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Supporting Information

Oxidation of cholesterol and *O*-protected derivatives by the environmental pollutant $NO_2^{\cdot}$

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Contents

1. Experimental Section

1.1 General procedures

^{1}H and ^{13}C spectra were recorded on either an Agilent MR-400 NMR spectrometer [400 MHz (^{1}H), 100.55 (^{13}C)] or on a Varian Unity Inova 500 spectrometer [499.688 MHz (^{1}H), 125.646 (^{13}C)], in deuterated chloroform ($CDCl_3$). Chemical shifts are reported in ppm (δ) using residual $CHCl_3$ as reference (δ = 7.26 ppm for ^{1}H NMR, δ = 77.16 ppm for ^{13}C NMR). Where possible, the assignment of the chemical shifts was confirmed through other NMR techniques, including DEPT, HSQC and COSY.

HRMS was conducted by ionising the samples via ESI into a Thermo-Finnigan LTQ FT-ICR hybrid mass spectrometer or an Agilent 6520 LC/Q-TOF mass spectrometer with an electrospray ionising source.

IR spectra were recorded on a Perkin-Elmer Spectrum One FTIR spectrometer. Samples were recorded neat, without solvent or preparation.

The crude products were purified by silica column chromatography, utilising ca. 60 g of dry silica per 1 g of crude product mixture. The eluting solvent consisted of mixtures of petroleum ether and ethyl acetate. Melting points were recorded with EZ-Melt Automated Melting Point Apparatus MPA120 from Standford Research Systems.

1.2 General procedure for the reactions with $NO_2^\bullet$

The reactions were performed at room temperature by adding a measured excess amount of liquid $NO_2^\bullet$ (0.5 mL, ca. 16 mmol, condensed at -10 ^{o}C) to 0.75 – 1 mmol of the cholesterol substrate.

For the reactions performed in dichloromethane or acetonitrile (20 – 100 mL), liquid $NO_2^\bullet$ (0.5 mL) was injected directly into the solution containing the starting material (Figure S1, left). The mixture was stirred for 20 – 120 mins at room temperature. The reaction was quenched by addition of saturated aqueous $NaHCO_3$ solution, the layers separated and the aqueous phase was extracted with dichloromethane. The combined organic layers were dried over sodium sulphate and concentrated in vacuo to give a crude product as a yellow viscous oil, which was purified by column chromatography

For the reactions performed in the absence of solvent, the liquid $NO_2^\bullet$ was injected into an upright vial inside a 500 mL flask, which was sealed with a septum and a contained the solid substrate (Figure S1, right). Complete evaporation of $NO_2^\bullet$ created an atmosphere of $NO_2^\bullet$, which reacted with the starting material at the gas/solid interface. In some experiments, the starting material was moistened with 40 equivalents of water.

a) Dry work-up: Any unreacted $NO_2^\bullet$ was removed in a stepwise procedure by first blowing nitrogen gas over the oil/solid reaction mixture. The reaction mixture was then repeatedly taken up in dichloromethane and concentrated by blowing a stream of nitrogen through the solution or by rotary evaporation. The resulting oil was dried under high vacuum.

b) Aqueous work-up: The reaction was quenched by addition of saturated aqueous $NaHCO_3$ solution (20 mL), extracted with dichloromethane or ethyl acetate (3 x 20 mL). The combined organic fractions were washed with water (20 mL) and brine (20 mL), dried over magnesium sulphate and concentrated in vacuo.

Crude reaction mixtures were analyzed directly by TLC and NMR before being purified by silica column chromatography.

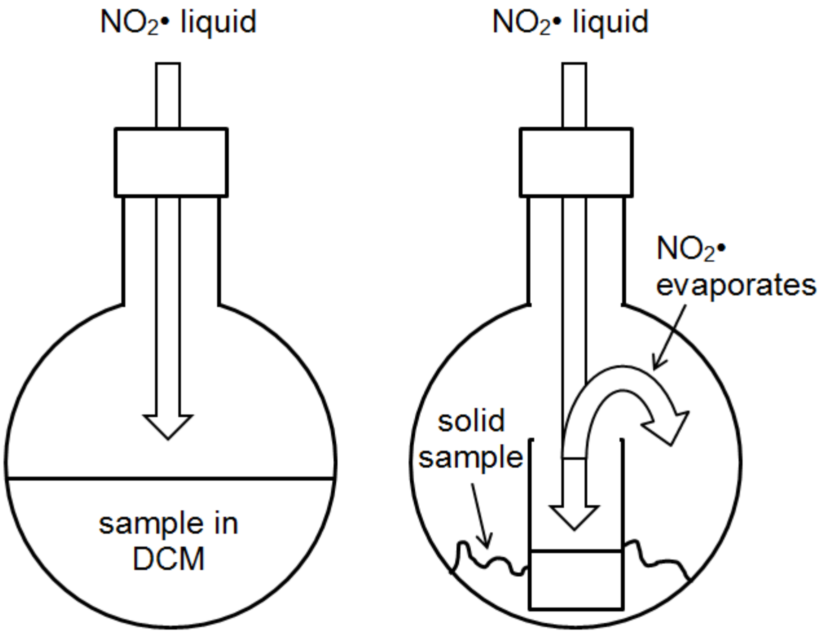


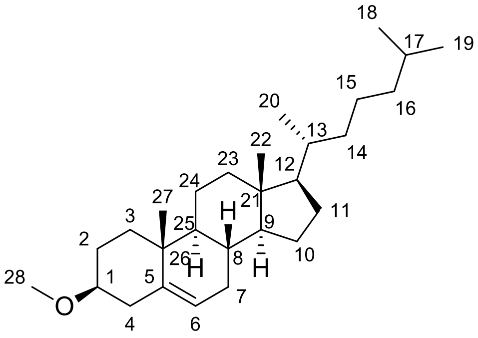
Figure S1. Experimental set-up for the reaction of *O*-protected cholesterol **1b** and **1c** with $NO_2^\bullet$. Left: reaction in dichloromethane; right: gas/solid reaction.

Cautionary Note: *N*-Nitroimines are known carcinogens. Proper precautions against inhalation of the vapours and contact with skin should always be maintaine

2 Spectroscopic data

2.1 Cholesterylmethyl ether (1b)

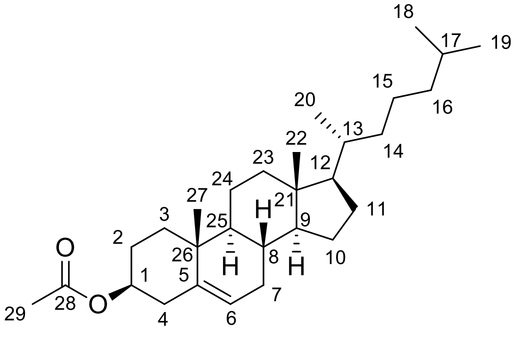
1b was synthesized according to literature procedures.[1]



^{1}H NMR (400 MHz, $CDCl_3$): δ 5.32 (dt, *J* = 5.2, 1.9 Hz, 1H, *H*-6), 3.32 (s, 3H, *H*-28), 3.03 (tt, *J* = 11.3, 4.4 Hz, 1H, *H*-1), 2.36 (ddd, *J* = 13.1, 4.7, 2.2 Hz, 1H, *H*-4), 2.18 – 2.09 (m, 1H, *H*-4), 2.02 – 1.95 (m, 1H, *H*-7), 1.95 – 1.75 (m, 5H, *H*-2, 3, 7), 1.61 – 0.99 (m, 13H), 0.97 (s, 3H, *H*-27), 0.89 (d, *J* = 6.5 Hz, 3H, *H*-20), 0.84 (dd, *J* = 6.6, 1.8 Hz, 6H, *H*-18, 19), 0.65 ppm (s, 3H, *H*-22). ^{13}C NMR (101 MHz, $CDCl_3$): δ 140.85 (*C*-5), 121.56 (*C*-6), 80.34 (*C*-1), 56.77, 56.16, 55.57, 50.19, 42.31, 39.78, 39.51, 38.69, 37.18, 36.87, 36.19, 35.78, 31.94, 31.88, 28.23, 28.00, 24.28, 23.83, 22.81, 22.55, 21.07, 19.36, 18.71, 11.85 ppm.

