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The Suzuki cross-coupling reaction: a powerful tool for the attachment of organometallic 'NCN'-pincer units to biological scaffolds☆

Gabriela Guillena [a], Cornelis A. Kruithof [a], Miguel A. Casado [a], Maarten R. Egmond [b], Gerard van Koten [a,*]

[a] Department of Metal-Mediated Synthesis, Debye Institute, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands
[b] Department of Enzymology and Protein Engineering, C.B.L.E., Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

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Abstract

Platinum(II) and palladium(II)–NCN {NCN is the terdentate coordinating monoanionic 'pincer' ligand $[C_6H_3(CH_2NMe_2)_2\text{-}2,6]^-$} complexes have been covalently bonded via their *para*-position to both the α-carbon of an α-amino acid and to the γ-position of an alkyl phosphonate by means of Suzuki cross-coupling reactions. The resulting platinum(II) complexes can be used as biomarkers, while the palladium(II) analogs are active Lewis-acid catalysts. Both the pincer–metal substituted α-amino acid and phosphonate can be used to introduce these organometallic units in biomolecules such as proteins or enzymes.

Keywords: Platinum; Palladium; α-Amino acid; Phosphonate

1. Introduction

The incorporation of organometallic complexes in large biomolecules requires the synthesis of small, stable and topologically predetermined organometallic units. In such building blocks, the metal is usually introduced at the end of the synthetic protocol, which normally involves several known organic transformations to attach the ligand moiety to its biological counterpart. Cross-coupling reactions, such as the Suzuki–Miyaura coupling reaction [1] between arylboron derivatives and alkyl halides or triflates catalyzed by palladium species, have shown to be powerful tools to incorporate aryl moieties in a large variety of molecules. Among these, various biologically relevant molecules such as α-amino acids [2], phosphonates [3], carbohydrates [4] and nucleosides [5] have been used as starting materials. The mild reaction conditions used, in principle, can help to preserve the enantiomeric purity of the starting biomolecule, which makes this an interesting approach to introduce any aryl moiety to the aforementioned molecules. Despite these facts, to the best of our knowledge, the Suzuki–Miyaura cross-coupling reaction has not been used to incorporate metal-binding ligands in such biomolecules which would enable the preparation of so-called 'bioorganometallic molecules'. In one case, the related Sonogashira cross-coupling reaction between terminal alkynyl derivatives and aryl halides or triflates catalyzed by copper–palladium species has recently been used for the attachment of possible coordination complexes, such as bypiridine and terpyridine-metal derivatives, onto α-amino acids [6] with promising results. Recent studies demonstrated the great versatility of 1-bromo-4-iodo-2,6-bis[(dimethylamino)methyl]benzene (**1**, I–NCN–Br, Fig. 1) as a multidentate organometallic ligand in organic and organometallic chemistry [7].

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* Corresponding author. Tel.: +31-30-2533120; fax: +31-30-2523615

E-mail address: g.vankoten@chem.uu.nl (G. van Koten).

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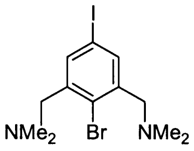

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Fig. 1. Ligand precursor I–NCN–Br.

Some of the transition metal complexes of such potentially terdentate, monoanionic 'NCN'-pincer ligands have a remarkable stability due to the presence of the bis ortho-chelation motif which makes the central σ-carbon–metal bond less sensitive towards electrophilic or nucleophilic attack [8]. In fact, this unusual high stability allowed us to perform various reaction protocols, which are commonly used for peptide chemistry. For example, we have prepared NCN-pincer platinum(II) [9] and palladium(II) [10] complexes which were covalently bonded, via the *para*-C position, to either *N*- or *C*-termini of different natural α-amino acids (Fig. 2). The resulting compounds are currently studied for applications as biomarkers and as homogeneous catalysts.

In this paper, we report the usefulness of the Suzuki–Miyaura cross-coupling approach for the attachment of NCN-pincer metal complexes to biologically relevant molecules such as α-amino acids and phosphonates [11].

