

**SYNTHESIS AND CHARACTERIZATION OF THIOCTIC ACID
POLYETHYLENE GLYCOL 3 N-HYDROXYSUCCINIMIDE (TA-PEG3-NHS)
AND THIOCTIC ACID POLYETHYLENE GLYCOL 600 N-
HYDROXYSUCCINIMIDE (TA-PEG600-NHS) AS A LINKER BETWEEN
QUANTUM DOT AND ANTIVIRAL PEPTIDE**

Muhammad Shaffiq Bin Zainal Osman Shah¹, Dejian Zhou² and Yuan Guo²

¹*Faculty of Applied Sciences, Universiti Teknologi Mara (UiTM),
Cawangan Perak, Kampus Tapah, 35400 Tapah Road, Perak, Malaysia.*

²*School of Chemistry, Faculty of Mathematics and Physical Sciences,
University of Leeds, Leeds LS2 9JT, United Kingdom.*

Corresponding author: mshaffiq@perak.uitm.edu.my

ABSTRACT

Nowadays, nanomaterials study is vastly expanding in the chemistry field with the discovery of quantum dot. The quantum dot is crucial because of their unique property which is be able to absorb and emit at specific wavelength determined by their radius also have the long last fluorescence lifetime, which enables the use of time gated-detection. The project plan for this study is important especially on the finding of therapeutic agent towards the human immunodeficiency virus (HIV) or hepatitis C virus (HCV) which could rupture or destroy the virus by doing the integration process on the quantum dot with the dendritic cell-specific intercellular adhesion molecule-3-grabbing non integrin (DC-SIGN) and antiviral peptide. The DC-SIGN which target, attach, and bind to the surface of HIV or HCV, meanwhile the anti-viral peptide will act to destroy the virus at the same time. The main objective of this research is to study, finding and synthesize an efficient linker which could bind between the quantum dot and antiviral peptide. In this research project, the synthesis involves the esterification between the carboxylic acid compounds with the *N*-Hydroxysuccinimide (NHS) to form carboxylic acid *N*-Hydroxysuccinimide ester compound. Herein, both compounds, thioctic acid polyethylene glycol 3 *N*-hydroxysuccinimide (TA-PEG3-NHS) and thioctic acid polyethylene glycol 600 *N*-hydroxysuccinimide (TA-PEG600-NHS) have been synthesized and identified by using nuclear magnetic resonance (NMR), liquid chromatography-mass spectrometry (LC-MS), and infrared (IR) instrumentations. These compounds can be used to covalently link between antiviral peptide and quantum dot. Hence, the variations of the polymer polyethylene glycol (PEG) length may change the presentation of the antiviral peptide on quantum dot surface and can be compared. This research is a milestone to the overall project objective by preparing the two of significant carboxylic acid *N*-Hydroxysuccinimide ester compounds which are TA-PEG3-NHS and TA-PEG600-NHS.

Keywords: TA-PEG3-NHS; TA-PEG600-NHS; Quantum dot; Antiviral peptide; Quantum dot antiviral peptide linker; Quantum dot peptide linker;

INTRODUCTION

Fluorescent semiconductor nanocrystals or commonly known as quantum dots (QDs), currently is one of the important research areas in bio-conjugated nanoparticle, nanobiotechnology, and also in bio-medical technology commonly for labelling or identifying specific biomolecules. Quantum dot has an unique structure, and can be manipulated in order to emit in a broad range of wavelengths by changing their shape, size and their composition, also have an electrical properties that can be exploited in a range of sensing, biomedical, and material applications [1,2]. These form an excellent Förster resonance energy transfer (FRET) donors from QD because of their exceptional brightness and high quantum yields [3,4,5] their capacity to bind multiple acceptor molecules [6] and has an unique characteristic excitation and emission spectra. The FRET are introduce into QD-based bioanalysis of molecular structure and protein-protein, protein-nucleic acid, and any other interactions which improve the sensitivity [7] and lead to the specificity and the intrinsic sensitivity of FRET to small changes in donor-acceptor distances. Quantum dot is said to have a broad absorption and narrow symmetric emission and, these are extremely well suited for FRET sensor based, and because of it characteristics, the spectral may enable a wide selection of excitation wavelength which would minimize excitation of the acceptor directly, reducing the background and the sensitivity will be improved [8,9,10,11].

The QD coating with dihydrolipoic acid (DHLA) are known to be more stable in alkaline solutions than at acidic pH [12] and also been reported the DHLA-QD are stable for 6-24 months [13]. However, by only capping the QD by DHLA is not sufficient because it tends to slowly aggregate when exposed to acidic conditions. The steric stabilization of these nanoparticles hinges on the deprotonating of the terminal carboxyl groups of DHLA are the major problem why it aggregated under acidic condition. The pH limitation of DHLA-capped QD had been overcome by designing a new (bidentate and multidentate) ligands are needed such as DHLA-PEG-QD. The readily available thioctic acid and poly(ethyleneglycol)s (PEG) had been utilized in simple esterification schemes, and this are followed by the reduction of the 1,2-dithiolane in order to produce multi gram quantities of PEG-terminated dihydrolipoic acid (DHLA-PEG) capping substrates [14].

