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Synthesis of Amidation Agents and Their Reactivity in Condensation Reactions

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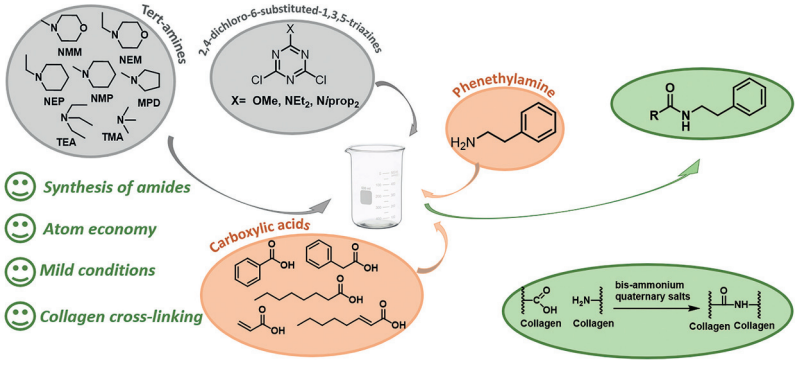

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Abstract Nowadays, the development of new approaches which smartly bypass the use of harsh reaction conditions and hazardous chemicals covers a pivotal role. In this research paper the synthesis, characterization, and application of novel libraries of triazine bis-quaternary ammonium salts, employed as coupling agents to produce amides is reported. Full characterization of the novel compounds by ^{1}H and ^{13}C NMR, FT-IR spectroscopy, ESI-HRMS, and elemental analysis is provided. Furthermore, a comparison in terms of activity of the preformed triazine compounds versus in situ formulations has been evaluated for the formation of amides in the presence of phenylethylamine and different aliphatic or aromatic acids. A possible correlation between the chemical structure of the triazine and their reactivity for the formation of the triazine bis-quaternary ammonium salts is also reported. Moreover, best performing condensation agents have been further tested for the cross-linking of collagen powder as possible wet white tanning systems, for sustainable and environmentally friendly leather tanning.

Key words 1,3,5-triazine bis-quaternary ammonium salts, amidation agents, collagen cross-linking, green chemistry, sustainable leather tanning

One of the most important reactions in organic chemistry and biochemistry is still today the synthesis of amides due to their wide application for the production of peptides, pharmaceuticals, biologically active compounds, and industrial polymers, detergents and lubricants.[1] Consequently, many different physical[2] and chemical protocols have been developed for the synthesis of amides by dehydro-condensation reaction of a carboxylic acid and an amine.[3] Within the chemical dehydro-condensation agents for amide synthesis, triazine-based quaternary ammonium salts such as 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) are gaining increasing interest for the synthesis of amides,[4] peptides,[5] collagen, cross-linked carboxymethylcellulose[2,6] and grafted amine.[7]

Albeit the strategic importance of amides, most methods commonly employed for their synthesis still lack both in economic and environmental sustainability, have low atom efficiency, generate large quantities of hazardous waste products, and require complicated purification steps.[8] Compared to other conventionally employed coupling agents such as carbodiimides, quaternary ammonium salts derived from 2-chloro-4,6-dialkoxy-1,3,5-triazines are known for their higher stability, lower cost, and reduced hazard.[2,4,6] Nevertheless, DMTMM, the only 2-chloro-4,6-dialkoxy-1,3,5-triazine-derived quaternary ammonium salt commercially available, should be improved to implement the use of these products on industrial scale. Our research group has long been involved in the study of innovative sustainable processes for fine chemistry.[9] Recently, our studies have focused on the development of new condensation agents.

This in mind, the scope of this work is to verify if the synthesis of bis-quaternary ammonium salts, synthesized by reaction of 6-substituted 2,4-dichloro-1,3,5-triazines with a tertiary amine (Ams), could generate new families of efficient condensation agents.

Compared to 4,6-disubstituted 2-chloro-1,3,5-triazine-derived quaternary ammonium salts, one of the major advantages of triazine bis-quaternary ammonium salts, is the presence of two active reaction sites for amide synthesis,

which should allow to reduce triazine consumption, synthetic costs, implementing atom efficiency and overall sustainability of the process.

The activity of the isolated triazine bis-quaternary ammonium salts will be compared with the activity of condensation agents prepared by adding directly to the reaction mixture a 6-substituted 2,4-dichloro-1,3,5-triazine and a tertiary amine. Advantages foreseen will be evaluated for the condensation of different aromatic, aliphatic, α,β-unsaturated carboxylic acids and an amine.

Finally, most efficient triazine bis-quaternary ammonium salts will be tested for bovine collagen powder (BCP) cross-linking. Easy to use and inexpensive, BCP has been previously employed as standard substrate for preliminary cross-linking (or tanning) tests to verify the efficacy of a coupling compound as tanning agent. This methodology has been demonstrated to be easily implementable from laboratory to industrial scale for bovine hides tanning, to produce metal, formaldehyde, and phenol-free leather.[6a,10]

The different 6-substituted 2,4-dichloro-1,3,5-triazines and derived bis-quaternary ammonium studied in this work are reported in Figure 1. Only the synthesis of 2,4-dichloro-6-methoxy-1,3,5-triazine (MMT) has been previously reported,[11] but, to the best of our knowledge, no bis-quaternary ammonium salt of MMT has yet been studied. Moreover, also the synthesis of 2,4-dichloro-6-(diisopropylamino)-1,3,5-triazine (MIAT) and 2,4-dichloro-6-(diethylamino)-1,3,5-triazine (MEAT), the preparation of the corresponding quaternary ammonium salts of MMT, MIAT, and MEAT and their use as condensation agents has never been reported. MMT, MIAT, and MEAT have been synthesized by reaction of cyanuric chloride with methanol, or a secondary amine, in the presence of an acid scavenger, according to the general synthetic strategy reported in Scheme 1. Bis-quaternary ammonium salts of the three different 6-substituted 2,4-dichloro-1,3,5-triazines were prepared similarly to triazine mono-quaternary ammonium salts such as DMTMM and other triazine derivatives.[11] In particular, all triazine bis-quaternary ammonium salts derived from MMT, MEAT, and MIAT have been prepared by reaction with *N*-ethyl- or *N*-methyl-substituted tertiary amines at 0 °C (Scheme 1). The seven different tertiary amines employed are *N*-methylmorpholine (NMM), *N*-ethylmorpholine (NEM), *N*-methylpiperidine (NMP), *N*-ethylpiperidine (NEP), *N*-methylpyrrolidine (MPD), trimethylamine (TMA), and triethylamine (TEA). All the triazine bis-quaternary ammonium salts isolated are reported in Figure 1 with the respective yields.

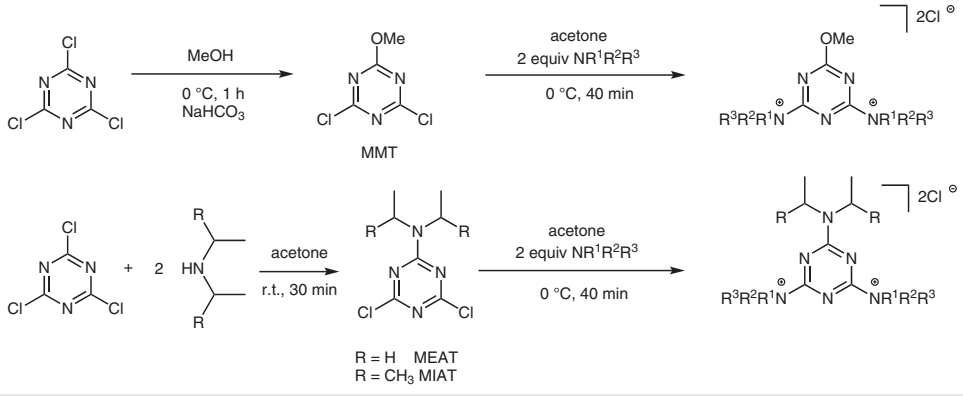
The stability of MMT-Ams, MIAT-Ams, and MEAT-Ams was monitored by ^{1}H NMR over a period of over 34 weeks both in pure form and in water solution (see Supporting Information). It is interesting to note that, except for MMT-MM, the stability of the triazine bis-quaternary ammonium salts synthesized in this work was highly superior compared to DMTMM.[12]

Yields and stability data highlight differences in the reactivity of MMT, MIAT, and MEAT in the presence of the *tert*-amines tested. For example, quaternarization of MIAT and MEAT took place only in the presence of *N*-methyl-substituted amines and in no case with *N*-ethyl-amines, while MMT was highly reactive both with NEM and NEP allowing to synthesize very stable MMTEM and MMTEP in high yields (89% and 94%, respectively).

