Solid-Phase Synthesis of (ω-Aminoalkyl)peptoids Using Azide Chemistry

Daniel Fritz, Stefan Bräse*

Karlsruhe Institute of Technology, Institute of Organic Chemistry, Fritz-Haber-Weg 6, 76131 Karlsruhe, Germany
Fax +49(721)6088581; E-mail: braese@kit.edu

Received 7 March 2010

Abstract: Functionalized (ω-aminoalkyl)peptoids – useful molecular transporters for drugs and probes into cells – have been synthesized by a combined microwave-assisted sub-monomer peptoid synthesis with 1-amino-ω-azidoalkanes and a subsequent reduction of the azide moiety on solid supports. This method enables the preparation of unprecedented chiral amino-functionalized and PEG-type peptoids. Both the scope and limitations of this process are presented herein.

Key words: peptoid, amines, azides, peptidomimetic transporters, solid-phase synthesis, microwave synthesis, protecting groups

Peptoids – oligo(*N*-substituted glycines) – belong to an emerging class of functionalized peptidomimetics.[1] Pioneered by the Zuckermann group,[2] the Bradley group,[3] our group[4] and others, it has been demonstrated that (ω-aminoalkyl)peptoids are useful carriers for drugs and probes into cells.[5,6] Functionalized peptoids are often prepared through classical peptide-like synthesis. Thus, a so-called sub-monomer method[7] is more easily accessible, since the synthesis of the building blocks delivers the sub-monomers in fewer overall steps. In this manuscript, we exploit the sub-monomer method with 1-amino-ω-azidoalkanes (see safety note[8]), followed by the reduction of the azide moieties on the bead (Scheme 1).[9]

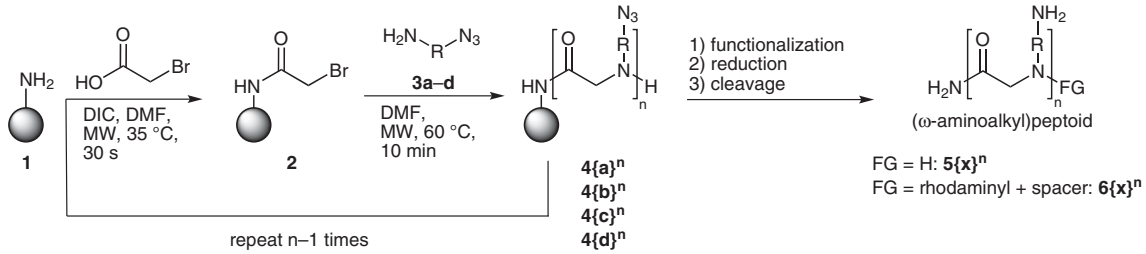
The use of azides in peptoid chemistry has been extensively demonstrated by the Kirshenbaum group[10] and, more recently, by Kodadek et al.[11] Despite the fact that reduction of azides into amines is well established in the literature,[9] to the best of our knowledge the conversion into amines has not been performed for the synthesis of peptoids.

The syntheses of the different 1-amino-ω-azidoalkanes **3a–d** are outlined in Scheme 2. Although 1-amino-6-azidoalkane **3a** is commercially available, it can easily be prepared by selective Staudinger reduction of 1,6-diazidohexane prepared in situ (46% overall yield).[12] Other amino azides **3b–d** were synthesized similarly. The chiral aminoazides **3b** and **3c** were prepared through a sequence of the Sonogashira coupling of enantiomerically pure 3-bromophenylethylamine (obtained from BASF) with 5-chloro-1-pentyne (**9**), subsequent optional reduction of the alkyne function, substitution of the chloride, and protecting group chemistry (54–55% overall yields).

The PEG-type azide[15] **3d** was prepared from triethylene glycol **10** via tosylation, double nucleophilic displacement with sodium azide and subsequent mono-Staudinger reduction (22% overall yield). The aminoazides **3a–d** are stable molecules that can be stored at 0 °C over a prolonged period of time.

