Dyes and Pigments 90 (2011) 233–238

Contents lists available at ScienceDirect

Dyes and Pigments

journal homepage: www.elsevier.com/locate/dyepig

Novel unsymmetrical leuco-TAM, (2*E*, 2′*E*)-2,2′-{(E)-4-phenylpent-2-ene-1, 5-diylidene}bis(1,3,3-trimethylindoline) derivatives: Synthesis and structure elucidation

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ARTICLE INFO

Article history:
Received 4 October 2010
Received in revised form
3 January 2011
Accepted 5 January 2011
Available online 13 January 2011

Keywords:
Unsymmetrical leuco-TAM dye
The Malachite green FB-analog
The Cy5 TAM$^+$ dye
The α, β, γ, δ-unsaturated imminium salts
COSY
HMBC
NOESY

ABSTRACT

Novel unsymmetrical leuco-TAM, (2*E*, 2′*E*)-2,2′-{(E)-4-phenylpent-2-ene-1,5-diylidene}bis(1,3,3-trimethylindoline) derivatives were synthesized from a reaction of commercially available 2-methylene-1,3,3-trimethylindoline (FB) and substituted cinnamaldehyde derivatives. The chemical structures of the resulting molecules were determined using 1D and 2D NMR spectroscopy experiments, including COSY, HMBC and NOESY. The compounds had the *EEE* configuration and were potent precursors for Cy5 TAM$^+$ dyes. This is different from the Malachite green FB-analogs, which generally have a *ZE* configuration and are precursors to Cy3 TAM$^+$ dyes. The formation of unsymmetrical LTAM molecules as the sole product suggests that the Michael-type addition of a FB molecule occurs on the δ-carbon of the α, β, γ, δ-unsaturated imminium salts, which were formed as an intermediate at the first step.

1. Introduction

Triarylmethane (TAM) compounds are used in a range of chemical, pharmaceutical and life science industries including thermal imaging and carbonless copying materials, etc. [1,2] They are sometimes called "leuco-TAM (LTAM)" and have been applied as either building-blocks or blocking groups in the synthesis of a range of functional organic compounds [3–6].

LTAM molecules can be oxidized to form TAM$^+$ dyes, which have medicinal activities e.g. photochemotheraphy agents in eye protection devices [7,8]. Among the TAM$^+$ dye molecules available, Malachite green (MG, 4-[(4-dimethylaminophenyl)-phenyl-methyl]-*N*, *N*-dimethyl-aniline), which is generally called "aniline green", is used primarily as a dye in the textile industry [9,10]. MG is also useful for treating fungal and bacterial infections in fish and fish eggs, even though it is toxic. Consequently, there is strong demand for substitutes for MG compounds. Many research groups [11–13], have examined the structural modification of leuco-TAM molecules. We previously reported the synthesis of symmetrical leuco-TAM molecules, (2*Z*, 2′*E*)-2,2′-(2-phenyl propane-1,3-diylidene) bis(1,3,3-trimethylindoline) derivatives prepared from a reaction of excess Fischer base and substituted salicylaldehydes [14–16].

Although significant progress has been made toward the synthesis of symmetric LTAM dyes, the synthesis of unsymmetrical LTAM dyes are still a challenge [17–19]. As part of an ongoing study on the structural modification of leuco-TAM molecules, we report the synthesis and structural elucidation of unsymmetrical leuco-TAM, (2*E*,2′*E*)-2,2′-{(*E*)-4-phenylpent-2-ene-1,5-diylidene}bis(1,3,3-trimethylindoline) derivatives, **Un-LTAM 1–4**, whose chemical structures are shown in Scheme 1.

2. Experimental section

2.1. Materials

Fischer base (2-methylene-1,3,3-trimethylindoline) and salicylaldehyde derivatives were purchased from Aldrich Chemical Co. and used as received. p-Nitrocinnamaldehyde was obtained from the nitration of cinnamaldehyde with sulfuric acid and fuming nitric acid.

The following procedure was used to prepare the Un-LTAM dyes (2Z,2′E)-2,2′-((E)-4-phenylpent-2-ene-1,5-diylidene)bis(1,3,3-trimethylindoline) derivatives, **1–4**. Fischer's base (3.26E−3 kg,

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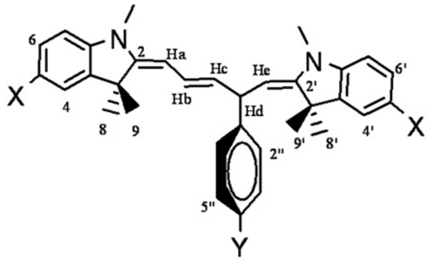
doi:10.1016/j.dyepig.2011.01.002



Compounds	Un-LTAM	X	Y
bis-(FB)PhPn	1	H	H
bis-(ClFB)PhPn	2	Cl	H
bis-(FB)(p-nitroPh)Pn	3	H	NO_2
bis-(ClFB)(p-nitroPh)Pn	4	Cl	NO_2

Scheme 1. Chemical structures of the unsymmetrical LTAM molecules.

