Accepted Manuscript

Design, synthesis and anticancer properties of 5-arylbenzoxepins as conformationally restricted *iso*Combretastatin A-4 analogues

Evelia Rasolofonjatovo, Olivier Provot, Abdallah Hamze, Jordi Rodrigo, Jérome Bignon, Joanna Wdzieczak-Bakala, Christine Lenoir, Déborah Desravines, Joëlle Dubois, Jean-Daniel Brion, Mouad Alami

PII: S0223-5234(12)00764-7

DOI: 10.1016/j.ejmech.2012.12.042

Reference: EJMECH 5913

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 25 October 2012

Revised Date: 18 December 2012

Accepted Date: 23 December 2012

Please cite this article as: E. Rasolofonjatovo, O. Provot, A. Hamze, J. Rodrigo, J. Bignon, J. Wdzieczak-Bakala, C. Lenoir, D. Desravines, J. Dubois, J.-D. Brion, M. Alami, Design, synthesis and anticancer properties of 5-arylbenzoxepins as conformationally restricted *iso*Combretastatin A-4 analogues, *European Journal of Medicinal Chemistry* (2013), doi: 10.1016/j.ejmech.2012.12.042.

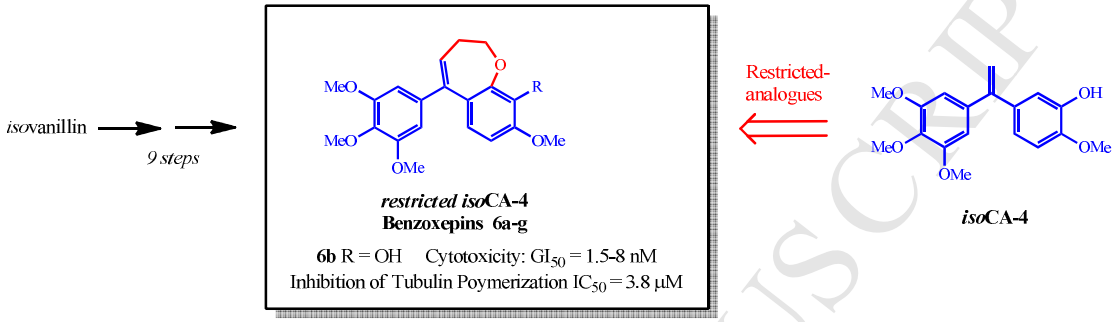
This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

Graphical abstract

Design, synthesis and anticancer properties of 5-arylbenzoxepins as conformationally restricted *iso*combretastatin A-4 analogues.

Evelia Rasolofonjatovo,[a] Olivier Provot,*[a] Abdallah Hamze,[a] Jordi Rodrigo,[a] Jérome Bignon,[b] Joanna Wdzieczak-Bakala,[b] Christine Lenoir,[b] Déborah Desravines,[b] Joëlle Dubois,[b] Jean-Daniel Brion,[a] Mouad Alami*[a]



ACCEPTED MANUSCRIPT

Design, synthesis and anticancer properties of 5-arylbenzoxepins as conformationally restricted *iso*Combretastatin A-4 analogues

Evelia Rasolofonjatovo,[a] Olivier Provot,*[a] Abdallah Hamze,[a] Jordi Rodrigo,[a] Jérome Bignon,[b] Joanna Wdzieczak-Bakala,[b] Christine Lenoir,[b] Déborah Desravines,[b] Joëlle Dubois,[b] Jean-Daniel Brion,[a] Mouad Alami*[a]

[a] *Univ. Paris-Sud, CNRS, BioCIS-UMR 8076, LabEx LERMIT, Laboratoire de Chimie Thérapeutique, Faculté de Pharmacie, 5 rue J.-B. Clément, Châtenay-Malabry, F-92296, France.*

[b] *Institut de Chimie des Substances Naturelles, UPR 2301, CNRS avenue de la terrasse, F-91198 Gif sur Yvette, France.*

[*] *Corresponding authors. Tel.: +33 1 46 83 58 47. Fax: +33 1 46 83 58 28. Email: olivier.provot@u-psud.fr (O.P.) or mouad.alami@u-psud.fr (M.A.).*

Abstract

A series of novel benzoxepins **6** was designed and prepared as rigid-*iso*CA-4 analogues according to a convergent strategy using the coupling of *N*-tosylhydrazones with aryl iodides under palladium catalysis. The most potent compound **6b**, having the greatest resemblance to CA-4 and *iso*CA-4 displayed antiproliferative activity at nanomolar concentrations against various cancer cell lines and inhibited tubulin assembly at a micromolar range. In addition, benzoxepin **6b** led to the arrest of HCT116, K562, H1299 and MDA-MB231 cancer cell lines in the G_2/M phase of the cell cycle, and strongly induced apoptosis at low concentrations. Docking studies demonstrated that benzoxepin **6b** adopt an orientation similar to that of *iso*CA-4 at the colchicine binding site on β-tubulin.

Keywords: Benzoxepin, Combretastatin, *iso*CA-4, Tubulin, Cytotoxicity, Apoptosis, Cancer

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

1. Introduction

Combretastatin A-4 (CA-4, Figure 1), a natural stilbene isolated by Pettit[1] from the South African tree *Combretum caffrum*, is potently cytotoxic against a variety of cancer cells, including multidrug-resistant cell lines.[2,3] CA-4, which binds on β-tubulin at the colchicine binding site, is also an exceptionally strong inhibitor of tubulin polymerization with an IC_{50} value of 1.0 μM.[4] Disruption of tubulin assembly results in rapid tumor endothelial cell damage followed by neovascular shutdown, and subsequent hemorrhagic necrosis.[5] A great interest in CA-4 is reflected in the fact that the water soluble phosphate prodrug CA-4P (fosbretabulin, **1b**)[6,7] is currently used for advanced anaplastic thyroid carcinoma, even if some vascular side effects were reported.[8,9] Despite its remarkable anticancer activity, CA-4 isomerizes into its inactive (*E*)-isomer[10] during storage, administration[11] and metabolism[12] leading to a significant loss of potency. The poor bioavailability and solubility in biological media associated with the *Z* to *E*-isomerization drawback,[11] together with the structural simplicity of CA-4, makes it very interesting from a medicinal chemistry point of view, thus stimulating the search for new and more potent compounds with improved pharmacological properties[13,14] Considerable effort has been gone into modifying the isomerizable *cis* carbon-carbon double bond. Several reviews outlined this vast array of chemistry focusing on the stabilization of the two aryl rings of CA-4 using one to three atom bridgeheads.[15-20]

<Figure 1>

Figure 1. Representative inhibitors of tubulin polymerization and rational drug design from CA-4 and *iso*CA-4 to dihydrobenzoxepin analogues **6**.

In our efforts to discover novel CA-4 analogues having non-isomerizable linkers between the A- and B-rings,[21-26], we recently synthesized a series of 1,1-diarylethylene derivatives[27-29] with general structure **2**. The most active molecules in this series, *iso*CA-4 (**2a**), *iso*NH_2CA-4 (**2b**), *iso*FCA-4 (**2c**) and **2d** displayed a nanomolar level of cytotoxicity against various cancer cell lines, inhibited tubulin polymerization (ITP) at a micromolar level, and arrested cancer cells in the G_2/M phase of the cell cycle (Figure 1).[30-33] These 1,1-diarylethylene compounds **2** were found to be as active as their (*Z*)-1,2-ethylene isomers (CA-4 **1a**, NH_2CA-4 **1c**, FCA-4 **1d**), clearly demonstrating that it is possible to replace the (*Z*)-double bond of CA-4 derivatives with a 1,1-ethylene unit with no loss of efficacy.

*Iso*CA-4 was utilized as a starting point to design cyclic and heterocyclic derivatives **3**-**5** with restricted rotation to identify novel compounds with improved anticancer activities. Inserting the double bond of *iso*CA-4 into 6-membered rings led to (dihydro)naphtalene **3**[34-36] and chromene **4**[37,38] derivatives endowed with interesting anticancer activities (Figure 1). In 2008, Pinney reported the synthesis of benzosuberenes of type **5**[39,40] as highly cytotoxic agents, demonstrating that the double bond in *iso*CA-4 could be successfully included in a 7-membered ring to maintain potent activity against various cancer cell lines. In continuation of our earlier work, a novel series of restricted *iso*CA-4 derivatives **6** containing a 2,3-dihydrobenzoxepin ring was designed. Herein we report the synthesis, the biological evaluation and the possible binding mode of 5-aryldihydrobenzo[*b*]oxepins **6**.

2. Results and discussion

2.1. Chemistry.

The retrosynthetic analysis of the target 5-arylbenzoxepins **6** is outlined in Figure 2. We envisionned that the 5-aryl moiety in **6** could be installed through palladium catalyzed coupling reactions starting from (*i*) vinylstannanes **7** (Stille coupling, path *a*), (*ii*) vinyl iodides **8** (Suzuki coupling, path *a*), or (*iii*) *N*-tosylhydrazones **11** (path *b*) using a recent methodology developed by Barluenga and us[41,42] (Figure 2). To build up the 7-membered heterocyclic ring of compounds **7** and **8**, a ring-closing metathesis (RCM) reaction was planned as the key step from the corresponding diene derivatives **9, 10** and **13**, respectively. An alternative route to **11** could be an intramolecular Mitsunobu reaction starting from phenol **12**.

ACCEPTED MANUSCRIPT

<Figure 2>

Figure 2. Retrosynthetic analysis of 5-aryldihydrobenzo[*b*]oxepins **6**.

At the outset of this work, and according to path *a* (Scheme 1), we first studied the synthesis of diene **9a** ($R^1 = R^2 = H$) through regioselective addition of Bu_3SnH on the alkyne triple bond[43] of terminal arylalkyne **14**.[44] As expected, we were pleased to observe a total regioselectivity in the palladium-catalyzed hydrostannylation of **14** leading exclusively to α-vinylstannane **9a** in a 80% yield. Iododestannylation of **9a** with molecular iodine in CH_2Cl_2 at room temperature afforded **10a** ($R^1 = R^2 = H$) in a modest and non-optimized yield of 45%. Unfortunately, despite attempting a plethora of RCM conditions using the Grubbs I, Grubbs II, and Hoveyda-Grubbs catalysts (from 2% to 20%) in CH_2Cl_2, toluene at different temperatures (from 20 °C to reflux) and various concentrations, no macrocyclization of dienes **9a** and **10a** was ever observed.

<Scheme 1>

Scheme 1. Synthesis of vinylstannane **9a** and vinyliodide **10a**. Reagent and conditions: a) $PdCl_2(PPh_3)_2$ (2 mol%), Bu_3SnH (1.2 equiv), THF, 20 °C; b) I_2, CH_2Cl_2, 20 °C.

The synthesis of benzoxepin derivatives **6** was next envisioned according to *path b* involving an intramolecular Mitsunobu reaction (Scheme 2). Initially, the compounds targeted **6b** and **6c** contain on the benzoxepin nucleus 9-hydroxy and 8-methoxy substituents which are present in the *iso*CA-4 B-ring, and that were proved to be essential for bioactivity (Scheme 2).

<Scheme 2>

Scheme 2. Synthesis of dihydrobenzoxepins **6b,c**. Reagent and conditions: a) MOMCl (2 equiv), *i*Pr_2NH (2 equiv), CH_2Cl_2; b) But-3-yn-1-ol (1.1 equiv), $PdCl_2(PPh_3)_2$ (8 mol%), CuI (15 mol%), Et_3N; c) PTSA (1 equiv), H_2O, 100 °C, sealed tube; d) DEAD (1.5 equiv), PPh_3 (1.5 equiv), THF; e) $TsNHNH_2$ (2.5 equiv), PTSA (20 mol%), EtOH reflux; f) Pd_2dba_3 (10 mol%), XPhos (20 mol%), *t*BuOLi (2 equiv), ArI (1 equiv), 90 °C, sealed tube; g) $AlCl_3$ (5 equiv), CH_2Cl_2.

Arylalkynol **17**[45] was prepared from iodophenol **15** through a sequence of MOM-ether protection followed by a Sonogashira-Linstrumelle coupling reaction[46,47] with homopropargylic alcohol. The triple bond of **17** was regioselectively hydrated in the presence of PTSA[48-52] in water, and a subsequent intramolecular Mitsonobu reaction furnished the required benzoxepan-5-one **19** in a 63% yield. This latter was mixed with $TsNHNH_2$ in the presence of PTSA to afford the key intermediate *N*-tosylhydrazone **11a**, which in turn was coupled with various aryl halides under Pd_2dba_3/XPhos as the catalytic system.[41,53] Accordingly, trimethoxyphenylbenzoxepin **6a** was formed in an excellent 98% yield.[54,55] Finally, the phenolic part of isopropylether **6a** was revealed by treatment with $AlCl_3$ to give **6b** but in a low 15% yield. Target molecule **6c**, with a 3,5-dimethoxyphenyl nucleus as A-ring, was also prepared from *N*-tosylhydrazone **11a,** and the resulting crude coupling product treated with $AlCl_3$ in CH_2Cl_2 furnished the desired benzoxepin derivative **6c** in a modest 25% yield.

For benzoxepin derivatives **6d-g** having different substituents on C9, we envisioned their synthesis *via* a convergent approach starting from *N*-tosylhydrazone **11b** having a bromine atom at the C9 position, useful for subsequent functionalizations under palladium catalysis (Scheme 3)[32,36,56]. To this end, we planned the construction of the 7-membered ring of **11b** using RCM of diene **13a**. Briefly, benzaldehyde **21** was prepared from *iso*vanillin through a sequence of regioselective *N*-methylpyrrolidin-2-one hydrotribromide (MPHT) bromination[57,58] and allyl ether formation. Further reaction with vinylmagnesium chloride afforded diene **13a**, which underwent ring-closing olefin metathesis using the Grubbs II catalyst in CH_2Cl_2.[59] The resulting dihydrobenzoxepin-5-ol **22** was subjected to a double bond reduction, PCC alcohol oxidation and then *N*-tosylhydrazone formation to give **11b**. Further palladium-catalyzed coupling reaction with 3,4,5-trimethoxyiodobenzene furnished the key 9-bromo-8-methoxybenzoxepin **6d** in a 68% yield. Having achieved efficient access to **6d**, we then turned studying various means of substitution on the C9-bromo atom. The bromo substituent of **6d** was first exploited in a coupling reaction using sodium azide as the amino source in the presence of a catalytic amount of CuI to give **6e** (75%).[60,61] Debromination of **6d** was achieved using catalytic amounts of $Pd(OAc)_2$, PPh_3 and K_2CO_3 in hot *n*BuOH and produced **6f** in 69% yield.[62] For the synthesis of **6g** having a

ACCEPTED MANUSCRIPT

butyn-1-ol chain, **6d** was coupled with but-3-yn-1-ol using $PdCl_2(PPh_3)_2$ and CuI catalysts under microwave irradiation at 120 °C in DMF (74%). Noteworthy that compound **6b** was also prepared in an acceptable yield of 55% by treating **6d** with KOH in the presence of Pd_2dba_3 and *t*BuXPhos in a dioxane-H_2O mixture according to a Buchwald protocol.[63] Finally, because the double bond present in *iso*CA-4 can be reduced to furnish *iso*erianin derivatives with no loss of anticancer properties,[23] we next focused our attention on the catalytic reduction of the carbon-carbon double bond of **6b** and **6g**. Thus, 5-aryltetrahydrobenzoxepins **25a** and **25b** were obtained in non-optimized yields using H_2 in the presence of Pd/C in MeOH (Scheme 3).

<Scheme 3>

Scheme 3. Synthesis of dihydrobenzoxepins **6b**, **6d-g** and tetrahydrobenzoxepins **25a,b**. Reagent and conditions: a) $Hg(OAc)_2$ (1 equiv), NaBr (1 equiv), 50 °C, EtOH; b) MPHT (1 equiv), CH_3CN; c) 3-bromoprop-1-ene (1.2 equiv), K_2CO_3 (1.2 equiv), CH_3CN; d) vinylmagnesium chloride (1.2 equiv), THF; e) Grubbs II catalyst, 5 mol%, CH_2Cl_2, 20 °C; f) H_2, Pd/C, MeOH, AcOH cat.; g) PCC, CH_2Cl_2; h) $TsNHNH_2$ (1 equiv), PTSA (10 mol%), $MgSO_4$ (1 equiv), EtOH, reflux; i) Pd_2dba_3 (10 mol%), XPhos (40 mol%), *t*BuOLi (2 equiv), 3,4,5-trimethoxyiodobenzene (1.2 equiv), 90 °C, sealed tube; j) Pd_2dba_3 (5 mol%), *t*BuXPhos (15 mol%), KOH (3 equiv), dioxane-H_2O, 90 °C, sealed tube; k) CuI (10 mol%), TMEDA (6 mol%), NaN_3 (2 equiv), potassium ascorbate (6 mol%) DMSO-H_2O, 90 °C, sealed tube; l) $Pd(OAc)_2$ (1 mol%), PPh_3 (2.5 mol%), *n*BuOH, 100 °C, sealed tube; m) *n*But-3-yn-1-ol (1.2 equiv), $PdCl_2(PPh_3)_2$ (5 mol%), PPh_3 (15 mol%), CuI (15 mol%), Et_2NH (2 equiv), DMF, 120 °C, MWI.

2.2. Biological results.

2.2.1 In vitro cell growth assay

All the synthesized compounds were tested in a preliminary cytotoxic assay on a human colon carcinoma (HCT116) cell line using CA-4,[64] and *iso*CA-4 as reference compounds.

<Table 1>

Table 1. Cytotoxic activity of dihydrobenzoxepins **6b-g** and tetrahydrobenzoxepins **25a,b** against HCT116[a]

In this assay, the benzoxepin derivatives **6** inhibited cellular growth to a varying degree. Except tetrahydrobenzoxepin **25b**, having a bulky butanol chain on C9 which was found inactive against HCT116 cells, all selected compounds displayed a nanomolar level of cytotoxicity. Highest GI_{50} values were found for both bromine derivative (**6d**, GI_{50} = 170 nM) and compound **6g** having a butyn-ol chain on the C9 position (**6g**, GI_{50} = 250 nM) (Table 1). These functional groups on C9 were bulky, so next we examined smaller substituents on the C9 position. The C9-OH benzoxepin derivative **6b**, having the greatest resemblance to *iso*CA-4 is undoubtedly the most cytotoxic compound in this series with a GI_{50} value of 1.5 nM slightly inferior to those of *iso*CA-4 (GI_{50} = 3 nM) and CA-4 (GI_{50} = 2 nM). Contrary to Ohsumi's finding,[65] it appears that the introduction of a NH_2 substituent on C9 result in weaker cytotoxicity (**6e**, GI_{50} = 22 nM). Compound **6f**, with no substitution on the C9 position showed a significative loss of activity with a GI_{50} value of 85 nM. Concerning the A-ring, it seems that a 3,4,5-trimethoxyphenyl ring is required for strong activity, as compound **6c** (GI_{50} = 25 nM) with only two MeO-substituents on the A-ring exhibited a lowest cytotoxicity in comparison with **6b**. Evaluation of tetrahydro-benzoxepin **25a** which was found to elicit a nanomolar level of cytotoxicity (GI_{50} = 20 nM) demonstrated that the presence of the double bond in dihydrobenzoxepin compounds **6** was important but not essential for antiproliferative property as we previously showed by reducing the double bond of *iso*CA-4.[23]

2.2.2 Cytotoxicity and Inhibition of Tubulin Polymerization for Selected Compounds

To further characterize the cytotoxicity profiles of these compounds, we investigated the effect of the more cytotoxic benzoxepins **6b**, **6c**, **6e**, **6f**, and **25a** (GI_{50}< 85 nM) on the proliferation of three human tumor cell lines: myelogenous leukemia (K562), non-small cell lung carcinoma (H1299) and hormone-independent breast cancer (MDA-MB231).

