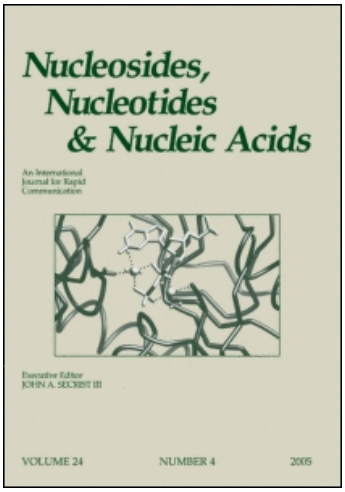

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Synthesis of Nucleoside Libraries on Solid Support. I. N^2,N^6-Disubstituted Diaminopurine Nucleosides[†]

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ABSTRACT

Starting with 2-iodo-6-chloro-9-(β-D-ribofuranosyl)purine, a library of more than 1,300 N^2,N^6-polysubstituted diaminopurine nucleosides was created. The starting material was condensed with a polystyrene monomethoxytrityl resin and a pool of primary and secondary amines was used to displace the 6-chloro atom with high regioselectivity. The 2-iodo was subsequently displaced by various primary amines. Nucleosides were cleaved from the resin with hexafluoroisopropanol solutions. A majority of compounds reached a purity of more than 80% without the need for any type of purification.

Key Words: Combinatorial library; Nucleoside; Purine.

[†]In honor and celebration of the 70th birthday of Professor Leroy B. Townsend.

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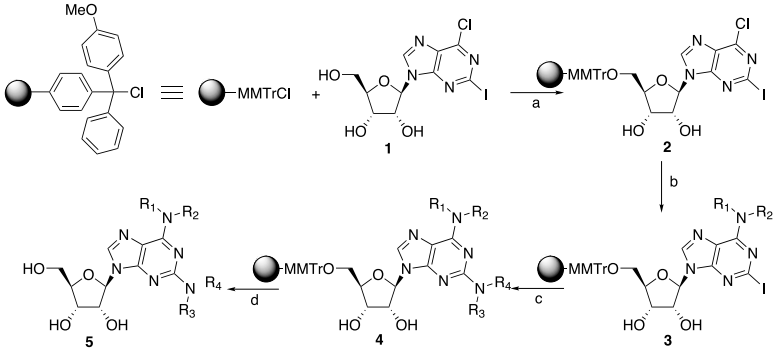
INTRODUCTION

Traditionally, the synthesis of nucleosides has always been a slow process that involved several steps with purification in each step.[1] This approach is still used by most nucleoside chemists.[2] We were interested in developing a synthetic scheme that would allow us to synthesize a large number of purine nucleoside analogues in a short period of time. Since purification steps are time consuming, one of our requirements was to obtain our target compounds pure enough for biological screening, without post-synthesis purification. To achieve this goal, each reaction had to be high yielding, which represented a challenge because of the known limitations of diaminopurine nucleosides in term of reactivity and/or stability.[3] We felt that the best way to achieve our goal was to link our starting nucleoside to a solid support. Solid-phase organic synthesis is well documented in the literature,[4,5] but to our knowledge, this chemistry was never applied toward the synthesis of a large number of purine nucleoside analogues.[6]

RESULTS AND DISCUSSION

The selection of a suitable solid support was the first of many challenges that we had to face. 2,6-Diamino purine nucleosides are not stable in the presence of an acid like TFA or even acetic acid, and one or more reaction steps involve the use of nucleophiles at high temperatures, which precluded the choice of ester bonded resins. We found that a polystyrene-methoxytrityl chloride (PS-MMTrCl) resin could be used throughout our synthetic scheme, and it did not require harsh conditions during the cleavage step. Our nucleoside products were cleaved using mild acidic conditions that would not lead to depurination. Our first attempt to condense 2-iodo-6-chloro-9-(β-D-ribofuranosyl)purine[7] with the resin using pyridine as solvent was unsuccessful, leading to a displacement of the chloro atom to give the 6-pyridinium derivative as a major side product. To overcome this issue, the reaction was run in THF in the presence of 2,6-lutidine, a hindered aromatic tertiary amine. The condensation most probably occurred at the 5'-OH group, the only primary alcohol in the molecule (Scheme 1). We did not try to determine the exact regiochemistry of the addition, since



Scheme 1. Reagents and conditions: (a) 2,6-lutidine, THF, rt; (b) R_1R_2NH, 25–60°C, NMP-toluene 1:1; (c) R_3R_4NH, 80–95°C, NMP-toluene 1:1; (d) HFIP 30%, DCE, 50°C.

