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A Quantitative Assay of Sodium Triacetoxyborohydride

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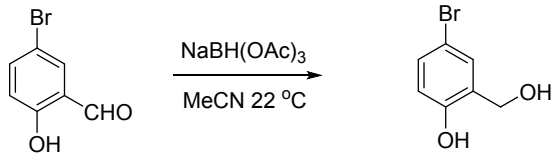


HPLCAssay yield of alcohol correlated to:
1) independent assay (H_2 evolution)
2) reductive amination yield

Abstract: Sodium triacetoxyborohydride (STAB) is a common reducing agent with potency that degrades over time and is not uniformly assigned. A simple assay has been developed to determine the active borohydride content of this reagent, based on an aldehyde reduction. The HPLC assay yield of a salicylaldehyde reduction has been shown to accurately determine this potency, and has been validated against the H_2 evolution method, as well as yields obtained from a reductive amination. Use of this assay data to adjust a STAB charge, as well to optimize a reductive amination, has been demonstrated.

Keywords: *sodium triacetoxyborohydride, potency assay, hydrogen evolution, reductive amination*

▪ INTRODUCTION

Sodium triacetoxyborohydride (STAB)[1] is a widely employed reducing agent for reductive amination reactions.[2] As a water- and air-sensitive reagent, the potency of a particular lot of reagent, once opened, is expected to gradually decrease over time. Accordingly, we have found the need to add more reagent to a given reaction at a fixed scale as the supply ages. A convenient assay for this reagent would alleviate the uncertainty associated with this charge.

An accurate potency measurement could have several advantages with respect to the large-scale use of STAB. Knowing the actual strength of the solid can help users predict an accurate minimal charge required for full conversion, as opposed to the "excess" typically recommended.[2] This has implications for the cost associated with the commercial reagent, as well as the thermal and H_2-evolution hazards that accompany quench of the excess charge. Furthermore, added control could be leveraged in cases where excess reagent is detrimental to batch performance due to either competing reactivity[3] or to product stability concerns in the presence of excess borate/borohydride salts.[4] Incorporation of a simple assay into acceptance criteria of a large-scale supply prior to use would also help ensure reagent quality under GMP conditions.

During a recent API synthesis campaign, we discovered that a 50 kg batch of STAB that was purchased based on a listed specification of 97% was in fact significantly less potent, at ~67%.[5] A subsequent survey of numerous suppliers revealed specifications of ≥80% or 95-97% purity based on "titration." Further investigation revealed a significant lack of information in many cases as to the type of titration used or the associated procedure, and in those cases where information was available, the titration method varied from vendor to vendor and few, if any, further details were available.[6]

STAB is prepared by the addition of solid $NaBH_4$ to a solution of AcOH in a solvent (typically DMAc or toluene), followed by filtration of the resulting solids.[7] The purity of the isolated solid depends on the efficiency of the reaction itself, as well as the washing and drying protocols. Our experiences suggest that purity assays based on titration do not necessarily correlate to the performance of the reagent during reductive amination reactions. For example, acid/base titration results reported "as boric acid" or "as acetic acid" do not specifically indicate the wt% of the active borohydride species. Additionally, the reactivity of the solid towards air and moisture necessitates periodic re-assignment of the potency (wt%) with use and/or sampling.[8]

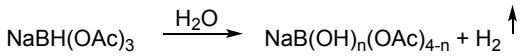
There are no known reports in the literature with regards to an analytical assay for STAB. By contrast, significant work has been reported regarding assay determination of sodium borohyride ($NaBH_4$),[9] and a wide variety of methods have been developed. These include, but are not limited to, H_2 evolution, quantitative FTIR, aqueous acid/base titrations, and the aqueous iodate method. We anticipated that adaptation of these assays would be complicated by the significant difference in reactivity between $NaBH_4$ and STAB and by the instability of the reagent to aqueous conditions.[10] Furthermore, our attempts to develop direct analytical techniques- such as elemental analysis and quantitative ^1H NMR- were unsuccessful.[11]

We therefore sought to develop an indirect assay. Specifically, we envisioned an aldehyde reduction as a measure of STAB potency (Scheme 1) whereby an alcohol is formed[12] and the yield of this reaction reflects the amount of active STAB that was present in the added sample. In principle, a reductive amination use test would also be useful, but we envisioned an independent assay that isolates the influence of a particular batch of STAB on a reductive amination.[13] Our requirements were that the reaction was high yielding and used inexpensive, readily available materials with no special equipment beyond an HPLC. We report herein the successful realization

of this goal, compare the results to the H_2 evolution standard, and demonstrate the applicability of STAB assay results towards a reductive amination.

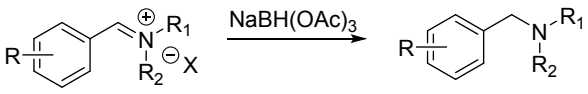
Scheme 1: Approaches to the measurement of STAB borohydride potency

a) H_2 evolution assay



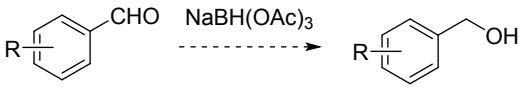
measure of borohydride wt%: detection of H_2

b) Reductive amination use test assay



measure of borohydride wt%: assay yield of amine

c) Aldehyde reduction assay (this work)

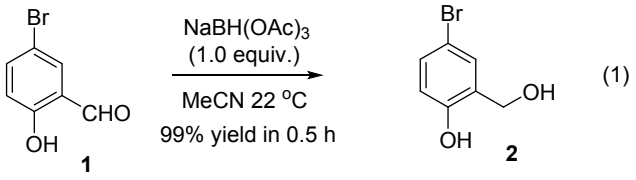


measure of borohydride wt%: assay yield of alcohol

We focused on aromatic aldehydes, since the chromophores would enable assay yield determination through quantitative HPLC analysis. STAB has been reported to reduce aldehydes, but closer examination of the data reveals that a large excess is typically required and often under forcing conditions.[1b] We confirmed these reports by observing inefficient reduction of simple benzaldehyde derivatives.[14, 15] The requirement for excess reducing agent complicated attempts to quantify the potency of the reagent, since inefficient reactivity needed to be decoupled from active borohydride content.

