Solid-Phase Synthesis of Tetrahydropyridazinedione-Constrained Peptides

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Supporting Information

ABSTRACT: The design and solid-phase synthesis of tetrahydropyridazine-3,6-dione (Tpd) peptidomimetics derived from backbone-aminated peptides is reported. The described protocol features the synthesis of chiral α-hydrazino acids suitable for chemoselective incorporation into growing peptide chains. Acid-catalyzed cyclization to form the Tpd ring during cleavage affords the target peptidomimetics in good yield and purity. The scope of Tpd incorporation is demonstrated through the synthesis of constrained peptides featuring nucleophilic/electrophilic side chains and sterically encumbered α-substituted hydrazino acid residues.

Peptide backbone tethering strategies have proven useful for the elucidation of potential bioactive conformations and for enhancing peptide stability. The Freidinger–Veber lactam1 and related structures1c,2 represent important examples of cyclic constraints suitable for conformational scanning of linear peptides. These motifs often retain the native composition of the peptide backbone while restricting rotation about the ω and ψ bonds across a dipeptide subunit. Given the resurgence of peptides as lead structures for chemical probe and drug discovery, there remains a need for new constraints to interrogate local conformation. The ability to readily incorporate covalent tethers using solid-phase peptide synthesis (SPPS) techniques will greatly enhance their utility in structure–activity relationship studies.

In an effort toward synthetically accessible rigidified peptides, we targeted a tetrahydropyridazine-3,6-dione (Tpd) scaffold derived from an N-aminated aspartyl dipeptide precursor (Figure 1).3 The peptide backbone amino substituent would provide not only a nucleophilic handle for intramolecular cyclization but also an additional site for derivitization or potential hydrogen-bonding interactions. Engaging the pro-R Ca substituent as the complementary tethering site affords a Tpd constraint favoring the ψ torsion typical of a β-strand, whereas tethering via the pro-S site would favor a −120° ψ dihedral angle reminiscent of a type II′ β-turn. Thus, changing the stereochernistry of the presumed aspartate precursor could provide access to distinct conformational probes.

In contrast to peptoid (N-alkyl glycine oligomer) synthesis, the assembly of peptides bearing substituents on both Nα and Cα remains a considerable challenge. The synthesis of peptide tertiary amide (PTA) libraries on solid support was recently reported based on a submonomer approach.4 The on-resin syntheses of some backbone aminated peptides have also been described, though these have so far been limited to N-amino glycine-containing structures (“azapeptoids”)5 or to natural products harboring piperazic acid derivatives.6 To the best of our knowledge, there exists no general methodology for preparing Ca-substituted N-amino peptide derivatives by conventional SPPS.

To assess the feasibility of hydrazino acid acylation, we first screened a variety of coupling reagents for the reaction between the known esters7 and Fmoc-protected aspartate derivatives in solution (Scheme 1). Even with the less hindered N-amino glycine ester 1a, most common condensation reagents failed to effectively promote dipeptide formation (entries 1–3 and 6–8). The use of HATU gave moderate yields of 2a but afforded none of the desired product when N-amino alanine derivative 1b was employed as the substrate (entries 4 and 5). Preactivation of Fmoc-D-Asp(Bu)-OH or Fmoc-D-Asp(Me)-OH as the mixed anhydride6c gave good yields of 2a–d.
however, lower conversions were again observed with the α-substituted coupling partner 1b (entries 9−12). Yields improved with in situ generation of the Fmoc-protected amino acid chloride using triphosphgene. Optimal yields (>80%) and cleaner reactions were obtained when the acid chloride was preformed in the presence of thionyl chloride and isolated prior to condensation (entries 15 and 16).8 We found Fmoc-Asp(Me)-Cl to be a remarkably shelf-stable solid that showed no appreciable erosion of enantiopurity during short-term storage or coupling at elevated temperatures.9 Formation of the tetrahydropyridazinedione ring was achieved in moderate yield using DIC/DIEA/DMAP following Boc and tert-butyl ester deprotection of 2a. Alternatively, ring closure was effected via acidolysis of 2c and subsequent heating in toluene to give 3 in 53% yield.

