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Hydrazone and Hydrazone-Containing *N*-Substituted Glycines as Peptoid Surrogates for Expedited Library Synthesis: Application to the Preparation of Tsg101-Directed HIV-1 Budding Antagonists

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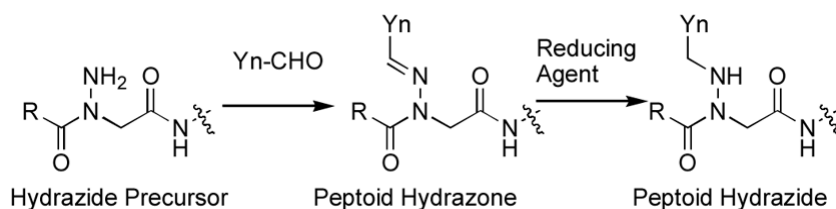
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Abstract



Replacing the Pro6 in the p6^{Gag}-derived 9-mer “P-E-P-T-A-P-E-E” with *N*-substituted glycine (NSG) residues is problematic. However, incorporation of hydrazone amides (“peptoid hydrazones”) can be readily achieved in library fashion. Furthermore, reduction of these hydrazones to *N*-substituted “peptoid hydrazides” affords a facile route to library diversification. This approach is demonstrated by application to Tsg101-binding compounds designed as potential HIV budding antagonists.

“Peptoids”¹ are polymers of *N*-substituted glycine residues (NSG **1**; Scheme 1), which have emerged as an important class of peptide mimetic that can retain bioactivity while exhibiting resistance to proteolytic degradation.^{2,3} Peptide-peptoid hybrids containing both peptide and NSG residues have also shown significant utility.⁴ One example is the replacement of key Pro residues with NSG residues in WW and SH3 domain-binding peptides. These signaling domains specifically recognize Pro-rich sequences and NSG residues can provide greater selectivity and affinity than achieved with parent Pro residues.⁵ This latter work is particularly relevant to recent findings that efficient budding of HIV from the plasma membrane requires binding of the host Tsg101 protein with the short peptide motif, “Pro-Thr/Ser-Ala-Pro” (“PTAP”) located within the p6 region of the viral Gag protein.⁶ An NMR solution structure of the PTAP-containing p6^{Gag}-derived 9-mer peptide, “P-E-P-T-A-P-E-E” bound to Tsg101 indicated that the most important interactions were derived from the “Ala-Pro”

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Supporting Information Available: Procedures and binding data for Tsg101 FA binding assays and synthetic experimentals for dipeptides **8a**, **16a** and **16b** and hydrazones **11a** – **11r** and hydrazides **12a** – **12r** are provided. This material is available free of charge via the Internet at <http://pubs.acs.org>.

residues, which are in deep depressions reminiscent of the “Xxx-Pro” pockets used by WW and SH3 domains.⁷ The similarities shared by Tsg101 and SH3 domains in their recognition of key Pro residues suggests that replacement of Pro residues with NSG constructs may also be beneficial for the preparation of PTAP-based Tsg101 binding inhibitors.⁷

The solid-phase synthesis of NSG-containing libraries is usually achieved by the “submonomer approach,” in which the amino terminus of the growing peptide chain is bromoacetylated, then reacted with amines to yield the corresponding NSG residues (Scheme 1).⁸ However, this approach can be disadvantageous for long or difficult sequences.⁹ We found such to be the case with the p6^{Gag}-derived 9-mer sequence, “P-E-**P**-T-A-**P**-P-E-E” where the Pro3 residue could be satisfactorily replaced by an NSG residue using the submonomer approach, but replacement of the critical Pro6 residue (changing P-E-**P**-T-A-**P**-P-E-E to P-E-**P**-T-A-[NSG]-P-E-E) proved to be problematic. Although the reasons for this difficulty appeared to be sequence-related and steric in nature, modifications that would be expected to ameliorate steric crowding, such as replacement of the Pro3 residue by Ala (ie, P-E-**P**-T-A-[NSG]-A-E-E) or resorting to a C-terminal pseudo-proline strategy¹⁰ (for P-E-**P**-T-A-[NSG]-P-E-E-S), failed to overcome the problems. In order to work around the impasse presented above, we envisioned substituting the traditional peptoid NSG unit (**1**) with hydrazone amides to yield “peptoid hydrazones” of type **4** (Scheme 2).

