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Solid Phase Synthesis of Purines from Pyrimidines

Raffaella Di Lucrezia,[†] Ian H. Gilbert,*[,†] and Christopher D. Floyd[‡]

Welsh School of Pharmacy, Cardiff University, Redwood Building, King Edward VII Avenue, Cardiff, CF10 3XF U.K., and British Biotech, Watlington Road, Cowley, Oxford, OX4 5LY U.K.

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In this paper the solid phase synthesis of various substituted purines is described starting from 4,6-dichloro-5-nitropyrimidine. The 4,6-dichloro-5-nitropyrimidine was coupled to Rink amide resin followed by displacement of the second chloride by an amino compound. Reduction of the nitro compound proved to be problematic but was achieved using lithium aluminum hydride/aluminum trichloride. The diamines (**13**) were then elaborated to purines by three different routes.

Introduction

Purines and pyrimidines are very important molecules in biology and have many applications in the area of therapeutics. For example, pyrimidines are interesting drug targets because they are found on some nucleosides, are well-known DHFR inhibitors, and present potential anti-viral, anti-fungal, anti-cancer, and anti-protozoan activity. Similarly, purines are found on some nucleosides and as such may represent important anti-infective and anti-cancer agents. In addition, adenosine receptors are important in regulation of a number of physiological processes. Selective agonists or antagonists[1] of specific adenosine receptors should have therapeutic value; for example, in cardiovascular or central nervous system complaints.[2] Various substituted adenosine analogues have been shown to act as selective adenosine receptor agonists and xanthine analogues as adenosine receptor antagonists. We were therefore interested in the solid phase synthesis of pyrimidines and purines as a prelude to preparation of libraries. Initially we focused on using pyrimidines as templates.

There have been a number of syntheses reported of pyrimidines and purines on solid phase. Thus various substituted pyrimidines have been prepared starting either by de novo synthesis of the pyrimidine[3] core or from the pyrimidine core itself.[4] In this latter case substitution was generated at the 2-, 4-, and 6-positions of the pyrimidine. Purines have also been prepared on solid phase by substitution of the halogenated purine core,[5] resulting in substitution at the 2-, 6-, and 9-positions.

In this paper we describe manipulation of the pyrimidine core on solid phase and subsequent elaboration to a purine nucleus. We were particularly interested in having amino substitution in the 5- and 6-positions of the pyrimidine nucleus as this should allow elaboration to a purine nucleus. When introducing substitution on the 5- and 6-positions of the pyrimidine, there is the potential problem of lack of activity of the 5-position to nucleophilic attack. Recently Srivastava et al.[6] have reported preparation of pyrimidopyrimidines by functionalization of the 5- and 6-positions of the pyrimidine, although the functionalization that they described was a 5-keto and 6-amino group.

Discussion

We first investigated the reactivity and coupling of pyrimidines on solid phase. 2,4-Dichloropyrimidine (**1**) was attached to Rink amide resin by shaking in diisoproylethylamine (DIPEA) in DMF (Scheme 1). This linker has also been used by Guillier et al.[4] for pyrimidines. Unfortunately, the reaction appeared to occur at both the 2- and 4-positions, giving rise to a mixture of products. One of the isomers predominated when compound **1** was reacted with nucleophiles in solution phase, and this was tentatively assigned to coupling at the 4-position.[7,8] The subsequent step, displacement of the second chlorine, proved very problematic owing to deactivation of the pyrimidine ring to nucleophilic attack following attachment to the resin. A variety of conditions were examined including the use of strong bases. However, in all cases the product, isolated following cleavage from the resin by TFA, contained varying amounts of either 4-amino-2-chloropyrimidine (**7**) or 2-amino-4-chloropyrimidine (**9**) in addition to the desired products **6** and **8**. The ratio of nonsubstituted compounds (**7** + **9**) to substituted compounds (**6** + **8**) was 7:1, and the ratio of **6**:**8** was about 4:1. As we were interested in coupling low molecular weight, volatile amines to the pyrimidine nucleus, this precluded the use of elevated temperatures. Instead the starting pyrimidine needed to be both more reactive and give better regioselective control.

