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Total synthesis of rubrolide M and some of its unnatural congeners

Fabio Bellina,* Chiara Anselmi and Renzo Rossi*

Dipartimento di Chimica e Chimica Industriale, Via Risorgimento 35, I-56126 Pisa, Italy

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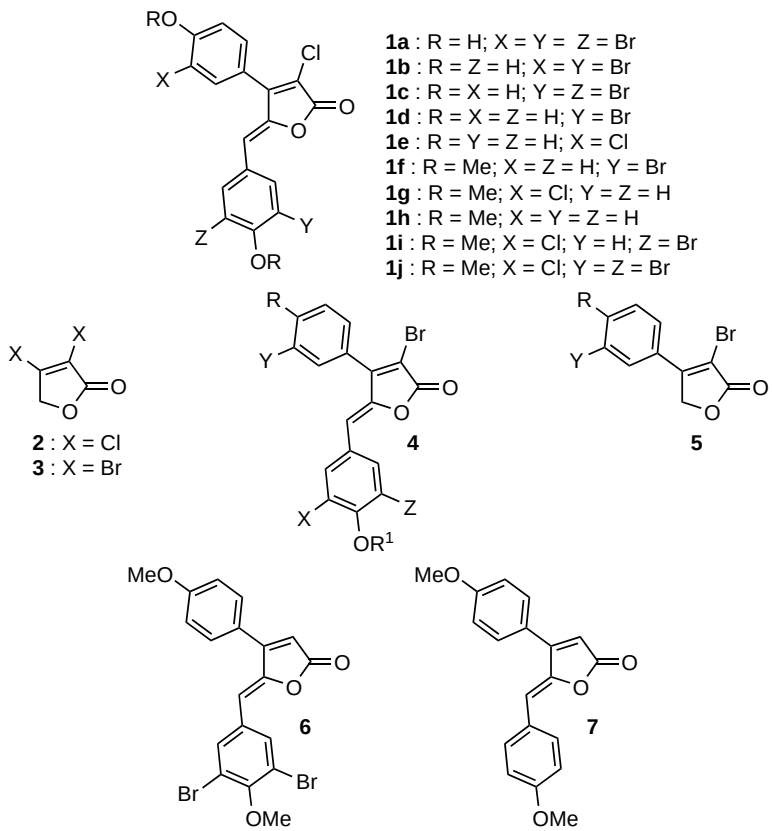
Abstract—Two protocols have been developed for the Pd-catalyzed regioselective synthesis of 4-aryl-3-chloro-2(5*H*)-furanones starting from 3,4-dichloro-2(5*H*)-furanone. These monochloro derivatives have then been used as precursors to (*Z*)-4-aryl-5-[1-(aryl)methylidene]-3-chloro-2(5*H*)-furanones including naturally-occurring rubrolide M.

Recently, four new (*Z*)-4-aryl-5-[1-(aryl)methylidene]-3-chloro-2(5*H*)-furanones, i.e. rubrolides I (**1a**), K (**1b**), L (**1c**) and M (**1d**), have been isolated from the red colonial tunicate *Synoicum blochmanni*.[1]

All these substances have been found to display significant cytotoxicities against four cancer cell lines, i.e. P-388 suspension culture of mouse limphoid carcinoma, the monolayer cultures of human lung carcinoma (A-



* Corresponding authors. Tel.: +39 50 918214; fax: +39 50 918260; e-mail: rossi@dcci.unipi.it

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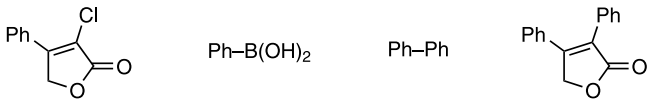
549), human colon carcinoma (HT-29) and human melanoma (MEL-28), but rubrolide M (**1d**) proved to be the most active compound.[1] To the best of our knowledge, no synthetic route towards compounds **1a–d** has been reported to date.

