國立台灣大學理學院化學研究所

碩士論文

Department of Chemistry

College of Science

National Taiwan University

Master Thesis

以結構與反應機轉設計岩藻醣轉移酶之抑制劑

Development of α-Fucosyltransferase Inhibitors by Structure- and Mechanism-Based Design

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中華民國 98 年 5 月

May, 2009

謝　誌

終於畢業了！幫助我完成這篇論文的幕後推手實在無法一一感謝，不如就謝天吧！有機會在這一刻寫下這篇謝誌，也就表示我的人生終於再往前跨一大步，現在的心情實在感動到無法用言語表示，興奮到想飛起來大叫，大海也無法澆熄我的熱情。

能夠順利完成這篇論文真的要很感謝我的指導老師林俊宏老師和方俊民老師，兩位老師細心地教導下，讓我從懵懵懂懂到現在順利的拿到碩士學位。感謝林俊宏老師對我的一切如此包容，在老師實驗室學習到許多化學合成和生化方面的學問，還有定期出遊讓實驗室氣氛變的很歡樂，讓我能在快樂中作實驗，希望老師作研究的同時也不要忘了要休息，身體健康才是活力的泉源喔！感謝方俊民老師讓我學習到對實驗謹慎小心的態度，有機合成的紮實更是我學習的對象，謝謝老師給我這麼好的實驗環境和研究氣氛讓我更無憂慮並全心的致力於實驗上。

感謝中研院基因體中心的楊文彬老師與鄭偉杰老師撥空擔任我的口試委員並細心的修改我的論文，口試時也給我許多意見讓我獲益良多，讓我知道學問知識是可以去懷疑挑戰的，而非全盤接收，這樣的思考模式在我往後的學習生涯上必定會深受其用。

幕後功臣真的太多太多，最先要感謝的是吳世雄老師，您真是我生命中的貴人，感謝老師那時給我擔任研究助理的機會，鼓勵我繼續申請研究所，我想，沒有您當時的一句話，我不會有機會從台大畢業，老師，真的非常感謝您！還有師大的黃文彰老師，謝謝老師當時幫我寫推薦函，還很體貼的先問了我的近況，這對那時的我而言是相當寶貴的安慰。還有佛心來的佳甫學長，在我裝滿食物的腦袋中塞了許多有關磷酸的知識，還有很多實驗上的小技巧，深厚的底子是我望塵莫及，學長，你的未來不是夢，一定可以做到的；跟我同甘共苦的育瑞同學，幫助我進行生物活性的測試，不厭其煩地講解到底 K_i 是怎麼得到的 (雖然我現在依然想不起來)，我也要祝你畢業順利；照片會騙人的鼎堅大叔，大大感謝你在口試時為我擋了好幾槍，從你這裡也學到了好多分子模擬界的精髓，希望你右邊和後方的女孩不會忽略你。還要感謝我的花友智傑，你的驚人記憶力總讓我嘖嘖稱奇，感謝人生路上可以遇到你這樣的好友，不管在專業知識或生活體驗上都給我莫大幫助，下次再遇到請叫我無敵大正妹、總愛男扮女裝大跳妞妞舞的崇冠，謝謝你常在我心情不好時還白目的惹我生氣讓我出氣、很會享受生活又擁有魔鬼身材的衝浪型男蛋頭怪文鴻，別忘了我們是 704 四大金釵、刀子嘴豆腐心又愛分享怪東西的書銘，記得下次要找我連線，我現在已經是馬力歐賽車的一把交椅，以及正經八百很想把我導向正途其實快被我帶壞的 hood 友効吾、美食品味家又同時致力於瘦身的順原學長、對我很好卻很愛亂花錢的冠蓉，很開心我們度過了許多充滿食物的下午茶時間，希望可以在國外與你相遇、很好勞役的學弟丹哥，在我最後階段任我使喚，但看到你還是想對你說：「對不起，我有事耽擱了」、原來是運動健將同時又是好媽媽的 Manjusha，你是我第一個外國朋友，知道你一定看不懂中

文，決定要翻譯送給你，「Dear Manman, hard to express how I feel about you, but I'm really happy to be a friend of you!」，還有奇怪的學弟聖偉，居然已經是一個可愛娃兒的老爸了、好客的 Reddy、小不點姿潔、美工很厲害又有可愛蘋果臉的昶思、扮相很性感的孟容、怎麼吃都不會胖的馥檀，總讓我好生羨慕以及安全帽和你不成比例卻有漂亮小腿的 handsome boy 俊男，還有頭髮永遠歪一邊的小朱、擁有可愛外表確有大媽個性的琇琄和作風脫序說話無厘頭的伯平，我很確定我的眼睛比你大上好幾倍！中研院因為有了你們，我的回憶變的多采多姿，讓我即使沈浸在實驗失敗的痛苦中，還可以有宣洩的管道，謝謝你們和我一起走過這幾年的歲月，不敢說把青春花在你們身上，不過肯定在你們在任期間多了幾條細紋，不管是快樂還是難過，有你們真好！

還有方老師實驗室的聰哥，總秉持「活到老，學到老」的學習態度、對音樂很有品味的文賢、愛恨分明合成實力雄厚的小卓、雙主修生化和美食的超人大溫、幽質美聲笑話很高級的公政、很講義氣又很細心的流氓阿彬，paper 不要發太快，你一定可以找到 perfect match 的、與唷吶的合照會騙人的謝瑋哲，要感謝的人還有好多，真的無法一一提出，如果可以的話真想給每個你一個大大的擁抱，外加一個曲式風格的笑話，謝謝你們在我經歷挫折時為我加油打氣，給我支持與鼓勵，才不會忘記你呢！

還有我的 F6 伙伴們，真感謝能遇到你們這樣真誠以對的一群死黨，理性與感性的小美心文，你的演唱會我沒空去、和我分享很多美裝經驗的小圓怡淳，抱歉還是忍不住想起你的綽號、溫柔的大頭貢丸宛俐，總是不吝惜給我加油打氣，超愛你的！男子氣概比誰都足的怪力倍偲、思慮清晰的阿伯嬿融，我不會忘記和你同住時更加「瞭解」你，笑話比我還冷的曉雯，你們從不隱藏給我意見，我們一輩子都要像這樣，以後我會接你們的電話的。還有跟我一起熬夜的婉玲，感謝你的美食團購和無限量供應我這個食量超大的咬橘子動物。

謝謝凰祺在我念研究所這幾年來一路上給我的支持與陪伴，路途比別人漫長的感覺只有你最清楚，謝謝你快樂時陪我瘋狂去看海，夜深人靜時接起我的奪命連環 call，還能冷靜的給我安慰，我要把這份殊榮與你分享。

最重要的，我要大力感謝我的家人，謝謝你們讓我可以任性這麼久，每次打電話回家總可以聽聽今日菜單好安慰我不能回家吃飯的無奈，掛電話前的那句：「沒什麼事吧？」，聽了覺得好安心，好像有一座大山在我背後做我的依靠，除了給我的強力支持以外，你們賜給我的眾多「小朋友」們讓我可以在打拼論文的強大壓力下偷閒陷入網拍的迴圈中。還有我的豬寶拉麵、寶貝妹妹和機車多多，我要頒發十盒西莎感謝你們，因為看到你們的可愛讓我把煩惱的事情拋到腦後。

通往甜美果實的路上依然可能佈滿小石子試圖要絆倒我，但我帶了一把萬能鑰子要清光這些阻礙，度過了這幾年，現在的我更充滿勇氣去挑戰未來，一路上有你們的陪伴，讓我的人生到目前整個是部「開朗少女成功記」，任務圓滿成功！

盈曲　2009.6

於中研院生化所R704的賽車椅上

中文摘要

生物體中岩藻醣轉移酶常催化最後一個轉醣步驟，而形成如 Lewis y 與 sialyl Lewis x 含有岩藻醣的寡醣。由於這些寡醣的重要生理活性 (諸如與癌細胞轉移及細菌感染的關聯性)，使得這類酵素常被視為藥物開發的目標。此篇碩士論文即針對岩藻醣轉移酶抑制劑的設計、合成及其應用作探討。

根據先前所報導的 X-ray 晶體結構及其反應機轉，在抑制劑的設計上有三項特點。二磷酸鳥苷 (guanosine diphosphate) 在與岩藻醣轉移酶間的作用力上，扮演重要的角色；酵素反應的過渡態 (transition state) 具有正電荷的特徵；醣受體結合部位附近之疏水區域。針對這些特點，設計出具有比咯啶 (pyrrolidine)、吡咯烷 (piperidine)、嘧唑 (imidazole) 的二磷酸鳥苷衍生物為細菌及人類岩藻醣轉移酶抑制劑，並探討其分子結構與活性間的關係。

除此之外，本實驗室先前合成一系列具有二磷酸鳥苷的岩藻醣抑制劑，以篩選三唑環上不同衍生基團對生物活性的影響，將此結果中效果最好的衍生基團連結到比咯啶環抑制劑 **YCC-7** (化合物 **61**)。此化合物對胃幽門桿菌的岩藻醣轉移酶抑制效果最好，IC_{50} 及 K_i 值分別為 44.1 μM 及 29.5 μM。我們進一步以電腦模擬計算解釋抑制劑 **YCC-7** 與該酵素間的作用力。

Abstract

α-Fucosyltransferases (FucTs) usually catalyze the final steps in the biosynthesis of fucose-containing oligosaccharides. Owing to the related biological significance (such as tumor metastasis and bacterial infection), these enzymes are considered as the targets for therapeutic intervention. This thesis is mainly focused on the design, synthesis and evaluation of FucT inhibitors. On the basis of the reported x-ray crystal structures and mechanistic studies, the molecules were designed to include guanosine diphosphate (GDP) that offers major binding affinity, a negative-containing group to mimic the positive-charge character of the transition state, and a hydrophobic group to acquire additional affinity. Several GDP-conjugated pyrrolidines, piperidines and imidazoles were prepared and evaluated as the inhibitors against the FucTs from *Helicobacter pylori* and human. The structure and activity relationship was also discussed.

Furthermore, a series of GDP- and triazole-containing compounds were also developed as FucT inhibitors previously. Because 2'-(phenylsulfonyl-methyl)benzyl group was found to be the best hydrophobic group attached to the triazole, the same group was hens coupled with GDP-pyrrolidine to give **YCC-7** (**61**). **YCC-7** was found to be a potent inhibitor against *H. pylori* α-1,3-FucT. The corresponding IC_{50} and K_i values are 44.1 and 29.5 μM, respectively. Computational modeling was further

employed for the explanation at molecular basis.

目錄

表目錄

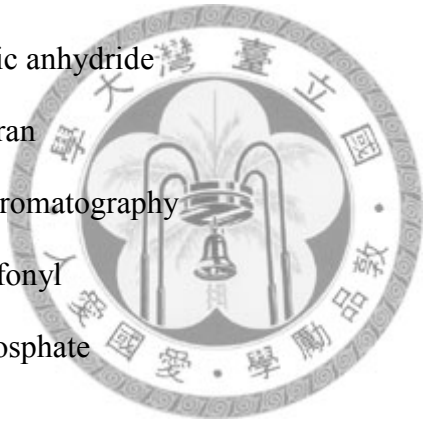
圖目錄

流程目錄

縮寫對照表

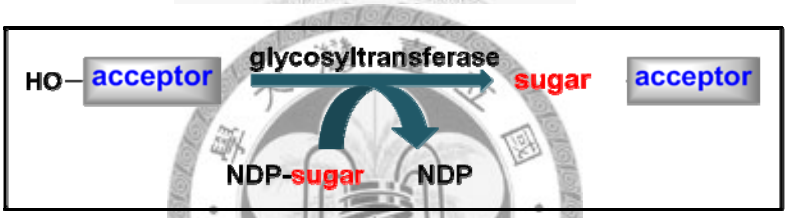
Arg	arginine
Asp	aspartate
Boc	*tert*-butoxycarbonyl
CAN	ceric ammonium nitrate
CAT	catalytic domain
Cbz	carbobenzoxy
CDI	1,1'-carbonyldiimidazole
DCC	dicyclohexyl carbodiimide
DMAP	4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
Et_3N	triethylamine
FucT	fucosyltransferase
Gal	galactose
GDP	guanosine diphosphate
GlcNAc	*N*-acetylglucosamine
Glu	glutamate
GMP	guanosine monophosphate
***h*FucT**	*Human* fucosyltransferase
HPLC	high performance liquid chromatography
***Hp*FucT**	*Helicobacter pylori* fucosyltransferase
LacNAc	galactose-β1,4-*N*-acetylglucosamine
Leu	leucine
Le	Lewis antigen

LPS	lipopolysaccharide
Lys	lysine
***m*-CPBA**	*meta*-chloroperoxybenzoic acid
MeCN	acetonitrile
NBS	*N*-bromosuccinimide
NMR	nuclear magnetic resonance
PDB	protein data bank
PPh_3	triphenylphosphine
SLe	sialyl Lewis antigen
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
TLC	thin-layer chromatography
Ts	4-toluenesulfonyl
UDP	uridine diphosphate



第一章 緒論

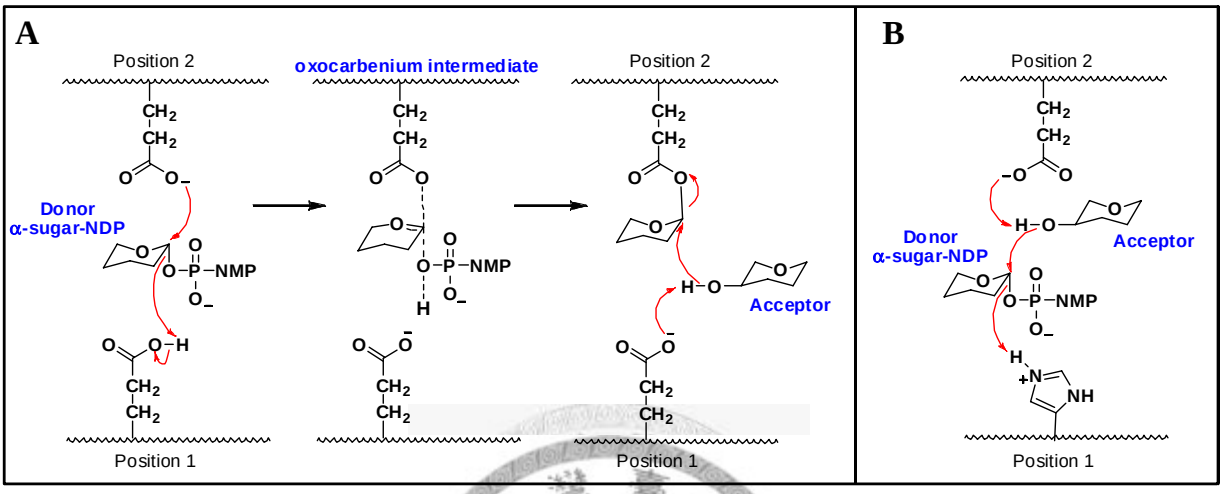
醣化反應 (glycosylation) 在生物體內需要許多酵素的參與，如醣類轉移酶 (glycosyltransferase)。醣類轉移酶催化各種醣共軛分子 (glycoconjugate)的醣化反應 (圖一)。[1,2] 醣類轉移酶將由核苷單磷酸或核苷二磷酸 (nucleoside mono- or diphosphate) 活化的醣予體 (sugar donor substrate) 轉移到適當的醣受體 (sugar acceptor substrate) 上，同時釋放出一分子的核苷單磷酸或核苷二磷酸，而所形成的醣類接和物，如醣脂質、醣蛋白等，在生物體內扮演多樣角色，如細胞間的辨識、細胞轉移、以及免疫反應，因此被視為藥物開發的目標。[3,4,5]



圖一、 醣類轉移酶作用示意圖 [1]

醣類轉移酶有兩種分類方式，可依據催化反應的受質以及催化機制來進行區分：以催化反應的醣予體受質來分，可分為常見的 galactosyltransferase、glucosyl-transferase、sialyltransferase 以及 fucosyltransferase 等；催化反應機制的分類可分為保留機制 (retention) 以及反轉機制 (inversion) 兩種，是依醣分子在轉移時變旋異構中心的立體化學組態是否改變而分別。在醣體轉移的催化區 (active site) 中，帶有羧基的胺基酸扮演重要的催化角色，包括為天門冬胺酸 (aspartate) 或是麩胺酸 (glutamate)。在保留機制中，Asp/Glu 擔任一般酸 (general acid)，將醣苷鍵(glycosidic bond) 質子化 (protonation)，另一個 Asp/Glu 擔任親核基 (nucleophile)，直接攻擊變旋異構中心 (anomeric center)，形成醣化中間體 (glycosyl-enzyme intermediate)，在醣受體進入酵素活化中心後，在一般鹼 (general base) 的作用下，形成立體組態

相同的產物 (圖二 A)，[6] 因此在保留機制中，會發生兩次反轉機制，使其立體組態保持不變；而反轉機制中，一個 Asp/Glu 去質子化醣受體，使醣受體直接對醣予體之變旋異構中心作親核性攻擊 (nucleophilic attack)，離去一分子核苷磷酸或二磷酸，而得到立體組態相反的產物 (圖二 B) 。[6]



圖二、 醣類轉移酶催化反應機轉中，醣予體之構型 A：保留；B：反轉 [6]

其中岩藻醣轉移酶催化合成許多具有岩藻醣的寡醣分子，如：sialyl Lewis x (SLe^x)。SLe^x 不只在細胞間作用或黏著上有貢獻，與發炎反應、胃潰瘍或是癌症轉移也有相關。[7] 因此本論文針對岩藻醣轉移酶抑制劑的設計、合成及其活性測試作探討。

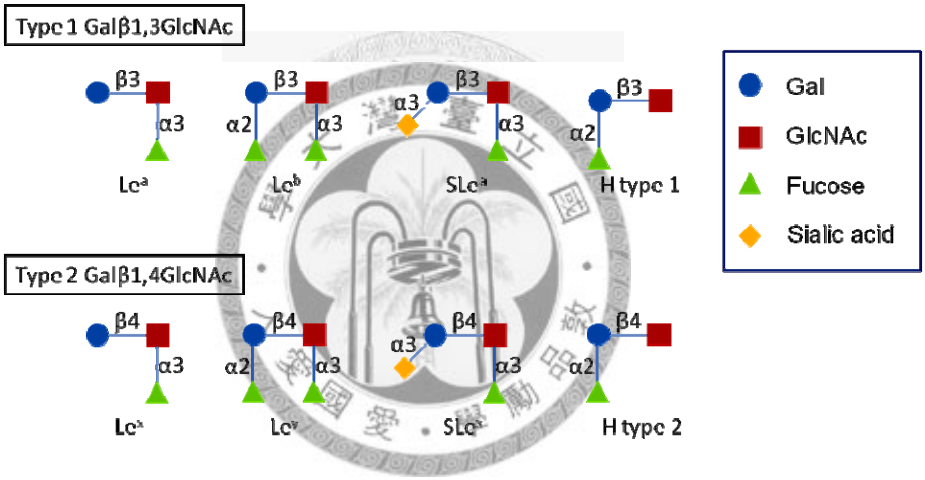
1. 岩藻醣轉移酶 (Fucosyltransferase；FucT) 的介紹

生物體中岩藻醣轉移酶參與許多生理行為，如受精作用、胚胎形成、[8] 淋巴細胞運行、免疫反應以及癌細胞的轉移。[2] 岩藻醣轉移酶廣泛地存在於植物、昆蟲、寄生蟲以及菌體中。[9,10] 岩藻醣轉移酶常催化醣生合成中的最後一個轉醣步驟，而形成具有岩藻醣的寡醣 (fucosylated oligosaccharide)。[11]

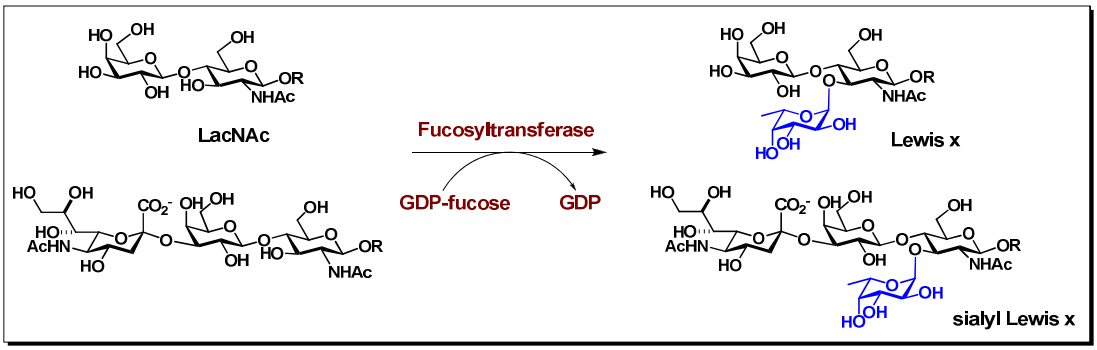
在岩藻醣轉移的過程中，岩藻醣轉移酶 (FucT) 將接有二磷酸鳥苷的岩藻醣 (GDP-fucose) 催化轉移到醣受體 (sugar acceptor substrate) 上，釋放出一分子核苷

二磷酸。[2] 依所形成的醣苷鍵 (glycosidic bond) 位置不同可區分成三個類別，分別為α1,2–、α1,3/4–、α1,6–FucT，[12] 而這三種岩藻醣轉移酶都屬於反轉催化機制。[13] 其中α1,2–、α1,3/4–FucT 與 Lewis 抗原 (如：Le^a、Le^b、Le^x、Le^y，圖三、圖四) 的生合成最後一個步驟中有關。

