An Unambiguous Synthesis of Adenylosuccinic Acid and its Constituent Nucleoside

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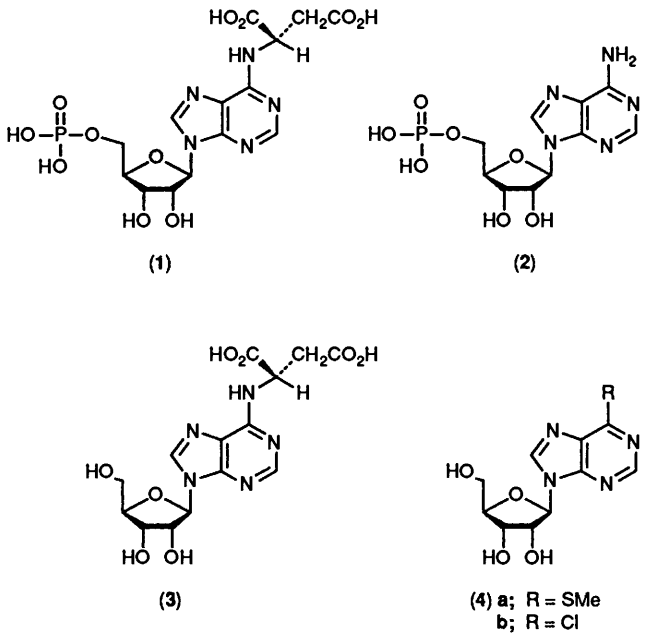
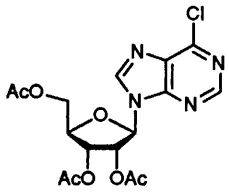
6-(4-Chlorophenylthio)-9-(2,3-*O*-cyclopentylidene-β-D-ribofuranosyl)-9*H*-purine (**7**) is prepared from 6-chloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine (**5**) in three steps and is then converted, by treatment with 3-chloroperbenzoic acid, into the corresponding sulphoxide (**9**) and crystalline sulphone (**8**) in *ca.* 88 and 65% isolated yield, respectively. When the sulphoxide (**9**) is heated with dibenzyl L-aspartate in *N,N*-dimethylacetamide solution at 70–75 °C for 28 hr, compound (**11**) is obtained in *ca.* 70% isolated yield. Removal of the protecting groups from compound (**11**) gives *N*-[9-(β-D-ribofuranosyl)-9*H*-purin-6-yl]-L-aspartic acid (**3**) as a crystalline solid in *ca.* 68% yield; phosphorylation of compound (**11**) with dibenzyl phosphorochloridate and removal of the protecting groups gives adenylosuccinic acid (**1**), isolated as its ammonium salt, in *ca.* 66% yield. When the sulphone (**8**) is converted *via* compound (**11**) into the diacids (**3**) and (**1**), the products obtained appear to be contaminated with the D-acids (**12**) and (**14**), respectively, which are diastereoisomers of compounds (**3**) and (**1**).

Despite the fact that {*N*-[9-(β-D-ribofuranosyl)-9*H*-purin-6-yl]-L-aspartic acid} 5′-phosphate [adenylosuccinic acid (**1**)] has been known[1] for a long time to be the intermediate in the biosynthetic conversion of inosine 5′-phosphate into adenosine 5′-phosphate (**2**), we are unaware of any previous report relating to its chemical synthesis. However, an enzymatic synthesis of adenylosuccinic acid (**1**), involving the adenylosuccinase-promoted addition of fumarate to the amino function of adenosine 5′-phosphate (**2**), has been reported.[2]. There are two accounts[3,4] in the literature which relate to the chemical synthesis of *N*-[9-(β-D-ribofuranosyl)-9*H*-purin-6-yl]-L-aspartic acid (**3**), the nucleoside component of adenylosuccinic acid (**1**). The first synthesis,[3] which presumably led to a mixture of compound (**1**) and its diastereoisomer (see below) derived from D-aspartic acid, was effected by heating unprotected 6-methylthio-9-(β-D-ribofuranosyl)-9*H*-purine (**4a**) together with unprotected (±)-aspartic acid in dilute aq. sodium hydroxide, and led to only a very small quantity of isolated product. The second synthesis,[4] which involved heating unprotected 6-chloro-9-(β-D-ribofuranosyl)-9*H*-purine (**4b**) and L-aspartic acid together under similar conditions, was also carried out on a small scale. As compound (**3**) is a polar, ionizable compound, anion-exchange chromatography was used in the course of its purification. Ion-exchange chromatography was similarly used in the purification of enzymatically synthesized[2] adenylosuccinic acid (**1**). It seemed to us that if the large-scale preparation of the L-aspartic acid derivatives (**1**) and (**3**) was to be a feasible proposition, it would be desirable to work with protected intermediates which were soluble in organic solvents and which could be purified by standard techniques (*i.e.*, chromatography on silica gel), and to choose protecting groups which could be removed cleanly under mild conditions. We have followed this strategy and we now report the unambiguous chemical synthesis of the ammonium salt of adenylosuccinic acid (**1**) and its constituent nucleoside (**3**)

6-Chloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine (**5**), which was prepared from inosine by the two-step literature procedure[5,6] (see Experimental section) in virtually quantitative yield, was allowed to react with a slight excess each of 4-chloro(thiophenol) and triethylamine in methanol solution at room temperature for 30 min to give the corresponding 6-(4-chlorophenylthio) derivative (**6a**). The latter compound was deacetylated by treatment with ammonia in methanol solution to give compound (**6b**) in *ca.* 72% overall yield. When compound (**6b**) was heated with an excess of 1,1-dimethoxycyclopentane in the presence of toluene-4-sulphonic acid monohydrate (0.1 mol. equiv.) in tetrahydrofuran (THF) solution, under nitrogen, at 50 °C for 1 h, the corresponding 2′,3′-*O*-cyclopentylidene derivative[7] (**7**) was obtained and was isolated as a foam in *ca.* 84% yield. Treatment of compound (**7**) with 3-chloroperbenzoic acid (*ca.* 3 mol equiv.) in dichloromethane solution at room temperature for 3 h gave the corresponding sulphone (**8**) which was isolated as a crystalline solid in 65% yield. When compound (**7**) was allowed to react with 3-chloroperbenzoic acid (*ca.* 1.05 mol equiv.) at − 5 °C, the corresponding sulphoxide (**9**) was obtained and was isolated as





