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Peptide Synthesis Using *o*-Nitrophenylsulfenyl *N*-Carboxy α-Amino Acid Anhydrides

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A derivative of *N*-carboxy α-amino acid anhydrides (NCAs) in which amino proton is substituted by *o*-nitrophenylsulfenyl (Nps) group has been prepared almost quantitatively. The reaction of the Nps–NCAs with amino acid esters proceeds rapidly to give the Nps-dipeptide esters with full optical activity in high yields. The chain lengthening of peptides by the reaction of the Nps–NCAs was accomplished with good results. The stepwise peptide synthesis using the Nps–NCAs was successfully applied to the synthesis of a C-terminal hexapeptide amide of eledoisin sequence. The results show that the Nps–NCAs are most useful compounds for stepwise synthesis of peptides.

Successful synthesis of peptides using *N*-carboxy α-amino acid anhydrides (NCAs) has been reported by us[1-4] and other groups.[5-16] The NCA method for peptide synthesis has a great advantage of rapid acylation of an amino acid by the NCAs so that the reaction completes in a few minutes[6] to a few hours[1] to give a peptide in very high yield. Another advantage of the NCA method is that the reaction of NCAs with amino acids or peptides can be accomplished without protection of the functional group of the amino acids or the peptides. The NCA method, therefore, gives directly a free peptide from an amino acid. The latter advantage, however, is counterbalanced by the defect that in the NCA method, a small amount of by-product is sometimes formed[7] and is difficult to remove from the desired peptide. This difficulty may be overcome by choice of a strategy of peptide synthesis using an N-substituted NCA. The N-substituted NCA having as high a reactivity as the NCA may be used as an acylating component in conventional solution state peptide synthesis to give a pure N-protected peptide in very high yield, perhaps above 90%, which is obtained by the NCA method of peptide synthesis.[1,6] The by-product accompanied by the reaction of the N-substituted NCA, if present, may be easily removed by the conventional washings of the product with an acid and a base.[17]

Recently, Kricheldorf[18] found that *o*-nitrophenylsulfenyl (Nps) chloride reacts almost quantitatively with NCAs to give the N-substituted NCAs by the Nps group. We considered that the Nps–NCAs may be successfully used for our strategy of peptide synthesis. We have studied synthesis and reaction of the Nps–NCAs and found that the Nps–NCAs are most useful compound for stepwise synthesis of Nps-protected peptides.[19] This paper reports the preparation of new Nps–NCAs and the synthesis of peptides including a hexapeptide amide having the C-terminal sequence of eledoisin[20] by the Nps–NCA method.

Synthesis of Nps–NCAs. The synthesis of Nps–NCA of glycine and L-phenylalanine was reported by Kricheldorf.[18] New Nps–NCAs of other amino acids were prepared by the method similar to that reported by Kricheldorf. The method involves the fast reaction of Nps-Cl with the 3-amino proton of the NCA to give the Nps–NCA and hydrogen chloride, which is trapped with triethylamine (Scheme I). The reaction was carried out at 0° and tetrahydrofuran (THF) was used as a suitable solvent. Triethylamine must be carefully added to the system. Hasty addition of the reagent caused the undesired polymerization of the NCA through the activated NCA mechanism.[21] The formation of the polymeric by-product, which is insoluble in ethyl acetate, makes it difficult to purify the Nps–NCA by recrystallization. Though the use of acetonitrile as the solvent can suppress the polymerization,[22] it is not suitable for the reaction because the resulting Nps–NCA is poorly soluble in acetonitrile.