By contrast, we found that salicylaldehyde derivatives react efficiently with STAB (eq. 1). One such compound, 5-bromosalicylaldehyde (**1**), is readily available in high purity (99%) as an

easily handled solid from numerous commercial suppliers at a low cost. The product **2** is also commercially available as a high purity solid (>98%), making analytical standard preparation straightforward. Therefore, we chose this reaction for assay development.



(1)

▪ RESULTS AND DISCUSSION

H_2 evolution studies as a benchmark for the aldehyde reduction assay. We initially assigned potency to various batches based on the amount of H_2 evolved when the STAB was exposed to water. The H_2 evolution method has been cited as a common method for analysis of $NaBH_4$,[9] though this method requires a complex and potentially expensive apparatus.[9, 16] Towards this end, we charged an RC-1 calorimeter with solid STAB from multiple manufacturers and of various ages, all of which were received with a CoA indicating 95-97% purity, followed by a fixed amount of H_2O. We elected to measure the pressure increase in a closed system, while accounting for reactor volume, vapor pressure, and the solubility of H_2 in water. The temperature of the system was maintained at 25°C by the circulating jacket on the vessel. The volume of the headspace was found by subtracting the volume of water (100 mL) in the system from the total volume of the system (determined to be 1250 mL). The final vessel pressure from the experiments was corrected to the partial pressure of hydrogen by subtracting the vapor pressure of water at 25 °C (0.0317 bar) and the pressure generated by dosing water into the closed empty system (0.0545 bar).[17] With this temperature, headspace volume and partial pressure of hydrogen, the moles of hydrogen gas

generated in the decomposition were calculated according to equation 2.[18] The solubility of hydrogen in water at 25°C and the pressures observed in these experiments was calculated according to Henry's law and found to be very low (approximately 0.0008 mol/kg), so the amount of hydrogen dissolved in the aqueous solution was ignored and it was assumed that all hydrogen generated by the decomposition of STAB is present in the gas phase.[17] The results are presented in Table 1.

$$P_{Hydrogen} = P_{System} - P_{Water,25°C} - P_{Water\ Addition} \quad (2)$$

Table 1. H_2 evolution experiments

entry	g STAB	mmol[a]	P(sys)[b]	P_{H_2}[c]
1	10.08	47.6	1.07	0.98
2	10.06	47.5	0.90	0.81
3	10.29	48.6	0.83	0.74
4	10.08	47.6	0.93	0.84
5	10.58	49.9	0.79	0.70

[a] mmol *uncorrected*. [b] Final pressure of the system (bar).
[c] Calculated partial pressure of H_2 (bar).

Using the ideal gas law, we calculated the mmol of H_2 liberated during these experiments. The potency (wt%) of STAB was calculated as the mmol of H_2 evolved divided by the theoretical mmol based on an assumed 100 wt%. The results are presented in Table 2.

$$Moles_{Hydrogen} = \frac{Volume_{Headspace} * Pressure_{Hydrogen}}{R * Temperature} \quad (3)$$

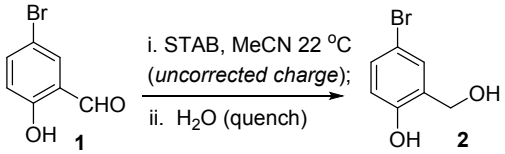
$$Potency\ of\ STAB = \frac{mmol_{Hydrogen}}{mmol_{STAB}(uncorrected)} \quad (4)$$

Table 2. Calculation of STAB active borohydride content (potency/wt%) based on H_2 data

entry	mmol (H_2)	potency (wt%)
1	45.5	95.6
2	37.6	79.2
3	34.3	70.6
4	39.0	81.9
5	32.5	65.1

Comparison of H_2 evolution data to the assay yield of the reduction of aldehyde 1. In parallel, we assigned a potency to the same batches of STAB used in Table 1 based on the reduction of **1** to **2**. STAB (1.0 equiv. *uncorrected* for wt% potency) was added to a MeCN solution of **1** (1.0 to 1.1 equiv.), and after ≥30 min., the reaction was quenched with H_2O and the resulting homogeneous solution was diluted in a volumetric flask and directly assayed using HPLC (see Experimental Section for details). The measured assay yield of **2** (in mmol) was divided by the theoretical mmol based on an assumed 100 wt% of STAB, and the resulting number was assigned as the potency of STAB. The results are presented in Table 3.

Table 3. STAB potency (wt%) assigned by the reduction of 1.

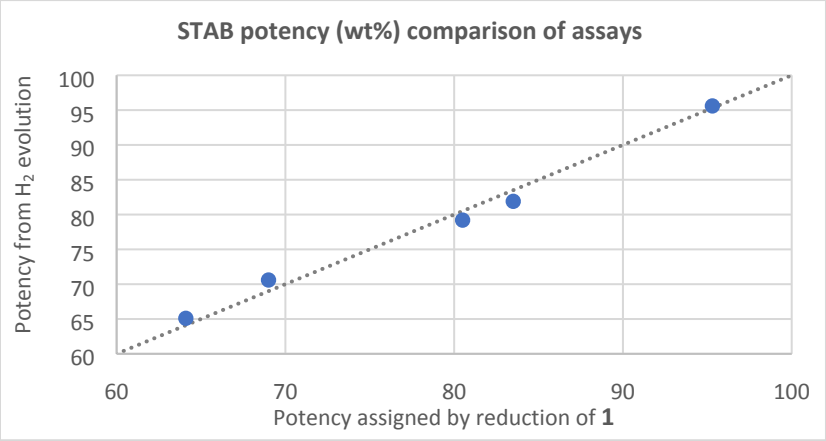


entry	equiv. **1**	% assay yield of **2**
1	1.1	95.3
2	1.1	80.5
3	1.1	69.0
4	1.1	83.5
5	1.1	64.1

Conditions: **1** (1.1 equiv. as a >99% solid**),** MeCN (18 x vol), then STAB (1.0 equiv. *uncorrected*)

By plotting the results in Table 2 vs. Table 3, we observed that the data fit well with the graph of perfect correlation (y = x, dashed line). The calculated $R^2 = 0.98$ gave us confidence that the assay yield of **2** was a competent measure of the potency of a particular batch of STAB (Figure 1). We have found that measured assay yield of the reduction of **1** to **2** was the same in DMAc, THF and MeCN, though the reaction was fastest in MeCN. There was no difference observed between adding STAB in a single portion or portion-wise. This procedure is convenient in that direct assay of the crude reaction was utilized, avoiding any workup procedure.

