One pot synthesis of selenocysteine containing peptoid libraries by Ugi multicomponent reactions in water†

Muhammad Abbas, John Bethke and Ludger A. Wessjohann*

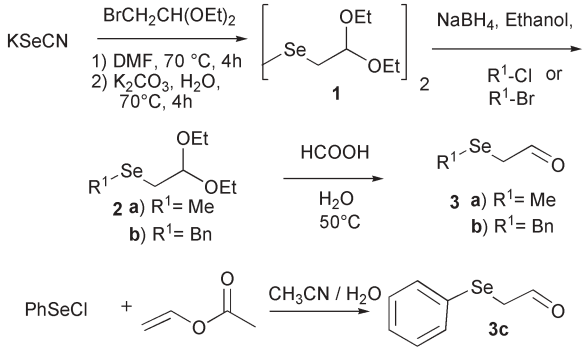
Received (in Cambridge, UK) 14th October 2005, Accepted 22nd November 2005
First published as an Advance Article on the web 15th December 2005
DOI: 10.1039/b514597j

Selenocysteine containing peptoids and peptide–peptoid conjugates were synthesized by combinatorial Ugi-MCRs (multicomponent reactions) in water: for the first time, an acetal (selenoacetal 2a) was used in Ugi-MCR to furnish selenocysteine peptoids in one step as model compounds for selenocysteine peptides and proteins.

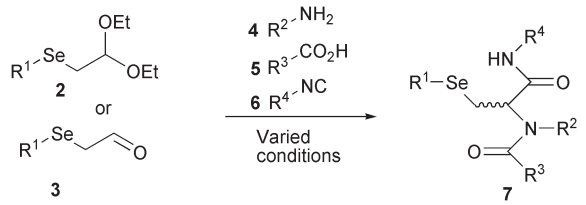
Selenium has been shown to be a nutritionally essential trace element for mammals, including humans.[1] It is an integral part of thioredoxin-reductases[2] and glutathione peroxidases (GPx), antioxidant enzymes, as well as of several other selenoproteins.[3,4] Until now, more than 30 selenoproteins and selenopeptides are identified but the function of several is still unknown. To examine the differences of cysteine (Cys, C) *versus* selenocysteine (Sec, U) in proteins, efforts have been made to replace cysteine with selenocysteine.[5,6] Unfortunately, the conditions necessary for selenocysteine incorporation by the cell's protein assembly mechanism are very specific. As a result, the preparation of non-natural selenocysteine containing peptides and proteins by molecular biology methods is difficult, although some recent successful approaches have been documented.[7] Complex proteins provide limited access for certain selective measurements (*e.g.* redox potential)[8] which need to be correlated to small changes in the selenocysteine vicinity. In proteins, other residues, buried Sec-sites, and folding can complicate direct measurements or data interpretation. Small Sec–peptide fragments can provide clearer answers, but often suffer from lengthy syntheses, or are not readily available as libraries from larger-scale preparations. There are only very few methods available for the synthesis of natural selenium compounds, especially of selenocysteine due to its lability towards oxidation.[5,9] This leads to quite limited chemical studies on biologically relevant organoselenium compounds. Most available data are exclusively based upon aromatic selenides (*i.e.* phenylselenenyl derivatives) in organic solvents, which is of limited relevance under physiological conditions.[10] Thus, syntheses of aliphatic selenols are rare and always designed for only one target compound (*e.g.* selenocysteine).[9]

In contrast to such singular approaches, in this communication a combinatorial one is reported, which produces a variety of selenocysteine analogs through a broadly applicable and fast method. For the first time, we apply the Ugi four component reaction (Ugi-4CR) to the synthesis of model selenopeptoids. These selenopeptoids are supposed to have a similar short-range environment and show similar properties as the selenoprotein portion but are easy to synthesize and to study.

In all studies, a strategy was followed in which the seleno moiety is embedded in the carbonyl building unit, which has to be one of the four components in the Ugi-4CR, and is the only one to place the methylselenol side chain in the α-position of the dipeptoid formed. Since unprotected 2-selenoacetaldehyde is not available and the corresponding diselenide is unreactive or misbehaved in Ugi reactions, other protected forms had to be used. Diselenodiacetal **1** was synthesized from KSeCN and 1,1-diethoxy-2-bromoacetate in DMF (Scheme 1).[11] Reductive alkylation of diselenide **1** gave the selenylacetals **2a** and **2b** which can be used directly in case of aqueous reaction medium. For the Ugi reaction in organic solvents the acetals **2a** and **2b** were deprotected under acidic conditions to obtain the selenylaldehydes **3a** and **3b**, respectively (Scheme 1).[12] The selenoaldehyde **3c** is more conveniently prepared from vinyl acetate and PhSeCl (Scheme 1).[13]



Scheme 1 Synthesis of 2-selenoacetaldehyde building blocks.



