

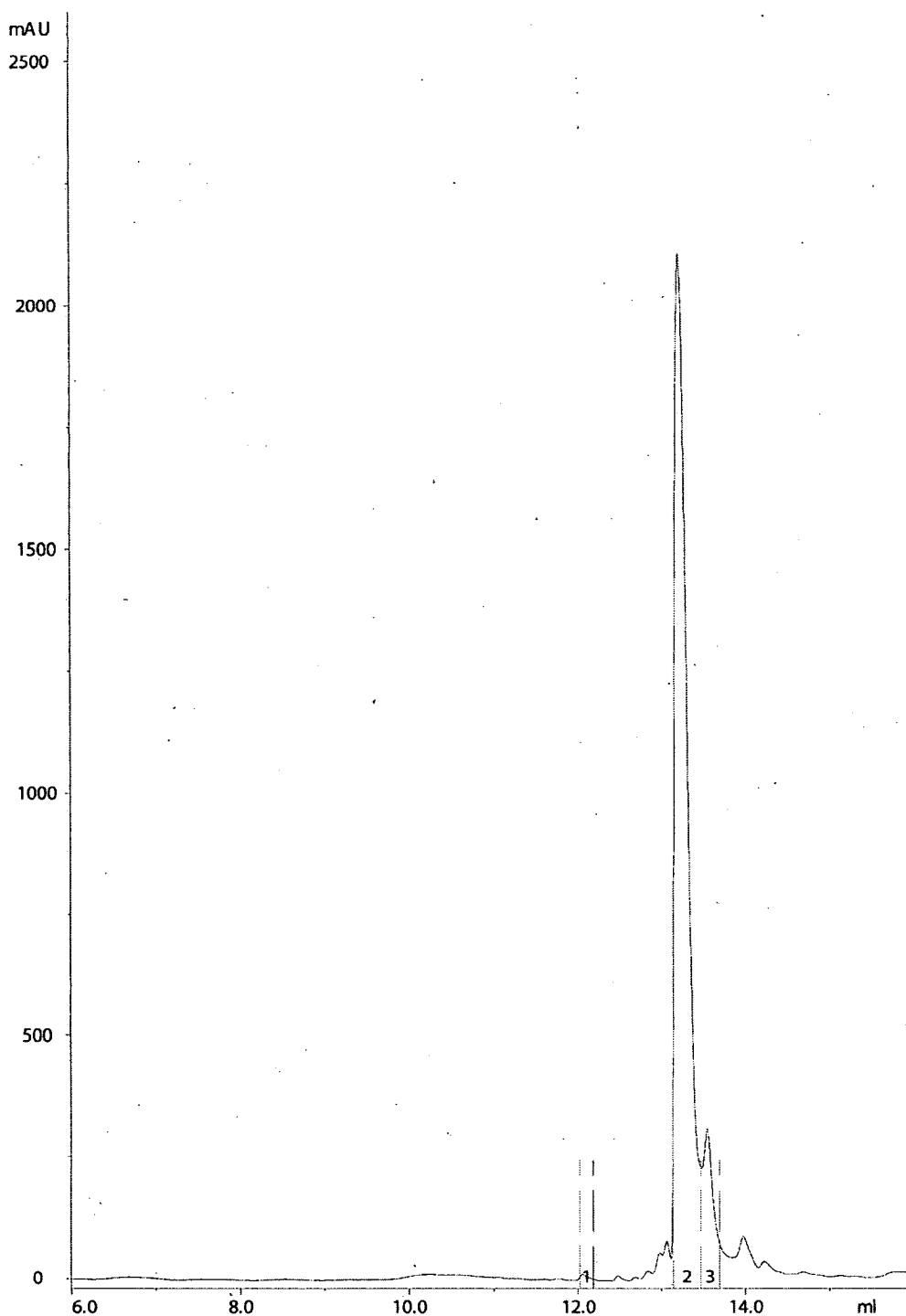


Figure 1. A typical HPLC run of TM2e. Peak 2 was collected for NMR study. Peak 3 originated from earlier termination of the expression.

