Anal. Calcd for $C_{27}H_{37}H_5O_7$: C, 59.65; H, 6.86; N, 12.89. Found: C, 59.74; H, 7.00; N, 12.63. Amino acid analysis: Gly, 1.01; Phe, 0.99; Leu, 1.00.

Glycyl-L-phenylalanyl-L-leucine (11, H-Gly-L-Phe-L-Leu-OH). c-NOPY-Gly-Phe-Leu-OH (**10**, 0.545 g, 1 mmol) was dissolved in 40 mL of methanol, which had been saturated with anhydrous ammonia. The solution was stirred for 2 h at room temperature, and then concentrated to volume of ca. 5 mL under reduced pressure in a rotary evaporator. Pyridine (20 mL) was added, and the solution was again concentrated to ca. 5 mL. Upon addition of a small amount of acetone and 30 mL of ether, the product separated as a light buff-colored precipitate and was collected by filtration, yield 0.297 g. Colored impurities were removed by extraction with 5 mL of hot ethanol to yield 0.187 g (56%) of white product, mp 220–223 °C. The tripeptide separated as clumps of slender white needles, mp 225–227 °C dec, when its solutions in ammonium hydroxide were maintained for a time under reduced pressure. The product was homogeneous by TLC, R_f 0.44 (1-butanol:acetic acid:water, 10:1:3) on Analtech silica gel G plates (lit.[7] R_f 0.45): IR (Nujol) 3.03, 6.00, 6.04, 6.13, 6.47, 7.26, 7.48, 7.58, 7.69, 7.93, 8.10, 8.73 μm; $[\alpha]^{25}_{589}$ −12.9° (*c* 1.06, HOAc) [lit.[7] $[\alpha]^{25}_{589}$ −12.5° (*c* 1.04, HOAc).

Anal. Calcd for $C_{17}H_{25}N_3O_4$: C, 60.87, H, 7.51; N, 12.53. Found: C, 60.68; H, 7.80; N, 12.53.

i-NOPY Glycylation of Lysozyme. To a solution of 120 mg of lysozyme (Sigma) in 2.2 mL of 6 M guanidine hydrochloride was added 85 mg of **8b** in 0.2 mL of acetonitrile. The mixture was made alkaline (pH 8–9) by addition of 0.03 mL of *N,N,-N',N'*-tetramethylethylenediamine (TMEDA) and stirred at room temperature for 2 h and then acidified to pH 2 by addition of 6 N hydrochloric acid. Additional water was added (ca. 12 mL), and the precipitate of modified lysozyme was centrifuged down and then washed twice with 10 mL of water, three times with 10 mL of warm absolute ethanol, and once with 10 mL of acetonitrile. The final ethanol and acetonitrile supernatants did not show the presence of the *N*-NOPY chromophore (λ_{max} 380 nm), indicating that excess *N*-NOPY-Gly containing reagent had been removed. The resulting modified lysozyme was obtained in the form of a tan powder. The material gave an ultraviolet spectrum in acetic acid–water (1:1 v/v) in which the absorbance of 0.9 at 380 mm from the *N*-NOPY-glycine residue exceeded that at 280 nm (0.7). Amino acid analysis of this material gave the results expected for lysozyme, except for an increased amount of glycine amounting to 4.8 residues.

Deblocking of a 23 mg. sample of this N-(i-NOPY)-glycylated lysozyme was carried out by suspending the material in a solution prepared from 2 mL of water, 2 mL of DMF, and 2 mL of concentrated ammonium hydroxide and stirring the mixture for 64 h. A small amount of solid that remained undissolved was centrifuged down. The ultraviolet spectrum of a sample of the supernatant solution diluted with ethanol indicated that it contained the deblocking product, i-NOPY-NH_2 (λ_{max} 355 nm). The residue obtained by evaporation of the supernatant was combined with the very small centrifuged pellet and mixed with a solution containing 6 mL of water, 6 mL of DMF, and 5 drops of 2,4-dinitrofluorobenzene (DNFB). The mixture was adjusted to a pH of 8–9 by addition of 6 drops of TMEDA and stirred overnight. Concentrated ammonium hydroxide (2 mL) was added to destroy any remaining DNFB and stirring was continued for 2 h. The mixture was dialyzed against distilled water (1 L) for ca. 2 h with two changes of water and then for 64 h with two additional changes. Samples of the resulting solution of DNFB-treated glycylated lysozyme were taken for amino acid analysis. Results of amino acid analyses are recorded in Table I.

