

Synthesis of Some Novel Organic Nitrates and Comparative in Vitro Study of Their Vasodilator Profile

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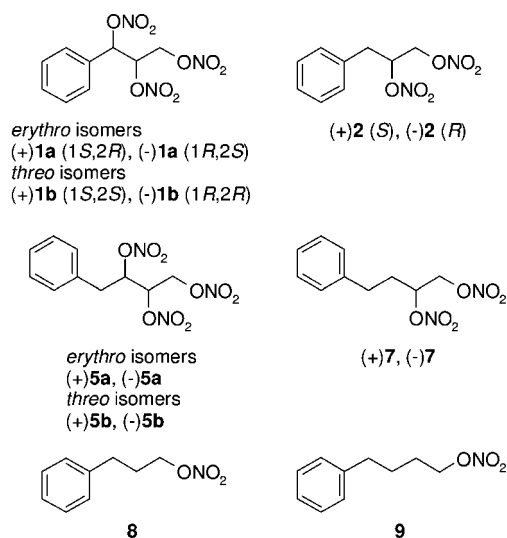
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Synthesis and structural characterization of the 4-phenylbutane-1,2-diyl dinitrate and of the *erythro* and *threo* diastereoisomers of 4-phenylbutane-1,2,3-triyl trinitrate as well as the HPLC chiral separation of the corresponding racemic mixtures are reported. Vasodilator activity of the single enantiomers of these products, of 4-phenylbutyl nitrate, and of the previously described phenylpropyl analogues were assessed on rat aorta strips precontracted with phenylephrine. The compounds were able to relax the contracted tissue in a concentration dependent manner. In the couples of antipodes, a complete lack of enantioselectivity was observed as far as the vasodilator potency is concerned. The concentration response curves of the products, with the exception of those of all the trinitrooxy substituted models, were rightward shifted in the presence of ALDH-2 inhibitors. Mono and dinitrates, but not trinitrates, displayed in vitro cross-tolerance with GTN. This new series of nitric acid esters is an interesting tool that can help to shed light on the unresolved puzzle of nitrate pharmacology. Selected members are worthy of additional study as potential drugs.

A variety of organic nitrates are commonly used in medicine because of their ability to display relaxing effects on vasculature smooth muscle.^{1,2} The generally accepted mechanism of this action involves their conversion in the smooth muscle cell into nitric oxide (NO^o), which is able to activate soluble guanylate cyclase (sGC). As a consequence, an increase in intracellular cGMP concentration occurs, triggering activation of a protein kinase (cGK-1) that phosphorylates Ca²⁺ transporters, causing Ca²⁺ sequestration and, consequently, vasodilation. The exact mechanism whereby NO is generated from organic nitrates is still a topic of dispute. It has been suggested that both nonenzymatic pathways, involving endogenous thiol groups or hemoglobin, and several enzymes play roles in the generation of NO.³ Frequently, repeated or continuous exposure to high doses of nitrates leads to a marked attenuation of many of their effects. This phenomenon, known as *tolerance*, sets a limit to the practical application of these drugs.⁴ The prototype of organic nitrate is glyceryl trinitrate (GTN). It was introduced in therapy for the relief of *angina pectoris* pain in 1876 and it is also the basis of Nobel's potent detonator.⁵ In light of the importance of nitrates in medicine, it is of undoubted interest to make new products available both as potential drugs which may increase the effectiveness of those employed in therapy and for use as probes to examine further the mode of action of drugs of this class. In a previous work,⁶ we described synthesis and configuration assignment of 1-phenylpropane-1,2,3-triyl trinitrate stereomers (+)**1a**, (–)**1a**, (+)**1b**, (–)**1b**, and of 3-phenylpropane-1,2-diyl dinitrate stereomers (+)**2**, (–)**2** (Chart 1). In this paper, we report synthesis and chiral HPLC resolution

Chart 1. Novel Organic Nitrates



of the related butyl derivatives (+)**5a**, (–)**5a**, (+)**5b**, (–)**5b**, (+)**7**, and (–)**7** (Chart 1). The in vitro vasodilating activity of all these products, together with the vasodilator profile of the simple 3-phenylpropyl nitrate (**8**) and 4-phenylbutyl nitrate (**9**) (Chart 1), was evaluated on rat thoracic aorta strips precontracted with phenylephrine and compared with GTN. Finally, the results of a preliminary in vitro study of cross-tolerance with GTN are reported.

