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Synthesis of Diazen-1-ium-1,2-diolates Monitored by the "*NO*tizer" Apparatus: Relationship between Formation Rates, Molecular Structure and the Release of Nitric Oxide[‡]

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Nitrogen-bound diazen-1-ium-1,2-diolates (diazeniumdiolates, "NONOates", "solid NO") are generally prepared from secondary amines and nitric oxide and are compounds of first choice for the direct release of nitric oxide (NO). First, we report on the relationships between the structures of the amines and the formation rates of the corresponding NONOates, second on the structures of the NONOates and the rate of NO release and finally between the rates of NONOate formation and NO release from these species. A series of differently sized and substituted cyclic and aliphatic amines were used to quantify the reactivity of amines towards NO by monitoring the decrease in NO pressure with the *NO*tizer, an apparatus developed for this study. The release of NO was measured amperometrically with an NO-sensitive electrode and the half-lives of novel diazeniumdiolates were determined by UV spectroscopy. It was found that steric hindrance and heteroatomic substituents in the amines' side chains reduce the formation rates of the sodium salts of the NONOates and also slow down NO release from them. Exceptions were found with piperidine-2-carboxylic acid derivatives which react to give NONOates more slowly than piperidine but release NO much faster despite steric hindrance. A secondary amine carrying an additional primary amine reacted quickly with NO, but the corresponding NONOate showed slower releaser of NO owing to the formation of an intramolecular NONOate salt as well as the sodium salt derivative. Azetidine reacts faster with NO than all of the other amines, but decomposition of the corresponding NONOate proved to be unexpectedly slow.

Introduction

Nitrogen-bound diazen-1-ium-1,2-diolates **2** (NONOates) are used as biomedical tools to mimic the physiological role of nitric oxide in vitro, but they are also potential drugs and have uses as molecular components in NO-releasing prodrugs, biomaterials and hybrid drugs.[2–6] NONOates imply the functional group $[N(O)NO]^-$ and in solution they are capable of donating NO directly into the media in a pH-, temperature- and structure-dependent manner. The release of NO is accelerated by lowering the pH and increasing the temperature.[7] Literature data demonstrate that decomposition rates of NONOates are mainly determined by their structure (Figure 1).[7–11] Zwitterionic oligoamine NONOates such as DETA/NO, SPER/NO and PAPA/NO have rather long half-lives, whereas sodium salt diazeniumdiolates like DEA/NO, PYRRO/NO, Na-PIPERAZI/NO and PIPECO/NO are comparatively quick releasers and within both groups the rate of decomposition is modulated by the individual structures.

NONOates are generally synthesized in a stainless steel standard hydrogenation apparatus or an autoclave in which the dissolved amine is exposed to several atmospheres of nitric oxide.[8,9] Sodium methoxide is used in the synthesis of sodium salt diazeniumdiolates [Equation (1)], whereas no additional base is needed in the formation of intermolecular ammonium salts [Equation (2)] or zwitterionic compounds [Equation (3)].

We recently developed the "*NO*tizer", a modified and improved hydrogenation apparatus for the specific synthesis of NONOates.[12] Besides several attributes that simplify the synthesis, the *NO*tizer features a PC-interface linked to a computer with appropriate software that allows the continuous on-line recording and graphical illustration of the pressure and temperature pattern in the reaction chamber. Further details are provided in the Experimental Section.

The effect of pH, temperature, light and the NONOates' molecular structure on dissociation rates has been described in previous publications.[8,9,13–23] The influence of these pa-

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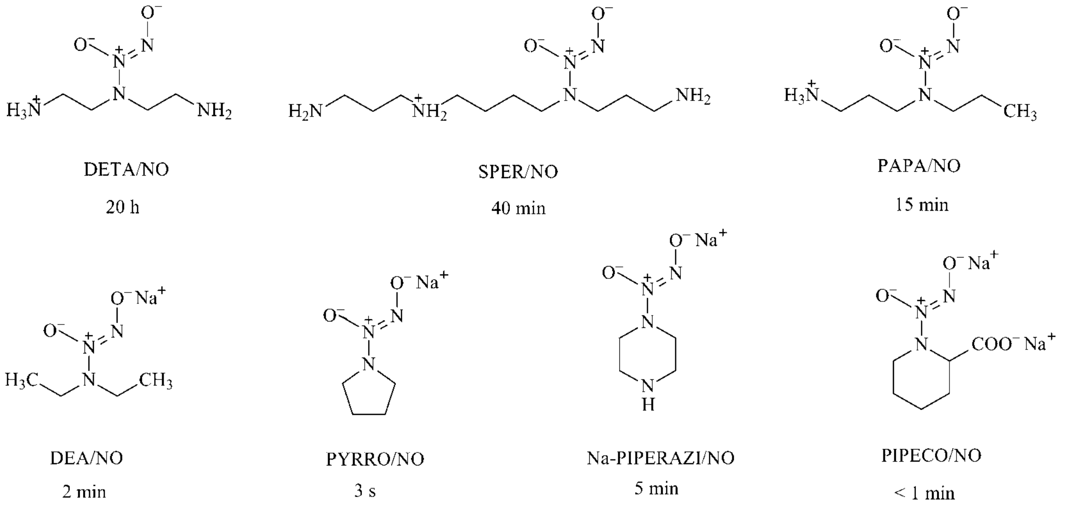
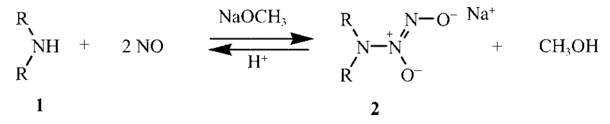


Figure 1. Zwitterionic and sodium salt NONOates with their corresponding half-lives at pH 7.4 and 37 °C.[7–11]



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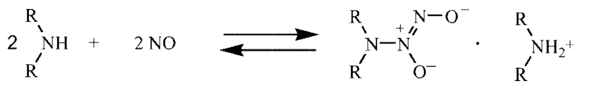

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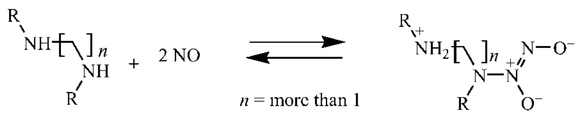

(3)

rameters on diazeniumdiolate formation, however, has not been investigated systematically to date. By using the *NO*tizer we have been able to study the relationships between the steric (i.e. constraints due to rehybridization of the amine nitrogen atom or congestion near to it) and electronic properties of the secondary amine acting as a nucleophile and the progress and rate of NONOate formation by measuring the amount (volume) of NO removed per unit time. Because we would rather focus on the "practical" reactivity of the amines under conditions that are commonly applied in diazeniumdiolate synthesis and not on the theoretical formation rates, and because all of the experiments were performed under identical standard conditions, comparison of the reactivities of different amines is meaningful. To simplify matters we use the term "formation rate" to describe the data we have obtained from the recording of NO consumed in the reaction with amines. Furthermore, we have investigated the correlation between the formation rates of NONOates and their dissociation in order to design compounds that allow controlled release of nitric oxide.

