Metabolites

DOI: 10.1002/ange.201106658

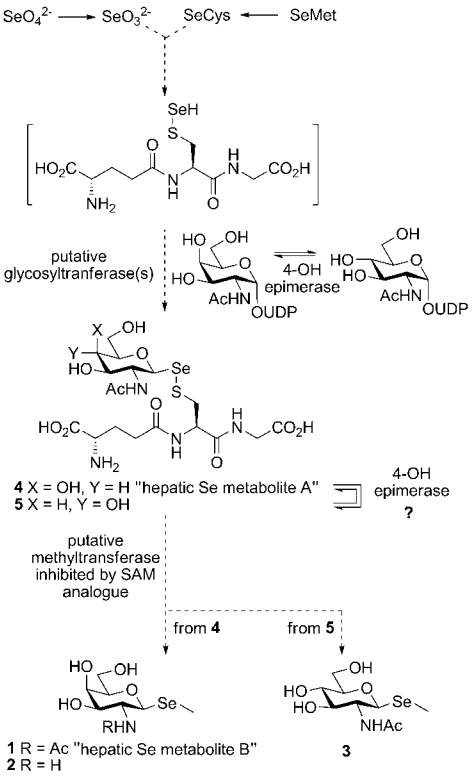
Selenenylsulfide-Linked Homogeneous Glycopeptides and Glycoproteins: Synthesis of Human "Hepatic Se Metabolite A"**

*Omar Boutureira, Gonçalo J. L. Bernardes, Marta Fernández-González, Daniel C. Anthony, and Benjamin G. Davis**

Selenium is a critical mammalian trace element found in proteins in the form of selenocysteine (SeCys) or selenomethionine (SeMet), but its role and metabolism are only partially understood.[1–3] Early studies[1,2] have surprisingly shown excretion of selenium as selenosugar **1**, so-called "hepatic Se metabolite B"; its presence in rats and humans (liver and urine) also suggests a common cross-species excretion mechanism (Scheme 1). These studies implicated **4**, so-called "hepatic Se metabolite A", which was neither isolated in pure form nor fully characterized, as the precursor on the basis of MS fragmentation. Selenosugars **2** and **3** have also been detected in urine, but, notably, glutathionyl precursors (e.g., **5**) of these trace metabolites have not yet been identified.[3] Indeed, the exact mechanism of selenium metabolism remains unclear, as do the roles of **4** and **5**, and the need for authentic standards has recently been emphasized.[4]

The putative biological roles as well as the intrinsic characteristics of selenium make it potentially useful as a structural, functional, and mechanistic probe for the detailed study of biological processes by ^{77}Se NMR[5–7] and X-ray spectroscopy,[8] and a tool for the determination of phase through the use of multiwavelength anomalous dispersion (MAD).[9] Because selenium is not present in detectable levels in the background and can be quantified at sub-μM concentrations, it also potentially allows sensitive estimation[10–12] of in vivo circulation lifetime and tissue distribution of seleno conjugates.

Selenenylsulfide-linked (glycosyl-SeS-Cys) glycopeptides, which form a class of conjugates that has not been synthesized previously, are therefore not only of fundamental interest as human metabolite natural products that might allow dissection of selenium utilization pathways, but also suggest potential as readily monitored labelled glycoconjugates. Herein, we present the first syntheses of such conjugates, including the identification of hepatic Se metabolite A, the creation of protein-conjugate variants, and an analysis of their serum-tracking potential and stability.



