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Lecture Transcripts

Preparation of Guanine PDE Inhibitors: Development of the Common Synthetic Route Strategy. A Case Study

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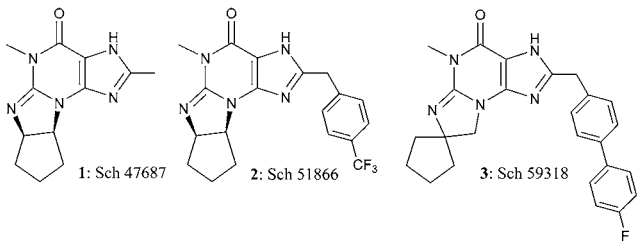
Abstract:

A single synthetic route, called the chloropurine route, capable of quickly delivering initial kilogram quantities of several chiral as well as achiral guanine phosphodiesterase inhibitors of increasing complexity is described. During the course of this work, unraveling the formation mechanism of chloropurines allowed for the scale-up of the key intermediates. The mechanism for the cyclization of the chiral five-membered amino alcohols and its implication on the enantiomeric purity needed for this step are also described.

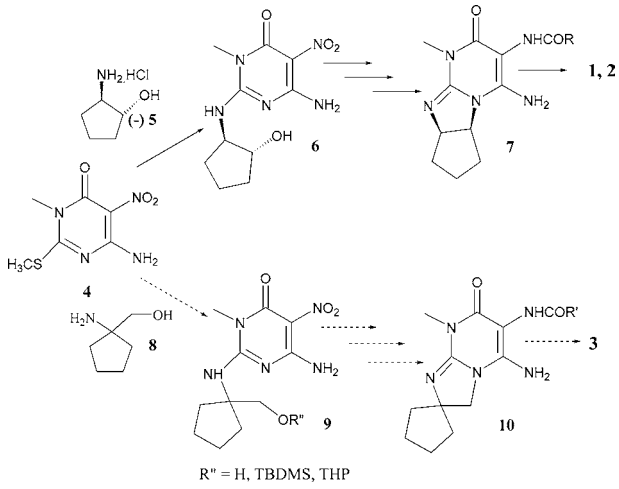
The timely availability of large quantities of a newly identified pharmaceutically active compound in early stages of its life cycle (Tox, Phase I) is important in its expedited development. This stage can often slow the development of an active pharmaceutical ingredient (API) if large quantities (kilograms) of this candidate are unavailable due to a lack of appropriate intermediates and/or synthetic methodologies.

To overcome such delays in the development of the phosphodiesterase (PDE) inhibitors **1**−**3** we envisaged two



approaches. The first involved use of an advanced intermediate, such as activated pyrimidine **4**, which can be converted to any of the above desired PDE inhibitors of varying complexities (Scheme 1). This work is described in the earlier publications.[1,2] This approach was well suited for the preparation of the PDE inhibitors **1**−**2** derived from a secondary amino alcohol **5**. However, the activated intermediate **4** proved resistant towards its reaction with tertiary amino alcohol cycloleucine **8**, limiting its utility for the synthesis of **3**.

Scheme 1. **Advanced activated pyrimidine route to PDE inhibitors**



As an alternate to the above approach we were also developing a common synthetic route strategy. Although such approach is common practice for medicinal chemists for a small-scale preparation of closely related drug analogues, significant development is required to make such anapproach viable on scale even for structurally related compounds. This manuscript describes the challenges encountered and the resolution of these challenges, leading to a speedy delivery of a diverse set of compounds. The approach described in this contribution was based on the route used by the medicinal chemists, but without the protecting groups (benzyl, TES) and chromatographic separations/purifications.[17,18] In this series of compounds the absence of protecting groups not only led to solubility difficulties, but it also led to previously unanticipated intermediates/reaction mechanisms and stereochemical outcomes compared to the above work.[17,18] This contribution describes the findings and the resolution of the above issues leading to the "chloropurine"[3] common synthetic pathway. This was the preferred route for an expedited delivery of kilogram quantities of all of the above PDE inhibitors.

The development of the chloropurine route started with Sch 47687 (**1**, Scheme 2), the first PDE inhibitor of interest to us. The conversion of commercially available 6-amino

(1) Gala, D.; DiBenedetto, D. J.; Kugleman, M.; Puar, M. S. *Tetrahedron Lett*. **2003**, *44*, 2717.