Results and Discussion

The structures of amines **1a**–**v** leading to corresponding diazeniumdiolates **2a**–**v** [see Equation (1)] are given in Figure 2.

Effect of Ring Size of Cyclic Amines/NONOates

A set of cyclic amines with 3–6 methylene groups was transformed into the corresponding NONOate sodium salts. The pressure–time plots were recorded in order to estimate the formation rates. The NO release from isolated NONOates was characterized with a nitric oxide sensor. The half-lives of the diazeniumdiolates were determined by UV spectroscopy at pH 7.4 at 37 °C. The results are illustrated in Figure 3 and Table 1(I). The decrease in ring size of the cyclic amine parallels the higher reactivity of the amine towards nitric oxide. The formation rate for compound **2a** is a factor of 1.73 higher than for **2c**, and for **2b** it is 1.44 higher than that of **2c**. The formation rates for **2c**, **2d** and **2e** are nearly identical. Steric ring strain due to abnormal bonding angles exists in **1a** but not significantly in **1b**. It can be concluded, therefore, that the reactivity of cyclic amines towards NO increases as the steric interference around the basic nitrogen atom decreases. This can be demonstrated impressively by comparing the reactivities of the sterically rigid pyrrolidine (**1b**) and the highly flexible diethylamine (**1l**) (see Figure 3). Furthermore, when comparing the mole ratio of NO consumed/amine for cyclic (Table 1, column D) and aliphatic amines (Table 2, column D) it is found that all of the cyclic amines, with the exception of compounds **1h** and **1k**, have a higher reactivity towards NO than the aliphatic and benzylamines.

Unexpectedly, **2a** turned out to be a rather slow releaser, whereas the other cyclic NONOates did not show significant differences in dissociation at pH 6.4. To verify this result, the decomposition of NONOates **2a**, **2b** and **2c** was monitored by UV spectroscopy. Figure 4(A) illustrates the decrease in UV absorption of these compounds dissolved

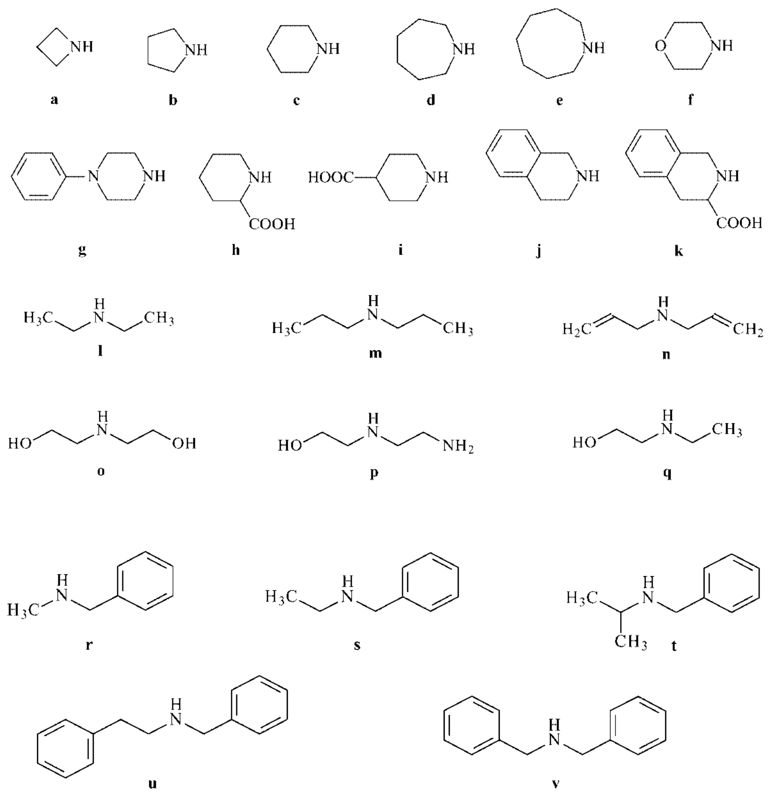

Figure 2. Amines **1a**–**v** used in the preparation of diazeniumdiolates **2a**–**v** [see Equation (1)].

in phosphate buffer at pH 7.4 at 37 °C. Again, compound **2a** decomposes much more slowly (k = 8.13×10^{-3}; half-life ca. 1.5 min) following a first-order kinetic curve [Figure 4(B)], as demonstrated previously for other NONOates.[8,14] It could be expected that the rate of decomposition of the NONOates increases with decreasing ring size; however, this only tends to be the case when comparing **2c**–**e** with **2b**. Possible reasons for the slower release of NO from **2a** are being explored.

Effect of Molecular Modifications in Six-Membered Cyclic Amines/NONOates

Not only steric but also electronic properties affect the formation and dissociation of NONOates. Some modified piperidine derivatives (**1f**–**k**) were selected to measure the progress of the reaction with NO and to compare it with the release of NO from corresponding NONOates **2f**–**k**. Figure 5 and Table 1(II) illustrate the results obtained using

Table 1. Formation and NO release data for cyclic amines **1/2a**–**k**.

	Compound	Formation				NO release	
		A[a]	B[b]	C[c]	D[d]	Maximum [s] (NO electrode)	Half-life [s] (UV spectr.)
I	**1a**[e]/**2a**	2.55 (1.73)	16:45	1.25	1.51	75.3 ± 2.8	85.2
	1b[e]/**2b**	2.11 (1.44)	19:24	1.45	1.39	29.3 ± 1.5	3.6
	1c[e]/**2c**	1.47 (1.00)	19:48	1.99	1.10	36.3 ± 3.2	18.9
	1d[e]/**2d**	1.43 (0.97)	31:15	1.82	1.08	29.3 ± 2.3	3.6
	1e[e]/**2e**	1.52 (1.03)	21:02	1.92	1.12	29.0 ± 2.6	4.7
II	**1f**[e]/**2f**	1.40 (0.95)	26:31	1.69	1.08	100.0 ± 11.9	152.3
	1g[e]/**2g**	1.17 (0.80)	24:32	2.16	1.00	82.0 ± 3.4	263.5
	1h[e]/**2h**	0.48 (0.33)	23:48	2.78	0.65	32.0 ± 1.0	6.1
	1i[e]/**2i**	1.24 (0.84)	20:14	2.19	0.98	39.6 ± 3.0	22.6
	1j[e]/**2j**	1.36 (0.93)	21:28	2.01	1.08	38.3 ± 2.8	29.3
	1k[e]/**2k**	0.45 (0.31)	45:48	2.65	0.61	27.3 ± 3.2	6.8

[a] Decrease in pressure (pressure descent) [bar] within the first 7 h (ratio compd./**1c**). [b] End of reaction [hh:mm]. [c] Remaining pressure [bar]. [d] Mole ratio of NO consumed/amine. [e] 0.05 mol amine was used in the synthesis.