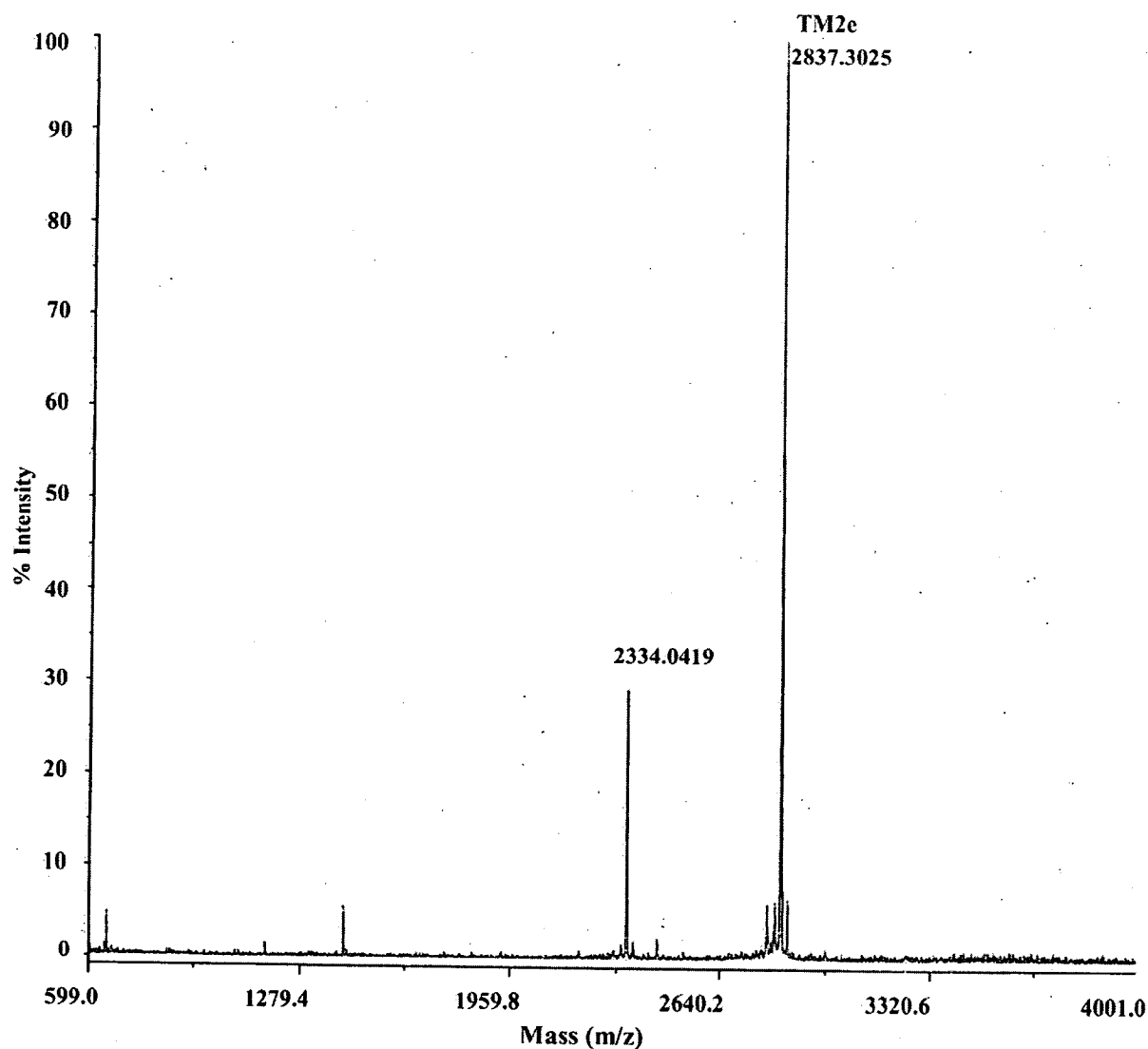


Figure 2. MALDI-TOF mass spectrum of [ $^{15}\text{N}$ ]TM2e preparation. TM2e appears at  $m/z=2837.3025$ . The peak with  $m/z=2334.0419$  and accounting for about 23% of total preparation is due to a TM2e fragment from A(-3) to R19. Other preparations used in this study contained significantly less contaminants than shown in this spectrum.

Table 1.  $^1\text{H}$  and  $^{15}\text{N}$  peak assignments for extended transmembrane domain 2 chain of the human neuronal GlyR  $\alpha_1$  subunit in SDS micelles at 30°C.

