Tetrahedron, 1959, Vol. 6, pp. 225 to 231. Pergamon Press Ltd. Printed in Northern Ireland

SYNTHESES WITH PYRIMIDINE-LITHIUM COMPOUNDS*†

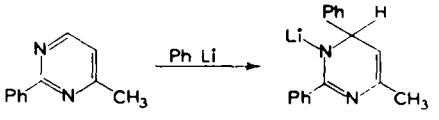
T. L. V. ULBRICHT‡
Department of Pharmacology, Yale University School of Medicine,
New Haven, Connecticut

(*Received* 27 *October* 1958; *in revised form* 24 *December* 1958)

Abstract—The principles of the preparation, reactivity and use of organolithium compounds is briefly discussed in relation to recent work with N-heterocyclic derivatives. The synthesis of thymine-$C^{14}H_3$ and 5-ethyluracil from a pyrimidine-lithium intermediate, and the separation of uracil and thymine by ion-exchange chromatography, are described. It is shown that such syntheses can be carried out with compounds containing active hydrogens by taking advantage of the higher reactivity of lithium-carbon bonds, and this is illustrated in a synthesis of thymidine from 5-bromodeoxyuridine.

THERE are several difficulties encountered in attempting to use in synthesis lithium derivatives of heterocyclic compounds containing the —C=N— group, since the use of organolithium compounds depends on their reactivity towards multiple bonds between carbon and hetero atoms. Thus in pyridines, at room temperature, organolithium compounds add very readily to the azomethine linkage,[1] but fully aromatic lithium pyridyls can be prepared from the bromocompounds and butyllithium by halogen-lithium exchange at low temperatures (−35°)[2] at which the rate of the azomethine addition reaction is greatly reduced. In certain compounds such as 4:5-dimethylthiazole,[3] 1-phenyl-3-methylpyrazole[4] and 1-substituted imidazoles,[5] lithium derivatives can be prepared at room temperature, and the azomethine linkage appears to be less reactive.

In pyrimidines, the azomethine linkages are *meta* to each other and the carbon atoms adjacent to nitrogen are therefore particularly susceptible to nucleophilic attack. This is seen when one compares the reaction of organolithium compounds with α- and β-picoline type derivatives and with methylpyrimidines respectively. Picolines metallate in the methyl group; for example, with phenyllithium, α-picoline gives 2-pyridylmethyllithium. On the other hand, pyrimidines with a methyl group in an analogous (2, 4 or 6) position usually react by addition, as in the following example:[6]



Pyrimidines would be expected to polymerise in attempted preparations of lithium derivatives, although the use of sufficiently low temperatures may minimise this, as

* This paper was presented in part, at the 131st National Meeting of the American Chemical Society, Miami, Fla., April (1957); and at the XVIth International Congress of Pure and Applied Chemistry, Paris, July (1957).

† This work was supported by a grant from the National Cancer Institute, U.S. Public Health Service.

‡ Present address: University Chemical Laboratory, Lensfield Road, Cambridge.

[1] K. Ziegler and H. Zeiser, *Ber.* **63**, 1847 (1930).
[2] H. Gilman and S. M. Spatz, *J. Amer. Chem. Soc.* **62**, 446 (1940).
[3] M. Erne and H. Erlenmeyer, *Helv. Chim. Acta* **31**, 652 (1948).
[4] H. R. Snyder, F. Verbanac and D. B. Bright, *J. Amer. Chem. Soc.* **74**, 3243 (1952).
[5] D. A. Shirley and P. W. Alley, *J. Amer. Chem. Soc.* **79**, 4922 (1957).
[6] T. D. Heyes and J. C. Roberts, *J. Chem. Soc.* 328 (1951).

was shown by Langley,[7] for example, in a synthesis of orotic acid-$C^{14}O_2H$ from 4-bromo-2:6-diethoxypyrimidine by halogen-lithium exchange and reaction with carbon dioxide.