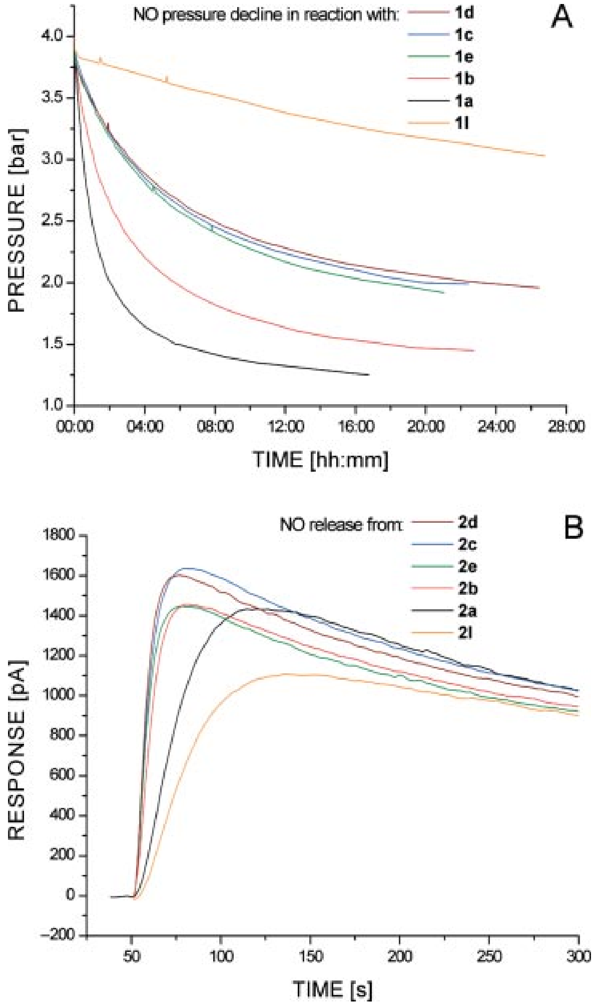

Figure 3. A) Pressure–time plot for the reaction of cyclic amines and **2l** with NO. B) NO release measured with an NO electrode.

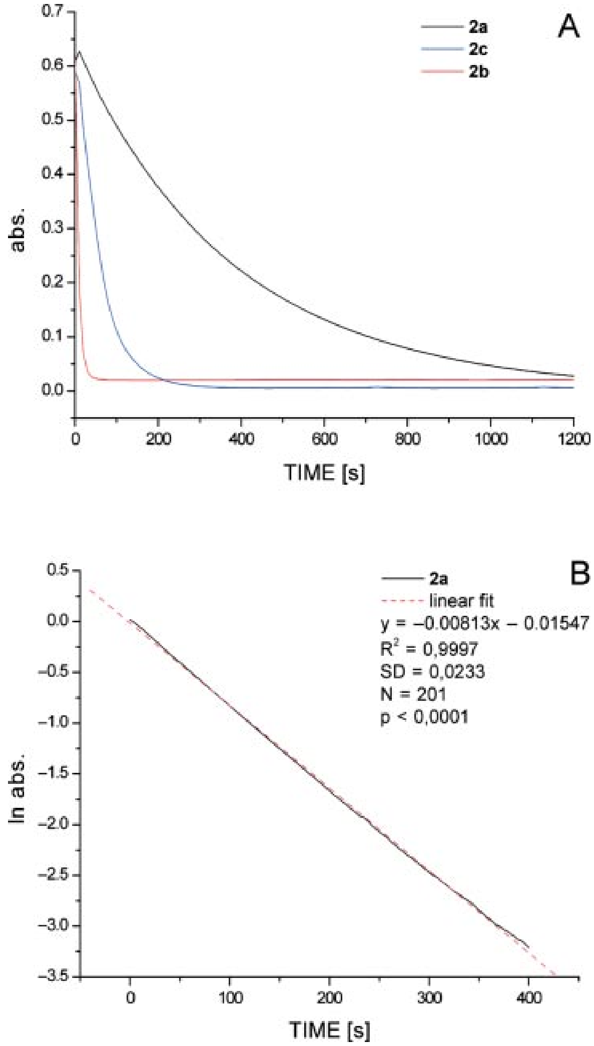

Figure 4. A) UV absorption for NONOates **2a**–**c** at 251 nm in phosphate buffer at ph 7.4 at 37 °C over 30 min. B) Linear fit of the natural logarithm of the UV absorption with time for compound **2a**.

2c as a reference. Substituents and heteroatoms attached or incorporated into the cyclic molecules cause a decrease in the rate of NONOate formation. This is especially pro-

Table 2. Formation and NO release data for aliphatic and benzylamines **1**/**2l**–**v**.

	Compound	Formation				NO release	
		A[a]	B[b]	C[c]	D[d]	Maximum [s] (NO electrode)	Half-life [s] (UV spectr.)
I	**1l**[e]/**2l**	1.04 (1.12)	37:29	1.87	0.57	85.5 ± 4.4	112.3
	1m[e]/**2m**	0.93 (1.00)	61:54	1.70	0.56	70.2 ± 9.8	93.2
	1n[e]/**2n**	1.20 (1.29)	38:01	1.68	0.63	131.2 ± 14.9	246.6
II	**1o**[e]/**2o**	0.73 (0.78)	44:04	2.11	0.59	266.0 ± 2.0	456.0
	1p[e]/**2p**	1.29 (1.39)	37:25	1.55	0.66	1765.0 ± 209.0	7498.9
	1q[e]/**2q**	1.04 (1.12)	62:48	1.56	0.65	116.4 ± 18.7	206.9
III	**1r**[e]/**2r**	2.11 (2.26)	36:54	0.94	0.84	50.6 ± 0.5	56.7
	1s[e]/**2s**	1.00 (1.07)	60:34	1.71	0.62	146.6 ± 16.6	302.0
	1t[e]/**2t**	0.55 (0.59)	69:39	2.66	0.37	911.0 ± 147.2	7036.1
	1u[e]/**2u**	0.82 (0.88)	65:57	1.88	0.56	233.0 ± 11.0	537.3
	1v[e]/**2v**	0.78 (0.84)	38:28	2.10	0.53	260.3 ± 5.0	679.5

[a] Decrease in pressure [bar] within the first 7 h (ratio compd./**1m**). [b] End of reaction [hh:mm]. [c] Remaining pressure [bar]. [d] Mole ratio of NO consumed/amine. [e] 0.1 mol amine was used in the synthesis.

nounced for amines with a carboxylic functional group in the *ortho*-position to the reactive nitrogen atom (**1h** and **1k**). The neighbouring carboxylic function slows down NONOate formation. On the other hand, the carboxylic group clearly catalyses the decomposition of the corresponding NONOates to produce NO very rapidly, which was first demonstrated by Saavedra et al. with PROLI/NO (proline).[17] The presence of heteroatoms, for example in **2f** or **2g**, slows down the release of NO relative to **2c** by a factor of two to three.

