Efficient microwave-assisted synthesis of multivalent dendrimeric peptides using cycloaddition reaction (click) chemistry†

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Multivalent dendrimeric peptides were synthesized *via* a microwave-assisted Huisgen 1,3-dipolar cycloaddition between azido peptides and dendrimeric alkynes in yields ranging from 46 to 96%.

Dendrimers are versatile constructs for the simultaneous presentation of especially biologically relevant ligands. These multivalent constructs are especially interesting for enhancing the interaction of weakly interacting individual ligands *e.g.* carbohydrates.[1] We have successfully explored this in the design, synthesis and biological evaluation of dendrimeric carbohydrates.[2] In addition, dendrimers might serve as promising molecular scaffolds for increasing effects merely by offering a number of ligands or by aligning these ligands.[3]

A crucial issue is the complete and efficient attachment of ligands to dendrimers. In the completely peptide-based dendrimers this was often achieved using peptide coupling chemistry.[3a] Nevertheless, in most cases peptides are attached to dendrimers by chemoselective reaction of sulfhydryl groups of peptides with maleimide or iodoacetamide functionalities.[4] However, new reactions with increased efficiency and chemoselectivity (preferably at physiological conditions) would be very welcome. The reaction between an acetylene and organic azides yielding the corresponding 1,4-disubstituted 1,2,3-triazoles, which was originally discovered by Huisgen,[5] and recently reinvestigated independently by Meldal[6] and Sharpless,[7] seems particularly suitable for chemoselective bioconjugation reactions.[8]

Here we show that peptides can be efficiently attached to a derivatized version of our earlier developed dendrimers[9] using a 1,3-dipolar cycloaddition ('click'-chemistry[10]), which was conveniently assisted by microwave irradiation[11] to ensure a complete modification of the alkyne endgroups. Thus we describe the synthesis of the required dendrimeric alkynes used in the attachment of azido peptides[12] either derived from the α-amino group, ε-amino group of lysine or an ω-aminohexanoyl spacer to obtain di-, tetra-, octa- and hexadecavalent dendrimeric peptides.

Our earlier developed approach for the convergent synthesis of amino acid based dendrimers[9,13] was adapted in order to obtain dendrimers with surface propargyl groups to enable a 1,3-dipolar cycloaddition ('click') reaction with amino acid/peptide derived azides. First generation dendrimer **1** was synthesized from 3,5-dihydroxybenzoic acid in an overall yield of 77% as shown in Fig. 1. Methyl ester **1** was saponified with Tesser's base[14] to obtain acid **2** and subsequently used in the synthesis of the second, third and fourth generation dendrimers **3**, **4** and **5**, respectively in 75–84% yields (Fig. 1). The azido peptides **6–14** used in this study were synthesized according to literature procedures[12] (Fig. 2).

Azide **6** was mixed with acetylene **1** in the presence of $CuSO_4$/Na-ascorbate/Cu-wire in different solvent systems for 16 h at room temperature. Monitoring by TLC showed that formation of the monovalent cycloadduct proceeded rapidly, and conversion to the divalent product was sluggish. Despite this **15** was isolated in fair yields (43–56%). A tremendous improvement was achieved by running this reaction under microwave irradiation. After 10 min at 100 °C using THF/H_2O (1/1) as a solvent in the presence of $CuSO_4$/Na-ascorbate, **15** was isolated in 93% yield (Fig. 2). A slight further improvement of the yield to 96% was achieved by performing the reaction in aqueous DMF.

Using the former solvent systems, divalent peptide cycloadducts **19–22** (Fig. 2) were obtained in fair to good yields (48–72%). The lower yields may be explained by the (significantly) lower solubility of **19–22** compared to **15**.

Encouraged by these results amino acid azide **6** and peptide azide **10**, respectively, were reacted with second generation dendrimer **3** for 5–10 min at 100 °C. Amino acid azide **6** gave a precipitate which was only soluble in DMF or DMSO and was characterized (^{1}H-, ^{13}C-NMR (APT, HMBC and HSQC), ESMS and MALDI-TOF) as tetravalent amino acid cycloadduct **16**‡ and obtained in excellent yield (91%). Tetravalent peptide cycloadduct **23** was isolated by extraction with EtOAc and obtained in a fair yield of 63% after crystallization from EtOAc/hexane. Even more rewarding was the successful preparation, using microwave irradiation, of octavalent peptide **24** (48%) as well as octavalent and hexadecavalent systems **17** and **18** in 69 and 94% yield, respectively (Fig. 2).

