Tetrahedron Letters 55 (2014) 4222–4226

Contents lists available at ScienceDirect

Tetrahedron Letters

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

Synthesis, chemical reactivity, and photophysical properties of 2′,7′ phenylated rhodamine dyes

Arunkumar Natarajan *, Eugene P. Boden, Andrew Burns, Patrick J. McCloskey, Michael J. Rishel

GE Global Research, 1 Research circle, Niskayuna, NY 12309, USA

ARTICLE INFO

Article history:
Received 22 April 2014
Revised 24 May 2014
Accepted 29 May 2014
Available online 17 June 2014

Keywords:
2′,7′-Diphenyl rhodamine dyes
Emission
Absorption
MSNT coupling
Lactonization

ABSTRACT

While exploring water soluble rhodamine based fluorescent polymeric systems for biological imaging applications we came across new rhodamine derivatives that possess interesting optical properties. We report the synthesis of three different 2′,7′-diphenylated rhodamine derivatives (**1**–**3**) with distinct photophysical properties. The three rhodamine derivatives differ by the number of methyl groups present on the nitrogens and their absorption maxima are red-shifted on increased methylation. We observed an unusual inertness of these compounds toward traditional DCC–DMAP esterification conditions, which we attribute to the ease of lactonization in the presence of even minute amounts of the nucleophile/base DMAP ($pK_a = 9.2$). Synthesis of acrylate esters was successfully accomplished using MSNT (1-(Mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole) coupling conditions using a much milder nucleophile/base, for example, *N*-methyl imidazole ($pK_a = 6.95$).

Introduction

Rhodamine dyes belong to the fluorone dye family of compounds and are widely used as laser gain media as well as for contrast enhancement in biotechnological applications such as fluorescence microscopy, flow cytometry, fluorescence correlation spectroscopy, and enzyme-linked immunosorbent assay (ELISA).[1] Other uses include tracer dyes for fluorescent detection applications in groundwater flow tracing,[2] leak detection, septic, and sewer inspections and similar applications.[3] The rhodamine family of dyes includes Rhodamine B, Rhodamine 6G, Rhodamine 123, Carboxytetramethylrhodamine (TAMRA), tetramethylrhodamine (TMR) and its isothiocyanate derivative (TRITC), sulforhodamine 101 (and its sulfonyl chloride form Texas Red), and Rhodamine Red and covers the spectral range from 450 to 650 nm emission with high quantum yields ranging from 0.65 to 0.95 depending on solvents and extinction coefficients of typically $\log \varepsilon_{max} > 8.8\ M^{-1}\ cm^{-1}$.[4]

A further application area in which rhodamine dyes are commonly used is monitoring the residual levels of polymeric coagulants in wastewater effluents. A common approach to monitoring the level of water soluble polymer coagulants is to blend fluorescent dyes in small amounts and to use fluorescence of the mixture to determine the concentration of the polymer in aqueous systems.[5] While exploring water soluble rhodamine based fluorescent polymeric systems as tracer dyes and bio-imaging applications we discovered some interesting chemical and photophysical properties in the 2′,7′-diphenylated rhodamine derivatives (**1**–**3**) in terms of their, (i) unusual ease of lactonization and therefore chemical inertness during DCC–DMAP esterification conditions; and (ii) changes in absorption and emission spectra based on the extent of methylation of the nitrogen groups.

Liu et al.,[6] first reported the synthesis of tetramethyl derivative of the 2′,7′-diphenylated rhodamine and used it as a benchmark system to compare the properties of the extended conjugation rhodamine based near-IR dyes they had synthesized and studied. We followed a similar synthesis protocol as described in Liu et al.[6] for the synthesis of 2′,7′-diphenylated rhodamine derivative **3**. However in addition to the tetramethyl derivative (**3**) we also observed the trimethyl- and dimethyl-diphenylrhodamine products (**2** and **1**, respectively) in reasonably larger yields compared to compound **3** under our synthesis conditions. All three compounds were easily purified using column chromatography and each of the products possessed interesting and distinct photophysical properties. We present here the five step synthesis procedure of 2′,7′-diphenylated rhodamines, formation of three different 2′,7′-diphenylated rhodamine products (*tetra*-, *tri*-, and *di*-methylated diphenylrhodamine) obtained during the condensation step, their photophysical properties and an exploration of their chemical inertness to DCC–DMAP (dicyclohexylcarbodiimide, dimethylaminopyridine) esterification conditions. Finally, we demonstrate an alternative esterification method using MSNT (1-(Mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole)[7] which delivers high esterification yields.