(2) Gala, D.; DiBenedetto, D. J.; Kugelman, M.; Mitchell, M. B. *Tetrahedron Lett*. **2003**, *44*, 2721.

(3) 2-Chloropurine is the key intermediate pathway approach.

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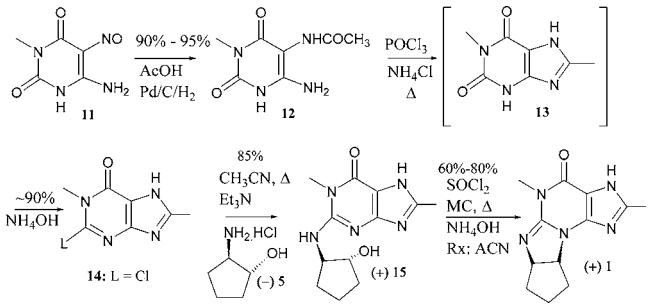
Scheme 2. **Synthesis of Sch 47687 via the chloropurine route**



uracil to caffeine metabolite A_1, i.e., **12**, via nitrosopyrimidine **11** developed and scaled up to kilogram scale in our laboratories and plant has been reported previously by us.[4] Insolubility of **12** in a variety of solvents posed a serious challenge to our initial goal of converting it to the xanthine **13**. It is worth noting that the research synthesis[17,18] utilized a benzyl protecting group at the 5-acetamido nitrogen which minimized the insolubility difficulties. It was also known that the removal of this benzyl group at the end of the sequence via catalytic hydrogenation was very difficult, often requiring a large amount of 20% $Pd(OH)_2/C$. This was undesirable from both the cost and "residual heavy metal (Pd) in the API" perspective. Our initial desire was to "activate" **13** to produce **14** by reacting it with an appropriate activating reagent (sulfonyl halides, anhydrides, etc. leading to the corresponding leaving group L in **14**) such that the activated purine **14** with a desirable leaving group L can then efficiently react with expensive chiral amino alcohol **5**.[5] Our continued work to form xanthine **13** led us to use $POCl_3$. When **12** was suspended in $POCl_3$ and heated (to ensure enough solubility for reasonable reaction rate), it led to the formation of xanthine **13** as well as the chloropurine **14**. We further found that **14** is formed under these conditions. As discussed later in this manuscript under the mechanism of formation of chloropurines, an addition of excess[6] NH_4Cl to the reaction mixture prior to heating **12** in neat $POCl_3$ as a solvent and a subsequent workup with NH_4OH allowed for an efficient one-pot, two-step conversion of **12** to **14**. In a typical plant procedure, the batch was gradually heated to reflux over 6−8 h and held at that temperature for 48 h for complete consumption of **12** and **13**. Attempts to minimize the use of $POCl_3$ via the use of high-boiling solvents typically lowered yields and/or increased impurities. Fortunately, after neutralization of HCl and $POCl_3$, the isolation of **14** lacking the protection of imidazole nitrogen(s) did not cause complications (dimerization, polymerization, N-oxidations, oiling of free base, etc.), allowing for good isolated yields of high-melting crude **14**. Crude **14** was then reacted with a stoichiometric amount of chiral amino alcohol **5** in refluxing acetonitrile containing an excess of TEA as an HCl scavenger. Typically, most of **14** was consumed after 72 h at which stage the reaction mixture was filtered to remove TEA·HCl as well as residual **13**. Addition of the reaction mixture to water allowed for precipitation of **15**. Crude **15**, after drying, was dissolved in methylene chloride and treated with a slight excess of thionyl chloride. A gentle warming (35−40 °C) of the reaction mixture led to essentially complete cyclization of **15** to **1** as its HCl salt. Neutralization of this reaction mixture with NH_4OH[7] allowed for precipitation of free base **1** which was recrystallized from acetonitrile. The two-step yield, although good, was slightly variable. This was traced to the degree of neutralization as well as the amount of water (introduced via aqueous NH_4OH). Insufficient neutralization caused the loss of **1·HCl** in water as well as CH_2Cl_2, whereas an excess of water removed free base **1** due to its solubility in water. Since the above preparation allowed for delivery of Tox and Phase I supplies of **1**, no further optimization work was done to improve the last-step yield.

