Testing the potential of the ferrocene chromophore as a circular dichroism probe for the assignment of the screw-sense preference of tripeptides

Marko Nuskol,a Petar Šutalo,b Marijana Đaković,b Monika Kovačević,a Ivan Kodrin*b and Mojca Čakić Semenčić*a

a Department of Chemistry and Biochemistry, Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, 10000 Zagreb, Croatia.
b Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, 10000 Zagreb, Croatia.
# Table of Contents

IR spectra .................................................................................................................. 2
NMR spectra .................................................................................................................. 5

- Compound 1a ........................................................................................................... 8
  - $^1$H NMR spectra .................................................................................................. 8
  - $^{13}$C NMR spectra ............................................................................................... 11
- Compound 2a .......................................................................................................... 15
  - $^1$H NMR spectra .................................................................................................. 15
  - $^{13}$C NMR spectra ............................................................................................... 18
- Compound 3a .......................................................................................................... 21
  - $^1$H NMR spectra .................................................................................................. 21
  - $^{13}$C NMR spectra ............................................................................................... 24
- Compound 4a .......................................................................................................... 28
  - $^1$H NMR spectra .................................................................................................. 28
  - $^{13}$C NMR spectra ............................................................................................... 30

*Cis-trans* isomerization of proline imide bonds of compounds 1a-4a .................. 32

NOESY spectra ............................................................................................................ 33
Single crystal X-ray crystallography ........................................................................... 34
CD spectroscopy ........................................................................................................... 36

HRMS spectra of compounds 1a-4a ........................................................................... 38

- Compound 1a ........................................................................................................... 38
- Compound 2a .......................................................................................................... 39
- Compound 3a .......................................................................................................... 40
- Compound 4a .......................................................................................................... 41

DFT study ...................................................................................................................... 42

Excited states ............................................................................................................... 48

- Compound 1a ........................................................................................................... 48
- Compound 2a .......................................................................................................... 51
IR spectra

Figure S1. The NH stretching vibrations of 1a–4a in CH$_2$Cl$_2$ ($c = 1 \times 10^{-3}$ mol dm$^{-3}$)

Figure S2. The NH stretching vibrations of compound 1a during dilution
Figure S3. The NH stretching vibrations of compound 2a during dilution

Figure S4. The NH stretching vibrations of compound 3a during dilution
Figure S5. The NH stretching vibrations of compound 4a during dilution

Table S1. NH and CO absorption frequencies of compounds 1a-4a in DCM (c = 1 mM)

| Compound | \( \nu \) NH (free) | \( \nu \) NH (assoc.) | amide I       | amide II       |
|----------|----------------------|-----------------------|---------------|----------------|
| 1a CH₂Cl₂| -                    | 3315                  | 1666, 1629    | 1558, 1525     |
| 2a CH₂Cl₂| -                    | 3323                  | 1666          | 1556, 1541, 1515|
| 3a CH₂Cl₂| 3420                 | 3326                  | 1672          | 1541, 1520     |
| 4a CH₂Cl₂| 3423                 | 3315                  | 1691, 1645    | 1556, 1541, 1514|
NMR spectra

Figure S6. NH region of the 'H NMR spectrum of 1a–4a (c = 2·10⁻³ mol·dm⁻³ at 25°C) showing signals corresponding to the major and minor isomers.
Figure S7. Solvent dependence of the amide proton chemical shifts of 1a–4a while increasing concentration of d6-DMSO in CDCl$_3$ ($c = 1 \times 10^{-3}$ mol·dm$^{-3}$).
Figure S8. Temperature dependence of the amide proton chemical shifts for 1a–4a in CDCl$_3$ ($c = 2 \times 10^{-3}$ mol·dm$^{-3}$).
Compound 1a

$^1$H NMR spectra

Figure S9. $^1$H NMR spectrum, full range
Figure S10. $^1$H NMR spectrum, downfield range
Figure S11. $^1$H NMR spectrum, upfield range
$^{13}$C NMR spectra

Figure S12. $^{13}$C NMR spectrum, full range
Figure S13. $^{13}$C NMR spectrum, downfield range
Figure S14. $^{13}$C NMR spectrum, upfield range
Figure S15. $^{13}$C NMR spectrum, upfield range
Compound 2a

