



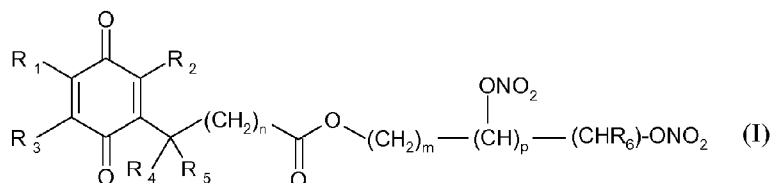
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(54) **Title:** QUINONE BASED NITRIC OXIDE DONATING COMPOUNDS

(57) **Abstract:** The present invention relates to nitric oxide donor compounds having a quinone based structure, to processes for their preparation and to their use in the treatment of pathological conditions where a deficit of NO plays an important role in their pathogenesis.

QUINONE BASED NITRIC OXIDE DONATING COMPOUNDS

The present invention relates to nitric oxide donor compounds, to processes for their preparation and to their use in the treatment of vascular diseases and in particular in the treatment of pathological conditions where a deficit of NO function plays an important role in their pathogenesis.

5 It is known that NO plays multiple physiological roles in regulating numerous and diverse organ functions, defects in the NO pathway lead to the development of many different pathological conditions. These disorders include hypertension, atherosclerosis, coronary artery diseases, cardiac failure, pulmonary hypertension, stroke, impotence, vascular complications in diabetes mellitus, gastrointestinal ulcers, asthma, and other
10 central and peripheral nervous system disorders.

Organic nitrates (esters of nitric acid) are proven medicinal substances for the treatment of dysfunctions of the circulatory system preferably cardiovascular and coronary dysfunctions. They display their effect both by relieving the heart via a reduction in the preload and after load and by improving the oxygen supply to the heart
15 via coronary dilatation.

However, it has been found that the classical organic nitrates used in therapy, such as glycerol trinitrate, isosorbide dinitrate or isosorbide 5-mononitrate, display, on continuous intake of high doses and within a short time, a distinct attenuation of the effect, the so-called nitrate tolerance or tachyphylaxis.

20 Nitrate tolerance develops despite an elevation in the drug plasma concentration reflecting a decrease in vascular sensitivity to previously therapeutic levels. This can be prevented or reduced by inclusion of a nitrate free period in the dosing schedule

Nitrate-tolerant individuals are more susceptible to enhanced vasoconstriction whenever the plasma nitrate concentration is allowed to fall, the so-called rebound effect.
25 This is reflected by increased sensitivity to a number of circulating vasoconstrictor substances such as catecholamines and angiotensin II. Clinically the rebound effect may

be more important than is currently recognized. Evidence suggests that even intermittent nitrate patch therapy results in increased vasoconstrictor sensitivity during the patch-off period. [Munzel T, Mollnau H, Hartmann M, et al. Effects of a nitrate-free interval on tolerance, vasoconstrictor sensitivity and vascular superoxide production. J Am Coll
5 Cardiol. 2000; 36:628-634].

Organic nitrates also cause unpleasant important side effects that include headache, hypotension, flush and nausea. Headache is the most prominent side effect and is caused by cerebral vasodilatation.

The nitrate tolerance and the other side effects have restricted the clinical use and
10 effectiveness of nitrates.

Therefore nitric oxide donor compounds which can produce extended release of NO and do not give rise to any nitrate tolerance are needed.

It is known that one way of reducing the tolerance of the nitrated organic compounds consists of introducing a thiol group in the molecule, for example by use of
15 sulphur containing amino acids. Accordingly EP 0 362 575 and EP 0 451 760 claim compounds which contain sulphydryl groups and prevent nitrate tolerance or diminish a nitrate tolerance which has already occurred.

Patent application WO-A-92/04337 discloses organic nitrated derivatives of the thiazolidine ring with vasodilating activity and a reduced tolerance.

20 US Patent 5,591,758 describes an enormous amount of different nitrated organic vasodilating compounds, with reduced tolerance, of highly variable structures.

Patent EP 1 120 419 discloses isosorbide mononitrates wherein the free hydroxyl group is esterified with either carboxylic acids or with thioacids wherein said ester groups are in trans position with respect to the nitrate group.

25 UK patent application no. GB 2 349 385 A discloses antioxidant nitrate or nitrite ester for use as vasodilator agents in the treatment of pathological conditions associated with endothelial dysfunction, in particular heart diseases.

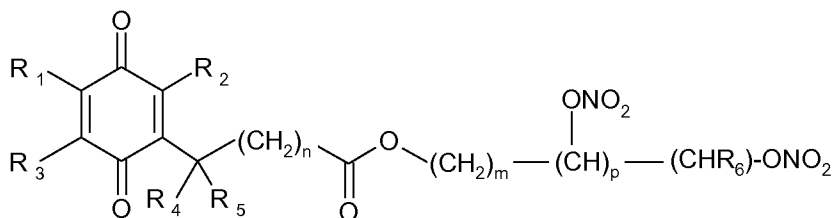
The disclosed compounds contain a superoxide scavenger moiety and a nitrate or

nitrite group and the two parts are stably linked in order to reduce the degradation of the molecules under physiological conditions. The stable link increases the activity of the anti-oxidant scavenger that can avert reactive oxygen species-mediated NO consumption of further production of deleterious species.

Published studies disclose that concomitant treatment with antioxidants preserves the sensitivity of the vasculature to organic nitrates in different experimental models. However the use of antioxidants in clinical practice is limited by the fact that oral administration of antioxidants leads to low bioavailability of the antioxidants and consequently lack of efficacy.

The present invention provides new nitric oxide donor compounds having a better pharmacological activity in term of less tolerance associated with the use of organic nitrates and a longer duration of action than that of nitric oxide donors described in the art.

The present invention relates to compounds of formula (I)



(I)

or stereoisomers thereof, wherein:

R₁ is methyl;

R₂ is H or methyl;

R₃ is methyl;

R₄ and R₅ are methyl and n is 1, or

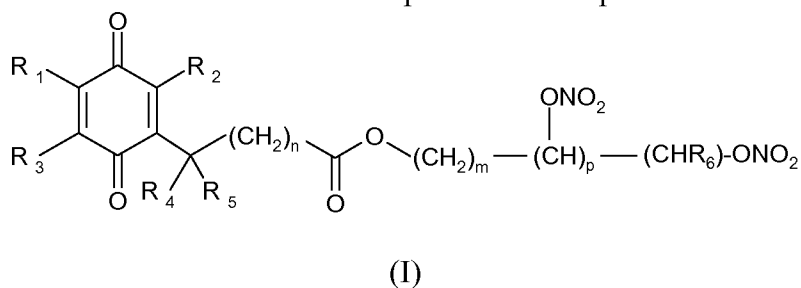
R₄ is H, R₅ is selected from phenyl, para-fluorophenyl, para-methoxyphenyl, para-isopropylphenyl, para-trifluoromethylphenyl and para-methylphenyl and n is 2;

m is an integer from 1 to 10, preferably m is an integer from 1 to 6;

p is 0 or 1;

R_6 is H or methyl.

Another embodiment of the invention provides a compound of formula (I)



5 or stereoisomers thereof, wherein:

R_1 , R_2 and R_3 are methyl;

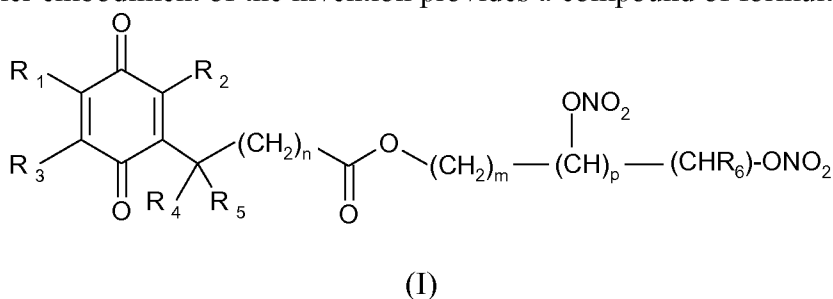
R_4 and R_5 are methyl and n is 1;

m is an integer from 1 to 10, preferably 1 to 6;

p is 0 or 1;

10 R_6 is H or methyl.

Another embodiment of the invention provides a compound of formula (I)



or stereoisomers thereof, wherein:

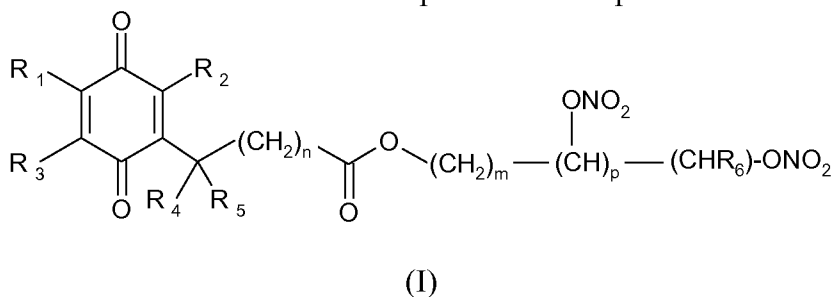
15 R_1 , R_2 and R_3 are methyl;

R_4 and R_5 are methyl and n is 1;

m is an integer from 1 to 10, preferably 1 to 6;

p is 0 and R_6 is H.

Another embodiment of the invention provides a compound of formula (I)



or stereoisomers thereof, wherein:

R_1, R_2, R_3 , are methyl;

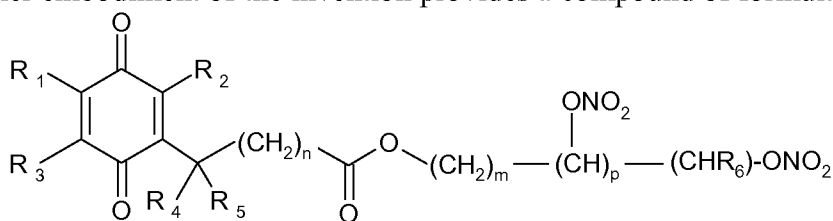
R_4 is H, R_5 is selected from phenyl, para-fluorophenyl, para-methoxyphenyl, para-isopropylphenyl, para-trifluoromethylphenyl and para-methylphenyl and n is 2;

5 m is an integer from 1 to 10, preferably 1 to 6;

p is 0 or 1;

R_6 is H or methyl.

Another embodiment of the invention provides a compound of formula (I)



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(I)

or stereoisomers thereof, wherein:

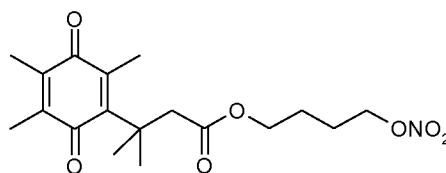
R_1, R_2, R_3 , are methyl;

R_4 is H, R_5 is selected from phenyl, para-fluorophenyl, para-methoxyphenyl, para-isopropylphenyl, para-trifluoromethylphenyl and para-methylphenyl and n is 2;

15 m is an integer from 1 to 10, preferably 1 to 6;

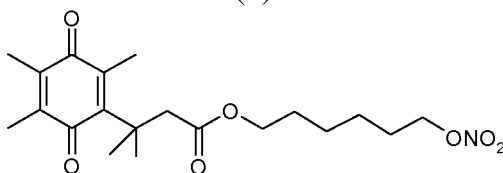
p is 0 and R_6 is H.

Another embodiment of the invention provides a compound of formula (I) selected from the group:



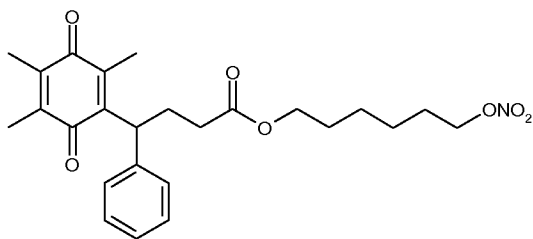
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(1)

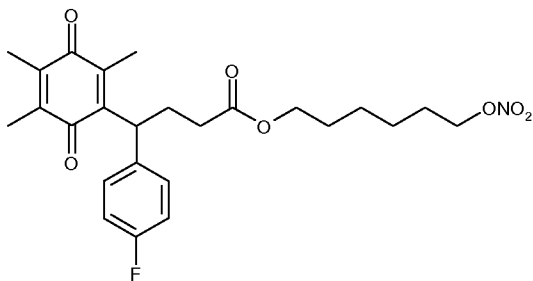


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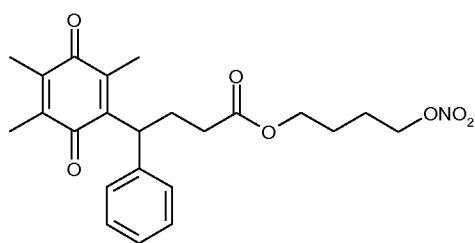


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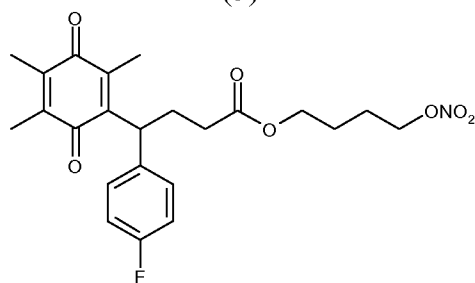


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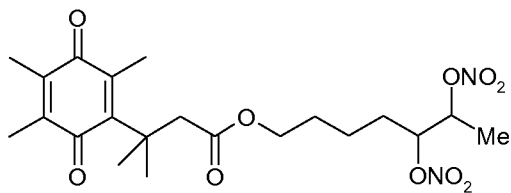


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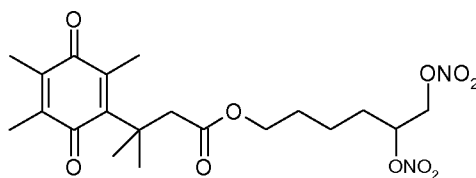
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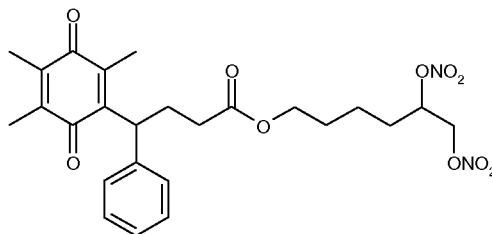


(7)

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(8)



(9)

5

and stereoisomers thereof.

After oral administration, the compounds of formula (I) increase the nitric oxide bioavailability and they are able to directly release NO and stimulate guanylate cyclase in the skeletal muscle.

10 The compounds of formula (I) have an antioxidant activity comparable to the antioxidant activity of well known antioxidant compounds like ferulic and caffeic acid or edaravone.