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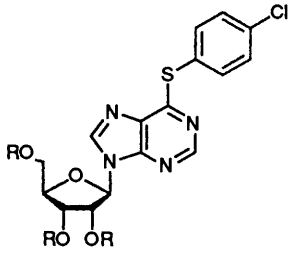
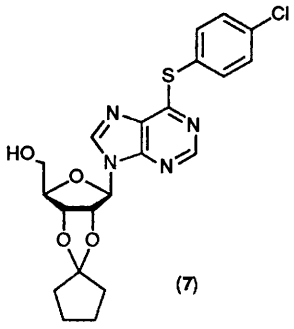


(6) a; R = Ac
b; R = H



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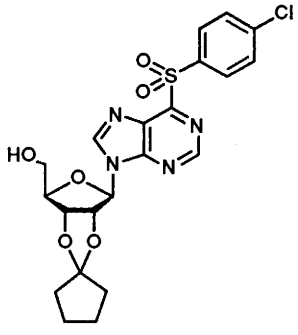

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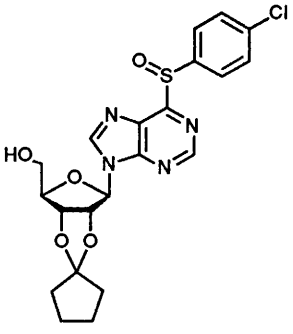

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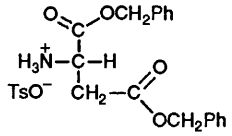

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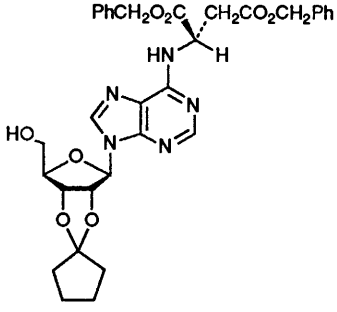


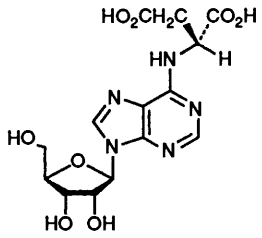
(11)

a foam in *ca.* 88% yield. It was easily possible to distinguish between the sulphone (**8**) and the slightly more polar sulphoxide (**9**) by TLC and by ^{13}C NMR spectroscopy. It was indeed clear from its ^{13}C NMR spectrum that compound (**9**) was obtained, as expected, as a mixture of diastereoisomers.

Purine nucleoside 6- and 8-sulphone derivatives have been shown[8,9] to be particularly susceptible to nucleophilic attack. However, although the sulphone (**8**) was found to react readily with cyclohexylamine at room temperature, much more drastic conditions were required to effect its reaction with esters of aspartic acid. Thus, it was necessary to heat compound (**8**) with dibenzyl L-aspartate (*ca.* 2.5 mol equiv.) [generated from its toluene-4-sulphonate salt (**10**) and di-isopropylethylamine] in *N*,*N*-dimethylacetamide (DMA) solution at 100 °C for 24 h to effect its conversion into dibenzyl *N*-[9-(2,3-*O*-cyclopentylidene-β-D-ribofuranosyl)-9*H*-purin-6-yl]-L-aspartate (**11**). The latter compound (**11**), which was later shown (see below) to be contaminated with a diastereoisomer, was isolated from the reaction products in 60% yield. The corresponding sulphoxide (**9**) proved to be somewhat more susceptible to nucleophilic attack[10] by the amino function of dibenzyl-L-aspartate. Thus, when compound (**9**) was heated with an excess each of the salt (**10**) and di-isopropylethylamine in DMA solution at 70–75 °C, the reaction proceeded almost to completion in 28 h, and the desired product (**11**) was obtained in *ca.* 70% isolated yield. Furthermore, this material was later shown (see below) to be virtually free from a diastereoisomeric contaminant.

The protecting groups were removed from a sample of dibenzyl *N*-[9-(2,3-*O*-cyclopentylidene-β-D-ribofuranosyl)-9*H*-purin-6-yl]-L-aspartate (**11**), prepared from the intermediate *sulphoxide* (**9**), by a two-step process. The cyclopentylidene group was first removed by treatment with formic acid–water (3:2 v/v) at room temperature for 3 h, and the two benzyl groups were then removed by hydrogenolysis in the presence of palladium–charcoal in ethanol–acetic acid–water (8:1:1 v/v), also at room temperature. The desired *N*-[9-(β-D-ribofuranosyl)-9*H*-purin-6-yl]-L-aspartic acid (**3**) was isolated from the products as a pure, crystalline solid in 68% yield, based on the original quantity of (**11**) used. When the protected intermediate (**11**), prepared from the *sulphone* (**8**), was unblocked in the same way, the unprotected nucleoside (**3**) was obtained in 61% isolated yield. Following the crystallization of the latter product from aq. ethanol, examination of the remaining mother liquors by liquid chromatography (LC) revealed, in addition to compound (**3**) [*ca.* 66%], the presence of a product with higher t_R [*ca.* 33%] believed (see below) to be its diastereoisomer (**12**). However, LC analysis of the crystalline material prepared from the sulphone (**8**) revealed that it was virtually pure L-compound (**3**), which was only very slightly (< 2%) contaminated with its D-diastereoisomer (**12**). Evidence for structure of the latter by-product was obtained by allowing the sulphone intermediate (**8**) to react with the racemic modification of dibenzyl aspartate[11] at 100 °C for 24 h. Following the removal of the protecting groups from the purified products, a 1:1 mixture [as indicated by LC] of diastereoisomers (**3**) and (**12**) was obtained in 36% overall yield, based on the original quantity of (**8**) used. The t_R-values of the major and minor components present in the above mother liquors corresponded to the t_R-values of compounds (**3**) and (**12**), respectively, observed in the course of the LC examination of the latter 1:1 mixture.