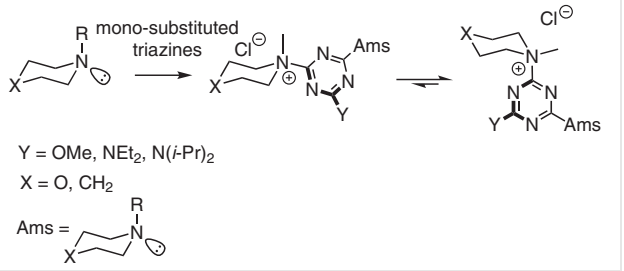
TMA gave very stable triazine bis-quaternary ammonium salts by reaction with MIAT and MEAT, while MMTTMA resulted very unstable.

MIATMM and MEATMM were considerably more stable than the corresponding MMTMM, respectively 15 and 13 weeks compared to few days for MMTMM (see Supporting Information), while in the presence of MPD, MMTMPD and MIATMPD were isolated in approximately 70% yield but no formation of MEATMPD was observed. It is interesting to note that almost a dozen stable products were achieved by reaction of 6-substituted 2,4-dichloro-1,3,5-triazine with two equivalents of a tertiary amine.

According to the literature, the stability of mono-quaternary ammonium salts derived from 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) and NMM strongly depends on



Scheme 1 Synthesis of MMT, MEAT, and MIAT and triazine bis-quaternary ammonium salts



Scheme 2 Reaction pathway for the formation of triazine bis-quaternary ammonium salts in the presence of cyclic *N*-methyl or *N*-ethyl-amine

the possibility of equatorial to axial conformational interchange with respect to the six-membered cyclic *tert*-amine ring (Scheme 2). In particular, ^{13}C NMR studies reported by Kunishima et al.[12] indicate that the reaction between NMM and CDMT may occur if the triazine ring is in equatorial position respect to the alkyl chain (CH_3) of NMM. Then, this rather strained structure spontaneously rearranges to a more stable chair conformation with the flattened triazinyl group in the axial position. Consequently, when the nucleophilic substitution and the conformational rearrangement are hindered by a β-alkyl group present on the *tert*-amine, the formation of the corresponding triazine mono-quaternary ammonium salt may be critical. For this reason, DMTMM may be easily synthesized, while DMTEM and DMTEP are more difficult to produce.

Since no data were available in the literature on triazine bis-quaternary ammonium salts reported in Figure 1, we

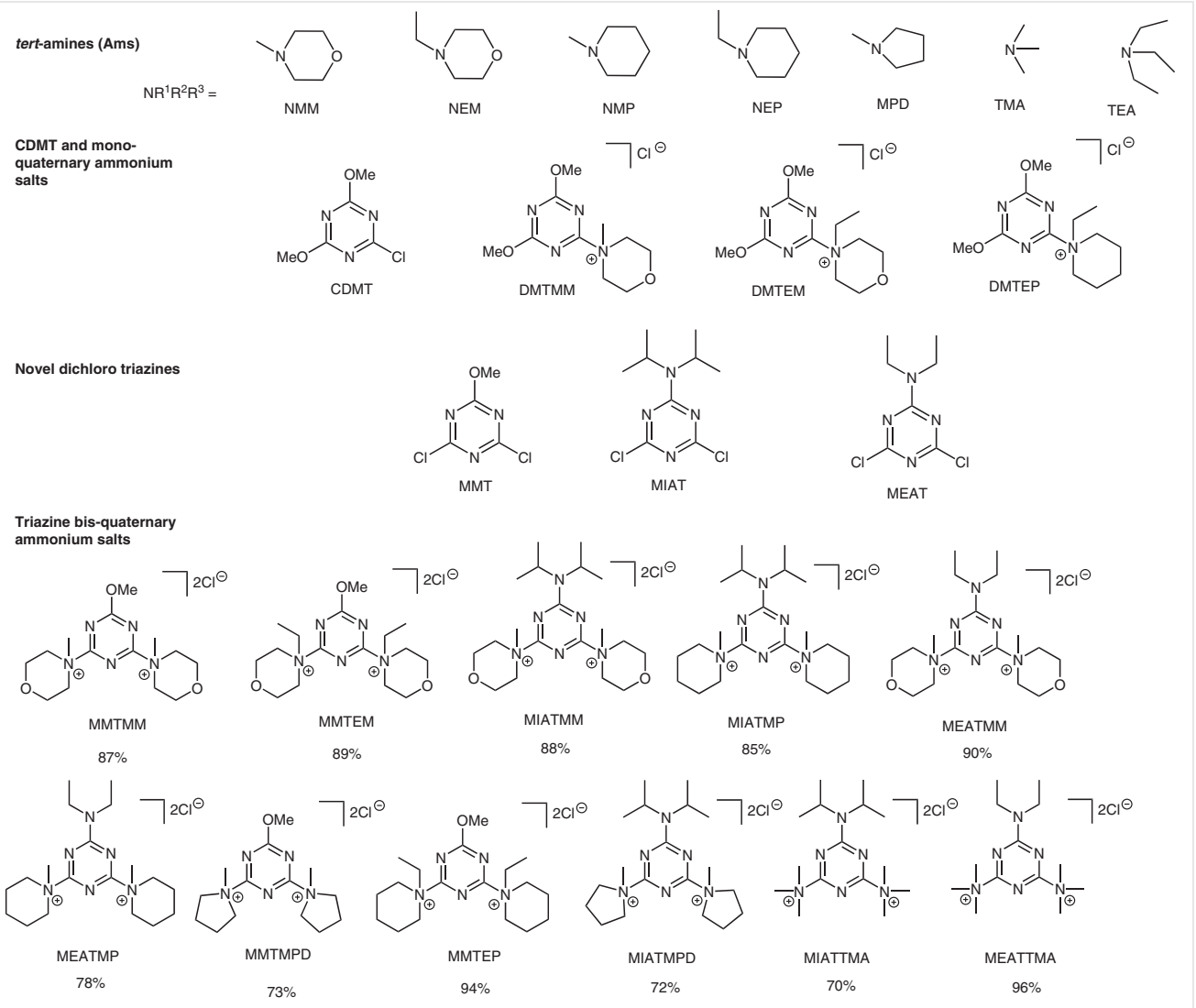


Figure 1 General formula of tertiary amines, mono-quaternary salts derived from CDMT and bis-quaternary ammonium salts derived from MMT, MIAT, and MEAT

deemed it interesting to verify if analogous conclusions on conformational stability could be inferred also for these compounds. This in mind, ^{13}C NMR characterization of the bis-quaternary ammonium salts of 6-substituted 2,4-dichloro-1,3,5-triazines was carried out. Chemical shifts of the carbon atoms of the *N*-$\mathbf{C}H_3$ methyl and secondary carbons (*N*-$\mathbf{C}H_2CH_3$) of the β-alkyl substituents present on the *tert*-amine in MMT-Ams, MIAT-Ams, and MEAT-Ams are reported in the Supporting Information (see Table 2 in the SI). Based on literature data,[12] from the δ value of the *N*-$\mathbf{C}H_3$ and *N*-$\mathbf{C}H_2CH_3$ their equatorial or axial position may be inferred[11] considering that generally, when the alkyl group present on the *tert*-amine is a -CH_3, a chemical shift below 50 ppm corresponds to an axial position, while δ values above 50 ppm are characteristic of an equatorial position.

