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**Original article**

# NO-Donors, part 3: nitrooxyacylated thiosalicylates and salicylates – synthesis and biological activities[#]

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**Abstract** – Organic nitrates release nitric oxide when incubated with thiosalicylic acid. S-Nitrooxyacylated esters and amides of thiosalicylic acid, together with the corresponding salicylates, were synthesized in order to perform a first in vitro evaluation of these new nitrate-thiol-hybrid prodrugs. These prodrugs might release NO in vivo after biotransformation without the use of endogenous reductives. None of these prodrugs released NO spontaneously when dissolved in buffer solution, but they did activate soluble guanylyl cyclase and induced vasodilatation of phenylephrine-pretreated male Wistar rat aorta in a potency range between that of isosorbiddinitrate and glycerole trinitrate. © 1999 Éditions scientifiques et médicales Elsevier SAS

**organic nitrates / nitric oxide / thiosalicylates / salicylates / vasodilatation**

## 1. Introduction

For more than a century organic nitrates have been used in the treatment of angina pectoris and congestive heart failure. The activity of these nitrates is mainly attributed to the release of nitric oxide (NO), an endogenous mediator with a rapidly growing list of physiological and pathophysiological functions [2]. The liberation of NO from organic nitrates in vivo is most likely an enzymatic metabolic reduction process [2, 3]. This does not however rule out the possibility of non-enzymatic reduction of nitrates by specific thiols. In general, all thiols reduce organic nitrates to inorganic nitrite, but only a very few of them are able to produce NO as well. The basic structural feature of these special thiols is a carbonyl function located two carbons away from a thiol group in a coplanar orientation as it is realized in cysteine, N-acetyl-cysteine and thiosalicylic acid [4–6]. Combining organic nitrate and a NO-liberating thiol in one molecule leads to prodrugs which might be endowed with typical properties of organic nitrates and may show a more facilitated mechanism of NO-release, which also might reduce the development of nitrate tolerance. Some promising approaches have already been made for nitrate-cysteine combinations, primarily N-nitrooxyacylated cysteines [7] but also cysteinamides, nitrooxyacylated at the amide nitrogen [8], and isosorbiddinitrate connected with a cyclized L-cysteinamide [9]. We established S-nitrooxyacylation both for various cysteines and thiosalicylic acid derivatives. Here we give a first report on the synthesis, stability and biological activities of S-nitrooxyacylthiosalicylates. To evaluate the influence of the thiosalicylate substructure the analogue salicylates were synthesized as well.

## 2. Chemistry

The target compounds (**SE**) could not be obtained from S-bromoacyl-thiosalicylates and silver nitrate. They were obtained instead by acylation [10] of various thiosalicylates and salicylates with nitratoacids, catalysed by carbonyldiimidazole (CDI) *(figure 1)*.

[#] For part 2 see [1]

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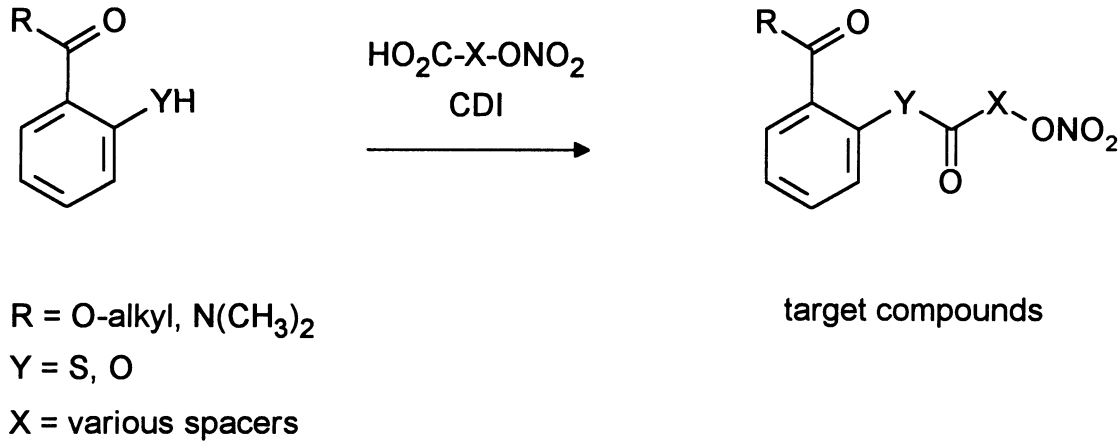
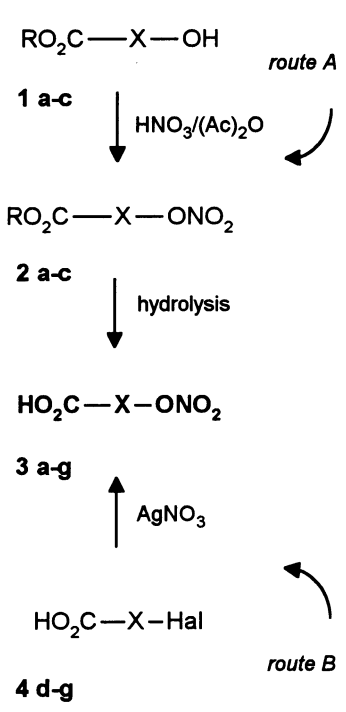



