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Synthesis and Hydrolysis of 4-Chloro-PyMTA and 4-Iodo-PyMTA Esters and Their Oxidative Degradation with Cu(I/II) and Oxygen

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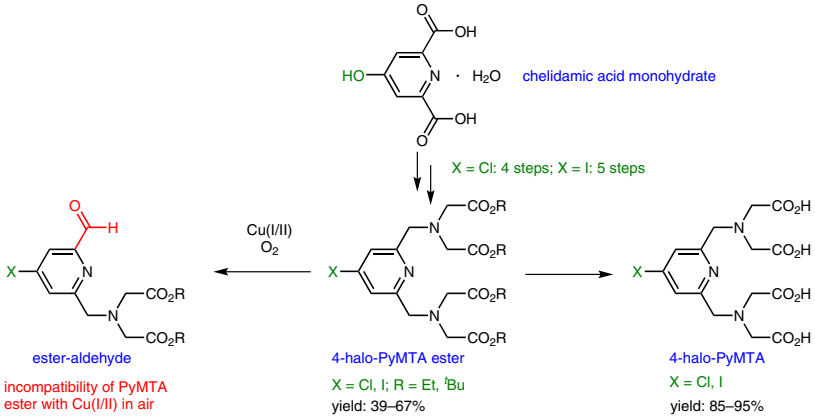

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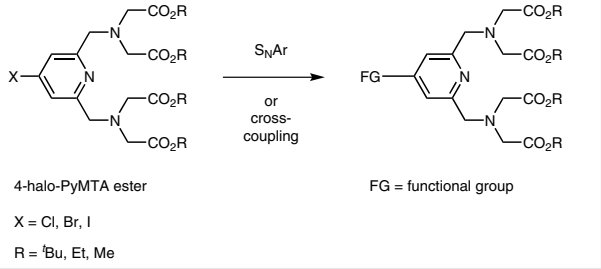
Abstract We disclose the syntheses of ethyl and *tert*-butyl esters of 4-chloro-PyMTA and 4-iodo-PyMTA from the commercially available chelidamic acid monohydrate in 39–67% overall yield. Additionally, ester hydrolyses with aqueous NaOH (ethyl esters) or trifluoroacetic acid (*tert*-butyl esters) are reported. The resulting materials contain 4-halo-PyMTA in mixture with partially deprotonated or partially protonated 4-halo-PyMTA. The ligand content expressed as the content of the common structural motifs of the present species, namely $[PyMTA - 4\ H^+]^{4-}$ (basic hydrolysis) and PyMTA (acidic hydrolysis), was determined to be 90–94 wt % by ^1H NMR spectroscopy using maleic acid as an internal standard. The *tert*-butyl esters were easily hydrolyzed with aqueous alkali hydroxide, with a decreasing rate in the series NaOH, KOH, LiOH. This finding indicates a Lewis acid assisted ester cleavage with the Na^+ ion fitting best to the multidentate ligand. Unexpectedly, PyMTA esters are incompatible with Cu(I/II) salts in the presence of oxygen. Under these conditions, one of the two aminomethyl groups is converted into a formyl group. This reaction not only limits the application of Cu(I/II)-catalyzed reactions but also necessitates trapping of any copper ions (e.g., with a metal ion scavenger) before the material is exposed to oxygen.

Key words dipicolinates, Ln(III) complexes, PyMTA, pyridine-based ligands, spin label

2,2′,2″,2‴-[(Pyridine-2,6-diyl)bis(methylenenitrilo)]tetrakis(acetic acid) (PyMTA) is one of the few ligands whose Ln(III) complexes possess concurrently attractive optical[1–13] and magnetic[12–23] properties, excellent chemical stability,[12,13,24] and biocompatibility.[5,8,13,16,20,22,25–29] It is the only ligand we know of whose Ln(III) complexes have already been applied in a wide scope: Its Gd(III) complex is used as a spin label for electron paramagnetic resonance spectroscopy[14–18] and as a contrast agent for magnetic resonance imaging.[12,13,19–21] PyMTA complexes with Tb(III), Dy(III), Tm(III), and Yb(III) are used as paramagnetic labels for nuclear magnetic resonance spectroscopy,[22,23] and its complexes with Eu(III), Tb(III), Sm(III), Nd(III), and Yb(III) are widely used because of their fluorescence[1–13] for time-resolved fluorescence spectroscopy,[8,26] fluorescence resonance energy transfer,[30–35] quenching resonance energy transfer,[25] immunoassays,[8,36–39] protein quantification,[28,34] minisequencing,[40] ligand binding assays,[26] and DNA analysis.[41,42]

Most of the functionalized PyMTAs used in the above-mentioned studies are prepared from 4-halo-PyMTA esters by employing either an aromatic nucleophilic substitution (S_NAr) reaction[13,22,27] or a Pd-catalyzed cross-coupling[6,9,10,16,18–21,23,41–46] (Scheme 1). The most-often found order of reactivity is aryl chloride > aryl bromide > aryl iodide in S_NAr reactions[47] and aryl iodide > aryl bromide > aryl chloride in cross-couplings.[48] In agreement with this, when 4-azido-PyMTA ester was prepared via S_NAr reaction of 4-chloro-PyMTA ester or 4-bromo-PyMTA ester with NaN_3 under comparable conditions, the yields were 100%[27] and 48%,[13] respectively, and in the cross-coupling with an alkyne 4-iodo-PyMTA ester was more reactive than 4-bromo-PyMTA ester, as reported by Takalo and co-workers[46] and confirmed by our own experiments.[49] Although the 4-chloro-PyMTA ester and the 4-iodo-PyMTA ester clearly are of advantage when it comes to reactivity, the 4-bromo-PyMTA ester has so far been used in most cases because of its much better accessibility via the procedure disclosed by Takalo and co-workers. Their well-established route starts from chelidamic acid and provides 4-bromo-PyMTA ester in up to 64% yield in four simple steps.[46] The situation is very different for 4-chloro-PyMTA ester and 4-iodo-PyMTA ester. The synthesis of 4-chloro-PyMTA ester starting from 2,6-bis(bromomethyl)-4-chloropyridine was reported to

give a yield of 96%;[27] however, the starting material is very difficult to obtain commercially and the only report on its preparation states a yield of 8% with 4-chloro-2,6-dimethylpyridine as the starting material.[50] The synthesis of 4-iodo-PyMTA ester was reported as a seven-step route starting from chelidamic acid, giving an overall yield of ca. 21%.[46,51,52] Two steps lack a detailed description; moreover, when we tried to repeat the synthesis, at the stage of converting the hydroxyl groups of (4-iodopyridine-2,6-diyl)dimethanol (**8**) into bromo substituents inseparable byproducts formed, which resulted in inseparable contaminants in the products of subsequent reactions.



Scheme 1 Synthesis of substituted PyMTA esters from 4-halo-PyMTA esters via S_NAr reaction or cross-coupling

Herein, we report an easy access to 4-chloro-PyMTA esters and 4-iodo-PyMTA esters starting from commercially available chelidamic acid monohydrate in overall yields of 39–42% and 60–67%, respectively. In addition, we closely examine the ester hydrolysis. The PyMTA *tert*-butyl esters are surprisingly labile towards alkali hydroxides, especially NaOH. Moreover, basic hydrolysis of the ethyl esters gives partially deprotonated PyMTA and acidic hydrolysis of the *tert*-butyl esters gives partially protonated PyMTA. We disclose procedures for the ester hydrolysis that give materials with a ligand content of a minimum 90 wt % expressed in terms of the content of the common structural motifs [PyMTA – 4 H^+]$^{4-}$ (basic hydrolysis) and PyMTA (acidic hydrolysis), and report a procedure for the quantification. Finally, we document the surprising lability of PyMTA esters towards oxidative degradation in the presence of Cu(I/II) salts and oxygen, and outline countermeasures.

