Report

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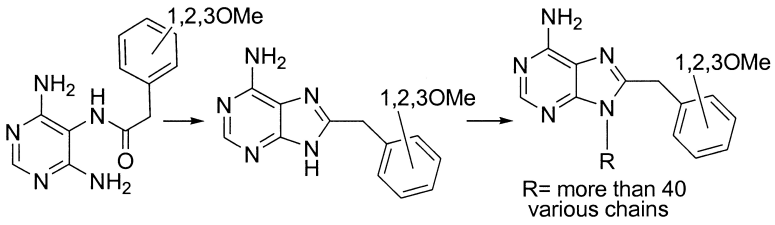
J. Comb. Chem., **2001**, 3 (6), 518-520• DOI: 10.1021/cc010017t • Publication Date (Web): 21 September 2001



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Reports

Facile Synthesis of a Library of 9-Alkyl-8-benzyl-9*H*-purin-6-ylamine Derivatives

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Received April 12, 2001

The Hsp90 family of chaperones plays a key role in regulating the physiology of cells exposed to environmental stress and in maintaining the malignant phenotype in tumor cells. Occupancy of the ATP/ADP N-terminal pocket of Hsp90 by the natural products geldanamycin (GM) and radicicol (RD) interferes with the activity of the chaperone and causes the degradation of a subset of key signaling proteins dependent on Hsp90 function. The Her2 transmembrane tyrosine kinase is one of the most sensitive targets of the drugs.[1] We have designed a novel Hsp90 inhibitor, PU3, a 9-alkyl-8-trimethoxybenzyl-purine, and demonstrated that this simple compound binds to Hsp90 and shares the important biological effects of GM and RD.[2] PU3 is less potent, but it is a first-generation lead compound. It is likely that further modifications will yield derivatives with increased binding affinity and activity. Here, we describe the development of a facile synthetic route that we employed for the parallel synthesis of over 40 derivatives of the PU3 class.

Syntheses of purine libraries have been described before;[3,4] however, there is no known efficient method for making 8-benzylpurine derivatives in a high-throughput manner. One could envision that an efficient strategy for attaining significant structural diversity in the PU3 class would be the cyclization of several 5-N-acylated 4,5,6-triaminopyrimidines, followed by 9-N-alkylation of the resulting purines. We found, however, that cyclization of the *N*-(4,6-diaminopyrimidin-5-yl)-2-(methoxyphenyl)acetamide derivatives (**PY-A**, **PY-B**, and **PY-C**) under published conditions yielded unsatisfactory results. Use of strong acidic conditions (PPA[4] or TsOH[5]) and high reaction temperatures resulted in a complex mixture, mostly due to decomposition of starting material. The method used by Dramisky[6] to cyclize 5-acetylamino-6-chloro-4-methylaminopyrimidine to the purine derivative, that is, heating in a steel cylinder at 100 °C in ammonia−methanol, yielded an unsatisfactory 5% in our hands.[7] Moreover, these harsh conditions are highly unsuitable for the preparation of a library of compounds using a parallel synthesizer.

Thus, we focused on developing an appropriate cyclization procedure and alkylation method that are amenable to high throughput. Our synthesis (Scheme 1) begins with the acylation of 4,5,6-triaminopyrimidine with several methoxyphenylacetyl fluorides (**A**, **B**, and **C**) under DMAP catalysis. The acid fluorides are conveniently generated just prior to the coupling step, treating the phenylacetic acid with cyanuric fluoride and pyridine.[8] The acylation reaction proceeded in high yields (60−80%) in DMF, resulting in the derivatives **PY-A**, **PY-B**, and **PY-C**. After precipitation from EtOAc, these materials were used in the cyclization step. We found that refluxing the 5-*N*-acyltriaminopyrimidine derivatives **PY-A**, **PY-B**, and **PY-C** in NaOMe/MeOH for 3−5 h resulted in high conversion of the starting material (80−90% yield) to the products **PU-A**, **PU-B**, and **PU-C**. In the last step, the purines were alkylated by the Mitsunobu methodology.[3] The use of 2.2 equiv of PPh_3 and 5 equiv of DEAD was necessary to bring the highly insoluble purines into the reaction solution of toluene and CH_2Cl_2. We did not observe satisfactory conversion by the use of THF as a solvent for the Mitsunobu step. Once all reagents were dissolved, the reaction proceeded rapidly: while in the case of primary unbranched alcohols the reaction was terminated in less than 15 min, bulkier alcohols required up to 1 h. In addition to the desired 9-N-alkylated purine, 3-N-alkylated products were observed up to 14−33% of the total recovered mass. The position of the alkyl chain was unambiguously determined by X-ray crystallography. This is an interesting observation because alkylation of adenine gives mainly 3-N-alkylated product in neutral conditions and $^7/_9$-N-alkylated products when a base is present.[9] However, alkylation of **PU-A**, **PU-B**, and **PU-C**, using the Mitsunobu reaction or NaH and the corresponding alkyl iodide,[4] gave 9-N- and 3-N-alkylated purines in similar ratios. The 7-N-alkyl product was isolated only in some instances as 1−3% of the product mix.[10]

Reactions were performed in parallel using the Agonaut Quest 210 synthesizer, and mixtures were purified on a ISCO CombiFlash system. The two isomers were easily separated because of a significant difference in R_f values (0.3−0.6). The yields of recovery ranged from 75% to 95%. In some cases, the formation of **PU-C-2** was detected as an impurity. This was caused by the presence of ethanol in some DEAD