Dibenzyl *N*-[9-(2,3-*O*-cyclopentylidene-β-D-ribofuranosyl)-9*H*-purin-6-yl]-L-aspartate (**11**), prepared from the *sulphoxide* (**9**), was then allowed to react with dibenzyl phosphorochloridate[12] (*ca.* 3.0 mol equiv.) in anhydrous pyridine solution at −40 °C for 2 h to give, following short-column chromatography of the products on silica gel, the corresponding fully protected 5′-phosphate (**13**). The protecting groups were removed from the latter compound by the two-step unblocking procedure described above in the conversion of diester (**11**) into diacid (**3**). An excess of conc. aq. ammonia was added to a solution of the resulting adenylosuccinic acid (**1**) in water. The solution obtained was then concentrated under reduced pressure to small volume and added to *ca.* one hundred times its volume of absolute ethanol to give the ammonium salt of adenylosuccinic



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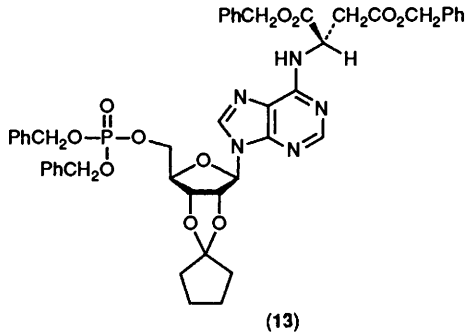

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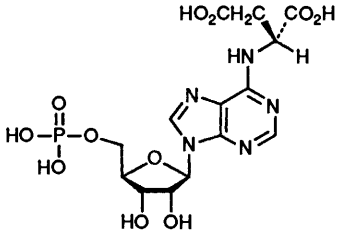

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acid (**1**) as a solid precipitate in *ca.* 66% overall yield for the three steps starting from compound (**11**). The latter material was found by LC to be *ca.* 99.5% pure, and to have the same t_R-value as a commercial sample of what was assumed to be enzymatically synthesized[2] adenylosuccinic acid (**1**); it was further characterized on the basis of its ^{1}H, ^{13}C, and ^{31}P NMR spectra, and was also found to undergo quantitative conversion into adenosine 5′-phosphate (**2**) when it was treated with adenylosuccinate lyase.[13] A sample of dibenzyl *N*-[9-(2,3-*O*-cyclopentylidene-β-D-ribofuranosyl)-9*H*-purin-6-yl]-L-aspartate (**11**), prepared from the *sulphone* (**8**), was also converted into the ammonium salt of adenylosuccinic acid (**1**). The latter product, which was obtained from compound (**11**) in 59% overall yield by the same three-step procedure, was found by LC analysis to consist of adenylosuccinate (*ca.* 91%) and a component with shorter t_R-value (9%), believed to be the ammonium salt of its diastereoisomer (**14**). A small quantity of this second material was isolated by preparative LC, and was then treated with bacterial alkaline phosphatase to give what appeared from LC analysis to be the diastereoisomeric impurity (**12**) found in samples of *N*-[9-β-D-ribofuranosyl)-9*H*-purin-6-yl]-L-aspartic acid (**3**), prepared from the *sulphone* (**8**).

We believe that the chemical synthesis of adenylosuccinic acid (**1**), starting from the *sulphoxide* (**9**), is superior to the enzymatic synthesis[2] and it would appear that our strategy (see above) has been successful: the fully protected intermediate (**13**) is freely soluble in organic solvents and can be purified by conventional chromatographic techniques. Furthermore, the latter intermediate (**13**) can then be cleanly unblocked under relatively mild conditions to give virtually pure adenylosuccinic acid (**1**) without any further purification steps. It would therefore seem likely that the present synthesis could readily be scaled up if large quantities of adenylosuccinic acid (**1**) were needed; for example, for the treatment of sufferers from Duchenne muscular dystrophy.[14] The enzymatic synthesis of compound (**1**) necessarily leads to an unprotected product. Although, as indicated above, the latter material may be purified by anion-exchange chromatography, it is quite possible that the scaling up of this process would present considerable practical difficulties. Finally, the unambiguous chemical synthesis of adenylosuccinic acid (**1**) from dibenzyl L-aspartate and the direct comparison of the chemically with enzymatically synthesized material confirms beyond doubt that the absolute configuration at C-2 of the succinate residue in adenylosuccinic acid (**1**) is *S*.

Experimental

^{1}H and ^{13}C NMR spectra were measured at 360 and 90.6 MHz, respectively, with a Bruker AM 360 spectrometer; tetramethylsilane was used as internal standard. Merck silica gel H was used for short-column chromatography. Merck silica gel 60 F_{254} TLC plates were developed in solvent system A [$CHCl_3$–EtOH (95:5 v/v)] or B [$CHCl_3$–EtOH (98:2 v/v)] unless otherwise stated. LC was carried out on a Jones APEX ODS 5μ column, which was eluted isocratically with mixtures of 0.1 M-triethylammonium acetate and acetonitrile. Pyridine, di-isopropylethylamine, and benzene were dried by heating, under reflux, with calcium hydride and were then distilled; THF was dried by heating, under reflux, first with sodium benzophenone and then with lithium aluminium hydride; DMA was dried over 4 Å molecular sieves. The toluene-4-sulphonate salt of dibenzyl L-aspartate, adenylosuccinic acid, and enzymes were purchased from the Sigma Chemical Co., Ltd.

