Supplementary Material

Edaravone-Encapsulated Agonistic Micelles Rescue Ischemic Brain Tissue by Tuning Blood-Brain Barrier Permeability

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Supplemental figures

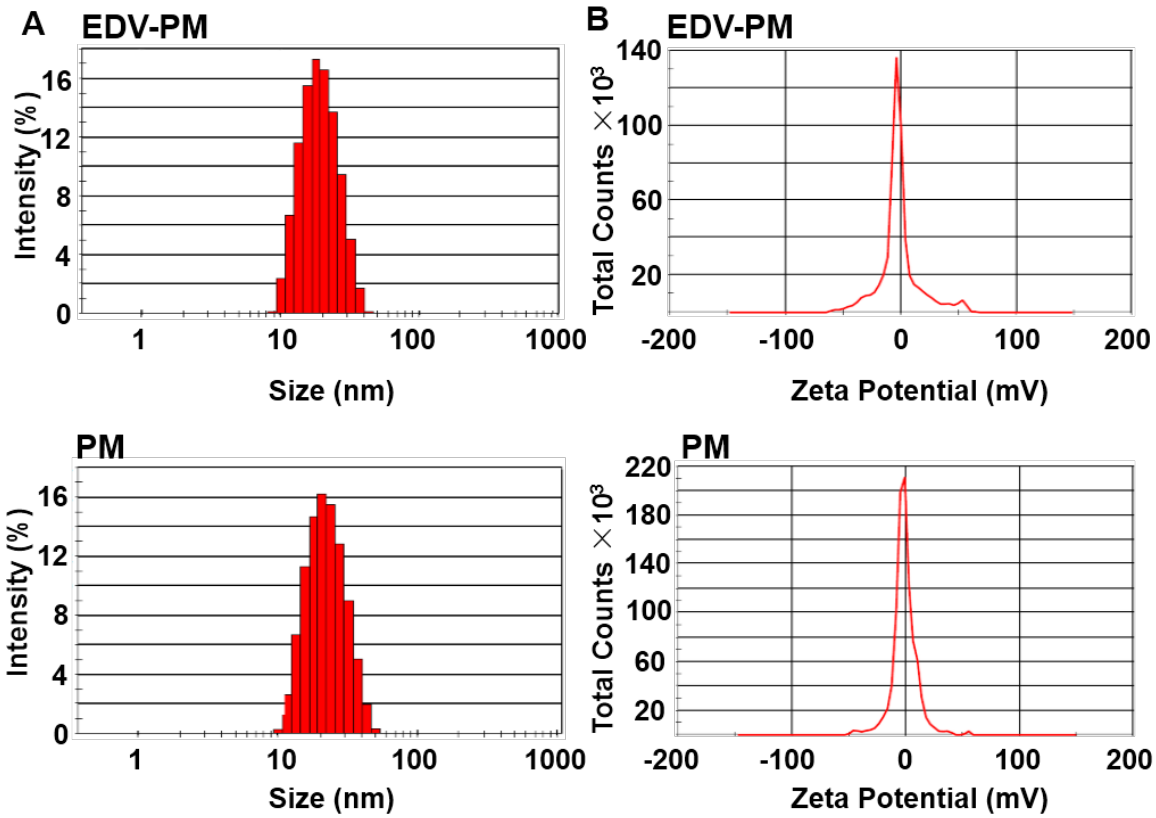


Figure S1. Characterization of the agonistic and control micelles. Hydrodynamic size distributions (A) and the zeta potentials (B) of **EDV-PM** and PM were measured by dynamic light scattering (DSL). The average diameters of **EDV-PM** and PM were determined as 21 and 19 nm. Their average zeta potentials were measured as -1.1 and -0.9 mV.