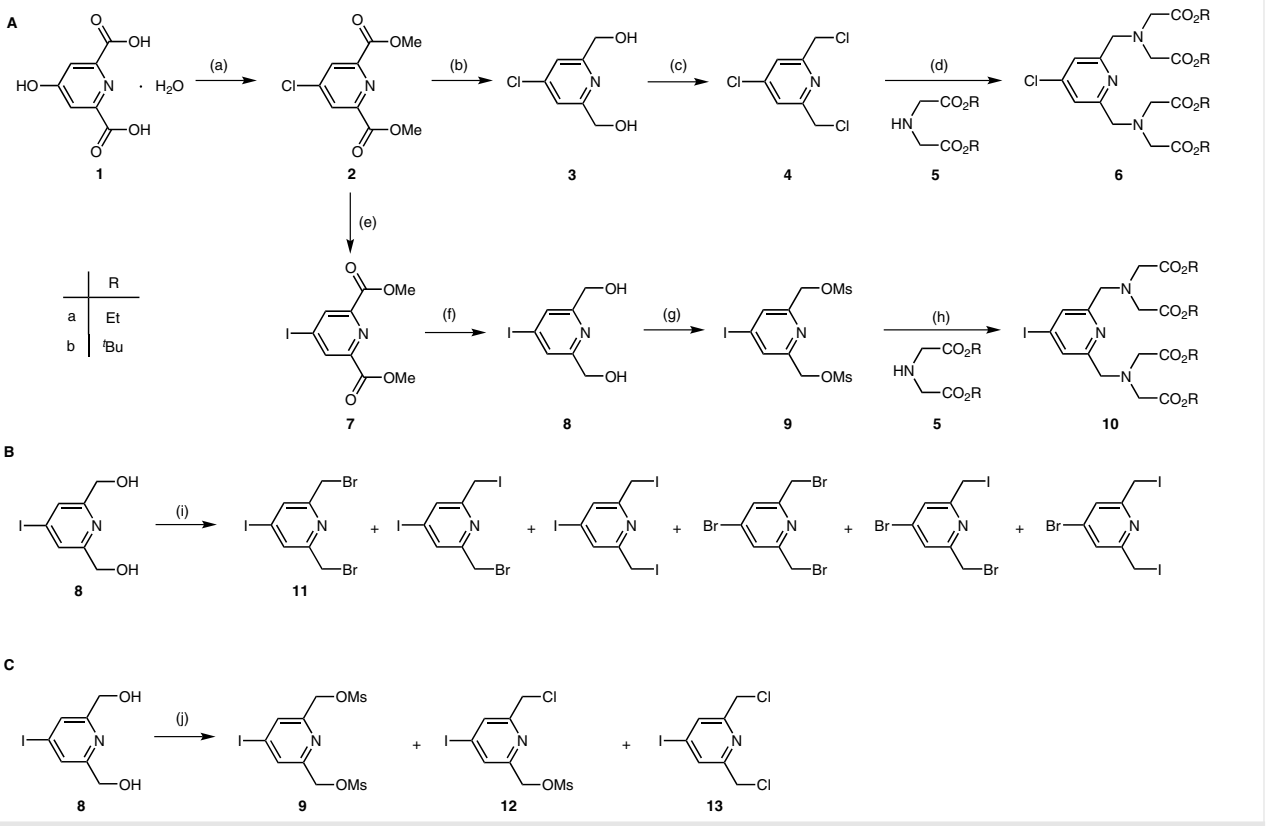
Our routes to 4-chloro-PyMTA esters **6** and 4-iodo-PyMTA esters **10** are depicted in Scheme 2A. Briefly, chelidamic acid monohydrate (**1**) was treated with thionyl chloride followed by the addition of MeOH to afford dimethyl 4-chlorodipicolinate (**2**), the common intermediate for the preparation of all the esters. To obtain the 4-chloro-PyMTA esters **6**, chloro diester **2** was reduced with $NaBH_4$ in the presence of $CaCl_2$ to chloro diol **3**. Then, the two hydroxyl groups of **3** were replaced by chlorine atoms via reaction with phosphoryl chloride and DMF, and these were subsequently exchanged for dialkyl nitrilodiacetate groups. For preparing the corresponding 4-iodo-PyMTA esters **10**, chloro diester **2** was converted into dimethyl 4-iododipicolinate (**7**) via treatment with NaI, acetyl chloride, and ultrasound.[53] Iodo diester **7** was reduced to iodo diol **8**, as per the corresponding chloro diol **3**. The hydroxyl groups of iodo diol **8** were converted into mesylate groups, which were exchanged for dialkyl nitrilodiacetate groups. In the following, essential details of the individual synthetic steps are outlined.

Dimethyl 4-chlorodipicolinate (dimethyl 4-chloropyridine-2,6-dicarboxylate, **2**) is a very important compound not only for the synthesis of 4-halo-PyMTA esters but also for the synthesis of other pyridine-based compounds.[54–66] Its first reported preparation started from chelidamic acid with PCl_5 and MeOH as the reagents.[66] This procedure was frequently used and gave yields between 26% and 71%.[59–65,67] Recently, $SOCl_2$ was introduced as an alternative chlorination reagent for chelidamic acid.[54–57,68] Using $SOCl_2$ is beneficial, because the two side products SO_2 and HCl easily escape as gases from the reaction mixture. However, the reports are contradictory: Müller and co-workers[56] and Hamada and co-workers[55] reported to have prepared chloro diester **2** by treatment of chelidamic acid with $SOCl_2$ followed by addition of MeOH, whereas Kupai and co-workers[54] and Bradshaw and co-workers[68] reported that dimethyl 4-hydroxydipicolinate formed under these conditions. Kupai and co-workers converted the dimethyl 4-hydroxydipicolinate into chloro diester **2** in a second, separate step by treatment with $SOCl_2$ in the presence of a catalytic amount ('2 drops') of DMF.[54] Bourdolle and co-workers[57] described a one-pot procedure for the synthesis of chloro diester **2** from chelidamic acid by treatment with $SOCl_2$ and DMF, followed by the addition of MeOH. Neither experimental details nor the yield was given. We treated chelidamic acid monohydrate (**1**) with $SOCl_2$ in the absence and in the presence of DMF, distilled off the residual $SOCl_2$, and then added MeOH. Isolation of chloro diester **2** was very simple: it precipitated as pure material from the methanolic reaction mixture, whereas dimethyl 4-hydroxydipicolinate and chelidamic acid remained in solution. Without DMF only dimethyl 4-hydroxydipicolinate was formed, whereas chloro diester **2** was formed in the presence of DMF. Clearly, the Vilsmeier reagent, generated in situ from $SOCl_2$ and DMF, is needed for the substitution of the hydroxyl group at the pyridine ring by a chlorine atom. Furthermore, we found that the amount of DMF played an important role. Experiments with 0.05–0.2 equivalents of DMF (relative to chelidamic acid) and a reaction time of 4 hours provided chloro diester **2** in 46–67% yield. The missing 54–33% was a mixture of dimethyl 4-hydroxydipicolinate and chelidamic acid. Increasing the reaction time to 21 hours had no effect; however, increasing the amount of DMF to 2 equivalents raised the yield to 85–87% (3 runs). For the regular reaction, in which DMF acts as a catalyst, a small amount of DMF is sufficient. Therefore, our results indicate that DMF decomposes during the reaction. A further

increase in the amount of DMF did not improve the yield. Using anhydrous chelidamic acid, which is more expensive than the hydrate, gave about the same yield.

The reduction of dimethyl 4-chlorodipicolinate (**2**) to chloro diol **3** and of dimethyl 4-iododipicolinate (**7**) to iodo diol **8** have been reported:[46,69,70] 3–5 equivalents of $NaBH_4$ were used and the reaction mixtures were refluxed for 15–18 hours; the workup was laborious as a continuous liquid–liquid extraction was run for several days. $NaBH_4$ in combination with $CaCl_2$[71] in MeOH allowed us to decrease the amount of $NaBH_4$ to 2 equivalents and, additionally, the reactions were complete after 4–5 hours at room temperature. The products were simply isolated via precipitation: The reaction mixture, a suspension, was filtered through silica gel and the solvent of the filtrate, i.e., MeOH, was removed. 1 M HCl was added to the residue to convert the diol into the corresponding hydrochloride salt; thereby, the hydrochloride salt of the diol as well as the inorganic components went into solution. The pH of the solution was raised to pH 12 by the addition of NaOH, and consequently the hydrochloride salt of the diol was converted back into the diol which precipitated from the solution. The NMR spectra of the precipitates show only the signals of the diol. However, the masses of the materials were more than those expected for a 100% yield. Clearly inorganic matter, possibly $Ca(OH)_2$, which is not detectable by NMR spectroscopy, also precipitated. This inorganic matter did not interfere with the subsequent reactions.

To introduce the dialkyl nitrilodiacetate groups, the hydroxyl groups of the diols needed to be converted into a good leaving group. In the case of chloro diol **3**, the hydroxyl groups were exchanged for chloro substituents: with $POCl_3$ and DMF, trichloride **4** was obtained in 63% yield (over 2 steps from **2**). The reaction of chloro diol **3** with PCl_3, in analogy to the conversion of the corresponding bromo diol into tribromide,[46] gave trichloride **4** in ca. 35% yield as a mixture with an unidentified product. The reported synthesis of trichloride **4** from chloro diol **3** using mesyl chloride[72] was not reproducible in our hands. The conversion of chloro diol **3** was far from complete, even at higher temperature and for a longer reaction time than given in the reported procedure. After 6 days at room temperature,



