Supporting Information

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Persulfate Reaction in a Hair-Bleaching Formula: Unveiling the Unconventional Reactivity of 1,13-Diamino-4,7,10-Trioxatridecane

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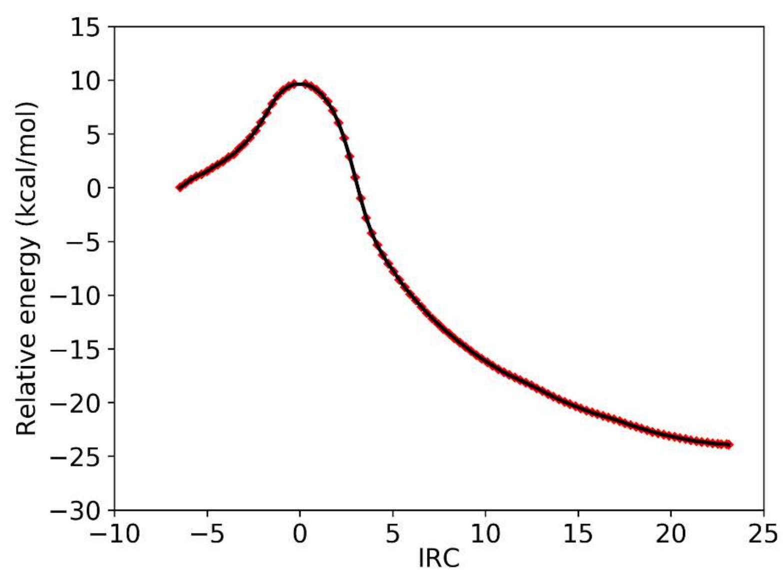


Figure S1. Intrinsic reaction coordinate (IRC) calculation for the transition state at B3LYP/6-311g++(d,p) level of theory

Experimental Details

General Information

All reagents and solvents were purchased from Sigma-Aldrich, Merck (Darmstadt, Germany) and Biosolve (Dieuze, France). All solvents used were LC-MS grade. water was obtained from a Milli-Q A10 water system (Merck) and acetonitrile was purchased from Merck. OLAPLEX Bond Multiplier Nº 1 was purchased from Amazon (WA, USA) and Blondor Multi-Blond powder, an ammonium and sodium persulfate-containing powder, and Welloxon Perfect 12%, a hydrogen peroxide-containing cream were obtained from Wella (Darmstadt, Germany). 1,13-diamino-4,7,10-trioxatridecane (ether-diamine) was purchased from BASF (Ludwigshafen, Germany) and further purified through distillation. Final purity was assessed via gas chromatography and resulted in 99,87%. High resolution mass spectrum was recorded on a LTQ Orbitrap XL instrument (ThermoFisher Scientific, MA, USA). NMR-spectra were recorded on a Bruker (MA, USA) instrument (^{1}H 300 MHz, ^{13}C 75.4 MHz, for the isolated azanyl ester derivative; ^{1}H 500 MHz for the reaction of the ether-diamine in model bleach).

General Instrument Operation for ether-diamine quantification

A liquid chromatography-electrospray ionization-tandem mass spectrometry method was developed for the determination of the ether-diamine. A Waters (CA, USA) Acquity UPLC was used for the isocratic separation of the analyte. Chromatographic separation was performed using a Waters Atlantis T3 3µm, 3.0x150 mm column. The injection volume was 5 µL. The autosampler and the column oven were kept at room temperature. Mobile phase A was Water containing 0.1% of trifluororacetic acid (TFA) and mobile phase B was acetonitrile containing 0.1% of TFA. Elution was isocratic 80% A and 20% B over the total run time of 4 minutes. The flow rate was 0.50 ml/min. A ratio of 95:5 water/MeOH was used as weak wash and 5:95 as strong wash.

Target analyte was detected and quantified by a Waters Acquity TQ detector, that was set to acquire in Multiple Reaction Monitoring (MRM) mode with a dwell time of 300 msec. The electro spray ionization source was operated in the positive mode. The analyte was detected in its protonated form ($M+H^{+}$) by the first quadrupole (Q1), and the suitable fragment ion was selected by the third quadrupole (Q3). The MS/MS, ion source and interface parameters were manually optimized for the ether-diamine. The transition 221.3/204.3 was used as primary channel and 221.3/164.3 as confirmatory. Collision energy was 20 V for both transitions. The following settings were applied for ion source and interface: Capillary 3.00 kV, Cone 30 V, Extractor 2 V, RF Lens 0.5 V, Source Temperature 120 ºC, Desolvation Temperature 350 ºC, Desolvation Gas Flow 900 L/hr, Cone 120 L/hr.

MassLynx v4.1 software (Waters) was used for acquisition and processing of the LC-MS/MS data. The HPLC/SRM peaks for the ether-diamine (primary and confirmatory) are integrated and evaluated against the calibration curve. Calibration curves were prepared from a minimum of 6 different dilutions. A 1/X-weighted linear regression analysis for the primary transition was performed for the observed peaks as a function of analyte concentration. The concentration of each analyte in the calibration standards, QC samples, and samples is then back-calculated using the generated calibration curve. The peak areas of the primary and the confirmatory transition were tabulated for the standards and samples. Confirmatory ratio (P/C) was calculated by the ratio of the area of the primary (P) and the confirmatory (C) transitions. ether-diamine quantification was verified, being the P/C ratio of a sample within ±5% of the mean P/C ratio of the associated calibration curve.

