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NODAPA-OH and NODAPA-$(NCS)_n$: Synthesis, ^{68}Ga-radiolabelling and in vitro characterisation of novel versatile bifunctional chelators for molecular imaging

Patrick J. Riss *, Carsten Kroll, Verena Nagel, Frank Rösch

Institute of Nuclear Chemistry, University of Mainz, Fritz-Strassmann-Weg 2, 55128 Mainz, Germany

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ABSTRACT

This report concerns synthesis, ^{68}Ga-radiolabelling and stability data of 1,4,7-triazacyclononane-1,4-diacetic acid-7-*p*-isothio-cyanatophenyl-acetic acid (NODAPA-NCS), 1,4,7-triazacyclononane-1-acetic acid-4,7-di-*p*-isothiocyanatophenyl-acetic acid (NODAPA-$(NCS)_2$) and 1,4,7-triazacyclononane-1,4-diacetic acid-7-*p*-hydroxyphenyl-acetic acid (NODAPA-OH), versatile bifunctional chelators with potential for molecular imaging. Protein binding and exemplified conjugation are also reported.

Non-invasive molecular imaging of biochemical mechanisms and pathologies in vivo is an emerging interdisciplinary field of research. Among the available techniques for a sensitive, quantitative visualisation of the biochemical and physiological function of biological tissue approached by suitable imaging probes, positron emission tomography (PET) provides great potential in terms of quantification, sensitivity, temporal, and lateral resolution. The commercially available ^{68}Ge/^{68}Ga radionuclide generator system and its recent improvement concerning on-line processing and labelling,[1] may provide a beneficial complement to nuclear imaging with established, cyclotron produced PET nuclides like ^{11}C and ^{18}F. ^{68}Ga provides a high positron abundance of 89% and an intermediate positron maximum energy. With its half-life (1.13 h) lying perfectly in between the half-lives of the most frequently used ^{11}C (0.33 h) and ^{18}F (1.82 h), it provides excellent decay characteristics as a PET-radiolabel. In addition, its most promising advantage is its availability via a radionuclide generator system. The mother nuclide ^{68}Ge has a half-life of 270.8 d, guaranteeing prolonged use of generator set-ups in action (ca. 1 year). Consequently, ^{68}Ga provides an economical alternative to the cyclotron produced radionuclides. On the other hand, radiolabelling of molecular probes with ^{68}Ga requires a completely different synthesis route. The main group metal rapidly forms chelate-complexes with hard donor functions of four to six coordinating chelators. Adequate radioligand precursors meeting the coordination chemistry of gallium(III) with versatile conjugation possibilities are of high interest. To reinforce the flow of novel tracer candidates to biological evaluation, a convenient, time efficient route to various chelator conjugated potential targeting vectors would be desirable.

The macrocyclic chelators DOTA and NOTA (Fig. 1) are established as frequently considered routes for the introduction of a ^{68}Ga-tag. Compared to open chain acyclic analogues, both provide complexes of superior kinetic and thermodynamic stability since gallium is irreversibly complexed at room temperature. DOTA remains the most frequently used chelator because of its better commercial availability and less challenging synthesis. Its six-coordinate nine-ring analogue NOTA forms slightly distorted octahedral complexes with gallium which display higher stabilities and faster incorporation of Ga(III) at lower temperatures.[2] Thus, we were interested in a time-saving and cost-effective access to a versatile NOTA-based gallium chelator allowing convenient conjugation to various targeting molecules.

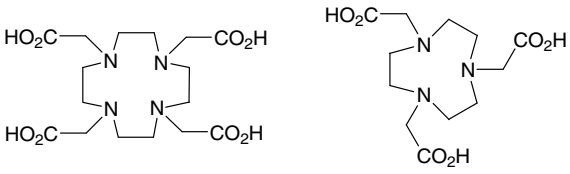

Figure 1. (4,7,10-*tris*-Carboxymethyl-1,4,7,10-tetraaza-cyclododec-1-yl)-acetic acid (DOTA) and (4,7-bis-carboxymethyl-[1,4,7]-triazanon-1-yl)-acetic acid (NOTA).

* Corresponding author. Tel.: +49 6131 3925701.
E-mail address: riss@uni-mainz.de (P.J. Riss).