Sch 51866, **2**, was chosen as the second PDE inhibitor of interest. Application of the chloropurine route for its preparation led to many dissimilar and some similar observations and required changes to the experimental conditions at every step for a successful ending. Unlike acetic acid or acetic anhydride, *p*-CF_3-Ph-CH_2COOH is a solid. Thus, the experimental conditions developed for the preparation of **12** via reductive amidation needed changes for the preparation of **17**. To this end it was found that **11** as an aqueous NaOH solution can be completely hydrogenated to **16**. This diamine is air sensitive, and its isolation was undesirable. Again, it was discovered that the treatment of a solution of this diamine in aqueous NaOH with only a slight excess of *p*-CF_3-Ph-CH_2COCl (prepared in a quantitative yield by treatment of the corresponding acid with $SOCl_2$) led to precipitation of high-quality amide **17** in very good isolated yield.[8] Similar to amide **12**, amide **17** could only be isolated in its hydrated form. Initially this appeared counterproductive for the next step where $POCl_3$ was used as a solvent, and hence, some effort was put into drying **17**. As discussed later in this contribution, difficulty in removing this water of hydration from **17** proved fortuitous.

Unlike amide **12**, initial attempts to convert **17** to xanthine **18** and chloropurine **19** led to irreproducible results. The rate as well as extent (temperature and time) of heating the suspension of **17** in $POCl_3$ led to formation of one major to many other products during its conversion to **19**. This had a significant impact on the yield as well as the quality of **19**.

(4) Gala, D.; DiBenedetto, D.; Günter, F.; Kugelman, M.; Maloney, D.; Cordero, M.; Mergelsberg, I. *Org. Process Res. Dev.* **1997**, *1*, 85.

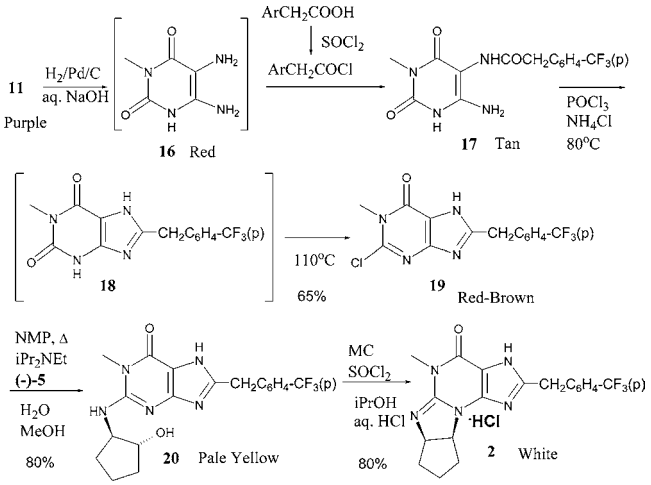
(5) (a) Barr, A.; Frencel, I.; Robinson, J. B. *Can. J. Chem.* **1977**, *55*, 4180. (b) Overman, L. E.; Sugai, S. *J. Org. Chem.* **1985**, *50*, 4154.

(6) Towards the development/optimization of 2-chloropurine formation/isolation in high yields with the least number of impurities, several metal halides, Vilsmeier reagents, phosphorous halides, phase-transfer reagents, amine HCl salts, cosolvents (toluene, xylenes, chlorobenzene, dichlorobenzenes, etc.), various workups (quenches with protic solvents, amines, inorganic bases), and various combination of the above were evaluated. Of these, the use of $POCl_3$ and NH_4Cl gave the best results. Since the latter is inexpensive, appeared to improve solubility/stirability of the reaction mixtures, and did not interfere with the workup, it was used in excess (3−5 mol).

(7) The use of a mild base was desirable in view of the instability of these PDE inhibitors to strong base such as aqueous NaOH. See Gala, D.; Puar, M. S.; Czarniecki, M.; Das, P. R.; Kugelman, M.; Kaminiski, J. *Tetrahedron Lett.* **2000**, *41*, 5025.

