JOC Note

pubs.acs.org/joc

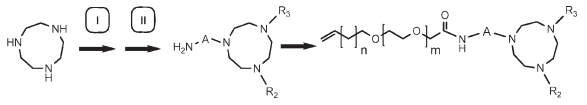
Double Selective Synthetic Approach to the N-Functionalized 1,4,7-Triazacyclononane Derivatives: Chelating Compounds for Controllable Protein Orientation

Tomasz D. Sobieściak* and Piotr Zielenkiewicz

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5a, Warsaw 02-106, Poland

tomasz_s@ibb.waw.pl

Received November 24, 2009



The double selective synthetic approach is proposed for easy preparation of asymmetrically N-substituted derivatives of 1,4,7-triazacyclononane (tacn, [9]aneN$_3$) with the same or different pendant arms. The synthetic routes simplify the synthesis of "tacn" ligands for functionalization of other carboxy terminated molecules/supports and also facilitate access to azamacrocycle functionalized self-assembled monolayer (SAMs) or nanopatterns for specific and stable Histidine-tagged protein immobilization.

The analysis of protein complexes and protein−protein interactions is currently a subject of great interest.[1] Studies of these interactions often require ligands which are linked to biocompatible surfaces and specifically and selectively interact with biomolecules.[2] Protein interactions can be detected by SPR measurement on biochips[3] that are prepared from SAM on gold,[4] and by the method that combines SAMs with MALDI-TOF-MS (also called as SAMDI).[5] Oligo(ethylene glycol)-terminated[6] SAMs are inert in nonspecific interactions with proteins.[7]

A selective, stable, and nondestructive immobilization of proteins on surfaces in a sterically defined form[8] is a prerequisite for the investigation of molecular interactions. Currently, the predominant strategy for spatially oriented immobilization of proteins is based on specific interaction between a metal chelator, usually nitrilotriacetic acid (NTA) functionalized SAMs, and histidine-tagged proteins.[3] NTA are very frequently employed for His-tag protein purification.[9a] More than 60% of the proteins produced for structural studies include His$_6$-tag[9b] and can be directly used for selective and oriented binding to chelator-functionalized surfaces. However, complexes between His-tag and NTA-Ni^{2+} are of very low affinity (K_d values in the 1−10 μM range).[10] SAMs presenting the Multivalent Chelators based on NTA were prepared with increased stability of the chelator-His-tag complexes.[11] The chips based on SAMs that present "tacn" ligand offer a major advantage over current NTA-based chelator systems, i.e., the triazamacrocyclic ligand forms stable (nondissociable) complexes with a Ni ion and forms stable complexes with His-tagged proteins.[12] The protein binding selectivity also has been examined with the "tacn"-modified sorbent.[13]

"Tacn" chelators also have been used for an affinity-capturing system of His-tagged proteins in SAMDI experiment[14] on SAMs prepared from maleimide-terminated OEG-alkyl disulfides. These SAMs were incubated with thiol-terminated "tacn" after formation of a monolayer. However, SAMs have more often been formed from OEG-alkanethiols than from unsymmetrical disulfides. The latter are known for reduced kinetics of chemisorption on gold.[15] SAMs with reactive carboxyls, often in "active ester" form, are the most widely used for coupling with desired ligands.[16] Formation of an amide bond mediated by NHS/EDC is simple but suffers from sensitivity of the transiently formed active NHS esters to hydrolysis,[17] which complicates application of this method, especially in microspotting. Hence,

(1) (a) Monti, M.; Cozzolino, M.; Cozzolino, F.; Vitiello, G.; Tedesco, R.; Flagiello, A.; Pucci, P. *Expert Rev. Proteomics* **2009**, *6*, 159–169. (b) Fuentes, G.; Oyarzabal, J.; Rojas, A. M. *Curr. Opin. Drug Discovery Dev.* **2009**, *12*, 358–366. (c) Piehler, J. *Curr. Opin. Struct. Biol.* **2005**, *15*, 4–14. (d) Lee, H. J.; Yan, Y.; Marriott, G.; Corn, R. M. *J. Physiol.* **2005**, *563*, 61–71.

