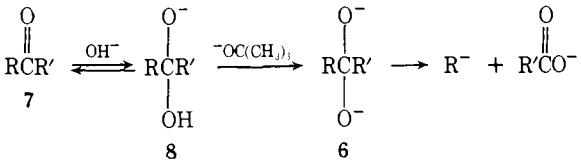
Table I. Yields Obtained in the Hydrolysis of Amides with Potassium *tert*-Butoxide–Water[a]

Amide	Amine	% yield	Acid	% yield	Time, h
$C_6H_5CON(CH_3)_2$	$(CH_3)_2NH$	85[b]	$C_6H_5CO_2H$	96	5
$CH_3CON(CH_3)C_6H_5$	$C_6H_5NHCH_3$	98	CH_3CO_2H	96[c]	3
$C_6H_5CONC_5H_{10}$	Piperidine	93[b]	$C_6H_5CO_2H$	96	48
$HCON(C_6H_5)_2$	$(C_6H_5)_2NH$	100	HCO_2H	55[d]	2
$CH_3CON(CH_3)_2$	$(CH_3)_2NH$	65[b]	CH_3CO_2H	88[c]	12
$(CH_3)_3CCON(CH_3)_2$	$(CH_3)_2NH$	82[b]	$(CH_3)_3CCO_2H$	88	27[e]

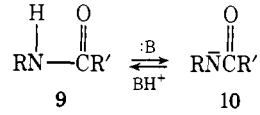
[a] All hydrolyses were carried out at room temperature in diethyl ether unless otherwise indicated. [b] Isolated as the *p*-toluenesulfonamide. [c] Acetic acid was determined titrimetrically after neutralization with Amberlite IR-120 ion-exchange resin. Analysis of standard sodium acetate by this method routinely gave 90–92% values. The values quoted in the table have been corrected for this tendency of the method to give values which are 9% low. [d] Isolated as the phenylhydrazide. [e] The hydrolysis was carried out in refluxing tetrahydrofuran.

In considering the requirement that **3** be an intermediate in the hydrolysis process, we were drawn to the analogous intermediacy of **6** in the cleavage of nonenolizable ketones by hydroxylic base.[6] The best conditions for this cleavage involved treatment of 1 equiv of the ketone in ether with the basic system derived from ca. 6 equiv of potassium *tert*-butoxide and 2 equiv of water in ether. Reaction of the water with the potassium *tert*-butoxide generated finely divided, essentially anhydrous potassium hydroxide (2 equiv) and *tert*-butyl alcohol (2 equiv). The strongly nucleophilic and poorly solvated hydroxide added to the carbonyl of **7** to produce **8.** The conversion of **8** into the dianion **6** was accomplished by the excess potassium *tert*-butoxide. Once **6** was generated, cleavage occurred with ease.[7] Thus, we felt that conditions which were applicable to the base-promoted cleavage of nonenolizable ketones should be sufficient to readily hydrolyze amides.



In a typical procedure, a slurry of potassium *tert*-butoxide (13.7 g, 0.122 M), water (0.67 g, 0.037 M), and the amide (0.0185 M) in diethyl ether was stirred vigorously at room temperature (ca. 24°), and the course of the reaction was followed by TLC analysis. When the amide was completely gone, the reaction was cooled in an ice bath and ice was added to the reaction mixture until two layers formed. The layers were separated and the products were isolated from the appropriate layers. Table I lists the yields of both the amine and acid fragments obtained in a series of hydrolyses. As can be noted from the table, the yields are excellent in those cases where the products do not require special methods of analysis. Both aliphatic and aromatic amides can be hydrolyzed at relatively low temperatures. Only in the case of the highly hindered *N,N*-dimethylpivalamide did the reaction require heating.

All of the examples listed in Table I are tertiary amides. This illustrates both a strength and a weakness of the method. Both secondary and primary amides are extremely resistant to hydrolysis under our conditions. For instance, acetamide was hydrolyzed to the extent of less than 10% after 45 h. Acetanilide showed no detectable hydrolysis products after 7 days. Presumably, this is due to the conversion of **9** into **10** in an acid–base reaction. This means that our method will allow the selective hydrolysis of a tertiary amide in the presence of a secondary or primary amide. In this regard, it offers an attractive counterpart to the procedure of White[8] for the cleavage of secondary amides in the presence of tertiary amides.



We believe that the ease with which tertiary amides can be hydrolyzed by our method offers certain distinct advantages relative to the protecting of secondary amines as amides. In principle, any secondary amine which has been protected via conversion to a tertiary benzamide derivative can be regenerated at room temperature under basic conditions.

Acknowledgment. We are indebted to the National Cancer Institute of the Public Health Service for Grant CA-17269 which supported this investigation. We are also grateful to Dr. H. B. Renfroe of the Ciba-Geigy Corporation for a stimulating discussion in relation to this study.

