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Synthesis of Pyrrolnitrin and Related Halogenated Phenylpyrroles

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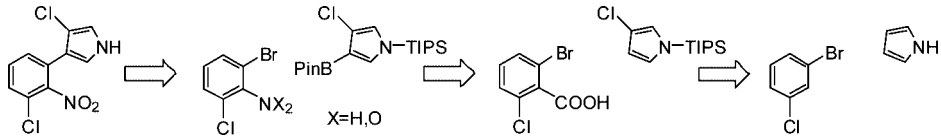
ABSTRACT

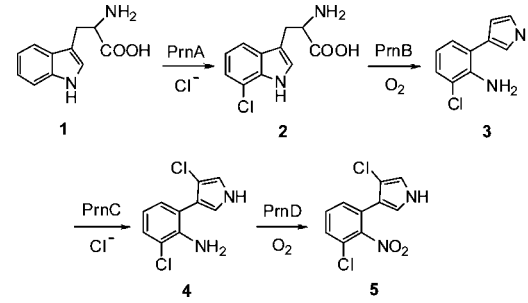


A general approach to halogenated arylpyrroles, including the antifungal natural product pyrrolnitrin, is described using newly synthesized halogenated pyrroles and 2,6-disubstituted nitrobenzenes or 2,6-disubstituted anilines.

Halogenations are a particularly interesting class of enzymatic reactions, especially in light of the high percentage of medicinal natural products and pharmaceuticals that bear halogen atoms.[1] Enzymatic incorporation of halogens during natural product biosynthesis allows for the fine-tuning of electronic and steric properties which determine the affinity and selectivity of a molecule's interactions with its biological target. Enzymes can install halogen atoms on aliphatic carbons, olefinic centers, and a wide variety of aromatic and heterocyclic rings.[2] For some time, it was thought that haloperoxidases were Nature's only halogenation catalysts,[3] but more recent work has uncovered two further classes of oxidative enzymatic catalysts: α-ketoglutarate-dependent[4] and flavin-dependent halogenases.[5]

Few of these enzymes have been structurally and/or mechanistically characterized and many questions surround their activities. Our interests lie in the mechanisms of the halogenation steps and the strategies employed by the enzymes to ensure regioselectivity; especially in cases where the "unexpected" regioisomer is formed. For example, the biosynthesis of the antifungal antibiotic pyrrolnitrin (**5**) from tryptophan (**1**) is believed to involve two regioselective chlorinations of unactivated positions carried out by the flavin-dependent halogenases PrnA and PrnC (Scheme 1).[6]

Scheme 1. Proposed Mechanism of Pyrrolinitrin Biosynthesis



The lack of commercial availability of the putative biosynthetic precursors to pyrrolnitrin or suitable substrate analogs thereof has precluded detailed studies of these enzymes beyond PrnA.[7] Although two total syntheses of **5** have been reported,[8,9] they are cumbersome, involving *de*

(1) Gribble, G. W. *J. Chem. Educ.* **2004**, *81*, 1441–1449.
(2) Vaillancourt, F. H.; Yeh, E.; Vosburg, D. A.; Garneau-Tsodikova, S.; Walsh, C. T. *Chem. Rev.* **2006**, *106*, 3364–3378.
(3) Morrison, M.; Schonbaum, G. R. *Annu. Rev. Biochem.* **1976**, *45*, 861–888.
(4) Vaillancourt, F. H.; Yin, J.; Walsh, C. T. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 10111–10116.
(5) Keller, S.; Wage, T.; Hohaus, K.; Holzer, M.; Eichhorn, E.; van Pee, K.-H. *Angew. Chem., Int. Ed.* **2000**, *39*, 2300–2302.
(6) Kirner, S.; Hammer, P. E.; Hill, D. S.; Altmann, A.; Fischer, I.; Weislo, L. J.; Lanahan, M.; van Pee, K.-H.; Ligon, J. M. *J. Bacteriol.* **1998**, *180*, 1939–1943.
(7) Dong, C. J.; Flecks, S.; Unversucht, S.; Haupt, C.; van Pee, K.-H.; Naismith, J. H. *Science* **2005**, *309*, 2216–2219.

