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Synthesis and Characterization of Dicobalthexacarbonyl-Alkyne Derivatives of Amino Acids, Peptides, and Peptide Nucleic Acid (PNA) Monomers

Gilles Gasser,[†,‡] Merja A. Neukamm,[†] Alexandra Ewers,[§] Oliver Brosch,[§] Thomas Weyhermüller,[§] and Nils Metzler-Nolte*[,†]

Lehrstuhl für Anorganische Chemie I - Bioanorganische Chemie, Fakultät für Chemie and Biochemie, Ruhr-Universität Bochum, Universitätsstrasse 150, D-44801 Bochum, Germany, and Max-Planck-Institut für Bioanorganische Chemie, Stiftstrasse 34-36, D-45470 Mülheim/Ruhr, Germany

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The reaction of $Co_2(CO)_8$ with alkyne-containing amino acids [**1a:** phenylalanine (Phe) and **1b:** methionine (Met)], two suitably alkyne-functionalized derivatives of the neuropeptide enkephalin (Enk) [**3:** Ac-Enk-Prop and **5:** Ac-Enk(Pgl)-NH_2 (Ac - Acetyl; Pgl - propargylglycine; Prop - propargylamine)], a thymine Peptide Nucleic Acid (T-PNA) monomer (**7**), and a PNA-like monomer (**9**) derivative gave the respective dicobalthexacarbonyl bioconjugates in very good yields. Two different sites for labeling of the biomolecules were successfully used: The organometallic moiety was reacted with the *C*-terminus of alkyne-containing amino acids, peptide or PNA thymine monomers, and alternatively the organometallic compound was complexed to an internal site in the peptide or PNA. To this end, a simple glycine was replaced by propargylglycine in peptides, and a new alkyne-containing PNA-like monomer, in which an alkyne chain replaces the nucleobase, was used for PNA chemistry. For the synthesis of the two alkyne-containing enkephalin derivatives **3** and **5**, two different resins, namely sulfamylbutyryl and Rink amid, were used as they allow to selectively insert, on the solid phase, an alkyne moiety at the *C*-terminus and on a side-chain of a peptide sequence, respectively. The identity and constitution of all cobalt complexes were confirmed by different analytical methods (IR, FAB, ESI-MS, and NMR). Most notably, IR spectroscopy shows intensive bands in the 2100−2000 cm^{-1} region because of the $Co_2(CO)_6$ moiety. In both ^1H NMR spectra of the dicobalthexacarbonyl PNA monomer derivatives **8** and **10**, all signals are doubled because of the *cis-trans* isomerism about the central amide bond. The X-ray structure of a dicobalthexacarbonyl phenylalanine derivative (**2a**) confirms the proposed composition of the bioconjugates and shows that, as anticipated, the alkyne group of **2a** is no longer linear upon complexation in comparison to the alkyne group of the bioconjugate precursor **1a**, as indicated by a C−C≡C angle of about 143° in **2a**. Moreover, the C≡C bond of **1a** was elongated by about 0.15 Å upon Co_2 coordination.

Introduction

The synthesis of new bioorganometallics is currently a field of active research because of the potential of such compounds in areas like radio-labeling, biosensing, or medicinal chemistry.[1−15] In particular, dicobalthexacarbonyl-alkyne bioconjugates, formed by reacting $Co_2(CO)_8$ with alkyne-containing biological molecules, have attracted much attention in the view of the development of novel metal-based anticancer drugs. For example, dicobalthexacarbonyl derivatives of non-

* To whom correspondence should be addressed. E-mail: nils.metzler-nolte@rub.de. Fax: +49-(0)234-321 4378. Phone: +49-(0)234 322 8152.
† Ruhr-Universität Bochum.
‡ Alexander von Humboldt Stipendiat, gratefully thanks the AvH for funding.
§ Max-Planck-Institut für Bioanorganische Chemie.

(1) Jaouen, G. *Bioorganometallics: Biomolecules, Labelling, Medicine*; Wiley-VCH, Weinheim, 2006.
(2) Metzler-Nolte N.; Severin, K. In *Concepts and Models in Bioinorganic Chemistry*; Kraatz, H.-B., Metzler-Nolte, N., Eds.; Wiley-VCH Verlag GmbH & Co: Weinheim, Germany, 2006, pp 113−136.
(3) Metzler-Nolte, N.; Salmain, M. In *Ferrocenes: Ligands, Materials and Biomolecules*; Stepnicka, P., Ed.; John Wiley & Sons Ltd.: Chichester, U.K., 2008, pp 499−639.
(4) Schatzschneider, U.; Metzler-Nolte, N. *Angew. Chem., Int. Ed.* **2006**, *45*, 1504–1507, and references therein.
(5) Severin, K.; Bergs, R.; Beck, W. *Angew. Chem., Int. Ed.* **1998**, *37*, 1634–1654, and references therein.
(6) Metzler-Nolte, N. *Angew. Chem., Int. Ed.* **2001**, *40*, 1040–1043.
(7) Metzler-Nolte, N. *Chimia* **2007**, *61*, 736–741.

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steroidal anti-inflammatory drugs (NSAIDs), in particular an acetyl salicylic acid (ASS, Aspirin) alkyne derivative, were found to be promising lead structures with potent antiproliferative activity.[16−18] For similar purposes, $Co_2(CO)_8$ was also reacted to alkyne-containing nucleosides and fructoses by Ott, Gust, Dembinski and co-workers.[19,20] Our group recently reported the first organometallic peptide conjugate, namely, a cobaltcarbonyl-alkyne enkephalin derivative, which showed significant toxicity against two different tumor cell lines.[21] In addition to the use of dicobalthexacarbonyl bioderivatives in drug development, the intense carbonyl bands of these bioconjugates make possible their selective detection and even quantification by IR spectroscopy (carbonyl metallo immuno assay, CMIA).[22−24] In this perspective, dicobalthexacarbonyl of hormones,[25−31] peptides,[32] proteins,[33] and mycotoxins[34] were synthesized and their spectroscopic properties, as well as their binding properties in the case of hormones, studied in-depth by Salmain, Jaouen, and co-workers.

To extend the range of biomolecules containing a dicobalthexacarbonyl unit, we embarked on a program to synthesize simple amino acids, small peptides, and peptide nucleic acid monomers (PNA)[35] with the $Co_2(CO)_6$ moiety, as previously undertaken by our group with ferrocene[36−41] or/and platinum(0) complexes.[42] To the best of our knowledge, only Burger et al. reported a α-trifluormethyl substituted α-ethinyl-amino acid cobalt complex, which was then reacted, under Pauson-Khand conditions, to give the cyclopentenone substituted α-trifluoromethyl amino acid derivative.[43]

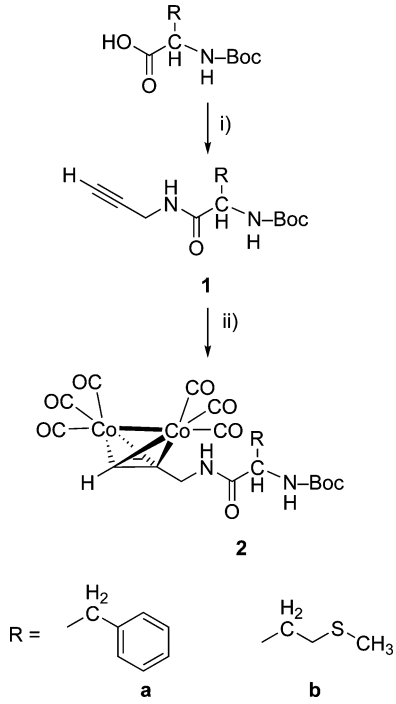
In this paper, we report our initial results on the preparation of dicobalthexacarbonyl derivatives of the amino acids phenylalanine (Phe) and methionine (Met), as well as the first crystallographic characterization of a dicobalthexacarbonyl amino acid derivative. Using solid phase peptide synthesis (SPPS) techniques, the formation of two dicobalthexacarbonyl derivatives of the neuropeptide Enkephalin (Enk) is described. Using the same chemistry, the synthesis of a dicobalthexacarbonyl thymine (T) PNA monomer is also reported. Finally, to further increase the scope of this research, the preparation of a $Co_2(CO)_6$ PNA monomer derivative starting from an alkyne-containing PNA monomer[44] is reported.

