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4-(9-Fluorenylmethyloxycarbonyl)phenylhydrazine (FmPH): A New Chromophoric Reagent for Quantitative Monitoring of Solid-Phase Aldehydes[1−3]

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A direct method for quantifying solid-phase aldehydes has been developed, using a new reagent, 4-(9-fluorenylmethyloxycarbonyl)phenylhydrazine (FmPH). The FmPH reagent was synthesized in three steps (24% overall yield) from commercially available *p*-hydrazinobenzoic acid. Resin-bound aldehydes reacted quantitatively with FmPH, in the presence of trimethylorthoformate (TMOF) as a dehydrating agent, to form a highly conjugated, immobilized FmPH-hydrazone. Next, mild treatment of the hydrazone with an excess of piperidine−*N*,*N*-dimethylformamide (1:1) released the piperidine−dibenzofulvene adduct chromophore (ϵ_{301nm} = 7800 M^{-1} cm^{-1}) from the support. FmPH quantitation of aldehydes proved to be a straightforward, sensitive, and reproducible technique for monitoring resin-bound aldehydes [albeit insufficiently reactive to allow reliable quantification of ketones]. The FmPH aldehyde assay is applicable to a range of solid supports, as demonstrated specifically for poly(ethylene glycol)-polystyrene (PEG-PS), aminomethylpolystyrene (AMP), and cross-linked ethoxylate acrylate resin (CLEAR).

Introduction

Methods for solid-phase synthesis[4−12] and combinatorial chemistry[12−18] have been established as essential tools for drug discovery in the pharmaceutical and biotechnology industries. As more and more solid-phase reactions are worked out, reliable and robust methods for solid-phase reaction monitoring[5,9,19−22] must also be developed. The solid-phase mode presents several challenges, because classical techniques for following the course of reactions, such as TLC, do not apply to polymer-supported intermediates. Additionally, on-bead NMR,[23−31] IR,[32−36] MS,[22,37,38] and more recently electrochemical impedance spectroscopy[39] (ESI) experiments are not straightforward. A viable way to probe the progress of a solid-phase reaction is to cleave the intermediate from the linker/support[40,41] and then use classical techniques for characterization and/or quantitation. However, such approaches may be less than advantageous to the solid-

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(3) Abbreviations used are as follows: AMP, aminomethylpolystyrene; BAL, backbone amide linker; Boc, *tert*-butyloxycarbonyl; CLEAR, cross-linked ethoxylate acrylate resin or poly(trimethylpropane ethoxylate (14/3 EO/OH) triacrylate-*co*-allylamine); DCC, *N*,*N*′-dicyclohexylcarbodiimide; DIEA, *N*,*N*-diisopropylethylamine; DIPCDI, *N*,*N*′-diisopropylcarbodiimide; DMAP, 4-(*N*,*N*-dimethylamino)pyridine; DMF, *N*,*N*-dimethylformamide; DNPH, 2,4-dinitrophenylhydrazine; ESI, electrochemical impedance spectroscopy; Fm, 9-fluorenylmethyl; Fmoc, 9-fluorenylmethoxycarbonyl; FmPH, 4-(9-fluorenylmethyloxycarbonyl)-phenylhydrazine; HRESI-MS, high-resolution electrospray ionization mass spectrometry; HATU, *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HOAc, acetic acid; HOBt, 1-hydroxybenzotriazole; *p*-PALdehyde, 4-formyl-(3,5-dimethoxyphenoxy)valeric acid; PEGA, poly(*N*,*N*-dimethacrylamide-*co*-bisacrylamido-poly(ethylene glycol)-*co*-monoacrylamido-poly(ethylene glycol)); PEG-PS, poly(ethylene glycol)-polystyrene (graft resin support); Purpald, 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole; TEA, triethylamine; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TMOF, trimethyl orthoformate. All amino acids denote the L-configuration unless indicated otherwise. Abbreviations used for amino acids follow the IUPAC-IUB Commission of Biochemical Nomenclature in the following: *J. Biol. Chem.* **1972**, *247*, 977−983.

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phase chemist because (i) not all intermediates are stable to cleavage conditions, (ii) low-load resins may not provide enough product for isolation and characterization, (iii) sophisticated equipment might be needed, and (iv) the process may entail an unacceptable time delay in developing the information needed to make informed decisions on how to proceed with the synthesis. Fortunately, a variety of rapid on- and off-bead analytical techniques are available for monitoring some solid-phase functional groups.[5,9,19−22]

We are currently interested in the qualitative/quantitative monitoring of solid-phase aldehydes using on-bead chemical derivatization methods.[42−45] Given the practical importance of on-resin reductive amination[46−48] in backbone amide linker (BAL) anchoring,[49,50] and other solid-phase aldehyde transformations involving redox chemistry, condensations, and C−C bond formation, among others (see various reviews[6−12,49−55]), companion methods to monitor these common conversions are needed. We recently reported on a qualitative colorimetric test to monitor solid-phase aldehydes using 2,4-dinitrophenylhydrazine (DNPH),[45,56] to go with literature methods that use *p*-anisaldehyde[43] or 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald).[44] Nevertheless, a simple and robust *quantitative* protocol for resin-bound aldehydes was still required.

Quantitative measurements of resin-bound functional groups can be valuable when calculating loadings, optimizing reactions, and establishing yields (extent of conversion) of solid-phase transformations. To the best of our knowledge, the only previously described method to quantitate solid-phase aldehydes uses fluorescence spectroscopy to measure the uptake of dansyl hydrazine from a supernatant solution by resin-bound aldehydes.[42] While this method is reported to be quite sensitive, it is also tedious and only serves *indirectly* for quantification.

