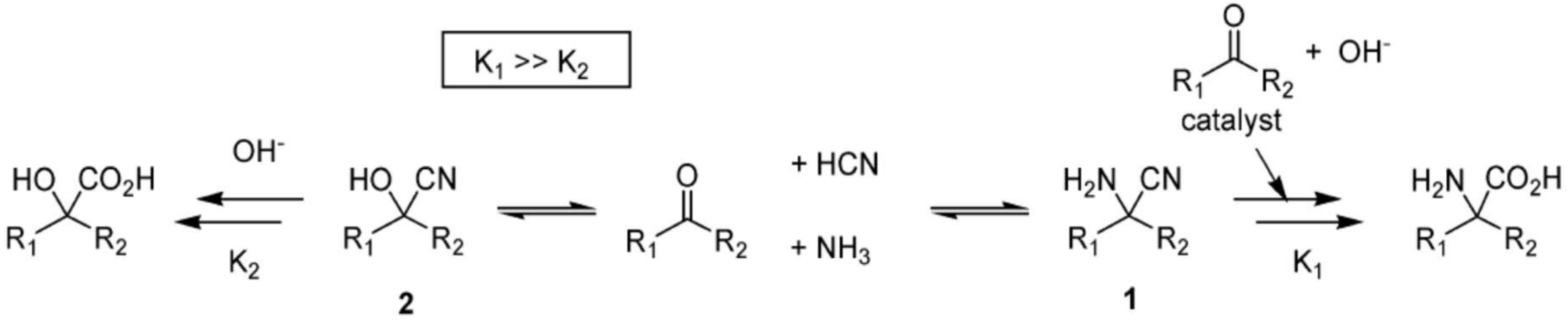


SIPF copper complex geometry with two glycine ligands, optimized by ab initio Hartree−Fock calculations.

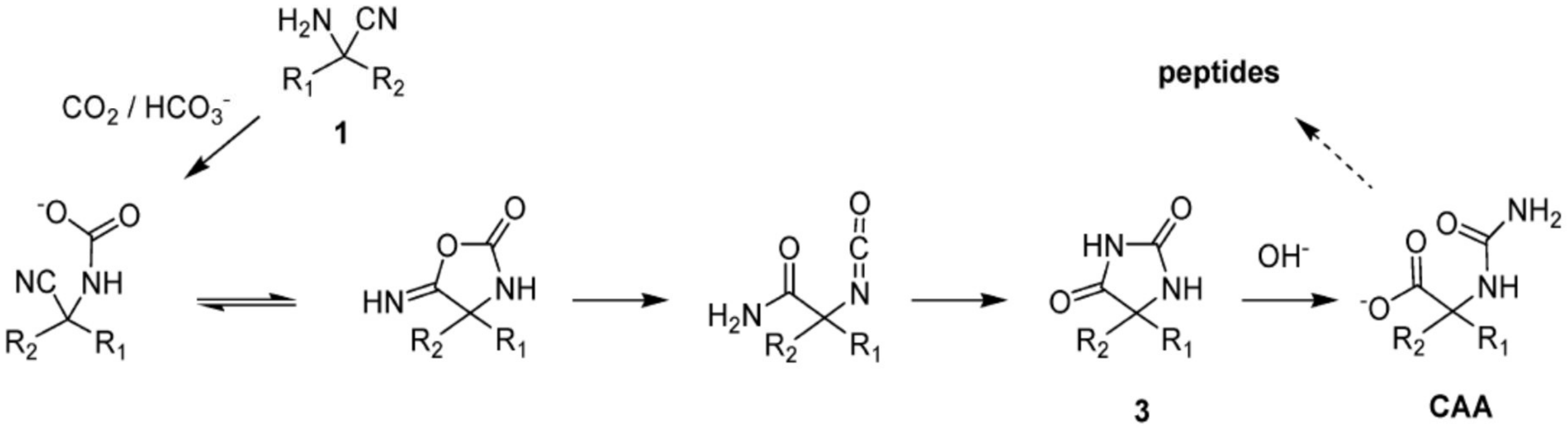
Rode, B. M.; Fitz, D.; Jakschitz, T. Chem. *Biodiversity* **2007**, *4*,2674.