These two properties of the compounds of the invention, which are the NO-donation and the antioxidant activity, make them useful drugs for the prevention and/or treatment of pathology in which not only a deficit of NO but also oxidative stress play an important role in their pathogenesis such as: pulmonary hypertension, the treatment and/or prevention of dysfunctions of the circulatory system involving vasculopathies preferably pulmonary arterial hypertension, Sickle cell disease, systemic sclerosis, scleroderma, muscular dystrophies such as Duchenne's muscular dystrophy and 15 Becker's muscular dystrophy, cardiac allograft vasculopathy, pathological conditions plays an important role in their pathogenesis, and/or tissue damage due to ischemia and/or 20 due to ischemia-reperfusion.

The compounds of formula (I) can be used in combination with at least a

therapeutic agent selected from non steroidal anti-inflammatory drugs, steroidal anti-inflammatory drugs, endothelin receptor antagonists, hydroxyurea.

The pharmaceutical compositions may be administered by different routes. For example, they may be administered orally in form of pharmaceutically preparations such as tablets, capsules, syrups and suspensions, parenterally in form of solutions or emulsions, etc. They may also be administered topically in form of creams, pomades, balsams, and transdermically for example through the use of patches or bandages. The preparations may comprise physiologically acceptable carriers, excipients, activators, chelating agents, stabilizers, etc. In case of injections there may be incorporated physiologically acceptable buffers, solubilizing agents or isotonic.

The pharmaceutical compositions according to the present invention may further comprise a non steroidal anti-inflammatory drug (NSAID), a steroid drug, a thrombolytic agent such as plasminogen activator urokinase, streptokinase, alteplase or anistreplase.

Alternately, the pharmaceutical compositions according to the invention may further comprise a hypolipidemic agent preferably simvastatin, lovastatin, atorvastatin, pravastatin, fluvastatin, eptastatin, lifibrol, acifran, acitemate, glunicate or rosuvastatin.

The daily dose may be varied depending on the specific symptoms, the age, the body weight of the patients, the specific mode of administration, etc., and a daily normal dose for an adult person could be between 0.1 to 1000 mg, and could be administered as one dose only or divided into several doses during the day.

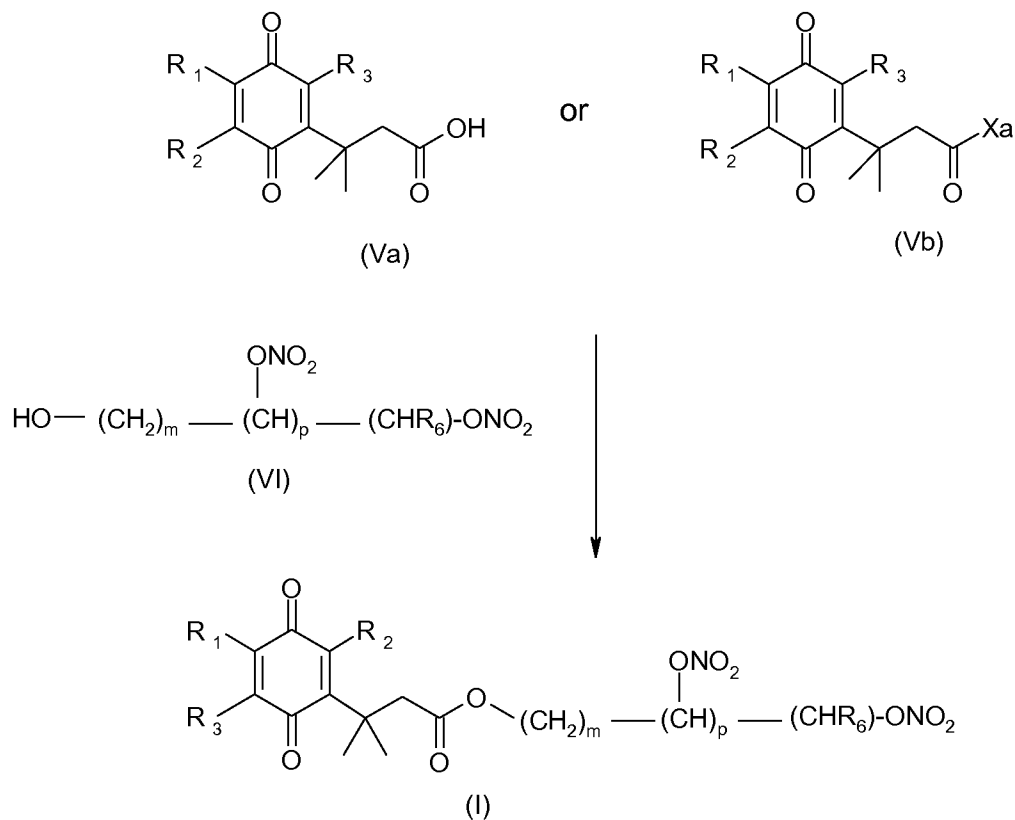
General synthesis

The compounds of formula (I) wherein R_1 , R_2 , R_3 , m and p are as above defined, R_4 and R_5 are methyl n is 1 can be prepared:

by reacting compounds (Va) with compounds of formula (VI) in presence of coupling reagents such as DCC, EDC, HBTU, HATU, and of catalytic amount of $Sc(OTf)_3$ as depicted in scheme 1; or

by reacting compounds of formula (Vb), wherein X_a is an activating group selected from N_3 , F, Cl, Br, preferably Cl, with compounds (VI) in presence of a base such as

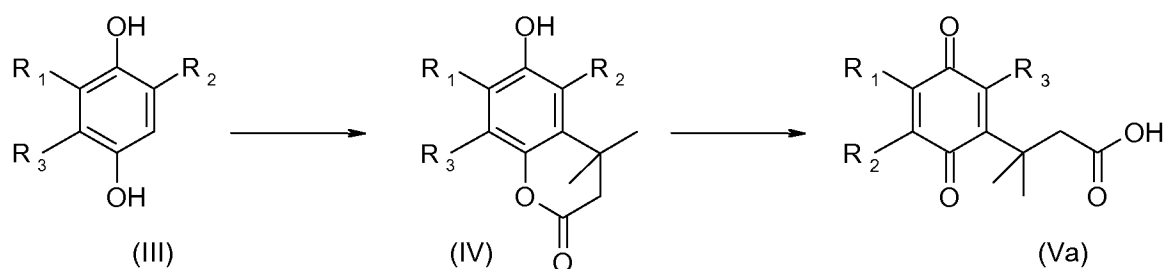
pyridine or triethylamine or K_2CO_3 , Cs_2CO_3 in an aprotic/non polar solvent such as THF, DMF or CH_2Cl_2 or at temperature ranging from $-80^\circ C$ to $60^\circ C$ for (Vb) as depicted in Scheme 1:



5

Scheme 1

Compounds of formula (Vb) can be prepared by known method from the corresponding compounds of formula (Va). Compounds of formula (Va) can be prepared as depicted in Scheme 2:



10

Scheme 2

Hydroquinones of general formula (III) are reacted with methanesulfonic acid and methyl 3-methylbut-2-enoate to obtain the lactones (IV) as described in literature by

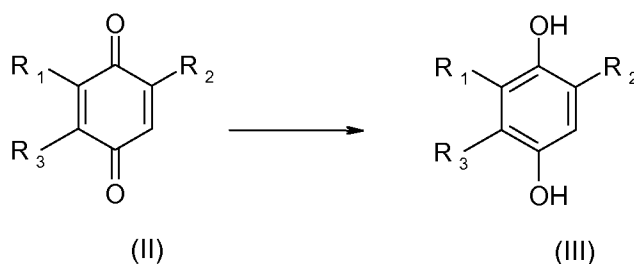
Carpino et al., *J. Org. Chem.*, 1989, 54, 3303-3310.

Compounds of formula (Va) are prepared by reacting compounds (IV) with freshly crystallized NBS according to conditions described by Borchardt et al., *J. Am. Chem. Soc.*, 1972, 94, 9175.

5 Compounds of formula (III) are commercially available or can be prepared by reduction of the corresponding quinones of formula (II), using reducing reagents such as, for example NaBH₄ in methanol (Scheme 3).

Quinones of formula (II) are commercially available or can be prepared by methods described in the literature.

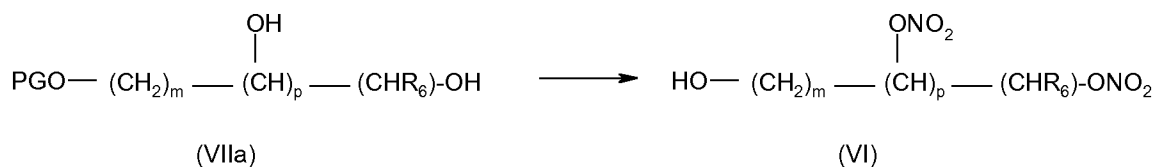
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Scheme 3

Compounds of formula (VI) wherein m, p and R₆ are as above defined can be synthesized starting from the corresponding alcohols of formula (VIIa)

15



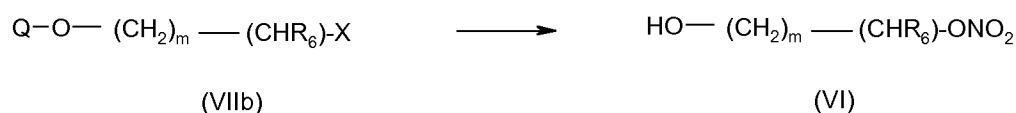
wherein PG is a suitable hydroxyl protective group and m, p and R₆ are as above defined by reaction with nitric acid and acetic anhydride in a temperature range from -50°C to 20°C, or by reacting with triflic anhydride/tetraalkylammonium nitrate salt in an aprotic polar/non polar solvent such as DMF, THF or CH₂Cl₂ at temperature ranging from -80°C to 65°C in the presence of a base as pyridine, lutidine, 2,6-di-tert-butyl-4-methylpyridine followed by the removal of the by known methods (see for example: T.W. Greene, P.G.M. Wuts "Protective groups in organic Synthesis", 4th edition, J. Wiley &

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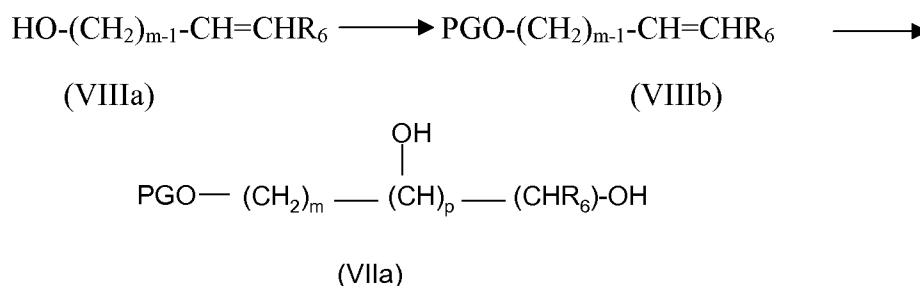
Sons, New York, 2006).

Alternatively, the hydroxyl group is first converted to the corresponding mesyl or tosyl or triflate group and then nitrated using known methods, as for example tetraalkylammonium nitrate and sodium nitrate followed by the removal of the protecting group by methods well known in the art.

Alternatively compounds of formula (VI) wherein m and R₆ are as above defined, p is 0 can be synthesized by reacting the corresponding halogen derivative (VIIb) wherein Q is H or PG wherein PG is as previously defined and X is an halogen atom as Cl, Br, I with a nitrating agent, for example, AgNO₃ in acetonitrile as known in the literature, optionally followed by the removal of the Q protecting group by methods well known in the art.



Compound of formula (VIIa) wherein m is as previously defined, p is 1 and R₆ is H or CH₃ can be synthesized by reacting the corresponding alkenyl-alcohol of formula (VIIIb) with a dihydroxylating reagent such as ADmix α or ADmixβ or KMnO₄, OsO₄ in a 1/1 mixture of protic/aprotic solvents like tBuOH, H₂O, optionally in the presence of an activator like methanesulfonamide at temperature ranging from -20 to 30°C, optionally followed by a chiral separation of the diols (VIIa)



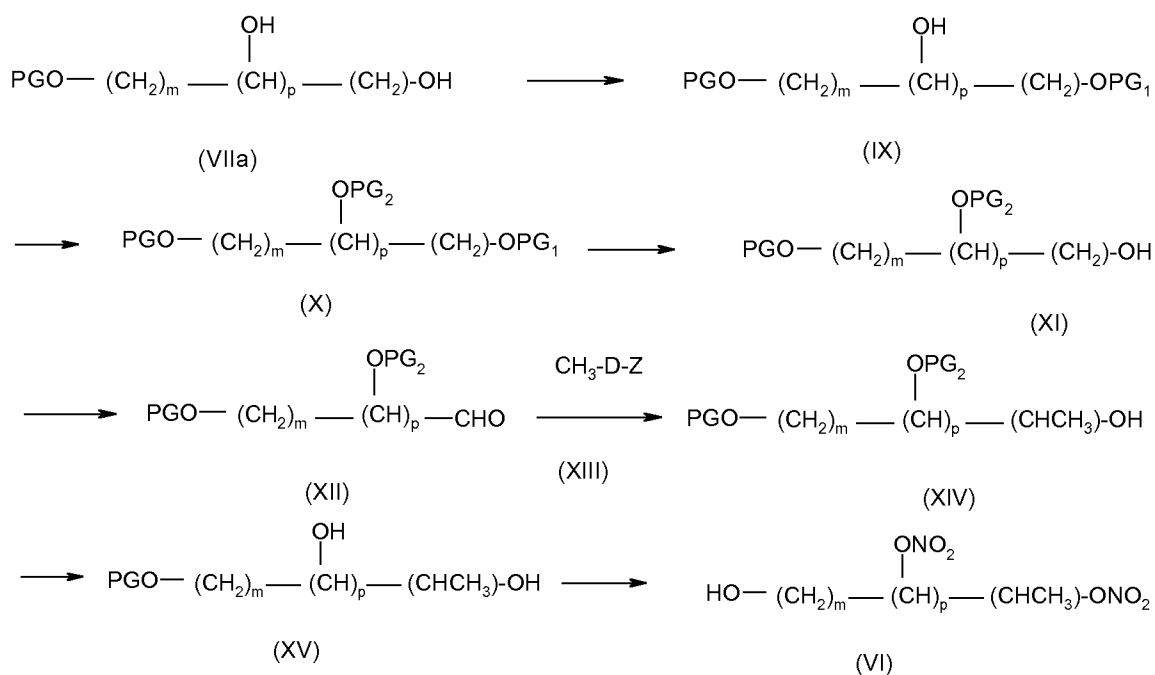
Compounds of formula (VIIIb) are prepared from compounds (VIIIa) by protecting the free hydroxyl group with a suitable PG group already defined with known methods (see for example: T.W. Greene, P. G. M. Wuts "Protective groups in organic Synthesis",

4th edition, J. Wiley & Sons, New York, 2006).

Compounds (VIIIa) are commercially available or can be prepared from known compounds using known methods.

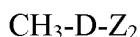
Alternatively compounds (VI) wherein m is as above defined, p is 1 and R₆ is H can be prepared by reacting compounds (VIIIb) with I₂ and AgNO₃ in acetonitrile as described in WO2009/098113.