A very interesting point, not previously discussed, is that pyrimidine-lithium compounds have a pronounced orange colour. This can perhaps be explained by analogy with the spectrum of pyridine, which shows in addition to the bands present in benzene, a band due to an $n \rightarrow \pi$ transition (the lone pair of electrons on nitrogen shifting to an anti-bonding orbital). In acid solution, of course, this band disappears. In the dimethoxy-pyrimidine-lithium compound, the electrons of the C—Li bond will be almost entirely localised on the C atom and easily excited, so that the colour may be ascribed to a $\sigma \rightarrow \pi$ transition. If so, replacement of the methoxyl groups in the ring by more electronegative groups should shift the absorption to longer wavelengths.*

New evidence on the mechanism of the halogen-metal exchange reaction, by which heterocyclic compounds are usually prepared, has been recently presented and discussed,[8] and is consistent with the idea that the exchange takes place via an addition complex and without any change in the configuration of the carbon atom at which reaction takes place.

The work to be described depends on two reactions, halogen-metal exchange (1), and the coupling reaction in which the organic radicals join and the lithium halide is formed (2):

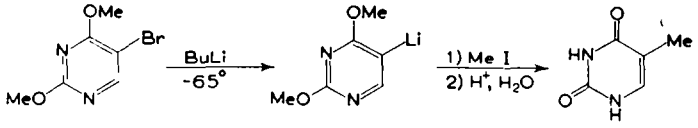
$$RLi + R'X \qquad (1)$$

$$RX + R'Li \rightleftharpoons R{-}R' + LiX \qquad (2)$$

Reaction (1), where X = H, depends on the relative acidities of RH and R'H; the initial reaction appears to be the nucleophilic attack on hydrogen (of RH) by the anion (R'^-) of the reagent.[9,10] When X = halogen, the reaction is an equilibration between the lithium salts of the two weak acids, RH and R'H, and therefore largely depends on the relative stabilities of R^- and R'^-. Pyrimidine-lithium compounds are therefore readily formed by reaction (1).

Reaction (2) depends mainly on the nature of the halogen. The reaction is negligible when X = Cl, and in general iodides have to be used, as in the present work.

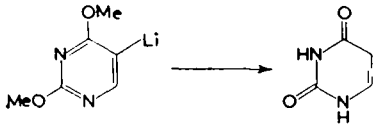
Thymine has now been synthesised employing these basic reactions by the conversion of 5-bromo-2:4-dimethoxypyrimidine to the lithium derivative, reaction with methyl iodide, and hydrolysis:



* The author thanks Prof. H. C. Longuet-Higgins for a discussion of this point.

[7] B. W. Langley, *J. Amer. Chem. Soc.* **78**, 2136 (1956).
[8] G. Wittig and U. Schollkopf, *Tetrahedron* **3**, 93 (1958).
[9] D. Bryce-Smith, *J. Chem. Soc.* 1079 (1954).
[10] D. Bryce-Smith, V. Gold and D. P. N. Satchell, *J. Chem. Soc.* 2473 (1954).

However, paper chromatography showed that the product was contaminated by uracil, formed by reaction of water with pyrimidine-lithium compound which had not reacted with methyl iodide:



By carbonation before treating the reaction mixture with water, the formation of uracil was much reduced; any uracil-5 carboxylic acid formed is easily separated from thymine. Thymine-$C^{14}H_3$, for which this synthesis was designed, was prepared in 40 per cent yield from $C^{14}H_3I$. Previously, methyl-labelled thymine was only available from a five-step synthesis, with a yield of purified material of 6·4 per cent.[11] From the same pyrimidine-lithium intermediate and ethyl iodide, 5-ethyluracil, otherwise not readily available, has been synthesised, and seems to merit biological testing as an analogue of thymine.

In order that very pure thymine could be prepared by this method, the separation of appreciable quantities of thymine and uracil was investigated. The method of Cohn[12] was found to give almost no separation, but when Dowex-1-chloride was used and elution begun with an ammonium hydroxide–ammonium chloride solution of high pH, uracil was eluted. After changing to ammonium chloride as eluant, pure thymine was obtained.

