Total Synthesis

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Total Synthesis of Chloptosin**

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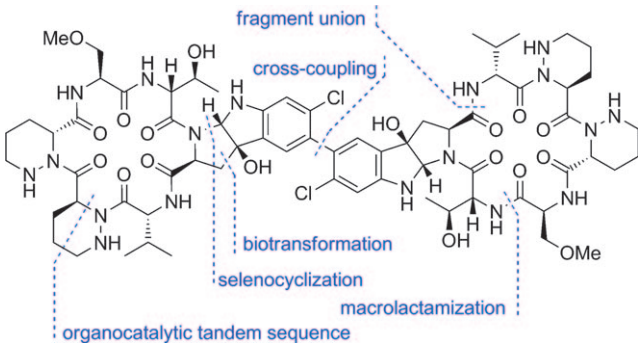
Chloptosin (**1**) is a structurally interesting anticancer agent that was isolated by Umezawa et al. from a culture broth of *Streptomyces*.[1] It was found to induce apoptosis in both the apoptosis-resistant human pancreatic adenocarcinoma cell line AsPC-1 and also in several apoptosis-sensitive cancer cell lines. In addition, chloptosin exhibits strong antimicrobial activity against Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA). Chloptosin represents a challenging synthetic target consisting of two identical cyclic hexapeptide subunits connected by a central biaryl bond. These subunits contain exclusively nonproteinogenic amino acids in alternating enantiomeric forms including the unusual (*R*)- and (*S*)-piperazic acids, in addition to a 6-chloropyrroloindole residue.[1,2]

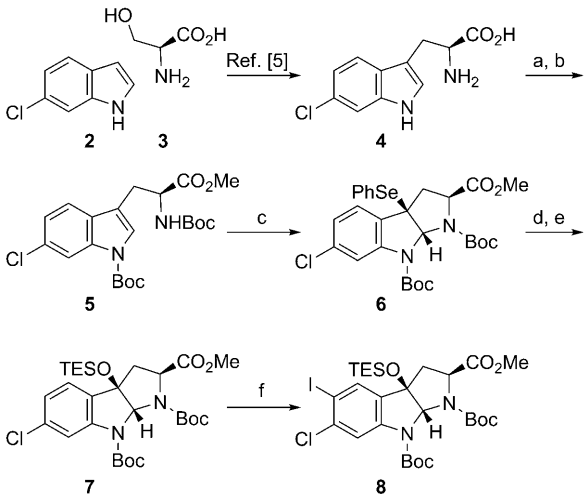
Herein we present some of our synthetic approaches to chloptosin (**1**) that eventually resulted in the successful preparation of the natural product using a new asymmetric organocatalytic route to prepare the embedded piperazic acids.

Although we considered several synthetic approaches, we have elected to use a convergent approach to chloptosin (**1**) in the first instance, in which the central biaryl linkage could be constructed by late-stage union of two hexapeptide monomers through a metal-catalyzed coupling reaction (Scheme 1). We anticipated that these monomer units could then be derived from a 6-chloropyrroloindole fragment by coupling with the nonproteinogenic amino acid derivatives D-threonine, L-serine methyl ether, (*R*)- and (*S*)-piperazic acids, and D-valine. A known selenocyclization reaction[3] would provide rapid access to the pyrroloindole fragment, while both piperazic acids could be prepared by an organocatalytic tandem reaction sequence.[4] Should this route fail, we anticipated that early dimerization of a 6-chloropyrroloindole core fragment would be necessary, which in turn would be followed by bidirectional incorporation of the requisite amino acids.[5]



Scheme 1. Chloptosin (**1**) and key disconnections.

To begin the synthesis, an appropriate precursor for the selenocyclization reaction had to be constructed. Thus, 6-chlorotryptophan **4** was prepared using an enzymatic biotransformation starting from 6-chloroindole (**2**) and L-serine (**3**; Scheme 2).[6] Esterification of **4** and N protection with Boc afforded the cyclization precursor **5**. Cyclization of **5** using *N*-(phenylseleno)phthalimide proceeded smoothly and furnished the expected pyrroloindole fragment **6** as a single