Alternatively compounds (VI) wherein m is as above defined, p is 1 and R₆ is CH₃ can be obtained from compounds (VIIa) as depicted in Scheme 4, according the following steps:



Scheme 4

- 1) protecting the primary hydroxyl group of compounds (VIIa)
- 2) protecting the secondary hydroxyl group to obtain compounds of formula (X) wherein PG₁ is the trityl group and PG₂ is TBDPS, TBDMS or TIPS.
- 3) removing the protecting group PG₁ and oxidizing compound (XI) to aldehyde with known methods to obtain compounds (XII)
- 4) reacting compound (XII) with compound (XIII)



(XIII)

wherein D is Zn, Mg, or Cu, preferably Zn; Z is R or a halogen, preferably Cl, in the presence of a chiral amino alcohol catalyst such as (1*S*,2*R*)-(-)-(dibutylamino)-1-phenyl-1-propanol or (1*R*,2*S*)-(+)-(dibutylamino)-1-phenyl-1-propanol or an achiral catalyst in an aprotic/ non polar solvent such as toluene, THF or Et₂O at temperature ranging from -80°C to 65°C

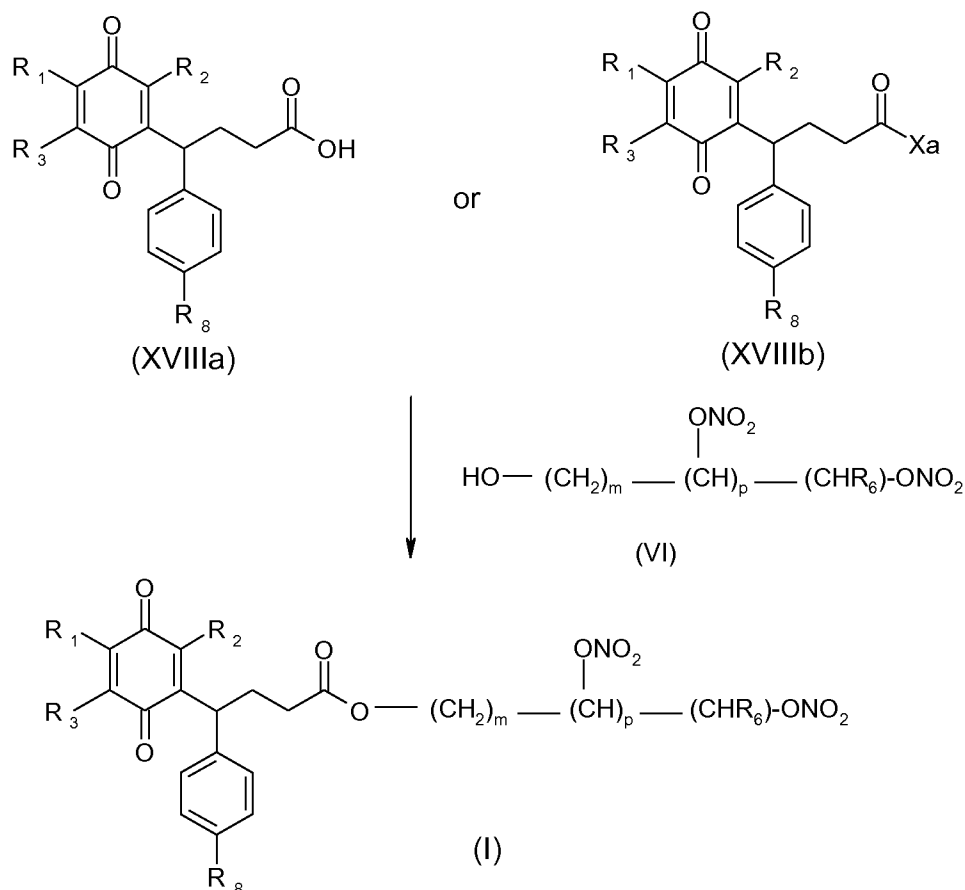
5) removing the protecting group PG₂ by methods well known in the art to obtain compound (VI).

Compounds of formula (I) wherein R₄ is H and R₅ is selected from phenyl, para-fluorophenyl, para-methoxyphenyl, para-isopropylphenyl, para-trifluoromethylphenyl and para-methylphenyl, n is 2 and R₁, R₂, R₃, R₆ m and p are as above defined, can be prepared:

by reacting compounds of formula (XVIIIa) wherein R₁, R₂, R₃ are as above defined, R₈ is H, F, CH₃O-, (CH₃)₂CH-, CF₃- or CH₃-, with compounds of formula (VI) in presence of a coupling reagent such as DCC, EDC, HBTU, HATU, and of catalytic amount of Sc(OTf)₃ or DMAP, as depicted in Scheme 5;

by reacting compounds of formula (XVIIIb) wherein R₁, R₂, R₃, R₈ are as above defined, Xa is an activating group selected from N₃, F, Cl, Br, preferably Cl, with compounds of formula (VI) in presence of a base such as pyridine or triethylamine or K₂CO₃, Cs₂CO₃ in an aprotic/non polar solvent such as THF, DMF or CH₂Cl₂ or at temperature ranging from -80°C to 60°C as depicted in Scheme 5:

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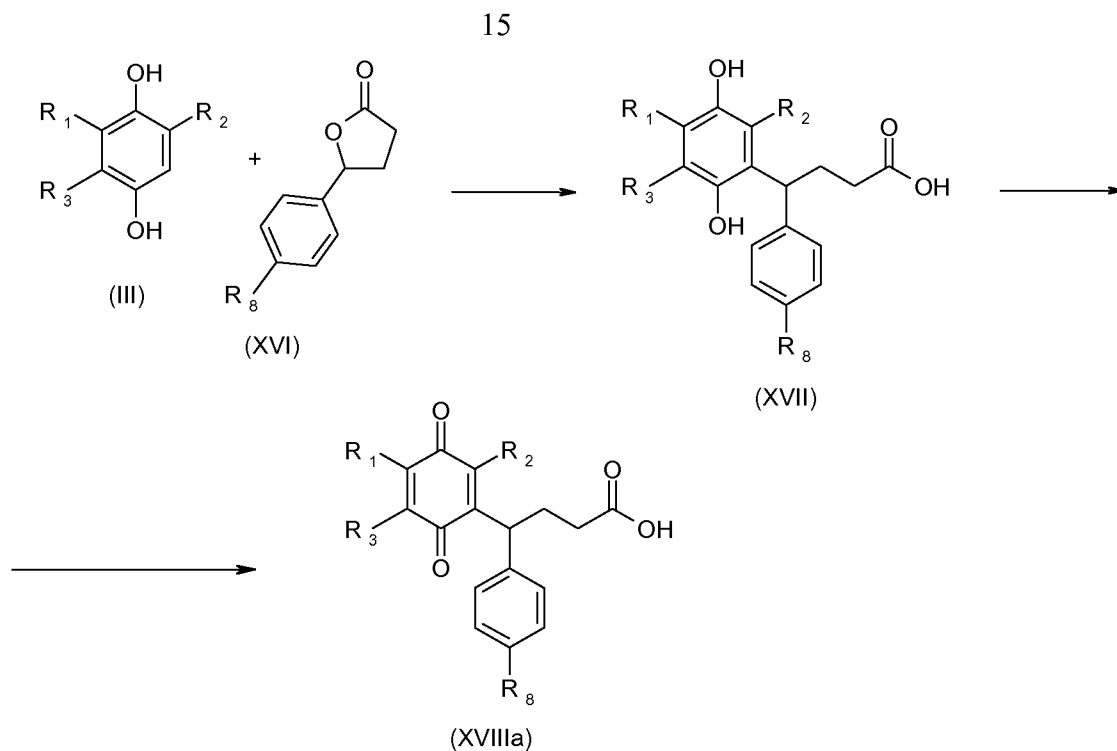


Scheme 5

Compounds of formula (XVIIIb) can be obtained using known methods starting from the corresponding compounds of formula (XVIIIa).

- 5 Compound of formula (XVIIIa) can be prepared by oxidation of carboxylic acids of formula (XVII) wherein R_1 , R_2 , R_3 and R_8 are as above described according to the procedure disclosed by Jurd and Wong, *Aust.J.Chem.* 1980, 33, 137, as depicted in Scheme 6.

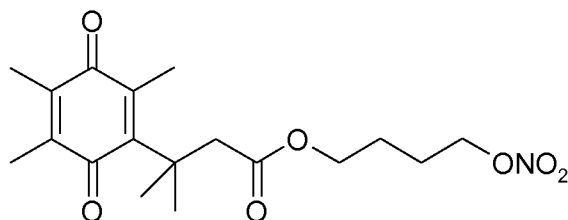
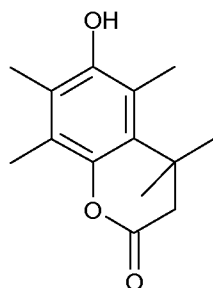
- Carboxylic acids of formula (XVII) can be prepared by acid-catalyzed coupling
 10 reaction between hydroquinone (III) and the commercially available γ -lactone (XVI)



Scheme 6

Example 1

Synthesis of 4-(nitrooxy)butyl 3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-
5 1,4-dienyl)butanoate (compound 1)

**Step 1: Synthesis of 6-hydroxy-4,4,5,7,8-pentamethylchroman-2-one**

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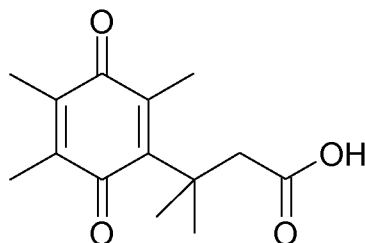
A synthetic procedure similar to the one described by Carpino et al., *J. Org. Chem.*, 1989, 54, 3303-3310 was used.

Methanesulfonic acid (20 mL) was heated at 70°C. In parallel,

2,3,5-trimethylbenzene-1,4-diol (2.0 g, 13.14 mmol) and methyl 3-methylbut-2-enoate (1.94 mL, 13.14 mmol, 1 eq) were added quickly and the reaction was heated for 2 h at this temperature. The reaction was then poured in water and, after cooling, was extracted with EtOAc (3 x 100 mL). The combined organic layers were washed successively with water, saturated NaHCO₃, water and brine, dried (Na₂SO₄), filtered and evaporated. The residue was crystallized from 30% CHCl₃ in n-Hexane to give the title compound as a pale grey solid (1.86 g, Yield: 60%). Melting point: 185°C.

¹H NMR (300 MHz, CDCl₃) δ 4.63 (s, 1H), 2.54 (s, 2H), 2.36 (s, 3H), 2.22 (s, 3H), 2.18 (s, 3H), 1.45 (s, 6H).

Step 2: Synthesis of 3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanoic acid

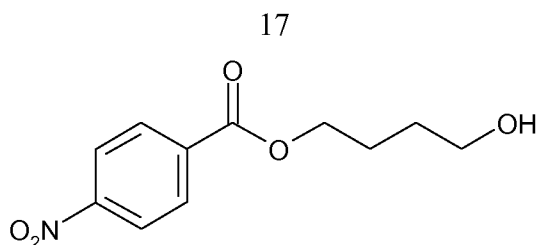


The reaction was performed according to conditions described by Borchardt et al., *J. Am. Chem. Soc.*, 1972, 94, 9175.

A stirred solution of 6-hydroxy-4,4,5,7,8-pentamethylchroman-2-one (2.0 g, 0.853 mmol) in 10% aqueous acetonitrile (100 mL) was added with a solution of freshly recrystallised NBS (1.6 g, 0.853 mmol, 1 eq) in acetonitrile (20 mL). The reaction was stirred for 1h and then diluted with water (100 mL) and extracted with Et₂O (3 x 100 mL). The combined organic layers were washed with water and brine, dried (Na₂SO₄), filtered and evaporated. The residue was crystallized from Et₂O/n-Hexane to give the title compound as a yellow solid (1.64 g, Yield: 77%).

¹H NMR (300 MHz, CDCl₃) δ 11.08 - 8.78 (m, 1H), 3.02 (s, 2H), 2.14 (s, 3H), 1.95 (s, 3H), 1.93 (s, 3H), 1.44 (s, 6H).

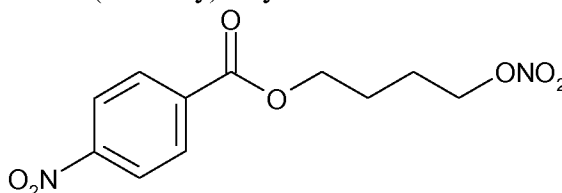
Step 3: Synthesis of 4-hydroxybutyl 4-nitrobenzoate



A stirred solution of 1,4-butanediol (3.0 g, 33.29 mmol, 1.1 eq) and 4-nitrobenzoyl chloride (5.56 g, 29.96 mmol) in EtOAc (100 mL) cooled to 0°C was added with dropwise triethylamine (4.6 mL, 33.3 mmol, 1.1 eq) and the reaction was stirred vigorously for 6h. The reaction was diluted with water and the organic layer separated, washed with HCl 0.1 M, water and brine. The organic phase was dried (Na₂SO₄), filtered and evaporated. The residue was triturated in cold Et₂O and the solid filtered off. The filtrate was evaporated to give the title compound as a viscous oil which solidified upon standing (2.86 g, Yield: 40%).

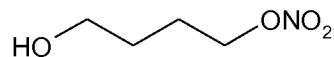
¹H NMR (300 MHz, CDCl₃) δ 8.30 (d, J = 8.8, 2H), 8.22 (d, J = 8.8, 2H), 4.44 (t, J = 6.5, 2H), 3.76 (t, J = 6.3, 2H), 1.93 (dt, J = 14.4, 6.7, 2H), 1.75 (dt, J = 13.2, 6.4, 2H).

Step 4: Synthesis of 4-(nitrooxy)butyl 4-nitrobenzoate



Concentrated nitric acid (1.9 mL, 45.15 mmol, 3 eq) was added dropwise to acetic anhydride (20 mL) cooled to 0°C. Then solid 4-hydroxybutyl 4-nitrobenzoate was added and the reaction was stirred at this temperature for 30 min then poured on ice. After melting, the organic oil was separated from the aqueous liquid and diluted with EtOAc. The organic layer was washed with NaHCO₃ (2x 30 mL), water and brine, dried (Na₂SO₄), filtered and evaporated. The residue was purified by flash chromatography (Biotage System, SNAP Cartridge silica 100 g, eluent: n-hexane/ethyl acetate 85/15 to n-hexane/ethyl acetate 75/25 during 8 CV) affording the title compound as a yellow oil (3.73g, Yield: 87%).

¹H NMR (300 MHz, CDCl₃) δ 8.34 - 8.29 (m, 2H), 8.24 - 8.19 (m, 2H), 4.56 (t, J = 5.9, 2H), 4.44 (t, J = 6.0, 2H), 1.99 - 1.90 (m, 4H).

