DOI: 10.1002/anie.200805782

Chemical Ligation

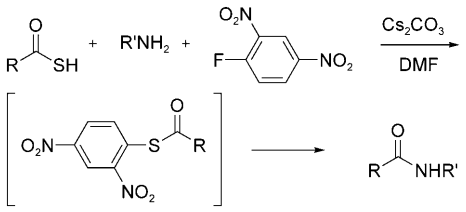
Epimerization-Free Block Synthesis of Peptides from Thioacids and Amines with the Sanger and Mukaiyama Reagents

David Crich and Indrajeet Sharma*

Dedicated to Professor Carl R. Johnson

The reaction of 2,4-dinitrobenzenesulfonamides with thioacids to give amides was described in 1998 by Tomkinson and co-workers.[1] We extended this reaction to the synthesis of peptides and their glycoconjugates by combining amino thioacids and their C-terminal-peptide congeners with N-terminal sulfonamides.[2a] In a three-component coupling, we used this process to capture thioacids generated in situ from the nucleophilic ring-opening of a variety of cyclic thioanhydrides.[2b] These reactions involve nucleophilic aromatic substitution of the thiocarboxylate group on the electron-deficient sulfonamide with the formation of a highly reactive *S*-(2,4-dinitrophenyl) thioester, and its subsequent condensation with the released amine.[2a,3–6] We show herein that amide bonds can be formed by the coupling of thioacids with amines under the aegis of 2,4-dinitrofluorobenzene and other electron-deficient arenes and heteroarenes at ambient temperature.

The Sanger reagent, 2,4-dinitrofluorobenzene, was traditionally used for the degradation of peptides and proteins through its reaction with N-terminal amines and for the identification of N-terminal amino acids.[7] Sanger noted, however, that thiols and other nucleophiles, including the side chains of histidine and tyrosine, competed with amines for capture by 2,4-dinitrofluorobenzene.[7a] We surmised that a thiocarboxylate salt would react significantly more rapidly than an amine with this reagent and thus enable the formation of the thioester and ultimately peptide synthesis. In this way, a peptide degradation reagent could effectively be turned into a reagent for their synthesis (Scheme 1).



Scheme 1. Amide-forming reaction with the Sanger reagent.

Table 1: Preparation of thioesters from protected amino acids and thiols.[a]

Reagent	Thioester	Yield [%]
DCC	Boc-L-Val-SFm	99
DCC	Boc-Aib-SFm	82
DCC	Boc-L-Asp-(α-OBn)-γ-SFm	97
DIC	Fmoc-L-Ala-STmob	98
DIC	Boc-L-Phe-STmob	96
DIC	Z-L-Val-STrt	95
PyBop	Z-L-Ala-L-Phe-SFm	72
PyBop	Boc-L-Lys(Boc)-L-Arg(Pbf)-L-Asn(Trt)-L-Arg(Pbf)-SFm	82

[a] Aib = 2-aminoisobutyric acid, Bn = benzyl, Boc = *tert*-butoxycarbonyl, DCC = dicyclohexylcarbodiimide, DIC = diisopropylcarbodiimide, Fmoc = 9-fluorenylmethoxycarbonyl, FmSH = 9-fluorenylmethylthiol, Pbf = 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl, PyBop = (1*H*-benzotriazol-1-yloxy)tris(pyrrolidino)phosphonium hexafluorophosphate, TmobSH = 2,4,6-trimethoxybenzylthiol, TrtSH = triphenylmethanethiol, Z = benzyloxycarbonyl.

A series of thioesters was prepared by the coupling of suitably protected amino acids with 9-fluorenylmethanethiol,[2a] 2,4,6-trimethoxybenzyl thiol,[8] or triphenylmethanethiol (Table 1). With simple carbamate-protected amino acids, carbodiimide coupling reagents were employed for these condensations; however, with peptides (Table 1, last two entries) we preferred to use the reagent (1*H*-benzotriazol-1-yloxy)tris(pyrrolidino)phosphonium hexafluorophosphate (PyBop) recommended by Kajihara and co-workers[9] to avoid the problem of epimerization in the thioesterification of all but simple amino acids. The final entry in Table 1 is noteworthy for both the sterically hindered nature of the reaction site and the fact that no epimerization was observed under the PyBop coupling conditions. When the same thioester was prepared under the carbodiimide–hydroxybenzotriazole conditions reported[10] for epimerization-free thioester synthesis, the product was obtained in good yield (77%) but as a 3:1 mixture of epimers. The requisite thioacids were released cleanly from the thioesters with piperidine[2a] in the case of the fluorenylmethyl thioesters, or with trifluoroacetic acid for the trimethoxybenzyl thioesters and triphenylmethyl thioesters,[11] and used immediately in the peptide-bond-forming reactions.