In search of a simpler and more *direct* route to quantify solid-phase aldehydes, we were interested in designing a stable chromophore-based "reagent" (**1**, Scheme 1) that would react quantitatively with the aldehyde to provide an immobilized intermediate (**2**). Subsequent mild and selective release of chromophore **3**, followed by its sensitive measurement by ultraviolet−visible (UV−vis) spectroscopy, would provide an accurate result that could be related directly to the absolute amount of aldehyde. Literature protocols for quantifying amines,[57−65] thiols,[66,67] and alcohols[68,69] during solid-phase peptide/organic synthesis follow this straightforward model.

The present article describes the synthesis of a new chromophoric hydrazine-based reagent, 4-(9-fluorenyl-

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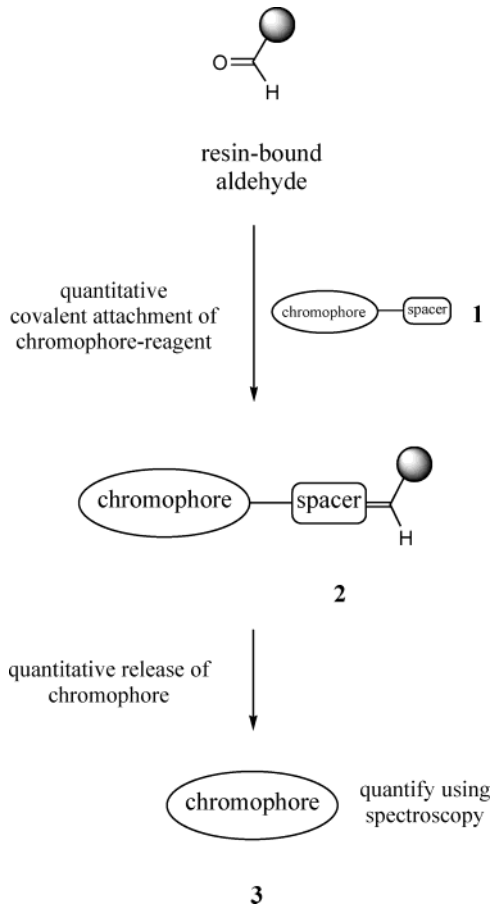
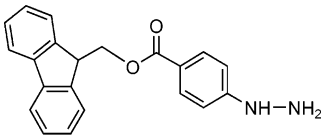
SCHEME 1. Principle of On-Bead Immobilization, Release of Chromophore, and Off-Bead Spectroscopic Analysis



methyloxycarbonyl)phenylhydrazine (FmPH) (**4**, Figure 1), and how it can be applied according to the aforementioned concept for the sensitive and *direct* quantification of resin-bound aldehydes. Both the reagent attachment and chromophore release step are readily made to go to completion. Note that in the FmPH assay, quantitative removal of the 9-fluorenylmethyl (Fm) group gives the same piperidine−dibenzofulvene adduct chromophore that is released in 9-fluorenylmethyloxycarbonyl (Fmoc) quantitation of amines in peptide synthesis.[59,65]

Results and Discussion

Pilot Studies and Rationale. Initial attempts to quantify resin-bound aldehydes involved the use of the chromophoric reagent, H-Gly-OFm·HCl (**5**), which was expected to serve for aldehyde quantification by way of *imine* formation with the BAL system[49,50,70] (**6**). Despite extensive efforts, coupling to form **7** occurred at best to 48% under near-forcing conditions [**5** (10 equiv) plus *N*,*N*-diisopropylethylamine (DIEA, 3 equiv), in *N*,*N*-dimethylformamide (DMF)−trimethyl orthoformate (TMOF) (1:1, 1 mL), for 2 h at 80 °C] (Scheme 2).

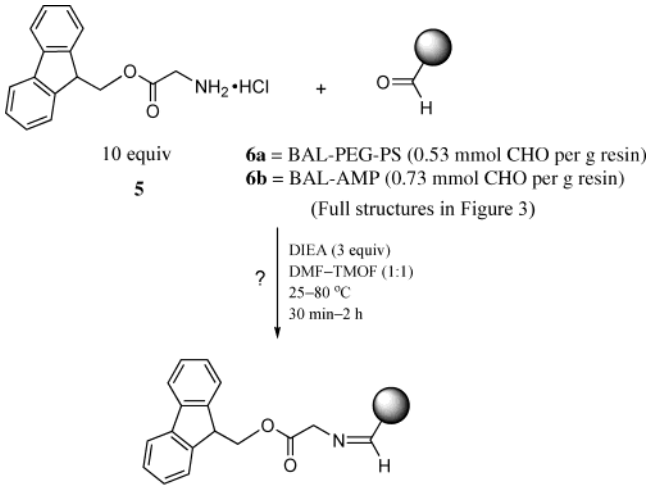


FmPH (**4**)

[used as its TFA salt]

FIGURE 1. 4-(9-Fluorenylmethyloxycarbonyl)phenylhydrazine (FmPH) (**4**). This new reagent contains a hydrazine group that can be made to react quantitatively with solid-phase aldehydes. It also contains a chromophoric 9-fluorenylmethyl group that can be released and quantified after FmPH immobilization on solid supports.

SCHEME 2. Proposed Reaction of H-Gly-OFm·HCl with BAL Supports



Consequently, efforts shifted toward design and synthesis of a suitable hydrazine-based reagent that would potentially form a *hydrazone* at much faster rates and with higher yields. The reactions of aldehydes with substituted hydrazines to form hydrazones are introductory textbook organic chemistry,[71,72] and have been applied in several solid-phase syntheses. For example, immobilized hydrazine linkers have been condensed with exogenous aldehydes to access α-branched amines.[73−75] Alternatively, hydrazines (e.g., dansyl hydrazine, or 2,4-dinitrophenylhydrazine) have been condensed with resin-bound aldehydes for other specialty applications.[42,45,56,76−78]

Preparation of FmPH (Scheme 3). Commercially available 4-hydrazinobenzoic acid (**8**) was treated with di-*tert*-butyl dicarbonate in the presence of catalytic 4-(*N*,*N*-dimethylamino)pyridine (DMAP) at 60 °C[79] to

