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Synthesis and anti-inflammatory activity of a series of N-substituted naproxen glycolamides: Nitric oxide-donor naproxen prodrugs☆

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Abstract—A series of glycolamide naproxen prodrugs containing a nitrate group as a nitric oxide (NO) donor moiety has been synthesized. These compounds were evaluated for their anti-inflammatory activity, naproxen release, and gastric tolerance. Compounds **4a**, **4b**, **5a**, **5b**, **7b**, and **7c** exhibited anti-inflammatory activity equivalent to that of the parent NSAID, naproxen-Na, in the rat carrageenan paw edema model. At equimolar doses relative to naproxen-Na, the NO-donor glycolamide derivatives **4a**, **4b**, **5a**, **5b**, **7b**, and **7c** were gastro-sparing in the rat. Naproxen formation from these NO-donor glycolamides varied among the structures examined, with the N-substituent on the amide group having a particular influence, and demonstrated their prodrug nature. Compound **7b** was selected for exemplary demonstration that the glycolamide nitrates can be bioactivated to release NO. These data open the possibility that naproxen glycolamide nitrates may represent a safer alternative to naproxen as anti-inflammatory medicines.

1. Introduction

Conventional nonsteroidal anti-inflammatory drugs (NSAIDs) that inhibit nonselectively both major cyclooxygenase (COX) isoforms are effective therapies against the signs and symptoms of inflammation.[1,2] Naproxen (Aleve™, Naprosyn™, Anaprox™, and Naprelan™) (**I**) is one of the most popular NSAIDs for relieving arthritic pain, although its use invites gastrointestinal (GI) complications ranging from stomach irritation to life-threatening GI ulceration and bleeding.[3–5] Selective COX-2 inhibitors (coxibs) possess the salutary analgesic and anti-inflammatory properties of NSAIDs with generally enhanced, but still not absolute, GI tolerance.[6] Yet in both healthy subjects[7] and the increasing number of patients concomitantly taking low-dose aspirin for cardiovascular prophylaxis[8], coxibs can exacerbate aspirin's GI toxicity. An apparent class-effect cardiovascular risk that predisposes patients to heart attack and stroke with chronic coxib use has recently been uncovered.[9] Thus, there remains compelling need for effective NSAIDs with improved safety. As an alternative to COX-2 selective inhibitors, we and others have developed COX-inhibiting nitric oxide (NO) donors (CINODs), a new class of anti-inflammatory agents utilizing the gastroprotective properties of NO to reduce the GI damage associated with nonselective COX inhibition.[10–19] Herein, we report the synthesis and pharmacological profiles of a series of glycolamide prodrugs of naproxen (**II**–**IV**) containing a nitrate group as a NO-donor moiety. These compounds exhibit analgesic and anti-inflammatory potencies similar to those of naproxen-Na and the prototypic CINOD AZD3582[19a] in rodent models, but with a significantly less gastric damage.

2. Chemistry

Recent recognition of a cardiovascular liability associated with coxib therapy in moderate to high-risk patients

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Keywords: Non-steroidal anti-inflammatory drug (NSAID); Nitric oxide; Naproxen; CINOD; Anit-inflammatory; Cyclo-oxygenase.

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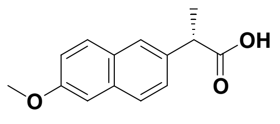

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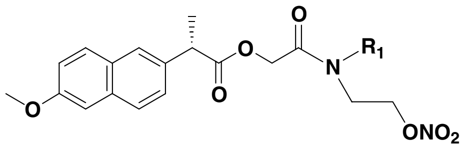

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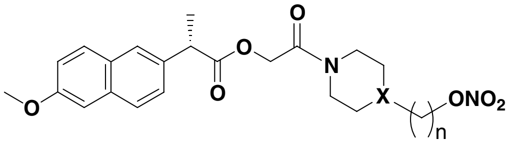

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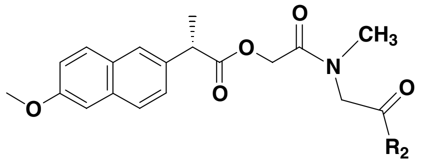

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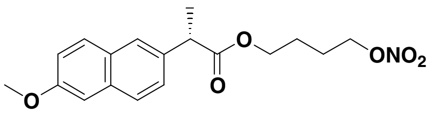

AZD3582

has refocused considerable attention on existing NSAIDs with the least clinical side effects such as naproxen.[10–19] Accordingly, we sought to design a naproxen NSAID derivative with an intrinsic NO-donating capability that would leverage NO's gastroprotective properties to promote GI safety. To facilitate covalent incorporation of an NO-donor group, the naproxen core structure should contain an appropriate functionality that can be derivatized easily to a NO-donating prodrug. Many types of bioreversible derivatives for diverse functional groups have been explored in prodrug design.[20–22] Glycolamide esters appeared particularly suitable for our purpose, since they are rapidly cleaved in human plasma.[23] Herein, we have prepared a series of NO-donating, N-substituted glycolamide esters of naproxen and have determined their biological (i.e., anti-inflammatory) activity and acute gastric tolerance.

The structures and synthesis of naproxen derivatives **2**–**5** are presented in Scheme 1. The naproxen glycolic ester **3** was prepared by esterification of **1** with *tert*-butylbromoacetate in the presence of $NaHCO_3$ in DMF at room temperature followed by hydrolysis of the *tert*-butyl ester with a mixture of TFA/CH_2Cl_2. Compounds (**4a**–**4d**, **5a**–**5d**) were prepared by direct amidation of the glycolic acid group of **3** with an appropriate amine nitrate salt (**4a-I**–**4d-I**, **5a-I**–**5c-I** in Scheme 1) using either EDAC or DCC in the presence of 1–2 equiv of DMAP in CH_2Cl_2 or DMF (Scheme 1). It has been found and characterized that compound **4d** slowly decomposes into compound **4d-II** at ambient temperature.[‡]

The synthesis of the *N*-methyl glycolamide ester of naproxen, **6**, is illustrated in Scheme 2. Compounds **7a** and **7b** were synthesized from **6** in a manner similar to those of **4** and **5** (Scheme 2).

3. Results and discussion

A series of NO-donor naproxen glycolamides was synthesized and evaluated for oral anti-inflammatory activity, gastric tolerance, and naproxen release relative to the sodium salt of the parent drug, naproxen (**I**). Compounds **4c**–**4d** and **7d** were not evaluated biologically due to their inherent instability at ambient temperature. The anti-inflammatory activities and gastric irritancies of naproxen-Na, AZD3582, and selected NO-donor naproxen glycolamide derivatives (**4a**–**4b**, **5a**–**5d**, and **7a**–**7c**) are given in Table 1. The rat paw-edema test was performed according to the standard method of Winter et al.[24] All compounds (**4a**–**4b**, **5a**–**5b** and **7b**–**7c**) showed significant anti-inflammatory activity in this test at an equimolar dose relative to 30 mg/kg naproxen-Na. Compound **7c** was the most potent anti-inflammatory agent in this series containing a sarcosine glycolamide linker with a three-carbon aliphatic nitrate ester group; its anti-inflammatory potency was comparable to that of AZD3582.[19a] Compound **5d**, a piperizine glycolamide, elicited a 44% reduction of paw edema, while the other naproxen glycolamides appreciably reduced inflammation by ~44% as well. The in vivo gastric tolerance of these compounds was characterized as described by Kitagawa et al.[25] and Al-Ghamdi et al.[26] Naproxen sodium at a high oral dose (30 mg/kg) caused significant stomach lesions, whereas at equimolar oral doses **4a**–**4b**, **5a**–**5b**, and **7a**–**7c** caused comparatively little gastric damage (Table 1), exhibiting a level of gastric tolerance comparable to that of AZD3582. Compounds

