A novel strategy for synthesis of 5-iodo (\(^{125/131}\)I)-1, 2, 3-triazoles via click chemistry

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Abstract: We report a facile and effective method for radioiodine-labeled radiopharmaceuticals via copper (I)-catalyzed click chemistry route. In the novel radioiodination method, 5-iodo (\(^{125/131}\)I)-1, 2, 3-triazoles were synthesized after a 24-h click reaction in organic solvent with a radiochemical yield of 13\%. However, in the aqueous phase, the radiochemical yield of the conjugation radioiodine to RGD via click chemistry was 0\%. This suggested an exchange between hydrogen ion and iodine ion in aqueous phase so that no enough radioiodine was left to conjugate with RGD. We propose different mechanisms of Cu (I)-catalyzed cycloaddition of organic azides and 1-iodoalkynes in organic phase and aqueous phase.

Key words: click chemistry; radioiodination; 5-iodo (\(^{125/131}\)I)-1, 2, 3-triazoles; mechanism

INTRODUCTION

Copper (I)-catalyzed azide-alkyne cycloaddition reaction (CuAAC) is one of the most widely used click chemistry approaches in bioorganic and medicinal studies\(^{[4-5]}\). This reaction can be reliably carried out under mild and tolerable conditions such as in aqueous media at neutral pH with also the merits of reasonable reaction time, no sensitivity towards moisture or oxygen, high yields, stereospecificity, absence of solvent contamination (or use of a benign one if required), and simple product isolation. In 2006, Marik et al.\(^{[6]}\) adopted CuAAC reaction for the preparation of \(^3\)F-labeled short peptide fragments and demonstrated its potential use in position emission tomographic (PET) studies. CuAAC has since been a popular method in radiopharmaceutical researches of, for instance, \(^3\)F-labeled small organic molecules and biomolecules for PET imaging, and \(^{125/131}\)I-labeled compounds for single-photon emission computed tomography (SPECT)\(^{[21-23]}\). Recent reports described radioiodination labeling of pharmaceutical molecules through CuAAC reaction\(^{[26-27]}\). Inspired by the synthesis of 1,4,5-triaryl-1,2,3-triazoles from 5-iodo-1, 2, 3-triazoles by Cu(I)-catalyzed azides-1-iodoalkynes reaction\(^{[28-33]}\), we designed a novel radioiodination route, in which 1-iodo (25/29)\(^I\)-2-p-methylphenylethynyl was conjugated with benzyl azides to produce 5-iodo (25/29)\(^I\)-1, 2, 3-triazole compound. We also used this method to conjugate a(RGDK)-N\(_2\) with 1-iodo(25/29)\(^I\)-alkynes in aqueous media but failed to obtain radiochemical yields. We propose a modified mechanism of CuAAC of organic azides and 1-iodoalkynes in organic and aqueous phase reaction.

MATERIALS AND METHODS

Reagents and instruments

All the experiments were carried out under specified temperature conditions. Solvents were distilled from appropriate drying agents and degassed before use. The melting points were determined using a WRS-IA apparatus and were uncorrected. High-resolution mass spectra were obtained with a Thermo-MAT95XP mass spectrometer under electron impact ionization conditions. Nuclear magnetic resonance (NMR) spectra were recorded using a Bruker Avance 400 or 500 MHz spectrometer. FT-IR spectra were measured in the forms of potassium bromide (KBr) tablets using a Thermo Nicolet Avatar 370 FTIR Spectrometer from 4000 to 400 cm\(^{-1}\) at a resolution of 4 cm\(^{-1}\) and 32 scans. High-performance liquid chromatography (HPLC) analyses of the compounds were performed using a Dionex P680 system equipped with a tunable absorption detector and a PDA-100 photodiode-array detector using a Hypersil BDS C-18 reversed-phase column (5 \(\mu\)m, 250 mm \(\times\) 4.6 mm). Trifluoroacetic acid (TFA) at the concentration of 0.14% (weight) in water (B) as well as in ACN (A) was used as the mobile phase (70: 30 water/ACN). The flow rate was 1 ml/min.

