Review
doi.org/10.1002/cmtd.202300053
Chemistry Europe
European Chemical Societies Publishing

www.chemistrymethods.org

Non-Conventional Methodologies for the Synthesis of *N*-Nitrosamines

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N-Nitrosamines acting as contaminants in our environment is a topic of increasing concern. Detection methods are required, necessitating analytical standards. Herein, we discuss some conventional methodologies to prepare *N*-nitrosamines and compare them with unconventional pathways towards their synthesis. These methods are often more environmentally benign and safer.

Introduction

N-Nitroso compounds are organic compounds bearing a nitroso functional group attached to a nitrogen atom. The delocalisation of the electron lone pair of the amine nitrogen atom into the π-electron system results in the formation of two conformational isomers (rotamers). This is due to the hindered rotation about the N–N bond caused by its partial double bond character. This zwitterionic structure is the key species responsible for the physical and chemical properties of *N*-nitrosamines. These conformational isomers can be observed in the NMR spectra of *N*-nitrosamines and such rotamers possess temperature-dependent properties.[1]

In symmetric secondary amines this results in the formation of one isomer with diastereotopic groups attached to the nitrogen. If the secondary amine is asymmetric, the resulting product will exist as *E* and *Z* isomers, where the most stable (major) isomer has least steric hindrance (Figure 1).

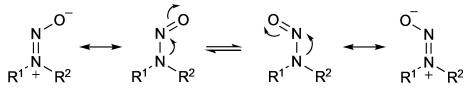

Figure 1. *E* and *Z* isomers of *N*-nitrosamines.

The toxicity of *N*-nitrosodimethylamine (NDMA) was studied in 1954 by Magee and Barnes following the suspicion of afflicting damage to the liver of humans. After experiments on several small mammals, it was proven to cause damage to the liver.[2] Since then, further studies have shown that *N*-nitrosamines display carcinogenic[3,4,5,6] and mutagenic[7,8] properties.

N-Nitrosamines are contaminants found throughout our environment, such as in air, soil, water, food, drinks, drugs, and diet.[5] In 2018, NDMA was detected in a batch of the drug valsartan (sold under the brand name of Diovan) at levels exceeding acceptable intake limits.[9] Therefore, it is vital to study how these compounds are formed as contaminants and to understand how to remediate, detect, and prevent their occurrence. Detection of *N*-nitrosamines is especially important and requires analytical studies to be performed on these compounds. Therefore, the synthesis of model substrates is necessary, but conventional methodologies suffer from the use of harsh reaction conditions, toxic reagents, and are environmentally polluting. Thus, it is desirable to synthesise such compounds in a safe and clean manner, and omitting the use of certain chemicals, such as concentrated acids, which could pose difficulties if there are acid-sensitive functional groups present in the product.

Electrosynthesis is an alternative approach towards traditional reagent-based redox reactions. Electrochemistry was first incorporated in organic reactions in 1848 by Kolbe,[10] and since then this field has become a key discipline in organic chemistry. Organic electrosynthesis has various advantages such as sustainability, of key concern in modern day society, mild reaction conditions, and high functional group tolerance. An important topic in new science is green methodologies, which requires the need for developing alternative approaches in chemistry. Toxic redox reagents can be omitted and replaced with electrons, directly from a current source. Additionally, late-stage functionalisation of complex molecules is also possible and important in the pharmaceutical industries and material sciences.

Photochemistry is also a vital route to synthetic organic chemists. In photoinduced chemical reactions, photons are delivered to the substrate or photocatalyst, which then reach an excited state, and ultimately lead to the formation of the product through a series of redox processes.[11] As with electrosynthesis, milder reaction conditions are achievable, and photochemical approaches require the use of (visible) light as a potential source of photons, hazardous reagents can be avoided, and high functional group tolerance can be achieved.

