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Synthesis of Triple-Bond-Containing 1-Hydroxy-1,1-bisphosphonic Acid Derivatives To Be Used as Precursors in "Click" Chemistry: Two Examples

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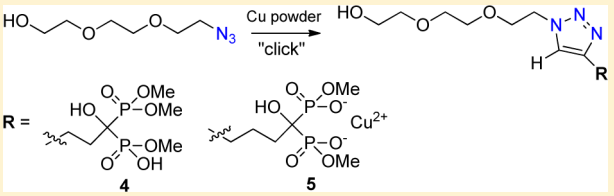
Supporting Information

ABSTRACT: The synthesis of novel (ω-alkynyl-1-hydroxy-1,1-diyl)bisphosphonic acid tetramethyl esters (**1a**–**c**), their *P*,*P*′-dimethyl esters (**2a**–**c**), and two trimethyl ester derivatives (**3a** and **3b**) is reported. The prepared compounds can be attached to many kinds of molecules containing azide ($-N_3$) functionalities using a "click" chemistry approach. As an example, bisphosphonate trimethyl ester **3a** and *P*,*P*′-dimethyl ester **2b** were attached to triethylene glycol to form triethylene glycol–bisphosphonate conjugates **4** and **5** as model compounds for further studies in, for example, nanoparticle targeting.



Bisphosphonates (BPs), which have been studied intensively more than 50 years, are analogues of the naturally occurring pyrophosphate, and they can be characterized by their P–C–P backbone (Figure 1).[1,2] During that time,

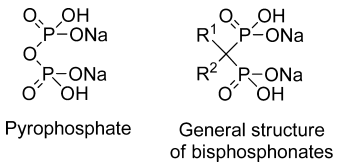


Figure 1. Structure of pyrophosphate and general structure of bisphosphonates.

according to a SciFinder search, approximately 17 000 different BP structures in approximately 34 000 studies have been published. At the beginning BPs were used as water softeners since they inhibit the formation of calcium and magnesium crystals, but as known in general, their very high affinity for the bone mineral hydroxyapatite represents the basis for their current main use today as drugs for the treatment of different kinds of bone diseases, such as osteoporosis and Paget's disease. In the literature there are hundreds of different BP applications, such as combatting against parasitic diseases,[3−8] the treatment of atherosclerosis,[9] and use as bone-imaging agents[10] and bone-targeting promoieties (e.g., as anti-inflammatory drugs[11] and bone metastases[12]). They can also be used in extraction of actinide ions,[13] as a new class of herbicides,[14] and in studies for crystal engineering.[15] Furthermore, BPs have exhibited interesting anticancer properties[16] and have been claimed to be important precursors in the preparation of nucleotide analogues.[17,18]

Our group has extensive experience related to BP synthesis in recent years,[19−21] and a while ago we devised three novel applications for BPs: (1) a method for effective removal of chromium(III) from tannery effluents and aqueous solutions based on solid BPs,[22] (2) the first bisphosphonate hydrogelators as potential composers of biocompatible gels,[23] and (3) novel unexpected effects of BPs on plant growth that are nonherbicidal.[24] In addition, we discovered a method for collecting metals from aqueous solutions using an insoluble BP material.[25] These highlight the enormous potential of BPs for not only their medical uses but also their utilization in many other unexpected fields.

According to a literature search with SciFinder, there are generally few "click" chemistry examples related to BPs,[26−29] and only two examples of (ω-alkynyl-1-hydroxy-1,1-diyl)-bisphosphonic acids have been reported.[30,31] There are three papers describing the conjugation of 1-hydroxy-1,1-bisphosphonates using the "click" chemistry approach, one of which describes conjugation of azide-containing BP to antibacterial agents.[31−33] There are no examples of the synthesis of (ω-alkynyl-1-hydroxy-1,1-diyl)bisphosphonic acid esters or their conjugation via "click" chemistry. Tetraesters of 1-hydroxy-1,1-bisphosphonic acids are sensitive toward undergoing a rearrangement to form tetraalkyl phosphono phosphates if any base and/or too-high temperatures are used in their syntheses, as has been reported in the case of tetraesters of etidronic acid[34−38] (Figure 2). In fact, this was also a problem

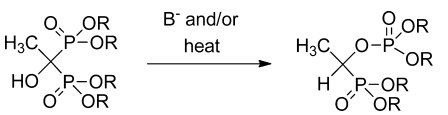


Figure 2. Rearrangement of etidronic acid tetraesters to tetraalkyl phosphono phosphates.