<Table 2>

Table 2. Cytotoxic activity and inhibition of tubulin polymerization of selected compounds

The results depicted in Table 2 revealed that all selected compounds which retain a high level of cytotoxicity against HCT116 were also strongly cytotoxic with no significant disparity against K562, H1299 and MDA-MB231 cancer cell lines. Again, benzoxepin **6b** was the most active compound inhibiting the growth of K562, H1299 and MDA-MB cancer cell lines with GI_{50} values inferior ranging from 1.5 to 8 nM comparable to that of *iso*CA-4 (GI_{50} = 3-5 nM), thus clearly indicating an antiproliferative activity regardless of the origin of the tumor cells. To confirm that the antiproliferative activities of these derivatives, like those of CA-4 and *iso*CA-4, were related to an interaction with the microtubule system, benzoxepins **6b**,**c**, **6e**,**f** and **25a** were evaluated for their inhibitory effects on tubulin assembly (Table 2). As expected, all tested compounds strongly inhibited tubulin assembly with comparable IC_{50} values of 3.2-3.9 μM, which were about two times greater than that of *iso*CA-4 (IC_{50} = 1.5 μM). Consistent with earlier observations[66], the replacement of the 4-methoxy group in **6b** (CI_{50} = 3.8 μM) with an hydrogen atom in **6c** (CI_{50} = 3.2 μM) had no significant impact on tubulin assembly but reduced cytotoxicity by approximately 10 orders of magnitude.

2.2.3 Cell Cycle Analysis and Apoptosis

Because molecules exhibiting activity on tubulin should cause the alteration of cell cycle parameters leading to a preferential G_2/M blockade,[67] the effect of the most potent benzoxepin **6b** on the cell cycle of K562, MDA-MB231, H1299 and HCT116 cells was then investigated. Cancer cells were cultured without or with **6b** (Figure 3) and cell cycle distribution was analyzed by flow cytometry after 24 h of treatment using the standard propidium iodide procedure.

<Figure 3>

Figure 2. Effects of benzoxepin **6b** at a concentration of 5.0 nM on cell cycle distribution in various cancer cell lines.

After treatment with 5 nM of **6b**, a net increase in the number of cells arrested at the G_2/M growth phase was observed. Cell-cycle arrest in the G_2/M phase is frequently followed by DNA fragmentation and other morphological features of apoptosis. The potency of benzoxepin **6b** to induce apoptosis was further characterized by a specific apoptosis assay. Cleavage of pro-caspases to active caspases is one of the hall marks of apoptosis. HCT116 and H1299 cells were incubated with 1, 5, and 10 nM of **6b** for 24 h and caspases-3 and -7 activities were evaluated using the standard caspases assays.

<Figure 4>

Figure 3. Apoptotic effects of **6b** in HCT116 and H1299 cells. The results are expressed in the percentage of apoptotic cells detected following 24 h of treatment with **6b** at different concentrations.

The results depicted in Figure 4 show a significant dose-dependent increase in proteolytic activity of caspases in HCT116 and H1299 cells treated with **6b** at low concentrations of 5 and 10 nM. These data suggest that benzoxepin **6b**, in addition to its antiproliferative properties, also induced apoptosis in the tested cancer cell lines.

2.3. Docking study.

To rationalize the potential binding mode of benzoxepins **6** in tubulin, a docking study was carry out with **6b** as the most potent derivative in this series. For this purpose, the X-ray structure of tubulin-DAMA-colchicine complex (Code PDB: 1sa0)[68] was used.

<Figure 4>

Figure 5. Docked pose of **6b** (gray) overlayed with *iso*CA-4 (blue) in the tubulin binding site.

The docking-derived superimposition of **6b** and *iso*CA-4 was illustrated in Figure 5, and benzoxepin **6b** showed a binding pose similar to the one observed with *iso*CA-4. As it was previously observed with CA-4 and *iso*CA-4, the trimethoxyphenyl ring of **6b** was placed in proximity of Cys241 and the benzoxepin ring in proximity of Val181 to establish a hydrogen bond with its C9-OH substituent.

3. Conclusion

We have designed and synthesized a series of benzoxepin compounds **6** as rigid-*iso*CA-4 analogues and evaluated their biological activities. Compound **6b**, the hybrid most closely resembling *iso*CA-4 in structure, have potent antimitotic and cytotoxic properties. Benzoxepin **6b**, which was prepared from isovanillin according to a convergent synthetic strategy in 9 steps (7.8% overall yield), displayed a nanomolar level of cytotoxicity against various cancer cell lines (GI_{50} = 1.5-8 nM), and showed similar antitubulin

ACCEPTED MANUSCRIPT

activities than that of *iso*CA-4. Flow cytometry analysis indicated that benzoxepin **6b** at a low concentration of 5 nM, arrested the cell cycle in the G_2/M phase in H1299, K562, HCT116 and MDA-MB231 cells. Furthermore, **6b** was characterized as a strong apoptotic agent by inducing the activation of procaspases in HCT116 and H1299 cells. All these results are well supported by molecular modelling studies, where it was observed that benzoxepin **6b** and *iso*CA-4 can be perfectly superimposed in the tubulin binding-site. On the basis of its excellent properties, compound **6b** is worthy of further *in vitro* and *in vivo* evaluations.

4. Experimental

4.1 General considerations

The synthesis of **6g** was achieved using a Biotage Initiator microwave synthesizer. NMR spectra were performed on a Bruker AVANCE 300 (^{1}H, 300 MHz; ^{13}C, 75 MHz)or Bruker AVANCE 400 (^{1}H, 400 MHz; ^{13}C, 100 MHz). Unless otherwise stated, $CDCl_3$ was used as solvent. Chemical shifts δ are in ppm, and the following abbreviations are used: singlet (s), doublet (d), triplet (t), multiplet (m), doublet of triplet (dt), quintuplet (qt), broad singlet (br s). Elemental analyses (C, H, N) were performed with a Perkin-Elmer 240 analyzer and were within 0.4% of the theorical values otherwise stated. Mass spectra were obtained using a Bruker Esquire electrospray ionization apparatus. High resolution mass spectra were recorded on a MicrotofQ Bruker Daltonics. Thin-layer chromatography was performed on silica gel 60 plates with a fluorescent indicator and visualized under a UVP Mineralight UVGL-58 lamp (254 nm) and with a 7% solution of phosphomolybdic acid in ethanol. Flash chromatography was performed using silica gel 60 (40-63 μm, 230-400 mesh ASTM) at medium pressure (200 mbar). All solvents were distilled and stored over 4 Å molecular sieves before use. All reagents were obtained from commercial suppliers unless otherwise stated. Organic extracts were, in general, dried over magnesium sulphate ($MgSO_4$) or sodium sulphate (Na_2SO_4).

4.2 Synthesis of (1-(2-(but-3-en-1-yloxy)phenyl)vinyl)tributylstannane **9a**

To a solution of 1-(but-3-en-1-yloxy)-2-ethynylbenzene[69] (600 mg, 3.5 mmol) in THF (10 mL) were successively added $PdCl_2(PPh_3)_2$ (246 mg, 0.35 mmol, 10 mol%) and Bu_3SnH (1.15 mL, 4.2 mmol, 1.2 equiv). The mixture was stirred at room temperature for 1 h and concentrated. Purification by flash chromatography on silica gel (neutralized with 1% of Et_3N) afforded compound **9a** as a colorless oil (1.25 g; 2.8 mmol; 80%). R_f (cyclohexane/EtOAc : 9/1) = 0.77. ^{1}H NMR: ($CDCl_3$, 300 MHz): 7.15 (dt, 1H, *J* = 1.8 Hz, *J* = 7.6 Hz), 7.03 (dd, 1H, *J* = 7.5 Hz, *J* = 1.8 Hz), 6.89 (m, 1H), 6.79 (d, 1H, *J* = 7.6 Hz), 5.89-5.86 (m, 2H), 5.39 (m, 1H), 5.13 (m, 2H), 4.01 (t, 2H, *J* = 7.3 Hz), 2.65-2.55 (m, 2H), 1.55-1.30 (m, 12H), 1.20-0.65 (m, 15H). ^{13}C NMR (100 MHz, $CDCl_3$): 155.6, 131.2, 128.3, 126.1, 120.2, 116.6, 113.8, 111.5, 110.8, 67.1, 33.3, 28.7 (3), 26.8 (3), 13.3 (3), 9.8 (3), one C missing. IR (neat): 2955, 2926, 1577, 1464, 1237, 1117, 1030 cm^{-1}. MS (APCI) $[M+H]^+$ = 465.0.

4.3 Synthesis of 1-(but-3-en-1-yloxy)-2-(1-iodovinyl)benzene **10a.**

To a CH_2Cl_2 (10 mL) solution containing **9a** (597 mg, 1.29 mmol) was added in one portion I_2 (326 mg, 1.29 mmol) at 0°C. The mixture was then stirred at rt for 1 h. Then a saturated KF solution (10 mL) was added to the mixture which was extracted with Et_2O, washed with a $Na_2S_2O_3$ solution (10 mL). The combined organic layers were dried over $MgSO_4$, filtered, and the solvent was then removed. Purification by flash chromatography afforded compound **10a** as a brown oil (213 mg; 0.71 mmol; 45%). R_f (cyclohexane/EtOAc : 9/1) = 0.54. ^{1}H NMR: ($CDCl_3$, 300 MHz): 7.28-7.24 (m, 2H), 6.92-6.82 (m, 2H), 6.28 (s, 1H), 6.14 (s, 1H), 5.94 (m, 1H), 5.19-5.11 (m, 2H), 4.07 (m, 2H), 2.62 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 134.6, 130.2, 130.1, 129.9, 120.2, 117.7, 117.3, 117.1, 112.1, 100.5, 67.8, 33.7. IR (neat): 29272927, 1661, 1594, 1487, 1447, 1283, 1243, 1162, 1113 cm^{-1}. MS (APCI) $[M+H]^+$ = 301.0.

4.4 Synthesis of 1-iodo-3-isopropoxy-4-methoxy-2-(methoxymethoxy)benzene **16**.

Phenol **15**[**Error! Bookmark not defined.**] (1.5 g, 4.86 mmol) was mixed with Et_2NH (1.41 mL; 9.72 mmol) and MOMCl (0.75 mL; 9.72 mmol) in CH_2Cl_2 (15 mL) for 1 h at room temperature. The medium was quenched with H_2O (45 mL) and extracted with CH_2Cl_2 (3 x 15 mL). The combined organic layers were dried over $MgSO_4$, filtered, and the solvent was then removed. Purification by flash chromatography afforded compound **16** as a colorless oil (1.67 g; 4.77 mmol; 98%). R_f (cyclohexane/EtOAc: 8/2) = 0.68. ^{1}H NMR: ($CDCl_3$, 300 MHz): 7.36 (d, 1H, *J* = 8.8 Hz) 6.42 (d, 1H, *J* = 8.8 Hz), 5.14 (s, 2H), 4.33 (m, 1H), 3.75 (s, 3H), 3.60 (s, 3H), 1.21 (d, 6H, *J* = 6.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 155.0, 140.1, 132.8, 124.0, 109.7, 99.3, 81.5, 75.9, 58.6, 56.1, 22.4 (2C). MS (ESI) $[M+Na]^+$ = 375.0.

ACCEPTED MANUSCRIPT

4.5 Synthesis of 4-(3-isopropoxy-4-methoxy-2-(methoxymethoxy)phenyl)but-3-yn-1-ol **17**.

A solution of **16** (700 mg; 2 mmol), but-3-yn-1-ol (0.2 mL, 2.42 mmol), $PdCl_2(PPh_3)_2$, (112 mg; 0.16 mmol), CuI (44 mg; 0.30 mmol) was stirred in Et_3N (10 mL) for 2 h at room temperature. EtOAc (20 mL) was added to the crude mixture which was washed with a saturated NH_4Cl solution. After extraction with CH_2Cl_2, the combined extracts were dried ($MgSO_4$) and concentrated. Purification by flash chromatography on silica gel afforded alkyne **17** as a brown oil (538 mg; 1.80 mmol; 90%). R_f (cyclohexane/EtOAc: 7/3) = 0.22. ^{1}H NMR: ($CDCl_3$, 300 MHz): 7.09 (d, 1H, *J* = 8.6 Hz), 6.61 (d, 1H, *J* = 8.6 Hz), 5.23 (s, 2H), 4.39 (m, 1H), 3.83 (s, 3H), 3.80 (t, 2H, *J* = 6.2 Hz), 3.61 (s, 3H), 2.69 (t, 2H, *J* = 6.2 Hz), 1.27 (d, 6H, *J* = 6.2 Hz), OH not seen. ^{13}C NMR (100 MHz, $CDCl_3$): 154.6, 127.7, 114.9, 110.8, 107.4, 99.0, 97.3, 88.9, 78.9, 75.6, 61.1, 57.6, 55.9, 24.1, 22.4 (2C). IR (neat): 3894, 3807, 3634, 3489, 2976, 2106, 2021, 1594, 1491, 1437, 1381, 1290, 1207, 1157, 1099, 1060, 968, 921, 803, 774, 693 cm^{-1}. MS (ESI) $[M+Na]^+$ = 317.0.

4.6 Synthesis of 4-hydroxy-1-(2-hydroxy-3-isopropoxy-4-methoxyphenyl)butan-1-one **18**.

To a solution of **17** (400 mg; 1.36 mmol) in EtOH (272 µL) was added PTSA (260 mg; 1.36 mmol) and H_2O (2.45 mL). The solution was then stirred at 100° C in a sealed tube for 0.5 h. After cooling, the crude mixture was extracted with CH_2Cl_2. The organic layers were dried over $MgSO_4$, filtered, and the solvent was then removed. Purification by flash chromatography afforded compound **18** as a brown oil (228 mg; 0.84 mmol; 62%). R_f (cyclohexane/EtOAc: 5/5) = 0.30. ^{1}H NMR: ($CDCl_3$, 300 MHz): 12.53 (brs, 1H), 7.53 (d, 1H, *J* = 9.1 Hz), 6.46 (d, 1H, *J* = 9.1 Hz), 4.43 (m, 1H), 3.88 (s, 3H), 3.71 (t, 2H, *J* = 6.1 Hz), 3.05 (t, 2H, *J* = 7.1 Hz), 1.97 (m, 2H), 1.29 (d, 6H, *J* =6.2 Hz), OH not seen. ^{13}C NMR (100 MHz, $CDCl_3$): 205.2, 158.9,. 157.6, 134.5, 126.0, 114.7, 102.8, 75.1, 61.9, 55.9, 34.5, 27.1, 22.4 (2C). IR (neat): 2976, 1631, 1505, 1429, 1261, 1131, 1092, 944, 685, 619 cm^{-1}. MS (APCI) $[M+H]^+$ = 269.0.

4.7 Synthesis of 9-isopropoxy-8-methoxy-3,4-dihydrobenzo[b]oxepin-5(2H)-one **19**.

Compound **18** (300 mg; 1.11 mmol) was stirred for 1 h with DEAD (0.27 mL; 1.68 mmol) and PPh_3 (441 mg; 1.68 mmol) in THF (30 mL). After concentration under reduced pressure, the crude mixture was purified by flash chromatography to afford benzoxepin-5-one **19** as a pale yellow oil (174 mg; 0.69 mmol; 63%). R_f (cyclohexane/EtOAc: 5/5) = 0.60. ^{1}H NMR: ($CDCl_3$, 300 MHz): 7.51 (d, 1H, *J* = 8.9 Hz), 6.66 (d, 1H, *J* = 8.9 Hz), 4.34 (m, 1H), 4.22 (t, 2H, *J* = 6.7 Hz), 3.85 (s, 3H), 2.83 (t, 2H, *J* = 6.9 Hz), 2.15 (m, 2H), 1.28 (d, 6H, *J* = 6.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 199.4, 157.7, 156.6, 137.9, 124.4, 123.9, 106.4, 75.7, 72.8, 55.9, 40.2, 25.9, 22.4 (2C). IR (neat): 2975, 2023, 1674, 1587, 1493, 1433, 1372, 1270, 1218, 1196, 1169, 1093, 1008, 964 cm^{-1}. MS (ESI) $[M+Na]^+$ = 273.1.

4.8 Synthesis of N'-(9-isopropoxy-8-methoxy-3,4-dihydrobenzo[b]oxepin-5(2H)-ylidene)-4-methylbenzenesulfonohydrazide **11a**.

To a solution of benzoxepin-5-one **19** (173 mg; 0.69 mmol) and PTSA (28 mg; 0.14 mmol) in ethanol (5 mL) was added 4-methylbenzenesulfonohydrazide (321 mg; 1.72 mmol). The resulting mixture was stirred under reflux for 1 h. The medium was diluted with EtOAc and filtered over a pad of Celite. The solvent was next removed and the residue was purified by crystallization in ethanol to afford **11a** as a white solid (147 mg; 0.35 mmol; 51%). R_f (cyclohexane/EtOAc: 5/5) = 0.60. F = 192-194 °C. ^{1}H NMR: ($CDCl_3$, 300 MHz): 7.76 (d, 2H, *J* = 8.1 Hz), 7.60 (brs, 1H), 7.31 (d, 2H, *J* = 8.1 Hz), 6.87 (d, 1H, *J* = 8.3 Hz), 6.53 (d, 1H, *J* = 8.3 Hz), 4.20 (m, 1H), 4.10 (m, 1H), 3.80 (m, 1H), 3.76 (s, 3H), 2.60 (t, 2H, *J* = 6.8 Hz), 2.37 (s, 3H), 1.80 (m, 2H), 1.19 (d, 6H, *J* = 6.3 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 157.1, 155.3, 151.6, 144.1, 138.8, 135.5, 129.7 (2C), 128.2 (2C), 123.9, 122.8, 107.1, 75.8, 71.0, 56.1, 26.3, 24.9, 22.5 (2C), 21.7. IR (neat): 3803, 3676, 2972, 1595, 1494, 1435, 1373, 1321, 1288, 1219, 1164, 1097, 911, 812, 728, 665 cm^{-1}. MS (APCI) $[M+H]^+$ = 419.0.