Lewis 抗原為具有岩藻醣的寡醣分子，分為第一型及第二型抗原。由 Galβ1,3GlcNAc 雙醣片段組成的寡醣分子為第一型抗原，如 Le^a，Le^b 與 sialyl Le^a; Galβ1,4GlcNAc 組成的則為第二型抗原，例如 Le^x，Le^y，sialyl Le^x 等。雖然某些哺乳動物細胞表面已報導存在 Lewis 抗原，但腫瘤細胞上往往會大量表現 (圖三)；例如肺癌及乳癌細胞上都發現有大量表現的 sialyl Le^x 及 sialyl Le^a。[14]



圖三、 第一型及第二型 Lewis 抗原



圖四、 以 FucT 催化合成之 Lewis 抗原

1.1 人類岩藻醣轉移酶的介紹

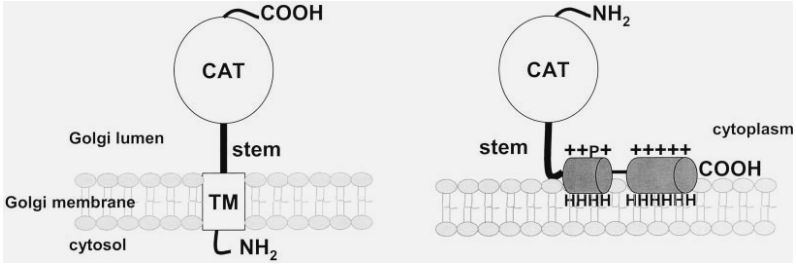
人體中的岩藻醣轉移酶依照胺基酸序列相似度不同，可分成十一大類。[15]FucT 1 及 FucT 2 屬於α1,2-FucT，將岩藻醣轉移至 galactose-β(1,4)-*N*-acetylglucosamine (Galβ1,4GlcNAc；LacNAc) 的 galatose 二號位置上，而形成不同組織中 H 抗原；FucT 8 屬於 α1,6-FucT，會將接在天冬醯胺上的 GlcNAc 岩藻醣化；FucT 3、4、5、6、7、9 屬於 α1,3/4-FucT，此六種則依醣受體、特性及分佈的不同作歸類。[16]

FucT 3 在消化道及腸胃組織的內皮細胞大量表現，這類酵素合成所有第一型 Lewis 抗原，包含 Lewis a、Lewis b 及 sialyl Lewis a，因此被稱為 Lewis 酵素。FucT 3 的胺基酸序列與 FucT 5 及 FucT 6 很相似，其中 FucT 5 對第二型受質做α1,3 位置轉移具相當大的活性，FucT 3 則對α1,4 位置轉移較有活性；而 FucT 6 只對第二型受質做催化，被認為是內皮細胞以及癌細胞上合成 sialyl Lewis x 的主要酵素。[15]

FucT 4 及 FucT 7 則在骨髓細胞中大量表現，分別催化內部 LacNAc[17] 及末端的 α2,3-sialylated LacNAc 的岩藻醣轉移。[18]FucT 9 也屬於 α1,3-FucT，它的胺基酸序列與其他岩藻醣轉移酶差異較大，只表現在粒性白血球 (granulocytes)、自然殺手細胞、B 細胞中，而且是存在腦中含量最多的 α1,3-FucT。相較於其他型岩藻醣轉移酶對內部 LacNAc 做岩藻醣轉移，FucT 9 較常對 polyLacNAc 末端的 GlcNAc 作用。FucT 10 及 FucT 11 也屬於 α1,3/4-FucT，但缺乏分辨第一型及第二型受質的氨基酸序列，而 FucT 11 不具有與細胞膜嵌合的區域。[19]

不同的岩藻醣轉移酶會在細胞表面上合成不同的 Lewis 抗原，因而影響細胞間的辨識，但由於每種人類岩藻醣轉移酶會有部分相似的功能，使得利用岩藻醣連接的位置來區別各型的酵素活性會一定的困難度。

目前所有真核生物的岩藻醣轉移酶都是第二型膜蛋白，具有一個典型的結構，其 N 端位於細胞質中，接有與細胞膜嵌合的區域，而 C 端為一催化區域 (圖五)。[20,21] 真核生物的 FucT 上的 C 端構型穩定，N 端則屬於高變異區且被認為與醣受體的辨識有關。[20]



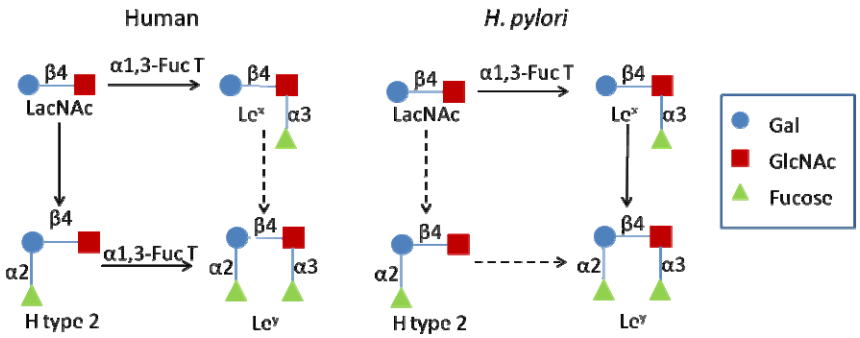
圖五、真核生物(左)與幽門螺旋桿菌(右)的岩藻醣轉移酶示意圖 [20,21]

1.2 幽門螺旋桿菌岩藻醣轉移酶的介紹

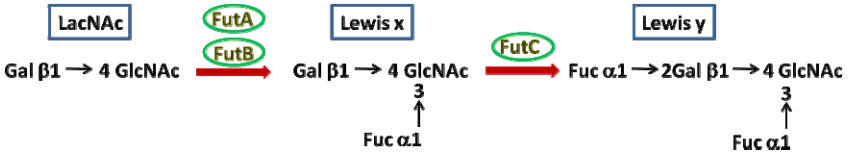
相較於人類的岩藻醣轉移酶，幽門螺旋桿菌的岩藻醣轉移酶缺少與細胞膜嵌合的區域，其活性催化區域則為在 N 端 (圖五)。其 C 端具有七重覆組合 (heptad repeats region)，此區域由七個胺基酸為一個單位 (D(D/N)LR(V/I)NY))，重複二到十個片段，再連接一段帶大量正電及疏水區域的片段，這種雙性分子在功能上被認為可以仿造真核生物岩藻醣轉移酶上與細胞膜嵌合的部分，前者所形成的亮胺酸鏈 (leucine zipper) 則推測可能使得岩藻醣轉移酶形成二聚體，而後者推測是用來與磷脂細胞膜結合的區域 (圖五)。[22,23,24]

幽門螺旋桿菌的α1,2 及α1,3/4-Fuc T 都已證明可催化合成 Lewis 抗原，[25,26,27] 有別於人類 FucT ，幽門螺旋桿菌的α1,2-Fuc T 會以 Le^x 為受質合成 Le^y 而非直接利用 LacNAc (圖六 A)，[26] 而α1,3/4-Fuc T 則是由兩種基因來的，*fut*A 及 *fut*B，形成的兩種異形體 Fut A 及 Fut B，Fut A 偏好將內部的 LacNAc 岩藻醣化，而 Fut B 則會將末端的 LacNAc 岩藻醣化 (圖六 B)。[25]

A



B



圖六、A：人類與幽門螺旋桿菌 FucT 合成 Lewis 抗原路徑。

B：幽門螺旋桿菌 FucT 合成 Lewis 抗原路徑。[25]

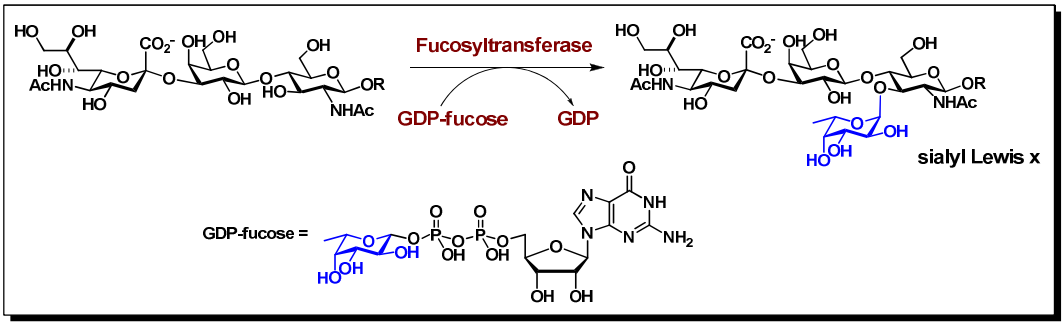
2. 岩藻醣轉移酶的功能

總括而言，岩藻醣轉移酶及催化合成各種具有岩藻醣之醣蛋白及醣脂質。不同類型的岩藻醣轉移酶其醣受體不盡相同，但其醣予體都為岩藻醣二磷酸鳥苷 (GDP-fucose)。

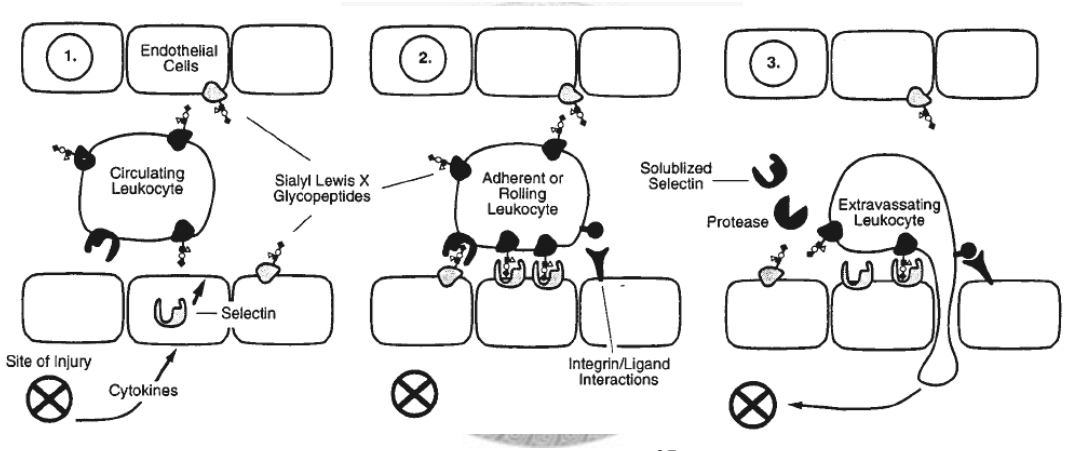
岩藻醣基化 (fucosylation) 在生物體內是寡醣分子上最常見的修飾，是將岩藻醣連接在多醣分子的氧或氮原子上。據報導，像發炎或是癌症等的致病環境都與岩藻醣基化的增多有關係，再者，岩藻醣基化是癌症中一種常見的醣化反應，因此岩藻醣基化的蛋白已被用來作為癌症標記分子。[28]

具有唾液酸的 Lewis 抗原 (sialyl Lewis groups；圖七) 是一種常見的 selectin 受體，為淋巴細胞膜表面上醣化神經磷脂或醣蛋白的一部份。[29] 從生物體內抗發炎反應的流程中可以知道，受損傷的組織會釋放出細胞因子促使內皮細胞表現 E-及 P-selectin，此兩種蛋白質會辨識白血球上的 SLe^x 及其他寡醣分子 [30] 而使白血球可黏著在內皮細胞表面 [31,32,33]，白血球表面則表現 L-selectin 去辨識內皮細胞上相似

結構的醣類受質[34,35]。利用這些酵素及其受質結合使白血球滾動，[36]使白血球上的整合蛋白 (integrin) 和內皮細胞上的蛋白質結合，促使白血球進入發炎部位 (extravasation；圖八)。[37,38]



圖七、合成具有唾液酸之 Lewis 抗原

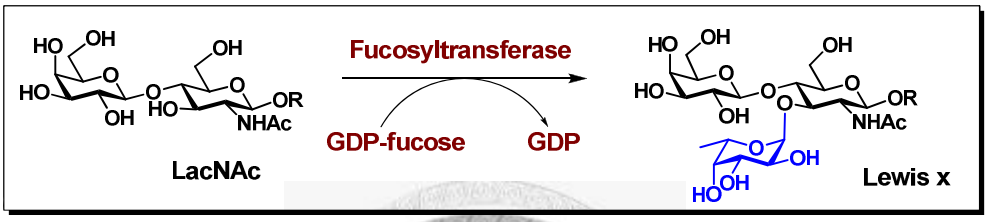


圖八、發炎反應流程[37]

在白血球黏著缺乏症這種遺傳疾病中發現，由於無法進行 SLe^x 的生合成，使得白血球無法進行黏著在內皮細胞上，進而無法進到發炎部位，此類患者常會重複受到嚴重的細菌感染。[29]

另一方面，也有相當多急性或慢性疾病是因為太多白血球停留在感染或致病因形成的部位，如心因性休克、中風、血栓形成、風濕、皮膚炎、腦炎等。除此之外，癌症的轉移也與 SLe^x 或其他癌細胞表面的寡醣分子的表現量以及其與內皮細胞的 selectin 作用有關。因此目前著重在如何阻擋細胞進行黏著，以發展抗血栓劑、免疫抑制劑或癌症轉移阻抗的研發藥物。[29]

另外，幽門螺旋桿菌的岩藻醣轉移酶也在最後一步醣化作用中將岩藻醣轉移至 LacNAc 而形成 Le^x (圖九) 及其他的 Lewis 抗原，包括 Le^y、Le^a 及 Le^b，而 Le^x 形成 oligomer 或 polymer 存在幽門螺旋桿菌中脂多醣 (LPS, lipopolysaccharide) 的 *O*-antigen 中，此分子模擬寄主細胞表面上的 Lewis 抗原使寄主細胞的免疫系統無法分辨這類分子，造成幽門螺旋桿菌長期在胃黏膜表面上長期感染，已被推測與胃潰瘍、胃癌有所關連。[39]



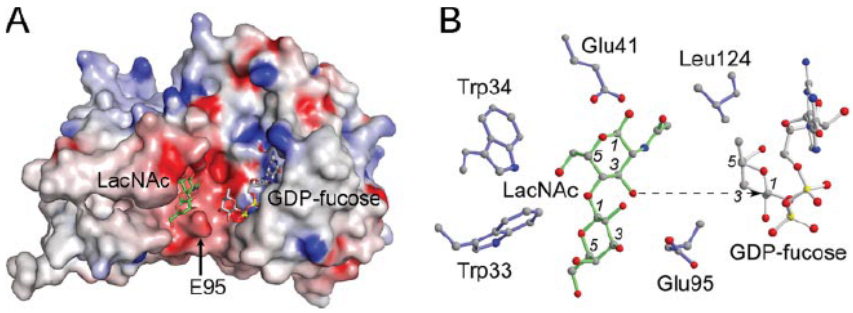
圖九、Le^x 抗原之合成

3. 岩藻醣轉移酶的催化機制

目前所有的岩藻醣轉移酶已被證實都屬於醣反轉轉移催化機制 (inverting glycosyltransferase)，[13] 由於許多這類酵素的晶體結構已知，[40] 可由此推測出岩藻醣轉移酶的催化機制。

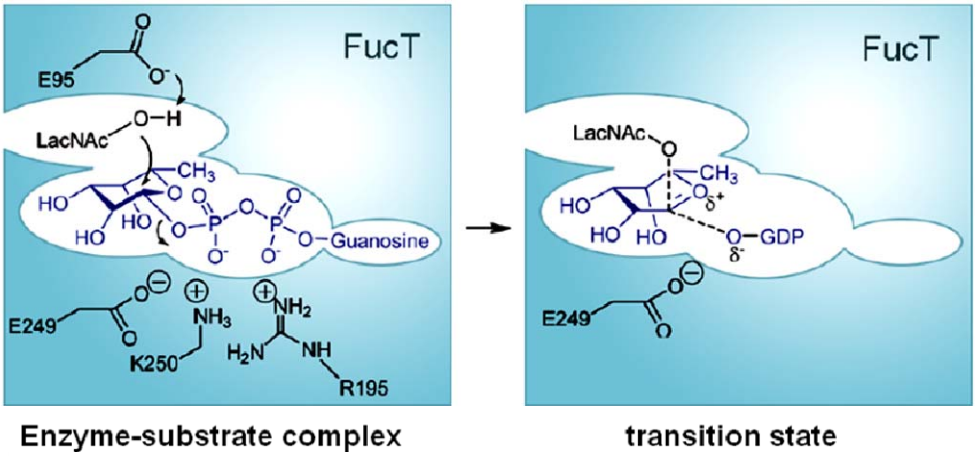
醣反轉轉移酶中最為熟知的β-glucosyltransferase 就是利用 Asp100 當作一般鹼，相對於岩藻醣轉移酶中的 Glu95。而 Glu95 位在 N 端區中推測與醣受體有關，除此之外，Glu95 的支鏈正好落在 GDP-fucose 中岩藻醣上的變旋異構中心附近 (圖十 A)，加上定點突變的實驗結果，佐證了此胺基酸擔任一般鹼的角色。[39]

利用一般鹼及醣予體的位置、酵素中活化位置的構形及方位可以模擬出醣受體 LacNAc 在酵素中的位置，由此圖可以看出 GlcNAc 的三號羥基與岩藻醣轉移酶的變旋異構中心以及 Glu95 的羧酸在鄰近位置 (圖十 B)。[39] 以此岩藻醣轉移酶與受質的結構，可推測出與人類岩藻醣轉移酶相似的催化機制。[41]



圖十、A：模擬醣受體 LacNAc 在岩藻醣轉移酶中的環境。B：模擬 LacNAc、GDP-fucose 及鄰近胺基酸相對位置，其中虛線表 GlcNAc 對 fucose 做親核性攻擊。[39]

LacNAc 會與岩藻醣轉移酶活化區域中 N 端帶負電的區域作用，其 GlcNAc 的三號位置羥基被 Glu95 去質子化後，氧原子對 GDP-fucose 中岩藻醣的變旋異構中心作親核性攻擊，形成醣苷鍵，並具有反轉立體結構的寡醣分子 Le^x，放出一分子 GDP (圖十一)。[39] 由於此變旋異構中心位在 GlaNAc 三號羥基及離去基 GDP 中間，推測應產生 S_N2-like 的立體化學逆轉。



圖十一、FucT 催化機制之推測 [39]

在此催化機制中，我們可以將各個步驟歸納出以下特點：醣受體進入酵素活化區後二磷酸鳥苷的磷酸部分，會與鄰近胺基酸 Lys250 及 Arg195 有靜電作用力；當醣受體去質子化對醣予體做親核性攻擊後，將會使岩藻醣本身在過渡狀態

(transition state) 中，由原本的椅型構形 (chair form) 扭轉成 half-chair 構形，另外，原本與二磷酸鳥苷鍵結的醣苷鍵變弱，使岩藻醣變成帶有 oxocarbenium ion 的過渡態，周圍帶有負電的胺基酸如 Glu249 則會穩定過渡狀態中帶正電的部分，如 oxocarbenium ion 及離去基二磷酸鳥苷。

從 X-ray 結晶結果中可知岩藻醣轉移酶活性區中鄰近胺基酸與二磷酸鳥苷有 13 個氫鍵作用力，而與岩藻醣本身只有 5 個氫鍵作用力，得知岩藻醣轉移酶與二磷酸鳥苷間作用力更甚岩藻醣，因此推測在設計岩藻醣轉移酶之抑制劑時，二磷酸鳥苷會扮演相當重要的角色。由以上催化機制中的幾個特點，將可以此對不同的受質及狀態設計岩藻醣轉移酶之抑制劑 (表一)。[39]

H-bonds between GDP/GDP-fucose and FucT residues

FucT residues	H-bond distance of	
	GDP	GDP-fucose
	Å	
Val-222-O	Base N1 (2.6)	Base N1 (2.7)
Lys-223-O	Base N2 (3.0)	Base N2 (3.2)
Ser-188-Oγ	Base N7 (2.8)	Base N7 (2.8)
Val-186-O	Ribose O2′ (2.8)	Ribose O2′ (2.8)
Lys-225-Nζ	Ribose O3′ (3.1)	Ribose O2′ (2.7)
Lys-225-Nζ		Ribose O3′ (2.9)
Glu-249-Oϵ2	Ribose O3′ (2.8)	Ribose O3′ (2.9)
Ser-188-N	α-Phosphate O2A (2.7)	α-Phosphate O1A (2.9)
Asn-189-N	α-Phosphate O2A (2.8)	α-Phosphate O1A (3.0)
Arg-195-NH1	β-Phosphate O3B (2.7)	α-Phosphate O2A (3.2)
Asn-189-Nδ2		β-Phosphate O1B (2.7)
Lys-250-Nζ	β-Phosphate O2B (2.6)	β-Phosphate O2B (3.1)
Arg-195-NH2	β-Phosphate O3B (2.7)	β-Phosphate O3B (2.6)
Asn-240-Nδ2	β-Phosphate O1B (2.6)	Fucose O2 (2.4)
Tyr-246-OH		Fucose O2 (2.8)
Gly-94-O		Fucose O3 (2.5)
Tyr-246-OH		Fucose O3 (2.5)
Glu-249-Oϵ1		Fucose O4 (2.7)

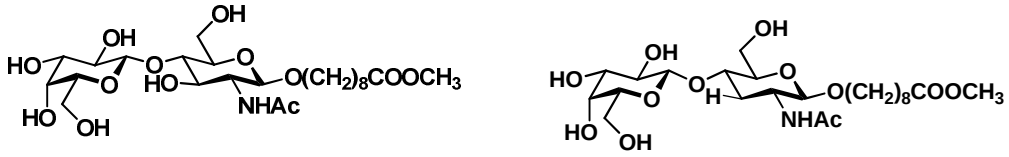
表一、受質 GDP-fucose 在 FucT 活性區域中所具有的氫鍵作用力 [39]

4. 岩藻醣轉移酶抑制劑的文獻回顧

醣類轉移酶的抑制劑可區分成三個類型：醣受體類似物 (acceptor substrate analogues)、醣予體類似物 (donor substrate analogues) 及過渡狀態類似物 (transition-state analogues)。

4.1 醣受體類似物 (acceptor substrate analogues)

