

### Asymmetric Vacuum UV photolysis of the Amino Acid Leucine in the Solid State\*\*

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The origin of biomolecular asymmetry, the fact that biological molecules such as amino acids show the same chirality, still requires an explanation and might be traced back to interstellar low-temperature processes.<sup>[1–6]</sup> Under interstellar conditions, chiral solid-state amino acids might have been subjected to circularly polarized vacuum ultraviolet (VUV) electromagnetic radiation before and during their space journey to Earth. However, to date, experimental data on chiroptical properties of amino acids were limited to aqueous

solutions, which is not relevant in terms of astrophysical conditions.

Amino acids, the chiral building blocks of proteins, probably played a key role in both the appearance of life on Earth and the emergence of biomolecular asymmetry. Some of these amino acids were discovered in carbonaceous meteorites,<sup>[1,2]</sup> with enantiomeric excesses (*ee* values) and isotopic composition<sup>[3]</sup> indicating an extraterrestrial origin of biomolecular asymmetry. Amino acids have also been synthesized in the laboratory by simulating interstellar/circumstellar ice photochemistry.<sup>[4,5]</sup> Proteins are exclusively based on L-amino acid monomer units. The use of homochiral monomers is generally considered as advantageous for the prebiotic construction of their structure.<sup>[7–9]</sup> Interestingly, even small *ee* values can be amplified by autocatalytic reactions leading to near homochirality.<sup>[10,11]</sup>

Numerous asymmetric mechanisms have been suggested and tested experimentally, including the influence of circularly polarized light (CPL) as an external chiral force.<sup>[12–14]</sup> Astronomical sources of CPL,<sup>[15,16]</sup> which might produce enantiomeric selection in interstellar organic molecules, were observed in the IR range.<sup>[17]</sup> Electromagnetic radiation from magnetic white dwarfs and white-dwarf binaries (Polars) can be circularly polarized up to 12% and 50%, respectively.<sup>[18]</sup> CPL might also be produced by dust grains aligned by a magnetic field in the reflection nebulae in star-formation regions.

Since the pioneering work of Kuhn et al. who studied the enantioselective photodecomposition of ethyl- $\alpha$ -bromopropionate and *N,N*-dimethyl- $\alpha$ -azidopropionamide via electronically excited states,<sup>[19,20]</sup> asymmetric photolysis experiments on several chiral compounds have been reported.<sup>[21,22]</sup> In 1977, the amino acids alanine<sup>[23]</sup> and leucine<sup>[24]</sup> were decomposed enantioselectively in aqueous solution by excitation of the ( $\pi^*$ ,n)-transition at 213 nm, and gave *ee* values of 2.5%.<sup>[24]</sup> The corresponding photolysis mechanism was recently examined.<sup>[25,26]</sup> To date, no higher *ee* value has been generated in amino acids.

So far, experiments using CPL have been performed in solution, which is less relevant with respect to astrophysical conditions. Because of the solvent absorption below 200 nm, such studies have been restricted to excitations of the ( $\pi^*$ ,n)-electronic transition (213 nm on leucine), so that only part of the UV/VUV absorption spectrum was accessible. This situation is unfortunate since in general amino acids exhibit stronger absorption cross-sections for the ( $\pi^*$ , $\pi_1$ )-electronic transitions, which are therefore interesting to investigate. It is noteworthy that Inoue et al.<sup>[27]</sup> irradiated pentane solutions of racemic (*E*)-cyclooctene with left circular polarized synchrotron radiation (*l*-CPSR) or right-CPSR (*r*-CPSR) at 190 nm and observed an enantioselective photoisomerization effect. Herein we report on chiroptical properties and asymmetric photolysis of D,L-leucine that was exposed, for the first time in the solid state, to VUV *l*-CPSR and *r*-CPSR at 170 nm and 182 nm, inducing asymmetric photochemistry via ( $\pi^*$ , $\pi_1$ )-electronic transitions.

The amino acid leucine was chosen for the study of solid-state asymmetric photodecomposition experiments for the following reasons: 1) The photochemistry of the ( $\pi^*$ ,n)-

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[\*\*] This work was funded by the EU, CNES, FNAD, Région Centre, and the Département du Cher (France). We acknowledge the support of Mai Julie Nguyen, Romain Jacquet, Bertrand Pilette, and the synchrotron-teams of ASTRID and LURE. We thank Wolfram H.-P. Thiemann, Henri B. Kagan, and Odile Dutuit. U.J.M. is grateful to Le Studium for a C.B.M. postdoctoral position, to the DFG, Bonn (Germany) for funding his research group at the University of Bremen, and for a CNRS position at the University of Nice.

