523. *Experiments on the Synthesis of Purine Nucleosides. Part XXVI.* 9-D-*Glucopyranosido*isoguanine.

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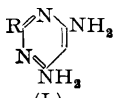
A variety of theoretically possible methods for the adaptation, to the synthesis of guanine and *iso*guanine glycosides, of the general synthetic route to purine nucleosides developed in earlier papers of this series has been investigated. Direct preparation of guanine glycosides by modification or replacement of groups at position 2 in other substituted purine glycosides proved impracticable. In model experiments, the preparation of *iso*guanine and 9-*methyl*isoguanine was, however, effected from 2-*methylsulphonyladenine* and 2-*methylsulphonyl*-9-*methyladenine* by replacement of the methylsulphonyl residue by hydroxyl, either directly by sodium hydroxide or indirectly by preliminary conversion into the corresponding 2-*ethoxy*- or 2-*benzyloxy*-compounds. Application of these findings to 2-*methylsulphonyl*-9-(*tetra-acetyl* D-*glucopyranosido*)*adenine* resulted in a synthesis of 9-D-*glucopyranosido*isoguanine.

The first step in the general synthesis of purine nucleosides developed in earlier papers of this series is the formation of a derivative of 6-amino-4-glycosidaminopyrimidine. In its original form, the method employed was simple condensation of a sugar with a suitable 4 : 6-diaminopyrimidine (Baddiley, Lythgoe, and Todd, *J.*, 1943, 571); the later method, designed to ensure the production of furanoside, in which a Schiff base is first prepared from an aldehydosugar and a 4 : 6-diaminopyrimidine (Kenner, Lythgoe, and Todd, *J.*, 1948, 957; Kenner, Rodda, and Todd, this vol., p. 1613; Kenner, Taylor, and Todd, *ibid.*, p. 1620) is a variant of the original, and the pyrimidine derivatives employed are subject to the same structural limitations. It was pointed out (Baddiley, Lythgoe, and Todd, *loc. cit.*) that only those 4 : 6-diaminopyrimidines (I) would condense with sugars which bore in position 2 a substituent R incapable of taking part in prototropic change (*e.g.*, H, Me, SMe). This was attributed to the fact that in such cases one of the groups at positions 4 and 6 would show in considerable degree the properties of a true amino-group, whereas if a group capable of participating in prototropic change (*e.g.*, OH, SH, NH_2) were present in position 2 both groups in positions 4 and 6 would react mainly in the tautomeric imino-form and would fail to condense with a sugar. This being so, it was clear that the successful application of the method to nucleosides containing in position 2 such groups as NH_2 or OH (*e.g.*, glycosides of guanine or *iso*guanine) would depend on the introduction of these groups either by direct substitution or by replacement of another group at some stage after attachment of the sugar residue. Experiments were early initiated to find a suitable solution of this problem, but it proved more elusive than was at first expected. The present paper presents the results of these studies and their successful outcome exemplified by the synthesis of 9-D-*glucopyranosido*isoguanine (6-*amino*-2-*hydroxy*-9-D-*glucopyranosidopurine*) (II; R = OH, R′ = D-glucosidyl).

Since it is known that a chlorine atom at position 2 in a purine glucoside can be replaced directly by an amino-group (Fischer and Helferich, *Ber.*, 1914, **47**, 210), the simplest solution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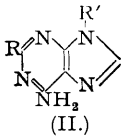
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of the problem appeared to be to use 2-chloro-4 : 6-diaminopyrimidine as starting material in nucleoside syntheses and replace the chlorine atom in the final product. This chloro-compound is not described in the literature and we could find no convenient method of preparing it. 4 : 6-Diamino-2-hydroxypyrimidine did not dissolve in boiling phosphoryl chloride, and addition of dimethylaniline to the mixture brought about breakdown of the pyrimidine ring system. Partial amination of 2 : 4-dichloro-6-aminopyrimidine gave only 4-chloro-2 : 6-diaminopyrimidine. The possibility of introducing an amino-group at position 2 in an adenine glycoside was examined in model experiments with adenine and 9-methyladenine, but under conditions which readily yield 2-aminopyridine from pyridine no appreciable reaction occurred between either of these compounds and sodamide in dimethylaniline.