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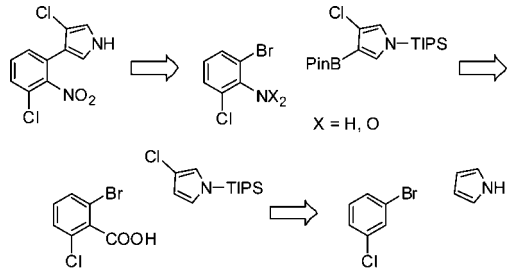
novo construction of the substituted pyrrole ring and are not easily amenable to access related derivatives. We therefore developed a new approach to **5**, as well as its biosynthetic precursors **3** and **4**, in parallel with our efforts to heterologously overexpress the four individual members of the pyrrolnitrin biosynthetic gene cluster in *E. coli*[10] to facilitate in vitro mechanistic and structural studies of these enzymes. We report our synthetic efforts here.

Since 3-arylpyrroles have been constructed by Pd-catalyzed cross-coupling of appropriate 3-boronated pyrroles with aryl halides,[13] we settled on the retrosynthetic approach to **5** shown in Scheme 2. This would allow us to more easily

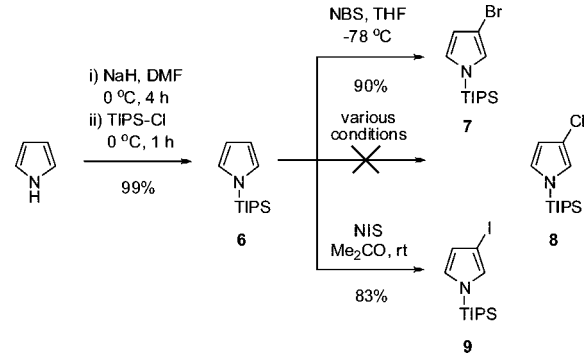
Scheme 2. Retrosynthetic Approach to Pyrrolnitrin



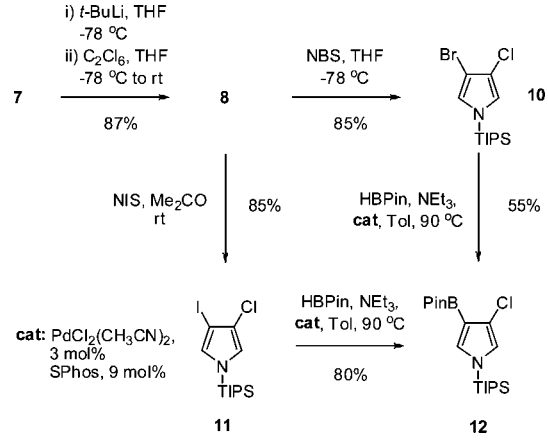
prepare **5**, its putative biosynthetic precursors, and substrate analogs by simply changing the substitution on the pyrrole or aryl halide undergoing cross-coupling.

Unfortunately, a regioselective preparation of 3-chloropyrrole has hitherto not been described in the literature.[14,15] While regioselective bromination and iodination of pyrrole can be directed to the 3-position conveniently using the procedure of Muchowski and co-workers (Scheme 3),[14] this yields a mixture of products when attemepted with common electrophilic chlorinating agents. However, we found that by first installing bromine regioselectively as in Scheme 3, subsequent lithium-halogen exchange followed by quenching of the organolithium with an electrophilic chlorine source (e.g., hexachloroethane) allowed access to new halogenated pyrroles in good yields, such as the 3-chloropyrrole **8** as well as the 3-bromo-4-chloropyrrole **10** and 3-chloro-4-iodopyrrole **11**, following treatment with NBS and NIS, respectively. The pinacolboronate ester **12** could then be easily prepared from **10** or **11** using the procedure of Billingsley and Buchwald (Scheme 4).[16]