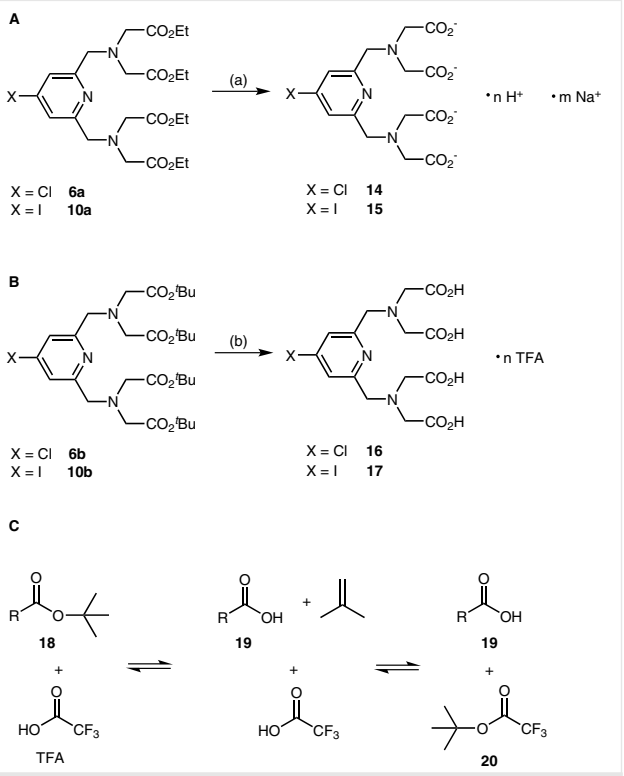
Scheme 2 *Reagents and conditions*: A: Preparation of 4-halo-PyMTA esters. (a) 1) $SOCl_2$, DMF, reflux, 22 h, 2) MeOH, 87%; (b) $NaBH_4$, $CaCl_2$, MeOH, r.t., 4 h; (c) $POCl_3$, DMF, 50 °C, 40 min, 63% over 2 steps; (d) Na_2CO_3, KI, MeCN, r.t., 23 h, 71–76%; (e) NaI, AcCl, MeCN, ultrasound, r.t., 30 min, 94%; (f) $NaBH_4$, $CaCl_2$, MeOH, r.t., 5 h; (g) MsCl, Et_3N, CH_2Cl_2, 0 °C, 10 min, then r.t., 15 min (a mixture of iodo dimesylate **9**, monochloride **12**, and iodo dichloride **13** was obtained; see Scheme 2C); (h) Na_2CO_3, KI, MeCN, r.t., 20 h, **10a** (73% over 3 steps), **10b** (82% over 3 steps). B: Preparation of iodo dibromide **11** from iodo diol **8**. (i) PBr_3, $CHCl_3$, reflux, 8 h. C: Mesylation of iodo diol **8**. (j) MsCl, Et_3N, CH_2Cl_2, 0 °C, 10 min, then r.t., 15 min, **9** (69%), **12** (5%), and **13** (2%), yields over 2 steps from **7**.

only 17–38% (4 runs) of chloro diol **3** had reacted and the products were the chloro dimesylate, the dichloride, and trichloride **4**. Raising the temperature to 40 °C and extending the reaction time to 9 days gave a conversion of only 25%. Most probably, the low solubility of chloro diol **3** in CH_2Cl_2 hindered the reaction; supporting this assumption, mesylation of the much better soluble iodo diol **8** was possible (see below). The precipitating triethylamine hydrochloride may also have contributed to the low reactivity by coating the undissolved chloro diol **3**.

For the conversion of iodo diol **8** into the iodo-PyMTA esters **10**, we tried to prepare iodo dibromide **11** by refluxing iodo diol **8** with PBr_3 in $CHCl_3$ according to a published procedure.[46] Unfortunately, the halogen atom at the pyridine ring and the two benzylic halogen atoms interchanged during the reaction and a mixture of all possible halides was the result (Scheme 2B; for details, see Supporting Information). The desired iodo dibromide **11** was the major product, but the other products could not be removed by either column chromatography or crystallization. Using this mixture as the starting material to prepare the PyMTA ester gave an inseparable mixture of 4-iodo-PyMTA ester and 4-bromo-PyMTA ester. Additionally, the compounds with an iodomethyl group decompose slowly in solution, a decomposition which starts with oxidation of the iodomethyl group to a formyl group. Having failed with a clean formation of iodo dibromide **11**, we turned to the mesylation of iodo diol **8** (Scheme 2C). Reactions of iodo diol **8** with mesyl chloride in CH_2Cl_2 at room temperature gave mixtures of iodo dimesylate **9**, monochloride **12**, and iodo dichloride **13**, with an iodo dimesylate **9** content of 69 to 93 mol% (3 runs). Lowering the temperature to ice bath temperature in order to gain higher selectivity resulted in an incomplete conversion. The three products are easily separable via chromatography, but when 4-iodo-PyMTA esters **10** are the goal, there is no need for a separation (see below).

To finally obtain 4-chloro-PyMTA esters **6** and 4-iodo-PyMTA esters **10**, we treated trichloride **4** and iodo dimesylate **9**, or the mixture of iodo dimesylate **9**, monochloride **12**, and iodo dichloride **13**, with dialkyl iminodiacetates **5**. The reactivities of the benzylic chloride and of the benzylic mesylate groups were quite different. Substitution of the mesylate group in MeCN using Na_2CO_3 as base went smoothly at room temperature. The benzylic chloride is inert under these conditions, and a reaction at room temperature was accomplished only upon the addition of KI.

The reported procedures for the hydrolyses of PyMTA ethyl ester and PyMTA methyl ester consist of an ester cleavage under basic conditions with LiOH, $Ba(OH)_2$, and NaOH as the reagents and a subsequent protonation of the carboxylates by the addition of HCl, H_2SO_4, or a proton-exchange resin.[4,13,16,23,42,73] The combination of LiOH with HCl was applied in the preparation of PyMTA-labeled lipids, which are insoluble in water, so that the water-soluble byproduct LiCl could be easily removed by washing with water.[4] The combination of $Ba(OH)_2$ with H_2SO_4 was applied in the preparation of water-soluble derivatives of PyMTA. In this case, the water-insoluble byproduct $BaSO_4$ was removed by filtration.[73] It is very doubtful that the Ba^{2+} ions could be completely removed because chelate ligands with aminoacetate groups [e.g., diethylenetriaminepentaacetic acid (DTPA) and ethylenediaminetetraacetic acid (EDTA)] are strong complexing agents for the Ba^{2+} ion and their aqueous solutions can even dissolve $BaSO_4$.[74] PyMTA is structurally very similar to EDTA and therefore we expect its aqueous solution to dissolve $BaSO_4$. PyMTA contaminated with Ba^{2+} ions may be problematic in the case of biological applications considering the high toxicity of Ba^{2+} ions. The combination of NaOH with proton-exchange resin has been recently used.[13,16,23] The advantage of this combination is that the only byproduct, the Na^+-loaded resin, is removed simply by filtration. We applied this procedure, namely treatment with NaOH, then acidification with a proton-exchange resin, to 4-chloro-PyMTA ethyl ester **6a** and 4-iodo-PyMTA ethyl ester **10a** in order to obtain 4-chloro-PyMTA and 4-iodo-PyMTA (Scheme 3A). The first step, the basic ester hydrolysis, proceeded smoothly, whereas the second step, the protonation with the proton-exchange resin, was not without pitfalls. We found that the amount of the proton-exchange resin needs to be carefully controlled. We added just enough needed to reduce the pH of the solution to pH 3; adding more resin barely changed the pH of the solution, but drastically decreased the yield. Obviously, protonation of the amino groups of 4-halo-PyMTA occurs and the corresponding protonated 4-halo-PyMTA is trapped as a counterion of the resin. The adhesion of 4-halo-PyMTA to the resin was reversible upon addition of NaOH. Minimizing the loss of 4-halo-PyMTA resulted in an incomplete exchange of Na^+ for H^+, such that mixtures of 4-halo-PyMTA and partially deprotonated 4-halo-PyMTA as sodium salts, $\{[4\text{-halo-PyMTA} - 4\ H^+]^{4-}\cdot n\ H^+\cdot m\ Na^+\}$ **14** and **15**, were obtained. Quantitative ^1H NMR spectroscopy[75] revealed the ligand content, which is expressed as the amount of the motif $[4\text{-halo-PyMTA} - 4\ H^+]^{4-}$ that is common to all species, to be 90–92 wt% (for details, see Supporting Information). The content varied slightly from batch to batch. Therefore, whenever uncomplexed Ln(III) ions are to be avoided, e.g., because of their high toxicity[76] and interference with physical measurements, it is necessary to quantify the amount of ligand in the individual materials, so that the stoichiometric amount of Ln(III) can be added.



Scheme 3 *Reagents and conditions*: A: Basic hydrolysis of 4-halo-PyMTA ethyl esters **6a** and **10a**. (a) 1) NaOH, H_2O, EtOH, r.t., 4 h, 2) proton-exchange resin, 85–95%. B: Hydrolysis of 4-halo-PyMTA *tert*-butyl esters **6b** and **10b** with TFA. (b) TFA (50 equiv), r.t., 1 h (treatment applied four times), 89–93%. C: Reversibility of the hydrolysis of *tert*-butyl esters with TFA.

All the reports on the hydrolysis of PyMTA *tert*-butyl esters[1,6,8,10,18,20,21,27,44–46,77,78] have applied the same procedure: the PyMTA *tert*-butyl ester is dissolved in ca. 400 equivalents of trifluoroacetic acid (TFA), the solution is stirred at room temperature for 1.5 hours, and finally all volatiles are removed to obtain PyMTA as the trifluoroacetate salt. We applied this protocol to hydrolyze 4-iodo-PyMTA *tert*-butyl ester **10b** (Scheme 3B) and found that ca. 1% of the ester groups had not been hydrolyzed. Taking into account that the PyMTA ester has four ester groups, this corresponds to ca. 4% of incompletely hydrolyzed PyMTA ester. Even after a reaction time of 22 days, 0.2% of the ester groups remained. The reaction with less TFA provided a product with more residual ester groups. These findings are in agreement with the reversibility of the hydrolysis of *tert*-butyl esters with TFA. Reaction of a *tert*-butyl ester **18** with TFA results in the formation of the corresponding acid **19** and isobutene (Scheme 3C). Isobutene reacts immediately with TFA giving *tert*-butyl trifluoroacetate (**20**), which can transfer the *tert*-butyl group back to the acid **19**.[79] Upon removal of the volatiles, the ratio of TFA and trifluoroacetate **20** changes in favor of trifluoroacetate **20** because of the higher volatility of TFA.[79] Therefore, the overall equilibrium shifts back to the side of the starting compound **18**. Adding anisole as a trap for the intermediately occurring *tert*-butyl carbenium ion makes the reaction irreversible, but this comes at the expense of the removal of the trapping agent and its product, such as anisole and 4-*tert*-butylanisole. A simple solution is to apply the process repeatedly: thus, the PyMTA *tert*-butyl ester was treated with 50 equivalents of TFA for 1 hour, then all volatiles, TFA and trifluoroacetate **20**, were evaporated. This treatment was applied again, allover for four times. This way, a complete ester hydrolysis was achieved. If 100 equivalents of TFA per run were used, two runs were sufficient. The materials obtained from this process were highly hygroscopic and well soluble in MeOH. They contained not only 4-chloro-PyMTA or 4-iodo-PyMTA but also a large amount of TFA which tenaciously resisted removal under reduced pressure. Dissolving the materials in MeOH and dropping this solution into a mixture of pentane and Et_2O gave {4-halo-PyMTA·n TFA} **16** and **17** with a content of the structural motif 4-halo-PyMTA common to all species of around 94 wt% as colorless precipitates (for details, see Supporting Information). These materials have a very limited solubility in MeOH.