Now the stage was set for the next challenge involving attachment of larger or bioactive peptides to these dendrimeric systems using this microwave-assisted cycloaddition chemistry. In view of our interest in antimicrobial peptides, azidopeptide **11**, representing the amino acid residues 12–23 of the antimicrobial peptide magainin I amide[15] was coupled under microwave assistance and divalent cycloadduct **25** was obtained in nearly quantitative yield (Fig. 2). Work-up of this water-soluble divalent peptide was easy as also was purification by HPLC or size exclusion chromatography. Then, azido-Leu-enkephalin (**12**), azide **13** (a fibronectin

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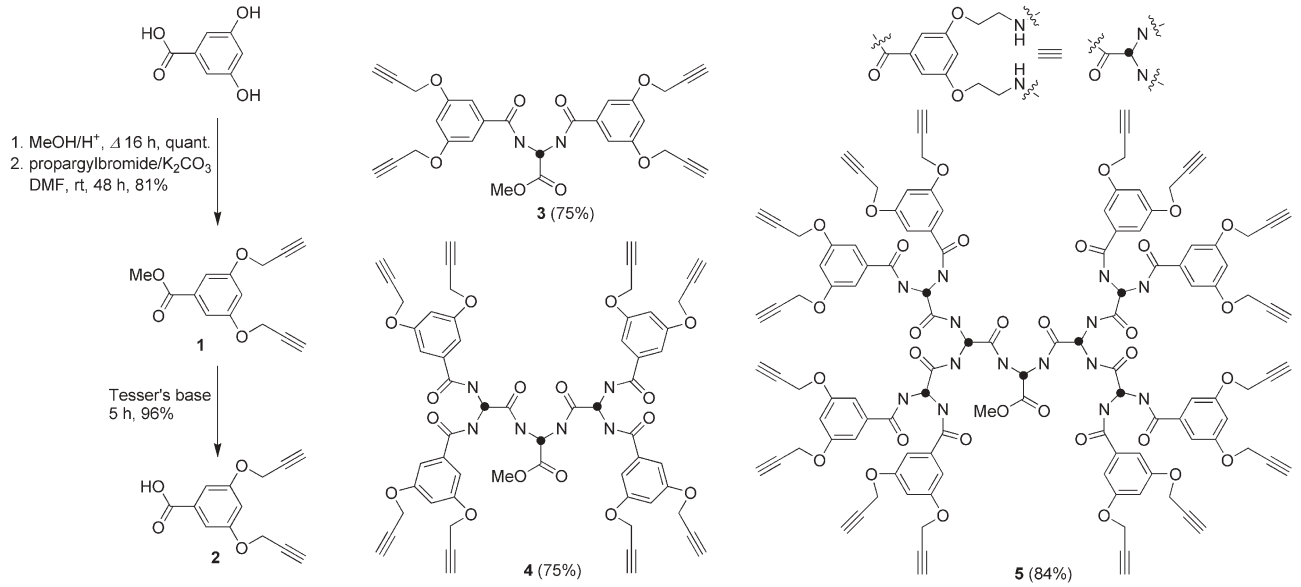

Fig. 1 Synthesis of 1st generation dendrimer **1** and alkyne building block **2**; Structures of the 2nd generation dendrimer (**3**), 3rd generation dendrimer (**4**) and the 4th generation dendrimer (**5**).

