Asian Journal of Chemistry; Vol. 30, No. 10 (2018), 2369-2372

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2018.21528

Development of Optimized MRI Contrast Agents for Copper(I) Click Reactions

MUSTAFA M. AL-YASSIRY*, FARAH K. ALORAIBI and S.J. ARCHIBALD

Department of Chemistry and Biochemistry, College of Medicine, University of Misan, Misan, Iraq

Corresponding author: E-mail: mman.alyassiry@yahoo.com

Received: 18 June 2018; *Accepted*: 23 July 2018; *Published online*: 31 August 2018; AJC-19077

This work has been focused on synthesizing gadolinium(III) 1,4,7-triacetic acid-1,4,7,10-tetraazacyclododecane (DO3A) macrocyclic complexes (GdAa1) as potential T1 MRI contrast agents. Alkyne derivatives chelators were synthesized with three different alkyne pendent arms (butyne) are suitable for Cu(I) catalyzed cycloaddition 'click' chemistry with azide derivatives to form 1,2,3-triazole rings. Triazole formation with benzyl azide was used as "proof of concept" to form chelators and gadolinium complexes (GdAa1) that could be assessed for their properties as MRI contrast agents. One of the complexes was then selected for conjugation with azide derivative of a thymidine DNA base (AZT, a clinically approved drug molecule) to link *via* triazole group and demonstrate the widespread utility of this approach. Relaxivity measurements were carried out on the synthesized gadolinium(III) complexes to evaluate their potential as MRI contrast agents with GdAa1 showing the highest T1 relaxivity result. Therefore, GdA2a (relaxivity, 5.74 m M^{-1} s^{-1}) was conjugated with DNA base derivatives (3′-Azido-3′-deoxythymidine, AZT) to give the desired product in an 89 % yield with a relaxivity of 4.33 m M^{-1} s^{-1} indicating the potential of these compounds for future *in vivo* applications in MRI studies.

Keywords: Gadolinium complexes, Click chemistry, MRI contrast agents, Azide derivatives.

INTRODUCTION

Magnetic resonance imaging (MRI) is a highly effective and common diagnostic imaging technique for clinical applications due to its capability to provide anatomical and functional images with high resolution and showing the deep structure of the internal body [1]. This imaging modality has some advantages compared to other medical imaging techniques (PET, CT, X-ray), such as that it does not require harmful ionizing radiation and can directly detect soft tissues. It also has high spatial resolution and provides good contrast between tissue types [2,3].

Click chemistry is a term presented for the first time by the Sharpless group in 2001 to describe the highly effective reaction of organic components that has significant advantages compared with the other slower and lower yielding methods [4]. The compounds generated in this research project are made by Cu(I) catalyzed azide-alkyne cycloaddition method. The Cu(I) catalyzed click chemistry is a Huisgen 1,3-dipolar cycloaddition which leads to 1,4-disubstituted 1,2,3-triazole and formed from a reaction between an azide and terminal alkyne in the presence of copper. This connection is rapidly formed, selective and produces high yields with no or few by products [5,6]. The Cu(I) catalyzed "click" reaction has been used previously to produce contrast agents for imaging based on lanthanide metal chelates. A lot of work has gone into development of alkyne or azide functionalized metal chelators which can be conjugated to biological molecules, such as peptides or sugars, the paramagnetic properties of Gd(III) enable its use as an MRI contrast agent. The choice of chelating moiety affects the kinetic and thermodynamic stability and selection of an appropriate biologically active molecule can be used to enable targeted delivery of agent [7].

EXPERIMENTAL

^{1}H NMR and ^{13}C NMR were obtained using a Jeol JNM-LA400 spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C in the solvents indicated, referenced against standard internal TMS or residual non deuterated solvent signal. Chemical shifts (δ) are quoted in parts per million (ppm). Deuterated solvents were purchased from either Goss Chemicals Ltd. or Cambridge Isotopes Ltd.