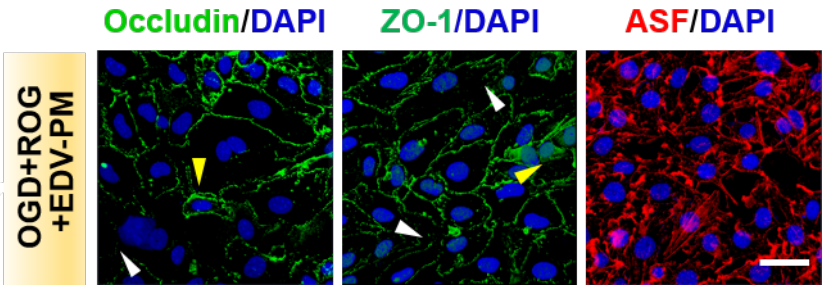


Figure S2. Fluorescence microscopic images of TJ associated proteins ZO-1, occludin and actinomyosin stress fiber (ASF) in endothelial bEnd.3 monolayer after the treatment of OGD (2 h) followed ROG (2 h) plus **EDV-PM** (10 μM). ASF stained by phalloidin was displayed in red, immunofluorescence of ZO-1 and occludin was in green, nuclei stained by DAPI was in blue. White and yellow arrow heads point to the disrupted paracellular TJ proteins and the translocated TJ proteins in the perinuclear area. Scale bar, 50 μm. OGD: oxygen and glucose deprivation. ROG: reoxygenation.

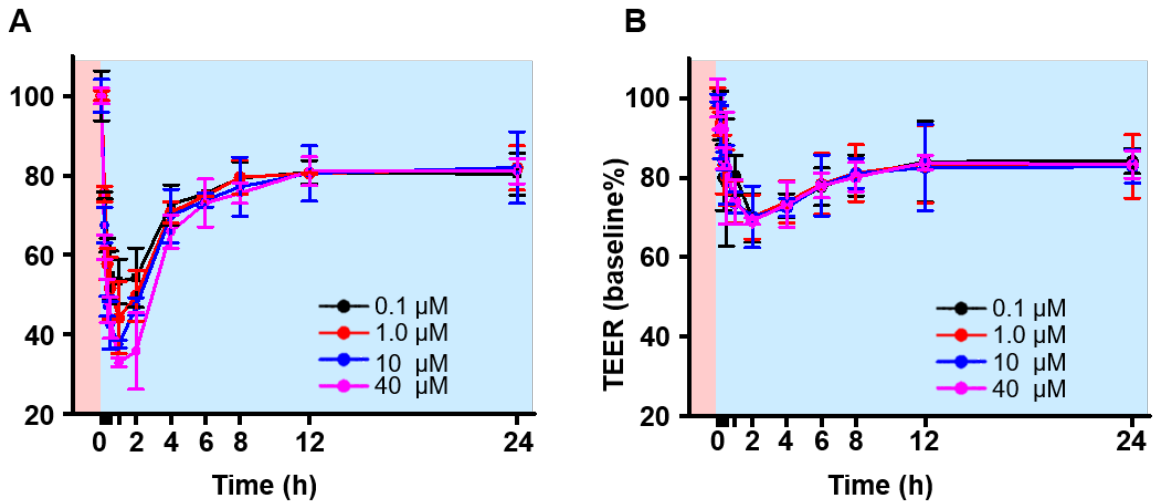


Figure S3. Time dependent TEER values of the OGD treated bEnd.3 cell monolayer after addition of **EDV-AM** or **EDV-PM** with different concentrations during the reoxygenation stage (n = 4 independent measurements). Panel A and B presenting time dependent TEER values after addition of 0.1, 1.0, 10 and 40 μM **EDV-AM** or **EDV-PM** in reoxygenation stage. Data are presented as mean ± SD. OGD: oxygen and glucose deprivation.