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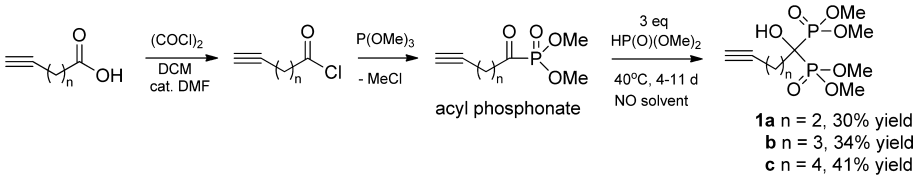
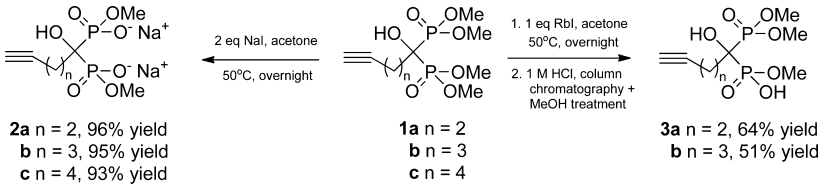
Scheme 1. Synthesis of Novel BP Tetramethyl Esters 1a−c



Scheme 2. Synthesis of Novel BP *P,P′*-Dimethyl Esters 2a−c and Trimethyl Esters 3a−b



encountered in the synthesis of (ω-alkynyl-1-hydroxy-1,1-diyl)bisphosphonic acid tetramethyl esters **1a−c** described in this study and will be discussed in more detail later in the paper.

The possibility of preparing BP tetraesters and partial esters is of major importance. Tetraesters can function as protecting groups when modifying the R^1 and R^2 groups of BPs[39] (see Figure 1), and if needed, they can be removed afterward to produce the corresponding bisphosphonic acids by either acid hydrolysis or a silylation/desilylation procedure under mild conditions.[40] In addition, these esters are much more lipophilic than BP acids or salts, and this makes it possible to perform syntheses and conjugations in organic solvents, which is mandatory in some cases. In our ongoing study,[41] we have observed that BP partial esters have different affinities for hydroxyapatite than the corresponding acids or salts, and there are some unexpected results that may also open new targeting possibilities (e.g., targeting those molecules to sites other than bone) because BP acids and salts are very rapidly transported to the bone surface when administered into the blood circulation.[42,43]

Ethylene glycols (EGs) of different sizes have been used as linkers to enhance and optimize the properties of molecules.[44−46] The most extensively used nonionic polymer in polymer-based drug delivery research is poly(ethylene glycol) (PEG).[47] The role of PEG has proven to be crucial in the evolution of drug delivery systems, especially for tumor targeting and treatment. PEG-coated liposomal nanoparticles have proved to be efficient drug delivery systems since they achieve high circulation times, increased stability, decreased systemic toxicity, and increased tumor accumulation.[48] The versatility that has been demonstrated with PEG will allow for the exploration of novel uses and continuous improvements of anticancer treatments.[49]

Gold nanoparticles (GNPs) are widely used in many fields, and the range of applications for GNPs is growing rapidly, including photodynamic therapy, therapeutic agent delivery, and diagnostics.[50] BP-functionalized GNPs have recently been reported to have the potential to improve clinical detection of breast microcalcifications by mammography.[51] We have research experience related to silicon nanoparticles in our group.[52−54] GNPs are commercially available in the form of PEGylated azides ($PEG-N_3$),[55] which makes them highly interesting particles for different purposes should they be conjugated with BPs by the "click" chemistry approach.[56]

Perhaps the most practical way to prepare tetraesters of 1-hydroxy-1,1-bisphosphonates is the reaction between equivalent amounts of acyl chloride, trialkylphosphite, and dialkylphosphite with or without a catalytic amount of base.[34,57,58] This was also the starting point when the compounds reported here were synthesized (Scheme 1). ω-Alkynoic acids were converted to the acid chlorides by the well-known method that uses oxalyl chloride as the chlorinating reagent, and subsequent addition of trimethylphosphite formed the corresponding acylphosphonates as intermediate products. Finally, 3 equiv of dimethylphosphite was added, and the reaction mixture was stirred for about 4−11 days without solvent at 40 °C to produce (ω-alkynyl-1-hydroxy-1,1-diyl)-bisphosphonic acid tetramethyl esters **1a−c**. The first synthesis strategy was to use the standard method with equivalent amounts of starting materials, but only a very small amount of the desired product was obtained. When the temperature was elevated to over 50 °C or a catalytic amount of base (dibutylamine) was added to the reaction mixture, there was isomerization of the product to the tetraalkyl phosphono phosphate as revealed by the ^{31}P NMR spectrum. (This is the same phenomenon as shown in Figure 2 for etidronic acid tetraalkyl ester; also see p S31 in the Supporting Information. The proposed reaction mechanism has been reported elsewhere.[38]) Therefore, the above-mentioned modified procedure was developed (for a more detailed procedure, see the Experimental Section).