Surprisingly, the PyMTA *tert*-butyl ester is also easily hydrolyzed under basic conditions, as shown in Figure 1 for 4-iodo-PyMTA *tert*-butyl ester **10b**. With NaOD, 78% of the ester groups were hydrolyzed within 7 hours at room temperature and after 24 hours the hydrolysis was nearly complete. Such an unusual reactivity of a *tert*-butyl ester has also been reported for 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) *tert*-butyl ester.[80] We assume complexation of the metal ion by the PyMTA ester causing an increase in electrophilicity of the ester groups and consequently the unusual lability of a *tert*-butyl ester group towards hydroxide. In line with this assumption, we found that the rate of the hydrolysis depends on the type of the alkali metal ion M^+ of MOD but does not follow the basicity of MOD (basicity: KOH > NaOH > LiOH). Only one of the three ions will best meet the demand of the ligand with respect to ion radius. The hydrolysis rate decreases in the order Na^+, K^+, Li^+ (Figure 1); clearly, Na^+ is a better fit than Li^+ or K^+.

When working with PyMTA esters, we noticed that one of the two aminomethyl substituents was slowly converted into a formyl group when the material was in contact with Cu(I/II), Pd(II), and air (for details, see Supporting Information).[49] To study this oxidation more closely, $PdCl_2$ or $PdCl_2(PPh_3)_2$ or CuI or a mixture of these salts was added to a solution of 4-iodo-PyMTA ethyl ester **10a** in THF and the reaction mixture (first a suspension, after a few hours a solution) was stirred in air for 240 hours (Table 1). No oxidation occurred in the presence of only $PdCl_2$ and/or $PdCl_2(PPh_3)_2$ (Table 1, entries 2–4). When CuI was added, 12 mol% of 4-iodo-PyMTA ethyl ester **10a** was converted into PyMTA ester aldehyde **21** (Table 1, entry 5). Additional introduction

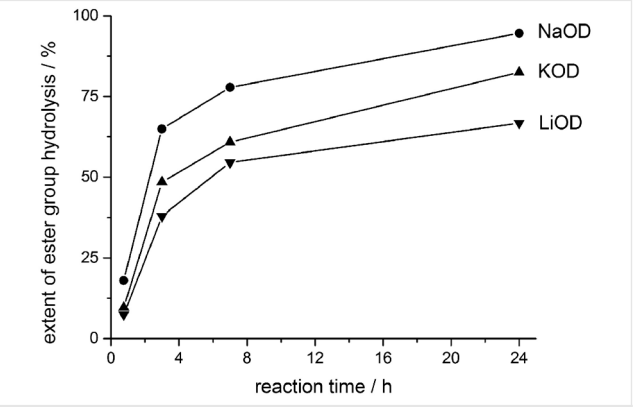


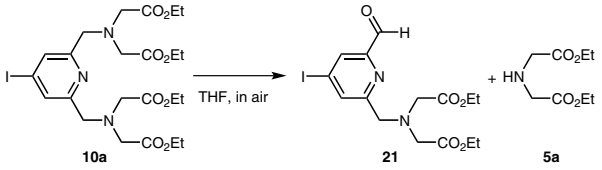
Figure 1 Hydrolysis of 4-iodo-PyMTA *tert*-butyl ester **10b** with 0.35 M alkali deuteroxide in D_2O/CD_3OD at r.t., as studied by ^1H NMR spectroscopy. The data points are connected purely as a visual aid.

of $PdCl_2$ or $PdCl_2(PPh_3)_2$ raised the conversion to 35 mol% and 42 mol%, respectively (Table 1, entries 6 and 7). When air was excluded by working under argon and degassing the solution, no oxidation occurred (Table 1, entries 8–10). Clearly, oxygen is the oxidant, Cu(I/II) the catalyst, and Pd(II) an accelerator. We assume that the complexation of Cu(II) triggers the oxidation. This assumption gains support from an ESI mass spectrum of the reaction solution of entry 6 (Table 1) which shows a signal at m/z = 705.0 which matches a Cu(II)–PyMTA ester complex with one chloride ion as counterion. We repeated some of these experiments using CH_2Cl_2 instead of THF as solvent or exchanging ethyl 4-iodo-PyMTA ethyl ester **10a** for 4-iodo-PyMTA *tert*-butyl ester **10b**. The results were comparable to the results with 4-iodo-PyMTA ester **10a** in THF, which indicates that the oxidation is neither specific to the solvent nor to the type of ester group.

If there is a need to bring Cu(I/II) in contact with PyMTA esters, according to our experience the following procedure inhibits the oxidation: The reaction is performed under oxygen-free conditions. After the reaction but before exposure to air during standard workup, metal scavenger QuadraPure™-TU is added to remove Cu(I/II). Note that column chromatography on silica gel gave material that became slowly oxidized, which means that this measure is insufficient to remove Cu(I/II) completely. Though the oxidation is very slow, Cu(I/II) causes oxidation during storage of the materials. Material that had been treated with metal scavenger was storable without change in air.

In summary, we have developed procedures for the syntheses of 4-chloro-PyMTA esters and 4-iodo-PyMTA esters from commercially available chelidamic acid monohydrate in overall yields of 39–67%. Hydrolysis of the 4-halo-PyMTA ethyl esters with aqueous NaOH and subsequent addition of proton-exchange resin in an amount that is just sufficient to adjust the pH to pH 3 gave materials with a content of

Table 1 Oxidative Degradation of 4-Iodo-PyMTA Ethyl Ester **10a** to PyMTA Ester Aldehyde **21**[a]



Entry	Ester and catalysts (μmol)				Conditions	Color of the solution	Product composition (%)	
	10a	CuI	$PdCl_2$	$PdCl_2(PPh_3)_2$			**10a**	**21**
1	20	–	–	–	air	colorless	100	0
2	20	–	5.1	–	air	colorless	100	0
3	20	–	–	5.0	air	yellow	100	0
4	20	–	5.1	5.1	air	yellow	100	0
5	20	5.3	–	–	air	yellow	88	12
6	20	5.5	5.0	–	air	yellow	65	35
7	20	5.5	–	5.0	air	yellow	58	42
8	20	5.5	–	–	argon	colorless	100	0
9	20	5.5	5.0	–	argon	brown	100	0
10	20	5.5	–	5.0	argon	yellow	100	0

[a] Reaction conditions: Entries 1–7: **10a** (20 μmol) was dissolved in THF (5 mL) in a 10-mL glass vial with a snap-on cap, which was punctured with a cannula. The catalysts were added and the reaction mixture was stirred at r.t. for 240 h. The solvent was removed under reduced pressure. Entries 8–10: **10a** (20 μmol) was dissolved in THF (5 mL). The colorless solution was degassed. Under argon, catalysts were added and the reaction mixture was stirred at r.t. for 240 h. Metal scavenger QuadraPure™-TU (10 mg for 1 μmol metal) was added. The suspension was stirred for 5 h, filtered, and the solvents of the filtrate were removed. For all entries, the ratio of **10a** and **21** in the residue was determined by ^1H NMR spectroscopy.

90–92 wt% of the common motif [PyMTA – 4 H^+]$^{4-}$. Adding more resin resulted in adhesion of PyMTA to the resin and a drastically decreased yield. The PyMTA *tert*-butyl esters were hydrolyzed by reaction with TFA. For complete hydrolysis, a twofold treatment with 100 equivalents of TFA is necessary, and some TFA remains in the final product. The content of the common motif PyMTA was determined to be around 94 wt%. In addition to the syntheses, we have disclosed the surprising lability of PyMTA *tert*-butyl esters towards alkali hydroxide and the oxidative degradation of PyMTA esters in the presence of Cu(I/II) and oxygen, two issues of importance when working with PyMTA esters.