Electrospray ionisation (ESI) mass spectra were recorded on low resolution Varian 500-MS LC-ion trap system. The comp-

ound to be analyzed was diluted in methanol and approximately 0.1 µL was added and run in 80 % MeOH:20 % H_2O. Accurate mass spectrometry measurements (HRMS) were recorded at EPSRC National Mass Spectrometry Service Centre, University of Swansea, UK using a LQT Orbitrap XL. The elemental CHN were analyzed using a CHN analyser EA1108 (Carlo Erba). Inductively coupled optical emission spectroscopy (ICP-OES) analysis was carried out using a Perkin Elmer Optima 5300 DV. All the samples were in solid state, digested with aqua-regia in glass sample vials on a hotplate. Calibration standards were prepared at 1 and 10 ppm from 1000 ppm concentrates of gadolinium which was purchased from commercial suppliers.

All solvents and reagents were purchased, primarily, from commercial suppliers and were used without further purification unless otherwise stated. When dry solvents were required, the solvent was either dried using 3 Å molecular sieves in the lab or taken from a sure-seal bottle to ensure the absence of water. TLC was used to monitor the reaction progress with F_{254} silica plates and visualization by UV light and stains. Silica gel (60A, 35-70 micron) was used to perform column chromatography.

Synthesis of 1,4,7,10-tetraazacyclododecane- tri-*tert*-butyl 2,2′, 2″-(10-(but-3-yn-1-yl)-1,4,7-triyl)triacetate (A_1): A modified method based on the reported procedure [10] was followed. A solution of 4,7-*tris*-(*tert*-butylacetate)-1,4,7,10-tetraazacyclododecane (100 mg, 0.194 mmol), 1-bromo 2-butyne (31 mg, 0.23 mmol) and Cs_2CO_3 (157 mg, 0.48 mmol) in dry MeCN (10 mL) was stirred under nitrogen at room temperature for 24 h. The obtained solid from the reaction mixture were filtered off and the solvent was removed. Product was purified on silica gel chromatography using DCM:Me_2CO:MeOH:: 6:3.5:0.5 as eluent to give compound **A_1** as light brownish oil Yield: 38 mg (34 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.43 (s, 9H, 3 × C$\underline{H}_3$), 1.48 (s, 18H, 6 × C$\underline{H}_3$), 2.25 (dt, 2H, C$\underline{H}_2$C=CH), 2.62 (s, 4H, ring C$\underline{H}_2$), 2.78 (s, 12H, ring C$\underline{H}_2$), 2.81-2.99 (m, 8H, ring C$\underline{H}_2$), 3.11-3.25 (m, 2H, C$\underline{H}_2$CH$_2$C=CH), 3.52 (m, 1H, C=C$\underline{H}$), 3.91 (s, 2H, C$\underline{H}_2$CO), 4.26 (s, 4H, C$\underline{H}_2$CO). ^{13}C NMR ($CDCl_3$): δ 28.21, 29.13, 29.25, 53.25, 54.01, 55.22, 56.71, 58.18, 68.73, 70.26, 171.54, 171.91. HRMS$^+$ for $C_{30}H_{54}N_4O_6$ 567.4116 *m/z* $[M+H]^+$; found 567.4102 *m/z* $[M+H]^+$.

Synthesis of 1,4,7,10-tetraazacyclododecane-2,2′,2″-(10-(but-3-yn-1-yl)-1,4,7-triyl)triacetic acid (A_2): Compound **A_1** (100 mg, 0.17 mmol) triacetate was dissolved in a mixture of dichloromethane (6 mL) and trifluoroacetic acid (6 mL). The solution was stirred overnight at room temperature. The volatiles were removed by rotary evaporation and the residue dissolved in the minimum amount of methanol. Addition of diethyl ether yielded the desired compound as a light yellow solid (47 mg) in a 67 % yield. The white fluffy solid was collected *via* centrifuge and dissolved in H_2O and freeze-dried. ^{1}H NMR (400 MHz, D_2O), 2.32 (s, 1H, C$\underline{H}_2$C=CH), 2.62-4.41(m, 24H, C$\underline{H}_2$), 4.61 (t, 1H, C≡C$\underline{H}$). ^{13}C NMR (400 MHz, D_2O): δ 31.28, 35.26, 42.37, 45.11, 45.89, 47.88, 50.60, 51.39, 52.56, 72.79, 74.18, 173.90, 174,84). Elemental analysis of $C_{18}H_{30}N_4O_5\cdot(CF_3CO_2H)_{3.1}(H_2O)$ calculated (found) (%): C 37.75 (38.21), H 4.60 (4.87), N 7.28 (7.55). ESMS$^+$ *m/z* $[M+H]^+$ 399.4.

