151. *Experiments on the Synthesis of Purine Nucleosides. Part III. 4-Glycosidaminopyrimidines.*

By J. Baddiley, B. Lythgoe, and A. R. Todd.

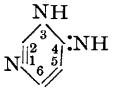
Direct glycosidisation of 4-aminopyrimidines is complicated by the tendency of such compounds to behave as though they were derivatives of 4-iminodihydropyrimidine. Theoretical considerations suggested that glycosidisation of one of the amino-groups in derivatives of type (II) should be possible. This view has been justified experimentally and 6-*amino*-4-d-*xylosidamino*-2-*methylthiopyrimidine*, 6-*amino*-4-d-*mannosidamino*-2-*methylthiopyrimidine*, and 6-*amino*-4-d-*xylosidamino*-2-*methylpyrimidine* have been synthesised.

The successful synthesis of purine nucleosides bearing a sugar residue at N_9 by the hypothetical route proposed in Part I (Baddiley, Lythgoe, McNeil, and Todd, this vol., p. 383) depends in the first place upon the availability of 5-amino-4-glycosidaminopyrimidines bearing appropriate substituents elsewhere in the molecule. Until now the only glycosidaminopyrimidines which have been synthesised are the 5-glucosidamino-compounds of Thannhauser and Dorfmüller (*Ber.*, 1914, **47**, 1304) obtained by direct condensation of 4 : 5-diaminopyrimidines with glucose in aqueous solution; under these conditions only the 5-amino-group reacts. It is evident from the ease of reaction in such cases that, for our purpose, either the 5-amino-group would have to be protected in some way or it would be necessary to glycosidise a 4-aminopyrimidine and subsequently to introduce an amino-group into position 5.

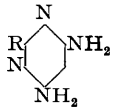
The glycosidisation of a 4-amino-group in a pyrimidine derivative, essential in either of the routes mentioned above, presents considerable difficulty and our early experiments in which we endeavoured to condense some readily available 4-amino-pyrimidines, *e.g.*, 4-amino-2-hydroxy-6-methylpyrimidine, 4-amino-5-aminomethyl-2-methylpyrimidine or their silver derivatives, with glucose or acetobromoglucose failed completely. These failures were not really surprising in view of the fact that, owing to the proximity of the annular nitrogen in position 3, such compounds in general behave as though they were derivatives of 4-iminodihydropyrimidine (I). We therefore next endeavoured, in a variety of uniformly unsuccessful experiments, to evade direct glycosidisation. Among these were several attempts to condense 1-aminoglucose (glucosimine) with 4-halogeno-

pyrimidines under various conditions; even with compounds such as 4-chloro-5-nitro-2-amino-6-methylpyrimidine and 2 : 4-dichloro-5-nitropyrimidine, in which the presence of a 5-nitro-group rendered the halogen in position 4 extremely reactive towards ammonia, no condensation could be effected. With the termination of these experiments it was decided to re-examine in more detail the problem of direct glycosidisation of substituted 4-aminopyrimidines.

Consideration of the tautomeric possibilities in substituted pyrimidines suggests that in a derivative of type (II) in which R represents H, alkyl or some substituent other than a group (*e.g.*, OH, SH, NH_2) capable of prototropic change, one double bond must occupy position 1 : 2 or 2 : 3 and hence one of the amino-groups at positions 4 and 6 should show, in considerable degree, the behaviour of a true amino-group. In this case one would expect direct glycosidisation of one amino-group in a compound of type (II) to be practicable. The validity of this argument appears to be borne out by our experimental results.



(I.)



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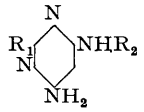

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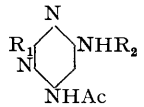

(IV.)

As a model substance we selected in the first instance the readily accessible 4 : 6-diamino-2-methylthiopyrimidine (II; R = SMe). Although attempts to glucosidise by the action of acetobromoglucose on this substance or its silver derivative failed, application of the procedure used by Kuhn and Ströbele (*Ber.* **1937**, **70**, 773) in preparing *N*-glycosides in the benzene series was successful. When refluxed in alcoholic solution with *d*-xylose or *d*-mannose in presence of ammonium chloride, (II; R = SMe) yielded crystalline glycosides which we regard as 6-*amino*-4-d-*xylosidamino*-2-*methylthiopyrimidine* (III; R_1 = SMe, R_2 = $C_5H_9O_4$) and 6-*amino*-4-d-*mannosidamino*-2-*methylthiopyrimidine* (III; R_1 = SMe, R_2 = $C_6H_{11}O_5$) respectively. Under similar conditions *d*-xylose and 4 : 6-diamino-2-methylpyrimidine (II; R = Me) gave 6-*amino*-4-d-*xylosidamino*-2-*methylpyrimidine* (III; R_1 = Me, R_2 = $C_5H_9O_4$).