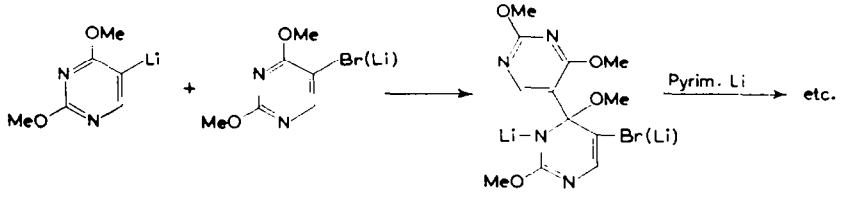
An unexpected finding was that the yield in the above type of reaction depended on the method of preparation of the butyl-lithium used (although in all cases this was estimated by double titration). The yield was higher when butyllithium was prepared from butyl chloride* than when it was prepared from butyl bromide. One possible explanation is that there is unreacted butyl bromide present, furthering a reaction of type (2):

$$\text{Pyrim. Li} + \text{BuBr} \rightarrow \text{Pyrim. Bu} + \text{LiBr}$$

which in any case must occur to some extent, since butyl bromide is a product of reaction (1):

$$\text{Pyrim. Br} + \text{BuLi} \rightarrow \text{Pyrim. Li} + \text{BuBr}$$

However, the principal side-reaction—perhaps, in fact, the major reaction—is the formation of polymeric compounds by the addition of the pyrimidine-lithium compound across an azomethine linkage in another molecule:



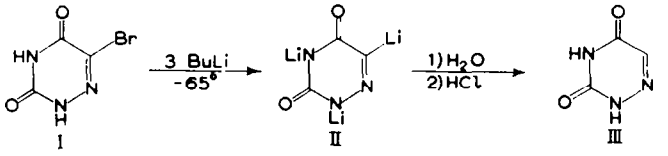
These reactions are very sensitive to traces of impurity. In the case of the lithium

* Sometimes this reaction fails completely, an observation also made by other investigators, though not recorded in the literature. Invariably the lithium metal was responsible.

[11] R. B. Henderson, R. M. Fink, and K. Fink, *J. Amer. Chem. Soc.* **77**, 6381 (1955).
[12] W. E. Cohn, *Science* **109**, 377 (1949).

alkenyls,[13,14] the important factor was the purity of the halide from which they were prepared. In the present instance, the purity of the halide (e.g. methyl iodide) used in the subsequent coupling reactions seems to be more important. Whereas Grignard reactions are catalysed by traces of iodine, the use of impure methyl iodide containing iodine greatly reduces the yield with pyrimidine-lithium compounds. (Radioactive methyl iodide decomposes very quickly, and should be supplied and used dissolved in an inert anhydrous solvent.) It was observed that iodine catalyses the formation of polymeric products from butyllithium in ether, and it seems to have a similar effect on pyrimidine-lithium compounds.

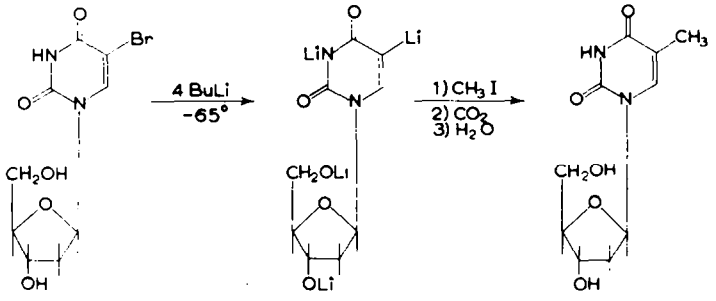
In the reactions so far described, alkoxypyrimidines have been used, with later hydrolysis to yield the products with active hydrogen atoms. But these reactions can be carried out without protecting the active hydrogens in the ring, as was first shown by Chang and Ulbricht[15] in the case of 5-bromo-6-azauracil (I):



I II III

6-Azauracil (III) was obtained in 17 per cent yield in this reaction, probably the first employing an intermediate containing more than two lithium atoms. (Some reactions have previously been carried out with compounds containing *one* active hydrogen atom, e.g. bromo-naphthols.[16]) Similarly, 5-bromouracil can be converted to uracil, or to thymine, as indicated by paper chromatography and ultra-violet spectra.

