nature portfolio

Peer Review File

Positional isomerisation of pyridine via nitrogen transposition

Corresponding Author: Professor Sungwoo Hong

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Referee #1

(Remarks to the Author)
This manuscript by Hong and colleagues describes a skeletal editing strategy that allows the interconversion of pyridine positional isomers through formal nitrogen transposition. The work introduces a conceptually innovative transformation with potentially important implications for scaffold editing and medicinal chemistry. The chemistry is mechanistically fascinating and, if fully validated in terms of scope and practicality, could represent a significant advance. However, several aspects require further clarification and experimental support to meet the standards expected for publication in Nature.

Strengths:

1. The study introduces a creative strategy for formal nitrogen-atom transposition within pyridines without altering peripheral substituents. Direct positional editing of heteroaromatic cores remains underdeveloped, and this approach addresses a long-standing synthetic limitation.
2. The transformation proceeds through a well-designed cascade involving aziridination, ring expansion, electrocyclization, and dinitrogen extrusion. The mechanistic proposal is logical and supported by isotopic labeling studies.
3. The reaction conditions are transition metal-free and do not rely on harsh oxidants. The transformation is conducted under relatively simple conditions, thereby enhancing its potential practical value.
4. A range of mono- and disubstituted pyridines is examined, including C2-, C3-, C4-, C5-, and several multi-substituted systems.
5. The method aligns well with current interest in skeletal editing and scaffold hopping. If generalizable, this strategy could provide a valuable alternative to de novo synthesis in medicinal chemistry.

Weaknesses and Concerns:

1. The transformation requires prior preparation of activated pyridinium salts through multiple steps (amination, tosylation, and methylation). The overall step economy should be more critically discussed. This diminishes the claim that the strategy accelerates SAR exploration, as substantial synthetic effort precedes the isomerization step.

The inclusion of relatively simple drug conjugates (e.g., paracetamol derivatives in Figure 4) appears less compelling. The manuscript does not provide examples involving structurally complex drug molecules, despite emphasizing the importance of pyridines as privileged scaffolds. This raises concerns about compatibility with drug-like molecules such as Omidenepag (bearing a free carboxylic acid), as highlighted in Figure 1 to underscore the significance of this work. I encourage the authors to demonstrate this chemistry on pyridine-containing drugs bearing reactive functionalities (e.g., amines, alcohols, carboxylic acids). If such substrates are incompatible, the introduction and associated claims should be moderated accordingly.

2. Several regioselectivity trends remain insufficiently explained:

• Why do C2,3-disubstituted systems yield exclusively C3,4-products?

• Why do certain substitution patterns produce only one positional isomer?
• Why do C3-substituted substrates sometimes react at the substituted site?
• Why do electronically related substituents show substantial yield differences (e.g., ester 7g, 74% vs ketone 7h, 33%; methyl 7b, 86% vs extended alkyl 7i, 42%)?

A clearer electronic and mechanistic rationale is required. The substrate scope does not sufficiently probe extreme electronic bias. Strongly electron-donating and strongly electron-withdrawing substituents are underrepresented, particularly in electronically polarized systems that would rigorously test regioselectivity. I suggest providing mechanistic and/or computational support to rationalize the observed isomer outcomes.

5. Can this methodology be extended to quinolines? This limitation should be discussed more clearly and contextualized experimentally.

• No inert-atmosphere control experiment is presented.
• No comprehensive base screening table is provided.
• Internal standards for NMR yield determination are not specified.
• Some NMR spectra lack full assignment or display impurities.
• Please clarify whether diazepine intermediates were observed or trapped. Can we stop this transformation at the diazepines, which are valuable scaffolds in medicinal chemistry?
• The mechanistic language should be refined. Specifically, is the final demethylation step proceeding through an SN2 pathway?

6. The term “positional isomerization” may imply intramolecular nitrogen migration, whereas the process involves external nitrogen insertion followed by extrusion. A more precise description, such as “formal nitrogen transposition,” would improve conceptual clarity.

7. Several typos are there. Figure 2: 15N-labeled 7a should be 7a’ or a different number. Also, I could not find how the authors measured the percentage of 15N incorporation (quantification) into the molecule. Line 45, N should be italic. Please correct the typo in Figure 3 (“C2,5-substituted”).

In summary, this manuscript introduces a creative and potentially impactful skeletal editing transformation. The conceptual advance is strong. However, the substrate scope, multi-step process, and lack of justification for regioselectivity, including computational calculations and supporting data, do not yet fully support the claims of broad applicability, practical utility, and mechanistic understanding at the level expected for publication in Nature.

Referee #2

(Remarks to the Author)
Hong and co-workers report a strategy for pyridine positional isomerization through sequential nitrogen insertion and deletion, enabling predictable N-atom transposition without altering substituent identity. The concept is intellectually appealing and provides access to positional isomers that would otherwise require multistep synthesis. However, in its current form, the methodology appears to have notable limitations in scope and mechanistic substantiation. Overall, the work remains preliminary and does not yet meet the conceptual depth and generality expected for publication in Nature.
The main concerns are outlined below:
1. Although described as a general strategy, the substrate scope appears structurally biased. The majority of successful examples involve ortho-substituted pyridines, while substrates bearing substitution at other positions (e.g., 3- or 4-disubstituted systems) perform poorly. What is the underlying reason for the diminished reactivity or selectivity in these cases? Have the authors explored structural or condition modifications to overcome these limitations? Without a clearer understanding or solution, the generality of the method remains uncertain.
2. The manuscript includes modifications of relatively simple drug-like derivatives. However, the impact of the strategy would be significantly strengthened by demonstrating its performance on genuinely complex pharmaceuticals or natural products containing sensitive functional groups.
3. The authors propose that solvent plays a decisive role in controlling regioselectivity. However, the solvent study appears limited. Beyond toluene, DMF, and THF, were additional solvents screened to validate this conclusion? Is the regioselectivity trend consistent across a broader polarity or donor-number range? The current dataset is insufficient to firmly establish solvent as the primary determinant.
4. In Supplementary Figure 6, two mechanistic pathways are proposed. While experimental data are presented, no computational support is provided. Have DFT calculations been performed to compare the energy profiles of the two pathways? Can theoretical analysis explain the regioselectivity trends? Given the mechanistic centrality to this work, computational validation would be highly valuable.
5. The proposed mechanism involves nitrogen insertion/deletion steps and the generation of N_2. However, experimental validation remains limited. Has N_2 formation been directly detected? Have intermediates I, II, or III been observed or trapped? Are kinetic studies available to support the proposed sequence? Without experimental corroboration, the mechanistic proposal remains largely speculative.
While the conceptual framework is intriguing, the current manuscript lacks sufficient mechanistic depth, general substrate applicability, and rigorous validation to justify publication in Nature. Substantial strengthening of scope and mechanistic

support would be required to meet the journal's standards

Referee #3

(Remarks to the Author)
The authors present an innovative strategy which would make a nice addition to the skeletal edit toolbox of transformations. (Please note that thermal hazards are a key concern for this methodology which the Authors should provide further comments on). This study outlines a methodology for synthesizing N-atom rearranged pyridines through ring expansion of pyridinium salts using hydroxylamine reagents, followed by nitrogen extrusion. The approach demonstrates broad substrate applicability, and a plausible mechanism is proposed. Data analysis revealed the presence of positional isomers, with efforts made to provide rational explanations. The experimental section details spectral data and reaction conditions.

•This reviewer would like to Invite the authors to revise their manuscript to address specific concerns before a final decision is reached. Main points to address are safety concerns, computational support for mechanism proposed & selectivity observed, along with mass balance considerations for reactions exhibiting low yields. In instances where isomers are present, the Authors must clearly label as such in the figures. More points can be found below.

•Originality and significance: This work is original and would be of interest to readers of this journal

•Data & methodology: validity of approach, quality of data, quality of presentation: see below

•Consistency needed between e.g. Line 8: "de novo" and Line 54: italized "de novo"
•Although vismodegib isomerization is present in Fig. 1, it is not mentioned in the text until much later in line 132. Please omit from Fig.1 or acknowledge in local text.
•Line 44: "Fig. 1d"- there is no such label in Fig.1- please add or omit from text.
•Line 65: "1a" – there is no compound 1a in this manuscript. Please include compound 1a or renumber appropriately.
•Provide details or references for synthesis of all N-protected amidopyridinium salts in the manuscript (including safety data/precautions). And provide details or references for experimental data of each N-protected amidopyridinium salt isolated (including safety data/precautions) in SI as most are currently missing from the SI.
•This methodology uses reagents and compounds with potential thermal hazards, the author should comment on the safety data or precautions advised for this chemistry in the manuscript and SI.
- The author should comment on safety precautions undertaken for the use of MSA in dioxane with 70% perchloric acid (potentially explosive) in the manuscript and SI. Note that compound 2a was used on 4.26 gram scale. Please also comment on the scale justification (no mention of gram scale in the text only in Fig. 3) and safety controls undertaken.
•Line 72: "…SN2-type elimination…" – Is a more accurate description, SN2-type displacement followed by elimination/rearrangement?
•Line 73-74: Is a correction for Fig. 2a right needed? O-(2,4-dinitrophenyl)hydroxylamine 59% yield or 72% yield as shown in SI (Line 80, entry 8).
•Line 78: Reference note is needed to direct the reader to SI for reaction optimization details.
•Line 73-78 systematic evaluation of reaction paraments discussion: Author should include more comments on key findings from reaction optimization in the manuscript:
- Do the Authors have any thoughts on why H2N-OPh.HCl gives N.D. product of 7a but O-(2,4-dinitrophenyl)hydroxylamine gives rise to 72% yield of 7a?
- Do the Authors have any thoughts on why sub-stoichiometric base (0.5 eq. Cs2CO3) gives rise to 73% yield of 7a?
- Alternative solvent Ethyl Acetate also gives rise to 84% yield of 7a and should be highlighted alongside Toluene in Line 74 and Fig. 2a right?
- Correct Fig. 2a right: "DMF instead of THP" is listed as 73% yield of 7a in manuscript but its 65% yield in SI (Line 86, entry 7)?
- Did the Authors assess the safety advantages of using 40 °C or 60 °C instead of 80 °C for reaction temperatures?
•Line 84: "THP (0.75ml)" should be "THP (1.0mL)" as its more consistent with entries in Fig. 2a right - see solvent screening in the SI (Line 86, entries 2-9)?
•Line 90-93: More detailed Mechanistic discussion is needed.
- Author should include Supplementary Figure 6 in the manuscript which provides more details than Fig. 2a, bottom.
- Do computational calculations align with "major pathway" and "minor pathway" denotation in the Supplementary Figure 6?
•Lines 102-103: "Across all major substitution classes, the reaction delivers controlled positional isomerization with high efficiency and fidelity" Author should adjust sentence to accommodate where there are lower yielding reactions e.g. 7j, 7k, 7m.
•Fig. 3: Author should comment on what else is observed with lower yielding reactions- mass balance? Isomers where possible?
•Fig. 3: correct heading for 7t-7z should be C2,5-substituted not "C2,4-substituted"?
•Fig. 3: Author should include discussion on substrate scope limitations in manuscript- any comments on why 3-CN substrate gives trace products but 7w gives 50% isolated yield?
•Line 106-107: "ketones (7h) and extended alkyl chains (7i), were readily accommodated" is this statement consistent with their isolated yields? What else is observed with these lower yielding reactions- mass balance? Isomers where possible?
•Line 108-109: "Pyridines substituted at the C3 position, which are typically challenging to manipulate selectively…" what is the context for this comment as 7n, 7o, 7ad and C2,5-substituted pyridines are high yielding reactions?
•Line 129: should be bipyridine (7ag) and not "bipyridine (7af)"
•Line 130: should be terpyridine (7ah) and not "terpyridine (7ag)"

•Fig. 4a: Author should recheck characterization data for compound 7ag - NMR spectra shows presence of minor impurity in the sample, are isomers present and does the yield need to be adjusted? If so, please comment accordingly in the manuscript as currently Line 130 states "…frameworks underwent efficient positional isomerization…".
•Line 136-142: For discussion on selectivity, please include "Supplementary Figure 4. Substrate scope demonstrating solvent-dependent positional selectivity" from the SI (Line 906-910) in the manuscript.
- Is the selectivity observed consistent with computational calculations and mechanistic rationalization across all substrates?
•Line 140-142: "The reduced regioselectivity observed in DMF is consistent with enhanced solvation of the hydroxylamine nucleophile, which may diminish its sensitivity to subtle differences in electrophilic character between the C2 and C4 positions of the pyridine core"
- Are the solvent effects observed consistent with computational assessments?
•Line 149: "…high fidelity across diverse substitution patterns and functional groups" Is this statement consistent with the observed presence of isomers and lower yields in instances?

SI
•Line 47: consistency between temperature in scheme and procedure, 80 oC vs 100 oC
•Line 59: consistency between temperature in scheme and procedure, 80 oC vs 100 oC

•Appropriate use of statistics and treatment of uncertainties: as detailed above

•Conclusions: robustness, validity, reliability: as detailed above

•Suggested improvements: experiments, data for possible revision: as detailed above

•References: appropriate credit to previous work? Line 35-36: "Skeletal editing strategies, which allow selective modification of aromatic frameworks, offer a powerful conceptual foundation for this approach" References for this statement should include: Blakemore DC et al. Organic synthesis provides opportunities to transform drug discovery. Nat. Chem 10, 383–394 (2018).

Version 1:

Reviewer comments:

Referee #1

(Remarks to the Author)
I thank the authors for their effort in revision. The mechanistic additions — the GC–MS $^{15}N/^{14}N$ isotope experiment, the ^{13}C-labeled solvent selectivity study, and the Curtin–Hammett DFT analysis — are genuine improvements and strengthen the paper's mechanistic foundation. However, the central concern I raised in Round 1 remains unresolved, and it goes to the core of what justifies publication in Nature.
Critical Concern: No Real Pyridine-Containing Drug Has Been Tested, and No Practical Advantage Over De Novo Synthesis Has Been Demonstrated
Figure 4a is presented as evidence of "synthetic utility" through "drug-derived substrates." Upon close reading, every compound in that figure is a synthetic conjugate in which a pyridine unit has been artificially appended to a fragment from a known drug or bioactive molecule. The pyridine rings being isomerized are not the native pharmacophoric elements of any parent drug — they are synthetic appendages installed specifically to enable this chemistry. Not one actual pyridine-containing drug has been subjected to the isomerization conditions with its native pyridine intact. This is not a presentational issue; it is a scientific one. Figure 4 does not demonstrate what it is claimed to demonstrate.
This concern is compounded by the compatibility limitations acknowledged in lines 154–156: substrates bearing free amines, alcohols, and carboxylic acids are incompatible with the pyridinium salt preparation step. These are precisely the functional groups present in the overwhelming majority of real pyridine-containing drugs. In practice, applying this method to an actual drug molecule would require protection of nucleophilic groups, three-step pyridinium salt preparation, the isomerization itself, and final deprotection — a minimum of five to six operations on a molecule already in hand. What is the overall yield across this sequence for any representative real substrate? If the isomerization step proceeds at 60–80% yield but the cumulative yield across the full sequence is 10–20%, the practical advantage of purchasing or independently synthesizing the target is unclear at best and negative at worst.
This raises a question the authors must address directly: who, in a pharmaceutical or medicinal chemistry setting, would realistically use this method? A drug discovery team conducting SAR exploration needs to access positional isomers rapidly and efficiently. If the method requires installing a synthetic pyridine handle, protecting multiple functional groups, executing a multi-step sequence, and then deprotecting — all on a molecule that already exists — what advantage does it offer over a straightforward de novo synthesis of the desired isomer? The manuscript's claims about accelerating SAR exploration and enabling late-stage diversification require a concrete answer to this question, not an implied one.
The authors must therefore provide at least one example using a genuine pyridine-containing drug or advanced pharmaceutical intermediate, reporting the full step count and overall yield from the drug to the isomerized product, alongside an honest comparison with the de novo route. If the method offers fewer steps, higher overall yield, or access to an otherwise synthetically inaccessible isomer, that would be a compelling and convincing case. If no such advantage can be shown, the pharmaceutical utility framing must be substantially revised.
To be clear, the positional isomerization chemistry in Figures 2 and 3 is creative and mechanistically well supported. It stands as a meaningful contribution to skeletal editing on its own merits. But the framing of Figure 4 as late-stage

pharmaceutical diversification, and the claims in the abstract and conclusion about accelerating drug discovery, are not supported by the data. Presenting synthetic pyridine conjugates as drug molecules and asserting practical pharmaceutical utility without demonstrating an efficiency advantage is a form of overclaiming that is increasingly prevalent in the skeletal editing literature and should not pass unchallenged in this journal.
I therefore ask the authors to take one of two paths:

Provide at least one genuine pyridine-drug example with the full sequence reported — step count, overall yield, and direct comparison to de novo synthesis; or
Revise the abstract, Figure 4 description, and conclusion to accurately characterize the substrates as synthetic pyridine conjugates of drug-derived fragments, remove the late-stage pharmaceutical applicability claims, and reframe the contribution as a powerful new method for pyridine positional isomerization demonstrated across complex molecular environments.

If neither path is taken, I do not consider this manuscript suitable for publication in Nature in its current form.
Overall Recommendation: Major Revision
The core chemistry is impressive, and the mechanistic support is now solid. But the pharmaceutical utility claims — which are central to the manuscript's framing and its case for Nature — remain unsupported. No real pyridine drug has been tested. No efficiency advantage over de novo synthesis has been demonstrated. The functional group incompatibilities are significant and underacknowledged. These gaps must be filled, or the claims revised to honestly reflect what has actually been shown.

Referee #2

(Remarks to the Author)
Although the authors have addressed most of the issues raised in the previous round of review, several concerns still remain.
1. A closely related pyridine nitrogen-transposition reaction has recently been reported by Greaney and co-workers, who achieved pyridine C–N transposition through a cycloaddition/cycloreversion (CACR) strategy using TsCN as the dienophile (Angew. Chem. Int. Ed. 2026, e5249878). Although the strategy differs from that described in the present manuscript, the overall synthetic objective is highly similar. This recent work should be cited and discussed to better clarify the novelty and distinctive features of the present approach.
2. The practical and atom-economic advantages of the present method remain insufficiently clear. In the Greaney protocol, pyridine activation and transposition can be achieved through a shorter sequence, whereas the present method requires several operations to generate the N–N salt intermediate before the final transposition step. In addition, MSH is known to be unstable and requires separate preparation, which further complicates the overall process. The authors should provide a more balanced discussion of the operational efficiency, step economy, and atom economy of their method relative to existing approaches.
3. Have the authors attempted to conduct the transformation in a one-pot manner? If feasible, such a procedure would significantly improve the practical appeal of the methodology. If unsuccessful, the authors should briefly discuss the main limitations or incompatibilities encountered.
4. From an application perspective, the current examples do not yet fully demonstrate the practical utility of the method. The drug-molecule derivatives appear to be prepared mainly through routine derivatization rather than through transformations that uniquely benefit from the present nitrogen-transposition strategy. Additional examples involving late-stage transposition of more complex pyridine-containing molecules, or a clearer explanation of how this approach provides access to otherwise difficult-to-obtain isomers, would substantially strengthen the manuscript.

Version 2:

Reviewer comments:

Referee #1

(Remarks to the Author)
Referee #1 (Remarks to the Author) — Round 3

I thank the authors for the further revisions and appreciate the effort that has clearly gone into reorganizing Figure 4 and softening the pharmaceutical-applicability language throughout the manuscript. Some progress has been made. However, having read the revised manuscript and response letter carefully, I must say that the core concern I raised in Round 1, and reiterated in Round 2, remains fundamentally unaddressed. I am somewhat frustrated to be raising the same point a third time, but I would rather do so clearly now than see this manuscript published with claims the data do not support.

The central question has still not been answered: who, in an industrial or medicinal chemistry setting, would use this method?

The authors have added yield numbers and reorganized figures, but they have not engaged with the substance of the question. A method's value for SAR exploration or late-stage diversification is determined by its overall efficiency relative to alternatives, not by whether a single step proceeds in reasonable yield. I asked specifically for a comparison against de novo synthesis. That comparison is still absent. Reporting that vismodegib, abiraterone acetate, and etoricoxib were converted in 44%, 31%, and 31% overall yield is a step forward, but without step counts for these sequences, and without

any benchmark for what an independent synthesis of the same isomer would require, the reader has no way to judge whether this method is actually advantageous. I do not think this is a difficult ask, and I am concerned that three rounds in, it has still not been addressed head-on.

Yield reporting throughout the manuscript is inconsistent and, frankly, appears to inflate the method's apparent efficiency.

This is a more specific and, I think, quite important issue. On close inspection of Figure 2, the yields reported there correspond to the isomerization step alone (step 2 of the sequence), not the overall yield from the pyridinium salt or the parent substrate. For a manuscript whose central claim is about the practicality of accessing positional isomers, reporting a single-step yield in the headline figure, without also stating the overall yield across the full sequence, gives a misleadingly favorable impression of the method's efficiency. Positional isomerization, as a concept being sold to the reader, should be judged by what it costs from starting material to final isomer, not by the yield of the most favorable individual step.

The same ambiguity persists in Figure 4. It is not clear from the figure or its legend whether the reported yields are truly overall isolated yields from the parent drug, or yields calculated from the pyridinium salt intermediate onward. The response letter states overall yields of 44%, 31%, and 31%, but if these figures do not match what is actually plotted or labeled in Figure 4 itself, that inconsistency needs to be resolved. I would ask that every yield reported anywhere in the manuscript, in the main figures, the SI, and the text, be labeled unambiguously as either "step yield" or "overall yield from [defined starting point]," with no exceptions. As it stands, a reader skimming the figures would reasonably come away with an inflated sense of how efficient this chemistry is in practice.

The omidenepag example in the introduction is never followed through on, and this is emblematic of a broader pattern.

The manuscript opens with omidenepag as its motivating example, specifically highlighting that its pyridine ring has multiple substitutable positions conferring distinct biological activities. This is precisely the scenario in which a positional isomerization platform would offer the clearest advantage over de novo synthesis: access to a family of isomers from one common intermediate. Yet omidenepag is never revisited as a substrate anywhere in the manuscript. None of its isomers are made. The compound is used to establish the significance of the work and then abandoned in favor of single-transformation examples on other scaffolds. I would ask the authors either to demonstrate at least a subset of omidenepag's positional isomers, which would substantially strengthen the paper's central claim, or to replace it in the introduction with an example that the manuscript actually delivers on. Using a compound to frame the paper's importance without ever testing it is, at best, an oversight, and at worst, it is the same overselling pattern that runs through the rest of the manuscript.

General impression.

I want to be fair to the authors: the core isomerization chemistry in Figures 2 and 3 is genuinely creative, and the mechanistic work is thorough and well executed. That was true in Round 1 and remains true now. My concern has never been with the fundamental chemistry. It is with the framing, and with what I perceive as a consistent pattern, across three rounds of revision, of addressing the presentational surface of my comments (reorganizing figures, softening specific phrases) without engaging with the substantive question underneath them. I do not think this is a matter of the authors misunderstanding what was asked. The requests have been specific and consistent across rounds. I would ask that this revision be treated with the care it requires, rather than as a checklist of phrase substitutions to satisfy a reviewer.

Requested revisions for this round:

Provide step counts alongside overall yields for the vismodegib, abiraterone acetate, and etoricoxib sequences, and include a direct, honest comparison to the de novo synthesis of at least one of these isomers.
Clearly and consistently label every yield in the manuscript (Figures 2, 3, and 4, and the SI) as either a step yield or an overall yield, with the starting point of the "overall yield" calculation explicitly defined.
Either demonstrate positional isomers of omidenepag consistent with the framing used in the introduction, or remove/replace the example with one the manuscript substantiates.

Overall Recommendation: Major Revision

The chemistry itself continues to merit publication in a strong venue. But the manuscript's central claim, that this method offers a practical advantage for accessing pyridine positional isomers relevant to drug discovery, is still not supported by the data as presented, and the yield reporting in its current form does not give readers an accurate picture of the method's efficiency. I would ask the authors to address these points directly and completely in the next round.

Referee #2

(Remarks to the Author)
The authors have adequately addressed the requested revisions and responded clearly to the reviewers' comments. The manuscript is now suitable for publication.

Version 3:

Reviewer comments:

Referee #1

(Remarks to the Author)
The authors have adequately addressed the requested revisions and clearly responded to the reviewers' comments. The manuscript is now suitable for publication.

Referees' comments:

Referee #1 (Remarks to the Author):
This manuscript by Hong and colleagues describes a skeletal editing strategy that allows the interconversion of pyridine positional isomers through formal nitrogen transposition. The work introduces a conceptually innovative transformation with potentially important implications for scaffold editing and medicinal chemistry. The chemistry is mechanistically fascinating and, if fully validated in terms of scope and practicality, could represent a significant advance. However, several aspects require further clarification and experimental support to meet the standards expected for publication in Nature.
Strengths:
1. The study introduces a creative strategy for formal nitrogen-atom transposition within pyridines without altering peripheral substituents. Direct positional editing of heteroaromatic cores remains underdeveloped, and this approach addresses a long-standing synthetic limitation.
2. The transformation proceeds through a well-designed cascade involving aziridination, ring expansion, electrocyclization, and dinitrogen extrusion. The mechanistic proposal is logical and supported by isotopic labeling studies.
3. The reaction conditions are transition metal-free and do not rely on harsh oxidants. The transformation is conducted under relatively simple conditions, thereby enhancing its potential practical value.
4. A range of mono- and disubstituted pyridines is examined, including C2-, C3-, C4-, C5-, and several multi-substituted systems.
5.The method aligns well with current interest in skeletal editing and scaffold hopping. If generalizable, this strategy could provide a valuable alternative to de novo synthesis in medicinal chemistry.
Response and Action: We are deeply grateful for the reviewer's careful evaluation and supportive feedback.

Weaknesses and Concerns:
1. The transformation requires prior preparation of activated pyridinium salts through multiple steps (amination, tosylation, and methylation). The overall step economy should be more critically discussed. This diminishes the claim that the strategy accelerates SAR exploration, as substantial synthetic effort precedes the isomerization step.
Response and Action: We thank the reviewer for raising this point. We acknowledge that the preparation of N-amidopyridinium salts requires a three-step sequence prior to the isomerization step, and we agree that this aspect of the overall step economy warrants more transparent discussion. We recognize that the synthetic effort associated with these intermediates should be factored into a balanced assessment of the strategy's practical utility for SAR exploration.
The N-amidopyridinium salts can be prepared from the corresponding pyridines through a three-step sequence: (i) N-amination using MSH, (ii) tosylation of the resulting N-aminopyridine, and (iii) alkylation to form the N-amidopyridinium salt (see the Supplementary Information). Each step proceeds in good efficiency, and the overall sequence delivers the desired salts in moderate to good overall yield from readily available pyridines, with only minor variations observed in select cases. Notably, the preparation is readily scalable, and gram-scale synthesis of these salts has been demonstrated without erosion of yield. In most instances, isolation is accomplished by simple recrystallization without chromatographic purification, further facilitating large-scale preparation and long-term storage.

Notably, the three-step activation sequence reflects a fundamental mechanistic requirement rather than a procedural necessity. A central challenge in this design is the conversion of the original aromatic ring nitrogen into a suitable leaving group for extrusion. The N-amidopyridinium salt addresses demands: it positions the ring nitrogen for release as N_2 gas—the thermodynamic sink that drives the overall transformation and renders deletion from an otherwise stable aromatic ring feasible.