Experimental details for the quantification of ether-diamine in Olaplex

Two stock solutions of ether-diamine were prepared in water containing 0.1% of TFA. Stock solution 1 (1006.9 ng/mL) was used to prepare a 7 points calibration curve, as described in table S1:

Table S1. Example of calibration curve preparation.

Name	Vol. of Stock Solution 1 (µL)	Vol. of LC-MS water 0.1% TFA (µL)	Final Concentration (ng/mL)
Standard 1	100	900	100.7
Standard 2	150	850	151.0
Standard 3	200	800	201.4
Standard 4	250	750	251.7
Standard 5	300	700	302.1
Standard 6	400	600	402.8
Standard 7	500	500	503.5

Stock Solution 2 (1026.2 ng/mL) was used to prepare three quality controls, as described in Table S2:

Table S2. Quality controls preparation.

Name	Volume of Stock Solution 2 (µL)	Volume of LC-MS water 0.1% TFA (µL)	Resulting Concentration (ng/mL)
QC A	150	850	153.9
QC B	200	800	205.2
QC C	400	600	410.5

Two solutions of OLAPLEX Bond Multiplier Nº 1 (1988.2 and 2036.2 ng/mL) in water 0.1% TFA were prepared for analysis. Two spiking solutions (ether-diamine spiked in Olaplex) were prepared in water 0.1% TFA in order to evaluate the recovery of the analytical method, at the final concentrations of Olaplex + ether-diamine: 1. 1958.8 ng/mL + 208.4 ng/mL; 2. 1966.6 ng/mL + 221.0 ng/mL.

In the end, to 1 mL solution of each analyte were added 200 μL of acetonitrile 0.1% TFA, in order to improve the chromatographic performance of the method. This last dilution is neglected in the calculations, since it has been applied to all measured analytes.

Quantification of standards, quality controls, spiking solutions and samples were performed as described above. The ether-diamine content in Olaplex resulted in 9.15±0.04%. The recovery, expressed as the ratio between the ether-diamine measured and the ether-diamine actually present in the spiking solutions (ether-diamine in Olaplex + spiked ether-diamine) in percent was 94.8±0.01%. The relative accuracy for the three quality controls was between 100.0-106.5%. The correlation coefficient for the calibration curve linear regression r^2 was 0.999.

Experimental details for the stability study of the ether-diamine contained in Olaplex in a model bleach

One stock solution of ether-diamine was prepared in water 0.1% TFA (1087.9 ng/mL), from which a 6 points calibration curve (108.8 – 544.0 ng/mL) was prepared, in a similar way as already described in Table S1. The stability test was conducted in a model bleach, prepared as described in Table S3.

Table S3. Model bleach preparation and experimental procedure in the analysis of the ether-diamine degradation.

Compound	Weight (g)	volume (mL)	mol weight	mmol	density (g/mL)
water +	38.2	38.2	18	2122	1.000
25% ammonia +	6.8	7.5	17.0	100	0.906
$(NH_4)_2S_2O_8$ +	21	33.9	228.2	92	solid
= Solution A	66	57.0	-	-	1.157*
In the single Erlenmeyer Flask					
Solution A	5.785	5.000	-	-	1.157
H_2O_2 50%	1.514	1.267	34	22.3	1.195
Olaplex	0.384	0.365	-	-	1.053
Ether-diamine (in Olaplex)	0.384*9.15%= 0.036	-	220.3	0.163	1.005
% Olaplex w/w		5%			
Olaplex concentration	57.901 mg/mL				
Ether-diamine concentration	57.901*9.15%= 5.30 mg/mL				

*Density of solution A was evaluated by weighting 1 mL of solution.

5.000 mL of a solution of $(NH_4)_2S_2O_8$ and ammonium hydroxide in water (Solution A) were added to 5 Erlenmeyer flasks (one for each time point: 0, 10, 20, 30 and 40 min), followed by 1.267 mL of H_2O_2. Each flask was capped and put on a multiple hotplate stirrer, set at room temperature and at a rotation speed of 500 rpm. It is important to conduct the experiment using different flasks in order to avoid ammonia evaporation, that would occur through repeated opening of a single flask, needed to withdraw aliquots of the reaction mixture.

For the time point 0 min, 5.000 mL of water 0.1% TFA were added to the mixture of Solution A and H_2O_2 under stirring, followed by 0.365 mL of Olaplex. The resulting solution was diluted 1:10000 in water 0.1% TFA. For time points 10, 20, 30 and 40 min, 0.365 mL of Olaplex were added to the mixture of Solution A and H_2O_2 under stirring. After the appropriate time 5.000 mL of water 0.1% TFA were added to the reaction mixture and the resulting solutions were diluted 1:10000 in water 0.1% TFA.

In the end, to 1 mL solution of each analyte were added 200μL of acetonitrile 0.1% TFA, in order to improve the chromatographic performance of the method. This last dilution is neglected in the calculations, since it was applied to all measured analytes.

The time point 0 min was used to evaluate the recovery of the analytical method, when applied to the reaction mixture. The calculated content of ether-diamine in the measured solution at 0 min is 302.06 ng/mL, considering 9.15% of ether-diamine in Olaplex. The measured amount of ether-diamine at 0 min was 294.8 ng/mL, thus resulting in a recovery of 97.6%. The correlation coefficient for the calibration curve linear regression r^2 was 0.998. Measured concentration of ether-diamine for the other time points, also depicted in Figure 5 of the manuscript, are described in the Table S4:

Table S4. Ether-diamine concentration between 0-40 min.