Thus 4,6-dichloro-5-nitropyrimidine (**10**) was used as the starting pyrimidine. This was readily coupled to Rink amide resin (Scheme 2). Solution phase studies had confirmed that the displacement of the second chloride was very rapid, even at room temperature. This second step was carried out on the resin, and the intermediate (**12**) could be cleaved from the resin (using TFA) to confirm structure. At this stage it was decided to investigate reduction of the nitro group to the amine which would allow subsequent elaboration of the structure.

* To whom correspondence should be addressed. Tel: +44 29 20 87 58 00. Fax: +44 29 20 87 41 49. E-mail: gilbertih@cf.ac.uk.

[†] Cardiff University.

[‡] British Biotech.

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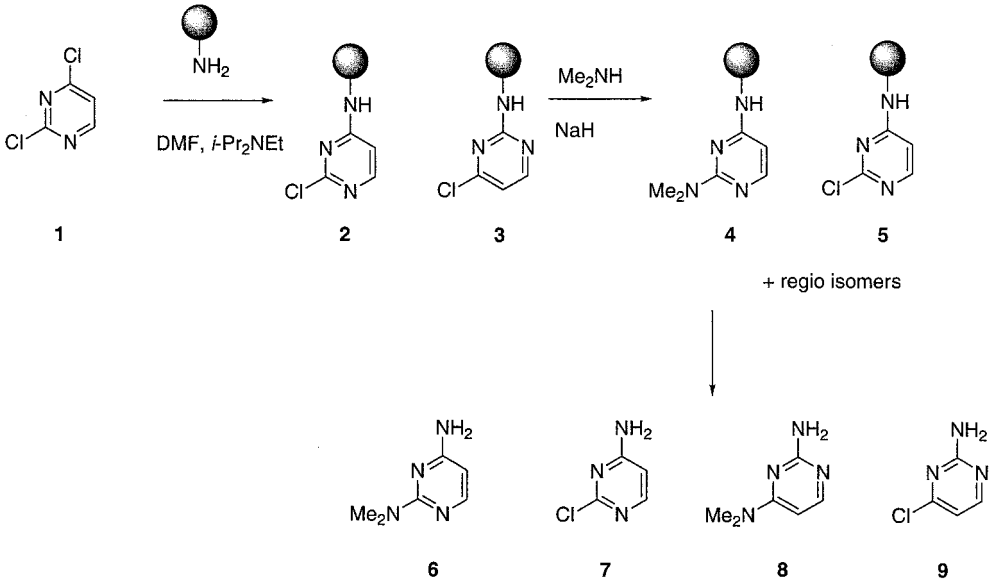
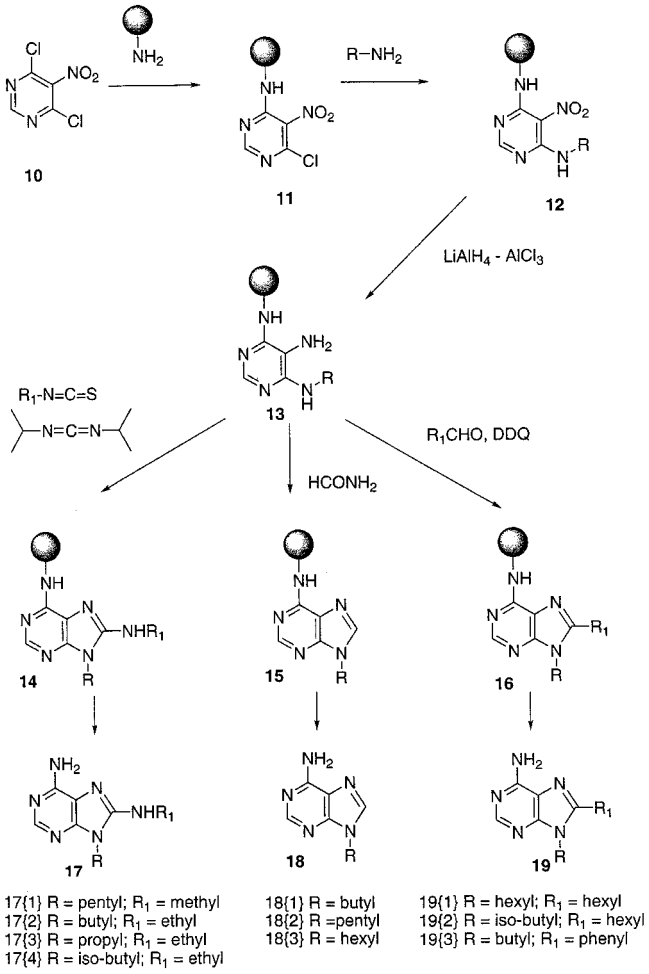
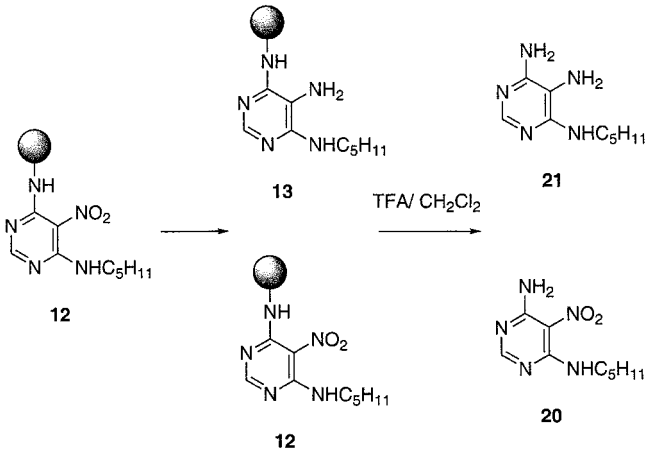
Scheme 1