Results and Discussion

Synthesis and Characterization of Cobalt-Containing Amino Acids 2a and 2b. To synthesize the amino acid organometallic derivatives **2a** and **2b**, propargylamine was first reacted with the activated *N*-Boc protected amino acid, namely, phenylalanine (Phe) and methionine (Met) (Scheme 1). An alternative synthetic procedure to that reported by Curran et al.[45] involving the activation of the carboxylic acid group of the amino acid with isobutylchloroformiate in the presence of *N*-methyl morpholine, was used to give the alkyne-containing amino acids **1a** and **1b**, respectively. **1a** and **1b** were then reacted with $Co_2(CO)_8$, in tetrahydrofuran (THF), to yield the desired cobalt-containing compounds **2a** and **2b**, respectively, in very good yields. The solubility of **2a** and **2b** in apolar solvents like pentane or heptane facilitates the separation of **2a** and **2b** from the more polar starting materials **1a** and **1b**. The formation of both complexes was confirmed by IR spectroscopy with three vibration

(8) Metzler-Nolte, N. In *Bioorganometallics: Biomolecules, Labeling, Medicine*; Jaouen, G., Ed.; Wiley-VCH: Weinheim, 2006.
(9) Yan, Y. K.; Melchart, M.; Habtemariam, A.; Sadler, P. J. *Chem. Commun.* **2005**, 4764–4776.
(10) Peacock, A. F. A.; Sadler, P. J. *Chem. Asian J.* **2008**, *3*, 1890–1899.
(11) Hartinger, C. G.; Dyson, P. J. *Chem. Soc. Rev.* **2009**, *38*, 391–401.
(12) Johnson, T. R.; Mann, B. E.; Clark, J. E.; Foresti, R.; Green, C. J.; Motterlini, R. *Angew. Chem., Int. Ed.* **2003**, *42*, 3722–3729.
(13) Allardyce, C. S.; Dorcier, A.; Scolaro, C.; Dyson, P. J. *Appl. Organomet. Chem.* **2005**, *19*, 1–10.
(14) Jaouen, G.; Dyson, P. J. In *Comprehensive Organometallic Chemistry III. - Applications III: Functional Materials, Environmental and Biological Applications*; O'Hare, D., Ed.; Elsevier: Amsterdam, 2007; Vol. 12, pp 445−464.
(15) Meggers, E. *Curr. Opin. Chem. Biol.* **2007**, *11*, 287–292.
(16) Jung, M.; Kerr, D. E.; Senter, P. D. *Arch. Pharm.* **1997**, *330*, 173–176.
(17) Schmidt, K.; Jung, M.; Keilitz, R.; Schnurr, B.; Gust, R. *Inorg. Chim. Acta* **2000**, *306*, 6–16.
(18) Roth, T.; Eckert, C.; Fiebig, H.-H.; Jung, M. *Anticancer Res.* **2002**, *22*, 2281–2284.
(19) Sergeant, C. D.; Ott, I.; Sniady, A.; Meneni, S.; Gust, R.; Rheingold, A. L.; Dembinski, R. *Org. Biomol. Chem.* **2008**, *6*, 73–80.
(20) Ott, I.; Koch, T.; Shorafa, H.; Bai, Z.; Poeckel, D.; Steinhilber, D.; Gust, R. *Org. Biomol. Chem.* **2005**, *3*, 2282–2286.
(21) Neukamm, M. A.; Pinto, A.; Metzler-Nolte, N. *Chem. Commun.* **2008**, 232–234.
(22) Salmain, M.; Vessières, A.; Brossier, P.; Butler, I. S.; Jaouen, G. *J. Immunol. Meth.* **1992**, *148*, 65–75.
(23) Salmain, M.; Vessieres, A.; Varenne, A.; Brossier, P.; Jaouen, G. *J. Organomet. Chem.* **1999**, *589*, 92–97.
(24) Vessieres, A.; Salmain, M.; Brossier, P.; Jaouen, G. *J. Pharm. Biomed. Anal.* **1999**, *21*, 625–633.
(25) Salmain, M.; Vessieres, A.; Top, S.; Jaouen, G.; Butler, I. S. *J. Raman Spectrosc.* **1995**, *26*, 31–38.
(26) Vessieres, A.; Tondu, S.; Jaouen, G.; Top, S.; Ismail, A. A.; Teutsch, G.; Moguilewsky, M. *Inorg. Chem.* **1988**, *27*, 1850–1852.
(27) Jaouen, G.; Vessières, A.; Top, S.; Savignac, M.; Ismail, A. A.; Butler, I. S. *Organometallics* **1987**, *6*, 1985–1986.
(28) Salmain, M.; Vessieres, A.; Jaouen, G.; Butler, I. S. *Anal. Chem.* **1991**, *63*, 2323–2329.
(29) Vessieres, A.; Top, S.; Vaillant, C.; Osella, D.; Mornon, J.-P.; Jaouen, G. *Angew. Chem., Int. Ed.* **1992**, *31*, 754–755.
(30) Philomin, V.; Vessieres, A.; Gruselle, M.; Jaouen, G. *Bioconjugate Chem.* **1993**, *4*, 419–424.
(31) Osella, D.; Galeotti, F.; Cavigiolio, G.; Nervi, C.; Hardcastle, K. I.; Vessières, A.; Jaouen, G. *Helv. Chim. Acta* **2002**, *85*, 2918–2925.
(32) Savignac, M.; Sasaki, A.; Potier, P.; Jaouen, G. *J. Chem. Soc., Chem. Commun.* **1991**, 615–617.
(33) Varenne, A.; Salmain, M.; Brisson, C.; Jaouen, G. *Bioconjugate Chem.* **1992**, *3*, 471–476.
(34) Gruselle, M.; Deprez, P.; Vessieres, A.; Greenfield, S.; Jaouen, G.; Larue, J.-P.; Thouvenot, D. *J. Organomet. Chem.* **1989**, *59*, C53−C56.
(35) Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, *254*, 1497–1500.
(36) Brosch, O.; Weyhermüller, T.; Metzler-Nolte, N. *Eur. J. Inorg. Chem.* **2000**, *2*, 323–330.
(37) Hess, A.; Metzler-Nolte, N. *Chem. Commun.* **1999**, 885–886.
(38) Brosch, O.; Weyhermüller, T.; Metzler-Nolte, N. *Inorg. Chem.* **1999**, *38*, 5308–5313.
(39) van Staveren, D. R.; Metzler-Nolte, N. *Chem. Rev* **2004**, *104*, 5931–5985, and references therein.
(40) Kirin, S. I.; Kraatz, H.-B.; Metzler-Nolte, N. *Chem. Soc. Rev.* **2006**, *35*, 348–354.
(41) Köster, S. D.; Dittrich, J.; Gasser, G.; Hüsken, N.; Hernandez, I.; Jios, J. L.; Metzler-Nolte, N. *Organometallics* **2008**, *27*, 6326−6332.
(42) Gasser, G.; Brosch, O.; Ewers, A.; Weyhermüller, T.; Metzler-Nolte, N. *Dalton Trans.* **2009**, DOI: 10.1039/b81969g.
(43) Sewald, N.; Gaa, K.; Burger, K. *Heteroat. Chem.* **1993**, *4*, 253–258.
(44) Gasser, G.; Hüsken, N.; Köster, S. D.; Metzler-Nolte, N. *Chem. Commun.* **2008**, 3675–3677.
(45) Curran, T. P.; Grant, A. L.; Lucht, R. A.; Carter, J. C.; Affonso, J. *Org. Lett.* **2002**, *4*, 2917–2920.

Scheme 1. Synthesis of **2a** and **2b**[a]



[a] (i) *N*-methylmorpholine, isobutylchloroformiate, propargylamine, r.t, 1h, 96% for **1a** and 82% for **1b**. (ii) $Co_2(CO)_8$, THF, 2h, 91% for **2a** and 90% for **2b**.

Table 1. Comparison of Characteristic Chemical Shifts of Compounds **1** and **2**

δ (in $CDCl_3$)/ppm	**1a**	**1b**	**2a**	**2b**
C*H*$_2$	3.96	4.02	4.63	4.59
C≡C*H*	2.16	2.19	5.99	6.03
C≡*C*H	71.6	71.7	73.0	72.1
C≡CH	79.0	79.2	92.9	91.9
Co−*C*≡O			200.0	199.2
N^{Boc}	−292	−292	−292	−293
N^{Prop}	−270	−270	−264	−265

bands corresponding to the carbonyl groups at 2096, 2056, and 2024 cm^{-1} for **2a**, and 2097, 2058, and 2036 cm^{-1} for **2b**, respectively. The complexation of **1a** and **1b** with the dicobalthexacarbonyl moiety was further ascertained by ^1H, ^{13}C, and ^{15}N NMR spectroscopy. All the proton signals of the propargylamine are shifted downfield, notably the alkyne proton which undergoes a shift by about 4 ppm (see Table 1 and Figure 1). This observation is due to the structural change of the $CH_2-C\equiv CH$ atom sequence, which is no longer linear but becomes bent upon complexation.[46,47] The C*H* proton is hence moved out of the shielding part of the anisotropy cone and shifts downfield in the ^1H NMR spectrum.

X-ray Crystal Structure of 2a. Single crystals suitable for an X-ray analysis were grown by slow crystallization from a pentane solution of **2a** at −30 °C. The unit cell contains three crystallographically independent molecules. An Oak Ridge Thermal Ellipsoid Plot (ORTEP) plot of one molecule is presented in Figure 2 with selected bond lengths and angles summarized in the caption. All structural parameters of the three molecules are rather similar. In the Figure, as in the following text, we therefore refer to this molecule only. As expected, the two amide bonds are planar and in a *trans* configuration. The C≡C bond is elongated by about 0.15 Å upon Co coordination to 1.329(6) Å in **2a** compared to 1.187(14) Å as found for a ferrocenyl propargylamine amino acid derivative recently reported by our group.[42] This bond now corresponds to a double bond as found by Howard et al. (1.36(1) Å) for dicobalthexacarbonyl diphenylacetylene.[48] This description is also supported by the fact that the alkyne group is no longer linear[46,47] as indicated by a C−C≡C angle of 142.9(4)° in **2a**. The Co atoms in **2a** are in a distorted octahedral environment with the cobalt atoms coordinated by three carbonyl groups, the two alkyne carbon atoms and the other cobalt atom. As expected, the two Co atoms and the two carbon atoms from the double bond form a distorted tetrahedron.[46] The bond lengths and angles of the complex **2a** are in accordance with similar complexes previously reported.[19,49,50] Notably, the Co−Co distance of 2.4617(9) Å in **2a** is in agreement with what was found by Ott, Gust, Dembinski et al. for a dicobalthexacarbonyl nucleoside derivative[19] (2.4682(5) Å) or by Nicholas et al. for a pinacol dicobalthexacarbonyl derivative (2.4647(9) and 2.4648(9) Å).[50]

Hydrogen bonds between residues are crucial for both the formation and the stability of secondary and tertiary structures in peptides. As recently found by our group for similar (bi)organometallic amino acid derivatives,[42] the packing of **2a** in the solid state is also governed by such interactions. Thus, each molecule of **2a** has two amide bonds that can be used for intermolecular hydrogen bonds. There are three crystallographically independent molecules in the unit cell, which pack by such hydrogen bonding interactions along the crystallographic *c* axis. Since the *c* axis also contains a crystallographic screw axis, six molecules are required for a full helical turn with a pitch of 26.13 Å, which corresponds to the length of the crystallographic *c* axis (Figure 3).