18.8 mmol) was added drop-wise to an ethanolic solution of cinnamaldehyde (1.0E−3 kg, 7.5 mmol). The reaction mixture was stirred in an ice bath for 5−6 h. The precipitate was filtered from the reaction mixture and washed thoroughly with cold ethanol. Purification was achieved by precipitation from acetone/hexane.

(2*E*,2′*E*)-2,2′-{(*E*)-4-phenylpent-2-ene-1,5-diylidene}bis(1,3,3-trimethylindoline) Un-LTAM 1, brown, Yield 42%, M.p. 191 °C, ^1H NMR(500 MHz, $CDCl_3$) δ 1.49(s, 3H), 1.57(s, 3H), 1.61(s, 3H), 3.03(s, 3H), 4.48(d, J = 10.8 Hz, 1H), 4.73(q, J = 5.4, 10.5 Hz, 1H), 5.28(d, J = 11.4 Hz, 1H), 5.63(q, J = 6.0, 15.0 Hz, 1H), 6.47(d, J = 7.5 Hz, 1H), 6.51(d, J = 7.8 Hz, 1H), 6.62(q, J = 11.4, 15.0 Hz, 1H), 6.71(t, J = 7.2 Hz, 1H), 6.74(t, J = 7.2 Hz, 1H), 7.05(d, J = 7.5 Hz, 1H), 7.10(t, J = 7.5 Hz, 1H), 7.20(t, J = 6.9 Hz, 1H), 7.35(d, J = 16.2 Hz, 1H), 7.35(t, J = 15.0, 15.9 Hz, 1H); ^{13}C NMR(125 MHz, $CDCl_3$) δ 28.3, 28.4, 28.7, 28.8, 28.9, 29.2, 44.3, 44.9, 45.1, 95.5, 97.5, 104.6, 105.2, 117.9, 118.6, 121.5, 121.5, 125.8, 126.0, 127.7, 127.8, 128.0, 128.5, 130.1, 138.3, 138.7, 144.9, 145.9, 146.4, 152.7, 154.8; ES-Mass for $C_{33}H_{42}N_2$, Mw: 466.7; 467 *m/z*; C, 84.93; H, 9.07; N, 6.00 obtained C, 84.87; H, 9.09; N, 5.89.

(2E,2′E)-2,2′-{(E)-4-phenylpent-2-ene-1,5-diylidene}bis(5-chloro-1,3,3-trimethylindoline) Un-LTAM 2, brown, Yield 46%, M.p. 186 °C, ^1H NMR(500 MHz, $CDCl_3$) δ 1.46(s, 3H), 1.54(s, 3H), 1.61(s, 3H), 3.00(s, 3H), 4.48(d, J = 10.8 Hz, 1H), 4.69(q, J = 6.6, 10.5 Hz, 1H), 5.26(d, J = 11.7 Hz, 1H), 5.62(q, J = 6.6, 14.4 Hz, 1H), 6.36(d, J = 8.4 Hz, 1H), 6.39(d, J = 6.3 Hz, 1H), 6.55(q, J = 11.7, 14.4 Hz, 1H), 6.97(d, J = 2.1 Hz, 1H), 6.98(d, J = 2.1 Hz, 1H), 7.21(t, J = 6.9 Hz, 1H), 7.34(d, J = 6.9 Hz, 1H), 7.35(t, J = 6.9 Hz, 1H); ^{13}C NMR(125 MHz, $CDCl_3$) δ 28.2, 28.3, 28.5, 28.7, 29.1, 29.3, 44.4, 45.0, 45.0, 96.1, 98.0, 105.3, 105.9, 121.2, 122.0, 123.0, 123.5, 126.6, 127.0, 127.3, 127.4, 127.5, 128.2, 128.3, 140.0, 140.3, 144.5, 144.9, 145.9, 151.1, 153.9; ES-Mass for $C_{33}H_{34}Cl_2N_2$, Mw: 529.5; 531 *m/z*; C, 74.85; H, 6.47; Cl, 13.39; N, 5.29 obtained C, 74.79; H, 6.38; N, 5.18.

