N-Benzyl cytisine undergoes an efficient C(6)-N(7) cleavage via directed C(6) lithiation, borylation and oxidation to provide a “privileged” heterocyclic core unit comprising a highly functionalised, cis-3,5-disubstituted piperidine in enantiomerically pure form. The potential offered by this unit as a means to explore chemical space has been evaluated and methods have been defined (and illustrated) that allow for selective manipulation of N(1), C(3'), and the pyridone N. The pyridone core can also be diversified via bromination (at C(3'') and C(5'')) which is complementary to direct C-H activation based on Ir-catalyzed borylation to provide access to C(4''). The use of a boronate-based 1,2-migration as an alternative trigger to mediate C(6)-N(7) cleavage of cytisine was evaluated but failed. However, the stability of the intermediate boronate opens a new pathway for the elaboration of cytisine itself using both Matteson homologation and Zweifel olefination.

Introduction

Contemporary “small molecule” drug discovery relies on a variety of strategies to both identify and develop “hits” into leads and then drug candidates. Clearly, the ability to define and explore novel “chemical space” plays a key role in terms of generating (and then protecting to enable further exploitation) intellectual property. For many years natural products provided a valued entry to enable structural variation but pharmaceutical industry interest in this area has, at points, waned in favor of, for example, combinatorial chemistry and related approaches as the preferred means by which to achieve molecular diversity. However, the enormous structural variation associated with natural products has, regardless, continued to provide a source of inspiration (and a guide) that has led to the development of, for example, diversity-oriented and “chemical genetics” approaches to lead discovery. Natural products, which are often available in enantiomerically pure form and may contain multiply stereogenic centers, do provide one important starting point for a diversity-oriented approach to drug discovery. Several natural products or core components of natural products, such as 1-O-acetylbritannilactone and maslinic acid have found application in this area (Scheme 1).

Taking this further, an ability to harness further the reactivity of a readily available natural product to widen the reach of the structural diversity available is well exemplified by Schwarz’s work on α-santonin. Here, both the natural product 1 and a readily available variant 2 (available from 1 by acid-catalysed rearrangement) provided analogues of both of these related scaffolds that were explored as inhibitors of 5-lipoxygenase.

Scheme 1 Top: Example of natural products providing a functionalised core scaffold. Bottom: derivatisation of α-santonin 1.
The role of “privileged scaffolds” serves as a further focus in this area, with many being based on or incorporating a heterocyclic core. Of these, piperidines are especially widely distributed and are found in a large number of bioactive and important pharmaceutical compounds. Our work has focussed on developing new and stereochemically-defined heterocyclic scaffolds based, in part, on nitrogen-based natural products that incorporate piperidine moieties. These include coerulescine, which led to the development of a range of stereochemically-defined spirocyclic bis(azacycles), such as the piperidine and the pyrrolidone-containing units and. Each of these spirocyclic scaffolds (within which the individuals enantiomers are accessible) offers three discrete functionalisation sites: two secondary but readily differentiated amines (blue and purple), and a primary alcohol (red), all of which are distributed across a spatially distinctive spirocyclic core.

We have, more recently, aimed to extend this chemistry by evaluating the opportunity to “release” a stereochemically complex and functionally-flexible piperidine core unit from a readily accessible precursor; this is related to but distinctive from the concept outlined in Scheme 1. Our initial focus for this has been the development of the chemistry based on (-)-cytisine, a readily accessible lupin alkaloid from Laburnum, and the results of this work are summarised in this paper.

(-)-Cytisine, which is a partial agonist of the nicotinic acetylcholine receptor (the high affinity nicotine binding site in brain), is marketed (as Tabex®) within Eastern and Central Europe for smoking cessation. While that specific nicotinic profile may not be required (or desired), we recognised that cytisine offered a readily available starting point from which to construct a series of structurally diverse libraries if, for example, the piperidine unit (coded blue) could be “released” from within the tricyclic heterocyclic core of Cytisine. Cytisine has already inspired a diversity-oriented synthesis approach to novel inhibitors of Bcl-2 and there has been extensive studies associated with the substitution of cytisine itself, driven largely by attempts to develop novel nicotinic ligands. Of particular relevance to the work described here are the studies of Rouden and co-workers on the site-specific lithiation of-N-acylated cytisines; this is illustrated by an N to C acyl transfer to functionalise at C(6) (Scheme 2). We had reasoned that using an appropriate electrophile, functionalisation of C(6) would allow for cleavage of the C(6)-N(7) link, and release of an intact piperidine unit. This was achieved as shown in Scheme 2 by C(6)-lithiation (via 8) and (in situ) silylation of (-)-N-methylcytisine (caulophylline) followed by C-Si → C-O oxidation followed by reduction. This sequence demonstrated the underlying concept by liberating the piperidine moity (highlighted in blue) and provided a direct entry to the related lupin piperidine alkaloid (+)-kuraramine in 18% overall yield from (-)-7.