醣受體類似物用於與原生的醣受體競爭進入酵素的活性區域，以防止醣類轉移至原生的醣受體上。Palcic *et al.* 在 1991 年及 1996 年分別發表利用受質 LacNAc 原有的構型做修飾，變換各個羥基位置做為抑制劑，發現將 GlcNAc 的三號位置(如圖十二中的化合物 **1**)的羥基以氫原子取代會使結合能力喪失，推測相較於其他位置的羥基，此位置的羥基對結合效果有顯著貢獻。但由於醣受體對酵素的結合能力很差(約 mM 等級)，在設計抑制劑時較少以結合能力不好的醣予體作修飾。

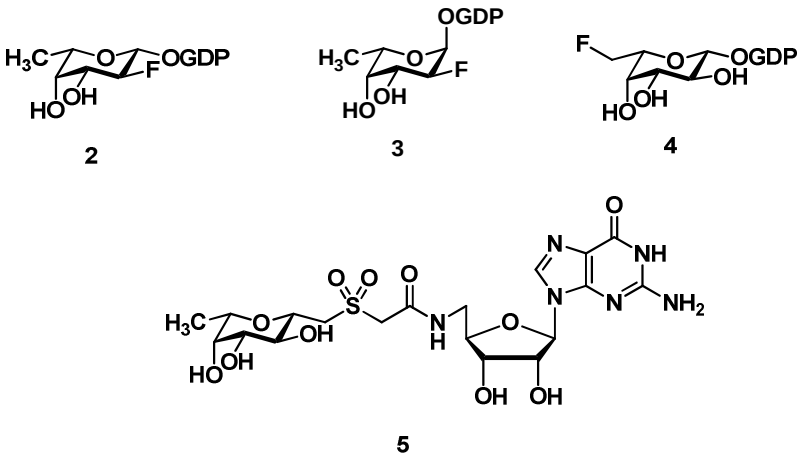


圖十二、FucT 之醣受體與其類似物結構

4.2 醣予體類似物 (donor substrate analogues)

醣予體類似物通常會具有二磷酸鳥苷或二磷酸的類似結構，同樣去競爭酵素的活性區域。Wong *et al.* 在 2000 年也曾利用岩藻醣轉移酶共同的醣予體 GDP-fucose 將其中的羥基修飾為氟原子 (如圖十三中化合物 **2**、**3**、**4**)，利用氟原子本身的強拉電子性質，使得形成 oxocarbenium ion 的過渡狀態不穩定，所以醣苷鍵 (glycosidic bond) 不會水解而成為抑制物，其抑制常數範圍約在μM 等級。[44,45]

另外，Chapleur *et al.* 在 2001 年將二磷酸鳥苷部分置換成硫醯基及氨基的組合(如圖十三中化合物 **5**)，發現其效果並不如預期，推測是因為引進不帶電的取代基，無法與酵素活化區域中的金屬離子作用，因此大大的減低其抑制效果。[46]



圖十三、FucT 之醣予體類似物之結構

4.3 過渡狀態類似物 (transition-state analogues)

過渡狀態類似物則是去仿造過渡狀態中岩藻醣本身具有的兩個特點：oxocarbenium ion 帶正電性質及 half-chair 構型。

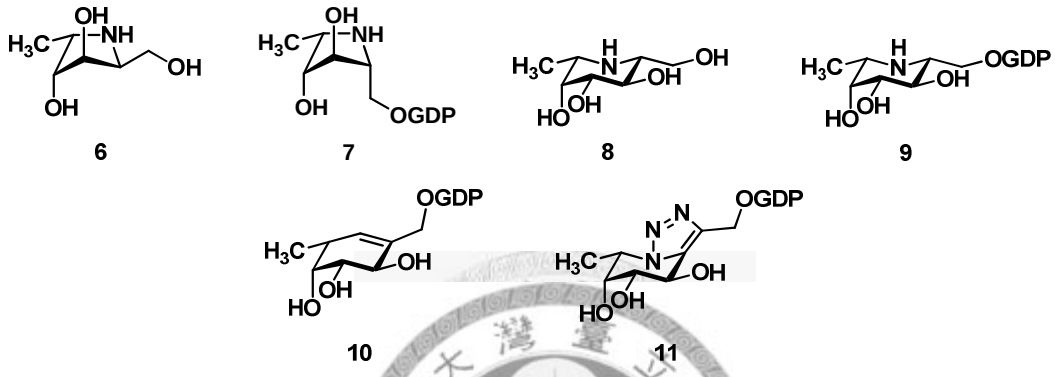
以往在設計醣水解酶抑制劑時，常利用 iminosugar 模擬過渡狀態，主要原因是在生理狀態中 iminosugar 會被質子化而帶有正電性質。所謂的 iminosugar 即是將醣分子環上的氧原子置換為氮原子的化合物，亦可稱為 iminocyclitol，由於醣類水解酶與醣類轉移酶催化過程的過渡狀態中的醣分子構型及性質很相似，因此在設計過渡狀態類似物的抑制劑時，也常利用五環或六環的 iminosugar 為基準做各種修飾。[47]

Wong et al. 在 1992 年及發表利用比咯啶環 (pyrrolidine) 為中心，同時具備過渡狀態中的兩個特點：帶正電性質及 half-chair 構型，且仿造岩藻醣上羥基的立體位置為抑制劑，並比較在過渡狀態類似物的設計中，二磷酸鳥苷的有無對抑制效果的影響，其中化合物 **6** 之抑制常數為 34 mM[44,47,48] 而化合物 **7** 的抑制常數為 45 μM，[49] 推測二磷酸鳥苷在與酵素結合時具有顯著的貢獻。

另外，Wong et al. 利用吡咯烷 (piperidine) 為中心，捨棄仿造其 half-chair 構型，同樣以與岩藻醣相同的羥基立體結構，在 1996 年及 2002 年中分別發表化合物 **8** 及 **9**，其抑制常數為 71.5 mM[50] 與 13 μM，[51] 同樣顯示二磷酸鳥苷的重要性。

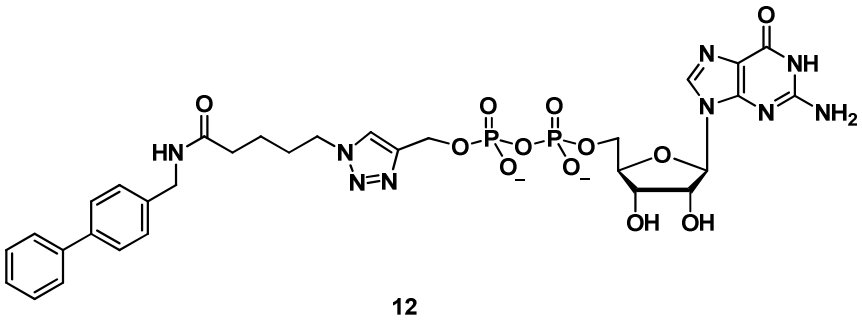
Wong et al. 同時發表另外兩種型態的類似物如化合物 **10** 及 **11**，[52] 此兩種類似物不具正電性質，但可由其抑制常數都為 8 μM 的結果中發現此兩種化合物的構型與過渡狀態中的岩藻醣相當類似，而有較好的抑制效果。

以上三種過渡狀態類似物都將原本較易斷的醣苷鍵置換成由碳原子衍生與醣分子鍵結，使岩藻醣類似環與二磷酸鳥苷間的鍵結更不易斷裂而失去結合能力。



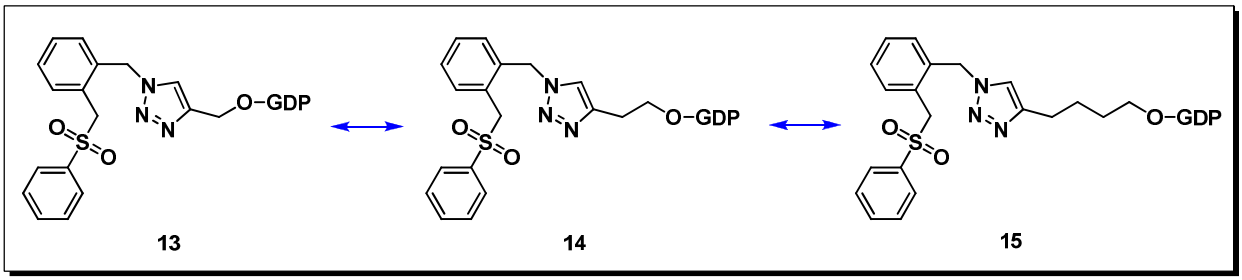
圖十四、過渡狀態類似物之抑制劑

目前文獻中活性最好的岩藻醣轉移酶抑制劑來自 Wong et al. 在 2003 年所發表的化合物 (圖十五)，保留原有的二磷酸鳥苷，利用點擊化學 (click chemistry)[53] 做鍵結，以篩選另一端疏水基團，其中效果最佳的為圖十五中的化合物 **12**，其抑制常數約為 62 nM，間接證明在沒有岩藻醣類似環的氫鍵作用下，二磷酸鳥苷與酵素的強結合力及疏水端對抑制效果的貢獻度。

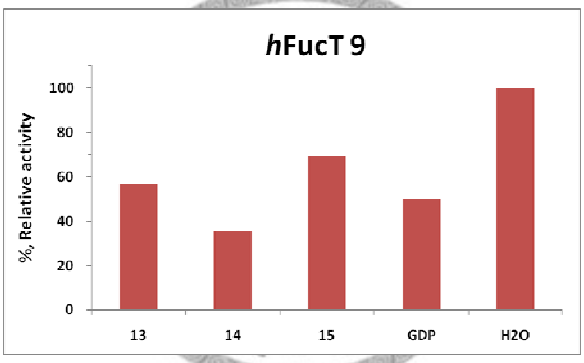


圖十五、以點擊化學合成之 FucT 抑制劑

本實驗室利用點擊化學合成一系列具有疏水基團的岩藻醣抑制劑。同樣以而二磷酸鳥苷為出發點，改變二磷酸鳥苷與 1,2,3-三唑環 (1,2,3-triazole ring) 間碳鏈長度，如圖十六，分別具一個碳 (**13**)、兩個碳 (**14**) 及四個碳 (**15**)，由這三個化合物的相對抑制百分比而言 (表二)，改變鏈長對岩藻醣轉移酶的抑制效果並無太大影響。



圖十六、本實驗室以點擊化學合成不同鏈長之抑制劑結構



表二、以點擊化學合成不同鏈長之抑制劑相對抑制百分比

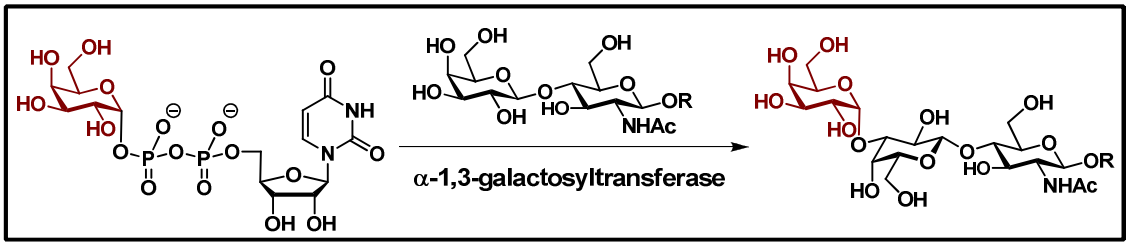
4.4 變旋異構中心上之立體化學對抑制效果的影響力

Schmidt *et al.* 曾在 2001 年發表半乳醣轉移酶 (α-1,3-galactosyltransferase) 之複合受質抑制劑 (bisubstrate analogue)，[54] 所謂的複合受質抑制劑即是將醣予體及醣受體作鍵結而成的化合物。

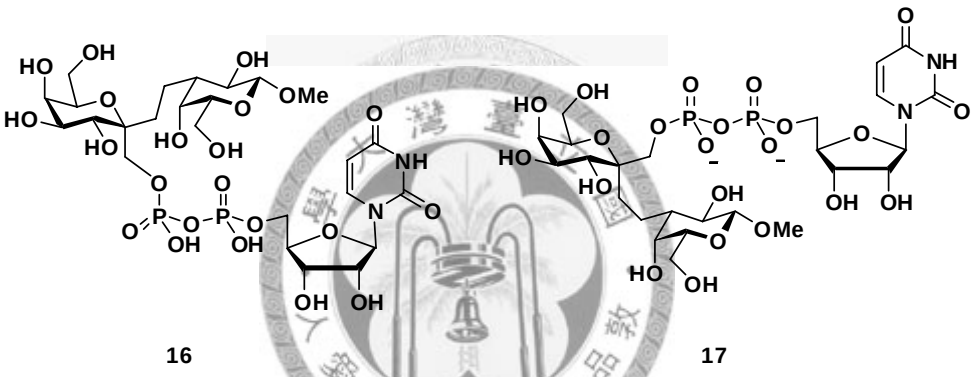
半乳醣轉移酶之受質為α-Gal-UDP，經此酵素轉醣化的催化保留機制 (retaining catalysis) 而形成α型產物 (圖十七A)，在文獻報導中，化合物 **16** 的抑制效果比化合物**17**佳 (圖十七B)，表示此抑制劑進入酵素活化區後，其二磷酸尿苷 (uridine diphosphate；UDP) 片段偏好在β側，與原始受質立體結構不符，因此作者推論在

此醣轉移酶催化過程中，醣予體 UDP-Gal 不只扮演了離去基的角色，在進入酵素活性區域後，二磷酸尿苷片段會離去而到原本醣予體的β側，與鄰近的胺基酸及過渡狀態中的 oxocarbenium ion 有正負電吸引而穩定整個過渡狀態，醣受體再從α側做親核性攻擊 (圖十七 C)。[54]

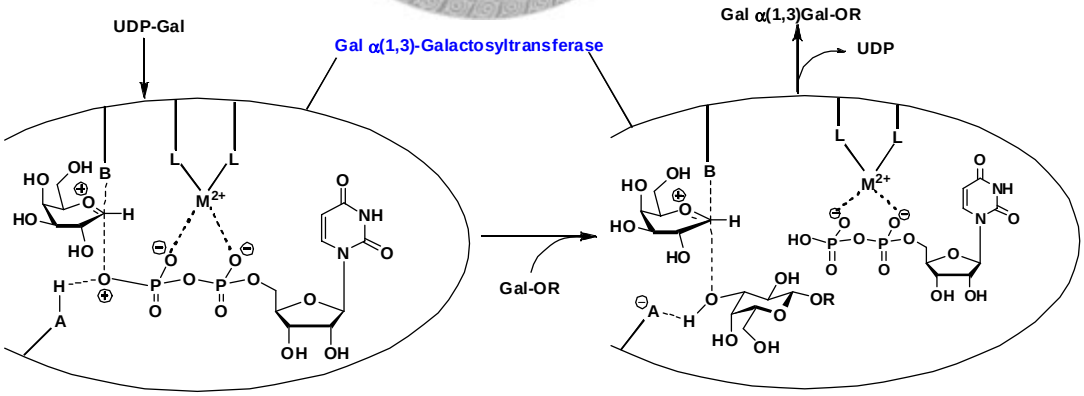
A



B



C



圖十七、A：半乳醣轉移酶之催化生合成反應。B：文獻中半乳醣轉移酶之複合受質抑制劑。C：半乳醣轉移酶之反應機制。

5. 岩藻醣轉移酶抑制劑之設計原則

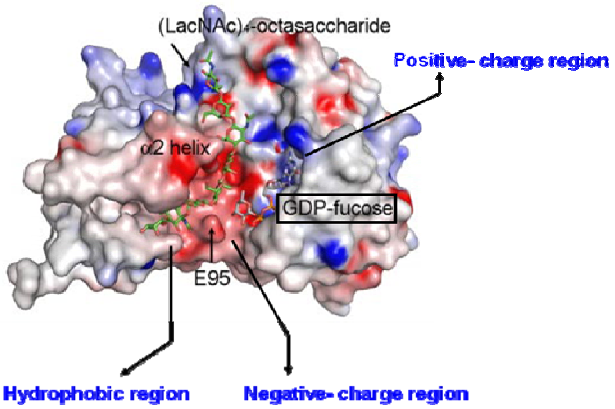
目前所有的岩藻醣轉移酶已被證實都屬於醣反轉轉移催化機制 (inverting glycosyltransferase)，[13] 過渡狀態也都具有相同的構型與帶電性質，因此設計酵素抑制劑時，盡可能的讓所設計的分子模擬催化過程中所產生的過渡狀態 (transition state)，[55] 由岩藻醣轉移酶的催化機制中 (圖十一)，[39] 可歸納出以下三點設計原則：

A. 岩藻醣轉移過程之電性特徵及構型

由岩藻醣轉移酶的催化機制中，[39] 可以知道受質在過渡狀態中會因為二磷酸鳥苷的先離去 [42] 而具有 oxocarbenium ion，也使得岩藻醣被扭曲成 half-chair 構型，因此要同時模擬此兩特徵，則可利用先前提到的比咯啶環 (pyrrolidine)，此結構由氮原子取代後的胺基在生理條件下 pH 7.5 中可被質子化 (protonation)，具有正電荷的特性，而五環化合物本身具有的特殊構型也可以模擬岩藻醣的 half-chair 構型。

B. 二磷酸鳥苷部分的保留及衍生之疏水區域

由幽門螺旋桿菌之岩藻醣轉移酶晶體結構正負電分佈可知（圖十八），當受質進入酵素活性區時，二磷酸的負電會與周圍帶正電胺基酸 (positive-charge region) 有靜電作用力，而受質岩藻醣周圍則處於帶負電胺基酸環境 (negative-charge region) 中，推測此環境可與過渡狀態的 oxocarbenium ion 作正負電吸引力，並且可以將醣受體上的羥基去質子化，使得容易對醣予體作親核性攻擊，因此在設計時保留受質中二磷酸鳥苷片段，另外，引進比咯啶環 (pyrrolidine) 取代岩藻醣，而比咯啶環在生理條件 pH 7.5 的環境中帶正電的性質有利於在此負電環境中存在。再者，可以看到鄰近 GDP-fucose 有疏水區域，推測利用比咯啶作疏水基團的衍生可以增加與酵素結合能力。



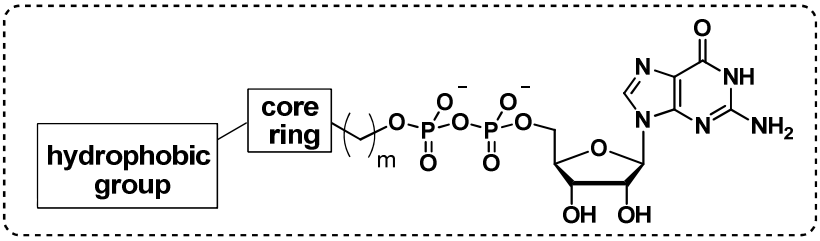
圖十八、*Hp*FucT 晶體結構[39]

C. 醣苷鍵之修飾

受質在催化過程中，岩藻醣與二磷酸鳥苷間的醣苷鍵會先斷裂，因此在設計抑制劑時，將原本較易斷的醣苷鍵置換成由碳原子衍生與醣分子鍵結，使岩藻醣類似環與二磷酸鳥苷間的鍵結更不易斷裂而失去結合能力。

6. 研究目標

根據先前所報導的 X-ray 晶體結構及其反應機轉[5]在抑制劑的設計有以上三項特點。如圖十九，針對這些特點，我們以二磷酸鳥苷為中心，選擇以比咯啶 (pyrrolidine)、吡咯烷 (piperidine)、嘧唑 (imidazole) 為岩藻醣模擬環分子，做環分子本身不同的構型及是否具有正電荷對抑制效果的影響力比較。除此之外，並利用效果較好的環分子做疏水區域的組合式化學 (combinatorial chemistry)，篩選對抑制效果貢獻度高的疏水基團，並探討分子結構與活性間的關係 (structure and activity relationship)。