References and Notes

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Magnetic Resonance Spectroscopy on Carbon-13 Labeled Uracil in Transfer Ribonucleic Acid[1]

Sir:

We would like to report results on the incorporation of carbon-13 uracil, labeled at the C-4 position, into transfer ribonucleic acid (tRNA) using a mutant strain of *Salmonella typhimurium.* The labeled bulk tRNA's were studied with carbon-13 nuclear magnetic resonance spectroscopy (^{13}C NMR).[2] The carbon-13 labeled uracil was prepared from ^{13}C enriched potassium cyanide. The strain of *S. typhimurium,* designated JL-1055,[3] has the genotype *pyrA, pyrG, cdd,* and *udp.* The bacteria were grown in 10-l. batches at 37 °C in a minimal media of glucose and salts, plus arginine, cytidine, and carbon-13 labeled uracil, these additional nutrients being required by JL-1055 for normal growth. The cells, harvested in the late log phase, were recovered by centrifugation and the tRNA was extracted[4] and purified by DEAE column chromatography.[5] The puri-

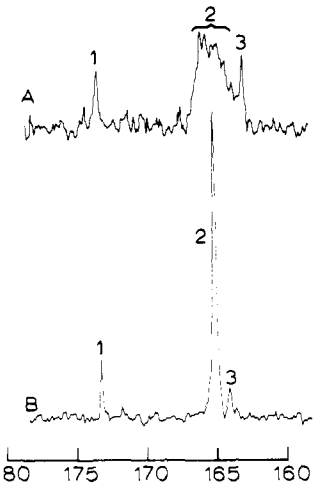

Figure 1. Carbon-13 NMR spectra of ^{13}C enriched tRNA. Spectrum A was taken at 37 °C. Spectrum B was taken at 82 °C. Chemical shifts are referenced to Me_4Si.

Table I. C-4 Chemical Shifts[a] and Base Ratios[b] of ^{13}C Labeled tRNA and Other Nucleosides

Compound	Line 1 (D)[c]	Line 2 (U/T)	Line 3 (ψ)
tRNA (37 °C)	173.8 (0.11)	164.6–166.9 (1.0)	163.6 (0.13)
tRNA (82 °C)	173.3 (0.12)	165.3 (1.0)	164.4 (0.10)
Uridine		165.8	
Dihydrouridine	173.5		
Thymidine		166.0	
Pseudouridine			165.0
Base Ratios[d]	(0.16)[e]	(1.0)	(0.13)[f]

[a] Chemical shifts are with respect to Me_4Si. [b] The values in parentheses are ratios of minor base line to the uridine/ribothymidine band or line and are calculated from the areas of the lines. [c] See text for line assignments. Key: D = dihydrouridine; U = uridine; T = ribothymidine; ψ = pseudouridine. [d] Base ratios are literature values for *E. coli*. [e] P. Ceruitti, J. W. Holt, and H. Miller, *J. Mol. Biol.*, 34, 505 (1968). The mole value for uridine and ribothymidine needed to calculate the ratio was taken from reference *f*. [f] D. B. Brown, J. D. Smith, and P. F. Spahr, *J. Mol. Biol.*, 2, 113 (1960).

fied tRNA had an absorbance of 17 A_{260} units per milligram and an A_{260} to A_{280} ratio of 1.9. Its activity, based on a tritiated methionine acceptance assay, was comparable to that of commercially prepared *E. coli* bulk tRNA. About 55 mg of carbon-13 labeled tRNA was dissolved in 2 ml of 0.02 M Tris buffer, 0.25 M sodium chloride, 0.01 M magnesium chloride, 0.001 M EDTA, and 0.02 M sodium thiosulfate. The estimated pH at 37 °C was 6.6[6] (pH at 5°C is 7.5). The solution was later lyophilized and redissolved in 1 ml of water. The pH of this solution was estimated to be 6.6 at 37 °C.[6]

The ^{13}C NMR spectra were obtained on a Varian XL-100-15 nuclear magnetic resonance spectrometer equipped with a Fourier transform accessory. Proton noise decoupling was used with all spectra. Figure 1 shows the 160–180 ppm portion of two spectra, one taken at 37°C (Figure 1A) and one taken at 82 °C (Figure 1B). Dioxane was used as an internal reference with the chemical shifts converted to Me_4Si by adding 66.3 ppm to the measured shifts. These results along with the ratios of the area of each line to the area of the uridine/thymidine band or line and the measured chemical shifts of the C-4 carbons of uridine and the three most abundant minor bases derived from uridine are tabulated in Table I.