Results and Discussion

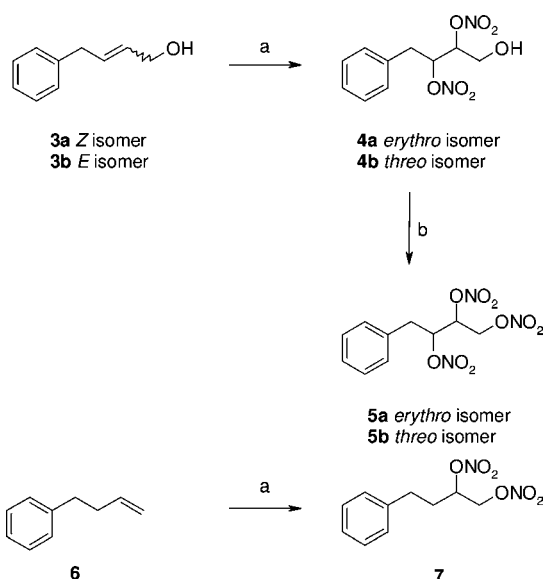
Chemistry. The new target compounds **5**, **7** were synthesized according to the pathway reported in Scheme 1. The racemic mixture **5a** was prepared starting from the *Z*-stereomer of 4-phenylbut-2-en-1-ol (**3a**) containing a small amount of the *E*-stereomer **3b** (less than 2%, HPLC detection). Following an old procedure to prepare nitrates,⁷ the product was treated with

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^o Abbreviations: ALDH-2, aldehyde dehydrogenase 2; GTN, glyceryl trinitrate; NO, nitric oxide; sGC, soluble guanylate cyclase; cGMP, cyclic guanosine monophosphate; cGK-1, cGMP-dependent protein kinase type I; ERP, energy release potential; ODO, 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinolin-1-one.

Scheme 1. Preparation of Organic Nitrates **5** and **7**^a

^a Reagent and conditions: (a) AgNO₃, I₂, CH₃CN, rt, then reflux; (b) AgNO₃, PPh₃, NBS, CH₃CN, -15 °C to rt.

AgNO₃ and I₂ in CH₃CN at room temperature, followed by reflux, to give the intermediate racemic mixture of the dinitroxy substituted alcohol **4a**. This product was only partly purified by column chromatography and then immediately treated with AgNO₃ and triphenylphosphine (PPh₃) in acetonitrile solution, in the presence of *N*-bromosuccinimide (NBS), to afford the expected racemic mixture **5a**. The racemic mixture **5b** was synthesized in a similar manner starting from the *E*-stereomer **3b** through the intermediate formation of **4b**. The *erythro* and *threo* configurations were assigned to **4a** and **4b**, and consequently to **5a** and **5b**, respectively, on the basis of the knowledge that the reaction in a suitable solvent of AgNO₃ and iodine with olefins affords *cis*-dinitrates.⁸ The racemic mixture of the dinitroxy substituted compound **7** was prepared from olefin **6** following the same procedure used to prepare **4** from **3**. Elemental analyses and ¹H- and ¹³C NMR spectra of these products are in keeping with the proposed structures. The racemic mixtures of **5a**, **5b**, and **7** were resolved into the corresponding enantiomers by chiral chromatography using a Chiralcel OD column to a high degree of optical purity (see Experimental Section). The overall energy release potential

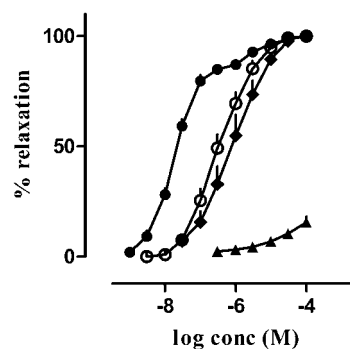


Figure 1. Concentration–response curves of GTN in the absence (●) and in the presence of inhibitors (1 μM benomyl (○), 1 mM chloral hydrate (◆), or 1 μM ODQ (▲)).

(ERP), a parameter that evaluates the explosive hazard of a compound, calculated with the ASTM Program CHEAT AH 8.0, was found to be HIGH for all these products. Consequently, we used only small quantities of these substances and even then treated them with great caution. We never observed violent decomposition induced by thermal or mechanical shock.