Supporting information for this article is available on the WWW under <http://www.angewandte.org> or from the author.

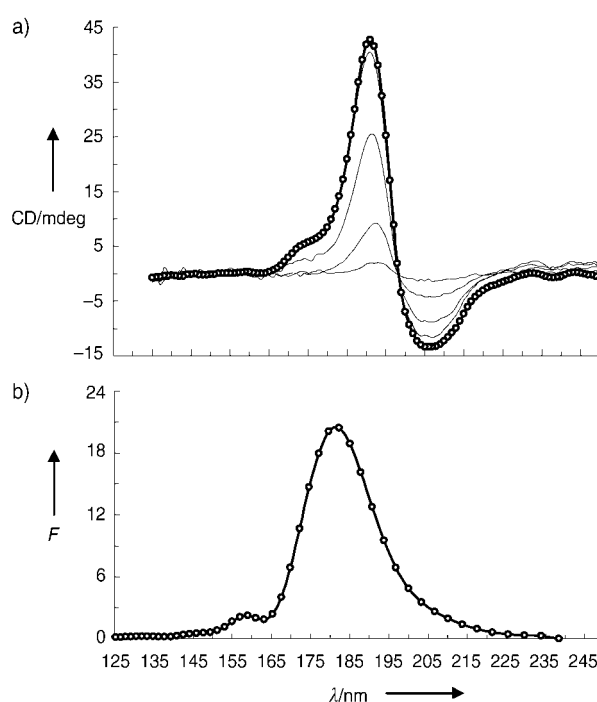
electronic transition is well-known. 2) Leucine shows the largest anisotropy factor  $g$  ( $g = \Delta\epsilon/\epsilon$ ) among proteinaceous amino acids.<sup>[24]</sup> It has been established that the gain in  $ee$  value achievable during photolysis of a racemate is a function of the magnitude of  $g$  and of the extent of reaction.<sup>[22]</sup> 3) Enantiomeric separation techniques provide a high separation factor  $\alpha$  and a high resolution  $R_s$ . 4) Leucine has a slow racemization rate compared to other amino acids.<sup>[28]</sup>

To establish the most suitable conditions for the enantioselective photodecomposition of solid-state leucine, electronic transitions and circular dichroism (CD) features were studied in the VUV photon energy range. VUV absorption spectra of solid films of leucine (1  $\mu\text{m}$  film thickness deposited on an  $\text{MgF}_2$  window) were recorded between 110 and 220 nm by synchrotron radiation (SR) absorption spectroscopy on beamline SA-61 of the Super-ACO storage ring of LURE in Orsay (France). A weak ( $\pi^*,n$ )-transition of leucine was detected at 211 nm, and a strong ( $\pi^*,\pi_1$ )-transition at 183 nm, about five-times more intense. Additional electronic transitions were determined below 150 nm.

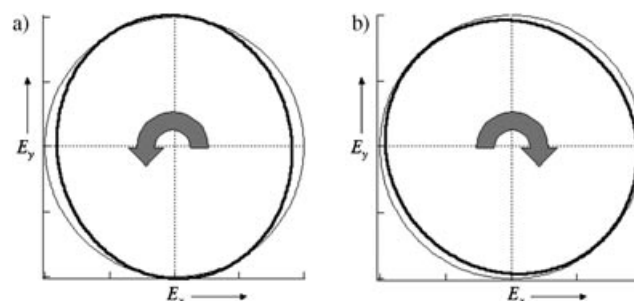
A VUV CD spectrum of L-leucine in solution in 1,1,1,3,3,3-hexafluoroisopropanol has been reported.<sup>[29]</sup> It shows a positive ( $\pi^*,n$ )-electronic transition at 194 nm and a negative ( $\pi^*,\pi_1$ )-electronic transition at 168 nm. As part of our study VUV-CD spectra of solid-state D-leucine films, with estimated thickness ranging from 0.2 to 1  $\mu\text{m}$  deposited on  $\text{MgF}_2$  windows, were run below 200 nm at the Institute for Storage Ring Facilities, University of Aarhus (Denmark) using the synchrotron beamline UV1-ASTRID. The obtained CD spectrum is given in Figure 1 (top). It shows a negative band at 207 nm and a positive band at 191 nm, noticeably different from the spectrum recorded in solution. Comparing the CD data with the absorption spectrum, we conclude that the ( $\pi^*,\pi_1$ )-electronic transition splits into three CD bands, a negative band above 200 nm which overlaps with the negative ( $\pi^*,n$ )-band, and two positive bands, at 191 nm and at 175 nm.