An excess of dimethyl phosphite was used to drive the reaction to completion so that no acyl phosphonate was left in the reaction mixture (this was monitored via the ^{31}P NMR spectrum; see, e.g., the spectrum on page S31 in the Supporting Information). Unfortunately, as can be seen in Scheme 1, the yields of **1a−c** were rather low as a result of partial isomerization of the desired products to tetraalkyl phosphono phosphates. It appeared that some of the product was isomerized during the column chromatography purification, which partly reduced the isolated yield. It was interesting to note that the reaction mixture of **1a** contained the greatest amount of the rearranged product and the reaction mixture of **1c** contained the least, even though the reactions were performed under the same conditions, so it may be concluded that there is some kind of effect of the triple bond on the rearrangement process and that the effect is stronger when it is closer to the BP part of the molecule.

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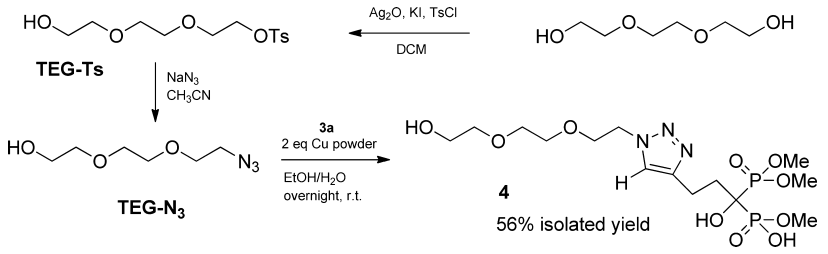
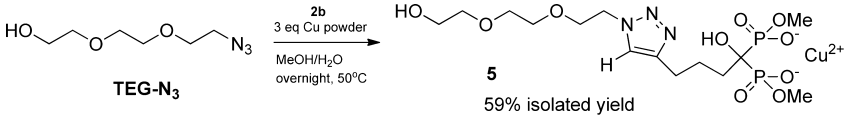
Scheme 3. Synthesis of TEG−N_3 and of Novel TEG-Conjugated BP 4 Using "Click" Chemistry



Scheme 4. Synthesis of Novel TEG-Conjugated BP 5 Using "Click" Chemistry



(*ω*-Alkynyl-1-hydroxy-1,1-diyl)bisphosphonic acid *P*,*P*′-dimethyl esters **2a**−**c** were prepared in very high yields from the corresponding tetramethyl esters **1a**−**c** using the NaI/acetone method[59] (Scheme 2). The synthesis of trimethyl esters **3a** and **3b** was much more challenging because one would need to find a selective agent capable of removing only one methyl group from tetramethyl esters **1a** and **1b**. We have reported earlier[59] about the selective synthesis of etidronic acid trimethyl and triethyl esters using the KI/acetone system, but in this case, this approach was not as selective as expected. The logical way was to test the RbI/acetone system, and this was observed to be more selective than KI/acetone, leading to very reasonable yields of trimethyl esters **3a** and **3b** after isolation (Scheme 2).

Since the raw product mixture in the case of **3a** contained an approximately 15% yield of the formed *P*,*P*′-dimethyl ester, a few percent yield of rearranged product, and a few percent yield of some monophosphorus species, it was purified by column chromatography. Unfortunately the *P*,*P*′-dimethyl ester eluted with the desired trimethyl ester **3a**, so a further purification step was required. The earlier experiences with the different solubilities of the BP *P*,*P*′-dimethyl and trimethyl esters suggested that it might be possible to separate trimethyl ester **3a** from the corresponding *P*,*P*′-dimethyl ester by dissolving the raw product in MeOH and crystallizing the *P*,*P*′-dimethyl ester out of the MeOH solution, hoping that **3a** would still remain dissolved in MeOH. In the attempt to dissolve the raw mixture into MeOH, another component was found to be totally insoluble in MeOH, and surprisingly, it was the desired product **3a**. One plausible explanation for this phenomenon is the presence of very strong intra- or intermolecular hydrogen bond(s) formed between the atoms capable of that in **3a** in solid form, so the methanol molecules could not solvate the molecules of **3a**, which is crucial for dissolution. It would be very interesting to compare the crystal structures of **3a** and the corresponding *P*,*P*′-dimethyl ester and determine the absolute reason for this unexpected situation. When trimethyl ester **3b** was synthesized the same procedure was also used successfully.

Since the ultimate goal was to prepare nanoparticles or related molecules loaded with medicine targeting bone (or any other calcium-containing target such as atherosclerotic plaques[9]), triethylene glycol (TEG) was chosen as the model compound to be conjugated with **3a** and **2b** using "click" chemistry for the reasons presented in the introductory discussed above. Commercially available TEG was readily converted to the monoazide form (TEG−N_3) via the tosyl form (TEG−Ts) using the known method[60] (Scheme 3) before it was conjugated with **3a** and **2b** using solid copper as a catalyst (Schemes 3 and 4).