The used of PEG had been reported that can promote dispersion of the inorganic particles in water [12,13,14,15]. The substrates make the cap exchange reactions of trioctylphosphine oxide (TOPO)-capped QD that producing water-soluble nano-crystals which are extended periods of time stable compound and relatively had a broad pH range, from weak acidic conditions to strongly basic conditions. The full synthesis and design of DHLA and DHLA-PEG600 had been described in the paper [14]. The model schematic compounds of the quantum dot with PEG segment together with the functional group variables are shown in the figure 1.

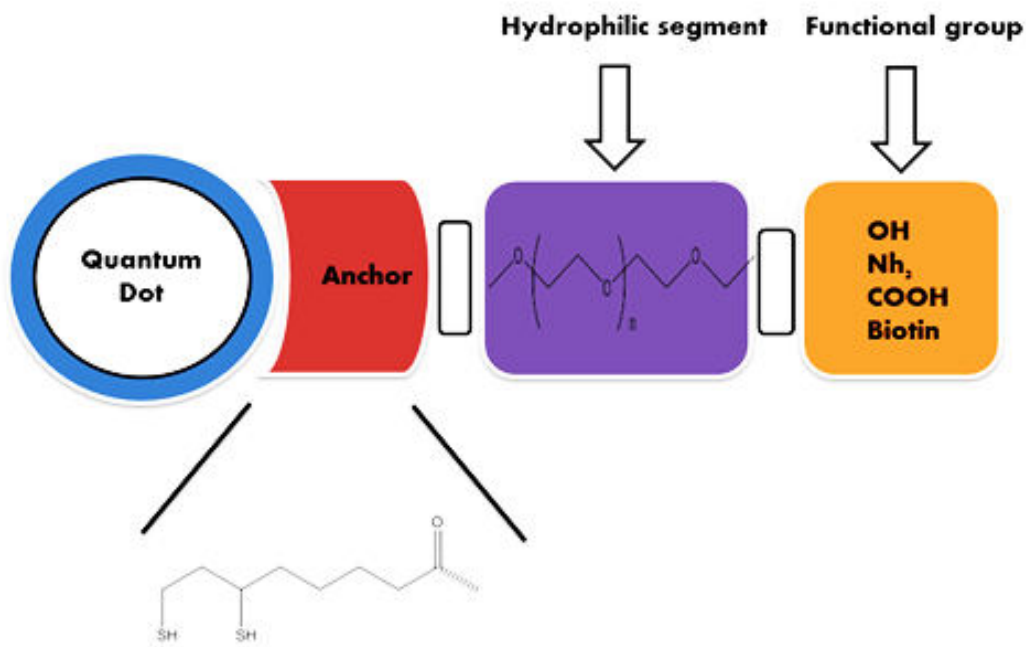


Figure 1: Schematic model compound of the quantum dot with functional group variations

And it was undergo the self-assembly of quantum dot bioconjugates as described in the paper [12] and the quantum dot was said to have the ability to self-assembled with a mixture of the protein. One of the technique is by FRET assays that may be perform to know at what extent the his-tag mediates self-assembly of fluorescent proteins to quantum dot and at under what conditions. It is used to confirm the proximity of the nanoparticle and biomolecule, and due to the strong distance dependence, allowing the derivation of a separation distance. This can be observed and can be determine for both overall conjugated structures and self-assembly.

In this project, the main aims are to synthesis and identify the potential linker which can bind the QD and the anti-viral peptide. TA-PEG600-NHS and TA-PEG3-NHS are the two interest compounds to synthesize and identification to bind the two components which are QD and anti-viral peptide. The TA-PEG600-NHS had been synthesized using the starting material TA-PEG600-COOH. Meanwhile for TA-PEG3-NHS, the starting material was from TA-PEG3-COOH. The thioctic acid uses in the project because of the availability compound, which can do the ring opening of the terminal dithiolane to produce a dithiol group, the resulting dihydrolipoic acid-appended ligands (DHLLA-PEG-OH) allowed effective cap exchange and also give strong anchoring to the QD. The length variation of the average number of PEG with PEG600 and PEG3 is to investigate the capability or efficiency the PEG at the different length to bind with the anti-viral peptide in the context of this project. All the synthesized compounds were

characterized by NMR, LC-MS and IR in this project.

EXPERIMENTAL

Materials and Instrumentations

Thioctic acid (TA, > 99%), dicyclohexylcarbodiimide (DCC, >99%), dimethylaminopyridine (DMAP, >99%), N-hydroxysuccinimide (NHS, 98%) and all other chemicals and reagents stated were all purchased from Sigma-Aldrich (Dorset, UK) and used without further purification unless stated otherwise. Solvents were obtained from Fisher Scientific (Loughborough, UK) and used as received. The entire synthesized yields sample was sent to the NMR Bruker Avance III 500 MHz Ultrashield machine, variable temperature, and an automated sample changing. All the products synthesized were sent to the LC-MS Bruker Daltonics – HCT. For the entire dried product that has been synthesized was sent to ALPHA's Platinum ATR FTIR without any solvents for dissolving sample.