Vasodilator Activity. The vasodilator activities of all nitroxyderivatives, focus of this work, as well as of GTN, taken as a reference, were assessed on endothelium denuded rat aorta strips precontracted with 1 μM phenylephrine. All the products were able to dilate the strips in a concentration-dependent manner. Their potencies as vasodilators, expressed as EC₅₀, are collected in Table 1. In our hands, the concentration–response curve for the GTN relaxant effects was biphasic with a distinct region of reduced responsiveness at 1 μM (Figure 1), as already observed by other authors.^{9–11} The EC₅₀ value was found to be 0.029 ± 0.004 μM. As previously discussed, it is commonly accepted that GTN induces vasorelaxation by generating NO in vascular smooth muscle cells, which in turn activates the target enzyme soluble guanylate cyclase (sGC) following interaction with its heme site. Indeed, the vasorelaxing response of GTN is nearly abolished by the presence of 1 μM ODQ (1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one), a well-known heme site inhibitor of sGC.¹² Both enzymatic and nonenzymatic mechanisms have been proposed for NO production from GTN.^{3,4b,c,13} Recently, it was shown that the mitochondrial isoform of aldehyde dehydrogenase (ALDH-2) catalyzes the transformation of GTN into 1,2-dinitrate and nitrite, which in turn is converted to NO through pathways not fully understood.

Table 1. Vasodilating Activity of Compounds **1**, **2**, **5**, **7**, **8**, **9**, and GTN

compd	EC ₅₀ [μM] ^a				EC ₅₀ beno/EC ₅₀	EC ₅₀ CH/EC ₅₀	EC ₅₀ tol/EC ₅₀
	+benomyl ^b	+chloral hydrate ^c	+ODQ ^d	in tolerant tissue ^e			
(+) 1a	0.29 ± 0.09	0.36 ± 0.07		21 ± 2	0.78 ± 0.06	1.2	2.7
(-) 1a	0.21 ± 0.06	0.32 ± 0.08		18 ± 2	0.61 ± 0.10	1.5	2.9
(+) 1b	0.28 ± 0.06	0.32 ± 0.06	0.21 ± 0.11	30 ± 1	1.0 ± 0.2	1.1	3.6
(-) 1b	0.22 ± 0.05	0.35 ± 0.08	0.27 ± 0.02	22 ± 1	0.65 ± 0.05	1.6	3.0
(+) 5a	0.18 ± 0.02	0.23 ± 0.06		21 ± 2	0.48 ± 0.09	1.3	2.7
(-) 5a	0.25 ± 0.05	0.33 ± 0.07		18 ± 2	0.68 ± 0.07	1.3	2.7
(+) 5b	0.11 ± 0.02 ^f	0.14 ± 0.04		10 ± 1	0.22 ± 0.02	1.3	2.0
(-) 5b	0.18 ± 0.02	0.23 ± 0.05		12 ± 1	0.49 ± 0.10	1.3	2.7
(+) 2	0.061 ± 0.010	0.60 ± 0.13	1.2 ± 0.2	>100	6.1 ± 1.0	9.8	100
(-) 2	0.063 ± 0.010	0.63 ± 0.13	1.2 ± 0.3	>100	6.4 ± 1.1	10	102
(+) 7	0.049 ± 0.003	0.16 ± 0.04	0.37 ± 0.09	30 ± 5	2.4 ± 0.7	3.3	49
(-) 7	0.049 ± 0.006	0.12 ± 0.02	0.27 ± 0.08	29 ± 3	1.6 ± 0.3	2.4	33
8	0.16 ± 0.03	1.5 ± 0.5	3.0 ± 0.6	>100	20 ± 4	9.4	125
9	0.15 ± 0.02	0.51 ± 0.11	1.1 ± 0.2	66 ± 4	4.5 ± 0.8	3.4	30
GTN	0.029 ± 0.004	0.42 ± 0.07	0.94 ± 0.30	>100	4.4 ± 1.3	14	152

^a Data expressed as means ± SE, *n* = 3–15. ^b Experiments performed in the presence of 1 μM benomyl. ^c Experiments performed in the presence of 1 mM chloral hydrate (CH). ^d Experiments performed in the presence of 1 μM ODQ. ^e Vessels were preincubated with 0.55 mM GTN for 1 h followed by washing. ^f *P* < 0.05 when compared to EC₅₀ of (-)**5b**; Student's *t* test for unpaired values.