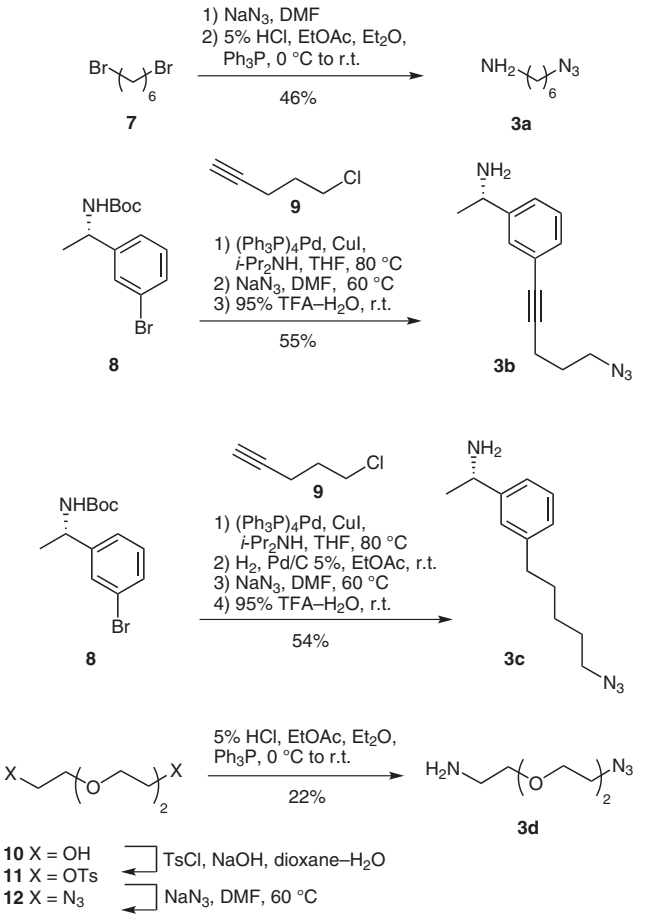
With the aminoazides **3a–d** in hand, various peptoids **4{a}**^{2}, **4{b}**^{3}, **4{c}**^{3}, **4{d}**^{1}, **4{d}**^{6} were assembled using the microwave-assisted protocol described by Gorske et al.:[16b] Rink-amide resin **1** (loading: 0.63 mmol/g), acylation with bromoacetic acid [*N*,*N*′-diisopropylcarbodiimide, *N*,*N*-dimethylformamide (DMF), MW] and subsequent amination with the amines **3a–d** (DMF, MW).[1,6] Microwave irradiation was used to accelerate the heating and reaction times and thus to improve the coupling efficiency of the more hindered α-branched amines **3b** and **3c**.[16] This sequence was repeated until the desired backbone length was reached. The efficiency of the coupling was monitored through cleavage of the azidopeptoids **4{x}**^{n} directly from the solid supports and subsequent analysis by MALDI-TOF mass-spectrometry.

In our case, Staudinger reduction conditions[9] [a) 1–2 M R_3P, THF, 3 h; b) H_2O–THF (1:8), r.t., 60 min] for the

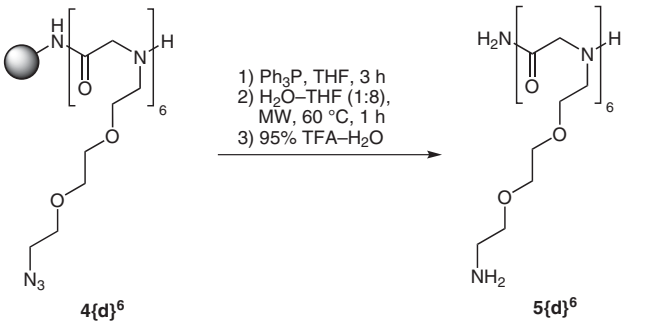


Scheme 1 Generic synthesis of aminoalkylpeptoids **5{x}**^{n} and **6{x}**^{n} (MW = microwave); for details, see Scheme 2