$^1$H NMR spectra

Figure S16. $^1$H NMR spectrum, full range
Figure S17. $^1$H NMR spectrum, downfield range
Figure S18. $^1$H NMR spectrum, upfield range
$^{13}$C NMR spectra

Figure S19. $^{13}$C NMR spectrum, full range
Figure S20. $^{13}$C NMR spectrum, downfield range
Figure S21. $^{13}$C NMR spectrum, upfield range
Compound 3a

$^1$H NMR spectra

Figure S22. $^1$H NMR spectrum, full range
Figure S23. $^1$H NMR spectrum, downfield range
Figure S24. \(^1\text{H} \text{ NMR spectrum, upfield range}\)
$^{13}$C NMR spectra

3a (Boc-L-Pro’-D-Pro-L-Ala-NHFc)

$^{13}$C NMR, CDCl$_3$, 100 MHz

Figure S25. $^{13}$C NMR spectrum, full range
Figure S26. $^{13}$C NMR spectrum, downfield range
Figure S27. $^{13}$C NMR spectrum, upfield range
Figure S28. $^{13}$C NMR spectrum, upfield range
Compound 4a

$^1$H NMR spectra

Figure S29. $^1$H NMR spectrum, full range
Figure S30. $^1$H NMR spectrum, upfield range
$^{13}$C NMR spectra

Figure S31. $^{13}$C NMR spectrum, full range
Figure S32. $^{13}$C NMR spectrum, upfield range
**Cis-trans** isomerization of proline imide bonds of compounds 1a-4a.

Table S2. Influence of temperature and volume fraction of DMSO on *cis-trans* isomerization of proline imide bonds of compounds 1a-4a.

| Temperature  | 298.15 K | 308.15 K | 318.15 K | 323.15 K |
|--------------|----------|----------|----------|----------|
| 1a           | trans-trans isomer (%) | other isomers (%) | trans-trans isomer (%) | other isomers (%) | trans-trans isomer (%) | other isomers (%) | trans-trans isomer (%) | other isomers (%) |
| 1a           | 76       | 13, 11   | 71       | 16, 12   | 66       | 18, 16   | 66       | 18, 16   |
| 2a           | 100      | -        | 100      | -        | 100      | -        | 100      | -        |
| 3a           | 87       | 13       | 85       | 15       | 82       | 18       | 83       | 17       |
| 4a           | 67       | 27, 6    | 52       | 26, 22   | 48       | 27, 25   | 45       | 30, 25   |

Table S3. Influence of temperature and volume fraction of DMSO on *cis-trans* isomerization of proline imide bonds of compounds 1a-4a.

| ϕ (DMSO) | 0.5 | 0.9 | 0.13 | 0.17 | 0.2 |
|----------|-----|-----|------|------|-----|
|          | trans-trans isomer (%) | other isomers (%) | trans-trans isomer (%) | other isomers (%) | trans-trans isomer (%) | other isomers (%) | trans-trans isomer (%) | other isomers (%) |
| 1a       | 83  | 17  | 63   | 25, 12 | 60   | 31, 9   | 60   | 31, 9   | 52  | 35, 13 |
| 2a       | 100 | -   | 100  | -     | 100  | -       | 100  | -       | 100 |
| 3a       | 75  | 25  | 62   | 34, 4 | 46   | 28, 14, 12 | 41 | 35, 14, 10 | 40 | 39, 12, 9 |
| 4a       | 67  | 33  | 63   | 37    | 64   | 36      | 58   | 42      | 58  | 42   |

S32
Figure S33. Long [C(CH$_3$)$_3$-H$_2$Fe/H$_5$Fe] and medium range [C(CH$_3$)$_3$-NH$_{Ala}$] NOE contacts in spectra of 1a-4a.
**Single crystal X-ray crystallography**

A single crystal was mounted on a glass fiber. Data collections were carried out on an Oxford Diffraction Xcalibur four-circle kappa geometry single-crystal diffractometer with Sapphire 3 CCD detector, using a graphite monochromated MoKα (λ = 0.71073 Å) radiation, and applying the CrysAlisPro Software system\(^1\) at 293(2) K. Data reduction, including absorption correction, was done by CrysAlisPro program. The structures were solved by the Superflip computer program.\(^2\) The coordinates and the anisotropic thermal parameters for all non-hydrogen atoms were refined by full-matrix least-squares methods based on \(F^2\) using the SHELXL program.\(^3\) The hydrogen atoms were generated geometrically using the riding model with the isotropic factor set at 1.5 \(U_{eq}\).