At the reviewer's suggestion, we have revised the manuscript to discuss the overall step and to present a balanced view of the strategy's practical scope.
"The requisite N-amidopyridinium salts are readily accessed from the corresponding pyridines in three steps and, in most cases, isolated by simple recrystallization without chromatographic purification (see Supplementary Information)."

The inclusion of relatively simple drug conjugates (e.g., paracetamol derivatives in Figure 4) appears less compelling. The manuscript does not provide examples involving structurally complex drug molecules, despite emphasizing the importance of pyridines as privileged scaffolds. This raises concerns about compatibility with drug-like molecules such as Omidenepag (bearing a free carboxylic acid), as highlighted in Figure 1 to underscore the significance of this work. I encourage the authors to demonstrate this chemistry on pyridine-containing drugs bearing reactive functionalities (e.g., amines, alcohols, carboxylic acids). If such substrates are incompatible, the introduction and associated claims should be moderated accordingly.

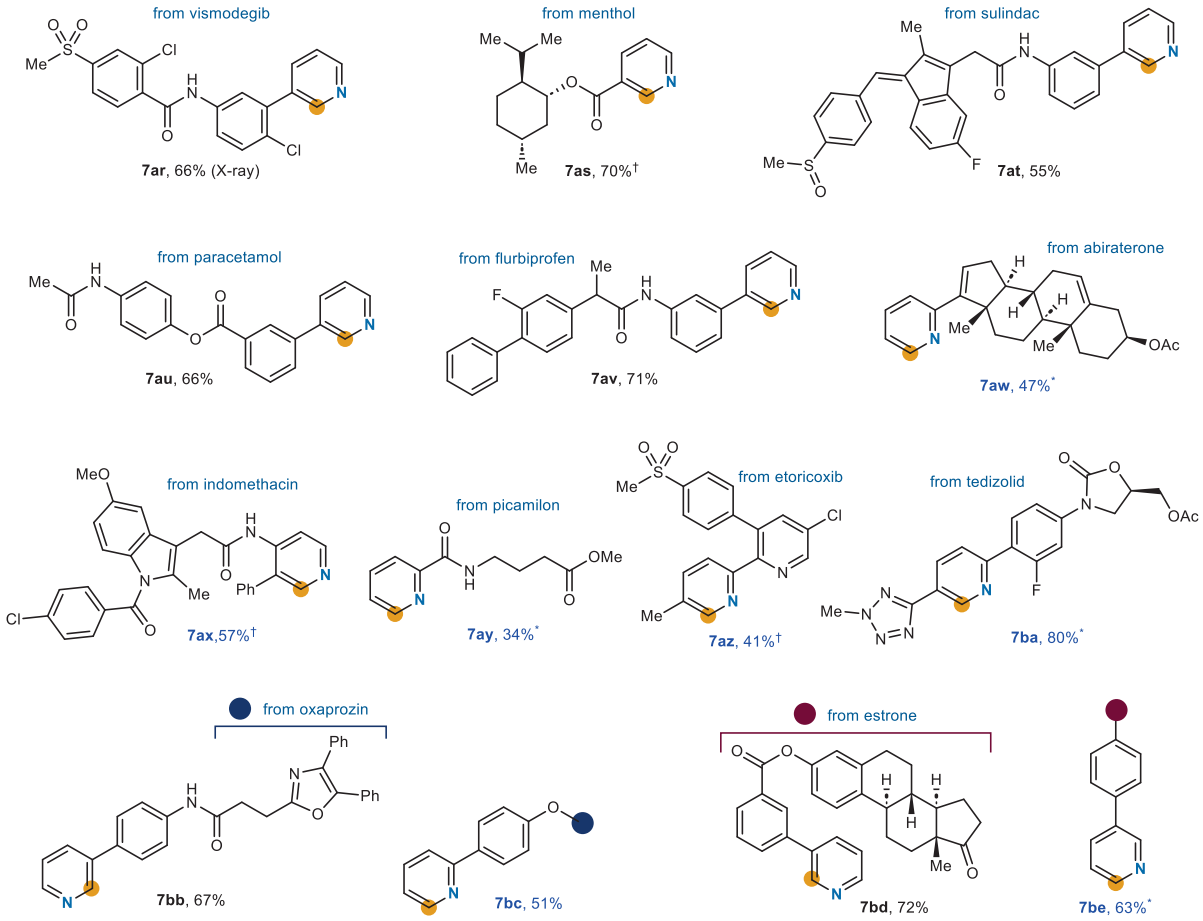
Response and Action: We thank the reviewer for this valuable suggestion. To further evaluate the applicability of the method to structurally complex, drug-like molecules, we substantially expanded the late-stage diversification studies and examined 14 pyridine-containing bioactive molecules and pharmaceuticals that were available to us in Korea. These substrates encompass a broad range of molecular architectures and functional-group combinations.

As shown in the revised Fig 4, the transformation proved compatible with derivatives of vismodegib, sulindac, flurbiprofen, indomethacin, abiraterone, tedizolid, etoricoxib, picamilon, oxaprozin, and estrone—spanning anti-cancer agents, NSAIDs, antibiotics, and steroid-based scaffolds. The reaction tolerated a wide array of functional groups, including sulfones, sulfoxides, esters, amides, halides, alkenes, indoles, tetrazoles, oxazoles, and steroid frameworks, underscoring the robustness of the method under structurally demanding conditions.

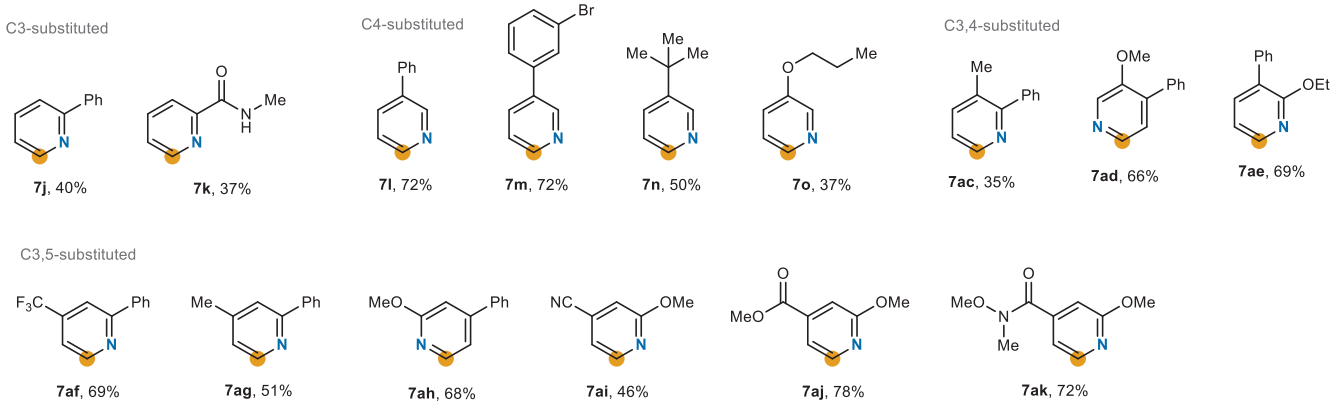
Substrates containing free nucleophilic functionalities—such as amines, alcohols, and carboxylic acids—were also investigated. These groups were found to be incompatible with the pyridinium salt preparation step, as they undergo competitive tosylation. Nevertheless, such functionalities can be readily accommodated by appropriate protection (e.g., acylation or esterification) prior to salt formation.

As suggested, the following sentence has been added to the revised manuscript to reflect this limitation:
"Substrates bearing free nucleophilic groups, such as amines, alcohols, and carboxylic acids, are not compatible with the pyridinium salt preparation step; such functionalities can, however, be accommodated by prior protection."

These additions provide a more transparent and comprehensive assessment of both the scope and current limitations of the method in the context of late-stage diversification of drug-like molecules.



In addition, we have further investigated the reactivity of more 3-, 4-, and disubstituted pyridine systems bearing a variety of substituents.



2. Several regioselectivity trends remain insufficiently explained:
•Why do C2,3-disubstituted systems yield exclusively C3,4-products?
•Why do certain substitution patterns produce only one positional isomer?
•Why do C3-substituted substrates sometimes react at the substituted site?
•Why do electronically related substituents show substantial yield differences (e.g., ester 7g, 74% vs ketone 7h, 33%; methyl 7b, 86% vs extended alkyl 7i, 42%)?

A clearer electronic and mechanistic rationale is required. The substrate scope does not sufficiently probe extreme electronic bias. Strongly electron-donating and strongly electron-withdrawing substituents are underrepresented, particularly in electronically polarized systems that would rigorously test regioselectivity. I suggest providing mechanistic and/or computational support to rationalize the observed isomer outcomes.

Response and Action: We thank the reviewer for this insightful comment regarding the origin of product selectivity. To address this point, we performed DFT calculations to interpret the observed regioselectivity within the Curtin–Hammett framework. As shown in the computed free energy profile (Fig. 5), the initial nucleophilic addition step leading to **Int2-*o*** and **Int2-*p*** proceeds through nearly isoenergetic transition states (**TS1-*o***: 22.2 kcal mol^{-1}; **TS1-*p***: 21.2 kcal mol^{-1}), and the resulting intermediates are likewise very similar in energy. These negligible differences indicate that **Int2-*o*** and **Int2-*p*** are in rapid equilibrium under the reaction conditions, consistent with Curtin–Hammett behavior. Under such circumstances, product distribution is governed not by the relative stabilities of the intermediates, but by the relative free energies of the subsequent cyclization transition states. Consistent with this interpretation, the calculated cyclization barrier for **TS2-*o*** ($\Delta G^{\ddagger}$ = 24.5 kcal mol^{-1} from **Int2-*o***) is significantly lower than that for **TS2-*p*** ($\Delta G^{\ddagger}$ = 27.8 kcal mol^{-1} from **Int2-*p***), giving a $\Delta\Delta G^{\ddagger}$ of approximately 3.2 kcal mol^{-1}. This energy difference is sufficient to account for the predominant formation of **Int3-*o*** under kinetic control. The lower barrier associated with **TS2-*o*** likely reflects a more favorable geometric alignment for intramolecular cyclization, together with more effective stabilization of the developing charge-separated character in the transition state, as suggested by the optimized geometries.

The calculations also rationalize the solvent-dependent selectivity observed experimentally. In the more polar solvent DMF, the charge-separated character of the transition state is more effectively stabilized relative to toluene, reducing the $\Delta\Delta G^{\ddagger}$ between the competing cyclization pathways — consistent with the diminished selectivity observed experimentally under DMF conditions.

Taken together, these results support a Curtin–Hammett-controlled mechanism in which rapidly equilibrating intermediates undergo irreversible, selectivity-determining cyclization. The preferential formation of **Int3-*o*** therefore arises from the intrinsically lower activation barrier of **TS2-*o***, rather than from the thermodynamic stability of the preceding intermediates.

These findings have been incorporated into the revised manuscript (Fig. 5 and Supplementary Fig. 6).

In addition, we have added a paragraph to further explain the origin of the observed selectivity.

“To probe the origin of regioselectivity, we performed DFT calculations within the Curtin–Hammett framework. The initial nucleophilic addition step leading to **Int2-*o*** and **Int2-*p*** proceeds through nearly isoenergetic transition states (**TS1-*o***: 22.2 kcal mol^{-1}; **TS1-*p***: 21.2 kcal mol^{-1}), indicating that these intermediates are in rapid equilibrium under the reaction conditions. Product distribution is therefore governed by the relative free energies of the subsequent cyclization transition states. The calculated barrier for **TS2-*o*** ($\Delta G^{\ddagger}$ = 24.5 kcal mol^{-1}) is substantially lower than that for **TS2-*p*** ($\Delta G^{\ddagger}$ = 27.8 kcal mol^{-1}), with a $\Delta\Delta G^{\ddagger}$ of 3.2 kcal mol^{-1}consistent with the observed *ortho* selectivity. The diminished selectivity in DMF is attributed to enhanced stabilization of the charge-separated transition state in the more polar medium, which reduces the $\Delta\Delta G^{\ddagger}$ between competing pathways (Fig. 5b,c and Supplementary Fig. 6).”

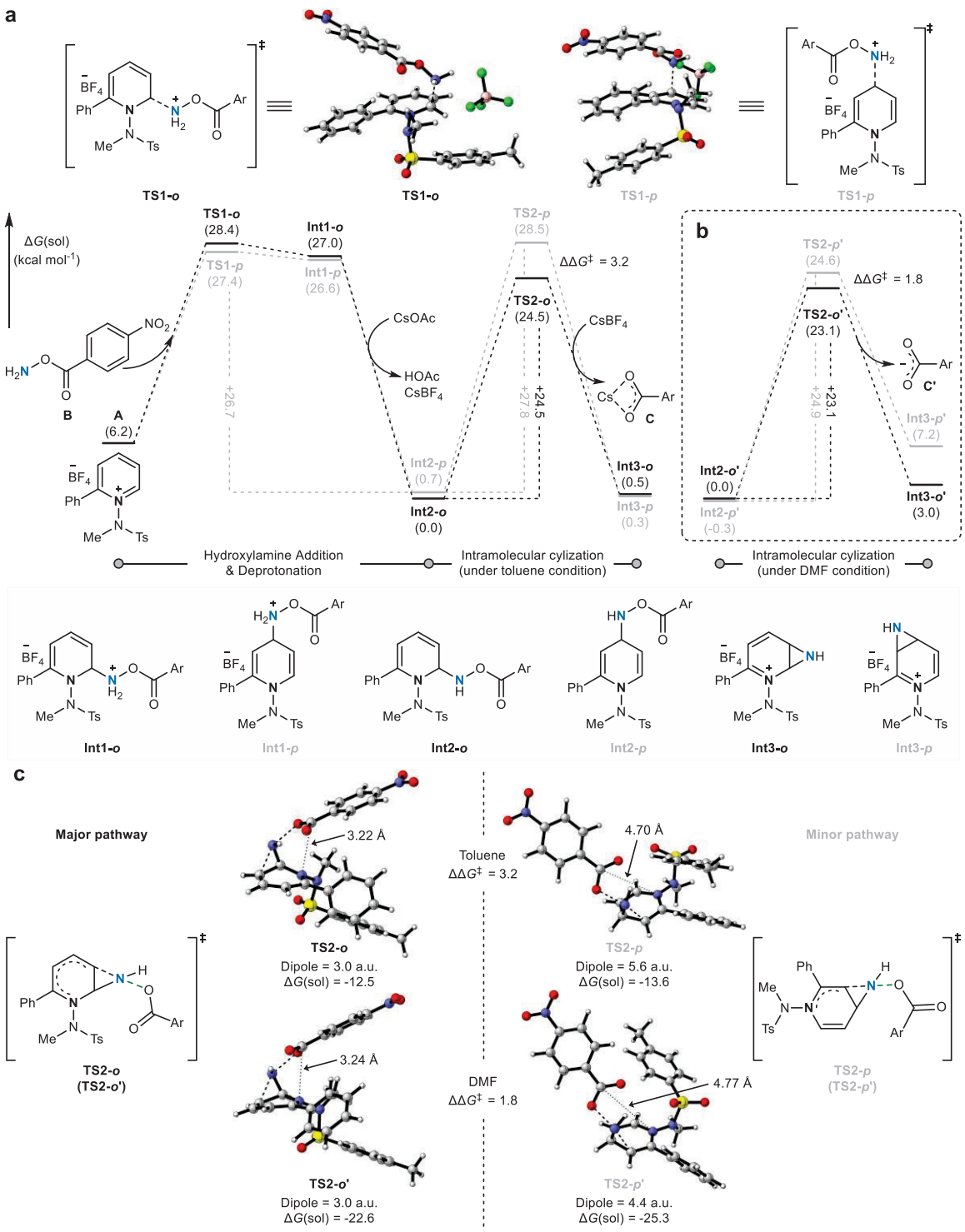
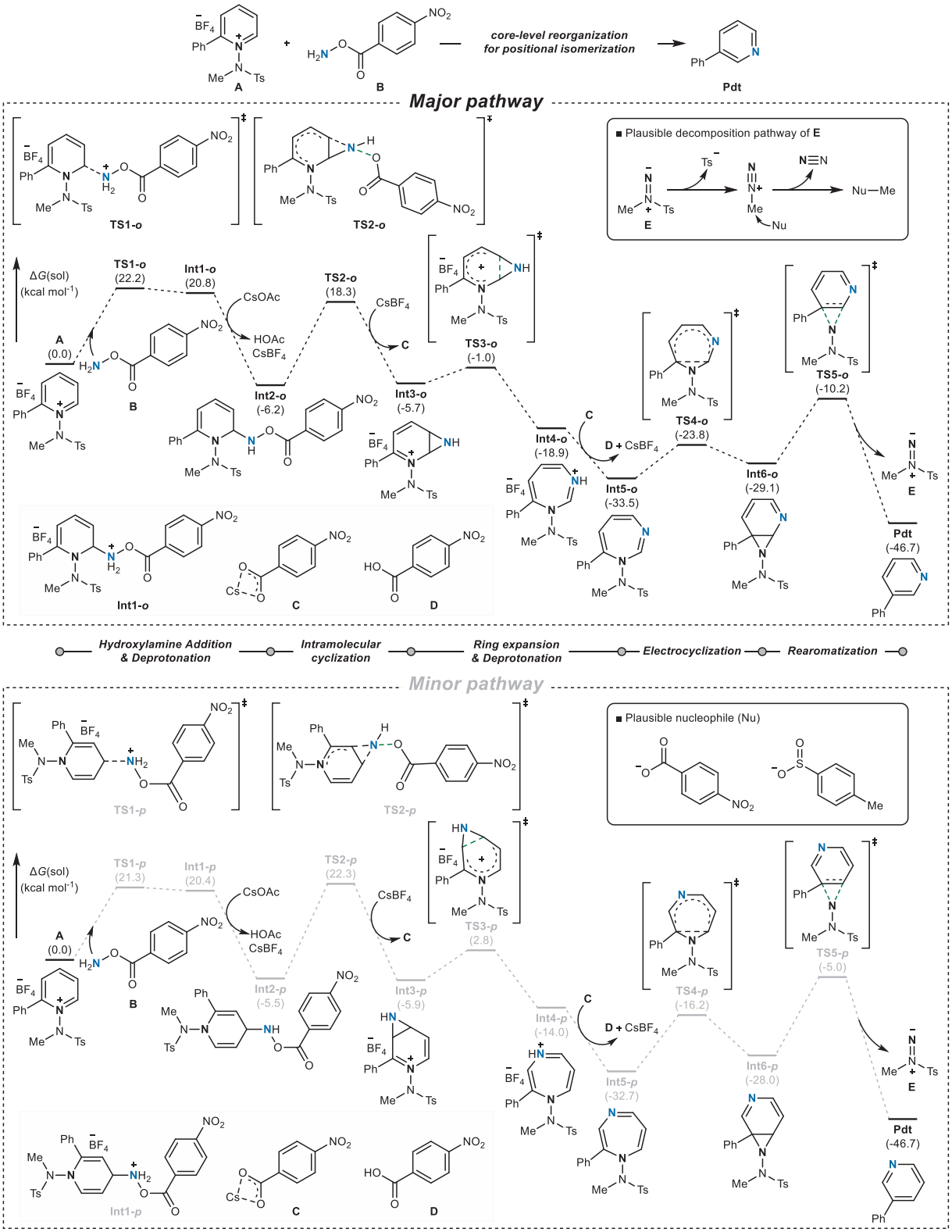


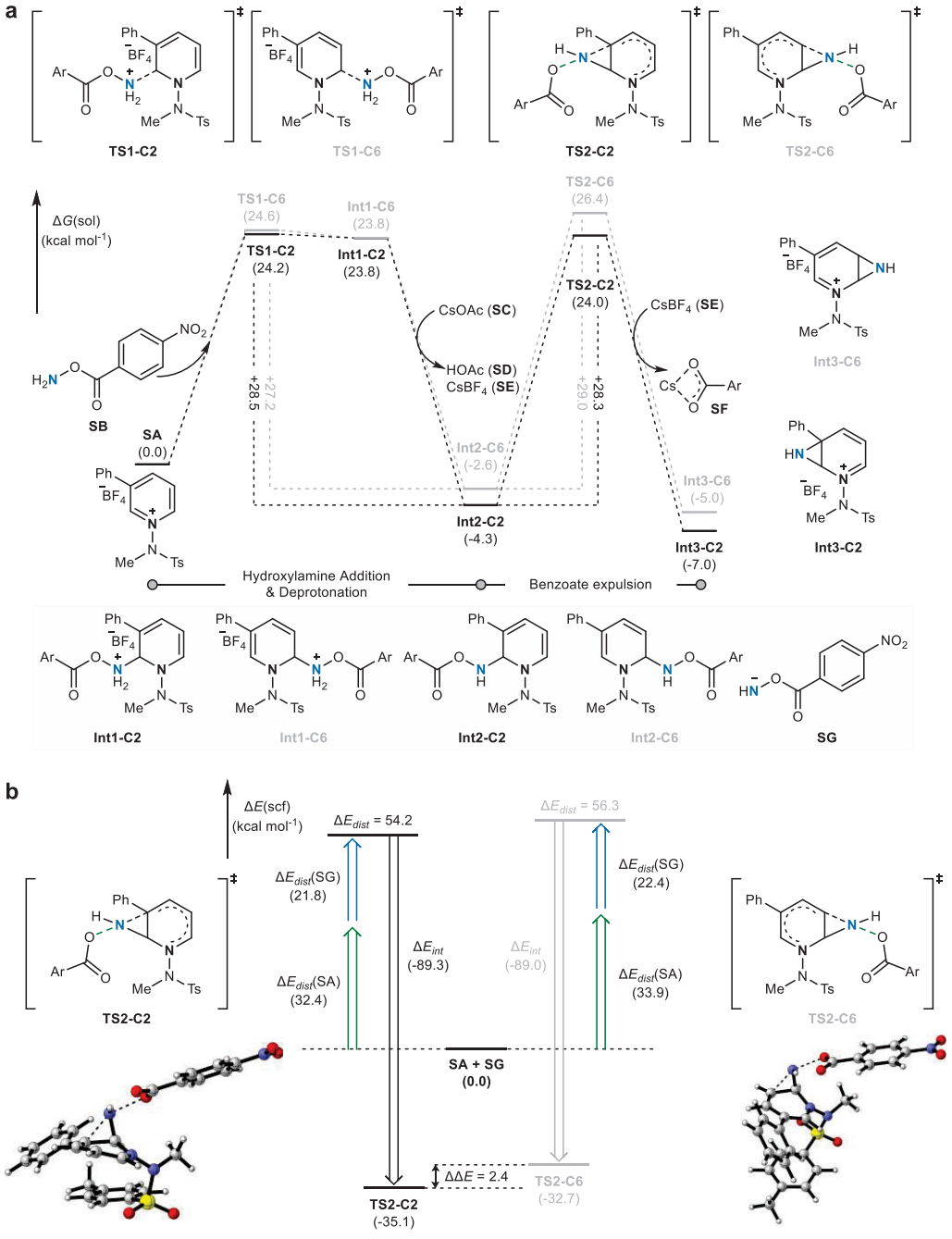
Fig. 5| DFT calculations rationalizing the regioselectivity of the intramolecular cyclization. **a,** Computed free energy profiles for the competing *ortho*- and *para*-selective pathways under toluene conditions. **b**, Free energy profile for the cyclization step under DMF conditions, illustrating the reduced $\Delta\Delta G^{\ddagger}$ relative to toluene. **c**, Optimized geometries of the selectivity-determining transition states **TS2-*o*** and **TS2-*p***.



Supplementary Figure 6. Computational studies of the C2-substituted substrate and proposed reaction mechanism.

Method: ωB97X-D3BJ/def2-TZVPP/CPCM(Toluene)//ωB97X-D3BJ/def2-SVP/CPCM(Toluene).

The reviewer also raised the question of why C3-substituted substrates sometimes react at the substituted site. To elucidate the origin of the site selectivity between the C2 and C6 positions in the C3-phenyl substituted system, computational studies were conducted. The results revealed that the reaction at the C2 (substituted) position has a lower-energy transition state (**TS2-C2**) compared with that at the C6 position (**TS2-C6**). To further investigate the origin of this energy difference, distortion–interaction analysis was conducted. Interestingly, the two transition states exhibited nearly identical interaction energies, whereas a 2.1 kcal mol^{-1} difference was observed in the structural distortion energies. These results indicate that the preferential reaction at the C2 position arises from the lower distortion energy required to access the corresponding transition state. For the OMe substituent, we attribute this behavior to a hydrogen-bonding interaction between the OMe group and the incoming H_2N–OR nucleophile, which likely serves to direct nucleophilic attack toward the OMe-substituted position.



Supplementary Figure 5. Computational studies of the C3-substituted substrate. a, DFT investigation of competitive site selectivity between the C2 and C6 pathways, including computed activation barriers. **b,** Distortion–interaction analysis of **TS2-C2** and **TS2-C6**. Method: ωB97X-D3BJ/def2-TZVPP/CPCM(THP)//ωB97X-D3BJ/def2-SVP/CPCM(THP).

-Why do electronically related substituents show substantial yield differences (e.g., ester 7g, 74% vs ketone 7h, 33%; methyl 7b, 86% vs extended alkyl 7i, 42%)?

Response and Action: We thank the reviewer for pointing this out. For the ester/ketone pair (**7g**, 74% vs. **7h**, 33%), we attribute the diminished yield of the ketone-containing substrate to competing oxime formation between the ketone moiety and the H_2N–OR nucleophile, a well-precedented side reaction. Although the corresponding oxime byproduct was not directly observed in our system, competition with this pathway likely accounts for the reduced reactivity of **7h** relative to the ester-containing substrate **7g**, which lacks this competing reaction pathway and is therefore well tolerated under the reaction conditions.

For the second comparison, we wish to clarify a potential source of confusion: **7b** does not bear a methyl substituent directly on the pyridine ring, but rather a phenyl group at the ring, with no benzylic proton adjacent to the pyridine core. In contrast, the extended alkyl-substituted substrate **7i** possesses a benzylic position directly connected to the pyridine ring, rendering it susceptible to facile deprotonation under the reaction conditions, consistent with precedent in the literature (*Angew. Chem. Int. Ed.* **2022**, *61*, e202204217). The absence of such a benzylic proton in **7b** therefore disfavors this competing deprotonation pathway, accounting for the substantially higher yield observed relative to **7i**.

7g, 74% **7h**, 33% **7b**, 86% **7i**, 42%

The following statement has been adjusted to clarify the scope limitations:

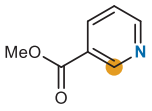
"Ketones (**7h**) and alkyl chains (**7i**) afforded somewhat lower yields, yet the breadth of compatible substrates establishes a general platform for *ortho*-to-*meta* transposition."

In addition, as suggested, we have examined additional substrates bearing electron-donating, electron-withdrawing, or both types of substituents simultaneously to more rigorously probe the electronic requirements of the transformation. Substrates bearing electron-donating groups (OMe, anilide) as well as electron-withdrawing groups (CF_3, CN, ester, ketone, amide) all participated successfully, demonstrating the broad electronic tolerance of the methodology. Notably, systems bearing both electron-donating and electron-withdrawing substituents simultaneously also underwent the transformation with high selectivity. However, when the pyridine ring became excessively electron-deficient, reactivity was compromised: 3-cyano- and 3,5-diester-substituted derivatives failed to undergo the desired transformation, and preparation of the NO_2-substituted starting material proved difficult, precluding its evaluation. The combined results are summarized below; unsuccessful substrates have been compiled in the Supplementary Information. The expanded substrate scope has been incorporated into the revised manuscript (Fig. 3).