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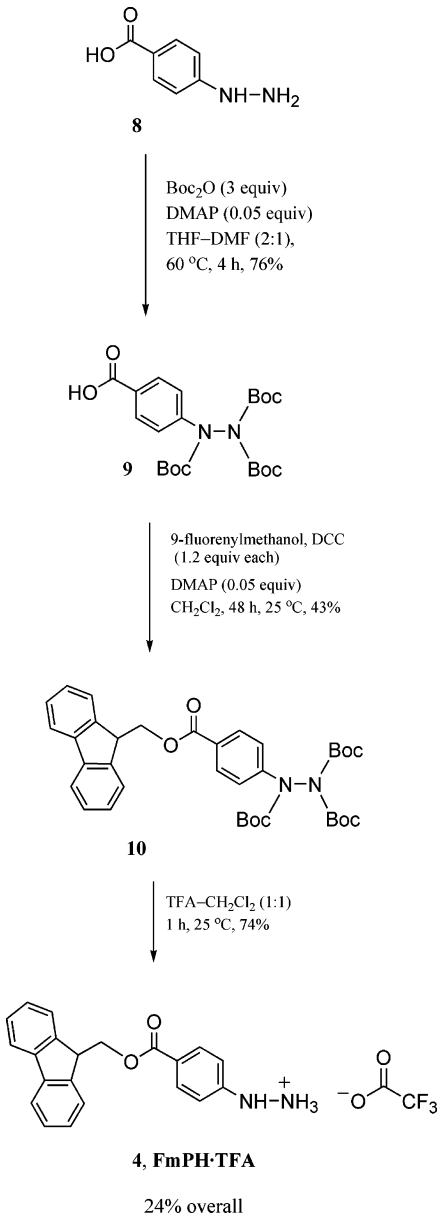
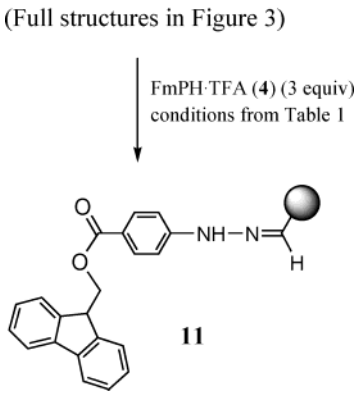
SCHEME 3. Preparation of FmPH·TFA



provide the *N*,*N*,*N*′-tris[*tert*-butyloxycarbonyl (Boc)]-protected hydrazino acid **9**,[80] which without purification was esterified with 9-fluorenylmethanol with use of *N*,*N*′-dicyclohexylcarbodiimide (DCC) in the presence of DMAP as a catalyst. Silica gel chromatography gave exclusively the *N*,*N*,*N*′-tris intermediate **10**, which was treated with trifluoroacetic acid (TFA) to remove all Boc groups and provided FmPH (**4**, isolated as its trifluoroacetate salt) in 24% overall yield.

SCHEME 4. Reaction of FmPH·TFA with BAL Supports To Form Resin-Bound Hydrazone



Reaction of FmPH·TFA with Resin-Bound Aldehydes. With the goal of achieving quantitative condensation, several solvent/catalyst systems were evaluated to facilitate the reaction of hydrazine **4** with BAL-PEG-PS (**6a**)[49,50] (Scheme 4 and Table 1). To evaluate the level of FmPH-hydrazone (**11**) formation, **6a** was exposed to the conditions listed, and at appropriate time periods, **11** was treated with an excess of piperidine−DMF (1:1) for 30 min at 25 °C to quantitatively remove the Fm ester[81−84] (Scheme 5). The resultant piperidine−dibenzofulvene adduct (**13**) released into solution was monitored by UV−vis spectroscopy[59,65] at 301 nm. Conditions that gave the value closest to the known loading of **6a** were considered optimal.

Optimal formation of **11** occurred when resin **6a** was condensed with a solution of **4** (3 equiv) plus DIEA, 3 equiv in DMF−TMOF (1:1) for 30 min at 80 °C. These conditions gave reproducible, near-quantitative loadings (Table 1, entry K). Other catalyst/solvent systems gave comparable results at 80 °C [e.g., DIEA (6 equiv) in MeOH−EtOH−TMOF (2:1:1), entry E; DIEA, 3 equiv in MeOH−EtOH−TMOF (2:1:1), entry H; or DIEA (6 equiv) in DMF−TMOF (1:1), entry L]. The use of TMOF as a dehydrating agent[85] was necessary to achieve quantitative results (see entries E, H, K, and L and compare to G and N). None of the acid-catalyzed protocols showed the full expected loading, presumably due to premature cleavage of the hydrazone over the extended reaction periods used (entries A, B, and C). However, in the presence of excess base, the full expected loading was observed (compare entry E with entries H, K, and L). At ambient temperatures, with reaction times between 2 and 24 h, the level of hydrazone formation was still incomplete (entries M, N, and O).

Kinetics of Reactions. To gain further insight into these reactions, the formation of hydrazone **11** and the release of dibenzofulvene adduct **13** were studied as a function of time (Figure 2). In summary, formation of