(2E,2′E)-2,2′-{(E)-4-(4-nitrophenyl)pent-2-ene-1,5-diylidene}bis(1,3,3-trimethylindoline) Un-LTAM 3, orange, Yield 53%, M.p. 182 °C, ^1H NMR(500 MHz, $CDCl_3$) δ 1.50(s, 3H), 1.54(s, 3H), 1.61(s, 3H), 3.05(s, 3H), 4.39(d, J = 11.1 Hz, 1H), 4.83(q, J = 6.3, 10.5 Hz, 1H), 5.28(d, J = 11.7 Hz, 1H), 5.56(q, J = 6.3, 14.7 Hz, 1H), 6.51(d, J = 7.2 Hz, 1H), 6.54(d, J = 7.5 Hz, 1H), 6.67(q, J = 11.7, 14.4 Hz, 1H), 6.75(t, J = 7.2 Hz, 1H), 6.77(t, J = 7.2 Hz, 1H), 7.07(d, J = 7.2 Hz, 1H), 7.13(t, J = 7.8 Hz, 1H), 7.55(d, J = 8.4 Hz, 1H), 8.19(d, J = 8.7 Hz, 1H); ^{13}C NMR(125 MHz, $CDCl_3$) δ 28.3, 28.5, 28.6, 28.7, 29.0, 29.2, 44.4, 45.1, 94.9, 95.6, 104.9, 105.4, 118.3, 118.8, 121.5, 121.6, 123.9, 126.9, 127.8, 128.6, 128.8, 138.0, 138.5, 145.7, 146.1, 146.3, 153.8, 154.4, 155.6; ES-Mass for $C_{33}H_{35}N_3O_2$, Mw: 505.7; 506 *m/z*; C, 78.38; H, 6.98; N, 8.31 obtained C, 78.29; H, 6.91; N, 8.24.

(2E,2′E)-2,2′-{(E)-4-(4-nitrophenyl)pent-2-ene-1,5-diylidene}bis(5-chloro-1,3,3-trimethylindoline) Un-LTAM 4, orange, Yield 55%, M.p. 177 °C, ^1H NMR(500 MHz, $CDCl_3$) δ 1.47(s, 3H), 1.52(s, 3H), 1.59(s, 3H), 3.02(s, 3H), 4.40(d, J = 10.5 Hz, 1H), 4.78(q, J = 6.3, 10.8 Hz, 1H), 5.26(d, J = 11.1 Hz, 1H), 5.56(q, J = 6.3, 14.7 Hz, 1H), 6.40 (d, J = 8.1 Hz, 1H), 6.42(d, J = 8.1 Hz, 1H), 6.62(q, J = 11.1, 14.7 Hz, 1H), 6.99(d, J = 2.4 Hz, 1H), 7.00(d, J = 2.1 Hz, 1H), 7.06(d, J = 8.1 Hz, 1H), 7.07(d, J = 8.1 Hz, 1H), 7.53(d, J = 8.7 Hz, 1H), 8.18(d, J = 8.7 Hz, 1H); ^{13}C NMR(125 MHz, $CDCl_3$) δ 28.2, 28.4, 28.5, 28.6, 29.1, 29.4, 44.6, 45.1, 95.6, 96.2, 105.6, 106.1, 122.1, 123.1, 123.7, 123.8, 126.6, 127.5, 128.3, 128.5, 139.6, 140.1, 144.3, 144.7, 146.4, 153.4, 153.9, 154.8; ES-Mass for $C_{33}H_{33}Cl_2N_3O_2$, Mw: 574.5; 575 *m/z*; C, 68.99; H, 5.79; Cl, 12.34; N, 7.31 obtained C, 68.83; H, 5. 68; N, 7.28.