Of the glycosidic nature of these products there can be no doubt; they are readily hydrolysed by dilute acids to the original constituents, and hence there can have been no Amadori rearrangement (Kuhn and Dansi, *Ber.*, 1936, **69**, 1745; Kuhn and Weygand, *ibid.*, 1937, **70**, 769) during their preparation. The possibility that they are Schiff bases is ruled out by the ready conversion of (III; R_1 = SMe, R_2 = $C_5H_9O_4$) into a *tetra-acetyl* derivative [IV; R_1 = SMe, R_2 = $C_5H_6O(OAc)_3$] in which one acetyl residue is attached to the 6-amino-group. Hydrolysis of this acetyl derivative with sodium methoxide in methanol gave 6-*acetamido*-4-d-*xylosidamino*-2-*methylthiopyrimidine* (IV; R_1 = SMe, R_2 = $C_5H_9O_4$), which underwent further hydrolysis with hot dilute acid to *d*-xylose and 4-*amino*-6-*acetamido*-2-*methylthiopyrimidine* (IV; R_1 = SMe, R_2 = H) identical with the product obtained on acetylation of (II; R = SMe) with acetic anhydride in pyridine solution at room temperature. In a similar manner (III; R_1 = SMe, R_2 = $C_6H_{11}O_5$) gave on acetylation a *penta-acetyl* derivative [IV; R_1 = SMe, R_2 = $C_6H_7O(OAc)_4$], hydrolysed successively to 6-*acetamido*-4-d-*mannosidamino*-2-*methylthiopyrimidine* (IV; R_1 = SMe, R_2 = $C_6H_{11}O_5$) and to 4-amino-6-acetamido-2-methylthiopyrimidine and *d*-mannose. The ready hydrolysis of the new glycosides with dilute acids is evidence for the location of the sugar residue, since pyrimidine derivatives in which sugar is linked to a nuclear nitrogen are known to be resistant to acid hydrolysis. In the experiments of Kuhn and Ströbele (*loc. cit.*) it was found that *N*-pentosides prepared by their method had a furanose structure. Whether the 4-glycosidamino-compounds here described have a furanose or a pyranose structure is not yet established, but experiments to this end are in progress.

In connection with the reasons, outlined above, which led to the selection of 4 : 6-diaminopyrimidines of type (II) for glycosidisation, it is interesting to note that we have so far failed to obtain any trace of glycoside formation under similar conditions with 4-amino-2 : 6-dimethylpyrimidine (cyanmethine), 4 : 6-diamino-2-thiolpyrimidine, 4 : 6-diamino-5-benzeneazopyrimidine, and 4 : 6-diamino-5-thioformamidopyrimidine. In the case of the first two compounds amidine-type tautomerism involving the amino-groups is unchecked. Failure to glycosidise 4 : 6-diamino-5-benzeneazopyrimidine might be explained in several ways; the benzeneazo-group may introduce a steric factor or new possibilities of tautomerism or the insolubility of the substance in alcohol might of itself be sufficient explanation. The failure to glycosidise 4 : 6-diamino-5-thioformamidopyrimidine was unexpected, but it is probably connected with the extremely low solubility of the compound in alcohol.

Experimental.

The activated aluminium oxide used in these experiments was prepared by heating "Alumina hydrate" (British Aluminium Co., Ltd.) from 20° to 360° with stirring during 5 hours.