The synthesis of thymidine from 5-bromodeoxyuridine has been studied with a view to developing a method for the preparation of C^{14}-labelled thymidine, otherwise only available by enzymatic synthesis.[17]



IV

The main difficulty in this synthesis is that it is a heterogeneous reaction. The tetra-lithium intermediate (IV) is insoluble in tetrahydrofuran (as is trilithium uracil) and also in other solvents which can be used for this reaction (ether, dioxan–ether, dioxan–tetrahydrofuran, dimethoxyethane), whereas the other lithium derivatives described, including the trilithiumazauracil (II), are soluble. Chromatography of the

[13] E. A. Braude and C. J. Timmons, *J. Chem. Soc.* 2000 (1950).
[14] E. A. Braude and J. C. Coles, *J. Chem. Soc.* 2014 (1950).
[15] P. K. Chang and T. L. V. Ulbricht, *J. Amer. Chem. Soc.* **80**, 976 (1958).
[16] S. V. Sunthankar and H. Gilman, *J. Org. Chem.* **16**, 8 (1951).
[17] M. Friedkin and D. Roberts, *J. Biol. Chem.* **207**, 257 (1954).

products showed the presence of considerable quantities of deoxyuridine and of unreacted 5-bromodeoxyuridine in addition to thymidine, and very little material having the properties of a compound containing N^1-methyl or O-methyl groups. The yield of thymidine was therefore essentially limited by (1) incomplete halogen-lithium exchange, and (2) incomplete reaction with methyl iodide, but nevertheless it appeared that at the temperature of the reaction a real distinction between the reactivity of lithium-carbon bonds as against that of lithium-nitrogen and lithium-oxygen bonds had been achieved. This is presumably due to the high nucleophilicity of a carbon atom having an appreciable negative charge.

Tetrahydrofuran proved to be the best solvent for this reaction. Formation of deoxyuridine was reduced by carbonation after allowing time for the methyl iodide to react, but could not be avoided completely. Various methods were tried for the separation of deoxyuridine and thymidine on ion-exchange columns, including gradient elution with formate and phosphate buffers on Dowex-1-formate, but satisfactory separation was not achieved. The purification was carried out in three stages: (1) removal of salts by washing on Dowex-1-formate; (2) separation from deoxyuridine, 5-bromodeoxyuridine, 5-carboxydeoxyuridine and other impurities by paper chromatography using the ethyl acetate–phosphate buffer system;[18] and (3) final chromatography to remove impurities from the paper and salts, on Dowex-1-formate.

The yield of thymidine, whose purity was also checked by biological assay, was 10 per cent. For a reaction of this type, this seemed satisfactory. A lower yield was obtained in one synthesis using C^{14}-methyl iodide. The conditions of this reaction were somewhat different. The radioactive methyl iodide was not supplied in solution and had partially decomposed, and the reaction had to be run on a larger scale without opportunity of a cold run. In a heterogeneous reaction, this may well affect the yield. On the scale originally used, with pure methyl iodide, the yield is reproducible, and this represents a convenient chemical method for the synthesis of thymidine-$C^{14}H_3$.

It is possible that the methods described in this paper could be used to synthesise pyrimidine (or other heterocyclic) C-glycosides from suitable lithium intermediates and glycosyl halides. The feasibility of such a method would depend on the relative reactivities of the halogen and the ester groups in the glycosyl halide at the low temperature of the reaction.