Scheme I

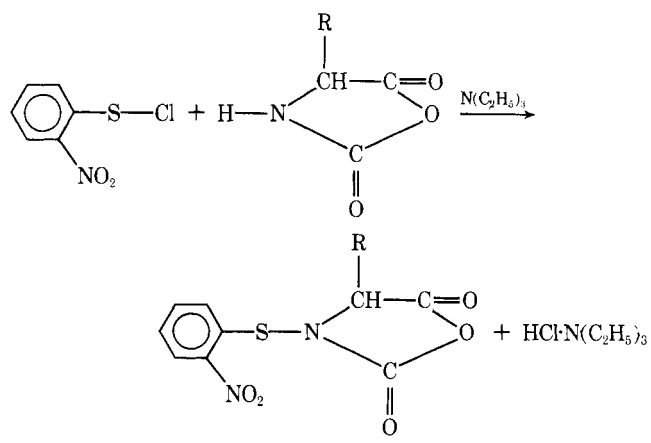


Table I
Results of Syntheses of Nps-NCAs

Registry no.	Nps-NCA[a]	Yield, %	Mp, °C	[α]D in THF, deg (c)	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N
40331-72-4	Gly	94	168–170[b]		48.66	2.72	12.61	48.72	2.66	12.65
52071-13-3	Ala	92	176–178	+22.7 (1.0)	50.85	3.41	11.86	50.69	3.49	11.90
52152-51-9	Val	94	150–152	+16.9 (1.0)	54.54	4.58	10.60	54.46	4.60	10.56
55903-68-9	Leu	82	97–99	+44.8 (1.0)	56.11	5.07	10.07	56.20	5.10	10.01
54745-13-0	Ile	92	101–103	+32.9 (1.0)	56.11	5.07	10.07	56.15	5.02	10.10
40331-74-6	Phe	92	151–153[c]	+31.2 (2.0)	61.54	3.87	8.97	61.60	3.90	8.92
54745-15-2	Lys (Z)	94	121–123	+23.5 (2.0)	59.01	4.95	9.83	59.05	4.88	9.85
54745-18-5	Glu (OMe)	93	113–115	+31.9 (2.0)	50.65	3.92	9.09	50.68	3.86	9.05
54743-96-3	Glu (OBzl)	94	Oil	+18.7 (2.5)			6.73			6.61
54743-91-8	Asp (OBzl)	94	137–138	+70.8 (2.0)	58.38	3.81	7.57	58.35	3.83	7.60

[a] All amino acid residues have the L configuration except for glycine. [b] 168–170°; see ref 17. [c] 151–153°; see ref 17.

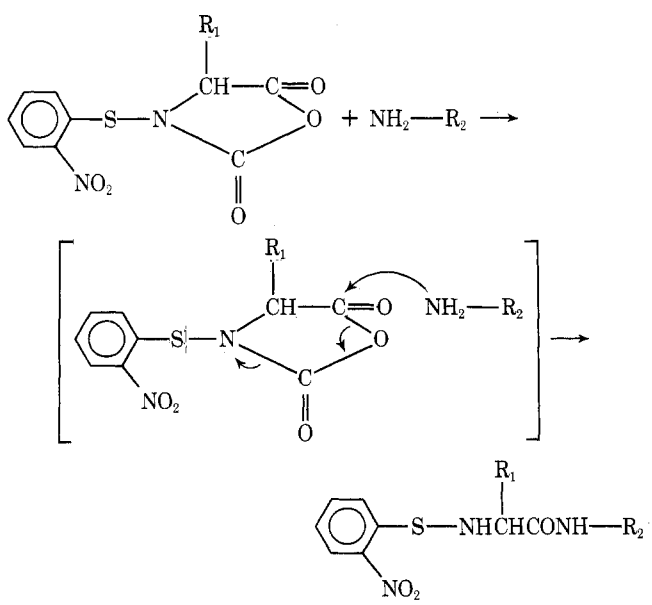
The product was obtained almost quantitatively and readily purified by recrystallization from ethyl acetate. The optical purity of the product was checked by acid hydrolysis and the product was found to have full optical purity.

The Nps–NCAs are conveniently characterized by infrared absorption bands at 1850–1860 and 1780–1790 cm^{-1} characteristic of the anhydride carbonyl strechings and 1600, 1570, and 1460 cm^{-1}, and some sharp bands near 750 cm^{-1} resulting from the substituted benzene ring. Kricheldorf[18] reported that theNps–NCAs have a broad infrared band near 3440–3540 cm^{-1}. We found no broad band near 3450 cm^{-1} in the infrared spectra of our samples of the Nps–NCAs. The contamination with water which comes from the KBr crystals gives the infrared absorption band. We consider that the band at 3440–3450 cm^{-1} reported by Kricheldorf may result from the presence of a small amount of water.

Results of syntheses of the Nps–NCAs are shown in Table I together with the physical properties and the elemental analysis. The synthesis of the Nps–NCA of other amino acids than those listed in Table I is now undertaken.

Synthesis of Peptides Using Nps–NCAs. The Nps–NCA is an intramolecularly activated amino acid derivative by the anhydride group and may be easily attacked at the C-5 carbonyl carbon by an amino compound to give the Nps-peptide with leaving carbon dioxide (Scheme II). We tried to synthesize Nps-dipeptide esters by the reaction of the Nps–NCAs with amino acid esters. The reaction was carried out by the same procedure as that of the conventional method of peptide synthesis, for 2 hr in tetrahydrofuran at room temperature. After the reaction, the solvent was removed at reduced pressure to give an oil, which was dissolved in ethyl acetate. Then the solution was treated by the same method as that of the conventional peptide synthesis, i.e., washings with aqueous solution of citric acid and of sodium bicarbonate and with water, isolation, and purification by recrystallization. The products, which were isolated in very high yields (above 85%), were identical with the authentic sample of Nps-dipeptide esters prepared by the conventional dicyclohexylcarbodiimide method. The reaction of the Nps–NCAs proceeded rapidly as expected from the rapid reaction of unsubstituted NCAs,[1,6] but Nps-L-valine NCA needed 3 hr for completing the acylating reaction. The slower reaction of the Nps–NCA may result from the steric hindrance of the methyl side chain branching at the β carbon of the amino acid residue.