(2) Rusmini, F.; Zhong, Z.; Feijen, J. *Biomacromolecules* **2007**, *8*, 1775–1789.

(3) Sigal, G. B.; Bamdad, C.; Barberis, A.; Strominger, J.; Whitesides, G. M. *Anal. Chem.* **1996**, *68*, 490–497.

(4) (a) Ulman, A. *Chem. Rev.* **1996**, *96*, 1533–1554. (b) Senaratne, W.; Andruzzi, L.; Ober, C. K. *Biomacromolecules* **2005**, *6*, 2427–2448.

(5) (a) Yeo, W.-S.; Min, D.-H.; Hsieh, R. W.; Greene, G. L.; Mrksich, M. *Angew. Chem., Int. Ed.* **2005**, *44*, 5480–5483. (b) Fukuzawa, S.; Asanuma, M.; Tachibana, K.; Hirota, H. *J. Mass. Spectrom. Soc. Jpn.* **2006**, *54*, 187–193.

(6) Oligo(ethylene glycol) (OEG) is also referred to as oligo(oxyethylene), oligo(ethylene oxide).

(7) Prime, K. L.; Whitesides, G. M. *Science* **1991**, *252*, 1164–1167.

(8) (a) Gamsjaeger, R.; Wimmer, B.; Kahr, H.; Tinazli, A.; Picuric, S.; Lata, S.; Tampé, R.; Maulet, Y.; Gruber, H. J.; Hinterdorfer, P.; Romanin, C. *Langmuir* **2004**, *20*, 5885–5890. (b) Merkel, J. S.; Michaud, G. A.; Salcius, M.; Schweitzer, B.; Predki, P. F. *Curr. Opin. Biotechnol.* **2005**, *16*, 447–452. (c) Cha, T.; Guo, A.; Zhu, X.-Y. *Proteomics* **2005**, *5*, 416–419.

(9) (a) Crowe, J.; Döbeli, H.; Gentz, R.; Hochuli, E.; Stüber, D.; Henco, K. *Methods Mol. Biol.* **1994**, *31*, 371–387. (b) Derewenda, Z. S. *Methods* **2004**, *34*, 354–363.

(10) (a) Nieba, L.; Nieba-Axmann, S. E.; Persson, A.; Hämäläinen, M.; Edebratt, F.; Hansson, A.; Lidholm, J.; Magnusson, K.; Karlsson, A. F.; Plückthun, A. *Anal. Biochem.* **1997**, *252*, 217–228. (b) Lata, S.; Reichel, A.; Brock, R.; Tampé, R.; Piehler, J. *J. Am. Chem. Soc.* **2005**, *127*, 10205–10215.

(11) Valiokas, R.; Klenkar, G.; Tinazli, A.; Reichel, A.; Tampe, R.; Piehler, J.; Liedberg, B. *Langmuir* **2008**, *24*, 4959–4967.

(12) Johnson, D. L.; Martin, L. L. *J. Am. Chem. Soc.* **2005**, *127*, 2018–2019.

(13) Jiang, W.; Prescott, M.; Devenish, R. J.; Spiccia, L.; Hearn, M. T. W. *Biotechnol. Bioeng.* **2009**, *103*, 747–756.

(14) Marin, V. L.; Bayburt, T. H.; Sligar, S. G.; Mrksich, M. *Angew. Chem., Int. Ed.* **2007**, *46*, 8796–8798.

(15) Bain, C. D.; Biebuyck, H. A.; Whitesides, G. M. *Langmuir* **1989**, *5*, 723–727.

(16) (a) Wagner, P.; Hegner, M.; Kernen, P.; Zaugg, F.; Semenza, G. *Biophys. J.* **1996**, *70*, 2052–2066. (b) Laurent, N.; Voglmeir, J.; Wright, A.; Blackburn, J.; Pham, N. T.; Wong, S. C. C.; Gaskell, S. J.; Flitsch, S. L. *ChemBioChem* **2008**, *9*, 883–887.

(17) Tournier, E. J. M.; Wallach, J.; Blond, P. *Anal. Chim. Acta* **1998**, *361*, 33–44.