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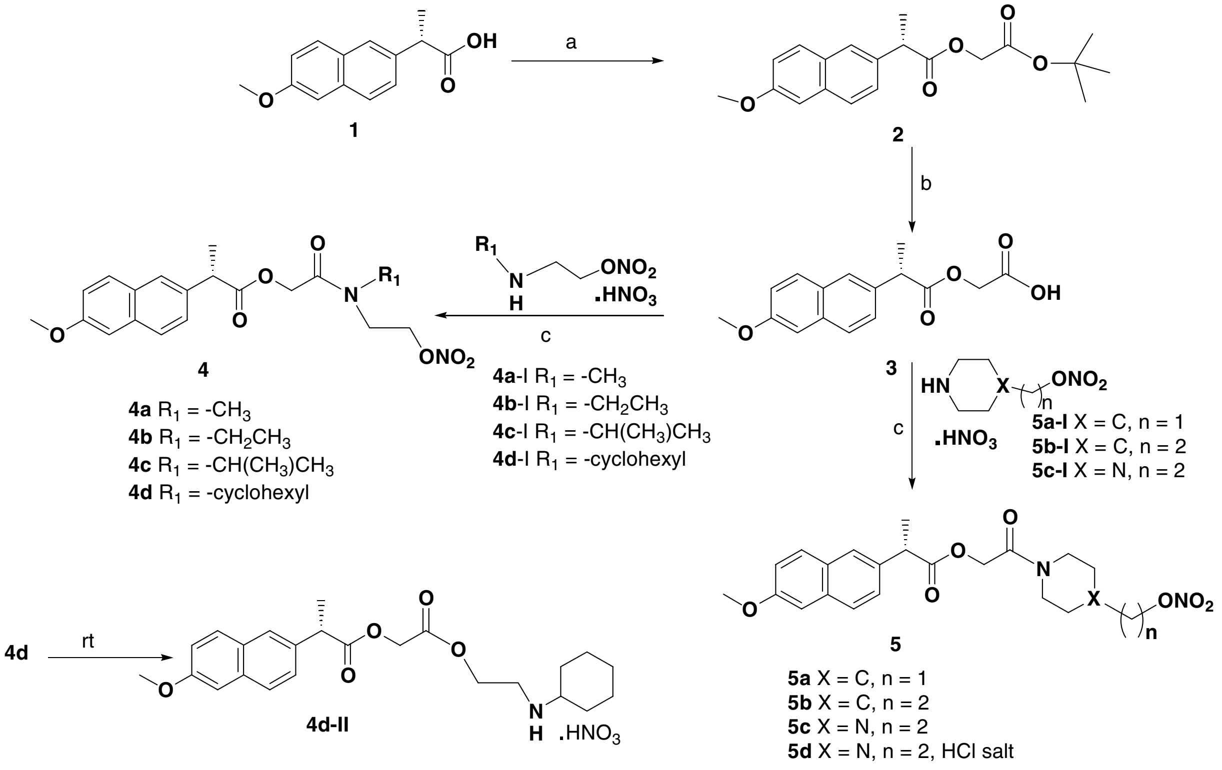
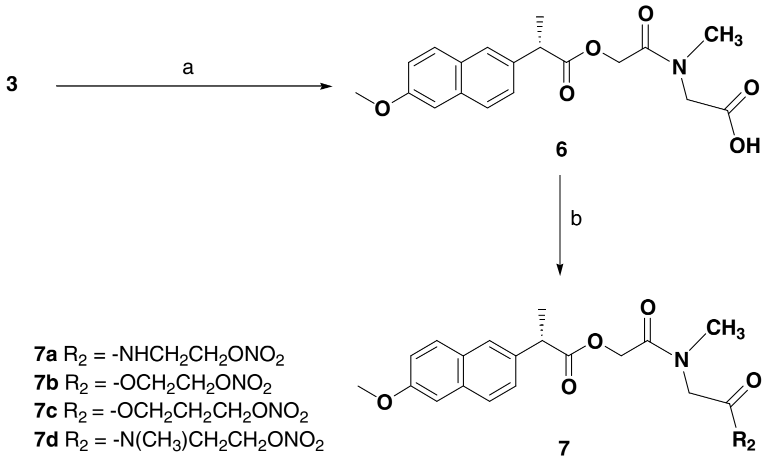
[‡] This could be attributed to both the electronic and steric properties of substitution on *N*-glycolamide. With bulky and more lipophilic branched alkyl groups, *iso*-propyl- and cylcohexyl-substituted compounds **4c** and **4d** are unstable at room temperature compared to the methyl- and ethyl-substituted derivatives **4a** and **4b**.



Scheme 1. Reagents: (a) *tert*-Butyl bromoacetate/$NaHCO_3$/DMF; (b) TFA/CH_2Cl_2; (c) EDAC/DMAP/CH_2Cl_2.

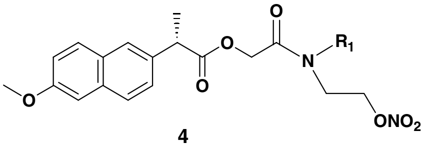


Scheme 2. Reagents: (a) (i) Sarcosine *tert*-butyl ester hydrochloride/TEA/DMAP/DCC/CH_2Cl_2; (ii) TFA/CH_2Cl_2; (b) nitrate tether salt/EDAC or DCC/DMAP/CH_2Cl_2.

5c and **5d** were less damaging to the stomach than naproxen-Na, but less well tolerated than most other compounds (**4a**–**4b**, **5a**–**5b**, and **7b**–**7c**) in this series and were accordingly not studied further. Based upon the combined criteria of in vivo oral anti-inflammatory activity and gastric safety, compounds **5b**, **7b**, and **7c** exhibited the best overall pharmacological profiles.

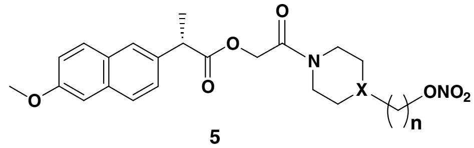
The prototypic CINOD AZD3582 is known to be metabolized as a prodrug, resulting in naproxen formation.[19a] The potential for naproxen generation from the lead anti-inflammatory, gastrosparing naproxen glycolamides was examined in vitro by incubating the compounds in human whole blood at a nominal concentration of 40 μM and quantifying the naproxen formed therein by LC. Naproxen itself was catabolized only slightly (by <20%) over 120 min of the incubation (Table 2). Production of naproxen from all NO-donor glycolamides tested demonstrates the prodrug nature of this compound class, whereby the ester bond of the NO-donor naproxen glycolamide derivative is hydrolyzed in human blood to liberate naproxen.

Table 1. Anti-inflammatory activity and gastric toxicity of NO-donating naproxen glycolamides in rats



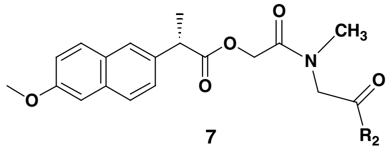
4

4a R_1 = $-CH_3$
4b R_1 = $-CH_2CH_3$



5

5a X = C, n = 1
5b X = C, n = 2
5c X = N, n = 2
5d X = N, n = 2, HCl salt



7

7a R_2 = $-NHCH_2CH_2ONO_2$
7b R_2 = $-OCH_2CH_2ONO_2$
7c R_2 = $-OCH_2CH_2CH_2ONO_2$

Compound[a]	Anti-inflammatory activity (% paw swelling reduction)[b]	Gastric toxicity (% lesion reduction)[c]
Vehicle	0	0
Naproxen	52 ± 0.5	0 ± 6.2
AZD3582	52 ± 0.05	88 ± 1.9
4a	38±0.06	77 ± 1.5
4b	40 ± 0.04	74 ± 3.7
5a	40 ± 0.07	70 ± 0.5
5b	39 ± 0.05	94 ± 0.5
5c	[d]	44 ± 6.2
5d	44 ± 0.08	28 ± 4.5
7a	[d]	74 ± 0.5
7b	37 ± 0.05	97 ± 0.5
7c	49 ± 0.08	81 ± 7.7

All values are means ± SEM and are significantly different from vehicle control ($P < 0.01$ by ANOVA followed by Dunnett's Multiple Comparisons Test).

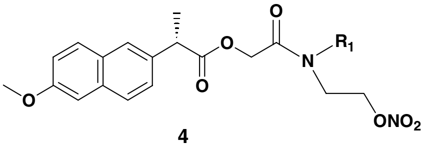
[a] Equimolar doses relative to naproxen-Na 30 mg/kg.

[b] Relative to vehicle.

[c] Relative to naproxen.

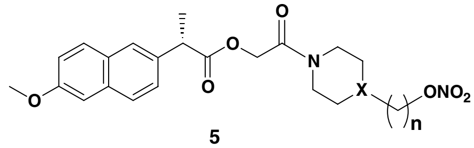
[d] Not determined.

Table 2. Naproxen production from NO-donating naproxen glycolamides in human whole blood



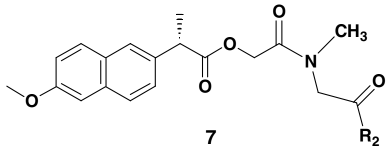
4

4a R_1 = $-CH_3$
4b R_1 = $-CH_2CH_3$



5

5a X = C, n = 1
5b X = C, n = 2



7

7b R_2 = $-OCH_2CH_2ONO_2$

Time (min)	Plasma naproxen concentration (μM)					
	Naproxen-Na	**4a**	**4b**	**5a**	**5b**	**7b**
0	0	0	0	0	0	0
5	32.3	9.9	2.9	[a]	[a]	12.9
20	33.1	31.2	23.3	14.4	9.9	19
40	31.9	29	35.1	23.9	11.8	25.3
60	32.1	33	37.9	36.2	21.4	25.4
120	32.1	33.2	37.9	37.5	28.1	24.7
240	[a]	32.8	[a]	[a]	[a]	34.3

[a] Not determined.

