Controlled aggregation properties of modified single amino acids

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Abstract

Herein, we report the self-assembled structure formed by Fmoc protected charge single amino acid Fmoc-L-glutamic acid 5-tert-butyl ester (**Fmoc-Glu(OtBu)-OH**), Fmoc-L-aspartic acid 4-tert-butyl ester (**Fmoc-Asp(OtBu)-OH**), and Na-Fmoc-Ne-Boc-L-lysine (**Fmoc-Lys(Boc)-OH**). The self-assembled architecture formed by the charge aliphatic amino acids were assessed under different conditions such as concentration, temperature and pH. **Fmoc-Glu(OtBu)-OH** assembled to spheres at both lower and higher concentration under room temperature condition. However, it forms a broom stick like morphology at both lower and higher concentration on heating. **Fmoc-Asp(OtBu)-OH** on the other hand formed rod like at both both low and high concentration and also on hhealing**. Fmoc-Lys(Boc)-OH** also self-assemble to sphere like morphology in all conditions irrespective of concentration and heating. Since these structure are very intriguing, our future endevorous is to study these structure through different microscopic techniques such as scanning electron microscopy (SEM), Transmission Electron microscopy (TEM). The mechanisms of the structure formation by these amino acids will be characterized by using solution state NMR, FTIR and TGA in future. The self-assembled structures formed by modified amino acids are easy and

facile route to design novel nanoarchitectures which may be potentially useful in future for various type of applications in the field of material chemistry, bioscience, biomedical.

Keywords

Self-assembly; Fmoc-variant modified single amino acid; sphere; rod; broom stick.

Introduction

Molecular self-assembly is consider to be the branch of nanotechnology which involve the study of the self-assembled architecture formed by the molecules without the presence of external forces.[1] The forces which may impart in the formation of self-assembled structure governed by electrostatic interaction,[2] pi-pi stacking,[3, 4] hydrogen bonding,[2, 5] Van der waals forces of interaction,[6] and hydrophobic interaction.[7] However, the study of the self-assembling properties of amino acids is very crucial due to its significant importance which may associated with the several disease which occur due to the aggregation of single amin acids.[2, 8-10] Gazit et al reported for the very first time self-assembled structure formed by Phe-Phe,[11] followed Phe,[10] Tyr[8] and Trp[9] and its implication in amyloid associated disease. The same group has also reported the nano/micro architecture formed by modified amino acids, such as Fmoc-Phe-Phe[12] and Fmoc-Phe show gel like properties.[13]

Modified single amino acids are potential candidates that are used in the many research area owing to its potent applications in the field of material science,[14] tissue engineering,[15] 3D-printing,[16] chemistry,[17] biomedicine,[18] nanotechnological applications.[19] Out of various modified single amino acids, fluorenylmethyloxycarbonyl (Fmoc) protected amino acids are of particular interest due to its hydrophobicity and planarity which is facilitated by the presence of three membered aromatic ring.[13, 20, 21] Previous literature reports suggest that Fmoc conjugated amino acids show gel like properties and hence could absorb higher amount water molecules,[22] These gels were found to be biocompatible[23] and hence may be used for further biomedical applications such as drug delivery,[24] tracking,[25] and sensing.[26]

Herein, this manuscript we report the self-assembled structure formed by the modified charge amino acids, **Fmoc-Glu(OtBu)-OH**, **Fmoc-Asp(OtBu)-OH**, and **Fmoc-Lys(Boc)-OH**. The structure formation of this compounds was thoroughly characterized by optical microscopy under varying concentration from 1, 3, 5 and 10 mM at room temperature and after heating at 70 ^{0}C for 30 minutes. Recently, our group has been reported the self-assembled structure formed by Fmoc variant of threonine N-(9-Fluorenylmethoxycarbonyl)-O-tert-butyl-L-threonine (Fmoc-Thr(tbu)-OH) and Fmoc variant of serine N-(((9H-fluoren-9-yl)methoxy)carbonyl)-O-(tert-butyl)-L-serine (Fmoc-Ser(tbu)-OH) under varying concentration and temperature.[27] Our group has also reported the self-assembled architecture formed by modified aromatic single amino acids.[4] Moreover, in past there were several group who have reported self-assembled structures formed by single amino acids and modified amino acids. In this direction Panda et al reported the self-assembled structure of Fmoc-Cysteine and its application in drug delivery,[28] Gazit et al demonstrated that fluorenyl-methoxy-carbonyl-β,β-diphenyl-Ala-OH (Fmoc-Dip-Ala) to opel gemstone-like structure.[29] Moreover Sato et al. reported the self-assemble structure formed by Fmoc-lysine in DMSO:water mixture.[30] Recently, Kundu et al. reported that Fmoc-L-lysine di-Fmoc-L-lysine shows the gel-like properties in different organic solvent–water mixtures. Moreover, Bai et al reported Fmoc-dipeptide and assessed its catalytic role as thermolysin.[31]