As part of our program aimed at developing concise and efficient protocols for the selective synthesis of natural and unnatural substances, which are potentially cytotoxic against human tumor cell lines, we decided to develop a general procedure for the synthesis of rubolide M (**1d**) and its congeners, such as compounds **1e–j**, and to evaluate the cytotoxic activities of these substances. It occurred to us that access to these compounds might be achieved starting from readily available 3,4-dichloro-2(5*H*)-furanone (**2**)[2] by a protocol similar to that, recently used successfully to prepare a variety of (*Z*)-4-aryl-5-[1-(aryl)methylidene]-3-bromo-2(5*H*)-furanones **4**[3] including the compound with the structure corresponding to that reported for naturally-occurring rubrolide N (**4a**) (R = OH; X = Br; Y = Cl; $R^1 = Z = H$)[1,4] starting from 3,4-dibromo-2(5*H*)-furanone (**3**).[5] This protocol involved the regioselective arylation of **3** at the bromine-bearing carbon atom 4 of this dibromo derivative followed by the appendage of a 1-(aryl)methylidene unit at C-5 of the resulting 4-aryl-3-bromo-2(5*H*)-furanone **5** by a furanolate chemistry similar to that previously used to synthesize the dimethyl ethers **6** and **7** corresponding to rubrolides C and E.[6]

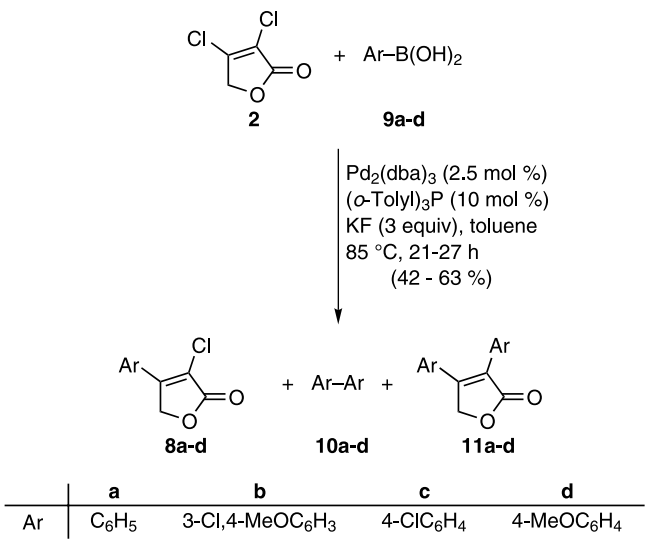
However, the selective synthesis of 4-aryl-3-chloro-2(5*H*)-furanones **8** starting from **2** proved to be problematic. In fact, in a preliminary attempt to prepare regioselectively 3-chloro-4-phenyl-2(5*H*)-furanone (**5a**) by reaction of **2** with 1.1 equiv. of phenylboronic acid (**9a**) under the conditions commonly employed for monoarylation of **3**,[3] i.e. in THF at 65°C in the presence of 5 mol% $PdCl_2(MeCN)_2$, 20 mol% $AsPh_3$ and 3.0 equiv. of Ag_2O, we found that the conversion of the reaction was very low (ca. 10%) and that the major reaction product was biphenyl (**10a**) derived from homocoupling of **9a**. Interestingly, **10a** was the major reaction product also when **2** was reacted with 1.1 equiv. of **9a** either in methanol at 40–60°C in the presence of 1.5 equiv. of AcONa and 3 mol% $PdCl_2(PPh_3)_2$ or in toluene at 80°C in the presence of 3.0 equiv. of Ag_2O and a catalyst system consisting of 2.5 mol% $Pd_2(dba)_3$ and 5 mol% *tert*-Bu_3P.[7] Nevertheless, we found that the formation of **10a** could be minimized by treatment of **2** with 1.05 equiv. of **9a** in toluene at 80°C in the presence of 3.0 equiv. of KF, 2.5 mol% $Pd_2(dba)_3$ and 5 mol% *tert*-Bu_3P. Under these conditions the conversion of the reaction was almost quantitative, but **8a** was obtained in a comparable amount with 3,4-diphenyl-2(5*H*)-furanone (**11a**) (52 and 41% yield, respectively). On the other hand, the use either of electron-rich phosphine ligands such as Cy_3P or (*o*-biphenyl)P(*tert*-Bu)$_2$ in place of *tert*-Bu_3P or of a base such as Cs_2CO_3 or CsF in place of KF did not allow to increase either the regioselectivity of the Pd-catalyzed reaction between **2** and **9a** or the yield of **8a**.



