

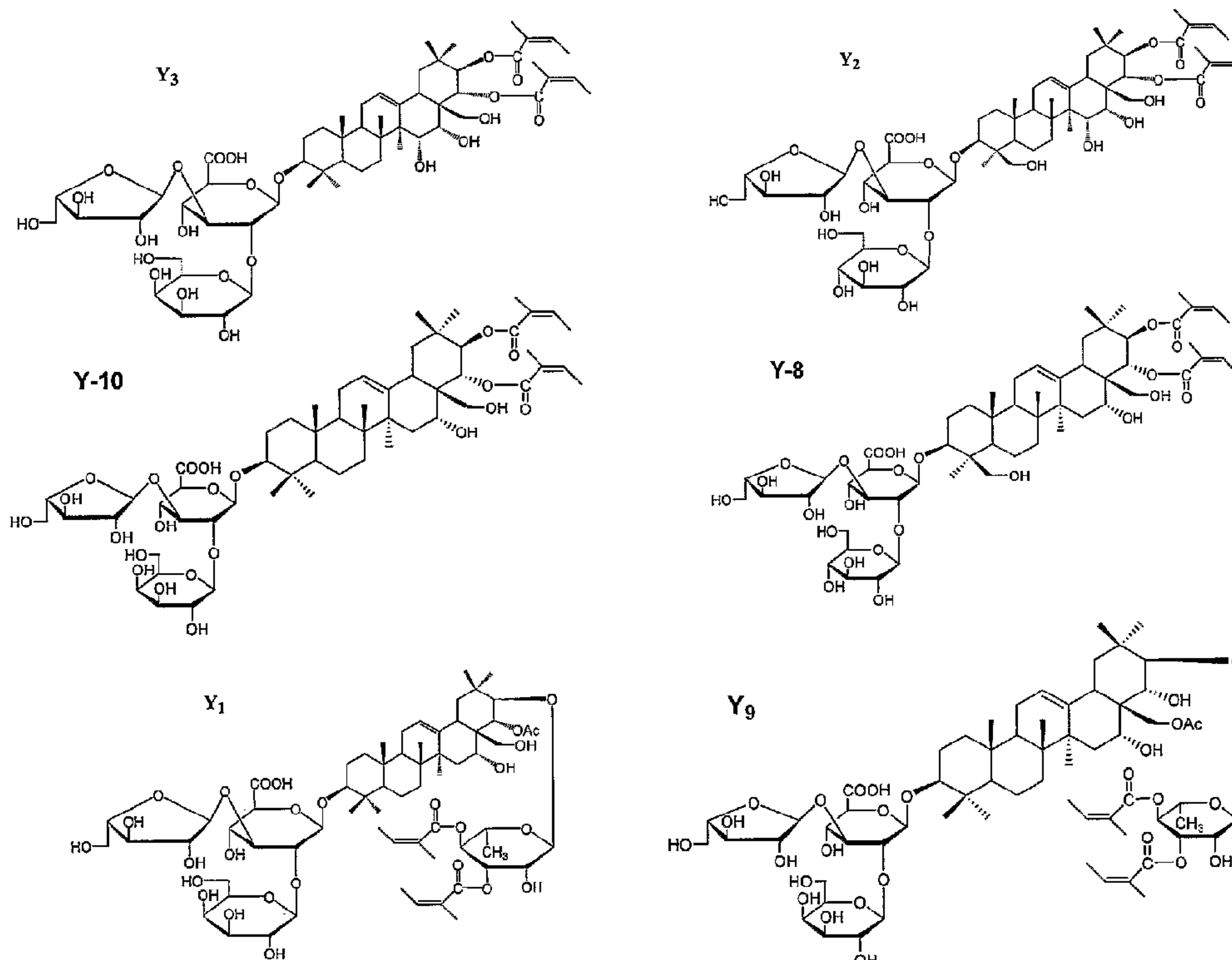


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(54) **Titre : COMPOSES ANTITUMORAUX COMPRENANT DES GROUPES ANGELOYL**
(54) **Title: ANTI-TUMOR COMPOUNDS WITH ANGELOYL GROUPS**

Structure of six anticancer Saponins with biangeloyl groups (Y3 (Y), Y2, Y8, Y10, Y1 and Y9)



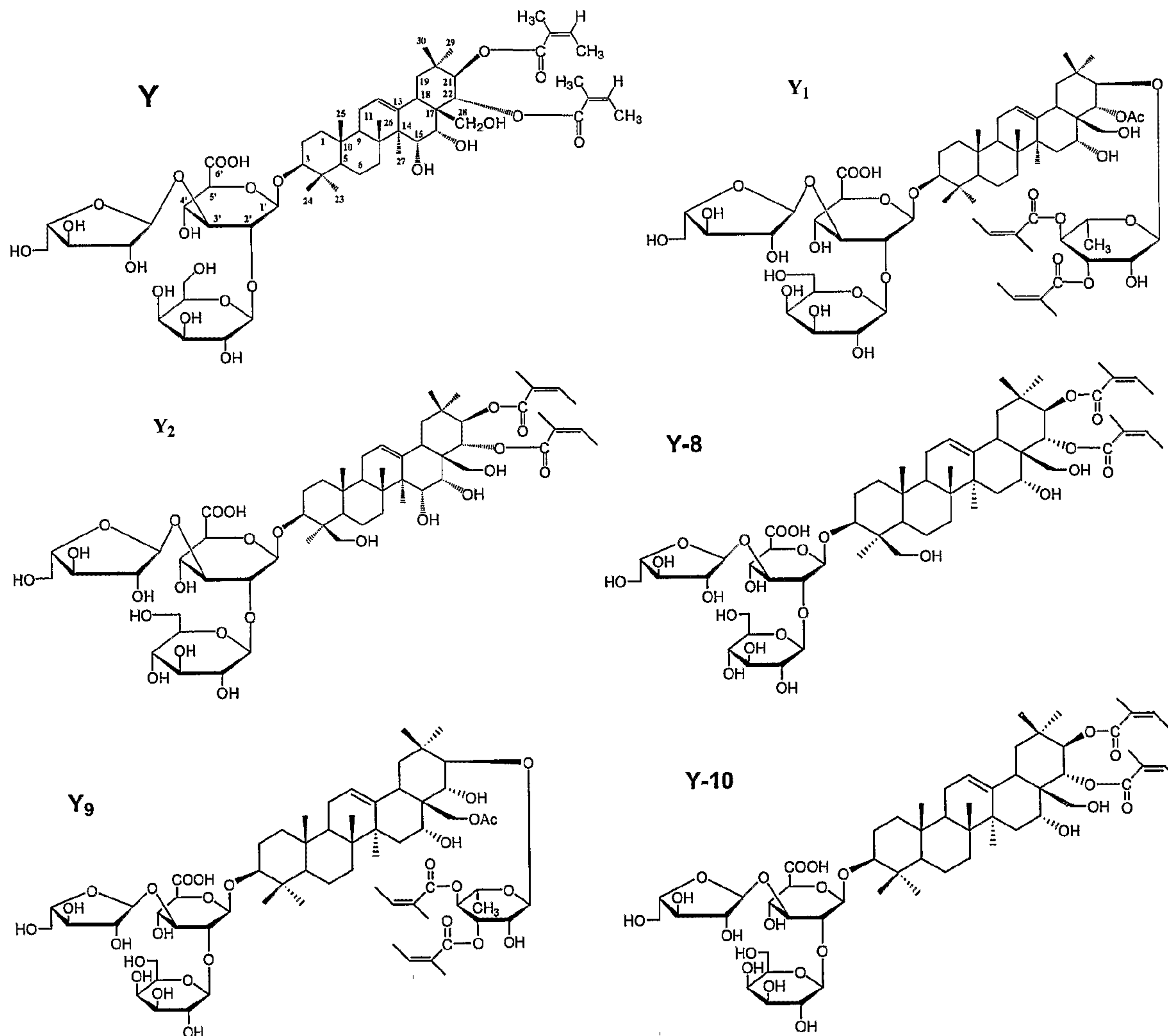
(57) **Abrégé/Abstract:**

Novel compounds such as compounds designated herein as Xanifolia-Y or -Y3, -Y1, -Y2, -Y8, -Y9 and -Y10 are disclosed. These compounds have anticancer activity. The compounds of the present invention are obtainable from plants in the sapindaceae family, such as Xanthoceras sorbifolia, or other natural sources or products. The compounds of the present invention may also be synthesized chemically. (See Formula Y) (See Formula Y1) (See Formula Y2) (See Formula Y-8) (See Formula Y9) (See Formula Y-10)

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2005/04/27 (US11/117,760); 2005/05/17 (US11/131,551)

ANTI-TUMOR COMPOUNDS WITH ANGELOYL GROUPS**ABSTRACT OF THE INVENTION**

Novel compounds such as compounds designated herein as Xanifolia-Y or -Y3, -Y1, -Y2, -Y8, -Y9 and -Y10 are disclosed. These compounds have anticancer activity. The compounds of the present invention are obtainable from plants in the sapindaceae family, such as *Xanthoceras sorbifolia*, or other natural sources or products. The compounds of the present invention may also be synthesized chemically.



ANTI-TUMOR COMPOUNDS WITH ANGELOYL GROUPS

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20 **FIELD OF THE INVENTION**

This invention relates to novel anti-tumor compounds obtainable from *Xanthoceras sorbifolia* and plants from the sapindaceae family.

BACKGROUND OF THE INVENTION

25 Wenguanguo is a species of the sapindaceae family. Its scientific name is *Xanthoceras sorbifolia* Bunge. Wenguanguo is the common Chinese name. Others are Wenguannguo, Wenguanmu, Wenguanhua, Xilacedeng, Goldenhorn and Yellowhorn. Wenguanguo is grown in Liaoning, Jilin, Hebei, Shandong, Jiangsu, Henan, Shanxi, Shaanxi, Gansu, Ningxia and Inner Mongolia, China. Its seeds, leaves and flowers are
30 edible and have been used as a folk or traditional medicine for centuries. Its branches and woods are also used as a folk or traditional medicine. For more detailed information and background or relevant art of the present invention, please refer to page 1, lines 25-38, to page 13 of International PCT Application No. PCT/US04/33359, filed October 8, 2004, and U.S. Serial No. 10/906,303, filed February 14, 2005

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Yingjie Chen, Tadahiro Takeda and Yukio Ogihara reported in Chem. Pharm. Bull 33(4)1387-1394(1985) describes a study on *Xanthoceras sorbifolia* Bunge. See Section V. Saponins from the Fruits of *Xanthoceras sorbifolia*. Four new saponin compounds were isolated from the fruits of *Xanthoceras sorbifolia* Bunge. These structures are bunkankasaponins A, B, C and D. Yingjie Chen, Tadahiro Takeda and Yukio Ogihara reported in Chem. Pharm. Bull 33(3)1043-1048(1985) is another a study focused on the *Xanthoceras sorbifolia* Bunge. See Section IV. Structures of the Minor Prosapogenin. Yingjie Chen, Tadahiro Takeda and Yukio Ogihara. Chem. Pharm. Bull 33(1)127-134(1985) describes yet another study on the *Xanthoceras sorbifolia* Bunge. See Section III. Minor Prosapogenins Saponins from the Fruits of *Xanthoceras sorbifolia* Bunge. Other studies which have focused on saponin compounds include: Laurence Voutquenne, Cecile Kokougan, Catherine Lavaud, Isabelle Pouny, Marc Litaudon. "Triterpenoid saponins and Acylated prosapogenins from *Harpullia austro-caledonica*." Phytochemistry 59 (2002) 825-832. Laurence Voutquenne et al. "Haemolytic acylated triterpenoid saponins from *Harpullia austro-caledonica*" Phytochemistry 66(2005)825-835. Zhong Jaing, Jean-francois Gallard, Marie-Therese Adeline, Vincent Dumontet, Mai Van Tri, Thierry Sevenet, and Mary Pais "Six Triterpenoid Saponins from *Maesa laxiflora*." J. Nat. Prod. (1999), 62, 873-876. Young Seo, John M. Berger, Jennine Hoch, Kim M Neddermann, Isia Bursuker, Steven W. Mamber and David G. Kingston. "A new Triterpene Saponin from *Pittosporum viridiflorum* from the Madagascar Rainforest ". J. Nat. Prod. 2002, 65, 65-68. Xiu-Wei Yang, Jing Zhao, Xue-Hui Lui, Chao-Mei Ma, Masao Hattori, and Li He Zhang "Anti-HIV-1 Protease Triterpenoid Saponins from the Seeds of *Aesculus chinensis*." J. Nat. Prod. (1999), 62, 1510-1513. Yi Lu, Tatsuya Umeda, Akihito Yagi, Kanzo Sakata, Tirthankar Chaudhuri, D.K. Ganguly, Secion Sarma. "Triterpenoid Saponins from the roots of the tea plant (*Camellia sinensis* var. *Assamica*)." Phytochemistry 53 (2000) 941-946. Sandra Apers, Tess E. De Bruyne, Magda Claeys, Arnold J. Viletinck, Luc A.C. Pieters. "New acylated triterpenoid saponins from *Maesa laceceolata*." Phytochemistry 52 (1999) 1121-1131. Ilaria D'Acquarica, Maria Cristina, Di Giovanni, Francesco Gasparrini, Domenico Misiti, Claudio D' Arrigo, Nicolina Fagnano, Decimo Guarnieri, Giovanni Iacono, Giuseppe Bifulco and Raffaele Riccio. "Isolation and

structure elucidation of four new triterpenoid estersaponins from fruits of the *Pittosporum tobira* AIT." *Tetrahedron* 58 (2002) 10127-10136.

5 However, these references do not disclose saponin compounds having the same structure as the compounds of this invention which are capable of inhibiting cancer or tumor cell growth.

Cancer cells are defined by two heritable properties: (1) they reproduce in defiance of normal restraints on cell division; and (2) they invade and colonize territories normally reserved for other cells. Cancers require mutations of one to many genes for its development, and they are classified according to the tissue and cell type from which they arise. Cancers arising from epithelial cells are named carcinomas; those arising from connective tissue or muscle cells are named sarcomas. In addition, there are cancers called leukemias, which are derived from hemopoietic cells. Cancers can also develop from cells of the nervous system.

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Ovarian cancer is the 5th leading cause of cancer death in women, and the leading cause of death from gynecologic malignancies. In the United States, females have a 1.4 to 2.5%, or 1 out of 40-60 women, lifelong chance of developing ovarian cancer. Older women are at highest risk. More than half of the deaths from ovarian cancer occur in women between 55 and 74 years of age, and approximately one quarter of ovarian cancer deaths occur in women between 35 and 54 years of age.

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25 Ovarian cancer is disproportionately deadly for a number of reasons. First, symptoms are vague and non-specific, so women and their physicians frequently attribute them to more common conditions. By the time the cancer is diagnosed, the tumor has often spread beyond the ovaries. Also, ovarian cancers shed malignant cells that frequently implant on the uterus, bladder, bowel, and lining of the bowel wall (omentum). These cells can begin forming new tumor growths before cancer is even suspected. Second, because no cost-effective screening test for ovarian cancer exists, more than 50 percent of women with ovarian cancer are diagnosed in the advanced stages of the disease.

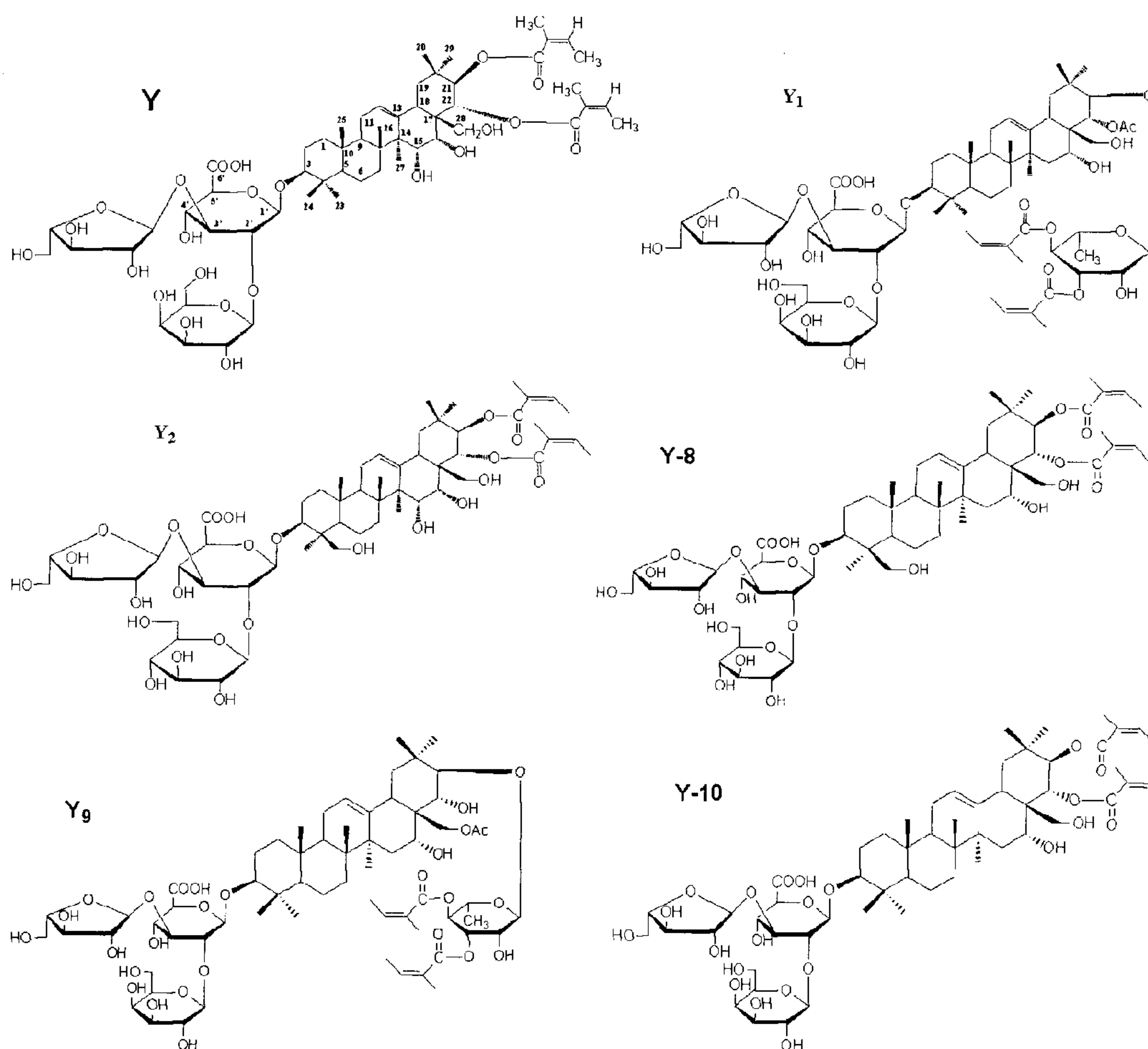
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Therefore, it is an object of this invention to provide compounds and/or compositions extracted from *Xanthoceras sorbifolia* or plants, or chemically or biochemically synthesized, which have substantial potency against ovarian cancer.

5 SUMMARY OF THE INVENTION

In accordance with these and other objects of the invention, a brief summary of the present invention is presented. Some simplifications and omission may be made in the following summary, which is intended to highlight and introduce some aspects of the present invention, but not to limit its scope. Detailed descriptions of a preferred
10 exemplary embodiment adequate to allow those of ordinary skill in the art to make and use the invention concepts will follow in later sections.

The invention provides six novel compounds of the structure (Y1, Y2, Y or Y3, Y8, Y9,
Y10) as shown in Figure 1. As used herein, "Structure Y" is also referred to as
15 "Structure Y3".



The formula, chemical name and common name of these compounds are presented in Table 1 below.

5 **Table 1. Formula, Chemical Name and Common Name of Six Novel Compounds of Structure (Y, Y1, Y2, Y8, Y9, Y10)**

Names	Formula	Chemical Name
Xanifolia- Y (Y3)	C ₅₇ H ₈₈ O ₂₃	3-O-[β-D-galactopyranosyl(1→2)]-α-L-arabinofuranosyl(1→3)-β-D-glucuronopyranosyl-21,22-O-diangeloyl-3β, 15α, 16α, 21β, 22α, 28-hexahydroxyolean-12-ene,
Xanifolia- Y1	C ₆₅ H ₁₀₀ O ₂₇	3-O-[β-D-galactopyranosyl(1→2)]-α-L-arabinofuranosyl(1→3)-β-D-glucuronopyranosyl-21-O-(3,4-diangeloyl)-α-L-rhamnopyranosyl-22-O-acetyl-3β,16α, 21β, 22α, 28-pentahydroxyolean-12-ene
Xanifolia- Y2	C ₅₇ H ₈₈ O ₂₄	3-O-[β-D-glucopyranosyl-(1→2)]-α-L-arabinofuranosyl(1→3)-(D-glucuronopyranosyl-21,22-O-diangeloyl-3β, 15α, 16α, 21β, 22α, 24β, 28-heptahydroxyolean-12-ene
Xanifolia- Y8	C ₅₇ H ₈₈ O ₂₃	3-O-[β-galactopyranosyl (1→2)]-α-arabinofuranosyl (1→3)-β-glucuronopyranosyl-21, 22-O-diangeloyl-3β, 16α, 21β, 22α, 24β, 28-hexahydroxyolean-12-ene
Xanifolia- Y9	C ₆₅ H ₁₀₀ O ₂₇	3-O-[β-galactopyranosyl (1→2)]-α-arabinofuranosyl (1→3)-β-glucuronopyranosyl-21-O-(3,4-diangeloyl)-α-rhamnopyranosyl-28-O-acetyl-3β, 16α, 21β, 22α, 28-pentahydroxyolean-12-ene
Xanifolia- Y10	C ₅₇ H ₈₈ O ₂₂	3-O-[β-galactopyranosyl (1→2)]-α-arabinofuranosyl (1→3)-β-glucuronopyranosyl-21, 22-O-diangeloyl-3β, 16α, 21β, 22α, 28-pentahydroxyolean-12-ene

10 The above six compounds (Y, Y1, Y2, Y8, Y9 and Y10) have anti-cancer effect. These compounds inhibit the growth of human ovarian and other cancer cells. See Figure 2, 3 and 4.

15 A consensus sub-structure was derived or identified from the bioactive compounds (Y, Y1, Y2, Y8, Y9 and Y10) of the invention. A consensus sub-structure of the compounds of the invention is a biangeloyl group located on adjacent carbons. For example, in structure Y, Y2, Y8 and Y10, the biangeloyl group is located at 21β and 22α of the triterpene backbone. See Figure 5. For structure Y1 and Y9, the biangeloyl group is

located at C3 and C4 of the sugar ring. See Figure 6. In an embodiment, the biangeloyl group of the bioactive compounds (Y, Y1, Y2, Y8, Y9 and Y10) of the invention is situated in a trans-position in adjacent carbons of a structure. See Figure 7. In another embodiment, a sugar moiety or sugar chain is located at C3 of the triterpene. In a further embodiment, the sugar moiety or sugar chain selected from the group consisting of: D-glucose, D-galactose, L-rhamnose, L-arabinose, D-xylose, alduronic acid, D-glucuronic acid and D-galacturonic acid.

Studies on the structural and functional relationship of the six compounds of the invention indicate that changes to or substitutions of the functional groups located at C15 and C24 of the triterpene do not affect anticancer activity. The compounds (Y, Y1, Y2, Y8, Y9 and Y10) of the invention have activity in inhibition of tumor growth. See Figure 2, 3 and 4. The compounds are prepared by a chromatography purification process involving FPLC and HPLC as described in Figure 8, 9, 10, 11, 12 and 13. Compound Y is purified using a procedure described in this application. Figure 11A shows the NMR peaks of compound Y. Comparison of Figure 2 and Figure 14 shows that the purified compound Y possesses potency ($IC_{50} = 1.5 \text{ ug/ml}$) 10 times higher than the original extract ($IC_{50} = 25 \text{ ug/ml}$). Compound Y also has a high selectivity toward ovarian cancer. See Figure 15.

The purified compound Y1, Y2, Y8, Y9, and Y10 also show inhibitory activity toward human cancer cells with a higher potency toward ovarian carcinoma. See Figure 3 and 4. The plant extract containing compound Ys also shows inhibitory activity toward human cancer cells. For example, studies were performed on eleven human cancer cell lines, and the plant extract of the invention shows a higher potency toward ovarian carcinoma. See Figures 14, 15 and 16 and Table 3.1 for a comparison of the activities of the plant extract of the invention among the different cancer cell lines. As used herein, "Ys" or "compound Ys" is used to denote compound Y or Y3, Y1, Y2, Y8, Y9, Y10, or other bioactive compounds obtainable from a *Xanthoceras sorbifolia* extract of the invention.

This invention provides an extract of *Xanthoceras sorbifolia* capable of inhibiting cancer growth. The cancer includes, but is not limited to ovary cancer, bladder cancer, prostate cancer, leukocytes cancer, and bone cancer. Compounds of the invention, shown to be effective against cancer, can be isolated from the plant called *Xanthoceras sorbifolia*,

synthesized chemically, or extracted from other biological sources, including plants from the sapindaceae family.

This invention provides a process of producing bioactive compounds from husks, leaves, branches or stems, fruit-stems, seed shell, roots and barks of the Wenguanguo. The extraction of the bioactive compounds of the invention from different parts of the Wenguangu plant can be performed separately or simultaneously. This invention further discloses methods of obtain the bioactive compounds of the invention. In addition to saponin comopunds, the extracts of the invention also contain saccharides, proteins, glycosides, flavonoids, curmarin extracts, alkaloid extracts, organic acid extracts, tannin and other bioactive compounds. The saponin compounds obtainable from the extract of the invention were investigated, and have been shown to possess inhibitory activity against cancer growth.

The compounds, extracts or compositions of the present invention is capable of regulating many cellular pathways, including the receptors or components of a cell, such as G-protein receptor, Fas protein, receptor Tyrosine Kinases, Mitogen, mitogen receptor. The compounds of the invention can be isolated from the plant called *Xanthoceras sorbifolia*, synthesized chemically, or extracted from other biological sources, including plants from the sapindaceae family.

This invention provides compounds, including compound of structures Y, Y1, Y2, Y8, Y9 and Y10, obtainable from *Xanthoceras sorbifolia* and capable of inhibiting cancer growth. In an embodiment, the cancer includes but is not limited to bladder cancer, cervix cancer, prostate cancer, lung cancer, breast cancer, leukocytes cancer, colon cancer, liver cancer, bone cancer, brain cancer, and ovary cancer. This invention provides a compound of oleanene triterpenoidal saponin comprising a side chain at Carbon 21 and Carbon 22 of said compound, wherein the side chain comprises angeloyl groups. In an embodiment, the compound comprises one or more sugars, wherein C3 and C4 of the sugar are acylated with angeloyl groups. This invention provides a triterpinoidal saponin compound comprising a triterpene backbone and a biangeloyl group, wherein the one angeloyl group is attached to 21 β and one angeloyl group is attached to 22 α of the triterpene backbone, and wherein the presence of the biangeloyl group produces anticancer activity. This invention provides a triterpenoidal saponin compound comprising a triterpene backbone and a sugar moiety or rhamnose,

wherein the sugar moiety or rhamnose is attached to the triterpene backbone, wherein the sugar moiety or rhamnose further comprises a biangeloyl group, and wherein the presence of the biangeloyl group produces anticancer activity. This invention provides a triterpenoidal saponin compound comprising a triterpene backbone, said triterpene backbone is acylated at either 21 β or 22 α position or at both 21 β and 22 α position with a sugar moiety or sugar chain, and wherein at least one sugar in the sugar moiety or sugar chain comprises angeloyl groups attached to the C3 and C4 position of said sugar. In an embodiment, the two angeloyl groups are in a trans-position on a structure, and the presence of the biangeloyl group produces anticancer activity. In an embodiment, the two angeloyl groups are in a trans-position in adjacent carbons on a structure, and the presence of the biangeloyl group produces anticancer activity. This invention provides a salt of the above-described compounds. This invention provides a pharmaceutical composition comprising an effective amount of the above-described compounds and a pharmaceutically acceptable carrier(s).

This invention provides a method for isolating compounds from *Xanthoceras sorbifolia* comprising the steps of: extracting *Xanthoceras sorbifolia* powder with an appropriate amount of an organic solvent for an appropriate amount of time to obtain an extract, identifying the bioactive compounds in the extract; purifying the bioactive compounds in the extract with FPLC to obtain a fraction of the bioactive compounds; and isolating the purified individual bioactive compound from the fraction of the bioactive compounds with preparative HPLC. This invention provides a compound having a structure verified by NMR spectral data derived from proton NMR, carbon NMR, 2D NMR of the Heteronuclear Multiple Quantum Correlation (HMQC), Heteronuclear Multiple Bond Correlation (HMBC), NOESY and COSY, and Mass spectral data derived from MALDI-TOF and ESI-MS.

This invention provides a compound and its derivatives which are effective against cancer. The compounds or compositions of the present invention are capable of regulating various cellular pathways, including but not limiting the following: receptors or components, such as G-protein receptor, Fas protein, receptor for Tyrosine Kinases, mitogens, mitogen receptors, TGF Beta-smad, FGF, TGF-beta and TGF-alpha, ras-GTPase-MAP kinase, jun-fos, Src-fyn, Jak-Jnk-STAT, BMP, Wnt, or myc-cell proliferation. The compounds and composition of the invention derived from *Xanthoceras Sorbifolia* is also capable of regulating the components and receptors

associated with cell death, and are capable of (re)activating the cell death program or pathways.

DETAILED DESCRIPTION OF THE FIGURES

5 **Figure 1** shows the structures of six bioactive anticancer saponin compound of the invention.

Figure 2 shows the anticancer activity of purified Compound Y. The experiment was performed on ovarian cancer cells (OCAR-3) and the inhibition activity was determined by MTT assay. For details, refer to Experiment 3. Abscissa: Concentration (ug/ml).

10 Ordinate: % Cell Growth. The IC50 is approximately 1-1.5 ug/ml. A: Point scale. B: Linear scale.

Figure 3 shows the inhibition of the purified Compound Y1 and Compound Y2 on the growth of ovarian cancer cells.

Figure 4 shows the anticancer activity of Y, Y8, Y9 and Y10 on ovarian cancer cells as
15 determined by MTT assay.

Figure 5 shows a consensus structure derived from four bioactive anticancer saponin compounds of the invention (i.e., Y, Y2, Y8 and Y10).

Figure 6 shows a consensus structure derived from two bioactive anticancer saponin compounds of the invention (i.e., Y1 and Y9).

20 **Figure 7** shows a general structural formular derived from the consensus structures of the six bioactive compounds of the invention (i.e., Y, Y1, Y2, Y8, Y9 and Y10). (A) A consensus active functional group is the biangeloyl group attached to 21 β and 22 α of the triterpene backbone. (B) A consensus active functional group is the biangeloyl group attached at C3 and C4 of a sugar ring (or rhamnose). In an embodiment, the
25 functional active structure is a biangeloyl group situated in a trans-position on a structure.

Figure 8 shows the separation of the components of Xanthoceras sorbifolia extract by HPLC with a μ bondapak C18 column. Details of the experiment were presented in Experiment 2.

30 **Figure 9** shows the elution profile of an extract of Xanthoceras sorbifolia in FPLC with 10-80% gradient. Ordinate: Optical density (at 245nm). Abscissa: Fractions (5ml/fraction).

Figure 10 shows the results of the screening of cell growth activity using fractions obtained from FPLC chromatography. The assay was conducted in bladder cells. The
35 fractions obtained from FPLC as shown in Figure 9 were used. As shown in this figure,

different components of *Xanthoceras sorbifolia* extracts cause either growth or inhibition effects on cells. Only fraction 5962 (Fraction Y) causes cell inhibition. Fractions 610, 1116 and 1724 cause minor stimulation of cell growth. Abscissa: concentration (ug/ml). Ordinate: % Cell Growth (determined by MTT assay).

5 **Figure 11** shows HPLC profile of Fraction Y with 45% acetonitrile isocratic elution in a preparative C18 column (Delta Pak C18). Under these conditions, fractions Y (Y3), Y1 and Y2 are well separated from each other and they are subsequently purified. A and B shows the purity of the collected Y3 and Y2 by HPLC under same conditions.

Figure 12 shows the separation profile of Y8, Y9 and Y10 with 45% acetonitrile isocratic
10 elution in a preparative C18 column (Delta Pak C18).

Figure 13 shows the HPLC profiles of purified Y8, Y9 and Y10.

Figure 14 shows the growth curves of ovarian cancer cells after treatment with the crude extract of *Xanthoceras sorbifolia* as determined by the MTT assay. The two curves in the figure are two experiments results. This study determined the efficacy of
15 the extract of *Xanthoceras sorbifolia* on cancer cells. In these experiments, cancer cell lines from 11 different human organs were employed. This figure shows that ovary cancer cells are the most sensitive cancer cells in responding to *Xanthoceras Sorbifolia* extract of the invention. Results of other cancer cells were represented in Figures 16A, 16B, 16C. Abscissa: concentration (ug/ml). Ordinate: % Cell Growth (determined by
20 MTT assay).

Figure 15 shows the comparison of potency of Compound Y in ovarian cancer cells and cervical cancer cells. Ovarian cancer cells are much more sensitive than the cervical cancer cells. The IC50 for Compound Y in ovary cells is about 1.5ug/ml while the IC50 in cervical cancer cells is over 20 ug/ml.

25 **Figures 16A** shows the growth curves of sensitive group of bladder and bone cancer cells as determined by MTT assay. The curves in the figure are the repeated experiments results. Abscissa: concentration (ug/ml). Ordinate: % Cell Growth (determined by MTT assay).

Figures 16B shows the growth curves of semi-sensitive group: leukocyte, liver;
30 prostate, breast and brain cancer cells as determined by MTT assay. The curves in the figure are the repeated experiments results. Abscissa: concentration (ug/ml). Ordinate: % Cell Growth (determined by MTT assay).

Figures 16C shows the growth curves of least sensitive: colon, cervix and lung cancer cells as determined by MTT assay. The curves in the figure are the repeated

experiments results. Abscissa: concentration (ug/ml). Ordinate: % Cell Growth (determined by MTT assay).

Figure 17 shows the structure of Compound Y having the formula of $C_{57}H_{88}O_{23}$ and the chemical name of 3-O-[β -D-galactopyranosyl(1 $\rightarrow$ 2)]- α -L-arabinofuranosyl(1 $\rightarrow$ 3)- β -D-glucuronopyranosyl-21,22-O-diangeloyl-3 β , 15 α , 16 α , 21 β , 22 α , 28-hexahydroxyolean-12-ene.

Figure 18 shows the spectrum of proton NMR of Compound Y.

Figure 19 shows 2D NMR (HMQC) results of Compound Y.

Figure 20 shows 2D NMR (HMBC) results of Compound Y.

10 Figure 21 shows the mass spectrum of compound Y with MALDI-TOF (high mass): Y + Matrix (CHCA) + Angiotensin 1 "two point calibration".

Figure 22 shows the mass spectrum of compound Y with ESI-MS.

Figure 23 shows the structure of Compound Y1 having the formula of $C_{65}H_{100}O_{27}$ and the chemical name of 3-O-[β -D-galactopyranosyl(1 $\rightarrow$ 2)]- α -L-arabinofuranosyl(1 $\rightarrow$ 3)- β -D-glucuronopyranosyl-21-O-(3,4-diangeloyl)- α -L-rhamnopyranosyl-22-O-acetyl-3 β , 16 α , 21 β , 22 α , 28-pentahydroxyolean-12-ene.

Figure 24 shows the Proton NMR spectrum of Compound Y1.

Figure 25 shows the 2D NMR (HMQC) results of Compound Y1.

Figure 26 shows the 2D NMR (HMBC) results of Compound Y1.

20 Figure 27 shows COSY-NMR profile of Compound Y1.

Figure 28 shows the chemical structure and the chemical name of Compound Y2.

Figure 29 shows the proton NMR spectrum of Y2.

Figure 30 shows the 2D NMR spectrum of Y2 (HMQC)-level-1.

Figure 31 shows the C13 NMR spectra of compound Y2.

25 Figure 32 shows the 2D NMR (HMBC)-level-1 spectra of compound Y2.

Figure 33 shows the 2D NMR HOHAHA (TOCSY)-level-1 spectrum of compound Y2.

Figure 34 shows the mass spectrum of compound Y2 +Matrix + Standards.

Figure 35 shows the chemical structure of Y8.

Figure 36 shows H-NMR spectrum of Y8.

30 Figure 37 shows C13-NMR spectrum of Y8.

Figure 38 shows 2D NMR HMQC (level 1) spectrum of Y8.

Figure 39 shows the chemical structure of Y9.

Figure 40 shows H-NMR spectrum of Y9.

Figure 41 shows 2D NMR HMQC (level 1) spectrum of Y9.

- Figure 42 shows 2D NMR HMBC (level1) spectrum of Y9.
- Figure 43 shows the chemical structure of Y10.
- Figure 44 shows H-NMR spectrum of Y10.
- Figure 45 shows C13 NMR spectrum of Y10.
- 5 Figure 46 shows 2D NMR HMQC (level 1) spectrum of Y10.
- Figure 47 shows the chemical structure and the chemical name of Compound R1.
- Figure 48 shows the Proton-NMR spectrum of compound R1.
- Figure 49 shows the 2D NMR (HMQC) spectrum of compound R1.
- Figure 50 shows the 2D NMR (HMBC) spectrum of compound R1.
- 10 Figure 51 shows the 2D NMR (COSY) spectrum of compound R1.
- Figure 52 shows the C13 NMR spectrum of compound R1.
- Figure 53 shows the chemical structure of Compound O54.
- Figure 54 shows the Proton-NMR spectra of compound O54.
- Figure 55 shows the 2D NMR (HMQC) spectra of compound O54.
- 15 Figure 56 shows the 2D NMR (HMBC) spectra of compound O54.
- Figure 57 shows the absorption spectrum of Xanthoceras sorbifolia extract of the invention. Abscissa: Wavelength in nm. Ordinate: Optical Density. The extract has three absorption maximum at 207nm, 278nm and 500nm.
- Figure 58 shows the proton NMR spectrum of Y4.
- 20 Figure 59 shows the 2D NMR (HMQC) spectrum of Y4.
- Figure 60 shows purification of component-R with HPLC. A: Extract from fraction #10 of FPLC (iso-30) was further separated by HPLC. B: Rechromatogram of the major component under same condition as described in A.
- Figure 61. Fractionation of Fraction-O with HPLC with 20% acetonitrile isocratic elution
- 25 (iso-20).
- Figure 62 shows rechromatography of O54, O28 and O34 (from iso-20).
- Figure 63A shows the chemical structure of a compound, wherein R1 represents angeloyl group; R2 represents angeloyl group; R3 represents OH or H; R4 represents H or OH or CH3 or CH2OR6 or COOR6; wherein R6=H or acetyl or sugar moiety;
- 30 Positions 23, 24, 25, 26, 27, 29, 30 of the compound independently comprise CH3 or CH2OH or CHO or COOH or alkyls group or acetyl group or derivative thereof; R6 represents Ac or H and R5 represents sugar moiety, wherein the sugar moiety comprises at least one sugar, or D-glucose, or D-galactose, or L-rhamnose, or L-arabinose, or D-xylose, or alduronic acid, or D-glucuronic acid or D-galacturonic acid, or

derivative thereof, or the combination thereof. In an embodiment, R5 represents a compound capable of performing the function of sugar moiety.

Figure 63B shows the chemical structure of a compound wherein R1 represents angeloyl group; R2 represents angeloyl group; R3 represents Ac or H; R4 represents H or OH; R6 represents Ac or H; R7 represents H or OH or CH3 or CH2OR6 or COOR6 wherein R6=H or acetyl or sugar moiety; Positions 23, 24, 25, 26, 27, 29, 30 of the compound independently comprise CH3 or CH2OH or alkyls group or acetyl group or derivative thereof and R5 represents sugar moiety, wherein the sugar moiety comprises at least one sugar, or D-glucose, or D-galactose, or L-rhamnose, or L-arabinose, or D-xylose, or alduronic acid, or D-glucuronic acid, or D-galacturonic acid, or derivative thereof, or the combination thereof. In an embodiment, R5 represents a compound capable of performing the function of the sugar moiety.

Figure 64 shows HPLC (iso-45) profiles of FPLC fractions #5657.

Figure 65 shows the effect of compound X on the growth of OCAR-3 cells.

Figure 66 shows H-NMR of compound X.

Figure 67 shows 2D NMR (HMQC) of compound X.

Figure 68 shows 2D NMR (HMBC) of compound X.

Figure 69 shows C13-NMR of compound X.

Figure 70 shows the chemical structure of compound X.

Figure 71 shows the mass spectrum 1 (WALDI-TOF) of compound X.

Figure 72 shows the mass spectrum 2 (WALDI-TOF) of compound X.

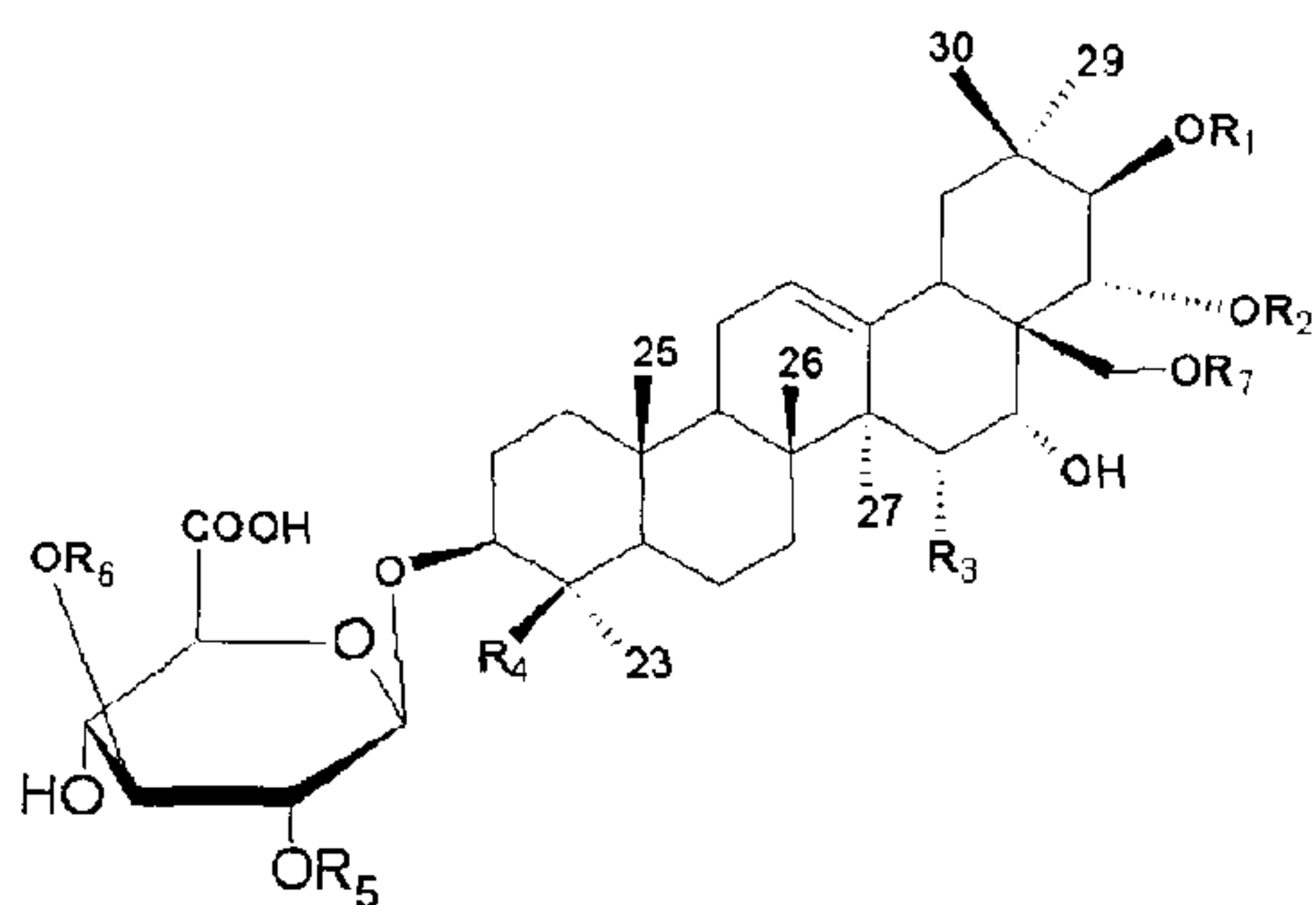
Figure 73 shows a comparison of MTT and Haemolytic activities of saponin compound and Compound Ys of the invention. (A) and (B) shows hemolytic activities. (C) and (D) show MTT activities.

Figure 74 (A) shows a compound of the invention without angeloyl groups. (B) shows a compound of the invention without sugar moiety.

Figure 75 shows the saponin compounds of the invention.

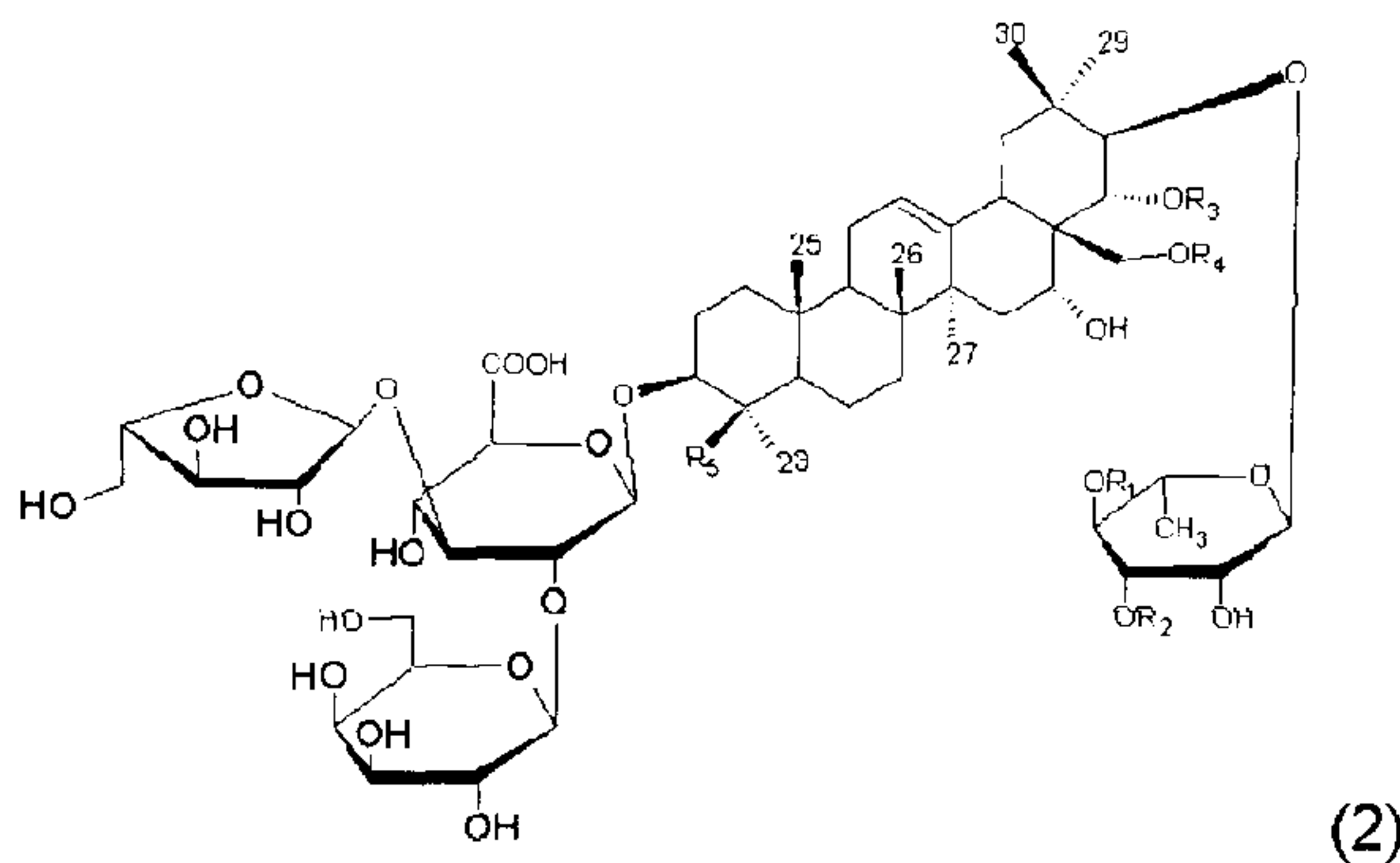
DETAILED DESCRIPTION OF THE INVENTION

This invention provides a compound selected from a compound of formula (1):



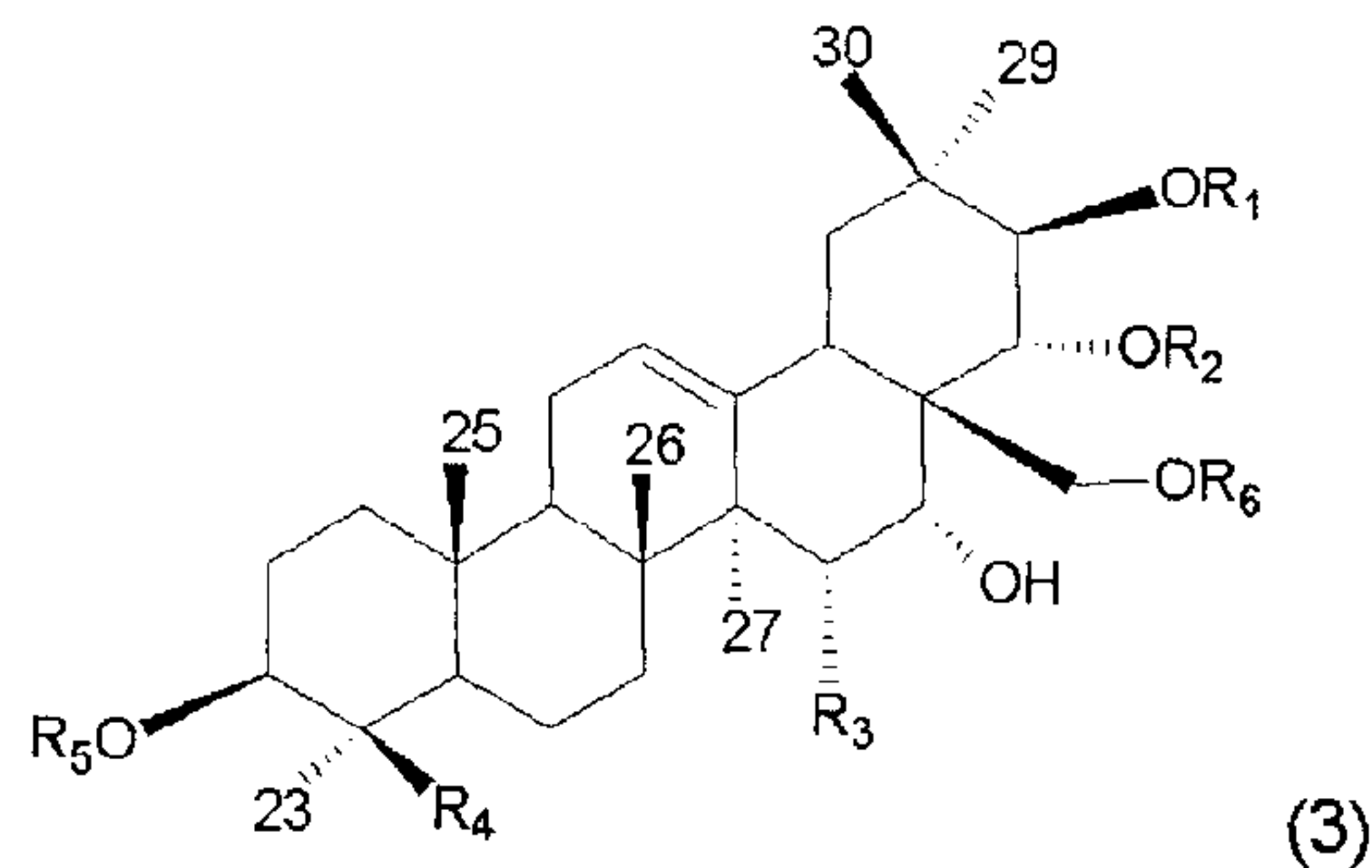
or a salt, ester or derivative thereof, wherein R1 represents angeloyl group; R2 represents angeloyl group; R3 represents OH or H; R4 represents CH3 or CH2OH; R7 represents H; and R5 represents D-glucose or D-Galactose; and R6 represents L-arabinose.

This invention provides a compound selected from a compound of formula (2):



or a salt, ester or derivative thereof, wherein R1 represents angeloyl group; R2 represents angeloyl group; R3 represents Ac or H; R4 represents H or Ac; and R5 represents CH₃ or CH₂OH.

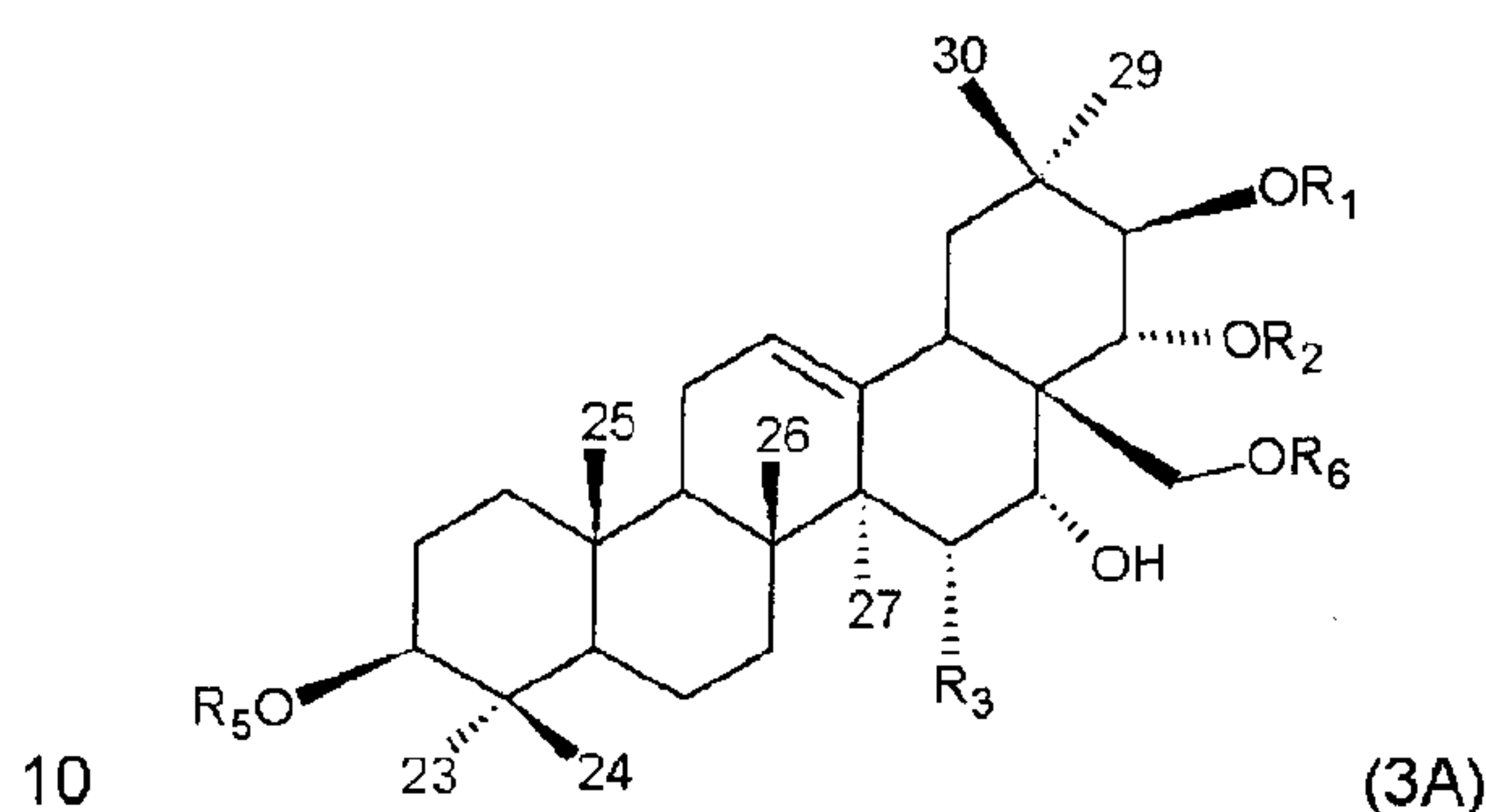
This invention provides a compound selected from a compound of formula (3):



or a salt, ester or derivative thereof, wherein R1 represents angeloyl group; R2 represents angeloyl group; R3 represents OH or H; R4 represents CH3 or CH2OH or

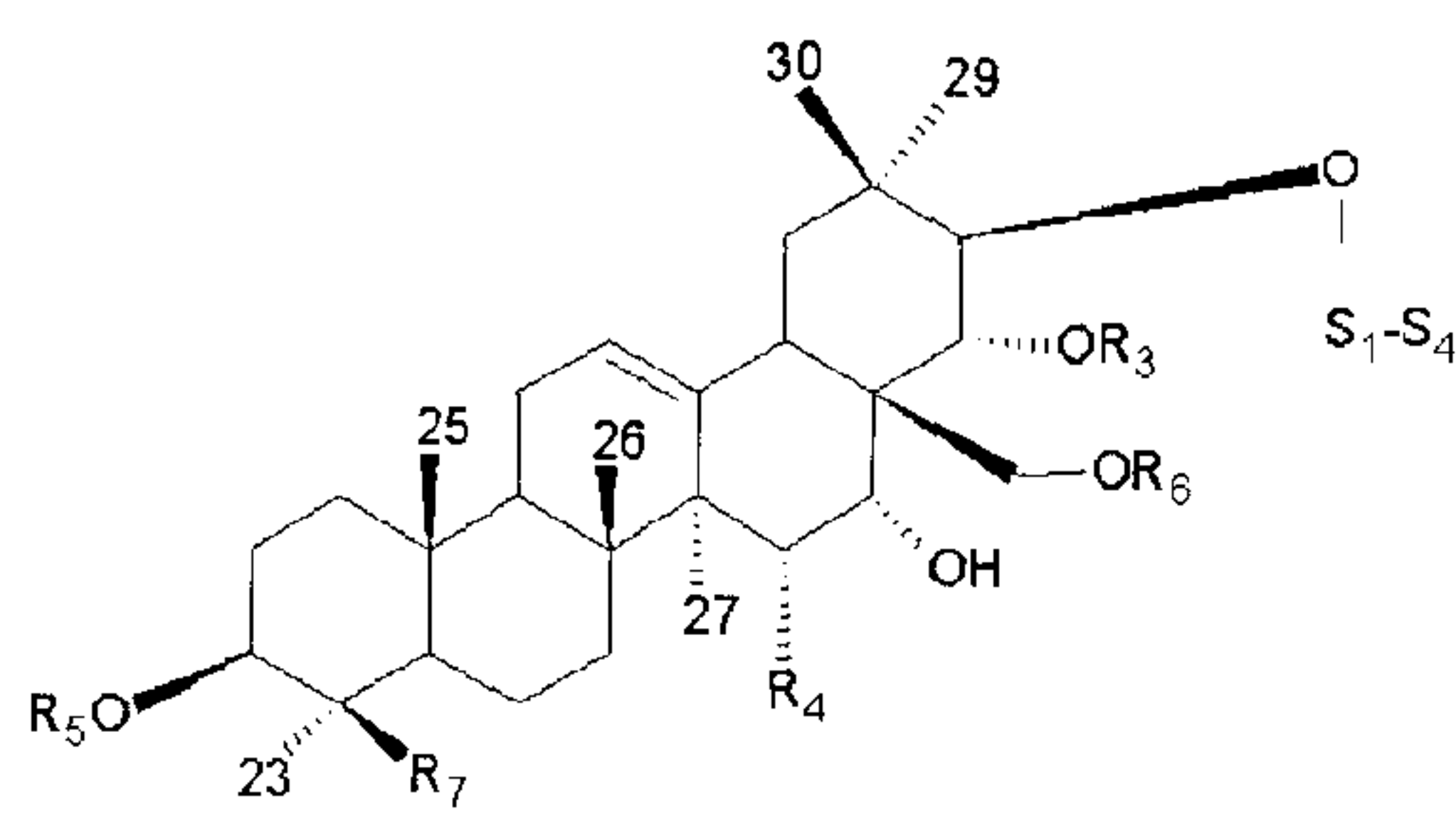
alkyls group or their derivatives; R6 represents Ac or H and R5 represents sugar moiety, wherein the sugar moiety comprises at least one sugar, or D-glucose, or D-galactose, or L-rhamnose, or L-arabinose, or D-xylose, or alduronic acid, or D-glucuronic acid, or D-galacturonic acid, or derivative thereof, or the combination thereof. In an embodiment, R5 represents a compound capable of performing the function of sugar moiety. In another embodiment the sugar moiety comprises L-arabinose, or D-glucose, or D-galactose, or combinations thereof.

This invention provides a compound selected from a compound of formula (3A):

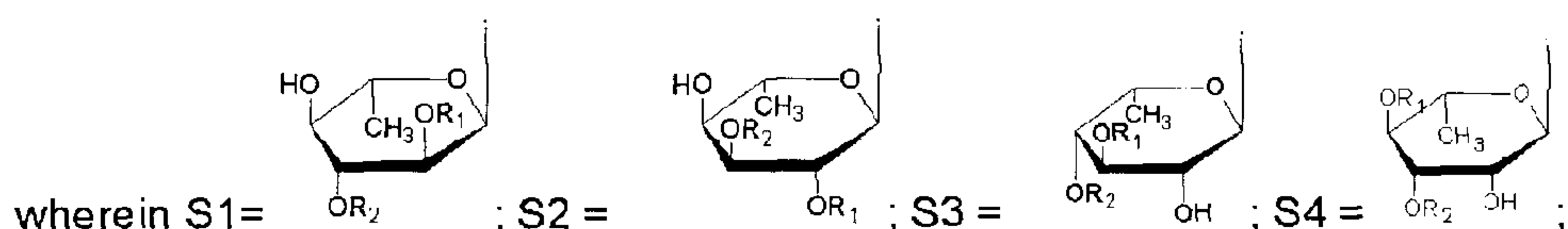


or a salt, ester or derivative thereof, wherein R1 represents angeloyl group; R2 represents angeloyl group; R3 represents OH or H; Positions 23, 24, 25, 26, 27, 29, 30 of the compound independently comprise CH₃, or CH₂OH, or CHO, or COOH, alkyls group, or acetyl group, or derivative; R6 represents Ac or H; and R5 represents sugar moiety, wherein the sugar moiety comprises at least one sugar, or D-glucose, or D-galactose, or L-rhamnose, or L-arabinose, or D-xylose, or alduronic acid, or D-glucuronic acid, or D-galacturonic acid, or their derivative thereof, or the combination thereof. In an embodiment, R5 represents a compound capable of performing the function of the sugar moiety. In another embodiment the sugar moiety comprises L-arabinose, D-glucose and/or D-galactose, or combinations thereof. In a further embodiment, any two of R1, R2 or R6 are angeloyl groups, or any one of R1, R2 or R6 is attached to a sugar moiety in which two angeloyl groups are attached to adjacent carbons of the monosaccharides. In a further embodiment, R1, R2, and R6 comprises angeloyl group, acetyl group, tigloyl group, senecioly group, or an acid with two to five carbons or combination thereof. In a further embodiment, at least one of R1, R2 or R6 is attached a sugar moiety or rhamnose, wherein sugar moiety or rhamnose comprises two angeloyl group, acetyl group, tigloyl group, senecioly group, acid having two to five carbons, or combinations thereof.