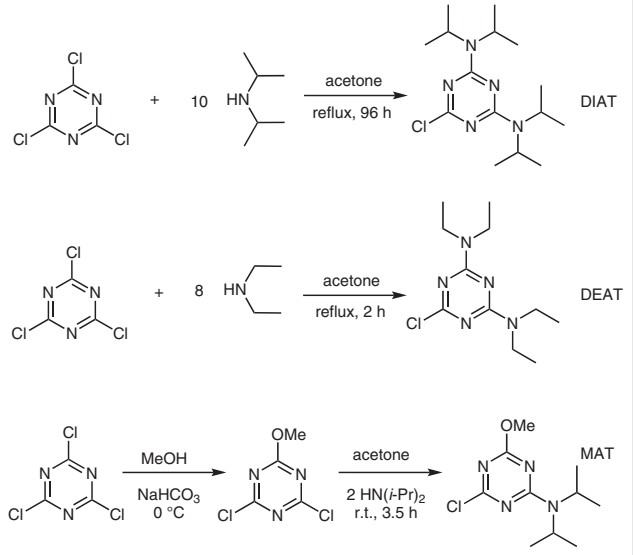
Additionally, the chemical shift of the secondary carbon atom of *N*-$\mathbf{C}H_2CH_3$ falls above 60 ppm when in equatorial position, while axial conformations have δ values below 60 ppm. According to the data reported in Table 2 of the Supporting Information, MMTEM and MMTEP have δ values of 65.0 and 64.7 ppm, respectively, indicating that also for these triazine bis-quaternary ammonium salts, the most stable conformation is the one with the *N*-alkyl chain in the equatorial position and the triazine ring in axial position, in agreement with the literature.[12]

It may be inferred that the presence of a less crowded substitution pattern on MMT compared to CDMT, MEAT, or MIAT allowed nucleophilic attack by both NEM and NEP on MMT, and consequently the rearrangement to the equatorial conformation giving MMTEM and MMTEP in good yields.

Moreover, all 6-substituted 2,4-dichloro-1,3,5-triazines bis-quaternary ammonium salts, having a tertiary amine with a -CH_3 in the β position, showed δ values for -CH_3 above 50 ppm, indicating a conformation with the methyl group in equatorial position and the triazine moiety in axial position.

In order to further highlight the reactivity of MMT, MIAT, and MEAT in comparison to mono-quaternary ammonium salts, disubstituted 2-chloro-4,6-(diisopropylamino)-1,3,5-triazine (DIAT), 2-chloro-4,6-(diethylamino)-1,3,5-triazine (DEAT), and 2-chloro-4-(diisopropylamino)-6-methoxy-1,3,5-triazine (MAT) were synthetized (Scheme 3). While CDMT is a very well known compound employed for the synthesis of DMTMM and other quaternary ammonium salts, DIAT, DEAT, and MAT had never been reported previously in the literature and were synthesized with a protocol analogous to the one adopted for other disubstituted triazine compounds. Moreover, MAT could be alternatively prepared first by nucleophilic substitution of methanol, followed by reaction with diisopropylamine or vice versa without any significant difference (yield 70%, see Supporting Information for further details).

The 4,6-disubstituted 2-chloro-1,3,5-triazine compounds, DIAT, DEAT, and MAT, were further reacted with



Scheme 3 Synthesis of DIAT, DEAT, and MAT

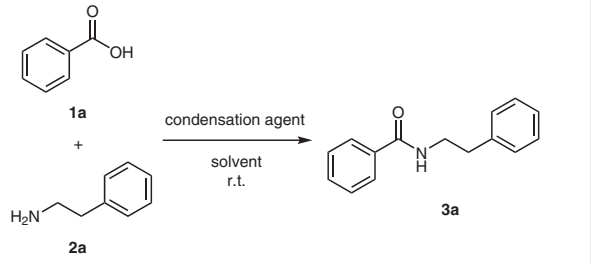
the seven different tertiary amines previously employed for the synthesis of bis-quaternary ammonium salts as above described, but without any success. Recently Kitamura et al.[13] reported that the presence of electron-withdrawing or electron-donating groups strongly influences the reactivity of triazine quaternary ammonium salts and correlated this behavior to the Hammett substituent constants. In particular, according to the Hammett substituent constants[14] -OCH_3 and -OCH_2CH_3 have a σm respectively of +0.12 and +0.10, while σm of -$N(CH_2CH_3)_2$ is –0.16 and –0.26 for -$N[CH(CH_3)_2]_2$, thus the decrease in reactivity of DEAT and MAT may be attributed to the reduced electron-withdrawing nature of the substituents present in DEAT and MAT, compared to, for example, CDMT and 2-chloro-4,6-diethoxy-1,3,5-triazine (CDET). As far as the mono-substituted MIAT and MEAT are concerned, the strong electron-withdrawing nature of the two Cl atoms (σm +0.37) efficiently counterbalances the negative Hammett substituent constants of -$N(CH_2CH_3)_2$ (σm –0.16) and -$N[CH(CH_3)_2]_2$ (σm –0.26) allowing to synthesize the bis-quaternary ammonium salts reported in Figure 1.

All synthesized triazine compounds, were tested in order to verify their activity for the synthesis of amides. Moreover, coupling agents prepared in situ by addition of 6-substituted 2,4-dichloro-1,3,5-triazines and a *tert*-amine in the reaction mixture, have also been tested. This methodology has seldom been employed in the literature and may constitute a valid alternative to the isolated quaternary ammonium salts allowing to reduce processing costs, solvents, and time. The activity of different MMT-Ams, MIAT-Ams, MEAT-Ams (preformed), MMT/*tert*-amine, MIAT/*tert*-amine, and MEAT/*tert*-amine (in situ formulation) was tested employing benzoic acid (**1a**) and phenylethylamine (**2a**)

Table 1 Dehydro-condensation Reaction between Benzoic Acid (**1a**) and Phenylethylamine (**2a**) by Isolated Bis-quaternary Ammonium Salts or MMT/*tert*-Amine, MIAT/*tert*-Amine, and MEAT/*tert*-Amine In Situ Protocol[a]

Entry	Coupling agent[b]	Yield[c] (%) of **3a** after 15 min/1 h	Entry	Coupling agent[b]	Yield[c] (%) of **3a** after 15 min/1 h
1	MMTMM	65/68	11	MIAT/NMP	17/20
2	MMTMM[d]	82/89	12	MIATMPD	38/42
3	MMT/NMM	48/52	13	MIAT/MPD	47/51
4	MMT/NMP	51/55	14	MEATMM	39/44
5	MMTMPD	49/57	15	MEAT/NMM	38/40
6	MMT/MPD	55/60	16	MEATMP	52/57
7	MMT/TMA	32/34	17	MEAT/NMP	26/28
8	MIATMM	47/51	18	MEAT/MPD	45/47
9	MIAT/NMM	25/33	19	MEATTMA	42/46
10	MIATMP	29/33	20	MEAT/TMA	17/19

[a] Reaction conditions: benzoic acid (1.2 mmol), phenylethylamine (1.2 mmol), MeOH (6 mL), 25 °C.
[b] Coupling agent: 6-substituted 2,4-dichloro-1,3,5-triazine (0.6 mmol) and tertiary amine (1.2 mmol) were added; for isolated MMT-Ams, MIAT-Ams, and MEAT-Ams (0.6 mmol) were used.
[c] Yield in **3a** was measured by GLC using mesitylene as internal standard after 15 min and 1 h.
[d] MMTMM (0.83 mmol).

