DOI: 10.1002/ejoc.200901326

Chiral Isocyanoazides: Efficient Bifunctional Reagents for Bioconjugation

Valentine G. Nenajdenko,*[a] Anton V. Gulevich,[a] Nadezhda V. Sokolova,[a] Andrey V. Mironov,[a] and Elizabeth S. Balenkova[a]

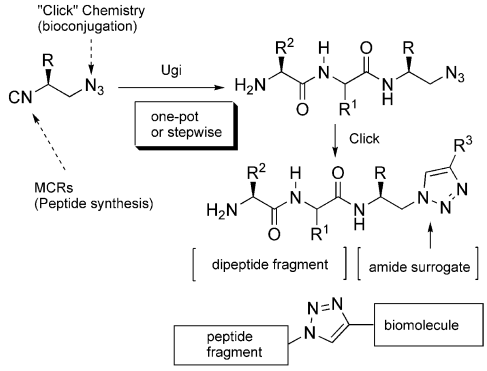
Keywords: Isocyanides / Azides / Multicomponent reactions / Click chemistry / Bioconjugation

Chiral scaffolds combining isocyanide and azide groups can be effectively synthesized to permit the efficient construction of both amino (hydroxy) acids and triazole derivatives, which enables the preparation of hybrid peptide molecules by Ugi/click or click/Ugi strategies.

Introduction

The unusual valence structure of the isocyanide group and the unique reactivity of isocyanides[1] lend themselves for the development of multicomponent reactions (MCRs),[2] such as the Ugi and Passerini reactions, which are especially effective for synthesis of amino and hydroxy acids, peptides, and depsipeptides.[3,4] The azide functional group is also highly reactive and has found many synthetic applications. In particular, organic azides serve as valuable intermediates in combinatorial chemistry and in the synthesis of peptides and other nitrogen-containing hetero- and alicyclic compounds.[5] The discovery of the "click" Cu^{I}-catalyzed Huisgen 1,3-dipolar cycloaddition reaction between acetylenes and azides opens a very simple and effective route to the regioselective synthesis of 1,2,3-triazoles.[6] Click chemistry was demonstrated to be useful for the assembly of functional units into one molecule by using the 1,2,3-triazole moiety as a fastening knot.[7]

The simultaneous incorporation of isocyanide and azide functionalities into one substrate permits one to combine the transformation of the isocyanide group into a peptide fragment with connection by an azide group to another valuable fragment (Scheme 1). Moreover, the 1,2,3-triazolyl moiety, readily available by the click chemistry protocol, was found to be an effective amide bond surrogate that can mimic peptides.[8] Therefore, triazolo-modified peptides can serve as efficient peptidomimetics with improved medicinal properties such as solubilization, hydro(lipo)philicity, enzyme binding, and transport of biomolecules in living systems (modified lipids, sugars, etc).[9]



Scheme 1. Isocyanoazides – precursors for the synthesis of "clickable" peptides.

Natural proteins such as glycoproteins, lipoproteins, and protein–porphyrin complexes (myoglobin, hemoglobin) often function as complex aggregates that incorporate other fragments. Artificial conjugated biopolymers consisting of a protein part connected with other biomolecules by a triazole moiety can be used as blood components,[10] anticancer medications,[11] and HIV protease inhibitors.[12] Therefore, a facile approach to the ligation of proteins, peptides, or their mimics with biologically important molecules or polymers is extremely desirable.[13]

Another prospective application of isocyanoazides is in supramolecular chemistry and related fields. Such common functional units as fullerenes, porphyrins, phthalocyanines, calixarenes, crown ethers, polyaromatics, carboranes, and ferrocene can be equipped with a chiral peptide backbone. These hybrid molecules would offer interesting possibilities for advanced structural design, such as self-assembly to chiral helices.