2. Results and discussion

The incorporation of an organometallic site at the α-position of an α-amino acid [12] would allow, after removal of the corresponding protective groups, the introduction of an organometallic site to any position of a peptidic chain. For this purpose, we have chosen the Suzuki–Miyaura cross-coupling reaction of the protected allyl glycine ester **2** [13] and the bifunctional precursor I–NCN–Br **1** (Scheme 1). The borane intermediate formed by reaction of **2** with 9-BBN (9-borabicyclo[3.3.1]nonane) was reacted with **1** using [PdCl$_2$(dppf)] as catalyst. After 12 h reflux, the NCN-bishomophenylalanine analog **3** was obtained in 72% yield. Metallation of **3** was carried out using [Pt(μ-SEt$_2$)(p-tol)$_2$]$_2$, yielding the expected protected NCN-pincer platinum(II) α-amino acid **4** in 79% yield. Alternatively, the palladium(II) analog **5** was obtained by oxidative addition of Pd$_2$(dba)$_3$·CHCl$_3$ in 66% yield. Deprotection, i.e. hydrolysis, of the α-labeled α-amino acid **4** to the corresponding free α-amino acid would enlarge the applicability of these bioorganometallic complexes in peptide chemistry [14]. Although most organometallic complexes do not tolerate basic or acidic conditions, NCN-pincer platinum halide complexes are stable enough to survive these conditions. Thus, the hydrolysis of **4** to the free α-amino acid **8** was accomplished in two steps. First, the methyl ester-protecting group of **4** was hydrolyzed to the corresponding acid **6** (isolated in 77% yield) by treatment with lithium hydroxide in a THF–H$_2$O solution [15]. Using a similar method the methyl ester of the palladated compound **5** could be hydrolyzed to the free amino acid **7** in 46% yield. In the second stage, removal of the Boc-protecting group was achieved by treatment of **6** with HBr–Et$_2$O, giving the free α-platinum(II) labeled α-amino acid **8** in 75% yield (Scheme 1).

Recently we also succeeded in extending the above-described procedure for the attachment of NCN-pincer metal complexes to phosphonates. To this purpose, allyl *N*,*N*-dimethyl phosphonate **9** [16] was synthesized from commercial available diethylchlorophosphate in several steps. Compound **9** was reacted with 9-BBN to generate a borane intermediate, which subsequently was coupled to I–NCN–Br **1**, using [PdCl$_2$(dppf)] as catalyst. After 3 h reflux the phosphonate derivative **10** was obtained in 90% yield. Metallation of **10** using [Pt(μ-SEt$_2$)(p-tol)$_2$]$_2$ yielded the expected NCN-pincer platinum(II) substituted phosphonate **11** in 78% yield. By means of an oxidative addition of **10** with Pd$_2$(dba)$_3$·CHCl$_3$, also the palladium(II) phosphonate analog **12** could be obtained in 73% yield (Scheme 2).

3. Conclusions

In summary, we have developed procedures allowing the successful attachment of the versatile I–NCN–Br ligand to α-position of an α-amino acid as well as to the terminal position (γ-position in the case of **11** and **12**) of the chain of an alkyl phosphonate. These derivatives can be directly metallated to give the platinum(II) or palladium(II) complexes in good to quantitative yields.

In our further research we are now using these bioorganometallics derivatives for different purposes.