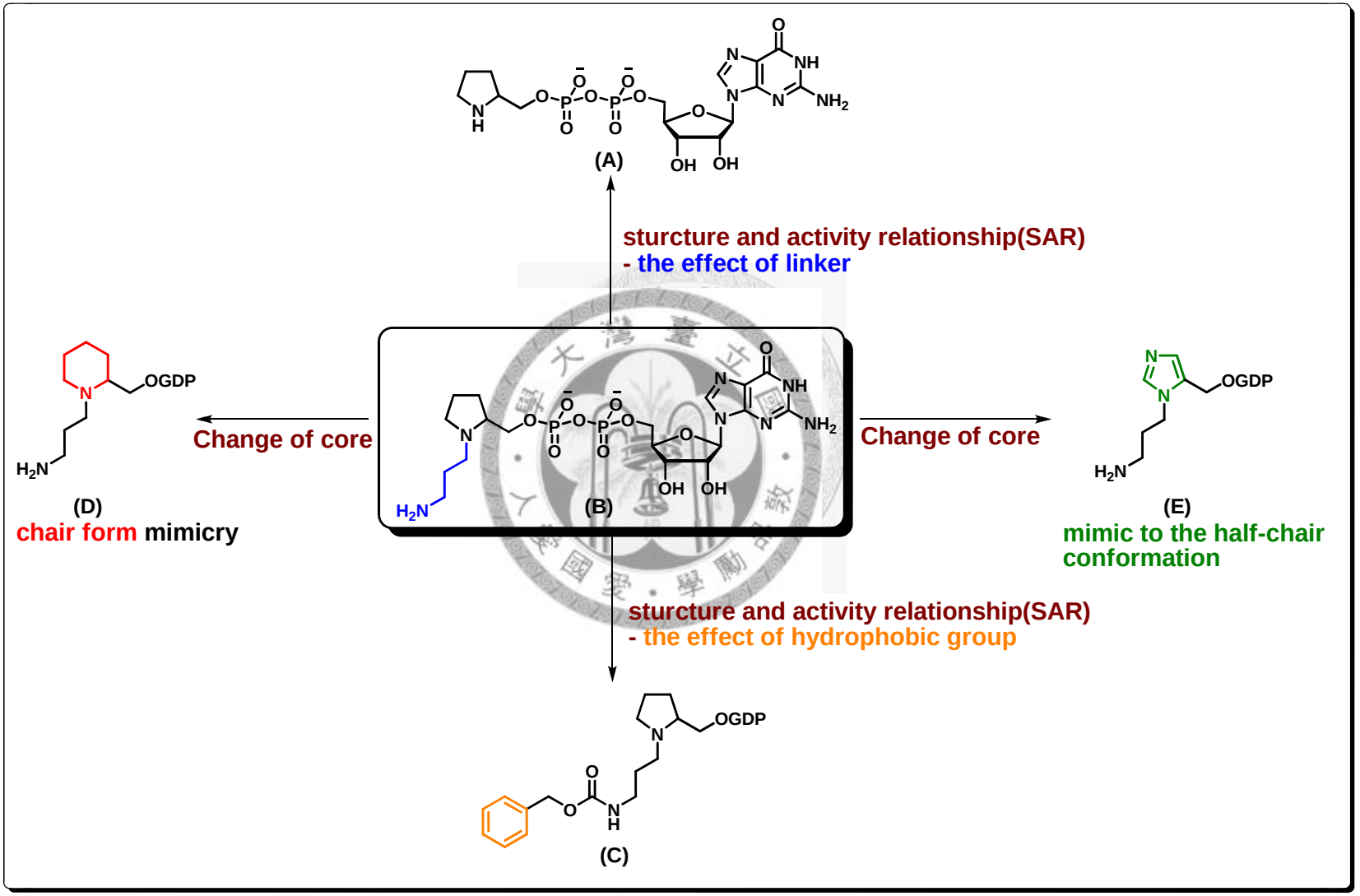
圖十九、FucT 抑制劑設計概念

圖十九中，以二磷酸鳥苷為中心，衍生以比咯啶 (pyrrolidine) 環模擬岩藻醣在過渡狀態中的兩個特點：具有正電性質及 half-chair 構型，並與一長鏈作鍵結，以利後續疏水基團的組合式化學。在比咯啶與長鏈的鍵結方法上，常見的有醯胺鍵 (amide bond) 或碳氮單鍵等的鍵結方式，若利用醯胺鍵作鍵結，所形成的比咯啶長鏈衍生物將無法具有帶正電性質，且碳鏈也無法順利擺動，可能會影響與酵素結合效果，因此選擇利用一般碳氮鍵的鍵結方式作碳鏈衍生。

由實驗室先前對於二磷酸鳥苷與環間鏈長的研究可知，鏈長為 1、2 或 4 個碳對抑制效果並無顯著影響，為了合成方便，我們選擇一個碳的鏈長；另外，由於酵素晶體結構中看出醣予體受質中岩藻醣周圍的疏水區域相當大，因此我們在設計比咯啶做疏水基團衍生的長鏈時，選擇先以較短的碳數為優先。

如圖二十的設計概念，這個構想向上延伸則單純利用比咯啶鍵結二磷酸鳥苷比較其長鏈與比咯啶環本身對抑制效果的貢獻度；向下延伸則將以組合式化學作疏水基團的鍵結，比較疏水端的貢獻度。

另外，橫向延伸則可將比咯啶還分別置換成吡咯烷 (piperidine) 環與嘧唑 (imidazole) 環。吡咯烷為椅型結構，在此例子中可比較在模擬岩藻醣的環中，half-chair 構型對抑制效果是否有其必要性；而嘧唑在此構想中則可利用其平面結構及不帶電性質，比較此兩種特質的貢獻度。

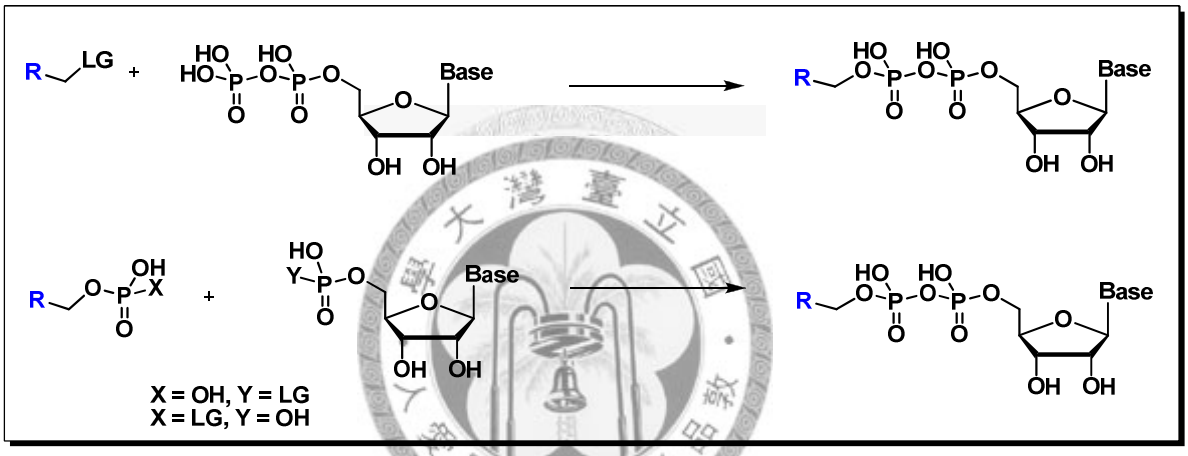


圖二十、FucT 抑制劑設計概念

第二章 結果與討論

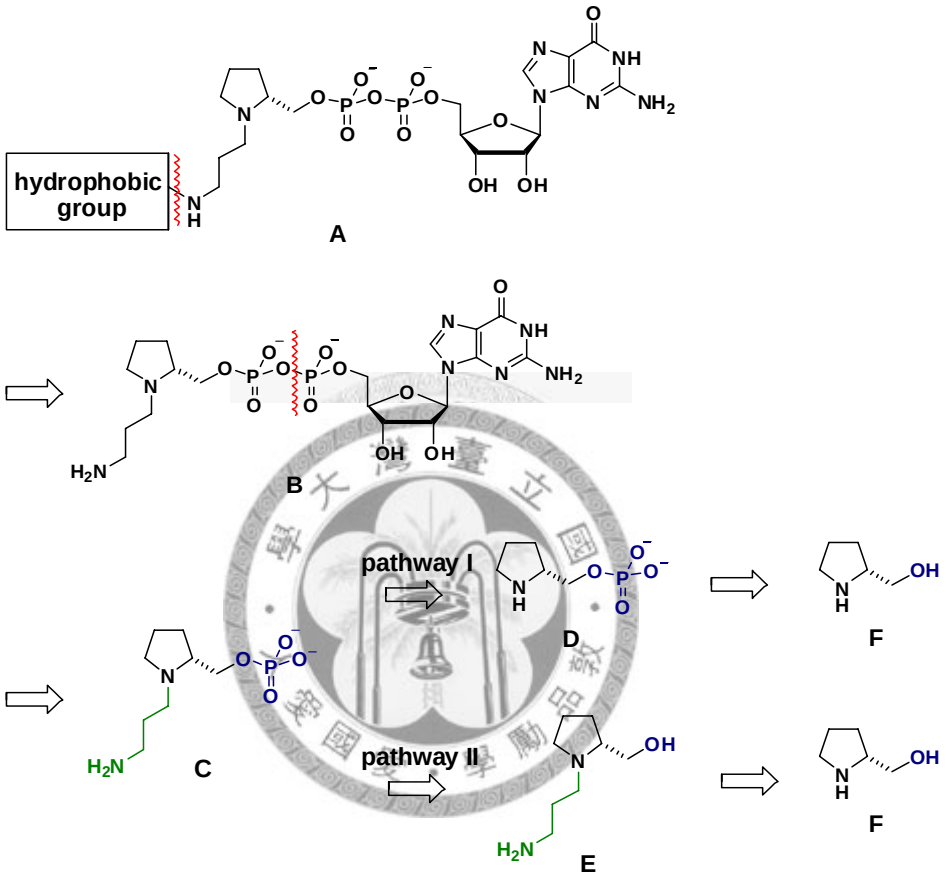
1. 目標產物的逆合成分析

合成二磷酸類的化合物主要有兩種合成策略 (圖二十一)，一是直接利用二磷酸作親核性攻擊至另一具有離去基之基團上;另一則是將其中一段單磷酸活化後，利用另一段單磷酸攻打而形成二磷酸化合物。



圖二十一、合成二磷酸化合物的策略

目標產物的逆合成如流程一所示，化合物 A 中的疏水基團可利用組合式化學作快速篩選，可裂解成片段 B，而磷酸酐鍵則可以利用單磷酸的親核性攻擊，可得到片段 C 以及磷酸鳥苷，在合成片段 C 時，由路徑一，先將片段 F 作磷酸化後，再接上碳鏈；或由路徑二，先由片段 F 作碳鏈鍵結，再作磷酸化，同樣可回推至片段 C。



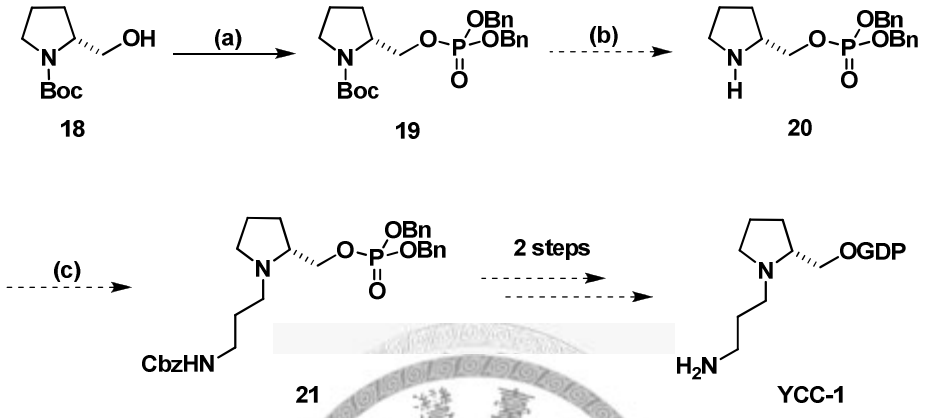
流程一、目標產物 A 之逆合成

2. 以路徑一合成岩藻醣轉移酶之抑制物

以 N-Boc-D-prolinol **18** 為起始物，進行如流程二的步驟，將羥基以三價磷化物做 S_N2 反應得到亞磷酸基團後，再氧化而得到具有苄基 (Bn; benzyl group) 保護的磷酸基團化合物 **19**，產率 94%。在步驟 b 中先以 10%鹽酸在甲醇溶液中，反應 30 分鐘後，產率為 15%；後又以 10 % TFA 在二氯甲烷溶液中，反應 30 分鐘，並沒有目標產物，以質譜追蹤，發現帶有苄基保護的磷酸基團在酸性條件下容易脫掉；

為了要使反應乾淨且高產率，又嘗試以氧化方式將 Boc 保護基脫去，利用 CAN 在常溫下反應，副產物相當多，同樣沒有目標產物。

在路徑一中發現，在具有苄基保護的磷酸基團化合物中，並不容易得到乾淨且高產率的反應，因此我們轉向路徑二，將磷酸化步驟放在最後進行。

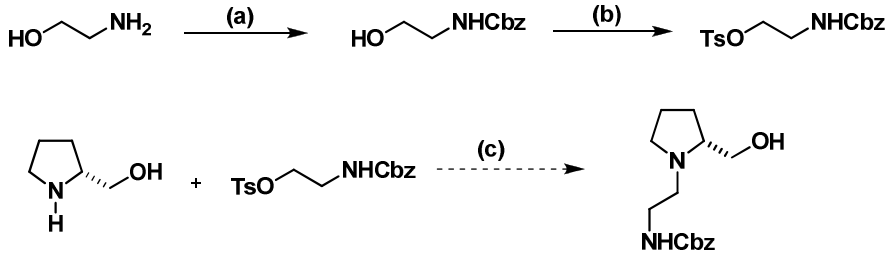


Reagents: (a) $(i\text{-Pr})_2NP(OBn)_2$, tetrazole, CH_2Cl_2; *m*-CPBA, CH_2Cl_2, 94%; (b) 10 % HCl, CH_3OH, 15-30%; (c) **24**, K_2CO_2, THF.

流程二、以路徑一合成目標產物 **29** (**YCC-1**)

3. 岩藻醣轉移酶抑制物 29 (YCC-1) 及 32 (YCC-3) 之合成

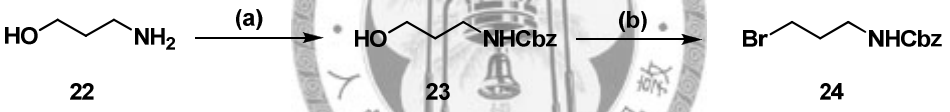
如路徑二的合成策略，以比咯啶 (pyrrolidine) 上的氮原子衍生碳鏈，以作組合式化學。起初我們選擇較短的兩個碳的鏈長做連接，以 2-aminoethanol 為起始物，先將胺基以 benzyl carbamate 保護後，再以甲苯磺酸基將羥基活化形成好的離去基後，再以比咯啶做 S_N2 反應，反應由三乙基胺為鹼，常溫下攪拌 12 小時後，我們並沒有發現比咯啶衍生長鏈產物。我們推測流程三中兩個碳的長鏈胺基保護基為一強拉電子基，使得一號位置的氫酸度變強，反應所加入的弱鹼使此長鏈做消去反應 (elimination)，而無法進行 S_N2 反應。



Reagent: (a) CbzCl, $NaHCO_3$, H_2O, rt, 13 hr, 90%; (b) TsCl, DMAP, Et_3N, CH_2Cl_2, 89%; (c) Et_3N, THF.
CbzCl = benzyl chloroformate; TsCl = 4-Toluenesulfonyl chloride; DMAP = N,N-dimethylaminopyridine

流程三、起初的碳鏈及比咯啶衍生碳鏈之合成

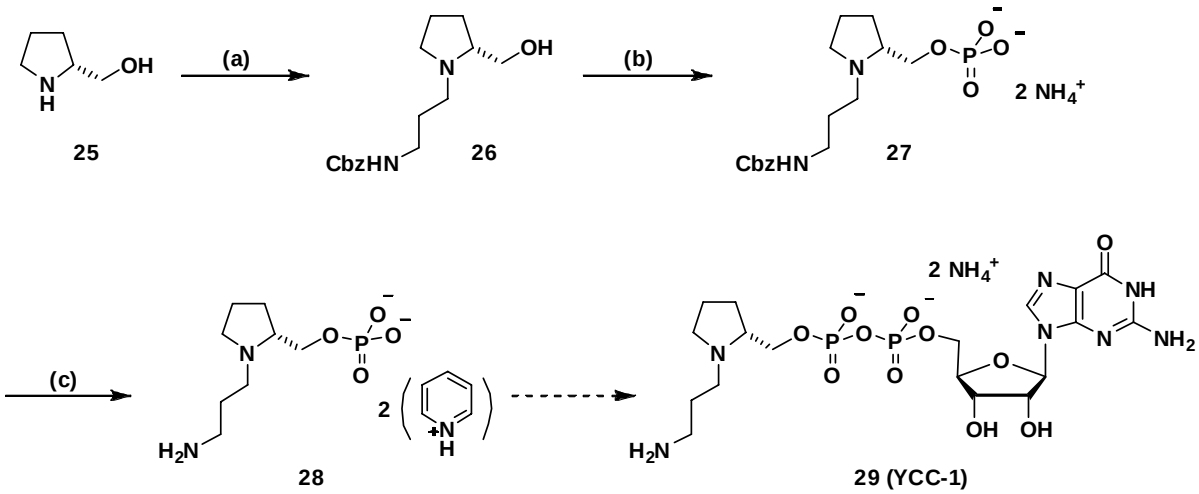
因此我們將長鏈系統改為流程四中的三個碳，並將離去基改為離去效果較好的溴。起始物 3-amino-1-propanol **22** 溶於水中，加入碳酸氫鈉待全溶後，再加入 benzyl chloroformate，劇烈攪拌 16 小時後，簡單萃取即可得到產物 **23**。再將化合物 **23** 溶於 THF 中，以 PPh_3 及 NBS 做溴化反應，可得到產物 **24**。



Reagents and conditions: (a) CbzCl, $NaHCO_3$, H_2O, rt, 16 h, 95%; (b) NBS, PPh_3, THF, rt, 2 h, 94%.
CbzCl = benzyl chloroformate; NBS = N-bromosuccinimide; PPh_3 = triphenyl phosphine

流程四、三個碳的碳鏈之合成

如路徑二的合成策略，我們以 D-(-)-prolinol **25** 為起始物，如流程五中，以碳酸鉀為鹼性條件下做 S_N2 反應，將長鏈鏈結至比咯啶 (pyrrolidine) 環上，得到產物 **26**，產率 97%。再利用化合物 **26** 進行磷酸化反應，選擇利用 trichloroacetonitrile 先將羥基活化後，再以 tetrabutylammonium phosphate 攻擊，常溫下反應 2 天後，得到磷酸化產物化合物 **27**，產率 71%。選擇以氫化 (hydrogenation) 方式將化合物 **27** 中胺基的保護基脫去，使用氫氣為 proton donor，在 Pd/C 為催化劑的條件下，反應兩個小時後，可得化合物 **28**。



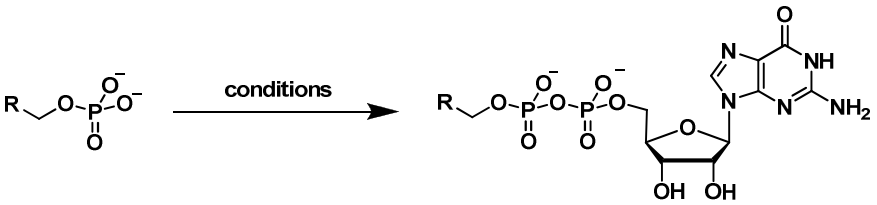
Reagents and conditions: (a) **24,** K_2CO_3, THF, 60 °C, 14h, 97%; (b) Cl_3CCN, $Bu_4NH_2PO_4$, CH_2Cl_2, rt, 2d, 73%; (c) H_2, Pd/C, H_2O/MeOH, 2h; pyridine.

流程五、以路徑二合成目標產物 **29 (YCC-1)**

以化合物 **28** 合成具有磷酸酐的化合物 **29** 時，多次嘗試了其他方法，但都沒有辦法得到目標產物。

流程六中根據 Thorson *et al.*[56] 在 1998 年的報導，利用 CDI (1,1'-carbonyldiimidazole) 來活化單磷酸，此反應只需數小時即可完成。當我們參考此文獻，將 GMP 在 triethylamine 下加入 CDI，常溫下攪拌 5 小時後，以 TLC 片追蹤至起始物耗完後，加入數滴甲醇將未反應之 CDI 淬熄，將此混合物在真空下抽乾後，加入另一磷酸化合物，在常溫下攪拌後，發現反應沒有進行，將溫度升高至 50 度後，由 TLC 片上可看到有許多副產物，且起始物無法耗完。

另外，Sinay *et al.*[57] 在 2003 年同樣利用 imidazole 類活化單磷酸，根據報導，反應同樣可在數小時內完成。流程六中將 GMP 在冰浴下慢慢滴加入 trifluoroacetic anhydride (TFAA)，反應十分鐘後，得到一黃綠色溶液，將過量的 trifluoroacetic anhydride 及 trifluoroacetic acid 抽乾後，在冰浴下加入 N-methylimidazole，同樣反應十分鐘後，由 TLC 片上可看到起始物消失後，將此混合物在零度下加入事先攪拌的另一磷酸化合物與分子篩，慢慢回到常溫後，由 TLC 片同樣發現許多副產物的產生。



	conditions	reaction time	result
1	i) CDI, Et_3N, DMF ii) monophosphate, DMF	23 h	messy
2	i) TFAA, Et_3N, MeCN ii) N-methylimidazole, Et_3N, DMF iii) monophosphate, 4A M.S.	14 h	messy
3	i) TFAA, Et_3N, MeCN ii) N-methylimidazole, Et_3N, DMF iii) monophosphate	14 h	messy
4	GMP-morpholidate, tetrazole, pyridine	2 ~ 4 d	27 ~ 42%

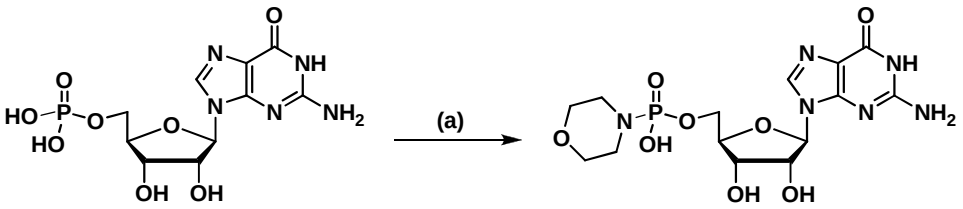
流程六、合成磷酸酐的方法

最後，Wong *et al.* [58] 在 1997 年發表利用 NDP-morpholidate 及 tetrazole 的條件下，可得到磷酸酐化合物。利用此法前我們必須先合成 GMP-morpholidate，我們發現直接以 guanosine 5'-monophosphate disodium slat 為起始物，抑或以離子交換樹脂交換成吡啶離子形式，其反應效率都相當差，因此我們選擇交換為氫離子形式的起始物。

首先將陰離子交換樹脂以水洗過後，再以 1N 鹽酸約五至十倍樹脂體積慢慢的流過樹脂後，再用水將樹脂表面的鹽酸洗掉，將鈉鹽起始物溶於樹脂水中，慢速攪拌約 12 小時後，以約 500 mg 起始物/600 mL 水的量以每分鐘三毫升的流速將樹脂上的起始物洗下來，將水溶液抽乾後即是氫離子形式的起始物。我們在這一步發現若樹脂活化不完全 (鹽酸量太少或流速太快) 將會使交換效果變差，而攪拌速度太快或攪拌太久則會使樹脂破裂，而使沖洗不易。最後以水將起始物沖洗時也需以慢速且大量的水進行，才可以收到產率高的起使物。而樹脂的活化程度將會影響下一步合成 GMP-morpholidate 的效率。

如流程七中，將起始物溶在水中，加入 *t*-butanol 及 morpholine，在 80 度油浴中回流約三十分鐘後，再將 dicyclohexyl dicarboiimide 的 *t*-butanol 溶液逐滴加入，

同樣在 80 度油浴中迴流，以 TLC 片觀察反應進行，我們發現反應時間為 24 小時為最佳狀態，在反應冷卻過濾後，以 silica gel 純化時，我們發現即使以含有氨水的沖堤液，GMP-morpholidate 在酸性管柱中相當容易水解，因此必須以較短的管柱快速將產物沖出。



Reagents and conditions: (a) morpholine, DCC, H_2O/*t*-BuOH = 1/1, 80 ^oC, 24 h, 84%.