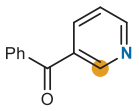
In addition, the following statement has been added to clarify the scope limitations:

"Despite the broad substrate scope, excessively electron-deficient pyridines remained challenging: 3-cyano- and 3,5-diester-substituted derivatives failed to undergo isomerization."

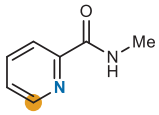
Electron-withdrawing (EWG)



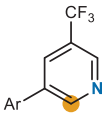
7g, 74%



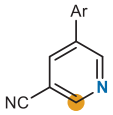
7h, 33%



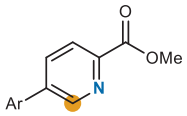
7k, 37%



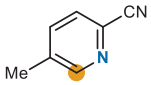
7s, 74%



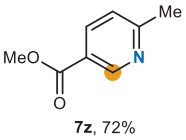
7u, 80%



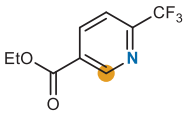
7v, 61%



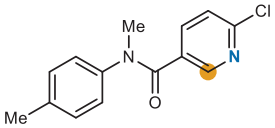
7y, 50%



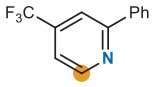
7z, 72%



7aa, 67%

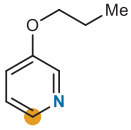


7ab, 50%

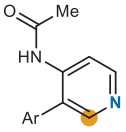


7af, 69%

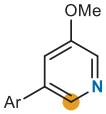
Electron-donating (EDG)



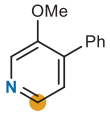
7o, 37%



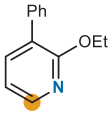
7q, 61%



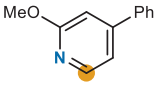
7t, 67%



7ad, 66%

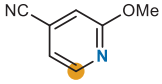


7ae, 69%

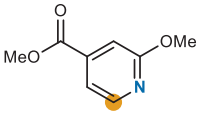


7ah,58%

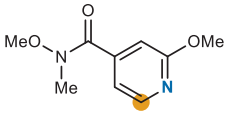
Electronically polarized systems (EWD+EDG)



7ai, 46%

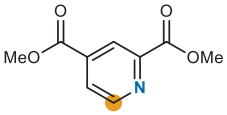


7aj, 78%



7ak, 72%

Unsuccessful



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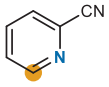

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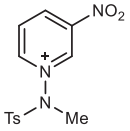


Difficulties in salt preparation

5. Can this methodology be extended to quinolines? This limitation should be discussed more clearly and contextualized experimentally.

Response and Action: We thank the reviewer for this insightful question. In pyridinium salts, the reaction proceeds through formation of a 1,3-diazepine intermediate (**II**), which undergoes 6π-electrocyclization to generate a bicyclic aziridinium intermediate (**III**). This strained intermediate subsequently undergoes retro-cycloaddition to furnish the isomerized pyridine product.

To examine whether this strategy could be extended to quinoline systems, we subjected quinolinium substrates to the standard reaction conditions; however, no skeletal rearrangement products were observed. We propose that this limitation arises from two energetic penalties inherent to the quinoline framework. First, progression from intermediate **II** to **III** would incur a substantial dearomatization penalty by disrupting the aromatic stabilization of the fused benzene ring, a cost absent in the monocyclic pyridine system. Second, the fused benzene ring imposes conformational rigidity that restricts the geometric flexibility required for productive 6π-electrocyclization. Together, these factors render the electrocyclization step energetically prohibitive for quinoline substrates, thereby precluding the skeletal reorganization pathway accessible in pyridinium systems.

II III

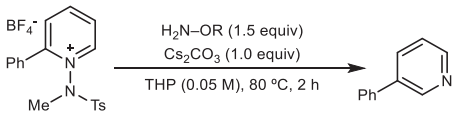
To clarify this limitation, we have added the following statement to the revised manuscript:

"Quinoline substrates were also unreactive under the standard conditions, likely because the key 6π-electrocyclization step is energetically disfavored owing to disruption of the fused benzene ring aromaticity."

•No inert-atmosphere control experiment is presented.
•No comprehensive base screening table is provided.
•Internal standards for NMR yield determination are not specified.
•Some NMR spectra lack full assignment or display impurities.

Response and Action: We thank the reviewer for this comment. Atmosphere control experiments and base screening data have been added to the Supplementary Information. The following statement has also been incorporated into the experimental procedures: "The crude residue was analyzed by ^{1}H NMR spectroscopy using mesitylene as an internal standard to determine the yield." Additionally, newly acquired NMR spectra have been included in the Supplementary Information.

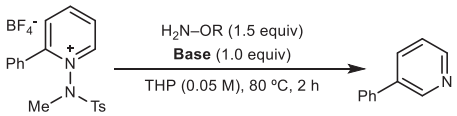
Atmosphere screening



2a 7a

Entry	Atmosphere	Yield (%)
1	Ar	85
2	N_2	86
3	O_2	87

Base screening



2a 7a

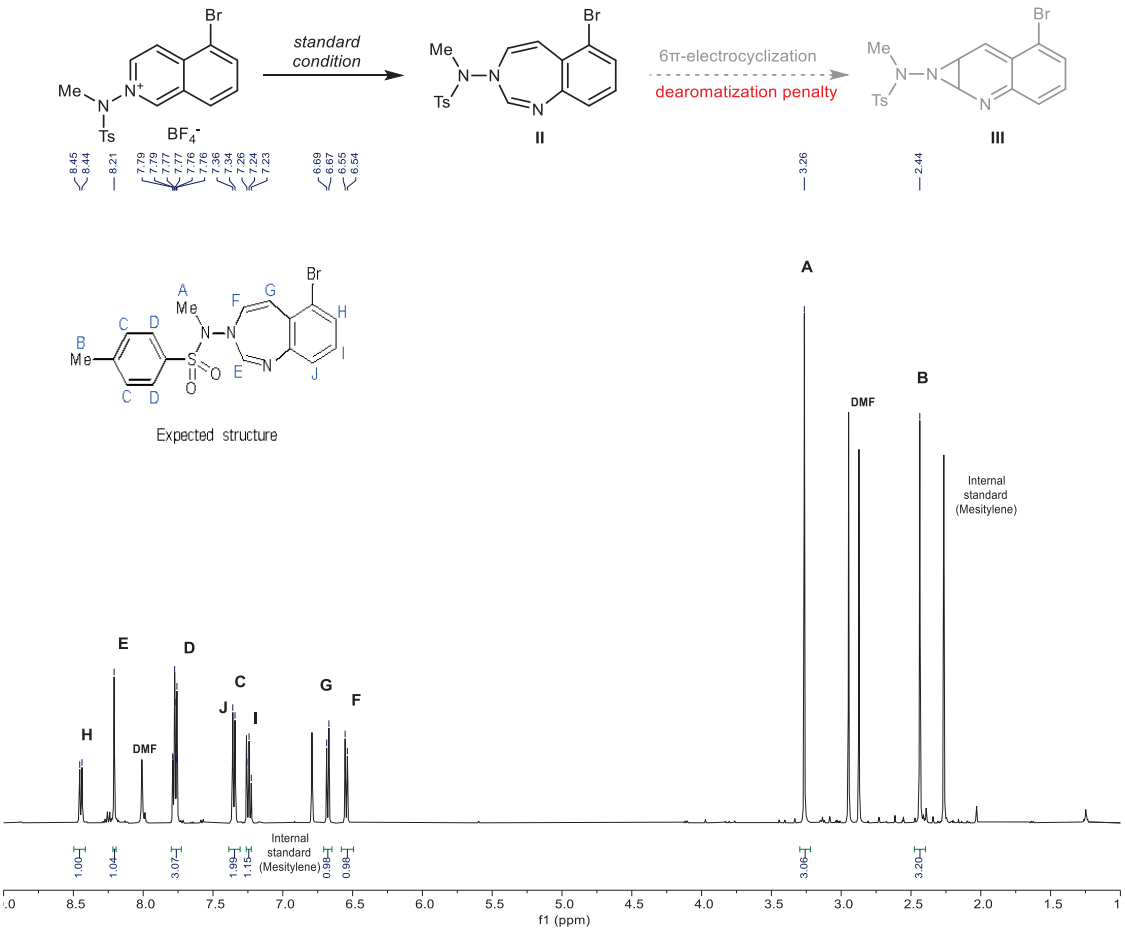
Entry	Base	Yield (%)
1	K_2CO_3	18
2	Na_2CO_3	10
3	$CsHCO_3$	20
4	CsOAc	86
5	K_3PO_4	82

6	KO^{t}Bu	81
7	2,4,6-Collidine	36
8	Triethylamine (TEA)	55
9	1,8-Diazabicyclo[5.4.0]-7-undecene (DBU)	52

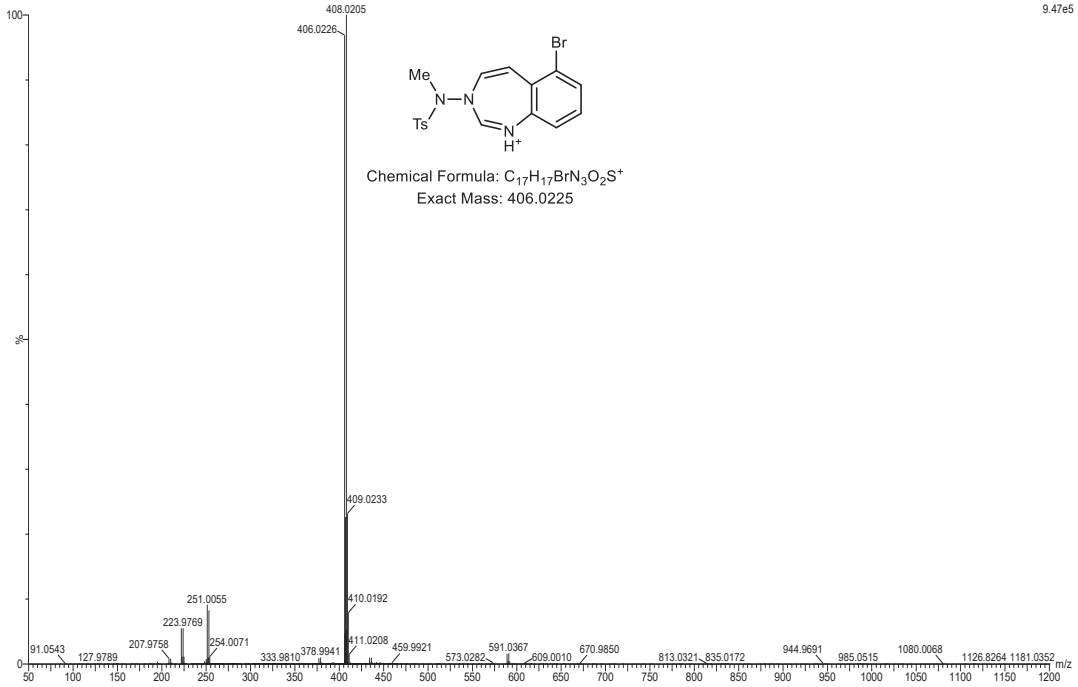
•Please clarify whether diazepine intermediates were observed or trapped. Can we stop this transformation at the diazepines, which are valuable scaffolds in medicinal chemistry?

Response and Action: We thank the reviewer for this important recommendation. In the current system, the reaction proceeds too rapidly to permit direct trapping of intermediates **I**, **II**, or **III**. We reasoned that the transformation might be arrested at the diazepine stage by employing isoquinoline-derived substrates, in which the subsequent 6π-electrocyclization step is disfavored owing to the additional dearomatization penalty associated with the fused benzene ring.

To intercept the putative diazepine intermediate **II**, we subjected an isoquinoline-derived substrate to the reaction conditions. Crude ^{1}H NMR analysis revealed signals consistent with formation of the proposed diazepine intermediate; however, this species proved unstable, and all attempts at isolation or crystallization for X-ray analysis resulted in decomposition. Nevertheless, the corresponding molecular ion peak was successfully detected by HRMS, providing supporting evidence for the existence of this intermediate. The crude ^{1}H NMR spectrum and HRMS data for the putative diazepine intermediate are below.



^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.45 (d, *J* = 8.1 Hz, 1H), 8.21 (s, 1H), 7.77 (m, 3H), 7.35 (d, *J* = 8.0 Hz, 2H), 7.24 (t, *J* = 7.9 Hz, 1H), 6.68 (d, *J* = 8.0 Hz, 1H), 6.55 (d, *J* = 8.0 Hz, 1H), 3.26 (s, 3H), 2.44 (s, 3H).



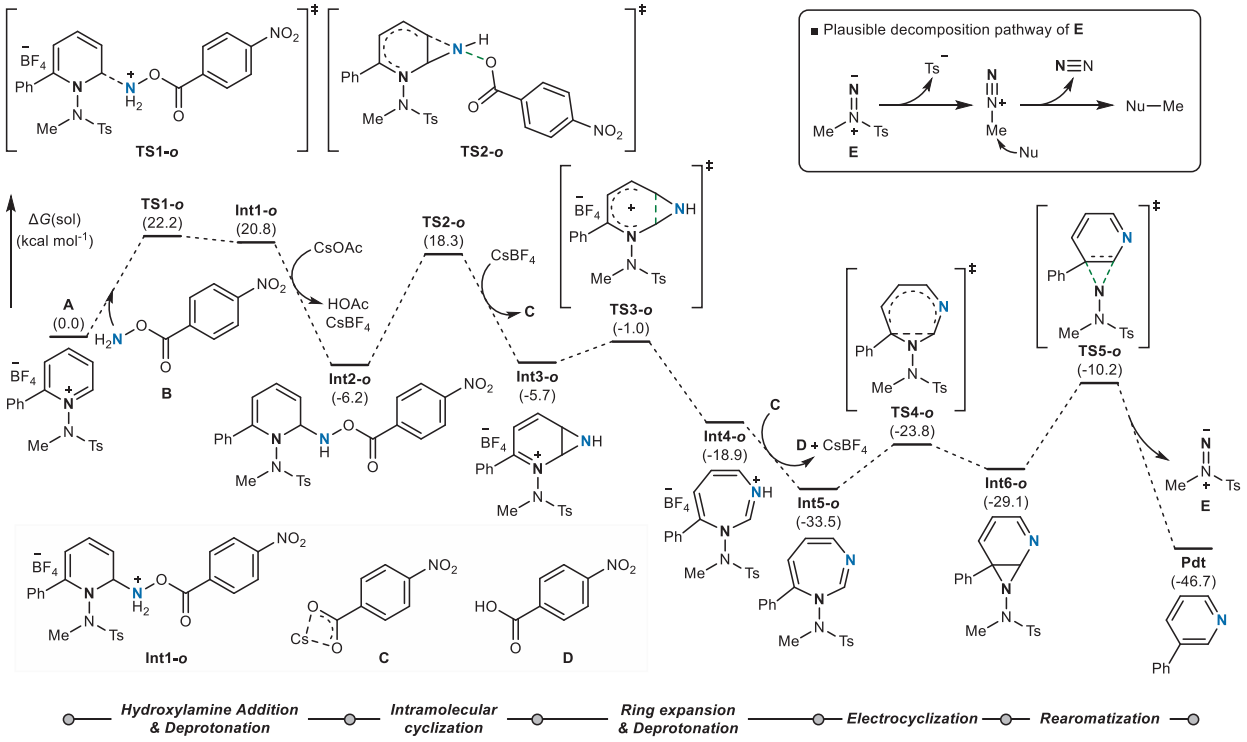
•The mechanistic language should be refined. Specifically, is the final demethylation step proceeding through an SN2 pathway?

Response and Action: We thank the reviewer for this helpful suggestion. We have made minor revisions to the mechanistic description to more accurately reflect the reaction pathway. Based on DFT calculations, the final demethylation step, initially described as a direct S_N2 process, is revised to proceed through a retro-cycloaddition generating an isodiazene intermediate **E**, which undergoes fragmentation to a tosyl anion and a methanediazonium species; the latter then undergoes nucleophile-assisted demethylation via an S_N2 pathway with concomitant extrusion of N_2. The revised mechanism has been updated in the manuscript accordingly.

As suggested, we have revised this sentence:

“These results demonstrate that only *N*-alkyl sulfonamide groups capable of nucleophilic displacement and fragmentation are effective, consistent with the proposed mechanism.”

.



6. The term "positional isomerization" may imply intramolecular nitrogen migration, whereas the process involves external nitrogen insertion followed by extrusion. A more precise description, such as "formal nitrogen transposition," would improve conceptual clarity.

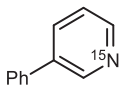
Response and Action: We appreciate this helpful suggestion. To clarify the conceptual nature of the transformation and avoid the possible implication of intramolecular nitrogen migration, we have revised the title of the manuscript to more explicitly reflect the nitrogen transposition process.

The title now reads:
"Positional isomerization of pyridines via nitrogen transposition"

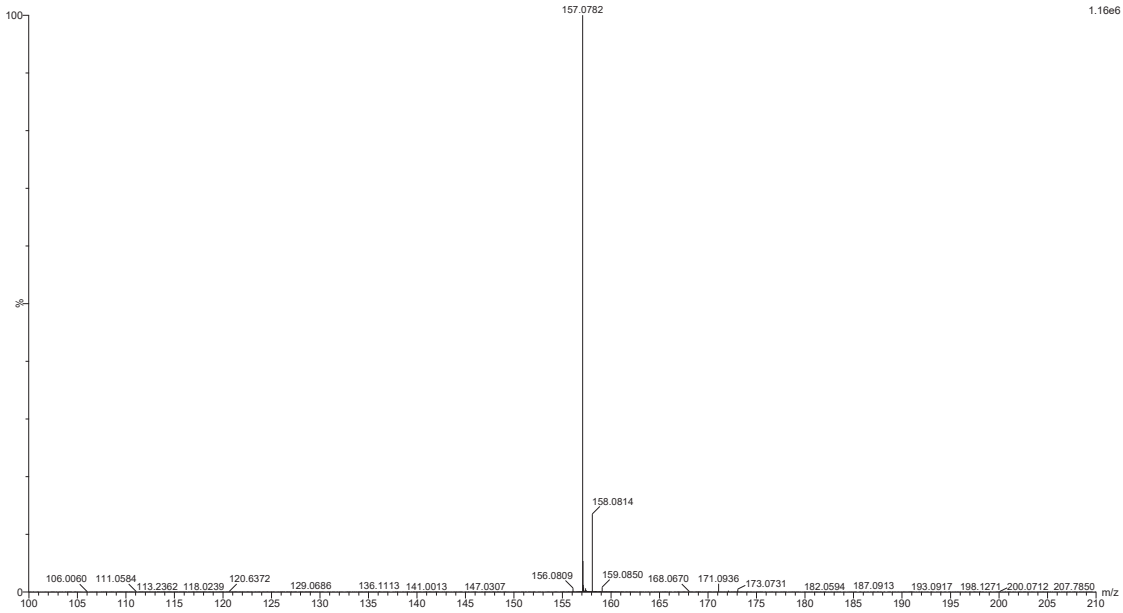
7. Several typos are there. Figure 2: 15N-labeled 7a should be 7a' or a different number. Also, I could not find how the authors measured the percentage of 15N incorporation (quantification) into the molecule. Line 45, N should be italic. Please correct the typo in Figure 3 ("C2,5-substituted").

Response and Action: We thank the reviewer for pointing out this error. The notation has been corrected, and ^{15}N-labeled **7a** has been revised to **^{15}N-7a'** in the manuscript. And the ^{15}N incorporation data have been added to the Supplementary Information. The errors have been corrected in the revised manuscript.

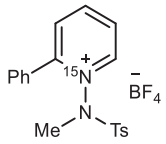
3-phenylpyridine-1-^{15}N (^{15}N-7a')



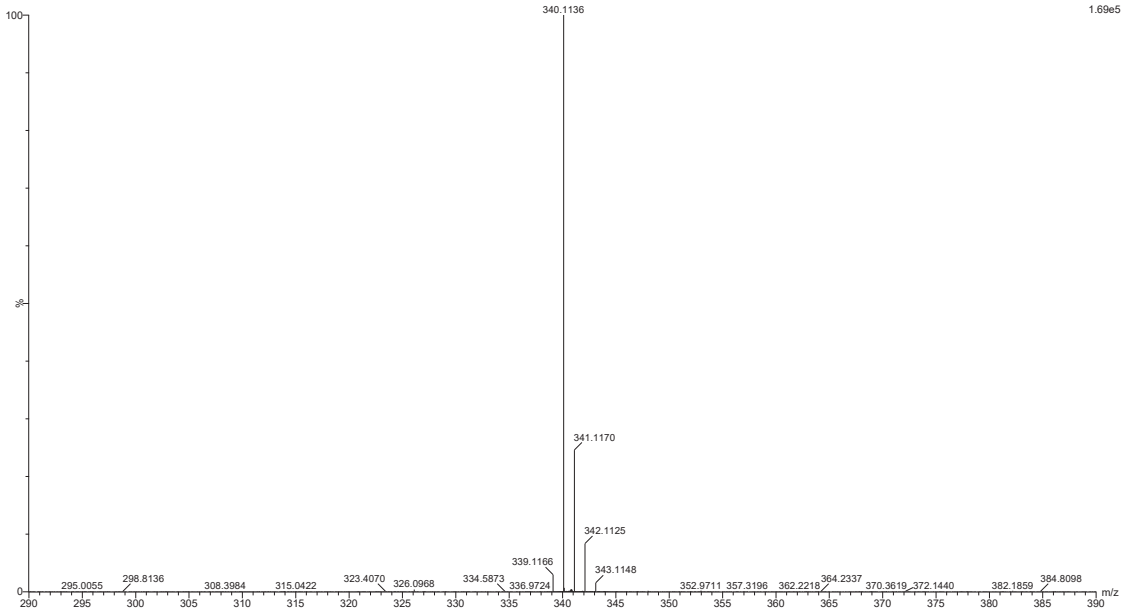
$$\textbf{>99\% }^{15}\textbf{N incorporation} = \frac{1160000-\left((0.0107\times11)\times7523\right)+156300}{7523+1160000+156300}\times 100\%$$



2-phenyl-1-[(*N*-methyl-4-methylbenzenesulfonamido)]pyridin-1-ium-1-^{15}N tetrafluoroborate (^{15}N-2a″)



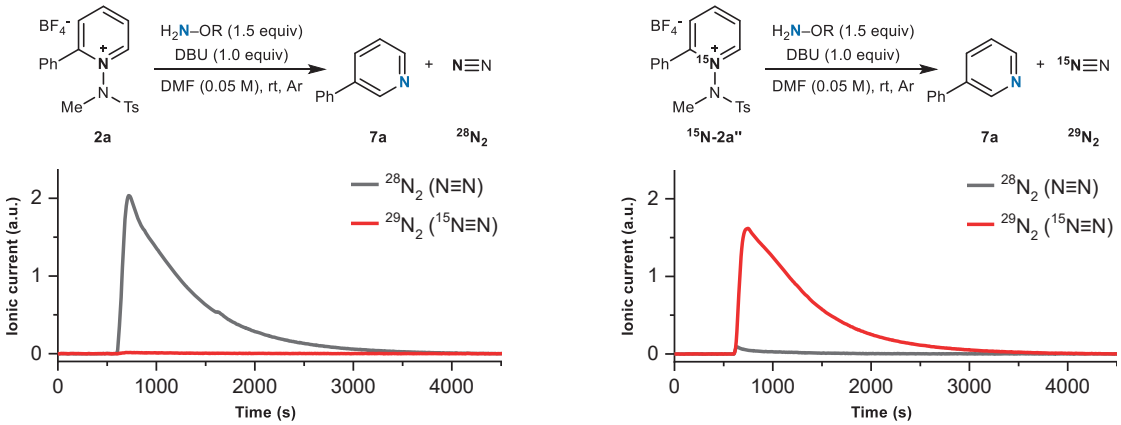
97% ^{15}N incorporation = $\frac{169100-\left((0.0107\times19)\times4946\right)+41500}{4946+169100+41500}$ × 100%



In addition, to directly probe N_2 formation, the reaction was conducted using **2a** and a ^{15}N-labeled substrate (**^{15}N-2a″**) in an Ar-purged reaction chamber, with nitrogen gas evolution monitored by GC–MS (Supplementary Information, Section VI). In the experiment using **2a**, formation of $^{14}N^{14}N$ (*m/z* = 28) was clearly observed. To further establish the origin of the evolved nitrogen gas, a parallel experiment employing

^{15}N-2a″ was performed; under these conditions, $^{14}N^{14}N$ (*m/z* = 28) was negligible, whereas $^{15}N^{14}N$ (*m/z* = 29) was unambiguously detected. These results provide direct experimental evidence that the observed nitrogen gas originates from the pyridine nitrogen itself.

We have added the following statement to the revised manuscript:
"Formation of $^{14}N^{14}N$ (*m/z* = 28) was observed with **2a**, whereas $^{15}N^{14}N$ (*m/z* = 29) was detected with **^{15}N-2a″**, providing direct evidence that the evolved nitrogen gas originates from the pyridine nitrogen itself (Fig. 2d)."



In summary, this manuscript introduces a creative and potentially impactful skeletal editing transformation. The conceptual advance is strong. However, the substrate scope, multi-step process, and lack of justification for regioselectivity, including computational calculations and supporting data, do not yet fully support the claims of broad applicability, practical utility, and mechanistic understanding at the level expected for publication in Nature.

Referee #2 (Remarks to the Author):

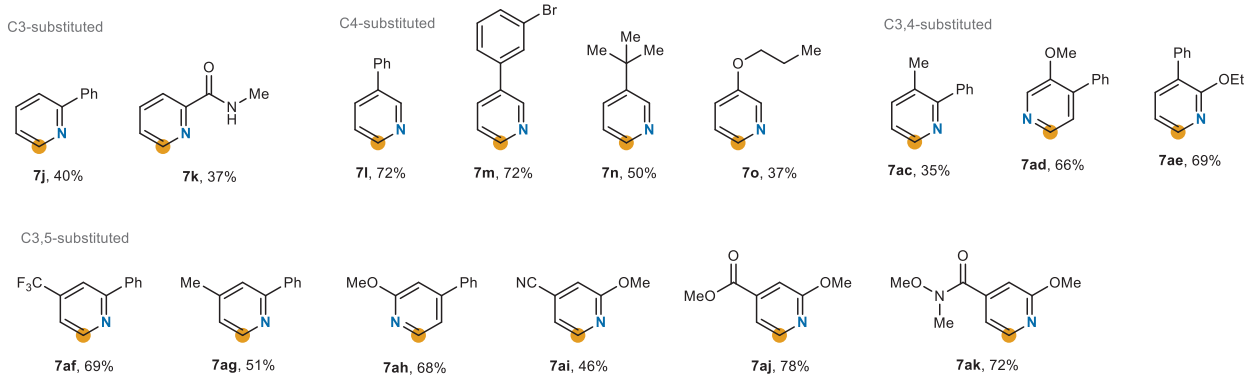
Hong and co-workers report a strategy for pyridine positional isomerization through sequential nitrogen insertion and deletion, enabling predictable N-atom transposition without altering substituent identity. The concept is intellectually appealing and provides access to positional isomers that would otherwise require multistep synthesis. However, in its current form, the methodology appears to have notable limitations in scope and mechanistic substantiation. Overall, the work remains preliminary and does not yet meet the conceptual depth and generality expected for publication in Nature.

