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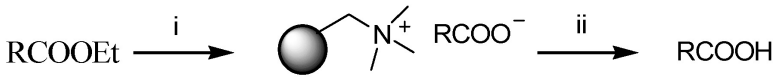

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High-Throughput Ester Hydrolysis with Catch-and-Release Isolation of Carboxylic Acids

Tina M. Morwick*

Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Road, Ridgefield, Connecticut 06801-0368

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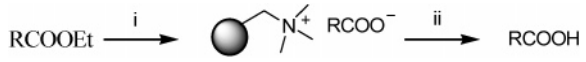
Introduction. The carboxylic acid functionality manifests a ubiquitous presence in biologically active molecules. It is found in a number of drugs and has been identified as a privileged fragment.[1] Numerous endogenous ligands that function as signaling molecules incorporate carboxylic acid functionality, as well. Examples include various eicosanoids, retinoic acid, thyroid hormone, γ-aminobutyric acid, and numerous peptide hormones and growth factors. Therefore, high-throughput synthesis of carboxylic acids for the generation of compound libraries for drug discovery or chemical genomics studies is quite relevant.

Typically, preparation of carboxylic acids develops via an ester-protected synthon, which is unmasked late in the synthesis. This is generally accomplished by aqueous hydrolysis, which is difficult to incorporate into a parallel method.[2] Therefore, a protocol whereby the product acids are captured directly onto a solid support would be highly advantageous.

Use of polymer-supported reagents and scavengers has undergone continual expansion in recent years.[3] We have explored the utility of the ion-exchange resin Amberlyst A26 (OH^- form) for ester hydrolysis. Sporadic reports of the use of this reagent for hydrolysis have appeared in the literature.[4] The hydroxide-bound Amberlyst and related reagents have also been used as scavengers; however, we have not found a description of the use of this or related reagents for ester hydrolysis in combination with catch-and-release methodology. This protocol offers significant utility in terms of high-throughput synthesis, because it provides an efficient laboratory procedure for ester hydrolysis as well as product isolation and purification. Other strategies for parallel ester hydrolysis and resin-facilitated product isolation have been reported;[5] however, the hydroxide ion-exchange resin offers a more direct approach. Therefore, we have investigated its use in this regard, and herein the preliminary results are reported (Scheme 1).

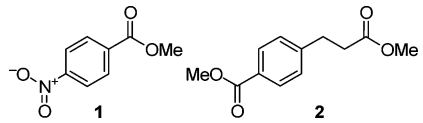
Results and Discussion. Initially, solvent effects were investigated. The Amberlyst polymer is a macroreticular resin with a permanent pore structure, and therefore, swelling was not expected to have a significant impact. To explore this premise, solvents which have demonstrated diverse resin solvation/swelling effects and having variable associated physicochemical properties were examined.[6] Table 1 shows results from these comparisons. Esters were treated with the resin and agitated overnight in the designated solvent. The resin-captured products were isolated by filtration and washed with additional solvent. The product acids were then eluted from the resin with formic acid in methanol.[7] (See Supporting Information for experimental procedures.) For hydrolysis of ester **1**, THF, DMF, methanol, and acetonitrile demonstrated comparable efficiency with good yield of products recovered from the eluant in high purity (>95%). For dichloromethane, however, the reaction was apparently sluggish with significant quantities of unreacted ester isolated from the wash fraction. No starting ester was observed in the wash solutions for the other four solvents; however, a negligible amount of the acid was seen in the wash fraction with THF and acetonitrile only. For the diester **2**, the expectation was that following hydrolysis of an initial ester, the monoacid would be captured onto the resin, thereby rendering it unavailable for further hydrolysis. In contrast, the eluted products were found to contain pure diacid, with the exception of the reactions in methanol and DMF, in which cases a small percentage of the monoacid was isolated. Additionally, the wash solutions were found to contain exclusively the starting diester. Hydrolysis of the monoacid esters can potentially occur via release from the resin and recapture during the second hydrolysis or by an intraresin reaction,[8] whereby second stage hydrolysis results from reaction of the resin-bound monoacid ester with nearby resin-bound hydroxide. A second experiment in methanol was carried out using only 0.9 equiv of resin-bound hydroxide. As can be seen in the table, the quantity of isolated product was diminished, and the diacid was still favored, albeit less exclusively. Again, the wash solution was found to contain pure starting diester.

Scheme 1[a]



[a] (i) Amberlyst A26 (OH^- form), solvent; (ii) formic acid, methanol.