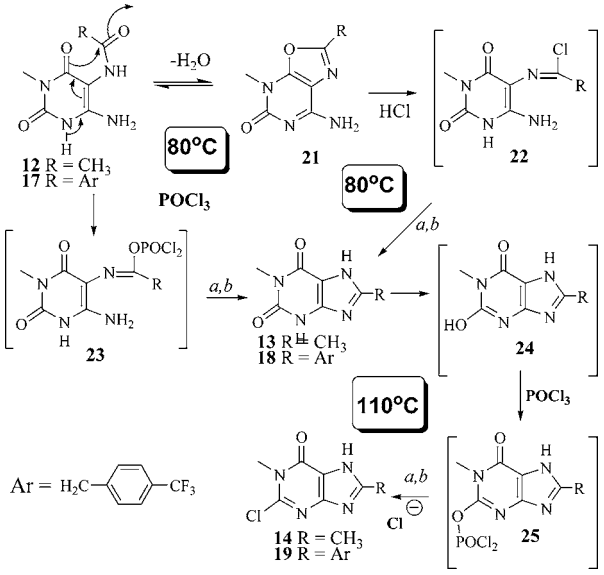
(8) Such favorable faster amidation compared to the hydrolysis of this acid chloride under such basic conditions was unexpected. This procedure was applicable to several aliphatic as well as aromatic acid chlorides (unpublished results).

Scheme 3. **Preparation of Sch 51866 (2) via the chloropurine route**



Conversion of **17** to **18**, due to the insolubility of the latter, typically resulted in a very thick, difficult-to-stir slurry. This necessitated use of more $POCl_3$ compared to **13** and required longer reaction time for its conversion to **19**. After unraveling the mechanism of conversion of **17** to **19** via the intermediacy of oxazole **21**, a two-stage heating (75−80° for 24 h followed by reflux for 3 days) led to 65% isolated yield of **19** (with 5−10% **18** in it). An incomplete conversion of **18** to **19** and some hydrolysis of **19** during neutralization/isolation were the causes for contamination of **19** with **18**. In the next set of dissimilarities, it was discovered that unlike **14**, the reaction of chloropurine **19** with chiral **5** was very sluggish in refluxing acetonitrile, requiring over a week for ∼90% conversion. Development work for this reaction led to the conditions described in Scheme 3 where the reaction of **19** with **(−)5** in *N*-methyl-2-pyrrolidinone (NMP) at ∼110 °C was complete in ∼18 h. At the end of reaction, the mixture was filtered to remove **18** and most of $(^{i}Pr)_2Net\cdot$HCl salt. The reaction mixture was then added at room temperature to water containing ∼3% MeOH. The presence of MeOH allowed for the removal of traces of polar impurity and led to a clean precipitation of **20** in 80% yields. As in the case of **15**, crude **20**, after drying, was dissolved in methylene chloride (MC), treated with thionyl chloride, warmed to ∼40 °C for its complete cyclization, and then treated with aqueous NH_4OH, as above, to form free base **2**. This free base was not soluble in water; hence, it was isolated reproducibly in 85−90% yield. This free base could be crystallized from a variety of solvents. To improve the solubility and bioavailability of this compound in water, a salt-selection program ensued which identified the mono HCl salt as the preferred salt. In the plant, free base **2** was suspended in warm (60−65 °C) 2-propanol and treated with a stoichiometric amount of concentrated HCl at which stage the entire mixture became a homogeneous solution from which the **2·HCl** crystallized as a white solid when the solution was cooled. As noted in Scheme 3, in the initial stages of this developmental work of **2** when analytical methods were being developed, chemists monitored the progress of various reactions by following the color changes and kept this program on track.

Scheme 4. **Proposed chloropurine(s) formation mechanism**[a]

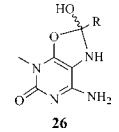


[a] Conditions - a: Elimination. b: Addition:elimination to the nitriliums followed by addition may be preferred; however, addition followed by elimination cannot be ruled out.

Several interesting mechanistic insights were made during the discovery of the N-unprotected chloropurine route and its application to the preparation of PDE inhibitors **1** and **2**. One pertained to the formation of chloropurines **14** and **19** from amides **12** and **17**, respectively, and the other pertained to cyclization of purine amino alcohols **15** and **20** to **1** and **2**, respectively.

The conversion of **17** to **19** proved difficult compared to that of **12** to **14**. Had the latter conversion been difficult, this route might never have materialized because trouble shooting without a chromophore on **12**−**14** would have been difficult! As stated above the one major and many minor temperature-dependent compounds (in addition to **18**) were noticed during the conversion of **17** to **19**. The presence of the aromatic chromophore facilitated monitoring of the reaction profile. The major new compound was isolated and was determined (NMR, MS) to be oxazole **21**.[9] On the basis of work done on isolated **21**, the following conclusions were made. Treatment of **17** with $POCl_3$ leads to the formation of **18** as well as **21**. At temperatures of 80 °C or less, **21** converts to **18**. In this case the amount of HCl and, hence, water in **17** (i.e. water of hydration) is important. If the

(9) As seen with the bicyclic azetidinone hemiketals,[10] it is quite likely that one or two of the of the compounds observed in this conversion are stable bicyclic aminals **26**. In view of facile 4-chlorination of pyrimidines,[1] 4-chloropyrimidine derivative of **17** and or **18** can also be the sources of the minor compounds.