Gadolinium(III) complex of 1, 4, 7, 10-tetraazacyclododecane-2,2′,2″-(10-(but-3-yn-1-yl)-1,4,7-triyl)triacetic acid (GdA_2): A modified method based on the reported procedure [10] was adapted to synthesize GdA_2. Compound **A_2** (100 mg, 0.13 mmol) was dissolved in a mixture of 10 mL of methanol and triethylamine (0.15 mL), and then $GdCl_3\cdot6H_2O$ (55 mg, 0.15 mmol) was added to the solution. The solution was stirred for two days at 50 ºC, the residue was redissolved in methanol and triturated with diethyl ether to give white powder (35 mg) in 48 % yield. Elemental analysis of $C_{18}H_{27}N_4O_6Gd.(CH_3OH)(H_2O)_{1.5}$ calculated (found) (%): C 39.09 (39.45), H 5.34 (5.64), N 9.58 (9.78). ESMS$^+$ $[M+H]^+$ 540.1.

Gadolinium(III) complex of 1,4,7,10-tetraazacyclododecane-(3-benzyl-5-propyl-1,2,3-triazole1)-1,4,7-triyl) triacetic acid (GdA_{2a}): A modified method is adapted [12] for the synthesis of **GdA_{2a}** complex. Complex **GdA_2** (100 mg, 0.18 mmol) and benzyl azide (30 mg, 0.22 mmol) were dissolved in water:*tert*-butanol (1:1), then $CuSO_4\cdot5H_2O$ (2.3 mg, 0.009 mmol) was added to reaction mixture. Sodium ascorbate (3.5 mg, 0.018 mmol) was dissolved in a separate flask and introduced to the reaction mixture by syringe in dropwise manner. The solvent was removed under reduced pressure after the reaction was completed, forming a white powder (72 mg) in 59 % yield. ESMS$^+$ $[M+H]^+$ 687.1, ICP: Gd: 22.93; found 18.623.

RESULTS AND DISCUSSION

The main aim of this work is to synthesize alkyne-functionalized DO3A chelator suitable for click chemistry with biologically relevant azide functionalized molecule. The chelating ligand (**A_1**) was synthesized and functionalized with alkyne arm (butyne) as first stage (**Scheme-I**). And then synthesis was performed using DO3A and halogenated alkynyl precursor was optimized for reaction temperature and time. The arm selected from the possible alkyne derivatives was 4-bromo-1-butyne, which is commercially available and bromine is good leaving group for the substitution reaction. This reaction was carried out under the same conditions as described above to form the desired product **A_1**. The reaction was left to stir for 21 days with regular monitoring by silica TLC and mass spectrometry (Fig. 1), which showed the formation of product at *m/z* 567 $[M+H]^+$. The product still showed the presence of unreacted starting materials (*t*-Bu-DO3A at *m/z* 515) after this reaction time [8].

