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IDENTIFICATION OF IMPURITIES AND FORCED DEGRADATION CHARACTERIZATION OF PROTECTED-H_3MN-16ET, A PRECURSOR FOR RADIOTHERAPY OF HEPATOMA USING LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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ABSTRACT

The purity and impurities of protected-H_3MN-16ET, a homemade precursor for the radiopharmaceutical ^{188}Re-MN-16ET applicable to hepatoma treatment, was determined by monolithic C18 reversed-phase high-performance liquid chromatography coupled with triple quadrupole tandem mass spectrometry(HPLC-QqQMS) for drug safety and efficacy assessment. The chromatographic purity amounted to 96.3%, exceeding the quality criteria, and three impurities were found in the material. The major impurity with an abundance of 2.15% was the transesterification byproduct of 2-mercapto ethanol. In addition, in order to assay stability and decomposed causes, protected-H_3MN-16ET under acidic / basic hydrolysis, oxidation, thermal- and photo- induced stress conditions were investigated by analyzingthe forced degradation products through the same HPLC-tandem MS method. The radiochemical precursordid not withstandacidic / basic hydrolytic conditions and was easily transformed into carboxylic acid. It was also decomposed under UV and thermal exposures. However, it may resist oxidation, because it did not bear any oxygen-susceptible functional group. The sulfur-bonded protecting group CPh_3 may be UV sensitive and the major cause to account for photo degradation. Heat could induce the chemical to fracture C-N bonding of the long alkyl side chain.

Keywords *–Identification of impurity; Stress test; Forced degradation; H_3MN-16ET; LC-QqQ MS*

1. INTRODUCTION

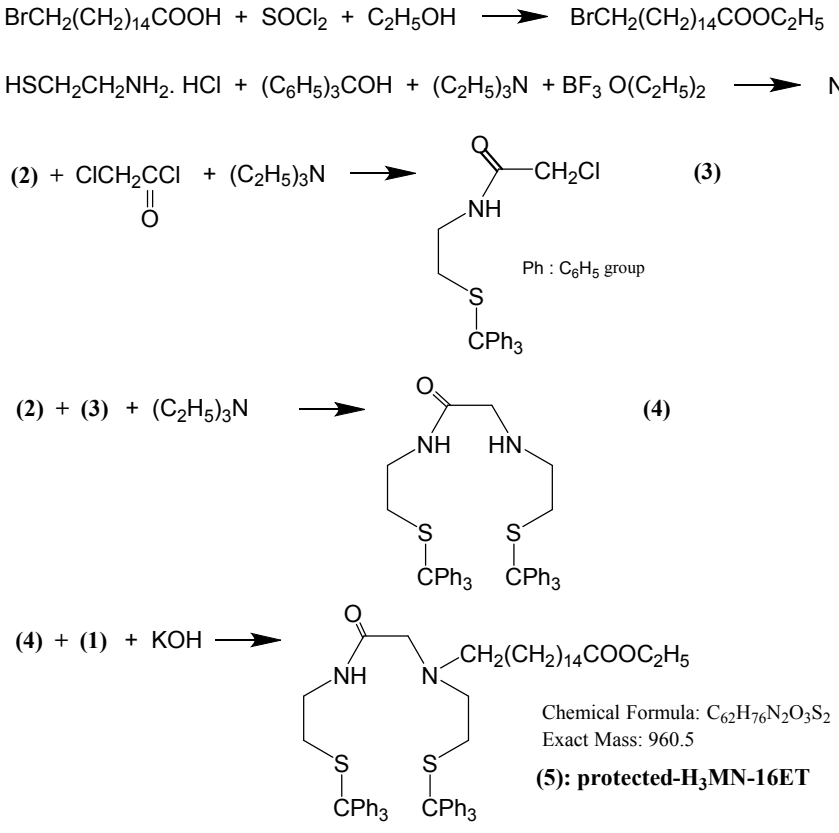
Roughly 33,000 new cases of primary liver cancer and 23,000 deaths have been recorded in the United States since 2014 [1]. On the other hand, according to statistics from Taiwan's Ministry of Health and Welfare, hepatic tumor led to approximately 7,000 deaths per year in Taiwan and was the second leading cause of cancer deaths in 2013 [2]. Approaches to liver tumor treatment include surgery, ablation, tumor arterial embolization, radiation therapy, targeted therapy, and chemotherapy depending on stage, size and location [3]. Hepatoma treatment methods combining embolization and radiotherapy have been extensively investigated [4-7]. One of these studies, an amino-amido-(protected)dithiol (N_2S_2) tetradentate ligand bearing a hexadecyl carboxylate ethyl ester side chain, protected-H_3MN-16ET, was synthesized and labeled with the γ- and β- radionuclide, Re-188 (half-life of 17.0 h) before its dissolution in lipiodol, ^{188}Re-MN-16ET /

lipiodol has been served for hepatoma preclinical trial involving animal model [8-11]. The results indicated that ^{188}Re-MN-16ET / lipiodol is a potential candidate for therapy of hepatocellular carcinoma. The radiopharmaceutical is going to apply for phase I clinical studyapprovalfrom Food and Drug Administration, Taiwan.

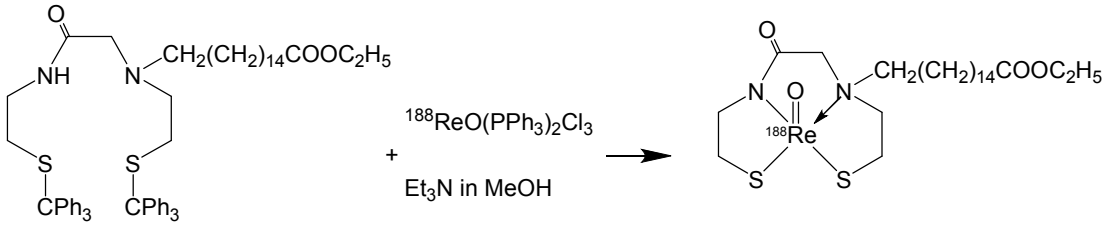
The synthetic routes of ^{188}Re-MN-16ET and its precursor, protected-H_3MN-16ET are outlined in Fig. 1 [8]. According to the International Conference on Harmonisation(ICH) Guidelines states that it is mandatory to determine purity level and identification of impurities in new drug substances produced by chemical syntheses which intend for human use to assure drug quality and safety[12]. Impurities—which include inorganic or metallic ions, moisture, and volatile as well as nonvolatile organic impurities—result from agents, solvents and catalyst residues, side products, reaction intermediates, and degradation products related to processes[12]. Nonvolatile organic impurities are typically analyzed by (ultra-) high performance liquid chromatography (U-HPLC) coupled with mass spectrometry.

