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Nitric Oxide-Releasing Cyclodextrins

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ABSTRACT: A series of secondary amine-modified cyclodextrin (CD) derivatives were synthesized with diverse exterior terminal groups (*i.e.*, hydroxyl, methyl, methoxyl, and primary amine). Subsequent reaction with nitric oxide (NO) gas under alkaline conditions yielded *N*-diazeniumdiolate-modified CD derivatives. Adjustable NO payloads (0.6–2.4 μmol/mg) and release half-lives (0.7–4.2 h) were achieved by regulating both the amount of secondary amine precursors and the functional groups around the NO donor. The bactericidal action of these NO-releasing cyclodextrin derivatives was evaluated against *Pseudomonas aeruginosa*, a Gram-negative pathogen with antibacterial activity proving dependent on both the NO payload and exterior modification. Materials containing a high density of NO donors or primary amines exhibited the greatest ability to eradicate *P. aeruginosa*. Of the materials prepared, only the primary amine-terminated hepta-substituted CD derivatives exhibited toxicity against mammalian L929 mouse fibroblast cells. The NO donor-modified CD was also capable of delivering promethazine, a hydrophobic drug, thus demonstrating potential as a dual-drug releasing therapeutic.

INTRODUCTION

Nitric oxide (NO), an endogenously produced diatomic free radical, is associated with a wide range of physiological roles, including platelet aggregation and adhesion, vasodilation, wound repair, the immune response, and carcinogenesis.[1-4] Deficiency in endogenous NO production has been linked to certain health disorders and disease, such as diabetes and cystic fibrosis.[5] Low levels of exhaled NO are associated with impaired lung function in cystic fibrosis.[6]

Exogenous NO delivery has been used as a potential therapeutic to treat cardiovascular disorders, cancer and bacterial infection.[7-10] Gaseous NO from high pressure cylinders is used clinically to treat pulmonary hypertension.[11] Other research has focused on the synthesis and use of NO donors (*e.g.*, *N*-diazeniumdiolates, *S*-nitrosothiols, metal nitrosyls, organic nitrates) to lower the risk of systemic NO exposure and facilitate both local and sustained NO delivery.[12] Low molecular weight *N*-diazeniumdiolates (NONOates) NO donors, in particular, are among the most widely employed NO release compounds due to straightforward preparation, long-term stability if appropriately packaged/stored, and spontaneous degradation to NO into solution in physiological media.[12] Unfortunately, the concentration of low molecular weight NO donors necessary to illicit a biological response often is toxic to mammalian cells and tissue.

Macromolecular-based NO-storage systems, including linear and dendritic polymers,[13-16] silica nanoparticles,[17,18] chitosan oligosaccharides,[19] liposomes,[20,21] and metal organic frameworks[22] have been developed to increase NO payloads without compromising cell/tissue viability. While possessing attractive (i.e., therapeutically relevant) NO payloads, the synthetic burden of these systems, limited water solubility, and/or restricted control over release kinetics represent a significant challenge in their further development for clinical use.

Cyclodextrins (CDs) are a family of naturally produced, highly water-soluble cyclic oligosaccharides, composed of (α-1,4)-linked α-D-glucopyranose residues.[23,24] Their cyclic structure consists of a hydrophobic central cavity and hydrophilic exterior that enhances both water solubility and protects encapsulated hydrophobic drugs against peripheral stimulants (*e.g.*, light, heat, oxygen, enzyme).[25] To date, CDs have found wide utility as agrochemicals, fragrances, food additives, and gene/drug delivery carriers.[26,27] Cyclodextrins have also been used to fabricate supramolecular devices (*e.g.*, polyrotaxane, molecular shuttle),[28,29] assemblies (*e.g.*, micelle, vesicle, tube, sheet, hydrogel),[30-33] and polymers.[34,35] These favorable properties make CDs intriguing as NO-release/drug delivery vehicles. Two prior reports have described *S*-nitrosothiol-modified CD systems to achieve NO release.[36,37] However, a CD-based scaffold with tunable NO-release payloads and kinetics that could be applied clinically as a therapeutic remains elusive.

