Report

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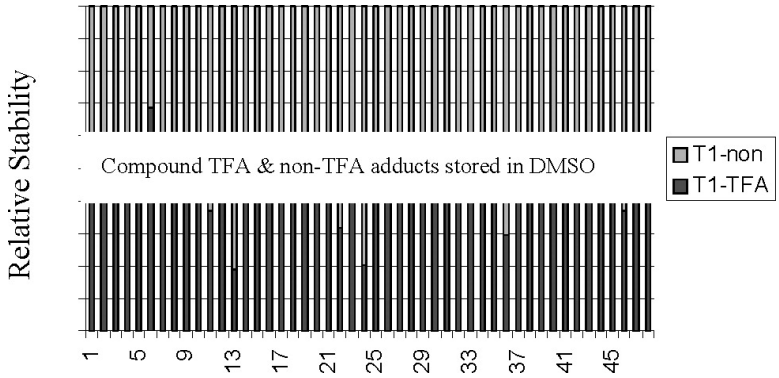

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Reports

Studies of the Relative Stability of TFA Adducts vs Non-TFA Analogues for Combinatorial Chemistry Library Members in DMSO in a Repository Compound Collection

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The stability during storage and handling of compounds in large Pharmaceutical Discovery repository collections is a topic of great importance to those involved in both archiving and high-throughput screening of these materials. A few recent studies have discussed the quality and purity of libraries immediately post synthesis.[1,2] After archiving of these compounds, however, for ease of assay, such collections are frequently stored in DMSO either as liquid libraries or frozen stock. Preliminary studies in our laboratories[3] indicated that general repository samples could be maintained at an acceptable level of integrity for approximately 1 year under a humidity-controlled atmosphere. Similar conclusions have been reached by other workers, as disclosed at a recent conference session[4−9] dedicated to the topic.

A high-throughput organic synthesis (HTOS) group at Abbott Laboratories routinely utilizes highly automated parallel synthesis platforms, standardized chemical protocols, and preformatted monomer collections to generate both diverse libraries for hit explosion and focused libraries for lead optimization.[10] These compounds are purified by the high-throughput purification (HTP) group by either high performance liquid chromatography (HPLC) or supercritical fluid chromatography (SFC), the former of which generates TFA (trifluoroacetic acid) salts or adducts in the process of purification. The libraries generated via the HTOS group and purified by the HTP group are utilized by all of the therapeutic areas and represent a substantial portion of the compounds being added to the corporation's compound collection. As a result, the question as to the relative stability during storage in DMSO for high-throughput screening of the TFA adducts vs the non-TFA containing analogues had to be addressed. Potential problems might be encountered either as a result of the elevated solution-phase pH resulting from excess TFA present or by direct reaction between trifluoroacetic acid and the library compound. Researchers from Discovery Partners International reported[6] one compound showing reduced stability in the TFA-adduct form. Since approximately one-third of all materials currently submitted for storage and screening are generated within HTOS, these samples represent an important part of the company's repository screening collection. Experiments were therefore undertaken to ascertain the relative stability of TFA adducts specifically for the structural types and functionalities as generated in the high-throughput synthesis format.

To obtain an appropriate representative collection of compounds for the study, a series of diverse cores was selected from the Abbott repository collection. Cores were selected such that they were amenable to modification via one or more of the standardized chemistries employed, they were of an appropriate molecular weight range such that reaction products were within the target MW range desired, and were chosen utilizing computational diversification protocols so as to statistically represent the compound collection as a whole. Finally, care was taken to include compounds containing structural fragments with potential acid lability within the study. Cores were derivatized via standardized HTOS chemistry protocols and represent a majority of the standardized transformations performed by the HTOS group. Examples of the standardized transformations utilized include acylations, reductive alkylations, sulfonylations, Mitsunobu reactions, Suzuki couplings, urea formations, and nucleophilic substitution reactions. A specific example of the development and utilization of one of the