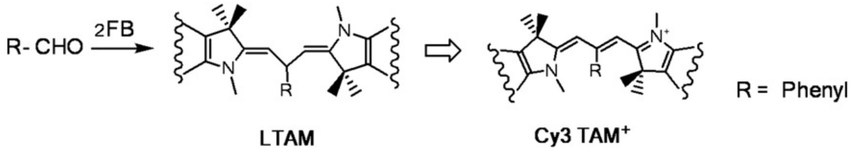
2.2. Measurements

The melting points were determined using a Nikon Labophot-2 polarizing microscope equipped with a Mettler FP82HT hot stage and were uncorrected. All NMR data was acquired on a Varian UNITY-INOVA 500 MHz spectrometer equipped with a 5 mm PFG H {C, N} triple-resonance probe and a 5 mm C−N broadband probe. Samples of the Un-LTAM **1**−**5** were dissolved in $CDCl_3$. The NMR experiments included ^1H and ^{13}C 1D NMR, DEPT, 2D COSY, 2D NOESY, HETCOR, 2D HSQC, and 2D HMBC. The HSQC spectra and gradient HMBC were acquired in phase-sensitive mode and magnitude mode, respectively. The pulse conditions were as follows: for the ^1H NMR spectra, spectrometer frequency (SF) = 500.13 MHz, acquisition time (AQ) = 2.936 s, relaxation delay (RD) = 2.0 s, 90° pulse width = 6.0 μs, spectral width (SW) = 5580.4 Hz, and Fourier transform (FT) size = 16 384; for the ^{13}C NMR spectra, SF = 125.75 MHz, AQ = 1.042 s, RD = 2.0 s, 90° pulse width = 6.0 μs, SW = 31446.5 Hz, line broadening (LB) = 3.0 Hz, and FT size = 32 768; for the ^1H−^1H COSY and NOESY spectra, AQ = 0.092 s, RD = 1.5 s, SW = 5580.4 Hz, 90° pulse width = 6.0 μs, number of points (NP) = 1024, number of increments (NI) = 256, and the mixing time of NOESY = 0.8 s; and for the HMQC and HMBC spectra, AQ = 0.092 s, RD = 1.8 s, SW = 5580.4 (^1H) and 31253.9 (^{13}C) Hz, NP = 1024, and FT size = 1024 × 1024.

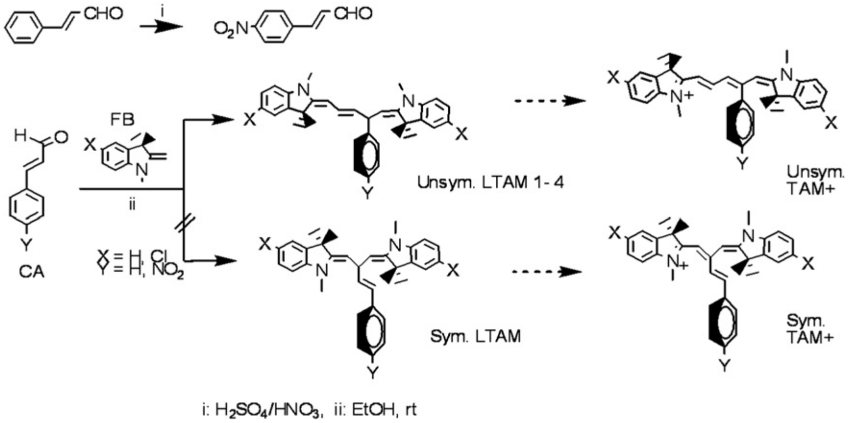
3. Results and discussion

3.1. Synthesis of unsymmetrical leuco-TAM dyes

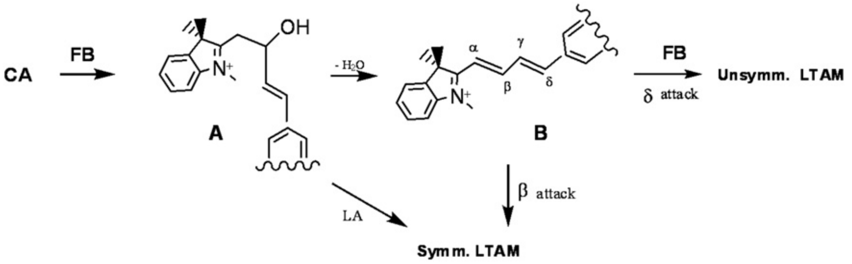
Generally, leuco-TAM (LTAM) dyes can be obtained from a reaction of a molar excess of Fischer base and substituted aryl



Scheme 2. General scheme for the formation of a Cy3 TAM$^+$, via a LTAM molecule.



Scheme 3. Synthetic scheme of the symmetric and unsymmetrical LTAM molecules.



Scheme 4. Mechanistic processes of the Michael-type addition of a FB molecule to the β and δ carbon of the α, β, γ, δ-unsaturated imminium salts **B** to form symmetrical and unsymmetrical LTAM dyes, respectively.

aldehydes according to the known procedure [14,15]. The prepared symmetric LTAM dyes are the precursor of the Cy3 TAM$^+$ dyes [20], where three carbon units are connected to two symmetric FB groups, as shown in Scheme 2.

For the structural modification of leuco-TAM molecules, an attempt was made to prepare a styryl ring pendant LTAM (viz. R = styryl in Scheme 2) instead of the phenyl ring, which was expected to have elongated conjugation from the N$^+$ center of the FB ring to the phenyl ring.

Surprisingly, unsymmetrical LTAM **1**–**4** were obtained from a reaction of excess Fischer's base with the substituted cinnamaldehydes (CA), as shown in Scheme 3. The symmetrical LTAM consisted of two symmetric FB rings on the central carbon, where a styryl ring was located, whereas unsymmetrical LTAM had two different FB derivatives on the central carbon, a 1,3,3-trimethyl-2-methyleneindoline and a 2-allylidene-1,3,3-trimethylindoline group.