Synthesis and Characterization of Cobalt-Containing Enkephalin Derivatives 4 and 6. The neuropeptide leucine enkephalin ([Leu^5]-Enk has the primary amino acid sequence Tyr-Gly-Gly-Phe-Leu. It was first isolated in 1975 from the brain of a pig and is a natural ligand to the opiate receptor.[51] This peptide was chosen in this study because it can be easily synthesized on the solid phase in high yield and purity, as well as allowing the subsequent attachment of metal complexes, as shown previously by our group[7,21,41,52−57] and

(46) Sly, W. G. *J. Am. Chem. Soc.* **1958**, *81*, 18–20.
(47) Dickson, R. S.; Fraser, P. J. In *Advances in Organometallic Chemistry 12*; Academic Press: New York, 1974, p 323.
(48) Gregson, D.; Howard, J. A. K. *Acta Crystallogr.* **1983**, *C39*, 1024–1027.
(49) Seyferth, D.; Nestle, M. O.; Wehman, A. T. *J. Am. Chem. Soc.* **1975**, *97*, 7417–7425.
(50) Lake, K.; Dorrell, M.; Balckman, N.; Khan, M. A.; Nicholas, K. M. *Organometallics* **2003**, *22*, 4260–4264.
(51) Koenig, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 2024–2033.
(52) van Staveren, D. R.; Metzler-Nolte, N. *Chem. Commun.* **2002**, 1406–1407.
(53) Kuchta, M. C.; Gross, A.; Pinto, A.; Metzler-Nolte, N. *Inorg. Chem.* **2007**, *46*, 9400–9404.
(54) Kirin, S. I.; Noor, F.; Metzler-Nolte, N.; Mier, W. *J. Chem. Educ.* **2007**, *84*, 108–111.

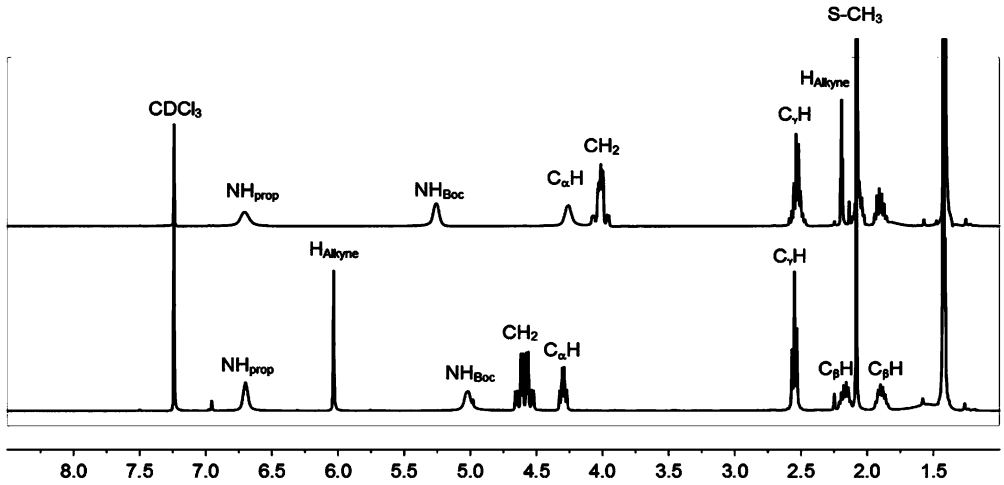


Figure 1. Comparison of the ^{1}H NMR spectra of **1b** (above) and **2b** (below). Note the downfield shift of the alkyne proton from 2.2 ppm to 6.03 ppm.

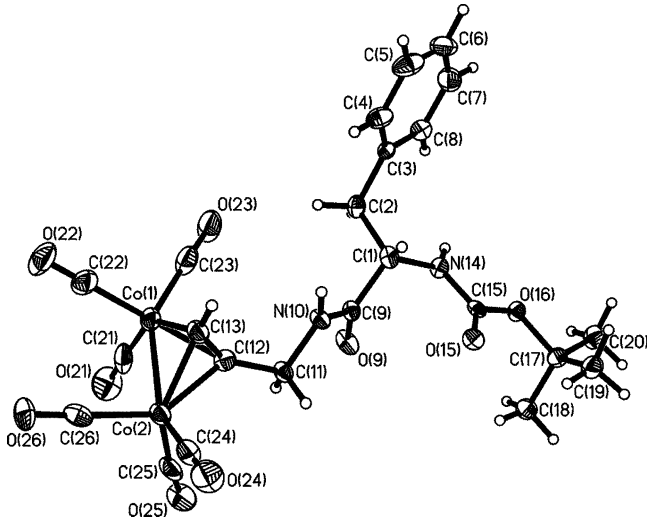


Figure 2. ORTEP plot of one molecule of **2a** with numbering scheme, 40% probability. Pertinent structural parameters for the other two crystallographically independent molecules are very similar, see text. Selected bond lengths [Å] and angles [deg]: Co(1)−Co(2) 2.4617(9); Co(1)−C(13) 1.941(4); Co(1)−C(12) 1.976(4); Co(2)−C(12) 1.954(4); Co(2)−C(13) 1.964(4); Co(1)−C(23) 1.792(6; Co(1)−C(21) 1.803(6); Co(1)−C(22) 1.810(6); Co(2)−C(24) 1.784(6); Co(2)−C(26) 1.799(5); Co(2)−C(25) 1.820(6); C(13)−C(12)−C(11) 142.9(4); O(9)−C(9)−N(10) 122.2(4); O(15)−C(15)−N(14) 123.5(4).

recently reviewed by König et al.[58] Two different SPPS procedures were used to prepare the two alkyne-containing enkephalin derivatives **3** and **5** (Schemes 2 and 3). The sulfamylbutyryl resin developed by Ellman and co-workers,[59−61] itself based on Kenner's acylsulfonamide *safety-catch* linker,[62] was employed to form **3**. Starting from the commercially available leucine preloaded sulfamylbutyryl resin, the peptide was prepared using standard Fmoc SPPS procedures (see Experimental Section for further details). The *N*-terminus of the peptide was first acetylated. The 1-chlorotrityl (1-Cltrt) protecting group of Tyr was then removed using a cocktail of CH_2Cl_2:TIS:TFA 94:5:1 (v/v/v). Afterward, the *N*-acylsulfonamide linker was alkylated with excess iodoacetonitrile (ICH_2CN) in the presence of DIPEA in dry DMF to give a very reactive *N,N*-cyanomethyl-acylsulfonamide (Scheme 2). The expected alkyne-containing peptide **3** was finally cleaved from the resin by nucleophilic substitution with 5 equiv of propargylamine to finally obtain **3** in 22% yield (see Experimental Section for full synthetic details). No subsequent purification was undertaken as an HPL chromatogram of the crude product already indicated >95% purity, which was considered sufficient for the next synthetic step (Supporting Information, Figure S1). The low yield for **3** is probably due to partial acetylation of the linker group during the acetylation of the *N*-terminus of the peptide. Nevertheless, the formation of **3** was ascertained by ESI-MS with a peak at $m/z = 657.3$, corresponding to $[M+Na]^+$. Furthermore, the ^{1}H NMR spectrum of **3** shows the expected triplet of the terminal alkyne proton at 3.0 ppm ($^4J = 2.5$ Hz), while a pseudoquartet corresponding to NH−CH_2−C≡CH is observed at 3.9 ppm ($^3J = 5.5$ Hz, Supporting Information, Figure S2). In ^{13}C spectroscopy, the tertiary and quaternary carbons of the alkyne group appear at 72.8 and 80.9 ppm, respectively.

