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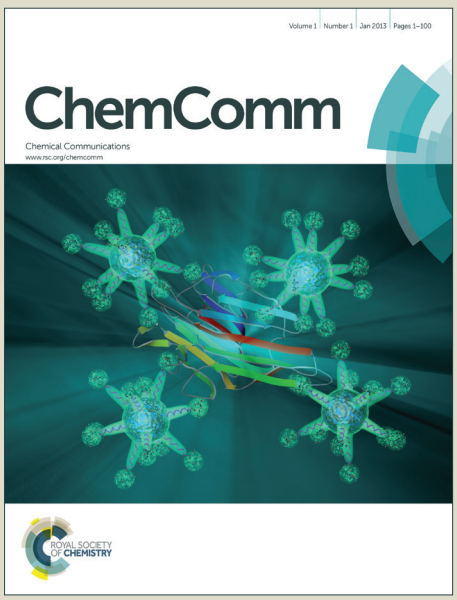

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ARTICLE TYPE

A small molecule peptidomimetic of spider silk and web†

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A peptidomimetic compound containing leucine, tyrosine and malonic acid self-assembles through various noncovalent interactions to form spider silk like fiber at ambient temperature. From the high-density liquid, a liquid–solid phase transition has initiated at 20°C and solidification occurs upon contact with air. The fiber has comprehensive mechanical strength and optical properties like spider silk to mimic a natural spider web.

Peptidomimetic -small protein like molecules designed to mimic natural peptides or proteins- compounds continue to grow in interest due to their potential applications in material science, biochemistry and medicinal chemistry.[1] These designer compounds should be able to recognize the targets like natural counterpart or should have same biological effects and material properties.[2] The organization of peptidomematic compounds by noncovalent interactions such as hydrogen bonding, π–π stacking and hydrophobic interactions, in a particular fashion is the key step towards the fabrication of new materials.[3] In addition, the study of functional diversities of small peptidomimetic compounds, containing chiral amino acids and non chiral spacers are relatively rare.[4]

Spider silks are protein polymers and glue that are naturally spun into fibers and threads by spiders.[5] The spider silk have extremely high mechanical strength, stability, biocompatibility, smoothness, and thinness in comparison to other available materials, which make them especially interesting.[6] The ambience conditions like temperature, humidity, acidity, UV radiation have significant effect on the mechanical properties of the spider silk.[7] However, there are little approaches to develop synthetic materials having spider silk like properties.

Herein, we have synthesized a series of peptidomimetic compounds containing leucine, tyrosine and malonic acid and investigated their structures and functions.

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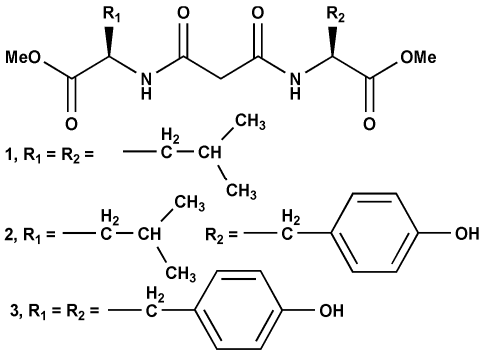
Interestingly the non symmetric compound **2** formed spider silk like soft fibers. The soft fiber has comprehensive mechanical strength and exhibits violet-blue birefringence under cross polarized light which is a characteristic of natural spider silk. The soft fibers are very stable at ambient temperature even to spin and mimic a natural spider web.

For peptidomimetic compounds **1, 2** and **3** the design principle explored was how to use a central nonchiral spacer between two terminal chiral amino acids with hydrophobic adjustment (Scheme 1). There are two hydrophobic leucine residues in compound **1**. For compounds **2** and **3**, we have used the Tyr residue to introduce additional hydrogen bonds. Compound **2** is non symmetric. Target compounds **1, 2** and **3** were synthesized by functionalizing malonic acid with the appropriate chiral amino acid methyl ester following a high purity, as confirmed by ^{1}H-NMR (nuclear magnetic resonance), ^{13}C-NMR, Fourier-transform infrared (FTIR) and mass spectrometry (MS) analysis (see Supplementary Information).