Unless otherwise stated, reactions were performed under ambient atmosphere, using commercial solvents and reagents. The solvents used for extraction and chromatography were of technical grade and were distilled prior to use. The proton-exchange resin (Dowex® 50WX4 hydrogen form, Sigma-Aldrich, 91 g) was sequentially washed with THF (3 × 200 mL), EtOH (2 × 100 mL), H_2O (2 × 150 mL), and EtOH (200 mL), and then dried over P_4O_{10} at 0.05 mbar for 5 d to obtain a pure and dry proton-exchange resin (30 g). The temperature given for the reactions refers to the bath temperature. Solvents were removed under reduced pressure at a bath temperature of ca. 40 °C. The products were dried at room temperature at ca. 0.05 mbar. The pH values of solutions were determined using pH indicator strips (resolution: 0.3 pH, Merck). Column chromatography was carried out on silica gel 60 M (Acros), applying slight pressure. The size of columns is given as diameter × length. Material was loaded onto the column dissolved in a small quantity of the eluent. TLC was performed on silica gel coated aluminum foil (Merck, 60 F254). The spots were detected with UV light (λ = 254 nm). The compositions of solvent mixtures are given in volume ratios. For centrifugation, a VWR CompactStar CS4 centrifuge (centrifuging radius: 8.5 cm, relative centrifugal force: 4000g, rotor angle: 30°) and VWR metal-free centrifuge tubes (15 mL, overall length: 118 mm, inside diameter: 15 mm, outside diameter: 17 mm, polypropylene, sterile) were used. NMR spectra were recorded at room temperature on a Bruker Avance 500 500-MHz instrument. The spectra were calibrated using the solvent signal as an internal standard [$CDCl_3$: δ(^{1}H) = 7.25, δ(^{13}C) = 77.0; CD_2Cl_2: δ(^{1}H) = 5.32, δ(^{13}C) = 53.8; DMSO-d_6: δ(^{1}H) = 2.49, δ(^{13}C) = 39.5; CD_3OD: δ(^{1}H) = 3.31, δ(^{13}C) = 49.0; D_2O: δ(^{1}H) = 4.79]. For ^{13}C NMR experiments in D_2O, a drop of MeOH was added as the internal standard [$\delta(^{13}C)_{MeOH}$ = 49.5]. Signal assignments are supported by DEPT-135, COSY, HMBC, and HMQC experiments. ESI mass spectra were recorded using an Esquire 3000 ion trap mass spectrometer (Bruker Daltonik) equipped with a standard ESI source. EI mass spectra were recorded using an Autospec X magnetic sector mass spectrometer with EBE geometry (Vacuum Generators, Manchester, UK) equipped with a standard EI source. HRMS analyses were performed using an FT-ICR mass spectrometer (APEX III, Bruker Daltonik) interfaced to an external ESI ion source. The ratio of components in a mixture was determined by ^{1}H NMR spectroscopy and is given as the molar ratio.

Chloro Diester (Dimethyl 4-Chlorodipicolinate, 2)

Anhydrous DMF (27.6 mL, 358 mmol) was added slowly to a suspension of chelidamic acid monohydrate (**1**) (36.0 g, 179 mmol) in $SOCl_2$ (230 mL, 3.17 mol) (caution: exothermic reaction and vigorous gas evolution). After 1 h of stirring at room temperature, the suspension was slowly heated to reflux (oil bath temperature 95 °C) and stirred at this temperature for 22 h (caution: vigorous gas evolution). During this time the suspension turned into a pale yellow solution. The excess $SOCl_2$ was distilled off under reduced pressure. To the cooled (ice bath) residual orange-red solid, MeOH (500 mL) was added slowly (caution: exothermic reaction and vigorous gas evolution). The resulting suspension was heated to reflux (oil bath temperature 70 °C) and MeOH was added until all of the solid was dissolved. Upon slowly cooling to room temperature, colorless needles formed. The suspension was cooled with an ice-water bath and the needles were collected by filtration and washed with cold MeOH to obtain chloro diester **2** (35.6 g, 87%); mp 139–140 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.28 (s, 2 H, ArH), 4.02 (s, 6 H, Me).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 163.9 (C=O), 149.3 (C_{Ar} *meta* to Cl), 146.6 (C_{Ar}Cl), 128.1 (C_{Ar}H), 53.3 (CH_3).

MS (ESI): *m*/*z* = 480.9 [2 M + Na]$^+$, 251.9 [M + Na]$^+$, 229.9 [M + H]$^+$.

HRMS (EI, 70 eV): *m*/*z* [M]$^{+\bullet}$ calcd for $C_9H_8ClNO_4^{+\bullet}$: 229.01364; found: 229.01373.

Anal. Calcd for $C_9H_8ClNO_4$: C, 47.08; H, 3.51; N, 6.10. Found: C, 47.18; H, 3.51; N, 6.07.

Chloro Diol 3

This reaction was performed under argon. $NaBH_4$ (3.28 g, 86.7 mmol) was added portionwise within 35 min to a suspension of chloro diester **2** (10.0 g, 43.6 mmol) and finely powdered $CaCl_2$ (4.84 g, 43.6 mmol) in MeOH (250 mL) under cooling with a water bath (about room temperature). The colorless suspension was stirred at room temperature and the reaction was followed by TLC [R_f(**2**) = 0.40, R_f(**3**) = 0.10 (Et_2O)]. The reaction was complete after 4 h and the suspension was filtered through silica gel (3 cm × 6 cm, rinsing with MeOH). Removal of the solvents from the filtrate afforded a yellow solid (21.4 g) which was dissolved in 1 M HCl (180 mL). The pH of the solution was raised to pH 12 by addition of 1 M aqueous NaOH, whereupon a colorless solid precipitated. The precipitate was isolated by filtration and dried under reduced pressure over P_4O_{10} for 3 d and in air for 7 d until the weight of the product stayed constant. A colorless powder (8.01 g, consisting of chloro diol **3** and inorganic salts) was obtained.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.24 (s, 2 H, ArH), 4.76 (d, ^3J = 5.4 Hz, 4 H, CH_2), 2.99 (t, ^3J = 5.4 Hz, 2 H, OH).

^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.35 (s, 2 H, ArH), 5.28 (t, ^3J = 5.7 Hz, 2 H, OH), 4.52 (d, ^3J = 5.7 Hz, 4 H, CH_2).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 163.5 (C_{Ar} *meta* to Cl), 144.0 (C_{Ar}Cl), 118.0 (C_{Ar}H), 63.7 (CH_2).

MS (ESI): *m*/*z* = 195.9 [M + Na]$^+$, 174.0 [M + H]$^+$.

HRMS (ESI): *m*/*z* [M + Na]$^+$ calcd for $C_7H_8ClNO_2Na^+$: 196.0136; found: 196.0138.

Trichloride 4

The material obtained from the reduction of chloro diester **2** (2.00 g, consisting of chloro diol **3** and inorganic salts) was suspended in DMF (50 mL). $POCl_3$ (2.3 mL, 24.7 mmol) was added slowly to the suspension (caution: slightly exothermic reaction), whereupon the precipitate dissolved completely. The pale yellow solution was stirred at 50 °C for 40 min. During this time the color of the solution changed from pale yellow to orange. The solution was cooled to room temperature and saturated aqueous $NaHCO_3$ (50 mL) was added (caution: exothermic reaction and vigorous gas evolution). Et_2O (50 mL) was added, the organic phase was separated, and the aqueous phase was

extracted with Et_2O (2 ×). The combined organic phases were washed with H_2O (3 ×) and dried over $MgSO_4$. Removal of the solvents gave trichloride **4** (1.54 g, 63% over 2 steps) as a pale yellow solid.

^1H NMR (500 MHz, $CDCl_3$): δ = 7.46 (s, 2 H, ArH), 4.62 (s, 4 H, CH_2).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 157.8 (C_{Ar} *meta* to Cl), 145.9 ($C_{Ar}Cl$), 122.3 ($C_{Ar}H$), 45.7 (CH_2).

MS (ESI): *m*/*z* = 209.8 $[M + H]^+$.

HRMS (EI, 70 eV): *m*/*z* $[M]^{+\bullet}$ calcd for $C_7H_6Cl_3N^{+\bullet}$: 208.95603; found: 208.95683.

4-Chloro-PyMTA Ethyl Ester (6a)

Trichloride **4** (749 mg, 3.56 mmol) was dissolved in MeCN (30 mL). Na_2CO_3 (3.78 g, 35.7 mmol), KI (1.18 g, 7.13 mmol), and diethyl iminodiacetate (**5a**) (2.2 mL, 12.6 mmol) were added successively. The suspension was sonicated in an ultrasound bath at room temperature for 35 min and then stirred at room temperature for 23 h. Et_2O and H_2O were added. The organic phase was separated and the aqueous phase was extracted with Et_2O (2 ×). The solvents of the combined organic phases were removed. Chromatography (4.5 cm × 28 cm; pentane–Et_2O, 1:2) of the residual yellow oil (2.09 g) gave ethyl ester **6a** (1.30 g, 71%) as a colorless oil; R_f = 0.35.

^1H NMR (500 MHz, $CDCl_3$): δ = 7.52 (s, 2 H, ArH), 4.13 (q, ^3J = 7.2 Hz, 8 H, CH_2CH_3), 3.98 (s, 4 H, $ArCH_2$), 3.56 (s, 8 H, CH_2CO), 1.23 (t, ^3J = 7.2 Hz, 12 H, CH_3).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.0 (CO), 160.3 (C_{Ar} *meta* to Cl), 145.6 ($C_{Ar}Cl$), 121.4 ($C_{Ar}H$), 60.5 (CH_2CH_3), 59.5 ($ArCH_2$), 54.9 (CH_2CO), 14.2 (CH_3).

MS (ESI): *m*/*z* = 538.3 $[M + Na]^+$, 516.3 $[M + H]^+$.

HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{23}H_{34}ClN_3O_8Na^+$: 538.1927; found: 538.1928.

Anal. Calcd for $C_{23}H_{34}ClN_3O_8$: C, 53.54; H, 6.64; N, 8.14. Found: C, 53.51; H, 6.75; N, 8.07.