Scheme 2. Preparation of the pyrroloindole fragment **8**. Reagents and conditions: a) $SOCl_2$, MeOH, 0 to 65 °C, 99%; b) Boc_2O, NaOH, $Bu_4N{\cdot}HSO_4$, CH_2Cl_2, RT, quant.; c) *N*-PSP, PPTS, Na_2SO_4, CH_2Cl_2, RT, 89%; d) *m*CPBA, wet CH_2Cl_2, 0 °C to RT, 80%; e) TESCl, DBU, DMF, 50 °C, 95%; f) ICl, 2,6-di-*tert*-butylpyridine, CH_2Cl_2, 55 °C, sealed tube, 88%. Boc = *tert*-butoxycarbonyl, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, DMAP = *N*,*N*-dimethylaminopyridine, DMF = *N*,*N*-dimethylformamide, *m*CPBA = *meta*-chloroperbenzoic acid, PPTS = pyridinium *para*-toluenesulfonate, *N*-PSP = *N*-(phenylseleno)phthalimide, TES = triethylsilyl.

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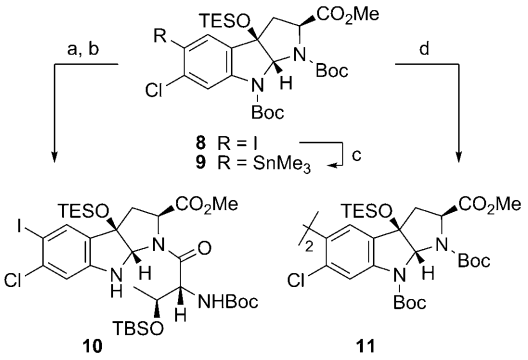
diastereoisomer. Oxidative deselenation of **6** with wet *m*-chloroperbenzoic acid generated the corresponding alcohol, which was then protected as its silyl ether **7**. Finally, regioselective iodination of **7** was achieved by the action of iodine monochloride.

Intermediate **8** was used as a test substrate for both peptide coupling and for the crucial dimerization process (Scheme 3). Treatment of **8** with trifluoroacetic acid yielded



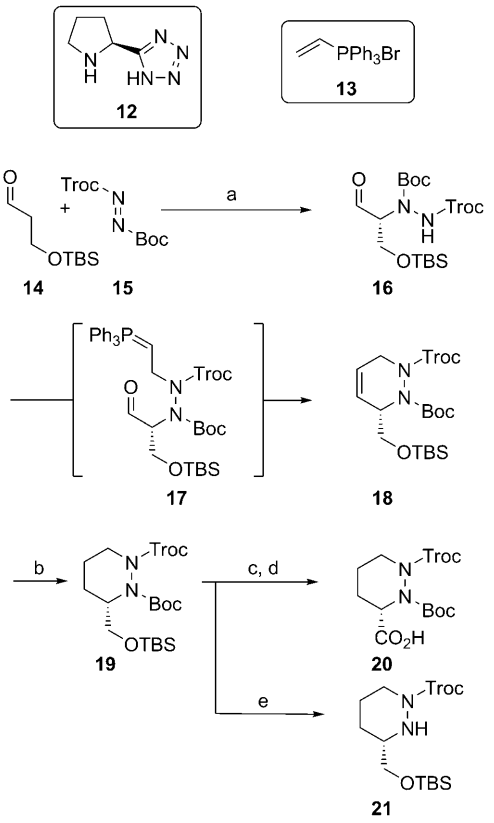
Scheme 3. Dimerization and peptide coupling studies. Reagents and conditions: a) TFA, 0 °C to RT; b) BocThr(OTBS)OH, HATU, collidine, CH_2Cl_2, −10 °C to RT, 85 % (over 2 steps); c) Me_6Sn_2, [$Pd(PPh_3)_4$], toluene, 55 °C, 60 %; d) **8**, [$Pd_2(dba)_3$], Ph_3As, CuI, DMF, 100 °C (μw), 73 %. dba = dibenzylideneacetone, HATU = *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, TBS = *tert*-butyldimethylsilyl, TFA = trifluoroacetic acid.

the free diamine, which underwent selective peptide bond formation with *N*-Boc threonine and gave dipeptide **10**, a motif present in the natural product. To examine the feasibility of a late-stage coupling of two chloptosin monomers through metal-catalyzed coupling reaction, iodide **8** was converted into its pendant trimethylstannane derivative **9** (Scheme 3). Coupling of **9** with iodide **8** yielded the dimerized core structure **11** in good yield.