Response and Action: We are deeply grateful for the reviewer's careful evaluation and supportive feedback.

The main concerns are outlined below:

1.Although described as a general strategy, the substrate scope appears structurally biased. The majority of successful examples involve ortho-substituted pyridines, while substrates bearing substitution at other positions (e.g., 3- or 4-disubstituted systems) perform poorly. What is the underlying reason for the diminished reactivity or selectivity in these cases? Have the authors explored structural or condition modifications to overcome these limitations? Without a clearer understanding or solution, the generality of the method remains uncertain.

Response and Action: We appreciate this recommendation. As suggested, beyond *ortho*-substituted pyridines we have investigated the reactivity of 3-, 4-, and disubstituted pyridine systems bearing a variety of substituents.

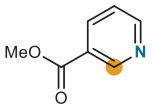


In addition, we have examined additional substrates bearing electron-donating, electron-withdrawing, or both types of substituents simultaneously to more rigorously probe the electronic requirements of the transformation. Substrates bearing electron-donating groups (OMe, anilide) as well as electron-withdrawing groups (CF_3, CN, ester, ketone, amide) all participated successfully, demonstrating the broad electronic tolerance of the methodology. Notably, systems bearing both electron-donating and electron-withdrawing substituents simultaneously also underwent the transformation with high selectivity. However, when the pyridine ring became excessively electron-deficient, reactivity was compromised: 3-cyano- and 3,5-diester-substituted derivatives failed to undergo the desired transformation, and preparation of the NO_2-substituted starting material proved difficult, precluding its evaluation. The combined results are summarized below; unsuccessful substrates have been compiled in the Supplementary Information. The expanded substrate scope has been incorporated into the revised manuscript (Fig. 3).

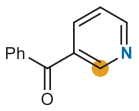
In addition, the following statement has been added to clarify the scope limitations:

"Despite the broad substrate scope, excessively electron-deficient pyridines remained challenging: 3-cyano- and 3,5-diester-substituted derivatives failed to undergo isomerization."

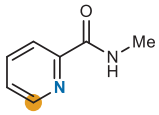
Electron-withdrawing (EWG)



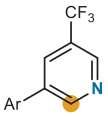
7g, 74%



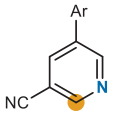
7h, 33%



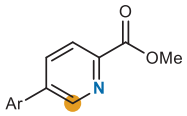
7k, 37%



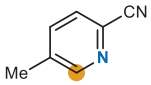
7s, 74%



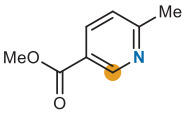
7u, 80%



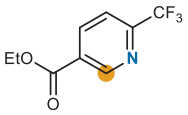
7v, 61%



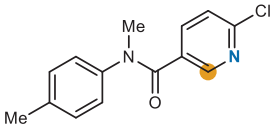
7y, 50%



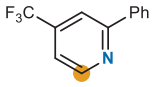
7z, 72%



7aa, 67%

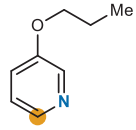


7ab, 50%

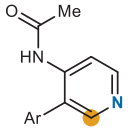


7af, 69%

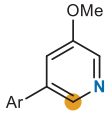
Electron-donating (EDG)



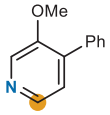
7o, 37%



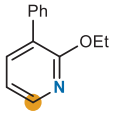
7q, 61%



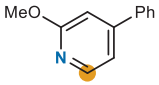
7t, 67%



7ad, 66%

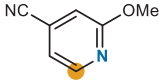


7ae, 69%

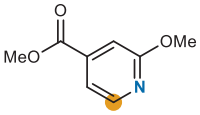


7ah,58%

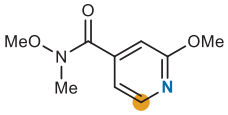
Electronically polarized systems (EWD+EDG)



7ai, 46%

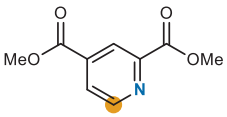


7aj, 78%



7ak, 72%

Unsuccessful



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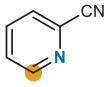

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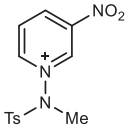


Difficulties in salt preparation

2.The manuscript includes modifications of relatively simple drug-like derivatives. However, the impact of the strategy would be significantly strengthened by demonstrating its performance on genuinely complex pharmaceuticals or natural products containing sensitive functional groups.

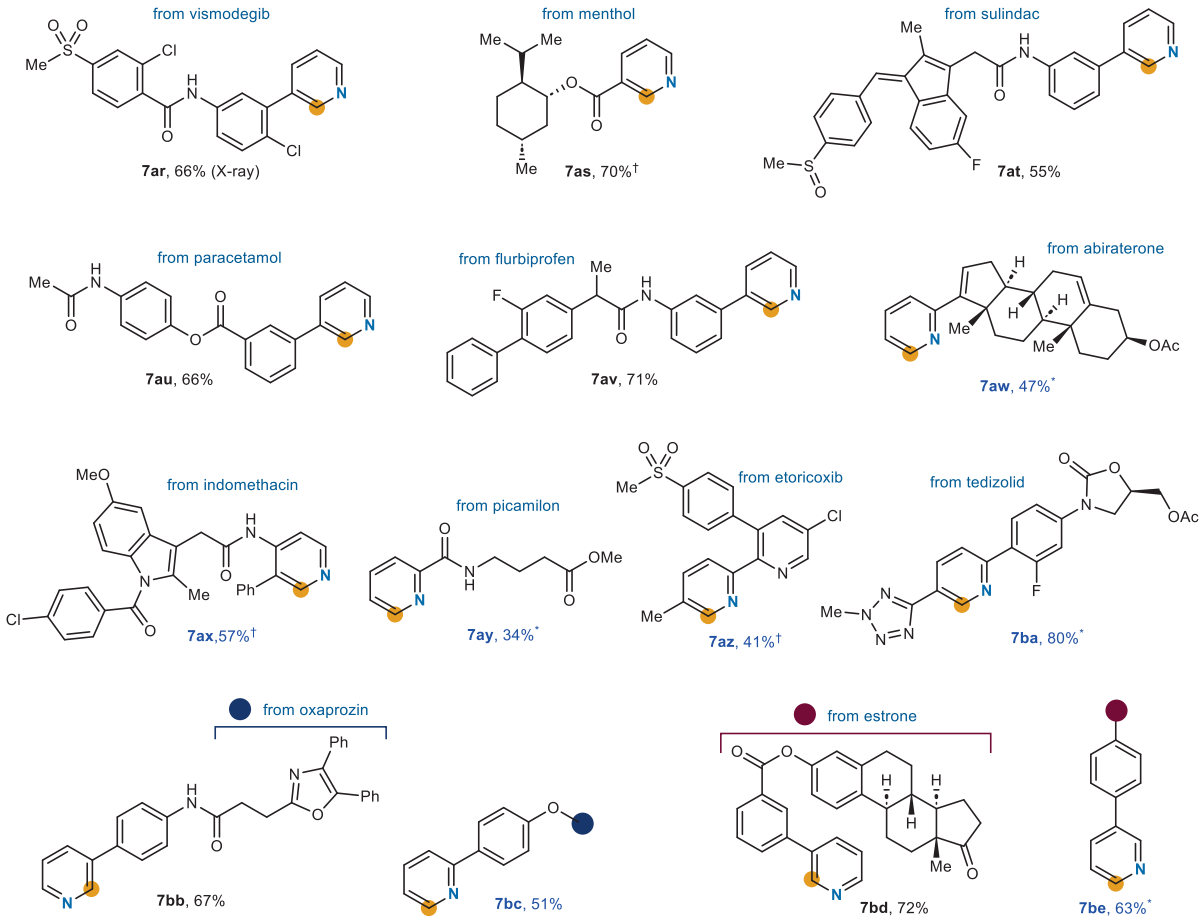
Response and Action: We thank the reviewer for this valuable suggestion. To further evaluate the applicability of the method to structurally complex, drug-like molecules, we substantially expanded the late-stage diversification studies and examined 14 pyridine-containing bioactive molecules and pharmaceuticals that were available to us in Korea. These substrates encompass a broad range of molecular architectures and functional-group combinations.

As shown in the revised Fig 4, the transformation proved compatible with derivatives of vismodegib, sulindac, flurbiprofen, indomethacin, abiraterone, tedizolid, etoricoxib, picamilon, oxaprozin, and estrone—spanning anti-cancer agents, NSAIDs, antibiotics, and steroid-based scaffolds. The reaction tolerated a wide array of functional groups, including sulfones, sulfoxides, esters, amides, halides, alkenes, indoles, tetrazoles, oxazoles, and steroid frameworks, underscoring the robustness of the method under structurally demanding conditions.

Substrates containing free nucleophilic functionalities—such as amines, alcohols, and carboxylic acids—were also investigated. These groups were found to be incompatible with the pyridinium salt preparation step, as they undergo competitive tosylation. Nevertheless, such functionalities can be readily accommodated by appropriate protection (e.g., acylation or esterification) prior to salt formation.

As suggested, the following sentence has been added to the revised manuscript to reflect this limitation: "Substrates bearing free nucleophilic groups, such as amines, alcohols, and carboxylic acids, are not compatible with the pyridinium salt preparation step; such functionalities can, however, be accommodated by prior protection."

These additions provide a more transparent and comprehensive assessment of both the scope and current limitations of the method in the context of late-stage diversification of drug-like molecules.

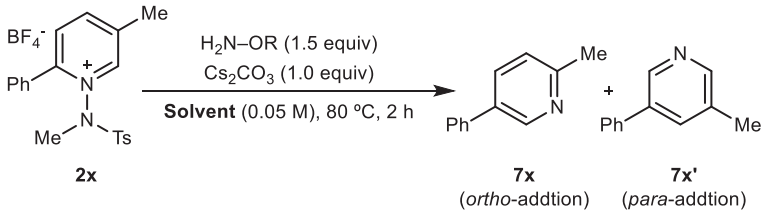


3.The authors propose that solvent plays a decisive role in controlling regioselectivity. However, the solvent study appears limited. Beyond toluene, DMF, and THF, were additional solvents screened to validate this conclusion? Is the regioselectivity trend consistent across a broader polarity or donor-number range? The current dataset is insufficient to firmly establish solvent as the primary determinant.

Response and Action: We thank the reviewer for this important comment. In response, we conducted an expanded solvent screening study to more rigorously evaluate the role of solvent in controlling regioselectivity. As shown in the revised Supplementary Information, a broad range of solvents was examined, encompassing a wide span of dielectric constants (ε = 2.25–80.1) and solvent classes, including nonpolar (toluene, dioxane), moderately polar aprotic (EtOAc, DCE, acetone), highly polar aprotic (DMF, DMA, MeCN, propylene carbonate), and protic solvents (MeOH, EtOH, iPrOH, TFE). Across this diverse solvent set, a consistent trend in regioselectivity is observed. Nonpolar and weakly polar solvents (e.g., toluene, EtOAc, DCE) strongly favor formation of **7x**, whereas highly polar aprotic solvents (e.g., DMF, DMA, MeCN) lead to a significant increase in **7x′** formation or diminished selectivity. Interestingly, protic solvents exhibited distinct and unusual reactivity patterns. In methanol, the reaction proceeded only

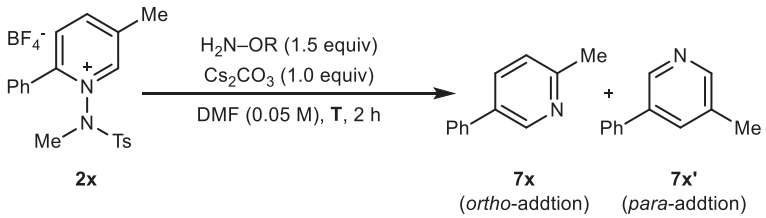
minimally, whereas ethanol resulted in a marked decrease in regioselectivity, with **7x′** becoming the major product. In contrast, larger alcohol solvents such as propanol and isopropanol restored the original selectivity trend, while the acidic solvent TFE proved unsuitable for the reaction. To further investigate the unusual selectivity reversal observed in ethanol, the ethanol conditions were applied to a broader range of substrates. However, productive reactivity was observed only in a very limited number of cases, whereas most substrates exhibited little or no conversion. Consequently, DMF was identified as the most suitable solvent for accessing the alternative regioisomer. Importantly, the solvent-dependent selectivity trend was consistently observed across a broad range of solvent environments rather than being limited to a narrow set of conditions. These results substantially strengthen our conclusion that solvent plays a decisive role in governing the regioselectivity of the reaction. The expanded solvent-screening data and corresponding discussion have been added to the revised Supplementary Information.
In addition, we investigated the effects of temperature and base on regioselectivity. Lower temperatures led to improved selectivity, whereas higher temperatures increased the formation of **7x′**, although at the expense of overall reaction efficiency. By contrast, variation of the base had little influence on the observed selectivity.

Solvent effects



Entry	Solvent	Dielectric constant	**7x** (%)	**7x′** (%)
1	H_2O	80.1	21	9
2	*N*,*N*-Dimethylacetamide (DMA)	37.78	55	20
3	Acetonitrile (MeCN)	37.5	53	21
4	*N*,*N*-Dimethylformamide (DMF)	36.71	57	23
5	Methanol (MeOH)	32.70	trace	trace
6	Ethanol (EtOH)	24.55	24	42
7	Propanol	20.33	39	14
8	Isopropanol	19.92	67	9
9	2,2,2-Trifluoroethanol (TFE)	27.0	trace	trace
10	Dichloroethane (DCE)	10.36	69	3
11	Ethyl acetate (EA)	6.02	80	3
12	Toluene	2.38	88	trace
13	1,4-Dioxane	2.25	27	trace

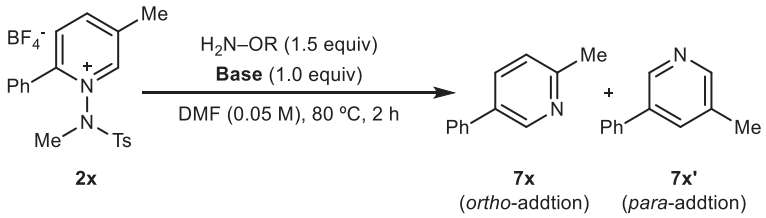
Temperature effects



2x **7x** (*ortho*-addtion) **7x'** (*para*-addtion)

Entry	Temperature (T)	**7x** (%)	**7x′** (%)
1	25 ºC	63	9
2	50 ºC	58	16
3	80 ºC	57	23
4	120 ºC	47	28

Base effects



2x **7x** (*ortho*-addtion) **7x'** (*para*-addtion)

Entry	Base	**7x** (%)	**7x′** (%)
1	Cs_2CO_3	57	23
2	K_2CO_3	51	20
3	CsOAc	56	21
4	K_3PO_4	53	17
5	2,4,6-Collidine	36	6
6	1,8-Diazabicyclo[5.4.0]-7-undecene (DBU)	39	20

4. In Supplementary Figure 6, two mechanistic pathways are proposed. While experimental data are presented, no computational support is provided. Have DFT calculations been performed to compare the energy profiles of the two pathways? Can theoretical analysis explain the regioselectivity trends? Given the mechanistic centrality to this work, computational validation would be highly valuable.

Response and Action: We thank the reviewer for this insightful comment regarding the origin of product selectivity. To address this point, we performed DFT calculations to interpret the observed regioselectivity within the Curtin–Hammett framework. As shown in the computed free energy profile (Fig. 5), the initial nucleophilic addition step leading to **Int2-*o*** and **Int2-*p*** proceeds through nearly isoenergetic transition states (**TS1-*o***: 22.2 kcal mol^{-1}; **TS1-*p***: 21.2 kcal mol^{-1}), and the resulting intermediates are likewise very similar in energy. These negligible differences indicate that **Int2-*o*** and **Int2-*p*** are in rapid equilibrium under the reaction conditions, consistent with Curtin–Hammett behavior. Under such circumstances, product distribution is governed not by the relative stabilities of the intermediates, but by the relative free energies of the subsequent cyclization transition states. Consistent with this interpretation, the calculated cyclization barrier for **TS2-*o*** ($\Delta G^{\ddagger}$ = 24.5 kcal mol^{-1} from **Int2-*o***) is significantly lower than that for **TS2-*p*** ($\Delta G^{\ddagger}$ = 27.8

kcal mol^{-1} from **Int2-*p***), giving a $\Delta\Delta G^{\ddagger}$ of approximately 3.2 kcal mol^{-1}. This energy difference is sufficient to account for the predominant formation of **Int3-*o*** under kinetic control. The lower barrier associated with **TS2-*o*** likely reflects a more favorable geometric alignment for intramolecular cyclization, together with more effective stabilization of the developing charge-separated character in the transition state, as suggested by the optimized geometries.
The calculations also rationalize the solvent-dependent selectivity observed experimentally. In the more polar solvent DMF, the charge-separated character of the transition state is more effectively stabilized relative to toluene, reducing the $\Delta\Delta G^{\ddagger}$ between the competing cyclization pathways — consistent with the diminished selectivity observed experimentally under DMF conditions.
Taken together, these results support a Curtin–Hammett-controlled mechanism in which rapidly equilibrating intermediates undergo irreversible, selectivity-determining cyclization. The preferential formation of **Int3-*o*** therefore arises from the intrinsically lower activation barrier of **TS2-*o***, rather than from the thermodynamic stability of the preceding intermediates.
These findings have been incorporated into the revised manuscript (Fig. 5 and Supplementary Fig. 6).

In addition, we have added a paragraph to further explain the origin of the observed selectivity.

“To probe the origin of regioselectivity, we performed DFT calculations within the Curtin–Hammett framework. The initial nucleophilic addition step leading to **Int2-*o*** and **Int2-*p*** proceeds through nearly isoenergetic transition states (**TS1-*o***: 22.2 kcal mol^{-1}; **TS1-*p***: 21.2 kcal mol^{-1}), indicating that these intermediates are in rapid equilibrium under the reaction conditions. Product distribution is therefore governed by the relative free energies of the subsequent cyclization transition states. The calculated barrier for **TS2-*o*** ($\Delta G^{\ddagger}$ = 24.5 kcal mol^{-1}) is substantially lower than that for **TS2-*p*** ($\Delta G^{\ddagger}$ = 27.8 kcal mol^{-1}), with a $\Delta\Delta G^{\ddagger}$ of 3.2 kcal mol^{-1}consistent with the observed *ortho* selectivity. The diminished selectivity in DMF is attributed to enhanced stabilization of the charge-separated transition state in the more polar medium, which reduces the $\Delta\Delta G^{\ddagger}$ between competing pathways (Fig. 5b,c and Supplementary Fig. 6).”

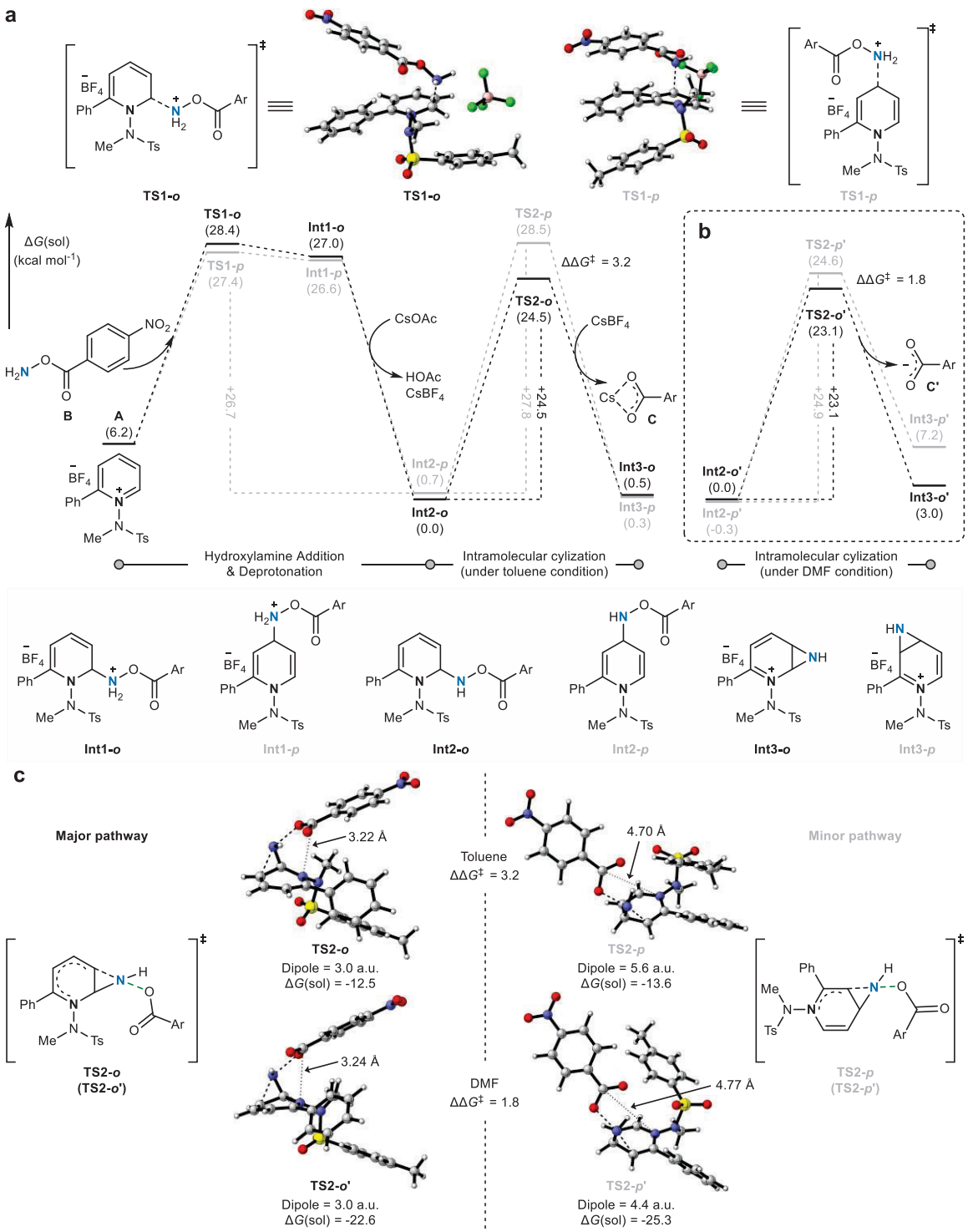
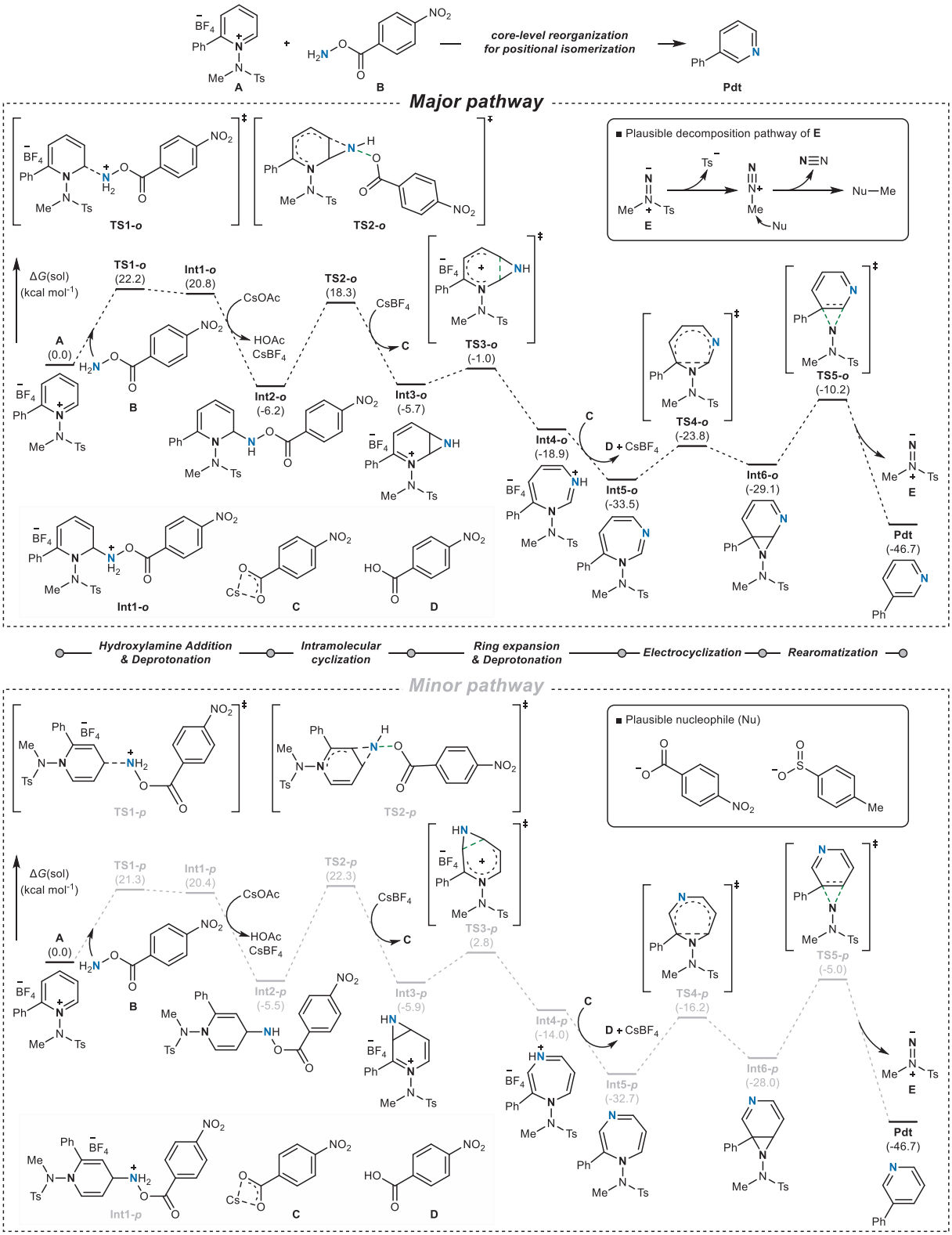


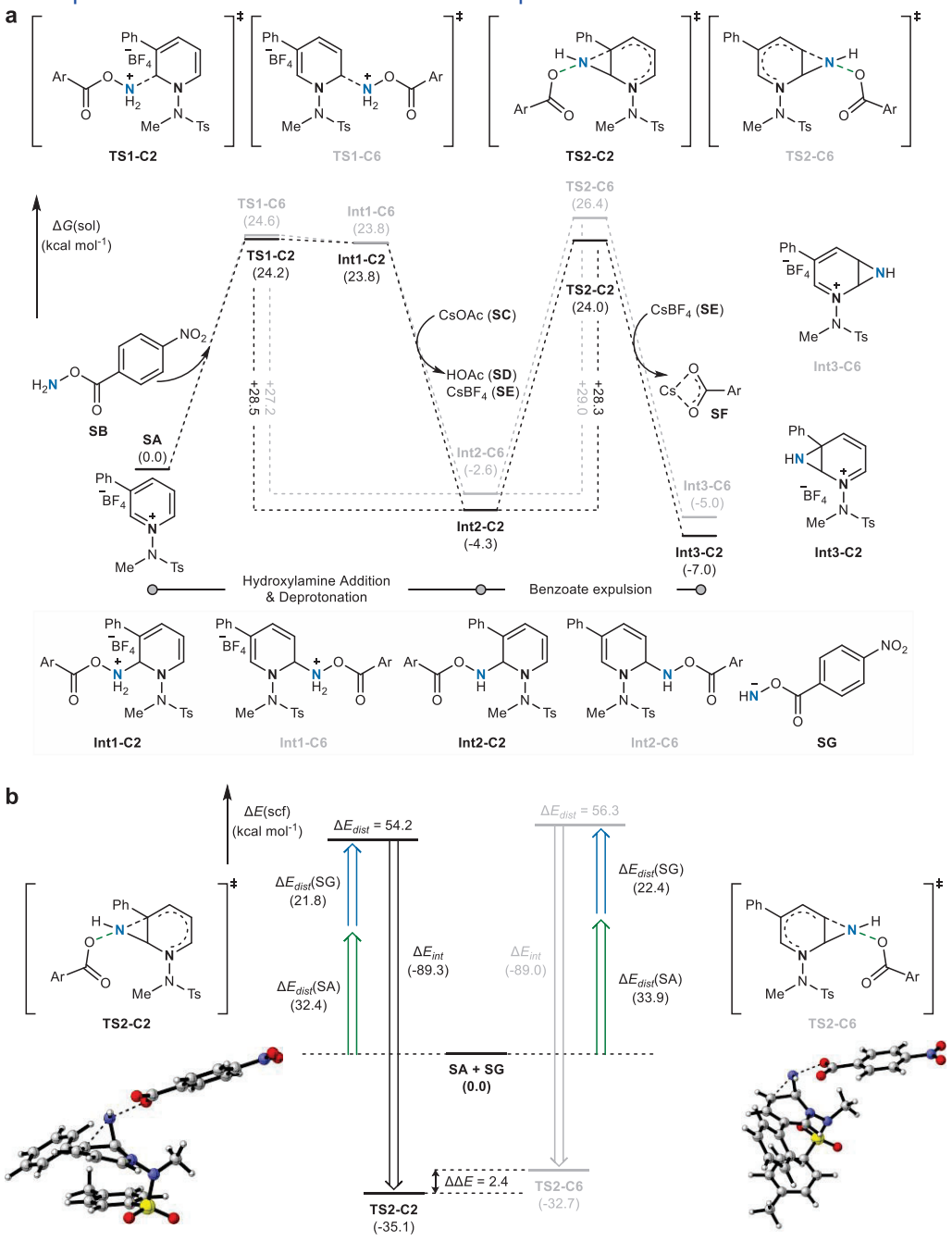
Fig. 5 | DFT calculations rationalizing the regioselectivity of the intramolecular cyclization. **a,** Computed free energy profiles for the competing *ortho*- and *para*-selective pathways under toluene conditions. **b**, Free energy profile for the cyclization step under DMF conditions, illustrating the reduced $\Delta\Delta G^{\ddagger}$ relative to toluene. **c**, Optimized geometries of the selectivity-determining transition states **TS2-*o*** and **TS2-*p***.