DOI: 10.1021/jo902504d Published on Web 02/25/2010

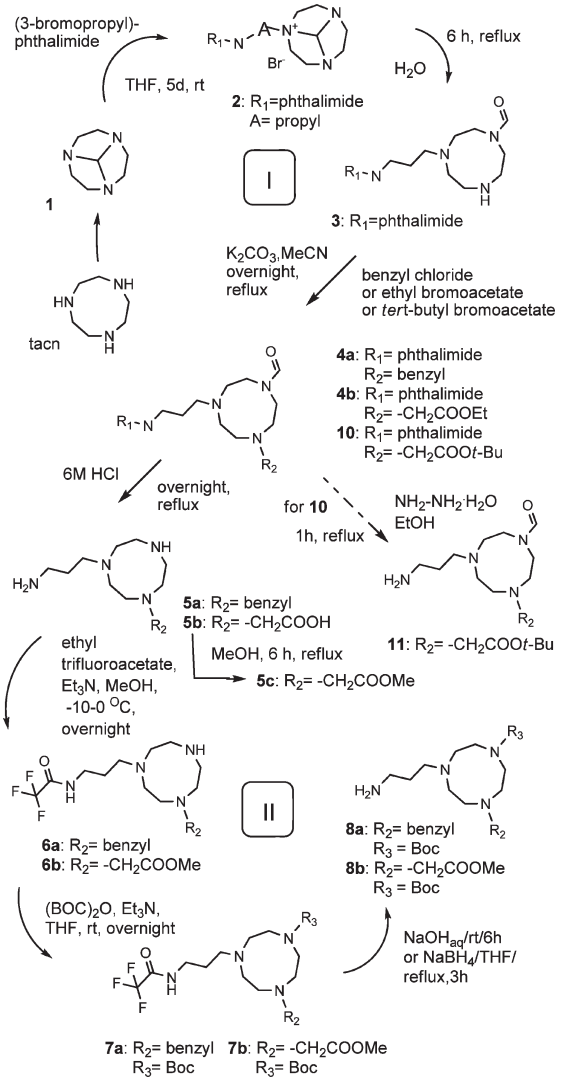
separate synthesis of fully ligand-functionalized OEG-alkane-thiols and formation of SAMs from compounds which are already ready for interaction with their affinity counterparts is the best way.[18]

"Tacn" and its derivatives coordinate facially to metal ions and form a very stable[19a] and diverse range of metal complexes.[19b] The preparation of special "tacn" derivatives for functionalization of other molecules/supports is not simple. This results from synthetic difficulties involved in the differentiation of three equivalent secondary amino groups on azacrown. Even the synthesis of pure "tacn" is low-yielding and troublesome. Syntheses of monosubstituted or asymmetrically functionalized "tacn" derivatives with more than one type of pendant donor generally yielded a mixture of products.[20]

Thus, we looked for a convenient, inexpensive, facile, and efficient synthesis of "tacn" derivatives from commercially available precursors which does not require special reaction conditions, exclude the occurrence of the statistically substituted derivatives, and offer the possibility of simple modulation of coordination properties of azamacrocycle. The introduction of specific, various functionalities at the periphery of the coordinating moiety may allow for fine-tuning of the properties of the complex.[21] The choice of "tacn orthoamide" as a starting compound provides efficient routes to "tacn" derivatives incorporating different functional groups. The "tacn-orthoamide" was initially used effectively for the synthesis of monofunctionalized "tacn" derivatives.[22] Then, many applications of this general reaction scheme apeared with a simple change in the type of azacrown substituent.[23] Subsequent simple modifications of this procedure involve introduction of the monoamidinium salt of phthalimide-tacn as precursors to "tacn" with two different pendant arms with a potential for intermolecular cross-linking.[24] These derivatives we found to be basic model compounds for the search for an expanded generally applicable synthetic approach.