The coupling of thioacids with amines in the presence of the Sanger reagent was first investigated by using thioacetic

[*] Prof. Dr. D. Crich, I. Sharma
Department of Chemistry, Wayne State University
5101 Cass Avenue, Detroit, MI 48202 (USA)
Prof. Dr. D. Crich
Centre de Recherche CNRS de Gif-sur-Yvette
Institut de Chimie des Substances Naturelles
Avenue de la Terrasse, 91198 Gif-sur-Yvette (France)
Fax: (+33) 1-6907-7752
E-mail: dcrich@icsn.cnrs-gif.fr

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200805782.

Table 2: Coupling of thioacids with amines with the Sanger reagent.[a]

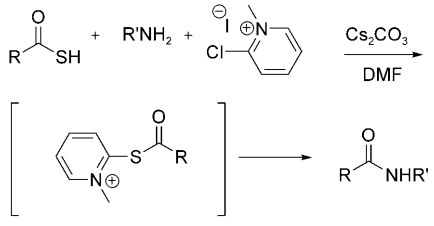
Entry	Thioester	Release reagent[b]	Thioacid	Amine	Product	Yield [%]
1	–	–	AcSH	L-Trp	Ac-L-Trp	76
2	–	–	AcSH	L-Trp-OMe·HCl	Ac-L-Trp-OMe	99
3	Boc-L-Val-SFm	Pip	Boc-L-Val-SH	L-Phe-OMe·HCl	Boc-L-Val-L-Phe-OMe	84
4	Boc-L-Val-SFm	Pip	Boc-L-Val-SH	D-Phe-OMe·HCl	Boc-L-Val-D-Phe-OMe	82
5	Boc-L-Val-SFm	Pip	Boc-L-Val-SH	L-Trp-OMe·HCl	Boc-L-Val-L-Trp-OMe	76
6	Boc-Aib-SFm	Pip	Boc-Aib-SH	L-Trp-OMe·HCl	Boc-Aib-L-Trp-OMe	95
7	Boc-Aib-SFm	Pip	Boc-Aib-SH	Gly-OEt·HCl	Boc-Aib-Gly-OEt	96
8	Boc-L-Asp-(α-OBn)-γ-SFm	Pip	Boc-L-Asp-(α-OBn)-γ-SH	L-Phe-OMe·HCl	Boc-L-Asp-(α-OBn)-γ-L-Phe-OMe	93
9	Fmoc-L-Ala-STmob	TFA	Fmoc-L-Ala-SH	Gly-OEt·HCl	Fmoc-L-Ala-Gly-OEt	60
10	Fmoc-L-Ala-STmob	TFA	Fmoc-L-Ala-SH	L-Phe-OMe·HCl	Fmoc-L-Ala-L-Phe-OMe	59
11	Fmoc-L-Ala-STmob	TFA	Fmoc-L-Ala-SH	L-Tyr-OMe·HCl	Fmoc-L-Ala-L-Tyr-OMe	64
12	Z-L-Ala-L-Phe-SFm	Pip	Z-L-Ala-L-Phe-SH	Gly-Gly-OMe·HCl	Z-L-Ala-L-Phe-Gly-Gly-OMe	66

[a] In all reactions, the amine hydrochloride (0.1 M) was used in *N*,*N*-dimethylformamide (DMF) with the thioacid (1.2 equiv), and with Cs_2CO_3 (1.5 equiv) as the base (see the Supporting Information). [b] Pip = piperidine, TFA = trifluoroacetic acid.

acid with L-tryptophan and its methyl ester (Table 2, entries 1 and 2). The acetamide was obtained in excellent yield when the three components were mixed at room temperature in the presence of cesium carbonate. A series of dipeptides was then accessed by coupling of the released thioacids with other amino esters and peptide esters. Our premise that deprotonated thioacids would be more nucleophilic toward the Sanger reagent than amines was validated by the yields of the dipeptides (Table 2); 2,4-dinitrofluorobenzene is an effective reagent for the formation of amide bonds between thioacids and amines. The preparation of both the L,L and L,D isomers of Boc-Val-Phe-OMe (Table 2, entries 3 and 4) verified the absence of epimerization, as also deduced by the inspection of "high-field" NMR spectra. The suitability of this method for the formation of hindered peptide bonds is evident from entries 6 and 7 of Table 2.