2.2 Cholesteryl acetate (1c)

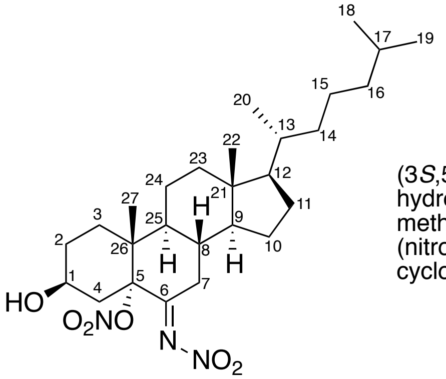
1c was synthesized according to literature procedures.[2]



^{1}H NMR (400 MHz, $CDCl_3$): δ 5.39 – 5.34 (m, 1H, *H*-6), 4.64 – 4.56 (m, 1H, *H*-1), 2.38 – 2.23 (m, 2H, *H*-4), 2.02 (s, 3H, *H*-29), 2.01 – 1.03 (m, 26H), 1.01 (s, 3H), 1.00 – 0.92 (m, 3H), 0.91 (d, *J* = 6.5 Hz, 3H), 0.86 (dd, *J* = 6.6, 2.2 Hz, 6H, *H*-18, 19), 0.67 ppm (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$):

δ 170.47 (*C*-28), 139.63 (*C*-5), 122.62 (*C*-6), 73.96 (*C*-1), 56.68, 56.13, 50.03, 42.30, 39.73, 39.51, 38.12, 36.99, 36.58, 36.18, 35.79, 31.89, 31.86, 28.23, 28.01, 27.77, 24.28, 23.83, 22.82, 22.56, 21.42, 21.03, 19.30, 18.71, 11.85 ppm.

2.3 5-Nitrato-6-nitroimino cholesterol (2a)



(3*S*,5*R*,8*S*,9*S*,10*R*,13*R*,14*S*,17*R*,*E*)-3-hydroxy-10,13-dimethyl-17-((*R*)-6-methylheptan-2-yl)-6-(nitroimino)hexadecahydro-1*H*-cyclopenta[*a*]phenanthren-5-yl nitrate

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.95 (tt, *J* = 11.4, 4.8 Hz, 1H, *H*-1), 2.47 (ddd, *J* = 15.1, 4.7, 2.0 Hz, 1H, *H*-4), 2.40 (dd, *J* = 13.2, 4.1 Hz, 1H, *H*-4), 2.09 – 1.92 (m, 4H), 1.91 – 1.57 (m, 6H), 1.56 – 1.43 (m, 4H), 1.40 – 1.18 (m, 8H), 1.19 – 1.10 (m, 4H), 1.08 (s, 3H), 1.04 – 0.97 (m, 1H), 0.89 (d, *J* = 6.5 Hz, 3H), 0.86 (dd, *J* = 6.6, 1.8 Hz, 6H), 0.66 ppm (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 176.13 (*C*-6), 93.14 (*C*-5), 66.48 (*C*-1), 56.08, 55.99, 46.56, 45.53, 43.49, 39.59, 39.36, 38.98, 36.17, 35.78, 33.30, 32.60, 31.13, 30.07, 28.15, 28.08, 23.92, 22.94, 22.70, 21.83, 18.72, 15.02, 12.24 ppm. HRMS (ESI): $C_{27}H_{46}N_3O_6^+$ ($[M+H]^+$), calcd. 508.3387, found 508.3401, $C_{27}H_{49}N_4O_6^+$ ($[M+NH_4]^+$), calcd. 525.3652, found 525.3637. IR (neat): ν_{max} 831, 1290, 1310, 1574, 1638, 2869, 2948 cm^{-1}. Colourless powder, m.p. 75 – 78 ^{o}C.

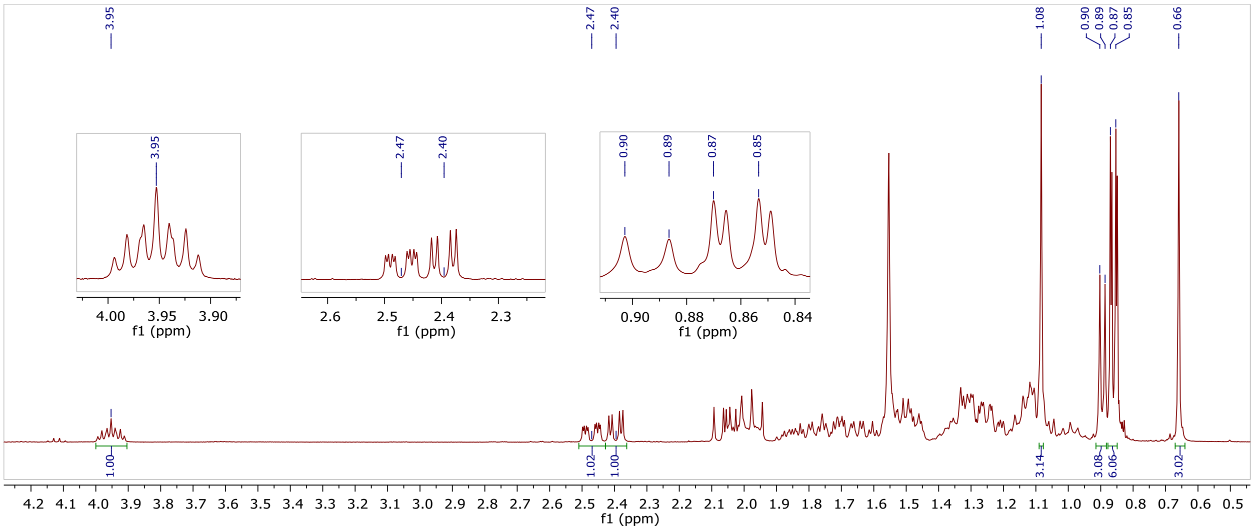


Figure S2. ^{1}H NMR spectrum of 5-nitrato-6-nitroimino cholesterol (**2a**).