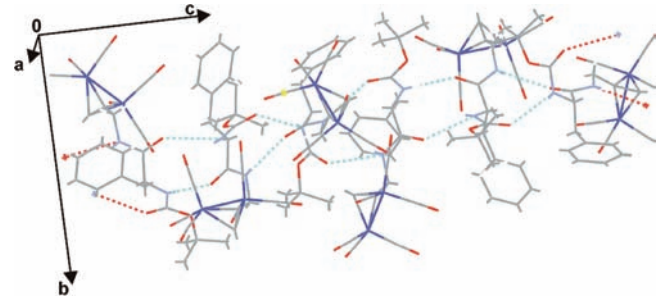
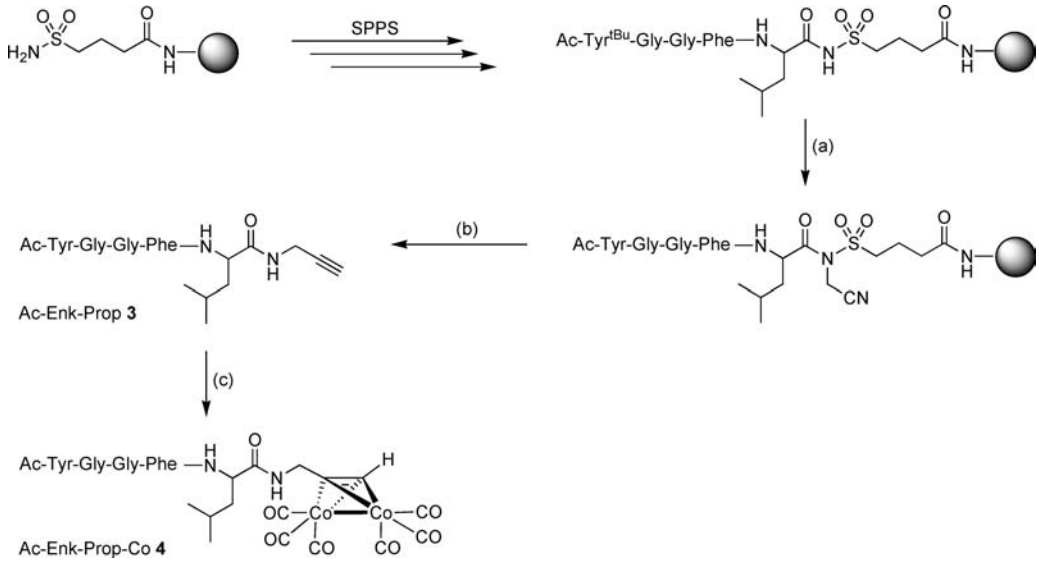


Figure 3. Helical arrangement of **2a** along the crystallographic *c* axis, see text for details.

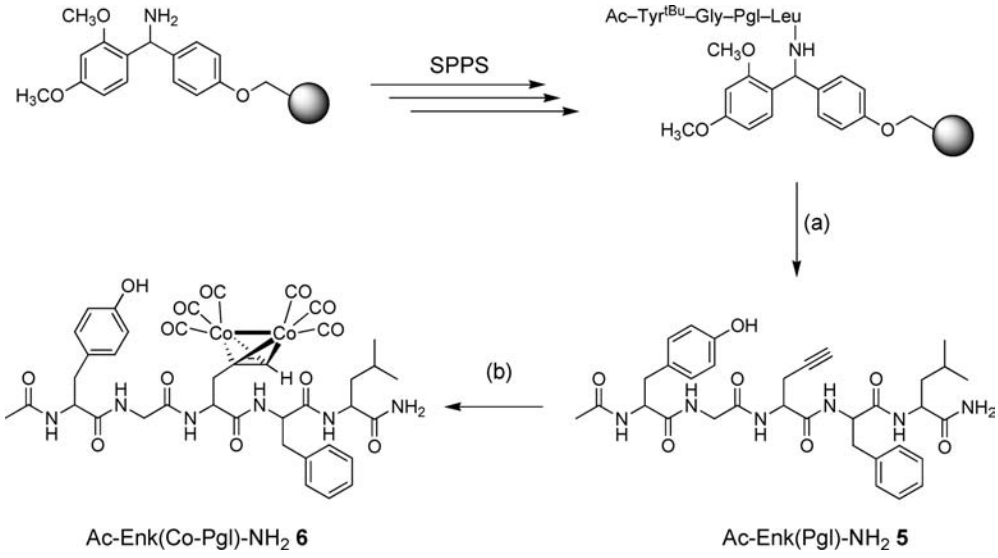
(55) Caddy, J.; Hoffmanns, U.; Metzler-Nolte, N. *Z. Naturforsch.* **2007**, *62B*, 460–466.
(56) Lemke, J.; Metzler-Nolte, N. *Eur. J. Inorg. Chem.* **2008**, *21*, 3359–3366.
(57) Zagermann, J.; Kuchta, M. C.; Merz, K.; Metzler-Nolte, N. *J. Organomet. Chem.* **2009**, *694*, 862−867.
(58) Dirscherl, G.; König, B. *Eur. J. Org. Chem.* **2008**, 597–634.
(59) Backes, B. J.; Ellman, J. A. *J. Am. Chem. Soc.* **1994**, *116*, 11171–11172.
(60) Backes, B. J.; Virgilio, A. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1996**, *118*, 3055–3056.
(61) Backes, B. J.; Ellman, J. A. *J. Org. Chem.* **1999**, *64*, 2322–2330.
(62) Kenner, G. W.; McDermott, J. R.; Sheppard, R. C. *Chem. Commmun.* **1971**, 636–637.

Scheme 2. Synthesis of Ac-Enk-Prop-Co **4**[a]



[a] (a) (i) CH_2Cl_2:TIS:TFA 94:5:1 (v/v/v); (ii) ICH_2CN, DIPEA, DMF, 18h; (b) Propargylamine, THF, 23h; (c) $Co_2(CO)_8$, THF, 1h.

Scheme 3. Synthesis of Ac-Enk(Co-Pgl)-NH_2 **6**[a]



[a] (a) TFA:TIS:H_2O 95:2.5:2.5 (v/v/v); (b) $Co_2(CO)_8$, THF.

For the formation of Ac-Tyr-Gly-Pgl-Phe-Leu-NH_2 [(Ac-Enk(Pgl)-NH_2, **5**, Pgl - propargylglycine), a standard SPPS procedure on a Rink resin was employed, except that Gly-3 after Phe was replaced by the artificial amino acid Fmoc-propargylglycine (Fmoc-Pgl-OH). Again, the crude product obtained was pure enough to be used for the next synthetic step. The presence of **5** was confirmed by ESI-MS with a peak at $m/z = 657.7$, corresponding to $[M+Na]^+$. As for **3**, ^{1}H NMR spectroscopy ascertained the presence of compound **5** with the expected pattern for the C_β protons of Phe, Tyr and Pgl as shown in Figure 4. As expected, the $CH_2-C\equiv CH$ protons of the propargylglycine in **5** appeared separately as two multiplets at 2.47 and 2.30 ppm, respectively, because of their nonequivalence, contrary to **3** where they are equivalent and appear as a pseudoquartet at 3.9 ppm.

With **3** and **5** in hand, the respective dicobalthexacarbonyl enkephalin derivatives Ac-Enk-Prop-Co **4** and Ac-Enk(Co-Pgl)-NH_2 **6** could be easily prepared by adding a solution of $Co_2(CO)_8$ in THF to a suspension of **3** and **5** in THF, respectively (Schemes 2 and 3). After evaporation of the solvent, compounds **4** and **6** could be isolated quantitatively in good purity (see Experimental Section and Supporting Information, Figures S3 and S5 for HPL chromatograms of **4** and **6**, respectively). The presences of **4** and **6** were confirmed unambiguously by ESI-MS with a molecular peak for both compounds at m/z 943.0 corresponding to $[M+Na]^+$. Furthermore, the fragmentation of the molecular peaks in the mass spectrometer (MS^2) of **4** and **6** shows the appearance of six peaks separated by $\Delta m/z = 28$, corresponding to the sequential loss of all carbonyl ligand(s) in **4** and **6**

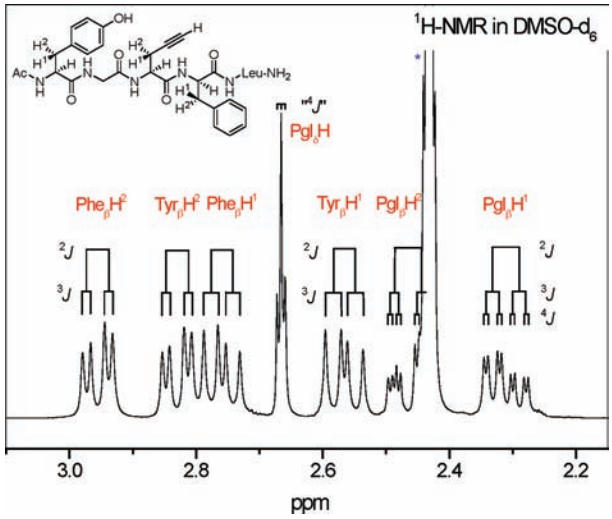
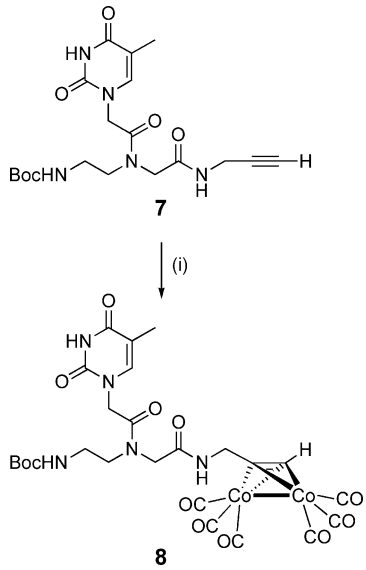


Figure 4. Section of the 400 MHz ^{1}H NMR spectrum of Ac-Enk(Pgl)-NH_2 **5** in DMSO-d_6, showing the C_βH proton range (3.0−2.2 ppm).

Scheme 4. Synthesis of **8**[a]

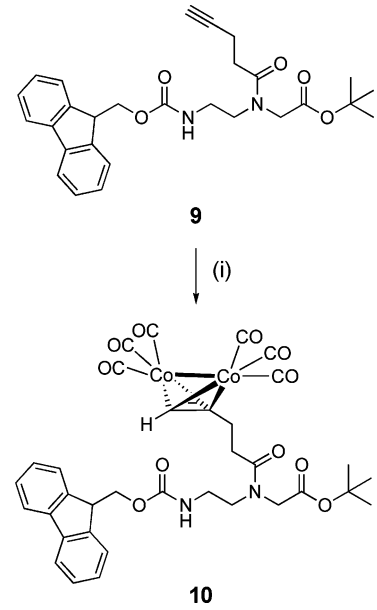


[a] (i) $Co_2(CO)_8$, THF, 1h, 32%.