Flow chemistry is another particularly advantageous approach, especially when chemical processes pose high risks. With flow chemistry, one can readily control pressure and temperature, take advantage of flow chemistry's natural efficiency in homogenous mixing, and the fast dissipation of

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heat for exothermic reactions. Moreover, there is only a small quantity of compounds present at any point in the reactor as the formation of product is continuous through the reactor during synthesis. Thus, flow chemistry in combination with electrochemistry or photochemistry is therefore an invaluable tool to chemists.[12]

Model substrates are an ideal way to approach the issue of detection by creating analytical standards. If these compounds can be directly accessed through synthesis, they can be utilised as references and standards for the detection of such products as contaminants in commercial production processes. Additionally, having a wide range of methods to access *N*-nitrosamines is advantageous as certain model substrates may have complex functional groups that are poorly tolerated under traditional reaction conditions. Thus, this review will focus on the comparison of non-conventional synthetic techniques and conventional synthetic methodologies.

Conventional *N*-Nitrosamine Formation

Prior to the use of unconventional synthetic techniques, the synthesis of *N*-nitrosamines has been well explored using conventional chemical syntheses. The first report of the synthesis of nitrosamines was in 1933 by Hartman *et al.*,[13] where the authors outline a procedure which uses sodium nitrite, a now commonly used nitrosating reagent, in an acidic aqueous medium such as hydrochloric acid, to produce nitrosomethylaniline from methylaniline. A limited scope was investigated for this initial methodology.

Since then, this process has been improved, with authors such as Borikar *et al.*[14] refining the process in 2010, with the inclusion of *p*-toluenesulfonic acid and anisole as supporting reagents, alongside sodium nitrite. The use of dichloromethane as a solvent also enabled a larger scope of organic substrates to be explored as an aqueous solution is no longer necessary.

Other nitrosating agents include alkyl nitrites, such as *tert.*-butyl nitrite. Chaudhary *et al.* reported a variety of conditions for the *N*-nitrosation of *N*-methyl aniline including solvent-free conditions.[15] Of note is that aprotic solvents gave a faster conversion (20–30 min) than protic solvents (45–90 min), although full conversion and high yields were observed in all cases. Under solvent-free conditions, quantitative yields were observed.

Looking to a wider scope of substrates, the efficiency of *tert.*-butyl nitrite was diminished, being most effective with secondary aryl amines and secondary alkyl amines being the least effective substrate, with some substrates requiring heating up to 45 °C and reaction times of up to 8 hours.

N-Nitrosation of tertiary amines requires a preceding *N*-dealkylation, which occurred as part of the *N*-nitrosation with *tert.*-butyl nitrite as reported by Guo *et al.*[16] In contrast to the findings of Chaudhary *et al.* when working with tertiary alkanes, the reactions proceeded best in *tert.*-butanol, unlike the aprotic solvents which were favoured in the secondary amine examples.

Challis *et al.* reported the use of bromonitromethane to form *N*-nitroso compounds from secondary alkanes.[17] Due to the hazardous nature of nitromethanes as explosives they are generally undesirable as solvents in an industrial setting. The mechanistic understanding of the decomposition of nitromethanes is that homolysis occurs by generating NO_2/N_2O_4. This is another approach to use nitromethanes directly in aqueous solvents, which was investigated by the same group.[18]

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Another major conventional synthetic route to *N*-nitrosamines is the use of nitrosyl halides and nitrosonium salts. Examples of this work include the work of Sather *et al.* employing $NOBF_4$,[19] and Dewynter's *et al.* work using NOCl.[20] Sather *et al.* made use of basic conditions, specifically employing triethylamine at 0 °C, and investigated the decay of the resulting nitrosamide products, whilst Dewynter's optimum conditions made use of LiOH in dichloromethane at 0 °C. The advantage of these conditions, although harsh and difficult for scale-up, is the high reactivity of the *in situ* generated NO^+ ions making these conditions ideal for an access of more challenging substrates, such as secondary amines with significant steric bulk and sulfonamides.