Table 1. Comparison of Solvent Effects for Hydrolysis of **1** and **2**[a]



solvent	ester	wash% rec ester	eluant % rec acid
DCM	**1**	30	50
THF	**1**	<1	81
DMF	**1**	<1	90
MeOH	**1**	<1	81
ACN	**1**	<1	84
DCM	**2**	53	18(0)[b]
THF	**2**	23	41(0)[b]
DMF	**2**	15	39(4)[b]
MeOH	**2**	9	51(1)[b]
MeOH[c]	**2**	51	23(12)[b]
ACN	**2**	9	34(0)[b]

[a] See Supporting Information for the general method. Unless noted, ~1.85 equiv of OH^- was used. [b] Yield of diacid (monoacid). [c] 0.9 equiv of OH^- was used.

* Corresponding author. Phone: 203-798-5751. Fax: 203-798-5297. E-mail: tmorwick@rdg.boehringer-ingelheim.com.

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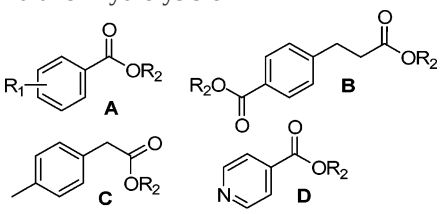
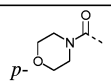
Table 2. Parallel Hydrolysis of **1**−**12**[a]



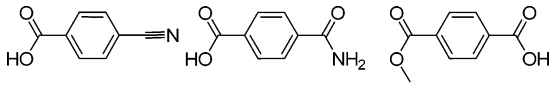
Ester (core)	R_1	R_2	Wash % rec ester	Eluant % rec acid
1 (A)	*p*-NO_2	Me	<1	62
2 (B)		Me	23	53(3)[b]
2(B)[c]		Me	4	69(0)[b]
3(C)		Et	1[d]	78
4(A)	*p*-Cl	Et	5[d]	66
4(A)[e]	*p*-Cl	Et	2	64
5(A)	*p*-Cl	Me	<1	72
5(A)[e]	*p*-Cl	Me	< 1	62
6(A)	*p*-CN	Me	< 1	72[f]
7(A)		Me	11	72
8(A)	*p*- NMe_2	Et	90[d]	3[g]
9(A)	*p*-OEt	Et	49[d]	28
10(A)[h]	*p*- CH_2NH_2	Me	43	51[i]
11(A)	2-NO_2-4-Cl	Me	2	33
12(D)		Me	<1	19
11(A)[j]	2-NO_2-4-Cl	Me	<1	97
12(D)[k]		Me	<1	93[l]

[a] See Supporting Information for the general method. Unless noted, ~1.67 equiv of OH^- was used. [b] Yield of diacid (monoacid). [c] 3 equiv of OH^- was used. [d] Mixture of ethyl and methyl esters. [e] Reaction run in acetonitrile. [f] Product is 83% acid plus two byproducts (see Figure 1). [g] Mixture of product, starting ester, methyl ester. [h] HCl salt; 3 equiv of hydroxide was used. [i] Formate salt. [j] Eluant is 1 M HCl water/acetonitrile (See Supporting Information for method modification). [k] Eluant is 1 N HCl aqueous (see Supporting Information for method modification). [l] HCl salt.

Results of these experiments indicate the protocol is effective using a variety of solvents with variable properties. Ester **1** is efficiently hydrolyzed, but the reagent is not capable of selective hydrolysis of diester **2** to the monoacid ester.

Next, an investigation of the general utility of the method for various ester substrates was executed. Methanol was chosen as the reaction solvent on the basis of its use as eluant and substrate solubilities. These reactions were run in parallel in a Bohdan block, and results are shown in Table 2. Again, unless otherwise noted, wash solutions contain only starting esters, and eluants deliver pure (>95%) product acids.

Results for **1** and **2** show some reduction in yield for **1** when the reaction is run in the Bohdan block protocol, as compared to the vial reactions (62 vs 81%), but similar product formation was observed for **2** using the different protocols (53 vs 51%). Increasing the number of equivalents of hydroxide to 3.0 enhanced the yield of the diacid **2**, which was now isolated without contamination of the monoacid. Substrate **3**, an aliphatic ethyl ester performed efficiently. It was noteworthy, however, that the wash solution contained both ethyl and methyl esters, resulting from transesterification with the reaction solvent. Basic catalysis is known to promote transesterification, and this phenomenon was observed for other examples of ethyl esters, as well. Substrates **4** and **5** were used to compare hydrolysis of ethyl vs methyl esters and demonstrate similar efficiencies with a small advantage in the case of methyl (72 vs 66%); however, transesterification to the methyl ester prior to hydrolysis could not be ruled out. A second comparison was therefore made by repeating the process with **4** and **5** using acetonitrile as solvent. As shown in the table, a slight reduction in the quantity of isolated acid was observed, as compared to the reactions run in methanol, but the results confirm that both esters show similar reactivity. For the nitrile derivative **6**, two byproducts were observed spectrally but were not isolated. Mass analysis and NMR indicated hydrolysis of the nitrile was a competing process (see Figure 1). Hydrolysis of heterocyclic nitriles with hydroxide exchange resins has been previously reported.[9]

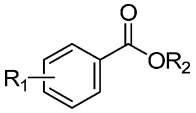


6a 83% **6b$_1$** 9% **6b$_2$** 8%

Figure 1. Results from hydrolysis of **6** indicating relative proportions of desired acid and two proposed byproducts.