# *Condensation of aminoacids into peptides*

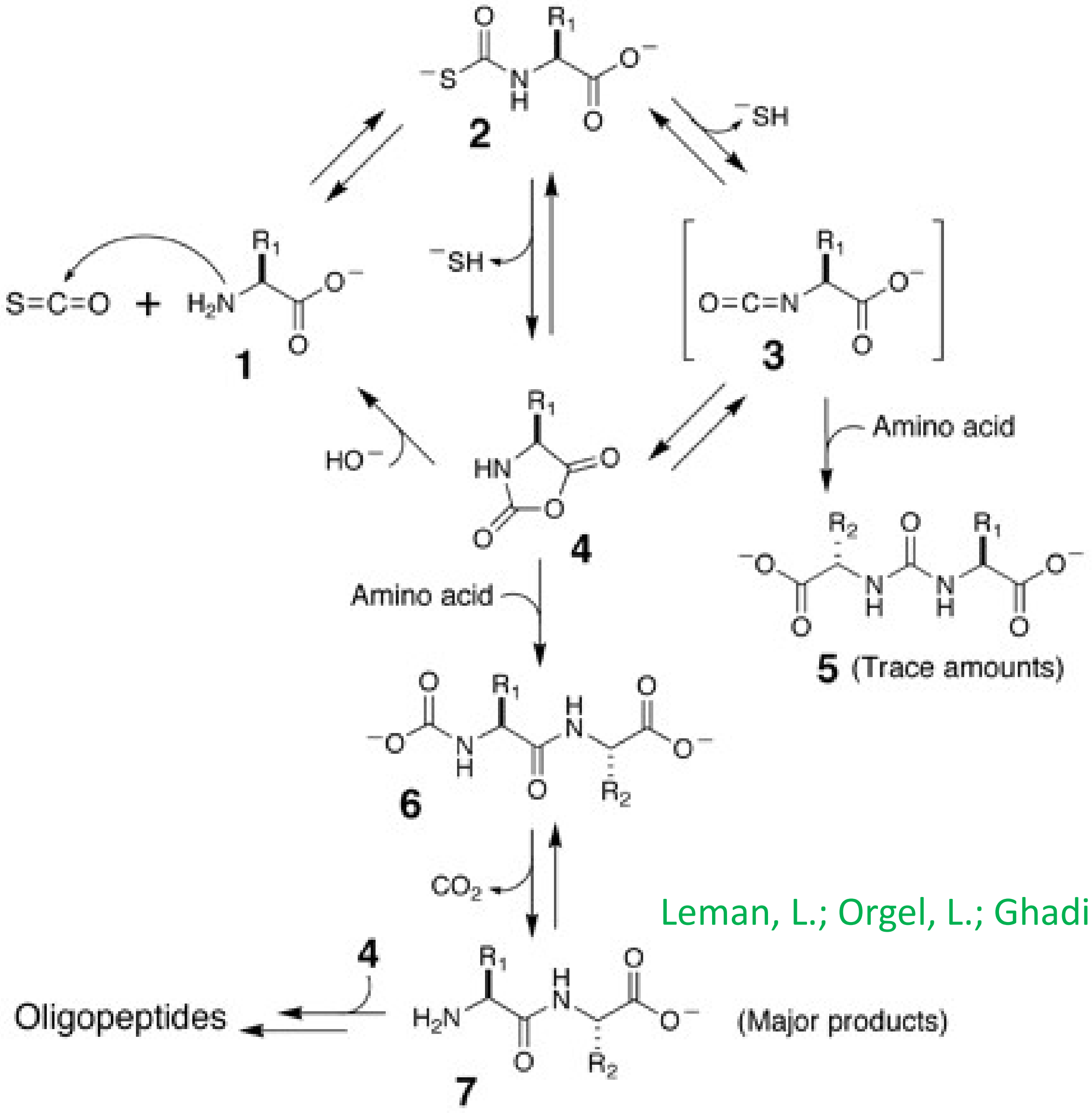
Scheme 1. Synthesis of $\alpha$-Amino Acids through the Strecker Reaction



Scheme 2. Bücherer–Bergs Hydrolysis of $\alpha$-Aminonitriles

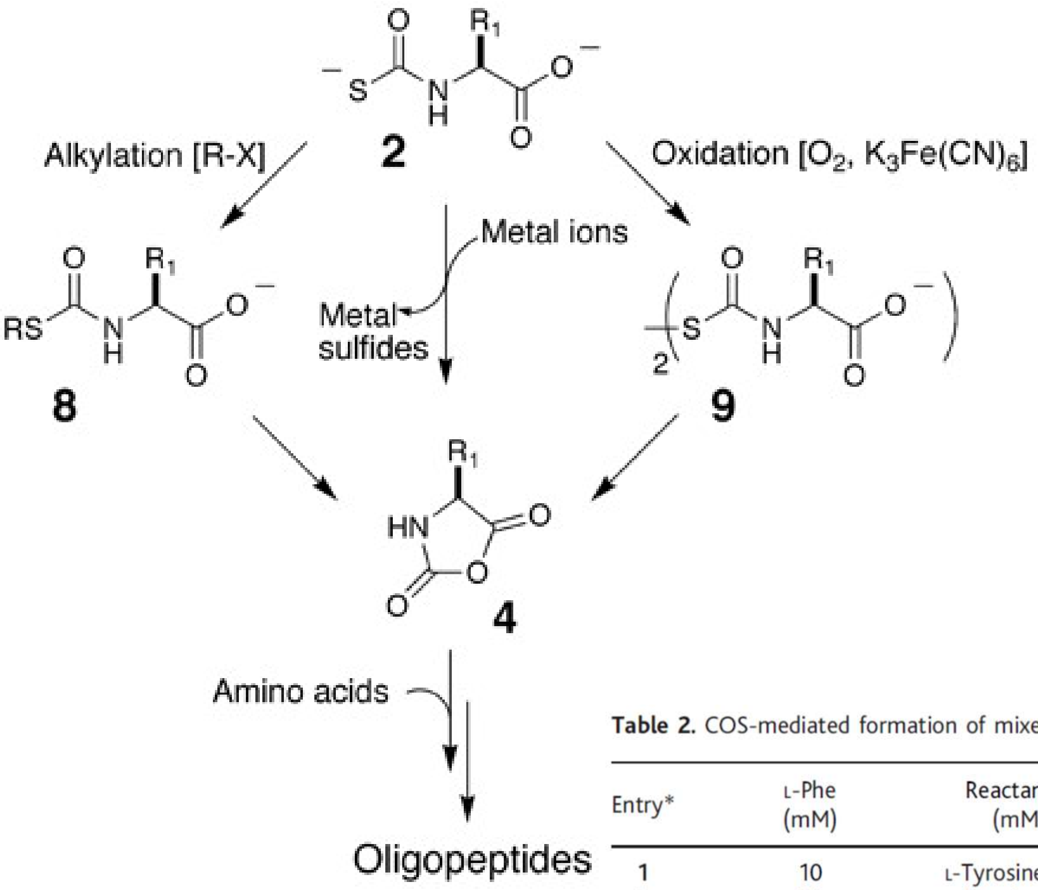


# *Carbonyl sulfide – condensing agent*



Leman, L.; Orgel, L.; Ghadiri, M. R. *Science* **2004**, *306*, 283-286.

