Communication

Separation of Alkyne Enantiomers by Chiral Column HPLC Analysis of Their Cobalt-Complexes

Qiaoyun Liu 1, Jing Wang 1, Junfei Li 1, Xiaolei Wang 2, Shichao Lu 2, Xuan Li 2, Yaling Gong 2,* and Shu Xu 2,∗

1 School of Chemistry and Material Science, Shanxi Normal University, 1 Gongyuan Street, Linfen, Shanxi 041004, China; liuqy@sxnu.edu.cn (Q.L.); 214111062@stu.sxnu.edu.cn (J.W.); lijunfei@sxnu.edu.cn (J.L.)
2 State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Beijing Key Laboratory of Active Substances Discovery and Drugability Evaluation, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, 2A NanWei Road, Xicheng District, Beijing 100050, China; wangxiaolei@imm.ac.cn (X.W.); lushichao@imm.ac.cn (S.L.); lanthanum1979@gmail.com (X.L.)
* Correspondence: ylgong@imm.ac.cn (Y.G.); xushu@imm.ac.cn (S.X.); Tel.: +86-10-6316-5243 (S.X.)

Abstract: Separation of the enantiomers of new chiral alkynes in strategic syntheses and bioorthogonal studies is always problematic. The chiral column high-performance liquid chromatography (HPLC) method in general could not be directly used to resolve such substrates, since the differentiation of the alkyne segment with the other alkane/alkene segment is not significant in the stationary phase, and the alkyne group is not a good UV chromophore. Usually, a pre-column derivatization reaction with a tedious workup procedure is needed. Making use of easily-prepared stable alkyne-cobalt-complexes, we developed a simple and general method by analyzing the in situ generated cobalt-complex of chiral alkynes using chiral column HPLC. This new method is especially suitable for the alkynes without chromophores and other derivable groups.

Keywords: alkyne; cobalt complex; HPLC; chiral column; resolution

1. Introduction

Alkyne is one of the fundamental groups in organic chemistry, which exists widely in natural products and unnatural functionalized molecules [1]. The development of modern transition-metal-catalyzed reactions, such as cross coupling [2], Pauson-Khand reaction [3], click chemistry [4], etc., has caused alkynes to play a more and more important role in strategic syntheses and bioorthogonal design [1].

Enantiomeric purity is vital for chiral alkynes to function. The enantiomeric excess (ee) of chiral alkynes with unknown optical rotation could be determined generally in two ways: (1) using another chiral reagent to derivate diastereomers and then measure the diasteromeric ratio; (2) using enantioselective chromatography to directly measure the enantiomeric excess. Recently, in the course of our total synthesis of natural products, we prepared the known alkyne 1a (Figure 1), and found that two methods were used in the literatures to determine its ee [5–13]. Most literature used Mosher’s methodology [14] which undertook an esterification with expensive Mosher’s chiral reagent, and determined the enantiomeric purity by NMR analysis of the resultant diasteromeric mixture [5–12]. One study used high-performance liquid chromatography (HPLC) with chiral column to analyze the 3,5-dinitrobenzoate derivative of 1a [13]. Since both methods need a derivatization reaction with tedious workup procedure, we initially attempted a direct analysis of 1a with chiral column HPLC.
method. However, no good result was achieved, which may be due to two causes: (1) the alkyne- and alkene-chains in 1a are similar in size, and therefore might be difficult to be differentiated by chiral stationary phase. In fact, no enantiomeric separation could be found in all our attempted chiral columns and HPLC conditions; (2) alkyne and other functional groups in 1a are all poor chromophores. As a result, 1a showed very low UV-absorption, and it was difficult to characterize under normal HPLC concentration using a UV detector.

Figure 1. Preparation of 2a and the comparison of its properties with those of 1a. (a) Transformation from 1a to 2a; (b) The UV-Vis spectra of 1 mM solution of 1a in n-hexane; (c) The UV-Vis spectra of 0.01 mM solution of 2a in n-hexane; (d) The high-performance liquid chromatography (HPLC) chromatogram of injected 1a (20 µL, 1 M in n-hexane), with CHIRALPAK-IB column, 2-PrOH/n-hexane 0.4:99.6 eluting-solvent system, 1 mL·min⁻¹ flow rate, and 200 nm detection wavelength at 25 °C; (e) The HPLC chromatogram of racemic 2a (20 µL, 1 mM in n-hexane) with the same conditions as (d) except the detection wavelength of 350 nm; (f) The HPLC chart of enantiomeric enriched 2a with the same conditions as (e).
Aware of the insufficiency of the present ee determination methodology for chiral alkynes such as 1a, we decided to develop and report herein a simple and general method by analyzing the in situ generated cobalt-complex of chiral alkynes using chiral column HPLC. This new method is especially suitable for the alkynes without chromophores and other derivable groups.

