



Cite this: *Chem. Commun.*, 2024, **60**, 9428

Received 18th July 2024,
Accepted 5th August 2024

DOI: 10.1039/d4cc03596h

rsc.li/chemcomm

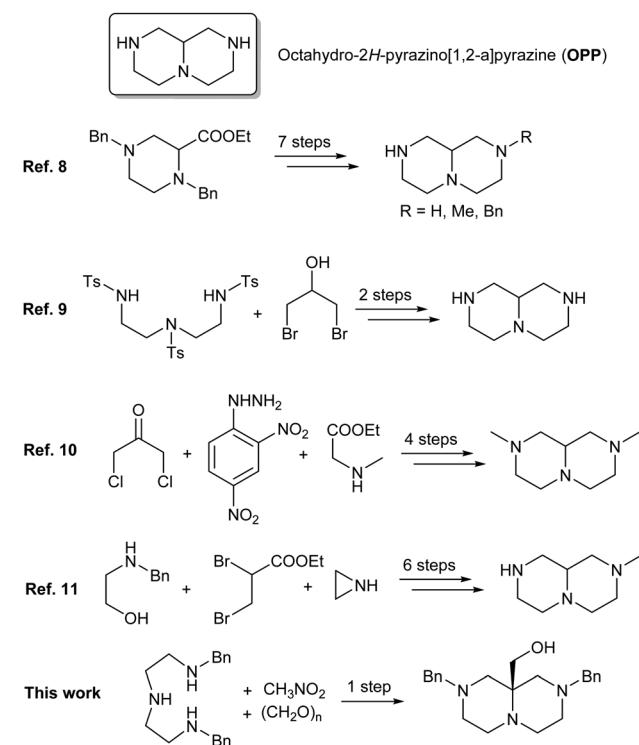
An unexpected nitro group displacement during a nitro-Mannich reaction led to the one-pot formation of the octahydro-2*H*-pyrazino[1,2-*a*]pyrazine core, representing the shortest access to date to this pharmacologically relevant heterobicyclic system. A mechanistic hypothesis is suggested and supported by specific experiments and HRMS analysis of reaction mixtures.

The heterobicyclic core of octahydro-2*H*-pyrazino[1,2-*a*]pyrazines (OPPs) (Scheme 1) is the subject of an increasing number of medicinal chemistry studies, focused on its pharmacophoric properties. OPPs have been extensively investigated as putative β -turn mimetics^{1,2} and as 5-HT_{2C} receptor agonists.³ OPP derivatives were patented as IgE inhibitors,⁴ while inhibitor activity was ascribed to OPPs against renal outer medullary potassium channel (ROMK),⁵ ubiquitin specific peptidase 30 (USP30)⁶ and the important lung adenocarcinoma-related mutant oncogene KRAS^{G12C}.⁷

The synthesis of the OPP framework is not straightforward, and the few procedures reported in the literature usually require several steps. Gubert *et al.*⁸ described a 7-step synthesis of the unsubstituted OPP, starting from ethyl 1,4-dibenzylpiperazine-2-carboxylate, while Wu *et al.* reported a transannular reaction leading to the unsubstituted OPP during the HBr-mediated detosylation of 1,4,7-tritosyl-1,4,7-triazadecan-9-ol for which fractional crystallization is required to separate the native OPP from the 10-membered monocyclic isomeric byproduct.⁹

Different approaches have been developed for the preparation of octahydro-2*H*-pyrazino[1,2-*a*]pyrazinones (including

-diones and -triones), with the eventual need for an additional reduction step to access the fully reduced OPP. An early approach involves 4 steps, starting from 1,3-dichloroacetone, 2,4-dinitrophenylhydrazine and sarcosine ethyl ester and leading to *N*-methyl-octahydro-2*H*-pyrazino[1,2-*a*]pyrazine.¹⁰ A 6-step preparation of the same derivative starting from *N*-benzylethanolamine was reported in 1977 by Beim and Day.¹¹ Peptide synthesis approaches were employed for the preparation of the OPP core, sequentially forming the piperazine rings by *ex novo* lactamization of α -amino acid precursors, either by solution-phase¹² or by solid-phase² protocols.