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the following sequence of reactions would not be affected by the position of the linker on the sugar moiety.

The loaded resin was dispersed into fourteen 96-well plates (total of 1344 wells), each well containing approximately 70 mg of resin. The plates were placed on Advanced ChemTech Vanguard semi-automatic synthesizers. Primary and secondary amines in 1:1 solutions of 1-methyl-2-pyrrolidone (NMP) and toluene were used to displace the 6-chloro atom with high regio-selectivity. This reaction was run at temperatures ranging from room temperature for the most reactive amines to 60°C for the least reactive ones. It was critical to maintain the temperature of the reaction wells within a few degree centigrade range to avoid any side reaction with the 2-iodo atom. After 12 hours, all reaction vessels were flushed and washed with NMP, methylene chloride and methanol. Displacement of the 2-iodo was carried out at higher temperatures (between 80°C and 95°C for 72 hours) with various primary amines. We found that most linear secondary amines were not reactive enough to displace the iodo atom, even at elevated temperatures. However, cyclic secondary amine like piperidine, piperazine and morpholine reacted efficiently. Interestingly, hydrazines did not give us satisfactory results, probably because of decomposition at high temperatures; most final products after reaction with hydrazines showed the desired molecular weight minus fifteen, which corresponds to the deaminated product. The last step was the cleavage of our nucleosides from the resin. We first used a solution of acetic acid in water or methanol (50–80%), but it led to a significant amount of deglycosylated product. The same occurred with a 1.5–5% solution of TFA in dichloromethane. However, using a 30% solution of hexafluoroisopropanol in 1,2-dichloroethane,[8] we did not notice any deglycosylation. This reaction was performed at 45°C for 24 hours. After evaporation of the solvents, each compound was weighed and characterized by LC/MS. Out of the 1300 compounds synthesized, more than 70% reached a purity over 60% without purification. The main impurities were due to a double substitution of the same amine at the 2- and 6-positions, as well as some unreacted 2-iodo compounds. This solid-phase synthetic approach proved successful in obtaining a very large number of 2,6-disubstituted purine ribonucleosides in a short period of time. This library is currently being evaluated against various viruses and other targets. It will also be evaluated for its effect as adenosine receptor agonists or antagonists.

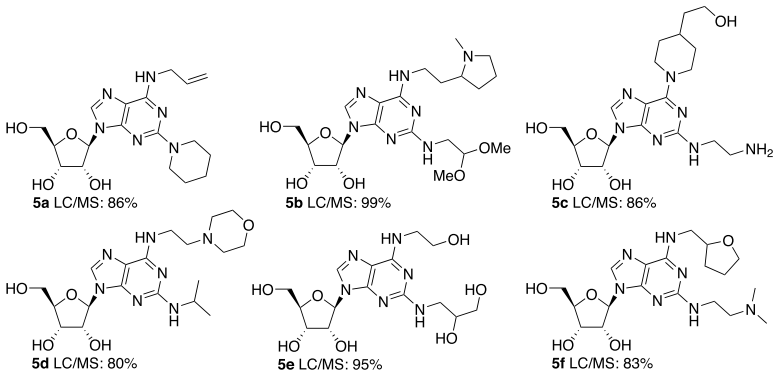

Figure 1. Selected compounds and their purity by LC/MS at 220 nm.

Examples of compounds obtained by this method are shown in Figure 1. The percentage indicates the purity by LC/MS at 220 nm.