2. Results and Discussion

To overcome the above-mentioned two weak points of alkynes, our idea was to make use of its metal complex. It is well known that Co$_2$(CO)$_6$ can easily react with alkynes to lose two carbon monoxides and form stable Co$_2$(CO)$_6$–alkyne complexes. Figure 1a showed this transformation from alkyne 1a to its complex 2a. By this simple complexation, (1) the alkyne-chain became significantly bulky, which was very different in size from the other side-chain; (2) the Co-complexation substructure was a good chromophore, making the UV absorption of 2a more than 100 times stronger than 1a (Figure 1b,c), and a wide range of detection wavelength (200–400 nm) could be selected in the HPLC experiment. As a result, comparison with the unsuccessful result of 1a (Figure 1d), 2a was easily resolved by HPLC using a CHIRALPAK IB column with 0.4:99.6 2-PrOH/n-hexane eluting-solvent system, 1 mL·min$^{-1}$ flow rate, and 350 nm detection wavelength at 25 °C (Figure 1e). Under the same conditions, an enantioenriched 2a was also tested (Figure 1f), and the detected enantiomeric ratio was consistent with that determined by the modified Mosher’s methodology [11].

After confirmation of our idea on compounds 1a and 2a, we next prepared a series of Co$_2$(CO)$_6$–alkyne complexes 2b–k (Table 1) from none-chromophore alkynes, and checked their resolution by HPLC chiral column. As a result, all the enantiomeric pairs of Co-complexes were successfully baseline-separated. For alcohol substrates, the size of another side-chain (butyl, allyl, ethyl, methyl) or the distance between the C≡C triple bond and the hydroxyl group did not show any notable effect for the enantiomeric separation (2a–e). Even a tertiary alkynol-complex 2f could be well resolved, although a CHIRALPAK IA column was used in this case. Internal alkyne-complexes 2g and 2h also gave good separation. Replacing the hydroxyl group with halogen atoms might change the interaction between the substrates and the stationary phase of the column. Fortunately, the chloride 2i and the fluoride 2j were both resolved, although lower polar eluting-solvent systems were needed for the low polarity of these substrates. Notably, the tetrahydropyran (THP)-protected alkynol-complex 2k could also been enantiomerically separated. Actually, compounds such as 1i–k, are not good substrates for classical pre-column derivatization methods.

Table 1. Scope of alkynes for HPLC resolution of their Co-complexes.
Table 1. Cont.

| Alkyne (1) | Co-Complex (2) | HPLC Chart | HPLC Conditions (CHIRALPAK-IB Column 350 nm, 25 °C) |
|------------|----------------|------------|--------------------------------------------------|
| 2d         | Co(CO)₃(OC)₃Co | ![Chart](image1) | 2-PrOH/n-hexane = 0.4:99.6 1 mL·min⁻¹             |
| 2e         | Co(CO)₃(OC)₃Co | ![Chart](image2) | 2-PrOH/n-hexane = 0.4:99.6 1 mL·min⁻¹             |
| 2f         | Co(CO)₃(OC)₃Co | ![Chart](image3) | 2-PrOH/n-hexane = 0.4:99.6 1 mL·min⁻¹             |
| 2g         | Co(CO)₃(OC)₃Co | ![Chart](image4) | 2-PrOH/n-hexane = 0.5:99.5 1 mL·min⁻¹             |
| 2h         | Co(CO)₃(OC)₃Co | ![Chart](image5) | 2-PrOH/n-hexane = 0.3:99.7 1 mL·min⁻¹             |
| 2i         | Co(CO)₃(OC)₃Co | ![Chart](image6) | EtOAc/n-hexane = 0.05:99.95 0.3 mL·min⁻¹           |
| 2j         | Co(CO)₃(OC)₃Co | ![Chart](image7) | CH₂Cl₂/n-hexane = 0.05:99.95 0.4 mL·min⁻¹         |
| 2k         | Co(CO)₃(OC)₃Co | ![Chart](image8) | 2-PrOH/n-hexane = 0.1:99.9 1 mL·min⁻¹             |

*a CHIRALPAK-IA column was used; b The HPLC was measured at 0 °C; c The HPLC was measured at 10 °C.*
It was noted that most of the above HPLC experiments were carried out with the same chiral
column using the most general 2-PrOH/n-hexane eluting-solvent system. Except for changing the
2-PrOH/n-hexane ratio to make the retention time between 10–20 min, no more optimization was done
for HPLC conditions. The simple setup of the HPLC conditions implied the easiness and generality of
our Co-complexation method in monitoring the asymmetric alkyne-synthesis reactions.

Although the above HPLC analysis was all carried out with purified Co-complexes, since the
CHIRALPAK immobilized-polysaccharide type columns can tolerate a wide range of solvents, we next
attempted to directly measure the reaction system of 1a, Co2(CO)8, and CH2Cl2 (Figure 1a). To our
delight, by just diluting a small amount of the reaction mixture with n-hexane and then injection
into HPLC, the in situ generated 2a could be monitored without any problems (Figure 2). Since the
manipulation was rather simple without the tedious workup procedure like those of Mosher’s or other
carbonyl chloride methods [5–13], this result implied a promising application of our Co-complexation
method in monitoring the asymmetric alkyne-synthesis reactions.