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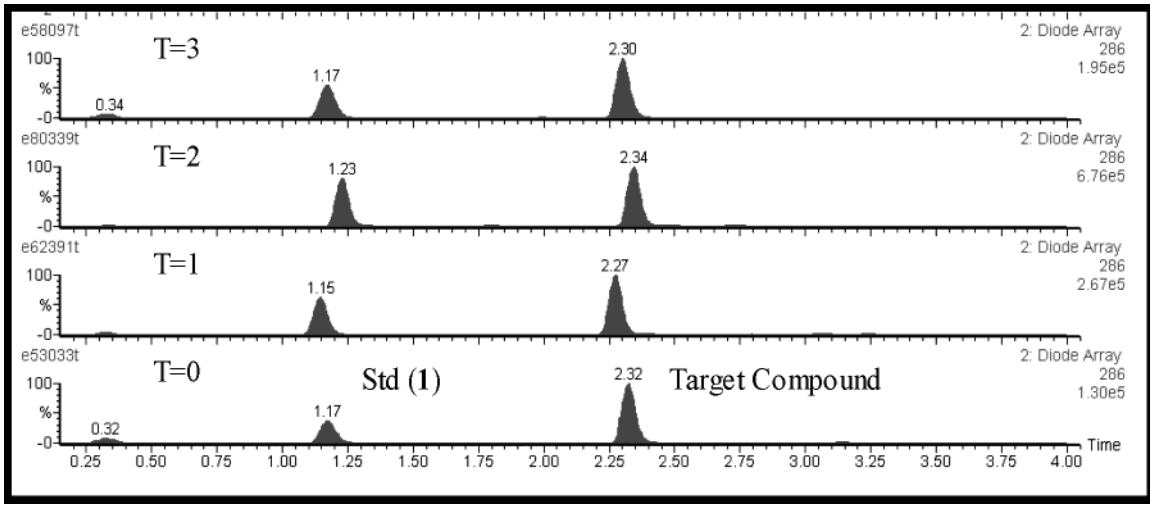
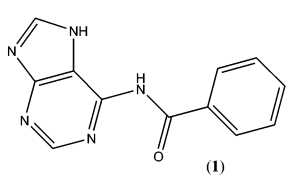


Figure 1. Analytical HPLC trace for a single compound at multiple time points. To obtain decomposition information on individual compounds, the target compound UV peak is integrated relative to the internal standard UV peak at a predetermined wavelength (286 nm in this case).

standardized chemistries, the Mitsunobu protocol, has been previously reported.[11] The monomers utilized for the study were selected from internally developed "diversity" collections that are organized by reactive functionality and were chosen to complement the chemistry employed for each sublibrary prepared. Diversity collections were designed to include hydrophobic and hydrophilic aliphatic, aromatic, and heterocyclic moieties. The utilization of the diverse monomer sets further increases the degree of molecular diversity for the study and includes functional groups commonly used to generate structure/activity relationships. These synthetic efforts thus provided a set of functionally diverse compounds representative of those to be stored in our repository and subjected to high-throughput screening.

Upon completion of the chemical synthesis, each crude mixture was split in half and purified one-half each by both semipreparative HPLC (thus generating TFA salts and adducts for structural types where possible) and by semipreparative SFC (generating the corresponding nonadduct analogues). Purification conditions for SFC and HPLC chromatography varied slightly according to the structural types of samples processed. Typical SFC purification conditions were Diol column (21.2 × 150 mm, 6-μm particle size, Berger Instruments), gradient elution of 0.5% Et_3N/MeOH in carbon dioxide, 5−60% over 6 min and hold at 60% for 2 min. Fraction collection was triggered by UV at 220, 240, or 254 nm, preset depending upon compound structure. Typical HPLC purification conditions were Symmetry C8 column (40 × 100 mm, 7-μm particle size, radial compression format, Waters), gradient elution of 10−95% aqueous 0.1% TFA/CH_3CN at 40 mL/min over 10 min. Fraction collection triggered by UV at 220, 240, or 254 nm, preset depending upon compound structure. Postpurification analytical HPLC/MS/UV/ELSD was thereafter employed to validate the identity and purity of compounds to be included within the study. To determine the amount of TFA residual in samples upon completion of HPLC purification, F NMR spectra were acquired on compounds containing a fluorine atom within the structure. This allowed the relative integration of fluorine signals within each sample, giving molar percentages of TFA salt or adduct. Results indicate that in addition to the anticipated TFA salt equivalent for protonatable functional groups such as amines, excess TFA was present as an "adduct" form within all samples. The amount of nonsalt form residual TFA varied from ∼0.2 to ∼0.6 equivalents, thus resulting in variations in the acidity of the final samples when dissolved in DMSO.