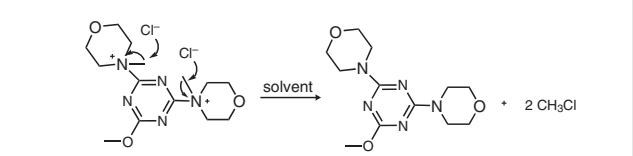


Scheme 4 Dehydro-condensation reaction for the synthesis of amide **3a**

as standard substrates (Scheme 4 and Table 1). All data reported in Table 1 are the mean values achieved for a set of at least three experiments monitored after 15 min and 1 h.

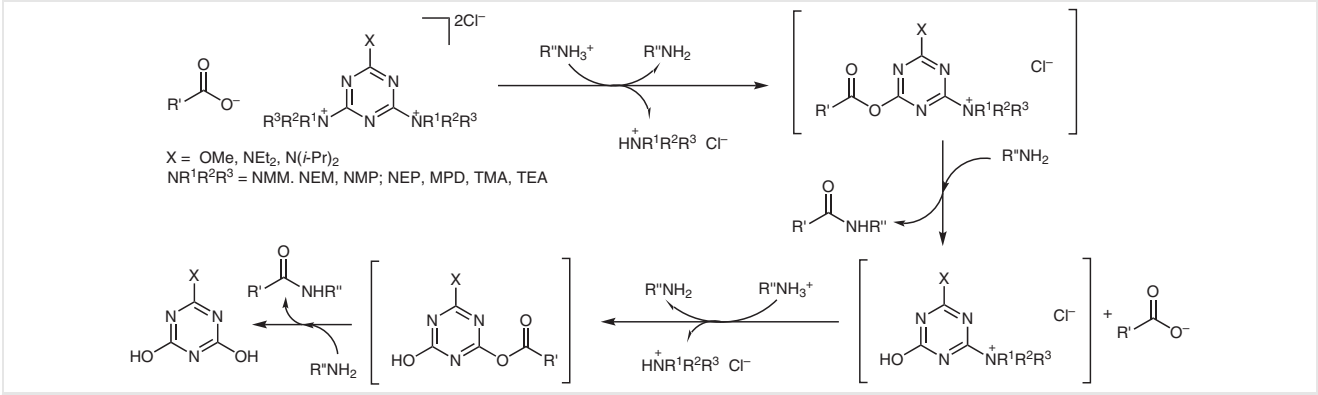
For example, the in situ formulation in the presence of MMT/*tert*-amine foresees: dissolution of 1.2 mmol of benzoic acid (**1a**) in the reaction solvent, followed by addition of 0.6 mmol of MMT, 1.2 mmol of *tert*-amine NMM, and 1.2 mmol of phenylethylamine (**2a**) (Table 1, entry 3). Analogous conditions were employed for the formulation of MIAT/*tert*-amine and MEAT/*tert*-amine. The yield of amide **3a** was monitored by GLC after 15 min and 1 h. For a set of preliminary experiments, the reaction mixture was monitored by GLC also after 24 h; since no significant difference was observed between the yield in amide **3a** after 1 h and 24 h, all further reactions were monitored within 1 h.

It is well known that depending on the *tert*-amine used, the corresponding triazine quaternary ammonium salt may be unstable and decompose leading to the formation of triazine derivatives which are totally inactive as dehydro-condensation agents (see Scheme 5).[4a,15] On the contrary, for the triazine bis-quaternary ammonium salts reported in Figure 1, most of the isolated MMT-Ams, MIAT-Ams, and MEAT-Ams showed good stability, allowing to compare the two different protocols (preformed and in situ formulation), with the exception of MMTMP, MMTTMA, and MEAT-MPD which were not stable enough to be isolated. Additionally, the synthesis of bis-quaternary ammonium salts prepared from 6-substituted 2,4-dichloro-1,3,5-triazine and *N*-ethyl tertiary amines was generally unsuccessful, except for MMTEM and MMTEP, thus *N*-ethyl *tert*-amines were not further investigated in this work.



Scheme 5 Demethylation of DMTMM

The isolated triazine bis-quaternary ammonium salts and 6-substituted 2,4-dichloro-1,3,5-triazine/*tert*-amine in situ formulation allowed to obtain **3a** in comparable yields (see, for example, Table 1, entries 1, 3 and 5, 6 or entries 14, 15). MMT/*tert*-Ams gave better yields in amide **3a** compared to MIAT/*tert*-Ams (cf. Table 1, entries 3 and 9) or MEAT/*tert*-Ams (cf. Table 1, entries 3 and 15). Isolated MMTMM was the most efficient amidation agent for the synthesis of **3a** reaching yields up to 68% in 1 h (Table 1, entry 1). It is important to consider that, in the literature amidation reactions carried out in the presence of DMT-Ams or CDMT/*tert*-amine employ a molar ratio between the carboxylic acid and the coupling agent of 1:1 (mol/mol). In this work the ratio between **1a** and the triazine derivative was 2:1 (mol/mol), reducing by half the amount of condensa-



Scheme 6 Formation of the mono and bis-ester intermediate

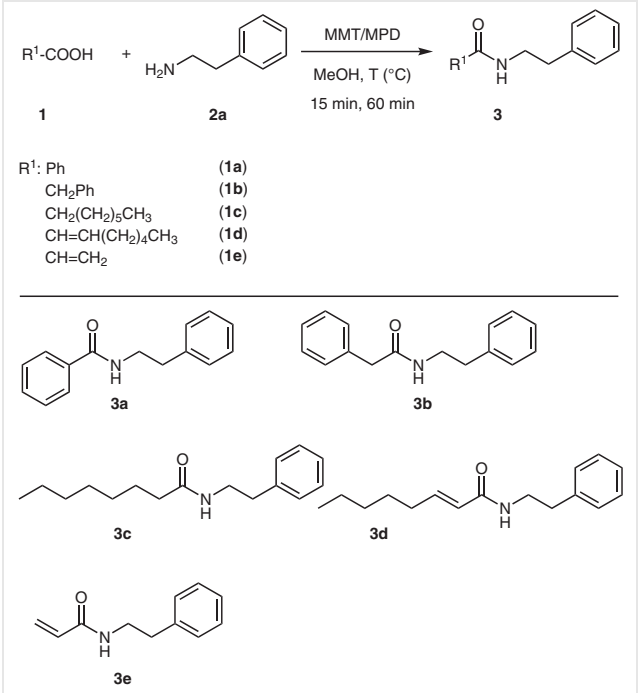
tion agent employed. These data may be rationalized considering that the dichloro-triazine derivatives such as MMT-Ams, MIAT-Ams, or MEAT-Ams, may originate two intermediate active ester species allowing to activate up to two equivalents of carboxylic acid for each equivalent of triazine present in the reaction mixture (Scheme 6). Although part of the triazine bis-quaternary ammonium salts tested give conversions in **3a** below 50% by 1 h (see, for example, Table 1, entries 7, 9, or 10–12), when conversions above 50% are achieved it indicates that, for each equivalent of triazine present, more than one equivalent of **1a** reacted. In other words, considering, for example, Table 1, entry 1, 1.2 mmol of **1a** were reacted with 0.6 mmol of MMTMM, producing 0.82 mmol of **3a** (yield 68%). This implies that one of the chlorine atoms (0.60 mmol) was 100% active in the reaction conditions tested, while the other chlorine atom was efficient for over 36% (0.22 mmol), substantiating that lower consumption of these quaternary ammonium salts may be achieved, compared to 4,6-disubstituted 2-chloro-1,3,5-triazine derivatives. Moreover, if the molar ratio between **1a** and MMTMM was slightly increased, the yield in **3a** raised to 89% in 1 h (Table 1, entry 2), which is comparable to values obtained with DMTMM or CDMT/NMM system.[12]

To widen the scope of the coupling agents synthesized, dehydro-condensation reactions for the synthesis of different amides were tested (Scheme 7) ranging from aliphatic, aromatic, sterically hindered, and α,β-unsaturated acids. Only MMT/MPD was further tested since, comparing data reported in Table 1, this coupling system showed comparable activity to MMTMM, higher than MMT/NMM and moreover could be easy formulated in the reaction mixture, without isolation of the triazine quaternary ammonium salt.