Scheme 3. Regioselective Halogenation of Pyrroles



Scheme 4. Preparation of 3-Chloropyrroles



Preparation of the desired 2-bromo-6-chloroaniline **17** (and 2-bromo-6-chloronitrobenzene **18**) was somewhat challenging. While it has been reported that **17** can be prepared in 71% yield by hydrolysis of the anilide produced from the treatment of *N*-(2-bromophenyl) benzohydroxamic acid with thionyl chloride,[17] this reaction proved difficult to reproduce in our hands.[18] We next attempted the preparation of **17** using directed ortho metalation of 2-chloroaniline. Both *tert*-butoxycarbonyl (Boc)[19] and pivaloyl (Piv)[20] were used as directed metalation groups on the aniline moiety. Unfortu-

(8) Nakano, H.; Umio, S.; Kariyone, K.; Tanaka, K.; Kishimot, T.; Noguchi, H.; Ueda, I.; Nakamura, H.; Morimoto, Y. *Tetrahedron Lett.* **1966**, *7*, 737–740.

(9) Gosteli, J. *Helv. Chim. Acta* **1972**, *55*, 451–460.

(10) To date, overexpression of PrnA[7] and PrnB[11] in *Pseudomonas fluorescens* and PrnD[12] in *E. coli* has been reported, but enzyme activity has only been reconstituted *in vitro* for PrnA and PrnD.

(11) De Laurentis, W.; Khim, L.; Anderson, J. L.; Adam, A.; Johnson, K. A.; Phillips, R. S.; Chapman, S. K.; van Pee, K. H.; Naismith, J. H. *Biochemistry* **2007**, *46*, 12393–12404.

(12) Lee, J.; Simurdiak, M.; Zhao, H. *J. Biol. Chem.* **2005**, *280*, 36719–36728.

(13) Alvarez, A.; Guzman, A.; Ruiz, A.; Velarde, E.; Muchowski, J. M. *J. Org. Chem.* **1992**, *57*, 1653–1656.

(14) Bray, B. L.; Mathies, P. H.; Naef, R.; Solas, D. R.; Tidwell, T. T.; Artis, D. R.; Muchowski, J. M. *J. Org. Chem.* **1990**, *55*, 6317–6328.

(15) 3-Chloropyrrole has been isolated and characterized from mixtures obtained upon acidification of *N*-chloropyrrole (De Rosa, M. *J. Org. Chem.* **1982**, *47*, 1008−1010) and photolysis of 3-chloropyridine *N*-oxide (Bellamy, F.; Streith, J.; Fritz, H. *Nouv. J. Chim.* **1979**, *3*, 115−122).

(16) Billingsley, K.; Buchwald, S. L. *J. Am. Chem. Soc.* **2007**, *129*, 3358–3366.

(17) Ayyangar, N. R.; Kalkote, U. R.; Nikrad, P. V. *Ind. J. Chem. B* **1983**, *22*, 872–877.

(18) Attempts carried out at low temperature in Et_2O, THF, or benzene gave no reaction, whereas reactions carried out at elevated temperatures or with excess $SOCl_2$ gave complex product mixtures.

(19) Muchowski, J. M.; Venuti, M. C. *J. Org. Chem.* **1980**, *45*, 4798–4801.

(20) Fuhrer, W.; Gschwend, H. W. *J. Org. Chem.* **1979**, *44*, 1133–1136.

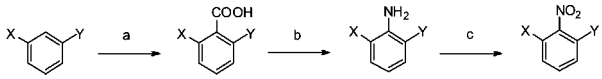
nately, while the 2-chloropivanilide could be metallated and halogenated in excellent yield, the subsequent removal of the Piv group proved difficult. In contrast, the Boc group could be easily removed, but the metalation/halogenation proceeded in poor yields.