4.9 Synthesis of 3-bromo-2-hydroxy-4-methoxybenzaldehyde **20**[70]

To a solution of *iso*vanillin (2.5 g; 21.55 mmol) in EtOH (25 mL) were added a drop of AcOH and $Hg(OAc)_2$ (6.85 g; 21.55 mmol). After 20 min of stirring at 50°C, an aqueous solution of NaBr (2.21 g; 21.55 mmol) was added and the precipitate was filtered. This latter was dissolved in CH_3CN and MPHT (8.45 g; 21.55 mmol) was added in one portion. After 20 min of stirring at room temperature, the medium was concentrated and hydrolyzed with a saturated aqueous $NaHCO_3$ solution. After extraction with EtOAc (3 x 100 mL), the combined extracts were dried ($MgSO_4$) and concentrated. Purification by flash chromatography on silica gel afforded **20** as a beige solid (2.34 g; 12.05 mmol; 56%).

4.10 Synthesis of 2-(allyloxy)-3-bromo-4-methoxybenzaldehyde **21**.

To a CH_3CN solution of **20** (4.6 g; 20 mmol) was added K_2CO_3 (3.32 g; 24 mmol) and allylbromide (2.08 mL; 24 mmol). The mixture was stirred for 1 h under reflux and after cooling to room temperature, the medium was filtered, concentrated and hydrolyzed with H_2O. After extraction with EtOAc, the combined extracts were dried ($MgSO_4$) and concentrated. Purification by flash chromatography on silica gel afforded **21** as a colorless oil (5.2 g; 19.2 mmol, 96%). R_f (cyclohexane/EtOAc: 8/2) = 0.48. ^{1}H NMR: ($CDCl_3$, 300 MHz): 10.15 (s, 1H), 7.77 (d, 1H, *J* = 8.8 Hz), 6.74 (d, 1H, *J* = 8.8 Hz), 6.06 (m, 1H), 5.36-5.26 (m, 2H), 4.55 (m, 2H), 3.91 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 188.3, 162.2, 160.5, 132.2, 128.8, 124.6, 119.6, 107.9, 107.5, 76.6, 56.8. IR (neat): 3082, 2938, 2847, 2362, 1675, 1584, 1485, 1452, 1437, 1414, 1391, 1359, 1291, 1246, 1199, 1178, 1127, 1073 cm^{-1}. MS (APCI) $[M+H]^+$ = 271.0 (^{79}Br), 273.5 (^{81}Br).

4.11 Synthesis of 1-(2-(allyloxy)-3-bromo-4-methoxyphenyl)prop-2-en-1-ol **13a**.

To a solution of **21** (1.0 g; 3.7 mmol) in THF (10 mL) was added at 0° C 4.5 mL of a 1N solution of vinylmagnesium bromide (4.5 mmol) in THF. After stirring for 1 h at room temperature, the medium was hydrolyzed with H_2O. After extraction with CH_2Cl_2, the combined extracts were dried ($MgSO_4$) and concentrated. Purification by flash chromatography on silica gel afforded **13a** as a yellow oil (1.05 g; 3.51 mmol; 95%). R_f (cyclohexane/EtOAc: 7/3) = 0.50. ^{1}H NMR: ($CDCl_3$, 300 MHz): 7.22 (d, 1H, *J* = 8.7 Hz), 6.64 (d, 1H, *J* = 8.7 Hz), 6.16-5.90 (m, 2H), 5.43-5.13 (m, 5H), 4.47 (m, 2H), 3.81 (s, 3H), 2.40 (d, 1H, *J* = 5.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 156.8, 154.8, 139.7, 133.2, 129.9, 127.0, 118.2, 114.9, 107.8, 107.3, 74.7, 69.8, 56.5. IR (neat): 2947, 1675, 1584, 1485, 1319, 1291, 1246, 1199, 1178, 1127, 998 cm^{-1}. MS (APCI) $[M+H]^+$ = 299.1 (^{79}Br), 301.1 (^{81}Br).

4.12 Synthesis of 9-bromo-8-methoxy-2,5-dihydrobenzo[b]oxepin-5-ol **22**

Compound **13a** (828 mg; 2.79 mmol) was stirred under argon with Grubbs II catalyst ([1,3-bis-(2,4,6-trimethylphenyl)-2-imidazolidinylidene]dichloro(phenylmethylene)(tricyclohexylphosphine)ruthenium, 231 mg; 0.27 mmol) in CH_2Cl_2 (60 mL) for 1 h at room temperature. The crude mixture was concentrated and the residue was purified by flash chromatography on silica gel to afford **22** as a colorless oil (558 mg; 2.04 mmol; 73%). R_f (cyclohexane/EtOAc: 7/3) = 0.30. ^{1}H NMR: ($CDCl_3$, 300 MHz): 7.11 (d, 1H, *J* = 8.6 Hz), 6.55 (d, 1H, *J* = 8.6 Hz), 5.87 (m, 1H), 5.34-5.30 (m, 2H), 4.50 (m, 2H), 3.80 (s, 3H), 2.80 (d, 1H, *J* = 7.0 Hz, OH). ^{13}C NMR (100 MHz, $CDCl_3$): 156.4, 153.6, 133.0, 131.8, 127.1, 124.2, 107.2, 106.5, 69.8, 68.5, 56.5. IR (neat): 2362, 1675, 1584, 1291, 1246, 1199, 1178, 1127, 1073, 956, 873 cm^{-1}. MS (APCI) $[M+H]^+$ = 271.3 (^{79}Br), 273.0 (^{81}Br).

4.13 Synthesis of 9-bromo-8-methoxy-2,3,4,5-tetrahydrobenzo[b]oxepin-5-ol **23**

A mixture of **22** (480 mg, 1.76 mmol), Pd/C (48 mg), MeOH (20 mL) and 1 drop of AcOH was stirred under an H_2 atmosphere for 1 h at room temperature. The mixture was then filtered over a pad of Celite and concentrated. Purification by flash chromatography on silica gel afforded **23** as a colorless oil (318 mg; 1.16 mmol; 66%). R_f (cyclohexane/EtOAc: 9/1) = 0.33. ^{1}H NMR: ($CDCl_3$, 300 MHz): 7.24 (d, 1H, *J* = 7.2 Hz), 6.61 (d, 1H, *J* = 7.2 Hz), 4.86 (m, 1H), 4.17-3.99 (m, 2H), 3.87 (s, 3H), 2.50-2.15 (m, 2H), 2.10-1.93 (m, 2H), OH not seen. ^{13}C NMR (100 MHz, $CDCl_3$): 156.3, 156.1, 131.3, 126.3, 106.7, 76.4, 73.3, 72.5, 56.6, 34.2, 27.0. IR (neat): 2978, 1595, 1490, 1435, 1355, 1321, 1288, 1231, 1206, 1164, 812, 730, 665, 641 cm^{-1}. MS (APCI) $[M+H]^+$ = 273.0 (^{79}Br), 275.1 (^{81}Br).

4.14 Synthesis of 9-bromo-8-methoxy-3,4-dihydrobenzo[b]oxepin-5(2H)-one **24**

To a solution of **23** (240 mg; 0.87 mmol) in CH_2Cl_2 (15 mL) was added in 3 portions PCC (192 mg) at the begining of the reaction, a second drop of PCC (192 mg) was next added to the mixture after 1 h of stirring and a last drop of PCC (96 mg) was finally added to complete the oxidation reaction after 3 h of stirring. The mixture was filtered over a pad of Celite and concentrated. Purification by flash chromatography on silica gel afforded **24** as brown oil (228 mg; 0.84 mmol; 96%). R_f (cyclohexane/EtOAc: 8/2) = 0.60.^{1}H NMR: ($CDCl_3$, 300 MHz): 7.83 (d, 1H, *J* = 8.8 Hz), 6.77 (d, 1H, *J* = 8.8 Hz), 4.38 (t, 2H, *J* = 6.8 Hz), 4.02 (s, 3H), 2.94 (t, 2H, *J* = 6.8 Hz), 2.27 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 198.8, 160.6, 159.5, 129.6, 124.0, 106.4, 104.7, 73.0, 56.7, 40.1, 25.6. IR (neat): 2813, 1795, 1490, 1462, 1399, 1321, 1287, 1219, 1164, 812, 728, 652 cm^{-1}. MS (APCI) $[M+H]^+$ = 271.4 (^{79}Br), 273.5 (^{81}Br).

4.15 Synthesis of N'-(9-bromo-8-methoxy-3,4-dihydrobenzo[b]oxepin-5(2H)-ylidene)-4-methylbenzenesulfonohydrazide **11b**

A solution of **24** (665 mg; 2.5 mmol), PTSA (53 mg; 0.25 mmol), $MgSO_4$ (296 mg; 2.5 mmol) 4-methylbenzenesulfonohydrazide (460 mg; 2.5 mmol) was stirred under reflux for 2 h. The medium was filtered over a pad of Celite, concentrated, diluted with EtOAc and washed with H_2O (3 x 10 mL). After extraction, the crude was precipitated in EtOH to afford **11b** as a white solid (0.98 g; 2.22

ACCEPTED MANUSCRIPT

mmol, 88%). R_f (cyclohexane/EtOAc: 7/3) = 0.30. F = 201-203 °C. ^{1}H NMR: ($CDCl_3$, 300 MHz): 8.14 (s, 1H, NH), 7.71 (d, 2H, *J* = 8.0 Hz), 7.24 (d, 1H, *J* = 8.8 Hz), 7.13 (d, 2H, *J* = 8.0 Hz), 6.46 (d, 1H, *J* = 8.8 Hz), 3.95 (t, 2H, *J* = 6.6 Hz), 3.71 (s, 3H), 2.43 (t, 2H, *J* = 6.6 Hz), 2.23 (s, 3H), 1.75 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 158.2, 155.9, 154.2, 144.2, 135.3, 129.6, 128.0, 127.9, 124.3 (2C), 107.0, 105.4, 70.8, 56.5, 26.1, 24.2, 21.6, one C missing. IR (neat): 2967, 1595, 1494, 1435, 1373, 1321, 1288, 1219, 1164, 812, 728, 665 cm^{-1}. MS (APCI) $[M+H]^+$ = 439.0 (^{79}Br), 441.0 (^{81}Br).

4.16 Synthesis of 9-isopropoxy-8-methoxy-5-(3,4,5-trimethoxyphenyl)-2,3-dihydrobenzo[b]oxepin **6a**

To a dioxane (2 mL) solution of *N*-tosylhydrazone **11a** (60 mg, 0.14 mmol), *t*BuOLi (23 mg; 0.28 mmol)), Pd_2dba_3 (13 mg; 0.014 mmol), and XPhos (13 mg; 0.028 mmol) was added 3,4,5-trimethoxyphenyliodide (42 mg, 0.14 mmol). The mixture was stirred at 90 °C in a sealed tube for 1 h. After cooling at room temperature, the crude mixture was filtered over a pad of Celite and concentrated. The residue was purified by silica gel chromatography to yield **6a** as a brown oil (55 mg; 0.137 mmol; 98%). R_f (cyclohexane/EtOAc: 7/3) = 0.60. ^{1}H NMR: ($CDCl_3$, 400 MHz): 6.65 (d, 1H, *J* = 8.8 Hz), 6.56 (d, 1H, *J* = 8.8 Hz), 6.50 (s, 2H), 6.14 (t, 1H, *J* = 5.9 Hz), 4.48-4.43 (m, 3H), 3.87 (s, 3H), 3.83 (s, 3H), 3.81 (s, 6H), 2.50 (m, 2H), 1.35 (d, 6H, *J* = 5.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 153.3, 152.8 (2C), 152.2, 141.2, 139.7 (2C), 138.9, 137.3, 126.3, 125.3, 106.5, 106.0 (2C), 76.5, 75.8, 60.9, 56.1 (2C), 55.9, 30.4, 22.7 (2C). IR (neat): 2936, 1710, 1659, 1582, 1549, 1493, 1411, 1232 cm^{-1}. MS (APCI) $[M+H]^+$ = 401.0.

4.17 Synthesis of 8-Methoxy-5-(3,4,5-trimethoxyphenyl)-2,3-dihydrobenzo[b]oxepin-9-ol **6b**

According to Scheme 2: A solution of **6a** (110 mg; 0.28 mmol) in CH_2Cl_2 (5 mL) was stirred with $AlCl_3$ (92 mg; 0.28 mmol) for 1 h at room temperature. Then another portion of $AlCl_3$ (92 mg) was added to the crude mixture. After 1 h of stirring, the medium was hydrolyzed by H_2O (10 mL). After extraction with CH_2Cl_2 (3 x 10 mL), the combined extracts were dried ($MgSO_4$) and concentrated. Purification by flash chromatography on silica gel afforded benzoxepin **6b** as a pale yellow oil (16 mg; 0.04 mmol; 15%).

According to Scheme 3: A solution of **6d** (100 mg; 0.24 mmol), KOH (5 mmol), Pd_2dba_3 (46 mg; 0.005 mmol) and *t*BuXPhos (8 mg; 0.015 mmol) in a mixture of dioxane/H_2O: 1/1 (2 mL) was stirred at 90 °C in a sealed tube. After 1 h, the medium was cooled to room temperature, quenched with NH_4Cl (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were then dried over $MgSO_4$, filtered and evaporated to dryness. Purification by flash chromatography afforded compound **6b** as a pale yellow oil **(**47 mg, 0.13 mmol, 55%). R_f (cyclohexane/EtOAc: 7/3) = 0.26. ^{1}H NMR: ($CDCl_3$, 300 MHz): 6.51 (d, 1H, *J* = 8.8 Hz), 6.42 (m, 3H), 6.08 (t, 1H, *J* = 5.9 Hz), 5.73 (br s, 1H, OH), 4.47 (t, 2H, *J* = 5.9 Hz), 3.83 (s, 3H), 3.80 (s, 3H), 3.74 (s, 6H), 2.49 (q, 2H, *J* = 5.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 152.8 (2C), 146.7, 144.7, 141.2, 138.6 (2C), 137.6, 126.2, 125.0, 121.3, 106.3, 106.0 (2C), 76.6, 60.9, 56.1 (3C). 30.3, IR (neat): 3853, 3618, 3441, 2937, 1580, 1504, 1444, 1411, 1333, 1233, 1124, 1089, 1007 cm-1. MS (APCI) $[M+H]^+$ = 359.2. HRMS calcd for $C_{20}H_{23}O_6$ $[M+H]^+$ 359.1495, obsd 359.1510.

4.18 Synthesis of 5-(3,5-dimethoxyphenyl)-8-methoxy-2,3-dihydrobenzo[b]oxepin-9-ol **6c**

To a dioxane (1 mL) solution of *N*-tosylhydrazone **11a** (100 mg, 0.24 mmol), *t*BuOLi (38 mg; 0.48 mmol)), Pd_2dba_3 (20 mg; 0.02 mmol), and XPhos (20 mg; 0.04 mmol) was added 3,5-dimethoxyphenyliodide (52 mg, 0.24 mmol). The mixture was stirred at 90 °C in a sealed tube for 1 h. After cooling, the crude mixture was diluted in CH_2Cl_2 and stirred for 2 h with $AlCl_3$ (22 mg; 0.24 mmol). The medium was then hydrolyzed by H_2O (10 mL). After extraction with CH_2Cl_2, the combined extracts were dried ($MgSO_4$) and concentrated. Purification by flash chromatography on silica gel afforded benzoxepin **6c** as a pale yellow oil (20 mg; 0.06 mmol; 25%). R_f (cyclohexane/EtOAc: 7/3) = 0.40. ^{1}H NMR: ($CDCl_3$, 300 MHz): 6.41 (d, 1H, *J* = 8.9 Hz), 6.33 (d, 1H, *J* = 8.9 Hz), 6.28 (m, 3H), 6.01 (t, 1H, *J* = 5.9 Hz), 5.66 (s, 1H), 4.37 (t, 2H, *J* = 5.9 Hz), 3.74 (s, 3H), 3.61 (s, 6H), 2.42 (q, 2H, *J* = 5.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 160.5 (2C), 146.7, 145.1, 144.9, 141.0, 137.6, 126.7, 124.8, 121.4, 107.0, 106.4 (2C), 99.5, 76.3, 56.2, 55.4 (2C), 30.6. IR (neat): 3876, 2938, 1590, 1450, 1204, 1153, 1090 cm^{-1}. MS (APCI) $[M+H]^+$ = 329.6. Compound **6c**, however, does not give completely satisfactory HRMS analyses despite repeated attempts at purification, but all other spectral data were consistent with this structure.

4.19 Synthesis of 9-bromo-8-methoxy-5-(3,4,5-trimethoxyphenyl)-2,3-dihydrobenzo[b]oxepin **6d**

To a dioxane (3 mL) solution of *N*-tosylhydrazone **11b** (800 mg, 1.84 mmol), *t*BuOLi (296 mg; 3.68 mmol), Pd_2dba_3 (168 mg; 0.16 mmol), and XPhos (344 mg; 0.72 mmol) was added 3,4,5-trimethoxyphenyliodide (632 mg, 2.16 mmol). The mixture was stirred at 90 °C in a sealed tube for 1 h. After cooling at room temperature, the crude mixture was filtered over SiO_2 and concentrated. The

residue was purified by silica gel chromatography to yield **6d** as brown oil (536 mg; 1.26 mmol; 68%). R_f (cyclohexane/EtOAc: 8/2) = 0.33. ^{1}H NMR: ($CDCl_3$, 300 MHz): 6.86 (d, 1H, *J* = 8.7 Hz), 6.56 (d, 1H, *J* = 8.7 Hz), 6.41 (s, 2H), 6.19 (t, 1H, *J* = 6.3 Hz), 4.58 (t, 2H, *J* = 6.1 Hz), 3.84 (s, 3H), 3.80 (s, 3H), 3.74 (s, 6H), 2.37 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 156.3, 155.1, 152.9 (2C), 141.2, 138.0, 137.4, 130.0, 127.2, 126.5, 106.7, 106.6, 105.6 (2C), 78.0, 60.9, 56.4, 56.1 (2C). 29.3, IR (neat): 2874, 1985, 1579, 1485, 1445, 1441, 1386, 1333, 1233, 1122, 1004, 975 cm^{-1}. MS (APCI) $[M+H]^+$ = 421.0 (^{79}Br), 423.5 (^{81}Br). HRMS calcd for $C_{20}H_{22}BrO_5$ $[M+H]^+$ 421.0651, obsd 421.0663.