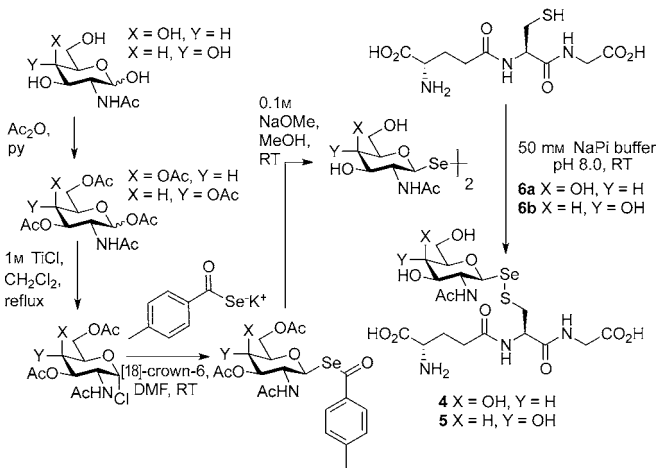
Scheme 1. Putative pathway for the biosynthesis of selenium-containing natural products **1**–**3**. Methyltransferase activity has been inferred through inhibition studies.[2] OH epimerase in mammalian cells[13] interconverts GlcNAcUDP and GalNAcUDP, but is unknown for the other substrates shown here.

[*] Dr. O. Boutureira, Dr. G. J. L. Bernardes, Dr. M. Fernández-González, Prof. B. G. Davis
Department of Chemistry, University of Oxford
Chemistry Research Laboratory
Mansfield Road, OX1 3TA (UK)
E-mail: ben.davis@chem.ox.ac.uk
Homepage: http://users.ox.ac.uk/~dplb0149
Dr. D. C. Anthony
Department of Pharmacology, University of Oxford
Mansfield Road, Oxford, OX1 3QT (UK)

[**] We thank the European Commission (Marie Curie Intra-European Fellowship for O.B.), FCT, Portugal (G.J.L.B.), and Xunta de Galicia, Spain (M.F.G.) for financial support. B.G.D. is a recipient of a Royal Society Wolfson Merit Award and is supported by an EPSRC LSI Platform grant. We thank Dr. J. S. O. McCullagh and M. K. Patel for technical assistance.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201106658.

reactions with cysteine (Scheme 2).[17] Notably, the relative stability of diselenides does not lead to a reduced reactivity,[18] and we previously noted that kinetic control may be applied

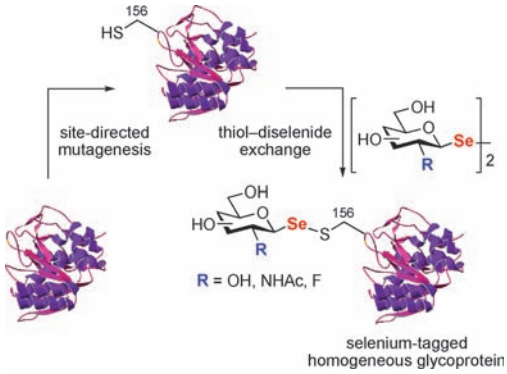


Scheme 2. Synthesis of putative Se-metabolism precursors **4** and **5**. DMF = *N*,*N*-dimethylformamide, py = pyridine.

