rent and a platinum working electrode cell [L. P. Rigdon and J. E. Harrar, *Anal. Chem.*, **46**, 696 (1974)] with aqueous pH 4 acetate buffer–methanol (1:1 v/v) in the salt bridge and the cathode compartment.

(4) NMR also shows the presence of exchangeable amide proton, thus ruling out other tautomeric structures.

Selective Reduction of the Amide Carbonyl Group in Dipeptides by Borane[1]

Roger W. Roeske,* Frederick L. Weitl, Kari U. Prasad, and Richard M. Thompson

Departments of Biochemistry and Pediatrics, Indiana University School of Medicine, Indianapolis, Indiana 46202

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The facile reduction of amides by borane[2,3] led us to attempt the reduction of the peptide carbonyl group in *N*-alkoxycarbonyl dipeptide esters **1** in order to obtain the corresponding diamino esters **2.** The products, after hydrolysis of the ester and suitable protection of the newly generated amino group, can be regarded as derivatives of diamino acids **4** and could be incorporated into synthetic peptides. This would amount to selective replacement of a peptide bond by an aminoethylene unit (–CONH → –CH_2NH–) and would be useful in structure–activity studies in peptide hormones. The amino group also offers a point of attachment of a reactive group for affinity labeling of enzymes and receptors.

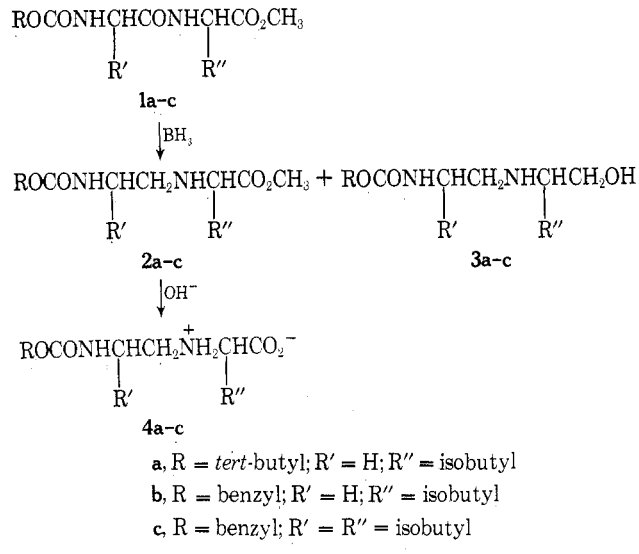
Since the relative ease of reduction of isolated carbonyl groups by BH_3 is $-CO_2H > -CONR_2 > CONHR > -CONH_2 > -CO_2R >$ ROCONHR,[2-4] it was a priori possible to reduce selectively the amide bond in **1.** However, an ester group located α to an amide is more easily reduced to the corresponding alcohol than is an isolated ester. Thus when benzoyl glycine ethyl ester was treated with borane, the amino ester and amino alcohol were produced in yields of 11 and 85%, respectively.[3]

We have found that concurrent reduction of the ester in dipeptides is minimized by carrying out the reaction at −20 °C with 2 mol of BH_3 for 4–5 h. Under these conditions, a considerable amount of starting material remains unreacted, but it is easily separated from the basic products and does not diminish greatly the synthetic usefulness of the procedure. Thus, reduction of 3.0 g of Boc-Gly-Leu-OMe[5] under the above conditions gave 1.24 g of starting material and 0.64 g of pure **2a,** after separation from other products by chromatography on silica gel. Alkaline hydrolysis of **2a** gave the amino acid **4a** in 73% yield. In general, more vigorous reaction conditions led to more complex mixtures (TLC) from which only the amino alcohols **3** could be isolated.

We have found that the severe acid treatment used to hydrolyze the amine–borane complexes formed after reduction of simple amides[2] can be replaced by treatment with 0.5 N HCl in methanol at room temperature overnight, which is compatible with the benzyloxycarbonyl group. In fact, reductions of the acid-sensitive Boc dipeptide esters were quenched by treatment with aqueous methanol overnight.

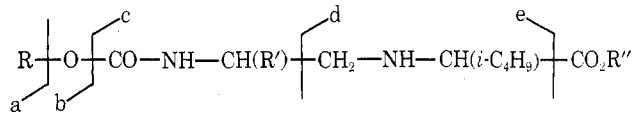
Although we expect the reduction to proceed with complete retention of asymmetry at the chiral centers, we have not proved this point. Perhaps the best evidence for retention is that the products from several reductions of the same material have the same optical rotations and have sharp melting points.