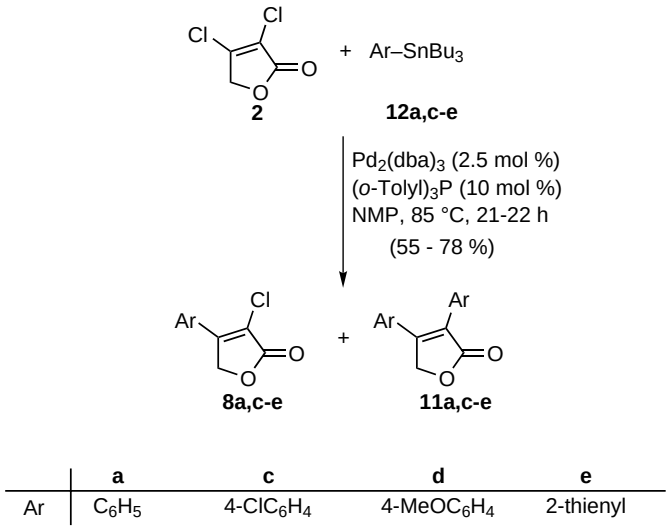
At last we found that either the **8a**:**11a** molar ratio or the yield of **8a** could be increased when the reaction between **2** and 1.05 equiv. of **9a** was performed in toluene at 85°C for 24 h in the presence of 2.5 mol% $Pd_2(dba)_3$, 10 mol% (*o*-Tolyl)$_3$P and 3.0 equiv. of KF (Scheme 1). In fact, the crude mixture derived from this reaction contained **8a** contaminated by less than 15 and 7% of **10a** and **11a**, respectively. On the other hand, purification of this crude mixture by MPLC on silica gel allowed to obtain pure **8a** in 63% yield. These reaction conditions were then used to prepare compounds **8b**, **8c** and **8d** in 57, 42 and 61% yields, respectively (Scheme 1).

It should be mentioned that similarly to what observed in the preparation of **8a**, the crude reaction mixtures corresponding to the preparations of compounds **8b**, **8c** and **8d** proved to be contaminated by less than 7% of the corresponding 3,4-diaryl-2(5*H*)-furanones **11b**, **11c** and **11d**, respectively, and by less than 15% of the biaryls **10b–d** derived from homocoupling of the arylboronic acids **9b–d**.

Even though these results were satisfactory, we searched for a new and more efficient route to compounds **8** starting from dichloride **2**. Thus, we found that compounds **8** could be alternatively synthesized by treatment of **2** with 1.1 equiv. of aryl(tributyl)stannanes **12** in NMP at 85°C for 21–22 h in the presence of the catalyst system consisting of 2.5 mol% $Pd_2(dba)_3$ and 10 mol% (*o*-Tolyl)$_3$P. This protocol allowed the preparation of compounds **8a**, **8c**, **8d** and **8e** in 53, 61, 78 and 55% yields, respectively (Scheme 2). Notably, the crude mixtures which derived from these cross-coupling reac-



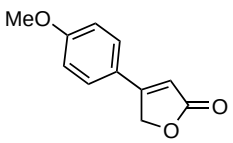
Scheme 1.



Scheme 2.

tions proved to be contaminated by less than 5% of compounds **11**.

Having set up two different protocols for the regioselective synthesis of compounds **8** we then established the feasibility of using these 2(5*H*)-furanone derivatives in the preparation of a precursor to rubrolide M, i.e. compound **1f**, and for its structure of general formula **1**, which are characterized by methoxyaryl moieties, by application of a procedure similar to that recently used to prepare dimethyl ethers **6** and **7** corresponding to rubrolides C and E, respectively, starting from 4-(4-methoxyphenyl)-2(5*H*)-furanone (**13**).[6]



