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Encapsulated Reagents for Nitrosation

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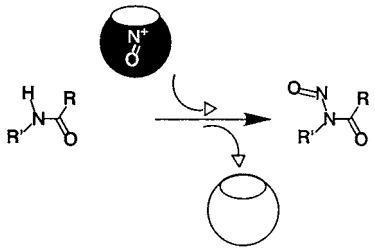
ABSTRACT



A novel class of stable, mild, and size–shape-selective nitrosating agents for secondary amides is introduced. These are based on reversible entrapment and release of reactive nitrosonium species by calix[4]arenes. The NO^+ encapsulation controls the reaction selectivity.

Among the challenges of modern synthetic chemistry are the control of kinetics and selectivity of reactions and the creation of reagents that modify a portion of a molecule without protecting other reactive sites. "Molecule-within-molecule", or encapsulation complexes,[1] offer an exciting breakthrough here as they can at will entrap and release guest species, under subtle chemical or physical control. For such complexes, stabilization of reactive species within the interiors,[2] controlled chemical reactivity,[3] and catalysis[4] have been impressively demonstrated. For example, a self-assembling capsule was used to entrap dicyclohexylcarbodiimide (DCC) and dibenzoyl peroxide (DBPO).[5] The chemical stability of these reagents was greatly improved within the shielded interior. Controlled release and displacement of reactants within a capsule resulted in autocatalysis in the amide bond formation.[3c,d]

We define *encapsulated reagents* as highly reactive species reversibly entrapped within the host cavity that can be released into the reaction mixture under subtle control. The cavity offers protection from the bulk environment and thus controls the reaction rates. Chemical transformations with encapsulated reagents may occur either within the cavity interior, or outside, upon release.[2−4] As far as the delicate, noncovalent forces holding the molecule-within-molecule complex together are concerned, temperature, solvent polarity, and substrate−cavity size−shape complementarity are the critical factors responsible for the reagent release and the occurrence of the reaction. In this communication, we introduce a novel class of encapsulated reagents, stable, mild and selective nitrosating reagents, that are based on reversible encapsulation of reactive nitrosonium species by calixarenes.

In organic chemistry, nitrosation holds a special place. Alkylnitrites (RO−NO), nitrosoamines/amides, and nitrosothiols are used in biomedicine as NO-releasing drugs.[6] In total synthesis, −N═O is an important activating group,

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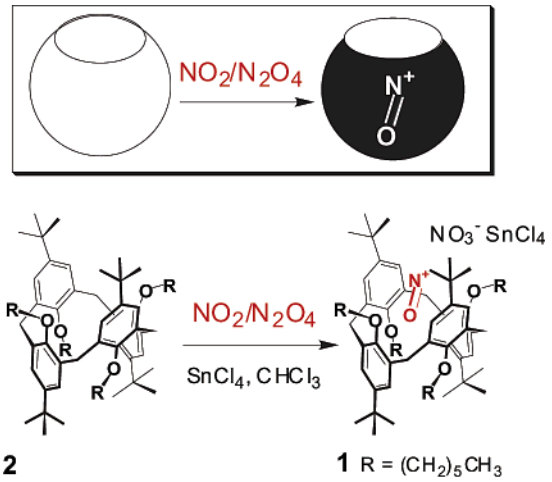

Figure 1. Chemical fixation of NO_2/N_2O_4 with calix[4]arenes. Generation of encapsulated nitrosating reagents.

allowing elegant transformations of amides to carboxylic acids and their derivatives.[7] In addition, nitrosation mimics interactions between biological tissues and environmentally toxic NO_X gases, NO, N_2O_3 and NO_2/N_2O_4, which generate mutagenic nitrosoamines/peptides and nitrosate and deaminate DNA.[8]

We recently reported that simple calix[4]arenes reversibly interact with NO_2/N_2O_4 and entrap highly reactive NO^+ cation within their cavities.[9] NO^+ is generated from N_2O_4, which is known to disproportionate to $NO^+NO_3^-$.[10] Stable calixarene−nitrosonium complexes were quantitatively isolated upon addition of a Lewis acid. Only one NO^+ cation was found per cavity; very high K_{ass} values $\gg 10^6$ M^{-1} were determined.[11]