Figure 2. The HPLC chromatogram of the reaction mixture of 1a, Co2(CO)8, and CH2Cl2, with
CHIRALPAK-IB column, 2-PrOH/n-hexane 0.4:99.6, 1 mL·min−1 flow rate, and 350 nm detection
wavelength at 25 °C. Sample preparation: 20 µL of the reaction mixture was taken 5 min after the
reaction started, diluted with 0.5 mL n-hexane, and passed through a disposable syringe filter (Nylon 66,
0.22 µm, 13 mm); 5 µL of the filtration was injected.

3. Materials and Methods

3.1. General Methods

All reactions were carried out under an atmosphere of Ar unless otherwise indicated. 1H-, and
13C-NMR spectra were acquired on Mercury-300 (Agilent, Santa Clara, CA, USA), AVANCE III-400
(Bruker, Billerica, MA, USA), WNMR-I-500 (Zhongke Niujin Co., Ltd., Wuhan, China), or VNMRS-600
spectrometers (Agilent). Chemical shifts are indicated in parts per million (ppm) downfield from
tetramethylsilane (TMS, δ = 0.00) with residual undeuterated solvent peaks as internal reference for
1H-NMR and deuterated solvent peaks shifts for 13C-NMR. Multiplicities are reported as s (singlet),
d (doublet), t (triplet), q (quartet), m (multiplet), br (broad) or combinations of those. For NMR
analysis of the Co2(CO)8-alkyne complexes, the sample solution in CDCl3 should pass through a
disposable syringe filter (Nylon 66, 0.22 μm, 13 mm) immediately before the NMR experiment, to remove the small amount of paramagnetic material. Mass spectra (MS) are electron ionization (EI) or electrospray ionization (ESI). EI-MS data were measured on GCT mass spectrometer (Micromass, Manchester, UK). ESI-MS data were measured on Thermo-Fisher Accela liquid chromatography system coupled with Exactive Plus Orbitrap mass spectrometer (Thermo-Fisher, Bremen, Germany). Reagents and compounds 1c-f, 1k were purchased from commercial suppliers and used as received. Compound 1a was prepared according to the literature procedure [8]. Compounds 1b, 1g-j, and 2a-k were synthesized as following. See Supplementary Materials for spectra and chromatograms of the prepared products.

3.2. Preparation of Alkynes and Compound Characterization

Non-1-yn-5-ol (1b). To dry THF (254 mL) was added n-BuLi (2.5 M in n-hexane, 9.74 mL, 24.4 mmol) at −78 °C. 4-pentyn-1-al [8] (1.00 g, 12.2 mmol) was added slowly at −78 °C. Then the mixture was stirred for 3 h while its temperature reached 23 °C. Methanol (50 mL) was added to quench excess n-BuLi. The mixture was concentrated. The residue was dissolved in 10% aqueous HCl and the resulting mixture was extracted with CH2Cl2. The combined organic layers were dried over Na2SO4, concentrated, and purified by silica gel column chromatography. Elution with cyclohexane/acetone (50/1) gave a pale yellow oil (0.38 g, 22%); 1H-NMR (300 MHz, CDCl3) δ 3.75 (br, 1H, CHOH), 2.34 (t, J = 6.0 Hz, 2H), 1.98 (s, 1H, ≡CH), 1.72–1.60 (m, 2H), 1.67 (br, 1H, OH), 1.46–1.33 (m, 6H), 0.91 (t, J = 6.3 Hz, 3H, CH3). 13C-NMR (75 MHz, CDCl3) δ 84.5 (C=CH), 71.0, 68.9, 37.3, 35.9, 28.0, 22.9, 15.2, 14.3. HRMS (ESI) m/z calcd. for C9H17O+ [M + H]+: 141.1274; found: 141.1269.

