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A convenient semicarbazide resin for the solid-phase synthesis of peptide ketones and aldehydes

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Abstract—Use of a semicarbazide resin for the solid-phase preparation of peptide ketones and aldehyde led to optimal results in terms of both purity of the final product and overall yield. This resin was prepared without complication by activation of the commercial available aminomethyl polystyrene with CDI at room temperature, followed by treatment with *tert*-butyl carbazate. Furthermore, the TNBSA colorimetric assay has been adapted for checking the incorporation of the carbonyl moiety onto hydrazine-based resins.

1. Introduction

Cysteine proteases can be traced to the era when life was based exclusively on sulfur. They are implicated in many essential biological processes, and are targets of great pharmaceutical interest due to their role in the pathogenesis of many diseases.[1,2] All cysteine proteases are characterized by a well-conserved active site with a Cys-His dyad. Simple inhibition of these enzymes can be achieved by blocking the active site Cys residue using a peptide.[3–7] Reversibly or irreversibly binding molecules can be used as cysteine protease inhibitors, which usually feature an electrophilic functionality such as a carbonyl group or Michael acceptor that can react with the nucleophilic cysteine residue. Thus, ketones,[4] fluoroketones,[6] and aldehydes[3,5] have been reported as cysteine protease inhibitors.

Caspases, a family of cysteine proteases that are implicated in the apoptotic cascade, are of particular interest. Pharmacological caspase inhibition has been demonstrated to prevent neuronal cell death,[8] thereby fueling the syntheses of a great variety of caspase inhibitors.[9] While solid-phase combinatorial chemistry has been an important tool in the discovery of caspase inhibitors, its efficacy in this domain requires novel building blocks such as amino ketones[10] as well as appropriate solid supports.

In previous work, aldehyde or ketone-based caspase inhibitors were prepared on solid-phase, taking advantage of the β-carboxyl group of a related Asp C-terminal moiety present in the majority of these inhibitors. Thus, the first building block was attached to the solid support through the β-carboxylate moiety.[11] A fundamental shortcoming of this approach is the instability of the chiral center adjacent to the aldehyde or ketone, though this can be overcome by protecting the carbonyl moiety as a ketal, enol ether or enamine before attachment to the solid support.[12] Nonetheless, these protection strategies in turn have inherent drawbacks, namely low yields of attachment and/or final cleavage.

In contrast, hydrazine-based linkers have appeared as a better alternative, as suggested by publications of syntheses whereby the carbonyl moiety is protected in solution by an imine bond presented in a bifunctional linker, and the linker is subsequently attached to a polystyrene-based solid support.[13,14] Detachment of target compounds from the resin is achieved under acidic conditions. More recently, hydrazine linkers have been developed in which aldehydes and ketones were attached directly to the solid support.[15] One of these is based on aromatic oximes,[15c] and the authors proposed that by careful selection of

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the aromatic substitution (i.e., electron-donating/withdrawing), it is possible to control the degree of sensitivity of the resin-bound aryloxime to hydrolysis. Ellman et al. developed an elegant semicarbazone resin for the solid-phase preparation of ketones.[16,17]

As the Ellman strategy was based on a hydroxymethyl ArgoGel resin, which involves the use of an expensive polyethylenglycol resin and a hydroxy function much less reactive than amino functions toward carbonic acid derivatives,[18] our group decided to evaluate the performance of various polystyrene based resins in the anchoring of carbonyl moieties.

2. Results and discussion

The resins that were screened comprised semicarbazone **1**, hydrazinomethyl **2** and two semicarbazides, **3** and **4** (Fig. 1). The resins were evaluated for the incorporation of phenylpropyl ketone of an aspartidyl moiety **5**.[10a]

Resin **1** was prepared by following a slightly modified version of Ellman's protocol (Scheme 1).

Thus, hydroxymethyl polystyrene resin was activated with CDI, but *tert*-butyl carbazate was used instead of anhydrous hydrazine. Although, the semicarbazone is less nucleophilic than the hydrazine, it was thought that the monoprotected hydrazine derivative would avoid some extra cross-linking of the solid support, which would in turn lower the functionalization of the resin and could change its physical properties to make it more reticulate. The derivatization of the resin was deemed satisfactory using colorimetric assays[19] as well as IR spectroscopy (i.e., disappearance of OH signal and appearance of carbonyl signal). After removal of the Boc protecting group with TFA–DCM (1:1), followed by neutralization with DIEA–DCM (1:19), the resin was treated with a solution of the phenylpropyl ketone of Asp **5**[10a] in DCM for 16 h at 25 °C. The loading was determined after elimination of the Fmoc group with piperidine–DMF (1:4) by UV analysis of the piperidine adduct (ε_{301}: 7800 M^{-1} cm^{-1}). Under those conditions, and even after several trials, the ketone was never incorporated with a yield greater than 40% (Table 1).[20]