Preparation of TA-PEG600-NHS from TA-PEG600-COOH

TA-PEG600-COOH (0.0897 g, 0.097 mmol), DMAP (0.005 g, 0.045 mmol), DCC (0.098 g, 0.48 mmol) and 10 mL CH₂Cl₂ was added into 100 mL round bottomed flask equipped with magnetic stirring bar. The mixture was kept at 0°C in an ice bath. NHS (0.05 g, 0.44 mmol) and 5 mL CH₂Cl₂ was added in addition funnel and was drop wise into the mixture for 30 mins under N₂ while stirring. When addition completed, the mixture was cooled to room temperature and was let stirred for 3 nights to ensure the starting material fully reacted. Then, the mixture had been filtered off through celite and rinsed the celite plug with CH₂Cl₂. The mixture was evaporated using rotary evaporator to remove an excess solvent. TLC was conducted to check the purity of the product with eluent (CHCl₃:MeOH = 10:1 (Vol/Vol)), R_f (TA-PEG600-NHS) = 0.36, R_f (TA-PEG600-COOH) = 0.1. Then, flash chromatography using the same ratio had been conducted and selected fractions were collected. The product was dried off using rotary evaporator under vacuum and pale yellow crystal ester compound obtained, weight 0.3092 g, 0.30 mmol, and yield: 28%.

¹H NMR (500 MHz, CDCl₃): δ 6.35 (br, s, 1H), 6.20 (br, s, 1H), 3.95 (t, 2H), 3.55 (br, s, 2H), 3.45 (t, 2H), 3.35 (t, 2H), 3.20 (m, 2H), 3.15 (m, 2H), 2.65 (br, s, 4H), 2.60 (m, 2H), 2.48 (m, 2H), 2.40 (m, 1H), 2.15 (t, 2H), 1.85 (m, 1H), 1.25 (m, 2H). IR (neat) ν_{max}/cm⁻¹: 3328.62, 2867.27, 1701.19, 1650.45, 1540.61, 1089.81, 843.17. m/z (ES): found 5.6%, MNa⁺, 507.2. C₄₄N₃S₂O₁₉H₈₀ requires MNa, 509.13.

Preparation of TA-PEG3-NHS from TA-PEG3-COOH

TA-PEG3-COOH (0.1 g, 0.21 mmol), DMAP (0.0026 g, 0.021 mmol), DCC (0.047 g, 0.23 mmol) and 2.5 mL CH₂Cl₂ was added into 50 mL round bottomed flask equipped with magnetic stirring bar. The mixture was kept at 0°C in an ice bath. NHS (0.024 g, 0.21 mmol) and 3 mL CH₂Cl₂ was added in addition funnel and was dropwise into the mixture for 30 mins under N₂ while stirring. When addition completed, the mixture was cooled to room temperature and was let stirred for overnight to ensure the starting

material fully reacted. Then, the mixture had been filtered off through celite and rinsed the celite plug with CH_2Cl_2 . The mixture was evaporated using rotary evaporator to remove an excess solvent. TLC was conducted to check the purity of the product with eluent ($\text{CHCl}_3:\text{MeOH} = 10:1$ (Vol/Vol)), R_f (TA-PEG3-NHS) = 0.49, R_f (TA-PEG3-COOH) = 0.19. Then, flash chromatography using the same ratio had been conducted and selected fractions were collected. The product was dried off using rotary evaporator under vacuum and pale yellow crystal ester compound obtained, weight 0.0838 g, 0.15 mmol and yield: 67.58%.

^1H NMR (500 MHz, CDCl_3): δ 6.35 (br, s, 1H), 6.10 (br, s, 1H), 4.05 (t, 2H), 3.55 (br, s, 2H), 3.50 (t, 2H), 3.40 (t, 2H), 3.15(m, 2H), 3.04 (m, 2H), 2.92 (t, 2H), 2.78 (br, s, 4H), 2.55 (t, 2H), 2.40 (m, 1H), 2.10 (t, 2H), 1.60 (m, 2H), 1.40 (m, 1H). IR (neat) $\nu_{\text{max}}/\text{cm}^{-1}$: 3313.54, 2925.71, 2862.50, 1735.97, 1646.17, 1538.39, 1360.69, 1203.49, 1083.18, 728.33. m/z (ES): found 82.3%, MNa^+ , 578.2. $\text{C}_{24}\text{N}_3\text{S}_2\text{O}_9\text{H}_{39}$ requires MNa , 577.64.

RESULTS AND DISCUSSIONS

The synthetic method in order to produce the TA-PEG600-NHS and TA-PEG3-NHS, was conducted based on the journal paper [16], since these are the two new synthetic compounds, in this paper the experimental method for synthesis of lipoic acid NHS-ester (LA-NHS) had been described and shown. This had been adapted for the methodology for this experiment to produced TA-P600-NHS and TA-PEG3-NHS from the starting material of TA-PEG600-COOH and TA-PEG3-COOH. Because of the relationship of the two same reactants carboxylic acid group and NHS used, this can be applied by using the same methods that had been described in the paper. But some amendment had been made depending on the suitability and again availability of solvents and compounds to conduct the experiment. The usage of DCC and DMAP solvents are important in this experiment in order to do the esterification process in the experiment. To date, there is no literature on methodology to synthesize TA-PEG600-NHS and TA-PEG3-NHS.

