Nitrous Oxide

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Synthesis of Triazenes with Nitrous Oxide**

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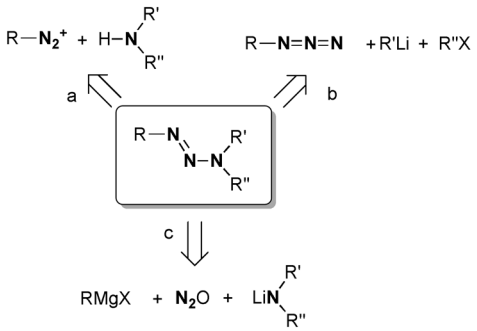
Abstract: *Triazenes are valuable compounds in organic chemistry and numerous applications have been reported. Furthermore, triazenes have been investigated extensively as potential antitumor drugs. Here, we describe a new method for the synthesis of triazenes. The procedure involves a reagent which is rarely used in synthetic organic chemistry: nitrous oxide (N_2O, "laughing gas"). Nitrous oxide mediates the coupling of lithium amides and organomagnesium compounds while serving as a nitrogen donor. Despite the very inert character of nitrous oxide, the reactions can be performed in solution under mild conditions. A key advantage of the new procedure is the ability to access triazenes with alkynyl and alkenyl substituents. These compounds are difficult to prepare by conventional methods because the required starting materials are unstable. Some of the new alkynyltriazenes were found to display high cytotoxicity in in vitro tests on ovarian and breast cancer cell lines.*



Scheme 1. Retrosynthetic analysis of trisubstituted triazenes. Classical synthetic routes involve the coupling of diazonium salts with amines (a) or the reaction of azides with organolithium compounds followed by alkylation (b). The new synthetic procedure is based on organomagnesium compounds, nitrous oxide, and lithium amides (c).

Triazenes represent "a versatile tool in organic synthesis".[1] For example, they have been used as multifunctional linkers in solid-phase synthesis,[2] as removable directing groups for C–H activation,[3] as protecting groups for the synthesis of complex natural products,[4] in shape-persistent phenylacetylene-based systems,[5] and for the controlled desaturation of unactivated aliphatic compounds.[6] In addition to their synthetic utility, triazenes are of importance because of their biological activity. Two triazenes, dacarbazine and temozolomide, are currently used in the clinic as chemotherapy drugs, the latter may even be administered orally. Numerous other triazenes have also been tested for their antitumor activity.[7]

The main synthetic route for the synthesis of trisubstituted triazenes relies on the coupling of diazonium salts with secondary amines (Scheme 1 a).[8] Trialkyltriazenes have been prepared by the reaction of aliphatic azides with organolithium compounds, followed by alkylation with organohalides (Scheme 1 b).[9] These standard procedures have an important shortcoming: triazenes with alkynyl or alkenyl substituents in the 1-position are difficult to access because the required starting materials are highly unstable. 1-Azido-1-alkynes, for example, decompose at low temperatures and the first spectroscopic characterization of such a compound was only achieved recently.[10] Diazonium salts of alkenes and alkynes are likewise not very stable.[11] Herein, we describe a novel synthetic route for the preparation of trisubstituted triazenes. The reaction involves the coupling of lithium amides with nitrous oxide and organomagnesium compounds (Scheme 1 c). The new procedure allows access to the elusive 1-alkenyl and 1-alkynyltriazenes in good yields, thereby opening the way to new, putative drug structures.

Chemical reactions with N_2O typically proceed by transfer of an oxygen atom and liberation of N_2.[12] In contrast, there are very few examples of reactions involving the utilization of nitrous oxide as a nitrogen donor, in particular in the context of synthetic organic chemistry. Some cyclic alkynes react with nitrous oxide at elevated pressure to give diazo ketones.[13] Phenylcalcium iodide was shown to react with N_2O to give azobenzene in moderate yield.[14] In a related fashion, lithiated ferrocenes were coupled with N_2O to give azo-bridged ferrocene oligomers.[15] These transformations can be regarded as homocoupling reactions of C nucleophiles with insertion of N_2. We hypothesized that triazenes could be obtained in a related fashion by cross-coupling/N_2 insertion of a C nucleophile and an N nucleophile.