This invention provides a compound selected from a compound of formula (4):

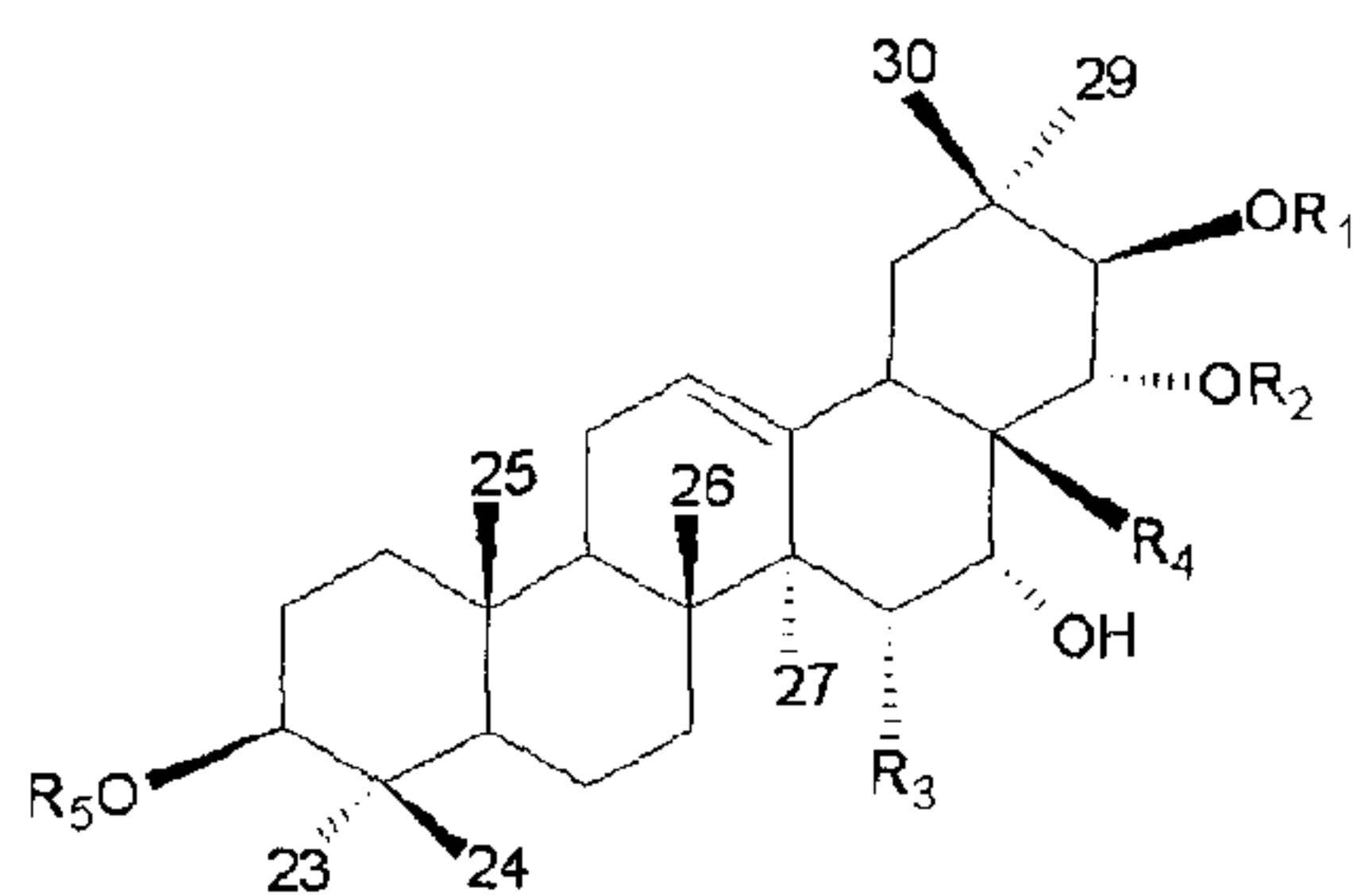


or a salt, ester or derivative thereof,



- 5 Wherein S1-S4 =S1 or S2 or S3 or S4; R1 represents angeloyl group; R2 represents angeloyl group; R3 represents Ac or H; R4 represents H or OH; R6 represents Ac or H; R7 represents CH₃ or CH₂OH or alkyl group or their derivatives and R5 represents sugar moiety or sugar chain selected from the group consisting of D-glucose, D-galactose, L-rhamnose, L-arabinose, D-xylose, alduronic acid, D-glucuronic acid, D-galacturonic acid and their derivatives. In an embodiment, R1 represents angeloyl group; R2 represents angeloyl group; R3 represents Ac or H; R4 represents H or OH; R6 represents Ac or H; Positions 23, 24, 25, 26, 27, 29, 30 of the compound independently comprise CH₃, CH₂OH, CHO, COOH, alkyls group, acetyl group or derivative thereof; R5 represents sugar moiety, wherein the sugar moiety comprises at
- 10 least one sugar, or D-glucose, or D-galactose, or L-rhamnose, or L-arabinose, or D-xylose, or alduronic acid, or D-glucuronic acid or D-galacturonic acid, or their derivative thereof, or the combination thereof. In an embodiment, R5 represents a compound capable of performing the function of the sugar moiety. In another embodiment, R1, R2, R3, R6 comprises an angeloyl group, acetyl group, tigloyl group, senecioly group, or an
- 15 acid having two to five carbons. In a further embodiment, the sugar moiety comprises L-arabinose or/ and D-glucose or/and D-galactose, or combinations thereof.
- 20

This invention provides a compound selected from a compound of formula (5):



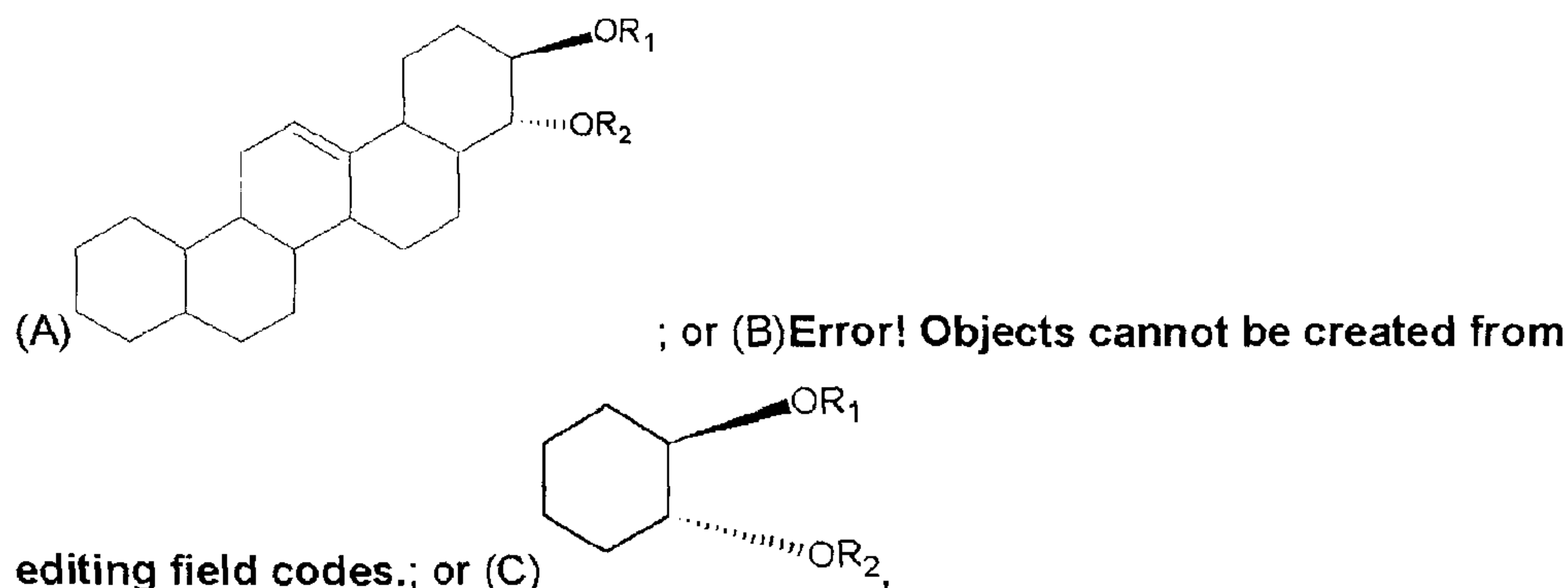
Or a salt, ester, metabolite or derivative thereof, wherein R1 and R2 represent angeloyl group; R3 represents H or OH; R4 represent CH₂OR₆; and wherein R6 is H; R5 represents at least one sugar moiety or its derivatives. In an embodiment, R1 and R2 represent angeloyl group; R3 represents H or OH; R4 represents COOR₆ wherein R6 is H; R5 represents at least one sugar moiety or its derivatives. In an embodiment, R1 represents H; R2 represents angeloyl group; R3 represents H or OH; R4 represents CH₂OR₆ or COOR₆; wherein R6 is an angeloyl group; and R5 represents at least one sugar moiety or its derivatives. In another embodiment, at least two of R1, R2, and R6 comprise an angeloyl group or acid having five carbons; R3 represents H or OH; R4 represents CH₂OR₆ or COOR₆; and wherein R6 is angeloyl group; R5 represents at least one sugar moiety or its derivatives. In a further embodiment, at least one angeloyl of R1 or R2 is replaced by acetyl group, tigloyl group, senecioly group, or an acid having two to five carbons; R3 represents H or OH; R4 represents CH₂OR₆ or COOR₆; and wherein R6 is angeloyl group; R5 represents at least one sugar moiety or its derivatives. In a further embodiment, at least one of R1, R2, and R6 is a sugar moiety or rhamnose comprising at least two angeloyl groups, acetyl group, tigloyl group, senecioly group, or an acid having two to five carbons or combination thereof. In a further embodiment, positions 23, 24, 25, 26, 29, 30 of the compound independently comprise CH₃, CH₂OH, CHO, COOH, alkyls group, acetyl group or derivative thereof. In a further embodiment, R5 represents sugar moiety comprising glucose, galactose or arabinose. In a further embodiment, R5 represents sugar moiety, wherein the sugar moiety comprises at least one sugar, or D-glucose, D-galactose, or L-rhamnose, or L-arabinose, or D-xylose, or alduronic acid, or D-glucuronic acid or D-galacturonic acid, or derivative thereof, or the combination thereof. In an embodiment, R5 represents a compound capable of performing the function of the sugar moiety. In a further embodiment, the R5 represents H. In a further embodiment, R4 represents H or OH or CH₃.

Substitution, deletion and/or addition of any group in the above-described compounds will be apparent to one of ordinary skill in the art based on the teaching of this

application. In a further embodiment, the substitution, deletion and/or addition of the group(s) in the compound of the invention does not substantially affect the biological function of the compound. In a further embodiment, the angeloyl groups are in a trans-position on a structure.

5

This invention provides a compound comprising the following structure:



wherein R1 represents angeloyl group and R2 represents angeloyl group. In an embodiment, the biangeloyl group is acylated in trans-position. In another embodiment, the biangeloyl group is acylated in trans-position on adjacent carbons. In a further embodiment, the biangeloyl group is acylated in a structure. In a further embodiment, R1, R2 comprises angeloyl group, tigloyl group, senecioly group, an acid having two to five carbons, or combinations thereof. In a further embodiment, the compound comprises at least two angeloyl groups, tigloyl groups, senecioly groups, an acid having two to five carbons, or combinations thereof. In a further embodiment, the compound further comprises a sugar moiety. In a further embodiment, the sugar moiety comprises glucose, galactose or arabinose or combination thereof. In a further embodiment, the sugar moiety comprises at least one sugar, or D-glucose, or D-galactose, or L-rhamnose, or L-arabinose, or D-xylose, or alduronic acid, or D-glucuronic acid, or D-galacturonic acid, or their derivative thereof, or the combination thereof. In a further embodiment, the structure A, B or C comprises a compound capable of performing the function of the sugar moiety. Substitution, deletion and/or addition of any group in the above-described compounds will be apparent to one of ordinary skill in the art based on the teaching of this application. In a further embodiment, the substitution, deletion and/or addition of the group(s) in the compound of the invention does not substantially affect the biological function of the compound. In a further embodiment, the angeloyl groups are in a trans-position on a structure.

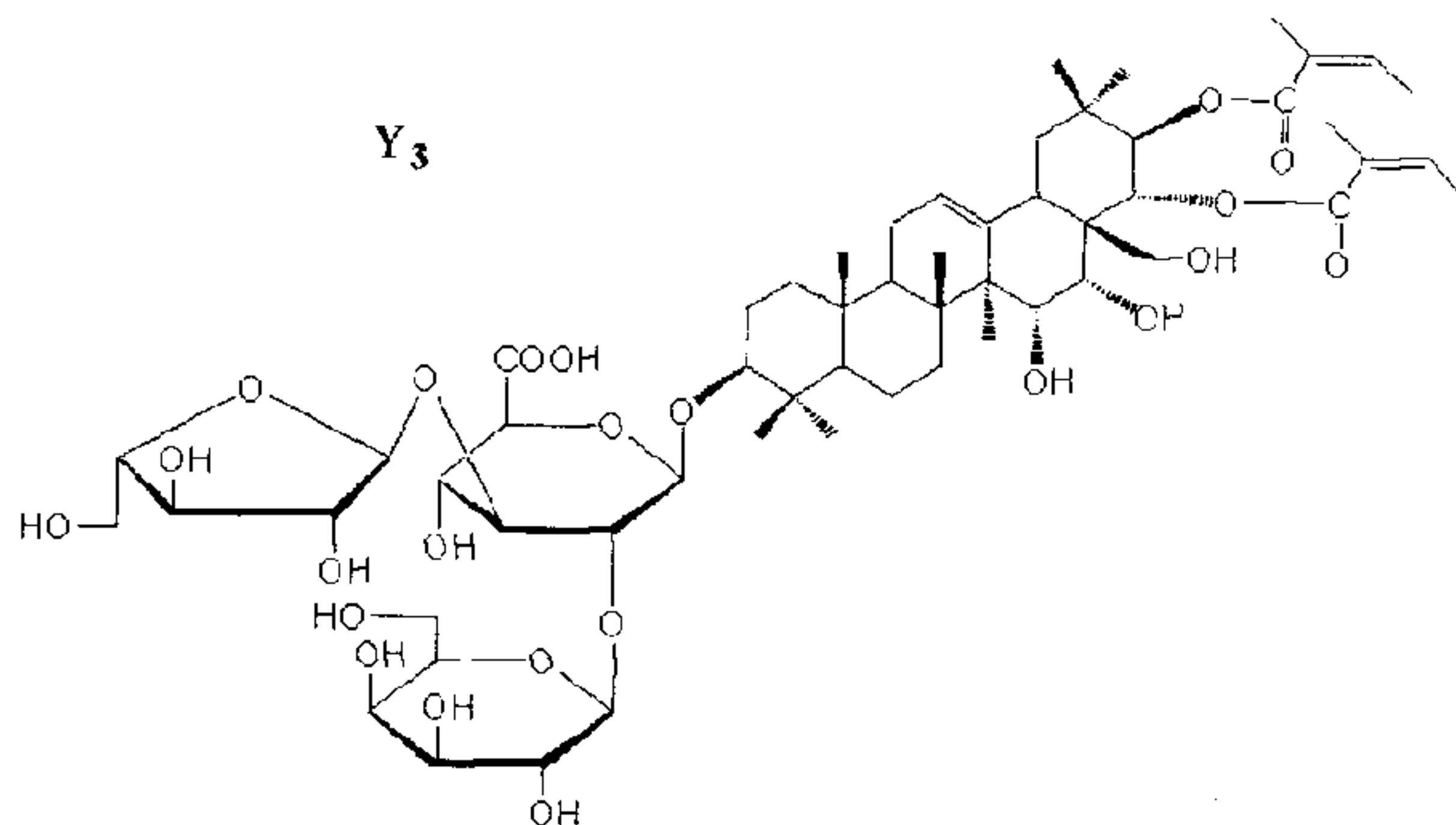
A composition for treating cancers or inhibiting virus, comprising a compound which comprises at least two side chains which comprise angeloyl groups, wherein the side chains are at adjacent carbon in *trans* position. In an embodiment, the side chains are at alternative carbon in *cis* position. In an embodiment, the side chains are at alternative carbon in *trans* position. In an embodiment, the side chains are in non-adjacent carbon *cis* or *trans* position. In an embodiment, the side chains comprise a functional group capable of performing the function of angeloyl group.

This invention provides a composition for inhibiting tumor cell growth, comprising the above-described compounds. In an embodiment, the composition comprises a suitable carrier. In another embodiment, the composition comprises a pharmaceutically suitable carrier. This invention provides a method for treating ovarian cancer in a subject, comprising administering to said subject an effective amount of the above-described compositions.

This invention provides a method for isolating compounds from *Xanthoceras sorbifolia* herb, or plants from the sapindaceae family comprising the steps of: (a) extracting *Xanthoceras sorbifolia* or plant powder with organic solvents to obtain an organic extract; (b) collecting the organic extract; (c) refluxing the organic extract to obtain a second extract; (d) removing the organic solvent from the second extract; (e) drying and sterilizing the second extract to obtain a crude extract powder; (f) fractionating the crude extract powder into components using HPLC and FPLC chromatography with silica gel, C18 and other equivalent solid phase materials; (g) monitoring absorption wavelength at 207nm or 254nm; (h) identifying the bioactive components of the crude extract powder; (i) purifying one or more bioactive components of the crude extract powder with FPLC to obtain one or more fraction of the bioactive component; and (j) isolating the desired fraction of the bioactive component with preparative HPLC.

Compound Y or Y3

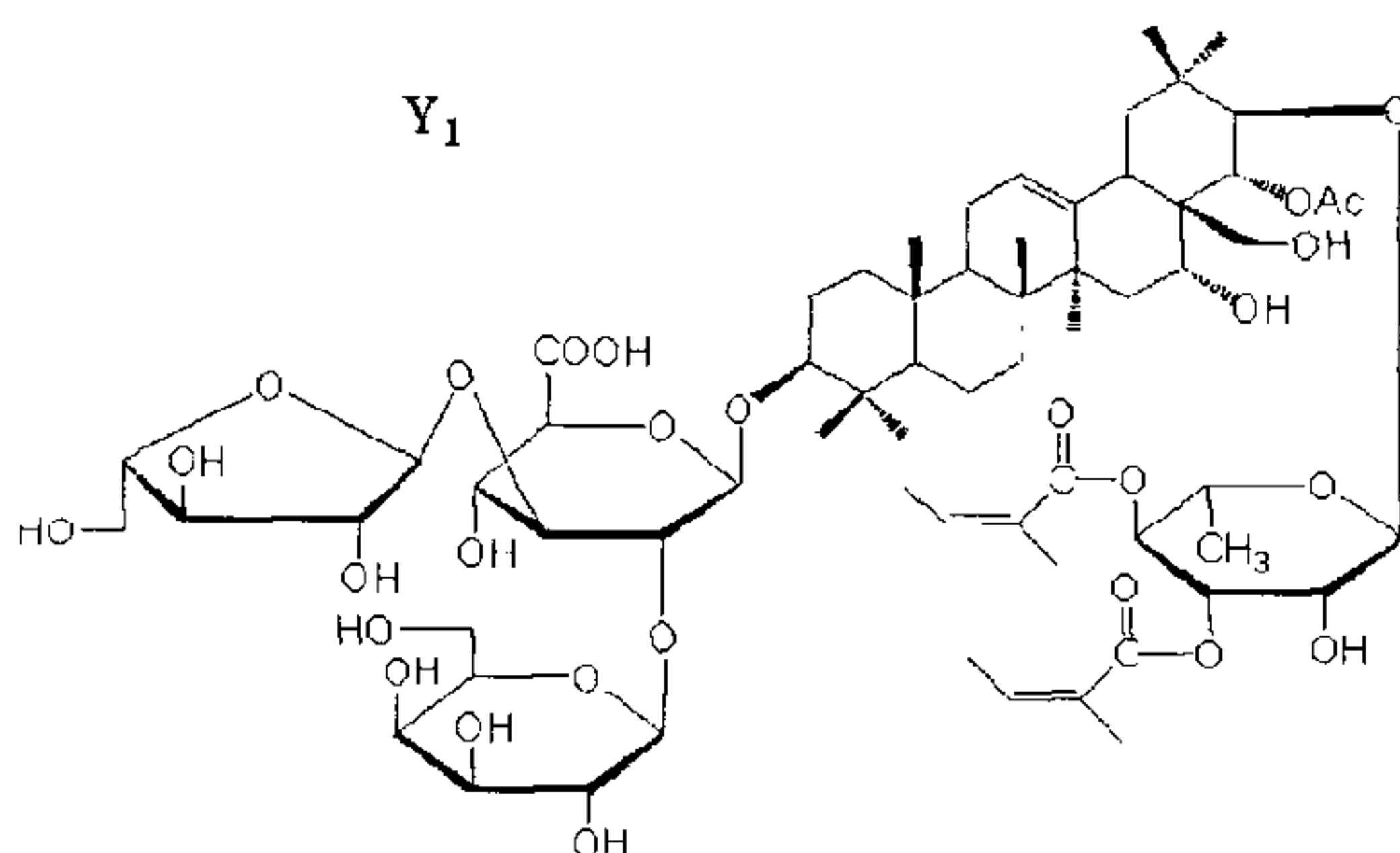
This invention provides a compound comprising the following structure, i.e., see Figure 17, having the formula of $C_{57}H_{88}O_{23}$ and the name of 3-O-[β -D-galactopyranosyl(1 $\rightarrow$ 2)]- α -L-arabinofuranosyl(1 $\rightarrow$ 3)- β -D-glucuronopyranosyl-21,22-O-diangeloyl-3 β , 15 α , 16 α , 21 β , 22 α , 28-hexahydroxyolean-12-ene, also known as Xanifolia-Y. This compound is obtainable from *Xanthoceras sorbifolia* or plants from the sapindaceae family.



This compound belongs to an oleanene triterpenoidal saponin with a trisaccharide chain attached at C-3 of the aglycone and two angeloyl groups acylated at C-21 and C-22. This compound has anti-cancer activity. The assignment of this structure is supported by spectral data, i.e., H-NMR, 2D NMR (HMBC, HMQC), and MS (MALDI-TOF, EMS). Accordingly, this compound has the characteristic property as shown in Figures 18-22 or Table 5.1.

Compound Y1

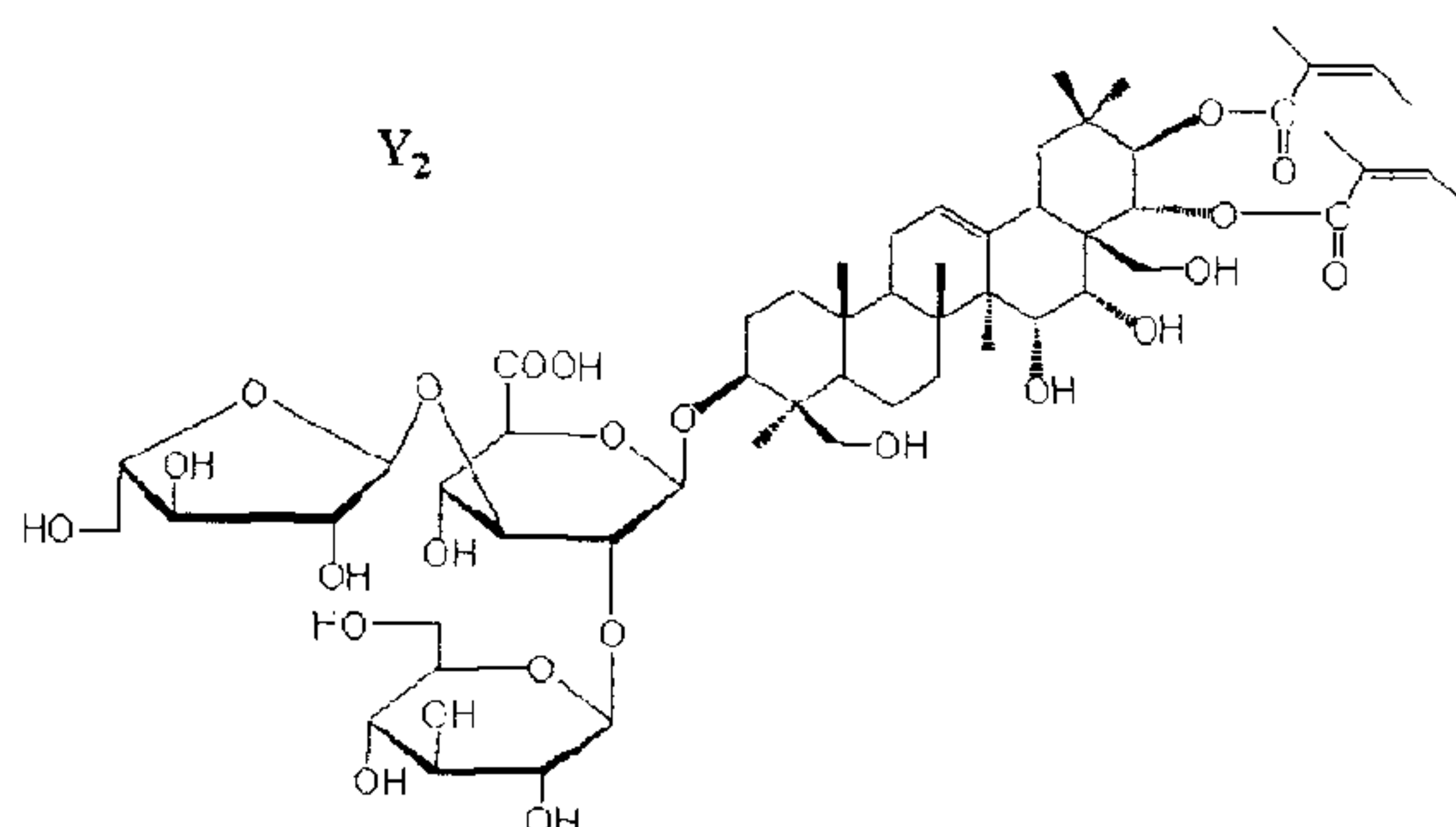
This invention provides another compound comprising the following structure, i.e., see Figure 23, having the formula of $C_{65}H_{100}O_{27}$ and the name of 3-O-[[β -D-galactopyranosyl(1 $\rightarrow$ 2)]- α -L-arabinofuranosyl(1 $\rightarrow$ 3)- β -D-glucuronopyranosyl-21-O-(3,4-diangeloyl)- α -L-rhamnopyranosyl-22-O-acetyl-3 β ,16 α , 21 β , 22 α , 28-pentahydroxyolean-12-ene, also known as Xanifolia-Y1.



This compound is a bisdesmosidic polyhydroxyoleanene triterpenoidal saponin with a trisaccharide chain at C-3 of the backbone and a monosaccharide moiety at C-21 where two angeloyl groups were acylated at C-3 and C-4 position. This compound has anti-cancer activity. The assignment of this structure is supported by spectral data, i.e., H-NMR, 2D NMR (HMBC, HMQC, COSY), and MS (MALDI-TOF). Accordingly, this compound has the characteristic property as shown in Figures 24-27.

Compound Y2

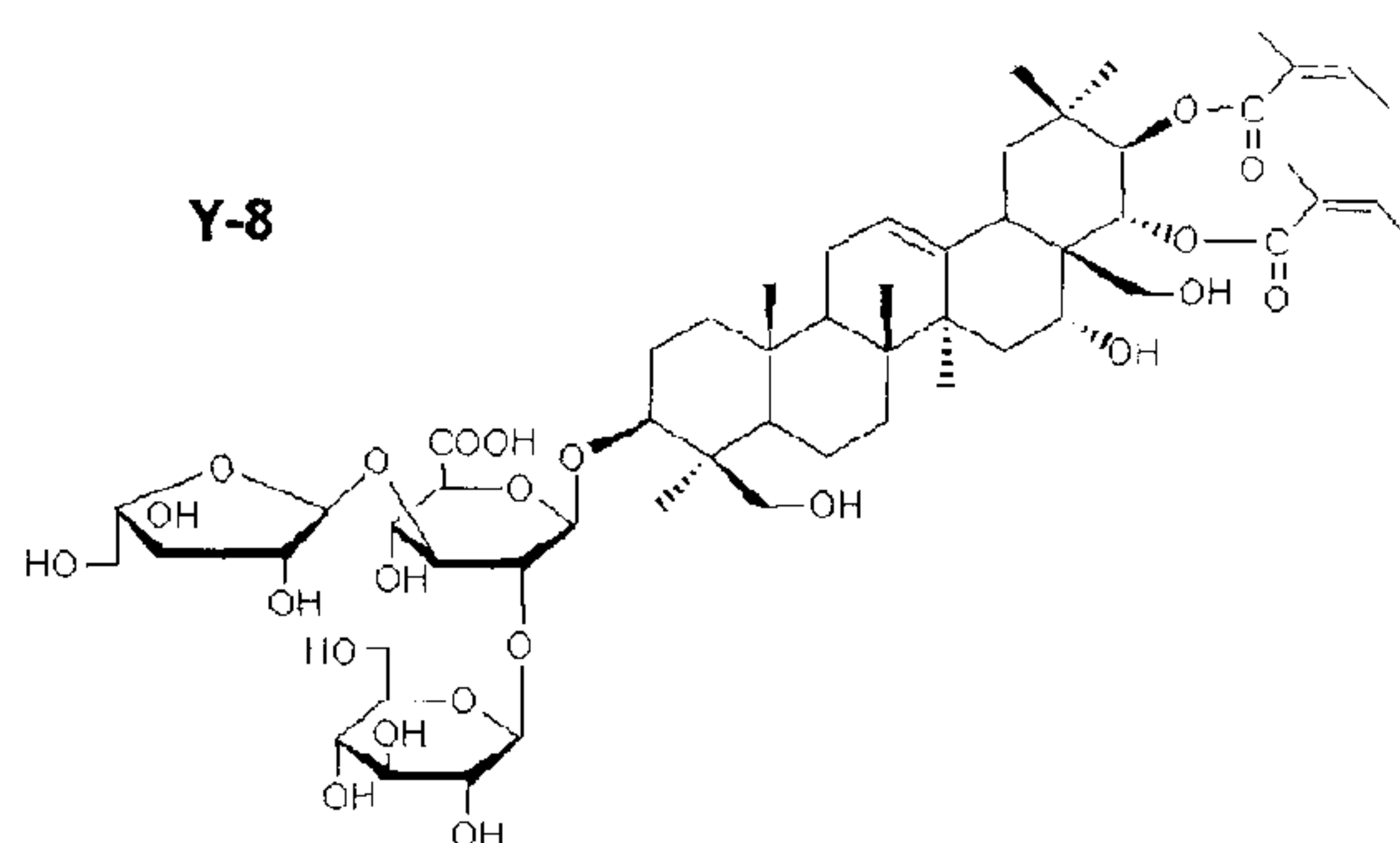
This invention provides a third compound comprising the following structure, i.e., see Figure 28, having the formula of $C_{57}H_{88}O_{24}$ and chemical name of 3-O-[[β -D-glucopyranosyl-(1 $\rightarrow$ 2)]- α -L-arabinofuranosyl(1 $\rightarrow$ 3)- β -D-glucuronopyranosyl-21,22-O-diangeloyl-3 β , 15 α , 16 α , 21 β , 22 α , 24 β , 28-heptahydroxyolean-12-ene, also known as Xanifolia-Y2.



10 This compound (Y2) belongs to saponins comprising a triterpene, a sugar moiety and angeloyl groups linked to the backbone. The angeloyl groups are linked to the backbone at C21 and C22 positions. This compound has anti-cancer activity. The assignment of this structure is supported by spectral data, i.e., H-NMR, C-NMR, 2D NMR (HMBC, HMQC, TOCSY), and MS (MALDI-TOF). Accordingly, this compound has the
15 characteristic property as shown in Figures 29-34.

Compound Y8

This invention provides a fourth active compound Y8 and the structure was determined by 1D NMR, 2D NMR, and MS analysis. The compound comprises the following
20 structure, i.e. see Figure 35, having the formula of $C_{57}H_{87}O_{23}$ and chemical name of 3-O-[[β -glucopyranosyl (1 $\rightarrow$ 2)]- α -arabinofuranosyl (1 $\rightarrow$ 3)- β -glucuronopyranosyl-21, 22-O-diangeloyl-3 β , 16 α , 21 β , 22 α , 24 β , 28-hexahydroxyolean-12-ene, also known as Xanifolia-Y8.



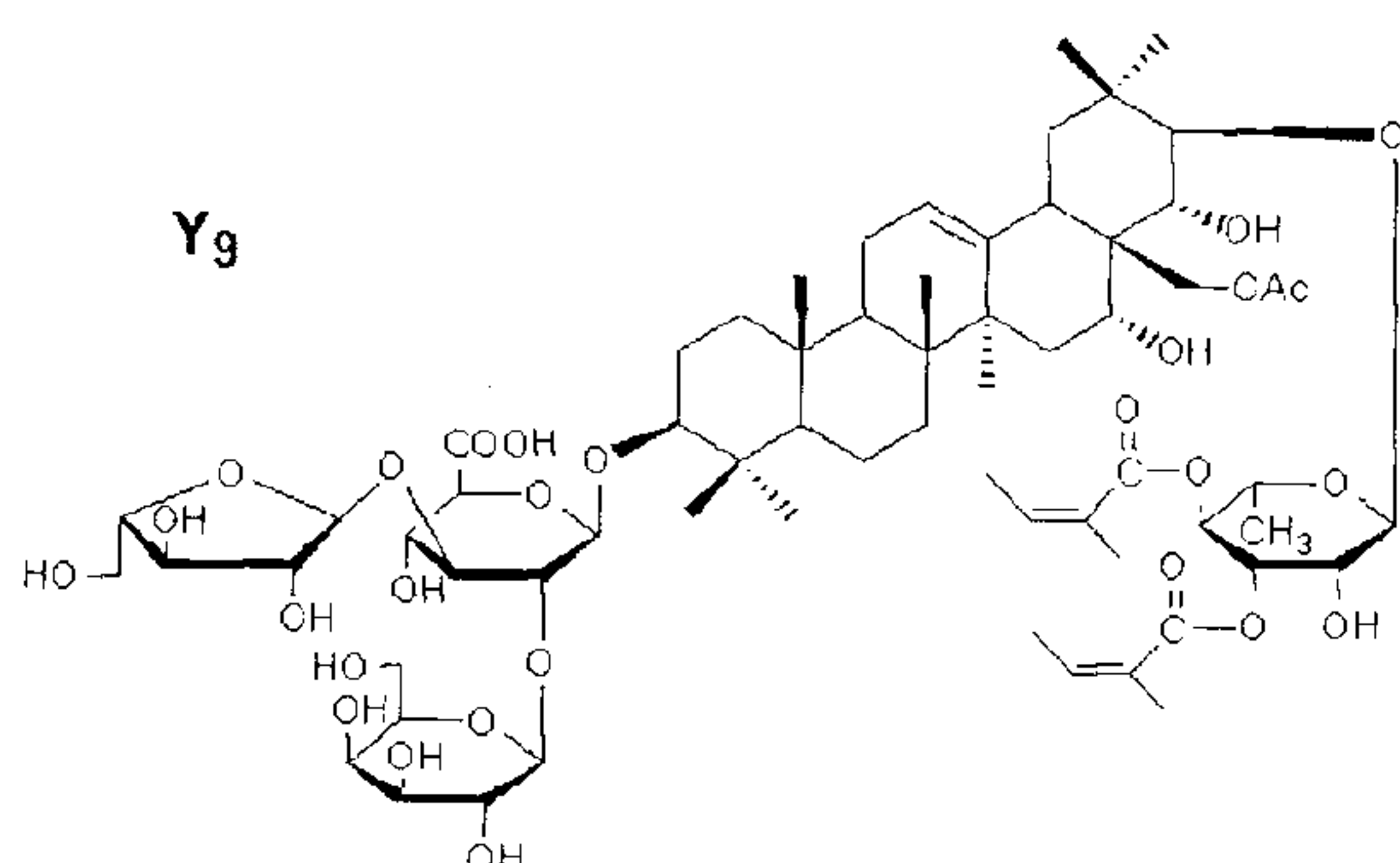
The assignment of this structure is supported by spectral data, i.e., H-NMR, C13-NMR and 2D NMR (HMQC). Accordingly, this compound has the characteristic property as shown in figures 36-38.

5

Compound Y9

This invention provides a fifth active compound Y9 and the structure was determined by 1D NMR, 2D NMR, and MS analysis. The compound comprises the following structure, i.e., see Figure 39, having chemical name 3-O- $[\beta$ -galactopyranosyl (1 $\rightarrow$ 2)]- α -arabinofuranosyl (1 $\rightarrow$ 3)- β -glucuronopyranosyl-21-O-(3,4-diangeloyl)- α -rhamnopyranosyl-28-O-acetyl-3 β , 16 α , 21 β , 22 α , 28-pentahydroxyolean-12-ene, also known as Xanifolia-Y9.

10



The assignment of this structure is supported by spectral data, i.e., H-NMR, 2D NMR (HMQC and HMBC). Accordingly, this compound has the characteristic property as shown in Figures 40-42.

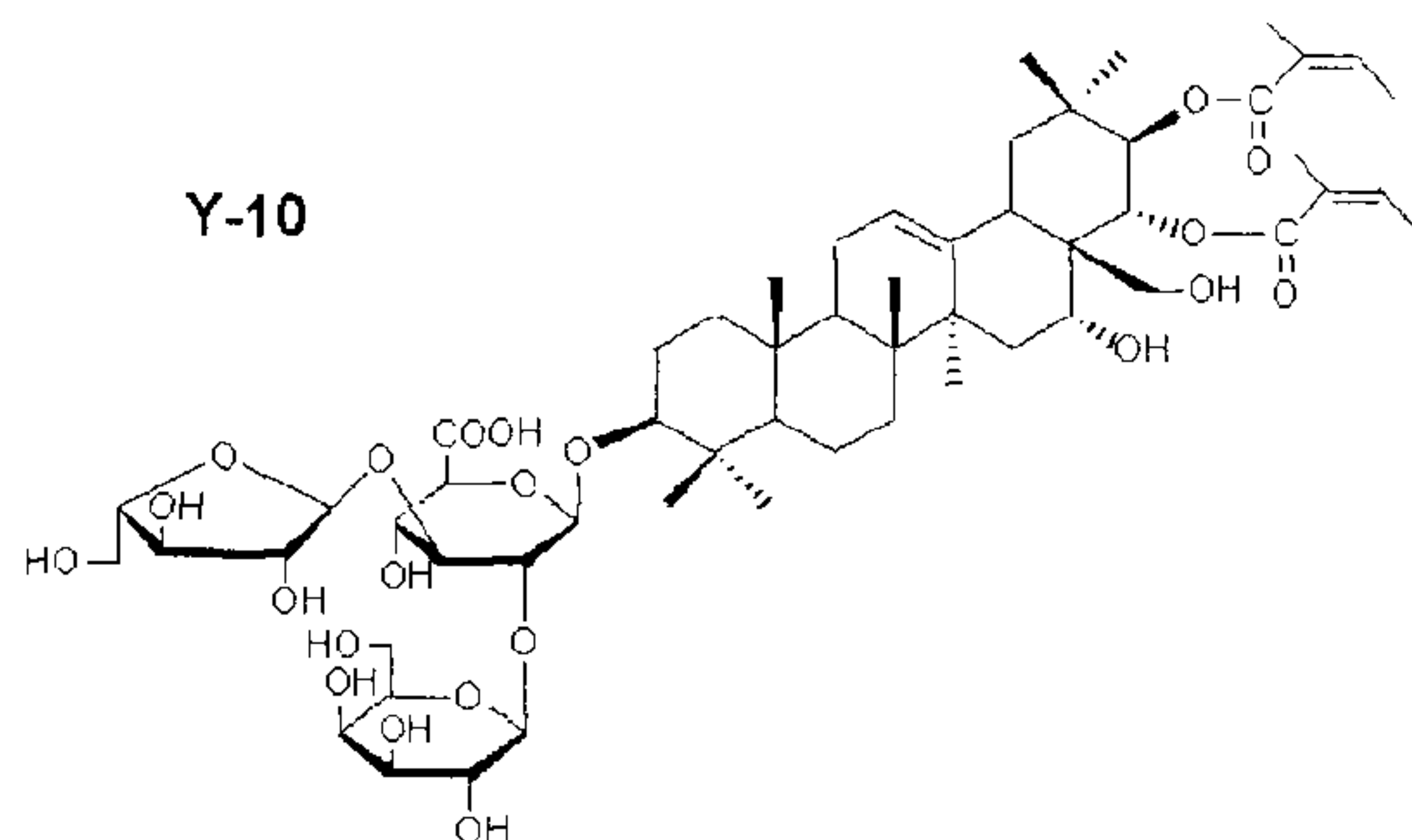
15

Compound Y10

This invention provides a sixth active compound Y10 and the structure was determined by 1D NMR, 2D NMR and MS analysis. The compound comprises the following structure, i.e., see Figure 43, having the formula of C₅₇H₈₇O₂₂ and chemical name of 3-O- $[\beta$ -galactopyranosyl (1 $\rightarrow$ 2)]- α -arabinofuranosyl (1 $\rightarrow$ 3)- β -glucuronopyranosyl-21, 22-

20

O-diangeloyl-3 β , 16 α , 21 β , 22 α , 28-pentahydroxyolean-12-ene, also known as Xanifolia-Y10.



The assignment of this structure is supported by spectral data, i.e., H-NMR, C13-NMR
5 and 2D NMR (HMQC). Accordingly, this compound has the characteristic property as shown in figures 44-46.

This invention provides a compound comprising a sugar moiety and a triterpene or Sapogenin, wherein the triterpene or sapogenin is acylated at Carbon 21 and 22 with
10 angeloyl groups. In an embodiment, the triterpene or sapogenin is acylated at Carbon 21 and/or 22 with a sugar moiety which comprises two angeloyl groups. In another embodiment, the compound comprises one or more sugars. In another embodiment, the compound comprises at least two angeloyl groups. In a further embodiment, the sugar moiety comprises at least one sugar, or D-glucose, or D-galactose, or L-rhamnose, or L-
15 arabinose, or D-xylose, or alduronic acid, or D-glucuronic acid or D-galacturonic acid, derivative thereof, or the combination thereof. In a further embodiment, a compound capable of performing the function of the sugar moiety is attached to the triterpene or sapogenin. In a further embodiment, the angeloyl group may be replaced by tigloyl group, senecioly group, an acid having two to five carbons, or combinations thereof.
20 Substitution, deletion and/or addition of any group in the above-described compounds will be apparent to one of ordinary skill in the art based on the teaching of this application. In a further embodiment, the substitution, deletion and/or addition of the group(s) in the compound of the invention does not substantially affect the biological function of the compound. In a further embodiment, the angeloyl groups are in a trans-
25 position on a structure. In a further embodiment, the angeloyl groups are in an adjacent trans-position on a structure.

Extracts obtained from Xanthoceras sorbifolia having anticancer activity is disclosed. Experiments for determining anti-cancer activity of the extract of the invention employ

human cells lines derived from eleven human organs, i.e., (HTB-9 (bladder), HeLa-S3 (cervix), DU145 (prostate), H460 (lung), MCF-7 (breast), K562 (leukocytes), HCT116 (colon), HepG2 (liver), U2OS (bone), T98G (brain) and OVCAR-3 (ovary)). The response of the 11 cell lines toward the extract of the invention can be categorized into four groups: (A) most sensitive: Ovary, see Figure 14; (B) Sensitive: bladder, bone, (C) Semi-sensitive: prostate, leukocyte, liver, breast, and brain; and (D) least sensitive: colon, cervix, and lung. See Figure 16A, B, C. The respective IC50 values are listed in Table 3.1.

Table 3.1. IC50 values of Xanthoceras Sorbifolia Extract Determined in Different Cancer Cells

Cancer cells from different organs	IC50 determined by MTT assay (ug/ml)
Ovary (most sensitive)	15-15
Bladder (sensitive)	45-50
Bone	40-55
Prostate (semi-sensitive)	40-50
Leukocyte	45-50
Liver	45-65
Breast	65
Brain	70-85
Colon (least sensitive)	90
Cervix	115
Lung	110

In order to identify the active compounds of Xanthoceras sorbifolia, the extracts from Xanthoceras sorbifolia were separated by chromatography comprising FPLC (Fast Protein Liquid Chromatography) and HPLC (High Performant Liquid Chromatography). Multiple fractions were obtained by FPLC procedures, i.e., see Figure 9, and HPLC, i.e., see Figure 8. Analysis of the fractions by HPLC shows that the extract comprises 26 identifiable fractions, designated as a to z, which are shown in Figure 8. Anti-cancer activities of these fractions were determined by the MTT assay.

FPLC fraction 5962, i.e., see Figure 10, which corresponds to fraction Y in HPLC, i.e., see Figure 8, has anti-cancer activity. Compound Y can be purified from fraction Y. Fraction 5962 was further separated into 4 components, designated as Y1 to Y4. See

Figure 11. Compounds Y1-Y4 can be purified from the fraction 5962. Fraction 6365 was further separated into 5-6 components, designated as Y5-Y10. See Figure 12. Compounds Y5-Y10 can be purified from Fraction 6365. Compounds Y or Y3, Y1 and Y2 show strong anti-tumor activity, i.e., see Figure 2-3, and were therefore isolated and purified. Similarly, compounds Y8, Y9 and Y10 also show strong anti-tumor activity, i.e., see Figure 4, and were therefore isolated and purified. See Figure 13. Accordingly, the structures of these active compounds, i.e., Y, Y1, Y2, Y8, Y9 and Y10 and their uses are the subject of this application.

The inhibition effects of the compounds of the present invention on ovarian cancer cells were evaluated with the MTT assay. Compound Y shows at least 10 times higher potency ($IC_{50} = 1.5 \text{ ug/ml}$), i.e., see Figure 2, than the original crude extract as shown in Figure 14 ($IC_{50} = 20 \text{ ug/ml}$). The selectivity of compound Y toward different cell lines was tested, and it was found that compound Y has a much higher potency toward ovarian cancer cells as compared to the cervical cancer cells. See Figure 15.

This invention provides a method for identifying and isolating the bioactive compounds from plants, herbs or plant extracts. In an embodiment, the extracts include extracts of *Xanthoceras sorbifolia* or of plants from the sapindaceae family. This invention provides the chemical structure of six bioactive compounds isolated from *Xanthoceras sorbifolia* or plants from the sapindaceae family. The structure of the compounds of the invention is shown in Figure 1. This invention provides spectral data including H-NMR, C-13-NMR, 2D NMR (HMBC, HMQC, COSY, TOCSY), and MS (MALDI-TOF, ESI-MS) in supporting the assigned structures.

This invention provides a consensus sub-structure or functional group derived from the bioactive compounds purified from fraction Y. The compounds, such as Y or Y3, Y1, Y2, Y8, Y9 and Y10, isolated from fraction Y are collectively referred to as "Ys" and their common name is Xanifolia-Ys. The consensus sub-structure or functional group of these compounds is the biangeloyl group located on adjacent carbons. For example, in compound Y, Y2, Y8 and Y10, the biangeloyl group is located at 21β and 22α of the triterpene backbone. See Figure 5. In compound Y1 and Y9, the biangeloyl group is located at C3 and C4 of the sugar ring. See Figure 6. Accordingly, the biangeloyl group of the bioactive compounds of the invention is situated in a trans-position with respect to each other on a structure. See Figure 7. It has been shown that the bioactive functional

group of the compounds of the invention is a biangeloyl group attached in-trans to adjacent carbons located in a structure. See Figure 7. This invention provides a salt of the above-described compounds.

- 5 This invention provides a composition comprising the above-described compounds and a suitable carrier. This invention provides a pharmaceutical composition comprising an effective amount of the above-described compounds and a pharmaceutically acceptable carrier. This invention provides an anti-ovarian cancer agent or composition comprising the above-described compositions. This invention provides a composition effective
10 against cancer growth. The cancer includes but is not limited to bladder cancer, bone cancer and ovary cancer. This invention provides a composition effective against skin cancer and KB cancer growth. This invention provides a composition comprising the above-described compounds and their salts, esters, derivatives or metabolites capable of inhibiting tumour growth. This invention provides a composition for inhibiting virus
15 growth and/or activities comprising the above-described compounds and their salts, esters, derivatives or metabolites.

This invention provides a composition comprising the above-described compounds and their salts, esters, derivatives or metabolites capable of hemolytic activities. See Figure
20 73. It has been shown that a compound of the invention having two angeloyl groups has a stronger anticancer activity than a compound with one angeloyl group. See Figure 65, 2, 3. A compound with two angeloyl groups shows more haemolytic activity than a compound with one angeloyl group. See Figure 73 A. The compound loses hemolytic activity when the angeloyl groups are removed See Figure 73 B. The compounds of the
25 invention or their derivatives are also obtainable by chemical synthesis or isolated from natural or biological sources, including plants from the sapindaceae family. It has also been shown that saponin compounds of the invention having two angeloyl groups are more potent than Escin for treating diseases relating to hamoylic activities. See Figure 73A.

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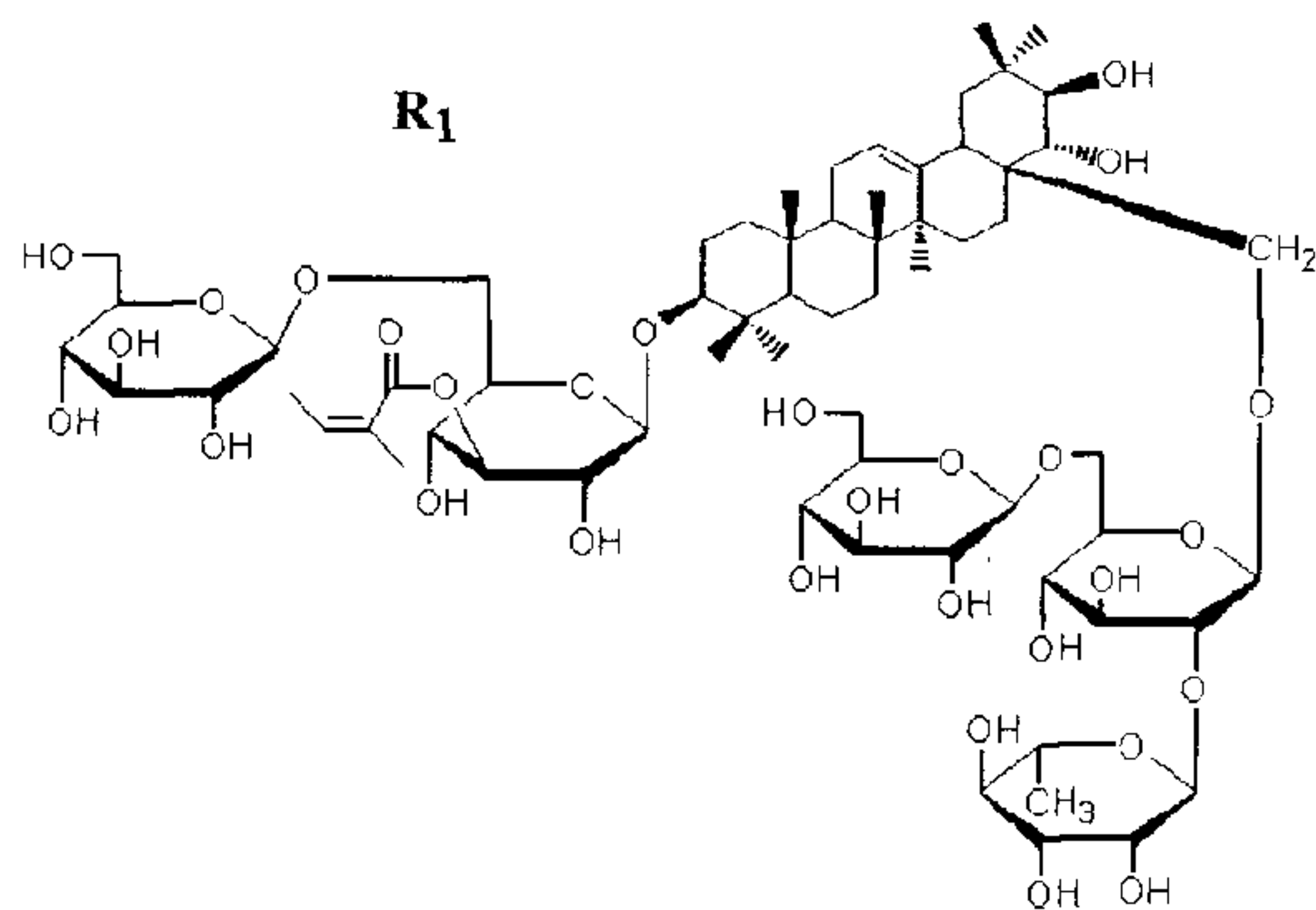
This invention provides a composition for treating chronic venous insufficiency, peripheral edema, antilipemic, chronic venous disease, varicose vein disease, varicose syndrome, venous stasis, Expectorant, cerebro-organic convulsion, cerebral circulation disorder, cerebral edema, psychoses, dysmenorrheal, hemorrhoids, episiotomies,
35 haemonhoids, peripheral oedema formation or postoperative swelling; for reducing

symptoms of leg pain, pruritis, lower leg volume, thrombosis, thrombophlebitis; and for preventing gastric ulcers antispasmodic. This invention provides a composition for AntiMS, antianeurysm, antiasthmatic, antibradykinic, anticapillarihemorrhagic, anticephalagic, anticervicobrachialgic, antieclamptic, antiedemic, antiencephalitic, antiepiglottitic, antiexudative, antifu, antifracture, antigingivitic, antihematoma, antihistaminic, antihydrarthritic, antimeningitic, antioxidant, antiperiodontic, antiphlebitic, antipleuritic, antirauco, antirhinitic, antitonsillitic, antiulcer, antivaricose, antivertiginous, cancerostatic, corticosterogenic, diuretic, fungicide, hemolytic, hyaluronidase inhibitor, lymphagogue, natriuretic, pesticide, pituitary stimulant, thymolytic, vasoprotective, venotonic.

Other compounds were also isolated from fraction R and fraction O of the extract of *Xanthoceras sorbifolia*, which are designated herein as R1 and O54, respectively. Their structures were determined. Both compounds are triterpenoidal saponins. Both compounds lack biangeloyl attachment in the triterpene backbone or in the sugar rings. Preliminary experiments indicate both R1 and O54 do not have anticancer activity.

Compound R1

The structure of compound R1 is shown below and in Figure 47. Compound R1 has a chemical formula of $C_{65}H_{106}O_{29}$ and chemical name of 3-O-[angeloyl-(1→3)-β-D-glucopyranosyl-(1→6)]-β-D-glucopyranosyl-28-O-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-3β, 21β, 22α, 28-tetrahydroxyolean-12-ene, also known as Xanifolia-R1.



The assignment of this structure is supported by spectral data, i.e., H-NMR, C-13-NMR, 2D NMR (HMBC, HMQC, COSY), and MS (MALDI-TOF, EMS). Accordingly, this compound has the characteristic property as shown in Figures 48-52.

Compound-O54

This invention provides a compound O54 isolated from the extract of *Xanthoceras sorbifolia*. The structure of O54 was determined and has a formula of $C_{60}H_{100}O_{28}$.

The Structure of Compound O54 is shown below. See also Figure 53.

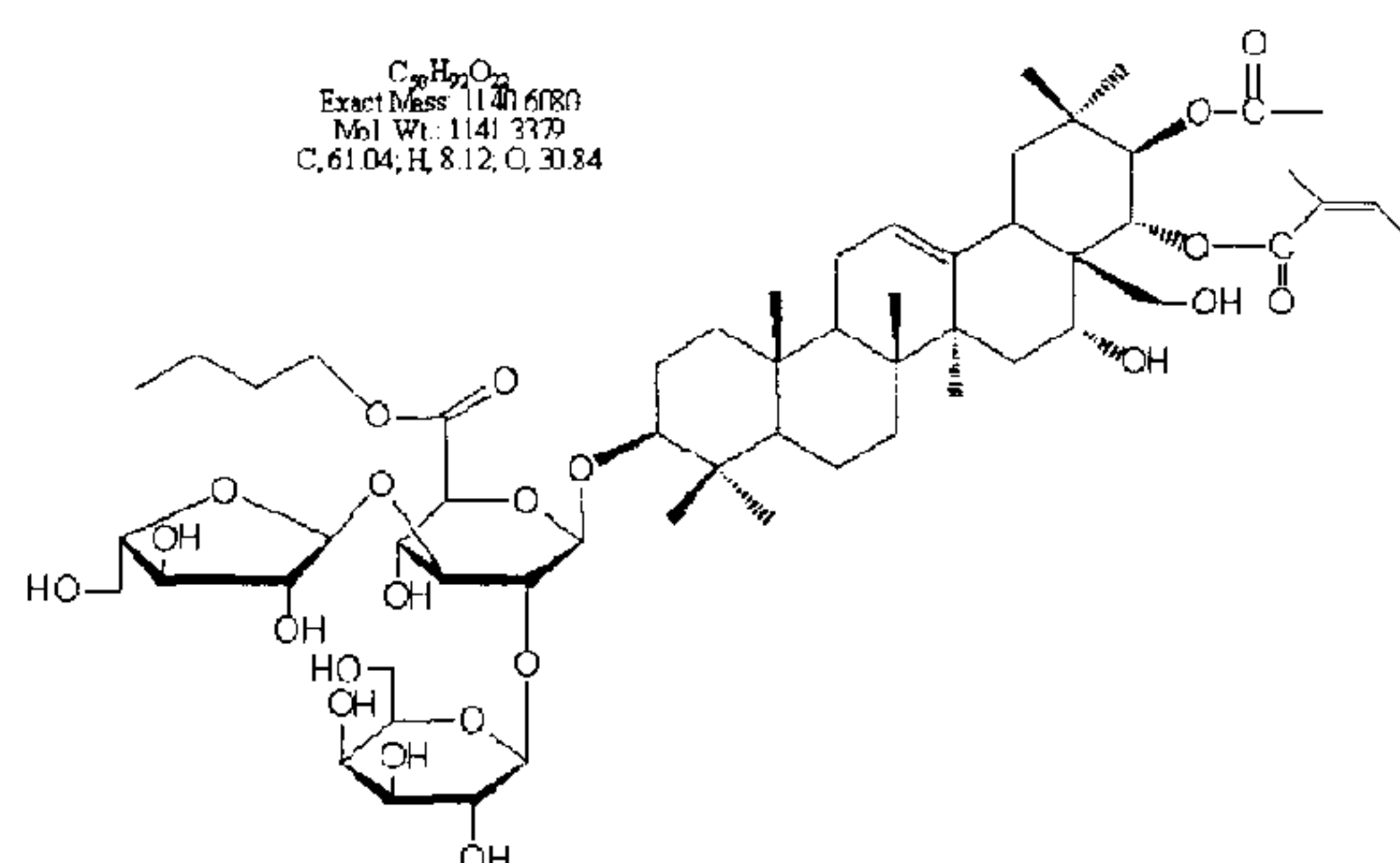
5 Error! Objects cannot be created from editing field codes.

The chemical name of compound-O54 is: 3-O- β -D-glucopyranosyl-(1 $\rightarrow$ 6)]- β -D-glucopyranosyl-28-O-[α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- β -D-glucopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranosyl-3 β , 21 β , 22 α , 28-tetrahydroxyolean-12-ene, also known as Xanifolia-O54. The assignment of this structure is supported by spectral data, i.e., ^1H -NMR, 2D
10 NMR (HMBC, HMQC). Accordingly, this compound has the characteristic property as shown in Figures 54-56.

In addition to the compound Ys, R1 and O54, other compounds were also isolated from fraction X of the extract of *Xanthoceras sorbifolia*, which are designated herein as X. Its
15 structure was determined. The compound is a triterpenoidal saponin with an angeloyl group attached at C22 of the triterpene.

Compound-X

This invention provides an active compound, designated as "compound X", isolated
20 from extract of *Xanthoceras Sorbifolia*. Compound X is an oleanene triterpenoidal saponin with a trisaccharide chain attached at C-3 of the aglycone and one angeloyl group acylated at C-22. The formula of compound X is $C_{58}H_{92}O_{22}$, and the chemical name of compound X is: 3-O-[[β -D-galactopyranosyl (1 $\rightarrow$ 2)]-[α -L-arabinofuranosyl (1 $\rightarrow$ 3)]- β -D-glucuronopyranoside butyl ester}-21-O-acetyl-22-O-angeloyl-
25 3 β ,16 α ,21 β ,22 α ,28-pentahydroxyolean-12-ene. The chemical structure of compound X is shown below. See also Figure 70.



This invention provides a composition comprising the compounds of the invention for treating enuresis and frequency micturition, and for improving the functions of the central nervous system including signaling the bladder to wake up from deep sleep or to relax the bladder so that it can store more urine. The compounds of the invention can
5 be used to relax the detrusor tension caused by aging, stress, nervousness, over-activity, instability, hyper-reflexia, and uninhibited bladder. In another embodiment, the compounds may be used for relaxing the contracted bladder tissue induced by acetylcholine (Ach). The compounds identified and isolated from extract of *Xanthoceras sorbifolia* may be used as acetylcholinesterase, an AChE inhibitor, for regulating
10 Antidiuretic hormone (ADH), which reduces the volume of urine, and as an anti-inflammatory agent.

The compounds of the invention can be used for accelerating the growth of bladder, for suppressing deep sleep, for increasing alertness in a sleeping subject, for modulating
15 the release, breakdown and uptake of Antidiuretic hormone (ADH) and its receptors, for modulating the secretion, breakdown and uptake of Adrenocorticotrophic hormone (ACTH) and its receptors, for modulating the release, breakdown and uptake of 5-hydroxytryptamine and its receptors, for modulating the release, breakdown and uptake of Acetylcholine (Ach) and its receptors, for modulating the release, breakdown and
20 uptake of Adrenaline (AD) and its receptors, for modulating the release, breakdown and uptake of Dopamine (DA) and its receptors, for modulating the release, breakdown and uptake of Norepinephrine (NE) and its receptors, for preventing sleep paralysis, for modulating the formation, release, breakdown and activity of neuropeptides and their receptors.

25

This invention provides a composition comprising the compounds of the invention for treating cancers; for inhibiting virus; for preventing cerebral aging; for improving memory; improving cerebral functions, for curing enuresis, frequent micturition, urinary incontinence, dementia, Alzheimer's disease, autism, brain trauma, Parkinson's disease
30 or other diseases caused by cerebral dysfunctions; for treating arthritis, rheumatism, poor circulation, arteriosclerosis, Raynaud's syndrome, angina pectoris, cardiac disorder, coronary heart disease, headache, dizziness, kidney disorder; cerebrovascular disease; inhibiting NF-Kappa B activation; for treating brain edema, severe acute respiratory syndrome, respiratory viral diseases, chronic venous insufficiency,
35 hypertension, chronic venous disease, anti-oedematous, anti inflammatory,

haemonhoids, peripheral oedema formation, varicose vein disease, flu, post traumatic edema and postoperative swelling; for inhibiting ethanol absorption; for lowering blood sugar; for regulating the adreocorticotropin and corticosterone level; and for treating impotence or premature ejaculation or diabetes. See U.S. Serial No. 10/906,303, filed
5 February 14, 2005, International Application No. PCT/US04/43465, filed December 23, 2004, International Application No. PCT/US04/33359, filed October 8, 2004, and U.S. Serial No. 11/131551, filed May 17, 2005.

This invention provides a composition capable of regulating the enzymatic activities of
10 bladder cell. The Xanthoceras Sorbifolia derived compounds and/or compositions of the invention are capable of regulating the various components or receptors of a cell and strengthening the cell growth process. The Xanthoceras Sorbifolia derived compound and/or composition regulates the activities of cell's enzymes. See U.S. Serial No.60/675,284, filed April 27, 2005. This invention provides compounds isolated from
15 Xanthoceras Sorbifolia, or its derivatives or metabolites capable of regulating pathways involved in cell proliferation. See U.S. Serial No. 10/906,303, filed February 14, 2005, International Application No. PCT/US04/43465, filed December 23, 2004, International Application No. PCT/US04/33359, filed October 8, 2004.

20

This invention provides the methods and uses of triterpenoidal saponins purified and isolated from plants. This invention provides compositions comprising the triterpenoidal saponins or their derivatives for inhibition of tumor growth. The compounds of the
25 invention comprise angeloyl group(s) or tigloyl group(s) or senecioyl group(s) or combinations thereof which are attached to carbon 21 and 22 of their saponogenines. In an embodiment, the compounds may comprise any two angeloyl groups or tigloyl groups or senecioyl groups or combinations thereof attached to a sugar moiety which bonds to carbon 21 or 22 of their saponogenines. The bioactive compounds can be
30 isolated from natural plants, including plants in the Sapindaceae family, which has between about 1400-2000 species with 140-150 genera. See U.S. Serial No.60/675,282, filed April 27, 2005.

Summary

This invention provides methods for identifying and purifying bioactive compounds from the plant extract of *Xanthoceras sorbifolia*. Eight bioactive compounds have so far been identified and purified, and six of them have been shown to have anticancer activity. These compounds are collectively referred to as triterpenoidal saponins. A consensus
5 sub-structure was identified or derived from the structure of the bioactive compounds of the invention. The consensus sub-structure or active functional groups of the bioactive compounds is the biangeloyl group located on adjacent carbons. The angeloyl groups are located at 21β and 22α of the triterpene backbone, i.e., see Figure 5, or located at C3 and C4 of the sugar ring, i.e., see Figure 6. Accordingly, the biangeloyl group of
10 these bioactive compounds is acylated in a trans-position in adjacent carbons of a structure. See Figure 7. The compound with single angeloyl group shows weaker anticancer activity than a compound with two angeloyl groups, i.e., see Figure 65, 2, 3. The compound with single angeloyl group shows less haemolytic activity than a compound with two angeloyl groups, i.e., see Figure 73. The structures or derivatives of
15 the compounds of the present invention are also obtainable by chemical synthesis or from biological sources.

This invention will be better understood from examples which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed
20 are merely illustrative of the invention as described more fully in the claims which follow thereafter.

EXPERIMENTAL DETAILS

25 Experiment 1: Herb Extraction

(a) extracting *Xanthoceras sorbifolia* powder of husks or branches or stems or leaves or kernels or roots or barks with organic solvent at ratio of 1:2 for 4-5 times for 20-35 hours each time to form an organic extract; (b) collecting the organic extract; (c) refluxing the organic extract for 2-3 times at 80°C to form second extract; (d) removing the organic
30 solvent from the second extract; and (e) drying and sterilizing the second extract to form a *Xanthoceras sorbifolia* extract powder.

Experiment 2: Analysis of *Xanthoceras Sorbifolia* Extract Components by HPLC Chromatography

35

Methods

HPLC. A C-18 reverse phase μ bondapak column (Water P/N 27324) was equilibrated with 10% acetonitrile, 0.005% Trifluoroacetic acid (equilibration solution). An extract of *Xanthoceras sorbifolia* prepared using the methods described in Experiment 1 was dissolved in equilibration solution (1 mg/ml) before applying into the column. 20ug of samples was applied into column. Elution conditions: Fractions were eluted (with flow rate 0.5 ml/min.) with acetonitrile gradient from 10% to 80% in 70 min, and then remains at 80% for 10 min. The acetonitrile concentration then decreased to 10% and remained at 10% for 25 min. The fractions were monitored at 207 nm and recorded in chart with a chart speed of 0.25 cm/min and with OD full scale of 0.128.

Instruments. Waters Model 510 Solvent Delivery System; Waters 484 tunable Absorbance Detector; Waters 745/745B Data Module.

Absorbance analysis. The absorption profile of *Xanthoceras Sorbifolia* extract at various wavelengths was determined. An extract of *Xanthoceras sorbifolia* of the present invention was dissolved in 10% acetonitrile/TFA and scanned at 200-700 nm with a spectrophotometer [Spectronic Ins. Model Gene Sys2].

Results

HPLC. About 60-70 peaks can be accounted for in the profile. Among them four are major peaks, 10 are of medium size and the rest are small fractions. The peaks are labelled with a to z following increased concentration of acetonitrile elution. See Figure 8.

Absorption maximum. Three absorption maximum were identified for *Xanthoceras sorbifolia* plant extract; 207nm, 278nm and 500nm. See Figure 57.

Experiment 3: Determination of the cell-growth activity effected by *Xanthoceras Sorbifolia* Extract with Cancer Cells Derived from Different Human Organs using MTT Assay

Methods and Materials

Cells. Human cancer cell lines were obtained from American Type Culture Collection: HTB-9 (bladder), HeLa-S3 (cervix), DU145 (prostate), H460 (lung), MCF-7 (breast), K562 (leukocytes), HCT116 (colon), HepG2 (liver), U2OS (bone), T98G (brain) and OVCAR-3 (ovary). Cells were grown in culture medium (HeLa-S3, DU145, MCF-7, Hep-G2 and T98G in MEN (Earle's salts); HTB-9, H460, K562, OVCAR-3 in RPMI-

1640; HCT-116, U2OS in McCoy-5A) supplemented with 10% fetal calf serum, glutamine and antibiotics in a 5% CO₂ humidified incubator at 37°C.

MTT assay. The procedure for MTT assay followed the method described in (Carmichael et al., 1987) with only minor modifications. Cells were seeded into a 96-wells plate at concentrations of 10,000/well (HTB-9, HeLa, H460, HCT116, T98G, OVCAR-3), 15,000/well (DU145, MCF-7, HepG2, U2OS), or 40,000/well (K562), for 24 hours before drug-treatment. Cells were then exposed to drugs for 48 hours (72 hours for HepG2, U2OS, and 96 hours for MCF-7). After the drug-treatment, MTT (0.5 mg/ml) was added to cultures for an hour. The formation of formazan (product of the reduction of tetrazolium by viable cells) was dissolved with DMSO and the O.D. at 490nm was measured by an ELISA reader [Dynatech. Model MR700]. The MTT level of cells before drug-treatment was also measured (T₀). The % cell-growth (%G) is calculated as:

$$\%G = (TD - T_0 / TC - T_0) \times 100 \quad (1)$$

where TC or TD represent O.D. readings of control or drug-treated cells. When T₀ > TD, then the cytotoxicity (LC) expressed as % of the control is calculated as:

$$\%LC = (TD - T_0 / T_0) \times 100. \quad (2)$$

Results

Among the 11 cell lines studies, inhibition of cell-grwoth after exposure of plant extract was observed. However, their sensitivity toward *Xanthoceras sorbifolia* extract is different. The response of the cell lines to the *Xanthoceras* extract can be categorized into four groups: Most sensitive, i.e., Ovary; Sensitive, i.e., bladder, bone; Semi-sensitive, i.e., prostate, leukocyte, liver, breast, and brain; and least sensitive, i.e., colon, cervix, and lung. See Figure 14, 15 and 16 A-D. Their IC₅₀ values are listed in Table 3.1 above.