Attention was next turned to the possibility of using as starting material in the general synthesis a 2-substituted 4 : 6-diaminopyrimidine (I) in which R was a substituted amino-group such that condensation of one of the free amino-groups with a sugar would succeed and from which the substituent(s) could later be removed giving finally a purine glycoside with a 2-amino-group. The use of an acylamino-group at $C_{(2)}$ was not investigated as attempts to prepare 4 : 6-diamino-2-acetamidopyrimidine for model experiments failed. Failure to obtain the desired 2-chloro-4 : 6-diaminopyrimidine also balked our efforts to use a substituted hydrazino-group at $C_{(2)}$ which might have been split later by hydrogenation. 4 : 6-*Dichloro*-2-(2′-*phenyl*-2′-*methylhydrazino*)*pyrimidine* was prepared by interaction of 2 : 4 : 6-trichloropyrimidine with 1-phenyl-1-methylhydrazine, but attempts to replace the chlorine atoms by amino-groups failed; direct ring synthesis of a suitable compound appeared impracticable as substituted amidinohydrazines did not condense with malononitrile to give pyrimidines. We were similarly unable to prepare 4 : 6-diamino-2-benzylaminopyrimidine by amination of 4 : 6-*dichloro*-2-*benzylaminopyrimidine* which was itself prepared by interaction of 2 : 4 : 6-trichloropyrimidine with benzylamine or by treatment of 2-*benzylamino*-4 : 6-*dihydroxypyrimidine* with phosphoryl chloride. 4-*Amino*-2-*benzylamino*-6-*hydroxypyrimidine*, prepared by condensation of benzylguanidine with ethyl cyanoacetate, could not be converted into a glycoside. This fact suggested that it might be necessary to have a disubstituted amino-group in position 2 if condensation with a sugar were to succeed, and experiments to prepare and examine such compounds were undertaken. Although the most suitable group seemed to be 2-dibenzylamino-, we also prepared compounds containing 2-dimethylamino- and 2-4′-morpholino-groups for comparison. 4 : 6-*Diamino*-2-*dibenzylaminopyrimidine* and 4-*amino*-2-*dibenzylamino*-6-*hydroxypyrimidine* were synthesised by condensation of *NN*-dibenzylguanidine (prepared as its *hydrochloride*) with malononitrile and ethyl cyanoacetate respectively. In similar fashion, 4 : 6-*diamino*-2-4′-*morpholinopyrimidine*, 4-*amino*-2-4′-*morpholino*-6-*hydroxypyrimidine*, 4 : 6-*diamino*-2-*dimethylaminopyrimidine*, and 4-*amino*-2-*dimethylamino*-6-*hydroxypyrimidine* were prepared by use of the same esters and 4-amidinomorpholine (isolated as its *hydrochloride*) and *NN*-dimethylguanidine. In trial experiments, it was observed that 4 : 6-diamino-2-dibenzylaminopyrimidine, 4 : 6-diamino-2-4′-morpholinopyrimidine, and 4 : 6-diamino-2-dimethylaminopyrimidine condensed with glucose in boiling ethanolic solution in presence of an acidic catalyst to give syrupy products which were undoubtedly glycosidic. In the case of the 2-dimethylamino-derivative, the nature of the condensation product was confirmed by coupling it with diazotised 2 : 5-dichloroaniline, the crystalline 6-*amino*-4-D-*glucosidamino*-2-*dimethylamino*-5-(2′ : 5′-*dichlorobenzeneazo*)*pyrimidine* being obtained. Work on the 2-dibenzylamino-compounds was not further pursued, however, since it was found impossible to remove the benzyl residues by hydrogenation either from 4 : 6-diamino-2-dibenzylaminopyrimidine or from 2-*dibenzylaminoadenine* synthesised from it.

The failure to find any solution to the basic problem by the above methods caused us to reconsider the possibility of replacing a 2-methylthio-substituent by NH_2 or OH. This we had considered at an early stage because of its obvious advantages should the replacement be easily effected; in particular it had the advantage that 4 : 6-diamino-2-methylthiopyrimidine

was more reactive in condensations with sugar derivatives than any of the other 4 : 6-diaminopyrimidines we had used in nucleoside syntheses. This approach had, however, been temporarily suspended when it was found that direct replacement of the methylthio-group in 4 : 6-diamino-2-methylthiopyrimidine by amino could not be effected with aqueous or alcoholic ammonia even at high temperatures. Several related compounds, in which the alkylthio-group was modified or the pyrimidine ring so substituted in position 5 that reactivity towards ammonia would be increased without rendering the compounds themselves worthless in nucleoside synthesis, had also been examined. Thus 4 : 6-*diamino*-2-*methylsulphonylpyrimidine*, 4-*amino*-6-*hydroxy*-2-*carbethoxymethylthiopyrimidine*, 4-*amino*-6-*hydroxy*-2-(*dicarbethoxymethylthio*)*pyrimidine*, and 5-*nitro*-4-*amino*-6-*hydroxy*-2-*methylthiopyrimidine* all failed to undergo the desired reaction with ammonia under a variety of conditions. These failures were at the time unexpected and we have no satisfactory explanation to offer. This unaccountable difficulty in replacement of alkylthio-groups in some pyrimidine derivatives has, of course, been noted on more than one occasion (cf. Curd and Rose, *J.*, 1946, **343**; Hull, Lovell, Openshaw, and Todd, *J.*, 1947, **41**). In taking up the problem afresh, it seemed desirable to carry out experiments on the replacement of the methylthio-group in 2-methylthioadenine and 2-methylthio-9-methyladenine as models for the 2-methylthioadenine glycosides. An exhaustive series of experiments designed to replace the methylthio-group directly with an amino- or substituted amino-group was carried out but without any success. No reaction occurred with saturated alcoholic ammonia below about 230°, and above this temperature only brown decomposition products were obtained. No replacement could be effected when sodamide was used under various conditions, with aniline or with methylamine + methylamine hydrochloride at 170°, or with ammonium chloride or acetamide at 160°; a mixture of aniline and aniline hydrochloride at 170° caused decomposition with formation of an unidentified substance (m. p. 255°) of empirical formula $C_8H_{10}N_4S$. Indirect methods of bringing about the replacement of $CH_3S\cdot$ by $NH_2\cdot$ such as conversion into an *iso*thiocyanate or a sulphone before treatment with ammonia were also unsuccessful.

The general conclusion drawn from all these experiments was that, although very attractive from the standpoint of easy production of guanine glycosides, the simple replacement of an acceptable substituent (other than halogen) in position 2 by $NH_2\cdot$ in suitable pyrimidine or purine derivatives was impracticable; we therefore examined the conversion of 2-methylthioadenine derivatives to *iso*guanine (2-hydroxyadenine) derivatives as a reasonable alternative, since the further conversion of *iso*guanine into guanine derivatives, although perhaps laborious, seemed feasible. Boiling with chloroacetic acid under reflux had no effect on 2-methylthio-9-methyladenine, although Johnson and Hill (*J. Amer. Chem. Soc.*, 1914, **36**, 372) used this method for replacing a 2-ethylthio-group in pyrimidine derivatives by hydroxyl. Sprague and Johnson (*J. Amer. Chem. Soc.*, 1935, **57**, 423) showed that a 2-methylsulphonyl-group in several pyrimidine derivatives resembled a halogen atom in its ease of replacement by OEt, OH, or NH_2. 2-*Methylsulphonyladenine* (II; R = $MeSO_2$, R′ = H) was readily prepared by the action of chlorine in aqueous solution on 2-methylthioadenine; although it did not react with ammonia, it was smoothly converted into *iso*guanine (II; R = OH, R′ = H) by boiling it under reflux with dilute aqueous sodium hydroxide.

