lene), 1.11 (3, s, C-1, OCH_3), 3.17 (3, s, C-1, OCH_3), and 3.14–3.37 ppm (1, m, C-5, H)], in 60% overall yield from the methoxy ketone **4**. Under the same acidic conditions employed above for protolysis and dehydration of the methoxycyclopropane **7**, the present cyclopropyl ether **8** was converted to the desired hydroxy ketone **10**[8] [mp 158–159°; nmr ($CDCl_3$) δ 0.83 (3, s, C-10,CH_3), 1.46 (3, s, C-9,CH_3), and 3.50–3.65 ppm (1, m, C-5,H)] in 80% yield without any evidence of dehydration of the secondary alcohol. Again conclusive proof for the structure and stereochemistry of this hydroxy ketone **10** was readily provided when Wolff–Kishner reduction and then oxidation of the resulting alcohol afforded the known ketone **12**[13] [mp and mmp 108–110° (sealed capillary)] in 50% overall yield. Aside from the utility of these intermediates for further synthetic exploration, the described approach toward angular methylation of polycyclic systems in the trans manner, together with the earlier results of Wenkert[5] that lead to the corresponding cis-fused systems, makes this methoxycyclopropane scheme a versatile method.

(15) Predoctoral Fellow of the National Science Foundation, 1968–present.

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Racemization During Peptide Couplings Using the Mixed Anhydride, *N*-Hydroxysuccinimide Ester, 8-Hydroxyquinoline Ester, and Acyl Azide Methods

Sir:

We have recently described a[1] method for assessing racemization with sensitivity in the 1–0.001% range. We wish to report results of applying this assay to four peptide coupling procedures.

The Mixed Anhydride Method. Though long regarded as racemization prone, this procedure has been found recently by Anderson and coworkers[2] to maintain optical integrity when used under defined conditions. Our observations confirm their findings in every detail.

When a THF solution, 0.2 *M* in *Z*-[1-^{14}C]-Gly-L-PheOH and triethylamine, was treated at −15° with 1 equiv of isobutyl chloroformate, followed 1 min later by 1 equiv of ethyl glycinate, then allowed to stand for 3 min and warmed to 22°, tripeptide was obtained in 30% yield, 1.5% of which was racemic. Use of *N*-methylmorpholine as base gave 98% yield, 0.20% DL, and with very carefully weighed equivalents of acid and methylmorpholine, 0.01% DL was observed. A Young coupling of [7-^{14}C]benzoyl-L-leucine with ethyl glycinate under the above conditions gave 1.4% DL with 1 equiv of triethylamine, and 39% DL with 2 equiv of base. Young couplings with 1.0, 1.1, and 2.0 equiv of *N*-methylmorpholine gave, respectively, 0.38, 2.4, and 15.7% DL.

The *N*-Hydroxysuccinimide (NHS) Ester Method. First studied by Anderson and coworkers,[3] these esters appear to couple without racemization and, owing to the high nucleophilicity of NHS toward acyl carbon, appear to be formed in optically pure state when NHS is combined with racemization prone peptide activated species.[4] We sought to test both these assertions quantitatively.

When *Z*-[1-^{14}C]-Gly-L-PheOH (0.5 *M*) and 1.1 equiv of NHS in DMF were combined at −10° with 1.1 equiv of dicyclohexylcarbodiimide, allowed to remain 4 hr at −10° and 12 hr at 2°, and then treated for 48 hr with 1.5 equiv of ethyl glycinate, tripeptide was obtained which was 3–7% racemic. When NHS was added in THF solution 1 min after the addition of chloroformate, under the mixed anhydride conditions of the preceding section, then followed 1 min later by ethyl glycinate, no reduction in racemization level was observed. For the coupling using *Z*-Gly-L-PheOH and triethylamine, addition of NHS changed the racemate level from 1.6 to 1.5%; for couplings with *N*-methylmorpholine, the figures were 0.19% without NHS, 0.14% with.

The optically pure NHS ester of *Z*-[1-^{14}C]-Gly-L-PheOH can best be prepared by reaction in chloroform of triethylamine and the HBr salt of the NHS ester of L-Phe[5] with the mixed anhydride derived from *Z*-[1-^{14}C]-GlyOH and isobutyl chloroformate. The resulting glass was identified by spectroscopic comparison with the characterized DL ester, mp 90–92°. When a chloroform solution of L ester, prepared without isolation, was combined at 22° with ethyl glycinate, tripeptide was obtained which contained 0.86% racemate. When any labeled D ester was selectively removed[1] by addition and recovery of unlabeled DL ester from the initial chloroform solution of L ester, coupling with ethyl glycinate again yielded 0.6–1.0% racemate.