All the products was successfully synthesized as described in the characterization section, the products for all the compounds sample was sent to NMR, LC-MS and IR for identification and recognition to get the right product. The data gained from these identification methods are essential in order to see whether the compounds products are correct and high in purity. The reason of the usage of three machines is to give an accurate and reliable data interpretation of each product compound, the NMR result would tell every peak also will determine the proton in the compound and gave the specific peaks or profile for each compounds. Despite that, the use of LC-MS machine would tell about the total molecular weight correlate with the molecular weight of each product produced for each compound synthesizes based on the mass to charge ration (m/z). But the mass spectrum that been produced by the LC-MS cannot give an exact molecular weight since the PEG used in the experiment is a mixture of different PEG units and the resultant of the mass spectra show separated series of peaks by 44 Daltons.

Meanwhile, the IR instrument would tell us about the functional groups that presence in each compound produced.

Characterization of TA-PEG600-NHS

The TA-PEG600-COOH was used to produce TA-PEG600-NHS which the esterification reaction took placed, and 28% yield pale yellow crystal was obtained. The compound structure for TA-PEG600-NHS had been drawn and identified as shown as in figure 2 before the data results had been analyzed and characterized using the instrumentations. Hence, the proton NMR data had been described as in figure 3, based on the NMR data result at the δ 2.65 ppm the sharp singlet peak been recognized since it is symmetrically carbon-carbon in the NHS compound it tend to shows only singlet peak and this showed for the proton hydrogen located at the NHS unit and from the experiment conducted in the journal paper [16], the NMR for NHS is in the range of δ 2.84 ppm (s, 4H), this is closer to the singlet peak, 4H, gained in this experiment.

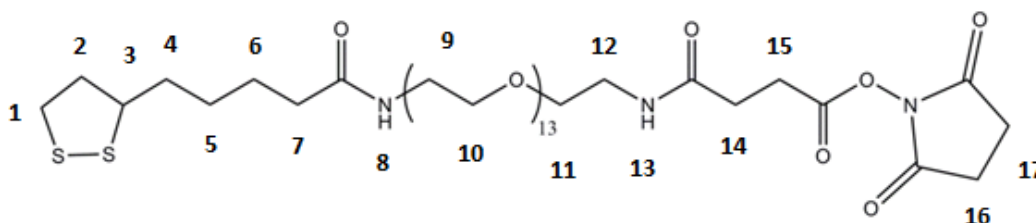


Figure 2: Synthesized compound structure of TA-PEG600-NHS

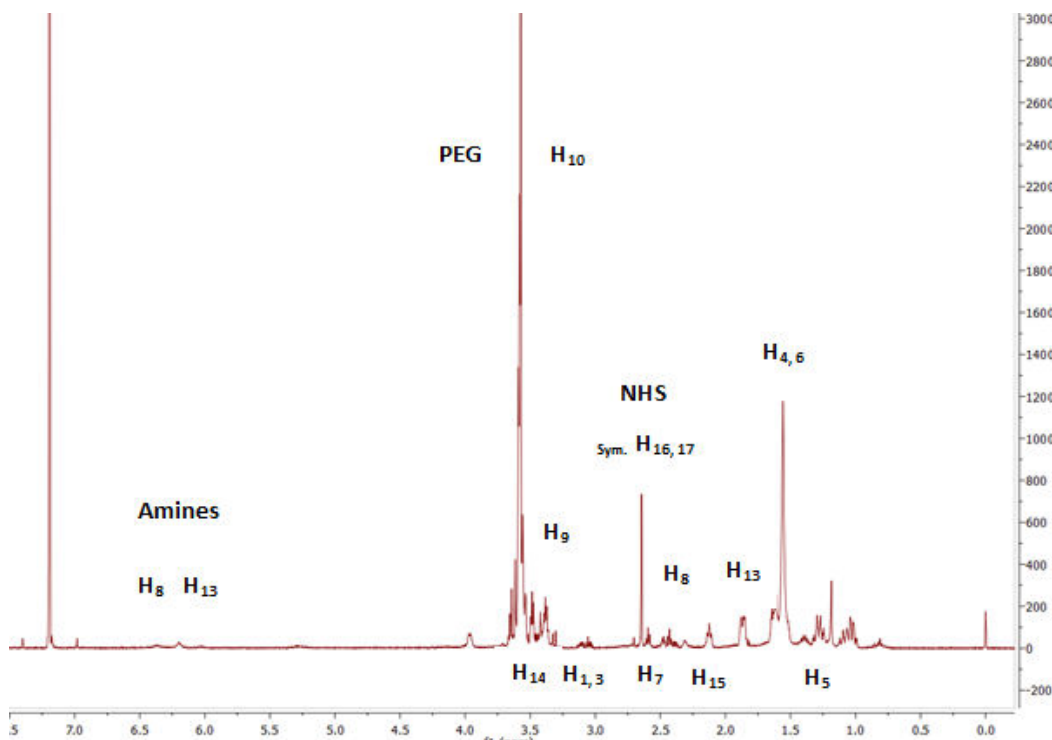


Figure 3: ^1H NMR result interpretation for compound TA-PEG600-NHS

Meanwhile for the mass spectrum from LC-MS data exposed the maximum m/z 490.3 at the retention time 1.70 minutes from the raw data but this not gave the fully correlation with the molecular weight for TA-PEG-600-NHS which is 1018.25 g/mol in molecular mass, but after the peak at the near retention time had been selected, the possible m/z values for the interest compound has been detected with the value 507.2 m/z within the range of 1.63-1.76 minutes as showed in the figure 4 and the value was existed for the double charged compound.