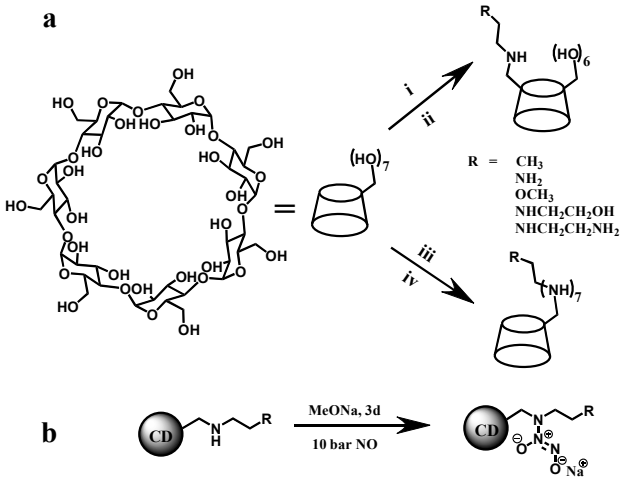
Herein, we report the synthesis of *N*-diazeniumdiolate-functionalized β-CD derivatives as NO-releasing biopolymers with variable NO payloads and highly tunable NO-release kinetics, the largest NO payloads for sugar-like biopolymer to date, and the ability to co-deliver a hydrophobic drug.

RESULTS AND DISCUSSION

β-CD was modified with tunable percentages of secondary amines using the synthetic strategy shown in Scheme 1a.[38-41] First, β-CD was reacted with tosyl chloride under basic conditions to yield mono-6-tosyl-β-cyclodextrin (CD-OTs), a mono-substituted intermediate.[38] The tosyl groups were further substituted with *N*-(2-Hydroxyethyl)ethylenediamine (HEDA), propylamine (PA), 2-methoxyethylamine (MA), ethylenedia-

mine (EDA), or diethylenetriamine (DETA) to yield secondary amine-modified mono-substituted β-CD derivatives.[39] These CD derivatives were given the following nomenclatures: CD-HEDA, CD-PA, CD-MA, CD-EDA, or CD-DETA, based on the primary amines employed in the reaction. To potentially improve NO loading, the secondary hydroxyl groups of the β-CD were converted into bromo groups to yield heptakis-6-bromo-6-deoxyl-β-cyclodextrin (CD-Br7).[40] Secondary amine-modified hepta-substituted β-CD derivatives were then synthesized by displacing bromide with primary amines to form CD-HEDA7, CD-PA7, CD-MA7, CD-EDA7, and CD-DETA7.[41] Synthetic details and analytical characterization are provided in Supporting Information (SI).

Scheme 1. Synthesis of secondary amine- and *N*-diazeniumdiolate-functionalized CD derivatives. (a) Preparation of secondary amine-modified CDs; reagents and conditions: (i) TsOCl, NaOH, H_2O/CH_3CN, room temp.; (ii) Primary amine (RNH_2), 75 °C; (iii) Bromine, $P(Ph)_3$, DMF, 80 °C; (iv) Primary amine (RNH_2), DMF, room temp. (b) Subsequent *N*-diazeniumdiolate formation.



The resulting secondary amine-modified CD derivatives (mono- and hepta-substituted) were reacted with NO gas at high pressures (10 bar) under strong alkaline conditions to yield *N*-diazeniumdiolate-modified CD derivatives (Scheme 1b). The representative synthesis and sequent ^{1}H NMR and UV-Vis characterization of CD-HEDA7/NO are provided in Figure 1. Of note, only one –NH- group is sufficiently facile to react with NO, resulting from steric hindrance (Figure 1a). Proton NMR indicated evidence for *N*-diazeniumdiolate NO donor-modification on the CD-HEDA7 backbone (Figure 1b). Specifically, proton signals in the range of 2.72–3.05 ppm corresponding to methylene groups bound to secondary amines were shifted downfield (2.90–3.11 ppm), owing to formation of hydrogen bonds between the terminal hydroxyl groups and *N*-diazeniumdiolate functional groups. Similar downfield shifts were also observed in the ^{1}H NMR spectra of other hydroxyl- or primary amine-terminated CD-NONOates (Figures S1–S5 for CD-HEDA/NO, CD-EDA/NO, CD-DETA/NO, CD-EDA7/NO and CD-DETA7/NO, respectively). Of note, the ^{1}H NMR spectra of methyl- and methoxyl-terminated CD-NONOates (*i.e.*, CD-MA/NO, CD-MA7/NP, CD-PA/NO and CD-PA7/NO) revealed upfield shifts for their methylene groups around the *N*-diazeniumdiolates after *N*-diazeniumdiolate formation (Figures S6–S9), possibly attributable to the absence of hydrogen bonding. Additional evidence for the formation of CD-NONOates was provided by the strong absorption peak at ~252 nm in the UV-Vis spectra of CD-HEDA7/NO (Figure 1c). A similar absorption peak (around ~255 nm) was observed for each of the other CD-NONOates (Figure S10 and S11). Of note, an absorption peak around 330–360 nm, characteristic peaks of carcinogenic *N*-nitrosamine species, was not observed, suggesting that these CD derivatives did not form *N*-nitrosamines during NO donor synthesis. During the *N*-diazeniumdiolation step, NO first reacts with a secondary amine to yield a nitrosamine radical anion intermediate; subsequently, this intermediate reacts with another molecule of NO to form the *N*-diazeniumdiolate.[42,43] High pressures (*i.e.*, 10 bar) of NO are known to drive the reaction to the desired *N*-diazeniumdiolate product.

