methylacetamide is added, increasing amounts of the N7Gua adduct are obtained.[45,48]

Electrochemical oxidation of 7,12-DMBA never yields any trace of N^2dG aralkylation but does give adducts involving the N-7 of Gua and the C-8 of dG. In contrast, the C8dG adduct is never obtained from benzylic carbenium ion intermediates, as reported here and in the literature.[48-51] These data tend to exclude a carbenium ion intermediate in the electrochemical oxidation.

Reaction to form an adduct can also occur between the 12-benzylic radical produced by loss of a proton from the radical cation (Scheme IIA) and, for example, the C-8 position of dG. The rate of adduct formation in anodic oxidation of 7,12-DMBA and dG, however, is not affected by the presence of 2,6-di-*tert*-butylpyridine, which is a base but not a nucleophile, suggesting that the benzylic radical is not formed as a discrete intermediate.

Therefore, we favor reaction between the N-7 and C-8 of the Gua moiety and the 7- or 12-CH_3 group of the 7,12-DMBA radical cation (Scheme IIB). At the present time, mechanistic details of this reaction are not established.

Electrochemical oxidation of 7,12-DMBA also produces N-7 and N-3 adducts of Ade at the 12-CH_3 and 7-CH_3, respectively. In contrast, reaction of adenosine with 7-CH_2BrBA or other benzylating agents produces the N-1 and exocyclic amino-substituted N^6 adducts but not the N-7 and N-3 adducts.[48,51] Thus, it is logical to assume that the same radical cation intermediate described above for reaction with dG operates in the adduction to dA.

Covalent binding of 7,12-DMBA to DNA by horseradish peroxidase-catalyzed and cytochrome P-450-catalyzed one-electron oxidation yields 7-MBA-12-CH_2-N7Gua and 7-MBA-12-CH_2-N7Ade, whereas 12-MBA-7-CH_2-N7Gua and 12-MBA-7-CH_2-N3Ade are not formed.[52] This study provides additional evidence that these two adducts are obtained via a radical cation intermediate, forging a link between the electrochemical and enzymatic experiments. Furthermore, the 12-CH_3 group is critical in the binding of the 7,12-DMBA to the nucleophiles of DNA in biological systems.

Conclusions

The radical cation of 7,12-DMBA reacts with dG to produce 7-MBA-12-CH_2-C8dG, 7-MBA-12-CH_2-N7Gua, and 12-MBA-7-CH_2-N7Gua. The 7-MBA-12-CH_2-C8Gua is a secondary product arising from electrochemical oxidation of the corresponding C8dG adduct, whereas 7,12-$(CH_2OH)_2$-BA is formed by electrochemical oxidation of 12-MBA-7-CH_2-N7Gua to form an N7Gua diadduct, which is rapidly hydrolyzed to 7,12-$(CH_2OH)_2$-BA during HPLC. With dA, the two adducts formed, in approximately equal amounts, are 7-MBA-12-CH_2-N7Ade and 12-MBA-7-CH_2-N3Ade. No detectable adducts are formed with dC or T. The synthesis is not only a demonstration of the reactivity of nucleosides and 7,12-DMBA under oxidizing conditions but also a source for necessary reference materials for studying the 7,12-DMBA-DNA adducts formed in biological systems.

Of particular importance to future biological studies is the ability of FAB MS/MS, as well as FLNS, to distinguish between the adducts of 7,12-DMBA at the 7- and 12-CH_3 groups and the N-7 and C-8 positions of Gua. FLNS also possesses the necessary selectivity to distinguish between 12-MBA-7-CH_2-N3Ade and 7-MBA-12-CH_2-N7Ade. On the other hand, the distinction between 7-MBA-12-CH_2-C8Gua and 7-MBA-12-CH_2-C8dG is straightforward by FAB MS/MS but very difficult by FLNS. Thus, the two techniques complement each other very nicely.

A mechanism of adduction is proposed in which a radical cation is formed by anodic oxidation and reacts via the methyl groups with various nucleophilic groups of dA and dG.

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Communications to the Editor

Control of Chemoselectivity in Catalytic Carbenoid Reactions. Dirhodium(II) Ligand Effects on Relative Reactivities

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Rhodium(II) acetate has become the catalyst of choice for reactions of diazo compounds that result in a broad selection of metal carbene transformations including cyclopropanation, carbon-hydrogen insertion, ylide generation, and aromatic cycloaddition.[1-4] High product yields and significant regio- and/or stereocontrol can generally be achieved.[5-9] However, there are few examples which permit evaluation of chemoselectivity for these catalytic reactions, and those that have been reported suggest

