(3) R. D. G. Cooper, P. V. DeMarco, and D. O. Spry, J. Amer. Chem. Soc., 91, 1528 (1969). According to a personal communication of R. D. G. Cooper the chemical shifts of the methyl groups of compound 4 in $CDCl_3$ and of compound 6 in C_6D_6 should be inverted in Table II of this publication. After correction of Δ_4 and Δ_6 for these groups, one obtains $\Delta_5 - \Delta_4$ for 2β-Me −0.07 and for 2α-Me +0.21 and $\Delta_6 - \Delta_4$ for 2β-Me +0.29 and for 2α-Me −0.04. The values for the methyl groups in Table III also should be corrected.
(4) G. Barbieri, M. Cinquini, S. Colonna, and F. Montanari, J. Chem. Soc. C, 659 (1968).
(5) D. H. R. Barton, D. G. T. Greig, G. Lucente, S. G. Sammes, M. V. Taylor, C. M. Cooper, C. Hewitt, and W. G. E. Underwood, J. Chem. Soc. C, 3540 (1971).
(6) D. O. Spry, J. Org. Chem., 37, 793 (1972).
(7) D. O. Spry, J. Amer. Chem. Soc., 92, 5006 (1970).
(8) R. D. G. Cooper, P. V. DeMarco, J. C. Cheng, and N. D. Jones, J. Amer. Chem. Soc., 91, 1408 (1969).
(9) R. A. Archer and P. V. DeMarco, J. Amer. Chem. Soc., 91, 1530 (1969).
(10) E. H. Flynn, Ed., "Cephalosporins and Penicillins: Chemistry and Biology," Academic Press, New York, N. Y., 1972, p 342.
(11) D. H. R. Barton, F. Comer, and P. G. Sammes, J. Amer. Chem. Soc., 91, 1529 (1969), and references cited therein.
(12) R. B. Morin, B. G. Jackson, R. A. Mueller, E. R. Lavagnino, W. B. Scanlon, and S. L. Andrews, J. Amer. Chem. Soc., 91, 1401 (1969).
(13) R. D. G. Cooper, J. Amer. Chem. Soc., 92, 5010 (1970).
(14) P. Yates and B. L. Shapiro, J. Org. Chem., 23, 759 (1958).

o-Nitrophenyl Esters of Benzyloxycarbonylamino Acids and Their Application in the Synthesis of Peptide Chains by the *in Situ* Technique[1]

Miklos Bodanszky,* Michio Kondo,[2] Cynthia Yang Lin, and Gerald F. Sigler

Department of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106

Received September 4, 1973

Synthesis of *S*-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (*S,S'*-dibenzyloxytoceine) is described. The partially protected tetrapeptide derivative, *S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide, was acylated with the *o*-nitrophenyl ester of benzyloxycarbonyl-L-asparagine and the chain was lengthened in the same manner, with *o*-nitrophenyl esters of benzyloxycarbonylamino acids, until the fully protected nonapeptide was secured. The latter was partially deprotected with HBr in acetic acid and *S,S'*-dibenzyloxytoceine was obtained as the hydrobromide. All of the operations were carried out in the same vessel from which the intermediates were not removed throughout the synthesis. The preparation and properties of *o*-nitrophenyl esters of benzyloxycarbonylamino acids are also reported.

In a recent publication[3] from this laboratory, the preparation of *o*-nitrophenyl esters of *tert*-butyloxycarbonylamino acids and their application in the synthesis of a protected nonapeptide, corresponding to the C-terminal sequence of a secretin analog, was described. An additional characteristic feature of the synthesis was that the intermediates remained in the same vessel throughout the chain lengthening steps. The expression "*in situ* peptide synthesis" was proposed for the new technique. This report deals with an extension of the new approach to benzyloxycarbonylamino acid *o*-nitrophenyl esters and includes also the preparation and properties (Table I) of a number of such active esters. The intriguing questions, why *o*-nitrophenyl esters are more reactive[4] than their para isomers, why their reaction rates in aminolysis are less solvent dependent,[5] and why these rates remain less effected by steric hindrance, such as encountered in solid phase peptide synthesis,[6] are the subject of a separate study.[7]

The preparation of *o*-nitrophenyl esters of benzyloxycarbonylamino acids followed the procedure used for the para isomers.[8,9] However, in the case of hindered amino acids such as isoleucine or valine, pyridine rather than ethyl acetate had to be applied as solvent. Otherwise, the relatively poor reactivity of *o*-nitrophenol resulted in the formation of appreciable amounts of *N*-acyldicyclohexylureas and it was necessary to purify the products by chromatography. In pyridine, the nucleophilic character of *o*-nitrophenol is more pronounced; the esterification proceeds at a faster rate as shown by the disappearance of the 4.8-μ band of DCC in the ir spectra. Details of the preparation of the active esters are described in the Experimental Section, their properties in Table I.

