CHEMISTRY
A EUROPEAN JOURNAL

ChemPubSoc
Europe

DOI: 10.1002/chem.201303347

Epimerization-Free C-Terminal Peptide Activation

Stanimir Popovic,[a] Hans Bieräugel,[a] Remko J. Detz,[b] Alexander M. Kluwer,[b] Jelmer A. A. Koole,[a] Dieuwertje E. Streefkerk,[a] Henk Hiemstra,[a] and Jan H. van Maarseveen*[a]

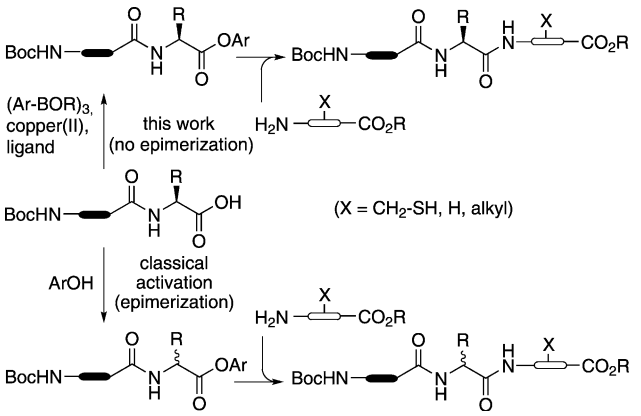
Peptides constitute a very important compound class in drug research.[1] Thus, racemization-free peptide synthesis is of key importance to acquire the required peptide chains in diastereomerically pure form. In general, the way to synthesize peptides with full stereointegrity is by elongation at the N terminus.[2] On the other hand, methods that allow epimerization-free C-terminal peptide activation would greatly enhance the available routes and would be a highly valuable synthetic tool for obtaining the desired peptides.[3] Especially for the convergent solution-phase synthesis of peptides by segment coupling a reliable and epimerization-free C-terminal activation methodology is required.[4] Recently, Danishefsky showed the utility of peptide 4-nitrophenyl esters in peptide segment couplings.[5] These esters were prepared by EDCI/HOBt-mediated coupling of the amino acid 4-nitrophenylesters to the C terminus of peptides.[5b] Due to otherwise inevitable epimerization this methodology is restricted by the requirement of a glycine residue at the C-terminal penultimate position. Herein, we disclose our initial results on the development of a racemization-free C-terminal peptide activation through the copper(II)-mediated Chan–Lam-type coupling between peptides and arylboroxines and subsequent amine-coupling reactions (Scheme 1).[6]

Recently, the group of Cheng reported the $Cu(OTf)_2$-mediated reaction of benzoic acids with arylboronic acids to provide facile access to arylesters.[7] The single example of an aliphatic acid, that is, phenylacetic acid, that reacted efficiently with phenylboronic acid to give the ester in near quantitative yield prompted us to expand this chemistry towards the peptide series. The suggested mechanistic course of this reaction and the mild conditions make epimerization very unlikely.[8] Therefore, the Chan–Lam approach may be a suitable method for activation of peptides bearing a chiral C-terminal amino acid.

[a] S. Popovic, H. Bieräugel, J. A. A. Koole, D. E. Streefkerk, Prof. Dr. H. Hiemstra, Dr. J. H. van Maarseveen
Van 't Hoff Institute for Molecular Sciences
University of Amsterdam, Science Park 904
1098XH Amsterdam (The Netherlands)
E-mail: j.h.vanmaarseveen@uva.nl
Homepage: http://hims.uva.nl/soc

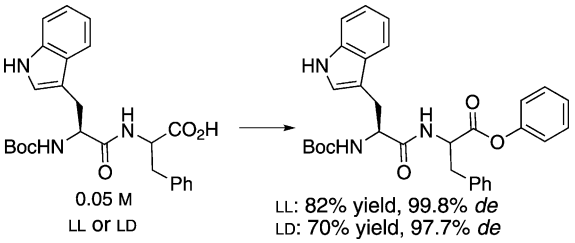
[b] Dr. R. J. Detz, Dr. A. M. Kluwer
InCatT, Science Park 904, 1098XH Amsterdam (The Netherlands)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201303347.



Scheme 1. Copper(II)-mediated and classical peptide arylester synthesis and subsequent elongation.