Isocyanides[14] and azides[15] also play important roles in coordination chemistry. Therefore, isocyanoazides can be used as efficient ligands for the synthesis of new promising

[a] Department of Chemistry, Moscow State University, Leninskije Gory, 199991 Moscow, Russia
Fax: +7-495-9328846
E-mail: nen@acylium.chem.msu.ru
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.200901326.

complexes. Moreover, polymerization of isocyanoazides opens broad possibilities for preparation of azido-modified polymeric materials.[16]

Several examples of azido-substituted amino acids[17] and azido acids[18] appeared recently in the literature as prospective building blocks for the synthesis of peptides. However, use of isocyanoazides opens the way to a more diverse approach owing to the possibility of constructing modified peptide molecules with both natural and unnatural amino acids in one step by divergent synthesis. Currently, there are only two literature examples of a molecule having both isocyano and azide groups. Zhu et al. developed a molecule in which isocyanide and azide groups are connected by an eight-atom chain.[19] Michelin et al. used 2-(azidomethyl)-phenyl isocyanide as a ligand for transition metals.[20]

In this paper, we report the synthesis of chiral scaffolds with isocyanide and azide groups in one molecule as a new type of linker permitting the construction of both amino (hydroxy) acids and triazole derivatives and the preparation of peptide-modified molecules by Ugi/click or click/Ugi strategies.

Results and Discussion

The starting point of the work was to develop an efficient, universal, and atom-economic strategy for synthesis of isocyanoazides from commercially available L-amino alcohols **1** (Scheme 2). The key transformation is a "one-pot" sequence in which the formation of mesylates is followed by dehydration of the formamide moiety to give isocyanides **4** in excellent yields. Isocyanides **4** containing a good leaving group undergo facile nucleophilic substitution with sodium azide to afford target isocyanoazides **5** in good yields.

It is noteworthy that, despite their high nitrogen content (for **5a**: $C_3H_4N_4$, N 58.3%), isocyanoazides are quite stable compounds at the room temperature and can be distilled in vacuo below 50 °C.[21] NMR spectroscopic and GC analyses of isocyanide **5d** prepared from L-isoleucinole (containing an additional stereocenter) showed a stereochemical purity higher than 99% *de*, indicating that no racemization had occurred during the steps of the synthesis.

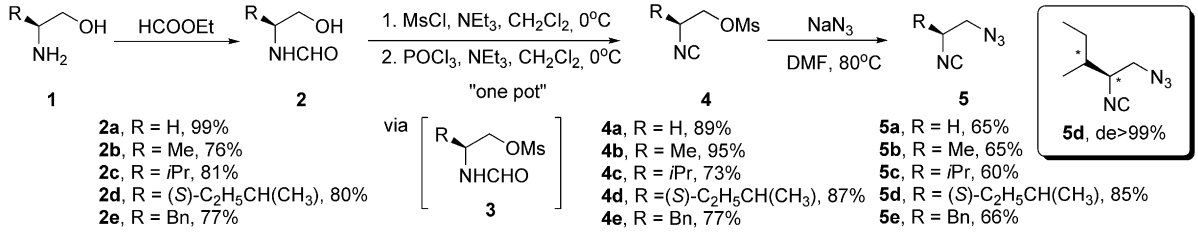
The optical purity of the isocyanoazides was further confirmed by Ugi reactions with D-α-phenylethylamine (Table 1). Presence of a single set of signals in ^1H and ^{13}C NMR spectra of products **6b**–**e** (Table 1, entries 2–5) indicates the formation of a single diastereomer. The Ugi and Passerini reactions were performed with different substrates to demonstrate the high efficiency and synthetic utility of isocyanoazides **5a**–**e**. In all cases, the corresponding products **6** and **8** were obtained in good yields (Table 1, Scheme 3).

The presence of an azido group in the products **6** of the Ugi reaction allows subsequent triazole formation affording 1,2,3-triazolyl-substituted peptides **7**. The click reaction proceeds regioselectively in high yields with a model acetylene (phenyl propargyl ether). The best yield was obtained with $CuI{\cdot}P(OEt)_3$ as a catalyst. Consequently, we have developed an "Ugi/click" strategy for the synthesis of triazolo-modified peptide molecules **7**. It is noteworthy that the Ugi reaction and subsequent cycloaddition can be performed in a "one-pot" tandem reaction sequence without decreasing the yields (as an example, **7a** can be obtained in 50% yield).

Isocyanoazides **5** themselves are promising compounds for the preparation of chiral isocyanotriazoles by click chemistry. However, we found that it is impossible to involve **5** in a click reaction directly (triazole **11** is not formed): apparently, formation of stable Cu^I complexes **9** takes place under these reaction conditions. To confirm this suggestion, we have synthesized complexes **9a**,**b** in excellent yields. This observation demonstrates the bidentate nature of **5** as a new type of chiral ligand (Scheme 4).