The yields observed with the Fmoc-protected thioacids (Table 2, entries 9–11) were lower than those observed with Boc-protected thioacids as a result of partial cleavage of the Fmoc group by the fluoride anion released in the course of the nucleophilic aromatic substitution step.[12] Accordingly, we turned to 2,4-dinitroiodobenzene and 2-chloro-1-methylpyridinium iodide (the Mukaiyama reagent)[13a] as condensing agents (Scheme 2, Table 3).

The Mukaiyama reagent and its analogues have been employed previously to effect peptide coupling reactions between amines and simple carboxylic acids.[13b,c] However, the yields of these coupling reactions are modest, except when secondary and sterically hindered amines are used.[13b,c,14] In contrast, the method described herein is applicable to all



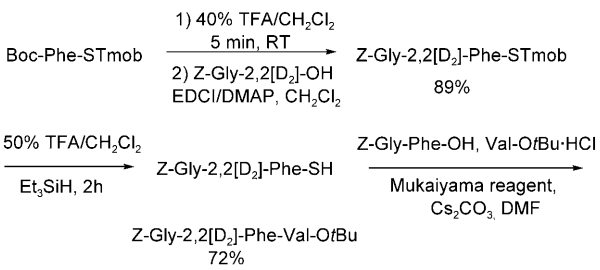
Scheme 2. Amide-forming reaction with the Mukaiyama reagent.

Table 3: Comparison of the Sanger reagent with 2,4-dinitroiodobenzene and with the Mukaiyama reagent for the construction of amide bonds between amines and Fmoc-L-Ala-SH.[a]

Amine	Dipeptide yield [%]		
	Sanger reagent	2,4-dinitroiodo-benzene	Mukaiyama reagent
Gly-OEt·HCl	60	83	80
L-Phe-OMe·HCl	59	79	86
L-Tyr-OMe·HCl	64	86	85
L-Ala-OMe·HCl	–	78	82
L-Cys(Trt)-OEt·HCl	–	82	88

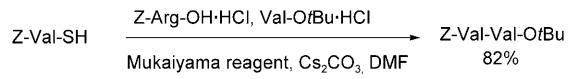
[a] In all reactions, the amine hydrochloride (0.1 M) was used in DMF with the thioacid (1.2 equiv), and with Cs_2CO_3 (1.5 equiv) as the base (see the Supporting Information).

amines owing to the greater nucleophilicity of the thiocarboxylate moiety. The increased reactivity of thiocarboxylate groups over simple carboxylate groups is illustrated by a competition reaction (Scheme 3),[15] in which the reactivity of a carboxylic acid was compared directly with that of its thio analogue. The tripeptide product isolated from this reaction was shown by mass spectrometry to be fully deuterium-labeled in the glycine residue, a result indicating the superiority of the thioacid in this chemistry.



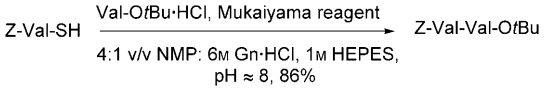
Scheme 3. Carboxylate/thiocarboxylate competition reaction. DMAP = 4-dimethylaminopyridine, EDCI = 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide hydrochloride.

The coupling of a valine-derived thioacid with Val-O*t*Bu in the presence of Z-L-Arg-OH resulted in the formation of a single dipeptide in high yield. This result reinforces the superior reactivity of the thioacid over the simple acid and also demonstrates the compatibility of the method with the guanidine and carboxylic acid moieties of the arginine derivative (Scheme 4).[16]



Scheme 4. Functional-group compatibility.

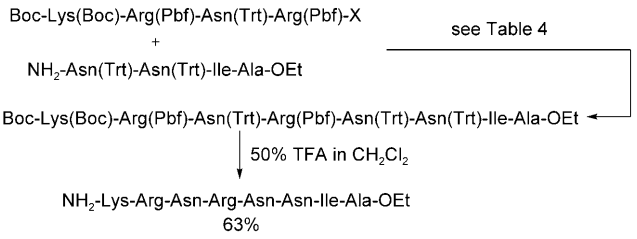
A dipeptide was also formed in good yield in a coupling reaction of a valine-derived thioacid with the Mukaiyama reagent in aqueous buffer (Scheme 5).[21]



Scheme 5. Coupling under aqueous conditions. HEPES = 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, NMP = *N*-methylpyrrolidone, Gn = guanidine.