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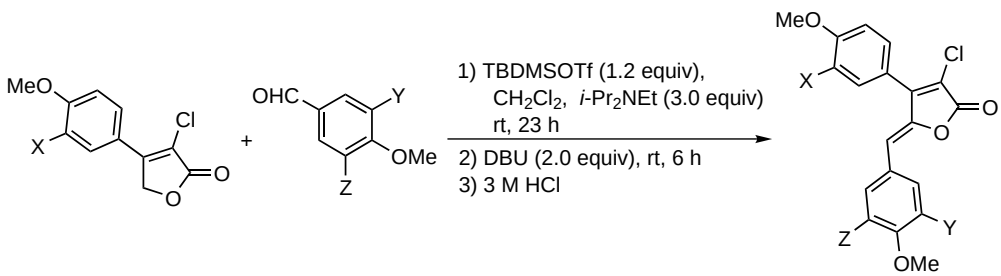
Thus, in this one-pot protocol a CH_2Cl_2 solution of a compound **8** was treated with 1.2 equiv. of *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBDMSOTf), 3.0 equiv. of *i*-Pr_2NEt and 1.0 equiv. of an arylaldehyde **14**. After stirring for 23 h at room temperature the reaction mixture was treated with 2.0 equiv. of DBU at 20°C for 6 h and then with 3 M HCl to give stereoselectively the required compound **1** (Scheme 3).

This procedure was employed to prepare compounds **1f** and **1i** in 46 and 43% yields starting from aldehyde **14a** and the 4-aryl-3-chloro-2(5*H*)-furanones **8d** and **8b**, respectively, and compounds **1h** and **1g** in 65 and 51% yields starting from aldehyde **14b** and the 4-aryl-3-chloro-2(5*H*)-furanones **8d** and **8b**, respectively.[8] However, an attempt to prepare **1j** starting from **14c** and **8b** was fruitless, the desired product being obtained in a negligible yield. On the other hand, another attempt to synthesize **1j** using a procedure very similar to that previously employed to prepare (*Z*)-21-benzylidene-3β-(*tert*-butyldimethylsilyl-oxy)-14-trimethylsilyloxy-5β,14β-card-20(22)-enolide from 3β-(*tert*-butyldimethylsilyloxy)-14-trimethylsilyloxy-5β,14β-card-20(22)-enolide,[9] i.e. by treatment of **8b** with less than 1.0 equiv. of NaH in NMP at 20°C for 0.5 h followed by addition of **14c**, was also unsuccessful. In fact, no desired compound **1j** was obtained under these reaction conditions and only the loss of **8b** was observed.

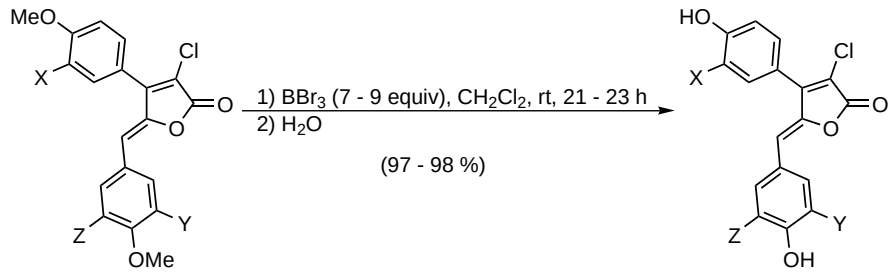
We then completed the first total synthesis of rubrolide M (**1d**) by reaction of **1f** with 9.0 equiv. of BBr_3 in CH_2Cl_2 at room temperature for 23 h followed by hydrolysis. This demethylation provided in 97% yield, compound **1d** having spectral properties in good agreement with those reported for the natural product[1] (Scheme 4). Under similar reaction conditions **1g** gave compound **1e** in 98% yield (Scheme 4)[10].

Finally, compounds **1d**–**i** were evaluated in the NCI 3-cell line, one dose primary anticancer assay at a 1.00×10^{-4} M concentration. This 3-cell line panel consisted of the MCF-7 (breast), NCI-H460 (lung), and SF-268 (CNS). The results for each test agent are reported in Table 1 as the percent of growth of the treated cells when compared to the untreated control cells.