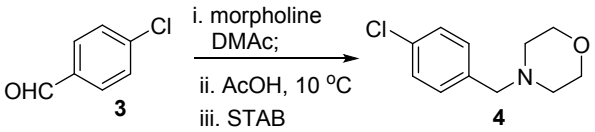
Figure 1: STAB potency comparison between H_2 evolution and reduction of 1



Comparison of reductive amination yield to the assay yield of the reduction of aldehyde 1. We further interrogated the validity of this assay by comparing the potency assigned by the salicylaldehyde reduction to that determined from the yield of a reductive amination. Towards this end, we subjected an iminium species (formed *in situ*) to a small excess (1.13 equiv., *vide infra*) *uncorrected* charge of STAB (Table 4). The batches of STAB were different from those represented in Table 1.[19] We selected DMAc as the solvent for these studies since it was the preferred solvent for our API- related process. The STAB supplies were also assayed using DMAc.

The data showed that the assay yield of **6** correlated well to the potency of the STAB as assigned by the reduction of **1**.

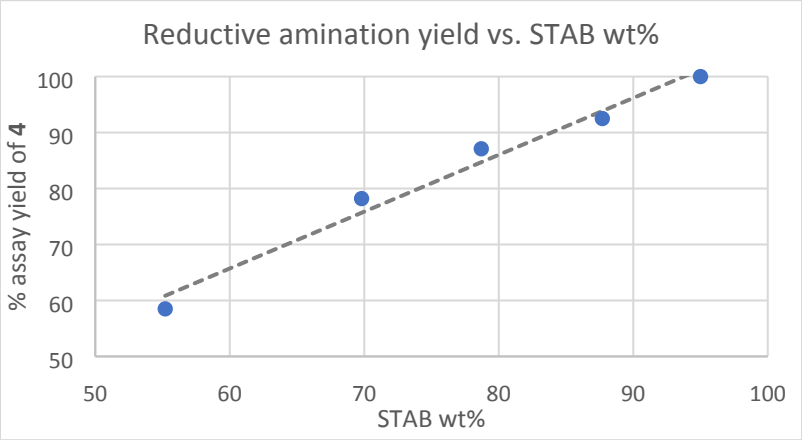
Table 4: Reductive amination yield compared to STAB potency (wt%)



entry	% assay yield of **4**	wt% of STAB
1	100	95.0
2	92.5	87.7
3	58.5	55.2
4	78.2	69.8
5	87.1	78.7

Conditions: **3**. 1.08 equiv. morpholine, DMAc (7.7 x vol), AcOH (1.65 equiv.), STAB (1.13 equiv. *uncorrected*)

Figure 2: STAB potency compared to reductive amination assay yield.

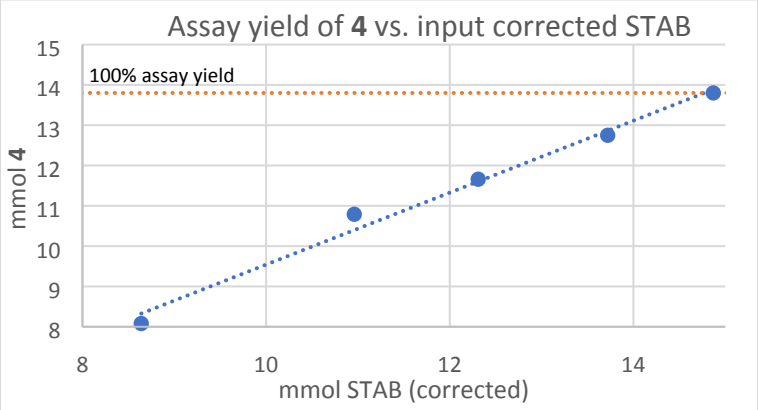


The choice of 1.13 equivalents of STAB (as an *uncorrected* charge) reflects the observation from our experience that some degree of STAB decomposition occurs over the course of the reaction. This may be due at least in part to the equivalent of water produced as a result of iminium formation. The reaction temperature addressed this, as we have found that the reaction of STAB

with water is significantly slower at 0 ˚C than at ambient temperature.[20] Nonetheless, the range of STAB potencies explored, regardless of the fixed uncorrected charge, ensured that a wide range of stoichiometries was explored. The slope of ~1.02 for a linear best fit reinforces the conclusion that the assay yield of the reductive amination for a fixed charge of STAB (*uncorrected for wt%*) reflects the potency of the reagent.[21]

Optimization of a reductive amination using STAB potency (wt%) and a wt%-adjusted charge. Plotting the data from Table 4 to reflect the yield of **4** (in mmol) as a function of the *corrected actual* STAB charge (in mmol), the linear best fit equation allowed us to predict the minimum STAB charge required to achieve 99% assay yield (Figure 3). For a reaction employing 2.0 g of **3** (14.23 mmol), we calculated that 15.08 mmol (1.06 equivalents) of wt% corrected- STAB was required for this reaction under these conditions to meet this target yield (14.08 mmol).[22]

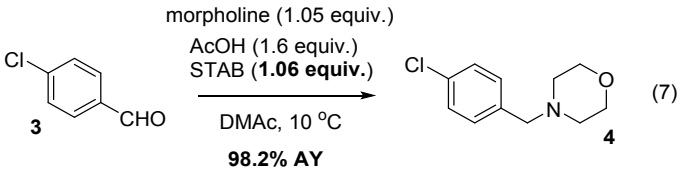
Figure 3: Assay yield (in mmol) of 6 compared to corrected input STAB charge (in mmol)



$$\text{x (mmol STAB } \textit{uncorrected}) = \frac{mmol\ \mathbf{4} - 0.6178}{0.8924} = 15.08 \text{ mmol (for 14.08 mmol of } \mathbf{4}) \quad (5)$$