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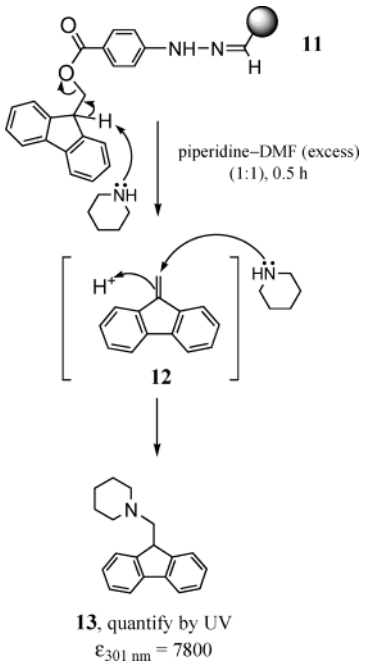
TABLE 1. Optimization of Protocol to Prepare Hydrazone 11

entry[a]	condensation conditions: catalyst (equiv)[b]	solvent (1 mL)[c]	temp (°C)	time (h)	loading (mmol/g)[d]
A	HOAc (5%, v/v)	MeOH−EtOH−TMOF (2:1:1)	80	0.5	0.40
B	H_2SO_4 (5%, v/v)	MeOH−EtOH−TMOF (2:1:1)	80	0.5	0.35
C	HCl (5%, v/v)	MeOH−EtOH−TMOF (2:1:1)	80	0.5	0.43
D	TEA (6)	MeOH−EtOH−TMOF (2:1:1)	80	0.5	0.43
E	**DIEA (6)**	**MeOH−EtOH−TMOF (2:1:1)**	**80**	**0.5**	**0.50**
F	pyridine (6)	MeOH−EtOH−TMOF (2:1:1)	80	0.5	0.21
G	DIEA (3)	DMF	80	0.5	0.27
H	**DIEA (3)**	**MeOH−EtOH−TMOF (2:1:1)**	**80**	**0.5**	**0.51**
I	no additional	DMF−TMOF (1:1)	80	0.5	0.25
J	no additional	MeOH−EtOH−TMOF (2:1:1)	80	0.5	0.18
K	**DIEA (3)**	**DMF−TMOF (1:1)**	**80**	**0.5**	**0.51**
L	**DIEA (6)**	**DMF−TMOF (1:1)**	**80**	**0.5**	**0.51**
M	DIEA (6)	DMF−TMOF (1:1)	25	2	0.34
N	DIEA (3)	DMF	25	2	0.18
O	DIEA (3)	DMF−TMOF (1:1)	25	24	0.39

[a] For each entry, **6a** (~10 mg, 0.53 mmol of CHO per g of resin) was condensed with **4** (3 equiv), using the listed catalyst, solvent, temperature, and time. All bold entries represent optimal conditions. Abbreviations are defined in footnote 2. [b] For all acid-catalyzed protocols (entries A, B, and C), the designated v/v ratio was used with respect to 1 mL of solvent. For all other catalysts, parentheses indicate the number of equivalents with respect to the amount of resin. [c] For each solvent system, the total volume was 1 mL. In cases where TMOF was present, **4** was *first* dissolved in the other cosolvent(s) (e.g., MeOH, EtOH, and/or DMF), followed by addition of the catalyst (i.e., acid or base), and then TMOF. [d] Loadings were calculated following the procedure under "FmPH quantitation" described in the Experimental Section.

SCHEME 5. Release of Piperidine−Dibenzofulvene Adduct from Resin-Bound Hydrazone



hydrazone **11** proceeded quantitatively within 10−20 min (Figure 2A, squares), and piperidine−dibenzofulvene adduct **13** was released quantitatively from hydrazone **11** within 10 min (Figure 2B, circles). Studies A and B combined show that FmPH quantitation of aldehydes could ideally be carried out in approximately 30 min. Also of significance, it was shown that as low as 2 μmol of aldehyde could be quantified by FmPH, with a reproducibility of ±3%.

In addition, hydrazine **4** and amine **5** were condensed with BAL supports in side-by-side experiments, to compare the rates of hydrazone versus imine formation (Figure 2, part A vs part C). Since the formation of imine

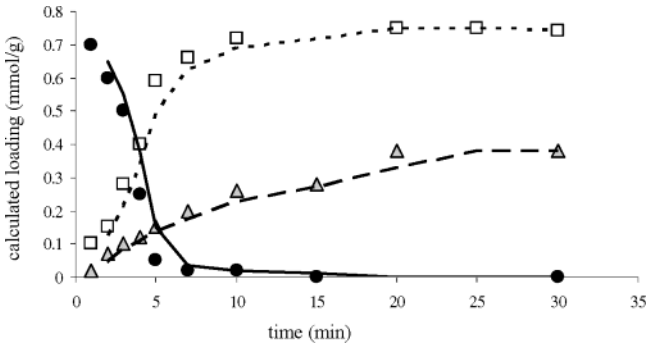


FIGURE 2. (A) Kinetics of formation (□) of hydrazone **11** with support **6b** (Scheme 4). (B) Kinetics of release (●) of dibenzofulvene adduct **13** from hydrazone **11** (Scheme 5). (C) Kinetics of formation (△) of imine **7** with support **6b** (Scheme 2). All calculations are described in the Experimental Section. Note: BAL-AMP (0.73 mmol of CHO per g of resin) (**6b**) was used instead of BAL-PEG-PS (**6a**) for all experiments described in this figure (see Schemes 2, 4, and 5).

7 took nearly 2 days to go to completion, it is clear that the title approach with FmPH is the superior way to quantify solid-phase aldehydes.

FmPH Quantitation versus Fmoc Quantitation. Our previous work described the qualitative monitoring of polymer-supported aldehydes with DNPH.[45,56] The lower limit of sensitivity for the DNPH test was established after testing several partially functionalized aldehyde supports (**16**, Scheme 6), which were prepared by coupling mixtures of 4-formyl-3,5-dimethoxyphenoxy)-valeric acid (PALdehyde)[86] (**14**) and Fmoc-Gly-OH (**15**) [combined 3 equiv carboxylic acid function in prespecified ratios] to commercially available amino-functionalized supports [poly(ethylene glycol)polystyrene (PEG-PS), 0.53 mmol/g;[87,88] aminomethylpolystyrene (AMP), 0.73 mmol/