SYNLETT 2010, No. 10, pp 1544–1548
Advanced online publication: 10.05.2010
DOI: 10.1055/s-0029-1219925; Art ID: G08110ST
© Georg Thieme Verlag Stuttgart · New York



Scheme 2 Synthesis of functionalized 1-amino-ω-azidoalkanes **3a–d**[13]



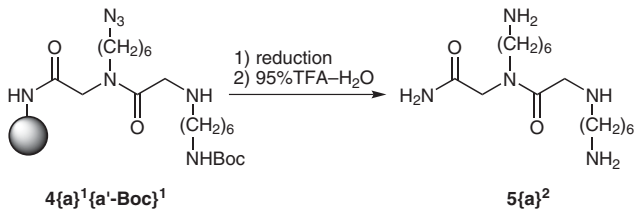
Scheme 3 Example of a synthesis of a functionalized aminopeptoid[14]

reduction of the azido moiety were only successful with hydrophilic and water-soluble azidopeptoids **4{d}^{n}** (Scheme 3). Peptoids having sidechains other than the ethyleneoxy unit, such as the more hydrophobic alkyl or aromatic groups, did not deliver the anticipated amines in acceptable yields. Unreacted starting material and the formation of the corresponding iminophosphorane were detected, but subsequent hydrolysis with water was limited, even after cleavage from the resin. In order to optimize the reduction in a Boc-orthogonal fashion, we screened for optimized reduction conditions, using the dipeptoid system **4{a}^{1}\{a′-Boc\}^{1}** with an azido and a Boc-protected amine moiety (Scheme 4, Table 1).

Table 1 Reduction of Azido Peptoids **4{a}^{1}\{a′-Boc\}^{1}** as Shown in Scheme 4

Entry	Reduction conditions	Temp (°C)	Time (h)	Comment[a]
1	a) Bu_3P, THF b) H_2O–THF (1:8)	r.t. r.t.	3 0.5	 A
2	a) Bu_3P, THF b) H_2O–THF (1:8), MW	r.t. 60	3 0.5	 A
3	a) Ph_3P, THF b) H_2O–THF (1:8)	r.t. r.t.	3 0.5	 A
4	a) Ph_3P, THF b) H_2O–THF (1:8), MW	r.t. 60	3 0.5	 A
5	a) Bu_3P, THF b) H_2O–THF (1:8)	r.t. r.t.	4 12	 A
6	a) Bu_3P, THF b) H_2O–THF (1:8), MW	r.t. 60	4 1	 A
7	a) Bu_3P, THF b) H_2O–DMF (1:4)	r.t. r.t.	4 12	 A
8	a) Bu_3P, THF b) H_2O–DMF (1:4), MW	r.t. 60	4 1	 A
9	a) Bu_3P, THF b) concd NH_4OH–DMF (1:4)	r.t. r.t.	4 12	 A
10	a) Bu_3P, THF b) H_2O–AcOH–DMF (1:2:8), MW	r.t. 60	4 1	 A
11	1 M $SnCl_2$ in DMF, MW	60	1	B
12	1 M $SnCl_2$ in DMF	r.t.	12	B
13	0.1 M $SnCl_2$, 0.4 M PhSH, 0.5 M Et_3N in THF	r.t.	1	C
14	0.1 M $SnCl_2$, 0.4 M PhSH, 0.5 M Et_3N in THF, MW	60	1	C

[a] According to MALDI-TOF analysis. A: No product or low content: Starting material and intermediate iminophosphoranes were strongly detected. B: Product visible but starting material strongly detected. C: Quantitative conversion; no starting material detected.