Scheme II



Racemization during the peptide bond formation by the Nps–NCAs was studied by the method reported by Muraoka and coworkers.[23] The method involves separation of diastereomeric isomers of a glycyl tripeptide containing a racemic amino acid residue such as glycyl-DL-alanyl-L-valine or glycyl-DL-alanyl-L-leucine[24] by using an amino acid analyzer.[25] The method can detect racemization of 0.01%. We prepared glycyl-L-alanyl-L-leucine by a method including the synthesis of Nps-L-alanyl-L-leucine benzyl ester by the reaction of Nps-L-alanine NCA with L-leucine benzyl ester (Scheme III). If the racemization occurs in the reaction of the Nps–NCA, examination of the tripeptide by the amino acid analyzer detects the presence of the diastereomeric isomer glycyl-D-alanyl-L-leucine. The diastereomeric isomer, however, could not be detected in our peptide. This result suggests that the Nps–NCA method of peptide synthesis is free from racemization. The optical rotation of an isolated dipeptide derivative was also examined and the result supported the conclusion that the peptide synthesis was free from racemization.

Scheme III

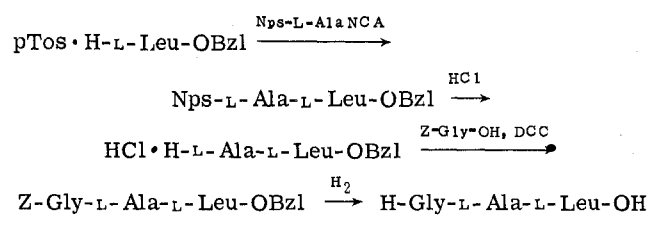


Table II
Results of Syntheses of Nps-Dipeptide Esters by the Nps-NCA Method

Registry no.	Dipeptide	Yield, %	Mp, °C	[α]D (c 1.0, THF)	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N
54743-90-7	Nps-Asp(OBzl)Leu-OBzl	98	112–113	−25.6	62.16	5.74	7.25	62.28	5.83	7.18
55871-22-2	Nps-Val-Val-OEt	84	85–86	−71.1	54.40	6.85	10.57	54.50	6.78	10.49
54743-92-9	Nps-Val-Ala-OBzl	88	134.5–135.5	−103.1	58.46	5.84	9.74	58.44	5.88	9.80
39741-15-6	Nps-Ile-Gly-OEt	91	121–122	−78.7	52.02	6.28	11.38	52.11	6.35	11.45
7754-66-7	Nps-Phe-Gly-OEt	86	122–123	−4.9	56.57	5.25	10.42	56.60	5.30	10.36
55871-23-3	Nps-Phe-Pro-OBzl	88	53–54	−30.8	64.15	5.38	8.31	64.08	5.48	8.35
55871-24-4	Nps-Leu-Met-OEt	86	93–94	−57.6	51.46	6.59	9.48	51.55	6.63	9.36
6234-24-8	Nps-Ala-Gly-OEt	89	101–102	−74.2	47.71	5.23	12.84	47.66	5.30	12.77
55871-25-5	Nps-Ala-Phe-OEt	94	116–117	+4.2	57.55	5.55	10.07	57.57	5.60	10.03
55871-26-6	Nps-Glu(OMe)Phe-OEt	90	90–91	+14.0	56.43	5.56	8.59	56.40	5.67	8.48

Table III
Results of Syntheses of Nps-Tripeptide and Nps-Tetrapeptide Esters by the Nps-NCA Method

Registry no.	Peptide	Yield, %	Mp, °C	[α]D, deg	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N
54743-93-0	Nps-Lys(Z)Asp(OBzl)Leu-OBzl	84	128–130	−16.4[a]	62.62	6.09	8.54	62.48	6.15	8.39
54743-94-1	Nps-Glu(OBzl)Val-Ala-OBzl	82	165–167	−11.0[a]	60.91	5.89	8.61	60.98	5.92	8.58
55871-27-7	Nps-Gly-Leu-Met-OEt	88	142–143	−7.0[b]	50.39	6.44	11.20	50.55	6.45	11.08
55871-28-8	Nps-Phe-Ile-Gly-OEt	94	167–169	−11.5[b]	58.13	6.24	10.85	58.20	6.32	10.88
55871-29-9	Nps-Val-Val-Val-OEt	80	180–181	−90.5[b]	55.63	7.31	11.28	55.58	7.45	11.18
54743-97-4	Nps-Val-Phe-Lys(Z)Ala-OBzl	89	190–192	−13.8[a]	62.84	6.23	9.99	62.75	6.30	9.85

[a] *c* 1.0, DMF. [b] *c* 1.0, THF.

Results of syntheses of the Nps-dipeptide esters are shown in Table II. The satisfactory result of the peptide synthesis results from the use of the Nps–NCAs, which can rapidly acylate the amino component. An advantage of the use of the Nps–NCAs is to suppress completely side reactions in the conventional NCA method of peptide synthesis. In the NCA method, occurrence of some side reactions of the NCAs has been elucidated.[7] One is "overreaction" resulting from premature decarboxylation of the product carbamate which is formed by the reaction of the NCA with an amino component. The other is oligomerization of the NCA via the NCA anion generated by N-proton abstraction in the anhydride. The *o*-nitrophenylsulfenyl protecting group of the Nps–NCAs prevents above both side reactions. Another feature with great advantage of the Nps–NCA method is the use of highly purified Nps–NCAs. In conventional peptide synthesis by the mixed anhydride method, which uses the same activated carboxyl component by the anhydride group as the Nps–NCA method, a mixed anhydride is prepared in situ between an acylated amino acid and an alkylchlorocarbonate and used without purification for the coupling reaction with the amino component. The coexistence of the by-product accompanied by the activation of the acylated amino acid decreases the yield of the coupling reaction. In contrast to the conventional mixed anhydride method, the new method using the Nps–NCAs can use generally the highly purified crystalline NCAs to increase the yield of the coupling reaction. If the reaction of the Nps–NCA with the amino component proceeds quantitatively, perhaps it is true that the by-product accompanied by the reaction is only carbon dioxide, which leaves as a gas from the reaction system. The feature makes it easy to purify the product and increases more the yield.