Here, we have demonstrated a new and practical synthetic method utilizing the "double selective asymmetric functionalization" of "tacn" (Scheme 1). This was done in the first selective stage (I) of synthesis, by adapting "tacn orthoamide" and taking advantage of self-protection of azacrown after revealing a masked formyl group resulting from the

SCHEME 1. Application of the Methodology to the Synthesis of 1,4,7-Triazonane Derivatives with Aminoalkyl Linker and Two Pendant Arms



At each stage of the synthesis: 100% conversion. I and II denote the two major selective stages of synthesis.

alkylation reaction. In the second selective stage (II) of synthesis, this was done by differentiation of amine groups by trifluoroacetylation, i.e., by selective monotrifluoroacetylation of primary over secondary amine and subsequent attachment of the third pendant arm, or by protection of the secondary amine. Our synthetic methodology allows the synthesis of a new type of triazamacrocyclic chelate that can possess an amino-terminated linker of variable length, introduced into the structure in order to increase the mobility of the final molecule and retain protein functionality, and one or two pendant arms. In our approach the "tacn" derivative is the key reaction product at every step of

(18) Luk, Y.-Y.; Tingey, M. L.; Hall, D. J.; Israel, B. A.; Murphy, C. J.; Bertics, P. J.; Abbott, N. L. *Langmuir* **2003**, *19*, 1671–1680.

(19) (a) Yang, R.; Zompa, L. J. *Inorg. Chem.* **1976**, *15*, 1499–1502. (b) Wainwright, K. P. *Coord. Chem. Rev.* **1997**, *166*, 35–90.

(20) (a) Kovacs, Z.; Sherry, A. D. *Tetrahedron Lett.* **1995**, *36*, 9269–9272. (b) Pulacchini, S.; Watkinson, M. *Tetrahedron Lett.* **1999**, *40*, 9363–9365. (c) Ellis, D.; Farrugia, L. J.; Peacock, R. D. *Polyhedron* **1999**, *18*, 1229–1234. (d) Biot, C.; Dessolin, J.; Ricard, I.; Dive, D. *J. Organomet. Chem.* **2004**, *689*, 4678–4682.

(21) Nonat, A.; Gateau, C.; Fries, P. H.; Mazzanti, M. *Chem.—Eur. J.* **2006**, *12*, 7133–7150.

(22) (a) Weisman, G. R.; Vachon, D. J.; Johnson, V. B.; Gronbeck, D. A. *J. Chem. Soc., Chem. Commun.* **1987**, *12*, 886–887. (b) Alder, R. W.; Mowlam, R. W.; Vachon, D. J.; Weisman, G. R. *J. Chem. Soc., Chem. Commun.* **1992**, *6*, 507–508. (c) Schulz, D.; Weyhermüller, T.; Wieghardt, K.; Nuber, B. *Inorg. Chim. Acta* **1995**, *240*, 217–229. (d) Stockheim, C.; Hoster, L.; Weyhermüller, T.; Wieghardt, K.; Nuber, B. *J. Chem. Soc., Dalton Trans.* **1996**, *23*, 4409–4416. (e) Blake, A. J.; Fallis, I. A.; Gould, R. O.; Parsons, S.; Ross, S. A.; Schröder, M. *J. Chem. Soc., Dalton Trans.* **1996**, 4379–4387.

(23) For example: (a) Ellis, D.; Farrugia, L. J.; Hickman, D. T.; Lovatt, P. A.; Peacock, R. D. *Chem. Commun.* **1996**, *15*, 1817–1818. (b) Blake, A. J.; Fallis, I. A.; Heppeler, A.; Parsons, S.; Ross, S. A.; Schröder, M. *J. Chem. Soc., Dalton Trans.* **1996**, *1*, 31–43. (c) Creaser, S. P.; Lincoln, S. F.; Pyke, S. M. *J. Chem. Soc., Perkin Trans. 1* **1999**, *9*, 1211–1213. (d) McGowan, P. C.; Podesta, T. J.; Thornton-Pett, M. *Inorg. Chem.* **2001**, *40*, 1445–1453.

(24) (a) Warden, A.; Graham, B.; Hearn, M. T. W.; Spiccia, L. *Org. Lett.* **2001**, *3*, 2855–2858. (b) Belousoff, M. J.; Battle, A. R.; Graham, B.; Spiccia, L. *Polyhedron* **2007**, *26*, 344–355.