Scheme 4 Reduction of the (ω-azido)peptoid **4{a}^{1}\{a′-Boc\}^{1}** for screening of conditions as described in Table 1[14]

After this screening, $SnCl_2$ indisputably generated the best results. Standard Staudinger reduction conditions primarily yielded the corresponding iminophosphorane, which could not be hydrolyzed in satisfactory quantities. Changing the solvent system from THF to DMF, heating under microwave irradiation (60 °C), prolonging hydrolysis time, use of alkyl or aryl phosphines and application of

basic or acidic conditions did not significantly improve the hydrolysis.

Better results were achieved by using $SnCl_2$ in DMF. Moreover, more satisfactory results were obtained through the addition of thiophenol and base (Et_3N), leading to the formation of the highly selective Bartra reagent[17] $[Sn(SPh)_3]^-$, which gave good and selective conversion into the amines.

Cleavage from the solid support yielded the (ω-aminoalkyl)peptoids **5{x}^{n}** in generally good purities, according to MALDI-TOF analysis (Table 2, Figure 1).

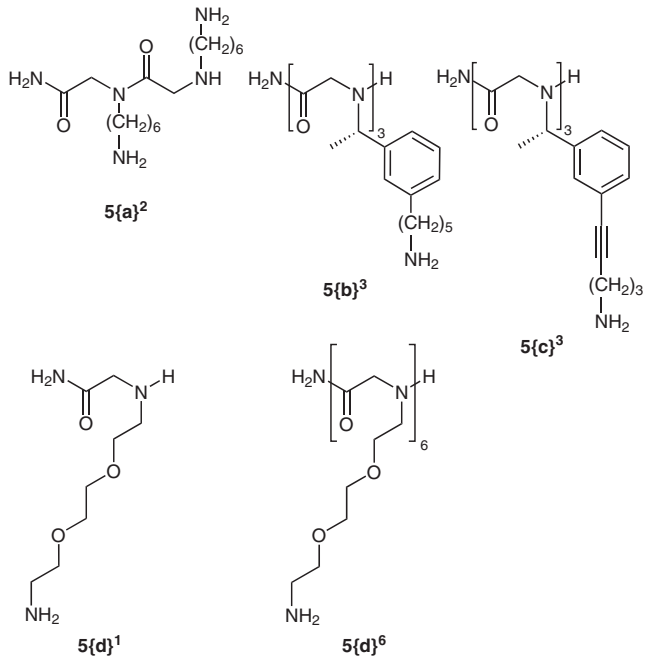


Figure 1 Synthesized peptoids **5{x}^{n}**.[14]

Table 2 Analytical Data of the Synthesized (ω-Aminoalkyl)peptoids

Entry	Peptoid	Amine used in synthesis	*m/z* $[M]^+$ calcd	*m/z* $[M + H]^+$ found[a]
1	**5{a}^{2}**	**3a**	329	330
2	**5{b}^{3}**	**3b**	755	756
3	**5{c}^{3}**	**3c**	743	744
4	**5{d}^{1}**	**3d**	233	234
5	**5{d}^{6}**	**3d**	1145	1146
6	**6{d}^{6}**	**3d**	1684	1684[b]

[a] According to MALDI-TOF analysis.
[b] Found mass: $[M]^+$.

The peptoids **5{b}^{n}** and **5{c}^{n}** represent a new class of chiral amino-functionalized peptoids, which might have an impact in their secondary structure[18] and therewith differ in their properties as molecular transporters.[4] The amino-PEG-type peptoids **5{d}^{n}**, which are unprecedented in structure,[19,20] will be explored for their delivery abilities.

After exploration of the reducing methods, we attached functionalized cargo, such as a rhodamine B dye, to the azidopeptoids. Subsequent reduction [a) Ph_3P, THF, 4 h; b) H_2O–THF (1:8), MW, 60 °C, 30 min] and cleavage from the solid support (95% TFA–H_2O) furnished novel rhodaminyl molecular transporters, for example **6{d}^{6}**, in good purities and overall yields up to 70% (Figure 2).