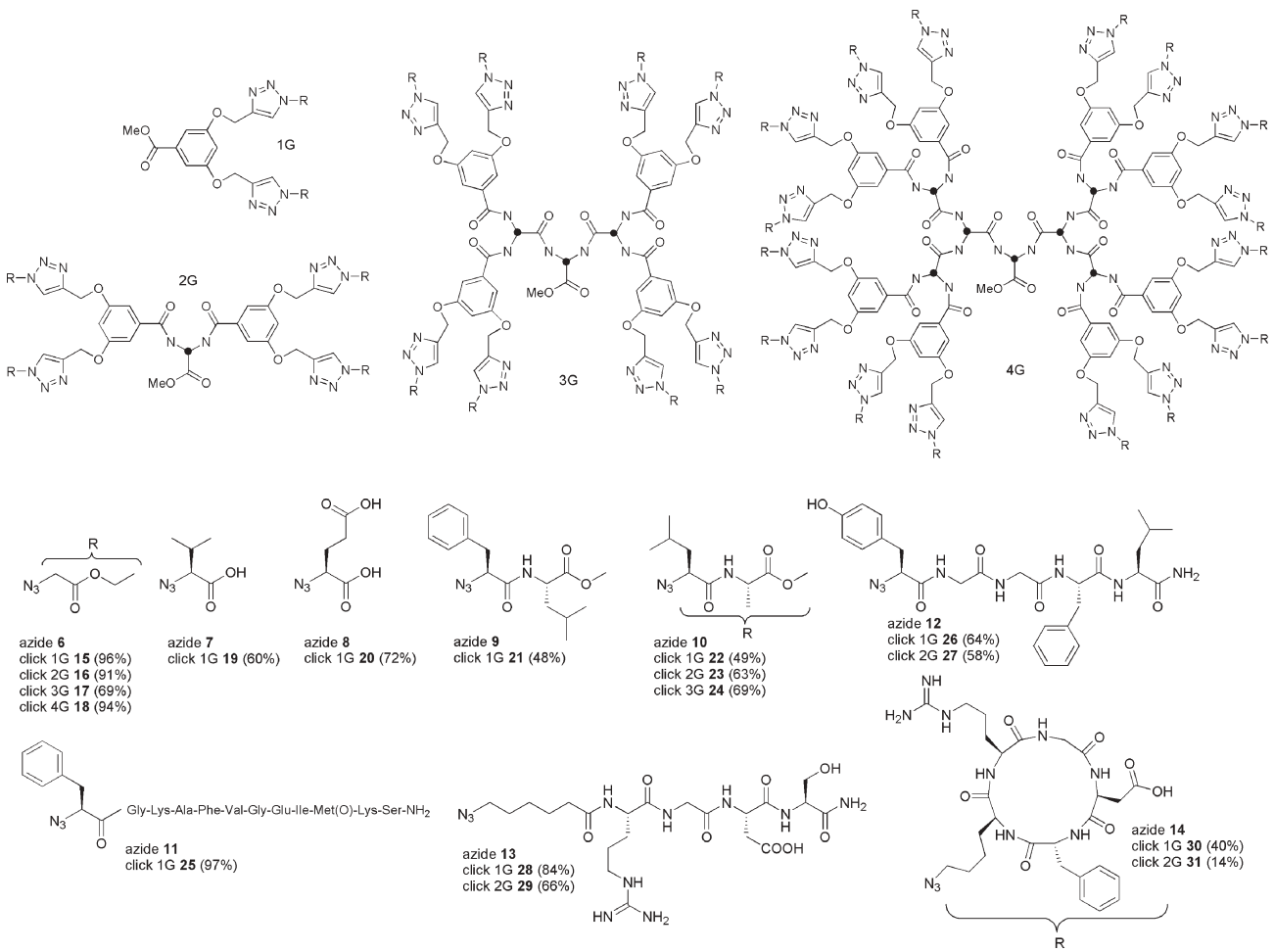

Fig. 2 Structures of the azides (**6–14**) and the corresponding peptide dendrimers: 1st generation ("click 1G"): **15**, **19–22**, **25–26**, **28** and **30**; 2nd generation ("click 2G"): **16**, **23**, **27**, **29** and **31**; 3rd generation ("click 3G"): **17** and **24**, and 4th generation ("click 4G"): **18**.

active fragment, connected to an ω-aminohexanoic acid spacer) and the cyclo RGD azido peptide **14** (an $\alpha_V\beta_3$ integrin binding RGD peptide for tumor targeting[16,17]) were coupled to alkynes **2** and **3**. The divalent and tetravalent peptides **26**, **28**, **30** and **27**, **29**, **31**, respectively (Fig. 2) were obtained after purification by HPLC in yields ranging from 14 to 84%. These peptides were identified by MALDI-TOF analysis. Most challenging was the coupling of 4th generation dendrimer (16 end-groups) **5** with cyclo RGD azido peptide **14** in order to obtain a hexadecavalent peptide dendrimer. HPLC analysis after work-up showed that the starting materials were consumed. However, so far we have been unable to characterize this construct by MALDI-TOF mass spectrometry. By SDS-PAGE bands were visible corresponding to a molecular weight ranging between 53000–65000 Da, corresponding to an aggregate in a tetra- or pentamer.