In order to draw up storage conditions and shelf life for drug substance, the chemical stability of pharmaceutical molecule and its deterioration causes and pathways are another issue of great concerned as it affects the safety and costs of storage and transportation for the chemical [13-15]. The stability of new drug substance was surveyed by stress testing which help identify the likely degradation products and figure out the degradation pathways. The forced degradation behaviors of drug material were characterized by stress testsinvolving high- and low-pH hydrolyses, oxidation, thermal and photo induced decompositions[16]. Degradation products, especially nonvolatile organic chemicals, are identified by HPLC-tandem mass spectrometry (HPLC-MS/MS) in general.

The purity of protected-H_3MN-16ET is imperative for preparation of ^{188}Re-MN-16ET, since the presence of impurities in the activate pharmaceutical ingredient may impact on the quality, safety and ^{188}Re labeling yield. Except for process-related residues, impurities in protected-H_3MN-16ET may arise from deterioration during improper storage conditions or end of shelf life. However, no study has addressed to impurities and forced degradation behavior of protected-H_3MN-16ET been published. In this study, the chromatographic purity and impurities of protected-H_3MN-16ET were evaluated by HPLC coupled with triple quadrupole tandem mass spectrometry (QqQ MS) along with its forced degradation character under acidic / basic hydrolysis, oxidation, thermal and photo irradiative conditions.



(a)



(b)

Fig. 1: Synthesis of protected-H_3MN-16ET (a) and ^{188}Re-MN-16ET (b).

2. MATERIALS AND METHODS

2.1 Materialsand reagents

Analytical-grade chemicals for LC-MS were used as received, without further purification. Methanol (HPLC and MS grade), acetonitrile (HPLC and MS grade), and ammonium acetate were all purchased from Merck (Darmstadt, Germany). Ultrapure water (total organic carbon < 5 ppb, resistivity≧18.2 MΩ-cm) was prepared using a Smart DQ3 reverse osmosis reagent water system (Merck Millipore, Billerica, MA, USA) fitted with a 0.22 μmpolyvinylidene fluoride filter and a UV light source. *N*-[2-(triphenylmethyl)thioethyl]-3-aza-19-ethyloxycarbonyl-3-[2-(triphenylmethyl)thioethyl]octadecanoate (protected-H_3MN-16ET) was prepared as a yellowish, amorphous material by the Chemistry Division of the Institute of Nuclear Energy Research (INER, Taiwan) and was covered with aluminum foil before storage at -18 ± 2°C under vacuum (<5 torr). It was identified by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy using a 300 MHz Gemini 2000 instrument (Varian Inc.) and infrared spectroscopy (FT-IR, Bio-Rad FTS-40). Its purity criteria is above 95% for quality control (QC) purposes using HPLC (Hitachi, L-7000).

2.2 Apparatus and equipment

2.2.1 HPLC-tandem mass spectrometer instrumentation

Protected-H_3MN-16ET quantification and degradation behavior under stress conditions were assessed by HPLC-QqQ MS. The HPLC system (Agilent 1100/1200 series, Palo Alto, CA, USA) comprised an online degasser, a quaternary pump, an autosampler, a thermostat column oven maintained at 25°C, and a diode array detector (DAD) set at 250 nm. Data were acquired and processed using the Agilent ChemStation software (ed. 10.02).

The MS analysis of protected-H_3MN-16ET dissolved in acetonitrile was initially conducted using a 4000 QTRAP® LC-MS/MS system with Analyst software 1.6.2 (AB Sciex, Concord, ON, Canada) to obtain its appropriate MS parameters and fragments *m/z*. Samples solutions (10 ppm) were introduced into the spectrometer using a syringe pump at a flow rate of 10 μL min^{-1} (Harvard Apparatus Inc., Holliston, MA, USA). Impurities and degradation products were identified by coupling the mass spectrometer with HPLC. Evaporated high purity liquid nitrogen (99.999%) was used as nebulization, curtain and collision gas in all the 4000 QTRAP LC-MS/MS studies.

2.2.2 Other equipments

Buffer solution pH values were measured and adjusted using a pH meter (Sartorius AG, Goettingen, Germany). Thermal decomposition was evaluated using an annually calibrated (to checked for accuracy and reliability) temperature programmable (±1°C) digital oven (FD 53, Binder GmbH, Tuttlingen, Germany) equipped with a forced convection unit. Photo-degradation was studied under UV irradiation using a low-pressure mercury lamp as the light source (253.7 nm, 20 W).

2.3 Methods

2.3.1 Purity level determination and impurity identification

Impurities were separation on a reversed phase column (Chromolith® RP-18e end capped monolithic, Merck, Germany, 4.6 mm × 100 mm, and guard column) using gradient programmed composition of mobile phase A (ammonium acetate 2 mM/acetic acid 0.1% buffer, pH 4.5, with acetonitrile 1% in aqueous solution) and B (acetic acid 0.1% in acetonitrile). The eluent flow at 0.7 mL min^{-1}according to

the gradient program shown in Table 1. The total turnaround time amounted to 60 min. In order to find the trace impurities in the drug material (while impurity exceeding 0.1% it should be identified according to the International Conference on Harmonisation (ICH) guidance [12]), the concentration of protected-H_3MN-16ET for determination of purity and impurities structure was prepared around 1750 ppm and an injection volume of 3 µL. Upon exiting the analytical column, the eluate was split into two streams (10:1 volume ratio) flowing to the DAD and MS/MS detector, respectively, using a T-connector. Parameters of 4000 QTRAP®were summarized in Table 1. The analyte was ionized using a turbo spray ion source (electrospray ionization, ESI) in the positive-ion mode at 5500 V and 400°C. Mass spectra were recorded between 100 and 1000 Dalton with unit resolutions in Q1 and Q3.

2.3.2 Forced degradation study

HPLC and 4000 QTRAP LC-MS/MS parameters for the degradation of protected-H_3MN16ET were similar to those used in the purity assessment, with minor modifications (Table 1).