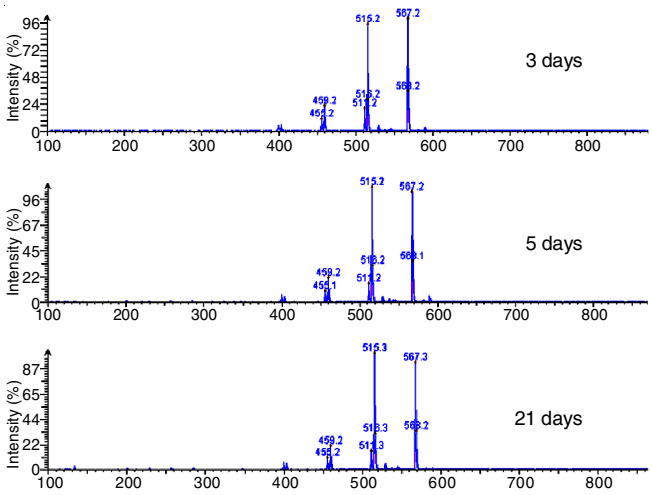
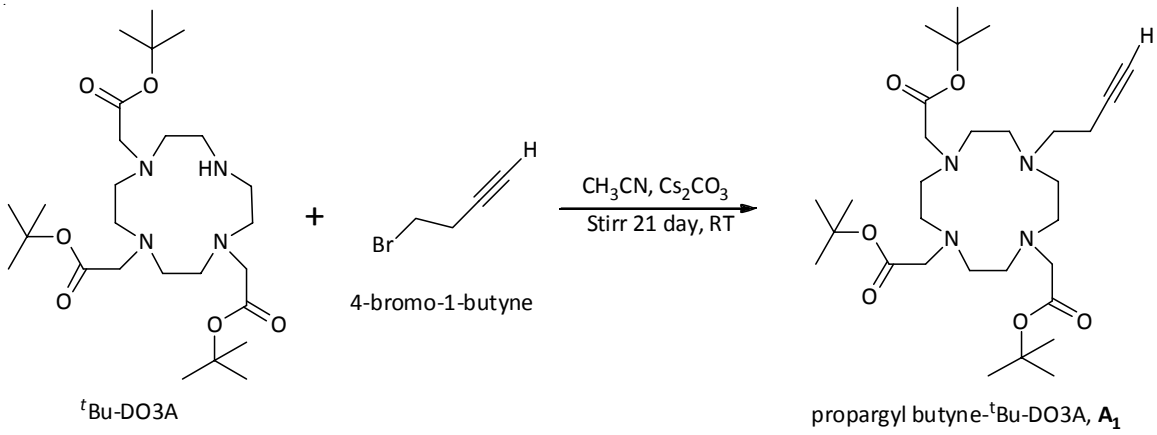


Fig. 1. Mass spectrum of **A_1** contained starting materials DO3A at *m/z* 515 after 3, 5 and 21 days



Scheme-I: Synthetic route to form N-functionalized tBu-DO3A with propargyl butyne arm (5)

The reaction was monitored by TLC and MS at three different interval of times (3, 5, and 21 days) and shows no significant change by continuing the stirring for a longer time period. At this stage, the reaction was terminated and the synthesized ligand $\mathbf{A_1}$ purified by silica gel chromatography with DCM (97 %):MeOH (3 %) as eluents. TLC and MS analysis results showed that desired product was present after column chromatography but still had impurities of starting materials present (product at *m/z* 567 $[M+H]^+$, (DO3A) at *m/z* 515) (Fig. 2).

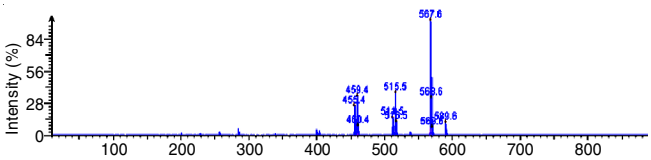


Fig. 2. Mass spectrum of purified with small percentage of starting materials DO3A at *m/z* 515

The synthesized ligand $\mathbf{A_1}$ was then deprotected from acetate arms to get $\mathbf{A_2}$ as novel ligand in this research work, (Fig. 3). Identity and purity of this product ($\mathbf{A_2}$) was confirmed by ^{1}H NMR, MS and CHN analyses.

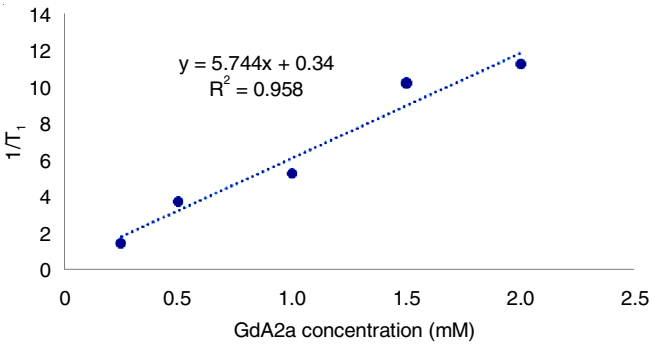
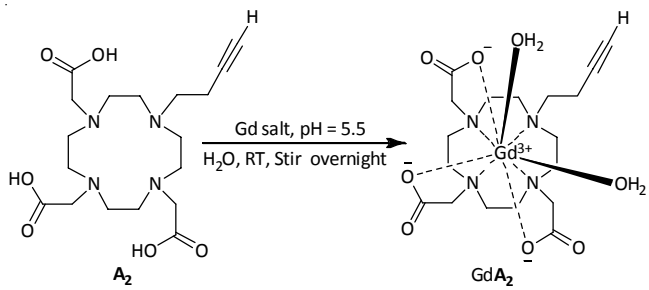


