Pergamon

SCIENCE DIRECT®

Tetrahedron 59 (2003) 8947–8954

TETRAHEDRON

Methods to initiate synthetic re-structuring of peptides

Qi Wei, Susan Harran† and Patrick G. Harran*

Department of Biochemistry, University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd, Dallas, TX 75390-9038, USA

Received 21 March 2003; accepted 2 April 2003

Abstract—Sculpture during assembly is an appropriate description of procedures used to transform unprotected peptide **i** into complex macrocycle **ii**. These methods appear a general means to begin manipulating the form and characteristics of common polyamides. Herein we demonstrate initial phases of a type of synthesis that has the potential to produce large numbers of novel structural classes beginning with machine-made heteropolymers.

1. Introduction

We are attempting to develop synthetic procedures able to incrementally re-cast the form and properties of common peptides. In particular, reaction sequences which begin by incorporating a hydrophobic 'reaction nucleus' (**N** in Scheme 1A) within peptide chains. Doing so, the aim is to offset polarity and restrict conformational flexibility while generating intermediates poised for additional manipulation. The long-term goal is to systematically move beyond peptidomimetics with a form of synthesis that molds new structural types into existence as much as it builds them from parts.[1] Herein we describe our initial results.

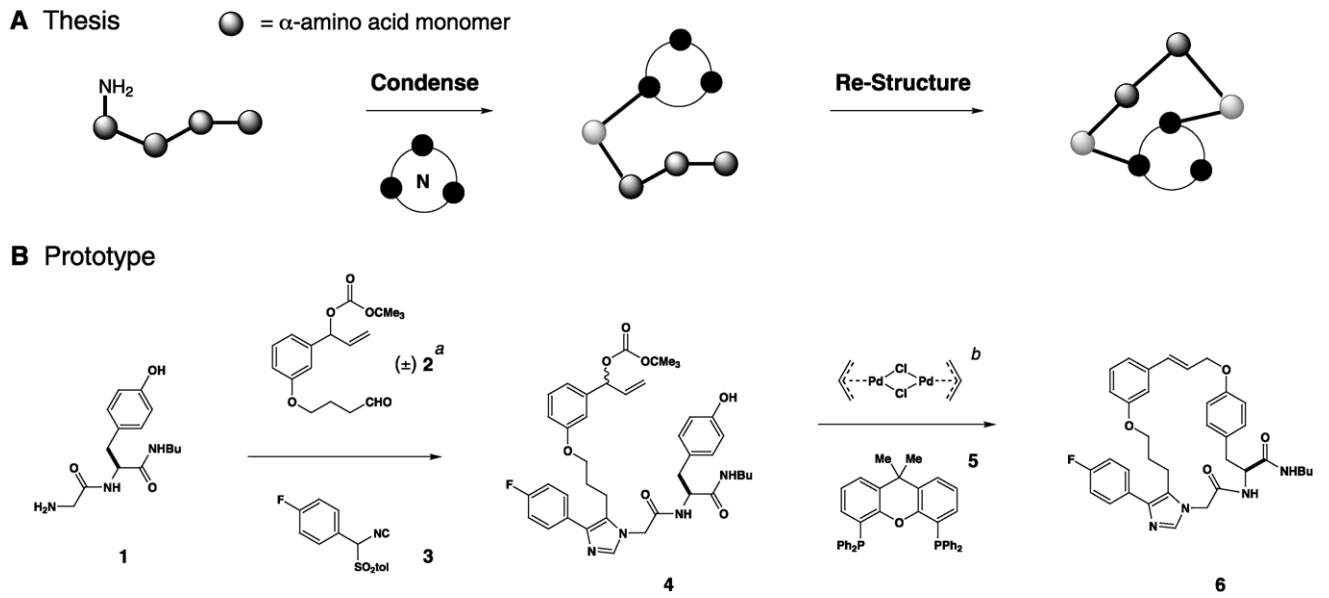
2. Results and discussion

Compound **2** (Scheme 1B) represents a minimal prototype of reaction nucleus **N**. Conventional peptide synthesis occurs such that a free N-terminus is a feature available on all sequences prepared and aldehyde **2** is designed to exploit this commonality. For example, mixing **2** and dipeptide **1** ligates the two via Schiff-base formation. This joining is then made irreversible by treatment in situ with α-(*p*-toluenesulfonyl)-4-fluorobenzylisonitrile (**3**).[2] The product of the resulting cycloaddition/sulfinate elimination[3] (**4** isolated simply by precipitation from a concentrated EtOAc solution[4]) integrates the peptide amino terminus into a heterocyclic ring that now displays tethered functionality able to advance our processing sequence. In particular, the allylic carbonate present in **4** can be decomposed by catalytic amounts of soluble palladium salts.[5] If there is a nucleophile of appropriate pK_a in proximity, a putative intermediate π-allyl palladium complex is trapped internally and a large ring is formed. Among the set of proteinogenic amino acid side-chain functional groups, we find the phenol of tyrosine (pK_a∼10.1) uniquely competent in such cyclizations. However, generality and efficiency in the transformation were achieved only after extensive experimentation. Numerous combinations of metal pre-catalyst and stabilizing ligand were either poisoned by substrate or led, slowly and inefficiently, to a mixture of desired cyclic cinnamyl ether **6** and oligomeric material[6]—the latter dominating in most instances. The situation changes considerably with the use of a catalyst formed by pre-treating $[(\eta^3\text{-allyl})PdCl]_2$ with van Leeuwen's Xantphos[7] (2:1 **5**/Pd atom). In this instance, the conversion of **4** to **6** is complete within minutes at rt (DMF, 5 mM in **4**) and competing oligomerization is minimized. We are aware neither of large rings being formed in this manner previously nor of the $[(\eta^3\text{-allyl})PdCl]_2$/**5** combination finding use in catalyzed allylic etherification—despite its marked effectiveness here.

As shown in Table 1, the protocol of multi-component condensation/metal-catalyzed cycloetherification appears a general means to begin manipulating the structure of linear, unprotected peptides.[8] In fact, we have yet to identify a sequence displaying a free N-terminus and a tyrosine residue that will not participate.[9] The catalyzed cycloetherification step tolerates free carbinols and carboxamides, thioethers, and selected heteroaromatics. Sequences containing multiple tyrosine residues ring-close with comparable efficiency although, in the case examined (entry 5), regioselectivity is modest.[10] Minor technical demands of the

Keywords: peptides; heterocycles; palladium catalysis; rearrangements; solid-phase synthesis.
* Corresponding author. Tel.: +1-214-648-3612; fax: +1-214-648-6455; e-mail: pharra@biochem.swmed.edu
† Present Address: Cumbre Inc., 1502 Viceroy Drive, Dallas, TX 75235-2304, USA.