4.20 Synthesis of 8-methoxy-5-(3,4,5-trimethoxyphenyl)-2,3-dihydrobenzo[b]oxepin-9-amine **6e**

A solution of **6d** (100 mg; 0.24 mmol), NaN_3 (31 mg; 0.48 mmol), CuI (3 mg; 0.02 mmol), DMEDA (0.13 mg; 0.012 mmol) and sodium ascorbate (8 mg; 0.04 mmol) in a mixture of DMSO/H_2O: 5/1 (2 mL) was stirred at 60 °C in a sealed tube. After 12 h, the medium was cooled, quenched with NH_4Cl and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated to dryness. Purification by flash chromatography afforded **6e** as a colorless oil (64 mg; 0.18 mmol, 75%). R_f (cyclohexane/EtOAc: 7/3) = 0.26. ^{1}H NMR: ($CDCl_3$, 300 MHz): 6.64-6.43 (m, 3H), 6.37 (d, 1H, *J* = 8.6 Hz), 6.17 (t, 1H, *J* = 6.0 Hz), 4.60-4.45 (t, 2H, *J* = 6.0 Hz), 3.88 (s, 6H), 3.81 (s, 6H), 2.48 (q, 2H, *J* = 6.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 152.9 (2C), 147.6, 144.9, 141.9, 138.9, 137.3, 128.1, 125.7, 119.9, 106.0 (2C), 105.5, 76.6, 61.0, 56.2 (2C), 55.8, 30.1, 1C missing. IR (neat): 2444, 1985, 1579, 1485, 1415, 1333, 1233, 1122, 1004, 975 cm^{-1}. MS (APCI) $[M+H]^+$ = 358.0. HRMS calcd for $C_{20}H_{24}NO_5$ $[M+H]^+$ 358.1654, obsd 358.1665.

4.21 Synthesis of 8-methoxy-5-(3,4,5-trimethoxyphenyl)-2,3-dihydrobenzo[b]oxepin **6f**

A *n*BuOH (2 mL) solution of **6d** (105 mg; 0.24 mmol), $Pd(OAc)_2$ (1.5 mg; 0.0015 mmol), PPh_3 (7.5 mg; 0.006 mmol), was stirred for 1 h at 100 °C in a sealed tube. The medium was cooled, H_2O (8 mL) was added to the crude mixture. After extraction with EtOAc (3 x 5 mL), the combined organic layers were dried over $MgSO_4$, filtered and evaporated to dryness. Purification by flash chromatography afforded **6f** as a brown oil (57 mg; 0.165 mmol, 69%). R_f (cyclohexane/EtOAc: 7/3) = 0.64. ^{1}H NMR: ($CDCl_3$, 300 MHz): 6.82 (d, 1H, *J* = 6.9 Hz), 6.61 (m, 1H), 6.48 (m, 1H), 6.38 (s, 2H), 6.06 (t, 1H, *J* = 6.7 Hz), 4.38 (t, 2H, *J* = 6.7 Hz), 3.81 (s, 3H), 3.74 (s, 9H), 2.47 (q, 2H, *J* = 6.7 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 159.8, 159.2, 152.9 (2C), 140.9, 139.1, 137.1, 132.2, 126.5, 109.3, 106.7, 105.9 (2C), 76.6, 60.9, 56.1, 55.4 (2C), 30.6, 1C missing. IR (neat): 2937, 1607, 1580, 1499, 1461, 1411, 1356, 1234, 1125, 1007, 826 cm^{-1}. MS (APCI) $[M+H]^+$ = 343.0. HRMS calcd for $C_{20}H_{23}O_5$ $[M+H]^+$ 343.1545, obsd 343.1552.

4.22 Synthesis of 4-(8-methoxy-5-(3,4,5-trimethoxyphenyl)-2,3-dihydrobenzo[b] oxepin-9-yl)but-3-yn-1-ol **6g**

A DMF (1 mL) solution of **6d** (80 mg; 0.19 mmol), but-3-yn-1-ol (0.17 mL; 0.23 mmol), $PdCl_2(PPh_3)_2$ (7 mg; 0.001 mmol), PPh_3 (7 mg; 0.03 mmol), CuI (2 mg; 0.03 mmol) and Et_2NH (0.4 mL; 0.38 mmol) was stirred at 120 °C under microwave irradiation. After 25 min, the medium was cooled down to room temperature, quenched with NH_4Cl and extracted with EtOAc (3 x 10 mL). The combined organic layers were then dried over $MgSO_4$, filtered and evaporated to dryness. Purification by flash chromatography afforded compounds **6g** as a colorless oil (59 mg; 0.14 mmol; 74%). R_f (cyclohexane/EtOAc: 4/6) = 0.42. ^{1}H NMR: ($CDCl_3$, 300 MHz): 6.90 (d, 1H, *J* = 8.9 Hz), 6.57 (d, 1H, *J* = 8.9 Hz), 6.46 (s, 2H), 6.20 (t, 1H, *J* = 6.0 Hz), 4.56 (t, 2H, *J* = 6.0 Hz), 3.93-3.82 (m, 8H), 3.80 (s, 6H), 2.80 (t, 2H, *J* = 6.0 Hz), 2.49 (q, 2H, *J* = 6.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 160.1, 159.4, 152.9 (2C), 140.8, 138.5, 137.3, 131.2, 126.7, 125.3, 105.7 (2C), 105.3, 94.6, 77.3, 76.0, 60.9 (2C), 56.1 (3C), 29.9, 24.6, 1C missing. IR (neat): 3531, 2939, 1582, 1504, 1486, 1463, 1412, 1356, 1333, 1283, 1236, 1126, 1102, 1055, 1007, 911, 829, 731, 696. MS (APCI) $[M+H]^+$ = 411.0. HRMS calcd for $C_{24}H_{27}O_6$ $[M+H]^+$ 411.1808, obsd 411.1808.

4.23 Synthesis of 8-methoxy-5-(3,4,5-trimethoxyphenyl)-2,3,4,5-tetrahydrobenzo[b]oxepin-9-ol **25a**

Compound **6b** (72 mg, 0.2 mmol) in MeOH (8 mL) was stirred under a H_2 pressure of 20 bars for 1 h at room temperature. The mixture was then filtered over a pad of Celite and concentrated. Purification by flash chromatography on silica gel afforded **25a** as a pale brown oil (46 mg; 0.126 mmol; 63%). R_f (cyclohexane/EtOAc: 7/3) = 0.30. ^{1}H NMR: ($CDCl_3$, 300 MHz): 6.48 (d, 1H, *J* = 6.7 Hz). 6.40 (s, 2H), 6.21 (d, 1H, *J* = 6.7 Hz), 5.83 (brs, 1H, OH), 4.22-4.10 (m, 2H), 4.02-3.95 (m, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 3.81 (s, 6H), 2.25-2.10 (m, 2H), 2.05-1.95 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 153.0 (2C), 146.4, 146.1, 139.4, 137.7, 136.2, 130.2, 119.3, 106.6, 105.4 (2), 73.8, 60.9, 56.1 (3C), 48.8, 33.1, 29.7. IR (neat): 3374, 2932, 2160, 1998, 1590, 1462, 1238, 1127, 1085, 1044 cm^{-1}. MS (APCI) $[M+H]^+$ = 361.0. Compound **25a**, however, does not give completely satisfactory HRMS analyses despite repeated attempts at purification, but all other spectral data were consistent with this structure.

4.24 Synthesis of 4-(8-methoxy-5-(3,4,5-trimethoxyphenyl)-2,3,4,5-tetrahydrobenzo[b]oxepin-9-yl)butan-1-ol **25b**

A mixture of **6g** (90 mg, 0.21 mmol), Pd/C (9 mg), MeOH (30 mL) and 3 drops of AcOH was stirred under a H_2 atmosphere for 3 h at room temperature. The mixture was then filtered over a pad of Celite and concentrated. Purification by flash chromatography on silica gel afforded **25b** as a colorless oil (30 mg; 0.007 mmol; 34%). R_f (cyclohexane/EtOAc: 9/1) = 0.33. ^{1}H NMR: ($CDCl_3$, 300 MHz): 6.47 (d, 1H, *J* = 8.5 Hz), 6.39 (m, 3H), 4.11 (m, 2H), 3.80-3.75 (m, 1H), 3.78 (s, 3H), 3.74 (s, 6H), 3.70 (s, 3H), 3.62 (t, 2H, *J* = 5.8 Hz), 2.64 (t, 2H, *J* = 6.9 Hz), 1.99 (m, 4H), 1.54 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): 158.3, 157.0, 153.2 (2C), 140.2, 136.4, 130.7, 127.1, 123.6, 105.7 (2C), 105.5, 73.1, 63.1, 61.0, 56.2 (2C), 55.7, 48.6, 33.2, 32.7, 30.1, 26.1,. 23.3. IR (neat): 2938, 1590, 1450, 1153, 1090 cm^{-1}. MS (APCI) $[M+H]^+$ = 417.0. HRMS calcd for $C_{24}H_{33}O_6$ $[M+H]^+$ 417.2277, obsd 417.2288.

4.25 Cell Culture and Proliferation Assay

Cancer cell lines were obtained from the American type Culture Collection (Rockville, MD) and were cultured according to the supplier's instructions. Briefly MDA-MB231 and H1299 cells were grown in Dulbecco minimal essential medium (DMEM) containing 4.5 g/L glucose supplemented with 10% FCS and 1% glutamine. Human K562 leukemia and HCT116 colorectal carcinoma cells were grown in RPMI 1640 containing 10% FCS and 1% glutamine. Cell lines were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. Cell viability was assessed using Promega CellTiter-Blue TM reagent according to the manufacturer's instructions. Cells were seeded in 96-well plates (5 × 103 cells/well) containing 50 µL growth medium. After 24 h of culture, the cells were supplemented with 50 µL of the tested compound dissolved in DMSO (less than 0.1% in each preparation). After 72 h of incubation, 20 µL of resazurin was added for 2 h before recording fluorescence (λex = 560 nm, λem = 590 nm) using a Victor microtiter plate fluorimeter (Perkin-Elmer, USA). The GI_{50} corresponds to the concentration of the tested compound that caused a decrease of 50% in fluorescence of drug treated cells compared with untreated cells. Experiments were performed in triplicate. The GI_{50} values for all compounds were compared to the GI_{50} of CA-4 and *iso*CA-4 and were measured the same day under the same conditions.

4.26 Tubulin Binding Assay

Sheep brain tubulin was purified according to the method of Shelanski[71]by two cycles of assembly-disassembly and then dissolved in the assembly buffer containing 0.1 M MES, 0.5 mM $MgCl_2$, 1 mM EGTA, and 1 mM GTP, pH 6.6 (the concentration of tubulin was about 2-3 mg/mL). Tubulin assembly was monitored by fluorescence according to reported procedure[72] using DAPI as fluorescent molecule. Assays were realized on 96-well plates prepared with Biomek NKMC and Biomek 3000 from Beckman Coulter and read at 37°C on Wallac Victor fluorimeter from Perkin Elmer. The IC_{50} value of each compound was determined as the concentration which decreased the maximum assembly rate of tubulin by 50% compared to the rate in the absence of compound. The IC_{50} values for all compounds were compared to the IC_{50} of isoCA-4 and measured the same day under the same conditions.

4.27 Cell Cycle Analysis

Exponentially growing cancer cells (K562, H1299, HCT116, MDA-MB231) were incubated with benzoxepine **6b** at a concentration of 5 nM or DMSO for 24 h. Cell-cycle profiles were determined by flow cytometry on a FC500 flow cytometer (Beckman-Coulter, France) as described previously.[73]

4.28 Apoptosis Assay

Apoptosis was measured by the Apo-one homogeneous caspase-3/7 assay (Promega Co, WI) according to the manufacturer's recommendations. Briefly, HCT116 and H1299 cells were subcultured on a 96-well plate with 5×10^4 cells/well in 100 µL medium. After 24 h of incubation, the medium in the 96-well plate was discarded and replaced with medium containing different concentrations of benzoxepin **6b** (1, 5, and 10 nM) or 0.1% DMSO (as negative control). The treated cells were incubated for 24 h, each well then received 100 µL of a mixture of caspase substrate and Apo-one caspase 3/7 buffer. After 1 h of incubation, the fluorescence of sample was measured using a Victor microtiter plate fluorimeter (Perkin-Elmer, USA) at 527 nm.

4.29 Molecular modeling

For ligand preparation, compound **6b** was built using Chemdraw and converted to 3D using the dbtranslate program from Unity module of Sybyl (Tripos International, S.L., Missouri, USA). The 3D structure was minimized and a conformational analysis was

performed (systematic search) selecting all possible rotatable bonds. The most stable structure of each compound was selected and used for docking. For the receptor preparation, the x-ray structure of tubulin-colchicine complex (PDB: 1sa0) was used for this study downloaded from the Protein Data Bank. Hydrogen atoms were added and an energy minimisation using AMBER 8.0[74] was performed keeping the α-carbons constrained by a constraint-force of 50 kcal/mol $Å^2$, which permitted to side-chains free to move and kept the same secondary structures. Over the refined model we defined the active site of tubulin as 12 Å around the crystallized colchicine. We performed a docking of all compounds using the program GOLD 4.1.2.[75]

Acknowledgments

The CNRS (Centre National de la Recherche Scientifique) is gratefully acknowledged for financial support of this research. We thank the MRES (Ministère de la Recherche et de l'Enseignement Supérieur) for a financial fellowship to E.R. Our laboratory BioCIS-UMR 8076 is a member of the Laboratory of Excellence LERMIT supported by a grant from ANR (Agence Nationale de la Recherche, ANR-10-LABX-33). The work on tubulin was supported by a grant from ANR (ANR-09-BLAN-0071).

References

[1] G. R. Pettit, S. B. Singh, E. Hamel, C. M. Lin, D. S. Alberts, D. Garcia-Kendall, Experientia 45 (1989) 209-211.
[2] G. R. Pettit, M. R. Rhodes, D. L. Herald, E. Hamel, J. M. Schmidt, R. K. Pettit, J. Med. Chem. 48 (2005) 4087-4099.
[3] A. T. Mc Gown, B. W. Fox, Cancer Chemother. Pharmacol. 26 (1990) 79-81.
[4] G. R. Pettit, B. Toki, D. L. Herald, P. Verdier-Pinard, M. R. Boyd, E. Hamel, R. K. Pettit, J. Med. Chem. 41 (1998) 1688-1695.
[5] D. J. Chaplin, G. R. Pettit, S. A. Hill, Anticancer Res. 19 (1999) 189-195.
[6] D. J. Chaplin, S. A. Hill, Int. J. Radiat. Oncol. Biol. Phys. 54 (2002) 1491-1496
[7] G. R. Pettit, C. Temple, V. L. Narayanan, R. Varma, M. R. Boyd, G. A. Rener, N. Bansal, Anti-Cancer Drug Des. 10 (1995) 299-309.
[8] G. J. Rustin, S. M. Galbraith, H. Anderson, M. Stratford, L. K. Folkes, L. Sena, L. Gumbrell, P. M. Price, J. Clin. Oncol. 21 (2003) 2815-2822.
[9] A. Dowlati, K. Robertson, M. Cooney, W. P. Petros, M. Stratford, J. Jesberger, N. Rafie, B. Overmoyer, V. Makkar, B. Stambler, A. Taylor, J. Waas, J. S. Lewin, K. R. McCrae, S. C. Remick, Cancer Res. 62 (2002) 3408-3416.
[10] G. R. Pettit, M. R. Rhodes, D. L. Herald, D. J. Chaplin, M. R. L. Stratford, E. Hamel, R. K. Pettit, J.-C. Chapuis, D. Oliva, Anti-Cancer Drug Des. 13 (1998) 981-993.
[11] K. Ohsumi, T. Hatanaka, K. Fujita, R. Nakagawa, Y. Fukuda, Y. Nihei, Y. Suga, Y. Morinaga, Y. Akiyama, T. Tsuji, Bioorg. Med. Chem. Lett. 8 (1998) 3153-3158.
[12] S. Aprile, E. Del Grosso, G. C. Tron, G. Grosa, Drug Metab. Dispos. 35 (2007) 2252-2261.
[13] B. L. Flynn, G. S. Gill, D.W. Grobelny, J. H. Chaplin, D. Paul, A. F. Leske, T. C. Lavranos, D. K. Chalmers, S. A. Charman, E. Kostewicz, D. M. Shackleford, J. Morizzi, E. Hamel, M. K. Jung, G. Kremmidiotis, J. Med. Chem. 54 (2011) 6014-6027.
[14] N. H. Nam, Curr. Med. Chem. 10 (2003) 1692-1722 and references therein.
[15] G. C. Tron, T. Pirali, G. Sorba, F. Pagliai, S. Busacca, A. A. Genazzani, J. Med. Chem. 49 (2006) 3033-3044 and references therein.
[16] M. Marelli, F. Conforti, G. A. Statti, X. Cachet, S. Michel, F. Tillequin, F. Menichini, Curr. Med. Chem. 18 (2011) 3035-3081 and references therein.
[17] A. Chaudhary, S. N. Pandeya, P. Kumar, P. P. Sharma, S. Gupta, N. Soni, K. K. Verma, G. Bhardwaj, Mini-Rev. Med. Chem. 7 (2007) 1186-1205 and references therein.
[18] A. Cirla, J. Mann Nat. Prod. Rep. 20 (2003), 558-564 and references therein
[19] M. Arthuis, R. Pontikis, G. G. Chabot, J. Seguin, L. Quentin, S. Bourg, L. Morin-Allory, J.-C. Florent ChemMedChem 6 (2011) 1693-1705 and references therein.
[20] Y. Shan, J. Zhang, Z. Liu, M. Wang, Y. Dong, Curr. Med. Chem. 18 (2011) 523-538 and references therein.
[21] O. Provot, A. Giraud, J.-F. Peyrat, M. Alami, J.-D. Brion, Tetrahedron Lett. 46 (2005) 8547-8550.
[22] C. Mousset, A. Giraud, O. Provot, A. Hamze, J. Bignon, J. M. Liu, S. Thoret, J. Dubois, J.-D. Brion, M. Alami, Bioorg. Med. Chem. Lett. 18 (2008) 3266-3271.
[23] S. Messaoudi, A. Hamze, O. Provot, B. Tréguier, J. Rodrigo De Losada, J. Bignon, J. M. Liu, J. Wdzieczak-Bakala, S. Thoret, J. Dubois, J.-D. Brion, M. Alami, ChemMedChem 6 (2011) 488-497.
[24] C. Mousset, O. Provot, A. Hamze, J. Bignon, J.-D. Brion, M. Alami, Tetrahedron 64 (2008) 4287-4294.
[25] N. L'Hermite, A. Giraud, O. Provot, J.-F. Peyrat, M. Alami, J.-D. Brion, Tetrahedron 62 (2006) 1199-12002.
[26] E. Rasolofonjatovo, O. Provot, A. Hamze, J. Bignon, S. Thoret, J.-D. Brion, M. Alami, Eur. J. Med. Chem. 45 (2010) 3617-3626.
[27] A. Hamze, D. Veau, O. Provot, J.-D. Brion, M. Alami, J. Org. Chem. 74 (2009) 1337-1340.
[28] F. Liron, M. Gervais, J.-F. Peyrat, M. Alami, J.-D. Brion, Tetrahedron Lett. 44 (2003) 2789-2794.
[29] B. Tréguier, A. Hamze, O. Provot, J.-D. Brion, M. Alami, Tetrahedron Lett. 50 (2009) 6549-6552.
[30] S. Messaoudi, B. Tréguier, A. Hamze, O. Provot, J.-F. Peyrat, J. Rodrigo De Losada, J. M. Liu, J. Bignon, J. Wdzieczak-Bakala, S. Thoret, J. Dubois, J.-D. Brion, M. Alami, J. Med. Chem. 52 (2009) 4538-4542.
[31] A. Hamze, A. Giraud, S. Messaoudi, O. Provot, J.-F. Peyrat, J. Bignon, J. M. Liu, J. Wdzieczak-Bakala, S. Thoret, J. Dubois, J.-D. Brion, M. Alami, ChemMedChem 4 (2009) 1912-1924.
[32] A. Hamze, E. Rasolofonjatovo, O. Provot, C. Mousset, D. Veau, J. Rodrigo, J. Bignon, J. M. Liu, J. Wdzieczak-Bakala, S. Thoret, J. Dubois, J.-D. Brion, M. Alami, ChemMedChem 6 (2011) 2179-2191.
[33] M. A. Soussi, S. Aprile, S. Messaoudi, O. Provot, E. Del Grosso, J. Bignon, J. Dubois, J.-D. Brion, G. Grosa, M. Alami, ChemMedChem 6 (2011) 1781-1788.
[34] K. G. Pinney, V. P. Mocharla, Z. Chen, C. M. Garner, A. Ghatak, M. Hadimani, J. Kessler, J. M. Dorsey, K. Edvardsen, D. J. Chaplin, J. Prezioso, U. R. Ghatak, U.S. Patent Appl. Publ. (2004**)** 20040043969 A1.
[35] K. G. Pinney, V. P. Mocharla, P. Vani, Z. Chen, C. M. Garner, A. Ghatak, M. Hadimani, J. Kessler, J. M. Dorsey PCT Int. Appl. (2001), WO 2001068654 A2 20010920.
[36] E. Rasolofonjatovo, O. Provot, A. Hamze, J. Rodrigo, J. Bignon, J. Wdzieczak-Bakala, D. Desravines, J. Dubois, J.-D. Brion, M. Alami, Eur. J. Med. Chem. 52 (2012) 22-32.