流程七、GMP-morpholidate 的合成

在此反應中發現，GMP-morpholidate 及磷酸起始物都相當容易吸水而呈現黏稠狀，因此在反應前置準備必須先除水，也就是說，以酒精燈加熱在真空下放置五分鐘，重複三至四次直到兩者都呈現發泡狀，才能減低反應中的 GMP-morpholidate 水解比例，而提高反應效能。完成 GMP-morpholidate 的合成後，將磷酸化合物與吡啶共沸三次，另外將 GMP-morpholidate 與 tetrazole 在真空下抽 10 個小時後，加入吡啶在常溫下反應 2 至 3 天後起始物消耗完畢，可得磷酸酐產物，產率約 27～42%。

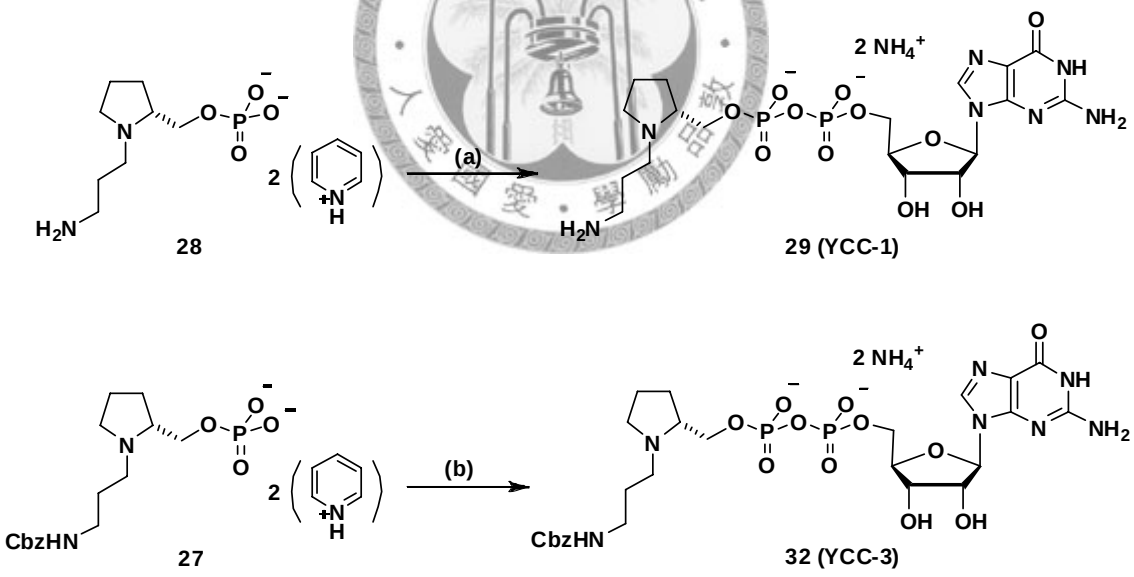
利用 NDP-morpholidate 及 tetrazole 可以成功合成出具有磷酸酐的化合物，但其缺點是反應時間相當久，產率沒有辦法突破，且這樣的磷酸酐反應對水氣相當敏感，即使是形成磷酸酐之後，也會因為保存不當 (酸性條件或水氣下) 而分解。

另外，我們在純化這類具有二磷酸的化合物時也遇到了困難。首先以 silica gel 純化，由於這類化合物極性很高，因此選擇以異丙醇、水及胺水的混合溶劑為沖堤液，利用水相條件純化時，耗時較久且二磷酸化合物在管柱中會有水解的現象。本論文中多數的二磷酸化合物極性與 GMP-mprpholidate 水解產物 GMP 相當接近，單純以 silica gel 純化很難得到純度高的產物，因此利用 Alkaline phosphatase

水解 GMP 末端的磷酸根，而形成與產物極性差異較大的磷酸與鳥苷 (guanosine) 片段，我們發現在以酵素作水解前要先作初步純化，將其他雜質如反應物 tetrazole 及溶劑 pyridine 去除，以免使酵素失去活性。

酵素水解後再利用高效能液相層析儀 (high performance liquid chromatography; HPLC) 作分離，以陰離子交換樹脂管柱 mono Q 及碳酸氫胺水溶液為沖堤液。利用 HPLC 純化可以得到高純化化合物，但同樣耗時很久。利用碳酸氫胺水溶液為沖堤液時，雖然此種鹽類可利用冷凍乾燥方法去除，但其離子交換效率不高，使得產率無法提高，且固定需要用氫氧化鈉水溶液清洗管柱以維持交換效能。

為初步比較疏水區對抑制效果是否有幫助，利用末端胺基未去保護的化合物 **27** 做磷酸鳥苷的鍵結，得到化合物 **32** 做初步活性測試。流程八中，將化合物 **27** 與吡啶共沸過，再與 GMP-morpholidate **31** 及 tetrazole 在常溫下攪拌兩天後，得到產物 **32**，產率 39%。

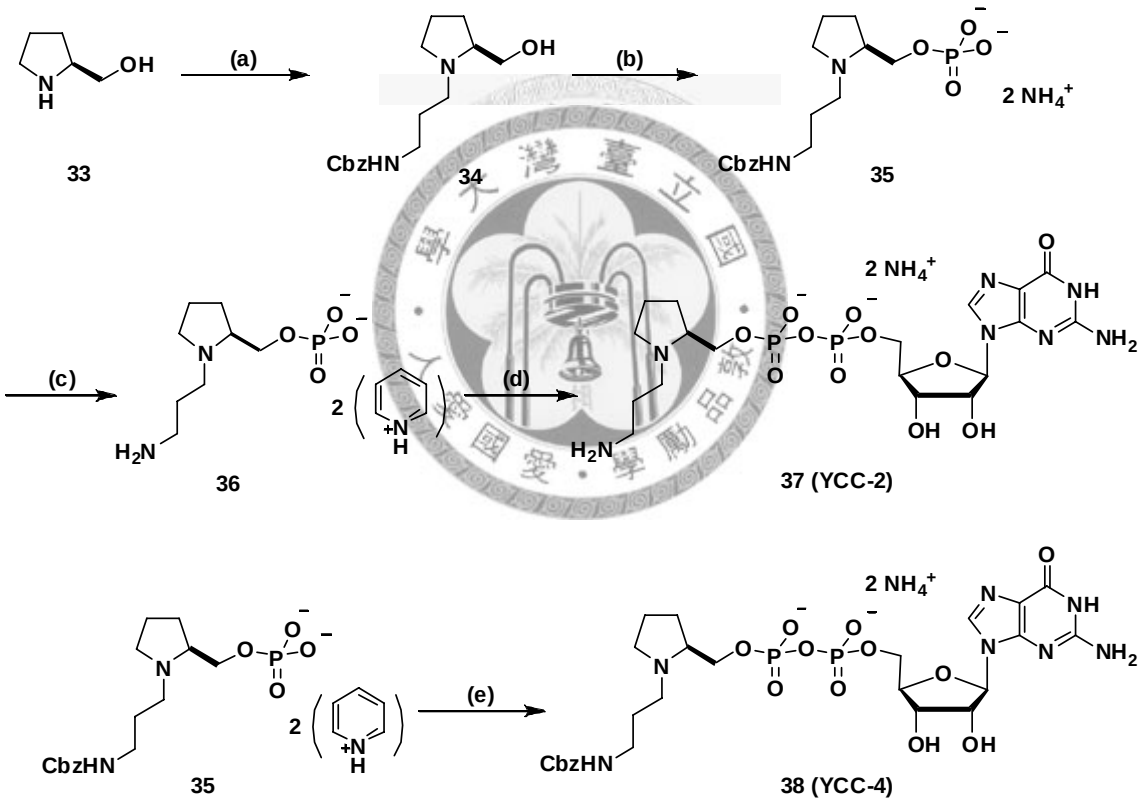


Reagents and conditions: (a) pyridine; GMP-morpholidate **31**, tetrazole, pyridine, rt, 3d, 31% in two steps.
(b) pyridine; GMP-morpholidate, tetrazole, pyridine, rt, 3d, 39%

流程八、以路徑二合成目標產物 **29 (YCC-1)** 及 **32 (YCC-3)**

4. 岩藻醣轉移酶抑制物 37 (YCC-2) 及 38 (YCC-4) 之合成

同樣依路徑二合成，我們以 L-(+)-prolinol **33** 為起始物，如流程九所示，先以比咯啶 (pyrrolidine) 環上的胺基做 S_N2 反應，與長鏈鏈結，得到產物 **34**，產率 93%。再進行磷酸化反應，以 trichloroacetonitrile 將羥基活化，加入 terabutylammonium phosphate 得一橘色澄清溶液攪拌 2 天後，得到磷酸化產物化合物 **35**，產率 72%。將化合物 **35** 去保護後，可得化合物 **36**。再將化合物 **36** 與吡啶 (pyridine) 共沸過三次後，先後加入 GMP-morpholidate **31** 及 tetrazole，以無水吡啶 (pyridine) 為溶劑，在常溫下反應 3 天後，得到目標產物 **37**，產率為 39%。



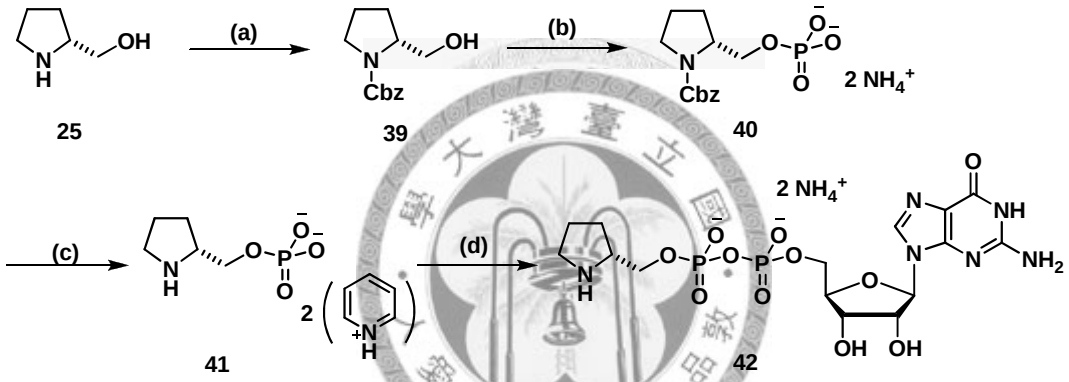
Reagents and conditions: (a) **24,** K_2CO_3, THF, 60 °C, 14h, 93%; (b) Cl_3CCN, $Bu_4NH_2PO_4$, CH_2Cl_2, rt, 2d, 72%; (c) H_2, Pd/C, H_2O/MeOH, 2h; pyridine; (d) GMP-morpholidate **31**, tetrazole, pyridine, rt, 3d, 39% in two steps; (e) pyridine; GMP-morpholidate, tetrazole, pyridine, rt, 2d, 36%

流程九、以路徑二合成目標產物 **37 (YCC-2)** 及 **38 (YCC-4)**

同樣在化合物 **35** 中加入吡啶，共沸三次後，再與 GMP-morpholidate **31** 及 tetrazole 於常溫下攪拌兩天，可得產物 **38**，產率 39%。

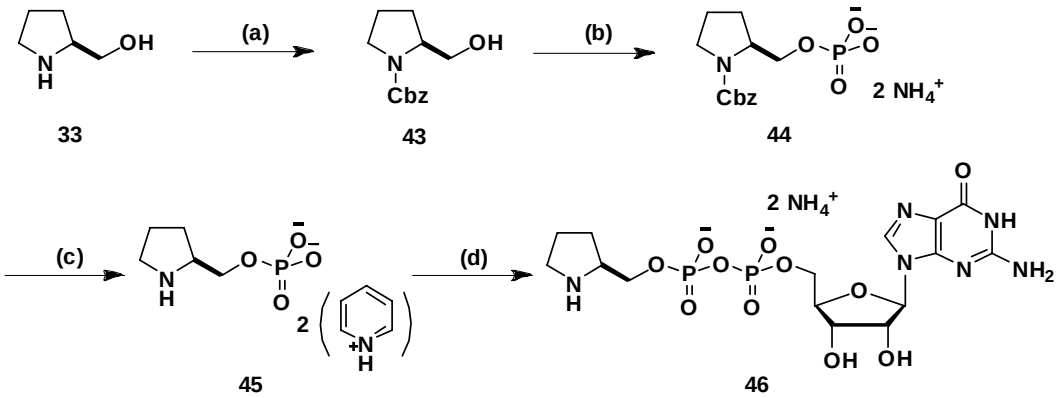
我們另外合成了不具有碳鏈衍生的比咯啶環帶有二磷酸鳥苷的化合物以比較碳鏈對抑制效果是否有影響。

依相同合成方法，分別以 D-(-)-prolinol **25** 及 L-(+)-prolinol **33** 為起始物，將比咯啶的胺基以 benzyl cloroformate 保護後，以 trichloroacetonitrile 將羥基活化，加入 terabutylammonium phosphate 攪拌 2 天後，得到磷酸化產物化合物 **40** 及 **44**。氫化去保護後，可得化合物 **41** 和 **45**。再分別將化合物 **41** 和 **45** 與吡啶 (pyridine) 共沸過三次後，先後加入 GMP-morpholidate **31** 及 tetrazole，以無水吡啶 (pyridine) 為溶劑，在常溫下反應 3 天後，得到目標產物 **42** 和 **46**。



Reagents and conditions: (a) CbzCl, $NaHCO_3$, H_2O, rt, 15 hr, 76%; (b) Cl_3CCN, $Bu_4NH_2PO_4$, CH_2Cl_2, rt, 2d, 86%; (c) H_2, Pd/C, H_2O/MeOH, 2h; pyridine; (d) GMP-morpholidate **31**, tetrazole, pyridine, rt, 3d, 39% in two steps.

流程十、化合物 **42** 的合成

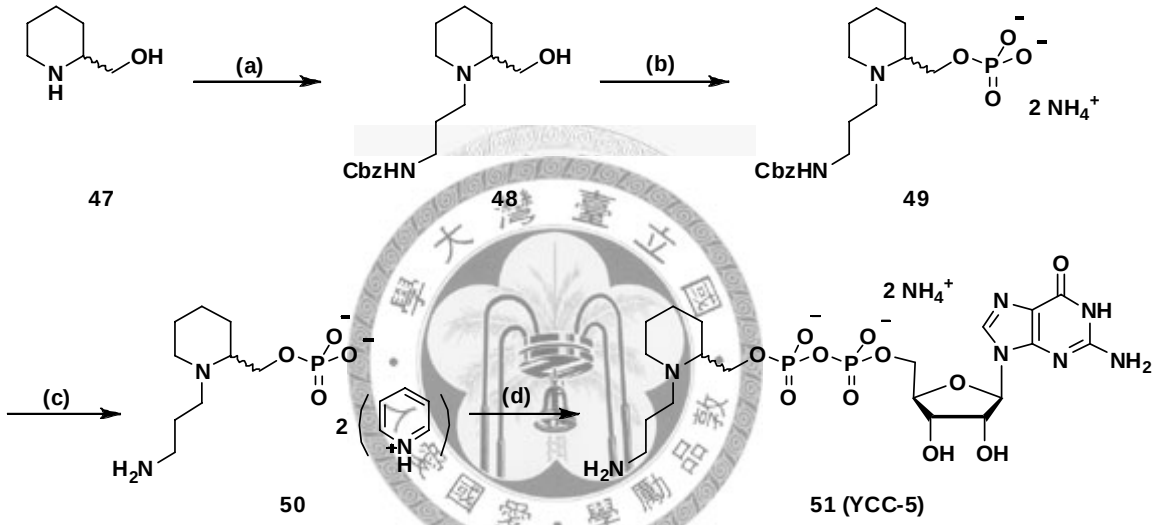


Reagents and conditions: (a) CbzCl, $NaHCO_3$, H_2O, rt, 15 hr, 70%; (b) Cl_3CCN, $Bu_4NH_2PO_4$, CH_2Cl_2, rt, 2d, 81%; (c) H_2, Pd/C, H_2O/MeOH, 2h; pyridine; (d) GMP-morpholidate **31**, tetrazole, pyridine, rt, 3d, 46% in two steps.

流程十一、化合物 **46** 的合成

5. 岩藻醣轉移酶抑制物 51 (YCC-5) 之合成

以 2-piperidinemethanol **47** 為起始物，進行 N-alkylation 反應，將長鏈鍵結至吡咯烷 (piperidine) 環上，可得產物 **48**，產率 89%。以五價磷酸鹽化合物 tetrabutylammonium phosphate 進行磷酸化反應，可得化合物 **49**，產率 76%。以 Pd/C 為催化劑的條件下做氫化反應，兩個小時後，可得化合物 **50**。再將化合物 **50** 與吡啶 (pyridine) 共沸過三次後，先後加入 GMP-morpholidate **31** 及 tetrazole，以無水吡啶 (pyridine) 為溶劑，在常溫下反應 3 天後，得到目標產物 **51**，產率 29%。



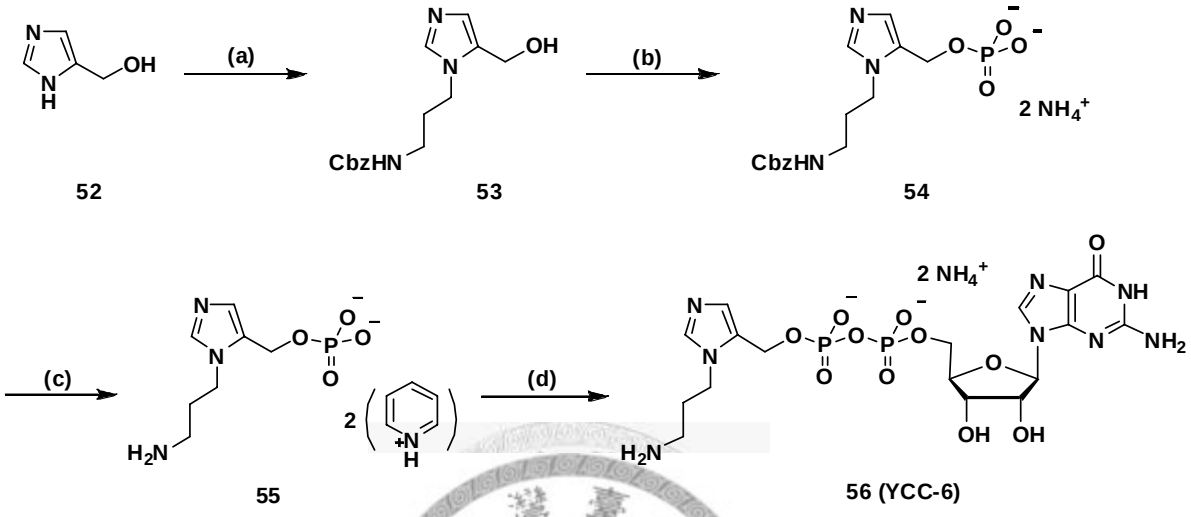
Reagents and conditions: (a) **24,** K_2CO_3, THF, 60 °C, 14h, 89%; (b) Cl_3CCN, $Bu_4NH_2PO_4$, CH_2Cl_2, rt, 2d, 76%; (c) H_2, Pd/C, H_2O/MeOH, 2h; pyridine; (d) GMP-morpholidate **31**, tetrazole, pyridine, rt, 3d, 29% in two steps;

流程十二、化合物 **51 (YCC-5)** 的合成步驟

6. 岩藻醣轉移酶抑制物 56 (YCC-6) 之合成

在路徑二的合成策略中，我們以 5-hydroxymethyl-imidazole **52** 為起始物，有別於之前二級胺基的親核性能力，化合物 **52** 中的胺基攻擊能力較弱，為避免羥基在加熱條件下也會做 S_N2 反應，此步驟在室溫下進行，攪拌 16 小時後，在 TLC 片上看到兩個主點，其中極性較小的為主產物，化合物 **53**，產率 56%。同樣以 trichloroacetonitrile 先將羥基活化後，加入 tetrabutylammonium phosphate 在常溫下反應 2 天，得到化合物 **54**，產率 66%。再以氫化 (hydrogenation) 方式將化合物

54 中胺基的保護基脫去，反應兩個小時後，可得化合物 **55**。再將化合物 **55** 與吡啶共沸過三次後，先後加入 GMP-morpholidate **31** 及 tetrazole，以無水吡啶 (pyridine) 為溶劑，在常溫下反應 3 天後，得到目標產物 **56**，產率為 27%。



52　53　54　55　56 (YCC-6)

Reagents and conditions: (a) **24,** K_2CO_3, DMF, 16h, 56%; (b) Cl_3CCN, $Bu_4NH_2PO_4$, CH_2Cl_2, rt, 2d, 66%; (c) H_2, Pd/C, H_2O/MeOH, 1.5h; pyridine; (d) GMP-morpholidate **31**, tetrazole, pyridine, rt, 3d, 27% in two steps;