For the examination of the usefulness of the new group of active esters for chain lengthening, especially by the *in situ* technique, the partially protected nonapeptide *S*-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (*S,S'*-dibenzyloxytoceine)[10] was selected. This choice was dictated in part by the familiarity of one of the authors (M. B.) with the synthesis of this peptide, including the properties of the intermediates. The *in situ* approach[3] requires a suitable solvent for the acylation reaction and a precipitant for the selective separation of the intermediate acyl peptides from the by-products, such as salts of triethylamine or diisopropylamine,[11] nitrophenol, and also from the excess[12] of the acylating reagent, the active ester. For some peptide derivatives it may be difficult to find a proper solvent–precipitant combination, and information on the solubility properties of the expected products is indeed desirable. In the present synthesis of *S,S'*-dibenzyloxytoceine, the protected tetrapeptide derivative *N*-benzyloxycarbonyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide[13] was used as starting material, because the shorter chain intermediates are too soluble in common organic solvents that can keep the by-product, etc., in solution. The benzyloxycarbonyl group was removed with HBr in acetic acid, the hydrobromide precipitated and washed with ether, and the resulting amine allowed to react with the *o*-nitrophenyl ester of the next residue, benzyloxycarbonyl-L-asparagine, in dimethylformamide in the presence of tertiary base. The protected pentapeptide was isolated by dilution of the reaction mixture with 95% ethanol. The same combination, dimethylformamide as solvent and 95% ethanol as precipitant, was used through the rest of the synthetic procedure. The reactions were carried out in a centrifuge tube, *from which the intermediates were not removed.* The yields in this facile technique were found to be comparable with those of the conventional approach in which the intermediates were transferred from the reaction vessel to a filter, etc. These intermediates (Table II) were obtained mostly in satisfactory purity; yet, when necessary, they could be purified by recrystallization, still *in situ*, in the same centrifuge tube.

Because of some obvious limitations, such as the availability of a suitable solvent–precipitant combination or

Table I
o-Nitrophenyl Esters of Benzyloxycarbonylamino Acids

Registry no.	Benzyloxycarbonyl-amino acid o-nitrophenyl ester	Mp, °C	$[\alpha]^{25}D$ (c 2, DMF containing 1% AcOH)	Tlc R_f(A)	Tlc R_f(B)	Formula	Mol wt	Elemental analyses, % Calcd C	Calcd H	Calcd N	Found C	Found H	Found N
40777-53-5	Ala	94	−51	0.72	0.60	$C_{17}H_{16}N_2O_6$	344.3	59.3	4.7	8.1	59.0	4.7	8.0
49844-60-2	Asp(Bzl)	74–75	−32	0.73	0.62	$C_{25}H_{22}N_2O_8$	478.5	62.8	4.6	5.8	62.7	4.7	5.8
41446-00-8	Asn	162	−42	0.62	0.36	$C_{18}H_{17}N_3O_7$	387.4	55.8	4.4	10.8	55.9	4.5	10.6
23180-02-1	Cys(Bzl)	98–99	−105	0.70	0.56	$C_{24}H_{22}N_2O_6S$[a]	466.5	61.8	4.7	6.0	61.8	4.7	6.2
49689-66-9	Glu(Bzl)	83–84	−43	0.71	0.62	$C_{26}H_{24}N_2O_8$	492.5	63.4	4.9	5.7	63.2	4.8	5.6
49689-67-0	Gln	135–136	−39	0.66	0.40	$C_{19}H_{19}N_3O_7$	401.4	56.9	4.8	10.5	56.6	4.8	10.6
6154-41-2	Gly	75		0.71	0.62	$C_{16}H_{14}N_2O_6$	330.3	58.2	4.3	8.5	57.9	4.4	8.5
49689-69-2	Ile	Oil		0.84	0.88	$C_{20}H_{22}N_2O_6$	386.4	62.2	5.7	7.3		[b]	
49689-70-5	Leu	87–89	−47	0.83	0.90	$C_{20}H_{22}N_2O_6$	386.4	62.2	5.7	7.3	62.3	5.7	7.5
49689-71-6	Lys(Boc)	76–78	−32	0.75	0.65	$C_{25}H_{31}N_3O_8$	501.5	59.9	6.2	8.4	59.8	6.2	8.3
49689-72-7	Met	65–67	−56	0.77	0.89	$C_{19}H_{20}N_2O_6S$[c]	404.4	56.4	5.0	6.9	56.4	4.9	6.9
49689-73-8	Phe	109–110	−63	0.80	0.67	$C_{23}H_{20}N_2O_6$	420.4	65.7	4.8	6.7	65.9	4.9	6.8
49689-74-9	Pro	Oil		0.77	0.83	$C_{19}H_{18}N_2O_6$	370.4	61.6	4.9	7.6		[b]	
49689-75-0	Ser(Bzl)	55–57	−18	0.82	0.90	$C_{24}H_{22}N_2O_7$	450.5	64.0	4.9	6.2	64.2	5.1	6.2
49689-76-1	Trp	116–118	−65	0.70	0.57	$C_{25}H_{21}N_3O_6$	459.5	65.4	4.6	9.2	65.6	4.7	9.2
49689-77-2	Tyr(Bzl)	137	−55	0.83	0.87	$C_{30}H_{26}N_2O_7$	526.6	68.4	5.0	5.3	68.5	5.2	5.3
49689-78-3	Val	Oil		0.75	0.85	$C_{19}H_{20}N_2O_6$	372.4	61.3	5.4	7.5		[b]	