[37] E. Rasolofonjatovo, B. Tréguier, O. Provot, A. Hamze, E. Morvan, J.-D. Brion, M. Alami, Tetrahedron Lett. 52 (2011) 1036-1040.
[38] E. Rasolofonjatovo, B. Tréguier, O. Provot, A. Hamze, J.-D. Brion, M. Alami, Eur. J. Org. Chem. (2012) 1603-1615.
[39] M. Sriram, J. J. Hall, N. C. Grohmann, T. E. Strecker, T. Wootton, A. Franken, M. L. Trawick, K. G. Pinney, Bioorg. Med. Chem. 16 (2008) 8161-8171.
[40] K. G. Pinney, M. Sriram, U.S. PCT Appl. WO (2006), 2006138427 A2.
[41] J. Barluenga, P. Moriel, C. Valdés, F. Aznar, Angew. Chem. Int. Ed. 46 (2007) 5587-5590.
[42] J. Barluenga, C, Valdés, Angew. Chem. Int. Ed. 50 (2011) 7486-7500.
[43] M. Alami, F. Liron, M. Gervais, J.-F. Peyrat, J.-D. Brion, Angew. Chem., Int. Ed. 41 (2002), 1578-1580.
[44] A. Hamze, D. Veau, O. Provot, J.-D. Brion, M. Alami, J. Org. Chem. 74 (2009), 1337-1340.
[45] G. S. Gill, D. W. Grobelny, J. H. Chaplin, B. L. Flynn, J. Org. Chem. 73 (2008) 1131-1134.
[46] K. Sonogashira, Y. Tohda, N. Hagihara, Tetrahedron Lett. 16 (1975) 4467-4470.
[47] M. Alami, F. Ferri, G. Linstrumelle, Tetrahedron Lett. 34 (1993) 6403-6406.
[48] N. Olivi, E. Thomas, J.-F. Peyrat, M. Alami, J.-D. Brion, Synlett (2004) 2175-2179.
[49] G. Le Bras, O. Provot, J.-F. Peyrat, M. Alami, J.-D. Brion, Tetrahedron Lett. 47 (2006) 5497-5501.
[50] M. Jacubert, O. Provot, J.-F. Peyrat, A. Hamze, J.-D. Brion, M. Alami, Tetrahedron 66 (2010) 3775-3787.
[51] M. Jacubert, A. Hamze, O. Provot, J.-F. Peyrat, J.-D. Brion, M. Alami, Tetrahedron Lett. 50 (2009) 3588-3592.
[52] G. Le Bras, A. Hamze, S. Messaoudi, O. Provot, P.-B. Le Calvez, J.-D. Brion, M. Alami, Synthesis (2008) 16071611.
[53] E. Brachet, A. Hamze, J.-F. Peyrat, J.-D. Brion, M. Alami, Org. Lett. 12 (2010) 4042-4045.
[54] For the synthesis of 5-arylbenzoxepins according to Suzuki couplings, see ref 44 and I. Barrett, M. J. Meegan, R. B. Hughes, M. Carr, A. J. S. Knox, N. Artemenko, G. Golfis, D. M. Zisterer, D. G. Lloyd, Bioorg. Med. Chem. 16 (2008) 9554-9573.
[55] I. Barrett, M. Carr, N. O'Boyle, L. M. Greene, A. J. S. Knox, D. G. Lloyd, D. M. Zisterer, M. J. Meegan, J. Enzym. Inhib. Med. Chem. 25 (2010) 180-194.
[56] M. Jacubert, A. Tikad, O. Provot, A. Hamze, J.-D. Brion, M. Alami, Eur. J. Org. Chem. (2010), 4492-4500.
[57] A. Bekaert, O. Provot, O. Rasolojaona, M. Alami, J.-D. Brion, Tetrahedron Lett. 46 (2005) 4187-4191.
[58] M. Jacubert, A. Tikad, O. Provot, A. Hamze, J.-D. Brion, M. Alami, Eur. J. Org. Chem. 2010, 4492-4500.
[59] During the preparation of this manuscript, a similar strategy for the synthesis of benzosuberene derivatives was reported, see: Z. Chen, C. J. O'Donnell, A. Maderna, Tetrahedron Lett. 53 (2012) 64-66.
[60] S. Messaoudi, J.-D. Brion, M. Alami, Adv. Synth. Catal. 352 (2010) 1677-1687.
[61] S. Messaoudi, J.-D. Brion, M. Alami, Mini-Rev. Org. Chem. 8 (2011) 448-454.
[62] J. Chen, Y. Zhang, L. Yang, X. Zhang, J. Liu, L. Li, H. Zhang, Tetrahedron 63 (2007) 4266-4270.
[63] K. Anderson, T. Ikawa, R. E. Tundel, S. L. Buchwald, J. Am. Chem. Soc. 128 (2006) 10694-10695.
[64] A. Giraud, O. Provot, A. Hamze, J.D. Brion, M. Alami, Tetrahedron Lett., 49 (2008) 1107-1110.
[65] K. Ohsumi, R. Nakagawa, Y. Fukuda, T. Hatanaka, Y. Morinaga, Y. Nihei, K. Ohishi, Y. Suga, Y. Akiyama, T. Tsuji, J. Med. Chem. 41 (1998) 3022-3032.
[66] M. Cushman, D. Nagarathnam, D. Gopal, H.-M. He, C. M. Lin, E. Hamel, J. Med. Chem. 35 (1992) 2293-2314.
[67] Y. G. Tong, X. W. Zhang, M. Y. Geng, J. M. Yue, X. L. Xin, T. Fang, S. Xu, L. J. Tong, M. H. Li, C. Zhang, W. H. Li, L. P. Lin, J. Ding, Mol. Pharmacol. 69 (2006) 1226-1233.
[68] R. B. Ravelli, B. Gigant, P. A. Curmi, I. Jourdain, S. Lachkar, A. Sobel, M. Knossow, Nature 428 (2004) 198-202.
[69] M. Rosillo, G. Dominguez, L. Casarrubios, U. Amador, J. Pérez-Castells, J. Org. Chem. 69 (2004), 2084-2093.
[70] A.R. Pereira, W.K. Strangman, F. Marion, L. Feldberg, D. Roll, R. Mallon, I. Hollander, R.J. Anderson, J. Med. Chem. 53 (2010) 8523-8533.
[71] M. L. Shelanski, F. Gaskin, C. R. Cantor, Proc. Natl. Acad. Sci. U.S.A. 70 (1973) 765-768.
[72] D. M. Barron, S. K. Chatterjee, R. Ravindra, R. Roof, E. Baloglu, D. G. I. Kingston, S. Bane, Anal. Biochem. 315 (2003) 49-56.
[73] C. Venot, M. Maratrat, C. Dureuil, E. Conseiller, L. Bracco, L. Debussche, EMBO J. 17 (1998) 4668-4679.
[74] D. A. Case, T. A. Darden, T. E. Cheatham, III, C. L. Simmerling, J. Wang, R. E. Duke, R. Luo, K. M. Merz, B. Wang, D. A. Pearlman, M. Crowley, S. Brozell, V. Tsui, H. Gohlke, J. Mongan, V. Hornak, G. Cui, P. Beroza, C. Schafmeister, J. W. Caldwell, W. S. Ross, P. A. Kollman, AMBER8: University of California, San Francisco, (2008).
[75] M. L. Verdonk, J. C. Cole, M. J. Hartshorn, C. W. Murray, R. D. Taylor, Proteins 52 (2003) 609-623.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Legends to Figures, Schemes and Tables

Figure 1. Representative inhibitors of tubulin polymerization and rational drug design from CA-4 and *iso*CA-4 to dihydrobenzoxepin analogues **6**.

Figure 2. Retrosynthesis of 5-arylbenzoxepins **6**.

Scheme 1. Synthesis of vinylstannane **9a** and vinyliodide **10a**. Reagent and conditions: a) $PdCl_2(PPh_3)_2$ (2 mol%), Bu_3SnH (1.2 equiv), THF, 20 °C; b) I_2, CH_2Cl_2, 20 °C.

Scheme 2. Synthesis of dihydrobenzoxepins **6b,c**. Reagent and conditions: a) MOMCl (2 equiv), *i*Pr_2NH (2 equiv), CH_2Cl_2; b) But-3-yn-1-ol (1.1 equiv), $PdCl_2(PPh_3)_2$ (8 mol%), CuI (15 mol%), Et_3N; c) PTSA (1 equiv), H_2O, 100 °C, sealed tube; d) DEAD (1.5 equiv), PPh_3 (1.5 equiv), THF; e) $TsNHNH_2$ (2.5 equiv), PTSA (20 mol%), EtOH reflux; f) Pd_2dba_3 (10 mol%), XPhos (20 mol%), *t*BuOLi (2 equiv), ArI (1 equiv), 90 °C, sealed tube; g) $AlCl_3$ (5 equiv), CH_2Cl_2.

Scheme 3. Synthesis of dihydrobenzoxepins **6b**, **6d-g** and tetrahydrobenzoxepins **25a,b**. Reagent and conditions: a) (i) $Hg(OAc)_2$ (1 equiv), NaBr (1 equiv), 50 °C, EtOH; (ii) MPHT (1 equiv), CH_3CN; b) 3-bromoprop-1-ene (1.2 equiv), K_2CO_3 (1.2 equiv), CH_3CN; c) vinylmagnesium chloride (1.2 equiv), THF; d) Grubbs II catalyst, 5 mol%, CH_2Cl_2, 20 °C; e) H_2, Pd/C, MeOH, AcOH cat.; f) PCC, CH_2Cl_2; g) $TsNHNH_2$ (1 equiv), PTSA (10 mol%), $MgSO_4$ (1 equiv), EtOH, reflux; h) Pd_2dba_3 (10 mol%), XPhos (40 mol%), *t*BuOLi (2 equiv), 3,4,5-trimethoxyiodobenzene (1.2 equiv), 90 °C, sealed tube; i) Pd_2dba_3 (5 mol%), *t*BuXPhos (15 mol%), KOH (3 equiv), dioxane-H_2O, 90 °C, sealed tube; j) CuI (10 mol%), TMEDA (6 mol%), NaN_3 (2 equiv), potassium ascorbate (6 mol%) DMSO-H_2O, 90 °C, sealed tube; k) $Pd(OAc)_2$ (1 mol%), PPh_3 (2.5 mol%), *n*BuOH, 100 °C, sealed tube; l) *n*But-3-yn-1-ol (1.2 equiv), $PdCl_2(PPh_3)_2$ (5 mol%), PPh_3 (15 mol%), CuI (15 mol%), Et_2NH (2 equiv), DMF, 120 °C, MWI.

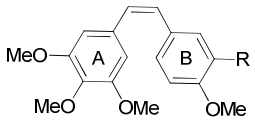
Table 1. Cytotoxic activity of dihydrobenzoxepins **6b-g** and tetrahydrobenzoxepins **25a,b** against HCT116[a]

Table 2. Cytotoxic activity and inhibition of tubulin polymerization of selected compounds

Figure 3. Effects of benzoxepin **6b** at a concentration of 5.0 nM on cell cycle distribution in various cancer cell lines.

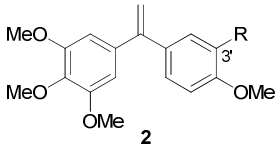
Figure 4. Apoptotic effects of **6b** in HCT116 and H1299 cells. The results are expressed in the percentage of apoptotic cells detected following 24 h of treatment with **6b** at different concentrations.

Figure 5. Docked pose of **6b** (gray) overlayed with *iso*CA-4 (blue) in the tubulin binding site.



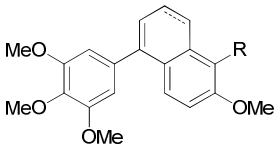
1

CA-4	**1a**	R = OH
CA-4P	**1b**	R = OPO_3Na_2
NH_2CA-4	**1c**	R = NH_2
FCA-4	**1d**	R = F

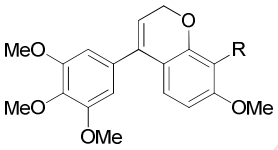


2

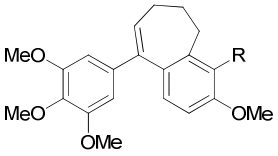
***iso*CA-4**	**2a**	R = OH
***iso*NH_2CA-4**	**2b**	R = NH_2
***iso*FCA-4**	**2c**	R = F
	2d	R = C≡C-CH_2OH



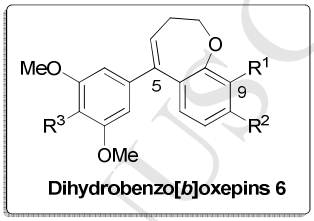
(Dihydro)naphtalenes 3



Chromenes 4[17]