0040–4020/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2003.04.008



Scheme 1. *Reaction conditions*: (a) 1 equiv. **2**, K_2CO_3, DMF, rt, 5 h; then add **3** (1.05 equiv.), rt, 17 h (80%). (b) 10 mol% $[(\eta^3\text{-allyl})PdCl]_2$, 40 mol% **5**, degassed DMF, rt, 1 h (63%).

cyclization include a need for oxygen-free media and workup with aqueous NaCN[11] to avoid partial destruction of allylic ether products during isolation.

In line with our original design criteria, the cinnamyl ether unit formed via cycloetherification is a staging ground for further chemoselective modifications. Scheme 2 outlines two simple demonstrations of this potential. Following desiccation,[12] cyclization product **13** undergoes diastereoselective[13] dihydroxylation using a modified Sharpless protocol.[14] This transforms its allylic ether substructure into a monoaryl glycerol (namely **18**, Scheme 2A)—a moiety likely amenable to derivatization with, for example, fatty acids and/or sugars. A second perturbation available in the allylic ether series is Claisen rearrangement. For example, thermolysis of **8** provides phenolic α-olefin **19**—a change accompanied by four-atom ring-contraction of the macrocycle (Scheme 2B). As currently executed,[15] the sigmatropic rearrangement is not stereoselective. However, this is viewed as an asset given the diversity-oriented goals of the program. In addition, the functionality in **19** produced as a result of rearrangement can be modified in direct and useful ways. Mitsunobu etherification with allyl carbinol followed by ruthenium catalyzed ring-closing olefin metathesis[16] annulates a hydrophobic, 7-membered ring onto the existing 21-membered macrocycle-affording yet another novel polycyclic structure (namely **21**).

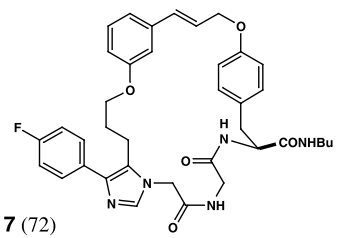
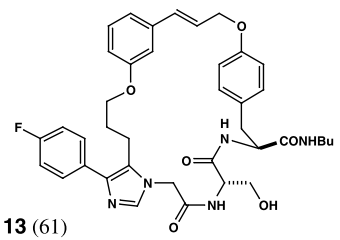
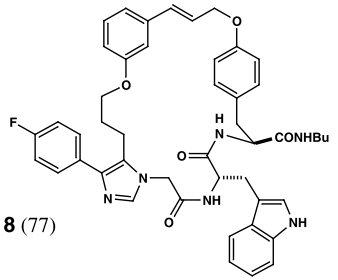
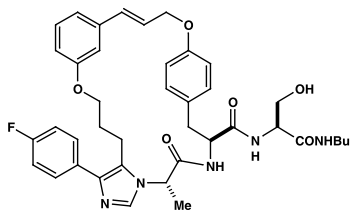
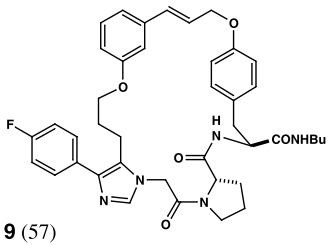
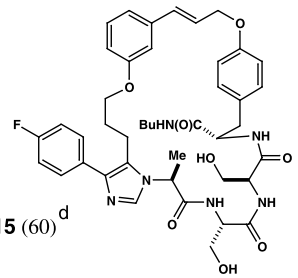
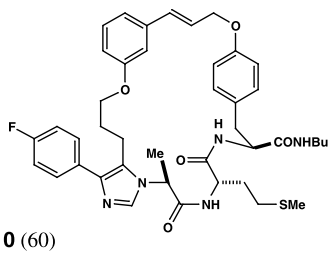
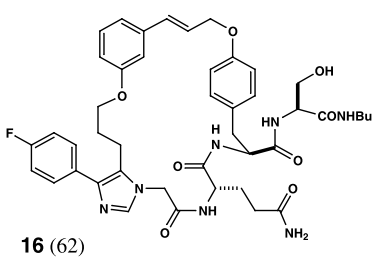
The elaborations above are a small sampling of perturbations intended to impart increasing 'alkaloid-like'[17] character to initially formed macrocycles. As the methodology is refined and expanded, and our collection of polycyclic compounds grows, the opportunity to discover members having new biochemical functions likewise increases. It is conceivable, given the complexity and functional group content of the compounds in question, that specificity in, for example, protein binding, will be observed at a frequency higher than that typical of conventional pharmaceutical collections. To facilitate testing this hypothesis, we have adapted initial stages of our processing sequence to a solid-phase format (Scheme 3). This will permit use of automated parallel synthesis techniques during library construction. To date, we have found an amine-presenting form of Foley's silane-functionalized polystyrene resin (**23**)[18] most well-suited for our needs. Fmoc-based peptide synthesis[19] is facile in this format and subsequent isonitrile-based imidazole synthesis and catalyzed cycloetherification have both been performed successfully. While optimization continues,[20] the solid-phase synthesis of **26** suggests we now have superior means to quickly widen our starting material base (to include N-alkylated, D-configured, and unnatural residue-containing peptides), to incorporate and evaluate second generation reaction nuclei **N** with enhanced functionality, and to screen for new dehydrogenative (ideally, biaryl-forming) oxidation methods as part of more complete processing sequences-those able to close the gap between abundant oligopeptides and novel natural product-like substances.