* Corresponding author. Tel.: +1 518 387 4772; fax: +1 518 387 5592.
E-mail address: natararu@ge.com (A. Natarajan).

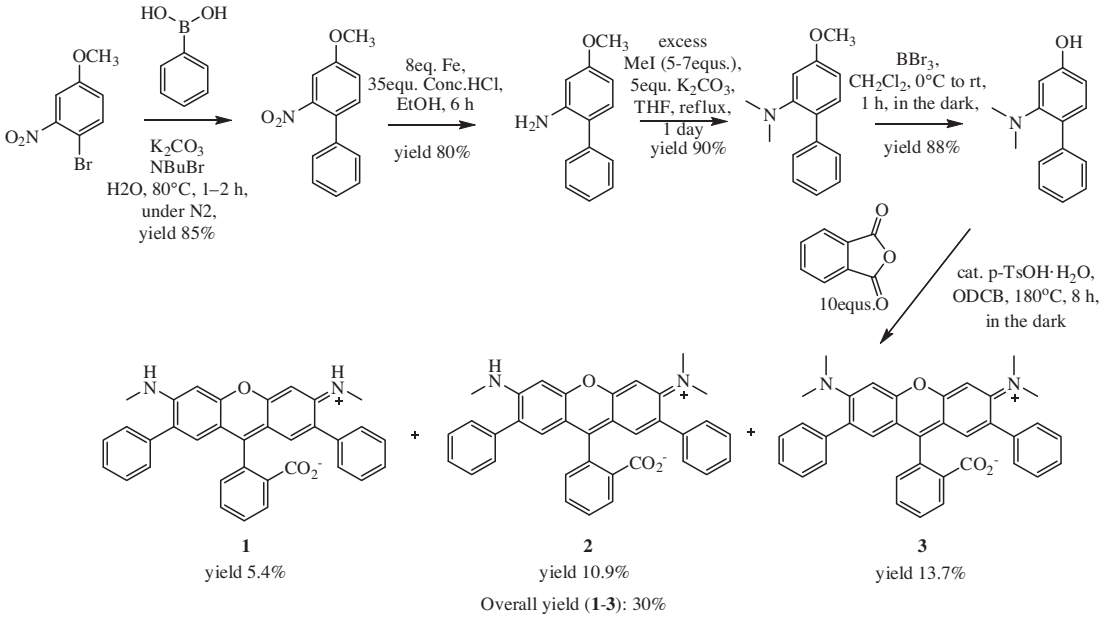
http://dx.doi.org/10.1016/j.tetlet.2014.05.124
0040-4039/