Scheme 2 One pot combinatorial synthesis of selenocysteine peptoids by Ugi-4-component reaction of 2-selenoacetal building blocks.

Leibniz Institute of Plant Biochemistry, Department of Bioorganic Chemistry, Weinberg 3, D-06120 Halle (Saale), Germany. E-mail: wessjohann@ipb-halle.de; Fax: +49 345 5582 1309; Tel: +49 345 5582 1301

† Electronic supplementary information (ESI) available: Procedure for the synthesis of selenocysteine peptoids under water and microwave conditions and spectral data of some selected products. See DOI: 10.1039/b514597j

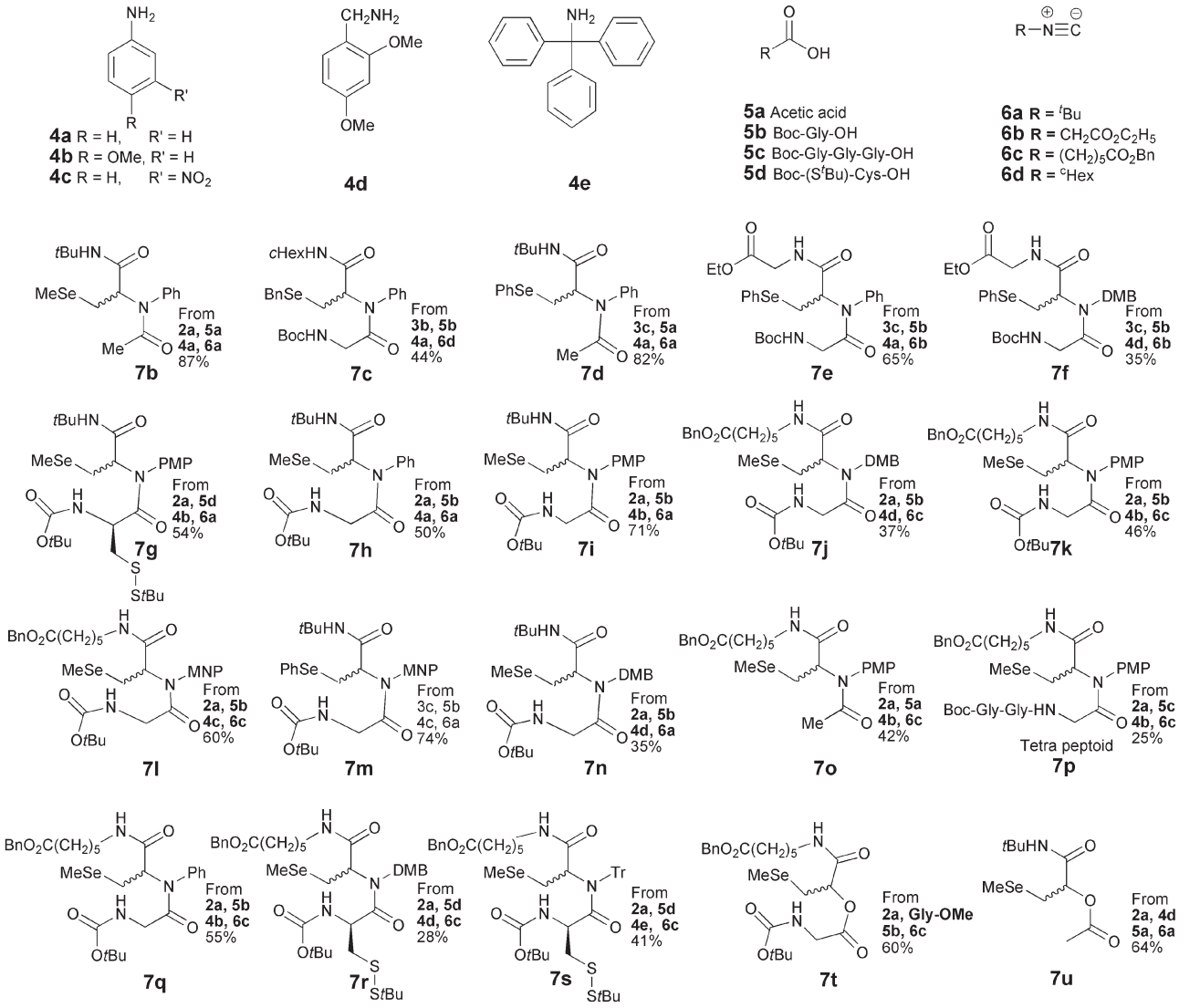
Table 1 Selected Se-Ugi-4CRs under microwave or aqueous conditions