When treated with chlorine in aqueous solution, preferably at room temperature, 2-methylthio-9-methyladenine yielded a crystalline product containing chlorine, considered to be 6-*chloroamino*-2-*methylsulphonyl*-9-*methylpurine*, since it gave 2-*methylsulphonyl*-9-*methyladenine* (II; R = $MeSO_2$, R′ = Me) by dissolution in boiling water and addition of aqueous ammonia, sodium hydrogen sulphite, or sodium dithionite. 2-Methylsulphonyl-9-methyladenine was also obtained, albeit in poorer yield, from the corresponding 2-methylthio-compound by treatment with hydrogen peroxide in acetic acid; oxidation of the 2-methylthio-compound with chlorine in dioxan gave the unstable 2-*methylsulphinyl*-9-*methyladenine* (II; R = MeSO, R′ = Me). On being heated with dilute aqueous sodium hydroxide, 2-methylsulphonyl-9-methyladenine yielded 9-*methyliso*guanine (II; R = OH, R′ = Me); this replacement proceeded much more readily than with the unsubstituted 2-methylsulphonyladenine. The same product was obtained by converting the sulphone into 2-ethoxy-9-methyladenine by heating it with ethanolic sodium ethoxide and dealkylating the product with hydrobromic acid, or with ethyl chloroformate in ethanolic solution (reaction in the latter case proceeding by initial formation of an unstable quaternary salt). Since a benzyloxy-group which would undergo fission by hydrogenation was likely to be of more general use than an ethoxy-group, 2-*benzyloxy*-9-*methyladenine* (II; R = $CH_2Ph\cdot O$, R′ = Me) was prepared by treating the 2-methylsulphonyl-compound with one molecular proportion of sodium benzyloxide. When excess of sodium

benzyloxide was used, the product obtained appeared to be 6-*benzylamino*-2-*benzyloxy*-9-*methylpurine*, the 6-amino-group being simultaneously benzylated; treated with hydrochloric acid in acetic acid this compound yielded 6-*benzylamino*-2-*hydroxy*-9-*methylpurine*. Catalytic hydrogenation of 2-benzyloxy-9-methyladenine in an acid medium yielded, as expected, 9-methyl*iso*guanine.

The next step was to apply these methods to the synthesis of an *iso*guanine glycoside so as to test their validity in the nucleoside field, and 9-D-glucopyranosido-2-methylthioadenine was selected as a suitable starting material. This substance was first prepared by Holland, Lythgoe, and Todd (*J.*, 1948, 965), who obtained it, with difficulty and in poor yield as an amorphous powder by applying the standard synthetic procedures of nitrosation, reduction, thioformylation, and ring-closure to 6-amino-4-D-glucosidamino-2-methylthiopyrimidine. The reason for this difficulty is unknown, but repetition of the method described furnished the crystalline glucoside (m. p. 269—271°) in excellent yield. On acetylation it yielded a crystalline *tetra-acetate*, whose identity was confirmed by treatment in ethanolic solution with Raney nickel followed by deacetylation, to yield 9-D-glucopyranosidoadenine. Oxidising agents such as chlorine and hydrogen peroxide attacked both the glucose residue and the methylthio-group in 9-D-glucopyranosido-2-methylthioadenine, but the tetra-acetyl derivative was oxidised readily with hydrogen peroxide in acetic acid to give 9-(*tetra-acetyl* D-*glucopyranosido*)-2-*methylsulphonyladenine* [II; R = $MeSO_2$, R′ = $C_6H_7O(OAc)_4$]. When this sulphone was heated under reflux for a short time with aqueous sodium hydroxide, 9-D-*glucopyranosido*iso-*guanine* (II; R = OH, R′ = $C_6H_{11}O_5$) was obtained. Attempts to prepare 9-D-glucopyranosido-2-benzyloxyadenine were less successful. From the model experiments on 2-methylthio-9-methyladenine discussed above, it was clear that only one molecular equivalent of sodium benzyloxide should be used if benzylation of the 6-amino-group were to be avoided. To avoid complications, the tetra-acetyl sulphone [II; R = $MeSO_2$, R′ = $C_6H_7O(OAc)_4$] was first deacetylated with sodium methoxide in the cold, and the product treated with one equivalent of sodium benzyloxide in dimethylformamide. An amorphous material was obtained which was free of sulphur and, from its ultra-violet absorption spectrum, appeared to consist largely of the benzyloxy-compound, but it could not be purified to give a homogeneous product.

Experimental.

4 : 6-*Dichloro*-2-(2′-*phenyl*-2′-*methylhydrazino*)*pyrimidine*.—2 : 4 : 6-Trichloropyrimidine (30 g.; prepared according to Baddiley and Topham, *J.*, 1945, 678) was dissolved in ethanol (60 c.c.), and 1-phenyl-1-methylhydrazine (43 c.c.) added. The mixture was set aside for 2 days, a crystalline precipitate gradually forming. This was collected and recrystallised from 2-ethoxyethanol; the *product* was obtained as colourless prisms (17 g.), m. p. 163—165° (Found: C, 49·2; H, 3·8; N, 20·0. $C_{11}H_{10}N_4Cl_2$ requires C, 49·1; H, 3·7; N, 20·8%). Replacement of the chlorine atom at position 2 is assumed, since this is known to be the most reactive halogen atom in 2 : 4 : 6-trichloropyrimidine (cf. Büttner, *Ber.*, 1903, **36**, 2227).

2-*Benzylamino*-4 : 6-*dihydroxypyrimidine*.—Benzylguanidine hydrochloride (9·3 g.) was added to ethanolic sodium ethoxide (3·5 g. of sodium in 45 c.c. of ethanol), and the mixture filtered from sodium chloride. Ethyl malonate (8 g.) was added to the filtrate which was then heated under reflux for 4 hours. The precipitate which had formed was collected and the mother-liquor evaporated, leaving a residue which was combined with the precipitate. Recrystallisation from aqueous acetic acid (50 c.c. of water + 6 c.c. of acetic acid) gave 2-*benzylamino*-4 : 6-*dihydroxypyrimidine* as colourless needles (5·7 g.), m. p. 270—272° (decomp.) (Found: C, 60·5; H, 5·3; N, 19·4. $C_{11}H_{11}O_2N_3$ requires C, 60·8; H, 5·1; N, 19·3%).