Interestingly, no symmetrical LTAM dyes were formed. In terms of the mechanism, symmetrical LTAM molecules could be formed from the Michael-type addition of the second Fischer base molecule onto the β carbon of α, β, γ, δ-unsaturated imminium salts (B). On the other hand, unsymmetrical LTAM molecules are expected from δ carbon-attack of α, β, γ, δ-unsaturated imminium salts (B). Another possible route for the formation of symmetric LTAM might be direct nucleophilic attack of a FB molecule toward the carbinol carbon of the carbinol intermediate (A) formed in the presence of a Lewis acid. Scheme 4 shows the mechanistic processes for the formation of symmetric and unsymmetrical LTAM dyes.

Experimentally, unsymmetrical LTAM molecules were formed as the sole product. This suggests that the Michael-type addition of a FB molecule occurs on the δ -carbon and not on the β-carbon of the α, β, γ, δ-unsaturated imminium salts.

3.2. Structural elucidation of unsymmetrical LTAM molecules by ^{1}H NMR spectroscopy

According to earlier reports [14], Malachite green FB-analogs, (2*Z*, 2′*E*)-2,2′-(2-phenyl propane-1,3-diylidene) bis(1,3,3- trimethylindoline) derivatives (Fig. 1), show the very characteristic ^{1}H NMR resonance patterns of the three adjacent protons, two N-methyl and diastereotopic gem-dimethyl groups. Therefore, these N-methyl and three consecutive protons (Ha, Hb and Hc), or sometimes gem-dimethyl peaks can be used to discriminate between the diastereomers. Namely the methylene protons Ha and Hb resonate at ~4.3 and ~4.4 ppm, respectively, as two doublets and the central proton Hc is observed as a doublet of doublets at ~5.4 ppm. Two N-methyl groups are well separated in the range of 1.3–1.4 and 1.5–1.7 ppm, for the *E*- and *Z*- indoline groups, respectively. The four diastereotopic methyl groups are also separated distinctively in the range of 1.32–1.44 and 1.50–1.70 ppm for the *E*- and *Z*- indoline groups, respectively.

The spectrum of the *EE* diastereomer shows a doublet and triplet at ~4.5 and ~5.1 ppm for the Ha/Hb and Hc, respectively. The N-methyl groups are collapsed into a single resonance at 3.0 ppm, and two, instead of four, diastereotopic methyl groups are shown at 1.4 and 1.6 ppm, for the *EE* diastereomer.

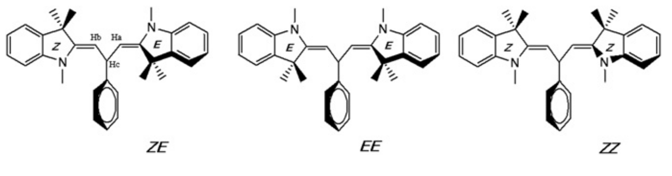


Fig. 1. The *ZE*, *EE* and *ZZ* diastereomers of the Malachite green FB-analogs.

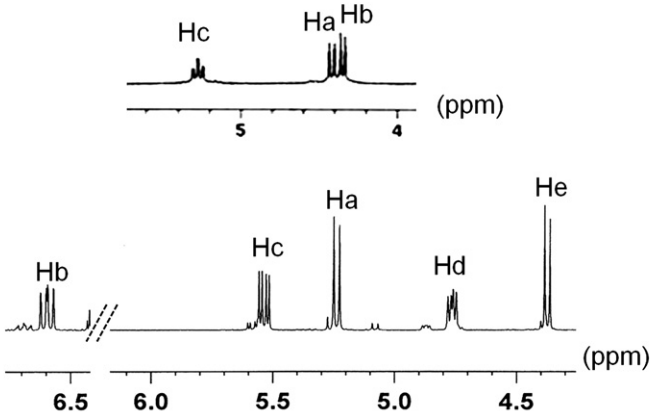


Fig. 2. The five correlated protons (B) of unsymmetric **Un-LTAM 1–4** examined, compared to the three correlated protons (A) of the symmetric FB-analog.