Fig. 3. Graphs to show concentration *vs.* 1/T for GdA2a

The second stage of this project consists of synthesis of gadolinium(III) complex with the previously prepared alkyne functionalized DO3A ($\mathbf{A_1}$). As synthesized ligand ($\mathbf{A_1}$) contains DO3A component, they can form up to seven coordination bonds and chelate di- or trivalent metal cations of suitable size. Gadolinium(III) is paramagnetic in nature and has a symmetric S electronic state which is ideal for use as an MRI contrast agent [8,9] thus, it was chosen as preferred lanthanide metal to coordinate with chelators $\mathbf{A_2}$ to produce gadolinium(III) complex (GdA_2) (**Scheme-II**).

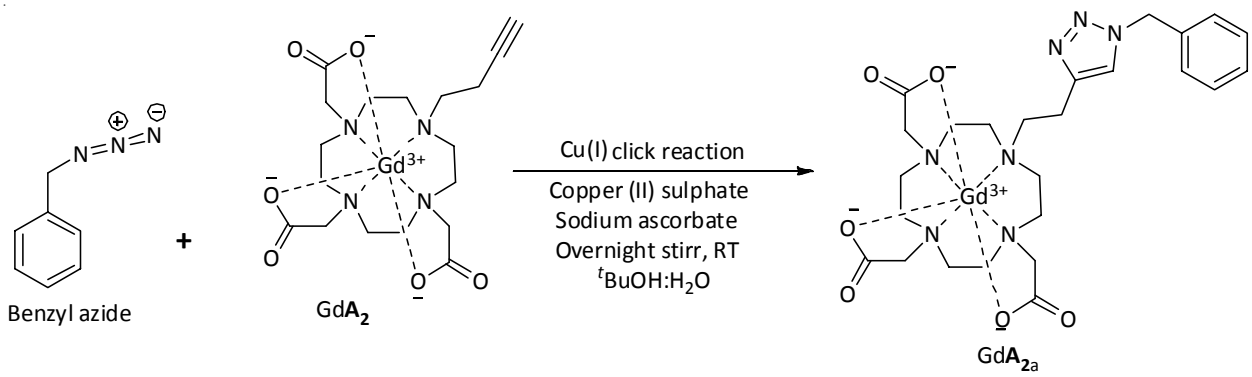


Scheme-II: Gd(III) complexation reaction with synthesised ligand $\mathbf{A_2}$

The aim in formation of Gd^{3+} complex prior to "clicking" DO3A macrocycle with an azide functionalized derivative is to prevent Cu^{2+} ions (catalyst) from complexing with these macrocyclic ligands, complicating purification and compromising Gd(III) chelation necessary for MRI contrast agent activity. Thus, complexation reactions were carried following a modified literature method [10]. Once alkyne functionalized chelator $\mathbf{GdA_2}$ was prepared, a simple organic azide was required in order to investigate the chelate reactivity in Cu(I) catalyzed "click" reaction. Due to its stability and the ease of preparation from benzyl bromide, benzyl azide was selected [11].

"Click" reaction between butyne-functionalised DO3A ($\mathbf{GdA_2}$) and benzyl azide was carried out by conjugate $\mathbf{GdA_2}$ obtained using the standard protocol described by Sharpless *et al*. [12] as a white powder product ($\mathbf{GdA_{2a}}$) with 85 % yield, (**Scheme-III**).

T_1 relaxation studies for GdA_{2a}: Relaxivity rates was determined for $\mathbf{GdA_{2a}}$ across range of concentration values (0.5, 1, 1.5, 2 and 2.0 mM) at pH 7, which means that all the N and O donors are expected to be deprotonated and coordinated to gadolinium(III) metal centre [13]. Fig. 3 shows graph of MRI relaxivity measurements for the synthesized $\mathbf{GdA_{2a}}$ indicating the linear relationship between relaxation rates $1/T_1$ (r_1) *vs.* various concentrations of these complexes to give high T_1 relaxivity value 5.744 m M^{-1} s^{-1} compare to the other clinical MRI contrast agents, with correlation coefficienent 0.9581. This indicates its potential use as T_1 weighted contrast agent in MRI applications [5,14].



