LiI·2H_2O (1.2 equiv, 2,6-lutidine, reflux, 36 h) gave 81% of the γ-keto ester **12**.

Regioselective generation of the requisite silyl enol ether **13** was uniquely achieved in 80% yield by reaction of **12** with 0.95 equiv of LDA (THF, −78 → 0 °C, 30 min), trapping with Me_3SiCl, dilution with hexane, and filtration through Celite and then Florisil. Reaction of the silyl enol ether **13** under our standard cycloalkenylation conditions (1.0 equiv of $Pd(OAc)_2$, CH_3CN, room temperature, 8 h) gave after flash chromatography an 8:1 mixture of the bicyclic olefins **15a** and **14**, which was directly saponified (1 N KOH, aqueous MeOH, reflux, 2 h) to give 55% of the methylene acid **15b**, mp 121–122 °C.[12] Formation of the third ring proceeded through the acid chloride **15c** (from **15b**, 2 equiv of ClCOCOCl, Et_2O, room temperature, 4 h), which was reacted with Me_4Sn (2 equiv) and a catalytic amount of $PhCH_2Pd(Ph_3P)_2Cl$ in HMPA (65 °C, 3 days)[13] to give 82% of the desired diketone **16**, accompanied by traces of the pseudo-acid chloride (mp 75–76 °C, IR: ν_{CO} 1812 cm^{-1}), which was inert to further reagent. When diketone **16** was stirred with NaH (4 equiv., toluene, reflux, 2 h), the tricyclic dienone **17**,[14] mp 56–57 °C, was obtained as the sole product in 83% yield. *We had thus attained the complete carbocyclic framework of the target molecule in 16% yield over nine steps.*

Conversion of the exocyclic methylene group of **17** to an axial COOH group was necessary to complete the synthesis, since Danishefsky had transformed keto acid **18a** to (±)-quadrone in three steps.[8] Initial forays to this end by using 1 equiv of MCPBA (CH_2Cl_2, 25 °C, 8 h) showed no stereoselection at the methylene group, leading to both diastereomeric epoxy enones in a 1:1 ratio.[15] After much exploration it was found that reaction of dienone **17** with 2.0 eq of thexylborane (THF, 0 → 25 °C, 4 h), followed by dichromate oxidation (10 equiv, 25 °C, 12 h), gave 50% of the axial acid **18a** (mp 143–146 °C) and 16% of the equatorial acid **18b** (mp 168–71 °C).[16] Acid **18a** from our sequence was identical in all respects with a sample kindly provided by Professor Danishefsky.

Our formal total synthesis of quadrone, which corresponds to a 14-step sequence in 2.4% overall yield, provides a compelling illustration of the power of a Pd(II)-mediated cycloalkenylation strategy in natural products synthesis. Further development of this reaction is in progress.

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Registry No. (±)-**1**, 74807-65-1; **2a**, 82951-15-3; **2b**, 82951-16-4; **2c**, 82951-17-5; **2d**, 82951-18-6; **3c**, 73741-66-9; **4a**, 82951-19-7; **4b**, 82951-20-0; **5** (R = $CH_2CH{=}CH_2$), 82951-21-1; **5** (R = CH_3), 6553-64-6; **7a**, 82951-22-2; **7b**, 82951-23-3; **7b**, 82951-23-3; **8a**, 82951-24-4; **8b**, 82951-26-6; **9**, 60924-91-6; **10** (R = R′ = H), 82951-29-9; **10** (R = $COOCH_3$, R′ = H), 82951-30-2; **11**, 82951-31-3; **12**, 82951-32-4; **13**, 82951-33-5; **14**, 82951-34-6; **15a**, 82951-35-7; **15b**, 82951-36-8; **15c**, 82951-37-9; **15c** pseudo-acid chloride, 82951-40-4; **16**, 82951-38-0; **17**, 82951-39-1; **18a**, 82978-37-8; $Pd(OAc)_2$, 3375-31-3; 1,4-dimethylbicyclo[3.3.1]non-2-en-9-one, 80954-09-2; 1-methyl-4-methylenebicyclo[3.3.1]nonan-9-one, 82951-25-5; 2,6,6-trimethylbicyclo[3.2.1]-oct-2-en-8-one, 82951-27-7; 2,6,6-trimethylbicyclo[3.2.1]oct-3-en-8-one, 82951-28-8.

