

PREPARATION OF TERTIARY - (BUTYL - ^3H) - AZIDOFORMATE

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t-Butylazidoformate (t-butyloxycarbonylazide) has many advantages in the synthesis and study of the chemistry of peptides and proteins, both as an easily removable blocking agent and as a diagnostic reagent for amino groups.

Where biologically active molecules are the subject of interest, biological activity determinations are often the primary means of confirmation that a diagnostic reaction has taken place. The method used for modification must fulfill certain conditions viz: (1) the reaction must proceed under fairly mild conditions of pH and temperature, otherwise the biological molecule may be decomposed, (2) the reaction should take place in aqueous medium, particularly if large tertiary structures are involved, (3) the yield from the reaction must be very high to avoid the need for separating the reacted and unreacted molecules, otherwise the results will be indecisive, (4) the reactants must be easily removed, or be non-toxic under the conditions of assay, (5) the reaction should be reversible under mild conditions so that activity lost on modification can be regenerated, thus proving that the modification did occur and thereby providing the diagnosis. t-Butylazidoformate is a reagent which fulfills these requirements. Since it is also often desirable to follow the course

of a modified molecule in a biological system e.g., by autoradiography, a tracer is necessary.

The various requirements, and the fact that use of available fluorescent reagents such as dansyl chloride and labelled reagents such as 1-fluoro-2,4-dinitrobenzene-³H do not permit removal of the derivatising group, prompted us to investigate and establish a method for the synthesis of labelled t-butylazidoformate. Therefore, a method of synthesis of t-(butyl-³H)-azidoformate is described in this paper. The reaction scheme is shown in Figure 1. An adapted version of the synthetic route to the unlabelled compound (1-3) has been employed.

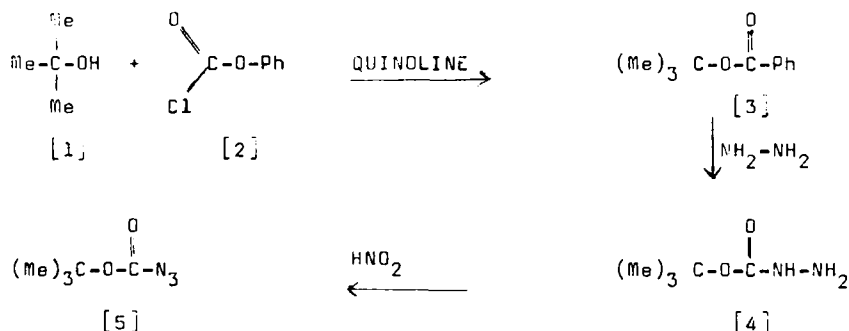


Figure 1 REACTION SCHEME

t-(Butyl-³H)-phenylcarbonate

t-Butyl alcohol [1] (12ml) purified by re-distillation, was exchanged with tritium gas (7Ci) at the Radiochemical Centre, Amersham, using the Wilzbach technique. Tritium incorporated into the hydroxyl group was exchanged for hydrogen by treatment with water to give t-(butyl-³H)-alcohol (approx. 1Ci/mole). The t-(butyl-³H)-alcohol (3ml) was diluted with t-butylalcohol (77ml),

and quinoline (98ml) and dichloromethane (125ml) were added. The mixture was stirred at 40°C and phenylchloroformate [2] (100ml) was added dropwise over four hours during which the temperature was maintained at 40-41°C. The solution was then allowed to stand at 25°C for 24 hours, after which distilled water (150ml) was added to dissolve the precipitated salt. The organic layer was washed with distilled water (4 x 100ml), 5% w/v hydrochloric acid (4 x 100ml) and finally with distilled water (200ml lots) until the washings were neutral. The organic liquid was then dried with anhydrous sodium sulphate and filtered off in a closed system. The solvent was removed by distillation in a closed system, and the remaining product purified by distillation under reduced pressure (b.p. 120-124°C at 1.0 mm. Hg; yield 109.1g = 66.1%). Infrared spectroscopy of the product confirmed it to be t-(butyl-³H)-phenylcarbonate [3].

t-(Butyl-³H)-carbazate

64% v/v Hydrazine hydrate solution (59g) was added to t-(butyl-³H)-phenylcarbonate (109g), and the mixture was stirred and heated to 96°C. After 10 min at this temperature, the mixture was allowed to cool to 25°C, and was maintained at this temperature for a further 24 hours with continual stirring. Ether (300ml) was then added and the ethereal solution shaken well with sodium hydroxide solution (30g in 100ml of water). The mixture was extracted with ether for 24 hours in a continuous extractor, and the ether was subsequently removed by distillation with the usual precautions. The remaining product was purified by distillation under reduced pressure (b.p. 80-82°C at 1.0 m.m. Hg; yield 72.8g = 98.5%). Infrared spectroscopy of the product confirmed it to be t-(butyl-³H)-carbazate [4].

t-(Butyl-³H)-azidoformate

t-(Butyl-³H)-carbazate (72.8g) was dissolved in redistilled glacial acetic acid (68ml) and distilled water (96ml). The mixture was cooled in an ice-salt bath to -5°C and a solution of sodium nitrite was added to the vigorously stirred solution over a period of one hour; the temperature was not allowed to rise above -5°C. Distilled water (125ml) was then added and the solution was extracted with ether (6 x 50ml). The ether layer was washed with distilled water (3 x 50ml) and 0.1 M sodium bicarbonate (3 x 100ml), dried over magnesium sulphate, and filtered off in a closed system. The ether was distilled off at 0°C, 4 m.m Hg and the remaining product was distilled at room temperature, in a closed system, using a trap immersed in solid carbondioxide-acetone as a condenser (b.p. 20°C at 1.0 m.m. Hg; yield 57.4g = 72.9%). Infrared spectroscopy of the pale yellow product confirmed it to be t-(butyl-³H)-azidoformate [5]. The specific activity of the t-(butyl-³H)-azidoformate was determined using an Intertechnique Liquid Scintillation spectrometer SL40 and butyl PBD [2-(4'-t-butylphenyl)-5-⁴"-biphenyl)-1,3,4 oxadiazole] in toluene as a liquid scintillator (366 mCi/mole).

When the diazotisation of the t-butylazidoformate is carried out, it is essential to keep the temperature at the value stated. Elevation of the temperature results in extensive formation of undesirable side-products which are dark brown or black. Several laboratories have commented on the explosive character of t-butyl-azidoformate when distilled under reduced pressure at 75°C. Utilisation of the carbon dioxide-acetone bath and trap as condensing system avoided the use of high temperature, and no problem was

encountered in obtaining the pure product. Clearly higher specific activities of t-(butyl-³H)-azidoformate may be obtained using initially higher specific activities of t-(butyl-³H)-alcohol.

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