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Scalable Synthesis of Diazeniumdiolates: Application to the Preparation of MK-8150

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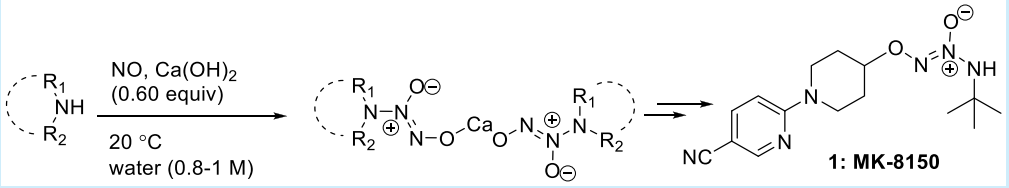
Supporting Information

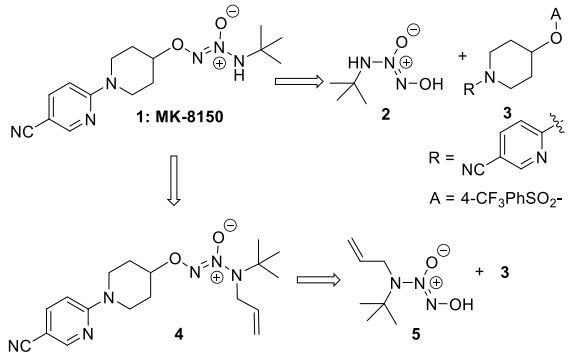


ABSTRACT: Synthetic diazeniumdiolate (DAZD)-based nitric oxide is utilized to modulate the nitric oxide (NO) concentration in cellular environments and to control physiological processes, yet chemists are still struggling to find efficient and scalable methodologies that will enable them to access sufficient quantities of the high-energy diazeniumdiolate intermediates for biological studies. Now, a general, scalable, safer, and high-yielding new methodology adaptable to the large-scale synthesis of DAZDs has been developed.

The field of nitric oxide (NO) donors has made phenomenal progress in the past decades.[1] Rather than simply being considered a toxic pollutant, nitric oxide (NO) is now recognized as playing a key role in the regulation of cardiovascular, immune, and nervous systems.[2] In addition to NO being an active signal-inducing molecule in biological systems, it exhibits a wide variety of well-documented biological properties, such as antimicrobial,[3] antitumor,[4] and antiplatelet[5] properties. Furthermore, it plays a role in the regulation of blood pressure,[6] immune response,[7] and neurotransmission.[8] Diazeniumdiolate (DAZD)-based NO donors, which are typically stable as the respective salt or the derivatized O^2-alkylated diazeniumdiolates, or with a polymeric coating, release 2 equiv of nitric oxide either under physiological conditions (pH 7.4 at 37 °C), or when metabolized by esterase via a known mechanism.[9] However, when NO is released from *N*,*N*-dialkylated DAZDs, their corresponding amine precursor (e.g., dimethylamine, diethylamine, pyrrolidine, piperidine) is generated and the released amines can back-react with an oxidative intermediate of nitric oxide to generate carcinogenic *N*-nitrosamines.[10] However, the carcinogenicity of nitrosamines will significantly decrease by increasing the substitution at the α-position of the *N*-nitroso group. This led to the discovery of MK-8510 (**1**), which is a novel O^2-alkylated diazeniumdiolate NO donor, as a potent and significant blood-pressure-lowering drug candidate in humans[11] and was selected for further development.

Two potential approaches to the chemical synthesis of MK-8150 (**1**), using either an unprotected diazeniumdiolate (**2**) or an allyl-protected diazeniumdiolate (**5**), are summarized in Scheme 1. Within the field of DAZD synthesis, there are significantly less reports for the successful preparation of

Scheme 1. Key Synthetic Strategy for the Synthesis of MK-8150 (1)



DAZDs from primary amines, compared to secondary amines. Not surprisingly, DAZDs and their salt derivatives are both thermally unstable and unstable to moisture. Primary amines have been successfully converted to the corresponding DAZD salts at cryogenic temperature; however, decomposition rapidly occurred at room temperature. Indeed, DAZD (**2**) and its salt have not been prepared.[12] In view of these potential challenges for the synthesis of **1**, we selected the allyl-protected high-energy diazeniumdiolate (**5**) used in the medicinal chemistry route to begin our investigation.[10a]

Although DAZD was discovered over 50 years ago, synthetic methods to access DAZD are lacking, with the only general method being direct reaction of a *N*,*N*-di-*n*-alkylamine with