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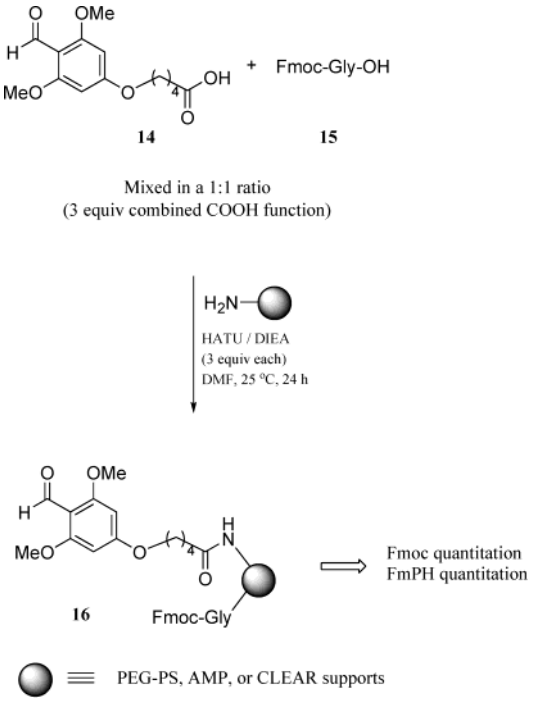
SCHEME 6. Preparation of Partially Substituted Aldehyde Supports



g;[89] cross-linked ethoxylate acrylate resin (CLEAR, 0.46 mmol/g);[90] and poly(*N*,*N*-dimethacrylamide-*co*-bisacrylamido-poly(ethylene glycol)-*co*-monoacrylamido-polyethylene glycol) (PEGA, 0.26 mmol/g)[91,92]]. This experimental design makes it possible to determine *indirectly* the absolute amount of PALdehyde present on **16**, by Fmoc quantitation.[45,59,65]

For the present work, a goal was to compare the *indirect* Fmoc quantitation[59,65] versus the *direct* FmPH quantitation as methods to quantify aldehydes. Thus, a 1:1 ratio of **14** and **15** was coupled to PEG-PS, AMP, and CLEAR supports giving rise to resins **16** with approximately 50% loading of PALdehyde. The absolute amount of aldehyde was determined to be between 49% and 57% (Table 2), proving that both methods give consistent and accurate results. Nevertheless, FmPH quantitation is probably more accurate because it involves direct reaction with residual resin-bound aldehydes, as opposed to the indirect procedure with Fmoc-Gly-OH.

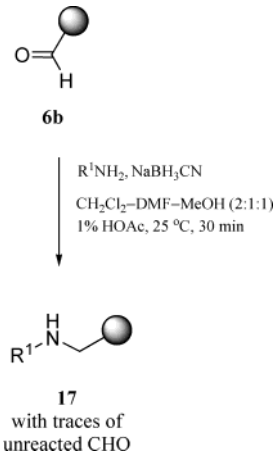
Application of FmPH Quantitation to a Range of Aldehyde Supports. During a recent BAL solid-phase synthesis of lidocaine and procainamide analogues,[56] the conversion of BAL to secondary amines via reductive amination was a critical step that significantly influenced the overall yield and purity of the final product mixture after cleavage. We revisited this step, using FmPH

TABLE 2. Comparison between Fmoc and FmPH Quantitation of Aldehydes on PEG-PS, AMP, and CLEAR Supports

partial aldehyde support (**16**)[a]	quantity of aldehyde (%)	
	Fmoc[b]	FmPH[c]
BAL-PEG-PS	57	54
BAL-AMP	50	51
BAL-CLEAR	49	50

[a] See Experimental Section and Scheme 6 for the procedure to prepare partially substituted aldehyde supports. [b] Indicates average of three separate experiments. Starting with ~10 mg of **16**, Fmoc quantitation[59,65] allowed loading of Fmoc-Gly to be determined. Theoretical loadings of commercially available amino-functionalized supports were determined by coupling Fmoc-Gly-OH to the support, and then submitting to Fmoc quantitation. Percent Fmoc-Gly-OH was determined by taking the calculated loading of Fmoc-Gly-OH and dividing by the theoretical loading of the respective support. Percent aldehyde was then calculated *indirectly* by subtracting the percent of Fmoc-Gly-OH from 100%. [c] Indicates average of three separate experiments. **16** (~10 mg) was treated by using the protocol of note footnote *b* to release Fmoc and provide the free NH_2. The remaining aldehydes were quantified *directly* by using FmPH quantitation.

SCHEME 7. Reductive Amination of Amines with BAL-AMP



quantitation to monitor $NaBH_3CN$-mediated reductive aminations of **6b** with a range of primary aliphatic and aromatic amines. The aforementioned reaction provided secondary amines **17**, and in some cases, residual unreacted aldehydes (Scheme 7). After 30 min at 25 °C, percent conversions for the reductive amination were largely in the range of 70−97%, as calculated by subsequent FmPH quantitation of the unreacted resin-bound aldehyde (Table 3). As predicted, conversions improved as the amine was changed from 2,6-dimethylaniline (entry P) to aniline (entry Q) to benzylamine (entry R); this could be due to either steric or electronic effects to the amino functionality. Unhindered primary aliphatic amines, including amino acid *tert*-butyl esters (entries S, T, and U), converted relative to the same extent under the conditions evaluated. Conversion with H-Phe-O*t*Bu·HCl (entry V) was slightly lower than that with the other aliphatics, again probably due to steric interferences from the benzyl group.

FmPH quantitation was extended to other aldehyde supports related to our research (Figure 3). Supports were quantified that contained aromatic aldehydes with activating groups [methoxy (**6a/b**), hydroxy (**18**)] in the

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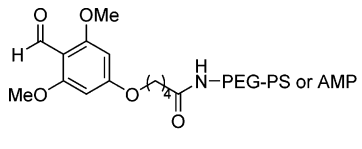
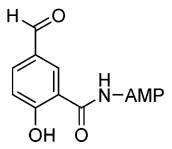
TABLE 3. Percent Conversion of Resin-Bound Aldehydes after Reductive Amination with BAL-AMP (6b)

entry	primary amine	% aldehyde remaining[a]	% conversion[b]
P	2,6-dimethylaniline	68	32
Q	aniline	30	70
R	benzylamine	6	94
S	butylamine	5	95
T	cyclohexylamine	4	96
U	H-Gly-O*t*Bu·HCl	3	97
V	H−Phe-O*t*Bu·HCl	11	89

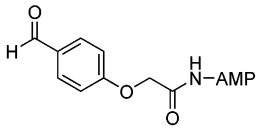
[a] This value was determined by first submitting **17** (~5 mg) to FmPH quantitation from which the loading of unreacted aldehyde was calculated. Percent aldehyde was then determined by taking the calculated loading and dividing by the theoretical loading of BAL-AMP (0.73 mmol of aldehyde per g of resin). [b] Percent conversion was calculated by subtracting the percent of aldehyde from 100%.