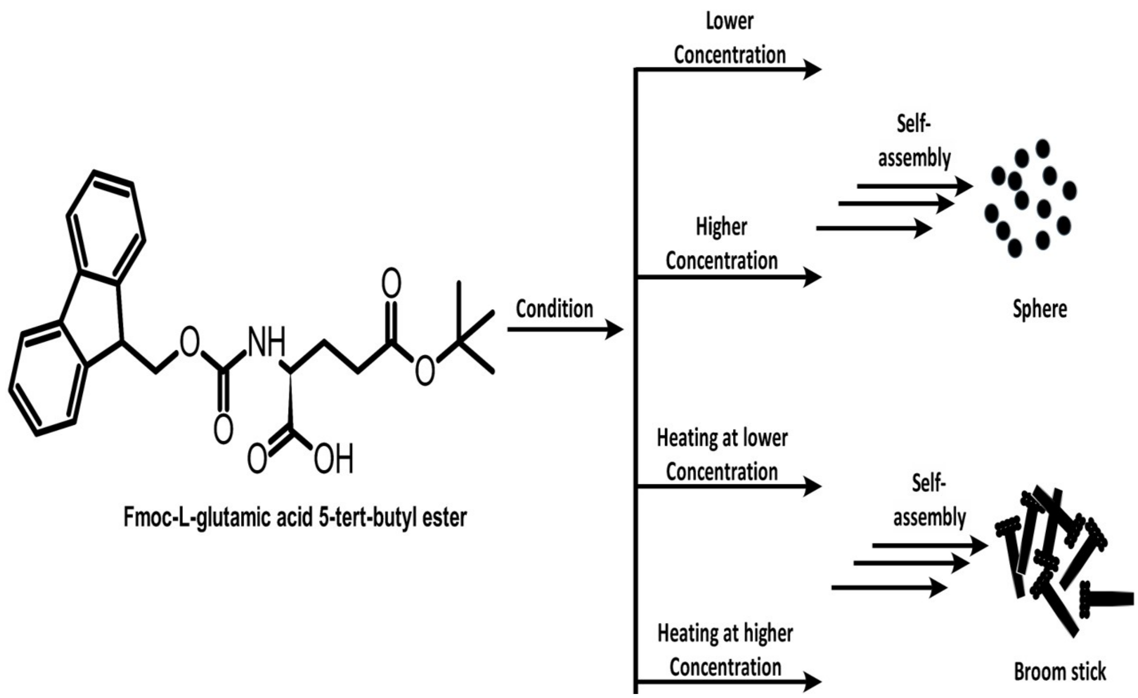


Figure 1. Controlled morphological changed in the self-assembled structures of **Fmoc-Glu(OtBu)-OH** under varying concentration and temperature.

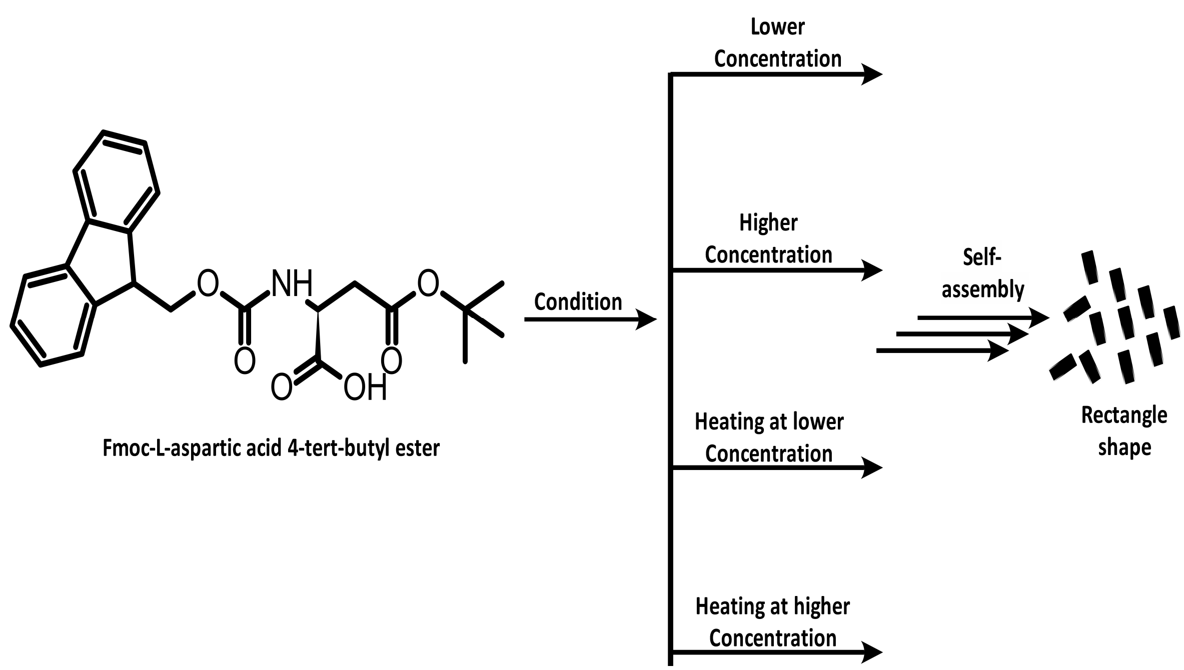


Figure 2. Controlled morphological changed in the self-assembled structures of **Fmoc-Asp(OtBu)-OH** under varying concentration and temperature.