When a solution of lithium diisopropylamide (LDA) and phenylmagnesium bromide (1:1) in tetrahydrofuran (THF) was allowed to react with N_2O (99.999%) at room temperature, the desired coupling product, 1-phenyl-3,3-diisopropyltriazene (**1**), was formed in 20% yield after 18 h. Optimization of the reaction conditions revealed that an excess of Grignard reagent (1.5–2.0 equiv) and slightly elevated temperatures (50°C) were beneficial for the coupling reaction. Furthermore, we were able to improve the yield by performing the reaction in a stepwise fashion. Thus, a solution of LDA in THF was first subjected to an atmosphere of N_2O. A reaction between the gas and the amide occurred, as evident

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by the formation of a white precipitate. After 4 h, the N_2O atmosphere was replaced by dry dinitrogen and a solution of phenylmagnesium bromide in THF (2.0 equiv) was added to the suspension. The resulting clear solution was then heated for 4 h at 50 °C. This procedure resulted in the near-quantitative formation of triazene **1** (isolated in 94 % yield).

The scope of the coupling reaction was examined using different arylmagnesium compounds and various lithium dialkylamides. A range of 1-aryl-3,3-dialkylltriazenes was obtained in good yields. Selected products are depicted in Figure 1 (**1**–**5**; further examples are shown in Figure S1 in the Supporting Information). Reactions with the aromatic amides $LiNPh_2$ and LiNMePh were not successful, presumably because of the lower nucleophilicity of the amides.

Having established that aryl Grignard reagents can be used as C nucleophiles, we turned our attention to alkynyl-

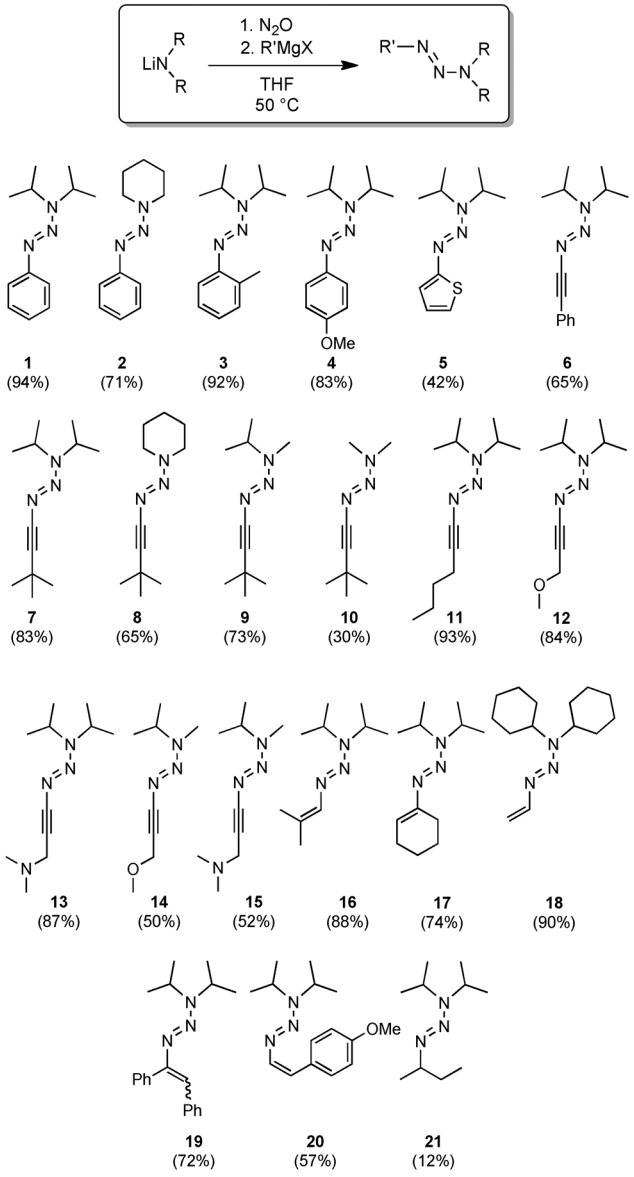


Figure 1. Synthesis of triazenes by reactions of lithium dialkylamides with N_2O and organomagnesium compounds. The values below the product numbers refer to the yields of the isolated products.

