Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 1063–1066
Bioorganic & Medicinal Chemistry Letters

Acylguanidine inhibitors of β-secretase: Optimization of the pyrrole ring substituents extending into the S_1 and S_3 substrate binding pockets

Derek C. Cole,[a,*] Joseph R. Stock,[a] Rajiv Chopra,[b] Rebecca Cowling,[a] John W. Ellingboe,[a] Kristi Y. Fan,[c] Boyd L. Harrison,[c] Yun Hu,[d] Steve Jacobsen,[d] Lee D. Jennings,[a] Guixian Jin,[a] Peter A. Lohse,[e] Michael S. Malamas,[c] Eric S. Manas,[f] William J. Moore,[f] Mary-Margaret O'Donnell,[e] Andrea M. Olland,[b] Albert J. Robichaud,[c] Kristine Svenson,[b] JunJun Wu,[b] Eric Wagner[d] and Jonathan Bard[d]

[a]*Chemical & Screening Sciences, Wyeth Research, 401 N. Middletown Road, Pearl River, NY 10965, USA*
[b]*Chemical & Screening Sciences, Wyeth Research, 200 Cambridge Park Drive, Cambridge, MA 02140, USA*
[c]*Chemical & Screening Sciences, Wyeth Research, 865 Ridge Road, Monmouth Junction, NJ 08852, USA*
[d]*Discovery Neuroscience, Wyeth Research, 865 Ridge Road, Monmouth Junction, NJ 08852, USA*
[e]*ArQule, 19 Presidential Way, Woburn, MA 01801, USA*
[f]*Chemical & Screening Sciences, Wyeth Research, 500 Arcola Road, Collegeville, PA 19426, USA*

Received 22 October 2007; revised 5 December 2007; accepted 6 December 2007
Available online 26 December 2007

Abstract—Proteolytic cleavage of amyloid precursor protein by β-secretase (BACE-1) and γ-secretase leads to formation of β-amyloid (Aβ) a key component of amyloid plaques, which are considered the hallmark of Alzheimer's disease. Small molecule inhibitors of BACE-1 may reduce levels of Aβ and thus have therapeutic potential for treating Alzheimer's disease. We recently reported the identification of a novel small molecule BACE-1 inhibitor *N*-[2-(2,5-diphenyl-pyrrol-1-yl)-acetyl]guanidine (**3.a.1**). We report here the initial hit-to-lead optimization of this hit and the SAR around the aryl groups occupying the S_1 and $S_{2'}$ pockets leading to submicromolar BACE-1 inhibitors.

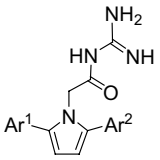
Alzheimer's disease (AD) is a progressive neurodegenerative disease that is the leading cause of dementia.[1] Varying estimates suggest that from 5% to 50% of early onset AD cases result from a variety of genetic mutations, meaning the remainder are sporadic with the risk increasing with age.[2] β-Amyloid plaques are one of the key hallmarks of AD and are formed by aggregation of amyloid fibrils, which in turn are formed from the neurotoxic amyloid β-peptide (β-amyloid, $A\beta_{40,42}$). Proteolytic cleavage of amyloid precursor protein (APP) by β-secretase (also referred to as β-site APP cleaving enzyme-1, BACE-1, Asp-2, and memapsin-2) generates the membrane-bound β-C-terminal fragment (βCTF, also referred to as C99) which in turn is cleaved by γ-secretase to generate Aβ.[3–5] Thus, BACE-1 inhibitors may decrease levels of Aβ and have therapeutic benefit in the treatment of AD.[6,7] A high throughput screening campaign identified *N*-[2-(2,5-diphenyl-pyrrol-1-yl)-acetyl]guanidine **3.a.1** (Ar^1 & Ar^2 = Ph, Table 1) as an inhibitor of BACE-1 with low micromolar activity (BACE-1 IC_{50} = 3.7 μM, K_d = 2.8 μM).[8] Compound **3.a.1** inhibited Aβ formation in a cellular assay with an IC_{50} of 8.9 μM and consistent with a BACE-1 mediated inhibition mechanism caused a dose dependent reduction of βCTF and Aβ levels in a radiolabeled immunoprecipitation assay without affecting α-secretase amyloid precursor protein (α-sAPP) levels.[8] Compound **3.a.1** was selective for BACE-1 over cathepsin D (IC_{50} = 60 μM), pepsin (IC_{50} > 50 μM), and BACE-2 (IC_{50} > 50 μM).

