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Protected Aminooxyprolines for Expedited Library Synthesis: Application to Tsg101-Directed Proline-Oxime Containing Peptides

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Abstract

The stereoselective synthesis of aminooxy-containing proline analogues bearing Fmoc/Boc or Fmoc/Mtt protection that renders them suitable for incorporation into peptides using Fmoc protocols is reported. Acid-catalyzed unmasking at the completion of peptide synthesis yields free aminooxy–functionalities for oxime formation through reaction with libraries of aldehydes. This allows post solid-phase diversification strategies that may facilitate structure activity relationship studies.

The presence of proline residues in peptides and proteins can induce β-turn secondary structures that provide specific bioactivities.[1] This is exemplified by "proline rich motifs" (PRMs) that are involved with the formation of protein-protein complexes.[2] Libraries of proline analogues could serve as valuable tools for studying these interactions. However, the preparation of peptides bearing 3- and 4-substituted proline congeners has been limited by the need to synthesize each individual proline derivative in protected form prior to peptide synthesis.[3]

The incorporation of suitably protected aminooxy groups into orthogonally-protected proline residues could result in highly useful biochemical tools amenable to diversification through oxime formation by conjugation with libraries of aldehydes following the completion of peptide synthesis.[4–6] The aminooxy-containing proline analogues[7] **1** and **3**, bearing Fmoc and Boc or **2**, having Fmoc and Mtt protection, represent examples of proline derivatives that may be suitable for post solid-phase diversification through oxime formation (Figure 1). Reported herein are the synthesis of these new proline analogues and their application in a physiologically-relevant context where proline residues serve as key recognition elements.

Synthesis

Commercially available (4*R*)-4-hydroxy-*L*-proline (**4**) was protected as its *N*-Cbz, *O*-Bn derivative (4*R*)-**5** (Scheme 1).[8,9] Inversion of the (4*R*)-stereocenter was achieved in two steps involving the 4-nitrobenzoate ester (**6**)[10] under Mitsunobu conditions.[11] The high cost of (4*S*)-4 makes its preparation from the relatively inexpensive (4*R*)-**4** attractive.[12]

Mitsunobu transformation of the protected isomeric (4*R*)- and (4*S*)-4-hydroxyprolines (**5**)[13] was achieved smoothly with inversion of C4 stereochemistry to give the corresponding (4*S*)-

and (4*R*)- *N*-phthalimidoaminooxy compounds (**7**) in nearly quantitative yield (Scheme 2).[14,15]

The phthalimido groups of 7 were cleaved at room temperature using aqueous hydrazine and the free aminooxy primary amines were directly protected by treatment with Boc anhydride and triethylamine. Hydrogenation of the resulting derivatives (**8**)[16,17] over Pd•C removed both the *O*-Bn and *N*-Cbz protecting groups to yield the free amino acids, which were converted in situ to their N-Fmoc forms (**1**).[18,19] In this fashion starting from the commercially available free amino acid **4**, (4*S*)-**1** was obtained in 82% overall yield (7 steps) and (4*R*)-**1** was obtained in 53% overall yield (9 steps).

N-Boc-protection of aminooxy functionality is suitable for Fmoc-based solid-phase protocols used in combination with moderately labile acid-sensitive resins, where removal of the Boc groups is concomitant with cleavage of the peptide from the resin. However, when deprotection of the aminooxy group is desired without cleavage of the peptide from the solid-phase resin, more highly acid-labile methyltrityl (Mtt) protection (**2**) is appropriate. Toward this objective, the isomeric phthalimido-protected aminooxy intermediates (4*R*)-**7** and (4*S*)-**7** were treated with methylhydrazine, then directly reacted with 4-methyltrityl chloride to give the Mtt protected compounds (4*R*)-**9** and (4*S*)-**9** (Scheme 3).[20,21] Simultaneous hydrogenolytic cleavage of both the amino and carboxylic protecting groups (Pd•C/H_2 in EtOAc : MeOH) and subsequent treatment with Fmoc-OSu provided the final products (4*S*)-**2**[22] and (4*R*)-**2**[23] in 77% overall yield (7 steps) and 63% overall yield (9 steps) respectively, starting from commercially available **4**.