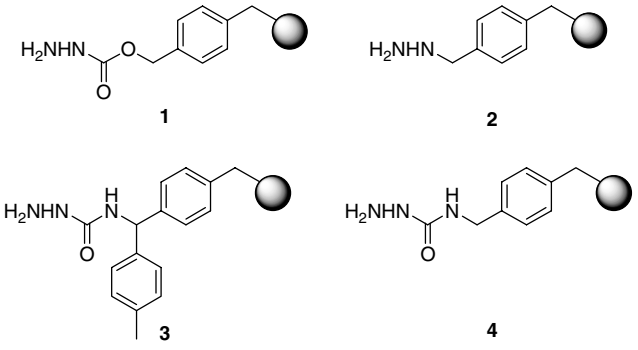
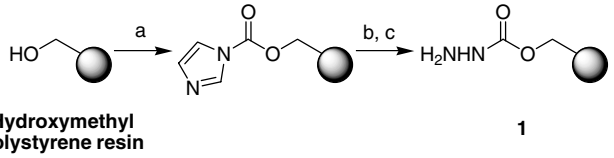


Figure 1. Hydrazine-based resins explored.



Scheme 1. Preparation of semicarbazone resin. Reagents and conditions: (a) CDI, DMF, 3 h; (b) Boc-NH-NH_2, DMF, 3 h; (c) TFA–DCM (1:1), 1 h.

The hydrazinomethyl resin **2** was obtained by treating a Merrifield resin with aq hydrazine in DMF for 16 h, and the synthesis was evaluated at both 25 and 80 °C (Table 2). Incorporation of the ketone was carried out as above and the best results were obtained when resin **2** was synthesized at 80 °C with a 84% yield.

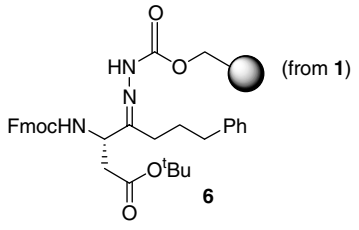
A potential problem of using hydrazinomethyl resin **2** is the presence of the rather reactive NH group, which can lead to the formation of side products.

The same protocol used for the preparation of semicarbazone **1** was employed for the two semicarbazide resins **3** and **4** using *p*-methylbenzhydrylamine and aminomethyl polystyrene resins, respectively. Again, the resins **3** and **4** were treated with a solution of the phenylpropyl ketone of Asp **5** in DCM during 16 h at 25 °C (Table 3).

The lower performance of the *p*-methylbenzhydryl amine polystyrene **8** with respect to the aminomethyl polystyrene **9** can be attributed to the lower nucleophilicity of the amino function in resin **8**. This result reinforces the idea of choosing the most TFA stable aminomethyl in front of the *p*-methylbenzhydrylamine as base solid support to avoid undesirable cleavage at the methylbenzhydryl site.[21]

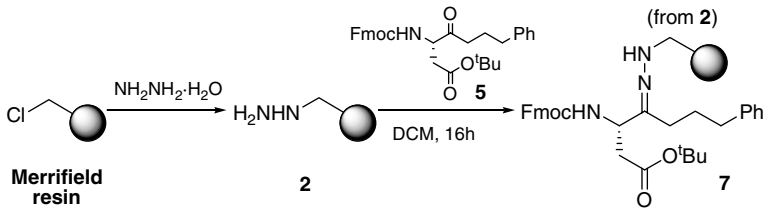
The incorporation of the ketone onto all of these resins was also monitored using the TNBSA colorimetric assay, which was first developed for the detection of first and secondary amines.[22] Resins containing a free hydrazine moiety turn black, whereas those containing no free hydrazine are visualized as colorless.