Time (min)	Ether-diamine (ng/mL)
0	294.8
10	277.5
20	255
30	252.4
40	243.3

Experimental details for the stability study of ether-diamine contained in Olaplex in a commercial bleach (Blondor+Welloxon)

One stock solution of ether-diamine was prepared in water 0.1% TFA (1087.9 ng/mL), from which a 6 points calibration curve (108.8 – 544.0 ng/mL) was prepared, in a similar way as already described in Table S1.

The stability test was conducted according to the usage conditions of OLAPLEX Bond Multiplier Nº 1. [1] The content of ether-diamine was analyzed at 0 and 40 minutes in duplicate. An accurately weighted amount of Blondor and Welloxon (see Table S5), were put into an hairdresser´s bowl and vigorously mixed with an hairdresser´s brush until homogeneity. A volume of ca. 3.75 mL of Olaplex was then added to the bowl and the weight accurately noted. The mixture was again vigorously mixed until homogeneity and a stopwatch was started at this point to count 40 min. For time point 0 min, a volume of ca. 3 mL was withdrawn from the bowl, using a 5 mL syringe. From this aliquot ca. 1 g is transferred to a 50 mL falcon tube and the exact weight noted. Since the mixture tends to capture humidity and swell, the syringe was immediately capped and stored at room temperature and the remaining content used later for the 40 min analysis. Subsequently, 10.0 mL of water were added to the falcon tube and vortexed for 1 min. Then ca. 0.250 mL of the mixture was filtered through a 37 mm acrodisc syringe filter with 1 µm glass fiber media (Pall Corporation). The filtered solution was then diluted 1:1000 in water 0.1% TFA and used for analysis. When the stopwatch counted 40 min, ca. 1 g of the previously stored mixture was transferred to a falcon tube and the sample processed in the same way described previously for time 0 min.

Prior to analysis, to 1 mL solution of each analyte were added 200µL of acetonitrile 0.1% TFA, in order to improve the chromatographic performance of the method. This last dilution is neglected in the calculations, since it has been applied to all measured analytes.

Table S5. Weights of Blondor, Welloxon and Olaplex used to asses stability in a commercial bleach and calculated concentrations.

Time (min)	Blondor (g)	Welloxon (g)	Olaplex (g)	Ether-diamine/Mixture (mg/g)= [(9.15%*Olaplex)/ (Blondor+ Welloxon+Olaplex)]	Mixture in 50mL falcon (g)	Theoretical Conc. Of Ether-diamine in injection vial (ng/mL)
Experiment 1						
0 min	30.059	45.157	3.737	4.331	1.042	451.29
40 min					1.051	455.19
Experiment 2						
0 min II	30.422	45.199	3.598	4.156	1.030	428.07
40 min II					1.028	427.24

The amount of ether-diamine contained in the mixture of OLAPLEX Bond Multiplier Nº 1, Blondor and Welloxon was calculated as described in the Table S5, and then used to calculate the theoretical concentration of ether-diamine in the measured solution.

The ether-diamine degradation over 40 minutes was evaluated by calculating the difference in the concentrations measured at 0 and 40 min, normalized for the weight of the mixture transferred in the falcon tube and expressed as percent of measured ether-diamine at 0 minute:

$$Percent\ of\ Degradation = \frac{X}{(Diamine\ Conc.\, 0\ \mathrm{min} \div Aliquot\ in\ falcon\ tube\ at\ 0\ \mathrm{min})} \times 100$$

Where:

Experiment 1:

$$X = \frac{Diamine\ Conc.\ 0\ min}{Aliquot\ in\ falcon\ tube\ at\ 0\ min} - \frac{Diamine\ Conc.\ 40\ min}{Aliquot\ in\ falcon\ tube\ at\ 40\ min} = \frac{331.5}{1.042} - \frac{313.6}{1.051} = 19.76$$

$$Percent\ of\ Degradation = \frac{19.76}{(331.5\ \div 1.042)} \times 100 = 6.21\%$$

Experiment 2:

$$X = \frac{Diamine\ Conc.\ 0\ min}{Aliquot\ in\ falcon\ tube\ at\ 0\ min} - \frac{Diamine\ Conc.\ 40\ min}{Aliquot\ in\ falcon\ tube\ at\ 40\ min} = \frac{310.7}{1.030} - \frac{285.4}{1.028} = 24.02$$

$$Percent\ of\ Degradation = \frac{24.02}{(310.7\ \div 1.030)} \times 100 = 7.96\%$$

The two time points 0 min were used to evaluate recovery, that was calculated by comparing the measured ether-diamine at time 0 and the calculated theoretical concentration of ether-diamine (Table S5):

Experiment 1: (331.5/451.29)*100= 73.5%; Experiment 2: (310.7/428.07)*100= 72.6%. The correlation coefficient for the calibration curve linear regression r^2 was 0.999.

