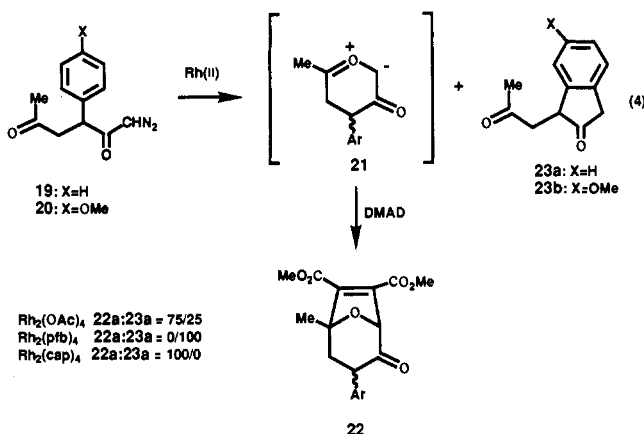
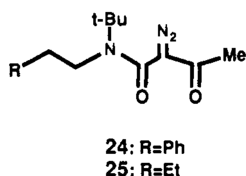


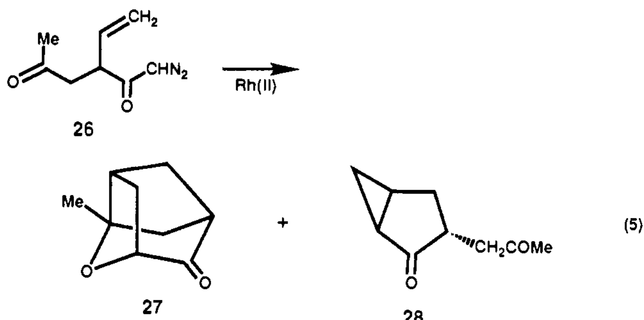
with $Rh_2(pfb)_4$, ketone **23** was formed (85%) to the virtual exclusion of cycloadduct **22**. Dipole formation (i.e., **22a** (90%)) rather than formal insertion is the only process which occurs when $Rh_2(cap)_4$ is used as the catalyst.

Chemoselectivity in these competitive transformations depends on the inherent electron demand from ligands of the rhodium(II) carbene intermediate, with that derived from $Rh_2(pfb)_4$ being more electrophilic than that from $Rh_2(acam)_4$ or $Rh_2(cap)_4$.[1,2,6,15,16] Electrophilic aromatic substitution occurs to the exclusion of alkene cyclopropanation or carbonyl ylide generation with the carbene generated with $Rh_2(pfb)_4$, and this selectivity is reversed with the use of $Rh_2(cap)_4$. In addition, cyclopropanation excludes C–H insertion, which in turn precludes aromatic cycloaddition in competitive metal carbene reactions catalyzed by $Rh_2(cap)_4$; the reversed product control occurs with $Rh_2(pfb)_4$. *What is so remarkable about these results is the degree to which chemoselectivity can be achieved over such a broad spectrum of carbene transformations by simply changing the dirhodium(II) ligands from perfluorobutyrate to carboxamide.*

Not all competitive carbenoid reactions can be effectively controlled with ligand replacement on the dirhodium(II) framework. With diazoacetoacetamides **24** and **25**, both β- and γ-



lactam C–H insertion products are obtained, and their ratio changes from 60:40 with $Rh_2(pfb)_4$ to 40:60 with $Rh_2(acam)_4$. Similarly, treatment of diazo ketone **26** with $Rh_2(OAc)_4$ led to a 1:1 mixture of the internal dipolar cycloadduct **27** as well as the cyclopropanated product **28** (eq 5). In this case, replacement



of the acetate ligand with perfluorobutyrate or caprolactam did not significantly alter the chemoselectivity of the reaction. Investigations are underway to further demonstrate the potential of dirhodium(II) ligand changes on selectivity.

Acknowledgment. A.P. acknowledges the National Cancer Institute (CA-26751) for their support of this research. M.P.D. is grateful to the National Science Foundation for their support and to Hoan Q. Pho and Jack Taunton for their preliminary results.

Supplementary Material Available: Spectral data for **1–18** and **22–28** and selected intermediates (10 pages). Ordering information is given on any current masthead page.