[a] S: Calcd 6.7, found 6.6. [b] No satisfactory analyses were obtained; the oily products were characterized by their uv, ir, and nmr spectra. [c] S: Calcd 7.9, found 7.7.

Table II
Properties of the Protected Intermediates of the *in Situ* Synthesis of *S,S'*-Dibenzyloxytoceine

Intermediate	Registry no.	Yield,[a] %	Tlc R_f(A)	Mp,[a] °C
Z-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2	14485-83-7	82[b] (79)	0.58	211–213[b] (211–212)
Z-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2	21688-11-9	95 (80)[c]	0.43	202–203 (210–212)[c,d]
Z-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2	14485-85-9	79 (80)	0.52	229–232 (228–230)
Z-Tyr(Bzl)-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2	39005-18-0	96 (92)	0.65	237–239 (240–242)
Z-Cys(Bzl)-Tyr-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2	3274-73-5	92 (92)	0.68	239–242 (239–242)

[a] In parentheses, yields and melting points of the intermediates prepared in a control experiment by conventional technique are reported. [b] After recrystallization from 90% EtOH. [c] After recrystallization from 80% EtOH. [d] This protected hexapaptide amide might be dimorphic. Previously reported melting points: 209° [R. A. Boissonnas, S. Guttmann, P. A. Jaqenoud, and J. P. Waller, *Helv. Chim. Acta*, **38**, 1491 (1955)]; 209–210° [J. Rudinger, J. Honzl, and M. Zaoral, *Collect. Czech. Chem. Commun.*, **21**, 202 (1956)]; and 247–248° [M. Bodanszky and V. du Vigneaud, *J. Amer. Chem. Soc.*, **81**, 5688 (1959)].

the need for different equipment for different size batches, we cannot propose that the *in situ* technique should be used as the exclusive approach for peptide synthesis in solution. Moreover, it is our general view that each individual objective requires careful considerations before a decision on the approach is made. Nevertheless, the experience reported here suggests that o-nitrophenyl esters of protected amino acids are useful tools and the *in situ* technique a convenient approach in peptide synthesis.

Experimental Section

Capillary melting points are reported uncorrected. On thin-layer chromatograms, the protected peptides were revealed by *tert*-butyl hypochlorite–KI reagents.[14,15] Active esters were detected by their uv absorption and through exposure to ammonia. The following solvent systems were applied for development: A, *n*-BuOH–AcOH–H_2O (4:1:1); B, $CHCl_3$–MeOH (9:1); C, EtOAc–pyridine–AcOH–H_2O (60:20:6:11) [in paper chromatography: *n*-BuOH–pyridine–AcOH–H_2O (30:24:6:20)].[16] Reagent grade commercial solvents were used without purification, but DMF was dried over a molecular sieve (Linde 4A 4AXH). For amino acid analysis, samples were hydrolyzed with constant boiling 6 *N* HCl in evacuated, sealed ampoules at 110° for 16 hr and analyzed by the Spackman–Stein–Moore method.[17]