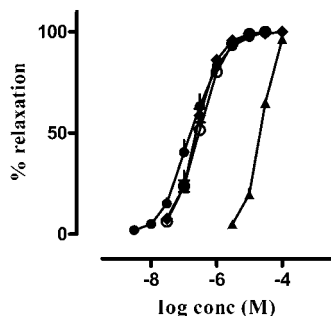


Figure 2. Concentration–response curves of (–)**1b** in the absence (●) and in the presence of inhibitors (1 μ M benomyl (○), 1 mM chloral hydrate (◆), or 1 μ M ODQ (▲)).

It seems that NO can be produced directly by ALDH-2 also.^{4c} Inhibitors of ALDH-2, such as benomyl (methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate) and chloral hydrate, shift the concentration–response curve of GTN to higher concentration.^{4b,c,13} Because in ALDH-2 deficient animals or in the presence of ALDH-2 inhibitors the GTN maximal response is only shifted but not suppressed,¹¹ two independent pathways of GTN bioactivation have been postulated: the high affinity pathway dependent on ALDH-2 activity and the low affinity one dependent on P450 enzyme(s).^{4b,11}

The introduction of a phenyl group into the C-1 of GTN modifies the in vitro vasodilator profile of the drug. In fact, both the *erythro* enantiomers (+)**1a**, (–)**1a** and *threo* enantiomers (+)**1b**, (–)**1b** show a monophasic concentration–response curve. An example of this behavior is reported in Figure 2. In the two pairs of antipodes, a complete lack of enantioselectivity is observed as far as the vasodilating potency is concerned, and the *erythro* enantiomers are as potent as the *threo* ones (Table 1). EC₅₀ values of the products are about 10-fold higher than the GTN value. No shift or a nonsignificant shift of the concentration–response curves occurred when the experiments were repeated in the presence of 1 μ M benomyl and for (+)**1b** and (–)**1b** also in the presence of 1 mM chloral hydrate (see Figure 2 for an example). This suggests that the GTN high affinity pathway (ALDH-2) is not responsible for the bioactivation of these products. A strong rightward shift of the concentration–response curves was observed when the experiments were repeated in the presence of 1 μ M ODQ. Furthermore, the maximal response was maintained and no further changes occurred, even raising ODQ concentration to 100 μ M. This finding suggests that these nitrates, unlike GTN, can elicit vasodilation at high concentration by a pathway independent of the activation of the heme site of sGC. The trinitrooxy substituted stereomers of the butyl series (+)**5a**, (–)**5a**, (+)**5b**, and (–)**5b** behave very similarly to those of the propyl series (see Table 1). The principal difference is only a slightly higher potency of the *threo* stereomer (+)**5b** compared with all the other trinitrooxy substituted products. 3-Phenylpropane-1,2-diyl dinitrate stereomers (+)**2**, (–)**2** behave in a different manner. They show the same biphasic concentration–response curves as GTN (Figure 3). The two enantiomers display the same potency, and they are about 2-fold less potent than GTN and 4-fold more potent than the phenyl trinitrooxy stereomers (Table 1). Also, in the presence of the inhibitors used in our experiments, they display behavior reminiscent of GTN. Indeed, in tissues preincubated with ODQ, the vasodilator response is largely suppressed while in the presence of benomyl the concentration–response curve is shifted rightwards about 10-fold. When chloral hydrate was used to inhibit ALDH-2, this

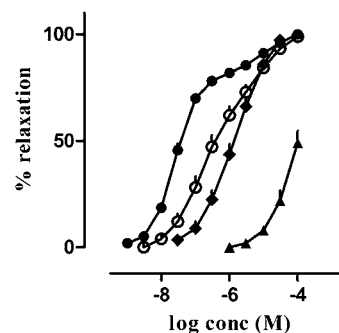


Figure 3. Concentration–response curves of (+)**2** in the absence (●) and in the presence of inhibitors (1 μ M benomyl (○), 1 mM chloral hydrate (◆), or 1 μ M ODQ (▲)).