2.3.3 Forced degradation procedures

Forced degradation studies under acidic/basic hydrolysis, oxidation, and photoirradiationconditions were conducted using methanolicprotected- H_3MN-16ETsolutions (1000 ppm). In contrast,thermal degradation was performed in the solid state in vial.[16] The conditions are described as follows.

1. Acidic / basic hydrolysis: The tested solution (200 µL) was mixed with 0.1 M HCl or NaOH (200 µL) in seven vials and incubated for specific time periods (1, 2, 4, 8, 16 and 24 h) in a water bath shaker thermostat at 37°C. At each specified time, a vial was removed from the bath and the reaction mixture was neutralized using 0.1 M NaOH or HCl (200 µL) for HPLC-MS/MS analysis.
2. Oxidation: Tested solutions (200 µL) were mixed with 3% H_2O_2 (200 µL) and incubated under the same conditions as for the hydrolysis. At each specified time, a vial was removed from the bath and the oxidation was stopped by diluting the reaction mixture with methanol (600 µL) for HPLC-MS/MS analysis.
3. Photolysis: The test solution (200 µL) was placed in a transparent vial, irradiated using UV light at a distance of 20 cm for specific time periods, and analyzed by LC-MS/MS.
4. Thermolysis: Solid protected-H_3MN-16ET (5 mg) was weighed into a vial, maintained in a preheated oven (80°C) for specific time periods, cooled, and dissolved in methanol (5 mL, 1000 ppm) for LC-MS/MS analysis.

Table 1: HPLC and MS/MS parameters for the determination of impurities and forced degradation products of protected-MN-16ET

HPLC Parameter	
Stationary phase	Chromolith® RP-18e,End capped monolithic, 4.6 ID × 100 mm, thermostat at 25°C
Injection volume, µL	3
Detector	DAD at 250 nm
Mobile phase	
Flow rate, mL min^{-1}	Always 0.7 *0.7 during 0-15 and 30-45 min but 0.8 during 15-30min
Composition	A: ammonium acetate 0.2mM / 0.1% acetic acid aqueous with 1% CH_3CN B: 0.1% acetic acid in CH_3CN
Gradient program	0-3.0 min, 30%B isocratic 3.0-15.0 min, 30% → 100% B 15.0-30.0 min, 100% B isocratic 30.0-45.0 min, 100% → 30% B *30.0-35.0 min, 100% → 30% B 45.0-60.0 min, 30% B isocratic *35.0-45.0 min, 30% B isocratic
Turnaround time, min	60, *45

Mass Spectrometric Parameters	
Source temperature (°C)	400
Polarity	Positive
Resolution, Q1 and Q3	Unit
Nebulizer gas, NEB (psi)	40
Curtain gas, CUR (psi)	10
Turbo gas	15
Collision gas, CAD (psi)	Medium
Ion spray voltage, IS (V)	5500
Ion energy 1, IE1 (V)	0.4
Ion energy 3, IE3 (V)	0.3
Declustering potential, DP (V)	60; *40
Entrance potential, EP (V)	10

**Minor modified instrument parameters of HPLC and MS/MS for forced degradation study to reduce chromatographic time*

3. RESULTS AND DISCUSSION

3.1HPLC-MS/MS determination of protected-H_3MN-16ET purity and its impurities

A reversed phase HPLC method was developed to determine the purity of protected-H_3MN-16ET and isolate its impurities. The compound was dissolved in acetonitrile (1750 ppm) and analyzed on a monolithic silica column exhibiting a characteristic porous structure, for which the surface was modified with C18 group and fully end-capped with methyl group using an aqueous ammonium acetate buffer / acetonitrile mixture for gradient elution. The detection wavelength was set to 250 nm, which corresponds to the absorption wavelength of the triphenylmethyl (CPh_3) protective groups. The HPLC chromatogram of protected-H_3MN-16ET is shown in Fig. 2. The precursorappeared at aretention time (R_t) of 29.04± 0.05 min for 20,300theoretical plates, symmetry factor of 0.40, width of 0.48 min, and selectivity of 1.55. The baseline drifted as a result of the mobile phase gradient. In the absence of a commerciallyavailable standard,no calibration curve was plottedto quantitate protected-H_3MN-16ET.Therefore, the purity was estimatedas the ratio betweenthe peak area of thetarget compoundand thesum of all peak areasin the chromatogram, excluding blank background peaks [17,18]. The chromatographic purity of prepared protected-H_3MN-16ET amounted to 96.3%, surpassing the standard QC qualification criterion of 95%. The chromatogram also displaced three impurity peaks, defined as Imp.1–3at R_t values of 11.47, 14.07 and 31.99 min, respectively. Imp. 1–3 presented chromatographic abundances of 0.77%, 0.74% and 2.15%, respectively. Their structures were identified by MS/MS.

The protected-H_3MN-16ET solution in acetonitrile was introduced into the mass spectrometer by syringe infusion for enhanced MS (EMS) and MS/MS scans in the positive ion mode. Instrument parameters are listed in Table 1. EMS scan spectra displayed an*m/z* value of 961 attributable to the $[M+H]^+$ ion. Secondary mass spectra obtained in the "enhanced product ion (EPI) scan mode" for *m/z* 961 (Fig. 3) presented a single product ion at *m/z* value of 243, which corresponded to the stable organic cation triphenylmethylium, $(Ph)_3C^+$ acting as a thiol-protecting group. The absence of other *m/z* fragment peaks suggested that these fragments are neutral and undetectable.

HPLC-MS/MS spectra of Imp. 1 - 3 were studied to ascertain their structures (Table 2). The spectrum of Imp. 1 (R_t = 11.47 min) only displayed a meaningful *m/z* signal at 243 in addition to background noise, corresponding to an unknown molecule bearing $(Ph)_3C$, denoted as $(Ph)_3CX$, where X represents an unknown fragment, in the absence of *m/z* data for the protonated molecular ion. Despite its possible presence in the protected-H_3MN-16ET solution, the cation $(Ph)_3C^+$ was not retained in the C18 column, and consequently, was expected to appear before 4 min.