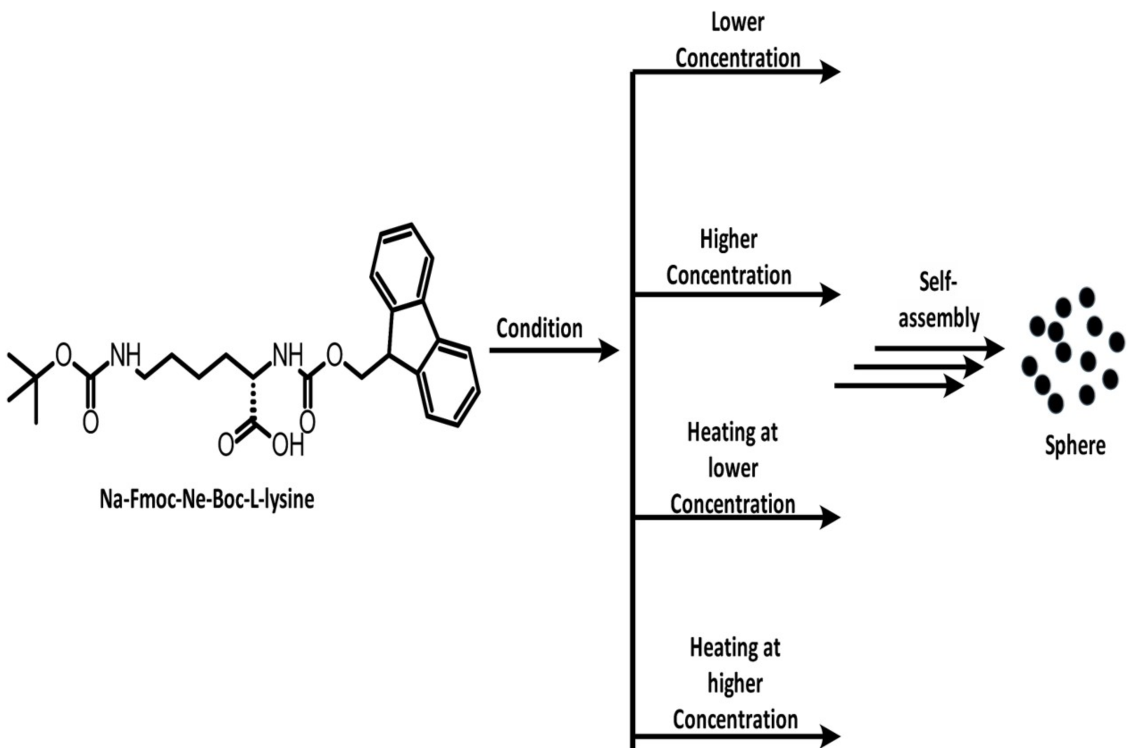
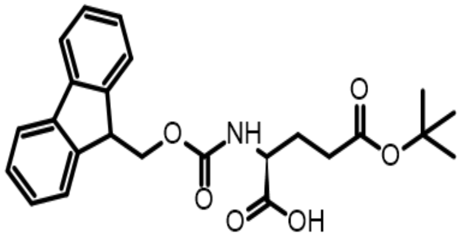


Figure 3. Controlled morphological changes in the self-assembled structures formed by (**Fmoc-Lys(Boc)-OH**) under varying concentration and temperature.

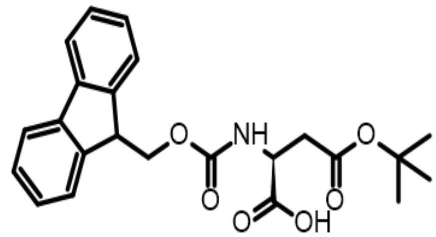
Figure 1 describe the graphical depiction of self-assembled structure formed by **Fmoc-Glu(OtBu)-OH.** The figure shows that **Fmoc-Glu(OtBu)-OH** self-assemble to sphere like structure at both low and high concentrations in room temperature conditions. When this solution was heated at 70 ^{0}C for 30 minutes their was a morphological transition from sphere to t broom stick like morphologies. Similar studies were also performed on **Fmoc-Asp(OtBu)-OH** (Figure 2). **Fmoc-Asp(OtBu)-OH** shows a irregular rectangular rod like self-assembled structure at low and high concentration under both room temperature and after heating at at 70 ^{0}C for 30 minutes. The **Fmoc-Lys(Boc)-OH** shows sphere like self-assembly in all condition such as low and highconcentration and also on heating.