Step 5: Synthesis of 4-hydroxybutyl nitrate

A stirred solution of 4-(nitrooxy)butyl 4-nitrobenzoate (2.13 g, 7.49 mmol) in a 3/1
5 THF/EtOH mixture (40 mL) cooled to 0°C was added with NaOH 1M (7.5 mL, 1 eq).
The reaction was stirred at this temperature for 3 h then diluted with EtOAc and water.
The organic layer was separated, washed with water and brine, dried (Na₂SO₄), filtered
and evaporated. The residue was purified flash chromatography (Biotage System, SNAP
Cartridge silica 100 g, eluent: n-hexane/ethyl acetate 85/15 to n-hexane/ethyl acetate
10 75/25 during 8 CV) affording the title compound as a colourless oil (0.48g, Yield: 49%).

¹H NMR (300 MHz, CDCl₃) δ 4.52 (t, J = 6.5, 2H), 3.72 (t, J = 6.2, 2H), 1.87 (dt, J
= 14.2, 6.5, 2H), 1.75 - 1.63 (m, 1H).

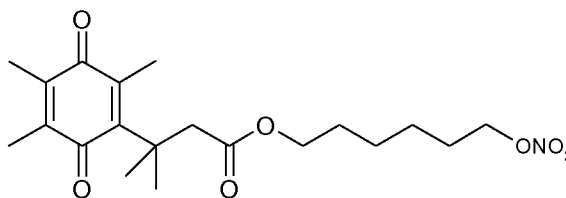
Step 6: Synthesis of 4-(nitrooxy)butyl 3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dienyl)butanoate (Compound (1))

15 A stirred solution of 4-hydroxybutyl nitrate (2.0 g, 14.8 mmol) and 3-methyl-3-
(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanoic acid (3.70 g, 14.8 mmol) in
dry CH₂Cl₂ cooled to 0°C was added with EDC (3.12 g, 16.28 mmol, 1.1 eq) and a
catalytic amount of DMAP (0.05 g). The reaction was stirred for 5 h at this temperature
and then washed with water, HCl 1M, water and brine, dried (Na₂SO₄), filtered and
20 evaporated. The residue was purified by flash chromatography (Biotage System, 2 SNAP
Cartridge silica 340 g, eluent: n-hexane/ethyl acetate 85/15 to n-hexane/ethyl acetate
70/30 during 8 CV) affording the title compound as a yellow oil (5.02 g, Yield: 92%).

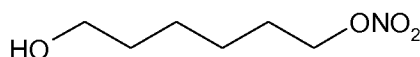
¹H NMR (300 MHz, CDCl₃) δ 4.45 (t, J = 6.2, 2H), 4.01 (t, J = 6.1, 2H), 2.98 (s,
2H), 2.14 (s, 3H), 1.94 (s, 6H), 1.82 - 1.62 (m, 4H), 1.42 (s, 6H).

25 **Example 2**

Synthesis of 6-(nitrooxy)hexyl 3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-
1,4-dienyl)butanoate (Compound 2)



Step 1: Synthesis of 6-Nitrooxy-hexan-1-ol



5 A solution of 6-bromohexan-1-ol (2.2 mL, 16.6 mmol) in CH₃CN (100 mL) was added with silver nitrate (5.95 g, 35 mmol, 2 eq). The reaction was stirred at room temperature for 3 days. The reaction was quenched by addition of a solution of brine. After 15 min of stirring, the solution was filtered, extracted with ethyl acetate, washed with H₂O, brine, dried over sodium sulfate, filtered and evaporated. The residue was
10 purified by column chromatography (Biotage System, SNAP Cartridge silica 100 g, eluent: n-hexane/ethyl acetate 80/20 to n-hexane/ethyl acetate 50/50 during 12 CV) to give the desired product as a colorless oil (2.34 g, Yield: 86%).

¹H NMR (300 MHz, CDCl₃) 4.47 (t, *J* = 6.6 Hz, 2H), 3.68 (t, *J* = 6.1 Hz, 2H), 1.77 (m, 2H), 1.62 (m, 2H), 1.48 (m, 4H), 1.27 (s, 1H).

15 **Step 2:** Synthesis of 6-(nitrooxy)hexyl 3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dienyl)butanoate

A stirred solution of 6-hydroxyhexyl nitrate (164 mg, 1.0 mmol) and 3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanoic acid (250 mg, 1.0 mmol) in dry CH₂Cl₂ cooled to 0°C was added with EDC (202 mg, 1.1 mmol, 1.1 eq) and a
20 catalytic amount of DMAP (0.02g). The reaction was stirred for 16 h from 0°C to rt. The reaction was washed with water, HCl 1M, water and brine, dried (Na₂SO₄), filtered and evaporated. The residue was purified by flash chromatography (Biotage System, SNAP Cartridge silica 100 g, eluent: n-hexane/ethyl acetate 85/15 to n-hexane/ethyl acetate 70/30 during 8 CV) affording the title compound as a yellow oil (286 mg, Yield:72%).

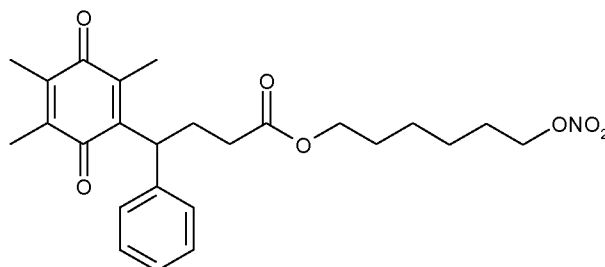
25 ¹H NMR (300 MHz, CDCl₃) δ 4.44 (t, *J* = 6.6, 2H), 3.97 (t, *J* = 6.6, 2H), 2.97 (s, 2H), 2.12 (s, 3H), 1.94 (d, *J* = 10.4, 6H), 1.77 - 1.65 (m, 2H), 1.63 - 1.50 (m, 2H), 1.47

- 1.41 (m, 6H), 1.41 - 1.30 (m, 4H).

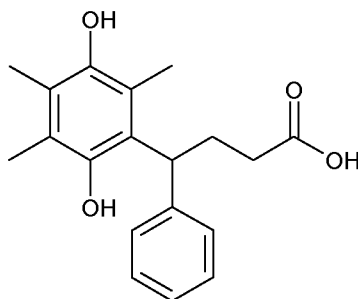
Example 3

Synthesis of 6-(nitrooxy)hexyl 4-phenyl-4-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dienyl)butanoate (Compound 3)

5



Step 1: Synthesis of 4-(2,5-dihydroxy-3,4,6-trimethylphenyl)-4-phenylbutanoic acid



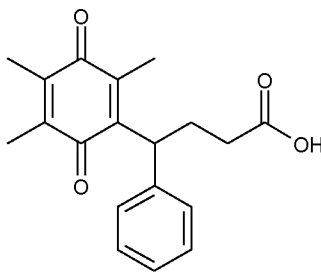
10

The reaction was performed according to conditions described by Mitsuru et al., *J. Med. Chem. Soc.*, 1989, 32, 2214-2221.

Boron trifluoride etherate (0.25 ml; 1.99 mmol) was added dropwise to a mixture of trimethylhydroquinone (1.0 g; 6.57 mmol) and γ -phenyl- γ -butyrolactone (1.1 g; 6.57 mmol) in Toluene (70 ml) at 60°C during 10 minutes. The mixture was stirred for further 2 hours and then the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography (Biotage system, SNAP Cartridge silica 100 g, EtOAc in n-hexane from 9% to 60% in 10 CV) affording the title compound (0.74 g; Yield: 36%) as an orange solid.

¹H NMR (300 MHz, CDCl₃) δ 7.39 - 7.07 (m, 5H), 4.72 - 4.25 (m, 3H), 2.74 - 2.25 (m, 4H), 2.25 (s, 3H), 2.08 (s, 3H), 1.98 (m, 2H).

Step 2: Synthesis of 4-phenyl-4-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanoic acid



To a solution of 4-(2,5-dihydroxy-3,4,6-trimethylphenyl)-4-phenylbutanoic acid
5 (0.74 g; 2.33 mmol) in CH₃CN:H₂O 1:1 (50 ml), Ammonium cerium nitrate (3.3 g; 5.87 mmol) was added. The mixture was stirred 3 hours at room temperature then was poured into H₂O (30 ml). Et₂O (20 ml) was added, the two phases were separated and the organic layer was extracted with Et₂O (2X20 ml). The combined organic layers were washed with
10 brine, dried on Na₂SO₄ and concentrated affording 560 mg of the title compound without any further purification.

¹H NMR (300 MHz, CDCl₃) δ 7.40 - 7.06 (m, 5H), 4.35 (t, *J* = 7.6, 1H), 2.77 - 2.25 (m, 4H), 2.15 - 2.03 (m, 3H), 1.97 (m, 6H).

Step 3: Synthesis of 6-(nitrooxy)hexyl 4-phenyl-4-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dienyl)butanoate (Compound 3)

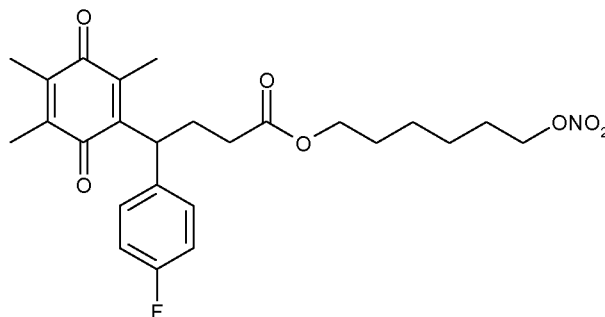
15 To a solution of 4-phenyl-4-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanoic acid (0.29g; 0.92 mmol) and 6-Nitrooxy-hexan-1-ol (synthesised as in example 2, step 1) (0.17mg; 0.92 mmol) in CH₂Cl₂ (5 ml), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) (0.29g; 1.38 mmol) and DMAP cat. were added. The solution was stirred 30 minutes at 0°C and 4 hours at room temperature then
20 washed with a solution of NaH₂PO₄ 5% (5 ml), H₂O (5 ml) and brine. The organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The residue was purified by flash chromatography (Biotage SP1 instrument, SNAP cartridge silica 50 g, Hex/EtOAc 9:1, 10 CV) affording the title compound (0.35 g; Yield:83%)

¹H NMR (300 MHz, CDCl₃) δ 7.37 - 7.08 (m, 5H), 4.44 (t, 2H), 4.38 - 4.27 (m,

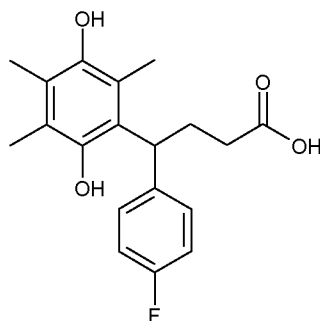
1H), 4.06 (t, 2H), 2.68 - 2.52 (m, 1H), 2.52 - 2.35 (m, 1H), 2.35 - 2.26 (m, 2H), 2.07 (s, 3H), 1.97 (m, 6H), 1.80 - 1.66 (m, 2H), 1.66 - 1.52 (m, 2H), 1.50 - 1.29 (m, 4H).

Example 4

Synthesis of 6-(nitrooxy)hexyl 4-(4-fluorophenyl)-4-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dienyl)butanoate (Compound 4)



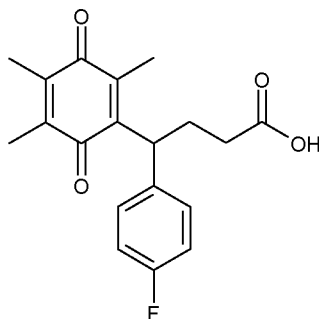
Step 1: Synthesis of 4-(2,5-dihydroxy-3,4,6-trimethylphenyl)-4-(4-fluorophenyl)butanoic acid



The reaction was performed according to conditions described by Mitsuru et al., *J. Med. Chem. Soc.*, 1989, 32, 2214-2221.

Boron trifluoride etherate (0.21 ml; 1.65 mmol) was added dropwise to a mixture of trimethylhydroquinone (0.50 g; 3.30 mmol) and γ -(4-fluorophenyl)- γ -butyrolactone (0.59 g; 3.30 mmol) in toluene (10 ml) at 60°C during 10 minutes. The mixture was stirred for further 2 hours and then the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography (Biotage system, SNAP Cartridge silica 50 g, EtOAc in n-hexane from 9% to 60% in 10 CV) affording the title compound (0.48 g; Yield: 43%) as an orange solid.

Step 2: Synthesis of 4-(4-fluorophenyl)-4-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanoic acid



To a solution of 4-(2,5-dihydroxy-3,4,6-trimethylphenyl)-4-(4-fluorophenyl)butanoic acid (0.48 g; 1.44 mmol) in CH₃CN:H₂O 1:1 (40 ml), Ammonium cerium nitrate (2.04 g; 3.60 mmol) was added. The mixture was stirred 3 hours at room temperature then was poured into H₂O (30 ml). Et₂O (20 ml) was added, the two phases were separated and the organic layer was extracted with Et₂O (2X20 ml). The combined organic layers were washed with brine, dried on Na₂SO₄ and concentrated affording 430 mg of the title compound without any further purification.

¹H NMR (300 MHz, CDCl₃) δ 7.32 - 7.19 (m, 3H), 6.97 (m, 2H), 4.29 (t, *J* = 7.6, 1H), 2.70 - 2.25 (m, 4H), 2.10 (s, 3H), 2.03 - 1.89 (m, 6H).

Step 3: 6-(Nitrooxy)hexyl 4-(4-fluorophenyl)-4-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dienyl)butanoate (Compound 4)

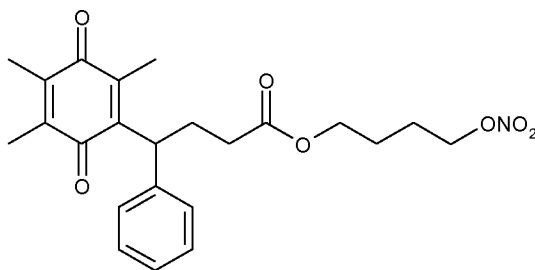
To a solution of 4-(4-fluorophenyl)-4-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanoic acid (0.22g; 0.66 mmol) and 6-Nitrooxy-hexan-1-ol (synthesised as in example 2, step 1) (0.12 mg; 0.66 mmol) in CH₂Cl₂ (5 ml), 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) (0.19 g; 0.98 mmol) and DMAP cat. were added. The solution was stirred 30 minutes at 0°C and 4 hours at room temperature then washed with a solution of NaH₂PO₄ 5% (5 ml), H₂O (5 ml) and brine. The organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The residue was purified by flash chromatography (Biotage SP1 instrument, SNAP cartridge silica 50 g, Hex/EtOAc 9:1, 10 CV) affording the title compound (0.18 g; Yield: 58%)

¹H NMR (300 MHz, CDCl₃) δ 7.36 - 7.17 (m, 3H), 7.05 - 6.88 (m, 2H), 4.44 (t,

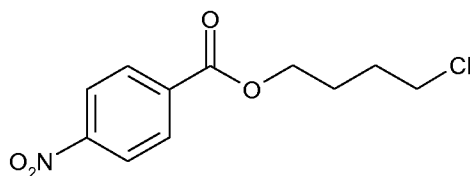
2H), 4.28 (t, 1H), 4.06 (t, 2H), 2.67 - 2.33 (m, 2H), 2.33 - 2.22 (m, 2H), 2.11 (s, 3H), 2.00 (s, 3H), 1.95 (s, 3H), 1.80 - 1.55 (m, 4H), 1.49 - 1.30 (m, 4H).