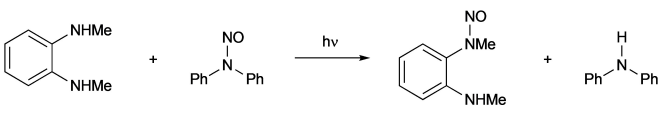
All these conventional approaches (Table 1) share the common issue of posing high risks, requiring the use of harsh reagents and reaction conditions, and as a result, safer and greener options for the synthesis of such compounds are preferred.

Photochemical Batch Synthesis of *N*-Nitrosamines

Xiang and co-workers proposed a photochemical methodology for the synthesis of *N,N'*-dimethyl-*N*-nitrosobenzene using a *trans*-nitrosation reaction, where the nitroso functionality of *N*-nitrosodiphenylamine is transferred onto *N,N'*-dimethylbenzene-1,2-diamine under photochemical conditions as shown in Scheme 1.[21] This did not undergo further cyclization to a benzotriazole product. Unfortunately, photochemical routes towards the synthesis for a wide scope of *N*-nitrosamines is an underexplored area.

Table 1. Summary of conventional methods for the nitrosation of amines.

Year	Conditions	Yield
1933[13]	$NaNO_2$ (1 equiv.), HCl, 10 °C, 1 h	87–93 %
1978[18]	N_2O_4	ca. 50 %
1990[17]	bromonitromethane, MeCN, 70 °C, 1–4 days	25–80 %
1996[20]	NOCl (2.5 equiv.), $LiOH_{(aq)}$ (2 equiv.)	32–94 %
2010[14]	$NaNO_2$, *p*-TsOH, CH_2Cl_2, rt	85–97 %
2011[19]	$NOBF_4$ (4 equiv.), TEA (2 equiv.)	60–80 %
2016[15]	*t*-BuONO (1-2 equiv.), neat, 5 min–8 h	82–97 %
2019[16]	*t*-BuONO (1.5 equiv.), solvent (varies by desired outcome), rt–60 °C, 1–26 h	63–99 %

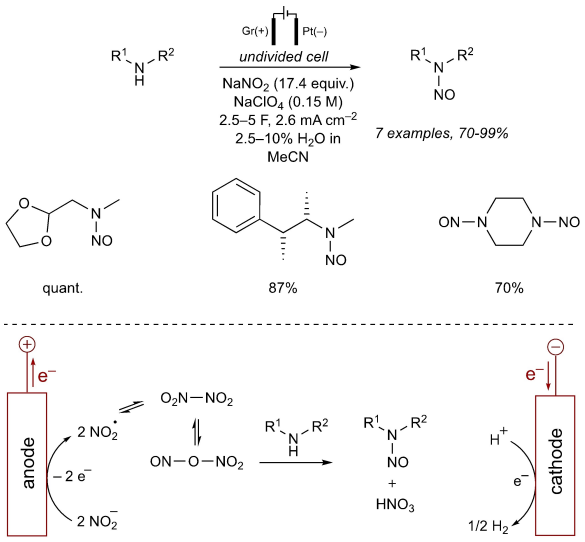


Scheme 1. Photochemical *trans*-nitrosation of *N,N'*-dimethylbenzene-1,2-diamine.

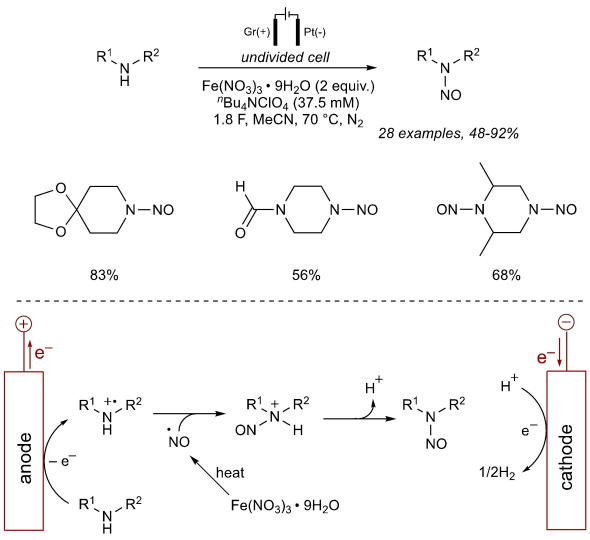
Electrochemical Batch Synthesis of *N*-Nitrosamines