The CD band splitting is assumed to arise from an excitation coupling based on coupling processes between leucine molecules in the solid state. We deduce from the CD data that the ( $\pi^*,n$ )-electronic transition of D-leucine is induced preferentially by right-handed circularly polarized light ( $r$ -CPL) and the two positive ( $\pi^*,\pi_1$ )-electronic transitions of D-leucine are preferentially excited by  $l$ -CPL. Since VUV electronic excitations lead to leucine photolysis, mainly by decarboxylation, irradiation of D,L-leucine with  $r$ -CPL at the ( $\pi^*,n$ )-electronic transition should theoretically produce an enantiomeric excess of L-leucine. Conversely, irradiation at the positive ( $\pi^*,\pi_1$ )-electronic transitions with  $r$ -CPL should lead to an enantiomeric excess of D-leucine by photodecomposition of the L-leucine. Opposite enantiomerichments are expected when using the opposite photon helicity  $l$ -CPL.

To achieve VUV asymmetric photodecomposition of solid state D,L-leucine, intense and quasi-perfect CPSR (Figure 2) as emitted by the OPHELIE Undulator<sup>[30]</sup> of the VUV SU5 beamline<sup>[31]</sup> was used to irradiate the samples. The photon energy was first set at 6.8 eV (182 nm) (see Figure 1 bottom) and then at 7.3 eV (170 nm), so that information about the energy-dependence of the process could be gained. The first



**Figure 1.** Spectral dependence of leucine CD and SR-irradiation. a) VUV CD spectrum of solid-state D-leucine recorded at the Synchrotron Facility ISA, University of Aarhus (Denmark). High-resolution measurement of D-leucine, film thickness 1  $\mu\text{m}$  (bold line); low-resolution measurements of D-leucine, film thickness 0.2, 0.4, 0.6, and 0.8  $\mu\text{m}$  (thin line); leucine films were immobilized on  $\text{MgF}_2$ . b) Spectral flux ( $F$ ) of the OPHELIE undulator of the SU5 beamline at LURE (Orsay, France) in arbitrary units used for irradiation with CPSR centered at 6.8 eV (182 nm).

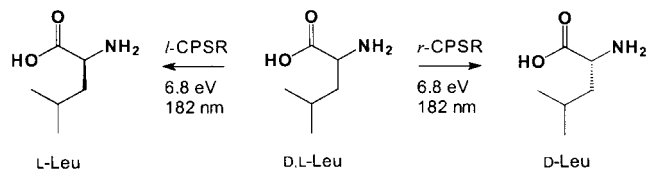


**Figure 2.** Measured polarization ellipses (thick lines) at the sample location. They were measured for a SR centered at 6.8 eV (182 nm), for a)  $r$ -CPSR and b)  $l$ -CPSR, as produced by the OPHELIE undulator of the SU5 beamline. Absolute circular polarization rates are of 91% for  $r$ -CPSR and 94% for  $l$ -CPSR. Thin lines are guide to the eye illustrating the perfect CP light case and the dashed lines indicate the normal ellipse axes corresponding to the two components ( $E_x$ ,  $E_y$ ) of the electric field associated with the light. The curved arrows indicate the helicity of the photons as seen by an observer facing the beam coming out of the plane of the Figure.

energy closely matches the middle of the two CD positive bands of the ( $\pi^*,\pi_1$ )-electronic transitions, while the second corresponds to the almost absent CD contribution on the short-wavelength side. Solid films of D,L-leucine were irradiated with  $r$ -CPSR and with  $l$ -CPSR in separate experiments. After photodecomposition, the remaining leucine was

extracted and analyzed by enantioselective CG-MS. As summarized in Table 1, five samples were used: sample 1 was not irradiated and was used as a normalization sample, while the other samples were irradiated with different photon

**Table 1:** Enantiomeric excesses (averaged over three to six samples for the same irradiation conditions) obtained after irradiation of D,L-leucine with *l*-CPSR and *r*-CPSR.<sup>[a]</sup>