**Figure 1.** General route to the target compounds.

The nitratoacids **3** were obtained by following two different methods. Treatment of the hydroxyesters **1** with a mixture of fuming nitric acid/acetanhydride followed by acid or base catalysed hydrolysis of the esters **2** (route A, *figure 2*), or treatment of the halogenated acids **4** with silver nitrate in dry acetonitrile (route B).

These nitratoacids were linked with thiosalicylates and salicylates via S- resp. O-acylation. Not all of the planned combinations of nitratoacids with thioles or phenoles were possible. Two nitratoacids (**3a** and **d**) underwent decomposition when activated with carbonyldiimidazole. **3b** could be activated but decomposed as soon as a thiole



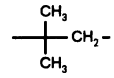
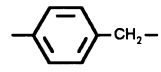
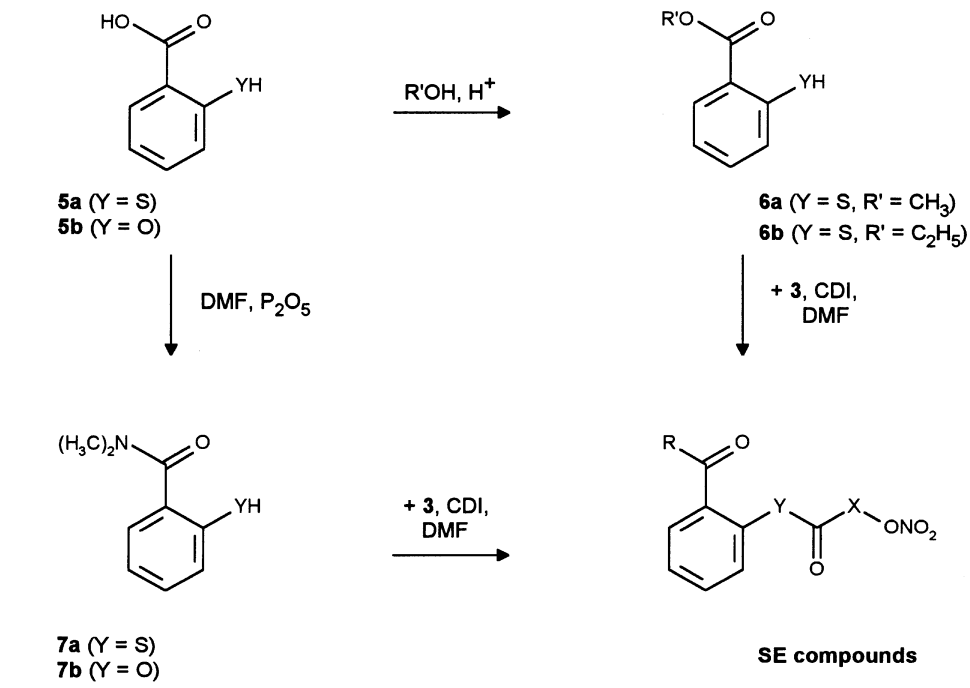

| 1-4 | X | R | Hal | Route | Lit. |
|---|---|---|---|---|---|
| a |  | —$C_2H_5$ | – | A | [7] |
| b |  | —$C_2H_5$ | – | A | [11] |
| c |  | —$CH_3$ | – | A | [7] |
| d | —$CH_2$- | – | Br | B | [12] |
| e | —$CH_2CH_2$- | – | I | B | [12] |
| f |  | – | Br | B | – |
| g | —$(CH_2)_{10}$— | – | Br | B | – |