Scheme 2



Scheme 3



The use of some conventional reagents for the reduction of aromatic nitro groups to amino groups (for example, iron or tin in dilute hydrochloric acid, or palladium catalyzed hydrogenation) was probably precluded because the reaction was being undertaken on solid phase. These conditions would also increase the risk of loss of compound or cleavage of the linker from the resin. We decided to investigate lithium aluminum hydride as a reducing agent. In solution phase, lithium aluminum hydride carries out a reductive dimerization of aromatic nitro groups to form azo compounds.[9] However, we reasoned that under solid phase conditions, dimerization would be unlikely to occur. Initial reactions were very promising, and lithium aluminum hydride reduced the nitro group to an amino group, as was detected by cleavage of the product from the resin followed by mass spectrometry. Unfortunately these reaction conditions proved to be somewhat variable; in particular the age of the solution of lithium aluminum hydride seemed to be important, with older (less pure) solutions giving better results.

Therefore, a number of other conditions for reduction of the nitro group were tried (Scheme 3). Essentially the resin bound pentyl derivative (**12**) was subject to various reducing conditions (Table 1), and the product was cleaved from the resin using TFA. The product was then examined by mass spectrometry for the unreduced compound **20** and the reduced compound **21**. The data presented in Table 1 show the relative peak heights of unreduced (**20**) and reduced (**21**) compounds according to electrospray mass spectrometry. The only reduction condition which appeared to consume all of the starting material was lithium aluminum hydride–

Table 1. Reduction Conditions for the Nitro Group Showing the Ratio of the Peak Heights in Electrospray Mass Spectrometry of Unreduced (**20**) to Reduced (**21**) Compound