Among the NCAs, L-proline NCA has no N proton to be substituted by the Nps protecting group. Thus the synthesis of peptides containing proline residue cannot be accomplished by the Nps–NCA method and the introduction of Nps-proline residue to peptide chain must be done by conventional coupling with Nps-proline.[19]

The Nps–NCA method was developed for the stepwise synthesis of higher oligopeptides. The Nps protecting group of the dipeptide derivatives was easily removed[26] by action of hydrogen chloride in dioxane and the resulting dipeptide ester hydrochloride was allowed to react with the Nps–NCA. The Nps-tripeptide ester thus obtained was isolated and purified. Results of syntheses of Nps-tripeptide esters and an Nps-tetrapeptide ester are shown in Table III. The Nps-NCAs react as easily with dipeptides and a tripeptide esters as with amino acid esters to give the higher peptide esters in high yields. These results demonstrate that the Nps–NCA method can be successfully used for stepwise synthesis of Nps-peptides.

In order to further demonstrate the usefulness of the Nps–NCA method, a C-terminal hexapeptide amide of eledoisin, L-alanyl-L-phenylalanyl-L-isoleucylglycyl-L-leucyl-L-methionine amide, was synthesized by two approaches, a stepwise method and a fragment condensation method. In the stepwise approach, L-methionine ethyl ester hydrochloride was allowed to react with Nps-L-leucine NCA to give Nps-L-leucyl-L-methionine ethyl ester in 86% yield. The dipeptide ester was amidated by action of ammonia in methanol. The Nps protecting group of the dipeptide amide was removed by treating with hydrogen chloride to yield quantitatively the dipeptide amide hydrochloride, which was allowed to react with Nps-glycine NCA. The elongation of the peptide chain of the resulting tripeptide amide was carried out by the sequential reactions with Nps–NCAs of L-isoleucine and L-phenylalanine. The intermediate peptide amides were isolated in above 90% yield after purification. The removal of the Nps group was accomplished almost quantitatively. Nps-L-phenylalanyl-L-isoleucylglycyl-L-leucyl-L-methionine amide was obtained in 65.7% yield from the starting L-methionine ethyl ester. The pentapeptide amide was also prepared in 66% yield by

Table IV
Intermediates of Synthesis of Eledoisin Related Peptide

Registry no.	Intermediate	Yield, %	Mp, °C	[α]D, (c 1.0, DMF)	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N
55871-30-2	Nps-Gly-Leu-Met-NH_2	91	197–198	−8.4	48.40	6.20	14.86	48.51	6.28	14.80
55871-31-3	Nps-Ile-Gly-Leu-Met-NH_2	94	226–228	−63.0	51.36	6.90	14.38	51.28	6.85	14.40
55871-32-4	Nps-Phe-Ile-Gly-Leu-Met-NH_2	93	232–234	+21.3	55.79	6.75	13.40	55.70	6.82	13.48
55871-33-5	Nps-Ala-Phe-Ile-Gly-Leu-Met-NH_2	92	246–247	−22.3	55.34	8.02	13.96	55.38	8.10	14.05

the fragment condensation of Nps-L-phenylalanyl-L-isoleucylglycine with L-leucyl-L-methionine ethyl ester hydrochloride which had been prepared stepwise by the Nps–NCA method, followed by amidation. The pentapeptide amide obtained by the fragment condensation method was identical with that synthesized stepwise. After the Nps group of the pentapeptide amide was removed, the resulting peptide amide hydrochloride was treated with Nps-L-alanine NCA to give Nps-L-alanyl-L-phenylalanyl-L-isoleucylglycyl-L-leucyl-L-methionine amide in 92% yield. By stepwise synthesis, the yield of the hexapeptide amide of the C-terminal sequence of eledoisin was 60.3% calculated from L-methionine ethyl ester. Table IV shows the results of syntheses of the intermediate peptides.

The new method for peptide synthesis using the Nps–NCAs described here has some characteristic advantages which result from the use of the Nps–NCAs. The active anhydride derivative, Nps–NCA, of amino acids can be used in highly purified state for peptide synthesis. The rapid acylation of the amino component by the Nps–NCAs proceeds without formation of by-product to give the Nps-peptides in high yields. This rapid method may be used with great advantages for the stepwise synthesis of many other peptides.