The sub-monomer method is particularly useful for the generation of highly functionalized side chains. In the case of the chiral protected sub-monomers **3b** and **3c**, at least two steps can be saved and therewith the overall yield is up to 35% higher compared to the synthesis of the corresponding monomers (data not shown).

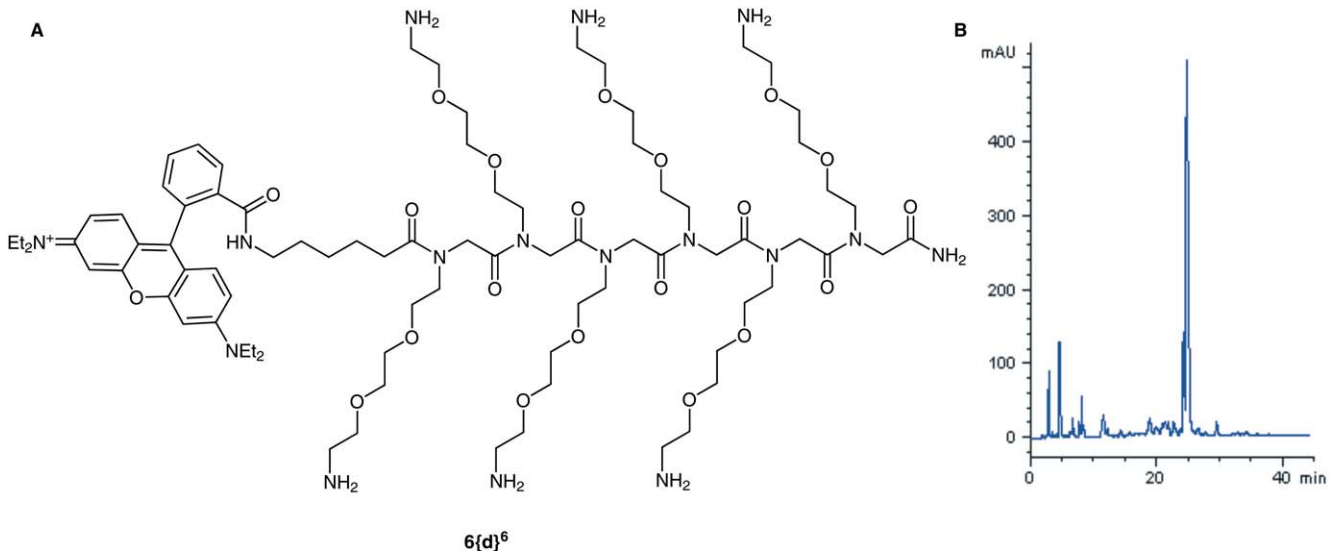


Figure 2 (A) Example of a synthesized aminopeptoid functionalized with the fluorescent dye rhodamine B. Only one rhodamine isomer is shown.[21] (B) HPLC-Chromatogram (A/B = H_2O/MeCN 0.1% TFA; 2 min at 37% B, then 37 to 77% B in 40 min) of crude **6{d}^{6}** at 256 nm.

Introducing ω-aminoalkyl chains into peptoids through the reduction of the ω-azidoalkyl species opens new possibilities for inserting protected amines. This method is orthogonal to the frequently used Boc- and Nosyl-protecting groups. Besides the useful Click reaction, this method can also be an effective tool for creating new bioconjugates, with more than one active element bound through a peptide bond in cell-penetrating transporter systems. Compared to the Boc-, Fmoc- and Nosyl-protected amines so far predominantly used,[1–7] this method is cheaper and, in various cases such as with the chiral peptoids **5{b,c}^{n}**, also leads to the desired product in fewer reaction steps with better overall yields.