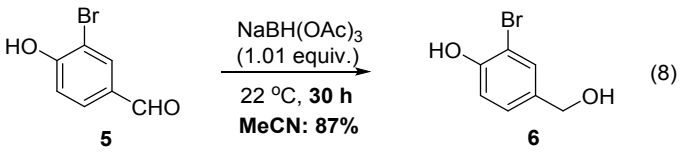
$$\text{charge of STAB, } \textit{corrected} = \frac{(mmol\ STAB)(MW\ STAB)}{potency\ (wt\%)} \quad (6)$$

We elected to demonstrate this concept using a supply of STAB that assayed at 84.0 wt%. This was the same STAB source used for entry 2 in Table 4, re-assayed after an additional 2 months of use. Solving for y = 14.08 mmol (99% of 14.23 mmol), 1.06 equivalents corresponded to a STAB charge of 3.80 g. When we performed that experiment, we observed >99% conversion of **3** and obtained **4** in 98.2 % assay yield (eq. 7).[23] The yield was nearly identical to that obtained using a higher *corrected* charge (1.2 equiv.) with higher wt% STAB (96.0 wt%; see Experimental Section). This represents a significant reduction in the STAB charge beyond "excess" or the suggested minimal 1.4-1.5 equivalents.[2]



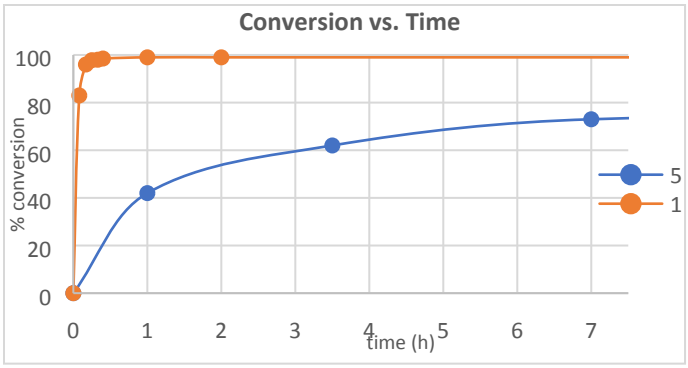
(7)

Mechanistic considerations. The facile reduction of **1** appears to be due to the phenol moiety. Furthermore, the *ortho*-relationship of the phenol to the aldehyde is important. For example, the isomeric 2-bromo-4-hydroxybenzaldehyde (**5**) reacted slowly with STAB under otherwise identical conditions (eq. 8). Figure 4 illustrates the different conversion kinetics of aldehydes **1** and **5** under otherwise identical conditions. Use of 1.00-1.01 equiv. of STAB (*corrected charge*) reduced **1** to **2** within 30 min., while the reduction of **5** to **6** required 30 h to reach 87% conversion.[24]

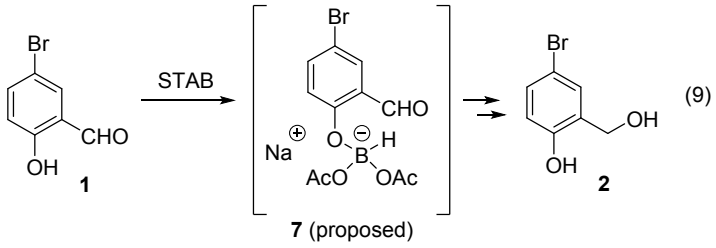


(8)

Figure 4: Conversion profiles of 1 and 5



This difference in reactivity may result from an intermediate mixed borohydride **7** as an active intermediate in the case of **1** that facilitates the reduction (eq. 9). Support for this proposal was secured when ^{1}H NMR experiments revealed phenol/acetic acid exchange when STAB and phenol are present in solution[25] and when a new signal was detected in the ^{11}B NMR spectrum upon addition of phenol to STAB.[26] Furthermore, a ^{1}H NMR spectrum acquired when STAB was added to a solution of **5** in CD_3CN showed a splitting of aromatic and aldehydic peaks, as well as free AcOH, suggestive of a borate analogous to **7**.



▪ CONCLUSION

In conclusion, we have developed a rapid, accurate, and predictive assay to determine the potency (wt%) of STAB. The HPLC assay yield of a salicyladehyde reduction quantifies the active borohyrdride content of a sample of the reagent. This assay was validated by comparison to H_2 evolution data obtained from the aqueous decomposition of STAB, as well as by data obtained from a reductive amination reaction. The utility of the assay data was demonstrated by accurately

predicting the minimum charge of STAB for a given reductive amination required for nearly quantitative conversion. As direct measurements and color-indicating titrations[27] are not yet viable, this assay can help guide users to quickly and easily ascertain the potency of STAB supplies in their labs.

▪ EXPERIMENTAL SECTION

General. Reagents and solvents were obtained from commercial sources and were used as received. Aldehydes **1, 3,** and **5**, and alcohols **2** and **6** were purchased from commercial sources as solids and were used as received. **1** was assayed by the vendor and labeled as 99% pure; **2** was assayed by the vendor and labeled as ≥98% pure; **3** was assayed by the vendor and labeled as 97% pure; **5** was assayed by the vendor and labeled as 97% pure; **6** was assayed by the vendor and labeled as 98% pure. HPLC area under the curve (AUC) for each compound was as follows: 99.8% of **1**, 100% of **2**, 97% of **3**, 97% of **5**, and 98% of **6**. The HPLC purities were used as working purities. Unless otherwise noted, the charges described below are the purity-adjusted amount $\left[(actual\ charge) * \frac{purity\%}{100}\right]$.

^{1}H NMR spectra were recorded using a 300 or 500 MHz Bruker Avance spectrometer, using the d_6-dmso resonance (2.50 ppm) as an internal standard. ^{13}C NMR spectra were recorded on a 125 MHz Bruker spectrometer using the d_6-dmso resonance (39.5) ppm as an internal standard measured. High-resolution mass spectrometry (HRMS) was performed using a HPLC-TOFMS mass spectrometer in electrospray ionization (ESI) mode. Melting points were recorded on a TA Instruments Q1000 system which applied a heating ramp of 10 ˚C/min from 30 to 320 ˚C. Melting points are recorded as peak temperatures.