流程十三、化合物 **56 (YCC-6)** 的合成步驟

7. 生物活性測試

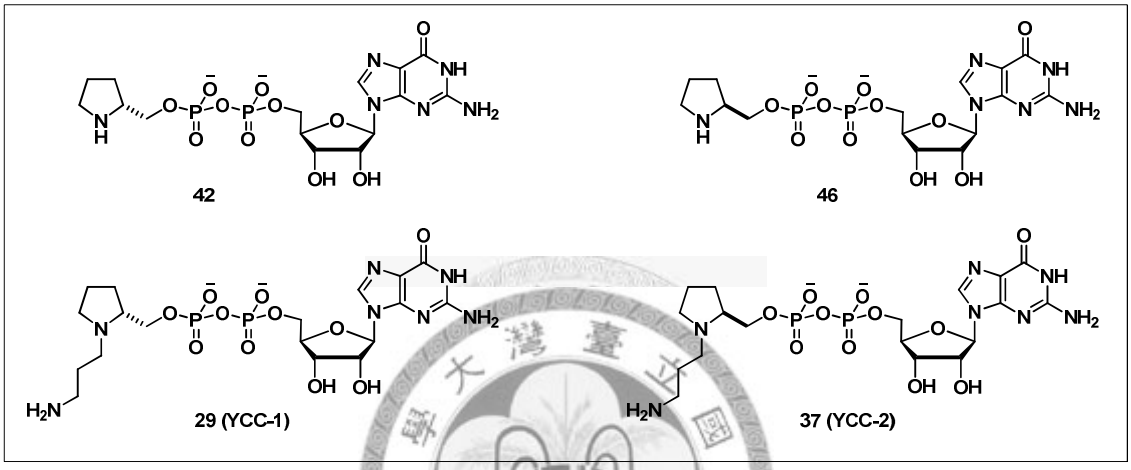
7.1 放射線標定法 (Radiolabeled Assay)[59]

這個方法是讓抑制劑及酵素受質競爭與酵素作用的能力。同時將 FucT 受質 GDP-L-[U-^{14}C]fucose、抑制劑、醣受體 (LacNAc)、及岩藻醣轉移酶放入含有 $MnCl_2$ 的緩衝溶液中，在 37 度水浴中反應，反應的產物再以 TLC 片分離，觀察形成具有 ^{14}C 標記的 Le^x 量多寡，評估化合物對酵素活性的抑制程度。抑制劑與酵素結合能力越強，則形成的 Le^x 越少;當產物 Le^x 越多，則表示抑制劑與酵素結合能力越弱。

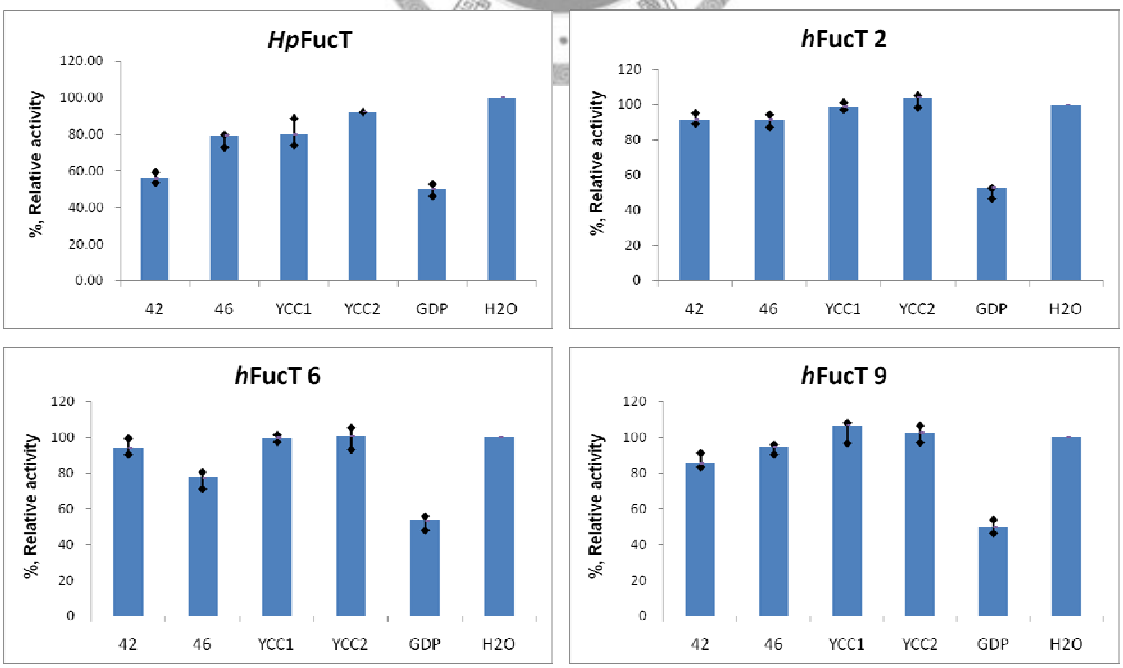
7.2 生物活性測試結果

我們將所設計的轉醣酶抑制劑化合物 **42**、**46**、**29** (**YCC-1**) 至 **56** (**YCC-6**) 利用此檢測系統進行轉醣酶活性之檢測。

首先我們針對帶有二磷酸鳥苷的比咯啶環 (如圖二十二)，具有碳鏈衍生及不具有碳鏈衍生的化合物 **42**、**46**、**29 (YCC-1)** 及 **37 (YCC-2)** 作岩藻醣轉移酶活性測試。 其中 GDP 是已知抑制劑，IC_{50} 約 50~250 μM。此活性測試實驗中，以水為正向控制組 (positive control)，以抑制劑 GDP 相對抑制活性 50%下的濃度定為化合物加入的濃度，比較化合物和 GDP 的抑制效果。



圖二十二、具有二磷酸鳥苷的比咯啶類 FucT 抑制劑

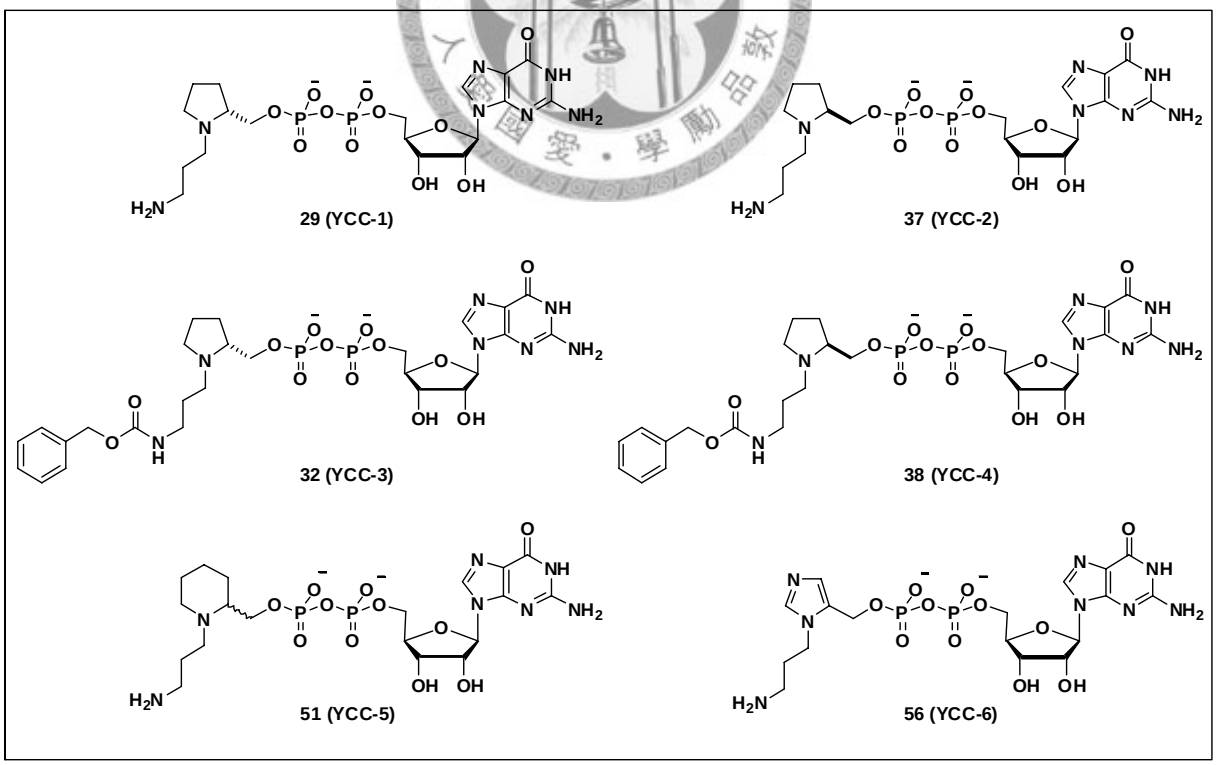


圖二十三、比咯啶環抑制物對 FucT 之抑制效果 (由本實驗室莊育瑞提供)

(GDP 為已知抑制劑，IC_{50} = 50~250 μM；n = 3)

如圖二十三中，比較化合物 **42**、**46**、**29** (**YCC-1**) 及 **37** (**YCC-2**) 對岩藻醣轉移酶的抑制效果發現，對於人類岩藻醣轉移酶，比咯啶環上的立體中心及碳鏈的有無對於酵素活性並無顯著差異，也沒有一定趨勢；但對於胃幽門螺旋桿菌的岩藻醣轉移酶而言，D 型抑制劑效果比 L 型抑制劑好，且不具碳鏈的比咯啶環抑制物化合物 **42** 及 **46** 對酵素活性抑制也比具有碳鏈的比咯啶環抑制物化合物 **29** (**YCC-1**) 及 **37** (**YCC-2**) 好。

另一方面，比較比咯啶環及另外兩種不同環，吡咯烷 (piperidine) 抑制劑化合物 **51** (**YCC-5**) 與嘧唑 (imidazole) 化合物 **56** (**YCC-6**) (圖二十四) 對酵素活性的影響，此兩種環與比咯啶的性質不同 (如表三)，其中吡咯烷環的構型為 chair 構型，具有正電性質，而嘧唑環則為平面結構且為不帶電結構。針對化合物 **29** (**YCC-1**)、**37** (**YCC-2**)、**32** (**YCC-3**) 及 **38** (**YCC-4**) 利用此檢測系統進行轉醣酶活性之檢測。如圖二十五，由檢測結果中發現在同時具有二磷酸鳥苷的抑制劑中，變換不同環取代岩藻醣並不會對酵素活性有影響。

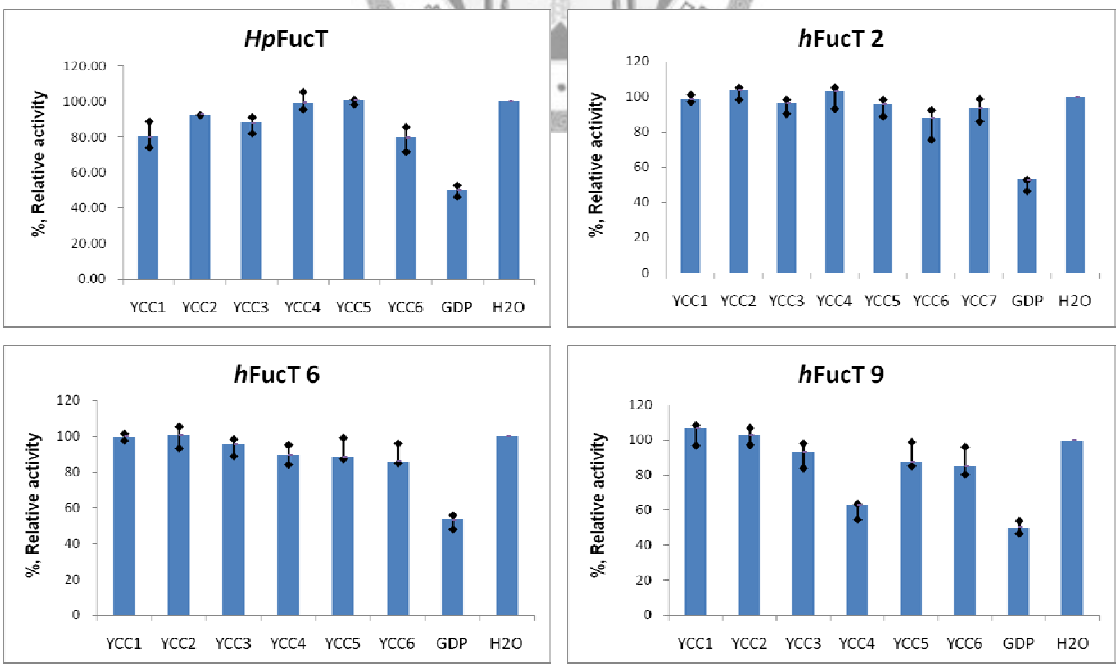


圖二十四、具有二磷酸鳥苷的 FucT 抑制物

piperidine	pyrrolidine	imidazole
chair conformation	half chair-like conformation	plane conformation
protonated at pH 7.5 to mimic positive charge	protonated at pH 7.5 to mimic positive charge	neutral at pH 7.5

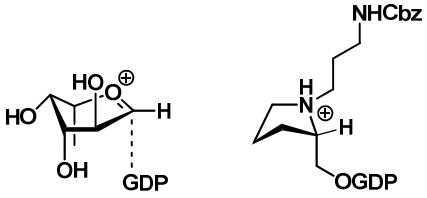
表三、比咯啶 (pyrrolidine)、吡咯烷 (piperidine) 與咪唑 (imidazole) 的性質

針對帶有二磷酸鳥苷的比咯啶環，比較碳鏈末端具有疏水基團的抑制劑化合物 **32** (**YCC-3**) 及 **38** (**YCC-4**) 及不具有疏水基團的抑制劑化合物 **29** (**YCC-1**) 及 **37** (**YCC-2**) 的活性測試結果 (圖二十五)。我們發現 **38** (**YCC-4**) 對酵素抑制效果有較明顯的影響力，這樣的結果說明，在比咯啶環有碳鏈衍生時，由於 GDP 與酵素間有許多氫鍵作用力，若將 GDP 結合位置固定，發現 L 型的化合物在構型上與受質 GDP-fucose 在過渡狀態中的構型與環境比較相似 (圖二十六)。除了立體中心的影響，推測碳鏈末端的疏水基團也與 X-ray 結構中所看到的疏水區域有疏水作用力，使化合物 **38** (**YCC-4**) 與酵素的結合能力較好。



圖二十五、各類抑制物對 FucT 之抑制效果 (由本實驗室莊育瑞提供)

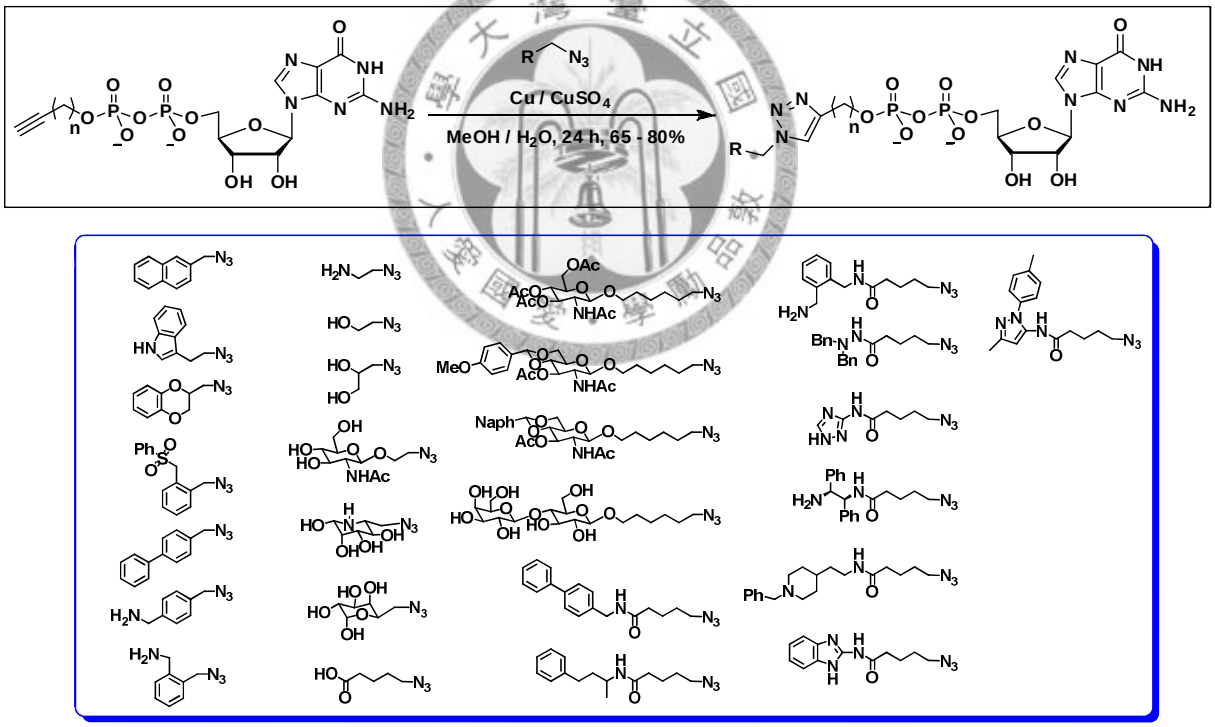
(GDP 為已知抑制劑，IC_{50} = 50~250 μM；n = 3)



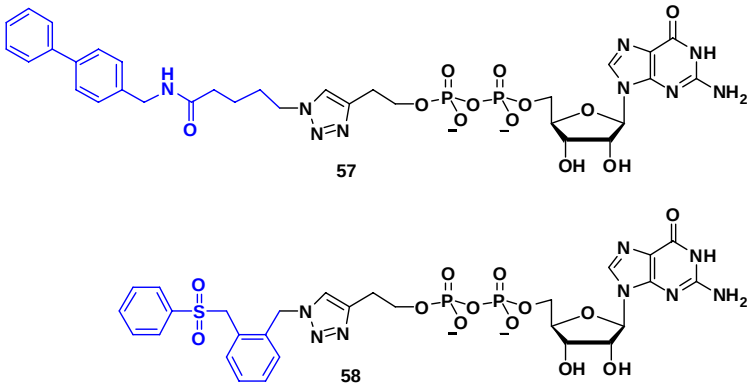
圖二十六、GDP-fucose 的過渡狀態與 **38** (**YCC-4**) 之構型比較

8. 疏水基團之衍生

本實驗室利用點擊化學 (click chemistry) 合成出各種具二磷酸鳥苷的岩藻醣轉移酶抑制劑 (圖二十七)，這類化合物以 triazole 環模擬 GDP-fucose 中岩藻醣的位置，且末端接和各種不同類型的官能基以比較對抑制效果的影響力。



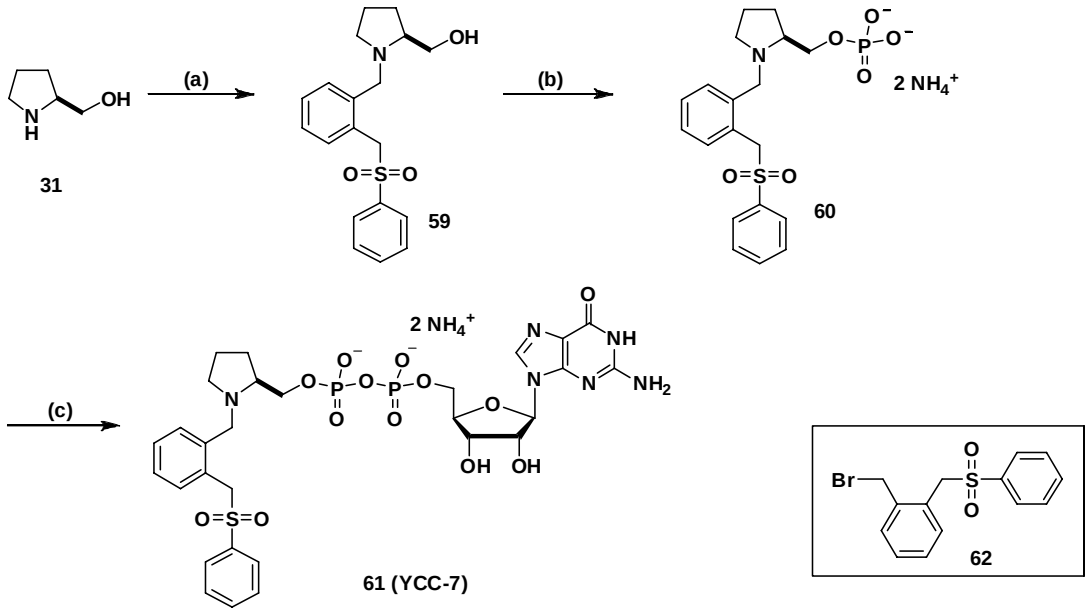
圖二十七、本實驗室以點擊化學合成 FucT 抑制物 (由本實驗室潘佳甫提供)



圖二十八、本實驗室合成之 FucT 抑制物

從酵素的活性測試結果發現化合物 **57** 及 **58** 抑制效果較佳 (圖二十八)，此類抑制劑都具有 triazole 環，因此推測所產生不同的抑制效果是來自環上所衍生的官能基之貢獻，利用化合物 **58** 上的疏水基團，接和到本篇論文所使用的比咯啶環上，來測試抑制效果。

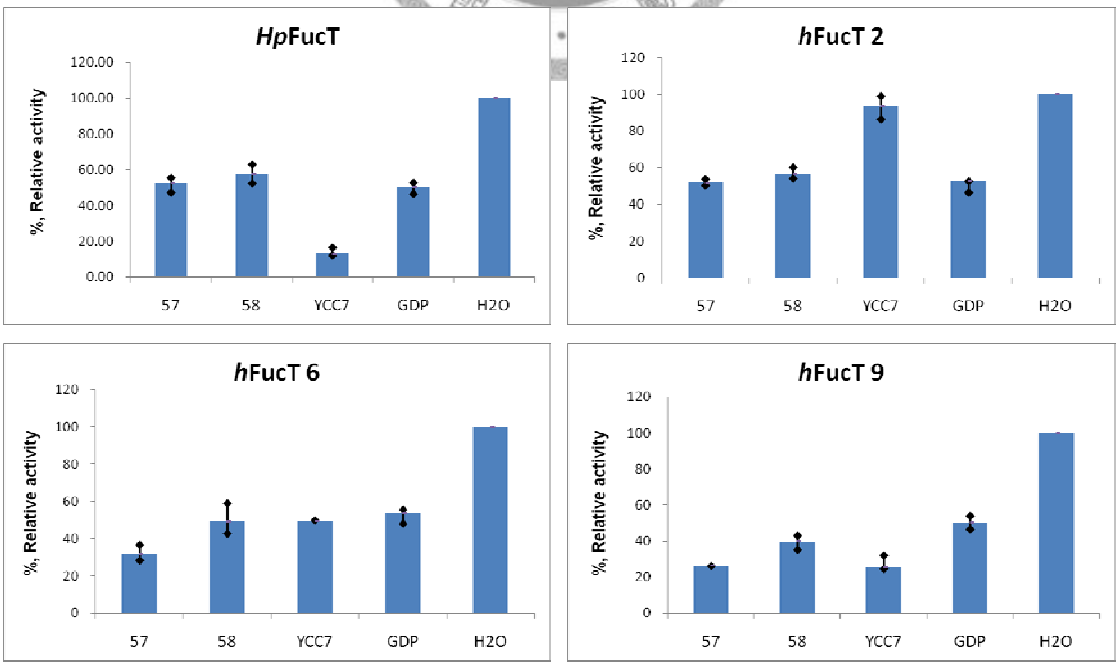
以 L-(+)-prolinol **33** 為起始物，如流程十四所示，在比咯啶環上的胺基做 S_N2 反應，在碳酸鉀的鹼性條件下與疏水基團作鍵結，在結晶可得到產物 **59**，產率 76%。再進行磷酸化反應，以 trichloroacetonitrile 將羥基活化，加入 terabutylammonium phosphate 得一橘色澄清溶液攪拌 2 天後，得到磷酸化產物化合物 **60**，產率 75%。再將化合物 **60** 與吡啶 (pyridine) 共沸過三次後，先後加入 GMP-morpholidate **31** 及 tetrazole，以無水吡啶 (pyridine) 為溶劑，在常溫下反應 3 天後，得到目標產物 **61**，產率為 41%。



Reagents and conditions: (a) **62,** K_2CO_3, THF, rt, 15h, 76%; (b) Cl_3CCN, $Bu_4NH_2PO_4$, CH_2Cl_2, rt, 2d, 75%; (c) GMP-morpholidate **31**, tetrazole, pyridine, rt, 3d, 41%.