Our group are interested to study the self-assembly of single amino acids,[2, 5, 32, 33] modified single amino acids,[4, 27] peptides,[34-37] and heterocyclic compounds.[38-42] Recently, our group has been reported the self-assembly of non-aromatic single amino acids cysteine and methionine formed a amyloid-like fibrillar structures.[2] In another studies our group has also reported the self-assembled structure formation by Proline (Pro), hydroxyproline (Hyp), and lysine.HCl (Lys) to globular, fibrillar, and tape-like self-assembled structure at various ageing time and increasing concentration.[5] In addition to these our group has also studied the self-assembled structure formation by the heterocyclic compounds and assessed its implications on their photophysical characteristics. In this direction, we have also studied the self-assembled structure formation and aggregation properties of pyridothiazole conjugate (PTC1) and its application as aggregation-induced emission enhancement dye (AIEE) for sensing amyloid fibrillation.[39] In other work, we reported the self-assembly property of acyl thiourea based organic molecule and its applications for the sequential detection of copper and lactic acid.[41] Recently, we also reported lotus-like self-assembled structures formed by a new AIEE dye andits application in sensing and bioimaging.[40] Hence, from our previous studies, we motivated to study the self-assembly of modified single amino acids to assess their applications as functional materials in the future.

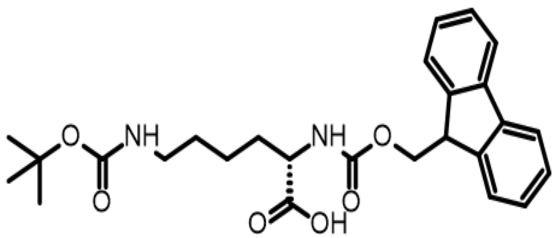
Result and Discussion



Fmoc-L-glutamic acid 5-tert-butyl ester



Fmoc-L-aspartic acid 4-tert-butyl ester



Na-Fmoc-Ne-Boc-L-lysine

Scheme 1: Chemical structure of **Fmoc-Glu(OtBu)-OH**, **Fmoc-Asp(OtBu)-OH**, and **Fmoc-Lys(Boc)-OH**

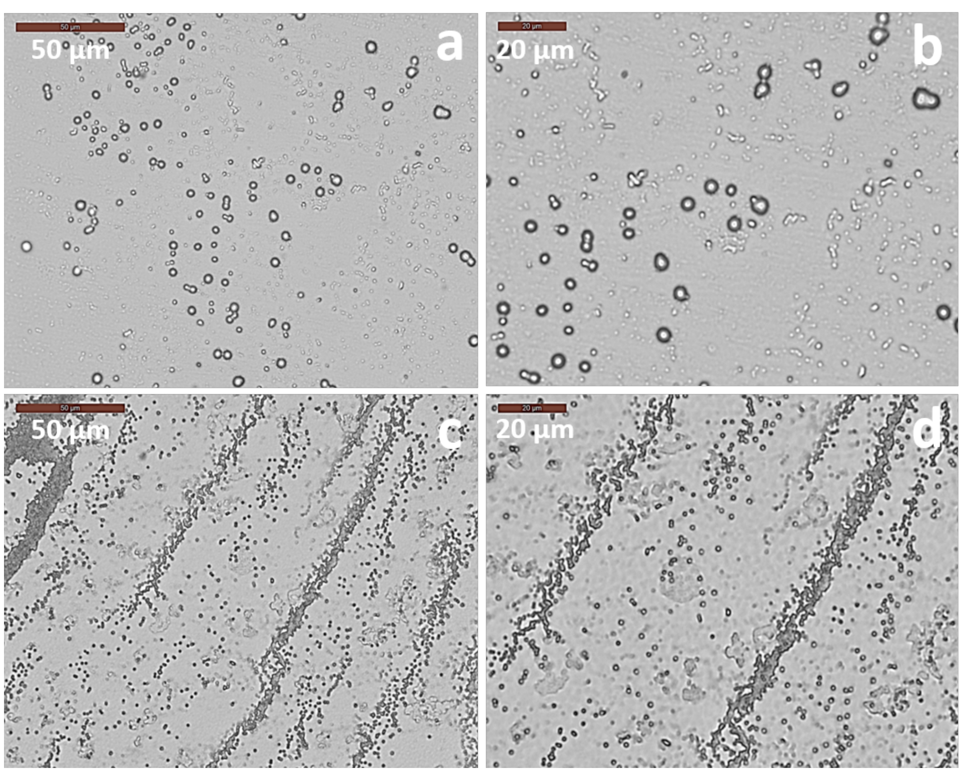


Figure 4: Self-assembled structures formed by **Fmoc-Glu(OtBu)-OH** at room temperature (a) Optical microscopy images at 3 mM concentration under 40X; (b) Optical microscopy images at 1 mM concentration under 63X; (c) Optical microscopy images at 10 mM concentration under 40X; (d) Optical microscopy images at 10 mM concentration under 63X.