Scheme 1 Octahydro-2*H*-pyrazino[1,2-*a*]pyrazine (OPP) and former synthetic accesses.

^a Dipartimento di Scienze del Farmaco (DSF), Università del Piemonte Orientale, Largo Donegani 2, I-28100 Novara, Italy. E-mail: fabio.travagin@uniupo.it, claudio.maestri@uniupo.it

^b PRC Ticinum Lab srl, Via Bovio 6, 28100 Novara, NO, Italy

^c CAGE Chemicals srl, Via Bovio 6, 28100 Novara, NO, Italy

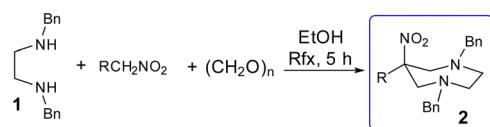
^d Dipartimento di Chimica, Università degli Studi di Milano, Via Golgi 19, 20133 Milano, Italy

^e Bracco Imaging SpA, Via Egidio Folli 50, 20134 Milano, Italy

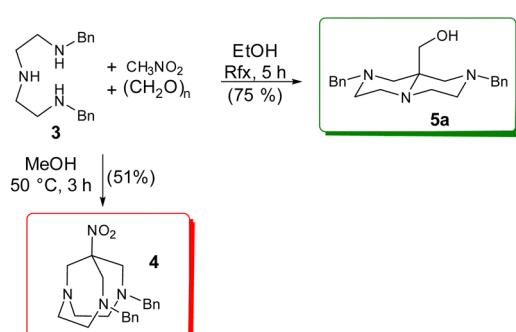
[†] Electronic supplementary information (ESI) available. CCDC 2327990 (5a). For ESI and crystallographic data in CIF or other electronic format see DOI: <https://doi.org/10.1039/d4cc03596h>



Previous work



This work



Scheme 2 Preparation of compounds **2**, **4** and **5a** by nitro-Mannich reaction.

The first piperazine ring of OPPs was alternatively accessed through the Castagnoli–Cushman reaction of cyclic anhydrides with imines.¹

The nitro-Mannich reaction is a well-established C–C bond forming transformation leading to useful synthons bearing vicinal nitrogen-based functional groups.¹³ Our research group has routinely employed the nitro-Mannich reaction for the synthesis of precursors of mesocyclic chelating agents, by reacting a vicinal secondary diamine (e.g.: *N,N'*-dibenzyl-1,2-ethylenediamine, **1**) with formaldehyde and a suitable nitroalkane (Scheme 2).¹⁴

In continuation of our studies on functionalized analogues of mesocyclic chelating agents, we explored the reactivity of a secondary triamine, observing an unexpected behaviour, dependent on the reaction conditions. When *N,N'*-dibenzylidethylenetriamine **3**¹⁵ is refluxed in ethanol with nitromethane and *para*-formaldehyde, a vigorous evolution of a red-brown gas (identified as NO₂ by absorption in aqueous NaOH solution, the latter tested positive to nitrite by semiquantitative nitrite-test strips) occurred and the formation of a main product was observed by TLC analysis. This product was isolated in 75% yield as a white solid and to our surprise its NMR and MS spectra are not compatible to the expected nitro-Mannich product **4** (Scheme 2). The NMR spectra suggest a symmetric structure, lacking the nitro group as confirmed by IR and HRMS analysis, the latter providing the molecular formula C₂₂H₂₉N₃O (Fig. S7–S12, S27 and S29, ESI[†]).

Crystallization from diethyl ether provided single crystals suitable for X-ray diffractometric analysis (Fig. 1 and Table S3, ESI[†]), which identified the new compound as (2,8-dibenzyl-octa-hydro-9aH-pyrazino[1,2-*a*]pyrazin-9-yl)methanol **5a** (Scheme 2 and Fig. 1).