3. Experimental

3.1. Data for compounds 2

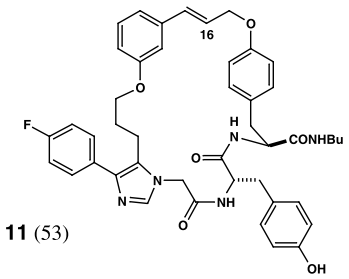
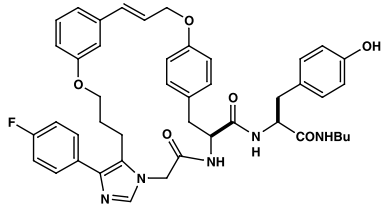
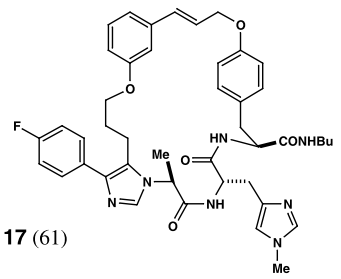
3.1.1. *tert*-Butyl-{1-[3-((4-oxo)butoxy)phenyl]-2-propenyl} carbonate (2). Anhydrous Cs_2CO_3 (103.2 g, 317 mmol) and 3-hydroxybenzaldehyde (32.2 g, 264 mmol) are suspended in 600 mL DMF. 4-(*t*-Butyldimethylsiloxy)-1-chlorobutane (21.6 g, 106 mmol) is added and the mixture brought to 115°C with vigorous stirring. Beginning at 4 h, two additional portions (21.6 g each) of alkyl chloride are added at 1.5 h intervals. The mixture is cooled to rt, diluted with 1.5 L EtOAc, washed with H_2O (2×500 mL), dried over $MgSO_4$, and concentrated. Filtration through a column of silica gel (5% EtOAc/hexanes) provides a light yellow oil (48.6 g, 60%). A portion of this material (38.9 g, 126 mmol) is dissolved in 500 mL dry THF and cooled to −70°C under N_2. A solution of vinyl magnesium bromide (Aldrich, 1.0 M

Table 1. Macrocyclic cinnamyl ethers prepared by sequential three-component condensation/palladium-catalyzed cycloetherification

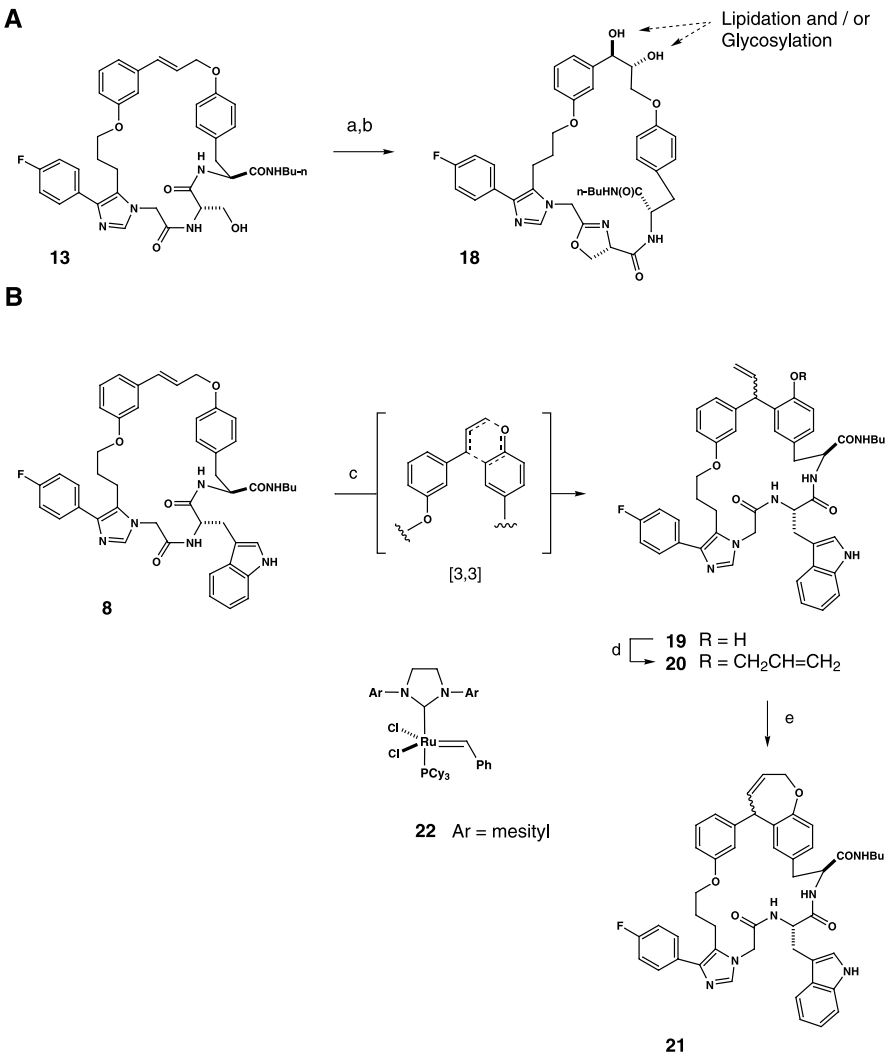
Entry	Peptide[a]	Condensation[b] product (%)	Cycloetherification[c] product (%)	Entry	Peptide	Condensation product (%)	Cycloetherification product (%)
1	GGY	(64)	 **7** (72)	6	GSY	(65)	 **13** (61)
2	GWY	(80)	 **8** (77)	7	AYS	(42)	 **14** (53)
3	GPY	(65)	 **9** (57)	8	ASSY	(49)	 **15** (60)[d]
4	ACY	(55)	 **10** (60)	9	GQYS	(60)	 **16** (62)

(*continued on next page*)

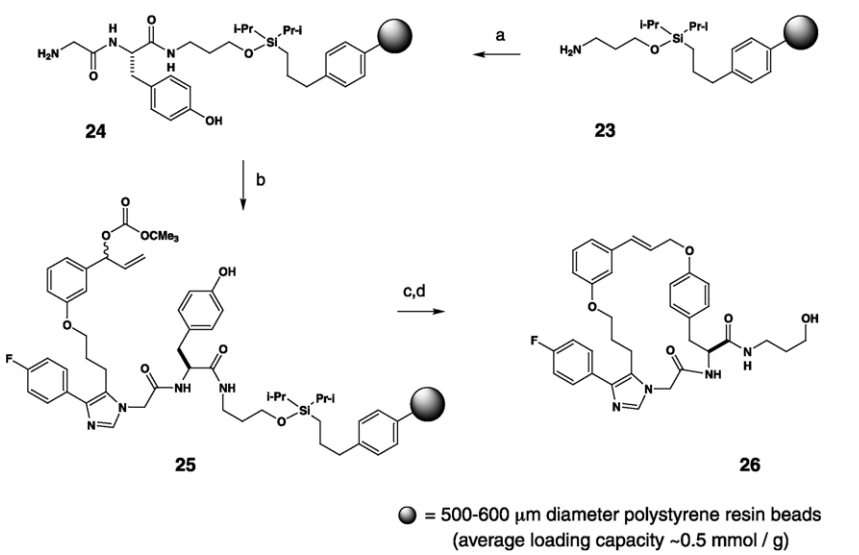
Table 1 (*continued*)