Instrument operation and experimental details for the semi-quantitative analysis of the azanyl ester derivative

A liquid chromatography-electrospray ionization-tandem Mass Spectrometry method was developed for the determination of the azanyl ester derivative. A Shimadzu Nexera X2 ultra high performance chromatograph (Shimadzu Corp., Kyoto, Japan) that was equipped with two LC-30AD pumps, DGU-20A5r degasser, SIL-30AC autosampler and CTO-30A column oven was used for the gradient separation of the analyte. Chromatographic separation was performed using a Waters Acquity UPLC BEH C18, 1.7 µm, 2.1x150 mm column. The injection volume was 1 µL. The autosampler was kept at 15 ºC and the column oven at 40 ºC. Mobile phase A was water containing 20 mM of ammonium formate and mobile phase B was pure acetonitrile. Elution was isocratic 35% B until 1.8 min, followed by a linear gradient to 95% B at 2.25 min, kept until 5.25 min and then back to 35% B in 0.75 min. Between 6.0 and 8.0 min, 35% B was kept for equilibration. The flow rate was 0.25 mL/min until 1.8 min, after which a linear flow gradient was performed to speed up the column flush and equilibration. The flow rate was increased to 0.40 mL/min at 2.25 min and kept steady for 4.35 min. The flow rate was returned to 0.25 mL/min in 0.15 min and kept steady until 8.0 min. water/MeOH (50:50) was used as needle wash and as pump seal wash. The needle wash was performed before and after aspiration (500 µL).

Target analyte was detected and quantified by a QTRAP 6500+ (Applied Biosystem, Ontario, Canada) detector, that was set to acquire in Multiple Reaction Monitoring (MRM) mode with a dwell time of 100 msec. The electro spray ionization source was operated in the negative mode. The analyte was detected in its de-protonated form ($M-H^-$) by the first quadrupole (Q1), and the suitable fragment ion was selected by the third quadrupole (Q3). The transition 315.0/97.0 was used as primary channel and 315.0/80.0 as confirmatory. The collision energy was -23 V for both transitions. The following settings were applied for ion source and interface: Curtain Gas 20 units, Collision Gas High, Ion Spray Voltage -3500 V, Temperature 700 ºC, Ion Source Gas1 70 units, Ion Source Gas2 70 units, Declustering Potential -30.0 V, Entrance Potential -10.0 V, Collision Cell Exit Potential -9.0 V.

The Analyst 1.6.3 software (Sierra Analytics, CA, USA) was used for the acquisition and the processing of the LC-MS/MS data. The HPLC/SRM peaks for the ether-diamine (primary and confirmatory) was integrated and the peak areas plotted against time. No difference between the profile of the primary and the confirmatory channel was observed.

The experiment was conducted in a model bleach, prepared as described in the following table:

Table S6. Model bleach preparation and Ether-diamine content in the analysis of the azanyl ester derivative.

Compound	Weight (g)	volume (mL)	mol weight	mmol	density (g/mL)
water +	38.2	38.2	18	2122	1.000
25% ammonia +	6.8	7.51	17.0	100	0.906
$(NH_4)_2S_2O_8$+ +	21	33.9	228.2	92	solid
= Solution A					1.157
In the single Erlenmeyer Flask					
Solution A	5.785	5.000			1.157
H_2O_2 50%	1.514	1.267	34	22.3	1.195
Ether-diamine	0.035	0.035	220.3	0.159	1.005
% Ether-diamine w/w		0.48%			
Ether-diamine concentration		5.55 mg/mL			

First, 5.000 mL of a solution of $(NH_4)_2S_2O_8$ and ammonium hydroxide in water (Solution A) were added to 5 Erlenmeyer flasks (one for each time point: 0, 10, 20, 30 and 40 min), followed by 1.267 mL of H_2O_2. Each flask was capped and put on a multiple hotplate stirrer, set at room temperature and at a rotation speed of 500 rpm.

For the time point 0 min, 5.000 mL of water 0.1% formic acid (FA) were added to the mixture of Solution A and H_2O_2 under stirring, followed by 0.035 mL of ether-diamine. The resulting solution was diluted 1:1000 in water 0.1% FA. For time points 10, 20, 30 and 40 min, 0.035 mL of ether-diamine were added to the mixture of Solution A and H_2O_2 under stirring. After the appropriate time 5.000 mL of water 0.1% FA were added to the reaction mixture and the resulting solutions were diluted 1:1000 in water 0.1% FA. In the end, to 1 mL solution of each analyte were added 200µL of acetonitrile, in order to improve the chromatographic performance of the method.

Instrument operation and experimental details for the collection of the mass spectrum of the azanyl ester derivative

A liquid chromatography-electrospray ionization-tandem mass spectrometry method was developed for the determination of the ether-diamine. A Shimadzu Nexera X2 ultra high performance chromatograph equipped as already described above was used for the gradient separation of the analyte. Chromatographic separation was performed using a Waters Acquity UPLC BEH C18, 1.7 µm, 2.1x100 mm column. The injection volume was 2 µL. The autosampler was kept at 15 ºC and the column oven at 40 ºC. Mobile phase

A was water containing 20 mM of ammonium formate and mobile phase B was pure acetonitrile. Elution was isocratic 35% B until 1.2 min, followed by a linear gradient to 95% B at 1.5 min, kept until 3.5 min and then back to 35% B in 0.5 min. Between 4.0 and 5.0 min, 35% B was kept for equilibration. The flow rate was 0.25 mL/min until 1.2 min, after which a linear flow gradient was performed to speed up the column flush and equilibration. The flow rate was increased to 0.50 mL/min at 1.5 min and kept steady for 2.9 min. The flow rate was returned to 0.25 mL/min in 0.10 min and kept steady until 5.0 min. water/MeOH (50:50) was used as needle wash and as pump seal wash. The needle wash was performed before and after aspiration (500 µL).

Mass spectrum was recorded by a QTRAP 6500+ (Applied Biosystem, Ontario, Canada) detector, that was set to acquire in Product Ion (MS2) mode, with 315.0 as parent mass. The electro spray ionization source was operated in the negative mode. The collision energy was -23 V. The following settings were applied for ion source and interface: Curtain Gas 20 units, Collision Gas High, Ion Spray Voltage -3500 V, Temperature 700 ºC, Ion Source Gas1 70 units, Ion Source Gas2 70 units, Declustering Potential -30.0 V, Entrance Potential -10.0 V, Collision Cell Exit Potential -9.0 V.

