(2-methoxyethyl) ether. When more than 1 equiv of alkyllithium compound is present, a slow second-step proton transfer apparently takes place to give the dianions **1b** and **2b**. *The dianions, but not the monoanions, rearrange to their 1,2-disubstituted isomers*, **3b** and **4b**.

In typical reactions, measured amounts of hydrazine substrate, solvent (usually diethyl ether), and methyllithium in diethyl ether were injected through a septum into a carefully dried vessel, under nitrogen. The reaction was allowed to proceed with rapid stirring. Samples were removed using syringe technique and quenched in water; the dried organic layer was analyzed either by gas chromatography or nmr spectroscopy, or both. The identity of the rearranged isomers was confirmed by comparison with authentic samples.

Compound **1** is recovered unchanged upon treatment with 1.0 equiv of methyllithium after 90 hr at 30° in diethyl ether and even after heating at 114° in bis(2-methoxyethyl) ether for 26 hr. With more than 1 equiv of methyllithium, slow rearrangement takes place at 30° in ether, as shown by the data in Table I. Complete isomerization to **3** took place in 71 hr in the presence of 8 equiv of methyllithium.

Table I. Rearrangement of 1,1-Diphenylhydrazine Dianions at 30° in Diethyl Ether

Molar ratio, CH_3Li:**1**	Reaction time, hr	% rearr
1.0	90	0
1.8	90	11
2.0	44	29
	90	43
3.0	44	42
	90	61
8.0	47	85
	71	>95

Compound **2** likewise undergoes no rearrangement after 48 hr when treated with 1.0 equiv of methyllithium in ether at 30°. Heating of the reaction to 112° in bis(2-methoxyethyl) ether led to partial decomposition with formation of aniline and N-methylaniline, but no rearrangement of the recovered hydrazine. When **2** was treated with 2.5 equiv of methyllithium in ether solution for 22 hr and then quenched, 35% of **4** was found. Very long reaction times led to considerable decomposition with formation of aniline and N-methylaniline.

That dianions of the hydrazines are actually formed was shown by observing the disappearance of the CH_3Li proton resonance at τ 11.91 in unquenched reaction solutions containing excess methyllithium. One equivalent of CH_3Li reacts with **1** within the time of mixing, and a second equivalent of CH_3Li disappears over several hours. The decrease of CH_3Li resonance is accompanied by a decrease in the N–H resonance at τ 6.1–6.2 as the dianion is formed. The nmr studies suggest that, although reaction of **1a** with a second equivalent of CH_3Li to form **1b** is slow, the rearrangement of **1b** to **3b** is even slower, so that rearrangement is ultimately rate determining.

These experiments show that phenyl-substituted hydrazines will undergo anionic rearrangement, but only as their dianions and then only very slowly.[5] In contrast, anions of silyl-substituted hydrazines appear to rearrange with a half-time of less than 10^{-3} sec at −80°. The striking differences between these classes of compounds emphasizes the unique mobility of the organosilyl moiety in anionic rearrangements. The course of anionic rearrangement may be quite different for silyl-[4] and arylhydrazines; the mechanism of the latter reactions is under investigation.

(5) 1,2 migration of phenyl in arylhydrazine anions appears to be much slower than the corresponding rearrangement of aryl groups in ethanes. See E. Grovenstein, Jr., and G. Wentworth, *J. Am. Chem. Soc.*, **89**, 1852 (1967).

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Measurement of Racemization in Peptide Synthesis by Nuclear Magnetic Resonance Spectroscopy

Sir:

A major problem in the synthesis of peptides is associated with the degree of racemization at the coupling stage.[1] For example, the combination of two optically active, suitably blocked amino acids in an idealized system affords a dipeptide whose steric purity is ascertained by the isolation of any enantiomers or diastereoisomers, employing such techniques as countercurrent distribution,[2] fractional crystallization,[3-5] and gas–liquid partition,[6,7] paper,[8,9] and thin layer[10] chromatography. Many experiments have evaluated the effect of changing activating agents, acyl protecting groups, amino components, bases, salts, solvents, and temperatures on the optical purity of the condensation reaction.[11-13] It is still difficult to untangle these entwined factors in detail due to the paucity of separable model peptide pairs.