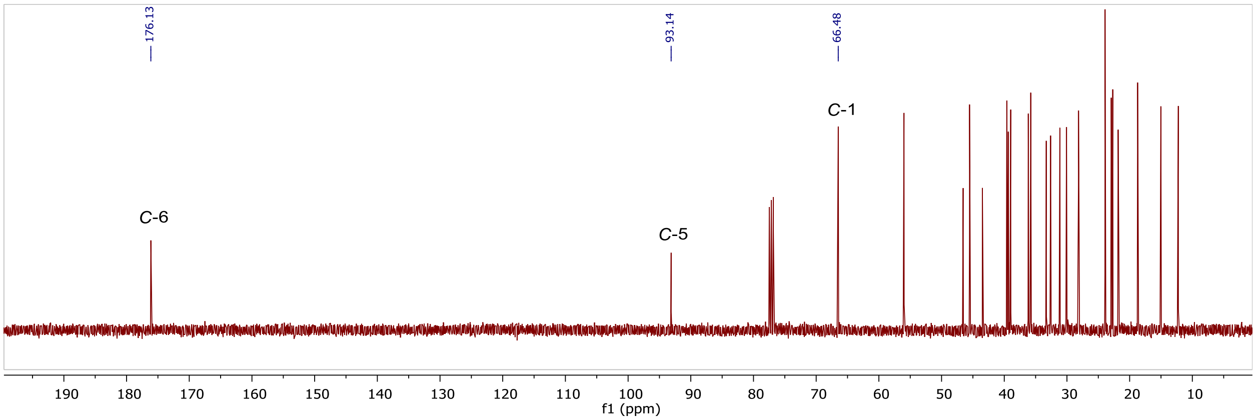
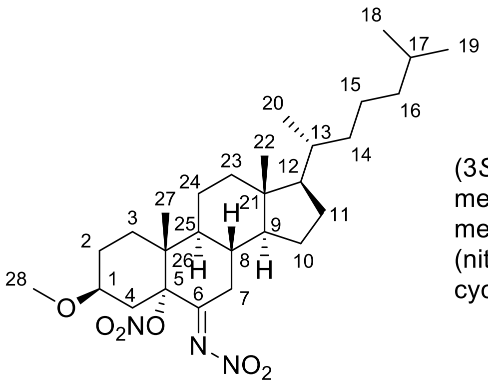


Figure S3. ^{13}C NMR spectrum of 5-nitrato-6-nitroimino cholesterol (**2a**).

2.4 5-Nitrato-6-nitroimino cholesterylmethyl ether (2b)



(3*S*,8*S*,9*S*,10*R*,13*R*,14*S*,17*R*,*E*)-3-methoxy-10,13-dimethyl-17-((*R*)-6-methylheptan-2-yl)-6-(nitroimino)hexadecahydro-1*H*-cyclopenta[*a*]phenanthren-5-yl nitrate

^{1}H NMR (400 MHz, CDCl$_3$): δ 3.42 (tt, *J* = 11.4, 4.8 Hz, 1H, *H*-1), 3.33 (s, 3H, *H*-28), 2.55 (ddd, *J* = 15.2, 4.6, 1.8 Hz, 1H), 2.39 (dd, *J* = 13.3, 4.1 Hz, 1H), 2.07 – 1.89 (m, 4H), 1.88 – 1.56 (m, 5H), 1.54 – 1.39 (m, 4H), 1.38 – 1.16 (m, 7H), 1.16 – 1.06 (m, 4H), 1.04 (s, 3H), 1.03 – 0.93 (m, 2H), 0.87 (d, *J* = 6.5 Hz, 2H), 0.83 (dd, *J* = 6.7, 1.8 Hz, 3H), 0.63 ppm (s, 3H). ^{13}C NMR (101 MHz, CDCl$_3$): δ 176.01 (*C*-6), 92.99 (*C*-5), 74.90 (*C*-1), 56.39 (*C*-28), 56.15, 56.05, 46.84, 45.63, 43.54, 39.65, 39.42, 39.02, 36.23, 35.83, 33.38, 31.02, 29.39 (*C*-4), 28.20, 28.14, 26.79, 23.97, 23.00, 22.75, 21.86, 18.78, 15.01, 12.28 ppm. HRMS (ESI): $C_{28}H_{48}N_3O_6^+$ ([M+H]$^+$), calcd. 522.35373, found 522.35370. IR (neat): ν_{max} 1290, 832, 1104, 1310, 1574, 1639, 2865, 2950 cm^{-1}. Colourless crystals, m.p. 123 - 124 ^{o}C.

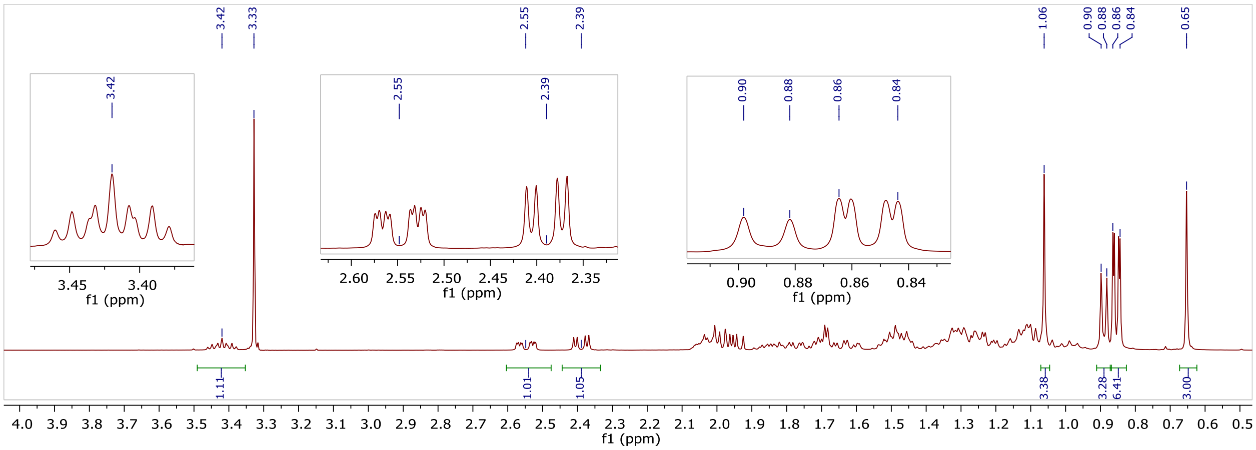


Figure S4. ^{1}H NMR spectrum of 5-nitrato-6-nitroimino cholesterylmethyl ether (**2b**).

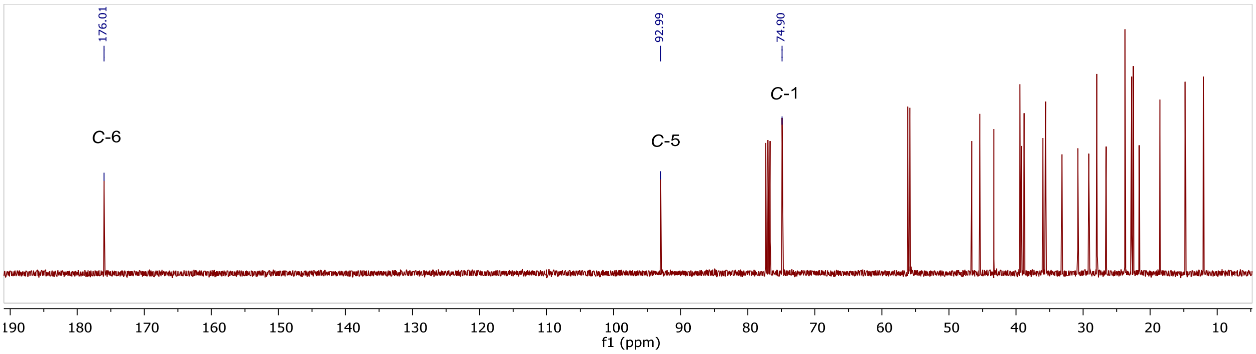
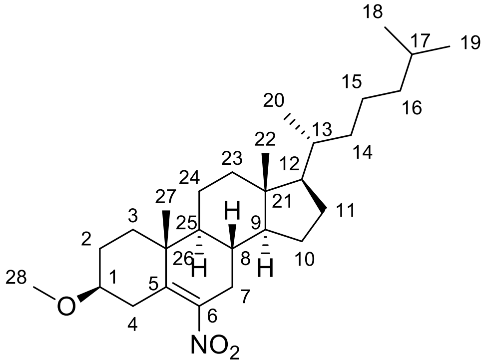


Figure S5. ^{13}C NMR spectrum of 5-nitrato-6-nitroimino cholesterylmethyl ether (**2b**).