4-Chloro-PyMTA *tert*-Butyl Ester (6b)

Trichloride **4** (765 mg, 3.63 mmol) was dissolved in MeCN (30 mL). Na_2CO_3 (3.85 g, 36.4 mmol), KI (1.21 g, 7.30 mmol), and di-*tert*-butyl iminodiacetate (**5b**) (3.05 g, 12.4 mmol) were added successively. The suspension was sonicated in an ultrasound bath at room temperature for 35 min and then stirred at room temperature for 23 h. The suspension was filtered through silica gel (3.0 cm × 4.0 cm, rinsing with Et_2O). The solvents of the filtrate were removed. Chromatography (4.5 cm × 28 cm; pentane–Et_2O, 1:1) of the residual yellow oil gave *tert*-butyl ester **6b** (1.74 g, 76%) as a colorless oil; R_f = 0.45.

^1H NMR (500 MHz, $CDCl_3$): δ = 7.54 (s, 2 H, ArH), 3.96 (s, 4 H, $ArCH_2$), 3.43 (s, 8 H, CH_2CO), 1.43 (s, 36 H, CH_3).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.3 (CO), 160.7 (C_{Ar} *meta* to Cl), 145.6 ($C_{Ar}Cl$), 121.1 ($C_{Ar}H$), 81.0 (CMe_3), 59.4 ($ArCH_2$), 55.8 (CH_2CO), 28.1 (CH_3).

MS (ESI): *m*/*z* = 650.4 $[M + Na]^+$, 628.4 $[M + H]^+$.

HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{31}H_{50}ClN_3O_8Na^+$: 650.3179; found: 650.3162.

Anal. Calcd for $C_{31}H_{50}ClN_3O_8$: C, 59.27; H, 8.02; N, 6.69. Found: C, 59.14; H, 8.09; N, 6.56.

Iodo Diester (Dimethyl 4-Iododipicolinate, 7)

The published procedure[53] was followed. NaI (39.2 g, 261 mmol) was added to a solution of chloro diester **2** (6.0 g, 26.1 mmol) in MeCN (200 mL). The suspension was sonicated for 20 min at room temperature. AcCl (6.05 mL, 78.2 mmol) was added slowly to the colorless suspension, whereupon the color of the suspension changed to dark red. The dark red suspension was sonicated for 30 min at room temperature. CH_2Cl_2 (200 mL) and saturated aqueous Na_2CO_3 (100 mL) were added. The organic phase was separated and washed firstly with saturated aqueous $Na_2S_2O_3$ until the color of the organic phase had changed from red to colorless and then with saturated aqueous NaCl (2 × 50 mL) and H_2O (50 mL). The organic phase was dried over Na_2SO_4 and filtered. Removal of the solvents gave pale yellow crystals which were recrystallized in MeOH (280 mL) giving iodo diester **7** as colorless needles (7.85 g, 94%); mp 170–171 °C.

^1H NMR (500 MHz, $CDCl_3$): δ = 8.65 (s, 2 H, ArH), 4.01 (s, 6 H, Me).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 163.7 (C=O), 148.2 (C_{Ar} *meta* to I), 137.0 ($C_{Ar}H$), 106.9 ($C_{Ar}I$), 53.3 (CH_3).

MS (ESI): *m*/*z* = 664.7 $[2 M + Na]^+$, 343.9 $[M + Na]^+$, 321.9 $[M + H]^+$.

HRMS (EI, 70 eV): *m*/*z* $[M]^{+\bullet}$ calcd for $C_9H_8INO_4^{+\bullet}$: 320.94925; found: 320.94928.

Anal. Calcd for $C_9H_8INO_4$: C, 33.67; H, 2.51; N, 4.36. Found: C, 33.74; H, 2.54; N, 4.23.

Iodo Diol 8

This reaction was performed under argon. $NaBH_4$ (946 mg, 25.0 mmol) was added portionwise within 10 min to a suspension of iodo diester **7** (4.03 g, 12.5 mmol) and finely powdered $CaCl_2$ (1.39 g, 12.5 mmol) in anhyd MeOH (100 mL) at room temperature, whereupon the color of the suspension changed to orange. The orange suspension was stirred at room temperature and the reaction was followed by TLC [R_f(**7**) = 0.53, R_f(**8**) = 0.1 (Et_2O)]. The reaction was complete after 5 h and the colorless suspension was filtered through silica gel (3 cm × 2 cm, rinsing with MeOH). The solvents of the filtrate were removed and the residual colorless solid was dissolved in 1 M HCl (60 mL). The pH of the solution was raised to pH 12 by addition of 1.0 M aqueous NaOH. Thereupon, a colorless solid precipitated. The precipitate was isolated by filtration and dried under reduced pressure over P_4O_{10} for 3 d. A white powder (3.95 g, consisting of iodo diol **8** and inorganic salts) was obtained.

^1H NMR (500 MHz, DMSO-d_6): δ = 7.69 (s, 2 H, ArH), 5.48 (t, ^3J = 5.9 Hz, 2 H, OH), 4.47 (d, ^3J = 5.9 Hz, 4 H, CH_2).

^{13}C NMR (125 MHz, DMSO-d_6): δ = 162.2 (C_{Ar} *meta* to I), 127.0 ($C_{Ar}H$), 107.4 ($C_{Ar}I$), 63.6 (CH_2).

MS (ESI): *m*/*z* = 552.8 $[2 M + Na]^+$, 287.9 $[M + Na]^+$, 265.9 $[M + H]^+$.

HRMS (ESI): *m*/*z* $[2 M + Na]^+$ calcd for $C_{14}H_{16}I_2N_2O_4Na^+$: 552.90916; found: 552.90706.

Iodo Dimesylate 9; Experiment A

The material obtained from the reduction of iodo diester **7** (2.52 g, consisting of iodo diol **8** and inorganic salts) was suspended in CH_2Cl_2 (25 mL) and Et_3N (3.5 mL, 25.1 mmol). MsCl (1.85 mL, 23.9 mmol) was added within 5 min to the cooled (ice bath) suspension. After the suspension was stirred for 10 min under cooling, the ice bath was removed and the suspension was stirred for 15 min at room temperature. H_2O (15 mL) and saturated aqueous $NaHCO_3$ (15 mL) were added. The suspension was filtered. The filtrate consisted of two liquid phases. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (6 × 15 mL). The organic phases were com-

bined, dried over $MgSO_4$, and the solvents were removed. A white solid consisting of iodo dimesylate **9**, monochloride **12**, and iodo dichloride **13** in a molar ratio of 90:7:3 was obtained. The components of the colorless solid were separated by column chromatography (5 cm × 46 cm; Et_2O–CH_2Cl_2, 1:4). Iodo dimesylate **9** (2.34 g, 69% over 2 steps; R_f = 0.42), monochloride **12** (130 mg, 5% over 2 steps; R_f = 0.65), and iodo dichloride **13** (61 mg, 2% over 2 steps; R_f = 0.80) were obtained as colorless solids.

Iodo Dimesylate 9

Mp 129–130 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.83 (s, 2 H, ArH), 5.24 (s, 4 H, CH_2), 3.11 (s, 6 H, CH_3).

^{1}H NMR (500 MHz, CD_2Cl_2): δ = 7.86 (s, 2 H, ArH), 5.24 (s, 4 H, CH_2), 3.10 (s, 6 H, CH_3).

^{13}C NMR (125 MHz, CD_2Cl_2): δ = 154.9 (C_{Ar} *meta* to I), 131.4 (C_{Ar}H), 107.4 (C_{Ar}I), 70.5 (CH_2), 38.3 (CH_3); recorded in CD_2Cl_2 because of the poor solubility of **9** in $CDCl_3$.

MS (ESI): *m*/*z* = 459.8 $[M + K]^+$, 443.9 $[M + Na]^+$, 421.9 $[M + H]^+$.

HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_9H_{12}INO_6S_2Na^+$: 443.90429; found: 443.90533.

Anal. Calcd for $C_9H_{12}INO_6S_2$: C, 25.66; H, 2.87; N, 3.33; S, 15.22. Found: C, 25.71; H, 2.81; N, 3.46; S, 14.94.

Monochloride 12

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.85 and 7.78 (2 s, 1 H each, ArH), 5.24 (s, 2 H, CH_2O), 4.57 (s, 2 H, CH_2Cl), 3.11 (s, 3 H, CH_3).

MS (ESI): *m*/*z* = 744.7 $[2 M + Na]^+$, 399.8 $[M + K]^+$, 383.9 $[M + Na]^+$, 361.9 $[M + H]^+$.

Iodo Dichloride 13

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.82 (s, 2 H, ArH), 4.58 (s, 4 H, CH_2).

MS (EI, 70 eV): *m*/*z* = 300.8 (100.0) $[M]^{+\bullet}$, 265.9 (38.6) $[M - Cl]^+$, 173.9 (6.5) $[M - I]^+$, 139.0 (11.5) $[M - I - Cl]^{+\bullet}$, 104.0 (18.6) $[M - I - 2Cl]^+$.

