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Isobornyloxycarbonyl Function, a New Convenient Amino-Protecting Group in Peptide Synthesis. I. Synthesis and Properties of Isobornyloxycarbonylamino Acids[1]

Masahiko Fujino, Susumu Shinagawa, Osamu Nishimura and Tsunehiko Fukuda

Chemical Research Laboratories, Research and Development Division, Takeda Chemical Industries, Ltd.[2]

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Isobornyloxycarbonyl chloride (IBOC-Cl), which is a satisfactorily stable oil, was prepared from isoborneol and phosgene. The chloride, when allowed to react with amino acids, gave the corresponding isobornyloxycarbonyl (IBOC-)-amino acids. In most cases, the IBOC-amino acids could be obtained in crystalline form and in good yields.

The IBOC-amino acids are smoothly cleaved by acid-catalyzed solvolysis with trifluoroacetic acid to yield the free amino acids. Since the IBOC-Cl is much easier to prepare than *t*-butyloxycarbonyl chloride, the new amino-masking method is considered to be of great use in the synthesis of complicated peptides.

The *t*-butyloxycarbonyl (BOC-) group[3] has been widely used in the preparation of complicated peptides because of its readily removable nature under solvolytic conditions in mild acid solutions.[4]

Although a wide variety of acylating agents has been recommended for the amino-masking purpose,[3,5] none of them appear to be superior to *t*-butyloxycarbonyl chloride.[6] The only drawback of this reagent, however, is that the reagent is very unstable and has to be prepared immediately before its use. Occasionally, *t*-amyloxycarbonyl chloride[7] can be used for introducting the *t*-amyloxycarbonyl group which is easily removed by trifluoroacetic acid as the BOC-group, however, the chloride is also too unstable for a general use under oridinary conditions.

In view of the well-known property of isobornyloxy-derivatives which tend to undergo solvolysis by the effect of an anchimeric assistance,[8] it was assumed that the isobornyloxy-

1) After completion of our manuscript a patent specification which deals with the preparation of IBOC-amino acids has appeared [G. Jäger and R. Geiger, Japan Patent 3217 1971 (Oct. 27, 1971)].
2) Location: *Juso-Nishinocho, Higashiyodogawa-ku, Osaka.*
3) F.C. Mckay and N.F. Anderson, *J. Am. Chem. Soc.*, **79**, 4686 (1957); G.W. Anderson and A.C. McGregor, *ibid.*, **79**, 6180 (1957).
4) H. Kappeler and R. Schwyzer, *Helv. Chim. Acta*, **44**, 1136 (1961).
5) L.A. Carpino, B.A. Carpino, P.J. Crowley, C.A. Giza and P.H. Terry, *Org. Syn.*, **44**, 15 (1964); M. Frankel, D. Ladkany, C. Gilon and Y. Wolman, *Tetrahedron Letters*, **1966**, 4765; M. Fujino and C. Hatanaka, *Chem. Pharm. Bull.* (Tokyo), **15**, 2015 (1967); W. Broadkent, J.S. Morley and B.E. Stone, *J. Chem. Soc. C.*, **1967**, 2632; D.S. Tarbell and M.A. Insalaco, *Proc. Natl. Acad. Soc. U.S.*, **57**, 235 (1967); M. Itoh and D. Morino, *Experientia*, **24**, 1011 (1968); B. Reszotarska and S. Wiejak, *Ann. Chem.*, **716**, 2161 (1968); E. Schnabel, H. Herzog, P. Hoffmann, E. Klauke and I. Ugi, *ibid.*, **716**, 175 (1968); L.A. Carpino, K.N. Parameswaran, R.K. Kikley, J.W. Spiewak and E. Schmitz, *J. Org. Chem.*, **35**, 3291 (1970); E. Guibe-Jampel and M. Wakselman, *Chem. Comm.*, **1971**, 267.
6) R.B. Woodward, K. Heusler, H. Gosteli, P. Naegeli, W. Oppoler, R. Ramage, S. Ranganathan and H. Vorbruggen, *J. Am. Chem. Soc.*, **88**, 852 (1966); S. Sakakibara, I. Honda, K. Takada, M. Miyoshi, T. Ohnishi and K. Okumura, *Bull. Chem. Soc. Japan*, **42**, 809 (1969).
7) S. Sakakibara, M. Shin, M. Fujino, Y. Shimonishi, S. Inoue and N. Inukai, *Bull. Chem. Soc. Japan*, **38**, 1522 (1965); S. Sakakibara and M. Fujino, *ibid.*, **39**, 947 (1966).
8) E.S. Gould, "Mechanism and Structure in Organic Chemistry," Henry Holt and Company, New York, N.Y., 1960, pp. 594—599.