**Figure 2.** Synthesis of the nitratoacids.



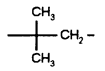
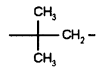
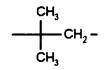
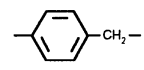
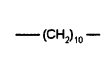
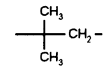
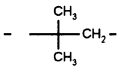

| | SE 145 | SE 152 | SE 158 | SE 175 | SE 85 | SE 136 | SE 157 |
|---|---|---|---|---|---|---|---|
| X |  |  |  |  |  |  |  |
| Y | S | S | S | S | S | O | O |
| R | —OCH3 | —OC2H5 | —N(CH3)2 | —OCH3 | —OC2H5 | —OCH3 | —N(CH3)2 |

**Figure 3.** Synthesis of the target compounds (part 1).

was added and compound **3e** showed neither decomposition nor acylation. Thiosalicylic acid itself, as well as the N-unsubstituted thiosalicylamide, suffered rapid decomposition with all nitratoacids. Obviously there is a competition between reduction of the nitrate by the thiole [2–4] and formation of the thioesters. *Figure 3* gives these target compounds which we have obtained successfully as stable solid compounds.

O-Nitratoacylation of salicylamid did not yield the expected compound **8** but rather a mixture of the N-acylated **10** and the diacylated **SE 161**. A rearrangement producing N-acetyl- from O-acetyl-salicylamid is described in the literature [11]. In order to obtain a completely O-acylated target compound we treated **9** with two equivalents of **3c** and obtained pure **SE 161**. Finally, we nitrooxylated thiophenol (a thiole which is unable to release NO from organic nitrates) and obtained **SE 135** *(figure 4)*.

### *2.1. Stability*

Stability of the target compounds under simulated physiological conditions was investigated. Substances were dissolved in 50 mM phophate-buffer pH =

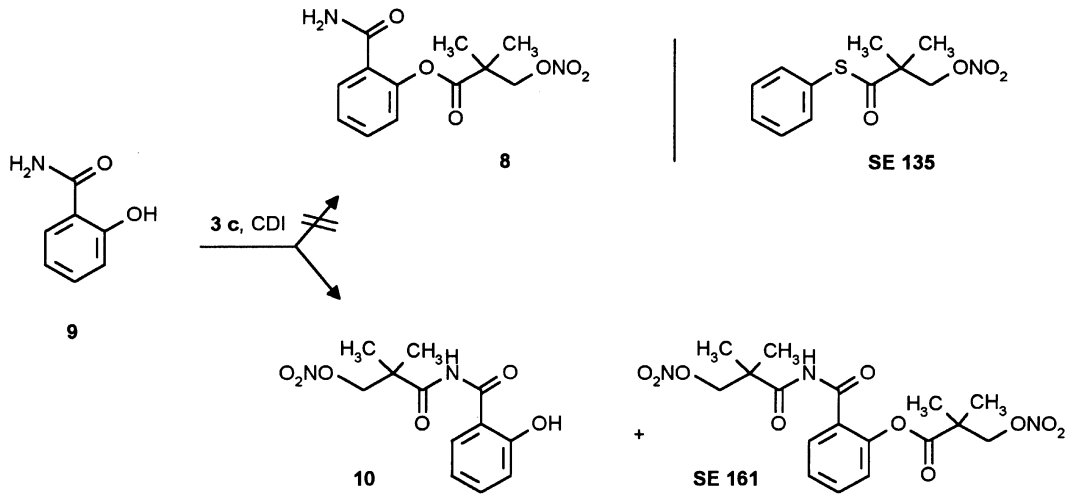



**Figure 4.** Synthesis of the target compounds (part 2).

7/acetonitrile (5:1) and stored at 37 °C for 24 h. Before and after that period, HPLC analysis using an RP-8 column with UV-detector was performed to detect possible instabilities due to the combination of organic nitrates and NO releasing thioles resp. analogues. The diminishment of the starting material at 37 °C/24 h ranged between 0–3.7% with the exception of **SE 145** (16%) and **SE 161** (18%) By means of its lipophilic character, **SE 85** could not be chromatographed on this column. A sufficient thermal stability of this compound was certified by NMR-spectroscopy after 24 h at 37 °C in DMSO.