We then came across the work of Roe,[21] which showed that 1,3-difluorobenzene and 1,3-dichlorobenzene could be converted to 2,6-difluorobenzoic acid and 2,6-dichlorobenzoic acid in 81% and 75% yield, respectively, via an aryllithium intermediate subjected to carboxylation and acidification.[21a] A subsequent Schmidt rearrangement was shown to yield 2,6-difluoroaniline in 86% yield.[21b]

While Roe's route was encouraging, Hickey and coworkers[22] have shown that benzyne-type decomposition of the 2-bromo-6-chlorophenyl lithium intermediate occurs under these conditions. Therefore, following Hickey et al., we switched from *n*-butyllithium to lithium di-*iso*-propyl amide as the base and from −50 to −78 °C as the temperature for the lithiation step and were very pleased to see quantitative formation of 2-bromo-6-chlorobenzoic acid following quenching with CO_2 and acidification.

Indeed, these conditions improve on Roe's yields of 2,6-difluorobenzoic acid and 2,6-dichlorobenzoic acid (not shown) and also allowed the peparation of 2,6-dibromobenzoic acid from 1,3-dibromobenzene. We found that dropping the reaction temperature further to −100 °C allowed the lithiation/carboxylation sequence to be carried out on even 1-bromo-3-iodobenzene.[23] Subjecting these dihalobenzoic acids to Schmidt conditions afforded the 2,6-dihaloanilines in good to excellent yields, including the desired 2-bromo-6-chloroaniline **17**. Furthermore, oxidation of the 2,6-dihaloanilines with *m*-chloroperbenzoic acid in dichloroethane afforded the 2,6-dihalonitrobenzenes in good yield, including the desired 2-bromo-6-chloronitrobenzene **18**. The results of the lithiation-carboxylation-Schmidt-oxidation sequence are summarized in Table 1.

Table 1. 2,6-Dihaloanilines (and 2,6-Dihalonitrobenzenes) by the Lithiation-Carboxylation-Schmidt (Oxidation) Sequence

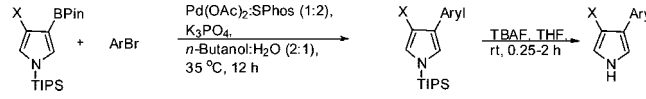


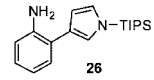
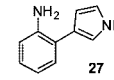
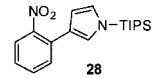
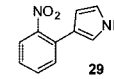
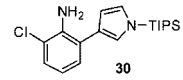
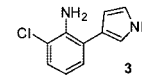
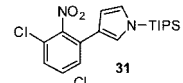
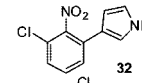
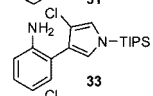
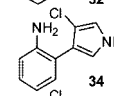
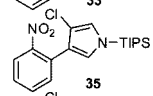
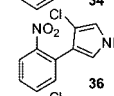
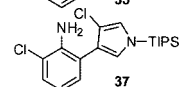
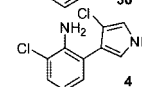
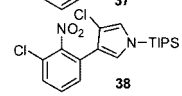
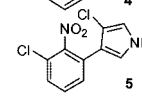
aryl halide	yield (product)	yield (product)	yield (product)
X, Y = Br, F	92 (**13**)	86 (**14**)	88 (**15**)
X, Y = Br, Cl	99 (**16**)	92 (**17**)	94 (**18**)
X, Y = Br, Br	85 (**19**)	78 (**20**)	82 (**21**)
X, Y = Br, I	80 (**22**)	83 (**23**)	78 (**24**)

[a] (i) LDA, THF, −78 °C, 1 h; (ii) CO_2, −78 °C to rt, 2 h (−100 °C to rt for **22**). [b] (i) H_2SO_4, 60 °C, 1.5 h; (ii) NaN_3, rt, 42 h. [c] *m*-CPBA, DCE, 70 °C, 2 h.

With the necessary coupling partners for **3**, **4**, and **5** as well as related halogenated pyrroles in hand, Suzuki−Miyaura cross-couplings of the various pyrrole pinacolboronate esters and aryl bromides were carried out. We began with a series of reactions of the unchlorinated pyrrole pinacolboronate ester (**25**, prepared from **9** in 80% yield via the analogous conditions in Scheme 4) with commercially available 2-bromoaniline and 2-bromonitrobenzene as well as our newly prepared **17** and **18**. The results are shown in Table 2.