The unexpected elimination of the nitro group and the concomitant formation of compound **5a** prompted us to investigate the mechanism of this unprecedented reaction.

The nitro group is known to act as a leaving group,^{16,17} for example in β -eliminations,¹⁸ generally triggered by the presence of an acidic hydrogen in the β -position, and in S_{RN1} substitutions,¹⁹ requiring a radical-stabilising substitution pattern.

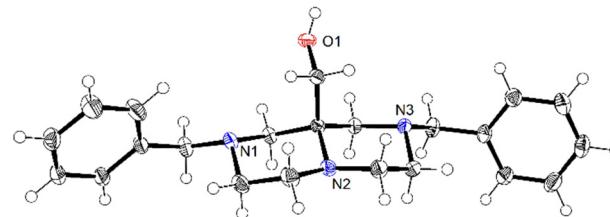


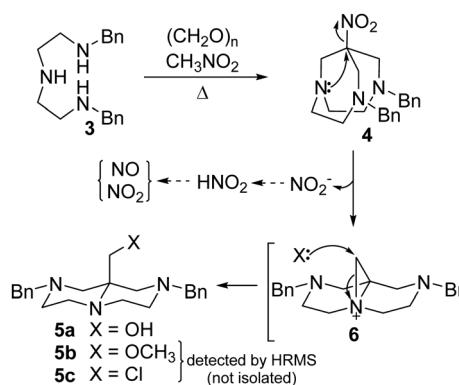
Fig. 1 ORTEP representation of the crystal structure of compound **5a**, obtained by SC-XRD analysis. Ellipsoids are represented with 50% probability. Color codes: C, gray; N, blue; O, red; H, light gray.

Compound **5a** may be originated by the loss of the nitro group from the expected nitro-Mannich product **4** preformed under the reaction conditions or directly by an alternative pathway. The expected nitro-Mannich product **4** is in fact detected in the reaction mixture and can be isolated in 51% yield by running the reaction under milder conditions (methanol, 50 °C), along with minor amounts of **5a** (Scheme 2).

The comparison between the molecular formula of compounds **4** and **5a** points to the loss of the nitro group and the following introduction of a hydroxyl group. The clear change in connectivity may be traced back to a rearrangement process, likely concomitant with the loss of the nitro group. The complete loss of the nitro group strongly suggested by the copious evolution of gaseous NO₂ points to an intermolecular origin of the incoming OH group, presumably ascribed to H₂O, either adventitious or produced by the initial nitro-Mannich steps (3 eq. of H₂O are generated in the condensation 3 + 3CH₂O + CH₃NO₂ → **4** + 3H₂O).

A possible reaction mechanism for this unprecedented transformation is proposed in Scheme 3.

The nitrotriamine **3** is initially formed by a classic nitro-Mannich condensation. On heating, compound **4** undergoes an intramolecular nucleophilic displacement of the nitro group by the central nitrogen atom, leading to the intermediate tricyclic aziridinium ion **6**. Nucleophilic ring opening of the strained three-membered ring takes place at its unhindered methylene group, leading to compound **5a**, with the role of the nucleophile played by water formed during the condensation step. The nitro



Scheme 3 Proposed mechanism for the formation of compound **5a** and two side products **5b** and **5c**, detected by HRMS.



group acts as the leaving group and is lost as NO_2^- , the latter in equilibrium with the highly unstable nitrous acid, finally undergoing decomposition to nitrogen oxides (Scheme 3).