## 3. Biological results and discussion

### *3.1. Spontaneous liberation of NO*

Liberation of NO was determined electrochemically by a Clark-type NO-sensitive electrode (Iso-NO, World Precision Instruments Inc., Berlin, Germany). Measurements were performed with $10^{-8}$ up to $10^{-4}$ M solutions of the compounds in Krebs-Henseleit buffer (pH 7.4) with constant stirring at 37 °C. No release of NO was found with the **SE**-compounds. This confirms that an in vivo biotransformation of these prodrugs is indispensable.

### *3.2. Guanylyl cyclase activation*

The most active compound, **SE 175,** was chosen to verify that the vasorelaxation observed with the **SE** compounds was mediated by sGC-stimulation. After treatment with 100 mM **SE 175** the cGMP level in a rat aorta segment increased significantly from 2.8 ± 0.6 ($n$ = 10) to 8.8 ± 2.8 ($n$ = 5) pmol cGMP/mg protein ($P$ = 0.012).

### *3.3. Vasorelaxation of isolated vessel segments*

Vasorelaxation after precontraction with phenylephrine was measured using aorta segments of rats. All of the nine investigated nitrates proved to be potent vasodilators. *Figure 1* gives the relaxation curves for the three most potent, and the activities ($EC_{50}$ values) for all compounds in decreasing order. The benzylnitrate derivative showed to be the most active compound. The ‘carrier’ of the nitrato group (thiosalicylic acid ester, thiosalicylic amide, thiophenol, salicylic acid ester or salicylic amide) does not seem to have any influence on the biological activity in this in vitro assay. In vivo investigations, like decreasing blood pressure under repeated treatment, will be necessary to evaluate this influence *(figure 5).*

## 4. Experimental protocols

### *4.1. Vasorelaxation of isolated vessel segments*

Method: after cervical translocation, the aorta of male Wistar rats (250–300 g) was dissected free and rapidly immersed in cold oxygenated Krebs-Henseleit solution (pH 7.4). Four ring segments (5 mm) of the thoracic aorta were suspended in individual organ chambers (10 mL)

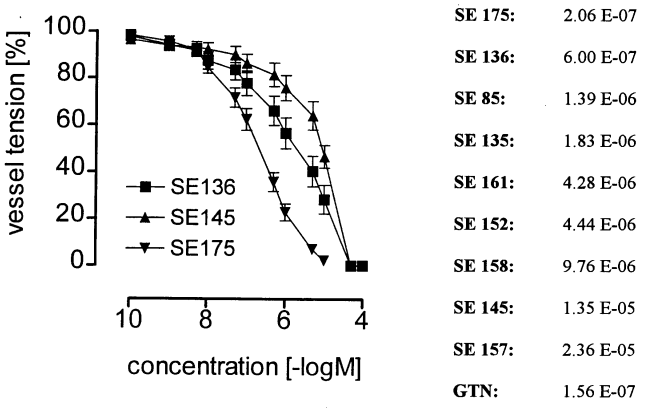



**Figure 5.** Relaxation-dose curves ($n$ = 9–10) and halfmaximal vasorelaxing activity ($EC_{50}$ values, given in mol/L, $n$ = 2–10) of nitrooxyacylated thiosalicylates and salicylates in thoracic aorta vessels of rats.

filled with Krebs buffer. The solution was aerated continuously with 95% $O_2$ and 5% $CO_2$ maintained at 37 °C. A linear force transducer (Statham force displacement transducer) recorded the actual tension of the aortic rings. After equilibration at a resting tension of 4 g (1 h) contractile function of segments was tested by application of KCl (60 mM). The vasorelaxing activity was studied by cumulative application of the compounds after precontraction with a single dose of phenylephrine (1 μM).

*4.2. Activity of soluble guanylyl cyclase*

Activity of soluble guanylyl cyclase was measured as reported earlier [12] by formation of [$^{32}$P]-cGMP from [α-$^{32}$P]-GTP.