The spectra in Figure 1 are characterized by a band (164.6–166.9 ppm) in Figure 1A and by an intense line (165.3 ppm) in Figure 1B belonging to the carbon-13 labeled uridine incorporated into the tRNA, plus two additional lines belonging to minor bases synthesized in vivo during the maturation process of the molecule.[7] In agreement with this assignment, the chemical shift of the C-4 carbon of uridine in water is seen to be at the midpoint of the band in Figure 1A and close to the major line in Figure 1B (Table I). Ribothymidine, one of the more prevalent minor pyrimidine bases in tRNA, has a chemical shift close to uridine and would not be resolvable in these spectra. The band of lines belonging to uridine in Figure 1A indicates a wide difference in the environments seen by the individual bases in tRNA in its native state. When tRNA is heated to 82 °C it assumes a random coil configuration, and the local environmental effects are diminished leaving only a single sharp line. The different environments experienced by the uridines are explainable by the cloverleaf model of Holley et al.[8] and by the tertiary model of Kim et al.[9] Higher magnetic fields plus the partial cleavage techniques of Kearns and Schulman[10] would allow a detailed examination of the secondary and tertiary structure of tRNA.

The first line in the spectra is well separated from the uridine region and, as it is very close to the C-4 shift of dihydrouridine in water (Table I), may be assigned to that carbon. The ratio of dihydrouridine to uridine plus ribothymidine, while slightly low, is comparable to the ratio reported for *E. coli* (Table I). The dihydrouridine shift is seen to change very little in going from 37 to 82 °C, suggesting that there is little change in the environment of dihydrouridine upon denaturation of tRNA. The model of Kim et al.[9] does not propose base interaction for dihydrouridine in the native state and therefore these bases may exhibit random coil behavior. Our data support such a conclusion.

The most difficult line to assign is the line at 163.6 ppm at 37 °C and at 164.4 ppm at 82 °C. At 82 °C the line is seen to have a shift close to the C-4 shift of pseudouridine. Also its ratio to uridine plus ribothymidine is close to the value found in the literature for *E. coli* pseudouridine (Table I). The next most abundant minor base derived from uridine found in *E. coli* and *Salmonella* tRNA is 4-thiouridine,[11] which has a C-4 shift 26.4 ppm downfield from uridine C-4 in Me_2SO.[12] All other minor bases derived from uridine are less than 1% of the uridine present in tRNA[11] and are not observed. It therefore is reasonable to assign the line at 164.4 ppm in Figure 1B to pseudouridine while, pending further study, the assignment of the line at 163.6 ppm in Figure 1A can only be tentative. Most of the pseudouridine in tRNA, according to the model of Kim et al.,[9] is involved in hydrogen bonded base pairing, and hydrogen bonding of an oxygen attached to a carbon can cause an upfield shift of about 1 ppm. Thus, the ratio of the line at 163.6 ppm to the uridine/ribothymidine band, the hydrogen bond shift, and the negligible percentage of other minor bases derived from uridine suggest its assignment to the C-4 carbon of pseudouridine.

References and Notes

(1) This work was supported in part by the National Institutes of Health of the U.S. Public Health Service under awards GM08521, GN01374, RR00574, AM00803, and RR07092. Synthesis of Carbon-13 labeled uracil was supported by ERDA Award E(11-1)-2451. One of the authors (W.D.H.) is indebted to the National Science Foundation for support in the form of a predoctoral fellowship. We also wish to acknowledge helpful discussions with Dr. Baldomero Olivera and thank Dr. John Ingraham for supplying the *Salmonella* culture.
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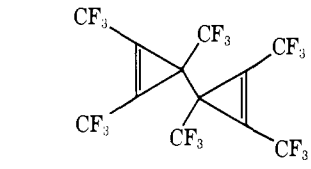
Perfluorohexamethylbicyclopropenyl

Sir:

We wish to describe a synthesis and some chemistry of the title compound (**1**), the final member of the first complete set of benzene valence isomers.[1,2] This family is shown below, numbered in order of diminishing energy content.[2b,3]



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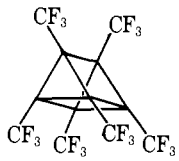

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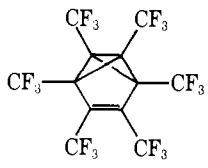

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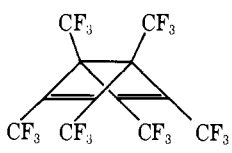

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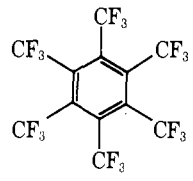


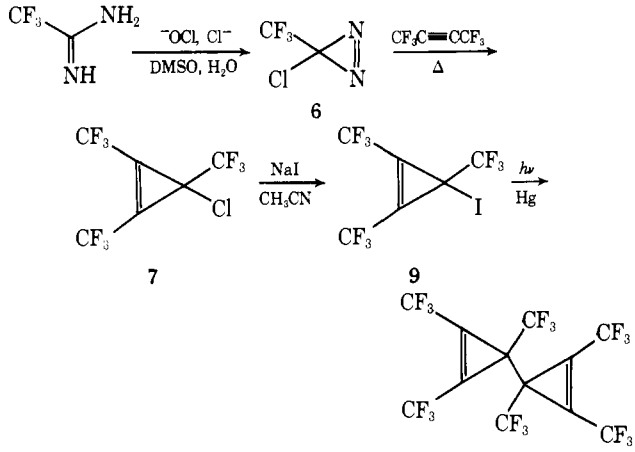
5

The synthetic strategy entailed coupling of a suitably functionalized perfluoro-1,2,3-trimethylcyclopropene, prepared in turn via addition of a carbene to perfluoro-2-butyne (PFB).