Preparation of the *N*-Boc derivatized isomeric 3-substituted products (**3**) began by protection of commercially available (3*S*)-3-hydroxy-*L*-proline (**10**) using the conditions described above for the conversion of **4** to (4*R*)-**5**. The attempted Mitsunobu transformation of the resulting (3*S*)-**11**[24] using *N*-hydroxyphthalimide under a variety of reaction conditions predominately gave the corresponding α,β-elimination material with no desired product. With the epimeric (3*R*)-**11**, prepared in near quantitative yield from (3*S*)-**11** by a two-step *p*-nitrobenzoyl ester stereo-inversion sequence,[25,26] a low yield (10%) of the desired 3-phthalimidooxy analogue (3*S*)-**13** could be obtained.[27,28] This allowed subsequent conversion to the final product (3*S*)-**3** (Scheme 4).[29]

Application of Aminooxy-Proline Analogues to Peptide Library Synthesis

In order to demonstrate the utility of post solid-phase diversification using aminooxy-proline analogues, we employed a Tsg101-binding peptide system. The UEV (ubiquitin E2 variant) domain of the human protein Tsg101 (tumor susceptibility gene 101) is recruited by major structural proteins of HIV-1 to facilitate viral budding. This involves the direct interaction of the Tsg101 UEV domain with a Pro-Thr-Ala-Pro ("PTAP") sequence in the viral Gagp6 protein.[30,31] Using a p6 – derived sequence, we had previously replaced critical Pro residues with hydrazone- and hydrazide-containing *N*-substituted glycines as peptoid surrogates that allowed the expedited synthesis of libraries of Tsg101-directed binding antagonists.[32] In our current study, we utilized the wild-type p6-derived "PEPTAPPEE" sequence to examine libraries of peptides having proline-oximes situated in place of the Pro7 residue (underlined).[33,34]

Libraries were generated from parent aminooxy-proline containing peptides prepared using the orthogonally protected residues described above. TFA-mediated cleavage of peptides from the resin gave the globally-deprotected aminooxy-proline containing peptides **15** – **17** (Table 1) which were purified by HPLC. Condensation of **15** – **17** with a series of aldehydes (**18a – t**) was conducted overnight in DMSO with a catalytic amount of AcOH and a molar ratio of peptide to aldehyde of 1:1. As indicated by HPLC, the reactions went to completion, yielding

proline-oxime containing peptides **19** – **21** in sufficient purity (>90%) for direct biological evaluation (Table 1).[35] Certain of these peptides (for example, **21o**; IC_{50} = 11 μM) exhibited up to five-fold higher Tsg101-binding affinity than wild-type peptide (K_d = 54 μM),[36] indicating the potential utility of the approach.

Conclusions

Reported herein are the design and synthesis of 3- and 4-aminooxy-substituted praline analogues suitably protected for Fmoc-based peptide synthesis. As demonstrated by application in a Tsg101-binding system, these non-natural amino acid analogues may be highly useful for post solid-phase diversification strategies that could facilitate rapid structure-activity relationship studies, potentially leading to new biologically active motifs.

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9. **(4R)-5**: $[\alpha]^{20}_D$ −53.2 (c 2.02, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 – 7.18 (m, 10 H), 5.23 – 5.11 (m, 2 H), 5.01 – 4.95 (m, 2 H), 4.53 (m, 1 H), 4.40 (m, 1 H), 3.65 – 3.51 (m, 2 H), 2.91 (brs, 1 H), 2.26 (m, 1 H), 2.02 (m, 1 H).
10. **6**: $[\alpha]^{20}_D$ −63.8 (c 1.36, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (AB, J_{AB} = 8.8, 2.0 Hz, 2 H), 8.03 – 7.97 (m, 2 H), 7.38 – 7.28 (m, 5 H), 7.22 – 7.15 (m, 5 H), 5.58 (m, 1 H), 5.25 – 5.02 (m, 4 H), 4.73 (dd, *J* = 9.2, 1.6 Hz, 0.5 H), 4.63 (dd, *J* = 9.2, 1.6 Hz, 0.5 H), 3.92 – 3.85 (m, 2 H), 2.66 – 2.46 (m, 2 H). ESI-MS (+VE) *m/z*: 527.1(M+Na)$^+$. HR-ESI/APCI MS cacld for $C_{27}H_{25}N_2O_8$ (M +H) $^+$: 505.1611, Found: 505.1606.