Supplementary Figure 6. Computational studies of the C2-substituted substrate and proposed reaction mechanism.

Method: ωB97X-D3BJ/def2-TZVPP/CPCM(Toluene)//ωB97X-D3BJ/def2-SVP/CPCM(Toluene).

To elucidate the origin of the site selectivity between the C2 and C6 positions in the C3-phenyl substituted system, computational studies were conducted. The results revealed that the reaction at the C2 (substituted) position has a lower-energy transition state (**TS2-C2**) compared with that at the C6 position (**TS2-C6**). To further investigate the origin of this energy difference, distortion–interaction analysis was conducted. Interestingly, the two transition states exhibited nearly identical interaction energies, whereas a 2.1 kcal mol^{-1} difference was observed in the structural distortion energies. These results indicate that the preferential reaction at the C2 position arises from the lower distortion energy required to access the corresponding transition state. For the OMe substituent, we attribute this behavior to a hydrogen-bonding interaction between the OMe group and the incoming H_2N–OR nucleophile, which likely serves to direct nucleophilic attack toward the OMe-substituted position.



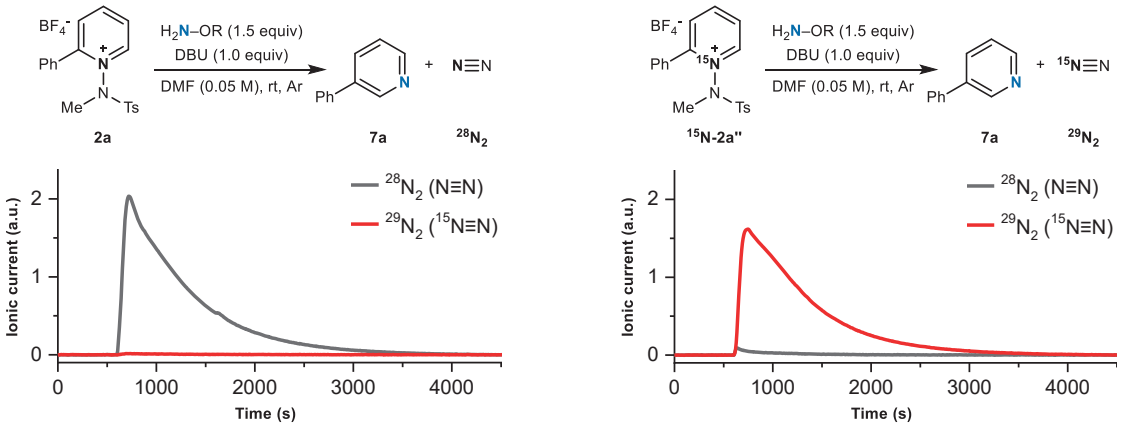
Supplementary Figure 5. Computational studies of the C3-substituted substrate. a, DFT investigation of competitive site selectivity between the C2 and C6 pathways, including computed activation barriers. **b,**

Distortion–interaction analysis of **TS2-C2** and **TS2-C6**. Method: ωB97X-D3BJ/def2-TZVPP/CPCM(THP)//ωB97X-D3BJ/def2-SVP/CPCM(THP).

5. The proposed mechanism involves nitrogen insertion/deletion steps and the generation of N_2. However, experimental validation remains limited. Has of N_2 formation been directly detected? Have intermediates I, II, or III been observed or trapped? Are kinetic studies available to support the proposed sequence? Without experimental corroboration, the mechanistic proposal remains largely speculative.

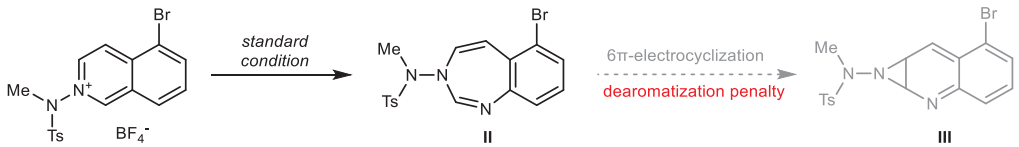
Response and Action: We thank the reviewer for this important recommendation. To directly probe N_2 formation, the reaction was conducted using **2a** and a ^{15}N-labeled substrate (**^{15}N-2a″**) in an Ar-purged reaction chamber, with nitrogen gas evolution monitored by GC–MS (Supplementary Information, Section VI). In the experiment using **2a**, formation of $^{14}N^{14}N$ (*m/z* = 28) was clearly observed. To further establish the origin of the evolved nitrogen gas, a parallel experiment employing **^{15}N-2a″** was performed; under these conditions, $^{14}N^{14}N$ (*m/z* = 28) was negligible, whereas $^{15}N^{14}N$ (*m/z* = 29) was unambiguously detected. These results provide direct experimental evidence that the observed nitrogen gas originates from the pyridine nitrogen itself.

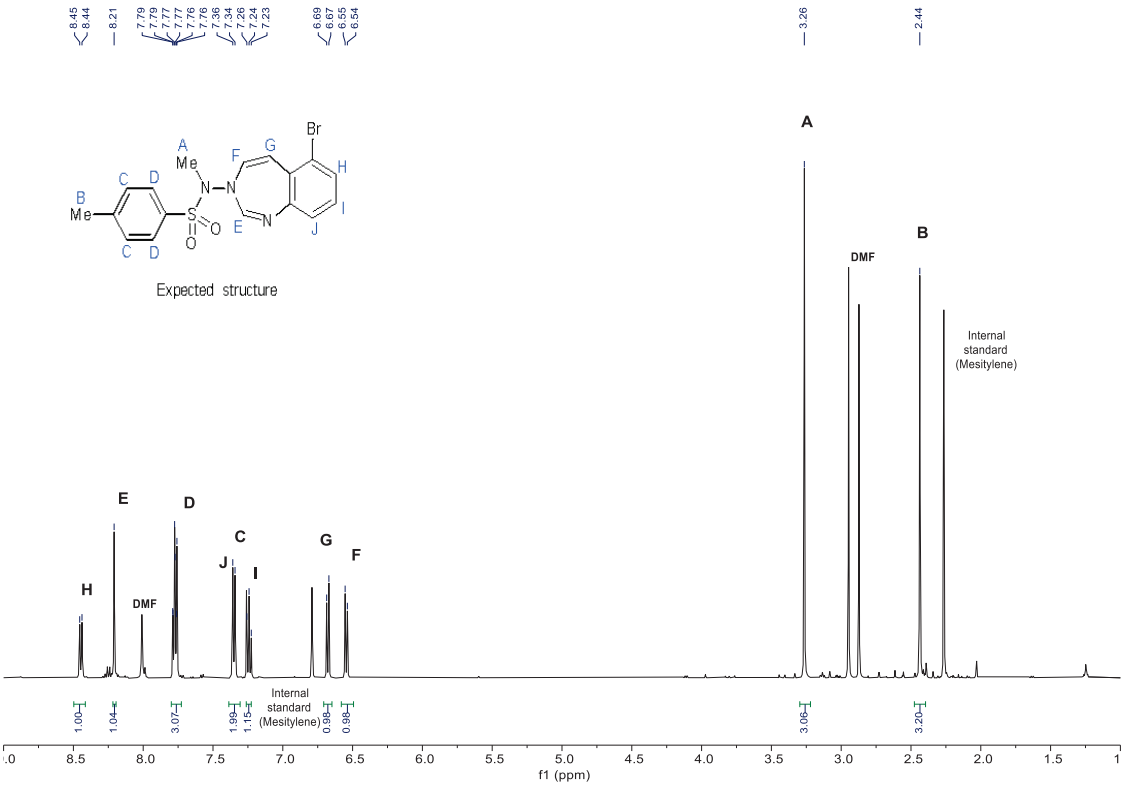
We have added the following statement to the revised manuscript:
"Formation of $^{14}N^{14}N$ (*m/z* = 28) was observed with **2a**, whereas $^{15}N^{14}N$ (*m/z* = 29) was detected with **^{15}N-2a″**, providing direct evidence that the evolved nitrogen gas originates from the pyridine nitrogen itself (Fig. 2d)."



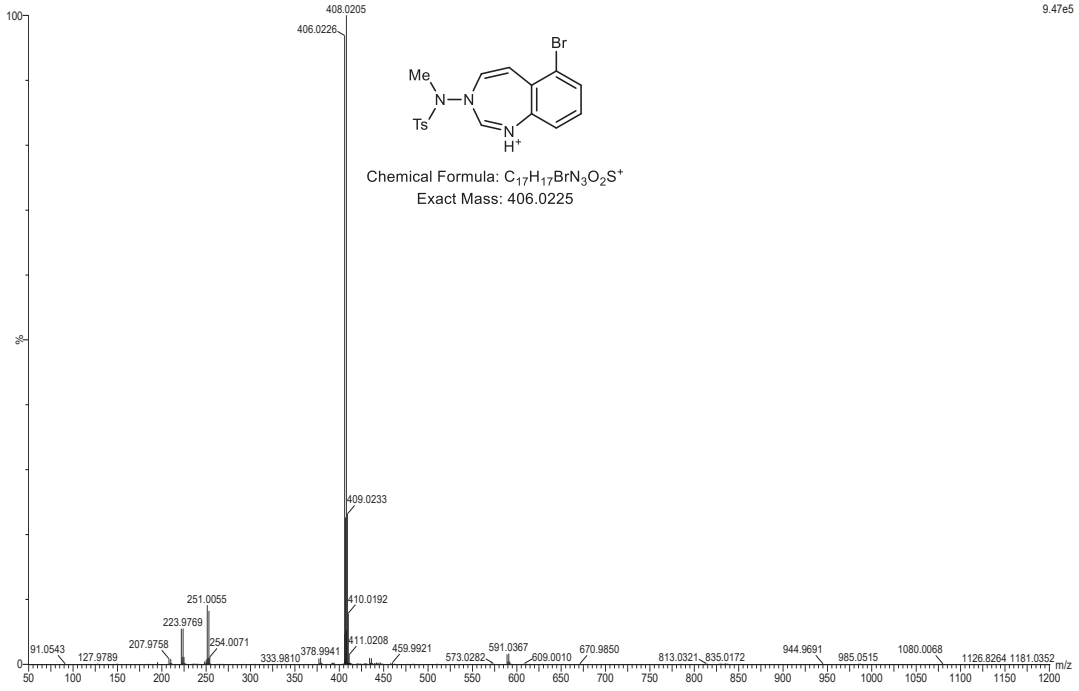
In the current system, the reaction proceeds too rapidly to permit direct trapping of intermediates **I**, **II**, or **III**. We reasoned that the transformation might be arrested at the diazepine stage by employing isoquinoline-derived substrates, in which the subsequent 6π-electrocyclization step is disfavored owing to the additional dearomatization penalty associated with the fused benzene ring.

To intercept the putative diazepine intermediate **II**, we subjected an isoquinoline-derived substrate to the reaction conditions. Crude ^{1}H NMR analysis revealed signals consistent with formation of the proposed diazepine intermediate; however, this species proved unstable, and all attempts at isolation or crystallization for X-ray analysis resulted in decomposition. Nevertheless, the corresponding molecular ion peak was successfully detected by HRMS, providing supporting evidence for the existence of this intermediate. The crude ^{1}H NMR spectrum and HRMS data for the putative diazepine intermediate are below.





^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.45 (d, *J* = 8.1 Hz, 1H), 8.21 (s, 1H), 7.77 (m, 3H), 7.35 (d, *J* = 8.0 Hz, 2H), 7.24 (t, *J* = 7.9 Hz, 1H), 6.68 (d, *J* = 8.0 Hz, 1H), 6.55 (d, *J* = 8.0 Hz, 1H), 3.26 (s, 3H), 2.44 (s, 3H).



While the conceptual framework is intriguing, the current manuscript lacks sufficient mechanistic depth, general substrate applicability, and rigorous validation to justify publication in Nature. Substantial strengthening of scope and mechanistic support would be required to meet the journal's standards

Referee #3 (Remarks to the Author):

The authors present an innovative strategy which would make a nice addition to the skeletal edit toolbox of transformations. (Please note that thermal hazards are a key concern for this methodology which the Authors should provide further comments on). This study outlines a methodology for synthesizing N-atom rearranged pyridines through ring expansion of pyridinium salts using hydroxylamine reagents, followed by nitrogen extrusion. The approach demonstrates broad substrate applicability, and a plausible mechanism is proposed. Data analysis revealed the presence of positional isomers, with efforts made to provide rational explanations. The experimental section details spectral data and reaction conditions.
•This reviewer would like to Invite the authors to revise their manuscript to address specific concerns before a final decision is reached. Main points to address are safety concerns, computational support for mechanism proposed & selectivity observed, along with mass balance considerations for reactions exhibiting low yields. In instances where isomers are present, the Authors must clearly label as such in the figures. More points can be found below.
•Originality and significance: This work is original and would be of interest to readers of this journal
Response and Action: We are deeply grateful for the reviewer's careful evaluation and supportive feedback.

•Data & methodology: validity of approach, quality of data, quality of presentation: see below
•Consistency needed between e.g. Line 8: “de novo” and Line 54: italized “de novo”
Response and Action: We thank the reviewer for pointing out this inconsistency. The formatting of “*de novo*” has been standardized throughout the manuscript, and the term is now consistently presented in italics.

•Although vismodegib isomerization is present in Fig. 1, it is not mentioned in the text until much later in line 132. Please omit from Fig.1 or acknowledge in local text.
Response and Action: We thank the reviewer for this helpful suggestion. To ensure consistency between Fig. 1 and the main text, we have added the sentence to explicitly mention the vismodegib example in the revised manuscript.

“For example, in vismodegib, nitrogen transposition converts the pyridine *ortho*-substituent into the corresponding *meta*-substituted isomer.”

•Line 44: “Fig. 1d”- there is no such label in Fig.1- please add or omit from text.
Response and Action: We thank the reviewer for pointing out this error. The error has been corrected in the revised manuscript.

•Line 65: “1a” – there is no compound 1a in this manuscript. Please include compound 1a or renumber appropriately.
Response and Action: We thank the reviewer for pointing out this error. The error has been corrected in the revised manuscript.

•Provide details or references for synthesis of all N-protected amidopyridinium salts in the manuscript (including safety data/precautions). And provide details or references for experimental data of each N-protected amidopyridinium salt isolated (including safety data/precautions) in SI as most are currently missing from the SI.
Response and Action: We appreciate this recommendation. Detailed experimental information regarding the preparation of *N*-amidopyridinium salts has been added to the Supplementary Information VI. In addition, the following safety data/precautions have been included in Supplementary Information VI.

•This methodology uses reagents and compounds with potential thermal hazards, the author should comment on the safety data or precautions advised for this chemistry in the manuscript and SI.
- The author should comment on safety precautions undertaken for the use of MSA in dioxane with 70% perchloric acid (potentially explosive) in the manuscript and SI. Note that compound 2a was used on 4.26 gram scale. Please also comment on the scale justification (no mention of gram scale in the text only in Fig. 3) and safety controls undertaken.

Response and Action: We thank the reviewer for raising these important safety considerations and address each point below.
Thermal hazards of the aminating reagent: O-(4-nitrobenzoyl)hydroxylamine may present thermal hazards and should be handled with appropriate caution, including storage at low temperature. Although the reaction was conducted at 80 °C, the short reaction time meant that no safety issues were encountered under the reported conditions. We have nevertheless added explicit handling and storage precautions for this reagent to the revised manuscript and Supplementary Information.

*MSA / perchloric acid conditions***:** We agree that the combination of MSA in dioxane with 70% perchloric acid warrants careful attention owing to its potential thermal and explosive hazards. We have added a detailed description of the precautions undertaken to the revised Supplementary Information: all operations involving perchloric acid were carried out in a well-ventilated fume hood with appropriate personal protective equipment and continuous temperature monitoring to guard against uncontrolled exotherms, the setup was designed to avoid accumulation of hazardous intermediates, and all procedures followed our institutional safety guidelines.

Gram-scale experiment and scale justification: The gram-scale reaction (**2a**, 4.26 g) was performed to demonstrate the practicality and scalability of the method. It was conducted with enhanced temperature control and continuous monitoring, and no abnormal exothermic behavior or safety incidents were observed. We have revised the main text to describe this experiment explicitly (previously shown only in Fig. 3) and to state its rationale.

We have added the corresponding handling precautions to the revised manuscript and Supplementary Information.

CAUTION
- MSH is a highly unstable solid and should be used directly in solution immediately after synthesis. If solvent removal is necessary, the solvent should be evaporated at temperatures below 20 ºC, and complete removal of the solvent should be avoided.
- 70% Perchloric acid ($HClO_4$) presents a potential explosion hazard, particularly in contact with organic solvents or oxidizable materials. All operations should be carried out in a well-ventilated fume hood with appropriate personal protective equipment.
- Trimethyloxonium tetrafluoroborate can generate HBF_4 on contact with moisture; exposure to moisture should therefore be minimized during handling.
- *N*-amidopyridinium salts are generally stable and can be stored for extended periods. In rare cases, however, exposure to moisture causes them to transform into a sticky, gum-like material, so storage under moisture-free conditions is recommended whenever possible.

•Line 72: "…SN2-type elimination…" – Is a more accurate description, SN2-type displacement followed by elimination/rearrangement?
Response and Action: We thank the reviewer for this helpful suggestion. We have made minor revisions to the mechanistic description to more accurately reflect the reaction pathway. Based on DFT calculations, the final demethylation step, initially described as a direct S_N2 process, is revised to proceed through a retro-cycloaddition generating an isodiazene intermediate **E**, which undergoes fragmentation to a tosyl anion and a methanediazonium species; the latter then undergoes nucleophile-assisted demethylation via an S_N2 pathway with concomitant extrusion of N_2. The revised mechanism has been updated in the manuscript accordingly.

As suggested, we have revised this sentence:
"These results demonstrate that only *N*-alkyl sulfonamide groups capable of nucleophilic displacement and fragmentation are effective, consistent with the proposed mechanism."
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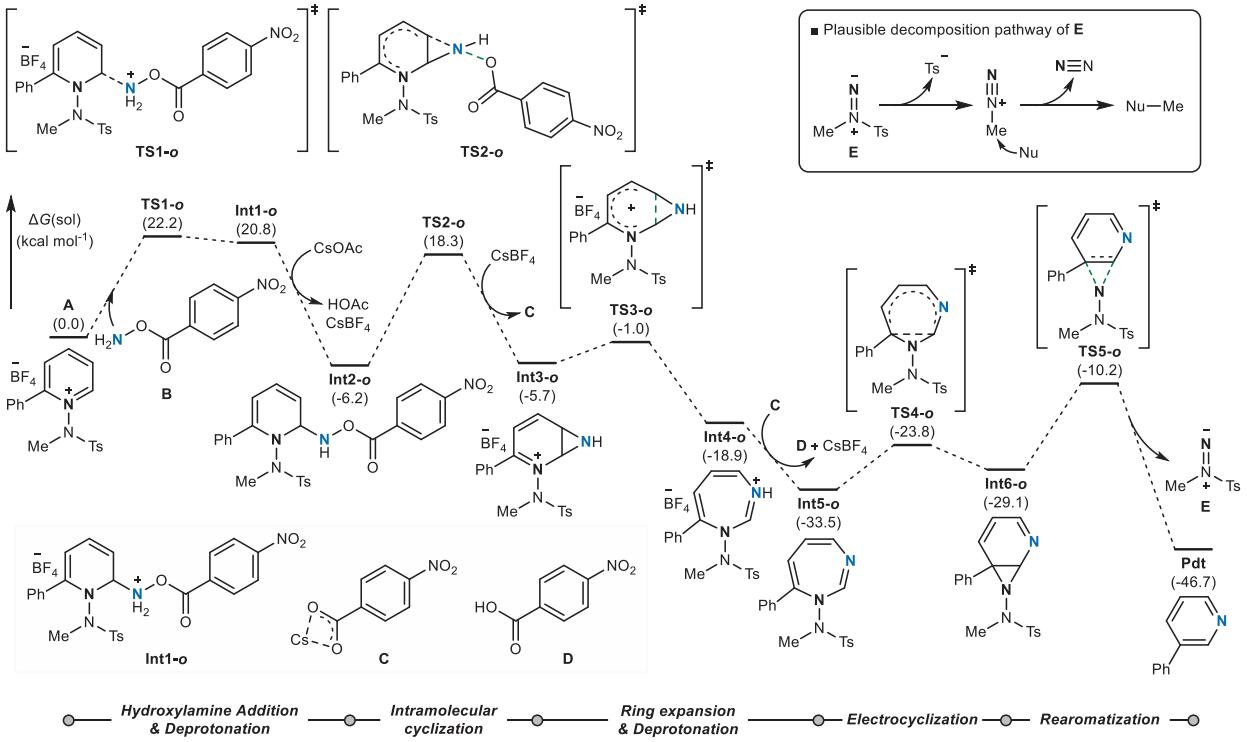
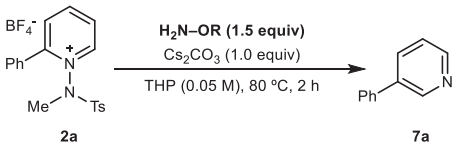


•Line 73-74: Is a correction for Fig. 2a right needed? O-(2,4-dinitrophenyl)hydroxylamine 59% yield or 72% yield as shown in SI (Line 80, entry 8).

Response and Action: We thank the reviewer for pointing out this issue. We acknowledge that our original description may have been unclear. The conditions used in Fig. 2a and those reported in the Supplementary Information (Line 80, entry 8) are not identical, which accounts for the observed difference in yields. To clarify this point, we have added an additional optimization table to the Supplementary Information.

Hydroxylamine screening



Entry	Hydroxylamine (H_2N–OR)	Yield (%)
1	*O*-(2,4-dinitrophenyl)hydroxylamine	59
2	*O*-(mesitylsulfonyl)hydroxylamine	trace

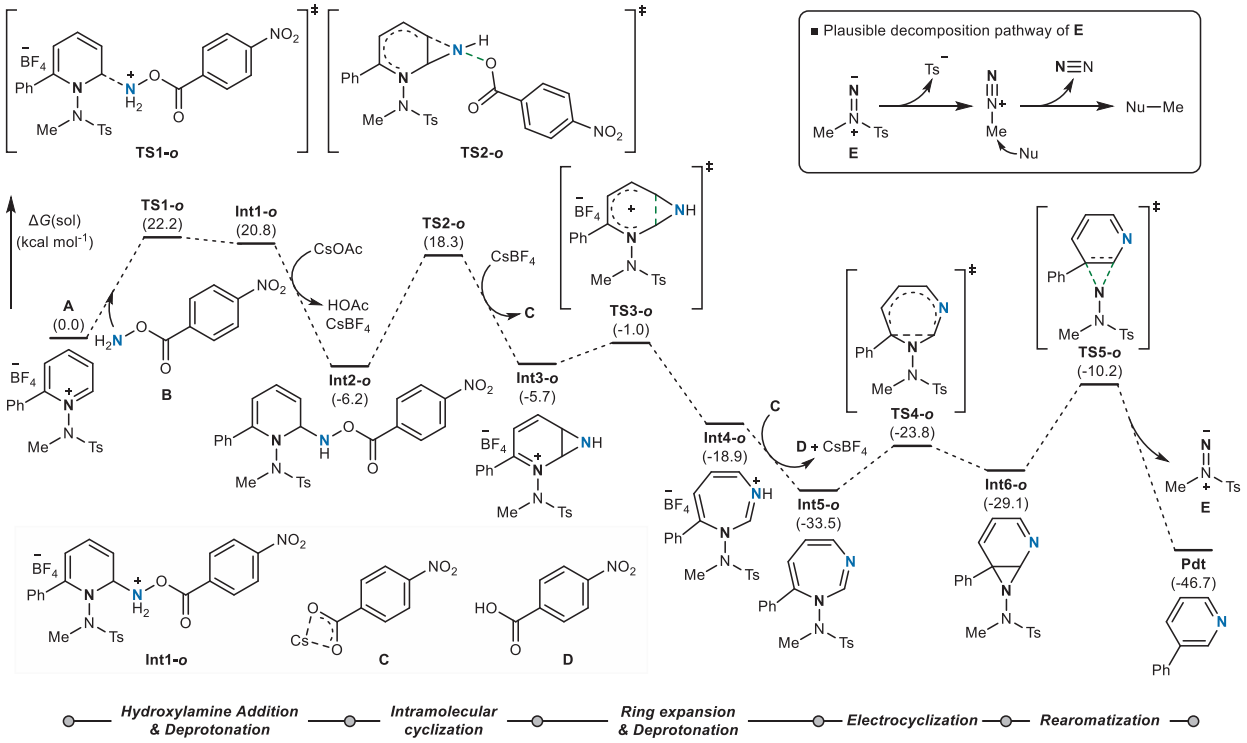
•Line 78: Reference note is needed to direct the reader to SI for reaction optimization details.