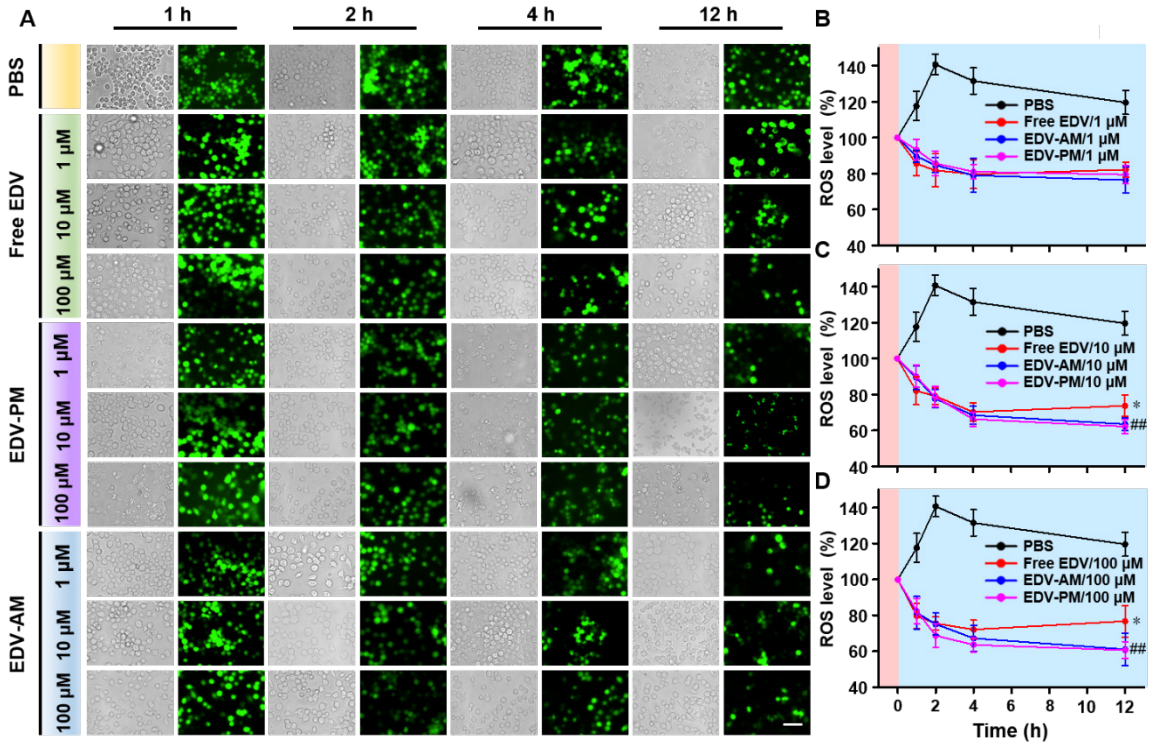


Figure S4. Agonistic micelle efficiently eradicating OGD induced intracellular ROS. (A) White light and fluorescence images of OGD treated live RAW264.7 microphage cells at selected time-points post addition of PBS, free EDV, **EDV-PM** or **EDV-AM**. (B−D) Normalized intracellular ROS level as a function of incubation time post addition of PBS, free EDV, **EDV-PM** or **EDV-AM** with a final concentration of 1.0, 10 and 100 μM. * $P < 0.05$, **EDV-AM** vs. free EDV group; ## $P < 0.01$, **EDV-AM** vs. PBS group. OGD: oxygen and glucose deprivation. ROG: reoxygenation.