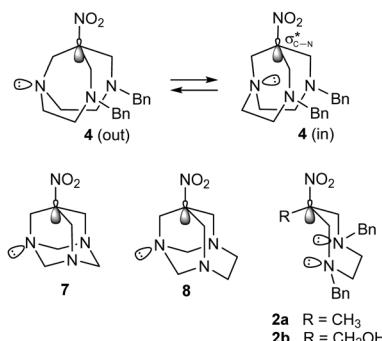
To assess the role of water, triamine 3, *para*-formaldehyde and nitromethane were heated in an aprotic solvent (THF), kept dry by an excess of preactivated 3 Å molecular sieves. TLC analysis of the reaction mixture clearly showed the formation of 4, the total absence of 5a and the formation of minor amounts of highly polar compounds. The reaction mixture was then cooled to room temperature and filtered under vacuum to remove the molecular sieves. Water was added, and the solution refluxed for additional 4 hours. TLC analysis showed the progressive formation of 5a, confirming the direct role of water in this reaction.

Careful analysis by HPLC-HRMS of the reaction mixture run in methanol (Scheme 2) and leading to 4 allowed to detect two additional compounds (5b and 5c), identified on the base of their molecular weight and fragmentation patterns (Fig. S16, ESI[†]). The methoxy derivative 5b likely arises from solvolytic ring opening of aziridinium 6. The formation of compound 5c could be ascribed to the presence of residual chloride ions in the starting triamine 3, as the latter is prepared and isolated as the trihydrochloride following a literature procedure.¹⁵

A rational underlying the unusual nucleophilic displacement may be proposed on the base of stereoelectronic effects. The instability of nitrotriamine 4 on heating could arise from the possibility for this flexible bicyclic structure to undergo a pyramidal inversion of the (central) nitrogen atom, leading its lone pair free to flip outward/inward (Scheme 4). The lone pair, once directed inward, can approach the carbon atom bearing the nitro group to start the nucleophilic displacement *en route* to the intermediate aziridinium 6.

Obviously, pyramidal inversion of the nitrogen atom is precluded in the nitrotriamines 7²⁰ and 8²¹ (Scheme 4), previously reported in the literature, as they are stable at temperatures higher than those involved in the formation of 5 (compound 7 decomposes only at $T > 260$ °C while compound 8 withstands recrystallization from boiling 1-butanol (bp 117.7 °C) and sublimes unchanged at 191–192 °C).

A similar nitro group displacement could be expected in monocyclic nitrodiamines 2a–b^{22,23} (Scheme 4), as they are free to undergo pyramidal inversion of the nitrogen atoms. However, compounds 2a–b are stable under the reaction conditions



Scheme 4 Nitrodiamines and nitrotriamines discussed in the text.

(*i.e.* refluxing ethanol), actually employed for their synthesis in almost quantitative yield. Nevertheless, HRMS of 2a–b clearly shows fragmentation peaks corresponding to the formation of the bicyclic aziridinium ion with loss of the nitro group in the mass spectrometer ion source (Fig. S17 and S18, ESI[†]), supporting the possibility of this reaction pathway. The lower propensity of the monocyclic nitrodiamines 2a–b to the loss of the nitro group could be explained by the need for assuming high energy conformations to allow the nitrogen lone pair to approach the $\text{C}-\text{NO}_2$ carbon atom, while the bicyclic structure of 4, when in the “in” conformation, forces the lone pair correctly pointed towards the $\sigma_{\text{C}-\text{N}}^*$ orbital.

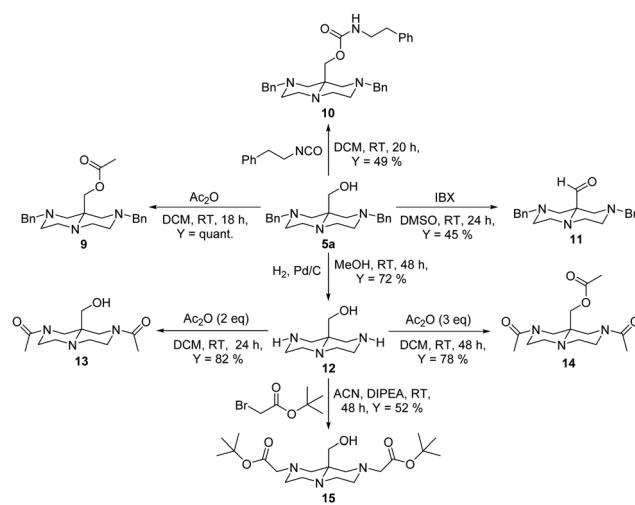
The easy access to the OPP core represented by this unexpected transformation paves the way for the preparation of a wide range of OPP derivatives, taking advantage of the functionalization pattern of compound 5a.