Example 5

Synthesis of 4-(nitrooxy)butyl 4-phenyl-4-(2,4,5-trimethyl-3,6-dioxocyclohexa-
5 1,4-dienyl)butanoate (Compound 5)



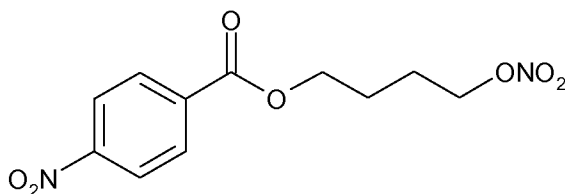
Step 1: Synthesis of 4-chlorobutyl 4-nitrobenzoate



To a solution of 4-Chlorobutanol (1.09 g; 10.04 mmol) and TEA (1.7 ml; 12.05 mmol) in CH₂Cl₂ (25 ml) cooled at 0°C, 4-Nitrobenzoyl chloride (2.23 g; 12.05 mmol) was added portionwise. The mixture was stirred 2 hours at room temperature then was washed with NaH₂PO₄ (25 ml), H₂O and brine. The residue was
15 purified by flash chromatography (Biotage SP1 instrument, SNAP cartridge silica 100 g, n-Hexane/EtOAc 9:1, 10 CV) affording the title compound (2.48 g; Yield: 96%)

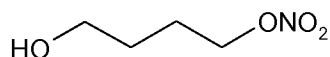
¹H NMR (300 MHz, CDCl₃) δ 8.38-8.25 (m, 2H), 8.25-8.14 (m, 2H), 4.55-4.33 (m, 2H), 3.73-3.53 (m, 2H), 2.13-1.85 (m, 4H).

Step 2: Synthesis of 4-(nitrooxy)butyl 4-nitrobenzoate



To a solution of 4-chlorobutyl 4-nitrobenzoate (2.48 g; 9.62 mmol) in CH₃CN (40 ml), NaI (5.77 g, 38.30 mmol) was added. The mixture was heated in a microwave apparatus (40 minutes; 120°C) then the salts were filtered off and the solvent evaporated under reduced pressure. EtOAc (50 ml) was added and the solution was washed with a solution of Na₂S₂O₅ 5% (50 ml), H₂O and brine. The organic layer was dried on Na₂SO₄ and concentrated under reduced pressure. The residue was dissolved in CH₃CN (40 ml) and AgNO₃ (1.97 g; 11.54 mmol) was added. The mixture was heated at the mw for 15 minutes at 120°C then the salts were filtered off and the solvent evaporated under reduced pressure. EtOAc (30 ml) was added, the precipitate was removed again by filtration and the solvent was evaporated. This procedure was repeated three times then the organic layer was washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure. The residue was purified by flash chromatography (Biotage SP1 instrument, SNAP cartridge silica 100 g, EtOAc in n-hexane from 5% to 40% in 10 CV) affording the title compound (2.50 g; Yield:93%) as a clear oil.

Step 3: Synthesis of 4-hydroxybutyl nitrate



To a solution of 4-(nitrooxy)butyl 4-nitrobenzoate (2.5 g; 8.76 mmol) in THF (30ml) cooled at 0°C, NaOH 2M (8.7 ml; 17.53 mmol) was added dropwise. The solution was stirred 4 hours at room temperature then was diluted with NaHCO₃ saturated solution (20 ml) and extracted with CH₂Cl₂ (3x30ml). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The residue was purified by flash chromatography (Biotage SP1 instrument, SNAP cartridge silica 50 g, EtOAc in n-hexane from 10% to 100% in 10 CV) affording the title compound (1.0 g; Yield:85%).

¹H NMR (300 MHz, CDCl₃) δ 4.50 (td, *J* = 6.5, 2H), 3.70 (t, *J* = 6.2, 2H), 1.95 - 1.76 (m, 2H), 1.76 - 1.59 (m, 2H).

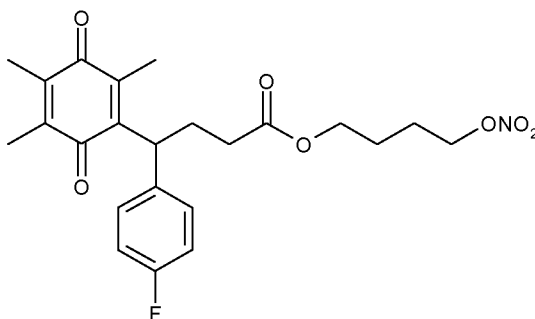
Step 4: Synthesis of 4-(nitrooxy)butyl 4-phenyl-4-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dienyl)butanoate (Compound 5)

To a solution of 4-phenyl-4-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanoic acid (synthesized as in Example 3, steps 1 and 2) (0.27 g; 0.86 mmol) and 4-hydroxybutyl nitrate (0.15 mg; 0.86 mmol) in CH_2Cl_2 (4 ml), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (0.25 g; 1.30 mmol) and DMAP cat. were added. The solution was stirred 30 minutes at 0°C and 4 hours at room temperature then washed with a solution of NaH_2PO_4 5% (5 ml), H_2O (5 ml) and brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (Biotage SP1 instrument, SNAP cartridge silica 50 g, Hex/EtOAc 9:1, 10 CV) affording the title compound (0.23 g; Yield: 62%) as an orange oil.

^1H NMR (300 MHz, CDCl_3) δ 7.38 - 7.11 (m, 5H), 4.47 (m, 2H), 4.34 (t, $J = 7.7$, 1H), 4.09 (t, $J = 6.0$, 2H), 2.73 - 2.52 (m, 1H), 2.52 - 2.23 (m, 3H), 2.07 (s, 3H), 1.97 (m, 6H), 1.89 - 1.65 (m, 4H).

Example 6

Synthesis of 4-(nitrooxy)butyl 4-(4-fluorophenyl)-4-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dienyl)butanoate (Compound 6)



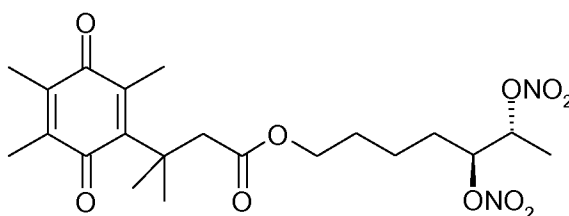
To a solution of 4-(4-fluorophenyl)-4-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanoic acid (synthesized as in Example 4, steps 1 and 2) (0.19g; 0.57 mmol) and 4-hydroxybutyl nitrate (synthesised as in Example 5, steps 1, 2 and 3) (0.10 mg; 0.57 mmol) in CH_2Cl_2 (4 ml), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (0.16 g; 0.86 mmol) and DMAP cat. were added. The solution was stirred 30 minutes at 0°C and 4 hours at room temperature then washed with a solution of NaH_2PO_4 5% (5 ml),

H₂O (5 ml) and brine. The organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The residue was purified by flash chromatography (Biotage SP1 instrument, SNAP cartridge silica 50 g, n-Hexane/EtOAc 9:1, 10 CV) affording the title compound (0.15 g; Yield: 59%) as an orange oil.

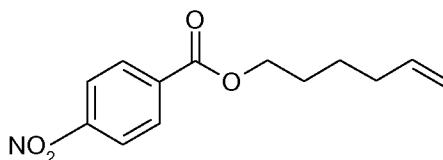
¹H NMR (300 MHz, CDCl₃) δ 7.35 - 7.15 (m, 2H), 7.06 - 6.87 (m, 2H), 4.47 (m, 2H), 4.28 (t, *J* = 7.7, 1H), 4.09 (t, *J* = 6.0, 2H), 2.68 - 2.22 (m, 4H), 2.08 (s, 3H), 1.97 (m, 6H), 1.86 - 1.65 (m, 4H).

Example 7

Synthesis of (5*S*,6*R*)-5,6-bis(nitrooxy)heptyl 3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dienyl)butanoate ((5*S*,6*R*)-isomer of Compound 7)



Step 1: Synthesis of hex-5-enyl 4-nitrobenzoate



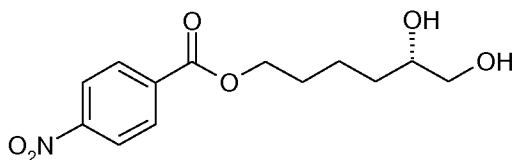
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At 0°C, a solution of 5-hexen-1-ol (19.4 mL; 161.54 mmol) in dichloromethane (513 mL), was added with p-nitrobenzoyl chloride (35.97 g, 193.85 mmol) followed by a solution of triethylamine (27.0 mL, 193.85 mmol) in dichloromethane (150 mL) dropwise. The mixture was stirred at ambient temperature for 21 hours, then washed with water, 1M aqueous HCl, brine. The organic layer was dried (Na₂SO₄) and the solvent removed under reduced pressure. The residue was purified by flash chromatography (Biotage System, two SNAP Cartridge silica 340 g, eluent: n-hexane/ethyl acetate 90/10 to n-hexane/ethyl acetate 50/50 during 12 CV) to give the title compound as a yellow oil (40.00 g, 99%).

¹H NMR (300 MHz, CDCl₃) δ 8.30 (dt, *J* = 9.0, 3.0 Hz, 2H), 8.22 (dt, *J* = 9.0, 3.0 Hz, 2H), 5.83 (1H, ddt, *J* = 16.9, 10.2, 6.7 Hz), 4.95-5.11 (2H, m), 4.39 (2H, t, *J* = 6.6 Hz), 2.15 (2H, m), 1.84 (2H, m) 1.50-1.66 (2H, m).

Step 2: Synthesis of (5S)-5,6-dihydroxyhexyl 4-nitrobenzoate

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To a vigorously stirred solution of commercially available “AD mix α” (112.5 g) in 1:1 water/*t*-butanol (822 mL), at 0 °C, hex-5-enyl 4-nitrobenzoate was added A (20.00 g, 80.23 mmol). The mixture was stirred vigorously at 4°C (cold room) for 21 hours. The mixture was cooled to 0 °C and ethyl acetate (450 mL) was added, followed by slow portionwise addition of sodium metabisulfite (33.1 g). The mixture was stirred at 0 °C for 30 minutes, then at ambient temperature for 1 hour. The organic phase was separated and the aqueous extracted with ethyl acetate. The combined organic extracts were washed with brine, dried (Na₂SO₄) and the solvent removed under reduced pressure. Purification by filtration over a short pad of silica gel, eluting with ethyl acetate, gave the title compound as an off-white solid (21.90 g, 96%).

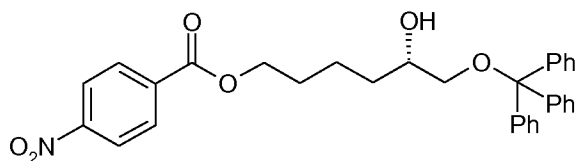
10

¹H NMR (300 MHz, CDCl₃) δ 8.30 (dt, *J* = 9.0, 2.0 Hz, 2H), 8.21 (dt, *J* = 9.0, 2.0 Hz, 2H), 4.39 (t, *J* = 6.6 Hz, 2H), 3.80-3.62 (2H, m), 3.47 (1H, m), 2.59 (bs, 1H), 2.42 (bs, 1H), 1.90-1.75 (2H, m), 1.73 - 1.45 (4H, m).

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Step 3: Synthesis of (5S)-6-triphenylmethyloxy-5-hydroxyhexyl 4-nitrobenzoate

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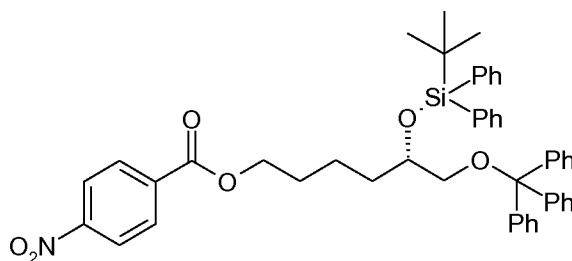
A solution of (5S)-5,6-dihydroxyhexyl 4-nitrobenzoate (13.46 g, 47.53 mmol) in anhydrous N,N-dimethylformamide (123 mL), under N₂, was added with triphenylchloromethane (14.57 g, 52.28 mmol), followed by triethylamine (7.29 mL,

25

52.28 mmol) and 4-dimethylaminopyridine (581 mg, 4.75 mmol). The resulting solution was stirred at ambient temperature for 23 hours. The mixture was poured into water and extracted with diethyl ether ($\times 3$). The combined organic extracts were washed with saturated aqueous NH_4Cl and water, then dried (Na_2SO_4) and the solvent removed under reduced pressure. Purification by flash chromatography, eluting with 20% ethyl acetate/hexane to 50% ethyl acetate/n-hexane gave the title compound as pale yellow oil (20.90 g, 84%).

^1H NMR (300 MHz, CDCl_3) δ 8.28 (d, $J = 8.8$ Hz, 2H), 8.20 (d, $J = 8.8$ Hz, 2H), 7.49-7.42 (m, 6H), 7.36-7.23 (m, 9H), 4.36 (t, $J = 6.5$ Hz, 2H), 3.82 (dp, $J = 10.9, 3.1$ Hz, 1H), 3.21 (dd, $J = 9.3, 3.3$ Hz, 1H), 3.07 (dd, $J = 9.2, 7.7$ Hz, 1H), 2.36 (d, $J = 2.9$ Hz, 1H), 1.86 - 1.72 (m, 2H), 1.56 - 1.38 (m, 4H).

Step 4: Synthesis of (5S)-6-triphenylmethyloxy-5-*tert*-butyldiphenylsilyloxyhexyl 4-nitrobenzoate

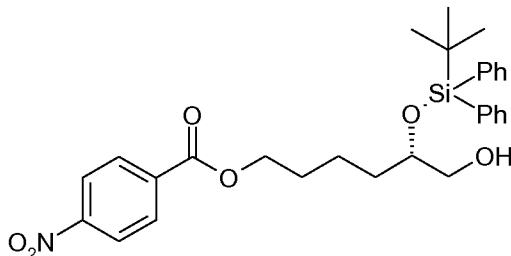


A solution of (5S)-6-triphenylmethyloxy-5-hydroxyhexyl 4-nitrobenzoate (7.10 g, 13.51 mmol) in anhydrous N,N-dimethylformamide (65 mL), under N_2 , was added with imidazole (1.84 g, 27.02 mmol) and the solution cooled to 0 °C. *tert*-Butyldiphenylsilyl chloride (7.03 mL, 27.02 mmol) was added and the solution stirred at 0 °C for 10 minutes, then at ambient temperature for 15 hours. The mixture was poured into water and extracted with diethyl ether. The combined organics were dried (Na_2SO_4) and the solvent removed under reduced pressure. Purification by flash chromatography, eluting with 5% ethyl acetate/n-hexane gave the title compound as off-white foam (5.29 g, 51%).