The protonated molecular ion of Imp. 2 (R_t = 14.07 min) was detected at an *m/z* value of 300 along with fragments at *m/z* values of 283 ($[M+H]^+$ 17) and 254 ($[M+H]^+$ 46), indicating that Imp. 2 corresponded to ethyl 16-amino-hexadecanoate ($C_{18}H_{37}NO_2$, MW = 299.3), an amination byproduct of ethyl 16-bromo-hexadecanoate (the intermediate **1**).

With a peak area of 2.15%, Imp. 3 (R_t = 31.99 min) was the most significant impurity and appeared at a longer retention time than protected-H_3MN-16ET. Its MS^1 presented an *m/z* signal at 993 (= 961 + 32) and gave MS^2 fragmentation peaks at 750 (= 993 243), 507 (= 993 – 2 × 243) and 243 ($(Ph)_3C^+$). Furthermore, the tertiary mass spectra (MS^3) of *m/z* peaks observed at 750 and 507 displayed fragment ion mass groups (507, 447, 372, 342, 243) and (447, 372, 342, 312, 266), respectively. The potential fragmentation scheme to assign the MS/MS data were figured out in Fig. 4, suggested that Imp. 3 was protected-H_3MN-16-COO-$(CH_2)_2$SH, a transesterification byproduct of 2-mercaptoethanol with protected-H_3MN-16ET.

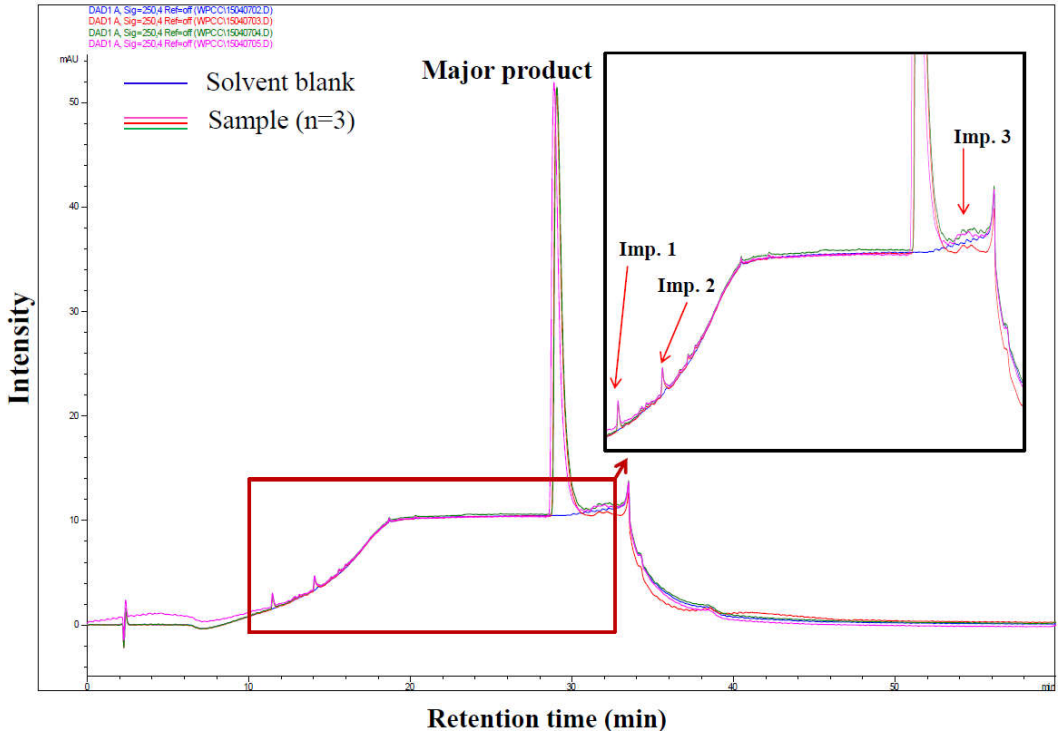
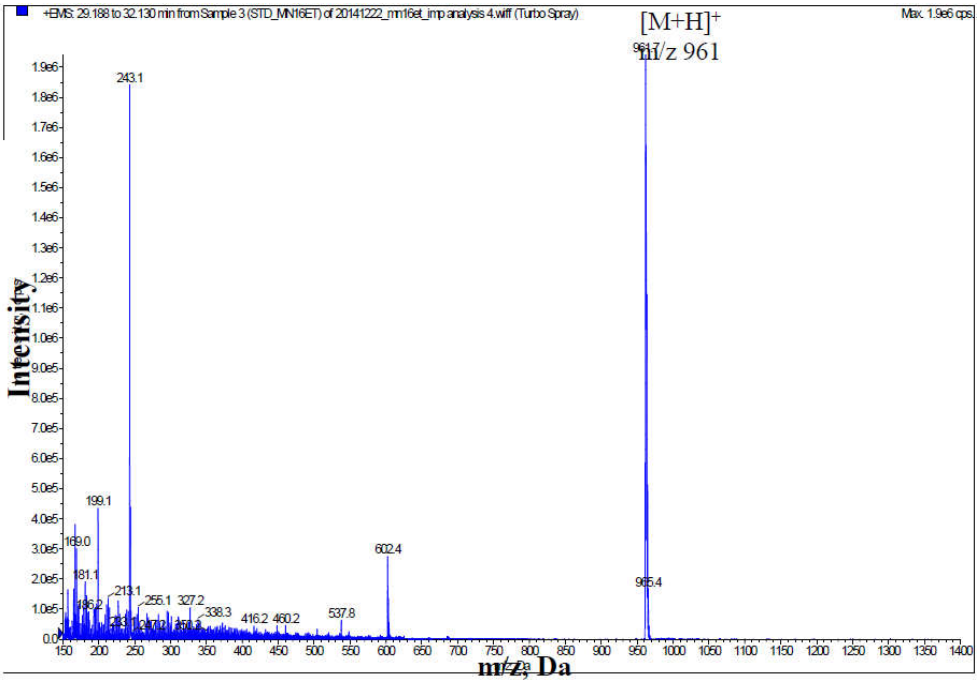
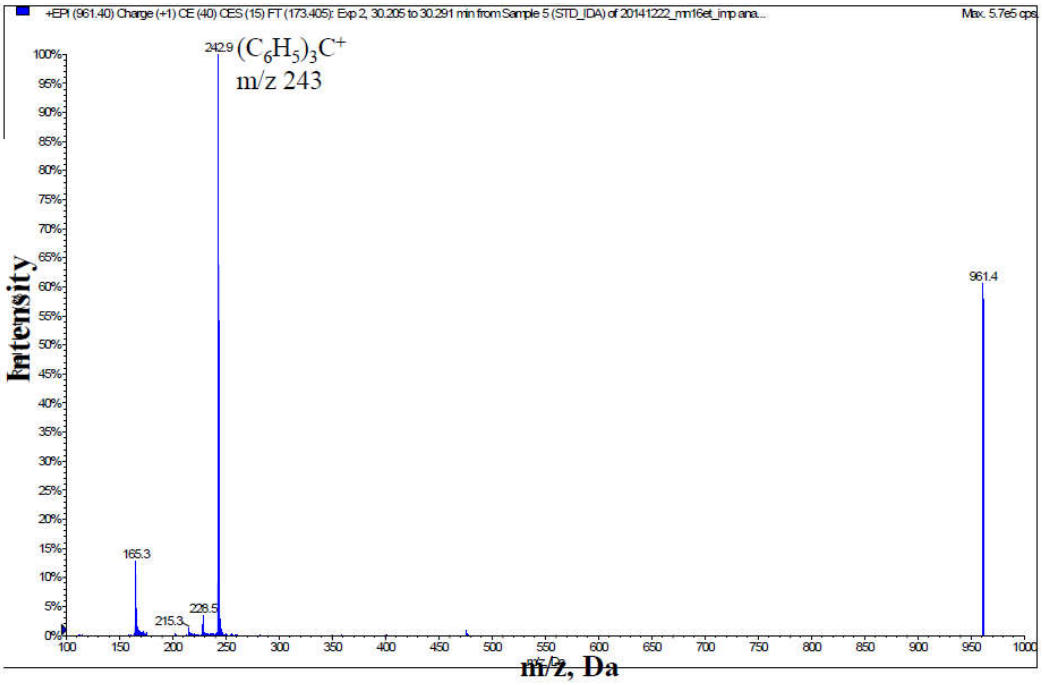