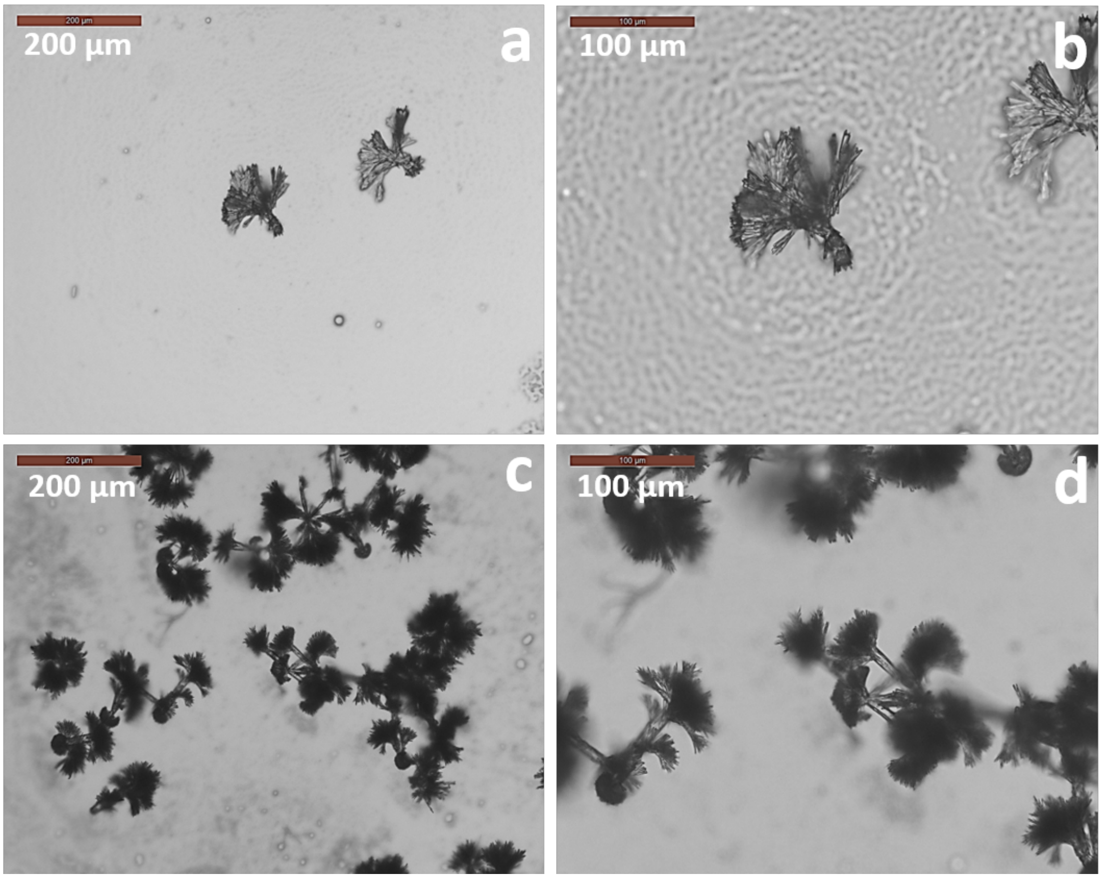


Figure 5: Self-assembled structures formed by **(Fmoc-Glu(OtBu)-OH)** on heating at 70 ^{0}C (a, b) Optical microscopy images at 3 mM concentration under 40X; (c, d) Optical microscopy images at 10 mM concentration under 40X.

The **Fmoc-Glu(OtBu)-OH** shows a sphere like morphology at both lower and higher concentration at room temperature (Figure 4). When the same sample were heated the sphere changes to the broom stick like morphology.