8d : X = H **14a** : Y = Br; Z = H **1f** : X = Z = H; Y = Br
8b : X = Cl **14b** : Y = Z = H **1g** : X = Cl; Y = Z = H
8d : X = H **14b** : Y = Z = H **1h** : X = Y = Z = H
8b : X = Cl **14a** : Y = H; Z = Br **1i** : X = Cl; Y = H; Z = Br
8b : X = Cl **14c** : Y = Z = Br **1j** : X = Cl; Y = Z = Br

Scheme 3.



1f : X = Z = H; Y = Br
1g : X = Cl; Y = Z = H

1d : X = Z = H; Y = Br
1e : X = Cl; Y = Z = H

Scheme 4.

Table 1. Results of the cytotoxicity tests for compounds **1d–i** in the NCI 3-cell line, one close primary anticancer assay[a]

Entry	Compound	Growth percentage			Activity
		NCI-H460 (lung)	MCF-7 (breast)	SF-268 (CNS)	
1	**1d**	1	0	0	Active
2	**1e**	0	0	1	Active
3	**1f**	67	88	97	Inactive
4	**1g**	10	20	20	Active
5	**1h**	15	89	32	Active
6	**1i**	65	62	3	Active

[a] In the protocol used, each line was inoculated and preincubated for 48 h on a microtiter plate. Test agents were then added at a 1.00×10^{-4} M concentration in DMSO and the culture incubated for 48 h. End-point determinations were made with alamar blue.

As shown in Table 1, compounds **1d**, **1e** and **1g** proved to be significantly cytotoxic (entries 1, 2 and 4), even though the cytotoxicity values for **1d** and **1e** were higher than those for **1g**. On the other hand, compound **1f** was found to be inactive and compounds **1h** and **1i** exhibited limited cytotoxicity. In fact, **1h** reduced the growth only of the SF-268 and the MCF-7 cell lines to 32% or less (entry 5) and compound **1i** reduced significantly (to 3%) the growth only of the SF-268 cell line (entry 6). Thus, these data seem to suggest that, as previously observed for (*Z*)-4-aryl-5-[1-(aryl)-methylidene]-3-bromo-2(5*H*)-furanones,[3] the presence of phenol subunits is necessary in order for (*Z*)-4-aryl-5-[1-(aryl)methylidene]-3-chloro-2(5*H*)-furanones to exhibit high cytotoxicities. These data also show that (*Z*)-4-aryl-5-[1-(aryl)methylidene]-3-chloro-2(5*H*)-furanones containing methoxyaryl subunits are less potent than the corresponding substances characterized by phenol subunits.

In conclusion, we have developed two protocols for the Pd-catalyzed regioselective synthesis of 4-aryl-3-chloro-2(5*H*)-furanones. We have also shown that these monochloro derivatives represent useful precursors to (*Z*)-4-aryl-5-[1-(aryl)methylidene]-3-chloro-2(5*H*)-furanones including naturally-occurring rubrolide M. Interestingly, some of these last compounds including rubrolide M have been found to be active in the NCI 3-cell line, one dose primary anticancer assay.

Acknowledgements

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(b) Littke, A. F.; Dai, C.; Fu, G. C. *J. Am. Chem. Soc.* **2000**, *122*, 4020–4028; (c) Littke, A. F.; Fu, G. C. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 2411–2413; (d) Wolfe, J. P.; Tomori, H.; Sadighi, J. P.; Yin, J.; Buchwald, S. L. *J. Org. Chem.* **2000**, *65*, 1158–1174.

