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DOI: 10.1039/C7CC00399D

COMMUNICATION

Methionine and seleno-methionine type peptide and peptoid building blocks by five-component five-center reactions

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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A first example of 5-component 5-center reactions with isonitriles [Ugi-5CRs] is described. The extended Ugi type reactions involve selenoaldehydes as well as ammonia, both challenging reactants in multicomponent (MCR) systems, to generate methionine and Se-methionine moieties and derivatives as protected building blocks or for direct ligation in peptides or peptoids. The peptoid/peptide building blocks proved not cytotoxic but increased expression of genes encoding for stress protective selenoproteins (Gpx1).

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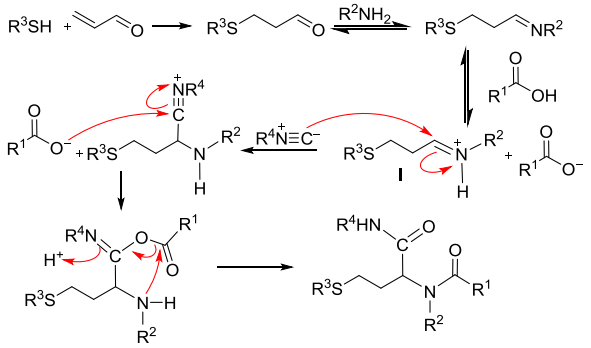
Sulfur and selenium amino acids play a crucial and very special role in biochemistry and protein chemistry.[1–3] For example, methionine is not only the starter amino acid in peptide synthesis, among other peptide functions, it also is crucial as methyl donor in form of its derivative *S*-adenosyl methionine (SAM).[4] Its selenium analogue is not canonic, but it is the most relevant organic source of the trace element selenium. It also exhibits different properties compared to other selenium supplements, e.g. changing cellular ROS status or DNA methylation, which affects epigenetic controls and represents an ideal tool for biochemical and protein structural studies.[5,6] Replacement of methionine with Se-methionine in the biosynthesis can solve the phase-problem, known as Patterson method,[7] and found application in determination of protein structures via x-ray analysis.[8] However, due to the elements special properties, selenium reactions are not always well behaved, and the element with its bound moieties easily eliminates or oxidizes, or reacts with other electrophiles, something definitely to expect during Se-methionine syntheses too.

Thus, based on our previous experience producing bioactive selenium compounds and selenocysteine (Sec) peptides by multi-component reactions (MCRs),[9–11] we set out to try the more complex syntheses of Se-methionine and thereby methionine as mother compound too. MCRs underlie the most fruitful concepts for the rapid generation of high structural variety and molecular complexity in a single conversion.[12–14] During the last decades, MCRs have expanded rapidly, especially since they permit quick access to a defined chemical space or allow multiple assemblies of several moieties. They also can improve the total synthesis of natural products and related structures[15–17] including the build-up, coupling or cyclization of peptides, peptoids and pseudo-peptides, specifically by Ugi reactions.[17,18] On the other hand, higher-order MCRs, in which five or more components are combined in a single reaction, are scarce, with the Ugi-7-component reaction being an exception.[19] Most higher MCRs, however, consist of additive combinations of 3- or 4-CRs at a poly-functional or an in-between deprotected molecule rather than being a single MCR.[12,20,21]



Scheme 1. Putative reaction mechanism of Ugi 5CRs. The order of events for the formation of intermediate **I** may be inverted (i.e. first iminium ion formation followed by Michael-addition). Since the reaction runs in alcohol under slightly acidic conditions, up to **I** all O/O-, O/S-, N/O-, N/N- and N/S-(hemi-)acetal species of the various combinations might be formed in equilibrium (not shown for clarity).