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13. **(4*S*)-5**: $[\alpha]^{20}_D$ −17.1 (c 2.56, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.24 (m, 10 H), 5.30 – 5.00 (m, 4 H), 4.49 (dd, *J* = 9.6, 1.6 Hz, 0.4 H), 4.42 (dd, *J* = 9.6, 1.6 Hz, 0.6 H), 4.34 (m, 1 H), 3.72 – 3.57 (m, 2 H), 3.27 (brs, 1 H), 2.29 (m, 1 H), 2.12 (m, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.12, 174.04, 154.98, 154.31, 136.36, 136.25, 135.23, 135.04, 128.58, 128.48, 128.44, 128.37, 128.27, 128.15, 128.08, 128.03, 127.90, 127.84, 70.97, 70.00, 67.46, 67.42, 67.38, 67.34, 67.25, 58.25, 57.88, 55.91, 55.57, 38.68, 37.80.
14. **(4*S*)-7**: Mp 130–132 °C. $[\alpha]^{20}_D$ −4.20 (c 0.88, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 – 7.81 (m, 2 H), 7.77 – 7.74 (m, 2 H), 7.45 – 7.23 (m, 10 H), 5.33 – 4.99 (m, 4 H), 4.94 (m, 1 H), 4.65 (dd, *J* = 10.0, 2.0 Hz, 0.5 H), 4.58 (dd, *J* = 9.6, 2.0 Hz, 0.5 H), 4.05 (m, 1 H), 3.92 (m, 1 H), 2.70 (m, 0.5 H), 2.66 (m, 0.5 H), 2.45 (m, 1 H). ESI-MS (+VE) *m/z*: 523.1(M+Na)$^+$. HR-ESI/APCI MS cacld for $C_{28}H_{24}N_2NaO_7$ (M+Na) $^+$: 523.1481, Found: 523.1475.
15. **(4*R*)-7**: Mp. 124–126°C. $[\alpha]^{20}_D$ −44.1 (c 1.09, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 – 7.82 (m, 2 H), 7.77 – 7.75 (m, 2 H), 7.40 – 7.18 (m, 10 H), 5.26 – 5.14 (m, 3 H), 4.99 – 4.96 (m, 2 H), 4.78 (m, 1 H), 4.08 (m, 1 H), 3.75 (dd, *J* = 12.8, 4.0 Hz, 1 H), 2.71 (m, 1 H), 2.15 (m, 1 H). ESI-MS (+VE) *m/z*: 523.1 (M+Na)$^+$. HR-ESI/APCI MS cacld for $C_{28}H_{24}N_2NaO_7$ (M+Na)$^+$: 523.1481, Found: 523.1473.
16. **(4*S*)-8**: $[\alpha]^{20}_D$ −37.6 (c 1.27, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.23 (m, 10 H), 6.82 (s, 0.50 H), 6.74 (s, 0.50 H), 5.23 – 4.99 (m, 4 H), 4.56 - 4.45 (m, 2 H), 3.78 (d, *J* = 12.8 Hz, 0.5 H), 3.73 (d, *J* = 12.8 Hz, 0.5 H), 3.59 (dd, *J* = 12.8, 4.8 Hz, 0.5 H), 3.56 (dd, *J* = 12.8, 4.8 Hz, 0.5 H), 2.51 (d, *J* = 4.8 Hz, 0.5 H), 2.48 (d, *J* = 4.8 Hz, 0.5 H), 2.21 (ddd, *J* = 18.8, 9.2, 4.8 Hz, 0.5 H), 2.16 (ddd, *J* = 18.8, 9.2, 4.8 Hz, 0.5 H), 1.41 (s, 4.5 H), 1.40 (s, 4.5 H). ESI-MS (+VE) *m/z*: 493.1(M +Na)$^+$. HR-ESI/APCI MS cacld for $C_{25}H_{30}N_2NaO_7$ (M+Na) $^+$: 493.1951, Found: 493.1955.
17. **(4*R*)-8**: $[\alpha]^{20}_D$ −30.4 (c 1.40, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (s, 0.56 H), 7.67 (s, 0.44 H), 7.30 – 7.10 (m, 10 H), 5.19 – 5.09 (m, 2 H), 5.02 – 4.94 (m, 2 H), 4.55 – 4.45 (m, 2 H), 3.93 (d, *J* = 12.4 Hz, 0.56 H), 3.82 (d, *J* = 12.4 Hz, 0.44 H), 3.56 (m, 1 H), 2.55 (m, 1 H), 1.99 (m, 1 H), 1.41 (s, 9H). ESI-MS (+VE) *m/z*: 493.1(M+Na)$^+$. HR-ESI/APCI MS cacld for $C_{25}H_{30}N_2NaO_7$ (M +Na)$^+$: 493.1951, Found: 493.1968.