6-*Amino*-4-d-*xylosidamino*-2-*methylthiopyrimidine*.—*d*-Xylose (4 g.), 4 : 6-diamino-2-methylthiopyrimidine (12 g.), and ammonium chloride (0·75 g.) were refluxed with ethyl alcohol (100 c.c.; dried over magnesium ethoxide) for 4 hours, and the cooled solution passed through a column of activated aluminium oxide (500 g.). The column was washed with alcohol (2·5 l.) and the filtrate and washings were united and evaporated, giving unchanged 4 : 6-diamino-2-methylthiopyrimidine (8 g.). Elution of the column with ice-cold water (2·5 l.) or with a pyridine–water–methanol mixture (1 : 1 : 2; 2·5 l.) and evaporation of the eluate in a vacuum at 20° gave a pale amber gum (dry weight 8 g.). This was freed from inorganic matter by dissolution in absolute alcohol; the solution was evaporated in a vacuum to 40 c.c., and ethyl acetate (40 c.c.) added. After 5 days the crystalline crust of 6-*amino*-4-d-*xylosidamino*-2-*methylthiopyrimidine* was collected, m. p. 190—192° (decomp.). Yield, 0·6 g. (Found: C, 41·5; H, 5·6; S, 11·7. $C_{10}H_{16}O_4N_4S$ requires C, 41·7; H, 5·5; S, 11·1%). Evidence is presented below showing that the gummy reaction product contains further considerable

quantities of the same material, but its separation in crystalline condition is attended with great difficulty. The substance is moderately readily soluble in water and pyridine, sparingly soluble in alcohol, and insoluble in other common organic solvents. Fehling's solution is not reduced below 60°; at higher temperatures reduction takes place slowly.

Hydrolysis. The xyloside (0·2 g.) was refluxed with N/10-hydrochloric acid (5 c.c.) for ½ hour, and the solution cooled, treated with N/10-sodium hydroxide (5 c.c.), and concentrated to 5 c.c. in a vacuum. Addition of a hot concentrated solution of picric acid (0·2 g.) and cooling gave 4 : 6-*diamino*-2-*methylthiopyrimidine picrate*, needles, m. p. 212° (decomp.), undepressed on admixture with a specimen prepared from the authentic diamine. Yield, 0·2 g. (Found : C, 34·2; H, 2·5. $C_5H_8N_4S,C_6H_3O_7N_3$ requires C, 34·3; H, 2·9%).

A second portion of xyloside was hydrolysed in similar fashion, and the solution heated for 1 hour with phenylhydrazine in acetic acid. On cooling, *d*-xylosazone separated, m. p. 159° alone or mixed with an authentic specimen.

6-*Acetamido*-4-*triacetyl*-d-*xylosidamino*-2-*methylthiopyrimidine*.—(a) *From crystalline* 6-*amino*-4-d-*xylosidamino*-2-*methylthiopyrimidine*. The crystalline xyloside (0·1 g.) in dry pyridine (10 c.c.) was treated at room temperature with acetic anhydride (1 c.c.) and acetyl chloride (0·1 c.c.), and the solution heated on the steam-bath for 1 hour. After cooling, ethyl alcohol (5 c.c.) was added to destroy the excess of acetic anhydride, the solution kept for 1 hour, and solvents removed in a vacuum. The residue, freed from pyridine by evaporation in a vacuum with ethyl alcohol, was crystallised from ethyl alcohol, giving 6-*acetamido*-4-*triacetyl*-d-*xylosidamino*-2-*methylthiopyrimidine* in needles (30 mg.), m. p. 226° (decomp.), $[\alpha]_D^{18°}$ + 57° (pyridine, c = 5·1%) (Found : C, 47·3; H, 5·3; N, 12·5. $C_{18}H_{24}O_8N_4S$ requires C, 47·3; H, 5·3; N, 12·3%).

(b) *From the gummy eluate material* (Experiment by Mr. A. Topham). A portion (5 g.) of this material, from which the crystalline xyloside had been separated, acetylated in pyridine (40 c.c.) with acetic anhydride (12 c.c.) and acetyl chloride as described for the crystalline xyloside, gave the same tetra-acetyl compound (2·25 g.), m. p. 226°, undepressed in admixture with a specimen prepared as in (a) above. This shows that there is present in the eluate gum considerably more 6-amino-4-*d*-xylosidamino-2-methylthiopyrimidine than can be readily separated in crystalline form.

6-*Acetamido*-4-d-*xylosidamino*-2-*methylthiopyrimidine*.—To the above tetra-acetyl compound (6 g.) in dry chloroform (60 c.c.), methyl-alcoholic sodium methoxide (0·7 g. of sodium in 100 c.c. of methyl alcohol) was added at room temperature. After 15 minutes water (400 c.c.) was added, the mixture well shaken, and the aqueous layer removed, neutralised by addition of sulphuric acid, and concentrated in a vacuum to 100 c.c.. The 6-*acetamido*-4-d-*xylosidamino*-2-*methylthiopyrimidine* which separated crystallised from water in needles, m. p. 95—100°, containing varying quantities of water of crystallisation, or from ethyl alcohol in rosettes (also hydrated), m. p. 192—193°. Yield, 2·5 g. (Found in material dried over phosphoric oxide at 100°/0·1 mm. : C, 43·6; H, 5·5; N, 16·7. $C_{12}H_{18}O_5N_4S$ requires C, 43·6; H, 5·45; N, 17·0%). The anhydrous substance had $[\alpha]_D^{20°}$ + 23° (pyridine, c = 2·1%).