Scheme 1. The peptidomimetic compounds **1, 2** and **3**.

The compound **1** is solid but compound **2** and **3** are highly viscous liquid at 25°C. However, for compound **2**, a liquid–solid phase transition has initiated at 20°C and solidification occurs upon contact with air. Compound **3** does not exhibit such properties under same condition and remain highly viscous even below 20°C. The phase transition and solidification upon contact with air at 20°C facilitate to form soft fiber from highly viscous compound **2**. The fibers have prepared using a spatula (inset of Figure 1a) or a glass rod deep inside highly viscous compound **2** and pull it out. An

injection syringe can provide soft fiber with uniform diameter like a spigot of a spider. The soft fibers of compound **2** have adhesive glue property like a glue covered spider silk fiber. The soft fibers are very stable at ambient temperature even to spin a web (Figure 1a) that can mimic a natural spider web (Figure 1b). The polarized optical microscope based on an Olympus platform with a rotatable stage was used to study the nature of the soft fibers. For better contrast and magnification, a "dry" 50× long distance objective was used. The use of the "dry" objective allowed high-resolution measurements without contaminating the soft fibers with either oil or water, which may alter the nature of the fibers. We have also studied the natural spider silk under same condition as reference. The soft fiber obtained from peptidomimetic compound **2** exhibits violet-blue birefringence under cross polarizer (Figure 1c). The reference natural spider silk also shows same type of violet-blue birefringence under cross polarized light (Figure 1d).[8] We conclude that the optical properties of the native spider silk and the soft fibers from the peptidomimetic compound **2** are very close.

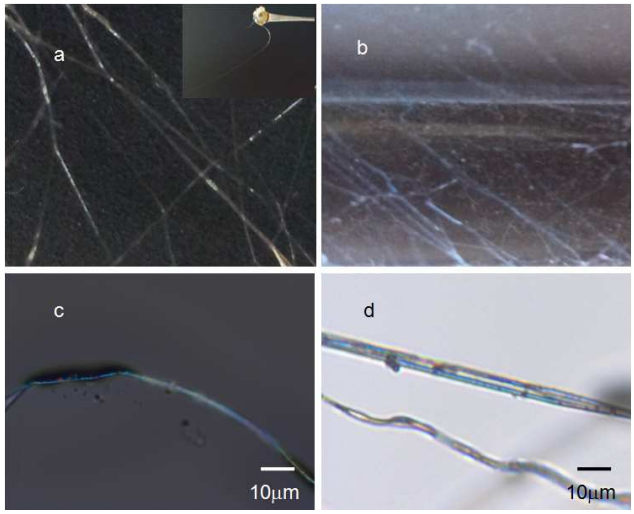


Fig. 1 (a) Photograph of synthetic web made by soft fiber of compound **2**. Inset: the development of fiber from a highly viscous liquid using a spatula (b) Photograph of a natural spider web. (c) Soft fiber of compound **2** under polarized-light showing violet-blue birefringence. (d) Polarized-light micrograph of natural spider silk showing violet-blue birefringence.

The viscoelastic properties of peptidomimetic compounds **2** and **3** were measured with a commercial rheometer (AR-G2, TA Instruments, New Castle, USA). The surface between the inner ring and the circular wall of the container was oscillatory sheared with constant maximum strain. Flow theory between concentric cylinders is applied and both the storage modulus (G´, a measure of the elastic response of the material) and the loss modulus (G´´, a measure of the viscous response) were measured at 25°C as a function of time. The storage modulus (G´) of the compound **2** was found to be approximately an order of magnitude lower than the loss modulus (G´´), indicative of an viscous rather than elastic material (Figure 2a).[9] Figure 2b shows that the viscoelastic properties of compound **3** are significantly different from that of compound **2**. This may be due to difference in hydrophobic and pi-pi stacking interactions among the reported compounds.