Finally, we describe the application of this new peptide-bond-forming reaction to the 4+4 block synthesis of L-Lys-L-Arg-L-Asn-L-Arg-L-Asn-L-Asn-L-Ile-L-Ala. This octapeptide is the C-terminal sequence of oxyntomodulin from porcine jejunoileum and is responsible for the inhibitory properties of this extended version of glucagon towards the secretion of gastric acid.[17] This octapeptide was selected as a target because of the widespread current interest in oxyntomodulin derivatives as appetite suppressants and as potential therapeutics for obesity,[18] and because the concatenation of its multiple functionalized side chains provides a true test of the methodology.

Two tetrapeptides were synthesized by standard manual solution-phase techniques (see the Supporting Information).[19] An allyl ester protecting group was then removed from the C-terminal arginine residue, and the resulting acid was converted into the 9-fluorenylmethyl thioester, as set out in the final entry of Table 1. The 4+4 fragment coupling was carried out by liberation of the thioacid functionality with piperidine, followed by combination with the N-terminal tetrapeptide in the presence of the Mukaiyama reagent. The protected target octapeptide was isolated in 66% yield as a single epimer (Scheme 6, Table 4). This block synthesis provided an opportunity for the comparison of the thioacid methodology with more common methods. To this end, the coupling of the tetrapeptide carboxylic acid was effected with PyBop, HATU, and EDCI[20] under standard conditions with the results indicated in the Table 4. The PyBop and HATU methods gave the protected octapeptide in comparable yields to that observed with the thioacid method but required significantly longer reaction times for a similar result, and some epimerization occurred. The reaction was not complete with the carbodiimide method after 14 h and resulted in



Scheme 6. Comparative block syntheses of an octapeptide (see Table 4 for conditions and yields for the coupling step).

Table 4: Comparative block syntheses of an octapeptide (coupling step in Scheme 6).[a]

X	Coupling reagent[b]	*t* [h]	Yield [%]	Epimer ratio[c]
SFm	1) Pip; 2) Mukaiyama	1	66	>99:1
OH	PyBop	8	69	93.7:6.3
OH	HATU	6	70	93.1:6.9
OH	EDCI	14	54	83:17
OH	EDCI-HOBt	8	63	94.2:5.8

[a] All coupling reactions were conducted in DMF under standard conditions (temperature and concentration) with epimerization-free, all-L tetrapeptides. [b] HATU = *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridine-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide, HOBT = *N*-hydroxybenzotriazole. [c] Epimerization ratios were determined by comparison with an authentic sample of Boc-Lys(Boc)-Arg(Pbf)-Asn(Trt)-D-Arg(Pbf)-Asn(Trt)-Asn(Trt)-Ile-Ala-OEt.

significantly more epimerization.[21,22] Finally, the treatment of this octapeptide with trifluoroacetic acid in dichloromethane removed the complete suite of acid-labile protecting groups and afforded the octapeptide as a single epimer in 63% yield after purification by reversed-phase HPLC.

The high nucleophilicity of thiocarboxylates enables their preferential reaction with electron-deficient aromatic halides by nucleophilic substitution in the presence of an amine. This method generates highly reactive thioesters in situ, which then react with the amine to form peptide bonds, and tolerates the presence of all proteinogenic amino acid side chains, except for cysteine and lysine,[2a] which must be protected. No particular amino acid is required for coupling, in contrast to native chemical ligation and its variants,[6a,23] and peptide-bond formation at hindered residues functions efficiently, unlike peptide ligation by direct aminolysis.[24]

Received: November 27, 2008
Published online: February 19, 2009

Keywords: aromatic substitution · chemical ligation · peptides · thioacids · thioesters

[1] a) T. Messeri, D. D. Sternbach, N. C. O. Tomkinson, *Tetrahedron Lett.* **1998**, *39*, 1669–1672; b) T. Messeri, D. D. Sternbach, N. C. O. Tomkinson, *Tetrahedron Lett.* **1998**, *39*, 1673–1676.
[2] a) D. Crich, K. Sana, S. Guo, *Org. Lett.* **2007**, *9*, 4423–4426; b) D. Crich, A. A. Bowers, *Org. Lett.* **2007**, *9*, 5323–5325.