shift about doubled. The related butyl derivatives (+)**7** and (–)**7** show a vasodilator profile near that of propyl analogues. They are slightly more potent, but the principal difference is a less pronounced shift of the concentration–response curves under the action of benomyl, chloral hydrate, and ODQ. In addition, in the presence of this latter inhibitor, the products are able to trigger almost entirely the maximal relaxation. Finally 3-phenylpropyl and 4-phenylbutyl nitrates (**8**) and (**9**), respectively, are about three times less potent than the related dinitrooxy analogues but they show a similar vasodilator profile. Recently it was suggested, on the basis of a study of a limited number of nitrates, that three or more nitrooxy groups must be present in the vasodilator in order for it to be bioactivated by ALDH-2 because organic nitrates with less than three nitrate groups should not be sufficiently reactive to undergo nucleophilic attack by thiol groups present in the active cleft of enzyme.¹¹ All the data here reported clearly shows that the presence of three nitrooxy groups is a condition neither necessary nor sufficient for the bioactivation of the nitrovasodilator by ALDH-2. Therefore, other molecular descriptors, in addition to the electrophilicity of the –ONO₂ group, must play important roles in making bioactivation by this enzyme possible.

Cross-Tolerance. In vitro cross tolerance with GTN of all the nitrates described in the present work was evaluated by studying their vasodilator activity after incubation of the aortic strips with a high concentration (0.55 mM) of GTN for 1 h followed by washout. The results expressed as EC_{50tol} are reported in Table 1. The two stereomers (+)**2**, (–)**2** and the mononitrooxy substituted compound **8**, whose vasodilator potencies are reduced by about 10-fold and 20-fold in the presence of benomyl and chloral hydrate, respectively, display similar behavior to that of GTN. Indeed, they show high EC_{50tol}/EC₅₀ values, which range from 100 to 125 (see Figure 4 for an example). The related butyl derivatives (+)**7**, (–)**7**, and **9**, which were less sensitive to benomyl and chloral hydrate inhibition, show lower EC_{50tol}/EC₅₀ values that range from 30 to 49. Finally, all trinitrooxy substituted products (+)**1a**, (–)**1a**, (+)**1b**, (–)**1b**, (+)**5a**, (–)**5a**, (+)**5b**, and (–)**5b**, whose potency is not significantly influenced by the presence of benomyl, show a very modest GTN cross-tolerance (see Figure 5 for an example). To conclude, in this study, only the products that exhibit high potencies as vasodilators and whose activity is decreased in the presence of ALDH-2 inhibitors display in vitro cross-tolerance with GTN. These results support the hypothesis that ALDH-2 plays a central role in the development of in vitro tolerance.¹⁴

Conclusion

This work describes the vasodilator profiles of new esters of nitric acid, obtained by introducing at C-1 of GTN a benzyl or

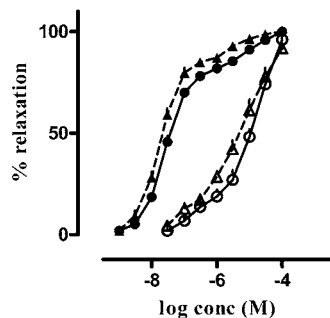


Figure 4. Concentration–response curves of (+)2 (circles) and GTN (triangles) in vessels with or without treatment with 0.55 mM GTN for 1 h followed by washout (open or solid symbols, respectively).

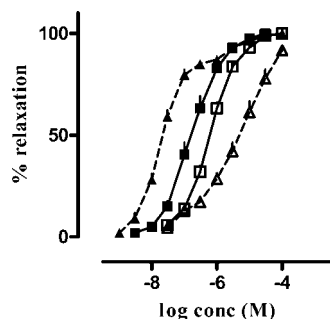


Figure 5. Concentration–response curves of (-)1b (squares) and GTN (triangles) in vessels with or without treatment with 0.55 mM GTN for 1 h followed by washout (open or solid symbols, respectively).

a phenyl group. Further compounds, formally derived from these leads by elimination of one or two nitroxy functions, were studied as well. All trinitroxy substituted compounds are about 4–10 fold less potent than GTN as vasodilators when tested on rat aorta strips precontracted with phenylephrine and their vasodilator profile in the presence of ODQ, benomyl and chloral hydrate is quite different from that of GTN. By contrast, all the related dinitroxy and mononitroxy substituted products display a vasodilator profile similar to that of GTN, but their potencies are 2-fold (dinitroxy series) and 5-fold (mononitroxy series) lower than that of GTN. Unlike the trinitroxy analogues, they display evidence of in vitro cross-tolerance with GTN. These new series of nitrates are interesting tools for shedding new light on the unresolved puzzle of nitrate pharmacology. Selected members of these compounds are worthy of additional study as potential drugs.