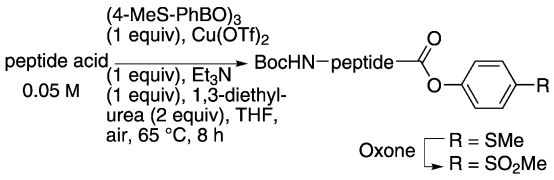
To evaluate and optimize the Chan–Lam-type esterification of peptides, Boc-Trp-Phe-OH was selected as the substrate. The first coupling attempt was performed using the conditions reported by Cheng and co-workers, that is, stirring of the carboxylic acid, phenylboronic acid (3 equiv), $Cu(OTf)_2$ (0.4 equiv), and urea (1 equiv) at elevated temperature (60 °C) under air using EtOAc as the solvent. Gratifyingly, after running the reaction for 12 h, Boc-Trp-Phe-OPh could be isolated in a yield of 40 %. The reaction proceeded in a rather clean fashion, and besides the recovered peptide phenol and diphenyl ether were isolated as the main side products. It should also be noted that all three nitrogen atoms bearing acidic protons, that is, the indole-NH, amide-NH and Boc-NH, showed no reactivity under these mild conditions. After screening several other bidentate N-centered ligands, ureas stood out as the ligand class of choice. Replacing urea with 1,3-diethylurea gave a homogeneous reaction mixture and the yield further improved to 50 %. The formation of phenol could be suppressed by starting from phenylboroxine, further enhancing the yield to 69 %. By decreasing the concentration of the carboxylic acid to 0.05 M, slightly increasing the temperature to 65 °C, and adding Et_3N as the base (1 equiv), yields of up to 82 % were obtained, albeit now requiring 1 equiv of $Cu(OTf)_2$ (Scheme 2). To check the stereointegrity of the copper(II)-catalyzed peptide esterification, Boc-Trp-D-Phe-OPh was prepared in 70 % isolated yield, and after comparison of the ^1H NMR spectra and chiral HPLC traces with Boc-Trp-Phe-



Scheme 2. Optimized copper(II)-mediated peptide arylester synthesis. Reagents and conditions: $(PhBO)_3$ (1 equiv), $Cu(OTf)_2$ (1 equiv), Et_3N (1 equiv), 1,3-diethylurea (2 equiv), air, THF or EtOAc, 65 °C, 8 h.

OPh no sign of epimerization was observed (*de* values of 99.8 and 97.7 % for the LL- and LD-diastereoisomers, respectively). This result is in stark contrast with the loss of stereocontrol observed after esterification of Boc-Trp-D-Phe-OH with phenol using HATU—especially known for its low racemization tendency—as the carboxyl activating agent, providing a 2:1 mixture of Boc-Trp-D-Phe-OPh and Boc-Trp-Phe-OPh (data not shown).

Although unsubstituted arylesters have been used directly in peptide ligation reactions, their mediocre reactivity requires large amounts of activating additives lowering the practicability.[9] Unfortunately, so far, all attempts to react electron-poor arylboroxines that should deliver highly activated esters, such as the pentafluorophenyl or 4-nitrophenyl, have given low yields. To overcome this, the 4-methylthiophenyl ester was considered, which can be transformed into the 4-(methylsulfonyl)phenyl ester by oxidation. Previous work has shown that 4-(methylsulfonyl)phenyl esters are very suitable as optically stable activated esters in peptide elongation chemistry.[10] $Cu(OTf)_2$-mediated coupling of Boc-Trp-Phe-OH with 4-(methylthio)phenyl boroxine using the optimized conditions gave the ester in the isolated yield of 57 % (Scheme 3, entry 1). Partial oxidation of the thioether to the sulfoxide was performed in quantitative yield using *m*CPBA as the oxidizing agent. However, further oxidation to the sulfone was not possible without affecting the tryptophan indole moiety. Fortunately, the synthesis of Boc-Phe-Phe-4-(methylsulfonyl)phenyl ester proceeded without problems in 77 % overall yield (entry 2), now using oxone as the oxidizing agent, the remainder of which was easily removed by non-basic aqueous extraction. To rule out the occurrence of epimerization in the Chan–Lam/oxidation reaction sequence, the diastereomeric Boc-Phe-D-Phe-4-(methylsulfonyl)phenyl ester was also prepared (entry 3). The exceptional stereointegrity of this sequence was further demonstrated by the synthesis of two epimeric dipeptides containing the very racemization-prone phenylglycine at the peptide C terminus. The optical purity of both Boc-Phe-Phg-4-(methylsulfonyl)phenyl ester and Boc-Phe-D-Phg-4-(methylsulfonyl)-phenyl ester remained 99.9 % (entries 4 and 5). Couplings at sterically hindered positions offer another major challenge in peptide synthesis. Recently, Danishefsky and co-workers showed the efficiency of arylesters in sterically congested cases, such as elongation of peptides bearing a C-terminal valine.[5] According to the postulated mechanism of the Chan–Lam reaction, the ester is obtained via σ-bond formation between a carboxylic acid O atom and the aryl C atom connected to the copper(III) species, which is one bond further away from a potentially sterically hindered amino acid α-carbon atom compared with classical ester formation using peptide coupling reagents. Copper(II)-mediated reaction of Boc-Phe-Val-OH with 4-(methylthio)phenyl boroxine using the optimized conditions gave the corresponding ester in 68 % yield (entry 6). Next, oxidation of the Chan–Lam product with oxone gave Boc-Phe-Val-4-(methylsulfonyl)phenyl ester in quantitative yield. Also, Boc-Trp(Boc)-Val-4-(methylsulfonyl)phenyl ester was prepared in 70 % overall yield (entry 7). It is worth mentioning that due to the presence of the indole-*N*-Boc protective group oxidation to the sulfone proceeded uneventfully. Boc-Phe-Ala-4-(methylsulfonyl)phenyl ester was prepared in 83 % overall yield (entry 8). Finally, also the tripeptide Boc-Asp(*t*Bu)-Phe-Phe-OH was esterified and oxidized in an overall yield of 55 % (entry 9).