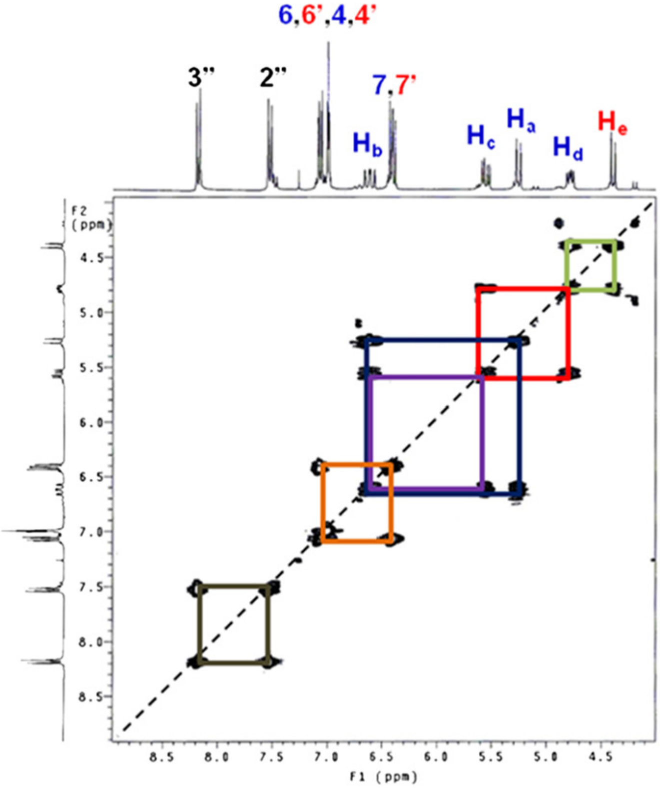


Fig. 3. A 1H–1H COSY spectrum of Un-LTAM **4** in the range of 4.0–8.5 ppm.

The symmetrical vinyl-analog LTAM (in Scheme 2) are expected to have a similar pattern for the three consecutive protons in A in Fig. 2, in addition to the vinyl protons of the styryl group. However, the ^{1}H NMR spectra of the synthesized unsymmetrical LTAM **1**–**4** dyes displays very distinct signals for the five closely-correlated protons of Ha, Hb, Hc, Hd and He, as indicated by B in Fig. 2. They are well-separated over a wide range, ~4.4–6.8 ppm.

COSY was used to identify the protons belonging to the aromatic rings, particularly the five consecutive protons on the connecting carbon chain between the two indoline groups. Hb was correlated with the two Ha and Hc protons simultaneously. Similarly, Hd was also correlated with Hc and He. This correlation pattern could not be expected from the structure of symmetrical LTAM. Fig. 3 presents a typical COSY spectrum of Un-LTAM **2** in the range, 4.0–8.5 ppm.

HMBC experiments identify which protons belong to which ring component. The remaining chemical shift assignments within the FB rings, A and B, of the molecules were mostly made from the HMBC and NOESY data. Since the A and B rings are not identical, HMBC was needed to differentiate between the two different FB groups of the molecules. As a representative example, the HMBC experiment for Un-LTAM 4 showed the C2′ correlated with Hd, H8′, H9′, and H10′ of ring A and C2 correlated with Hb, H8, H9, and H10 in ring B. Similarly, C3 in ring A correlated with Ha, H-4, H-8 and H-9, and C3′ in ring B correlated with He, H4′, H8′, and H9′. HMBC correlated C3a of ring A with H7, H8 and H9 of ring A, and C3a′ of ring B with H7′, H8′, and H9′ in ring B.

The gem-dimethyl peaks of ring B resonated in the range of 1.52–1.57 and 1.59–1.61 ppm. This suggests that ring B has an *E* configuration and those gem-dimethyl protons are located in proximity to the aromatic ring and are thus deshielded (Δppm = ~0.15) compared to the reported value [14] of the *Z*-configuration of the FB-analog.

Fig. 4 shows the possible structures with five consecutive protons and one FB unit with an *E* configuration.

Among the three possible structures of LTAM molecules in Fig. 4, symmetrical structure A could be ruled out from the 1H NMR and COSY experiment, as mentioned above.

The final question remains as to whether the configuration of the double bonds at the position 2–7″ is *E* or *Z*. NOESY was used to solve this problem. Fig. 5 shows the 2D NOESY of Un-LTAM **4** with the spatial correlations of Ha-H10, Hb-H8/H9, H8′/H9′-H2″/6″, and H10′–He. In addition, an unexpected correlation Hb–He was found. This NOE phenomenon indicates an *EEE* geometry counting from the A to B rings.

All data obtained from the COSY, NOE and NOESY showed that the structure of the reaction product is coincident with unsymmetrical (*EEE*)-LTAM, which is C in Fig. 5.