All manipulations were carried out under a nitrogen atmosphere. STAB samples were weighed in a ventilated balance enclosure), then added without delay to reaction solutions under positive N_2 pressure. HPLC assays were performed using a method with the following conditions: Ascentis Express C18 column; 4.6 mm × 100 mm, 2.7 μm particle size, 40 °C, flow rate of 1.5 mL/min consisting of a mobile phase comprised of MeCN and 0.1% by volume aqueous H_3PO_4; gradient 10% MeCN ramp to 95% MeCN over 5 min, then isocratic 95% MeCN for 2 min, with integration based on spectra recorded at the 210 nm wavelength. Assay yields were determined by quantitative dilution of the crude product stream to a known volume, followed by subjection to HPLC analysis. Comparison of the area under the curve of the diluted sample to that of a sample of known concentration prepared from the analytically pure authentic product allowed calculation of the total amount of the desired product. Alternatively, when the product was isolated via crystallization, the assay yield was determined by combining the mass of the analytically pure isolate with the desired product lost to the combined liquors (filtered supernatant plus washes). The loss was determined as described above for the assay yield, utilizing a standard prepared from the isolate.

H_2 evolution studies: Experiments using the selected method to measure hydrogen gas generation were carried out on the Mettler Toledo RC1mx instrument in a MP10-1.0-RTC glass pressure reactor. This system was equipped with a LEO 3 digital pressure gauge, to measure and record the pressure in the reactor, and a gravimetric dosing setup that can dose liquids into a closed system under pressure. This gravimetric dosing system was set up on the RC1mx with a ProMinent Beta 4 dosing pump and Mettler Toledo XS4002S balance.

The reactor atmosphere was purged three times with vacuum, followed by N_2 backfill. Under a positive pressure of N_2, STAB (~10 g) was charged as a solid (see Table 1). The reactor

was sealed under N_2 at atmospheric pressure, and the cooling fluid in the vessel jacket was circulated at 25 ˚C. Water (100 mL) was added via the dosing pump over 8 minutes, and the pressure was recorded over 20 minutes (the pressure increase was verified to be complete by the end of the water addition).

*Table 3 assay of STAB via reduction of **1** to 4-bromo-2-(hydroxymethyl)phenol (**2**)*. 5-Bromo-salicylaldehyde **1** (1.10 g, 5.5 mmol) was dissolved in MeCN (20 mL) at T_i = 20-25 ˚C. STAB (1.06 g, 5.0 mmol *uncorrected*) was added under mild positive N_2 pressure, and the resulting slurry was agitated for at least 30 min. *Note: a mild, delayed exotherm of ≤ 5 ˚C was typically observed on this scale without external cooling.* The reaction slurry was quenched with H_2O (20 mL). The resulting homogeneous solution was transferred to a 50 mL volumetric flask, diluting with 1:1 v:v MeCN:H_2O to 50 mL total volume. A 50x dilution sample was prepared and analyzed according to the described HPLC method. Comparison of the area under the curve (AUC) to that of a previously prepared analytical standard commercially available **2** allowed for determination of the assay yield of **2** (analytical standard solution prepared was 40.30 mg dissolved in 100 mL of 1:1 v:v MeCN:H_2O). At this scale, the theoretical yield of **2**, corresponding to 5.0 mmol, was 1.015 g.

$$assay\ yield\ of\ \mathbf{2}\ (in\ mg) = \left[\frac{area\ sample}{area\ standard}\right] * \left[\frac{mg}{mL} of\ standard\right] * \left[\frac{50\ mL}{mL\ rxn}\right] * 50\ mL\ rxn$$

$$STAB\ wt\% = \frac{mg\ \mathbf{2}\ (by\ assay)}{1015\ mg\ theoretical\ yield\ \mathbf{2}}$$

Entry 1, Table 3: AUC analytical standard (40.30 mg/mL) was 1113574. AUC for 2 in assay sample was 1068246. Using the equations above, this corresponded to 967 mg of **2**.

Entry 2, Table 3: AUC analytical standard (40.30 mg/mL) was 1113574. AUC for 2 in assay sample was 903557. Using the equations above, this corresponded to 817 mg of **2**.

Entry 3, Table 3: AUC analytical standard (40.30 mg/mL) was 1113574. AUC for 2 in assay sample was 773607. Using the equations above, this corresponded to 700 mg of **2**.

Entry 4, Table 3: AUC analytical standard (40.30 mg/mL) was 1113574. AUC for 2 in assay sample was 937034. Using the equations above, this corresponded to 848 mg of **2**.

Entry 5, Table 3: AUC analytical standard (40.30 mg/mL) was 1113574. AUC for 2 in assay sample was 719878. Using the equations above, this corresponded to 651 mg of **2**.

*Preparation of an analytical standard of N-(4-chlorobenzyl)morpholine (**4**).* A 250 mL jacketed vessel was charged with 4-chloro-benzaldehyde (10.0 g, 71.14 mmol) and DMAc (40.0 mL). The resulting solution was agitated ambient temperature under an atmosphere of N_2. Morpholine (6.50 g, 6.53 mL, 74.70 mmol) was added and the resulting mixture was aged for 1 h. *Note: the addition of morpholine was mildly exothermic.* After 1 h, the mixture was cooled to T_i = 10 °C, and AcOH (6.84 g, 6.51 mL, 113.82 mmol) was added, followed by two portions of STAB (2 x 9.45 g of 96.0 wt% solid, separated by 45 minutes) under positive N_2 pressure. The resulting slurry was agitated for 16-18 h at T_i = 10 °C. The reaction was quenched with H_2O (40 mL) at T_i = 10 °C, forming a slurry in which a portion of the product had crystallized. The slurry was warmed to T_i = 20 °C, followed by the addition of 20 wt% aq. K_2CO_3 (80 mL) over 1 h. The resulting slurry was agitated for an additional hour, and then was filtered. The cake was displacement-washed with 1:3 v:v DMAc:H_2O (40 mL), followed by H_2O (40 mL). The cake was dried via vacuum/N_2 sweep to afford 14.43 g of **4** as a white solid. The combined filtrates were assayed for 0.41 g of **4**. This corresponded to 96% isolated yield and 98.5% assay yield of **4**. Mp = 66.0 ˚C (DSC); ^{1}H NMR (500 MHz, d_6-DMSO): δ 7.40-7.35 (m, 2H), 7.43-7.30 (m, 2H), 3.60-3.53 (m, 4H), 3.46-3.43-7.30 (s, 2H), 2.37-2.30 (m, 4H); ^{13}C{^{1}H} NMR (125 MHz, d_6-dmso): δ 137.4, 132.0, 131.1, 128.6, 62.0,