Dec-1-yn-4-ol (1g). To a solution of tert-butylimethyl(oct-1-en-7-yn-4-yl)oxy)silane [8] (3.00 g, 12.6 mmol) in dry THF (15 mL) cooled at −78 °C added dropwise lithium diisopropylamide (LDA, 10.064 mL, 25.16 mmol) via syringe. The resulting mixture was stirred at −78 °C for 1 h followed by addition of iodoethane (5.056 mol, 62.9 mmol). The reaction solution allowed to warm to room temperature. After being stirred for 2 h, the mixture was passed through a silica-gel pad (eluted with CH2Cl2), and concentrated in vacuum. To the residue was added tetrabutylammonium fluoride (1 M in THF, 39.4 mL, 39.4 mmol) at room temperature. The reaction mixture was stirred for 10 h, and then was quenched with saturated aqueous NH4Cl. The aqueous layer was extracted with CH2Cl2. The organic phase was then dried over Na2SO4, concentrated, and purified by silica gel column chromatography. Elution with cyclohexane/CH2Cl2 (30/1) gave a pale yellow oil (0.98 g, 51%); 1H-NMR (500 MHz, CDCl3) δ 5.82 (m, 1H, CH=CH2), 5.14–5.11 (m, 2H, CH=CH2), 3.80 (m, 1H, CH–OH), 2.32–2.26 (m, 3H), 2.21–2.11 (m, 3H), 2.00 (br, 1H, OH), 1.66 (m, 1H), 1.60 (m, 1H), 1.10 (t, J = 7.5 Hz, 3H, CH3), 13C-NMR (125 MHz, CDCl3) δ 134.6 (CH=CH2), 118.0 (CH=CH2), 82.4, 78.8, 70.0 (CH–OH), 41.8, 35.6, 15.3, 14.2, 12.3. HRMS (ESI) m/z calcd. for C10H19O+ [M + H]+: 153.1274; found: 153.1269.

8-(Trimethylsilyl)oct-1-yn-7-yn-4-ol (1h). To a solution of 5-(trimethylsilyl)pent-4-ynal [15] (9.17 g, 59.4 mmol) in CH2Cl2 (400 mL) cooled at −78 °C added dropwise allylboronic acid pinacol ester (12.26 mL, 65.4 mmol). The resulting mixture was stirred at −78 °C for 1 h, and then at 0 °C for 3 h. The reaction was quenched by addition of water, extracted with CH2Cl2, and washed with brine. The organic layer was dried with Na2SO4 and concentrated. To the residue was added CH2Cl2 (100 mL) and triethanolamine (15 mL, 112 mmol) at room temperature. The mixture was stirred for 3 h, then passed through a silica gel pad (eluted with CH2Cl2), concentrated, and purified by silica gel column chromatography. Elution with cyclohexane/EtOAc (100/1) gave a yellow oil (11.13 g, 95%); 1H-NMR (400 MHz, CDCl3) δ 5.82 (m, 1H, CH=CH2), 5.17–5.12 (m, 2H, CH=CH2), 3.79 (m, 1H, CH–OH), 2.38 (t, J = 7.2 Hz, 2H), 2.30 (m, 1H), 2.20 (m, 1H), 1.91 (br, 1H, OH), 1.74–1.61 (m, 2H), 0.14 (s, 9H, CH3 × 3), 13C-NMR (100 MHz, CDCl3) δ 134.5 (CH=CH2), 118.2 (CH=CH2), 106.9 (≡Si–C), 85.3 (C≡C–Si), 70.0 (CH–OH), 41.8, 35.2, 16.5, 0.08 (SiCH3 × 3). HRMS (ESI) m/z calcd. for C11H21OSi+ [M + H]+: 197.1356; found: 197.1357.
4-Chlorooct-1-en-7-yne (1I). To a solution of oct-1-en-7-yn-4-ol (1a, 300 mg, 2.4 mmol) in CH₂Cl₂ (18 mL) at 0 °C, pyridine (0.384 mL, 4.8 mmol) was then added, followed by triphosgene (356 mg, 1.2 mmol) in one portion. The solution was stirred for 5 min and then warmed to gentle reflux. After 6 h, the reaction mixture was poured into a separatory funnel containing 1 M aqueous HCl (20 mL), and the biphasic mixture was shaken vigorously. Upon separation of layers, the aqueous layer was re-extracted with CH₂Cl₂. The organic phase was dried over Na₂SO₄, concentrated, and purified by silica gel column chromatography. Elution with cyclohexane gave a pale yellow oil (213.6 mg, 62%); ¹H-NMR (300 MHz, CDCl₃) δ 5.85 (m, 1H, CH=CH₂), 5.18–5.13 (m, 2H, CH=CH₂), 4.10 (m, 1H, CHCl), 2.56 (m, 2H), 2.41 (m, 2H), 2.05–1.82 (m, 2H), 1.98 (s, 1H, CH=N). ¹³C-NMR (75 MHz, CDCl₃) δ 133.7 (CH=CH₂), 118.2 (CH=CH₂), 82.8 (C≡CH), 69.1 (C≡CH), 60.8 (CH=CH₂), 42.5, 36.2, 15.8. HRMS (ESI) m/z calcd. for C₈H₁₂Cl⁺ [M + H]⁺: 143.0622; found: 143.0618.