Recently, a series of diastereoisomeric N-acylalanylphenylalanine methyl esters and N-acylphenylalanylalanine methyl esters were shown to possess different

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Table I. Degree of Racemization during Peptide Bond Formation

Component activated[a,b]	Condensed with[a,b]	% D–L or L–D in product with coupling agent[c] CDI	K	DCC	EDC	Methyl resonance[d] L–L	D–L or L–D
For-L-Ala	L-Phe-OMe	<3	<3	<3	<3	80.5	74.3
For-L-Phe	L-Ala-OMe	<3	<3	<3	<3	79.5	72.5
Ac-L-Ala	L-Phe-OMe	<3	<3	35	27	79.0	71.5
Ac-L-Phe	L-Ala-OMe	16	6	50	41	81.0	73.5
Bz-L-Ala	L-Phe-OMe	19	<3	..	..	84.5	79.5
Bz-L-Phe	L-Ala-OMe	35	<3	..	..	79.5	74.5
Z-Gly-L-Ala	L-Phe-OMe	<3	5	10	12	78.0	71.0
Z-Gly-L-Phe	L-Ala-OMe	<3	9	17	17	77.5	70.5
Z-Gly-Gly-L-Phe	L-Ala-OMe	10	15	25	20	81.5	72.0

[a] For = formyl; all other abbreviated designations of compounds follow IUPAC–IUB rules. [b] The optical purity of the starting materials was verified by gas–liquid partition chromatography: N-acetyl and N-benzoyl compounds were converted to the menthyl ester derivatives, while the N-formyl compounds were hydrolyzed and analyzed as the N-trifluoroacetyl-L-prolyl peptide esters; the methyl ester compounds were assayed similarly.[7] [c] The limit of measurement was generally 3%, although in some cases a more accurate value was obtained by duplicate procedures. [d] All spectra were determined on a Varian A-60 spectrometer with the center of gravity of the chemical shift given in hertz downfield from tetramethylsilane (J = 7.2 ± 0.3 Hz). The compounds were dissolved in deuteriochloroform (deuterioethanol for the tetrapeptide).

nuclear magnetic resonance (nmr) spectra.[14a] The methyl doublet signal in an L–L (or D–D) compound was at lower field than the equivalent signal for the D–L (or L–D) analog due to deshielding and offered a potentially convenient means for the quantitative analysis of such mixtures. We have now employed this technique to examine the influence of several coupling agents and N-acyl protecting groups on the extent of racemization during peptide synthesis.

In a typical experiment, N-acetyl-L-phenylalanine (0.207 g, 1 mmole), carbonyldiimidazole (0.162 g, 1 mmole), and methylene chloride or acetonitrile (5 ml) was stirred at −5° for 1 hr. The hydrochloride of L-alanine methyl ester (0.139 g, 1 mmole) and triethylamine (0.14 ml, 1 mmole) were added and the solution was kept at 0° for 15 hr. After suitable washings, the solution was dried and evaporated; the solid residue was redissolved in deuteriochloroform and the solution was used for nmr analysis.

It is evident (Table I) that carbonyldiimidazole (CDI) and 2-ethyl-5-phenylisoxazolium-3′-sulfonate (K) are preferable here to dicyclohexycarbodiimide (DCC) and 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide (EDC). Optical purity is lower in the peptides derived from N-benzoyl- and N-acetylamino acids, but the N-formyl group is noted as a potentially useful amino protecting group.[14b] The strongly electron-withdrawing N-acyl functions obviously cause a decrease of steric homogeneity in the products, while the phenylalanyl derivatives seem more prone to racemize than the alanyl derivatives.

Among the advantages of the nmr procedure are convenience, generality, and rapidity, while sensitivity is comparable to previous methods. As a test, an artificial mixture of N-acetylalanylphenylalanine methyl ester (L–L, 9.862 mg; D–L, 0.201 mg) was readily resolved at 100 MHz with the aid of a computer of average transients. At 60 MHz both sets of methyl doublets are separable in the racemic N-benzoyl and N-benzyloxycarbonyl peptides but overlap to give a triplet in the corresponding N-acetyl and N-formyl peptides. Area integration is simplified in this latter pattern, which can be internally standardized by the presence of the acetyl or methyl ester singlets. Nmr is extensively used for the gathering of kinetic data, and it is suggested that such an approach in combination with the method outlined here may have immediate application to work concerned with the mechanisms of racemization in amino acids,[15] peptides,[16–18] and active esters.[19,20]

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Enzyme–Substrate Interaction by Nuclear Magnetic Resonance

Sir:

We should like to report the study of enzyme–substrate (or inhibitor) interaction by nmr techniques using the changes in chemical shifts of hydrogen or fluorine atoms of the substrate (or inhibitor) exchanging rapidly between solution and the active site of the enzyme.[1] The magnitude and direction of such shifts can potentially give a detailed understanding of the manner in which various parts of a substrate interact with the active site of an enzyme. If δ is the observed change in

(1) This method has also recently been applied to lysozyme: E. W. Thomas, *Biochem. Biophys. Res. Commun.*, 24, 611 (1966); M. A. Raftery, F. W. Dahlquist, S. Parsons, and S. I. Chan, unpublished observations.