(25) (a) For example: Pale-Grosdemange, C.; Simon, E. S.; Prime, K. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1991**, *113*, 12–20. (b) For example: Hoffmann, Ch.; Tovar, G. E. M. *J. Colloid Interface Sci.* **2006**, *295*, 427–435. (c) Yam, C. M.; Lopez-Romero, J. M.; Gu, J.; Cai, C. *Chem. Commun.* **2004**, 2510–2511. (d) Lasseter, T. L.; Clare, B. H.; Abbott, N. L.; Hamers, R. J. *J. Am. Chem. Soc.* **2004**, *126*, 10220–10221.

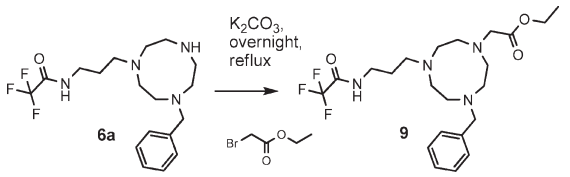
synthesis, without byproduct with another distribution pattern of substituents. The usefulness of these ligands has been further demonstrated by the synthesis of a "tacn" analogue linked to OEG-alkene, which is commonly used as a precursor in the synthesis of OEG terminated alkanethiols[25a] or silanes[25b] for SAMs formation and can be directly and readily used for functionalization of hydrogen-terminated silicon[25c,d] and diamond surfaces[25d] for SAMs formation.

We assume that all synthetic steps in our approach should meet some of the demands of "positional synthesis" (mechanosynthesis), i.e., the synthetic route should minimalize unwanted reactions and strongly favor desired reactions and should yield exclusively the desired product in a highly controlled fashion. The synthesis of "tacn"[26] and "tacn-orthoamide" (**1**)[27] is readily accomplished by standard procedures. Compund **8a** was prepared according to the synthetic strategy outlined in Scheme 1, starting with "tacn orthoamide" (**1**). The compounds **2**−**5a** were synthesized following known methods, i.e., "tacn" monofunctionalization method,[22] azacrown amine alkylation after revealing the formyl group.[22e,23,35] Compounds **2**−**5a** have been previously described.[24]

Selective protection of primary amine in the presence of secondary amines without requirement for a chromatographic separation of mono- and diprotected mixtures of "tacn" compounds was best accomplished by the reaction of **5a** with ethyl trifluoroacetate[28] in methanol solution in the presence of an amine base. No trace of a ditrifluoroacetylated byproduct was detected after reaction at −10 to 0 °C. The Boc protected "tacn" derivative **7a** was prepared by reaction of **6a** with Boc anhydride in THF. The resulting compound was treated with NaOH in water/MeOH at rt in order to remove the trifluoroacetyl protecting group, leading to free aminopropyl linker substituted "tacn" derivative **8a**.[29]

Removal of the trifluoroacetyl group requires treatment with a base[30a−c] or reductive deprotection with $NaBH_4$ in ethanol.[30a,d] The "tacn" derivative bearing the methyl ester pendant arm **7b** was synthesized in search of appropriate deprotection conditions for the trifluoroacetyl moiety in the presence of acid/base sensitive methyl ester group. The sodium borohydride/ethanol system,[31] similar to the K_2CO_3/water/methanol system,[32] is inappropriate in the case of the methyl ester group. A similar procedure starting with amidinium salt **2**, but differing in the use of ethyl bromoacetate instead of benzyl chloride, was adopted for preparation of **8b** (Scheme 1). Accordingly, the secondary amino group in azacrown **3** was alkylated with ethyl bromoacetate yielding compound **4b**. Compound **7b** was obtained in three consecutive steps: hydrolysis, monotrifluoroacetylation, and Boc protection. We found that sodium borohydride in THF/absolute ethanol removed the trifluoroacetyl group effectively in compound **7b**, but also reduced the methyl ester pendant arm group to alcohol.[33] Therefore, deprotection was performed by using $NaBH_4$ in THF at reflux, which afforded compound **8b**. These conditions did not affect the methyl ester functionality in the molecule.[34]

SCHEME 2. Alternative Functionalization Route



To check the possibility of derivatization of **6a** via an alkylation reaction with ethyl bromoacetate,[35] the route presented in Scheme 2 was performed. The "tacn" derivative **6a** was functionalized with the methylenecarboxylate group, instead of the Boc group introduced on the "tacn" ring previously, giving compound **9**.[36] This approach illustrated the suitability of monotrifluoroacetylated "tacn" derivatives as precursors to asymmetrically N-functionalized azamacrocycles with different pendant arms.