Acknowledgment. We gratefully acknowledge support from a grant from the Health Research and Services Foundation of Pittsburgh and technical assistance from William E. Harte and Joseph J. Nunes.

Registry No. **1a**, 16206-17-0; **1b**, 16206-02-3; **2a**, 88767-00-4; **2b**, 88767-01-5; **5a**, 52555-23-4; **5b**, 88767-02-6; **6b**, 88767-03-7; **7a**, 52555-24-5; **7b**, 88767-04-8; **8a**, 88767-05-9; **8b**, 88767-06-0; **9a**, 88767-07-1; **9b**, 88767-08-2; **10a**, 88767-09-3; **11**, 15373-56-5; $H_2NCH_2CO_2H$, 56-40-6; H-L-Phe-L-Leu-OEt·HCl, 88767-10-6; H-L-Phe-L-Leu-OH, 3303-55-7; lysozyme, 9001-63-2.

On Charge Distribution in Diazenium Salts

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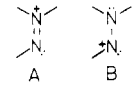
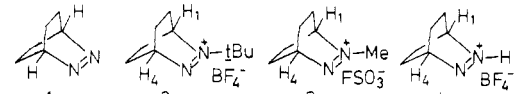
McBride and Bens[1] pointed out in 1959 that diazenium cations should be resonance hybrids of structures A and B and that the all-octet structure A should be preferred.



A B

Nelsen and Landis[2] reported that both bridgehead hydrogens for *tert*-butylated bicyclic azo compounds are shifted substantially downfield of the bridgehead hydrogens of the azo compounds. Bridgehead hydrogen chemical shifts (all in CD_3CN, relative to residual CD_2HCN at δ 1.93) are reported below for 2,3-diazabicyclo[2.2.2]oct-2-ene (**1**) and three cations derived from it. The upfield



1	2	3	4
$\delta(H_{1,4})$ 5.00	5.64, 5.84	5.27, 5.72	5.58(ave)

bridgehead hydrogens of both **2** and **3** appear as broadened triplets, and the downfield peaks are broadened singlets, suggesting that they correspond to the same hydrogen in both compounds. Replacement of the *tert*-butyl group of **2** by methyl as in **3** should principally affect the shift of H_1, causing it to move upfield because of a decrease in steric compression. This suggested the assignment of H_1 as the δ 5.64 peak in **2**, and the δ 5.27 peak in **3**. An NOE experiment verified this assignment of **3**, because decoupling the methyl group caused an 8.7% enhancement of the δ 5.27 peak compared to a 0.3% enhancement of the δ 5.72 peak. The result that H_1, on a carbon attached to the formally positive nitrogen, comes δ 0.45 upfield of H_4 surprised us and made us wonder about the charge distribution in these molecules.

Geometry-minimized MNDO calculations[3] were run on $(H_2N{=}NH)^+$ (**5**) and $(Me_2N{=}NMe)^+$ (**6**) as models. Both were constrained to be planar and the methyl groups were idealized by being held tetrahedral, with all CH distances the same (1.112 Å was obtained). **6** is calculated as being stablest in the geometry shown in Figure 1, with one CH bond of each methyl group in the molecular plane. The total charge densities at each atom are also shown in Figure 1; 76% of the charge is calculated to be at the hydrogens of **5** and 99% at the methyl groups of **6**. The charge density at the trisubstituted nitrogen (N_2) is calculated to be more negative than that at the disubstituted nitrogen (N_3) in both cases. This is principally a result of N=N π-bond polarization. The calculated p_z electron densities at N_2 and N_3 are given at the bottom of Figure 1, where it may be seen that the π-electron distributions are similar for **5** and **6**, with greater electron density at the formally positive nitrogen, as expected. The π-electron distributions at N_2:N_3 are calculated as 62:38 in **5**, and 60:40 in **6**. The calculated charges at the hydrogens of **5** and the carbons

(1) McBride, W. R.; Bens, E. M. *J. Am. Chem. Soc.* **1959**, *81*, 5546.
(2) Nelsen, S. F.; Landis, R. T. *J. Am. Chem. Soc.* **1974**, *96*, 1788.
(3) Dewar, M. J. S.; Thiel, W. *J. Am. Chem. Soc.* **1977**, *99*, 4489, 4907. Program 353, Quantum Chemistry Program Exchange, Indiana University, Bloomington, IN.