*4.3. Chemistry*

Melting points were determined in open capillary tubes on a digital Gallenkamp melting point apparatus and are not corrected. The IR spectra were recorded on a Perkin-Elmer 1420 using KBr pellets for solids and NaCl plates for liquid substances. The $^1$H-NMR spectra were obtained on a Bruker WM 200 (200 MHz) spectrometer using DMSO-$d_6$ as the solvent. Chemical shifts are reported in δ = ppm relative to tetramethylsilane as the internal standard. Elemental analyses were carried out with a Heräus CHN-O-Rapid or an Elementar Vario EL and were within ± 0.4% of the calculated values.

*4.3.1. Methyl thiosalicylate* (**6a**)

A mixture of 20.0 g (0.13 mol) thiosalicylic acid, 5.0 g 4-toluenesulfonic acid, 30 mL methanol and 500 mL toluene was refluxed under argon for 48 h, washed with water, saturated $NaHCO_3$ solution, and water again (100 mL each), then dried ($Na_2SO_4$) and evaporated. The residue was distilled. 15.9 g (73%) colourless oil; b.p. = 67–69 °C, 0.03 mm Hg ( [13]: 87–93 °C, 0.40 mm Hg); IR (KBr) cm$^{-1}$: 1 700 (C=O); $^1$H-NMR δ 3.83 (s, 3H, $CH_3$), 5.26 (s, 1H, SH), 7.1–7.5 (m, 3H, aromat. H-3,4,5), 7.92 (dd, $J$ = 7.1/2.4 Hz, 1H, aromat. H-6).

*4.3.2. Ethyl thiosalicylate* (**6b**)

As described for **6a**, using 15 mL ethanol. 16.7 g (70%) colourless oil; b.p. = 69–72 °C, 0.04 mm Hg ( [14]: b.p. not given); IR (KBr) cm$^{-1}$: 1 700 (C=O); $^1$H-NMR δ 1.28 (t, $J$ = 8.5 Hz, 3H, $CH_3$), 4.41 (q, $J$ = 8.5 Hz, 2H, $CH_2$), 7.2–7.7 (m, 3H, aromat. H-3,4,5), 8.05 (dd, $J$ = 7.2/2.2 Hz, 1H, aromat. H-6).

The nitrooxycarboxylic acids were prepared as reported (**3a** [7, 15], **3c** [7], **3d** [15], **3e** [15]), with the exception of the following:

*4.3.3. 2-Nitrooxyisobutyric acid* (**3b**)

6.28 mL of fuming $HNO_3$, followed by 14.3 mL acetic acid anhydride were dropped into a stirred solution of 5.0 g (76 mmol) ethyl 2-hydroxyisobutyrate and 0.2 g urea in 500 mL $CH_2Cl_2$, maintaining a temperature < 10 °C. After stirring at room temperature for 24 h, the mixture was poured into 800 mL of icewater. The organic layer was separated, washed with water, saturated $NaHCO_3$ solution, and water again, then evaporated at < 40 °C. Distillation of the oily residue produced 8.9 g (66%) of colourless ethyl 2-nitrooxyisobutyrate (b.p. = 33–34 °C, 0.04 mm Hg). For hydrolysis, 5.0 g (28 mmol) of this ester were dissolved in 250 mL of $CH_3OH$ and added at 10 °C to a solution of 3.17 g (56 mmol) KOH in 20 mL of water. After stirring at room temperature for ≅ 2 h (TLC control) the mixture was acidified with concentrated hydrochloric acid and the solvent was evaporated. 100 mL water were added and the mixture was extracted with $CH_2Cl_2$ (2 × 200 mL). The organic layer was dried ($Na_2SO_4$), evaporated, and the residue crystallized from n-hexane. 2.80 g (67%) colourless crystals; m.p. = 76 °C ( [16]: 78–79 °C); IR (KBr) cm$^{-1}$: 1 715 (C=O), 1 630 and 1 290 (N=O); $^1$H-NMR δ 1.59 (s, 6H, 2 × $CH_3$); 13.50 (s, 1H, COOH). Anal. $C_4H_7NO_5$ (C, H, N).