In summary, azidopeptoids with hydrophilic side chains can be readily reduced under standard Staudinger conditions, while reduction of more hydrophobic peptoids only generates high yields when using the Bartra reagent for the reduction of the azido moiety. A new class of chiral and amino-PEG-type peptoids has been synthesized through an efficient, combined microwave-assisted submonomer synthesis, starting from 1-amino-ω-azidoalkanes and using the aforementioned reduction conditions on solid supports. Structural and biological studies of these new chiral and achiral molecular transporters will remain our focus in the near future.

Acknowledgment

We acknowledge the financial support of the Evangelisches Studienwerk e.V. Villigst and the DFG-Centre for functionalized nanostructures (CFN, project E1.1). The chiral phenylethylamines were gifts from BASF (Prof. Dr. Ditrich, ChiPros). We greatly appreciate the dedicated help received from Karolin Niessner and Dr. Michael Gartner.

References and Notes

(1) Simon, R. J.; Kania, R. S.; Zuckermann, R. N.; Huebner, V. D.; Jewell, D. A.; Banville, S.; Ng, S.; Wang, L.; Rosenberg, S.; Marlowe, C. K. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 9367.

(2) Zuckermann, R.; Dubois-Stringfellow, N.; Dwarki, V.; Innis, M. A.; Murphy, J. E.; Cohen, F.; Tetsuo, U. (Chiron Corporation, USA) PCT Int. Appl. WO 9806437, **1998**.

(3) (a) Peretto, I.; Sanchez-Martin, R. M.; Wang, X.-h.; Ellard, J.; Mittoo, S.; Bradley, M. *Chem. Commun.* **2003**, 2312. (b) Diaz-Mochon, J. J.; Fara, M. A.; Sanchez-Martin, R. M.; Bradley, M. *Tetrahedron Lett.* **2008**, *49*, 923. (c) Fara, M. A.; Diaz-Mochon, J. J.; Bradley, M. *Tetrahedron Lett.* **2006**, *47*, 1011.

(4) (a) Schröder, T.; Niemeier, N.; Afonin, S.; Ulrich, A. S.; Krug, H. F.; Bräse, S. *J. Med. Chem.* **2008**, *51*, 376. (b) Schröder, T.; Schmitz, K.; Niemeier, N.; Balaban, T. S.; Krug, H. F.; Schepers, U.; Bräse, S. *Bioconjugate Chem.* **2007**, *18*, 342.

(5) Tan, N. C.; Yu, P.; Kwon, Y.-U.; Kodadek, T. *Bioorg. Med. Chem.* **2008**, *16*, 5853.

(6) For a different approach, see: Hahn, F.; Schmitz, K.; Balaban, T. S.; Bräse, S.; Schepers, U. *ChemMedChem* **2008**, *3*, 1185.

(7) Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H. *J. Am. Chem. Soc.* **1992**, *114*, 10646.

(8) CAUTION: Short chained 1-halo-ω-azidoalkanes might be explosive. We recommend the use of alkanes with at least six carbon atoms in case diazidoalkanes are formed. For a discussion, see ref. 9

(9) For a review concerning azide chemistry, see: Bräse, S.; Gil, C.; Knepper, K.; Zimmermann, V. *Angew. Chem. Int. Ed.* **2005**, *44*, 5188; *Angew. Chem.* **2005**, *117*, 5320.

(10) (a) Jang, H.; Fafarman, A.; Holub, J. M.; Kirshenbaum, K. *Org. Lett.* **2005**, *7*, 1951. (b) Holub, J. M.; Jang, H.; Kirshenbaum, K. *Org. Biomol. Chem.* **2006**, *4*, 1497. (c) Shin, S. B. Y.; Yoo, B.; Todaro, L. J.; Kirshenbaum, K. *J. Am. Chem. Soc.* **2007**, *129*, 3218. (d) Holub, J. M.; Jang, H.; Kirshenbaum, K. *Org. Lett.* **2007**, *9*, 3275. (e) Holub, J. M.; Garabedian, M. J.; Kirshenbaum, K. *QSAR Comb. Sci.* **2007**, *26*, 1175. (f) Shah, N. H.; Kirshenbaum, K. *Macromol. Rapid Commun.* **2008**, *29*, 1134.