Results and discussion

The rhodamine derivatives (**1**–**3**) were prepared from commercially available 4-bromo-3-nitroanisole, as shown in Scheme 1. The first step involved the synthesis of the 4-methoxy-2-nitrobiphenyl via Suzuki coupling of 4-bromo-3-nitroanisole with the phenyl boronic acid and gave 85% yield.[8] The second step was the reduction of the nitro-group to amine using conventional Fe/concentrated HCl which produced an 80% yield of the 4-methoxybiphenyl-2-amine. The third step was the methylation of the amino group using K_2CO_3/MeI to obtain 4-methoxy-*N*,*N*-dimethylbiphenyl-2-amine in 90% yield. It is important to emphasize here that the excess MeI (5–7 equiv) was used and the reaction was monitored by TLC until the *N*,*N* dialkylation was complete. We observed that isolation of the product without base wash yielded the protonated form (yellow crystals) of the *N*,*N*-dimethylbiphenyl-2-amine due to HI by-product formation during the alkylation reaction. Therefore, after the reaction went to completion, the product was washed twice with $NaHCO_3$ to deprotonate the product, ultimately yielding *N*,*N*-dimethylbiphenyl-2-amine (yellow oil). By allowing the reaction to run to completion and isolating only the dialkylated product (based on ^{1}H NMR analysis) we are confident that no monomethylated product was carried forward to the reaction with phthalic anhydride. The fourth step was the dealkylation of the methoxy group using BBr_3 to obtain 2-(dimethylamino)biphenyl-4-ol in 88% yield. After the reaction was complete, the product was washed thrice with $NaHCO_3$ to prevent isolation of the protonated form of the 2-(dimethylamino)biphenyl-4-ol. Finally, condensation of 2-(dimethylamino)biphenyl-4-ol with 10 equiv of phthalic anhydride, catalytic amount of *p*-toluenesulfonic acid, and heating at 180 °C for 8 h in dichlorobenzene gave a mixture of products (**1**–**3**) with an overall yield of 30% and individual yields of 5.4%, 10.9%, and 13.7%, respectively. In contrast to Liu et al.,[6] which reports only the tetramethyl rhodamine (**3**) in 15% yield, we obtained three rhodamine derivatives (**1**–**3**) with overall yield of 30%. Due to the differing polarities of the *di*, *tri*, and *tetra*-methylated rhodamine derivatives, the products **1**–**3** eluted with different R_f values of 0.7, 0.5, and 0.2 in 2/8 Ethyl acetate/Hexane on silica gel TLC plate. The products were easily isolated in pure forms using silica gel chromatography and the same Ethyl acetate/Hexane = 2:8 as elution solvent. The ^{1}H NMR analysis confirmed the formation of three compounds **1**–**3**. It is interesting to note that the major difference between the three compounds is seen on the *N*-methyl groups in ^{1}H NMR, one signal at 2.88 ppm (s, 6H′s) for compound **1**, showing two signals for compound **2,** 2.6 ppm (s, 6H′s), 2.87 ppm (s, 3H′s), and only one signal at 2.6 ppm (s, 12H′s) for compound **3**.[9] LC-MS analysis of the compounds showed E^+ with molecular mass 512 (for compound **1**), 526 (for compound **2**), and 540 (for compound **3**) in the positive ion mode when eluted using a reverse phase C_{18} column with H_2O–CH_3CN solvent system.

The UV–vis absorption spectra of compounds **1**–**3** show that each molecule has a distinct absorption spectrum (in methanol) with the tetramethyl derivative (**3**) being the most red shifted λ_{max} at 578 nm (bathochromic shift) followed by compounds **2** and **1** which are 557 nm and 539 nm respectively. The trend in the shift of absorption spectrum we see here is very similar to the trend seen in the commercial molecules Rhodamine 6G (tetraethyl derivative) having λ_{max} in ethanol at 540 nm (533 nm in methanol) and Rhodamine B (dimethyl derivative) having λ_{max} at 526 nm in ethanol, respectively.[10] We believe that the extended conjugation conferred by diphenylation of derivatives (**1**–**3**) is responsible for the red-shifts seen in their absorptions when compared to the non-phenylated versions. As shown in Figure 1, the absorption spectrum of Rhodamine 6G (tetraethyl derivative) has a similar absorption spectrum to the dimethyl diphenyl rhodamine derivative (**1**) and the tetramethyl diphenyl rhodamine derivative is red-shifted by ~18 nm. This shows that the phenylated rhodamine derivatives can effectively extend the spectral range of the rhodamine compounds to access longer wavelengths.

Next, we measured the fluorescence of compounds (**1**–**3**) and found that these compounds had distinct emission profiles and quantum yields. Specifically, increasing methylation correlated with decreases in quantum yield. Rhodamine 6G in methanol was used as a standard for relative quantum yield analysis,[11] each dye was diluted to the same peak absorbance (0.25 OD) and excited at their respective maximum absorbances (1 nm slit width). The acid form of the dimethyl, diphenyl derivative (**1**) exhibits an absolute quantum yield of >90% (comparable to Rhodamine 6G) as shown in Figure 1, while the trimethyl derivative (**2**)



Scheme 1. Synthesis route for 2′,7′-diphenyl rhodamine derivatives (**1**–**3**).

