Synlett R. Pierre et al. Letter

Identification of 1,5,7-Triazabicyclododecene and Polystyrene-Supported Superbases as Efficient Hydroxylaminolysis Agents of Sterically Hindered and Epimerizable Esters

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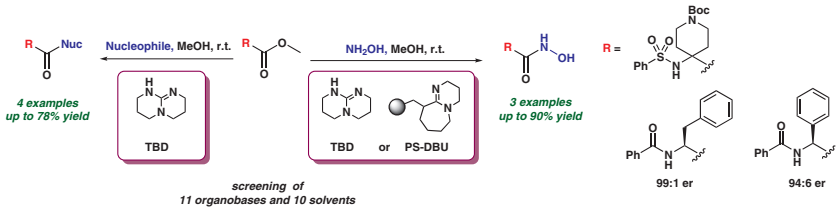

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Abstract In modern pharmaceutical research, the need for reliable protocols for the preparation of chemical libraries in a controlled manner is quintessential to driving the Design-Make-Test cycle in drug discovery programs. In this letter, we communicate the identification of 1,5,7-triazabicyclododecene and polystyrene-supported superbases as efficient hydroxylaminolysis agents of sterically hindered and epimerizable esters and, to some extent, amides, using iChem Explorer® as the conditions scouting tool.

Key words hydroxylaminolysis, hydroxamic acid, TBD, polymer-supported superbases, superbases, non-racemizing process

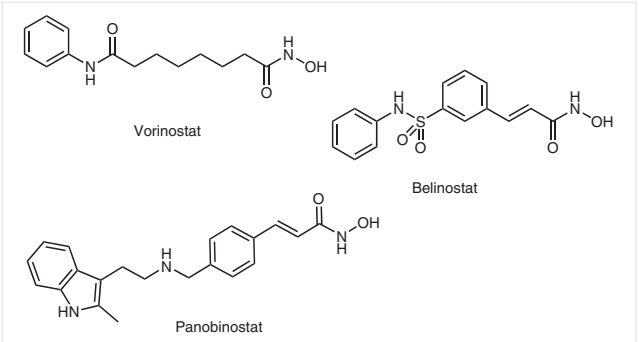


Figure 1 Pan-HDAC inhibitors having the hydroxamic acid functionality recently approved by the FDA

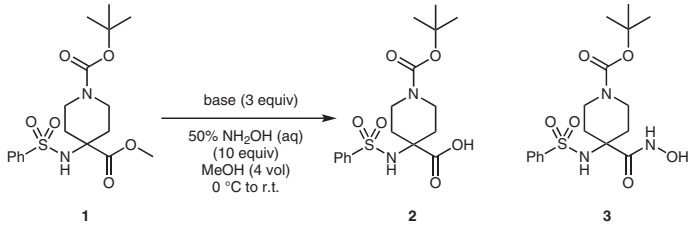
With the recent approvals of the histone deacetylase (HDAC) inhibitors[1] Voronistat,[2] Belinostat,[3] and Panobinostat[4] for the treatment of cutaneous T cell lymphoma (CTCL), peripheral T-cell lymphoma and multiple myeloma, the hydroxamic acid group has come under the spotlight (Figure 1).[5] The hydroxamic acid group binds to the zinc ion present in the active site of HDAC proteins. Inhibition of HDAC modifies cellular events such as proliferation, differentiation, and apoptosis, thereby making it a particularly attractive target for cancer therapies.[6]

In a recent letter, we disclosed the advantages of using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in solution to reduce reaction times and increase yields of hydroxamic acids from carboxylic acid esters.[7] In this letter, we describe the discovery that 1,5,7-triazabicyclododecene (TBD) significantly accelerates the hydroxylaminolysis of hindered esters even more and report its utilization for common aminolysis reactions. Moreover, we show that the hydroxylaminolysis process using polymer-supported superbases, in particular polymer-supported DBU, proceeds with minimal in situ epimerization while significantly accelerating the reaction rate compared with the standard protocol.