In addition to cell-growth inhibition, the *Xanthoceras sorbifolia* plant extract also stimulate a minor cell growth at low concentrations in bladder, bone and lung cells. Results indicate that there is a cell or tissue stimulation component(s) in the extract. See Figures 16A and 16D.

To investigate the inhibition components of the *Xanthoceras sorbifolia* plant extract, the plant extract was fractionated. Figure 10 shows the results of the screening of fractions obtained after FPLC chromatography for cell growth-inhibition activity. The assay was conducted with bladder cells. The fractions obtained from FPLC, as shown in Figure 9, were used. As shown in Figure 9, different components of *Xanthoceras sorbifolia* extract caused either growth or inhibition effects on cells. Only fractions 5962, designated as Fraction Y, cause cell growth inhibition. Abscissa: concentration (ug/ml). Ordinate: % Cell Growth (determined by MTT assay).

10 **Experiment 4: Purification of the Inhibition Components in the *Xanthoceras Sorbifolia* Extract.**

(A) Fractionation of plant extracts with FPLC

Methods

- 15 Column. Octadecyl functionalized silica gel. Column dimension: 2cm x 28cm; equilibrated with 10% acetonitrile – 0.005% TFA before use.
Sample loading: 1-2 ml, concentration: 100mg/ml in 10% acetonitrile/TFA.
Gradient elution condition: 10-80% acetonitrile in a total volume of 500 ml.
Monitor absorption wavelength: at 254nm.
- 20 Fraction Collector: 5 ml/fractions (collect from 10% to 72% acetonitrile)
Instrument: AKTA-FPLC, P920 pump; Monitor UPC-900; Frac-900.

Results

- The elution profile of the chromatography shows 4-5 broad fractions. See Figure 9.
- 25 These fractions were analyzed with HPLC. Specific components, corresponding to a-z as specified in Figure 8, are then assigned in these FPLC fractions. FPLC fractions are then grouped into 7 pools and analyzed for cell growth activity in bladder cells with MTT assay. See Experiment 3. It was found that only pool #5962, corresponding to fraction Y in HPLC, contains inhibition activity. See Figure 10. It was also found in later
- 30 experiments that fractions beyond 62 also show inhibition activity. The components isolated from fractions 63-65 showed inhibition activities. See Figure 4, 12 and 13.

(B) Isolation of Component Ys with Preparative HPLC

Methods

- 35 Column: A preparative HPLC column (Waters Delta Pak C18-300A);

Elution conditions: 45% acetonitrile isocratic elution with flow rate of 1 ml/min.

Fractions are monitored at 207nm and were collected and lyophilized.

Results

- 5 Final separation of Y fractions was achieved by HPLC with a preparative column. See Figure 11 and 12. These fractions, which include compound Y1, Y2, Y or Y3 and Y4, were collected. Re-chromatography of compound Y showed a single peak in HPLC with a C18 reverse phase column. See Figure 11A and 11B. Re-chromatography of the compound Y8, Y9 and Y10 showed a single peak in HPLC with a C18 reverse phase
10 column. See Figure 13.

(C) Appearance and solubility

The pure compound Ys is an amorphous white powder, soluble in aqueous alcohol, i.e., methanol or ethanol, 50% acetonitrile and 100% pyridine.

15

(D) Inhibition analysis of Compound Ys with MTT assay

Inhibition analysis of compound Y was determined with MTT assay. Figure 2 shows that compound Y has activity against ovarian cancer cells (OCAR-3) with IC50 value of 1.5 ug/ml which is 10-15 times more potent than the unpurified extract shown in Figure 14.

- 20 Figure 15 shows the selectivity of compound Y to ovarian cancer cells compared with cervical cancer cells (HeLa). Figure 3 shows the inhibition activities of compound Y1 and Y2 on the growth of ovarian cancer cells (OCAR-3). Figure 4 shows the inhibition activities of compound Y, Y8, Y9 and Y10 on the growth of ovarian cancer cells (OCAR-3).

25

Experiment 5: Determination of the Chemical Structure

Methods

- NMR analysis. The pure compound Y of *Xanthoceras sorbifolia* was dissolved in pyridine-D5 with 0.05% v/v TMS. All NMR spectra were acquired using a Bruker Avance
30 600 MHz NMR spectrometer with a QXI probe (¹H/¹³C/¹⁵N/³¹P) at 298 K. The numbers of scans for 1D ¹H spectra were 16 to 128, depending on the sample concentration. 2D HMQC spectra were recorded with spectral widths of 6000 × 24,000 Hz and data points of 2024 × 256 for t2 and t1 dimensions, respectively. The number of scans was 4 to 128. 2D HMBC were acquired with spectral widths of 6000 × 30,000 Hz

and data points of 2024×512 for t_2 and t_1 dimensions, respectively. The number of scans was 64. The 2D data were zero-filled in t_1 dimension to double the data points, multiplied by cosine-square-bell window functions in both t_1 and t_2 dimensions, and Fourier-transformed using software XWIN-NMR. The final real matrix sizes of these 2D spectra are 2048×256 and 2048×512 data points ($F_2 \times F_1$) for HMQC and HMBC, respectively.

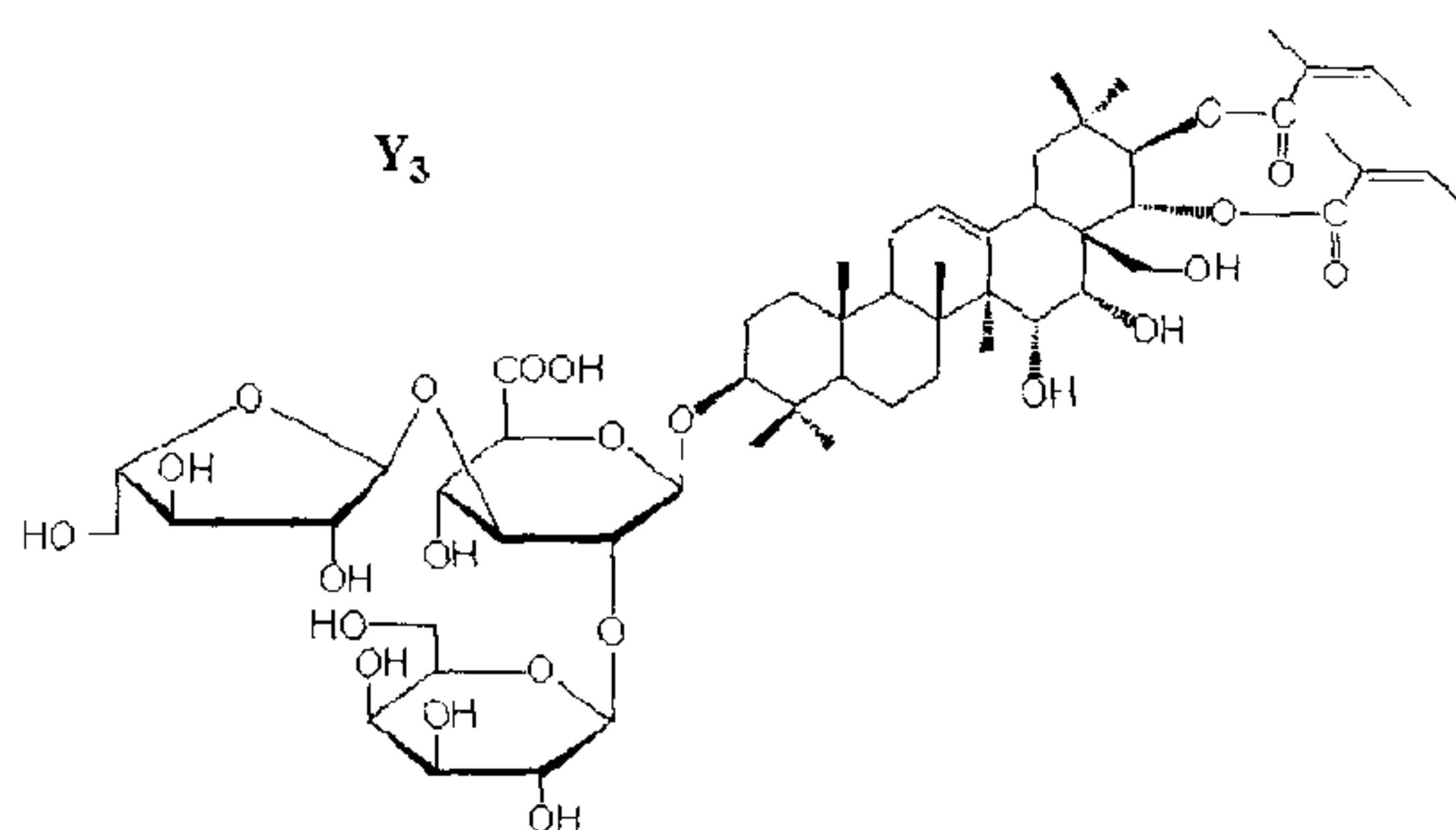
Mass spectral analysis. The mass of samples was analyzed by (A) MALDI-TOF Mass Spectrometry and by (B) ESI-MS Mass spectrometry. (A) Samples for MALDI-TOF were first dissolved in acetonitrile, and then mixed with the matrix CHCA, i.e., Alpha-cyano-4-hydroxycinnamic acid, 10mg CHCA/mL in 50:50 water/acetonitrile and 0.1% TFA in final concentration. The molecular weight was determined by the high resolution mass spectroscope analysis with standards. (B) For ESI, the sample was analyzed with LCQ DECA XP Plus machine made by Thermo Finnigan. It is ionized with ESI source and the solvent for the compound is acetonitrile.

15

Results

The profile of the proton NMR is presented in Figure 18. The 2D NMR profiles of HMQC and HMBC are shown in Figures 19 and 20, respectively. Table 5.1 summarizes the 2D NMR chemical shift data and the assignment of functional groups derived from these data. Based on these data and analysis, the structure of compound Y (Y3) is assigned as shown below.

Structure of Compound Y (See also Figure 17)



25 The chemical name of compound Y is: 3-O-[β -D-galactopyranosyl(1 $\rightarrow$ 2)]- α -L-arabinofuranosyl(1 $\rightarrow$ 3)- β -D-glucuronopyranosyl-21,22-O-diangeloyl-3 β , 15 α , 16 α , 21 β , 22 α , 28-hexahydroxyolean-12-ene. The mass spectrum of compound Y as determined by MALDI-TOF and ESI-MS, i.e., see Figure 21, 22, indicates that the mass of compound Y is 1140.57 which agree with the theoretical mass of the compound Y.

Conclusion

The active compound Y isolated from extract of *Xanthoceras sorbifolia* is an oleanene triterpenoidal saponin with a trisaccharide chain attached at C-3 of the aglycone and two angeloyl groups acylated at C-21 and C-22. The formula of Y is $C_{57}H_{88}O_{23}$, and the chemical name of Compound Y is: 3-O-[β -D-galactopyranosyl(1 $\rightarrow$ 2)]- α -L-arabinofuranosyl(1 $\rightarrow$ 3)- β -D-glucuronopyranosyl-21,22-O-diangeloyl-3 β , 15 α , 16 α , 21 β , 22 α , 28-hexahydroxyolean-12-ene.

Experiment 6: Determination of the Chemical Structure of Compound Y1 of *Xanthoceras Sorbifolia* Extract

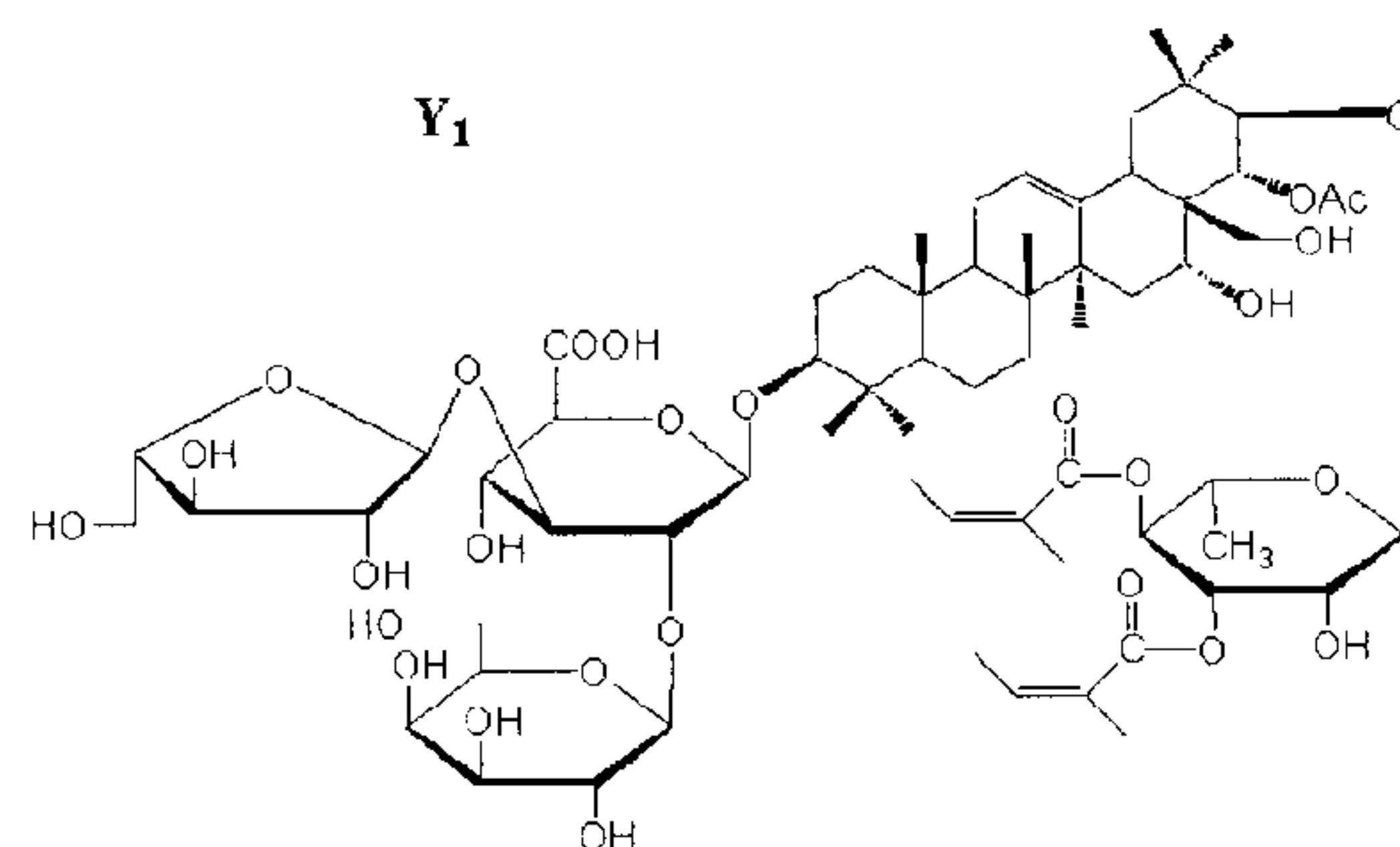
Methods

The method for NMR and MS analysis for compound Y1 is similar to the method described in Experiment 5.

Results

The spectrum of the H-NMR is presented in Figure 24. The 2D NMR spectra of HMQC, HMBC and COSY are shown in Figures 25, 26 and 27, respectively. Table 6.1 summarizes the chemical shift data and the assignment of functional groups derived from these data. Based on these data and analysis, the structure of compound Y1 is assigned and shown below.

Structure of Y1 (See also Figure 23)



The chemical name of Y1 is: 3-O-[β -D-galactopyranosyl(1 $\rightarrow$ 2)]- α -L-arabinofuranosyl(1 $\rightarrow$ 3)- β -D-glucuronopyranosyl-21-O-(3,4-diangeloyl)- α -L-rhamnopyranosyl-22-O-acetyl-3 β , 16 α , 21 β , 22 α , 28-pentahydroxyolean-12-ene.

Conclusion

Compound Y1 isolated from extract of *Xanthoceras sorbifolia* is a bisdesmosidic polyhydroxyoleanene triterpenoidal saponin with a trisaccharide chain at C-3 of the backbone and a monosaccharide moiety at C-21 where two angeloyl groups were acylated at C-3 and C-4 position. The formula of Y1 is $C_{65}H_{100}O_{27}$,

5

Experiment 7: Determination of the Chemical Structure of Compound Y2 of *Xanthoceras Sorbifolia* Extract.

Methods

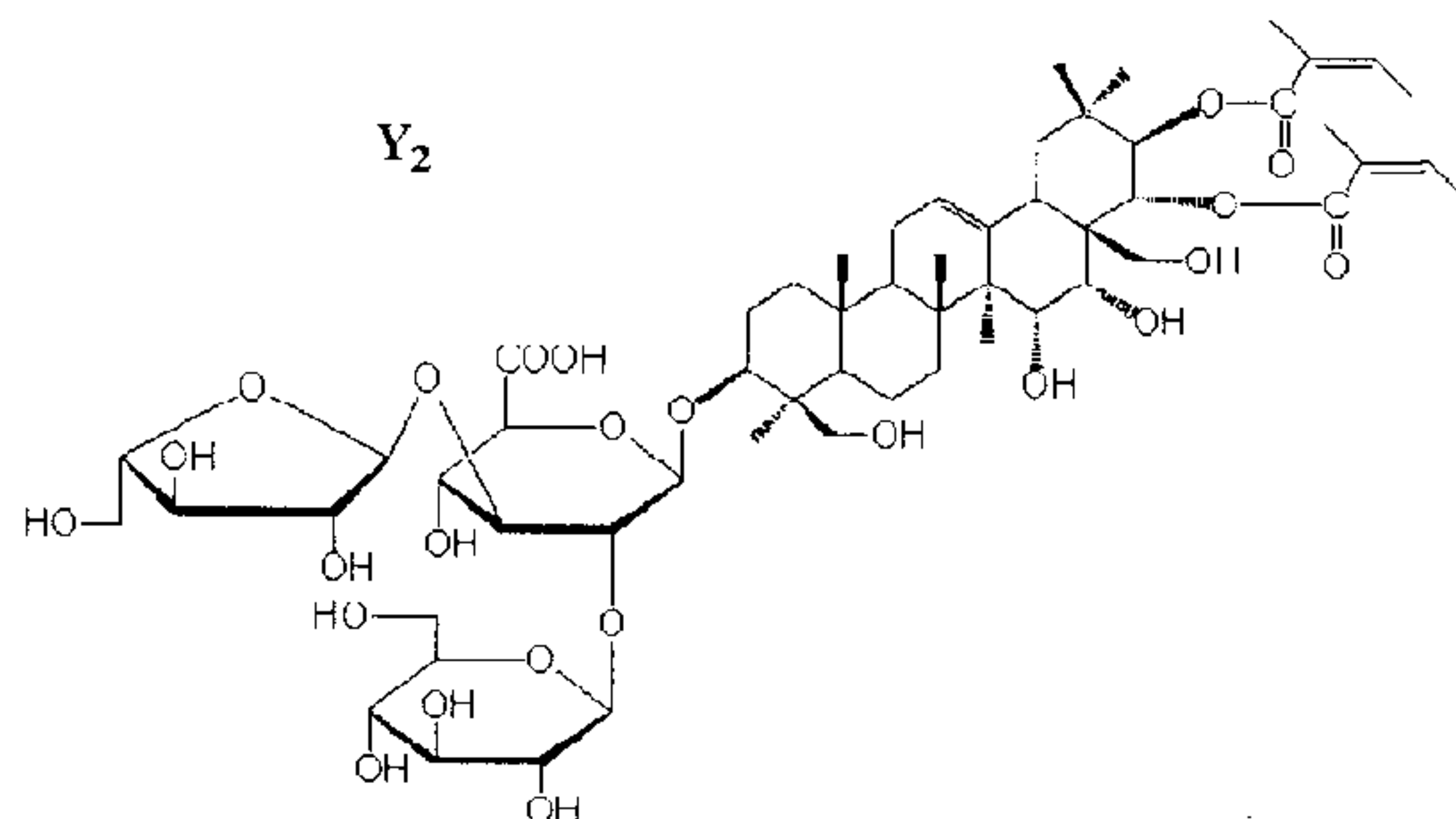
- 10 The method for NMR and MS analysis for compound Y2 is similar to the method described in Experiment 5.

Results

- 15 The 1D and 2D NMR spectra of ^1H -NMR, ^{13}C -NMR, HMQC, HMBC and (TOCSY) and MS (MALDI-TOF) of Y2 are showed in Figures 29-34. Table 7.1 summarizes the 1D and 2D NMR chemical shift data and the assignment of functional groups derived from these data.

Conclusion

- 20 Based on these data and analysis, the compound Y2 isolated from extract of *Xanthoceras sorbifolia* is an oleanene triterpenoidal saponin with a trisaccharide chain attached at C-3 of the aglycone and two angeloyl groups acylated at C-21 and C-22. The chemical structure of Y2 is shown below. See also Figure 28.



- 25 The formula of Y2 is $C_{57}H_{88}O_{24}$, and the chemical name of Compound Y2 is: 3-O-[β -D-glucopyranosyl-(1 $\rightarrow$ 2)]- α -L-arabinofuranosyl(1 $\rightarrow$ 3)- β -D-glucuronopyranosyl-21,22-O-diangeloyl-3 β , 15 α , 16 α , 21 β , 22 α , 24 β , 28-heptahydroxyolean-12-ene.

Experiment 7B. Chemical structure analysis of Y4

Results of Y4 analysis

The profile of the proton NMR of Y4 is presented in Figure 58. The profiles of 2D NMR (HMQC) of Y4 is presented in Figure 59.

5

Experiment 8. Purification of the Inhibition Components Y8-Y10 in the Xanthoceras Sorbifolia Extract

(A) Fractionation of Xanthoceras Sorbifolia extracts components with FPLC

10 Methods

The methods for this experiment are similar to the methods described in Experiment 4 Section (A) and (B).

Results

15 The elution profile shows 4-5 broad fractions. See Figure 9. These fractions were analyzed with HPLC. FPLC fractions 63, 64 and 65 are further separated on 45% isocratic analysis, 4-5 major components were separated. See Figure 12. These fractions were assigned designations Y8, Y9 and Y10. These fractions were collected. Re-chromatography of the compound Y8, Y9 and Y10 showed a single peak in HPLC
20 with a C18 reverse phase column. See Figure 13.

(B) Inhibition analysis with MTT assay.

Inhibition analysis of purified compounds was determined with the MTT assay. Results indicate that compound Y8, Y9 and Y10 has activity against ovarian cancer cells
25 (OCAR-3) with IC50 values of 3, 4 and 1.5 ug/ml, respectively. See Figure 4.

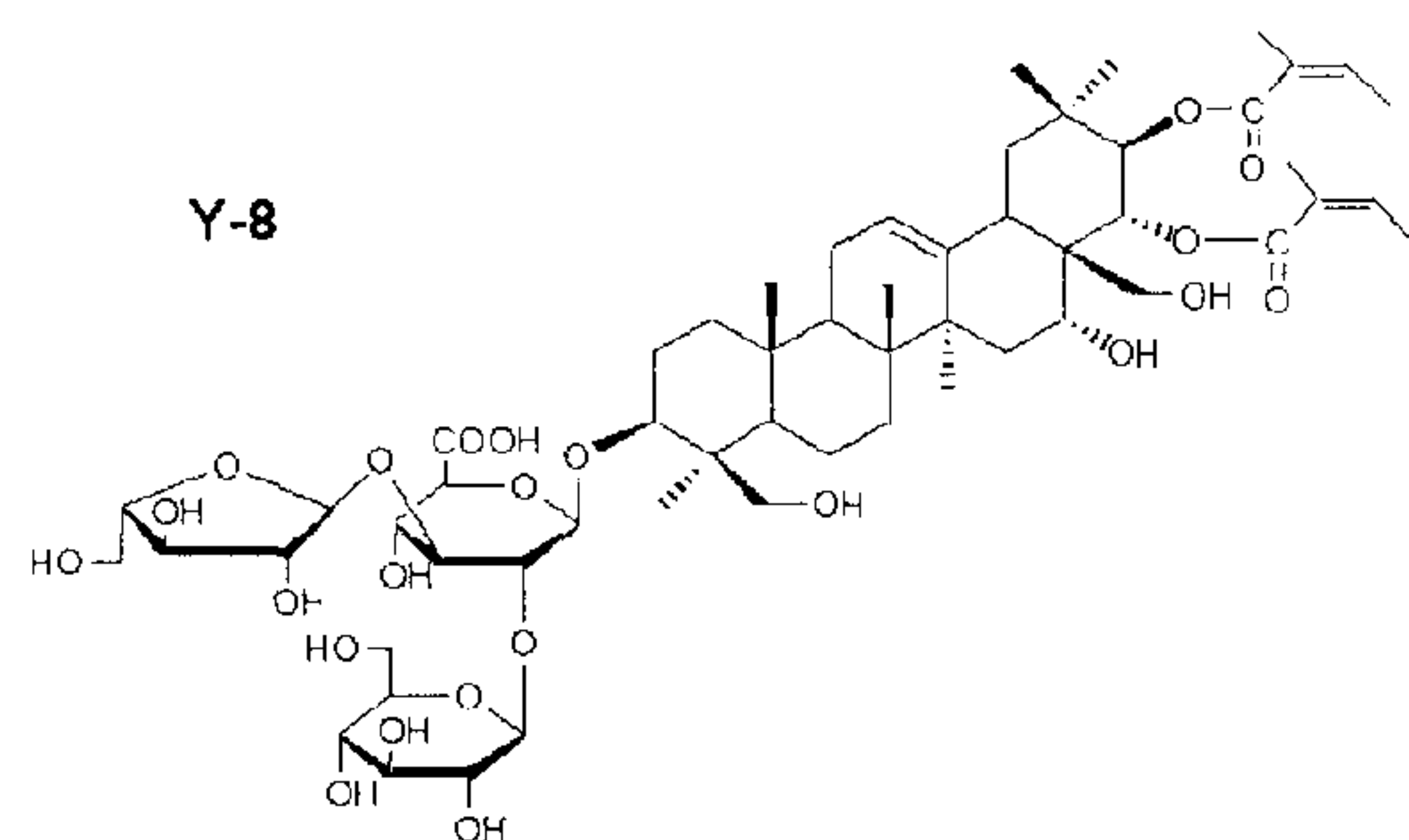
Experiment 9. Determination of the Chemical Structure of Compound Y8 of Xanthoceras Sorbifolia Extract

30 Methods

The method for NMR and MS analysis for compound Y8 is similar to the method described in Experiment 5.

Results

The spectral profiles of the H-NMR, C13-NMR and 2D NMR (HMQC) of compound Y8 are presented in Figures 36-38. Table 9.1 summarizes the 1D and 2D NMR chemical shift data and the assignment of functional groups derived from these data. Based on these data and analysis, the compound Y8 isolated from extract of *Xanthoceras sorbifolia* is an oleanene triterpenoidal saponin with a trisaccharide chain attached at C-3 of the aglycone and two angeloyl groups acylated at C-21 and C-22. The formula of compound Y8 is $C_{57}H_{88}O_{23}$, and the chemical name of Y8 is: 3-O-[β -glucopyranosyl (1 $\rightarrow$ 2)]- α -arabinofuranosyl (1 $\rightarrow$ 3)- β -glucuronopyranosyl-21, 22-O-diangeloyl-3 β , 16 α , 21 β , 22 α , 24 β , 28-hexahydroxyolean-12-ene. The chemical structure of compound Y8 is presented in the following figure. See also Figure 35.



Experiment 10. Determination of the Chemical Structure of Compound Y9 of *Xanthoceras Sorbifolia* Extract

15

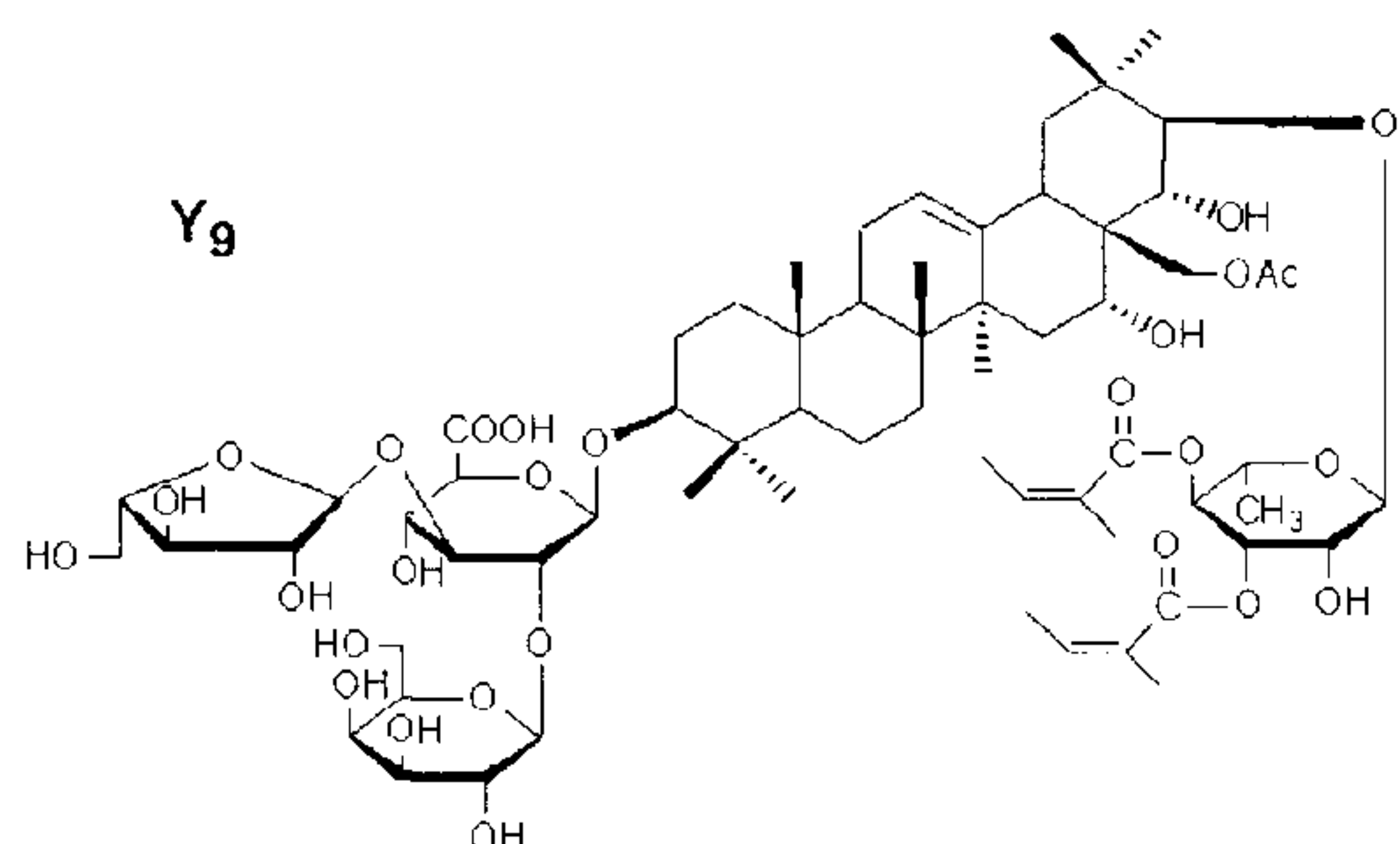
Methods

The method for NMR and MS analysis for compound Y9 is similar to the method described in Experiment 5.

20 Results

The spectral profiles of the H-NMR and 2D NMR, i.e., HMQC and HMBC, of Y9 are shown in Figures 40-42. Table 10.1 summarizes the 1D and 2D NMR chemical shift data and the assignment of functional groups derived from these data. Based on these data and analysis, compound Y9 isolated from extract of *Xanthoceras sorbifolia* is a bisdesmosidic polyhydroxyoleanene triterpenoidal saponin with a trisaccharide chain at C-3 of the backbone and a monosaccharide moiety at C-21 where two angeloyl groups were acylated at C-3 and C-4 position. The formula of compound Y9 is $C_{65}H_{100}O_{27}$ and the chemical name of Y9 is: 3-O-[β -galactopyranosyl (1 $\rightarrow$ 2)]- α -arabinofuranosyl (1 $\rightarrow$ 3)- β -glucuronopyranosyl-21-O-(3,4-diangeloyl)- α -rhamnopyranosyl-28-O-acetyl-3 β , 16 α ,

21 β , 22 α , 28-pentahydroxyolean-12-ene. The chemical structure of Compound Y9 is presented in the following figure. See *also* Figure 39.



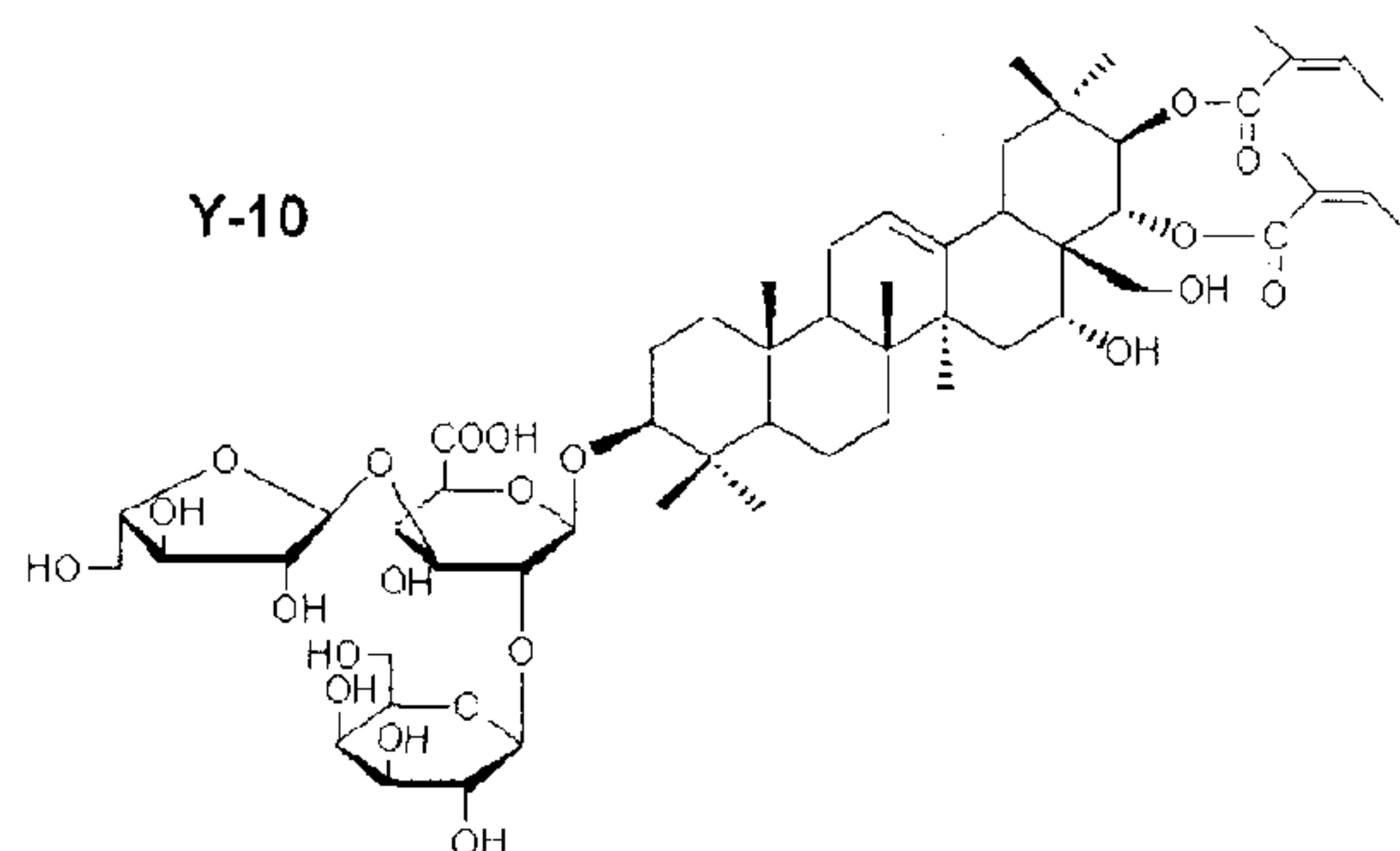
5 Experiment 11. Determination of the Chemical Structure of Compound Y10 of Xanthoceras Sorbifolia Extract

Methods

The method for NMR and MS analysis for compound Y10 is similar to the method described in Experiment 5.

Results

The profile of the H-NMR, C13-NMR and 2D NMR (HMQC) are shown in Figures 44-46. Table 11.1 summarizes the 1D and 2D NMR chemical shift data and the assignment of functional groups derived from these data. Based on these data and analysis, compound Y10 isolated from extract of Xanthoceras sorbifolia is an oleanene triterpenoidal saponin with a trisaccharide chain attached at C-3 of the aglycone and two angeloyl groups acylated at C-21 and C-22. The formula of compound Y10 is C₅₇H₈₈O₂₂, and the chemical name of Y10 is: 3-O-[β -galactopyranosyl (1 $\rightarrow$ 2)]- α -arabinofuranosyl (1 $\rightarrow$ 3)- β -glucuronopyranosyl-21, 22-O-diangeloyl-3 β , 16 α , 21 β , 22 α , 28-pentahydroxyolean-12-ene. The chemical structure of Compound Y10 is presented in the following figure. See *also* Figure 43.



Experiment 12. Purification of Component R from Xanthoceras Sorbifolia Extract

(A) Purification of Xanthoceras Sorbifolia extracts components with FPLC and HPLC

5 Methods

The methods used are similar to the methods described in Experiment 4, section (A) and (B) except a 30% acetonitrile isocratic elution was used in HPLC for isolation of the Compound R.

10 Results

Fraction No. 39-41 from gradient elution of FPLC were pooled and further purified with an open ODS-C18 column with isocratic 30% acetonitrile elution. Six identifiable fractions in two groups were collected. Fractions 6-13 were further characterized with HPLC. These fractions were further separated into 4-5 components with the 30%
15 acetonitrile isocratic elution in a DeltaPak column. The fraction designated herein as "R1", is the major component. See Figure 60A. The pure R1 was subsequently collected from the column elution. See Figure 60B.

(B) Appearance and solubility

20 The pure R1 appears as an amorphous white powder, soluble in aqueous alcohol, i.e., methanol or ethanol, 50% acetonitrile and 100% pyridine.

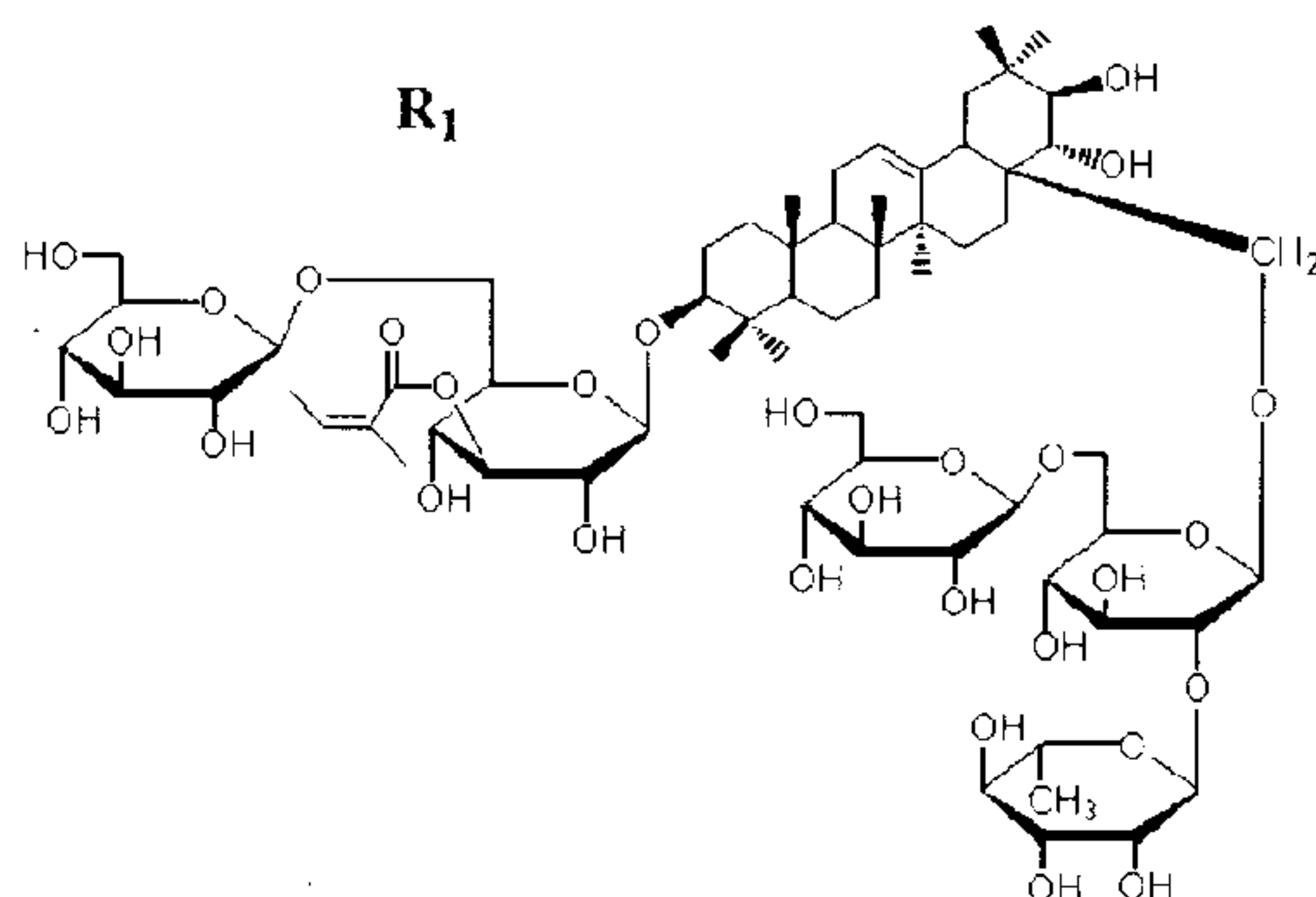
(C) Determination of the chemical structure of R1

Methods

25 The NMR and MS Analysis of R1 is similar to the method described in Experiment 5.

Results

The NMR spectra of pure R1 are presented in Figures 48-52. Based on chemical shift analysis, compound R1 isolated from extract of Xanthoceras sorbifolia is a triterpenoid
30 saponins with five sugars and one angeloyl group attached to the sugar moiety. The chemical structure of R1 is shown in following figure. See *also* Figure 47.



The formula of Compound R1 is $C_{65}H_{106}O_{29}$, and the chemical name of R1 is: 3-O-[angeloyl-(1→3)-β-D-glucopyranosyl-(1→6)]-β-D-glucopyranosyl-28-O-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-3β, 21β, 22α, 28-tetrahydroxyolean-12-ene.

Experiment 13: Purification of Component-O from *Xanthoceras Sorbifolia* extract

(A) Fractionation of *Xanthoceras Sorbifolia* extracts components with FPLC and HPLC

Methods

The methods used are similar to the methods described in Experiment 4, section (A) and (B) except a 20% acetonitrile isocratic elution was used in HPLC for isolation of the Compound O.

Results

Fractions obtained from FPLC were analyzed with HPLC. By comparison with the profiles of the original sample, a specific component, in this case fraction O, was identified (#28-30). Fraction O was collected for further purification. Sixteen identifiable HPLC fractions were observed in the elution profiles. See Figure 61. Fractions 28, 34 and 54 were further purified. See Figures 62. These purified components are named as compound O28, O34 and O54, respectively.

(B) Appearance and solubility

The purified compound O23 and O34 are light yellow amorphous powder, soluble in aqueous alcohol, i.e., methanol, ethanol, 50% acetonitrile and 100% pyridine. The purified compound O54 is a white amorphous powder, soluble in aqueous alcohol, i.e., methanol, ethanol, 50% acetonitrile and 100% pyridine.

(C) Structure analysis of Compound O54

Methods

The NMR and MS analysis of O54 is similar to the method described in Experiment 5.

5

Results

The NMR spectra of compound O54 is presented in Figures 54-56. Based on the chemical shift analysis, compound O54 isolated from extract of *Xanthoceras sorbifolia* is a bisdesmosidic polyhydroxyoleanene triterpenoidal glycoside with a disaccharide chain [β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside] affixed to C-3 and a trisaccharide chain [α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester] attached to C-28. The chemical structure of compound O54 is presented in the following figure. See also Figure 53.

10

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- 15 The formula of compound O54 is C₆₀H₁₀₀O₂₈, and the chemical name of O54 is: 3-O-β-D-glucopyranosyl-(1→6)]-β-D-glucopyranosyl-28-O-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-3β, 21β, 22α, 28-tetrahydroxyolean-12-ene.

Experiment 14: Purification of Component-X from *Xanthoceras Sorbifolia* extract

20

(A) Fractionation of *Xanthoceras Sorbifolia* extracts components with FPLC and HPLC

Methods

- 25 The methods used are are similar to the methods described in Experiment 4, section (A) and (B) except collect FPLC fractions 56 and 57 which contain compound X were further separated with preparative HPLC.

Results

- 30 Five major and seven minor peaks were observed in the chromatogram. Compound X was eluted near the middle of the elution profile (Figure 64). Compound X was collected and lyophilized.

(B) Appearance and solubility

The purified Compound X is white powder and is soluble in 50% acetonitrile.

(C) Inhibition analysis of Compound X with MTT assay

MTT analysis of compound X in OVCAR-3 cells indicates some inhibition activity. The
5 IC₅₀ of compound X is approximately 27 ug/ml. See Figure 65.

Experiment 15. Determination of the Chemical Structure of Compound X of Xanthoceras Sorbifolia Extract

10 Methods

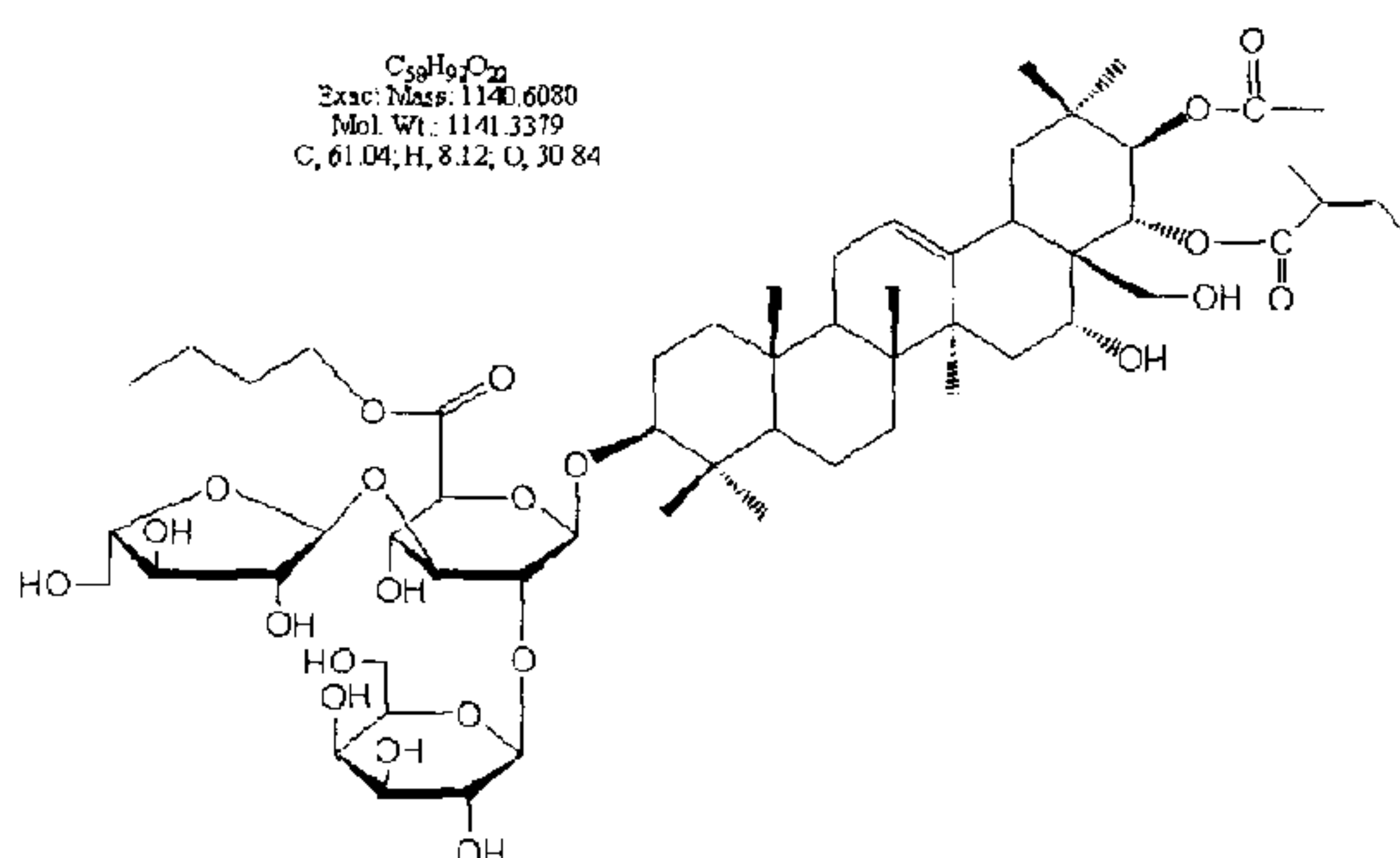
The method for NMR and MS analysis for compound X is similar to the method described in Experiment 5.

Results

15 The profile of the proton and C13 NMR are presented in Figure 66 and 69, respectively. The 2D NMR profiles of HMQC and HMBC are shown in Figure 67 and 68. Table 15.1 summarizes the 2D NMR chemical shift data and the assignment of functional groups derived from these data. Based on these data and analysis, the structure of compound X is assigned as shown below. The mass spectrum of Compound X as determined by
20 MALDI-TOF (Figure 71 and 72) indicates the mass of compound Y is 1140.60 which agree with the theoretical mass of the compound X.

Conclusion

The active compound X isolated from extract of Xanthoceras Sorbifolia is an oleanene
25 triterpenoidal saponin with a trisaccharide chain attached at C-3 of the aglycone and one angeloyl group acylated at C-22. The formula of X is C₅₈H₉₂O₂₂, and the chemical name of Compound X is: 3-O-[[β-D-galactopyranosyl (1→2)]-[α-L-arabinofuranosyl (1→3)]-β-D-glucuronopyranoside butyl ester}-21-O-acetyl-22-O-angeloyl-3β,16α,21β,22α,28-pentahydroxyolean-12-ene. The chemical structure of compound X
30 is presented in the following figure. See *also* Figure 70.



Experiment 16. Determination the haemolytic activities of Compound Y of Xanthoceras Sorbifolia

5

Methods:

- Human whole blood was obtained from Houston Gulf Coast Blood Center
- Red blood cells were isolated by the following method: Human blood (in EDTA) was diluted 1:1 with PBS, underlay with 4 ml of Histopaque-1077 (SIGMA) and was centrifuged at 400g for 30 min.
- Red blood cells (RBC) were collected and washed three times with PBS.
- 10% suspension of RBC were prepared with PBS before use.
- 50 ul of RBC suspension was added to 2 ml of Saponins with different concentration.
- The suspension was mixed by vortexing and sit at room temperature for 60 minutes.
- The suspension was centrifuged at 3000 rpm for 5 min. Absorbance of the supernatant was measured at 540 nm.

10

15

Results:

- In this experiment, hemolytic activity of human red blood cells by Xanifolia-Y (#63Y), Escin and SIGMA saponin standard were compared. Y contains two angeloyl groups, Escin has one angeloyl group and SIGMA saponin standard is a mixture of saponins from Quillaia bark. The results show that #63Y (compound Y) has higher hemolytic activity (IC₅₀=1 ug/ml) than Escin or SIGMA saponin standard (IC₅₀=5 ug/ml). See Figure 73 A.

25

Experiment 17. Determination the hemolytic and MTT activities of Compound Y after removal of the angeloly or the sugar moiety by alkaline or acid hydrolysis, respectively

Methods:

(A) Alkaline Hydrolysis of Xanifolia-Y. 20 mg of Xanifolia-Y was dissolved in 0.5 ml of 1M NaOH. The solution was incubated in 80C water bath for 4 hours. It was cooled to room temperature before neutralized with 0.5 ml 1 N HCl (adjust pH to about 3). The mixture was extracted with 2 ml 1-butanol 3 times. The butanol fractions were collected and lyophilized. The hydrolyzed saponin with further purified with HPLC in a C-18 column eluted with 25% acetonitrile. (B) Acid Hydrolysis of Xanifolia-Y. 15 mg Xanifolia-Y was dissolved in 1 ml of Methanol. 1 ml of 2N HCl was then added. The mixture was refluxed in 80C water bath for 5 hours. The solution was then neutralized by adding 2 ml of 1N NaOH (to final pH 3-4). The aglycone was then extracted with ethylacetate 3 ml x 3. The extracts were collected and pooled. Further isolation of aglycone (sugar-removed Xanifolia-Y) was achieved by HPLC with isocratic elution of 80% acetonitrile.

Results:

The angeloly groups or the sugar moiety of the compound Y, were removed by alkaline or acid hydrolysis, respectively. The hemolytic activities of the hydrolysed products were then analyzed. Results of these studies indicate that removing sugars reduce hemolytic activity, but removing angeloyl groups destroy the hemolytic activity. It also suggested that sugars are helpful but not essential for hemolytic activity. See Figure 73 B. The experiment results show that compound-Y lost MTT activities if the angeloyl groups were removed. However, the MTT activities were very weak when the sugar moiety of the compound was removed. See Figure 73 C, 73 D. Results of comparison of hemolytic activities between Compound Y, Escin and Saponin standard from SIGMA are shown in Figure 73 A and 73 B. Results of Comparison of hemolytic activities between Compound Y, compound Y without sugar moiety or angeloly groups are shown in Figure 73 B. Chemical structures of compound Y without sugar moiety or angeloly groups are shown in Figure 74 A and 74B respectively.

Experiment 18. Determination the anti virus activities of Compound Y

The major procedures for the determination of antiviral activity of Compound Y are:

A. Determine the production of HIV virus after a non-lethal dosage of Compound Y is added to the viral culture system.

B. Determine the growth activity of HIV virus after contact to compound Y. The steps for these experiments are:

1. Pre-treat HIV virus with different dosages of Compound Y for variable length of time.

2. Remove compound Y from virus.
 3. Mix treated virus with cells.
 4. Measure Virus production.
 5. Negative control:-no virus in cell.
 - 5 6. Positive control – untreated virus mixed with cell.
- Result: The virus growth will be inhibited after treatments of Compound Y.

TABLES OF EXPERIMENTAL RESULTS:

Table 5.1. ¹³C and ¹H NMR Data for Compound Y (in Pyridine-d₅)^a

Position	C	H	Key HMBC correlations
1	38.7	0.83, 1.40	C-3, C-5, C-9
2	26.4	1.81, 2.14	–
3	89.6	3.25, 1H, dd, 12.0/4.0 Hz	C-23, C-24, GlcA C-1'
4	39.4	–	–
5	55.3	0.78	–
6	18.5	1.55, 1.59	C-8, C-10
7	36.5	2.00, 2.10	C-5, C-9
8	41.2	–	–
9	47.0	3.06	C-7, C-8, C-12, C-14, C-26
10	37.2	–	–
11	23.7	1.74, 1.89	–
12	125.2	5.49, 1H, br s	C-9, C-11, C-14, C-18
13	143.4	–	–
14	47.5	–	–
15	67.3	4.21	C-8, C-27
16	73.6	4.45	C-14, C-15, C-18
17	48.3	–	–
18	40.8	3.07	C-12, C-13, C-14, C-16, C-19, C-20, C-28,
19	46.8	1.41, 1.69	–
20	36.2	–	–
21	79.3	6.71, 1H, d, 10 Hz	C-20, C-22, C-29, C-30, 21-O-Ang C-1'''
22	73.5	6.32, 1H, d, 10 Hz	C-16, C-17, C-21, C-28, 22-O-Ang C-1'''
23	27.7	1.26, 3H, s	C-3, C-4, C-5, C-24
24	16.5	1.16, 3H, s	C-3, C-4, C-5, C-23
25	16.0	0.81, 3H, s	C-1, C-5, C-9, C-10
26	17.3	0.99, 3H, s	C-7, C-8, C-9, C-14
27	21.0	1.85, 3H, s	C-8, C-13, C-14, C-15
28	62.9	3.50, 1H, d, 11.0 Hz, 3.76, 1H, d, 11.0 Hz,	C-16, C-17, C-18, C-22
29	29.2	1.09, 3H, s	C-19, C-20, C-21, C-30
30	20.0	1.32, 3H, s	C-19, C-20, C-21, C-29
GlcA			
1'	104.9	4.89, 1H, d, 7.8 Hz	C-3
2'	79.1	4.38	GlcA C-1', C-3', Gal C-1''

3'	86.1	4.20	GlcA C-2', C-4', Ara C-1'''
4'	71.5	4.42	GlcA C-3', C-5', C-6'
5'	78.0	4.52	GlcA C-4', C-6'
6'	171.9	–	–
Gal			
1''	104.6	5.32, 1H, d, 7.7 Hz	GlcA C-2'
2''	73.6	4.42	Gal C-1'', C-3''
3''	74.9	4.10	Gal C-2''
4''	69.5	4.56	Gal C-2'', C-3''
5''	76.4	3.94	Gal C-4'', C-6''
6''	61.6	4.43, 4.52	Gal C-4'', C-5''
Ara-f			
1'''	110.6	6.03, 1H, br s	GlcA C-3', Ara C-2''', C-4'''
2'''	83.4	4.94	Ara C-3'''
3'''	78.3	4.78	Ara C-2'''
4'''	85.2	4.82	Ara C-5'''
5'''	62.2	4.12, 4.28	Ara C-3'''
21-O-Ang			
1''''	167.7	–	–
2''''	129.6	–	–
3''''	137.2	5.96, 1H, dq, 7.0/1.5 Hz	Ang C-1''', C-4''', C-5'''
4''''	15.5	2.10, 3H, dq, 7.0/1.5 Hz	Ang C-2''', C-3'''
5''''	20.8	2.00, 3H, s	Ang C-1''', C-2''', C-3'''
22-O-Ang			
1''''	167.9	–	–
2''''	129.8	–	–
3''''	136.3	5.78, 1H, dq, 7.0/1.5 Hz	Ang C-1''', C-4''', C-5'''
4''''	15.5	1.93, 3H, dq, 7.0/1.5 Hz	Ang C-2''', C-3'''
5''''	20.5	1.74, 3H, s	Ang C-1''', C-2''', C-3'''

^a The data were assigned based on HMQC and HMBC correlations.

Table 6.1. ¹³C and ¹H NMR Data for Compound Y1 (in Pyridine-d₅)

Position	C	H
1	38.6	0.85, 1.33
2	26.3	1.86, 2.10
3	89.7	3.25 (1H, m)
4	39.5	–
5	55.5	0.75
6	18.3	1.40, 1.43
7	33.1	1.20, 1.50
8	40.0	–
9	46.7	1.69
10	36.5	–
11	23.5	1.75, 1.91
12	123.6	5.37 (1H, br s)

13	143.0	—
14	41.8	—
15	34.7	1.53, 1.73
16	68.5	4.45
17	48.2	—
18	39.9	3.04
19	47.6	1.30, 3.05
20	36.7	—
21	85.3	5.05 (1H, d, $J = 9.6$ Hz)
22	73.8	6.17 (1H, d, $J = 9.6$ Hz)
23	27.7	1.29 (3H, s)
24	16.5	1.16 (3H, s)
25	15.5	0.78 (3H, s)
26	17.1	0.82 (3H, s)
27	27.3	1.83 (3H, s)
28	63.7	3.42, 3.60 (each, 1H, d, $J = 10.6$ Hz)
29	29.9	1.42 (3H, s)
30	19.9	1.37 (3H, s)
3-O-GlcA- <i>p</i>		
1	105.5	4.93 (1H, d, $J = 7.8$ Hz)
2	78.6	4.37
3	86.0	4.20
4	71.6	4.43
5	78.0	4.50
6	171.8	—
Gal- <i>p</i>		
1	104.5	5.33 (1H, d, $J = 7.8$ Hz)
2	73.5	4.43
3	74.9	4.10
4	69.5	4.57
5	76.3	3.95
6	61.1	4.44, 4.53
Ara- <i>f</i>		
1	110.9	6.04 (1H, br s)
2	83.3	4.95
3	78.3	4.78
4	85.2	4.82
5	62.0	4.13, 4.31
21-O-Rham- <i>p</i>		
1	105.1	4.88 (1H, d, $J = 1.5$ Hz)
2	70.5	4.25
3	74.0	5.59
4	71.5	5.70
5	68.5	3.89
6	17.6	1.18 (3H, d, $J = 6.6$ Hz)
Rham-3-Ang		
1	167.3 ^a	—
2	128.2 ^b	—
3	138.5 ^c	5.98 ^f (1H, q, $J = 7.2$ Hz)
4	15.7 ^d	2.02 ^g (3H, d, $J = 7.2$ Hz)

5	20.6 ^e	1.92 ^h (3H, s)
Rham-4-Ang		
1	167.2 ^a	–
2	128.0 ^b	–
3	138.2 ^c	5.88 ^f (1H, q, $J = 7.2$ Hz)
4	15.5 ^d	1.96 ^g (3H, d, $J = 7.2$ Hz)
5	20.5 ^e	1.85 ^h (3H, s)
22-O-Acetyl		
1	171.4	–
2	21.8	2.31 (3H, s)

^{a-h} The data with the same labels in each column may be interchangeable.

Table 7.1: ¹³C and ¹H NMR data for Y2 (in Pyridine-d₅)^a

Position	C	H
1	38.4	0.83, 1.36
2	26.4	1.89, 2.25
3	91.3	3.39, 1H, m
4	43.4	–
5	56.7	0.87, 1H, d, 12.0 Hz
6	18.6	1.31, 1.57
7	36.3	1.97, 2.12
8	40.7	–
9	46.7	1.63
10	36.6	–
11	23.9	1.69, 1.89
12	125.1	5.48, 1H, br s
13	143.4	–
14	47.5	–
15	67.1	4.18, 1H, d, 4.1 Hz
16	73.2	4.43
17	48.1	–
18	41.4	3.06
19	46.6	1.40, 3.08
20	36.1	–
21	78.3	6.69, 1H, d, 10.2 Hz
22	73.1	6.30, 1H, d, 10.2 Hz
23	22.0	1.29, 3H, s
24	62.9	3.28, 1H, d, 11.2 Hz; 4.32
25	15.6	0.64, 3H, s
26	17.1	0.94, 3H, s
27	20.8	1.84, 3H, s
28	63.1	3.48, 3.72 (each, 1H, d, 10.6 Hz)
29	29.3	1.09, 3H, s
30	20.0	1.32, 3H, s
3-O-GlcA		
1	104.5	4.87, 1H, d, 7.2 Hz
2	78.6	4.31

3	86.5	4.23
4	71.6	4.45
5	77.4	4.53
6	171.9	
Glc		
1	103.7	5.48, 1H, d, 7.8 Hz
2	75.3	4.02
3	78.0	4.31
4	69.3	4.52
5	78.2	3.62
6	61.5	4.33, 4.50
Ara		
1	110.1	6.05, 1H, br s
2	83.5	4.97
3	77.8	4.74
4	85.0	4.84
5	62.2	4.18, 4.33
21-O-ang		
1	167.5	–
2	128.7	–
3	137.2	5.95, 1H, dd, 14.4/7.2 Hz
4	16.7	2.08, 3H, d, 7.2 Hz
5	20.6	2.00, 3H, s
22-O-ang		
1	167.9	–
2	128.9	–
3	136.3	5.76, 1H, dd, 14.4/7.2 Hz
4	15.6	1.95, 3H, dd, 7.2 Hz
5	20.4	1.74, 3H, s

^a The data were assigned based on COSY, HMQC and HMBC correlations.

Table 9.1. ¹³C and ¹H NMR Data for Y₈ (in pyridine-*d*₅)

Position	¹³ C	¹ H
1	38.2	0.74, 1.30
2	26.3	1.85, 2.26 (1H, m)
3	91.1	3.30 (1H, m)
4	43.4	–
5	55.9	0.82
6	18.2	1.22, 1.48
7	32.9	1.24, 1.49
8	39.8	–
9	46.5	1.67
10	36.2	–
11	23.8	1.70, 1.83
12	123.3	5.39 (1H, br s)
13	142.5	–
14	41.4	–
15	34.6	1.64, 1.83

16	68.4	4.53
17	47.8	—
18	39.7	3.09
19	47.0	1.39, 3.11
20	36.2	—
21	78.5	6.68 (1H, d, $J = 10.2$ Hz)
22	73.4	6.30 (1H, d, $J = 10.2$ Hz)
23	22.1	1.32 (3H, s)
24	63.2	3.28, 4.31 (each, 1H, d, $J = 10.8$ Hz)
25	15.4	0.62 (3H, s)
26	16.4	0.78 (3H, s)
27	27.3	1.82 (3H, s)
28	63.3	3.39, 3.62 (each, 1H, d, $J = 10.8$ Hz)
29	29.3	1.08 (3H, s)
30	20.0	1.32 (3H, s)
3-O-Glc A- <i>p</i>		
1	104.5	4.93 (1H, d, $J = 7.2$ Hz)
2	78.0	4.23
3	86.2	4.25
4	71.6	4.44
5	77.3	4.53
6	171.9	—
Glc- <i>p</i>		
1	103.7	5.48 (1H, d, $J = 7.2$ Hz)
2	75.3	4.04
3	77.8	4.27
4	69.3	4.48
5	78.2	3.61
6	61.1	4.38, 4.48
Ara- <i>f</i>		
1	111.1	6.04 (1H, br s)
2	83.5	4.97
3	77.4	4.84
4	85.2	4.86
5	62.1	4.12, 4.37
21-O-Ang		
1	167.5	—
2	128.9	—
3	137.0	5.93 (1H, q, $J = 7.2$ Hz)
4	15.7	2.07 (3H, d, $J = 7.2$ Hz)
5	20.8	2.00 (3H, s)
22-O-Ang		
1	167.9	—
2	128.9	—
3	136.2	5.87 (1H, q, $J = 7.2$ Hz)
4	15.6	2.03 (3H, d, $J = 7.2$ Hz)
5	20.6	1.88 (3H, s)

^{a-g} The data with the same labels in each column may be interchangeable.