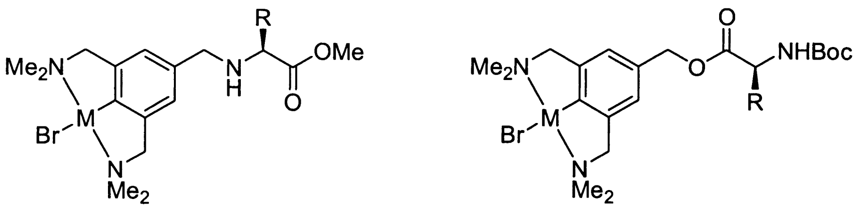
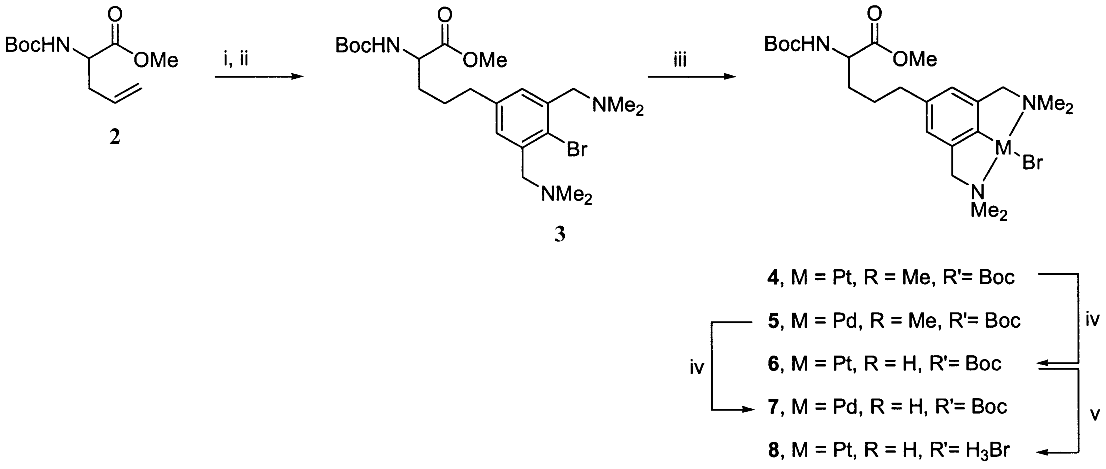


Fig. 2. *N*- and *C*-metallated α-amino acids (M = Pt or Pd).



Scheme 1. *Reagents and conditions*. (i) 9-BBN, THF, 2 h r.t.; (ii) K_3PO_4, DMF, 10% [$PdCl_2$(dppf)], **1**, Δ, 12 h; (iii) [Pt(μ-SEt_2)(*p*-tol)$_2$]$_2$ or $Pd_2(dba)_3 \cdot CHCl_3$, C_6H_6, 50 °C, 3 h; (iv) LiOH, THF–H_2O, r.t., 12 h; (v) HBr–Et_2O, r.t., 1 h.

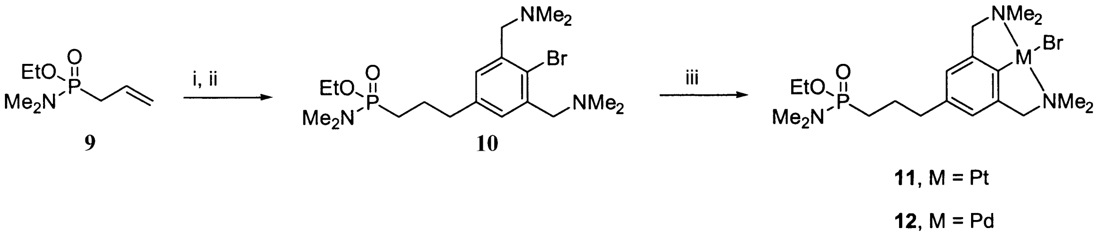
The free organometallic amino acids **6**, **7** and **8** are currently included at various positions in the chains of polypeptides. In one example, the NCN-pincer platinum complex acts as a color biomarker in combinatorial synthetic peptide libraries.

The γ-NCN-pincer platinum substituted allylphosphonate is highly interesting because phosphonates are commonly used as selective inhibitors of enzymes such as lipases [17]. The use of phosphonates of type **11** and **12** would allow the single-site directed incorporation of this bioorganometallic unit into a biomolecule of great complexity as an enzyme. In this way, interesting properties of such a biomolecule, e.g. water solubility, nano-size, chiral environment of the inhibited pocket, would be transferred to the organometallic site. Current studies already showed that a phosphonate derivative of **11** is an active inhibitor of the enzyme cutinase (from *Fusarium solani pisi*, [18]) and that the inhibited lipase-NCN-pincer construct can be isolated. Presently full characterization of this construct is underway.