Our first screening, using iChem Explorer® as the scouting tool,[8] focused on the choice of base to examine whether we could improve further the crude reaction profiles compared with that obtained with DBU using hindered ester **1** as the substrate.[7] Table 1 summarizes the results obtained from a selection of common nucleophilic and non-nucleophilic organobases with a large range of pK_a.[9]

Nucleophilic aromatic bases such as pyridine and DMAP give no trace of product. Both DIPEA and DABCO also resulted in no conversion (entries 4 and 5). Perhaps surprisingly, the weaker amidine base DBN (entry 7) afforded just 35% of hydroxamic acid product **3** compared with 70% when DBU was used (entry 6). However, cyclic guanidine bases, 1,5,7-triazabicyclododecene (TBD) and 7-methyl-1,5,7-triazabicyclo(4.4.0)dec-5-ene (MTBD) proved superior to DBU, completing the reaction after just 30 h vs. 48 h and gave the best ratio of hydroxamic acid product to carboxylic acid byproduct (entries 8, 9 vs. 6). Finally, acyclic guanidine bases

Table 1 Effect of Base on Hydroxamic Acid Formation



1 2 3

Entry	Base	pK_a[a]	Time(h)	Ratio (%)[b]			Yield of **3** (%)
				1	**2**	**3**	
1	none		48	100	–	–	–
2	pyridine	12.5	48	100	–	–	–
3	*N*,*N*-dimethylaminopyridine (DMAP)	17.9	48	100	–	–	–
4	*N*,*N*-diisopropylethylamine (DIPEA)	18.5[c]	48	100	–	–	–
5	1,4-diazabicyclo[2.2.2]octane (DABCO)	18.3	48	100	–	–	–
6	1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)	24.3	48	–	30	70	59
7	1,5-diazabicyclo[4.3.0]non-5-ene (DBN)	23.8	48	–	65	35	–
8	1,5,7-triazabicyclododecene (TBD)	26.0	30	–	22	78	64
9	7-methyl-1,5,7-triazabicyclo[4.4.0]dec-5-ene (MTBD)	25.4	30	–	31	69	–
10	1,1,3,3-tetramethylguanidine (TMG)	23.3	48	–	71	29	–
11	2-*tert*-butyl-1,1,3,3-tetramethylguanidine	25.3	48	–	75	25	–
12	*tert*-butylimino-tris(dimethylamino)phosphorane (P_1-*t*-Bu)	26.9	48	–	35	65	–

[a] Values in MeCN.
[b] Conversion determined by UPLC-MS.
[c] Actual value not found; estimated value.

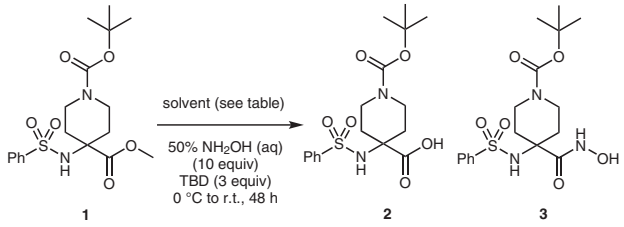
(entries 10 and 11) afforded less than 30% conversion to the desired hydroxamic acid product but the phosphazene base P_1-*t*-Bu afforded an acceptable conversion to the desired product **3** (entry 12).

With these results in hand, we chose TBD as the base and looked at the effect of changing the solvent on product distribution. From Table 2, among the dipolar aprotic solvents, only dimethyl sulfoxide (DMSO) afforded a positive ratio of **3** to **2** (entry 1) whereas *N*,*N*-dimethylformamide (DMF) and sulfolane gave more carboxylic acid by-product (**2**) (entries 2 and 3). All ethereal solvents screened favored conversion to the hydroxamic product **3** (entries 4–6). In terms of protic solvents, as expected, water gave a poor ratio (entry 7), which we presume is due to saponification in situ to the undesired carboxylic acid. However, alcoholic solvents (entries 8 and 9) afforded the best ratios, with MeOH clearly giving the best conversion (entry 9). We also carried out the reaction in an ionic liquid with the aim of recovering the solvent after the reaction was complete but, unfortunately, the carboxylic acid impurity was the major product (entry 10).