We next explored a submonomer approach to prepare a model peptidomimetic containing a Tpd-Gly subunit on solid support. Analogous to well-established methods for peptoid synthesis,10 we employed α-bromoacetic acid as a building block and carried out subsequent SN2 displacement with tert-butyl carbazate (Scheme 2). Condensation with 3 equiv of Fmoc-D-Asp(Me)-Cl in the presence of 9 equiv of collidine (50 °C, 1 h × 3) was followed by standard peptide elongation and cleavage from the resin with 95:5 TFA/H2O. Under these conditions, acidolysis was attended by Tpd ring closure to give peptidomimetic 5 as the major product in 23% overall yield after RP-HPLC purification.

In the course of extending the submonomer approach to Cα-substituted (nonglycine) variants, we observed the formation of diastereomeric mixtures of Tpd peptidomimetics following cleavage. After ruling out potential racemization of the D-Asp chiral center, we confirmed the configurational instability of the intermediate chiral α-bromoacetamide under the conditions required for efficient SN2 displacement (Scheme 3). Reaction of 6a with 2 M tert-butyl carbazate in DMF at 50 °C followed by acidic cleavage from the resin afforded a 1:2:1 diastereomeric mixture of products (7), as judged by LCMS (acetone was added to the cleavage cocktail to provide more well-resolved hydrazine derivatives). The limitations of a bromoacetamide submonomer protocol for Tpd synthesis were further highlighted in our attempt to prepare the corresponding N-amino phenylalanine derivative on solid support. Incubation of 6b with tert-butyl carbazate gave rise to cinnamide 8 as the major product. Presumably, the competing elimination pathway would also complicate the synthesis of other Tpd-Xaa dipeptides capable of forming extended conjugated systems (Xaa = Tyr, Trp, His, Asp/Asn).

To circumvent these issues, we opted to incorporate Boc-protected hydrazino acid building blocks into growing peptide chains. Chiral α-hydroxy esters 9 were prepared by diazotization of the corresponding α-amino acids11 followed by esterification (Scheme 4). Installation of the tert-butyl carbazate group via the triflate and subsequent saponification gave acids 11a−f. Remarkably, solutions of 11 in 0.5 M aq. HCl partitioned readily into ethyl acetate, suggesting that the α-
The amino group is not easily protonated even at low pH. Previous failed acylation attempts (see Scheme 2) confirmed the poor nucleophilicity of this nitrogen and prompted us to explore chemoselective amidation at the C-terminus. Reaction of 11a with H-Phe-OMe in the presence of HATU afforded the desired N-amino dipeptide 12 in 84% yield without any detectable racemization or self-condensation of the α-hydrazino acid. Encouraged by this result, we reacted 5 equiv of 11a with resin-bound Tyr in the presence of 5 equiv of HATU, and 10 equiv of DIEA in DMF at 50 °C to give intermediate 13. Elaboration of the model peptide and tandem cleavage/cyclization as described above gave diastereomerically pure Tpd-containing tetrapeptide mimic 14 in 27% overall yield following RP-HPLC purification.

To demonstrate the broad utility of our solid-phase protocol, we carried out the synthesis of a variety of Tpd-containing peptidomimetics as shown in Table 1. The described methodology is tolerant of both D- and L-Tpd subunits, and the six-membered ring closure is not adversely affected by the presence of other electrophilic or nucleophilic side chains within the peptide. In addition, hindered α-hydrazino acids such as N-amino-Ile, -Val, and -Leu can be readily incorporated. Analysis of the crude HPLC traces for various Tpd peptidomimetics revealed that the principle impurity is the trifluoroacetylated N-amino peptide. However, this byproduct is typically a minor component of the crude mixture (<15%) and is readily removed during preparative RP-HPLC purification. Only in the case of N-amino serine-derivative 21 did we observe inefficient ring closure. Although we were able to isolate the desired Tpd derivative in low yield, LCMS revealed the major product to be the uncyclized N-amino peptide. The nucleophilicity of Nβ appeared to be generally lower in this case, as the trifluoroacetylated byproduct was also conspicuously absent from the crude mixture.