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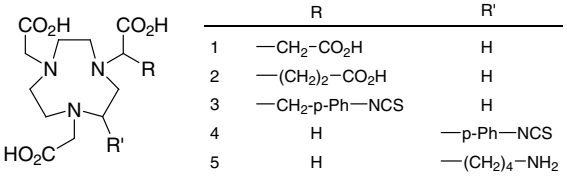
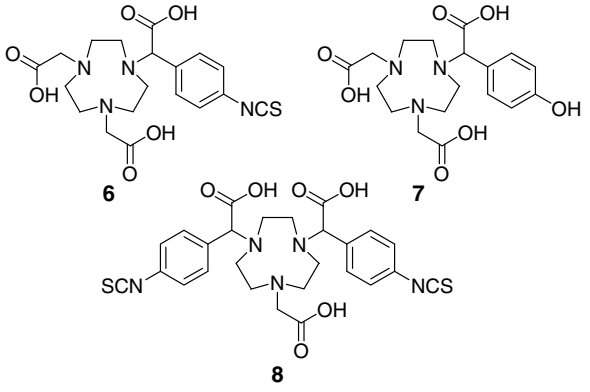
Specific bifunctional derivatives of NOTA, published so far, are surveyed in Figure 2. NODAGA (**1**) and NODASA (**2**) are limited to coupling peptides through an amide bond.[3,4] The value of NODAGA has been demonstrated convincingly. The isothio-cyanatophenyl derivative **3** has been introduced by Brechbiel et al. but no further use has been reported yet.[5] The C-substituted analogue **4** is commercially available. The synthesis of C-substituted NOTA derivative **5** was reported by Parker et al. in 7.7% yield.[6] Brechbiel et al. also reported very low cyclisation yields of C-substituted nine-membered rings due to transannular condensation.[5] Consequently, we were interested in a cost-effective concept for an easy access to a bifunctional derivative of NOTA, starting from bulk chemicals.

Therefore a straightforward and economic synthesis of a bifunctional chelator was developed. Orthogonally reactive phenol as well as isothiocyanate functions were included for conjugation. The intended bifunctional chelators are illustrated in Scheme 1. The gallium core forms stable five-ring chelates with the Nitrogen and the adjacent carboxylate donors in NOTA and its analogues. Introduction of a conjugation functionality in a pendant arm branch into NOTA–analogue chelators following nucleophilic N-alkylation, requires a secondary leaving group in α-position to the carboxylate function. In addition, the planar phenylen subunit provides a non-flexible initial spacer, complementary to **2**, that can be combined with a variety of additional spacer functions via the included conjugation functionality. Both linkers, 2-bromo-(4-acetoxy-phenyl)-*tert*-butylacetate (**9**) and 2-bromo-2-(*p*-nitrophenyl)-*tert*-butylacetate (**10**) were easily synthesised from the corresponding acetic acid derivatives via *tert*-butyl protection under Steglich conditions (i) in up to 80% yield, followed by Wohl–Ziegler bromination (ii) in 90% yield (Scheme 2).[7–10,14] Cyclisation of 1,4,7-tritosyl-1,4,7-triazacyclononane (**11**) was achieved according to the well-known procedure reported by Richman and Atkins.[11] Full detosylation (a) was performed in concentrated sulphuric acid (110 °C, 2 h; Scheme 2).[12] The polyhydrogensulfate of 1,4,7-triazacyclononane (TACN, **12**) was precipitated in ethanol and diethylether,[13] dissolved in a small amount of water and basified (pH 13) with sodium hydroxide. After exposure to activated carbon under reflux followed by extraction with predistilled 1-butanol, **12** was obtained as colourless crystals displaying sufficient purity for all further reactions. Statistical alkylation (b) with **9** or **10** was carried out in the presence of potassium carbonate over three days in dichloromethane, using a threefold excess of TACN (**12**). Employing a lower excess of **12**, (d) the yield of the dialkylated product **15** increased. Thereby, a convenient access to multivalent [^{68}Ga]chelates is provided. The trialkylated product was not observed. Introduction of the *tert*-butyl protected acetic acid donor functions (c) was performed in acetonitrile with stoichiometric amounts of 2-bromo-*tert*-butyl acetate and potassium carbonate as base to afford **14**, **16** or **18** in a yield of up to 90%.[4] Although all NODAPA derivatives presented herein were successfully synthesised via statistical alkylation, the latter remains somewhat unfavourable for the synthesis of mono-functionalised derivatives **14**. Furthermore, an unreasonable excess of **12** has to be employed. Therefore, an alternative route was examined. The key step involved selective detosylation of two ring-nitrogens to facilitate stoichiometrical alkylation. First, 1,4,7-tritosyl-TACN (**11**) was reacted with HBr in glacial acetic acid containing an eightfold excess of phenol (Scheme 2, e). The reaction proceeded straightforward to afford 88% of 1-tosyl-1,4,7-triazacyclononane (**17**), as determined by ESI-MS and proton-NMR. Subsequent exposure of **17** to *tert*-butyl bromoacetate in acetonitrile followed by SET-reduction (f) with sodium naphtalenide in dimethoxyethane or lithium in propylamine/ethylendiamine gave secondary amine **19**. The latter was converted into the protected chelators **14a** and **14b** by reaction with bromides **9** and **10**, respectively, in 27% overall yield. Catalytic hydrogenation of **16** was conducted under basic conditions (g) to give the desired aniline **20** in good yield (90%) as shown in scheme 3.[8]