Analytical HPLC/UV/MS was selected as the technique by which decomposition over preselected time intervals was monitored. Mass spectrometry was employed for validation of the target compound, and ultraviolet spectroscopy, for the quantitation of the same. At the beginning of the study, each purified compound was dissolved in DMSO at a 10 mM concentration, and several replicate 96-well microtiter plates were generated from these stock solutions. Aliquots were taken from the daughter plates at several time points, including $T = 0$, diluted with MeOH to an analytically appropriate concentration, and transferred to microtiter plates for LC/UV/MS analysis. Internal standardization was found to give the most accurate results; therefore, sample preparation for each time point was accomplished by the addition of a UV-active standard (**1**) to the sample aliquot. Integration of the internal standard to the "target" compound for each compound at each time point could then be compared for TFA vs non-TFA analogues. Triplicate samples were analyzed for each time point. Figure 1 shows representative data for an individual compound at multiple time points. Further details of the experimental procedure may be found in an earlier publication.[3]



(**1**)

A small set of 96 compounds (48 pairs of TFA adducts and non-TFA adducts) were selected for a preliminary study. This allowed sufficient instrument capabilities to perform triplicate results and ensure good quality data. Intentions were

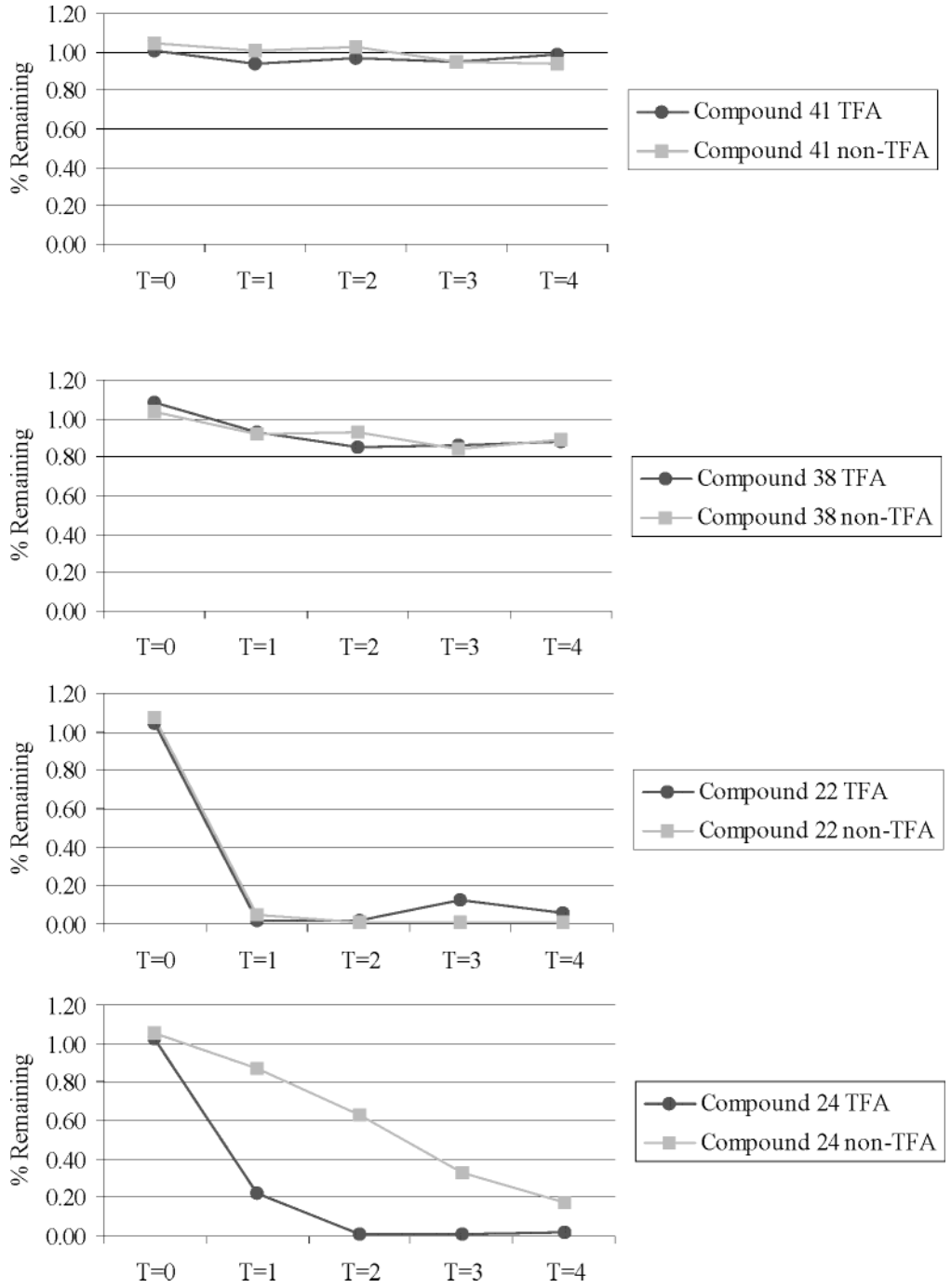


Figure 2. Decomposition curves for TFA adducts and nonadducts to individual compounds over four time points equally spaced over 4 months at 40 °C. Percentage remaining is an average value for three samples analyzed.