4 : 6-*Dichloro*-2-*benzylaminopyrimidine*.—(*a*) 2-Benzylamino-4 : 6-dihydroxypyrimidine (1 g.) was heated under reflux for 15 minutes with phosphoryl chloride (10 c.c.). The solution was evaporated to dryness under reduced pressure, and the residue recrystallised from ethanol, forming colourless needles (0·12 g.), m. p. 129—131° (Found: C, 52·0; H, 3·7; N, 16·2; Cl, 28·0. $C_{11}H_9N_3Cl_2$ requires C, 52·0; H, 3·6; N, 16·5; Cl, 27·9%).

(*b*) 2 : 4 : 6-Trichloropyrimidine (4·3 g.) was added to a solution of benzylamine (5 g.) in ethanol (8 c.c.), and the mixture set aside for 5 hours. The product, recrystallised from 80% ethanol, had m. p. 126—129°. The mixed m. p. with material prepared by method (*a*) was 128—131°.

4-*Amino*-2-*benzylamino*-6-*hydroxypyrimidine*.—Ethanolic sodium ethoxide (5 g. of sodium in 100 c.c. of alcohol) was added to a solution of benzylguanidine hydrochloride (20 g.) and ethyl cyanoacetate (12·2 g.) in ethanol (100 c.c.), and the mixture heated under reflux for 6 hours. Worked up in the usual way the *pyrimidine* crystallised from ethanol in colourless prisms (9·8 g.), m. p. 202—204° (Found: C, 61·2; H, 5·6; N, 26·0. $C_{11}H_{12}ON_4$ requires C, 61·1; H, 5·6; N, 25·9%).

NN-*Dibenzylguanidine Hydrochloride*.—Dibenzylamine hydrochloride (122 g.) was dissolved in boiling 2-ethoxyethanol (1530 c.c.), cyanamide (61 g.; dried over phosphoric oxide) and a solution of hydrogen chloride in 2-ethoxyethanol (12 c.c.; saturated at room temperature) were added, and the whole was heated under reflux for 4 hours. The solution was concentrated to a viscous syrup, and ethyl acetate (600 c.c.) was added. The colourless crystalline precipitate which separated was collected and recrystallised by heating it under reflux with *n*-butanol (110 c.c.), filtering, and adding ethyl acetate

(600 c.c.) to the hot filtrate. The *hydrochloride* separated as colourless prisms (64·2 g.), m. p. 193—195°, which were collected, washed with ether, and dried at 90° (Found: C, 65·2; H, 6·8; N, 15·3. $C_{15}H_{18}N_3Cl$ requires C, 65·3; H, 6·6; N, 15·2%).

4-*Amidinomorpholine Hydrochloride*.—*S*-Methyl*iso*thiourea sulphate (139 g.), water (367 c.c.), and morpholine (90 c.c.) were placed in a flask fitted with a reflux condenser to which was attached an efficient mercaptan trap. The mixture was slowly heated to boiling and then caused to reflux for 5 minutes. A hot solution of barium chloride (122 g.) in water (250 c.c.) was added. The whole was next heated on the steam-bath for 30 minutes and filtered through kieselguhr. Evaporation of the filtrate under reduced pressure gave a gum which was dissolved in hot ethanol (100 c.c.), and acetone (750 c.c.) was added. The *hydrochloride* which separated (84 g.) was recrystallised from a mixture of 2-ethoxyethanol and ethyl acetate and formed colourless prisms whose m. p. varied from 130° to 160° according to rate of heating (Found: C, 36·1; H, 7·2; N, 25·1. $C_5H_{12}ON_3Cl$ requires C, 36·3; H, 7·3; N, 25·4%).

4 : 6-*Diamino*-2-*dibenzylaminopyrimidine*.—Ethanolic sodium ethoxide (1·3 g. of sodium in 25 c.c. of alcohol) was added to a solution of *NN*-dibenzylguanidine hydrochloride (15·4 g.) and malononitrile (3·7 g.) in ethanol (45 c.c.), and the mixture heated under reflux for 6 hours. The product was isolated in the usual manner and purified by sublimation at $100°/10^{-3}$ mm. This *pyrimidine* formed colourless prisms (8·7 g.), m. p. 124·5—125·5° (Found: C, 70·5; H, 6·2; N, 23·2. $C_{18}H_{19}N_5$ requires C, 70·8; H, 6·3; N, 22·9%).

4 : 6-*Diamino*-2-4′-*morpholinopyrimidine*.—Prepared by the same method as the preceding compound from 4-amidinomorpholine hydrochloride (16·55 g.) and malononitrile (6·6 g.), this *compound* formed colourless needles (8·2 g.), m. p. 205—206°, from ethanol or water (Found: C, 49·2; H, 6·6; N, 36·2. $C_8H_{13}ON_5$ requires C, 49·2; H, 6·7; N, 35·9%).

4 : 6-*Diamino*-2-*dimethylaminopyrimidine*.—Prepared as above from *NN*-dimethylaminoguanidine hydrochloride (12·35 g.) and malononitrile (6·6 g.), the *product*, recrystallised from ethanol or purified by sublimation at $100°/10^{-4}$ mm., formed colourless prisms (6·5 g.), m. p. 261—263° (Found: C, 47·2; H, 6·9; N, 46·3. $C_6H_{11}N_5$ requires C, 47·0; H, 7·2; N, 45·7%).