18. **(4S)-1**: $[\alpha]^{20}_D$ −26.4 (c 0.65, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 – 7.55 (m, 6 H), 7.42 – 7.35 (m, 2 H), 7.33 – 7.29 (m, 2 H), 4.61 – 4.33 (m, 4 H), 4.22 (m, 1 H), 3.92 (d, *J* = 12.8 Hz, 0.4 H), 3.78 (d, *J* = 12.4 Hz, 0.6 H), 3.50 (m, 1 H), 2.79 (d, *J* = 14.8 Hz, 0.6 H), 2.50 (d, *J* = 14.4 Hz, 0.4 H), 2.36 (m, 0.4 H), 2.16 (m, 0.6 H), 1.46 (s, 9 H). ESI-MS (+VE) *m/z*: 491.1(M+Na)$^+$. HR-ESI/ APCI MS cacld for $C_{25}H_{28}N_2NaO_7$ (M+Na) $^+$: 491.1794, Found: 491.1788.
19. **(4*R*)-1**: $[\alpha]^{20}_D$ −41.2 (c 0.97, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.6 Hz, 1 H), 7.69 (d, *J* = 7.6 Hz, 1 H), 7.59 – 7.51 (m, 2 H), 7.41 – 7.25 (m, 4.35 H), 7.18 (0.65 H), 4.53 – 4.33 (m, 4 H), 4.24 (t, *J* = 6.8 Hz, 0.65 H), 4.13 (t, *J* = 6.8 Hz, 0.35 H), 3.96 (d, *J* = 12.6 Hz, 0.35 H), 3.85 (d, *J* = 12.6 Hz, 0.65 H), 3.53 (m, 1 H), 2.23 (m, 0.65 H), 2.10 (m, 0.35 H), 1.49 (s, 5.5 H), 1.46 (s, 3.5 H). ESI-MS (+VE) *m/z*: 491.1 (M+Na)$^+$. HR-ESI/APCI MS cacld for $C_{25}H_{28}N_2NaO_7$ (M+Na)$^+$: 491.1794, Found: 491.1800.
20. **(4*R*)-9**: $[\alpha]^{20}_D$ −30.5 (c 0.94, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 – 7.19 (m, 19 H), 7.16 (m, 1 H), 7.10 (d, *J* = 9.0 Hz, 2 H), 7.04 (d, *J* = 9.0 Hz, 2 H), 6.44 (s, 0.5 H), 6.41 (s, 0.5 H), 5.19 – 4.93 (m, 4 H), 4.35 (t, *J* = 8.0 Hz, 0.5 H), 4.24 (t, *J* = 8.0 Hz, 0.5 H), 4.10 (m, 1 H), 3.85 (d, *J* = 12.0 Hz, 0.5 H), 3.67 (d, *J* = 12.0 Hz, 0.5 H), 3.47 (dd, *J* = 8.0, 4.4 Hz, 0.5 H), 3.44 (dd, *J* = 8.0, 4.4 Hz, 0.5 H), 2.90 (s, 3 H), 2.25 (m, 1 H), 1.87 (dd, *J* = 8.2, 5.0 Hz, 0.5 H), 1.83 (dd, *J* = 8.2, 5.0 Hz, 0.5 H). ESI-MS (+VE) *m/z*: 649.3 (M+Na)$^+$.HR-ESI/APCI MS cacld for $C_{40}H_{38}N_2NaO_5$ (M+Na)$^+$: 649.2678, Found: 649.2674.
21. **(4*S*)-9**: $[\alpha]^{20}_D$ −25.5 (c 1.00, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.30 (m, 2 H), 7.25 – 7.18 (m, 16 H), 7.15 – 7.02 (m, 6 H), 6.40 (s, 0.5 H), 6.38 (s, 0.5 H), 5.18 – 5.05 (m, 2 H), 4.96 (d, *J* = 11.6 Hz, 1 H), 4.87 (d, *J* = 12.8 Hz, 0.5 H), 4.75 (d, *J* = 12.4 Hz, 0.5 H), 4.51 (dd, *J* = 8.0, 1.2 Hz, 0.5 H), 4.40 (dd, *J* = 8.0, 1.2 Hz, 0.5 H), 4.07 (m, 0.5 H), 4.03 (m, 0.5 H), 3.70 (d, *J* = 12.4 Hz, 0.5 H), 3.62 (d, *J* = 12.0 Hz, 0.5 H), 3.45 (dd, *J* = 12.4, 4.8 Hz, 0.5 H), 3.40 (dd, *J* = 12.4, 4.8 Hz, 0.5 H), 2.39 (t, *J* = 12.0 Hz, 1 H), 2.28 (s, 3 H), 2.00 (m, 1 H). ESI-MS (+VE) *m/z*: 649.2 (M +Na)$^+$.HR-ESI/APCI MS cacld for $C_{40}H_{38}N_2NaO_5$ (M+Na)$^+$: 649.2678, Found: 649.2665.