magnesium compounds. These coupling partners are particularly interesting because the putative products, 1-alkynyltriazenes, cannot be accessed by standard procedures (see above). By using a similar procedure as for arylmagnesium compounds, we were indeed able to synthesize 1-alkynyl-3,3-dialkyltriazenes (**6**–**15**, Figure 1). Aromatic as well as aliphatic alkynes can be employed and variations of the alkyl substituents on the amine group are likewise possible (further examples are shown in Figure S1 in the Supporting Information). Notably, alkynes containing functional groups such as ethers (**12**, **14**) or tertiary amine groups (**13**, **15**) are suitable as substrates. Generally, amides with bulky alkyl substituents (e.g isopropyl) were found to give higher yields than did amides with small substituents (e.g. methyl).

Alkenyl Grignard reagents can also be used as the coupling partners. As in the case of aryl and alkynyl Grignard reagents, the procedure appears to be rather general, as evident by the successful formation of the triazenes **16**–**20** (Figure 1) and **S8**–**S10** (see Figure S1 in the Supporting Information).

All the triazenes were characterized by NMR spectroscopy and high-resolution mass spectrometry. In addition, we have analyzed the alkynyltriazenes **6** and **10**, as well as the alkenyltriazenes **17** and **20** by single-crystal X-ray crystallography. As expected, the compounds display a *trans* geometry (Figure 2). The N–N bond lengths observed for **6**, **10**, **17**, and **20** are similar to those found in aromatic triazenes, such as *p*-(3,3-dimethyl-1-triazeno)benzonitrile.[16]

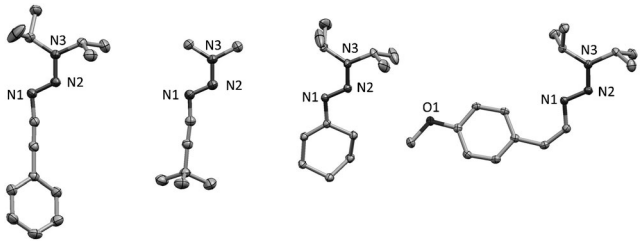
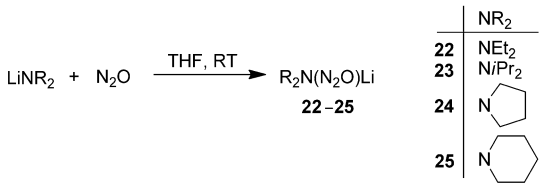


Figure 2. The molecular structures of the triazenes **6**, **10**, **17**, and **20** (from left to right) in the solid state. Hydrogen atoms are not shown for clarity.

Trialkyltriazenes can also be prepared by our method, as evident by the synthesis of 1-isobutyl-3,3-diisopropyltriazene **21**. However, side products were formed along with **21**, and the yield of **21** was low (12 %). Similar results were observed for attempted coupling reactions with other alkyl Grignard reagents such as *i*PrMgCl: the product could be detected by GC-MS, but in low yields along with several side products. An optimization of the reaction conditions for trialkyltriazenes was not undertaken because these compounds can be prepared by alternative procedures.[9]

The reaction of lithium dialkylamides with N_2O was examined in more detail to obtain information about the mechanism of the reaction. In the literature, one finds only sparse data about this type of reaction. In 1953, Meier reported that $LiNEt_2$ is able to react with N_2O.[17] The addition of N_2O to an equimolar mixture of PhLi and Et_2NH in diethyl ether was reported to result in the formation of a black

reaction mixture. After workup, tetraethyltetrazene was isolated in low yield (identified by its boiling point). Meier speculated about intermediates with the formula $R_2N(N_2O)Li$, but isolation of such an aminodiazotate was not attempted. As mentioned above, we have observed white precipitates upon reaction of lithium dialkylamides with N_2O in THF. The precipitates from the reactions of four different amides have been isolated and analyzed (Scheme 2).