Entry	Peptide[a]	Condensation[b] product (%)	Cycloetherification[c] product (%)	Entry	Peptide	Condensation product (%)	Cycloetherification product (%)
5	GYY	(64)	 **11** (53) +  **12** (25)	10	AH(3-Me)Y	(48)	 **17** (61)

[a]Prepared in the form H2N-AA1-AAn-CONHBu-*n*; where amino acid residues (AA) are listed in one-letter codes. [b]See Section 3 and Ref. 4. Yields quoted refer to >90% pure (^{1}H NMR) precipitated powders. [c]See Section 3. Yields quoted are for isolated, chromatographically homogeneous material. [d]Isolates contaminated with ~15% unidentified by-products.



Scheme 2. *Reaction conditions*: (a) $[MeO(CH_2)_2]_2NSF_3$, CH_2Cl_2, −20°C (62%). (b) $K_2Fe(CN)_6$, 1.5 mol% $K_2OsO_2(OH)_4$, 15 mol% (DHQD)PHAL, K_2CO_3, $MeSO_2NH_2$, *t*-BuOH/H_2O, rt (61%). (c) 180°C (microwave), silica gel, CH_3CN, 40 min (74%). (d) allyl alcohol, DIAD, PPh_3, THF (81%). (e) 20 mol% **22**, CH_2Cl_2, 40°C (65%).



Scheme 3. *Reaction conditions*: (a) Ref. 19. (b) **2** (4 equiv.), 1:1 THF/$(MeO)_3CH$, rt, 4 h; THF wash (3×); **3** (5 equiv.), piperazine (5 equiv.), DMF, rt, 24 h. (c) $[(\eta^3\text{-allyl})PdCl]_2$/**5** complex (~10 mol%), degassed DMF, rt, 2×1 h. (d) HF/pyridine, THF, rt, 3 h.

in THF, 150 mL) is added over 30 min via dropping funnel. When complete, the cooling bath is removed and the solution brought to rt over 45 min. Sat. aqueous NH_4Cl (100 mL) is added and the mixture partitioned between EtOAc and H_2O. The layers are separated, the aqueous layer is extracted with EtOAc (1×), and the combined organics are dried over Na_2SO_4 and concentrated. The residue is dissolved in 350 mL CH_2Cl_2 and cooled in an ice bath under N_2. Di-*t*-butyldicarbonate (27.5 g, 126 mmol) is added followed by *p*-dimethylaminopyridine (15.4 g, 126 mmol) in 5 equal portions over 20 min. The solution is stirred at rt for 3.5 h and quenched with sat. aqueous NH_4Cl. The organic layer is separated and washed with H_2O and brine, dried over Na_2SO_4, and concentrated. Flash chromatography on silica gel (5% EtOAc/hexanes) provides a homogenous carbonate product (46.0 g, 84% yield-two steps). This material is transferred to a polypropylene bottle and dissolved in 600 mL THF. Anhydrous pyridine (75 mL) and HF/pyridine complex (Aldrich, ~70% HF, 30 mL) are added and the solution stirred at rt for 4 h. The reaction is concentrated to ~1/3 its original volume, diluted with 500 mL EtOAc, and washed with sat. $CuSO_4$ (3×150 mL), H_2O, sat. aqueous $NaHCO_3$, and brine. Rotary evaporation provides an oil that is chromatographed on silica gel (30% EtOAc/hexanes) to afford an alcohol product (26.1 g, 77%). A portion of this substance (21.43 g, 66.4 mmol) is dissolved in CH_2Cl_2 (250 mL), cooled in an ice bath, and treated successively with pyridine (8.1 mL, 0.10 mol) and solid Dess–Martin periodinane (42.3 g, 0.10 mol). The mixture is stirred for 1 h at 4°C and 2 h at rt. A solution of sat. aqueous $NaHCO_3$ containing $Na_2S_2O_3$ (80 mL) is added and the two-phase system is mixed vigorously until the organic layer clears (~20 min). The layers are separated and the organics are washed with sat. aqueous $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated. Flash chromatography on silica gel (10→20% EtOAc/hexanes) gives aldehyde **2** (15.73 g, 74%) as a cream colored, pasty solid. **2**: R_f=0.40 (20% EtOAc/hexanes). IR (film): 2981, 2936, 2827, 2726, 1742, 1602, 1587, 1370, 1254, 1161, 1087, 941, 854, 792 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 9.86 (s, 1H), 7.27 (dd, *J*=5.4, 4.8 Hz, 1H), 6.96 (d, *J*=5.4 Hz, 1H), 6.89 (s, 1H), 6.83 (d, *J*=4.8 Hz, 1H), 5.90–6.18 (m, 2H), 5.24–5.35 (m, 2H), 4.01 (t, *J*=4.5 Hz, 2H), 2.68 (t, *J*=4.9 Hz, 2H), 2.10–2.16 (m, 2H), 1.49 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 201.6, 158.8, 152.6, 140.3, 136.1, 129.6, 119.3, 117.1, 114.1, 112.9, 82.2, 78.9, 66.6, 40.5, 27.7, 21.9. LRMS (positive electrospray) calcd for $C_{18}H_{24}O_5$: $[M+H]^+$ 321.17. Found: 321.14.

3.1.2. General condensation and cyclization procedures (1→4→6). A mixture of dipeptide **1** (413 mg, 1.4 mmol), aldehyde **2** (451 mg, 1.4 mmol), powdered anhydrous K_2CO_3 (486 mg, 3.5 mmol), and 4 Å molecular sieves (2.0 g) is suspended in 4 mL DMF. The mixture is stirred under N_2 for 5 h (rt) and then treated with solid isonitrile **3** (428 mg, 1.478 mmol). Stirring is continued for 17 h. The mixture is filtered through a plug of celite and concentrated. The residue remaining is dissolved in EtOAc (200 mL) and washed with H_2O and brine. Concentration of the organic layer to ~20 mL induces precipitation of **4** which is pelleted by centrifugation (6 min at 3500 rpm). The supernatant is decanted and the solid (670 mg) washed with 30% EtOAc/hexanes (3×5 mL). The washings and supernatant are combined and the precipitation procedure repeated once more to afford additional **4** (152 mg, combined yield=80%) as a light yellow powder. ES-MS: calcd for $C_{41}H_{49}FN_4O_7$ $[M^+ + H]$: 729.37. Found: 729.37.