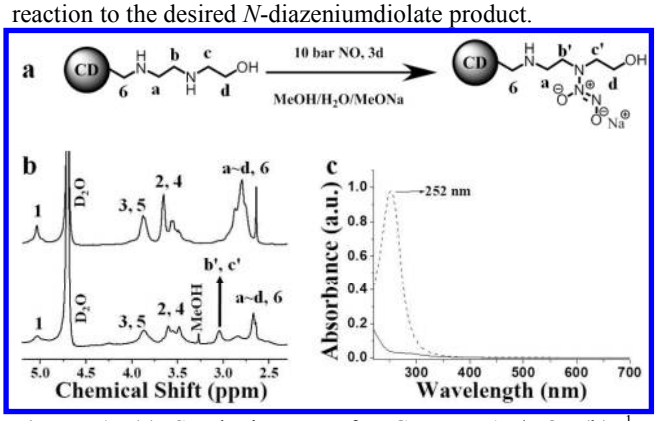
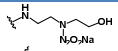
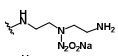
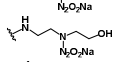
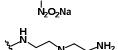


Figure 1. (a) Synthetic route for CD-HEDA7/NO. (b) ^{1}H NMR spectra for CD-HEDA7 (top line) and CD-HEDA7/NO (bottom line). (c) UV-Vis spectra for CD-HEDA7 (solid line) and CD-HEDA7/NO (dash line).

As shown in Figure 2a, the degradation of the *N*-diazeniumdiolate upon protonation yields two moles of NO and the parent secondary amine. This degradation is pH-dependent, and results in more rapid release at lower pH. Real-time NO release was measured using a chemiluminescence-based nitric oxide analyzer (NOA). NO payloads and release kinetics of the CD-NONOates were measured under physiological condition (pH 7.40, 37 °C). The resulting NO-release parameters (*i.e.*, NO payload, half-life of NO release, and conversion efficiency) are provided in Table 1. Representative cumulative NO-release profiles of the *N*-diazeniumdiolate-modified CD derivatives are shown in Figures 2b and S12. In general, the CD-NONOates exhibited tunable NO payload capabilities (*e.g.*, NO payloads from ~0.6 to ~2.4 μmol/mg) and variable NO-release kinetics (*e.g.*, half-lives spanning 0.7 to 4.2 h), depending on the type and amount of secondary amines, and exterior chemical modification. The achievable NO payloads with these NO donor-modified CD derivatives are significantly greater than previously reported NO-releasing biopolymers (*e.g.*, ~0.9 μmol/mg for chitosan).[19] Using the NO payload data, we calculated secondary amine (NO precursor) to *N*-diazeniumdiolate NO donor conversion efficiencies to span 12–41%. The less than expected conversion efficiency is attributed to the proximity between the NO donor precursors (*i.e.*, secondary amines) and oligosaccharide ring, sterically hindering complete formation of the *N*-diazeniumdiolate NO donor on each secondary amine.

Table 1. Nitric oxide-release properties for CD-NONOates in PBS (pH 7.4) at 37 °C.