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**Table 1. Tpd Peptidomimetics Prepared by SPPS**

| compound | sequence | isolated yield (%) | [M+H]_{obs} | [M+H]_{calc} |
|----------|----------|-------------------|-------------|-------------|
| 5        | Ac-Phe-(D)Tpd-Gly-Tyr-NH₂ | 23 | 539.2249 | 539.2242 |
| 14       | Ac-Phe-(D)Tpd-Ala-Tyr-NH₂ | 27 | 553.2405 | 553.2397 |
| 15       | Ac-Leu-(D)Tpd-Ala-Tyr-NH₂ | 21 | 503.2613 | 503.2613 |
| 16       | Ac-Leu-(L)Tpd-Ala-Tyr-NH₂ | 34 | 503.2613 | 503.2594 |
| 17       | Ac-Leu-(D)Tpd-Phc-Phe-NH₂ | 18 | 579.2926 | 579.2904 |
| 18       | Ac-Leu-(D)Tpd-Ile-Phe-NH₂ | 26 | 545.3082 | 545.3087 |
| 19       | Ac-Leu-(D)Tpd-Leu-Phe-NH₂ | 42 | 545.3082 | 545.3076 |
| 20       | Ac-Leu-(D)Tpd-Val-Phe-NH₂ | 30 | 531.2926 | 531.2930 |
| 21       | Ac-Leu-(D)Tpd-Ser-Phe-NH₂ | 3 | 519.2562 | 519.2579 |
| 22       | Ac-Ser-Leu-(D)Tpd-Ala-Tyr-NH₂ | 21 | 606.2822 | 606.2886 |
| 23       | Ac-Ser-Leu-(L)Tpd-Ala-Tyr-NH₂ | 16 | 590.2933 | 590.2926 |
| 24       | H-Gly-Leu-(D)Tpd-Ala-Ser-Phe-NH₂ | 18 | 605.3042 | 605.3036 |
| 25       | H-Gly-Leu-(D)Tpd-Phe-Ser-Ala-NH₂ | 13 | 605.3042 | 605.3032 |
| 26       | H-Gly-Leu-(L)Tpd-Phe-Ser-Ala-NH₂ | 13 | 605.3042 | 605.3043 |
| 27       | Ac-Val-Lys-Asn-Pro-Asp | Gly-(D)Tpd-Ala-Thr-NH₂ | 22 | 954.4634 | 954.4638 |

In summary, we have described the efficient synthesis of cyclic N-amino peptide derivatives for conformational scanning of bioactive lead structures. Chiral α-hydrazino acid building blocks were synthesized in solution and chemoselectively incorporated on solid phase. Cleavage from the resin and concomitant ring closure gave rise to rigidified peptidomimetics. Notably, our methodology allows for the synthesis of Tpd-constrained peptides bearing various native and sterically hindered side chains. Given that this protocol is operationally simple and amenable to combinatorial synthesis, we anticipate the Tpd motif will find broad application as a probe of local peptide conformation. Efforts toward structurally defined and biologically active N-amino peptide derivatives are currently underway in our laboratory.

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**ASSOCIATED CONTENT**

Supporting Information

Full experimental details and copies of NMR and LCMS spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

We gratefully acknowledge the National Institutes of Health (R21CA167215) and the Anna Valentine Cancer Fund (FIG award) for financial support. Additional support of the Chemical Biology Core Facility was provided by the NIH through the Moffitt Cancer Center support grant (P30CA076292).

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(12) The diastereomeric purity of 12 was verified by 1H NMR (see Supporting Information).

(13) Synthesis of 14 using the bromoacetamide submonomer approach yielded a mixture of diastereomers following cleavage. The route in Scheme 4 afforded 14 with >20:1 dr as judged by LCMS and NMR (see Supporting Information).