Fig. 2: Typical chromatogram of protected-H_3MN-16ET.



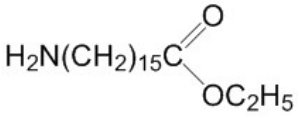
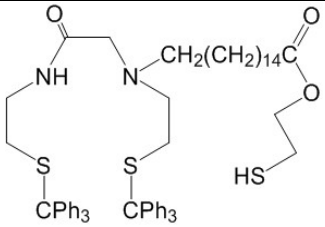
(a)



(b)

Fig. 3: Tandem mass spectra, MS^1 (a) and MS^2 (b) of protected-H_3MN-16ET

Table 2:MS/MS identification of impurities in protected-H_3MN-16ET

*Imp. #	R_t, min	Chromatographic abundance (%)	Exact mass (Da) / Fragments ion *m/z*	Identity
Imp. 1	11.47	0.77	ND / 243	X-CPh_3 X: unknown
Imp. 2	14.07	0.75	299 / 283, 254	 $C_{18}H_{37}NO_2$
Imp. 3	31.99	2.15	992 / 750, 507, 243	 $C_{62}H_{76}N_2O_3S_3$

*: *Impurity*

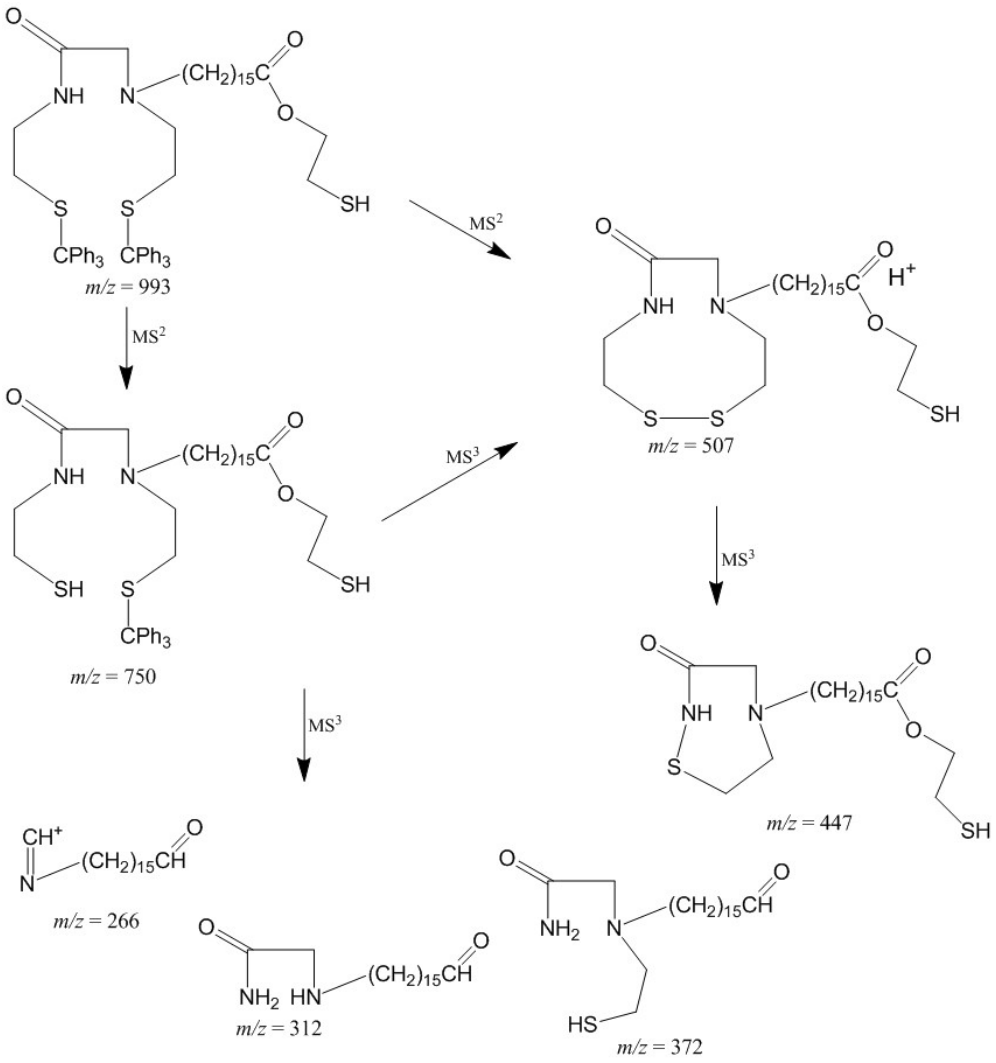


Fig. 4: Scheme of Imp.3 fragmentation pattern of to illustrate its MS/MS data

3.2 HPLC-MS/MS characterization of the forced degradation behavior of protected-H_3MN-16ET

In addition to identifying impurities, the newly developed HPLC-MS/MS method was also used to separate forced degradation products of protected-H_3MN-16ET at a turnaround time of 45 min per injection instead of 60 min. Modified parameters are marked by an asterisk in Table 1. R_t for protected-H_3MN-16ET was detected at 28.13 ± 0.05 min with 23,500theoretical plates, symmetry factor of 0.39, width of 0.44 min, and a selectivity of 1.80.