In contrast to singular multistep synthetic approaches,[22] a novel combinatorial method is reported in this study. Using this broadly applicable and fast method, a variety of peptoid building blocks consisting of methionine or Se-methionine analogues is generated through Ugi five component five center reactions (Ugi-5CRs, see Scheme 1 and Tables). In the literature 4C5C-Rs (four-component five-center reactions) are

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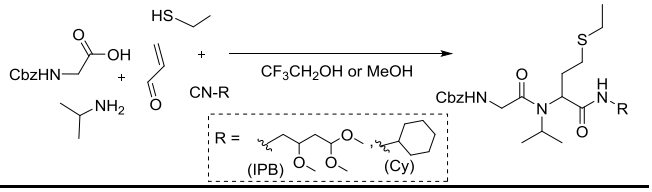
described,[23,24] but to the best of our knowledge there are no reports on true Ugi-type 5CRs (five-component, five-center reactions). As depicted in Scheme 1, the classical Ugi 4 component reaction comprised of an oxo, amino, carboxylic acid, and isonitrile component is extended by the in-situ Michael-addition of a nucleophile as fifth component to an α,β-unsaturated aldehyde as oxo component. For reaction optimization, a model system consisting of ethylthiol (as less volatile Michael nucleophile leading to the methionine homolog ethionine), acrolein (Michael acceptor and oxo component), *N*-protected glycine (carboxylic acid component, C-terminal peptide model), and an isonitrile (IPB: 4-isocyanopermethylbutane-1,1,3-triol[25,26] or cyclohexylisonitrile) was used with different isopropylamine (Table 1). These components were reacted in different molar ratios and the most successful one (Table 1) was selected for compound library syntheses. As found in our previous synthetic attempts and also herein, trifluoroethanol turned out to be the most suitable solvent.

Table 1. Optimization the Ugi-5CRs (values are molar equivalents of the reactants)



Entry no.	Cbz-Gly	Acrolein	EtSH	*i*PrNH$_2$	Isonitrile	Yield (%)
1	1.0	1.5	1.5	1.0	1.25[b]	32
2	1.0	1.0	1.0	1.0	1.00[b]	20
3[a]	1.0	1.5	1.5	1.0	1.25[b]	15[a]
4	1.0	1.5	1.5	1.5	1.25[b]	55
5	1.0	1.5	1.5	2.5	1.25[b]	50
6	1.0	1.5	2.2	1.5	1.25[b]	67
7	1.0	1.5	2.2	2.5	1.25[c]	37
8	1.0	1.5	2.2	1.5	1.25[c]	78

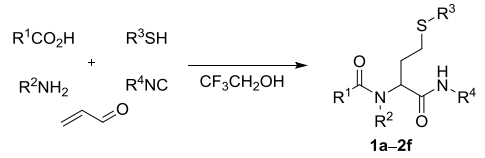
[a]solvent MeOH; [b]IPB; [c]cyclohexylisonitrile.

For the preparation of various homomeric L-methionine containing peptoids, ethylthiol, propylthiol and allylthiol were used as sulfur nucleophiles and allowed to react with acrolein (Table 2). Successively, isopropylamine or aniline were added followed by a carboxylic acid and an isocyanide (cyclohexylisonitrile or the convertible isonitrile and peptide-coupling-anker reagent IPB).[25] Applying this procedure, the first peptoids using an Ugi-5CR were generated.

The products **1a**–**1g** and **2a**–**2d** were obtained in moderate to good yields, while **2e** and **2f** (*S*-allyl moiety each) gave low yields. As expected, better yields were obtained when isopropylamine was used instead of aniline. The convertible isonitrile IPB is a masked C-terminal activator, which after acidic triggering allows coupling to the free *N*-terminus of another peptide (or intramolecular cyclization).[25]

In order to form classical peptide bonds at the newly formed methionine site, the amine component has to be ammonia. Such reactions are usually not feasible in the acid catalysed Ugi reaction, mostly because complex product mixtures and stable *N*-acetal-intermediates (cf. urotropine) are obtained.[14–17]

Table 2. Ugi-5CRs to homomeric methionine peptoids: amine component *i*PrNH$_2$ or aniline