Table 10.1 ^{13}C and ^1H NMR Data for Y_9 (in pyridine- d_5)

Position	^{13}C	^1H
1	38.5	0.83, 1.36
2	26.3	1.80, 2.08 (1H, m)
3	89.5	3.26(1H, m)
4	39.5	—
5	55.6	0.71
6	18.4	1.23, 1.46
7	32.8	1.23, 1.52
8	40.0	—
9	46.7	1.67
10	36.5	—
11	23.7	1.77, 1.88
12	123.5	5.41 (1H, br s)
13	142.8	—
14	41.7	—
15	34.5	1.56, 1.88
16	67.8	4.81
17	46.6	—
18	40.2	2.80 (1H, m)
19	47.5	1.36, 3.10 (1H, m)
20	36.7	—
21	91.8	4.83
22	71.3	4.37
23	27.7	1.26 (3H, s)
24	16.5	1.13 (3H, s)
25	15.5	0.79 (3H, s)
26	16.9	0.95 (3H, s)
27	27.3	1.82 (3H, s)
28	65.9	4.22, 4.33 (each, 1H, d, $J = 10.2$ Hz)
29	29.9	1.49 (3H, s)
30	20.0	1.33 (3H, s)
3-O-Glc A-p		
1	105.9	4.93 (1H, d, $J = 7.2$ Hz)
2	78.5	4.36
3	86.1	4.20
4	71.6	4.40
5	77.6	4.51
6	171.9	—
Gal-p		
1	104.5	5.31 (1H, d, $J = 7.6$ Hz)
2	73.5	4.42
3	74.9	4.09
4	69.5	4.57
5	76.3	3.95
6	61.6	4.40, 4.54
Ara-f		
1	111.0	6.03 (1H, br s)
2	83.3	4.93

3	78.0	4.76
4	85.2	4.81
5	62.1	4.12, 4.29
21-O-Rham- <i>p</i>		
1	105.1	4.87 (1H, d, $J = 1.5$ Hz)
2	70.5	4.39
3	74.0	5.58
4	71.1	5.70
5	69.0	3.89
6	17.0	1.11 (3H, d, $J = 6.6$ Hz)
Rham-3-O-Ang		
1	167.6 ^a	—
2	128.3 ^b	—
3	138.6 ^c	5.93 ^f (1H, q, $J = 7.2$ Hz)
4	15.7 ^d	1.95 (3H, m)
5	20.7 ^e	1.94 ^g (3H, s)
Rham-4-O-Ang		
1	167.5 ^a	—
2	128.0 ^b	—
3	138.5 ^c	5.87 ^f (1H, q, $J = 7.2$ Hz)
4	15.6 ^d	1.95 (3H, m)
5	20.6 ^e	1.85 ^g (3H, s)
28-O-Acetyl		
1	170.1	—
2	20.5 ^e	1.84 ^g (3H, s)

^{a-g} The data with the same labels in each column may be interchangeable.

Table 11.1 ¹³C and ¹H NMR Data for Y₁₀ (in pyridine-*d*₅)

Position	¹³ C	¹ H
1	38.5	0.87, 1.38
2	26.4	1.86, 2.12 (1H, m)
3	89.7	3.24 (1H, dd, $J = 12.0/4.2$ Hz)
4	39.8	—
5	55.6	0.75
6	18.2	1.29, 1.49
7	32.9	1.27, 1.54
8	39.8	—
9	46.7	1.68
10	36.5	—
11	23.6	1.70, 1.83
12	123.3	5.40 (1H, br s)
13	142.5	—
14	41.4	—
15	34.8	1.60, 1.83
16	68.4	4.49
17	47.8	—
18	39.7	3.06
19	47.0	1.40, 3.10
20	36.1	—

21	78.5	6.69 (1H, d, $J = 10.2$ Hz)
22	73.5	6.31 (1H, d, $J = 10.2$ Hz)
23	27.7	1.30 (3H, s)
24	16.5	1.17 (3H, s)
25	15.4	0.80 (3H, s)
26	16.7	0.83 (3H, s)
27	27.3	1.83 (3H, s)
28	63.4	3.40, 3.64 (each, 1H, d, $J = 10.8$ Hz)
29	29.3	1.09 (3H, s)
30	20.1	1.33 (3H, s)
3-O-Glc A- <i>p</i>		
1	104.9	4.91 (1H, d, $J = 7.8$ Hz)
2	78.7	4.40
3	86.1	4.23
4	71.5	4.44
5	77.1	4.53
6	171.8	–
Gla- <i>p</i>		
1	104.6	5.34 (1H, d, $J = 7.8$ Hz)
2	73.4	4.50
3	74.9	4.11
4	69.6	4.58
5	76.4	3.98
6	61.6	4.47, 4.52
Ara- <i>f</i>		
1	110.9	6.05 (1H, br s)
2	83.4	4.95
3	77.5	4.78
4	85.2	4.83
5	62.1	4.16, 4.39
21-O-Ang		
1	167.5	–
2	128.8	–
3	137.9	5.92 (1H, q, 7.2 Hz)
4	15.7	2.07 (3H, d, 7.2 Hz)
5	20.8	2.00 (3H, s)
22-O-Ang		
1	167.9	–
2	128.8	–
3	136.8	5.87 (1H, q, 7.2 Hz)
4	15.6	2.03 (3H, d, 7.2 Hz)
5	20.6	1.88 (3H, s)

Table 15.1. ^{13}C and ^1H NMR data for Compound-X (in $\text{MeOH-}d_4$)

Position	C	H
1	38.5	1.00, 1.64
2	25.7	1.70, 1.85
3	90.9	3.18 (1H, dd, $J = 11.4/3.6$ Hz)
4	39.1	–
5	55.6	0.78 (1H, d, $J = 11.4$ Hz)

6	17.9	1.44, 1.58
7	33.5	1.40, 1.69
8	39.8	-
9	46.4	1.67
10	36.4	-
11	23.3	1.89, 1.93
12	123.9	5.38 (1H, br s)
13	143.7	-
14	40.9	-
15	32.5	1.38, 1.60
16	68.8	3.99
17	48.5	-
18	39.6	2.64 (1H, m)
19	46.6	1.20, 2.68 (1H, m)
20	35.8	-
21	80.6	6.00 (1H, d, $J = 10.2$ Hz)
22	72.4	5.88 (1H, d, $J = 10.2$ Hz)
23	27.0	1.08 (3H, s)
24	15.0	0.98 (3H, s)
25	15.4	0.88 (3H, s)
26	15.9	0.94 (3H, s)
27	26.3	1.49 (3H, s)
28	63.0	2.91, 3.25 (each, 1H, d, $J = 11.4$ Hz)
29	28.4	0.90 (3H, s)
30	18.8	1.11 (3H, s)
3-O-GlcA- <i>p</i>		
1	104.1	4.55 (1H, d, $J = 7.8$ Hz)
2	77.3	3.74
3	85.0	3.68
4	70.7	3.62
5	75.0	3.86
6	170.3	-
butyl-1	59.8	3.02 (2H, m)
butyl-2	29.3	1.05
butyl-3	18.4	1.51
butyl-4	12.5	1.16
3-O-Gal- <i>p</i>		
1	103.1	4.65 (1H, d, $J = 7.8$ Hz)
2	72.0	3.54
3	73.6	3.50
4	69.1	3.82
5	75.8	3.46
6	61.3	3.59, 3.70
Ara- <i>f</i>		
1	109.4	5.24 (1H, d, $J = 1.8$ Hz)
2	82.0	4.10
3	76.4	3.87
4	83.9	4.06
5	61.5	3.59, 3.70
21-O-Acetyl		

1	169.9	—
2	21.6	2.14 (3H, s)
22-O-Ang		
1	167.8	—
2	127.5	—
3	140.0	6.17 (1H, q, $J = 7.2$ Hz)
4	14.7	2.02 (3H, d, $J = 7.2$ Hz)
5	19.6	1.85 (3H, s)

5

10

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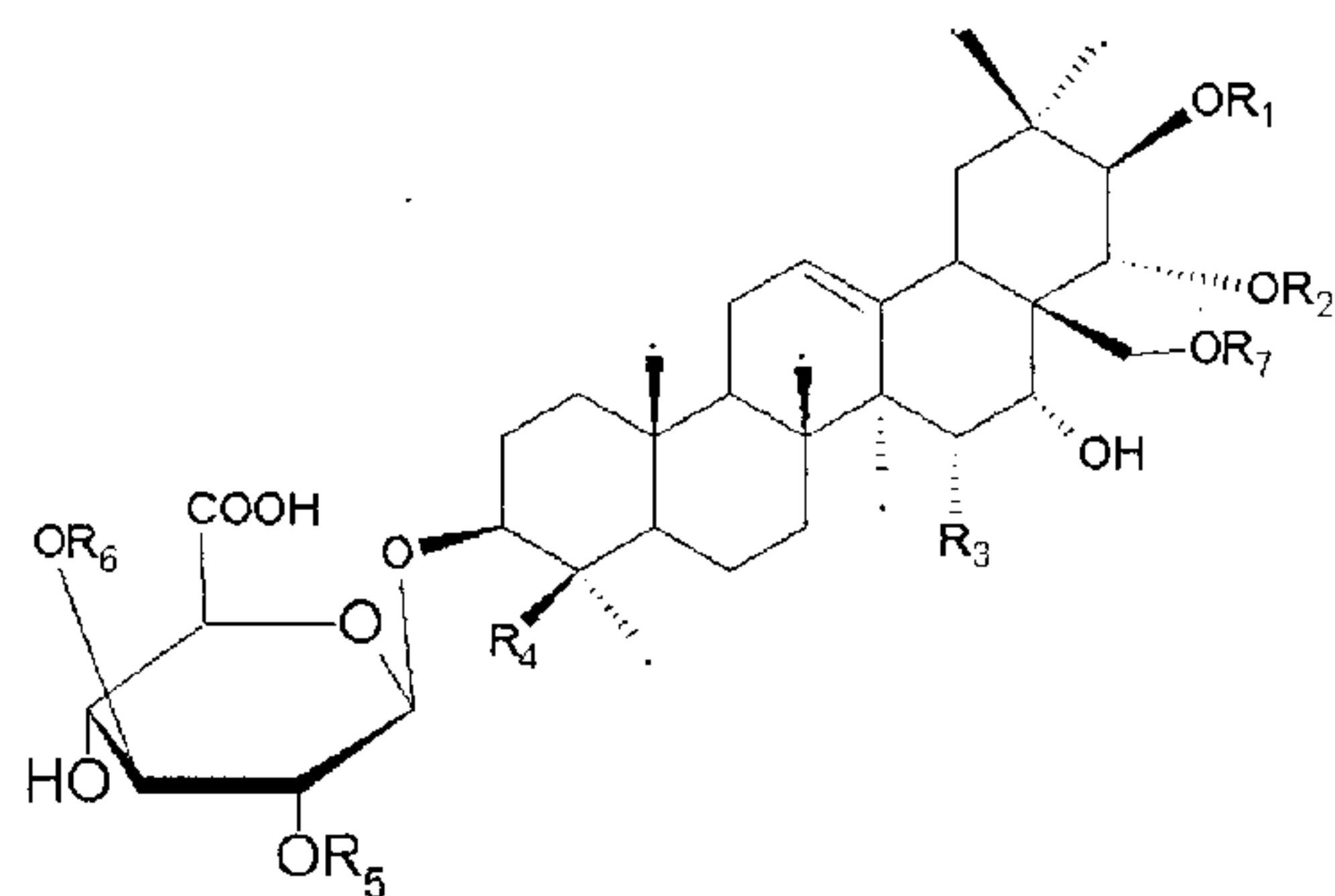
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What is claimed is:

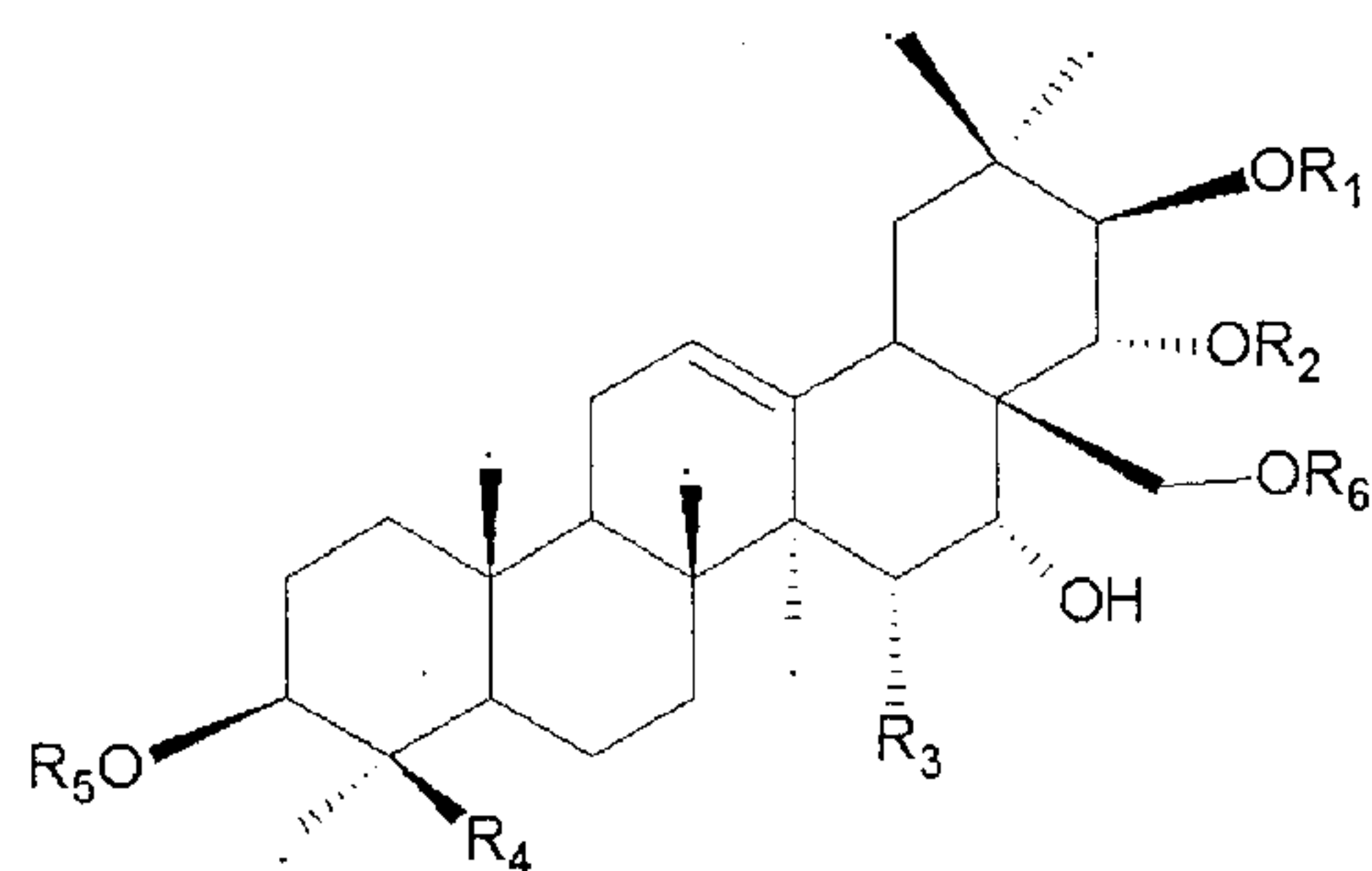
1. A compound for use in treating cancer having the following formula (1) or (3):



(1),

5 or a salt, ester thereof, wherein R1 is angeloyl group; R2 is angeloyl group; R3 is OH or H; R4 is CH₂OH; R7 comprises H; and R5 is glucose; and R6 is arabinose;

or:



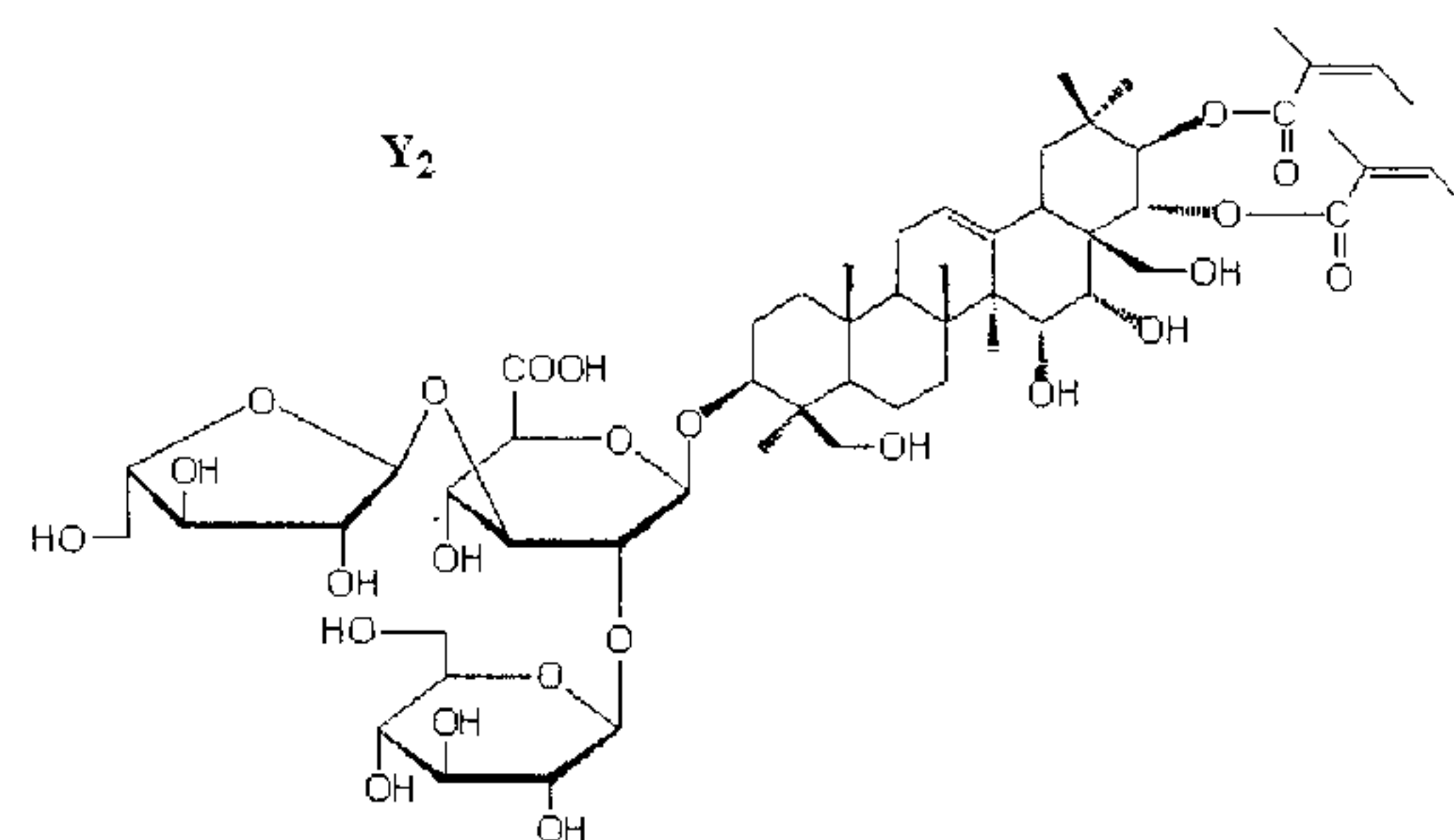
(3),

10 or a salt, ester thereof, wherein R₁ is angeloyl group; R₂ is angeloyl group; R₃ is OH or H; R₄ is CH₂OH; R₆ is H; and R5 are sugar moieties, wherein the sugar moieties are glucopyranosyl, arabinofuranosyl, and glucuronopyranosyl.

2. The compound for use in treating cancer according to claim 1, comprising the following structure:

15

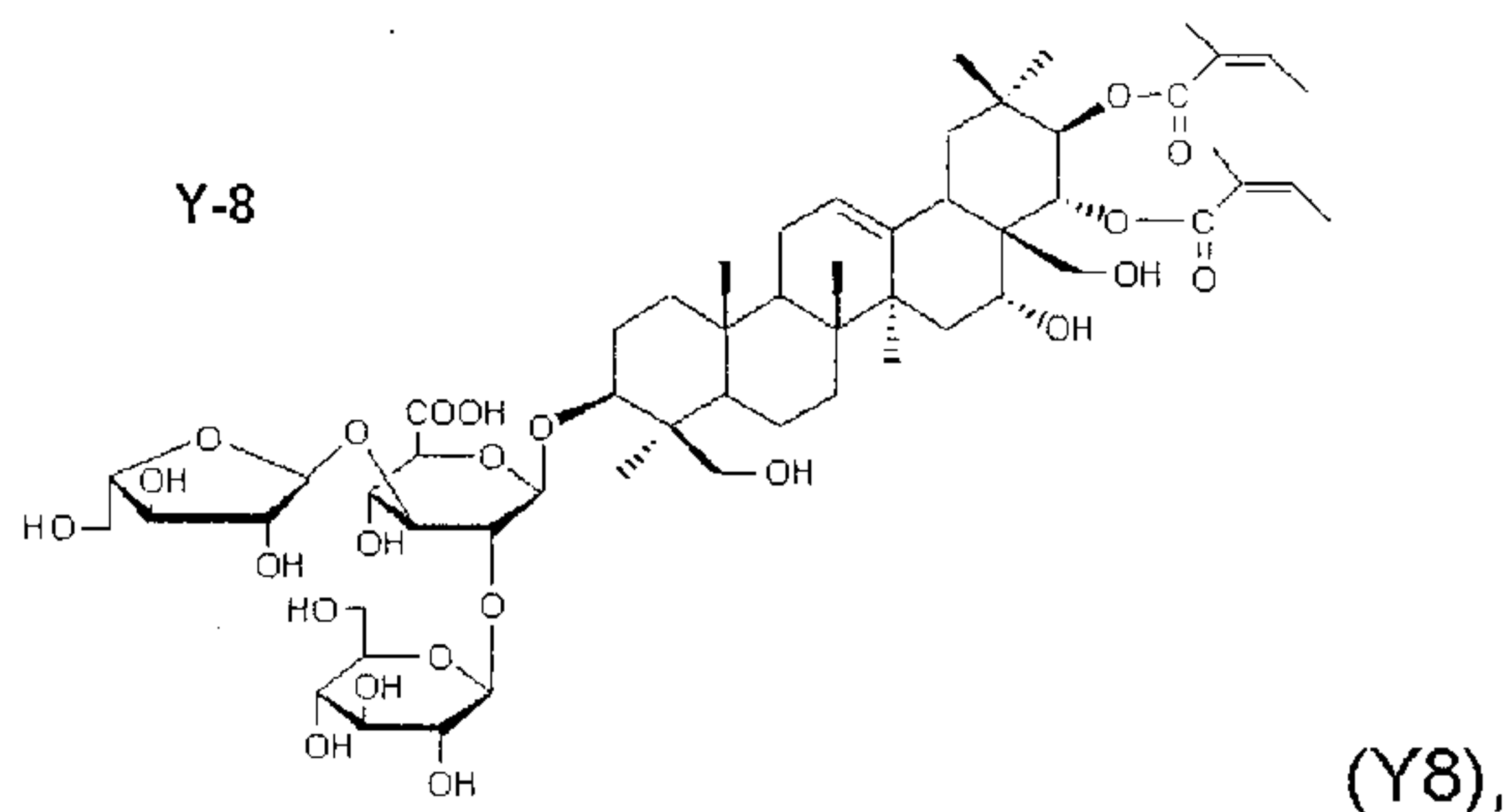
(a) A compound comprising structure (Y2):



(Y2),

or chemical name: 3-O-[β -D-glucopyranosyl-(1 $\rightarrow$ 2)]- α -L-arabinofuranosyl(1 $\rightarrow$ 3)- β -D-glucuronopyranosyl-21,22-O-diangeloyl-3 β , 15 α , 16 α , 21 β , 22 α , 24 β , 28-heptahydroxyolean-12-ene, or Xanifolia-Y2; or

- 5 (b) A compound comprising structure (Y8):



or chemical name: 3-O-[β -glucopyranosyl (1 $\rightarrow$ 2)]- α -arabinofuranosyl (1 $\rightarrow$ 3)- β -glucuronopyranosyl-21, 22-O-diangeloyl-3 β , 16 α , 21 β , 22 α , 24 β , 28-hexahydroxyolean-12-ene, or Xanifolia-Y8 .

10

3 . The compound for use in treating cancer according to any one of claims 1 and 2 which is in the form of a salt.

4. The compound for use in treating cancer of any one of claims 1 and 2, the structure of which being synthesized or isolated from natural sources.

15

5. The compound of any one of claims 1 and 2 for use in treating cancer wherein the cancer is breast cancer, leukocyte cancer, liver cancer, ovarian cancer, bladder cancer, prostate cancer, skin cancer, bone cancer or brain cancer.

20

6 . A composition comprising an effective amount of the compound of any one of claims 1 and 2 and a carrier for use in inhibiting tumor or cancer cell growth.

7. The composition of claim 6, wherein the cancer is breast cancer, leukocyte cancer, liver cancer, ovarian cancer, bladder cancer, prostate cancer, skin cancer, bone cancer or brain cancer.

25

8. A use of compound according to any one of claims 1 and 2 for manufacture of medicament for inhibiting cancers.

9. A use of compound according to any one of claim 1 and 2 for inhibiting cancers.

10. The use of compound of claim 9, wherein the cancer is breast cancer, leukocyte
5 cancer, liver cancer, ovarian cancer, bladder cancer, prostate cancer, skin cancer, bone cancer or brain cancer.

11. The compound for use according to any one of claims 1 and 2, wherein the compound is obtained by a method comprising the steps of:

- 10 (a) extracting *Xanthoceras sorbifolia* or plant powder with organic solvents to obtain an organic extract; wherein the powder is prepared from the husks, branches, stems, leaves, kernels, roots, barks or seed shells of the herb or plant;
- (b) collecting the organic extract;
- (c) refluxing the organic extract to obtain a second extract;
- 15 (d) removing the organic solvent from the second extract;
- (e) drying and sterilizing the second extract to obtain a crude extract powder;
- (f) fractionating the crude extract powder into components using HPLC and FPLC chromatography with silica gel, C18 or other equivalent solid phase materials;
- (g) monitoring absorption wavelength at 207nm or 254nm;
- 20 (h) identifying the bioactive components of the crude extract powder;
- (i) purifying the bioactive components of the crude extract powder with FPLC to obtain a fraction of the bioactive components; and
- (j) isolating the compound from the fraction of the bioactive component with preparative HPLC.

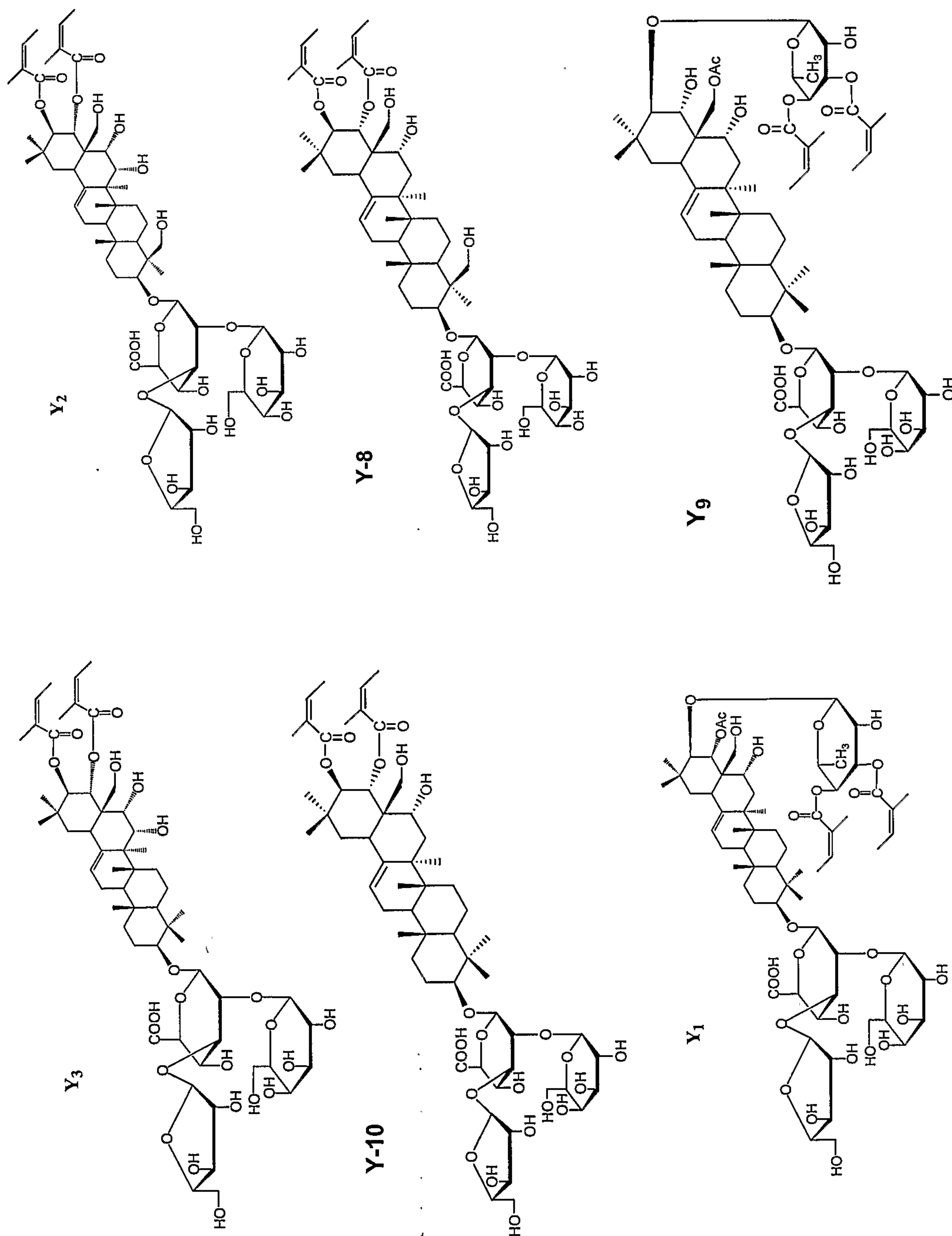
25

12. A pharmaceutical composition use in treating cancer comprising the compound of any one of claims 1 and 2 and a pharmaceutical acceptable carrier.

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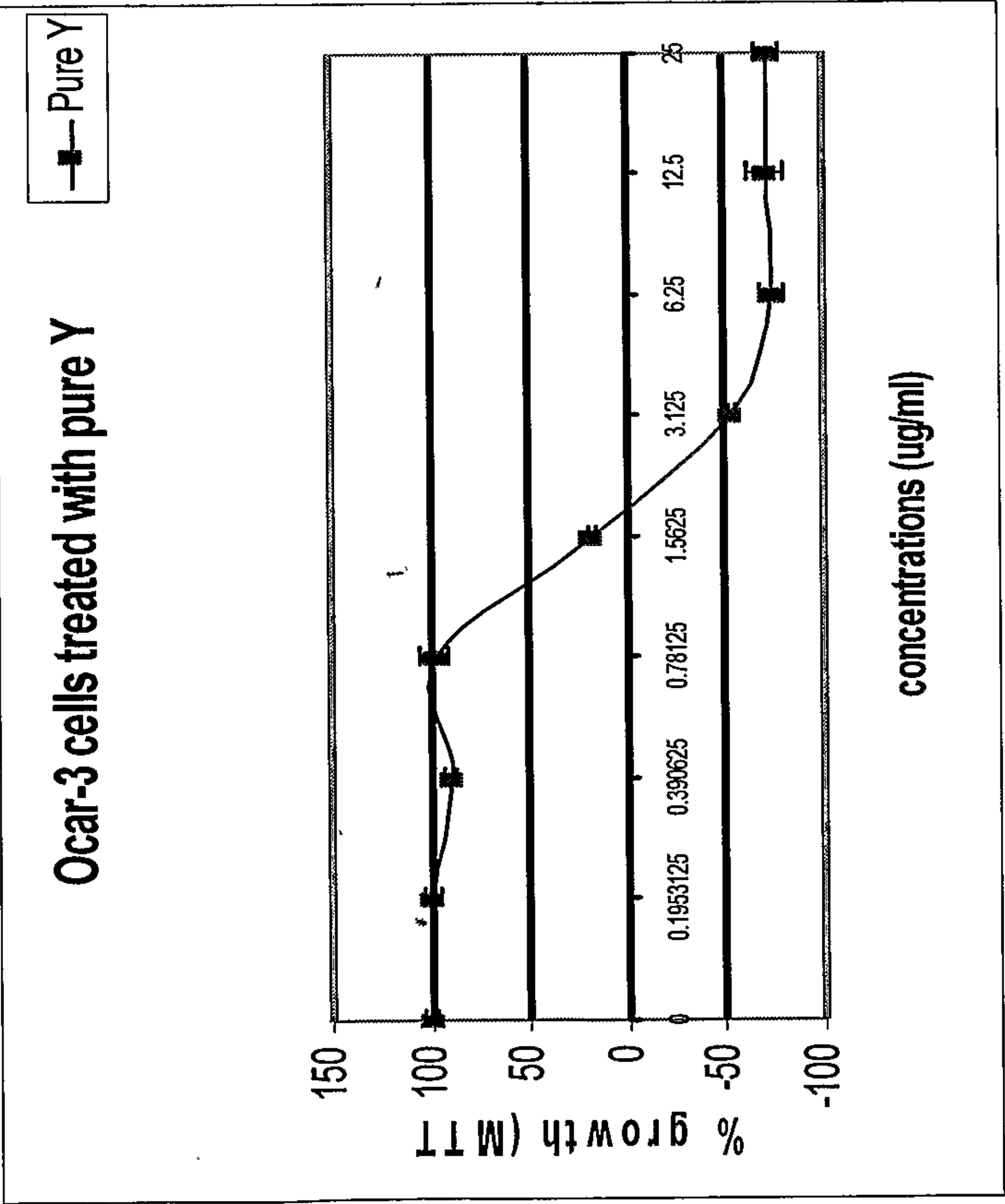
Figure 1

Structure of six anticancer Saponins with biangeloyl groups (Y3 (Y), Y2, Y8, Y10, Y1 and Y9).

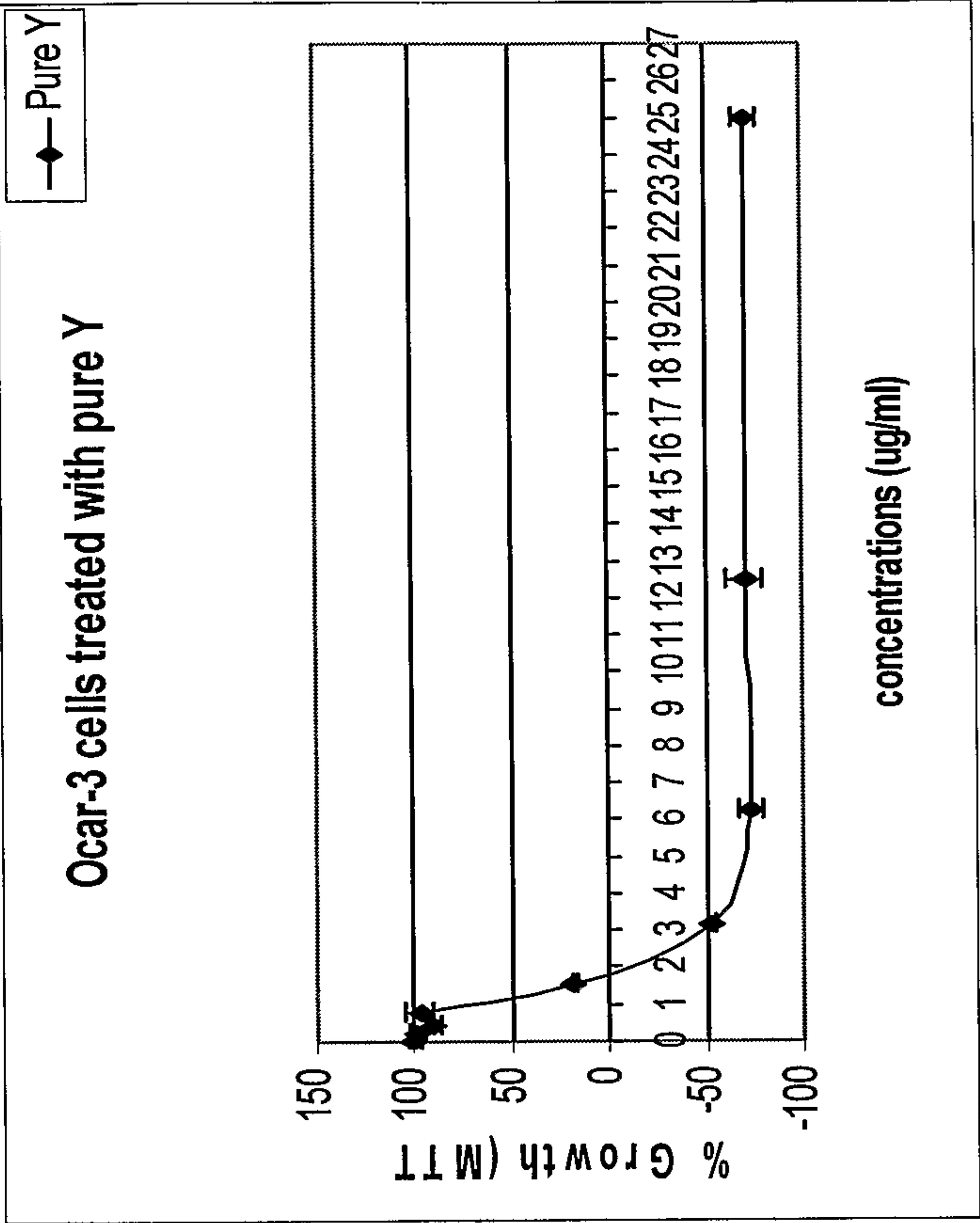


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Figure 2
Anticancer activity of Compound Y

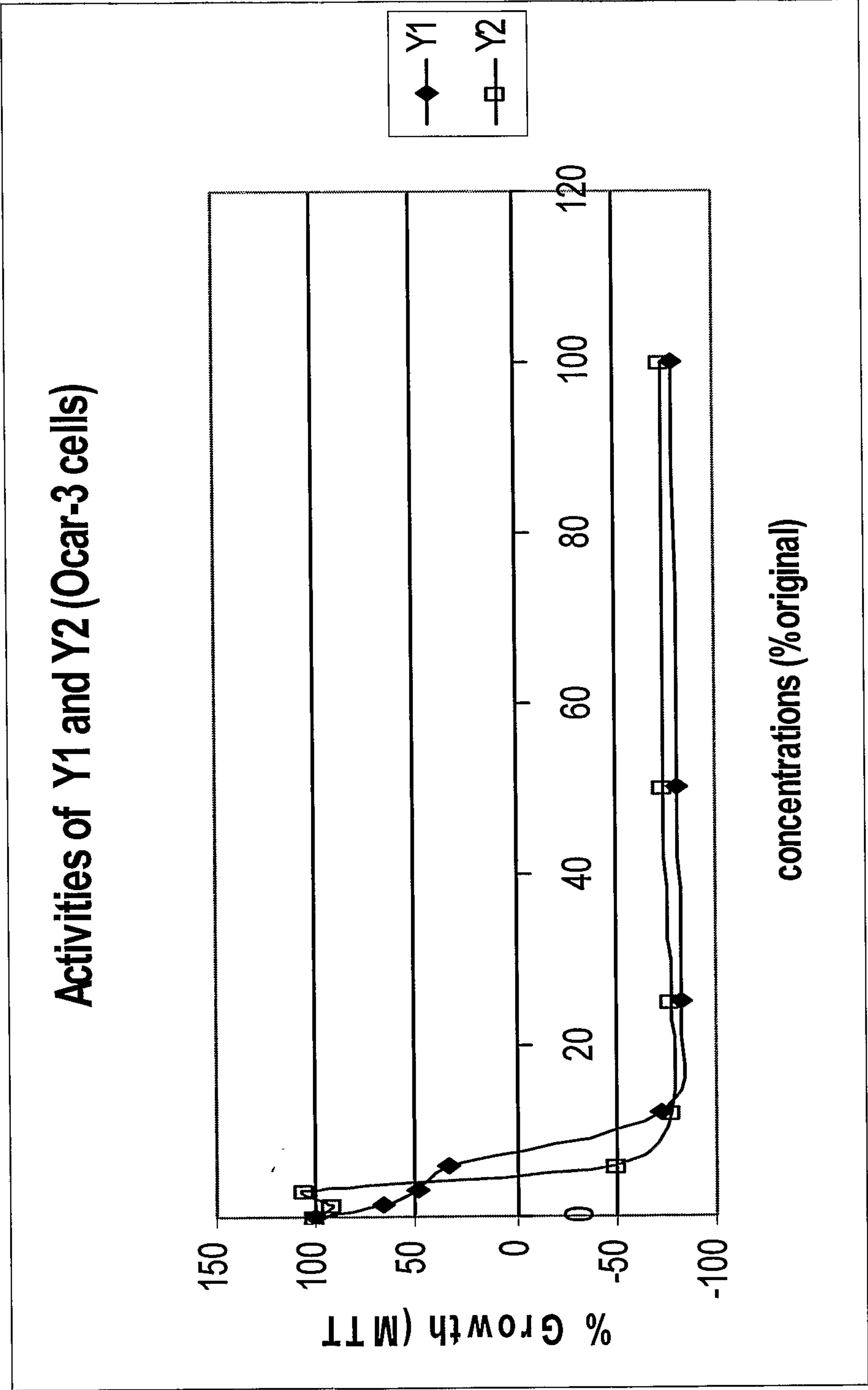
(A) Points graph



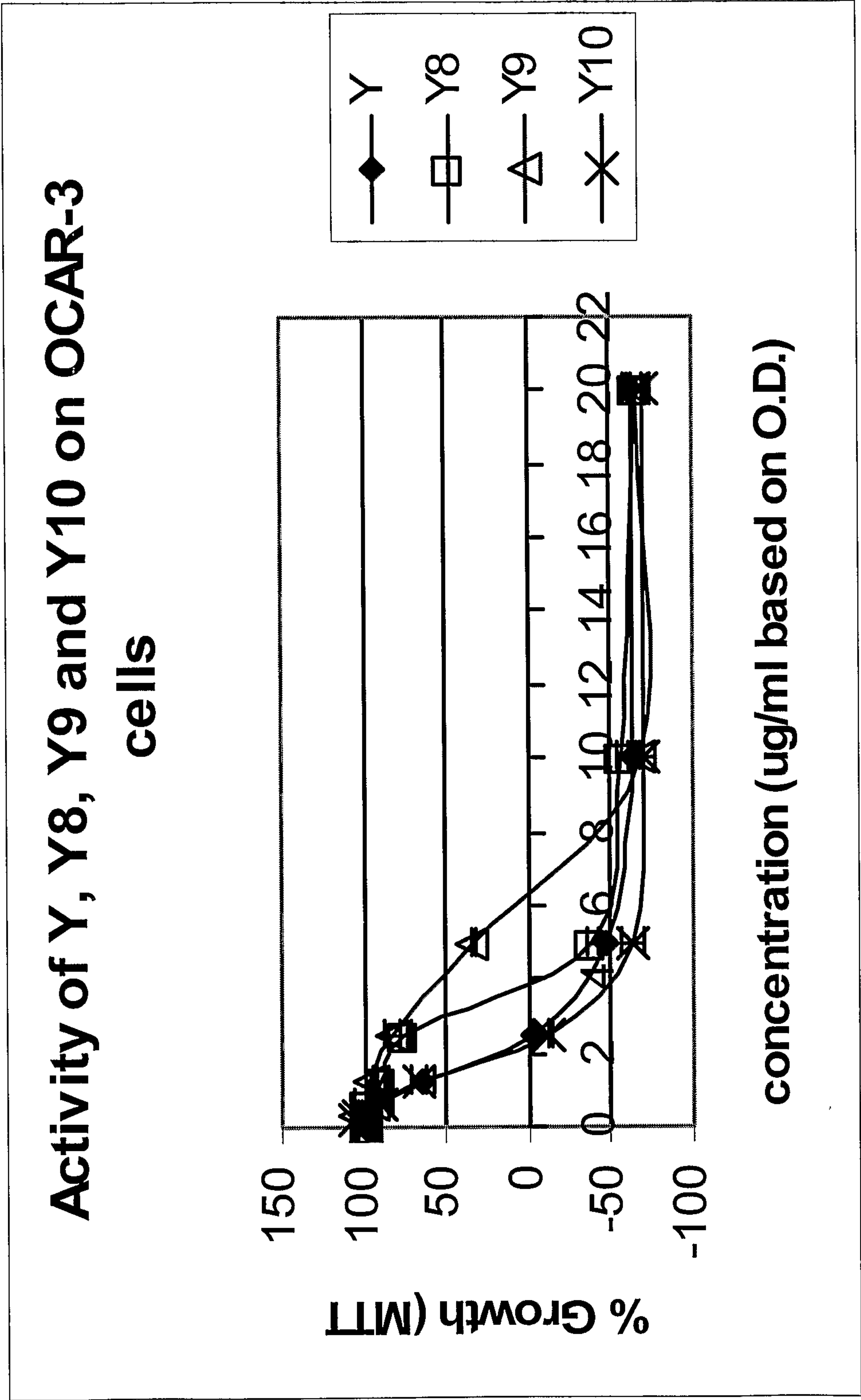
(B) X,Y linear graph



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Figure 3
Y1 and Y2 activity on Ovarian cancer cells



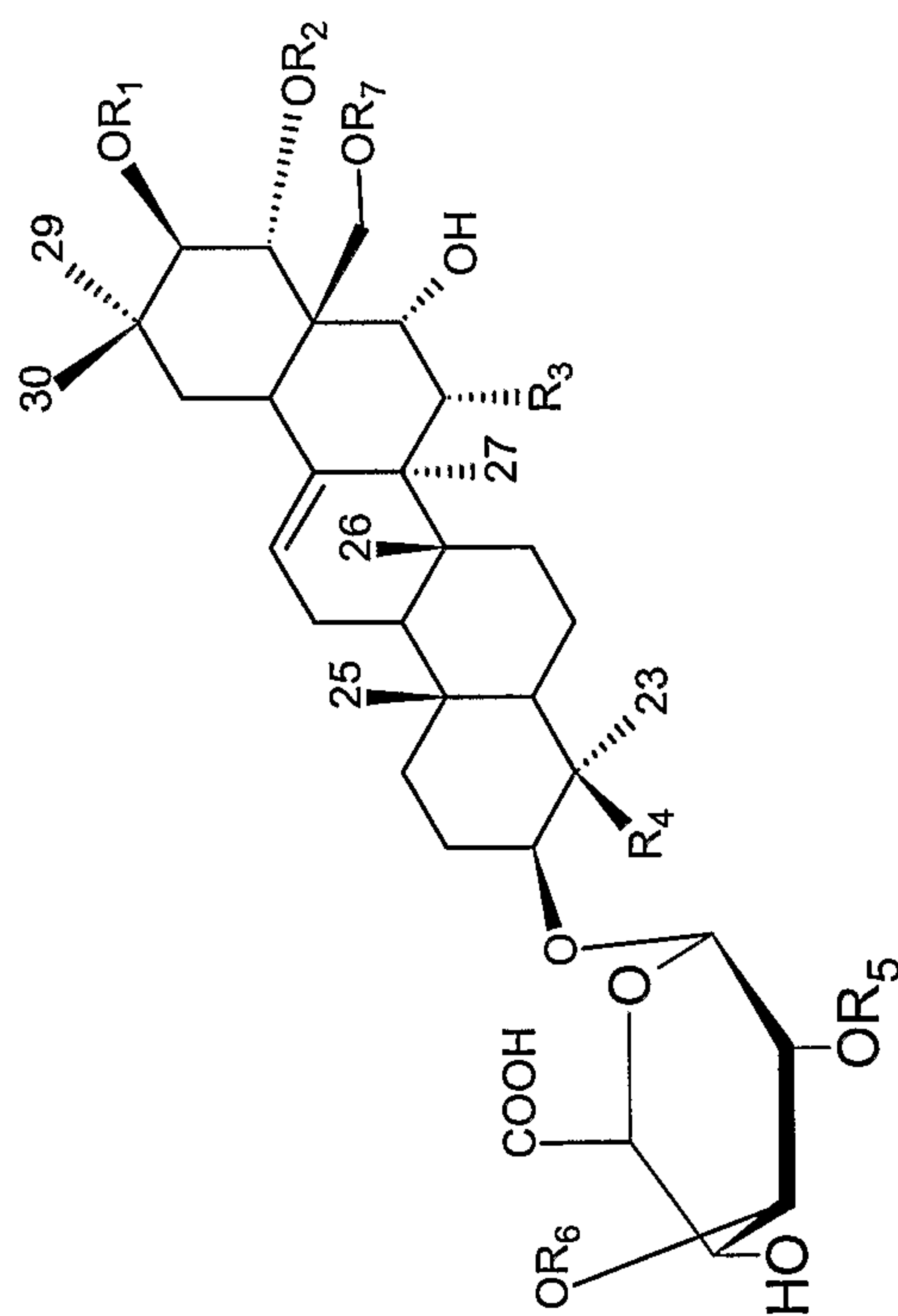
4/77
Figure 4
Anticancer activity of Compounds Y, Y8, Y9 and Y10.



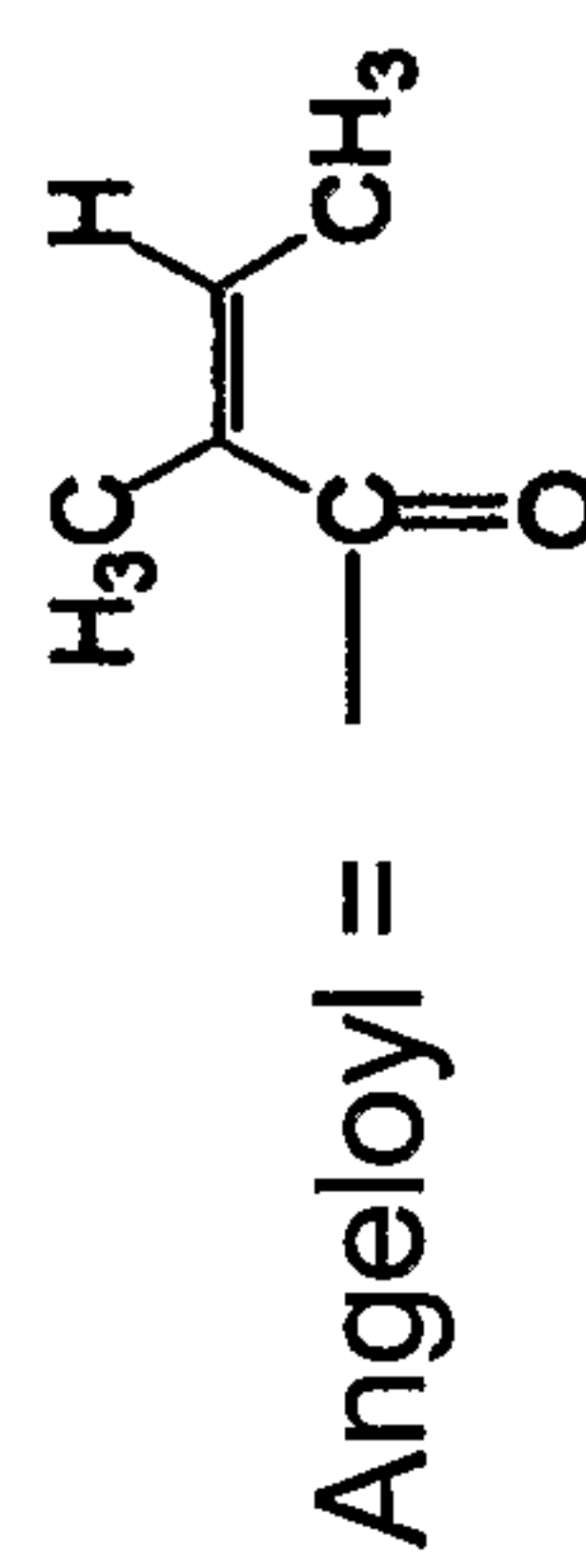
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Figure 5

Consensus structure of compound Ys
(Y, Y2, Y8 and Y10)

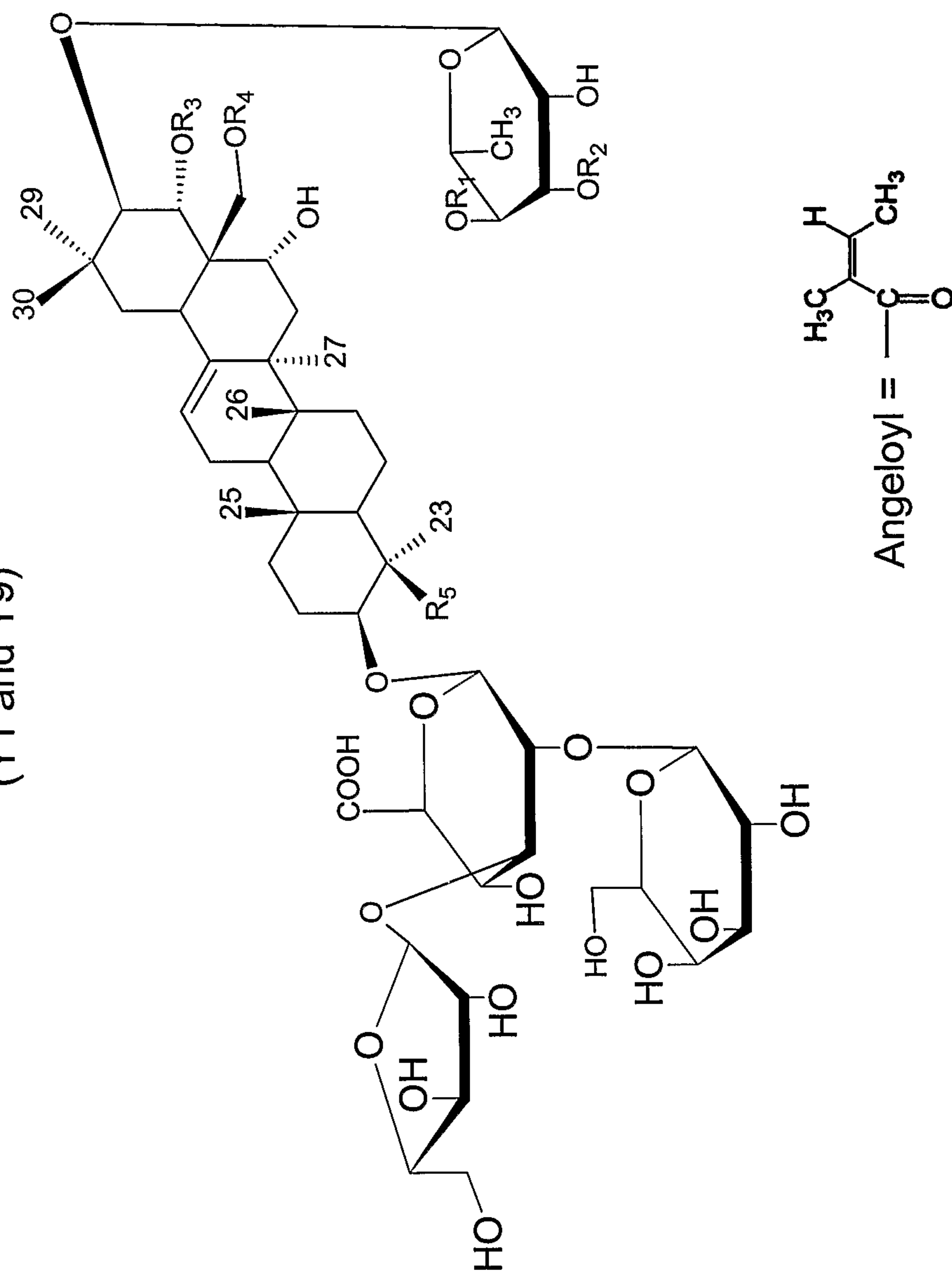


R1 = Angeloyl
R2 = Angeloyl
R3 = OH or H
R4 = CH₃ or CH₂OH
R5 = D- glucose, D-galactose
R6 = L- arabinose
R7 = H,



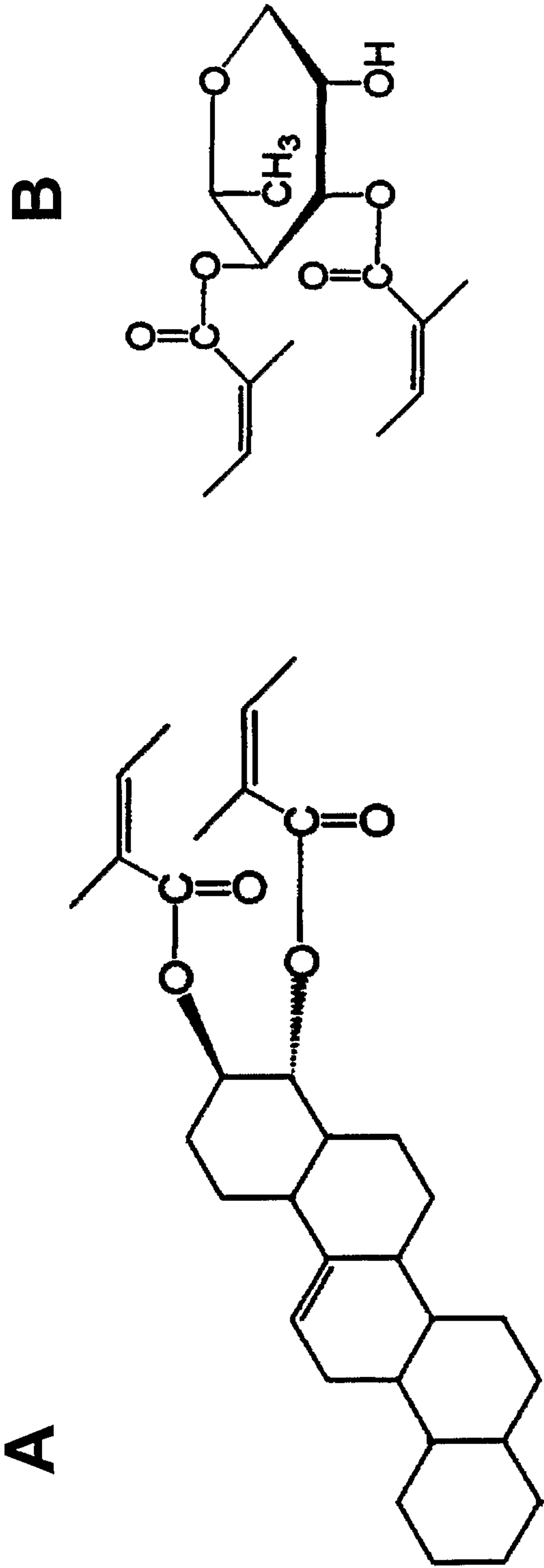
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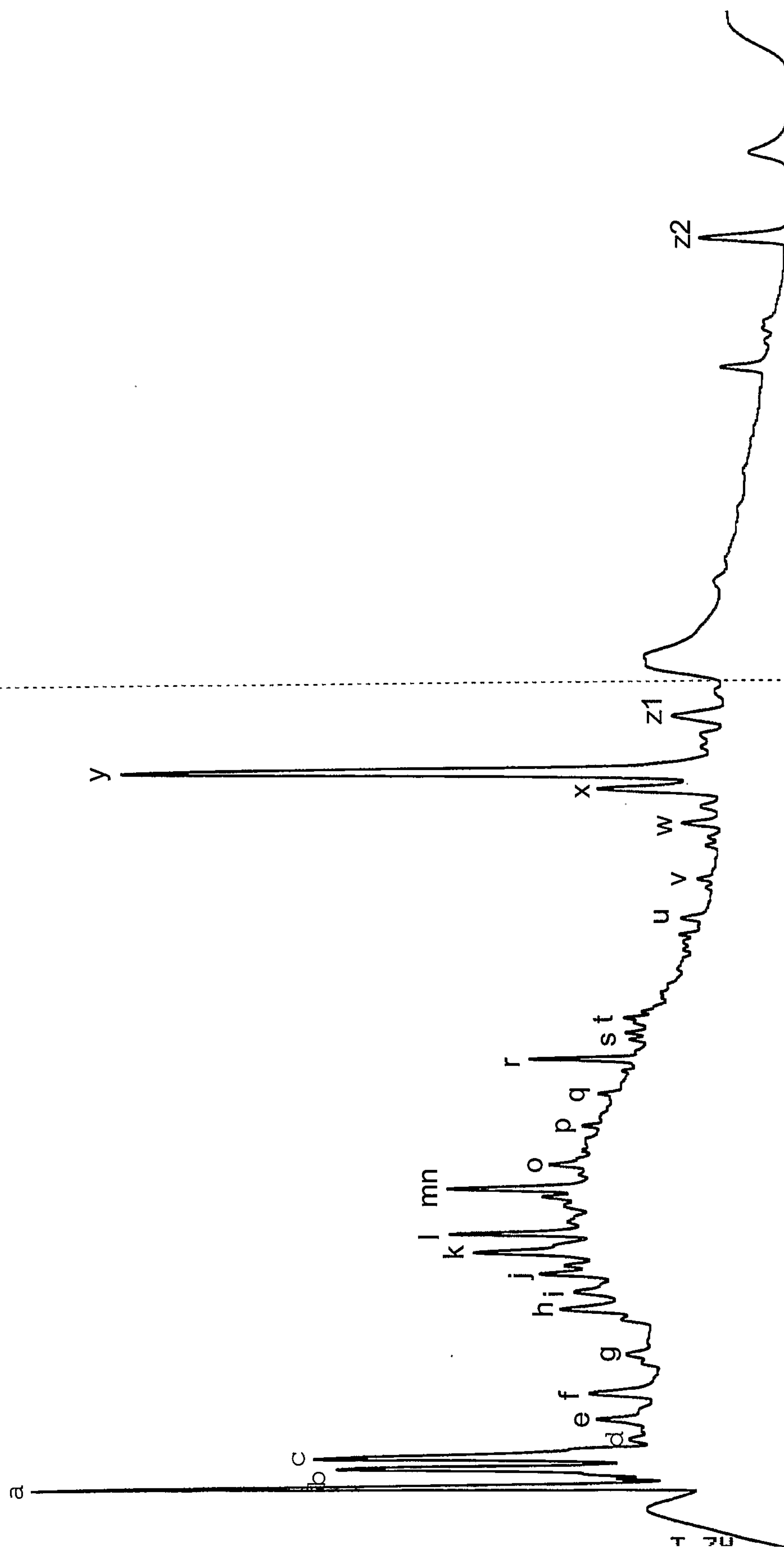
Figure 6
Consensus structure of Ys
(Y1 and Y9)



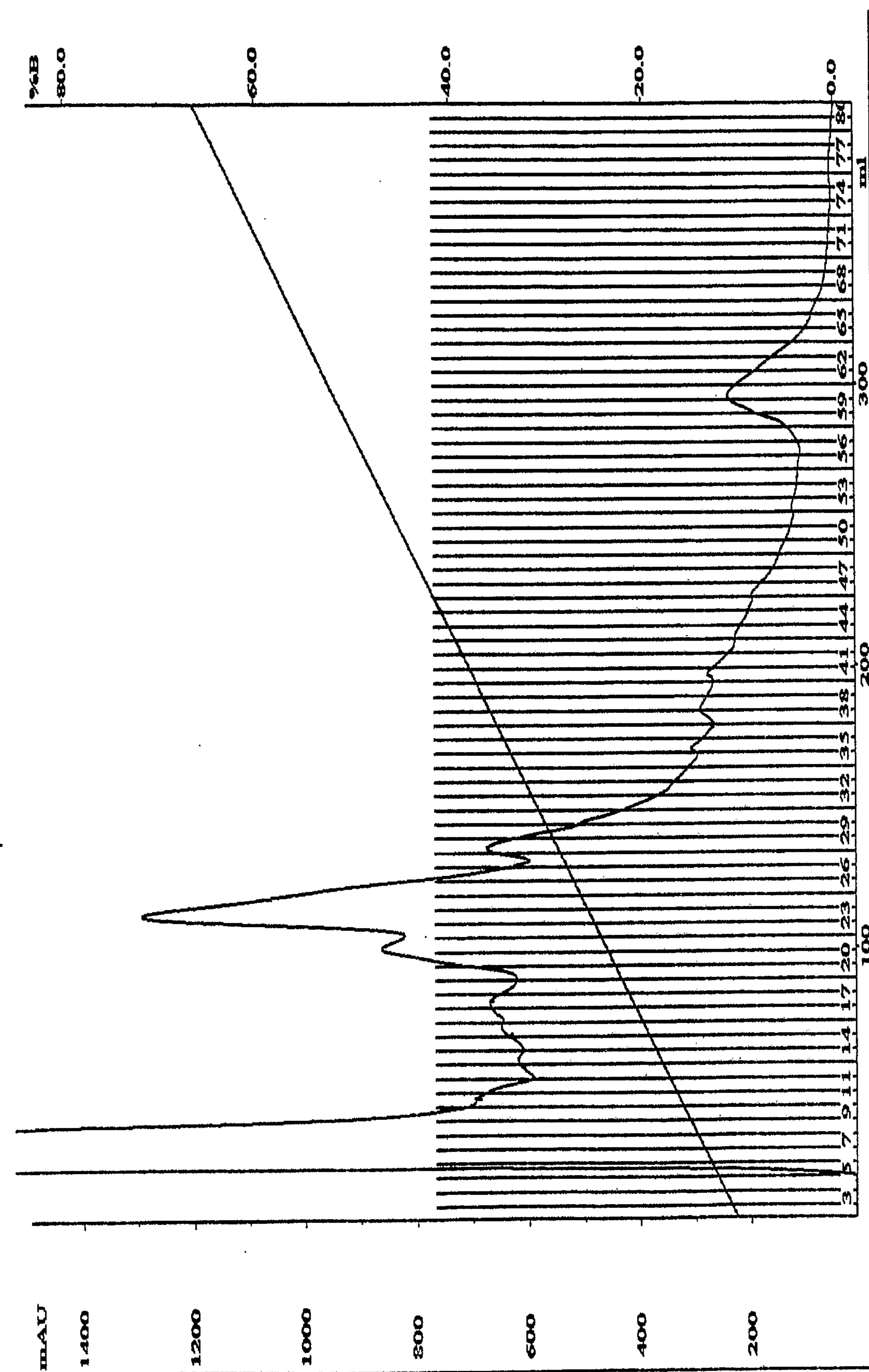
R1 = Angeloyl
R2 = Angeloyl
R3 = Ac or H
R4 = Ac or H
R5 = CH₃ or CH₂OH

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Figure 7
Biangeloyl group situated in trans-position on a structure.