After undergoing forced degradation for specified time periods, protected-H_3MN-16ET solutions were analyzed by HPLC-DAD and MS/MS. The degradation tendencies of the drug precursor under various conditions were determined by monitoring its chromatographic peak area as a function ofreaction duration (Fig. 5).

The results demonstrated that protected-H_3MN-16ET was susceptible to hydrolysis by OH^- and H^+ ions (0.1 M). Especially, more than 95% protected-H_3MN-16ET was hydrolyzed by OH^- ions in less than 1 h in a higher pH solution. The acidic / basic hydrolysis generated a unique noticeable product (R_t = 25.3 min), giving rise to an *m/z* peak at 933 for the protonated molecular ion. This peak corresponded to the carboxylic acid derivative of protected-H_3MN-16ET (*m/z* = -28, ethyl ester → carboxylic acid). Although protected-H_3MN-16ET can undergo OH / H^+-catalyzed methyl transesterification in methanolic solution (Le Chatelier's principle), however,no *m/z*signal was

observed at 947 by LC-MS.This may result from the weaker nucleophilicity ofthe methyl group compared to itsethylcounterpart, and methyl transesterification would happen at higher temperature [19]. Neitherchromatographic peak area of protected-H_3MN-16ET reduced significantly nor degradation product peaks were detected within 24 h under oxidative conditions in the presence of 3% H_2O_2, indicating its inertness to oxidizing agent because the absence of oxidizable functional group in the tested material [16].

Protected-H_3MN-16ET almost totally decomposed within 16 h under UV irradiation (254 nm). Five photolytic productsDP_p. 1–5 were detected at $R_t$values of 14.7, 15.9, 17.8, 20.9, and 24.1 mincorresponding to protonated molecular ion *m/z*peaks at 659, 417, 475, 701, and 719, respectively (Fig. 6a). On the other hand, only 45% degradation was observed within 24 h under thermal exposure (80°C) (Fig. 6b). Threethermolytic products DP_t.1–3appeared in at R_t values of 16.0, 18.5, and 25.3 min, giving protonated molecular ion *m/z* peaks at 767, 679, and 933, respectively.Themolecular formulaeand structures of DP_p. 1–5 and DP_t.1–3were derived from the tandem MS datawas summarized in Table 3.

The peak with *m/z* value at 475 and R_t at 20.3 min had been present before the material was irradiated with UV and without obviously difference after exposed to UV for hours hence it had not been examined deeply. For DP_p.1, 2, and 5, there were two possible isomeric structures respectively with mercaptoethyl bonded with either amine or amide. It is impossible to tell from which one is present based on tandem mass spectra. But C-N bonding on amide is more labile than amine based on acid-base principle of organic chemistry [19] therefore it is reasonable to figure out the structures listed in Table 3. After UV irradiation for 16 and 24 h, the peak area of DP_P. 3 was declined and disappeared, respectively. In the meanwhile, the peak with R_t =14.2, *m/z* value at 417 was emerged and ascended. It is believed that DP_p 3 was further decomposed into DP_p. 2', the other isomer of DP_p. 2.

Thermal degradation product DP_t. 2 may arise from the decomposition of the alkyl side chain in the ethyl hexadecanoate moiety to form intermediate **4** in the synthetic route of protected-H_3MN-16ET (Fig. 1a). Compound DP_t. 3 corresponded to the same carboxylic acid as the hydrolysis product.Data suggested that DP_t. 1 was derived from DP_t. 3 by the departure of two phenyl groups concomitant with the amide reduction into amine.

The degradation pathways and causes for protected-H_3MN-16ET were shown in Fig. 7.