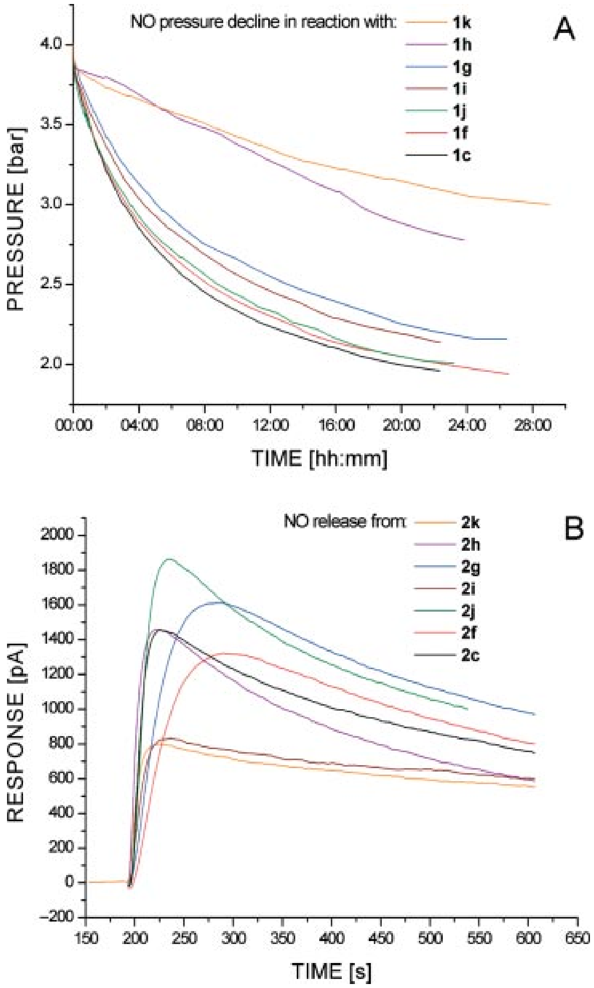

Figure 5. A) Pressure–time plot for the reaction of substituted cyclic amines with NO. B) NO release measured with an NO electrode.

Influence of Substitution in Noncyclic Derivatives

A series of noncyclic amines with different alkyl chains were transformed into NONOates. Diethyl- (**1l**), dipropyl- (**1m**) and diallylamine (**1n**) served as simple alkylamines, the latter to study the effect of double bonds in the β–γ position with respect to the secondary amine, whereas different hydroxy-, amino- and benzyl-substituted amines were studied to exemplify other electronic and steric effects. Figures 6, 7 and 8 as well as Table 2 show the kinetics for the synthesis of NONOates and the release of NO.

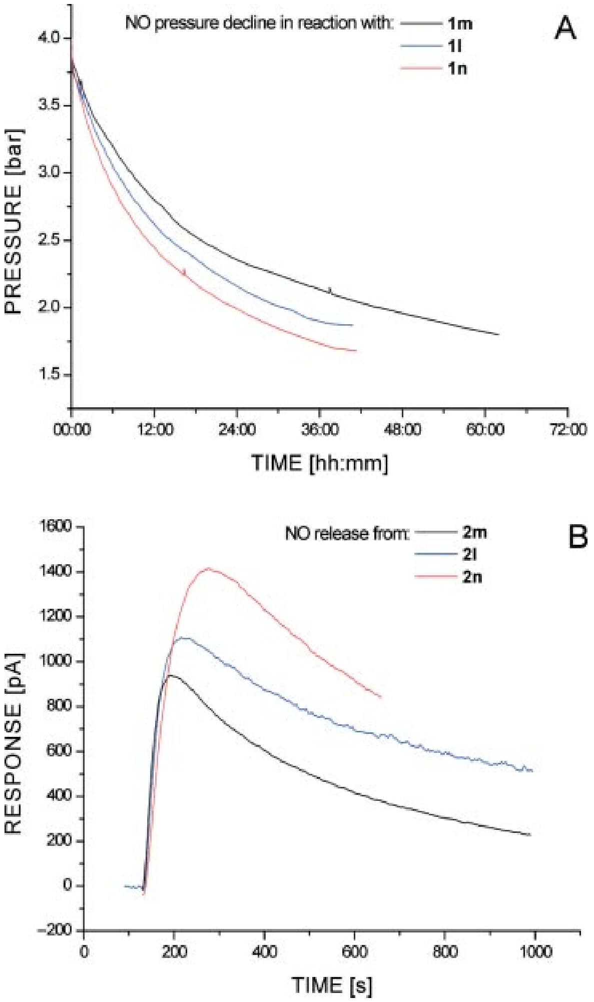

Figure 6. A) Pressure–ime plot for the reaction of alkylamines with NO. B) NO release measured with an NO electrode.

The data in Figure 6 and Table 2(I) demonstrate that the double bonds in **1n** accelerate the synthesis relative to saturated analog **1m**, whereas **1l** has an intermediate reactivity. The same sequence, but reversed, appears in the rate of liberation of NO, with **2n** being the slowest releaser and **2m** the fastest liberator. The results presented in Figure 7 and Table 2(II) show that hydroxy groups (**1o** compared with **1l** and **1m**) generally hamper the formation of NONOates. In contrast, the formation of **2q** is faster than the formation of non-hydroxylated **2m** since ethyl is less bulky than propyl (see Figure 6). The NO release rate is generally reduced by the presence of hydroxy groups. Accordingly, the maximum NO release is shifted to the right to a greater extent with two hydroxy groups (**2o**) than with just one (**2q**). By far the slowest NO releaser is **2p**. It is likely that **1p** and NO produce the usual NONOate sodium salt but also the intramolecular ammonium salt as well, which is a slow releaser, as outlined in the Introduction section. The ^{1}H NMR spectrum of **2p** exhibits a minor triplet (20 mol-%) at $\delta = 2.6$ ppm for the methylene group neighbouring the protonated amine and a major triplet at $\delta = 2.27$ ppm for the corresponding methylene group in the so-