Experimental Section

General Procedure for the Synthesis of Nps–NCAs. An NCA of amino acid (0.1 mol) was dissolved in 300 ml of tetrahydrofuran and the solution was cooled to 0° by an ice bath. Crystals of Nps-Cl, 19 g (0.1 mol), were added with stirring to the solution. Then 14 ml of triethylamine was slowly dropped with vigorous stirring into the solution. After the addition of triethylamine, the system was stirred at 0° for 15 min. The resulting crystals of triethylamine hydrochloride were removed by filtration. The filtrate was concentrated under reduced pressure at 35°. The residual oil was crystallized by adding *n*-hexane. The crystals of Nps–NCA were dissolved in a small amount of ethyl acetate and undissolved material was removed by filtration. Addition of *n*-hexane to the solution and cooling in a refrigerator gave crystalline pale yellow product. Recrystallization of the product from ethyl acetate gave a pure Nps–NCA. The product was collected by filtration and dried over P_2O_5.

Nps-L-Leucine NCA could not be crystallized from ethyl acetate. Then the Nps–NCA was dissolved in diethyl ether. The solution was gradually diluted with diisopropyl ether and *n*-hexane (1:1) until the system became cloudy. The system was cooled at −20° in a refrigerator for 2 days. The resulting crystals were collected and dried. Nps-γ-benzyl L-glutamate NCA failed to crystallize from any solvents.

Acid Hydrolysis of Nps-L-Valine NCA. Nps-L-valine NCA (0.29623 g, 1 mmol) was hydrolyzed by dissolving in 20 ml of methanol containing 1 ml of concentrated hydrochloric acid. The solution gave an optical rotation αD of 0.229°. An authentic sample of Nps-L-valine (0.27024 g, 1 mmol) was analogously hydrolyzed and the solution gave an optical rotation of 0.232°. These results are identical within the experimental errors.

General Procedure for the Synthesis of Peptide Using Nps–NCAs. An amino acid ester hydrochloride or *p*-toluenesulfonate (0.1 mol) was dissolved in 200 ml of tetrahydrofuran or acetonitrile. Triethylamine (14 ml, 0.1 mol) was added to the solution. The resulting salt was removed by filtration. To the solution, an Nps–NCA (0.105 mol) was added and allowed to react with stirring at room temperature. After 2 hr, the solvent was evaporated under reduced pressure at 35°. The residual oil was dissolved in 400 ml of ethyl acetate and the solution was washed with 5% citric acid, 5% sodium bicarbonate, and water and dried over sodium sulfate. The solution was concentrated in vacuo at 40° to give an oil, which was crystallized by adding *n*-hexane. The product was recrystallized from ethyl acetate.

Check of Racemization in the Nps–NCA Method by Muraoka Method. L-Leucine benzyl ester *p*-toluenesulfonate (1.42 g, 3.6 mmol) was dissolved in 30 ml of tetrahydrofuran and 0.51 ml (3.64 mmol) of triethylamine was added. Nps-L-alanine NCA (1.07 g, 3.6 mmol) was added to the reaction system and allowed to react for 2 hr at room temperature. After the reaction, the system was filtered and the filtrate was concentrated under reduced pressure to give an oil of Nps-L-alanyl-L-leucine benzyl ester. The product was dissolved in 10 ml of 1 *N* hydrochloric acid in methanol. The solution was concentrated at 30°. To the residual oil, 200 ml of diethyl ether was added to precipitate the dipeptide ester hydrochloride. The solvent was removed by decantation and the residue was repeatedly washed with diethyl ether until the yellow color of the residue disappeared. Then the product was dissolved in 50 ml of acetonitrile and the hydrochloride of the dipeptide was converted into the free ester. The solution was cooled to 0° and 0.75 g (3.6 mmol) of benzyloxycarbonylglycine and 0.72 g (3.8 mmol) of dicyclohexylcarbodiimide was added to the solution. The reaction system was allowed to stand for 2 days at −5°. The resulting dicyclohexylurea was removed by filtration and the filtrate was concentrated to give an oil. The oil was dissolved in 30 ml of ethyl acetate and undissolved urea was removed. The filtrate was concentrated again under reduced pressure. The residual oil was crystallized by adding *n*-hexane followed by cooling at −20° in a refrigerator. The product was isolated and dried.

A part of the crude product (50 mg) was hydrogenated in 90% acetic acid, and the filtrate was evaporated. The residue was dissolved in 0.2 *M* citric buffer at pH 4.25 (10 ml). Ten milliliters of the solution was analyzed by a Hitachi amino acid analyzer Model KLA-2 under the same conditions reported by Muraoka et al.[24] Glycine was eluted at 32 ml of effluent volume and glycyl-L-alanyl-L-leucine was eluted at 130 ml of the effluent volume. An elution peak corresponding to glycyl-D-alanyl-L-leucine, which was reported to elute at 159 ml, was not found in the analysis.

Comparison of the Samples of Nps-L-valyl-L-alanine Benzyl Ester Prepared by the Nps–NCA Method and the DCC Method. The optical rotation of the samples was examined by a Jasco automatic polarimeter Model DIP-SL. The dipeptide obtained by our method gave the optical rotation αD of −1.031° at 1.00 g dl^{-1} in tetrahydrofuran and −1.763° at 2.00 g dl^{-1} in ethyl acetate. The authentic sample shows the αD of −1.029° in tetrahydrofuran and −1.763° in ethyl acetate under the same conditions.