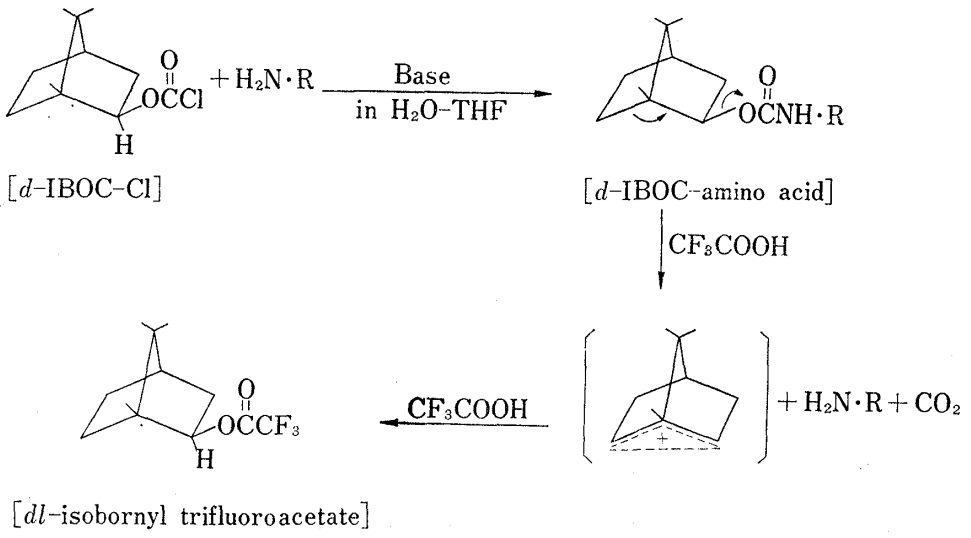

Chart 1

carbonyl (IBOC-) group might be useful for the same purpose as the BOC-group in the peptide synthesis.

Isobornyloxycarbonyl chloride (IBOC-Cl) is easily obtained in its *d*- or *dl*-form in excellent yield as a satisfactorily stable oily substance by the treatment of *d*- or *dl*-isoborneol with slight excess of phosgene in an ether solution.

d-IBOC-derivatives of amino acids were prepared from the corresponding amino acids by the reaction with *d*-IBOC-Cl under the Schötten–Baumann conditions using either NaOH or $NaHCO_3$. In most cases, the *d*-IBOC-derivatives were obtained in crystalline forms and

