## 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.<sup>1.2</sup> 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.<sup>17,18</sup> 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.<sup>17,18</sup> This contribution describes the findings and the resolution of the above issues leading to the "chloropurine"<sup>3</sup> 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

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

<sup>(2)</sup> Gala, D.; DiBenedetto, D. J.; Kugelman, M.; Mitchell, M. B. Tetrahedron Lett. 2003, 44, 2721.

<sup>(3) 2-</sup>Chloropurine is the key intermediate pathway approach.

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.<sup>4</sup> 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<sup>17,18</sup> 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)<sub>2</sub>/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<sub>3</sub>. 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<sup>6</sup> NH<sub>4</sub>Cl to the reaction mixture prior to heating 12 in neat  $POCl_3$  as a solvent and a subsequent workup with NH<sub>4</sub>OH 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<sub>3</sub> via the use of high-boiling solvents typically lowered yields and/or increased impurities. Fortunately, after neutralization of HCl and POCl<sub>3</sub>, 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<sub>4</sub>OH<sup>7</sup> 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<sub>4</sub>OH). Insufficient neutralization caused the loss of 1·HCl in water as well as CH<sub>2</sub>Cl<sub>2</sub>, 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<sub>3</sub>-Ph-CH<sub>2</sub>COOH 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<sub>3</sub>-Ph-CH<sub>2</sub>COCl (prepared in a quantitative yield by treatment of the corresponding acid with SOCl<sub>2</sub>) led to precipitation of high-quality amide 17 in very good isolated yield.<sup>8</sup> Similar to amide 12, amide 17 could only be isolated in its hydrated form. Initially this appeared counterproductive for the next step where POCl<sub>3</sub> 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<sub>3</sub> 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.

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

 <sup>(5) (</sup>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.

<sup>(6)</sup> 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<sub>3</sub> and NH<sub>4</sub>Cl 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).

<sup>(7)</sup> 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.

<sup>(8)</sup> 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<sub>3</sub> 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  $(75-80^{\circ} \text{ for } 24 \text{ h followed})$ 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  $\sim$ 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  $\sim$ 110 °C was complete in  $\sim 18$  h. At the end of reaction, the mixture was filtered to remove 18 and most of (<sup>i</sup>Pr)<sub>2</sub>Net· HCl salt. The reaction mixture was then added at room temperature to water containing  $\sim$ 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  $\sim 40$  °C for its complete cyclization, and then treated with aqueous NH<sub>4</sub>OH, 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<sup>a</sup>



<sup>*a*</sup> 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**.<sup>9</sup> On the basis of work done on isolated **21**, the following conclusions were made. Treatment of **17** with POCl<sub>3</sub> 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

<sup>(9)</sup> As seen with the bicyclic azetidinone hemiketals,<sup>10</sup> 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,<sup>1</sup> 4-chloropyrimidine derivative of 17 and or 18 can also be the sources of the minor compounds.



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





reaction mixture is rapidly heated to reflux, this HCl is removed (presumably via  $N_2$  stream and/or azeotropic distillation with POCl<sub>3</sub>) 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<sup>6</sup> were evaluated. Of these, the use of NH<sub>4</sub>Cl as an additional chloride source and reaction quench with NH<sub>4</sub>OH 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<sub>4</sub>Cl 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<sub>4</sub>OH 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<sup>1</sup> 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<sub>2</sub> 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<sub>2</sub>Cl 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.<sup>11</sup> 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.<sup>13</sup> 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<sup>1</sup>). The latter is suitable for the favorable "cis" fivemembered ring closure.<sup>14</sup> 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.<sup>15</sup> When the biphenyl acid chloride (via reaction of **33** with

<sup>(11)</sup> 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.

<sup>(12)</sup> 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.

<sup>(13)</sup> These conclusions were made after thorough <sup>1</sup>H NMR, <sup>13</sup>C NMR, HPLC, and MS studies of the isolated compounds.

<sup>(14)</sup> 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<sub>2</sub>, 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.<sup>16–18</sup> As expected, for the POCl<sub>3</sub> 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

- (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.

treated with  $\sim$ 5% MeOH/CH<sub>2</sub>Cl<sub>2</sub>. 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  $\sim$ 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<sub>2</sub>Cl<sub>2</sub> (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.

Received for review September 22, 2003.

OP030212R

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

<sup>(16)</sup> 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.