Iodo Dimesylate 9; Experiment B

The material obtained from the reduction of iodo diester **7** (1.83 g, consisting of iodo diol **8** and inorganic salts) was suspended in CH_2Cl_2 (80 mL). This colorless suspension was sonicated in an ultrasound bath at room temperature for 20 min. Ice was added to cool the ultrasound bath to 3 °C. Et_3N (2.4 mL, 17.2 mmol) and MsCl (1.30 mL, 16.8 mmol) were added at 3 °C successively to the suspension. The suspension was stirred and sonicated in the cold water bath for 100 min, whereupon the bath temperature increased slowly to 10 °C. H_2O (100 mL) and saturated aqueous $NaHCO_3$ (20 mL) were added. The suspension was filtered. The filtrate consisted of two liquid phases. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 40 mL). The combined organic phases were filtered through silica gel (4 cm × 4 cm; rinsing with CH_2Cl_2–Et_2O, 4:1). Removal of the solvents from the filtrate gave a colorless solid (2.15 g) consisting of iodo dimesylate **9** (79% yield over 2 steps), monochloride **12** (10% yield over 2 steps), and iodo dichloride **13** (1% yield over 2 steps) in a molar ratio of 88:11:1. For analytical data, see experiment A.

4-Iodo-PyMTA Ethyl Ester 10a; Experiment A

A suspension of iodo dimesylate **9** (5.22 g, 12.4 mmol), diethyl iminodiacetate (**5a**) (8.23 g, 43.5 mmol), and Na_2CO_3 (15.67 g, 147.8 mmol) in MeCN (130 mL) was stirred at room temperature for 23 h. The reaction mixture was filtered through silica gel (4 cm × 4 cm, rinsing with Et_2O). The solvents of the filtrate were removed. Chromatography (6 cm × 35 cm; pentane–Et_2O, 1:2) of the residual pale yellow oil yielded 4-iodo-PyMTA ethyl ester **10a** (5.88 g, 78%) as a colorless oil; R_f = 0.60. For analytical data, see experiment B.

4-Iodo-PyMTA Ethyl Ester 10a; Experiment B

The material obtained in experiment B for the preparation of iodo dimesylate **9** [1.17 g, consisting of iodo dimesylate **9** (2.49 mmol), monochloride **12** (0.31 mmol), and iodo dichloride **13** (0.03 mmol)] was dissolved in MeCN (30 mL). Na_2CO_3 (3.00 g, 28.4 mmol), KI (936 mg, 5.64 mmol), and diethyl iminodiacetate (**5a**) (1.8 mL, 10.3 mmol) were added successively. The suspension was sonicated in an ultrasound bath at room temperature for 1 h and then stirred at room temperature for 20 h. Et_2O and H_2O were added successively to the suspension. The organic phase was separated and the aqueous phase was extracted with Et_2O (2 ×). The combined organic phases were washed with H_2O (2 ×). The solvents were removed. Chromatography (4.5 cm × 28 cm; pentane–Et_2O, 1:2) of the residual orange oil gave 4-iodo-PyMTA ethyl ester **10a** (1.25 g, 73% over 3 steps) as a colorless oil; R_f = 0.60.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.91 (s, 2 H, ArH), 4.16 (q, ^3J = 7.1 Hz, 8 H, CH_2CH_3), 3.97 (s, 4 H, $ArCH_2$), 3.58 (s, 8 H, CH_2CO), 1.26 (t, ^3J = 7.1 Hz, 12 H, CH_3).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.9 (CO), 159.3 (C_{Ar} *meta* to I), 130.3 (C_{Ar}H), 107.3 (C_{Ar}I), 60.5 (CH_2CH_3), 59.2 ($ArCH_2$), 54.9 (CH_2CO), 14.2 (CH_3).

MS (ESI): *m*/*z* = 630.2 $[M + Na]^+$, 608.2 $[M + H]^+$.

HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{23}H_{34}IN_3O_8Na^+$: 630.12828; found: 630.12686.

Anal. Calcd for $C_{23}H_{34}IN_3O_8$: C, 45.48; H, 5.64; N, 6.92. Found: C, 45.62; H, 5.72; N, 6.91.

4-Iodo-PyMTA *tert*-Butyl Ester 10b; Experiment A

A suspension of iodo dimesylate **9** (1.50 g, 3.56 mmol), di-*tert*-butyl iminodiacetate (**5b**) (2.59 g, 10.6 mmol), and Na_2CO_3 (3.78 g, 35.6 mmol) in MeCN (50 mL) was stirred at room temperature for 24 h. Et_2O (25 mL) and H_2O (25 mL) were added successively. The organic phase was separated and the aqueous phase was extracted with Et_2O (3 × 15 mL). The solvents of the combined organic phases were removed. Chromatography (5 cm × 32 cm; pentane–Et_2O, 1:1) of the residual colorless viscous oil provided 4-iodo-PyMTA *tert*-butyl ester **10b** (2.30 g, 90%) as a colorless viscous oil; R_f = 0.50. For analytical data, see experiment B.

4-Iodo-PyMTA *tert*-Butyl Ester 10b; Experiment B

The material obtained in experiment B for the preparation of iodo dimesylate **9** [959 mg, consisting of iodo dimesylate **9** (2.04 mmol), monochloride **12** (0.26 mmol), and iodo dichloride **13** (0.02 mmol)] was dissolved in MeCN (30 mL). Na_2CO_3 (2.46 g, 23.2 mmol), KI (769 mg, 4.63 mmol), and di-*tert*-butyl iminodiacetate (**5b**) (2.11 g, 8.61 mmol) were added successively. The suspension was too viscous to be stirred. Therefore, additional MeCN (10 mL) was added. The colorless suspension was sonicated in an ultrasound bath at room temperature for 1 h and then stirred at room temperature for 20 h. Et_2O and H_2O were added successively. The organic phase was separated

and the aqueous phase was extracted with Et_2O (2 ×). The combined organic phases were washed with H_2O (2 ×). The solvents were removed. Chromatography (4.5 cm × 29 cm; pentane–Et_2O, 1:2) of the residual orange oil gave 4-iodo-PyMTA *tert*-butyl ester **10b** (1.37 g, 82% over 3 steps) as a colorless oil; R_f = 0.50.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.89 (s, 2 H, ArH), 3.94 (s, 4 H, $ArCH_2$), 3.44 (s, 8 H, CH_2CO), 1.44 (s, 36 H, CH_3).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.3 (CO), 159.8 (C_{Ar} *meta* to I), 130.1 ($C_{Ar}H$), 107.5 ($C_{Ar}I$), 81.1 (CMe_3), 59.2 ($ArCH_2$), 55.8 (CH_2CO), 28.2 (CH_3).

MS (ESI): *m*/*z* = 742.3 [M + Na]$^+$, 720.3 [M + H]$^+$.

HRMS (ESI): *m*/*z* [M + Na]$^+$ calcd for $C_{31}H_{50}IN_3O_8Na^+$: 742.25348; found: 742.25518.

Anal. Calcd for $C_{31}H_{50}IN_3O_8$: C, 51.74; H, 7.00; N, 5.84. Found: C, 51.85; H, 7.04; N, 5.82.

{[4-Chloro-PyMTA – 4 H$^+$]$^{4-}$·n H$^+$·m Na$^+$} (14)

4-Chloro-PyMTA ethyl ester (**6a**) (199 mg, 386 µmol) was dissolved in EtOH (2 mL). A solution of NaOH (125 mg, 3.12 mmol) in H_2O (2 mL) was added whereupon a mixture of two liquid phases formed, which turned into one colorless phase within the next 10 min of stirring. This was stirred at room temperature for 4 h. Then, proton-exchange resin was added in an amount needed to reduce the pH of the solution to pH 3. The solution was separated from the proton-exchange resin by filtration, and the resin was washed with a 1:1 mixture of EtOH and H_2O (3 × 1.5 mL). The solvents of the combined filtrates were removed. {[4-Chloro-PyMTA – 4 H$^+$]$^{4-}$·n H$^+$·m Na$^+$} (**14**) (148 mg, 85%) was obtained as a colorless solid. The content of the structural motif [4-chloro-PyMTA – 4 H$^+$]$^{4-}$ was determined by quantitative ^{1}H NMR spectroscopy to be 89.5 wt% (for details, see Supporting Information).

^{1}H NMR (500 MHz, CD_3OD): δ = 7.52 (s, 2 H, ArH), 4.25 (s, 4 H, $ArCH_2$), 3.62 (s, 8 H, CH_2CO).

^{13}C NMR (125 MHz, CD_3OD): δ = 173.5 (CO), 158.1 (C_{Ar} *meta* to Cl), 147.3 ($C_{Ar}Cl$), 124.6 ($C_{Ar}H$), 59.9 ($ArCH_2$), 57.8 (CH_2CO).

MS (ESI): *m*/*z* = 401.9 [M – H]$^-$, 200.4 [M – 2 H]$^{2-}$.

HRMS (ESI): *m*/*z* [M – H]$^-$ calcd for $C_{15}H_{17}ClN_3O_8^-$: 402.07097; found: 402.06984.

Anal. Calcd for {[4-chloro-PyMTA – 4 H$^+$]$^{4-}$·n H$^+$·m Na$^+$} with 89.5 wt% of [4-chloro-PyMTA – 4 H$^+$]$^{4-}$ ($C_{15}H_{14}ClN_3O_8$): C, 40.33; H, 3.16; N, 9.40. Found: C, 40.61; H, 4.32*; N, 9.53. *H comes from [4-chloro-PyMTA – 4 H$^+$]$^{4-}$ as well as from '·n H$^+$'.