Compounds **4a**, **4b** and **5a** were also rapidly metabolized to naproxen over 120-min incubation with similar kinetics. Production of naproxen from compounds **5b** and **7b** was somewhat slower than from the other glycolamides tested, yet reached comparable level in the case of **7b** by 240 min.

To illustrate the potential of this series of nitrated naproxens to donate NO, **7b** was incubated for up to 2 h with cultured human liver parenchymal cells (hepatocytes) under physiological conditions. This test system was chosen because of the high intrinsic capacity of mammalian liver to biotransform organic nitrate vasodilators to NO.[27] By 1 h incubation with the hepatocytes, **7b** released 60% of the available NO-equivalents as nitrate and nitrite products of NO oxidative decomposition. Since in living systems NO and NO-derived metabolites participate in other reactions not necessarily leading to nitrate/nitrite formation,[27] this figure most likely underestimates the extent of acute bioactivation of **7b** to NO, but nonetheless shows that an exemplary nitrated naproxen glycolamide can be catabolized to NO and thus acts as a NO donor.

4. Conclusion

We have synthesized a series of N-substituted glycolamides of naproxen that contain a nitrate functionality as a NO-donor group. Compounds of this class were demonstrated to be NO-donating, naproxen-releasing prodrugs. Compounds **4a**, **4b**, **5a**, **5b** **7b**, and **7c** possessed oral anti-inflammatory activity equivalent to that of the conventional NSAID, naproxen-Na, in the rat carrageenan paw edema model. Naproxen generation from these NO-donor glycolamides varied, with the N-substituent of the glycolamide group having a particular influence. *N*-Methyl glycolamides appeared to be the best prodrug substituents in this series. At equimolar doses relative to naproxen-Na, compounds **4a**, **4b**, **5a**, **5b**, **7b**, and **7c** were gastrosparing in rats. Our data suggest that these naproxen glycolamide nitrate prodrugs may afford a safer alternative to naproxen (**I**) for the treatment of inflammatory disease and pain.

5. Experimental

5.1. Naproxen formation in human blood

Freshly obtained human whole blood was aliquoted (1 mL/well) into 24-well plates and pre-equilibrated to 37 °C. To each well, test compound in DMSO was added to a final concentration of 40 μM, and the samples were incubated at 37 °C for varying periods of time up to 240 min. At the end of each incubation, blood was de-proteinated by mixing it with acetonitrile in a 1:1 volume ratio and centrifuging the mixture at 1500 rpm for 10 min at 4 °C. The resulting supernatant was recovered, filtered, and analyzed by LC for its naproxen content.[16]

5.2. Anti-inflammatory activity

The rat paw edema test was used to assess anti-inflammatory activity.[24] Male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 180–220 g were fasted overnight with water ad libitum. Twelve animals per treatment group were used. Test compound or vehicle (PEG 400/Methocel) was administered by oral gavage 1 h prior to carrageenan (Sigma, St. Louis, MO) injection (50 μL of 1% carrageenan in saline) into the subplantar region of right hind paw at a dose volume of 1 mL/kg. Paw volume was determined using the water-displacement method as the average of two measurements differing by less than 0.2 mL. Paw volume measurements were assessed immediately as prior to and 3 h post carrageenan injection. Results are expressed as the mean % inhibition of the change in paw volume produced by carrageenan injection relative to vehicle ± standard error of the mean (SEM).

5.3. Gastric irritancy

The rat gastric lesion test, described by Kitagawa et al.[25] and Al-Ghamdi et al.[26], was used to evaluate the gastrosparing activity of test compounds. Male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 230–250 g were used for the experiments. Twelve animals per treatment group were used. The rats were fasted for 24 h with free access to water and then dosed by oral gavage with vehicle (PEG 400/Methocel) or test compound given at a dosing volume of 1.0 mL/kg. Food was withheld after dosing. The rats were euthanized by CO_2 asphyxiation 3 h after dosing. Their stomachs were extirpated, dissected along the greater curvature, washed with 0.9% saline, pinned open, and digitally photographed. The images were analyzed for visible hemorrhagic lesions using Image-J software (National Institute of Health, Bethesda, MD) by investigators blinded to treatment. The total length of all lesions for each stomach was summed to obtain a mean total lesion score, which was expressed as a percent lesion reduction relative to the lesion score from naproxen-Na ± SEM.

5.4. NO-donating capability

Compound **7b** was incubated at a final concentration of 70 μM in 0.4 mL of medium with a monolayer of 3.9×10^5 viable human hepatocytes cultured under standard conditions prescribed by the supplier (Clonetics, San Diego, CA). After 1 h, the culture medium was recovered and analyzed for net nitrate/nitrite formation relative to control incubations without test compound by using a diazotization-based colorimetric assay (Cayman, Ann Arbor, MD).

5.4.1. General procedures. All reagents and anhydrous solvents were generally used as received from the commercial supplier. Reactions were routinely performed under a nitrogen atmosphere in oven-dried glassware. Melting points were determined with an electrothermal heating block (Metler) and are uncorrected. ^1H and ^{13}C spectra were recorded on Varian 300 and 75 MHz, respectively. NMR spectra were recorded in $CDCl_3$ unless otherwise specified, and chemical shifts are reported relative to tetramethylsilane as an internal standard. Low resolution mass spectra were obtained using atmospheric pressure-turbo ion spray ionization. Elemental analyses were performed by Robertson Microlit Laboratories Inc., Madison, N.J. Gravity and flash column chromatographies were performed using EM Science silica gel 60 (230–400 mesh). TLC was performed on 250 μm precoated EM Science silica gel 60 F_{254} aluminum sheets. Preparative TLC was performed using 20 × 20 cm 1000 μm precoated silica gel plates (Whatman). Spots were visualized under 254 nm light or after staining with phosphomolybdic acid.

5.4.2. ((*tert*-Butyl)oxycarbonyl)methyl (2*S*)-2-(6-methoxy(2-naphthyl))propanoate (2). A mixture of (2*S*)-2-(6-methoxy(2-naphthyl))propanoic acid (naproxen, 3 g, 13.0 mmol), $NaHCO_3$ (1.4 g, 16.7 mmol), and *tert*-butyl bromoacetate (5.08 g, 26.0 mmol) in DMF (30 mL) was stirred at room temperature for 2 days, and the solvent was evaporated in high vacuo. The residue was recrystallized from a mixture of CH_2Cl_2/EtOAc/hexane to give **2** as a white solid in quantitative yield. Mp 85–86 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69–7.70 (m, 3H), 7.43 (dd, $J = 1.6$ and 8.6 Hz, 1H), 7.10–7.15 (m, 2H), 4.47 (ABq, $J_{AB} = 15.7$ Hz, $\Delta\nu_{AB} = 40.7$ Hz, 2H), 3.97 (q, $J = 7.2$ Hz, 1H), 3.90 (s, 3H), 1.63 (d, $J = 7.2$ Hz, 3H), 1.42 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.2, 166.9, 157.8, 135.4, 133.9, 129.5, 129.1, 127.3, 126.5, 126.3, 119.1, 105.8, 82.5, 61.7, 55.5, 45.3, 28.1, 18.8. MS (API-TIS) m/z 345 (MH^+), 362 (MNH_4^+).

5.4.3. 2-((2*S*)-2-(6-Methoxy(2-naphthyl))propanoyloxy)-acetic acid (3). Trifluoroacetic acid (8.4 mL) was added dropwise to a solution of the product of **2** (4.8 g, 14 mmol) in CH_2Cl_2 (20 mL). The reaction mixture was stirred at room temperature for 2 days, and the volatile material was removed in vacuo. The residue after evaporation was recrystallized from EtOAc/hexane to give **3** (2.7 g, 66%) as a white solid. Mp 122–123 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67–7.73 (m, 3H), 7.42 (d, $J = 9.1$ Hz, 1H), 7.12–7.17 (m, 2H), 4.65 (ABq, $J_{AB} = 16.4$ Hz, $\Delta\nu_{AB} = 34.3$ Hz, 2H), 3.98 (q, $J = 7.1$ Hz, 1H), 3.92 (s, 3H), 1.63 (d, $J = 7.1$ Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.2, 173.0, 157.9, 135.1, 133.9, 129.5, 129.1, 127.4, 126.4, 126.3, 119.2, 105.8, 60.5, 55.5, 45.2, 18.7. MS (API-TIS) m/z 289 (MH^+), 306 (MNH_4^+).