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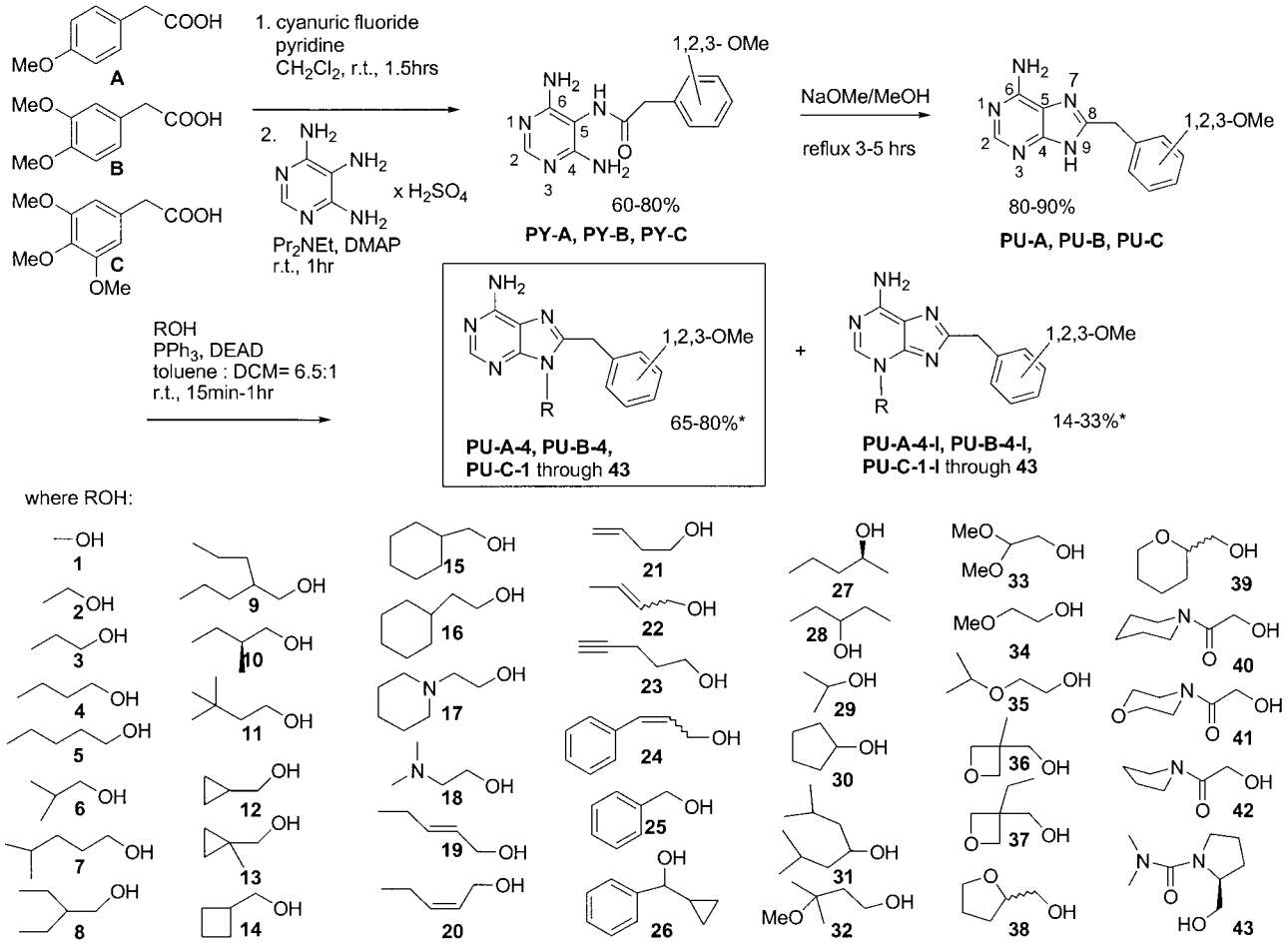
10.1021/cc010017t CCC: $20.00
Published on Web 09/21/2001

Scheme 1. Schematic Representation for the Synthesis of Purine Library and a Listing of the Alcohols Employed in the Mitsunobu Step[a]



[a] The asterisk represents the percentage of the two isomers in the reaction mixture.

batches. Because the impurity eluted very close to the main product, it was difficult to remove and caused a decrease in the recovery yields. We were thereby forced to replace DEAD with di-*tert*-butyl azodicarboxylate. The Mitsunobu reaction proceeded in comparable yields using this reagent, and its use is advisable.

Although we were only interested in applying this reaction scheme to mono-, di-, or trimethoxybenzyl derivatives, we feel that it will be useful in the synthesis of various other 8-benzylpurines. The Mitsunobu reaction can accommodate a high array of primary and secondary alcohols in the alkylation of purines. We have employed a variety of unbranched and branched, linear and cyclic, saturated and unsaturated primary alcohols and several secondary alcohols. Only two alcohols, neopentyl alcohol and cyclohexanol, were found to give no product in this reaction, probably because of steric effects. The synthesis is amenable for high throughput and can generate a variety of derivatives in good yields.

Acknowledgment. This research was supported in part by the Leukemia Research Foundation (G.C.). X-ray crystallography was performed in the laboratory of Prof. Gerard Parkin at Columbia University.

Supporting Information Available. General synthetic procedures, listing of the names and physical properties of all characterized compounds, and crystallographic information files for the 9-N- and 3-N-alkylated products. This material is available free of charge via the Internet at http://pubs.acs.org.

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