53.6; HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ calc'd for $C_{11}H_{15}ClNO$ 212.0837; found 212.0843. This was consistent with published data.[28]

*Table 4 assay of STAB via reduction of **1** to 4-bromo-2-(hydroxymethyl)phenol (**2**)*. 5-Bromo-salicylaldehyde **1** (5.0 mmol, 1.0 g) was dissolved in DMAc (10 mL), to which was added STAB (5.0 mmol, 1.06 g uncorrected, or based on a theoretical assay of 100 wt%) in a single portion under positive N_2 pressure. After 3 h, the reaction was quenched with H_2O (5 mL), then diluted with MeCN to 25 mL total volume in a volumetric flask. A 100x dilution sample was prepared and analyzed according to the described HPLC method. Comparison of the area under the curve (AUC) to that of a previously prepared analytical standard from isolated material allowed for determination of the assay yield of **2**.

Entry 1, Table 4: AUC analytical standard (40.30 mg/mL) was 1142537. AUC for **2** in assay sample was 1093024. Using the equations above, this corresponded to 964 mg of **2**.

Entry 2, Table 4: AUC analytical standard (40.30 mg/mL) was 1142537. AUC for **2** in assay sample was 1008704. Using the equations above, this corresponded to 817 mg of **2**.

Entry 3, Table 4: AUC analytical standard (40.30 mg/mL) was 1142537. AUC for **2** in assay sample was 626983. Using the equations above, this corresponded to 553 mg of **2**.

Entry 4, Table 4: AUC analytical standard (40.30 mg/mL) was 1142537. AUC for **2** in assay sample was 821870. Using the equations above, this corresponded to 709 mg of **2**.

Entry 5, Table 4: AUC analytical standard (40.30 mg/mL) was 1142537. AUC for **2** in assay sample was 925984. Using the equations above, this corresponded to 799 mg of **2**.

*Table 4 assay yield determination of N-(4-chlorobenzyl)morpholine **4***. A 50 mL EasyMax vessel was charged with 4-chloro-benzaldehyde (1.94 g, 13.80 mmol) and DMAc (10.0 mL). The resulting solution was agitated at 300-500 rpm at ambient temperature under an atmosphere of N_2.

Morpholine (1.30 g, 1.31 mL, 14.97 mmol) was added, resulting in a mild exotherm. After 1 h, the mixture was cooled to T_i = 10 °C over 10 minutes. AcOH (1.37 g, 1.30 mL, 22.77 mmol) was added, followed by three equal portions of STAB (3 x 1.11 g *uncorrected*), under positive N_2 pressure and separated by 30 minutes each. The resulting slurry was agitated for 16-18 h at T_i = 10 °C. The solution was then warmed to T_i = 20-22 °C over 10 minutes. The reaction was quenched with H_2O (10 mL), and the resulting solution was transferred to a 25 mL volumetric flask, rinsing and diluting with MeCN to a total volume of 25 mL. A 1.0 mL aliquot of the resulting homogeneous solution was diluted to 200 mL or 250 mL with 1:1 v:v MeCN:H_2O and analyzed according to the described HPLC method. Comparison of the area under the curve (AUC) to that of a previously prepared analytical standard from isolated material allowed for determination of the assay yield of **4**.

$$assay\ yield\ of\ \mathbf{4}\ (in\ mg) = \left[\frac{area\ sample}{area\ standard}\right] * \left[\frac{mg}{mL} of\ standard\right] * [dilution\ factor] * 25\ mL\ rxn$$

$$assay\ yield\ of\ \mathbf{4} = \frac{g\ \mathbf{4}\ (by\ assay)}{2.92\ g\ theoretical\ yield\ \mathbf{4}}$$

Entry 1, Table 4: AUC analytical standard (40.07 mg/mL) was 1034537. AUC for **4** in assay sample was 1210823, (250 x sample dilution factor). Using the equations above, this corresponded to 2.92 g of **4**.

Entry 2, Table 4: AUC analytical standard (40.07 mg/mL) was 1034537. AUC for **4** in assay sample was 1115268, (250 x sample dilution factor). Using the equations above, this corresponded to 2.70 g of **4**.

Entry 3, Table 4: AUC analytical standard (40.03 mg/mL) was 1043300. AUC for **4** in assay sample was 708271, (250 x sample dilution factor). Using the equations above, this corresponded to 1.70 g of **4**.

Entry 4, Table 4: AUC analytical standard (40.03 mg/mL) was 1043300. AUC for **4** in assay sample was 1189743, (200 x sample dilution factor). Using the equations above, this corresponded to 2.28 g of **4**.

Entry 5, Table 4: AUC analytical standard (40.03 mg/mL) was 1043300. AUC for **4** in assay sample was 1324739, (200 x sample dilution factor). Using the equations above, this corresponded to 2.54 g of **4**.