Scheme 2. Synthesis of the N_2O adducts **22–25**.

The products **22–25** display low solubility in noncoordinating organic solvents such as toluene or chloroform. Upon the addition of water, the corresponding amine formed along with liberation of N_2O, as shown by NMR spectroscopy and gas chromatography, respectively (see Figure S2 in the Supporting Information). The products display moderate solubility in THF, with the exception of **22**, which is poorly soluble. The solubility in THF can be enhanced by the addition of LiCl (0.5 M). For compound **22**, for example, the solubility increases from about 1 mM to 59 mM, and the solubility of **25** is increased from about 76 to 328 mM. This observation suggests that **22–25** form aggregates in THF, which are cleaved by the addition of LiCl. **22–25** are all soluble in $[D_6]$DMSO and decomposition is sufficiently slow to allow analysis (see Figure S3 in the Supporting Information). The NMR spectra of **22–25** showed a single set of signals for the alkyl substituents. The simple NMR spectra are in line with the elemental analyses, which indicated that adducts had formed with the formula $R_2N(N_2O)Li$. Compounds **22–25** were isolated in yields between 68 and 95%. Examination of the crude reaction mixtures by ^1H NMR spectroscopy revealed that the reactions are very clean, with negligible amounts of side products and almost quantitative conversion.

A crystallographic analysis was deemed crucial to establish the connectivity and the geometry of the products. Despite extensive screening of the crystallization conditions, we were unfortunately not able to obtain single crystals for any of the products. The formation of aggregates presumably hampered the crystallization of **22–25**. Therefore, we examined the co-crystallization of the compounds with known Li^+ ionophores. Finally, we were able to obtain single crystals of **22** and **24** bound to the metallacrown complex $[\{(cymene)Ru(C_5H_3NO_2)\}_3]$ (**26**). This organometallic ionophore was developed previously in our research group,[18] and displays a very high affinity and selectivity for Li^+ ions. The structures of the complexes [**26**×**22**] and [**26**×**24**] are depicted in Figure 3.

In both cases, the lithium cation is coordinated to three oxygen atoms of the metallacrown complex. The fourth binding site is occupied by the oxygen atom of the $R_2N(N_2O)^-$ ion. The latter adopts a *cis* conformation with respect to the central N1–N2 bond. Two independent molecules in the asymmetric unit ("A" and "B") can be observed for both [**26**×**22**] and [**26**×**24**]. The bond lengths observed in the two independent $R_2N(N_2O)Li$ guests are different. Form "A" shows a very short O1–N1 bond and a long N1–N2 bond, whereas the opposite trend is observed for form "B" (see Table S2 in the Supporting Information). One should note that form "B" is less defined because of crystallographic disorder, and differences in the bond lengths should be taken with care.

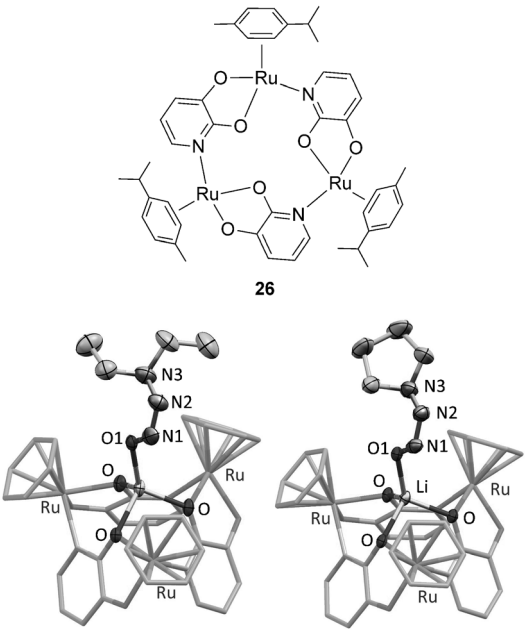
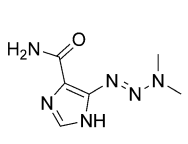
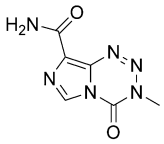