Peptide Amidation by Chemical Protein Engineering. A Combination of Enzymatic and Photochemical Synthesis

D. B. Henriksen,[†] K. Breddam,[‡] J. Møller,[§] and O. Buchardt*,[†]

Research Center for Medical Biotechnology
Chemical Laboratory II, The H. C. Ørsted Institute
University of Copenhagen, Universitetsparken 5
DK-2100 Copenhagen Ø, Denmark
Department of Chemistry, Carlsberg Laboratory
Gl. Carlsbergvej 10, DK-2500 Valby, Denmark
Department of Chemistry, University of Odense
Campusvej 55, DK-5230 Odense M, Denmark

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Peptide hormones often terminate in carboxamido groups, which are essential for full biological activity.[1] Apart from their use in various biological studies, such peptide amides and analogues are also of considerable interest as drugs, e.g., calcitonin against various bone disorders.[2] This has led to a brisk interest in their procurement in new ways because it is too time-consuming and expensive to synthesize larger quantities by standard chemical methods.[3]

They cannot be expressed in microorganisms, which lack the necessary enzymatic machinery for production of C-terminal amides, and they are thus not produced by gene technology. It is generally agreed that the in vivo generation of peptide amides takes place from peptide precursors with glycine as the C-terminus. The precursors are enzymatically hydroxylated[4] and subsequently hydrolyzed to the relevant amides, presumably also enzymatically.[4]

Serine carboxypeptidase catalyzed transpeptidations using peptide substrates and amino acid amides as nucleophiles have resulted in high yields of peptide amides. However, none of the serine carboxypeptidases accept prolinamide or glutamic or as-

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[†] University of Copenhagen.
[‡] Carlsberg Laboratory.
[§] University of Odense.

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Scheme I

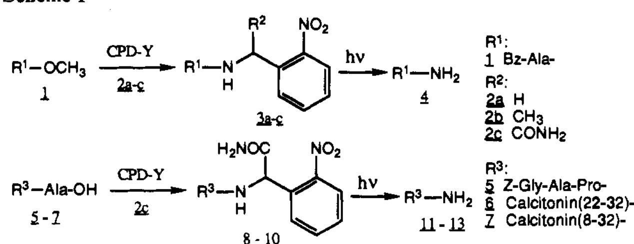

Table I. Enzymatic (CPD-Y) and Photochemical Transformations[a]

substrate	nucleophile	enzyme product (%)[b]	photochemical product (%)[c]
1	**2a**	**3a** (85)	**4** (90)
1	**2b**	**3b** (25)	**4** (95)
1	**2c**	**3c** (95)	**4** (95)
5	**2c**	**8** (95)	**11** (95)
6[d]	**2c**	**9** (91)	**12**[d] (95)
7[e]	**2c**	**10** (90)	**13**[e] (95)

[a] Identification procedures were the following. **2b**, **2c**, and **3a–c**: elemental analyses, IR, ^{1}H and ^{13}C NMR. **5** and **8**: FAB MS, ^{1}H NMR. **6** and **7**: FAB MS, amino acid analysis. **9–13**: FAB MS, retention time, UV identical with those of authentic sample. [b] Transacylation: 70 nmol of enzyme (CPD-Y)/mmol of *N*-benzoylalanine methyl ester (**1**), nucleophile (**2a–c**) concentration 0.15–0.25 M in 5 mM EDTA; pH 8.0, 20 °C. Products (**3a–c**) were purified by HPLC. Transpeptidation: 1.0 μmol of CPD-Y/mmol of substrate (**5–7**), nucleophile (**2c**) concentration 0.2 M in 5 mM EDTA; pH 6.5, 20 °C. Products (**8–10**) were purified by HPLC. [c] Photolysis: peptide concentrations 1.0 mM, $NaHSO_3$ concentration 40 mM in 50% methanol (purged with nitrogen). pH adjusted with sodium hydroxide to 8.0 for peptides **3a–c** and 9.5 for **8–10**. Irradiation: 1–2 h, using a PS 200 Osram medium-pressure mercury lamp at a distance of 20 cm. A 40% copper sulfate solution was employed to filter out wavelengths below 320 nm. The peptide amides (**4**, **11**, **12**, and **13**) were purified by HPLC. [d] H-Pro-Gln-Thr-Ala-Ile-Gly-Val-Gly-Ala-Pro-X; **6**, X = Ala-OH; **12**, X = NH_2. [e] H-Leu-Gly-Thr-Tyr-Thr-Gln-Asp-Phe-Asn-Lys-Phe-His-Thr-Phe-Pro-Gln-Thr-Ala-Ile-Gly-Val-Gly-Ala-Pro-X; **7**, X = Ala-OH; **13**, X = NH_2. The substrate was chosen to resemble human calcitonin, but to avoid the methionine and the disulfide bridge.

partic acid α-amide as nucleophiles, which makes such peptide amides unavailable by this method,[5] e.g., calcitonin, which terminates in prolinamide. Furthermore, transpeptidation using ammonia as nucleophile has also led to peptide amides, albeit in very moderate yields.[5,6]

We now report an alternative method for the preparation of peptide amides, including those terminating in prolinamide, by transpeptidation with protected nucleophiles followed by photochemical deprotection.