	R	R'
1	—CH_2–CO_2H	H
2	—$(CH_2)_2$–CO_2H	H
3	—CH_2-p-Ph—NCS	H
4	H	—p-Ph—NCS
5	H	—$(CH_2)_4$–NH_2

Figure 2. NOTA-based bifunctional chelators.



Scheme 1. Novel NOTA-derived bifunctional chelators NODAPA-NCS (**6**), NODAPA-OH (**7**) and NODAPA-$(NCS)_2$ (**8**).

Subsequent conversion to an isothiocyanate **21** was carried out using thiophosgene in 85% yield (Scheme 3h).[15c] The *tert*-butyl esters were deprotected in trifluoroacetic acid and the trifluoroacetic acid salts were removed using an ion exchange resin, to afford **6** and **8** in up to 23% overall yield.[15a,c] Compound **7** was obtained in similar yield after deacetylation in 10% KOH in methanol, prior to deprotection in TFA.[15b] The desalted precursors were directly used for radiolabelling without further purification. In order to analyse whether the chain branch, containing the coupling moiety in one pendant arm, affects the kinetic and thermodynamic characteristics of [^{68}Ga]NOTA-complex formation, labelling of NODAPA-NCS (**6**), NODAPA-OH (**7**), NODAPA$(NCS)_2$ (**8**), and NODAPA-NO_2 (**14b**) with generator produced and purified gallium-68 was carried out in aqueous solution at pH 2.8.

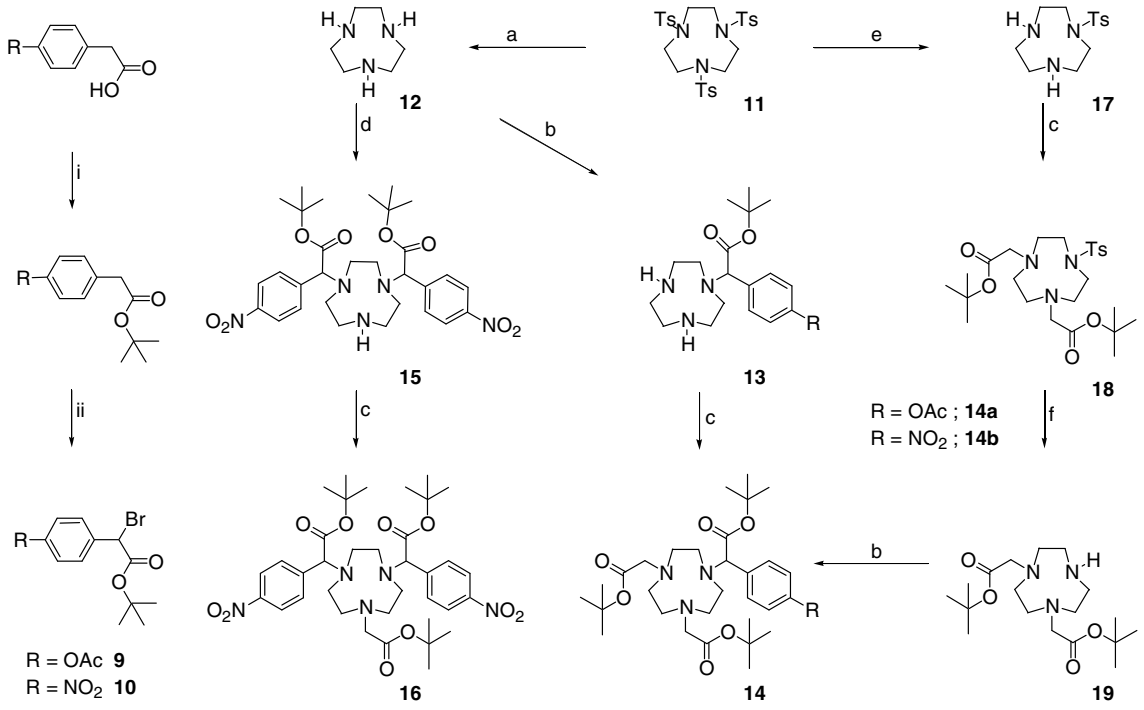
Quality control was performed using an Agilent Zorbax C 8 column using 50 mM phosphate buffer and MeOH as eluent at 0.5 ml/min.