Table I. Yield and Properties of *d*-IBOC-amino Acids

N^{α}-*d*-Isobornyloxycarbonyl derivatives of	Yield[a] (%)	mp[b] (°C)	$[\alpha]_D^{25}$ ($c=1$, EtOH)	Formula[c]
L-Alanine	84	108—110	−57.9	$C_{14}H_2O_{34}N$
β-Alanine	98	oil		
β-Alanine, DCHA[d]	82	120—121	−23.9	$C_{20}H_{36}O_4N_2$
L-Aspargine	91	169—170(dec.)	−37.5	$C_{15}H_{24}O_5N_2$
L-Arginine, H_2O[e]	60	200—204(dec.)	−14.8	$C_{17}H_{32}O_5N_4$
L-Glutamine	89	60—62(amorph.)	−39.4	$C_{16}H_{26}O_5N_2$
Glycine	86	176—177	−42.7	$C_{13}H_{21}O_4N$
L-Isoleucine	79	97—98	−39.0	$C_{17}H_{29}O_4N$
L-Leucine, H_2O[e]	90	75—76	−53.2	$C_{17}H_{31}O_5N$
L-N^{ε}-IBOC-Lysine	100	oil		
L-Methionine	100	oil		
L-Methinone,DCHA[d]	82	130—131	−10.3	$C_{28}H_{50}O_4N_2S$
L-Nitroarginine	80.4	153—153.5	−28.7	$C_{17}H_{29}O_6N_5$
L-Phenylalanine	93	110—117	−30.3	$C_{20}H_{27}O_4N$
L-Phenylalanine DCHA[d]	98	168.5—169.5	+ 7.7	$C_{32}H_{50}O_4N_2$
L-Proline	90	135.5—136	−95.6	$C_{16}H_{25}O_4N$
L-Serine	90	oil		
L-Serine, DCHA[d]	76	153—154	−14.0	$C_{26}H_{46}O_5N_2$
L-Threonine	92	oil		
L-Threonine, DCHA[d]	86	128—130	−16.0	$C_{27}H_{48}O_5N_2$
L-Tryptophan	83	180—181(dec.)	−36.4	$C_{22}H_{28}O_4N_2$
L-Valine	80	137.5—138	−49.3	$C_{15}H_{27}O_4N$

a) Based on amino acids; *b*) Melting points were determined by the capillary tube method and were uncorrected; *c*) Analytical results for C, H, N, S were within ±0.4% of the theoretical values; *d*) dicyclohexylamine salt; *e*) monohydrate

in good yields. Some of oily products were converted to the corresponding dicyclohexylamine salts.

The yield and physical constants of the *d*-IBOC-amino acids prepared are listed in Table I.

The removal of the IBOC-group from the amino nitrogen proceeds *via* a nonclassical carbonium ion mechanism[9] by the treatment with trifluoroacetic acid at room temperature within 30 min as shown in Fig. 1, or also by treatment with 20% hydrogen bromide in acetic acid for 1—2 min, whereas the IBOC-group is stable under ordinary conditions for catalytic hydrogenation, hydrazinolysis and alkaline saponification.

Moreover, it might be worthy of note that IBOC-derivatives are rather stable under the treatment with 1—2N hydrogen chloride in organic solvents. This indicates that the IBOC-protecting method could have an advantage for the selective protection of the ε-amino function of lysine in the peptides derived from N^{α}-carbobenzoxy-amino[10] or N^{α}-*o*-nitrophenylsulfenyl-amino acids[11] over the known synthesis with the BOC-protecting group.

The aforementioned selectivity in the reactions and removal of this new protecting group together with its reasonable stability of the reagent (IBOC-Cl) for storage would demonstrate that the new amino masking method could be of great use in the synthesis of complicated peptides.

Further applications of this new protecting group in the classical and the solid-phase peptide synthesis are now under progress in our laboratory.

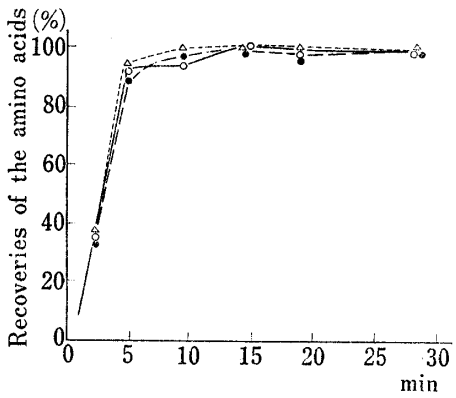

Fig. 1. Removals of IBOC-group by the Treatment with Trifluoroacetic Acid at Room Temperature
—○—: IBOC-glycine
—△—: IBOC-alanine
—●—: IBOC-leucine
IBOC-Alanine (134.6 mg), IBOC-glycine (127 mg) and IBOC-leucine monohydrate (164 mg) were dissolved in trifluoroacetic acid (12.0 ml) containing anisole (0.3 ml), and a 0.5 ml of portion was taken in the each intervals.
The trifluoroacetic acid was removed by flash evaporation under a high vaccum, and the residues were directly analyzed by a Hitachi model KLA-3B autoanalyzer.