However, this initial solution involved the use of a silyl electrophile which necessitated a stoichiometric amount of a mercury-based oxidant to achieve the key Fleming acyl migration (Rouden et al). Bottom: C(6) Oxidative functionalisation of N-methylcytisine; a biomimetic synthesis of (+)-kuraramine (Gallagher et al).

![Diagram](https://example.com/diagram.png)

**Scheme 2.** Top: C(6) Lithiation triggering N(3)→C(6) acyl migration (Rouden et al.). Bottom: C(6) Oxidative functionalisation of N-methylcytisine; a biomimetic synthesis of (+)-kuraramine (Gallagher et al.).
piperidine and the pyridone moieties respectively); an ability to manipulate the C(3′) methylene unit; further functionalisation of the pyridone moiety via either electrophilic bromination (which occurs predominantly at C(3″)) or, and of more utility and novelty, Ir-catalysed C-H activation which selectively targets C(4″).

This program has been driven in part by an increased awareness of the power and potential within medicinal chemistry associated with cost effective in silico assessment of ligands against protein targets of interest. Indeed, the goal would be to enable a full evaluation of the “effectiveness” of a ligand or family of ligands prior to embarking on an expensive program of chemical synthesis and evaluation by biological assay. As the dependability of these in silico methods increases so will their value as a reliable predictive tool. Those methodologies must, necessarily, encompass versatile, drug-like scaffolds such as A.

Results and discussion

To improve the efficiency of the fragmentation of cytisine, we have explored the chemistry illustrated in Scheme 2 in a number of ways: (i) the conditions for C(6) lithiation must avoid metalation at other sites, see below. (ii) the variation of the N(3) substituent that is possible; this was N-methyl earlier but this is less attractive for downstream synthetic utility. (iii) the choice of electrophile (ideally employed in situ during the deprotonation step) used to trap the C(6) lithiated species (i.e. 8) in order facilitate C(6)-N(7) cleavage. (iv) the conditions needed to achieve that critical transformation that also avoid heavy metals such as Hg(II).

Revisiting C(6) lithiation of N-substituted cytisines.

The original conditions used to lithiate N-methycytisine 7 (which was trapped with Me$_2$PhSiCl to give 9) had proceeded in 48% yield. Relatively straightforward variation of some of the basic reaction parameters allowed us to optimise this basic transformation to 83% in favour of C(6) lithiation and trapping.

Although we did observe yields of 9 of up to 90%, 83% reflected the yield obtained when competing lithiation (and trapping) at C(10) was suppressed. The latter led to a C(10) silylation adduct 12 (and this was exacerbated in the absence of TMEDA) and we also observed a (known)$^{18}$ dimerization product 13 (Scheme 3). While LDA provided cleanly lithiation at C(6), Rouden observed C(10) (as well as competing C(9)) metalation and (in situ) silylation of N-methylcytisine 7 when a particularly bulky base (LiTMP) was used. We did see some level of C(10) lithiation with LDA and an analogous metalation (cf 12) within a simple pyridone (i.e. at C(4), pyridone numbering) has been reported by Katritzky.$^{17}$ We evaluated N-Boc cytisine as a substrate for C(6) metalation but analysis of the crude reaction mixture (following reaction with LDA and silylation) indicated substitution at C(2) and/or (C(4) had occurred which is associated with Boc-directed metalation.$^{18}$

Use of N-benzyl cytisine 14 was more attractive both for downstream manipulations and because we did not observe competitive metalation (as 12) or dimer formation (as 13). Although Rouden has examined lithiation of 14, in our hands and using a more synthetically useful aryl-based chlorosilane (Me$_2$PhSiCl), TMEDA (1 equiv.) did have an impact in terms of reaction efficiency and in particular suppressed a small amount of competing lithiation at C(10); 15 was isolated in 84% yield free of competing side products, with the mass balance accounted for by recovered 14 (Scheme 4).