Scheme I



a, R = *tert*-butyl; R′ = H; R″ = isobutyl
b, R = benzyl; R′ = H; R″ = isobutyl
c, R = benzyl; R′ = R″ = isobutyl

The mass spectra of **2a–c** exhibit characteristic fragmentations which can be generalized as



Other ions may appear at M^+, M + 1, b + H, and e − (b + H), or may be due to losses of C_3H_6 (42 amu) or C_4H_8 (56 amu) via McLafferty rearrangements. The most interesting ion in these spectra is ion d. It was previously reported[6] that for polyamino alcohols formed by exhaustive reduction of peptides by $LiAlH_4$, the amine fragment formed by carbon–carbon cleavage was a significant ion in the mass spectra. However, in the present case the charge remains only on the carboxyl fragment.

Experimental Section

Melting points are uncorrected. TLC was carried out on silica gel plates (E. Merck) in BAW (*n*-BuOH–AcOH–H_2O, 4:1:1), TCW (tetrahydrofuran–cyclohexane–H_2O, 93:7:5), EAE (EtOAc–EtOH–AcOH, 9:1:1), and CMA ($CHCl_3$–MeOH–AcOH, 90:30:5); spots were located with ninhydrin and by chlorination followed by starch–iodide spray. Column chromatography was carried out on silica gel 60 (E. Merck) in a column 18 × 1 in. ^1H NMR spectra were taken in a Varian T-60 spectrometer. Mass spectra were determined on an LKB-9000-S at an ionization potential of 70 eV. Tetrahydrofuran was dried by distillation from CaH_2. Borane was obtained from the Aldrich Chemical Co. as a 1 M solution in THF containing 5 mol % $NaBH_4$ stabilizer. All evaporations were done at ≤40 °C. Microanalyses were by Midwest Microlabs, Indianapolis, Ind.

***N*-[2-[(*tert*-Butyloxycarbonyl)amino]ethyl]-L-leucine Methyl Ester Hydrochloride (2a).** A solution of 3.0 g (10 mmol) of Boc-Gly-Leu-OMe in 20 ml of THF was cooled to −20 °C in a flask flushed with N_2 and fitted with a rubber septum. A solution of 20 ml of 1 M BH_3 in THF (Aldrich Co.) was added with a syringe and the reaction was allowed to stir at −20 °C for 4 h under N_2. Residual BH_3 was quenched by cautious addition of 10 ml of MeOH at −20 °C (caution, $H_2\uparrow$) and the mixture was stirred overnight at room temperature; the solution was evaporated under vacuum and treated with MeOH (3 × 50 ml) with evaporation to dryness after each addition, to remove boric acid as trimethyl borate. The residue was suspended in water, the pH was adjusted to 3.0 with HCl, and unreacted starting material (1.2 g) was removed by extraction with ether. The aqueous solution (mixture of **2a** and **3a**) was evaporated to dryness and chromatographed on a column of silica gel. **2a** was eluted with 5% MeOH in $CHCl_3$, followed closely by **3a.** Crystallization from MeOH–Et_2O gave 0.97 g of **2a,** mp 162–164 °C, in 30% yield (51% based on consumed **1a**): $[\alpha]^{25}D$ +9.1° (*c* 1.0, MeOH); TLC (EAE) R_f 0.42; ^1H NMR ($CDCl_3$, Me_4Si) δ 3.83 (s, 3 H, $COOCH_3$), 1.45 [s, 9 H, $-NHCOOC(CH_3)_3$],

0.95 [d, 6 H, $-CH_2CH(CH_3)_2$]; mass spectrum (70 eV) *m/e* (rel intensity) 289 (M + 1, 0.7), 288 (M, 0.3), 229 (42) 215 (15), 173 (100), 158 (77), 155 (60), 113 (23), 102 (94), 99 (23), 73 (1.7), 57 (47). Anal. Calcd for $C_{14}H_{29}N_2O_4Cl$: N, 8.62. Found: N, 8.40.