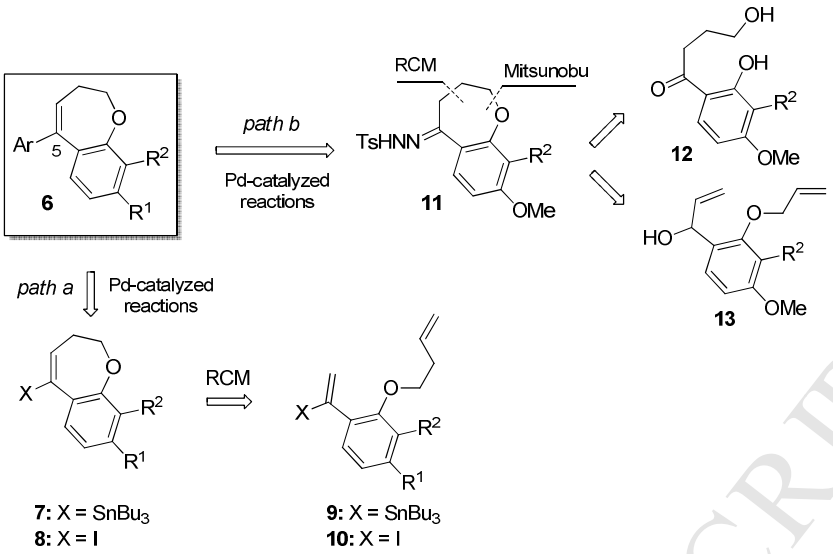
Benzosuberenes 5



Dihydrobenzo[*b*]oxepins 6

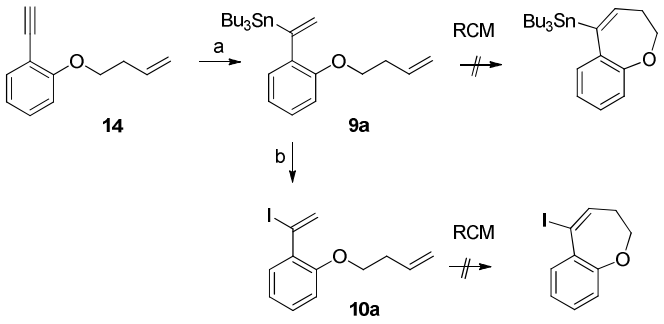
<Figure 1>

ACCEPTED MANUSCRIPT



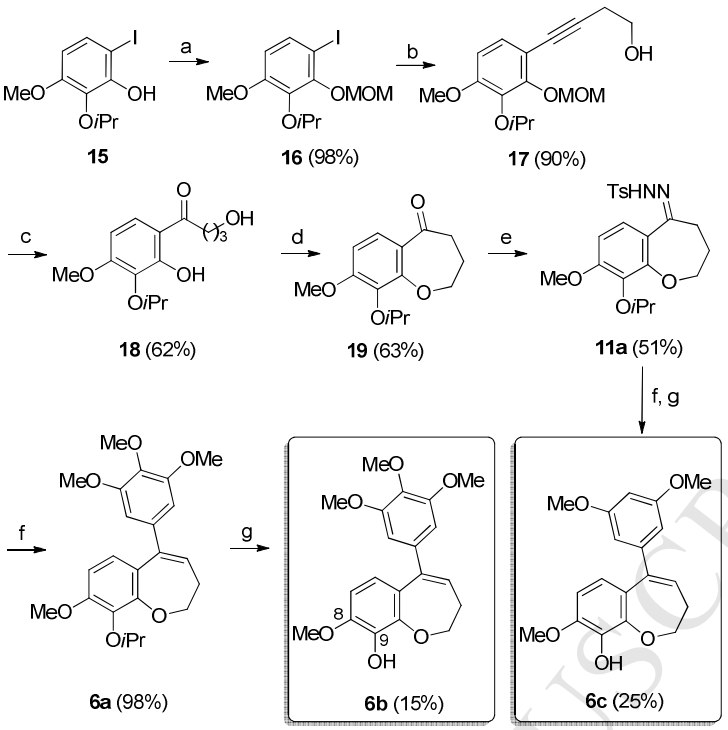
<Figure 2>

ACCEPTED MANUSCRIPT



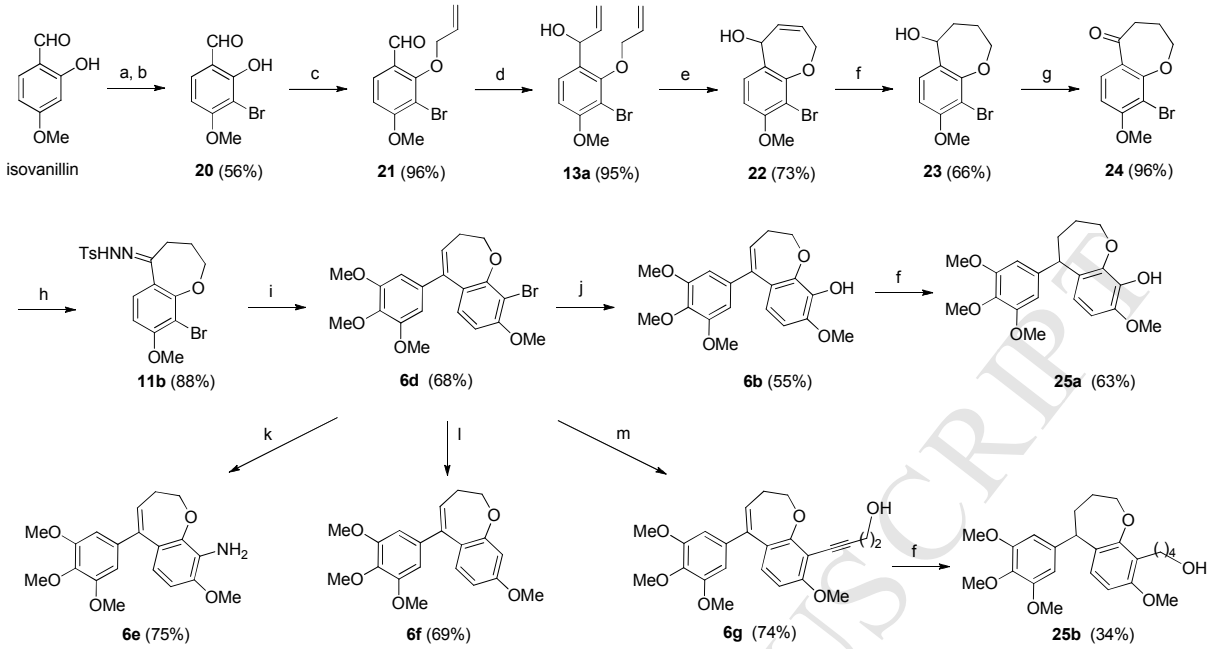
<Scheme 1>

ACCEPTED MANUSCRIPT



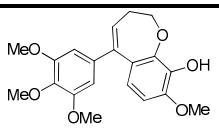
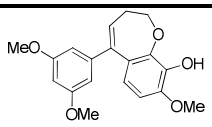
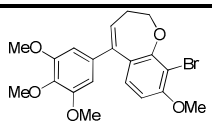
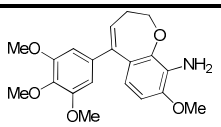
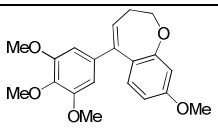
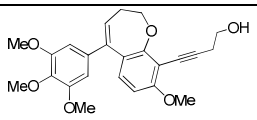
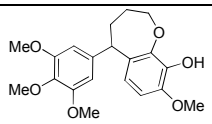
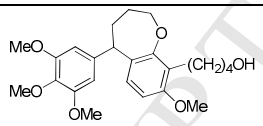
<Scheme 2>

ACCEPTED MANUSCRIPT



<Scheme 3>

ACCEPTED MANUSCRIPT

Cytotoxicity against HCT-116[a] cells				
Cpnd				
GI_{50}[b] [nM]	**6b**: 1.5 ± 0.1	**6c**: 25 ± 3	**6d**: 170 ± 18	**6e**: 22 ± 3
Cpnd				
GI_{50}[b] [nM]	**6f**: 85 ± 10	**6g**: 250 ± 22	**25a**: 20 ± 3	**25b**: Na[c]
GI_{50}[b] [nM]	***iso*CA-4**: 3 ± 0.2[d]	**CA-4**: 2 ± 0.2[d]		

[a]HCT-116 Human colon carcinoma. [b]GI_{50} is the concentration of compound needed to reduce cell growth by 50% following 72 h cell treatment with the tested drug (average of three experiments). [c]Non active. [d]The GI_{50} values for CA-4, *iso*CA-4 were determined in this study.

<Table 1>

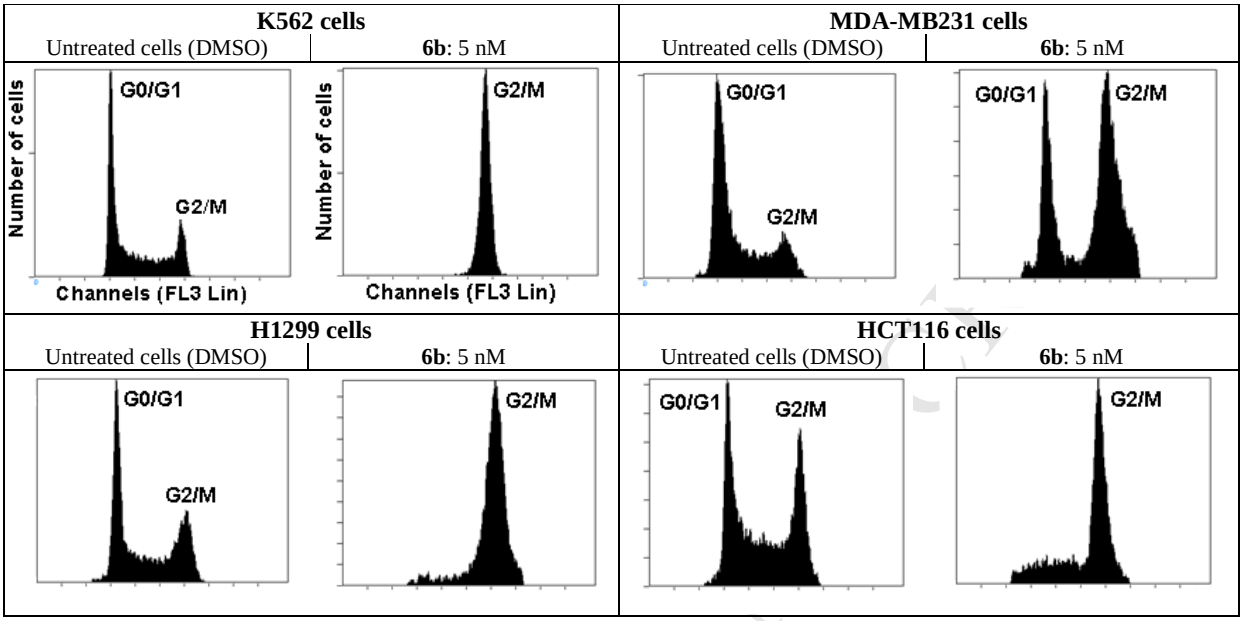
ACCEPTED MANUSCRIPT

Cpnd	Cytotoxicity GI_{50}[a] [nM]				Inhibition of tubulin polymerization IC_{50}[c] [μM]
	HCT116[b]	K562[b]	H1299[b]	MDA-MB231[b]	
6b	1.5 ± 0.1	8 ± 1	4.5 ± 0.3	3 ± 0.2	3.8 ± 0.4
6c	25 ± 3	30 ± 4	32 ± 3	28 ± 4	3.2 ± 0.3
6e	22 ± 3	40 ± 4	30 ± 2	8 ± 1	3.6 ± 0.4
6f	85 ± 10	100 ± 9	180 ± 15	250 ± 23	3.9 ± 0.4
25a	20 ± 3	50 ± 5	7 ± 1	15 ± 2	3.3 ± 0.3
***iso*CA-4**[d]	3 ± 0.2	5 ± 0.3	3 ± 0.2	4 ± 0.3	1.5 ± 0.2

[a]GI_{50} is the concentration of compound needed to reduce cell growth by 50% following 72 h cell treatment with the tested drug (average of three experiments). [b]HCT116, colon carcinoma; K562, myelogenous leukaemia; H1299, non-small cell lung carcinoma; MDA-MB231 hormone-independent breast cancer. [c]ITP, Inhibition of Tubulin Polymerization; IC_{50} is the concentration of compound required to inhibit 50% of the rate of microtubule assembly (average of three experiments). [d]The GI_{50} and IC_{50} values (cytotoxicity and ITP, respectively) for *iso*CA-4 were determined in this study.

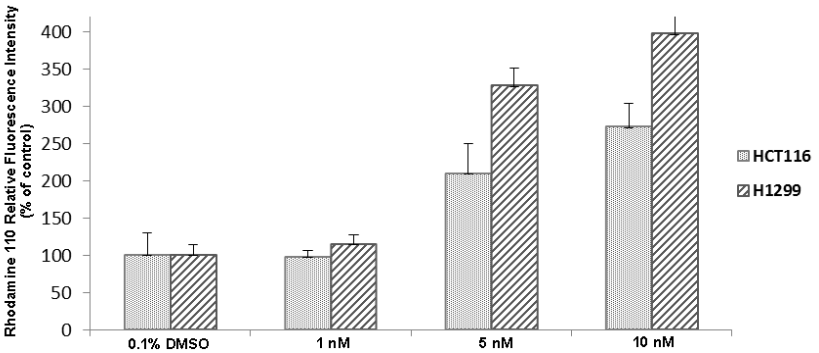
<Table 2>

ACCEPTED MANUSCRIPT



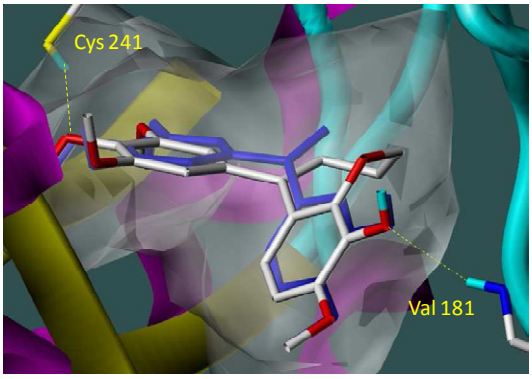
<Figure 3>

ACCEPTED MANUSCRIPT



<Figure 4>

ACCEPTED MANUSCRIPT



<Figure 5>

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Highlights

Benzoxepins **6** as restricted-analogues of *iso*CA-4 have been synthesized.

Highly cytotoxic benzoxepin **6b** inhibited tubulin assembly at a micromolar level.

Compound **6b** arrested the cellular cycle in the G_2/M phase and induced apoptosis.

Presumptive binding mode of **6b** and *iso*CA4 are similar on β-tubulin.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Supplementary data

Design, synthesis and anticancer properties of 5-arylbenzoxepins as conformationally restricted *iso*Combretastatin A-4 analogues

Evelia Rasolofonjatovo,[a] Olivier Provot,*[a] Abdallah Hamze,[a] Jordi Rodrigo,[a] Jérome Bignon,[b] Joanna Wdzieczak-Bakala,[b] Christine Lenoir,[b] Déborah Desravines,[b] Joëlle Dubois,[b] Jean-Daniel Brion,[a] Mouad Alami*[a]

[a] Univ. Paris-Sud, CNRS, BioCIS-UMR 8076, LabEx LERMIT, Laboratoire de Chimie Thérapeutique, Faculté de Pharmacie, 5 rue J.-B. Clément, Châtenay-Malabry, F-92296, France.

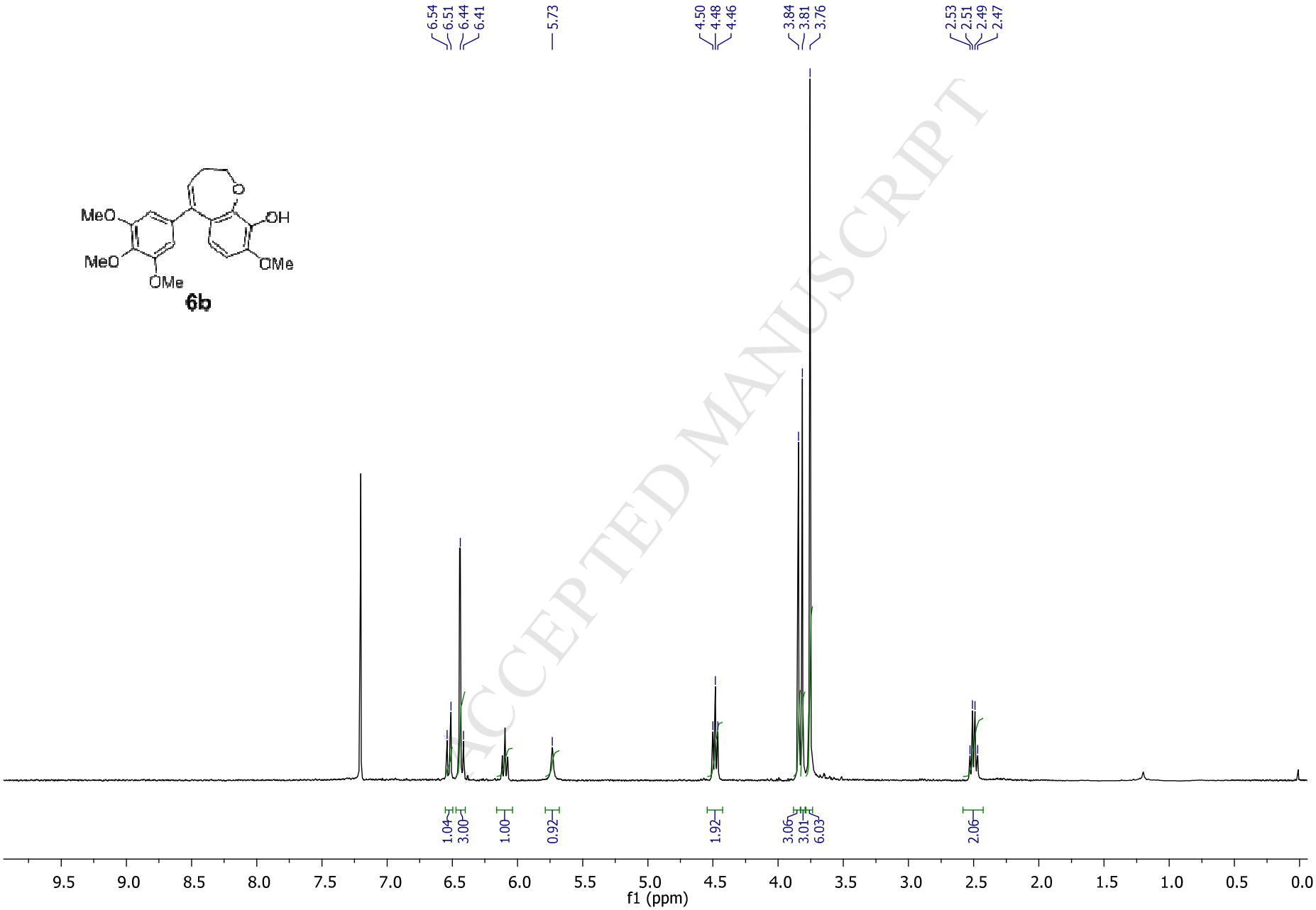
[b] Institut de Chimie des Substances Naturelles, UPR 2301, CNRS avenue de la terrasse, F-91198 Gif sur Yvette, France.

[*] Corresponding authors. Tel.: +33 1 46 83 58 47. Fax: +33 1 46 83 58 28. E-mail adress: olivier.provot@u-psud.fr (O.P.) or mouad.alami@u-psud.fr (M.A.).