Masui and co-authors established the first electrochemical batch approach towards the synthesis of *N*-nitrosamines (Scheme 2) utilising a large excess of readily available $NaNO_2$ as the nitroso source, which had limited solubility in the reaction solution.[22] They synthesised a total of seven *N*-nitrosamines, including a dinitrosated substrate, however only one product was isolated due to safety concerns, while the rest was determined by gas-liquid chromatography analysis. With the support of existing literature,[23,24] they proposed that the nitrite anion undergoes an one electron oxidation, and combines with another nitrite radical species, forming dinitrogen tetroxide (N_2O_4). Following this, the dinitrogen tetroxide combines with an aliphatic secondary amine, forming the corresponding *N*-nitrosamine, and releasing nitric acid. Simultaneously, hydrogen gas is generated from the reduction of protons at the cathode (Scheme 2).

Lu and co-workers subsequently described the batch electrochemical synthesis of *N*-nitrosamines using iron nitrate nonahydrate $Fe(NO_3)\cdot 9H_2O$ as the nitroso source at elevated temperatures (Scheme 3).[25] They successfully synthesised 28 substrates, including two dinitrosated examples. It was proposed that the reaction was initiated by the one-electron oxidation of aliphatic secondary amines, forming the radical cation. Since the reaction solution is heated to 70 °C, this results in the thermal decomposition of iron nitrate, releasing $NO^{\bullet}$, which combines with the radical cation through a biradical coupling reaction, and forms the resulting *N*-nitrosamine accompanied by the release of one proton. At the same time,



Scheme 2. First batch electrochemical *N*-nitrosation using $NaNO_2$.



Scheme 3. Batch electrochemical *N*-nitrosation using $Fe(NO_3)_3 \bullet 9H_2O$ at 70 °C.

the cathodic reduction of protons forms hydrogen. This mechanism was supported by DFT-studies and control experiments. Both of these synthetic routes utilise perchlorate salts as the supporting electrolyte, which may be explosive under certain circumstances, for example at high scales.

Another acid-free batch electrochemical procedure for the synthesis of *N*-nitrosamines from aliphatic secondary amines was developed by Wang *et al.* as shown in Scheme 4.[26] In contrast to previous work, they utilised five equivalents of potassium or sodium nitrite and performed the reaction in dichloromethane. Additionally, this method allowed for a late-stage functionalisation of six drug molecules, and the corresponding *N*-nitroso compounds are useful for analytical detection purposes of *N*-nitroso impurities present in drug molecules. Using potassium nitrite and sodium nitrite as the nitrosating agents, they propose a nearly identical mechanism as Masui, where a nitrite radical was generated *via* the anodic oxidation of the nitrite anion. This is followed by the release of a nitrosonium cation (NO^+) which combines with a secondary amine and forms the corresponding *N*-nitrosamine with the release of a proton. This is accompanied by the cathodic reduction of protons forming hydrogen.