Scheme-III: Cycloaddition click reaction between benzyl azide and GdA_2 forming 1,2,3-triazole (GdA_{2a})

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. W.P. Kluijfhout, S. Venkatesh, T. Beninato, M.R. Vriens, Q.Y. Duh, D.M. Wilson, T.A. Hope and I. Suh, *Surgery*, **160**, 747 (2016); https://doi.org/10.1016/j.surg.2016.05.003.
2. D.W. Park, S.-H. Ye, H.B. Jiang, D. Dutta, K. Nonaka, W.R. Wagner and K. Kim, *Biomaterials*, **35**, 7851 (2014); https://doi.org/10.1016/j.biomaterials.2014.05.088.
3. V.P. Mocharla, B. Colasson, L.V. Lee, S. Roper, K.B. Sharpless, C.H. Wong and C. Kolb, *Angew. Chem.*, **44**, 116 (2005); https://doi.org/10.1002/anie.200461580.
4. V.V. Rostovtsev, L.G. Green, V.V. Fokin and K.B. Sharpless, *Angew. Chem.*, **41**, 2596 (2002); https://doi.org/10.1002/1521-3773(20020715)41:14<2596::AID-ANIE2596>3.0.CO;2-4.
5. S.W. Millward, H.D. Agnew, B. Lai, S.S. Lee, J. Lim, A. Nag, S. Pitram, R. Rohde and J.R. Heath, *Integr. Biol.*, **5**, 87 (2013); https://doi.org/10.1039/C2IB20110K.
6. L.N. Goswami, L.X. Ma, P.J. Kueffer, S.S. Jalisatgi and M.F. Hawthorne, *Molecules*, **18**, 9034 (2013); https://doi.org/10.3390/molecules18089034.
7. M. Zhang, P.A. Rupar, C. Feng, K.X. Lin, D.J. Lunn, A. Oliver, A. Nunns, G.R. Whittell, I. Manners and M.A. Winnik, *Macromolecules*, **46**, 1296 (2013); https://doi.org/10.1021/ma302054q.
8. S. Biju, M. Harris, L.V. Elst, M. Wolberg, C. Kirschhock and T.N. Parac-Vogt, *RSC Adv.*, **6**, 61443 (2016); https://doi.org/10.1039/C6RA09450C.
9. M. Jauregui, W.S. Perry, C. Allain, L.R. Vidler, M.C. Willis, A.M. Kenwright, J.S. Snaith, G.J. Stasiuk, M.P. Lowe and S. Faulkner, *Dalton Trans.*, 6283 (2009); https://doi.org/10.1039/b911588a.
10. R.F.H. Viguier and A.N.J. Hulme, *Am. Chem. Soc.*, **128**, 11370 (2006); https://doi.org/10.1021/ja064232v.
11. P.A. Sukerkar, K.W. MacRenaris, T.R. Townsend, R.A. Ahmed, J.E. Burdette and T.J. Meade, *Bioconjug. Chem.*, **22**, 2304 (2011); https://doi.org/10.1021/bc2003555.
12. P. Wu, A.K. Feldman, A.K. Nugent, C.J. Hawker, A. Scheel, B. Voit, J. Pyun, J.M.J. Frechet, K.B. Sharpless and V.V. Fokin, *Angew. Chem. Int. Ed.*, **43**, 3928 (2004); https://doi.org/10.1002/anie.200454078.
13. S. Aime, M. Botta, R.S. Dickins, C.L. Maupin, D. Parker, J.P. Riehl and J.A.G. Williams, *J. Chem. Soc., Dalton Trans.*, 881 (1998); https://doi.org/10.1039/a708667i.
14. P.A. Furman, J.A. Fyfe, M.H. Stclair, K. Weinhold, J.L. Rideout, G.A. Freeman, S.N. Lehrman, D.P. Bolognesi, S. Broder, H. Mitsuya and D.W. Barry, *Acad. Sci. U.S.A.*, **83**, 8333 (1986); https://doi.org/10.1073/pnas.83.21.8333.