5.4.4. (*N*-Methyl-*N*-(2-(nitrooxy)ethyl)carbamoyl)methyl (2*S*)-2-(6-methoxy(2-naphthyl))propanoate (4a)

5.4.4.1. Methyl(2-(nitrooxy)ethyl)ammonium nitrate (4a-I). Methyl(2-(hydroxy)ethyl)amine (1.5 g, 20 mmol) in EtOAc (65 mL) was added dropwise to a mixture of fuming HNO_3 (6.3 g, 4.2 mL, 100 mmol) and Ac_2O (16.3 g, 15.1 mL, 160 mmol) at −10 °C. The reaction mixture was stirred at −10 °C for 30 min and diluted with EtOAc and hexane. The precipitate was collected by filtration and washed with hexane to give **4a-I** (2.79 g, 82%) as an off-white solid. Mp 58–62 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.59–4.67 (m, 2H), 4.64–4.73 (m, 2H), 2.61 (br s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 59.8, 38.7, 24.4. MS (API-TIS) m/z 121 (MH^+).

5.4.4.2. (*N*-Methyl-*N*-(2-(nitrooxy)ethyl)carbamoyl)methyl(2*S*)-2-(6-methoxy(2-naphthyl))propanoate (4a). A mixture of **3** (1.03 g, 3.57 mmol), **4a-I** (0.72 g, 3.93 mmol), and DMAP (0.44 g, 3.57 mmol) in CH_2Cl_2 (20 mL) at 0 °C was treated with 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (0.75 g, 3.93 mmol). The reaction mixture was stirred at 0 °C for 3 h, diluted with more CH_2Cl_2, washed with water, brine, and dried over Na_2SO_4. The residue after filtration and evaporation was chromatographed on silica gel eluting with 1:3 to 1:2 to 1:1 EtOAc/hexane to give **4a** (0.71 g, 51%). Mp 80–81 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67–7.74 (m, 3H), 7.45 (dd, $J = 1.5$ and 8.5 Hz, 1H), 7.08–7.18 (m, 2H), 4.70 (ABq, $J_{AB} = 14.5$ Hz, $\Delta\nu_{AB} = 58.2$ Hz, 2H), 4.53–4.67 (m, 2H), 4.02 (q, $J = 7.1$ Hz, 1H), 3.92 (s, 3H), 3.56–3.77 (m, 2H), 2.97 (s, 3H), 1.65 (d, $J = 7.2$ Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.4, 167.1, 157.8, 135.3, 133.9, 129.4, 129.0, 127.3, 126.4, 126.3, 119.1, 105.7, 71.3, 61.7, 55.4, 46.3, 45.3, 36.0, 18.7. MS (API-TIS) m/z 391 (MH^+), 408 (MNH_4^+). Anal. Calcd for $C_{19}H_{22}N_2O_7$: C, 58.46; H, 5.68; N, 7.18. Found: C, 58.32; H, 5.55; N, 6.94.

5.4.5. (*N*-Ethyl-*N*-(2-(nitrooxy)ethyl)carbamoyl)methyl (2*S*)-2-(6-methoxy(2-naphthyl))propanoate (4b)

5.4.5.1. Ethyl(2-(nitroxy)ethyl)ammonium nitrate (4b-I). Ethyl(2-(hydroxy)ethyl)amine (5 g, 56 mmol) in EtOAc (60 mL) was added dropwise to a mixture of fuming HNO_3 (17.7 g, 11.8 mL, 280 mmol) and Ac_2O (45.8 g, 42.3 mL, 448 mmol) at −10 °C. The reaction mixture was stirred at −10 °C for 30 min and diluted with EtOAc and hexane. The oil layer was separated and dried in high vacuo to give **4b-I** (8.4 g, 76%) as a low-melting solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.60–8.95 (br s, 2H), 4.84 (m, 2H), 3.37–3.49 (m, 2H), 3.02–3.16 (m, 2H), 1.24 (t, $J = 7.2$ Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 69.1, 43.6, 42.6, 11.0. MS (API-TIS) m/z 135 (MH^+).

5.4.5.2. (*N*-Ethyl-*N*-(2-(nitrooxy)ethyl)carbamoyl)methyl(2*S*)-2-(6-methoxy(2-naphthyl))propanoate (4b). A mixture of **3** (1.0 g, 3.47 mmol), **4b-I** (0.75 g, 3.82 mmol), and DMAP (0.42 g, 3.47 mmol) in CH_2Cl_2 (20 mL) at 0 °C was treated with 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (0.73 g, 3.82 mmol). The reaction mixture was stirred at 0 °C for 3 h, diluted with more CH_2Cl_2, washed with water, brine, and dried over Na_2SO_4. The residue after filtration and evaporation was chromatographed on silica gel eluting with 1:2 to 1:1 EtOAc/hexane to give **4b** (0.4 g, 29%) as a white solid. Mp 60–61 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65–7.74 (m, 3H), 7.44 (dd, $J = 1.5$ and 8.5 Hz, 1H), 7.06–7.17 (m, 2H), 4.70 (ABq, $J_{AB} = 14.4$ Hz, $\Delta\nu_{AB} = 64.9$ Hz, 2H), 4.54–4.65 (m, 2H), 4.01 (q, $J = 7.1$ Hz, 1H), 3.91 (s, 3H), 3.50–3.72 (m, 2H), 3.26 (q, $J = 7.2$ Hz, 2H), 1.64 (d, $J = 7.2$ Hz, 3H), 1.17 (t, $J = 7.1$ Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.5, 166.9, 157.8, 135.3, 133.9, 129.4, 129.0, 127.3, 126.4, 126.3, 119.1, 105.7, 71.0, 61.5, 55.4, 45.3, 43.9, 43.3, 18.7, 14.0. MS (API-TIS) m/z 405 (MH^+), 422 (MNH_4^+). Anal. Calcd for $C_{20}H_{24}N_2O_7$: C, 59.40; H, 5.98; N, 6.93. Found: C, 59.28; H, 5.81; N, 6.70.

5.4.6. (*N*-*i*-Propyl-*N*-(2-(nitrooxy)ethyl)carbamoyl)methyl(2*S*)-2-(6-methoxy(2-naphthyl))propanoate (4c)

5.4.6.1. *i*-Propyl(2-(nitroxy)ethyl)ammonium nitrate (4c-I). *i*-Propyl(2-(hydroxy)ethyl)amine (5 g, 48 mmol) in EtOAc (60 mL) was added dropwise to a mixture of fuming HNO_3 (15.2 g, 10.1 mL, 242 mmol) and Ac_2O (39.6 g, 36.6 mL, 387.7 mmol) at −10 °C. The reaction mixture was stirred at −10 °C for 30 min and diluted with EtOAc and hexane. The oil layer was separated and triturated with ice-cold EtOAc. The solid was filtered and dried in high vacuo to give **4c-I** (2.4 g, 23%) as a white solid. Mp 102–105 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.50-8.72 (br s, 2H), 4.77 (t, $J = 4.9$ Hz, 2H), 3.32–3.40 (m, 3H), 1.23 (d, $J = 6.5$ Hz, 6H).

^{13}C NMR (75 MHz, DMSO-d_6) δ 69.2, 50.2, 41.4, 18.5. MS (API-TIS) *m/z* 149 (MH$^+$).

5.4.6.2. (*N*-*i*-Propyl-*N*-(2-(nitrooxy)ethyl)carbamoyl)-methyl(2*S*)-2-(6-methoxy(2-naphthyl))propanoate (4c). A mixture of **3** (1.5 g, 5.2 mmol), **4c-I** (1.1 g, 5.2 mmol), and DMAP (0.64 g, 5.2 mmol) in CH_2Cl_2 (10 mL) at 0 °C was treated with 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (1 g, 5.2 mmol). The reaction mixture was stirred at 0 °C for 3 h, diluted with more CH_2Cl_2, washed with water, brine, and dried over Na_2SO_4. The residue after filtration and evaporation was chromatographed on silica gel eluting with 1:3 EtOAc/hexane to give **4c** (0.49 g, 22%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72–7.69 (m, 3H), 7.44 (dd, J = 1.6 and 8.5 Hz, 1H), 7.11–7.16 (m, 2H), 4.74 (ABq, J_{AB} = 14.3 Hz, $\Delta\nu_{AB}$ = 65.4 Hz, 2H), 4.54–4.65 (m, 2H), 4.01 (q, J = 7.2 Hz, 1H), 3.91 (s, 3H), 3.75–3.82 (m, 1H), 3.43–3.52 (m, 2H), 1.64 (d, J = 7.1 Hz, 3H), 1.15 (d, J = 6.7 Hz, 3H), 1.14 (d, J = 6.7 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.4, 166.9, 157.7, 135.3, 133.8, 129.4, 129.0, 127.3, 126.4, 126.2, 119.1, 105.6, 70.1, 61.9, 55.4, 47.7, 45.2, 38.5, 21.1, 18.7. MS (API-TIS) *m/z* 419 (MH$^+$), 436 (MNH_4^+). LCMS 97%.