Graphical work has been performed by Mercury 4.3.1.\(^4\) The thermal ellipsoids were drawn at the 30 % probability level. General and crystal data with the summary of intensity data collection and structure refinement for compound 2a are given in Table S3. CCDC 2035760 contains the supplementary crystallographic data for this paper.

The compound 2a crystallizes in the chiral orthorhombic P2₁2₁2₁ space group, with the stereogenic carbon atoms C12 (Ala), C15 (Pro), and C20 (Pro) being of expected configurations, namely S, S, and R, respectively (Figure 7). The bond lengths in two cyclopentadienyl (Cp) rings, the bond lengths within the ferrocene core, as well as the bond lengths in the attached tripeptide strand present no unexpected features and are all within the range typically found for the analogous classes of compounds. The Cp rings are in the eclipsed conformation with a pseudo torsion angle C1–Cg1–Cg2–C6 of the ferrocene being approx. 1.75° (where Cg1 and Cg2 are centroids of the cyclopentadienyl rings C1–C5 and C6–C12, respectively). The Cp rings are coplanar with each other, displaying a tilt angle of only 2.3(3)°.

Table S4. Details on hydrogen bond geometry for 2a.

| D–H···A   | d(H···A) / Å | d(D···A) / Å | \(\angle(D–H···A) / ^\circ\) |
|-----------|------------|-------------|-------------------------|
| N1–H11···O3 | 2.35(3)    | 3.142(4)    | 158(3)                 |
| N2–H12···O4 | 2.24(3)    | 2.961(4)    | 159(3)                 |

\(^1\) CrysAlisPRO, Oxford Diffraction/Agilent Technologies UK Ltd, Yarnton, England.
\(^2\) L. Palatinus and G. Chapuis, Superflip - a computer program for the solution of crystal structures by charge flipping in arbitrary dimensions. *J. Appl. Cryst.* **2007**, *40*, 786.
\(^3\) M. Sheldrick, Crystal structure refinement with SHELXL. *Acta Cryst.* **2015**, *C71*, 3.
\(^4\) C. F. Macrae, I. J. Bruno, J. A. Chisholm, P. R. Edgington, P. McCabe, E. Pidcock, L. Rodriguez-Monge, R. Taylor, J. van de Streek and P. A. Wood, New features for the visualization and investigation of crystal structures. *J. Appl. Crystallogr.* **2008**, *41*, 466.
### Table S5. Crystal data and details of the structure determination for 2a.

| **Compound** | **2a** |
|--------------|--------|
| **Formula**  | C\textsubscript{28} H\textsubscript{38} Fe N\textsubscript{4} O\textsubscript{5} |
| **\(M_r\)**  | 566.47 |
| **Colour and habit** | Orange, block |
| **Crystal system, space group** | Orthorombic, \(P2_12_12_1\) |
| **Crystal dimensions (mm\(^3\))** | 0.59 x 0.52 x 0.50 |
| **\(a\) (Å)** | 10.4815(5) |
| **\(b\) (Å)** | 15.8006(8) |
| **\(c\) (Å)** | 17.2486(11) |
| **\(\alpha\) (˚)** | 90 |
| **\(\beta\) (˚)** | 90 |
| **\(\gamma\) (˚)** | 90 |
| **\(V\) (Å\(^3\))** | 2856.6(3) |
| **\(Z\)** | 4 |
| **\(D_{\text{calc}}\) (g cm\(^{-3}\))** | 1.317 |
| **\(\mu\) (mm\(^{-1}\))** | 0.571 |
| **\(F(000)\)** | 1200 |
| **\(\theta\) range for data collection (˚)** | 4.38 – 27.00 |
| **\(h, k, l\) range** | \(-13:12, -20:13, -22:15\) |
| **Scan type** | \(\omega\) |
| **No. measured reflections** | 9022 |
| **No. independent reflections (\(R_{\text{int}}\))** | 5753 (0.0426) |
| **No. observed reflections, \(I \geq 2\sigma(I)\)** | 4436 |
| **No. refined parameters** | 351 |
| **\(R, wR[I \geq 2\sigma(I)]\)** | 0.0425, 0.0900 |
| **\(R, wR[\text{all data}]\)** | 0.0632, 0.0987 |
| **Goodness of fit on \(F^2, S\)** | 0.981 |
| **Max., min. electron density (e Å\(^{-3}\))** | 0.220, −0.166 |
| **CCDC number** | 2035760 |
### CD spectroscopy