For this project, nitrosonium complex **1** was employed as an encapsulated nitrosating reagent for secondary amides (Figure 1). Complex **1** was quantitatively prepared upon bubbling NO_2/N_2O_4 through the $CHCl_3$ solution of tetrakis(*O*-*n*-hexyloxy)calix[4]arene **2** in the presence of $SnCl_4$ and characterized by UV−vis, FTIR, ^{1}H NMR spectroscopy, and CHN elemental analysis.[12]

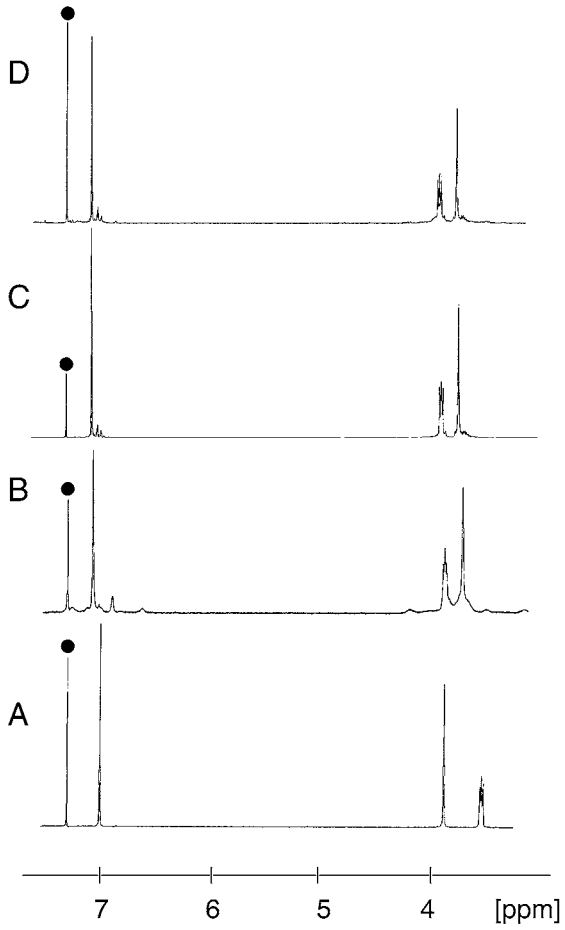

Figure 2. Portions of the ^{1}H NMR spectra (500 MHz, $CDCl_3$, 295 ± 1 K) of (A) calix[4]arene **2**; (B) calix[4]arene−nitrosonium complex **1** prepared from **2**, NO_2/N_2O_4, and $SnCl_4$; (C) a mixture of **2** and NO_2/N_2O_4; and (D) nitrosonium complex, prepared upon addition of $NO^+SbF_6^-$ to calixarene **2**. The residual $CHCl_3$ signals are marked (●).

Specifically, the UV−vis spectrum showed a broad charge-transfer[13] band at $\lambda_{max} \approx 578$ nm. The complex is deeply colored. The FTIR spectrum exhibited characteristic[13] arene−NO^+ stretching at $\nu = 1934$ cm^{-1}. The ^{1}H NMR spectrum of **1** showed new sets of the calixarene signals, different from **2** (Figure 2). In particular, aromatic CH protons of guest-free **2** were seen as a singlet at 6.95 ppm. In nitrosonium complex **1**, this signal was transformed into a singlet at 7.02 ppm. The methylene bridge CH_2 protons of **2** were recorded as a singlet at 3.73 ppm. In complex **1**, this was seen at 3.60 ppm.

Complex **1** can be cleanly obtained from calixarene **2** and NO_2/N_2O_4 even without $SnCl_4$ (Figure 2C). Finally, inde-

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(11) Kochi and Rathore recently described charge-transfer complexes between NO^+ cation and structurally similar calix[4]arenes. The cation was found encapsulated within the cavity (X-ray analysis). See: Rathore, R.; Lindeman, S. V.; Rao, K. S. S.; Sun, D.; Kochi, J. K. *Angew. Chem., Int. Ed.* **2000**, *39*, 2123−2127.