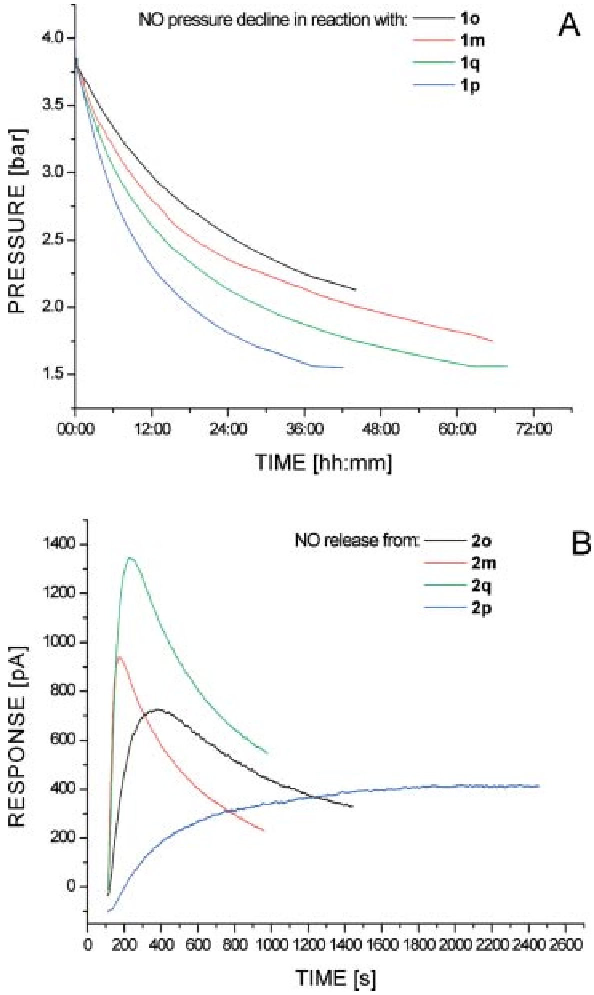

Figure 7. A) Pressure–time plot for the reaction of substituted alkylamines with NO. B) NO release measured with an NO electrode.

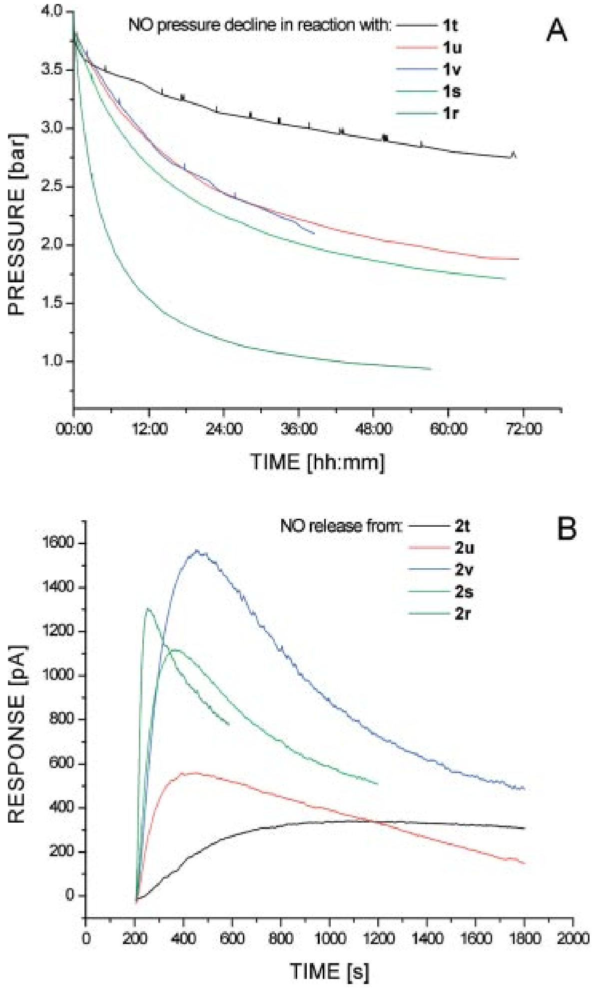

Figure 8. A) Pressure–time plot for the reaction of benzylamines with NO. B) NO release measured with an NO electrode.

dium salt. Nevertheless, the additional primary amino function in **1p** accelerates the formation of the corresponding NONOate.

Furthermore, the effect of steric hindrance in the secondary aliphatic amines reacting with NO can be demonstrated with the benzylamine derivatives. As shown in Figure 8 and Table 2(III), an isopropyl moiety (**1t**) causes a total NO consumption of just over 1.3 bar within a reaction time of 70 h, while the amine with a methyl moiety (**1r**) consumes more than 3 bar of NO in total and an amount four-fold higher in the first 7 h. Only minor differences in the reaction rates of **1u**, **1v** and **1s** could be observed. Analysis of the release of NO again demonstrates that an increase in steric hindrance decelerates the rate of NO liberation from the NONOates in solution (compare **2r** with **2s**, **2s** with **2t** and **2u** with **2v**). The synthesis of diazeniumdiolates derived from amines like diisopropylamine, di-*sec*-butylamine, dicyclohexylamine or 2,2,6,6-tetramethylpiperidine was not successful, as evidenced by an NO consumption that was close to zero.

Correlation between NONOate Formation and Decomposition

The correlation between NONOate formation and decomposition is visualized in Figure 9. It can be observed that steric hindrance around the secondary amine as well as heteroatomic substitutions in the amine's backbone clearly hamper the bonding of NO to the amine's nitrogen atom and the formation of the NONOate is slowed. Because of their reduced conformational flexibility, cyclic amines show much higher reaction rates than aliphatic amines, whereas the effect of heteroatomic substituents on slowing the reaction progress can be observed in both cyclic and aliphatic amines. Phenyl substituents in the α position are well-tolerated with regard to NONOate formation (compare **1l** with **1s** and **1u** with **1v** in Table 2), whereas the introduction of a second substituent in the α position – even a small methyl group giving an isopropyl derivative (**1t**) – inhibits the reaction with NO dramatically (compare **1c** with **1h** and **1j** with **1k** in Table 1 and **1s** with **1t** in Table 2).