Response and Action: We thank the reviewer for this suggestion. A reference directing the reader to the Supplementary Information for reaction optimization details has now been added in the revised manuscript. "(see Supplementary Information for optimization details)"

•Line 73-78 systematic evaluation of reaction paraments discussion: Author should include more comments on key findings from reaction optimization in the manuscript:

- Do the Authors have any thoughts on why H2N-OPh.HCl gives N.D. product of 7a but O-(2,4-dinitrophenyl)hydroxylamine gives rise to 72% yield of 7a?

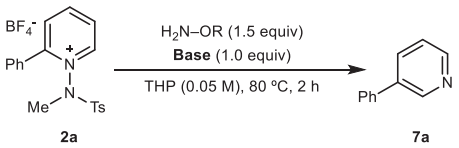
Response and Action: We thank the reviewer for this question. In our proposed mechanism, the highest-energy transition state corresponds to **TS2-*o***, in which N–O bond cleavage of the hydroxylamine is coupled with intramolecular cyclization. The leaving-group ability is therefore expected to be a key determinant of reaction efficiency at this step. Consistent with this rationale, *O*-(2,4-dinitrophenyl)hydroxylamine—in which the electron-withdrawing nitro groups stabilize the developing oxyanion character—showed good reactivity. By contrast, H_2N–OPh•HCl would generate phenolate as the leaving group, which is a poorer leaving group than 2,4-dinitrophenolate; this difference likely accounts for the lack of reactivity observed in this case.



- Do the Authors have any thoughts on why sub-stoichiometric base (0.5 eq. Cs2CO3) gives rise to 73% yield of 7a?

Response and Action: We thank the reviewer for this question. Our base screening provides a plausible explanation. Both $CsHCO_3$ (a partially protonated form of Cs_2CO_3, entry 3) and 2,4,6-collidine (entry 8) promote the reaction, indicating that these species can themselves function as bases under the reaction conditions. We therefore propose that Cs_2CO_3 sustains reactivity at sub-stoichiometric loading because it continuously generates additional basic species in situ—including $CsHCO_3$ and the pyridine product itself—thereby maintaining a sufficient effective base concentration throughout the reaction. Consistent with this interpretation, mono-basic CsOAc at 0.5 equiv (entries 4 and 5) gave markedly lower reactivity than Cs_2CO_3. We attribute this to the inability of CsOAc to generate additional basic species in situ, which prevents it from maintaining an adequate effective base concentration over the course of the reaction.

Base screening



Entry	Base	Yield (%)
1	K_2CO_3	18
2	Na_2CO_3	10
3	$CsHCO_3$	20
4	CsOAc	86
5	CsOAc (0.5 equiv)	45
6	K_3PO_4	82
7	KO^{t}Bu	81
8	2,4,6-Collidine	36
9	Triethylamine (TEA)	55
10	1,8-Diazabicyclo[5.4.0]-7-undecene (DBU)	52

- Alternative solvent Ethyl Acetate also gives rise to 84% yield of 7a and should be highlighted alongside Toluene in Line 74 and Fig. 2a right?
Response and Action: We thank the reviewer for pointing out this issue. At the reviewer's suggestion, we have added a new entry for ethyl acetate in Fig. 2a right and the following sentence has been included in the revised manuscript:

"Toluene and ethyl acetate served as a viable alternative to tetrahydropyran (THP) with minimal impact on efficiency (entry 2 and 3)"

- Correct Fig. 2a right: "DMF instead of THP" is listed as 73% yield of 7a in manuscript but its 65% yield in SI (Line 86, entry 7)?
Response and Action: We thank the reviewer for pointing out this error. The error has been corrected in the revised manuscript.

- Did the Authors assess the safety advantages of using 40 °C or 60 °C instead of 80 °C for reaction temperatures?
Response and Action: We evaluated reaction temperatures of 40°C, 60°C, and 80°C. While lower temperatures (40 and 60°C) offer safety advantages and reduced energy consumption, they resulted in diminished yields (60% and 75%, respectively). The optimal balance of efficiency and practicality was achieved at 80°C (88%).

•Line 84: "THP (0.75ml)" should be "THP (1.0mL)" as its more consistent with entries in Fig. 2a right - see solvent screening in the SI (Line 86, entries 2-9)?
Response and Action: We appreciate this recommendation. At the reviewer's suggestion, we have revised "THP (0.75ml)" to "THP (1.0mL)" in Fig. 2a.

•Line 90-93: More detailed Mechanistic discussion is needed.
- Author should include Supplementary Figure 6 in the manuscript which provides more details than Fig. 2a, bottom.

- Do computational calculations align with “major pathway” and “minor pathway” denotation in the Supplementary Figure 6?

Response and Action: We thank the reviewer for this insightful comment regarding the origin of product selectivity. To address this point, we performed DFT calculations to interpret the observed regioselectivity within the Curtin–Hammett framework. As shown in the computed free energy profile (Fig. 5), the initial nucleophilic addition step leading to **Int2-*o*** and **Int2-*p*** proceeds through nearly isoenergetic transition states (**TS1-*o***: 22.2 kcal mol^{-1}; **TS1-*p***: 21.2 kcal mol^{-1}), and the resulting intermediates are likewise very similar in energy. These negligible differences indicate that **Int2-*o*** and **Int2-*p*** are in rapid equilibrium under the reaction conditions, consistent with Curtin–Hammett behavior. Under such circumstances, product distribution is governed not by the relative stabilities of the intermediates, but by the relative free energies of the subsequent cyclization transition states. Consistent with this interpretation, the calculated cyclization barrier for **TS2-*o*** ($\Delta G^{\ddagger}$ = 24.5 kcal mol^{-1} from **Int2-*o***) is significantly lower than that for **TS2-*p*** ($\Delta G^{\ddagger}$ = 27.8 kcal mol^{-1} from **Int2-*p***), giving a $\Delta\Delta G^{\ddagger}$ of approximately 3.2 kcal mol^{-1}. This energy difference is sufficient to account for the predominant formation of **Int3-*o*** under kinetic control. The lower barrier associated with **TS2-*o*** likely reflects a more favorable geometric alignment for intramolecular cyclization, together with more effective stabilization of the developing charge-separated character in the transition state, as suggested by the optimized geometries.

The calculations also rationalize the solvent-dependent selectivity observed experimentally. In the more polar solvent DMF, the charge-separated character of the transition state is more effectively stabilized relative to toluene, reducing the $\Delta\Delta G^{\ddagger}$ between the competing cyclization pathways — consistent with the diminished selectivity observed experimentally under DMF conditions.

Taken together, these results support a Curtin–Hammett-controlled mechanism in which rapidly equilibrating intermediates undergo irreversible, selectivity-determining cyclization. The preferential formation of **Int3-*o*** therefore arises from the intrinsically lower activation barrier of **TS2-*o***, rather than from the thermodynamic stability of the preceding intermediates.

These findings have been incorporated into the revised manuscript (Fig. 5 and Supplementary Fig. 6).

In addition, we have added a paragraph to further explain the origin of the observed selectivity.

“To probe the origin of regioselectivity, we performed DFT calculations within the Curtin–Hammett framework. The initial nucleophilic addition step leading to **Int2-*o*** and **Int2-*p*** proceeds through nearly isoenergetic transition states (**TS1-*o***: 22.2 kcal mol^{-1}; **TS1-*p***: 21.2 kcal mol^{-1}), indicating that these intermediates are in rapid equilibrium under the reaction conditions. Product distribution is therefore governed by the relative free energies of the subsequent cyclization transition states. The calculated barrier for **TS2-*o*** ($\Delta G^{\ddagger}$ = 24.5 kcal mol^{-1}) is substantially lower than that for **TS2-*p*** ($\Delta G^{\ddagger}$ = 27.8 kcal mol^{-1}), with a $\Delta\Delta G^{\ddagger}$ of 3.2 kcal mol^{-1}consistent with the observed *ortho* selectivity. The diminished selectivity in DMF is attributed to enhanced stabilization of the charge-separated transition state in the more polar medium, which reduces the $\Delta\Delta G^{\ddagger}$ between competing pathways (Fig. 5b,c and Supplementary Fig. 6).”

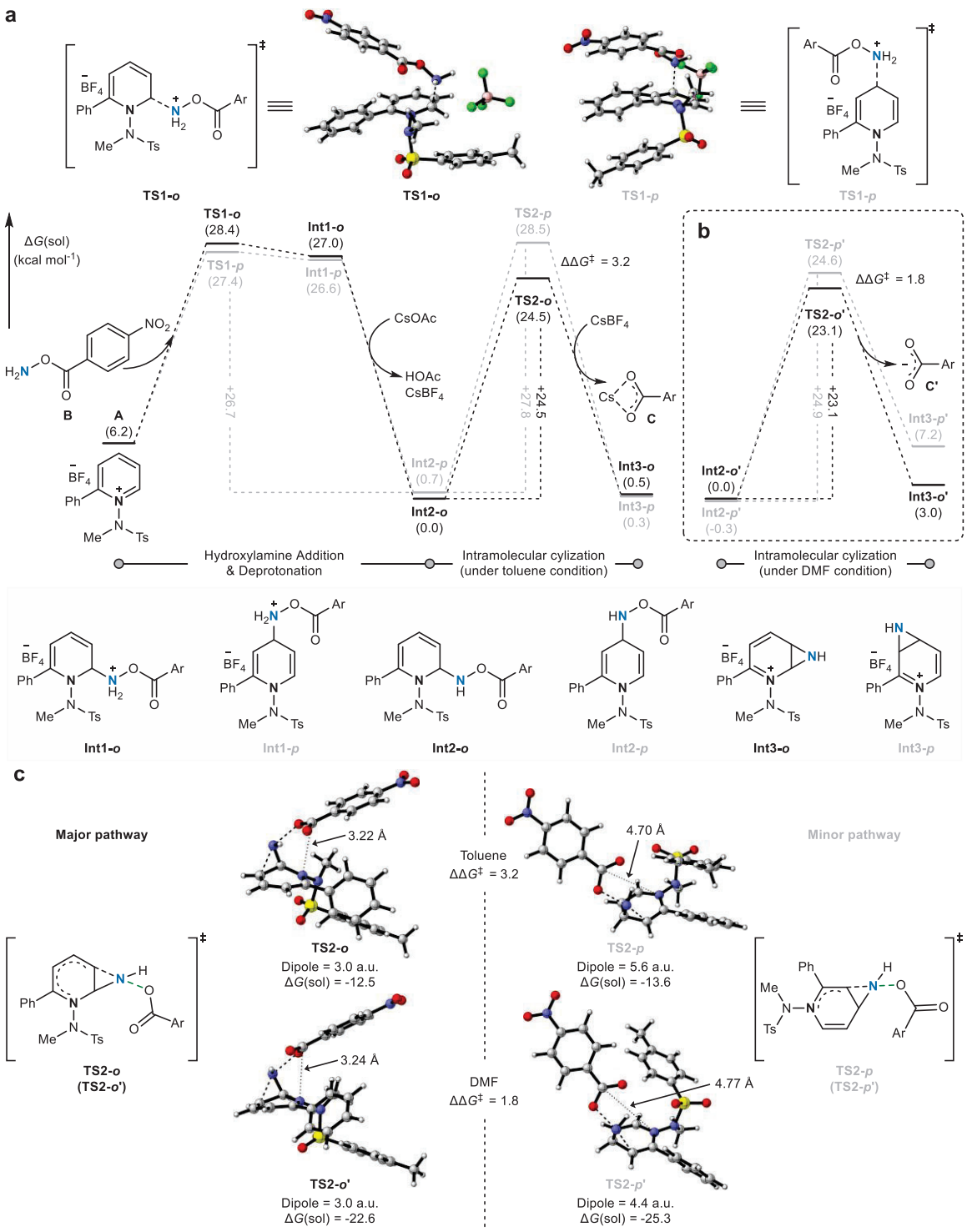
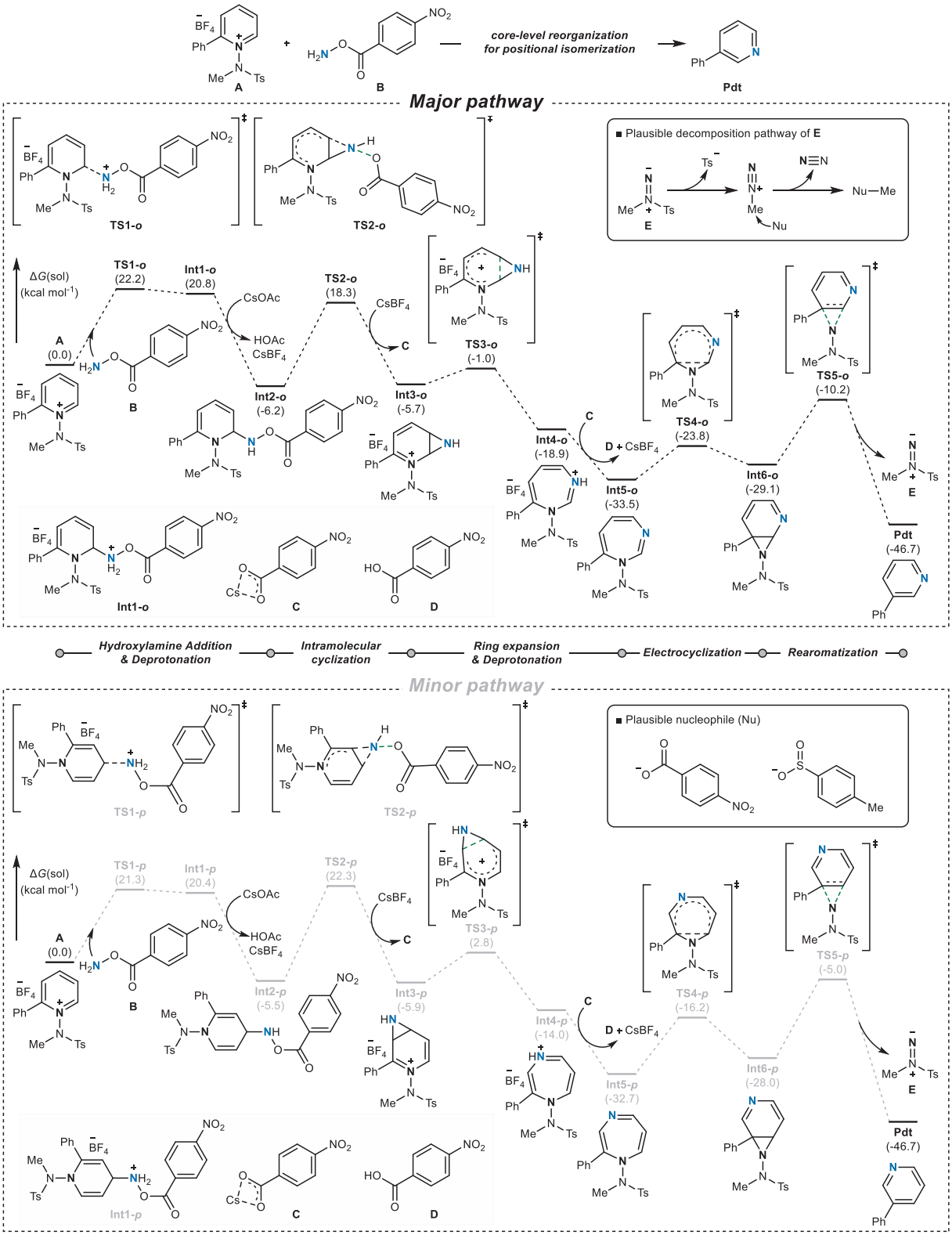


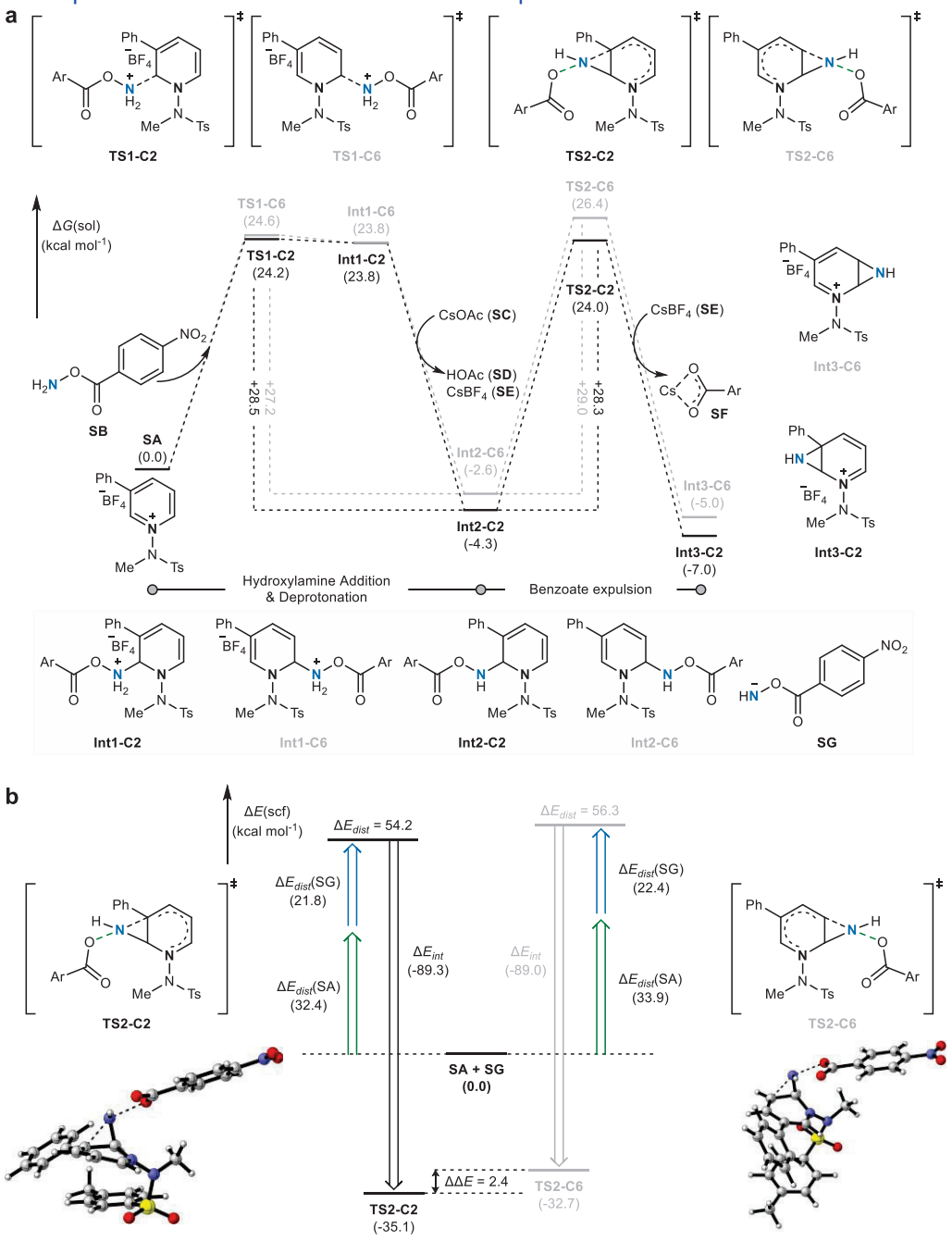
Fig. 5| DFT calculations rationalizing the regioselectivity of the intramolecular cyclization. **a,** Computed free energy profiles for the competing *ortho*- and *para*-selective pathways under toluene conditions. **b**, Free energy profile for the cyclization step under DMF conditions, illustrating the reduced $\Delta\Delta G^{\ddagger}$ relative to toluene. **c**, Optimized geometries of the selectivity-determining transition states **TS2-*o*** and **TS2-*p***.



Supplementary Figure 6. Computational studies of the C2-substituted substrate and proposed reaction mechanism.

Method: ωB97X-D3BJ/def2-TZVPP/CPCM(Toluene)//ωB97X-D3BJ/def2-SVP/CPCM(Toluene).

To elucidate the origin of the site selectivity between the C2 and C6 positions in the C3-phenyl substituted system, computational studies were conducted. The results revealed that the reaction at the C2 (substituted) position has a lower-energy transition state (**TS2-C2**) compared with that at the C6 position (**TS2-C6**). To further investigate the origin of this energy difference, distortion–interaction analysis was conducted. Interestingly, the two transition states exhibited nearly identical interaction energies, whereas a 2.1 kcal mol^{-1} difference was observed in the structural distortion energies. These results indicate that the preferential reaction at the C2 position arises from the lower distortion energy required to access the corresponding transition state. For the OMe substituent, we attribute this behavior to a hydrogen-bonding interaction between the OMe group and the incoming H_2N–OR nucleophile, which likely serves to direct nucleophilic attack toward the OMe-substituted position.



Supplementary Figure 5. Computational studies of the C3-substituted substrate. a, DFT investigation of competitive site selectivity between the C2 and C6 pathways, including computed activation barriers. **b,**

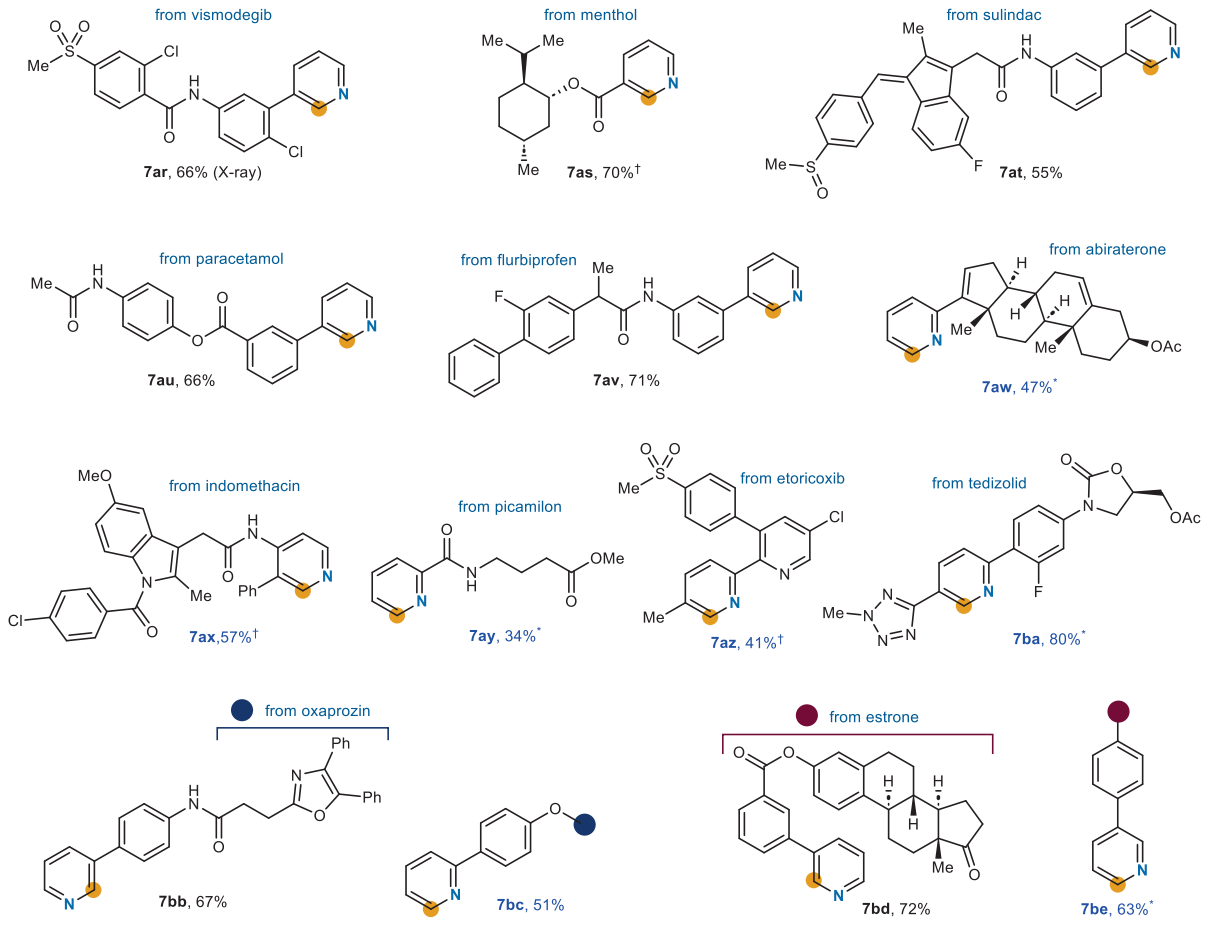
Distortion–interaction analysis of **TS2-C2** and **TS2-C6**. Method: ωB97X-D3BJ/def2-TZVPP/CPCM(THP)//ωB97X-D3BJ/def2-SVP/CPCM(THP).

•Lines 102-103: “Across all major substitution classes, the reaction delivers controlled positional isomerization with high efficiency and fidelity” Author should adjust sentence to accommodate where there are lower yielding reactions e.g. 7j, 7k, 7m.

Response and Action: We thank the reviewer for this helpful comment. To better reflect the experimental data, we have revised the wording in the manuscript to more accurately describe the overall reaction performance.

“The transformation proceeds with generally good fidelity across diverse substitution patterns and functional groups,”

To further evaluate the applicability of the method to structurally complex, drug-like molecules, we substantially expanded the late-stage diversification studies and examined 14 pyridine-containing bioactive molecules and pharmaceuticals that were available to us in Korea. These substrates encompass a broad range of molecular architectures and functional-group combinations. As shown in the revised Fig 4, the transformation proved compatible with derivatives of vismodegib, sulindac, flurbiprofen, indomethacin, abiraterone, tedizolid, etoricoxib, picamilon, oxaprozin, and estrone—spanning anti-cancer agents, NSAIDs, antibiotics, and steroid-based scaffolds. The reaction tolerated a wide array of functional groups, including sulfones, sulfoxides, esters, amides, halides, alkenes, indoles, tetrazoles, oxazoles, and steroid frameworks, underscoring the robustness of the method under structurally demanding conditions.



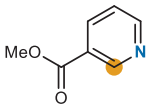
•Fig. 3: correct heading for 7t-7z should be C2,5-substituted not “C2,4-substituted”?

Response and Action: We thank the reviewer for pointing out this error. The errors have been corrected in the revised manuscript.

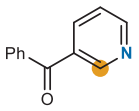
•Fig. 3: Author should comment on what else is observed with lower yielding reactions- mass balance? Isomers where possible?
•Fig. 3: Author should include discussion on substrate scope limitations in manuscript- any comments on why 3-CN substrate gives trace products but 7w gives 50% isolated yield?
•Line 106-107: “ketones (7h) and extended alkyl chains (7i), were readily accommodated” is this statement consistent with their isolated yields? What else is observed with these lower yielding reactions- mass balance? Isomers where possible?
Response and Action: We thank the reviewer for this insightful comment. we address them together below. For substrates that observed reduced yields, the major issue is generally not the formation of multiple isomers, but rather formation of intractable byproducts that could not be fully characterized. In most cases, no significant amounts of alternative isomers were detected by LC–MS analysis of the crude reaction mixtures.
In the case of the 3-CN substrate, only trace product formation was observed, which is consistent with the general trend of this reaction, where highly electron deficient systems exhibit significantly reduced reactivity. This is attributed to excessive electronic deactivation of the pyridine core by the strongly electron-withdrawing cyano group. In contrast, substrate **7w** (**7y** in revised manuscript) afforded a synthetically useful isolated yield (50%). This improvement is likely due to the presence of the *ortho*-methyl substituent, which modulates the electronic distribution of the pyridine ring and partially counterbalances the strong electron-withdrawing effect of the cyano group, thereby maintaining sufficient reactivity for productive transformation.