Experimental Section

Chemistry. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 instrument, with $\text{Si}(\text{CH}_3)_4$ as an internal standard. The following abbreviations were used to indicate the peak multiplicity: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet. Low resolution mass spectra were recorded with a Finnigan-Mat TSQ-700 instrument. IR spectra were recorded on a Shimadzu FT-IR 8101 M. Flash column chromatography was performed on silica gel (Merck Kieselgel 60, 230–400 mesh ASTM) using the indicated eluents. Petroleum ether 40–70 °C (PE) was used as coeluent. The progress of the reactions was followed by thin layer chromatography (TLC) on 5 cm \times 20 cm plates with a layer thickness of 0.2 mm. Anhydrous magnesium sulfate was used as a drying agent for the organic phases. Organic solvents were removed under vacuum at 35–40 °C. Analysis (C, H, N) of the new compounds dried at 20 °C, pressure < 10 mmHg for 24 h, were performed at the University of Geneva, and the results are within $\pm 0.4\%$ of the theoretical values. Structures **1**,⁶ **2**,⁶ **3a**,¹⁵ **8**,¹⁶ and **9**¹⁷

were synthesized according to published methods. Compound **3b** was obtained by the same procedure used to obtain **3a** starting from *trans*-but-2-en-1,4-diol,¹⁸ and its spectral characteristics correspond to those published.¹⁹

Chiral Separation. Analytical chiral HPLC experiments on Chiralcel OD-H column (250 mm \times 4.6 mm, 5 μm) (Daicel Co., Tokyo) were performed with LaChrom (Merck) screening unit equipped with an L-7100 pump, an L-7200 autosampler, an L-7360 oven that accommodates 12 columns alimeted by a Valco valve, an L-7400 UV detector, and a Jasco OR-1590 polarimeter detector. Analyses were performed at 1 mL/min, at a controlled temperature (25 °C) with UV (254 nm) and polarimetric detection, eluting with hexane/*i*PrOH 7/3 v/v. Retention times R_t in minutes, retention factor $k_i = (R_{t_i} - R_{t_0})/R_{t_0}$ and enantioselectivity factor $\alpha = k_2/k_1$ are given. Semipreparative separations were performed with a Merck-Hitachi LiChrograph model L-6000 HPLC pump and a Merck-Hitachi LiChrograph L-4000 UV detector (254 nm). For semipreparative separations, a Chiralcel OD column (250 mm \times 10 mm, 10 μm) was used, eluting with hexane/*i*PrOH 7/3 v/v at a flow rate of 4.5 mL/min. The solvents were HPLC grade from SDS (Peypin, France) and were filtered on a Millipore membrane of 0.45 μm and degassed before use.