8-Hydroxyquinoline Esters. Although available in optically pure form only by a Goodman inverse synthesis,[6,7] peptide esters of 8-hydroxyquinoline are of theoretical interest for their racemization-free coupling behavior.[7] Using D-labeled racemate[1] we have established the clean recovery of the DL 8-HQ ester of *Z*-Gly-PheOH (mp 125–128°) from excess L ester (mp 78–80°). By racemate recovery[1] we have prepared labeled L ester containing less than 0.001% of its activity as labeled D and have coupled it with ethyl glycinate in DMF, 40 hr, at either 0 or 20°. In either case, the isolated tripeptide contained 0.16% racemate.

Acyl Azides. Subsequent to our earlier azide results,[1] we have observed that the bicarbonate extraction which usually follows diazotization in an azide procedure has a substantial affect on the racemization level.

An ethereal solution of [7-^{14}C]benzoyl-L-leucyl azide, freshly extracted from an aqueous acetic–

(1) D. S. Kemp, S. W. Wang, G. Busby III, and G. Hugel, *J. Amer. Chem. Soc.*, **92**, 1043 (1970).

(2) G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *ibid.*, **89**, 5012 (1967).

(3) J. E. Zimmerman and G. W. Anderson, *ibid.*, **89**, 7151 (1967); G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *ibid.*, **85**, 3039 (1963); G. W. Anderson, F. M. Callahan, and J. E. Zimmerman, *ibid.*, **89**, 178 (1967).

(4) M. Goodman and C. Glaser in "Peptides: Chemistry and Biology," Marcel Dekker, New York, N. Y., 1970.

(5) Satisfactory elemental analyses were obtained for new compounds, excepting the NHS ester of *Z*-Gly-L-PheOH.

(6) M. Goodman and K. G. Steuben, *J. Org. Chem.*, **27**, 3409 (1962); *J. Amer. Chem. Soc.*, **81**, 3980 (1959).

(7) H. D. Jakubke and A. Voigt, *Chem. Ber.*, **99**, 2419 (1966).

hydrochloric acid diazotization mixture, was divided into four portions. The first was dried and treated directly with ethyl glycinate at 2°; from it was isolated dipeptide, 0.10% racemic. The remaining portions were extracted at 2° with several portions of aqueous sodium bicarbonate; of these, one was treated at 2° with ethyl glycinate, another was allowed to warm to 20° 1 hr after addition of amine, and a fourth, treated at 2° with 1 equiv of acetic acid and 2 equiv of ethyl glycinate. The respective levels of racemate were 0.36, 0.31, and 0.27%. The inability of added acid to decrease the racemate level suggests that racemization occurs in part during extraction.

Acyl azide preparations which have been freed of traces of acid are particularly vulnerable to base-catalyzed racemization. After 10 min at 3° in ether containing 0.03 *M* triethylamine, the Young azide is racemized to the extent of 2.5%; the Anderson azide, after 15 min at 3° in DMF containing 0.2 *M* triethylamine, is racemized to the extent of 50.3%! *Extensive racemization may therefore occur whenever peptide azides are placed in strongly basic media, and reaction conditions which combine acyl azides and excesses of tertiary amines should be avoided.*[8]

Summary. The optically pure *N*-hydroxysuccinimide and 8-hydroxyquinoline esters have been found to be roughly comparable to the *p*-nitrophenyl esters[1] in extent of racemization during coupling. In our hands the addition of *N*-hydroxysuccinimide does not reduce the extent of racemization for carbodiimide couplings to an acceptable level, and addition of NHS is found to have an insignificant effect on racemization level for mixed anhydride couplings. Although these results have been obtained under a limited range of conditions for one model peptide, we feel they argue strongly against the use of *N*-hydroxysuccinimide as a racemization suppressor in fragment condensations involving these coupling agents. Under carefully defined conditions, the mixed anhydride procedure can rival the acyl azides and 3-acyloxy-2-hydroxy-*N*-ethylbenzamides[1] in yielding peptides of better than 99.9% chiral purity, but slight deviations from exact stoichiometry can result in an order of magnitude increase in racemate level.

(8) For earlier reports of tertiary amine catalyzed racemization of azides, see ref 1 and G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *J. Amer. Chem. Soc.*, **88**, 1338 (1966).

(9) A. P. Sloan Fellow, 1968–1970. Financial support from National Institutes of Health Grant No. GM 13453 is gratefully acknowledged. Author to whom correspondence should be addressed.