The following statement has been added to clarify the scope limitations:
"Despite the broad substrate scope, excessively electron-deficient pyridines remained challenging: 3-cyano- and 3,5-diester-substituted derivatives failed to undergo isomerization."

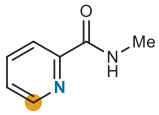
Electron-withdrawing (EWG)



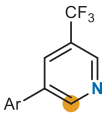
7g, 74%



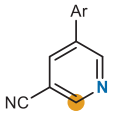
7h, 33%



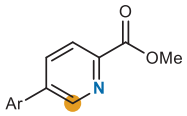
7k, 37%



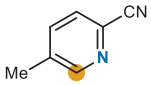
7s, 74%



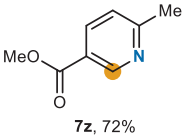
7u, 80%



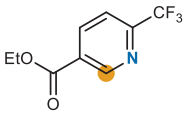
7v, 61%



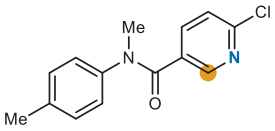
7y, 50%



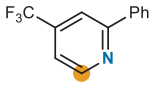
7z, 72%



7aa, 67%

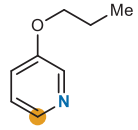


7ab, 50%

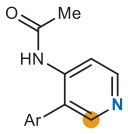


7af, 69%

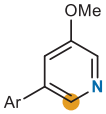
Electron-donating (EDG)



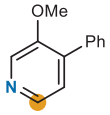
7o, 37%



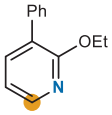
7q, 61%



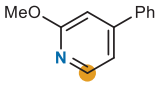
7t, 67%



7ad, 66%

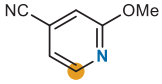


7ae, 69%

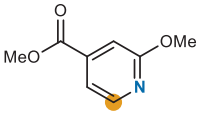


7ah,58%

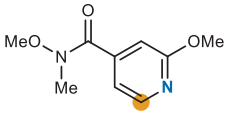
Electronically polarized systems (EWD+EDG)



7ai, 46%

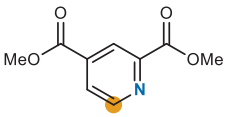


7aj, 78%

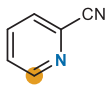


7ak, 72%

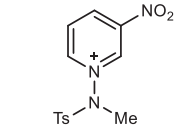
Unsuccessful



trace



trace



Difficulties in salt preparation

Regarding ketone-containing substrate **7h** and extended alkyl substrate **7i**, to better reflect the experimental data, we have revised the wording in the manuscript to more accurately describe the overall reaction performance. In these cases, reduced yields are primarily attributed to competing side reactions, imine formation in the case of ketones and benzylic deprotonation pathways for extended alkyl systems, rather than poor regioselectivity or formation of alternative isomers. Importantly, no significant amounts of alternative isomers were detected by LC–MS analysis of the crude reaction mixtures.

For the ketone (**7h**, 33%), we attribute the diminished yield of the ketone-containing substrate to competing oxime formation between the ketone moiety and the H_2N–OR nucleophile, a well-precedented side reaction. Although the corresponding oxime byproduct was not directly observed in our system, competition with this pathway likely accounts for the reduced reactivity of **7h**.The extended alkyl-substituted substrate **7i** possesses a benzylic position directly connected to the pyridine ring, rendering it susceptible to facile deprotonation under the reaction conditions, consistent with precedent in the literature (*Angew. Chem. Int. Ed.* **2022**, *61*, e202204217).

“Ketones (**7h**) and alkyl chains (**7i**) afforded somewhat lower yields, yet the breadth of compatible substrates establishes a general platform for *ortho*-to-*meta* transposition.”

•Line 108-109: “Pyridines substituted at the C3 position, which are typically challenging to manipulate selectively…” what is the context for this comment as 7n, 7o, 7ad and C2,5-substituted pyridines are high yielding reactions?
Response and Action: We thank the reviewer for this helpful comment. To avoid ambiguity, the phrase “which are typically challenging to manipulate selectively” has been removed from the sentence in the revised manuscript.

“*meta*-Substituted pyridines also participated in the transformation, albeit with modestly reduced efficiency (**7j**, **7k**).”

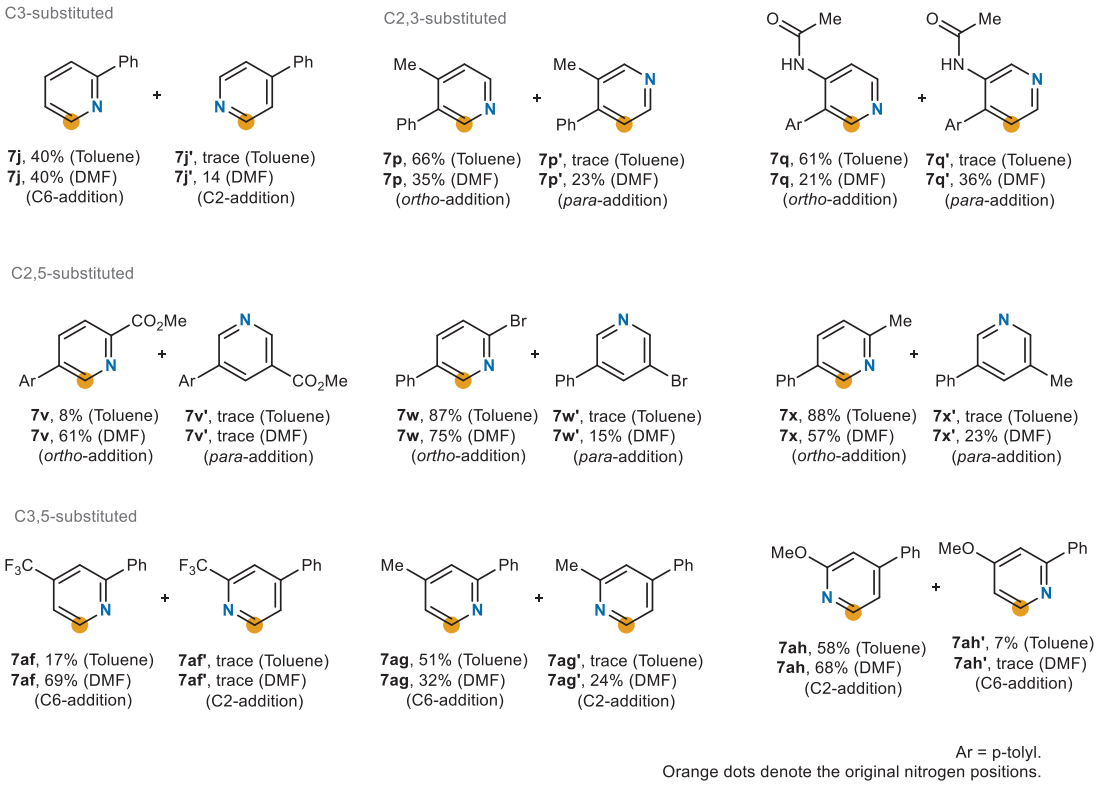
•Line 129: should be bipyridine (7ag) and not “bipyridine (7af)”
•Line 130: should be terpyridine (7ah) and not “terpyridine (7ag)”
Response and Action: We thank the reviewer for pointing out this error. The errors have been corrected in the revised manuscript.

•Fig. 4a: Author should recheck characterization data for compound 7ag - NMR spectra shows presence of minor impurity in the sample, are isomers present and does the yield need to be adjusted? If so, please comment accordingly in the manuscript as currently Line 130 states “…frameworks underwent efficient positional isomerization…”.
Response and Action: We thank the reviewer for pointing out this error. Bipyridine **7ag** (**7ap** in the revised manuscript) did not show the formation of regioisomers. The observed impurity is considered to originate from *O*-(4-nitrobenzoyl)hydroxylamine. The product was purified, and the yield was recalculated (60% to 53%). The corresponding new NMR data have been added to the Supplementary Information.

•Line 136-142: For discussion on selectivity, please include “Supplementary Figure 4. Substrate scope demonstrating solvent-dependent positional selectivity” from the SI (Line 906-910) in the manuscript.
- Is the selectivity observed consistent with computational calculations and mechanistic rationalization across all substrates?
Response and Action: We appreciate this valuable suggestion. As recommended, we expanded the analysis by including additional substrates to further demonstrate the solvent-dependent positional selectivity (see revised Supplementary Fig. 5).

Accordingly, the following sentence was revised:
“This solvent-dependent behavior was consistently observed across additional substrates (**2j**, **2p**, and **2w**; see Supplementary Fig. 5 for further examples).”

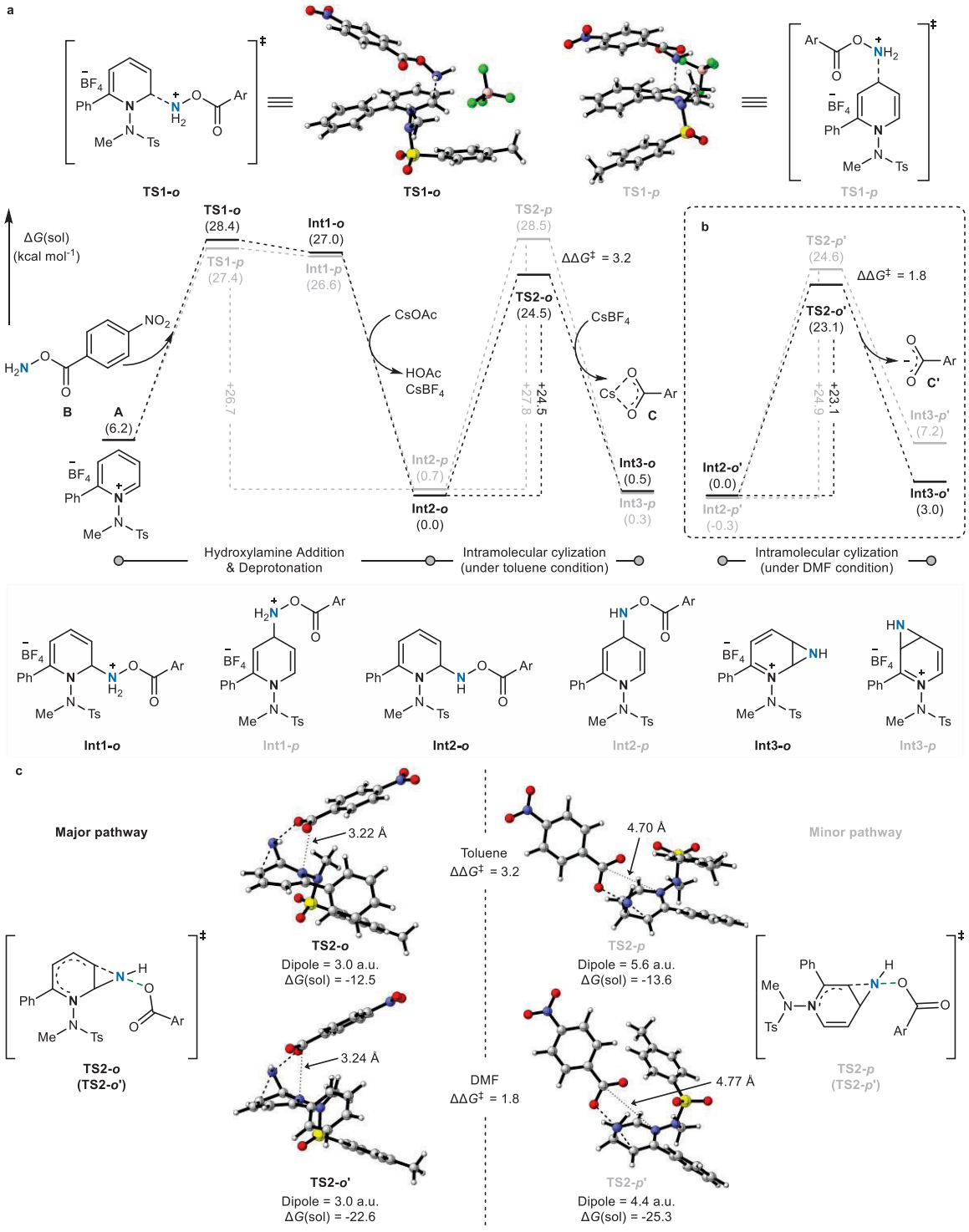
In addition, we added the following discussion to further clarify the origin of the selectivity:
“The diminished selectivity in DMF is attributed to enhanced stabilization of the charge-separated transition state in the more polar medium, which reduces the $\Delta\Delta G^{\ddagger}$ between competing pathways (Fig. 5b,c and Supplementary Fig. 6).”



•Line 140-142: "The reduced regioselectivity observed in DMF is consistent with enhanced solvation of the hydroxylamine nucleophile, which may diminish its sensitivity to subtle differences in electrophilic character between the C2 and C4 positions of the pyridine core"

- Are the solvent effects observed consistent with computational assessments?

Response and Action: We thank the reviewer for this question. As shown earlier, the computed solvent effects are indeed consistent with our experimental observations. In the more polar solvent DMF, the charge-separated character that develops at the selectivity-determining transition state is more effectively stabilized than in toluene, which reduces the $\Delta\Delta G^{\ddagger}$ between the competing cyclization transition states (Fig. 5b,c and Supplementary Fig. 6). This smaller energetic difference accounts for the diminished regioselectivity measured under DMF conditions.Taken together, these results support a Curtin–Hammett-controlled mechanism in which rapidly equilibrating intermediates undergo irreversible, selectivity-determining cyclization. The preferential formation of **Int3-*o*** therefore arises from the intrinsically lower activation barrier of **TS2-*o***, rather than from the relative thermodynamic stability of the preceding intermediates. These computational results have been added to the revised manuscript as Fig. 5 and Supplementary Information.



•Line 149: "…high fidelity across diverse substitution patterns and functional groups" Is this statement consistent with the observed presence of isomers and lower yields in instances?

Response and Action: We thank the reviewer for this helpful comment. To avoid overstatement and better reflect the experimental data, we have revised the wording in the manuscript accordingly.

The sentence now reads: “The transformation proceeds with generally good fidelity across diverse substitution patterns and functional groups.”

SI
•Line 47: consistency between temperature in scheme and procedure, 80 oC vs 100 oC
•Line 59: consistency between temperature in scheme and procedure, 80 oC vs 100 oC
Response and Action: We thank the reviewer for pointing out this error. We revised both to 100 ºC

•Appropriate use of statistics and treatment of uncertainties: as detailed above
•Conclusions: robustness, validity, reliability: as detailed above
•Suggested improvements: experiments, data for possible revision: as detailed above

•References: appropriate credit to previous work? Line 35-36: “Skeletal editing strategies, which allow selective modification of aromatic frameworks, offer a powerful conceptual foundation for this approach” References for this statement should include: Blakemore DC et al. Organic synthesis provides opportunities to transform drug discovery. Nat. Chem 10, 383–394 (2018).
Response and Action: We thank the reviewer for this helpful suggestion. The recommended reference (Blakemore et al., Nat. Chem. 2018) has now been added to in the revised manuscript (Ref 15)

Referees' comments:

Referee #1 (Remarks to the Author):

I thank the authors for their effort in revision. The mechanistic additions — the GC–MS $^{15}N/^{14}N$ isotope experiment, the ^{13}C-labeled solvent selectivity study, and the Curtin–Hammett DFT analysis — are genuine improvements and strengthen the paper's mechanistic foundation. However, the central concern I raised in Round 1 remains unresolved, and it goes to the core of what justifies publication in Nature.

Critical Concern: No Real Pyridine-Containing Drug Has Been Tested, and No Practical Advantage Over De Novo Synthesis Has Been Demonstrated

Figure 4a is presented as evidence of "synthetic utility" through "drug-derived substrates." Upon close reading, every compound in that figure is a synthetic conjugate in which a pyridine unit has been artificially appended to a fragment from a known drug or bioactive molecule. The pyridine rings being isomerized are not the native pharmacophoric elements of any parent drug — they are synthetic appendages installed specifically to enable this chemistry. Not one actual pyridine-containing drug has been subjected to the isomerization conditions with its native pyridine intact. This is not a presentational issue; it is a scientific one. Figure 4 does not demonstrate what it is claimed to demonstrate.

This concern is compounded by the compatibility limitations acknowledged in lines 154–156: substrates bearing free amines, alcohols, and carboxylic acids are incompatible with the pyridinium salt preparation step. These are precisely the functional groups present in the overwhelming majority of real pyridine-containing drugs. In practice, applying this method to an actual drug molecule would require protection of nucleophilic groups, three-step pyridinium salt preparation, the isomerization itself, and final deprotection — a minimum of five to six operations on a molecule already in hand. What is the overall yield across this sequence for any representative real substrate? If the isomerization step proceeds at 60–80% yield but the cumulative yield across the full sequence is 10–20%, the practical advantage of purchasing or independently synthesizing the target is unclear at best and negative at worst.

This raises a question the authors must address directly: who, in a pharmaceutical or medicinal chemistry setting, would realistically use this method? A drug discovery team conducting SAR exploration needs to access positional isomers rapidly and efficiently. If the method requires installing a synthetic pyridine handle, protecting multiple functional groups, executing a multi-step sequence, and then deprotecting — all on a molecule that already exists — what advantage does it offer over a straightforward de novo synthesis of the desired isomer? The manuscript's claims about accelerating SAR exploration and enabling late-stage diversification require a concrete answer to this question, not an implied one.

The authors must therefore provide at least one example using a genuine pyridine-containing drug or advanced pharmaceutical intermediate, reporting the full step count and overall yield from the drug to the isomerized product, alongside an honest comparison with the de novo route. If the method offers fewer steps, higher overall yield, or access to an otherwise synthetically inaccessible isomer, that would be a compelling and convincing case. If no such advantage can be shown, the pharmaceutical utility framing must be substantially revised.

To be clear, the positional isomerization chemistry in Figures 2 and 3 is creative and mechanistically well supported. It stands as a meaningful contribution to skeletal editing on its own merits. But the framing of Figure 4 as late-stage pharmaceutical diversification, and the claims in the abstract and conclusion about accelerating drug discovery, are not supported by the data. Presenting synthetic pyridine conjugates as drug molecules and asserting practical pharmaceutical utility without demonstrating an efficiency advantage is a form of overclaiming that is increasingly prevalent in the skeletal editing literature and should not pass unchallenged in this journal.

I therefore ask the authors to take one of two paths:

Provide at least one genuine pyridine-drug example with the full sequence reported — step count, overall yield, and direct comparison to de novo synthesis; or

Revise the abstract, Figure 4 description, and conclusion to accurately characterize the substrates as synthetic pyridine conjugates of drug-derived fragments, remove the late-stage pharmaceutical applicability claims, and reframe the contribution as a powerful new method for pyridine positional isomerization demonstrated across complex molecular environments.

If neither path is taken, I do not consider this manuscript suitable for publication in Nature in its current form.

Overall Recommendation: Major Revision

The core chemistry is impressive, and the mechanistic support is now solid. But the pharmaceutical utility claims — which are central to the manuscript's framing and its case for Nature — remain unsupported. No real pyridine drug has been tested. No efficiency advantage over de novo synthesis has been demonstrated.

The functional group incompatibilities are significant and underacknowledged. These gaps must be filled, or the claims revised to honestly reflect what has actually been shown.

Response and Action: We thank the reviewer for this feedback. We would like to clarify that the previous revision already included three marketed pyridine-containing drugs—vismodegib, abiraterone acetate, and etoricoxib—as representative examples. We recognize, however, that these compounds were presented together with other structurally complex substrates, making this point insufficiently apparent in both the revised figure and our response letter. We have therefore reorganized the presentation to clearly distinguish these marketed drug examples (Fig. 4, bottom), making this aspect unambiguous, as detailed below.

We also agree that our original presentation overstated the pharmaceutical implications of the method. This stemmed in part from an insufficiently clear distinction between two conceptually different substrate classes: *drug-derived pyridine conjugates*, in which a pyridine unit is appended to a bioactive scaffold, and *pyridine-containing drugs*, in which the pyridine ring is embedded within the drug's core structure. Conflating these under a single "late-stage functionalization" framing created ambiguity regarding what the method actually demonstrates.

First, we have removed statements throughout the Abstract, the Figure 4 description, main text, and the Conclusion that implied broad “late-stage pharmaceutical applicability”. The study is now framed as a strategy for pyridine positional isomerization across structurally complex molecular environments, a claim we believe is fully supported by the experimental results without overstating pharmaceutical relevance.

Specifically, we have made the following revisions:

"~is compatible with pharmaceutically relevant molecules" to *"~operates across structurally complex molecular environments"*

"~accommodates complex pharmaceuticals~" to *"~applies to structurally complex molecular architectures~"*

"To demonstrate the synthetic utility of this platform, a diverse set of drug-derived substrates were subjected to the standard conditions (Fig. 4a)." to *"To evaluate the method in structurally complex settings, a diverse set of elaborated pyridine substrates was subjected to the standard conditions (Fig. 4a)."*

“~across diverse substitution patterns and functional groups, enabling application to structurally complex, medicinally relevant scaffolds.” to *“~across diverse substitution patterns and functional groups, and extends to structurally complex molecular scaffolds.”*

“By eliminating the need for independent de novo syntheses of each positional isomer, this protocol enables late-stage diversification and accelerates SAR exploration in drug discovery.” to *“This method thus provides a unified route to positional isomers that are otherwise difficult to access, obviating the need for independent de novo syntheses.”*

Second, to directly address the reviewer's concern regarding authentic pyridine-containing pharmaceuticals, we have reorganized Figure 4 to clearly present three such drugs—vismodegib, abiraterone acetate, and etoricoxib—in which the pyridine is embedded within the core structure. To more transparently illustrate the practical efficiency of the overall sequence starting from the parent drugs, the figure has also been redesigned to explicitly display the overall isolated yields for each transformation. The corresponding overall isolated yields from the parent drugs were 44% for vismodegib, 31% for abiraterone acetate and etoricoxib for 31%, which are now explicitly reported in the revised manuscript. Each was carried through the complete sequence, and the corresponding discussion has been revised as follows:

*"We next applied the method to pyridine-containing drugs, in which the pyridine is embedded within the core structure. Vismodegib (**1bc**), a Hedgehog pathway inhibitor used to treat advanced basal cell carcinoma, was cleanly converted to **7bc** in 44% overall yield over the sequence (X-ray data in the Supplementary Information). Abiraterone acetate (**1bd**), an androgen biosynthesis inhibitor used for the*

*treatment of prostate cancer, was similarly transformed into **7bd** in 31% overall yield. Etoricoxib (**1be**), a selective COX-2 inhibitor used for the treatment of inflammatory disorders, likewise underwent efficient isomerization to afford **7be** in 31% overall yield."*

*"Substrates bearing free nucleophilic groups, such as amines, alcohols, and carboxylic acids, are incompatible with the pyridinium salt preparation step, but these functionalities are readily accommodated by prior protection. For example, tedizolid (**7aw**) and picamilon (**7ax**) were successfully converted once masked as their corresponding acyl and ester derivatives, respectively."*

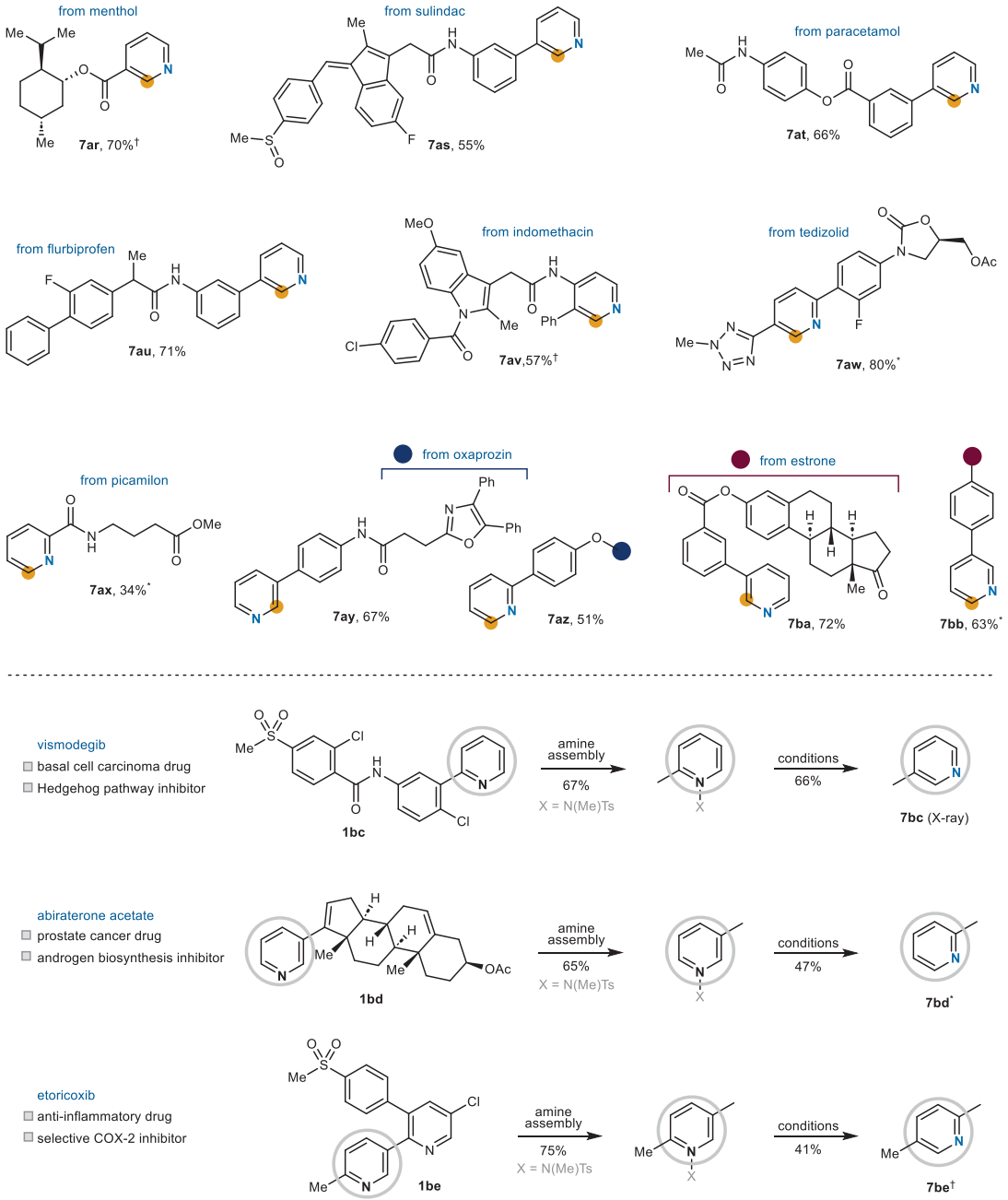


Fig. 4| Application to structurally complex pyridines. Orange dots denote the original nitrogen positions. †Reaction in toluene (0.05 M). *Reaction in DMF (0.05 M).