0022-3263/84/1949-1134$01.50/0

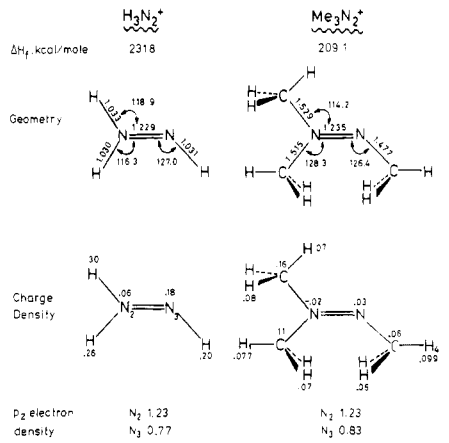


Figure 1. Summary of MNDO results for $H_3N_2^+$ and $Me_3N_2^+$.

of **6** are also in the order we had expected, i.e., more positive charge on the atoms attached to the formally positive N_2 than on those attached to N_3. Nevertheless, MNDO predicts H_1 of **6** to have a smaller positive charge than H_4. This agrees with the NMR result of H_1 appearing upfield of H_4 in **3**. Electron density is generally accepted as being an important factor in determining ^1H NMR chemical shifts, although it is not the only factor. In an attempt to see if the rather small charge difference calculated for the hydrogens of **6** could lead to the observed shift differences for **2** and **3**, we calculated the in-plane hydrogen charge densities for 1,2-*cis*-dimethyldiazene (as a model for **1**), obtaining 0.034 and for 1-protio-1,2-*cis*-dimethyldiazene (as a model for **4**) and obtaining 0.086 and 0.104 for H_1 and H_4, respectively (average 0.096). We note that the MNDO hydrogen charge densities are in the same order as the proton chemical shifts, although a plot of one against the other is not linear. Obviously the models are not perfect, and even if MNDO charge densities were perfect, and charge density were the only factor influencing chemical shift, a straight line would not be expected. The MNDO calculations emphasize the importance of N=N π-bond polarization in determining charge distribution and do predict a downfield shift for H_4 compared to H_1 of **3**, as is observed experimentally. We presume that the presence of the coplanar, formally sp^2 lone pair at N_3 is important in determining the charge distribution in diazenium salts.[4]

Experimental Section

1 and **2** were prepared as previously described.[5]

2-Methyl-2,3-diazabicyclo[2.2.2]oct-2-enylium fluorosulfonate (3) was generated by treating the azo compound with 1 equiv of methyl fluorosulfonate in CD_3CN: ^1H NMR (CD_3CN) δ 5.72 (bs, 1 H), 5.27 (bt, 1 H), 4.49 (s, 3 H), 2.0–2.2 (m, 4 H), 1.6–1.8 (m, 2 H), 1.4–1.6 (m, 2 H).

2-Protio-2,3-diazabicyclo[2.2.2]oct-2-enylium fluoroborate (4) was generated by addition of 1 equiv of an ether solution of HBF_4 to the azo compound in CD_3CN: ^1H NMR (CD_3CN) δ 5.58 (bs, 2 H), 2.12 (bd, 4 H), 1.54 (bd, 4 H).

Acknowledgment. We thank the National Science Foundation for generous support of this research and Frank Weinhold for helpful discussion.

Registry No. **1**, 3310-62-1; **2**, 43008-10-2; **3**, 88868-48-8; **4**, 88868-49-9; $H_3N_2^+$, 51943-19-2; $Me_3N_2^+$, 88868-50-2; methyl fluorosulfonate, 421-20-5.

(4) One could presumably construct a "shielding cone" model as a framework for rationalizing the chemical shifts.