Recently, we have developed an organocatalytic, one-pot procedure for the enantioselective synthesis of 3,6-dihydropyridazines from achiral aldehydes and ketones using tetrazole catalyst **12**. This process proceeds with good to excellent yields and enantioselectivities through α-amination, base-promoted conjugate addition to a vinylphosphonium salt, and subsequent Wittig ring-closure (Scheme 4).[4,7]

In the new application reported here, we envisaged that the initially formed 3,6-dihydropyridazine could act as a versatile precursor for the preparation of a number of piperazic acid derivatives. To provide selective access to each nitrogen atom in the piperazic acid, an orthogonally protected azodicarboxylate was necessary, which would lead directly to readily differentiable doubly protected piperazic acid precursors.

The required starting material **15** was prepared in quantitative yield in two steps from *tert*-butyl carbazate by protection with Troc and oxidation.[8] More importantly, when **15** was subjected to the organocatalytic α-amination conditions with **12** as the catalyst, hydrazino aldehyde **16** was formed as the sole product from the reaction mixture in high

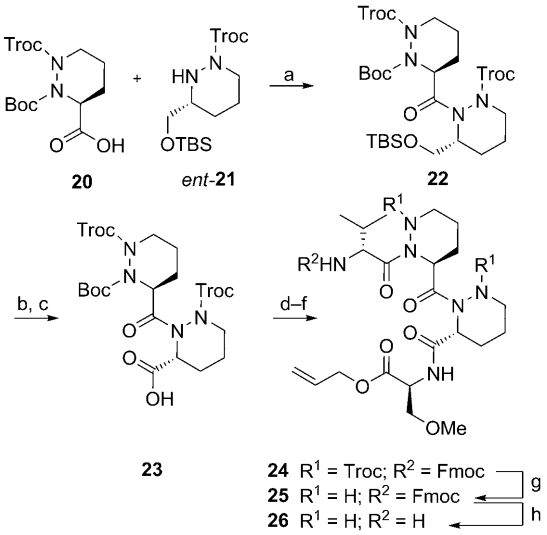


Scheme 4. Preparation of piperazic acids **20** and **21**. Reagents and conditions: a) **12**, CH_2Cl_2, −5 °C; then **13**, NaH, THF, −5 °C to RT, 90 %, e.r. = 92:8; b) PtO_2, H_2 (10 bar), THF, RT, 98 %; c) HCl (4 M in dioxane), CH_2Cl_2, THF, 0 °C to RT; d) $NaIO_4$, $RuCl_3.nH_2O$, water, acetone, MeCN, RT, 94 % (over 2 steps); e) TESOTf, 2,6-lutidine, CH_2Cl_2, 0 °C to RT, 94 %. Tf = trifluoromethanesulfonyl, THF = tetrahydrofuran, Troc = 2,2,2-trichloroethyloxycarbonyl.

yield and e.r. value (92:8; Scheme 4). Aldehyde **16** was trapped in situ by base-promoted addition of the secondary carbamate of **16** to vinyl phosphonium bromide **13**, which provided the transient ylid **17** that in turn immediately cyclized to the desired 3,6-dihydropyridazine **18**. *Ent*-**18** was obtained in similar yield and e.r. value by employing the corresponding tetrazole catalyst *ent*-**12**. Hydrogenation of the double bond present in **18** using platinum oxide gave key intermediate **19** in good yield. The functional groups present in **19** could be selectively unmasked as required for further conversion into piperazic acid coupling partners **20** and **21**. Treatment of **19** with hydrochloric acid led exclusively to cleavage of the silyl protecting group and, after oxidation using ruthenium periodate, provided **20**, the required precursor for peptide coupling at the C terminus. Recrystallization of protected piperazic acid **20** further improved the enantiomeric purity to > 200:1. Selective removal of the Boc carbamate from **19** could be achieved using triethylsilyl triflate and 2,6-lutidine to give **21**.[9] Each manipulation of hydrogenated intermediate **19** proceeded in excellent yield with no loss of enantiopurity and without affecting the remaining protecting groups present. Following this route, gram quantities of the requisite piperazic acid derivatives