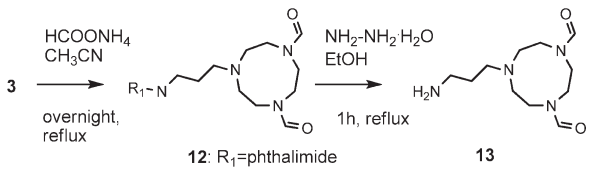
In exploring the alternative synthetic strategy to compounds with better solubility in aqueous buffers usually used for protein maintenance, we propose a reaction sequence in which we take advantage of self-protection of "tacn" by a formyl group. The synthesis of **11** followed the shortened route presented in Scheme 1. Thus, monoformyl "tacn" with phthalimide-protected aminopropyl linker and methylenecarboxylic acid *t*-Bu ester pendant arm **10** was obtained after four steps. Removal of the phthalimide protecting group by hydrazine afforded the "tacn" derivative with free unprotected aminopropyl linker **11**.[37] These conditions did not affect the more stable *t*-Bu ester function in the molecule, but suffer from the possibility of deprotection of the formyl group by hydrazine derivatives.[38]

"Bis-formyl-tacn" derivative **13** was obtained[39] in a similar way after convenient formylation with ammonium formate[40] and subsequent hydrazinolysis (Scheme 3).

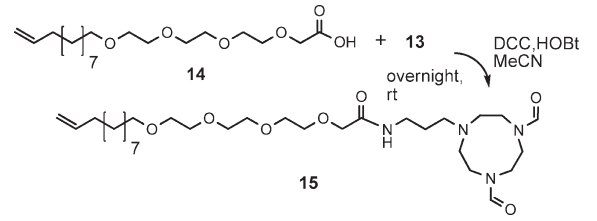
The aminoalkyl functionalized "tacn" ligands are important intermediates for the synthesis of azamacrocycle terminated

(26) Richman, J. E.; Atkins, T. J. *J. Am. Chem. Soc.* **1974**, *96*, 2268–2270.
(27) Atkins, T. J. *J. Am. Chem. Soc.* **1980**, *102*, 6364–6365.
(28) Xu, D.; Prasad, K.; Repic, O.; Blacklock, T. J. *Tetrahedron Lett.* **1995**, *36*, 7357–7360.
(29) In NMR spectra characteristic peaks are observed (see the Supporting Information). The ^{1}H NMR spectrum indicates that **7a** is still present (5.3 (br s, CONH)). The deprotection of the *N*-trifluoroacetyl group with 1 M NaOH was sluggish; see: Rosso, V. W.; Pazdan, J. L.; Venit, J. J. *Org. Proc. Res. Dev.* **2001**, *5*, 294–298.
(30) For example: (a) Calmès, M.; Escale, F.; Rolland, M.; Martinez, J. *Tetrahedron: Asymmetry* **2003**, *14*, 1685–1689. (b) Boger, D. L.; Yohannes, D. *J. Org. Chem.* **1989**, *54*, 2498–2502. (c) Jackson, R. F. W.; Rilatt, I.; Murray, P. J. *Chem. Commun.* **2003**, 1242–1243. (d) Weygand, F.; Frauendorfer, E. *Chem. Ber.* **1970**, *103*, 2437–2449.
(31) (a) Seki, H.; Koga, K.; Matsuo, H.; Ohki, S.; Matsuo, I.; Yamada, S-i. *Chem. Pharm. Bull.* **1965**, *13*, 995–1000. (b) Brown, M. S.; Rapoport, H. *J. Org. Chem.* **1963**, *28*, 3261–3263.
(32) Kaestle, K. L.; Anwer, M. K.; Audhya, T. K.; Goldstein, G. *Tetrahedron Lett.* **1991**, *32*, 327–330.
(33) For example, CF_3CONH- and −COOMe groups are stable in the presence of $NaBH_4$/THF/H_2O; see ref 30c.
(34) Selective deprotection of the trifluoroacetyl group in the presence of intact methyl ester group was also achieved with NaOMe/MeOH (see ref 30c) and with K_2CO_3/H_2O/MeOH (see ref 30b). Nonetheless, Kudzin et al. reported that NaOMe-mediated deprotection is not effective due to the formation of stable amidate salts; see: Kudzin, Z. H.; Łyżwa, P.; Łuczak, J.; Andrijewski, G. *Synthesis* **1997**, 44–46.
(35) Schultz, R. A.; White, B. D.; Dishong, D. M.; Arnold, K. A.; Gokel, G. W. *J. Am. Chem. Soc.* **1985**, *107*, 6659–6668.
(36) In NMR spectra characteristic peaks are observed (see the Supporting Information).
(37) The structure of this compound was analyzed by 2D NMR, IR, and ESI-MS as well (see the Supporting Information).
(38) Geiger, R.; Siedel, W. *Chem. Ber.* **1968**, *101*, 3386–3391.
(39) The structure of this compound was analyzed by 2D NMR, IR, and ESI-MS as well (see the Supporting Information).
(40) Reddy, P. G.; Kumar, G. D. K.; Baskaran, S. *Tetrahedron Lett.* **2000**, *41*, 9149–9151.