Encouraged by our screening results on hindered substrate **1**, we carried out a comparative study of DBU and TBD both in solution and on solid support for hydroxylaminolysis on enantiomerically pure substrates rather than examining substrate scope, which we expected to be very similar to that with DBU.[7] We chose methyl benzoyl-L-phenylalaninate (**4**) and methyl benzoyl-L-phenylglycinate (**6**), which were readily prepared from benzoyl chloride and the corresponding α-amino ester. Little racemization of **4** or **6** was observed during amide bond formation as verified by chiral HPLC.[10] For **4**, DBU, TBD, and MTBD in solution accelerated greatly the reaction and there was no racemization in situ (entries 3–5 vs. 1). As expected, polymer-supported DBU and TBD also worked, but the reaction was significantly slower (entries 3, 4 vs. 6, 7). In all our experiments, full conversion was observed with very little formation of carboxylic acid side-product and even in the presence of NaOH, no erosion of the stereogenic center was observed (Table 3).

To differentiate between the reaction conditions, we turned our attention to methyl benzoyl-L-phenylglycinate (**6**), which we anticipated would be much more sensitive to epimerization because of the lower pK_a of the proton α to the phenyl group (Table 4). In summary, sodium hydroxide, DBU, TBD, and MTBD in solution all afforded **7** very rapidly

Table 2 Effect of Solvent on Hydroxamic Acid Formation

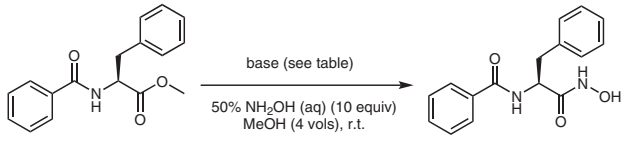


1 2 3

Entry	Solvent	Ratio (%)[a]	
		2	3
1	DMSO	41	59
2	DMF	53	47
3	sulfolane	57	43
4	tetrahydrofuran (THF)	45	55
5	MeTHF	43	57
6	1,2-dimethoxyethane (DME)	42	58
7	water	76	34
8	*i*-PrOH	38	62
9	MeOH	22	78
10	3-methyl-1-octylimidazolium hexafluorophosphate	71	29

[a] Conversion determined by LCMS.

Table 3 Epimerization of **4** during the Hydroxylaminolysis Process



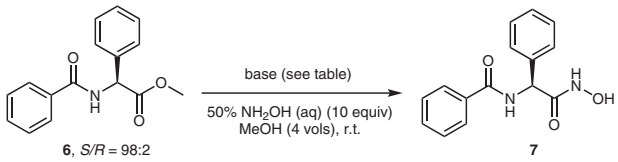
4, *S*/*R* = 99:1 **5**

Entry	Base (equiv)	Time (h)	Conv. (%)	Enantiomeric ratio
1	none	12	98	99:1
2	2N NaOH (aq) (3)	2	97	99:1
3	DBU (3)	0.5	95	99:1
4	TBD (3)	0.5	95	99:1
5	MTBD (3)	0.5	96	99:1
6	PS-DBU (3)[a]	1	93	99:1
7	PS-TBD (3)[a]	1	99	99:1

[a] Reaction carried out in the presence of 1 additional volume of CH_2Cl_2.