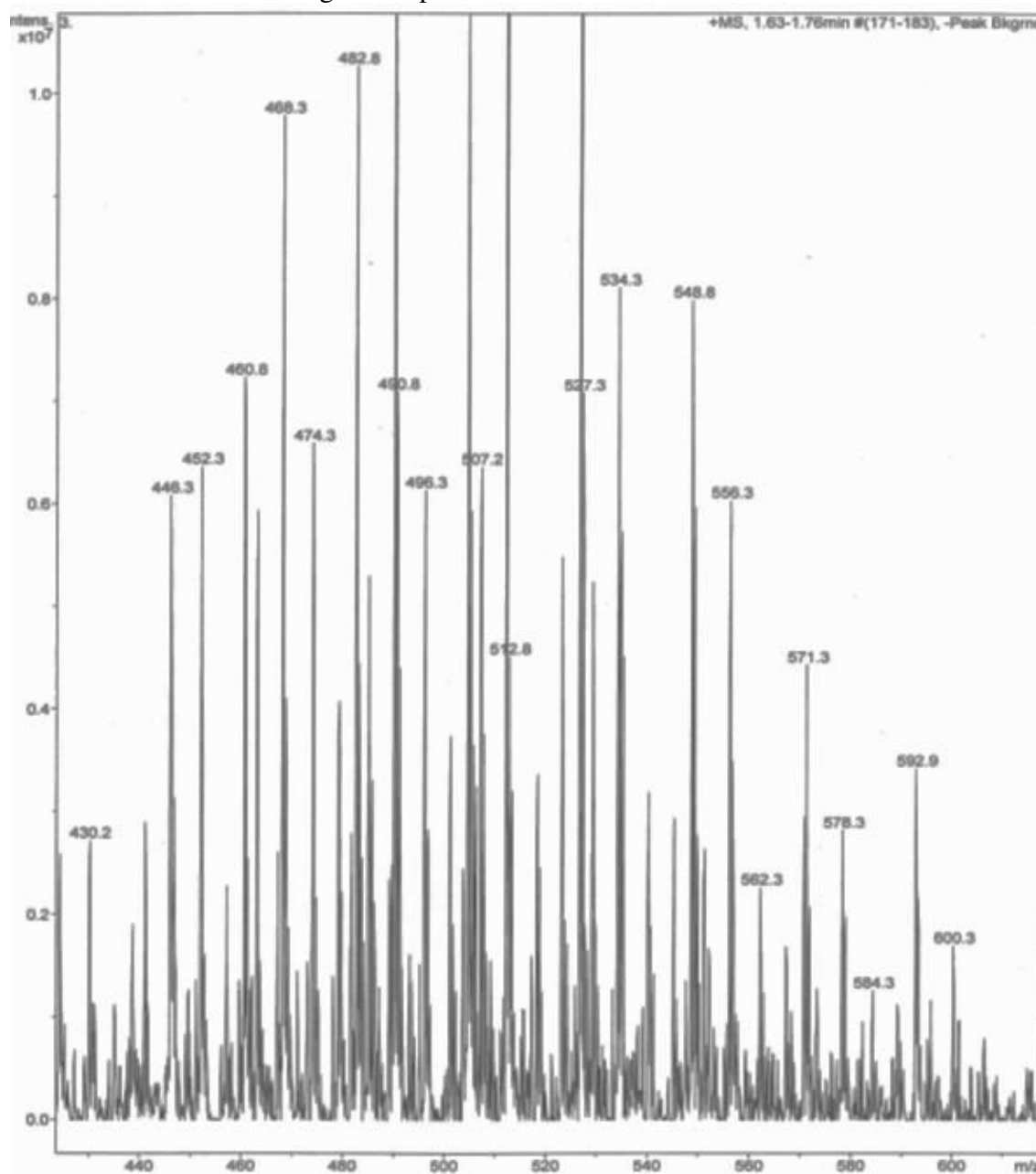


Figure 4: LC-MS data result interpretation for compound TA-PEG600-NHS

For the IR data as shown in the figure 5 and table 1 gave the ketone functional group with C=O stretch bond at 1701.19 cm^{-1} , and two secondary amines which one with N-H stretch bond at 3328.62 cm^{-1} and another one with N-H wag bond at 843.17 cm^{-1} .