that should pronounced affects be noted in a preliminary study, an expanded study would be immediately thereafter carried out on a larger sample set. Studies were carried out at 40 °C in order to predict longer term results, assuming a doubling of reaction decomposition rate per 10 °C rise in temperature.[12,13] (In fact, however, stability studies carried out in our laboratory[3] show that the rate of decomposition for a diverse set of structural types proceeds much faster than would be predicted upon the basis of this simple model.) Time points taken over four months of the study are anticipated to predict the stability of compounds over one year maintained at room temperature. Upon the basis of preliminary results[3] of our repository compound stability studies in which water was demonstrated to exhibit a deleterious effect, humidity controlled conditions were maintained throughout the experiment. During the present study, four time points were taken over the course of 4 months. Figure 2 depicts typical decomposition curve results for compounds within the study. Each compound set was found to follow one of several decomposition profiles. Either both TFA and non-TFA forms were stable during the time frame in which the study was undertaken (compound 41), both forms showed slight decomposition (compound 38) to substantial decomposition (compound 22) during the time frame, or the TFA adduct was less stable than the non-TFA adduct (compound 24).

Preliminary results from the small diverse set of TFA and non-TFA adducts studied are summarized in Figures 3 and

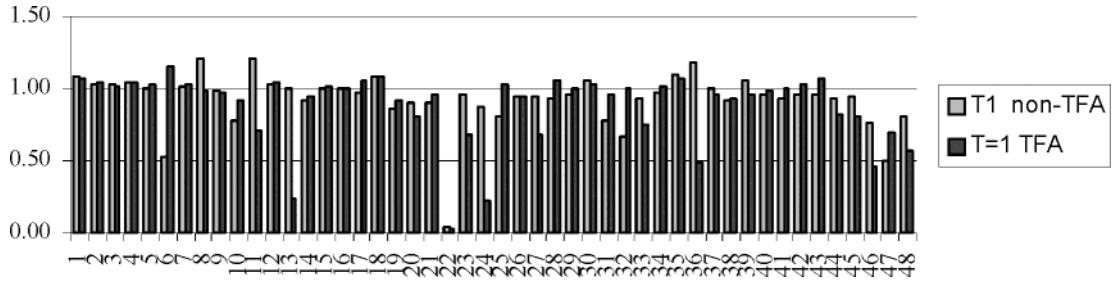


Figure 3. Percentage remaining after a 2-week accelerated stability study at 40 °C for a matched set of 48 TFA adducts and non-TFA analogues. Remaining percent determined by relative integration of UV for target compound vs an internal standard at preselected wavelength and normtalized to $T = 0$.