Peptoid hydrazones would be advantageous over traditional *N*-alkyl NSG residues for library synthesis, since variation of each *N*-alkyl NSG residue requires the separate elaboration of the entire peptide sequence amino-terminal of the NSG residue. On the other hand, libraries of peptoid hydrazones could be constructed by reacting a series of aldehydes (Yⁿ-CHO) with a single completed peptide bearing an unsubstituted hyrazide (**3**). Furthermore, subjecting the resulting peptoid hydrazones **4** to reducing conditions could lead to library diversification by formation of the corresponding *N*-substituted peptoid hydrazides (**5**). Although several variations on traditional peptide amide bonds have been reported, including azapeptoids,¹¹ urea peptoids,¹² amino-oxypeptoids,¹³ beta peptoids,¹⁴ hydrazine (retro)-peptoids,¹⁵ and azapeptides,¹¹ hydrazone amides have found very limited use.¹⁵ The current work represents one of the first systematic examinations against an important biological target of mixed peptide-peptoid constructs utilizing hydrazone (**2**) and hydrazide (**4**) based NSG residues.

The objective of the current study was to prepare a library of Tsg101-directed peptides of the sequence “FTPP-E-P-T-A-**X**-P-E-E-amide,” where “X” represents “N(R-C=N)CH₂C(O)” and “FTP” stands for amino-terminal “fluorescein thiourea pentanoyl” functionality. The latter would be required for analysis of Tsg101 binding affinities using fluorescence anisotropy.¹⁶

The first attempt at solid-phase synthesis used a modified sub-monomer approach that involved reacting *tert*-butyl carbazate with the *N*-bromoacetylated peptide chain. However, as we found previously with the unsuccessful sub-monomer synthesis of the corresponding *N*-alkyl NSG-containing peptides (see above), coupling failed with the next amino acid, *N*-Fmoc Ala. Therefore, the preformed dipeptide, *N*-Fmoc Ala-N(NHBoc)CH₂CO₂H (**9**), was prepared by solution chemistries (Scheme 3) and incorporated into the growing peptide chain. The remainder of the peptide sequence was constructed using standard Fmoc solid-phase protocols to provide the unsubstituted hydrazide **10** following cleavage from the resin and HPLC purification. This served as a common precursor for hydrazone library construction (Figure 1). Although a similar dipeptide protocol could be used to circumvent problems associated with *N*-alkyl NSG incorporation (see below), the approach would be extremely tedious by requiring unique dipeptide construction, incorporation and subsequent chain elongation for each *N*-alkyl NSG-containing final product.

With the unsubstituted hydrazide **10** in hand, condensation with a variety of aromatic and aliphatic aldehydes was achieved by gently shaking **10** with excess aldehyde (10 equivalents) in 50% aqueous acetonitrile (overnight). Direct purification by reverse phase HPLC

(0.03% TFA acetonitrile/H₂O) gave the peptide hydrazones **11a** – **11q** (Figure 1). Adequate yields were obtained for all aldehydes examined and good HPLC separation of product hydrazones **11** from other reaction components was achieved in facile fashion. The peptoid hydrazones were stable when stored at –20° C and stability was adequate for biochemical analysis of Tsg101 binding affinities using a fluorescence anisotropy assay.^{17,18} Treatment of the peptoid hydrazones **11** in 50% aqueous acetonitrile (0.1% TFA) with a 20-fold excess of NaBH₃CN at room temperature overnight yielded the hydrazides **12a** – **12q** (Figure 1), which were evaluated for their Tsg101-binding affinities.¹⁷

The Tsg101 binding affinity of the parent PTAP-containing 9-mer is relatively weak ($K_d = 50 \mu\text{M}$, Table 1). Binding constants for the highest affinity peptoid-hydrazones (**11q** and **11p**) and peptoid-hydrazides (**12m**, **12k**, **12q** and **12p**) are also shown in Table 1. The highest affinity peptoid-hydrazone was found to be the *n*-butyl-containing **11p**, which exhibited a roughly 5-fold enhancement in affinity relative to the parent PTAP 9-mer ($K_d = 10 \mu\text{M}$, Table 1). The highest affinity peptoid-hydrazide was **12q**.