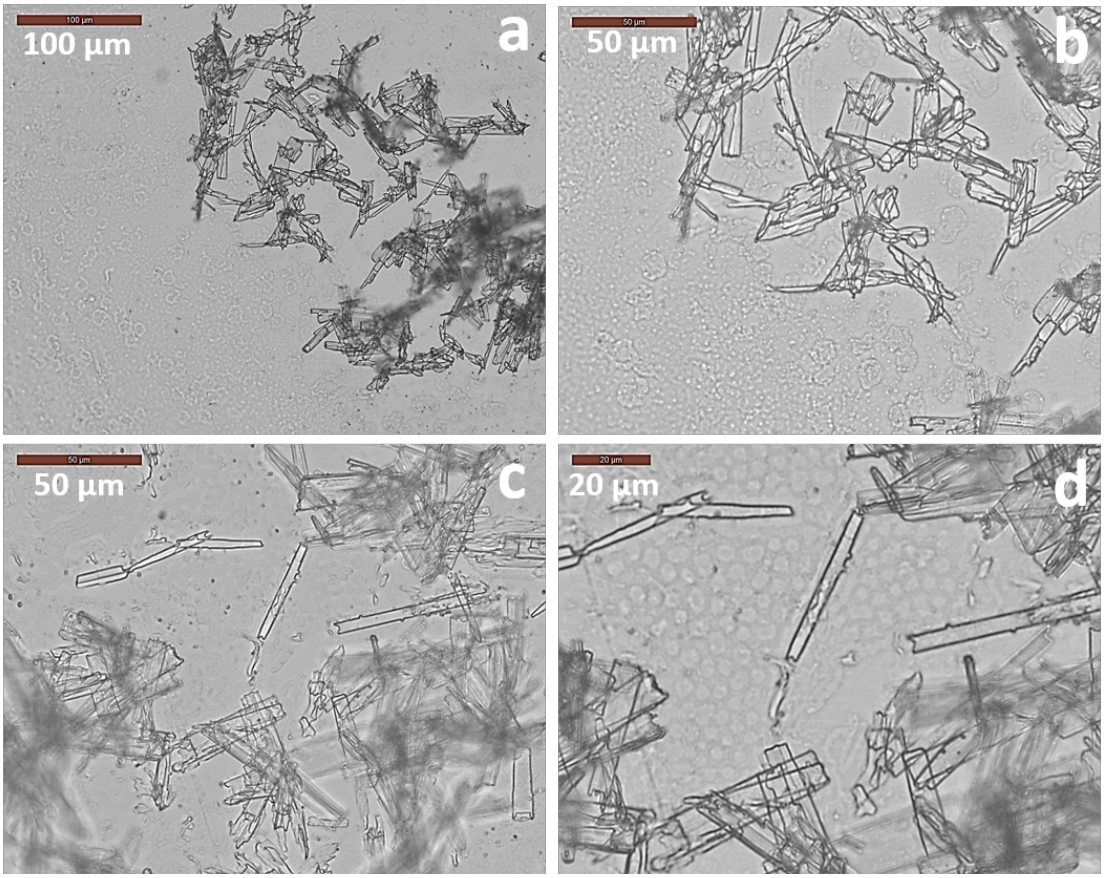


Figure 6. Self-assembled structure formed by **Fmoc-Asp(OtBu)-OH** at room temperature (a) Optical microscopy image at 3 mM concentration under 20X; (b) Optical microscopy image at 3 mM concentration under 40X; (c) Optical microscopy image at 10 mM concentration under 40X; and (d) Optical microscopy image at 10 mM concentration under 63X.