Nps-γ-benzyl L-glutamyl-L-valyl-L-alanine Benzyl Ester as an Example for Stepwise Synthesis of Higher Oligopeptides. Nps-L-valyl-L-alanine benzyl ester was prepared in 88% yield by the general procedure for dipeptide synthesis described above. The Nps-dipeptide benzyl ester (8.6 g, 0.02 mol) was dissolved in 50 ml of 1 *N* hydrochloric acid in dioxane. The solution was concentrated. Diethyl ether was added to the residue to give the crystals of dipeptide ester hydrochloride. The crystals were collected on a glass filter and washed with diethyl ether until the yellow color disappeared and dried in vacuo over P_2O_5. Then the product was dissolved in 100 ml of tetrahydrofuran and 2.8 ml (0.02 mol) of triethylamine was added to give the crystals of triethylamine hydrochloride, which were removed by filtration. To the filtrate was added 8.7 g (0.021 mol) of Nps-γ-benzyl L-glutamate NCA and this was allowed to react with stirring for 2 hr at room temperature. The solvent of the system was evaporated under reduced pressure. The resulting residue was dissolved in 400 ml of ethyl acetate. The solution was washed with 5% citric acid, 5% sodium bi-

carbonate, and water, and dried over sodium sulfate. The solution was concentrated and n-hexane was added to crystallize the product. The Nps-tripeptide benzyl ester was recrystallized from ethyl acetate to give 10.7 g (82%) of pure Nps-L-Glu(OBzl)-L-Val-L-Ala-OBzl.

Nps-L-phenylalanyl-L-isoleucylglycyl-L-leucyl-L-methionine Amide. A. Stepwise Approach. L-Methionine ethyl ester hydrochloride (10.7 g, 0.05 mol) was dissolved in 200 ml of tetrahydrofuran and treated with 17.0 g (0.055 mol) of Nps-L-leucine NCA by the general procedure for dipeptide synthesis described above. The purified Nps-L-leucyl-L-methionine ethyl ester (13.3 g, 0.03 mol) was dissolved in 150 ml of methanol saturated with ammonia and the solution was allowed to stand for 3 days. The solvent was evaporated to give a yellow solid, which was again dissolved in 150 ml of methanol. The solution was concentrated and diethyl ether was added to the residue. The resulting crystals of the Nps-dipeptide amide were obtained in 96% yield. The Nps-dipeptide amide was dissolved in 50 ml of 1 N hydrochloric acid in methanol. After the solvent was evaporated, 300 ml of diethyl ether was added. The resulting solid of the hydrochloride was isolated and washed with diethyl ether until the yellow color disappeared. The dipeptide amide hydrochloride (8.35 g, 0.028 mol) was dissolved in 150 ml of tetrahydrofuran and treated with 4.2 ml (0.03 mol) of triethylamine. After the crystals of the salt were removed by filtration, 7.6 g (0.03 mol) of Nps-glycine NCA was added and allowed to react for 2 hr at room temperature. The solvent was evaporated at 35°. The residue was dissolved in 400 ml of ethyl acetate, washed with 5% citric acid, 5% sodium bicarbonate, and water, and dried over sodium sulfate. The filtrate was concentrated to crystallize the Nps-tripeptide amide. The product was recrystallized from warm tetrahydrofuran. The Nps protecting group of the tripeptide amide was removed by dissolving in 50 ml of 1 N hydrochloric acid in methanol. Glycyl-L-leucyl-L-methionine amide hydrochloride was isolated by adding 400 ml of diethyl ether, followed by filtration, and washed with diethyl ether. The tripeptide amide hydrochloride was dissolved in 200 ml of tetrahydrofuran and treated with triethylamine, followed by 9.3 g (0.03 mol) of Nps-L-isoleucine NCA. The isolation and purification of the product were done by the same procedure of Nps-tripeptide amide to give a pure Nps-tetrapeptide amide. The Nps group of 11.7 g (0.02 mol) of Nps-L-isoleucylglycyl-L-leucyl-L-methionine amide was removed by action of hydrochloric acid. The tetrapeptide amide hydrochloride was treated in the presence of 3 ml (0.0214 mol) of triethylamine with 7.8 g (0.022 mol) of Nps-L-phenylalanine NCA in 300 ml of tetrahydrofuran. The solvent was removed by evaporation and the residue was diluted with 400 ml of water to crystallize the Nps-pentapeptide amide. The product was collected by filtration, and washed with 5% citric acid, 5% sodium bicarbonate, and water, and dried in vacuo over P_2O_5. The yield and physical properties of the intermediates described here are shown in Table IV.