*4.3.4. 11-Nitrooxyundecanoic acid* (**3g**)

5.0 g (18.85 mmol) 1l-bromoundecanoic acid in 30 mL of dry acetonitrile were added to 3.52 g (20.72 mmol) of $AgNO_3$ dissolved in 30 mL acetonitrile and stirred for 3 h at 60 °C. The filtrate of the reaction mixture was poured into 250 mL of icewater and the precipitated product was separated, washed with water and dried. 3.38 g (73%) white powder; m.p. = 40–41 °C; IR (KBr) cm$^{-1}$: 1 700

(C=O), 1 620 and 1 280 (N=O); $^1$H-NMR δ 1.20–1.80 (m, 16H, 8 × $CH_2$); 2.20 (t, *J* = 7.8 Hz, 2H, CO-$CH_2$); 4.50 (t, *J* = 7.8 Hz, 2H, $CH_2$-$ONO_2$); 12.00 (s, 1H, COOH). Anal. $C_{11}H_{21}NO_5$ (C, H, N).

*4.3.5. 4-Nitrooxymethylbenzoic acid* (**3f**)

5.38 g 4-bromomethylbenzoic acid in 30 mL of dry acetonitrile were added to 4.95 g (29.0 mmol) silver nitrate in acetonitrile and stirred overnight. The filtrate of the mixture was poured into 500 mL of icewater. The precipitated crude product was separated, dried in vacuo and recrystallized from diisopropylether. 4.12 g (84%) white powder; m.p. = 165 °C; IR (KBr) cm$^{-1}$: 1 690 (C=O), 1 670 and 1 270 (N=O); $^1$H-NMR δ 5.65 (s, 2H, $CH_2$-$ONO_2$); 7.58 (d, *J* = 8.1 Hz, 2H, aromat. H-3,5); 7.98 (d, *J* = 8.1 Hz, 2H, aromat. H-2,4). Anal. $C_8H_7NO_5$ (C, H, N).

*4.3.6. Ethyl S-(11-nitrooxyundecanoyl)-thiosalicylate* (**SE 85**)

3.0 g (12.1 mmol) of 11-nitrooxyundecanoic acid (**3g**) and 2.21 g (12.1 mmol) ethyl thiosalicylate (**6b**) were dissolved in 30 mL of dry $CH_2Cl_2$. 2.73 g (13.23 mmol) DCC, dissolved in 20 mL $CH_2Cl_2$, were added with protection by argon and stirred for 24 h. The solid was separated, the filtrate washed with 0.1 N hydrochloric acid (3 × 30 mL) and dried ($Na_2SO_4$). After evaporation, the crude product was chromatographed on a silica gel column with ethylacetate. 0.86 g (17%) yellow paste; IR (NaCl) cm$^{-1}$: 1 710 (C=O), 1 630 and 1 725 (N=O); $^1$H-NMR δ 1.25–1.75 (m, 19H, 8 × $CH_2$, $CH_2$-$CH_3$), 2.65 (t, *J* = 7.2 Hz, 2H, CO-$CH_2$-$(CH_2)_8$), 4.25 (q, *J* = 7.1 Hz, 2H, $CH_2$-$CH_3$), 4.50 (t, *J* = 6.6 Hz, 2H, -$CH_2$-$ONO_2$), 7.50–7.70 (m, 3H, aromat. H-3,4,5); 8.82 (dd, *J* = 6.8/2.2 Hz, 1H, aromat. H-6). Anal. $C_{20}H_{29}NO_6S$ (C, H, N).

*4.3.7. S-(3-Nitrooxypivaloyl)-thiophenole* (**SE 135**)

1.63 g (10.0 mmol) of **3c** in 50 mL of dry DMF were cooled to –10 °C and 1.78 g (11.0 mmol) of CDI were added. After stirring (argon) for 2 h, 1.10 g (10 mmol) of thiophenol were added and the reaction mixture was stirred for another 2 h at –10 °C. 50 mL ethylacetate were added, the mixture washed with saturated NaCl solution (3 × 30 mL), dried over $Na_2SO_4$ and evaporated. The residue was chromatographed on a silica gel column with petrolether/acetone (7:1). 0.7 g (28%) colourless oil; IR (NaCl) cm$^{-1}$: 1 690 (C=O), 1 640 and 1 275 (N=O); $^1$H-NMR δ 1.35 (s, 6H, C-$CH_3$), 4.68 (s, 2H, $CH_2$-$ONO_2$), 7.34–7.52 (m, 5H, aromat. H). Anal. $C_{11}H_{13}NO_4S$(C, H, N).

