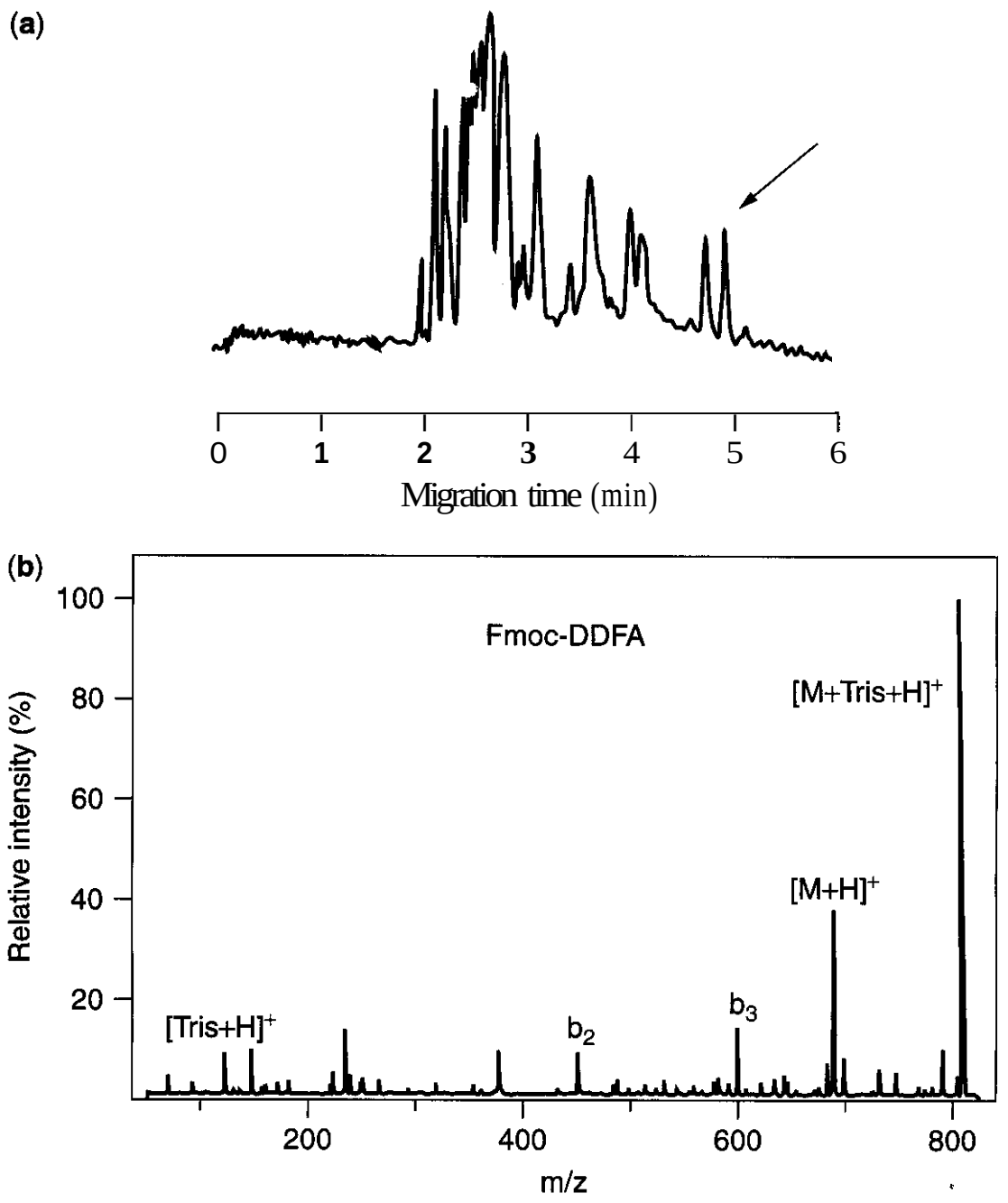

Figure 13.9. Affinity capillary electrophoresis–UV-mass spectrometry of a 100-tetrapeptide library screened for binding to vancomycin (104 μM in the electrophoresis buffer). (a) The elution of peptides was monitored with UV absorbance during capillary electrophoresis, and the elution time increased with increasing affinity for vancomycin. (b) Positive ion electrospray mass spectrum with CID of the Tris adduct of the protonated peptide detected at ~5 min in the electropherogram shown in a (Reproduced from Ref. 52 by permission of the American Chemical Society.)

identification, affinity constants for multiple compounds can be measured in a single analysis (51). Recognizing that on-line mass spectrometric detection was helpful for the identification of each ligand, Chu et al. (52) extended this approach to include the screening of combinatorial libraries as a means of drug discovery. The data in Fig. 13.9 show the results of screening a 100-tetrapeptide library for affinity to vancomycin using affinity capillary electrophoresis–mass spectrometry. Without vancomycin in the electrophoresis buffer, all the peptides eluted within 3 min. When vancomycin was present, the peptides eluted in order of affinity, with the highest affinity compounds being detected between 4.5 and 5 min. Positive ion electrospray tandem mass spectrometry was used to identify the highest affinity ligands (see Fig. 13.9b).

Note that some peptide ligands such as Fmoc-DDFA were detected as adducts with Tris, which was used in the electrophoresis buffer. Although the identification of this peptide was not prevented by the formation of this adduct, some buffers used during electrophoresis might interfere with mass spectrometric ionization and detection. Also, the types of libraries that have been screened using this approach have contained modest numbers of synthetic analogs such as peptides. Libraries exceeding 400 members required preliminary purification using affinity chromatography to reduce the number of compounds (52). As a result, this approach is probably not ideal for screening libraries containing molecularly diverse compounds or for screening natural product extracts. However, affinity capillary electrophoresis–mass spectrometry is fast; each analysis requires less than 10 min. Also, it may be used to measure affinity constants for ligand–receptor interactions.