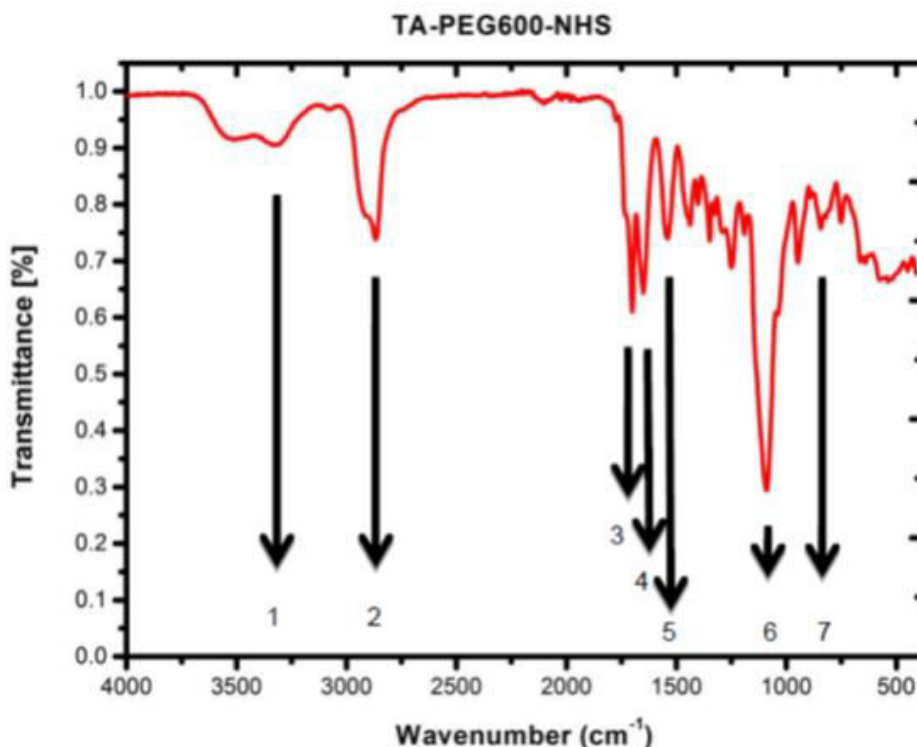


Figure 5: IR data result for compound TA-PEG600-NHS

Table 1: Summary of data result interpretation using IR instrument

Number	Frequency [cm^{-1}]	Bond
1	3328.62	N-H stretch
2	2867.27	C-H stretch
3	1701.19	C=O stretch
4	1650.45	N-H bend
5	1540.61	N-O asymmetric stretch
6	1089.81	C-N stretch
7	843.17	N-H wag

Characterization of TA-PEG3-NHS

The TA-PEG3 with terminal carboxylic acid had been reacted with NHS to formed TA-PEG3-NHS by the esterification reaction and the pale yellow crystal was obtained with the percentage yield of 67.58 %. The compound structure for TA-PEG3-NHS had been drawn and identified as shown as in figure 6 before the data result had been interpreted using the instrumentations. The interpretation of the proton NMR been identified and

showed the crucial peak for the hydrogen located at the NHS unit the value is 2.78 ppm that has sharp and singlet peak, this can be seen in the figure 7. The singlet peak produced is because of the symmetry of carbon-carbon at the NHS compound, hence gave singlet peak for hydrogen located in the NHS terminal of the compound. The interpretation was based on the experiment conducted in the journal paper [16] which in the paper, the proton NMR for NHS was assigned at δ 2.84 ppm, singlet with 4H, and this value are closer the value gained in the experiment.