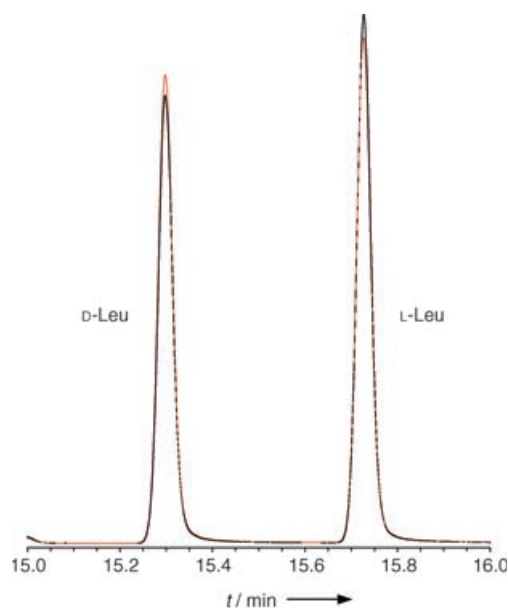


Sample	$E_{\text{photon}}$ [eV]/ $\lambda$ [nm]	Irradiation	$ee$ (D-leucine) [%]	$Cl_{95}$ <sup>[b]</sup>
1 <sup>[c]</sup>	–	–	0.00	0.38
2	6.8/182	<i>l</i> -CPSR	–0.88	0.28
3	6.8/182	<i>r</i> -CPSR	2.60	0.16
4	7.3/170	<i>r</i> -CPSR	0.75	0.36
5	7.3/170	<i>l</i> -CPSR	0.53	0.48

[a] Complete set of data in the Supporting Information. [b]  $Cl_{95}$  = confidence interval at 95 % determined by multiple analyses of each sample. [c] Not irradiated, control sample.

energies and CPL helicities. Sample numbers 1, 3, and 4 were analyzed six times in parallel, sample number 2 was analyzed three times, and sample number 5 was analyzed four times (see Table S1 in the Supporting Information). Overlaid chromatograms of the irradiated and separated leucine enantiomers are presented in Figure 3. No decomposition products of leucine could be identified with the GC-MS technique used, the expected volatile decomposition products,  $\text{CO}_2$  and primary amines, would be evacuated in the high-vacuum synchrotron beamline and avoid detection.

The highest gain in  $ee$  value was found to be +2.6 % in D-leucine when irradiating with *r*-CPSR at 6.8 eV (182 nm), that is, near the two positive ( $\pi^*$ ,  $\pi_1$ )-electronic transitions. Irradiation with *l*-CPSR at 6.8 eV resulted in an  $ee$  value of +0.88 % in L-leucine. Samples were irradiated for 3 h each with a decreasing photon flux, owing to the decrease of the electron current in the Super-ACO storage ring. The photon flux was measured during the irradiation by monitoring the photoemission current from a highly transparent gold mesh inserted in the SR beam just upstream of the samples. From this measurement, we can determine that the total dose of irradiation (flux  $\times$  duration) received by sample 2 is about 25 % lower than for sample 3. This result explains parts of the observed difference between the absolute  $ee$  value for the two light helicities. The remaining part could be explained by a likely difference in the spatial transverse flux density and pointing between *r*-CPSR and *l*-CPSR owing to non-perfect symmetry of the OPHELIE undulator.<sup>[32]</sup> The result of these effects being that irradiation would not have the same efficiency for the different helicities. Beside this, tiny inhomogeneities of the solid-state leucine samples 2 and 3 resulting in a nonsymmetrical alignment within the synchrotron beamline may account for some of the differences.



**Figure 3.** Overlaid gas chromatograms of irradiated D,L-leucine depicting the separated enantiomers after irradiation at 6.8 eV (182 nm) with *l*-CPSR (black line) and *r*-CPSR (red line) on the SU5 beamline at the Synchrotron Facility LURE, Orsay (France). The first eluting enantiomer is D-leucine. D,L-Leucine irradiated with *r*-CPSR, shows a marked enantioselective photodecomposition of L-leucine, the  $ee$  value for D-leucine being 2.60 %. Chromatograms were measured in the single-ion monitoring mode at  $m/z$  158 amu. For chromatographic overlay, the area of the sample irradiated with *r*-CPSR was multiplied by a factor of 1.61.  $t$  = retention time.

The results demonstrate that a significant enantiomeric enrichment can be induced in solid-state D,L-amino acids by irradiation with energies closely matching the positive ( $\pi^*$ ,  $\pi_1$ )-electronic transitions. Irradiation of solid-state D,L-leucine with CPSR at a higher energy, 7.3 eV (170 nm), did induce a minor  $ee$  value, which in addition does not change sign by switching photon helicity. This observation is coherent with the fact that the CD signal of leucine (see Figure 1) at 170 nm is vanishing.

In conclusion, enantiomeric excesses were induced in samples of leucine by VUV asymmetric photochemical reactions, such as enantioselective photolysis, under realistic interstellar/circumstellar conditions. These experimental data support the assumption that tiny interstellar/circumstellar ice grains can experience asymmetric reactions when irradiated by circularly polarized ultraviolet light. Various families of amino acids have been identified in carbonaceous meteorites,<sup>[1,2,33]</sup> with  $ee$  values and isotopic composition<sup>[3]</sup> indicating an extra-terrestrial origin of biomolecular asymmetry. A systematic study of asymmetric VUV photolysis in the solid state for different amino acids would help us to better understand the processes leading to their asymmetric formation and their role in prebiotic chemistry. Based on the present data, it is possible to conclude that asymmetric interstellar/circumstellar solid-state photochemical processes in the VUV might have triggered the appearance of biomolecular asymmetry and thus could have assisted the start of life.