4-Fluorodec-1-en-7-yne (1j). Dec-1-en-7-yn-4-ol (1g, 300 mg, 1.96 mmol) was added to a previously cooled (−45 °C) solution of diethylaminosulfur trifluoride (594 mg, 3.69 mmol) in dry CH₂Cl₂ (2.84 mL) with vigorous stirring over a 10-min period. The solution was allowed to come to room temperature overnight after which it was transferred into a separatory funnel containing water and CH₂Cl₂. The organic phase was then dried over Na₂SO₄, concentrated, and purified by silica gel chromatography. Elution with cyclohexane/CH₂Cl₂ (50/1) gave a yellow oil (15 mg, 5.0%); ¹H-NMR (300 MHz, CDCl₃) δ 5.82 (m, 1H, CH=CH₂), 5.16–5.10 (m, 2H, CH=CH₂), 4.66 (brd, J = 51.3 Hz, 1H, CH-F), 2.43–2.29 (m, 4H), 2.15 (q, J = 6.9 Hz, 2H, CH₂CH₃), 1.85–1.69 (m, 2H), 1.11 (t, J = 7.2 Hz, 3H, CH₃). ¹³C-NMR (75 MHz, CDCl₃) δ 133.0 (d, J = 6.0 Hz, CH=CH₂), 117.9 (CH=CH₂), 92.0 (d, J = 168.9 Hz, CH-F), 82.3, 78.1, 39.3 (d, J = 21.2 Hz), 34.0 (d, J = 20.9 Hz), 14.7 (d, J = 5.2 Hz), 14.2, 12.4. LRMS (ESI) m/z calcd. for C₁₀H₁₆F⁺ [M + H]⁺: 155.1; found: 155.1.

3.3. General Procedure for Preparation of Co₂(CO)₆-Alkyl Complexes and Compound Characterization

A mixture of alkylene (2 mmol, 1.0 equiv.), Co₂(CO)₆ (2.2 mmol, 1.1 equiv.) in CH₂Cl₂ (0.5 mL) was stirred at room temperature under atmosphere (balloon) until thin-layer chromatography (TLC) monitoring showed all alkylene consumed (about 1 h). The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography to provide Co₂(CO)₆-alkyl complex 2.

Hexacarbonyl[µ-(7,8-η:7,8-η)oct-1-en-7-yn-4-ol]-dicobalt-(Co-Co) (2a): Dark red oil, yield 57%; ¹H-NMR (500 MHz, CDCl₃) δ 6.02 (s, 1H, =CH), 5.83 (m, 1H, CH=CH₂), 5.19–5.15 (m, 2H, CH=CH₂), 3.79 (m, 1H, CH-OH), 3.09 (m, 1H), 2.92 (m, 1H), 2.36 (m, 1H), 2.22 (m, 1H), 1.80 (m, 2H), 1.66 (d, J = 4.0 Hz, 1H, OH). ¹³C-NMR (125 MHz, CDCl₃) δ 199.9 (br, CO × 6), 134.2 (C=C), 118.8 (CH=CH₂), 97.1 (C≡CH), 73.1 (C≡CH), 69.9 (CHOH), 42.1, 38.9, 30.4. HRMS (EI) m/z calcd. for C₁₃H₁₆Co₂O₆⁺ [M⁺ – CO]: 381.9292; found: 381.9300.

Hexacarbonyl[µ-(1,2-η:1,2-η)non-1-yn-5-ol]-dicobalt-(Co-Co) (2b): Dark red oil, yield 58%; ¹H-NMR (300 MHz, CDCl₃) δ 6.01 (s, 1H, =CH), 3.92 (m, 1H, CHO), 3.07 (dd, J = 15.6, 9.6, 6.3 Hz, 1H), 2.89 (ddd, J = 15.6, 9.9, 6.3 Hz, 1H), 1.76 (m, 1H), 1.49–1.43 (m, 4H), 1.38–1.33 (m, 3H), 0.92 (t, J = 6.6 Hz, 3H, CH₃). ¹³C-NMR (75 MHz, CDCl₃) δ 200.0 (br, CO × 6), 97.3 (C=C), 73.0 (C≡CH), 71.3 (CHOH), 39.5, 37.2, 30.3, 27.7, 22.7, 14.0 (CH₃). HRMS (EI) m/z calcd. for C₁₄H₁₆Co₂O₆⁺ [M⁺ – CO]: 397.9605; found: 397.9615.

Hexacarbonyl[µ-(4,5-η:4,5-η)pent-4-yn-2-ol]-dicobalt-(Co-Co) (2c): Dark red oil, yield 67%; ¹H-NMR (300 MHz, CDCl₃) δ 6.10 (s, 1H, =CH), 3.95 (m, 1H, CHO), 3.01 (d, J = 4.8 Hz, 2H, CH₂), 1.57 (d, J = 6.3 Hz, 1H, OH), 1.35 (d, J = 5.4 Hz, 3H, CH₃). ¹³C-NMR (75 MHz, CDCl₃) δ 199.8 (br, CO × 6), 92.0 (C≡CH), 73.9 (C=C), 68.7 (CHOH), 43.7 (CH₂), 23.7 (CH₃). HRMS (EI) m/z calcd. for C₁₀H₈Co₂O₆⁺ [M⁺ – CO]: 341.8979; found: 341.8987.