*4.3.8. Methyl O-(3-nitrooxypivaloyl)-salicylate* (**SE 136**)

1.52 g (10.0 mmol) methyl salicylate were treated with 1.63 g (10.0 mmol) **3c** and 1.78 g (11.0 mmol) of CDI as described for **SE 135**. The crude product was chromatographed on a silica gel column with petroether/ethylacetate (5:1). 2.37 g (80%) white powder; m.p. = 47–50 °C; IR (KBr) cm$^{-1}$: 1 750 (C=O), 1 730 (C=O); 1 625 and 1 280 (N=O); $^1$H-NMR δ 1.39 (s, 6H, C-$CH_3$), 3.80 (s, 3H, O-$CH_3$), 4.75 (s, 2H, $CH_2$-$ONO_2$), 7.20 (dd, *J* = 8.1/1.0 Hz, 1H, aromat. H-3), 7.42 (ddd, *J* = 8.1/1.0 Hz, 1H, aromat. H-5), 7.68 (ddd, *J* = 8.1/1.5 Hz, 1H, aromat. H-4), 7.93 (dd, *J* = 8.1/1.5 Hz, 1H, aromat. H-6). Anal. $C_{13}H_{15}NO_7$ (C, H, N).

*4.3.9. Methyl S-(3-nitrooxypivaloyl)-thiosalicylate* (**SE 145**)

2.58 g (15.3 mmol) **5a** were treated with 2.50 g (15.3 mmol) **3c** and 2.73 g (16.86 mmol) CDI as described for **SE 135**. The crude product was chromatographed on a silica gel column with petrolether/ethylacetate (5:1). 3.19 g (67%) white crystals; m.p. = 38–39 °C (crystallized from petrolether in the cold); IR (KBr) cm$^{-1}$: 1 730 (C=O), 1 690 (C=O), 1 630 and 1 275 (N=O); $^1$H-NMR δ 1.35 (s, 6H, C-$CH_3$), 3.79 (s, 3H, O-$CH_3$), 4.66 (s, 2H, $CH_2$-$ONO_2$), 7.50–7.70 (m, 3H, aromat. H-3,4,5), 7.88 (dd, *J* = 7.6/2.0 Hz, 1H, aromat. H-6). Anal. $C_{13}H_{15}NO_6S$ (C, H, N).

*4.3.10. Ethyl S-(3-nitrooxypivaloyl)-thiosalicylate* (**SE 152**)

Synthesized from 2.0 g (10.81 mmol) ethyl thiosalicylate (**6b**), 1.76 g (10.81 mmol) **3c** and 1.93 g (11.89 mmol) CDI as described for **SE 135**. The crude product was chromatographed on a silica gel column with petroleum ether/ethylacetate (4:1). 2.42 g (68%) yellowish oil; IR (NaCl) cm$^{-1}$: 1 720 (C=O), 1 700 (C=O), 1 635 and 1 280 (N=O); $^1$H-NMR δ 1.28 (t, *J* = 7.1 Hz, 2H, $CH_2$-$CH_3$), 1.35 (s, 6H, C-$CH_3$), 4.26 (q, *J* = 7.1 Hz, 2H, $CH_2$-$CH_3$), 4.65 (s, 2H, $CH_2$-$ONO_2$), 7.50–7.65 (m, 3H, aromat. H-3,4,5), 7.87 (dd, *J* = 7.1/2.0 Hz, 1H, aromat. H-6). Anal. $C_{14}H_{17}NO_6S$ (C, H, N).

*4.3.11. O-(3-Nitrooxypivaloyl)-salicylic acid dimethylamide* (**SE 157**)

Synthesized from 1.65 g (10.0 mmol) salicylic acid dimethylamide (**7b**) [17], 1.63 g (10.0 mmol) **3c** and 1.78 g (11.0 mmol) CDI as described for **SE 135**. The crude product was chromatographed on a silica gel column with ethylacetate. 3.0 g (96%) colourless oil; IR (NaCl) cm$^{-1}$: 1 750 (C=O), 1 650 (C=O), 1 640 and 1 280 (N=O); $^1$H-NMR δ 1.32 (s, 6H, C-$CH_3$); 2.76 (s, 3H, N-$CH_3$); 2.94 (s, 3H, N-$CH_3$); 4.68 (s, 2H, $CH_2$-$ONO_2$); 7.19 (d, *J* = 8.1 Hz, 1H, aromat. H-3); 7.33–7.36 (m, 2H,

aromat. H-4,5); 7.48 (m, 1H, aromat. H-6). Anal. $C_{14}H_{18}N_2O_6$ (C, H, N).