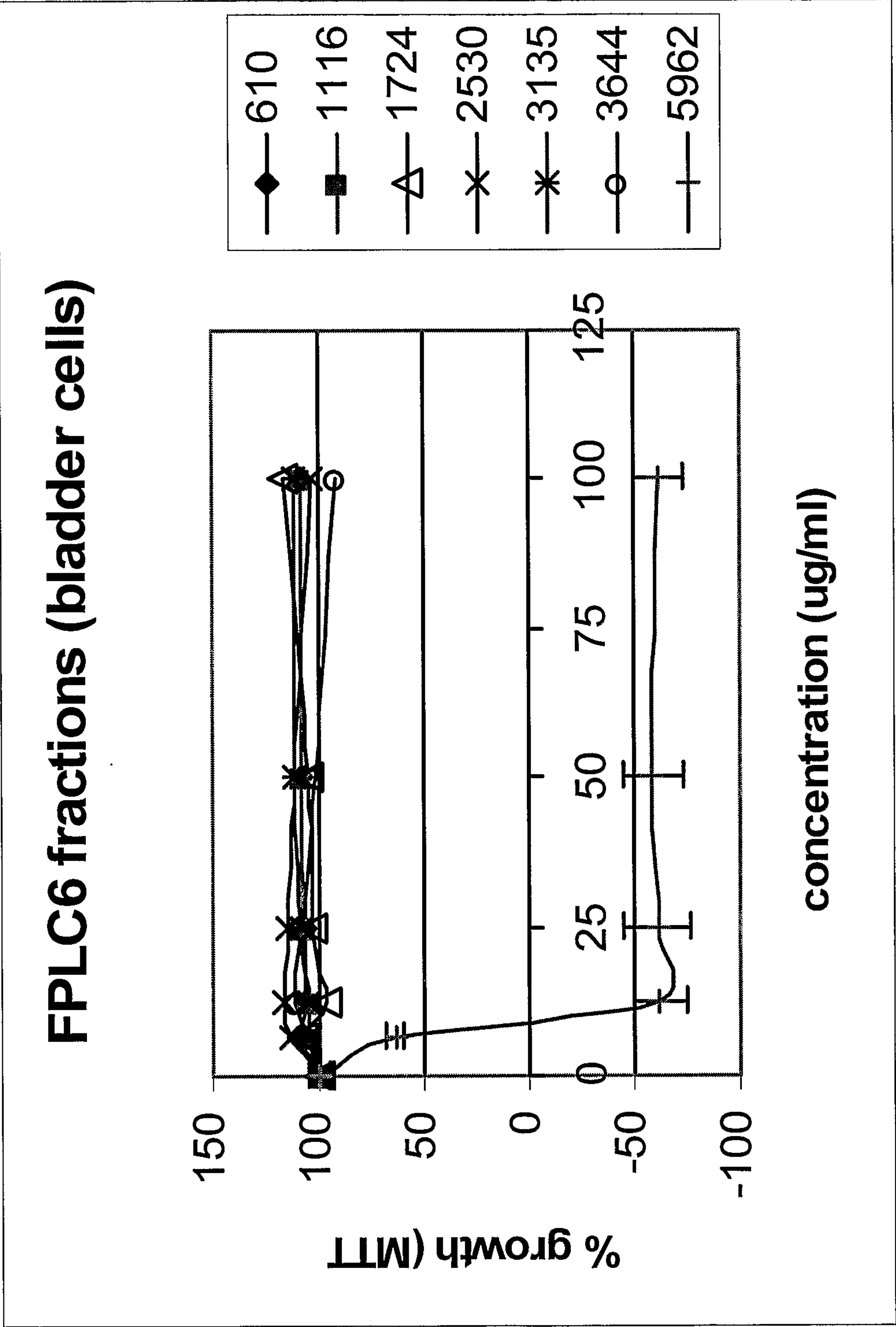


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Figure 8

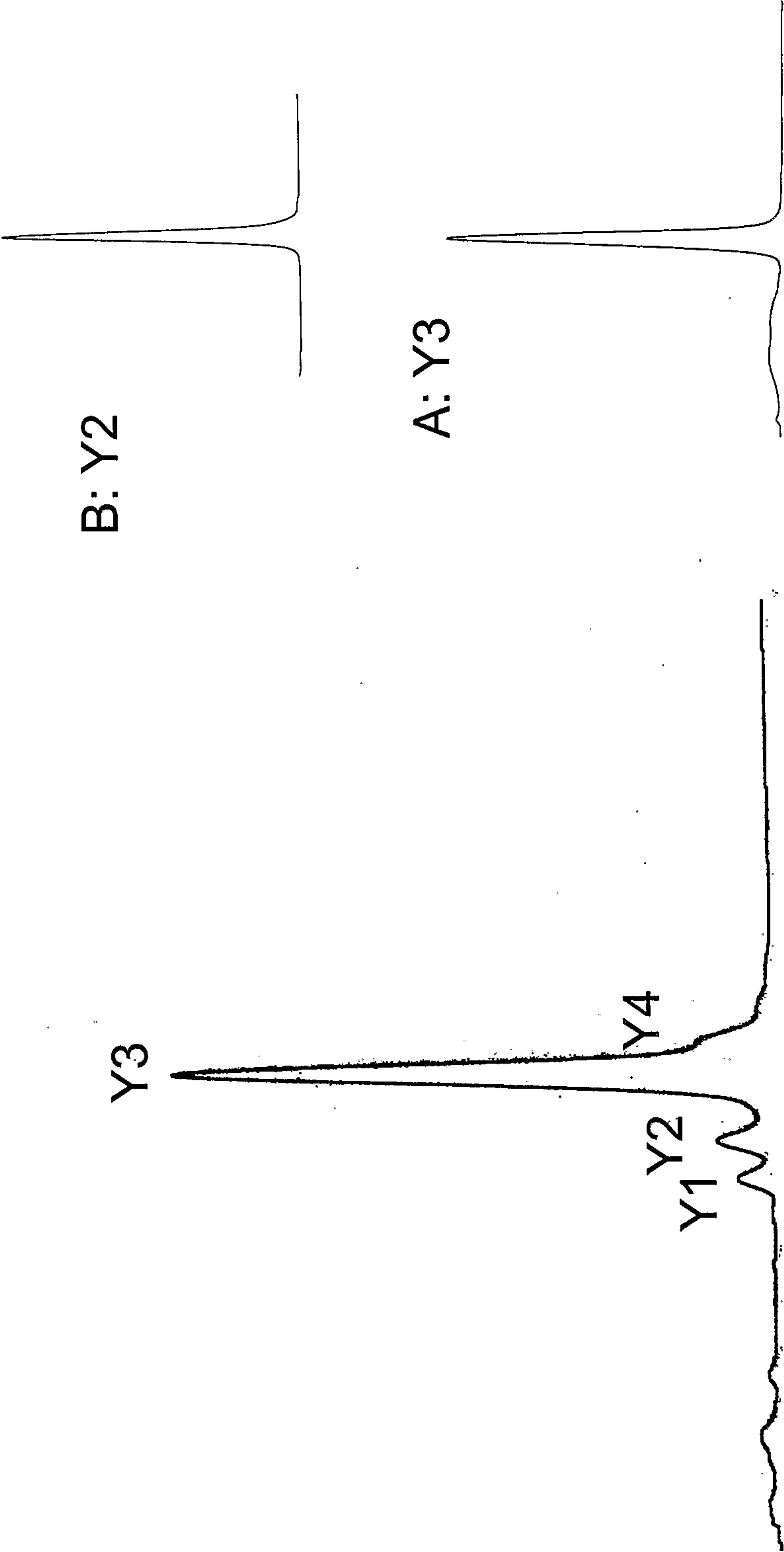
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Figure 9
FPLC profile of Plant extract



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Figure 10
Cell growth activity of fractions of plant extract



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Figure 11
Purification of Factions Ys



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Figure 12
Purification of Y8-Y10 with HPLC (iso-45)

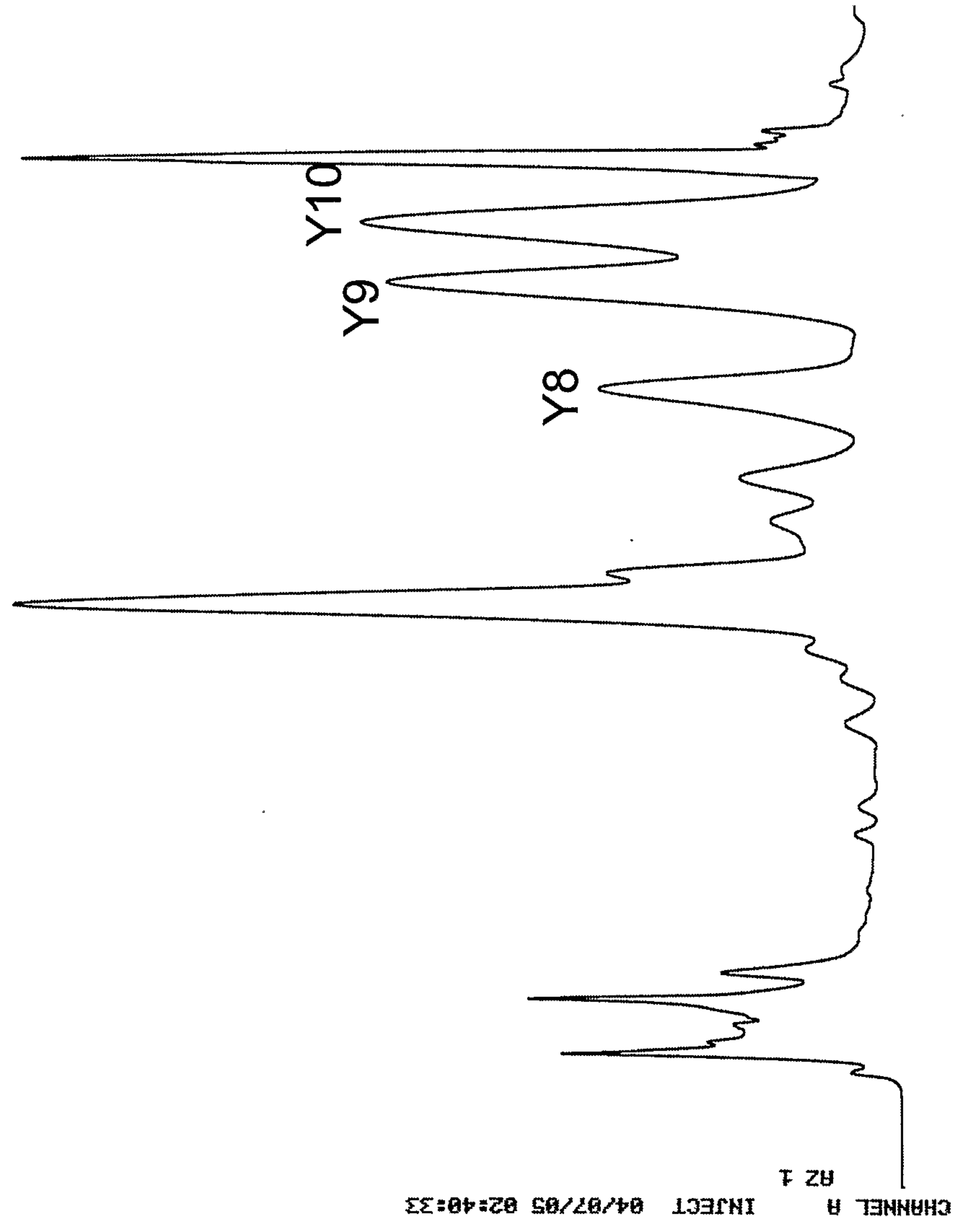
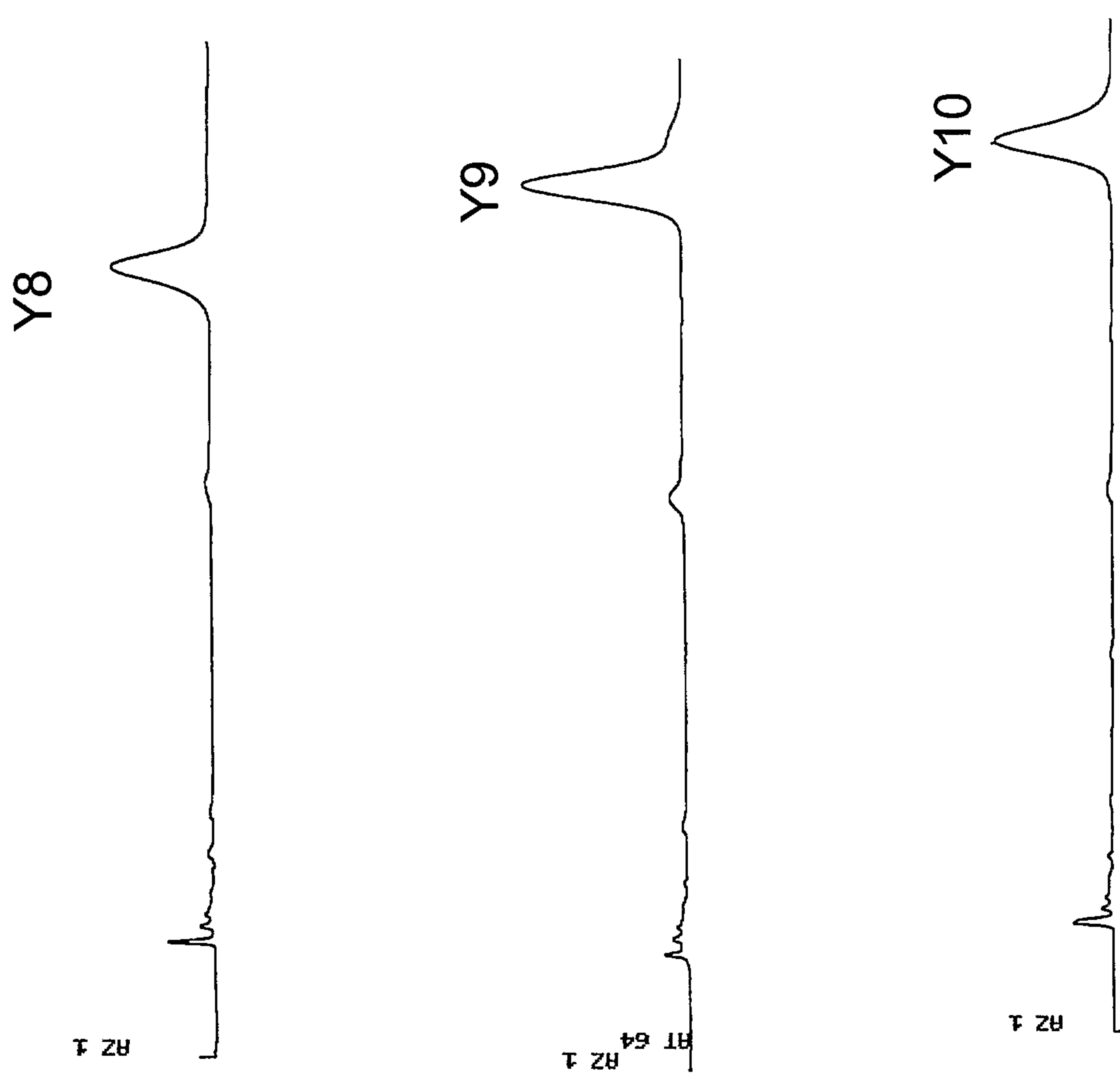
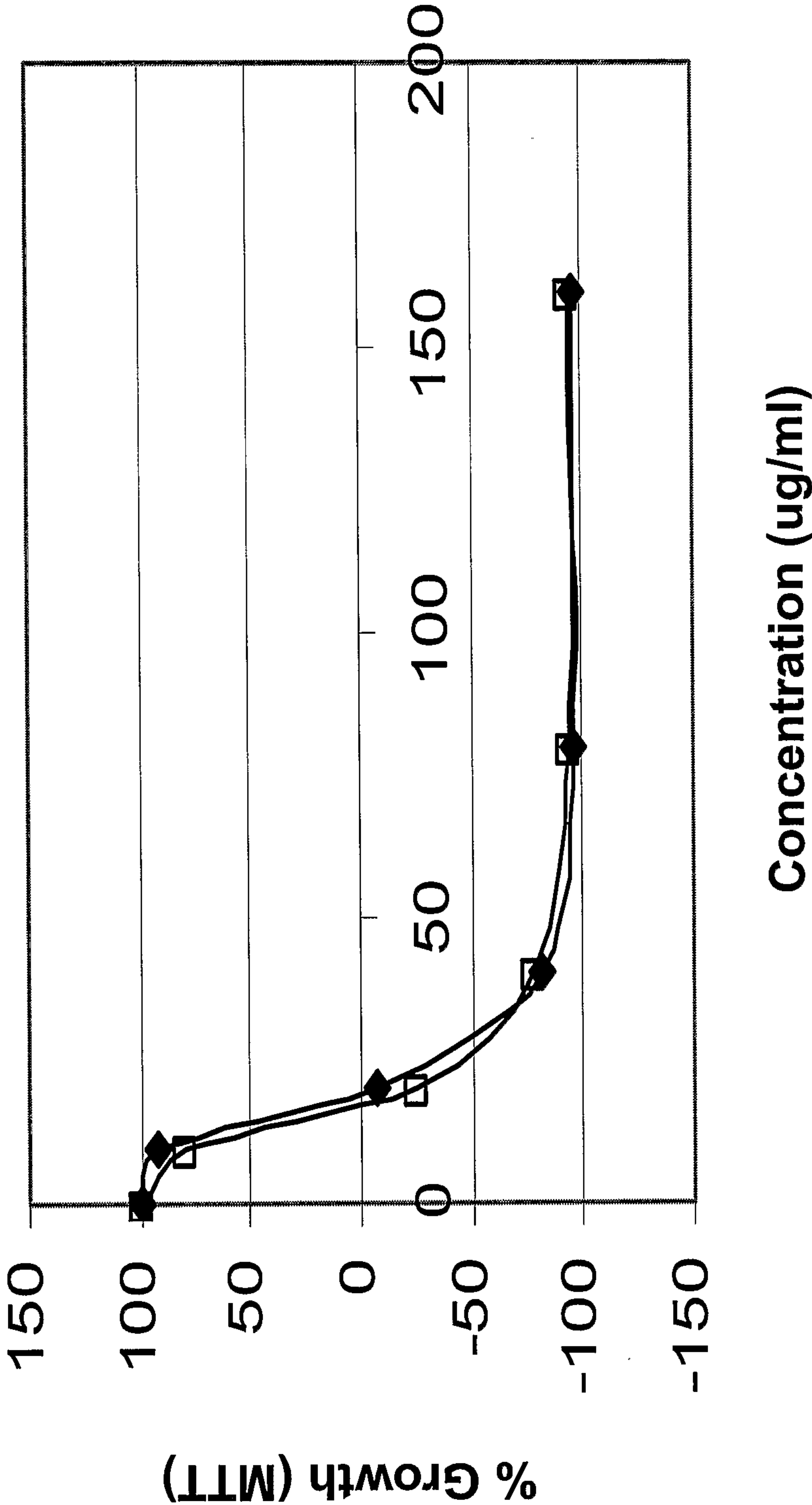


Figure 13
Purity of Y8, Y9 and Y10

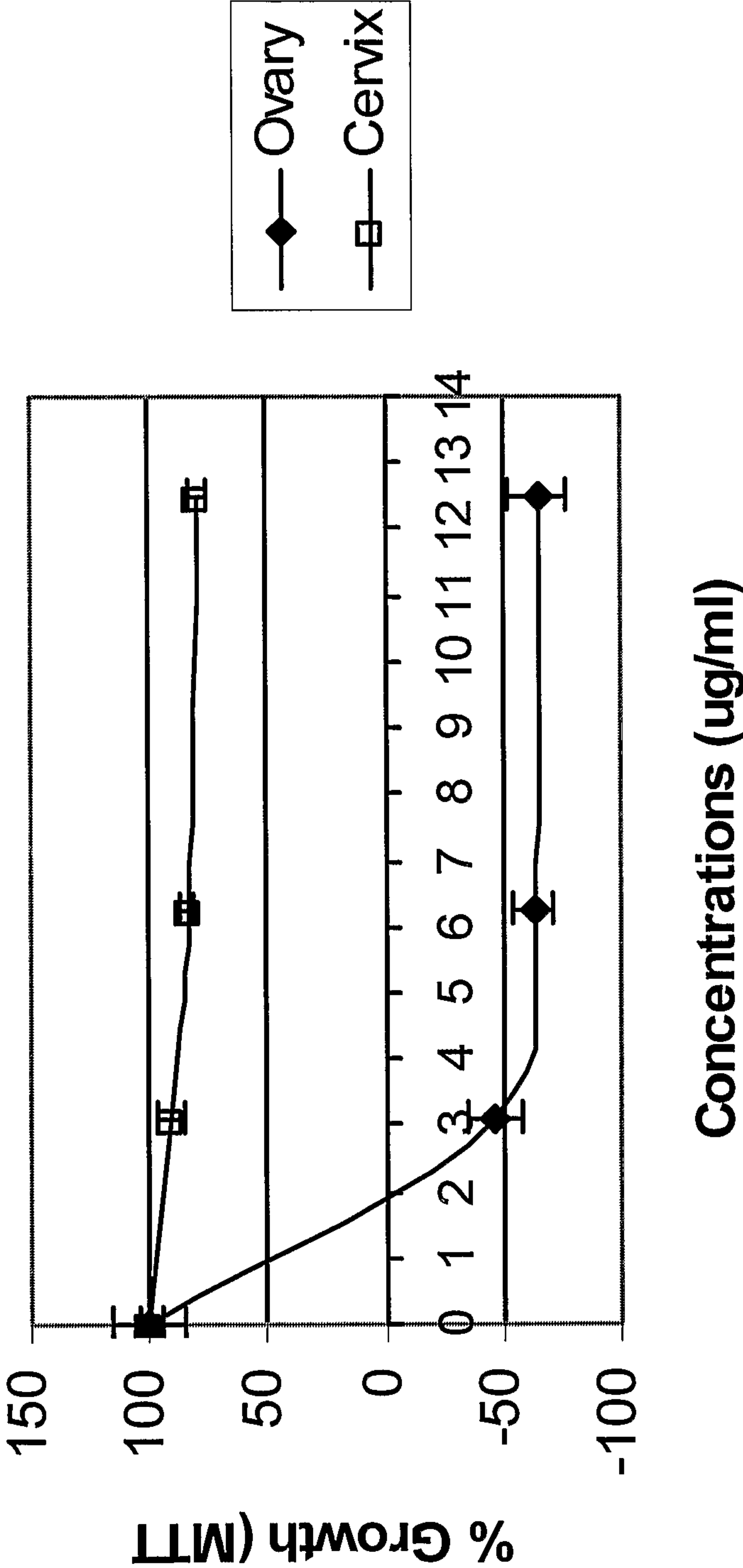


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Figure 14
Inhibition of Ovarian cancer growth by plant extract

Most Sensitive: Ovary



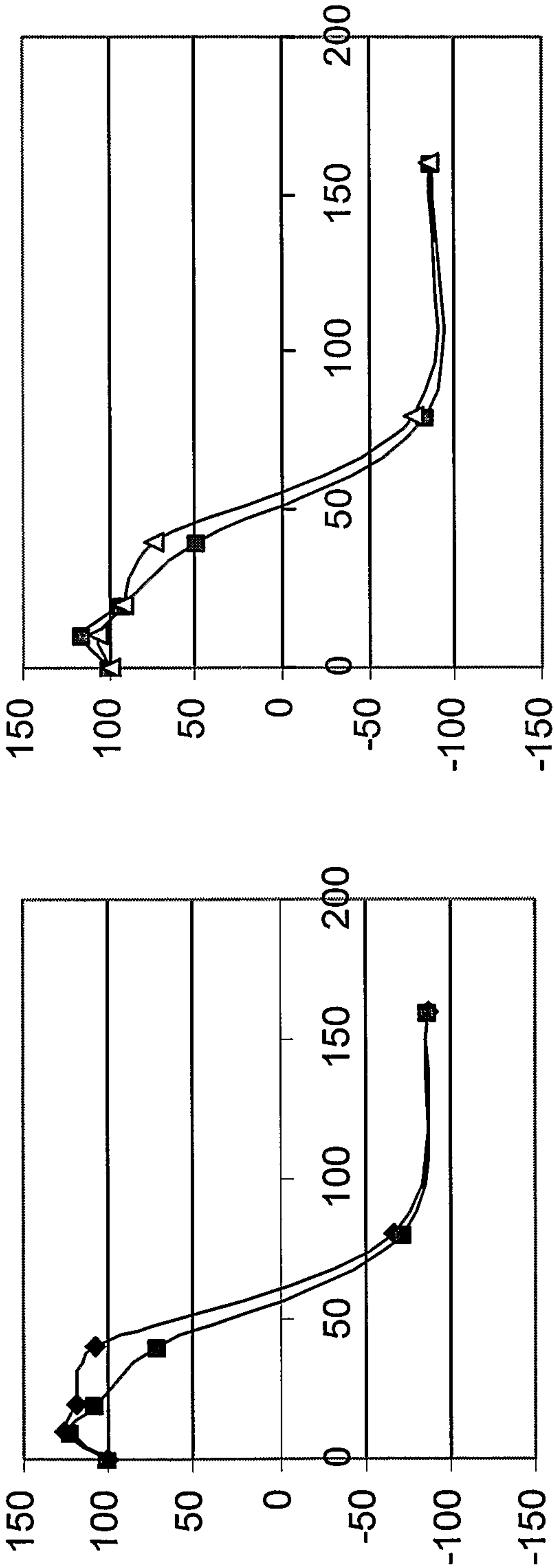
15/77
Figure 15
Comparison of Potency of Y in ovary and cervix cancer cells



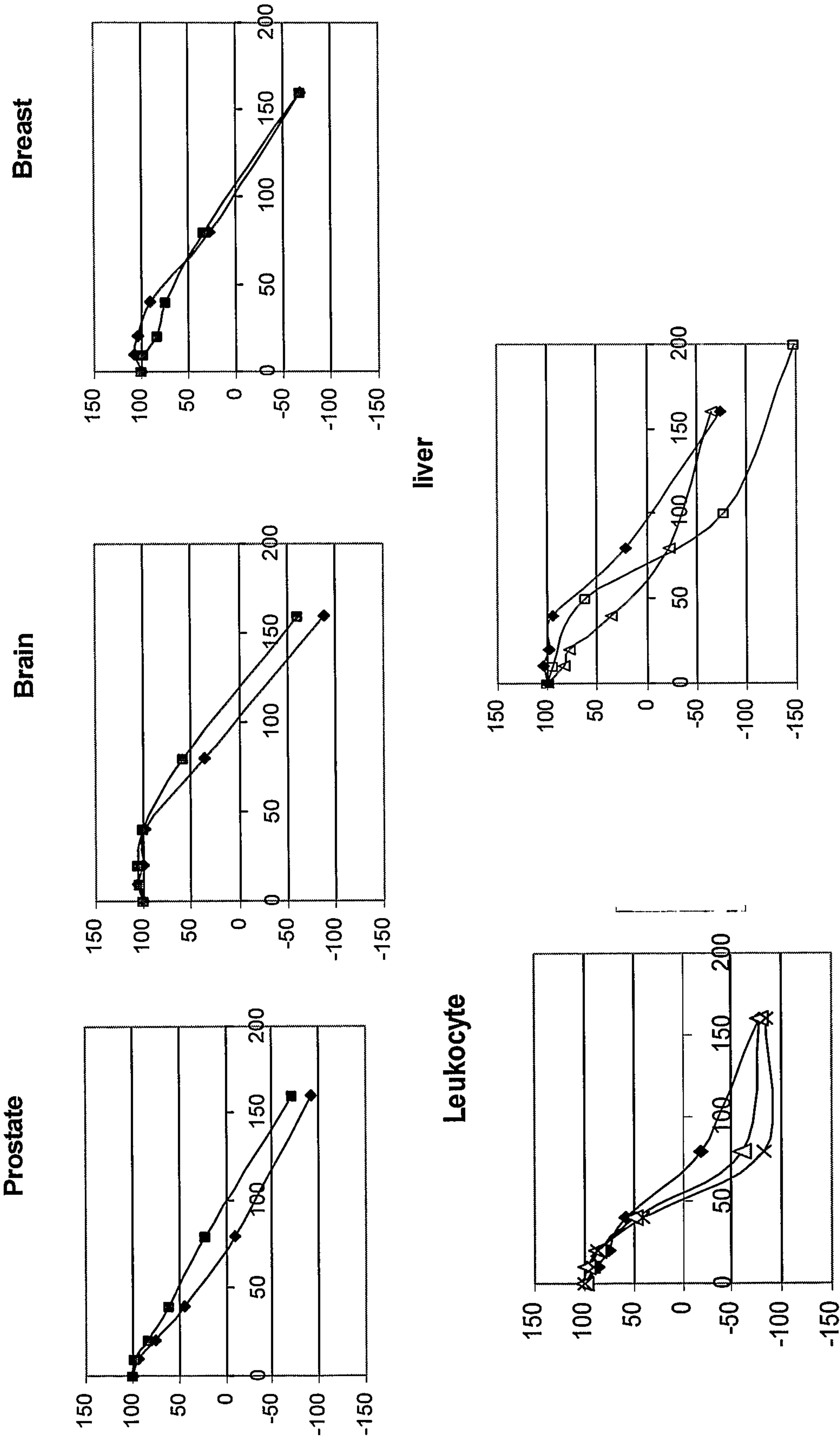
16/77
Figure 16A
Growth curve of cancer cells after treatment with extract

Sensitive

Bladder **Bone**



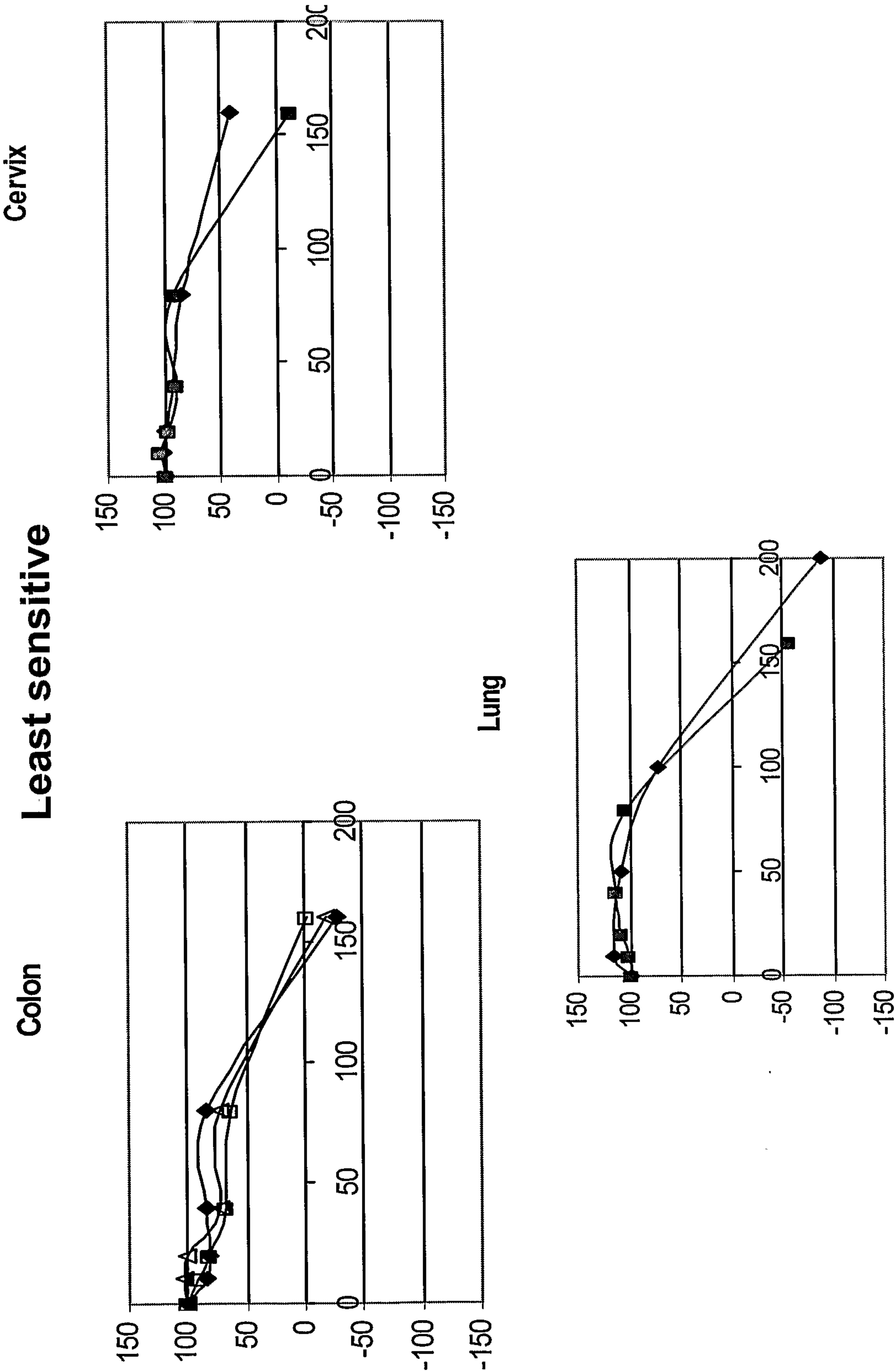
17/77
Figure 16B
Growth curve of cancer cells after treatment with extract
Semi-sensitive



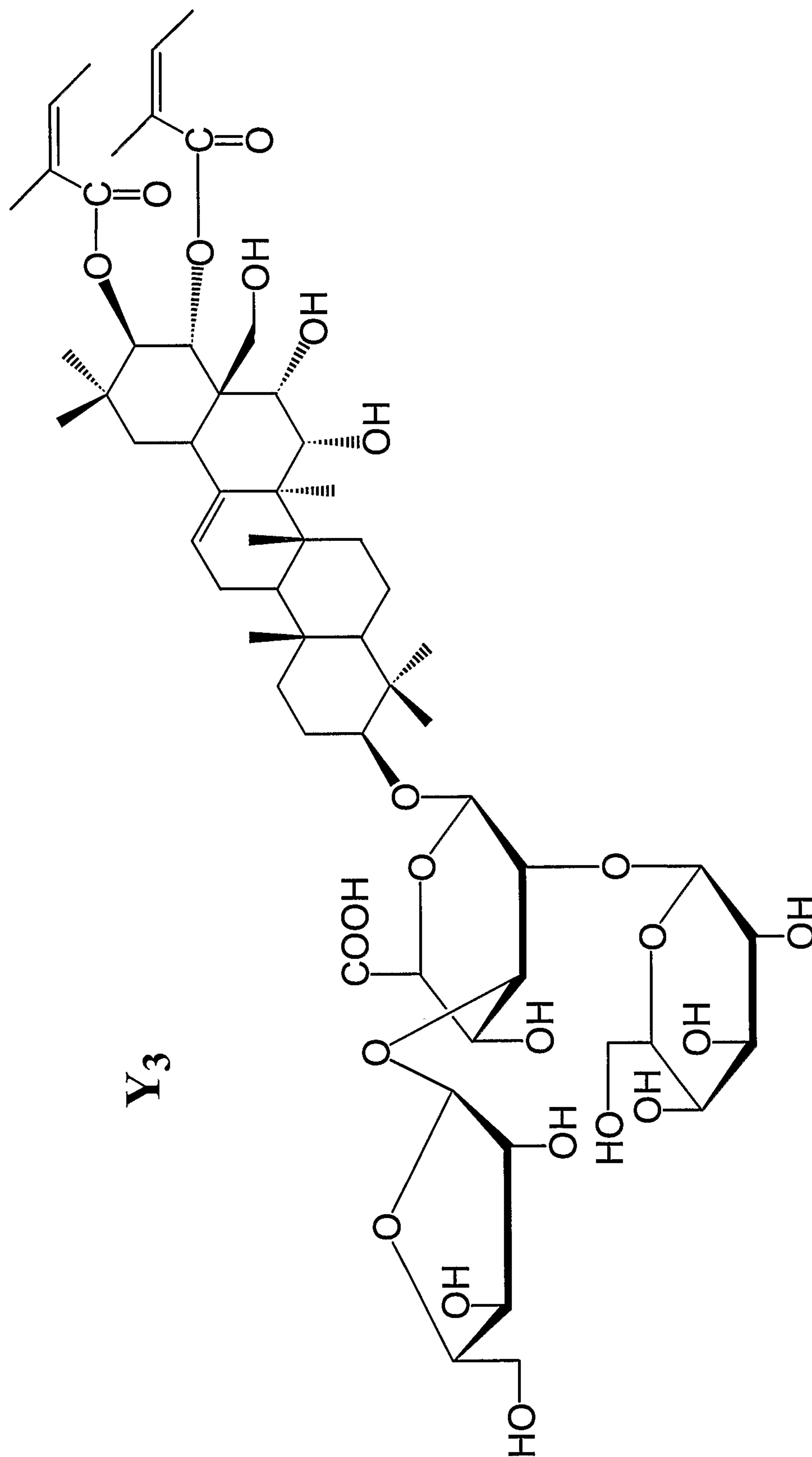
18/77

Figure 16C

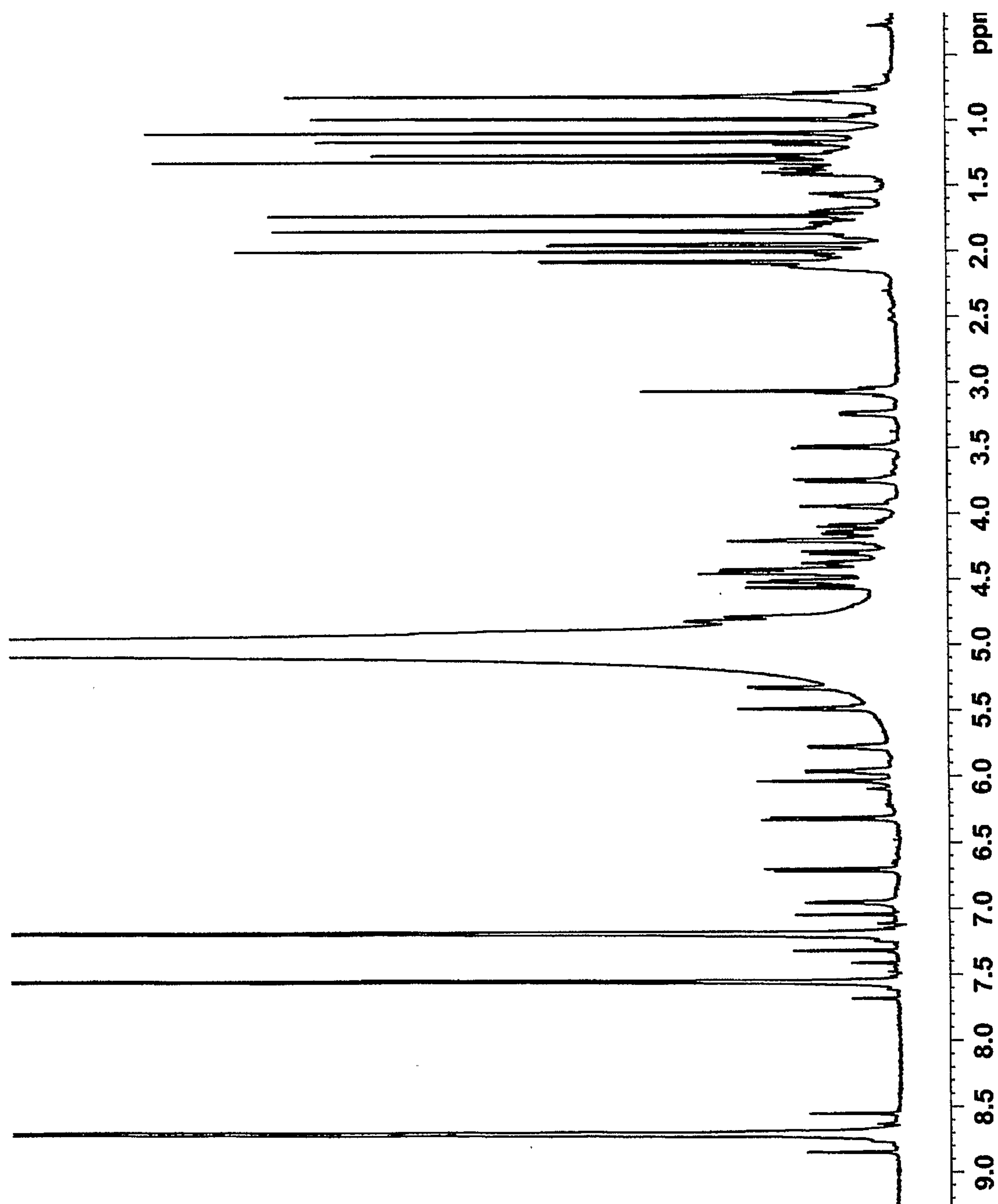
Growth curve of cancer cells after treatment with extract



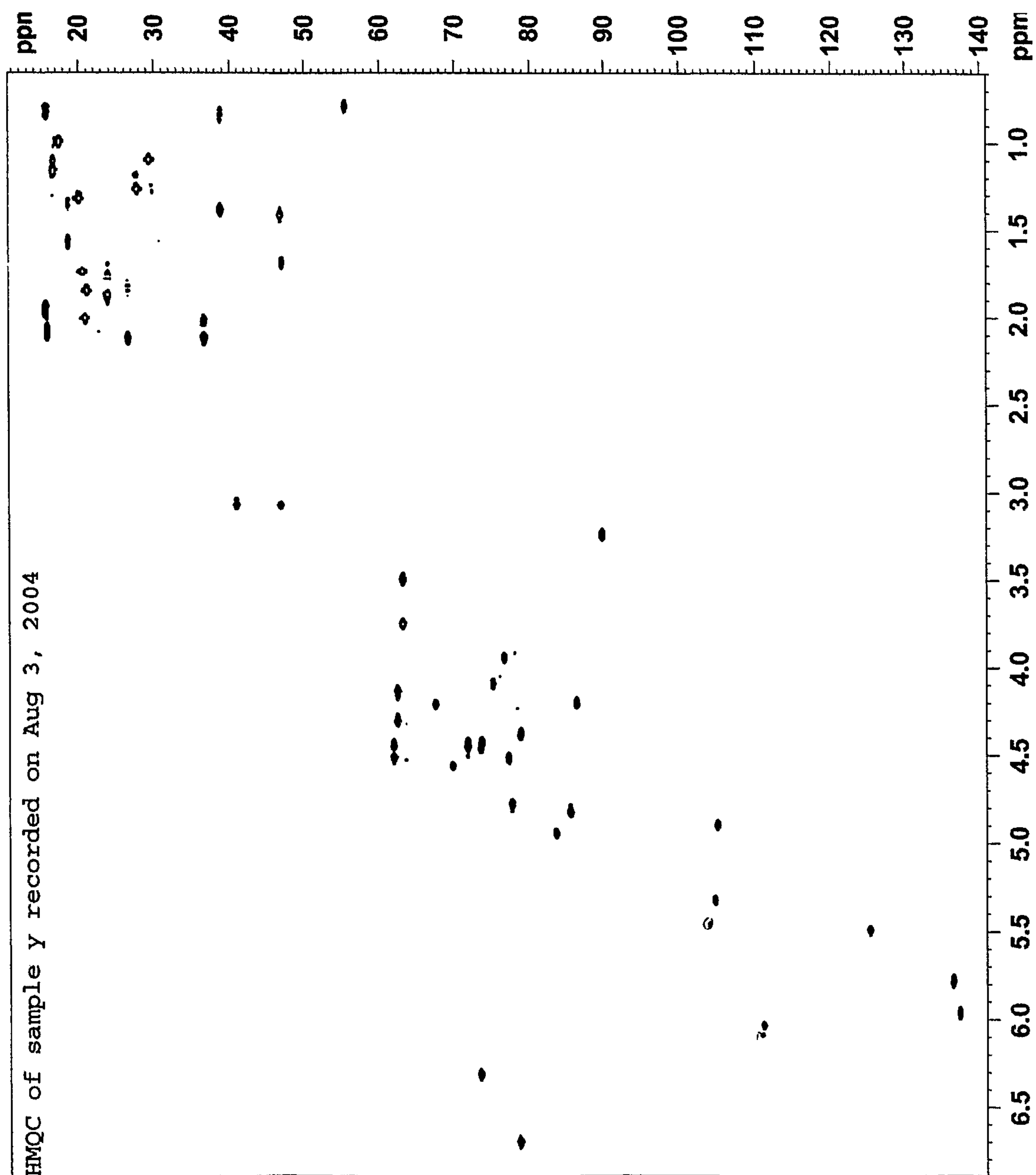
19/77
Figure 17
Chemical Structure of compound Y (Y3)



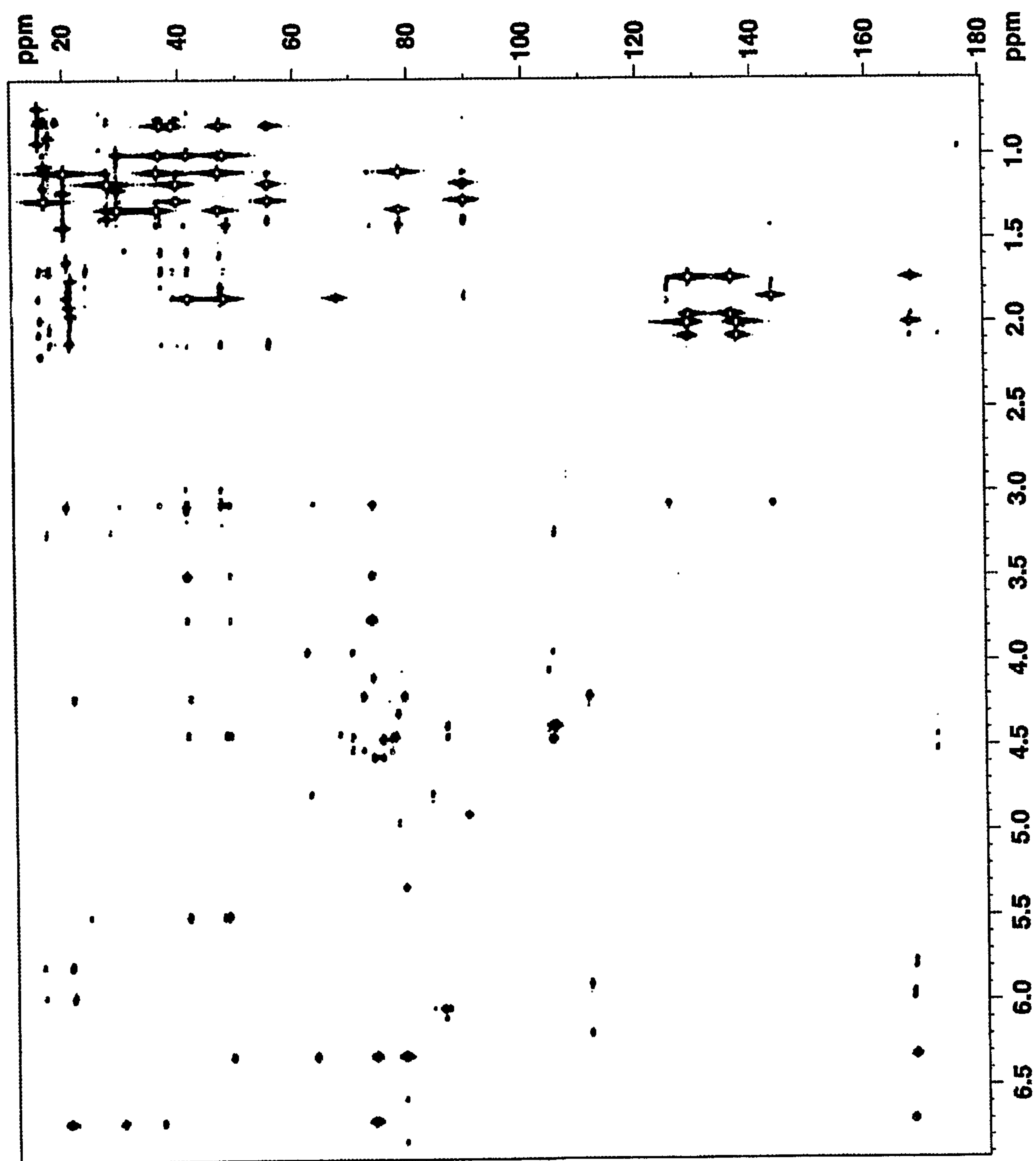
20/77
Figure 18
Proton NMR of Y



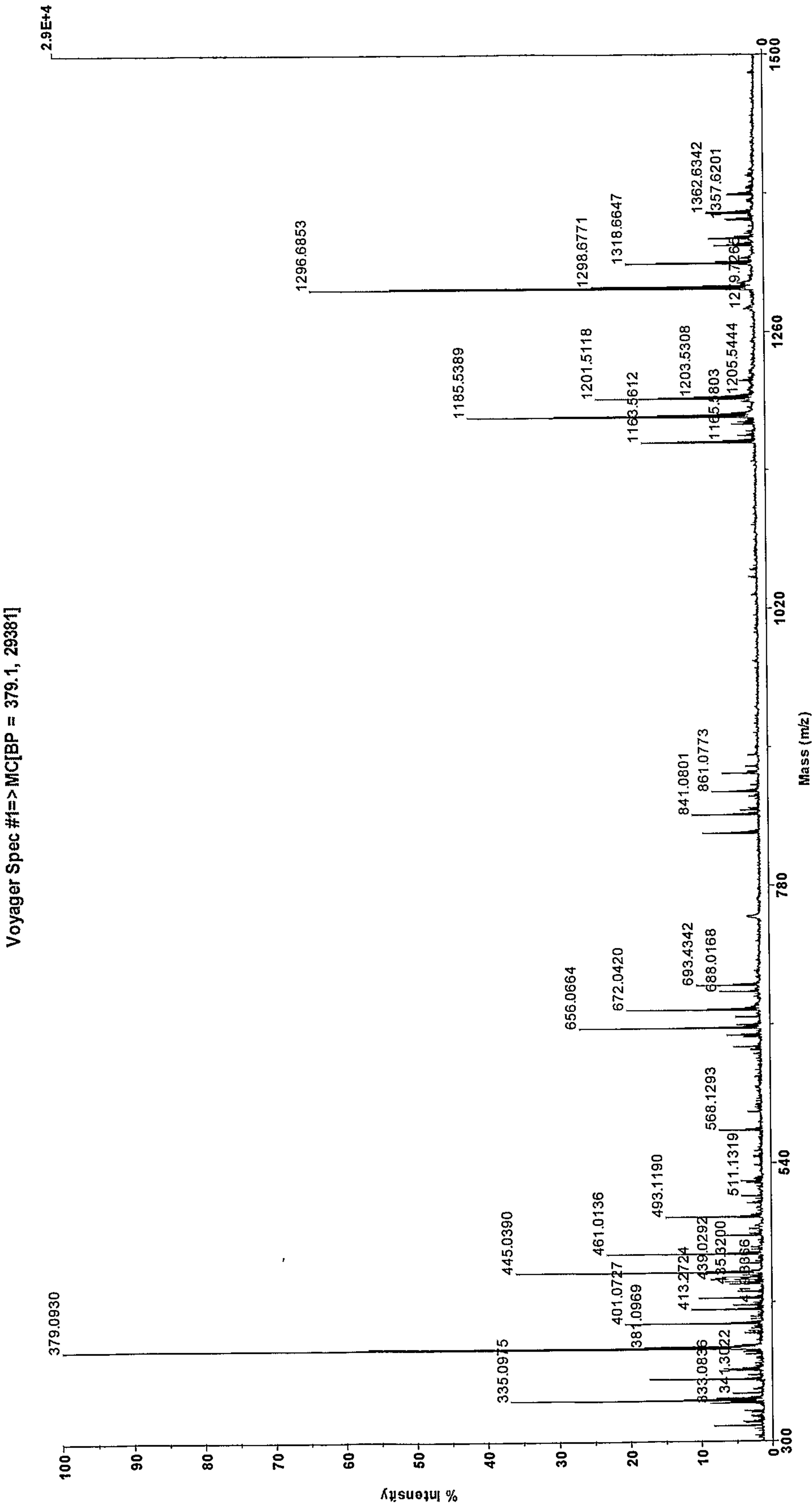
21/77
Figure 19
2D NMR (HMQC) of Y

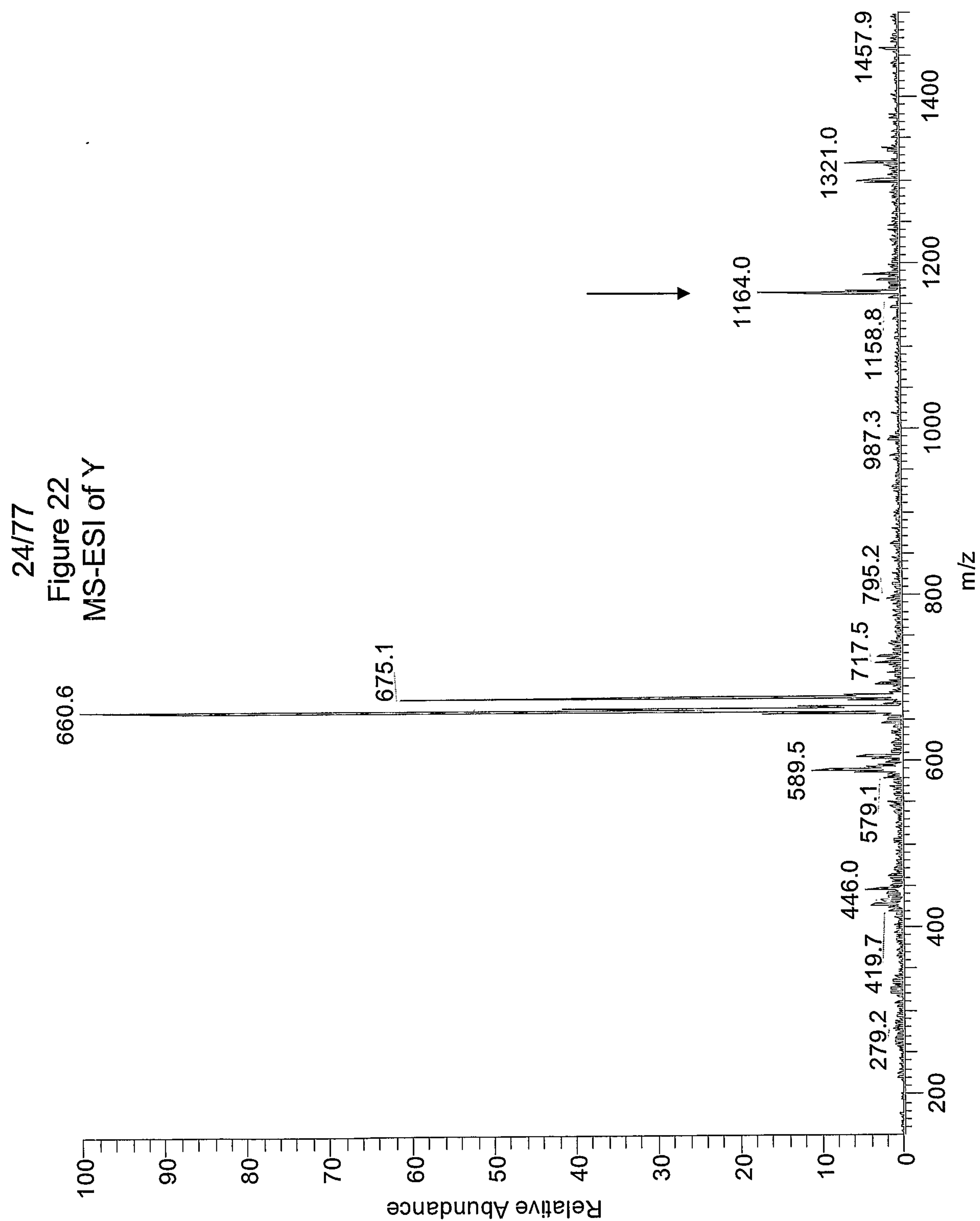


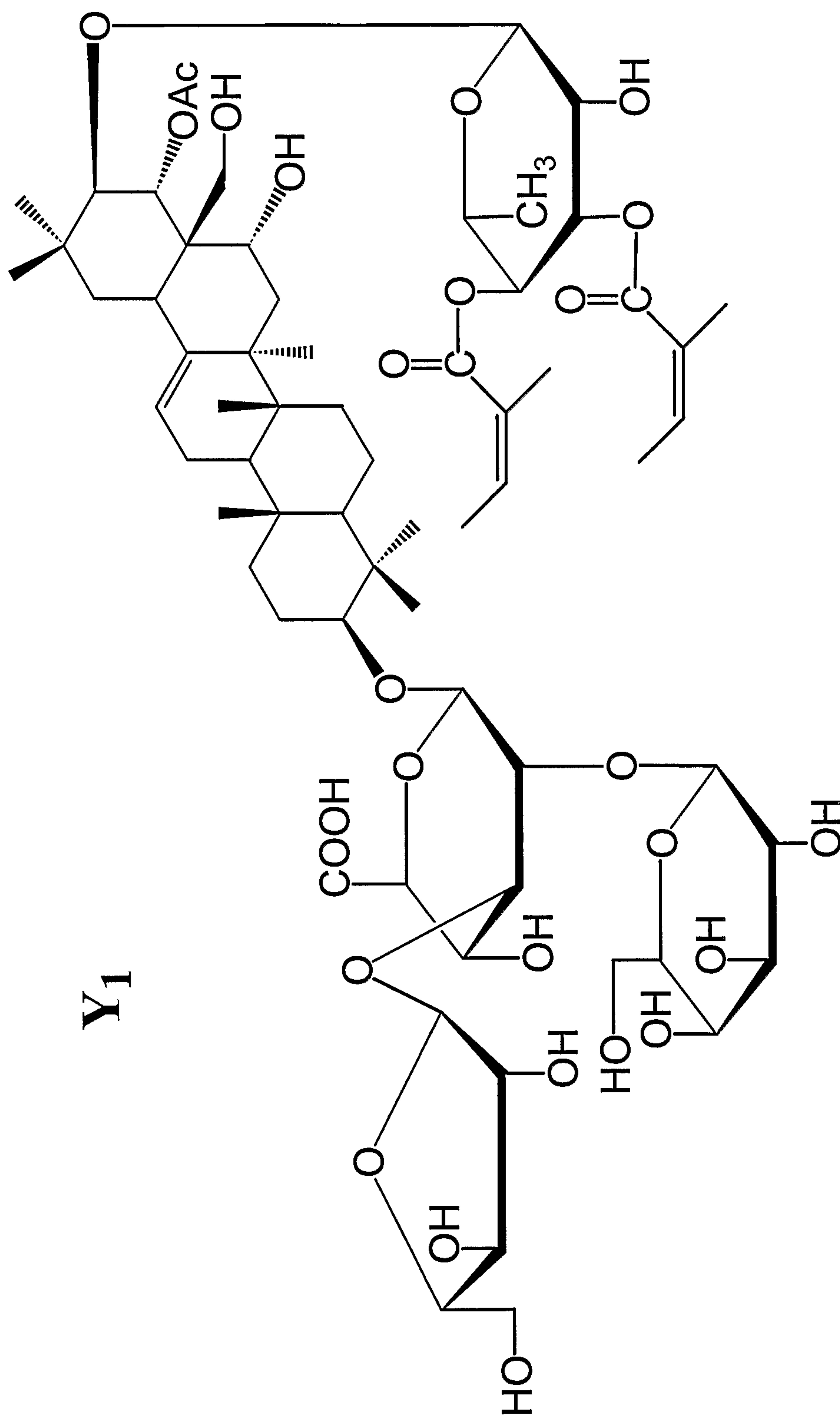
22/77
Figure 20
HMBC of Y



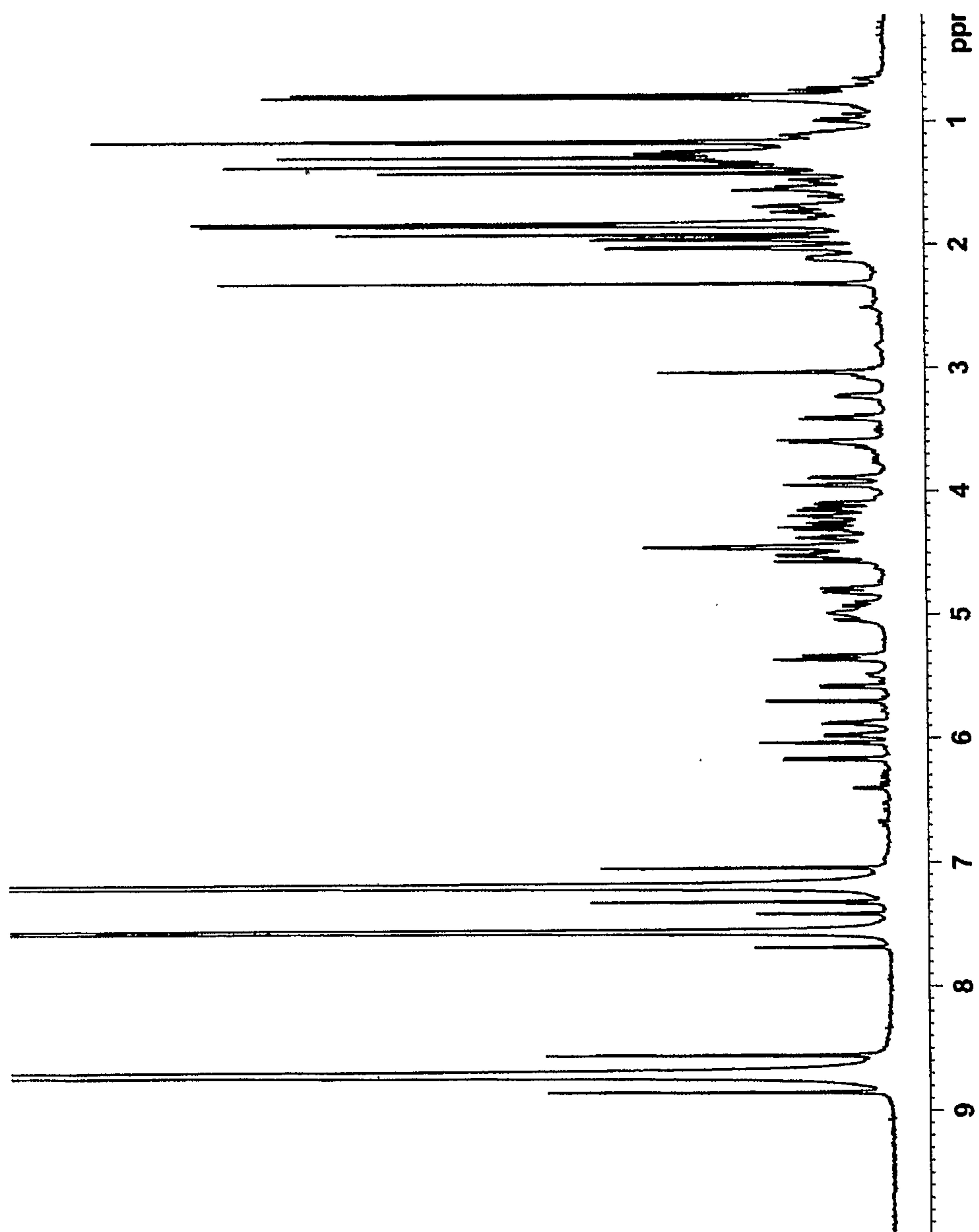
23/77
Figure 21
MS (MALDI-TOF) of Y
Y + Matrix (CHCA) + Angiotensin 1 “two point Calibration”