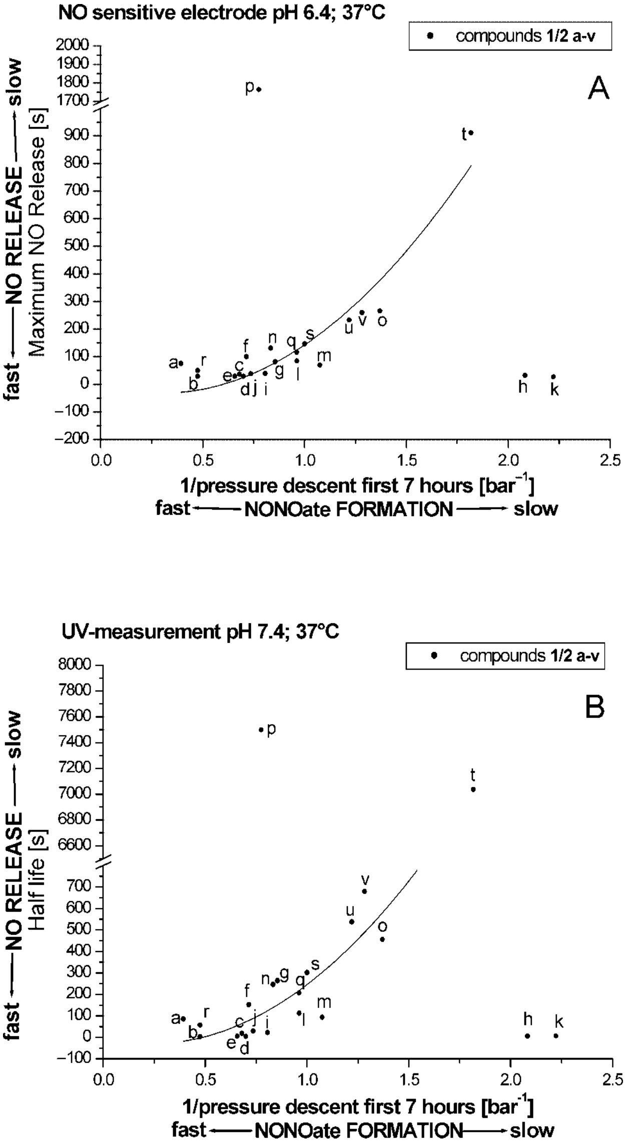

Figure 9. Relationship between NONOate formation and NO release for compounds **1/2a**–**v**. A) Ordinate: maximum release [s] measured with an NO-sensitive electrode at pH 6.4 at 37 °C. B) Ordinate: half-lives from UV measurements at pH 7.4 at 37 °C.

In general, a slow formation of NONOates yields slow NO releasers and vice versa, but remarkable exceptions, for example, **1/2h**,**k**,**p**, are evident. Sterically demanding substitution by carboxy or dimethyl (i.e. the isopropyl moiety) groups in the position α to the amine, as found in compounds **1h**, **1k** and **1t**, causes slow NONOate formation, but only compound **2t** shows the expected slow NO release. The *ortho*-carboxy function in **2h** and **2k** is certainly considered to be bulky, but on the other hand it clearly catalyzes the decomposition of the NONOates. Compounds **1/2p** are exceptional as well; the partial formation of the intramolecular salt contributes to a rather fast formation the NONOate, but this product is the slowest NO releaser of all the compounds investigated. Less obvious, owing to the scaling in Figure 9, is the exceptional pair of compounds **1/2a**. Compound **1a** was found to be the fastest reacting amine, but **2a** was not the fastest NO releaser. In fact it was found to be the slowest releaser of the unsubstituted cyclic NONOates.

Significance of the Results

The direct controlled release of nitric oxide from water-soluble compounds is of great interest not only for their use as tools to simulate physiological conditions in vitro, but also in drugs and biomaterials with therapeutic roles.[4,24] The "*NO*tizer" represents a modified hydrogenation apparatus which simplifies the synthesis of NONOates and allows the decrease in NO pressure to be recorded on-line. The rates of formation of the NONOates and the end of the reaction can be easily determined by this approach. The collected data can be used to predict NONOate formation and decomposition rates for novel compounds and therefore simplify their design.

We have also demonstrated that both amperometry and UV spectroscopy can be used to determine the liberation profile of different NONOates. Whether the pH is 6.4 or 7.4, the order of decreasing rate of NO release from NONOates is nearly identical [compare Figure 9(A) and (B)]. The advantage of the NO-sensitive electrode lies in its higher throughput which allows the rate of NO release from the tested NONOate to be assessed. However, the half-life cannot be determined by this method which can be regarded as a disadvantage.

Experimental Section

Acquisition of Pressure-Time Plots in the Formation of Nonoates: Chemicals were purchased from VWR International Merck KGaA, Darmstadt, and Linde AG, Unterschleissheim, Germany (NO gas 2.5 UN–Nr. 1660), and used without further treatment unless noted otherwise. All reactions in the *NO*tizer were conducted without any heating or cooling. The structures of amines **1a**–**v**, leading to the corresponding diazeniumdiolates **2a**–**v** [see Equation (1)], are given in Figure 2. To ensure comparability, all reactions were performed in methanol, which was dried before use and kept under an atmosphere of dry nitrogen. Elementary sodium was dissolved in methanol and the solution was filtered and added to an equimolar amount of amine, although two-fold molar quantities of sodium were added to amines **1h, 1i** and **1k**. The initial quantity of amine was 0.05 mol for cyclic amines **1a**–**k** and 0.1 mol for alkyl- and benzylamines **1l**–**v**. The final volume for every experiment was adjusted to 100 mL by adding methanol and the solution poured into the reaction chamber and tightly sealed. In order to provide an anaerobic atmosphere, the chamber was evacuated and flushed with dry nitrogen four times and then evacuated again. Finally an initial pressure of 4.0 bar of nitric oxide was applied, all the regulatory valves were closed, the solution stirred magnetically at 700 rpm and the pressure was recorded on-line producing the plots shown in Figures 3(A), 5–8(A).

The complete NO pressure–time plots were recorded and we also defined a further four reaction parameters. The end of the reaction [hh:mm] was fixed at the point when a drop in pressure of 0.05 bar takes longer than 3 h. The remaining pressure [bar] at this point was also used to describe the extent of the reaction. The remaining pressure at the end of the reaction is due to unreacted NO, but side reactions such as the disproportionation of NO cannot be excluded. Furthermore, we compared the reduction in pressure (pressure descent) [bar] within the first 7 h and the mol ratio of NO consumed by the amine. Because 2 mol of NO bind to 1 mol of amine the maximum theoretical ratio is 2. Note that after recording the pressure–time plots the pressure was raised back to high pressure several times until the NO consumption in the reaction was close to zero to improve the yield of the product as reported previously.[12]

Isolation and Characterization of the NONOates: The NONOates precipitated as solids during the reaction or after concentrating the final solution. Compounds **2o, 2p** and **2q** formed as resin-like residues after solvent evaporation. The solids were separated by filtration, washed with diethyl ether and dried in a desiccator under reduced pressure overnight. Melting points were measured with a Mel-Temp II, Laboratory Devices Inc., USA. The identity and purity of the products were verified by ^{1}H NMR spectroscopy (Bruker Advance 250 spectrometer) and elemental analyses were performed with a Hereaus Vario EL apparatus. Compounds **2b**,**c**,**f**–**i**,**l**,**m**,**q**,**r** have previously been reported in the literature and analytical data were confirmed.[9,11,25–30]

Sodium 1-(Azetidin-1-yl)diazen-1-ium-1,2-diolate (2a): Prepared from **1a** (2.86 g, 0.05 mol). Yield: 6.41 g (92%) as white crystals. M.p. 173–175 °C (dec.). ^{1}H NMR (250 MHz, D_2O/NaOD): δ = 1.93 (q, ^3J = 7.5 Hz, 2 H, NCH_2CH_2), 3.75 (t, ^3J = 7.5 Hz, 4 H, 2 × NCH_2) ppm. $C_3H_6N_3NaO_2$ (139.09): calcd. C 25.91, H 4.35, N 30.21; found C 25.59, H 4.25, N 29.88.