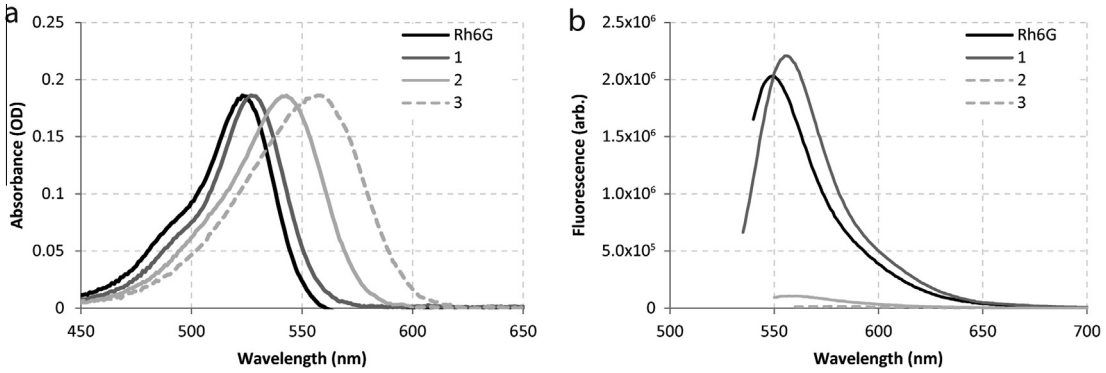


Figure 1. Absorption and emission profiles in methanol of 2′,7′-diphenyl rhodamine derivatives (**1**–**3**) compared to Rhodamine 6G (Rh6G).

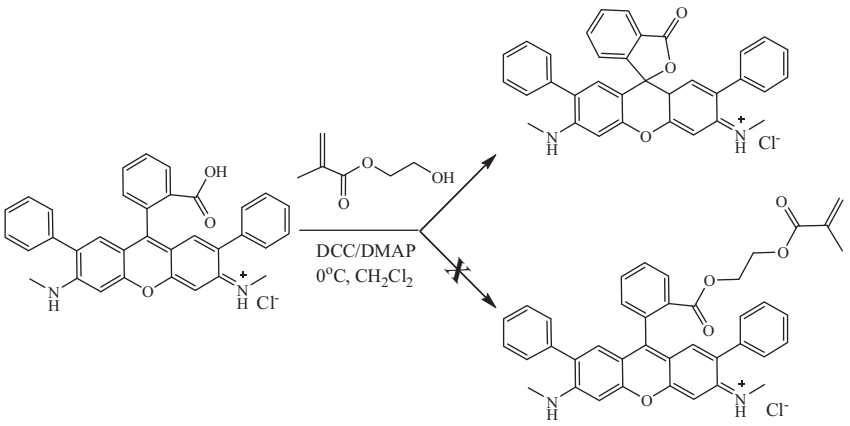
exhibits a relative quantum yield of ~4.5% and the tetramethyl derivative (**3**) exhibits a relative quantum yield of approximately 1% or less. These quantum yields highlight the differences between the diphenylated rhodamine systems and traditional rhodamine dyes which generally exhibit quantum yields >90% across a range of N-alkylations (e.g., Rhodamine B, Rhodamine 6G, etc.).[11] However, we have not yet deciphered the reason for the dramatic decrease in quantum yields for the *tri*- and *tetra*-methyl derivatives, especially when the dimethyl derivative **1** shows fluorescence quantum yields close to Rhodamine 6G, though.

Having demonstrated the optical properties of these dyes, attempts were then made to attach a methacrylate monomer via esterification in order to have a polymerizable moiety. Conventional rhodamine molecules have been converted to methacrylate esters in the literature using DCC–DMAP coupling chemistry via the carboxylic acid with near quantitative yields. However, to our surprise, when we used a similar synthesis protocol to make the corresponding methacrylate esters of compounds **1**–**3**, none of the compounds would react with the 2-hydroxyethyl methacrylate to form the desired products (Scheme 2). In all the cases, the ^{1}H NMR resonances indicated a chemical reaction had occurred, but the proton resonances did not correspond to the desired ester products. Instead, all of the ^{1}H NMR spectra showed signs of lactonization from the open acid form, which were confirmed, based on the Rhodamine B-lactone proton resonance as a model system. Further, LC-MS study shows no new product (ester) formation.