(12) **15b**: ^1H NMR ($CDCl_3$) δ 4.80 (d, J = 2 Hz, 1 H), 4.66 (d, J = 2 Hz, 1 H), 2.88 (d, J = 16 Hz, 1 H), 2.72 (d, J = 16 Hz, 1 H), 2.66 (m, 1 H), 2.25 (dd, J = 7, 14 Hz, 1 H), 2.10 (d, J = 14 Hz, 1 H), 2.06 (m, 2 H), 1.89 (d, J = 14 Hz, 1H), 1.82 (m, 1 H), 1.22 (s, 3 H), 1.01 (s, 3 H). No conjugated enone was found in this cyclization.

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(14) **17**: ^1H NMR ($CDCl_3$) δ 5.68 (s, 1 H), 4.74 (d, J = 2 Hz, 1 H), 4.60 (d, J = 2 Hz, 1 H), 2.99 (d, J = 18 Hz, 1 H), 2.55 (m, 1 H), 2.28 (dd, J = 7, 15 Hz, 1 H), 2.19 (d, J = 18 Hz, 1 H), 2.05 (m, 1 H), 1.90 (d, J = 14 Hz, 1 H), 1.75 (m, 1 H), 1.46 (d, J = 14 Hz, 1 H), 1.26 (s, 3 H), 0.99 (s, 1 H).

(15) Lewis acid catalyzed rearrangement of these epoxy enones gave mixtures of aldehydes in which skeletal rearrangement predominated.

(16) The acids **18a** and **18b** were readily separated by preparative TLC on silica gel with 60:1:1 CH_2Cl_2–CH_3OH–HOAc. We are grateful to Professor M. Goldstein (Cornell) for suggesting the dichromate procedure of Brown et al. (Brown, H. C.; Rothberg, I.; Van der Jagt, D. L. *J. Org. Chem.* **1972**, *37*, 4098), which was superior to the use of H_2O_2–OH^- and Jones reagent in this system.

^1H NMR Studies of ^{15}N-Labeled *Escherichia coli* $tRNA_f^{Met}$. Use of $^1J_{^1H-^{15}N}$ Couplings to Identify Imino Resonances of Uridine-Related Bases

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Since the pioneering experiments of Kearns and Shulman,[1,2] high-resolution proton nuclear magnetic resonance spectroscopy (^1H NMR) has become the preferred technique for studying the structure of tRNA in solution. The richest source of information is the region between 11 and 15 ppm,[3] where the imino protons of bases involved in stable secondary and tertiary interactions resonate. When properly assigned, these signals give important information about tertiary structure and conformational dynamics. Several techniques,[4-7] including analysis of tRNA fragments, empirical calculations of chemical shifts based on shielding interactions, chemical modification, comparative studies of different tRNAs, and most recently, nuclear Overhauser effects (NOEs),[7-11] have been used to assign chemical shifts. However, a typical class I tRNA contains 23–27 individual, often overlapping, peaks between 11 and 15 ppm, and assignment of resonances has proved to be a difficult and controversial task. The problems associated with making assignments would be simplified if peaks could be unambiguously assigned to imino resonances in uridine or guanosine. An approach that offers this possibility is the regiospecific introduction of ^{15}N into N(3) of uridine and structurally related bases. Replacement of ^{14}N by this isotope introduces a ^1H–^{15}N coupling interaction which should be clearly visible in the ^1H spectra of labeled tRNAs. In this and the following communication we report the first experiments with tRNA regiospecifically labeled with ^{15}N.

E. coli $tRNA_f^{Met}$ labeled with ^{15}N at N(3) in uridine and all bases derived biosynthetically from uridine (D, rT, Ψ, and s^4U) was isolated from the SΦ-187 auxotroph of the bacterium grown on medium containing [3-^{15}N]uracil.[12-15] Substantial enrichments

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(3) Abbreviations used: NOE, nuclear Overhauser effect; EDTA, ethylenediaminetetraacetic acid; ppm, parts per million; D, dihydrouridine; Ψ, pseudouridine; rT, ribothymidine; s^4U, 4-thiouridine; m^7G, 7-methylguanosine; A, adenosine; U, uridine; G, guanosine; C, cytosine; P, phosphate.