***N*-[2-[(Benzyloxycarbonyl)amino]ethyl]-L-leucine Methyl Ester Hydrochloride (2b).** Z-Gly-Leu-OMe (10 mmol, 3.38 g) was treated with 20 ml of 1 M BH_3 in THF according to the procedure for **2a** except that the reaction was stopped after 5 h by the addition of 15 ml of 2 N HCl in MeOH. Work-up gave 1.4 g of starting material and 0.94 g of **2b** after recrystallization from MeOH–Et_2O: yield 26% (44% on basis of recovered **2a**); mp 160–162.5 °C; $[\alpha]^{25}D$ +15.9° (*c* 1, MeOH); TLC (EAE) R_f 0.46; ^1H NMR ($CDCl_3$, Me_4Si) δ 7.33 (5 H, C_6H_5), 5.13 (s, 2 H, $C_6H_5CH_2$), 3.77 (s, 3 H, $COOCH_3$), 0.95 [d, 6 H, $CH_2CH(CH_3)_2$]; mass spectrum (70 eV) *m/e* (rel intensity) 323 (M + 1, 1.9), 322 (M, 1), 263 (100), 215 (1.2), 158 (76), 155 (88), 113 (52), 107 (28), 102 (77), 99 (47), 91 (80). Anal. Calcd for $C_{17}H_{27}N_2O_4Cl$: N, 7.83. Found: N, 7.71.

***N*-[L-2-[(Benzyloxycarbonyl)amino]-4-methylpentyl]-L-leucine Methyl Ester Hydrochloride (2c).** Z-Leu-Leu-OMe (10 mmol, 3.92 g) was treated with 20 mmol of BH_3 according to the procedure for **2b**. The ether extracts gave 3.1 g of starting material **1c**. Chromatography of the residue from the aqueous layer by the usual method gave **2c**, 0.30 g after recrystallization from MeOH–Et_2O: yield 7% (35% based on **1c** recovered); mp 162–164 °C; $[\alpha]^{25}D$ +2.0° (*c* 1, MeOH); TLC (EAE) R_f 0.74; ^1H NMR ($CDCl_3$, Me_4Si) δ 7.33 (5 H, C_6H_5), 5.10 (s, 2 H, $C_6H_5CH_2$), 3.75 (s, 3 H, $COOCH_3$), 0.93 [broad, 12 H, $CH_2CH(CH_3)_2$]; mass spectrum (70 eV) *m/e* (rel intensity) 379 (M + 1, 0.6), 378 (M, 0.4), 319 (37), 271 (1.2), 211 (100), 169 (22), 158 (58), 155 (29), 107 (27), 102 (55), 91 (56). Anal. Calcd for $C_{21}H_{35}N_2O_4Cl$: N, 6.75. Found: N, 6.35.

***N*-[2-(*tert*-Butyloxycarbonyl)amino]ethyl]-L-leucine (4a).** A solution of 1.62 g of **3a** in 30 ml of MeOH and 11.0 ml of 1 N NaOH was allowed to stand at room temperature for 6 h, then concentrated under vacuum to a syrup, diluted with water, and adjusted to pH 6.5 with HCl. The precipitate was collected and washed with a little cold water, wt 1.00 g (73%). Anal. Calcd for $C_{13}H_{26}N_2O_4$: N, 10.21. Found: N, 9.97.

Registry No.—**1a**, 7535-69-5; **1b**, 17331-93-0; **1c**, 3504-37-8; **2a** HCl, 57901-23-2; **2b** HCl, 57901-24-3; **2c** HCl, 57901-25-4; **3a**, 57901-26-5; **4a**, 57901-27-6; BH_3, 13283-31-3.

References and Notes

(1) (a) Presented in part at the 65th Annual Meeting of the American Society of Biological Chemists, Minneapolis, Minn., June 1974. (b) We acknowledge support from the Grace M. Showalter Residuary Trust, the James Whitcomb Riley Memorial Association, and from Research Grant PHS RO1 ES 942-01.

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(3) M. J. Kornet, P. A. Thio, and S. E. Tan, *J. Org. Chem.*, **33**, 3637 (1968).

(4) H. C. Brown, S. Krishnamurthy, T. P. Stocky, N. M. Yoon, and C. S. Pak, *J. Org. Chem.*, **38**, 2786 (1973).

(5) Boc = *tert*-butyloxycarbonyl; Z = benzyloxycarbonyl.

(6) K. Biemann, F. Gapp, and J. Seibl, *J. Am. Chem. Soc.*, **81**, 2274 (1959); H. J. Förster, J. A. Kelley, H. Nau, and K. Biemann in "Chemistry and Biology of Peptides: Proceedings of the 3rd American Peptide Symposium", J. Meienhofer, Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1973, pp 679–686.