The sample analyzed was prepared exactly as described in the semi-quantitative analysis of the azanyl ester derivative for the 40 min time point.

Instrument operations and experimental details for the isolation of *in-situ* generated azanyl ester derivative.

A PrepStar SD-1 (Agilent, California, USA) system, equipped with 2X200 mL pump heads, ProStar 325 UV-Vis Detector (Agilent) with high pressure flow cell 4-0.15 mm and a fraction collector FC 204 (Gilson, Wisconsin, USA) was used for the purification of the molecule of interest. Chromatographic separation was performed using a Machery-Nagel (Düren, Germany) NUCLEODUR® VarioPrep, 5µm, 32x250 mm column. The injection volume was 2 mL. Mobile phase A was water containing 0.05% FA and mobile phase B was pure acetonitrile. A linear gradient was applied between time 0.0 and 23.0 min, going from 2% to 35% B, followed by 2 min equilibration at 2% B. The flow rate was 72 mL/min. UV wavelength was 210 nm. The column was kept to room temperature.

To a 5.000 mL aliquot of Solution A, prepared as described in Table S6, were added 1.267 mL of H_2O_2 50%, followed by 0.350 mL of ether-diamine. The reaction mixture was neutralized to pH 4-5 after 2.5h by adding 0.60 mL of FA. The neutralized reaction mixture was filtered through a filter plug (Macherey-Nagel, PA-45/25, 0.45µm) prior to injection and the azanyl ester derivative isolated by preparative HPLC. The collected fraction were concentrated using a rotary evaporator. The final product appeared as a yellow oil.

Experimental details for ^{1}H-NMR analysis of ether-diamine reactivity in a model bleach.

The molar ratio of the different components in the control solution and in the model bleach reaction have been presented in Table 1 of the manuscript. Control solution was prepared by mixing 44.3 mL of deuterated water, 7.51 mL of 25% ammonia solution and 3.62 mL of ether-diamine. An aliquot of this solution was transferred to a NMR tube and analyzed. For the reaction in a model bleach, 28 mL of D_2O were added to a beaker, followed by 7.51 mL of 25% ammonia solution and $(NH_4)_2S_2O_8$ under stirring. After all the solid was dissolved in solution, 16.2 mL of H_2O_2 30% and 3.62 mL of ether-diamine were added. After 1-2 minutes slight gas generation and a light brown coloration were observed. Aliquots from the reaction mixture were transferred to an NMR tube at time 0, 1, 12, 48 and 144 h and the spectrum recorded.

Computational details

All DFT calculation were performed using the Gaussian16 package[2] available at the Marconi HPC cluster of Cineca. Molecular geometries of reactants, products as well as of the transition state (TS) were optimized employing the B3LYP exchange and correlation functional[3] and the 6-311++G(d,p) basis set. Solvent (water) and dispersion effects were taken into account through the Integral Equation Formalism of the Polarizable Continuum Model (PCM)[4] and the GD3BJ model[5] respectively. Harmonic vibrational frequency calculations were performed to verify that the optimized structures were minima (geometries with all positive frequencies) or TS (a geometry with just one negative frequency) and to evaluate the Gibbs free energy. The intrinsic reaction coordinate procedure (IRC) [6] starting from the TS structure was applied to confirm that the reactant and product structures were connected through the TS by the forward and reverse reactions.

References

[1] https://help.olaplex.com/detail/lightener-bleach-foils (accessed as of 09 January 2018)

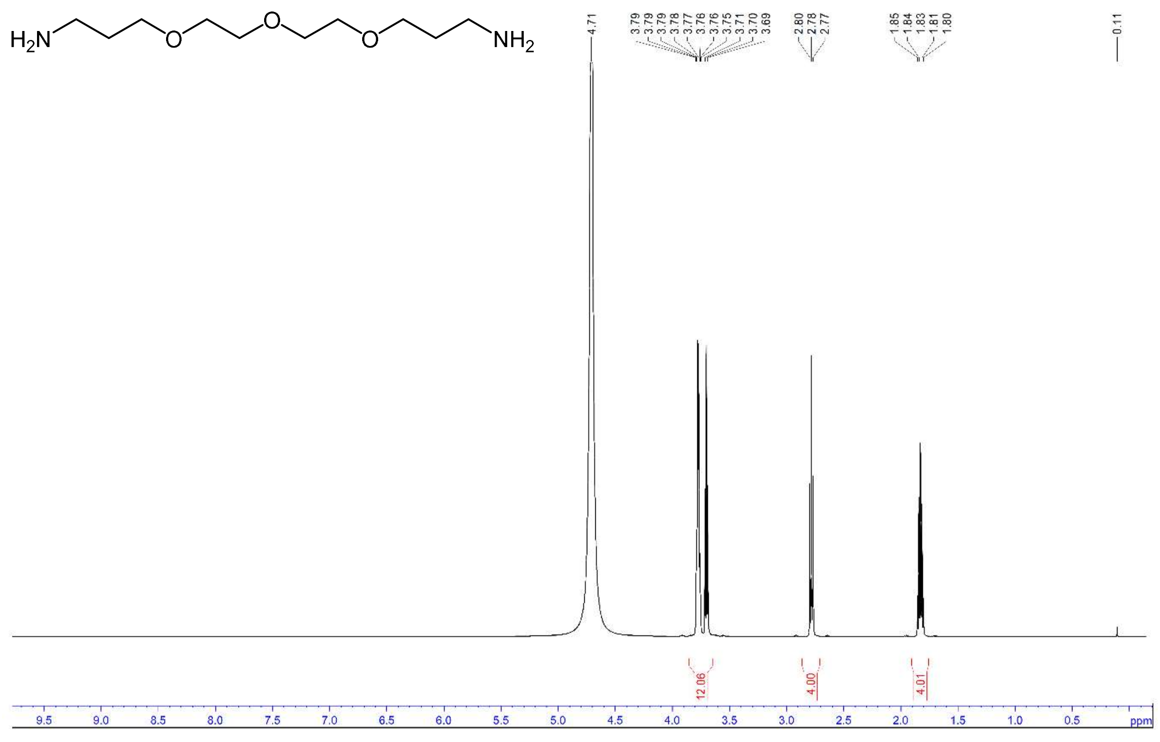
[2] *Gaussian 16, Revision A.03*, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. V. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. J. Bearpark, J. J. Heyd, E. N. Brothers, K. N. Kudin, V. N. Staroverov, T. A. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. P. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2016.