We demonstrated the versatility of the latter in accessing the OPP chemical space with a series of simple reactions, summarized in Scheme 5.

The OH-group of compound 5a shows the expected reactivity of a primary alcohol. Quantitative acetylation to 9 is obtained by treatment with acetic anhydride in DCM at r.t., while reaction in the same conditions with 2-phenethyl isocyanate provides the carbamate 10. The reaction of 5a with IBX in DMSO leads to the oxidation to the interesting triaminoaldehyde 11 in 45% yield.

Synthetic work on the distal nitrogen atoms of 5a requires their preliminary debenzylation, cleanly obtained by catalytic hydrogenolysis in methanol at r.t., to give compound 12. Selective acylation of the resulting secondary amines is achieved by treatment with stoichiometric acetic anhydride in DCM at r.t., quantitatively yielding diacetamide 13. An additional equivalent of acetic anhydride in the same experimental conditions allows exhaustive acylation of the secondary amines and of the primary alcoholic group to give the diamidoester 14 in 78% yield.

Treatment of compound 12 with an alkylating agent (*t*-butyl bromoacetate) in acetonitrile in the presence of DIPEA at r.t.



Scheme 5 OPP derivatives obtained by post-synthetic transformations of compound 5a.



leads to the alkylation of the secondary amines, yielding the highly functionalized triaminohydroxydiester **15** in 52% yield.

Finally, the reaction of 1,4,7-trimethyldiethylenetriamine with nitromethane and *para*-formaldehyde in refluxing ethanol for 5 h, the same conditions leading to the formation of compound **5a**, formed a complex mixture, possibly due to an extensive polymerization.

The reaction described in this manuscript represents an unprecedented, simple and efficient approach to pharmacologically relevant octahydro-2*H*-pyrazino[1,2-*a*]pyrazines (OPPs).

A possible mechanism for this transformation involving a combination of nitro-group displacement-rearrangement is proposed and supported by preliminary experimental data.

The functionalized OPP obtained by this unexpected transformation has been converted to a series of OPP derivatives, demonstrating its versatility.

The nitro-Mannich-rearrangement sequence described in this manuscript represents a short and efficient synthesis to OPPs core and a useful entry into the chemical space of this pharmacologically relevant heterobicyclic compounds.

Data availability

The data supporting this article have been included as a part of ESI.† CCDC 2327990 (**5a**) contain the supplementary crystallographic data for this paper.

Conflicts of interest

There are no conflicts to declare.

Notes and references

1. L. Usmanova, D. Dar'in, M. S. Novikov, M. Gureev and M. Krasavin, *J. Org. Chem.*, 2018, **83**, 5859–5868.
2. A. Golebiowski, J. Jozwik, S. R. Klopfenstein, A.-O. Colson, A. L. Grieb, A. F. Russell, V. L. Rastogi, C. F. Diven, D. E. Portlock and J. J. Chen, *J. Comb. Chem.*, 2002, **4**, 584–590.