	Aldehyde R^1	Amine (**4**) R^2	Acid (**5**) R^3	Isocyanide (**6**) R^4	Solvent	Isolated Yield (%) of Ugi product (**7**)
7a	Me (**3a**)	Ph	Me	*c*-C_6H_{12}	$CHCl_3$	61[a]
	Me (**2a**)				H_2O	85[b]
7b	Me (**3a**)	Ph	Me	*t*-Bu	$CHCl_3$	58[a]
	Me (**2a**)				H_2O	82[b]
7c	Bn (**3b**)	Ph	*N*-Boc–Gly	*c*-C_6H_{12}	$CHCl_3$	4[a]
	Bn (**3b**)				H_2O	44[b]
7d	Ph (**3c**)	Ph	Me	*t*-Bu	$CHCl_3$	43[a]
	Ph (**3c**)				H_2O	87[b]
7e	Ph (**3c**)	Ph	*N*-Boc–Gly	CH_2CO_2Et	$CHCl_3$	27[a]
	Ph (**3c**)				H_2O	65[b]
7f	Ph (**3c**)	DMB[d]	*N*-Boc–Gly	CH_2CO_2Et	$CHCl_3$	6[a]
	Ph (**3c**)				H_2O	35[c]

[a] Microwave, 160 °C, $CHCl_3$. [b] Water, rt. [c] Water, $Yb_2(SO_4)_3$. [d] DMB = 2,4-dimethoxy benzyl.

Compounds **2a**, **3b**, and **3c** were used as model carbonyl building blocks for the Ugi-4CR (Scheme 2). The other components (*i.e.* amines, acids, and isonitriles) are commercially available, except isonitrile **6c** which was prepared from the corresponding formamide.[14]

In preliminary experiments, we used aldehydes **3a–c** under classical Ugi-4CR conditions (stirring the four components in methanol or $CHCl_3$ at room temperature). The yields of the products were very low. However, under conventional and especially microwave heating, the products could be obtained in up to 61% yield (Table 1). Because the results were still unsatisfactory, we carried out the Ugi-4CR in different solvents. Interestingly, we found that in water the reaction worked smoothly at room temperature and gives better yields (Table 1, **7b–f**).[15] Excess water can have a negative impact on the Ugi reaction because of a less favorable equilibrium for the initial Schiff-base formation, and thus the competing Passerini reaction of unreacted aldehyde may dominate. This was not observed here, instead the



Scheme 3 A selection of starting materials (acids, amines and isonitriles) and protected selenopeptoid products with isolated yields of purified products. Ph = phenyl, PMP = 4-methoxy phenyl, MNP = 3-nitro phenyl, Tr = trityl.

formation of solid reaction products, which separate from the aqueous solution is likely to drive peptoid product formation.‡

Also, under these conditions protected aldehyde **2a** can be used directly in the Ugi-4CR. This is an important advantage, as a selenofunctionality in an unprotected aldehyde is detrimental for Ugi reactivity. The yields obtained with aniline as the amine in water, varying the acid and the isonitrile components (65–87%) were superior to the ones obtained earlier. In the case of 2,4-dimethoxybenzyl (DMB) amine, however, no reaction could be observed. In order to activate the intermediate imines, different Lewis acids were tested of which $Yb_2(SO_4)_3 \times 8H_2O$ (10 mol%) was the most suitable one for this purpose.