to such reaction manifolds. Although we had exploited this to favor selective mixed-disulfide formation,[14] we reasoned that the reaction might also allow access to selenenylsulfide-linked intermediates if correct conditions could be found for these to be trapped.[19] Sugar diselenides **6a–f** were prepared in two steps from the corresponding anomeric halides in good overall yields[20] (see Scheme 2 for **6a**,**b**, and the Supporting Information for **6c–f**). Conditions for the reaction of diselenides with L-glutathione (GSH) were tested by varying several parameters, but were limited where possible to those in aqueous biologically compatible buffers at a nearly neutral pH value, so that the reaction might then be applied to the investigation of other more complex SeS-linked glycoconjugates (Scheme 2). The optimization of reaction conditions was facilitated by direct monitoring of the reaction by using MS (negative mode TOF-ES). Signals that indicated the conversion of GSH (*m*/*z* 306) to product **4** (*m*/*z* 589) and a minor signal attributed to the formation of $(GS)_2$ (*m*/*z* 611) were monitored directly; the latter signal increased with longer reaction times (1–3 h, see the Supporting Information). Thus, reaction of GSH with $(GalNAcSe)_2$ **6a** in sodium phosphate (NaPi) buffer (50 mM, pH 8.0) at room temperature for 30 min afforded hepatic Se metabolite A (GalNAc-SeS-G, **4**) in 62% yield (based on the limiting peptide, > 95% conversion, see the Supporting Information); **4** was isolated both in its pure form and as trifluoroacetate salt **4**·TFA with GS_2 by using size-exclusion chromatography and RP-HPLC, respectively (see the Supporting Information). Notably, kinetic control of the thiol–diselenide exchange is sufficient to result not only in reasonably efficient formation of the product, but also in the stereoselective formation of the β anomer (d.r. > 98%, $^1J_{H1-Cl} = 159.7$ Hz), thus out-competing any glycosylselenyl mutarotation. The product was characterized by 1D and 2D (COSY, HSQC) ^1H and ^{13}C NMR experiments, thus allowing full assignment of all resonances. In particular, diagnostic signals of diastereotopic protons corresponding to Cys-CH_2 appeared in the ^1H NMR spectrum at 3.37 ppm ($J_{A,B} = 14.3$ Hz, $J_{A,H\alpha} = 4.8$ Hz) and 3.13 ppm ($J_{A,B} = 14.3$ Hz, $J_{B,H\alpha} = 9.5$ Hz), and in the ^{13}C NMR spectrum at 39.1 ppm ($C_\beta H_2SSe$). ESI-MS spectra showed the expected isotope distribution that is characteristic for Se also for the molecular ion. ESI-MS/MS measurements allowed fragmentation analysis of the synthetic GalNAc-SeS-G (**4**), which showed essentially identical patterns for both molecular (including isotopic distribution) and fragment ions to those reported previously[2] (see the Supporting Information).

The GlcNAc 4-OH epimer **5** was targeted next, both to investigate its role as a putative precursor of **3** and to test other possible configurations for hepatic Se metabolite A. The synthesis of **5** proved more challenging; direct monitoring of the thiol–diselenide exchange (see the Supporting Information) indicated complete conversion of GSH and $(GlcNAcSe)_2$ (**6b**) to expected product **5** after 30–45 min. However, compound **5** proved to be significantly more labile than compound **4**. Only traces of **5**·TFA were detected after purification by reverse-phase (RP) HPLC. Purification by size-exclusion chromatography afforded recovered diselenide **6b** (56%) along with a second, mixed fraction containing **6b**/**5**/$(GS)_2$ in a ratio of 3:1:3, as determined by ^1H NMR spectroscopy. The presence of the three different dichalcogenide moieties (Se_2, SeS, and S_2) in the same fraction can be tentatively attributed to comproportionation of the Se–S bond in **5** under these conditions; mixed samples that were incubated in D_2O at a lower temperature of 4°C for 7 days showed no signs of additional degradation/comproportionation.[15,21,22] Structural analyses of the product mixtures by mass spectrometry and NMR spectroscopy allowed characterization of putative glutathionyl precursor GlcNAc-SeS-G (**5**) as its β anomer (d.r. > 98%, $^1J_{H1-Cl} = 159.1$ Hz, see the Supporting Information). The structure of **5** was confirmed also by HRMS and a distinct characteristic isotope pattern for the molecular ion in ESI-MS. Importantly, ESI-MS/MS analyses showed different fragmentation patterns for **4** and **5**. The trends in the dependence of the fragmentation behavior on both collision energy and configuration were also consistent with those observed by Ogra et al.[23] for various selenomethyl *N*-acetylhexosamines (including those of GalNAc and GlcNAc).

Having gained valuable insight into reactivity and stability of sugar-SeS-G conjugates from their investigation under biologically compatible conditions, we next investigated more complex polypeptide substrates. It cannot be discounted[2] that metabolites, such as **1–3**, might also arise from methyltransferase activity (Scheme 1) both on sugar-SeS-glutathione conjugates and on sugar-SeS-protein conjugates. We reasoned that investigation of polypeptide substrates would also test the generality of a potentially straightforward convergent site-specific protein-labeling method for the synthesis of SeS-linked homogeneous glycopeptides and glycoproteins (Scheme 3). If successful, this method would allow the ready combination of flexible site-specific glycoconjugation[24,25] with efficient chemical incorporation of selenium into proteins as an additional tag-and-modify[26,27] strategy.