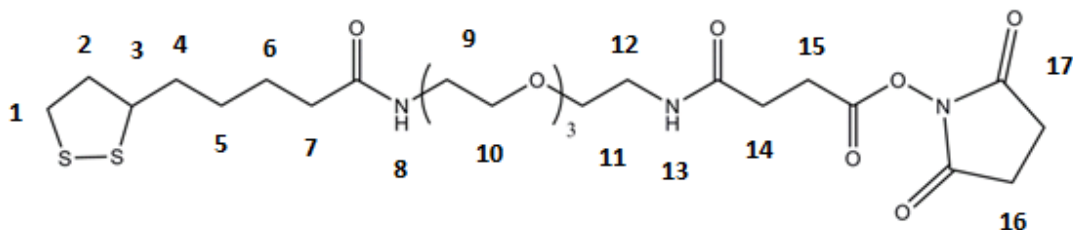


Figure 6: Synthesized compound structure of TA-PEG3-NHS

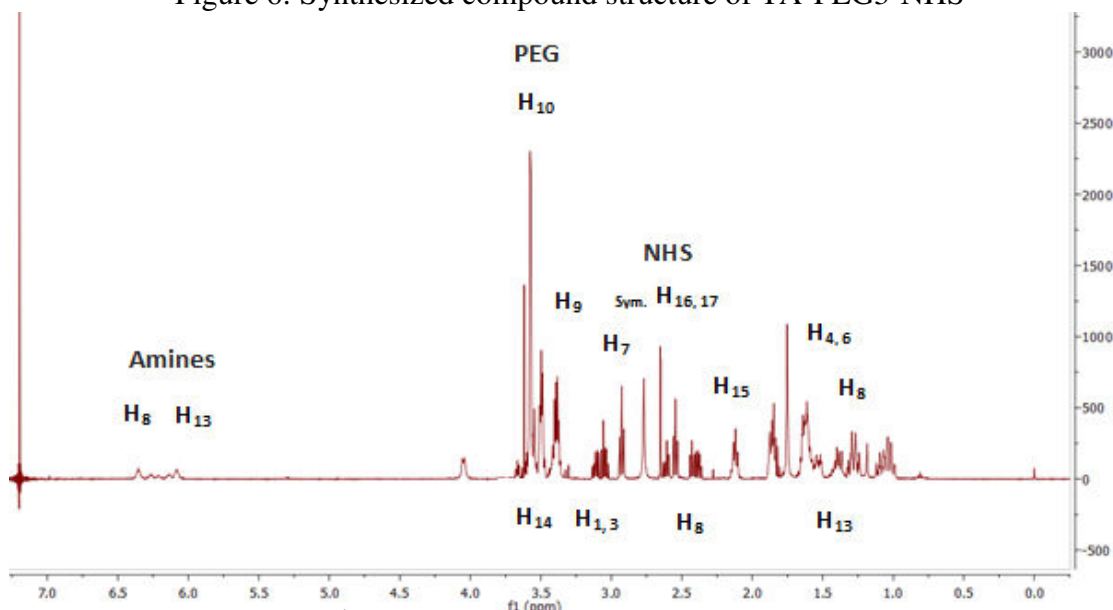


Figure 7: ^1H NMR result interpretation for TA-PEG3-NHS

The mass spectrum data gave promising result, which the total molecular weight for compound TA-PEG3-NHS is 577.64 g/mol, at peak with retention time 1.65 minutes gave maximum m/z 517.2 with the 82.3 % of area as shown in the figure 8, the second significant peak with the values of m/z 578.2 also been identified. This second peak with m/z 578.2 value is closer to the molecular weight of the ester compound, TA-PEG3-NHS and shows the interest product is present.

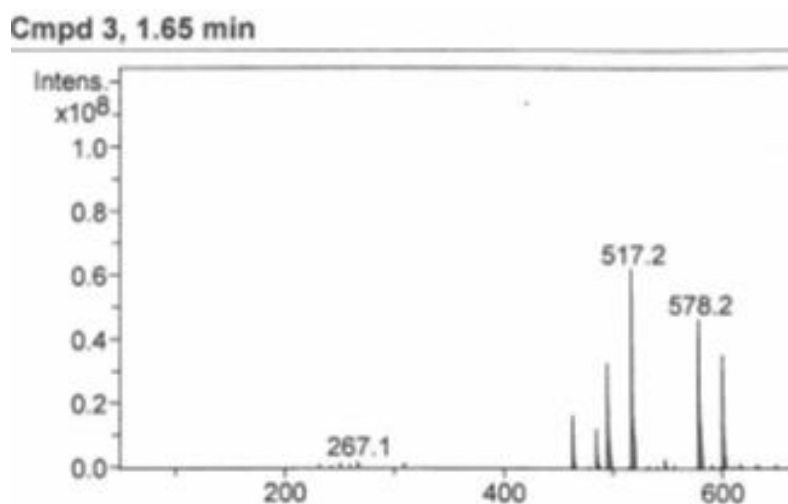


Figure 8: LC-MS data result interpretation for TA-PEG3-NHS

Hence, the IR data for the TA-PEG3-NHS had been described as in figure 9 and table 2, shows the presence of secondary amines functional group; one at frequency 3313.54 cm^{-1} with N-H stretch bond and another one was at frequency 728.33 cm^{-1} with N-H wag bond.

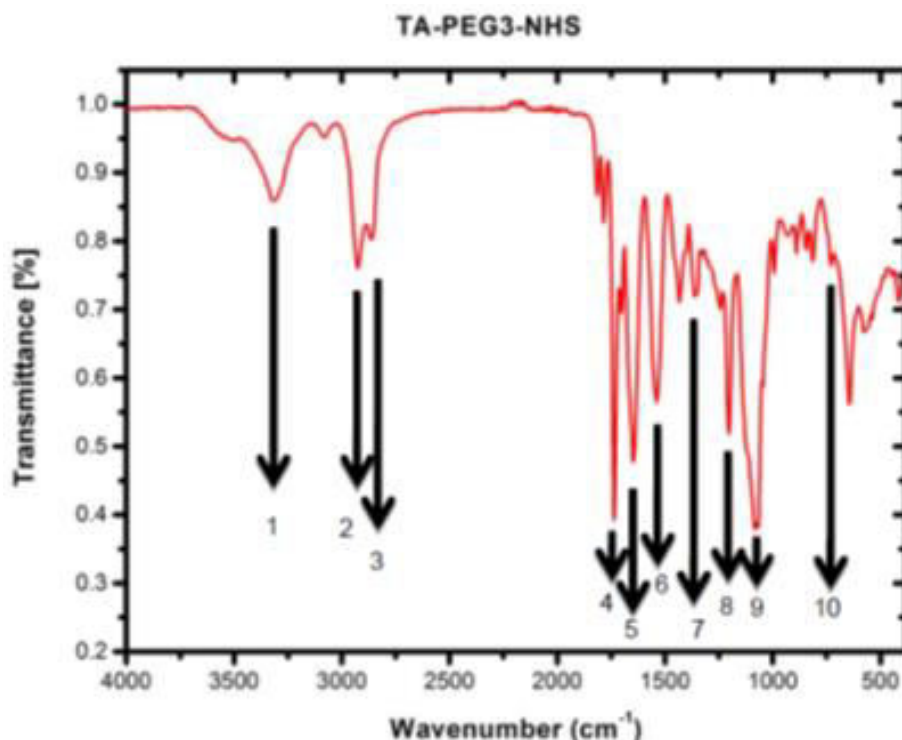


Figure 9: IR data result for TA-PEG3-NHS

Table 2: Summary of data result interpretation using IR instrument

Number	Frequency [cm^{-1}]	Bond
1	3313.54	N-H stretch
2	2925.71	C-H stretch
3	2862.50	C-H stretch
4	1735.97	C=O
5	1646.17	N-H bend
6	1538.39	N-O asymmetric stretch
7	1360.69	N-O asymmetric stretch
8	1203.49	C-N stretch
9	1083.18	C-N stretch
10	728.33	N-H wag

CONCLUSIONS

From the experiments conducted, we successfully synthesized the TA-PEG600-NHS and TA-PEG3-NHS these finding were supported with the data obtained from three different instruments to give the indication for the synthesis of these 2 compounds. The novel synthesis of TA-PEG600-NHS and TA-PEG600-NHS had been discovered which is vital and as a milestone of the overall project since there is no methodology to synthesize these compounds in the literature. All the data obtained, for the synthesis of TA-PEG600-NHS from the TA-PEG600-COOH and TA-PEG3-NHS from TA-PEG3-COOH had been recorded and collected in this experiment.