Table 2. Suzuki-Miyaura Cross-Couplings of the Pyrrole Pinacolboronote Ester **25** and its Chlorinated Derivative **12** with Relevant 2-Bromoanilines and 2-Bromonitrobenzenes



pyrrole	ArBr	product	yield	product	yield
X=H **25**		 **26**	85[a]	 **27**	79
X=H **25**		 **28**	81	 **29**	81
X=H **25**	**17**	 **30**	82	 **3**	80
X=H **25**	**18**	 **31**	87[b]	 **32**	64
X=Cl **12**		 **33**	81[c]	 **34**	75
X=Cl **12**		 **35**	86	 **36**	78
X=Cl **12**	**17**	 **37**	82	 **4**	73
X=Cl **12**	**18**	 **38**	89	 **5**	74

[a] Carried out at 100 °C for 16 h. [b] Carried out at 0 °C for 16 h. [c] Carried out at 100 °C for 5 h.

We found the conditions recently reported by Billingsley and Buchwald,[16] which employ the newer SPhos ligand in a 2:1 ratio with $Pd(OAc)_2$ to be superior to those reported by Muchowski et al.[13] for the preparation of simple arylpyrroles some time ago, which employed $Pd(PPh_3)_4$. The use of a butanol:water cosolvent system was key to minimize the amount of reduced aryl halide formed and maximize the overall yield. While couplings of 2-bromoaniline required 100 °C to achieve respectable yields for an overnight reaction, 2-bromonitrobenzene, **17** and **18** coupled with good yield overnight when heated to only 35 °C. Fluoro-desilylations also proceeded smoothly to yield the aryl pyrroles

(21) (a) Roe, A. M.; Burton, R. A.; Reavill, D. R. *Chem. Commun.* **1965**, 52. (b) Roe, A. M.; Burton, R. A.; Willey, G. L.; Baines, M. W.; Rasmussen, A. C. *J. Med. Chem.* **1968**, *11*, 814–819.

(22) Hickey, M. R.; Allwein, S. P.; Nelson, T. D.; Kress, M. H.; Sudah, O. S.; Moment, A. J.; Rodgers, S. D.; Kaba, M.; Fernandez, P. *Org. Proc. Res. Dev.* **2005**, *9*, 764–767.

(23) While some lithium-halogen exchange does occur at this temperature, the desired 2-bromo-6-iodobenzoic acid is the major product, formed in a 5:1 ratio with the 3-bromobenzoic acid arising from initial lithium-halogen exchange.

27,[27] **29**,[27] and **32**,[25] as well as monodechloroaminopyrrolnitrin **3**[24] in good yields.

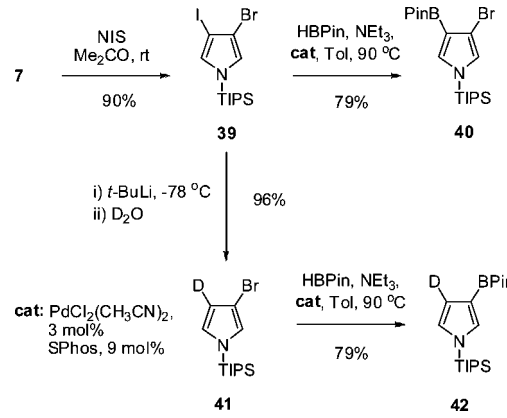
It is interesting to note that the cross-coupling of **18** with **25** had to be carried out at 0 °C, since at 35 °C, a significant amount of 2,6-dipyrrole substituted nitrobenzene is formed. Thus, cross-coupling of both the aryl bromide and the aryl chloride are possible under these conditions. This was not observed in cross-couplings of **25** with the less reactive dihalogenated aniline **17**.