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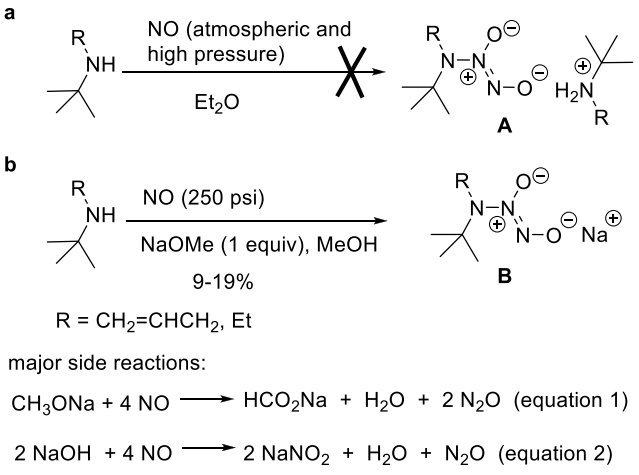
Org. Lett. XXXX, XXX, XXX–XXX

Scheme 2. Current Standard Methods for the Synthesis of DAZDs



Scheme 3. An Ideal Process for the Synthesis of DAZD

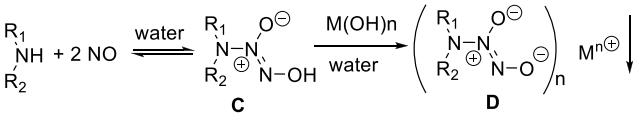
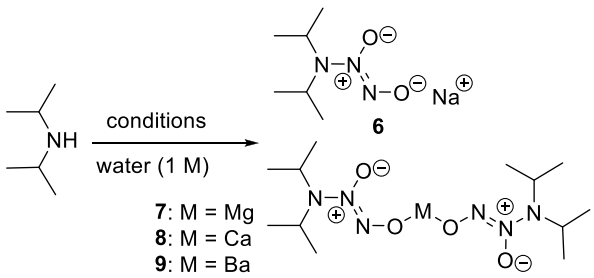


Table 1. Selected Results for the DAZD Formation



entry[a]	base	equiv of base	NO (psi)	temperature, T (°C)	reaction time, t (h)	DAZD yield (%)
1	$Mg(OH)_2$	0.60	250	20	120	36[b]
2	$Ca(OH)_2$	0.60	250	20	120	67[c]
3	$Ba(OH)_2$	0.60	250	20	120	25[b]
4	NaOH	1.20	250	20	120	16[b]
5	$Ca(OH)_2$	0.60	150	20	160	20[b]
6	$Ca(OH)_2$	0.60	250	40	160	52[b]
7	$Ca(OH)_2$	0.60	250	10	160	42[b]
8	$Ca(OH)_2$	0.60	250	0	160	38[b]
9	$Ca(OH)_2$	0.60	350	20	160	78[c]

[a]Reactions were performed on a 6.92 mmol scale. [b]NMR yield determined using *N*,*N*-dimethylpryidin-4-amine as an internal standard. [c]Isolated yield.

high-pressure NO gas to form DAZD. As shown in Scheme 2, employing this method to prepare DAZD from *N*-(*tert*-butyl)-*N*-alkylamines under both atmospheric and high-pressure conditions results in no detectable product **A** (see Scheme 2a).[13] However, under modified conditions, employing sodium methoxide in methanol and NO gas at 250 psi, the desired product **B** was isolated in 9%−19% yield with 50−60 wt % purity (Scheme 2b).[10a,14] In the course of this development work, two unexpected explosions were reported in a 500 mL autoclave reactor (during venting), because of the rapid depressurization of high-pressure nitric oxide (NO) from the headspace through Teflon-lined steel-braided tubing to atmospheric pressure.[15]

Therefore, the goal of our development work was to develop a practical, efficient, and scalable synthetic methodology to access sufficient quantities of the high-energy DAZDs intermediates in a safe manner. In addition to supporting process development, the identification of a general method to access DAZD intermediates and their derivatives should facilitate the investigation of DAZD-based NO donors for biological and medical studies. Although the reaction of NO with carbanions in the presence of sodium methoxide to prepare carbon-bound DAZDs was discovered over 100 years ago,[16] the side reaction of NO with sodium methoxide to generate sodium formate and potential hazardous nitrous oxide was just recently identified by Hrabie (see eq 1 in Scheme 2).[17] The NO gas can undergo a relatively quick disproportionation reaction that can lead to significant quantities of N_2O and NO_2 in a short period of time. Sodium hydroxide can also react with NO, leading to the formation of nitrous oxide and sodium nitrite (see eq 2 in Scheme 2). Hrabie[16] proposed dioxane and sodium trimethylsilanoate as the ideal combination of solvent and base for the formation of DAZDs. However, we did not see any advantage with the proposed conditions in our hands, nor did we find any successful report in the literature using this method. We believe that an ideal reaction to form DAZD would (a) minimize background reactions that generate hazardous nitrous oxide, by avoiding the use of strong bases such as sodium methoxide or sodium hydroxide; (b) avoid the use of any organic solvent to minimize the potential for ignition in the headspace (preferably run the reaction in water); and (c) involve precipitation of the DAZD salt from water toward end of the reaction in order to drive the reaction to completion and simplify isolation of product **D** (Scheme 3).