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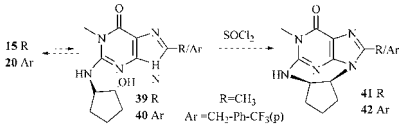
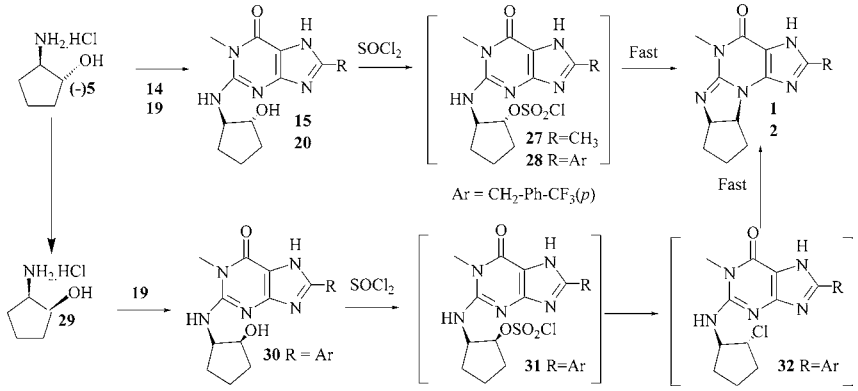
(10) Kugelman, M.; Gala, D.; Jaret, R. S.; Nyce, P. L.; McPhail, A. T. *Synlett* **1990**, 431.



Scheme 5. **Mechanism of purine-amino alcohols cyclization to guanines**



reaction mixture is rapidly heated to reflux, this HCl is removed (presumably via N_2 stream and/or azeotropic distillation with $POCl_3$) from the reaction mixture, resulting in degradation of **21** into a variety of compounds along with formation of **18**. This was proven in laboratory experiments on isolated **21**.

Thus, first-stage heating below 80 °C ensured a near quantitative conversion of **17** to **18**. In the case of **12** to **14**, less steric demand (methyl versus *p*-trifluoro-phenylmethyl) in the corresponding oxazole for its conversion to **13** and sufficient water of hydration in **12** probably made conversion of **12** to **14** very facile and did not require a prolonged first-stage heating.

Isolated xanthines **13** and **18** are very stable and insoluble. These properties required yet more energy for their conversion to chloropurines **14** and **19**, respectively. Our hypothesis is that the enolization of insoluble **13**/**18** to **24** was the most energy-demanding step. The trapped enolate **24**, i.e., **25**, could then benefit from extra chloride ions for its conversion to **19** (see Scheme 4). For this conversion (**18**, **24**, **25**, **19**) a variety of reaction conditions, cosolvents, metal chlorides, amine hydrochloride salts, phase-transfer catalysts (PTC) in combination with many divergent reaction-quenching and isolation procedures[6] were evaluated. Of these, the use of NH_4Cl as an additional chloride source and reaction quench with NH_4OH were found optimum. The former acted as an easy-to-remove "PTC" and actually improved the solubility of **18** (contrary to expectation, the addition of NH_4Cl made the reaction mixture much easier to stir), lowered the reaction time (to 3 days from 6 days), and posed no difficulties in the workup and isolation of **19**. The use of NH_4OH ensured pH control (limited hydrolysis of chloropurines to xanthines), and water solubility of its phosphate salts led to facile phase splits during workup.