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and stoichiometric Hg(OAc)$_2$ followed by a reductive ring opening of hemiaminal 10 (Scheme 2). Closer inspection of this process indicated two competing reactions: desilylation of 9 to regenerate 7 and (ii) possible competing N-oxidation of the tertiary (N(3)) amine was suggested by MS analysis. These oxidation conditions were modified to give a small increase in yield of 11 (from 38% to 47% from 8) by using a combination of H$_2$O$_2$ and peracetic acid (rather than peracetic acid alone) but this transformation still required Hg(OAc)$_2$. All attempts, however, to achieve the conversion of 9 to 10 by other means e.g. KBr, AcOOH/ AcOH; HBF$_4$, then H$_2$O$_2$; KH, tBuOOH then TFAB, thereby avoiding mercury salts, failed. This forced consideration of an electrophile that would offer an alternative cleavage mechanism. Lithiation followed by halogenation at C(6) was evaluated using a variety of halogen sources (Br$_2$, I$_2$, N-bromosuccinimide, BrCCl$_3$ClBr, CBr$_4$) proved unproductive. However, diphenyl disulfide (not shown, but see Supplementary Information) and boron-based electrophiles were effective in situ traps for the C(6) lithio intermediate derived from 7 (Scheme 5). In the boron series, further optimisation using both N-methyl and N-benzylcytisine 7 and 14 respectively led to (OPr)Bpin as the electrophile of choice, and adducts 16 and 17 were obtained in 93 and 89% yields respectively. While boronate esters 16 and 17 were relatively tolerant of chromatography, further purification was unnecessary. Direct oxidation of the crude boronates (using NaBO$_2$) followed by reduction (NaBH$_4$) gave the piperidine core structures, (+)-kuraramine 11 and the synthetically more flexible N-benzyl variant 18 in 32% and 52% overall yields (from 7 and 14) respectively.

![Scheme 5. C(6) borylation and ring cleavage.](image)

In summary, cytisine 6 is easily derivatised to the N-methyl and N-benzyl variants 7 and 14, each of which provides efficient access to the corresponding enantiomerically pure cis-3,5-disubstituted piperidines 11 and 18 respectively. The results reported here serve to improve significantly the synthesis of (+)-kuraramine 11 from N-methylecytisine and avoid heavy metal oxidants to mediate the key C(6)-N(7) bond cleavage. Use of a boron-based electrophile, and specifically (OPr)Bpin to trap the C(6) lithiated intermediate, was applicable to both the N-methyl and N-benzyl series.

Elaboration of the piperidine scaffold; identification of protecting group arrays that enable selective site manipulation.

Heterocyclic scaffolds, such as 11 and 18, offer significant potential with the N-Bn residue in particular cleavable under a variety of different reaction conditions. Consequently, the majority of the remainder of this paper is focused on developing the potential of the N-benzyl piperidine 18 as the basis of a flexible heterocyclic core structure. Our aim here is to demonstrate how the periphery of 18 can be functionalised in ways that would make this unit well suited to a general fragment-based drug discovery strategy, for the reasons articulated above. We have explored chemistry around four specific sites illustrated in general scaffold A: via the piperidine nitrogen (N(1)), at C(3’) (in 18, a primary alcohol), and in three areas of the pyridone moiety, at C(3’’) and C(4’’) and across the lactam unit (N(1’’) and C=O). The latter is especially important to protect (deactivate) when pursuing functionalization of C(3’’) (see below). Given the array of functional groups present within 18, we have determined methods for differentiating at these different sites. One consequence is that a series of protecting group arrays have been assessed and this is illustrated in Scheme 6.

Selective N- and O-Boc protection in combination with O-silylation provided a comprehensive set of options, which has allowed us to generate a number of differentially protected variants 19-24; this includes the fully deprotected variant 22. Additionally, the structure of the N,O-bis-Boc protected alcohol 20 was solved by X-ray crystallographic analysis (see Supplementary Information). The pyridone lactam underwent O-acetylation in the presence of an excess of Boc$_2$O, a protecting group array that is then cleavable under basic/nucleophilic conditions, see below. Further, the ability to manipulate selectively the pyridone nitrogen N(1’’) is illustrated by N-methylation of 24 to give adduct 25.