4-*Amino*-2-*dibenzylamino*-6-*hydroxypyrimidine*.—Ethanolic sodium ethoxide (0·85 g. of sodium in 15 c.c. of ethanol) was added to *NN*-dibenzylguanidine hydrochloride (5 g.) and ethyl cyanoacetate (2·05 c.c.) in ethanol (15 c.c.), and the mixture boiled under reflux for 2 hours. The mixture was evaporated to dryness, the residue taken up in dilute sodium hydroxide and filtered from sparingly soluble material and the filtrate (*ca.* 150 c.c.) acidified with acetic acid. The precipitated *pyrimidine* recrystallised from water in colourless prisms (2·4 g.), m. p. 221—222° (Found: C, 70·7; H, 5·9; N, 18·2. $C_{18}H_{18}ON_4$ requires C, 70·6; H, 5·9; N, 18·3%). An unidentified crystalline substance, m. p. 183—185°, was obtained from the residue left on dissolving the crude reaction product in dilute sodium hydroxide.

4-*Amino*-2-4′-*morpholino*-6-*hydroxypyrimidine*, colourless prisms, m. p. 274—277°, from water (Found: C, 49·5; H, 6·1; N, 28·5. $C_8H_{12}O_2N_4$ requires C, 49·0; H, 6·2; N, 28·6%) and 4-*amino*-2-*dimethylamino*-6-*hydroxypyrimidine*, colourless needles, m. p. 289—293°, from water (Found: C, 46·9; H, 6·1; N, 35·9. $C_6H_{10}ON_4$ requires C, 46·7; H, 6·5; N, 36·3%) were prepared in similar fashion by using 4-amidinomorpholine and dimethylaminoguanidine, respectively.

6-*Amino*-4-D-*glucosidamino*-2-*dimethylamino*-5-(2′ : 5′-*dichlorobenzeneazo*)*pyrimidine*.—A mixture of 4 : 6-diamino-2-dimethylaminopyrimidine (15 g.), D-glucose (17·7 g.), ammonium chloride (0·5 g.), ethanol (100 c.c.), and benzene (100 c.c.) was stirred and heated in a flask fitted with a 2-ft. Fenske column and reflux ratio head so that slow distillation occurred, 100 c.c. distillate being collected during 43 hours. At the end of this time the solution was cooled and poured on a short column of activated alumina (1500 g.), the column being washed with ethanol (5 l.). The column was now eluted with water (4 l.), and the eluate evaporated under reduced pressure. The residual resin (20 g.) was dissolved in water (100 c.c.), and pyridine (24 c.c.) was added. To this solution cooled in ice was added a solution of diazotised 2 : 5-dichloroaniline (prepared in the usual manner from 10 g. of amine) until the diazonium salt was in slight excess. After 15 minutes, the precipitated *azo-glycoside* was collected and recrystallised from pyridine–ethanol, forming small golden yellow prisms, m. p. 258° (Found: C, 44·5; H, 5·0; N, 20·3. $C_{18}H_{23}O_5N_7Cl_2$ requires C, 44·3; H, 4·8; N, 20·1%).

4 : 6-*Diamino*-2-*dibenzylamino*-5-*nitrosopyrimidine*.—Aqueous sodium nitrite (10 c.c. of 10%) was added slowly with shaking to 4 : 6-diamino-2-dibenzylaminopyrimidine (2 g.), dissolved in a mixture of water (100 c.c.) and acetic acid (3 c.c.) at 15°. After 20 minutes the precipitate was collected and recrystallised from ethanol. Thus obtained, the *nitroso*-compound formed violet prisms (1·45 g.), m. p. 189° (Found: C, 64·4; H, 5·2; N, 25·4. $C_{18}H_{18}ON_6$ requires C, 64·7; H, 5·4; N, 25·1%).

4 : 6-*Diamino*-2-*dimethylamino*-5-*nitrosopyrimidine* was prepared in similar fashion from 4 : 6-diamino-2-dimethylaminopyrimidine. It crystallised from 2-ethoxyethanol in reddish-violet needles, m. p. 281—283° (Found: C, 40·1; H, 5·6; N, 45·9. $C_6H_{10}ON_6$ requires C, 39·6; H, 5·5; N, 46·1%).

4 : 6-*Diamino*-2-*dibenzylamino*-5-*thioformamidopyrimidine*.—4 : 6-Diamino-2-dibenzylamino-5-nitrosopyrimidine (1·3 g.) in ethanol (50 c.c.) was hydrogenated at atmospheric pressure with a platinum oxide catalyst. After 1 hour absorption of hydrogen became slow (uptake, 196 c.c. at N.T.P.; theoretical requirement for reduction of nitroso-group, 175 c.c.). The solution was diluted by adding ethanol (100 c.c.), catalyst removed by filtration, and aqueous sodium dithioformate (0·6 g. in 80 c.c. water) added. The mixture was set aside overnight and the yellowish *thioformamido*-compound, which separated (1·0 g.) was collected and recrystallised from ethanol in colourless needles, m. p. 199° (decomp.) (Found: C, 62·8; H, 5·6; N, 22·9; S, 9·0. $C_{19}H_{20}N_6S$ requires C, 62·6; H, 5·5; N, 23·1; S, 8·8%).

4 : 6-*Diamino*-2-*dimethylamino*-5-*thioformamidopyrimidine* was prepared in similar fashion from 4 : 6-diamino-2-dimethylamino-5-nitrosopyrimidine, save that hydrogenation was carried out with a Raney nickel catalyst at 100° and 100 atm. pressure. Recrystallised from water, the product formed colourless prisms, m. p. 180° (decomp.) after some sintering at *ca.* 120° (Found: C, 40·0; H, 5·6; N, 39·4; S, 15·4. $C_7H_{12}N_6S$ requires C, 39·6; H, 5·7; N, 39·6; S, 15·1%).

2-*Dibenzylaminoadenine*.—A solution of 4 : 6-diamino-2-dibenzylamino-5-thioformamidopyrimidine (0·4 g.) in quinoline (5 c.c.) was boiled until evolution of hydrogen sulphide ceased (15 minutes). The

solution was cooled and light petroleum (40 c.c.; b. p. 40—60°) added. The oil which first separated set to a mass of crystals which were collected. Recrystallised from *n*-butanol–benzene, the *purine* formed colourless prisms, m. p. 225° (Found: C, 69·4; H, 5·8; N, 25·7. $C_{19}H_{18}N_6$ requires C, 69·1; H, 5·5; N, 25·5%).