# *Carbonyl sulfide – condensing agent*



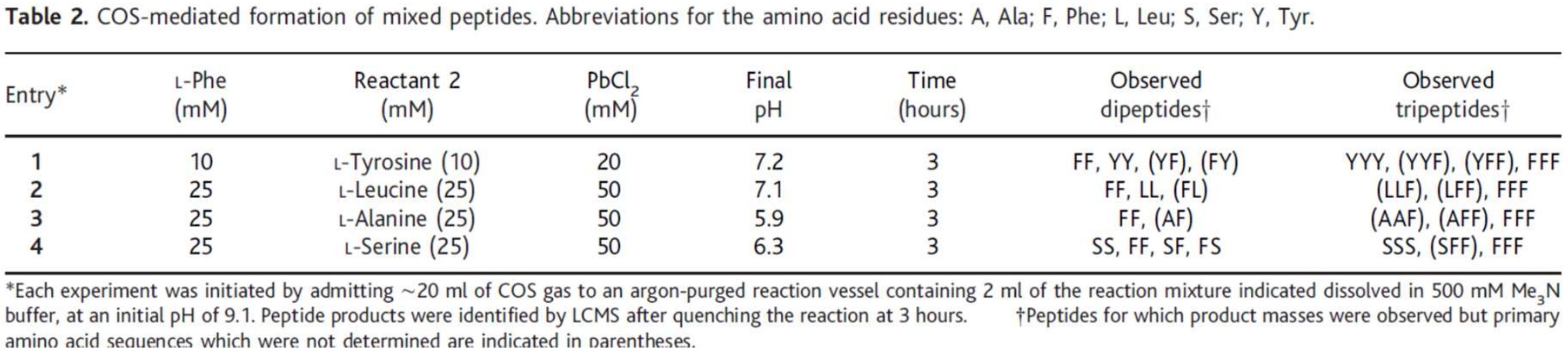


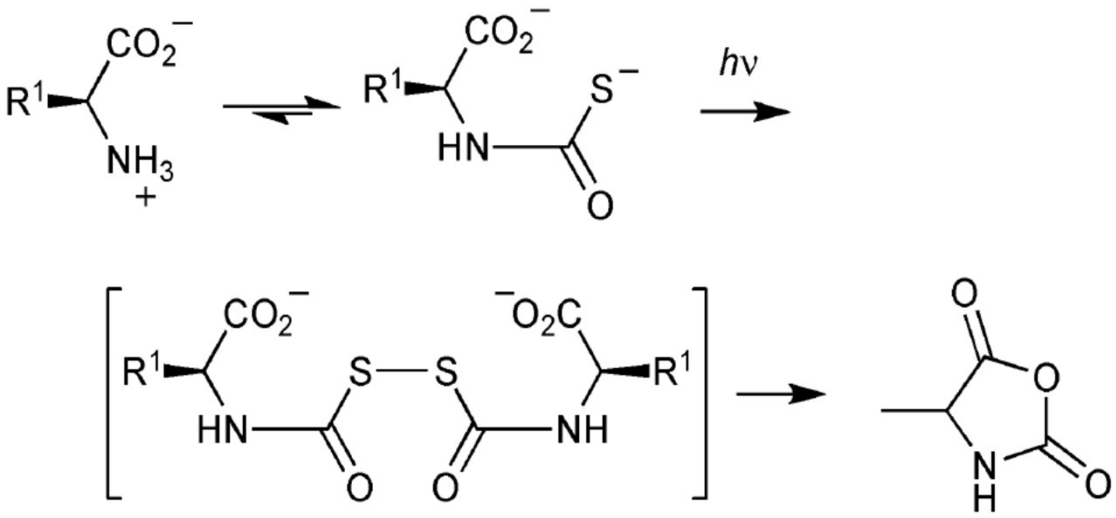
**Table 2.** COS-mediated formation of mixed peptides. Abbreviations for the amino acid residues: A, Ala; F, Phe; L, Leu; S, Ser; Y, Tyr.

| Entry* | L-Phe (mM) | Reactant 2 (mM) | $PbCl_2$ (mM) | Final pH | Time (hours) | Observed dipeptides† | Observed tripeptides† |
|---|---|---|---|---|---|---|---|
| 1 | 10 | L-Tyrosine (10) | 20 | 7.2 | 3 | FF, YY, (YF), (FY) | YYY, (YYF), (YFF), FFF |
| 2 | 25 | L-Leucine (25) | 50 | 7.1 | 3 | FF, LL, (FL) | (LLF), (LFF), FFF |
| 3 | 25 | L-Alanine (25) | 50 | 5.9 | 3 | FF, (AF) | (AAF), (AFF), FFF |
| 4 | 25 | L-Serine (25) | 50 | 6.3 | 3 | SS, FF, SF, FS | SSS, (SFF), FFF |

*Each experiment was initiated by admitting ~20 ml of COS gas to an argon-purged reaction vessel containing 2 ml of the reaction mixture indicated dissolved in 500 mM $Me_3N$ buffer, at an initial pH of 9.1. Peptide products were identified by LCMS after quenching the reaction at 3 hours. †Peptides for which product masses were observed but primary amino acid sequences which were not determined are indicated in parentheses.

Leman, L.; Orgel, L.; Ghadiri, M. R. *Science* **2004**, *306*, 283-286.