EXPERIMENTAL

General Methods

NMR spectra were recorded at 300 MHz and the chemical shifts are expressed relative to TMS. The libraries were enumerated by Afferent TeamWorks 3.0, labeled and weighted by label Automador, and synthesized on ACT Vanguard semi-automated synthesizers. Libraries were analyzed on a LC-MS system, consisting of Waters 2790 HPLC, Waters 996 photodiode array (PDA) detector, and micromass/Waters ZQ mass spectrometer. Luna C18 columns from Phenomenex were used for compound separation. Mass spectra from 100 to 1000 were acquired using electrospray ionization with positive and negative ion detections. UV spectra were recorded at 200–400 nm by the PDA, and the compound purity was monitored based on UV absorbency at 220 nm. The LC/MS operation was controlled by MassLynx software, and the LC/MS data were processed by OpenLynx software. Polystyrene monomethoxytrityl chloride resin was purchased from Novabiochem. Other reagents were purchased from Aldrich, Fluka, Acros and other companies, and used directly.

Resin **2**. A suspension of monomethoxytrityl resin (18 g, 32 mmol), 2,6-lutidine (7.5 mL, 64 mmol) and 2-iodo-6-chloro-9-(β-D-ribofuranosyl)purine (**1**, 20 g, 48 mmol) in THF (145 mL) was shaken at rt for 3 days. The resin was filtered and washed alternatively 3 times with DCM and methanol (excess of **1** was recovered from the filtrate). Resin **2** was dried under vacuum to yield 27.7 g of loaded material. Yield of loading: 80%.

Resins **3**. Resin **2** was dispersed into a 96 well-plate (75 mg per well) and solutions of amines R_1R_2NH (1 mL, 0.5 M in toluene-NMP 1:1) were added in each well. The temperature was set to 40°C (set A) or 60°C (set B) and the apparatus was shaken for 18 hours. The wash sequence was programmed as follow: 3 × 3 mL DMF, 3 × 3 mL methanol, 3 × 3 mL DCM. Resins **3** were used without further purification.

Resins **4**. Solutions of amines R_3R_4NH (1 mL, 2 M in toluene-NMP 1:1) were added in each well. The temperature was set to 95°C (set C) or 80°C (set D) and the apparatus was shaken for 64 hours. The wash sequence was programmed as follow: 3 × 3 mL DMF, 3 × 3 mL methanol, 3 × 3 mL DCM. Resins **4** were used without further purification.

Compounds **5**. A solution of hexafluoroisopropanol (30% in DCE, 1 mL) was added in each well, the temperature was set to 40°C, and the apparatus was shaken for 24 hours. Methanol (1 mL) was added in each well and the mixtures were shaken an additional 20 minutes. The resin was filtered and washed with DCE (0.5 mL) and methanol (0.5 mL). The filtrates were collected in individual vials, dried under high vacuum and analysed by LC/MS.

Proton NMR of selected compounds:

***N*^{6}-Allyl-2-Iodoadenosine** (**3a**, cleaved from resin to verify structure): ^{1}H NMR (DMSO-d_6): δ 8.4 (bs, 1H), 8.31 (s, 1H), 5.95–5.85 (m, 1H), 5.80 (d, J = 6.0

Hz, 1H), 5.49 (d, J = 6.0 Hz, 1H), 5.24 (d, J = 4.5 Hz, 1H), 5.2–5.0 (m, 3H), 4.51 (m, 1H), 4.10(m, 1H), 4.03 (m, 1H), 3.93 (m, 1H), 3.7–3.5 (m, 2H).

N^6-Allyl-2-(piperidin-1-yl)adenosine (5a): ^{1}H NMR (DMSO-d_6): δ 7.91 (s, 1H), 7.5 (bs, 1H), 5.98–5.85 (m, 1H), 5.74 (d, J = 6.0 Hz, 1H), 5.35 (d, J = 6.0 Hz, 1H), 5.16–5.10 (m, 2H), 5.02 (dd, J = 10.2 Hz, 1.5 Hz, 1H), 4.90 (bs, 1H), 4.62 (m, 1H), 4.13(m, 1H), 4.02 (m, 1H), 3.86 (m, 1H), 3.68–3.46 (m, 3H), 3.35–3.25 (m, 2H), 1.61–1.35 (m, 2H). MS(ES) *m/e* 391 (M + H).

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