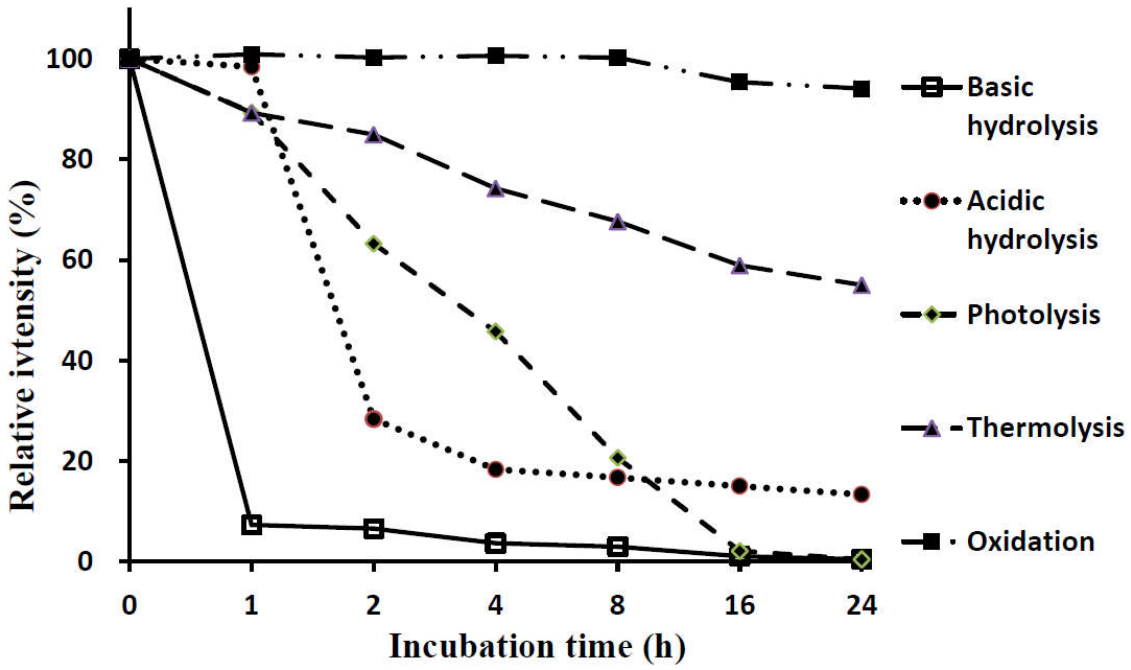
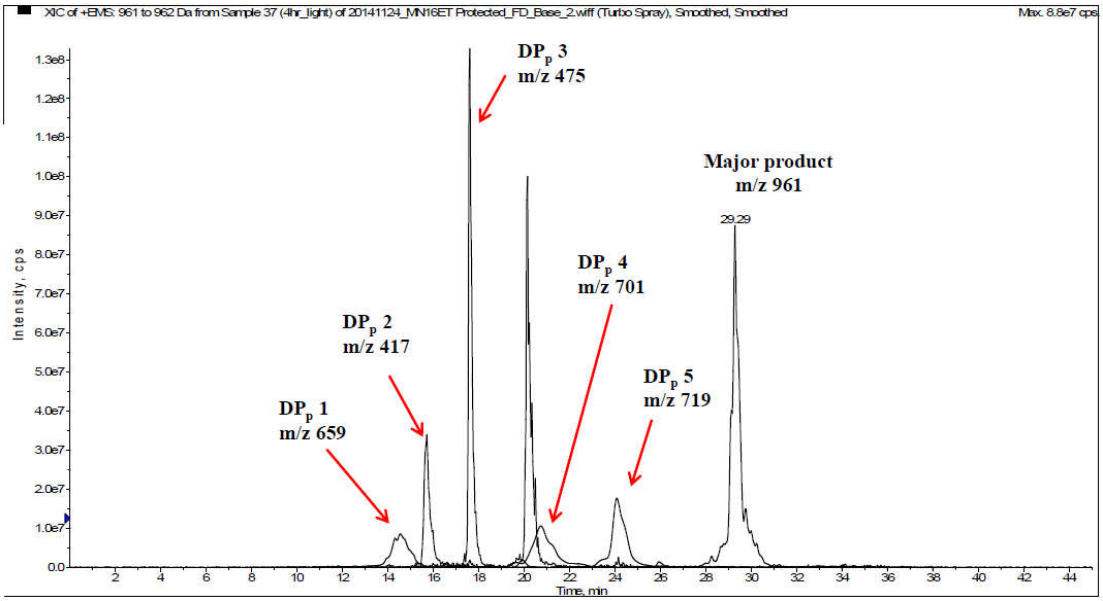
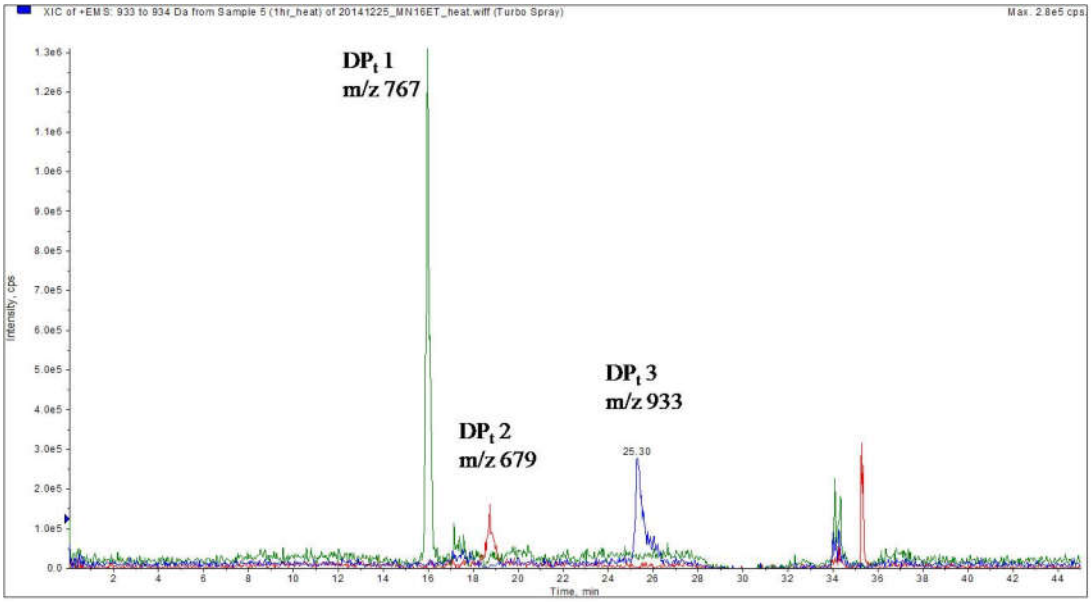


Fig. 5: Forced degradation tendencies of protected-H_3MN-16ET under acidic / basic hydrolysis, photolysis, thermolysis and oxidation conditions.



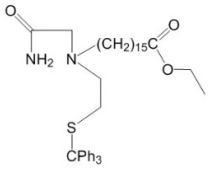
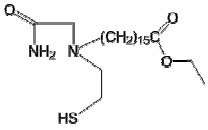
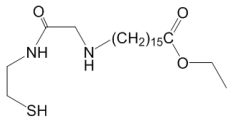
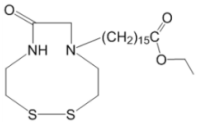
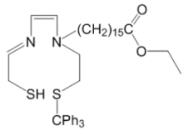
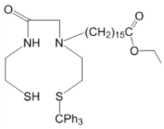
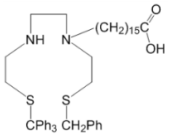
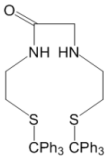
(a)

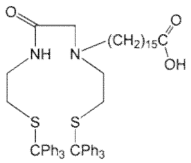


(b)

Fig. 6: HPLC-MS chromatograms for photolysis (a) and thermolysis (b) forced degradation studies of protected-H_3MN-16ET