(12) Complex **1**: *Procedure 1*. NO_2/N_2O_4 gas, generated from copper wire and concentrated HNO_3, was bubbled for 20 s through the solution of calixarene **2** (25 mg, 2.5×10^{-5} mol) and $SnCl_4$ (3 μL, 2.6×10^{-5} mol) in dry $CHCl_3$ (1.0 mL). The solvent was evaporated. The dark-blue solid was dissolved in dry $CHCl_3$ (0.5−1.0 mL) and used for further reactions. *Procedure 2*. Stock solution of NO_2 (~3 equiv) in $CHCl_3$ was added to the solution of **2** (1 equiv) and $SnCl_4$ (1.5 equiv) in $CHCl_3$ at room temperature. After 1 h, complex **1** was precipitated upon addition of hexanes, filtered, washed with hexanes, and dried in vacuo: ^{1}H NMR ($CDCl_3$) δ 7.02 (s, 8 H), 3.77 (t, $J = 7.5$ Hz, 8 H), 3.60 (s, 8 H), 1.38 (m, 32 H), 1.30 (s, 36 H), 0.92 (t, $J = 7.5$ Hz, 12 H); UV−vis ($CHCl_3$) $\lambda_{max} = 578$ nm; FTIR ($CDCl_3$) ν (NO^+) = 1934 cm^{-1}. Anal. Calcd for $C_{68}H_{104}Cl_4N_2O_8Sn$: C, 61.04; H, 7.83; N, 2.09. Found: C, 60.96; H, 7.88; N, 2.09.

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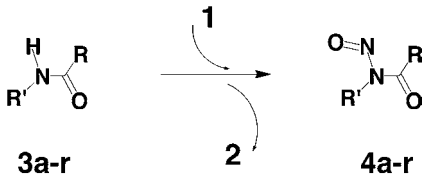

Figure 3. N-Nitrosation of amides **3** with encapsulated reagent **1**.

pendent structural evidence for **1** came from the complexation experiments between calixarene **2** and commercially available $NO^+SbF_6^-$ salt ($CDCl_3$, 295 K). The corresponding UV−vis, FTIR, and ^1H NMR complexation-induced changes were in agreement with the data presented above for complex **1** (Figure 2).

Chemical properties of the encapsulated NO^+ are *different* from those in bulk solution and are controlled by the cavity. We found the following:

1. Highly reactive NO^+ species are protected from the bulk environment. Complex **1** is quite stable toward moisture and oxygen and can be handled, for at least 30 min, without drybox conditions and/or a nitrogen/argon atmosphere. On the other hand, it can be decomposed within a few minutes by addition of H_2O or alcohols, recovering free calixarene **2**. Such stability of arene−nitrosonium complexes is remarkable.[13]

2. Complex **1** acts as a mild nitrosating reagent. When added to the equimolar solution of amide RC(O)NHR′ **3a**−**e** in freshly distilled $CHCl_3$, it reacted instantly at room temperature, yielding *N*-nitrosoamides **4a**−**e** in 50−95% (Figure 3, Table 1). Dark-blue solutions of **1** quickly discharged upon addition of **3a**−**e**, which is a reasonable *visual test* for the reaction. In the ^1H NMR spectra of the reaction mixtures, signals for amides **3a**−**e** and complex **1**

Table 1. Nitrosation of Amides **3a**−**q** with Encapsulated Reagent **1**. Yields of *N*-Nitrosoamides **4a**−**q**

compound	R	R′	yield, %
4a	C_2H_5	CH_3	50
4b	$CH_3(CH_2)_2$	CH_3	68
4c	$CH_3(CH_2)_3$	CH_3	53
4d	$CH_3(CH_2)_4$	CH_3	95
4e	$CH_3(CH_2)_6$	CH_3	63
4f	$C(CH_3)_3$	CH_3	0
4g	C_2H_5	C_2H_5	0
4h	$CH_3(CH_2)_2$	C_2H_5	0
4i	$CH_3(CH_2)_3$	C_2H_5	0
4j	$CH_3(CH_2)_4$	C_2H_5	0
4k	C_2H_5	$CH_3(CH_2)_2$	0
4l	$CH_3(CH_2)_2$	$CH_3(CH_2)_2$	0
4m	$CH_3(CH_2)_3$	$CH_3(CH_2)_2$	0
4n	$CH_3(CH_2)_4$	$CH_3(CH_2)_2$	0
4o	$CH_3(CH_2)_3$	$CH(CH_3)_2$	0
4p	$C(CH_3)_3$	$C(CH_3)_3$	0
4q	$CH_3(CH_2)_3$	$CH_2C_6H_5$	0