2.4 6-Nitrocholesterylmethyl ether (3b)



(3*S*,8*S*,9*S*,10*R*,13*R*,14*S*,17*R*)-3-methoxy-10,13-dimethyl-17-((*R*)-6-methylheptan-2-yl)-6-nitro-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthrene

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.33 (s, 3H, *H*-28), 3.12 (tt, *J* = 11.1, 4.3 Hz, 1H, *H*-1), 2.87 (ddd, *J* = 14.0, 4.6, 2.1 Hz, 1H, *H*-4), 2.52 (ddd, *J* = 17.1, 5.5, 2.9 Hz, 1H, *H*-7), 2.17 – 0.93 (m, 23H), 0.90

(d, *J* = 6.5 Hz, 3H, C*H*$_3$), 0.85 (dd, *J* = 6.6, 1.8 Hz, 6H, *H*-18, 19), 0.67 ppm (s, 3H, *H*-22). ^{13}C NMR (101 MHz, CDCl$_3$): δ 146.20 (*C*-5), 138.47 (*C*-6), 78.84 (*C*-1), 56.10, 55.97, 55.91, 49.11, 42.28, 39.45, 39.33, 38.14, 36.55, 36.09, 35.69, 33.28, 31.61, 31.56, 28.08, 27.97, 27.46, 24.12, 23.80, 22.79, 22.53, 20.98, 19.52, 18.66, 11.77 ppm. HRMS (ESI): $C_{28}H_{47}NO_3Na$ ([M+Na]$^+$), calcd. 468.34482; found 468.34366. IR (neat): ν_{max} 1100, 1381, 1518, 2869, 2944 cm^{-1}. Yellow oil.

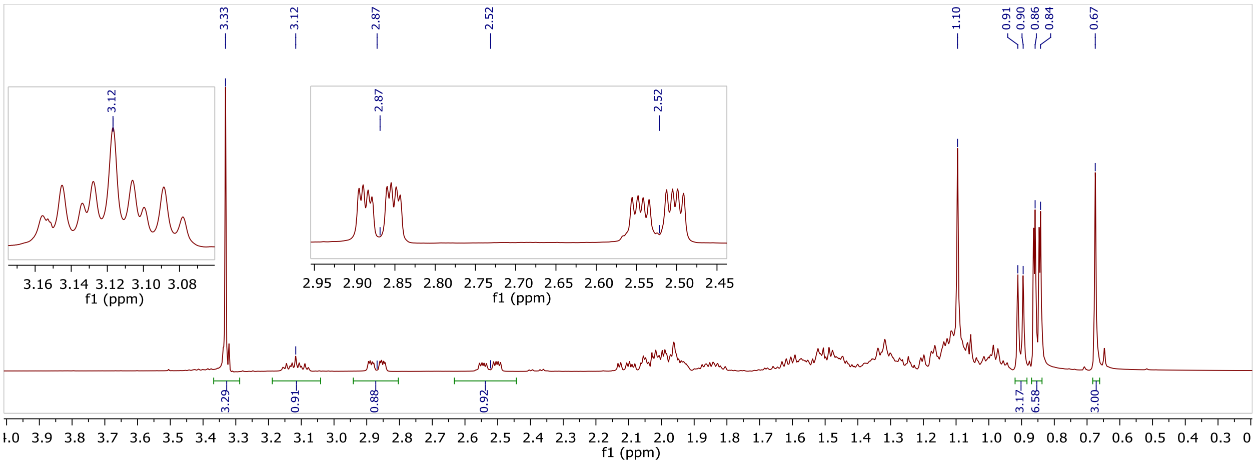


Figure S6. ^{1}H NMR spectrum of 6-nitrocholesterylmethyl ether (**3b**).

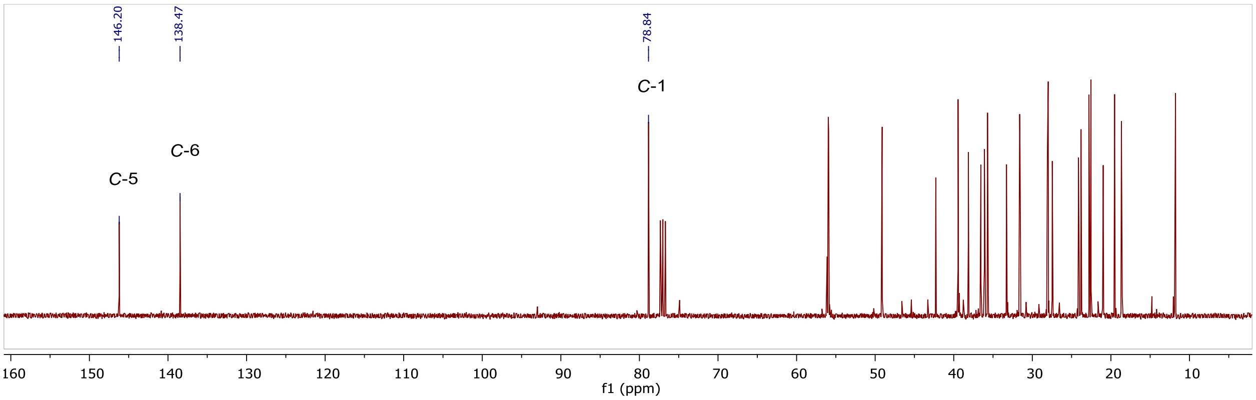
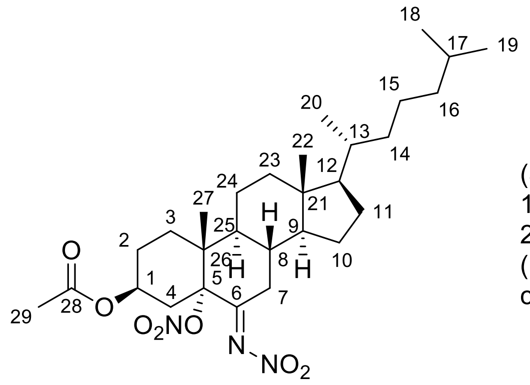


Figure S7. ^{13}C NMR spectrum of 6-nitrocholesterylmethyl ether (**3b**).