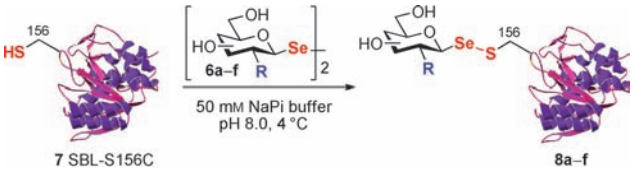
Scheme 3. General strategy for the chemical site-specific incorporation of selenoglycosides into proteins/peptides with different groups at the C-2 position in the carbohydrate moiety.

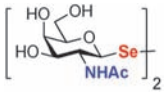
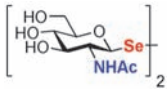
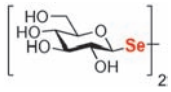
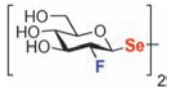
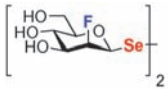
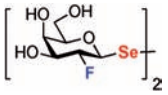
Chemical site-specific protein modification is a potentially powerful method to access homogeneous posttranslationally modified proteins and to introduce synthetic probes that are now key tools for understanding complex biological processes.[27–29] Selenium can provide the basis for structural determination and monitoring of protein surfaces and/or active-site events.[30–33] Most methods for the incorporation of selenium into proteins use the biosynthetic cell machinery.[34–40] Chemical methods (beyond the limits of SeCys and SeMet) have also been exploited as an alternative to genetic techniques, but to date the reported methods suffer from low efficiency (2–50%) and are restricted to active-site residues of proteins.[41] Peptide modifications are mainly focused on solid-phase techniques in combination with native-chemical-ligation strategies.[30,41–47] We envisioned the glycoselenenylation method developed in this work to be possibly valuable as it would allow the simultaneous incorporation of glycans, which is a widespread type of protein modification.[48]

A single-cysteine mutant (SBL-S156C, **7**) of subtilisin from *Bacillus lentus* was used as a model protein substrate. To test the glycan breadth of this Se–S bond-forming reaction, we reacted **7** with a range of reagents, which were prepared by using essentially analogous routes to those shown in Scheme 2 (see the Supporting Information and Table 1). Thus, thiol–diselenide exchange was successfully carried out by simply mixing sugar diselenide reagents **6a–c** with **7** in phosphate buffer at pH 8.0; the reactions proceeded to essentially full conversion within 30 min–2 h (Table 1, entries 1–3). The formation and isolation of these sugar-SeS-protein conjugates, which contain natural sugars that are known to be processed by glycosyltransferases (Gal, GlcNAc, and GalNAc), suggest that, at least in principle, such protein adducts would also be potentially viable intermediates in equivalent pathways to that shown in Scheme 1 for **4** and **5**.

To explore the scope of this method, we decided to apply the conditions described above to the preparation of glycoconjugates that bear different sugars, including non-natural derivatives, such as 2-fluoro-substituted analogues (Table 1, entries 4–6). There is a growing interest in the synthesis of fluorinated carbohydrates and their corresponding fluoro-substituted glycoconjugates.[49–52] The synthesis of SeS-linked 2-deoxy-2-fluoro-substituted β-D-glucosyl (FDG), β-D-mannosyl (FDM), and β-D-galactosyl (FDGal) glycoproteins **8d–f** was also achieved with excellent conversions (>95%; Table 1, entries 4–6).