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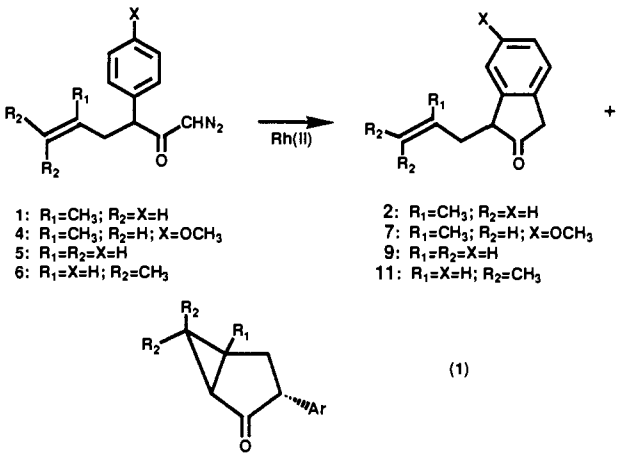
significant limitations for $Rh_2(OAc)_4$.[10-14] In contrast to other catalysts that are suitable for carbenoid reactions of diazo compounds, those constructed with the dirhodium(II) framework are most amenable to ligand modifications that could influence reaction selectivity.[15] Such influences have been reported for stereocontrol in cyclopropanation reactions[6] and for regiocontrol in selected C–H insertion reactions,[16] but the ability of dirhodium(II) ligands to determine reaction preference toward two different functional groups has not been thoroughly investigated.[17] We now report that, by changing the dirhodium(II) ligand from perfluorobutyrate (pfb) to acetamide (acam) or caprolactam (cap), the dominant course of the carbenoid reaction can be transformed from aromatic substitution to cyclopropanation, from carbon–hydrogen insertion to cyclopropanation, from aromatic cycloaddition to carbon–hydrogen insertion, and from aromatic substitution to carbonyl ylide formation.

We have found that the choice of dirhodium(II) ligand markedly influences the product distribution from substrates in which intramolecular cyclopropanation and aromatic substitution are competitive. This is illustrated by the treatment of α-diazo ketone **1** with a catalytic quantity of rhodium(II) acetate at 25 °C in benzene, which produced a 1:1 mixture of the aromatic substitution (**2**, 48%) and cyclopropane (**3**, 44%) products (eq 1).



1: R_1=CH_3; R_2=X=H
4: R_1=CH_3; R_2=H; X=OCH_3
5: R_1=R_2=X=H
6: R_1=X=H; R_2=CH_3

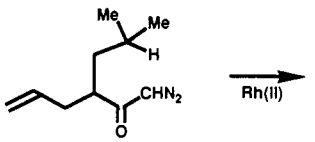
2: R_1=CH_3; R_2=X=H
7: R_1=CH_3; R_2=H; X=OCH_3
9: R_1=R_2=X=H
11: R_1=X=H; R_2=CH_3

(1)

3: R_1=CH_3; R_2=H; Ar=Ph
8: R_1=CH_3; R_2=H; Ar=$C_6H_4OCH_3$
10: R_1=R_2=H; Ar=Ph
12: R_1=H; R_2=CH_3; Ar=Ph

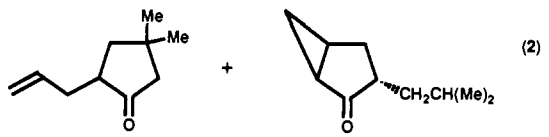
$Rh_2(OAc)_4$ 2:3 = 52/48
$Rh_2(pfb)_4$ 2:3 = 100/0
$Rh_2(cap)_4$ 2:3 = 0/100

However, changing the catalyst from $Rh_2(pfb)_4$ to $Rh_2(cap)_4$ causes a significant alteration of the product distribution.[17] The only compound that could be isolated from the $Rh_2(pfb)_4$-catalyzed decomposition of **1** was **2** (86%), which arose from formal insertion of the metal carbene into an ortho C–H bond of the aromatic ring.[18] In contrast, when $Rh_2(cap)_4$ was used as the catalyst, the major reaction path was cyclopropanation (**3**, 75%); product **2** was not evident in the crude reaction mixture. The rhodium(II)-catalyzed reactions of the closely related α-diazo ketones **4–6** were also examined (eq 1). Complete control of chemoselectivity could be achieved through the use of perfluorobutyrate or caprolactam ligands. In all cases, use of the electrophilic $Rh_2(pfb)_4$ resulted in exclusive formation of the aromatic substitution product. By changing the dirhodium(II) ligand to caprolactam, only cyclopropanation occurred. Rhodium(II) acetate, however, gave rise to a 1:1 mixture of both products. Similar results were obtained from the competition between cyclopropanation and aliphatic C–H insertion (eq 2), where $Rh_2(pfb)_4$ directed the



13

$Rh_2(OAc)_4$ 14:15 = 56/44
$Rh_2(pfb)_4$ 14:15 = 100/0
$Rh_2(cap)_4$ 14:15 = 0/100



(2)

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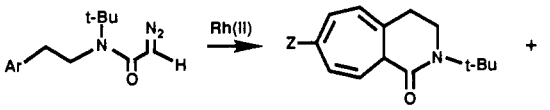
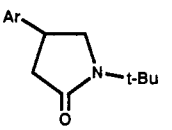
reaction of diazo ketone **13** exclusively to the tertiary C–H insertion product **14**, and use of $Rh_2(cap)_4$ produced only cyclopropane **15**.