Yields were very high (85 ± 5% after 3 min) and comparable to those achieved for NOTA (Fig. 3).

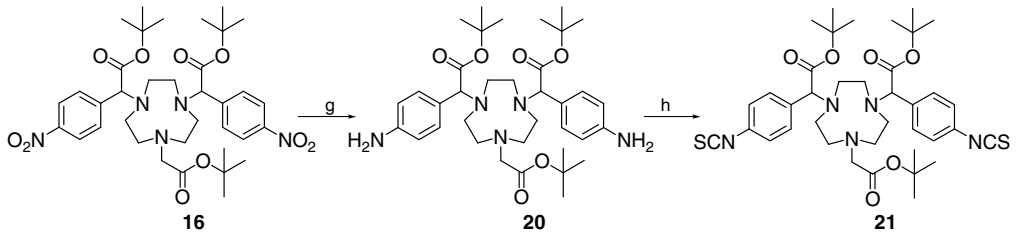
The stability of both novel ^{68}Ga chelates was determined in a DTPA-challenge study at 25 °C and 37 °C employing 1 mM, 10 mM and 100 mM solutions of DTPA in water, indicating >94% complex stability, in a similar range as the congener NOTA (Fig. 4).

Plasma protein binding and transchelation to serum proteins in vitro was examined under physiological conditions in rat plasma. Four MegaBequerels [^{68}Ga]NODAPA-OH were incubated in 300 μL of rat plasma from male adult Wistar rats, obtained via centrifugation of full blood. Samples of 50 μL were withdrawn after 1, 30, 60, 90, and 180 min and analysed by radio-TLC (silica gel 60, 5% aq. NaCl–EtOH, 3:1).

In correlation to the DTPA-challenge, less than 2% of non-[^{68}Ga]NODAPA-OH radioactivity was observed after 3 h.



Scheme 2. Synthesis route to mono-functionalised and di-functionalised NOTA derivatives. Reagents and conditions: (a) H_2SO_4, 2 h, 110 °C; (b) *tert*-butyl 2-bromo-2-(4′-nitrophenyl) acetate, CH_2Cl_2, K_2CO_3, 3 d; (c) *tert*-butyl bromoacetate, MeCN, K_2CO_3; (d) 2 equiv *tert*-butyl 2-bromo-2-(4′-nitrophenyl) acetate, CH_2Cl_2, K_2CO_3, 3 d; (e) PhOH, HBr/AcOH, 1.5 d, 90 °C; (f) dimethoxyethane, Na, $C_{10}H_8$; i—*t*-BuOH, CH_2Cl_2, DCC, DMAP, 3 h; ii—CCl_4 (distilled from P_4O_{10}), NBS, AIBN.



Scheme 3. Synthesis of di-functionalised NOTA derivatives. Reagents and conditions: (g) 2 mM KOH in MeOH, 10% Pd/C, H_2, rt, 4 h; (h) Ca_2CO_3, Me_2CO, H_2O, thiophosgene.

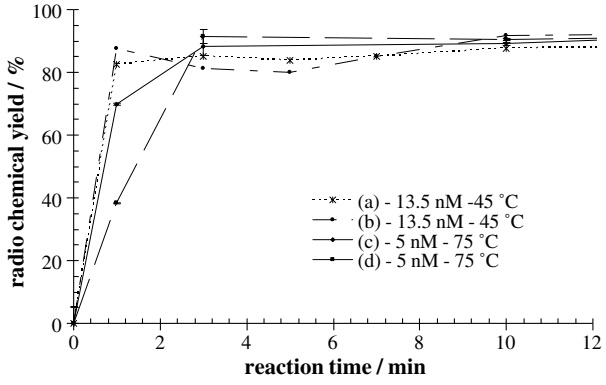


Figure 3. Time-dependency of ^{68}Ga-labelling of (a) NOTA, (b) NODAPA-OH (**7**), (c) NODAPA-NCS (**6**) and (d) NODAPA(NCS)$_2$ (**8**) pH 2.8, 5–13.5 nmol ligand, 5 ml H_2O, 400 μL [^{68}Ga]$GaCl_3$ in N2-solution.[1]