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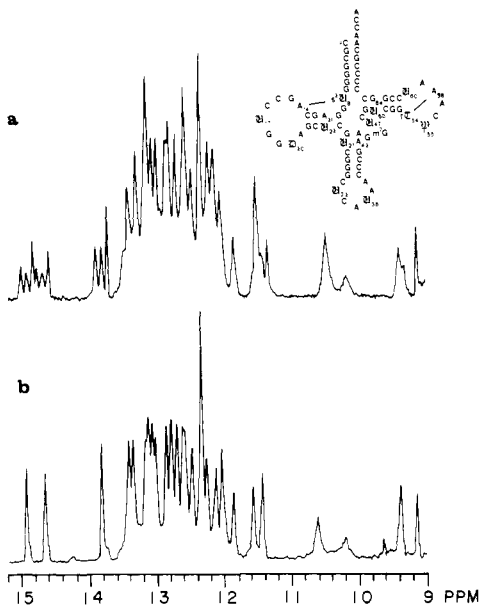

Figure 1. ^1H 500-MHz NMR spectra of *E. coli* $tRNA_f^{Met}$: (a) ^{15}N-labeled $tRNA_f^{met}$ at 30 °C; (b) unlabeled $tRNA_f^{met}$ at 35 °C.

were found in U (60%), D (64%), and Ψ (61%), and no label was detected in G (the only purine base with an imino proton).[16] Material obtained by this procedure had a specific activity of 1.5 nmol/A_{260} unit.

E. coli $tRNA_f^{Met}$ has 12 uridine-related bases that should contain ^{15}N. From the X-ray structure of the molecule[17] and previous NMR studies, ^1H-^{15}N couplings are expected for at least three secondary interactions involving U24, U27, and U50 (a wobble interaction with G64) and perhaps as many as three couplings for tertiary interactions involving s^4U8, rT54, and Ψ55. Additional ^1H-^{15}N pairs may also arise from "free" imino protons that are buried in the interior of the molecule and exchange slowly with water.

Samples for NMR spectra were dialysed against 0.1 mM sodium thiosulfate, lyophilized, and dissolved in 10 mM sodium cacodylate buffer containing 4% deuterium oxide, 50 mM sodium chloride, 10 mM magnesium chloride, and 1 mM EDTA at pH 7.0. Spectra were obtained on a Nicolet 500-MHz NMR spectrometer using a modified Redfield pulse sequence to minimize the signal from water.[18] ^1H NMR spectra of labeled and natural $tRNA_f^{Met}$ are shown in Figure 1, parts a and b, respectively. It is immediately apparent that the well-resolved 1b peaks at 13.82, 14.68, and 14.90 ppm in Figure 1b are approximately 1:1:1 trios[19] in Figure 1a. This is the pattern expected with a central peak due to ^1H-$^{14}N(3)$ flanked by the ^1H-$^{15}N(3)$ doublet ($^1J_{H\text{-}N} \sim 90$ Hz)[20] when the level of incorporation of label is 60-65%.

The resonance at 14.90 ppm has been attributed to the imino proton in the s^4U8-A11 tertiary pair in *E. coli* $tRNA_f^{Met}$ by several groups[21-25] and is regarded as one of the least controversial assignments in the region between 11 and 15 ppm.[7] As expected, that peak appears as a trio in Figure 1a. The assignment of the resonance at 14.68 ppm to the imino proton of m^7G in the m^7G46-C13-G22 triple on the basis of chemical shift comparisons among several tRNAs and chemical modification experiments[26] is also generally regarded as reliable.[7] It is clear, however, from the trio seen in Figure 1a that this is incorrect. A logical alternative more consistent with chemical shift patterns reported for other tRNAs is the rT54 imino proton in the rT54-A58 double,[24] although it should be noted that this is not a firm assignment since chemical shifts of imino protons in secondary A-U pairs have been reported as high as 14.6 ppm.[9] At present we cannot reliably choose among the imino protons of U24, U27, or rT54 for the peaks at 14.68 and 13.90 ppm, although NOE experiments planned for the future may permit a distinction.

The selective incorporation of ^{15}N into the uridine-derived bases of $tRNA_f^{Met}$ permits us to unambiguously locate resonances for three of the six potential hydrogen bonds involving s^4U8, U24, U27, U50, rT54, and Ψ55. In the following communication we describe variable-temperature and NOE studies that allow us to uncover trio patterns in more congested regions of the spectrum.

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^1H NMR Studies of ^{15}N-Labeled *Escherichia coli* $tRNA_f^{Met}$. An Unambiguous Assignment for the G-U Pair and Detection of a Uridine Resonance at 11.4 ppm

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The secondary G-U[1] wobble interaction is found in the stems of several tRNAs and may perturb the normal helical pattern in adjacent base pairs.[2] In a series of elegant NOE experiments, Johnston and Redfield[3,4] located resonances for two spatially

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