(Supporting Information, Figures S4 and S6). As for **2a** and **2b**, three intense carbonyl bands were observed by IR spectroscopy at 2095, 2053, and 2022 cm^{-1} and 2094, 2051, and 2021 $cm^{-1}$for **4** and **6**, respectively.

Synthesis and Characterization of a Cobalt-Containing Thymine PNA Monomer 8. For the synthesis of the dicobalthexacarbonyl thymine PNA monomer **8**, the commercially available *N*-Boc-protected thymine PNA monomer (Boc-T-PNA-OH) was turned into the propargylamide **7** as previously reported by our group.[42] **7** was then complexed with $Co_2(CO)_8$, in THF to give, after HPLC purification, the desired dark red cobalt-containing compound **8** (Scheme 4). The ^{1}H NMR spectrum of **8** shows the doubling of all proton signals in a 60:40 ratio. This is due to the presence of *cis*−*trans* isomers about the central amide bond, as commonly observed in PNA monomers (the isomers being denoted *major* and *minor*). Similar to the amino acid Co complexes discussed above, the alkyne proton is down-

Scheme 5. Synthesis of **10**[a]



[a] (i) $Co_2(CO)_8$, THF, 2h, 78%.

field shifted by nearly 4 ppm from 2.48/2.44 ppm (ma/mi) in **7** to 6.28/6.23 ppm in **8**. The formation of **7** is further confirmed by ESI-MS with peaks at *m*/*z* 730, 746, and 702 corresponding to molecular ions $[M+Na]^+$, $[M+K]^+$, and $[M+Na-CO]^+$ respectively. As for **2a** and **2b**, intense bands between 2097−2027 cm^{-1} were observed in the IR spectrum of **8** corresponding to the carbonyl groups of **8.** The presence of **8** was further ascertained by Raman spectroscopy with the disappearance of the ν(C≡C) vibration band present in **7** at 2125 cm^{-1} and the appearance of CO bands at 2093 and 2018 cm^{-1} in **8**.

Synthesis of a Cobalt-Containing PNA-like Monomer 10. Encouraged by the successful synthesis of the dicobalthexacarbonyl peptide **6**, we were eager to further explore the versatility of this labeling strategy with PNA. Thus, similarly to compound **6**, we were aiming to attach organometallic moieties not only at the *C* or *N* termini of a PNA monomer (respectively PNA oligomer) as reported above and previously by our group,[37,42,63,64] but also on a side chain of a PNA monomer (respectively PNA oligomer). To this end, we have reacted the alkyne-containing PNA monomer (**9**, Scheme 5), which was recently reported by our group[44] with $Co_2(CO)_8$. It is interesting to note that azidoferrocene was already successfully attached to **9** using the "Click Chemistry" methodology.[44] In comparison, the simple reaction of dicobalt octacarbonyl with **9** to yield **10** provides an even more direct way towards organometal-PNA derivatives.

The reaction was carried out using a similar procedure as for **2**, **4**, **6**, and **8**, that is, stirring a 1:1 mixture (organometallic: **9**) in deoxygenated dry THF for 2 h at room temperature. The cobalt carbonyl compound **10** was easily

(63) Verheijen, J. C.; van der Marel, G. A.; van Boom, J. H.; Metzler-Nolte, N. *Bioconjuguate Chem.* **2000**, *11*, 741–743.
(64) Maurer, A.; Kraatz, H.-B.; Metzler-Nolte, N. *Eur. J. Inorg. Chem.* **2005**, 3207–3210.

isolated in good yield after purification by column chromatography on silica. It was found very stable, even in the presence of oxygen and moisture from air. Compound **10** exhibits the characteristic metal−CO bands in the IR spectrum at 2090, 2048, and 2005 cm^{-1}. As for **8**, all signals are doubled in the ^1H NMR spectrum of **10** because of the *cis-trans* isomerism about the central amide bond (ratio ca. 50:50 for **10**). As expected, the alkyne C*H* signal at 1.72/1.88 ppm[44] in **9** shifts upon complexation with cobalt carbonyl to 6.10 ppm in **10**. Furthermore, the Co complexation of **9** is further corroborated by ^{13}C NMR spectroscopy with the ^{13}C NMR signals of the metal carbonyls appearing as a broad signal at about 201 ppm. ESI-MS gives indubitable evidence of the formation of **10** with a major peak at m/z = 784.9 corresponding to $[M+Na]^+$, as well as two peaks at m/z = 757 and 729 corresponding to $[M+Na-CO]^+$ and $[M+Na-2CO]^+$ respectively.

Conclusion

In this work, we have presented the facile labeling of alkyne-containing biomolecules with dicobalt octacarbonyl to give dicobalthexacarbonyl amino acid (**2a** and **2b**), enkephalin (**4** and **6**), T-PNA monomer (**8**), and PNA-like monomer (**10**) derivatives in very good yields. These compounds have relatively good stability in aqueous media and in air and can be handled without special precautions. Addition of an alkyne group, for example, as pentynoic acid, to the *N*-terminus of a peptide as the last step of an SPP synthesis is the most straightforward way to introduce such a functionality into a peptide.[21,41] Internal, and especially *C*-terminal labeling is more challenging in comparison. To achieve this task, two different resins, namely sulfamylbutyryl and Rink amid, were used to prepare the two alkyne-containing enkephalin derivatives **3** and **5**, respectively. These types of resins enabled us to insert, on the solid phase, an alkyne moiety selectively at the *C*-terminus and on a side-chain of the enkephalin peptide, respectively. The presence of the cobalt carbonyl complexes was ascertained by appropriate analytical methods, notably IR spectroscopy with intensive bands in the 2100−2000 cm^{-1} region for all new metal complexes. Electrospray mass spectrometry is a powerful tool not just for identification of the metal bioconjugates. Sequential loss of carbonyl ligands leads to a characteristic succession of signals with $\Delta m/z = 28$, which was readily observed in most of the new compounds. As expected, in both ^1H NMR spectra of the PNA monomer derivatives **8** and **10**, all signals are doubled because of the *cis-trans* isomerism about the central amide bond. Furthermore, to the best of our knowledge, the first X-ray structure of a dicobalthexacarbonyl amino acid derivative is reported. As anticipated, the former triple bond is lengthened by about 0.15 Å to a double bond and the alkyne group of **2a** is no longer linear upon complexation, as indicated by a C−C≡C angle of about 143°.

In conclusion, the facile synthesis of different dicobalthexacarbonyl alkyne bioconjugates is presented in this contribution. Two equally successful approaches to label biomolecules with a cobalt complex were reported: one that allows the organometallic moiety to be reacted to the *C*-terminus of alkyne-containing amino acids, peptide, or PNA thymine monomer, and another one which allows the organometallic to be complexed to an internal position in a peptide. To this end, propargylglycine replaces one glycine in peptides and is readily reacted with dicobalt hexacarbonyl. Interestingly, the reaction of more heavily substituted *para*-ethynyl-phenylalanine, which was obtained by our group from a Sonogashira coupling reaction of peptides,[65] does not yield the respective dicobalt alkyne complexes, possibly because of steric constraints. Finally, an alkyne-containing PNA-like monomer, in which the alkyne group replaces the usual nucleobase, again readily forms the dicobalt alkyne complex. Transfer of this labeling approach to other peptides and PNA oligomers is currently in progress in our laboratory.

Experimental Section

Materials. All reactions were carried out in ordinary glassware, and solvents were used without further precautions except if indicated. Chemicals were purchased from commercial suppliers and used as received. Only enantiomerically pure L amino acids were used. *N*-Boc-protected T-PNA Monomer was purchased from PE Applied Biosystems GmbH, Weiterstadt, Germany. PNA monomers are now available from ASM (Hannover, Germany) or Link Technologies (Lanarkshire, Scotland). Sulfamylbutyryl and Rink resins were purchased from Novabiochem.