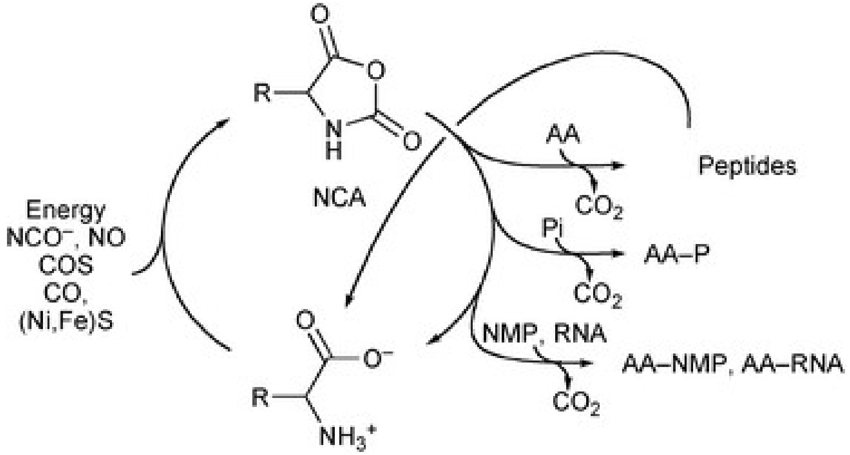
# *Carbonyl sulfide – photochemical activation*



A slow formation of NCAs from free amino acids and COS in the absence of oxidizing or alkylating agents has been reported and studied through theoretical chemistry investigations. However, it seems unlikely that COS ($\Delta G_0$ = 16.9 kJ/mol) could be able to generate NCA ($\Delta G_0$ = 60 kJ/mol) in spite of its cyclic structure.

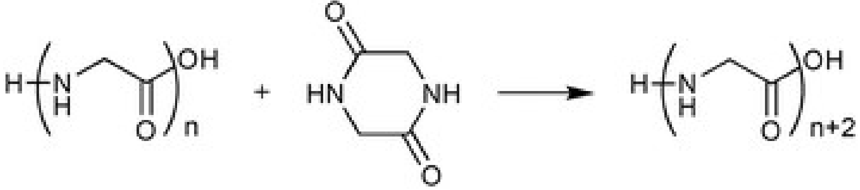
A photochemical activation of thiocarbamate that could take place in a way similar to that of thioacetate in aqueous solution may provide an explanation to this observation. This potential photochemical reaction may also constitute an efficient pathway for the prebiotic formation of NCAs.

# *Carbonyl sulfide – photochemical activation*

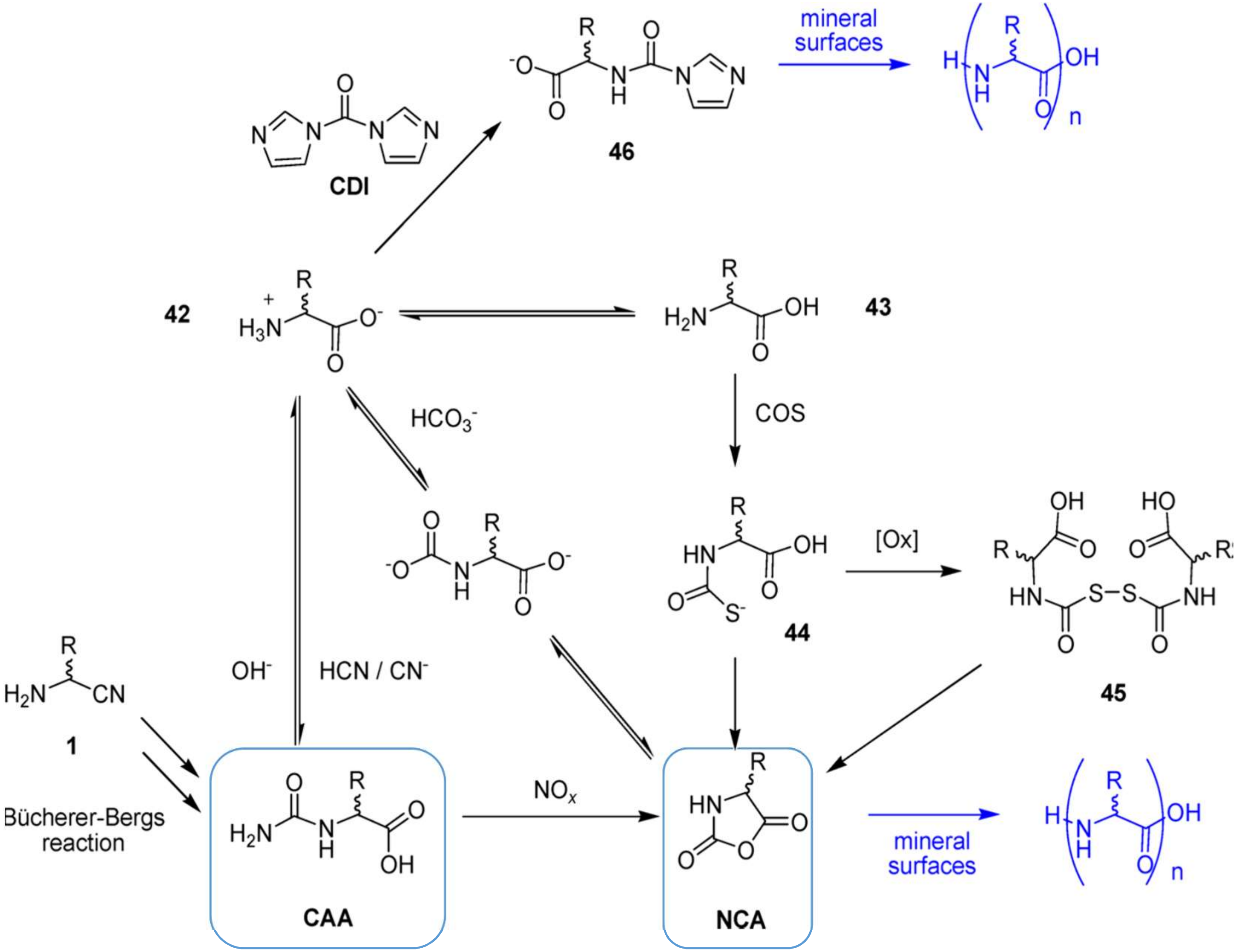


Pathways for the formation of NCAs and
further reactions including polymerization and interactions
with inorganic phosphate (Pi), nucleotides (NMP), and RNA.