[3] For peptide synthesis by "superarmed" esters synthesized by nucleophilic aromatic substitution, see: Z. J. Kamiński, B. Kolesińska, J. Kolesińska, G. Sabatino, M. Chelli, P. Rovero, M. Błaszczyk, M. L. Główka, A. M. Papini, *J. Am. Chem. Soc.* **2005**, *127*, 16912 – 16920.

[4] For peptide synthesis with nitrophenyl thioesters formed by the conventional condensation of thiols with acids, see: A. Ishiwata, T. Ichiyanagi, M. Takatani, Y. Ito, *Tetrahedron Lett.* **2003**, *44*, 3187 – 3190.

[5] For previous applications of thioacids in peptide synthesis, see: a) D. Yamashiro, J. F. Blake, *Int. J. Pept. Protein Res.* **1981**, *18*, 383 – 392; b) J. Blake, *Int. J. Pept. Protein Res.* **1981**, *17*, 273 – 274; c) Y. V. Mitin, N. P. Zapevalova, *Int. J. Pept. Protein Res.* **1990**, *35*, 352 – 356.

[6] For the use of thioacids as precursors to thioesters for native chemical ligation, see: a) P. E. Dawson, T. W. Muir, I. Clark-Lewis, S. B. H. Kent, *Science* **1994**, *266*, 776 – 779; b) J. P. Tam, Y.-A. Lu, C. F. Liu, J. Shao, *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 12485 – 12489; c) P. E. Dawson, S. B. H. Kent, *Annu. Rev. Biochem.* **2000**, *69*, 923 – 960.

[7] a) F. Sanger, *Biochem. J.* **1945**, *39*, 507 – 515; b) H. N. Eisen, S. Belman, M. E. Carston, *J. Am. Chem. Soc.* **1953**, *75*, 4583 – 4585; c) G. T. Hermanson, *Bioconjugate Techniques*, Academic Press, San Diego, **1996**.

[8] S. Vetter, *Synth. Commun.* **1998**, *28*, 3219 – 3223.

[9] T. J. Hogenauer, Q. Wang, A. K. Sanki, A. J. Gammon, C. H. L. Chu, C. Kaneshiro, Y. Kajihara, K. Michael, *Org. Biomol. Chem.* **2007**, *5*, 759 – 762.

[10] a) P. Wang, L. P. Miranda, *Int. J. Pept. Res. Ther.* **2005**, *11*, 117 – 123; b) H. Yang, H. Li, R. Wittenburg, M. Egi, W. Huang, L. S. Liebeskind, *J. Am. Chem. Soc.* **2007**, *129*, 1132 – 1140; c) H. Yang, L. S. Liebeskind, *Org. Lett.* **2007**, *9*, 2993 – 2995; d) S. Ficht, R. J. Payne, R. T. Guy, C.-H. Wong, *Chem. Eur. J.* **2008**, *14*, 3620 – 3629.

[11] N. Shangguan, S. Katukojvala, R. Greenberg, L. J. Williams, *J. Am. Chem. Soc.* **2003**, *125*, 7754 – 7755.

[12] For the cleavage of Fmoc groups with tetrabutylammonium fluoride, see: a) Y. Rew, D. Shin, I. Hwang, D. L. Boger, *J. Am. Chem. Soc.* **2004**, *126*, 1041 – 1043; b) W. R. Li, J. Jiang, M. M. Joullie, *Synlett* **1993**, 362; c) M. Ueki, M. Amemiya, *Tetrahedron Lett.* **1987**, *28*, 6617 – 6620.

[13] a) T. Mukaiyama, *Angew. Chem.* **1979**, *91*, 798 – 812; *Angew. Chem. Int. Ed. Engl.* **1979**, *18*, 707 – 808; b) P. Li, J. C. Xu, *Tetrahedron* **2000**, *56*, 8119 – 8131; c) S. Crosignani, J. Gonzalez, D. Swinnen, *Org. Lett.* **2004**, *6*, 4579 – 4582.

[14] As is implicit in the results of Sanger, simple peptide-based primary amines are more nucleophilic than carboxylic acids toward electron-deficient arenes. Steric bulk reduces the nucleophilicity of the amine and enables the carboxylate group to compete. The poor yields observed in the coupling of less hindered amines with carboxylic acids in the presence of the Mukaiyama reagent are readily shown by mass spectrometry to arise from competing attack of the amine on the reagent.