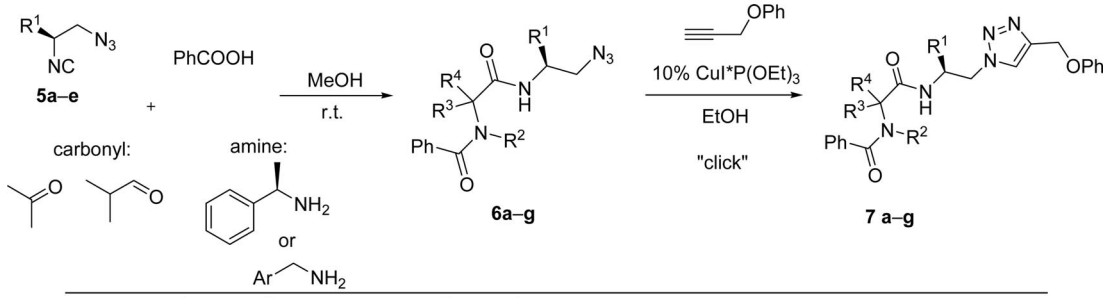
According to the X-ray data, **9a** packs as a coordination polymer in which copper is coordinated strongly to both the isocyanide carbon and the α-nitrogen of the azide group (Figure 1). It is noteworthy that azides usually coordinate Cu^I through a terminal γ-nitrogen with a bond angle of approximately 180°,[15] whereas, in the case of **9a**, coordination by α-nitrogen takes place. This can be attributed to the effect of chelation with a second isocyanoazide molecule via a 16-membered cycle. Therefore, due to their bifunctional nature, isocyanoazides **5** are promising ligands for transition metals.

Copper complexes **9** by themselves are not effective self-catalysts for the Huisgen 1,3-dipolar cycloaddition. However, formation of isocyanotriazoles **10** from azide complexes **9** readily occurs under standard conditions with external Cu^I catalysis. Triazoles **10** can be isolated quantitatively as copper complexes. Deprotected isocyanotriazoles **11** are obtained by treatment with a strong Cu^I complexation agent. We found $Na_2S_2O_3$ to be a very practical new reagent for the deprotection, which is superior to the highly toxic cyanides used in the literature. Consequently, com-



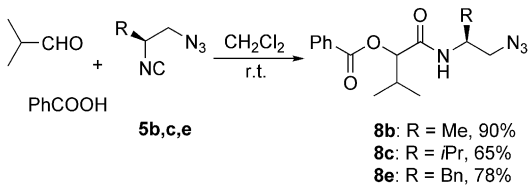
Scheme 2. Synthesis of isocyanoazides.

Table 1. Synthesis of modified amides.

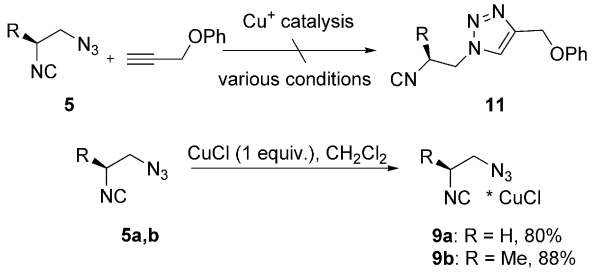


Entry	R^1	R^2	R^3	R^4	Product	Yield [%]	Product	Yield [%]
1	H	*(S)*-Ph(Me)CH	Me	Me	**6a**	65	**7a**	80
2[a]	Me	*(S)*-Ph(Me)CH	Me	Me	**6b**	51	**7b**	70
3[a]	*i*Pr	*(S)*-Ph(Me)CH	Me	Me	**6c**	48	**7c**	75
4[a]		*(S)*-Ph(Me)CH	Me	Me	**6d**	63	**7d**	80
5[a]	Bn	*(S)*-Ph(Me)CH	Me	Me	**6e**	53	**7e**	70
6[b]	H	4-$MeOC_6H_4CH_2$	H	*i*Pr	**6f**	93	**7f**	88
7[b]	Me	Bn	H	*i*Pr	**6g**	80	**7g**	79

[a] *de* > 99%. [b] *dr* ≈ 1:1.



Scheme 3. Passerini reaction with isocyanoazides.



Scheme 4. Synthesis of Cu isocyanoazide complexes.