(11) (a) Lim, H.-S.; Archer, C. T.; Kim, Y.-C.; Hutchens, T.; Kodadek, T. *Chem. Commun.* **2008**, 1064. (b) See also: Disney, M. D. PCT Int. Appl. WO 20081034898, **2008**.

(12) Lee, J. W.; Jun, S. I.; Kim, K. *Tetrahedron Lett.* **2001**, *42*, 2709.

(13) Analytical data for selected products: (*S*)-1-[3-(5-Azidopentyl)phenyl]ethylamine (**3b**): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.41 (tt, *J* = 7.2, 7.2 Hz, 2 H, $N_3CH_2CH_2CH_2$), 1.44 (d, *J* = 6.4 Hz, 3 H, $CHCH_3$), 1.62 (tt, *J* = 7.6, 7.2 Hz, 2 H, CH_2CH_2-Ar), 1.63 (tt, *J* = 7.2, 7.2 Hz, 2 H, $CH_2CH_2N_3$), 2.60 (t, *J* = 7.6 Hz, 2 H, CH_2-Ar), 3.25 (t, *J* = 7.2 Hz, 2 H, N_3CH_2), 4.12 (q, *J* = 6.4 Hz, 2 H, $CHCH_3$), 4.52 (br s, 2 H, NH_2), 7.08 (d, *J* = 7.2 Hz, 1 H, Ar_6-*H*), 7.14 (s, 1 H, Ar_2-*H*), 7.15 (d, *J* = 7.2 Hz, 1 H, Ar_4-*H*), 7.24 (dd, *J* = 7.2, 7.2 Hz, 1 H, Ar_5-*H*) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 23.4 ($CHCH_3$), 26.4 ($N_3CH_2CH_2CH_2$), 28.7 ($N_3CH_2CH_2$), 30.9 (CH_2CH_2-Ar), 35.7 (CH_2-Ar), 51.3 (N_3CH_2), 51.4 (*C*H), 123.5 (C_6-Ar-H), 126.2 (C_4-Ar-H), 127.8 (C_2-Ar-H), 128.7 (C_5-Ar-H), 142.9 [C_q-Ar(CH_2)], 143.5 [C_q-Ar(CH)] ppm. FTIR (film on KBr): 2936, 2860, 2097, 1678, 1542, 1490, 1452, 1385, 1256, 1203, 1136, 897, 835, 799, 721, 706, 558, 461 cm^{-1}; MS (FAB, matrix: *m*NBA): *m/z* (%) = 233(100) $[M + H]^+$, 205(6) $[M – N_2]^+$; HRMS: *m/z* calcd: 233.1766; found: 233.1769. (*S*)-1-[3-(5-Azidopent-1-enyl)phenyl]-ethylamine (**3c**): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.38 (d, *J* = 6.8 Hz, 3 H, $NCHCH_3$), 1.87 (tt, *J* = 6.8, 6.8 Hz, 2 H, $CH_2CH_2CH_2$), 2.44 (t, *J* = 6.8 Hz, 2 H, $CH_2C{\equiv}C$), 3.91 (t, *J* = 6.8 Hz, 2 H, N_3CH_2), 4.04 (q, *J* = 6.8 Hz, 1 H, $CHCH_3$), 5.62 (br s, 2 H, NH_2), 7.18 (dd, *J* = 4.0, 3.6 Hz, 1 H, Ar_5-*H*), 7.18 (d, *J* = 4.0 Hz, 1 H, Ar_6-*H*), 7.20–7.28 (m, 1 H, Ar_4-*H*), 7.31 (s, 1 H, Ar_2-*H*) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 16.7 ($CH_2C{\equiv}C$), 21.9 ($CHCH_3$), 27.8 (N_3CH_2), 50.2 ($CH_2CH_2CH_2$), 51.2 (*C*H), 81.1 (C≡*C*-Ar), 88.8 (*C*≡C-Ar), 124.2 [C_q-Ar(C≡C)], 125.8 (C_6-Ar-H), 128.9 (C_5-Ar-H), 129.5 (C_4-Ar-H), 131.4 (C_2-Ar-H), 141.3 [C_q-Ar(CH)] ppm. FTIR (film on KBr): 2932 (m), 2099, 1678, 1602, 1483, 1430, 1367, 1293, 1256, 1202, 1134, 897, 834, 798, 721, 700, 476; MS (FAB, matrix: *m*NBA): *m/z* (%) = 229(50)$[M + H]^+$, 212(100) $[M – NH_2]^+$; HRMS: *m/z* calcd: 229.1453; found: 229.1449. 2-[2-(2-Azidoethoxy)ethoxy]ethylamine (**3d**): Analytical data are consistent with those reported by: Klein, E.; DeBonis, S.; Thiede, B.; Skoufias, D. A.; Kozielski, F.; Lebeau, L. *Bioorg. Med. Chem.* **2007**, 6474.