Table 1: Site-specific syntheses of homogeneous SeS-linked glycoproteins **8a–f**.[a]



Entry	Selenenylating Reagent		t [h]	Product (Sugar, R)	Conv. [%] ESI-MS Found (Calcd)
1[b]		**6a**	2	**8a** (Gal, NHAc)	>95 26998 (26998)
2[b]		**6b**	2	**8b** (Glc, NHAc)	>95 26996 (26998)
3		**6c**	1	**8c** (Glc, OH)	>95 26955 (26956)
4		**6d**	2	**8d** (Glc, F)	>95 26960 (26958)
5		**6e**	2	**8e** (Man, F)	>95 26959 (26958)
6		**6f**	2	**8f** (Gal, F)	>95 26960 (26958)

[a] Reactions carried out at 4 °C with 100 equiv of sugar diselenide. [b] Reactions carried out at RT; no product formation at 4 °C.

The functional aspects of such SeS-linked glycoproteins were explored next. A key prerequisite is the preservation of the protein state. Importantly, modified glycoproteins retained inherent (enzymatic peptidase) activity (see the Supporting Information). Thus, the selenenylating method allows attachment of different potential probes (Se and glycan) without compromising protein function. Moreover, the stability of the newly formed Se–S bond was sufficient to permit the subsequent modification to more complex glycans through endoglycosidase-catalyzed transglycosylation.[53]

The ready introduction of a selenium tag into proteins has the potential to be advantageously employed for the detection, quantification, and tracking, for example during biodistribution studies by atomic detection methods. When compared to, for example, more standard ELISA or radiolabeling methods, such methods have advantages, including low detection limits, absence of radiation hazards, and the ability to distinguish between exogenous and endogenous selenoproteins by using different Se isotopes. We were delighted to find that selenoglycoprotein SBL-C156-SSe-Glc **8c** could be detected at a detection limit of as low as 20 ppb, even when greatly diluted in rat plasma, by using inductively coupled

plasma atomic emission spectrometry (ICP-AES), thus providing an important proof-of-principle for the use of such selenoconjugates as putative biological probes.

We next investigated the stability of the conjugate, with a view especially to potential in vivo applications. Moreover, we reasoned that the relative stability of SeS-linked intermediates might play a role in the suggested biosynthetic pathway outlined in Scheme 1. Notably, the higher stability of selenenylsulfide bonds as compared to disulfides has been exploited for solving peptide-folding problems and in protein engineering;[54,55] in addition to the favorable formation of SeS over S_2,[56] the former possesses a lower redox potential (typically 70 mV lower than the corresponding disulfides).[19] Glutathionyl-sugar conjugates **4** and **5**, and glycoprotein SBL-C156-SSe-Glc **8c** were incubated in rat plasma. No breakdown of either of the SeS-linked conjugates **4** and **8c** was detected over 30 h at 37 °C, thus suggesting that both are resistant and stable in vitro under these conditions (see the Supporting Information). The stability of SeS-linked glycoprotein conjugate SBL-C156-SSe-Glc **8c** is consistent with previous observations for BSA-ebselen complexes.[57] Putative biosynthetic intermediate GalNAc-SeS-G **4** proved stable even over prolonged periods of up to 95 h at 37 °C in rat plasma; in striking contrast, epimer GlcNAc-SeS-G **5** proved insufficiently stable even in aqueous buffer (see above) and could not be observed in plasma. Notably, all of the SSe-linked protein conjugates showed good stability regardless of configuration; the enhanced stability is tentatively attributed to reduced accessibility of the SSe linkage.

In summary, the first target synthesis and full characterization of human hepatic Se metabolite A has been accomplished by using a robust, efficient, and Cys-specific selenenylation protocol, which also allowed the first synthesis of the putative intermediate GlcNAc-SeS-G **5**. Although this metabolite has not yet been identified, it seems a logical precursor (Scheme 1) to minor natural product **3** of the human Se-metabolism pathway. The substrate scope and efficiency of this Se–S bond-forming method was demonstrated through its extension to proteins and to several other glycan types (both natural and non-natural), some of which allowed simultaneous incorporation of selenium and fluorine into proteins at predefined sites. To the best of our knowledge, this strategy also constitutes the first site-specific incorporation of selenoglycosides into proteins. The integrity of the covalent Se–S bond not only allows incorporation of selenium into proteins but also enzymatic elaboration of conjugates, furthermore, it permits quantification of selenium in biological fluids over time, including quantification by highly sensitive atomic detection methods. Given the ability to incorporate SeS-linked fluorosugars, it may be possible to create suitably (dual) labeled conjugates for detection by positron emission tomography (e.g., using ^{18}F labeling).[49–52] It is interesting to note that while our constructs are stable in plasma, Se–S bond cleavage can occur intracellularly[57] under much higher concentrations of reductant (GSH $\approx$ 10 μM); this could enable, for example, specific sugar-mediated intracellular delivery and then release mechanisms for such glycoconjugates.