In conclusion, we have developed an efficient microwave-assisted Huisgen 1,3-dipolar cycloaddition reaction for the synthesis of di-, tetra-, octa- and hexadecavalent dendrimeric peptides. Not only small peptide-based azides but also unprotected biologically relevant larger - even cyclic - azido peptides are efficiently converted into the corresponding multiple cycloaddition products. These multivalent dendrimeric peptides may be useful in the preparation of synthetic vaccines or for example in the diagnosis and treatment of infections, where by multivalency the biological activity can be enhanced significantly. In addition, this methodology may provide access to the rapid synthesis of highly functionalized dendrimers as possible protein mimics. The synthesis and biological evaluation of multivalent dendrimeric peptides including protein mimics is under current investigation in our laboratory.§

Notes and references

‡ General procedure as illustrated for **16**: Alkyne **3** (57 mg, 84 μmol, 1 equiv) and azide **6** (70 mg, 545 μmol, 1.6 equiv per arm) were dissolved in THF/H_2O (2 mL, 1/1) and Na-ascorbate (8 mg, 40 μmol, 50 mol%) followed by $CuSO_4)\cdot 5H_2O$ (1 mg, 4 μmol, 5 mol%) were added. The reaction mixture was placed in the microwave reactor (Biotage) and irradiated at 100 °C during 5 min. The precipitate was filtered off, washed with ice-cold MeOH and dried in a desiccator. Compound **16** was obtained as a white solid in 91% yield. R_f($CHCl_3$/MeOH/AcOH 95/20/3): 0.68; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.21 (t, 12H, J = 7.14 Hz), 3.59 (m, 4H), 3.81 (s, 3H), 4.17 (q, 8H, J = 7.14 Hz), 4.21 (m, 4H), 5.21 (s, 8H), 5.42 (s, 8H), 6.82 (m, 1H), 6.91 (m, 2H), 7.09 (m, 2H), 7.15 (m, 4H), 8.24 (s, 4H), 8.63 (t, 2H); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 14.2, 40.5, 50.6, 52.5, 61.5, 61.7, 66.6, 104.5, 106.6, 107.8, 126.3, 131.8, 136.5, 142.7, 159.2, 159.9, 166.1, 167.4; MALDI-TOF: calcd for $C_{54}H_{62}N_{14}O_{18}$: 1194.437, found: $[M + H]^+$ 1195.597, $[M + Na]^+$ 1217.578; elemental analysis calcd for $C_{54}H_{62}N_{14}O_{18}$: C 54.27%, H 5.23%, N 16.41%, found: C 54.16%, H 5.17%, N 16.22%.
§ Patent pending.

1 M. Mammen, S.-K. Choi and G. M. Whitesides, *Angew. Chem. Int. Ed.*, 1998, **37**, 2754.

2 R. Autar, A. S. Khan, M. Schad, J. Hacker, R. M. J. Liskamp and R. J. Pieters, *ChemBioChem*, 2003, **4**, 1317; J. A. F. Joosten, V. Loimaranta, C. C. M. Appeldoorn, S. Haataja, F. Ait El Maate, R. M. J. Liskamp, J. Finne and R. J. Pieters, *J. Med. Chem.*, 2004, **47**, 6499; D. Arosio, I. Vrasidas, P. Valentini, R. M. J. Liskamp, R. J. Pieters and A. Bernardi, *Org. Biomol. Chem.*, 2004, **2**, 2113; S. André, R. J. Pieters, I. Vrasidas, H. Kaltner, I. Kuwabara, F.-T. Liu, R. M. J. Liskamp and H.-J. Gabius, *ChemBioChem*, 2001, **2**, 822; J. A. F. Joosten, N. T. H. Tholen, F. Ait El Maate, A. J. Brouwer, G. W. van Esse, D. T. S. Rijkers, R. M. J. Liskamp and R. J. Pieters, *Eur. J. Org Chem.*, 2005, 3182.

3 (*a*) G. R. Newkome, C. N. Moorefield and F. Vögtle, *Dendrimers and Dendrons: Concepts, Syntheses, Applications*, Wiley, New York, 2001; (*b*) J. M. J. Fréchet, *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 4782.

4 G. T. Hermanson, *Bioconjugate Techniques*, Academic Press Limited, London, UK, 1996; A. Garman, *Non-radioactive Labelling: A Practical Approach*, Academic Press Limited, London, UK, 1997. For recent examples of dendrimer modification using chemical ligation and/or sulfhydryl groups see: I. Van Baal, H. Malda, S. A. W. Reulen, T. M. Hackeng, M. Merkx and E. W. Meijer, Abstracts of Papers, 229th ACS National Meeting, San Diego, CA, United States, March 13–17, 2005 (2005); A. Herrmann, G. Mihov, G. W. M. Vandermeulen, H.-A. Klok and K. Müllen, *Tetrahedron*, 2003, **59**, 3925; G. Mihov, D. Grebel-Koehler, A. Lübbert, G. W. M. VanderMeulen, A. Herrmann, H-A. Klok and K. Müllen, *Bioconjugate Chem.*, 2005, **16**, 283; I. Van Baal, H. Malda, S. A. Synowsky, J. L. T. van Dongen, T. M. Hackeng, M. Merkx and E. W. Meijer, *Angew. Chem., Int. Ed.*, 2005, **44**, 5052.

