

Solid-Phase Synthesis of Tetrahydropyridazinedione-Constrained Peptides

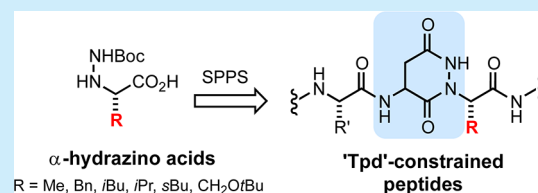
Chang Won Kang,[†] Sujeewa Ranatunga,[†] Matthew P. Sarnowski,[‡] and Juan R. Del Valle^{*,†,‡}

[†]Drug Discovery Department, H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida 33612, United States

[‡]Department of Chemistry, University of South Florida, Tampa, Florida 33620, United States

S 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 structures^{1c,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* α 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 stereochemistry 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 *N* α and *C* α 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

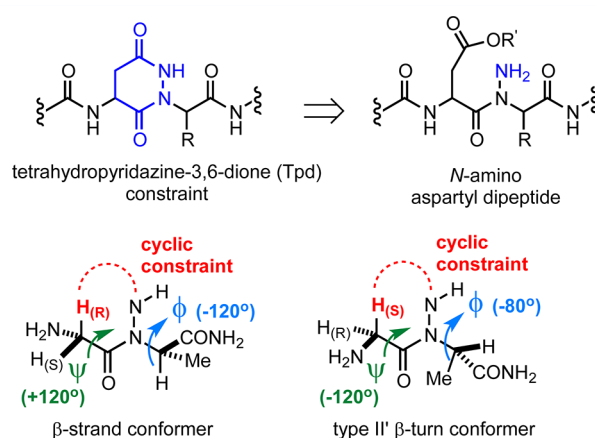


Figure 1. Design of Tpd-constrained peptides.

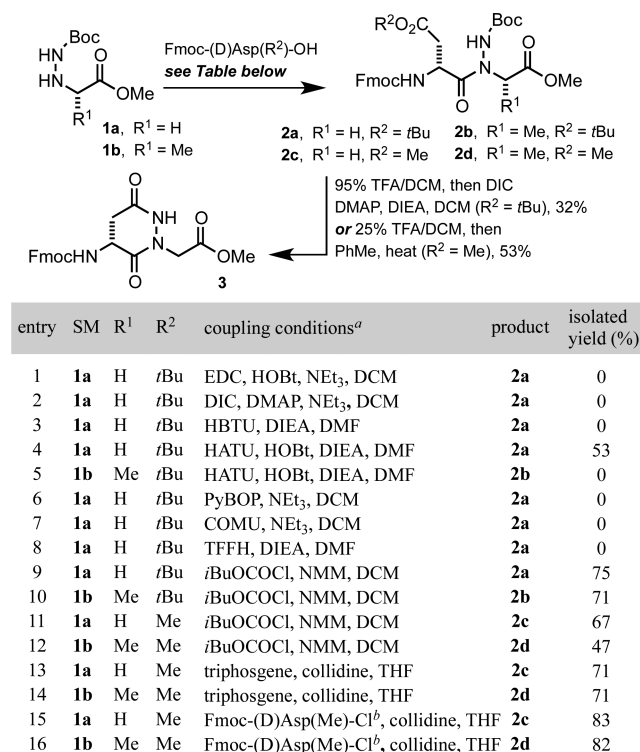
products harboring piperazic acid derivatives.⁶ To the best of our knowledge, there exists no general methodology for preparing α -substituted *N*-amino peptide derivatives by conventional SPPS.

To assess the feasibility of hydrazino acid acylation, we first screened a variety of coupling reagents for the reaction between the known esters **1**⁷ 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(*t*Bu)-OH or Fmoc-D-Asp(Me)-OH as the mixed anhydride^{5c} gave good yields of **2a–d**;