6-*Chloro*-9-(2,3,5-*tri*-O-*acetyl*-β-D-*ribofuranosyl*)-9H-*purine* (**5**).—Acetic anhydride (145 ml, 1.54 mol) was added to a stirred suspension of inosine (14.5 g, 54.1 mmol) in anhydrous pyridine (175 ml). The reactants were maintained at 40 °C for 1 h, and the resulting clear solution was evaporated under reduced pressure. After the solid residue obtained had been triturated with anhydrous diethyl ether (75 ml), it was filtered off, washed with diethyl ether (2 × 75 ml) and dried *in vacuo* over P_2O_5 at 50 °C (21.1 g) R_f 0.20 (system A).

The latter material (12.0 g) was added in small portions to a solution of freshly distilled phosphoryl trichloride (60.0 ml, 0.64 mol) and *N*,*N*-dimethylaniline (4.0 ml, 25.1 mmol). The resulting suspension was stirred under nitrogen and immersed in a preheated (to 115 °C) oil-bath. After *ca.* 4 min, a clear, homogeneous solution, which started to boil, was obtained. After a further period of 3 min, the products were concentrated under reduced pressure (bath temperature *ca.* 40 °C). The residue was cooled (ice-bath) and crushed ice (*ca.* 100 g) was added. The stirred mixture was maintained at 0 °C (ice-bath) for 1 h, and was then extracted with chloroform (3 × 75 ml). After the combined organic extracts had been washed first with M-hydrochloric acid (5 × 70 ml) and then with water (2 × 70 ml), they were dried ($MgSO_4$), and evaporated under reduced pressure. Repeated evaporation of the residue from chloroform gave the title compound as a pale yellow foam (12.6 g, quantitative yield for the two steps, starting from inosine); R_f 0.43 (system A), 0.28 (system B); $\delta_H(CDCl_3)$ 2.10 (3 H, s), 2.14 (3 H, s), 2.17 (3 H, s), 4.35–4.55 (3 H, m), 5.66 (1 H, t, *J* 5.1 Hz), 5.96 (1 H, t, *J* 5.3 Hz), 6.25 (1 H, d, *J* 5.1 Hz), 8.33 (1 H, s), and 8.79 (1 H, s); $\delta_C(CDCl_3)$ 20.29, 20.44, 20.66, 62.81, 70.37, 73.02, 132.26, 143.56, 151.16, 151.53, 152.23, 169.28, 169.49, and 170.19.

6-(4-*Chlorophenylthio*)-9-(2,3,5-*tri*-O-*acetyl*-β-D-*ribofuranosyl*)-9H-*purine* (**6a**).—4-Chloro(thiophenol) (0.87 g, 6.0 mmol)

and triethylamine (0.84 ml, 6.0 mmol) were added to a stirred solution of 6-chloro-9-(2,3,5-tri-*o*-acetyl-β-D-ribofuranosyl)-9*H*-purine (2.06 g, *ca.* 5.0 mmol) in methanol (35 ml) at room temperature. After 30 min, the products were concentrated under reduced pressure and the residue was partitioned between chloroform (200 ml) and saturated aq. sodium hydrogen carbonate (100 ml). The dried ($MgSO_4$) organic layer was concentrated under reduced pressure and the residue was chromatographed on silica gel. Fractions (eluted with $CHCl_3$) containing the desired product were combined and evaporated under reduced pressure to give the title compound as a glass (2.27 g, *ca.* 87% yield); R_f 0.50 (system A), 0.32 (system B); $\delta_H(CDCl_3)$ 2.09 (3 H, s), 2.13 (3 H, s), 2.15 (3 H, s), 4.38 (1 H, dd, *J* 5.1, 12.9 Hz), 4.42–4.50 (2 H, m), 5.66 (1 H, t, *J* 5.0 Hz), 5.96 (1 H, t, *J* 5.3 Hz), 6.22 (1 H, d, *J* 5.2 Hz), 7.45 (2 H, d, *J* 8.3 Hz), 7.59 (2 H, d, *J* 8.3 Hz), 8.18 (1 H, s), and 8.63 (1 H, s); $\delta_C(CDCl_3)$ 20.40, 20.54, 20.78, 62.97, 70.57, 73.10, 80.43, 86.53, 125.40, 129.61, 131.36, 136.10, 136.87, 141.58, 148.60, 152.52, 160.82, 169.34, 169.58, and 170.31.

6-(4-*Chlorophenylthio*)-9-β-D-*ribofuranosyl*-9H-*purine* (**6b**).—(*a*) 6-(4-Chlorophenylthio)-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine (2.27 g, *ca.* 4.4 mmol) was dissolved in 8M-methanolic ammonia (20 ml) and the solution was kept at room temperature overnight. The products were concentrated under reduced pressure and the residue was purified by short-column chromatography on silica gel. The appropriate fractions, eluted with $CHCl_3$–EtOH (95:5 v/v), were combined and evaporated under reduced pressure to give the title compound as glass (1.67 g, *ca.* 97%); R_f 0.24 (system A), 0.05 (system B); $\delta_H[(CD_3)_2SO]$ 3.58 (1 H, m), 3.70 (1 H, m), 3.99 (1 H, m), 4.19 (1 H, m), 4.60 (1 H, m), 5.12 (1 H, t, *J* 5.5 Hz), 5.25 (1 H, d, *J* 5.0 Hz), 5.55 (1 H, d, *J* 5.9 Hz), 6.01 (1 H, d, *J* 5.5 Hz), 7.57 (2 H, d, *J* 8.4 Hz), 7.67 (2 H, d, *J* 8.4 Hz), 8.61 (1 H, s), and 8.79 (1 H, s); $\delta_C[(CD_3)_2SO]$ 61.13, 70.17, 73.81, 85.65, 87.86, 125.63, 129.35, 130.56, 134.67, 137.16, 143.81, 148.66, 151.62, 158.46, and 171.57.