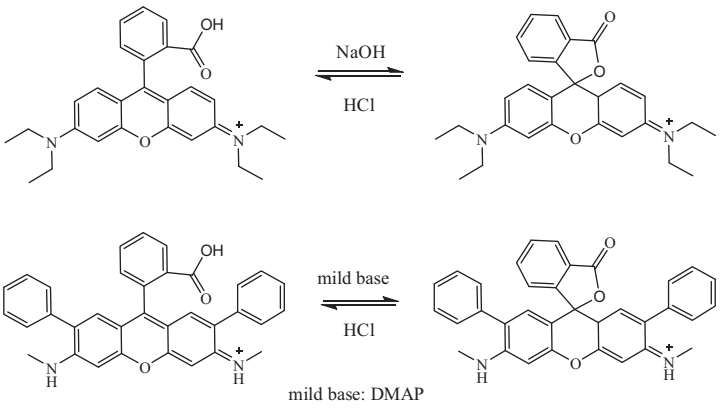
Therefore we conducted UV–vis analysis of compounds **1**–**3** upon addition of DMAP alone without other reagents to see if change in the basicity of the solution affected the diphenyl rhodamine molecule's absorption properties. As anticipated, upon excess DMAP addition, the solution absorbance was seen to dramatically decrease, virtually removing the visible wavelength absorptions seen above in Figure 1a suggesting lactone formation. This phenomenon indicates the lactonization of the dye which is known to dramatically decrease the visible wavelength absorption of xanthene species.[12]

It became apparent that the ease of lactonization of the diphenylated rhodamine derivatives (**1**–**3**) under even under mildly basic conditions was the reason for the lack of esterification reaction (Scheme 3). Use of DMAP as an acylation catalyst in the DCC–DMAP coupling reaction with a conjugate acid pK_a of 9.2 was enough to shift the equilibrium to the lactone and thereby render the acid group unavailable for DCC coupling. In the case of conventional Rh-B (without the phenyl derivatization at 2′ and 7′ position) type dyes, esterification happens easily with the DCC–DMAP coupling conditions[13,14] as lactonization requires a much stronger base like NaOH or triethylamine (pK_a = 10.78), potassium *tert*-butoxide (pK_a = 18).[15]

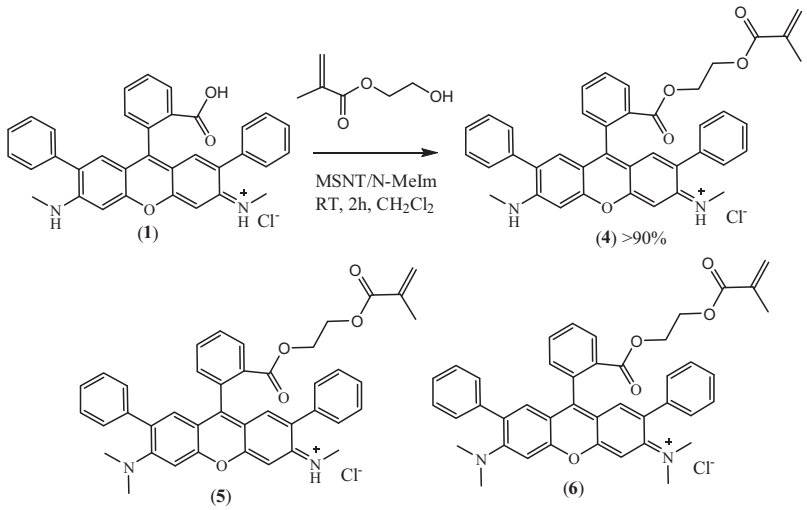
Finally, in order to accomplish the synthesis of the methacrylate ester monomers of compounds **1**–**3**, we sought a coupling reaction that used a milder base for the esterification. Our hypothesis was that if coupling reagents were available that used much milder bases and/or nucleophilic acylation catalysts with pK_a values lower than that of DMAP (pK_a = 9.2), then we could prevent lactonization and thereby make the acid group available for esterification. We found the 1-(Mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole (MSNT) coupling chemistry developed by Reese et al.[7] which uses *N*-methyl imidazole as base/acylation catalyst, which has a pK_a of 6.95. We tested this coupling chemistry following a similar procedure reported in Reese et al., wherein, one equivalent of diphenyl



Scheme 2. DCC–DMAP mediated esterification 2′,7′-diphenyl rhodamine derivatives (**1**–**3**) reaction resulted only in lactonization (only the schematic for derivative **1** shown).



Scheme 3. Lactonization conditions for Rhodamine 6G and diphenylated rhodamine (**1**).