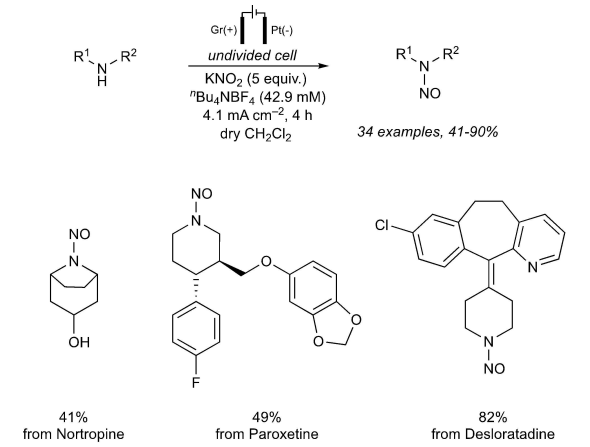
Flow Synthesis of *N*-Nitrosamines

Thompson *et al.* studied the synthesis of Lomustine, [1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea], which is used for the treatment of Hodgkin's lymphoma and brain tumours.[27] They accessed this compound using a two-step continuous-flow methodology.[28] The second step in this synthesis is the *N*-nitrosation of the amide 1-(2-chloroethyl)-3-cyclohexylurea in a microfluidic reactor. The authors investigated two different pathways for this nitrosation. The first one required the pumping of three components, the substrate dissolved in THF, neat formic acid, and $NaNO_2$ in aqueous methanol at 0 °C, with a residence time of 5 minutes. This produced Lomustine in 75%–79% yield depending on the purification methodology employed. The second method, shown in Scheme 5, introduced the two components, namely the substrate and 3 equivalents of *tert*.-butyl nitrite at 25 °C to a flow reactor with a residence time of 8 minutes, giving Lomustine in 91% yield. This work was followed up by Gerogiorgis *et al.* who investigated the kinetics for this flow synthesis.[29]

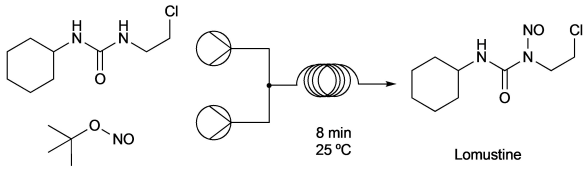
Additionally, Monbaliu *et al.* prepared anhydrous dinitrogen trioxide (N_2O_3) under flow conditions from O_2 and NO gas. The resulting N_2O_3 was pumped at a concentration of 51.5 mol%, combined with *N*-phenylglycine derivatives at 20 °C, a pressure of 5 bar, and a residence time of 10 min in a reactor which allowed the synthesis of the corresponding *N*-nitrosamines. Without isolation, the *N*-nitrosamines were then transformed into *N*-heterocycles under batch conditions.[30]

Electrochemical Flow Synthesis of *N*-Nitrosamines

Recently, Wirth and co-workers reported an electrochemical flow procedure.[31] In contrast to the previous batch electrochemical procedures where the salts are insoluble in the reaction media, a homogenous reaction mixture such as aqueous acetonitrile was employed to address solubility issues. The products were obtained in short reaction times in comparison to the batch procedures. This methodology allowed for the successful synthesis of 27 products, including one dinitrosated example, which were isolated in low to excellent