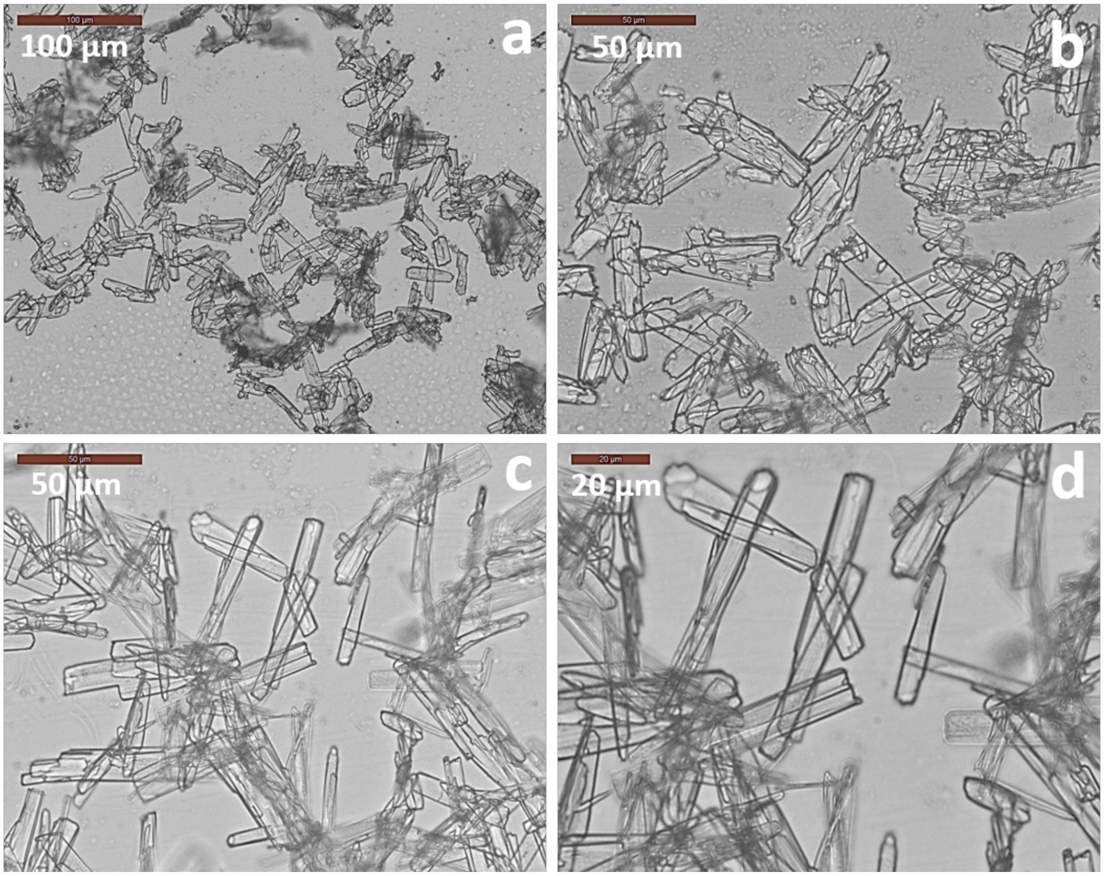


Figure 7. Self-assembled structure formed by **Fmoc-Asp(OtBu)-OH** on heated at 70 ^{0}C (a) Optical microscopy image at 3 mM concentration under 20X; (b) Optical microscopy image at 3 mM concentration under 40X; (c) Optical microscopy image at 10 mM concentration under 40X; and (d) Optical microscopy image at 10 mM concentration under 63X.

On the other hand **Fmoc-Asp(OtBu)-OH** shows a fractal like self-assembled structure lower and higher concentration. When the same samples were heated at 70 ^{0}C the self-assembled structure are not affected by heating and shows the same fractal like self-assembled structure at both lower and higher concentration on heating.

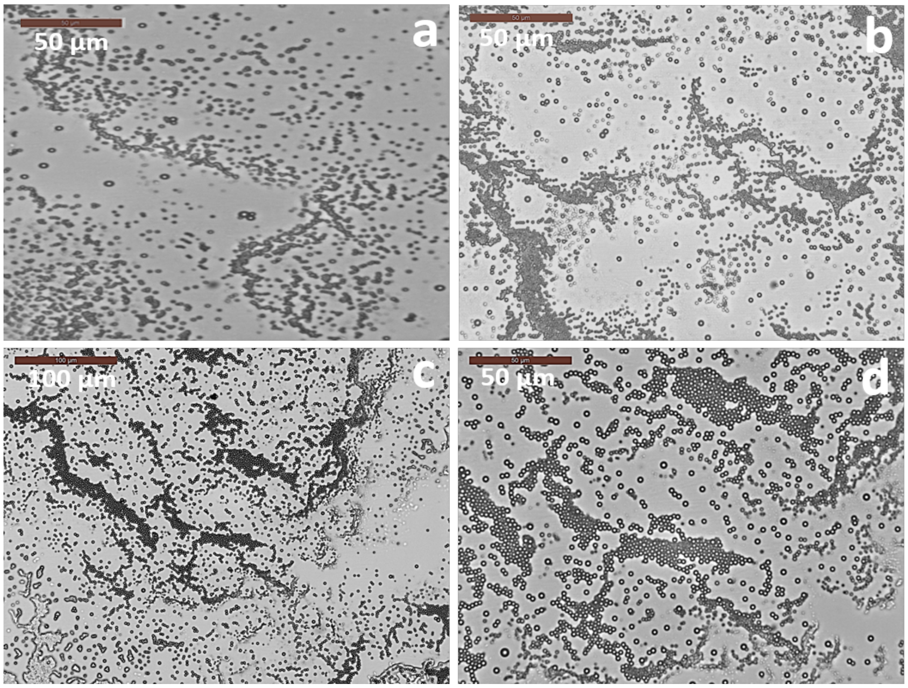


Figure 8. Self-assembled structures formed by **Fmoc-Lys(Boc)-OH** at room temperature. (a, b) Optical microscopy image at 3 mM concentration under 40X; (c) Optical microscopy image at 10 mM concentration under 20X; (d) Optical microscopy image at 10 mM concentration under 40X.