4. Experimental

4.1. General

Suzuki and metallation reactions were carried out under nitrogen atmosphere using standard Schlenk techniques. THF and C_6H_6 were dried from Na–benzophenone and distilled prior to use. DMF was flash-distilled from CaH_2 and stored on molecular sieves. The Pt salt [Pt(μ-SEt_2)(*p*-tol)$_2$]$_2$ [19], the ligand precursor [I–NCN–Br] **1** [7], and compounds **2** [13], **4** [9], **6** [9], **8** [9], **9** [16], **10** [16], **11** [16] were prepare according to published procedures. All other reagents were obtained commercially and used without further purification. The ^{1}H- and ^{13}C-NMR spectra were recorded at 300 and 75 MHz, respectively, at 25 °C and were referenced to external $SiMe_4$ ($\delta = 0.00$, J in Hz). Elemental analyses were performed by Kolbe, Mikroanalytisches Laboratorium (Mülheim, Germany). MALDI-TOF-MS spectra were acquired using a Voyager-DE BioSpectrometry Workstation (PerSeptive Biosystems Inc., Framingham, MA) mass spectrometer equipped with a nitrogen laser emitting at 337 nm. The instrument was operated in the linear mode at an accelerating voltage in the range 22 000 V. External calibration was performed using C_{60}/C_{70}, and detection was performed by means of a linear detector and digitizing oscilloscope operating at 500 MHz. Sample solutions with ca. 10 mg ml^{-1} in THF were used, and the matrix were 3,5-dihydroxybenzoic acid or 5-chlorosalycilic acid in THF (10 mg ml^{-1}). A solution of silver(I) trifluoroacetate in THF was in some cases added to the sample in order to improve the peak resolution. The sample solution (0.2 μl) and the matrix



Scheme 2. *Reagents and conditions*. (i) 9-BBN, THF, 3 h r.t.; (ii) K_3PO_4, DMF, 10% [$PdCl_2$(dppf)], **1**, Δ, 3 h; (iii) [Pt(μ-SEt_2)(*p*-tol)$_2$]$_2$ or $Pd_2(dba)_3 \cdot CHCl_3$, C_6H_6, Δ, 1 h.

solution (0.2 μl) were combined and placed on a gold MALDI target and analyzed after evaporation of the solvents.

*4.2. Synthesis of compound **5***

A mixture of **3** (187 mg, 0.38 mmol) and [$Pd_2(dba)_3 \cdot CHCl_3$] (207 mg, 0.20 mmol) in dry C_6H_6 (15 ml) was refluxed for 2 h. After cooling to room temperature (r.t.) the reaction mixture was filtered over Celite and the volatiles were removed at reduced pressure. The resulting oil was purified by column chromatography (SiO_2) using as eluent CH_2Cl_2 until all dba came out of the column and then a mixture of CH_2Cl_2–acetone (1:1) was used. The palladium-containing fractions were collected and evaporated to dryness, yielding **5** as a yellow solid (150 mg, 66%). ^{1}H-NMR ($CDCl_3$): δ 1.38 (s, 9H; OC(C*H*$_3$)$_3$), 1.53–1.76 (m, 4H; C*H*$_2$C*H*$_2$), 2.42–2.48 (m, 2H; C*H*$_2$CH), 2.91 (s, 12H; NC*H*$_3$), 3.67 (s, 3H; OC*H*$_3$), 3.91 (s, 4H; C*H*$_2$NMe$_2$), 4.24–4.26 (m, 1H; NH–C*H*–CO), 4.97 (d, $^3J_{HH}=7.9$, 1H; N*H*), 6.53 (s, 2H; Ar*H*); ^{13}C-NMR ($CDCl_3$): δ 27.3 (CH_2CH_2), 28.1 (OC(*C*H$_3$)$_3$), 32.2 (*C*H$_2$CH$_2$), 35.2 (*C*H$_2$CH), 52.1 (O*C*H$_3$), 53.1 (NH–*C*H–CO), 53.5 (N*C*H$_3$), 74.3 (*C*H$_2$NMe$_2$), 79.7 (O*C*(CH$_3$)$_3$), 119.7, 138.2 (C), 143.8 (C), 154.2 (C), 155.1 (NH*C*O), 173.1 (*C*O$_2$); MS (MALDI-TOF): *m/z* 524.6 $[M-Br]^+$ (Calc. 526.2); Anal. Calc. for $C_{23}H_{39}BrN_3O_4Pd$ (605.11): C, 45.52; H, 6.31; N, 6.92. Found: C, 45.68; H, 6.37; N, 6.86%.