SCHEME 3. Synthetic Route to the Bis-Formyl "tacn" Derivatives



SCHEME 4. Representative Synthesis of "Tacn"-Terminated OEG-Alkene



oligo(oxyethylene)alkyl derivatives. The synthesized aminopropyl linker functionalized azamacrocyclic ligand **13** was coupled to tri(ethylene glycol)-alkene with carboxyl functionality **14** via amide linkage to give the corresponding structure **15**.[41] Couplings of the "tacn" ligand **13** to carboxylic acid (**14**) was carried out with standard reagents DCC (or EDC) and HOBt in acetonitrile (Scheme 4).

ESI-MS and $^{1}H-^{13}C$ gHSQC NMR spectroscopy were applied to monitor the synthesis process. In the HSQC spectra, characteristic signals are clearly distinguishable from resonances of impurities. The room temperature ^{1}H NMR spectra of formyl protected "tacn" derivatives have double and/or broad signals due to the slow NMR time scale of hindered rotation around the exocyclic N−CO bond.[22e,24a] We observed that trifluoroacetyl- and Boc-protected "tacn" derivatives exhibit similar multiplication and broadening of signals. The restricted rotation about the N-CO/N=CO bond[42] and occurrence of conformational isomerism was described for trifluoroacetamides[43] and Boc-protected amines.[44] These conformers of "tacn" derivatives exist in solution and therefore assignment by NMR was not easy. Broadening of NMR signals can also be attributed at least in part to the heterogenity of the sample. Moreover, the structural analysis by NMR is complicated by inversion of the azamacrocycle ring,[21,45] signals broadening and affecting chemical shifts upon complexation of the cations,[46] formation of inter- and/or intramolecular hydrogen bonds,[47] splitting/broadening of signals caused by long-range fluorine coupling (J_{FC} and J_{FH}) in trifluoroacetamide derivatives,[48] the NMR shielding effect of a benzene ring, occurrence of CH-π interaction between the benzene ring and −CH protons,[49] and appearance of *t*-Bu in a Boc group as two singlets (or three, two of which may coincide) in the ^{1}H NMR spectrum.[50] Thus, the structure of synthesized compounds was determined in a straightforward manner by using one-bond ($^{1}H-^{13}C$) correlation NMR spectra and confirmed by the results of ESI mass spectrometric analysis. Nevertheless, for unambiguous assignment of all resonances to proper conformers, additional NMR correlation spectra, especially NOESY, and 1D-NOE difference NMR spectra are desirable.