[3] A. D. Becke, *J. Chem. Phys.* **1993**, *98*, 5648-5652.

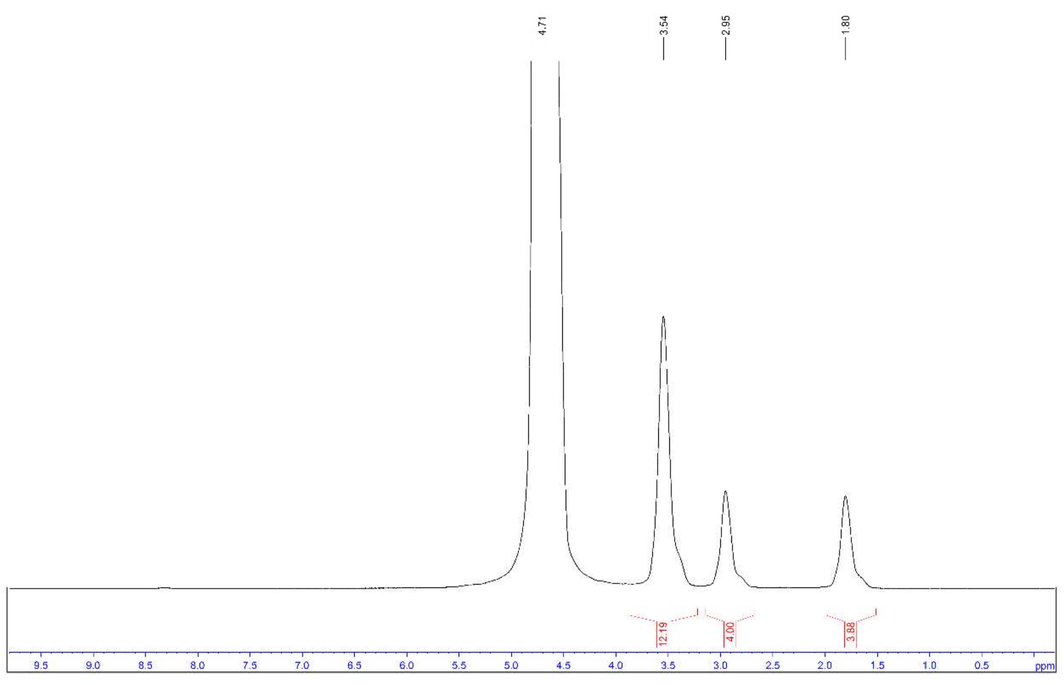
[4] E. Cancès, B. Mennucci, J. Tomasi, *J. Chem. Phys.* **1997**, *107*, 3032-3041.

[5] S. Grimme, S. Ehrlich, L. Goerigk, *J. Comp. Chem.* **2011**, *32*, 1456-1465.

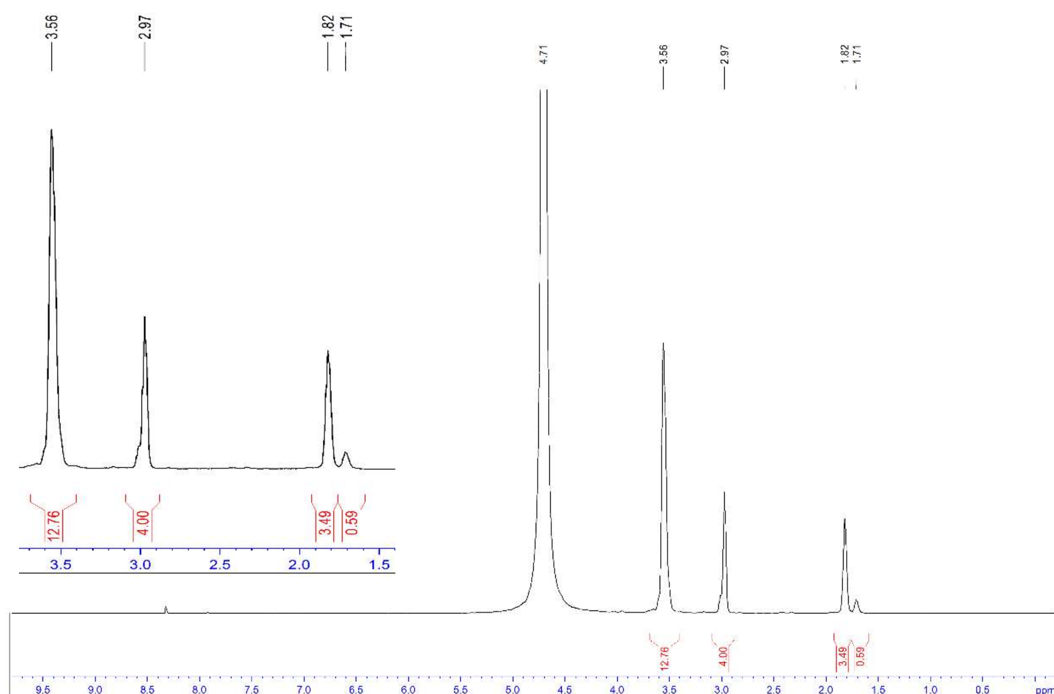
[6] K. Fukui, *Acc. Chem. Res.* **1981**, *14*, 363-368.