2.5 5-Nitrato-6-nitroimino cholesterylacetate (2c)

*Note: This compound was very difficult to obtain in pure form. It is likely that the starting material (cholesteryl acetate **1c**) was contaminated with trace amounts of water, resulting in rapid conversion to 6-nitrocholesteryl acetate (**3c**) in the presence of* NO_2*•.*



(3*S*,5*R*,8*S*,9*S*,10*R*,13*R*,14*S*,17*R*,*E*)-
10,13-dimethyl-17-((*R*)-6-methylheptan-
2-yl)-6-(nitroimino)-5-
(nitrooxy)hexadecahydro-1*H*-
cyclopenta[*a*]phenanthren-3-yl acetate

^{1}H NMR (400 MHz, $CDCl_3$): δ 4.94 (tt, *J* = 11.4, 5.0 Hz, 1H, *H*-1), 2.48 (ddd, *J* = 15.0, 4.8, 1.9 Hz, 1H, *H*-4), 2.36 (dd, *J* = 13.3, 4.0 Hz, 1H, *H*-2), 2.25 – 0.90 (m, 32H), 0.86 (dd, *J* = 6.4, 2.8 Hz, 3H, *H*-20), 0.82 (dt, J = 6.7, 2.2 Hz, 6H, *H*-18, 19), 0.73 – 0.55 ppm (m, 3H, C*H*$_3$). ^{13}C NMR (101 MHz, $CDCl_3$): δ 175.79 (*C*-6), 170.16 (*C*-28), 92.27 (*C*-5), 68.69 (*C*-1), 55.86, 55.70, 46.40, 45.20, 43.27, 39.40, 39.11, 38.75, 35.98, 35.58, 33.01, 30.60, 28.84, 27.93, 25.84, 23.72, 23.69, 22.76, 22.74, 22.50, 21.55, 21.06, 18.51, 14.69, 11.97 ppm. HRMS (ESI) *m/z* (%): $C_{29}H_{48}N_3O_7$ ([M+H]$^+$), calcd. 550.34868; found 550.34955. IR (neat): ν_{max} 834, 1060, 1244, 1288, 1310, 1567, 1642, 1762, 2950 cm^{-1}. Colourless oil.

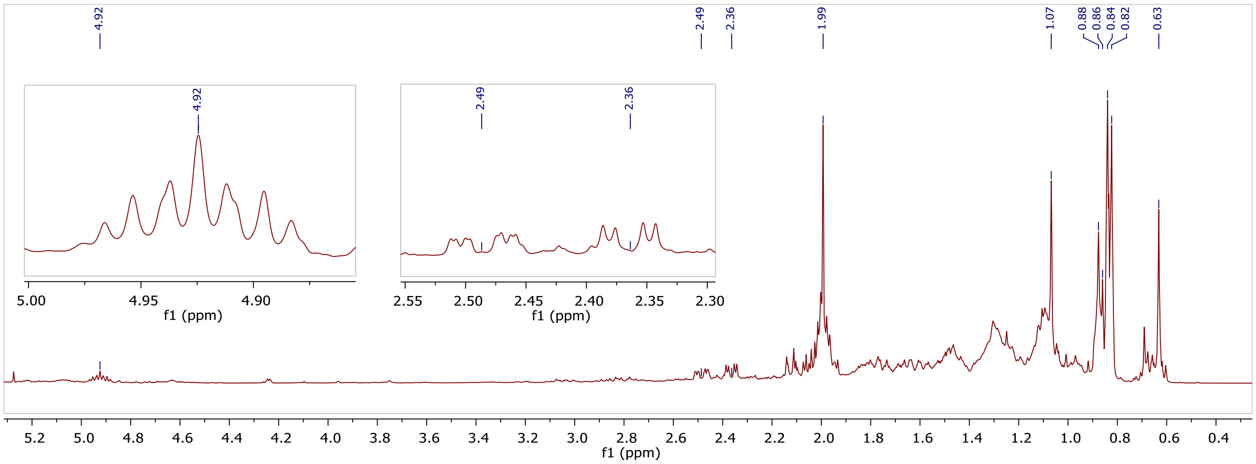


Figure S8. ^{1}H NMR spectrum of 5-nitrato-6-nitroimino cholesterylacetate (**2c**).

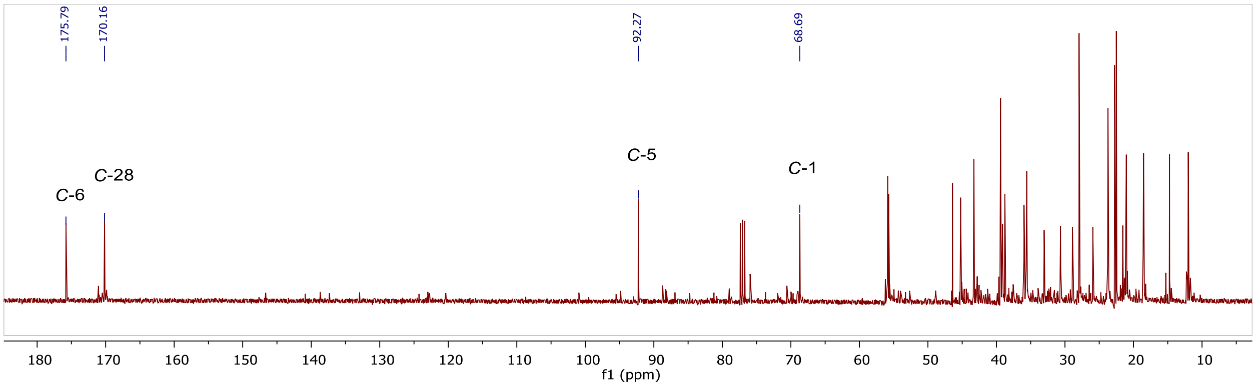
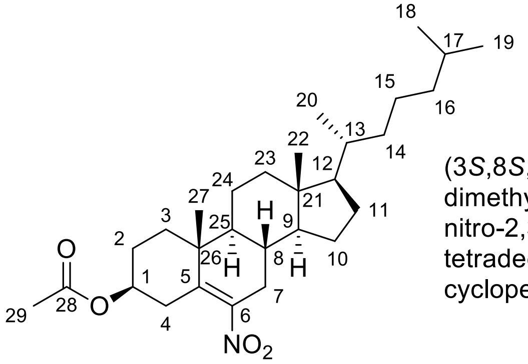


Figure S9. ^{13}C NMR spectrum of 5-nitrato-6-nitroimino cholesterylacetate (**2c**).

2.6 6-Nitrocholesteryl acetate (3c)



(3*S*,8*S*,9*S*,10*R*,13*R*,14*S*,17*R*)-10,13-dimethyl-17-((*R*)-6-methylheptan-2-yl)-6-nitro-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl acetate

^{1}H NMR (400 MHz, CDCl$_3$): δ 4.72 – 4.58 (tt, 1H, *H*-1), 2.77 (ddd, *J* = 14.0, 4.8, 2.0 Hz, 1H, *H*-4), 2.47 (ddd, *J* = 17.3, 5.5, 2.8 Hz, 1H, *H*-7), 2.35 – 0.94 (m, 20H), 0.90 (d, *J* = 6.5 Hz, 3H, C*H*$_3$), 0.85 (dd, *J* = 6.6, 2.0 Hz, 6H, *H*-18, 19), 0.68 ppm (s, 3H, C*H*$_3$). ^{13}C NMR (101 MHz, CDCl$_3$): δ 170.07 (*C*-28), 146.68 (*C*-6), 137.37 (*C*-5), 71.92 (*C*-1), 56.00, 55.93, 48.86, 42.27, 39.45, 39.25, 37.81, 36.08, 35.69, 33.27, 31.55, 31.03, 28.07, 27.98, 26.89, 24.09, 23.79, 22.79, 22.53, 21.25, 20.92, 19.68, 18.65, 11.78 ppm. HRMS (ESI); $C_{58}H_{94}N_2O_8Na$ [2M+Na]$^+$, calcd. 969.69024, found 969.68402; $C_{29}H_{48}NO_4Na$ [M+Na]$^+$, calcd. 496.34756, found 496.33850. IR (neat): ν_{max} 1034, 1239, 1365, 1583, 1708, 2868, 2950 cm^{-1}. Yellow oil.