The first attempt was to conjugate tetramethyl ester **1a** to TEG−N_3 using perhaps the most common "click" chemistry catalyst, $CuSO_4$, but that was not successful under the conditions used. When copper powder was added to the reaction mixture of **1a** and TEG−N_3, the "click" reaction worked well, but as expected, a yield of about 13% of the formed conjugate was rearranged (the BP moiety became isomerized as discussed earlier) according to the ^{31}P NMR spectrum, probably as a result of formation of the triazole ring which could act as a base to catalyze the rearrangement process.

Since the goal was to produce lipophilic BP conjugates as model compounds, one can conclude that conjugates **4** and **5** are excellent examples. Furthermore, it was very interesting to note that conjugate **5** was able to chelate copper into its structure, although this was not surprising because according to our experience, even BP *P*,*P*′-diesters can coordinate metal cations into their structures. This is not the case for BP triesters, which chelate metals very poorly or not at all, as observed for the case of conjugate **4**. It is a well-known phenomenon that when the metal cation is strongly coordinated into the molecule, then the atoms involved in the coordination have such long relaxation times that they are hardly visible or not visible at all in NMR spectra.[61] This was observed when attempts were made to measure the ^{31}P NMR spectrum for **5**, as no peaks were found. In addition, in the ^{13}C NMR spectrum, the middle carbon (P−C−P), which already in general has a long relaxation time, was not visible, and no carbon−phosphorus couplings were detected. Interestingly, the carbons from the triazole ring also were not visible, even when tested with a 28 s relaxation delay and a very concentrated sample, indicating that the triazole ring must take part in the chelation process. The ^{1}H NMR spectrum of **5** was also very simple, and no couplings were observed, but it was fair enough to confirm the structure when combined with MS and ^{13}C NMR results, especially when they were compared with the NMR and MS spectra of **4**. In the MS spectrum, a copper complex peak for **5** was also observed. It would be very interesting to determine the 3D crystal structure of **5** in the future, if the compound could be crystallized. The ^{13}C NMR spectrum of **5** revealed six small carbon signals that did not belong to conjugate **5** and probably originated from compound

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TEG−NH_2, which was formed in the reaction from TEG−N_3 (reduction reaction); in the ^{1}H NMR spectrum of **5** there was a visible signal at ca. 3.05 ppm that was probably attributable to the methylene protons next to the amino group (−$OCH_2CH_2NH_2$). On the basis of the signal intensities, it can be said that the purity of compound **5** was approximately 85%, but because it was prepared as a model compound, that is not very important in the further studies. Finally, it is worth mentioning that the color of conjugate **5** in CD_3OD in the NMR tube was slightly fluorescent yellow, not blue or green as could have been expected, and the same color remained even when 7.6 M DCl was added and the pH was measured to be ≤1, which is evidence of the very strong copper complex of **5**. Actually, when attempts were made to measure, for example, the ^{31}P NMR spectrum for the above-mentioned acidic sample, no visible signals could be observed.

In conclusion, three novel (ω-alkynyl-1-hydroxy-1,1-diyl)-bisphosphonic acid tetramethyl esters (**1a−c**) were synthesized and isolated in reasonable yields when their sensitivity to isomerization is taken into account. In addition, three novel (ω-alkynyl-1-hydroxy-1,1-diyl)bisphosphonic acid *P*,*P*′-dimethyl esters (**2a−c**) and two trimethyl esters (**3a** and **3b**) have been reported. All of the prepared compounds are highly useful as precursors for "click" chemistry, but if tetramethyl esters are used in reactions, the tertiary hydroxyl group maybe protected [e.g., by acylation (esterification)][37,38] against undergoing rearrangement. Novel triethylene glygol−BP conjugates **4** and **5** were synthesized by the "click" chemistry approach as model compounds for further studies (e.g., bone-targeted delivery systems).

EXPERIMENTAL SECTION

General Methods. ^{1}H, ^{31}P, and ^{13}C NMR spectra were recorded on a 500 MHz spectrometer operating at 500.1, 202.5, and 125.8 MHz, respectively. The solvent residual peaks were used as standards for ^{1}H and ^{13}C measurements in $CDCl_3$ and CD_3OD (7.26 and 77.16 ppm for $CDCl_3$ and 3.31 and 49.00 ppm for CD_3OD, respectively),[62] in D_2O in ^{1}H measurements (4.79 ppm), and trimethylsilylpropionic acid sodium salt (TSP) in ^{13}C measurements (0.00 ppm); 85% H_3PO_4 was used as an external standard for ^{31}P measurements. The $^nJ_{HP}$ couplings were calculated from proton spectra and all *J* values are given in hertz. The $^nJ_{CP}$ couplings were calculated from carbon spectra, and the coupling constants in hertz are given in parentheses. Mass spectra were recorded on a quadrupole time-of-flight mass spectrometer using electrospray ionization (ESI) with positive ionization mode for compounds **1a−c** and negative ionization mode for **2a−c**, **3a**, **3b**, **4**, and **5**. The purity of the products was determined from the ^{1}H and ^{31}P NMR spectra and was ≥95% unless stated otherwise.