A Selective Phosphorylation by Means of Bis(*O*-thiocarbonyl) Disulfides and Triphenylphosphine

Hiroshi Takaku,* Machiko Yamana, and Yasuhiro Enoki

Laboratory of Organic Chemistry, Chiba Institute of Technology, Narashino-shi, Chiba-ken 275, Japan

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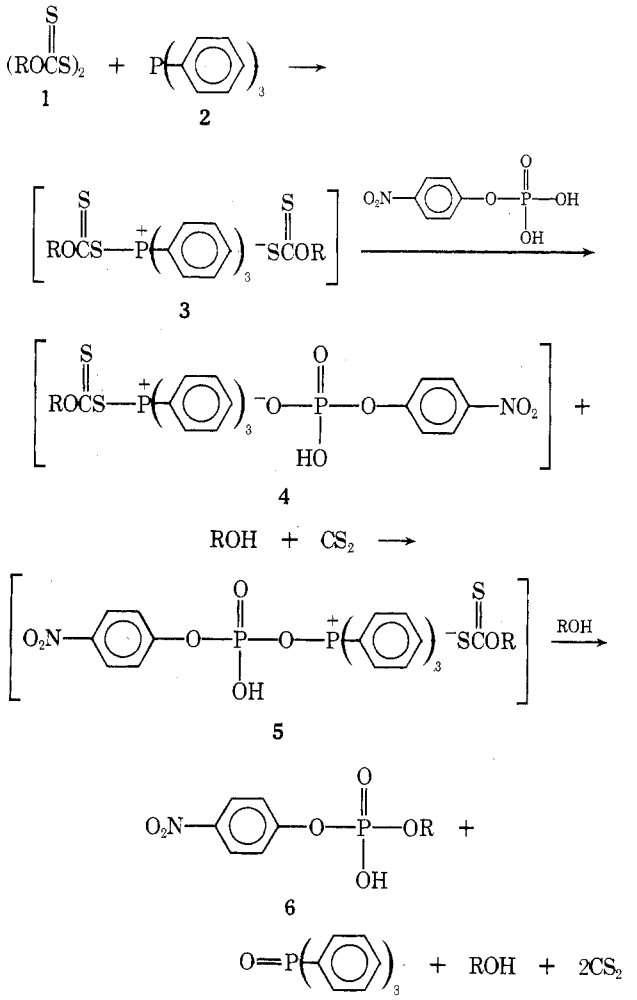
A number of phosphorylating systems have been devised with a view to preparing mixed diesters of phosphoric acid by intermolecular dehydration between monophosphates and alcohols. In order to bring the phosphate into reaction with an alcohol most systems employed initial activation of monophosphates by coupling reagents such as dicyclohexylcarbodiimide,[1] trichloroacetonitrile,[2] or 2,2′-dipyridyl disulfide and triphenylphosphine.[3] In these cases, mixed diesters of phosphoric acid, monophosphate, and pyrophosphate were formed so that the isolation of the expected mixed diesters of phosphoric acid became more difficult.



Recently, it has been demonstrated in this laboratory that phosphorylation of alcohols, phosphates, and nucleosides by the use of 8-quinolyl phosphates gave the corresponding mixed diesters of phosphoric acid, pyrophosphates, and nucleotides in good yields.[4]

The present paper describes a new method for the preparation of mixed diesters of phosphoric acid from monophosphate by the use of bis(*O*-thiocarbonyl) disulfides (**1**) and triphenylphosphine (**2**). The reaction seems to proceed through a phosphonium salt (**3**) which in turn reacts with monophosphate to form an intermediate (**4**), alcohol, and carbon disulfide. The intermediate (**4**) is further converted to phosphoryloxyphosphonium salt (**5**) by intramolecular oxidation–reduction reaction. The intermediate (**5**) reacts with alcohol to give mixed diester of phosphoric acid and triphenylphosphine oxide.

It is known that xanthic acid decomposes under acidic condition to give the corresponding alcohol and carbon disulfide.[5]

When *p*-nitrophenyl phosphate was treated with **1** and **2** at room temperature for 2 h, mixed diesters of phosphoric acid (**6**) were obtained in high yields without the formation of by-product such as symmetrical P^1,P^2-bis(*p*-nitrophenyl) pyrophosphate.

When *p*-nitrophenyl phosphate was treated with 5 equiv each of bis(*n*-butyl) dithiobis(thioformate) (**1c**) and **2** in dry tetrahydrofuran, *n*-butyl *p*-nitrophenyl phosphate (**6c**) was obtained in 85% yield.