5.4.7. (*N*-Cyclohexyl-*N*-(2-(nitrooxy)ethyl)carbamoyl)-methyl(2*S*)-2-(6-methoxy(2-naphthyl))propanoate (4d)

5.4.7.1. *i*-Cyclohexyl(2-(nitroxy)ethyl)ammonium nitrate (4d-I). *i*-Cyclohexyl(2-(hydroxy)ethyl)amine (25 g, 174 mmol) in EtOAc (200 mL) was added dropwise to a mixture of fuming HNO_3 (55 g, 36.7 mL, 0.87 mol) and Ac_2O (143 g, 131.7 mL, 1.4 mol) at −10 °C. The reaction mixture was stirred at −10 °C for 30 min and diluted with EtOAc and hexane. The solid was filtered, washed with EtOAc, and dried in high vacuo to give **4d-I** (27 g, 62%) as a white solid. Mp 127–129 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.50–8.60 (br s, 2H), 4.77 (t, J = 4.9 Hz, 2H), 3.32–3.45 (m, 2H), 2.48–3.14 (m, 1H), 1.90–2.05 (m, 2H), 1.65–1.80 (m, 2H), 1.50–1.61 (m, 1H), 1.00–1.30 (m, 5H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 69.2, 50.2, 41.4, 18.5. MS (API-TIS) *m/z* 149 (MH$^+$). Anal. Calcd for $C_8H_{17}N_3O_6$: C, 38.25; H, 6.82; N, 16.73. Found: C, 38.47; H, 7.05; N, 16.76.

5.4.7.2. (*N*-Cyclohexyl-*N*-(2-(nitrooxy)ethyl)carbamoyl)methyl(2*S*)-2-(6-methoxy(2-naphthyl))propanoate (4d). A mixture of **3** (1.5 g, 5.2 mmol), **4d-I** (1.3 g, 5.2 mmol), and DMAP (0.64 g, 5.2 mmol) in CH_2Cl_2 (10 mL) at 0 °C was treated with 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (1 g, 5.2 mmol). The reaction mixture was stirred at 0 °C for 3 h, diluted with more CH_2Cl_2, washed with water, brine, and dried over Na_2SO_4. The residue after filtration and evaporation was chromatographed on silica gel eluting with 1:3 EtOAc/hexane to give **4d** (1 g, 42%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66–7.74 (m, 3H), 7.44 (dd, J = 1.7 and 8.6 Hz, 1H), 7.08–7.17 (m, 2H), 4.74 (ABq, J_{AB} = 14.3 Hz, $\Delta\nu_{AB}$ = 68.5 Hz, 2H), 4.52–4.63 (m, 2H), 4.01 (q, J = 7.2 Hz, 1H), 3.91 (s, 3H), 3.41–3.62 (m, 2H), 3.18–3.32 (m, 1H), 1.65–1.88 (m, 5H), 1.64 (d, J = 7.2 Hz, 3H), 0.90–1.48 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.4, 167.0, 157.7, 135.3, 133.9, 129.4, 129.0, 127.3, 126.4, 126.2, 119.1, 105.7, 70.3, 62.0, 56.5, 55.4, 45.2, 39.4, 31.6, 25.8, 25.0, 18.7. MS (API-TIS) *m/z* 459 (MH$^+$), 476 (MNH_4^+).

5.5. {[2-(Cyclohexylamino)ethyl]oxycarbonyl}methyl (2*S*)-2-(6-methoxy(2-naphthyl))propanoate, nitric acid salt 4d-II

Mp 135–137 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.50–8.75 (br s, 2H), 7.70 (d, J = 8.7 Hz, 3H), 7.40 (dd, J = 1.7 and 8.4 Hz, 1H), 7.06–7.17 (m, 2H), 4.63 (ABq, J_{AB} = 16.1 Hz, $\Delta\nu_{AB}$ = 18.9 Hz, 2H), 4.27–4.43 (m, 2H), 3.96 (q, J = 7.2 Hz, 1H), 3.90 (s, 3H), 3.04–3.20 (m, 2H), 2.82–2.98 (m, 1H), 1.78–2.02 (m, 2H), 1.68–1.80 (m, 2H), 1.59 (d, J = 7.2 Hz, 3H), 1.00–1.47 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.4, 167.6, 157.8, 135.1, 133.8, 129.4, 129.0, 127.3, 126.4, 126.2, 119.2, 105.7, 60.8, 60.4, 58.0, 55.4, 45.1, 43.4, 29.0, 24.8, 24.5, 18.6. MS (API-TIS) *m/z* 414 (MH$^+$). Anal. Calcd for $C_{24}H_{32}N_2O_8$: C, 60.49; H, 6.77; N, 5.88. Found: C, 60.47; H, 6.54; N, 5.78.

5.5.1. 2-(4-((Nitrooxy)methyl)piperidyl)-2-oxoethyl (2*S*)-2-(6-methoxy(2-naphthyl))propanoate (5a)

5.5.1.1. Nitrooxy(4-piperidylmethyl)hydrogen nitrate (5a-I). 4-Piperidylmethan-1-ol (2.5 g, 21.7 mmol) in a mixture of EtOAc (25 mL) and CH_2Cl_2 (5 mL) was added dropwise to a mixture of fuming HNO_3 (6.83 g, 4.6 mL, 109 mmol) and Ac_2O (17.7 g, 16.3 mL, 174 mmol) at −10 °C. The reaction mixture was stirred at −10 °C for 30 min and diluted with EtOAc and hexane. The precipitate was collected by filtration and washed with hexane to give **5a-I** (2.5 g, 52%) as a pale green solid. Mp 51–53 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.45–8.75 (br s, 1H), 8.10–8.40 (br s, 1H), 4.44 (d, J = 6.4 Hz, 2H), 3.22–3.35 (m, 2H), 2.80–2.98 (m, 2H), 1.96–2.15 (m, 1H), 1.75–1.89 (m, 2H), 1.40–1.52 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 76.4, 42.7, 31.2, 24.9. MS (API-TIS) *m/z* 161 (MH$^+$). Anal. Calcd for $C_6H_{13}N_3O_6$: C, 32.29; H, 5.87; N, 18.83. Found: C, 32.03; H, 5.78; N, 18.73.

5.5.1.2. 2-(4-((Nitrooxy)methyl)piperidyl)-2-oxoethyl (2*S*)-2-(6-methoxy(2-naphthyl))propanoate (5a). A mixture of **3** (1.5 g, 5.21 mmol), **5a-I** (1.16 g, 5.21 mmol), and DMAP (0.63 g, 5.21 mmol) in CH_2Cl_2 (30 mL) at 0 °C was treated with 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (1.0 g, 5.21 mmol). The reaction mixture was stirred at 0 °C for 3 h, diluted with CH_2Cl_2, washed with water, brine, and dried over Na_2SO_4. The residue after filtration and evaporation was chromatographed on silica gel eluted with 1:2 to 1:1 EtOAc/hexane to give the title compound (1.74 g, 78%) as a white solid. Mp 94–95 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.75–7.82 (m, 3H), 7.44 (dd, J = 1.3 and 8.5 Hz, 1H), 7.30 (d, J = 2.5 Hz, 1H), 7.16 (dd, J = 2.5 and 8.9 Hz, 1H), 4.65–4.89 (m, 2H), 4.35 (bd, J = 6.4 Hz, 2H), 4.21–4.32 (m, 1H), 4.01 (q, J = 7.1 Hz, 1H), 3.86 (s, 3H), 3.55–3.73 (m, 1H), 2.81–3.02 (m, 1H), 2.45–2.63 (m, 1H), 1.87–2.03 (m, 1H), 1.55–1.72 (m, 2H), 1.51 (d, J = 7.1 Hz, 3H), 0.90–1.22 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ

173.5, 164.3, 157.2, 135.5, 133.3, 129.1, 128.4, 126.9, 126.5, 125.8, 118.7, 105.6, 76.9, 61.8, 55.1, 44.3, 43.2, 33.2, 28.2, 27.5, 18.6. MS (API-TIS) *m*/*z* 431 (MH^+), 448 ($MNH_4{}^+$). Anal. Calcd for $C_{22}H_{26}N_2O_7$: C, 61.39; H, 6.09; N, 6.51. Found: C, 61.24; H, 5.98; N, 6.40.