22. **(4*S*)-2**: $[\alpha]^{20}_{D}$ −17.9 (c 0.79, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 – 7.62 (m, 2 H), 7.50 – 7.36 (m, 2 H), 7.36 – 7.28 (m, 2 H), 7.28 – 7.12 (m, 14 H), 7.10 – 6.6 (m, 5 H), 4.40 – 4.00 (m, 4 H), 3.90 (m, 1 H), 3.40 (m, 1 H), 3.26 (m, 1 H), 2.60 (m, 1 H), 2.27 (s, 3 H) 1.90 (m, 1 H). ESI-MS (+VE) *m/z*: 647.2 $(M + Na)^+$. HR-ESI/APCI MS cacld for $C_{40}H_{36}N_2NaO_5$ $(M+Na)^+$: 647.2522, Found: 647.2518.
23. **(4*R*)-2**: $[\alpha]^{20}_{D}$ −35.6 (c 0.64, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, *J* = 7.8 Hz, 1 H), 7.66 (d, *J* = 7.8 Hz, 1 H), 7.54 (d, *J* = 7.2 Hz, 0.5 H), 7.48 (d, *J* = 7.2 Hz, 0.5 H), 7.42 (dd, *J* = 12.6, 7.4 Hz, 1 H),7.35 (t, *J* = 7.6 Hz, 1 H), 7.30 (dd, *J* = 11.8, 2.2 Hz, 1 H), 7.25 – 7.16 (m, 13 H), 7.13 (d, *J* = 8.4 Hz, 2 H), 7.08 – 7.02 (m, 3 H), 6.92 (d, *J* = , 0.5 H), 6.82 (d, *J* = , 0.5 H), 4.31 – 3.99 (m, 5 H), 3.80 (d, *J* = 12.0 Hz, 0.3 H), 3.60 (d, *J* = 12.0 Hz, 0.7 H), 3.32 (dd, *J* = 11.8, 4.2 Hz, 0.7 H), 3.24 (dd, *J* = 12.2, 4.2 Hz, 0.3 H), 2.30 (s, 3 H), 2.20 (m, 1 H), 1.87 (m, 0.7 H), 1.73 (m, 0.3 H). ESI-MS (+VE) *m/z*: 647.2 $(M+Na)^+$. HR-ESI/APCI MS cacld for $C_{40}H_{36}N_2NaO_5$ $(M+Na)^+$: 647.2522, Found: 647.2511.
24. **(3*S*)-11**: $[\alpha]^{20}_{D}$ −24.7 (c 1.64, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.20 (m, 10 H), 5.22 – 5.13 (m, 2 H), 5.09 – 5.00 (m, 2 H), 4.45 (m, 1 H), 4.42 (m, 0.5 H), 4.33 (m, 0.5 H), 3.75 – 3.62 (m, 2 H), 2.24 (brs, 1 H), 2.08 (m, 1 H), 1.91 (m, 1 H). ESI-MS (+VE) *m/z*: 356.1 $(M+H)^+$. HR-ESI/APCI MS cacld for $C_{20}H_{21}NNaO_5$ $(M+Na)^+$: 378.1317, Found: 378.1316.
25. **12**: $[\alpha]^{20}_{D}$ −91.4 (c 0.80, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (t, *J* = 9.2 Hz, 2 H), 7.93 (t, *J* = 9.2 Hz, 2 H), 7.41 – 7.26 (m, 5 H), 7.15 – 7.07 (m, 5 H), 5.75 (m, 1 H), 5.24 – 5.05 (m, 3 H), 4.98 – 4.78 (m, 2 H), 3.85 – 3.69 (m, 2 H), 2.38 – 2.25 (m, 2 H). ESI-MS (+VE) *m/z*: 527.1 $(M+Na)^+$. HR-ESI/APCI MS cacld for $C_{27}H_{24}N_2NaO_8$ $(M+Na)^+$: 527.1430, Found: 527.1428.
26. **(3*R*)-11**: $[\alpha]^{20}_{D}$ −33.0 (c 1.10, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 – 7.22 (m, 10 H), 5.25 – 5.00 (m, 4 H), 4.56 (m, 1 H), 4.48 (d, *J* = 6.8 Hz, 0.4 H), 4.44 (d, *J* = 6.8 Hz, 0.6 H), 3.68 (m, 1 H), 3.51 (m, 1 H), 2.85 (d, *J* = 5.0 Hz, 0.6 H), 2.79 (d, *J* = 5.0 Hz, 0.4 H), 2.04 (m, 2 H). ESI-MS (+VE) *m/z*: 378.1 $(M+Na)^+$. HR-ESI/APCI MS cacld for $C_{20}H_{21}NNaO_5$ $(M+Na)^+$: 378.1317, Found: 378.1312.
27. **13**: $[\alpha]^{20}_{D}$ + 1.25 (c 1.65, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 – 7.81 (m, 2 H), 7.78 – 7.75 (m, 2 H), 7.41 – 7.25 (m, 9 H), 7.16 (m, 1 H), 5.21 – 4.83 (m, 6 H), 3.87 (m, 1 H), 3.78 (m, 1 H), 2.32 (m, 1 H), 2.17 (m, 1 H). ESI-MS (+VE) *m/z*: 523.1 $(M+Na)^+$. HR-ESI/APCI MS cacld for $C_{28}H_{24}N_2NaO_7$ $(M+Na)^+$: 523.1481, Found:523.1481.
28. **14**: $[\alpha]^{20}_{D}$ −17.2 (c 0.74, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 – 7.19 (m, 11 H), 5.20 – 5.10 (m, 2H), 5.06 – 4.98 (m, 2 H), 4.71 (s, 0.45 H), 462 (s, 0.55 H), 4.48 (m, 1 H), 3.70 – 3.56 (m, 2 H), 2.13 (m, 1 H), 2.02 (m, 1 H), 1.41 (s, H). ESI-MS (+VE) *m/z*: 493.1 $(M+Na)^+$.HR-ESI/APCI MS cacld for $C_{25}H_{30}N_2NaO_7$ $(M+Na)^+$: 493.1951, Found: 493.1968.
29. **(3*S*)-3**: $[\alpha]^{20}_{D}$ −33.0 (c 0.60, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) 8.85 (brs, 1 H), δ 7.77 – 7.70 (m, 2.6 H), 7.56 – 7.50 (m, 2.4 H), 7.37 – 7.30 (m, 2 H), 7.29 – 7.23 (m, 2 H), 4.67 – 4.52 (m, 2 H), 4.44 (m, 1 H), 4.35 (m, 1 H), 4.21 (t, *J* = 7.2 Hz, 0.6 H), 4.12 (t, *J* = 7.2 Hz, 0.4 Hz), 3.69 – 3.58 (m, 2 H), 2.23 – 2.00 (m, 2 H), 1.44 (s, 9 H). ESI-MS (+VE) *m/z*: 491.1 $(M+Na)^+$. HR-ESI/APCI MS cacld for $C_{25}H_{28}N_2NaO_7$ $(M+Na)^+$: 491.1794, Found: 491.1800.
30. Demirov DG, Freed EO. Virus Res 2004;106:87. [PubMed: 15567490]
31. Mazze FM, Degreve L. Acta Virologica (English Edition) 2006;50:75.
32. Liu F, Stephen AG, Adamson CS, Gousset K, Aman MJ, Freed EO, Fisher RJ, Burke TR Jr. Org. Lett 2006;8:5165. [PubMed: 17048869]
33. Substitution of the Pro7 residue by alanine results in significant reduction of Tsg101-binding affinity, indicating the importance of the 7-position.
34. *N*-Terminal labeling with fluorosceine isothiocyanate linked via an aminovaleric acid spacer (FITC-Ava-) was employed.
35. A mixture of HPLC-purified aminooxy-proline containing peptide (**15** – **17**) (15 mM in DMSO, 10 μL), aldehdye (**18a – 18t**) (15 mM in DMSO, 10 μL) and acetic acid (70 mM in DMSO, 10 μL) was gently agitated at room temperature (overnight). Examination by HPLC showed the reactions had gone to completion to produce oxime products in ≥90% purity. Crude reaction mixtures were used directly for biological evaluation.
36. Based on a fluorescence anisotropy binding assay requirement of 20 mL of 5 mM peptide inhibitor solution, 0.20 mmol of TGR solid-phase resin is theoretically sufficient to prepare a 740 member oxime library.