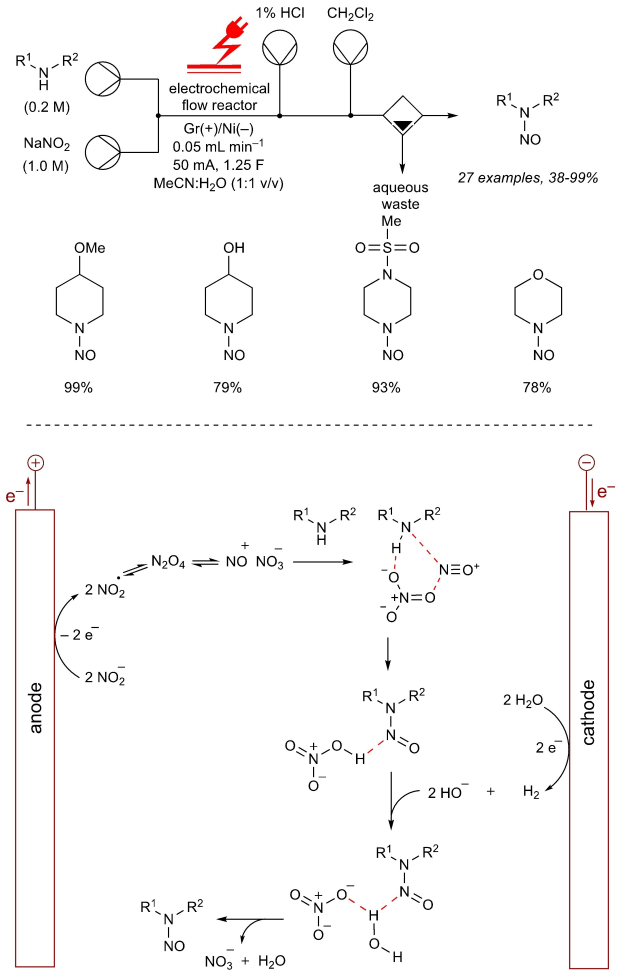


Scheme 4. Late-stage *N*-nitrosation of drug molecules using KNO_2.



Scheme 5. Synthesis of Lomustine through flow nitrosation.

yields (30–99%). The use of additional supporting electrolyte could be omitted due to the short interelectrode distance of 500 μm. Furthermore, the setup also contained an in-line liquid-liquid extraction procedure based on an acidic work-up, which allowed the direct purification of several compounds, eliminating the need for column chromatography (Scheme 6). An intrinsic advantage of flow chemistry is scalability and combining it with the environmentally benign properties of electrochemistry make this an invaluable tool in laboratories and industries. DFT calculations were performed for further insight into the mechanism, and it was shown that the *trans*-intermediate of N_2O_4 forms an adduct with the substrate, which, in the presence of a hydroxide anion released from the reduction of water, forms the corresponding *N*-nitrosamine and is accompanied with the release of a nitrate anion and one water molecule. In all electrochemical methodologies established, superstoichiometric (2–17 equiv.) amounts of nitrite/nitrate salt are necessary to obtain acceptable yields as compared to conventional routes where generally only 1–4 equivalents of nitrosating agents are employed.



Scheme 6. Electrochemical flow synthesis of *N*-nitrosamines using $NaNO_2$.

Summary and Outlook

Recently reported non-conventional methodologies for the synthesis of *N*-nitrosamines are discussed, namely batch and flow electrochemical, photochemical and flow syntheses, which are compared with conventional synthetic pathways. The procedures have developed over the years, along with a more detailed understanding of the mechanism in the electrochemical route. These are promising methodologies which can be superior alternatives to obtain *N*-nitroso analogues of drug molecules and related compounds. These can be used as analytical standards to monitor contaminants which may be present as impurities in pharmaceutical products and in other chemical products made for human consumption.

Acknowledgements

The authors acknowledge financial support provided by Cardiff University, BAE Systems (R.A.) and dstl (C.S.W.).

Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Keywords: amines · electrochemistry · flow synthesis · nitrosation · nitrosamines

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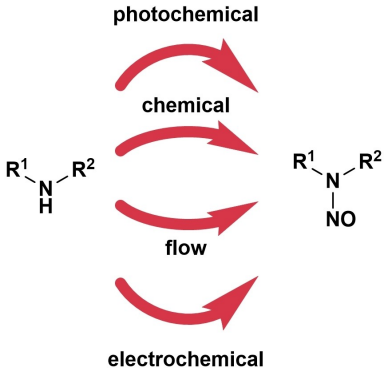
Manuscript received: November 16, 2023
Version of record online: ■■, ■■

REVIEW

Herein we highlight key concepts behind unconventional synthetic routes towards the synthesis of *N*-nitrosamines in comparison to traditional chemical routes. These include electrochemical, photochemical, and flow methodologies. Easier and cleaner access to *N*-nitrosamines is desirable for their use as intermediates and as analytical standards, due to their carcinogenic nature and low-tolerance thresholds in medicinal compounds.



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