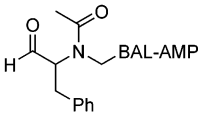
6a, 0.51 mmol/g, PEG-PS
6b, 0.71 mmol/g, AMP



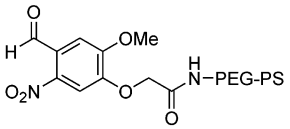
18, 0.64 mmol/g



19, 0.72 mmol/g



20, 0.48 mmol/g

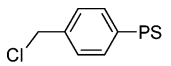


21, 0.30 mmol/g

FIGURE 3. Structures of various resin-bound aldehydes assayed by FmPH quantitation.

ortho or para positions, unsubstituted *p*-alkoxybenzaldehydes (**19**), aliphatic aldehydes (**20**), or aromatic aldehydes with deactivating groups, such as the nitro functionality in support **21**. Again, a range of different supports (e.g., PEG-PS, AMP, CLEAR) were tolerated. The nucleophilicity of FmPH·TFA under the given conditions did not cause any substantial interference with other electrophilic carbon centers such as alkyl halides, as demonstrated on Merrifield resin (**22**) and supported chloroethylamine (**23**) (Figure 4). Furthermore, interference from other functional groups [e.g., methoxy (**6** and **21**), phenolic (**18**), amide (**24**), and nitro (**21** and **25**)] was negligible (Figure 4).

Representative resin-bound ketones **26**−**28** (Figure 5) were reacted with FmPH under the same conditions that were optimized for aldehydes. Release and quantitation of the piperidine−dibenzofulvene adduct in the usual way revealed that FmPH incorporation had been at best in



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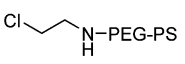

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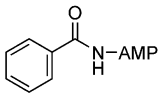

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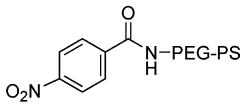

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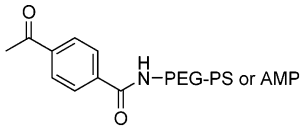
FIGURE 4. Structures of various resin-bound functional groups that were taken through the assay procedure. After FmPH quantitation, none of the supports showed any residual dibenzofulvene absorbance, suggesting no interference with FmPH.



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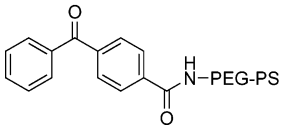

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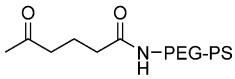

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FIGURE 5. Structures of various resin-bound ketones assayed by FmPH quantitation.

the 30−50% range. These preliminary results suggest that FmPH is not reactive enough to be useful for reliable quantification of resin-bound ketones.

Conclusions

A simple, reproducible, and direct method for quantifying solid-phase aldehydes has been developed and investigated thoroughly. A new reagent, FmPH, is synthesized and isolated as its stable TFA salt in a 27% overall yield for three easy steps. FmPH quantitation is a useful research tool for monitoring the progress of a number of solid-phase conversions, including reductive aminations and the attachment of aldehyde linkers to solid supports. Results are obtained by UV−vis, ideally in 30 min, with detection of aldehydes at levels as low as 2 μmol and reproduciblility to ±3%. We envision that this test could also be applied to other solid-phase aldehyde transformations, e.g., reduction of Weinreb amides, reduction of aldehydes to alcohols, or oxidation of alcohols to aldehydes, and will be a significant addition to the practical quantitative tools available for solid-phase synthesis.

Experimental Section

General Considerations. Materials, solvents, instrumentation, and general methods were essentially as described in previous publications from our laboratories,[49,56,93,94] as detailed further in the Supporting Information.

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***N*$^{\alpha}$-(*tert*-Butoxycarbonyl)glycine 9-fluorenylmethyl Ester.** Boc-Gly-OH (1.0 g, 5.7 mmol) was taken up in freshly distilled CH_2Cl_2 (25 mL), and DCC (1.4 g, 6.9 mmol), 9-fluorenylmethanol (1.3 g, 6.9 mmol), and DMAP (34 mg, 0.29 mmol) were added in order with stirring. The resultant suspension was then mixed for 48 h at 25 °C, filtered while cold, and concentrated to a brown oil. Purification by silica gel column chromatography (hexanes−EtOAc, 3:1) provided the title compound, Boc-Gly-OFm, as a clear colorless film; yield 609 mg (30%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.76 (d, J = 7.0 Hz, 2H), 6.58 (d, J = 7.0 Hz, 2H), 7.41 (t, J = 7.4 Hz, 2H), 7.3−7.4 (m, 2H), 5.12 (br s, 1H), 4.43 (d, J = 7.2 Hz, 2H), 4.23 (t, J = 7.2 Hz, 1H), 4.02 (d, J = 5.6 Hz, 2H), 1.47 (s, 9H). ESI-MS: *m*/*z* calcd for $C_{21}H_{23}NO_4$ 353.41, found 376.3 ([M + Na]$^+$).