To limit the background reaction, the concentration of hydroxide in the reaction solution must be maintained at a low level. If a base can slowly release hydroxide into the aqueous solution during the reaction, it will satisfy the demand for a base needed to form the DAZD salt during DAZD formation while decreasing the side reaction of eq 2 in Scheme 2. Keeping this in mind, alkali-metal hydroxides, such as LiOH, NaOH, KOH, and CsOH are likely not suitable bases for the reaction, because of their high solubility in water. The solubility of alkaline-earth metal hydroxides, such as calcium hydroxide, magnesium hydroxide, and barium hydroxide, in water at 20 °C are 0.165 g/100 mL (0.022 M), 0.000816 g/100 mL (1.4×10^{-4} M), and 3.89 g/100 mL (0.23 M), respectively. These three bases should be more suitable for the proposed DAZD formation than alkali-metal hydroxides. We hypothesized that DAZD salts of calcium, magnesium and barium could potentially precipitate out of the aqueous solution, driving the reaction to completion and allowing for direct isolation of the DAZD salts from water.

The DAZD sodium salt of diisopropylamine (**6**) was previously prepared in a 4% overall yield in a two-step sequence by conditions that required 27 atm (397 psi) pressure of NO, highlighting this substrate as one of the most challenging for DAZD formation.[10a] We began our investigation by using alkaline-earth metal hydroxides as bases and diisopropylamine as a model substrate to examine our proposed DAZD formation. Sodium hydroxide was used as the base for a control experiment. Each equivalent of alkaline-

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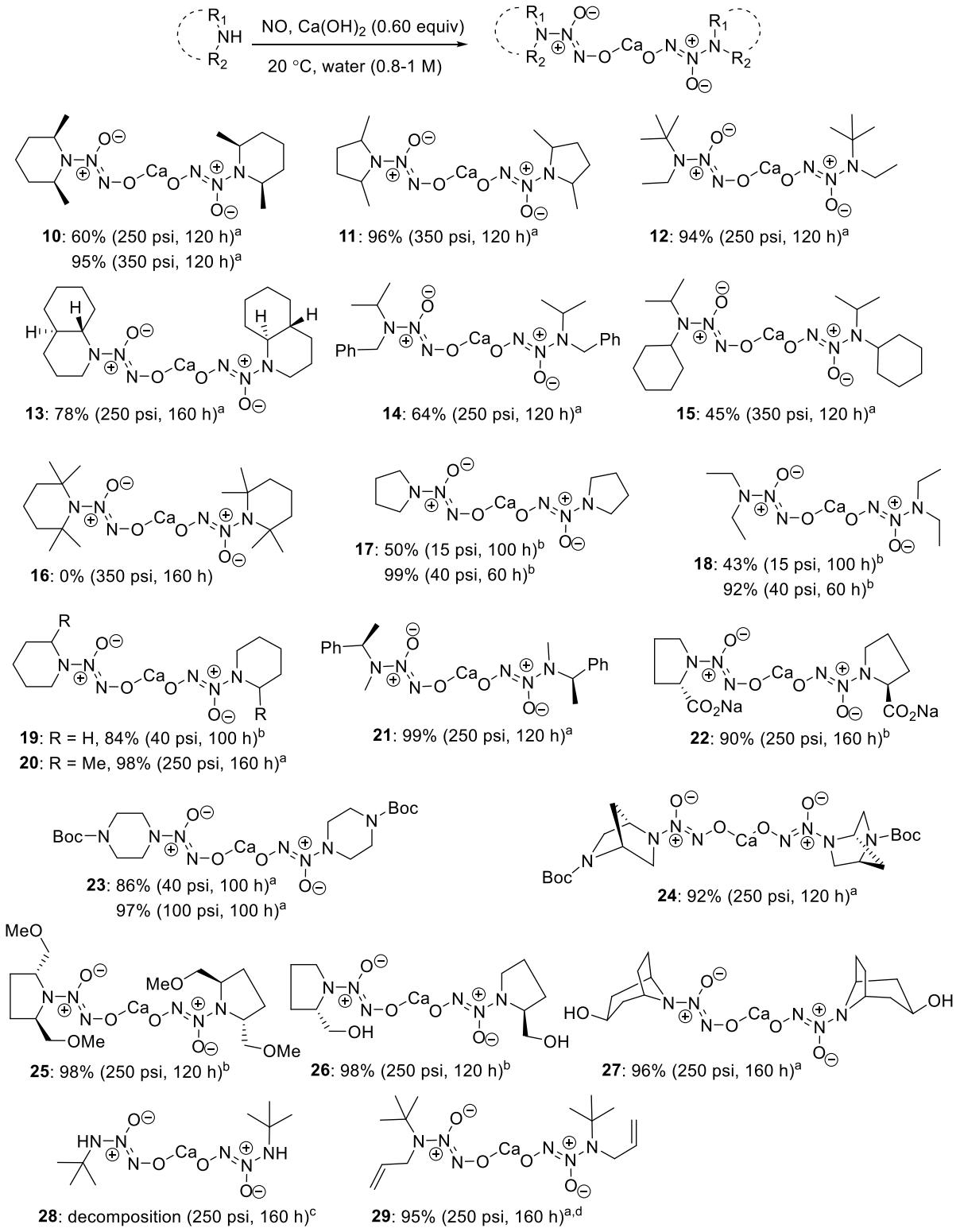