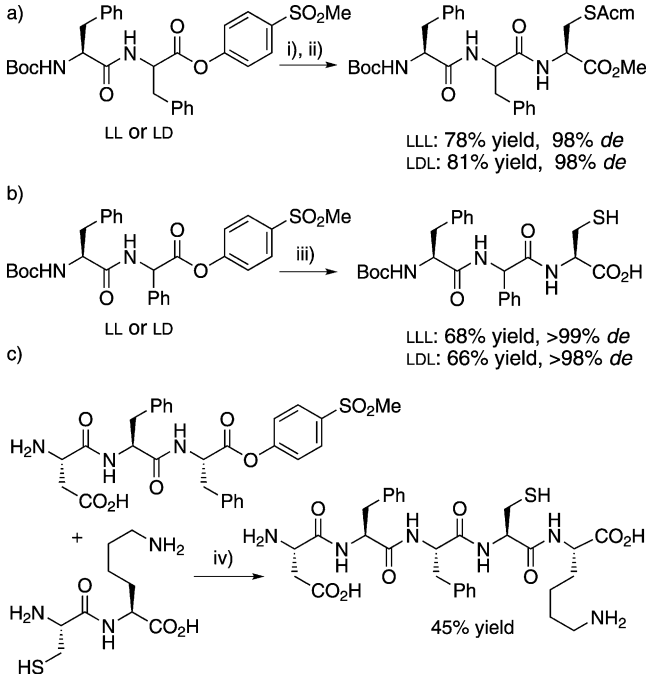


Entry	Peptide acid	R = SMe yield [%]	R = SMe *de* [%]	R = SO_2Me yield [%]	R = SO_2Me *de* [%]
1	Boc-Trp-Phe-OH	57	n.d.	-	-
2	Boc-Phe-Phe-OH	77	99.9	97[a]	99.9
3	Boc-Phe-D-Phe-OH	75	99.9	quant.	99.9
4	Boc-Phe-Phg-OH	70[b]	99.9	quant.	99.9
5	Boc-Phe-D-Phg-OH	73[b]	99.9	95[a]	99.9
6	Boc-Phe-Val-OH	68	n.d.	quant.	n.d.
7	Boc-Trp(Boc)-Val-OH	70	n.d.	quant.	n.d.
8	Boc-Phe-Ala-OH	83	n.d.	quant.	n.d.
9	Boc-Asp(*t*Bu)-Phe-Phe-OH	55	n.d.	quant.	n.d.

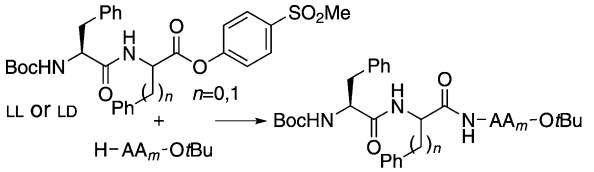
[a] Corrected for partial hydrolysis as determined by ^{1}H-NMR spectrocopy. [b] Et_3N (0.5 equiv) was used.