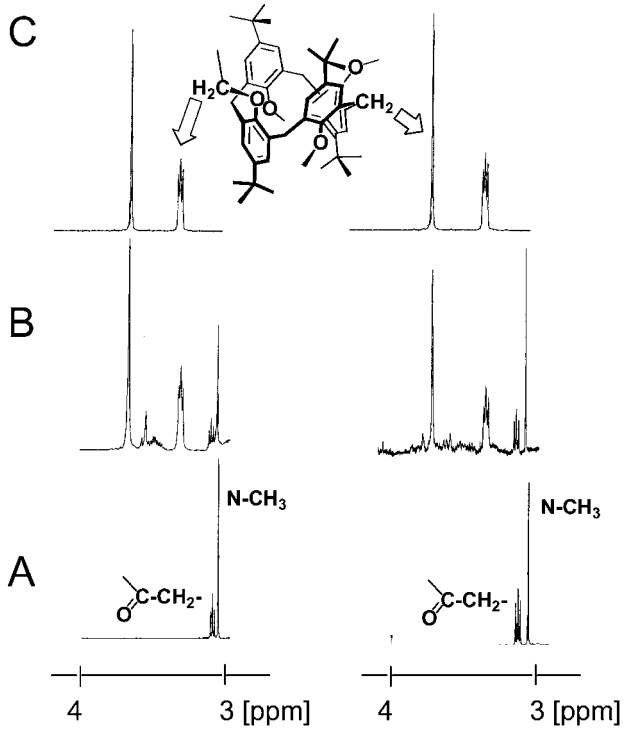

Figure 4. ^1H NMR analysis of nitrosation reactions (500 MHz, $CDCl_3$, 295 ± 1 K): (A) *N*-nitrosoamides **4b** (left) and **4c** (right), independently obtained from **3b**,**c** and NO_2/N_2O_4, respectively; (B) reaction mixtures **1** + **3b** (left) and **1** + **3c** (right) after ∼1 h; and (C) guest-free calixarene **2**.

disappeared and novel, characteristic signals for *N*-nitrosoamides at ∼3.2 ppm (2 H, q for **4a** and t for **4b**−**e**, C(O)-C*H*$_2$) and ∼3.1 ppm (s, 3 H, N(NO)−C*H*$_3$) and for nitrosonium-free calixarene **2** were detected (Figure 4, Supporting Information).[14,15]

3. In reaction with a variety of amides **3a**−**q**, only those possessing *N*−CH_3 substituents were transformed to the corresponding *N*-nitrosoamides **4a**−**e**. No reaction occurred for substrates **3f**−**q** (Table 1, NMR analysis). Accordingly, no color discharge was observed for these reaction mixtures.

Such delicate selectivity of N-nitrosation by complex **1** was unexpected and may be due to steric effects. Clearly, the unreacted subtrates were those possessing *N*-Alk groups bulkier than CH_3.

NO^+ is an aggressive electrophile and is typically not selective.[16,17] Mechanistically, nitrosation of secondary amides and peptides incorporates an electrophilic attack of NO^+

(14) Complex **1** (1 equiv) was added to the solution of amide **3a**−**q** (1−3 equiv) in freshly distilled $CHCl_3$, and the reaction mixture was stirred at room temperature for 5 h. The solvent was evaporated, and the residue was analyzed by ^1H NMR spectroscopy and, in some cases, separated by preparative TLC. All runs were performed at least in duplicate.