Sodium 1-(Azepan-1-yl)diazen-1-ium-1,2-diolate (2d): Prepared from **1d** (4.96 g, 0.05 mol). Yield: 4.23 g (47%) as a white solid. M.p. 198 °C (dec.). ^{1}H NMR (250 MHz, D_2O/NaOD): δ = 1.39–1.67 (m, 8 H, 4 × CH_2), 3.01 (t, ^3J = 5.5 Hz, 4 H, 2 × NCH_2) ppm. $C_6H_{12}N_3NaO_2$ (181.17): calcd. C 39.78, H 6.68, N 23.19; found C 39.50, H 6.46, N 22.79.

Sodium 1-(Azocan-1-yl)diazen-1-ium-1,2-diolate (2e): Prepared from **1e** (5.66 g, 0.05 mol). Yield: 7.13 g (73%) as a white solid. M.p. 238 °C (dec.). ^{1}H NMR (250 MHz, D_2O/NaOD): δ = 1.36–1.65 (m, 10 H, 5 × CH_2), 2.98 (t, ^3J = 5.5 Hz, 4 H, 2 × NCH_2) ppm. $C_7H_{14}N_3NaO_2$ (195.20): calcd. C 43.07, H 7.23, N 21.53; found C 43.05, H 7.22, N 21.41.

Sodium 1-(1,2,3,4-Tetrahydroisoquinolin-2-yl)diazen-1-ium-1,2-diolate (2j): Prepared from **1j** (6.66 g, 0.05 mol). Yield: 3.74 g (35%) as a pale yellow solid. M.p. 171 °C (dec.). ^{1}H NMR (250 MHz, D_2O/NaOD): δ = 2.95 (t, ^3J = 6.0 Hz, 2 H, NCH_2CH_2), 3.25 (t, ^3J = 6.0 Hz, 2 H, NCH_2), 4.17 (s, 2 H, NCH_2), 6.97–7.20 (m, 4 H, Ar–H) ppm. $C_9H_{10}N_3NaO_2$ (215.18): calcd. C 50.23, H 4.68, N 19.53; found C 50.00, H 4.93, N 19.21.

Disodium 1-(3-Carboxylato-1,2,3,4-tetrahydroisoquinolin-2-yl)diazen-1-ium-1,2-diolate (2k): Prepared from **1k** (8.86 g, 0.05 mol). Yield: 6.46 g (46%) as a white solid. M.p. 282–286 °C (dec.). ^{1}H NMR (250 MHz, D_2O/NaOD): δ = 2.60–3.16 (m, 2 H, $NCHCH_2$), 3.73–3.95 (m, 2 H, NCH_2), 4.14–4.38 (m, 1 H, NCH), 6.92–7.20 (m, 4 H, Ar–H) ppm. $C_{10}H_9N_3Na_2O_4$ (281.18): calcd. C 42.72, H 3.23, N 14.94; found C 43.02, H 3.23, N 14.95.

Sodium 1-(*N*,*N*-Diallylamino)diazen-1-ium-1,2-diolate (2n): Prepared from **1n** (9.7 g, 0.1 mol). Yield: 11.47 g (64%) as a beige solid. M.p. 147 °C (dec.). ^{1}H NMR (250 MHz, D_2O/NaOD): δ = 3.36 (d, ^3J = 6.63 Hz, 4 H, 2 × NCH_2), 4.96–5.19 (m, 4 H, 2 × NCH_2CHCH_2), 5.52–5.74 (m, 2 H, 2 × NCH_2CHCH_2) ppm. $C_6H_{10}N_3NaO_2$ (179.15): calcd. C 40.23, H 5.63, N 23.46; found C 39.96, H 5.60, N 23.15.

Sodium 1-[*N*,*N*-Bis(2-hydroxyethyl)amino]diazen-1-ium-1,2-diolate (2o): The use of **1o** (10.5 g, 0.1 mol) did not produce a solid compound but rather a viscous fluid which yielded a colourless highly hygroscopic solid after prolonged drying under reduced pressure. ^{1}H NMR (250 MHz, D_2O/NaOD): δ = 2.89 (t, ^3J = 5.74 Hz, 4 H, 2 × NCH_2), 3.48 (t, ^3J = 5.74 Hz, 4 H, 2 × NCH_2CH_2) ppm. $C_4H_{10}N_3NaO_4$ (187.13): calcd. C 25.67, H 5.39, N 22.46; found C 25.85, H 5.60, N, 22.12.

Sodium 1-[*N*-(2-Aminoethyl)-2-hydroxyethylamino])diazen-1-ium-1,2-diolate + 1-[*N*-(2-Ammonioethyl)-2-hydroxyethylamino]diazen-1-ium-1,2-diolate (2p): The use of **1p** (10.4 g, 0.1 mol) produced a highly viscous and hygroscopic amber fluid. ^{1}H NMR (250 MHz, D_2O/NaOD): δ = 2.27 (t, ^3J = 5.93 Hz, 2 H, $NCH_2CH_2NH_2$), 2.75 (t, ^3J = 5.93 Hz, 2 H, $NCH_2CH_2NH_2$), 2.81 (t, ^3J = 5.93 Hz, 2 H, NCH_2CH_2OH), 3.30 (t, ^3J = 5.93 Hz, 2 H, NCH_2CH_2OH) + δ = 2.60 (t, ^3J = 5.93 Hz, 2 H, $NCH_2CH_2NH_3^+$), 2.75 (t, ^3J = 5.93 Hz, 2 H, $NCH_2CH_2NH_3^+$), 2.81 (t, ^3J = 5.93 Hz, 2 H, NCH_2CH_2OH), 3.30 (t, ^3J = 5.93 Hz, 2 H, NCH_2CH_2OH) ppm.