2-*Dimethylaminoadenine* was prepared in the same manner from the appropriate thioformamido-compound. It crystallised from water as colourless needles, m. p. 295° (Found: C, 47·3; H, 5·5; N, 47·0. $C_7H_{10}N_6$ requires C, 47·2; H, 5·7; N, 47·2%).

4 : 6-*Diamino*-2-*methylsulphonylpyrimidine*.—4 : 6-Diamino-2-methylthiopyrimidine (15 g.) was dissolved in dilute hydrochloric acid (50 c.c. of *d* 1·16, diluted with 400 c.c. water), the solution cooled to 0°, and sodium hypochlorite solution (600 c.c. of N.) added with shaking. Aqueous sodium carbonate was added after 2 minutes until alkaline, and the precipitated *sulphone* collected after a further 5 minutes. Recrystallised from aqueous ethanol (85%) or from water, the product (3·3 g.) formed colourless plates, m. p. 197—198° (Found: C, 31·8; H, 3·8; N, 30·1. $C_5H_8O_2N_4S$ requires C, 31·9; H, 4·3; N, 29·8%).

4-*Amino*-6-*hydroxy*-2-*carbethoxymethylthiopyrimidine*.—Ethyl chloroacetate (2·3 c.c.) was added to a solution of 4-amino-6-hydroxy-2-mercaptopyrimidine (3·2 g.) in aqueous sodium hydroxide (20 c.c. of N.). The mixture was shaken at room temperature for 10 minutes, heated on the steam-bath for 10 minutes, and then cooled, and the precipitated product collected. Recrystallised from ethanol, the *thioether* formed colourless needles (3·9 g.), m. p. 180° (decomp.) (Found: C, 41·9; H, 4·9; N, 18·5. $C_8H_{11}O_3N_3S$ requires C, 41·9; H, 4·8; N, 18·3%).

4-*Amino*-6-*hydroxy*-2-(*dicarbethoxymethylthio*)*pyrimidine*.—Prepared in similar fashion from 4-amino-6-hydroxy-2-mercaptopyrimidine (6·44 g.) and ethyl bromomalonate (9·56 g.), the *thioether* crystallised from aqueous ethanol in colourless plates (7 g.), m. p. 173° (decomp.) (Found: C, 44·0; H, 5·1; N, 14·6. $C_{11}H_{15}O_5N_3S$ requires C, 43·8; H, 5·0; N, 13·9%).

5-*Nitro*-4-*amino*-6-*hydroxy*-2-*methylthiopyrimidine*.—Fuming nitric acid (35 c.c.) was decolorised with urea and then cooled in an ice bath, and 4-amino-6-hydroxy-2-methylthiopyrimidine (25 g.) added in small portions with stirring during 1¼ hours. After 1 hour at room temperature, water (100 c.c.) was added. The precipitate was collected, washed with hot water, dissolved in warm aqueous sodium hydroxide, and cooled. The yellow sodium salt which crystallised on cooling [8·6 g.; m. p. 333° (decomp.)] was collected and redissolved in hot water, and the solution acidified with acetic acid. On cooling, the free *nitro*-compound separated as colourless needles. Recrystallised from hot water it had m. p. 299° (decomp.) (Found: C, 30·0; H, 2·9; N, 27·5. $C_5H_6O_3N_4S$ requires C, 29·7; H, 3·0; N, 27·7%).

2-*Methylsulphonyladenine*.—2-Methylthioadenine (0·5 g.; Baddiley, Lythgoe, McNeil, and Todd, *J.*, 1943, 383) was suspended in water (20 c.c.) at 40°. Chlorine was now passed through the suspension for 15 minutes, during which the temperature rose slightly (to 43°). The mixture was cooled to 0° and the flocculent precipitate collected. The product was dissolved in boiling water, the solution made weakly alkaline with aqueous ammonia (evolution of chlorine), treated with charcoal, and filtered. The *sulphone* (0·3 g.) separated on cooling and was recrystallised from water; it formed colourless plates which did not melt below 350° (Found, in material dried at 120°/4 mm.: C, 33·7; H, 3·7; N, 32·5. $C_6H_7O_2N_5S$ requires C, 33·9; H, 3·3; N, 32·9%).

2-*Methylsulphonyl*-9-*methyladenine*.—(*a*) A rapid stream of chlorine was passed through a suspension of 2-methylthio-9-methyladenine (1·2 g.; Baddiley, Lythgoe, McNeil, and Todd, *loc. cit.*) in water (50 c.c.) at room temperature for 30 minutes with occasional shaking. 6-*Chloroamino*-2-*methylsulphonyl*-9-*methyladenine* separated as a flocculent precipitate. A portion was recrystallised from methanol, forming colourless fine needles, m. p. 248—250°, after softening at 140° (Found: C, 32·6; H, 3·4; Cl, 13·9. $C_7H_8O_2N_5SCl$ requires C, 32·1; H, 3·1; Cl, 13·6%).

The remainder of the crude chloro-compound was dissolved in boiling water, and sodium hydrogen sulphite added till the yellow colour of the solution was discharged. The hot solution was treated with charcoal, filtered, and cooled. 2-*Methylsulphonyl*-9-*methyladenine* separated; recrystallised from water it formed colourless needles, m. p. 313—314° (decomp.) (Found, in material dried at 120°/4 mm.: C, 37·0; H, 4·0; N, 30·8. $C_7H_9O_2N_5S$ requires C, 37·0; H, 4·0; N, 30·8%). The same product was obtained by boiling the chloro-compound with ammoniacal water or by treatment with sodium dithionite.

(*b*) Hydrogen peroxide (0·45 c.c. of 100 vol.) was added to a solution of 2-methylthio-9-methyladenine (0·3 g.) in acetic acid (10 c.c.), and the mixture warmed on the steam-bath for 1 hour. The solution was evaporated to dryness under reduced pressure, and the residue (0·12 g.) recrystallised several times from ethanol containing a little ammonia. The purified product had m. p. 304° and the mixed m. p. with material prepared by route (*a*) was 308°.