*4.3. Hydrolysis of **5**. Synthesis of compound 7*

A solution of **5** (100 mg, 0.16 mmol) and $LiOH \cdot H_2O$ (32 mg, 0.8 mmol, 56%) in THF–H_2O (4:1.5 ml) was stirred at r.t. overnight. The reaction mixture was subsequently treated with a 1 M HCl (aq.). The aqueous phase was extracted with EtOAc (3×20 ml) and the collective organic layer were washed with brine and dried over $MgSO_4$ to afford **20** (40 mg, 42%). ^{1}H-NMR (CD_3OD): δ 1.37 (s, 9H; OC(C*H*$_3$)$_3$), 1.59–1.81 (m, 4H; C*H*$_2$C*H*$_2$), 2.42–2.46 (m, 2H; C*H*$_2$CH), 2.89 (s, 12H; NC*H*$_3$), 3.90 (s, 4H; C*H*$_2$NMe$_2$), 4.18–4.25 (m, 1H; NH–C*H*–CO), 5.15 (d, $^3J_{HH}=8.0$, 1H; N*H*), 6.54 (s, 2H; Ar*H*); ^{13}C-NMR ($CDCl_3$): δ 27.3 (CH_2CH_2), 28.1 (OC(*C*H$_3$)$_3$), 32.1 (*C*H$_2$CH$_2$), 35.3 (*C*H$_2$CH), 53.4 (N*C*H$_3$), 65.6 (NH–*C*H–CO), 74.2 (*C*H$_2$NMe$_2$), 83.4 (O*C*(CH$_3$)$_3$), 119.7, 138.3 (C), 144.8 (C), 145.3 (C), 155.5 (NH*C*O), 177.3 (*C*O$_2$).

*4.4. Synthesis of compound **11***

Solid [Pt(μ-SEt$_2$)(*p*-tolyl)$_2$]$_2$ (1.06 g, 1.14 mmol) was added to a solution of **10** (1.02 g, 2.27 mmol) in (45 ml) C_6H_6 and heated to reflux for 1 h. The solvent was removed and the remaining dark brown oil dissolved in (15 ml) CH_2Cl_2 and filtrated through a path of Celite. The filtrate was concentrated under reduced pressure leaving a brown oil which was subsequently washed with (3×30 ml, stirring 30 min) C_5H_{12} and (2×30 ml, stirring 30 min) Et_2O. The residue was dried in vacuo to obtain the product as an off-white solid (1.11 g, 78%). ^{1}H-NMR (C_6D_6): δ 6.52 (s, Ar*H*, 2H), 3.91 (m, CH$_2$O, 2H), 3.33 (s, ArCH$_2$N, $^3J_{HPt}=44.56$ Hz, 4H), 2.77 (s, N(CH$_3$)$_2$, $^3J_{HPt}=37.54$ Hz, 12H), 2.60 (t, *C*H$_2$Ar, $^3J_{HH}=7.32$ Hz, 4H), 2.43 (d, PN(CH$_3$)$_2$, $^3J_{HP}=8.85$ Hz, 6H), 2.02 (m, CH$_2$, 2H), 1.71 (t, CH$_2$,$^3J_{HH}=7.63$ Hz, 2H), 1.06 (t, CH$_3$CH$_2$O, $^3J_{HH}=7.02$ Hz, 3H). ^{31}P-NMR (C_6D_6): δ 36.26 (s). ^{13}C-NMR (75.468 MHz, C_6D_6): δ 145.52, 136.38, 134.52, 119.54 (s, Ar*C*), 77.13 (s, $^2J_{CPt}=32.96$ Hz, ArCH$_2$N), 58.82 (d, CH$_2$O, $^2J_{CP}=7.33$ Hz), 54.62 (s, N(CH$_3$)$_2$), 37.44 (d, CH$_2$CH$_2$P, $^2J_{CP}=14.65$ Hz), 35.81 (d, PN(CH)$_3$, $^2J_{CP}=4.27$ Hz), 25.33 (d, CH$_2$P, $^1J_{CP}=130.62$ Hz), 16.65 (d, *C*H$_3$CH$_2$O, $^3J_{CP}=5.49$ Hz); MS (MALDI-TOF, CSA): *m/z*: 561.1 $[M-Br]^+$, 467.2 $[M-Br-Pt]^+$; Anal. Calc. for $C_7H_{16}NO_2P$: C, 42.30; H, 9.42; N, 6.69. Found: C, 42.48; H, 9.36; N, 6.76%.