An X-ray structure of **3.a.1** with BACE-1 was solved and revealed key binding interactions between the

Keywords: BACE-1; β-Secretase; Inhibitor; Acylguanidine.
* Corresponding author. Tel.: +1 845 602 4619; fax: +1 845 602 5561; e-mail: coledc@wyeth.com

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.12.010

Table 1. BACE-1 inhibitory activities of compounds **3.(a–l).(1–13)**



Ar^1	Ar^2 Inhibition (%) at 10 μM/IC_{50} (μM)	**a** Ph	**b** 2-Cl-Ph	**c** 2-MeO-Ph	**d** 3-F-Ph	**e** 3-Cl-Ph	**f** 3-MeO-Ph	**g** 3-CN-Ph	**h** 4-F-Ph	**i** 4-MeO-Ph	**j** 4-CF3-Ph	**k** 1-Naphthyl	**l** 3-Benzothienyl
1	Ph	**3.7**		51%	65%		78%	34%	66%		63%	56%	
2	2-Cl-Ph	**3**	56%	62%	69%		39%	52%	**2**	85%	81%	35%	40%
3	3-Cl-Ph	69%	58%	51%	56%		37%	38%	66%	59%	75%	34%	28%
4	3-Br-Ph	68%	69%	46%	47%			41%	63%	66%	64%	25%	9%
5	3-Me-Ph	**4.6**	70%	56%	63%		57%	22%	72%	41%	71%	37%	38%
6	4-Me-Ph	75%	43%	48%	70%		62%	3%	65%	39%	66%	61%	
7	4-MeO-Ph	53%		32%	58%		61%	23%	53%	12%	43%	9%	16%
8	2,5-di-Cl-Ph	3.5	48%	78%	68%		65%	60%	**2**	83%	92%	51%	
9	4-PhO-Ph	**1.3**	**1.1**	**1.3**	**1.4**	**1.3**	**1.5**	**0.6**	**1.4**	**0.5**	**0.6**		**0.7**
10	4-(4-Ac-PhO)-Ph	**1.0**	**0.7**	**1.6**	**1.2**		**1.3**	**0.8**	**1.3**	**0.6**	**0.9**		
11	4-BnO-Ph	64%	84%		58%		79%	41%	47%		25%		7%
12	2-Naph	56%	70%	53%	50%		64%	39%	50%	44%	72%	29%	40%
13	6-Me-2-Naph	60%	84%	53%	61%		79%	46%	58%	17%	49%		

acylguanidine moiety and the two catalytic aspartic acids, Asp32 and Asp228 (Fig. 1).[8–10] The pyrrole ring points toward the flap region, making a π-edge stacking interaction with Tyr71 and stabilizing the flap in an 'open' conformation relative to published peptidomimetic co-structures.[11] The two phenyl groups on the pyrrole ring extend into the S_1 and $S_{2'}$ substrate binding pockets. The *para*-position of the P_1 phenyl group projects directly toward the unoccupied S_3 pocket, indicating an opportunity to add substituents to the P_1 phenyl extending into the S_3 pocket and thereby potentially increasing binding affinity. This manuscript describes the initial hit-to-lead optimization of this series of BACE-1 inhibitors.

Initial SAR investigation of this lead began with the synthesis and assay of a 12×13 combinatorial library. The selection of target molecules was carried out interactively with molecular modelers and was based on the anticipated ability of different substituent groups to be accommodated into the S_1–S_3 and/or $S_{2'}$ pockets. Analogs were synthesized from α-bromomethyl ketones and methyl ketones as starting materials as shown in Scheme 1.