(*b*) The above experiment was repeated on a larger scale by keeping a solution of 6-(4-chlorophenylthio)-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine (26.85 g, *ca.* 51.5 mmol) in 8M-methanolic ammonia (150 ml) at room temperature overnight. The products were then concentrated under reduced pressure, then redissolved in chloroform (250 ml), and saturated aq. ammonium chloride (250 ml) was added. The precipitated solid at the interface between the two layers was collected by filtration and was suspended in distilled water (700 ml) at room temperature for 1 h. The solid was filtered off, washed with water (2 × 100 ml), and dried *in vacuo* over P_2O_5 to give the title compound (16.9 g, *ca.* 83%).

6-(4-*Chlorophenylthio*)-9-(2,3-O-*cyclopentylidene*-β-D-*ribofuranosyl*)-9H-*purine* (**7**).—1,1-Dimethoxycyclopentane[15] (16.6 g, 0.128 mol) and toluene-4-sulphonic acid monohydrate (0.81 g, 4.26 mmol) were added to a stirred solution of 6-(4-chlorophenylthio)-9-β-D-ribofuranosyl-9*H*-purine (16.8 g, *ca.* 42.5 mmol) in dry THF (250 ml) at room temperature. The reactants were heated at 50 °C under nitrogen for 1 h, cooled to room temperature, and were then treated with triethylamine (0.7 ml, 5.0 mmol). The products were evaporated under reduced pressure and the residue was partitioned between chloroform (400 ml) and saturated aq. sodium hydrogen carbonate (400 ml). The aq. layer was back-extracted with chloroform (2 × 100 ml), and the combined organic layers were dried ($MgSO_4$), and concentrated under reduced pressure. The residue obtained was fractionated by short-column chromatography on silica gel. The appropriate fractions, eluted with $CHCl_3$–EtOH (100:0–99:1 v/v), were combined and evaporated under reduced pressure to give the title compound as a foam (16.5 g, *ca.* 84%); R_f 0.37 (system A), 0.30 (system B); $\delta_H[(CD_3)_2SO]$ 1.55–1.80 (6 H, m), 1.98 (2 H, m), 3.54 (2 H, m), 4.29 (1 H, m), 4.93 (1 H, dd, *J* 2.3, 6.2 Hz), 5.11 (1 H, t, *J* 5.2 Hz), 5.37 (1 H, dd, *J* 2.5, 6.2 Hz), 6.25 (1 H, d, *J* 2.4 Hz), 7.57 (2 H, d, *J* 8.6 Hz), 7.66 (2 H, d, *J* 8.6 Hz), 8.62 (1 H, s), and 8.73 (1 H, s); $\delta_C[(CD_3)_2SO]$ 23.09, 23.65, 36.57, 36.71, 63.25, 81.85, 82.71, 85.93, 93.65, 123.73, 125.13, 129.64, 132.18, 136.21, 136.87, 143.01, 147.51, 151.67, and 161.70.

6-(4-*Chlorophenylsulphonyl*)-9-(2,3-O-*cyclopentylidene*-β-D-*ribofuranosyl*)-9H-*purine* (**8**).—3-Chloroperbenzoic acid (*ca.* 55%; 32.0 g, *ca.* 0.10 mol) was added to a stirred solution of 6-(4-chlorophenylthio)-9-(2,3-*O*-cyclopentylidene-β-D-ribofuranosyl)-9*H*-purine (15.7 g, *ca.* 34 mmol) in dichloromethane (600 ml) at room temperature. After 3 h, the products were diluted with dichloromethane (300 ml) and were then extracted successively with 5% aq. sodium hydrogen sulphite (2 × 300 ml) and saturated aq. sodium hydrogen carbonate (4 × 300 ml). The dried ($MgSO_4$) organic layer was concentrated under reduced pressure to give a glass (15.1 g). Crystallization of this material from absolute ethanol gave the *title compound* (10.8 g, *ca.* 65%) (Found: C, 51.4; H, 4.2; N, 11.1. $C_{21}H_{21}ClN_4O_6S$ requires C, 51.2; H, 4.3; N, 11.4%), m.p. 168 °C; R_f 0.28 (system B); $\delta_H[(CD_3)_2SO]$ 1.55–1.80 (6 H, m), 1.97 (2 H, m), 3.55 (2 H, m), 4.34 (2 H, m), 4.91 (1 H, dd, *J* 2.2, 6.2 Hz), 5.12 (1 H, m), 5.37 (1 H, dd, *J* 2.1, 6.2 Hz), 6.31 (1 H, d, *J* 2.0 Hz), 7.77 (2 H, d, *J* 8.5 Hz), 8.12 (2 H, d, *J* 8.5 Hz), 9.03 (1 H, s), and 9.11 (1 H, s); $\delta_C[(CD_3)_2SO]$ 23.10, 23.65, 36.49, 36.59, 63.11, 81.74, 83.44, 86.39, 93.16, 123.95, 129.66, 130.97, 131.10, 136.51, 141.58, 147.64, 151.57, 153.70, and 154.91.