As well as acting as possible probes of glycoprotein function, we believe such sugar-SeS-R conjugates might shed further light on the reactivity, stability, and metabolism of selenosugar. Our full data for GalNAc-SeS-G **4** are consistent with the partial data obtained previously for the isolated natural product hepatic Se metabolite A[2] and suggest that the previously proposed identity is correct. Moreover, the stabilities of **4** and **5** vary dramatically according to the configuration of the attached sugar. Although unexpected, this result allows us to propose an explanation for the observation that the more unusual glycosyl moiety GalNAc is the dominant sugar product of this human biosynthetic pathway. Regardless of the exact mechanism and the pathway(s) of its formation, GalNAc compound **4** is stable under conditions (aqueous and plasma, see above) under which GlcNAc **5** is not stable. These observations are consistent with prior suggestions that the stability of the SeS linkage is influenced by stereoelectronics and linkage environment (as well as by the pH value of the medium).[55] The difference in the stability of **4** and **5** mirrors the eventual proportions of Se metabolites that are found in human urine: the concentration of minor GlcNAc Se metabolite **3** is less than 2% of the concentration of GalNAc variant **1**.[58] **4** and **5** as well as **1** and **3** are pairs of 4-OH epimers; 4-OH sugar epimerase activity, which interconverts GalNAc with GlcNAc derivatives, is prominent in mammalian cells.[13] It may be that promiscuous methyltransferase activity (Scheme 1) processes available substrates and, from our results gathered here, is likely to favor GalNAc-SeS-R as a precursor on the basis of stability. These putative biosynthetic intermediates **4** and **5** will therefore be valuable in ongoing studies to isolate the associated human glycosyl- and methyl-transferases.

Received: September 19, 2011
Published online: December 30, 2011

Keywords: glycoproteins · metabolites · posttranslational modifications · selenium · selenoglycosides

[1] K. A. Francesconi, F. Pannier, *Clin. Chem.* **2004**, *50*, 2240.
[2] Y. Kobayashi, Y. Ogra, K. Ishiwata, H. Takayama, N. Aimi, K. T. Suzuki, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 15932.
[3] Y. Ogra, Y. Anan, *J. Anal. At. Spectrom.* **2009**, *24*, 1477.
[4] Y. Tsuji, N. Suzuki, K. T. Suzuki, Y. Ogra, *J. Toxicol. Sci.* **2009**, *34*, 191.
[5] C. A. Bayse, *Inorg. Chem.* **2004**, *43*, 1208.
[6] P. Gettins, S. A. Wardlaw, *J. Biol. Chem.* **1991**, *266*, 3422.
[7] H. Duddeck, *Prog. Nucl. Magn. Reson. Spectrosc.* **1995**, *27*, 1.
[8] D. M. Hatch, J. O. Boles, Z. Li, L. A. Pete Silks, *Curr. Org. Chem.* **2004**, *8*, 47.
[9] A. M. Deacon, S. E. Ealick, *Structure* **1999**, *7*, R161.
[10] A. Kussrow, E. Kaltgrad, M. L. Wolfenden, M. J. Cloninger, M. G. Finn, D. J. Bornhop, *Anal. Chem.* **2009**, *81*, 4889.
[11] D. E. Prasuhn, P. Singh, E. Strable, S. Brown, M. Manchester, M. G. Finn, *J. Am. Chem. Soc.* **2008**, *130*, 1328.
[12] E. Block, R. S. Glass, N. E. Jacobsen, S. Johnson, C. Kahakachchi, R. Kaminski, A. Skowronska, H. T. Boakye, J. F. Tyson, P. C. Uden, *J. Agric. Food Chem.* **2004**, *52*, 3761.
[13] H. M. Holden, I. Rayment, J. B. Thoden, *J. Biol. Chem.* **2003**, *278*, 43885.