*4.3.12. S-(3-Nitrooxypivaloyl)-thiosalicylic acid dimethylamide* (**SE 158**)

Synthesized from 1.81 g (10.0 mmol) **7a** [17], 1.63 g (10.0 mmol) **3c** and 1.78 g (11.0 mmol) CDI as described for **SE 135**. The crude product was chromatographed on a silica gel column with ethylacetate. 1.79 g (55%) thick brown oil; IR (NaCl) $cm^{-1}$: 1 685 (2 × C=O), 1 630 and 1 280 (N=O); $^1$H-NMR δ 1.32 (s, 6H, C-$CH_3$); 2.64 (s, 3H, N-$CH_3$); 2.95 (s, 3H, N-$CH_3$); 4.65 (s, 2H, $CH_2$-$ONO_2$); 7.34–7.60 (m, 4H, aromat. H). Anal. $C_{14}H_{18}N_2O_5S$ (C, H, N).

*4.3.13. N, O-Di-(3-nitrooxypivaloyl)-salicylamide* (**SE 161**)

Synthesized from 1.0 g (7.29 mmol) salicylamide, 2.38 g (14.58 mmol) **3c** and 2.48 g (15.31 mmol) CDI as described for **SE 135**. The crude product was chromatographed on a silica gel column with petroleum ether/acetone (1:1). 1.12 g (36%) white powder; m.p. = 73–75 °C; IR (KBr) $cm^{-1}$: 1 760 (C=O), 1 675 (2 × C=O), 1 630 and 1 275 (N=O); $^1$H-NMR δ 1.27 (s, 6H, C-$CH_3$); 1.33 (s, 6H, C-$CH_3$); 4.67 (s, 2H, $CH_2$-$ONO_2$); 4.68 (s, 2H, $CH_2$-$ONO_2$); 7.21 (d, $J$ = 7.6 Hz, 1H, aromat. H-3); 7.36 (dd, $J$ = 7.6/7.1 Hz, 1H, aromat. H-5); 7.48 (dd, $J$ = 7.1/1.5 Hz, 1H, aromat. H-6); 7.56 (ddd, $J$ = 7.6/7.6/1.5 Hz, 1H, aromat. H-4); 10.93 (s, 1H, NH). Anal. $C_{17}H_{21}N_3O_{10}$ (C, H, N).

*4.3.14. Methyl S-(4-nitrooxymethylbenzoyl)-thiosalicylate* (**SE 175**)

Synthesized from 0.50 g (2.97 mmol) **6a**, 0.55 g (2.97 mmol) **3f** and 0.53 g (3.27 mmol) CDI as described for **SE 135**. The crude product was chromatographed on a silica gel column with petroleum ether/acetone (1:1). 0.53 g (51%) white powder; m.p. = 61–64 °C; IR (KBr) $cm^{-1}$: 1 715 (C=O), 1 660 (C=O), 1 620 and 1 270 (N=O); $^1$H-NMR δ 3.76 (s, 3H, O-$CH_3$); 5.70 (s, 2H, $CH_2$-$ONO_2$); 7.60–7.75 (m, 5H, aromat. H-3,4,5,3′,5′); 7.90–8.08 (m, 3H, aromat. H-6,2′,6′). Anal. $C_{16}H_{13}NO_6S$ (C, H, N).

*4.3.15. Stability*

The HPLC equipment used was a Consta Metric 3200 (LCD-Analytical) pump with a Spherisorb 5 ODS 2 pre- (20 × 4 mm) and main-column (250 × 4 mm), a Spektro Monitor 3200 (LCD-Analytical) UV detector and an HP 4496 Series II (Hewlett Packard) integrator. Eluent: acetonitrile/phosphoric acid 0.1% (50:50); samples: 3 mg substance in 10 mL 50 mM phosphate buffer pH 7.4/ acetonitrile (1:5; flow: 1 mL/min; column temperature: 20–25 °C; detection wavelength: 190 mm. Samples were heated at 37 °C for 24 h in a common dry oven.

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