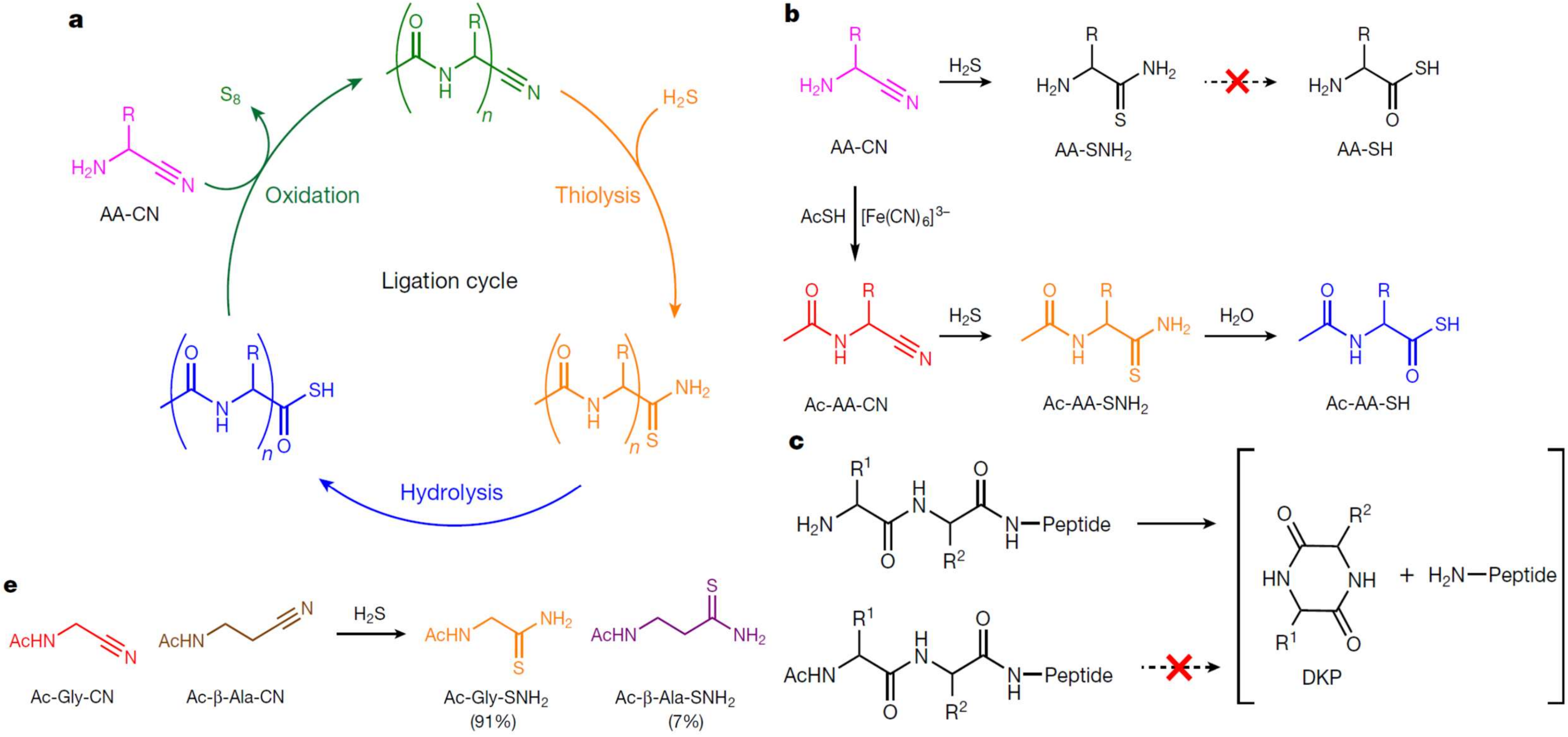
# *Diketopiperazines as intermediates for peptide condensation*