Hydrolysis. The above xyloside (0·2 g.) in N/10-sulphuric acid (10 c.c.) was heated under reflux at 95° for ½ hour. The solution on cooling and neutralisation with N-sodium hydroxide (1 c.c.) gave 4-amino-6-acetamido-2-methylthiopyrimidine, which formed colourless crystals (50 mg.) from hot water, m. p. 225—226°, undepressed in admixture with material prepared as described below.

Acetylation of 4 : 6-*Diamino*-2-*methylthiopyrimidine*.—(a) 4 : 6-Diamino-2-methylthiopyrimidine (1 g.) in pyridine (10 c.c.) was kept overnight at room temperature with acetic anhydride (10 c.c.). Removal of solvents and crystallisation of the residue from water gave the acetyl derivative, m. p. 225—226°.

(b) 4 : 6-Diamino-2-methylthiopyrimidine (0·5 g.) in ethyl acetate (5 c.c.) was refluxed for 1 hour with acetyl chloride (1·5 c.c.). Removal of solvents and crystallisation of the residue from alcohol gave the *hydrochloride*, m. p. 213—214° (Found : C, 33·8; H, 5·2; N, 22·7. $C_7H_{10}ON_4S,HCl,H_2O$ requires C, 33·5; H, 5·2; N, 22·3%). Yield, 90%. Dissolution in water and addition of alkali gave the free base, m. p. 225—226°, undepressed in admixture with material prepared by the routes described above.

6-*Amino*-4-d-*mannosidamino*-2-*methylthiopyrimidine*.—*d*-Mannose (8 g.), 4 : 6-diamino-2-methylthiopyrimidine (24 g.), and ammonium chloride (0·75 g.) were refluxed together in dry alcohol (250 c.c.) for 3½ hours, and the solution cooled. The separated solid was washed thoroughly with alcohol and crystallised from hot water. 6-*Amino*-4-d-*mannosidamino*-2-*methylthiopyrimidine* separated in tiny hydrated needles (12 g.), m. p. 213—214° (decomp.). On drying, part of the water of crystallisation was readily removed, but the remainder appeared to cling tenaciously (Found in material dried for 1 hour over phosphoric oxide at 100°/0·1 mm. : C, 38·7; H, 5·9; N, 16·7. $C_{11}H_{18}O_5N_4S,1·5H_2O$ requires C, 38·3; H, 6·1; N, 16·2%).

Hydrolysis. The above mannoside (1 g.) and N/10-sulphuric acid (70 c.c.) were heated under reflux at 95° for 40 minutes, and the solution cooled, neutralised with N-sodium hydroxide (7 c.c.), and evaporated to dryness in a vacuum. The residue was extracted with hot alcohol (100 c.c.), and the extract passed through a column of activated aluminium oxide (50 g.), which was then washed with alcohol (150 c.c.). Filtrate and washings, united and evaporated, gave 4 : 6-diamino-2-methylthiopyrimidine (0·3 g.), m. p. 186°, undepressed on admixture with an authentic specimen. The column was eluted with cold water (250 c.c.), and the eluate united with the residue from the alcohol extraction and evaporated to 7 c.c., a little inorganic material separating being removed by filtration. Addition of anhydrous sodium acetate (1 g.) and phenylhydrazine hydrochloride (0·5 g.) to the solution gave *d*-mannose phenylhydrazone, separating from dilute alcohol in colourless crystals (0·5 g.), m. p. 189—191° (Fischer, *Ber.*, 1887, **20**, 821, gives m. p. 188—190°).

