INSTRUMENTATION

High performance liquid chromatography (Analytical)

Waters HPLC (717 plus auto sampler) equipped with 515 pump and Waters 2996-photodiode array detector (Waters Corporation, Milford, USA).

High performance liquid chromatography (Preparative)

Waters system equipped with W 600 quaternary solvent delivery module Delta prep 2487 dual wavelength UV detector with auto liquid sampler handling system fitted with 5000μL loop. The data was collected and processed using Millennium software.

HPLC-ESI Mass Spectrometry

The electrospray ionization and MS-MS studies were performed on a triple quadrupole mass spectrometer PE Sciex model API 3000. The positive electrospray MS data was obtained by switching the capillary voltage between +5000 and -4500V respectively. The MS-MS data was generated with the collision energy ramping from 30 to 60 volts in nitrogen atmosphere.

Ultra Performance Liquid Chromatography (UPLC) -TOF MS

LCT premier XE (Water Corporation, Manchester, UK). Leucine Enkephalin was used as Lock spray for accurate mass measure.

NMR spectroscopy
The NMR experiments were performed on Varian spectrometer operating at 200, 400 and 500 MHz, Gemini, Mercury plus and Unity INOVA respectively. Standard pulse sequences provided by Varian were used.

**LC-NMR spectroscopy**

LC–NMR was performed on a Varian LC–NMR instrument (Varian Associates, Inc., Palo Alto, CA) using a Pro Star pump system, a Pro Star UV detector, an Unity INOVA 500 MHz NMR spectrometer and a micro flow LC–NMR probe. The probe has \(^1\)H\(^{13}\)C\) channels (\(^1\)H observed with \(^{13}\)C decoupling) with pulsed-field gradient along \(z\) axis. The active sample volume of the probe was approximately 60 µl and the transfer time from the UV cell to the active volume was calibrated to be 21s at a flow rate of 1.0 ml/min. Proton NMR experiments were performed in ‘stop-flow’ mode, where the HPLC flow was halted after the sample elution fraction was transferred to the NMR probe which was equilibrated at 25°C. Pulse sequence ‘\textit{lc1d}’ was used.