yellow crystals, mp 265°(decomp.)(ϕH) and the second [elution with ϕH–$CHCl_3$ (50:1–2:5)] was an oil [IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1690 (shoulder, α,β–unsaturated ketone), 1670 (=NCHO), 1600 (C=C)] respectively in 1:3 ratio. Refluxing of the oil with $LiAlH_4$ in ab. ether–ab. tetrahydrofuran for 4 hours and chromatography on silicic acid [elution with $CHCl_3$–MeOH(100:1–100:2)] afforded 4–hydroxy compound (*ca.* 12% yield), mp 124—126° from ϕH–*n*–hexane.

Identity of the 4–hydroxy compound and V from I was shown by comparison of their IR spectra ($CHCl_3$) and GLC (5% SE–30, 175°) and by mixed mp determination.

Thus V was undoubtedly 4–hydroxy compound and also tetrahydroisoquinolines could be prepared from glycine derivatives though in low yield.

Mechanistic details of the unusual rearrangement described above and synthesis of *dl*–gigantine (VII)[5] from *dl*–N–methylisosalsoline as an application of this method will be published in the near future.

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5) *d*–Gigantine [mp 151—152°, $[\alpha]_D$ 27.1°($CHCl_3$)] is isolated from *Carnegia gigantea* and the planar structure is assigned as VII. (J.E. Hodgkins, S.D. Brown, and J.L. Massingill, *Tetrahedron Letters*, **1967**, 1321).

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A Convenient Method for the Preparation of *tert*-Butyl Azidoformate

Progress in the peptide chemistry is accumulating data indicating the usefulness of the *tert*–butoxycarbonyl group (*t*–BOC) as a reversible protecting group for the amino function of amino acids and peptides, because of the ease of removal by mild acidolysis.[1,2] For the synthesis of complex peptides, such an acid labile protecting group is essential in addition to the historical benzyloxycarbonyl group.

Introduction of *t*–BOC group into amino acids requires multiple steps of reaction due to the unstability of *tert*–butyl chloroformate. For example, *t*–BOC amino acids are prepared by the reaction of the corresponding isocyanate with *tert*–butanol,[3] or by *tert*–butyl *p*–nitrophenyl carbonate[4] or *tert*–butyl cyanoformate[5,6] or *tert*–butyl azidoformate.[7–9] These pro-

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cedures are rather tedious and its amino acid derivatives are expensive. Recently, these situations have been improved immensely by the invention of new *t*-BOC reagents, such as *tert*-butoxycarbonylimidazole[10] or *tert*-butoxycarbonyl N-hydroxysuccinimide ester[11] or *tert*-butyl pentachlorophenyl carbonate.[12]

Prior to the introduction of these reagents, *tert*-butyl azidoformate[7-9] derived from the corresponding hydrazide, has been widely employed for the preparation of *t*-BOC amino acids. This procedure was examined in detail by Schnabel.[13] Though the reaction of the azide with amino acids requires certain period of time (approximately 24 to 40 hr at 45° to 50°) in the presence of a base, such as magnesium oxide or triethylamine or sodium bicarbonate, this azide is more advantageous than the other reagents, since the reaction mixture contains no extra reagents besides an appreciable amount of the base. Therefore purification of the products is simple and the compounds so obtained are highly pure. However, as has been mentioned above, the preparation of this azide requires multiple steps of reaction *via* phenyl chloroformate, phenoxycarbonyl *tert*-butylate and *tert*-butoxycarbonylhydrazide.[7-9] Conversion of *tert*-butyl *p*-nitrophenyl carbonate to the hydrazide is also possible.[14] Recently, Ovchinnikov, *et al.*[15] reported more convenient method to synthesize this hydrazide in an overall yield of 33% by the reaction of hydrazine and *tert*-butyl chloroformate, prepared directly with excess phosgen and the complex of potassium *tert*-butoxide and *tert*-butanol.

Taking advantage of the fact that the chloroformate in ether, rather than methylene chloride,[16] is more stable under cooling in dry-ice aceton, we have succeeded in preparing *tert*-butyl azidoformate directly from the reaction of *tert*-butyl chloroformate and hydrazoic acid in nearly 35% yield. Identity of the azide prepared by the present method with the compound prepared by the original Carpino method[7,8] was confirmed by comparison of their IR spectra. The azide can be distilled under reduced pressure and can be stored in cold for certain period.[8] Our present procedure, besides newly introduced *t*-BOC reagents, appears to reduce serious disadvantage hitherto suffered in the preparation of the *t*-BOC amino acids which are so important in the present peptide chemistry.