Changing from benzoic acid to phenylacetic acid (**1b**), no substantial variation in the overall yield of amide **3b** was observed (cf. Table 1, entry 1 and Table 2, entry 1). For the synthesis of **3c** and **3d** slightly lower yields were obtained, 42% and 54% respectively, probably due to reduced nucleophilicity of the carboxyl group of **1c** and **1d** compared to **1a**, which disfavors the formation of the active ester (Table 2, entries 3 and 4).

Data reported in Tables 1 and 2 confirmed that 2,4-dichloro-6-methoxy-1,3,5-triazine and a *tert*-amine allow to achieve reasonably good yields in amides **3a–e** with a significant reduction in consumption of triazine and solvents required for the synthesis and recovery of isolated bis-quaternary ammonium salts. These features are particularly important for some specific applications as for example, for their use as collagen cross-linking agents.

In a recent paper, we reported a study on the economic and environmental sustainability of DMTMM as a tanning



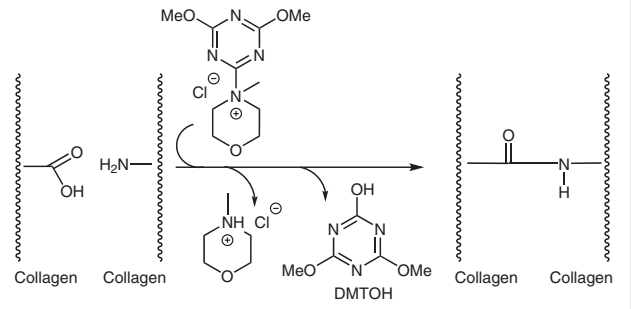
Scheme 7 Condensation reaction between phenylethylamine (**2a**) and carboxylic acids **1a–e** with MMT/MPD system

Table 2 Condensation Reaction between **2a** and Acids **1b–1e** in the Presence of MMT/MPD[a,b]

Entry	Acid	Amide	Yield[c] (%) after 15 min/1 h
1	**1b**	**3b**	59/65
2	**1c**	**3c**	33/42
3	**1d**	**3d**	52/54
4	**1e**	**3e**	33/34

[a] Reaction conditions: acid **1b–1e** (1.2 mmol), phenylethylamine (**2a**; 1.2 mmol), MeOH (6 mL).
[b] Coupling agent: 2,4-dichloro-6-methoxy-1,3,5-triazine (MMT; 0.6 mmol) and MPD (1.2 mmol).
[c] Yields were measured by GLC using mesitylene as internal standard after 15 min and 1 h.

agent.[6a] When these chemical agents are employed to stabilize the collagen structure, cross-linked amide bonds are formed between the pendant carboxylic and amine residues present in the protein chains (Scheme 8).[7,16,17] As far as this specific application is concerned, the efficiency of these agents in terms of condensation activity is only one of the features which have to be taken into consideration together with ease of use, versatility, low cost, and environmental impact. Thus, further experiments were carried out in order to verify the efficiency of the triazine derived bis-quaternary ammonium salts reported in Figure 1 as collagen cross-linking agents. In particular, bovine collagen powder (BCP) was selected as standard substrate for the preliminary cross-linking (or tanning) tests. According to the literature, 1.0 g of BCP contains 1.2 mmol of free carboxylic acid groups ($COOH_{coll}$) and 0.8 mmol of free amine groups (NH_{2Coll})[16b] and these functional groups will be cross-linked by an amidation agent to form covalent amide bonds, influencing the shrinking temperature (Ts) of the treated collagen. Generally, collagen Ts should increase from 50–55 °C for native collagen to Ts above 75 °C, to be sufficiently stabilized for further processing.[18,19]



Scheme 8 Chemistry likely involved in collagen cross-linking promoted by DMTMM

Variations in BCP shrinking temperature (Ts) were measured before and after the reaction to evaluate the cross-linking efficiency of the triazine bis-quaternary ammonium salt (preformed or in situ formulation), as previously reported for DMTMM.[10,17]

Cross-linking experiments were carried out according to the protocol optimized in our previous work,[10,18] simply by adding to a water suspension of BCP, the triazine bis-quaternary ammonium salts or a 6-substituted 2,4-dichloro-1,3,5-triazine and a *tert*-amine in 1:2 molar ratio.

Preliminary tests were carried out with a molar ratio between $COOH_{coll}$ and 6-substituted 2,4-dichloro-1,3,5-triazine of 4:1 (mol/mol), corresponding to half the amount normally employed with DMTMM.[10,18] In Table 3 only the results achieved for amidation agents giving Ts values above 70 °C are summarized.

Table 3 Shrinkage Temperature of Collagen Cross-linking by Different Triazine Bis-quaternary Ammonium Salts[a]

Entry	Coupling agent[b]	Ts (°C)[c]
1	–	55
2	MMTMM	70
3	MMT/NMM	71
4	MMT/NMP	80
5	MIATMM	75
6	MEATMM	79
7	MEAT/NMM	71

[a] Reaction conditions: collagen (250 mg, 0.3 mmol COOH), r.t., 4 h.
[b] Coupling agent: 6-substituted 2,4-dichloro-1,3,5-triazine (0.075 mmol) and tertiary amine (0.150 mmol); for isolated MMT-Ams, MIAT-Ams, and MEAT-Ams (0.075 mmol) were used.
[c] Shrinkage temperature was calculated by DSC (see experimental section).

From data in Table 3, it is possible to observe that Ts of collagen samples cross-linked by isolated MIATMM (entry 5) and MEATMM (entry 6) were higher compared to MMTMM (entry 2). Moreover, MMT/NMM (entry 3), MMT/NMP (entry 4), and MEAT/NMM (entry 7) were also efficient for collagen cross-linking reaching values as high as 80 °C, comparable to some of the most efficient synthetic tanning agents industrially employed today.[20] It should be further underlined that from an industrial point of view, the possibility to reduce triazine consumption by half is a great advantage since lower chemical consumption not only implies reducing material costs, but also expenses due to the treatment of wastewater after tanning.

In conclusion, in this work three different new families of triazine bis-quaternary ammonium salts have been synthesized and fully characterized. Isolated MMT-Ams, MIAT-Ams, and MEAT-Ams have been studied as well as a simplified methodology for the use of these compounds for coupling reactions. In particular, both isolated and in situ triazine bis-quaternary ammonium salts proved to be efficient for the synthesis of amides, reaching, in best reaction conditions, yields in **3a** of up to 89%. The activity of MMT/MPD was further investigated for the synthesis of ali-

phatic, aromatic, sterically hindered, and α,β-unsaturated acids reaching yields in the different amides up to 65%. Main advantage of these compounds relies in the presence of two active condensation sites present on the 6-substituted 2,4-dichloro-1,3,5-triazine, which, in best reaction conditions, may reduce the consumption of triazine up to almost 50%. To assess the possible industrial application of these new amidation agents, cross-linking experiments of bovine collagen powder were carried out and increase in collagen shrinking temperature measured to evaluate the efficiency of the different triazine agents used. MMT/NMP gave very good Ts up to 80 °C. This data is comparable to some of the most efficient tanning agents employed industrially today, such as, for example, aldehydes and natural or synthetic tannins. The possibility to stabilize collagen without the use of chrome salts, formaldehyde, or phenols constitutes a great advantage of triazine derivatives from a sustainability point of view.[6a,19,21,22] Moreover, the amount of MMT/NMP employed to reach Ts of 80 °C is by far lower compared to other triazine based cross-linking agents known to date for the tanning of hides, reducing both costs in reagents and chemical load in wastewater.

All chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA). Bovine collagen powder was purchased from Filk (Research Institute of Leather and Plastic Sheeting, Freiberg, Germany). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance AC 300 spectrometer (Billerica, MA, USA) operating at 300.21 and 75.44 MHz, respectively. FT-IR spectra were recorded using a Perkin-Elmer Spectrum-One spectrophotometer using KBr disks. Melting points were measured with a Buchi 235 model. Differential scanning calorimetry (DSC) of collagen samples was performed on a Netzsch STA 409 cell (Selb, Germany) fitted with an air-cooling compressor at r.t. and a controller Netzsch TASC 414/3. The instrument temperature was calibrated using indium as the standard. Collagen samples (about 7.0 mg) were weighed (about 0.1 mg) into aluminum pan melting pots and sealed. Samples were heated from 30 °C to 120 °C at a scanning rate of 10 °C/min. A sealed melting pot (about 7.0 mg) filled with Al_2O_3 (about 7.0 mg) was used as the reference. Gas-chromatography analysis were recorded with an Agilent Technologies 6850 gas chromatograph equipped with FID detector and capillary column HP-5 (cross-linked 5% phenylmethylsilicone) or HP-1 (100% dimethylsiloxane). Analyses were registered with following temperature ramps: HP-1 column: Ti: 50 °C × 1 min, rate: 20 °C/min, Tf: 230 °C × 35 min. HP-5 column: Ti: 50 °C × 4 min, rate: 20 °C/min, Tf: 230 °C × 30 min. ESI-MS analyses were performed using a Finnigan LCQ-Duo ion-trap instrument, operating in positive ion mode (sheath gas N_2, source voltage 4.0 KV, capillary voltage 21 V, capillary temperature 200 °C). All mass spectra were recorded on freshly prepared solutions. Elemental analyses (C, H, N) were carried out using a Fison EA1108 microanalyzer.

2,4-Dichloro-6-methoxy-1,3,5-triazine (MMT)

To a 100-mL flask, equipped with magnetic stirrer, was added $NaHCO_3$ (0.91 g, 10.8 mmol) in MeOH (30 mL). The suspension was cooled to 0 °C with an ice-cold water bath and cyanuric chloride (2.0 g, 10.8 mmol) was slowly added. The reaction stirred at 0 °C for 1 h (monitored by GC), and then the solvent was removed by reduced pressure evaporation. Acetone (30 mL) was added and the suspension filtered to remove NaCl. The acetone was evaporated to give MMT (10.5 mmol, 97%) as a white solid; mp 88 °C.

FT-IR (KBr): 1528, 1258, 815 cm^{-1}.

^{1}H NMR (300 MHz, acetone-d_6): δ = 4.17 (s, 3 H).

^{13}C NMR (75 MHz, acetone-d_6): δ = 172.7, 172.7, 57.4.

2,4-Dichloro-6-(diisopropylamino)-1,3,5-triazine (MIAT) and 2,4-Dichloro-6-(diethylamino)-1,3,5-triazine (MEAT)

To a 100-mL flask, equipped with magnetic stirrer, was added cyanuric chloride (2.0 g, 10.8 mmol) in acetone (20 mL). Then, the amine (21.6 mmol, 2 equiv) was added dropwise to the solution and the reaction was stirred at r.t. for 30 min (monitored by GC). The suspension was filtered to remove ammonium chloride and mother liquors were concentrated to dryness. The obtained solid was characterized by ^{1}H NMR, ^{13}C NMR, FT-IR, and mp.

MIAT

Yield: 10.26 mmol (95%); yellow solid; mp 103 °C.

FT-IR (KBr): 1525, 1333, 1229, 808 cm^{-1}.

^{1}H NMR (300 MHz, acetone-d_6): δ = 4.51 (m, 2 H), 1.39 (d, 12 H).

^{13}C NMR (75 MHz, acetone-d_6): δ = 169.2, 164.2, 47.7, 19.4.

ESI-HRMS: m/z [M – Cl]$^+$ calcd: 213.0907; found: 213.0712.

Anal. Calcd for $C_9H_{14}Cl_2N_4$ (249.14): C, 43.39; H, 5.66; N, 22.49. Found: C, 44.12; H, 6.54; N, 23.71.

MEAT

Yield: 9.94 mmol (92%); white solid; mp 77 °C.

FT-IR (KBr): 1521, 1336, 1235, 811 cm^{-1}.

^{1}H NMR (300 MHz, acetone-d_6): δ = 3.71 (m, 4 H), 1.22 (d, 6 H).

^{13}C NMR (75 MHz, acetone-d_6): δ = 169.9, 164.4, 42.7, 12.2.

ESI-HRMS: m/z [M – Cl]$^+$ calcd: 185.0594; found: 185.0612.

Anal. Calcd for $C_7H_{10}Cl_2N_4$ (221.09): C, 38.03; H, 4.56; N, 25.34. Found: C, 37.53; H, 4.89; N, 24.11.

2-Chloro-4,6-bis(diisopropylamino)-1,3,5-triazine (DIAT)

To a two-necked 100-mL flask, equipped with magnetic stirrer and bubble condenser, was added cyanuric chloride (1 g, 5.42 mmol) in acetone (30 mL). The system was cooled to 0 °C and a solution of diisopropylamine (7.6 mL, 54.20 mmol) in acetone (30 mL) was added. The solution was heated to reflux and stirred for 96 h (monitored by GC). Every 24 h, acetone (10 mL) was added. At the end of this period, the precipitate was filtered off and organic phase was evaporated to dryness. Finally, in order to remove unreacted amine, the obtained oil was washed and dried with CH_2Cl_2 (×4) to give DIAT (4.33 mmol, 80%) as a yellowish solid; mp 111 °C.

FT-IR (KBr): 1521, 1330, 1236, 810 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.38 (m, 4 H), 1.33 (d, 24 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 167.4, 163.6, 45.7, 20.5, 20.2.

ESI-HRMS: m/z [M + H]$^+$ calcd: 313.2033; found: 313.1943; m/z [M + Na]$^+$ calcd: 336.1082; found: 336.1985.

Anal. Calcd for $C_{15}H_{28}ClN_5$ (313.87): C, 57.40; H, 8.99; N, 22.31. Found: C, 56.92; H, 8.12; N, 23.01.

2-Chloro-4,6-bis(diethylamino)-1,3,5-triazine (DEAT)

To a two-necked 100-mL flask, equipped with magnetic stirrer and bubble condenser, was added cyanuric chloride (1 g, 5.42 mmol) in acetone (30 mL). The system was cooled to 0 °C and a solution of diethylamine (4.5 mL, 43.36 mmol) in acetone (30 mL) was slowly added. The solution was heated to reflux and stirred for 2 h (monitored by GC). At the end of this period, the precipitate was filtered off and organic phase was evaporated to dryness. Finally, in order to remove unreacted amine, the obtained oil was washed and dried with CH_2Cl_2 (×4) to give DEAT (4.33 mmol, 80%) as a dark yellow oil; mp 94 °C.

FT-IR (KBr): 1519, 1334, 1231, 813 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 3.52 (q, 8 H), 1.14 (t, 12 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 168.9, 164.0, 41.6, 41.3, 13.3, 12.7.

ESI-HRMS: *m*/*z* [M + H]$^+$ calcd: 257.1406; found: 257.1501; *m*/*z* [M + Na]$^+$ calcd: 280.1323; found: 280.4873.

Anal. Calcd for $C_{11}H_{20}ClN_5$ (257.77): C, 51.26; H, 7.82; N, 27.17. Found: C, 52.06; H, 8.37; N, 26.71.