Benzyloxycarbonylglycine o-Nitrophenyl Ester. Benzyloxycarbonylglycine (4.2 g, 20 mmol) and o-nitrophenol (5.56 g, 40 mmol) were dissolved in pyridine (100 ml). The solution was cooled to 5° in an ice bath and DCC (3.7 g, 18 mmol) was added to the stirred solution with an additional 35 ml of pyridine. The solution was kept at 5° for 0.5 hr and at room temperature for 1.0 hr. The pyridine was evaporated *in vacuo* and the residue was treated with ether (100 ml). The precipitate was filtered and washed with ether (100 ml). The combined filtrate and washings were evaporated and the residue was taken up in chloroform (250 ml). The chloroform solution was extracted successively with 0.1 *N* HCl (100 ml), H_2O (100 ml), and 0.1 *N* NaOH (6 × 50 ml). The organic layer was washed with water (2 × 100 ml), dried over $MgSO_4$, and filtered, and the solvent was evaporated *in vacuo*. Trituration of the crude oil with hexane precipitated a solid which was collected on a filter and washed with hexane. The air-dried material (5.44 g) was recrystallized from warm 95% ethanol (20 ml). Colorless prisms were collected, washed with cold ethanol, and dried *in vacuo* to give 3.77 g (mp 75°). Concentration of the mother liquors gave a second crop (0.84 g) with the same melting point; total yield, 74%.

Benzyloxycarbonyl-L-alanine o-nitrophenyl ester was prepared according to the method described for Z-Gly-ONO. The crude solid product was recyrstallized from 95% ethanol to give 4.4 g of colorless needles, mp 93–94°. A trace of DCU was detected on tlc (system B) and this impurity was removed by dissolving the material in a small amount of ether, filtering, and evaporating to dryness. The final product (4.2 g, 64%) melted at 94°.

Benzyloxycarbonyl-L-valine o-nitrophenyl ester was prepared according to the procedure described for Z-Gly-ONO. The product so far failed to crystallize even after chromatography on a column of silica gel with $CHCl_3$ as eluent, but it was homogeneous

on tlc. This active ester was characterized by its uv (λ_{max}^{alc} 257 nm), ir (active ester CO 5.65 μ), and nmr (the expected resonances) spectra.

Benzyloxycarbonyl-L-leucine *o*-Nitrophenyl Ester. The method used for Z-Gly-ONO was followed. The crystalline residue left after evaporation of the chloroform solution was purified by recrystallization from 95% ethanol. The purified material (74%) melted at 87–89°.

Benzyloxycarbonyl-L-isoleucine *o*-nitrophenyl ester was obtained as an oil. (The procedure described for Z-Gly-ONO was followed.) After chromatography on a column of silica gel in $CHCl_3$, the product was homogeneous on tlc (λ_{max}^{alc} 257 nm, active ester CO at 5.66 μ) and gave the expected nmr spectrum.

Benzyloxycarbonyl-L-aspartic Acid α-*o*-Nitrophenyl Ester, β-Benzyl Ester. Benzyloxycarbonyl-L-aspartic acid β-benzyl ester (3.86 g, 10.8 mmol) and *o*-nitrophenol (2.78 g, 20 mmol) were dissolved in pyridine (50 ml); the solution was cooled in an ice bath to 5°. DDC (2.06 g, 10 mmol) was added to the stirred solution. The reaction was allowed to proceed at 5° for 1.5 hr, at which time the ir spectrum showed disappearance of the carbodiimide band at 4.8 μ. The reaction mixture was concentrated to *ca.* 25 ml *in vacuo*. The *N,N'*-dicyclohexylurea (DCU) was collected on a filter and washed with pyridine (20 ml). The filtrate and washings were combined and evaporated *in vacuo*. Ether (150 ml) was added to the solid residue, and additional DCU was removed. The filtrate was evaporated *in vacuo*, the residue dissolved in chloroform (100 ml), and the solution extracted with 5% citric acid (100 ml), water (100 ml), 0.1 *N* NaOH (2 × 100 ml), and water (100 ml). The organic layer was dried over $MgSO_4$, filtered, and evaporated *in vacuo*. The crude oil which solidified on cooling in an ice bath was dissolved in hot 95% ethanol containing 1% acetic acid (35 ml). Upon cooling, crystals separated. They were collected on a filter, washed with cold ethanol, and dried *in vacuo*, 3.36 g (70%), mp 74–75°.