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References

[1] (a) A. Suzuki, J. Organomet. Chem. 576 (1999) 147;
(b) N. Miyaura, A. Suzuki, Chem. Rev. 95 (1995) 2457.
[2] (a) P.N. Collier, A.D. Campbell, T.M. Patel, T.M. Raynham, R.J.K. Taylor, J. Org. Chem. 67 (2002) 2121;
(b) M. Isaac, A. Slassi, K. Da Silva, T. Xin, Tetrahedron Lett. 42 (2001) 2957;
(c) S. Collet, R. Danion-Bougot, D. Danion, Synth. Commun. 31 (2001) 249;
(d) F. Firooznia, C. Gude, K. Chan, N. Narcopulos, Y. Satoh, Tetrahedron Lett. 40 (1999) 213.
[3] I. Pergament, M. Srebnik, Org. Lett. 3 (2001) 217.
[4] (a) C.R. Johnson, B.A. Johns, Tetrahedron Lett. 38 (1997) 7977;
(b) C.R. Johnson, B.A. Johns, Synlett (1997) 1406.
[5] (a) N. Amann, H.A. Wagenknecht, Synlett (2002) 687;
(b) M.K. Lakshman, J.H. Hillmer, J.Q. Martin, J.C. keeler, Y.Q.V. Dinh, F.N. Ngassa, L.M. Russon, J. Am. Chem. Soc. 123 (2001) 7779;
(c) M. Hocek, A. Holy, I. Votruba, H. Dvorakova, J. Med. Chem. 43 (2000) 1817.
[6] A. Khatyr, R. Ziessel, Org. Lett. 3 (2001) 1857.
[7] G. Rodríguez, M. Albrecht, J. Schoenmaker, A. Ford, M. Lutz, A.L. Spek, G. van Koten, J. Am. Chem. Soc. 124 (2002) 5127.
[8] (a) M. Albrecht, G. van Koten, Angew. Chem. Int. Ed. Engl. 40 (2001) 3750;
(b) M.H.P. Rietveld, D.M. Grove, G. van Koten, New J. Chem. 21 (1997) 751.

[9] (a) G. Guillena, G. Rodríguez, M. Albrecht, G. van Koten, Chem. Eur. J. (2002) 5368;
(b) M. Albrecht, G. Rodríguez, J. Schoenmaker, G. van Koten, Org. Lett. 2 (2000) 3461.

[10] G. Guillena, G. Rodríguez, G. van Koten, Tetrahedron Lett. 43 (2002) 3895.

[11] (a) P. Kielbansinki, J. Omalanczuk, M. Mikolajczyk, Tetrahedron: Asymmetry 9 (1998) 3283;
(b) C.D.S. Tomlin, The Pesticide Manual, British Crop protection Council, Farnham, Surrey, UK, 1997.

[12] R.M. Williams, Advances in Asymmetric Synthesis, vol. 1, Jai Press, Greenwich, 1995, p. 45.

[13] S. Collet, R. Danion-Bougot, D. Danion, Tetrahedron: Asymmetry 9 (1998) 2121.

[14] Gabriela Guillena, Koen Halkes, Gema Rodríguez, Gerard van Koten, Johannis P. Kamerling, manuscript in preparation.

[15] W. Oppoltzer, P. Lienard, Helv. Chim. Acta 75 (1992) 2572.

[16] C.A. Kruithof, M.A. Casado, G. Guillena, M.A. Egmond, G. van Koten, manuscript in preparation.

[17] (a) F. Björkling, A. Dahl, S. Patkar, M. Zundel, Bioorg. Med. Chem. 2 (1994) 697;
(b) P.E. Kolattukudy, Lipases, Elsevier, Amsterdam, 1984, pp. 471–501.

[18] (a) K. Faber, Biotransformations in Organic Chemistry, Springer-Verlag, Berlin-Heildeberg, 2000, p. 453;
(b) C. Martínez, P. Geus, M. Lauwereys, M. Matthyssens, C. Cambillau, Nature 356 (1992) 615.

[19] (a) B.R. Steele, K. Vrieze, Transition Met. Chem. 2 (1977) 140;
(b) A.J. Canty, J. Patel, B.W. Skelton, A.H. White, J. Organomet. Chem. 599 (2000) 195.