2-Chloro-4-methoxy-6-(diisopropylamino)-1,3,5-triazine (MAT)

To a 100-mL flask, equipped with magnetic stirrer, was added $NaHCO_3$ (0.23 g, 2.71 mmol) in MeOH (30 mL). Then, the system was cooled to 0 °C and cyanuric chloride (0.5 g, 2.71 mmoL) was slowly added. The solution was stirred for 1 h (monitored by GC). At the end of this period, solvent was removed by reduced pressure evaporation, then acetone (30 mL) was added, and the suspension obtained was filtered to remove NaCl. Next, diisopropylamine (0.76 mL, 5.42 mmol) was added to the solution and the reaction was stirred at r.t. for 3.5 h. Finally, the suspension was filtered, and mother liquors were evaporated to dryness to give MAT (1.62 mmol, 60%) as a white solid; mp 81 °C.

FT-IR (KBr): 1521, 1336, 1261, 1232, 803 cm^{-1}.

^1H NMR (300 MHz, acetone-d_6): δ = 4.61–4.39 (m, 2 H), 3.93 (s, 3 H), 1.38–1.31 (d, 12 H).

^{13}C NMR (75 MHz, acetone-d_6): δ = 170.8, 170.0, 165.5, 54.8, 47.1, 46.8, 19.7, 19.6.

ESI-HRMS: *m*/*z* [M + H]$^+$ calcd: 244.1091; found: 244.1783; *m*/*z* [M + Na]$^+$ calcd: 267.0988; found: 267.0823.

Anal. Calcd for $C_{10}H_{17}ClN_4O$ (244.72): C, 49.08; H, 7.00; N, 22.89. Found: C, 48.67; H, 8.50; N, 22.16.

Preparation of Triazine Bis-quaternary Ammonium Salts Derived from 6-Substituted 2,4-Dichloro-1,3,5-triazine; General Procedure

To a 50-mL flask, equipped with magnetic stirrer, was added 6-substituted 2,4-dichloro-1,3,5-triazine (MMT, MIAT, MEAT; 2.0 mmol) in acetone (20 mL). The solution was cooled to 0 °C and the amine (4.0 mmol, 2 equiv) was slowly added (in the case of TMA, a 45% wt. aq solution was used). The reaction was stirred for 40 min, then the precipitate was filtered and dried under vacuum. The solid product obtained was characterized by ^1H NMR, FT-IR, and mp, when possible the ^{13}C NMR spectrum was acquired as well. ^{13}C NMR spectrum of MMTMM was not acquired due to its low stability.

4,4′-(6-Methoxy-1,3,5-triazine-2,4-diyl)bis(4-methylmorpholin-4-ium) Dichloride (MMTMM)

Yield: 1.74 mmol (87%); white solid; mp 73 °C.

FT-IR (KBr): 1521, 1336, 1288, 1261, 1129 cm^{-1}.

^1H NMR (300 MHz, D_2O): δ = 4.53 (m, 4 H), 4.16 (m, 4 H), 3.87 (m, 8 H), 3.39 (s, 3 H), 2.87 (s, 6 H).

ESI-HRMS: *m*/*z* [M – Cl]$^+$ calcd: 346.1646; found: 346.1622.

Anal. Calcd for $C_{14}H_{25}Cl_2N_5O_3$ (382.28): C, 43.99; H, 6.59; N, 18.32. Found: C, 43.44; H, 6.01; N, 18.07.

4,4′-(6-Methoxy-1,3,5-triazine-2,4-diyl)bis(4-ethylmorpholin-4-ium) Dichloride (MMTEM)

Yield: 1.78 mmol (89%); white solid; mp 87 °C.

FT-IR (KBr): 1571, 1370, 1288, 1263, 1135 cm^{-1}.

^1H NMR (300 MHz, D_2O): δ = 4.57 (d, 4 H), 4.20 (d, 4 H), 3.96 (q, 4 H), 3.85 (m, 8 H), 3.39 (s, 3 H), 1.25 (t, 6 H).

^{13}C NMR (75 MHz, D_2O): δ = 168.8, 167.6, 65.0, 62.0, 57.9, 48.8, 7.0.

ESI-HRMS: *m*/*z* [M – Cl]$^+$ calcd: 374.1959; found: 374.1967.

Anal. Calcd for $C_{16}H_{29}Cl_2N_5O_3$ (410.34): C, 46.83; H, 7.12; N, 17.07. Found: C, 46.55; H, 6.88; N, 16.84.

1,1′-(6-Methoxy-1,3,5-triazine-2,4-diyl)bis(1-ethylpiperidin-1-ium) Dichloride (MMTEP)

Yield: 1.88 mmol (94%); white solid; mp 73 °C.

FT-IR (KBr): 1579, 1371, 1288, 1259 cm^{-1}.

^1H NMR (300 MHz, D_2O): δ = 4.48 (d, 4 H), 3.82 (q, 4 H), 3.54 (t, 4 H), 3.38 (s, 3 H), 1.95 (m, 4 H), 1.75 (m, 8 H), 1.20 (t, 6 H).

^{13}C NMR (75 MHz, D_2O): δ = 170.2, 168.9, 64.7, 59.9, 49.3, 21.5, 21.3, 7.8.

ESI-HRMS: *m*/*z* [M – Cl]$^+$ calcd: 370.2373; found: 370.2367.

Anal. Calcd for $C_{18}H_{33}Cl_2N_5O$ (406.39): C, 53.20; H, 8.19; N, 17.23. Found: C, 53.09; H, 7.98; N, 17.02.

1,1′-(6-Methoxy-1,3,5-triazine-2,4-diyl)bis(1-methylpyrrolidin-1-ium) Dichloride (MMTMPD)

Yield: 1.46 mmol (73%); white solid; mp 63 °C.

FT-IR (KBr): 1551, 1343, 1289, 1275 cm^{-1}.

^1H NMR (300 MHz, D_2O): δ = 3.56 (m, 4 H), 3.26 (s, 3 H), 2.97 (m, 4 H), 2.83 (s, 6 H), 2.07 (m, 4 H), 1.92 (m, 4 H).

^{13}C NMR (75 MHz, D_2O): δ = 155.8, 151.5, 57.0, 56.2, 41.0, 23.3.

ESI-HRMS: *m*/*z* [M – Cl]$^+$ calcd: 314.1747; found: 314.1735.

Anal. Calcd for $C_{14}H_{25}Cl_2N_5O$ (350.29): C, 48.00; H, 7.19; N, 19.99. Found: C, 47.89; H, 6.91; N, 19.85.

4,4′-[6-(Diisopropylamino)-1,3,5-triazine-2,4-diyl]bis(4-methylmorpholin-4-ium) Dichloride (MIATMM)

Yield: 1.76 mmol (88%); yellowish solid; mp 68 °C.

FT-IR (KBr): 1518, 1341, 1271, 1231, 1134 cm^{-1}.

^1H NMR (300 MHz, D_2O): δ = 4.59 (m, 2 H), 4.59 (m, 4 H), 4.21 (d, 4 H), 3.95 (m, 8 H), 3.60 (s, 6 H), 1.42 (d, 12 H).

^{13}C NMR (75 MHz, D_2O): δ = 169.0, 164.2, 62.2, 60.4, 55.2, 49.9, 19.2.

ESI-HRMS: *m*/*z* [M – Cl]$^+$ calcd: 415.2588; found: 415.2591.

Anal. Calcd for $C_{19}H_{36}Cl_2N_6O_2$ (451.44): C, 50.55; H, 8.04; N, 18.62. Found: C, 50.32; H, 7.98; N, 18.21.

1,1′-[6-(Diisopropylamino)-1,3,5-triazine-2,4-diyl]bis(1-methylpiperidin-1-ium) Dichloride (MIATMP)

Yield: 1.70 mmol (85%); white solid; mp 70 °C.

FT-IR (KBr): 1532, 1351, 1267, 1222 cm^{-1}.