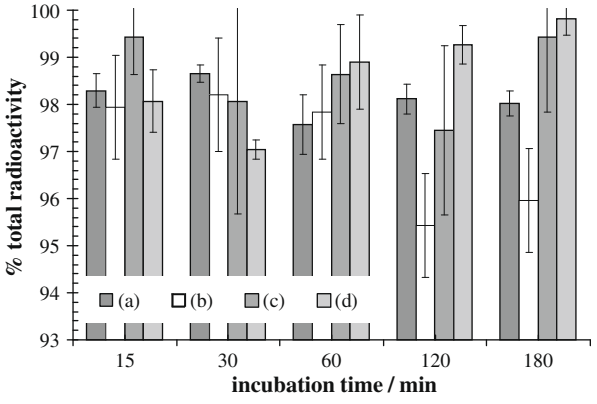
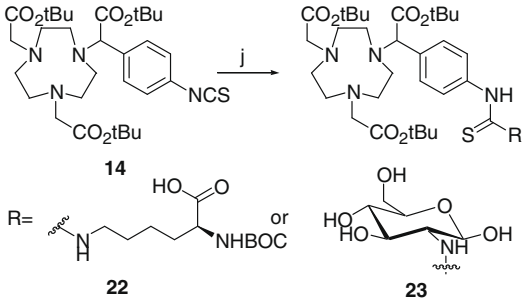


Figure 4. Stability of [^{68}Ga]NOTA (a), [^{68}Ga]NODAPA-OH (b), [^{68}Ga]NODAPA-NCS (c), and [^{68}Ga]NODAPA-(NCS)$_2$: (d); 10 mM DTPA in DPBS (pH 7.4) at 37 °C (n = 3).

In conclusion, three novel NOTA-based bifunctional chelators have been obtained via a simple and efficient synthesis route. **6**, **7**, and **8** provide excellent ^{68}Ga labelling and stability parameters. While offering –NCS and –OH functionalities, covalent coupling to various potential targeting vectors is possible. As a proof-of-concept, NODAPA-NCS was conjugated to L-lysine (**22**) and glucosamine (**23**) (Scheme 4).[15d] The latter symbolise the first bioconjugates with the promising novel NOTA-based bifunctional chelators introduced in the present work.



Scheme 4. Conjugation of NODAPA-NCS to model substrates. Reagents: (j) Ca_2CO_3, $MeOH/H_2O/NEt_3$, 7:1:1 (65–73%).