**Elaboration at C(3’); variation of C(3’’) oxidation level.**

That there is a requirement to protect/deprotect easily the pyridone moiety is exemplified attempts to derivatise the C(3’’) primary alcohol (Scheme 7). Reaction of N-Boc piperidine 19 with phthalimide under Mitsunobu conditions in an attempt to access the 3’ amino variant led cleanly (and in essentially quantitative yield) to N-Boc cytisine 25. Of course, cis-disubstituted piperidine 19 is well set up to undergo intramolecular alkylation, a possibility that was both recognized and exploited elegantly in one of the very early syntheses of cytisine carried out 60 years ago by van Tamelen and Baran, and subsequently used in related contexts by others.
We attempted to circumvent this issue via double mesylation of 19 to generate intermediate 27 (not isolated, but assignment of a double mesylate was based on MS). However, cyclisation still occurred with 27 the only product observed on exposure of 27 to azide or phthalate. Clearly, the O-mesylate is labile in the presence of a good nucleophile but O-Boc protection solves this issue. Using intermediate 20, activation and phthalimide displacement at C(3′) followed by imide cleavage provided the C(3′) amino derivative 28 (Scheme 8). Here, hydrazine also cleaves the O-Boc residue liberating the pyridone unit, but this is presumed to occur after nucleophilic displacement at C(3′).

In addition, the scope of the C(3′) amino substituent can be extended using aldehyde 29 derived from 20 using Swern oxidation. Aldehyde 29 was not routinely isolated (to minimize any risk of epimerization) but used directly for reductive amination or further oxidation (see below). Reductive amination using nucleophilic amines was generally accompanied by O-Boc cleavage under the reaction conditions (see 30a and 30b), and where (as in the case of an α-amino ester) this does not occur, O-Boc cleavage is then readily achieved using aqueous ammonia (leading to 30c). The latter is a generally applicable deprotection method and is illustrated further below. Alcohol 20 is also readily converted to the corresponding carboxylic acid 31 in excellent overall yield and acid 31 offers access to a representative library of C(3′) derivatives (Scheme 9).

Again, the pyridone unit is unmasked by O-Boc cleavage under nucleophilic conditions without disruption of other functionality to provide pyridones 32a-c. In addition, global deprotection of 31 is readily achieved to give the heterocyclic β-amino acid 33. Acid 33 is related to (S)-nipecotic acid (the parent piperidine-based β-amino acid), where 5-aryl substituted nipecotic acid derivatives have attracted medicinal chemistry interest. We had recognized a risk associated with C(3) epimerization of e.g. aldehyde 29. For that reason we secured the X-ray crystal structure of acid 31 (see Supplementary Information), which
confirmed both the (intact) cis-configuration and the presence of the O-Boc protected pyridine moiety.

Scheme 8. Successful displacement at C(3'). Yields of 30a-c are from alcohol 20.

Scheme 9. Ester/amide library based on carboxylic acid 31; global deprotection to provide acid 33.

Elaboration at N(1).

The final target site for substitution on this piperidine scaffold was at N(1). This can be carried out in a number of ways, and we have simply illustrated the accessibility of this key position by N-debenzylation of 18 to give 22 (Scheme 6) as an isolable and versatile intermediate, followed by a representative reductive amination leading to the bis(piperidine) 34 (Scheme 10). This does, however, serve to show that protection elsewhere is not necessary in order to access N(1).

Scheme 10. Reductive amination at N(1).

Regioselective functionalisation of the pyridone moiety; electrophilic bromination and Ir-catalyzed borylation.

The remaining region of the piperidine scaffold that was of interest is associated with the pyridone unit. Electrophilic halogenation of pyridones is well-established and leads predominately to 3-substitution, with the 5-halo isomer as (usually) the minor product. Using N-bromosuccinimide, this pattern was observed with pyridone 19 (Scheme 11). Bromides 35 and 36 are readily separated (and easily differential by 1H NMR) and provide an obvious and very useful handle at C(3'″) and C(5″). Access to C(4″) is also achievable using Ir-catalyzed borylation, as has been reported recently. In our hands, however, NH pyridones (e.g. 19) are not viable substrates for this C-H activation method. N-Alkyl pyridones (e.g. 7) are, though, generally reactive and with O-Boc protected pyridines (i.e. 23) highly selective borylation at C(4″) is observed.

The scope and potential of this chemistry is illustrated in Scheme 11, and this includes two representative transformations of the intermediate boronate esters: Suzuki coupling of adduct 37, followed by O-Boc cleavage (using basic ammonia) gave 38; Cu-mediated bromination and deprotection of adduct 39a gave 40, offering a reactant that complements the corresponding Bpin esters. It is useful to note that the low yield of 40 reflects the instability in solution of the intermediate bromide prior to O-Boc cleavage but this transformation has not been optimized.