^1H NMR (300 MHz, CDCl_3) δ 8.28 - 8.20 (m, 2H), 8.19 - 8.09 (m, 2H), 7.67 - 7.53 (m, 4H), 7.48 - 7.11 (m, 21H), 4.22 (t, $J = 6.4$ Hz, 2H), 3.97 - 3.87 (m, 1H), 3.15 (dd, $J =$

9.3, 4.8 Hz, 1H), 3.03 (dd, $J = 9.2, 6.4$ Hz, 1H), 1.78 - 1.44 (m, 4H), 1.27 (m, 2H), 1.02 (s, 9H).

Step 5: Synthesis of (5S)-5-tert-butyldiphenyloxy-6-hydroxyhexyl 4-nitrobenzoate



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A solution of (5S)-6-triphenylmethyloxy-5-tert-butyldiphenyl silyloxyhexyl-4-nitrobenzoate (4.06 g, 5.32 mmol) in dichloromethane (15 mL) was added with methanol (157 mL) and *p*-toluenesulfonic acid monohydrate (202 mg, 1.06 mmol). The solution was stirred at ambient temperature for 17 hours. The solvent was removed under reduced pressure and the residue dissolved in ethyl acetate and washed with saturated aqueous NaHCO₃, water, then brine. The organic layer was dried (Na₂SO₄) and the solvent removed under reduced pressure. Purification by flash chromatography (Biotage System, SNAP Cartridge silica 340 g, eluent: n-hexane/ethyl acetate 90/10 to n-hexane/ethyl acetate 70/30 during 12 CV) gave the title compound as a pale yellow oil (1.33 g, 48%).

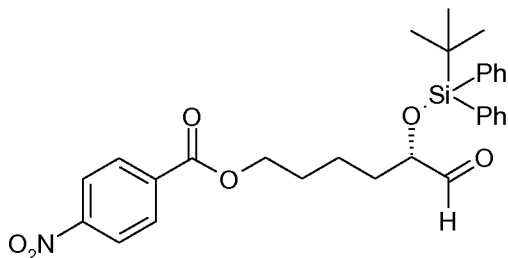
10

¹H NMR (300 MHz, CDCl₃) δ 8.32 - 8.26 (m, 2H), 8.20 - 8.15 (m, 2H), 7.73 - 7.66 (m, 5H), 7.48 - 7.35 (m, 5H), 4.25 (t, $J = 6.5$ Hz, 2H), 3.83 (dt, $J = 10.3, 5.3$ Hz, 1H), 3.52 (ddd, $J = 11.4, 5.9, 3.7$ Hz, 1H), 3.58 (ddd, $J = 11.4, 4.8, 3.1$ Hz, 1H), 1.79 (bs, 1H), 1.70 - 1.46 (m, 4H), 1.36 (dd, $J = 15.0, 7.4$ Hz, 2H), 1.09 (s, 9H).

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Step 6: Synthesis of (5S)-5-tert-butyldiphenylsilyloxy-6-oxohexyl 4-nitrobenzoate

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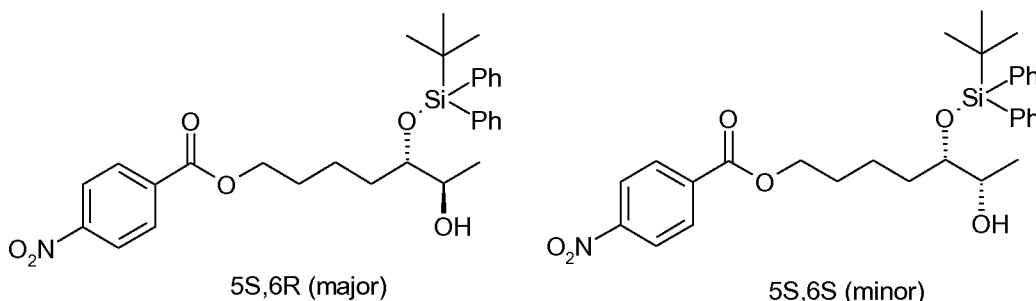


A 0.4 M solution of (5S)-5-tert-butyldiphenyloxy-6-hydroxyhexyl 4-nitrobenzoate

(9.29 g, 17.81 mmol) in dichloromethane (44.5 mL) was added with silica-supported TEMPO (307 mg; 0.178 mmol), followed by a 0.5 M solution aqueous KBr (3.53 mL). The mixture was cooled to 0 °C and stirred vigorously. A 0.37 M solution of NaOCl (10-15% active Cl) (13.73 mL) in water (46.30 mL) was added and the mixture buffered with solid NaHCO₃. The mixture was stirred vigorously at 0 °C for 3.5 hours. The solids were removed by filtration and washed well with dichloromethane and water. The organic layer was separated and the aqueous extracted with dichloromethane. The combined organics were dried (Na₂SO₄) and the solvent removed under reduced pressure to give the title compound as crude pale yellow oil (9.09 g, 98%) for use directly without further purification.

¹H NMR (300 MHz, CDCl₃) δ 9.64 (d, *J* = 1.4 Hz, 1H), 8.30 (dt, *J* = 9.0, 2.0 Hz, 2H), 8.19 (dt, *J* = 9.0, 2.0 Hz, 2H), 7.75-7.60 (m, 4H), 7.55-7.35 (m, 6H), 4.30 (t, *J* = 6.4 Hz, 2H), 4.09 (td, *J* = 5.6, 1.4 Hz, 1H), 1.90-1.35 (m, 6H), 1.12 (9H, s).

Step 7: Synthesis of (5*S*,6*R*)-6-hydroxy-5-terbutyldiphenyl silyloxyheptyl 4-nitrobenzoate and (5*S*,6*S*)-6-hydroxy-5-terbutyldiphenylsilyloxyheptyl 4-nitrobenzoate



A 250 mL schlenk flask (dried and flushed with N₂) was added with (1*R*,2*S*)-(+)-(dibutylamino)-1-phenyl-1-propanol (3.28 g, 12.45 mmol, 1 eq) followed by a 2 M solution of dimethylzinc in toluene (37.35 mL, 74.7 mmol, 6 eq). The resulting yellow solution was cooled to 0 °C and a solution of (5*S*)-5-tert-butyldiphenylsilyloxy-6-oxohexyl 4-nitrobenzoate (6.47 g, 12.45 mmol) in anhydrous toluene (40 mL) was added slowly. The solution was stirred at 0°C for 10 minutes then allowed to warm to ambient temperature and stirred for 18 hours. The solution was cooled to 0°C and slowly quenched by addition of saturated aqueous NH₄Cl (75 mL). The mixture was allowed to warm to

ambient temperature and extracted with ethyl acetate. The combined organics were dried (Na₂SO₄) and the solvent removed under reduced pressure. Purification by flash chromatography, eluting with 15% ethyl acetate/n-hexane to 25% ethyl acetate/n-hexane gave the title compound, a yellow oil (4.31 g, 65%), as an inseparable mixture of the

5 diastereoisomers 5*S*,6*R* (major) and 5*S*,6*S* (minor).

(5*S*,6*R*)-major diastereoisomer

¹H NMR (300 MHz, CDCl₃) δ 8.29 (dt, *J* = 9.0, 2.0 Hz, 2H), 8.16 (dt, *J* = 9.0, 2.0 Hz, 2H), 7.75-7.65 (4H, m), 7.50-7.30 (6H, m), 4.20 (t, *J* = 6.4 Hz, 2H), 3.83 (m, 1H), 3.72 (m, 1H), 2.09 (d, *J* = 4.9 Hz, 1H), 1.65-1.20 (m, 6H), 1.12 (d, *J* = 6.5 Hz, 3H), 1.09

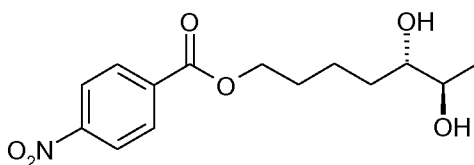
10 (s, 9H).

(5*S*,6*S*)-minor diastereoisomer:

¹H NMR (300 MHz, CDCl₃) δ 8.29 (dt, *J* = 9.0, 2.0 Hz, 2H), 8.16 (dt, *J* = 9.0, 2.0 Hz, 2H), 7.75-7.65 (m, 4H), 7.30-7.50 (m, 6H), 4.20 (t, *J* = 6.4 Hz, 2H), 3.71 (1H, m), 3.60 (1H, m), 2.21 (d, *J* = 6.1 Hz, 1H), 1.65-1.20 (m, 6H), 1.16 (d, *J* = 6.3 Hz, 3H), 1.09

15 (s, 9H).

Step 8: Synthesis of (5*S*,6*R*)-5,6-dihydroxyheptyl 4-nitrobenzoate



A solution of (5*S*,6*R*)-6-hydroxy-5-terbutyldiphenyl silyloxyheptyl 4-nitrobenzoate

20 (805 mg, 1.50 mmol) in diethyl ether (50 mL) was added with a 3% solution of HCl in methanol, dropwise (made from addition of acetyl chloride (2.00 mL) to methanol (50 mL)). The solution was stirred at ambient temperature for 41 hours. Amberlite IRA 400 (OH) resin was added and the mixture stirred for 1 hour, with further addition of the resin until the pH = 7 / 8. The resin was filtered off and washed with ethyl acetate, then

25 methanol. The solvent was removed under reduced pressure and the residue partitioned between ethyl acetate and saturated aqueous NaHCO₃ and the aqueous extracted with

ethyl acetate. The combined organics were dried (Na_2SO_4) and the solvent removed under reduced pressure. Purification by flash chromatography, eluting with 10% ethyl acetate/hexane to 90% ethyl acetate/n-hexane, gave the title compound, a pale yellow oil (189 mg, 42%), as a mixture of the diastereoisomers *5S,6R* (major) and *5S,6S* (minor).

5 Diastereomeric excess (*5S,6R*) = 56.4%.

The diastereoisomers were separated by preparative HPLC (conditions: column Phenomenex Gemini phenyl-hexyl 100x21.2 mm/5m.

Mobile phase: A: water + 0.1% Formic acid; B: methanol + 0.1% formic acid.

Flow rate: 25 mL/min.

10 Gradient profile: time 0 min: 45% A/ 55% B; 5.5 min: 40% A/ 60% B; 5.6 min: 0% A/ 100% B; 7.6 min: 0% A/ 100% B; 7.7 min: 45% A/ 55% B. Detector: δ : 254 nm) to give compound H as a white solid (135 mg). Enantiomeric excess /diastereomeric excess = 72.1%.

15 ^1H NMR (300 MHz, CDCl_3) δ 8.32 (dt, J = 9.0, 2.0 Hz, 2H), 8.23 (dt, J = 9.0, 2.0 Hz, 2H), 4.42, (t, J = 6.5 Hz, 2H), 3.84 (m, 1H), 3.66 (m, 1H), 1.42-2.0 (m, 8H), 1.19 (d, J = 6.4 Hz, 3H).

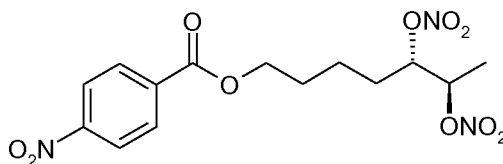
Alternative deprotection procedure

A stirred solution of (*5S,6R*)-6-hydroxy-5-terbutyldiphenyl silyloxyheptyl 4-nitrobenzoate (2.96 g, 5.52 mmol) in acetonitrile (60 mL) was added at 0°C with borontrifluoride - diethyletherate (3.5 mL, 5 eq) and the reaction was stirred at RT for 6h. The reaction was cooled to 0°C before quenching with a saturated solution of sodium bicarbonate. The reaction was diluted with ethyl acetate (50 mL) and the organic layer was separated, washed successively with water and brine (5 mL each), dried (Na_2SO_4), filtered and the solvent removed under reduced pressure. The residue was purified by
25 flash chromatography (Biotage System, 2 x SNAP Cartridge silica 100 g, eluent: gradient n-hexane/ethyl acetate 35/65 to n-hexane/ethyl acetate 30/70 during 7 CV) to give the title compound as a colourless oil (1.47 g, 90%) as a mixture of the diastereoisomers *5S,6R* (major) and *5S,6S* (minor).

The diastereoisomers were separated by preparative HPLC (conditions: column Phenomenex Gemini phenyl-hexyl 100x21.2 mm/5m to give the major diastereoisomer as a white solid (1.09 g, 66%).

Step 9: Synthesis of (5S,6R)-5,6-bis(nitrooxy)heptyl 4-nitrobenzoate

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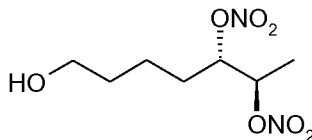


A stirred solution of (5S,6R)-5,6-dihydroxyheptyl 4-nitrobenzoate (400 mg, 1.34 mmol), tetrabutylammonium nitrate (863 mg, 2.82 mmol, 2.1 eq) and 2,6-di-tert-butyl-4-methylpyridine (580 mg, 2.82 mmol, 2.1 eq) in dry CH₂Cl₂ cooled to -78°C was added dropwise with triflic anhydride (0.778 g, 2.75 mmol, 2.05 eq) and the reaction was stirred for 1h at -78°C and left to turn back to rt. The reaction was then quenched with water and the organic layer was separated, washed with water and brine, dried on sodium sulfate, filtered and evaporated. The residue was purified by flash chromatography (Biotage SP4, SNAP 100 column, EtOAc in n-hexane from 20% to 30% in 10 CV) affording the title compound as an yellow oil (406 mg, Yield: 77%).

¹H NMR (300 MHz, CDCl₃) δ 8.32 (dt, *J* = 9.0 2.0 Hz, 2H), 8.22 (dt, *J* = 9.0, 2.0 Hz, 2H), 5.30 (m, 1H), 4.42 (td, *J* = 6.4 Hz, 1H), 1.95-1.55 (m, 6H), 1.43 (d, *J* = 6.7 Hz, 3H).

Step 10: Synthesis of (1R,2S)-6-hydroxy-1-methyl-2-(nitrooxy) hexyl nitrate

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A solution of (5S,6R)-5,6-bis(nitrooxy)heptyl 4-nitrobenzoate (163 mg, 0.42 mmol) in tetrahydrofuran (1.27 mL) and ethanol (1.27 mL), was added with a 1 M aqueous NaOH solution (546 μL, 0.546 mmol). The resulting yellow solution was stirred at ambient temperature for 1.5 hours. The solvent was concentrated under reduced

pressure and the aqueous residue partitioned between ethyl acetate and saturated aqueous NaHCO₃. The organic layer was washed with saturated aqueous NaHCO₃ and the aqueous back-extracted with ethyl acetate. The combined organics were dried (Na₂SO₄) and the solvent concentrated to a small volume (2 mL). The product was carefully purified by
5 flash chromatography (Biotage System, SNAP Cartridge silica 100 g, eluent: gradient n-hexane/ethyl acetate 75/25 to n-hexane/ethyl acetate 50/50 during 8 CV) to give the separation of the two diastereoisomers and the title compound as a colourless oil (0.147 g, 90%).