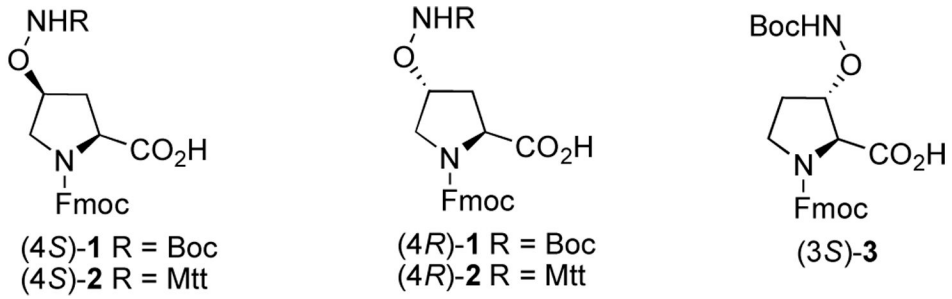
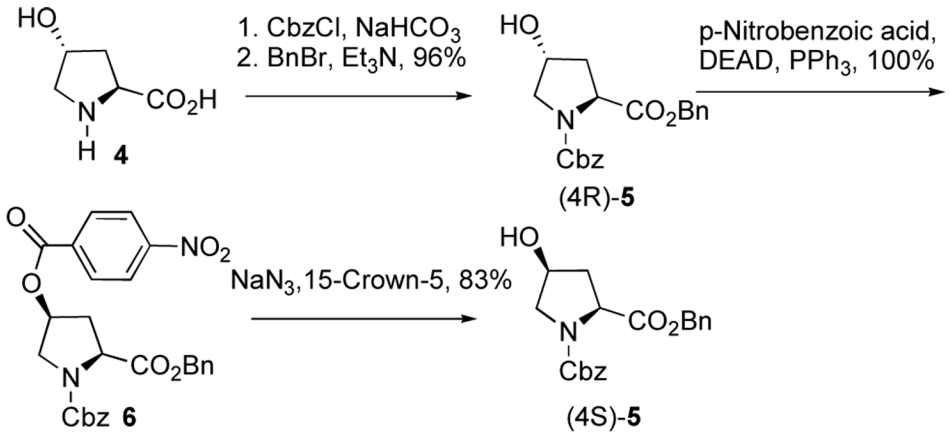
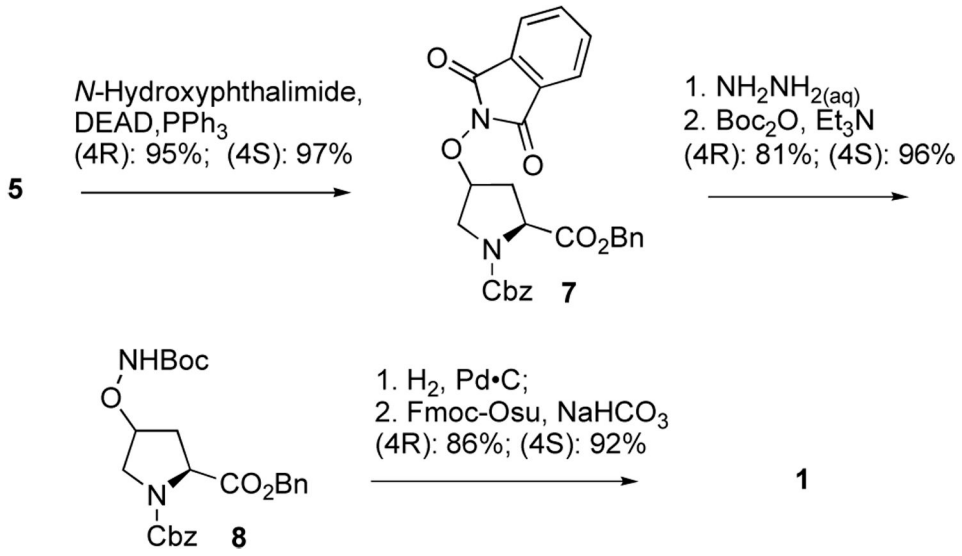