The chemistry outlined above serves to demonstrate the ability to manipulate the piperidine scaffold aligned to general structure A. This scaffold can be manipulated in a variety of distinct locations, using different transformations, and the only major requirement is the engagement of a suitable protecting group array that is primarily associated with moderating the reactivity of the pyridone unit. That moderation, largely based on N- and O-(Boc) protection, is readily achieved. Thus, we believe that the ready availability of (-)-cytisine and the efficiency with which the piperidine core unit can be revealed and then differentially manipulated makes this a potentially attractive starting point for a fragment-based discovery approach.
Enhancing the complexity of (-)-cytisine via C(6) lithiation; Matteson homologation and Zweifel olefination.

A key aspect of a fragment-based approach must be an ability to increase rapidly molecular complexity. We have also explored additional avenues to address that issue where the goal has been to identify methods to introduce additional (and novel) complexity to the cytisine-based (tricyclic) precursor prior to any fragmentation and further functionalisation. While these reactions do not contribute to extending the scope of the piperidine scaffold discussed earlier, they do provide access to novel cytisine variants that can be considered as alternative scaffold configurations and progenitors.

Of particular relevance here was the potential associated with trapping a C(6) lithiated cytisine (see Scheme 2, intermediate 8) with a secondary boronate ester (e.g. EtBpin). Our goal here had been to trigger a 1,2-migration sequence that would also serve to fragment the tricycle of cytisine and simultaneously create an addition stereocenter(*) at a key position on the periphery. The concept, which draws on previous work done within the Aggarwal group,23 is illustrated in Scheme 12.

Using N-benzyl cytisine 14, lithiation and trapping with EtBpin appeared to generate the requisite borate intermediate 41 based on 11B NMR which showed a signal at 6.3 ppm. However, all attempts to achieve 1,2-migration using a range of Lewis acids failed. Given that Bpin esters are sterically demanding, we also evaluated Et3B as a less demanding electrophile. Again, “ate” formation (analogous to 41) occurred (as judged by 11B NMR) but no migration products could be detected. The most plausible explanation for these outcomes is that the pyridone N is not a sufficiently good leaving group to drive the migration step. However, an appreciation of the stability of the pyridone moiety with respect to a 1,2-migration provided an opportunity to assess other “ate”-based chemistries available using boronate ester 17.

Boronate 17 undergoes efficient Matteson homologation24 to give boronate 42 in 89% yield. Oxidation of 42 provided primary alcohol 43 and the structure of the corresponding 4-nitrobenzoate 44 confirmed the configuration at C(6) (see Supplementary Information). A detailed 1H NMR analysis of 44 was also carried (see Supplementary Information) which aided in the assignment of adduct 46 (see below). Zweifel olefination25 also involves generation of a stable borate intermediate and using boronate 17 proceeds very efficiently. Using a modified procedure developed in the Aggarwal group,25c exposure of 17 to vinyl Grignard (and this likely generates the triethenyl borate intermediate 45), followed by exposure to I2 in methanol gave the C(6) ethenyl adduct 46 in 94% yield (Scheme 12). The configuration at C(6) was confirmed by NOESY experiments (using 44 for comparison) and details of these studies are also available in the Supplementary Information.
Conclusions

In summary, C(6) metalation of N-alkyl cytisine derivatives provides a direct and versatile method of functionalization that allows for ring fragmentation to provide a synthetically flexible, enantiomerically pure piperidine based heterocyclic scaffold. Given the privileged nature of the piperidine ring within medicinal chemistry, we suggest that this is an attractive unit for the development of more complex heterocyclic ligands within the context of a fragment-based approach to drug discovery. The potential of this functionalised piperidine scaffold has been exemplified using the N-benzylated variant. Critically it is possible to differentiate all key elements and, using C-H activation, to provide an additional functional handle within the pyridine unit. Attempts to induce a B-based 1,2-migration as a means to increase the molecular complexity within the piperidine scaffold failed, however, the relative stability of the key intermediate (identified by a lack of reactivity) can be exploited. Derivatives such as 43 and 46 that arise from this offer new opportunities for modification of the established nicotinic ligand profile of cytisine and studies in this area are underway.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

† When we applied a more conventional oxidation protocol (NaOH, H₂O₂) to the N-methyl boronate ester 16, we only observed decomposition.
§ It is noteworthy that the corresponding O-Boc protected variant 23 was unreactive under these same conditions.