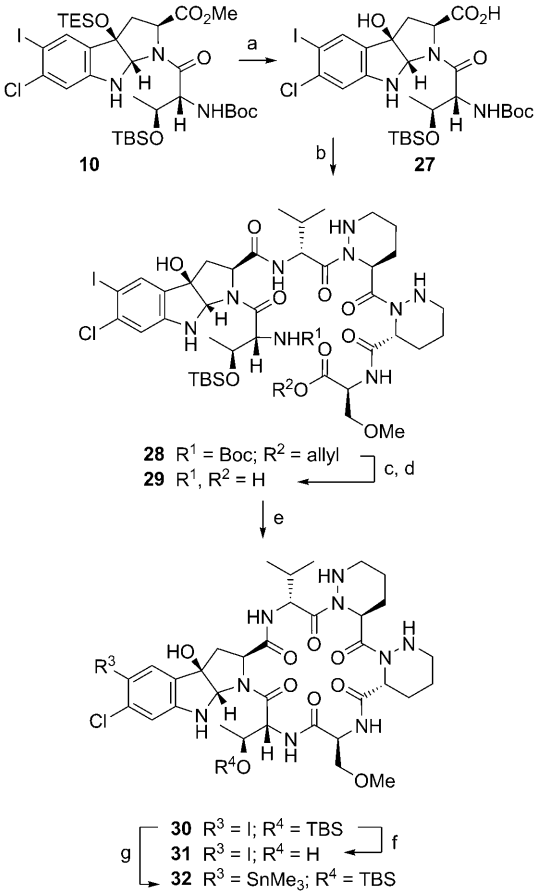
required for the projected total synthesis were readily accessible.

During the assembly of the peptide fragment **26**, it was anticipated that the nucleophilicity of the α nitrogen would be increased, relative to the corresponding acid, by maintaining the alcohol oxidation level on the C terminus of the piperazic alcohol, thereby facilitating peptide bond formation.[10] Thus, *ent*-**21** was treated with the acid chloride of **20** and the desired dipeptide could be isolated in 71% yield (Scheme 5). The latter was then converted into acid **23** by a short sequence involving cleavage of the silyl group and ruthenium-catalyzed oxidation. The peptide chain was further elongated by peptide coupling with *O*-methyl serine allyl ester, removal of the remaining Boc group and acylation using valine protected with Fmoc to give tetrapeptide fragment **24**.



Scheme 5. Preparation of tetrapeptide fragment **26**. Reagents and conditions: a) Ghosez' reagent, CH_2Cl_2, 0°C; then *ent*-**21**, pyridine, CH_2Cl_2, 0°C to RT, 71%; b) HCl (4 M in dioxane), CH_2Cl_2, THF, 0°C to RT, 77%; c) $NaIO_4$, $RuCl_3.nH_2O$, water, acetone, RT, 83%; d) HSer(OMe)Oallyl, HATU, iPr_2NEt, DMF, 0°C to RT, 79%; e) TFA, toluene, 0°C to RT; f) FmocValCl, AgCN, benzene, 80°C, 84% (over 2 steps); g) Pb/Cd, THF, aq NH_4OAc, RT, quant.; h) 10% piperidine in DMF (degassed), RT, quant. Fmoc = fluorenylmethyloxycarbonyl, Ghosez' reagent = 1-chloro-*N*,*N*-2-trimethyl-1-propenylamine.

We reasoned that a final macrocyclization reaction between the threonine and serine units would provide the greatest opportunity for success owing to the steric accessibility of both the nucleophilic and electrophilic reaction partners, and this therefore determined our eventual coupling strategy. Pyrroloindole threonine dipeptide **10** was available by standard peptide coupling (cf. Scheme 3). As anticipated, the desired fragment union between the crude sample of **26** and **27** afforded linear monomer **28** in good yield (Scheme 6). The allyl ester was cleaved in presence of the aryl iodide functionality using a $[Pd(PPh_3)_4]$ and morpholine mixture and the remaining Boc group was removed upon treatment with TFA. Treatment with the peptide coupling reagent HATU