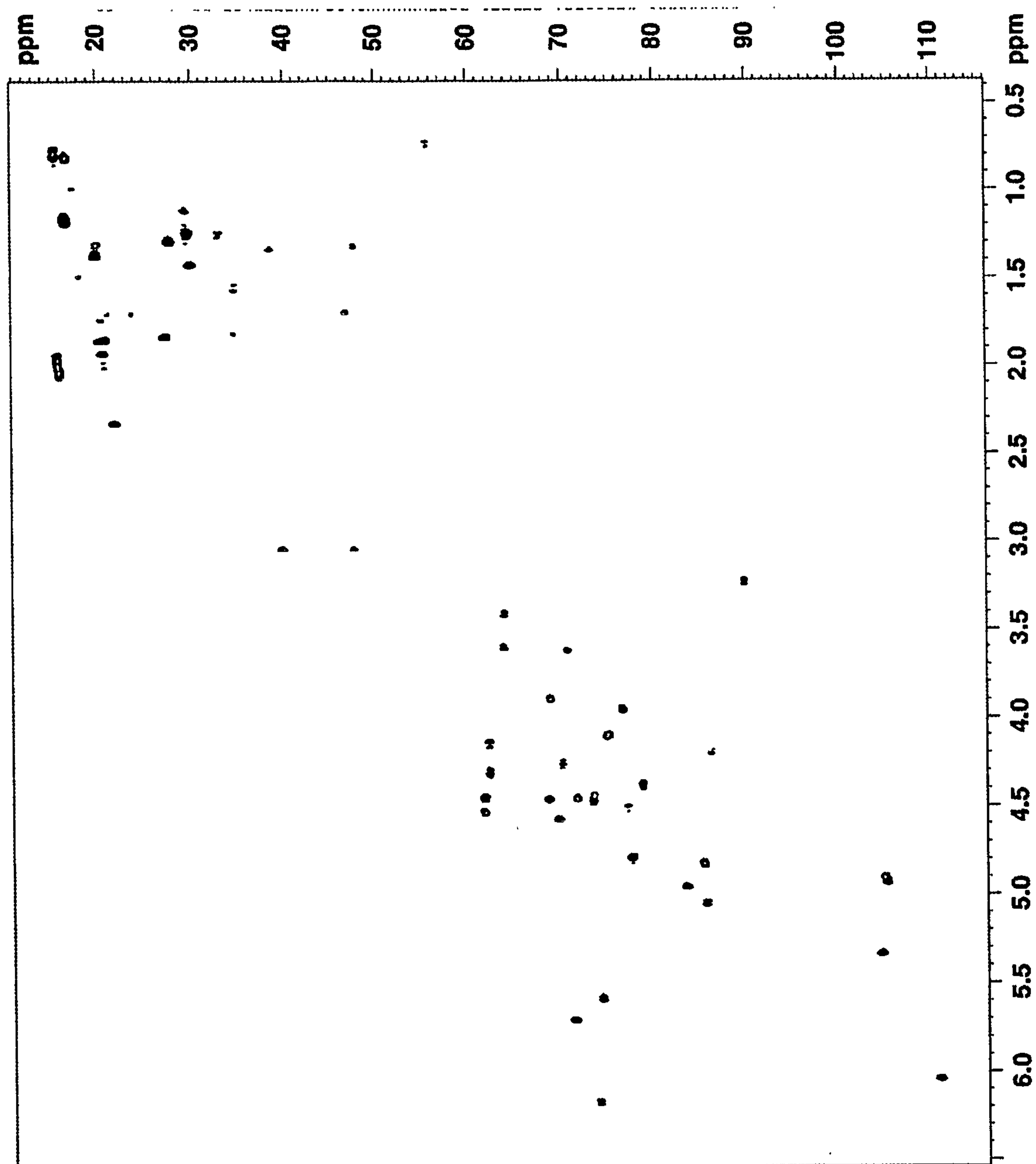




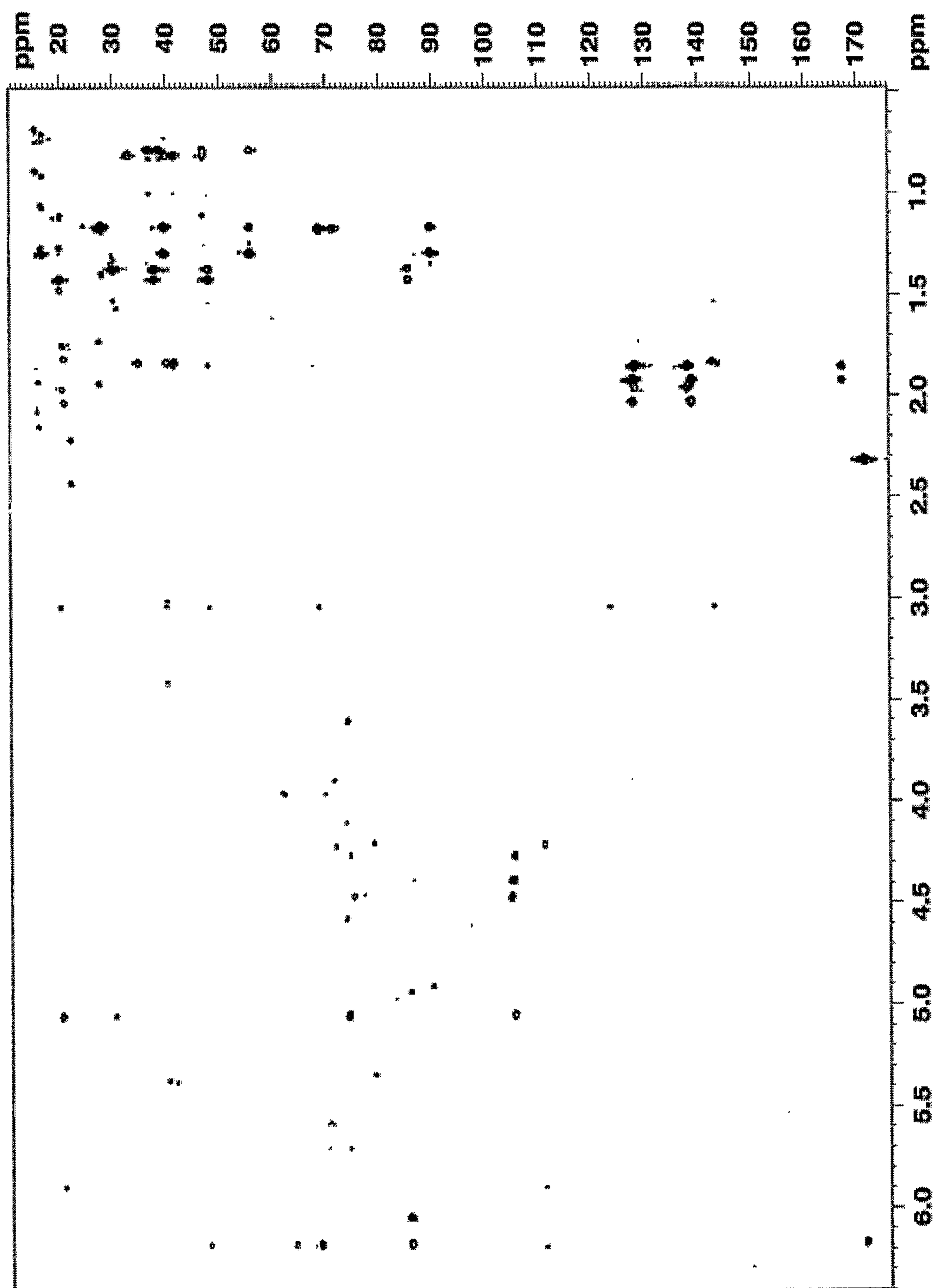
26/76
Figure 24
Proton NMR of Y1



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Figure 25
HMQC of Y1



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Figure 26
HMBC of Y1



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Figure 27

2D NMR (COSY) of Y1

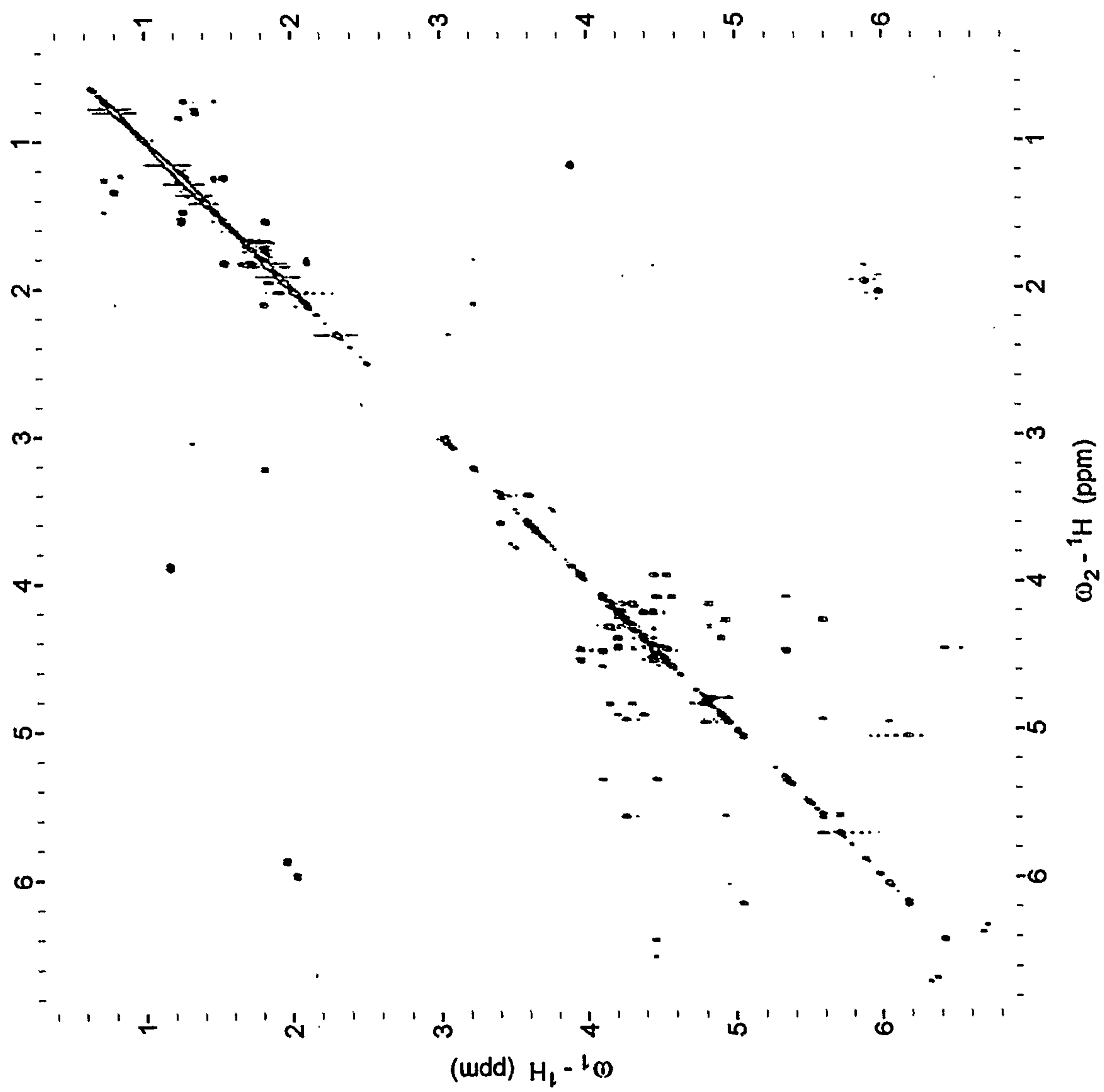
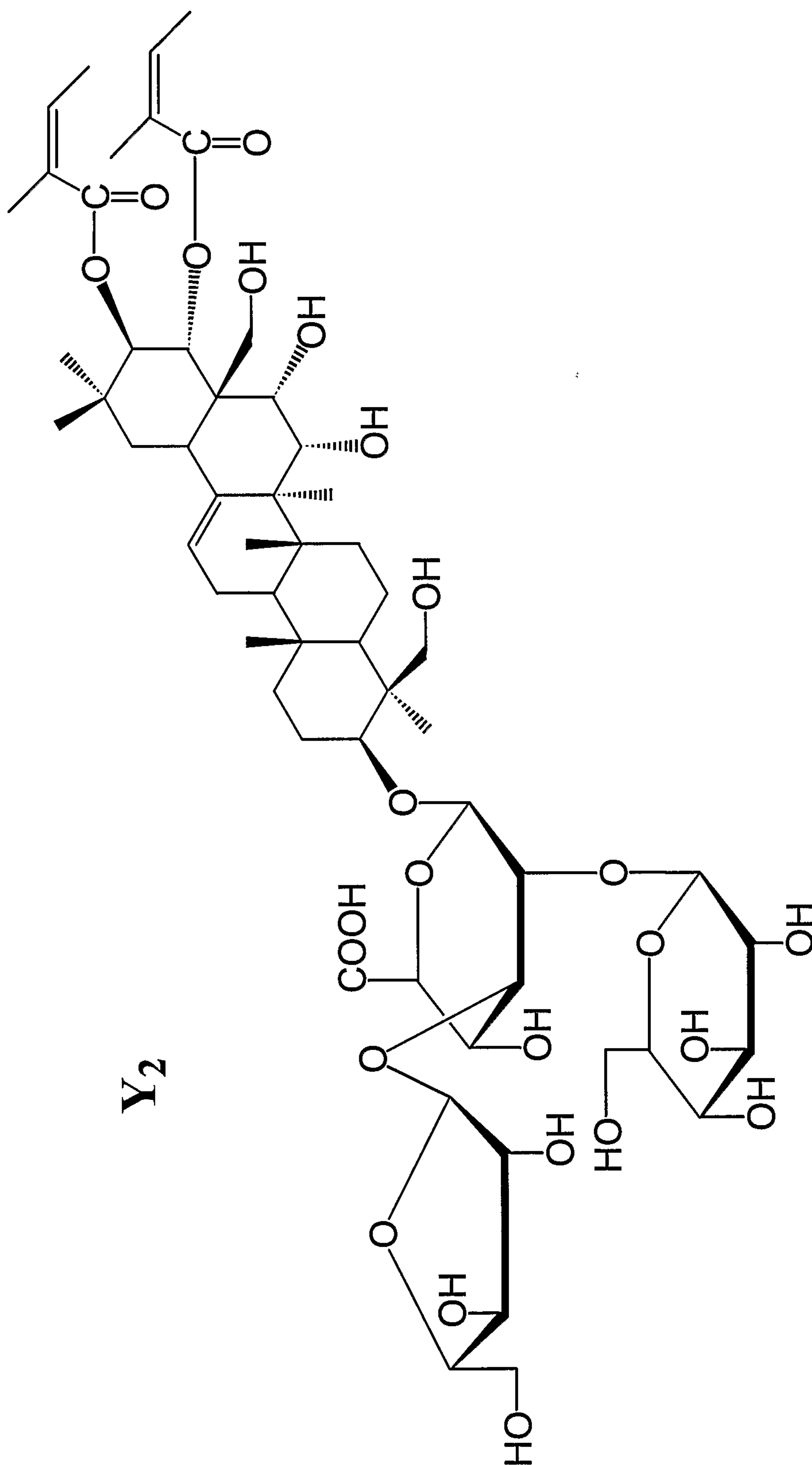
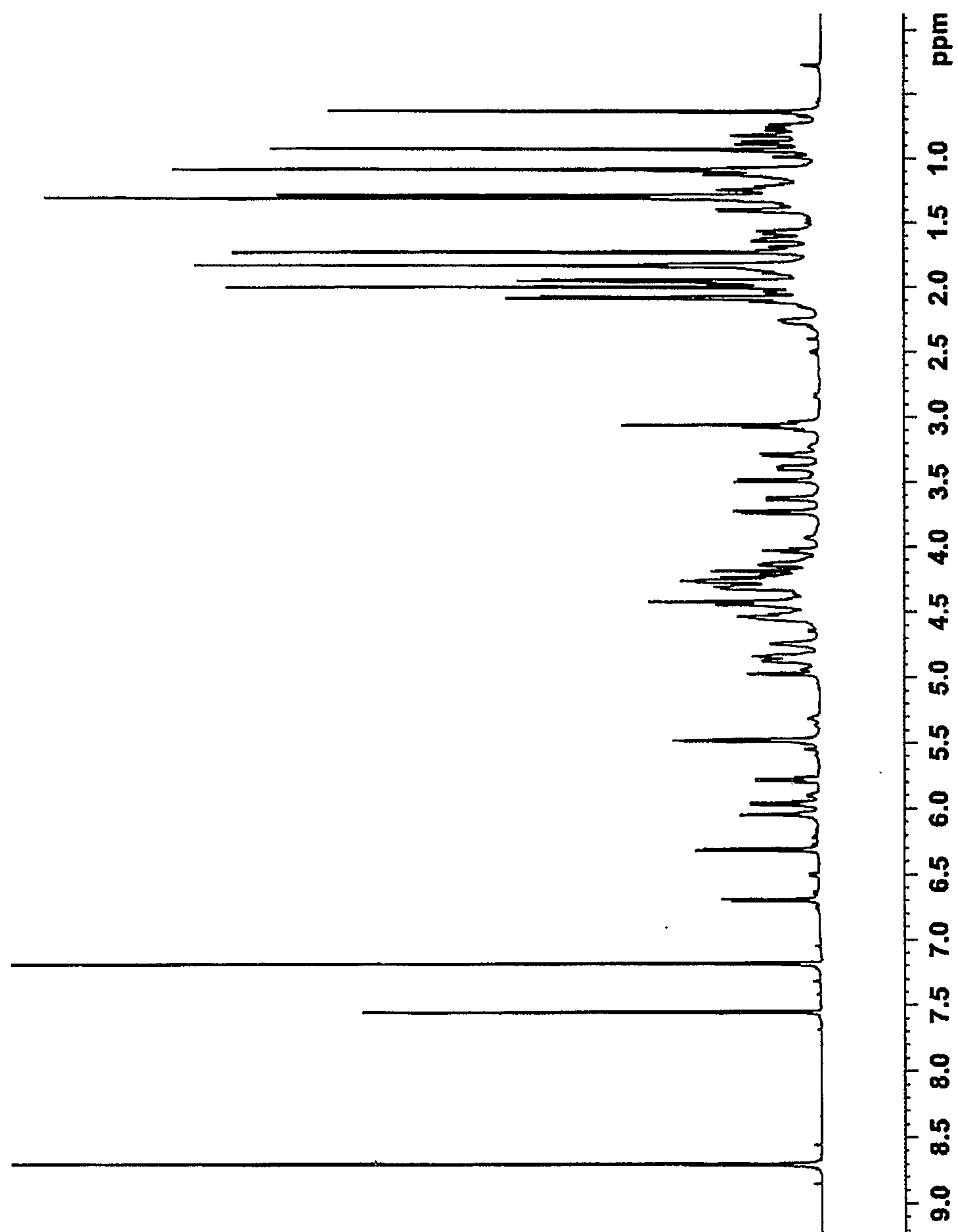


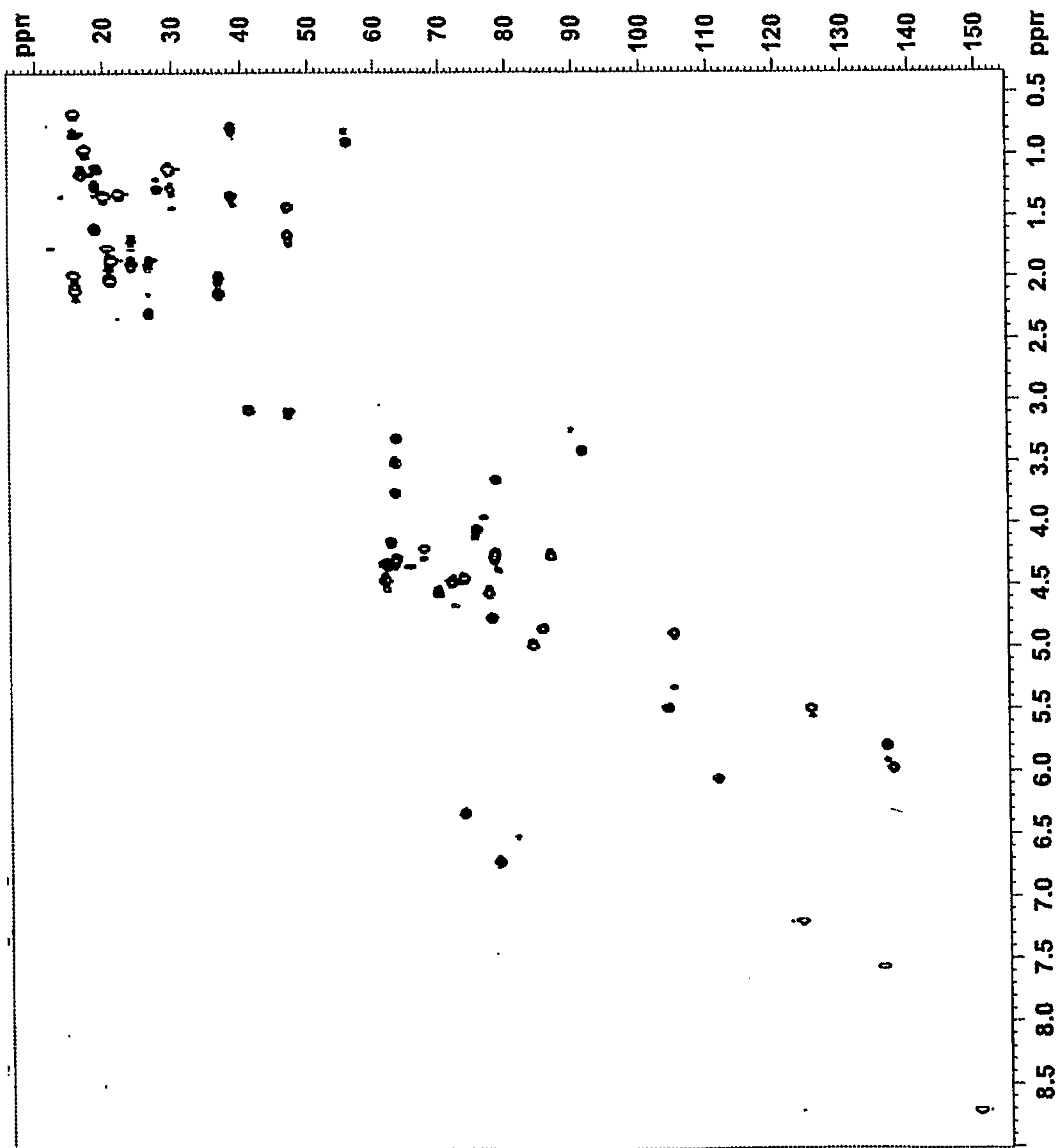
Figure 28. Chemical Structure of Y2



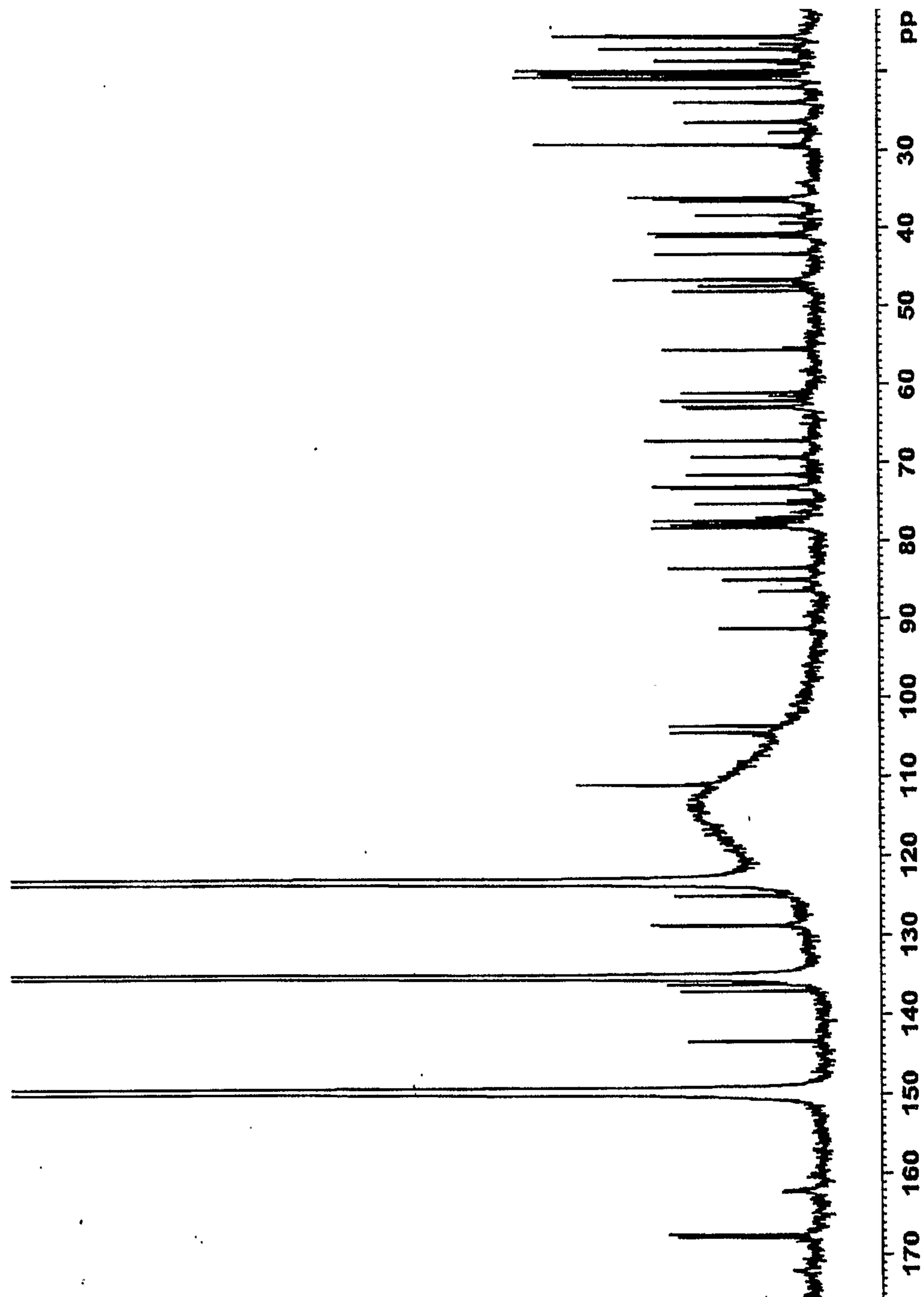
31/77
Figure 29
H-NMR Spectrum of Y2



32/77
Figure 30
Y2 HMQC-level-1



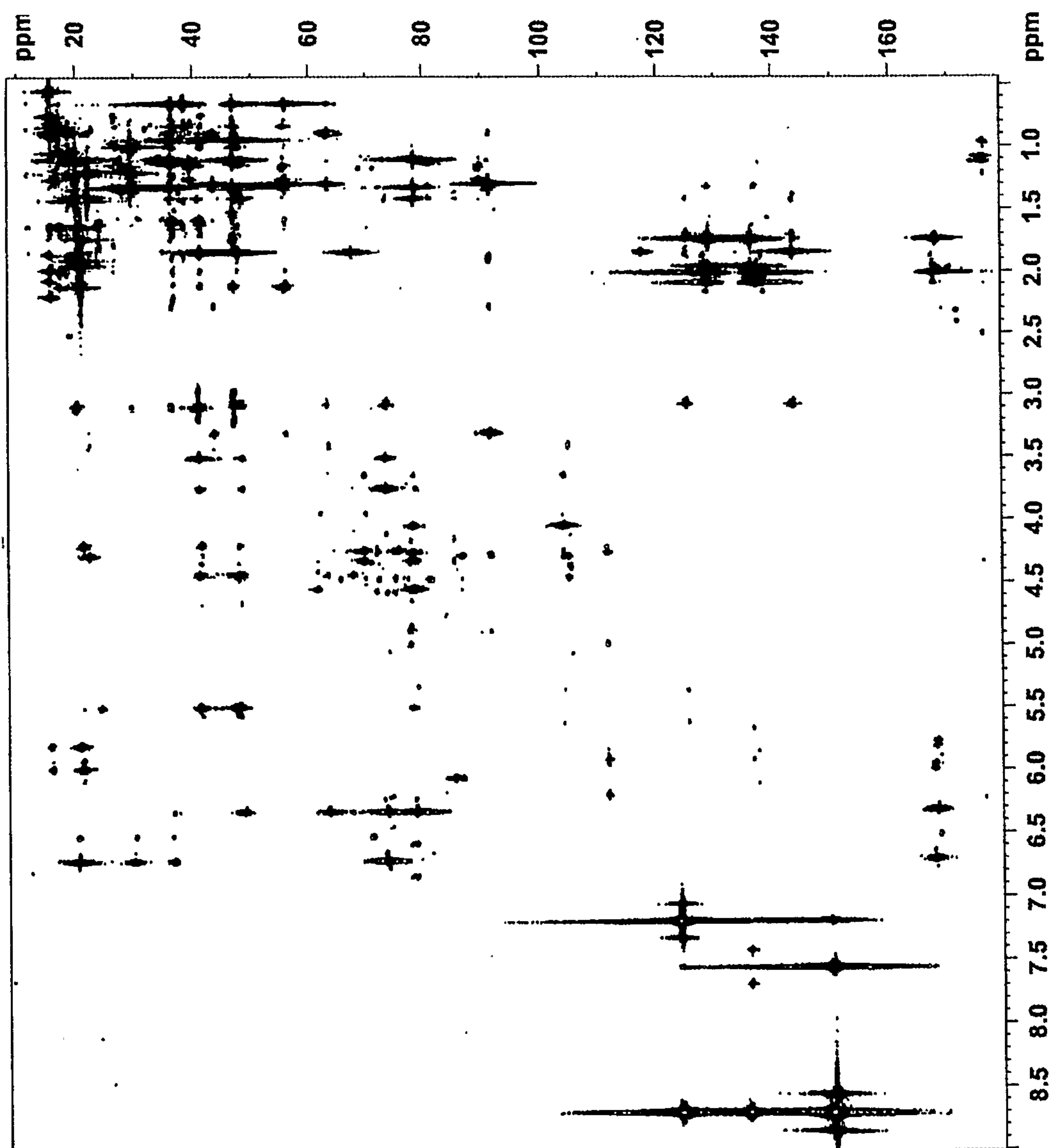
33/77
Figure 31
Y2 C13 H-NMR spectrum



34/77

Figure 32

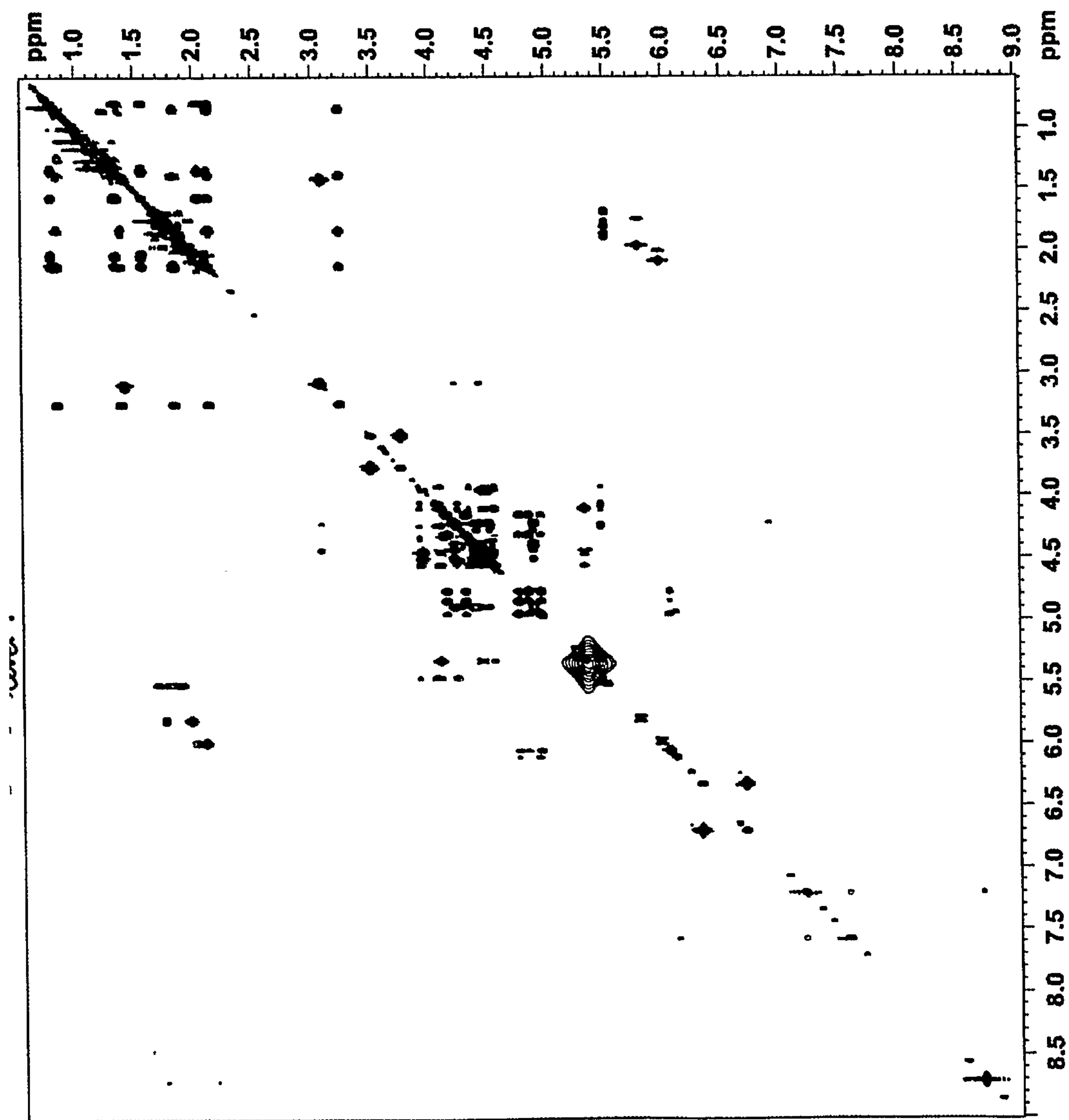
Y2-HMBC-level 1



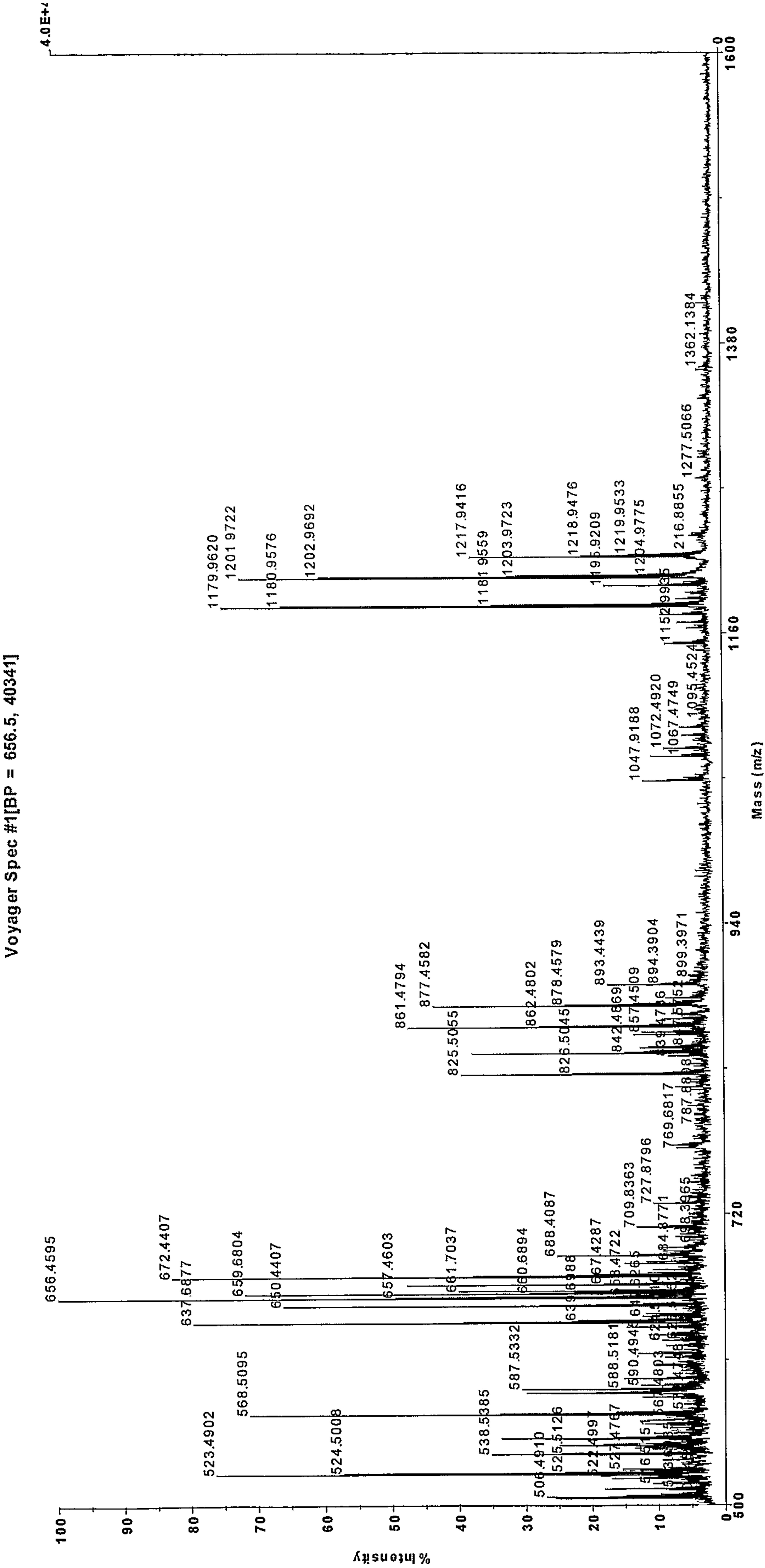
35/77

Figure 33

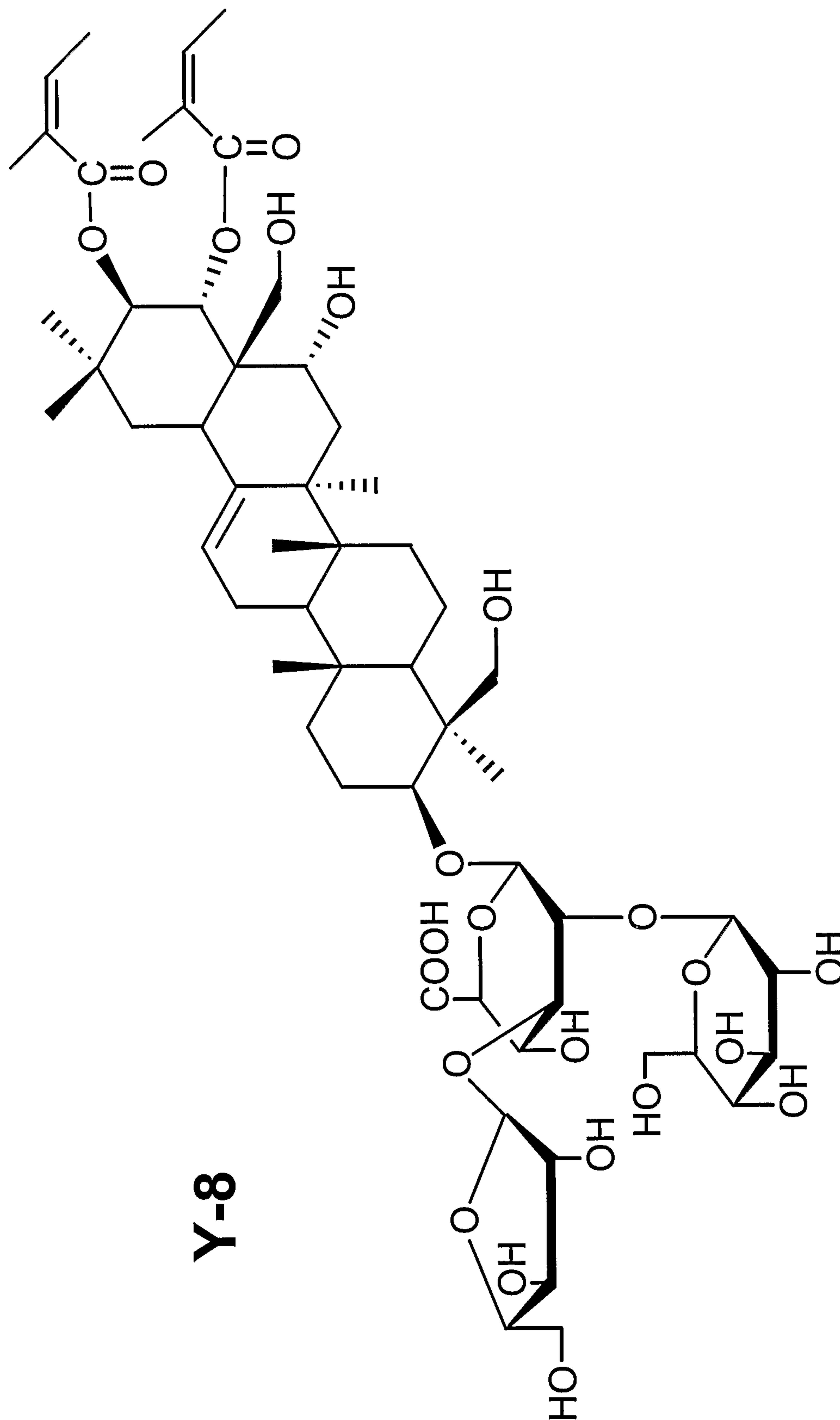
Y2-HOHAHA-level-1



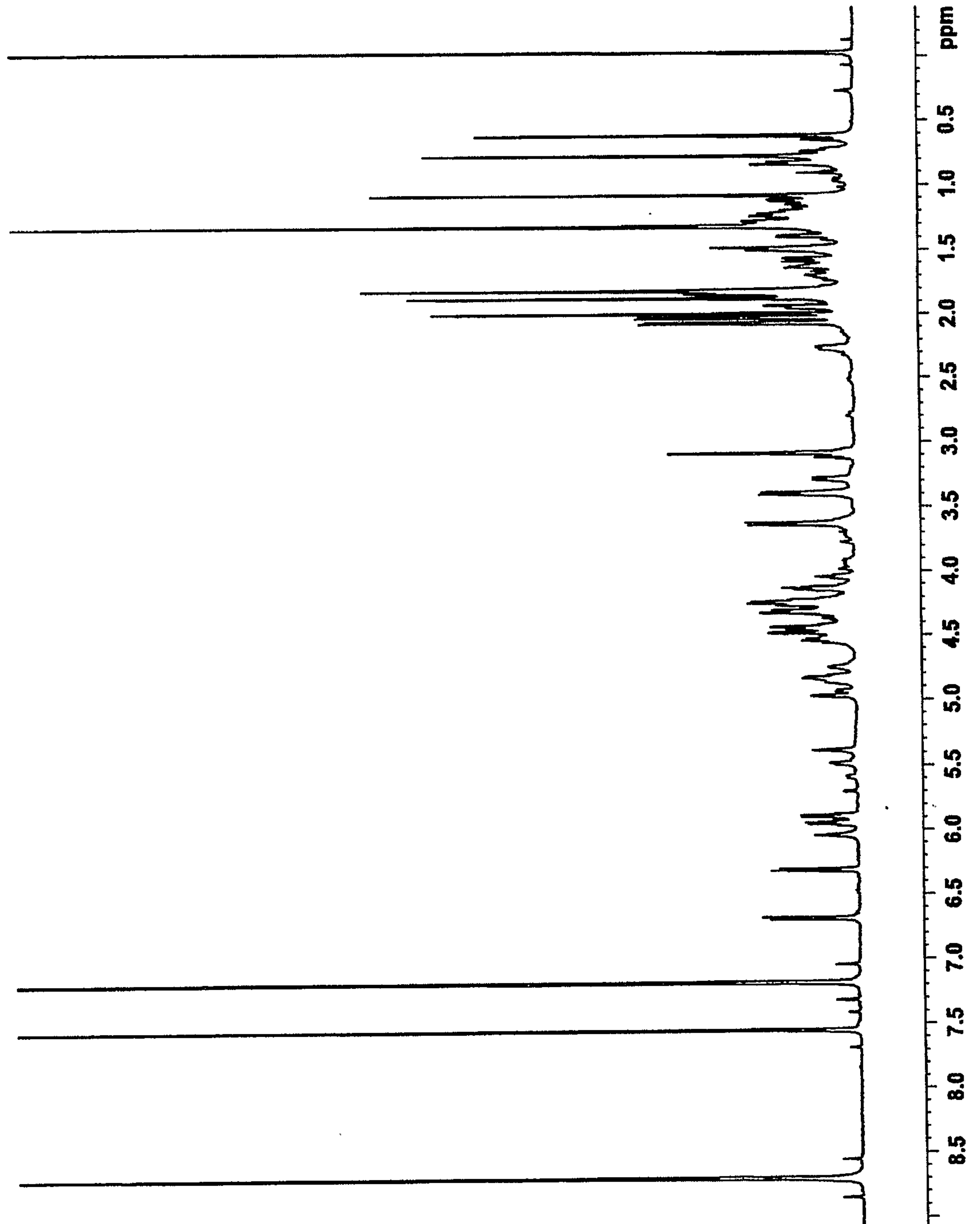
36/77
Figure 34
Mass spectrum of Y2 + Matrix + Standards



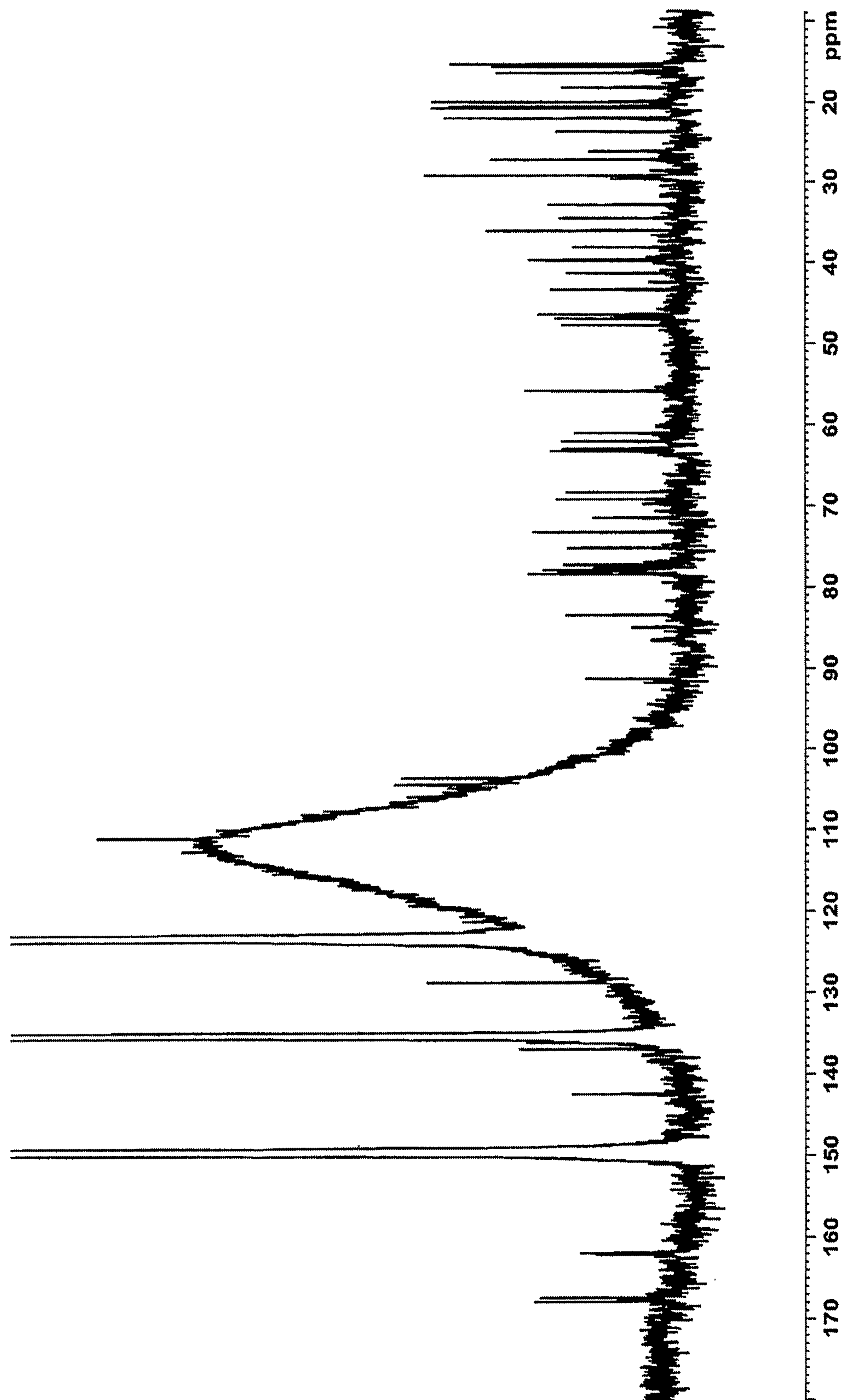
37/77
Figure 35
Structure of Y8



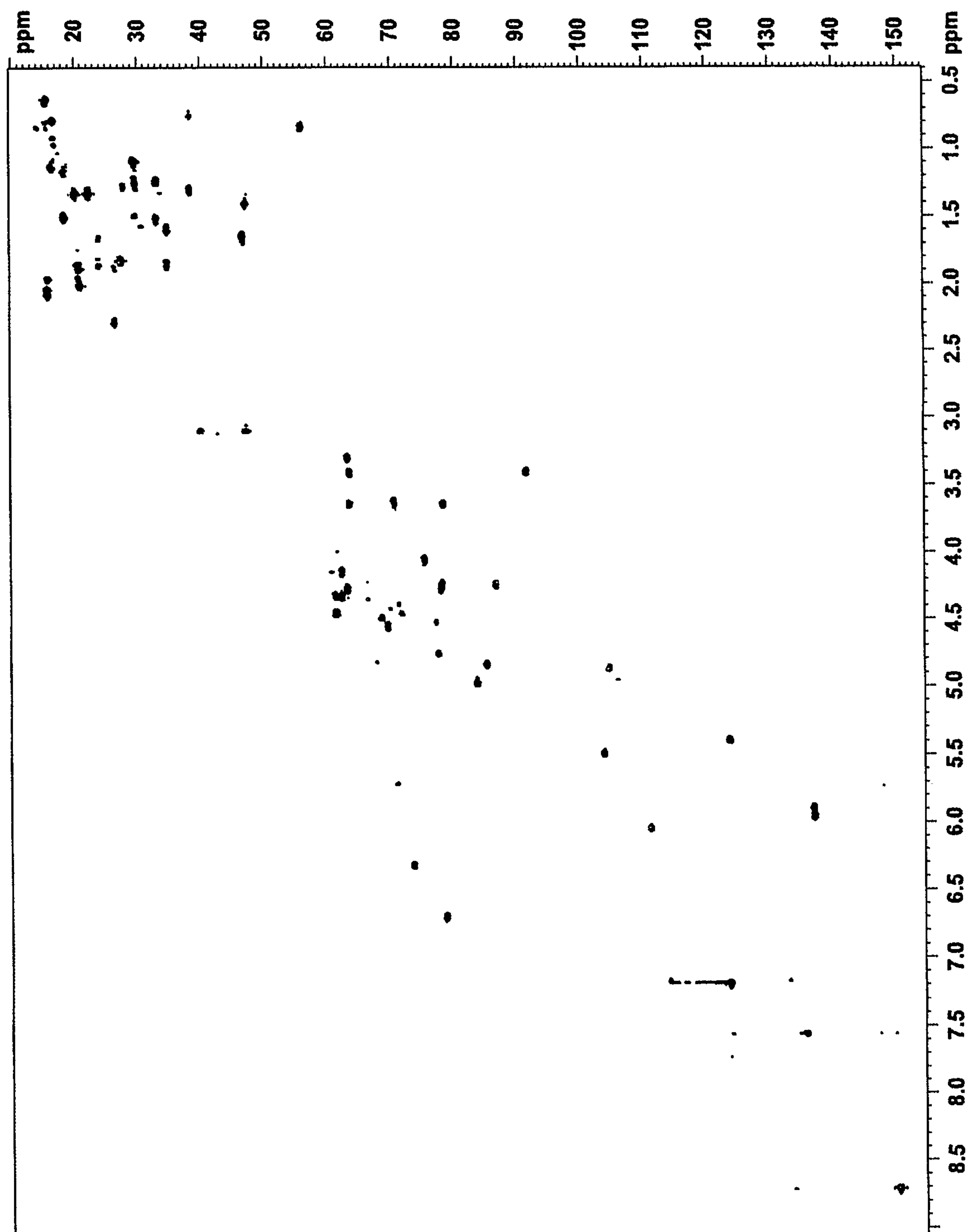
38/77
Figure 36
Y8-H-NMR-full



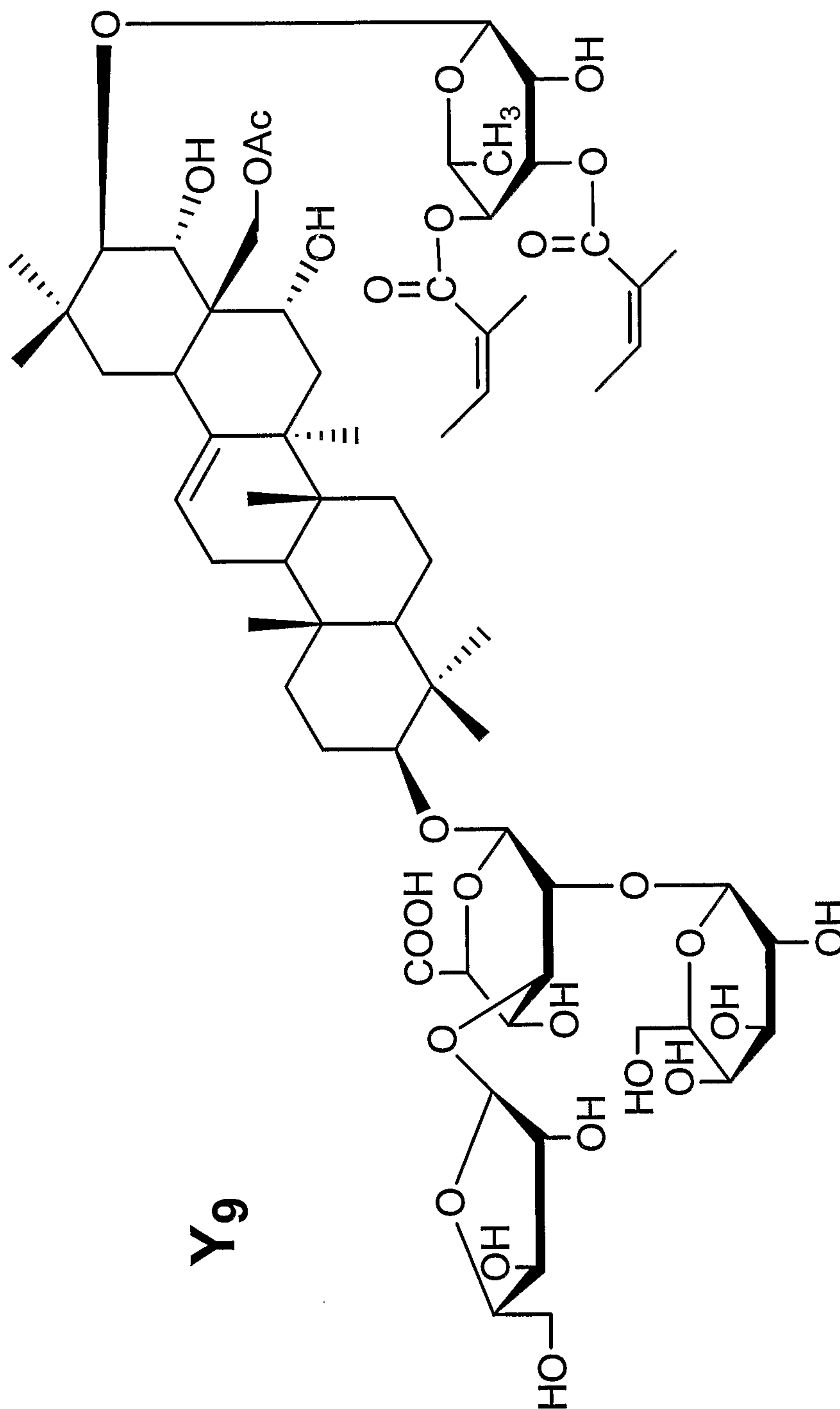
39/77
Figure 37
Y8-C13 NMR



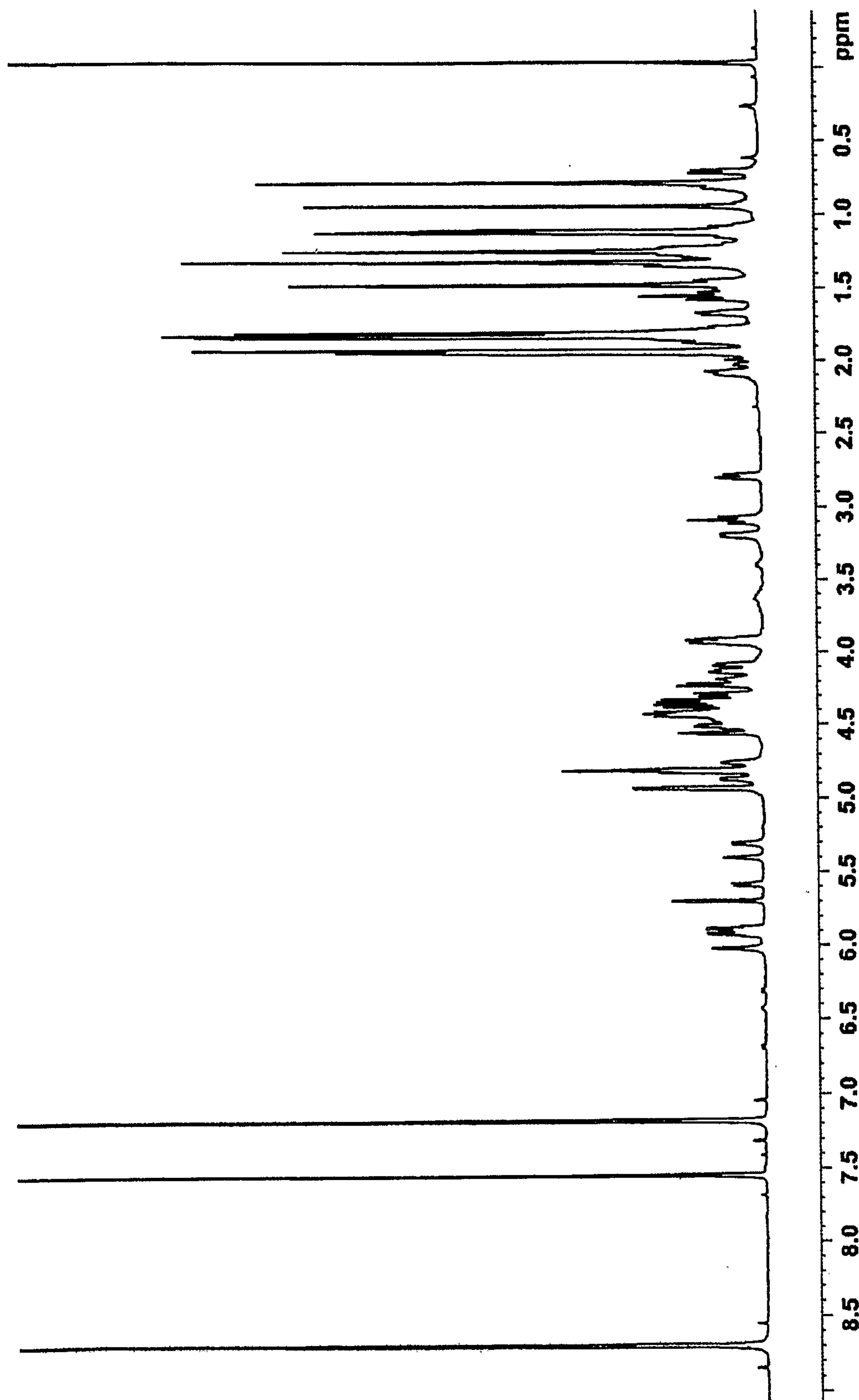
40/77
Figure 38
Y8-HMQC-level-1



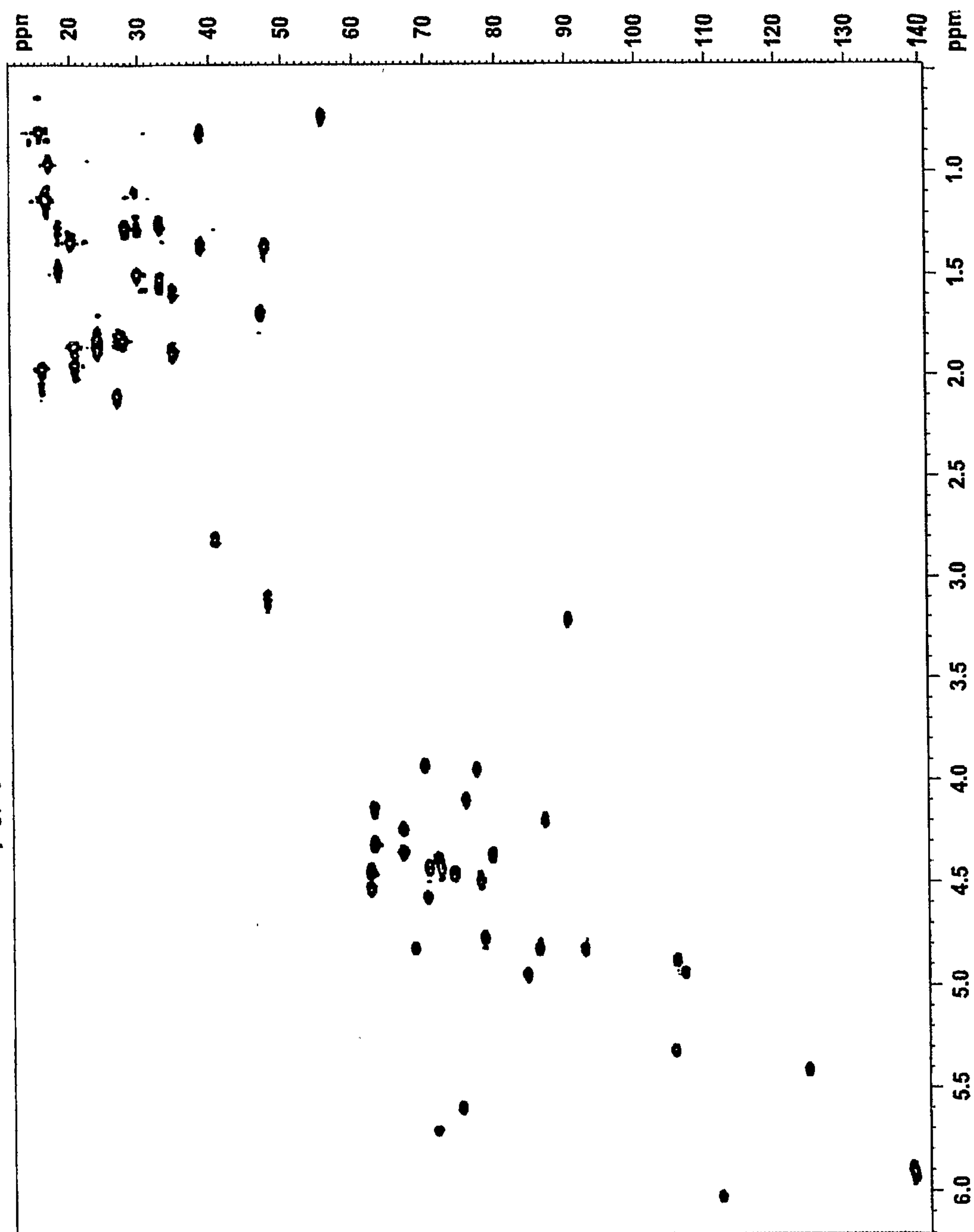
9Y



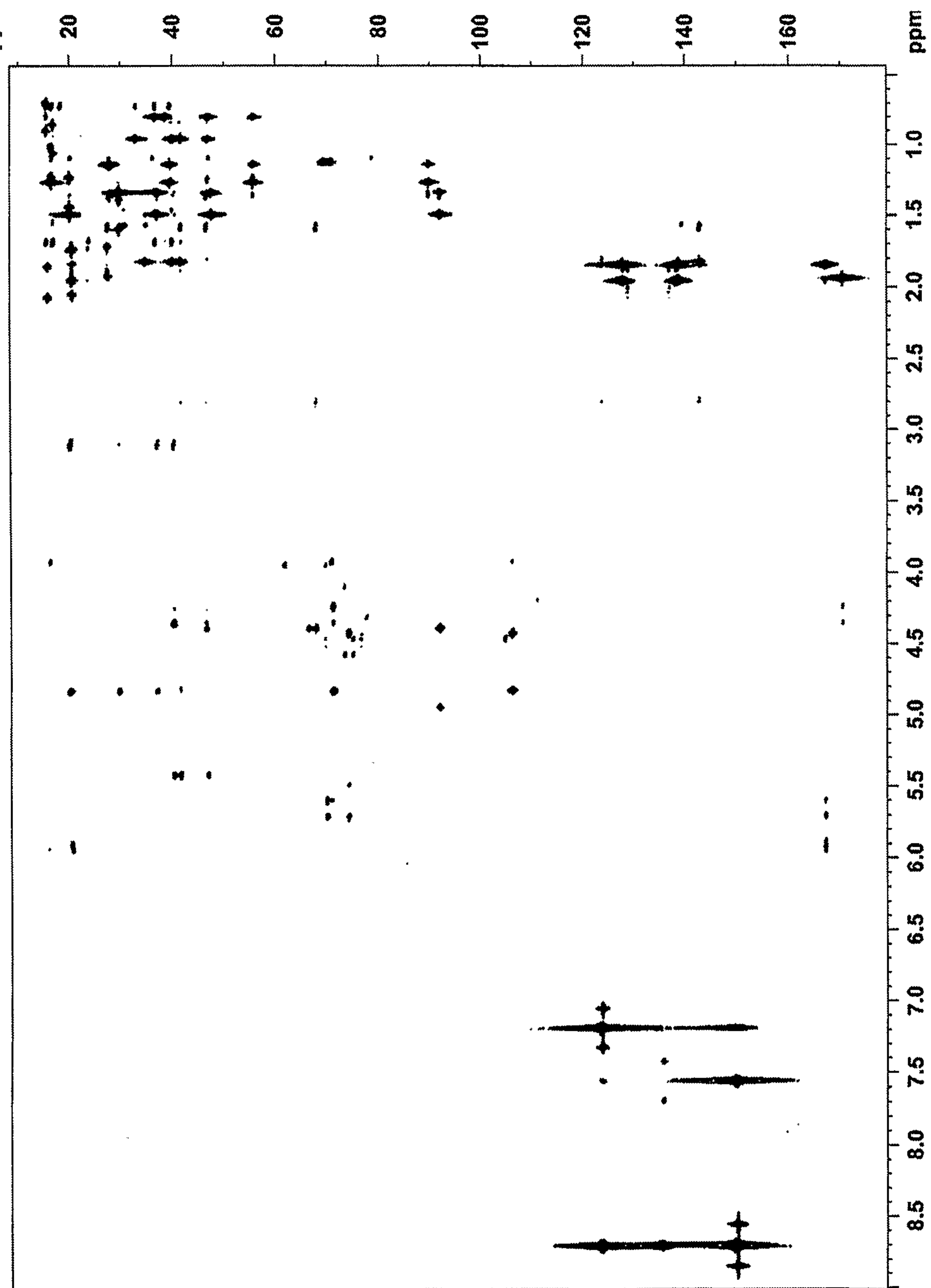
42/77
Figure 40
Y9-H-NMR



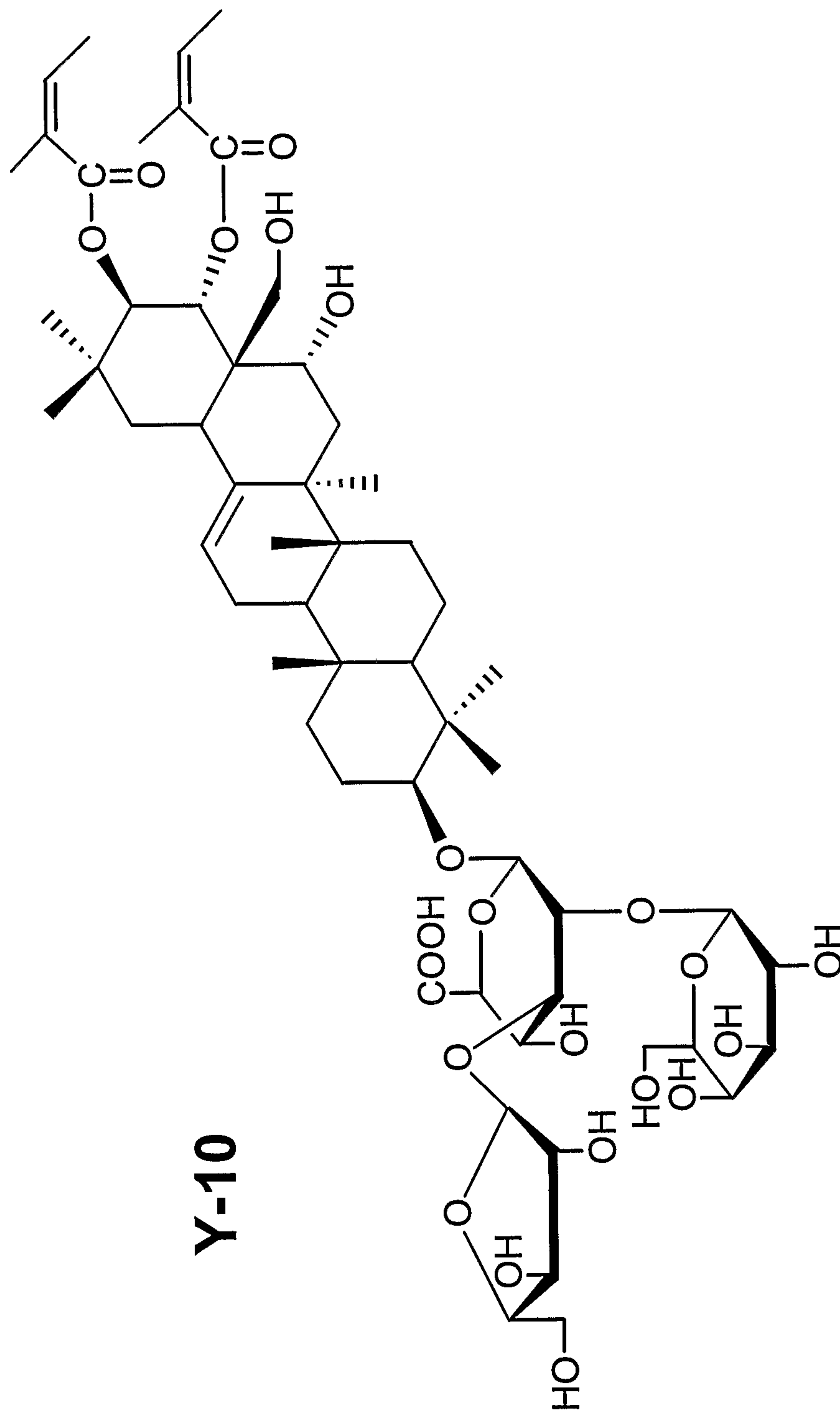
43/77
Figure 41
Y9-HMQC-level-1



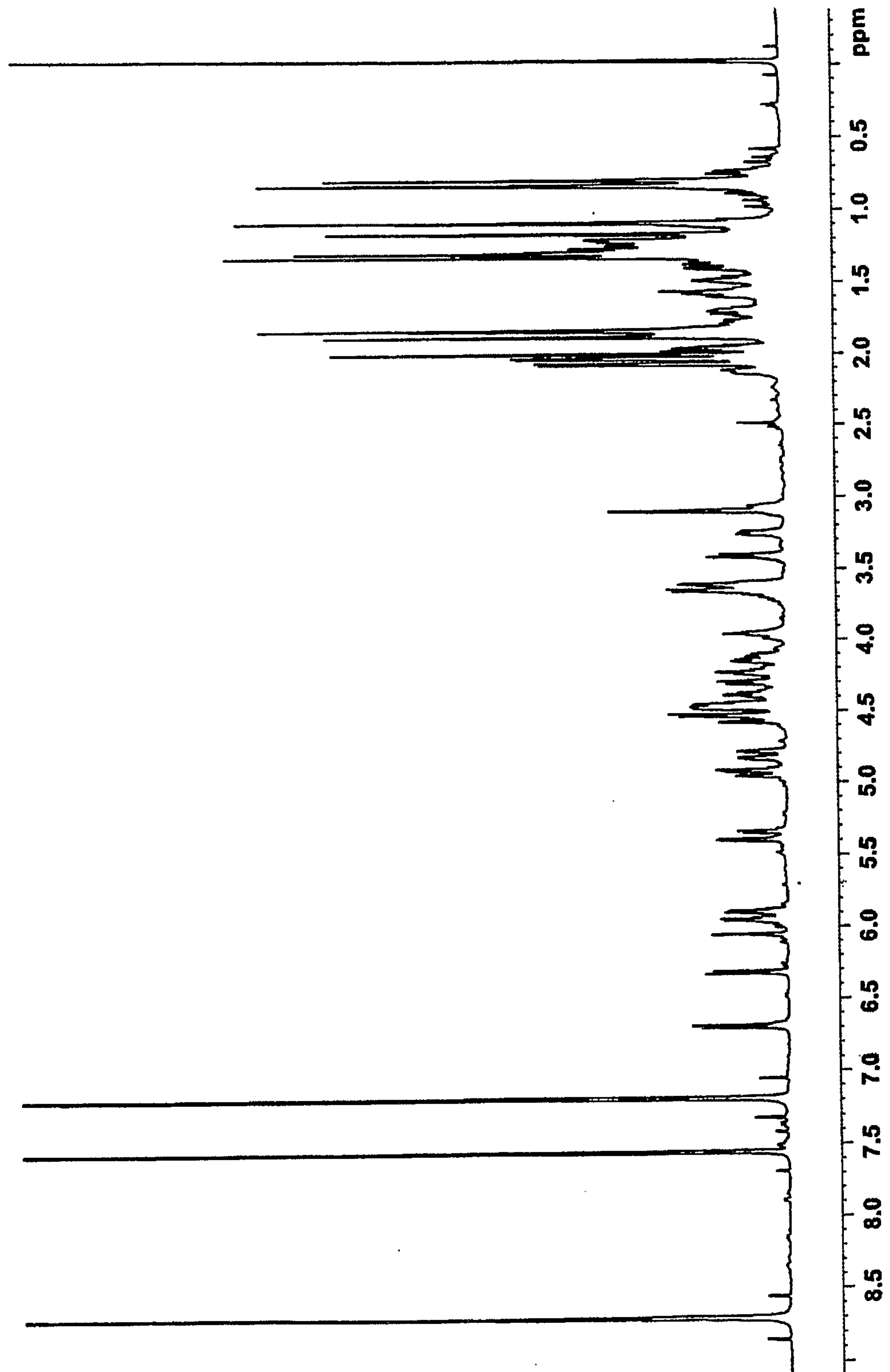
44/77
Figure 42
Y9 2D NMR (HMBC)