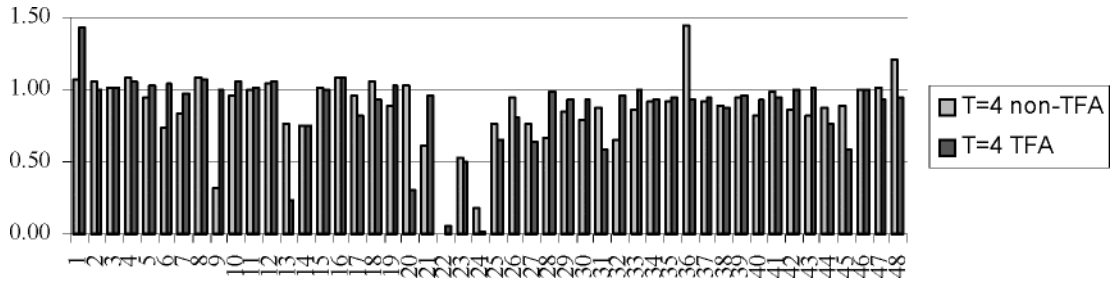
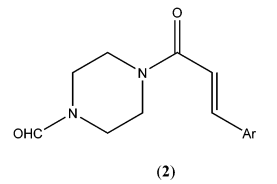


Figure 4. Percentage remaining after a 16-week accelerated stability study at 40 °C for a matched set of 48 TFA adducts and non-TFA analogues. Remaining percent determined by relative integration of UV for target compound vs an internal standard at preselected wavelength and normalized to $T = 0$.

4. Analysis of material remaining at each time point showed that more compounds were unstable as the TFA adducts than as nonadducts. Of the 96 compounds studied (pairs of 48 TFA adducts and 48 non-TFA adducts), 2 compounds were unstable in either form, 6 compounds were clearly more unstable in the TFA form, and 1 showed a slightly accelerated decomposition rate as the non-TFA adduct. A few preliminary conclusions can be drawn from these initial studies. Structures that one might expect to show some acid lability do, indeed, show this tendency in the presence of TFA. Structural fragments for compounds found to be more unstable (**2**, **3**, **4**, **5**) as the TFA adducts include acid-sensitive groups, such as ketals, aminals, alkoxy or dialkoxy benzyl ethers, and amines. Interestingly, but perhaps not surprisingly, it was noted that the presence of basic groups in the same molecule slowed the rate of decomposition of the TFA adduct in one instance. Although these results are only preliminary and are derived from a very small set of structures, it can be suggested that compounds containing acid-labile groups should not be routinely stored as TFA salts or adducts in DMSO solution in compound collections used for high-throughput screening and archiving purposes. On the basis of these preliminary results, an expanded TFA study has been initiated on a larger sample set and wider range of structural diversity. In this second study, 25 diverse cores were selected, and each was modified with a set of 48 monomers. The aim of the expanded TFA stability study is to ascertain if specific functional or structural groups are more or less stable in DMSO as TFA adducts. If such is found to be the case, purification methods can be selected so as to ensure that compounds entering the repository are submitted in the most stable format possible. The results from the follow-up study will be discussed in a future paper. Additionally, studies are presently underway to ascertain if excess TFA removal can be easily accomplished as part of a sample dry-down or postpurification protocol.



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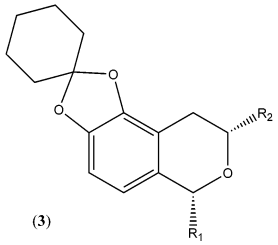

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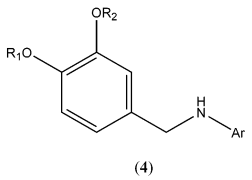

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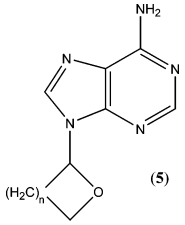

(**5**)

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