Scheme 3. Peptide activated arylester synthesis.

Next, the peptide 4-(methylsulfonyl)phenyl esters were evaluated in native chemical ligation (NCL) reactions.[11] Treatment of Boc-Phe-Phe-4-(methylsulfonyl)phenyl ester with excess cysteine only needed 1 h to reach full conversion (Scheme 4 a). This high rate is in accordance with results obtained by Danishefsky starting from peptide 4-nitrophenyl esters.[10] To overcome oxidative side reactions with the cysteine thiol moiety, after NCL capping by iodoacetamide was performed allowing facile purification giving Boc-Phe-Phe-Cys(Acm)-OMe in an isolated yield of 78 %. Similarly, Boc-Phe-D-Phe-Cys(Acm)-OMe was obtained in 81 % yield. Chiral HPLC and ^{1}H NMR analysis revealed 98 % *de* for both diastereoisomers. Gratifyingly, also phenylglycine 4-(methylsulfonyl)phenyl esters gave smooth NCL reactions with cysteine and both Boc-Phe-Phg-Cys-OH and Boc-Phe-D-Phg-Cys-OH could be isolated in yields of 68 and 66 %,



Scheme 4. NCL reactions from peptide arylesters. Conditions and reagents: i) HCl·H-Cys-OMe, 0.2 M Na_2HPO_4, 50 mM TCEP, pH 6.5, MeCN; ii) 0.2 M Na_2HPO_4, 50 mM TCEP, iodoacetamide, pH 7.5; iii) cysteine, 0.2 M Na_2HPO_4, 50 mM TCEP, pH 6.5, MeCN (final pH 6.75); iv) 0.2 M Na_2HPO_4, 50 mM TCEP, 6 M Gu·HCl, pH 7.5, MeCN (final pH 6.9).



Entry	Product	Conditions	Yield [%]	*de* [%]
1	Boc-Phe-Phe-**Gly-O*t*Bu**	**A**	90	86
2	Boc-Phe-Phe-**Gly-O*t*Bu**	**B**	91	99
3	Boc-Phe-D-Phe-**Gly-O*t*Bu**	**B**	85	99
4	Boc-Phe-Phe-**Gly-O*t*Bu**	**C**	87	99
5	Boc-Phe-Phe-**Gly-O*t*Bu**	**D**	83	52
6	Boc-Phe-Phg-**Gly-O*t*Bu**	**B**	86	81
7	Boc-Phe-D-Phg-**Gly-O*t*Bu**	**B**	75	81
8	Boc-Phe-Phe-**Val-O*t*Bu**	**B**	83	99
9	Boc-Phe-D-Phe-**Val-O*t*Bu**	**B**	80	99
10	Boc-Phe-Phe-**Val-O*t*Bu**	**C**	77	99
11	Boc-Phe-Phe-**Val-O*t*Bu**	**D**	90	85
12	Boc-Phe-Phe-**Ala-Gly-Trp-Val-O*t*Bu**	**E**	85	99
13	Boc-Phe-D-Phe-**Ala-Gly-Trp-Val-O*t*Bu**	**E**	80	99

Scheme 5. Direct peptide arylester aminolysis. A) H-AA-O*t*Bu, THF, RT, 2–3 d; B) H-AA-O*t*Bu (2.1 equiv, slow addition), THF, RT, 2–3 d; C) HCl·H-AA-O*t*Bu (1.1 equiv), DiPEA (2 equiv, slow addition), THF, RT, 2–3 d; D) H-AA-O*t*Bu (2.1 equiv), DMF, RT, 45 min (entry 5) or 18 h (slow addition, entry 11); E) tetrapeptide *tert*-butyl ester (1.1 equiv, slow addition), THF, RT, 3–4 d.

respectively, in diasteromerically pure form (Scheme 4b). Because peptide 4-(methylsulfonyl)phenyl esters only react slowly with amines under neutral aqueous coupling conditions (vide infra), they allow the presence of a free N terminus for a short time. This facilitates their use as the N-terminal fragment in NCL of fully unprotected peptides. To show this, H-Asp-Phe-Phe-4-(methylsulfonyl)phenyl ester was ligated with H-Cys-Lys-OH to give, after purification by preparative RP-HPLC, the pentapeptide H-Asp-Phe-Phe-Cys-Lys-OH in 45% yield (Scheme 4c).