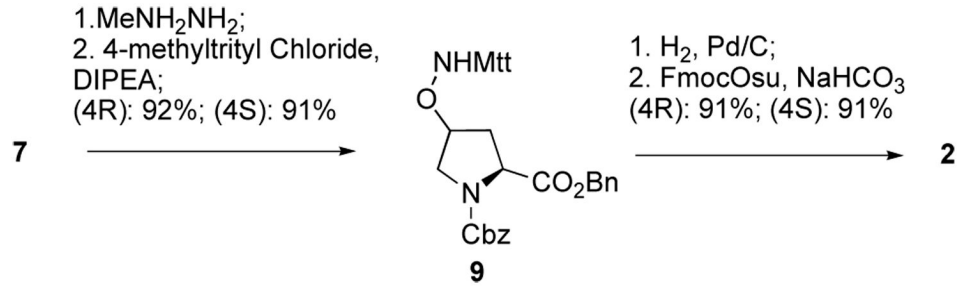
Figure 1.
Structures of orthogonally-protected proline analogues.



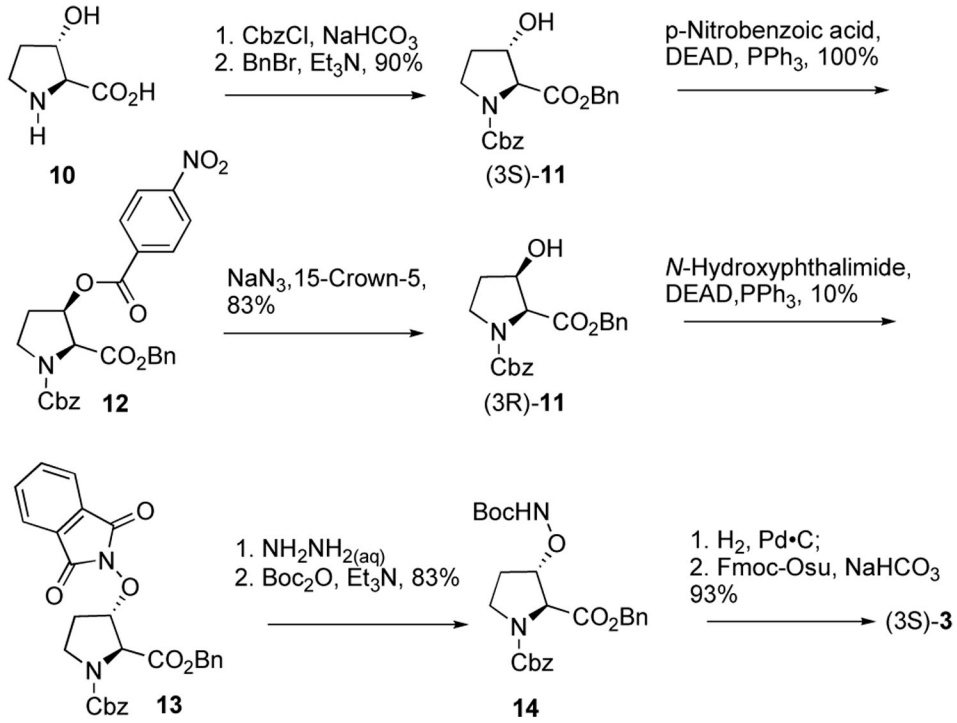
Scheme 1.