Instrumentation and Methods. Elemental analyses were carried out by H. Kolbe, Analytisches Laboratorium, Mülheim or on an Analytic Jena multi EA 3100. IR spectra were recorded on a Perkin-Elmer System 2000 instrument as KBr disks, additionally in CH_2Cl_2 solution if indicated, or on a ATR unit using a Bruker Tensor 27 FTIR spectrophotometer at 4 cm^{-1} resolution. Frequencies ν are given in cm^{-1}. Melting points (uncorrected) were determined on a Tottoli apparatus (Büchi, Switzerland). Mass spectra were recorded by the mass spectrometry service group, Mülheim, on a MAT 8200 (Finnigan GmbH, Bremen) instrument (EI, 70 eV) or on a MAT95 (Finnigan GmbH, Bremen) instrument (ESI, CH_3OH solution, positive ion detection mode) or on a Bruker Esquire 6000 (ESI mass spectra). Only characteristic fragments are given with intensities (%) and possible composition in brackets. RP-HPLC for compound **8** was carried out on a Nucleosil-N-7-C_{18} column (Nr. 312014, Size 250 × 20 mm) with a Merck C 6200 pump and a Shimadzu SPD-6-A V-detector at 260 and 420 nm. The eluents were a mixture of methanol/water in different ratio. RP-HPLC for compound **3**−**6** was carried out on a Varian Pro Star apparatus with a Dynastar C-18 colum (250 × 8 mm, analytic, 250 × 10 mm semipreparative and 250 × 21 mm preparative. Water (A) and acetonitrile (B) (HPLC grade) with 0.1% TFA were used as the eluents. In both cases, the flow rate was 1 mL min^{-1} (analytical) or 4 mL min^{-1} (preparative). HPLC gradient: 5% to 90% B over 18 min. NMR spectra were recorded at room temperature using a Bruker ARX 250 (^1H at 250.13 MHz and ^{13}C) or a DRX 400 (^1H at 400.13 MHz, ^{13}C and 2D spectra) or DRX 500 (^1H at 500.13 MHz, ^{13}C, ^{15}N, 2D) spectrometer. ^1H and ^{13}C spectra were referenced to TMS, using the ^{13}C signals or the residual proton signals of the deuterated solvents as internal standards. Positive chemical shift values δ (in ppm) indicate a downfield shift from the standard, only the absolute values of coupling constants are given in Hz. ^{15}N spectra were referenced to the absolute frequency

(65) Hoffmanns, U.; Metzler-Nolte, N. *Bioconjugate Chem.* **2006**, *17*, 204–213.

of 50.6969910 MHz, which was the resonance frequency of neat nitromethane under the same experimental conditions. All resonances were assigned by 2D NMR (H-H-COSY and ^{1}H-^{13}C-HMQC for ^{1}J and long-range couplings). Where unambiguous or proven spectroscopically, the following conventions are used: δ/δ' denotes pairs of signals originating from *cis*/*trans* isomers, integration "nH/2" indicates one signal of one rotational isomer only. ^{15}N chemical shifts and coupling constants were taken from the F1 projection of indirect detection ^{1}H−^{15}N correlated 2D spectra with 1024/256 data points in F1/F2, processed after applying a matched cosine function and zero filling in both dimensions.

Synthesis and Characterization

Propargylamide Derivatives of Phe^{Boc} (1a) and Met^{Boc} (1b). Both compounds were synthesized using an alternative method to that published by Curran et al.[45] but the analytical data match those reported previously.[45]

The respective *N*-Boc protected amino acids (10 mmol) were solubilized in THF (100 mL) at room temperature. *N*-methylmorpholine (1.01 g, 10 mmol) was then added to the clear amino acid solution, followed by the further addition of isobutylchloroformiate (10 mmol, 1.37 g). During the addition of the isobutylchloroformiate, a white precipitate of *N*-methylmorphonine hydrochloride was formed. Propargylamine (10 mmol, 0.55 g) was then added to the reaction mixture. A slight formation of CO_2 could be observed. The mixture was further stirred for 1 h at room temperature. The hydrochloride salt was then filtered, and the clear solution evaporated to dryness. To the residue was added diethyl ether and water, and the organic and aqueous phases were separated. The aqueous phase was then back-extracted with diethyl ether (3×). The combined organic phases were washed with water (1×), dried over Na_2SO_4, filtered and evaporated to dryness. The obtained product could be then recrystallized in heptane/THF mixture to give **1a** and **1b** in 82% and 96% yield, respectively.

Dicobalthexacarbonyl Complexes of Propargylamide Derivatives of Phe^{Boc} (2a) and Met^{Boc} (2b). The respective compounds **1a** and **1b** (0.6 mmol) were dissolved in dry and degassed THF (40 mL). To this solution was added slowly, under a atmosphere of argon, a solution of $Co_2(CO)_8$ (225 mg, 0.66 mmol) in dry and degassed THF. The color of the solution changed at this stage from brown to dark red. At the end of the gaz formation, the reaction mixture was stirred for another 1 h. The solution was then evaporated to dryness with the help of a Schlenk line. The residue was dissolved in dry and degassed pentane (50 mL), and the solution filtrated through canule. The solution was then evaporated to dryness to give a red solid. Yield: 181 mg (91%) for **2a** and 181 mg (90%) for **2b**.

Characterization Data for 2a. Analyses Found (%): C, 46.8; H, 3.9; N, 4.6. Calcd for $C_{23}H_{22}N_2O_9Co_2$ (%): C, 47.0; H, 3.8; N, 4.8. Values calculated for $C_{24.67}H_{26}N_2O_9Co_2$, which corresponds to the crystallographically determined composition of the single crystals: C 48.4; H 4.3; N 4.6. The samples submitted for elemental analysis and spectroscopic investigation were not all of the single crystal batch, the amount of solvent may therefore vary. Major IR bands: 3440 (br, m), 3310 (br, m), 2096 (m), 2056 (s), 2024 (br, s), 1660 (m). Mp 95 °C. ^{1}H NMR ($CDCl_3$): 7.31−7.18 (5H, m, H_{Phe}), 6.34 (1H, s, br, NH_{Prop}), 5.99 (1H, s, Co_2−CH), 4.80 (1H, s, br, NH_{Boc}), 4.64−4.59 (1H, m, Co_2−C−CH_2), 4.47−4.35 (2H, m, Co_2−C−CH_2 and $C_\alpha H$), 3.17−3.05 (2H, m, $C_\beta H$), 1.35 (9H, s, $C_{Boc}H_3$). ^{13}C NMR ($CDCl_3$): 200.0 (Co−C≡O), 171.4 (C═O), 155.9 (C_{Boc}═O), 137.6, 129.7 129.0, 127.2 (C_{Phe}), 92.9 (Co_2−C−CH_2), 80.4 ($C_{Boc}(CH_3)_3$), 73.0 (Co_2−CH), 56.0 (C_α), 42.0 (Co_2−C−CH_2), 38.5 (C_β), 28.1 ($C_{Boc}(CH_3)_3$). ^{15}N NMR ($CDCl_3$): −292 (NH_{Boc}), −264 (NH_{Prop}). MS: *m*/*z* 532 ([M−tBu]$^+$) (1), 420 ([M−6CO]$^+$) (34), 364 ([M−tBu−6CO]$^+$) (22), 28 ([CO]$^+$) (100).

Table 2. Crystallographic Data for **2a**

	2a
chem. formula	$C_{24.67}H_{26}Co_2N_2O_9$
crystal size, mm^3	0.56 × 0.46 × 0.32
Fw	612.33
space group	$P2_12_12_1$, No. 19
a, Å	14.253(2)
b, Å	22.896(3)
c, Å	26.133(4)
V, Å	8528(2)
Z	12
T, K	100(2)
ρ calcd, g cm^{-3}	1.431
refl. collected/$2\Theta_{max}$	37299/51.08
unique refl./$I > 2\sigma(I)$	14448/10401
no. of params/restr.	1060/355
λ, Å /μ(Kα), cm^{-1}	0.71073/12.17
R1[a]/goodness of fit[b]	0.0430/ 0.941
*w*R2[c] ($I > 2\sigma(I)$)	0.0691
residual density, e Å^{-3}	+0.42/−0.28

[a] Observation criterion: $I > 2\sigma(I)$. $R1 = \sum||F_o| - |F_c||/\sum|F_o|$. [b] $GOF = \{\sum[w(F_o^2 - F_c^2)^2]/(n - p)\}^{1/2}$. [c] $wR2 = \{\sum[w(F_o^2 - F_c^2)^2]/\sum w(F_o^2)^2\}^{1/2}$ where $w = 1/\sigma^2(F_o^2) + (aP)^2 + bP$, $P = (F_o^2 + 2F_c^2)/3$.

Characterization Data for 2b. Analyses Found (%): C, 39.9; H, 3.9; N, 4.8. Calcd for $C_{19}H_{22}N_2O_9SCo_2$ (%): C, 39.9; H, 3.9; N, 4.8. Major IR bands: 3299 (br, m), 2097 (m), 2058 (s), 2036 (br, s), 1698 (m), 1659 (m). Mp 86 °C. ^{1}H NMR ($CDCl_3$): 6.70 (1H, s, br, NH_{Prop}), 6.03 (1H, s, Co_2−C*H*), 5.01 (1H, s, br, NH_{Boc}), 4.64−4.52 (2H, m, Co_2−C−CH_2), 4.32−4.27 (1H, m, $C_\alpha H$), 2.56−2.54 (2H, m, $C_\gamma H$), 2.25−2.12 (1H, m, $C_\beta H$), 2.08 (3H, s, CH_3), 1.93−1.86 (1H, m, $C_\beta H$), 1.42 (9H, s, $C_{Boc}H_3$). ^{13}C NMR ($CDCl_3$): 199.2 (Co−*C*≡O), 171.2 (*C*═O), 155.7 (C_{Boc}═O), 91.9 (Co_2−*C*−CH_2), 80.2 ($C_{Boc}(CH_3)_3$), 72.1 (Co_2−*C*H), 53.6 (C_α), 42.0 (Co_2−C−*C*H_2), 31.2 (C_β), 30.2 (C_γ), 28.1 ($C_{Boc}(CH_3)_3$), 15.1 (S−*C*H_3). ^{15}N NMR ($CDCl_3$): −292 (NH_{Boc}), −264 (NH_{Prop}). MS: *m*/*z* 516 (2), 432 (34), 404 (21), 348 (15), 28 (100).