The second interesting observation was the stereochemical outcome in the cyclization of **15** and **20**. In a previous publication[1] we have reported that the substrate in which "trans" amino alcohol (−)**5** was incorporated led to the formation of "cis" chloro amine when subjected to the $SOCl_2$ reaction. A formation of such "cis" chloro amine of **5** can potentially lead to the "trans" five-membered cyclized isomer of the PDE inhibitor. To determine the extent of this reaction via a quantitative analytical method the following work was undertaken. In our hands, cyclization of **15** and **20** remained efficient and led only to the desired compounds (**1**, **2**). In this reaction "trans" five-membered PDE inhibitor products or the "trans" chloro amines (via an intra- or intermolecular displacement of OSO_2Cl group) were not detected. It appears that chlorosulfonylated **15** and **20** (**27** and **28**, respectively) are well situated for a rapid, thermodynamically favored "cis" five-membered ring cyclization, leading to the desired products. Also noteworthy is that even though these "trans"-substituted chlorosulfonylamines are well suited for the formation of other compounds, such compounds remained well below the detection limits in this series.[11] As shown in Scheme 5, when **5** was converted to "cis" amino alcohol **29**[12] and subjected to the same reaction sequence, it too led to the desired "cis" five-membered ring product.[13] In this case the chlorosulfonylated intermediate **31** does not undergo thermodynamically less favored "trans" cyclization which then allows for its conversion to the "trans" chloro amine **32** (a similar observation is reported by us in a previous article[1]). The latter is suitable for the favorable "cis" five-membered ring closure.[14] Thus, the chirality at the carbon bearing the amine is very important, and the chirality of the carbon bearing the hydroxyl group is unimportant for the formation of the above PDEs. This was an important observation for finding alternatives to expensive (−)**15**.

Finally, the lessons learned from the above two PDE inhibitors allowed for a rapid application and scale-up of the chloropurine route to the spirocyclic PDE inhibitor **3** (Scheme 6). One of the major issues with the unavailability of the biphenyl acetic acid (**33**) was resolved by discovering a novel, efficient, and scalable Pd/C coupling procedure as an alternate to classical but laborious and impractical for scale-up Wilgerödt−Kindler biphenyl acetic acid synthesis.[15] When the biphenyl acid chloride (via reaction of **33** with

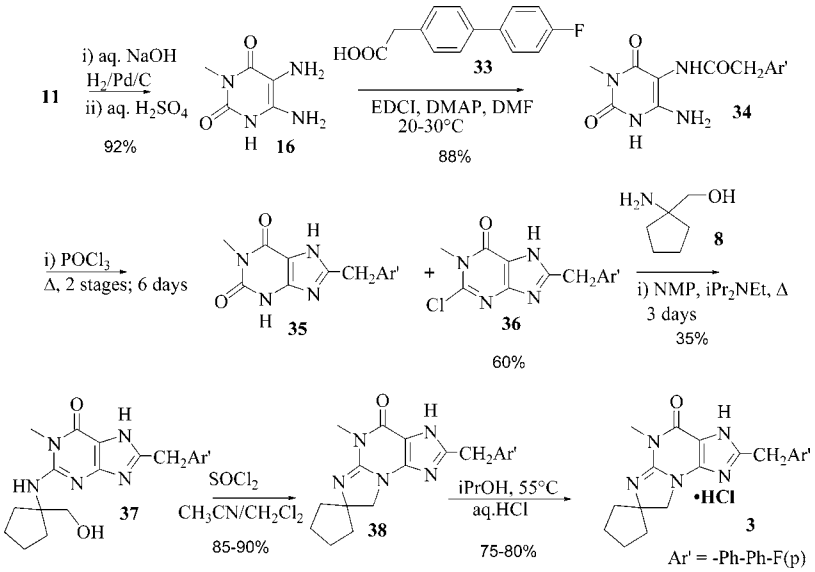
(11) Trans-substituted chlorosulfonylamines are suitable intermediates to form aziridines, but the latter were not detected in these reactions. In other reactions we have noticed that tautomerism in imidazole ring led to the reactions at either of the nitrogen atoms. In this series tautomers of **15** and **20** (i.e. **39** and **40**, respectively), if present, did not lead to **41** and **42**. This may be due to the fact that **39** and **40** would lead to seven-membered strained ring products which are far less favorable than the desired five-membered products, **1** and **2**.

(12) Enantiopure cis isomer was prepared by using the literature procedure for the conversion of *trans*-α-amino alcohols to *cis*-α-amino alcohols: Barnard, R. A. B.; Parkkari, J. H. *Can. J. Chem.* **1970**, *48*, 1377.

(13) These conclusions were made after thorough ^1H NMR, ^{13}C NMR, HPLC, and MS studies of the isolated compounds.

(14) Incidentally, this observation can be used for the next generation of inexpensive amino alcohol where the chirality at only the amino carbon would be important, and chirality at the alcohol carbon would be less important.