Hexacarbonyl[µ-(5,6-η:5,6-η)hex-5-yn-3-ol]-dicobalt-(Co-Co) (2d): Dark red oil, yield 78%; ¹H-NMR (300 MHz, CDCl₃) δ 6.09 (s, 1H, =CH), 3.66 (m, 1H, CHO), 3.00 (m, 2H, =CCH₂), 1.76 (d, J = 3.3 Hz,
1H, O) Hexacarbonyl[\eta^2-(\eta^1-\eta^3-3-\text{ol})]dicobalt-(Co–Co) (2e): Dark red oil, yield 84%; 1H-NMR (600 MHz, CDCl₃) δ 6.06 (d, J = 0.6 Hz, 1H, ≡CH), 4.63 (ddd, J = 7.8, 5.4, 5.4 Hz, 1H, CHOH), 1.84 (d, J = 5.4 Hz, 1H, OH), 1.76 (m, 1H of CH₂), 1.70 (m, 1H of CH₂), 1.09 (t, J = 7.2 Hz, 3H, CH₃). 13C-NMR (150 MHz, CDCl₃) δ 199.6 (br, CO × 6), 99.8 (C≡CH), 73.9, 71.6, 32.9 (CH₂), 10.6 (CH₃). HRMS (EI) m/z calcd. for C₁₂H₁₀Co₂O₆⁺ [M⁺ – CO]: 355.9136; found: 355.9142.

Hexacarbonyl[\eta^2-(\eta^1-\eta^3-3-\text{ol})]dicobalt-(Co–Co) (2f): Dark red oil, yield 78%; 1H-NMR (300 MHz, CDCl₃) δ 6.06 (s, 1H, ≡CH), 1.78 (m, 2H, CH₂), 1.73 (s, 1H, OH), 1.49 (s, 3H, CH₃), 1.03 (t, J = 7.2 Hz, 3H, CH₂CH₃). 13C-NMR (75 MHz, CDCl₃) δ 199.6 (br, CO × 6), 105.2 (C≡CH), 74.9, 72.1, 37.8 (CH₂), 29.8 (CH₃), 8.7 (CH₂CH₃). HRMS (EI) m/z calcd. for C₁₀H₈Co₂O₆⁺ [M⁺]: 369.8929; found: 369.8933.

Hexacarbonyl[\eta^2-(\eta^1-\eta^3-3-\text{ol})]dicobalt-(Co–Co) (2g): Dark red oil, yield 69%; 1H-NMR (300 MHz, CDCl₃) δ 5.82 (m, 1H, CH≡CH₂), 5.20-5.15 (m, 2H, CH≡CH₂), 3.79 (brd, J = 3.9 Hz, 1H, CH-OH), 3.08 (m, 1H), 2.91 (m, 1H, 2.86 (q, J = 7.2 Hz, 2H, CH₂CH₂), 2.36 (m, 1H), 2.22 (m, 1H), 1.82 (m, 2H), 1.69 (d, J = 3.9 Hz, 1H, OH), 1.29 (t, J = 7.2 Hz, 3H, CH₃). 13C-NMR (75 MHz, CDCl₃) δ 199.6 (br, CO × 6), 134.2 (CH=CH₂), 118.8 (CH=CH₂), 101.8, 99.0, 70.0 (CH-OH), 42.0, 38.3, 30.1, 27.5, 15.6 (CH₃). HRMS (EI) m/z calcd. for C₁₃H₁₅Co₂O₆⁺ [M⁺ – CO]: 409.9605; found: 409.9607.

Hexacarbonyl[\eta^2-(\eta^1-\eta^3-\eta^3-3-\text{ol})]dicobalt-(Co–Co) (2h): Dark red oil, yield 49%; 1H-NMR (300 MHz, CDCl₃) δ 5.82 (m, 1H, CH≡CH₂), 5.21-5.16 (m, 2H, CH≡CH₂), 3.80 (m, 1H, CH-OH), 3.20 (m, 1H), 2.96 (m, 1H), 2.40 (m, 1H), 2.23 (m, 1H), 1.82 (m, 2H), 1.68 (s, 1H, OH), 0.30 (s, 3H, CH₃×3). 13C-NMR (75 MHz, CDCl₃) δ 200.6 (br, CO × 6), 134.1 (CH=CH₂), 118.8 (CH=CH₂), 112.3 (≡C-Si), 97.1 (C≡C-Si), 70.0 (CH-OH), 42.0, 39.2, 31.4, 0.65 (SiCH₃×3). HRMS (EI) m/z calcd. for C₁₄H₂₀Co₂O₆Si⁺ [M⁺ – CO – SiH₄]: 453.9688; found: 453.9693.