Table 1. Preparation of semicarbazone resin **1**



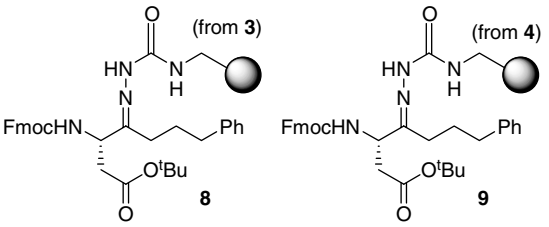
Entry	CDI/equiv	Boc-NH-NH_2/equiv	Yield/%
6a	5	5	32
6b	10	5	35
6c	10	10	36

Table 2. Synthesis of hydrazinomethyl polystyrene resin **3**



Entry	Hydrazine/equiv	Temperature/°C	Yield/%
7a	20	rt	26
7b	20	80	84

Table 3. Synthesis of semicarbazide resins



Entry	CDI/equiv	Boc-NH-NH_2/equiv	Yield/%
8[a]	5	5	43
9[b]	5	5	99

[a] From *p*-methylbenzhydrylamine PS (1% DVB) from NovaBiochem.
[b] From aminomethyl PS (1% DVB) from NovaBiochem.

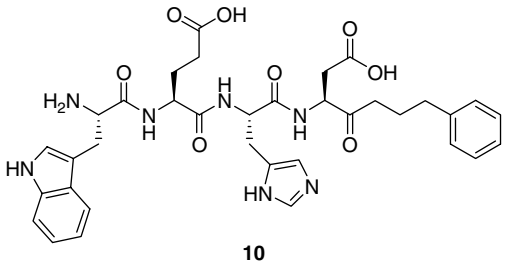


Figure 2. Chemical structure of tetrapeptide ketone **10**.

The two best resins prepared in this study, **7b** and **9** were further evaluated by employing them to synthesize tetrapeptide ketone **10** (Fig. 2), a known caspase-1 inhibitor.[23]

All of the amino acids were incorporated employing a Fmoc-^{t}Bu strategy, and the final product was cleaved using TFA–H_2O (4:1). Figure 3 shows the HPLC chromatogram of crude material obtained from each resin. The best purity was for the peptide prepared from resin **9** with an overall yield of 75% (Table 3).

Resin semicarbazide **4** was also used to incorporate with quantitative yields both the aldehyde and the chloromethylketone of the Fmoc-aspartidyl residue with the β-carboxyl group protected as *t*-Bu ester. The corresponding resins were used for the preparation of peptide libraries containing those motives as C-terminal functions.

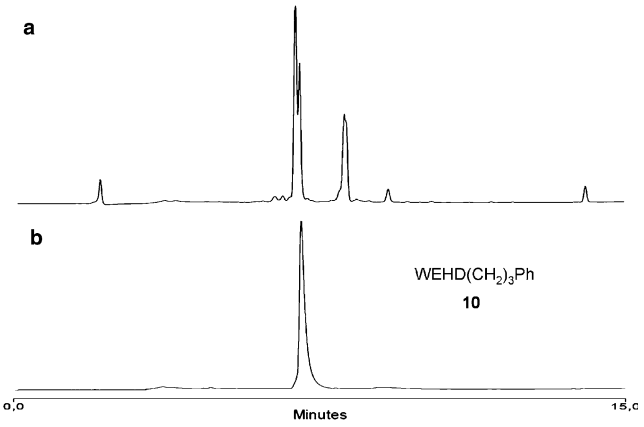


Figure 3. Chromatograms obtained analyzing the crude after cleavage of resins **6b** (a) and **9** (b). Conditions: symmetry C_{18} (5 μm, 4.4 × 150 mm) column, linear gradient 0–100% B in 15 min, followed by 5 min, flow 1 mL/min, detection 220 nm, (A: H_2O + 0.045% TFA, B: MeCN + 0.036% TFA).

3. Conclusions

Various types of hydrazine-based polystyrene solid support were synthesized through optimized protocols and tested for the anchoring of carbonyl functions. Among the resins, semicarbazide **4** demonstrated superior performance regarding both purity of the final product and overall yield. This resin is smoothly prepared at room temperature by activation of the commercial available aminomethyl polystyrene with CDI, followed by treatment with *tert*-butyl carbazate, which can avoid an extra cross-linking of the resin. In addition, the TNBSA colorimetric assay has been adapted for checking the incorporation of the carbonyl moiety to the hydrazine-based resin. In summary, resin **4** should facilitate the preparation of libraries of ketone or aldehyde containing molecules for the discovery of caspase inhibitors or other compounds of biological interest.

4. Experimental procedures

4.1. General

The DMF used was of peptide synthesis grade and the DCM was passed through basic alumina before use.

Other solvents and reagents were used as received directly from the vendor without any special treatment. ^{1}H NMR spectra were obtained on a Varian Gemini 200 MHz spectrometer in $CDCl_3$ unless stated otherwise. Chemical shifts are reported in ppm (δ units) downfield from internal tetramethylsilane or the appropriate solvent signal [$(CD_3)_2CO$].