5.5.2. 2-(4-(2-(Nitrooxy)ethyl)piperidyl)-2-oxoethyl (2*S*)-2-(6-methoxy(2-naphthyl))propanoate (5b)

5.5.2.1. Nitrooxy(2-(4-piperidyl)ethyl)hydrogennitrate (5b-I). 4-Piperidylethan-1-ol (10 g, 77.3 mmol) in a mixture of EtOAc (90 mL) and CH_2Cl_2 (5 mL) was added dropwise to a mixture of fuming HNO_3 (24.4 g, 16.3 mL, 387 mmol) and Ac_2O (63.2 g, 58.4 mL, 619 mmol) at −10 °C. The reaction mixture was stirred at −10 °C for 30 min and then diluted with EtOAc and hexane. The precipitate was collected by filtration and washed with hexane to give **5b-I** (7.5 g, 41% yield) as a white solid. Mp 86–88 °C. ^1H NMR (300 MHz, DMSO-d_6) δ 8.20–8.30 (br s, 1H), 8.05–8.20 (br s, 1H), 4.57 (t, J = 6.2 Hz, 2H), 3.15–3.30 (m, 2H), 2.70–2.90 (m, 2H), 1.75–1.90 (m, 2H), 1.55–1.72 (m, 3H), 1.19–1.37 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 71.6, 43.2, 31.9, 30.0, 28.1. Mass spectrum (API-TIS) *m*/*z* 175 (MH^+). Anal. Calcd for $C_7H_{15}N_3O_6$: C, 35.44; H, 6.37; N, 17.71. Found: C, 35.62; H, 6.39; N, 17.65.

5.5.2.2. 2-(4-(2-(Nitrooxy)ethyl)piperidyl)-2-oxoethyl (2*S*)-2-(6-methoxy(2-naphthyl))propanoate (5b). A mixture of **3** (1.5 g, 5.21 mmol), **5b-I** (1.2 g, 5.21 mmol), and DMAP (0.63 g, 5.21 mmol) in CH_2Cl_2 (30 mL) at 0 °C was treated with 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (1.0 g, 5.21 mmol). The reaction mixture was stirred at 0 °C for 3 h, diluted with CH_2Cl_2, washed with water, brine, and dried over Na_2SO_4. The residue after filtration and evaporation was chromatographed on silica gel eluted with EtOAc/hexane (1:3 to 1:2 to 1:1) to give **5b** (1.0 g, 43% yield) as a white solid. Mp 83–85 °C. ^1H NMR (300 MHz, DMSO-d_6) δ 7.65–7.75 (m, 3H), 7.44 (d, J = 8.5 Hz, 1H), 7.07–7.18 (m, 2H), 4.46–4.81 (m, 3H), 4.30–4.44 (m, 2H), 3.99 (q, J = 6.9 Hz, 1H), 3.91 (s, 3H), 3.50–3.59 (m, 1H), 2.75–2.93 (m, 1H), 2.41–2.60 (m, 1H), 1.63 (d, J = 7.9 Hz, 3H), 1.32–1.74 (m, 5H), 0.69–1.20 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.3, 164.8, 157.8, 135.5, 133.9, 129.4, 129.0, 127.3, 126.5, 126.4, 119.1, 105.7, 70.7, 62.3, 55.4, 45.4, 44.8, 42.2, 33.0, 32.6, 32.0, 31.4, 18.4. Mass spectrum (API-TIS) *m*/*z* 445 (MH^+), 462 ($MNH_4{}^+$). Anal. Calcd for $C_{23}H_{28}N_2O_7$: C, 62.15; H, 6.35; N, 6.30. Found: C, 62.21; H, 6.42; N, 6.26.

5.5.3. 2-(4-(2-(Nitrooxy)ethyl)piperazinyl)-2-oxoethyl (2*S*)-2-(6-methoxy(2-naphthyl))propanoate (5c)

5.5.3.1. Nitrooxy(2-piperazinylethyl)dihydrogennitrate (5c-I). 2-Piperazinylethan-1-ol (15 g, 115 mmol) in a mixture of EtOAc (132 mL) and CH_2Cl_2 (5 mL) was added dropwise to a mixture of fuming HNO_3 (36.3 g, 24.2 mL, 576 mmol) and Ac_2O (94.1 g, 87 mL, 921 mmol) at −10 °C. The reaction mixture was stirred at −10 °C for 30 min and diluted with EtOAc and hexane. The oil was separated and dried under vacuum to give **5c-I** (5.1 g, 19% yield) as a sticky oil. The crude product was used without further purification. Mass spectrum (API-TIS) *m*/*z* 176 (MH^+).

5.5.3.2. 2-(4-(2-(Nitrooxy)ethyl)piperazinyl)-2-oxoethyl (2*S*)-2-(6-methoxy(2-naphthyl))propanoate (5c). A mixture of the product of **3** (2.67 g, 9.27 mmol), **5c-I** (2.2 g, 9.27 mmol), and DMAP (3.39 g, 27.8 mmol) in CH_2Cl_2 (20 mL) and DMF (9.3 mL) at 0 °C was treated with 1-(3-(dimethylamino) propyl)-3-ethylcarbodiimide hydrochloride (1.78 g, 9.27 mmol). The reaction mixture was stirred at 0 °C for 3 h, diluted with CH_2Cl_2, washed with saturated $NaHCO_3$, and dried over Na_2SO_4. The residue after filtration and evaporation was chromatographed on silica gel eluted with MeOH/EtOAc/CH_2Cl_2 (1:25:25) to give **5c** (1.2 g, 29% yield) as an oil. ^1H NMR (300 MHz, DMSO-d_6) δ 7.65–7.72 (m, 3H), 7.43 (dd, J = 1.6 and 8.5 Hz, 1H), 7.07–7.16 (m, 2H), 4.66 (ABq, J_{AB} = 14.0 Hz, $\Delta\nu_{AB}$ = 28.0 Hz, 2H), 4.43 (t,J = 5.5 Hz, 2H), 3.98 (q, J = 7.1 Hz, 1H), 3.89 (s, 3H), 3.46–3.61 (m, 2H), 3.14–3.23 (m, 2H), 2.49–2.56 (m, 2H), 2.32–2.44 (m, 2H), 2.12–2.20 (m, 2H), 1.63 (d, J = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.2, 172.2, 164.9, 157.8, 135.4, 135.8, 129.4, 129.0, 127.3, 126.4, 126.3, 119.1, 105.7, 70.1, 62.1, 55.4, 54.8, 52.9, 45.3, 44.6, 41.9, 18.7. Mass spectrum (API-TIS) *m*/*z* 446 (MH^+). Anal. Calcd for $C_{22}H_{27}N_3O_7$: C, 59.32; H, 6.11; N, 9.43. Found: C, 59.11; H, 5.86; N, 9.24.

5.5.4. 2-(4-(2-(Nitrooxy)ethyl)piperazinyl)-2-oxoethyl(2*S*)-2-(6-methoxy(2-naphthyl))propanoate hydrogen chloride (5d).

To a solution of **5c** (0.3 g, 0.67 mmol) in EtOAc (4.3 mL) at 0 °C was added dropwise a solution of HCl gas in Et_2O (24 mg, 0.33 mL, 2 M solution, 0.67 mmol). The cloudy solution was dissolved in excess EtOAc and hexane was added. The solvent was evaporated to a small volume. The precipitate was filtered and washed with hexane to give **5d** (0.3 g, 92% yield) as a white solid. Mp 129–131 °C. ^1H NMR (300 MHz, DMSO-d_6) δ 11.65–12.00 (br s, 1H), 7.73–7.82 (m, 3H), 7.44 (dd, J = 1.4 and 8.6 Hz, 1H), 7.28–7.32 (m, 1H), 7.16 (dd, J = 2.5 and 8.9 Hz, 1H), 4.78–4.97 (m, 4H), 4.22–4.45 (m, 1H), 4.03 (q, J = 7.1 Hz, 1H), 3.87 (s, 3H), 3.80–4.05 (m, 1H), 3.35–3.67 (m, 5H), 2.90–3.30 (m, 3H), 1.52 (d, J = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.5, 165.0, 157.2, 135.5, 133.3, 129.1, 128.4, 126.9, 126.5, 125.8, 118.7, 105.7, 67.4, 61.4, 55.2, 50.8, 18.7. Mass spectrum (API-TIS) *m*/*z* 446 (MH^+). Anal. Calcd for $C_{22}H_{28}ClN_3O_7$: C, 54.83; H, 5.86; N, 8.72. Found: C, 54.78; H, 5.89; N, 8.65.