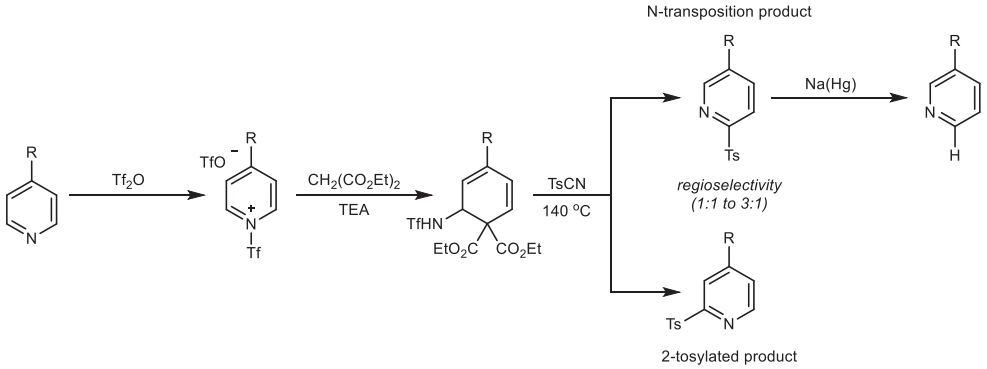
Referee #2 (Remarks to the Author):
Although the authors have addressed most of the issues raised in the previous round of review, several concerns still remain.

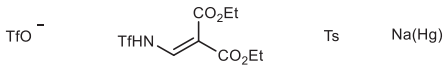
1. A closely related pyridine nitrogen-transposition reaction has recently been reported by Greaney and co-workers, who achieved pyridine C–N transposition through a cycloaddition/cycloreversion (CACR) strategy using TsCN as the dienophile (Angew. Chem. Int. Ed. 2026, e5249878). Although the strategy differs from that described in the present manuscript, the overall synthetic objective is highly similar. This recent work should be cited and discussed to better clarify the novelty and distinctive features of the present approach.
2. The practical and atom-economic advantages of the present method remain insufficiently clear. In the Greaney protocol, pyridine activation and transposition can be achieved through a shorter sequence, whereas the present method requires several operations to generate the N–N salt intermediate before the final transposition step. In addition, MSH is known to be unstable and requires separate preparation, which further complicates the overall process. The authors should provide a more balanced discussion of the operational efficiency, step economy, and atom economy of their method relative to existing approaches.

Response and Action: We thank the reviewer for this valuable suggestion. During the revision of our manuscript, two related studies on pyridine positional editing were reported by Greaney and co-workers and by Chiba and co-workers, further underscoring the growing interest in pyridine isomerization. We have cited both studies (15 and 42) and expanded the Introduction to more clearly distinguish the scope and characteristics of the present strategy.

Greaney and co-workers reported a cycloaddition/cycloreversion (CACR) strategy for pyridine C–N transposition using TsCN as the dienophile. This transformation is effective primarily for C4-substituted pyridines, with reaction selectivity strongly dependent on the steric demand of the substituent; no examples of *ortho*-substituted substrates were reported. The CACR process also generally affords mixtures of the desired N-transposition product and a competing 2-tosylated regioisomer. Furthermore, use of TsCN results in replacement of both the ring nitrogen and a native ring carbon with newly introduced atoms, and the products retain an *ortho*-tosyl substituent, thereby precluding direct access to the corresponding positional isomers of the parent pyridines.

With respect to the overall efficiency, we compared our sequence with the representative example reported by Greaney and co-workers using 4-*tert*-butylpyridine as a common benchmark. Based on the reported isolated yields, the overall yield of the Greaney sequence is **22%** (99% for substrate preparation × 32% for the cycloaddition/cycloreversion step × 70% for the Na(Hg) reduction). In contrast, our method provides an overall yield of **45%** (90% for N–N salt synthesis × 50% for the isomerization step). Thus, our sequence delivers approximately a twofold higher overall yield from the same pyridine starting material. It is also noteworthy that the Greaney protocol requires a high-temperature cycloaddition/cycloreversion step (140 °C) followed by a sodium amalgam reduction to remove the tosyl group, which requires the use of a mercury-containing reducing agent.





Chemical Formula: $C_{17}H_{19}F_6HgNNaO_{11}S_3^-$
Exact Mass: 847.9634

In terms of stoichiometric waste generation, the Greaney sequence produces substantially larger amounts of byproducts. Even excluding the sodium amalgam reduction step, the total mass of stoichiometric byproducts is approximately 100 Da greater than that of our protocol. When the Na(Hg) reduction is also taken into account, the difference becomes even more pronounced.

We have also discussed the recent work by Chiba and co-workers, who developed a radical-mediated aryl migration strategy for pyridines. While this method enables selective migration of *ortho*- or *para*-aryl groups to the *meta* position, it remains limited to aryl substituents and therefore does not constitute a general positional isomerization strategy applicable to diverse substituent classes.

Specifically, the following sentences have been added to the Introduction of the revised manuscript:

"Recently, Chiba elegantly demonstrated a radical-mediated aryl migration strategy enabling selective meta-relocation of ortho- or para-aryl substituents on pyridines, though the approach remains limited to aryl groups.[15]"

"Most recently, Greaney reported a cycloaddition/cycloreversion strategy for pyridine C–N transposition using TsCN as the dienophile.[42] The method is effective primarily for C4-substituted pyridines and typically affords mixtures of the desired N-transposition product and a competing 2-tosylated regioisomer while retaining an ortho-tosyl substituent."

3. Have the authors attempted to conduct the transformation in a one-pot manner? If feasible, such a procedure would significantly improve the practical appeal of the methodology. If unsuccessful, the authors should briefly discuss the main limitations or incompatibilities encountered.

Response and Action: We thank the reviewer for raising this important point. We attempted to perform the sequence in a one-pot manner. However, the tosylation step requires an aqueous work-up before the subsequent isomerization can proceed; when this work-up was omitted, residual reagents and byproducts interfered with the isomerization and the reaction did not proceed efficiently. Although a one-pot protocol was not feasible, the salts can, in most cases, be isolated by simple recrystallization and used directly in the subsequent isomerization step without chromatographic purification. We have briefly clarified this limitation in the revised manuscript. "The requisite *N*-amidopyridinium salts are readily accessed from the corresponding pyridines in three steps and, in most cases, isolated by simple recrystallization without chromatographic purification (see Supplementary Information).[45,46]"

To more transparently illustrate the practical efficiency of the overall sequence starting from the parent drugs, the figure has also been redesigned to explicitly display the overall isolated yields for each transformation. The corresponding overall isolated yields from the parent drugs were 44% for vismodegib, 31% for abiraterone acetate and etoricoxib for 31%, which are now explicitly reported in the revised manuscript. Each was carried through the complete sequence, and the corresponding discussion has been revised as follows:

*"We next applied the method to pyridine-containing drugs, in which the pyridine is embedded within the core structure. Vismodegib (**1bc**), a Hedgehog pathway inhibitor used to treat advanced basal cell carcinoma, was cleanly converted to **7bc** in 44% overall yield over the sequence (X-ray data in the Supplementary Information). Abiraterone acetate (**1bd**), an androgen biosynthesis inhibitor used for the treatment of prostate cancer, was similarly transformed into **7bd** in 31% overall yield. Etoricoxib (**1be**), a selective COX-2 inhibitor used for the treatment of inflammatory disorders, likewise underwent efficient isomerization to afford **7be** in 31% overall yield."*

Further clarification of this limitation is provided in our response to Comment 4 below.

4. From an application perspective, the current examples do not yet fully demonstrate the practical utility of the method. The drug-molecule derivatives appear to be prepared mainly through routine derivatization rather than through transformations that uniquely benefit from the present nitrogen-transposition strategy. Additional examples involving late-stage transposition of more complex pyridine-containing molecules, or a clearer explanation of how this approach provides access to otherwise difficult-to-obtain isomers, would substantially strengthen the manuscript.

Response and Action: We thank the reviewer for this feedback. We would like to clarify that the previous revision already included three marketed pyridine-containing drugs—vismodegib, abiraterone acetate, and etoricoxib—as representative examples. We recognize, however, that these compounds were presented together with other structurally complex substrates, making this point insufficiently apparent in both the revised figure and our response letter. We have therefore reorganized the presentation to clearly distinguish these marketed drug examples (Fig. 4, bottom), making this aspect unambiguous, as detailed below.

We also agree that our original presentation overstated the pharmaceutical implications of the method. This stemmed in part from an insufficiently clear distinction between two conceptually different substrate classes: *drug-derived pyridine conjugates*, in which a pyridine unit is appended to a bioactive scaffold, and *pyridine-containing drugs*, in which the pyridine ring is embedded within the drug's core structure. Conflating these under a single "late-stage functionalization" framing created ambiguity regarding what the method actually demonstrates.

First, we have removed statements throughout the Abstract, the Figure 4 description, main text, and the Conclusion that implied broad “late-stage pharmaceutical applicability”. The study is now framed as a strategy for pyridine positional isomerization across structurally complex molecular environments, a claim we believe is fully supported by the experimental results without overstating pharmaceutical relevance.

Specifically, we have made the following revisions:

"~is compatible with pharmaceutically relevant molecules" to *"~operates across structurally complex molecular environments"*

"~accommodates complex pharmaceuticals~" to *"~applies to structurally complex molecular architectures~"*

"To demonstrate the synthetic utility of this platform, a diverse set of drug-derived substrates were subjected to the standard conditions (Fig. 4a)." to *"To evaluate the method in structurally complex settings, a diverse set of elaborated pyridine substrates was subjected to the standard conditions (Fig. 4a)."*

“~across diverse substitution patterns and functional groups, enabling application to structurally complex, medicinally relevant scaffolds.” to *“~across diverse substitution patterns and functional groups, and extends to structurally complex molecular scaffolds.”*

“By eliminating the need for independent de novo syntheses of each positional isomer, this protocol enables late-stage diversification and accelerates SAR exploration in drug discovery.” to *“This method thus provides a unified route to positional isomers that are otherwise difficult to access, obviating the need for independent de novo syntheses.”*

Second, to directly address the reviewer's concern regarding authentic pyridine-containing pharmaceuticals, we have reorganized Figure 4 to clearly present three such drugs—vismodegib, abiraterone acetate, and etoricoxib—in which the pyridine is embedded within the core structure. To more transparently illustrate the practical efficiency of the overall sequence starting from the parent drugs, the figure has also been redesigned to explicitly display the overall isolated yields for each transformation. The corresponding overall isolated yields from the parent drugs were 44% for vismodegib, 31% for abiraterone acetate and etoricoxib for 31%, which are now explicitly reported in the revised manuscript. Each was carried through the complete sequence, and the corresponding discussion has been revised as follows:

*"We next applied the method to pyridine-containing drugs, in which the pyridine is embedded within the core structure. Vismodegib (**1bc**), a Hedgehog pathway inhibitor used to treat advanced basal cell*

*carcinoma, was cleanly converted to **7bc** in 44% overall yield over the sequence (X-ray data in the Supplementary Information). Abiraterone acetate (**1bd**), an androgen biosynthesis inhibitor used for the treatment of prostate cancer, was similarly transformed into **7bd** in 31% overall yield. Etoricoxib (**1be**), a selective COX-2 inhibitor used for the treatment of inflammatory disorders, likewise underwent efficient isomerization to afford **7be** in 31% overall yield."*

*"Substrates bearing free nucleophilic groups, such as amines, alcohols, and carboxylic acids, are incompatible with the pyridinium salt preparation step, but these functionalities are readily accommodated by prior protection. For example, tedizolid (**7aw**) and picamilon (**7ax**) were successfully converted once masked as their corresponding acyl and ester derivatives, respectively."*

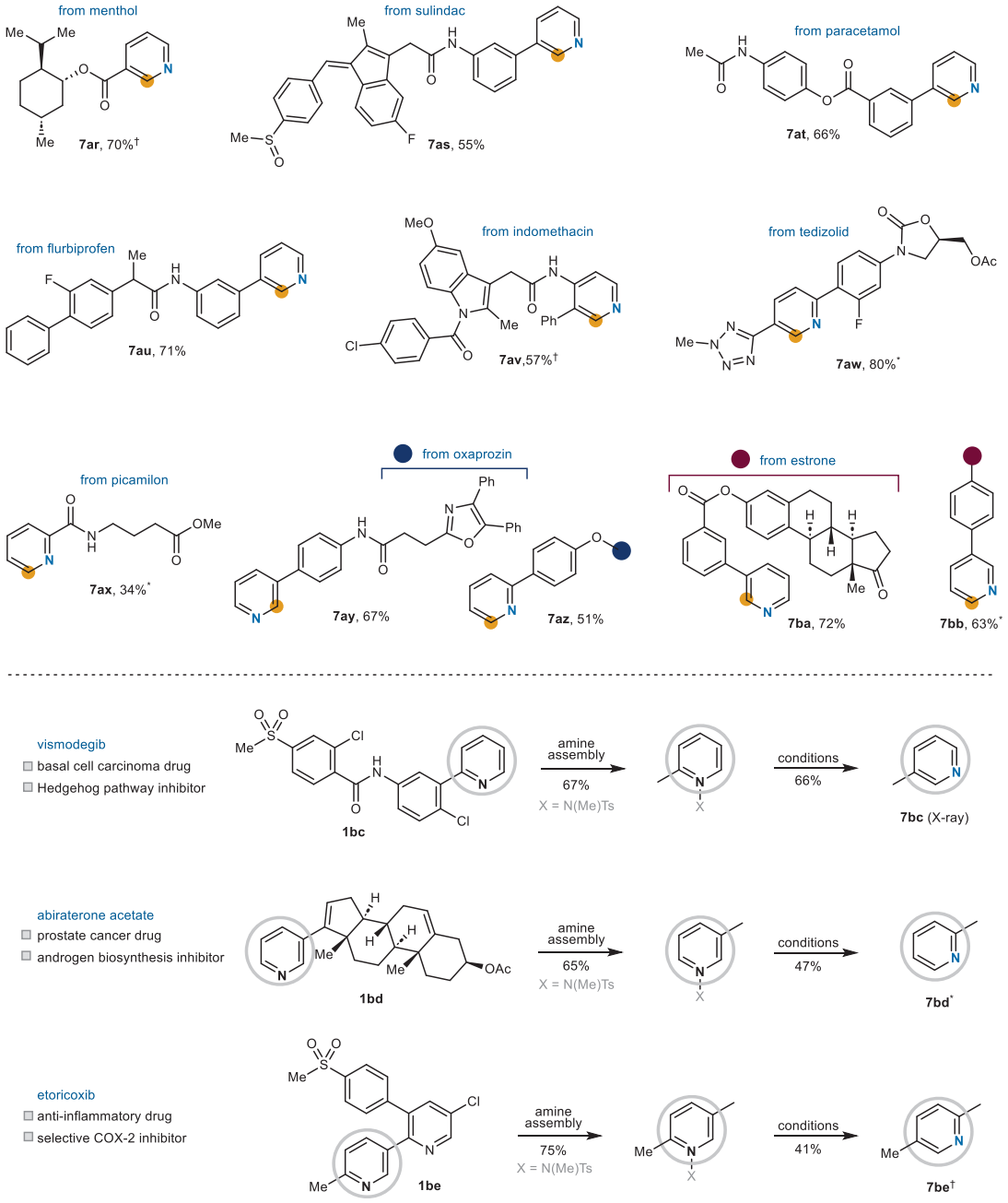


Fig. 4| Application to structurally complex pyridines. Orange dots denote the original nitrogen positions. †Reaction in toluene (0.05 M). *Reaction in DMF (0.05 M).

Referee #1

The chemistry itself continues to merit publication in a strong venue. But the manuscript's central claim, that this method offers a practical advantage for accessing pyridine positional isomers relevant to drug discovery, is still not supported by the data as presented, and the yield reporting in its current form does not give readers an accurate picture of the method's efficiency. I would ask the authors to address these points directly and completely in the next round.

Requested revisions for this round:

1. Provide step counts alongside overall yields for the vismodegib, abiraterone acetate, and etoricoxib sequences, and include a direct, honest comparison to the de novo synthesis of at least one of these isomers.

Response and Action: We thank the reviewer for this constructive suggestion. As suggested, in Fig. 4, where the practical application of the overall sequence to drugs is the central point, we report overall yields from the parent drug together with the individual step yields, with the starting point of each calculation explicitly defined in the figure and its legend.

-Fig. 4: Black values denote overall yields from pyridine **1**; parenthetical values denote step yields for salt formation (**1**→**2**, gray) and isomerization (**2**→**7**, blue). The general scheme has also been revised to depict the salt-formation step (**1**→**2**) explicitly, clarifying the two-stage sequence.

Each conversion of vismodegib, abiraterone acetate, and etoricoxib is now presented as a two sequence—salt formation (**1**→**2**) followed by isomerization (**2**→**7**). The individual step yields are shown, and the product labels report the overall yields from the respective parent drugs.

The corresponding text has been revised as follows:

"Vismodegib (**1bc**), a Hedgehog pathway inhibitor used to treat advanced basal cell carcinoma, was cleanly converted to **7bc** in 44% overall yield from the parent drug **1bc** (X-ray data in the Supplementary Information). Abiraterone acetate (**1bd**), an androgen biosynthesis inhibitor used for the treatment of prostate cancer, was similarly transformed into **7bd** in 31% overall yield from **1bd**. Etoricoxib (**1be**), a selective COX-2 inhibitor used for the treatment of inflammatory disorders, likewise afforded **7be** in 31% overall yield from **1be**."

To provide a transparent *de novo* comparison, we have also added to Fig. 4 a plausible *de novo* route to the etoricoxib-derived isomer **7be**. Because these positional isomers of marketed drugs are, to our knowledge, previously unreported, no direct synthetic precedent exists for the exact targets. We have therefore outlined a plausible *de novo* route based on a key intermediate and coupling transformations reported in the synthesis of the parent drug (ref. 47). The main text now states:

"For comparison, a plausible *de novo* route to the same etoricoxib-derived isomer is shown in Fig. 4, proceeding from 2-hydroxypyridine through two palladium-catalyzed couplings.[47] Because the proposed route has not been experimentally evaluated, its overall yield cannot be meaningfully compared with that of the editing sequence. The two strategies are complementary: skeletal editing offers a concise, palladium-free entry directly from the parent drug, whereas the *de novo* route draws on established transformations from readily available precursors. More broadly, skeletal editing accesses positional isomers from an existing scaffold without designing a separate *de novo* synthesis for each target."

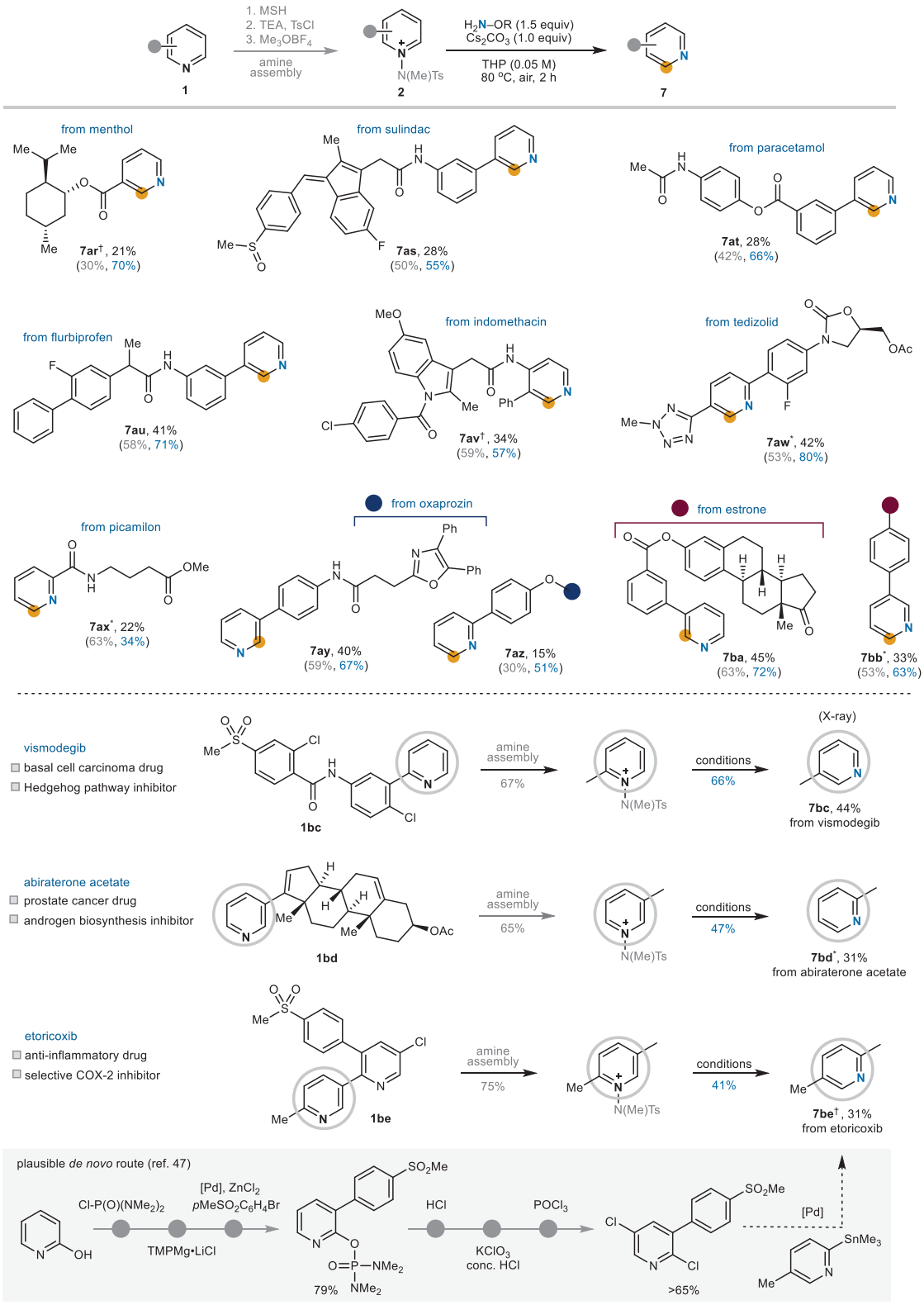


Fig. 4| Application to structurally complex pyridines. Orange dots denote the original nitrogen positions. †Isomerization reaction in toluene (0.05 M). *Isomerization reaction in DMF (0.05 M). Black values denote overall yields from pyridine **1**; parenthetical values denote step yields for salt formation (**1**→**2**, gray) and isomerization (**2**→**7**, blue). MSH, O-(mesitylsulfonyl)hydroxylamine.

2. Clearly and consistently label every yield in the manuscript (Figures 2, 3, and 4, and the SI) as either a step yield or an overall yield, with the starting point of the "overall yield" calculation explicitly defined.

Response and Action: We thank the reviewer for this important point and have clarified the yield reporting throughout the manuscript. As suggested, every yield in the revised manuscript, main figures, and Supplementary Information is now labeled unambiguously as either a step yield or an overall yield, with the starting point specified.

In Fig. 4, where the practical application of the overall sequence to drugs is the central point, we report overall yields from the parent drug together with the individual step yields, with the starting point of each calculation explicitly defined in the figure and its legend (see our response to Comment 1).

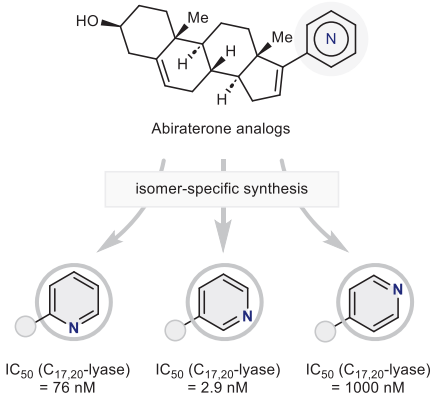
-Fig. 4: Black values denote overall yields from pyridine **1**; parenthetical values denote step yields for salt formation (**1**→**2**, gray) and isomerization (**2**→**7**, blue). The general scheme has also been revised to depict the salt-formation step (**1**→**2**) explicitly, clarifying the two-stage sequence.

Figures 2 and 3 are dedicated to the development, mechanistic study, and substrate scope of the new isomerization reaction, where the isomerization step itself is the focus of methodological interest. For clarity, these schemes depict only the pyridinium salts **2** and the isomerized products **7**. The salts **2** are prepared by a well-established procedure; full experimental details and yields are provided in the Supplementary Information. Accordingly, the yields reported in these figures are step yields for the isomerization, which we have now labeled explicitly and consistently as follows:

-Fig. 2: Yield of **7a** is a step yield from pyridinium salt **2a**.
-Fig. 3: Yields of **7** are step yields from pyridinium salts **2**.
-Supplementary Information: Yields of **7** are step yields from pyridinium salts **2**.

3. Either demonstrate positional isomers of omidenepag consistent with the framing used in the introduction, or remove/replace the example with one the manuscript substantiates.

Response and Action: We thank the reviewer for this suggestion. As suggested, we have replaced omidenepag with abiraterone (Fig 1a). The introduction now frames the significance of positional isomer access using abiraterone, and a corresponding reference has been added (ref. 2) in the revised manuscript.



Referee #2

The authors have adequately addressed the requested revisions and responded clearly to the reviewers' comments. The manuscript is now suitable for publication.

Response and Action: We sincerely thank the reviewer for the careful evaluation of our manuscript and for the supportive recommendation. We are grateful for the constructive comments provided throughout the review process, which have helped us strengthen the manuscript.

Referee #1

1. Please ensure your abstract is fully referenced, subsuming introductory material into it if needed.

Response and Action: We confirm that the Abstract is fully referenced.

2. Please ensure that the Supplementary Information is mentioned specifically throughout the article (eg. "see Supplementary Information Section/Figure/Table XY") and not generically as "see Supplementary Information"

Response and Action: We have revised the manuscript accordingly to refer specifically to the relevant Supplementary Information sections, figures, and tables throughout.

3. Please ensure graphical elements in the Figures (dividing lines between panels and boxes, for example) are kept only where they confer meaning.

Response and Action: We have retained graphical elements in the Figures only where they are necessary to convey meaning.

4. We typically include 4 modest figure displays in articles. Please consider moving the DFT calculations to an Extended Data Figure.
Response and Action: We have moved the DFT calculations to an Extended Data Figure, as suggested.

Extended Date Fig. 1| DFT calculations rationalizing the regioselectivity of the intramolecular cyclization.

5. Figures: Please only keep italics in the figures if chemically required. Also, bold formatting should only be kept for compound numbers/labels.

Response and Action: We have revised the Figures accordingly.

6. Please provide a definition for all the abbreviations used in the different panels in the figure caption.

Response and Action: We have defined all abbreviations used in the corresponding figure captions.

7. Please note that, per our updated guidelines, the acknowledgements and funding sections are now separate.

Response and Action: We Thank you for pointing this out. As requested, we have separated the acknowledgments and funding into two distinct sections.

Referee #1

The authors have adequately addressed the requested revisions and clearly responded to the reviewers' comments. The manuscript is now suitable for publication.

Response and Action: We sincerely thank the reviewer for the careful evaluation of our manuscript and for the supportive recommendation. We are grateful for the constructive comments provided throughout the review process, which have helped us strengthen the manuscript.