| Residue | NH   | H $\alpha$ 1 | H $\alpha$ 2 | H $\beta$ 1 | H $\beta$ 2 | H $\gamma$ 1 | H $\gamma$ 2 | H $\delta$ 1 | H $\delta$ 2 | H $\epsilon$ | H $\zeta$ | N     |
|---------|------|--------------|--------------|-------------|-------------|--------------|--------------|--------------|--------------|--------------|-----------|-------|
| A(-3)   | 8.88 | 4.37         | -            | 1.55        | -           | -            | -            | -            | -            | -            | -         | -     |
| P(-2)   | -    | 4.54         | -            | 2.52        | 1.98        | 2.10         | -            | 3.45         | -            | -            | -         | -     |
| A(-1)   | 8.48 | 4.24         | -            | 1.48        | -           | -            | -            | -            | -            | -            | -         | 125.5 |
| R0      | 8.23 | 4.26         | -            | 1.91        | 1.77        | 1.63         | -            | 3.21         | -            | 7.13         | -         | 116.0 |
| V1      | 7.61 | 3.92         | -            | 2.20        | -           | 1.03         | 0.97         | -            | -            | -            | -         | 117.3 |
| G2      | 8.17 | 4.04         | 3.86         | -           | -           | -            | -            | -            | -            | -            | -         | 108.6 |
| L3      | 7.91 | 4.23         | -            | 1.72        | -           | 1.68         | -            | 0.92         | -            | -            | -         | 121.3 |
| G4      | 8.38 | 3.87         | -            | -           | -           | -            | -            | -            | -            | -            | -         | 108.9 |
| I5      | 8.30 | 3.79         | -            | 2.00        | -           | 1.20         | 0.97         | 0.91         | -            | -            | -         | 120.8 |
| T6      | 8.16 | 3.95         | -            | 4.32        | -           | 1.29         | -            | -            | -            | -            | -         | 113.2 |
| T7      | 8.05 | 4.00         | -            | 4.40        | -           | 1.25         | -            | -            | -            | -            | -         | 119.1 |
| V8      | 7.98 | 3.80         | -            | 2.20        | -           | 1.08         | 0.98         | -            | -            | -            | -         | 120.9 |
| L9      | 8.35 | 4.21         | -            | 1.85        | -           | 1.60         | -            | 0.90         | -            | -            | -         | 119.4 |
| T10     | 8.11 | 4.12         | -            | 4.33        | -           | 1.29         | -            | -            | -            | -            | -         | 113.9 |
| M11     | 7.99 | 4.37         | -            | 2.25        | -           | 2.66         | 2.72         | -            | -            | -            | -         | 120.0 |
| T12     | 7.98 | 4.34         | -            | 4.34        | -           | 1.29         | -            | -            | -            | -            | -         | 110.4 |
| T13     | 7.92 | 4.40         | -            | 4.40        | -           | 1.29         | -            | -            | -            | -            | -         | 113.3 |
| Q14     | 8.06 | 4.39         | -            | 2.07        | -           | 2.43         | -            | -            | -            | 6.80         | -         | 120.2 |
| S15     | 8.04 | 4.51         | -            | 3.98        | -           | -            | -            | -            | -            | -            | -         | 115.0 |
| S16     | 8.29 | 4.50         | -            | 3.96        | -           | -            | -            | -            | -            | -            | -         | 117.0 |
| G17     | 8.36 | 4.04         | -            | -           | -           | -            | -            | -            | -            | -            | -         | 110.8 |
| S18     | 8.13 | 4.50         | -            | 3.90        | -           | -            | -            | -            | -            | -            | -         | 115.1 |
| R19     | 8.25 | 4.32         | -            | 1.90        | 1.82        | 1.70         | -            | 3.20         | -            | 7.16         | -         | 121.5 |
| A20     | 8.02 | 4.33         | -            | 1.38        | -           | -            | -            | -            | -            | -            | -         | 122.7 |
| S21     | 7.85 | 4.54         | -            | 3.86        | -           | -            | -            | -            | -            | -            | -         | 112.7 |
| L22     | 7.90 | 4.20         | -            | 1.76        | 1.63        | 1.88         | -            | 0.90         | -            | -            | -         | 126.9 |
| P23     | -    | 4.47         | -            | 2.05        | -           | 2.26         | -            | 3.60         | -            | -            | -         | -     |
| K24     | 7.77 | 4.23         | -            | 1.86        | 1.84        | 1.40         | -            | 1.69         | -            | 3.02         | 7.43      | 124.5 |

## Extra peaks:

|      |      |      |   |      |      |      |   |   |   |      |   |       |
|------|------|------|---|------|------|------|---|---|---|------|---|-------|
| Q14' | 8.02 | 4.39 | - | 2.22 | -    | 2.43 | - | - | - | 6.80 | - | 120.0 |
| S15' | 8.09 | 4.51 | - | 3.98 | -    | -    | - | - | - | -    | - | 115.0 |
| S16' | 8.24 | 4.47 | - | 3.98 | 3.94 | -    | - | - | - | -    | - | 117.0 |
| G17' | 8.39 | 4.04 | - | -    | -    | -    | - | - | - | -    | - | 110.2 |
| S18' | 8.12 | 4.50 | - | 3.90 | -    | -    | - | - | - | -    | - | 115.4 |
| A20' | 7.97 | 4.52 | - | 1.73 | -    | -    | - | - | - | -    | - | 122.9 |