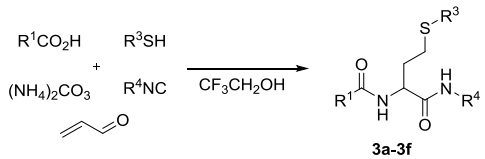


1a–2f

No.	R^1	R^2	R^3	R^4	Yield (%)
1a	Me	*i*Pr	Et	IPB	50
1b	CbzNHCH$_2$	*i*Pr	Et	IPB	60
1c	CbzNHCH$_2$	*i*Pr	Et	Cy	78
1d	CbzNHCH$_2$	*i*Pr	*n*Pr	IPB	55
1e	CbzNHCH$_2$	*i*Pr	*n*Pr	Cy	66
1f	CbzNHCH$_2$	*i*Pr	allyl	IPB	43
1g	CbzNHCH$_2$	*i*Pr	allyl	Cy	60
2a	CbzNHCH$_2$	Ph	Et	IPB	43
2b	CbzNHCH$_2$	Ph	Et	Cy	47
2c	CbzNHCH$_2$	Ph	*n*Pr	IPB	43
2d	CbzNHCH$_2$	Ph	*n*Pr	Cy	60
2e	CbzNHCH$_2$	Ph	allyl	IPB	20
2f	CbzNHCH$_2$	Ph	allyl	Cy	18

To avoid such side reactions, cleavable primary amines (i.e. ammonia synthons, like 1-amino sugars or photo labile 2-nitro benzyl amine) can be used, thereby adding additional steps and destroying the magic of the MCR concept.[27] Thus methods developed in Kazmaier's and our group for the direct use of ammonia or ammonium in a solvent of low nucleophilicity was tested.[9,27,28] Based on this, ammonium carbonate was successfully used as ammonia source also in the Ugi-5CR to directly obtain Se-methionine-peptide building blocks (Table 3), albeit in low yield requiring further, individual optimization if a single target molecule is selected.

Table 3. Ugi-5CRs to homomeric methionine peptides: amine component ammonia as $(NH_4)_2CO_3$



3a-3f

No.	R^1	R^3	R^4	Yield (%)
3a	CbzNHCH$_2$	Et	IPB	33
3b	CbzNHCH$_2$	Et	Cy	30
3c	CbzNHCH$_2$	*n*Pr	IPB	31
3d	CbzNHCH$_2$	*n*Pr	Cy	33
3e	CbzNHCH$_2$	allyl	IPB	24
3f	CbzNHCH$_2$	allyl	Cy	27

It is also well known from the literature, that not only ammonia is a problematic component in Ugi-type MCRs, but also selenoaldehydes as required for selenocysteine (Sec) peptides do not react properly.[9,29] However, in synthesis of the analogous Se-methionine derivatives, the problem did not

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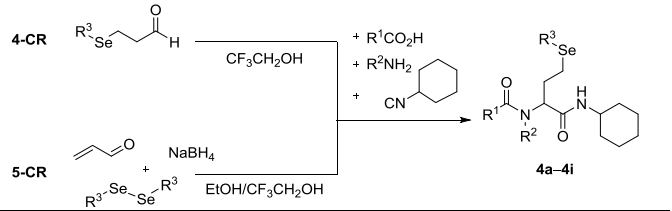
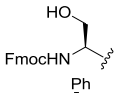
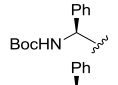
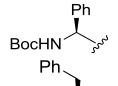
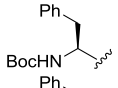
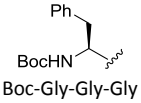
emerge, and both routes, a 4CR with a selenopropanal and the 5CR variant starting from simpler commercial building blocks worked out well (Table 4). Although the reaction yields of Ugi 5CRs with selenides appear modest at best this has to be put into perspective. While the 4CR version seems to give better yields, it should be considered that extra reactions and purifications are required to form the sensitive and smelly selenopropanals and thus the overall efficiency is not better at all. The model system (Table 1) shows that improving this Ugi-5CRs yields beyond the readily accessible 30-60% requires individual finetuning of conditions, because the very reactive component acrolein has to react with S/Se, N- and isonitrile nucleophiles in just the right regioselectivity and order. Alternative classical syntheses (Strecker etc.) additionally lack the flexibility to simultaneously incorporate peptide building blocks as amine or acid component (Table 4, entries **4b-e**) or ligation handles like IPB.[26,30] In this context, considering the formation of 5 new bonds in one step combined with the peptide ligation potential, the modest yields of the Se-Ugi-5CR become acceptable. As expected, even with chiral building blocks like in **4c-g**, no diastereoselection was observed. In fact, Ugi reactions are notoriously resistant to enantioselective synthesis, and so far no general method exists to reliable achieve even modest ee or de.[13] Fortunately, at least in nutrition, racemates of (Se-)methionine are as valuable as single enantiomers.[31]