Cinnamyl ether **6**. $[(\eta^3\text{-allyl})PdCl]_2$ (2.0 mg, 5.5 μmol) and bis-phosphine **5** (13.0 mg, 22 μmol) are dissolved in 1 mL degassed (N_2 purge) THF. The solution is stirred for 20 min, diluted with 8 mL degassed DMF and treated with **4** (40 mg, 55 μmol in 2 mL degassed DMF). Stirring is continued for 1 h and then 100 μL 1.0 M aq. NaCN is added. The solution is diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, and concentrated. Purification by flash chromatography (3→5% *i*-PrOH/$CHCl_3$) affords cyclic ether **6** (21 mg, 63%) as a white film. **6**: R_f=0.38 (10% *i*-PrOH/$CHCl_3$). $[\alpha]_D^{20}$=+65.7° (*c*=1.17, MeOH). IR (film): 3284, 2931, 1639, 1558, 1509, 1221, 1157, 839 cm^{-1}. ^1H NMR (400 MHz, CD_3OD): δ 7.56 (d, *J*=8.8 Hz, 1H), 7.55 (d, *J*=8.8 Hz, 1H), 7.52 (s, 1H), 7.22 (d, *J*=8.4 Hz, 2H), 7.17 (t, *J*=7.8 Hz, 1H), 7.11 (app t, *J*=8.8 Hz, 2H), 6.92 (m, 3H), 6.70 (dd, *J*=8.0, 2.4 Hz, 1H), 6.60 (s, 1H), 6.48 (d, *J*=16.2 Hz, 1H), 6.16 (dt, *J*=16.2, 5.6 Hz, 1H), 4.88 (d, *J*=5.6 Hz, 2H), 4.63 (d, *J*=16.4 Hz, 1H), 4.53 (dd, *J*=12.0, 3.6 Hz, 1H), 4.40 (d, *J*=16.4 Hz, 1H), 3.90 (sym 7 line m, 1H), 3.78 (sym 7 line m, 1H), 3.21 (sym 8 line m, 2H), 3.13 (dd, *J*=14.4, 3.2 Hz, 1H), 2.91 (app t, *J*=7.2 Hz, 2H), 2.79 (dd, *J*=14.4 Hz, 12.0, 1H), 1.93 (m, 1H), 1.77 (m, 1H), 1.48 (sym 7 line m, 2H), 1.34 (sym 6 line m, 2H), 0.92 (t, *J*=7.4 Hz, 3H). ^{13}C NMR (75 MHz, CD_3OD): δ 174.0, 169.5, 160.0, 157.9, 139.9, 138.4, 137.6, 134.5, 131.1, 131.0, 130.7, 130.2, 130.1, 129.5, 126.9, 120.1, 118.3, 116.5, 116.3, 114.9, 113.6, 68.6, 66.8, 57.0, 47.6, 40.4, 38.3, 32.6, 29.7, 25.4, 21.2, 14.2. ES-MS: calcd for $C_{36}H_{39}FN_4O_4$: $[M+H]^+$ 611.31. Found: 611.39.

3.1.3. Oxazoline-containing glycol 18. Alcohol **13** (45 mg, 0.64 mmol) is dissolved in 1 mL dry CH_2Cl_2, cooled to −20°C, and treated with bis(2-methoxyethyl)aminosulfur trifluoride (Deoxo-Fluor, 0.3 mL aliquot of CH_2Cl_2 stock solution, 0.96 mmol). The reaction is stirred at −20°C for 3 h, quenched with saturated $NaHCO_3$, and warmed to rt. The aqueous layer is washed with $CHCl_3$ (3×) and the combined organics dried over Na_2SO_4. Concentration and flash chromatography on silica gel ($CHCl_3$/EtOAc/MeOH, 8/1.7/0.3) affords an oxazoline product (25 mg, 57%) as a white solid. R_f=0.59 (10% MeOH/CH_2Cl_2). $[\alpha]_D^{20}$=+40.2° (*c*=0.33, MeOH). IR (film): 3280, 2960, 2927, 2873, 1657, 1513, 1293, 1223, 973, 840 cm^{-1}. ^1H NMR (300 MHz, CD_3OD): δ 7.70 (s, 1H), 7.48–7.58 (m, 2H), 7.02–7.22 (m, 5H), 6.82–6.96 (m, 4H), 6.67 (dd, *J*=9.0, 2.4 Hz, 1H), 6.56 (d, *J*=16.2 Hz, 1H), 6.33 (dt, *J*=15.9, 5.4 Hz, 1H), 4.80–4.96 (m, 2H), 4.52–4.64 (m, 2H), 4.27 (dd, *J*=10.5, 8.7 Hz, 1H), 4.04 (dd, *J*=8.7, 8.1 Hz, 1H), 3.80–3.92 (m, 2H), 3.22 (t, *J*=6.9 Hz, 2H), 3.13 (dd, *J*=14.1, 3.3 Hz, 1H), 2.95 (t, *J*=7.2 Hz, 2H), 2.81 (dd, *J*=14.1, 11.4 Hz, 1H), 1.80–2.00 (m, 2H), 1.44–1.60 (m, 2H), 1.30–1.44 (m, 2H), 0.94 (t, *J*=6.9 Hz, 3H). ^{13}C NMR (75 MHz, CD_3OD): δ 173.8, 173.4, 167.9, 160.3, 158.4, 139.5, 139.3, 138.4, 133.9, 131.3, 130.8, 130.5, 130.4, 130.3, 128.7, 126.4, 120.8, 116.6, 116.5, 116.2, 114.9, 113.4, 72.9, 69.6, 68.7, 67.0, 56.3, 42.7, 40.4, 38.2, 32.7, 29.5, 21.2, 20.6, 14.2. LRMS (positive electrospray) calcd for $C_{39}H_{42}FN_5O_5$: $[M+H]^+$ 680.33. Found: 680.53.