5 R. Huisgen, R. Knorr, L. Mobius and G. Szeimies, *Chem. Ber.*, 1965, **98**, 4014.

6 C. W. Tornøe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057.

7 V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem. Int. Ed.*, 2002, **41**, 2596.

8 For recent reviews and applications see: R. Breinbauer and M. Köhn, *ChemBioChem*, 2003, **4**, 1147; Q. Wang, R. Chan, R. Hilgraf, V. V. Fokin, K. B. Sharpless and M. G. Finn, *J. Am. Chem. Soc.*, 2003, **125**, 3192.

9 S. J. E. Mulders, A. J. Brouwer, P. G. J. van der Meer and R. M. J. Liskamp, *Tetrahedron Lett.*, 1997, **38**, 631; S. J. E. Mulders, A. J. Brouwer and R. M. J. Liskamp, *Tetrahedron Lett.*, 1997, **38**, 3085; A. J. Brouwer, S. J. E. Mulders and R. M. J. Liskamp, *Eur. J. Org. Chem.*, 2001, 1903; A. J. Brouwer and R. M. J. Liskamp, *Eur. J. Org. Chem.*, 2005, 487.

10 H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem. Int. Ed.*, 2001, **40**, 2004; B. Helms, J. L. Mynar, C. J. Hawker and J. M. J. Fréchet, *J. Am. Chem. Soc.*, 2004, **126**, 15020; P. Wu, A. K. Feldman, A. K. Nugent, C. J. Hawker, A. Scheel, B. Voit, J. Pyun, J. M. J. Frechet, K. B. Sharpless and V. V. Fokin, *Angew. Chem. Int. Ed.*, 2004, **43**, 2938.

11 K. A. Savin, M. Robertson, D. Gernert, S. Green, E. J. Hembre and J. Bishop, *Mol. Diversity*, 2003, **7**, 171; P. Appukkuttan, W. Dehaen, V. V. Fokin and E. Van der Eycken, *Org. Lett.*, 2004, **6**, 4223.

12 Azide **6** was prepared according to: S. G. Alvarez and M. T. Alvarez, *Synthesis*, 1997, 413. Azides **7–10**, **13** and **14** were synthesized according to: J. T. Lundquist, IV and J. C. Pelletier, *Org. Lett.*, 2001, **3**, 781. azido peptides **11** and **12** were synthesized according to: D. T. S. Rijkers, H. H. R. van Vugt, H. J. F. Jacobs and R. M. J. Liskamp, *Tetrahedron Lett.*, 2002, **43**, 3657.

13 For a recent review concerning peptide-based dendrimers: L. Crespo, G. Sanclimens, M. Pons, E. Giralt, M. Royo and F. Albericio, *Chem. Rev.*, 2005, **105**, 1663. Peptide-based dendrimers are also reviewed in reference 3*a*.

14 Tesser's base is a mixture of dioxane, methanol and aq. NaOH, see: G. I. Tesser and I. C. Balvert-Geers, *Int. J. Peptide Protein Res.*, 1975, **7**, 295.

15 M. Zasloff, *Proc. Natl. Acad. Sci. USA*, 1987, **84**, 5449.

16 R. Haubner, R. Gratias, B. Diefenbach, S. L. Goodman, A. Jonczyk and H. Kessler, *J. Am. Chem. Soc.*, 1996, **118**, 7461.

17 R. Haubner, H.-J. Wester, F. Burkhart, R. Senekowitsch-Schmidtke, W. Weber, S. L. Goodman, H. Kessler and M. Schwaiger, *J. Nucl. Med.*, 2001, **42**, 326; M. L. Janssen, W. J. Oyen, I. Dijkgraaf, L. F. Massuger, C. Frielink, D. S. Edwards, M. Rajopadhye, H. Boonstra, F. H. Corstens and O. C. Boerman, *Cancer Res.*, 2002, **62**, 6146; G. Thurmshirn, U. Hersel, S. L. Goodman and H. Kessler, *Chem. Eur. J.*, 2003, **9**, 2717.