ACCEPTED MANUSCRIPT

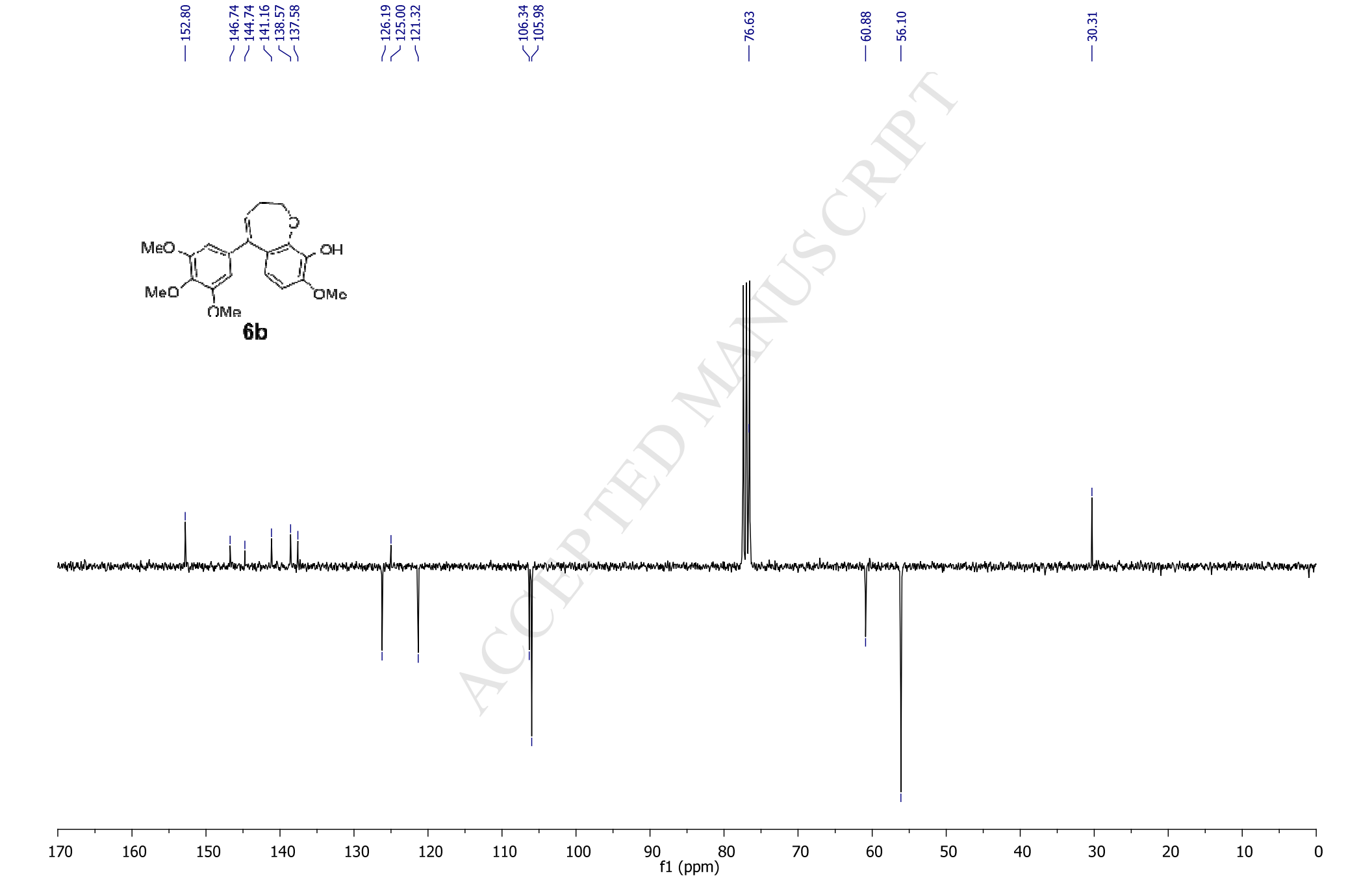
Contents

ACCEPTED MANUSCRIPT



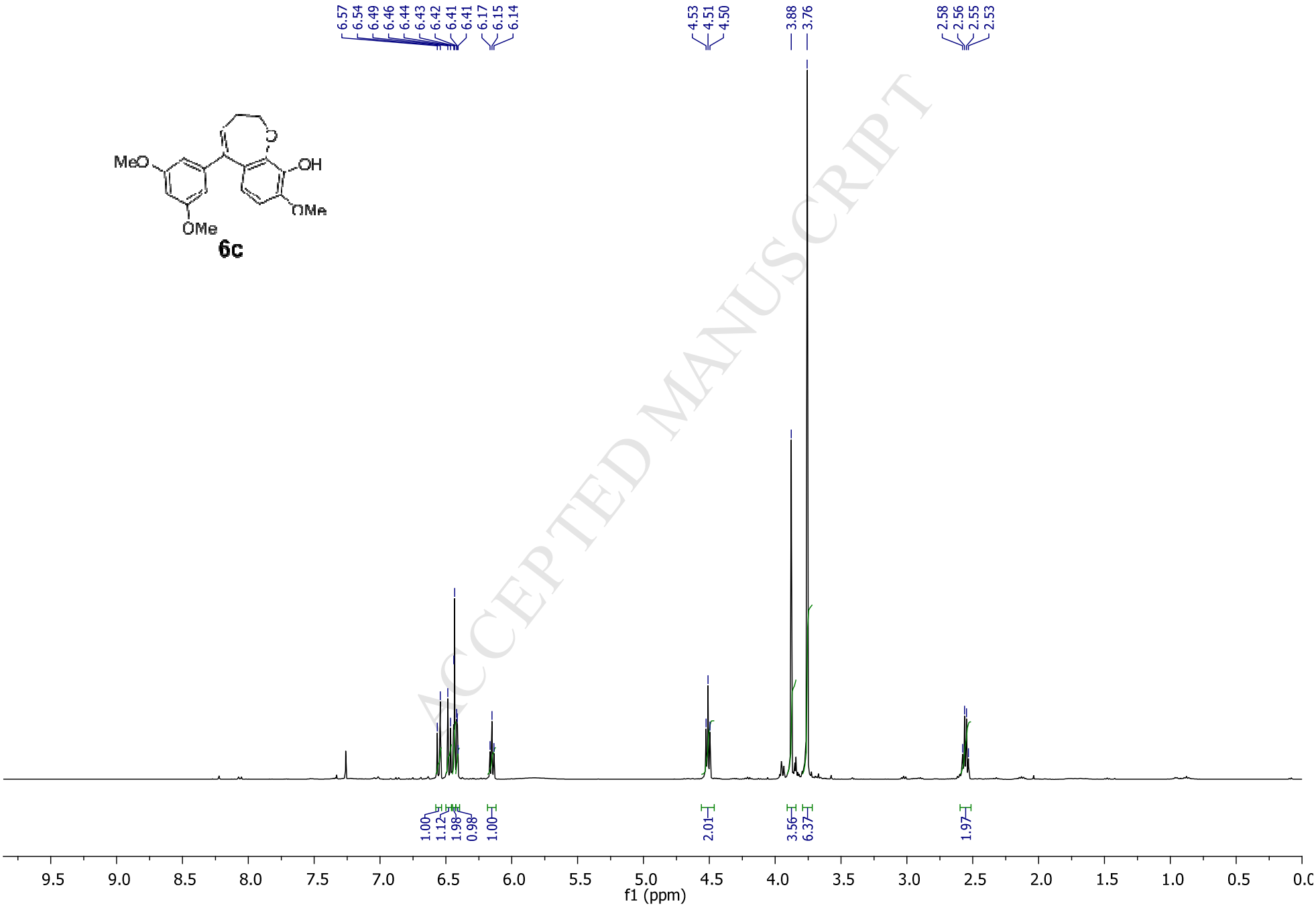
ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT



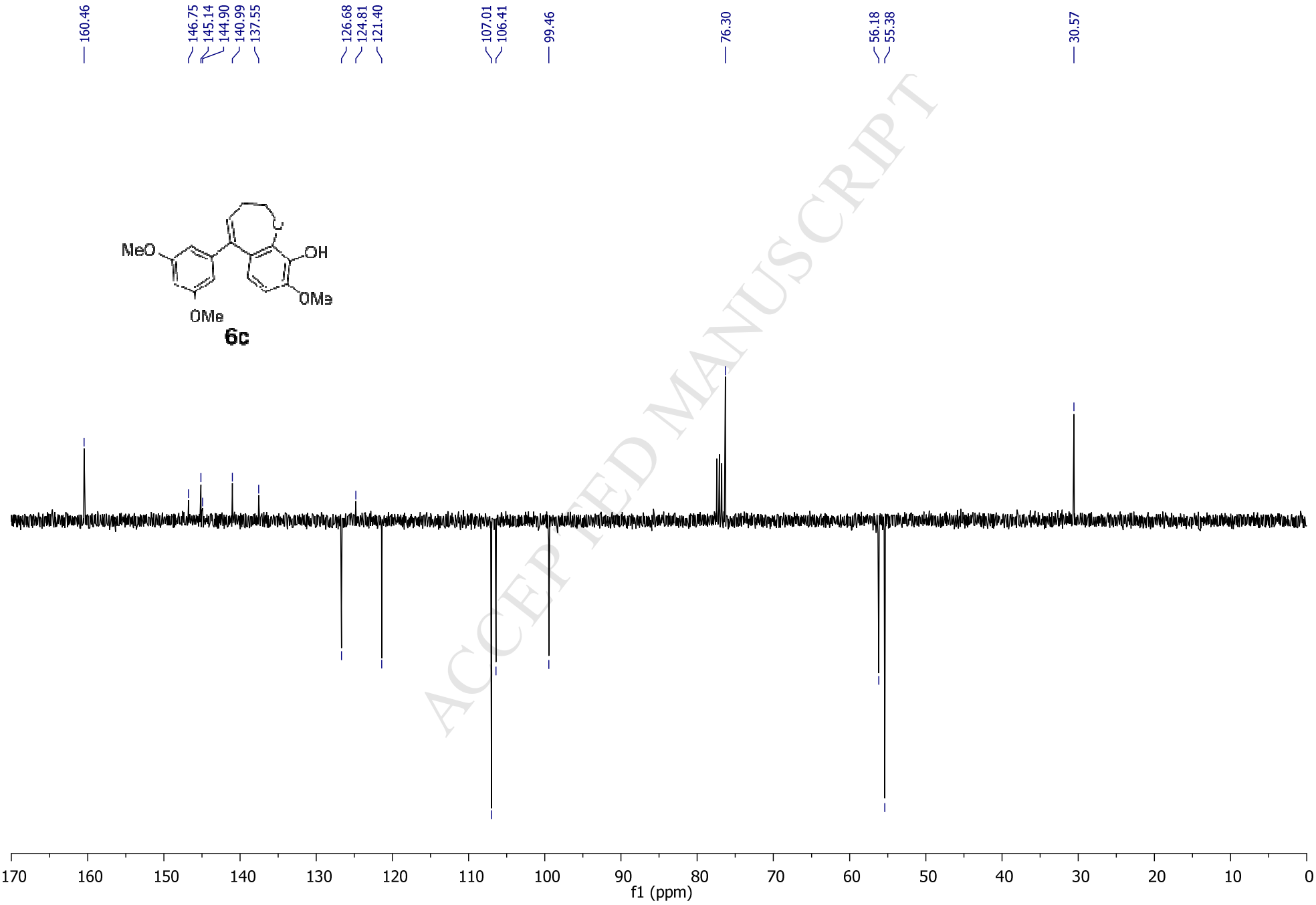
ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT



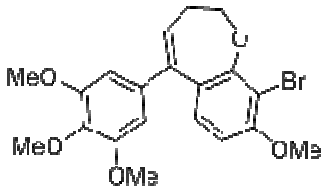
ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT



ACCEPTED MANUSCRIPT

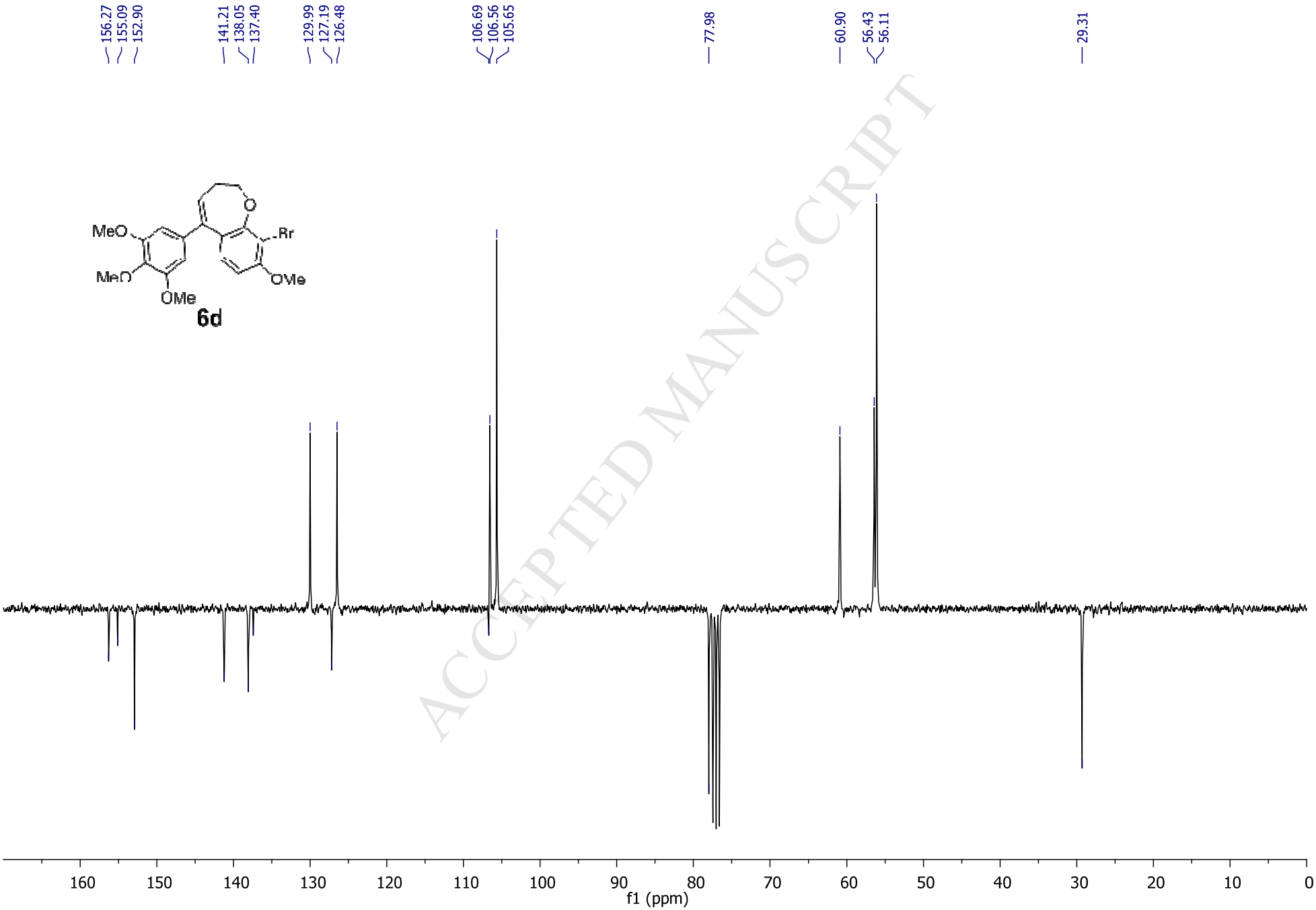
ACCEPTED MANUSCRIPT



6d

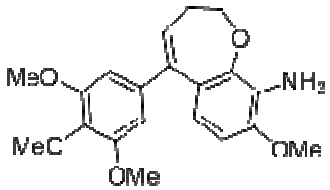
ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT



ACCEPTED MANUSCRIPT

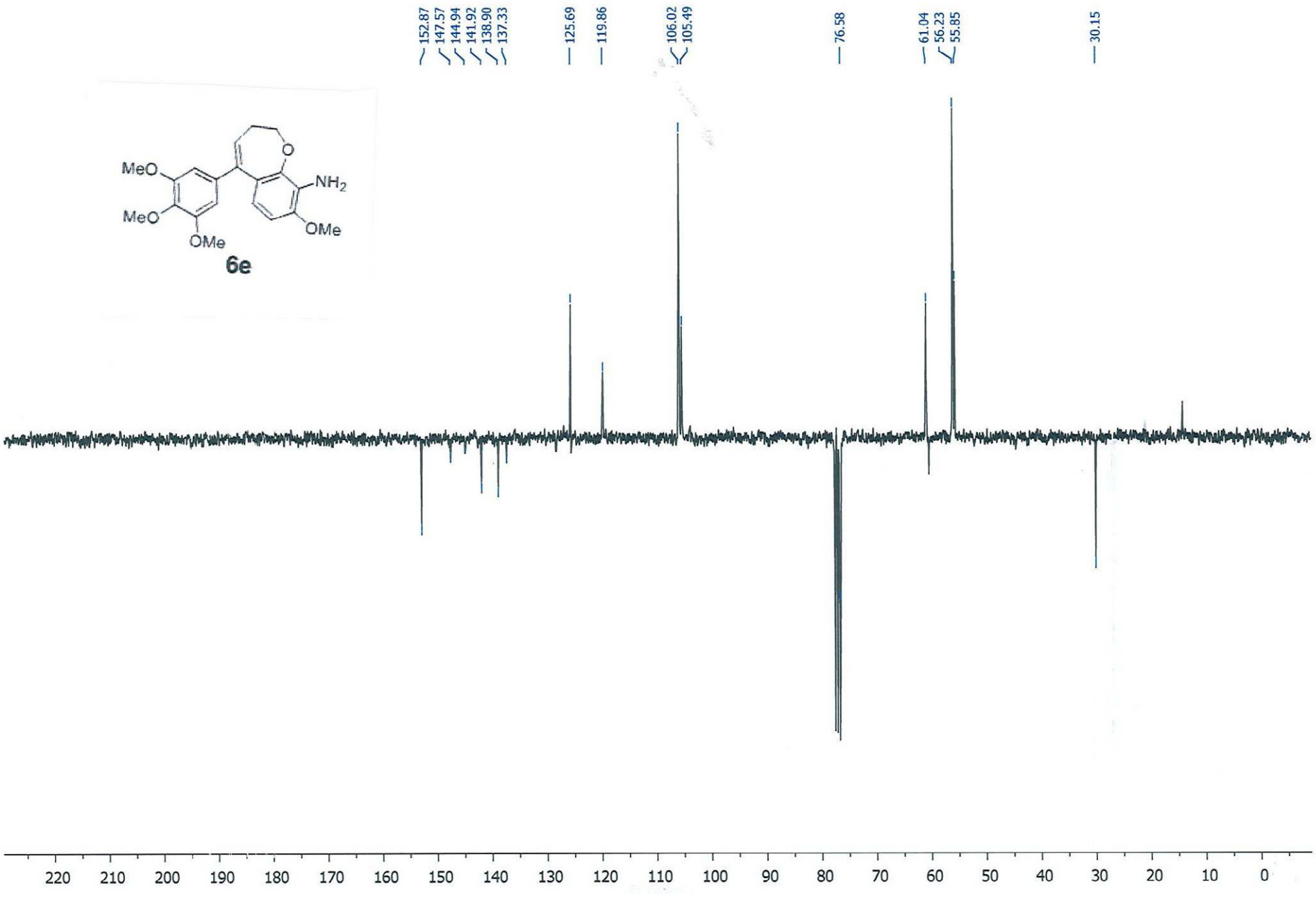
ACCEPTED MANUSCRIPT



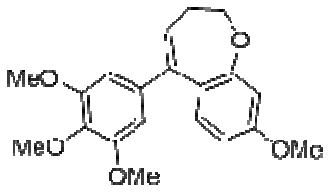
6e

ACCEPTED MANUSCRIPT

f1 (ppm)


152.87
147.57
144.94
141.92
138.90
137.33
125.69
119.86
106.02
105.49
76.58
61.04
56.23
55.85
30.15
MeO
MeO
OMe
NH2
OMe
O
6e
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