Glycine 9-Fluorenylmethyl Ester, Hydrochloride Salt (5). Boc-Gly-OFm (500 mg, 1.4 mmol) was taken up in 4 M HCl−dioxane (9 mL) and stirred for 30 min at 25 °C. The burgundy solution was concentrated under a stream of N_2 (to remove HCl) and subsequently under reduced pressure to provide a brown oil. After triturating with Et_2O (3 × 15 mL), compound **5** precipitated as a white solid (HCl salt), and was filtered and dried (2 mm, overnight, desiccator); yield 274 mg (77%) (23% overall from Boc-Gly-OH). ^{1}H NMR (500 MHz, CD_3OD-d_6): δ 7.73 (d, J = 12.0 Hz, 2H), 7.54 (d, J = 12.0 Hz, 2H), 7.33 (t, J = 12.0 Hz, 2H), 7.24 (t, J = 12.0 Hz, 2H), 4.53 (d, J = 11.0 Hz, 2H), 4.20 (t, J = 10.0 Hz, 1H), 3.75 (s, 2H). LRESI-MS: *m*/*z* calcd for $C_{16}H_{15}NO_2$ 253.30, found 267.3 ([M + Na]$^+$).

4-(9-Fluorenylmethyloxycarbonyl)[*N*,*N*,*N*-tris(*tert*-butyloxycarbonyl)phenylhydrazine (10). A solution of di-*tert*-butyl dicarbonate (8.6 g, 39 mmol) in tetrahydrofuran (THF, 10 mL) was added to a stirred solution of 4-hydrazinobenzoic acid (**8**) (1.9 g, 13 mmol) in THF−DMF (2:1, 50 mL), and the solution was mixed for 30 min at 25 °C. DMAP (39 mg, 0.33 mmol) was then added to the solution, following which the temperature was increased to 60 °C and stirring continued for 4 h.[79] The reaction mixture was then cooled, partially concentrated in vacuo (to remove THF), diluted with Et_2O (125 mL), and washed with 1 M aqueous $KHSO_4$−brine (1:1) (3 × 25 mL) and brine (3 × 15 mL), dried ($MgSO_4$), and concentrated to give **9** as a tan solid; yield 4.5 g (76%). Without further purification, **9** (2.0 g, 3.2 mmol) was taken up in freshly distilled CH_2Cl_2 (50 mL) and then DCC (1.1 g, 5.3 mmol), 9-fluorenylmethanol (1.0 g, 5.3 mmol), and DMAP (27 mg, 0.22 mmol) were added in sequence with stirring. The resultant suspension was then stirred for 48 h at 25 °C, after which it was cooled, filtered, concentrated to a brown oil, and purified by silica gel column chromatography (hexanes−EtOAc, 4:1) to provide **10** as a white solid; mp 125−127 °C; yield 1.2 g (43%) (33% overall for two steps). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.06 (d, J = 11.0 Hz, 2H), 7.81 (d, J = 12.5 Hz, 2H), 7.66 (d, J = 12.5 Hz, 2H), 7.55 (d, J = 11.5 Hz, 2H), 7.44 (t, J = 12.5 Hz, 2H), 7.34 (t, J = 12.5 Hz, 2H), 4.61 (d, J = 11.5 Hz, 2H), 4.41 (t, J = 12.0 Hz, 1H), 1.55 (s, 9H), 1.51 (s, 18H). HRESI-MS: *m*/*z* calcd for $C_{36}H_{42}N_2O_8$ 630.7274, found 653.2838 ([M + Na]$^+$).

Anal. Calcd for $C_{36}H_{42}N_2O_8$, MW 639.73: C, 68.55; H, 6.71; N, 4.44; O, 20.29. Found: C, 68.47; H, 6.74; N, 4.42.

4-(9-Fluorenylmethyloxycarbonyl)phenylhydrazine, Trifluoroacetate Salt (4). Compound **10** (350 mg, 0.55 mmol) was taken up in CH_2Cl_2−TFA (1:1, 10 mL) and stirred for 1 h at 25 °C. The burgundy solution was concentrated under a stream of N_2 (to remove TFA) and subsequently under reduced pressure to provide a brown oil. The oil was triturated with Et_2O (3 × 25 mL) while title compound **4** precipitated as a yellow solid (TFA salt), which was filtered and dried (2 mm, overnight, desiccator); mp 169−171 °C; yield 182 mg (74%) (24% overall from **8**). ^{1}H NMR (500 MHz, CD_3OD-d_6): δ 7.92 (d, J = 14.0 Hz, 2H), 7.80 (d, J = 9.0 Hz, 2H), 7.70 (m, 2H), 7.42 (t, J = 8.0 Hz, 2H), 7.34 (t, J = 8.0 Hz, 2H), 7.05 (d, J = 8.0 Hz, 2H), 4.55 (d, J = 6.5 Hz, 2H), 4.41 (t, J = 6.5 Hz, 1H). HRESI-MS: *m*/*z* calcd for $C_{21}H_{18}N_2O_2$ 330.1368, found 353.1246 ([M + Na]$^+$).

Anal. Calcd for $C_{23}H_{19}F_3N_2O_4$, MW 444.40: C, 62.16; H, 4.31; F, 12.83; N, 6.30; O, 14.40. Found: C, 62.49; H, 4.65; N, 6.27.

The title compound demonstrated excellent stability at ambient temperatures, but was nevertheless stored at 4 °C, as is typical for standard Fmoc-protected amino acids.