# *Condensation of aminoacids into peptides*

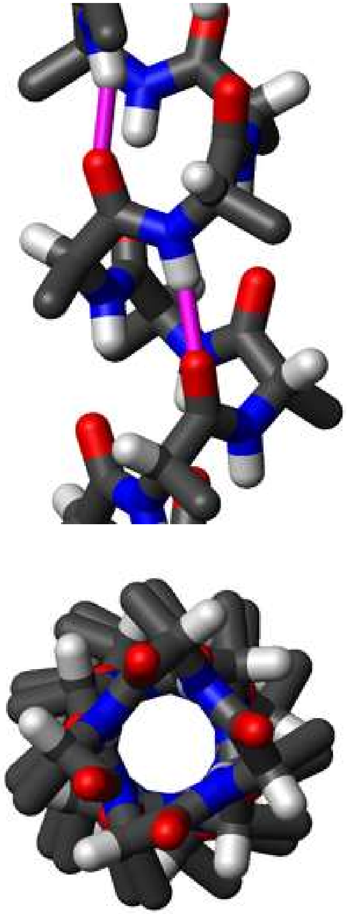


# *Prebiotic peptide condensation in water*

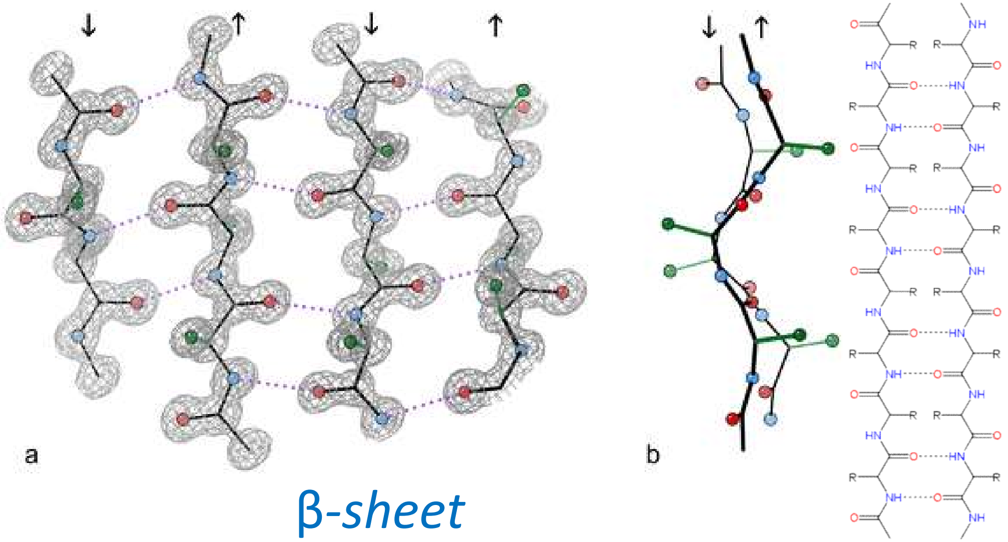


P. Canavelli, S. Islam & M. W. Powner *Nature* **2019**, *571*, 546-549.

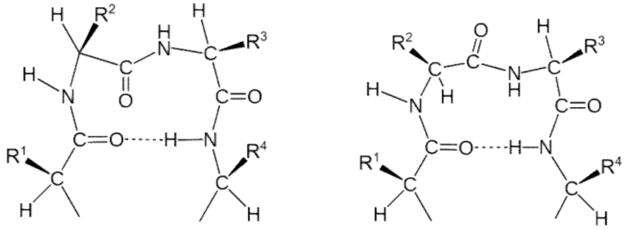
# *GADV-protein world*



*α-helix (Ala)*



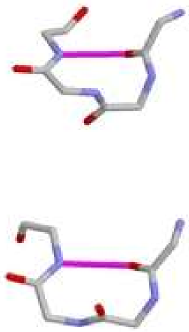
*β-sheet (Val)*



ß turn: Type I

ß turn: Type II

*β-turn (coil) (Gly)*



*hydrophilic and hydrophobic structures*

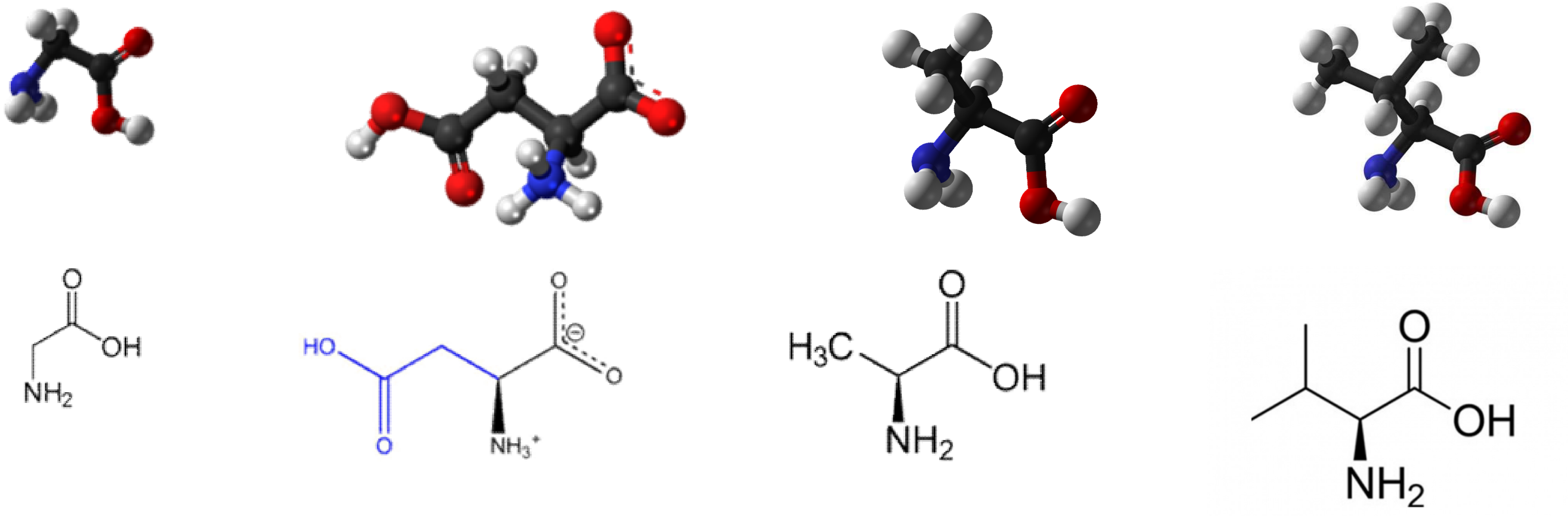
*globular structures*

*catalytic activity (Asp)*

# *Basic aminoacids for primitive genetic code?*

Primordial genetic code might have involved only 4 „GNC” codons:

- *GGC for glycine*
- *GCC for alanine*
- *GAC for aspartic acid*
- *GUC for valine*

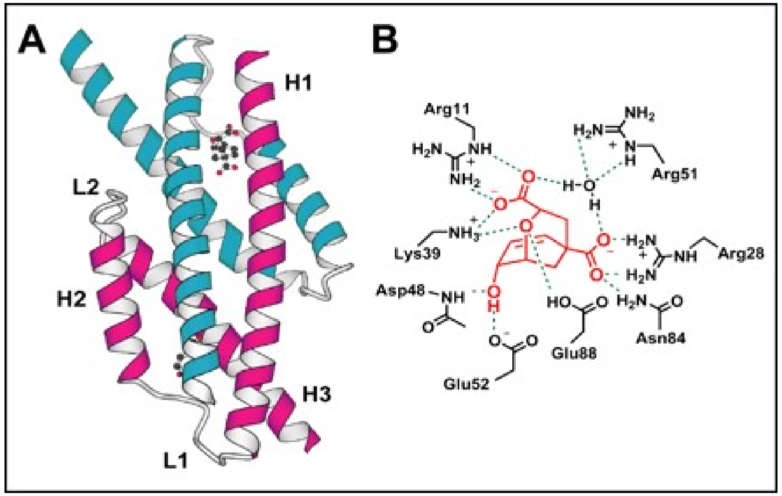


Later, the ‚GNC’ code probably evolved into ‚SNS’ code (S = G/C, N = A, U, G, C) – 16 codons encoding 10 basic aminoacids (Gly, Ala, Asp, Val, Glu, Leu, Pro, His, Glu, Arg)