***tert*-Butyl Azidoformate**——The entire reaction was carried out under cooling in dry-ice aceton. Phosgen (50 g) was introduced with the aid of a slow stream of nitrogen gas into a solution of *tert*-butanol (37 ml) and pyridine (39 ml) in an anhydrous ether (350 ml) during a period of 2 hr. To this solution, hydrazoic acid (prepared from 50 g of sodium azide according to the method of von Braun[17]) in ether (200 ml) and triethylamine (70 ml) were added dropwise for 1 hr. After stirring was continued for an additional 5 hr, the solution was kept in a refrigerator overnight. Water (300 ml) was added and the ether layer was separated. The organic phase was washed with 1 N HCl and a saturated solution of NaCl, dried over Na_2SO_4 and then evaporated with aspirator at 25°. The residue was distilled under reduced pressure; bp 47—50°/37 mm Hg. (lit.[8] bp 73—76°/70 mm Hg), yield 25 g (35%). IR spectra: 2100 cm^{-1} (–CO–N_3) and 1701 cm^{-1} (–O–CO–).

Note 1. For the preparation of *tert*-butyl chloroformate, the solvent system of ether and pyridine gave so far the best result than that of methylene chloride and pyridine or of ether and triethylamine.

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Note 2. Triethylamine in the above experiment can be substituted with pyridine (39 ml); yield 23 g (32%).

Note 3. According to the procedure of Breslow, *et al.*[18] in the preparation of *n*-octadecyl azidoformate, sodium azide (50 g) and H_2O (60 ml) were added to the solution of *tert*-butyl chloroformate prepared in ether in the presence of pyridine as stated above. Yield of the azide was 6.5 g to 11 g (9 to 15%).

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New Color Reaction of Hexosamines

The practical methods for the determination of hexosamines were the colorimetric methods developed by Elson,[1] Morgan[2] and Dische.[3] A new color reaction has been deviced for the microdetection and determination of hexosamines, based on a principle completely different from that of the Elson, Morgan or Dische method and having the advantage of being considerably more simple and selective. *p*-Nitrobenzaldehyde (*p*-NBA) reacted readily with hexosamines in pyridine solution to yield Schiff bases, and exhibited a blue color by addition of tetraethylammonium hydroxide solution. A possible mechanism of this color reaction was shown in Chart 1.

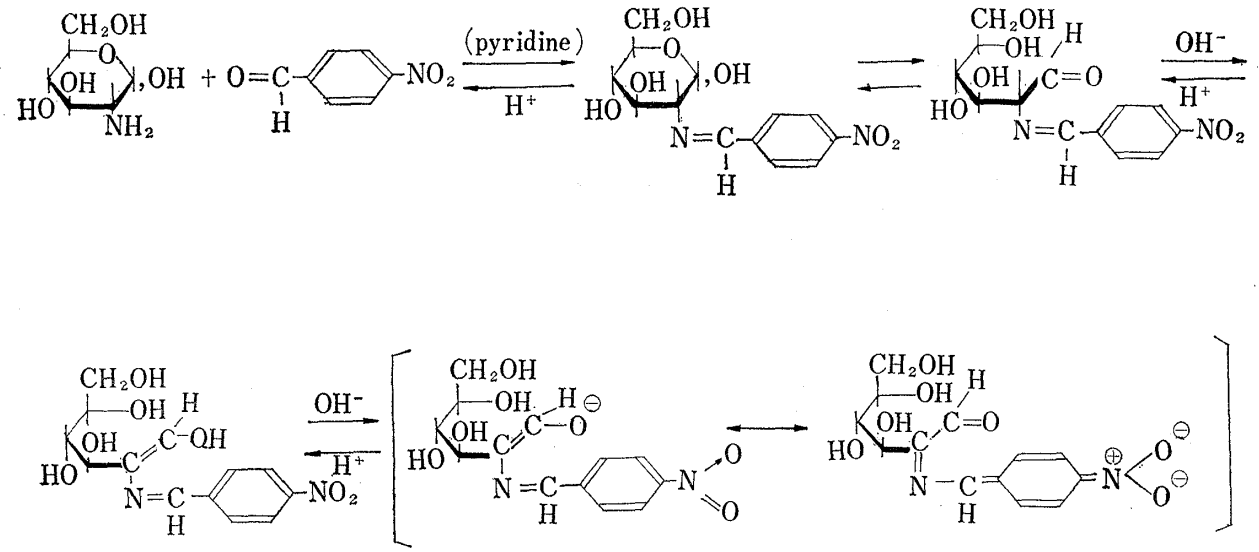

Chart 1. Mechanism of Color Reaction

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