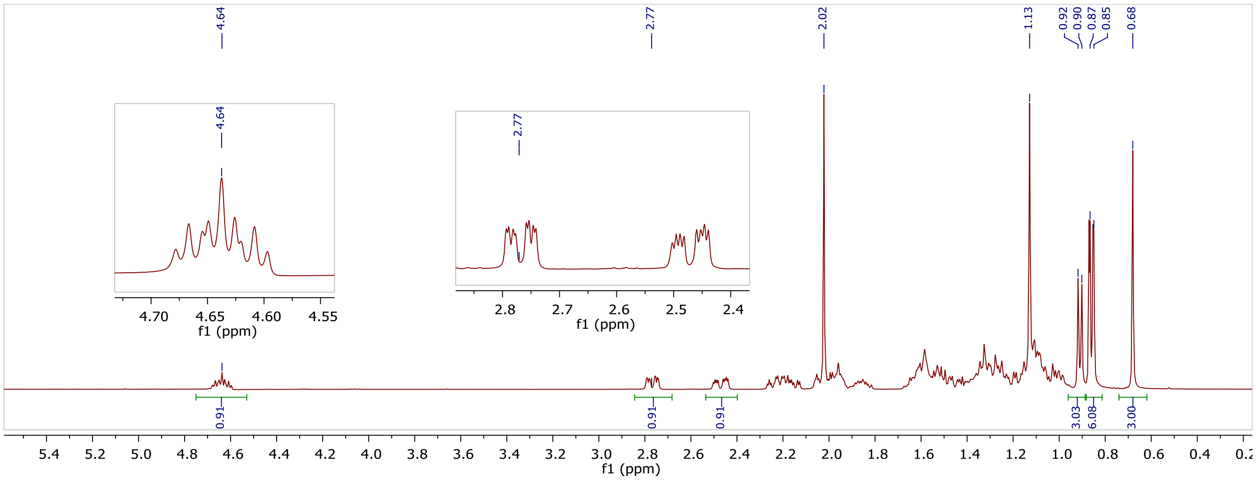


Figure S10. ^{1}H NMR spectrum of 6-nitrocholesteryl acetate (**3c**).

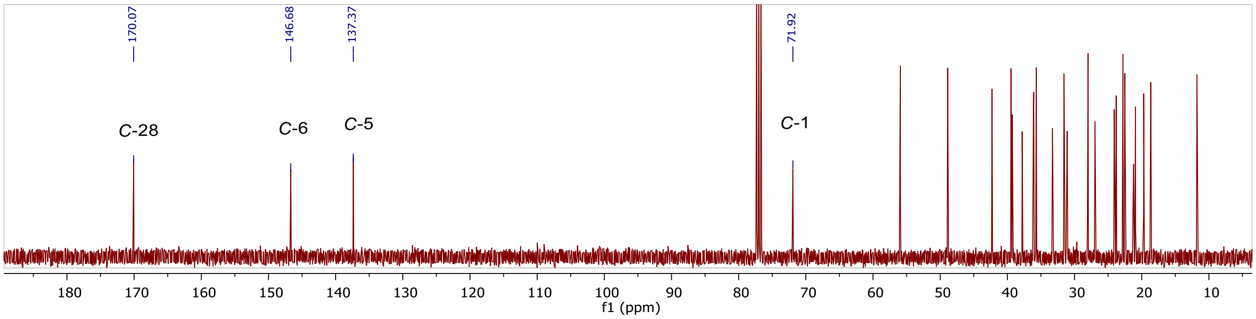


Figure S11. ^{13}C NMR spectrum of 6-nitrocholesteryl acetate (**3c**).

3. Mechanistic Studies

3.1 Formation of nitroimine nitrate 2b: Reaction of 1b in the NMR tube

Liquid $NO_2^{\bullet}$ (0.5 mL) was dissolved in acetonitrile (4.5 mL). From this solution 0.5 mL was taken and injected into an NMR tube containing cholesterylmethyl ether (**1b**) (0.075 mmol, 30 mg) in $CDCl_3$. The tube was inverted several times and directly analyzed by NMR after 20 minutes of reaction time. (Note that the reaction would have proceeded for these 20 minutes plus the time taken to run sufficient scans of the sample, e.g. about 10 minutes).

The results in Figure S12 show that the nitroimine nitrate **2b** is the only product formed under different experimental conditions (under exclusion of moisture).