6-(4-*Chlorophenylsulphinyl*)-9-(2,3-O-*cyclopentylidene*-β-D-*ribofuranosyl*)-9H-*purine* (**9**).—A solution of 3-chloroperbenzoic acid (*ca.* 55%; 1.38 g, *ca.* 4.4 mmol) in dichloromethane (40 ml) was added dropwise during 1 h to a cooled (ice–salt-bath, *ca.* −5 °C), stirred solution of 6-(4-chlorophenylthio)-9-(2,3-*O*-cyclopentylidene-β-D-ribofuranosyl)-9*H*-purine (1.94 g, *ca.* 4.2 mmol) in dichloromethane (60 ml). The reactants were maintained at *ca.* −5 °C for a further period of 2.5 h and were then extracted successively with 5% aq. sodium hydrogen sulphite (2 × 50 ml) and saturated aq. sodium hydrogen carbonate (2 × 100 ml). The dried ($MgSO_4$) organic layer was concentrated under reduced pressure and the residue was fractionated by short-column chromatography on silica gel. The appropriate fractions, eluted with $CHCl_3$–EtOH (99:1 v/v), were combined, and evaporated under reduced pressure to give the title compound as a foam (1.77 g, *ca.* 88%); R_f 0.23 (system B); $\delta_H[(CD_3)_2SO]$ 1.55–1.80 (6 H, m), 1.97 (2 H, m), 3.55 (2 H, m), 4.31 (1 H, m), 4.91 (1 H, m), 5.11 (1 H, m), 5.36 (1 H, m), 6.29 (1 H, d, *J* 2.3 Hz), 7.62 (2 H, m), 7.86 (2 H, m), 8.95 (1 H, s), and 9.07 (1 H, s); $\delta_C[(CD_3)_2SO]$ 23.07, 23.63, 36.51, 36.60, 63.08, 81.70, 81.73, 83.06, 83.15, 86.17, 86.21, 93.05, 93.17, 123.89, 123.92, 126.51, 129.74, 131.43, 131.48, 138.03, 140.66, 146.15, 146.20, 151.38, 151.44, 152.22, 152.24, 162.82, and 162.84.

Dibenzyl-N-[9-(2,3-O-*Cyclopentylidene*-β-D-*ribofuranosyl*)-9H-*purin*-6-*yl*]-L-*aspartate* (**11**).—(*a*) *N*,*N*-Di-isopropylethylamine (3.40 ml, 19.5 mmol) was added to a stirred solution of 6-(4-chlorophenylsulphonyl)-9-(2,3-*O*-cyclopentylidene-β-D-ribofuranosyl)-9*H*-purine (**8**) (2.40 g, *ca.* 4.9 mmol) and dibenzyl L-aspartate toluene-4-sulphonate (5.90 g, 12.15 mmol) in dry DMA (50 ml) and the resulting mixture was heated at 100 °C under nitrogen. After 24 h, the products were concentrated under reduced pressure and the residue was partitioned between chloroform (200 ml) and saturated aq. sodium hydrogen carbonate (200 ml). The organic layer was dried ($MgSO_4$), and evaporated under reduced pressure. The residual yellow oil obtained was redissolved in ethyl acetate (150 ml) and the solution was extracted with cold 3M-phosphoric acid (4 × 50

ml). The aq. layer was back-extracted with ethyl acetate (50 ml) and the combined organic layers were extracted with saturated aq. sodium hydrogen carbonate (100 ml), dried ($MgSO_4$), and concentrated under reduced pressure. The residual glass obtained was fractionated by short-column chromatography on silica gel. The appropriate fractions, eluted with $CHCl_3$–EtOH (99:1 v/v), were combined and concentrated under reduced pressure to give the title compound as a pale yellow glass (1.84 g, *ca.* 60%); R_f 0.30 (system B); $\delta_H[(CD_3)_2SO]$ 1.65 (4 H, m), 1.74 (2 H, m), 2.01 (2 H, m), 3.10 (2 H, m), 3.56 (2 H, m), 4.25 (1 H, m), 4.93 (1 H, dd, *J* 2.3, 6.2 Hz), 5.08 (2 H, s), 5.11 (2 H, s), 5.20 (1 H, m), 5.31 (1 H, m), 6.17 (1 H, d, *J* 2.7 Hz), 7.29 (10 H, m), 8.25 (1 H, s), 8.35 (1 H, m), and 8.42 (1 H, s); $\delta_C[(CDCl_3]$ 23.08, 23.66, 36.60, 36.75, 49.79, 63.36, 66.84, 67.48, 81.98, 82.54, 85.75, 93.76, 121.52, 123.43, 128.20, 128.36, 128.52, 135.20, 135.32, 140.16, 147.92, 152.28, 154.16, 170.39, and 170.62.

(*b*) *N,N*-Di-isopropylethylamine (1.40 ml, 8.0 mmol) was added to a stirred solution of 6-(4-chlorophenylsulphinyl)-9-(2,3-*O*-cyclopentylidene-β-D-ribofuranosyl)-9*H*-purine (**9**) (0.954 g, *ca.* 2.0 mmol) and dibenzyl L-aspartate toluene-4-sulphonate (2.40 g, 4.9 mmol) in dry DMA (20 ml), and the resulting mixture was heated at 70–75 °C under nitrogen. After 28 h, the products were worked up and purified as in section (*a*) above to give the title compound (0.88 g, *ca.* 70%), identical (TLC, ^1H and ^{13}C NMR) with the material obtained in section (*a*) above.

N-[9-(β-D-*Ribofuranosyl*)-9H-*purin*-6-*yl*]-L-*aspartic Acid* (**3**).—(*a*) Dibenzyl *N*-[9-(2,3-*O*-cyclopentylidene-β-D-ribofuranosyl)-9*H*-purin-6-yl]-L-aspartate [0.88 g, *ca.* 1.4 mmol; prepared from 6-(4-chlorophenylsulphinyl)-9-(2,3-*o*-cyclopentylidene-β-D-ribofuranosyl)-9*H*-purine] was dissolvd in formic acid–water (3:2 v/v; 30 ml) and the solution was stirred at room temperature. After 3 h, water (30 ml) was added and the products were extracted with chloroform (4 × 30 ml). The combined organic extracts were extracted with aq. sodium hydrogen carbonate (50 ml) and were then dried ($MgSO_4$), and concentrated under reduced pressure. The residue obtained was fractionated by short-column chromatography on silica gel. The appropriate fractions, eluted with $CHCl_3$–EtOH (96:4 v/v), were combined, and evaporated under reduced pressure to give a glass (0.65 g); R_f 0.22 (system A).