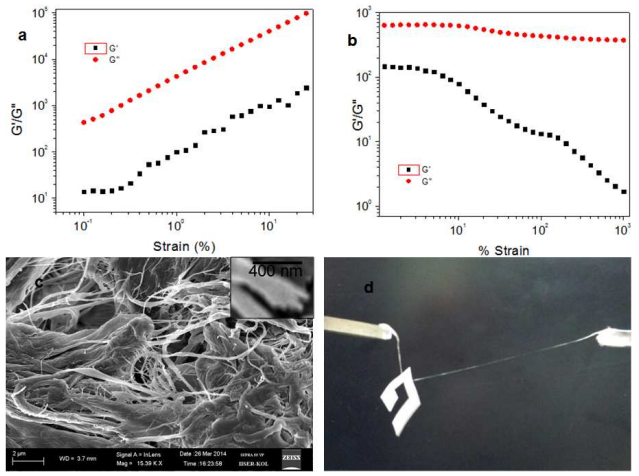


Fig. 2 Mechanical response of (a) compound **2** and (b) compound **3** at 25 °C with small oscillatory shear in the linear viscoelastic regime. Black circle: storage modulus (G´); red circle: loss modulus (G´´). (c) FE-SEM image of polydisperse long fibers of compound **2**. Inset: the enlarged fiber end showing multiple filaments bundle. (d) The hanging weight showing the mechanical strength of the fiber.

The morphology of the soft fibers obtained from compound **2** was observed by field emission scanning electron microscopy. The analysis of the morphology of compound **2** by FE-SEM reveals the formation of entangled polydisperse long unbranched fibers (Fig. 2c). The inset of Figure 2c shows a fiber with diameter about 200 nm. From the end of the fiber (Fig. 2c inset), the fiber is actually a thick bundle of several filaments with diameter *ca* 25 nm, which is a very common feature of natural spider silk.[10] The fiber has comprehensive mechanical strength. Further the mechanical strength of the fiber was studied by hanging weights (Figure 2d). A single fiber fabricated between two glass rod can allow 15 mg of weights.

FT-IR spectroscopy is an excellent technique to investigate the self-assembly of the peptidomimetic compounds. The region 3500–3200 cm^{-1} is important for the N–H stretching vibrations and 1800-1600 cm^{-1} corresponding to C=O stretching vibration of amide and ester groups. The intense bands at 3283 cm^{-1} for compound **1**, 3318 cm^{-1} for compound **2** and **3** indicate that all NH groups are hydrogen bonded (Fig. 3a).[11] The peaks at 1756 cm^{-1} for compound **1** and 1746 cm^{-1} for compound **2** and **3** indicate the C=O stretching vibrations of saturated non hydrogen bonded methyl ester (Fig. 3a). The compound **1** shows the amide I band at 1665 cm^{-1} and amide II band at 1540 cm^{-1}. Whereas compounds **2**, and **3** have the amide I band at 1662 cm^{-1} and amide II band at 1518 cm^{-1} (Fig. 3a). These results indicate that the hydrogen bonds mediated self-assembly pattern of the reported compounds **1**, **2** and **3** are very close.

Moreover, to investigate the nature of hydrogen bonding whether it is intramolecular or intermolecular, the NMR titration experiments were performed. To a separate solution of compounds **1**, **2** and **3** in $CDCl_3$ the hydrogen bond

accepting solvent DMSO-d_6 was gradually added by an amount of 10μL at room temperature and the NMR spectra were recorded. Generally, with the addition of small amount of $(CD_3)_2SO$ in $CDCl_3$ brings about monotonic downfield shifts of the solvent exposed NH functional groups, leaving the solvent-shielded NH groups mostly unaffected.[12] The results of the NMR titrations of compound **2** have been shown in the Figure 3b. The NMR titrations data show that the NH protons get exposed to the solvent and there are significant downfield shift of NH protons for the compound **2** with gradually increase in DMSO-d_6 concentration. Similar results have obtained for compounds **1** and **3** (ESI Figure 1). These results indicate that the compounds **1**, **2** and **3** form intermolecular hydrogen bonded structure.