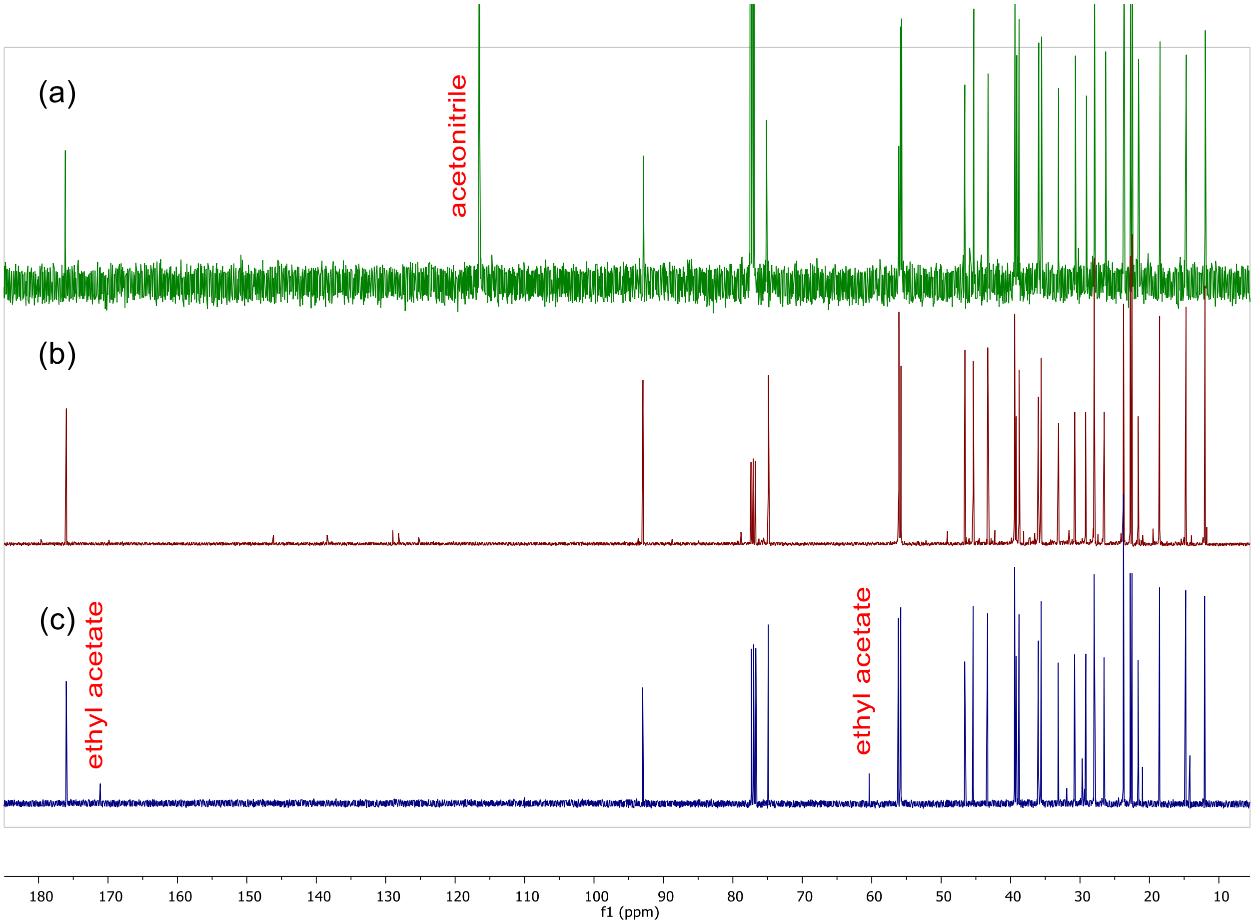


Figure S12. ^{13}C NMR spectra for the reaction of cholesterylmethyl ether (**1b**) with $NO_2^{\bullet}$ under different conditions. (a) Reaction in the NMR test tube after 20 minutes. (b) Reaction in dichloromethane after 10 minutes. (c) Reaction at the solid/gas interface after 3 days.

3.2 Formation of 6-nitrocholesterol 3c: Exposure of a mixture of 2c and 3c to $NO_2^{\bullet}$ in the presence of moisture

A 1:1 mixture of nitroimine nitrate **2c** (NIN) and nitro vinyl derivative **3c** (NV) totaling 0.75 mmol was re-exposed to 0.5 mL of liquid $NO_2^{\bullet}$ and 40 equivalents of water for 3 days, using the experimental set-up shown in Figure S1, right.

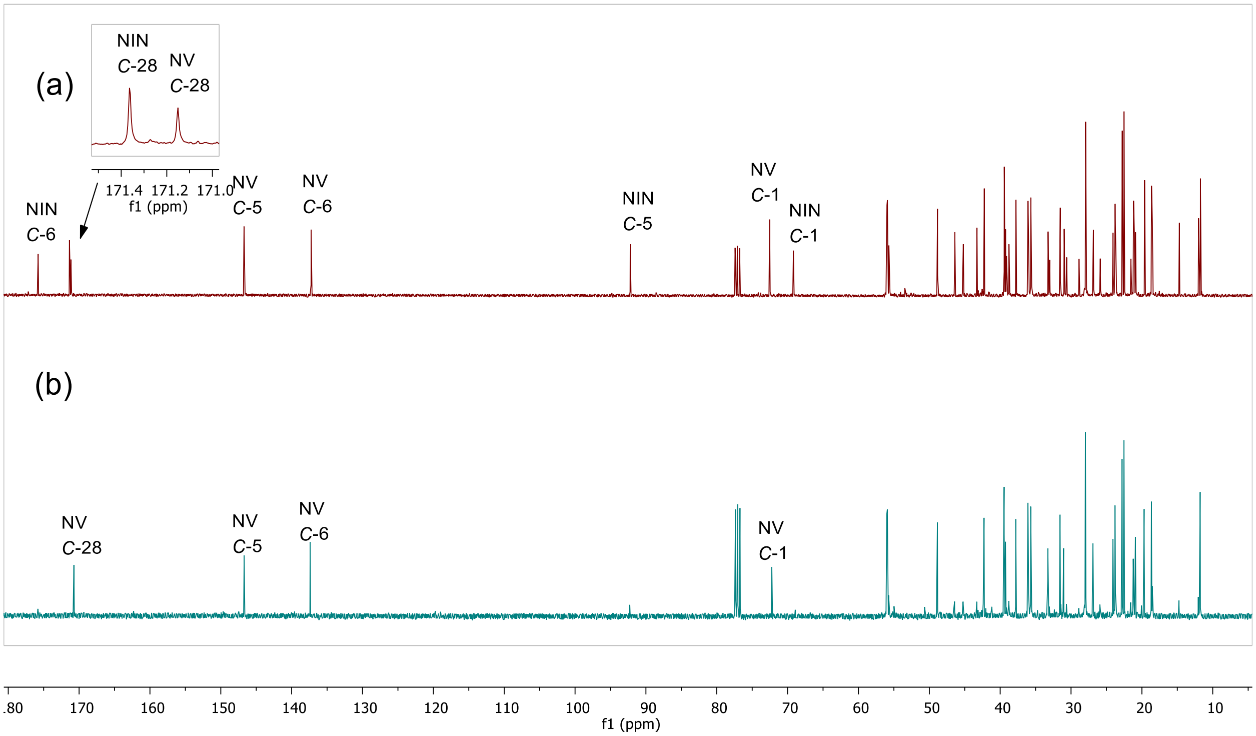


Figure S13. ^{13}C NMR spectra for the reaction of a 1:1 mixture of **2c** (NIN) and **3c** (NV) with $NO_2^{\bullet}$ in the presence of moisture. (a) Before exposure; (b) after exposure to $NO_2^{\bullet}$ and water for 3 days.

4. Crystallographic Data

Intensity data for **2b** were collected with an Oxford Diffraction SuperNova CCD diffractometer using Mo-Kα microsource radiation (graphite crystal monochromator λ = 0.71073). The temperature during the data collection was maintained at 130.0(1). Structure solution, and refinement were implemented within the WingX suite of programs. [3,4]

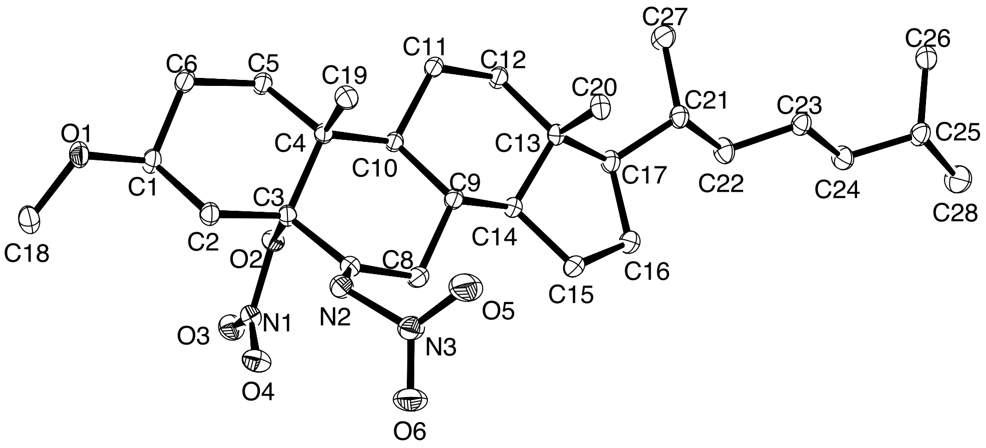


Figure S14. Thermal ellipsoid for **2b**, atoms are at the 20% probability level.

Crystal data for **2b** $C_{28}H_{47}N_3O_6$ *M* = 521.68, *T* = 130.0(2) K, λ = 0.71073 Å, Monoclinic, space group $P2_1$ *a* = 11.5615(3)*, b* = 10.1140(3) *c* =12.3397(3)*, Å*, β = 100.223(3)° *V* 1420.01(7) *Å*^{3}, Z = 4, D_c = 1.220 Mg M^{-3} μ(Mo-Kα) 0.085 mm^{-1}, F(000) = 568. Crystal dimensions 0.49 x 0.43 x 0.20 mm. 19248 reflections measured, 8954 independent reflections (R_{int} = 0. 03) the final R was 0.049 [I > 2σ(I)] and *w*R(F^2) was 0.1379 (all data).