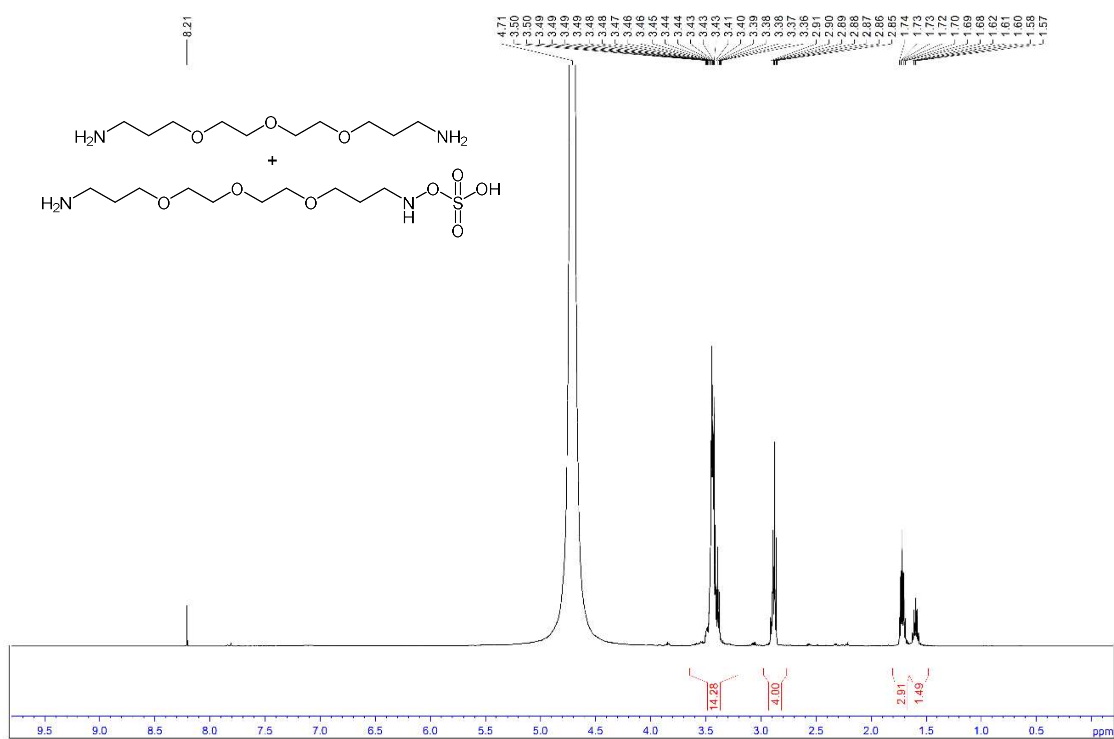
^{1}H-NMR of ether-diamine measured in D_2O/NH_3 (Solution A)



^{1}H-NMR of ether-diamine measured in $D_2O/NH_3/(NH_4)_2S_2O_8/H_2O_2$ at time $\underline{0}$ (Solution B)



^{1}H-NMR of ether-diamine measured in $D_2O/NH_3/(NH_4)_2S_2O_8/H_2O_2$ after 12h (Solution B)



^{1}H-NMR of ether-diamine measured in $D_2O/NH_3/(NH_4)_2S_2O_8/H_2O_2$ after 48h (Solution B)

Zoom of ^{1}H-NMR of ether-diamine measured in $D_2O/NH_3/(NH_4)_2S_2O_8/H_2O_2$ after 48h (Solution B)

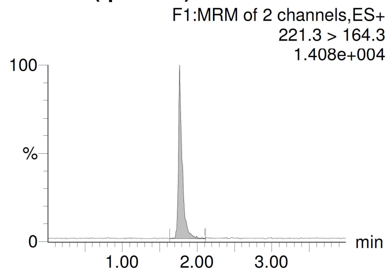
^{1}H-NMR of ether-diamine measured in $D_2O/NH_3/(NH_4)_2S_2O_8/H_2O_2$ after 144h (Solution B)

^{1}H-NMR of the isolated azanyl ester derivative measured in D_2O

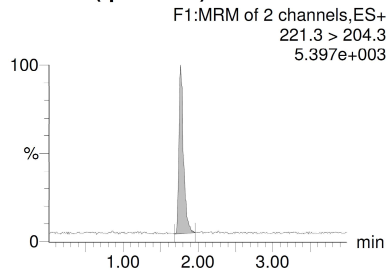
^{1}H-NMR of the isolated azanyl ester derivative measured in D_2O (zoom)

^{13}C-NMR of the isolated azanyl ester derivative measured in D_2O

Diamine (qualifier)