An optimal separation could be obtained using a Thar Investigator SFC system
10 using the following conditions:

Column: CHIRALPACK IB 250x10mm (5μm)

Cosolvent: n-Hexane/2-Propanol 1/1

Isocratic elution: CO₂/ co-solvent 90/1

Flow 10 ml/min T column: 40°C Injection volume: 80μl Detector wavelength:
15 210 nm

Run time: 8.5 min Cycle time: 3min Injected amount: 14-16 mg

(Sample preparation: 800mg of crude compound were solubilized in 4 ml of MeOH)

¹H NMR (300 MHz, CDCl₃) δ 5.25-5.34 (m, 2H), 3.70 (t, *J* = 5.9 Hz, 2H),
20 1.47-1.85 (m, 7H), 1.42 (d, *J* = 6.8 Hz, 3H).

Step 11: Synthesis of (5S,6R)-5,6-bis(nitrooxy)heptyl 3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dienyl)butanoate (compound 7)

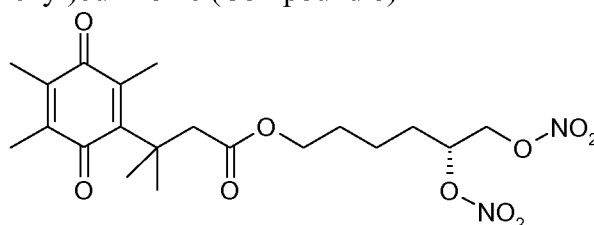
A stirred solution of 3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanoic acid (146 mg, 0.583 mmol, 1 eq) and (1R,2S)-6-hydroxy-1-methyl-2-
25 (nitrooxy)hexyl nitrate (140 mg, 0.583 mmol) in dry CH₂Cl₂ cooled to 0°C was added with EDC (117 mg, 0.612 mmol, 1.05 eq) and a catalytic amount of DMAP. The reaction was stirred at this temperature for 5h and then washed with water, HCl 0.1 M, water and brine. The organic solution was dried (Na₂SO₄), filtered and evaporated. The residue was

purified by flash chromatography (Biotage SP4 system, SNAP Cartridge silica 100 g, eluent: gradient n-hexane/ethyl acetate 80/20 to n-hexane/ethyl acetate 70/30 during 8 CV) to give the title compound as a yellow oil (210 mg, 76%).

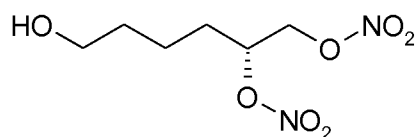
¹H NMR (300 MHz, CDCl₃) δ 5.32 - 5.17 (m, 2H), 3.99 (t, *J* = 6.2, 2H), 2.98 (s, 2H), 2.14 (s, 3H), 1.98 - 1.94 (m, 6H), 1.76 - 1.45 (m, 15H), 1.43 (s, 6H), 1.39 (d, *J* = 6.7, 3H).

Example 8

Synthesis of (R)-5,6-bis(nitrooxy)hexyl 3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dienyl)butanoate (Compound 8)

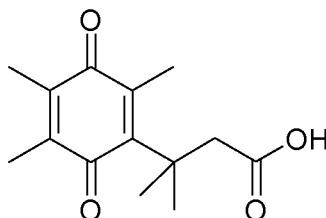


Synthesis of (2R)-6-hydroxyhexane-1,2-diyl dinitrate



(2R)-6-hydroxyhexane-1,2-diyl dinitrate was prepared as described in WO2005/070868.

Synthesis of 3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanoic acid



3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanoic acid

was prepared as described in Example 1 (setps1 and 2)

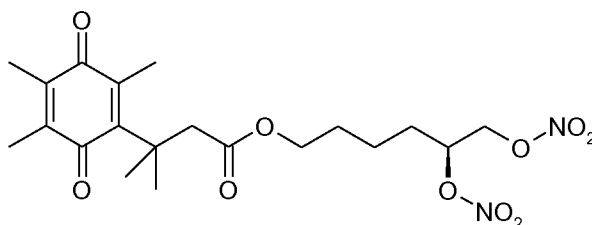
Step 1: Synthesis of (R)-5,6-bis(nitrooxy)hexyl 3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dienyl)butanoate

To a stirred solution of 3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanoic acid (237 mg; 0.95mmol) and (2R)-6-hydroxyhexane-1,2-diyl dinitrate (213 mg; 0.95 mmol) in DCM (5 ml) cooled to 0°C, EDAC (162 mg; 1.04mmol) and a catalytic amount of DMAP were added. The reaction was stirred overnight at 0°C. The crude was then washed with water, HCl 1N, water and brine, dried and evaporated under vacuum. The crude was purified by flash chromatography [Cy/EtOAc: 0% to 20% (1CV), 20% to 40% (7CV), 40% to 60% (2CV)]affording 338 mg of the title compound (Yield: 78.2%) as a yellow oil.

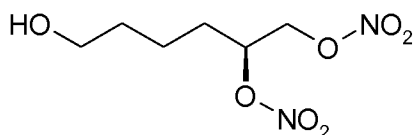
¹H NMR (300 MHz, CDCl₃) δ 5.30 – 5.21 (m, 1H), 4.74 (dd, *J* = 12.9, 3.0 Hz, 1H), 4.47 (dd, *J* = 12.9, 6.5 Hz, 1H), 3.99 (t, *J* = 6.3 Hz, 2H), 2.98 (s, 2H), 2.16 (d, *J* = 7.2 Hz, 3H), 1.96 (s, 6H), 1.81 – 1.69 (m, 2H), 1.66 – 1.56 (m, 2H), 1.52 – 1.31 (m, 8H).

Example 9

Synthesis of (S)-5,6-bis(nitrooxy)hexyl 3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dienyl)butanoate (Compound 8)



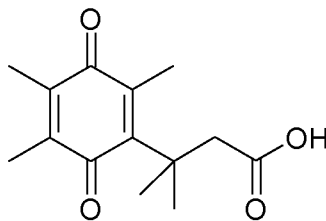
Synthesis of (2S)-6-hydroxyhexane-1,2-diyl dinitrate:



(2S)-6-hydroxyhexane-1,2-diyl dinitrate was prepared as described in WO2005/070868.

Synthesis of 3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanoic acid

38



3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanoic acid was prepared as described in example 1 (steps 1 and 2)

Synthesis of (S)-5,6-bis(nitrooxy)hexyl 3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dienyl)butanoate

To a stirred solution of 3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanoic acid (201 mg; 0.80 mmol) and (2S)-6-hydroxyhexane-1,2-diyl dinitrate (181 mg; 0.80 mmol) in DCM (5 ml) cooled to 0°C, EDAC (137 mg; 0.89 mmol) and a catalytic amount of DMAP were added. The reaction was stirred overnight at 0°C. The crude was then washed with water, HCl 1N, water and brine, dried and evaporated under vacuum. The crude was purified by flash chromatography [Cy/EtOAc: 0% to 20% (1CV), 20% to 40% (7CV), 40% to 60% (2CV)] affording 246 mg of the title compound (Yield: 67.1%) as a yellow oil.

¹H NMR (300 MHz, acetone) δ 5.49 (qd, *J* = 6.6, 2.6 Hz, 1H), 5.01 (dd, *J* = 13.0, 2.6 Hz, 1H), 4.73 (dd, *J* = 13.0, 6.3 Hz, 1H), 4.00 (t, *J* = 6.3 Hz, 2H), 2.95 (s, 2H), 2.13 (s, 3H), 1.94 (s, *J* = 6.3 Hz, 6H), 1.90 – 1.79 (m, 2H), 1.70 – 1.58 (m, 2H), 1.52 (dt, *J* = 8.0, 5.7 Hz, 2H), 1.43 (s, 6H).

Example 10: In vitro Antioxidant activity (TBARS test)

The antioxidant properties of compound (1) (Example 1), its precursor (3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanoic acid, (Intermediate 2) (Example 1, Step 2) and reference antioxidant compounds were assessed after NADPH-induced lipidic peroxidation of membrane lipids in rat hepatic microsomes using the detection of 2-thiobarbituric acid reactive substances (TBARS) by visible spectroscopy.

Hepatic microsomal membranes from male Wistar rats (200-250 g) were prepared by differential centrifugation (8000 g, 20 min; 120000 g, 1 h) in a HEPES/sucrose buffer

(10 mM, 250 mM, pH 7.4) and stored at -80°C. Incubation was performed at 37°C in a Tris-HCl/KCl (100 mM/150 mM, pH 7.4) containing microsomal membranes (2 mg prot/mL), sodium ascorbate (100 µM), and DMSO solutions of the tested compounds. Lipid peroxidation was initiated by adding ADP-FeCl₃ and NADPH (Method A) or 2.5 µM FeSO₄ (Method B) (as described by Boschi D. et al., J. Med. Chem. 2006, 49:2886-2897). Aliquots were taken from the incubation mixture at 5, 15, and 30 min and treated with trichloroacetic acid (TCA) 10% w/v. Lipid peroxidation was assessed by spectrophotometric (543 nm) determination of the TBARS consisting mainly of malondialdehyde (MDA). TBARS concentrations (expressed in nmol/mg protein) were obtained by interpolation with a MDA standard curve. The antioxidant activity of tested compounds was evaluated as the percent inhibition of TBARS production with respect to control samples, using the values obtained after 30 min of incubation. IC₅₀ values were calculated by nonlinear regression analysis.

The results reported in Table 1, showed that compound (1) inhibited in a concentration-dependent manner the generation of TBARS with a potency (IC₅₀ = 28µM) that is comparable to well known antioxidant compounds as ferulic or caffeic acids, edavarone or melatonin.

Table 1: In vitro Antioxidant activity (TBARS test)		
Compound	Antioxidant activity IC ₅₀ µM (CL 95%)	Method
Compound (1)	28 (25 - 31)	A
Intermediate 2	157 (79-309)	A
Ferulic acid	50.5 ± 0.4 ^a	B
Caffeic acid	33 (32-34)	B
Edavarone	17 (15-18) ^b	B
Melatonin	476 (442-512) ^c	B

Results are expressed as IC₅₀ of inhibition of TBARS production after 30 min incubation at 37°C

Method A: inhibition of rat hepatic lipid peroxidation induced by ADP-FeCl₃ and NADPH.

5 Method B: inhibition of rat hepatic lipid peroxidation induced by FeSO₄ and ascorbic acid

^a tested at 1mM concentration;

^bChegaev,K. et al. J. Med. Chem. 2009, 52:574-578:

^cChegaev,K. et al. J. Pineal Res. 2007, 42:371-385

10 **Example 11: Pharmacokinetic (PK) profile in rats**

The absorption and *in vivo* NO-donating property of the compounds of the invention were assessed by the evaluation of blood ¹⁵N-nitrite levels following oral administration.

Tested compounds:

- 15 ▪ 4-(nitrooxy)butyl-3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclo hexa-1,4-dienyl)butanoate (which corresponds to compound (1) containing nitrogen-15 (¹⁵N-Compound (1)));
- 6-(nitrooxy)hexyl-3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclo hexa-1,4-dienyl)butanoate (which corresponds to compound (2) containing nitrogen-15 (¹⁵N-Compound 2);
- 20 ▪ 6-(nitrooxy)hexyl-4-phenyl-4-(2,4,5-trimethyl-3,6-dioxocyclo hexa-1,4-dienyl)butanoate (which corresponds to compound (3) containing nitrogen-15 (¹⁵N-Compound 3);
- 6-(nitrooxy)hexyl-4-(4-fluorophenyl)-4-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dienyl)butanoate (which corresponds to compound (4) containing nitrogen-15 (¹⁵N-Compound 4);
- 25 ▪ 4-(nitrooxy)butyl-4-phenyl-4-(2,4,5-trimethyl-3,6-dioxocyclo hexa-1,4-dienyl)butanoate (which corresponds to compound (5) containing nitrogen-15

(¹⁵N-Compound 5);

- 4-(nitrooxy)butyl-4-(4-fluorophenyl)-4-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dienyl)butanoate (which corresponds to compound (6) containing nitrogen-15 (¹⁵N-Compound 6);
- 5 ▪ (5S,6R)-5,6-bis(nitrooxy)heptyl-3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dienyl)butanoate (which corresponds to compound (7) containing nitrogen-15 (¹⁵N-Compound 7);
- 5-isorbide mononitrate containing nitrogen-15 (5-ISM¹⁵N)

¹⁵N-labelled compounds (1)-(7) were dissolved in a mixture of 1% aqueous methocel and DMSO 98 / 2 (v/v) and orally administered to cannulated male SD rats at a dose of 30 mg/kg. At each time point (5, 10, 15, 30, 60, 180, 360 and 1440 minutes) 100 μL of blood sample were immediately protein crushed using using 300 μL of acetonitrile, vortex mixed and centrifuged 10 min at 4°C (3200g); The supernatant was transferred to a clean plate pending the nitrite quantification.

15 The quantification is based on conversion of ¹⁵NO₂⁻ to ¹⁵N-naphthotriazole using 1,2-diaminonaphthalene in acidic conditions, followed by quantification by LC-MS/MS. A group of samples of rat blood freshly spiked with known concentration of ¹⁵NO₂⁻ was added to evaluate the degree of conversion of nitrite to naphthotriazole.

A calibration curve (CC) for ¹⁵N-naphthotriazole in mouse blood was prepared in the range 0.01 - 30 μM. To do this, working solutions (WS) of ¹⁵N-naphthotriazole in DMSO were prepared by serial dilution of a 10mM stock solution to the following final concentrations:

WS (μM) 150 / 50 / 15 / 5 / 1.5 / 0.5 / 0.15 / 0.05

2 mL of mouse blood were protein precipitated using 6 mL of acetonitrile; 200 μL of deproteinized matrix were spiked with 10 μL of each working solution to create a calibration curve for ¹⁵N-naphthotriazole in the matrix to the following final nominal concentrations:

CC (μM) 30 / 10 / 3 / 1 / 0.3 / 0.1 / 0.03 / 0.01

Four working solutions of Na¹⁵NO₂ in water were prepared by serial dilution of a 10 mM stock solution to the following final concentrations:

WS (μM) 250 / 25 / 2.5 / 0

693 μL of deproteinized matrix were spiked with 7 μL of each working solution to
5 create spikes of ¹⁵N-naphthotriazole in the matrix to the following final nominal concentrations:

CC (μM) 10/ 1 / 0.1 / 0

To obtain ¹⁵N-nitrite to ¹⁵N-naphthotriazole conversion, 200 μL of deproteinized blood samples or spiked samples were spiked with 10 μL of 1,2-diaminonaphthalene
10 10⁻³M in HCl 0.5M and shaken for 20 minutes at room temperature. To stop conversion, 10 μL of NaOH 1M and 10 μL of DMSO were added to all the samples.