Received: September 9, 2014

Published: October 8, 2014

Scheme 1. Solution Phase Synthesis of Tpd Dipeptide Mimics



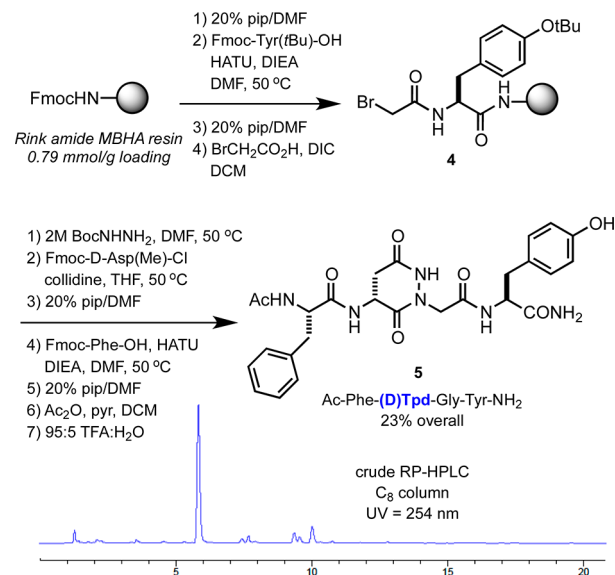
^aAll reactions carried out at rt for 24 h with 2–3 equiv of carboxylic acid and coupling reagent, and excess base. ^bThionyl chloride was used to generate Fmoc-(D)Asp(Me)-Cl. The isolated acid chloride was reacted with the hydrazo ester derivatives in the presence of collidine.

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 triphosphene. 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.

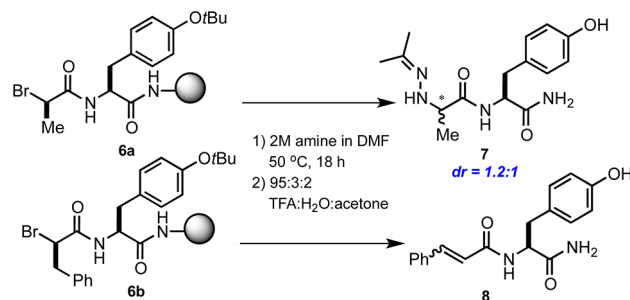
We next explored a submonomer approach to prepare a model peptidomimetic containing a Tpd-Gly subunit on solid support. Analogous to well-established methods for peptoid synthesis,¹⁰ we employed α -bromoacetic acid as a building block and carried out subsequent S_N2 displacement with *tert*-butyl carbazate (Scheme 2). Condensation with 3 equiv of Fmoc-D-Asp(Me)-Cl in the presence of 9 equiv of collidine (50 °C, 1 h \times 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 α -substituted (nonglycine) variants, we observed the formation of

Scheme 2. SPPS of Tpd-Gly Peptidomimetics

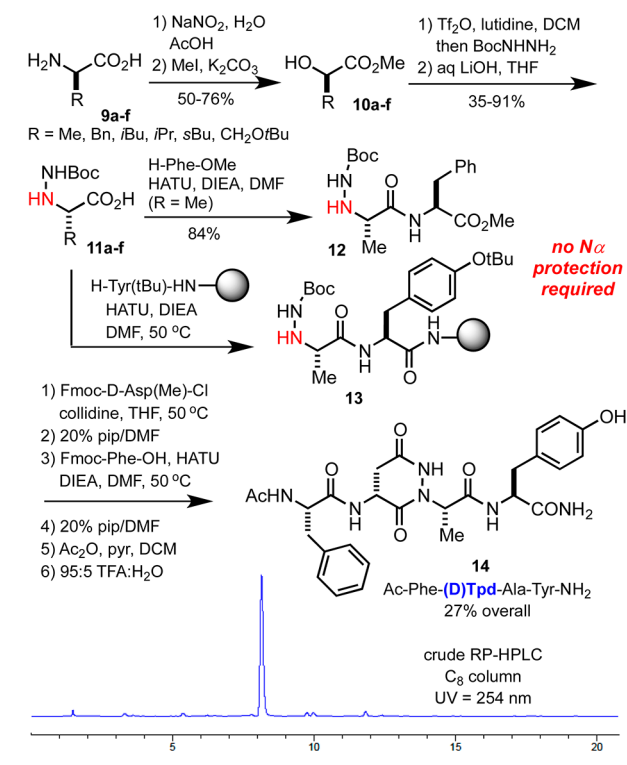


diastereomeric mixtures of Tpd peptidomimetics following cleavage. After ruling out potential racemization of the D-Asp chiral center, we confirmed the configurational instability of the intermediate chiral α -bromoacetamide under the conditions required for efficient S_N2 displacement (Scheme 3). Reaction of

Scheme 3. Attempted Submonomer-Based SPPS of α , α -Disubstituted Variants

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 hydrazone 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 D-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 -

Scheme 4. Synthesis of Chiral α -Hydrazino Acids and α -Substituted Tpd Peptidomimetics

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.¹² 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.¹³

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 $\text{N}\beta$ appeared to be generally lower in this case, as the trifluoroacetylated byproduct was also conspicuously absent from the crude mixture.