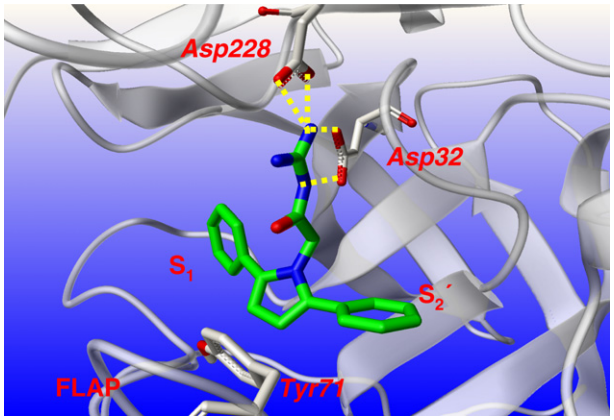
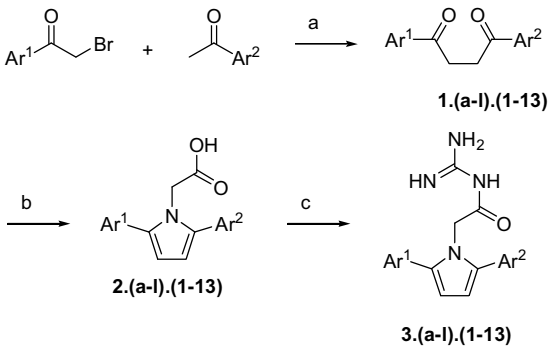


Figure 1. Complex of **3.a.1** with BACE-1 showing hydrogen bonding interactions between the acylguanidine moiety and Asp32 and Asp228 , the π-edge stacking interaction between the pyrrole ring and Tyr71 and the two phenyl groups projecting into S_1 and $S_{2'}$.[10]

The 1,4-diarylbutane-1,4-diones **1.(a–l).(1–13)** were prepared in a one-step cross-coupling reaction under the action of $ZnCl_2 \cdot t\text{-BuOH} \cdot Et_2NH$ using the procedure of Kulinkovich.[12] Reactions were carried out over 3–5 days at ambient temperature and gave modest yields of the diketones (36–50%). The crude diketones were coupled with glycine in an acid catalyzed condensation and subsequent CDI mediated coupling of the resultant pyrrole acetic acids with guanidine hydrochloride gave the target acylguanidines.

All compounds were assayed for BACE-1 inhibition in a fluorescence resonance energy transfer (FRET) assay[8] at a concentration of 10 μM, and IC_{50}'s were determined



Scheme 1. Reagents and conditions: (a) Et_2NH, *t*-BuOH, $ZnCl_2$, toluene, rt, 2–5 days; (b) glycine, *p*-TSA, EtOH, 80 °C, 3 days; (c) i—CDI, DMF, rt, 1 h; ii—guanidine·HCl, Et_3N, rt, 5 h.

for compounds showing >80% inhibition (Table 1). For acyl guanidines with smaller phenyl ring substituents (Table 1, rows 1–8), BACE-1 inhibition activity was not appreciably higher than for **3.a.1** (3.7 μM, 66% inhibition @10 μM). Because of the pseudo-symmetry of molecules with smaller phenyl ring substituents, the phenyl rings may be accommodated in either the S_1 and $S_{2'}$ pockets making interpretation of the SAR difficult. Improved potency was seen in all examples with the 4-phenoxyphenyl and 4-(4-acylphenoxy)phenyl substituent groups (Table 1, rows 9 and 10). These functional groups were included in the library design with the expectation that the phenoxy group on the P_1 phenyl would extend into the S_3 pocket. *para*-Benzyloxyphenyl analogs were poorly tolerated indicating this group may be too large to be accommodated (Table 1, row 11). Similarly the bicyclic ring systems were poorly tolerated (Table 1, row 12 and 13, and columns k and l). When Ar^1 is 4-(PhO)Ph or 4-(AcPhO)-Ph, the arylethers are locked into the S_1–S_3 pocket facilitating interpretation of the Ar^2 SAR. Somewhat surprisingly, no clear preference for *ortho*, *meta*, or *para*-substitution of the $P_{2'}$ phenyl was observed in this library (see Table 1, rows 9 and 10).

A co-crystal structure of compound **3.b.10** (BACE-1 IC_{50} = 0.7 μM), with BACE-1 (Fig. 2) shows the acylguanidine forming key H-bonding interactions with the two aspartic acids and the pyrrole ring π-stacking with the Tyr71 of the flap region.[10] The 4-(4-acetylphenoxy)-phenyl group occupies the S_1 and S_3 substrate binding pockets as predicted by our models and the 2-chloro group on the $P_{2'}$ phenyl is oriented toward the back of the $S_{2'}$ pocket, although this substituent does not appear to contribute significantly to potency (compare **3.a.10** and **3.b.10**, Table 1).