Benzyloxycarbonyl-L-glutamic acid α-*o*-nitrophenyl ester γ-benzyl ester was prepared following the procedure described above for the corresponding derivative of aspartic acid. The product was crystallized from 95% ethanol containing 1% acetic acid. The purified material was obtained in 60% yield, mp 83–84°.

Benzyloxycarbonyl-L-asparagine *o*-nitrophenyl ester was prepared on a 5-mmol scale, according to the procedure described in ref 9. The crude product was recrystallized from hot ethyl acetate; the purified material (0.87 g, 47%) melted at 162°.

Benzyloxycarbonyl-L-glutamine *o*-nitrophenyl ester was prepared from 20 mmol of Z-Gln as described for the corresponding *p*-nitrophenyl ester.[9] The purified active ester (1.9 g, 24%) melts at 138–139°. For analysis, a sample was recrystallized from hot ethyl acetate. This product had a somewhat lower melting point (135–136°).

N^{ϵ}-*tert*-Butyloxycarbonyl-N^{α}-benzyloxycarbonyl-L-lysine *o*-nitrophenyl ester was prepared from 45 mmol of the protected amino acid with 90 mmol of *o*-nitrophenol and 40 mmol of DCC as described for the active ester of Z-Gly. The crude product solidified under hexane; it was washed with hexane, dried in air (15.8 g), and then recrystallized from hot 95% ethanol containing 1% acetic acid. The purified active ester was collected in two crops which totaled 9.9 g (44%), mp 76–78°.

***N*-Benzyloxycarbonyl-*S*-benzyl-L-cysteine *o*-nitrophenyl ester** was obtained following the procedure used for Z-Gly-ONO. The crude product solidified on evaporation of the chloroform solution. It was recrystallized from hot 95% ethanol containing 1% acetic acid, from which, on cooling, it separated into colorless needles. From a 6-mmol experiment, 1.9 g (77%) pure active ester was secured, mp 98–100° (lit.[18] 101–102°).

***N*-Benzyloxycarbonyl-*O*-benzyl-L-serine *o*-nitrophenyl ester** was prepared in pyridine solution as described for Z-Gly-ONO, but about a day was necessary for the completion of the reaction (disappearance of the 4.8-μ band in the ir spectrum). The product was crystallized from ether–hexane, 61%, mp 55–57°.

Benzyloxycarbonyl-L-methionine *o*-nitrophenyl ester was prepared by the standard procedure described for Z-Gly-ONO. The crude material was crystallized under hexane, but was still not homogeneous on tlc. For final purification, it was chromatographed on a silica gel column (2.5 × 42 cm) with $CHCl_3$ as eluent. The purified material (3.77 g, 78%) melted at 65–67°.

Benzyloxycarbonyl-L-proline *o*-nitrophenyl ester was obtained by following the method described above, including chromatography on silica gel, but no crystals could be secured: λ_{max}^{alc} 257 nm, active ester CO 5.65 μ, nmr as expected.

Benzyloxycarbonyl-L-phenylalanine *o*-Nitrophenyl Ester. Ethyl acetate was used as a solvent (*cf.* ref 8) in this preparation. The purified product (57%) was obtained by crystallization from 95% ethanol containing 1% acetic acid, mp 109–110°.

Benzyloxycarbonyl-L-tryptophan *o*-Nitrophenyl Ester. Benzyloxycarbonyl-L-tryptophan (3.26 g, 10 mmol) and *o*-nitrophenol (1.67 g, 12 mmol) were dissolved in ethyl acetate (50 ml) and the solution was cooled to 5° in an ice bath. DCC (2.26 g, 11 mmol) was added to the stirred solution. The reaction was allowed to proceed 0.5 hr at 5° and an additional 5.5 hr at room temperature. Acetic acid (0.2 ml) was added and, after 15 min of stirring, the mixture was filtered. The precipitate was washed with ethyl acetate (20 ml), the filtrate and washings were evaporated *in vacuo*, and the residue redissolved in ether (100 ml). The ether solution was filtered and evaporated to give a crude oil which was dissolved in warm 95% ethanol containing 1% acetic acid (35 ml). Upon cooling, orange crystals formed. In two crops, a total of 2.83 g (59%) of active ester was secured, mp 116–118°.