For Un-LTAM **4**, the *gem*-dimethyl groups H8 & H9 are not diastereotopic and equivalent with a difference of $\Delta\delta = >0.01$ ppm because ring A is located adjacent to the chiral center C10″. Although ring B is located at the proximity of the chiral center, C10″, the *gem*-dimethyl groups, H8′ & H9′, are diastereotopic and two

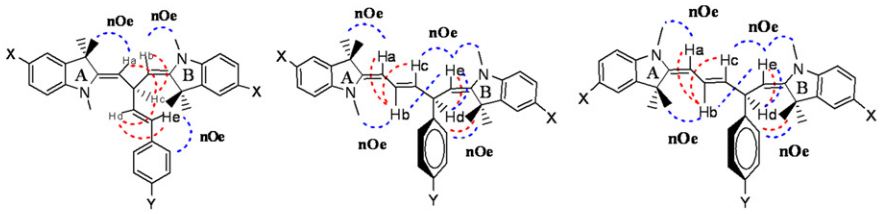


Fig. 4. 1H–1H NOE (blue-colored dot) and 1H–1H COSY (red-colored dot) correlation expected for symmetrical vinyl-analog (ZEE)-LTAM (A), unsymmetrical (*ZEE*)-LTAM (B) and unsymmetrical (*EEE*)-LTAM (C). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

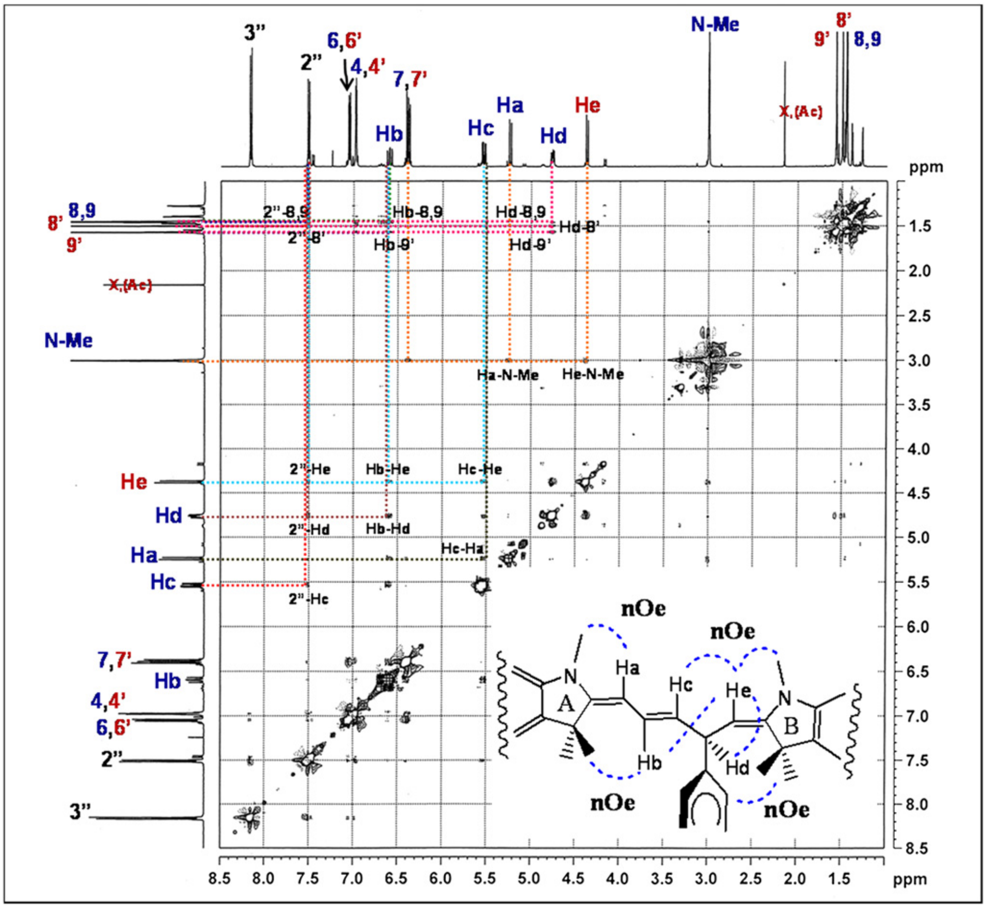


Fig. 5. NOESY of Un-LTAM **4**, showing the spatial correlations of Ha-H10, Hb-H8/H9, H8′/H9′-H2″/6″, and H10′–He.

different NMR signals are observed, i.e. 1.52 and 1.59 ppm. Two well-separated *gem*-dimethyl peaks in the range, 1.52–1.59 ppm, were also fully assigned from the strong NOE correlation between the protons, H8′/H9′ and H2″/H6″.

In summary, the prepared Un-LTAM **1**–**4** molecules belonging to Cy5 dyes have an *EEE* configuration, which is different from the Malachite green FB-analogs, which are Cy3 dyes with a *ZE* configuration as the major diastereomer.

Table 1 lists the selected ^{1}H resonances and coupling constants for Un-LTAM **1**–**4** in $CDCl_3$.