Because of the significant increase in potency observed for acyl guanidines with 4-phenoxy substituents on the P_1 phenyl, additional analogs were designed to further investigate substituents projecting into the S_3 pocket. *Meta* and *para*-acylaminophenyl and sulfonylaminophenyl (**4**–**7**), and *meta* and *para*-alkyloxyphenyl substituents (**8**–**9**) were prepared by modifications of the general route outlined in Scheme 1 (see Scheme 2).

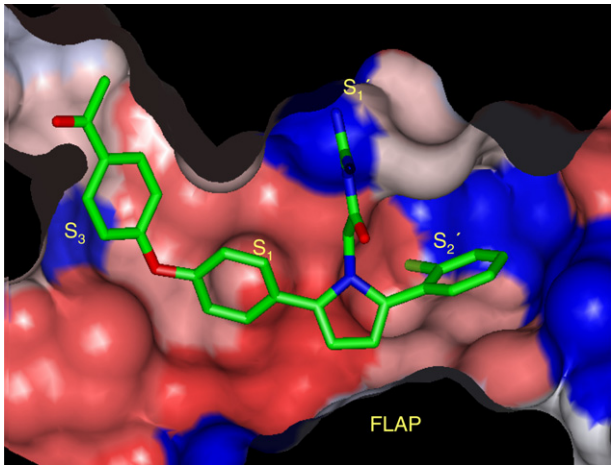
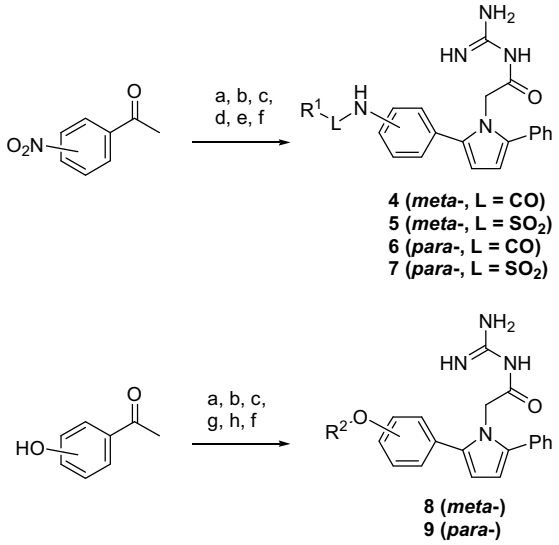


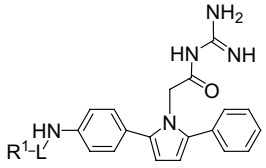
Figure 2. Complex of **3.b.10** with BACE-1. Polar/charged residues are shown in blue and lipophilic residues are shown in red.[10]



Scheme 2. Reagents and conditions: (a) Et_2NH, *t*-BuOH, $ZnCl_2$, toluene, rt, 2–5 days; (b) glycine, AcOH, 120 °C, 5 h; (c) TMSCl, MeOH, 70 °C, 2 h; (d) 10% Pd/C, MeOH–THF, 1 h; (e) R^1COCl or R^1SO_2Cl, Et_3N, DCM, 16 h; (f) guanidine, DMSO, rt, 2 h; (g) BBr_3, DCM, −78 °C, then rt, 5 h; (h) R^2Br, K_2CO_3, DMF, 65 °C, 16 h.

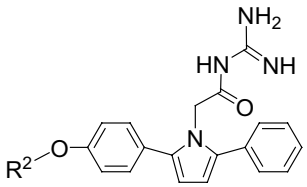
The amides and sulfonamides linked to the P_1 phenyl at the *meta*-position (**4** and **5**) all had weak activity (data not shown), while the *para*-alkyl, heteroaryl, and arylamides (Table 2, **6a**–**j**) generally had low μM activity with **6g** being the most potent analog (IC_{50} = 0.6 μM). The corresponding *para*-sulfonamides (**7**) had much weaker activity. Similarly, the set of *meta*-alkoxyphenyl analogs (**8**) had low activity (data not shown) while the *para*-alkoxyphenyl analogs (Table 3, **9a**–**g**) were surprisingly