but with significant epimerization in each case (entries 2–5) compared with the control reaction (entry 1). As expected, the rate of hydroxylaminolysis decreased when using polymer-supported DBU or TBD but epimerization was also significantly reduced to just 20% (entries 3 and 4 vs. 9 and 6). Reducing the quantity of polymer-supported base in the case of TBD had little effect on the enantiomeric ratio and reduced significantly the reaction rate (entries 6–8). In the case of polymer-supported DBU, however, a significant change was observed using just 0.5 equiv, whereby a final enantiomeric ratio of 89:11 was achieved (entries 9–11).[11] As we anticipated that the rate of hydroxylaminolysis was much greater than the rate of epimerization, and epimerization was occurring through deprotonation of the benzylic proton of the starting ester (**6**), we reversed the order of addition and added the ester to the reaction mixture. To our satisfaction, the enantiomeric ratio was improved from 78:22 to 85:15 and 94:6 at r.t. and at 0 °C, respectively, by using a syringe pump (entry 6 vs. entries 12 and 13).[12]

Table 4 Racemization of **6** during the Hydroxylaminolysis Process



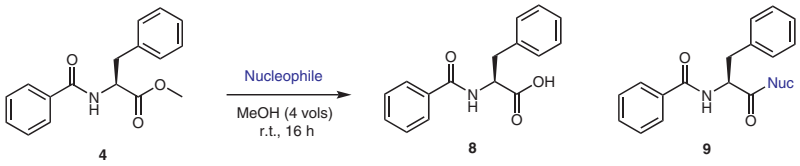
6, *S*/*R* = 98:2 **7**

Entry	Base	Equiv	Time	Conv. (%)[a]	Enantiomeric ratio
1	none	–	12 h	98	85:15
2	2N NaOH (aq)	3	2 h	97	59:41
3	DBU	3	<10 min	95	65:35
4	TBD	3	<10 min	99	54:46
5	MTBD	3	<10 min	96	54:46
6	PS-TBD	3	30 min	98	78:22
7	PS-TBD	1	1 h	97	81:19
8	PS-TBD	0.5	4 h	93	82:18
9	PS-DBU	3	30 min	93	78:22
10	PS-DBU	1	1 h	99	81:19
11	PS-DBU	0.5	4 h	88	89:11
12	PS-DBU[b]	3	20 min	88	85:15
13	PS-DBU[c]	3	1 h	91	94:6

[a] Based on LCMS analysis.
[b] Solution of **6** added at r.t. to a suspension of the resin and hydroxylamine over 2 min.
[c] Solution of **6** added at 0 °C *via* a syringe pump over 1 h.

Finally, we examined the use of other nucleophiles in the reaction to establish whether TBD could give access to simple amides under similar conditions directly from the carboxylic ester.[13] For this trial, we used the least hindered substrate, methyl benzoyl-L-phenylalaninate (**4**), as we anticipated that **1** would be far too hindered to observe any reaction. From Table 5, it is clear that there are significant advantages of having TBD present. Hydrazinolysis is significantly accelerated, as expected (entry 1 vs. 2), but TBD also significantly improved conversion to **9** for weaker nucleophiles such as piperidine (entries 3 vs. 4), *O*-benzylhydroxylamine (entries 5 vs. 6) and ammonia (entries 7 vs. 8), albeit with poor overall conversions and isolated yields.