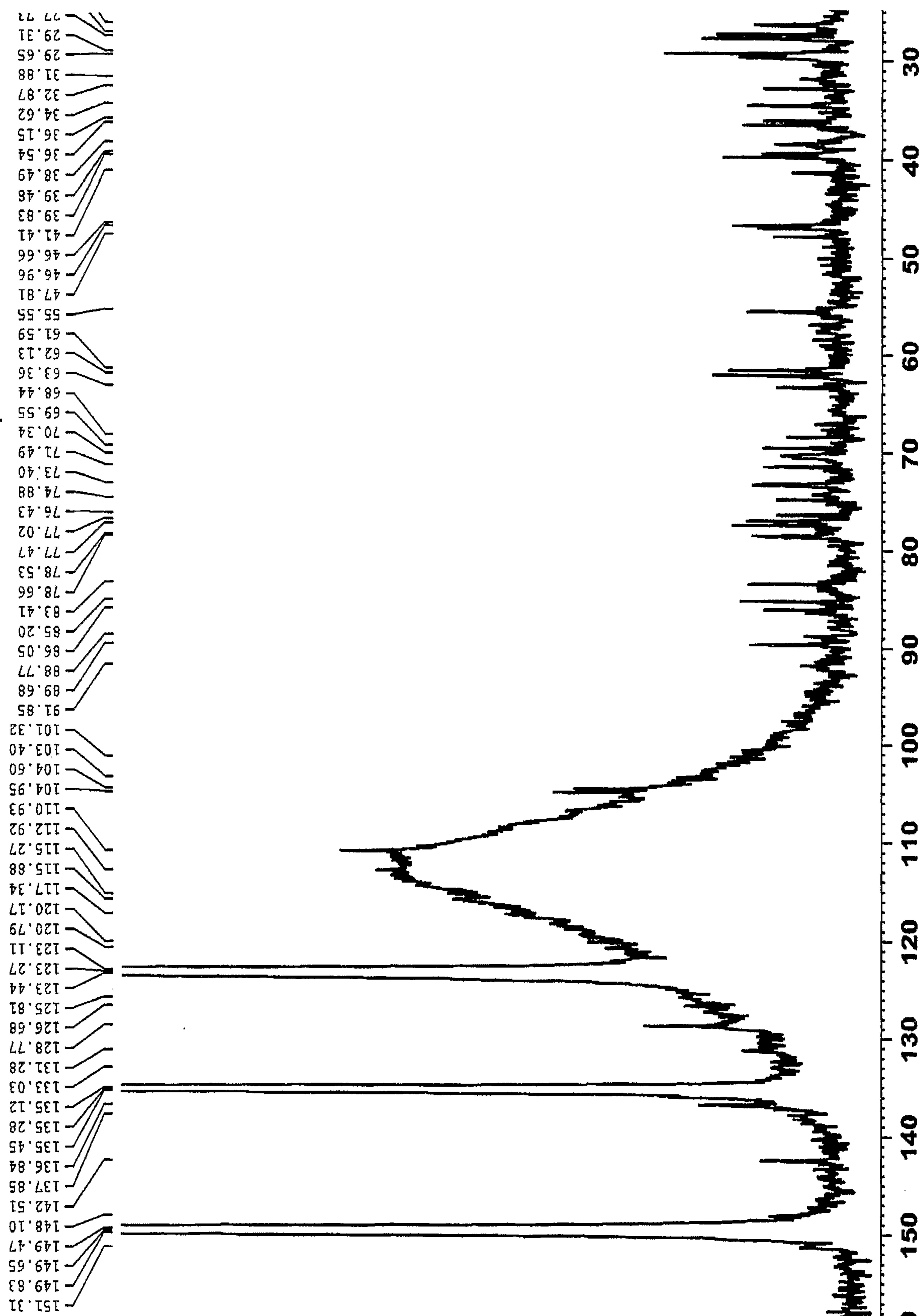
45/77
Figure 43
Structure of Y10



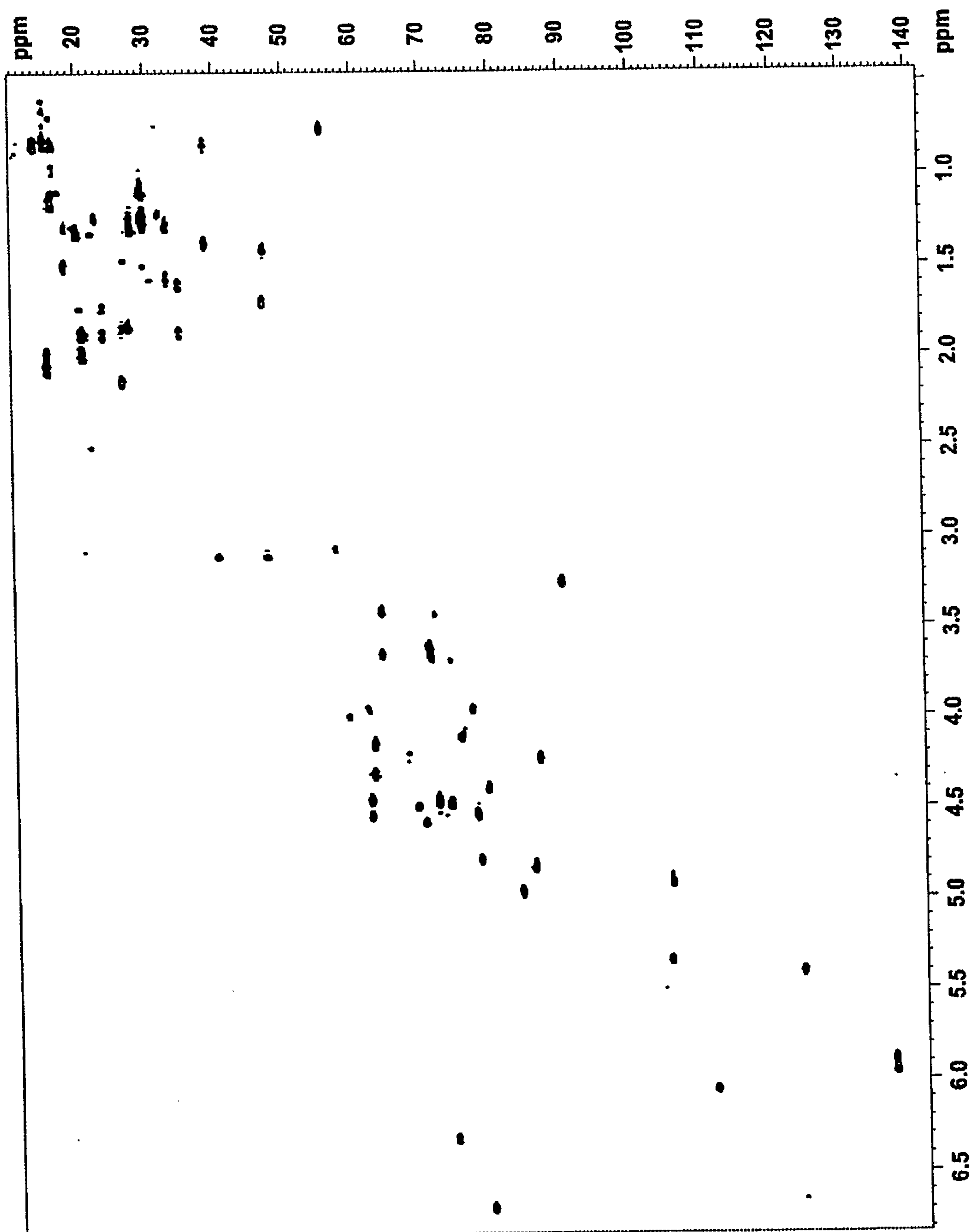
46/77
Figure 44
Y10-H-NMR



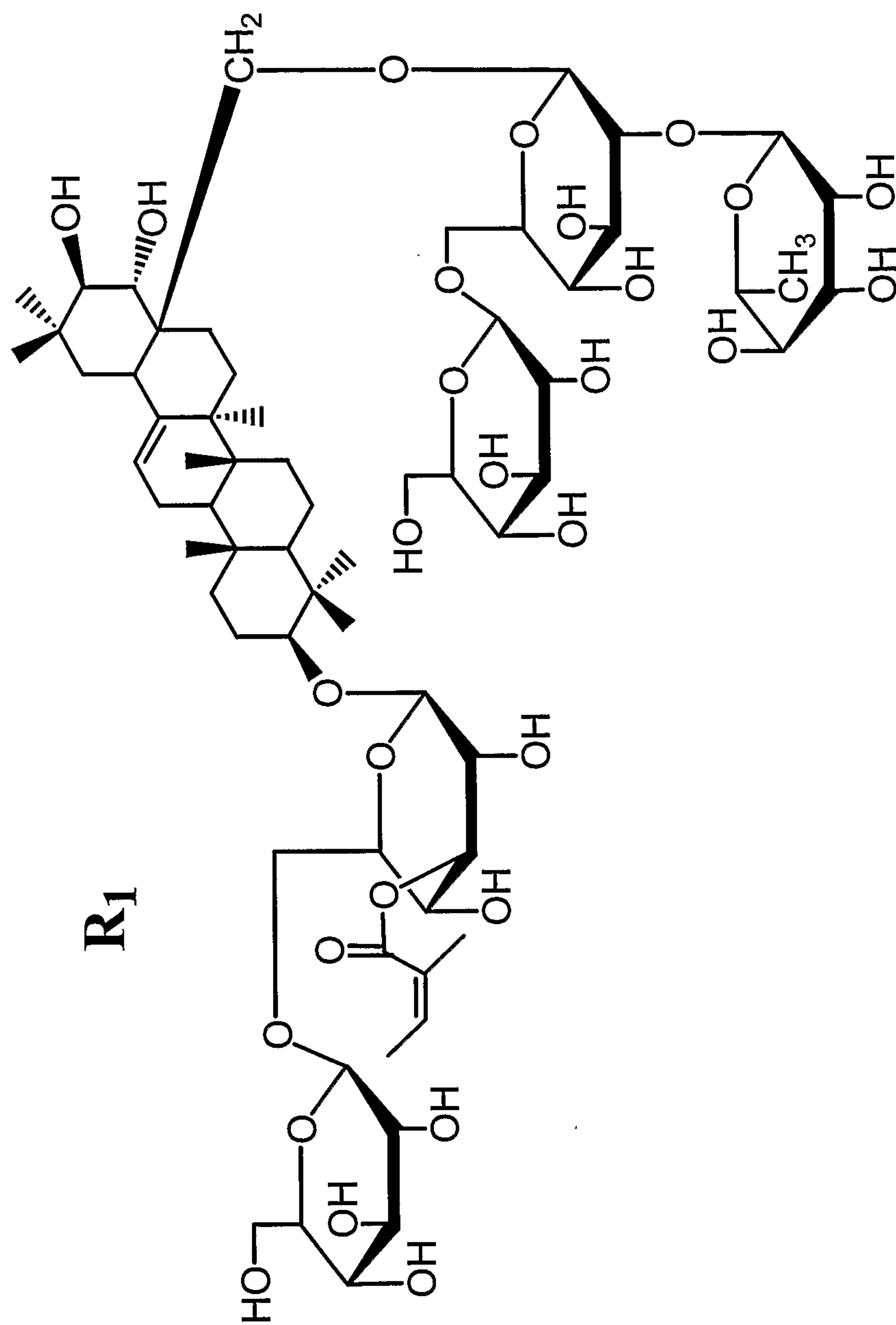
47/77
Figure 45
C13-NMR of Y10



48/77
Figure 46
Y10-HMQC-level-1

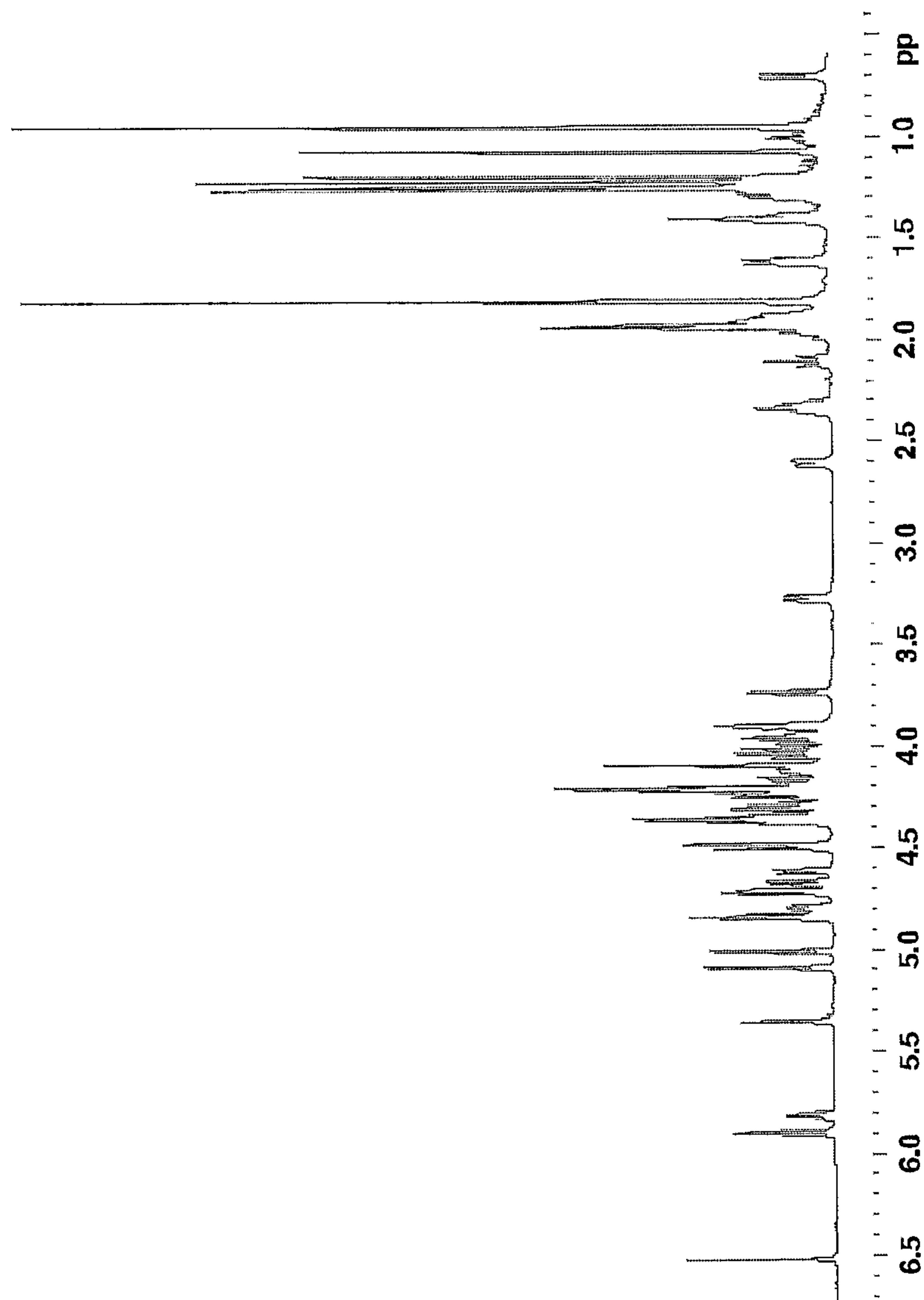


49/77
Figure 47
R1-Structure

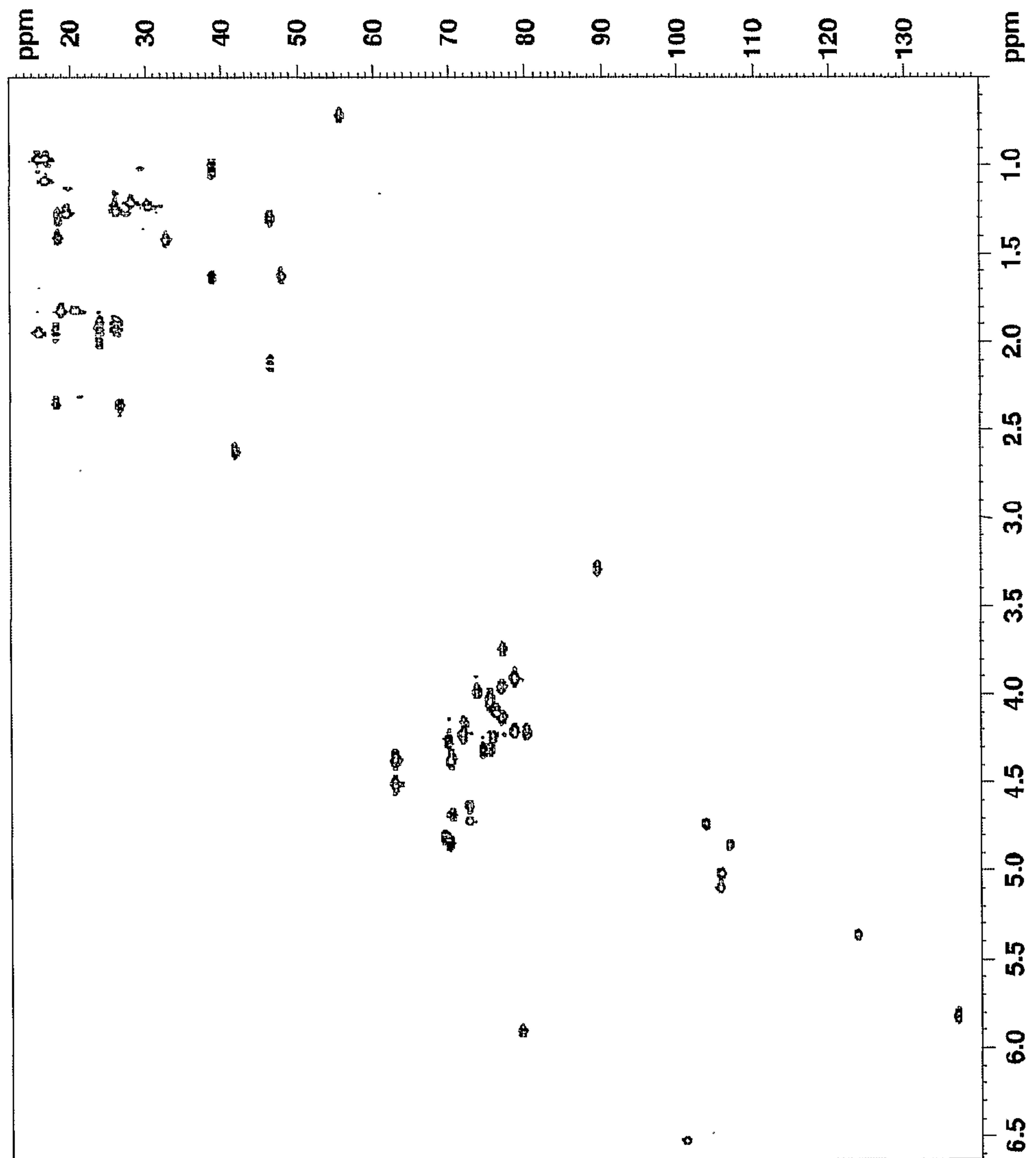


R1: 3-O-[angeloyl-(1→3)-β-D-glucopyranosyl-(1→6)]-β-D-glucopyranosyl-28-O-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→6)]-β-D-glucopyranosyl-3β, 21β, 22α, 28-tetrahydroxyolean-12-ene