(5) Snyder, J. P.; Heyman, M. L.; Gundestrup, M. *J. Chem. Soc., Perkins Trans. 1* **1977**, 1551.

Novel Synthesis of L-Phenylalanine

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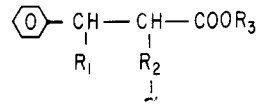
There has been considerable interest in the synthesis of phenylalanine (**1a**) since the discovery of the artificial sweetner aspartame.[1] Several synthetic routes to L-



	R_1	R_2	R_3
a.	H	NH_2	H
b.	OH	NH_2	H
c.	Cl	$NHCOCH_3$	C_2H_5
d.	$OCOCH_3$	$NH_2 \cdot HCl$	H
e.	H	$NHCOCH_3$	H
f.	$OCOCH_3$	$NHCOCH_3$	C_2H_5
g.	H	$NHCOCH_3$	C_2H_5
h.	OH	$NHCOCH_3$	H

phenylalanine have been reported in the past.[2] Among these, Vogler's procedure, to our knowledge, is the only known example demonstrating the transformation from *threo*-β-phenyl-L-serine (**1b**). This multistep procedure involved a hydrogenolysis of *N*-acetyl-β-chloro-L-phenylalanine ethyl ester (**1c**) in the presence of perchloric acid.

Now we report an efficient three-step synthesis of L-phenylalanine from *threo*-β-phenyl-L-serine[3] (Scheme I), which can be prepared by the enzymatic reaction of benzaldehyde and glycine.[3a] The key step is the hydrogenolysis of the hydrochloride salt of *threo*-*O*-acetyl-β-phenyl-L-serine (**1d**) to *N*-acetyl-L-phenylalanine (**1e**). We believe that this reaction sequence offers a synthetically useful alternative for the preparation of L-phenylalanine.

Results

This study was begun with the readily available DL materials. The ideal transformation of β-phenylserine (**1b**) to phenylalanine (**1a**) would be the direct removal of the OH group in **1b**. Cleavage of a benzylic C–O bond has been widely studied in the past.[4] The most versatile procedure for this manipulation is hydrogenolysis. However, our attempts at direct hydrogenolysis of β-phenylserine under various conditions ($HClO_4$,[5] HOAc, HCl, etc.; catalyst Pd/C, $Pd/BaSO_4$; pressure 1 atm–150 psi; temperature 25–80 °C) were unsuccessful. Efforts were then made to activate the C–O bond in β-phenylserine. *threo*-*O*-Acetyl-*N*-acetyl-β-phenylserine ethyl ester (**1f**),

(1) Mazur, K.; Schlatter, J. M.; Goldkamp, A. H. *J. Am. Chem. Soc.* **1969**, *91*, 2684.

(2) (a) Kaneko, T.; Izumi, Y.; Chibata, I.; Itoh, T. "Synthetic Production and Utilization of Amino Acids"; Wiley: New York, 1974; p 171. (b) Vineyard, B. D.; Knowles, W. S.; Sabacky, M. J.; Bachman, G. L.; Weinkauff, D. J., *J. Am. Chem. Soc.* **1977**, *99*, 5946. (c) U.S. Patent 4 220 590 to Monsanto Co., 1980; *Chem. Abstr.* **1975**, *83*, 164367*q*. (d) Johnson, J. C. "Amino Acids Technology"; Noyes Data Corporation: NJ, 1978; p 285. (e) Vogler, von K. *Helv. Chim. Acta* **1950**, *33*, 2111.

(3) (a) U.S. Patent 3 871 958 to Ajinomoto Co., 1975; *Chem. Abstr.* **1973**, *79*, 135298*e*. (b) Greenstein, J. P.; Winitz, M. "Chemistry of the Amino Acids"; Wiley: New York, 1961, Vol. 3, p 2603.

(4) Hartung, W.; Simonoff, R. "Organic Reactions"; Adams, R., Blatt, A., Cope, A., McGrew, F., Niemann, C., Snyder, H., Eds.; Wiley: New York, 1953; Vol. VII, p 263.

(5) Rosenmund, K.; Karg, E.; Marcus, F. *Ber.* **1942**, *75*, 1850.

0022-3263/84/1949-1135$01.50/0