Table 5 Use of TBD with Nucleophiles other than Hydroxamic Acid

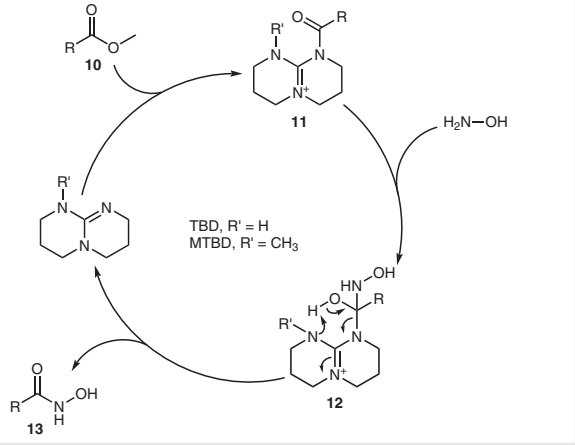


4 8 9

Entry	Nucleophile (equiv)	Time (h)	Ratio (%)[a]			Yield **9** (%)
			4	**8**	**9**	
1	hydrazine (10), TBD (2), MeOH	4	–	2	98	78
2	hydrazine (10), MeOH	18	2	5	92	–
3	piperidine (10), TBD (2), MeOH	8	44	15	41	35
4	piperidine (10), MeOH	8	88	12	–	–
5	*O*-benzylhydroxylamine (10), TBD (2), MeOH	16	39	26	35	23
6	*O*-benzylhydroxylamine (10), MeOH	16	91	8	1	–
7	NH_3 (7N in MeOH) (10), TBD (2), MeOH	24	44	20	36	31
8	NH_3 (7N in MeOH) (10), MeOH, r.t.	24	78	20	2	–

[a] Conversion determined by UPLC-MS.

Although we have not studied the mechanism in detail, we propose the possibility that TBD and MTBD act as nucleophilic acylation catalysts based on existing studies on DBU and TBD.[14] Nucleophilic attack of TBD or MTBD on the methyl ester **10** affords an activated intermediate **11**. Attack by hydroxylamine affords the tetrahedral intermediate **12**. Here, we speculate that the significant rate enhancement between DBU and TBD or MTBD could be explained by the presence of the extra nitrogen atom. We postulate that the nitrogen atom participates in the mechanism, accelerating the breakdown of **12** to the hydroxamic product **13** through intramolecular proton abstraction (Scheme 1).



Scheme 1 Proposed mechanism for the hydroxylaminolysis of esters using TBD or MTBD

In conclusion, we have extended our knowledge on superbase-mediated hydroxylaminolysis of esters and identified TBD and polymer-supported TBD and DBU as efficient bases for the formation of hydroxamic acids, hydrazides and, to some extent, amides. We also have shown that polymer-supported superbases can be employed for hydroxamic acid formation with little in situ racemization using substrates very prone to racemization.[15]

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Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/s-0036-1591551.

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(8) The iChem Explorer® is compatible with Agilent 1100 and 1200 HPLC systems. This heating/cooling stirring module allows the progress of reactions in up to 57 vials to be closely monitored during a single run (http://www.ichemexplorer.com/Articles/iChemExplorerFeatures.html).

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(10) Chiral HPLC conditions: PIC Solutions Chiral SFC; Column ID, 5 µm ×4.6×250 mm; $scCO_2$ / 25% *i*-PrOH; flow rate: 4 mL/min; wavelength: 210 nm; t_R = 3.4 (*R*), 4.5 (*S*) min.

(11) The authors recognize that a catalytic amount of base could be employed for this reaction, which is in accordance with the proposed mechanism. In the interest of our medicinal chemistry projects, whereby we were looking for high throughput process to prepare diverse libraries, we preferred to use an excess of superbase to assure rapid and complete hydroxylaminolysis.

(12) **Hydroxylaminolysis of methyl (*S*)-2-benzamido-2-phenylacetate (6); General Procedure**
To a stirred solution of 50 wt. % NH_2OH (aq) (216 µL, 3.53 mmol, 10 equiv) and the base (0.5–3 equiv) was added methyl benzoyl-D/L-phenylalaninate (100 mg, 0.35 mmol, 1 equiv) and the reaction mixture was stirred at r.t. until completion (<10 min to 12 h) and purified directly by mass-triggered preparative LCMS to afford *N*-(2-(hydroxyamino)-2-oxo-1-phenylethyl)benzamide (**7**; 90 mg, 90%) as a beige solid. LCMS (t_R = 1.01 min) purity: 100%; MS (ES^+): *m*/*z* = 285.04 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6): δ = 11.02 (s, 1 H), 9.01 (s, 1 H), 8.85 (d, *J* = 8.1 Hz, 1 H), 8.06–7.79 (m, 2 H), 7.62–7.20 (m, 9 H), 5.62 (d, *J* = 8.2 Hz, 1 H); ^{13}C NMR (101 MHz, DMSO): δ = 166.74, 166.27, 138.50, 133.91, 131.47, 128.30, 128.21, 127.79, 127.66, 127.45, 54.68.