[14] D. P. Gamblin, P. Garnier, S. van Kasteren, N. J. Oldham, A. J. Fairbanks, B. G. Davis, *Angew. Chem.* **2004**, *116*, 846; *Angew. Chem. Int. Ed.* **2004**, *43*, 828.
[15] L. A. Wessjohann, A. Schneider, M. Abbas, W. Brandt, *Biol. Chem.* **2007**, *388*, 997.
[16] G. J. L. Bernardes, D. P. Gamblin, B. G. Davis, *Angew. Chem.* **2006**, *118*, 4111; *Angew. Chem. Int. Ed.* **2006**, *45*, 4007.
[17] J. M. Chalker, G. J. L. Bernardes, Y. A. Lin, B. G. Davis, *Chem. Asian J.* **2009**, *4*, 630.
[18] J. Beld, K. J. Woycechowsky, D. Hilvert, *Biochemistry* **2007**, *46*, 5382.
[19] D. Steinmann, T. Nauser, W. H. Koppenol, *J. Org. Chem.* **2010**, *75*, 6696.
[20] Y. Kawai, H. Ando, H. Ozeki, M. Koketsu, H. Ishihara, *Org. Lett.* **2005**, *7*, 4653.
[21] L. A. Wessjohann, A. Schneider, *Chem. Biodiversity* **2008**, *5*, 375.
[22] A. Schneider, W. Brandt, L. A. Wessjohann, *Biol. Chem.* **2007**, *388*, 1099.
[23] Y. Ogra, K. Ishiwata, H. Takayama, N. Aimi, K. T. Suzuki, *J. Chromatogr. B* **2002**, *767*, 301.
[24] D. P. Gamblin, E. M. Scanlan, B. G. Davis, *Chem. Rev.* **2009**, *109*, 131.
[25] G. J. L. Bernardes, B. Castagner, P. H. Seeberger, *ACS Chem. Biol.* **2009**, *4*, 703.
[26] B. G. Davis, *Pure Appl. Chem.* **2009**, *81*, 285.
[27] J. M. Chalker, G. J. L. Bernardes, B. G. Davis, *Acc. Chem. Res.* **2011**, *44*, 730.
[28] S. Park, M.-R. Lee, I. Shin, *Chem. Soc. Rev.* **2008**, *37*, 1579.
[29] B. G. Davis, *Science* **2004**, *303*, 480.
[30] G. Roelfes, D. Hilvert, *Angew. Chem.* **2003**, *115*, 2377; *Angew. Chem. Int. Ed.* **2003**, *42*, 2275.
[31] M. Zhang, T. Yuan, J. M. Aramini, H. J. Vogel, *J. Biol. Chem.* **1995**, *270*, 20901.
[32] G. P. Mullen, R. B. Dunlap, J. D. Odom, *Biochemistry* **1986**, *25*, 5625.
[33] M. Mobli, A. D. de Araújo, L. K. Lambert, G. K. Pierens, M. J. Windley, G. M. Nicholson, P. F. Alewood, G. F. King, *Angew. Chem.* **2009**, *121*, 9476; *Angew. Chem. Int. Ed.* **2009**, *48*, 9312.
[34] C. Allmang, A. Krol, *Biochimie* **2006**, *88*, 1561.
[35] D. L. Hatfield, B. A. Carlson, X. Xu, H. Mix, V. N. Gladyshev, M. Kivie in *Progress in Nucleic Acid Research and Molecular Biology*, *Vol. 81*, Academic Press, New York, **2006**, p. 97.
[36] J. E. Squires, M. J. Berry, *IUBMB Life* **2008**, *60*, 232.
[37] X.-M. Xu, B. Carlson, Y. Zhang, H. Mix, G. Kryukov, R. Glass, M. Berry, V. Gladyshev, D. Hatfield, *Biol. Trace Elem. Res.* **2007**, *119*, 234.