Figure 1. The scope for the DAZD calcium salt formation. All the reactions were performed on a scale of 5−7 mmol, except noted otherwise. All yields are isolated yields. [Footnote "*a*" denoted that the product was directly isolated by filtration. Footnote "*b*" denotes that the product was isolated by lyophilization to remove water, because of the relative high solubility in water of the product. Footnote "*c*" denotes that the DAZD calcium salt was unstable after releasing the NO pressure and was not isolable. Footnote "*d*" denotes that the reaction was performed on a scale from grams to multiple kilograms.]

earth metal hydroxide can release 2 equiv of hydroxide. All the reactions were performed in water at a concentration of 1 M. Initial investigations focused on the evaluation of the bases while other conditions remained the same (Table 1, entries 1−4). Although both $Mg(OH)_2$ (Table 1, entry 1) and $Ca(OH)_2$ (Table 1, entry 2) were partially soluble in the reaction, significant amounts of solid in the $Ca(OH)_2$-mediated reaction were generated during the reaction. The solid was collected by

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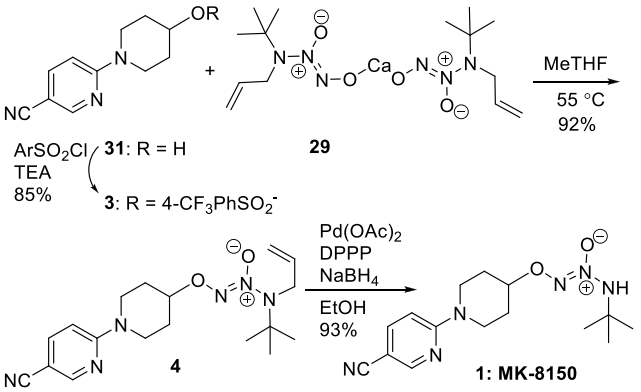
Scheme 4. Application of DAZD Calcium 29 for the Successful Synthesis of MK-8150 (1)



filtration and dried to give product **8** in 67% yield and in high purity. The reaction to form the calcium salt of DAZD was found to be high yielding and afforded the desired product in high purity (in terms of weight percentage). In contrast, the solids from reactions of $Mg(OH)_2$ and $Ba(OH)_2$ were significantly less. When sodium hydroxide was used as the base, a homogeneous solution was observed (Table 1, entry 4). Assay yields of the DAZD salts for these three reactions were performed by lyophilization to remove water and yields obtained by NMR analysis using *N*,*N*-dimethylpryidin-4-amine as an internal standard. Sodium nitrite was found to be the major component of the solid in the sodium-mediated reaction, which supported our hypothesis for the formation of side reactions in the presence of sodium hydroxide (eq 2 in Scheme 2). Further optimization of the $Ca(OH)_2$-mediated DAZD formation found that higher NO pressure gave a better yield of the desired product (Table 1, entry 9 vs entry 5). The optimal reaction temperature was found to be 20 °C. Both higher (e.g., 40 °C) or lower (e.g., 0 or 10 °C) temperature gave lower yield of the product (Table 1, entries 6−8).