Scaffold	Molecular Structure[a]	t[NO][b] (μmol/mg)	$t_{1/2}$ (h)	t_{4h}[NO][c] (μmol/mg)	Conversion Efficiency[c] (%)
CD-HEDA/NO		0.60±0.05	0.71±0.05	0.48±0.03	36±2
CD-MA/NO		0.58±0.04	1.46±0.18	0.43±0.03	35±3
CD-PA/NO		0.61±0.05	1.73±0.24	0.43±0.04	36±2
CD-EDA/NO		0.57±0.07	3.36±0.33	0.32±0.03	34±4
CD-DETA/NO		0.68±0.07	4.22±0.35	0.33±0.04	41±2
CD-HEDA7/NO		2.44±0.19	0.88±0.06	1.99±0.19	15±1
CD-MA7/NO		1.13±0.15	3.15±0.41	0.65±0.05	12±1
CD-PA7/NO		1.26±0.05	3.79±0.33	0.66±0.06	13±2
CD-EDA7/NO		1.24±0.06	3.20±0.30	0.64±0.08	13±1
CD-DETA7/NO		2.39±0.19	3.39±0.31	1.15±0.12	15±1

(a) The molecular structure segment of *N*-diazeniumdiolate in the backbone. (b) NO payload; (c) NO released over 4 h (μmol) per milligram of *N*-diazeniumdiolate-modified CD derivatives. Each parameter was analyzed in replicate ($n \geqslant 3$). (c) The theoretical maximum NO payloads were obtained by assuming that 1 mole secondary amine forms two moles of NO. Conversion efficiency was calculated by dividing the NOA data by the theoretical maximum NO payloads.

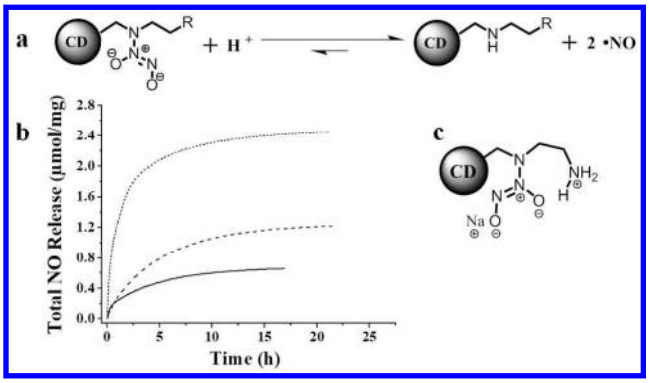


Figure 2. (a) Proton-initiated decomposition of *N*-diazeniumdiolate-modified CD derivatives. (b) Cumulative NO release given as t[NO] versus time for NO-releasing CD derivatives. Solid line represents CD-PA/NO; dash line represents CD-MA7/NO; dot line represents CD-HEDA7/NO. (c) Proposed structure for stabilization of *N*-diazeniumdiolate CD derivatives by neighboring cationic ammonium groups.

The NO payload for each of the mono-substituted CD-NONOates was ~0.6 μmol/mg. However, the NO-release kinetics of these CD-NONOates varied greatly depending on the identity of the polyamine NO donor precursor. For example, the NO-release half-lives for CD-HEDA/NO, CD-MA/NO and CD-PA/NO were 0.71, 1.46 and 1.73 h, respectively. The NO release kinetics correlated to the hydrophilicity of the polyamine with HEDA>MA>PA. Based on prior literature, *N*-diazeniumdiolate stabilization by adjacent cationic ammonium groups might extend NO release (Figure 2c).[13,44] We thus employed the utility of EDA and DETA to prepare primary amine-terminated CD derivatives (CD-EDA and CD-DETA). Both primary amine-terminated CD-NONOates led to significantly longer NO release (3.36 and 4.22 h NO-release half-lives for CD-EDA/NO and CD-DETA/NO, respectively), relative to the alkyl substituted systems.

With respect to substantially enhancing the NO payloads, hepta-substituted CD derivatives were synthesized thereby, increasing the NO donor precursor (*i.e.*, secondary amine) levels seven-fold compared to mono-substituted CD derivatives. Representative real-time NO-release profiles from hepta-substituted CD-NONOates are provided in Figure S12b. Hepta-CD derivatives exhibited significant NO payloads (Table 1), with short polyamine chains (MA, PA, and EDA) approaching ~1.2 μmol/mg, nearly two times greater than their mono-substituted CD-NONOates. The longer polyamine modifications (DETA and HEDA) exhibited four times more NO payload than their mono-substituted counterparts, owing to the greater secondary amine content. Although the percentage of secondary amine increased seven-fold, the increase of NO payload was only two-fold or four-fold, owing to the steric hindrance and repulsive interactions. Nevertheless, these biopolymers represent a notable advancement in NO loading on a sugar-like biopolymer. That is likely to be amenable for delivering therapeutic levels of NO in a water-soluble and non-toxic form.