Procedure for the Preparation of 2-(2-(2-Azidoethoxy)-ethoxy)ethanol (TEG−N_3). The procedure reported elsewhere was followed with slight modifications, and all of the ^{1}H and ^{13}C NMR spectra were comparable to those reported elsewhere.[60] Triethylene glycol (900 μL, 1.012 g, 6.74 mmol) was dissolved in dry DCM (30 mL) and cooled to about −5 °C. Ag_2O (2.34 g, 10.10 mmol, 1.5 equiv), TsCl (1.41 g, 7.40 mmol, 1.1 equiv), and dry KI (225 mg, 1.36 mmol, 0.2 equiv) were added, and the reaction mixture was stirred for 30 min at about −5 °C. Subsequently, the reaction mixture was filtered through Celite and evaporated to dryness. The crude product was purified by silica column chromatography using EtOAc as the eluent. 2-(2-(2-Hydroxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (TEG−Ts) (1.07 g, 52% yield) was present as a colorless oil. TEG−Ts (500 mg, 1.64 mmol) was dissolved in dry acetonitrile (10 mL), and NaN_3 (160 mg, 2.46 mmol, 1.5 equiv) was added. The reaction mixture was then refluxed for 40 h. After the mixture was cooled, water (10 mL) was added, and the mixture was extracted three times with DCM (10 mL). The organic phases were dried over $MgSO_4$ and evaporated to dryness. TEG−N_3 (262 mg, 91%) was present as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 4.76−4.71 (m, 2H), 3.70−3.65 (m, 6H), 3.64−3.59 (m, 2H), 3.39 (t, 2H, ^3J = 5.0), 2.28 (t, 2H, ^3J = 6.3, O*H*).

Procedure for the Preparation of Tetramethyl Ester 1a. 4-Pentynoic acid (1.11 g, 11.3 mmol) was dissolved in dry DCM (15 mL), and two drops of DMF were added. The reaction mixture was then stirred at 40−45 °C. Oxalyl chloride (1000 μL, 1.5 g, 1.05 equiv, 11.8 mmol) was added in portions into the reaction mixture, and after its total addition the reaction mixture was refluxed for 1 h. The reaction mixture was cooled to about 0 °C, and distilled trimethyl phosphite (1420 μL, 1.49 g, 12.0 mmol) was added. The reaction mixture stirred for 2 h at room temperature, and then all volatile fractions were removed by evaporation in vacuo. Distilled dimethyl phosphite (3250 μL, 3.9 g, ca. 3 equiv, 35.4 mmol) was added, and the reaction mixture was stirred for 7 days at 40 °C. Again all of the volatile fractions were removed by evaporation in vacuo. Diethyl ether (20 mL) and hexane (5 mL) were added to the residue with vigorous stirring, and the mixture was placed in the freezer (about −18 °C). After a few hours, two phases were separated, and after few days, compound **1a** crystallized out in the lower phase. The crystals were separated, washed with diethyl ether, and dried in vacuo. The product **1a** (1.02 g, 30%) was obtained as colorless crystals.

Procedure for the Preparation of Tetramethyl Ester 1b. This was prepared similarly to **1a**, starting from 5-hexynoic acid (1.26 g, 11.2 mmol), except that the reaction time was 4 days instead of 7 days and the crude product was purified by column chromatography using EtOAc/MeOH (8:2) as the eluent. **1b** (1.20 g, 34%) was obtained as a yellow viscous oil.

Procedure for Preparation of Tetramethyl Ester 1c. This was prepared similarly to **1a**, starting from 6-heptynoic acid (1.42 g, 11.3 mmol), except that the reaction time was 11 days instead of 7 days and the crude product was purified by column chromatography using EtOAc/MeOH (8:2) as the eluent. **1c** (1.51 g, 41%) was obtained as a slightly yellow viscous oil.

Procedure for the Preparation of *P*,*P*′-Dimethyl Ester 2a. Tetramethyl ester **1a** (200 mg, 0.67 mmol) was dissolved in dry acetone (6 mL), and dry NaI (205 mg, 2.05 equiv, 1.37 mmol) was added. The mixture stirred overnight at 50 °C, and the precipitate was filtered, washed with acetone, and dried in vacuo. The product **2a** (203 mg, 96%) was obtained as a white powder.