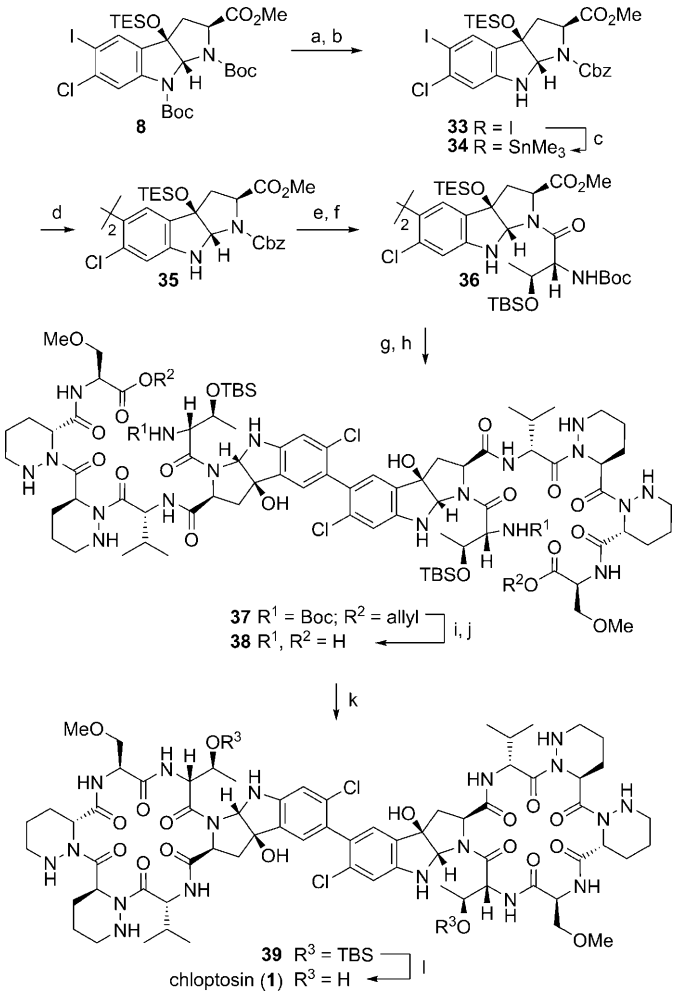


Scheme 6. Fragment union and cyclization studies. Reagents and conditions: a) LiOH, THF, water, MeOH, RT; b) PyAOP, HOAt, iPr_2NEt, **26**, CH_2Cl_2, 67% (over 2 steps); c) $[Pd(PPh_3)_4]$, morpholine, MeCN, 0°C; d) TFA, toluene, 0°C to RT; e) HATU, HOAt, iPr_2NEt, DMF, 0°C to RT, 79% (over 3 steps); f) HF·pyridine, pyridine, 0°C to RT, 44%; g) $[Pd(PPh_3)_4]$, Me_6Sn_2, toluene, 60°C, 30%. HOAt = 1-hydroxy-7-azabenzotriazole, PyAOP = (7-azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate.

cleanly converted the material into cyclic hexapeptide **30** in good yield. Final desilylation using HF in pyridine afforded chloptosin monomer **31**.

With chloptosin monomer **31** in hand, efforts were then focused on the dimerization process. Partial conversion of **30** into organostannane **32** proceeded cleanly. Indeed, when **30** and **32** were subjected to the dimerization conditions for **8** and **9** (cf. Scheme 3), a peak consistent with a dimer was observed by high-resolution mass spectrometry; however, this also indicated that an oxidation had occurred, presumably within the piperazic acid skeleton.[10–12]

In view of this result, it was necessary to return to the earlier dimerization approach. Accordingly, Boc-protected dimer **11** was treated with TFA to effect the liberation of the amine functionalities. Unfortunately, we were never able to remove all four Boc groups without substantial degradation. Therefore, we opted to use pyrroloindole **8** through initial conversion into Cbz-protected congener **33** (Scheme 7). Transformation of iodide **33** into its trimethylstannane analogue **34** and subsequent Stille reaction smoothly fur-



Scheme 7. Synthesis of chloptosin (**1**). Reagents and conditions: a) TFA, 0 °C to RT; b) CbzCl, K_2CO_3, THF, water, RT, 81 % (over 2 steps); c) $[Pd(PPh_3)_4]$, Me_6Sn_2, toluene, 55 °C, 84 %; d) **33**, $[Pd_2(dba)_3]$, Ph_3As, CuI, DMF, 100 °C (μw), 58 %; e) Pd/C, H_2, EtOAc, RT, quant.; f) BocThr-(OTBS)OH, HATU, HOAt, collidine, −10 °C to RT, 94 %; g) LiOH, water, THF, RT; h) **26**, BEP, HOAt, , iPr_2NEt, CH_2Cl_2, DMF, −10 °C to RT, 56 %; i) $[Pd(PPh_3)_4]$, Me_2NH, THF, 0 °C to RT; j) TFA, toluene, 0 °C to RT; k) PyBOP, HOAt, iPr_2NEt, CH_2Cl_2, DMF, 0 °C to RT, 43 % (over 3 steps); l) TBAF (1 M in THF), HOAc, THF, 0 °C to RT, 65 %. BEP = 2-bromo-1-ethylpyridinium tetrafluoroborate, Cbz = benzyloxycarbonyl, PyBOP = 1-benzotriazolyloxy-tris(pyrollidino)phosphonium, TBAF = tetrabutylammonium fluoride.

nished the required dimer **35**, which in turn could then be deprotected and coupled to Boc-D-threonine to give desired tetrapeptide **36**. This material was then successfully converted into the natural product following the route developed earlier through saponification, coupling with tetrapeptide **26**,[2b] allyl ester and Boc removal, macrocyclization, and desilylation. The synthetic material was identical in all respects to an authentic sample.