EXPERIMENTAL*

Solvents and reagents. Ether and tetrahydrofuran were distilled from lithium aluminium hydride, using a Vigreaux column. *n*-Butyl bromide and *n*-butyl chloride were dried for 30 min over phosphorus pentoxide, decanted and distilled. Methyl and ethyl iodide were freshly distilled.

n-*Butyllithium* was prepared from *n*-butyl bromide[19] or from *n*-butyl chloride.[20] In the latter case, freshly-prepared lithium wire had to be used to obtain consistent results. The butyllithium was estimated by double titration.[21]

Thymine, using butyllithium prepared from butyl bromide. An ethereal solution of butyllithium (0·53 N, 5 ml) was added in one min to a stirred solution of 5-bromo-2:4-dimethoxypyrimidine[22]

* Melting points are uncorrected. Analysis by Huffman Microanalytical Laboratories, Wheatridge, Colorado.

[18] W. H. Prusoff, *J. Biol. Chem.* **215**, 809 (1955).
[19] H. Gilman, J. A. Beel, C. G. Branner, M. W. Bullock, G. E. Dunn and L. S. Miller, *J. Amer. Chem. Soc.* **71**, 1499 (1949).
[20] H. Gilman, E. A. Zoellner and W. M. Selby, *J. Amer. Chem. Soc.* **54**, 1957 (1932).
[21] H. Gilman and A. H. Haubein, *J. Amer. Chem. Soc.* **66**, 1515 (1944).
[22] G. E. Hilbert and E. F. Jansen, *J. Amer. Chem. Soc.* **56**, 134 (1934).

(580 mg) in tetrahydrofuran (10 ml) at −65° (dry ice-*iso*propanol) in an atmosphere of nitrogen. Methyl iodide (380 mg) in ether (5 ml) was added to the orange-coloured solution, discharging most of the colour. After stirring for 10 min at −65°, excess dry ice was added, the cooling-bath removed, and stirring continued for 1 hr. The solution was poured into water (10 ml) and extracted with ether, sodium chloride and a few crystals of sodium thiosulphate being added to the aqueous layer. After drying over anhydrous magnesium sulphate, the ether was removed by blowing dry air onto the surface of the solution. The residue was refluxed with hydrochloric acid (6 N, 4 ml) for 2 hr, evaporated to dryness, and sublimed *in vacuo*, leaving a considerable residue of polymeric material. Resublimation at 200°/12 mm† gave thymine, m.p. and mixture m.p. 314–316°. Yield, 95 mg (29%). The yield in other runs was always 25–30%.

Thymine-$C^{14}H_3$, *using butyllithium prepared from butyl chloride.* The procedure was as above, using ethereal butyllithium (0·81 N, 3·4 ml, 2·7 m moles), 5-bromo-2:4-dimethoxypyrimidine (602 mg, 2·5 mM), tetrahydrofuran (10 ml), methyl iodide-C^{14} (New England Nuclear Co. 358 mg, 2·5 mM, 5 mC, 1·97 mC/mM) in ether (5 ml) transferred by pipette. Yield of resublimed product, 158 mg (40%). Specific activity, 1·95 mC/mM. UV spectrum: max/min 264/233 mμ (pH 7) (cf. thymine, 264/233; uracil, 259/227 mμ[23]). Paper chromatography confirmed the identity of thymine, and indicated the presence of a trace of (non-radioactive) uracil.

5-Ethyluracil. The same procedure was used, with ethereal butyllithium (0·62 N, 5 ml, from butyl bromide), 5-bromo-2:4-dimethoxypyrimidine (555 mg), tetrahydrofuran (10 ml), ethyl iodide (792 mg, 2 moles) in ether (5 ml). The yield of sublimed material was 110 mg (32%). A portion was recrystallised from methanol and resublimed, m.p. 301–303° dec.

(Found: C, 51·7; H, 5·8; N, 20·1. Calc. for $C_6H_8N_2O_2$: C, 51·4; H, 5·8; N, 20·0%).