4.2. Preparation of the semicarbazone resin 1

A solution of CDI (62 mg, 5 equiv) in DMF (3 mL) was added to a previously DMF-washed hydroxymethyl polystyrene resin (100 mg, 0.98 mmol/g) and the mixture was shaken at 25 °C for 3 h. The resins were washed with DMF (3×), a solution of Boc-NH-NH_2 (65 mg, 5 equiv) in DMF (3 ml) was added, and the mixture was left to shake for 3 h at 25 °C. Subsequent washings with DMF (3×) and DCM (3×) gave the Boc-protected resin ready for storage. Before the resins can be used, the Boc must be removed by treatment with a TFA–DCM (1:1) solution for 1 h at 25 °C.

4.3. Preparation of hydrazinomethyl resin 2

$NH_2NH_2{\cdot}H_2O$ (50 mL, 20 equiv) was added to a vessel containing Merrifield resin (100 mg, 0.5 mmol/g) swelled in DMF. The vessel was sealed and stirred with a shaker for 16 h at 80 °C. After this time, the resin was washed with DMF (3×) and DCM (3×).

4.4. General method for the preparation of semicarbazide resins 3 and 4

A solution of CDI (5 equiv) in DMF (3 mL) was added to a previously DMF-washed amino polystyrene resin (100 mg, 0.8 mmol/g and 1.1 mmol/g for **3** and **4**, respectively) and the mixture was shaken at 25 °C for 3 h. The resins were washed with DMF (3×), a solution of Boc-NH-NH_2 (5 equiv) in DMF (3 mL) was added, and the mixture was left to shake for 3 h at 25 °C, followed by washings with DMF (3×) and DCM (3×) to give the Boc-protected resins ready to be stored. Before the resins can be used, the Boc must be removed by treatment with a TFA–DCM (1:1) solution for 1 h at 25 °C.

4.5. Synthesis of *tert*-butyl-(3*S*)-3-[(9*H*-9-fluorenylmethoxy)carbonyl]amino-4-oxo-7-phenylheptanoate (5)

The synthesis was done following a previously described protocol[10a] to give the target compound as a yellow oil in 73% yield. ^{1}H NMR (200 MHz, $(CD_3)_2CO$) δ 7.76 (d, $J = 7.3$ Hz, 2H) 7.59 (d, $J = 7.3$ Hz, 2H) 7.48–7.09 (m, 9H) 5.88 (d, $J = 8.8$ Hz, 1H) 4.55–4.3 (m, 3H) 4.22 (t, $J = 6.6$ Hz, 1H) 2.88 (dd, $J = 4.4$, 16.8 Hz, 1H) 2.79–2.45 (m, 5H) 1.92 (q, $J = 7.3$ Hz, 2H) 1.42 (s, 9H). MS (ESI, positive ion): *m*/*z* Calculated: 514.3 $[M+1]^+$. Found: 514.6 $[M+1]^+$.

4.6. General method for the incorporation of ketones onto hydrazine based resins

Compound **5** (3 equiv) in DCM (3 mL) was added to the corresponding resin and the mixture was stirred in a shaker for 16 h at 25 °C, then the resin was washed with DCM (3×), DMF (3×) and DCM (3×).

4.7. H-WEHD-$(CH_2)_3$-Ph (10)

Fmoc-His(Trt)-OH, Fmoc-Glu(O*t*-Bu) and Fmoc-Trp(Boc)-OH (5 equiv each) were coupled to **9** with DIC (5 equiv) and HOBt (5 equiv) using a standard Fmoc-^{t}Bu protocol. consecutively. The final cleavage was done with a TFA–H_2O (4:1) solution. t_R: 7.0 min [conditions: symmetry C_{18} (5 μm, 4.4 × 150 mm) column, linear gradient 0–100%B in 15 min, followed by 5 min, flow 1 mL/min, detection 220 nm, (A: H_2O + 0.045% TFA, B: MeCN + 0.036% TFA)] MS MALDI-TOF: *m*/*z*. Found: 689.59 $[M+1]^+$, 711.60 $[M+Na]^+$, 728.59 $[M+K]^+$.

Abbreviations used and not defined in the text are: ^{t}-Bu, *tert*-butyl; Fmoc, 9-fluorenylmethyloxycarbonyl; Boc, *tert*-butoxycarbonyl; CDI, *N*,*N*′-carbonyldiimidazole; DCM, dichloromethane; DMF, *N*,*N*-dimethylformamide; Ph, phenyl; Amino acid symbols denote the L-configuration.

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