5.5.5. 2-(2-((2*S*)-2-(6-Methoxy(2-naphthyl))propanoyloxy)-*N*-methylacetylamino)acetic acid (6)

5.5.5.1. (*N*-(((*tert*-Butyl)oxycarbonyl)methyl)-*N*-methylcarbamoyl)methyl (2*S*)-2-(6-methoxy(2-naphthyl))propanoate (6a). A mixture of **3** (10 g, 34.7 mmol), sarcosine *tert*-butyl ester hydrochloride (6.3 g, 34.7 mmol), and DMAP (4.24 g, 34.7 mmol) in CH_2Cl_2 (125 mL) at 0 °C was treated with 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (6.7 g, 34.7 mmol). The reaction mixture was stirred at room temperature for 16 h, diluted with CH_2Cl_2, washed with water, brine, and dried over Na_2SO_4. The residue after filtration and evaporation was chromatographed on silica gel eluted with 1:9 to 1:1 EtOAc/hexane to give **6a** (11.8 g, 82% yield) as a white solid. Mp 103–104 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65–7.77 (m, 3H), 7.44 (dd, J = 1.3 and 8.9 Hz, 1H), 7.03–7.18 (m, 2H), 4.74 (ABq, J_{AB} = 14.5 Hz, $\Delta\nu_{AB}$ = 64.7 Hz, 2H), 3.72–4.19 (m, 3H), 3.90 (s, 3H), 2.93 (s, 3H), 1.63 (d, J = 7.1 Hz, 3H), 1.45 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.3, 168.0, 167.1, 157.8, 135.5, 133.9, 129.5, 129.1, 127.3, 126.5, 126.3, 119.0, 105.8, 82.2, 61.5, 55.4, 51.6, 45.3, 35.3, 28.2, 18.8. Mass spectrum (API-TIS) *m/z* 416 (MH^+), 433 (MNH_4^+), 438 (MNa^+).

5.5.5.2. 2-(2-((2*S*)-2-(6-Methoxy(2-naphthyl))propanoyloxy)-*N*-methylacetylamino)acetic acid (6). Trifluoroacetic acid (5 mL) was added dropwise to a solution of the product of **6a** (3.54 g, 8.5 mmol) in CH_2Cl_2 (15 mL). The reaction mixture was stirred at room temperature for 24 h, and the volatile material was removed *in vacuo*. The residue, after evaporation, was recrystallized from CH_2Cl_2/EtOAc/hexane to give **6** (2.55 g, 83% yield) as a white solid. Mp 174–175 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.76–7.80 (m, 3H), 7.42–7.45 (m, 1H), 7.27–7.29 (m, 1H), 7.15 (dd, J = 2.5 and 8.5 Hz, 1H), 4.84 (ABq, J_{AB} = 15.1 Hz, $\Delta\nu_{AB}$ = 26.9 Hz, 2H), 3.93–4.12 (m, 3H), 3.87 (s, 3H), 2.94 (s, 3H), 1.51 (d, J = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.4, 170.5, 166.6, 157.2, 135.5, 133.3, 129.2, 128.4, 126.9, 126.5, 125.8, 118.7, 105.8, 61.4, 55.2, 48.9, 44.3, 34.5, 18.7. Mass spectrum (API-TIS) *m/z* 360 (MH^+), 377 (MNH_4^+), 382 (MNa^+). Anal. Calcd for $C_{19}H_{21}NO_6$: C, 63.50; H, 5.89; N, 3.90. Found: C, 63.33; H, 5.90; N, 3.89.

5.5.6. (*N*-Methyl-*N*-((*N*-(2-(nitrooxy)ethyl)carbamoyl)methyl)carbamoyl)methyl(2*S*)-2-(6-methoxy(2-naphthyl))propanoate (7a)

5.5.6.1. 2-(Nitrooxy)ethylammonium nitrate (7a-I). 2-Hydroxyethylamine (5 g, 81.9 mmol) in EtOAc (40 mL) was added dropwise to a mixture of fuming HNO_3 (18 g, 12 mL, 0.29 mol) and Ac_2O (54 g, 50 mL, 0.53 mol) at -10 °C. The reaction mixture was stirred at 0 °C for 10 min and at room temperature for 10 min. The solvent was evaporated in high vacuo at 40 °C. The residue was sonicated with ether. The solid was filtered and washed with hexane to give **7a-I** as a white solid. Mp 92–94 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.71–8.25 (br s, 3H), 4.72 (t, J = 5.0 Hz, 2H), 3.23 (d, J = 5.0 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 70.9, 37.4.

5.5.6.2. (*N*-Methyl-*N*-((*N*-(2-(nitrooxy)ethyl)carbamoyl)methyl)carbamoyl)methyl(2*S*)-2-(6-methoxy(2-naphthyl))-propanoate (7a). A mixture of **6** (0.5 g, 1.39 mmol), **7a-I** (0.26 g, 1.53 mmol), and DMAP (0.17 g, 1.39 mmol) in CH_2Cl_2 (8 mL) at 0 °C was treated with 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (0.29 g, 1.53 mmol). The reaction mixture was stirred at 0 °C for 3 h, diluted with CH_2Cl_2, washed with water, brine, and dried over Na_2SO_4. The residue after filtration and evaporation was chromatographed on silica gel eluted with 1:2 EtOAc/hexane to 1:1:0.1 EtOAc/hexane/MeOH to give **7a** (0.61 g, 89% yield) as a white solid. Mp 79–81 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67–7.75 (m, 3H), 7.35–7.45 (m, 1H), 7.07–7.17 (m, 2H), 6.53–6.60 (br s, 1H), 4.68 (ABq, J_{AB} = 14.3 Hz, $\Delta\nu_{AB}$ = 39.6 Hz, 2H), 4.47 (t, J = 5.3 HZ, 2H), 3.93–4.07 (m, 3H), 3.91 (s, 3H), 3.46–3.58 (m, 2H), 3.01 (br s, 3H), 1.63 (d, J = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.0, 168.9, 167.9, 157.9, 135.2, 133.9, 129.4, 129.0, 127.3, 126.4, 126.3, 119.2, 105.8, 71.3, 61.6, 55.4, 52.3, 45.2, 37.0, 35.8, 18.6. Mass spectrum (API-TIS) *m/z* 448 (MH^+), 465 (MNH_4).

5.5.7. (*N*-Methyl-*N*-(((2-(nitrooxy)ethyl)oxycarbonyl)methyl)carbamoyl)methyl (2*S*)-2-(6-methoxy(2-naphthyl))propanoate (7b)

5.5.7.1. (*N*-(((2-Hydroxyethyl)oxycarbonyl)methyl)-*N*-methylcarbamoyl)methyl (2*S*)-2-(6-methoxy(2-naphthyl))propanoate (7b-I). To a solution of **6** (0.5 g, 1.4 mmol), DMAP (84.9 mg, 0.69 mmol), and ethylene glycol (1.6 g, 27.8 mmol) in CH_2Cl_2 (4 mL) was added dropwise a solution of DCC (0.36 g, 1.7 mmol) in CH_2Cl_2 (2 mL). The reaction mixture was stirred at room temperature for 16 h. The precipitate was filtered. The residue after evaporation of the solvent was redissolved in EtOAc, washed with water, brine, and dried over Na_2SO_4. The residue after filtration and evaporation was chromatographed on silica gel eluted with 1:1:0.1 EtOAc/hexane/MeOH to give **7b-I** (0.4 g, 71% yield) as a white solid. Mp 113–114 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67–7.73 (m, 3H), 7.44 (dd, J = 1.6 and 8.6 Hz, 1H), 7.11–7.15 (m, 2H), 4.74 (ABq, J_{AB} = 14.6 Hz, $\Delta\nu_{AB}$ = 55.9 Hz, 2H), 4.22–4.31 (m, 2H), 4.09–4.12 (br s, 2H), 4.01 (q, J = 7.2 Hz, 1H), 3.90 (s, 3H), 3.74–3.83 (m, 2H), 2.99 (s, 3H), 2.40 (br s, 1H), 1.64 (d, J = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.6, 168.9, 167.6, 157.8, 135.3, 133.9, 129.4, 129.0, 127.3, 126.5, 126.3, 119.1, 105.8, 67.1, 61.6, 60.9, 55.4, 50.2, 45.3, 35.5, 18.6. Mass spectrum (API-TIS) *m/z* 404 (MH^+), 421 (MNH_4^+). Anal. Calcd for $C_{21}H_{25}NO_7 \cdot 1$ mol H_2O: C, 59.85; H, 6.46; N, 3.32. Found: C, 59.94; H, 6.15; N, 3.39.