Hexacarbonyl[\eta^2-(\eta^1-\eta^3-\eta^3-3-\text{ol})]dicobalt-(Co–Co) (2i): Dark red oil, yield 74%; 1H-NMR (300 MHz, CDCl₃) δ 6.03 (s, 1H, ≡CH), 5.85 (m, 1H, CH=CH₂), 5.18–5.13 (m, 2H, CH=CH₂), 4.05 (m, 1H, CH-Cl), 3.11 (m, 1H), 2.97 (m, 1H), 2.57 (m, 2H), 2.05 (m, 2H). 13C-NMR (75 MHz, CDCl₃) δ 199.9 (br, CO × 6), 133.6 (CH=CH₂), 118.4 (CH=CH₂), 95.7 (C≡CH), 73.1 (C≡C), 61.2 (CH-Cl), 42.7, 39.5, 30.8. HRMS (EI) m/z calcd. for C₁₃H₁₂Co₂Cl₂O₆⁺ [M⁺ – CO]: 399.8954; found: 399.8960.

Hexacarbonyl[\eta^2-(\eta^1-\eta^3-\eta^3-3-\text{ol})]dicobalt-(Co–Co) (2j): Dark red oil, yield 90%; 1H-NMR (600 MHz, CDCl₃) δ 5.85 (ddt, J = 24.6, 17.4, 7.2 Hz, 1H, CH=CH₂), 5.17–5.13 (m, 2H, CH=CH₂), 4.66 (brd, J = 48.6 Hz, 1H, CH-F), 3.06 (m, 1H), 2.91 (m, 1H), 2.86 (q, J = 7.2 Hz, 2H, CH₂CH₂), 2.46 (m, 2H), 1.93 (m, 2H), 1.29 (t, J = 7.2 Hz, 3H, CH₃). 13C-NMR (150 MHz, CDCl₃) δ 199.0 (br, CO × 6), 132.6 (d, J = 6.3 Hz, CH≡CH₂), 118.3 (CH=CH₂), 101.8 (C≡C-Et), 98.2 (C≡C-Et), 92.5 (d, J = 170.1 Hz, CH-F), 39.4 (d, J = 21.3 Hz, CH₂CH₂CH₂), 36.2 (d, J = 20.7 Hz, CH₂CH₂C≡C), 29.3 (d, J = 4.1 Hz, C≡CH₂CH₂), 27.0 (CH₂CH₂), 15.6 (CH₃). HRMS (EI) m/z calcd. for C₁₅H₁₅Co₂F₂O₆⁺ [M⁺ – CO]: 411.9562; found: 411.9570.

Hexacarbonyl[\eta^2-(\eta^1-\eta^3-\eta^3-3-\text{ol})]dicobalt-(Co–Co) (2k): Dark red oil, yield 65%; 1H-NMR (500 MHz, CDCl₃) δ 6.03 (s, 1H, ≡CH), 4.63 (t, J = 4.0 Hz, 1H, O–CH–O), 3.99 (m, 1H), 3.88 (m, 1H), 3.60 (m, 1H), 3.53 (m, 1H), 3.15 (m, 2H), 1.84 (m, 1H), 1.72 (m, 1H), 1.62–1.51 (m, 4H). 13C-NMR (125 MHz, CDCl₃) δ 199.9 (br, CO × 6), 98.9 (O–CH–O), 92.9 (C≡CH), 73.7 (C≡C), 67.3, 62.4, 34.1, 30.6, 25.4, 19.5. HRMS (EI) m/z calcd. for C₁₄H₁₄Co₂O₂⁺ [M⁺ – CO]: 411.9398; found: 411.9400.
4. Conclusions

We have developed a simple and general method to separate the enantiomers of chiral alkynes using their in situ generated cobalt-complex by chiral column HPLC. HPLC analysis of enantiomeric purity of metal complexes with carbon-metal bonds is not usual [16]. Our method is particularly useful for the alkynes without chromophores and other derivable groups. Since the decomplexation of Co-alkyne complexes is well known [17], our method is also promisingly suitable for the preparative resolution of racemic alkynes.

Supplementary Materials: Supplementary materials are available online. (1) General Information; (2) UV-Vis spectra of 1a and 2a; (3) 1H- and 13C-NMR spectra of 1b, and 1g–j; (4) HPLC chart of 1a; (5) 1H-, 13C-NMR spectra and HPLC charts of 2a–k; (6) HPLC monitor of the reaction of 1a and Co2(CO)8.

Acknowledgments: This work was financially supported by the National Natural Science Foundation of China (21502234 and 21602256), Beijing Natural Science Foundation (2164074), Natural Science Foundation of Shanxi (2013011011-2), Research Funds from State Key Laboratory of Bioactive Substance and Function of Natural Medicines (GZTB201404), CAMS Innovation Fund for Medical Sciences (CIFMS, 2016-I2M-3-009), and Fundamental Research Funds for CAMS/PUMC (2016RC350004). We thank Li Li, IMM, CAMS/PUMC for the useful discussion and Leilei Zhang, IMM, CAMS/PUMC for high resolution mass spectrometric assistance.