Table 3:MS/MSidentification of photo and thermaldegradation products

DP. #	R_t, min	Exact mass (Da) Fragments ion m/z	Structure Formula
Photo degradation products			
DP_p. 1	14.6	658 641, 243	 $C_{41}H_{58}N_2O_3S$
DP_p. 2	15.9	416 312, 294, 266	 $C_{22}H_{44}N_2O_3S$
DP_p. 2’	14.2	416 312, 294, 266	 $C_{22}H_{44}N_2O_3S$
DP_p. 3	17.6	474 447, 358, 326, 312	 $C_{24}H_{46}N_2O_3S_2$
DP_p. 4	20.9	700 243	 $C_{43}H_{60}N_2O_2S_2$
DP_p. 5	24.2	718 243	 $C_{43}H_{62}N_2O_3S_2$
Thermal degradation products			
DP_t. 1	15.9	766 243, 165	 $C_{48}H_{66}N_2O_2S_2$
DP_t. 2	18.5	678 243, 165	 $C_{44}H_{42}N_2OS_2$

DP_t. 3	25.3	$\frac{932}{243}$	 $C_{60}H_{72}N_2O_3S_2$

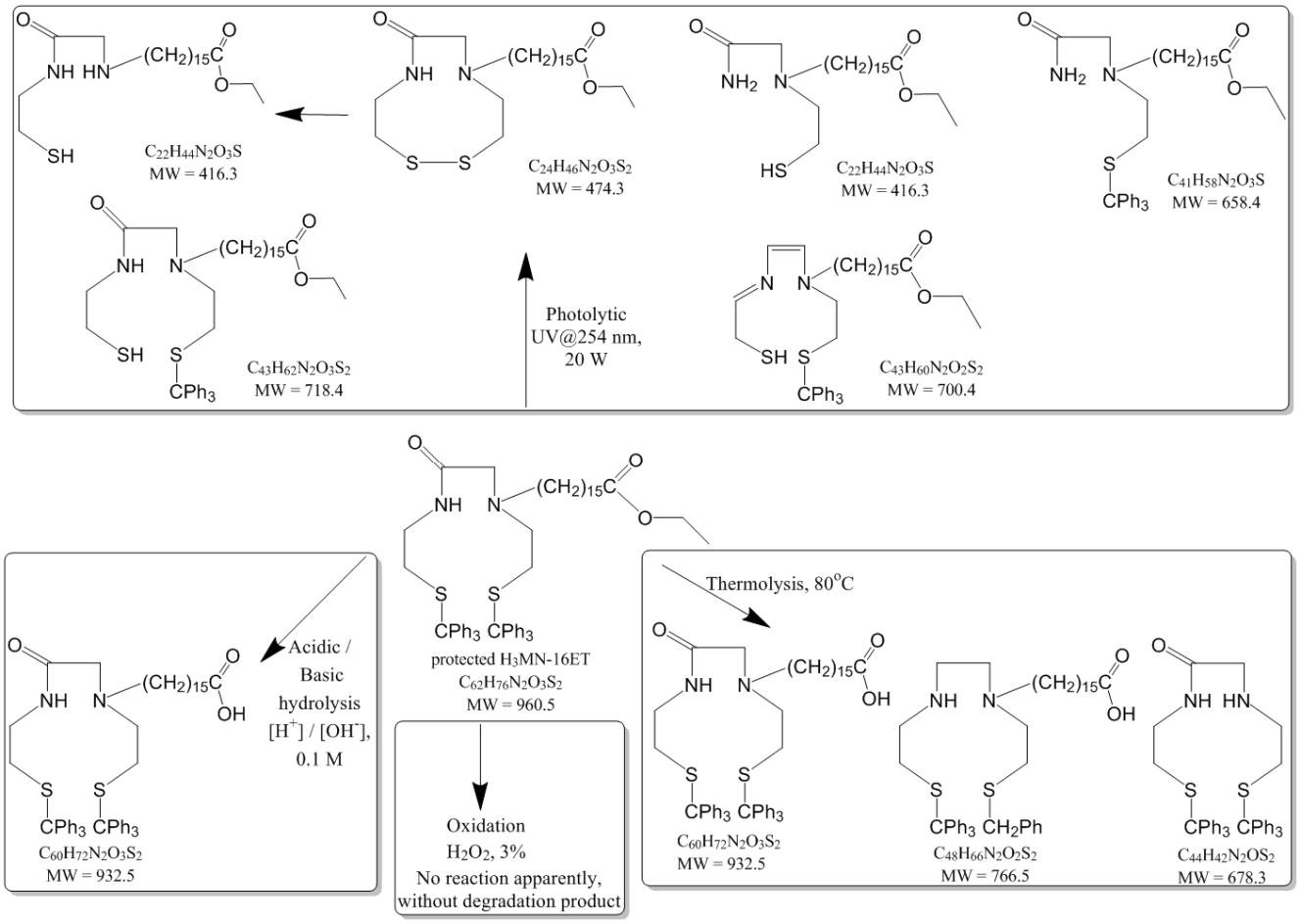


Fig.7: Degradation pathways of protected-H_3MN-16ET and their causes.

4. CONCLUSION

Synthesis and purification procedures are suitable for the production of high-purity product (> 95%) for $(CPh_3)_2$-MN-16ET and served for raw material to prepare ^{188}Re-MN-16ET. It was believedthat the main impurity in protected-H_3MN-16ET isthe 2-mercaptoethylester byproduct, which bears a thiol group that may dimerize through intermolecular disulfide bond. Minor impurities included $NH_2(CH_2)_{15}COOEt$ and an unknown compound containing a CPh_3 subgroup.

In the absence ofan oxidizablefunctional group,protected-H_3MN-16ET was inert to ambient oxygenas long as the protecting group CPh_3 remainedbound tosulfur.Itwaseasily hydrolyzed in basic andacidic solutions and formedits carboxylic acid derivative also at elevatedtemperature. Another possible thermolysispathwaywasits reverse reaction into asyntheticintermediate. Protected-H_3MN-16ET exhibited a complicated photolyticbehavior. The protecting group, CPh_3, was UV responsive because its aromaticity stabilized its charged or radical forms, producing an intramolecular disulfide bond. The other fracture bonding was C-N bond beside the amide group. For optimal long-term storage, protected-H_3MN-16ET needs tobe protected from sunlight exposureand stored in thedark in a moisture-free environmentbetween2 and 8°C.

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6. REFERENCES

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