no.	reduction conditions[a]	**20**	**21**
1	$SnCl_2 \cdot H_2O/H_2O$/70 °C	100	0
2[b]	$SnCl_2 \cdot 2H_2O$/EtOAc/70 °C	36	100
3	2 M $SnCl_2 \cdot 2H_2O$/DMF/rt	49	100
4	2 M $SnCl_2 \cdot 2H_2O$/DMF/60 °C	100	0
5	$NaBH_4/CoCl_2 \cdot 6H_2O$/MeOH/rt	100	0
6	$NaBH_4/SnCl_2 \cdot 2H_2O$/EtOH/rt	100	0
7	$NaBH_4/CuCl_2 \cdot 6H_2O$/MeOH/rt	100	0
8	$NaBH_4/CoCl_2 \cdot 6H_2O$/diglyme/100 °C	100	0
9[c]	$NaBH_4/Cu(acac)_2$/EtOH/rt	58	0
10	$Na_2S_2O_4$/EtOAc/70 °C	100	0
11	Zn/1 M HCl/MeOH/rt	100	10
12	Fe/1 M HCl/MeOH/rt	100	0
13	$NaBH_2S_3$/THF/rt	100	38
14[b]	$NH_2NH_2 \cdot xH_2O$/C/EtOH	88	0
15	$LiBH_4$/diglyme/THF/reflux	100	92
16	$LiAlH_4/AlCl_3$/THF/rt	0	100

[a] rt = room temperature. At least 5 equivalents of reagents were used in all cases. [b] On repetition on a large scale this gave only **20**. [c] The 100% peak in the spectrum was another compound.

aluminum chloride. Some other conditions gave partial reduction (reactions 2, 3, 13, 15), such as tin(II) chloride and lithium borohydride. However, these conditions were not consistently reproduceable.

The 4,6-diamino-5-nitropyrimidine (**12**) was remarkably resistant to reduction. This may be due to conjugation of the amino groups to the nitro group, making the latter resistant to reduction (Figure 1). In addition, there is significant intramolecular hydrogen bonding in the nitro compound which must be broken for reduction to occur. It is possible that the aluminum trichloride (or aluminum hydrides generated during the reaction) acts as a Lewis acid, complexing to the nitro group to facilitate reduction.

Therefore lithium aluminum hydride−aluminum trichloride was used as the reagent for reduction of the nitro group. The major drawback in its use was the formation of inorganic salts which contaminated the final products. Some of the inorganic salts could be removed by shaking with a saturated solution of Rochelle's salt and ammonium chloride in water/DMF following the reduction step. However, this treatment did not remove all the salts. During the final cleavage of the compounds from the resin, these salts were solubilized in the TFA and led to contamination of the product.

The reduction of the nitro group to an amino group opened up a number of possible further elaborations to purines. This could be achieved by ring formation using the amino groups on the 5- and 6-positions. A number of strategies to achieve this ring formation have been reported in the literature, and our results with several of these methods are described below.

It has been shown that 1,2-diaminobenzenes react with isothiocyanates in the presence of DCC to give five-membered imidazoles.[10,11] Thus reaction of the triamino intermediate **13** (R = pentyl) with isothiocyanates (R_1 = methyl) in the presence of DCC gave the 8-aminopurines, **14**. The product was cleaved from the resin and purified by ion-exchange chromatography to give the product **17**{**1**} in 45% yield. The products were unfortunately contaminated with dicyclohexylurea, a byproduct of the cyclization reaction. The presence of DCU could be clearly seen in the mass

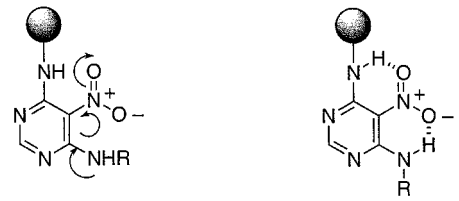


Figure 1.

spectra. Presumably the dicyclohexylurea was only partially soluble in the solvents used to wash the resin, but dissolved to some extent in the reagents used to cleave the compound from the resin. Subsequent investigations have shown use of diisopropylcarbodiimide instead of DCC removes this problem, owing to the greater solubility of the diisopropylurea in solvents used to wash the resin. Thus the diispropylurea was removed by washing the resin and did not appear as a contaminant in the product.

Formation of purines has been reported by cyclization of *ortho*-diaminoaromatic compounds with formamide.[12−14] Treatment of resin with formamide gave cyclization to give the simple unsubstituted purine. The product **18**{**1**} was isolated after cleavage from the resin in 7% yield. In addition, products with pentyl and hexyl substituents were prepared and detected by mass spectometry.