Comparing all the benzoate esters, electronic effects were found to be significant, with electron-withdrawing groups promoting optimal reactivity, as would be expected from a standard base-catalyzed hydrolysis of esters of substituted benzoic acids. For example, when nitro, chloro, nitrile, and amide functional groups were present at the 4-position, yields ranged from 62 to 72%. By contrast, progressively amplified electron-donating effects translated into increasingly retarded rates of reaction, as can be seen by the product recovery for **8**−**10**. The most electron-rich analog, the dimethylamino derivative **8**, gave the smallest recovery, and in this case only, the eluant was contaminated with the methyl and ethyl esters. (Again, wash solutions consisted of both methyl and ethyl esters for **8** and **9**.) Substrate **11** was an apparent anomaly to the electronic effects demonstrated for other benzoate esters using the standard protocol, because only a 33% yield of product was isolated. The poor recovery could have resulted from limited hydrolysis due to a steric effect imposed by the ortho substituent. However, the absence of significant isolated starting ester in the wash solution suggested that reactivity was not the problem. Solubility did not appear to be involved either, because an additional wash of the resin bed with DMF following elution was inconsequential. In this case, it was found that modification of the eluant was necessary to facilitate efficient product removal. A second experiment utilized HCl in water/acetonitrile and provided a substantial improvement in product isolation

Table 3. Results for microwave Hydrolysis of **8**−**10**[a]



ester	R_1	R_2	wash % rec ester	eluant % rec acid
8[b]	*p*- NMe_2	Et	41[c]	36
8[d]	*p*- NMe_2	Et	8[c]	79
9[d]	*p*-OEt	Et	<1[c]	94[e]
10[d,f]	*p*-CH_2NH_2	Me	5	85[g]

[a] See Supporting Information for the general method. Unless noted, ~1.67 eq OH^- was used. [b] Irradiation time is 15 minutes. [c] Mixture of ethyl and methyl esters. [d] Irradiation time is 40 minutes. [e] Standard elution followed by DMF wash of resin. [f] HCl salt; 3.3 eq OH^- was used. [g] Formate salt.

(97 vs 33%). Removal of the captured product from the anion exchange resin can be facilitated by using a more selective counterion and a stronger acid to ensure the released product acid is present predominantly in an uncharged form. HCl satisfied both of these conditions.[10] Elution of isonicotinic acid, derived from ester **12**, was similarly complicated, and in this case, aqueous HCl was effectively employed for product recovery.[11]

A third series of experiments was subsequetly performed to determine if reaction efficiency for the refractory electron rich examples could be improved using microwave irradiation (Table 3). The standard method was modified accordingly (see the Supporting Information). Analog **8** was chosen initially because it was the most resistant. The reaction mixture was irradiated at 130 °C for 15 min. A substantial improvement in product formation was observed as compared to the standard conditions, and pure 4-dimethylaminobenzoic acid was recovered in 36% yield. Increasing the reaction time by an additional 25 min provided a 79% yield of pure product, similar to recoveries for the best substrates. For the other electron-rich benzoates (**9**, **10**), the acids were recovered in good yield and purity (>95%) using the microwave conditions. For **9**, a second wash of the resin with DMF following elution was necessary.

Conclusion

In summary, we have studied the use of Amberlyst A26 (OH^- form) for high-throughput ester hydrolysis with catch-and-release isolation of the product carboxylic acids. Solvents with various resin solvating properties were efficacious. As expected, electronic effects on reactivity were significant; however, the modest conversions observed for electron-rich systems could be overcome using microwave irradiation. Additionally, it was observed that in some cases, a stronger acid eluant was required to achieve good recovery from the resin. Exploration of the general utility of this method in related processes and in multistep sequences is ongoing.

Supporting Information Available. Experimental methods for comparison of solvent effects, hydrolysis of **1**−**12**, modified procedures for **11** and **12**, and microwave reactions for **8**−**10** is provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) Bobbitt, J. M.; Scola, D. A. *J. Org. Chem.* **1960**, *25*, 560. It is noteworthy that although some hydrolysis of the aromatic nitrile was observed under the reaction conditions, for the reactions run in acetonitrile, no hydrolysis of the solvent aliphatic nitrile was observed.

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(11) It was observed that dilute HCl could not be used in methanol because re-esterification of product acids was noted. Formic acid did not demonstrate this liability for the examples described herein.

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