Table 4. Ugi-5CR versus Ugi-4CR: ammonia/aniline and selenoaldehyde components



No.	R^1	R^2	R^3	Yield (%) 4CR	Yield (%) 5CR
4a	Me	Ph	Ph	48	27
4b	$BocNHCH_2$	H	Me	30	34
4c		Ph	Ph	45	38
4d		H	Me	40	33
4e		H	Ph	43	36
4f		H	Me	64	55
4g		H	Ph	40	28
4h	Boc-Gly-Gly-Gly	H	Me	44	41
4i	Boc-Gly-Gly-Gly	H	Ph	35	31

To explore the toxicity of the obtained Se-methionine derivatives and peptides, three compounds were selected as model. First, unprotected and protected **4b**, and **4f** were tested against SW480 colon cancer cells using the XTT viability assay (24, 48 and 72 h). All three compounds were found to be non-toxic in the investigated dose range and period. Even the highest applied concentration (50 µM) did not affect cell viability (see Figure S1).

The various selenoproteins in mammals differentially respond to changes in selenium supply and compound type, with the oxidative stress protective protein GPx1 being the most sensitively responding selenoprotein.[32–34] Therefore, GPx1 mRNA expression in the human colon carcinoma cell line SW480 was analysed to quantify the effects of supplementation with the selected Se-methionine derivatives (unprotected and protected **4b**, and **4f**) and to compare their efficacy (see Figure S2). GPx1 mRNA expression levels were affected by both the dose and form of selenium. GPx1 mRNA was generally more expressed in the presence of low doses (5 µM) of unprotected and protected **4b** as well as **4f** after 72 h of action, i.e. more than untreated reference or than found for selenate or free Se-methionine, but less than under high selenite (see Figure S2). In mouse models, selenium-enriched foods (e.g. selenium enriched milk) exhibited cytoprotective properties on colon tumorigenesis due to increased GPx activity.[33] The herein synthesized compounds were of low toxicity, but equally effective in enhancing the selenium status, in particular the expression of genes encoding for a crucial selenoprotein (GPx1).

In this study we report the efficient, direct multi-component synthesis of various methionine and Se-methionine derivatives and small peptides from five commercial components in one pot, i.e. through the Ugi-5CRs. Noteworthy, the Se-methionine derivatives had no short-term cytotoxicity and thus are suitable for feeding and introduction as labels in cell based protein biosynthesis. In addition, they enhance the gene expression of the anti-oxidant selenoprotein Gpx1. Furthermore, this method allows to incorporate Se-methionine into proteins and peptides by chemical synthesis using one of our previously published Ugi ligation methods,[26,30] and it likely can be extended to other nucleophiles than thiols or selenols, e.g. such bearing labels or other groups relevant for applications, e.g. in cell biology.

This research was funded by the German Science Foundation (DFG grant numbers: LI1527/3-1, WE1467/13-1 and MU3275/3-1).

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