Table 1. Tpd Peptidomimetics Prepared by SPPS

compound	sequence	isolated yield (%)	$[\text{M}+\text{H}]_{\text{cal}}$	$[\text{M}+\text{H}]_{\text{obs}}$
5	Ac-Phe-(D)Tpd-Gly-Tyr-NH ₂	23	539.2249	539.2242
14	Ac-Phe-(D)Tpd-Ala-Tyr-NH ₂	27	553.2405	553.2397
15	Ac-Leu-(D)Tpd-Ala-Phe-NH ₂	21	503.2613	503.2613
16	Ac-Leu-(L)Tpd-Ala-Phe-NH ₂	34	503.2613	503.2594
17	Ac-Leu-(D)Tpd-Phe-Phe-NH ₂	18	579.2926	579.2904
18	Ac-Leu-(D)Tpd-Ile-Phe-NH ₂	26	545.3082	545.3087
19	Ac-Leu-(D)Tpd-Leu-Phe-NH ₂	42	545.3082	545.3076
20	Ac-Leu-(D)Tpd-Val-Phe-NH ₂	30	531.2926	531.2930
21	Ac-Leu-(D)Tpd-Ser-Phe-NH ₂	3	519.2562	519.2579
22	Ac-Ser-Leu-(D)Tpd-Ala-Tyr-NH ₂	21	606.2822	606.2886
23	Ac-Ile-(D)Tpd-Ala-Ser-Phe-NH ₂	16	590.2933	590.2936
24	H-Gly-Leu-(D)Tpd-Ala-Ser-Phe-NH ₂	18	605.3042	605.3036
25	H-Gly-Leu-(D)Tpd-Phe-Ser-Ala-NH ₂	13	605.3042	605.3032
26	H-Gly-Leu-(L)Tpd-Phe-Ser-Ala-NH ₂	13	605.3042	605.3043
27	Ac-Val-Lys-Asn-Pro-Asp-Gly-(D)Tpd-Ala-Thr-NH ₂	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.

■ 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>.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: juan.delvalle@moffitt.org.

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).

■ REFERENCES

- (1) (a) Freidinger, R. M.; Veber, D. F.; Perlow, D. S.; Brooks, J. R.; Saperstein, R. *Science* **1980**, *210*, 656. (b) Aube, J. *Synthetic Routes to Lactam Peptidomimetics In Advances in Amino Acid Mimetics and Peptidomimetics*, Vol. 1; Abell, A., Ed.; JAI Press Ltd.: London, 1997; pp 193–232. (c) Perdih, A.; Kikelj, D. *Curr. Med. Chem.* **2006**, *13*, 1525. (d) Freidinger, R. M.; Perlow, D. S.; Veber, D. F. *J. Org. Chem.* **1982**, *47*, 104.

(2) For additional reviews and selected examples, see: (a) Hanessian, S.; McNaughton-Smith, G.; Lombart, H. G.; Lubell, W. D. *Tetrahedron* **1997**, *53*, 12789. (b) Cluzeau, J.; Lubell, W. D. *Peptide Science* **2005**, *80*, 98. (c) Khashper, A.; Lubell, W. D. *Org. Biomol. Chem.* **2014**, *12*, 5052. (d) Scott, W. L.; Martynow, J. G.; Huffman, J. C.; O'Donnell, M. J. *J. Am. Chem. Soc.* **2007**, *129*, 7077. (e) Scott, W. L.; Alsina, J.; Kennedy, J. H.; O'Donnell, M. J. *Org. Lett.* **2004**, *6*, 1629. (f) Jamieson, A. G.; Boutard, N.; Beauregard, K.; Bodas, M. S.; Ong, H.; Quiniou, C.; Chemtob, S.; Lubell, W. D. *J. Am. Chem. Soc.* **2009**, *131*, 7917. (g) Ecija, M.; Diez, A.; Rubiralta, M.; Casamitjana, N.; Kogan, M. J.; Giralt, E. *J. Org. Chem.* **2003**, *68*, 9541. (h) Kumar, S.; Flamant-Robin, C.; Wang, Q.; Chiaroni, A.; Sasaki, N. A. *J. Org. Chem.* **2005**, *70*, 5946. (i) Doan, N.-D.; Hopewell, R.; Lubell, W. D. *Org. Lett.* **2014**, *16*, 2232. (j) Palomo, C.; Aizpurua, J. M.; Benito, A.; Miranda, J. I.; Fratila, R. M.; Matute, C.; Domercq, M.; Gago, F.; Martin-Santamaria, S.; Linden, A. *J. Am. Chem. Soc.* **2003**, *125*, 16243. (k) Proulx, C.; Lubell, W. D. *Org. Lett.* **2012**, *14*, 4552. (l) Sicherl, F.; Cupido, T.; Albericio, F. *Chem. Commun.* **2010**, *46*, 1266. (m) Robl, J. A.; Cimarusti, M. P.; Simpkins, L. M.; Weller, H. N.; Pan, Y. Y.; Malley, M.; DiMarco, J. D. *J. Am. Chem. Soc.* **1994**, *116*, 2348. (n) Sun, H.; Martin, C.; Kesselring, D.; Keller, R.; Moeller, K. D. *J. Am. Chem. Soc.* **2006**, *128*, 13761.