# *Reduced aminoacid alphabet*

9-aminoacid alphabet is sufficient to construct functional enzymes

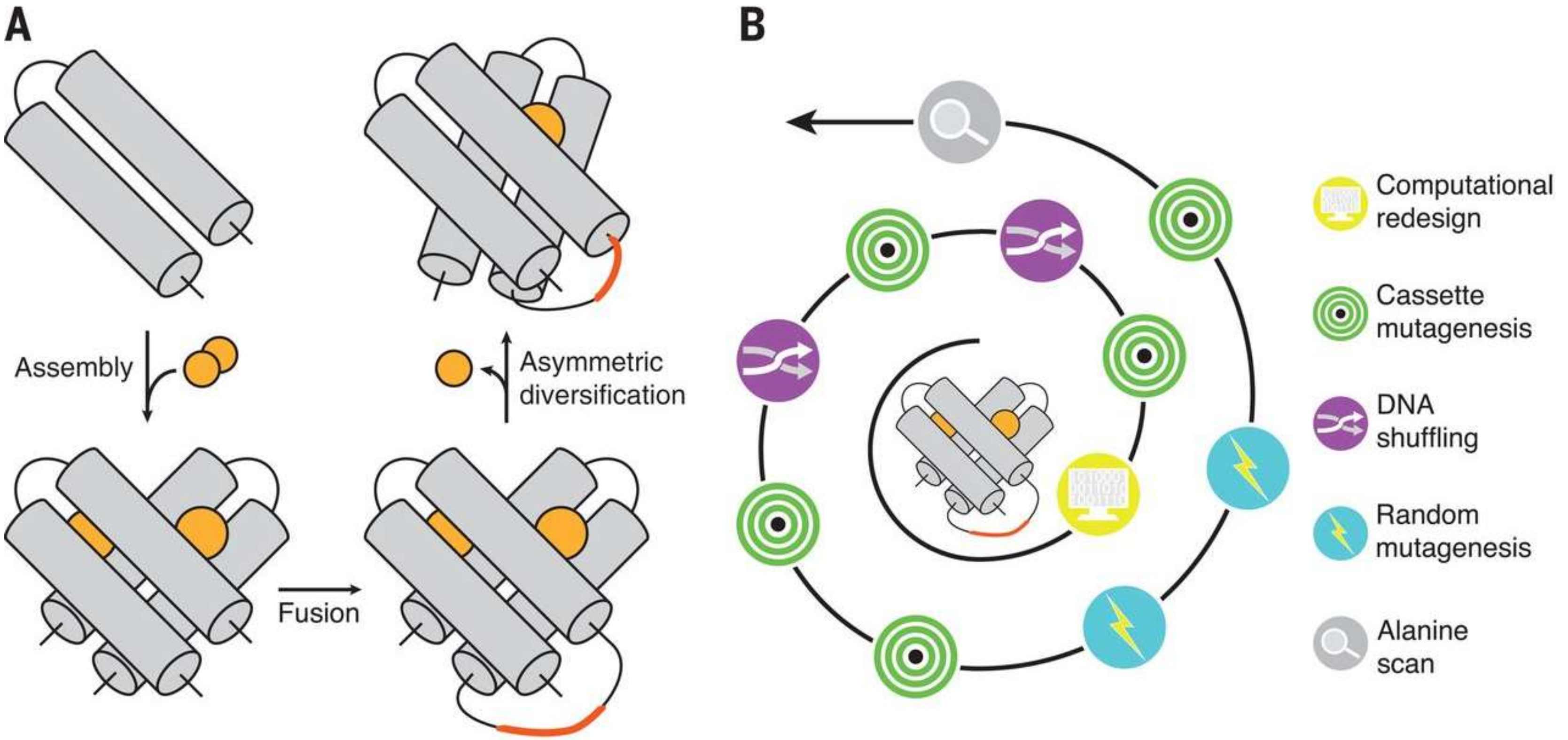
Aminoacids: Asp, Glu, Asn, Lys, Phe, Ile, Leu, Met, Arg



**AroQ structure and active site.** *A*, the homodimeric EcCM is shown with a transition state analog inhibitor bound at its active sites; the two identical polypeptide chains are colored *blue* and *pink* for clarity. *B*, proposed interactions between residues in the evolved active site of the simplified enzyme and the transition state analog inhibitor, compound **1** (*red*), based on the x-ray structure of EcCM. Residues Gln$^{88}$ and Ser$^{84}$ in EcCM are substituted with Glu$^{88}$ and Asn$^{84}$ in the 9-amino acid enzyme. Residue numbers are referenced to EcCM.

Walter, K. U., Vamvaca, K., Hilvert, D. *J. Biol. Chem.* **2005**, *280*,37742-37749.