The antibacterial activity of these CD-NONOates was evaluated against Gram-negative *P. aeruginosa*, a model pathogen associated with a number of serious medical infections (*e.g.*, traumatic burns, cystic fibrosis).[4,8] To start, planktonic bacterial viability assays were performed under static conditions. Minimum bactericidal concentrations over a 4 h exposure (MBC_{4h}) were used to quantify bactericidal activity. The MBC is defined as the minimum concentration required to eliminate bacteria viability by 3 logs (*i.e.*, 99.9% killing). The total NO amounts delivered by the CD derivatives over this period (*i.e.*, NO dose) was determined from the NO release data collected by the NOA. Both MBC_{4h} and required NO doses are provided

Table 2. Minimum bactericidal concentration (MBC_{4h}) and NO doses of NO-releasing CD derivatives to achieve 3-log reduction in planktonic *P. aeruginosa* viability.[a]

Mono-substituted CD derivatives	MBC_{4h} (μg/mL)	NO dose (μmol/mL)	Hepta-substituted CD derivatives	MBC_{4h} (μg/mL)	NO dose (μmol/mL)
CD-HEDA/NO	1000	0.48	CD-HEDA7/NO	250	0.50
CD-PA/NO	1000	0.43	CD-PA7/NO	500	0.33
CD-MA/NO	1000	0.43	CD-MA7/NO	500	0.33
CD-EDA/NO	500	0.16	CD-EDA7/NO	250	0.16
CD-DETA/NO	250	0.08	CD-DETA7/NO	100	0.11

(a) Results of n ⩾ 3 pooled experiments.

in Table 2. The bactericidal activity of both control and NO-releasing mono-substituted CD derivatives was tested to evaluate the effects of specific terminal groups on bactericidal action. At equivalent concentrations, mono-substituted control (without NO donor) CD derivatives did not influence bacterial viability regardless of the modification (Figure S13). As shown in Table 2, primary amine-terminated CD-NONOates required the lowest NO dose to eradicate *P. aeruginosa*. The methyl-, hydroxyl-, and methoxyl-terminated CD-NONOates required 2–4 times more NO to achieve similar action. Prior work has attributed enhanced antibacterial ability for primary amine-terminated NO-releasing macromolecules to faster association with bacteria and more localized NO delivery.[45,46] In this regard, the bactericidal action of mono-substituted CD-NONOates proves more dependent on specific exterior modifications.

Confocal laser scanning microscopy (CLSM) was utilized to further elucidate the impact of exterior modifications on the antibacterial action of primary amine-terminated CD-EDA/NO versus methyl-terminated CD-PA/NO. A NO-responsive fluorescent probe, 4,5-diaminofluorescein diacetate (DAF-2 DA) and standard nucleic acid-sensitive fluorescent dye propidium iodide (PI) were added to the *P. aeruginosa* and solution media, respectively.[46] Prior to introducing the NO-releasing CD-NONOates, no autofluorescence was measured from either DAF-2 or PI. Upon exposure to CD-PA/NO, green DAF-2 fluorescence (Figures 3b and S14) was observed, indicating NO accumulation inside the bacteria. Of note, green fluorescence was not noted upon exposing *P. aeruginosa* to CD-EDA/NO (Figures 3e and S15). In this case, intracellular NO accumulation was no longer measurable owing to cellular membrane damage. Red PI fluorescence, indicative of cell death was only observed for CD-EDA/NO at 1 h (Figure S15), but not for CD-PA/NO (Figure S14). Additionally, red PI fluorescence was measured after 2 h incubation (Figures 1c and 1f), with the intensity being greatest for CD-EDA/NO. These data indicate that the cellular damage rate achieved with CD-PA/NO was slower than CD-EDA/NO.