(13) **Evaluation of TBD as an acylation catalyst with other nucleophiles; General Procedure**
To a stirred solution of nucleophile (10 equiv) and 1,5,7-triazabicyclo[4.4.0]dec-5-ene (3 equiv) (supported or in solution) in MeOH (4 vols) at r.t., was added methyl benzoyl-L-phenylalaninate (**4**; 50 mg, 0.18 mmol, 1 equiv) and the crude reaction mixture was stirred for 1–24 h and purified directly by mass-trigger Prep LCMS to obtain **9**.
(*S*)-*N*-(1-Hydrazineyl-1-oxo-3-phenylpropan-2-yl)benzamide: Yield: 40 mg (78%); LCMS (t_R = 0.87 min) purity: 100%; MS (ES^+): *m*/*z* = 284.02 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6): δ = 9.30 (s, 1 H), 8.56 (d, *J* = 8.5 Hz, 1 H), 7.85–7.72 (m, 2 H), 7.56–7.47 (m, 1 H), 7.47–7.39 (m, 2 H), 7.39–7.30 (m, 2 H), 7.26 (dd, *J* = 8.3, 6.7 Hz, 2 H), 7.20–7.11 (m, 1 H), 4.67 (td, *J* = 8.7, 6.0 Hz, 1 H), 3.07–2.98 (m, 2 H).
(*S*)-*N*-(1-Oxo-3-phenyl-1-(piperidin-1-yl)propan-2-yl)benzamide: Yield: 21 mg (35%); LCMS (t_R = 1.16 min) purity: 100%; MS (ES^+): *m*/*z* = 337.12 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6): δ = 8.75 (d, *J* = 8.2 Hz, 1 H), 7.78–7.87 (m, 2 H), 7.47–7.58 (m, 1 H), 7.21–7.34 (m, 4 H), 7.13–7.21 (m, 1 H), 5.12 (td, *J* = 8.3, 6.4 Hz, 1 H), 3.43 (dd, *J* = 6.8, 4.4 Hz, 4 H), 2.89–3.13 (m, 2 H), 1.13–1.63 (m, 6 H).
(*S*)-*N*-(1-((Benzyloxy)amino)-1-oxo-3-phenylpropan-2-yl)benzamide: Yield: 15 mg (23%); LCMS (t_R = 1.13 min) purity: 100%; MS (ES^+): *m*/*z* = 375.10 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6): δ = 11.41 (s, 1 H), 8.68 (d, *J* = 8.2 Hz, 1 H), 7.73–7.96 (m, 2 H), 7.07–7.62 (m, 14 H), 4.67–4.83 (m, 2 H), 4.56 (td, *J* = 8.7, 6.2 Hz, 1 H), 2.93–3.09 (m, 2 H).
(*S*)-*N*-(1-Amino-1-oxo-3-phenylpropan-2-yl)benzamide: Yield: 15 mg (31%); LCMS (t_R = 0.91 min) purity: 100%; MS (ES^+): *m*/*z* = 269.05 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6): δ = 8.49 (d, *J* = 8.5 Hz, 1 H), 7.74–7.83 (m, 2 H), 7.48–7.61 (m, 2 H), 7.40–7.47 (m, 2 H), 7.34 (d, *J* = 7.2 Hz, 2 H), 7.25 (t, *J* = 8.5 Hz, 1 H), 7.13–7.20 (m, 1 H), 7.11 (s, 1 H), 4.64 (dd, *J* = 8.5, 4.1 Hz, 1 H), 2.90–3.20 (m, 2 H).

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