***N*-Benzyloxycarbonyl-*O*-benzyl-L-tyrosine *o*-Nitrophenyl Ester.** To a solution of Z-L-Tyr(Bzl)-OH (2.55 g, 6.2 mmol) in pyridine (6 ml), *o*-nitrophenol (1.68 g, 12 mmol) was added at 0° followed by DCC (1.24 g, 6 mmol). After 30 min, the reaction mixture was allowed to come to room temperature and was held there for 4.5 hr. Ethyl acetate (6 ml) was added to the mixture, the DCU which separated was filtered off and washed with ethyl acetate (5 ml). The combined filtrates and washings were evaporated to dryness, the crystalline residue dissolved in hot 95% ethanol (15 ml) containing 1% AcOH, and the solution left at room temperature overnight. The crystalline product was collected, washed with ethanol (5 ml), and dried over P_2O_5, 2.81 g (89%), mp 136–137°.

***S,S'*-Dibenzyloxytoceine (Hydrobromide).** A sample of the protected tetrapeptide derivative *N*-benzyloxycarbonyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide[13] (612 mg, 1 mmol) was dissolved in AcOH (1.5 ml) and treated with 4 *N* HBr in AcOH (1.5 ml) at room temperature for 1 hr. Dry ether (30 ml) was added and the tetrapeptide amide hydrobromide separated by centrifugation.[19] The salt was washed with ether (3 × 30 ml), dried *in vacuo* over NaOH for about 30 min, and dissolved in DMF (4 ml). Triethylamine was added to the solution until it was neutral (0.16 ml), and then an additional amount (0.14 ml) was added for the liberation of the free amine from its salt. After the addition of Z-L-Asn-ONO (*cf.* above, 580 mg, 1.5 mmol), the slight alkalinity[20] of the mixture was maintained with small amounts of triethylamine (a total of 0.14 ml was used for this purpose). After 1 day, a spot from the solution on filter paper gave no color with ninhydrin. Most of the solvent was removed *in vacuo* at 30° bath temperature, the residue taken up in 95% ethanol (20 ml) and the resulting suspension centrifuged. The protected pentapeptide amide was washed with 95% ethanol, ethyl acetate, and ether (30 ml each), dried *in vacuo*, and weighed. Small samples were used for tlc and for the determination of the mp (*cf.* Table II). The pentapeptide derivative was recrystallized from hot 90% ethanol (20 ml). The purified product was deprotected *in situ* with HBr in AcOH as described above and the chain was lengthened in the same manner until the nonapeptide derivative was secured. The *o*-nitrophenyl esters of Z-L-Gln, Z-L-Ile, Z-L-Tyr(Bzl), and Z-L-Cys(Bzl) were used for acylation, all in 50% excess.[12] In the final coupling step, during acylation with Z-L-Cys(Bzl)-ONO, diisopropylethylamine[11] rather than triethylamine was used as the acid-binding tertiary base. The yields on the individual protected intermediates, their melting point and behavior in tlc are summarized in Table II, which also contains the corresponding data on the same intermediates, prepared by the same series of steps but with conventional operations such as filtration, etc.

The major part (615 mg, 0.49 mmol) of the fully protected nonapeptide derivative, *N*-benzyloxycarbonyl-*S*-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide, was treated with HBr in AcOH as described above. The ether-washed and dried hydrobromide (750 mg)[21] suspended in 95% ethanol (3 ml) was treated with triethylamine (0.1 ml) and the solid separated by centrifugation. It was washed with 95% ethanol (3 × 2 ml) and with ether (3 ml) and dried *in vacuo* (526 mg), mp 214–216°. An aliquot (504 mg) of this material was dissolved in the upper phase (20 ml) of the solvent system *n*-BuOH–pyridine–AcOH–H_2O (4:2:1:7)[22] from which the peptide gradually separated. After 2 days at room temperature, the purified material was collected by centrifugation, washed with 95% ethanol, ethyl acetate, and ether (3 ml each), and dried *in vacuo* at room temperature. The purified product (316 mg), mp 237–239° (lit.[23] 220–225°), gave a single spot on tlc, R_f(A) 0.42, R_f(C) 0.23, and on paper chromatogram, R_f 0.91;

$[\alpha]^{25}D$ − 54° (c 2.3, 80% AcOH), $[\alpha]^{25}D$ − 34° (c 1.1, DMF). From the mother liquor, a second crop (73 mg, mp 231–234°) was obtained. Amino acid analysis: Asp, 1.05; Glu, 1.0; Pro, 0.95; Gly, 1.1; Ile, 1.0; Leu, 1.0; Tyr, 0.90; Cys(Bzl), 1.9.