50/77
Figure 48
R1-H-NMR



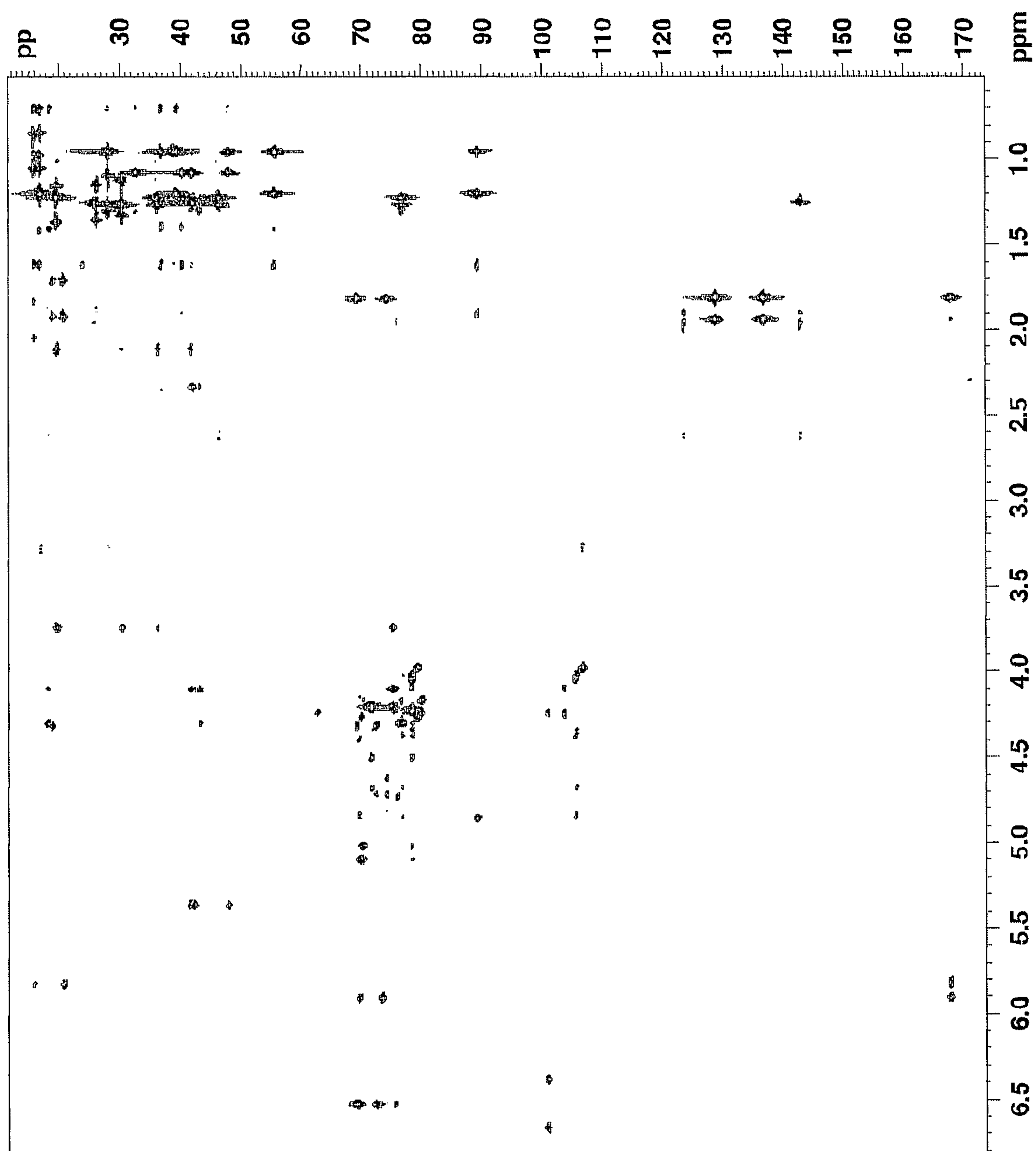
51/77
Figure 49
R1-HMQC



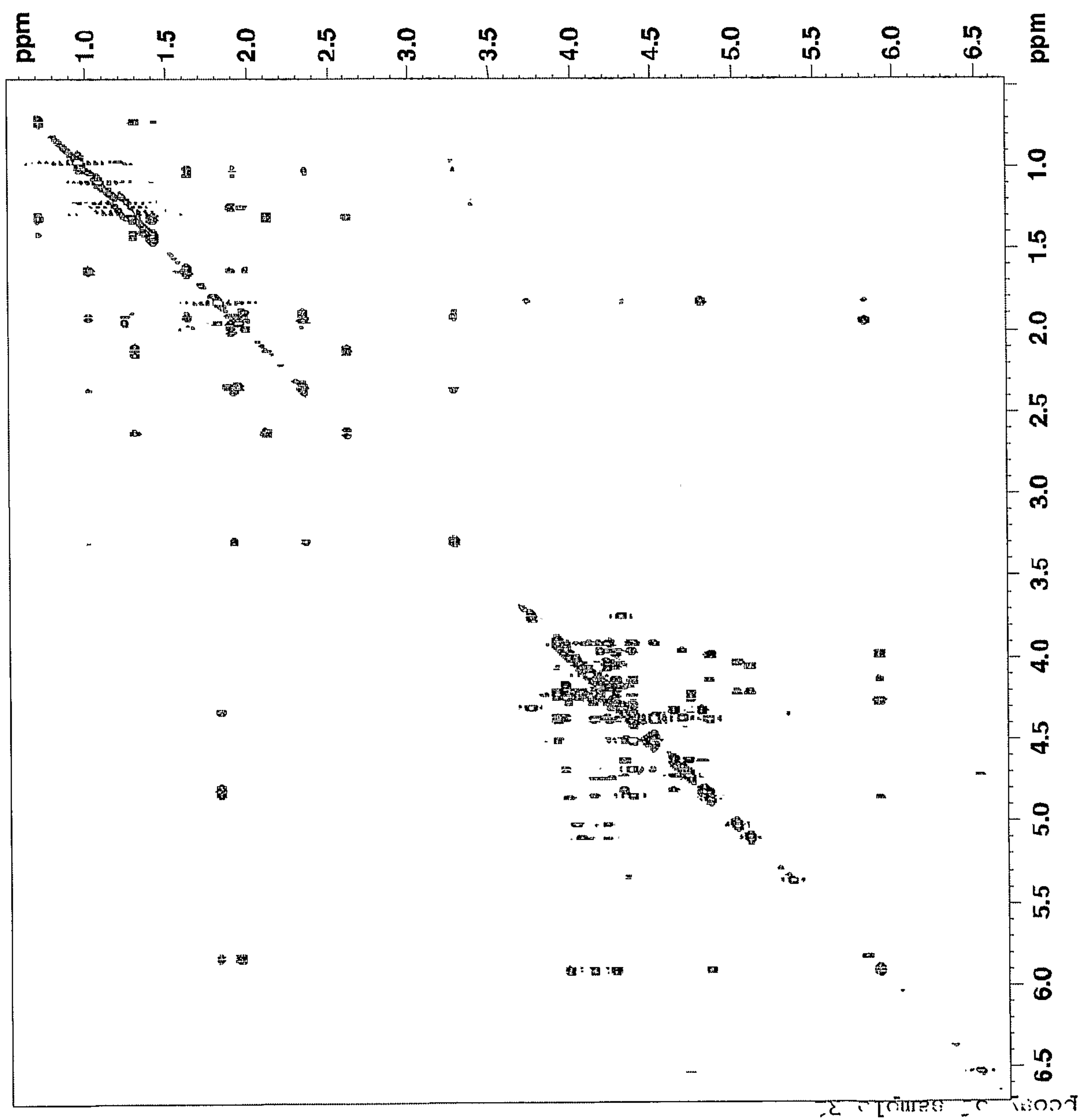
52/77

Figure 50

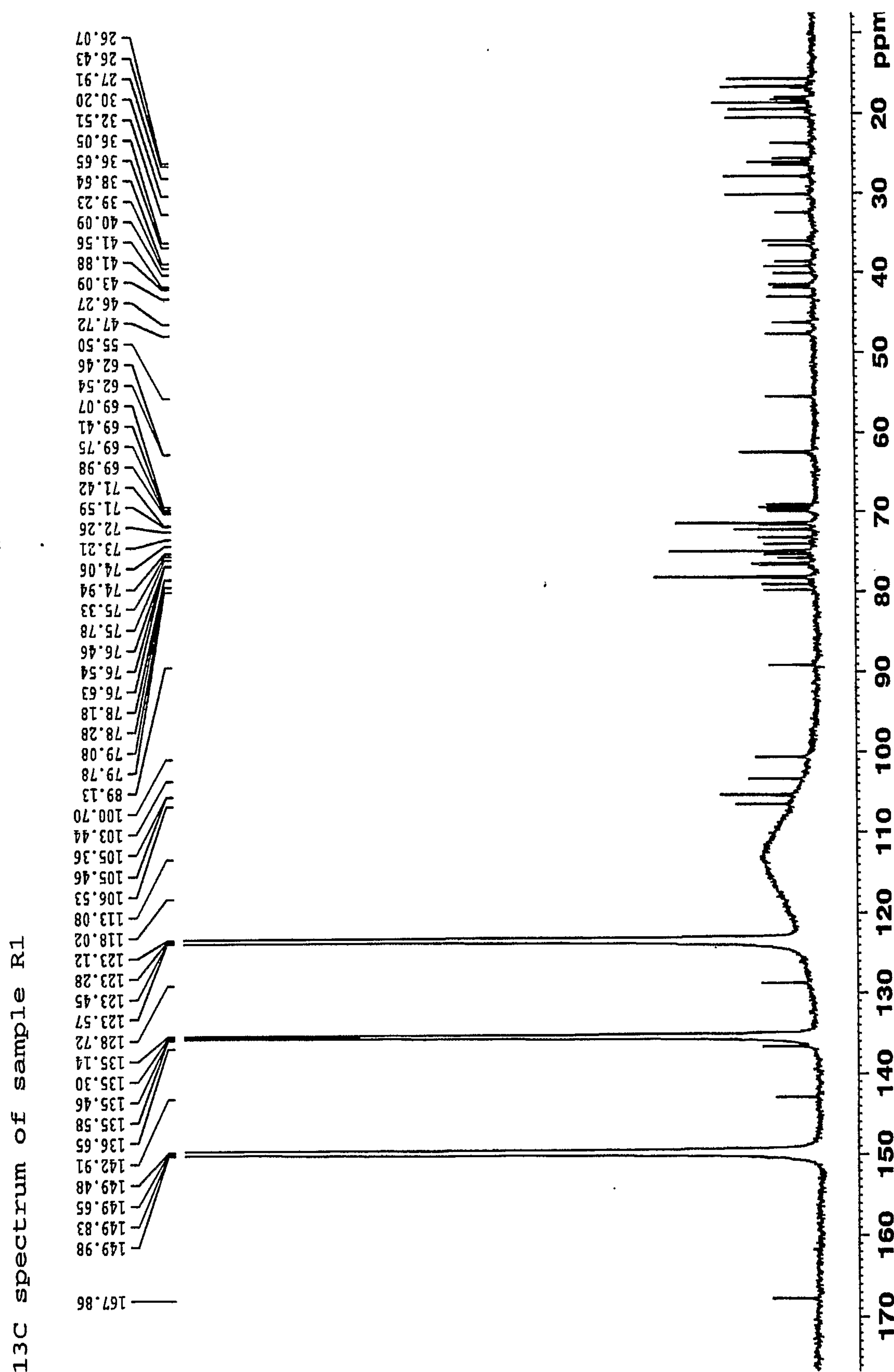
R1-HMBC-level 1



53/77
Figure 51
R1-COSY



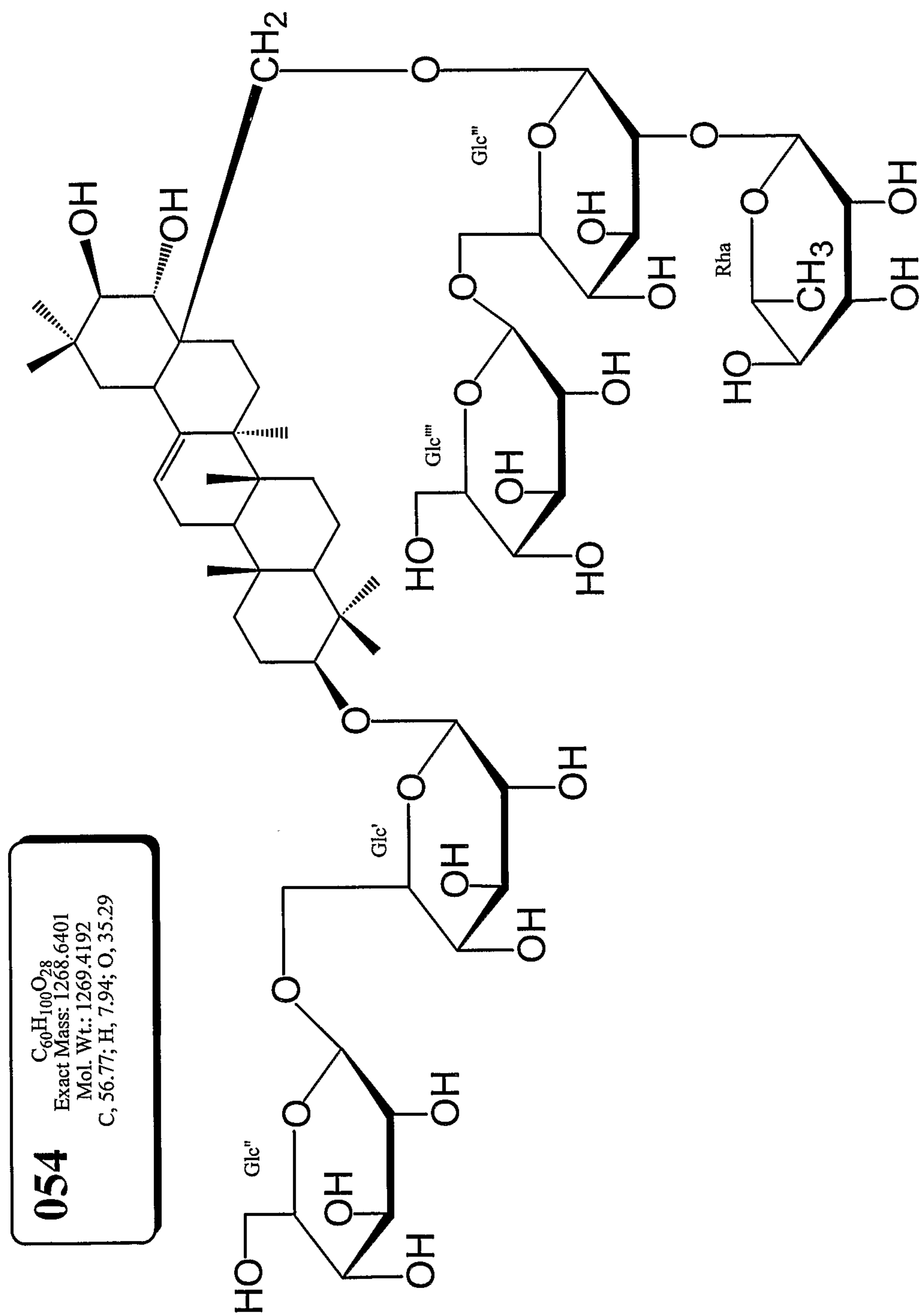
54/77
Figure 52
R1-C13-1



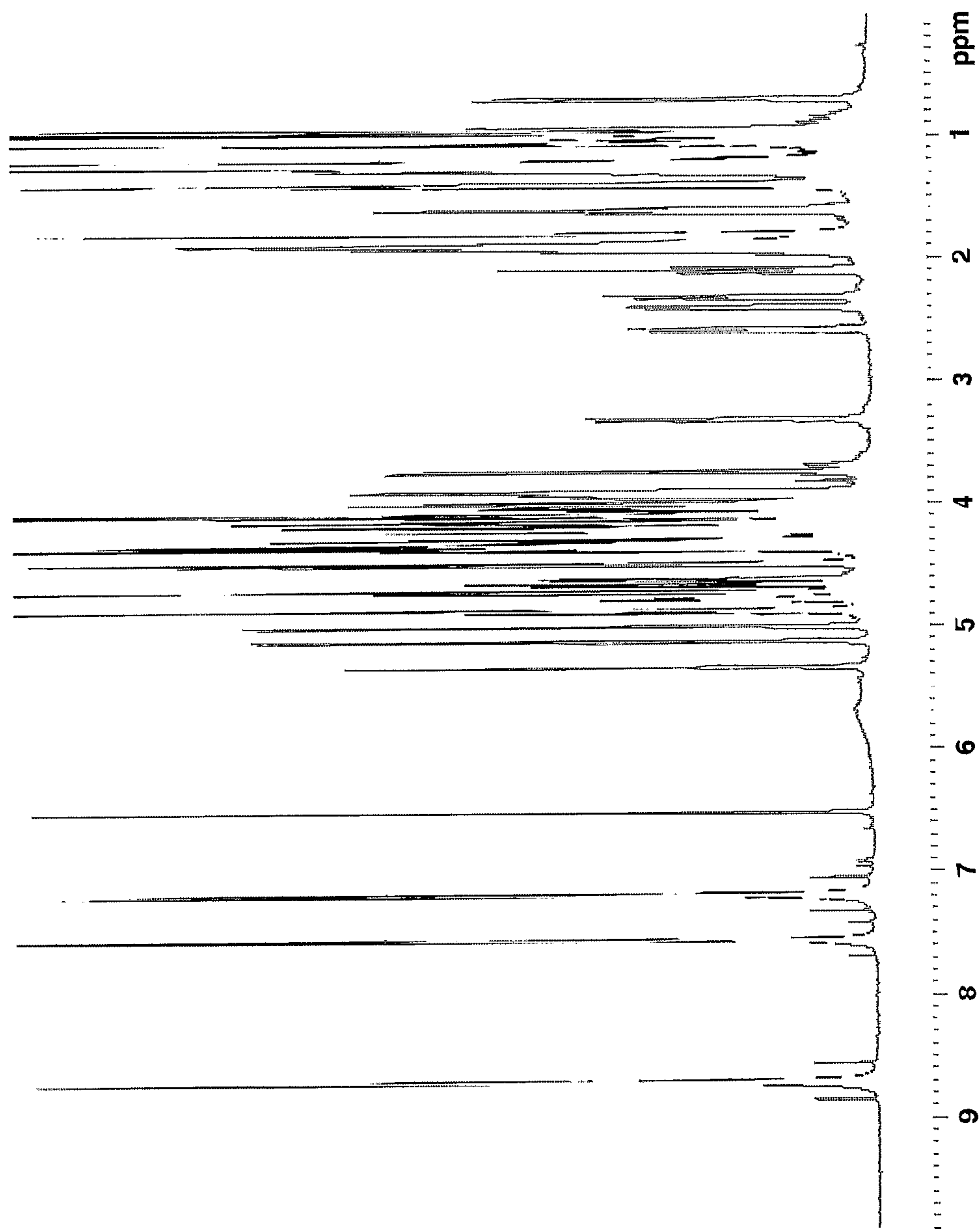
55/77

Figure 53

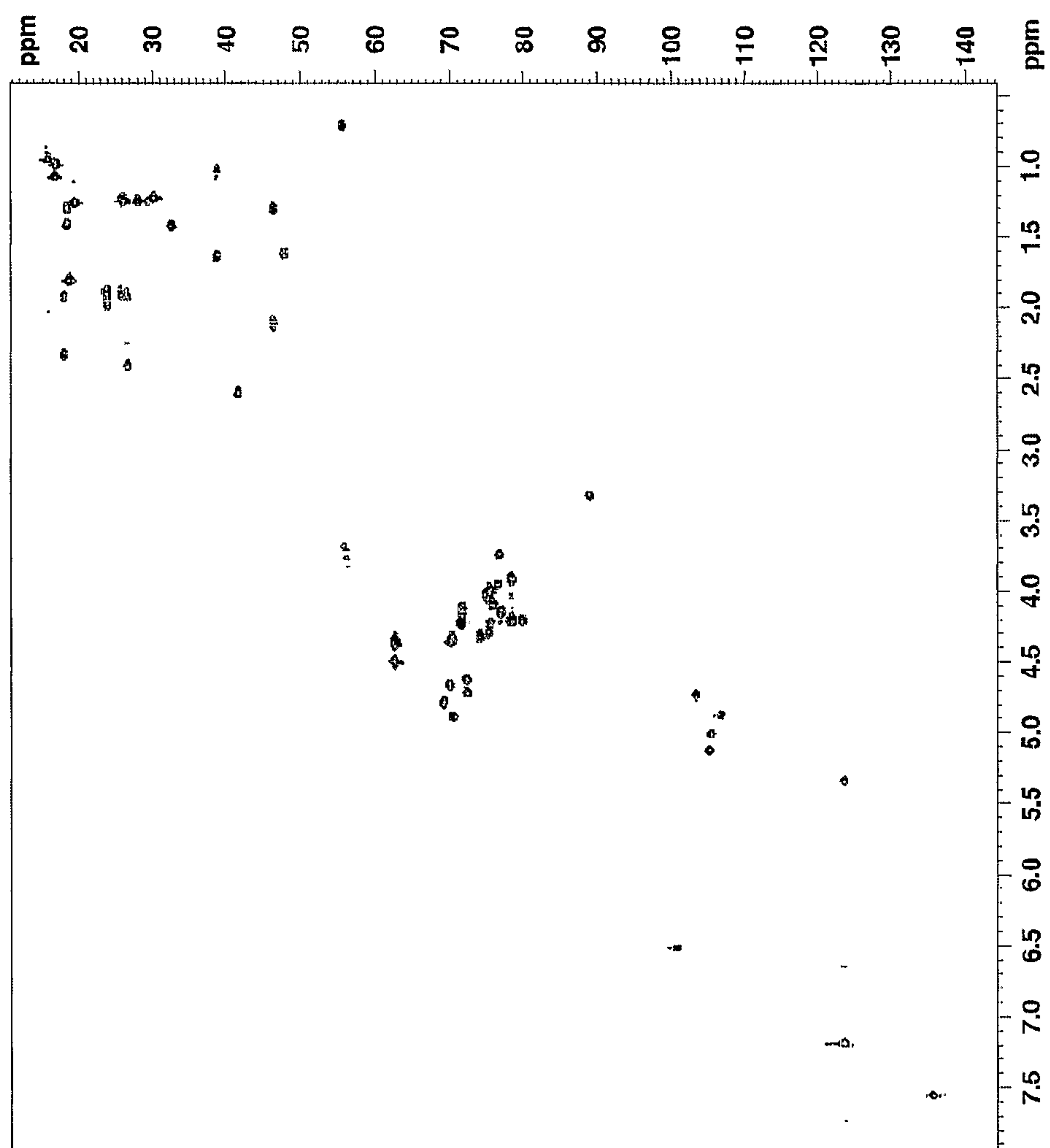
Structure of Compound O54



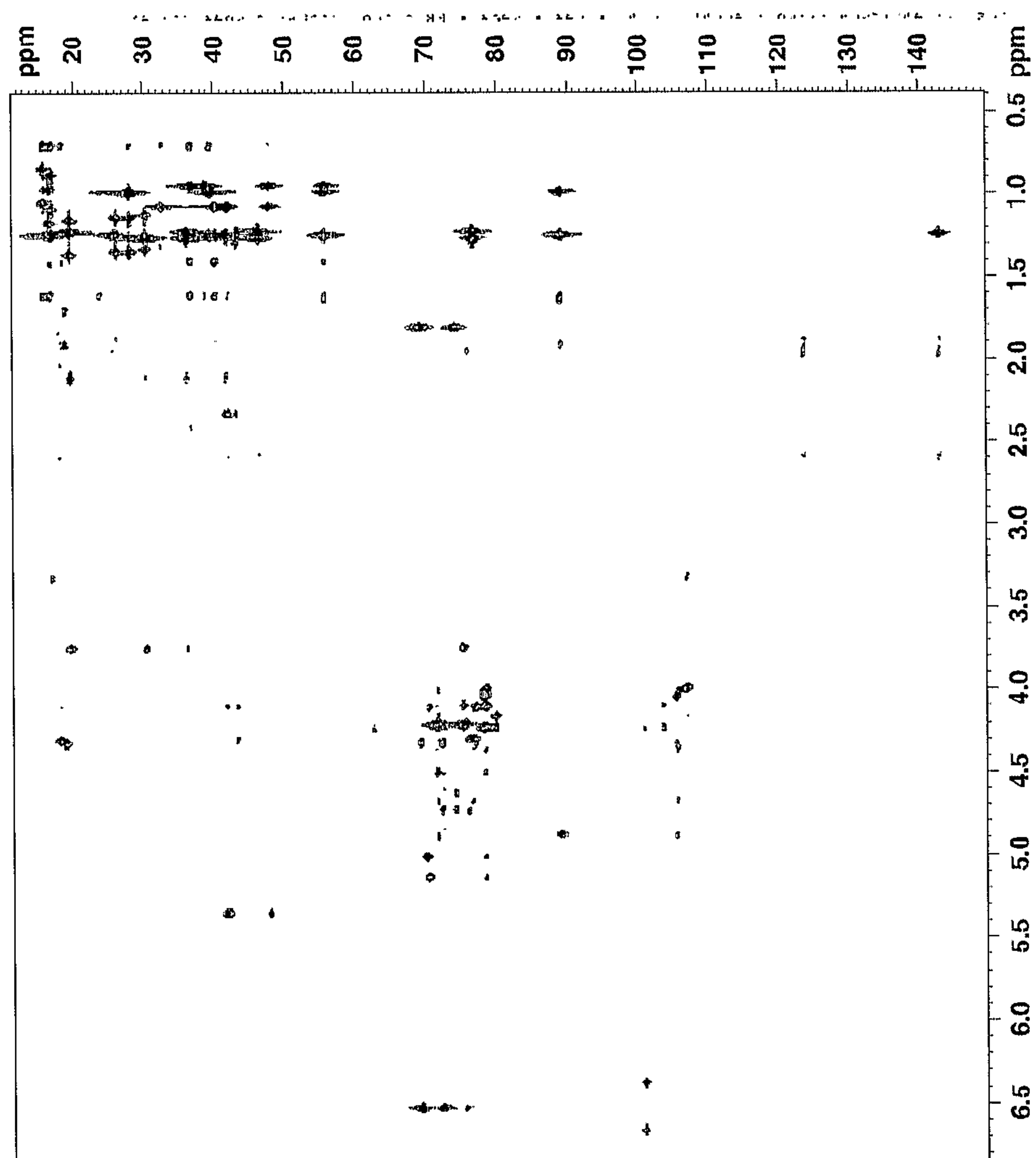
56/77
Figure 54
O54-H-NMR



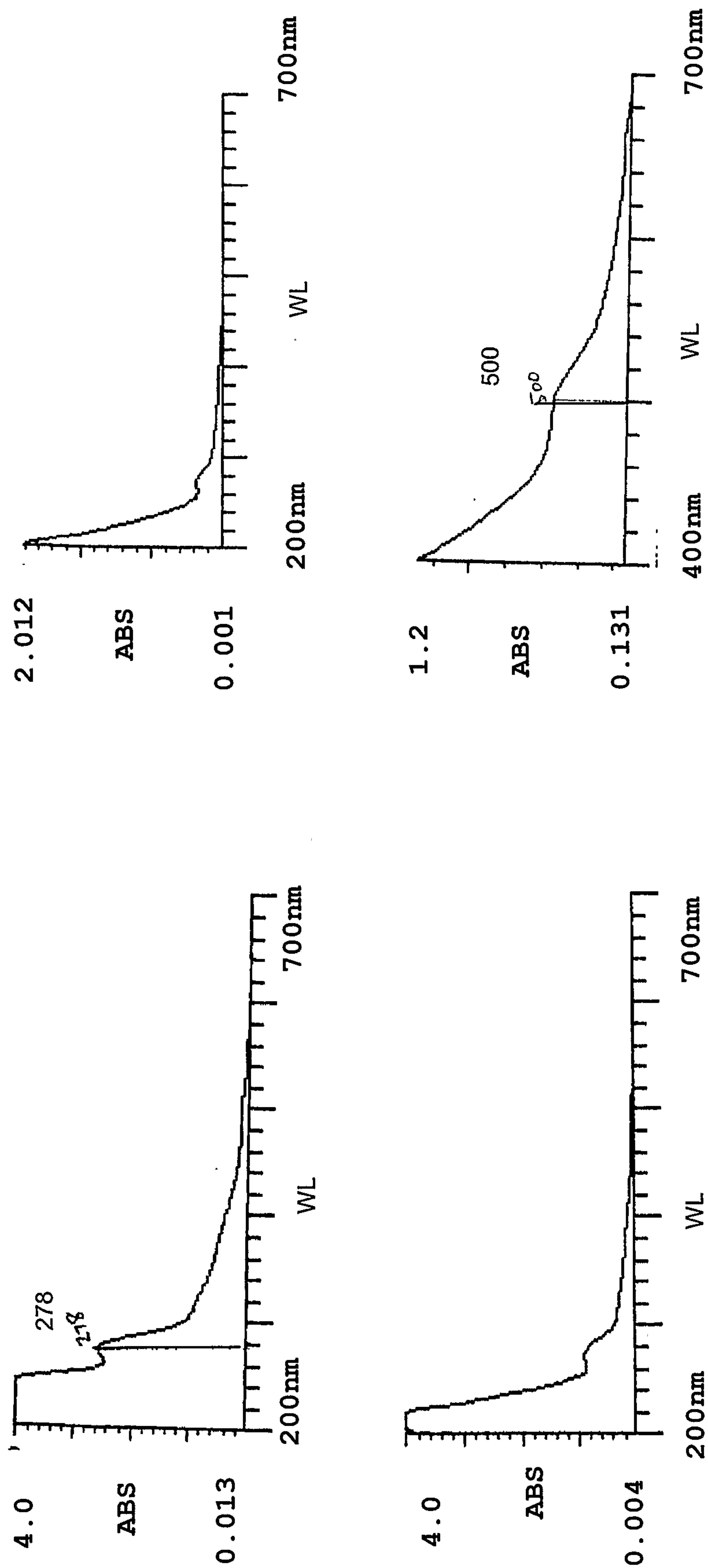
57/77
Figure 55
O54-HMQC



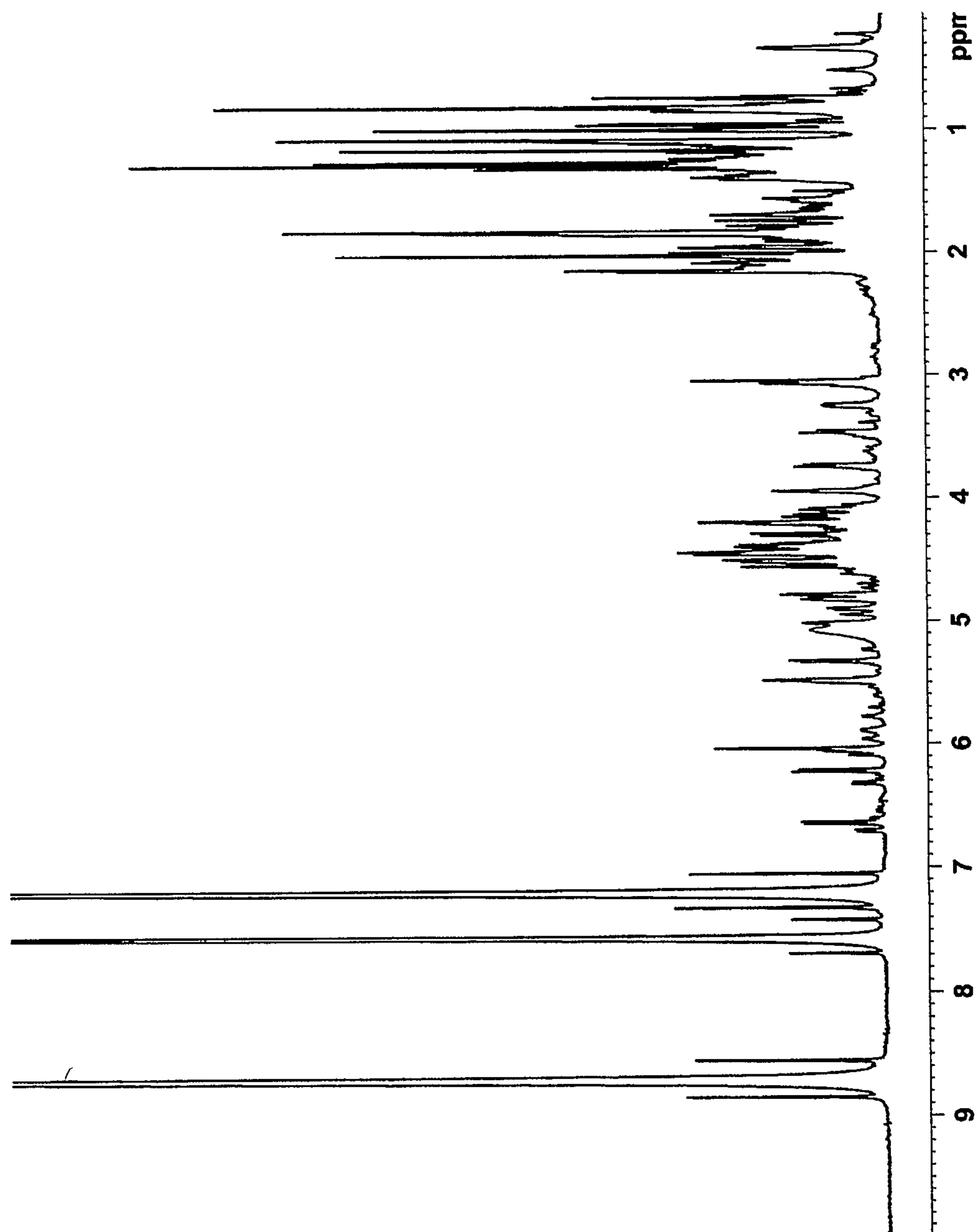
58/77
Figure 56
O54-HMBC



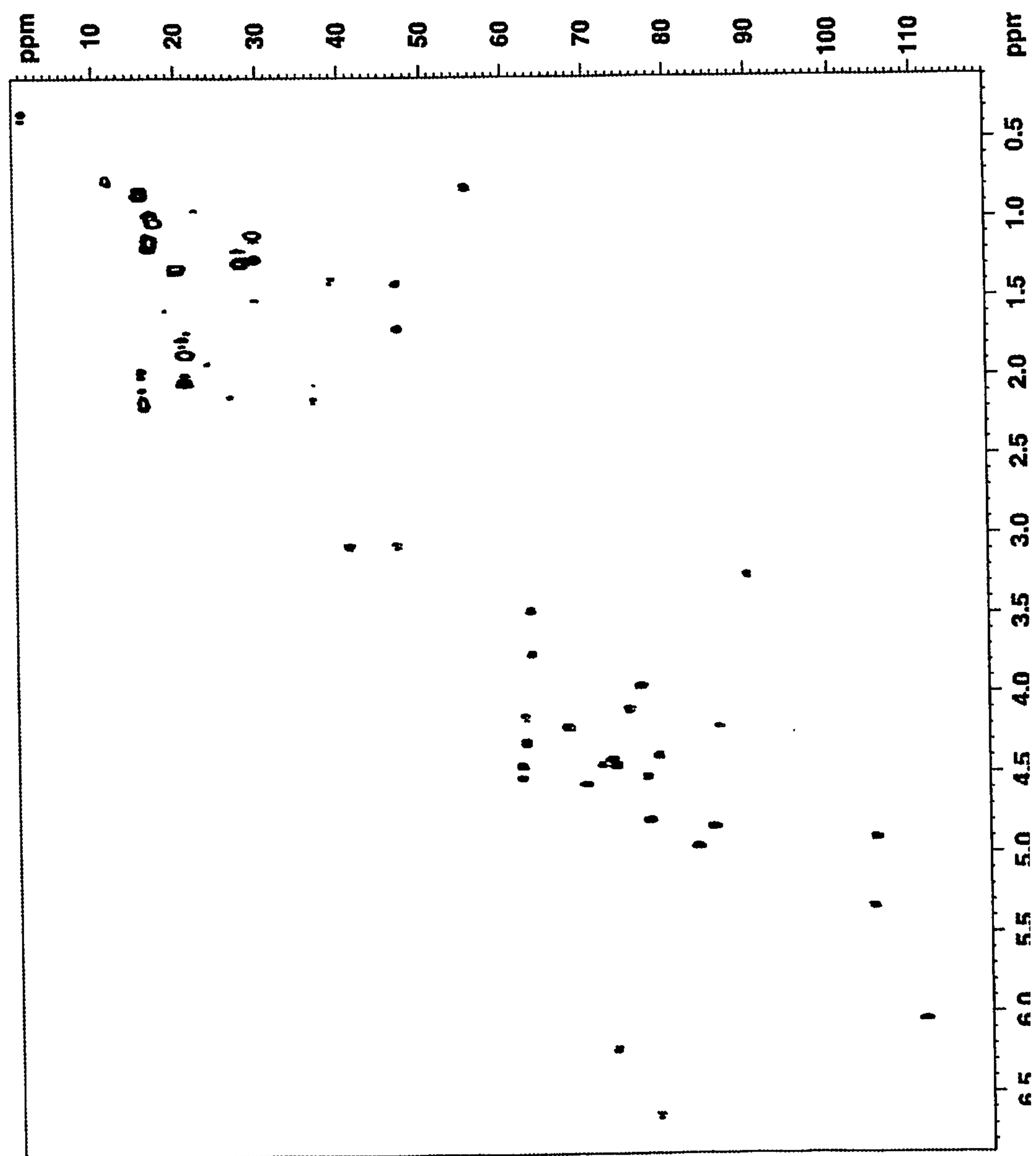
59/77
Figure 57



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Figure 58
H-NMR of Y4

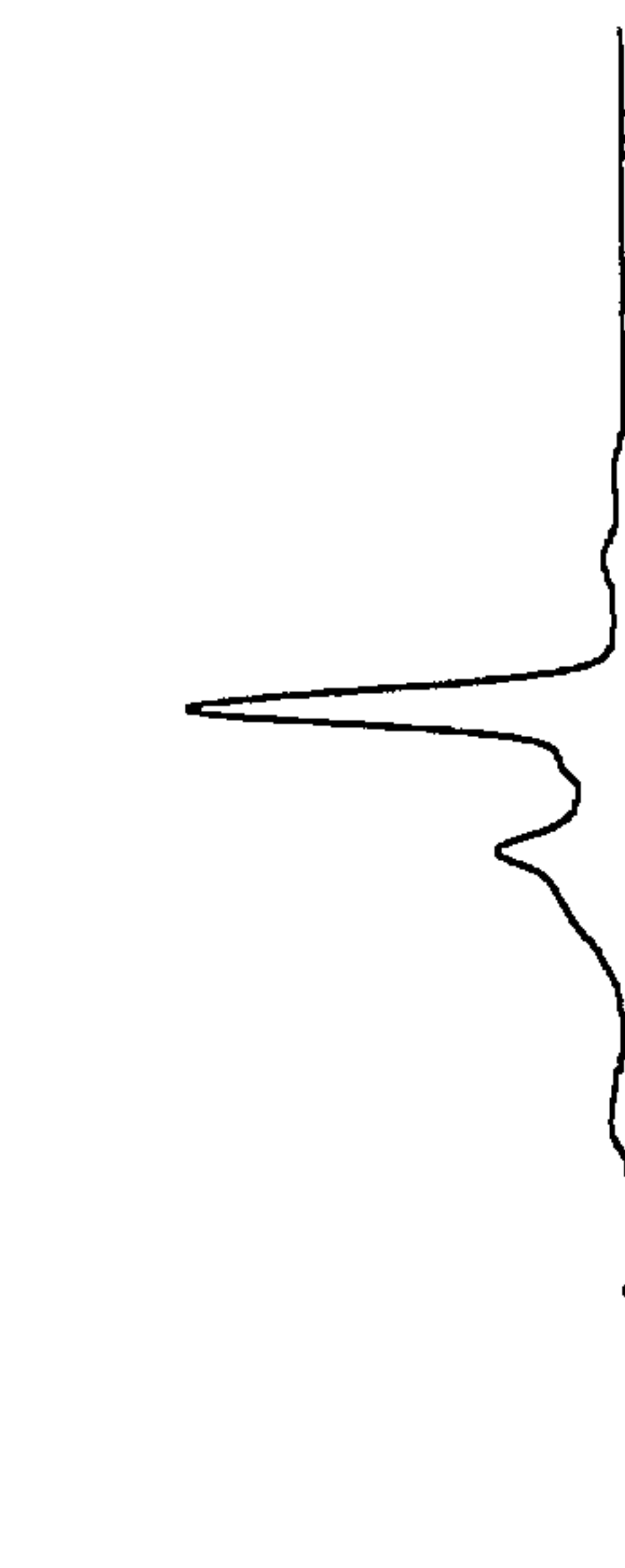


61/77
Figure 59
HMQC of Y4

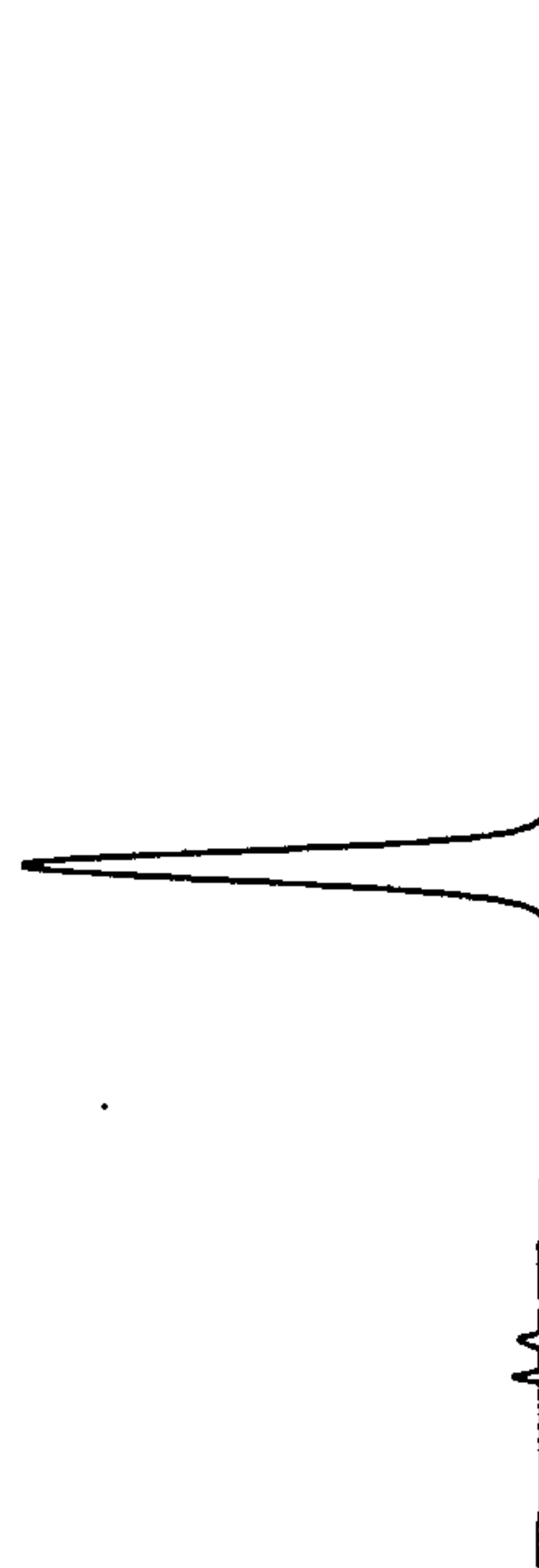


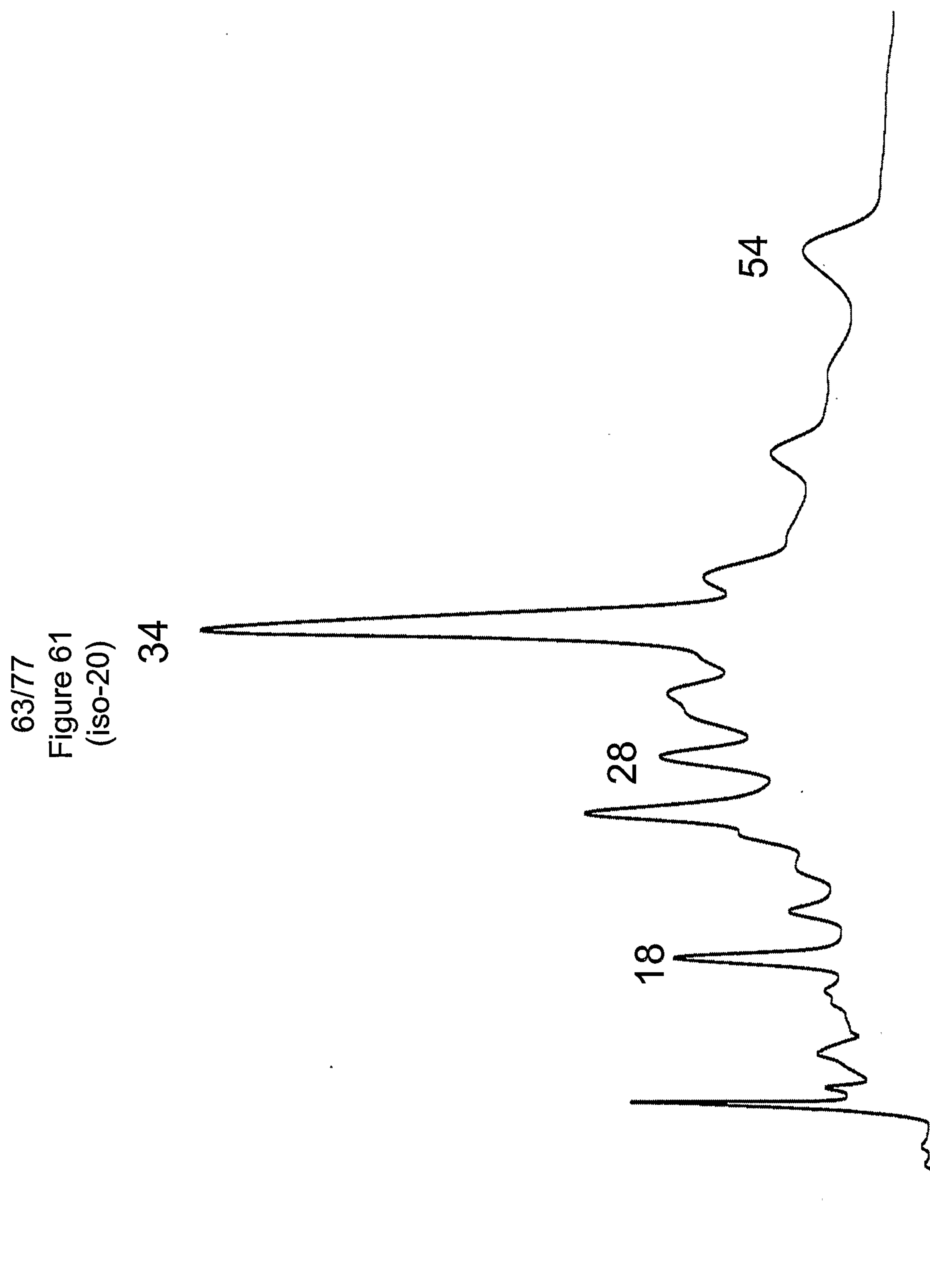
62/77
Figure 60

A

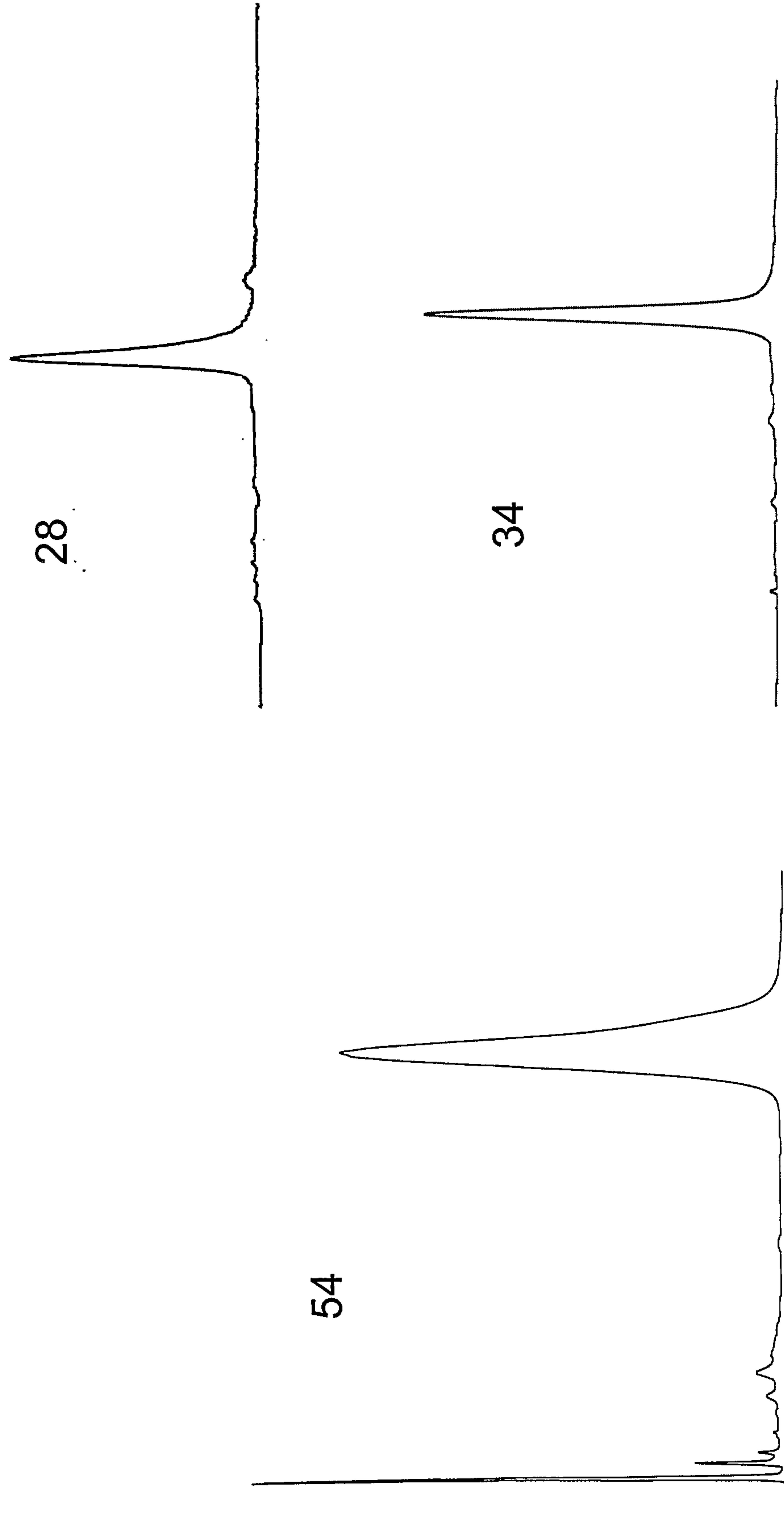


B

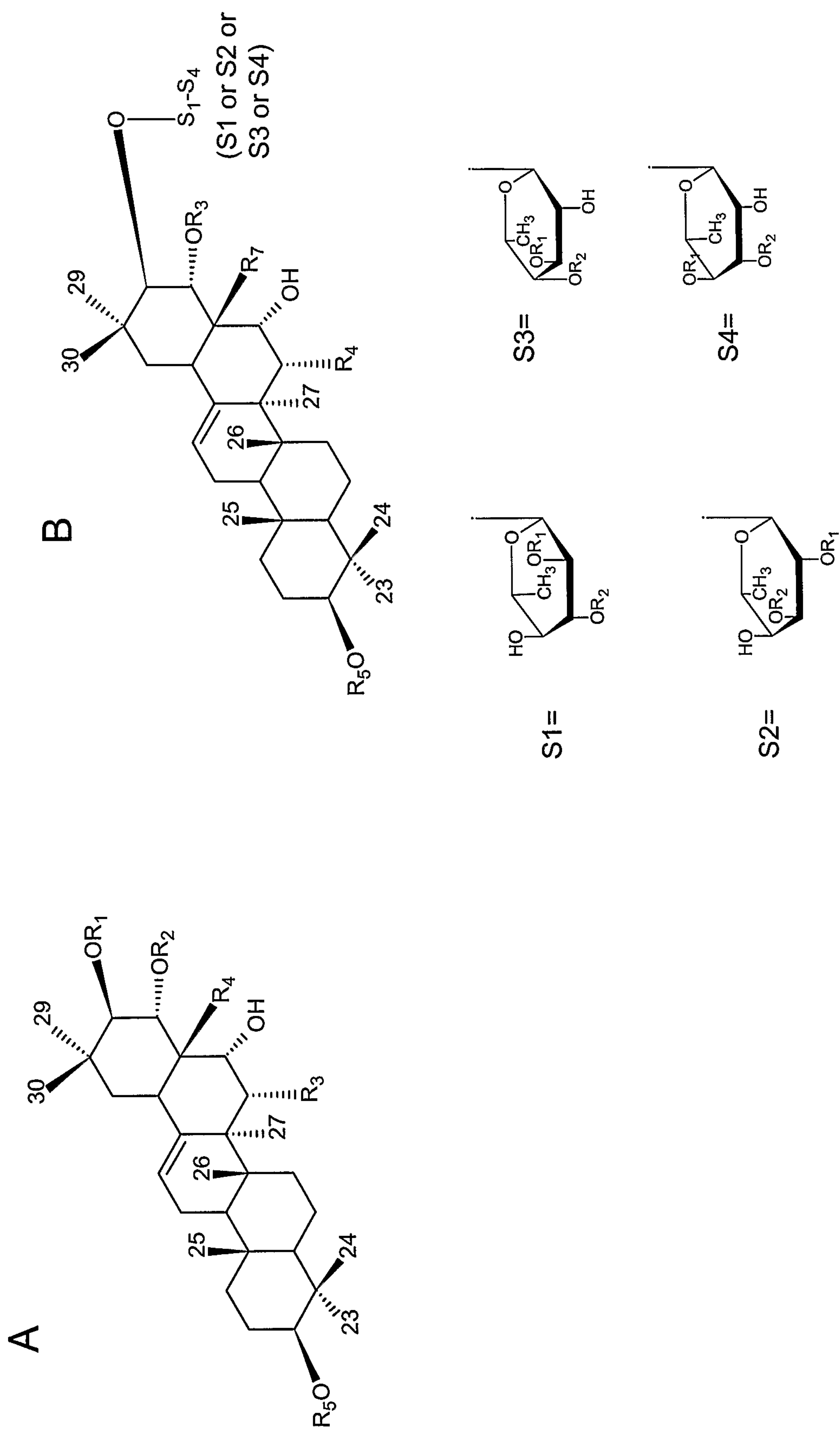




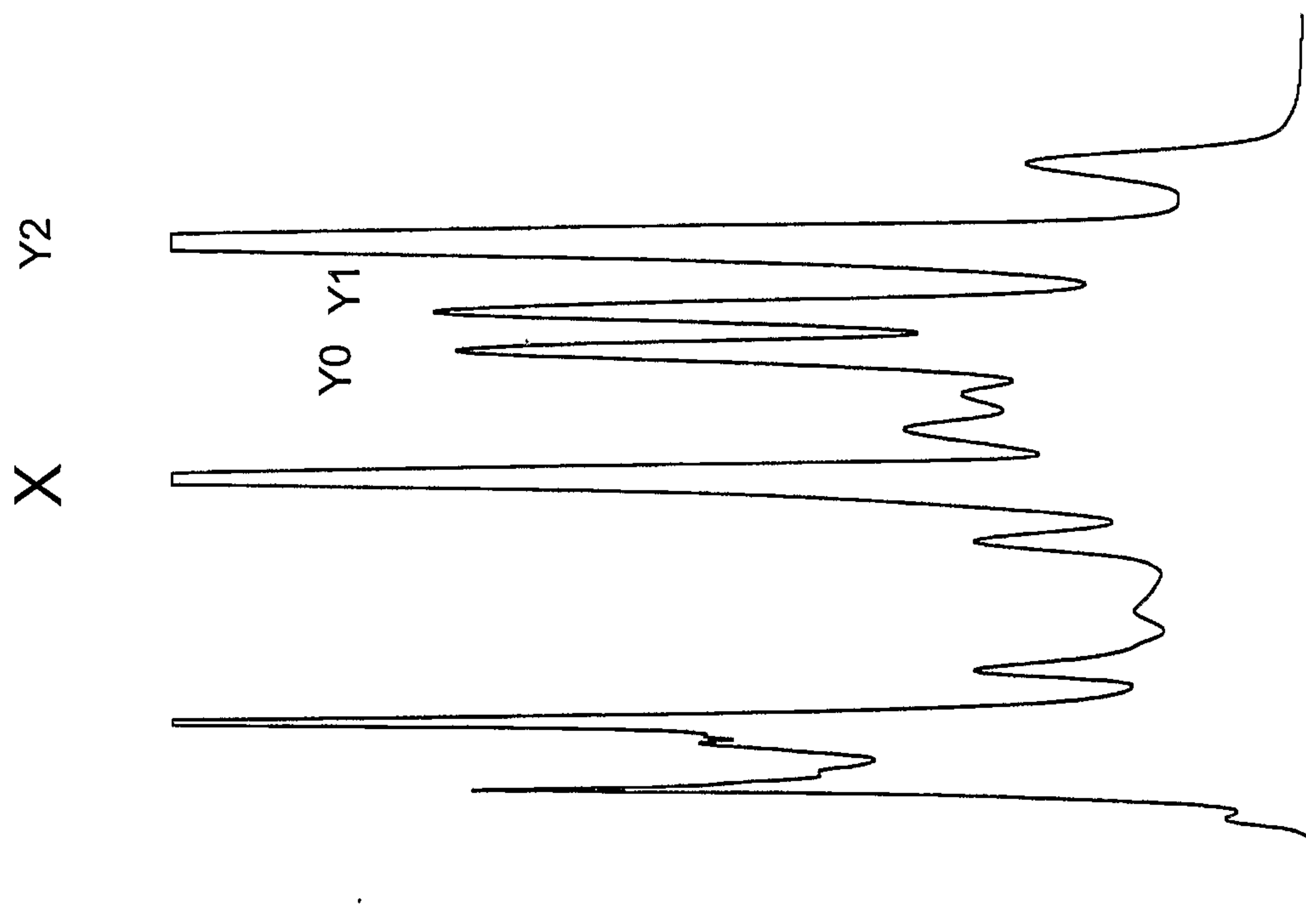
64/77
Figure 62
Re-chromatography of #54 #28 and #34 (from iso-20)



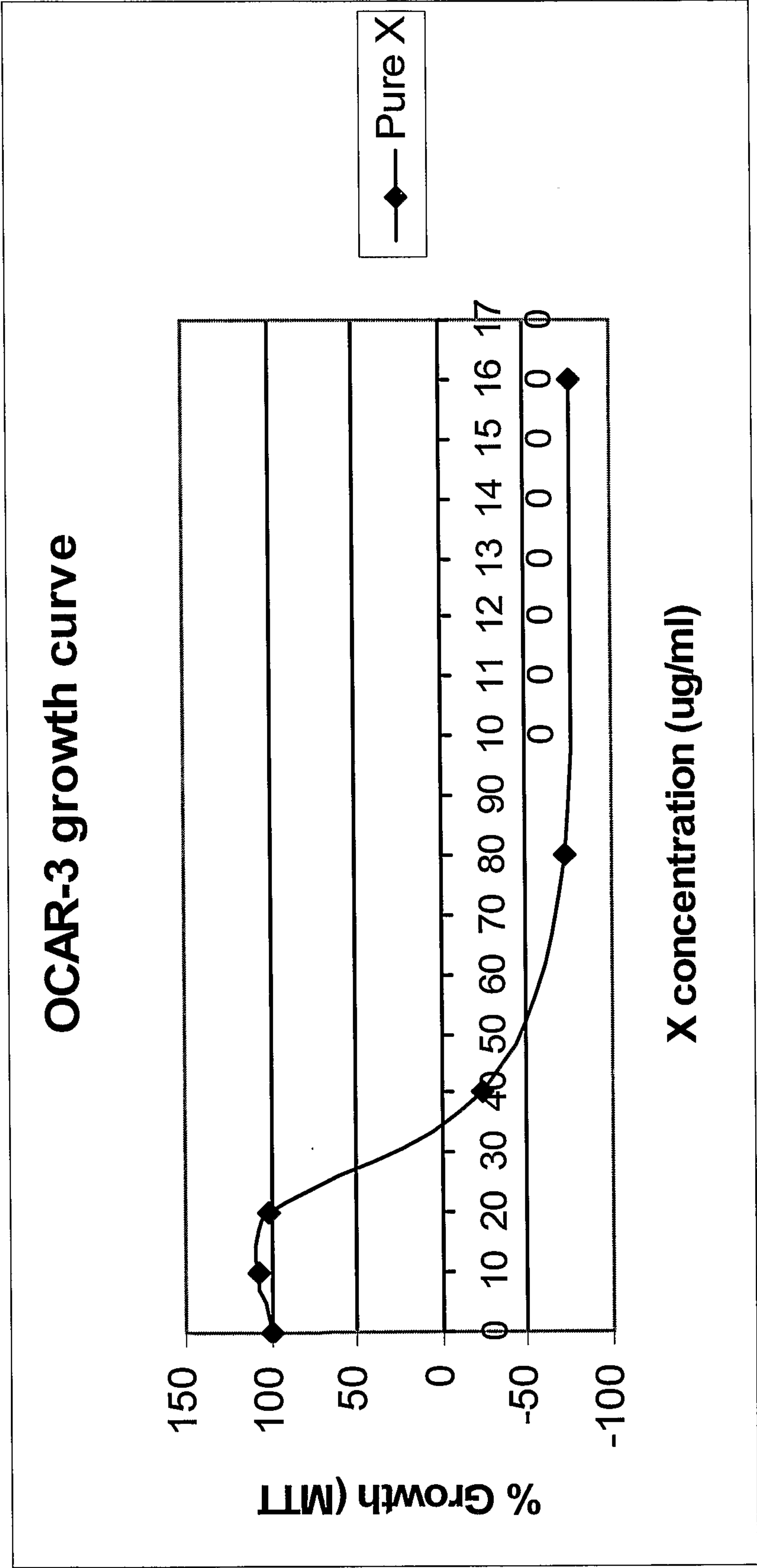
65/77
Figure 63



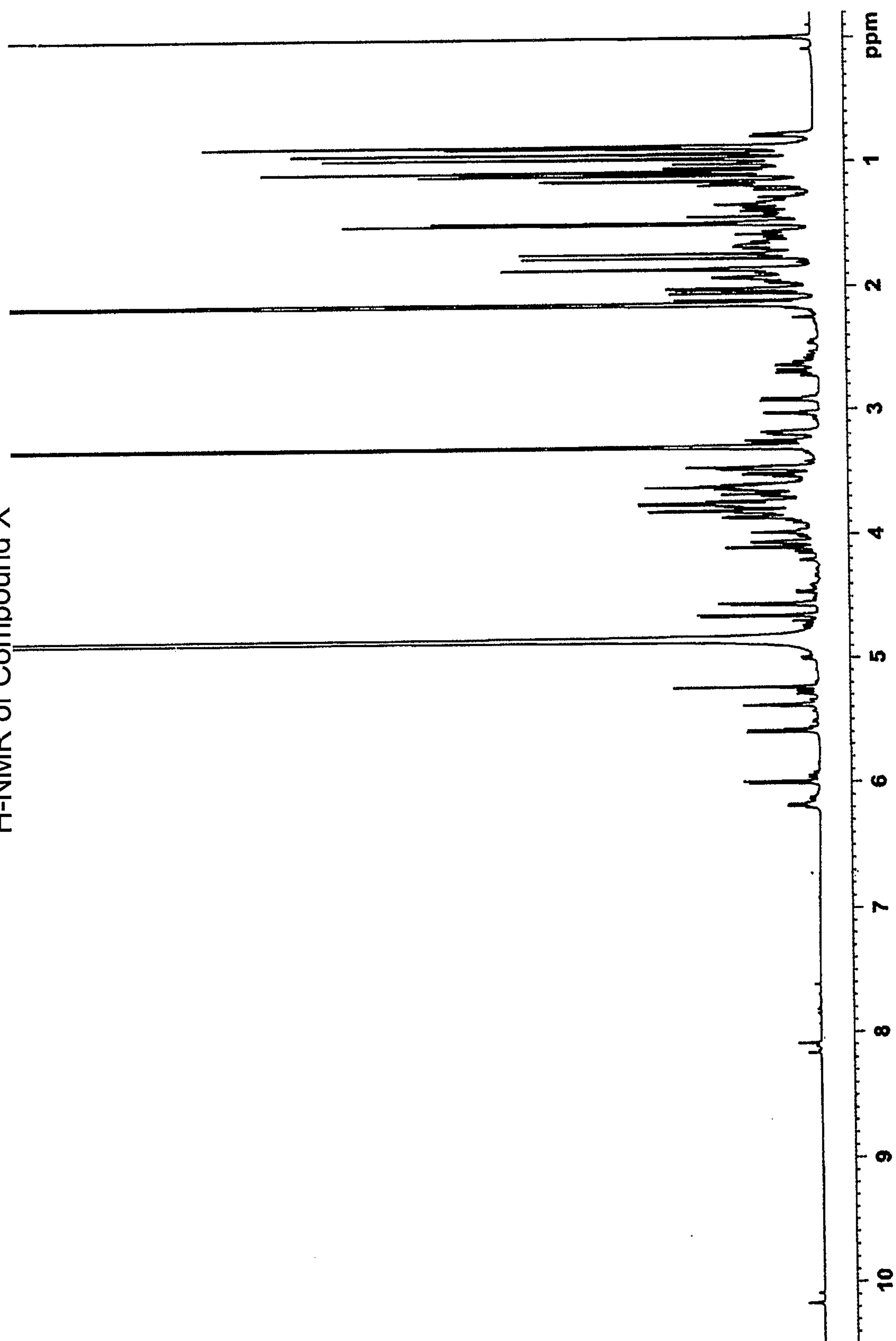
66/77
Figure 64
HPLC (iso-45) profile of FPLC fractions #5657



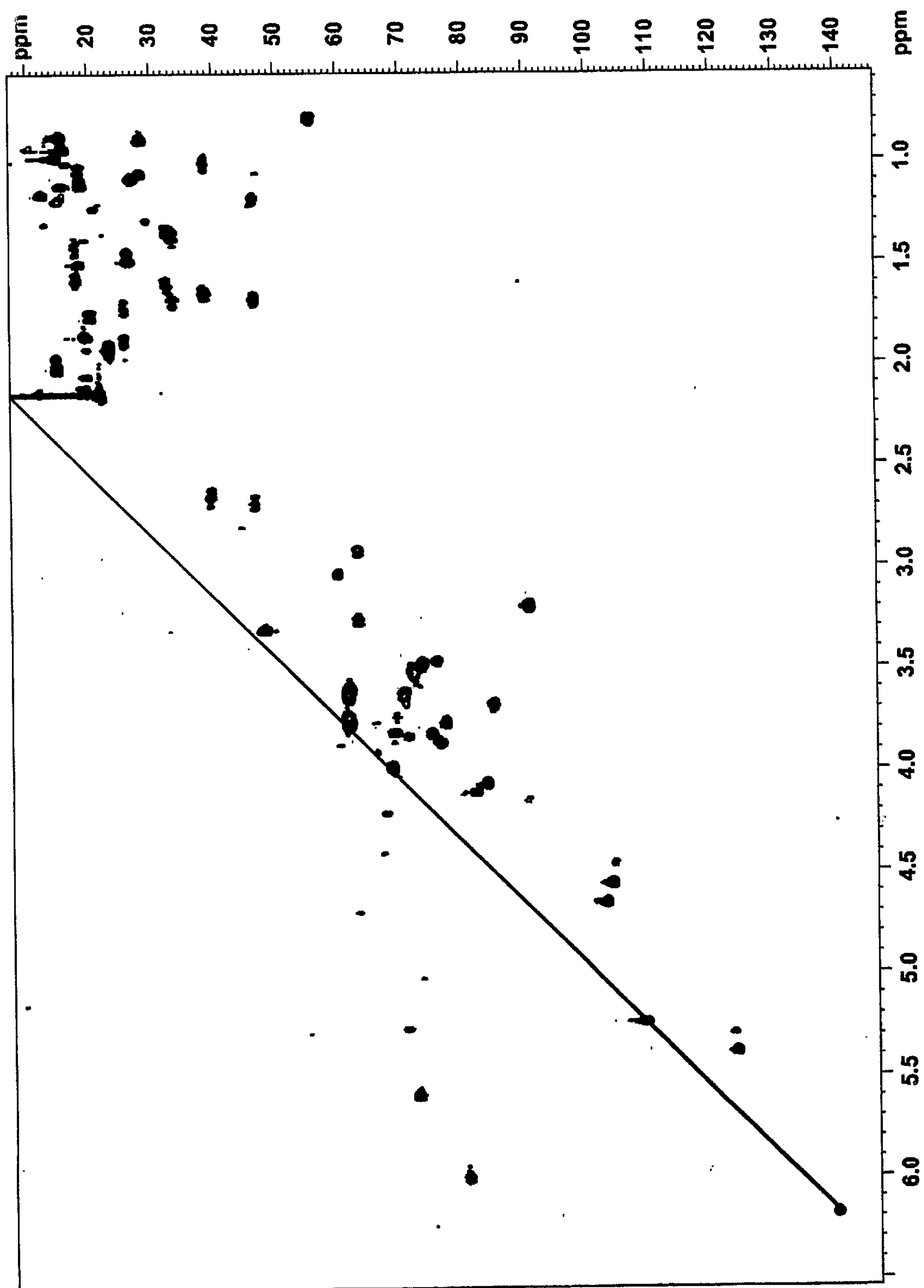
67/77
Figure 65
Effect of Compound X on the growth of OCAR-3 cells



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Figure 66
H-NMR of Compound X



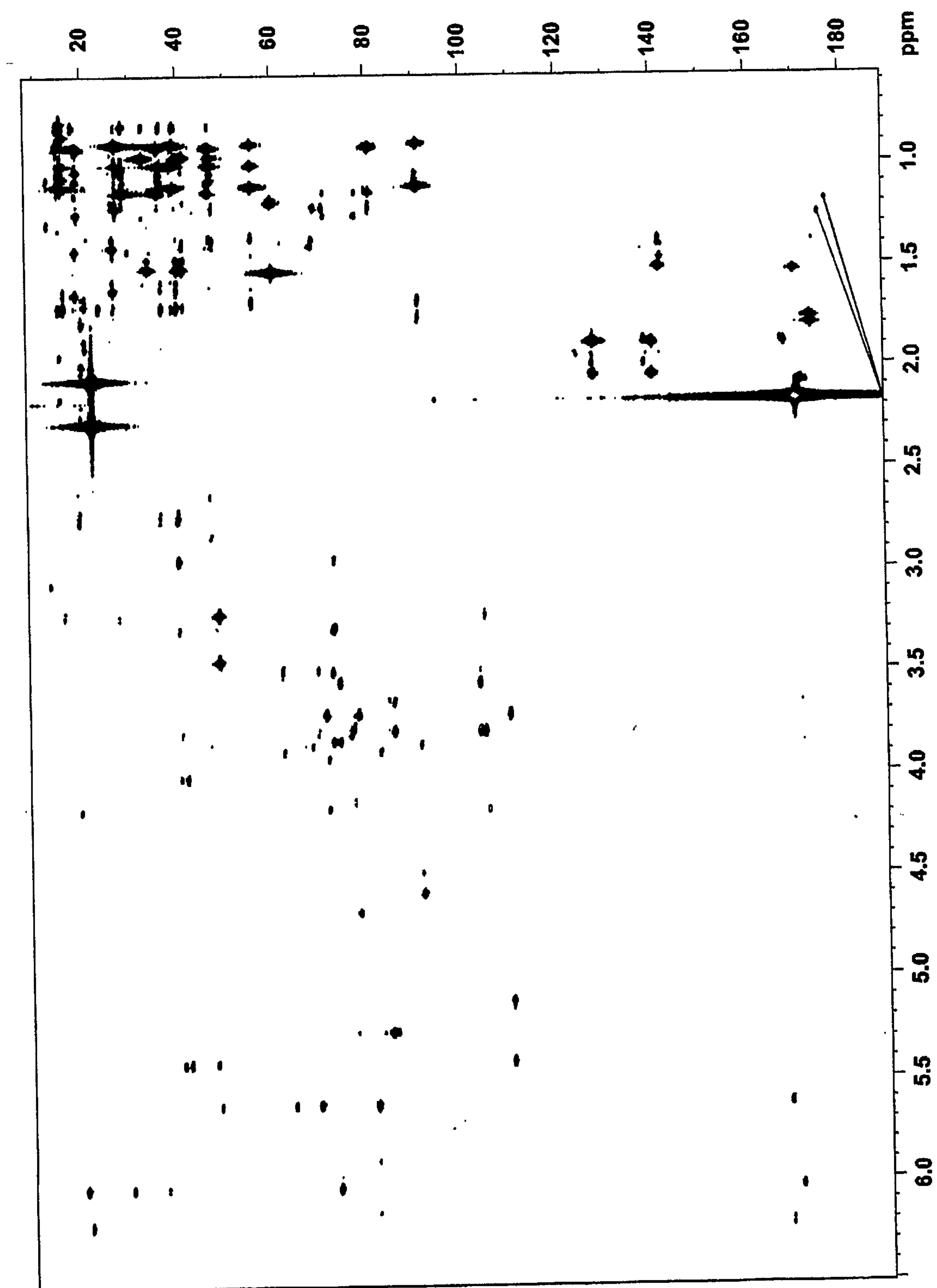
69/77
Figure 67
2D NMR (HMQC) of Compound X



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Figure 68

2D NMR (HMBC) of Compound X



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Figure 69
C13-NMR of Compound X

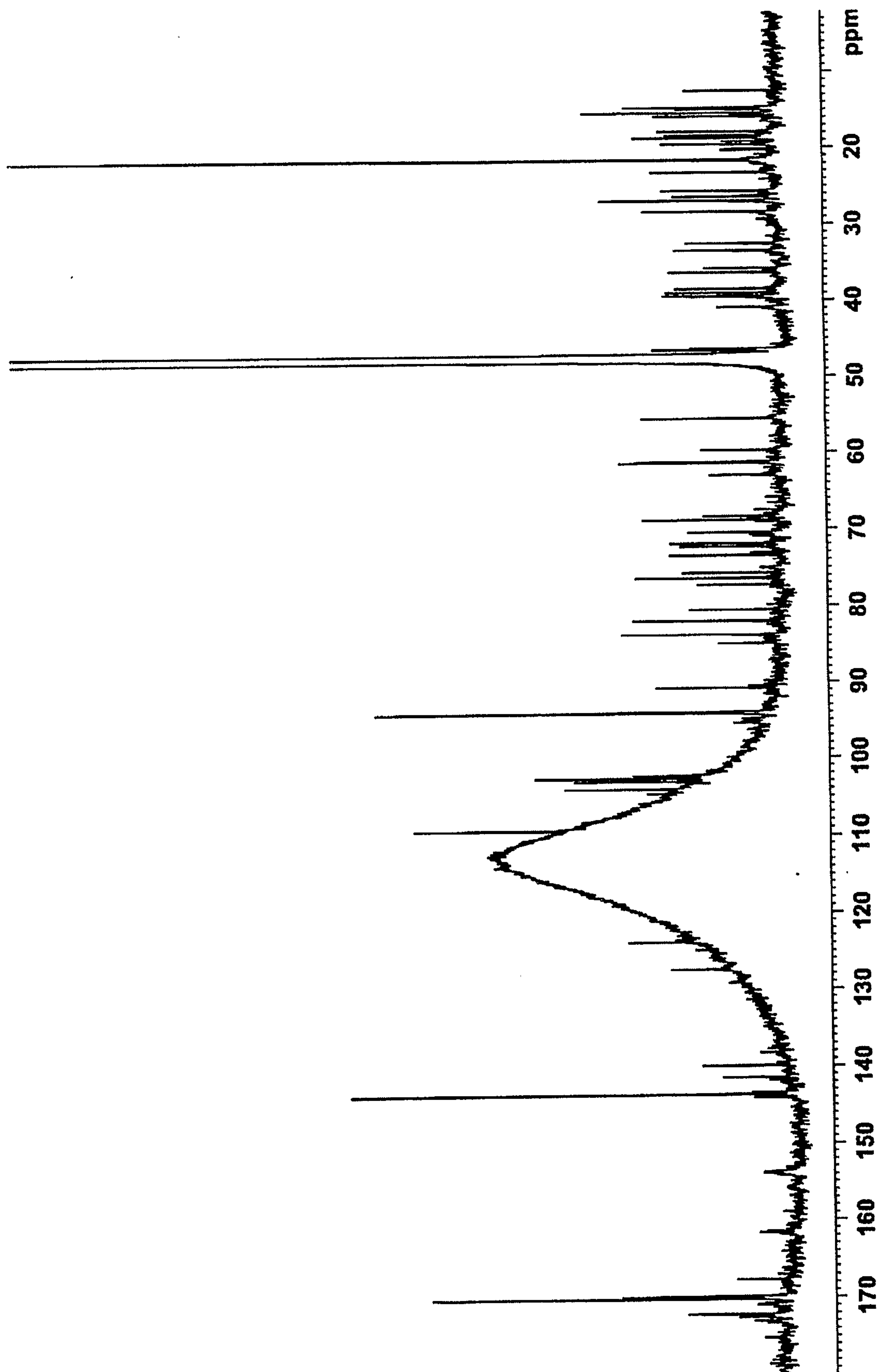
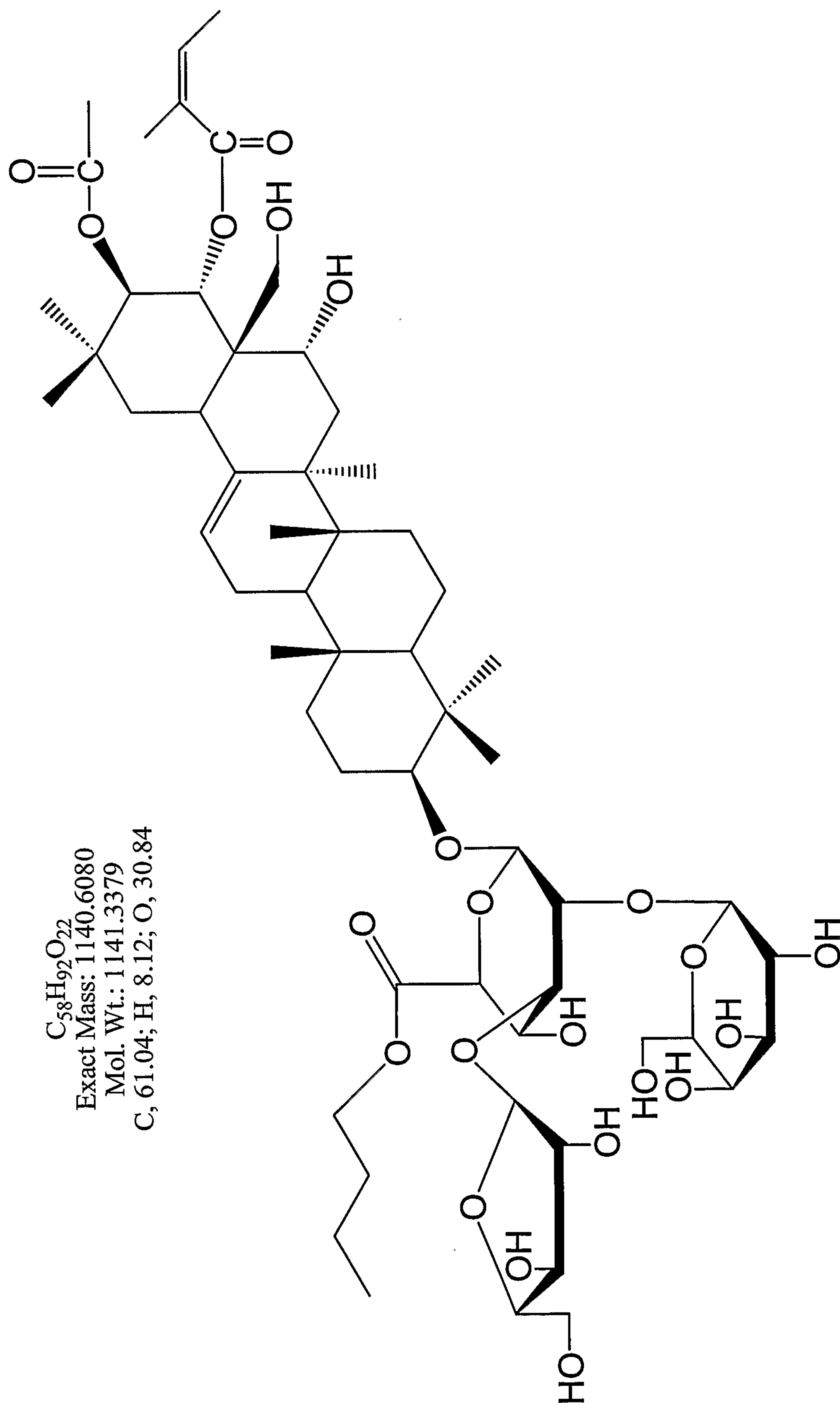


Figure 70

Chemical Structure of Compound X



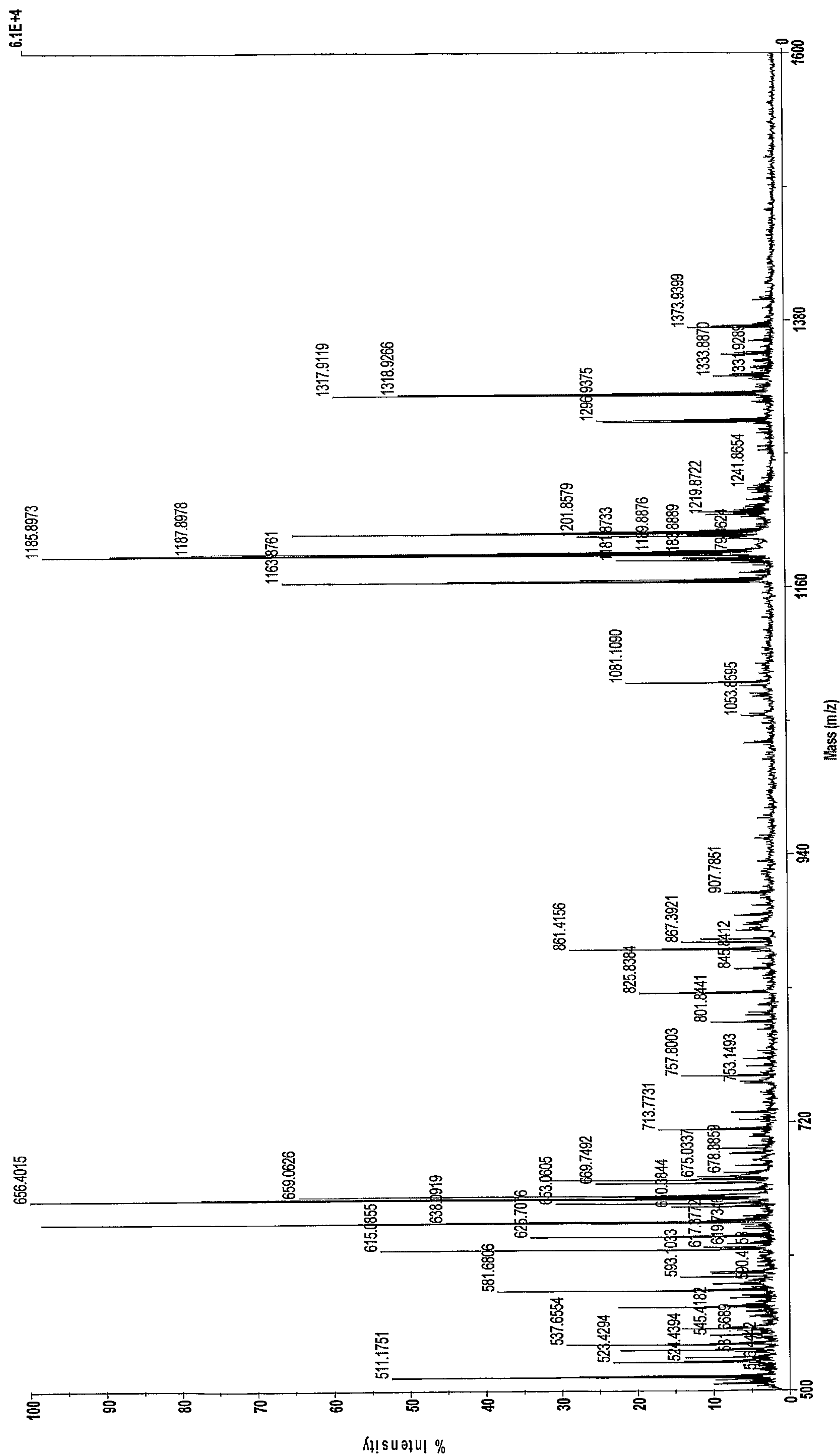
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Figure 71

Mass spectrum (WALDI-TOF) of Compound X

X+matrix(CHCA) without calibration

Voyager Spec #1[BP = 656.4, 61028]



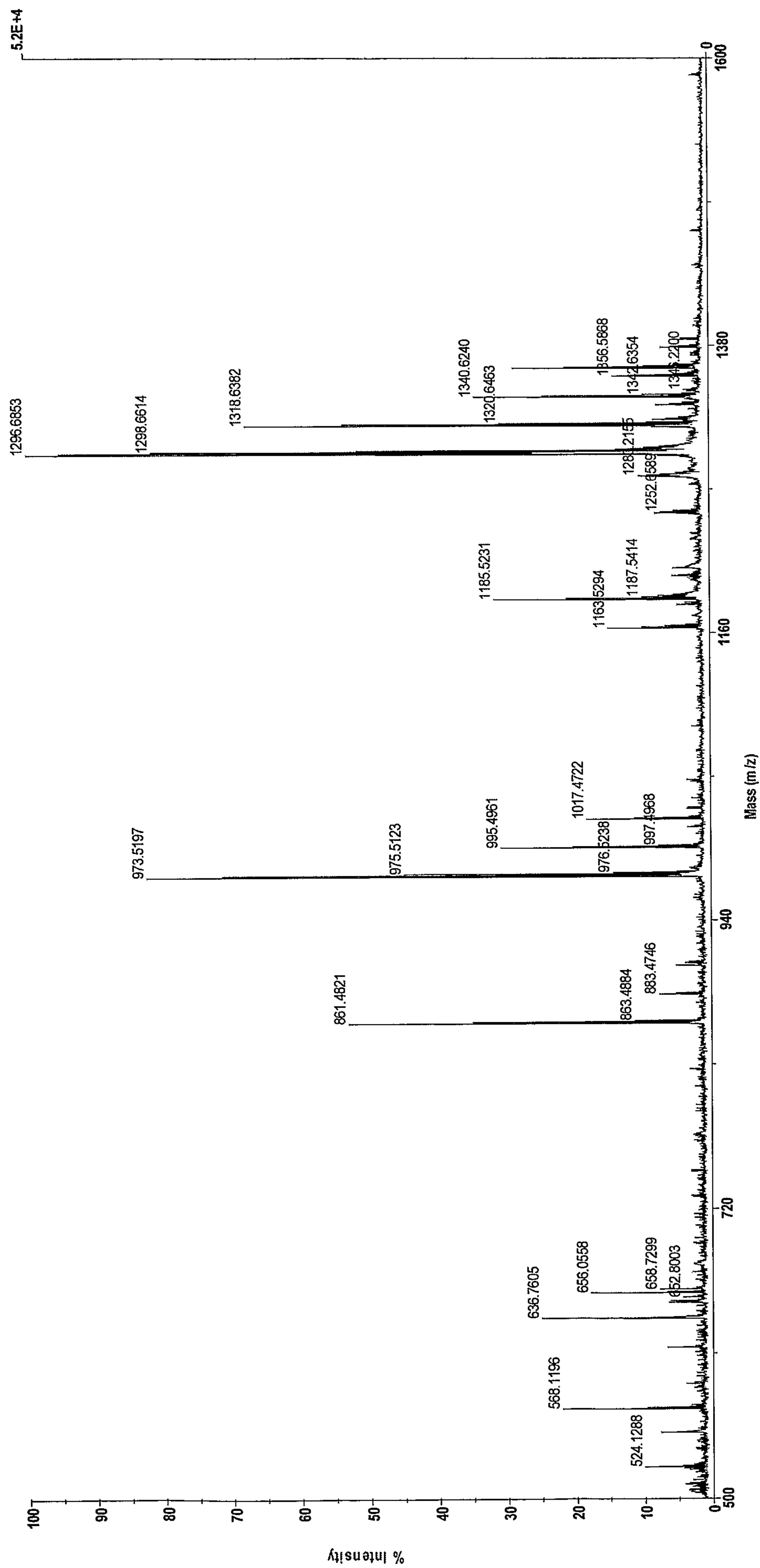
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Figure 72

Mass spectrum (WALDI-TOF) of Compound X

X+matrix(CHCA)+Angiotensin after calibration

Voyager Spec #1 =>MC [BP = 1296.7, 52168]



The mass of the major peak is (a) 1185.5231 and (b) 1163.5294

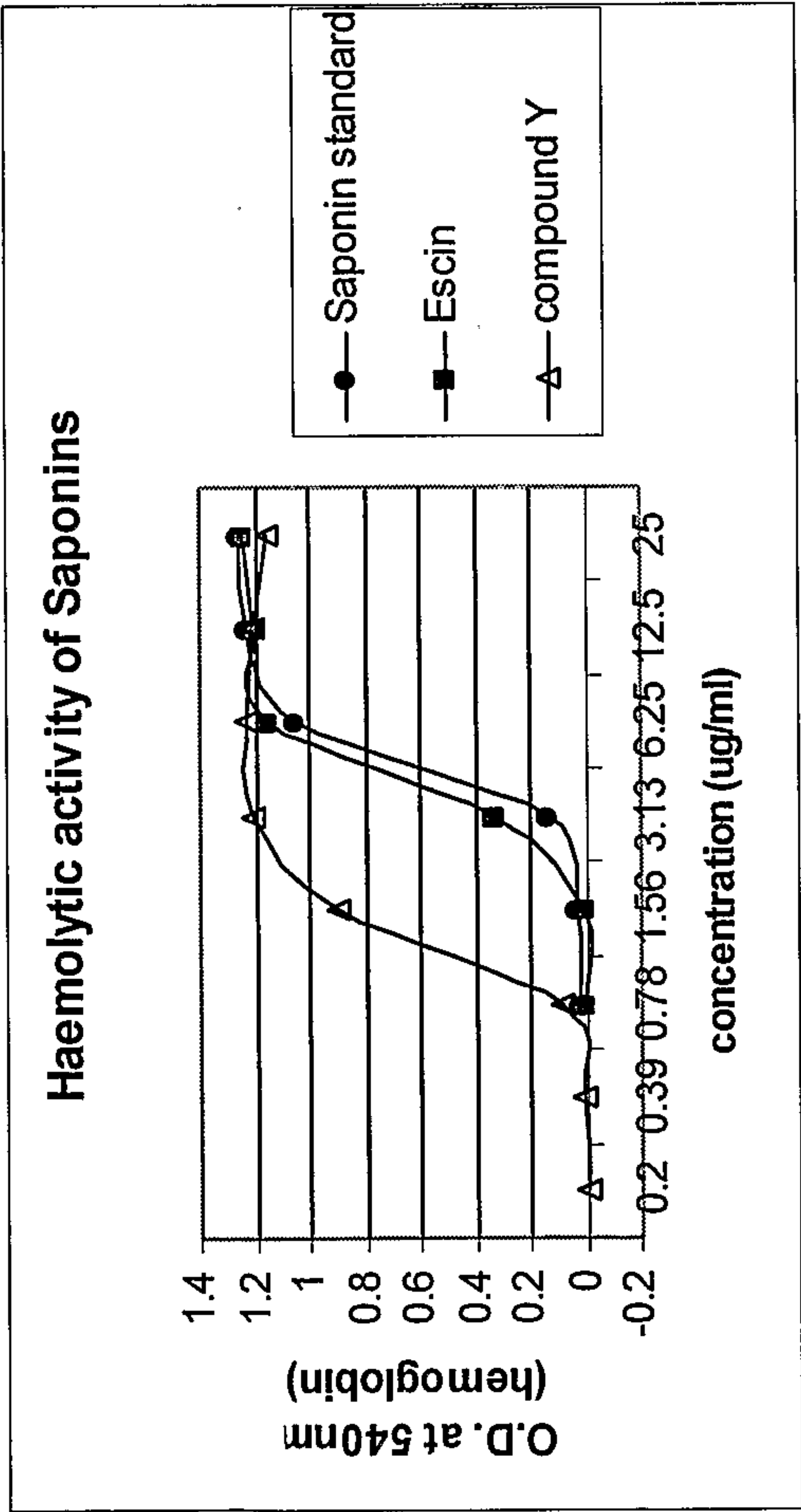
The theoretical mass of X is 1140.608

(a) $X + 2Na - H = 1140.608 + 45.9796 - 1 = 1185.5876$ (b) $X + Na = 1140.608 + 22.9898 = 1163.5978$

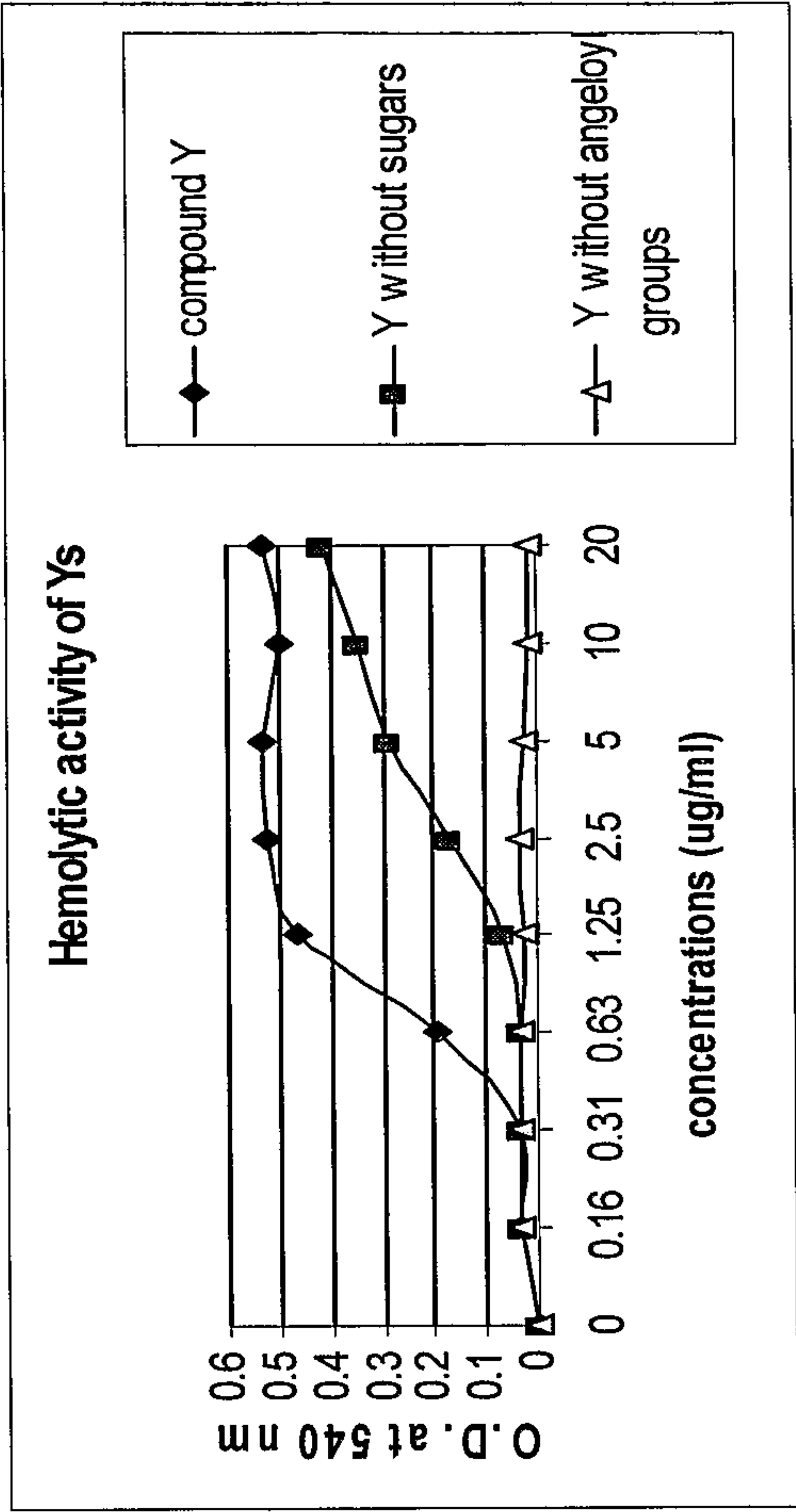
Figure 73

Haemolytic and Mtt activities of Compound Y

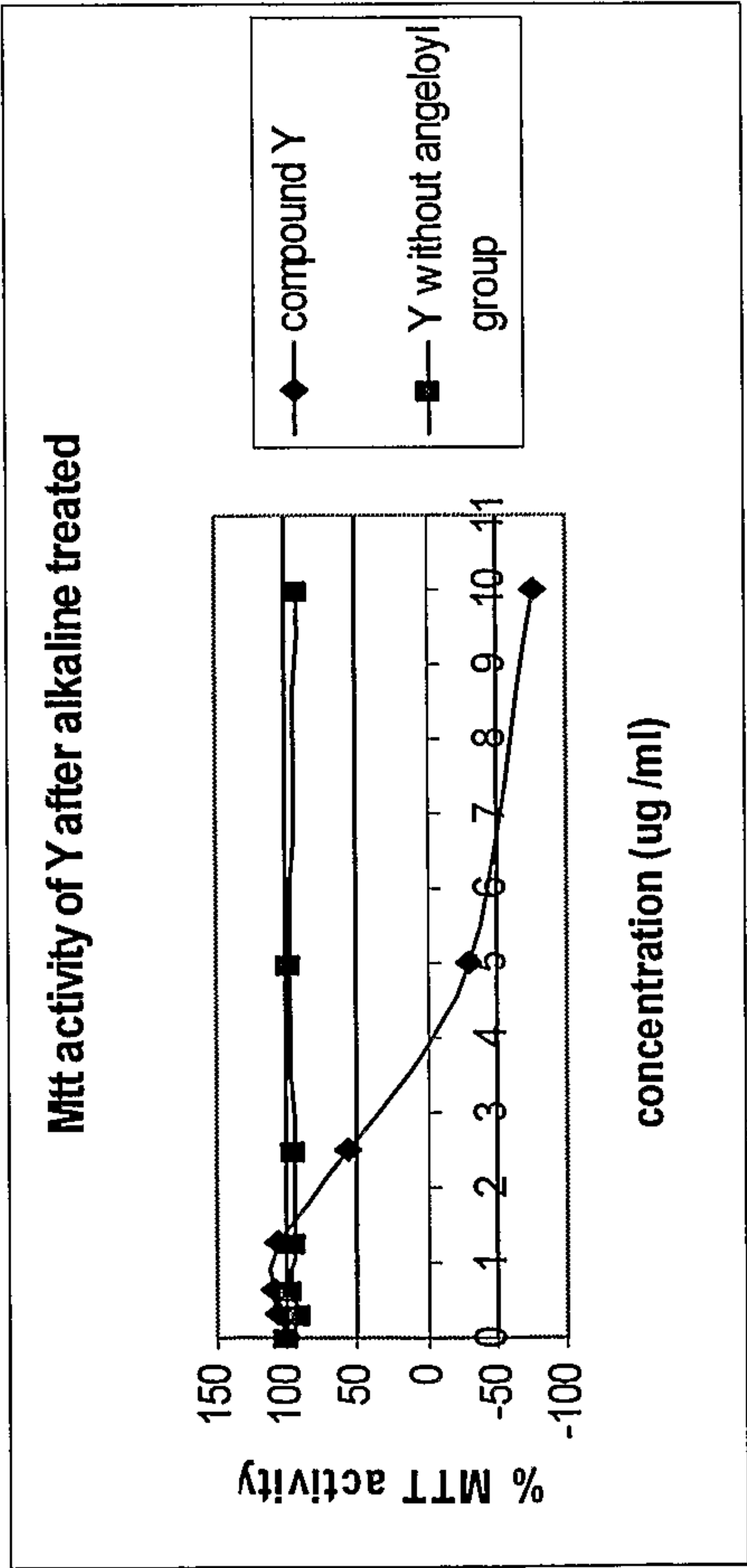
A



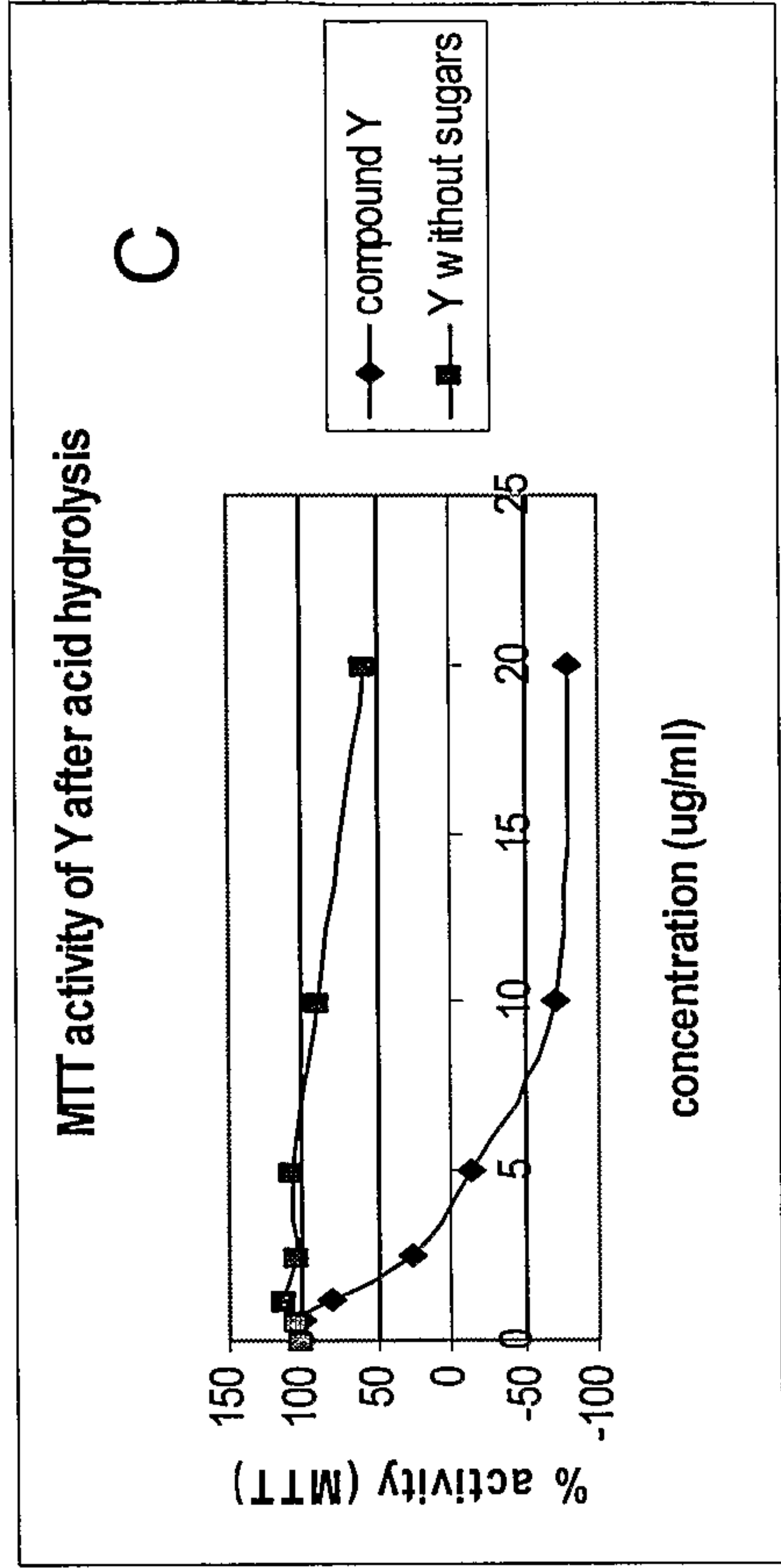
B

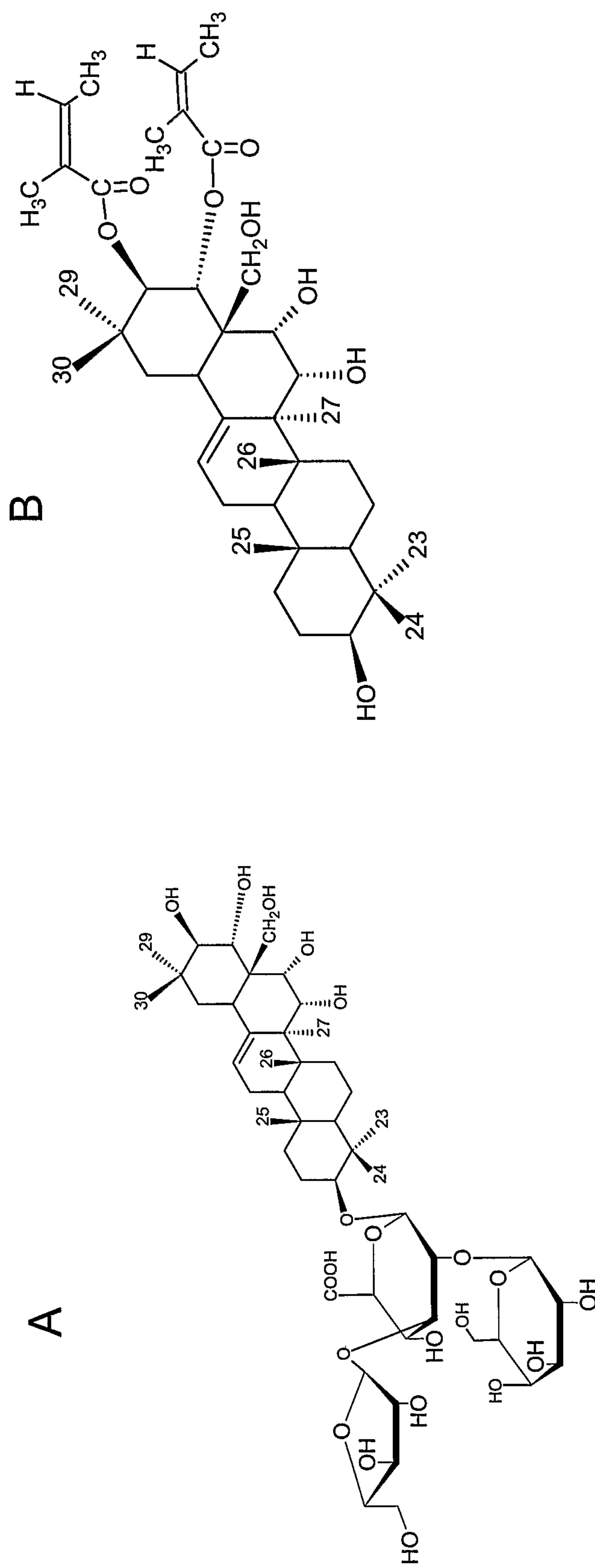


C



D

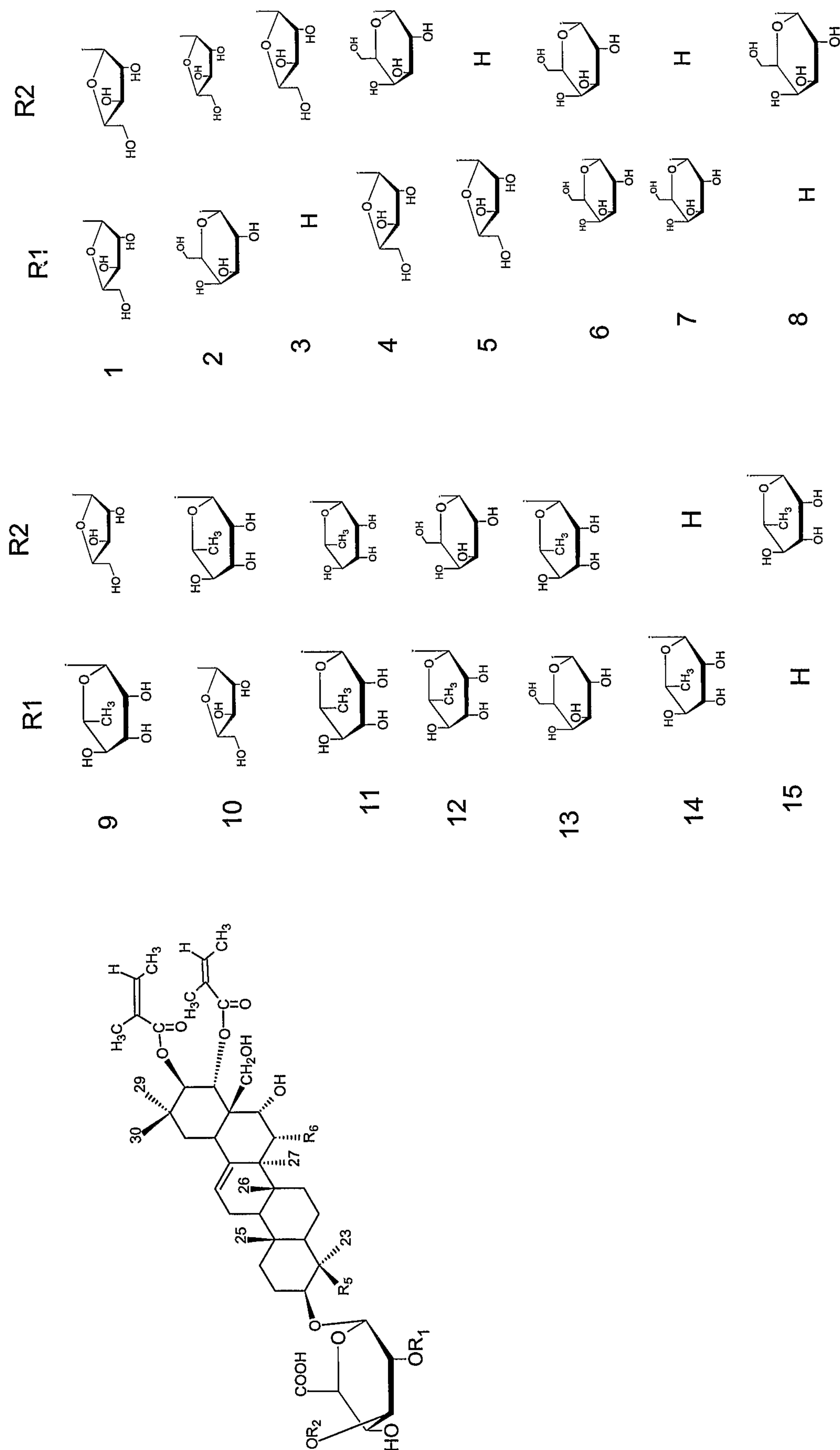


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Figure 74

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Figure 75

Structures of Compounds



R6=H or OH

R5=CH3 or CH2OH

R1= D- glucose or D-galactose or L-rhamnose or L-arabinose or, D-xylose or alduronic acid or D- glucuronic acid or D-galacturonic acid

R2=D- glucose or D-galactose or L-rhamnose or L-arabinose or, D-xylose or alduronic acid or D- glucuronic acid or D-galacturonic acid

Structure of six anticancer Saponins with biangeloyl groups (Y3 (Y), Y2, Y8, Y10, Y1 and Y9)