The analytical conditions were as follows:

	LC system	Agilent 1200 series				
	Column	Synergi Fusion-RP 50 x 2.0 mm (2.5μ) @ 40°C				
15	Mobile Phase	A. water + 0.1% formic acid				
		B. acetonitrile + 0.1% formic acid				
	Flow Rate	0.5mL/min				
	Gradient profile					
	Gradient profile:					
20	Time:	0.0	0.5	1.5	2.0	2.1
	A(%):	95	95	0	0	95
	B(%):	5	5	100	100	5
	Injection Volume	2 μL				
	Mass Spectrometer	API4000				
25	Ionization mode	ESI positive				
	Acquisition mode	MRM - m/z 171.10 / 115.00				

The raw data (average peak area at each time point) were interpolated using Analyst 1.4 software to obtain the concentration of ¹⁵N-naphthotriazole at each time

point. The concentration of ^{15}N -naphthotriazole in each sample was corrected by the ^{15}N -nitrite-to- ^{15}N -naphthotriazole conversion factor (CF) to obtain the original ^{15}N -nitrite concentration in the samples. The CF for each concentration of the spiked samples was calculated as:

5

$$\text{CF} = \frac{[^{15}\text{NAT}]_{\text{SPx}} - [^{15}\text{NAT}]_{\text{SP0}}}{C_x} * 100$$

where $[^{15}\text{NAT}]_{\text{SPx}}$ is ^{15}N -naphthotriazole concentration in the samples spiked at concentration x

$[^{15}\text{NAT}]_{\text{SP0}}$ is ^{15}N -naphthotriazole concentration in the unspiked samples

10

C_x is the nominal concentration of ^{15}N -nitrite in the sample

The ^{15}N -nitrite-to- ^{15}N -naphthotriazole conversion factor (CF) used is the average of the CF obtained at the three different concentrations of the spiked samples.

SigmaPlot 9.0 software was used to calculate the AUCs for the experiment using the extrapolated concentrations at each time point.

15

In Table 2 are reported ^{15}N -nitrite blood levels following oral administration of SD rats with 5-ISM ^{15}N (30 mg/kg) or with ^{15}N -labeled corresponding compounds of the invention (30 mg/kg).

All the compounds are able to release nitric oxide after oral administration in a range (C_{max}).

20

25

Table 2: Pharmacokinetic parameters for ^{15}N-nitrite blood levels following 30 mg/kg oral administration of ^{15}N-compound (1) to SD rats.			
Test compound	C _{max} (microM)	T _{max} (min)	AUC ₀₋₂₄ (microM*min)
^{15}N -Compound (1)	2.28±0.50	180	1855
^{15}N -Compound (2)	1.50±0.32	60	1009
^{15}N -Compound (3)	0.68±0.05	60	427
^{15}N -Compound (4)	0.39±0.32	15	218
^{15}N -Compound (5)	0.88±0.19	180	377
^{15}N -Compound (6)	2.55±0.22	60	1309
^{15}N -Compound (7)	6.68±0.55	60	2376
5-ISM ^{15}N	10.98±0.54	60	6647

Example 12: Skeletal muscle NO-release measurement

To assess the in vivo NO-release at the skeletal muscle level, cGMP and ^{15}N -labelled nitrites were measured in tibialis anterior muscle homogenates following a single oral administration with the ^{15}N -labeled Compound (1).

- 5 Fasted male CD1 mice (25-35 g, n=9 per group), obtained from Harlan Italy (Correzzana, Milan, Italy) were orally treated with tested compound (100 mg/kg) or vehicle (DMSO/Castor Oil/Tween80/Methocel 1% 2/5/5/88 v/v) in a total volume of 5 ml/kg by gavage. For the measurement of ^{15}N -labelled nitrites animals were treated with ^{15}N -labelled compounds. Then, 1h after oral treatment animals were sacrificed with
- 10 a lethal dose of tribromoethanol (Avertin® 250 mg/kg), given intraperitoneally, and tibialis anterior muscles were harvested, snap-frozen in liquid nitrogen and kept at -80°C until analysis. At the moment of analysis frozen muscles were homogenized in 10% trichloroacetic acid followed by centrifugation. The cGMP content was measured in the

soluble fraction by Cayman Chemical kit.

The results reported in Table 3 showed that compound (1) at the dose of 100 mg/kg significantly increases skeletal muscle cGMP compared to animals treated with vehicle, thus suggesting that the compound is able to release NO and stimulate guanylate cyclase in the skeletal muscle.

Table 3: Skeletal muscle cGMP evaluation in CD1 mice following oral treatment	
Treatment	cGMP content (pg/g of tissue)
Vehicle	9.1±0.6
Compound (1)	12.1±0.6*

Data are presented as mean ± SEM, *p<0.05 vs vehicle, n=9-17 mice/group.

¹⁵N-labelled nitrites were also quantified. The quantification is based on conversion of ¹⁵NO₂⁻ to ¹⁵N-naphthotriazole using 1,2-diaminonaphthalene in acidic conditions, followed by quantification by LC-MS/MS. A group of samples of rat skeletal muscle freshly spiked with known concentration of ¹⁵NO₂⁻ was added to evaluate the degree of conversion of nitrite to naphthotriazole.

Skeletal muscles were protein precipitated using 300 µL of acetonitrile, vortex mixed and centrifuged for 10 minutes at 4°C (3200g).

A calibration curve (CC) for ¹⁵N-naphthotriazole in mouse blood was prepared in the range 0.01 - 30 µM. To do this, working solutions (WS) of ¹⁵N-naphthotriazole in DMSO were prepared by serial dilution of a 10mM stock solution to the following final concentrations:

WS (µM) 150 / 50 / 15 / 5 / 1.5 / 0.5 / 0.15 / 0.05

2 mL of skeletal muscles were protein precipitated using 6 mL of acetonitrile; 200 µL of deproteinized matrix were spiked with 10 µL of each working solution to create a calibration curve for ¹⁵N-naphthotriazole in the matrix to the following final nominal concentrations:

CC (μM) 30 / 10 / 3 / 1 / 0.3 / 0.1 / 0.03 / 0.01

Four working solutions of $\text{Na}^{15}\text{NO}_2$ in water were prepared by serial dilution of a 10 mM stock solution to the following final concentrations:

WS (μM) 250 / 25 / 2.5 / 0

- 5 693 μL of deproteinized matrix were spiked with 7 μL of each working solution to create spikes of ^{15}N -naphthotriazole in the matrix to the following final nominal concentrations:

CC (μM) 10/ 1 / 0.1 / 0

- 10 To obtain ^{15}N -nitrite to ^{15}N -naphthotriazole conversion, 200 μL of skeletal muscles samples or spiked samples were spiked with 10 μL of 1,2-diaminonaphthalene 10^{-3}M in HCl 0.5M and shaken for 20 minutes at room temperature. To stop conversion, 10 μL of NaOH 1M and 10 μL of DMSO were added to all the samples.

The analytical conditions were as follows:

	LC system	Agilent 1200 series				
15	Column	Synergi Fusion-RP 50 x 2.0 mm (2.5 μ) @ 40°C				
	Mobile Phase	A. water + 0.1% formic acid				
		B. acetonitrile + 0.1% formic acid				
	Flow Rate	0.5mL/min				
	Gradient profile:					
20	Time:	0.0	0.5	1.5	2.0	2.1
	A(%):	95	95	0	0	95
	B(%):	5	5	100	100	5
	Injection Volume	2 μL				
	Mass Spectrometer	API4000				
25	Ionization mode	ESI positive				
	Acquisition mode	MRM - m/z 171.10 / 115.00				

The raw data (average peak area at each time point) were interpolated using Analyst 1.4 software to obtain the concentration of ^{15}N -naphthotriazole at each time

point. The concentration of ^{15}N -naphthotriazole in each sample was corrected by the ^{15}N -nitrite-to- ^{15}N -naphthotriazole conversion factor (CF) to obtain the original ^{15}N -nitrite concentration in the samples. The CF for each concentration of the spiked samples was calculated as:

5

$$\text{CF} = \frac{[^{15}\text{NAT}]_{\text{SPx}} - [^{15}\text{NAT}]_{\text{SP0}}}{C_x} * 100$$

where $[^{15}\text{NAT}]_{\text{SPx}}$ is ^{15}N -naphthotriazole concentration in the samples spiked at concentration x

$[^{15}\text{NAT}]_{\text{SP0}}$ is ^{15}N -naphthotriazole concentration in the unspiked samples

10

C_x is the nominal concentration of ^{15}N -nitrite in the sample

The ^{15}N -nitrite-to- ^{15}N -naphthotriazole conversion factor (CF) used is the average of the CF obtained at the three different concentrations of the spiked samples.

SigmaPlot 9.0 software was used to calculate the AUCs for the experiment using the extrapolated concentrations at each time point.

15

The results reported in Table 4 showed that compound (1) (dosed as (^{15}N -Compound 1) at the dose of 100 mg/kg generates a good amount of ^{15}N -labelled nitrites, which is a demonstration that the compound is able to release NO in the target organs.

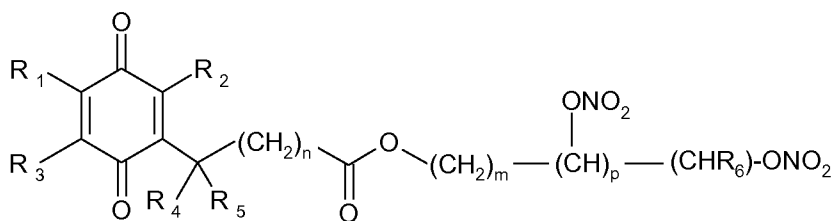
Table 4: ^{15}N-labelled nitrites measurement in CD1 mice tibialis anterior muscles following oral treatment	
Treatment	^{15}N -labelled nitrites content (μM)
15N-labelled Compound (1)	0.51 $\pm$ 0.19

20

Data are presented as mean $\pm$ SEM

CLAIMS

1. A compound of formula (I)



(I)

or stereoisomers thereof, wherein:

R₁ is methyl;

R₂ is H or methyl;

R₃ is methyl;

R₄ and R₅ are methyl and n is 1, or

R₄ is H, R₅ is selected from phenyl, para-fluorophenyl, para-methoxyphenyl, para-isopropylphenyl, para-trifluoromethyl phenyl or para-methylphenyl and n is 2;

m is an integer from 1 to 10;

p is 0 or 1;

R₆ is H or methyl.

2. A compound of formula (I) according to claim 1 wherein

R₂ is methyl;

R₄ and R₅ are methyl and n is 1.

3. A compound of formula (I) according to claim 2 wherein

p is 0;

R₆ is H.

4. A compound of formula (I) according to claim 2 wherein p is 1.

5. A compound of formula (I) according to claim 1 wherein

R₂ is methyl;

R₄ is H, R₅ is selected from phenyl, para-fluorophenyl,

para-methoxyphenyl, para-isopropylphenyl,

para-trifluoromethylphenyl or para-methylphenyl and n is 2.

6. A compound of formula (I) according to claim 5 wherein

p is 0;

5 R₆ is H.

7. A compound of formula (I) according to claim 5 wherein p is 1.

8. A compound of formula (I) according to any one of claims 1 to 7 wherein m is an integer from 1 to 6.

9. A compound of formula (I) according to claim 1 selected from:

10 4-(nitrooxy)butyl-3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclo hexa-1,4-dienyl)butanoate;

6-(nitrooxy)hexyl-3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclo hexa-1,4-dienyl)butanoate;

15 6-(nitrooxy)hexyl 4-phenyl-4-(2,4,5-trimethyl-3,6-dioxocyclo hexa-1,4-dienyl)butanoate;

6-(nitrooxy)hexyl-4-(4-fluorophenyl)-4-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dienyl)butanoate;

4-(nitrooxy)butyl-4-phenyl-4-(2,4,5-trimethyl-3,6-dioxocyclo hexa-1,4-dienyl)butanoate;

20 4-(nitrooxy)butyl-4-(4-fluorophenyl)-4-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dienyl)butanoate;

5,6-bis(nitrooxy)heptyl-3-methyl-3-(2,4,5-trimethyl-3,6-dioxo cyclohexa-1,4-dienyl)butanoate;

25 (5S,6R)-5,6-bis(nitrooxy)heptyl-3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dienyl)butanoate;

(S)-5,6-bis(nitrooxy)hexyl-3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dienyl)butanoate

(R)-5,6-bis(nitrooxy)hexyl-3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-

dienyl)butanoate

5,6-bis(nitrooxy)hexyl-4-phenyl-4-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-

dienyl)butanoate

and stereoisomers thereof.

- 5 10. A compound of formula (I) according to any of claims 1-9 for use as medicament.
11. A compound according to any one of claims 1 to 9 for use as nitric oxide donating agent.
12. A compound according to any one of claims 1 to 9 for use in the treatment of pulmonary arterial hypertension.
- 10 13. A compound according to any one of claims 1 to 9 for use in the treatment of Sick cell disease.
14. A compound according to any one of claims 1 to 9 for use in the treatment of systemic sclerosis and scleroderma.
15. A compound according to any one of claims 1 to 9 for use in the treatment of
- 15 muscular dystrophies.
16. A compound according to any one of claims 1 to 9 for use in the treatment of Duchenne's muscular dystrophy and Becker's muscular dystrophy.
17. A compound according to any one of claims 1 to 9 for use in the treatment of vascular dysfunctions.
- 20 18. A pharmaceutical formulation comprising a compound of formula (I) according to any one of claims 1 to 9 and a pharmaceutical acceptable carriers and/or excipients.
19. A pharmaceutical formulation comprising a compound of formula (I) according to any of claims 1 to 9 and a therapeutic agent selected from non-steroidal anti-inflammatory drugs, a steroidal anti-inflammatory drugs, an endothelin receptor antagonist or
- 25 hydroxyurea.

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2013/076555

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07C69/608 C07C203/04
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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A	GB 2 349 385 A (TEIJIN LTD [JP]) 1 November 2000 (2000-11-01) cited in the application figure 1; compound 5 page 6, line 12 - page 9, line 23 -----	1-19
A	DONATELLA BOSCHI ET AL: "NO-Donor Phenols: A New Class of Products Endowed with Antioxidant and Vasodilator Properties", JOURNAL OF MEDICINAL CHEMISTRY, vol. 49, no. 10, 1 May 2006 (2006-05-01), pages 2886-2897, XP055078079, ISSN: 0022-2623, DOI: 10.1021/jm0510530 Scheme 2,3; page 2888 page 2891; table 1; compounds 35,37,41 ----- -/-	1-19



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

17 April 2014

Date of mailing of the international search report

15/05/2014

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Patteux, Claudine

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2013/076555

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	WO 2012/037665 A1 (ORAL DELIVERY TECHNOLOGY LTD [CA]; FARBER MICHAEL [CA]) 29 March 2012 (2012-03-29) the whole document -----	1-19
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