Scheme 6. **Preparation of Sch 59318 (3) via the chloropurine route**



$SOCl_2$, quantitative yield) was reacted with the diamine **16** prepared in aqueous NaOH, it resulted in the hydrolysis of the acid chloride and recovery of **33**. This was unanticipated in view of the successful application of several other aliphatic and aromatic acid chlorides for the preparation of PDE inhibitors.[8] Hence, aqueous NaOH-free **16** was needed. This was achieved by neutralizing aqueous NaOH with 10% aqueous H_2SO_4 at 0−5 °C under inert atmosphere. This resulted in a precipitation of **16** which contained ∼1.5 mol of water. Even this amount of water competed with amidation, causing hydrolysis of **33** acid chloride and resulting in a mixture of **34** and **33**. In the initial phase of the project, treatment of **16** with **33** and EDCI resolved this issue, leading to good yield of isolated **34.**[16−18] As expected, for the $POCl_3$ reaction, due to the higher steric demand of **34** (vs **19**), each stage required approximately double the reaction time for its conversion to chloropuine **36**. The conversion yields were low as all of xanthine **35** could not be converted to **36**. After aqueous precipitation as described above, the mixture was treated with ∼5% MeOH/CH_2Cl_2. This treatment allowed for a recovery/removal of highly pure and insoluble **35** which was then recycled, effectively improving the overall yield. This recovery procedure proved useful, as in the next step the reaction of cycloleucine (**8**) with chloropurine **36** was very slow, requiring up to 72 h at 110 °C for only ∼35% isolated yield of **37**. In this reaction not only many highly colored impurities formed, but also most of **36** hydrolyzed to **35**. It appears that the hindered tertiary amine group of **8** requires harsh reaction conditions during which time the primary alcohol group in it displaces chlorine in **36**. The iminoether generated by the latter reaction then undergoes ready hydrolysis during TLC, HPLC, or workup, resulting in the formation of **35**. Again, highly insoluble **35** was recovered from this mixture as described above. Slurries in THF followed by *i*PrOH were then necessary to remove nonpolar and polar colored impurities, respectively, from **37**. Compound **37** in a mixture of acetonitrile/CH_2Cl_2 (to improve solubility) was converted to **38** in high yield with the use of thionyl chloride (35−40 °C, 4 h). This was then converted to its mono HCl salt in *i*PrOH (as for **2**) in an 80% yield.

In summary, a common synthetic route involving the preparation and use of 2-chloropurines as important intermediates was developed and utilized to deliver requisite quantities of three PDE inhibitors of differing structural complexity and of GMP quality. Interesting mechanistic and stereochemical findings were made during the course of this work.

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(15) Gala, D.; Stamford, A.; Jenkins, J.; Kugelman, M. *Org. Process Res. Dev.* **1997**, *1*, 163.

(16) Subsequently we discovered that treating **16** with the requisite quantity (necessary to consume water as determined by the Karl Fischer method) of either bis-trimethylsilylacetamide (BSA) or mono-silylmethylacetamide (MSA) prior to addition of acid chloride of **33** allowed for a successful preparation of **34** without the use of EDCI.

(17) Ahn, H.-S.; Bercovici, A.; Boykow, G.; Bronnenkant, A.; Chackalamannil, S.; Chow, J.; Cleven, R.; Cook, J.; Czarniecki, M.; Domalski, C.; Fawzi, A.; Green, M.; Gundes, A.; Ho, G.; Laudicina, M.; Lindo, N.; Ma, K.; Manna, M.; McKittrick, B.; Mirzai, B.; Nechuta, T.; Neustadt, B.; Puchalski, C.; Pula, K.; Silverman, L.; Smith, E.; Stamford, A.; Tedesco, R. P.; Tsai, H.; Tulshian, D.; Vaccaro, H.; Watkins, R. W.; Weng, X.; Witkowski, J. T.; Xia, Y.; Zhang, H. *J. Med. Chem.* **1997**, *40*, 2196.

(18) Vemulapalli, S.; Watkins, R. W.; Chintala, M.; Davis, H.; Ahn, H.-S.; Fawi, A.; Tulshian, D.; Chiu, P.; Chatterjeee, M.; Lin, C.-C.; Sybertz, E. J. *J. Cardiovasc. Pharmacol.* **1996**, *28*, 862.