^{1}H NMR (300 MHz, D_2O): δ = 4.61 (brm, 2 H), 4.51 (d, 4 H), 3.68 (t, 4 H), 3.44 (s, 6 H), 1.98 (m, 4 H), 1.73 (m, 8 H), 1.41 (d, 12 H).

^{13}C NMR (75 MHz, D_2O): δ = 169.5, 164.6, 62.2, 55.4, 49.6, 21.5, 20.8, 19.4.

ESI-HRMS: *m*/*z* [M – Cl]$^+$ calcd: 411.3002; found: 411.3001.

Anal. Calcd for $C_{21}H_{40}Cl_2N_6$ (447.49): C, 56.37; H, 9.01; N, 18.78. Found: C, 57.12; H, 9.21; N, 19.01.

1,1′-[6-(Diisopropylamino)-1,3,5-triazine-2,4-diyl]bis(1-methylpyrrolidin-1-ium) Dichloride (MIATMPD)

Yield: 1.44 mmol (72%); white solid; mp 68 °C.

FT-IR (KBr): 1540, 1345, 1279, 1215 cm^{-1}.

^{1}H NMR (300 MHz, D_2O): δ = 4.61 (m, 2 H), 4.43 (m, 4 H), 3.91 (d, 4 H), 3.56 (s, 6 H), 2.34 (m, 8 H), 1.42 (d, 12 H).

^{13}C NMR (75 MHz, D_2O): δ = 169.9, 163.7, 65.5, 51.3, 48.9, 21.9, 18.8.

ESI-HRMS: *m*/*z* [M – Cl]$^+$ calcd: 383.2689; found: 383.2685.

Anal. Calcd for $C_{19}H_{36}Cl_2N_6$ (419.44): C, 54.41; H, 8.65; N, 20.05. Found: C, 54.56; H, 8.12; N, 20.07.

6-(Diisopropylamino)-*N*^{2},*N*^{2},*N*^{2},*N*^{4},*N*^{4},*N*^{4}-hexamethyl-1,3,5-triazine-2,4-diaminium Dichloride (MIATTMA)

Yield: 1.40 mmol (70%); yellowish solid; mp 73 °C.

FT-IR (KBr): 1540, 1340, 1271, 1211 cm^{-1}.

^{1}H NMR (300 MHz, D_2O): δ = 4.63 (m, 2 H), 3.63 (s, 18 H), 1.42 (d, 12 H).

^{13}C NMR (75 MHz, D_2O): δ = 176.6, 170.0, 59.9, 50.0, 20.0.

ESI-HRMS: *m*/*z* [M – Cl]$^+$ calcd: 331.2376; found: 331.2371.

Anal. Calcd for $C_{15}H_{32}Cl_2N_6$ (367.36): C, 49.04; H, 8.78; N, 22.88. Found: C, 48.50; H, 8.31; N, 22.52.

4,4′-[6-(Diethylamino)-1,3,5-triazine-2,4-diyl]bis(4-methylmorpholin-4-ium) Dichloride (MEATMM)

Yield: 1.80 mmol (90%); white solid; mp 86 °C.

FT-IR (KBr): 1531, 1361, 1268, 1219, 1130 cm^{-1}.

^{1}H NMR (300 MHz, D_2O): δ = 4.49 (d, 4 H), 4.10 (d, 4 H), 3.82 (q, 8 H), 3.70 (q, 4 H), 3.48 (s, 6 H), 1.16 (t, 6 H).

^{13}C NMR (75 MHz, D_2O): δ = 169.1, 164.0, 61.8, 59.9, 55.1, 44.13, 11.4.

ESI-HRMS: *m*/*z* [M – Cl]$^+$ calcd: 387.2275; found: 387.2271.

Anal. Calcd for $C_{17}H_{32}Cl_2N_6O_2$ (423.38): C, 48.23; H, 7.62; N, 19.85. Found: C, 48.10; H, 6.89; N, 6.42.

1,1′-[6-(Diethylamino)-1,3,5-triazine-2,4-diyl]bis(1-methylpiperidin-1-ium) Dichloride (MEATMP)

Yield: 1.56 mmol (78%); white solid; mp 86 °C.

FT-IR (KBr): 1531, 1361, 1268, 1219, 1130 cm^{-1}.

^{1}H NMR (300 MHz, D_2O): δ = 4.53 (d, 4 H), 3.79 (q, 4 H), 3.65 (t, 4 H), 3.43 (s, 6 H), 1.96 (m, 4 H), 1.75 (m, 8 H), 1.26 (t, 6 H).

^{13}C NMR (75 MHz, D_2O): δ = 169.5, 164.4, 61.1, 55.1, 43.8, 20.9, 20.2, 11.5.

ESI-HRMS: *m*/*z* [M – Cl]$^+$ calcd: 383.2688; found: 383.2683.

Anal. Calcd for $C_{19}H_{36}Cl_2N_6$ (419.44): C, 54.41; H, 8.65; N, 20.04. Found: C, 54.10; H, 8.43; N, 8.56.

6-(Diethylamino)-*N*^{2},*N*^{2},*N*^{2},*N*^{4},*N*^{4},*N*^{4}-hexamethyl-1,3,5-triazine-2,4-diaminium Dichloride (MEATTMA)

Yield: 1.92 mmol (96%); yellowish solid; mp 72 °C.

FT-IR (KBr): 1563, 1368, 1268, 1215 cm^{-1}.

^{1}H NMR (300 MHz, D_2O): δ = 3.81 (q, 4 H), 3.62 (s, 18 H), 1.27 (t, 6 H).

^{13}C NMR (75 MHz, D_2O): δ = 171.1, 164.5, 54.4, 44.4, 11.1.

ESI-HRMS: *m*/*z* [M – Cl]$^+$ calcd: 303.2063; found: 303.2061.

Anal. Calcd for $C_{13}H_{28}Cl_2N_6$ (339.31): C, 46.02; H, 8.32; N, 24.77. Found: C, 45.98; H, 8.20; N, 24.52.

Coupling Reactions

Dehydro-condensation Reactions in the Presence of MMT/*tert*-Amine System; Typical Procedure

Acid **1a** (146.5 mg, 1.2 mmol) was dissolved in solvent (MeOH, 6 mL) and subsequently MMT (107.9 mg, 0.6 mmol), *tert*-amine (1.2 mmol), and amine **2a** (145.4 mg, 1.2 mmol) were added. The yield of amide **3a** was monitored by GLC using mesitylene as internal standard after 15 min and 1 h. To isolate **3a**, the mixture was filtered and the yellowish solid recovered was dissolved in CH_2Cl_2 and extracted with water (3 × 30 mL). The combined organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo to give **3a** as a white solid. Analogous reaction conditions were employed with MEAT and MIAT in the presence of a *tert*-amine.

Dehydro-condensation Reaction in the Presence of Isolated Quaternary Ammonium Salts; Typical Procedure

Acid **1a** (158.7 mg, 1.2 mmol) was dissolved in MeOH (6 mL) and the preformed amidation agent (0.6 mmol) and amine **2a** (145.4 mg, 1.2 mmol) were added. The yield of amide **3a** was monitored by GLC using mesitylene as internal standard after 15 min and 1 h. The procedure to isolate **3a** was the same as mentioned above.

Cross-Linking of Bovine Collagen Powder; Typical Procedure

The cross-linking of BCP in the presence of MMTMM is reported as an example (Table 3, entry 2): bovine collagen powder (300.0 mg, 0.36 mmol $COOH_{coll}$) was dispersed in water (30 mL) under magnetic stirring. After 30 min, MMTMM (199.2 mg, 0.72 mmol) was added to the suspension and stirring was continued for a further 4 h; the collagen powder was then recovered by filtration, air dried, and analyzed by DSC.

For MMT/NMM the same procedure was performed adding MMT (129.6 mg, 0.72 mmol) and NMM (145.6 mg, 1.44 mmol).

Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/a-1334-6916.

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