5.5.7.2. (*N*-Methyl-*N*-(((2-(nitrooxy)ethyl)oxycarbonyl)methyl)carbamoyl)methyl(2*S*)-2-(6-methoxy(2-naphthyl))-propanoate (7b). A suspension of **7b-I** (0.26 g, 0.64 mmol) in EtOAc (0.28 mL) was added dropwise to a mixture of fuming HNO_3 (61.3 mg, 41 µL, 0.97 mmol) and Ac_2O (148 mg, 137 µL, 1.45 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 3 h. Then an additional ice-cold mixture of fuming HNO_3 (61.3 mg, 41 µL, 0.97 mmol) and Ac_2O (148 mg, 137 µL, 1.45 mmol) was added dropwise to the reaction mixture at 0 °C. The stirring was continued for another 2 h. The reaction mixture was diluted with EtOAc, washed with ice-cold saturated $NaHCO_3$, water, and dried over Na_2SO_4. The residue after filtration and evaporation was chromatographed on silica gel eluted with 1:1:0.1 EtOAc/hexane/MeOH to give **7b** (0.2 g, 69% yield) as a white solid. Mp 71–73 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66–7.75 (m, 3H), 7.44 (dd, J = 1.6 and 8.5 Hz, 1H), 7.08–7.17 (m, 2H), 4.76 (ABq, J_{AB} = 14.6 Hz, $\Delta\nu_{AB}$ = 59.7 Hz, 2H), 4.64–4.69 (m, 2H), 4.39–4.42 (m, 2H), 4.14 (ABq, J_{AB} = 17.4 Hz, $\Delta\nu_{AB}$ = 32.0 Hz, 2H), 3.93–4.07 (m, 1H), 3.91 (s, 3H), 2.98 (s, 3H), 1.64 (d, J = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.3, 168.6, 167.4, 157.8, 135.4, 133.9, 129.5, 129.0, 127.3, 126.5, 126.3, 119.1, 105.7, 70.2, 61.5, 61.1, 55.4, 49.5, 45.3, 35.3, 18.7. Mass spectrum (API-TIS) *m/z* 449 (MH^+), 466 (MNH_4^+). Anal. Calcd for $C_{21}H_{24}N_2O_9$: C, 56.25; H, 5.39; N, 6.25. Found: C, 56.00; H, 5.17; N, 6.09.

5.5.8. (*N*-Methyl-*N*-(((3-(nitrooxy)propyl)oxycarbonyl)methyl)carbamoyl)methyl (2*S*)-2-(6-methoxy(2-naphthyl))propanoate (7c)

5.5.8.1. (*N*-(((3-Hydroxypropyl)oxycarbonyl)methyl)-*N*-methylcarbamoyl)methyl(2*S*)-2-(6-methoxy(2-naphthyl))propanoate (7c-I). To a solution of **6** (0.5 g, 1.4 mmol), DMAP (84.9 mg, 0.69 mmol), and 1,3-propanediol (2.1 g, 27.8 mmol) in CH_2Cl_2 (4 mL) was added dropwise a solution of DCC (0.36 g, 1.7 mmol) in CH_2Cl_2 (2 mL). The reaction mixture was stirred at room temperature for 16 h. The precipitate was filtered. The residue after evaporation of the solvent was redissolved in EtOAc, washed with water, brine, and dried over Na_2SO_4, filtered, and evaporated. The residue was chromatographed on silica gel eluted with 1:1:0.1 EtOAc/hexane/MeOH to give **7c-I** (0.35 g, 60% yield) as a white solid. Mp 80–82 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 7.40–7.73 (m, 3H), 7.43 (dd, $J = 1.5$ and 8.6 Hz, 1H), 7.07–7.15 (m, 2H), 4.75 (ABq, $J_{AB} = 14.6$ Hz, $\Delta\nu_{AB} = 57.7$ Hz, 2H), 4.28 (t, $J = 5.9$ Hz, 2H), 3.95–4.20 (m, 3H), 3.90 (s, 3H), 3.61–3.72 (m, 2H), 2.97 (s, 3H), 1.78–2.00 (m, 3H), 1.63 (d, $J = 7.2$ Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.4, 169.1, 167.3, 157.8, 135.3, 133.8, 129.4, 129.0, 127.2, 126.4, 126.2, 119.0, 105.7, 62.4, 61.5, 59.0, 55.4, 49.8, 45.2, 35.3, 31.6, 18.7. Mass spectrum (API-TIS) *m/z* 418 (MH^+), 435 (MNH_4^+), 440 (MNa^+). Anal. Calcd for $C_{22}H_{27}NO_7 \cdot 1/4$ mol H_2O: C, 62.62; H, 6.58; N, 3.32. Found: C, 62.35; H, 6.47; N, 3.44.

5.5.8.2. (*N*-Methyl-*N*-(((3-(nitrooxy)propyl)oxycarbonyl)methyl)carbamoyl)methyl (2*S*)-2-(6-methoxy(2-naphthyl))propanoate (7c). A suspension of **7c-I** (355 mg, 0.85 mmol) in EtOAc (7.6 mL) was added dropwise to a mixture of fuming HNO_3 (80.9 mg, 54 µL, 1.28 mmol) and Ac_2O (196 mg, 181 µL, 1.92 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 1.5 h. Then an additional ice-cold mixture of fuming HNO_3 (80.9 mg, 54 µL, 1.28 mmol) and Ac_2O (196 mg, 181 µL, 1.92 mmol) was added dropwise to the reaction mixture at 0 °C. The stirring was continued for an additional hour. The reaction mixture was diluted with EtOAc, washed with ice cold saturated $NaHCO_3$, water, and dried over Na_2SO_4. The residue after filtration and evaporation was chromatographed on silica gel eluted with 1:1:0.1 EtOAc/hexane/MeOH to give **7c** (0.34 g, 86% yield) as a white solid. Mp 41–43 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 7.60–7.73 (m, 3H), 7.45 (dd, $J = 1.7$ and 8.6 Hz, 1H), 7.09–7.17 (m, 2H), 4.76 (ABq, $J_{AB} = 14.6$ Hz, $\Delta\nu_{AB} = 59.0$ Hz, 2H), 4.52 (t, $J = 6.2$ Hz, 2H), 4.24 (t, $J = 6.0$ Hz, 2H), 4.10 (ABq, $J_{AB} = 17.4$ Hz, $\Delta\nu_{AB} = 30.4$ Hz, 2H), 3.93–4.04 (m, 1H), 3.91 (s, 3H), 2.98 (s, 3H), 2.02–2.11 (m, 2H), 1.64 (d, $J = 7.2$ Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.3, 168.8, 167.4, 157.7, 135.4, 133.8, 129.4, 129.0, 127.3, 126.5, 126.3, 119.1, 105.7, 69.9, 61.5, 61.3, 55.4, 49.7, 45.3, 35.3, 26.4, 18.7. Mass spectrum (API-TIS) *m/z* 463 (MH^+), 480 (MNH_4^+). Anal. Calcd for $C_{22}H_{26}N_2O_9$: C, 57.14; H, 5.67; N, 6.06. Found: C, 56.91; H, 5.86; N, 6.02.

5.5.9. [*N*-Methyl-*N*-({*N*-methyl-*N*-[2-(nitrooxy)ethyl]carbamoyl}methyl)carbamoyl]methyl(2*S*)-2-(6-methoxy(2-naphthyl))propanoate (7d). A mixture of **6** (1.5 g, 4.2 mmol), **4a-I** (0.71 g, 4.2 mmol), and DMAP (0.51 g, 4.2 mmol) in CH_2Cl_2 (24 mL) at 0 °C was treated with 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (0.88 g, 4.5 mmol). The reaction mixture was stirred at 0 °C for 3 h, diluted with CH_2Cl_2, washed with water, brine, and dried over Na_2SO_4. The residue after filtration and evaporation was chromatographed on silica gel eluted with 1:2 EtOAc/hexane to 1:1:0.1 EtOAc/hexane/MeOH to give **7d** (0.8 g, 42% yield) as a sticky white solid. ^1H NMR (300 MHz, $CDCl_3$) δ 7.67–7.75 (m, 3H), 7.35–7.45 (m, 1H), 7.11–7.17 (m, 2H), 4.78 (ABq, $J_{AB} = 14.6$ Hz, $\Delta\nu_{AB} = 59.5$ Hz, 2H), 4.59 (t, $J = 5.2$ HZ, 2H), 4.18 (ABq, $J_{AB} = 16.2$ Hz, $\Delta\nu_{AB} = 51.5$ Hz, 2H), 3.96–4.05 (m, 1H), 3.92 (s, 3H), 3.65–3.69 (m, 2H), 3.06 (br s, 3H), 2.98 (br s, 3H), 1.65 (d, $J = 7.2$ Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.4, 168.2, 167.3, 157.7, 135.4, 133.8, 129.4, 129.0, 127.3, 126.5, 126.3, 119.1, 105.7, 71.1, 61.6, 55.4, 49.4, 46.3, 45.3, 36.3, 35.3, 18.6. Mass spectrum (API-TIS) *m/z* 462 (MH^+), 479 (MNH_4^+). LCMS 99.4%.

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