Peptide-peptoid hybrids based on NSG hydrazones and hydrazides are highly advantageous over traditional *N*-alkyl NSG constructs in that libraries can be rapidly constructed following solid-phase peptide synthesis. This allows a large number of structural variations to be easily examined. Because peptoid-hydrazones and peptoid-hydrazides present structural features that are not contained in their *N*-alkyl NSG counterparts, their biological activities may be different. Accordingly, it was of interest to prepare the *N*-alkyl NSG-containing **13p** as a homologue of the most potent peptoid-hydrazone (**11p**) in order to compare their Tsg101-binding affinities. Peptoid **13p** has an *n*-pentyl-containing *N*-alkyl NSG residue, which is the same chain length as found with the *n*-butyl-hydrazone in **11p**. The analogue containing an *N*-3,4-dimethoxyphenethyl NSG residue (**13d**) was also prepared as a “negative control” based on the poor affinities of **11d** and **12d**.

Since the synthesis of **13d** and **13p** by sub-monomer methodology was not possible (see above), the more labor-intensive dipeptide approach was used as already described above. Here, the *N*-Fmoc-protected peptoid dimers **16a** and **16b** were first prepared by solution chemistries (Scheme 4), then these *N*-alkyl NSG-containing dipeptide units were inserted at the appropriate point during solid-phase peptide synthesis to yield the desired **13d** and **13p**. It was found that **13d** bound negligibly ($K_d \gg 500 \mu\text{M}$), consistent with the poor affinities of the corresponding peptoid hydrazone **11d** and the peptoid hydrazide **12d**. On the other hand, **13p** was found to bind with higher affinity ($K_d 200 \mu\text{M}$), than **13d**, consistent with the good affinities of **11p** and **12p**, although, the absolute affinity of **13p** was less than its hydrazone and hydrazide congeners (Table 1).

In conclusion, reported herein is the facile construction of libraries of peptide-peptoid hybrids based on NSG hydrazones and hydrazides. This approach has several advantages over traditional *N*-alkyl NSG-containing peptide-peptoid hybrids. First, the structure of the NSG residue is varied in a final step. This is in contrast to *N*-alkyl NSG-containing peptide-peptoid hybrids where variation of each *N*-alkyl NSG residue requires the separate elaboration of the entire peptide sequence amino-terminal of the NSG residue. Second, it affords an alternative approach for long or difficult sequences where insertion of *N*-alkyl NSG residues may be problematic. Finally, it provides products that present structural features not found in *N*-alkyl NSG-containing peptide-peptoid hybrids. As in the current Tsg101 study, this may result in

higher affinities than the corresponding *N*-alkyl NSG-containing homologues. This approach may be useful in a variety of biological applications.