Sodium 1-(*N*-Benzyl-*N*-ethylamino)diazen-1-ium-1,2-diolate (2s): Prepared from **1s** (13.5 g, 0.1 mol). Yield: 8.47 g (39%) as a white solid. M.p. 175 °C (dec.). ^{1}H NMR (250 MHz, D_2O/NaOD): δ = 0.60 (t, ^3J = 7.10 Hz, 3 H, NCH_2CH_3), 2.69 (q, ^3J = 7.10 Hz, 2 H, NCH_2CH_3), 3.67 (s, 2 H, NCH_2Ar), 6.87–7.10 (m, 5 H, Ar–H) ppm. $C_9H_{12}N_3NaO_2$ (217.20): calcd. C 49.77, H 5.57, N 19.35; found C 49.62, H 5.80, N 18.98.

Sodium 1-(*N*-Benzyl-*N*-isopropylamino)diazen-1-ium-1,2-diolate (2t): Prepared from **1t** (14.9 g, 0.1 mol). Yield: 3.4 g (15%) as a beige solid. The solid was filtered off from a large amount of unchanged **1t** and washed with diethyl ether. M.p. 298 °C (dec.). ^{1}HNMR (250 MHz, D_2O/NaOD): δ = 0.99 (d, ^3J = 6.31 Hz, 6 H, $NCH(CH_3)_2$), 3.90–4.00 (m, 1 H, NCH), 3.56 (s, 2 H, NCH_2Ar), 7.10–7.22 (m, 5 H, Ar–H) ppm. $C_{10}H_{14}N_3NaO_2$ (231.23): calcd. C 51.94, H 6.10, N 18.17; found C 51.63, H 6.15, N 17.84.

Sodium 1-[*N*-Benzyl-*N*-(2-phenylethyl)amino]diazen-1-ium-1,2-diolate (2u): Prepared from **1u** (21.1 g, 0.1 mol). Yield: 14.33 g (49%) as a white solid. M.p. 217 °C (dec.). ^{1}H NMR (250 MHz, D_2O/NaOD): δ = 2.45 (t, ^3J = 7.27 Hz, 2 H, NCH_2CH_2Ar), 2.99 (t, ^3J = 7.27 Hz, 2 H, NCH_2CH_2Ar), 3.86 (s, 2 H, NCH_2Ar), 6.99–7.25 (m, 10 H, Ar–H) ppm. $C_{15}H_{16}N_3NaO_2$ (293.30): calcd. C 61.43, H 5.50, N 14.33; found C 61.11, H 5.55, N 14.42.

Sodium 1-(*N*,*N*-Dibenzylamino)diazen-1-ium-1,2-diolate (2v): Prepared from **1v** (19.7 g, 0.1 mol). Yield: 9.2 g (33%) as a white voluminous solid. M.p. 201–203 °C (dec.). ^{1}H NMR (250 MHz, D_2O/NaOD): δ = 4.01 (s, 4 H, 2 × NCH_2), 7.20 (m, 10 H, Ar–H) ppm. $C_{14}H_{14}N_3NaO_2$ (279.27): calcd. C 60.21, H 5.05, N 15.05; found C 60.41, H 4.80, N 14.99.

NO Release Measurement: NO release was measured by using the Apollo 4000 free-radical analyser system with an ISO-NOP nitric oxide selective sensor built into a multiport measurement chamber by World Precision Instruments Inc., USA. The measurement chamber was tempered with a Lauda M3 circulating thermostat at 37 °C and stirred at 800 r.p.m. All experiments were started after the initial background signal had fallen below 7000 pA in a channel range of 10 nA for the electrode. The apparatus was calibrated daily with 2.0 mL of a 0.1 M KI/0.1 M H_2SO_4 solution in the chamber and an increasing amount of 50 μM $NaNO_2$ solution to produce nitric oxide by reduction. The nitrite solution was applied with an Eppendorf pipette (10, 20, 30, 40 and 60 μL) to give 249, 493, 728, 952 and 1388 nM solutions of NO, respectively. The dissociation of the diazeniumdiolates was measured by injecting a 50 μM NONOate/0.01 M NaOH solution (20 μL) into phosphate buffer (2.0 mL, pH 6.4) resulting in a 495 nM solution of NONOate. Because 1 mol NONOate produces 2 mol NO, the redox current flow of a 990 nM NO solution was measured. Note that the nitric oxide sensor measurement is not accumulative and only measures the arising current at any given time. For this reason the phosphate buffer at pH 6.4 was used to ensure a sharper signal for slower releasing NONOates. The maximum of the signal was chosen as a parameter with which to compare dissociation rates. Figure 10 shows a typical signal evolving from the decomposition of a diazeniumdiolate (here **2r**). This parameter represents the maximum concentration of nitric oxide reaching the electrode at a certain time. Every measurement was carried out at least three times and the mean value with the corresponding standard deviation was calculated.

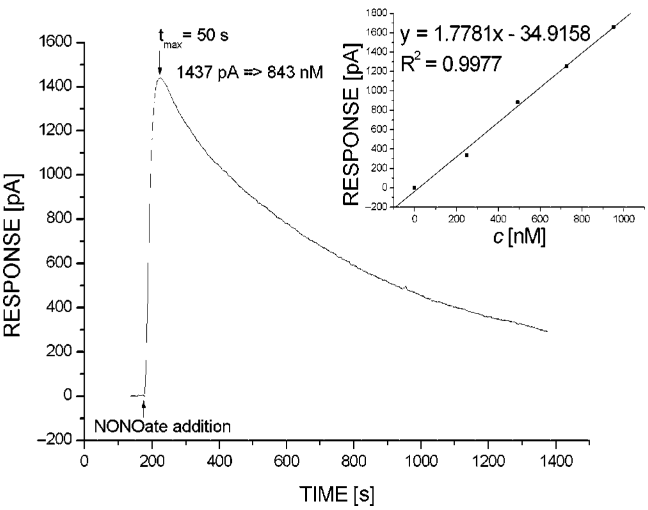

Figure 10. Calibration and NO release–time plot of NONOate **2r** in phosphate buffer at pH 6.4 at 37 °C.

The dissociation of the $–[N(O)NO]^–$ functional group rather than NO release was measured with a Jasco V-570 UV spectrometer in phosphate buffer at pH 7.4 at 37 °C with a NONOate concentration of $10^{–4}$M. The decrease in the absorption was measured at a wavelength of 251 nm over a period of 30 min. By plotting the absorption logarithmically and performing a linear regression analysis the rate constants (k) were determined and used to calculate the half-lives of the respective NONOates using the formula $t_{1/2} = \ln 2/k$. R^2 values were greater than 0.998 and every measurement was carried out at least three times to ensure reproducibility to within ±5%.[8]

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