Figure 3. Top: the metallacrown complex **26**. Bottom: the molecular structures of the complexes [**26**×**22**] (left) and [**26**×**24**] (right) in the solid state. Hydrogen atoms, solvent molecules (benzene), and the alkyl groups of the cymene π ligands are not shown for clarity.

The crystallographic analyses along with the NMR spectroscopic and elemental analyses data provide strong evidence that **22–25** are covalent N_2O adducts with the formula $R_2N(N_2O)Li$. So far, well-characterized covalent N_2O adducts of organic compounds with intact NNO groups have only been reported for N-heterocyclic carbenes[19] and frustrated Lewis pairs.[20] The ability of dialkylamides to form N_2O adducts in a clean and quantitative fashion is essential for the success of the overall coupling reaction. The subsequent reaction of the $R_2N(N_2O)Li$ adducts with Grignard reagents requires cleavage of the N–O bond, which is likely facilitated by the oxophilicity of Mg^{2+} ions.

As mentioned above, some 1-aryltriazenes are strongly cytotoxic. To evaluate if 1-alkynyl and 1-alkenyltriazenes exhibit relevant antitumor affects, we performed preliminary in vitro tests with two human cancer cell lines—ovarian cancer cells (A2780) and invasive breast cancer cells (MDA-MB-231)—as well as with model healthy cells (MCF-10a and HEK293). Ten alkynyltriazenes and four alkenyltriaznes were selected and, for comparison, we have included the clinically used triazenes dacarbazine (**27**) and temozolomide (**28**), as


dacarbazine (**27**)


temozolomide (**28**)

well as 1-phenyl-3,3-dimethyltriazene. The latter compound and closely related derivatives have been the subject of numerous biological studies.[7a,21]

Most of the tested alkynyl and alkenyltriazenes displayed high cytotoxicities (see Table S9 in the Supporting Information), and some are considerably more cytotoxic than those in clinical use. Importantly, the substitution of a phenyl group by an alkynyl group resulted in an increased cytotoxicity. The most potent compounds are the alkynyltriazenes **10**, **14**, and **15**, which displayed IC_{50} values in the low micromolar concentration range. Notably, the alkynyltriazenes **14** and **15** also showed a good selectivity for invasive breast cancer cells (MDA-MB-231) over healthy normal breast epithelial cells (MCF-10a). The cancer cells were about sixfold more sensitive towards these compounds, whereas the two compounds in clinical use are either not selective (**28**) or are more cytotoxic towards the healthy cell line (**27**).

To conclude, we have developed a new method for the synthesis of trisubstituted triazenes. The procedure involves the coupling of lithium amides with nitrous oxide and organomagnesium compounds. The following points are particularly noteworthy:

a) The new synthetic method allows access to triazenes with alkynyl and alkenyl substituents at the 1-position. Such compounds are difficult to prepare by conventional methods.
b) The results provide evidence that nitrous oxide can be used as an efficient nitrogen donor in organic synthesis. Previous attempts in this direction have been met with only limited success.
c) We have shown that dialkylamides react with N_2O to give covalent adducts. These compounds represent rare examples of well-characterized organic N_2O adducts with intact NNO groups.

It will be interesting to explore the chemical reactivity of the new alkynyl and alkenyltriazenes in more detail. Aryltriazenes can be converted into a plethora of different compounds by cleavage of the C–N or N–N bonds.[1,22] Synthetically useful transformations can likely also be found for alkynyl and alkenyltriazenes. A more detailed study of the biological activity of triazenes with alkynyl and alkenyl substituents is also worthwhile. It is conceivable that promising anticancer lead compounds can be discovered by a more thorough biological screening of these new triazenes.

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