3.3. *Potent unsymmetrical TAM$^+$ dyes*

Typical TAM dyes showed a range of structural forms, such as (a) leuco-, (b) carbinol-, and (c) chromatic- forms. Indeed, the structures of the TAM compounds are very important because they are related to their chemical and biological properties, such as how they are absorbed in the body and how reactive they are [20]. For example, malachite green (MG) is commonly in the chromatic form, and is generally called a green dye. However, it is converted to other forms when it is absorbed into the body. The first carbinol-form spreads across the cell membranes faster and then metabolizes into a leuco-form of MG.

In UV–Vis spectroscopy, MG shows absorption maxima at 620 and 430 nm for the x- and y-band, respectively, whereas the absorption maxima of the vinyl-log of MG are red-shifted for both the x- and y-bands, i.e. 651 and 488 nm, respectively. This suggests that the vinyl effects of a vinyl unit may, to a large extent, behave as an extended conjugation for both the x- and y-bands. The vinyl unit may behave as an extended conjugation of TAM$^+$ molecules, as indicated in Fig. 6.

Structurally, the symmetric and unsymmetric TAM$^+$ dyes in this work belong to a Cy3 and Cy5 dye, respectively, according to Ernst, et al. [21] for closed chain cyanines. It was reported that Cy3 is excited maximally at 550 nm and emits maximally at 570 nm in the

Table 1
Selected ^{1}H resonances (δ, in ppm) and coupling constants (Hz) of Un-LTAM **1**–**4** in $CDCl_3$.

Ring[a]	Proton	Un-LTAM 1	Un-LTAM 2	Un-LTAM 3	Un-LTAM 4
E^1	8-Me	1.49	1.46	1.50	1.47
	9-Me	1.49	1.46	1.50	1.47
	N-Me	3.03	3.00	3.05	3.02
E^2	8′-Me	1.61	1.61	1.61	1.59
	9′-Me	1.57	1.54	1.54	1.52
	N′-Me	3.03	3.00	3.05	3.02
2-pent enyl	Ha	5.28	5.26	5.28	5.26
	Hb	6.62	6.55	6.67	6.62
	Hc	5.63	5.62	5.56	5.56
	Hd	4.73	4.69	4.83	4.78
	He	4.48	4.48	4.39	4.40
J_{Ha-Hb}[b]		11.4	11.7	11.7	11.1
J_{Hb-Hc}[b]		15.0	14.4	14.4	14.7
J_{Hc-Hd}[b]		6.00	6.60	6.30	6.30
J_{Hd-He}[b]		10.7	10.7	10.8	10.7

[a] The E^1 and E^2 denote the left and right ring (in Scheme 1) of the Un-LTAM molecules, respectively.

[b] Coupling constants in Hz.

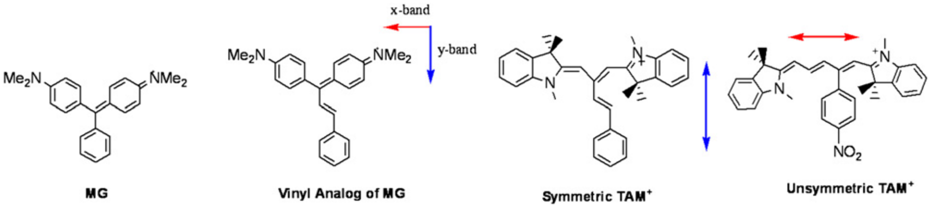


Fig. 6. Chemical skeleton for the **N**∼**N**$^+$ and C(phenyl)∼**N**$^+$ responsible for the x- and y-band, respectively, in the absorption spectrum.

orange–red part of the spectrum, whereas Cy5 is excited maximally at 649 nm and emits maximally at 670 nm, which is in the red part of the spectrum. Therefore, the x-band of the Un-TAM$^+$ are expected to be higher than 650 nm and 550 nm, for the y-band and x-band, respectively. A more detailed UV–Vis spectroscopic study of the Un-TAM$^+$ dyes will be needed.

4. Conclusion

Unsymmetrical LTAM **1**–**4** molecules were obtained as the sole product from a reaction of excess Fischer's base with substituted cinnamaldehydes. The chemical structures of these compounds were determined by 1D and 2D NMR experiments, including COSY, HMBC and NOESY. The prepared compounds had the *EEE* configuration and were potent precursors of a Cy5 TAM$^+$ dyes. This is different from the Malachite green FB-analogs, which generally have a *ZE* configuration. The formation of unsymmetrical LTAM molecules as the sole product suggests that the Michael-type addition of a FB molecule occurs on the δ-carbon of the α, β, γ, δ-unsaturated imminium salts, which were formed as an intermediate in the first step.

Acknowledgments

This study was supported by a Korea University Grant (2009).

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