All the starting materials were purchased from Aldrich or TCI chemical companies and used as received. Solvents were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai) and used as received (without extra drying, distillation or special handling). The radionuclide of Na\(^{22}\) was purchased...
from Ruijin Hospital, Shanghai Jiaotong University.

Potassium alkynyltrifluoroborates synthesis

A solution of a1 (3.06 g, 30 mmol) in 60 ml of dry THF was cooled to −78 °C in nitrogen. n-BuLi (18.75 ml, 1.6 mol/L in hexane, 30 mmol) was added dropwise, and the solution was stirred for 1 h at this temperature. Trimethylborate (4.68 g, 45 mmol) was then added dropwise at −78 °C. The solution was stirred at this temperature for 1 h and allowed to warm to −20 °C for 1 h. A saturated aqueous solution of potassium hydrogen difluoride (14.1 g, 180 mmol) was added to the vigorously stirred solution. The resulting mixture was stirred for 1 h at −20 °C, and then allowed to warm to room temperature for 1 h. All the solvents were removed under reduced pressure. The solid was then washed with hot acetone. The resulting organic solution was filtered with the solvent removed. The crude products was then dissolved in hot acetone and precipitated with diethyl ether, followed by cooling to −20 °C to complete the precipitation of the solid.

The yield of b, was 80%. 1H NMR: (400 MHz, CDCl3) δ = 2.82 (s, 3H), 7.06 (d, 2H), 7.22 (d, 2H); and the yield of b, was 82%. 1H NMR: (400 MHz, CDCl3) δ = 7.18-7.23 (m, 1H), 7.25-7.27 (m, 2H), 7.33-7.34 (m, 2H).

Synthesis of alkynyl iodides

In the mixture of 10 ml H2O and 10 ml THF, b1 (1.05 g, 5 mmol) and molecular iodine (1.54 g, 6 mmol) were added, and the reaction mixture was stirred at room temperature for 15 min. The solution was washed with aqueous Na2SO3, and THF was removed under reduced pressure followed then by addition of 10 ml H2O and extraction with dichloromethane (DCM, 20 mL×3). The organic layer was dried with Na2SO4 and DCM was removed on a rotary evaporator.

To the mixture of 1 (1.13 g) was synthetized as a yellow oil and the compounds were used without any further purification. 1H NMR: (400 MHz, CDCl3) δ = 7.30-7.33 (m, 3H), 7.42-7.45 (m, 2H).

c1 (1.25 g) was synthetized as a yellow oil and used without any further purification. 1H NMR: (400 MHz CDCl3) δ = 2.35 (s, 3H), 7.10-7.16 (m, 2H), 7.26-7.34 (m, 2H).

Synthesis of 5-iodotriazoles using Cu-TEA

1-benzyl-5-iodo-4-phenyl-1H-1,2,3-triazole (e1): The compounds c1 (228 mg, 1.00 mmol) and d1 (133 mg, 1.00 mmol) were dissolved in THF (5 ml). The solution was treated sequentially with CuI (9.52 mg, 0.05 mmol) and TEA (0.28 ml, 2.00 mmol) and stirred at room temperature for 6 h. The reaction was terminated by adding 1 ml of 10% NH4OH solution. The volatile components were removed by evaporation, and the resulting residue was dissolved in DCM. The target compounds were isolated by flash chromatography on a silica gel column, resulting in e1, as a fine white powder (292 mg, 81%). 1H NMR: (400 MHz CDCl3) δ = 5.68 (s, 2H), 7.26-7.42 (m, 6H), 7.44-7.48 (m, 2H), 7.93-7.96 (m, 2H); IR (υ [cm−1]) : 3032, 2929, 1600, 1447, 1348, 1231, 1069, 760, 724, 695; ESI-MS: [M+CH3OH+Na]+ 416.2.

1-benzyl-5-iodo-4-p-tolyldihydro-1H-1,2,3-triazole (e1): 315 mg, 84%; 1H NMR: (400 MHz CDCl3) δ = 2.40 (s, 3H), 5.63 (s, 2H), 7.24-7.28 (m, 5H), 7.32-7.36 (m, 2H), 7.80-7.84 (m, 2H); IR (υ [cm−1]) : 3027, 2921, 1603, 1451, 1226, 981, 713; ESI-MS: [M+CH3OH+Na]+ 464.2.