Scheme 2.



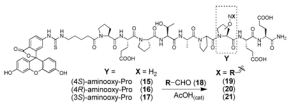
Scheme 3.

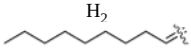
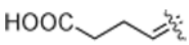
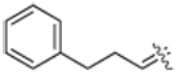
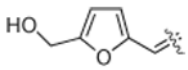
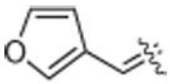
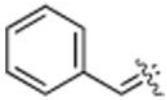
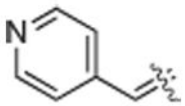
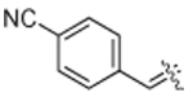


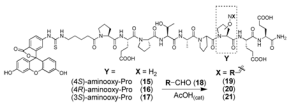
Scheme 4.

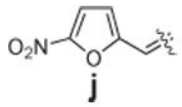
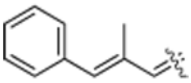
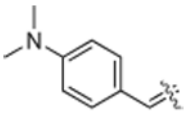
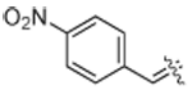
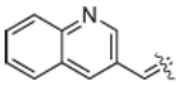
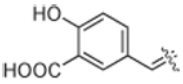
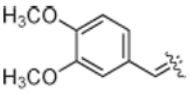
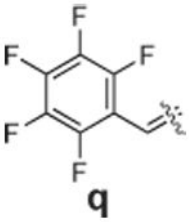
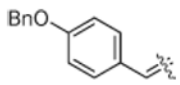
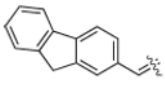
Table 1

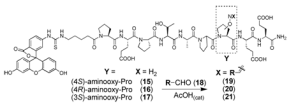
Tsg101-Binding Affinities of Proline-Oxime Containing Peptides Determined as in Reference 32.

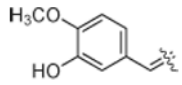


	IC_{50} (μM)		
X	**Peptide: 19**	**20**	**21**
H_2 	55 [**15**] nd*	43 [**16**] 54	21 [**17**] 41 *nd=Not determined
a 	42	41	23
b 	75	27	16
c 	83	26	20
d 	nd	26	25
e 	48	30	20
f 	64	26	15
g 	nd	29	22
h 	nd	20	22
i			



X	IC_{50} (μM) Peptide: 19	20	21
 j	nd	12	18
 k	58	13	12
 l	42	17	12
 m	91	21	16
 n	nd	25	19
 o	93	16	11
 p	114	16	22
 q	139	32	19
 r	54	30	18
 s	nd	27	17



	IC_{50} (μM)		
X	**Peptide: 19**	**20**	**21**
 **t**	nd	23	13