A solution of the latter material (0.65 g) in ethanol–acetic acid–water (8:1:1 v/v; 30 ml) and 10% palladium on activated carbon (0.13 g) were stirred together under hydrogen at room temperature and atmospheric pressure for 2 h. The products were then filtered and the residual catalyst was washed with ethanol–water (1:1 v/v; 20 ml). The combined filtrate and washings were evaporated under reduced pressure, and the residue was crystallized from aqueous ethanol to give the title compound (0.38 g, *ca.* 68% overall yield for the two steps) (Found: C, 42.1; H, 4.7; N, 17.2. $C_{14}H_{17}N_5O_8 \cdot H_2O$ requires C, 41.9; H, 4.8; N, 17.45%); m.p. 160 °C (decomp.); $[\alpha]_D^{20}$ −48 ° (*c* 1.6, DMA); t_R 5.3 min [0.1M-aq. triethylammonium acetate–MeCN (97:3 v/v)], 12.0 min [0.1M-aq. triethylammonium acetate–MeCN (98:2 v/v)]; λ_{max}(95% EtOH) 267 (ε 18 200); λ_{min} 232 nm (ε 2 000); λ_{max}(0.097M-aq. NaOH) 268 (ε 19 400); λ_{min} 233 nm (ε 2 550); $\delta_H[(CD_3)_2SO]$ 2.90 (2 H, m), 3.57 (1 H, m), 3.69 (1 H, m), 3.97 (1 H, m), 4.16 (1 H, m), 4.61 (1 H, m), 5.09 (1 H, m), 5.19 (1 H, d, *J* 4.2 Hz), 5.38 (1 H, m), 5.48 (1 H, d, *J* 5.8 Hz), 5.91 (1 H, d, *J* 6.1 Hz), 7.96 (1 H, d, *J* 7.9 Hz), 8.25 (1 H, s), and 8.41 (1 H, s); $\delta_C[(CD_3)_2SO]$ 35.88, 49.52, 61.65, 70.65, 73.60, 85.92, 88.07, 119.90, 140.29, 148.66, 152.06, 154.05, 172.13, and 172.89.

(*b*) Dibenzyl *N*-[9-(2,3-*O*-cyclopentylidene-β-D-ribofuranosyl)-9*H*-purin-6-yl]-L-aspartate [0.94 g, *ca.* 1.5 mmol; prepared from 6-(4-chlorophenylsulphonyl)-9-(2,3-*O*-cyclopentylidene-β-D-ribofuranosyl)-9*H*-purine] was treated with formic acid–water (3:2 v/v; 30 ml) as above, and the products were worked up and chromatographed to give a glass (0.67 g). A solution of a portion of the latter material (0.28 g) in ethanol–acetic acid–water (8:1:1 v/v; 15 ml) and 10% palladium on activated carbon (0.06 g) were stirred together under hydrogen at room temperature and atmospheric pressure for 2 h. The products were then worked up as above to give the title compound (0.152 g, *ca.* 61% overall yield for the two steps), identical with material described in section (*a*) above.

N-[9-(β-D-*Ribofuranosyl*)-9H-*purin*-6-*yl*]*aspartic Acid*.—Dibenzyl *N*-[9-(2,3-*O*-cyclopentylidene-β-D-ribofuranosyl)-9*H*-purin-6-yl] aspartate was prepared as above by allowing 6-(4-chlorophenylsulphonyl)-9-(2,3-*O*-cyclopentylidene-β-D-ribofuranosyl)-9*H*-purine (0.52 g, 1.05 mmol), (±)-dibenzyl aspartate[11] (0.83 g, 2.65 mmol), and *N,N*-di-isopropylethylamine (0.28 ml, 1.6 mmol) to react together in dry DMA (10 ml) at 100 °C for 24 h. Following the removal of the cyclopentylidene and benzyl protecting groups from a large portion (0.32 g) of this material by the procedures described above in the preparation of the L-aspartic acid-derived diastereoisomer, the title substance [0.13 g, *ca.* 36% overall yield based on the original quantity of (**8**) used] was obtained as a solid; $[\alpha]_D^{20}$ −31° (*c* 1.6, DMA); t_R 6.07 (*ca.* 50%), 8.15 (*ca.* 50% min [0.1M-aq. triethylammonium acetate–MeCN (97:3 v/v)]. Under the same elution conditions pure, crystalline *N*-[9-(β-D-ribofuranosyl)-9*H*-purin-6-yl]-L-aspartic acid had t_R 6.07 min, and the mother liquors remaining after the crystallisation of the latter compound [prepared from 6-(4-chlorophenylsulphonyl)-9-(2,3-*O*-cyclopentylidene-β-D-ribofuranosyl)-9*H*-purine (**8**)] had t_R 6.07 (*ca.* 66%) and 8.15 (*ca.* 33%) min.

Ammonium Salt of {N-[9-(β-D-*Ribofuranosyl*)-9H-*purin*-6-*yl*]-L-*aspartic Acid* 5′-*Phosphate*} (*Adenylosuccinic Acid*) (**1**).—(*a*) *N*-Chlorosuccinimide (0.50 g, 3.74 mmol) was added to a stirred solution of dibenzyl phosphonate[16] (1.00 g, 3.8 mmol) in dry benzene (15 ml) under nitrogen at room temperature. After 90 min, the products were filtered and the filtrate was concentrated under reduced pressure at 10–15 °C to give dibenzyl phosphorochloridate as an oil. The latter material was added by syringe to a stirred solution of dry [following evaporation from pyridine (2 × 8 ml) solution] dibenzyl *N*-[9-(2,3-*O*-cyclopentylidene-β-D-ribofuranosyl)-9*H*-purin-6-yl]-L-aspartate [0.80 g, *ca.* 1.27 mmol, prepared from 6-(4-chlorophenylsulphinyl)-9-(2,3-*O*-cyclopentylidene-β-D-ribofuranosyl)-9*H*-purine] in anhydrous pyridine (8 ml) at *ca.* −40 °C [acetone–solid CO_2-bath] under nitrogen. After 2 h, the reaction mixture was removed from the cooling bath and saturated aq. sodium hydrogen carbonate (20 ml) was added. The products were then concentrated under reduced pressure and the residual syrup was partitioned between chloroform (100 ml) and saturated aq. sodium hydrogen carbonate (100 ml). The aq. layer was back-extracted with chloroform (30 ml), and the combined, dried ($MgSO_4$) organic layers were evaporated under reduced pressure. The residue obtained was fractionated by short-column chromatography on silica gel. The appropriate fractions, eluted with $CHCl_3$–EtOH (99:1 v/v), were combined, and evaporated under reduced pressure to give a pale yellow glass (1.03 g); R_f 0.24 (system B); $\delta_P(CDCl_3)$ −0.2.