(3) For examples of bicyclic variants and Tpd homologues, see: (a) Rudolph, K.; Gerwin, N.; Verzijl, N.; van, d. K. P.; van, d. B. W. *Osteoarthritis Cartilage* **2003**, *11*, 738. (b) Chen, M. H.; Goel, O. P.; Hyun, J. W.; Magano, J.; Rubin, J. R. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1587. (c) Dolle, R. E.; Prasad, C. V. C.; Prouty, C. P.; Salvino, J. M.; Awad, M. M. A.; Schmidt, S. J.; Hoyer, D.; Ross, T. M.; Graybill, T. L.; Speier, G. J.; Uhl, J.; Miller, R.; Helaszek, C. T.; Ator, M. A. *J. Med. Chem.* **1997**, *40*, 1941. (d) Handy, E. L.; Totaro, K. A.; Lin, C. P.; Sello, J. K. *Org. Lett.* **2014**, *16*, 3488. (e) Hobbs, C. J.; Bit, R. A.; Cansfield, A. D.; Harris, B.; Hill, C. H.; Hilyard, K. L.; Kilford, I. R.; Kitas, E.; Kroehn, A.; Lovell, P.; Pole, D.; Rugman, P.; Sherborne, B. S.; Smith, I. E. D.; Vesey, D. R.; Walmsley, D. L.; Whittaker, D.; Williams, G.; Wilson, F.; Banner, D.; Surgenor, A.; Borkakoti, N. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1365. (f) Robl, J. A.; Sun, C.-Q.; Simpkins, L. M.; Ryono, D. E.; Barrish, J. C.; Karanewsky, D. S.; Asaad, M. M.; Schaeffer, T. R.; Trippodo, N. C. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2045.

(4) Gao, Y.; Kodadek, T. *Chem. Biol.* **2013**, *20*, 360.

(5) (a) Cabezas, E.; Satterthwait, A. C. *J. Am. Chem. Soc.* **1999**, *121*, 3862. (b) Kanta Sarma, B.; Yousufuddin, M.; Kodadek, T. *Chem. Commun.* **2011**, *47*, 10590. (c) Liu, F.; Stephen, A. G.; Adamson, C. S.; Gousset, K.; Aman, M. J.; Freed, E. O.; Fisher, R. J.; Burke, T. R., Jr. *Org. Lett.* **2006**, *8*, 5165. (d) Sarma, B. K.; Kodadek, T. *ACS Comb. Sci.* **2012**, *14*, 558.

(6) (a) Williams, P. D.; Bock, M. G.; Tung, R. D.; Garsky, V. M.; Perlow, D. S.; Erb, J. M.; Lundell, G. F.; Gould, N. P.; Whitter, W. L.; Hoffman, J. B.; Kaufman, M. J.; Clineschmidt, B. V.; Pettibone, D. J.; Freidinger, R. M.; Veber, D. F. *J. Med. Chem.* **1992**, *35*, 3905. (b) Oelke, A. J.; France, D. J.; Hofmann, T.; Wuitschik, G.; Ley, S. V. *Nat. Prod. Rep.* **2011**, *28*, 1445.

(7) (a) Acherar, S.; Salaun, A.; Le Grel, P.; Le Grel, B.; Jamart-Gregoire, B. *Eur. J. Org. Chem.* **2013**, *2013*, 5603. (b) Bonnet, D.; Margathe, J. F.; Radford, S.; Pflimlin, E.; Riche, S.; Doman, P.; Hibert, M.; Ganesan, A. *ACS Comb. Sci.* **2012**, *14*, 323.

(8) Carpino, L. A.; Cohen, B. J.; Stephens, K. E.; Sadat-Aalae, S. Y.; Tien, J. H.; Langridge, D. C. *J. Org. Chem.* **1986**, *51*, 3732.

(9) The integrity of the Fmoc-Asp(Me)-Cl chiral center was established by synthesis and analysis of the (S,S) and (R,S) diastereomers of **2d** by NMR and HPLC. See the Supporting Information for details.

(10) Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H. *J. Am. Chem. Soc.* **1992**, *114*, 10646.

(11) Deechongkit, S.; You, S.-L.; Kelly, J. W. *Org. Lett.* **2004**, *6*, 497.

(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).