X-ray Crystallographic Data Collection and Refinement of the Structure of 2a. A dark red single crystal of **2a** was coated with perfluoropolyether, picked up with a nylon loop, and was immediately mounted in the nitrogen cold stream of a Siemens SMART diffractometer. Graphite monochromated Mo Kα radiation (λ = 0.71073 Å) was used throughout. Final cell constants were obtained from least-squares fits of several thousand strong reflections. Intensity data were corrected for absorption using intensities of redundant reflections. The structure was readily solved by Patterson methods and subsequent difference Fourier techniques. The Siemens ShelXTL[66] software package was used for solution and artwork of the structures, and ShelXL97[67] was used for the refinement. All non-hydrogen atoms were anisotropically refined, and hydrogen atoms were placed at calculated positions and refined as riding atoms with isotropic displacement parameters. Crystallographic data of the compounds are listed in Table 2.

Compound **2a** was refined in the chiral space group $P2_12_12_1$ yielding the correct absolute configuration of the phenylalanine building block and a Flack parameter value of −0.023(9).[68] A pentane molecule originating from crystallization was found to be severely disordered. A split atom model with equal anisotropic displacement parameters of corresponding disordered atoms and

(66) *ShelXTL*, 6.14; Bruker AXS Inc.: Madison, WI, 2003.
(67) Scheldrick, G. M. *SHELXL-97, Program for the Refinement of Crystal Structures*; University of Göttingen: Göttingen, Germany, 1997.
(68) Flack, H. D. *Acta Crystallogr.* **1983**, *A39*, 876–881.

restrained bond distances was refined using the EADP and SADI instructions of ShelXL97. The occupation ratio of the refinement on three sites gave values of about 0.45, 0.34, and 0.22, respectively. A total of 355 restraints was used.

General Method for Manual SPPS. Resin-bound enkephalin derivatives were obtained by standard Fmoc-SPPS starting from the adequate resin. For a complete description on manual SPPS for metallocene-peptide bioconjugates, see a tutorial paper by Metzler-Nolte, Mier et al.[54]

Ac-Enk-Prop 3. After acetylation of the *N*-terminus of the desired peptide on the solid phase synthesized as described above, the resin (Sulfamylbutyryl, loading 20 mmol/g, 0.21 g) was washed with dry DMF. Twenty-five equivalents of iodoacetonitrile diluted in 3 mL of dry DMF were filtered through a small amount of basic aluminum oxide. Ten equivalents of DIPEA were then added to this solution. The acetylated resin was mixed to this solution and shaken, in the dark, under an atmosphere of argon, for 18 h. The resin was then washed with dry DMF and distilled THF. The resin was then shaken with 5 equiv of freshly distilled propargylamine in 2 mL of distilled THF, in the dark, under an atmosphere of argon, for 20 h. The resin was filtered, and the filtrate was evaporated to dryness to give a white cloudy oil. The peptide was precipitated with cold ether. The first cleavage gave a very low crude yield (15%). However, with new activation and repetition of the cleavage step on the residual resin, more crude product could be isolated. But, the analytical HPLC and ESI mass spectrum showed that the hydroxyl group of the Tyr was still partially protected with 1-Cltrt and a supplementary deprotection step using a mixture TFA:TIS:H_2O 95:2.5:2.5 (v/v/v) was necessary. Yield: 6 mg (22%).

Characterization Data of 3. $C_{33}H_{42}N_6O_7$ (634.72 g/mol): t_R = 14.04 min. MS (ESI, pos): *m/z* 657.3 $[M+Na]^+$. ^{1}H NMR (DMSO-d_6, 400.13 MHz): 8.20 (m, 2H, $N_{Gly,Prop}H$), 8.03 (m, 2H, $N_{Leu,Tyr}H$), 7.98 (d, 1H, J = 8.2 Hz, $N_{Phe}H$), 7.93 (t, 1H, J = 5.7 Hz, $N_{Gly}H$), 7.27−7.17 (m, 5H, $H_{Ar,\ Phe}$), 7.01 (d, 2H, J = 8.5 Hz, $H_{Ar,\ Tyr}$), 6.63 (d, 2H, J = 8.5 Hz, $H_{Ar,\ Tyr}$), 4.53 (m, $C_{\alpha,\ Phe}H$), 4.39 (m, $C_{\alpha,\ Tyr}H$), 4.27 (m $C_{\alpha,\ Leu}H$), 3.85 (pq, 2H, ^4J = 2.5 Hz, ^3J = 5.5 Hz, $C_{Prop}H_2$), 3.75−3.59 (m, 4H, $C_{\alpha,\ Gly}H_2$), 3.01 (pt, 1H, C≡CH), 3.02 (dd, 1H, ^3J = 4.5 Hz, ^2J = 13.9 Hz, $C_{\beta,\ Phe}H$), 2.90 (dd, 1H, ^3J = 4.6 Hz, ^2J = 13.9 Hz, $C_{\beta,\ Tyr}H$), 2.78 (dd, 1H, ^3J = 9.3 Hz, ^2J = 13.8 Hz, $C_{\beta,\ Phe}H$), 2.63 (dd, 1H, ^3J = 9.8 Hz, ^2J = 13.9 Hz, $C_{\beta,\ Tyr}H$), 1.76 (s, 3H, $C_{Ac}H_3$), 1.55 (m, 1H, $C_{\gamma,\ Leu}H$), 1.45 (m, 2H, $C_{\beta,\ Leu}H_2$), 0.85 (dd, 6H, ^3J = 6.5 Hz, ^2J = 19.0 Hz, $CH(C_{\delta,\ Leu}H_3)_2$). ^{13}C NMR (DMSO-$d_6$, 100.6 MHz): 171.8, 171.4, 170.5, 169.2, 168.8, 168.3 (*C*ON), 155.6 ($C_{Ar,\ Tyr}$OH), 137.5, ($CH_2C_{Ar,\ Phe}$), 129.8, 129.1 ($C_{Ar,\ Tyr}$), 127.9, 126.1 ($C_{Ar,\ Phe}$), 114.7 ($C_{Ar,\ Tyr}$), 80.9 (*C*≡CH), 72.8 (C≡*C*H), 54.3 ($C_{\alpha,\ Phe}$), 53.7 ($C_{\alpha,\ Tyr}$), 50.8 ($C_{\alpha,\ Leu}$), 41.9, 41.7 ($C_{\alpha,\ Gly}$), 40.8 ($C_{\beta,\ Leu}$), 37.3 ($C_{\beta,\ Phe}$), 36.5 ($C_{\beta,\ Tyr}$), 27.8 ($C_{Prop}H_2$) 24.0 ($C_{\gamma,\ Leu}$), 22.8 ($C_{\delta,\ Leu}$), 22.3 (C_{Ac}), 21.5 ($C_{\delta,\ Leu}$).

Ac-Enk-Prop-Co 4. In Schlenk conditions, 2.8 mg (0.004 mmol) of **3** were dissolved in dry THF (0.8 mL). A 0.25 mL quantity of a solution of 65 mg (0.19 mmol) of $Co_2(CO)_8$ in 10 mL of dry THF were slowly added with a syringe to the solution of **3**. The reaction was followed by HPLC. The solution was filtered and dried with a flux of nitrogen. The product precipitated as a brown-red powder. Yield: 4 mg (100%).

Characterization Data of 4. $C_{39}H_{42}Co_2N_6O_{13}$ (920.65 g/mol): t_R = 19.57 min. IR (cm^{-1}): 2095, 2053, 2022 (br, s), 1631 (br, s). MS (ESI, pos): *m/z* 943.0 $[M+Na]^+$.

Ac-Enk(Pgl)-NH_2 5. After the acetylation, the resin was washed with DMF, shrunk with MeOH and dried under high vacuum. The peptide was then cleaved from the resin with a mixture of TFA:TIS:H_2O 95:2.5:2.5 (v/v/v) (1.5 mL). The peptide was then precipitated with cold ether to give a white solid. Yield: 36 mg (62%).