Table S6. UV/Vis data of compounds 1a-4a in DCM and DCM/ DMSO mixtures (c = 1·10⁻³ M)

| Solvent    | 1a          | 2a          | 3a          | 4a          |
|------------|-------------|-------------|-------------|-------------|
| CH₂Cl₂     | 452 (883)   | 452 (672)   | 452 (1005)  | 452 (1006)  |
| DMSO = 5%  | 452 (862)   | 452 (664)   | 452 (978)   | 452 (954)   |
| DMSO = 9%  | 452 (846)   | 452 (660)   | 452 (953)   | 452 (933)   |
| DMSO = 13% | 452 (836)   | 452 (657)   | 452 (936)   | 452 (918)   |
| DMSO = 17% | 452 (826)   | 452 (653)   | 452 (922)   | 452 (907)   |
| DMSO = 20% | 452 (817)   | 452 (647)   | 452 (907)   | 452 (897)   |
| DMSO = 23% | 452 (810)   | 452 (644)   | 452 (892)   | 452 (885)   |

### CD data of compounds 1a-4a in DCM and DCM/ DMSO mixtures (c = 1·10⁻³ M)

| Solvent    | 1a          | 2a          | 3a          | 4a          |
|------------|-------------|-------------|-------------|-------------|
| CH₂Cl₂     | 471 (-1193)| 466 (-5463)| 469 (1352)  | 468 (1546)  |
| DMSO = 5%  | 467 (-680) | 467 (-5180)| 469 (761)   | 469 (684)   |
| DMSO = 9%  | 465 (-398) | 467 (-4901)| 468 (481)   | 470 (564)   |
| DMSO = 13% | 467 (-662) | 467 (-4666)| 465 (393)   | 465 (477)   |
| DMSO = 17% | 471 (-562) | 468 (-4434)| 466 (374)   | 465 (475)   |
| DMSO = 20% | 469 (-528) | 468 (-4189)| 472 (343)   | 457 (408)   |
| DMSO = 23% | 465 (-515) | 467 (-3966)| 468 (319)   | 461 (377)   |
Figure S34. CD curves of 1-4 in CH$_2$Cl$_2$ ($c = 1 \cdot 10^{-3}$ M, solid lines) with reduction of CD activity during unfolding induced by addition of up to 25% of DMSO (dashed lines).
HRMS spectra of compounds 1a-4a

Compound 1a

**Qualitative Analysis Report**

| Data Filename | OOS.d | Sample Name | PI-B5 |
|---------------|-------|-------------|-------|
| Sample Type   | Sample| Position    |       |
| Instrument Name | QTOF1 | User Name   |       |
| Acq Method    | metoda potvrda PMF.m | Acquired Time | 2/22/2017 6:33:25 AM |
| IRM Calibration Status | Success | DA Method | polyfenols.m |
| Comment       |       | Info.       |       |

**User Spectra**

| Fragmentor Voltage | Collision Energy | Ionization Mode | m/z       | z | Abund | Formula      | Ion       |
|--------------------|------------------|-----------------|-----------|---|-------|--------------|-----------|
| 175                | 0                | ESI             | 121.0509  | 1 | 473772.43 |             |           |
|                    |                  |                 | 467.1747  | 1 | 371223.82 |             |           |
|                    |                  |                 | 468.1771  | 1 | 105097.91 |             |           |
|                    |                  |                 | 564.223   | 1 | 129444.24 |             |           |
|                    |                  |                 | 566.2198  | 1 | 1237593.93|             |           |
|                    |                  |                 | 567.2244  | 1 | 1335097.14|             |           |
|                    |                  |                 | 568.2281  | 1 | 307929.13 |             |           |
|                    |                  |                 | 589.2082  | 1 | 98043.82  | C28 H38 Fe N4 O5 | (M+Na)^+ |
|                    |                  |                 | 922.0098  | 1 | 863316.48 |             |           |
|                    |                  |                 | 923.013   | 1 | 173370.39 |             |           |