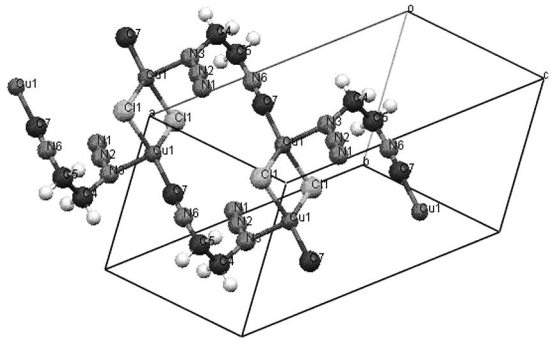
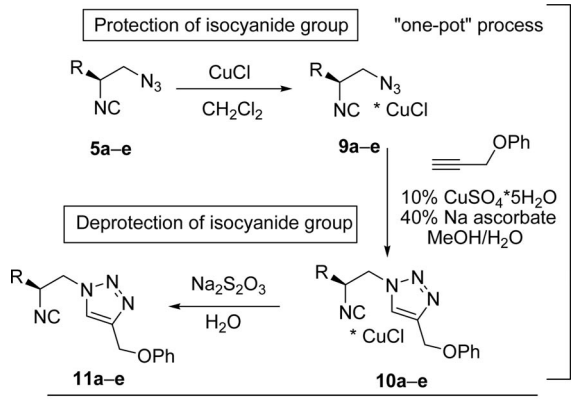


Figure 1. X-ray analysis of complex **9a**.[22]

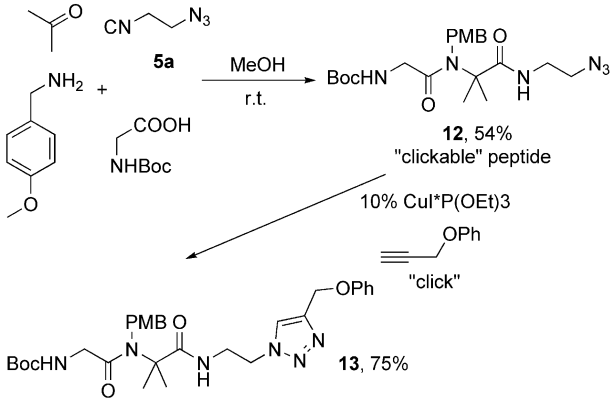
plexation of isocyanide with Cu^I can be used as a protection strategy for the isocyanide residue. A number of triazole-containing isocyanides **11** was obtained in good yields by this simple "one-pot" procedure (Table 2).

Table 2. Synthesis of modified amides.



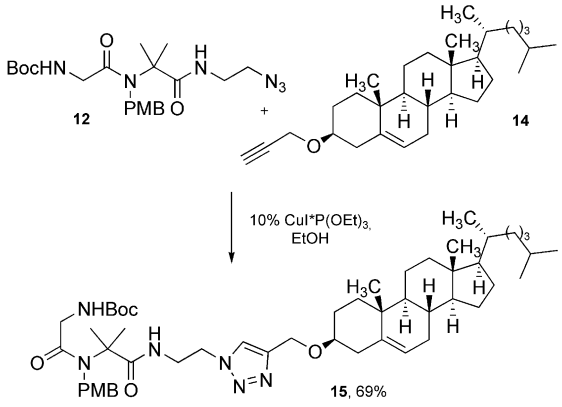
Entry	R	Product	Yield [%]
1	H	**11a**	77
2	Me	**11b**	89
3	*i*Pr	**11c**	50
4		**11d**	60
5	Bn	**11e**	60

The Ugi reaction with Boc-glycine allows one to obtain "clickable" peptide **12** containing the azide "fastener". Subsequent cycloaddition leads to triazole-containing peptide **13** in a good yield (Scheme 5). It should be noted that isocyanides **11** also can undergo multicomponent reactions to afford triazolo-modified peptide molecules like **13**.



Scheme 5. Synthesis of "clickable" and triazolo-modified peptides.

Furthermore, this strategy is effective for the conjugation of a peptide fragment with other biomolecules. As an example, we synthesized cholesterol derivative **15**, consisting of peptide and cholesterol, connected by a triazole linker, by cycloaddition of clickable peptide **12** to propargyl cholesterol **14**.[23] Binding of this peptide fragment to cholesterol changed the properties of this extremely important biomolecule dramatically, which can be useful for the creation of new potent drugs (Scheme 6).[24]



Scheme 6. Bioconjugation of peptide **12** and cholesterol.