[38] J. Wang, S. M. Schiller, P. G. Schultz, *Angew. Chem.* **2007**, *119*, 6973; *Angew. Chem. Int. Ed.* **2007**, *46*, 6849.
[39] A. J. de Graaf, M. Kooijman, W. E. Hennink, E. Mastrobattista, *Bioconjugate Chem.* **2009**, *20*, 1281.
[40] Y. Zhang, V. N. Gladyshev, *Chem. Rev.* **2009**, *109*, 4828.
[41] M. Muttenthaler, P. F. Alewood, *J. Pept. Sci.* **2008**, *14*, 1223.
[42] M. Iwaoka, R. Ooka, T. Nakazato, S. Yoshida, S. Oishi, *Chem. Biodiversity* **2008**, *5*, 359.
[43] R. Quaderer, D. Hilvert, *Chem. Commun.* **2002**, 2620.
[44] R. Quaderer, A. Sewing, D. Hilvert, *Helv. Chim. Acta* **2001**, *84*, 1197.
[45] M. D. Gieselman, L. Xie, W. A. van der Donk, *Org. Lett.* **2001**, *3*, 1331.
[46] N. Metanis, E. Keinan, P. E. Dawson, *Angew. Chem.* **2010**, *122*, 7203; *Angew. Chem. Int. Ed.* **2010**, *49*, 7049.
[47] L. Moroder, *J. Pept. Sci.* **2005**, *11*, 187.
[48] K. Ohtsubo, J. D. Marth, *Cell* **2006**, *126*, 855.
[49] O. Boutureira, F. D'Hooge, M. Fernandez-Gonzalez, G. J. L. Bernardes, M. Sanchez-Navarro, J. R. Koeppe, B. G. Davis, *Chem. Commun.* **2010**, *46*, 8142.
[50] O. Boutureira, G. J. L. Bernardes, F. D'Hooge, B. G. Davis, *Chem. Commun.* **2011**, *47*, 10010.
[51] R. Sackstein, C. J. Dimitroff, R. J. Bernacki, M. Sharma, K. L. Matta, B. Paul, U.S. Pat. Appl. Publ. US 2003148997A1 20030807, **2003**.
[52] S. A. Allman, H. H. Jensen, B. Vijayakrishnan, J. A. Garnett, E. Leon, Y. Liu, D. C. Anthony, N. R. Sibson, T. Feizi, S. Matthews, B. G. Davis, *ChemBioChem* **2009**, *10*, 2522.
[53] M. Fernández-González, O. Boutureira, G. J. L. Bernardes, J. M. Chalker, M. A. Young, J. C. Errey, B. G. Davis, *Chem. Sci.* **2010**, *1*, 709.
[54] M. Muttenthaler, S. T. Nevin, A. A. Grishin, S. T. Ngo, P. T. Choy, N. L. Daly, S.-H. Hu, C. J. Armishaw, C.-I. A. Wang, R. J. Lewis, J. L. Martin, P. G. Noakes, D. J. Craik, D. J. Adams, P. F. Alewood, *J. Am. Chem. Soc.* **2010**, *132*, 3514.
[55] N. Metanis, E. Keinan, P. E. Dawson, *J. Am. Chem. Soc.* **2006**, *128*, 16684.
[56] S. Pegoraro, S. Fiori, S. Rudolph-Böhner, T. X. Watanabe, L. Moroder, *J. Mol. Biol.* **1998**, *284*, 779.
[57] G. Wagner, G. Schuch, T. P. M. Akerboom, H. Sies, *Biochem. Pharmacol.* **1994**, *48*, 1137.
[58] B. Gammelgaard, L. Bendahl, *J. Anal. At. Spectrom.* **2004**, *19*, 135.