Table 2. BACE-1 inhibitory activities of compounds **6** and **7**



	R^1	L	% Inhibition at 10 μM/IC_{50} (μM)
6a	Me	–CO–	40%
6b	Et	–CO–	71%
6c	Cyclopropyl	–CO–	42%
6d	*t*-Bu	–CO–	79%
6e	cyclohexyl	–CO–	26%
6f	2,4-di-Cl-Ph	–CO–	**1.2**
6g	3-Br-Ph	–CO–	**0.6**
6h	3-MeO-Ph	–CO–	**1.8**
6i	4-Br-Ph	–CO–	**1.4**
6j	4-F-Bn	–CO–	54%
7a	Me	$–SO_2–$	65%
7b	Et	$–SO_2–$	75%
7c	*n*-Propyl	$–SO_2–$	71%
7d	Bn	$–SO_2–$	86%
7e	3-MeO-Ph	$–SO_2–$	50%
7f	3-Br-Ph	$–SO_2–$	65%
7g	3-Me-Ph	$–SO_2–$	43%

Table 3. BACE-1 inhibitory activities of compounds **9**



	R^2	% Inhibition at 10 μM/IC_{50} (μM)
9a	Et	**1.6**
9b	allyl	**1.7**
9c	Propargyl	83%
9d	3-CN-Propyl	**0.9**
9e	Butyl	**1.8**
9f	4-CN-Bn	**1.9**
9g	Phenethyl	68%

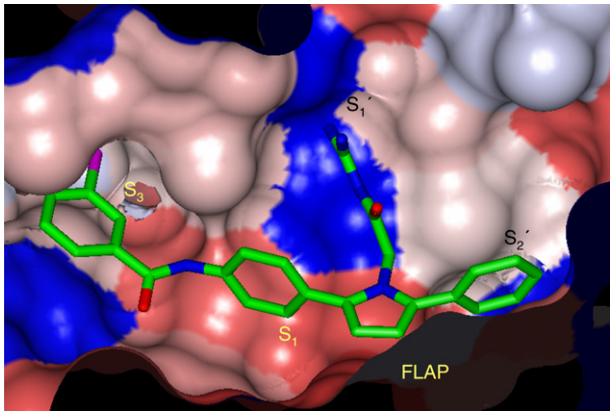


Figure 3. Complex of **6g** with BACE-1. Polar/charged residues are shown in blue and lipophilic residues are shown in red.[10]

active, indicating that sub-μM activity is possible with a smaller alkyl group occupying the S_3 pocket (see **9d**).

The crystal structure of the complex of acylguanidine **6g** with BACE-1 was subsequently solved and revealed that the 3-bromobenzoyl substituent was tightly packed into the S_3 pocket (Fig. 3).[10] Accommodation of this rather large substituent into the pocket was only possible with twisting of the amide bond out of plane with respect to the P_1 group. Presumably this twisting of the amide bond is unfavorable leading to reduced potency. In contrast, a model of **9d** indicates that the cyanopropylether extends into the S_3 pocket with minimal strain achieving similar potency with greater atom efficiency.

In general, these inhibitors are selective for BACE-1 over cathepsin-D (usually >50-fold, although **6g** is only 4-fold) and pepsin (data not shown) and have limited selectivity (<2-fold) over BACE-2[13] (Table 3). Most sub-micromolar BACE-1 inhibitors had low micromolar activity in the cellular $A\beta^{total}$ lowering ELISA (Table 4).

In summary, in this initial hit-to-lead optimization effort we have achieved up to 6-fold increase in potency and demonstrated the ability to extend directly from the S_1 pocket into the S_3 pocket with groups attached via the *para*-position of the P_1 group and with a variety of linkers. Future studies will focus on optimization of the interactions with S_3 while reducing the strain energy of the S_1–S_3 linker, and also incorporation of substituents on the acylguanidine extending into the $S_{1'}$ pocket.