Procedure for the Preparation of Trimethyl Ester 3a. Tetramethyl ester **1a** (200 mg, 0.67 mmol) was dissolved in dry acetone (5 mL), and RbI (142 mg, 1 equiv, 0.67 mmol) was added. The mixture was stirred overnight at 50 °C and then evaporated to dryness, dissolved in 1 M HCl (1.5 mL), and evaporated to dryness again. The crude product was first purified by column chromatography using EtOAc/MeOH (2:8) as the eluent, and if the collected product still contained the corresponding *P*,*P*′-dimethyl ester, then it was dissolved in MeOH and the white solids were separated and dried in vacuo. The product **3a** (122 mg, 64%) was obtained as a white solid.

Procedure for the Preparation of TEG−BP Conjugate 4. Trimethyl ester **3a** (40 mg, 0.14 mmol) was dissolved in 2 mL of EtOH/H_2O (1:1), and copper powder (19 mg, 0.30 mmol) and TEG-N_3 (25 mg, 1 equiv, 0.14 mmol) were added. The mixture was stirred overnight at room temperature and then evaporated to dryness. The residue was dissolved in DCM (ca. 5 mL) and filtered through Celite. Then MeOH (ca. 5 mL) was poured through the Celite and evaporated to dryness in vacuo. Product **4** (36 mg, 56%) was obtained as a slightly blue syrup (indicative of the presence of copper cations).

Procedure for the Preparation of TEG−BP Conjugate 5. Dimethyl ester **2b** (50 mg, 0.15 mmol) was dissolved in 2 mL of MeOH/H_2O (1:1), and copper powder (29 mg, 0.46 mmol) and TEG-N_3 (29 mg, 1.1 equiv, 0.17 mmol) were added. The mixture was stirred overnight at 50 °C and then cooled to room temperature, acidified with 2 M HCl, and evaporated to dryness. The residue was dissolved in DCM (ca. 5 mL) and filtered through Celite. Subsequently approximately 5 mL of DCM/MeOH (1:1) was poured through the Celite and evaporated to dryness in vacuo. The crude

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product was dissolved in MeOH (8 mL), and about 1 g of Dowex 50WX8-200 ion-exchange resin was added. The mixture was stirred for 0.5 h at room temperature. The Dowex was filtered and washed with MeOH before being stirred with 2 M HCl (ca. 5 mL) for 15 min at room temperature. The Dowex was filtered, and the filtrate was evaporated to dryness in vacuo. The product **5** (47 mg, 59%) was obtained as a yellow syrup, and its purity was approximately 85%.

Tetramethyl (1-Hydroxypent-4-yne-1,1-diyl)-bisphosphonate (1a). Colorless crystals, mp 62−64 °C. ^{1}H NMR ($CDCl_3$): δ 3.90−3.85 (m, 12H), 2.58−2.52 (m, 2H), 2.36−2.25 (m, 2H), 1.98 (t, 1H, ^{4}J = 2.5). ^{13}C NMR ($CDCl_3$): δ 84.0, 74.7 (t, $^{1}J_{CP}$ = 152.9, P−*C*−P), 69.0, 54.51 (t, $\sum^{2}J_{CP}$ = 6.2, 2C, OCH_3), 54.47 (t, $\sum^{2}J_{CP}$ = 6.8, 2C, OCH_3), 33.0, 13.4 (t, $^{2}J_{CP}$ = 7.0). ^{31}P NMR ($CDCl_3$): δ 22.07. MS (ESI^+): calcd for $C_9H_{19}O_7P_2$ $[M + H]^+$ 301.0606, found 301.0604.

Tetramethyl (1-Hydroxyhex-5-yne-1,1-diyl)bisphosphonate (1b). ^{1}H NMR ($CDCl_3$): δ 3.88−3.81 (m, 12H), 3.48 (t, 1H, $^{3}J_{HP}$ = 10.0, O*H*), 2.20 (td, 2H, ^{3}J = 7.0, ^{4}J = 2.5), 2.16−2.05 (m, 2H), 1.94 (t, 1H, ^{4}J = 2.5), 1.87−1.80 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 83.8, 75.0 (t, $^{1}J_{CP}$ = 152.0, P−*C*−P), 69.0, 54.4 (t, $\sum^{2}J_{CP}$ = 7.0, 2C, OCH_3), 54.3 (t, $\sum^{2}J_{CP}$ = 7.2, 2C, OCH_3), 33.4, 22.4 (t, $^{2}J_{CP}$ = 5.8), 18.9. ^{31}P NMR ($CDCl_3$): δ 22.77. MS (ESI^+): calcd for $C_{10}H_{21}O_7P_2$ $[M + H]^+$ 315.0763, found 315.0763.