3. G. S. Welmaker, J. A. Nelson, J. E. Sabalski, A. L. Sabb, J. R. Potoski, D. Graziano, M. Kagan, J. Coupet, J. Dunlop, H. Mazandarani, S. Rosenzweig-Lipson, S. Sukoff and Y. Zhang, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1991–1994.
4. Kowa Co. Ltd. (JP), *US Pat.*, US6340682B1, 2002.
5. Y. Zhu, R. K. de Jesus, H. Tang, S. P. Walsh, J. Jiang, X. Gu, N. Teumelsan, A. Shahripour, B. Pio, F.-X. Ding, S. Ha, B. T. Priest, A. M. Swensen, M. Alonso-Galicia, J. P. Felix, R. M. Brochu, T. Bailey, B. Thomas-Fowlkes, X. Zhou, L.-Y. Pai, C. Hampton, M. Hernandez, K. Owens, J. Ehrhart, S. Roy, G. J. Kaczorowski, L. Yang, E. R. Parmee, K. Sullivan, M. L. Garcia and A. Pasternak, *Bioorg. Med. Chem. Lett.*, 2016, **26**, 5695–5702.
6. Forma Therapeutics Inc. (US), *WO Pat.*, WO2019222468A1, 2019.
7. J. G. Kettle, S. K. Bagal, S. Bickerton, M. S. Bodnarchuk, J. Breed, R. J. Carbajo, D. J. Cassar, A. Chakraborty, S. Cosulich, I. Cumming, M. Davies, A. Eatherton, L. Evans, L. Feron, S. Fillery, E. S. Gleave, F. W. Goldberg, S. Harlfinger, L. Hanson, M. Howard, R. Howells, A. Jackson, P. Kemmitt, J. K. Kingston, S. Lamont, H. J. Lewis, S. Li, L. Liu, D. Ogg, C. Phillips, R. Polanski, G. Robb, D. Robinson, S. Ross, J. M. Smith, M. Tonge, R. Whitley, J. Yang, L. Zhang and X. Zhao, *J. Med. Chem.*, 2020, **63**, 4468–4483.
8. S. Gubert, C. Braojos, A. Sacristán and J. A. Ortiz, *Synthesis*, 1991, 318–320.
9. F. Liang, X. Wu, S. Zhang and C. Wu, *Synth. Commun.*, 2004, **34**, 845–851.
10. M. Rink and K. Feiden, *Arch. Pharm.*, 1962, **295**, 121–126.
11. H. J. Beim and A. R. Day, *J. Heterocycl. Chem.*, 1977, **14**, 307–308.
12. V. N. Belov, C. Funke, T. Labahn, M. Es-Sayed and A. de Meijere, *Eur. J. Org. Chem.*, 1999, 1345–1356.
13. A. Noble and J. C. Anderson, *Chem. Rev.*, 2013, **113**, 2887–2939.
14. F. Travagin, L. Lattuada and G. B. Giovenzana, *Coord. Chem. Rev.*, 2021, **438**, 213908–213931.
15. J. A. Scifani, M. T. Maranto, T. M. Sisk and S. A. Van Arman, *J. Org. Chem.*, 1996, **61**, 3221–3222.
16. N. Ono, *The Nitro Group in Organic Synthesis*, Wiley-VCH, New York, 2001, pp. 182–230.
17. R. Tamura, A. Kamimura and N. Ono, *Synthesis*, 1991, 423–434.
18. R. Ballini and A. Palmieri, *Adv. Synth. Catal.*, 2019, **361**, 5070–5097.
19. N. Kornblum, S. D. Boyd and F. W. Stuchal, *J. Am. Chem. Soc.*, 1970, **92**, 5783–5784.
20. E. B. Hodge, *J. Org. Chem.*, 1972, **37**, 320–321.
21. A. I. Kuznetsov, V. A. Kosmakov and A. S. Moskovkii, *Chem. Heterocycl. Compd.*, 1990, **26**, 578–581.
22. S. Aime, L. Calabi, C. Cavallotti, E. Gianolio, G. B. Giovenzana, P. Losi, A. Maiocchi, G. Palmisano and M. Sisti, *Inorg. Chem.*, 2004, **43**, 7588–7590.
23. G. Gugliotta, M. Botta, G. B. Giovenzana and L. Tei, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 3442–3444.