6-*Acetamido*-4-*tetra-acetyl*-d-*mannosidamino*-2-*methylthiopyrimidine*.—The above mannoside (1 g.) in dry pyridine (15 c.c.) was heated on the steam-bath for 1 hour with acetic anhydride (4 c.c.) and acetyl chloride (0·2 c.c.). After decomposition with ethyl alcohol and removal of solvents in the usual manner the residue was crystallised from ethyl alcohol, giving 6-*acetamido*-4-*tetra-acetyl*-d-*mannosidamino*-2-*methylthiopyrimidine* in needles, m. p. *ca.* 140—150°. Repeated crystallisation from alcohol, benzene and acetone–chloroform–petroleum gave crystals with the same characteristics. Yield, 1 g. (Found in material dried over calcium chloride at room temperature : C, 43·2; H, 5·4; N, 9·7; loss at 100°/0·1 mm., 9·3. $C_{21}H_{28}O_{10}N_4S,3H_2O$ requires C, 43·3; H, 5·8; N, 9·6; H_2O, 9·3%). The anhydrous substance had $[\alpha]_D^{20°}$ − 100° (pyridine, c = 16·0%).

6-*Acetamido*-4-d-*mannosidamino*-2-*methylthiopyrimidine*.—The above penta-acetyl derivative (7 g.) in dry chloroform (35 c.c.) was mixed with methyl-alcoholic sodium methoxide (1 g. of sodium in 50 c.c. of alcohol). After 15 minutes the resulting gel was shaken with ice-water (300 c.c.), and the aqueous layer separated, neutralised with acetic acid, and evaporated to 20 c.c. The 6-*acetamido*-4-d-*mannosidamino*-2-*methylthiopyrimidine* was collected and recrystallised from hot water, forming colourless needles (1·7 g.), m. p. 242—243° (decomp.), $[\alpha]_D^{20°}$ − 55° (pyridine, c = 0·7%) (Found : C, 43·4; H, 5·5; N, 15·3. $C_{13}H_{20}O_5N_4S$ requires C, 43·3; H, 5·6; N, 15·6%).

Hydrolysis. The above compound (0·2 g.) was heated with N/10-sulphuric acid (10 c.c.) under reflux at 95° for 45 minutes. The solution, cooled, neutralised with N-sodium hydroxide (1 c.c.), and evaporated to half its volume, gave 6-acetamido-4-amino-2-methylthiopyrimidine (50 mg.), m. p. 225—226°, undepressed in admixture with an authentic specimen.

6-*Amino*-4-d-*xylosidamino*-2-*methylpyrimidine*.—4 : 6-Diamino-2-methylpyrimidine (36 g.) (Baddiley, Lythgoe, McNeil, and Todd, *loc. cit.*), *d*-xylose (10 g.), anhydrous ethyl alcohol (500 c.c.), and ethyl-alcoholic hydrogen chloride

(4 c.c., saturated at 20°) were refluxed together for 72 hours in a flask fitted with a Fenske column and a reflux ratio head, water formed in the reaction being removed periodically as the ternary mixture by addition of a mixture of benzene and ethyl alcohol (2 : 1; total, 500 c.c.). The cooled solution was passed through a column of activated aluminium oxide (700 g.), which was then washed with ethyl alcohol (2 l.). Evaporation of filtrate and washings gave unchanged **4** : 6-diamino-2-methylpyrimidine (25 g.). Elution of the column with water (6 l.) and evaporation of the eluate in a vacuum below 30° to small volume gave crystalline 6-*amino*-4-d-*xylosidamino*-2-*methylpyrimidine*, m. p. 219° (decomp.), $[\alpha]_D^{20°}$ + 158° (water; c = 0·095%). Yield, 65% (Found: C, 46·6; H, 6·1; N, 21·4. $C_{10}H_{16}O_4N_4$ requires C, 46·9; H, 6·2; N, 21·8%).

Hydrolysis. (*a*) The above xyloside (0·3 g.) was refluxed with N/10-hydrochloric acid (25 c.c.) for 20 minutes, and the solution cooled, neutralised to Congo-red by addition of sodium hydroxide, and evaporated to dryness in a vacuum. The residue was extracted thrice with boiling alcohol, and the combined extracts (15 c.c.) passed through a column of activated aluminium oxide (15 g.), which was then washed with alcohol (70 c.c.). Evaporation of filtrate and washings gave **4** : 6-diamino-2-methylpyrimidine, m. p. 294°, undepressed in admixture with an authentic specimen.

(*b*) The xyloside (0·3 g.) was hydrolysed as above, and the neutralised solution concentrated to 12 c.c., treated with a solution of phenylhydrazine (0·3 g.) in acetic acid (2 c.c. of 40%), and heated at 100° for 1 hour. On cooling, *d*-xylosazone separated; it formed needles from aqueous alcohol, m. p. 158°, undepressed in admixture with authentic *d*-xylosazone (m. p. 158°).

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