流程十四、化合物 **61** (**YCC-7**) 的合成步驟

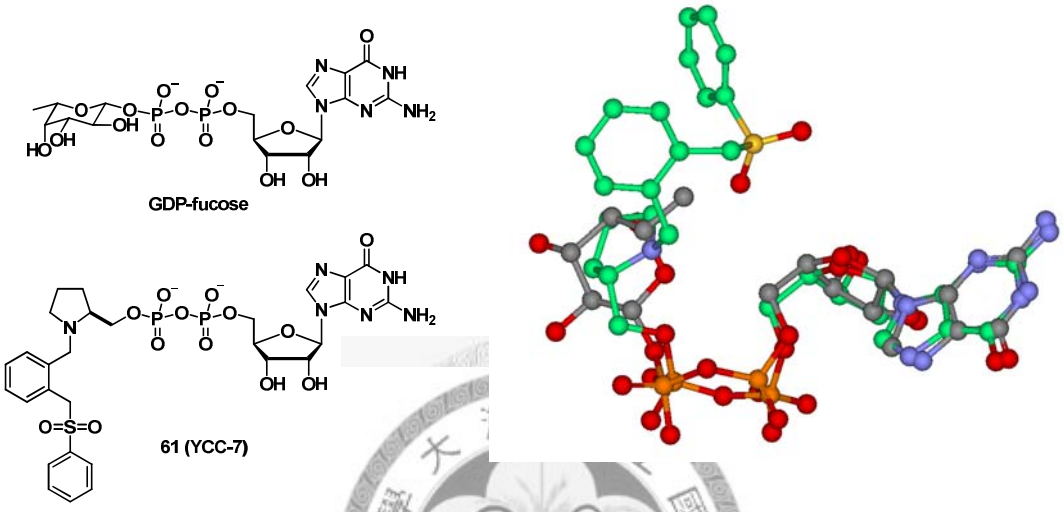
同樣將此化合物以放射線標定法測試對岩藻醣轉移酶之活性，與化合物 **57** 及 **58** 作比較，如圖二十九中，發現三者對 *h*FucT 6 及 *h*FucT 9 的抑制效果相差不大，對 *h*FucT 2 化合物 **61** (**YCC-7**) 則沒有抑制效果，較值得注意的是，**61** (**YCC-7**) 對幽門螺旋桿菌的岩藻醣轉移酶抑制效果最佳，IC_{50} 及 K_i 分別為 44.1 及 29.5 μM。



圖二十九、化合物 **57**、**58** 及 **61** (**YCC-7**) 的活性測試 (本實驗室莊育瑞提供)

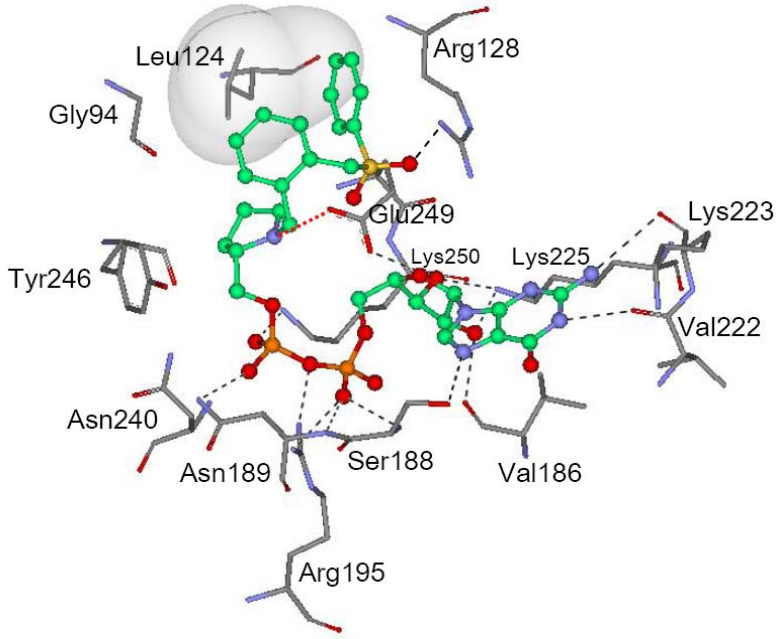
(GDP 為已知抑制劑，IC_{50} = 50~250 μM；n = 4)

為了探討 **61** (**YCC-7**) 的抑制活性與結構的關聯性，利用幽門螺旋桿菌的岩藻醣轉移酶與 GDP-fucose 的共結晶結構 (PDB code: 2NZY) 為藍本，進行 molecular docking 計算。由於化合物 **61** (**YCC-7**) 保留了 GDP 的部分，因此將此 GDP 部分採用共結晶結構中與酵素相同的結合模式固定，針對其餘的部分做結合模式最佳化的搜尋。



圖三十、GDP-fucose 及化合物 **61** (**YCC-7**) 的結構比較 (由本實驗室林鼎堅提供)

(紅：氧原子，橘：磷原子，黃：硫原子，藍：氮原子，綠：碳原子)



圖三十一、化合物 **61** (**YCC-7**) 與 FucT 電腦模擬計算結果 (本實驗林鼎堅提供)

(紅：氧原子，橘：磷原子，黃：硫原子，藍：氮原子，綠：碳原子)

在 Autodock 4.0 軟體中，[60,61] 可以藉由限制 GDP 部分的所有化學鍵轉動來達成此一目的，得到初步的 docking 結果。接著將此結果，使用 charmm force field[62] 進行 molecular dynamics 計算做結構上細部的調整，此部分的計算是以 Discovery Studio 2.1 軟體來完成。[63]

從圖三十中，GDP-fucose 及化合物 **61** (**YCC-7**) 的結構比較中，發現 **61** (**YCC-7**) 結構中的比咯啶環上的氮原子與 GDP-fucose 上岩藻醣的氧原子位置相近，正好可以模擬在過渡狀態中 oxocarbenium ion 所帶的正電區域相似，與我們當初的設計概念相符合。另外，由分子模擬得到的 binding mode (圖三十一)，推測化合物 **61** (**YCC-7**) 上的比咯啶環上的氮原子在質子化帶正電後會與鄰近的 Glu249 產生靜電作用力；疏水基團中的磺醯基 (sulfone) 則推測會與鄰近的 Arg128 有氫鍵產生；而疏水基團中的兩個苯環則同時與 Leu124 有疏水作用力產生。

我們推測相較於三唑環的平面構形，比咯啶環的好處是結構較有彈性，在進入酵素活性區後，胺基所衍生的苯環將有機會擺動到與其具有疏水作用力的胺基酸附近，而增加與酵素的結合能力。但嘧唑所衍生的抑制物則不具這樣的性質，其結構較剛性，末端的疏水基團無法與靠近疏水性胺基酸，而無法提升與酵素的結合能力。

第三章 實驗部分

1. General Method

All solvents and reagents were purchased from commercial sources and used without further purification unless otherwise specified. Tetrahydrofuran and dichloromethane were distilled from sodium, by using benzophenone as an indicator. NMR spectra were obtained on Bruker NMR AV400 (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR and 161 MHz for ^{31}P NMR). Presaturation experiment was done by using 1hpr pulse program and setting the decoupler frequency O1 parameter to the frequency of HOD. Chemical shifts (δ) were recorded in parts per million (ppm) relative to δ_H 7.27/δ_C 77.0 (central line of t) for $CDCl_3$, δ_H 4.80 for D_2O. The splitting patterns are reported as s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), m (multiplet) and br (broad). Coupling constant (*J*) are given in Hz. Analytical thin-layer chromatography (TLC) was performed on 0.25 mm Merck silica gel 60 F_{254} plates. Visualization of TLC plates was accomplished by UV light, *p*-anisaldehyde, or ninhydrin spray. Column chromatography was carried out with Kieselgel Si 60 (0.063-0.20 mm). The ESI-MS experiments were conducted on a JOEL JMS 700 high-resolution mass spectrometer. HPLC purification were performed using Tricon mono Q 10/100 GL column (10 × 100 mm) and water/ammonium dicarbonate as eluent. The optical rotation was measured at

the sodium D-line at 20 °C with a Perkin-Elmer Model 341 Polarimeter. The specific rotation was reported as $[\alpha]^{20}_{D}$ and the sample concentration *c* was in g/100 mL, the path length *l* was in dm. All reactions using air or moisture sensitive reagents were performed under an inert nitrogen atmosphere.

2. General Procedure for fucosyltransferase activity assay[59]

(This part was carried out by Mr. Yu-Reui Chuang, 莊育瑞)

The activity assay was detected by measuring the incorporation of radioactive label from GDP-L-[U-^{14}C]fucose (240 mCi/mmol, PerkinElmer Life and Analytical Sciences, Boston, MA) into reaction products. Reactions were conducted in assay buffer (ingredient shown below) in 37 °C, and initiated upon addition of fucosyltransferase, GDP-L-[U-^{14}C]fucose and fucosyltransferase inihibitors.

Assay condition:

Reaction buffer	Final concentration
KHepes pH 7.4	50 mM
KCl	150 mM
MnCl2	20 mM
LacNAc	1 mM

For radio thin layer chromatography (radio-TLC), samples were taken at defined points at times and spotted directly onto a Silica Gel 60 F_{254} TLC plate (Merck, Darmstadt, Germany). Following the development with a mixture of *i*-propanol/water/acetic acid (7:2:1), radioactivity was detected by imaging with a

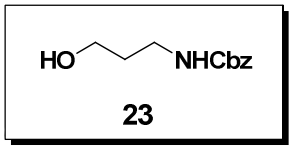
BAS-MS 2040 imaging plate and a BAS-1500 scanner (Fujifilm, Taipei, Taiwan). Signals could be quantified by means of the software Image Gauge V4.0 (Fujifilm).

3. Computational modeling

(This part was carried out by Mr. Ting-Chien Lin, 林鼎堅)

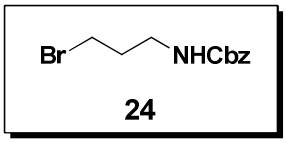
A two-step *in silico* simulation was carried out to understand how compound **61** (**YCC-7**) binds with *Hp*FucT. The complex structure of *Hp*FucT-GDP-fucose (PDB code: 2NZY) was utilized for the study. At the first stage, compound **YCC-7** was docked into the active site of the *Hp*FucT structure using Autodock 4.0.[60, 61] Because **YCC-7** and the substrate GDP-fucose both share GDP as the common motif, the GDP moiety of **YCC-7** was thus fixed to perform docking to obtain a preliminary binding mode. At the second stage, residues of *Hp*FucT within 10 Å from **YCC-7** and **YCC-7** itself were allowed to relax. CHARMM force field[62] was utilized in the MD (molecular dynamics) simulated annealing by using Discovery studio (DS) 2.1 (Accelrys Inc., San Diago).[63]

4. Synthetic Procedures and Spectral Data



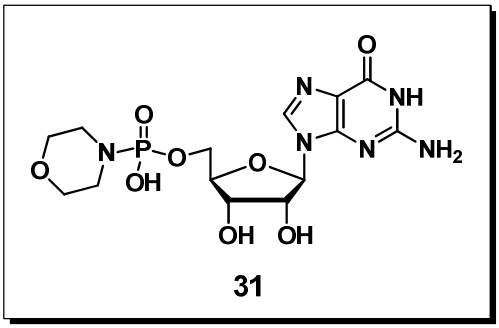
23

(3-hydroxypropyl)-carbamic acid benzyl ester (**23**).[64] A stirred solution of 3-amino-1-propanol **22** (5.0 g, 66.6 mmol) in water was added sodium bicarbonate (6.7 g, 79.9 mmol), followed by addition of benzyl chloroformate (11.4 mL, 79.9 mmol). The reaction mixture was stirred vigorously for 16 h, extracted with ethyl acetate for three times and dried over Mg_2SO_4. The organic layer was evaporated and coevaporated with ether to yield a crude white solid, which was further recrystallized with chloroform to give pure product (13.1 g, 95%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35 (5 H, m, ArH), 5.11 (2 H, s, $PhCH_2$), 3.67 (2 H, q, J = 5.7 Hz, CH_2), 3.34 (2 H, q, J = 6.1 Hz, CH_2), 2.82 (1 H, br, OH), 1.70 (2 H, p, J = 6.1 Hz, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 157.2 (C), 136.4 (C), 128.4 (CH), 128.0 (CH), 127.9 (CH), 66.7 (CH_2), 59.5 (CH_2), 37.7 (CH_2), 32.3 (CH_2); HRMS (ESI-TOF) calcd for $C_{11}H_{15}NO_3Na$ $[M+Na]^+$ 232.0944, found: 292.0946.



24

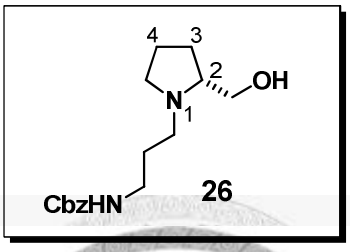
(3-bromopropyl)-carbamic acid benzyl ester (**24**).[65] A solution of NBS (0.8 g, 4.59 mmol) was added at 0 ºC to a stirred THF solution (15 mL) containing 0.8 g (3.83 mmol) of compound **23** and 1.2 g (4.59 mmol) of triphenylphosphine. Stirring was continued until TLC showed absence of starting material. The reaction was quenched by addition of 5 mL of methanol to the resulting pale orange mixture. The reaction mixture was evaporated and ethyl ether was added. The organic layer was washed with brine and evaporated to dryness. The resulting oil was purified by chromatography on silica gel using ethyl acetate/hexane = 2/8 as the eluent to yield a product appearing as an orange oil (1.1 g, 94%). R_f = 0.34 (ethyl acetate/hexane = 2/8); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.37 (5 H, m, ArH), 5.11 (2 H, s, $PhCH_2$), 4.91 (1 H, br, NH), 3.45 (2 H, t, J = 6.4 Hz, CH_2), 3.37 (2 H, q, J = 6.3 Hz, CH_2), 2.09 (2 H, t, J = 6.3 Hz, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.4 (C), 136.3 (C), 128.4 (CH), 128 (CH), 66.5 (CH_2), 39.3 (CH_2), 32.4 (CH_2), 30.5 (CH_2); HRMS (ESI-TOF) calcd for $C_{11}H_{14}BrNO_2Na$ $[M+H]^+$ 294.0100, found: 294.0129.



31

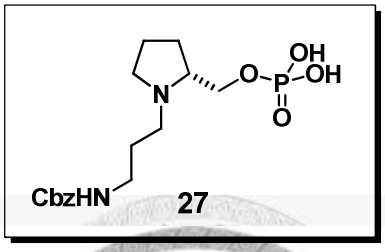
Guanosine-5' phosphoromorpholidate (31).[66] A solution of dicyclohexylcarbodiimide (0.4 g, 19.8 mmol) in *t*-butyl alcohol (15 mL) was added dropwisely to a refluxing solution of the guanosine 5'-monophosphate **30** (1.8 g, 2.96 mmol, free acid) in a mixture of water (30 mL), *t*-butyl alcohol (15 mL), and morpholine (1.7 mL, 19.8 mmol). The addition was completed in 3-4 hr and the mixture was further refluxed for 20 h until the TLC plate showed no starting material remained. The mixture was then cooled to room temperature and 5 mL of water was added into the mixture and kept stirring for 5 min. Any crystalline material present was removed by filtration and washed with water. The filtrate was evaporated in vacuo until the *t*-butyl alcohol was totally removed and the remaining aqueous phase was extracted three times with chloroform. The clear aqueous solution was evaporated to give the crude product, which was then purified by chromatography over silica gel with *i*-propanol/water/ammonium hydroxide = 7/2/1 to give compound **31** (1.8 g, 84%); R_f = 0.62 (*i*-propanol/water/ammonium hydroxide = 7/2/1); ^{1}H NMR (D_2O, 400 MHz) δ 8.04 (1 H, s, ArH), 5.90 (1 H, d, J = 4.8 Hz, CH), 4.80 (1 H, s, CH, HOD), 4.53 (1 H, t, J = 4.8 Hz), 4.32 (1 H, br, CH), 4.09-3.95 (2 H, m, CH_2), 3.59 (4 H, t, J = 4.2 Hz, CH_2), 2.96 (4

H, br, CH_2); ^{13}C NMR (D_2O, 100 MHz) δ 158.5 (C), 153.6 (C), 151.4 (C), 137.1 (CH), 116.0 (C), 87.2 (CH), 83.4 (1 C, d, $J_{C\text{-}P}$ = 8.8 Hz, CH), 73.6 (CH), 70.1 (CH), 66.7 (1 C, d, $J_{C\text{-}P}$ = 7.2 Hz, CH_2), 63.9 (CH_2), 44.5 (CH_2); ^{31}P NMR (D_2O, 161 MHz) δ 7.90 (1 P, s); HRMS (ESI-TOF) calcd for $C_{14}H_{20}N_6O_8P$ $[M-H]^-$ 431.1075, found: 431.1069.



(2R)-*N*-[*N'*-(benzyloxycarbonyl)-3'-aminopropyl]-2-hydroxymethyl pyrrolidine (**26**). To a stirring mixture of D-(-)-prolinol **25** (0.30 g, 2.97 mmol) and potassium carbonate (0.41 g, 2.97 mmol) in THF (8 mL) was added compound **24** (0.76 g, 2.82 mmol) at room temperature and then stirred at 60 °C for 14 h. The reaction mixture was cooled to room temperature, diluted with ethyl acetate (20 mL), washed with water twice and dried over Mg_2SO_4. Removal of the solvent afforded a pure product (0.84 g, 94%). R_f = 0.2 (methanol/chloroform = 1/20); $[\alpha]^{20}_D$ +26.13 (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.37-7.31 (5 H, m, ArH), 5.19 (1 H, br, OH), 5.11 (2 H, s, $PhCH_2$), 3.62 (1 H, dd, *J* = 10.9, 3.6 Hz, CH_2), 3.41 (1 H, dd, *J* = 10.9, 2.7 Hz, CH_2), 3.32-3.26 (2 H, m, CH_2), 3.17-3.16 (1 H, m, CH_2), 2.85-2.78 (1 H, m, CH_2), 2.57-2.53 (1 H, m, CH), 2.35-2.29 (1 H, m, CH_2), 2.24-2.17 (1 H, m, CH_2), 1.90-1.85 (1 H, m, CH_2),

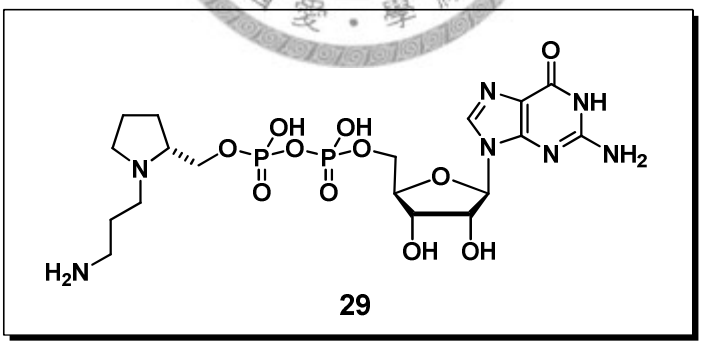
1.81-1.68 (5 H, m, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.5 (C), 136.3 (C), 128.4 (CH), 128.0 (CH × 2), 66.5 (CH), 65.1 (CH_2), 62.2 (CH_2), 54.1 (CH_2), 52.0 (CH_2), 39.3 (CH_2), 28.7 (CH_2), 27.4 (CH_2), 23.4 (CH_2); HRMS (ESI-TOF) calcd for $C_{16}H_{25}N_2O_3$ $[M+H]^+$ 293.1860, found: 293.1867.