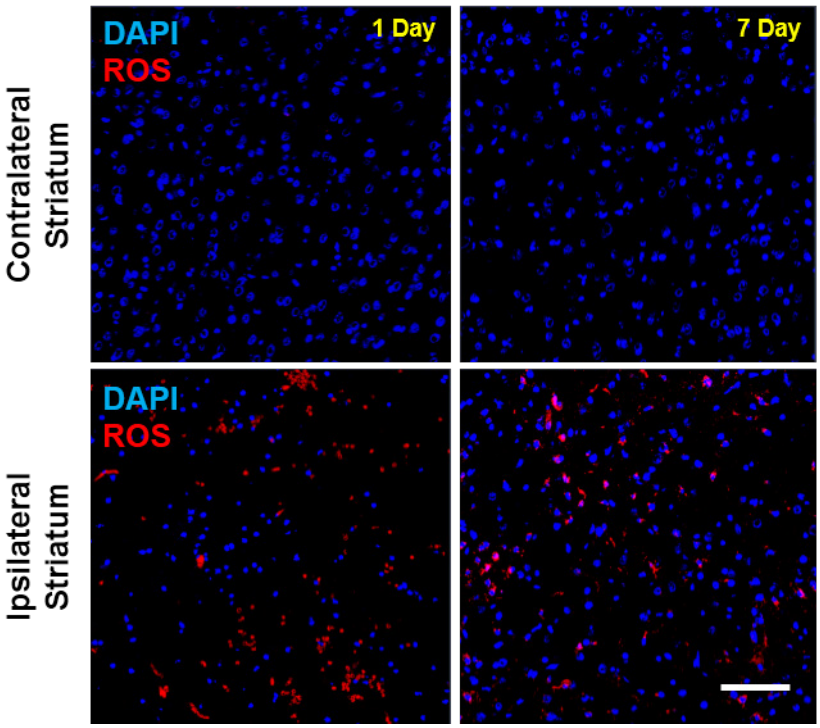
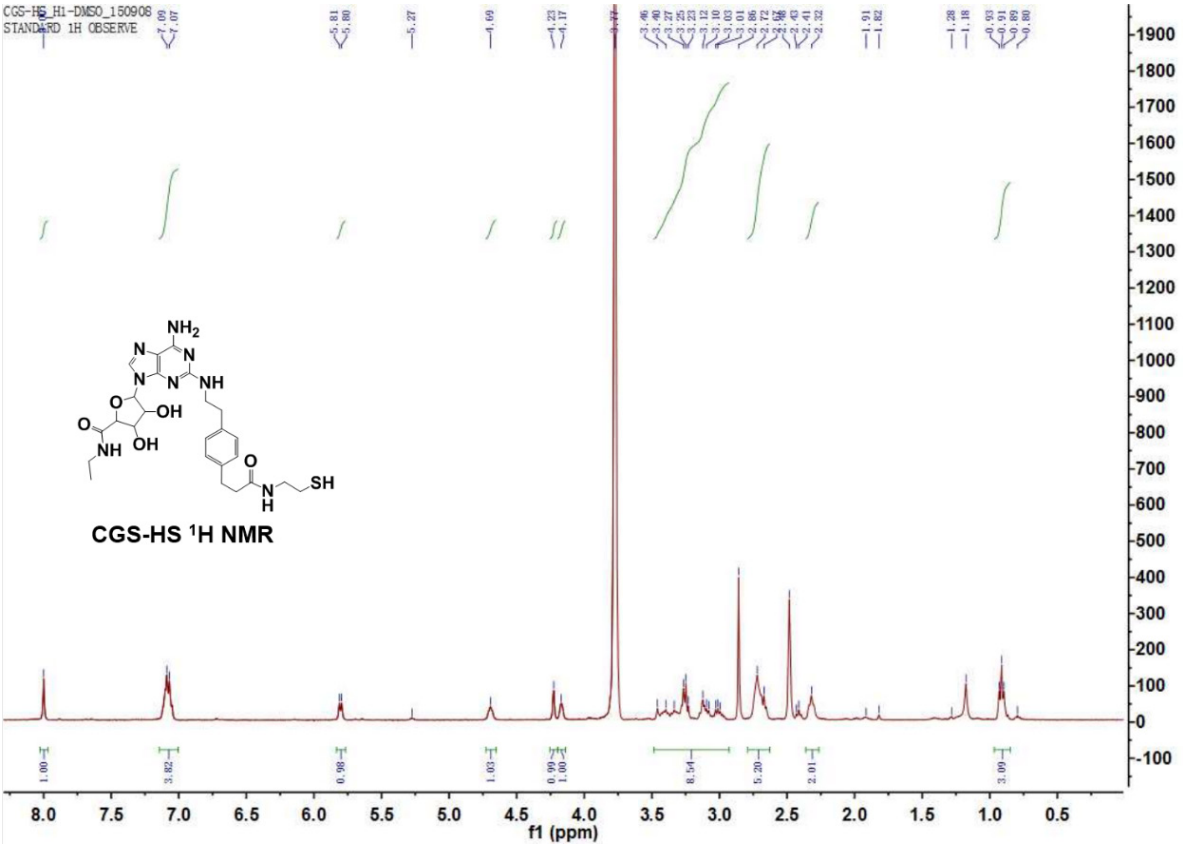