A third method reported to form the five-membered ring is condensation of a 1,2-diaminobenzene with an aldehyde in the presence of a mild oxidizing agent, in this case DDQ.[15] Treatment of intermediate **13** with an aldehyde in the presence of DDQ gave formation of an 8-alkylpurine or 8-arylpurine **16**. This was cleaved from the resin and purified by reverse phase chromatography to give the pure product **19**{**1**} in 7% yield.

In conclusion, we have prepared a series of purines on solid phase using a pyrimidine as a starting material. Purification of the products proved somewhat problematic. The main contaminant appeared to be inorganic salts. The main organic compound present was the desired compound. The methodology reported here describes substitution or variation at the 8- and 9-positions of the purine ring and could be extended to include substitution at the 2- and 6-positions.

Experimental Section

General Procedure for Preparation of Intermediate 13. Step 1: A DMF solution containing a large excess of 4,6-dichloro-5-nitropyrimidine (145 mg, 0.75 mmol) and DIPEA (97 mg, 0.75 mmol) was added to Rink amide MBHA (potential loading 0.15 mmol) and shaken for 2 h at room temperature, and then the resin was washed with DMF, H_2O, and CH_2Cl_2.

Step 2: A large excess of amine (0.75 mmol) and DIPEA (97 mg, 0.75 mmol) in DMF (4 mL) solution was added to the resin and the reaction was shaken for 2 h. The resin was then washed with DMF, H_2O, CH_2Cl_2, and Et_2O.

Step 3: A solution of lithium aluminum hydride (0.75 mL of a 1 M solution in THF, 0.75 mmol) and aluminum chloride (100 mg, 0.75 mmol) were premixed in dry THF (3 mL) and then added to the resin. The mixture was shaken overnight. The excess of lithium aluminum hydride was

quenched with ethyl acetate and the resin washed with water, a saturated solution of Rochelle's salt and ammonium chloride, DMF, water, methanol, dichloromethane, and ether. A few beads were then cleaved with TFA, and the product was examined by electrospray mass spectroscopy to check that reaction had occurred.

***N*8-Methyl-9-pentyl-9*H*-6,8-purinediamine (17{1}).** Step 4: A benzene (3 mL) solution of methylisothiocyanate (56 mg, 0.75 mmol) was added to the resin (**13**), and the mixture was refluxed for 15 min. Then DCC (155 mg, 0.75 mmol) was added. The reaction was refluxed at 80 °C overnight.

Step 5: The Rink amide resin (potential loading 0.1 mmol) was shaken with a 30% solution of TFA in CH_2Cl_2 for 20 min and then washed with CH_2Cl_2, H_2O, and MeOH. The solvents were reduced in vacuo, yielding 274 mg of crude compound. The product was purified by sulfonic ion-exchange resin (loading 4.4 mmol/g). The crude product was loaded in water (5 mL) and the resin eluted with water (30 mL), methanol (30 mL), and NH_4OH (0.2 g/mL, 4 × 10 mL) to yield the product (16 mg, 45%). δ_H (300 MHz, d^4-MeOH): 0.96 (3H, t, J = 6.6, C*H*$_3$), 1.33−1.48 (4H, m, CH_2CH_2CH$_3$), 1.77 (2H, m, CH_2CH$_2$N), 3.07 (3H, d, J = 9.4, CH_3NH), 4.03 (2H, t, J = 7.4, CH_2N), 8.04 (1H, s, C*H* aromatic). δ_C (75.5 MHz, D_2O): 11.5 (*C*H$_3$CH$_2$), 20.1 (*C*H$_2$), 25.8 (*C*H$_2$), 29.5 (*C*H$_2$), 26.4 (*C*H$_3$NH), 26.9 (*C*H$_2$), 47.3 (*C*H$_2$N), 114.0 (*C* aromatic 1), 147.2 (*C*H aromatic and C aromatic 2), 163.6 (*C*-NH$_2$), 169.5 (*C*-NH aromatic). *m*/*z* (ES$^+$): 235.0 (M + H$^+$, 100%).