In summary, a total synthesis of chloptosin (**1**) has been achieved in 30 steps (17 steps in the longest linear sequence) and in 4 % overall yield from azodicarboxylate **15**. Especially pleasing, during this work we developed a versatile enantioselective organocatalytic route to piperazic acids that employs a new differentially substituted (Boc and Troc) azodicarboxylate, to generate orthogonally protected piperazic acid building blocks in multigram quantities in a short and efficient sequence (> 80 % from cheap commercial achiral starting materials). Furthermore, a Stille reaction was used successfully to couple two sterically demanding *ortho*-chloropyrroloindoles. Following this readily modifiable route, a new generation of analogues for biological testing can be realized that could provide valuable new information concerning the mechanism of action of this structurally interesting molecule.

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[1] K. Umezawa, Y. Ikeda, Y. Uchihata, H. Naganawa, S. Kondo, *J. Org. Chem.* **2000**, *65*, 459.
[2] a) W.-X. Hong, L.-J. Chen, C.-I. Zhong, Z.-J. Yao, *Org. Lett.* **2006**, *8*, 4919; b) S.-M. Yu, W.-X. Hong, Y. Wu, C.-L. Zhong, Z.-J. Yao, *Org. Lett.* **2010**, *12*, 1124.
[3] a) S. P. Marsden, K. M. Depew, S. J. Danishefsky, *J. Am. Chem. Soc.* **1994**, *116*, 11143; b) S. V. Ley, E. Cleator, P. R. Hewitt, *Org. Biomol. Chem.* **2003**, *1*, 3492; c) P. R. Hewitt, E. Cleator, S. V. Ley, *Org. Biomol. Chem.* **2004**, *2*, 2415.
[4] a) A. J. Oelke, S. Kumarn, D. A. Longbottom, S. V. Ley, *Synlett* **2006**, 2548; b) S. Kumarn, A. J. Oelke, D. M. Shaw, D. A. Longbottom, S. V. Ley, *Org. Biomol. Chem.* **2007**, *5*, 2678.
[5] a) T. M. Kamenecka, S. J. Danishefsky, *Angew. Chem.* **1998**, *110*, 3164; *Angew. Chem. Int. Ed.* **1998**, *37*, 2993; b) T. M. Kamenecka, S. J. Danishefsky, *Angew. Chem.* **1998**, *110*, 3166; *Angew. Chem. Int. Ed.* **1998**, *37*, 2995; c) T. M. Kamenecka, S. J. Danishefsky, *Chem. Eur. J.* **2001**, *7*, 41.
[6] R. J. M. Goss, P. L. A. Newill, *Chem. Commun.* **2006**, 4924.
[7] a) D. A. Longbottom, V. Franckevicius, S. Kumarn, A. J. Oelke, V. Wascholowski, S. V. Ley, *Aldrichimica Acta* **2008**, *41*, 3; b) V. Aureggi, V. Franckevicius, M. O. Kitching, S. V. Ley, D. A. Longbottom, A. J. Oelke, G. Sedelmeier, S. E. Denmark, R. C. Smith, *Org. Synth.* **2008**, *85*, 72.
[8] See the Supporting Information for experimental details.
[9] M. Sakaitani, Y. Ohfune, *J. Org. Chem.* **1990**, *55*, 870.
[10] M. A. Ciufolini, N. Xi, *Chem. Soc. Rev.* **1998**, *27*, 437.
[11] Test reactions with tetrapeptide **25** are consistent with copper(I) iodide causing oxidation. Attempts to avoid the use of copper as an additive in Stille cross-couplings failed, as did Suzuki cross-coupling conditions.
[12] L. A. Paquette, M. Duan, I. Konetzki, C. Kempmann, *J. Am. Chem. Soc.* **2002**, *124*, 4257.