The latter material (1.03 g) was dissolved in formic acid–water (4:1 v/v; 25 ml) and the solution was kept at room temperature. After 3 h, water (25 ml) was added and the products were extracted with chloroform (4 × 30 ml). The combined extracts were washed with saturated aq. sodium hydrogen carbonate (50 ml), dried ($MgSO_4$), and concentrated under reduced pressure. The residue obtained was fractionated by short-column chromatography on silica gel. The appropriate fractions, eluted with $CHCl_3$–EtOH (97:3 v/v), were combined, and evaporated under reduced pressure to give a glass

(0.734 g); R_f 0.36 (system A), 0.13 (system B); $\delta_P(CDCl_3)$ −0.08.

A solution of this material (0.734 g) in ethanol–water (4:1 v/v; 50 ml) and 10% palladium on activated carbon (0.15 g) were stirred together under hydrogen at room temperature and atmospheric pressure for 2 h. The products were then filtered and the residual catalyst was washed with ethanol–water (1:1 v/v: 25 ml). Conc. aq. ammonia (*d* 0.88) was added to the combined filtrate and washings until the pH rose to *ca.* 9.0. The resulting solution was then evaporated under reduced pressure. The residual yellow glass obtained was redissolved in water (10 ml) and the solution was heated at 60 °C with activated charcoal for 30 min. The charcoal was removed by filtration and the filtrate was concentrated under reduced pressue to *ca.* 3 ml. Addition of the latter solution dropwise to stirred absolute ethanol (*ca.* 300 ml) gave the title compound as a precipitate (0.37 g, *ca.* 66% overall yield for the three steps); t_R 7.4 (*ca.* 99.5%), 5.9 (*ca.* 0.5%) min [0.1M-triethylammonium acetate–MeCN (98:2 v/v)]; $\delta_H(D_2O)$ 2.88 (1 H, dd, *J* 7.7, 16.1 Hz), 2.97 (1 H, dd, *J* 4.2, 16.0 Hz), 4.05 (2 H, m), 4.30 (1 H, m), 4.42 (1 H, t, *J* 4.4 Hz), 4.66 (1 H, t, *J* 5.4 Hz), 6.07 (1 H, d, *J* 5.6 Hz), 8.21 (1 H, s), and 8.38 (1 H, s); $\delta_C(D_2O)$ 39.65, 54.01, 65.17, 71.12, 75.17, 84.67 (d, *J* 8.3 Hz), 87.92, 119.74, 140.30, 148.94, 153.11, 154.41, 178.51, and 178.84; $\delta_P(D_2O)$ 1.13.

(*b*) Dibenzyl *N*-[9-(2,3-*O*-cyclopentylidene-β-D-ribofuranosyl)-9*H*-purin-6-yl]-L-aspartate [2.417 g, *ca.* 3.84 mmol, prepared from 6-(4-chlorophenylsulphonyl)-9-(2,3-*O*-cyclopentylidene-β-D-ribofuranosyl)-9*H*-purine] was phosphorylated with dibenzyl phosphorochloridate [prepared from dibenzyl phosphonate (3.54 g, 13.5 mmol)] in pyridine (30 ml) solution under the conditions described above in section (*a*). The reaction mixture was worked up and fractionated in the same way and the product obtained was treated as above with formic acid–water (4:1 v/v) to give, after work-up and chromatography, a glass (1.98 g). A large portion (1.68 g) of the latter material was hydrogenated in the presence of palladium–charcoal. Work-up, and neutralization of the products with conc. aq. ammonia, gave the title compound, which was isolated as above as a precipitate (1.024 g, *ca.* 59% overall yield for the three steps); t_R 7.5 (*ca.* 91%), 5.9 (*ca.* 9%) min [0.1M-triethylammonium acetate–MeCN (98:2 v/v)].

Enzyme-promoted Dephosphorylation of Impurity in Adenylosuccinic Acid (**1**) *Prepared from* 6-(4-*Chlorophenylsulphonyl*)-9-(2,3-*o-cyclopentylidene*-β-D-*ribofuranosyl*)-9H-*purine* (**8**).—The low-retention-time (t_R 5.9 min) impurity in the ammonium salt of adenylosuccinic acid obtained in section (*b*) above was isolated by preparative LC. A solution of the latter material (1.0 A_{260} unit) and bacterial alkaline phosphatase (2.0 units) in 0.05M-Tris hydrochloride buffer (pH 7.5; 0.2 ml) was maintained at 25 °C. After 15 min, complete digestion of the substrate to a product with t_R 8.38 min [0.1M-aq. triethylammonium acetate–MeCN (97:3 v/v)] had occurred. The product had the same t_R-value as the high-t_R diastereoisomer of *N*-[9-(β-D-ribofuranosyl)-9*H*-purin-6-yl] aspartic acid (see above).

Digestion of Adenylosuccinic acid (**1**) *with Adenylosuccinate Lyase.*—A solution of adenylosuccinate lyase (0.1 units) in 0.05M-potassium phosphate buffer (pH 7.0; 0.1 ml) was added to a solution of ammonium adenylosuccinate (0.0002 g) in the same buffer (0.2 ml) at 25 °C. After 17 h, the substrate (t_R 10.15 min) [0.1M-aq. triethylammonium acetate–MeCN (98.6:1.4 v/v)] was completely converted into adenosine 5′-phosphate (t_R 8.65 min).

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