"Clickable" peptides similar to **12** can be used for subsequent bioconjugation by using not only click chemistry but also Staudinger ligation and other reactions. Further investigations into the synthesis and modifications of "clickable" peptides will be published in due course.

Conclusions

In summary, we have suggested an efficient route to chiral isocyanides containing the azide functionality. Ugi and Passerini reactions of azidoisocyanide afford azide-containing derivatives of amino and hydroxy acids, which are suitable for the subsequent formation of corresponding triazole derivatives in good yields. It was found that azidoisocyanides in protected form in Cu^{I} complexes can still undergo Huisgen cycloaddition. Complexation of the isocyanide group with CuCl followed by the treatment with $Na_2S_2O_3$ was demonstrated to be an efficient protection/deprotection protocol for this valuable functionality. The chemistry described in this work opens access to "clickable" peptides equipped with the azide "fastener". We also demonstrated that azidoisocyanides are effective for the divergent synthesis of hybrid peptide molecules.

Supporting Information (see footnote on the first page of this article): Full experimental details and copies of NMR spectra.

Acknowledgments

This work was financially supported by Russian Federal Agency of Education, Grant P2063. We thank Dr. B. V. Lokshin for the optical rotation measurements.

[1] a) I. Ugi in *Isonitrile Chemistry*, Academic Press, New York and London, **1971**; b) I. A. O'Neil "*Isocyanides and their Heteroanalogues* (RZC)"; in *Comprehensive Organic Functional Group Transformations*, **2003**, Vol. 3, Part IV, 3.21, pp. 693–726.

[2] a) A. Dömling, I. Ugi, *Angew. Chem. Int. Ed.* **2000**, *39*, 3168–3270; b) J. Zhu, H. Bienamé (Eds.), *Multicomponent Reactions*, Wiley-VCH, Weinheim, **2005**; c) A. Dömling, *Chem. Rev.* **2006**, *106*, 17–89; d) J. Zhu, *Eur. J. Org. Chem.* **2003**, 1133–1144; e) L. El Kaim, L. Grimaud, *Tetrahedron* **2009**, *65*, 2153–2171; f) I. Akritopoulou-Zanze, *Curr. Opin. Chem. Biol.* **2008**, *12*, 324–331; g) C. Hulme, J. Dietrich, *Mol. Divers.* **2009**, *13*, 195–209; h) L. A. Wessjohan, D. G. Rivera, O. E. Vercillo, *Chem. Rev.* **2009**, *109*, 796–814.

[3] a) I. Ugi, D. Marquarding, R. Urban, *Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins* (Ed.: B. Weinstein), Marcel Dekker, New York **1982**, vol. 6, pp. 245–289; b) Ugi, A. Dömling, B. Werner, "*Synthesis of Peptides and Peptidomimetics*" in *Methods of Organic Chemistry (Houben-Weyl), Vol. E22* (Ed.: M. Goodman), ch. 4 (Multicomponent Reactions), **2002**, Thieme Verlag, Stuttgart.

[4] Recent examples of peptide and depsipeptide synthesis by MCR: a) A. V. Gulevich, N. E. Shevchenko, E. S. Balenkova, G.-V. Röschenthaler, V. G. Nenajdenko, *Synlett* **2009**, 403–406; b) M. Mroczkiewicz, R. Ostaszewski, *Tetrahedron* **2009**, *65*, 4025–4034; c) A. G. Zhdanko, A. V. Gulevich, V. G. Nenajdenko, *Tetrahedron* **2009**, *65*, 4629–4702; d) A. V. Gulevich, I. V. Shpilevaya, V. G. Nenajdenko, *Eur. J. Org. Chem.* **2009**, 3801–3808; e) A. G. Zhdanko, A. V. Gulevich, V. G. Nenajdenko, *J. Org. Chem.* **2009**, *74*, 884–887.

[5] S. Bräse, G. Gil, K. Knepper, V. Zimmermann, *Angew. Chem. Int. Ed.* **2005**, *44*, 5188–5240.

[6] a) H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem. Int. Ed.* **2001**, *40*, 2004–2021; b) V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem. Int. Ed.* **2002**, *41*, 2596–2599; c) C. W. Tornøe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057–3064; d) J. E. Moses, A. D. Moorhouse, *Chem. Soc. Rev.* **2007**, *36*, 1249–1262.