Hepta-substituted CD-NONOates exhibited far greater antibacterial capability versus mono-substituted CD-NONOates with identical terminal functional groups (Table 2). Although the hepta-substituted CD-NONOates had lower MBCs, the NO doses required to kill *P. aeruginosa* were similar with that of mono-substituted CD-NONOates when overall mass of the biopolymer was taken into account. In addition, hepta-substituted control (*i.e.*, without NO release) CDs with PA, HEDA, EDA and DETA modifications possessed enhanced inherent antibacterial action than the corresponding mono-substituted CDs (Figure S16). The greater density of alkyl and/or amine functional groups leads to faster membrane intercalation and cell membrane damage, respectively.[14,47,48]

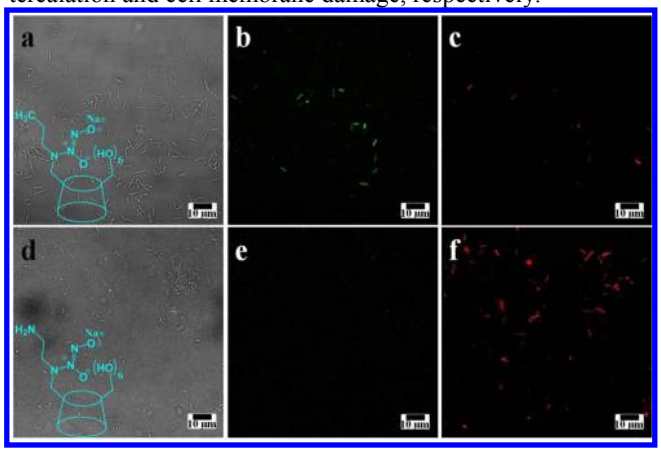


Figure 3. (a–c) Confocal laser scanning microscopy (CLSM) images of *P. aeruginosa* exposed to 300 μg/mL CD-PA/NO for 2 h. DAF-2 green fluorescence indicates intracellular NO delivery. The appearance of PI red fluorescence is attributed to cellular membrane destruction (cell death). (a) Bright field; (b) DAF-2; (c) PI. (d–f) CLSM images of *P. aeruginosa* exposed to 300 μg/mL CD-EDA/NO for 2 h. (d) Bright field; (e) DAF-2; (f) PI.

With respect to therapeutic potential, toxicity to mammalian cells is an equally important factor in the development of any new antibacterial agent. The cytotoxicity of CD-NONOates was evaluated by exposing L929 mouse fibroblast cells to 0–2000 μg/mL of both control and NO-releasing CD derivatives over a 4-hour period. Mouse fibroblast cell viability was maintained above 50% for both mono-substituted control and the corresponding NO-releasing CDs even at 2000 μg/mL (Figure 4a), regardless of the terminal functional group. While the hepta-substituted control CD derivatives were also not toxic, the cytotoxicity of the NO-releasing CD-NONOates proved to be dependent on the terminal functional group (Figure 4b). Both CD-PA7/NO and CD-MA7/NO were tolerable to the mouse fibroblasts even at 2000 μg/mL (63% and 73% cell viability for CD-PA7/NO and CD-MA7/NO, respectively). In contrast, cell viability fell to below 30% when treated with CD-EDA7/NO or CD-DETA7/NO, regardless of the tested concentrations, indicating considerable toxicity. This behavior is in part related to the effective delivery of NO induced by the fast cellular uptake of positively charged macromolecular sys-

tems.[49,50] These results were not unexpected given both the large level of terminal primary amines groups and considerable NO payloads. As reported by Knop et al., the presence of hydroxyl groups may mitigate cytotoxicity of certain macromolecular systems.[51] Hydroxyl-terminated CD-HEDA7/NO proved significantly more tolerable to the mouse fibroblasts up to 1000 μg/mL, despite having a high NO payload. With the exception of CD-EDA7/NO and CD-DETA7/NO, both the favorable toxicity profiles and noteworthy antibacterial activity against *P. aeruginosa* suggest high therapeutic utility for NO donor-modified CD derivatives for a range of clinical indications plagued by infections including wound healing and respiratory diseases.