Characterization Data of 5. $C_{33}H_{42}N_6O_7$ (634.72 g/mol): t_R = 13.58 min. IR (cm^{-1}) 1631, 1515 (br, s) υ_{CONH}. MS (ESI, pos): *m/z* 657.3 $[M+Na]^+$. ^{1}H NMR (DMSO-d_6, 400.13 MHz): 9.06 (s, 1H, $O_{Tyr}H$), 8.12 (t, 1H, J = 5.6 Hz, $N_{Gly}H$), 8.06 (t, 1H, J = 8.1 Hz, $N_{Phe}H$), 7.98 (m, 2H, $N_{Phe,\ Pgl}H$), 7.74 (d, 1H, J = 8.4 Hz, $N_{Leu}H$), 7.19−7.08 (m, 5H, $H_{Ar,\ Phe}$), 6.96 (d, 2H, J = 8.5 Hz, $H_{Ar,\ Tyr}$), 6.98, 6.88 (s, 1H, $CONH_2$), 6.96 (d, 2H, J = 8.5 Hz, $H_{Ar,\ Tyr}$), 4.44−4.30 (m, 3H, $C_{\alpha,\ Phe,\ Tyr,\ Pgl}H$), 4.15 (m, 1H, $C_{\alpha,\ Leu}H$), 3.64 (m, $C_{\alpha,\ Gly}H_2$), 2.96 (dd, 1H, ^3J = 5.0 Hz, ^2J = 13.9 Hz, $C_{\beta,\ Phe}H$), 2.83 (dd, 1H, ^3J = 4.6 Hz, ^2J = 13.8 Hz, $C_{\beta,\ Tyr}H$), 2.76 (dd, 1H, ^3J = 8.9 Hz, ^2J = 13.9 Hz, $C_{\beta,\ Phe}H$), 2.67 (pt, 1H, C≡CH), 2.57 (dd, 1H, ^3J = 9.9 Hz, ^2J = 13.9 Hz, $C_{\beta,\ Tyr}H$), 2.47 (m, 1H, ^4J = 2.7 Hz, ^3J = 5.1 Hz, ^2J = 16.9 Hz, $C_{\beta,\ Pgl}H$), 2.30 (m, 1H, ^4J = 2.5 Hz, ^3J = 8.5 Hz, ^2J = 16.8 Hz, $C_{\beta,\ Pgl}H$), 1.70 (s, 3H, $C_{Ac}H_3$), 1.49 (m, 1H, $C_{\gamma,\ Leu}H$), 1.38 (m, 2H, $C_{\beta,\ Leu}H_2$), 0.78 (dd, 6H, ^3J = 6.5 Hz, ^2J = 16.6 Hz, $CH(C_{\delta,\ Leu}H_3)_2$). ^{13}C NMR (DMSO-$d_6$, 100.6 MHz): 173.7 ($CONH_2$), 171.8, 170.1, 169.6, 169.2, 168.6 (CON), 155.6 ($C_{Ar,\ Tyr}$OH), 137.4, ($CH_2C_{Ar,\ Phe}$), 129.8 ($C_{Ar,\ Tyr}$), 129.1, 127.9, 126.1 ($C_{Ar,\ Phe}$), 114.7 ($C_{Ar,\ Tyr}$), 80.3 (C≡CH), 72.6 (C≡CH), 54.4 ($C_{\alpha,\ Phe}$), 54.1 ($C_{\alpha,\ Tyr}$), 51.3 ($C_{\alpha,\ Pgl}$), 50.8 ($C_{\alpha,\ Leu}$), 41.8, ($C_{\alpha,\ Gly}$), 40.8 ($C_{\beta,\ Leu}$), 37.0 ($C_{\beta,\ Phe}$), 36.5 ($C_{\beta,\ Tyr}$), 24.0 ($C_{\gamma,\ Leu}$), 22.9 ($C_{\delta,\ Leu}$), 22.3 (C_{Ac}), 21.5 ($C_{\delta,\ Leu}$,$C_{\beta,\ Pgl}$).

Ac-Enk(Co-Pgl)-NH_2 6. In Schlenk conditions, 11 mg (0.017 mmol) of **5** were suspended in dry THF (5 mL). A 0.5 mL quantity of a solution of 65 mg (0.19 mmol) of $Co_2(CO)_8$ in 5 mL of dry THF were slowly added with a syringe to the solution of **5**. The reaction color changed from yellow to red-brown. The reaction was followed by HPLC. For complete conversion of **5** to **6**, more of the $Co_2(CO)_8$ solution was added. The solution was then stirred for another 1 h. The solution was then filtered and dried with a flux of nitrogen. The product precipitated as a brown-red powder. Yield: about 18 mg (112% as obtained. The product likely contains residual solvent).

Characterization Data of 6. $C_{39}H_{42}Co_2N_6O_{13}$ (920.65 g/mol): t_R = 18.85 min. IR (cm^{-1}) 2094, 2051, 2021 (br, s), 1635 (br, s). MS (ESI, pos): *m/z* 943.1 $[M+Na]^+$.

***N*-Boc-Protected Propargylamide T-PNA Monomer (7). 7** was prepared following the procedure published by Metzler-Nolte et al.[42] The spectroscopic data of the products matched that reported previously.[42]

Dicobalthexacarbonyl Complex of N-Boc-Protected Propargylamide T-PNA Monomer (8). To a solution of **7** (0.10 g, 0.24 mmol) in dry and degassed THF (20 mL) was added slowly, under a atmosphere of argon, a solution of $Co_2(CO)_8$ (0.09 g, 0.26 mmol) in dry and degassed THF (20 mL). At the end of the gaz formation, the reaction mixture was stirred for another 1 h. The solution was then evaporated to dryness with the help of a Schlenk line. The residue was then purified by HPLC to give a dark-red solid. Yield: 0.05 g (32%).

Characterization Data of 8. ^{1}H NMR (CD_3CN): 8.97 (1H, s, br, NH_T), 7.40/7.21 (1H, s, br, N*H*), 7.04 (1H, s, C═C*H*), 6.28/6.23 (1H, s, CH_{alkyne}), 5.85/5.45 (1H, s, br, N*H*Boc), 4.59 (2H, s, CH_2−C_{alkyne}), 4.54/4.39 (2H, m, CH_2), 4.03/3.91 (2H, m, CH_2), 3.42 (2H, m, CH_2−CH_2−NH), 3.23 (2H, m, CH_2−CH_2−NH), 1.80 (3H, s, $CH_{3,T}$), 1.40 (9H, s, $C_{Boc}H_3$). ^{13}C NMR (CD_3CN): 199.4 (Co−*C*≡O), 168.5 (*C*═O), 167.8 (*C*═O), 164.2 (*C*═O_T), 164.1 (C═O_T), 156.2 (C═O_{Boc}), 151.1 (HC═*C*), 141.1 (H*C*═C), 92.5 (C_{alkyne}), 80.0 ($C_{Boc}(CH_3)_3$), 72.2 (HC_{alkyne}), 51.4 (CH_2), 49.1 (CH_2), 48.4 (CH_2), 42.0 (CH_2−C_{alkyne}), 38.9 (CH_2−CH_2), 28.4 ($C_{Boc}H_3$), 12.3 ($CH_{3,T}$). IR: 3448 (m), 2964 (s), 2097 (s), 2058 (s), 2027 (s),

1701 (s), 1654 (m), 1262 (m), 1095 (s), 1023 (s), 802 (s). Raman: 2093, 2018. ESI-MS (pos., THF): *m*/*z* 746 ($[M+K]^+$), 730 ($[M+Na]^+$), 746 ($[M-CO]^+$), 746 ($[M-2CO]^+$).

***tert*-Butyl 2-(*N*-(2-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)ethyl)pent-4-ynamido)acetate (9). 9** was prepared following the procedure published by Metzler-Nolte et al.[44] The spectroscopic data of the products matched that reported previously.[44]

Dicobalthexacarbonyl complex of 9 (10). $Co_2(CO)_8$ (79 mg, 0.21 mmol) dissolved in dry and degassed THF (5 mL) was added dropwise in 5 min, at room temperature, to a solution of *tert*-butyl *N*-[2-(*N*-9-fluorenylmethoxycarbonyl)aminoethyl)] glycinate hydrochloride (**9**) (100 mg, 0.21 mmol) in dry and degassed THF (10 mL). The dark red-brown mixture was stirred for 2 h at room temperature before the solvent was removed in vacuo to give a red solid. A purification by column chromatography on silica using ethyl acetate:hexane 1:2 as the eluent (Rf = 0.40) was performed to give a red sticky oil (124 mg, 78%).

Characterization Data of 10. IR bands (ν, cm^{-1}): 3328 br w, 3068 w, 2978 w, 2940 w, 2090 m, 2048 s, 2005 s, 1721 m, 1651 m, 1515 w, 1448 w, 1367 w, 1232 m, 1151 m, 1103 w, 1010 w, 942 w, 848 w, 758 w, 738 m, 643 w, 620 w. ^{1}H NMR Spectrum (CD_3OD): δ 7.77 (2H, m, C*H* Fmoc arom), 7.61 (2H, m, C*H* Fmoc arom), 7.37 (2H, m, C*H* Fmoc arom), 7.29 (2H, m, C*H* Fmoc arom), 6.26/6.32 (1H, br, s, $CH_2-NH-COO$), 4.35 (2H, m, CH Fmoc$-CH_2O$), 4.19 (1H, m, C*H* Fmoc$-CH_2O$), 3.99/4.08 (2H, m, $N-CH_2-COOC(CH_3)_3$), 3.49 (2H, m, CH_2-CH_2-N), 3.31 (under the solvent peak) (2H, m, $NH-CH_2-CH_2$), 3.16 (2H, m, CH_2-CH_2-CO), 2.58/2.76 (2H, m, $HC\equiv C-CH_2$), 1.47 (9H, br s, $O-(CH_3)_3$). ^{13}C NMR Spectrum (CD_3OD): δ 201.3 (br, Co$-CO$), 174.4/174.8 (CH_2-CON), 170.4/170.6 ($COOC(CH_3)_3$, 158.9 (NH-*C*OO), 145.4/145.5 (*C* Fmoc arom), 142.8 (br, *C* Fmoc arom), 128.9/128.9 (*C*H Fmoc arom), 128.3/128.3 (*C*H Fmoc arom), 126.2/126.3 (*C*H Fmoc arom), 121.1/121.1 (*C*H Fmoc arom), 97.8 (br, $HC\equiv C$), 83.2/84.0 ($O-C(CH_3)_3$), 74.9 (br, $HC\equiv C$), 67.9/67.9 (CH Fmoc$-CH_2O$), 50.5/52.3 ($N-CH_2-COOC(CH_3)_3$), 48.0−50.0 (under the solvent peak) (*C*H Fmoc$-CH_2O$ and CH_2-CH_2-N), 39.9/40.3 ($NH-CH_2-CH_2$), 35.7/36.4 ($HC\equiv C-CH_2$), 30.5/30.6 (CH_2-CH_2-CO), 28.4/28.5 ($OC(CH_3)_3$). ESI-MS (pos., CH_3OH): *m*/*z* 729.0 $[M+Na-2CO]^+$, 756.9 $[M+Na-CO]^+$, 784.9 $[M+Na]^+$, 800.9 $[M+K]^+$.

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Supporting Information Available: Crystallographic data (CIF file) of **2a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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