Peptides having a primary amide functionality at the carboxyl terminus are widely distributed in the animal kingdom and elicit a large variety of important physiological effects. In the cases studied, they are formed from precursors bearing a glycine residue at the carboxyl terminus through the agency of peptidyl-glycine α-amidating monooxygenases (PAM). These enzymes require oxygen, copper ion, and ascorbate to form the peptide amide and glyoxylate. A new assay for PAM has been developed based on reaction of glyoxylate with nitrosobenzene to form N-hydroxyformanilide and CO2. This assay allowed determination of the stereochemistry of hydrogen removal from the glycol residue of D-tyrosyl-L-valylglycine by purified PAM isolated from porcine pituitaries. The required stereospecifically tritium-labeled glycines were synthesized and analyzed by improved methods. The stereochemical outcome of the PAM reaction and the availability of the new assay permit examination of substrate specificity and design of specific inhibitors.