2-*Methylsulphinyl*-9-*methyladenine*.—Chlorine was passed through a solution of 2-methylthio-9-methyladenine (0·5 g.) in dioxan (25 c.c.) at 45° during 30 minutes. The mixture was cooled in ice, and the crystalline *sulphoxide* filtered off. After several recrystallisations from water in a nitrogen atmosphere it yielded colourless needles, m. p. 295° (decomp.) (Found: C, 39·1; H, 4·3; N, 33·1. $C_7H_9ON_5S$ requires C, 39·8; H, 4·3; N, 33·2%). The sulphoxide was readily oxidised yielding the sulphone, m. p. 313—314°, described above.

2-*Ethoxy*-9-*methyladenine*.—2-Methylsulphonyl-9-methyladenine (1 g.) was added to an ethanolic solution of sodium ethoxide (0·13 g. of sodium in 100 c.c. of ethanol), and the mixture heated under reflux for 5 hours during which time the sulphone dissolved completely giving a light-blue solution. The solution was evaporated under reduced pressure, and the residue recrystallised from water (charcoal) giving 2-ethoxy-9-methyladenine as colourless needles (0·7 g.), m. p. 214—215° (Found: C, 49·3; H, 6·0; N, 35·8. Calc. for $C_8H_{11}ON_5$: C, 49·7; H, 5·7; N, 36·2%). Falconer, Gulland, and Story (*J.*, 1939, 1784) claim to have prepared this substance by the action of sodium ethoxide on 2-chloro-9-methyladenine and describe it as a jelly-like mass; they record a m. p. 252—254°, but we obtained no material of this nature.

6-*Benzylamino*-2-*benzyloxy*-9-*methylpurine*.—2-Methylsulphonyl-9-methyladenine (0·25 g.) was heated on the steam-bath for 5 hours with a solution of sodium (75 mg., 3 atoms) in benzyl alcohol (15 c.c.).

The blue solution was evaporated under reduced pressure, and the residue recrystallised from water (charcoal). The *product* formed colourless needles (0·2 g.), m. p. 170—171° (Found : C, 70·0; H, 5·7. $C_{20}H_{19}ON_5$ requires C, 69·5; H, 5·5%). Ultra-violet absorption in ethanol : Max. at 2680 A. (ε, 24,300) with an inflection between 2580 and 2640 A. (ε, *ca.* 20,700).

When the substance (0·1 g.) was heated under reflux with a mixture of concentrated hydrochloric (1 c.c.) and acetic acid (1 c.c.) for 1 hour, a crystalline precipitate formed. This material was purified by dissolution in alkali and reprecipitation by addition of glacial acetic acid. A final recrystallisation from water gave colourless needles (0·47 mg.) of 6-*benzylamino*-2-*hydroxy*-9-*methylpurine*, m. p. 266—267° (Found : C, 61·2; H, 4·9; N, 27·2. $C_{13}H_{13}ON_5$ requires C, 61·2; H, 5·1; N, 27·4%).

2-*Benzyloxy*-9-*methyladenine*.—2-Methylsulphonyl-9-methyladenine (0·5 g.) was dissolved in hot benzyl alcohol (200 c.c.), and a solution of sodium (50 mg., 1 atom) in benzyl alcohol (10 c.c.) added. The mixture was heated on the steam-bath for 4 hours and worked up as above. The *product* crystallised from ethanol (charcoal) in long colourless needles (0·35 g.), m. p. 200° (Found : C, 61·3; H, 4·9; N, 27·7. $C_{13}H_{13}ON_5$ requires C, 61·2; H, 5·1; N, 27·4%). The substance was insoluble in alkali and its ultra-violet absorption spectrum in N/20-hydrochloric acid showed a maximum at 2750 A. (ε, 12,000) with an inflection between 2500 and 2560 A. (ε, *ca.* 7,000).

9-*Methyl*iso*guanine* (2-*Hydroxy*-9-*methyladenine*).—(a) *From* 2-*methylsulphonyl*-9-*methyladenine*. The sulphone (0·2 g.) was heated under reflux for 20 minutes with aqueous sodium hydroxide (20 c.c. of 10%). The cooled solution, acidified with acetic acid, yielded the crude 9-*methyl*iso*guanine* as a gelatinous precipitate. For purification, the base was converted into its sparingly soluble hydrochloride which crystallised readily from dilute hydrochloric acid solution. The free base was liberated from the hydrochloride by dissolving the latter in sodium hydroxide and making the solution acid again with acetic acid. It separated from hot water in a gelatinous form which fell to a powder on drying, but it could not be obtained in a crystalline condition; it had no m. p. but slowly darkened when heated and decomposed above 250°. Its ultra-violet absorption spectrum in N/20-hydrochloric acid showed a maximum at 2840 A. (ε, 8200) and a minimum at 2510 A. (ε, 1100). For analysis it was converted into the *picrate* which crystallised from water in long yellow needles which decomposed above 310° (Found : C, 36·8; H, 2·8; N, 28·3. $C_6H_7ON_5,C_6H_3O_7N_3$ requires C, 36·5; H, 2·5; N, 28·5%).

(b) *From* 2-*ethoxy*-9-*methyladenine*. (i) The ethoxy-compound (0·1 g.) was dissolved in a solution of hydrobromic acid in acetic acid (3 c.c. of 50%), and the solution set aside for 48 hours. Water was added to dissolve the precipitate, the mixture evaporated, and the residue dissolved in aqueous sodium hydroxide. After filtration from a little unchanged ethoxy-compound, the alkaline solution was acidified with acetic acid. 9-Methyl*iso*guanine, identical with the material obtained by method (*a*), was precipitated.

(ii) The ethoxy-compound (0·1 g.) was dissolved in absolute ethanol (20 c.c.), ethyl chloroformate (5 c.c.) added, and the solution heated under reflux for 6 hours, a small quantity of a white precipitate separating. Solvent was removed under reduced pressure, and the residue dissolved in aqueous sodium hydroxide. After filtration from unchanged ethoxy-compound (50 mg.), 9-methyl*iso*guanine was obtained from the filtrate by acidification with acetic acid.