Tetramethyl (1-Hydroxyhept-6-yne-1,1-diyl)-bisphosphonate (1c). ^{1}H NMR ($CDCl_3$): δ 3.89−3.81 (m, 12H, OCH_3), 3.19 (t, 1H, $^{3}J_{HP}$ = 10.5, O*H*), 2.22 (td, 2H, ^{3}J = 7.5, ^{4}J = 2.5), 2.07−1.96 (m, 2H), 1.93 (t, 1H, ^{4}J = 2.5), 1.75−1.66 (m, 2H), 1.59−1.52 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 84.3, 75.1 (t, $^{1}J_{CP}$ = 152.0, P−*C*−P), 68.4, 54.35 (t, $\sum^{2}J_{CP}$ = 7.1, 2C, OCH_3), 54.26 (t, $\sum^{2}J_{CP}$ = 7.2, 2C, OCH_3), 33.8, 29.1, 22.4 (t, $^{2}J_{CP}$ = 5.7), 18.3. ^{31}P NMR ($CDCl_3$): δ 22.95. MS (ESI^+): calcd for $C_{11}H_{22}O_7P_2Na$ $[M + Na]^+$ 351.0738, found 351.0740.

Dimethyl (1-Hydroxypent-4-yne-1,1-diyl)bisphosphonate Disodium Salt (2a). Mp >300 °C. ^{1}H NMR (D_2O): δ 3.70−3.65 (m, 6H, OCH_3), 2.58−2.53 (m, 2H), 2.39 (t, 1H, ^{4}J = 2.5), 2.27−2.17 (m, 2H). ^{13}C NMR (D_2O): δ 89.1, 77.6 (t, $^{1}J_{CP}$ = 143.4, P−*C*−P), 71.9, 55.3 (t, $\sum^{2}J_{CP}$ = 6.4, 2C, OCH_3), 36.9, 16.2 (t, $^{2}J_{CP}$ = 7.0). ^{31}P NMR (D_2O): δ 19.74. MS (ESI^-): calcd for $C_7H_{13}O_7P_2^-$ $[M - H]^-$ 271.0137, found 271.0130.

Dimethyl (1-Hydroxyhex-5-yne-1,1-diyl)bisphosphonate Disodium Salt (2b). Prepared similarly to **2a**, obtained as a white powder, 95% yield, mp >300 °C. ^{1}H NMR (D_2O): δ 3.67−3.62 (m, 6H, OCH_3), 2.38−2.36 (m, 1H), 2.28−2.22 (m, 2H), 2.06−1.95 (m, 2H), 1.85−1.78 (m, 2H). ^{13}C NMR (D_2O): δ 88.9, 78.2 (t, $^{1}J_{CP}$ = 143.5, P−*C*−P), 69.0, 55.2 (t, $\sum^{2}J_{CP}$ = 6.2, 2C, OCH_3), 37.2, 25.7 (t, $^{2}J_{CP}$ = 5.8), 21.2. ^{31}P NMR (D_2O): δ 20.38. MS (ESI^-): calcd for $C_8H_{15}O_7P_2^-$ $[M - H]^-$ 285.0293, found 285.0292.

Dimethyl (1-Hydroxyhept-6-yne-1,1-diyl)bisphosphonate Disodium Salt (2c). Prepared similarly to **2a**, obtained as a white powder, 93% yield, mp >300 °C. ^{1}H NMR (D_2O): δ 3.70−3.64 (m, 6H, OCH_3), 2.39 (t, 1H, ^{4}J = 2.5), 2.28 (td, 2H, ^{3}J = 7.5, ^{4}J = 2.5), 2.00−1.89 (m, 2H), 1.74−1.66 (m, 2H), 1.62−1.53 (m, 2H). ^{13}C NMR (D_2O): δ 89.5, 78.4 (t, $^{1}J_{CP}$ = 143.6, P−*C*−P), 71.9, 55.2 (t, $\sum^{2}J_{CP}$ = 6.4, 2C, OCH_3), 37.4, 31.7, 25.9 (t, $^{2}J_{CP}$ = 6.0), 20.4. ^{31}P NMR (D_2O): δ 20.54. MS (ESI^-): calcd for $C_9H_{17}O_7P_2^-$ $[M - H]^-$ 299.0450, found 299.0442.

Methyl Hydrogen (1-(Dimethoxyphosphoryl)-1-hydroxy-pent-4-yn-1-yl)phosphonate (3a). Mp ∼238 °C (decomposed). ^{1}H NMR (D_2O): δ 3.88 (d, 6H, $^{3}J_{HP}$ = 10.5, OCH_3), 3.70 (d, 3H, $^{3}J_{HP}$ = 10.5, OCH_3), 2.58−2.51 (m, 2H), 2.42−2.38 (m, 1H), 2.34−2.22 (m, 2H). ^{13}C NMR (D_2O): δ 88.0, 77.2 (dd, $^{1}J_{CP}$ = 144.1, $^{1}J_{CP'}$ = 151.4, P−*C*−P), 72.2, 57.12 (d, $^{3}J_{CP}$ = 7.7, OCH_3), 57.10 (d, $^{3}J_{CP}$ = 7.8, OCH_3), 55.8 (d, $^{3}J_{CP}$ = 6.7, OCH_3), 36.2, 15.9 (dd, $^{2}J_{CP}$ = 6.4, $^{2}J_{CP'}$ = 7.9). ^{31}P NMR (D_2O): δ 27.22 (d, $^{2}J_{PP}$ = 27.9), 15.29 (d). MS (ESI^-): calcd for $C_8H_{15}O_7P_2^-$ $[M - H]^-$ 285.0293, found 285.0282.