rac-erythro-4-Phenylbutane-1,2,3-triyl Trinitrate (5a). To the solution of **3a** (1.10 g, 7.6 mmol) and AgNO_3 (3.90 g, 23.0 mmol) in CH_3CN (50 mL), I_2 (1.90 g, 7.6 mmol) was added in one portion at rt. The reaction mixture was vigorously stirred until all iodine dissolved, and then it was heated at reflux for 12 h. The obtained mixture was filtered, the precipitate was washed with EtOAc, and the filtrate was diluted with EtOAc (100 mL). The organic phase was washed with H_2O (2×50 mL) and brine, dried and evaporated. The obtained dark-brown oil was partly purified by flash chromatography (eluent PE/EtOAc 9/1 v/v) to give crude **4a** (0.56 g) as a yellow oil. The obtained oil was dissolved in CH_3CN (15 mL), then PPh_3 (0.81 g, 3.1 mmol) and AgNO_3 (0.70 g, 4.1 mmol) were added. The resulting solution was cooled in an ice–salt bath, and NBS (0.55 g, 3.1 mmol) was added in small portions. The cooling bath was removed, and the reaction was vigorously stirred at rt for 2 h. The precipitate was filtered and washed with EtOAc. The filtrate was evaporated and purified by flash chromatography (eluent PE/ CH_2Cl_2 8/2 v/v) to give the title compound as a colorless oil, which solidifies at -24 °C and melts at rt. Yield 15%; mp < rt. IR (film) 3034, 2919, 1645, 1497, 1456, 1431, 1358, 1275, 1009, 899, 839, 750, 702 cm^{-1} . ^1H NMR (CDCl_3) δ : 3.02–3.18 (2H, m, PhCH_2), 4.55 (1H, dd, $^2J_{\text{HH}} = 13.3$ Hz, $^3J_{\text{HH}} = 7.4$ Hz, CHHONO_2), 4.77 (1H, dd, $^2J_{\text{HH}} = 13.3$ Hz, $^3J_{\text{HH}} = 3.1$ Hz, CHHONO_2), 5.36–5.40 (1H, m, CHONO_2), 5.52–5.58 (1H, m, CHONO_2), 7.23–7.39 (5H, m, C_6H_5). ^{13}C NMR (CDCl_3) δ : 35.6, 67.7, 77.3, 80.3, 128.2, 129.2, 129.4, 133.6. MS (EI): 317 (M^+). Anal. ($\text{C}_{10}\text{H}_{11}\text{N}_3\text{O}_9$) C, H, N.

The enantiomers (–)**5a** and (+)**5a** were separated by semipreparative HPLC ((+)**5a**, first eluted); 350 mg of racemic mixture were separated. The reported signs are the signs given by the Jasco OR-1590 polarimeter in the mobile phase used.²⁰ Optical purities ((+)**5a**, 100%; (–)**5a**, 99.99%) were assessed on analytical HPLC; $R_t(+)$ = 10.91, $R_t(-)$ = 17.55, $k(+)$ = 2.54, $k(-)$ = 4.70, α = 1.85, R_s = 6.34.

rac-threo-4-Phenylbutane-1,2,3-triyl Trinitrate (5b). The product was synthesized by the same procedure used to obtain **5a**, starting from **3b**. Colorless oil. Yield 13%. IR (film) 3034, 2921, 1651, 1645, 1497, 1456, 1485, 1358, 1269, 1030, 1001, 839, 750, 702 cm^{-1} . ^1H NMR (CDCl_3) δ : 3.08–3.12 (2H, m, PhCH_2), 4.58 (1H, dd, $^2J_{\text{HH}} = 12.9$ Hz, $^3J_{\text{HH}} = 6.9$ Hz, CHHONO_2), 4.77 (1H, dd, $^2J_{\text{HH}} = 12.9$ Hz, $^3J_{\text{HH}} = 3.9$ Hz, CHHONO_2), 5.37–5.39 (1H, m, CHONO_2), 5.49–5.51 (1H, m, CHONO_2), 7.20–7.36 (5H, m, C_6H_5). ^{13}C NMR (CDCl_3) δ : 35.6, 68.6, 75.9, 79.9, 128.0, 129.0, 129.3, 133.6. MS (EI): 317 (M^+). Anal. ($\text{C}_{10}\text{H}_{11}\text{N}_3\text{O}_9$) C, H, N.

The enantiomers (–)**5b** and (+)**5b** were separated by semipreparative HPLC ((–)**5b**, first eluted); 300 mg of racemic mixture were separated. The reported signs are the signs given by the Jasco OR-1590 polarimeter in the mobile phase used. Optical purities ((–)**5b**, 99.7%; (+)**5b**, 99.8%) were assessed on analytical HPLC;

Rt(-) = 9.96, Rt(+) = 11.92, $k(-)$ = 2.23, $k(+)$ = 2.87, α = 1.28, R_s = 2.38.