Next, the suitability of the Ugi-4CR protocol for selenocysteine incorporation into peptides was tested, using several amino acid residues as part of the different components of this four-component four-centre reaction. As can be seen from the results in Table 1 and Scheme 3, the yields of Ugi products are highly dependent on the amine used. In the case of anilines, the yields are satisfactory, whereas in the case of electron rich DMB-amine the yields are quite low (**7f**, **7j**, **7n** and **7r**). The reactivity of the amine unit plays a key role in the selenocysteine analog syntheses. Amine units from an amino acid mostly remain inactive under the reaction conditions used and usually give Passerini products (**7t**). However, the isonitrile derived from glycine ethyl ester (**6b**), usually problematic because of side reactions,[16] worked well in Ugi-4CR under aqueous conditions (in Ugi products **7e** and **7f**). PMP-amine (in Ugi products **7g**, **7i**, **7k**, **7o** and **7p**), DMB-amine (in Ugi products **7f**, **7j**, **7n** and **7r**), and trityl amine (in Ugi product **7s**) can be used in the selenopeptoid synthesis reactions as they can be removed under acidic (Tr)[17] or oxidative conditions (PMP[18] and DMB[19]) to give selenopeptides. Acetic acid (**7a**, **7b**, **7d** and **7o**) and *N*-Boc–Gly–OH (in Ugi products **7c**, **7e**, **7f**, **7h–n**, and **7q**) gave the best results. However *N*-Boc–(S–^{t}Bu)–Cys–OH (in Ugi product **7g**, **7r** and **7s**) gave moderate yields of cysteine–selenocysteine (–Cys-Sec–) dipeptoids. Cys-Sec-derivatives (in Ugi products **7g**, **7r** and **7s**) can lead to the formation of a selenenyl sulfide (–S–Se–) bridge which is a crucial intermediate in the catalytic cycle of some selenoproteins.[2,20] With a tripeptide (*N*-Boc–Gly-Gly-Gly–OH) as acid building block, the tetrapeptoid **7p** is obtained in reasonable yield.

In summary, we have developed a very straightforward and short synthesis of selenocysteine and/or selenomethionine peptoids in aqueous medium. These selenocysteine peptoids will be further used for electrochemical and physiological studies.

We gratefully acknowledge financial support from Deutsche Forschungsgemeinschaft as part of the Selenoprotein Schwerpunkt DFG-SPP 1087 (We-1467/4-1), and thank Prof. Dr. B. Westermann and Dr. W. Brandt for valuable suggestions and discussion.

Notes and references

‡ *Procedure for selenopeptoid synthesis in aqueous medium*: To selenylaldehyde **3b–c** (1.3 mmol) or acetal **2a** (2 mmol) in 5 ml of degassed water, amine (1.3 mmol) is added at room temp. At this point a non-reactive acid catalyst [*e.g.* $Yb_2(SO_4)_3$-hydrate] can be added. The mixture is stirred for 20 min. Then 1.3 mmol of isonitrile followed by 1.0 mmol of acid are added. The reaction mixture is stirred overnight. 10 ml Ethylacetate is added to dissolve the gummy product. The water layer is washed with ethylacetate three times, combined, dried over Na_2SO_4 and concentrated to gummy product which is purified by chromatography on silica gel, usually with petrol ether : ethyl acetate (*ca.* 3 : 1).