Because NCL with apolar peptides can be problematic due to the poor solubility in aqueous buffered solutions, also the direct aminolysis in organic solvents was investigated (Scheme 5). Based on published epimerization experiments of peptide esters, THF was selected as the solvent of choice.[12] At first, Boc-Phe-Phe-4-(methylsulfonyl)phenyl ester was treated with H-Gly-O*t*Bu to give, after standing for 1 day, Boc-Phe-Phe-Gly-O*t*Bu in an isolated yield of 90 and 86% *de* (entry 1). To avoid epimerization, the basic H-Gly-O*t*Bu (2 equiv) had to be added by slow addition to give the product in 91% yield and 99% *de* (entry 2). By using the same slow addition conditions, Boc-Phe-D-Phe-Gly-O*t*Bu was isolated in a slightly lower yield of 85% without any sign of epimerization (entry 3). Also, the HCl-salt could be used as the starting material for which the amine was liberated by slow addition of a base (DiPEA, 2 equiv; entry 4). The choice of the solvent is crucial indeed for the outcome of the reaction. By replacing THF with DMF, a dramatic aminolysis rate increase was observed giving full conversion in 45 min only. However, the tripeptide Boc-Phe-Phe-Gly-O*t*Bu was isolated in the unacceptable 52% *de* (entry 5). As could be expected, for peptide elongation through a direct aminolysis reaction phenylglycine proved to be sensitive to base-induced epimerization (entries 6 and 7). Both Boc-Phe-Phg-4-(methylsulfonyl)phenyl ester and Boc-Phe-D-Phg-4-(methylsulfonyl)phenyl ester gave some epimerization after treatment with H-Gly-O*t*Bu to produce the corresponding tripeptides in the yields of 86 and 75%, respectively, and a *de* of 81% for both compounds. We were glad to see that also the sterically congested H-Val-O*t*Bu could be used for the aminolysis reactions (entries 8–11). Both slow addition of H-Val-O*t*Bu to Boc-Phe-Phe-4-(methylsulfonyl)phenyl ester or liberation of the free amine of HCl·H-Val-O*t*Bu by slow addition of DiPEA gave the corresponding tripeptides in high yields and optical purities. Also, with H-Val-O*t*Bu loss of *de* to 85% was observed when using DMF as the solvent. Therefore, segment couplings of two short peptides were achieved in THF giving the fully protected hexapeptides Boc-Phe-Phe-Ala-Gly-Trp-Val-O*t*Bu and its epimer Boc-Phe-D-Phe-Ala-Gly-Trp-Val-O*t*Bu in the isolated yields of 85 and 80%, respectively, as pure stereoisomers (entries 12 and 13).

In conclusion, the copper(II)-mediated Chan–Lam reaction is a versatile methodology for epimerization-free C terminus peptide elongation and thus opens new horizons in peptide synthesis. The peptide carboxylic acids were transformed into peptide arylesters with complete stereointegrity. This robust method allowed the synthesis of 4-(methylthio)-

phenyl esters, which subsequently could be activated by oxidation to the sulfone. These peptide 4-(methylsulfonyl)phenyl esters served as excellent substrates for peptide segment couplings by NCL or direct amidation with peptide N termini. Further studies are in progress to allow the direct synthesis of electron-poor peptide esters and to expand this novel transition-metal mediated carboxylic activation method to larger peptides and, ultimately, cyclic peptides.

Acknowledgements

The National Research School Combination Catalysis is kindly acknowledged for a research grant to S.P. We also thank Dr. Steen Ingemann, Ms. Els Engelen-Goris and Mr. Nicolas Dailly (Pepscan, Lelystad, The Netherlands) for analytical assistance.

Keywords: aminolysis · aryl esterification · copper · peptide activation · peptides

[1] a) P. Vlieghe, V. Lisowski, J. Martinez, M. Khrestchatisky, *Drug Discovery Today* **2010**, *15*, 40–56; b) A. K. Sato, M. Viswanathan, R. B. Kent, C. R. Wood, *Curr. Opin. Biotechnol.* **2006**, *17*, 638–642; c) V. Hruby, *J. Nat. Rev. Drug Discovery* **2002**, *1*, 847–858.