Therefore, in order to clearly show the selectivity of the trifluoroacetylation stage, resulting in a lack of ditrifloroacetylated product, electrospray ionization mass spectrometry was applied (see the Supporting Information). ESI-MS is the technique of choice for solution mechanistic studies in chemistry and biochemistry.[51]

In summary, we developed a general method for the synthesis of selectively N-functionalized "tacn" with aminoalkyl linker. Our approach is not only effective, but also offers attractive features such as operational simplicity and convenience, starting material availability, mild reaction conditions, air-stability of all synthetic steps, and reduced number of chromatographic separations. The double selective synthetic method allows for the ordered multistep synthesis of "tacn" ligands. These ligands are ideal candidates for attachment, via the standard carbodiimide coupling, to carboxy-terminated oligo(oxyethylene)alkyl derivatives and facilitate access to "tacn"-functionalized SAMs.

Experimental Section

General Procedure for the Selective Trifluoroacetylation. Ethyl trifluoroacetate (1.2 equiv) was added dropwise to a solution of the aminoalkyl-"tacn" (1 equiv) and triethylamine (1 equiv) in anhydrous methanol at −10 to 0 °C. The resulting solution was stirred for 2 h. Subsequently, the reaction mixture was stirred overnight at ambient temperature. The solvent was then removed under reduced pressure to afford monotrifluoroacetylated compound. ESI-MS clearly shows the selectivity of the trifluoroacetylation reaction step.

Data for **6a**: HRMS (ESI) calcd for $C_{18}H_{28}F_3N_4O$ [$(M + H)^+$]:373.22152, found 373.22148.

Acknowledgment. ESI-MS and NMR experiments were performed by a local facility.

Supporting Information Available: Experimental procedures and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

(41) (a) The structure of this compound was analyzed by 2D NMR, IR, and ESI-MS as well (see the Supporting Information. (b) The "tacn"-OEG-alkene was further thioacetylated (AcSH, AIBN). Subsequent smooth deprotection of formyl and acetyl groups was performed under acidic conditions (see the Supporting Information).

(42) Stewart, W. E.; Siddall, T. H. *Chem. Rev.* **1970**, *70*, 517–551.

(43) (a) Suarez, C.; Nicholas, E. J.; Bowman, M. R. *J. Phys. Chem. A* **2003**, *107*, 3024–3029. (b) Graham, L. L. *Org. Magn. Reson.* **1971**, *4*, 335–342. (c) Massey, A. P.; Sigurdsson, S. Th. *Nucleic Acids Res.* **2004**, *32*, 2017–2022.

(44) Smith, B. D.; Goodenough-Lashua, D. M.; D'Souza, C. J. E.; Norton, K. J.; Schmidt, L. M.; Tung, J. C. *Tetrahedron Lett.* **2004**, *45*, 2747–2749.

(45) Kleinpeter, E.; Holzberger, A. *Tetrahedron* **2006**, *62*, 10237–10247.

(46) Benniston, A. C.; Gunning, P.; Peacock, R. D. *J. Org. Chem.* **2005**, *70*, 115–123.

(47) Sessler, J. L.; Sibert, J. W.; Burrell, A. K.; Lynch, V.; Markert, J. T.; Wooten, C. L. *Inorg. Chem.* **1993**, *32*, 4277–4283.

(48) Everett, J. R.; Davies, J. S.; Tyler, J. W. *J. Chem. Soc., Perkin Trans. II* **1986**, 349–353.

(49) (a) Suezawa, H.; Hashimoto, T.; Tsuchinaga, K.; Yoshida, T.; Yuzuri, T.; Sakakibara, K.; Hirota, M.; Nishio, M. *J. Chem. Soc., Perkin Trans. 2* **2000**, 1243–1249. (b) Evans, D. J.; Junk, P. C.; Smith, M. K. *New J. Chem.* **2002**, *26*, 1043–1048.

(50) (a) Dugave, C.; Demange, L. *Chem. Rev.* **2003**, *103*, 2475–2532. (b) Wróblewski, A. E.; Piotrowska, D. G. *Tetrahedron* **1998**, *54*, 8123–8132. (c) Wallen, E. A. A.; Christiaans, J. A. M.; Gynther, J.; Vepsalainen, J. *Tetrahedron Lett.* **2003**, *44*, 2081–2082.

(51) For a review see: (a) Santos, L. S.; Knaack, L.; Metzger, J. O. *Int. J. Mass Spectrom.* **2005**, *246*, 84–104. (b) Eberlin, M. N. *Eur. J. Mass Spectrom.* **2007**, *13*, 19–28.