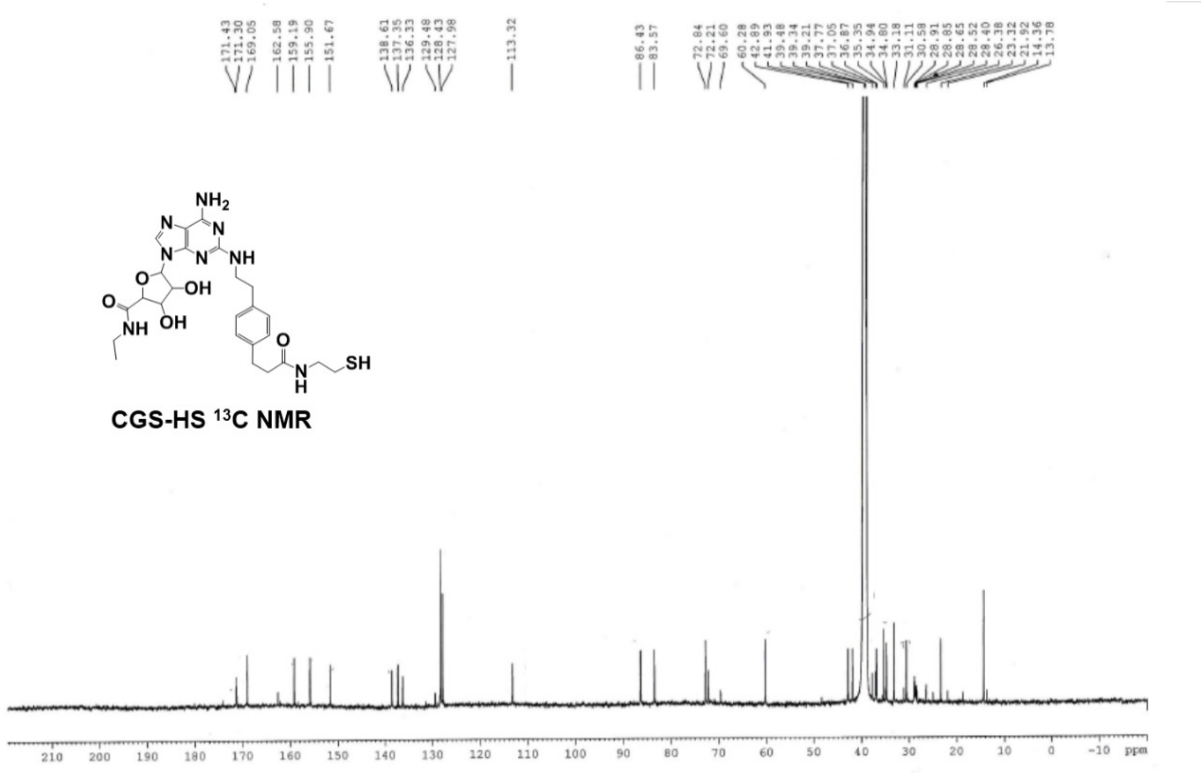
Figure S5. Fluorescence microscopic images presenting ROS levels in contralateral/ipsilateral hemisphere at 24 h and 7 days post ischemic model establishment. Cerebral ROS was visualized by the i.v. injected dihydroethidium (HEt) that was oxidized by ROS to ethidium (Et) with red color fluorescence. Scale bar, 100 μm.

Table S1. Neurological Severity Scores (NSS)