[7] M. Meldal, C. W. Tornøe, *Chem. Rev.* **2008**, *108*, 2952–3015.

[8] See for example: a) A. Brik, J. Alexandratos, Y.-C. Lin, J. H. Elder, A. J. Olson, *ChemBioChem* **2005**, *6*, 1167–1169; b) Ahsanullah, P. Schmieder, R. Kühne, J. Rademann, *Angew. Chem. Int. Ed.* **2009**, *48*, 5042–5045.

[9] See for example: a) H. C. Kolb, K. B. Sharpless, *Drug Discovery Today* **2003**, *8*, 1128–1137; b) N. G. Angelo, P. S. Arora, *J. Am. Chem. Soc.* **2005**, *127*, 17134–17135.

[10] N. Miller, G. M. Williams, M. A. Brimble, *Org. Lett.* **2009**, *11*, 2409–2412.

[11] Q. Wan, J. Chen, G. Chen, S. J. Danishefsky, *J. Org. Chem.* **2006**, *71*, 8244–8249.

[12] M. Whiting, J. C. Tripp, Y.-C. Lin, W. Lindstrom, A. J. Olson, J. H. Elder, K. B. Sharpless, A. V. Fokin, *J. Med. Chem.* **2006**, *49*, 7697–7710.
[13] C. P. R. Hackenberger, D. Schwarzer, *Angew. Chem. Int. Ed.* **2008**, *47*, 10030–10074.
[14] T. Saegusa, Y. Ito, *Synthesis* **1975**, 291–300.
[15] S. Cenin, E. Gallo, A. Caselli, F. Ragaini, S. Fantauzzi, C. Piangiolino, *Coord. Chem. Rev.* **2006**, *250*, 1234–1253.
[16] M. Suginome, Y. Ito, *Adv. Polym. Sci.* **2004**, *171*, 77–136.
[17] a) A. J. Link, M. K. S. Vink, D. A. Tirrell, *J. Am. Chem. Soc.* **2004**, *126*, 10598–10602; b) A. Le Chevalier Isaad, F. Barbetti, P. Rovero, A. M. D′Ursi, M. Chelli, M. Chorev, A. M. Papini, *Eur. J. Org. Chem.* **2008**, 5308–5314; c) A. Le Chevalier Isaad, A. M. Papini, M. Chorev, P. Rovero, *J. Pept. Sci.* **2009**, *15*, 451–454.
[18] See for example: a) V. D. Bock, D. Speijer, H. Hiemstra, J. H. van Maarseveen, *Org. Biomol. Chem.* **2007**, *5*, 971–975; b) E. Balducci, L. Belucci, E. Petricci, M. Taddei, A. Tafi, *J. Org. Chem.* **2009**, *74*, 1314–1321.
[19] T. Pirali, G. C. Tron, J. Zhu, *Org. Lett.* **2006**, *8*, 4145–4148.
[20] a) G. Facchin, R. A. Michelin, M. Mozzon, M. S. Pugliese, P. Sgarbossa, A. Tassan, *Inorg. Chem. Commun.* **2002**, *5*, 915–918; b) M. Basato, G. Facchin, R. A. Michelin, M. Mozzon, M. S. Pugliese, P. Sgarbossa, A. Tassan, *J. Organomet. Chem.* **2005**, *690*, 5414–5420.
[21] Attention: Heating isocyanoazides **5** above 60 °C can provoke an explosion. It is better to store isocyanoazides in a refrigerator at –20 °C to avoid decomposition.
[22] Suitable crystals of **9a** were obtained as colorless needles by crystallization from methanol under an argon atmosphere. $C_3H_4ClCuN_4$; M_r = 195.08 g/mol; crystal system: monoclinic; unit cell parameters: a = 14.6298(11) Å, b = 7.7331(11) Å, c = 6.2014(11) Å; space group: $P2_1/n$; volume: 660.7 Å^3. CCDC-743396 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
[23] Propargyl cholesterol **14** was obtained by the literature procedure in: J. A. Dunn, P. L. Pauson, *J. Organomet. Chem.* **1991**, *419*, 383–389.
[24] P. Inallinella, E. Bianchi, N. A. Ladwa, Y. J. Wang, R. Hrin, M. Veneziano, F. Bonelli, T. J. Ketas, J. P. Moore, M. D. Miller, A. Pessi, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 5801–5806.

Received: November 18, 2009
Published Online: February 1, 2010