rac-4-Phenylbutane-1,2-diyl Dinitrate (7). To the solution of **6** (0.40 mL, 2.7 mmol) and AgNO₃ (1.25 g, 7.3 mmol) in CH₃CN (15 mL), I₂ (0.68 g, 2.7 mmol) was added in one portion. The reaction mixture was vigorously stirred at rt until all iodine dissolved and then it was heated at reflux for 12 h. The obtained mixture was filtered, and the precipitate was washed with EtOAc. The filtrate was diluted with EtOAc (30 mL), and the organic phase was washed with H₂O (2 × 25 mL) and brine, dried and evaporated. The obtained oil was purified by flash chromatography (eluent PE/CH₂Cl₂ 8/2) to give the title compound as a pale-yellow liquid. Yield 57%. IR (film) 3088, 3065, 3030, 2930, 1954, 1659, 1651, 1645, 1634, 1497, 1456, 1435, 1383, 1271, 1180, 1157, 1113, 1045, 1001, 855, 752, 700 cm⁻¹. ¹H NMR (CDCl₃) δ : 1.97–2.18 (2H, m, PhCH₂CH₂), 2.67–2.89 (2H, m, PhCH₂), 4.45 (1H, dd, ²J_{HH} = 12.9 Hz, ³J_{HH} = 6.3 Hz, CHHONO₂), 4.71 (1H, dd, ²J_{HH} = 12.9 Hz, ³J_{HH} = 3.0 Hz, CHHONO₂), 5.20–5.28 (1H, m, CHONO₂), 7.15–7.34 (5H, m, C₆H₅). ¹³C NMR (CDCl₃) δ : 30.8, 31.1, 74.3, 78.4, 126.8, 128.3, 128.9, 139.4. MS (EI): 256 (M)⁺. Anal. (C₁₀H₁₂N₂O₆) C, H, N.

The enantiomers (-)**7** and (+)**7** were separated by semipreparative HPLC ((+)**7**, first eluted); 390 mg of racemic mixture were separated. The reported signs are the signs given by the Jasco OR-1590 polarimeter in the mobile phase used. Optical purities ((+)**7**, 100%; (-)**7**, 100%) were assessed on analytical HPLC; Rt(+) = 9.24, Rt(-) = 13.72, $k(+)$ = 2.00, $k(-)$ = 3.45, α = 1.73, R_s = 4.65.

Vasodilating Activity Assay. Thoracic aortas were isolated from male Wistar rats weighing 175–200 g that had been anaesthetised with CO₂ and killed by decapitation. All animals were treated humanely in accordance with recognized guidelines on experimentation. As few rats as possible were used. The purposes and the protocols of our studies have been approved by the Ministero della Salute, Rome, Italy. The endothelium was removed and the vessels were cut helically: three strips were obtained from each aorta. The tissues were mounted in organ baths containing 30 mL of Krebs-bicarbonate buffer with the following composition (mM): NaCl 111.2, KCl 5.0, CaCl₂ 2.5, MgSO₄ 1.2, KH₂PO₄ 1.0, NaHCO₃ 12.0, glucose 11.1, maintained at 37 °C and gassed with 95% O₂–5% CO₂ (pH = 7.4). The aortic strips were allowed to equilibrate for 120 min and then contracted with 1 μ M phenylephrine. When the response to the agonist reached a plateau, cumulative concentrations of the vasodilating agent were added. Results are expressed as EC₅₀ ± SE (μ M). The effects of 1 or 100 μ M ODQ, 1 μ M benomyl,¹⁴ and 1 mM chloral hydrate^{3a} on relaxation were evaluated in a separate series of experiments in which the selected inhibitor was added 5 min before the contraction. With this protocol, the inhibitor is preincubated for at least 30 min before the addition of the vasodilator compound. In vitro cross-tolerance with GTN was induced using a method described in literature.²¹ After 1 h equilibration, aortic strips were incubated in 0.55 mM GTN. Tissues were then extensively washed for 1 h at which time cumulative concentration–response curves for GTN or compounds under investigation were constructed. Responses were recorded by isometric transducer (1 g resting tension) connected to the MacLab System PowerLab (ADInstruments Ltd., Oxfordshire, U.K.). Compounds (+)**2**, (-)**2**, (+)**5a**, (-)**5a**, (+)**5b**, (-)**5b**, (+)**7**, (-)**7**, **8**, and **9** were dissolved in DMSO. The solution of compounds (+)**1a**, (-)**1a**, (+)**1b**, and (-)**1b** obtained after chiral separation⁶ were concentrated without completely removing the solvent to reduce the hazard of explosion and used after determination of their concentrations (HPLC) for the vasodilator experiments. Addition of the drug vehicle had no appreciable effect on contraction level.

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Supporting Information Available: Elemental analyses. IR spectra of **5a**, **5b**, and **7**. Concentration–response curves of all the products. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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