A portion of the above oxazoline (6.0 mg, 8.8 μmol) is dissolved in 1:1 *t*-BuOH/H_2O (0.2 mL). Solid $CH_3SO_2NH_2$ (2.5 mg, 26 μmol) and a pre-ground mixture of $K_3Fe(CN)_6$ (8.7 mg, 26 μmol), $K_2OsO_2(OH)_4$ (32.4 μg, 0.09 μmol), $(DHQD)_2PHAL$ (0.69 mg, 0.88 μmol), and K_2CO_3 (3.7 mg, 26 μmol) are added. The resultant mixture is stirred at rt for 20 h. Volatiles are removed in vacuo and the residue remaining is diluted with 5 mL H_2O, filtered, and concentrated. Purification by PTLC affords glycol **18** (4 mg, 63%) as a white film. **18**: R_f=0.38 (10% MeOH/CH_2Cl_2). $[\alpha]_D^{20}$=+46.5° (*c*=0.33, MeOH). IR (film): 3333, 2953, 2933, 1656, 1507, 1247, 1037, 977 cm^{-1}. ^1H NMR (300 MHz, CD_3OD): δ 7.75 (s, 1H), 7.52–7.62 (m, 2H), 7.27 (t, *J*=7.8 Hz, 1H), 7.12–7.02 (m, 3H), 6.94 (d, *J*=8.7 Hz, 2H), 6.82–6.72 (m, 2H), 6.62 (d, *J*=8.7 Hz, 2H), 4.96 (d, *J*=16.8 Hz, 1H), 4.84 (d, *J*=16.8 Hz, 1H), 4.71 (d, *J*=8.1 Hz, 2H), 4.47–4.60 (m, 2H), 4.27 (t, *J*=8.4 Hz, 1H), 3.78–3.92 (m, 3H), 3.73 (dd, *J*=10.2, 2.4 Hz, 1H), 3.47 (dd, *J*=10.2, 4.8 Hz, 1H). 3.12–3.24 (m, 2H), 3.05 (dd, *J*=14.4, 3.9 Hz, 1H), 3.00–2.80 (m, 3H), 1.82–2.04 (m, 2H), 1.42–1.54 (m, 2H), 1.26–1.40 (m, 2H), 0.92 (t, *J*=7.2 Hz, 3H). ^{13}C NMR (75 MHz, CD_3OD): δ 173.12, 173.10, 167.4, 159.7, 159.3, 144.5, 139.1, 138.0, 132.5, 131.5, 130.8, 130.3, 130.2, 129.0, 120.3, 116.6, 116.3, 116.2, 115.9, 114.1, 76.5, 76.0, 72.4, 70.6, 69.9, 67.5, 55.3, 42.5, 40.4, 38.1, 32.6, 29.6, 21.2, 20.8, 14.2. LRMS (positive electrospray) calcd for $C_{39}H_{44}FN_5O_7$: $[M+H]^+$ 714.33. Found: 714.44.

3.1.4. Claisen rearrangement products 19. Cinnamyl ether **8** (100 mg, 0.125 mmol) is placed in a 5 mL conical Smith Process Vial. Chromatography grade silica gel (500 mg) is added and the mixture is suspended in 4 mL CH_3CN. The tube is sealed under N_2 and inserted into the reaction chamber of a SmithCreator desktop microwave instrument. After heating (2.45 GHz) at 180°C for 40 min, the mixture is concentrated and the residue purified by flash chromatography (4→7% MeOH/CH_2Cl_2) to afford **19** (~1:1 diastereoisomeric mixture) as a pale yellow solid (74 mg, 74%). R_f=0.38 (10% MeOH/CH_2Cl_2). HRFAB calcd for $C_{47}H_{49}FN_6O_5$: $[M+H]^+$ 797.3827. Found: 797.3834.