Supplementary Material

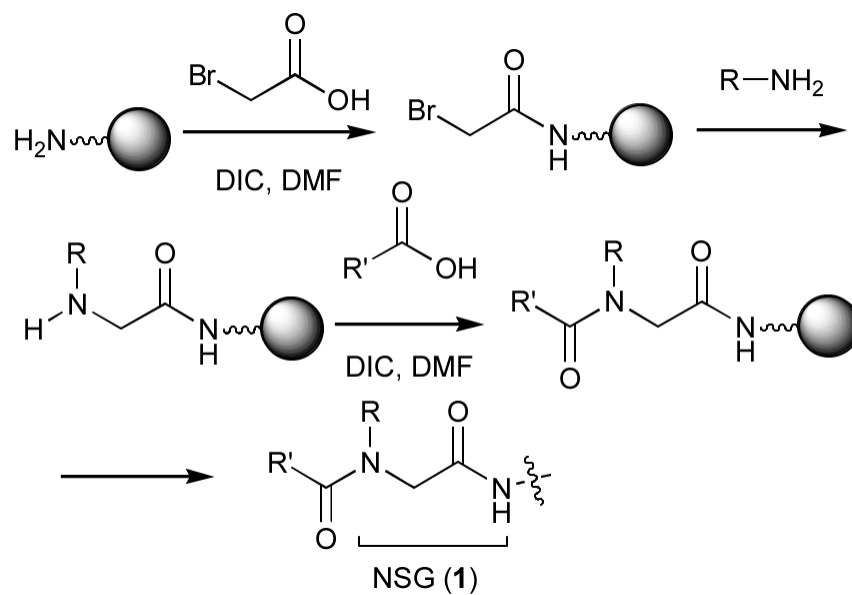
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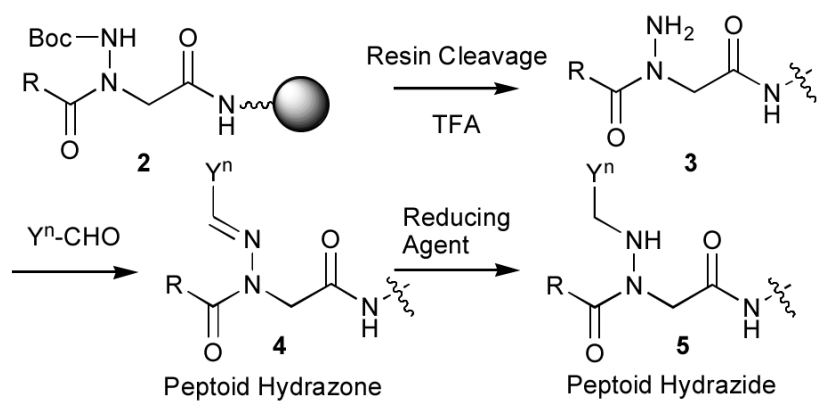
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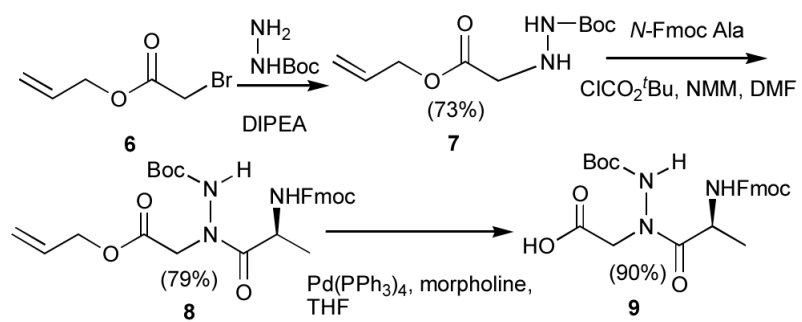
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17. A summary of the experimental procedure and a plot of FA binding data are provided in the Supporting Information.
18. Peptoid hydrazones **11** underwent slow decomposition when exposed to air at room temperature (approximately 50% decomposition after 4 days as indicated by HPLC analysis).



Scheme 1.
Peptoid synthesis by the “submonomer” approach.



Scheme 2.
Synthesis of peptoid hydrazones (2) and peptoid hydrazides (4) from a common hydrazide precursor (3).



Scheme 3.
Synthesis of protected dipeptide 9.