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Figure S35. HRMS spectrum of 1a
Figure S36. HRMS spectrum of 2a
Figure S37. HRMS spectrum of 3a
Figure S38. HRMS spectrum of 4a
## DFT study

Table S8. Relative energies of the most stable conformers of compounds 1a–4a optimized in chloroform at 298 K. Optimizations performed at the B3LYP-D3/6-311+G(d,p), LanL2DZ for Fe level of theory, PCM model for modelling solvent effects. Proline puckering modes of both prolines (Pro1 is closer to N-terminus), cis/trans isomers of proline peptide bonds, helicity, labels of intramolecular hydrogen bond patterns displayed in Figure S35 and X–Y distances [Å] of the selected X–H···Y hydrogen bonds connecting the n-membered rings.

| conformer | ΔE [kJ mol\(^{-1}\)] | χ angle | Pro1 ring pucker mode | Pro2 ring pucker mode | Boc-Pro1-Pro2 helicity | IHB pattern | NH···OC\(_{13}\) 13-membered | NH···OC\(_{10}\) 10-membered | NH···OC\(_{7}\) 7-membered | NH···N\(_{5}\) 5-membered | NH···OC\(_{10}\) 10-membered | NH···OC\(_{7}\) 7-membered |
|-----------|---------------------|--------|----------------------|----------------------|------------------------|------------|------------------|------------------|------------------|------------------|------------------|------------------|
| compound 1a |                     |        |                      |                      |                        |            |                  |                  |                  |                  |                  |                  |
| 1a-1      | 0.00                | −78.9  | endo                 | trans-cis            | P                      | A          | 2.92             |                  |                  |                  |                  | 3.02             |
| 1a-2      | 2.39                | −74.8  | endo                 | trans-cis            | P                      | A          | 2.88             |                  |                  |                  |                  | 3.05             |
| 1a-3      | 2.89                | 75.6   | endo                 | trans-trans          | P                      | B          | 3.02             | 2.74             | 2.87             |                  |                  | 2.85             |
| 1a-4      | 5.18                | 70.7   | exo                  | trans-cis            | P                      | C          | 2.90             | 2.74             | 2.87             |                  |                  | 2.85             |
| 1a-5      | 7.19                | 75.4   | exo                  | trans-trans          | P                      | B          | 2.99             | 2.74             | 2.87             |                  |                  | 2.85             |
| 1a-6      | 7.25                | 68.1   | endo                 | trans-cis            | P                      | C          | 2.90             | 2.74             | 2.87             |                  |                  | 2.84             |
| compound 2a |                     |        |                      |                      |                        |            |                  |                  |                  |                  |                  |                  |
| 2a-1      | 0.00                | −106.4 | endo                 | trans-trans          | P                      | B          | 3.01             | 2.72             | 3.05             |                  |                  | 3.05             |
| 2a-2      | 0.44                | −86.1  | endo                 | trans-trans          | P                      | B          | 3.02             | 2.72             | 3.07             |                  |                  | 3.07             |
| 2a-3      | 2.14                | −98.0  | exo                  | trans-trans          | P                      | B          | 2.95             | 2.75             | 3.09             |                  |                  | 3.09             |
| 2a-4      | 2.16                | −82.8  | exo                  | trans-trans          | P                      | B          | 2.97             | 2.73             | 3.07             |                  |                  | 3.07             |
| 2a-5      | 3.81                | −85.8  | endo                 | trans-trans          | P                      | B          | 3.06             | 2.72             | 3.15             |                  |                  | 3.15             |
| 2a-6      | 4.36                | −79.7  | exo                  | trans-trans          | P                      | B          | 2.97             | 2.74             | 3.15             |                  |                  | 3.15             |
| 2a-7      | 5.38                | −104.8 | endo                 | trans-trans          | P                      | B          | 3.00             | 2.73             | 3.15             |                  |                  | 3.15             |
| compound 3a |                     |        |                      |                      |                        |            |                  |                  |                  |                  |                  |                  |
| 3a-1      | 0.00                | 98.3   | exo                  | trans-trans          | M                      | B          | 2.94             |                  |                  |                  |                  | 3.03             |
| 3a-2      | 1.70                | 103.7  | endo                 | trans-trans          | M                      | B          | 2.98             | 2.75             | 3.08             |                  |                  | 3.08             |
| 3a-3      | 1.79                | −79.2  | endo                 | trans-trans          | P                      | D          | 2.95             |                  |                  |                  |                  | 2.85             |
| 3a-4      | 5.17                | −77.2  | endo                 | trans-trans          | P                      | D          | 2.92             |                  |                  |                  |                  | 2.86             |
| 3a-5      | 5.73                | 95.6   | endo                 | trans-trans          | M                      | B          | 3.00             | 2.75             | 3.02             |                  |                  | 3.02             |
| compound 4a |                     |        |                      |                      |                        |            |                  |                  |                  |                  |                  |                  |
| 4a-1      | 0.00                | 74.1   | endo                 | trans-cis            | M                      | A          | 2.88             |                  |                  |                  |                  | 3.07             |
| 4a-2      | 0.55                | 79.4   | endo                 | trans-cis            | M                      | A          | 2.93             |                  |                  |                  |                  | 3.04             |
| 4a-3      | 1.76                | 81.6   | endo                 | trans-trans          | M                      | E          | 2.88             |                  |                  |                  |                  | 2.88             |
| 4a-4      | 7.80                | 68.5   | exo                  | trans-trans          | P                      | C          | 2.92             | 2.91             |                  |                  |                  |                  |
| 4a-5      | 7.81                | −77.2  | endo                 | trans-cis            | P                      | C          | 2.84             | 2.86             |                  |                  |                  |                  |
Figure S39. The hydrogen bond patterns observed in the optimized geometries of the most stable conformers 1a – 4a. Numbering scheme for the selected n-membered hydrogen bonded rings.
Figure S40. DFT optimized geometries of the most stable conformers of 1a and 2a.
Figure S41. DFT optimized geometries of the most stable conformers of 3a and 4a.
Figure S42. Superposition of the calculated (2a-5, blue colour) and experimentally determined (from the crystal structure, orange colour) geometries of compound 2a. RMSD is 0.9489 Å.
Figure S43. TDDFT calculated ECD spectra of compounds 1a – 4a. The final Boltzmann-averaged spectrum at 298 K (red dashed line) is obtained by weighting each conformer spectrum (coloured solid lines) with the appropriate conformer Boltzmann weight factor for the final set of structures labeled in Table S6.
**Excited states**