Experimental[12]

***dl*-Isobornyl Chloroformate**——To a solution of liquid phosgene (200 g, 2.0 mole) in anhydrous THF (600 ml), a solution of *dl*-isoborneol (246.4 g, 1.6 mole) in anhydrous ether (800 ml) was added dropwise with stirring at about 5° over 60 min. The reaction mixture was allowed to stand at room temperature for additional 6 hr. The solvent was removed *in vacuo* to yield 323 g of sticky oil. The infrared (IR) spectrum (IR) $\nu_{max}^{CHCl_3}$ cm^{-1}: 1775) of the product supports the proposed structure.

Treatment of the chloroformate with aniline in a mixture of THF and water gave the carbanilate in yield 94.4%. mp 139—140°. *Anal.* Calcd. for $C_{17}H_{23}O_2N$: C, 74.69; H, 8.48; N, 5.12. Found: C, 74.75; H, 8.49; N, 5.30.

d-Isobornyl chloroformate was prepared in the same manner as described above. The yield of the *d*-chloroformate (oil) was practically quantitative.

General Procedure for the Preparation of Isobornyloxycarbonylamino Acids——a) NaOH procedure: An amino acid (50 mmole) was dissolved in ice-cold 1N NaOH (50 ml). To this were added a solution of chloroformate (13.0 g, 60 mmole) in THF or dioxane (40 ml) and 1N NaOH (50 ml) alternately in portions over a period of 60 min in an ice-bath with stirring. After stirring at room temperature for another 2—4 hr, the mixture was diluted with water (120 ml), and the solution was extracted two times with ether. The aqueous phase was acidified with 1N HCl under stirring and cooling. The precipitated product (solid or oil)

9) When *d*-IBOC-derivatives were treated with trifluoroacetic acid, trifluoroacetyl *dl*-isoborneol was a sole product obtained from an ethereal extract of the reaction mixture.
10) M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).
11) L. Zervas, D. Borovas and E. Gazis, *J. Am. Chem. Soc.*, **85**, 3660 (1963).
12) All melting points are uncorrected. Evaporations were all carried out with a rotary evaporator.

was extracted with AcOEt (120 ml) and the aqueous phase was extracted with two more portions of fresh AcOEt (80 ml). The combined extracts were dried over anhydrous Na_2SO_4 and the solvent was removed *in vacuo*. The residue was crystallized from a suitable solvent such as pet. ether, ether–pet. ether, AcOEt–pet. ether or AcOEt.

b) $NaHCO_3$ Procedure: *d*-Isobornyloxycarbonyl-L-Aspargine, as an Example: L-Aspargine monohydrate (3.00 g, 20 mmole) was suspended in water (50 ml), the mixture was stirred in an ice-bath, and $NaHCO_3$ (4 g) was added. *d*-Isobornyl chloroformate (5.2 g, 24 mmole) was dissolved in dioxane (20 ml) and added to the aspargine solution in three portions over 60 min with stirring in an ice-bath. The mixture was stirred at room temperature for another 5 hr when it was extracted two times with ether. After the aqueous phase was acidified with 0.5N HCl, the product separated was extracted into three portions of AcOEt (40 ml each). The combined extracts were dried over anhydrous Na_2SO_4, and the solvent was removed *in vacuo*. The residue was recrystallized from AcOEt to give fine platelets. Yield 5.70 g (96.6%), mp 169—170° (decomp.), $[\alpha]_D^{25}$ −37.5° (c=1.0, EtOH). *Anal.* Calcd. for $C_{15}H_{24}O_5N_2$: C, 57.67; H, 7.74; N, 8.97. Found: C, 57.75; H, 7.77; N, 8.79.

L-Serine, L-threonine, and L-glutamine were acylated in a similar manner and the data are given in Table I.