Scheme 4. MSNT mediated esterification reaction conditions shown for 2′,7′-diphenyl rhodamine derivatives (**1**) → (**4**). Similar protocols followed for synthesis of (**5**) and (**6**).

rhodamine acid derivative was dissolved in 2 mL of dry CH_2Cl_2 and mixed with 2 mL of dry CH_2Cl_2 containing 2-hydroxyethyl methacrylate/MSNT reagent/*N*-methyl imidazole (in molar ratios 2/2.2/2.2 with respect to the rhodamine acid). The solution was left to stir overnight at room temperature. This esterification chemistry generated quantitative reaction yields with compounds **1**–**3** to produce the 2-hydroxyethyl methacrylate derivatives **4**–**6** without producing the corresponding lactones (Scheme 4). This suggests that a *N*-methyl imidazole being a weaker base does not shift the equilibrium to the lactone form at least for the amounts used in our study thereby allowing the esterification reaction to proceed quantitatively. However, excess amounts of esterification reagents and the alcohol were needed because the competitive reaction of etherification of the alcohol by MSNT to form the sulfonate may occur otherwise. The crude reaction mixture was purified using column chromatography using silica gel and using 95% CH_2Cl_2 and 5% MeOH as eluents. The products were characterized using ^{1}H NMR[9] and LC-MS analysis. LC-MS analysis of the compounds **4**, **5**, and **6** showed molecular masses of 623, 637, and 651 in the E^+ positive ion mode when eluted using a reverse phase C_{18} column with H_2O–CH_3CN solvent system.

In summary, we have reported the synthesis and purification of three different 2′,7′-diphenylated rhodamine derivatives (**1**–**3**) exhibiting distinct photophysical properties. Additionally, these compounds showed unusual inertness toward ester formation using traditional DCC–DMAP esterification conditions due to their ease of lactonization in the presence of even minute quantities of DMAP, a nucleophile/base with $pK_a = 9.2$. We also demonstrated that these compounds could be easily esterified by using MSNT coupling conditions that use a much milder base, in this case *N*-methyl imidazole ($pK_a = 6.95$). We believe that the presence of 2′,7′-diphenyl substitution has a critical role to play in the reduction of quantum yields in **2** and **3**. Intriguingly, *tri*- and *tetra*-methyl substituted cases (**2** and **3**) shows markedly lower quantum yields of fluorescence while dimethyl substituted derivative (**1**) shows high quantum yields. We are currently pursuing further studies to understand the reasons for this behavior and would be included as a part of future publication.

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9. ^{1}H NMR (400 MHz, $CDCl_3$): Compound **1**: 2.88 (s, 6H), 4.0–4.6 (br s, 2H), 6.5 (s, 2H), 6.56 (s, 2H), 7.2–7.4 (m, 11H), 7.53 (t, 1H), 7.63 (t, 1H), 7.96 (d, 1H). Compound **2**: 2.6 (s, 6H), 2.87 (s, 3H), 4.1–4.25 (br s, 1H), 6.44 (s, 1H), 6.52 (s, 1H), 6.56 (s, 1H), 6.89 (s, 1H), 7.2–7.4 (m, 11H), 7.54 (t, 1H), 7.63 (t, 1H), 7.95 (d, 1H). Compound **3**: 2.6 (s, 12H), 6.56 (s, 2H), 6.88 (s, 2H), 7.2–7.4 (m, 11H), 7.55 (t, 1H), 7.62 (t, 1H), 7.96 (d, 1H). Compound **4**: 2.1 (s, 3H), 3.1 (s, 6H), 4.05 (m, 2H), 4.32 (m, 2H), 5.5 (s, 1H), 5.9 (s, 1H), 6.82 (s, 2H), 6.88 (s, 2H), 7.2–7.3 (m, 6H), 7.4–7.5 (m, 7H), 7.53 (t, 1H), 7.63 (t, 1H), 8.24 (d, 1H). Compound **5**: 2.1 (s, 3H), 2.95 (s, 6H), 3.1 (s, 3H), 4.05 (m, 2H), 4.3 (m, 2H), 5.5 (s, 1H), 5.9 (s, 1H), 6.85 (s, 1H), 6.9 (s, 1H), 6.92 (s, 1H), 7.1 (s, 1H), 7.2–7.4 (m, 11H), 7.78 (t, 1H), 8.05 (t, 1H), 8.25 (d, 1H). Compound **6**: 2.1 (s, 3H), 2.95 (s, 12H), 4.05 (m, 2H), 4.3 (m, 2H), 5.52 (s, 1H), 5.91 (s, 1H), 6.85 (s, 1H), 6.9 (s, 1H), 6.92 (s, 1H), 7.1 (s, 1H), 7.2–7.4 (m, 11H), 7.78 (t, 1H), 8.05 (t, 1H), 8.25 (d, 1H).
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