*Optimized synthesis of N-(4-chlorobenzyl)morpholine **4** (eq. 7)*. A 50 mL EasyMax vessel was charged with 4-chlorobenzaldehyde (2.0 g, 14.23 mmol) and DMAc (10 mL). The resulting solution was stirred under a nitrogen atmosphere at T_i = 20-22 ˚C. Morpholine (1.31 mL, 14.94 mmol) was added, resulting in a mild exotherm (3-5 ˚C). After 1 h, the solution was cooled to T_i = 10 °C over 10 minutes. AcOH (1.30 mL, 22.77 mmol) was added, followed by STAB (3 x 1.27 g, under positive N_2 pressure and separated by 30 minutes). The resulting slurry was agitated for 16-18 h at T_i = 10 °C. The solution was then warmed to T_i = 20-22 °C over 10 minutes. The reaction was quenched with H_2O (10 mL), followed by MeCN (10 mL) which was added to render the solution homogeneous. The crude reaction mixture was transferred to a 100 mL volumetric flask and diluted with 1:1 (v:v) MeCN:H_2O to 100 mL total volume. A 100x double dilution sample was prepared and assayed for 2.95 g desired product (98.2 % AY).

*Comparison of conversion profiles- Reduction of **1*** (*Figure 4 and eq. 1*): The experiment was performed with STAB that assayed at 92.5 wt%. A 50 mL three neck flask equipped with a stir bar and a N_2 inlet was charged with **1** (1015 mg , 5.05 mmol) and MeCN (20 mL, KF <100 ppm). The resulting homogeneous solution was stirred. Under a slight positive pressure, STAB (1160 mg total, *1.06 g corrected, 5.06 mmol corrected*) was added as a solid in a single portion. Assays were withdrawn at 5- minute intervals up to 25 minutes, then at 1 h and 2 h. After 2 h, the reaction slurry was quenched with H_2O (20 mL). The resulting homogeneous solution was transferred to a 50 mL

volumetric flask, diluting with 1:1 v:v MeCN:H_2O to 50 mL total volume. A 50x dilution sample was prepared and analyzed according to the described HPLC method. Calculation of the assay yield, as described above, resulted in an assay yield of 1012 mg, corresponding to 99% assay yield (AUC for 39.80 mg/100 mL **2** was 1127242, AUC of **2** in assay sample was 1146868).

The concentration of each component at the indicated time points was calculated using analytical standards prepared from commercial sources. The mmol of each component in the HPLC sample (not standardized) was calculated as follows:

$$mmol = \left[\frac{area\ sample}{area\ standard}\right] * \left[\frac{mg}{mL} standard\right] * [standard\ purity\%] * [\frac{1}{MW}]$$

The conversion was calculated as follows (in the case of **1**→ **2**):

$$conversion\% = \left[\frac{mmol\ \mathbf{2}}{mmol\ \mathbf{1} + mmol\ \mathbf{2}}\right] * 100$$

The standard solutions were assayed as follows (concentration reported here accounts for the charge of the solid; subsequent calculations account for purity in the actual calculations of mmol per sample):

AUC standard of **1** (37.76/100 mL): 2295432

AUC standard of **2** (39.80 mg/100 mL): 1127242

Reduction of **1**, AUC **1**: 257047 at 5 min., 16652 at 10 min., 19275 at 15 min., 12226 at 20 min., 17573 at 25 min., 13821 at 60 min.

Reduction of **1**, AUC **2**: 432966 at 5 min., 194005 at 10 min., 393306 at 15 min., 280038 at 20 min., 534189 at 25 min., 470549 at 60 min.

*Comparison of conversion profiles- Reduction of **5*** (*Figure 4*): The experiment was performed with STAB that assayed at 94.6 wt%. A 25 mL round bottom flask equipped with a stir bar and under a N_2 atmosphere was charged with **5** (2.0 mmol, 402 mg as a wt%-adjusted charge described

above) and MeCN (8 mL). STAB (2.02 mmol, 452 mg of 94.6% reagent) was charged in a single portion. Samples were withdrawn at the indicated time points and profiled using the HPLC method described above. The concentration of each component was calculated using analytical standards prepared from commercial sources.

AUC standard of **5** (19.86 mg/50 mL): 1724140

AUC standard of **6** (21.61 mg/50 mL): 1646468

Reduction of **5**, AUC **5**: 371946 at 1 h, 358215 at 3.5 h, 134702 at 7 h, 191522 at 23 h, 267342 at 30 h.

Reduction of **5**, AUC **6**: 235398 at 1 h, 513226 at 3.5 h, 324392 at 7 h, 714545 at 23 h, 1375476 at 30 h.

^{1}H NMR experiment: STAB and phenol in DMF-d_4. STAB (50 mg, 0.234 mmol) was dissolved in DMF-d_4 (3 mL) and a ^{1}H NMR spectrum was obtained. Phenol (18 mg, 0.191 mmol) was added, and after 12 h, the ^{1}H NMR spectrum was acquired. The latter spectrum showed a marked increase in the singlet resonance at 1.95 ppm, which was consistent with AcOH (measured at 1.98 ppm in DMF-d_4). Phenyl acetate, if formed, was predicted to have an acetate CH_3 singlet at 2.2 to 2.5 ppm but was not detected.[29]

*^{1}H NMR experiment: STAB and **5** in CD_3CN*. A mixture of **5** (38 mg, 0.191 mg) and CD_3CN (1 mL) was prepared, to which was added STAB (50 mg, 2.234 mmol). After 1 h, the product was observed (singlet at 4.53 ppm corresponding to the benzylic CH_2), along with two aldehyde resonances and splitting of the aromatic peaks.

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Supporting Information:

^{1}H and ^{13}C spectra for **4**, ^{1}H NMR studies spectra, H_2 evolution graphs, and discussion of unsuccessful alternative approaches. This material is available free of charge via the Internet at http://pubs.acs.org.

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[3] Gribble, G. W.; Abdel-Magid, A. F. Sodium Triacetoxyborohydride in the Encyclopedia of Reagents for Organic Synthesis, 2007. http://dx.doi.org/10

[4] Product instability in the presence of excess STAB was the genesis of this work. The details will be the subject of a forthcoming paper.

[5] The assay was initially assigned based on the amount of excess reagent required for full conversion in a reductive amination reaction compared to a batch from a different (more established) supplier whose stated purity was justifiably

assigned (based on performance) as a working standard consistent with 97%. The use-test assay described below subsequently confirmed this number.

[6] When we contacted vendors regarding specifics, we were unable to obtain any information prior to purchasing material. The subsequent details revealed that some titration procedures were rather complicated and subject to inaccuracies (large error bars).