Anal. Calcd for $C_{57}H_{81}N_{12}O_{12}S_2Br$: C, 53.9; H, 6.4; N, 13.2; S, 5.1; Br, 6.3. Found: C, 54.0; H, 6.5; N, 13.1; S, 5.2; Br, 6.6.

Registry No. *o*-Nitrophenol, 88-75-5; benzyloxycarbonylglycine, 1138-80-3; benzyloxycarbonyl-L-alanine, 1142-20-7; benzyloxycarbonyl-L-valine, 1149-26-4; benzyloxycarbonyl-L-leucine, 2018-66-8; benzyloxycarbonyl-L-isoleucine, 3160-59-6; benzyloxycarbonyl-L-aspartic acid β-benzyl ester, 3479-47-8; benzyloxycarbonyl-L-glutamic acid γ-benzyl ester, 5680-86-4; benzyloxycarbonyl-L-asparagine, 35264-96-1; benzyloxycarbonyl-L-glutamine, 2650-64-8; N^{ϵ}-*tert*-butyloxycarbonyl-N^{α}-benzyloxycarbonyl-L-lysine, 2389-60-8; *N*-benzyloxycarbonyl-*S*-benzyl-L-cysteine, 3257-18-9; *N*-benzyloxycarbonyl-*O*-benzyl-L-serine, 20806-43-3; benzyloxycarbonyl-L-methionine, 1152-62-1; benzyloxycarbonyl-L-proline, 1148-11-4; benzyloxycarbonyl-L-phenylalanine, 1161-13-3; benzyloxycarbonyl-L-tryptophan, 7432-21-5; *N*-benzyloxycarbonyl-*O*-benzyl-L-tyrosine, 16677-29-5; *S,S'*-dibenzyloxytoceine (hydrobromide), 17772-77-9.

References and Footnotes

(1) This study was supported by a grant from the U. S. Public Health Service (NIH AM-12473).
(2) Overseas Research Scholar, sponsored by the Ministry of Education in Japan.
(3) M. Bodanszky, K. W. Funk, and M. L. Fink, *J. Org. Chem.*, **38**, 3565 (1973).
(4) M. Bodanszky and R. J. Bath, *Chem. Commun.*, 1259 (1969).
(5) M. Bodanszky, R. J. Bath, A. Chang, M. L. Fink, K. W. Funk, S. M. Greenwald, and Y. S. Klausner in "Chemistry and Biology of Peptides. Proceedings of the 3rd American Peptide Symposium," J. Meienhofer, Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1972, p 203.
(6) M. Bodanszky and K. W. Funk, *J. Org. Chem.*, **38**, 1296 (1973).
(7) M. Bodanszky, M. L. Fink, K. W. Funk, C. Yang Lin, M. Kondo, and A. Bodanszky, *J. Amer. Chem. Soc.*, in press.
(8) M. Bodanszky and V. du Vigneaud, *Biochem. Prep.*, **9**, 110 (1962).
(9) M. Bodanszky, G. S. Denning, and V. du Vigneaud, *Biochem. Prep.*, **10**, 122 (1963).
(10) This partially protected form of reduced oxytocin was prepared from natural oxytocin by S. Gordon and V. du Vigneaud [*Proc. Soc. Exp. Biol. Med.*, **84**, 723 (1953)]. The name *S,S'*-dibenzyloxytoceine was proposed by V. du Vigneaud, P. S. Fitt, M. Bodanszky, and M. O'Connel [*ibid.*, **104**, 653 (1960)].
(11) M. Bodanszky and A. Bodanszky, *Chem. Commun.*, 591 (1967).
(12) M. Bodanszky in "Prebiotic and Biochemical Evolution," A. P. Kimball and J. Oro, Ed., North-Holland Publishing Co., Amsterdam, 1971, p 217.
(13) Prepared as described by M. Bodanszky and V. du Vigneaud, *J. Amer. Chem. Soc.*, **81**, 2504 (1959).
(14) D. P. Schwartz and M. J. Pallansch, *Anal. Chem.*, **30**, 219 (1958).
(15) R. H. Mazur, B. W. Ellis, and B. Cammarata, *J. Biol. Chem.*, **237**, 1619 (1962).
(16) S. G. Waley and J. Watson, *Biochem. J.*, **57**, 529 (1954).
(17) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).
(18) M. Bodanszky, *Acta Chim. Hung.* **10**, 335 (1957).
(19) The deprotection step and all subsequent operations were carried out in a centrifuge tube prepared from a 28 mm o.d. glass tube ending in a 24/40 standard tapered glass joint. The vessel formed in this way (*ca.* 40 ml) was provided with a drying tube containing cotton during the deprotection and with a 24/40 ground glass stopper during coupling reactions. A simple bench instrument (International Clinical Centrifuge, Model CL) was used for the separation of solids from the supernatant solutions. For evaporation of solvents, the tube was attached to all glass rotary evaporators. The intermediates were dried and weighed in the same tube in which also the next deprotection was performed. When purification by recrystallization became necessary, this too was done in the same vessel, without removing the peptide from it.
(20) The alkalinity of the mixture was checked by holding a piece of moist universal-indicator paper close above the surface of the solution.
(21) The weight corresponds to a polyhydrobromide; *cf.* footnote 23 in M. Bodanszky and V. du Vigneaud, *J. Amer. Chem. Soc.*, **81**, 5688 (1959).
(22) In preliminary experiments, *S,S'*-dibenzyloxytoceine hydrobromide was distributed in this solvent system in which it migrated with a *K* value of 4.0. Subsequently, however, the peptide separated from the system too readily and countercurrent distribution became impractical.
(23) H. C. Beyerman and R. A. In't Veld, *Recl. Trav. Chim. Pays-Bas*, **88**, 1019 (1969); *cf.* also H. C. Beyerman, C. A. M. Boers-Boonekamp, and H. Maassen van den Brink-Zimmermannova, *ibid.*, **87**, 257 (1968).