1-(4-chlorobenzyl)-5-iodo-4-phenyl-1H-1,2,3-triazole (e1): 315 mg, 84%; 1H NMR: (400 MHz CDCl3) δ = 5.64 (s, 2H), 7.25-7.27 (m, 2H), 7.33-7.36 (m, 2H), 7.37-7.42 (m, 2H), 7.44-7.49 (m, 2H), 7.92-7.95 (m, 2H), IR (υ [cm−1] ) : 3060, 2972, 1599, 1490, 1229, 1087, 772, 695; ESI-MS: [M+CH3OH+Na]+ 450.2.

1-(4-chlorobenzyl)-5-iodo-4-p-tolyldihydro-1H-1,2,3-triazole (e1): 315 mg, 84%; 1H NMR: (400 MHz CDCl3) δ = 2.40 (s, 3H), 5.67 (s, 2H), 7.25-7.38 (m, 6H), 7.81-7.83 (m, 2H); IR (υ [cm−1] ) : 3027, 2917, 1597, 1485, 1403, 1226, 1087, 983, 805, 765; ESI-MS: [M+CH3OH+Na]+ 485.2.

Synthesis of radio-alkynyl iodides

Alkynyltrifluoroborate b1 (100 μl of 0.05 mol/L in 50% aqueous tetrahydrofuran) was placed in an iodogenic vial containing no-carrier-added Na[125I] (40 μCi) aqueous solution. The iodogenic vial was shaken for 10 min on an oscillator. The radiochemical purity of the 1-[125I]iodo-2-phenylethylene, c1, was determined by radio-TLC (silica gel plate GF254, hexane).

Synthesis of 5-[125I]iodotriazole using Cu-TEA

A (without c1): 100 μl of (azidomethyl) benzene (1 mol/L) solution in THF was mixed with 100 μl of freshly prepared 1-[125I]iodo-2-p-methylphenylene, 100 μl of Cu-TEA [mixed CuI (9.52 mg) and TEA (0.28 ml) in 5 ml THF] solution in THF and incubated at room temperature for 24 h. The radiolabeling efficiency was determined by radio-HPLC.

B (with c1): 100 μl of 1-(iodoethynyl)-4-methylbenzene (1 mol/L) solution in THF and 100 μl of (azidomethyl) benzene (1 mol/L) solution in THF were mixed with 100 μl of freshly prepared 1-[125I]iodo-2-p-methylphenylene and 100 μl of Cu-TEA solution in THF and incubated at room temperature for 24 h. The radiolabeling efficiency was determined by radio-HPLC.

*One-pot* reaction with c(RGDfK)-N5

The mixture of 100 μl of c(RGDfK)5-N5 (0.2 mg solution, 100 μl of 1-(iodoethynyl)-4-methylbenzene (1 mol/L) solution in THF, 100 μl of b1 solution in THF, water, 100 μl of fresh Na[125I] solution, and 100 μl of Cu-TEA solution in THF were incubated at room temperature for 24 h. The radiolabeling efficiency was determined by radio-HPLC.
RESULTS AND DISCUSSION

Fig. 1 shows the synthesis of 5-iodo-1,2,3-triazoles. We adopted Molander’s method \(^{32}\) to avoid the use of any toxic reagents and obtain high yields of products appropriate for pharmaceutical purposes. The isolated yields of alkynyltrifluoroborates from the corresponding alkynes were more than 80% after a series of substitution reactions. But these reactions require the total absence of such active radicals as hydroxyl, carboxyl and amino groups because of the presence of n-BuLi. Nevertheless, these alkynyltrifluoroborates were stable as crystals in air, which makes them ideal “kit” for applications for pharmaceutical purpose.