## Experimental Section

Information on VUV circularly polarized synchrotron radiation and enantioselective GC-MS analyses are given in the Supporting Information. For the preparation of the solid-state leucine sample D,L-leucine (space group  $P\bar{1}$ , triclinic; 600  $\mu\text{g}$ ) were lyophilized and suspended in *m*-xylene (1,3-dimethylbenzene; 200  $\mu\text{L}$ ). A 2.5- $\mu\text{L}$  sample of the suspension was introduced into a 1  $\times$  5 mm cavity carved out at the surface of a VUV-transparent  $\text{MgF}_2$  window. The samples were dried in a desiccator filled with  $\text{P}_2\text{O}_5$  at ambient pressure. By this technique, a homogeneous film of leucine was deposited on the surface of the  $\text{MgF}_2$  window.

VUV CPSR was produced by the recently developed electromagnetic polarizing undulator OPHELIE<sup>[30]</sup> installed on the Super-ACO storage ring of LURE in Orsay (France), feeding the VUV high resolution variable polarization SU5 beamline,<sup>[31]</sup> whose monochromator was set to deliver only the zeroth order diffraction of its gratings. Under these conditions, the spectrum of the synchrotron radiation (SR) impinging on the sample, as depicted in Figure 1 (bottom), corresponds to a peak centered on the tunable central photon energy  $\epsilon_c$  with a 11% relative bandwidth ( $\Delta\lambda/\lambda_c$ ) proving an integrated flux in the range of  $10^{14}$  photon per second as derived from a VUV photodiode signal. Absolute circular polarization rates, measured at the sample location with a dedicated VUV polarimeter, vary from 90 to 100% over the 6 to 28 eV range.<sup>[32]</sup> For both photon energies, 6.8 eV and 7.3 eV, corresponding to two different undulator settings, they reach 91% for *r*-CPSR and 94% for *l*-CPSR. The corresponding polarization ellipses are shown in Figure 2.

After CPSR irradiation the remaining leucine was extracted from the  $\text{MgF}_2$  window cavity with 0.1M hydrochloric acid ( $3 \times 20 \mu\text{L}$ ). The extract was transferred into a reaction vial (1 mL volume) and an ethanol-pyridine mixture (3:1, v/v; 30  $\mu\text{L}$ ) was added. To this solution, ethyl chloroformate (6  $\mu\text{L}$ ) was added, the vial was capped tightly, and shaken vigorously for 10 s to form *N*-ethoxycarbonyl leucine ethyl esters (leucine-ECEE).<sup>[34,35]</sup> Chloroform (18  $\mu\text{L}$ ) was added and the vial was shaken again for extraction of the leucine-ECEE into the (lower) organic layer. Finally, 1  $\mu\text{L}$  of the organic phase was withdrawn by a microliter syringe and injected directly into the gas chromatographic Agilent 6890/5973 GC-MSD system equipped with a 25 m chiral stationary Chirasil-L-Val phase.

Received: April 14, 2005

Revised: May 27, 2005

Published online: ■ ■ ■ ■ ■, ■ ■ ■ ■ ■

**Keywords:** amino acids · astrochemistry · homochirality · photochemical reactions · synchrotron radiation

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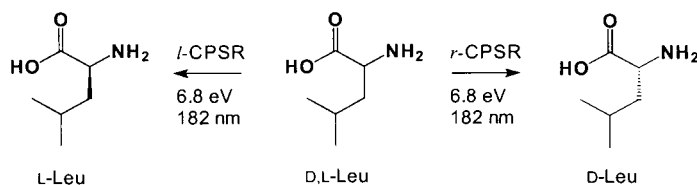
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## Communications

### Homochirality

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Asymmetric Vacuum UV photolysis of the  
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**One hand clapping:** Based on to solid-state circular dichroism data, the leucine ( $\pi^*$ ,  $\pi_1$ )-electronic transition is excited with right-circularly polarized synchrotron radiation (*r*-CPSR) at 182 nm (see scheme). This treatment induced an

enantiomeric excess of 2.6% of D-leucine. The results support the assumption that interstellar asymmetric photoreactions are capable of inducing significant enantiomeric enrichments in solid-state amino acids.