(15) For spectral comparison, representative *N*-nitrosoamides **4a**−**e**,**h**,**l**,**m**,**q** were quantitatively obtained employing NO_2/N_2O_4 in $CHCl_3$, similar to the protocol reported in ref 16a. From both methods, the spectral and TLC data for *N*-nitroso compounds **4a**−**e** were identical and in agreement with published data; see ref 16c−e and Supporting Information. **Caution:** *N*-Nitrosoamides are carcinogens and should be treated with extreme care.

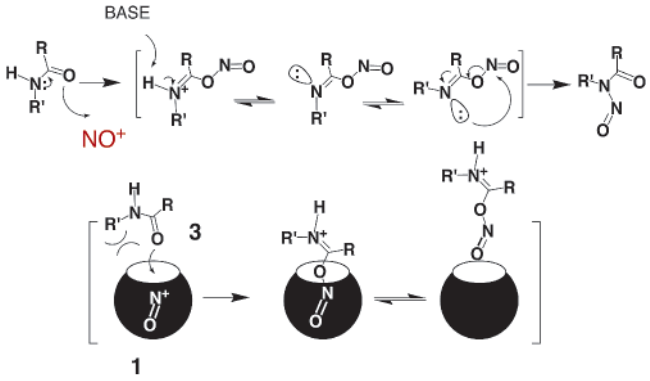

Figure 5. Top: the currently accepted mechanism of N-nitrosation of secondary amides/peptides.[17] Below: a proposed mechanism of nitrosation with encapsulated reagents.

(generated from NO^+−salts, N_2O_3, or NO_2/N_2O_4) on a nucleophilic carbonyl oxygen of the substrate, yielding the corresponding *O*-nitroso species (Figure 5).[17]

Rapid deprotonation, rotation around the C−O bond, and inversion through the nitrogen results in the intermediate, in which both the nitrogen lone pair and the NO group are properly oriented for the isomerization to the *N*-nitrosoamide.

Dimensions and shapes of R and R′ become much more crucial when encapsulated reagent **1** is employed. Molecules **3** approach the cavity **1**, facing it with the carbonyl oxygen (Figure 5). This places the N−R′ alkyl group in close proximity to the two *t*-Bu and two $O(CH_2)_5CH_3$ groups of calixarene. For sizable R′, this situation could be sterically unfavorable, so that the substrate C═O and the encapsulated NO^+ would not reach each other. As for the C(O)R alkyl group, it is obviously positioned farther away from the calixarene substituents and does not significantly interfere, except in the case with bulky amide **3f**.[18] Once formed, the *O*-nitroso intermediate leaves the interior and further collapses in bulk solution, as expected.[17] Due to the extremely strong binding of NO^+ by the calixarene, the rate-limiting formation of the O-nitrosation intermediates should take place within the cavity, *prior* to the NO^+ dissociation. Otherwise, all reactions should proceed with roughly the same rate, and no selectivity should be seen.

In summary, novel nitrosating reagents are now available that can be obtained upon fixation of NO_2/N_2O_4 with calix[4]arenes. These are encapsulated reagents, and their reactivity is controlled by the host cavity. They are stable, mild, and size−shape selective. It would be interesting to test the encapsulated reagents in the synthesis of peptide-based NO-releasing pharmaceuticals, including chiral derivatives, and also for regioselective activation of peptides through N-nitrosation. For this, synthetic modifications of the calix[4]arene cavity may be required.

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Supporting Information Available: Procedures and spectral data for complex **1**, amides **3**, and nitrosoamides **4** and ^{1}H NMR spectra of amides **3a**−**q** and nitrosoamides **4a**−**e**,**h**,**l**,**m**,**q**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) It is well-known that secondary *N*-alkyl amides similar to **3** exist mostly (>98%) as trans isomers; see, for example: Radzicka, A.; Pedersen, L.; Wolfenden, R. *Biochemistry* **1988**, *27*, 4538−4541 and references therein. In our experiments, no cis isomers **3** were detected by ^{1}H NMR. Moreover, if the nitrosation proceeded via the cis conformers, there should not be any preferences in reactivities for amides with different R′-groups, as they are positioned far away from the calixarene cavity.