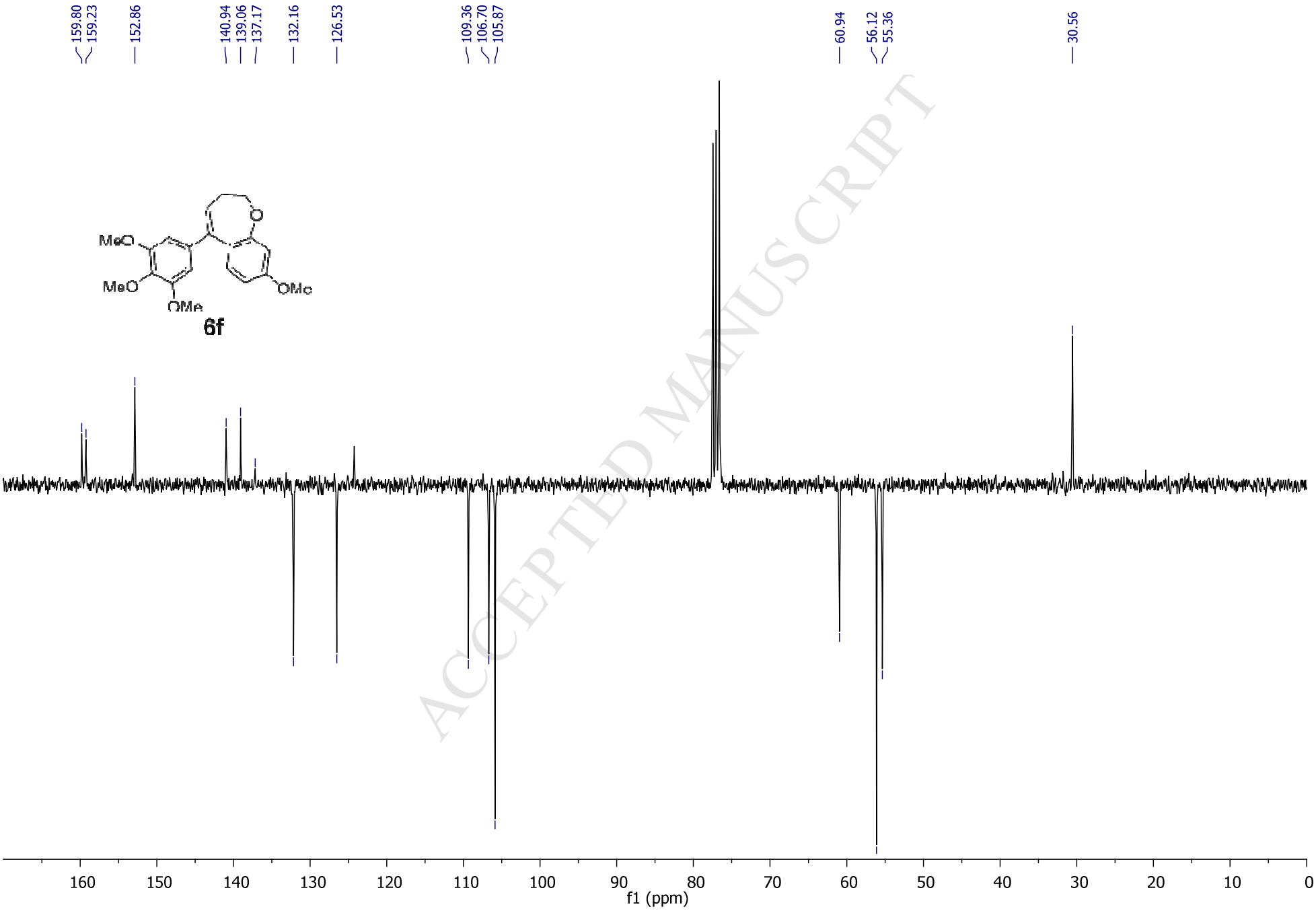
ACCEPTED MANUSCRIPT



6f

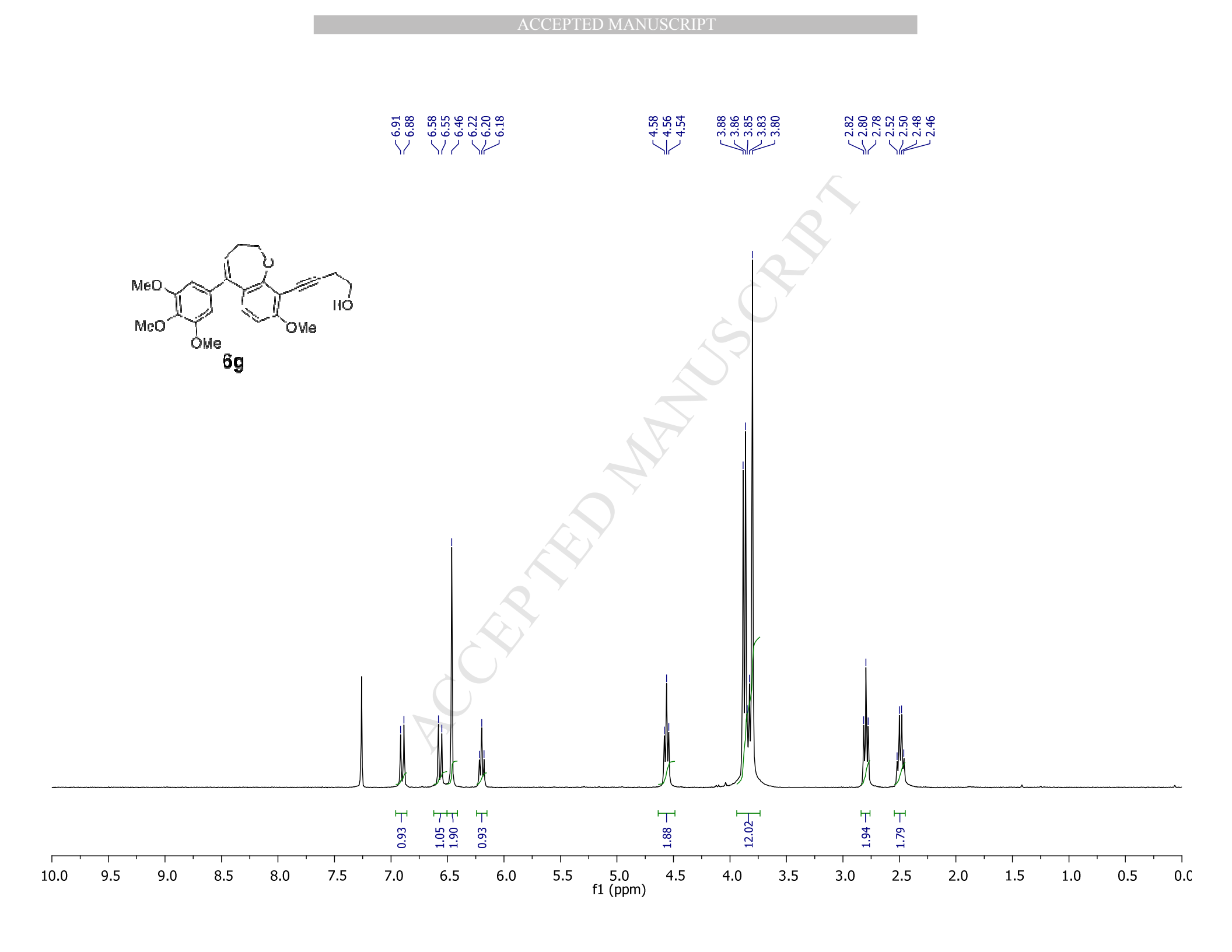
ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT



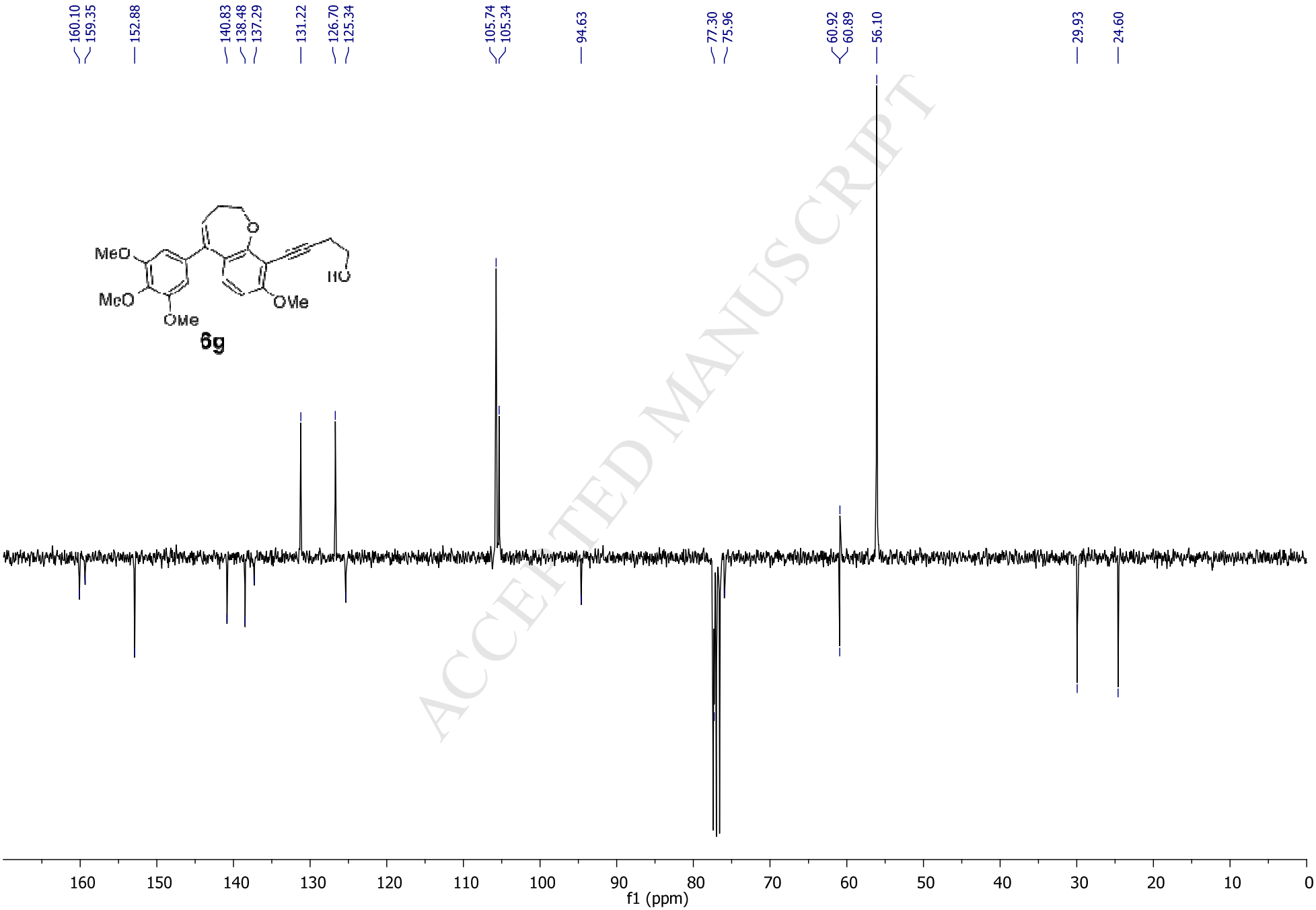
ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

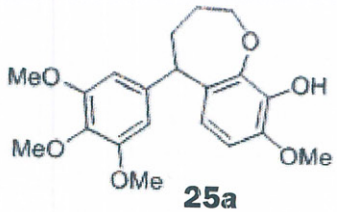


ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT



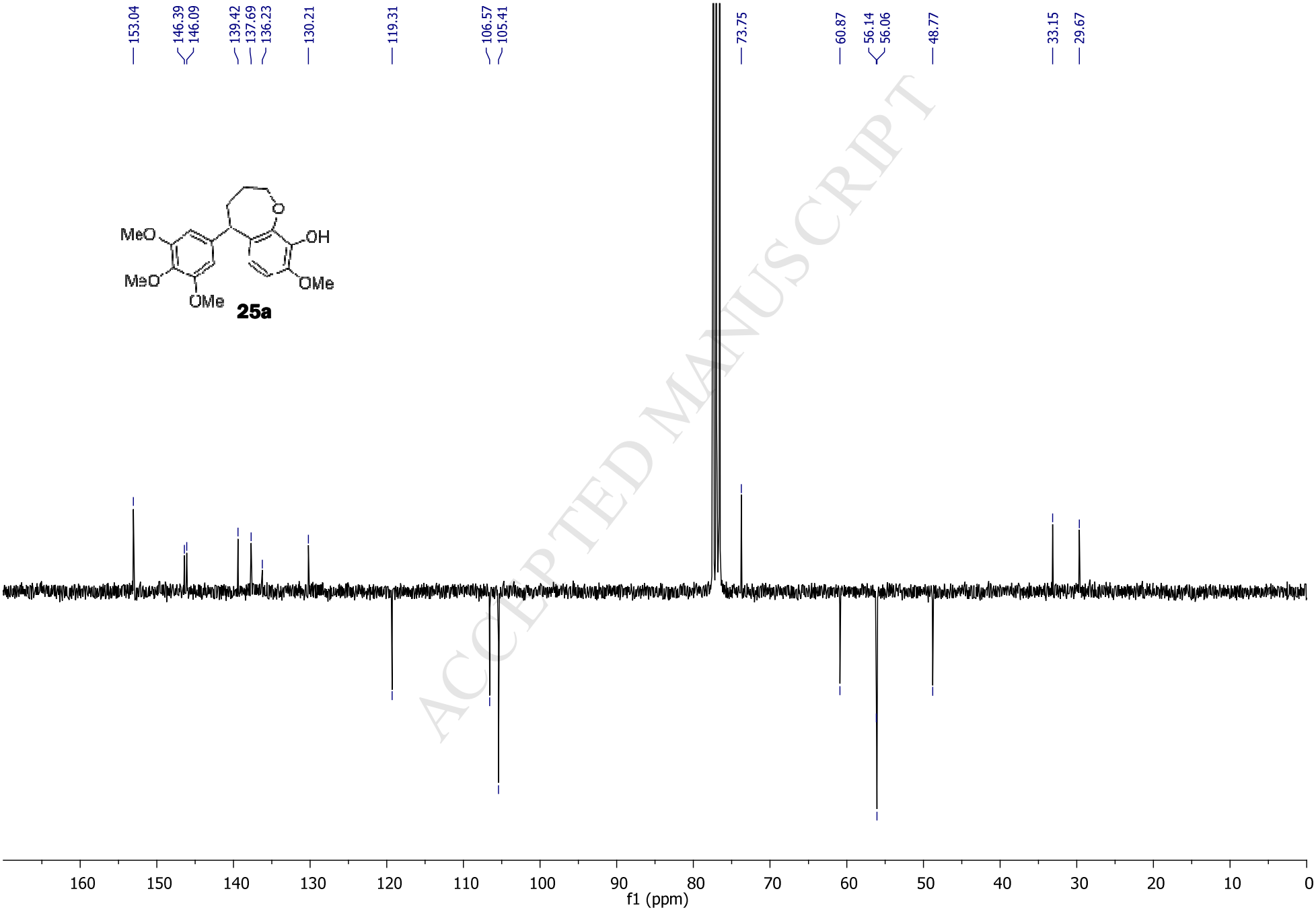
ACCEPTED MANUSCRIPT


MeO
MeO
OMe
O
OH
OMe

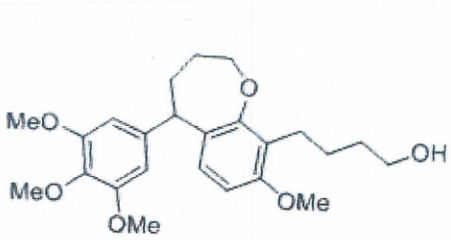
25a

6.52
6.49
6.44
6.26
6.23
5.82
4.19
4.02
3.86
3.85
3.81
2.13
2.04
1.03
2.00
0.96
0.97
2.04
1.03
6.41
6.17
2.32
1.94
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

ACCEPTED MANUSCRIPT



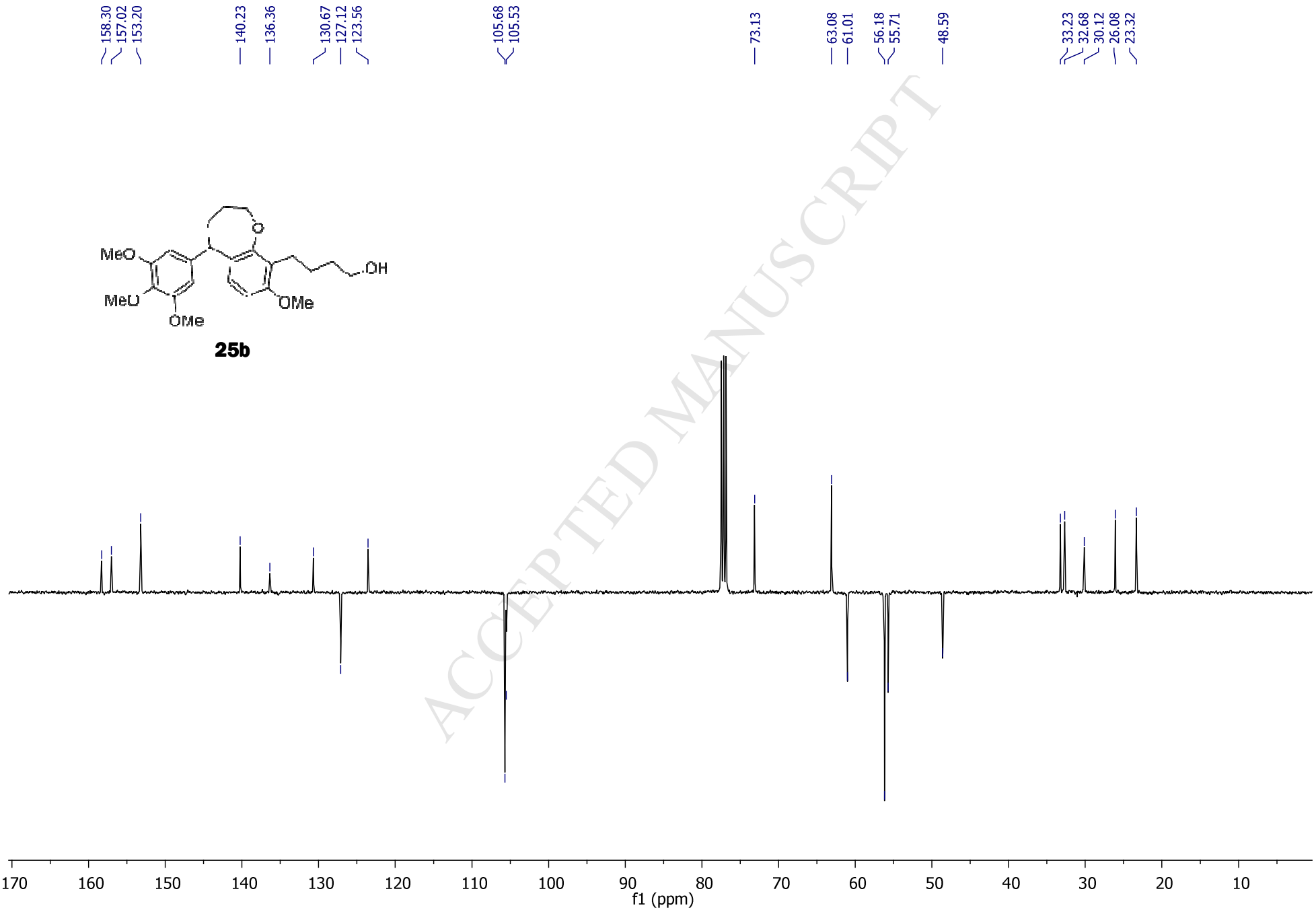
ACCEPTED MANUSCRIPT


MeO
MeO
OMe
O
OMe
OH

25b

6.56
6.53
6.48
6.45
4.20
4.17
3.85
3.81
3.77
3.72
3.70
3.68
2.71
2.12
2.01
1.63
1.56
1.00
3.09
2.09
3.21
5.98
3.19
3.02
2.11
5.05
4.06
f1 (ppm)

ACCEPTED MANUSCRIPT



ACCEPTED MANUSCRIPT