3.1.5. Mitsunobu etherification products 20. To phenols **19** (110 mg, 0.138 mmol) is added a solution of allyl alcohol (24 mg, 28 μL, 0.414 mmol) in anhydrous THF (0.37 mL) followed by PPh_3 (109 mg, 0.414 mmol in 92 μL PhH). The solution is cooled in an ice-water bath and treated with diisopropyl azodicarboxylate (84 mg, 82 μL, 0.414 mmol) dropwise via syringe. Stirring is continued at 0°C for 30 min and at rt for 30 min. Concentration and purification by flash chromatography ($CHCl_3$/EtOAc/MeOH 8/1.6/0.4→8/1.3/0.7) affords **20** as white solids (two separated diastereomers (1:1), 116 mg total, 81%). **20**: less polar diastereomer: R_f=0.53 ($CHCl_3$/EtOAc/MeOH=6/3/1). $[\alpha]_D^{24}$=-20.9° (*c*=0.46, MeOH). IR (film): 3296, 3065, 2927, 2855, 1648, 1509, 1375, 1245, 1156, 1104, 837, 741 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.86 (s, 1H), 7.47 (dd, *J*=8.8, 5.6 Hz, 2H), 7.35 (s, 1H), 7.34 (d, *J*=7.6 Hz, 1H), 7.21 (t, *J*=8.0 Hz, 1H), 7.10–7.19 (m, 2H), 6.96–7.06 (m, 4H), 6.92 (d, *J*=2.0 Hz, 1H), 6.84 (dd, *J*=8.4 Hz, 2.0, 1H), 6.68 (d, *J*=8.4 Hz, 1H), 6.47 (dd, *J*=8.4, 2.0 Hz, 1H), 6.36–6.44 (m, 3H), 6.22–6.34 (m, 2H), 6.16 (t, *J*=4.0 Hz, 1H), 5.92 (sym 10 line m, 1H), 5.29 (dd, *J*=17.2, 1.6 Hz, 1H), 5.20 (d, *J*=10.0 Hz, 2H), 5.02 (d, *J*=6.4 Hz, 1H), 4.91 (d, *J*=17.2 Hz, 1H), 4.61–4.70 (m, 2H), 4.49 (d, *J*=16.8 Hz, 1H), 4.41 (d, *J*=16.8 Hz, 1H), 4.33–4.40 (m, 2H), 3.70–3.78 (m, 1H), 3.30–3.38 (m, 1H), 3.13–3.30 (m, 3H), 3.01–3.13 (m, 2H), 2.89 (dd, *J*=14.4, 8.0 Hz, 1H), 2.54–2.68 (m, 2H), 1.60–1.74 (br m, 2H), 1.45–1.52 (m, 2H), 1.29–1.40 (m, 2H), 0.93 (t, *J*=6.8 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.79, 170.75, 167.6, 158.3, 155.0, 144.8, 140.1, 137.2, 136.3, 133.4, 132.2, 130.7, 129.4, 128.7, 128.6, 128.2, 127.4, 127.1, 122.9, 122.8, 122.5, 112.0, 118.6, 117.3, 116.7, 115.8, 115.6, 115.5, 112.4, 111.6, 110.9, 109.5, 69.1, 66.2, 54.2, 54.0, 48.8, 47.8, 39.7, 37.3, 31.6, 28.8, 27.2, 20.3, 19.9, 14.0. LRMS (positive electrospray) calcd for $C_{50}H_{53}FN_6O_5$: $[M+H]^+$ 837.42. Found: 837.15. **20**: more polar diastereomer: R_f=0.43 ($CHCl_3$/EtOAc/MeOH=6/3/1). $[\alpha]_D^{24}$=+9.9° (*c*=1.2, $CHCl_3$). IR (film): 3301, 3065, 2926, 2855, 1652, 1506, 1456, 1247, 1157, 840, 743 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 8.49 (s, 1H), 7.46 (dd, *J*=8.4, 6.0 Hz, 2H), 7.40 (d, *J*=8.0 Hz, 1H), 7.17–7.25 (m, 3H), 7.07–7.17 (m, 2H), 6.94–7.07 (m, 4H), 6.74 (s, 1H), 6.75 (d, *J*=8.4 Hz, 1H), 6.66 (d, *J*=8.4 Hz, 1H), 6.54–6.42 (m, 4H), 6.30 (sym 7 line m, 1H), 6.09 (s, 1H), 5.90 (sym 8 line m, 1H), 5.28 (d, *J*=17.2 Hz, 1H), 5.19 (t, *J*=9.6 Hz, 2H), 5.13 (d, *J*=7.2 Hz, 1H), 5.00 (d, *J*=17.2 Hz, 1H), 4.66 (dd, *J*=12.4, 6.8 Hz, 1H), 4.30–4.48 (m, 5H), 3.53–3.62 (m, 1H), 3.43–3.53 (m, 1H), 3.24–3.10 (m, 3H), 2.97–3.09 (m, 2H), 2.91 (dd, *J*=14.4, 7.2 Hz, 1H), 2.57–2.72 (m, 2H), 1.46–1.64 (m, 2H), 1.35–1.46 (m, 2H), 1.24–1.34 (m, 2H), 0.89 (t, *J*=7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.9, 170.7, 167.2, 160.4, 158.3, 155.2, 145.6, 140.3, 137.9, 137.4, 136.5, 133.5, 132.5, 131.2, 130.1, 129.2, 128.9, 128.8, 128.0, 127.3 127.1, 123.3, 122.7, 122.6, 120.0, 118.9, 117.0, 116.7, 115.81, 115.75, 115.5, 112.3, 111.6, 110.4, 109.9, 69.1, 66.3, 53.9, 48.0, 47.8, 39.7, 37.3, 31.7, 28.2, 27.9, 20.3, 20.2, 14.0. LRMS (positive electrospray) calcd for $C_{50}H_{53}FN_6O_5$: $[M+H]^+$ 837.42. Found: 837.25.

3.1.6. Dihydrobenzoxepins 21. To a dry reaction flask containing **20** (less polar diastereomer, 10 mg, 11.9 μmol) is added a degassed (N_2 purge) CH_2Cl_2 solution (0.8 mL) of ruthenium alkylidene **22** (Strem chemical, 1.0 mg, 10 mol%). The resultant solution is heated to reflux for a total of 10 h. Additional aliquots of catalyst solution (5 mol%) are added at 3 and 7 h. The mixture is cooled to rt, concentrated, and the residue purified by PTLC to afford **21** (6.2 mg, 65%) as a white film.

Compound **21**. ^1H NMR (400 MHz, CD_3OD): δ 7.54 (d, *J*=7.6 Hz, 1H), 7.41–7.47 (m, 3H), 7.31 (d, *J*=8.0 Hz, 1H), 7.20 (app t, *J*=8.0 Hz, 1H), 7.14 (d, *J*=8.0 Hz, 1H), 7.05–7.16 (m, 2H), 6.96–7.05 (m, 5H), 6.84 (d, *J*=7.6 Hz, 1H), 6.62 (dd, *J*=8.0 Hz, 1.6, 1H), 6.54 (s, 1H), 6.07–6.14 (m, 1H), 5.66 (app d, *J*=11.2 Hz, 1H), 4.58–4.66 (m, 4H), 4.52–4.58 (m, 2H), 4.35 (dd, *J*=19.0, 1.2 Hz, 1H), 3.70 (t, *J*=5.2 Hz, 2H), 3.26 (dd, *J*=14.8, 6.0 Hz, 1H), 3.07–3.15 (m, 3H), 2.98 (dd, *J*=14.8, 4.4 Hz, 1H), 2.89 (dd, *J*=14.4, 8.8 Hz, 1H), 2.62–2.74 (m, 2H), 1.60–1.71 (m, 1H), 1.47–1.60 (m, 1H), 1.37–1.46 (m, 2H), 1.28–1.37 (m, 2H), 0.92 (t, *J*=7.6 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.7, 170.3, 167.1, 158.3, 156.9, 145.1, 139.0, 137.7, 137.5, 136.3, 132.3, 131.4, 130.4, 129.9, 129.5, 129.3, 128.6,

128.5, 128.2, 126.8, 123.6, 122.9, 122.6, 121.3, 120.4, 118.7, 116.5, 115.8, 115.5, 111.7, 110.4, 109.2, 71.1, 65.5, 53.9, 53.4, 48.9, 47.5, 39.6, 36.9, 31.6, 28.7, 27.1, 20.2, 19.5, 14.0. LRMS (positive electrospray) calcd for $C_{48}H_{49}FN_6O_5$: $[M+H]^+$ 809.38. Found: 809.30. HRFAB calcd for $C_{48}H_{49}FN_6O_5$: $[M+H]^+$ 809.3826. Found: 809.3827.