With the optimized reaction conditions in hand, we investigated the scope of the DAZD formation protocol. We first examined another challenging sterically bulky substrate, (*cis*)-2,6-dimethylpiperidine, which was reported to be converted to the corresponding DAZD (sodium salt) in 5% yield.[10a] As shown in Figure 1, applying the conditions established with diisopropylamine, 67% yield of the DAZD calcium salt **10** was afforded under 250 psi NO. With an increased pressure of NO (350 psi), the reaction progressed to 95% yield. Under the same conditions, (mixture of *cis* and *trans*)-2,5-dimethylpyrrolidine afforded the corresponding DAZD calcium salt **11** in 95% yield. Both *N*-ethyl-2-methylpropan-2-amine and decahydroquinoline under 250 psi of NO smoothly generated the corresponding DAZD calcium salts **12** and **13** in high yields. Substrates with increasing the steric hindrance at the *N*-substituted alkyl group such as *N*-benzylpropan-2-amine and *N*-isopropylcyclohexanamine, provided their corresponding DAZD calcium salts **14** and **15** in 64% and 45% yields, respectively. In the case of an extremely sterically hindered amine, 2,2,6,6-tetramethylpiperidine, desired product **16** was not isolated under the current conditions, with only the starting material amine being recovered. Using unsubstituted pyrrolidine, diethylamine, and piperidine under 40 psi of NO, the corresponding DAZD calcium salts **17**−**19** were obtained in yields of 99%, 92%, and 84%, respectively. Even under 1 atm of nitric oxide, both pyrrolidine and diethylamine reacted with nitric oxide to provide the desired products **17** and **18** in moderate yields. (*R*)-*N*-methyl-1-phenylethan-1-amine performed well, yielding the corresponding DAZD calcium salt **21** in 99% yield. A variety of functional groups, including amino acid (**22**), Boc amides (**23** and **24**), ether amine (**25**), and alcohol (**26** and **27**), were nicely tolerated, providing the corresponding functionalized DAZD calcium salts in excellent yields. Under 250 psi of NO, *tert*-butylamine reacted with NO to generate a large amount of solid, which is likely the desired product **28**. However, the solid **28** was very unstable. The solid decomposed within minutes and released NO, which was immediately oxidized to a reddish brown gas NO_2 in the air. Finally, *N*-(*tert*-butyl)allylamine was converted to the corresponding DAZD calcium salt **29** under 250 psi of nitric oxide in 95% isolated yield. The preparation of **29** was successfully completed in multiple batches on the scale of multiple kilograms.

An investigation on the kinetic parameters for the DAZD calcium salt decomposition (Table S2 in the Supporting Information) showed an identical decomposition half-life, as reported for the DAZD sodium salt.[10a]

With key intermediate **29** in hand, development of a robust process for the synthesis of MK-8150 (**1**) was undertaken. Activation of the hydroxyl group of **31** with 4-(trifluoromethyl)benzenesulfonyl chloride in the presence of triethylamine afforded compound **3** in 85% yield (Scheme 4). S_N2 displacement of intermediate **3** by 0.70 equiv of **29** in 2-Me-THF gave the penultimate intermediate **4** in 92% yield and the eliminated byproduct (3,6-dihydro-2H-[1,2′-bipyridine]-5′-carbonitrile) in <1.5% yield, which was sufficiently rejected to undetectable levels after crystallization. Reductive allyl group deprotection with $NaBH_4$ in the presence of 1 mol % $Pd(OAc)_2$ and 1.3 mol % DPPP ligand afforded MK-8150 (**1**) in 93% yield and >99.5% purity.

In summary, we have developed a general, practical, efficient, greener, and safer methodology for the synthesis of diazeniumdiolate calcium. Application of the DAZD calcium salt **29** for the synthesis of MK-8150 (**1**) was achieved in 72.7% overall yield from commercially available alcohol **31**. This new methodology has several advantages over previous methods, such as significantly improved yields and easy isolation. The use of less-soluble $Ca(OH)_2$ as a base resulted in controlled release of hydroxide to minimize the background reaction with NO, a significant improvement over traditional methodology that has been used for past 50 years. We are confident this methodology will have an immediate and tangible impact on both academic and pharmaceutical research since NO is now recognized as playing a key role in the regulation of cardiovascular, immune, and nervous systems. A general methodology to access a wide range of NO donors should enable the investigation of NO signaling in biological systems and potentially lead to the discovery of new drugs.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.9b01401.

Experimental procedure, characterization data (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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