Author Contributions: X.L. and S.X. conceived the experiments; X.L., Y.G. and S.X. designed the experiments; J.W., X.L. and Y.G. performed the experiments; Q.L., J.L., Y.G., S.L., X.W. and S.X. analyzed the data; Q.L., J.L., X.W. and S.X. wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Trost, B.M.; Li, C.-J. Modern Alkyne Chemistry: Catalytic and Atom-Economic Transformations, 1st ed.; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2015.
2. Trost, B.M.; Masters, J.T. Transition metal-catalyzed couplings of alkynes to 1,3-enynes: Modern methods and synthetic applications. Chem. Soc. Rev. 2016, 45, 2212–2238. [CrossRef] [PubMed]
3. Yang, Z.; Shi, L.L. Exploring the complexity-generating features of the Pauson–Khand reaction from a synthetic perspective. Eur. J. Org. Chem. 2016, 14, 2356–2368.
4. Kacprzak, K.; Skiera, I.; Piasecka, M.; Paryzek, Z. Alkaloids and isoprenoids modification by copper(I)-catalyzed Huisgen 1,3-dipolar cycloaddition (click chemistry): Toward new functions and molecular architectures. Chem. Rev. 2016, 116, 2767–2770. [CrossRef] [PubMed]
5. Nagle, D.G.; Gerals, R.-S.; Yoo, H.-D.; Gerwick, W.H.; Kim, T.-S.; Nambu, M.; White, J.D. Absolute configuration of curacin A, a novel antimitic agent from the tropical marine cyanobacterium Lyngbya majuscula. Tetrahedron Lett. 1995, 36, 1189–1192. [CrossRef]
6. White, J.D.; Kim, T.-S.; Nambu, M. Synthesis of curacin A: A powerful antimitic from the cyanobacterium Lyngbya majuscula. J. Am. Chem. Soc. 1995, 117, 5612–5613. [CrossRef]
7. White, J.D.; Kim, T.-S.; Nambu, M. Absolute configuration and total synthesis of (+)-curacin A, an antiproliferative agent from the cyanobacterium Lyngbya majuscula. J. Am. Chem. Soc. 1997, 119, 103–111. [CrossRef]
8. Codesido, E.M.; Cid, M.M.; Castedo, L.; Mourinho, A.; Granja, J.R. Synthesis of vitamin D analogues with a 2-hydroxy-3-deoxy ring A. Tetrahedron Lett. 2000, 41, 5861–5864. [CrossRef]
9. García-Fandiño, R.; Aldagunde, M.J.; Codesido, E.M.; Castedo, L.; Granja, J.R. RCM for the construction of novel steroid-like polycyclic systems. 1. Studies on the Synthesis of a PreD3-D3 transition state analogue. J. Org. Chem. 2005, 70, 8281–8290. [CrossRef] [PubMed]
10. Nikolau, K.C.; Leung, G.Y.C.; Dethe, D.H.; Gusduru, R.; Sun, Y.-P.; Lim, C.S.; Chen, D.Y.-K. Chemical synthesis and biological evaluation of palmerolide A analogues. J. Am. Chem. Soc. 2008, 130, 10019–10023. [CrossRef] [PubMed]
11. Ngai, M.H.; Yang, P.-Y.; Liu, K.; Shen, Y.; Wenk, M.R.; Yao, S.Q.; Lear, M.J. Click-based synthesis and proteomic profiling of lipstatin analogues. Chem. Commun. 2010, 46, 8335–8337. [CrossRef] [PubMed]
12. Shibata, H.; Tsuchikawa, H.; Hayashi, T.; Matsumori, N.; Murata, M.; Usui, T. Modification of bafilomycin structure to efficiently synthesize solid-state NMR probes that selectively bind to vacuolar-type ATPase. Chem. Asian J. 2015, 10, 915–924. [CrossRef] [PubMed]
13. Iseki, K.; Mizuno, S.; Kuroki, Y.; Kobayashi, Y. A chiral formamide: Design and application to catalytic asymmetric synthesis. *Tetrahedron Lett.* **1998**, *39*, 2767–2770. [CrossRef]
14. Seco, J.M.; Quinoa, E.; Riguer, R. The assignment of absolute configuration by NMR. *Chem. Rev.* **2004**, *104*, 17–118. [CrossRef]
15. Miyamoto, H.; Hirano, T.; Okawa, Y.; Nakazaki, A.; Kobayashi, S. Stereoselective synthesis of spirocyclic oxindoles based on a one-pot Ullmann coupling/Claisen rearrangement and its application to the synthesis of a hexahydropyrrolo[2,3-b]indole alkaloid. *Tetrahedron* **2013**, *69*, 9481–9493. [CrossRef]
16. Wang, P.; Lee, H.K. Recent applications of high-performance liquid chromatography to the analysis of metal complexes. *J. Chromatogr. A* **1997**, *789*, 437–451. [CrossRef]
17. Nicholas, K.M.; Pettit, R. An alkyne protecting group. *Tetrahedron Lett.* **1971**, *12*, 9481–9493. [CrossRef]

**Sample Availability:** Samples of the compounds 2a–k are available from the authors.