{4-Chloro-PyMTA·n TFA} (16)

4-Chloro-PyMTA *tert*-butyl ester (**6b**) (99 mg, 158 µmol) was dissolved in TFA (613 µL, 8.0 mmol) and the solution was stirred at room temperature for 1 h. The volatile components were removed at room temperature by lowering the pressure to 10 mbar, giving a pale yellow solid. This procedure was applied all over four times. Then, the residual pale yellow solid was dissolved in MeOH (1 mL) and the solution was dropped into a mixture of Et_2O (1 mL) and pentane (2 mL), whereupon a colorless solid precipitated. Additional pentane was added slowly to the supernatant until no more precipitate formed. The precipitate was separated via centrifugation (5000 rpm, 1 min) and dried under reduced pressure over P_4O_{10}. {4-Chloro-PyMTA·n TFA} (**16**) (60 mg, 89%) was obtained as a colorless solid. The content of the structural motif 4-chloro-PyMTA was determined by quantitative ^{1}H NMR spectroscopy to be 94.2 wt % (for details, see Supporting Information).

^{1}H NMR (500 MHz, CD_3OD): δ = 7.72 (s, 2 H, ArH), 4.27 (s, 4 H, $ArCH_2$), 3.73 (s, 8 H, CH_2CO).

^{1}H NMR (500 MHz, D_2O): δ = 7.68 (s, 2 H, ArH), 4.74 (s, 4 H, $ArCH_2$), 4.14 (s, 8 H, CH_2CO).

^{13}C NMR (125 MHz, D_2O): δ = 169.3 (CO), 151.2 (C_{Ar} *meta* to Cl), 147.5 ($C_{Ar}Cl$), 125.7 ($C_{Ar}H$), 58.1 ($ArCH_2$), 56.0 (CH_2CO).

^{19}F NMR (470 MHz, D_2O): 75.39 ($F_3CCO_2^-$).

MS (ESI): *m*/*z* = 401.9 [M – H]$^-$, 200.3 [M – 2 H]$^{2-}$.

Anal. Calcd for {4-chloro-PyMTA·n TFA} with 94.2 wt% of 4-chloro-PyMTA ($C_{15}H_{18}ClN_3O_8$) not taking TFA into account: C, 42.03; H, 4.22; N, 9.81. Found: C, 43.24*; H, 4.65*; N, 9.80. *TFA contributes C and H.

{[4-Iodo-PyMTA – 4 H$^+$]$^{4-}$·n H$^+$·m Na$^+$} (15)

4-Iodo-PyMTA ethyl ester **10a** (200 mg, 329 µmol) was dissolved in EtOH (2 mL). A solution of NaOH (104 mg, 2.60 mmol) in H_2O (2 mL) was added whereupon a mixture of two liquid phases formed, which turned into one colorless phase within the next 10 min of stirring. This was stirred at room temperature for 4 h. Then, proton-exchange resin was added in an amount needed to reduce the pH of the solution to pH 3. The solution was separated from the proton-exchange resin by filtration, and the resin was washed with H_2O (5 × 2 mL). The solvents of the combined filtrates were removed. {[4-Iodo-PyMTA – 4 H$^+$]$^{4-}$·n H$^+$·m Na$^+$} (**15**) (168 mg, 95%) was obtained as a colorless solid. The content of the structural motif [4-iodo-PyMTA – 4 H$^+$]$^{4-}$ was determined by quantitative ^{1}H NMR spectroscopy to be 92.2 wt% (for details, see Supporting Information).

^{1}H NMR (500 MHz, CD_3OD): δ = 7.90 (s, 2 H, ArH), 4.25 (s, 4 H, $ArCH_2$), 3.67 (s, 8 H, CH_2CO).

^{13}C NMR (125 MHz, CD_3OD): δ = 173.4 (CO), 157.1 (C_{Ar} *meta* to I), 133.6 ($C_{Ar}H$), 108.8 ($C_{Ar}I$), 59.6 ($ArCH_2$), 57.6 (CH_2CO).

MS (ESI): *m*/*z* = 493.8 [M – H]$^-$, 246.3 [M – 2 H]$^{2-}$.

HRMS (ESI): *m*/*z* [M – H]$^-$ calcd for $C_{15}H_{17}IN_3O_8^-$: 494.00658; found: 494.00558.

Anal. Calcd for {[4-iodo-PyMTA – 4 H$^+$]$^{4-}$·n H$^+$·m Na$^+$} with 92.2 wt% of [4-iodo-PyMTA – 4 H$^+$]$^{4-}$ ($C_{15}H_{14}IN_3O_8$): C, 33.82; H, 2.65; N, 7.89. Found: C, 34.00; H, 3.66*; N, 7.93. *H comes from [4-iodo-PyMTA – 4 H$^+$]$^{4-}$ as well as from '·n H$^+$'.

{4-Iodo-PyMTA·n TFA} (17)

4-Iodo-PyMTA *tert*-butyl ester (**10b**) (100 mg, 139 µmol) was dissolved in TFA (535 µL, 7.0 mmol) and the solution was stirred at room temperature for 1 h. The volatile components were removed at room temperature by lowering the pressure to 10 mbar, giving a pale yellow solid. This procedure was applied all over four times. Then, the residual pale yellow solid was dissolved in MeOH (1 mL) and the solution was dropped into a mixture of Et_2O (1 mL) and pentane (2 mL), whereupon a colorless solid precipitated. Additional pentane was added slowly to the supernatant until no more precipitate formed. The precipitate was separated via centrifugation (5000 rpm, 1 min) and dried under reduced pressure over P_4O_{10}. {4-Iodo-PyMTA·n TFA} (**17**) (68 mg, 93%) was obtained as a colorless solid. The content of the structural motif 4-iodo-PyMTA was determined by quantitative ^{1}H NMR spectroscopy to be 93.9 wt% (for details, see Supporting Information).

^{1}H NMR (500 MHz, CD_3OD): δ = 8.03 (s, 2 H, ArH), 4.21 (s, 4 H, $ArCH_2$), 3.71 (s, 8 H, CH_2CO).

^{1}H NMR (500 MHz, D_2O): δ = 8.04 (s, 2 H, ArH), 4.69 (s, 4 H, $ArCH_2$), 4.11 (s, 8 H, CH_2CO).

^{13}C NMR (125 MHz, D_2O): δ = 169.3 (CO), 150.1 (C_{Ar} *meta* to I), 134.5 (C_{Ar}H), 108.9 (C_{Ar}I), 57.8 ($ArCH_2$), 56.0 (CH_2CO).

^{19}F NMR (470 MHz, D_2O): 75.39 ($CF_3CO_2^-$).

MS (ESI): m/z = 518.0 $[M + Na]^+$, 496.1 $[M + H]^+$.

Anal. Calcd for {4-iodo-PyMTA·n TFA} with 93.9 wt% of 4-iodo-PyMTA ($C_{15}H_{18}IN_3O_8$) not taking TFA into account: C, 34.16; H, 3.43; N, 7.97. Found: C, 35.15*; H, 3.91*; N, 8.10. *TFA contributes C and H.

Hydrolysis of 4-Iodo-PyMTA *tert*-Butyl Ester (10b) with LiOD, NaOD, and KOD

A 1.0 M solution of LiOD in D_2O was prepared from $LiOH·H_2O$ and D_2O as follows: $LiOH·H_2O$ (420.5 mg, 10.0 mmol) was dissolved in D_2O (5 mL) and the solvents were removed. This H–D-exchange process was applied all over three times. Then, D_2O was added to the residual colorless solid in a volume needed to obtain 10.0 mL of solution (i.e., 1.0 M LiOD in D_2O). The 1.0 M solutions of NaOD and KOD in D_2O were prepared by diluting 40 wt% solutions of NaOD or KOD in D_2O with D_2O.

Three samples of 4-iodo-PyMTA *tert*-butyl ester (**10b**) (30 µmol) dissolved in CD_3OD (440 µL) were prepared in NMR tubes. A solution of LiOD or NaOD or KOD (1.0 M in D_2O; 240 µL, 240 µmol) was added. Upon addition of the base, the solutions became turbid and 4-iodo-PyMTA *tert*-butyl ester (**10b**) separated as a viscous oil. The solutions were vigorously shaken and became homogeneous after a few hours. The extent of hydrolysis was determined by ^{1}H NMR spectroscopy. After the reaction mixture had become a homogeneous solution, the extent of the ester hydrolysis was calculated using the equation $I_{t\text{-}BuOD}/(I_{t\text{-}BuOD} + I_{RCOOt\text{-}Bu})$, where $I_{t\text{-}BuOD}$ is the integral of the signal of *t*-BuOD (δ = 1.23) and $I_{RCOOt\text{-}Bu}$ is the sum of the integrals of all *tert*-butyl ester groups (singlets between 1.35–1.50 ppm); several signals for *tert*-butyl ester groups appeared during the reaction process as **10b** has four ester groups and thus several partially hydrolyzed species occur as transient species. To determine the extent of the hydrolysis before the reaction mixture became a homogeneous solution figure $A = (I_{t\text{-}BuOD} + I_{RCOOt\text{-}Bu})/I_{MeOD}$ was determined after the reaction mixture had turned into a homogeneous solution. I_{MeOD} is the integral of the signal of the solvent MeOD which remains constant during the reaction. With figure *A* the extent of the hydrolysis was calculated using the equation $I_{t\text{-}BuOD}/(A{\cdot}I_{MeOD})$. The results of these experiments are shown in Figure 1.

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Supporting Information

Supporting information for this article is available online at http://dx.doi.org/10.1055/s-0035-1561660.

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