B. Fragment Condensation Approach. A carboxyl component of the fragment condensation, Nps-L-phenylalanyl-L-isoleucylglycine, was prepared stepwise. Glycine ethyl ester hydrochloride (7.0 g, 0.05 mol) was dissolved in 200 ml of tetrahydrofuran and treated with 7 ml (0.05 mol) of triethylamine, followed by 15.6 g (0.052 mol) of Nps-L-isoleucine NCA. Nps-L-isoleucylglycine ethyl ester was isolated in 91% yield after recrystallization from ethyl acetate. The Nps group of the dipeptide ester (14.7 g, 0.04 mol) was removed by treating with 50 ml of 2 N hydrochloric acid in dioxane. The resulting hydrochloride was treated in the presence of 6 ml of triethylamine with 15.3 g (0.044 mol) of Nps-L-phenylalanine NCA in 200 ml of tetrahydrofuran. Nps-L-phenylalanyl-L-isoleucylglycine ethyl ester was obtained in a yield of 19.4 g (94%). The tripeptide ester was saponified as follows. The crystals (15.5 g, 0.03 mol) were dissolved in 50 ml of methanol and 30 ml of acetone, and 30 ml of 1 N sodium hydroxide was added. The solution was allowed to stand for 1 hr at room temperature. Then the solvent was evaporated to give the residual aqueous solution, which was diluted with 100 ml of water. The aqueous solution was extracted with 40 ml of diethyl ether and acidified to pH 3 by 15% citric acid. The solution was extracted with ethyl acetate (2 × 300 ml). The extract was washed with water and dried over sodium sulfate. The solvent was evaporated to give an oil, which was crystallized by adding 300 ml of n-hexane. The Nps-tripeptide was recrystallized from ethyl acetate, 12.3 g (84% yield). An amino component, L-leucyl-L-methionine ethyl ester hydrochloride, was obtained as an oil by treating the Nps-dipeptide ester with hydrochloric acid in dioxane. The product (6.5 g, 0.02 mol) was dissolved in 50 ml of tetrahydrofuran and treated with 3 ml of triethylamine. A solution of the carboxyl component of the Nps-tripeptide free acid (9.8 g, 0.02 mol) in 50 ml of dimethylformamide was added to the solution of the amino component. The solution was cooled to −5°, and 4.6 g (0.04 mol) of N-hydroxysuccinimide and 4.18 g (0.022 mol) of dicyclohexylcarbodiimide were added with stirring. The reaction was allowed to stir for 5 hr at −5° and an additional 10 hr at 0°. The temperature of the solution was raised to room temperature and the reaction was continued for 10 hr at the temperature. The resulting crystals of dicyclohexylurea were removed by filtration and the filtrate was concentrated to give an oil. The oil was dissolved in 400 ml of ethyl acetate and undissolved crystals were removed by filtration. The filtrate was washed with 5% citric acid, 5% sodium bicarbonate, and water. The dried solution was concentrated. The residue was crystallized by adding 400 ml of n-hexane. Recrystallization of the product from warm ethyl acetate gave pure Nps-L-phenylalanyl-L-isoleucylglycyl-L-leucyl-L-methionine ethyl ester (10.6 g, 70% yield). The Nps-pentapeptide ester (7.6 g, 0.01 mol) was dissolved in 100 ml of methanol saturated by ammonia and the solution was allowed to stand for 5 days. Then the crystals were formed. The system was concentrated and 200 ml of diethyl ether was added. The crystals were collected by filtration and washed with diethyl ether. The product was recrystallized from hot methanol to give a pure amide, 6.9 g (94%).

Nps-L-alanyl-L-phenylalanyl-L-isoleucylglycyl-L-leucyl-L-methionine Amide. The pentapeptide amide was treated with hydrochloric acid. The resulting hydrochloride (6.15 g, 0.01 mol) was dissolved in 100 ml of dimethylformamide and allowed to react with 3.5 g (0.013 mol) of Nps-L-alanine NCA. The system was diluted with 500 ml of water and the precipitate was filtered off and washed with 5% citric acid, 5% sodium bicarbonate, water, and methanol. The dried product was recrystallized from 50 ml of warm dimethylformamide to give a pure product, 7.4 g (92%).

The final product was obtained by removal of the Nps group of the protected hexapeptide amide by treating with hydrochloric acid. The resulting hydrochloride was collected by filtration and washed with diethyl ether until the yellow color disappeared. The product was recrystallized from 80% ethanol to give a pure hexapeptide amide hydrochloride: 5.05 g (80% yield); mp 255–260 dec (lit.[27] mp 250–255 dec); $[\alpha]_D$ −12.0° (c 0.2, dimethylformamide). Anal. Calcd for $C_{31}H_{51}N_7O_6 \cdot HCl \cdot H_2O$: C, 52.86; H, 7.73; N, 13.92. Found: C, 52.91; H, 7.84; N, 13.86.

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Registry No.—Glycine NCA, 2185-00-4; alanine NCA, 2224-52-4; valine NCA, 24601-74-9; leucine NCA, 3190-70-3; isoleucine NCA, 45895-90-7; phenylalanine NCA, 14825-82-2; benzyloxycarbonyllysine NCA, 1676-86-4; methylglutamic acid NCA, 1663-47-4; benzylglutamic acid NCA, 3190-71-4; benzylaspartic acid NCA, 13590-42-6; Nps-Cl, 7669-54-7.