The ability of carboxypeptidase-Y (CPD-Y, from yeast) to catalyze transacylation reactions of peptide esters[5] was used to prepare a series of model substances terminating in *N*-(2-nitrobenzyl)amido groups. Bz-Ala-OMe (**1**) was used as substrate and 2-nitrobenzylamine (**2a**) and (2-nitrophenyl)glycinamide (**2c**) were efficiently transferred by CPD-Y at pH 8.0, whereas 1-(2-nitrophenyl)ethylamine (**2b**) was not, being too strong a base to be efficient as a nucleophile at this pH (Scheme I).[7] The reaction is very efficient, and with **2a** and **2c** as nucleophiles, more than 95% of the substrate had disappeared within 60 min. The reaction with **2b** was less efficient (Table I). CPD-Y also serves as a very efficient catalyst for transpeptidation, especially for C-terminal alanine containing peptides.[8]

This was used to prepare the transpeptidation products with **2c** from Z-Gly-Ala-Pro-Ala-OH (**5**) and the human calcitonin fragment precursors calcitonin (22–32)-Ala-OH (**6**) and calcitonin (8–32)-Ala-OH (**7**) in high yields (Table I).

Subsequently irradiation with long-wavelength UV light (λ > 320 nm) was used to remove the 2-nitrobenzyl protective ligand[9] (Scheme I) to give the warranted peptide amides in high yield for substrates derived from **1**, **5**, **6**, or **7** (Table I). The photochemical step in the transformation (Scheme I) mimics the in vivo transformation of the ultimate amino acid in the peptide amide precursors, i.e., causes hydroxylation.[10] If the pH is kept sufficiently high, but not high enough to otherwise effect the peptide, the final hydrolysis takes place, again mimicking the in vivo process, thus making it an attractive alternative model for the α-amidating action of pituitary enzymes.[4,11]

The photolysis frequently resulted in the generation of colored byproducts which might function as inner filters. This problem could be eliminated by the addition of sodium hydrogen sulfite to the photolysis mixture as a scavenger.

In conclusion, we have shown a high-yield pathway for peptide amide formation from peptide precursors, which could be made available in large amounts by gene technology.

Acknowledgment. This work was supported by Grant 1988-133/443-88.679 from the Danish National Agency of Industry and Trade, which is gratefully acknowledged.

Registry No. 1, 7244-67-9; **2a**, 1904-78-5; **2b**, 100311-54-4; **2c**, 138571-55-8; **3a**, 138571-56-9; **3b**, 138571-59-2; **3c**, 138571-60-5; **4**, 67711-04-0; **5**, 138571-57-0; **6**, 138571-58-1; **7**, 138605-26-2; **8**, 138605-27-3; **9**, 138605-28-4; **10**, 138605-29-5; **11**, 23927-09-5; **12**, 68801-56-9; **13**, 138605-30-8; CAD-Y, 9046-67-7.

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(7) Abbreviations used: Bz, *N*-benzoyl; Z, *N*-benzyloxycarbonyl; EDTA, ethylenediaminetetraacetic acid; HPLC, high-performance liquid chromatography; Other abbreviations of amino acids and peptides are according to guidelines of the IUPAC–IUB commission on Biochemistry Nomenclature.

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(9) Otherwise photolabile groups such as tryptophan, histidine, or tyrosine are not effected at wavelengths of >320 nm.[16a] No difference in yield or purity was observed at longer wavelength cutoff. 2-Nitrobenzoyl groups have previously been used in solid support synthesis of peptide amides as part of the linker which was photochemically cleaved to give peptide amides; cf. refs 3 and 10.

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In Situ Photoreactions of Proteins in Spectrometers Leading to Variations in Signal Intensities

D. Balasubramanian,* P. Guptasarma, and Manni Luthra

Centre for Cellular & Molecular Biology
Hyderabad 500 007, India

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Takeda and Moriyama[1] have recently reported that the circular dichroism (CD) signal of a protein decreases with an increase in the measurement time. They have wondered whether this might be due to some photoreaction of the protein, since the changes occurred only in the presence of light and not in the dark. We show here that the phenomenon is general and that these CD changes indeed arise due to photochemical reactions involving some of the protein chromophores that absorb in the chosen wavelength region.

Figure 1A shows that irradiation[2] of bovine serum albumin (BSA) at 222 nm leads to alterations in some of its aromatic side

* Please address correspondence to this author.
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(2) We chose to use fluorescence as the method to monitor the photoreaction, since this technique is more sensitive than absorption or CD, particularly in detecting small amounts of the photoproducts. Irradiation was done using the 150-W Xenon lamp of a Hitachi F-4000 spectrofluorimeter, with its monochromator set at the chosen wavelength and the entrance slit band width at 20 nm. The amount of light incident at the center of the 1-cm quartz cuvette holding the protein solution in the sample compartment of the fluorimeter was estimated to be 0.5 mW/cm^2, or about 10^{14} photons/s. The intensity of 220-nm radiation incident on the sample in a JASCO J-20 CD instrument (450-W Xe lamp, 2-nm slit) was smaller and caused photodamage to a BSA solution that was 60% of what was seen when an identical solution was irradiated in the fluorimeter above.

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