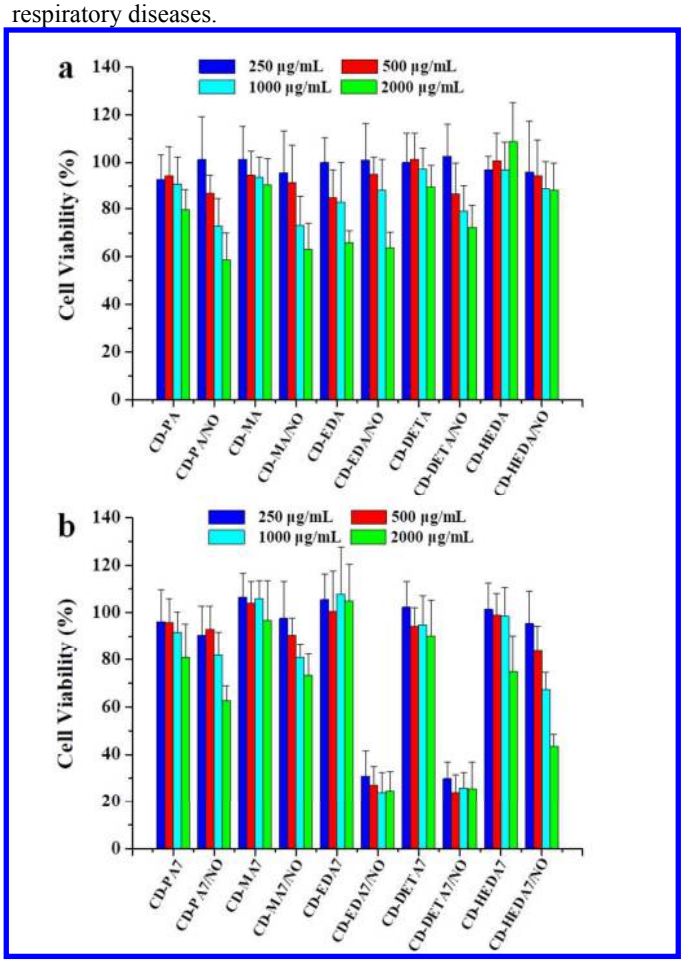


Figure 4. Cell viability (%) of L929 mouse fibroblasts following exposure to blank (buffer only), control (non-NO-releasing) and NO-releasing CD solutions at 250–2000 μg/mL for 4 h. The data represents the mean standard deviation of at least three determinations. (a) Mono-substituted CD derivatives; (b) Hepta-substituted CD derivatives.

Apart from exterior modifications to facilitate NO delivery, the interior cavity of cyclodextrin derivatives may be employed as a carrier of hydrophobic drugs.[25] Co-delivery of NO with a drug has proven effective in decreasing the required therapeutic concentration of the drug alone.[52-54] With this in mind, we investigated the ability of CD-NONOates to deliver both NO and a hydrophobic drug. As a proof-of-concept, promethazine (PM) was selected as a model hydrophobic drug in our study. PM is a neuroleptic mediation used as an antiemetic and remedy for motion sickness.[55] It has also been used off-label as an antibacterial agent.[56,57] Lutka previously reported the utility of CD as an effective carrier for PM,[55] noting both enhanced water-solubility and tolerability (Figure S17). We set out to investigate the antibacterial actions of PM, the complex of PM and CD-DETA, and the complex of PM and CD-DETA/NO against *P. aeruginosa*. As shown in Table 3 and Figure 5b, the MBC_{4h} for PM was 100 μg/mL, even when encapsulated within CD-DETA. The use of CD-DETA/NO to co-deliver NO and PM resulted in significant additive activity against *P. aeruginosa*, decreasing the MBC_{4h} of PM from 100 to 40 μg/mL. As CD-DETA forms an inclusive complex with PM at a molar ratio of 1:1 (Figure S18), the corresponding concentration of CD-DETA/NO was 162 μg/mL. Bacterial degradation of CD-DETA likely promotes the release of encapsulated PM initiating antibacterial action, in a similar manner to CD-capping silver nanoparticles.[58] Of note, the MBC_{4h} values of CD-DETA and CD-DETA/NO were 8 mg/mL and 250 μg/mL, respectively. Comparing these data, the combined delivery of NO and PM decreases the required MBC of each drug, with potential benefits for drug tolerability and avoiding/reducing potential adverse side-effects clinically. The cytotoxicity of PM, the complex of PM and CD-DETA, and the complex of PM and CD-DETA/NO was evaluated by exposing L929 mouse fibroblast cells to the respective MBC_{4h} (bacteria eradication) concentrations. As shown in Figure 5c, the PM at 100 μg/mL completely annihilated the mouse fibroblast cells. In contrast, the cell viability was 31% when using CD-DETA to deliver the PM, as a result of both the lower concentration of PM and its isolation to within the CD derivative. The co-delivery of NO and PM (via CD-DETA/NO) resulted in the least cell toxicity (viability of 52%), unequivocally demonstrating the utility of co-delivery with NO.