References and notes

1. Zhernosekov, K. P.; Filosofov, D. V.; Baum, R. P.; Aschoff, P.; Bihl, H.; Razbash, A.; Jahn, M.; Jennewein, M.; Rösch, F. *J. Nucl. Med.* **2007**, *48*, 1741.
2. Craig, A. S.; Parker, D.; Adams, H.; Bailey, N. A. *J. Chem. Soc. Chem. Commun.* **1989**, 1793.
3. Eisenwiener, K.-P.; Prata, M. I. M.; Buschmann, I.; Zhang, H. W.; Santos, A. C.; Wenger, S.; Reubi, J. C.; Mäcke, H. R. *Bioconjugate Chem.* **2002**, *13*, 530.
4. André, J. P.; Maecke, H. R.; Zehnder, M.; Macko, L.; Akyel, K. G. *Chem. Commun.* **1998**, 1301.
5. Brechbiel, M. W.; McMurry, T. J.; Gansow, O. A. *Tetrahedron* **1993**, *34*, 3691.
6. Cox, J.; Craig, A.; Helps, I. M.; Jankowski, K.; Parker, D.; Eaton, M.; Millican, A.; Millar, K.; Beeley, N.; Boyce, B. *J. Chem. Soc., Perkin Trans. 1* **1990**, 2567.
7. Ziegler, K.; Schenck, G.; Krockow, E. W.; Siebert, A.; Wenz, A.; Weber, H. *Liebigs Ann.* **1942**, *551*, 1.
8. Helps, I. M.; Parker, D.; Morphy, J. R.; Chapman, J. *Tetrahedron* **1989**, *45*, 219.
9. Neises, A.; Steglich, W. *Angew. Chem. Int., Ed. Engl.* **1978**, *17*, 52213.
10. Wohl, A. *Chem. Ber.* **1919**, *52*, 51.
11. Richman, J. E.; Atkins, T. J. *J. Am. Chem. Soc.* **1974**, *96*, 2268.
12. Raßhofer, W.; Vögtle, F. *Liebigs Ann. Chem.* **1977**, 1340.
13. Diril, H.; Chang, H. R.; Nilges, M. J.; Zhang, X.; Potenza, J. A.; Schugar, H. J.; Isied, S. S.; Hendrickson, D. N. *J. Am. Chem. Soc.* **1989**, *111*, 5102.
14. Horner, L.; Winkelmann, E. H. *Angew. Chem.* **1959**, *7*, 349.
15. (a) (**14b**) δ_H (300 MHz; $CDCl_3$) 1.40 (9H, s, *t*-Bu), 1.42 (18H, s, *t*-Bu), 2.78–3.25 (16H, m, N–CH_2, N–CH), 4.10 (1H, s, N–CH–Ar), 7.15 (2H, d, J = 8 Hz, *Ar*), 7.40 (2 H, d, J = 8 Hz, *Ar*); *m*/*z* (ESI) 606.32 ($[M+H]^+$ $C_{31}H_{48}N_4O_6S$ requires 606.33, 100%), 606.34 (96.1), 607.38 (58.0), 608.40 (12.9), 549.29.
(b) (**14a**) δ_H (300 MHz; $CDCl_3$) 1.41 (9H, s, *t*-Bu), 1.42 (9H, s, *t*-Bu), 1.43 (9H, s, *t*-Bu) 2.70–3.04 (12H, m, N–CH_2), 3.25 (2H, br s, CH_2–C(O)) 3.30 (2H, s, CH_2–C(O)) 4.41 (1H, dd, J = 6 Hz, N–CH–Ar), 6.74 (2H, d, J = 8 Hz, ArH), 7.20 (2H, d, J = 8 Hz, ArH); *m*/*z* (ESI) 564.37 ($[M+H]^+$ $C_{30}H_{49}N_3O_7$ requires 563.36, 100%), 565.37 (35%), 556.38 (8%).
(c) (**21**) δ_H (300 MHz; $CDCl_3$) 1.41 (s, 9H, *t*-Bu), 1.415 (s, 9H, *t*-Bu), 1.42 (s, 9H, *t*-Bu), 3.10–2.60 (m, 12H), 3.2 (m, 4H, N–*CH*$_2$–COO*t*-Bu), 4.41 (s, 1H, N–*CH*'–Ar), 4.41 (s, 1H, N–*CH*'–Ar), 4.42 (s, 1H, N*CH*–Ar); 7.57 (m, 4H, *Ar*); 8.16 (m, 4H, *Ar*) *m*/*z* (ESI) 738.30 (100%), 739.33 (947%), 740.35 (58.8%), 741.37 (10.8%), ($[M+H]^+$ $C_{38}H_{51}N_5O_6S_2$ requires 737.33, 100.0%), 738.33 (41,1%), 739.32 (9.0%), 739.33 (8.2%), 740.33 (37%).
(d) (**22**) δ_H (300 MHz; $CDCl_3$): 1.25 (m, 2H, Lys-g-CH_2), 1.34 (s, 9H, O*t*-Bu), 1.40 (s, 18H, O*t*-Bu) 1.42 (s, 9H, O*t*-Bu), 1.7–1.5 (m, 4H, β-*CH*$_2$, δ-*CH*$_2$), 3.0–2.65 (m, 12H, N–*CH*$_2$–*CH*$_2$), 3.25 (4H, s, N–*CH*$_2$–COO*t*-Bu); 3.50 (t, J = 5 Hz, 2H, ε-*CH*$_2$); 4.05 (s, 1H, N–*CH*–Ar); 4.34 (t, 1H, α-*CH*); 7.40–7.25 (m, 4H, *Ar*); *m*/*z* (ESI) 851.6 $[M+H]^+$ $C_{42}H_{70}N_6O_{10}S$ requires 850.49. (**23**) *m*/*z* (ESI): 784.43 $[M+H]^+$ $C_{37}H_{61}N_5O_{11}S$ requires 783.41.