[2] This is mainly because activation of the terminal carboxyl moiety of a peptide is followed by nucleophilic attack of the flanking amide bond leading to racemization prone oxazolone intermediates: a) C. Griehl, A. Kolbe, S. Merkel, *J. Chem. Soc. Perkin Trans. 2* **1996**, 2525–2529; b) S.-Y. Han, Y.-A. Kim, *Tetrahedron* **2004**, *60*, 2447–2467; c) A. El-Faham, F. Albericio, *Chem. Rev.* **2011**, *111*, 6557–6602.

[3] a) F. Weygand, W. Steglich, *Angew. Chem.* **1961**, *73*, 757; b) V. Declerck, P. Nun, J. Martinez, F. Lamaty, *Angew. Chem.* **2009**, *121*, 9482–9485; *Angew. Chem. Int. Ed.* **2009**, *48*, 9318–9321; c) S. Tsukiji, T. Nagamune, *ChemBioChem* **2009**, *10*, 787–798.

[4] a) A. Abdelmajeid, S. R. Tala, M. S. Amine, A. R. Katritzky, *Synthesis* **2011**, 2995–3005; b) B. K. Zambroń, S. R. Dubbaka, D. Marković, E. Moreno-Clavijo, P. Vogel, *Org. Lett.* **2013**, *15*, 2550–2553; c) H. Schröder, G. A. Strohmeier, M. Leypold, T. Nuijens, P. J. L. M. Quaedflieg, R. Breinbauer, *Adv. Synth. Catal.* **2013**, *355*, 1799–1807.

[5] a) Q. Wan, J. Chen, Y. Yuan, S. J. Danishefsky, *J. Am. Chem. Soc.* **2008**, *130*, 15814–15816; b) Q. Wan, S. J. Danishefsky, *Angew. Chem.* **2007**, *119*, 9408–9412; *Angew. Chem. Int. Ed.* **2007**, *46*, 9248–9252.

[6] J. X. Qiao, P. Y. S. Lam, *Synthesis* **2011**, 829–856.

[7] L. L. Zhang, G. Y. Zhang, M. L. Zhang, J. A. Cheng, *J. Org. Chem.* **2010**, *75*, 7472–7474.

[8] a) L. M. Huffman, S. S. Stahl, *J. Am. Chem. Soc.* **2008**, *130*, 9196–9197; b) A. E. King, T. C. Brunold, S. S. Stahl, *J. Am. Chem. Soc.* **2009**, *131*, 5044–5045; c) A. E. King, L. M. Huffman, A. Casitas, M. Costas, X. Ribas, S. S. Stahl, *J. Am. Chem. Soc.* **2010**, *132*, 12068–12073.

[9] J.-S. Zheng, H.-K. Cui, G.-M. Fang, W.-X. Xi, L. Liu, *ChemBioChem* **2010**, *11*, 511–515; b) G.-M. Fang, H.-K. Cui, J.-S. Zheng, L. Liu, *ChemBioChem* **2010**, *11*, 1061–1065.

[10] a) B. J. Johnson, P. M. Jacobs, *Chem. Commun.* **1968**, *2*, 73–75; b) B. J. Johnson, E. G. Trask, *J. Org. Chem.* **1968**, *33*, 4521–4524; c) B. J. Johnson, P. M. Jacobs, *J. Org. Chem.* **1968**, *33*, 4524–4526; d) B. J. Johnson, T. A. Ruettinger, *J. Org. Chem.* **1970**, *35*, 255–257; e) L. M. Siemens, F. W. Rottnek, L. S. Trzupek, *J. Org. Chem.* **1990**, *55*, 3507–3511; f) B. J. Johnson, D. S. Rea, *Can. J. Chem.* **1970**, *48*, 2509–2511.

[11] a) C. P. R. Hackenberger, D. Schwarzer, *Angew. Chem.* **2008**, *120*, 10182–10228; *Angew. Chem. Int. Ed.* **2008**, *47*, 10030–10074; b) D. Bang, B. L. Pentelute, S. B. H. Kent, *Angew. Chem.* **2006**, *118*, 4089–4092; *Angew. Chem. Int. Ed.* **2006**, *45*, 3985–3988; c) P. E. Dawson, T. W. Muir, I. Clak-Lewis, S. B. H. Kent, *Science* **1994**, *266*, 776–779.

[12] M. Bodanszky, A. Bodanszky, *Chem. Commun.* **1967**, 591–593.

Received: August 27, 2013
Published online: November 7, 2013