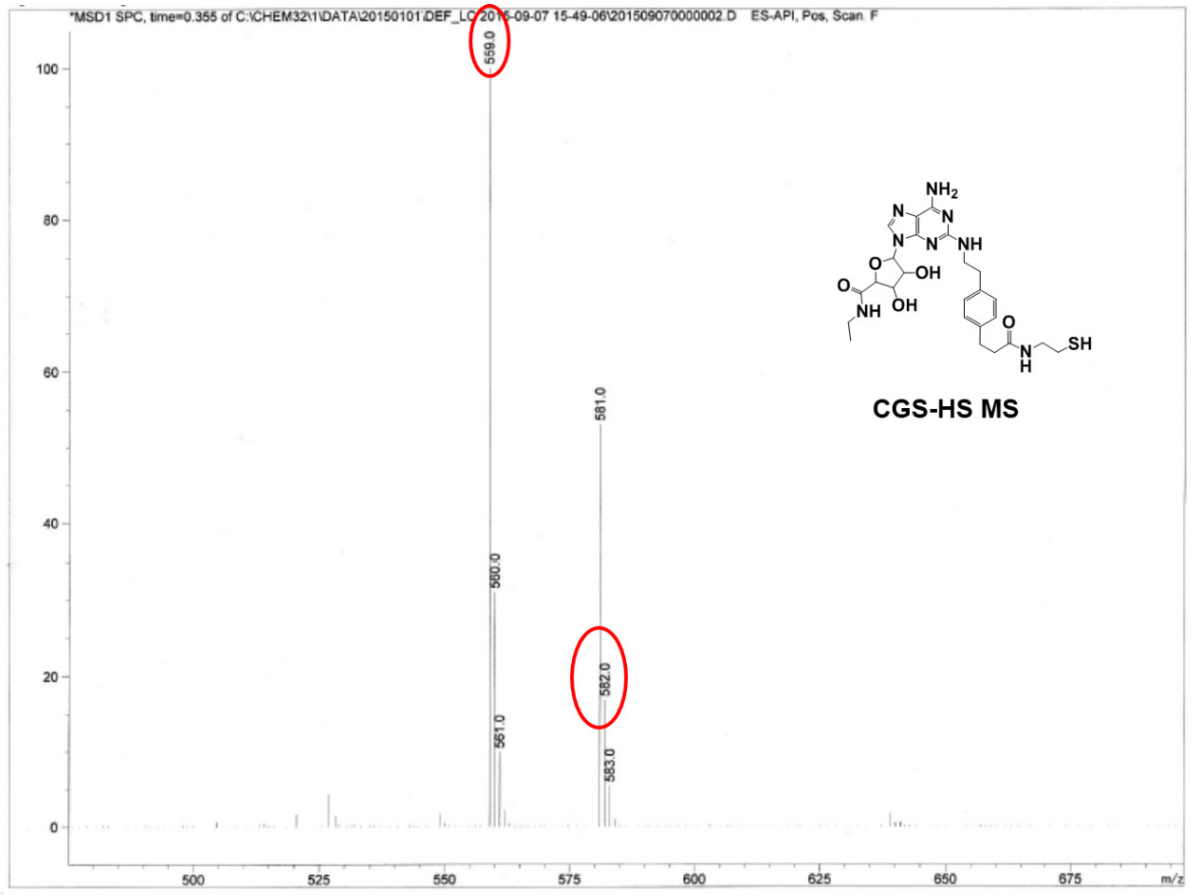
Points	**18**
Motor tests	**3**
Raising rat by the tail	**3**
Flexion of forelimb	1
Flexion of hindlimb	1
Head moved >10° to vertical axis within 30 s	1
Placing rat on the floor (normal=0; maximum=3)	**3**
Normal walk	0
Inability to walk straight	1
Circling toward the paretic side	2
Fall down to the paretic side	3
Sensory tests	**2**
Placing test (visual and tactile test)	1
Proprioceptive test (deep sensation, pushing the paw against the table edge to stimulate limb muscles)	2
Beam balance tests (normal=0; maximum=6)	**6**
Balances with steady posture	0
Grasps side of beam	1
Hugs the beam and one limb falls down from the beam	2
Hugs the beam and two limbs fall down from the beam, or spins on beam (>60 s)	3
Attempts to balance on the beam but falls off (>40 s)	4
Attempts to balance on the beam but falls off (>20 s)	5
Falls off: No attempt to balance or hang on to the beam (<20 s)	6
Reflexes absent and abnormal movements	**4**
Pinna reflex (head shake when touching the auditory meatus)	1
Corneal reflex (eye blink when lightly touching the cornea with cotton)	1
Startle reflex (motor response to brief noise from snapping a clipboard paper)	1
Seizures, myoclonus, myodystony	1



Spectrum 1. **^{1}H NMR spectrum of CGS-HS**. ^{1}H NMR (400 MHz, DMSO) δ 8.00 (s, 1H), 7.08 (d, *J* = 7.4 Hz, 4H), 5.80 (d, *J* = 6.6 Hz, 1H), 4.69 (s, 1H), 4.23 (s, 1H), 4.17 (s, 1H), 3.19 (tdd, *J* = 33.4, 31.5, 15.7 Hz, 9H), 2.70 (d, *J* = 20.4 Hz, 5H), 2.32 (s, 2H), 0.91 (t, *J* = 7.1 Hz, 3H).



Spectrum 2. **^{13}C NMR spectrum of CGS-HS**. ^{13}C NMR (151 MHz, DMSO) δ 171.43, 171.30, 169.05, 159.19, 155.90, 151.67, 138.61, 137.35, 136.33, 129.48, 127.98, 113.32, 86.43, 83.57, 72.84, 72.21, 69.60, 42.89, 41.93, 39.48, 39.34, 39.21, 37.77, 7.05, 36.87, 35.35, 34.94, 13.78.



Spectrum 3. MS spectrum of CGS-HS (MW: 558). $C_{25}H_{34}N_8O_5S$, $[M + H]^+$: 559; $[M + Na]^+$: 582.