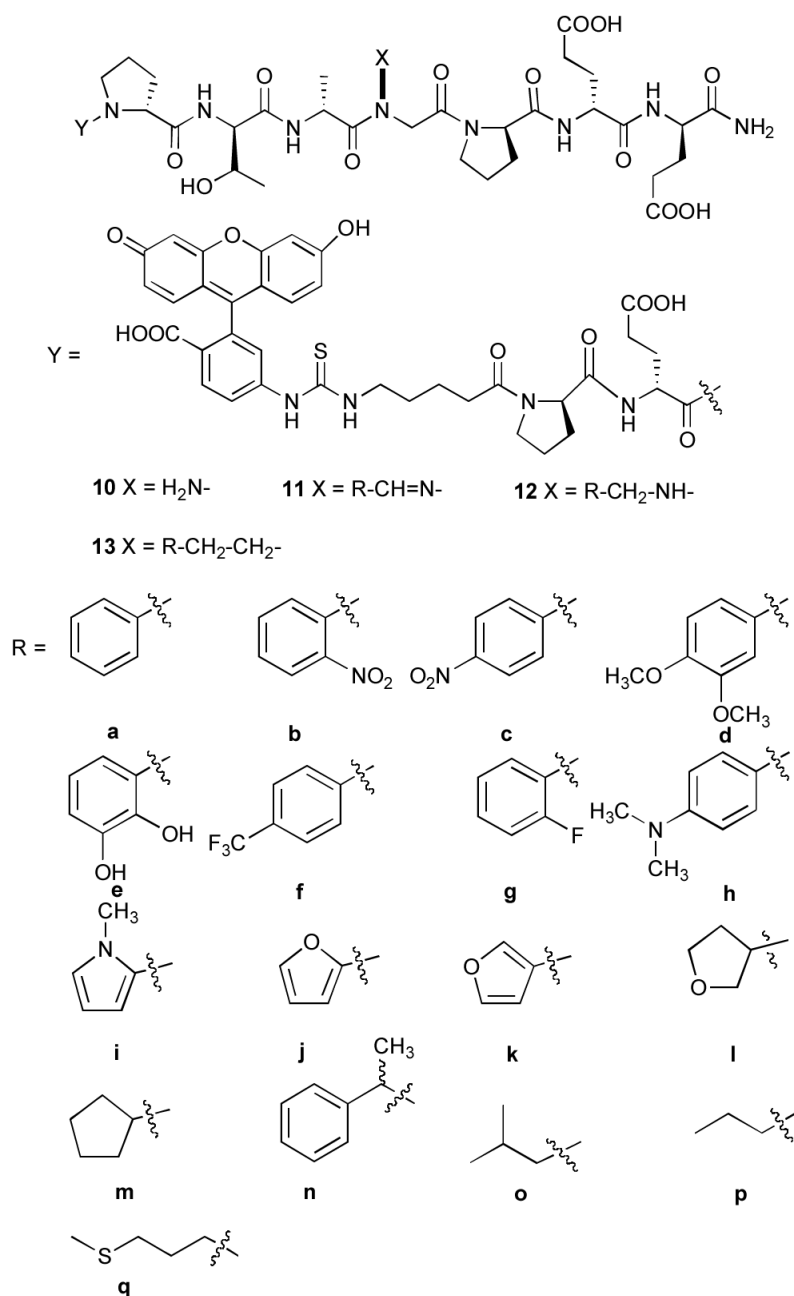
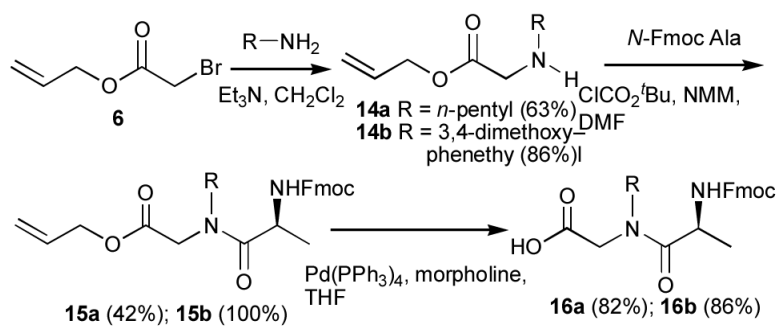


Figure 1.
intermediate hydrazide **10** and final products **12** and **13**.



Scheme 4.
Synthesis of peptoid intermediates **16a** and **16b**.

Table 1

Tsg101 binding affinities of select compounds.

compound	K_d (μM) ^a	compound	K_d (μM) ^a
11q	17.53 ± 1.81	12q	25.27 ± 8.06
11p	9.83 ± 1.25	13d	>>500
12k	46.30 ± 7.41	13p	200
12m	85.55 ± 13.08	PTAP ^b	53.5 ± 6.48
12p	60.3 ± 16.12		

^a Means ± SD determined from at least three independent experiments using at least two batches of protein.^b Wild-type peptide.