FmPH Quantitation: Direct Quantitation of Polymer-Supported Aldehydes. Approximately 5−10 mg of dry aldehyde resin (0.2−0.7 mmol of CHO per g of resin) was weighed into a glass vial with a Teflon-lined cap, and then a solution of FmPH·TFA (**4**)/DIEA (3 equiv each) in DMF (0.5 mL) was added. [For AMP aldehyde resins, DMF−CH_2Cl_2 (1:1, 0.5 mL) was used as solvent.] TMOF (0.5 mL) was added immediately and the resulting suspension was rotated on an orbital mixer for 30 min at 80 °C. The cooled suspension was transferred to a fritted plastic syringe and the resin was washed thoroughly with DMF (5 × 1.5 mL), MeOH (5 × 1.5 mL), and again with DMF (5 × 1.5 mL). The FmPH-derivatized support was treated with piperidine−DMF (1:1, 1 mL) for 30 min at 25 °C to release the piperidine−dibenzofulvene chromophore (**13**), and the filtrate was collected in a 25 mL volumetric flask. The resin was washed with piperidine−DMF (1:1, 5 × 2 mL), and on each wash, drained directly into the same 25-mL volumetric flask, followed by dilution up to 25 mL with piperidine−DMF (1:1). The absorbance of the solution was measured with an ultraviolet spectrometer [using piperidine−DMF (1:1) as a blank] at 301 nm, and incorporated in the following formula to calculate the loading of aldehyde:

$$\text{loading of CHO (mmol/g)} = [1000(A)(V)]/[\epsilon \times M]$$

where A is the average absorbance after three measurements, V is the dilution volume in mL, ϵ = 7800 M^{-1} cm^{-1} (the extinction coefficient for the dibenzofulvene chromophore), and M is the resin weight in mg.

Kinetic Study A: Formation of Hydrazone 11. Dry samples of BAL-AMP (5 mg, 0.73 mmol of CHO per g of resin) (**6b**) were weighed into glass vials (15 × 45 mm, 1 dram) and a solution of FmPH·TFA (**4**)/DIEA (3 equiv each) in DMF−CH_2Cl_2 (1:1, 0.5 mL) was added to each vial, followed immediately by TMOF (0.5 mL). The suspensions were mixed on an orbital mixer at 80 °C, and reactions were stopped individually after 1, 2, 3, 4, 5, 7, 10, 20, 25, and 30 min. The cooled suspensions were transferred to fritted plastic syringes, washed, and processed by the experimental protocol under the subsection FmPH Quantitation. The calculated loadings, as determined by the amount of **13** quantified, were plotted as a function of time (Figure 2A, squares).

Kinetic Study B: Release of Dibenzofulvene Adduct from Hydrazone 11. Dry samples of resin **11** (5 mg) [prepared from AMP-BAL (**6b**), 0.73 mmol of CHO per g of resin] were transferred to fritted plastic syringes (3 mL each), swollen in CH_2Cl_2 (1.5 mL, 5 min), and washed thoroughly with DMF (5 × 1.5 mL). The resins were then treated with piperidine−DMF (1:1, 1 mL) at 25 °C, and after 1, 2, 3, 4, 5, 7, 10, 15, 20, and 30 min, each reaction was stopped and the released piperidine−dibenzofulvene adduct (**13**) was quantified by the experimental protocol given under the subsection FmPH Quantitation. The amounts of Fm remaining on the support (calculated indirectly from the amount of **13** released subsequently) were plotted as a function of time (Figure 2B, circles).

Kinetic Study C: Formation of Imine 7. This experiment was conducted similar to what was described in the subsection Kinetic Study A, except that H-Gly-OFm·HCl (**5**)/DIEA (3 equiv each) was used instead of FmPH·TFA (**4**)/DIEA; reactions were stopped after 1, 2, 3, 4, 5, 7, 10, 15, 20, and 30 min, 24 h, and 48 h, respectively. The loadings of resins **7**, as determined by the amount of **13** quantified, were plotted as a function of time (Figure 2C, triangles).

Fmoc and FmPH Quantitation Comparison: Preparation of Partially Substituted PALdehyde Supports (16). PEG-PS (0.53 mmol of NH_2 per g), AMP (0.73 mmol of NH_2

per g), and CLEAR (0.46 mmol of NH_2 per g) supports [all obtained as the amine hydrochloride salts, approximately 50 mg each] were swollen in CH_2Cl_2 (1.5 mL, 5 min) and washed thoroughly with DMF−DIEA (4:1, 5 × 1.5 mL). PALdehyde[86] plus Fmoc-Gly-OH were mixed in a 1:1 ratio (3 equiv combined carboxylic acid function) with HATU/DIEA (3 equiv each), dissolved in DMF (1.5 mL), and the resultant solutions were added to the respective resins which were rotated on an orbital shaker for 24 h at 25 °C. The partially substituted aldehyde supports (**16**) were washed with DMF (5 × 1.5 mL), MeOH (5 × 1.5 mL), and DMF (5 × 1.5 mL). The resins were then processed first by Fmoc quantitation[45,59,65] to *indirectly* determine the percent aldehyde, and then following Fmoc, the same resins, which contained residual aldehydes, were subjected to FmPH quantitation. The percent loading of aldehydes were calculated as described in footnotes *b* and *c* of Table 2.

Solid-Phase Reductive Amination: Preparation of 17. BAL-AMP (**6b**, 0.73 mmol of CHO per g of resin) (5 mg) was swollen in CH_2Cl_2 (1.5 mL, 5 min) and washed thoroughly with DMF (5 × 1.5 mL). A solution of $NaBH_3CN$ (2 mg, 18 μmol, 5 equiv), the primary amine (18 μmol, 5 equiv), and 1% HOAc in CH_2Cl_2−DMF−MeOH (2:1:1; 1 mL) was added to the support and the sample was rotated on an orbital shaker for 30 min at 25 °C. The resultant secondary amine supports (**17**) were washed with DMF (5 × 1.5 mL), MeOH (5 × 1.5 mL), and CH_2Cl_2 (5 × 1.5 mL). The resins were checked by the chloranil test[95] and the DNPH test,[45] and then subjected to FmPH quantitation to determine the amount of residual aldehyde. Percent conversions were calculated by using the procedure described in footnotes *a* and *b* of Table 3. The primary amines used were 2,6-dimethylaniline, aniline, benzylamine, butylamine, cyclohexylamine, H-Gly-O*t*Bu·HCl, and H-Phe-O*t*Bu·HCl.

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Supporting Information Available: General procedures, ^{1}H NMR and HRESI-MS spectra, and elemental analysis for compounds **4** and **10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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