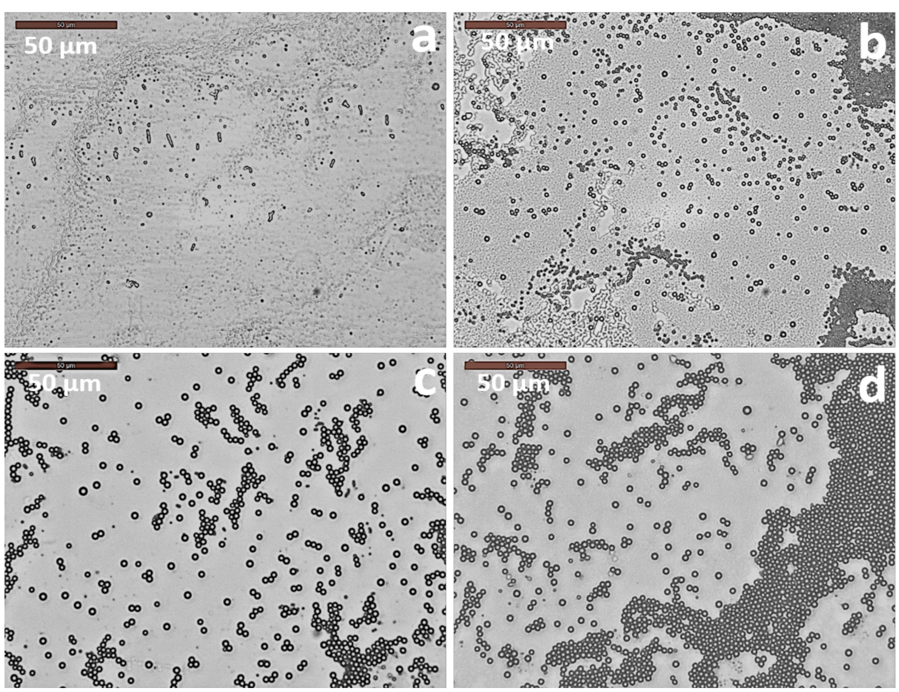


Figure 9: Self-assembled structures formed by **Fmoc-Lys(Boc)-OH** on heating at 70 ^{0}C temperature. (a, b) Optical microscopy images at 3 mM concentration under 40X; (c, d) Optical microscopy images at 10 mM under 40X.

Name of Sample	Concentration	Morphology	Condition
Fmoc-Glu(OtBu)-OH	Lower (3 mM)	Sphere	RT
Fmoc-Glu(OtBu)-OH	Higher (10 mM)	Sphere	RT
Fmoc-Glu(OtBu)-OH	Lower (3 mM)	Broom stick	On heating at 70 ^{0}C
Fmoc-Glu(OtBu)-OH	Higher (10 mM)	Broom stick	On heating at 70 ^{0}C
Fmoc-Asp(OtBu)-OH	Lower (3 mM)	Fractal	RT
Fmoc-Asp(OtBu)-OH	Higher (10 mM)	Fractal	RT
Fmoc-Asp(OtBu)-OH	Lower (3 mM)	Fractal	On heating at 70 ^{0}C
Fmoc-Asp(OtBu)-OH	Higher (10 mM)	Fractal	On heating at 70 ^{0}C
Fmoc-Lys(Boc)-OH	Lower (3 mM)	Sphere	RT
Fmoc-Lys(Boc)-OH	Higher (10 mM)	Sphere	RT
Fmoc-Lys(Boc)-OH	Lower (3 mM)	Sphere	On heating at 70 ^{0}C
Fmoc-Lys(Boc)-OH	Higher (10 mM)	Sphere	On heating at 70 ^{0}C

Conclusion

In conclusion, we have studied the self-assembling properties of modified charged amino acids **Fmoc-Glu(OtBu)-OH, Fmoc-Asp(OtBu)-OH,** and **Fmoc-Lys(Boc)-OH,** under different concentration and temperature. We observed these modified amino acids assemble to interesting architectures. Since modified single amino acids are very simple moleculaes which easy to synthesize, such bio-organic scaffolads may be potential interest to design nove nano/micro structures and may have immense importance in the field of nanotechnology, material science and healthcare.

Materials and method

General

All the chemicals used in these studies were of purity greater than 99%. All solvents and **Fmoc-Glu(OtBu)-OH, Fmoc-Asp(OtBu)-OH,** and **Fmoc-Lys(Boc)-OH** were purchased from the commercial suppliers and used without further purification. All studies were done using distilled solvents. Methanol was purchased from Merck. Ultrapure water was used for all the studies.

Optical Microscopy

A 20 mM stock solution of **Fmoc-Glu(OtBu)-OH, Fmoc-Asp(OtBu)-OH,** and **Fmoc-Lys(Boc)-OH, and** was prepared in 50% aqueous solution of methanol. The further dilution of all the charged amino acids was done by using Milli Q water to prepare four concentrations of 1 mM, 3 mM, 5 mM and 10 mM. A turbid solution has been observed on dilution with water. The self-assembling properties of these solutions were assessed under Optical Microscope (OM) by drop casting 20 μL solution of each sample on a clean glass slide. Furthermore, the same solution was heated at 70 °C and then drop casting 20 μL solution on a glass slide. For the self-assembly study always a fresh stock solution and fresh samples has been prepared. All optical microscopic images were visualized using a Leica DM2500 upright fluorescent microscope at various magnifications.

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Conflicts of interest

There is no conflict of interest to declare.

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