

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 Rh₂(pfb)₄ being more electrophilic than that from Rh₂(acam)₄ or Rh₂(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 Rh₂(pfb)₄, and this selectivity is reversed with the use of Rh₂(cap)₄. In addition, cyclopropanation excludes C-H insertion, which in turn precludes aromatic cycloaddition in competitive metal carbene reactions catalyzed by Rh₂(cap)₄; the reversed product control occurs with Rh₂(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.

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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

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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 hydroxylated⁴ 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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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 ^{<i>d</i>}	2c	9 (91)	12 ^d (95)
7 ^e	2c	10 (90)	13e (95)

^aIdentification procedures were the following. 2b, 2c, and 3a-c: elemental analyses, IR, ¹H and ¹³C NMR. 5 and 8: FAB MS, ¹H NMR. 6 and 7: FAB MS, amino acid analysis. 9-13: FAB MS, retention time, UV identical with those of authentic sample. ^bTransacylation: 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. 'Photolysis: peptide concentrations 1.0 mM, NaHSO₃ 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 ^dH-Pro-Gln-Thr-Ala-Ile-Gly-Val-Gly-Ala-Pro-X; 6, X = HPLC. Ala-OH; 12, X = NH₂. ^eH-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 esters⁵ 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

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 ($\lambda > 320$ nm) was used to remove the 2-nitrobenzyl protective ligand⁹ (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.

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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.

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

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Takeda and Moriyama¹ 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 irradiation² of bovine serum albumin (BSA) at 222 nm leads to alterations in some of its aromatic side

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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.^{6a} 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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⁽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 10¹⁴ 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.