From all the reliable data gained from the instruments such as NMR, LC-MS and IR machine the TA-PEG600-NHS and TA-PEG3-NHS had been synthetically produced in the lab. As a conclusion for this research project, the novel synthesis of TA-PEG600-NHS and TA-PEG3-NHS was discovered.

The possible compound produced in this experiment which may be able to link or ensemble the quantum dot and antiviral peptide is the milestone of the overall project. Hence, in this research project, the two novel compounds which are the TA-PEG600-NHS compound and TA-PEG600-NHS compound had been synthetically produced and identified. Further steps need to be optimize by varies the PEG compound such as PEG10, PEG50, or PEG100 *etc.* to find an efficient distance space or reaches the HIV or HCV from the anti-viral peptide to rupture the HIV or HCV by pore-formation process and increase the binding affinity, but must follow the Lipinski's rule for excellent drug discovery. The solublization properties of the PEG are depending on the number of PEG. The high number PEG will give high solublization in water this can be varies to get the stable compound.

The finding of the compounds needs further study especially on the stability and the ability to bind with the quantum dot and antiviral peptide. The next step needs to take into account is the compounds produced in this project have to undergo the conversion

from TA-PEG-NHS to DHLA-PEG-NHS in order to bind easily with the quantum dot and will give a strong binding affinity. Next, the primary amines of the anti-viral protein will react with NHS esters by nucleophilic attack and NHS is release as a byproduct and peptide bond will form.

Furthermore, if the linker success binds the quantum dot and antiviral peptide, the compound can be study towards the HIV/HCV in the future ahead. The integrated model of DC-SIGN, anti-viral peptide and quantum dot can be study in the future in one pool towards the HIV or HCV, if the linker between quantum dots with anti-viral peptide is succeed.

ACKNOWLEDGMENTS

The authors wish to thank Majlis Amanah Rakyat (MARA), Universiti Teknologi MARA (UiTM), University of Leeds, UK and Ministry of Education Malaysia (MOE) for funding this research.

REFERENCES

- [1]. P. Alivisatos, *Journal of Nature Biotechnology* **22** 47 (2004)
- [2]. X. Michalet, F. F. Pinaud, L. A. Bentolila, J. M. Tsay, S. Doose, J. J. Li, G. Sundaresan, A. M. Wu, S. S. Gambhir and S. Weiss, *Journal of Science* **307** 538 (2005)
- [3]. Sapsford, K. E., Pons. T., Medintz I. L., Mattoussi H., *Journal of Sensors* **6** 925–953 (2006)
- [4]. Qu L., Peng X., *J. Am. Chem. Soc.* **124** 2049–2055 (2002)
- [5]. Striolo A., Ward J., Prausnitz J. M., Parak W. J., Zanchet D., Gerion D., Milliron D., Alivisatos A. P., *J. Phys. Chem. B*, **106** 5500–5505 (2002)
- [6]. Medintz I. L., Clapp, A. R., Mattoussi, H., Goldman, E. R., Fisher, B., Mauro, J. M. *Nat. Mater.* **2** 630–638 (2003)
- [7]. L. Wang, R. Yan, Z. Huo, L. Wang, J. Zeng, J. Bao, X. Wang, Q. Peng, Y. Li, J. *Angew. Chem. Int. Ed.* **44** 6054–6057 (2005)
- [8]. I. L. Medintz, H. T. Uyeda, E. R. Goldman and H. Mattoussi, *J. Nat. Mater.* **4** 435 (2005)
- [9]. R. C. Somers, M. G. Bawendi and D. G. Nocera, *Chem. Soc. Rev.*, **36** 579 (2007)
- [10]. I. L. Medintz and H. Mattoussi, *J. Phys. Chem.* **11** 17 (2009)
- [11]. W. R. Algar, D. E. Prasuhn, M. H. Stewart, T. L. Jennings, J. B. Blanco-Canosa, P. E. Dawson and I. L. Medintz, *J. Bioconjugate Chem.* **22** 825 (2011)
- [12]. Igor L. Medintz, Thomas Pons, et al. *J. Bioconjugate Chem.* **19** 1789-1795 (2008)
- [13]. Ken-Tye Yong, Wing-Cheung Law, et al., *Chem. Soc. Rev.* **42** 1236-1250 (2013)
- [14]. Joshua A. Jackman, Goh Haw Zan, *J. Phys. Chem.* **117** 16117-16128 (2013)
- [15]. Rodahl, M., Hook, F., Fredriksson, C., Keller, C. A., Krozer, A., Brzezinski, P.,

- Voinova, M., and Kasemo, B., *Faraday Discuss.* **107** 229–246 (1997)
[16]. Wenhao Liu, Mark Howarth, Andrew B. Greytak et al., *J. Am. Chem. Soc.* **130**
1247- 1284 (2008)