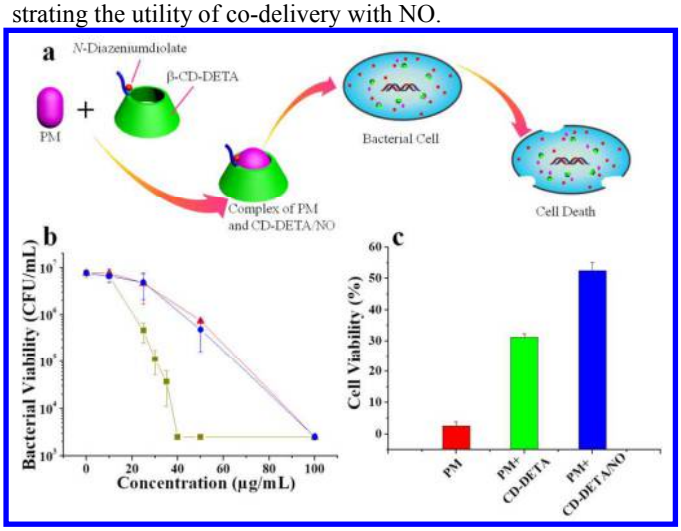


Figure 5. (a) Illustration of promethazine and NO co-delivery for antibacterial activity. (b) Bactericidal efficacy of PM (circle), the complex of PM and CD-DETA (triangle) and the complex of PM and CD-DETA/NO (square) against Gram-negative *P. aeruginosa*. PM and CD derivatives were delivered in a molar ratio of 1:1. The X-axis is the concentration of PM in different systems. (c) Cell viability (%) of L929 mouse fibroblasts following exposure to PM, the complex of PM and CD-DETA, and the complex of PM and CD-DETA/NO at the MBC_{4h} concentrations. Red bar was PM; green bar was the complex of PM and CD-DETA; blue bar was the complex of PM and CD-DETA/NO.

Table 3. MBC_{4h} for NO-releasing CD-DETA and PM against planktonic *P. aeruginosa*.[a]

	PM MBC_{4h} (μg/mL)	Corresponding carrier concentration (μg/mL)
PM	100	—
PM/CD-DETA complex	100	380
PM/CD-DETA/NO complex	40	162

(a) Results of n ⩾ 3 pooled experiments.

CONCLUSIONS

Herein, we report the synthesis of *N*-diazeniumdiolate-modified cyclodextrin derivatives with tunable NO payloads and NO-release kinetics based on the NO donor precursor structure and modification extent. CD derivatives modified fully with *N*-diazeniumdiolate precursors resulted in significant NO payloads and maximum bactericidal action against *P. aeruginosa*, regardless of terminal group modification. The antibacterial activity of primary amine-terminated CD derivatives displayed proved greater than any other terminal group functionalization of equivalent NO payload, attributed to their positive charge and ensuing ability to facilitate greater bacterial association with the negatively charged bacteria.[46] All of the CD-NONOates except CD-EDA7/NO and CD-DETA7/NO were nontoxic against L929 mouse fibroblast cells at their bactericidal doses. The combined action of NO and promethazine via PM/CD-DETA/NO demonstrates for the first time the potential of co-delivering NO with another drug from the same complex.

ASSOCIATED CONTENT

Supporting_Information

The Supporting Information is available free of charge on the ACS Publications website. Experimental details on the material synthesis and characterizations, antibacterial activity, cytotoxicity study; supplementary data including NMR data, UV-Vis spectra, Cumulative NO release curves, CLSM images, etc.

AUTHOR INFORMATION

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Notes

The authors declare the following competing financial interest(s): Mark H. Schoenfisch is a co-founder and maintains a financial interest in Novan, Inc. and Vast Therapeutics, Inc. Both companies commercialize macromolecular nitric oxide storage and release vehicles for clinical indications.

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