Table 1. Crystal data and structure refinement for **2b**.

Identification code	shelx	
Empirical formula	C28 H47 N3 O6	
Formula weight	521.68	
Temperature	130.0(1) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P 21	
Unit cell dimensions	a = 11.5615(3) Å	α= 90°.
	b = 10.1140(3) Å	β= 100.223(3)°.
	c = 12.3397(3) Å	γ = 90°.
Volume	1420.01(7) Å^{3}	
Z	2	
Density (calculated)	1.220 Mg/m^{3}	
Absorption coefficient	0.085 mm^{-1}	
F(000)	568	
Crystal size	0.4955 x 0.4323 x 0.2067 mm^{3}	
Theta range for data collection	3.338 to 36.636°.	
Index ranges	-18<=h<=19, -10<=k<=16, -20<=l<=19	
Reflections collected	19248	
Independent reflections	8954 [R(int) = 0.0322]	
Completeness to theta = 25.242°	99.6 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	1.00000 and 0.73209	
Refinement method	Full-matrix least-squares on F^{2}	
Data / restraints / parameters	8954 / 1 / 340	
Goodness-of-fit on F^{2}	1.061	
Final R indices [I>2sigma(I)]	R1 = 0.0490, wR2 = 0.1259	
R indices (all data)	R1 = 0.0642, wR2 = 0.1379	
Absolute structure parameter	1.4(6)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.477 and -0.353 e.Å^{-3}	

5. Computational data

The calculation for the reaction **10** → **11** were carried out using the Gaussian 09 program.[5] Geometry optimizations and vibrational frequency analyses for all ground and transition state structures were performed at the M06-2X/6-311++G** level of theory. The transition state showed only one imaginary frequency. Free energies given in this work include zero-point vibrational energy correction (ZPE), which was not scaled.

10

```
1\1\GINC-R107\FOpt\RM062X\6-311++G(d,p)\C3H7N2O3(1+)\ROOT\06-Nov-2015\
 0\\# M062X/6-311++G** scf=(qc,direct) nosymm freq=noraman opt=(z-matri
 x,calcfc,,maxcycle=500)\\freq&geom\\1,1\C,-2.5209410278,0.0963993506,-
 0.5704989302\H,-2.0413046776,-0.8269017706,-0.8692209476\H,-3.58178015
 16,0.2104462332,-0.7533596384\C,-1.8594480023,1.0932116413,0.004552344
 5\N,-0.4177720275,0.8960033636,0.1303233569\O,0.2553537815,0.088399244
 9,-0.7192490755\H,0.518415984,0.6320370614,-1.4819173426\O,-0.32421998
 27,1.5823167072,2.2227245229\N,0.2024096715,1.1581624286,1.2472672404\
 C,-2.3843049512,2.4283240603,0.4389775919\H,-3.3583243752,2.5729311964
 ,-0.0262436103\H,-2.5049941646,2.4922922074,1.5200557253\H,-1.72646341
 94,3.2361127266,0.1108164398\O,1.5184599615,0.9541319631,1.1875055594\
 H,1.8500586706,1.1772655963,2.08022283\\Version=ES64L-G09RevD.01\HF=-4
 53.1604518\RMSD=0.000e+00\RMSF=1.672e-05\Dipole=0.524208,0.2631401,-0.
 3801287\Quadrupole=4.5519717,-2.5158506,-2.0361211,-0.9376059,5.314360
 9,2.6471733\PG=C01 [X(C3H7N2O3)]\\@
```

Sum of electronic and thermal Free Energies = -453.076587 Hartrees

TS 10 → 11

```
1\1\GINC-R91\FTS\RM062X\6-311++G(d,p)\C3H7N2O3(1+)\ROOT\09-Nov-2015\0\
 \# M062X/6-311++G** scf=(qc,direct) nosymm freq=noraman opt=(ts,noeige
 ntest,calcfc,z-matrix,maxcycle=500)\\freq&geom\\1,1\C,-2.5369024345,0.
 0954246316,-0.5653284163\H,-2.0483904613,-0.7762074118,-0.9797231205\H
 ,-3.6196919853,0.1205883934,-0.5598884339\C,-1.8620373095,1.1525327568
 ,-0.1074590574\N,-0.4373405965,1.2011548737,-0.2746092483\O,0.06938405
 24,0.1198347883,-0.8411177063\H,0.9046346323,0.3890429748,-1.257396833
 \O,-0.2302624723,1.6730299109,2.2834098666\N,0.2255585342,1.1955123474
 ,1.3326916865\C,-2.4486890401,2.4248204743,0.4344616719\H,-3.452253710
 6,2.5535209537,0.0313154158\H,-2.5216640551,2.4113439802,1.5223746449\
 H,-1.8478996559,3.2845409317,0.1334477524\O,1.498984923,0.8718147803,1
 .2560500389\H,1.9317155793,1.0741746149,2.1137287387\\Version=ES64L-G0
 9RevD.01\HF=-453.1379565\RMSD=0.000e+00\RMSF=1.126e-05\Dipole=0.905824
 4,-0.1162132,0.1921098\Quadrupole=5.9885135,-4.212194,-1.7763196,-0.75
 2424,4.7200942,4.3033399\PG=C01 [X(C3H7N2O3)]\\@
```

ν_{imag} = -351.1580 cm^{-1}

Sum of electronic and thermal Free Energies = -453.057283 Hartrees

[11 – H_2O – NO^+]

```
1\1\GINC-R119\FOpt\RM062X\6-311++G(d,p)\C3H7N2O3(1+)\ROOT\11-Nov-2015\
 0\\# M062X/6-311++G** scf=(qc,direct) nosymm freq=noraman opt=(calcfc,
 z-matrix,maxcycle=500)\\freq&geom\\1,1\C,-2.6765996035,0.3087349849,-1
 .6766848063\H,-2.5840440275,-0.765945461,-1.785193353\H,-3.2447484275,
 0.8555674328,-2.419936429\C,-2.1199498445,0.9579558432,-0.6504687204\N
 ,-1.4041195304,0.1698092481,0.323692611\O,-1.1142731837,-0.9534983899,
 0.144928599\H,1.6159516671,0.6139929731,0.0052443273\O,-0.866175968,1.
 6554079764,2.5012100405\N,-0.5707378939,0.6952492342,2.0803833662\C,-2
 .1750061445,2.4171703911,-0.3277089457\H,-2.6745943766,2.9509510757,-1
 .1343026163\H,-2.7378752338,2.6020321481,0.5906150411\H,-1.1679184564,
 2.8248720537,-0.2200270816\O,1.0381303291,1.3330629858,0.2840977458\H,
 1.5610826927,2.1327095017,0.162380215\\Version=ES64L-G09RevD.01\HF=-45
 3.1921436\RMSD=0.000e+00\RMSF=6.389e-06\Dipole=0.2773776,0.6464077,0.1
 197029\Quadrupole=3.7044494,-3.5578538,-0.1465956,-2.8718498,2.6991249
 ,2.9602691\PG=C01 [X(C3H7N2O3)]\\@
```

Sum of electronic and thermal Free Energies = -453.118274 Hartrees

6. References

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