**Compound 1a**

Excited states 1 to 6 in the 1a-1 conformer. Left side displays pairs of natural transition orbitals and occupation numbers (above arrows) particular to the states of interests. Right side shows density difference plot for each transition, regions of increased (violet) and decreased (cyan) electron density.

*Figure S44. Excited states 1 in the 1a-1 conformer.*

*Figure S45. Excited states 2 in the 1a-1 conformer.*
Figure S46. Excited states 3 in the 1a-1 conformer.

Figure S47. Excited states 4 in the 1a-1 conformer.
Figure S48. Excited states 5 in the 1a-1 conformer.

Figure S49. Excited states 6 in the 1a-1 conformer.
Compound 2a

Excited states 1 to 6 in the 2a-1 conformer. Left side displays pairs of natural transition orbitals and occupation numbers (above arrows) particular to the states of interests. Right side shows density difference plot for each transition, regions of increased (violet) and decreased (cyan) electron density.

Figure S50. Excited states 1 in the 2a-1 conformer.

Figure S51. Excited states 2 in the 2a-1 conformer.
Figure S52. Excited states 3 in the 2a-1 conformer.

Figure S53. Excited states 4 in the 2a-1 conformer.
Figure S54. Excited states 5 in the 2a-1 conformer.

Figure S55. Excited states 6 in the 2a-1 conformer.