Table 4. IC_{50} values for inhibition of BACE-1, BACE-2, cathepsin-D and cellular Aβ production by **3.b.10** and **6g**

	BACE-1 IC_{50}, μM	BACE-2 IC_{50}, μM	CathD IC_{50}, μM	Cellular $A\beta^{total}$ ED_{50}, μM
3.b.10	0.7	2.3	18.9	3.8
6g	0.6	0.5	2.1	5.6

References and notes

1. Alzheimer's Association. http://www.alz.org.
2. Selkoe, D. J. *Physiol. Rev.* **2001**, *81*, 741.
3. Li, Y. M.; Xu, M.; Lai, M. T.; Huang, Q.; Castro, J. L.; DiMuzio-Mower, J.; Harrison, T.; Lellis, C.; Nadin, A.; Neduvelil, J. G.; Register, R. B.; Sardana, M. K.; Shearman, M. S.; Smith, A. L.; Shi, X. P.; Yin, K. C.; Shafer, J. A.; Gardell, S. J. *Nature* **2000**, *405*, 689.
4. Sinha, S.; Anderson, J. P.; Barbour, R.; Basi, G. S.; Caccavello, R.; Davis, D.; Doan, M.; Dovey, H. F.; Frigon, N.; Hong, J.; Jacobson-Croak, K.; Jewett, N.; Keim, P.; Knops, J.; Lieberburg, I.; Power, M.; Tan, H.; Tatsuno, G.; Tung, J.; Schenk, D.; Seubert, P.; Suomensaari, S. M.; Wang, S.; Walker, D.; Zhao, J.; McConlogue, L.; John, V. *Nature* **1999**, *402*, 537.
5. Wolfe, M. S.; Xia, W.; Ostaszewski, B. L.; Diehl, T. S.; Kimberly, W. T.; Selkoe, D. J. *Nature* **1999**, *398*, 513.
6. Ghosh, A. K.; Bilcer, G.; Hong, L.; Koelsch, G.; Tang, J. *Curr. Alzheimer Res.* **2007**, *4*, 418.
7. For recent reviews of small molecule BACE-1 inhibitors, see: (a) Durham, T. B.; Shepherd, T. A. *Curr. Opin. Drug Discov. Dev.* **2006**, *9*, 776; (b) Hills, I. D.; Vacca, J. P. *Curr. Opin. Drug Discov. Dev.* **2007**, *10*, 383.
8. Cole, D. C.; Manas, E. S.; Stock, J. R.; Condon, J. S.; Jennings, L. D.; Aulabaugh, A.; Chopra, R.; Cowling, R.; Ellingboe, J. W.; Fan, K. Y.; Harrison, B. L.; Hu, Y.; Jacobsen, S.; Jin, G.; Lin, L.; Lovering, F. E.; Malamas, M. S.; Stahl, M. L.; Strand, J.; Sukhdeo, M. N.; Svenson, K.; Turner, M. J.; Wagner, E.; Wu, J.; Zhou, P.; Bard, J. *J. Med. Chem.* **2006**, *49*, 6158.
9. For other BACE-1 inhibitors with basic amidine-like'warheads' see: Gerritz, S.; Zhai, W.; Shi, S.; Zhu, S.; Good, A. C.; Thompson, L. A. III, WO-2007002214 (2007); Mjalli, A. M.; Jones, D.; Gohimmukkula, D. R.; Huang, G.; Zhu, J.; Rao, M.; Andrews, R.C.; Ren, T. WO-2006099379 (2006); Murray, C. W.; Callaghan, O.; Chessari, G.; Cleasby, A.; Congreve, M.; Frederickson, M.; Hartshorn, M. J.; McMenamin, R.; Patel, S.; Wallis, N. *J. Med. Chem.* **2007**, *50*, 1116; Congreve, M.; Aharony, D.; Albert, J.; Callaghan, O.; Campbell, J.; Carr, R. A. E.; Chessari, G.; Cowan, S.; Edwards, P. D.; Frederickson, M.; McMenamin, R.; Murray, C. W.; Patel, S.; Wallis, N. *J. Med. Chem.* **2007**, *50*, 1124.
10. Coordinates for the BACE-1 complexes with **3.a.1**, **3.b.10**, and **6g** have been deposited in the Protein Data Bank under accession codes 2QU2, 2ZDZ, and 2ZE1, together with the corresponding structure.
11. Hong, L.; Koelsch, G.; Lin, X.; Wu, S.; Terzyan, S.; Ghosh, A. K.; Zhang, X. C.; Tang, J. *Science* **2000**, *290*, 150.
12. Nevar, N. M.; Kel'in, A. V.; Kulinkovich, O. G. *Synthesis* **2000**, 1259.
13. Chou, K. C. *J. Proteome Res.* **2004**, *3*, 1069.