A Novel Regioselective Protoberberine Synthesis by Thermolysis

Tetsuji Kametani,* Tamiko Takahashi, Toshio Honda, Kunio Ogasawara, and Keiichiro Fukumoto

Pharmaceutical Institute, Tohoku University, Aobayama, Sendai, Japan

Received April 10, 1973

Thermolytic intermolecular cycloaddition reaction of 3,4-dihydro-6,7-dimethoxyisoquinoline (**7**) with 1-cyanocyclobutenes (**5a** and **5b**) gave two stereoisomers **15**, **17** and **20**, **21**, respectively. Isomer **15** was found to be converted to the more stable trans isomer **17** by heating or keeping aside for a long time.

Recently we reported a novel synthesis of the protoberberine salts (**4**) through the intramolecular thermal rearrangement of the 1-(benzocyclobutenyl)-3,4-dihydroisoquinolines (**1**), in which *o*-quinodimethanes (**2**) and **3**[1] were postulated to be the intermediates (Chart I). It was thus of interest to examine the intermolecular condensation between the benzocyclobutenes (**5**) and the 3,4-dihydroisoquinoline (**7**),[2] the former of which would generate *o*-quinodimethane intermediates (**6**) thermally, thus leading to the formation of protoberberines (**8** and/or **9**) (Chart II).

Thermolysis of an equimolar amount of **5a** and **7** in bromobenzene at 150–160° gave two compounds in 80.4% total yield. Both products had the required composition for the protoberberines **8a** and **9a**.

Each of the two stereoisomers could be dehydrogenated with iodine in boiling ethanol[3] to give the same quaternary protoberberine iodide (**10**) which showed a deshielded one-proton singlet due to the C-8 hydrogen at 9.47 ppm, in addition to the signals for a pair of coupled methylene protons, four methoxyl groups, and four singlet aromatic protons in the nmr spectrum. Treatment of the quaternary protoberberine iodide (**10**) with sodium hydroxide provided a lactam (**11**) showing no C-8 proton in the nmr spectrum (Chart III). These results unequivocally allowed the assignment of a C-13 substituted structure **8a** to each of the isomers.

Interestingly, the first compound was transformed into the second one under the following conditions: (a) when heated at 150–160° for 15 min without solvent or kept aside at room temperature for 2 months and (b) when treated by filtration through silica gel eluted with methylene chloride. The reverse process could not be observed under the same conditions, or even under more severe conditions such as prolonged heating or heating at higher temperature. When the thermolysis was carried out without solvent, the second isomer was obtained in 88.0% yield as a major product accompanied by a small amount of the first isomer. These observations indicate that the compounds are stereoisomeric and that the former could be a kinetically formed product, while the latter could be the more stable thermodynamically formed product. It