Separation of thymine and uracil. Thymine (30 mg) and uracil (30 mg) were dissolved in aqueous sodium hydroxide (0·1 N, about 30 ml) and put on a column of Dowex-1-chloride (40 × 1 cm). The eluting solution was made up as follows: 1 volume of ammonium chloride (0·2 N) was diluted with 9 vols of ammonium hydroxide (0·23 N) to give a solution 0·02 N in chloride ion. This solution (pH ~ 10·4) was adjusted to pH 12·2 with sodium hydroxide (6 N). The eluate was collected at the rate of about 20 ml every 30 min. After 1 l. had been collected, uracil began to come off the column (this was followed by optical density at 280 mμ). When the uracil had come off, the eluting solution was changed to ammonium chloride (0·025 N), and after about 200 ml the thymine came off in about 60 ml. The recovery of thymine was 85 per cent, as estimated by optical density, and was purer than that used at the beginning of the experiment.

Uracil from 5-bromouracil. 5-Bromouracil (dried at 80° *in vacuo* 95·5 mg, 0·5 mM) was dissolved in hot dioxan (purified, 15 ml), ether (15 ml) and N-ethylmorpholine (purified, 10 ml) added, the solution cooled to −50° in an atmosphere of nitrogen, ethereal butyllithium (0·38 N, 4·3 ml, 1·65 mM, from butyl chloride) added, giving a white precipitate. Water (2 ml) was added, stirred vigorously, and the cooling bath removed. Paper chromatography and UV spectra showed the presence of uracil (~15%) and unchanged 5-bromouracil.

Thymine from 5-bromouracil. The same method was used, with tetrahydrofuran as solvent. After reaction with methyl iodide, water was added without prior carbonation. Paper chromatography revealed the presence of uracil, thymine (~15%), and 5-bromouracil.

Thymidine. 5-Bromodeoxyuridine (Cyclo Chemical Corp. dried at room temp *in vacuo*, 153·5 mg, 0·5 mM) was dissolved in hot tetrahydrofuran (15 ml) and cooled to −60° in an atmosphere of nitrogen. Ethereal butyllithium (0·71 N, 3·1 ml, 2·2 mM, from butyl chloride) was added to the stirred solution, giving a white precipitate. Methyl iodide (71 mg, 0·5 mM) in ether (5 ml) was added, and the mixture stirred for 20 min while the temp rose to −35°. Excess dry ice was added and the mixture stirred until the temp rose to 0°. The yellow solution was poured into water and dry air blown onto the surface to remove tetrahydrofuran. Ammonium hydroxide solution was added (pH 11, 30 ml) and put on a Dowex-1-formate column (10 × 2 cm) which was successively eluted with ammonium hydroxide (pH 11, 400 ml), water (250 ml), 0·001 N formic acid (200 ml) and finally 0·02 N formic acid. Elution was followed by optical density at 260 mμ and the pyrimidine nucleosides began to come off the column 250 ml after changing to 0·02 N formic acid. The solution containing the nucleosides was lyophilised, the residue dissolved in a little water and put on three 30-inch sheets of Whatman No. 3 paper and run in ethyl acetate–phosphate buffer for 6 hr. The thymididne

† Uracil-5-carboxylic acid does not sublime or decarboxylate under these conditions.

[23] D. Shugar and J. J. Fox, *Biochim. Biophys. Acta* **9**, 199 (1952).

areas were cut out, cut into small pieces, and extracted in a mixing blender with ammonium hydroxide (pH 11, 2 × 200 ml). The filtered solution was put on a Dowex-1-formate column and eluted with water and formic acid as before, to give 365 ml of solution with a maximum optical density of 1·37 at 267 mμ. This represents a yield of 10·3%. The product had the right UV spectrum, max/min 267/235 acid, 267/245 mμ base, and in five solvent systems (butanol–water, butanol–ammonia, *iso*propanol–hydrochloric acid, *iso*propanol–ammonia, ethyl acetate–phosphate buffer) gave a single spot, with the same R_F as a sample of authentic thymidine run concurrently. Biological assay (*Streptococcus faecalis*) indicated a purity of at least 90 per cent.

Acknowledgements—The author thanks Dr. A. D. Welch for his interest and encouragement, and his colleague Dr. W. H. Prusoff for advice concerning chromatographic methods, and for the biological assay of thymidine.