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Photochemical Syntheses of 2-Aza- and 2-Oxabicyclo[2.1.1]hexane Ring Systems[1]

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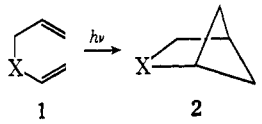
Irradiation of N-substituted 3-allylamino- and 3-allyloxy-5,5-dimethyl-2-cyclohexen-1-ones gives 2-aza- and 2-oxabicyclo[2.1.1]hexane derivatives, respectively, whose structures are assigned on the basis of the NMR spectral and chemical evidence. The photocycloaddition reaction of the *N*-methyl, *N*-allyl, and *N*-phenyl substituted allylamino and allyloxy derivatives produces exclusively or predominantly the thermodynamically unstable isomers, while the *N*-acetyl allylamino derivative gives a ca. 1:1 mixture of two possible isomers. It is suggested that the lone-pair electrons of the heteroatom play an important role in deciding the stereochemical course of this cycloaddition reaction.

Photochemical transformation of 1,5-hexadienes (**1**, X = CH_2) to bicyclo[2.1.1]hexanes (**2**, X = CH_2) has been extensively studied.[2] Several years ago, we initiated a photochemical study on 1,5-hexadienes containing a heteroatom (**1**, X = a heteroatom) with the hope that the reaction might be extended to the syntheses of the 2-heterobicyclo[2.1.1]hexane systems (**2**, X = a heteroatom). We now report the syntheses of the then unknown 2-azabicyclo[2.1.1]hexane ring system (**2**, X = NR)[3] from N-substituted 3-allylamino-5,5-dimethyl-2-cyclohexen-1-ones and the 2-oxabicyclo[2.1.1]hexane ring system (**2**, X = O)[4] from 3-allyloxy-5,5-dimethyl-2-cyclohexen-1-one. In addition, some chemical transformation reactions of the new heterocycles are described.

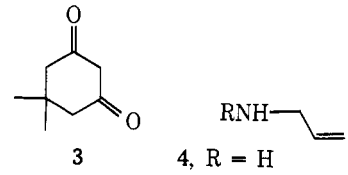


1 **2**

Results

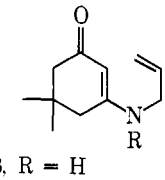
3-Allylamino-2-cyclohexen-1-ones (**8–11**) were readily obtained from dimedone (**3**) and the corresponding allylamines (**4–7**) in 75, 71, 46, and 64% yields, respectively. Acetylation of **8** with acetic anhydride and pyridine gave *N*-acetate **12** (43%), *C*-acetate **13** (8%), and an unidentified product (11%). The structural assignments of **8–13** are consonant with elemental analyses and ir, uv, NMR, and mass spectral data (see Experimental Section).

Irradiation of a 0.02 *M* cyclohexane solution of **9** with a 350-W high-pressure mercury lamp through a Pyrex filter for 10 hr resulted in the disappearance of **9** and the concomitant formation of a single photoproduct. The progress of the reaction was conveniently followed by TLC examination. The other aprotic solvents such as ether, benzene, acetone, and methylene chloride could be equally used, but the use of alcoholic solvents such as methanol or ethanol did not give a clear-cut result. The resulting photoproduct was isolated in 50–60% yield as a crystalline solid, mp 48.5–49.5°, by passing through a short alumina column after removal of the cyclohexane.

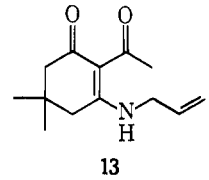


3

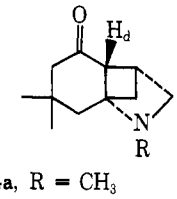
4, R = H
5, R = CH_3
6, R = $CH_2CH{=}CH_2$
7, R = C_6H_5



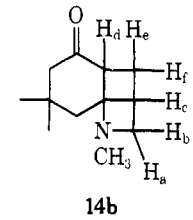
8, R = H
9, R = CH_3
10, R = $CH_2CH{=}CH_2$
11, R = C_6H_5
12, R = $COCH_3$



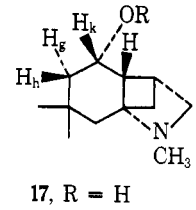
13



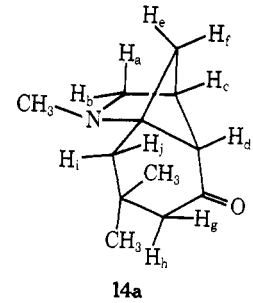
14a, R = CH_3
15, R = $CH_2CH{=}CH_2$
16, R = C_6H_5



14b



17, R = H
18, R = $COCH_3$



14a

The photoproduct was shown to be isomeric with **9** by elemental analysis and mass spectrometry. The ir (an absorption at 1710 cm^{-1} typical of a six-membered ketone) and NMR (no olefinic proton signal) spectrum show no unsaturation, and thus it must be tricyclic. It formed a crystalline hydrochloride, indicating the presence of a basic nitrogen. The lithium aluminum hydride reduction in ether