(c) *From* 2-*benzyloxy*-9-*methyladenine*. The benzyloxy-compound (0·15 g.) was hydrogenated in methanolic solution (75 c.c.) containing added hydrochloric acid (1 c.c. conc. + 20 c.c. of water) at room temperature and atmospheric pressure with as catalyst a mixture of palladous oxide and palladised charcoal. Hydrogen absorption ceased in 6 hours, and the filtered solution was then evaporated. Extraction of the residue with aqueous sodium hydroxide, followed by acidification of the alkaline solution with acetic acid, again yielded 9-methyl*iso*guanine.

iso*Guanine*.—A solution of 2-methylsulphonyladenine (80 mg.) in aqueous sodium hydroxide (5 c.c. of 10%) was heated under reflux for 3 hours, then cooled, and acidified with acetic acid. The precipitated *iso*guanine was converted into its sulphate, which crystallised from N-sulphuric acid in colourless plates which decomposed above 260° (Found, in material dried at 110°/1 mm. : N, 32·2. Calc. for $C_{10}H_{12}O_6N_5S,2H_2O$: N, 32·1%). Ultra-violet absorption spectrum in N/20-hydrochloric acid : Max. 2,840 A. (ε, 24,000); min. 2480 A. (ε, 3,200).

9-D-*Glucopyranosido*-2-*methylthioadenine*.—6-Amino-5-thioformamido-4-D-glucosidamino-2-methylthiopyrimidine (3·4 g.; m. p. 204—205°; prepared from 6 g. of 6-amino-4-D-glucosidamino-2-methylthiopyrimidine according to Holland, Lythgoe, and Todd, *loc. cit.*) was suspended in dry ethanol (300 c.c.), sodium methoxide (0·51 g.) added, and the mixture boiled under reflux in a nitrogen atmosphere for 5 hours, during which time the thioformamido-compound slowly dissolved. The resulting solution was cooled to 0° for 2 hours, filtered from a small amount of brown amorphous material, and concentrated under reduced pressure to small bulk (*ca.* 30 c.c.). Set aside at 0° for a few hours, a light-brown crystalline precipitate of the purine glucoside separated (2·7 g.). It was exceedingly soluble in water but crystallised from ethanol or dioxan in aggregates of small colourless plates, m. p. 269—271° (decomp.) (Found : C, 41·8; H, 4·8; N, 20·0. Calc. for $C_{12}H_{17}O_5N_5S$: C, 41·9; H, 4·9; N, 20·4%). On periodate titration, the glucoside took up 2·8 mols. of oxidant per mol. and liberated 1 mol. of formic acid per mol.

9-(*Tetra-acetyl* D-*glucopyranosido*)-2-*methylthioadenine*.—The above glucoside was acetylated with acetic anhydride in pyridine solution in the cold. The product was purified by dissolving it in benzene and passing the solution through a short column of neutral alumina. Recrystallised from benzene, the *acetate* formed yellowish plates, m. p. 141° (Found : C, 46·6; H, 4·6; N, 13·7. $C_{20}H_{25}O_9N_5S$ requires C, 46·9; H, 4·9; N, 13·7%). Desulphurised in the usual manner with Raney nickel, the tetra-acetate gave a product which, on deacetylation with sodium methoxide, furnished 9-D-glucopyranosidoadenine, m. p. 205—210°. The identity of the product was confirmed by conversion into the picrate which had m. p. 248—250° alone or mixed with an authentic specimen prepared by the method of Fischer and Helferich (*loc. cit.*).

9-(*Tetra-acetyl* D-*glucopyranosido*)-2-*methylsulphonyladenine*.—9-(Tetra-acetyl D-glucopyranosido)-2-methylthioadenine (0·5 g.) was dissolved in acetic acid (5 c.c.), hydrogen peroxide (0·7*b* c.c. of 100 vol.) added, and the solution set aside at room temperature for 36 hours, during which time the initially

yellowish solution had become almost colourless. Solvent was removed under reduced pressure at room temperature, and the resinous residue triturated with water and again evaporated. The residue, then a fine powder, was recrystallised from water. The *sulphone* formed long colourless plates (0·3 g.), m. p. 150—151° (Found : C, 44·1 ; H, 4·4 ; N, 12·8. $C_{20}H_{25}O_{11}N_5S$ requires C, 44·2 ; H, 4·6 ; N, 12·8%).

9-D-*Glucopyranosido*isoguanine.—The above sulphone (0·55 g.) was heated under reflux with aqueous sodium hydroxide (2 c.c. of 10%) for 20 minutes. The solution was cooled, acidified with acetic acid, and set aside overnight at 0°. The light-brown solid which separated was converted into a lead salt by treatment with lead acetate in the usual manner. Decomposition of the lead salt gave the *glucoside* which crystallised from water in colourless hydrated plates; it had no definite m. p. but decomposed at 265—270° (Found, in material dried at 120°/0·1 mm. for 10 hours : C, 41·8; H, 4·5; N, 22·1. $C_{11}H_{15}O_6N_5$ requires C, 42·1 ; H, 4·8 ; N, 22·4%). It yielded a *picrate* crystallising from water in yellow plates, m. p. 200—205° (decomp.) (Found, in material dried at 110°/0·1 mm. : C, 37·6 ; H, 3·5 ; N, 20·6. $C_{11}H_{15}O_6N_5,C_6H_3O_7N_3$ requires C, 37·6 ; H, 3·3 ; N, 20·6%).

Hydrolysed by heating it on the steam-bath for 3 hours with N-sulphuric acid, the glucoside yielded *iso*guanine sulphate, whose identity was confirmed by comparison of ultra-violet absorption and *X*-ray powder photographs (Found : C, 27·6 ; H, 4·0. Calc. for $C_{10}H_{12}O_6N_5S,2H_2O$: C, 27·5 ; H, 3·7%).

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