Competition between aromatic cycloaddition and C–H insertion is profoundly influenced by the choice of the dirhodium(II) ligand (eq 3). With diazoacetamide **16a**, $Rh_2(pfb)_4$ caused nearly



16a: Ar=C_6H_5
16b: Ar=p-$CH_3OC_6H_4$
16c: Ar=p-$NO_2C_6H_4$

17a: Z=H
17b: Z=OCH_3
17c: Z=NO_2



(3)

18a: Ar=C_6H_5
18b: Ar=p-$CH_3OC_6H_4$
18c: Ar=p-$NO_2C_6H_4$

$Rh_2(OAc)_4$ 17a:18a = 68/32
$Rh_2(pfb)_4$ 17a:18a = 95/5
$Rh_2(cap)_4$ 17a:18a = 3/97

exclusive formation of aromatic cycloaddition product **17a** (**17a**:**18a** = 95:5, 80% yield), whereas $Rh_2(cap)_4$ provided γ-lactam **18a** to the near exclusion of **17a** (**17a**:**18a** = 3:97, 82% yield). With $Rh_2(OAc)_4$, compounds **17a** and **18a** were formed in a 68:32 mixture (85% yield). Similar results were obtained with the *p*-methoxy derivative **16b**, where use of $Rh_2(pfb)_4$ gave the aromatic cycloaddition product **17b** with similar exclusion of the C–H insertion product, and with the *p*-nitro derivative **16c**, use of $Rh_2(acam)_4$ resulted in the γ-lactam **18c** in 90% yield. Mixtures of cycloaddition and insertion products were formed in reactions catalyzed by $Rh_2(OAc)_4$.

In earlier papers we described the formation of bridged oxabicyclo[3.2.1]heptanes from the dirhodium(II)-catalyzed reaction of 1-diazopentanediones.[19] This reaction involved cyclization of the electrophilic metal carbene onto the adjacent keto group to generate a cyclic carbonyl ylide, followed by a 1,3-dipolar cycloaddition.[4] As an extension of our interest in ligand effects and chemoselectivity, we prepared α-diazo ketones **19** and **20** since these substrates possess two competing sites for reaction (eq 4). They were treated with $Rh_2(OAc)_4$ and DMAD (1.2 mol), and the two products obtained corresponded to the dipolar cycloadduct **22** (60%) as well as benzocyclopentanone **23** (20%). In contrast,

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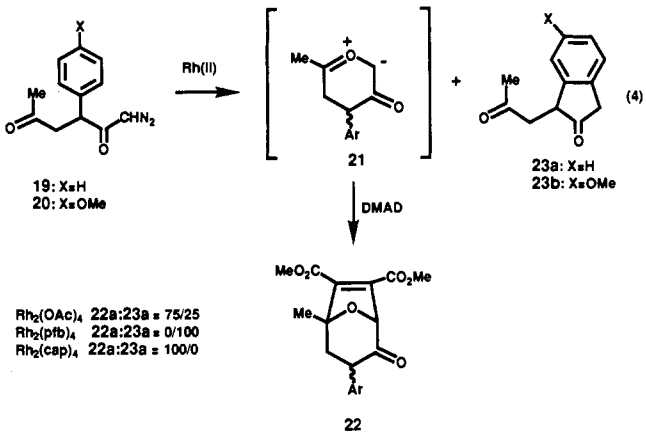
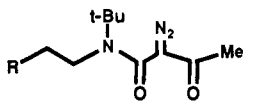


22

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.

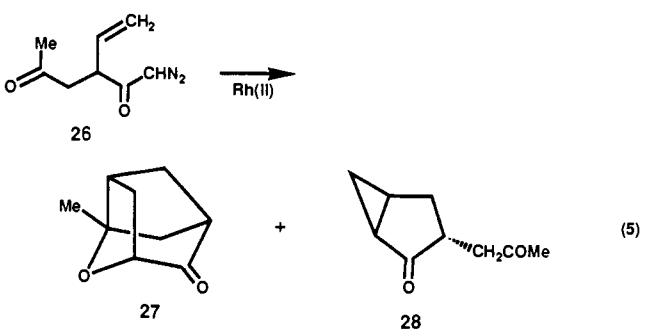
Chemoselectivity in these competitive transformations depends on the inherent electron demand from ligands of the rhodium(II) carbene intermediate, with that derived from $Rh_2(pfb)_4$ being more electrophilic than that from $Rh_2(acam)_4$ or $Rh_2(cap)_4$.[1,2,6,15,16] Electrophilic aromatic substitution 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-



24: R=Ph
25: R=Et

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



26

27 28

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 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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