Acknowledgements

Funding provided by the NIH (RO1-GM60591), the NSF (CAREER 9984282), the Howard Hughes Medical Institute, the Robert A. Welch Foundation, and an unrestricted research award from AstraZeneca. We thank Dr. Jia Zhou for experimental assistance. P.G.H. is a fellow of the Alfred P. Sloan Foundation.

References

1. Outlines of this general concept are also evident in Houghten's "Libraries from libraries experiments". See: Ostresh, J. M.; Husar, G. M.; Blondelle, S. E.; Dörner, B.; Weber, P. A.; Houghten, R. A. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 11138–11142.
2. Sisko, J.; Mellinger, M.; Sheldrake, P. W.; Baine, N. H. *Tetrahedron Lett.* **1996**, *37*, 8113–8116.
3. (a) Sisko, J.; Kassick, A. J.; Mellinger, M.; Filan, J. J.; Allen, A.; Olsen, M. A. *J. Org. Chem.* **2000**, *65*, 1516–1524. (b) van Leusen, A. M.; Wildeman, J.; Oldenziel, O. H. *J. Org. Chem.* **1977**, *42*, 1153–1159.
4. Imidazole **4** is produced as an inconsequential mixture of diastereomers. This material, and others like it examined in this study ('condensation' products in Table 1) are highly insoluble in most common organic solvents as well as water. Preparative scale purification therefore is a challenge. Precipitation is found most effective although, because recovery using this method is not complete, isolated yields (Table 1) underestimate the true efficiency of these reactions.
5. For palladium catalysis relevant to this work see: (a) Trost, B. M.; Toste, F. D. Int. Pat. App. (PCT) Publication number WO 00/14033, 2000. (b) Trost, B. M.; Toste, F. D. *J. Am. Chem. Soc.* **1998**, *120*, 9074–9075. (c) Trost, B. M.; Toste, F. D. *J. Am. Chem. Soc.* **1998**, *120*, 815–816. (d) Goux, C.; Lhoste, P.; Sinou, D. *Synlett* **1992**, 725–727.
6. A more polar by-product repeatedly generated in this reaction has been isolated and found to have ^{1}H NMR and mass spectral data consistent with a symmetrical dimer.
7. (a) Yin, J.; Buchwald, S. L. *J. Am. Chem. Soc.* **2002**, *124*, 6043–6048. (b) Kamer, P. C. J.; van Leeuwen, P. W. N. M.; Reek, J. N. H. *Acc. Chem. Res.* **2001**, *34*, 895–904. (c) Kranenburg, M.; van der Burgt, Y. E. M.; Kamer, P. C. J.; van Leeuwen, P. W. N. M. *Organometallics* **1995**, *14*, 3081–3089.
8. Peptides used in this study were prepared (Fmoc methodology) as C-terminal *n*-butyramides to approximate the size and hydrophobicity of a spacer used for solid-phase synthesis experiments (Scheme 3).
9. Synthetic peptides Gly-His, Gly-Thr, Gly-Pro-Ser, Gly-Pro-His, and Gly-Pro-Trp (each prepared as a C-terminal *n*-butyramide) readily undergo condensation with **2** followed by cycloaddition with **3**. The products (analogous to **4**), however, do not cyclize when treated with the $[(\eta^3\text{-allyl})PdCl]_2$/**5** complex. While initial studies have focused on peptides containing N-terminal Gly or Ala residues, we see no indication of a limited tolerance at this position.
10. The structures assigned to regioisomers **11** and **12** are tentative. The chemical shift (400 MHz, CD_3OD) of the β-styryl proton in **11** (C16H, δ 6.37) is more similar to that observed in 25-membered macrocycles (**7**–**10**, **13**, **16**, **17**—range: δ 6.29–6.45) than it is to the 22-membered ring series [namely **6** (δ 6.16) and **14** (δ 6.21)]. The minor isomer (assigned as **12**) has this resonance appearing at δ 6.15.
11. Isolated **6** decomposes ($t_{1/2}\sim$5 h) when re-subjected to the same conditions used for its synthesis. Aqueous cyanide minimizes product loss by poisoning the etherification catalyst.
12. Williams, A. J.; Uto, Y.; Wipf, P.; Reno, M. J.; Williams, D. R. *Org. Lett.* **2000**, *2*, 1165–1168.
13. Dihydroxylation using 'Super AD-mix-β' (Ref. 14) provides glycol **18** as a mixture (94:6) of diastereomers. The stereochemistry of the major isomer (shown) is assigned using an established mnemonic. The use of Super AD-mix-α provides the same two diols (confirmed by HPLC co-injection) but in a 92:8 ratio in which **18** is minor.
14. Nicolaou, K. C.; Yue, E. W.; La Greca, S.; Nadin, A.; Yang, Z.; Leresche, J. E.; Tsuri, T.; Naniwa, Y.; De Riccardis, F. *Chem. Eur. J.* **1995**, *1*, 467–494.
15. Aryl Claisen rearrangement is best performed by microwave heating (180°C, 40 min) solutions of **8** containing flash chromatography-grade silica gel (230–400 mesh, 4 g/mmol **8**). Added silica gel allows the reaction to proceed to full conversion without substantial degradation. Soluble Lewis-acidic additives have thus far failed in this regard.
16. Chatterjee, A. K.; Morgan, J. P.; Scholl, M.; Grubbs, R. H. *J. Am. Chem. Soc.* **2000**, *122*, 3783–3784.
17. In this discussion, 'alkaloid-like' is meant primarily to contrast 'peptide-like' Our goal is the systematic preparation, from peptides, of non-reactive molecules that passively diffuse into the cytoplasm of living cells from culture media.
18. Professor Matthew Shair (Harvard University) generously provided us with resin that fueled our initial experiments. See: Tallarico, J. A.; Depew, K. M.; Pelish, H. E.; Westwood, N. J.; Lindsley, C. W.; Shair, M. D.; Schreiber, S. L.; Foley, M. A. *J. Comb. Chem.* **2001**, *3*, 312–318.
19. *Fmoc Solid Phase Peptide Synthesis*; Chan, W. C., White, P. D., Eds.; Oxford University: Oxford, 2000; ISBN 0199637245.
20. At present, alcohol **26** (assignment supported by ^{1}H NMR and mass spectrometry) is isolated together with un-reacted H_2N-Gly-Tyr-NH$(CH_2)_3$OH. Achieving complete conversion in the synthesis of **25** from **24** is the subject of ongoing experiments.