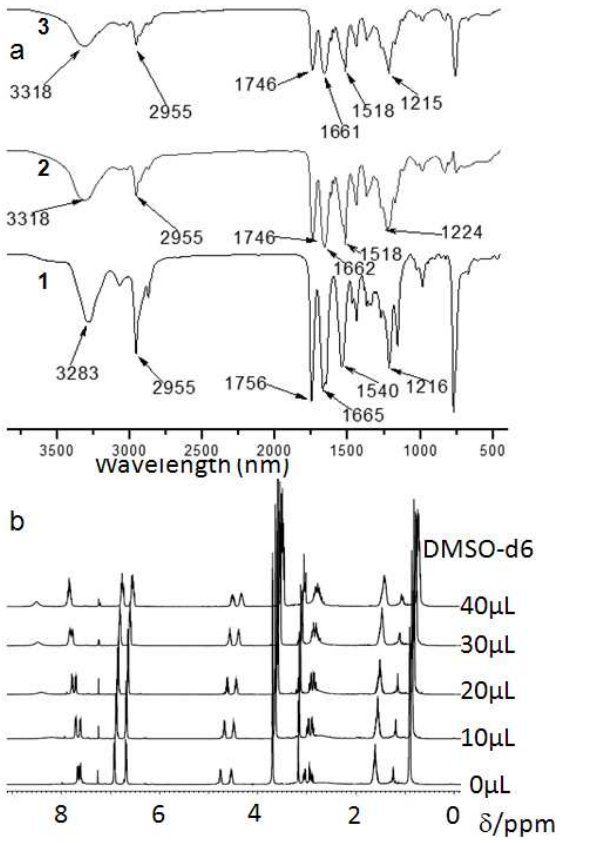


Fig. 3 (a) Solid state FT-IR spectra of compound **1**, **2** and **3**. (b) The solvent dependence of NH chemical shifts of compound **2** at varying concentrations of $(CD_3)_2SO$ in $CDCl_3$ solutions.

Solid state conformation of compound **1** has been studied by X-ray crystallography.[13] Compound **2** and **3** have failed to generate crystals. The torsion angle around the leucine residues (ϕ= -99.94 and ψ = 175.01) appears to play a critical role in dictating the overall extended structure of compound **1**. From the crystal structure of compound **1**, it is evident that there are two intermolecular hydrogen bonds (N1-H1....O6, 2.100Å, 2.938Å, 163°, x, 1+y, z and N2-H2....O3, 2.080Å, 2.917Å, 165°, x, -1+y, 1+z) between amide C=O and N-H leading to dimer structure (Fig. 4a). The dimeric assembly of compounds **1**, **2** and **3** also obtained from Mass spectrometry (see Supplementary Information). The dimeric units are further self-assembled by two cooperative hydrogen bonding interactions of amide groups to form a sheet like structure (Fig. 4b). For compound **1**, in higher order packing, a column like structure has been observed (Fig. 4c). From FTIR, Mass and NMR studies, the hydrogen bonds mediated self-assembly pattern of the compounds **1**, **2** and **3** are very close. Though the peptidomimetic has large surface-to-core ratio than the natural biopolymers with extended or globular domains, the molecular assembly pattern of compound **1**, is comparable with the packing pattern of hydrogen bonded strand and sheet in natural spider silk (Fig. 4d).[14]

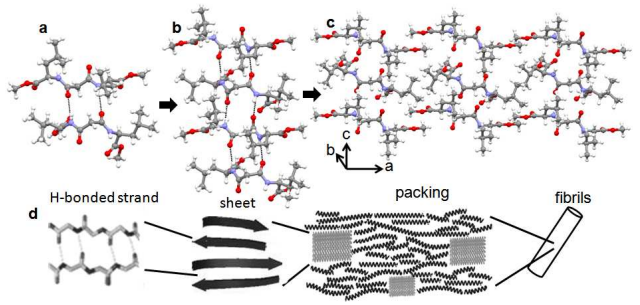


Fig. 4 (a) Hydrogen bonded dimer of compound **1** in crystal. (b) Sheet like assembly of compound **1**. (c) Higher order packing of compound **1** in crystal. (d) Schematic presentation of the packing of secondary structure elements in natural spider silk.

In summary, we have shown that the peptidomimetic compound containing leucine, tyrosine and malonic acid can mimic the structure and function of natural spider silk and web. The fiber has comprehensive mechanical strength and exhibits violet-blue birefringence under cross polarized light like natural spider silk. The results presented here may foster new studies to develop functionalized soft fibers with unconventional applications.

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