In the click reaction, the commonly used catalyst (CuSO₄/L-sodium ascorbate) \(^9\) failed in the iodination reaction because of the poor solubility of this water-soluble system in THF. Therefore, the Cul-TEA \(^{28}\) system was chosen as the catalyst in this click reaction, and the isolate yields were greater than 85% after 4 h (Fig. 3). Hein and Garcia-Alvarez’s work \(^{28, 30}\) was to conjugate the 1-iodoalkynes with azides, which was called “cold-click reaction”; in the radio-click reaction (hot-click reaction), however, the RCY was less than 13% in the presence of the “cold” 1-iodoalkynes (Fig. 4) and was almost zero in its absence (Tab. 1).

In the following experiment, the c(RGDK)-N₃ was chosen instead of the small organic azides. But the RCY of “one-pot” click reaction of c(RGDK)-N₃ and 1-[\(^{125}\)I] iodo-2-phenylethylene with the cold one was almost zero after a 24-h reaction when the mixed solvents contained water and THF. In this reaction, phenylacetylene and its click product with c(RGDK)-N₃ were formed. This indicates that there must be an interchange between the hydrogen ion and iodine cation with Cul as the catalyst. In the entire reaction process, other intermediates containing radio-iodine atoms were not detected by radio-HPLC except for iodide, which demonstrates that iodine atoms did not participate in the reaction till the last stage of electrophilic substitution reaction in the click reaction (Fig. 5).

Fig. 5 illustrates the proposed mechanisms for CuAAC of organic azides and 1-iodoalkynes in the...
organic and water phases. In the organic phase reaction, similar to the CuAAC reaction \([14, 34]\), the mechanism involves the formation of the \(\sigma\)-acetylide (complex 3), which is an irreversible process. As shown in Fig.5, the organic azide analogue 4 is transferred into the metal centre 5 with the \(\sigma\)-acetylide complex 3, followed by coordination of the azide through the proximal nitrogen center and subsequent cyclization to give rise to the product 6. The following reaction of complexes 1 and 6 generates 5-iodotriazole (complex 7). The radio-
1-iodoalkynes, as the starting material, reacts with the catalyst and is converted to α-acetylate. Because the amount of radio-1-iodoalkynes is much less than that of the catalyst, no radio-1-iodoalkynes is left to react with the complex 6. This explains the almost zero yields of radio-click reaction. However, when the starting materials contain non-radioactive 1-iodoalkynes, there can be an enough amount of 1-iodoalkynes (contending non-radioactive and radioactive) to react with complex 6 to result in a RCY above zero. Moreover, when water exists in the mixed solvents, an exchange between hydrogen ion and iodine cation occurs. The compound 1 can be converted into compound 8, which reacts with complex 6 to generate compound 9 in the last substitute stage. Therefore, the conjugate yield of 1-[125I]iodo-2-phenylethylene and e(RGD)-N, was almost zero in the "one-pot" click reaction regardless of the use of either "cold" or "hot" 1-iodoalkynes.

CONCLUSION

We show that "click chemistry" provides a simple, efficient, and reliable procedure for cold labeling of iodine with mild reaction condition and high yields. Moreover, the compounds b and b can be developed into "kits" for clinical application. Necessary modifications of the hot-click reaction need to be made before its clinical application. The mechanisms differ for CuAAC of azides and 1-iodoalkynes in organic and aqueous phase reaction. In the aqueous phase reaction, the hydrogen ion can exchange with iodine cation, which does not happen in organic solvents.

REFERENCES


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摘要:通过Cu(I)催化的点击化学合成一种放射性碘标记化合物,建立一套新的放射性碘标记的方法,该方法温和、快速、高效。结果显示在有机相反应中,通过点击化学反应5-碘(125/131I)-1,2,3-三唑化合物在24 h内合成,放射化学产率达到13%。但是在水相中,点击化学标记生物分子RGD的放射化学产率为0。原因是在水相中,Cu(I)催化作用使得氢离子和碘离子发生了交换反应,没有足够的放射性碘配体与RGD发生偶联。本文还提出了在反应溶剂为有机相和水相的条件下Cu(I)催化的环加成反应的不同反应机制。

关键词:点击化学;放射性碘标记;5-碘(125/131I)-1,2,3-三唑化合物;反应机制

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