(14) The final products were isolated as ammonium trifluoroacetate salts.

(15) For a different approach, see: (a) Natarajan, A.; Du, W.; Xiong, C.-Y.; DeNardo, G. L.; DeNardo, S. J.; Gervay-Hague, J. *Chem. Commun.* **2007**, 695. (b) Feau, C.; Klein, E.; Kerth, P.; Lebeau, L. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1499.

(16) (a) Olivos, H. J.; Alluri, P. G.; Reddy, M. M.; Salony, D.; Kodadek, T. *Org. Lett.* **2002**, *4*, 4057. (b) Gorske, B. C.; Jewell, S. A.; Guerard, E. J.; Blackwell, H. E. *Org. Lett.* **2005**, *7*, 1521.

(17) (a) Bartra, M.; Urpí, F.; Vilarrasa, J. *Tetrahedron Lett.* **1987**, *28*, 5941. (b) For general scope and limitation of this reagent, see: Bartra, M.; Romea, P.; Urpí, F.; Vilarrasa, J. *Tetrahedron* **1990**, *46*, 587. (c) For its application on solid supports, see: Zimmermann, V.; Avemaria, F.; Bräse, S. *J. Comb. Chem.* **2007**, *9*, 200.

(18) Kirshenbaum, K.; Barron, A. E.; Goldsmith, R. A.; Armand, P.; Bradley, E. K.; Truong, K. T. V.; Dill, K. A.; Cohen, F. E.; Zuckermann, R. N. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 4303.

(19) For the only report of PEG-type side chains in peptoids according to CAS, see: Barron, A. E. PCT Int. Appl. WO 2000043547, **2000**.

(20) Alkoxyethyl peptoids displaying PEG-like properties such as resistance to biofouling are known, for examples, see: (a) Kim, J. M.; Roy, R. *Carbohydr. Lett.* **1996**, *1*, 465. (b) Huang, C.-Y.; Uno, T.; Murphy, J. E.; Lee, S.; Hamer, J. D.; Escobedo, J. A.; Cohen, F. E.; Radhakrishnan, R.; Dwarki, V.; Zuckermann, R. N. *Chem. Biol.* **1998**, *5*, 345. (c) Statz, A. R.; Park, J. P.; Chongsiriwatana, N. P.; Barron, A. E.; Messersmith, P. B. *Biofouling* **2008**, *24*, 439. (d) Statz, A. R.; Barron, A. E.; Messersmith, P. B. *Soft Matter* **2008**, *4*, 131. (e) Messersmith, P. B.; Barron, A. E.; Statz, A.; Chongsiriwatana, N. P. PCT Int. Appl. WO 2010014940, **2010**.

(21) Adamczyk, M.; Grote, J. *Synth. Commun.* **2001**, *31*, 2681.

Copyright of Synlett is the property of Georg Thieme Verlag Stuttgart and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.