9-Butyl-9*H*-6-purineamine (18{1}). Step 4: $HCONH_2$ was added to the resin (**13**), and the mixture was heated to 160 °C overnight. The resin was then washed with DMF, H_2O, and CH_2Cl_2.

Step 5: The resin was shaken with a 30% solution of TFA in CH_2Cl_2 for 20 min and then washed with CH_2Cl_2, H_2O, and MeOH. The solvents were reduced in vacuo, yielding 36 mg of crude compound. ^{1}H NMR and ES$^+$-MS confirmed the presence of the target. The product was purified by preparative TLC to yield a transparent oil (2 mg, 7%). δ_H (300 MHz, d^4-MeOH): 0.92 (3H, t, J = 7.4, C*H*$_3$), 1.20−1.38 (4H, m, CH_2CH_2CH$_3$), 4.20 (2H, t, J = 7.0, CH_2N), 8.10 (1H, s, C*H* aromatic imid.), 8.19 (1H, s, C*H* aromatic pyrimidine). *m*/*z* (AP$^+$): 192.1 (M + H$^+$, 100%). *m*/*z* (ES$^+$): 192.0 (M+H$^+$, 100%); HRMS calcd for $C_9H_{14}N_5$ (M+H$^+$) 192.1249, found 192.1248.

8-Hexyl-9-hexyl-6-purinamine (19{1}). Step 4: DDQ (681 mg, 3 mmol) was added to the resin (potential loading 1.5 mmol) (**13**), and then heptanal (685 mg, 6 mmol) in DMF (12 mL) solution was added. The reaction was shaken at room temperature for 5 h. The resin was then washed with DMF, H_2O, and NH_4OH (at different concentrations).

Step 5: The resin was shaken twice with a 30% solution of TFA in CH_2Cl_2 for 20 min and then washed with CH_2Cl_2 yielding a black syrup.

ES$^+$-MS performed on the crude compound only showed the presence of the desired compound. *m*/*z* (ES$^+$): 304.2 (M + H$^+$).

Twenty percent of the crude was dissolved in 1 M NaOH (50 mL) and extracted with CH_2Cl_2 to yield 88 mg of yellow oil after filtration on Celite. Concentrated hydrochloric acid was added, and the solution was reduced in vacuo. The mixture was eluted through a 2 g C18-SPE column using a gradient of H_2O−MeOH−HCl (increasing concentrations of MeOH and maintaining an acidic medium using a few drops of concentrated HCl). The nonreduced intermediate *N*4-hexyl-5-nitro-4,6-pyrimidinediamine was eluted first [*m*/*z* (ES$^+$): 239 (M + H$^+$)], then the target compound [*m*/*z* (ES$^+$): 304.2 (M + H$^+$)]. Nonpolar impurities were not eluted. The hydrochloride salt of the expected compound was dissolved in CH_2Cl_2 and washed with a solution of 1 M NaOH to yield 6 mg (7% yield) of the pure target compound as a yellow oil. δ_H (300 MHz, d^4-MeOH): 0.88−0.93 (6H, m, 2 × C*H*$_3$), 1.27−1.92 (16H, m, 8 × C*H*$_2$), 2.94 (2H, t, J = 7.5, C*H*$_2$), 4.26 (2H, t, J = 7.4, CH$_2$N), 8.34 (1H, s, C*H* aromatic pyrimidine). δ_C (75.5 MHz, d^4-MeOH): 14.6 (*C*H$_3$), 14.8 (*C*H$_3$), 23.9 (*C*H$_2$), 24.0 (*C*H$_2$), 27.7 (*C*H$_2$), 28.3 (*C*H$_2$), 28.6 (*C*H$_2$), 30.4 (*C*H$_2$), 31.1 (*C*H$_2$), 32.8 (*C*H$_2$), 33.1 (*C*H$_2$), 45.0 (N-*C*H$_2$), 144.8, 161.7, 161.8. HRMS calcd for $C_{17}H_{30}N_5$ (M + H$^+$) 304.2501, found 304.2497.

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