Since the cross-couplings of **25** with the various aryl bromides in Table 2 proceeded as hoped, we next turned our attention to cross-couplings of the chlorinated pyrrole pinacolboronote ester **12**. The results are also shown in Table 2. Again, both the cross-couplings and the fluoro-desilylations proceeded smoothly and in good yield, allowing access to the arylpyrroles **34** and **36**,[28] as well as aminopyrrolnitrin **4**[29] and pyrrolinitrin **5**.

Since there is much interest[26] in whether chlorinating enzymes such as PrnA and PrnC can incorporate bromine into their substrates in lieu of chlorine, we also sought means to prepare brominated analogs of pyrrolnitrin in order to study the substrate scope of PrnC. This first required the synthesis of the brominated pyrrole pinacolboronate ester **40**, which we obtained in two steps from **7** as in Scheme 5. We also used an analogous approach to prepare the deuterated pyrrole **42** whose cross-coupling product with **18** could be used in mechanistic (kinetic isotope effect) studies with PrnC.

Not surprisingly, cross-couplings of **42** to the halogenated anilines and nitrobenzenes of Table 2 were good reactions, providing the 3-deuteriopyrrole analogs of pyrrolnitrin in essentially the same yields (e.g., 90% for **42** + 2-bromoaniline, see Supporting Information). However, the yields of the 3-bromopyrrole analogs from cross-couplings employing

Scheme 5. Preparation of Brominated and Deuterated Pyrroles



40 were much lower (e.g., 18% from **40** + 2-bromonitrobenzene, see Supporting Information) due to its competing self-reaction. Attempts to optimize the conditions to favor the cross-coupling over the homocoupling are underway.

In summary, we have developed a straightforward synthesis of the antifungal agent pyrrolnitrin, its biosynthetic precursors and related halogenated phenylpyrroles, which we are now using in kinetic and mechanistic studies of heterologously overexpressed pyrrolnitrin biosynthetic enzymes. Along the way, we have accessed 3-chloropyrroles by a tandem lithium-halogen exchange/electrophilic chlorination pathway, generalized a lithiation/carboxylation/Schmidt sequence for the preparation of 2,6-dihaloanilines (and 2,6-dinitrobenzenes following oxidation) bearing bromides and iodides, and determined optimal conditions for the cross-coupling conditions of chlorinated aryl bromides and boronated pyrroles.

Acknowledgment. This work was supported by grants from NSERC, Queen's University and the Canada Research Chairs program. D.A.P. and M.D.M. thank the Government of Ontario for an Early Researcher Award and Ontario Graduate Scholarship, respectively.

Supporting Information Available: Details of the preparation and characterization of all intermediate and final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL8026957

(24) Compound **3** has been prepared biosynthetically from 7-chlorotryptophan as in van Pee, K.-H.; Salcher, O.; Lingens, F. *Angew. Chem., Intl. Ed.* **1980**, *19*, 828–829.

(25) Compound **32** has been prepared by reductive dechlorination of **5** with iodotrimethylsilane. See: Sako, M.; Kihara, T.; Okada, K.; Ohtani, Y.; Kawamoto, H. *J. Org. Chem.* **2001**, *66*, 3610–3612.

(26) van Pee, K.-H.; Dong, C. J.; Flecks, S.; Naismith, J.; Patallo, E. P.; Wage, T. *Adv. Appl. Micro.* **2006**, *59*, 127–157.

(27) Compounds **27** and **29** have been synthesized with tritium at the 2-position of the pyrrole ring using methods similar to those described in ref 8. See: Chang, C. J.; Floss, H. G.; Hook, D. J.; Mabe, J. A.; Manni, P. E.; Martin, L. L.; Schroder, K.; Shieh, T. L. *J. Antibiot.* **1981**, *34*, 555–566.

(28) Compound **36** has been prepared using methods similar to those described in ref 8. See: UmioS. KariyoneK. TanakaK. Ueda I. MorimotoY. *Chem. Pharm. Bull.* 1969*17*588595.

(29) Compound **4** has been obtained by reduction of **5** as in: van Pee, K.-H.; Salcher, O.; Fischer, P.; Bokel, M.; Lingens, F. *J. Antibiot.* **1983**, *36*, 1735–1742.