Dicyclohexylamine Salt of d-Isobornyloxycarbonyl-amino Acids——As an Example: L-Methionine (2.99 g, 20 mmole) was acylated with *d*-isobornyl chloroformate by the general procedure: 6.6 g (100%) of an oil was obtained. The oil was dissolved in a small volume of ether and dicyclohexylamine (3.7 ml) was added. The crystals formed on standing in a refrigerator were collected by filtration and washed with ice-cold ether. Yield 9.10 g (89.2%), mp 130—131°, $[\alpha]_D^{25}$ −10.3 (c=1.0, EtOH). *Anal.* Calcd. for $C_{28}H_{50}O_4N_2S$: C, 65.85; H, 9.87; N, 5.49; S, 6.28. Found: C, 65.74; H, 10.02; N, 5.27; S, 6.20.

Dicyclohexylamine salts of L-serine, L-threonine and L-phenylalanine in Table I were prepared in a similar manner.

The Cleavage of Isobornyloxycarbonyl Group from Isobornyloxycarbonyl-amino Acids——a) *d*-Isobornyloxycarbonyl-L-alanine (134.6 mg), -glycine (127.0 mg) and -L-leucine (monohydrate, 164 mg) were dissolved in trifluoroacetic acid (12.0 ml) containing anisole (0.3 ml), and a 0.5 ml portion was taken at intervals as shown in Fig. 1. The trifluoroacetic acid was removed by flash evaporation under high vaccum, and the residues were analyzed by Hitachi autoanalyzer (see Fig. 1).

b) *d*-Isobornyloxycarbonyl-glycine (1275 g) was dissolved in trifluoroacetic acid (10 ml) and the solution was stirred for 20 min at room temperature (22°). The trifluoroacetic acid was removed by evaporation, and the residue was washed well with anhydrous ether. The residue was dissolved in EtOH (10 ml) and neutralized with triethylamine (0.75 ml). The resulting crystal was collected by filtration and washed with cold EtOH, and recrystallized from aqueous EtOH to give free glycine (354 mg, 94.0%) which was identical with authentic glycine by physicochemical criterions.

c) *dl*-Isobornyloxycarbonyl-glycine (5.11 g, 20 mmole) was dissolved in 20% hydrogen bromide in acetic acid (10 ml). After 2 min at room temperature, dry ether (100 ml) was added into the reaction mixture, and the resulting crystals were collected by filtration. The crystals were dissolved in water (50 ml), and the solution was passed through a column of Amberlite IRA-4B (AcO^-). The eluate was evaporated *in vacuo* to dryness. The residue was recrystallized from water–EtOH: The yield of free glycine was 1.38 g (92%).

On the other hand, *dl*-bornyloxycarbonyl-glycine (mp 143—144°) was treated with 20% anhydrous hydrogen bormide in acetic acid for 10 min at room temperature, no free glycine was detected.

d) *d*-Isobornyloxycarbonyl-L-aspargine (627 mg) was dissolved in 2 ml of acetic acid. To this was added 1 ml of 25% hydrogen bromide in acetic acid and the solution was stirred for 10 min at room temperature. The resulting mixture was diluted with anhydrous ether to yield fine crystals which were collected by filtration and washed with ether. The crystalline product was dissolved in 10 ml of aqueous acetone and the solution was neutralized with triethylamine (0.23 ml). The resulting crystals were collected by filtration to give aspargine monohydrate (282 mg, 94%), which was identical with authentic sample by IR spectrum and paper chromatography.

e) *d*-Isobornyloxycarbonyl-glycine (1.275 g) and anisole (1 ml) were dissolved in anhydrous hydrogen fluoride (*ca.* 4 ml) at −50°, and the solution was stirred for 10 min at 0°. The hydrogen fluoride was removed off by evaporation and the residue was dried *in vacuo* over NaOH pellets. The dried residue was washed well with anhydrous ether and dissolved in EtOH (12 ml). The solution was neutralized with triethylamine to yield crystals which were collected by filtration and washed with ice-cold EtOH. The washed product was recrystallized from aqueous EtOH to give free glycine in 96% yield (361 mg).

dl-Bornyloxycarbonyl-glycine was treated with hydrogen fluoride in a similar procedure, and this gave free glycine in 94% yield.

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