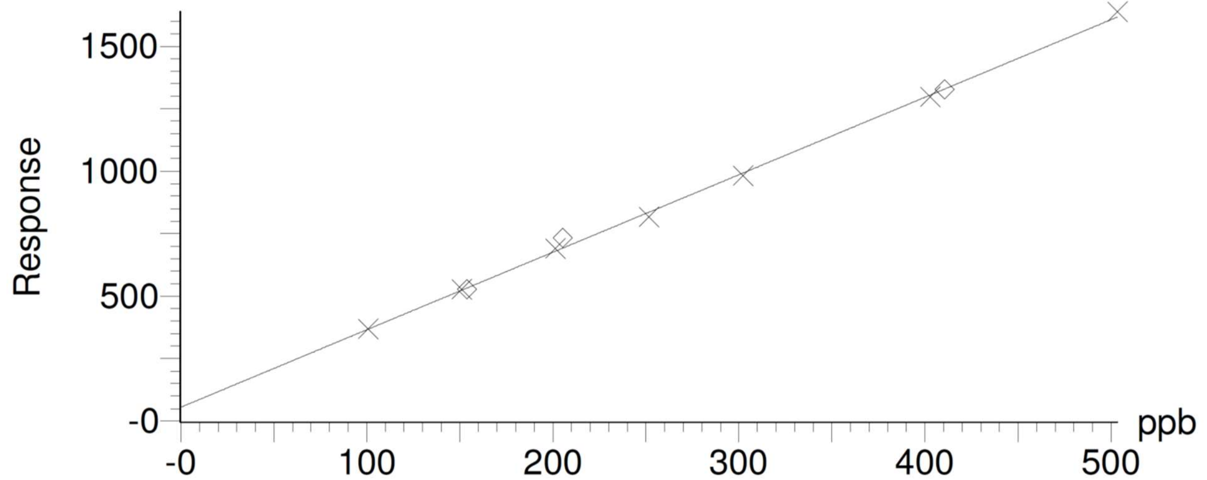


Diamine (quantifier)



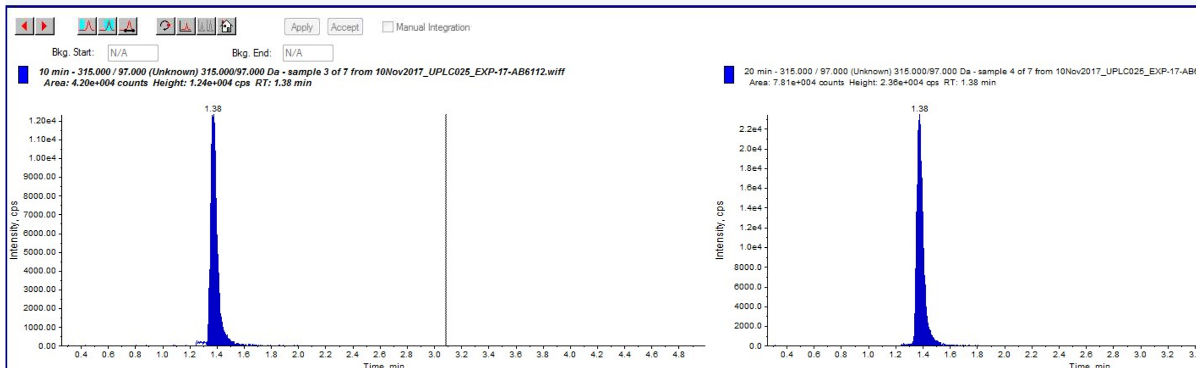
	# Name	Trace	RT	Area	IS Area	Response	Detecti...	ppb	%Dev
1	1 Diamine (qualifier)	221.3 > 164.3	1.77	815.129		815.129	MM	98.5	-2.2
2	2 Diamine (quantifier)	221.3 > 204.3	1.77	369.226		369.226	bb	101.1	0.4

Example of LC/MSMS chromatograms for the ether-diamine quantification



Plot of a seven points Calibration Curve (primary channel) with three quality controls for the ether-diamine quantification

	Sample Name	Sample ID	Sample Type	File Name	Analyte Peak Area (counts)	Analyte Peak Height (cps)	Analyte Concentration (ng/mL)	Standard Query Status	Use Record	Record Modified	Calculated Concentration (ng/mL)	Accuracy (%)
1	Blank		Unknown	10Nov2017_UPLC025_EXP-17-AB611	7.35e+001	6.02e+001	N/A	N/A			0.00	N/A
2	0 min		Unknown	10Nov2017_UPLC025_EXP-17-AB611	5.61e+003	1.97e+003	N/A	N/A			0.00	N/A
3	10 min		Unknown	10Nov2017_UPLC025_EXP-17-AB611	4.20e+004	1.24e+004	N/A	N/A			0.00	N/A
4	20 min		Unknown	10Nov2017_UPLC025_EXP-17-AB611	7.81e+004	2.36e+004	N/A	N/A			0.00	N/A
5	30 min		Unknown	10Nov2017_UPLC025_EXP-17-AB611	1.11e+005	3.35e+004	N/A	N/A			0.00	N/A
6	40 min		Unknown	10Nov2017_UPLC025_EXP-17-AB611	1.43e+005	4.23e+004	N/A	N/A			0.00	N/A
7	Blank		Unknown	10Nov2017_UPLC025_EXP-17-AB611	7.56e+001	7.06e+001	N/A	N/A			0.00	N/A



Example of LC/MSMS chromatogram for the azanyl ester quantification