(10) National Science Foundation Predoctoral Fellow, 1967–1970.

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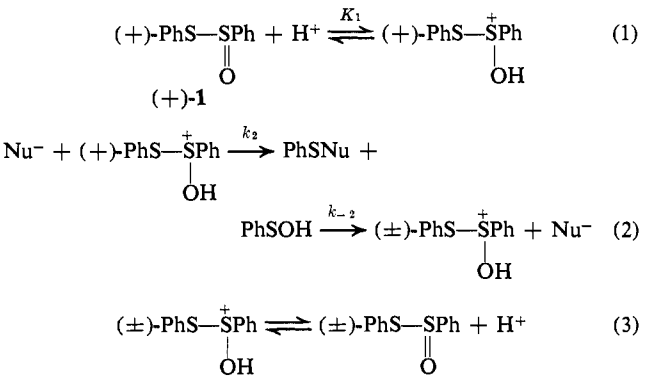
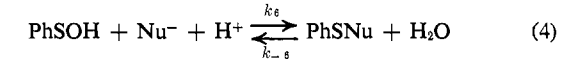
Acid- and Nucleophile-Catalyzed Oxygen-18 Exchange of Phenyl Benzenethiolsulfinate. New Insight into the Chemistry of Sulfenic Acids and Sulfenyl Derivatives[1]

Sir:

In aqueous dioxane optically active phenyl benzenethiolsulfinate, (+)-**1**, undergoes acid- and nucleophile-catalyzed racemization *via* the mechanism shown in eq 1–3, with k_2 rate determining.[2] One important



point unanswered previously was how rapidly PhSNu and PhSOH recombine to form thiolsulfinate (step k_{-2}) compared to the rate at which they are interconverted *via* the equilibrium shown in eq 4. We have



now investigated this point by studying the acid- and nucleophile-catalyzed ^{18}O exchange of PhS(^{18}O)SPh (**1**-^{18}O) under the same conditions. Our results, which reveal that exchange is generally considerably slower than racemization, provide some important new insights into the behavior of sulfenic acids and reactive sulfenyl derivatives in aqueous solution.

Labeled thiolsulfinate[3] (1.48 atom % ^{18}O) was subjected to exchange in 60% dioxane. Rates of exchange were determined by recovering the thiolsulfinate after varying periods of time and determining its ^{18}O content.[4] The results for the various nucleophiles studied as catalysts are shown in Table I. Besides the experi-

Table I. Rate of Oxygen-18 Exchange of Phenyl Benzenethiolsulfinate in 60% Dioxane[a]

Nucleophile	$[1]_0$, *M*	$[Nu^-] \times 10^2$, *M*	$[HClO_4]$, *M*	$k_{ex} \times 10^4$, sec^{-1}	(k_α/k_{ex})
Cl^-	0.050	10.0	0.40	1.0	2.3
Br^-	0.050	1.0	0.50	0.60	17
		3.0	0.50	1.53	19
n-Bu_2S	0.050	1.0	0.50	7.0	34
			0.10	1.5	31
		0.20	0.50	1.5	31
	0.025	0.20	0.10	0.63	15
	0.0125	0.20	0.10	0.83	11.3

[a] All runs at 39.6°. Ionic strength maintained constant at 0.5 by addition of lithium perchlorate where needed.

(1) This research was supported by the National Science Foundation, Grant No. GP-10732X.

(2) J. L. Kice and G. B. Large, *J. Amer. Chem. Soc.*, **90**, 4069 (1968).

(3) Prepared by reaction of thiophenol with labeled benzenesulfinyl chloride using the procedure of H. J. Backer and H. Kloosterziel, *Recl. Trav. Chim. Pays-Bas.*, **73**, 129 (1954). The labeled sulfinyl chloride was obtained by reaction of labeled acetic acid with $PhSCl_3$.

(4) Because the rates of acid- and nucleophile-catalyzed ^{18}O exchange are in several instances only about twice as fast as the rates of acid- and nucleophile-catalyzed disproportionation[5] of **1** to $PhSO_2SPh$ and PhSSPh under the same conditions,[6] some disproportionation occurs during the period while the exchange is being followed. Before analysis the recovered thiolsulfinate was separated from any thiolsulfonate and disulfide by preparative thin-layer chromatography on silica gel *via* a procedure shown in independent experiments not to lead to any exchange.

(5) J. L. Kice, C. G. Venier, G. B. Large, and L. Heasley, *J. Amer. Chem. Soc.*, **91**, 2028 (1969).

(6) J. P. Cleveland, unpublished results.