Methyl Hydrogen (1-(Dimethoxyphosphoryl)-1-hydroxy-hex-5-yn-1-yl)phosphonate (3b). Prepared similarly to **3a**, except that the eluent was EtOAc/MeOH (4:6). **3b** (97 mg, 51%) was obtained as a white solid, mp ∼220 °C (decomposed). ^{1}H NMR (D_2O): δ 3.854 (d, 3H, $^{3}J_{HP}$ = 11.0, OCH_3), 3.850 (d, 3H, $^{3}J_{HP}$ = 11.0, OCH_3), 3.68 (d, 3H, $^{3}J_{HP}$ = 13.0, OCH_3), 2.38 (t, 1H, ^{4}J = 2.5), 2.26 (td, 2H, ^{4}J = 2.5), 2.03−2.14 (m, 2H), 1.75−1.85 (m, 2H). ^{13}C NMR (D_2O): δ 88.2, 77.8 (dd, $^{1}J_{CP}$ = 144.9, $^{1}J_{CP'}$ = 151.2, P−*C*−P), 72.5, 57.04 (d, $^{3}J_{CP}$ = 7.8, OCH_3), 56.96 (d, $^{3}J_{CP}$ = 7.9, OCH_3), 55.7 (d, $^{3}J_{CP}$ = 6.7, OCH_3), 36.4, 25.3 (t, $\sum^{2}J_{CP}$ = 6.4), 21.0. ^{31}P NMR (D_2O): δ 27.76 (d, $^{2}J_{PP}$ = 30.5), 15.78 (d). MS (ESI^-): calcd for $C_9H_{17}O_7P_2^-$ $[M - H]^-$ 299.0450, found 299.0440.

Methyl Hydrogen (1-(Dimethoxyphosphoryl)-1-hydroxy-3-(1-(2-(2-(2-hydroxyethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)-propyl)phosphonate (4). ^{1}H NMR (D_2O/CD_3OD): δ 7.88 (s, 1H), 4.58 (t, 2H, ^{3}J = 4.8), 3.95 (t, 2H, ^{3}J = 4.8), 3.87 (d, 3H, $^{3}J_{HP}$ = 10.5), 3.86 (d, 3H, $^{3}J_{HP}$ = 10.5), 3.70 (d, 3H, $^{3}J_{HP}$ = 10.0), 3.67 (t, 2H, ^{3}J = 4.5), 3.66−3.59 (m, 4H), 3.54 (t, 2H, ^{3}J = 4.8), 3.09−2.95 (m, 2H), 2.38−2.22 (m, 2H). ^{13}C NMR (CD_3OD): δ 149.2, 123.9, 75.7 (dd, P−*C*−P, $\sum^{1}J_{CP \text{ and } CP'}$ = 142.9), 73.6, 71.43, 71.37, 70.4, 62.2, 51.3, 34.5, 20.9. ^{31}P NMR (CD_3OD): δ 28.45 (d, $^{2}J_{PP}$ = 43.3), 14.38 (d). MS (ESI^-): calcd for $C_{14}H_{28}N_3O_{10}P_2^-$ $[M - H]^-$ 460.1250, found 460.1230.

Dimethyl (1-Hydroxy-4-(1-(2-(2-(2-hydroxyethoxy)ethoxy)-ethyl)-1*H*-1,2,3-triazol-4-yl)butane-1,1-diyl)bisphosphonate Monocopper Salt (5). ^{1}H NMR (CD_3OD): δ 8.62 (br, 1H), 4.75 (br, 2H), 3.94 (br, 2H), 3.82−3.69 (br, 6H), 3.69−3.62 (br, 2H), 3.62−3.57 (br, 2H), 3.57−3.52 (br, 2H), 3.50−3.43 (br, 2H), 2.95−2.80 (br, 2H), 2.13−1.94 (br, 4H). ^{13}C NMR (CD_3OD): δ 73.7, 71.6, 71.4, 69.4, 62.1, 55.4, 34.2, 24.9, 23.6. MS (ESI^-): calcd for $C_{14}H_{28}N_3O_{10}P_2^-$ $[M - H]^-$ 460.1250, found 460.1265.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H, ^{13}C, and ^{31}P NMR spectra of **1a−c**, **2a−c**, **3a**, **3b**, **4**, and **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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