[7] Lam, T. T.; Bagner, C.; Tuma, L. Application of thermal analytical techniques in development of a safe and robust process for production of triacetoxyborohydride (STAB). *Thermochimica Acta* **2005**, *426*, 109-113.

[8] This is similar to organometallic reagents such as Grignard or organolithium species.

[9] For a recent review, see: Šljukić, B.; Santos, D. M. F.; Sequeira, C. A. C.; Bank, C. E. Analytical monitoring of sodium borohydride. *Anal. Methods*, **2013**, *5*, 829-839.

[10] The intrinsic instability of STAB under aqueous conditions is expected to lead to inaccurate results. Regarding acid/base titration, decomposition of STAB by aqueous acid results in the formation of acetic acid and boric acid (in addition toH_2) and the assay does not account for the background, or non-acid catalyzed, decomposition of STAB by water (analogous to known $NaBH_4$ assays). Back titration with base is complicated in terms of de-convoluting the contributions of newly formed acetic acid, boric acid, and any excess acid used/required to promote decomposition. A similar inaccuracy arises with the iodate method, in that the only reaction accounted for is between STAB and iodate under highly basic conditions while not accounting for reaction of STAB with water or the lability of the acetate groups under these conditions. For further discussion relevant to $NaBH_4$, see ref. 9.

[11] See Supporting Information for further details.

[12] An early example of $NaBH_4$ analysis involved reduction of acetone to isopropanol. See I. E. Lichtenstein, I. E.; Mras, J. S. Indirect Ultraviolet Spectrophotometric Determination of Borohydride Using Acetone. *J. Franklin Inst.*, **1966**, *281*, 481-485.

[13] A reductive amination is also more complex, involving imine formation (that would need to be quantitative) and the presence of water that can react with STAB. In the case of advanced substrates, use tests would consume valuable material as well.

[14] The observations span the range of both electron rich (piperonal) and electron deficient (4-nitrobenzaldehyde) Both substrates were reduced to <50% of the corresponding benzyl alcohol after 24 h in DMAc with 1 equivalent of STAB.

[15] The reduction of 4-chlorobenzaldehyde with STAB (1.1 equiv. of 90 wt% reagent) afforded only 10 LCAP of the corresponding alcohol in both DMAc and THF at 22 ˚C after 24 h, and 50 LCAP in MeCN. This result suggests that for reductive aminations with benzaldehyde derivatives where the imine/imminium equilibrium with the aldehyde does not heavily favor the former, that THF of DMAc would be preferred solvents over MeCN.

[16] *Sodium Borohydride Digest*, ed. J. Yamamoto, Rohm and Haas, USA, **2003**.

[17] Geankoplis, C. J. *Transport Processes and Separation Principles (Includes Unit Operations) 4th Edition,* Prentice Hall: Upper Saddle River, 2007.

[18] For our calculations in Table 1, the sum of -($P_{Water,25°C} + P_{Water\ Addition}$) = -(0.0317+0.0545) = -0.086 was rounded to -0.09.

[19] The difference arose from either a different bottle or a sub-divided lot stored under different conditions. For example, entry 1 used a new bottle from the same supplier as entry 1, Table 1; entry 2 used the STAB from entry 4 in Table 1, but one month earlier; entry 3 used STAB from the same supplier/manufacturing lot for entry 5 in Table 1, but from a different bottle that had been opened much earlier and had been more heavily sampled; entry 4 utilized the same STAB bottle from entry 3 in Table 1, but one month earlier; entry 5 utilized the same STAB bottle from entry 2 in Table 1, but one month earlier. As can be deduced, there was significant overlap in the STAB sources, but the experiments in Table 2 were performed earlier than those in Table 1, and some decomposition had occurred due to exposure of air and moisture.

[20] For similar observations with $NaBH_4$, see: Yu, L.; Matthews, M. A. Hydrolysis of sodium borohydride in concentrated aqueous solution *Int. J. Hydrogen Ener.* **20 11**, *36*, 7416-7426. The reductive amination rate also tended to decrease as the reaction temperature decreased.

[21] The non-zero y-intercept likely reflects the use of 1.13 equiv. of STAB (13 mol% “excess”) and reinforces the conclusion that this data only describes the reactivity spanning the examined STAB potencies and reaction conditions .

[22] This is not intended to be a general minimal charge, and the best fit linear equation applies only over the ranges represented by the acquired data. Each substrate and set of conditions (solvent, temp. etc.) will be subject to variation.

[23] The crude reaction HPLC profile contained 2 LCAP of the reduced aldehyde (4-chlorobenzyl alcohol). This represents an area under the curve ratio only and does not account for relative response factors at the observed wavelength.

[24] The data for the reduction of **1** was drawn from the experiment in eq.1, while the reduction of **5** was performed with 1.01 equiv. (*corrected*) of STAB. In a separate experiment, use of 1.01 equiv. of STAB resulted in quantitative conversion of **1**. The reduction of **1** reached similarly high conversion in DMAc after 16.5 h, while the reduction of **5** reached only 23% conversion in DMAc over the same time period.

[25] Increased levels of AcOH were observed, relative to the STAB reagent alone in solution, upon addition of phenol. For an alternative synthesis of analogues of **7**, see Joshi, R.; Sharma, G. S.; Kumar, V.; Hashmi, A. A.; Kumar, S.; Achila, R.; Hussain, M. E. Synthesis, Spectral and Biological Studies of Organotin(IV) Complexes of Heteroscorpionate. *Applied Organomet. Chem.* **2006**, *20*, 740-746.

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[27] Preliminary efforts showed proof of concept for color changes, but not accurate enough to serve as titrations. See Supporting Information for further information.

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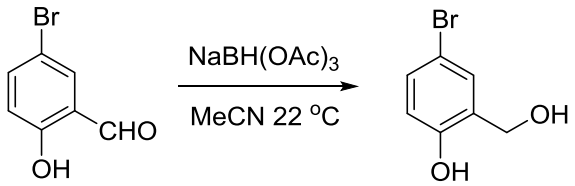

HPLC assay yield of alcohol correlated to STAB potency via:

1) independent assay (H_2 evolution)
2) reductive amination yield