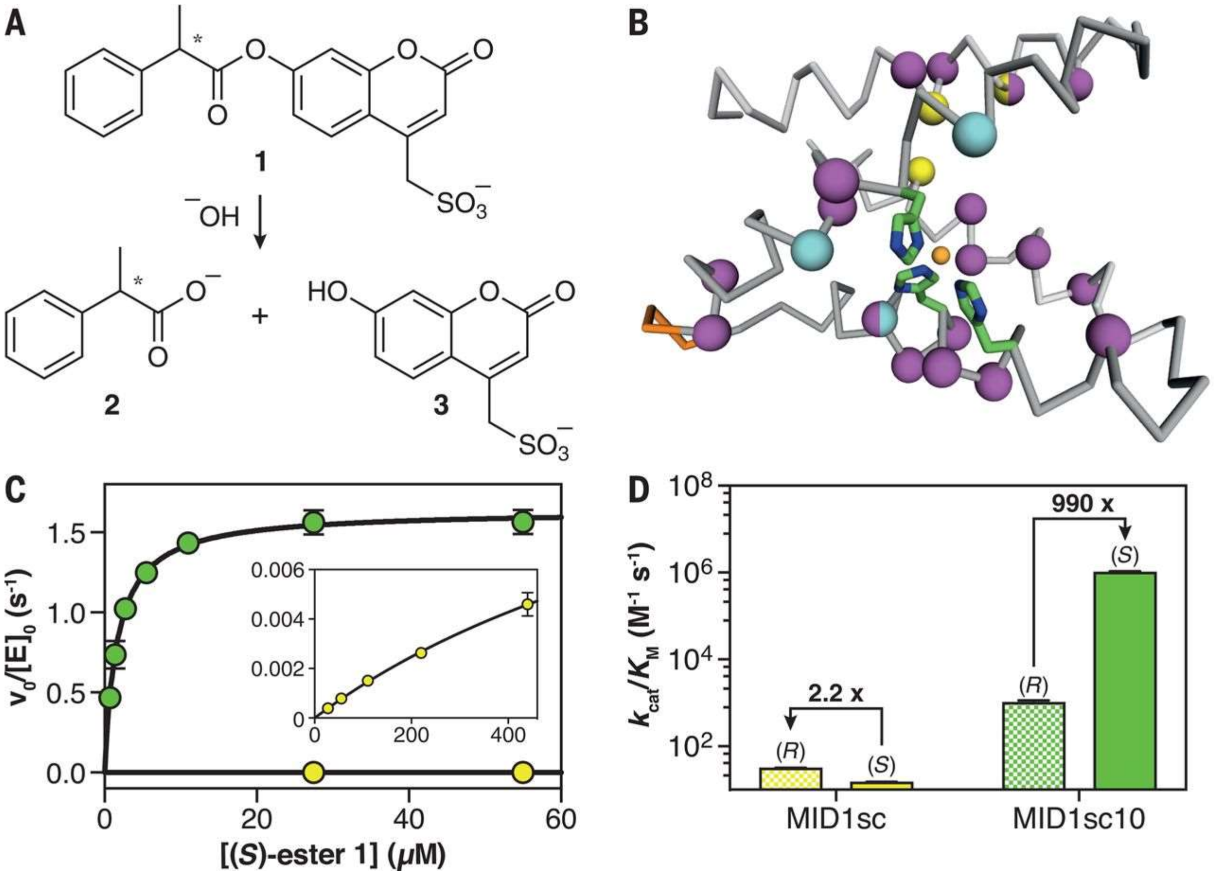
# *Evolution of a metalloenzyme from short peptides*



Zinc-mediated assembly of helix-turn-helix fragments, followed by fusion and asymmetric diversification, afforded MID1sc10, an efficient metalloesterase.

S. Studer, D.A. Hansen, Z. Pianowski, P.R.E. Mittl, A. Debon, S.L. Guffy, B.S. Der, B. Kuhlman, D. Hilvert *Science,* **2018**, *362,* 1285-1288

# *Evolution of a metalloenzyme from short peptides*



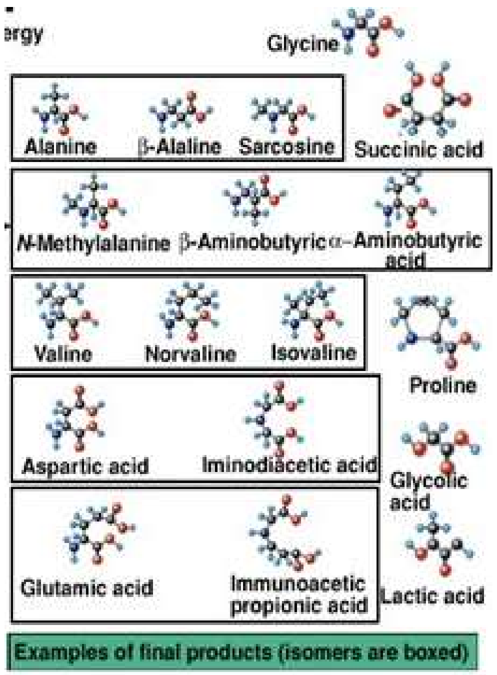
***Crystal structure of MID1sc10***
zinc ion - orange sphere,
coordinating histidines - green sticks
linkage of two polypeptides – orange sticks
beneficial mutations - magenta spheres,
residues replaced to prevent competitive zinc
binding modes - cyan spheres).

The evolved variant MID1sc10 is highly enantioselective as a consequence of a 2200-fold specificity switch from the modestly (R)-selective starting catalyst MID1sc

***Michaelis-Menten plots*** for **MID1sc** (yellow and inset) and **MID1sc10** (green) show a 70,000-fold improvement in hydrolysis efficiency for (S)-configured **1** after optimization.

S. Studer, D.A. Hansen, Z. Pianowski, P.R.E. Mittl, A. Debon, S.L. Guffy, B.S. Der, B. Kuhlman, D. Hilvert *Science,* **2018**, *362,* 1285-1288

# *Aminoacids - Summary*



Prebiotic generation plausible – variants of the Miller-Urey experiment
Strecker-type of chemistry likely

Aminoacids are good catalysts, can perform various chemical transformations

The origin of homochirality in the Universe caused by the pairity violation and stochastic fluctuations

Chirality amplification possible in numerous chemical reactions

Aminoacids can catalyse their own formation with chirality amplification and undergo physical enantioenrichment processes

Condensation of aminoacids into peptides plausible under prebiotic conditions using condensing agents

Simple peptides can exhibit broad structural variety,
catalytically active enzymes can be constructed with reduced aminoacid alphabet