1 K. Schwarz and C. M. Foltz, *J. Am. Chem. Soc.*, 1957, **79**, 3292.
2 W. Brandt and L. A. Wessjohann, *ChemBioChem*, 2005, **6**, 1; L. Flohé, W. A. Gunzler and H. H. Schock, *FEBS Lett.*, 1973, **32**, 132.
3 J. Rotruck, A. L. Pope, H. E. Ganther, A. B. Swanson, D. G. Hafeman and W. G. Hoekstra, *Science*, 1973, **179**, 588; M. Birringer, S. Pilawa and L. Flohé, *Nat. Prod. Rep.*, 2002, **19**, 693 (and ref. therein); G. C. Thomas and B. Ronald, *Chem. Rev.*, 2003, **103**, 1; G. Mugesh and H. B. Singh, *Chem. Soc. Rev.*, 2000, **29**, 347; C. Jacob, G. I. Giles, N. M. Giles and H. Sies, *Angew. Chem.*, 2003, **115**, 4890, *Angew. Chem., Int. Ed.*, 2003, **42**, 4742.
4 A. Kyriakopoulos and D. Behne, *Rev. Physiol. Biochem. Pharmacol.*, 2002, **145**, 1; D. Behne and A. Kyriakopoulos, *Annu. Rev. Nutr.*, 2001, **21**, 453.
5 I. Andreadou, W. Menge, J. Commandeur, E. Worthington and N. Vermeulen, *J. Med. Chem.*, 1996, **39**, 2040; B. K. Sarma and G. Mugesh, *J. Am. Chem. Soc.*, 2005, **127**, 11477.
6 Z. P. Wu and D. J. Hilvert, *J. Am. Chem. Soc.*, 1990, **112**, 5647; R. Syed, Z. P. Wu, J. M. Hogle and D. J. Hilvert, *Biochemistry*, 1993, **32**, 6157; R. J. Hondal, B. L. Nilsson and R. T. Raines, *J. Am. Chem. Soc.*, 2001, **123**, 5140; M. D. Gieselman and W. A. van der Donk, *Org. Lett.*, 2001, **3**, 1331; L. Moroder, *J. Pept. Sci.*, 2005, **11**, 187.
7 S. Boschi-Muller, S. Muller, V. Dorsselaer and B. Branlant, *FEBS Lett.*, 1998, **439**, 241; E. S. Arner, H. Sarioglu, F. Lottspeich, A. Holmgren and A. Böck, *J. Mol. Biol.*, 1999, **292**, 1003; K. E. Sandman, J. S. Benner and C. Noren, *J. Am. Chem. Soc.*, 2000, **122**, 960.
8 C. A. Collins, F. H. Fry, A. L. Holme, A. Yiakouvaki, A. Al-Qenaei, C. Pourzandc and C. Jacob, *Org. Biomol. Chem.*, 2005, **3**, 1541; S. Pariagh, K. M. Tasker, F. H. Fry, A. L. Holme, C. A. Collins, N. Okarter, N. Gutowski and C. Jacob, *Org. Biomol. Chem.*, 2005, **3**, 975; D. Besse, F. Siedler, T. Diercks, H. Kessler and L. Moroder, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 883.
9 A. Schneider, O. E. D. Rodrigues, M. W. Paixão, H. R. Appelt, A. L. Braga and L. A. Wessjohann, *Tetrahedron Lett.*, 2005/2006, DOI: 10.1016/j.tetlet.2005.11.101; A. L. Braga, D. S. Lüdtke, M. W. Paixão, E. E. Alberto, H. A. Stefani and L. Juliano, *Eur. J. Org. Chem.*, 2005, 4260; E. M. Stocking, J. N. Schwarz, H. Senn, M. Salzmann and L. A. Silks, *J. Chem. Soc., Perkin Trans. 1*, 1997, 2443.
10 L. A. Wessjohann and U. Sinks, *J. Prakt. Chem.*, 1998, **340**, 189; G. Mugesh, W. W. du Mont and H. Sies, *Chem. Rev.*, 2001, **101**, 2125.
11 A. Krief, W. Dumont and C. Delmotte, *Angew. Chem.*, 2000, **112**, 1735, *Angew. Chem., Int. Ed.*, 2000, **39**, 1669.
12 Ib. Johannsen, K. Lerstrup, L. Henriksen and K. Bechgaard, *J. Chem. Soc., Chem. Commun.*, 1984, 89.
13 C. J. Kowalski and J.-S. Dung, *J. Am. Chem. Soc.*, 1980, **102**, 7950.
14 B. Westermann and S. Dörner, *Chem. Commun.*, 2005, 2116.
15 M. C. Pirrung and K. Das Sarma, *J. Am. Chem. Soc.*, 2004, **126**, 445.
16 R. S. Bon, C. Hong, M. J. Bouma, R. F. Schmitz, F. J. J. de Kanter, M. Lutz, A. L. Spek and R. V. A. Orru, *Org. Lett.*, 2003, **5**, 3759.
17 G. C. Stelakatos, D. M. Theodoropoulos and L. Zervas, *J. Am. Chem. Soc.*, 1959, **81**, 2884.
18 P. Bravo, A. Farina, V. P. Kukhar, A. L. Markovsky, S. V. Meille, V. A. Soloshonok, A. E. Sorochinsky, F. Viani, M. Zanda and C. Zappalá, *J. Org. Chem.*, 1997, **62**, 3424; T. Kiyoshi, S. Maki, T. Daisuke, K. Motomu and I. Akira, *Heterocycles*, 1998, **47**, 77.
19 S. Susumi, H. Keiko and Y. Hisashi, *Tetrahedron*, 2001, **57**, 875.
20 L. D. Arscott, S. Gromer, R. H. Schirmer, K. Becker and C. H. Williams, *Proc. Natl. Acad. Sci. U. S. A.*, 1997, **94**, 3621; S. Gromer, J. Wissing, D. Behne, K. Ashman, R. H. Schirmer, L. Flohé and K. Becker, *J. Biochem.*, 1998, **332**, 591; S. Gromer, L. D. Arscott, C. H. Williams, R. H. Jr. Schirmer and K. Becker, *J. Biol. Chem.*, 1998, **273**, 20096; S. R. Lee, S. Bar-Noy, R. L. Kwon, J. Levine, T. C. Stadtman and S. G. Rhee, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 2521; L. Zhong, E. S. J. Arnér and A. Holmgren, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 5854.