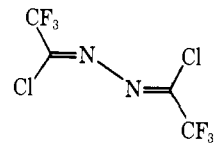
Dehydration[6] of trifluoroacetamide followed by addition of ammonia[7] to the resulting trifluoroacetonitrile gave trifluoroacetamidine.[8] Oxidative cyclization of trifluoroacetamidine by the method of Graham[9] (hypochlorite and chloride ion in aqueous dimethyl sulfoxide) gave diazirine **6** in 45% overall yield from trifluoroacetamide (Chart I). Chlorotrifluoromethyl-1-diazirine[10] is a gas (bp −19 to −18°) with the following spectroscopic features:[11] ir 1590, 1290, 1210, 960, 730 cm^{-1}; uv[11] 318 nm; NMR δ 18.37 (singlet); MS 114. The corresponding bromodiazirine was prepared in similar fashion by substitution of bromide for chloride ion in the Graham reaction.

When the chlorodiazirine[13] was decomposed in the gas phase at 120° in a large excess of PFB, two products resulted. That of shorter glc retention time was the desired chlorocyclopropene **7** (bp 42°; ir 1890, 1300, 1230, 1200, 1180, 1040, 910, 790, 730 cm^{-1}; NMR δ 7.59 (quartet) and 18.58 (septet), J = 0.7 Hz; MS 278). The product of longer

Chart I. Synthesis of Perfluorohexamethylbicyclopropenyl



retention time (ir 1630, 1270, 1220, 1180, 1010, 990, 750 cm^{-1}; NMR δ 17.16 (singlet); MS 260) proved to be the azine **8**, formed probably by carbene attack on diazirine.[14] Since the azine was a major product even in the presence of



8

a 30-fold excess of PFB, it is clear that addition of the electron-deficient carbene to the electron-deficient acetylene is not a facile process. To solve this problem, a stirred gas-phase reactor operating at 200° was charged with PFB (~0.5 atm), and diazirine was bled in very slowly from a reservoir maintained at ~1 atm with a carbon tetrachloride slush bath. The diazirine decomposed rapidly at 200°, with the result that its steady-state concentration in the reactor remained very low. Consequently, the azine/cyclopropene ratio dropped to ~0.05 with PFB/diazirine ratios of ~4. With this technique, pure **7** was obtained in 56% yield after GLC purification.

For coupling attempts to make the bicyclopropenyl, the iodocyclopropene (**9**) offered distinct advantages over its chloro counterpart. Nucleophilic displacement of the chlorine of **7** by iodide ion took place rapidly in solution at room temperature; the reaction also proceeded quite cleanly when gaseous chlorocyclopropene was contacted at 150° with excess sodium iodide supported on Chromosorb W. The last fact, together with the observation that methanolysis of **7** was fast even in dilute cyclohexane solution, argues strongly against the intermediacy of perfluorotrimethylcyclopropenium ion.[16] Displacement by iodide ion presumably occurs via an SN2′ process or even via a carbanion intermediate.[17] The iodocyclopropene, prepared in acetonitrile in 77% yield (GLC purified), was characterized by these properties: bp 71°; ir 1900, 1300, 1280, 1230, 1200, 1180, 1030, 870, 780, 710 cm^{-1}; uv 256 nm; NMR δ 8.39 (quartet, 6 F) and δ 15.45 (septet, 3 F), J = 0.8 Hz; MS 370.

Photolysis of **9** in the presence of mercury[18] in the gas phase at ~100° with a Pyrex-filtered medium-pressure mercury arc gave **1** in 84% yield after GLC purification. The fluorocarbon sublimes readily, yielding beautiful colorless radial clusters, mp 72.5–73.5° (ir 1890, 1290, 1230, 1220, 1200, 1180, 1160, 1050, 1010, 900, 820, 790, 700, 690 cm^{-1}; uv <190 nm;[19] NMR δ 6.01 (septet, 12 F) and δ 13.10 ("tridecet",[20] 6 F), J = 1.6 Hz; MS 486[21]).

The thermal stability of perfluorohexamethylbicyclopropenyl is remarkable: it aromatizes cleanly with $t_{1/2} \gtrsim 2$