[15] In the preparation of the dideuterated dipeptide thioacid, a Boc group was removed in the presence of the Tmob thioester.

[16] Parallel reactions of Val-O*t*Bu with Z-Gly-Phe-SH and Z-Gly-Phe-OH were also conducted with the Mukaiyama reagent. The tripeptide (Z-Gly-Phe-Val-O*t*Bu) was formed in 76% yield from the reaction of the thioacid, whereas the corresponding carboxylic acid gave the product in only 23% yield under identical conditions. No epimerization was observed in either experiment, as determined by comparison with an authentic sample of Z-Gly-D-Phe-Val-O*t*Bu (see the Supporting Information).

[17] C. Carles-Bonnet, J. Martinez, C. Jarrousse, A. Aumelas, H. Niel, D. Bataille, *Peptides* **1996**, *17*, 557 – 561.

[18] a) R. Nogueiras, S. J. Caton, D. Perez-Tilve, M. Bidlingmaier, M. H. Tschöp, *Drug Discovery Today Dis. Mech.* **2006**, *3*, 463 – 470; b) K. Wynne, S. R. Bloom, *Nat. Clin. Pract. Endocrinol. Metab.* **2006**, *2*, 612 – 620.

[19] a) *Houben-Weyl, Methods in Organic Chemistry: Synthesis of Peptides and Peptidomimetics, Vol. E22a–e* (Eds.: M. Goodman, A. F. Mahwah, L. Moroder, C. Toniolo), Thieme, Stuttgart, **2002**–**2003**; b) M. Bodanszky, *Peptide Chemistry: A Practical Textbook*, 2nd ed., Springer, Berlin, **1993**; c) M. Bodanszky, A. Bodanszky, *The Practice of Peptide Synthesis*, 2nd ed., Springer, Heidelberg, **1994**.

[20] a) S. Y. Han, Y. A. Kim, *Tetrahedron* **2004**, *60*, 2447 – 2467; b) C. A. G. N. Montalbetti, V. Falque, *Tetrahedron* **2005**, *61*, 10827 – 10852, and references therein.

[21] Although we have not conducted any confirmatory experiments, the rapidity of the in situ activation and coupling of thioacids reported herein stands in contrast to the synthesis of peptides by the two-step "superarmed"-ester protocol, which requires a relatively slow reaction of the acid with a 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium tetrafluoroborate salt, followed by addition of the amine.[3]

[22] As acids are activated, albeit more slowly, by the Mukaiyama reagent, protection of side-chain acid groups is advisable when the thioacid coupling method is used.

[23] a) D. Crich, A. Banerjee, *J. Am. Chem. Soc.* **2007**, *129*, 10064 – 10065; b) P. Botti, S. Tchertchian, WO 2006/133962, **2006**; c) R. Okamoto, Y. Kajihara, *Angew. Chem.* **2008**, *120*, 5482 – 5486; *Angew. Chem. Int. Ed.* **2008**, *47*, 5402 – 5406; d) R. Quaderer, D. Hilvert, *Chem. Commun.* **2002**, 2620 – 2621; e) R. J. Hondal, B. L. Nilsson, R. T. Raines, *J. Am. Chem. Soc.* **2001**, *123*, 5140 – 5141; f) C. Haase, H. Rohde, O. Seitz, *Angew. Chem.* **2008**, *120*, 6912 – 6915; *Angew. Chem. Int. Ed.* **2008**, *47*, 6807 – 6810; g) J. Chen, Q. Wan, Y. Yuan, J. Zhu, S. J. Danishefsky, *Angew. Chem.* **2008**, *120*, 8649 – 8652; *Angew. Chem. Int. Ed.* **2008**, *47*, 8521 – 8524; h) J. W. Bode, *Curr. Opin. Drug Discovery Dev.* **2006**, *9*, 765 – 775; i) D. Macmillan, *Angew. Chem.* **2006**, *118*, 7830 – 7834; *Angew. Chem. Int. Ed.* **2006**, *45*, 7668 – 7672.

[24] R. J. Payne, S. Ficht, W. A. Greenberg, C.-H. Wong, *Angew. Chem.* **2008**, *120*, 4483 – 4487; *Angew. Chem. Int. Ed.* **2008**, *47*, 4411 – 4415.