8. All new compounds were obtained in analytically pure form. Selected physical and spectral properties of compounds **1f–i** are as follows. Compound **1f**: mp 195–196°C. IR (KBr): *v* 1769, 1607, 1498, 1267, 859 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 7.91 (1H, d, $J=2.2$ Hz), 7.77 (1H, dd, $J=8.8$ and 2.2 Hz), 7.49 (2H, m), 7.07 (2H, m), 6.90 (1H, d, $J=8.8$ Hz), 6.04 (1H, s), 3.94 (3H, s), 3.90 ppm (3H, s). ^{13}C NMR (50.3 MHz, $CDCl_3$): δ 164.46, 161.29, 156.54, 149.43, 145.57, 135.39, 131.26, 130.57 (2C), 126.65, 120.00, 117.24, 114.41 (2C), 112.75, 112.00, 111.79, 56.32, 55.43 ppm. Compound **1g**: mp 148–150°C. IR (KBr): *v* 1764, 1605, 1498, 1273, 824 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 7.73 (2H, m), 7.56 (1H, d, $J=2.2$ Hz), 7.43 (1H, dd, $J=8.4$ and 2.2 Hz), 7.10 (1H, d, $J=8.4$ Hz), 6.91 (2H, m), 6.08 (1H, s), 4.00 (3H, s), 3.85 ppm (3H, s). ^{13}C NMR (50.3 MHz, $CDCl_3$): δ 164.44, 160.70, 156.50, 148.26, 144.46, 132.63 (2C), 130.69, 128.89, 125.23, 123.20, 121.01, 117.28, 114.59, 114.37 (2C), 112.11, 56.32, 55.36 ppm. Compound **1h**: mp 169–170°C. IR (KBr): *v* 1772, 1605, 1504, 1181, 826 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 7.73 (2H, m), 7.49 (2H, m), 7.07 (2H, m), 6.90 (2H, m), 6.12 (1H, s), 3.90 (3H, s), 3.84 ppm (3H, s). ^{13}C NMR (50.3 MHz, $CDCl_3$): δ 164.77, 161.11, 160.53, 149.57, 144.80, 132.53 (2C), 130.60 (2C), 125.40, 120.26, 116.53, 114.48, 114.33 (4C), 55.41, 55.32 ppm. Compound **1i**: mp 215–217°C. IR (KBr): *v* 1784, 1606, 1499, 1271, 818 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 7.92 (1H, d, $J=1.8$ Hz), 7.77 (1H, dd, $J=8.6$ and 1.8 Hz), 7.56 (1H, d, $J=8.8$ Hz), 7.42 (1H, dd, $J=8.7$ and 2.2 Hz), 7.10 (1H, d, $J=8.7$ Hz), 6.91 (1H, d, $J=8.8$ Hz), 6.01 (1H, s), 4.00 (3H, s), 3.94 ppm (3H, s). ^{13}C NMR (50.3 MHz, $CDCl_3$): δ 164.09, 156.70, 156.61, 148.11, 145.23, 135.48, 131.36, 130.66, 128.85, 126.46, 123.31, 120.74, 118.06, 112.82, 112.13 (2C), 111.83, 56.33 ppm (2C).

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10. Selected physical and spectral properties of compounds **1d** and **1e** are as follows. Compound **1d**: mp 227–230°C. IR (KBr): *v* 3473, 1750, 1611, 1418, 1288, 1163, 837 cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6): δ 9.29 (2H, br s), 8.06 (1H, d, $J=2.2$ Hz), 7.74 (1H, dd, $J=8.8$ and 2.2 Hz), 7.53 (2H, m), 7.06 (3H, m), 6.29 ppm (1H, s). ^{13}C NMR (50.3 MHz, DMSO-d_6): δ 164.62, 160.32, 155.88, 150.84, 146.27, 136.21, 132.35, 131.83 (2C), 127.33, 119.77, 117.47, 116.88, 116.65 (2C), 113.42, 110.74 ppm. Compound **1e**: mp 266–270°C. IR (KBr): *v* 3418, 1728, 1603, 1444, 1282, 1163, 829 cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6): δ 10.98 (1H, br s), 10.15 (1H, br s), 7.68 (2H, m), 7.58 (1H, d, $J=1.8$ Hz), 7.39 (1H, dd, $J=8.4$ and 1.8 Hz), 7.18 (1H, d, $J=8.4$ Hz), 6.89 (2H, m), 6.24 ppm (1H s). ^{13}C NMR (50.3 MHz, DMSO-d_6): δ 163.77, 158.94, 154.93, 148.58, 143.35, 132.71 (2C), 130.42, 129.23, 123.54, 120.07, 119.01, 116.78, 115.82 (2C), 115.10, 114.72 ppm.