27

(2R)-*N*-[*N*'-(benzyloxycarbonyl)-3'-aminopropyl]-2-phosphoxymethyl pyrrolidine (27). Compound **26** (0.81 g, 2.68 mmol) and tetrabutylammonium phosphate (2.32 g, 6.70 mmol) were coevaporated in vacuo for 10 h. To the mixture of compound **26** and tetrabutylammonium phosphate in dichloromethane (8 mL) was added trichloroacetonitrile (0.8 mL, 8.04 mmol) dropwisely, which resulted an orange solution. The reaction mixture was then stirred at room temperature for 2 days until the TLC plate showed absence of starting material. Removal of the solvent and excess trichloroacetonitrile carefully yielded a brown oil. The mixture was then added with 4 N HCl (10 mL) and methanol (2 mL), which was kept stirring at room temperature for 10 h. The acidic solution was adjusted to pH 8 with ammonium hydroxide. The reaction mixture was evaporated and extracted with chloroform. The aqueous layer was

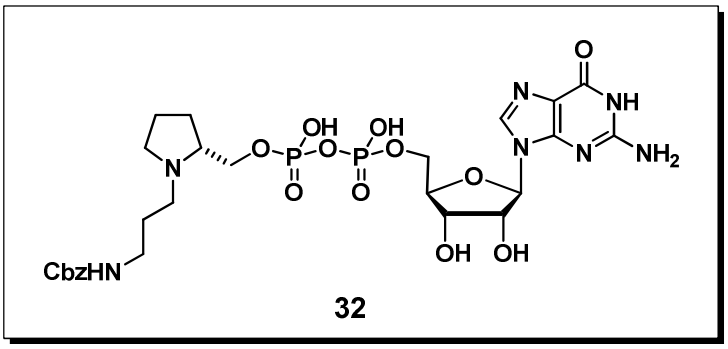
evaporated and further purified with chromatography in silica gel by using *i*-propanol/water/ammonium hydroxide = 7/2/1 as eluent to give compound **27** (0.71 g, 73%); R_f = 0.62 (*i*-propanol/water/ammonium hydroxide = 7/2/1); $[\alpha]^{20}_{D}$ +15.24 (*c* 2.1, H_2O); ^{1}H NMR (D_2O, 400 MHz) δ 7.43-7.41 (5 H, m, ArH), 5.08 (2 H, s, $PhCH_2$), 4.16-4.11 (1 H, m, CH_2), 3.93-3.86 (1 H, m, CH_2), 3.60 (2 H, br, CH_2), 3.34-3.16 (3 H, m, CH, CH_2), 3.00 (2 H, br, CH_2), 2.17 (1 H, br, CH_2), 2.04-1.90 (5 H, m, CH_2); ^{13}C NMR (D_2O, 100 MHz) δ 158.1 (C), 136.4 (C), 128.6 (CH), 128.2 (CH), 127.6 (CH), 68.4 (CH_2), 66.7 (CH_2), 61.8 (CH), 53.9 (CH_2), 52.4 (CH_2), 37.5 (CH_2), 25.5 (CH_2), 25.3 (CH_2), 22.9 (CH_2); ^{31}P NMR (D_2O, 161 MHz) δ 3.60 (1 P, s); HRMS (ESI-TOF) calcd for $C_{16}H_{24}N_2O_6P$ $[M-H]^-$ 371.1366, found: 371.1358.



29

(2R)-guanosine diphosphate-*N*-(3'-aminopropyl)-2-hydroxymethyl pyrrolidine (29; **YCC-1)**. To a solution of compound **27** (0.71 g, 1.87 mmol) and 10 % Pd/C (0.26 g) in water/methanol (10 mL, 1/1, v/v), hydrogen was passed through the stirred mixture for 2 h until the TLC showed no starting material remained. After filtration and

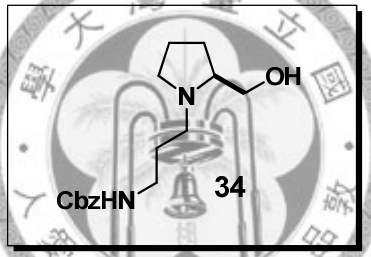
evaporation, the resulting product was then coevaporated with anhydrous pyridine (3 mL for three times). GMP-morpholidate **31** (1.42 g, 3.17 mmol) was added and coevaporated with anhydrous pyridine (3 mL for three times) to a minimum volume. The coupling reaction was initiated with the addition of tatrazole (1.11 g, 15.9 mmol) and pyridine (6 mL) and the reaction was stirred for two days under argon. Pyridine was then removed and the reaction mixture was washed three times with chloroform, evaporated to yield a crude product and purified by silica gel column chromatography by using *i*-propanol/water/ammonium hydroxide = 7/2/1 as eluent to give compound **29** (0.32 g, 31%); R_f = 0.23 (*i*-propanol/water/ammonium hydroxide = 7/2/1); $[\alpha]^{20}_{D}$ +7.2 (*c* 5.3, H_2O); ^{1}H NMR (D_2O, 400 MHz) δ 8.15 (1 H, s, CH), 5.97 (1 H, d, *J* = 5.2 Hz, CH), 4.80 (1 H, s, CH, HOD), 4.53 (1 H, br, CH), 4.38 (1 H, br, CH), 4.3-4.24 (3 H, m, CH, CH_2), 4.10-4.08 (1 H, m, CH_2), 3.87-3.85 (1 H, m, CH_2), 3.76-3.72 (1 H, m, CH_2), 3.56-3.49 (1 H, m, CH_2), 3.28-3.13 (4 H, m, CH, CH_2), 2.26-1.92 (6 H, m, CH_2); ^{13}C NMR (D_2O, 100 MHz) δ 158.8 (C), 153.9 (C), 151.6 (C), 137.3 (C), 116.1 (C), 86.9 (CH), 83.5 (1 C, d, J_{C-P} = 8.8 Hz, CH), 73.8 (CH_2), 70.3 (CH), 67.8 (CH), 65.3 (CH), 63.8 (1 C, d, J_{C-P} = 24.5 Hz, CH_2), 54.9 (CH_2), 52.0 (CH_2), 36.5 (CH_2), 25.5 (CH_2), 23.2 (CH_2), 22.5 (CH_2); ^{31}P NMR (D_2O, 161 MHz) δ −10.68 (1 P, d, J_{P-P} = 20.8 Hz), −11.16 (1 P, d, J_{P-P} = 20.9 Hz); HRMS (ESI-TOF) calcd for $C_{18}H_{30}N_7O_{11}P_2$ $[M-H]^-$ 582.1473, found: 582.1506.



32

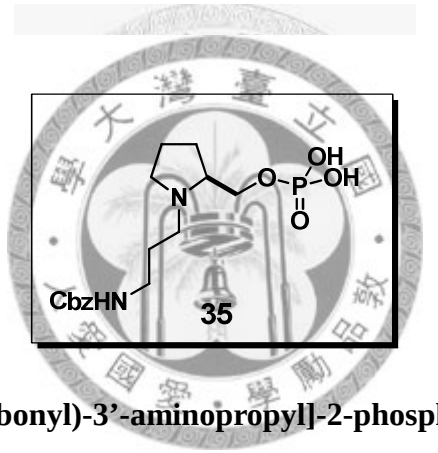
(2R)-guanosine diphosphate-*N*-[*N*'-(benzyloxycarbonyl)-3'-aminopropyl]-2-hydroxymethyl pyrrolidine (**32**; **YCC-3**). Compound **27** (0.33 g, 0.89 mmol) was coevaporated with anhydrous pyridine (3 mL for three times). GMP-morpholidate **31** (0.77 g, 1.77 mmol) was added and coevaporated with anhydrous pyridine (3 mL for three times) to a minimum volume. The coupling reaction was initiated with the addition of tatrazole (0.62 g, 8.86 mmol) and pyridine (6 mL) and the reaction was stirred for two days under argon. Pyridine was then removed and the reaction mixture was washed three times with chloroform, evaporated to yield a crude product and purified by silica gel column chromatography by using *i*-propanol/water/ammonium hydroxide = 7/2/1 as eluent to give compound **32** (0.25 g, 39%); R_f = 0.34 (*i*-propanol/water/ammonium hydroxide = 7/2/1); $[\alpha]^{20}_{D}$ +8.8 (*c* 2.5, H_2O); ^{1}H NMR (D_2O, 400 MHz) δ 8.07 (1 H, s, CH), 7.36- 7.28 (5 H, m, ArH), 5.89 (1 H, d, *J* = 5.6 Hz), 5.01 (2 H, s, $PhCH_2$), 4.70 (1 H, t, *J* = 5.4 Hz, CH), 4.48 (1 H, t, *J* = 4.5 Hz), 4.35 (1 H, br, CH), 4.24-4.21 (3 H, m, CH_2), 4.10-4.09 (1 H, m, CH_2), 3.75 (1 H, br, CH_2), 3.65 (1 H, br, CH_2), 3.37 (1 H, br, CH_2), 3.17 (2 H, br, CH, CH_2), 3.07 (2 H, br, CH_2), 2.20-2.18

(1 H, m, CH_2), 2.05- 1.94 (5 H, m, CH_2); ^{13}C NMR (D_2O, 100 MHz) δ 158.6 (C), 158.1 (C), 153.7 (C), 151.5 (C), 137.2 (CH), 136.2 (C), 128.5 (CH), 128.0 (CH), 127.2 (CH), 116.1 (C), 86.9 (CH), 83.5 (1 C, d, $J_{C\text{-}P}$ = 8.3 Hz, CH), 73.7 (CH_2), 70.2 (CH), 67.6 (CH), 66.6 (CH_2), 65.3 (CH), 63.6 (1 C, d, $J_{C\text{-}P}$ = 20.8 Hz, CH), 54.5 (CH_2), 53.0 (CH_2), 37.4 (CH_2), 25.5 (CH_2), 25.2 (CH_2), 22.6 (CH_2); ^{31}P NMR (D_2O, 161 MHz) δ −10.62 (1 P, d, $J_{P\text{-}P}$ = 21.1 Hz), −11.13 (1 P, d, $J_{P\text{-}P}$ = 20.9 Hz); HRMS (ESI-TOF) calcd for $C_{26}H_{36}N_7O_{13}P_2$ $[M-H]^-$ 716.1841, found: 716.1825.



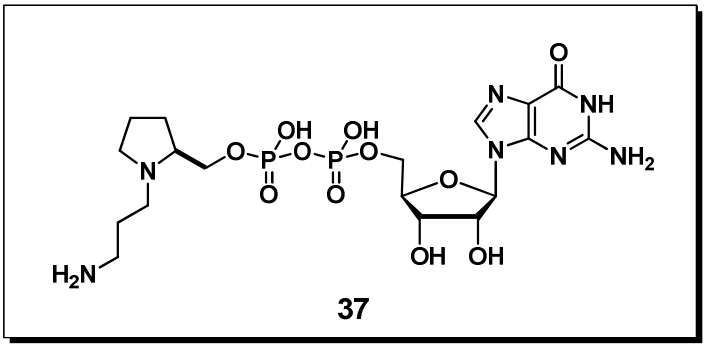
(2S)-*N*-[*N*'-(benzyloxycarbonyl)-3'-aminopropyl]-2-hydroxymethyl pyrrolidine (**34**). To a stirring mixture of L-(+)-prolinol **33** (0.71 mL, 6.92 mmol) and potassium carbonate (1.0 g, 6.92 mmol) in THF (15 mL) was added compound **24** (1.8 g, 6.57 mmol) at room temperature and then stirred at 60 ^oC for 14 h. The reaction mixture was cooled to room temperature, diluted with ethyl acetate (40 mL), washed with water twice and dried over Mg_2SO_4. Removal of the solvent afforded a pure product (1.9 g, 93%). R_f = 0.2 (methanol/chloroform = 1/20); $[\alpha]^{20}_D$ −25.35 (*c* 10.1, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.37-7.31 (5 H, m, ArH), 5.19 (1 H, br, NH), 5.10 (2 H, s, $PhCH_2$),

3.62 (1 H, dd, J = 10.9, 3.6 Hz, CH_2), 3.41 (1 H, dd, J = 10.9, 2.8 Hz, CH_2), 3.31-3.26 (2 H, m, CH_2), 3.18-3.17 (1 H, m, CH_2), 2.83-2.80 (1 H, m, CH_2), 2.56-2.54 (1 H, m, CH), 2.35-2.29 (1 H, m, CH_2), 1.90-1.85 (1 H, m, CH_2), 1.80-1.68 (5 H, m, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ156.5 (C), 136.7 (C), 128.5 (CH), 128.0 (CH × 2), 66.6 (CH), 65.2 (CH_2), 62.2 (CH_2), 54.1 (CH_2), 52.0 (CH_2), 39.4 (CH_2), 28.8 (CH_2), 27.4 (CH_2), 23.5 (CH_2); HRMS (ESI-TOF) calcd for $C_{16}H_{25}N_2O_3$ $[M+H]^+$ 293.1860, found: 293.1865.



(2S)-*N*-[*N*'-(benzyloxycarbonyl)-3'-aminopropyl]-2-phosphoxymethyl pyrrolidine (**35**). Compound **34** (0.81 g, 2.76 mmol) and tetrabutylammonium phosphate (2.42 g, 6.92 mmol) were coevaporated in vacuo for 10 h. To the mixture of compound **34** and tetrabutylammonium phosphate in dichloromethane (9 mL) was added trichloroacetonitrile (0.82 mL, 8.31 mmol) dropwisely, which resulted an orange solution. The reaction mixture was then stirred at room temperature for 2 days until the TLC plate showed absence of starting material. Removal of the solvent and excess trichloroacetonitrile carefully yielded a brown oil. The mixture was then added with 4 N

HCl (10 mL) and methanol (2 mL), which was kept stirring at room temperature for 10 h. The acidic solution was adjusted to pH 8 with ammonium hydroxide. The reaction mixture was evaporated and extracted with chloroform. The aqueous layer was evaporated and further purified with chromatography in silica gel by using *i*-propanol/water/ammonium hydroxide = 7/2/1 as eluent to give compound **35** (0.71 g, 72%); R_f = 0.62 (*i*-propanol/water/ammonium hydroxide = 7/2/1); $[\alpha]^{20}_{D}$ −13.67 (*c* 44.3, H_2O); ^{1}H NMR (D_2O, 400 MHz) δ 7.41-7.39 (5 H, m, ArH), 5.07 (2 H, s, $PhCH_2$), 4.14-4.09 (1 H, m, CH_2), 3.90-3.83 (1 H, m, CH_2), 3.56-3.53 (2 H, m, CH_2), 3.31-3.14 (3 H, m, CH, CH_2), 2.98 (2 H, br, CH_2), 2.15-2.10 (1 H, m, CH_2), 2.01-1.89 (5 H, m, CH_2); ^{13}C NMR (D_2O, 100 MHz) δ 157.9 (C), 136.3 (C), 128.6 (CH), 128.1 (CH), 127.5 (CH), 68.4 (CH_2), 66.6 (CH_2), 61.6 (CH), 53.7 (CH_2), 52.2 (CH_2), 37.4 (CH_2), 25.5 (CH_2), 25.2 (CH_2), 22.9 (CH_2); ^{31}P NMR (D_2O, 161 MHz) δ 3.60 (1 P, s); HRMS (ESI-TOF) calcd for $C_{16}H_{24}N_2O_6P$ $[M-H]^-$ 371.1366, found: 371.1338.

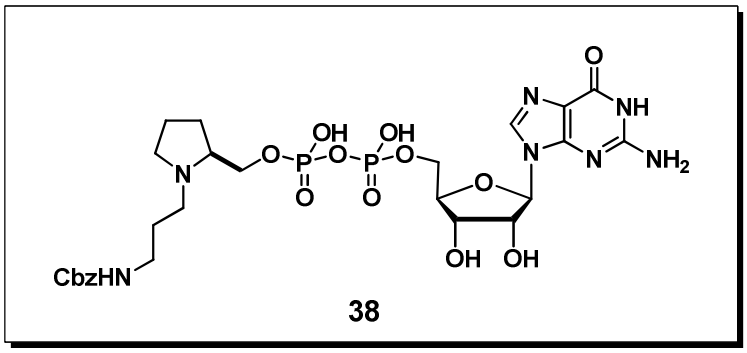


37

(2S)-guanosine diphosphate-*N*-(3'-aminopropyl)-2-hydroxymethyl pyrrolidine (**37**;

YCC-2). To a solution of compound **35** (0.16 g, 0.44 mmol) and 5 % Pd/C (0.31 g) in water/methanol (10 mL, 1/1, v/v), hydrogen was passed through the stirred mixture for 2 h until the TLC showed no more starting material remained. After filtration and evaporation, the resulting product was then coevaporated with anhydrous pyridine (3 mL for three times). GMP-morpholidate **31** (0.38 g, 0.88 mmol) was added and coevaporated with anhydrous pyridine (3 mL for three times) to a minimum volume. The coupling reaction was initiated with the addition of tatrazole (0.31 g, 4.41 mmol) and pyridine (6 mL) and the reaction was stirred for two days under argon. Pyridine was then removed and the reaction mixture was washed three times with chloroform, evaporated to yield a crude product and purified by silica gel column chromatography by using *i*-propanol/water/ammonium hydroxide = 7/2/1 as eluent to give compound **37** (0.10 g, 29%); R_f = 0.23 (*i*-propanol/water/ammonium hydroxide = 7/2/1); $[\alpha]^{20}_{D}$ — 10.12 (*c* 0.2, H_2O); ^{1}H NMR (D_2O, 400 MHz) δ 8.06 (1 H, s, CH), 5.89 (1 H, d, *J* = 5.8 Hz, CH), 4.80 (1 H, s, CH, HOD), 4.45 (1 H, br, CH), 4.29 (1 H, br, CH), 4.22-4.16 (3 H, m, CH, CH_2), 4.01-3.98 (1 H, m, CH_2), 3.78-3.76 (1 H, m, CH_2), 3.65-3.63 (1 H, m, CH_2), 3.41 (1 H, br, CH_2), 3.12-3.04 (4 H, m, CH, CH_2), 2.17-1.80 (6 H, m, CH_2); ^{13}C NMR (D_2O, 100 MHz) δ 159.0 (C), 154.1 (C), 151.8 (C), 137.5 (CH), 116.3 (C), 86.9 (CH), 83.6 (1 C, d, $J_{C\text{-}P}$ = 7.0 Hz, CH), 73.8 (CH_2), 70.4(CH), 67.9 (CH), 65.4 (CH), 63.8 (CH_2), 54.9 (CH_2), 52.2 (CH_2), 36.6 (CH_2), 25.6 (CH_2), 23.4 (CH_2), 22.7 (CH_2); ^{31}P

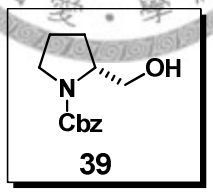
NMR (D_2O, 161 MHz) δ −10.66 (1 P, d, $J_{P\text{-}P}$ = 21.1 Hz), −11.14 (1 P, d, $J_{P\text{-}P}$ = 20.8 Hz); HRMS (ESI-TOF) calcd for $C_{18}H_{30}N_7O_{11}P_2$ $[M-H]^-$ 582.1426, found: 582.1473.



38

(2S)-guanosine diphosphate-*N*-[*N'*-(benzyloxycarbonyl)-3'-aminopropyl]-2-hydroxymethyl pyrrolidine (**38**; **YCC-4**). Compound **35** (0.43 g, 0.99 mmol) was coevaporated with anhydrous pyridine (3 mL for three times). GMP-morpholidate **31** (0.92 g, 1.99 mmol) was added and coevaporated with anhydrous pyridine (3 mL for three times) to a minimum volume. The coupling reaction was initiated with the addition of tatrazole (0.71 g, 9.94 mmol) and pyridine (6 mL) and the reaction was stirred for two days under argon. Pyridine was then removed and the reaction mixture was washed three times with chloroform, evaporated to yield a crude product and purified by silica gel column chromatography by using *i*-propanol/water/ammonium hydroxide = 7/2/1 as eluent to give compound **38** (0.30 g, 36%); R_f = 0.34 (*i*-propanol/water/ammonium hydroxide = 7/2/1); $[\alpha]^{20}_D$ −20.32 (*c* 1.9, H_2O); ^1H NMR (D_2O, 400 MHz) δ 8.05 (1 H, s, CH), 7.39-7.25 (5 H, m, ArH), 5.87 (1 H, d, *J* =

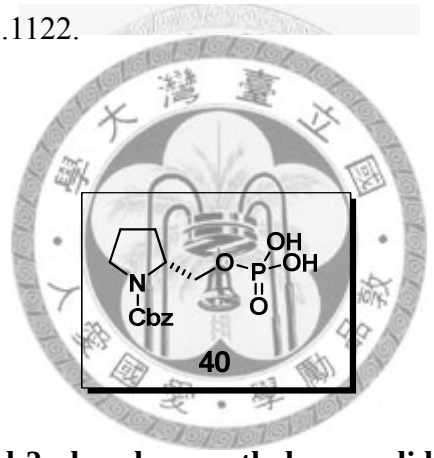
5.4 Hz, CH), 4.97 (2 H, s, $PhCH_2$), 4.66 (1 H, t, J = 5.2 Hz, CH), 4.48 (1 H, t, J = 4.8 Hz, CH), 3.34 (1 H, br, CH), 4.26-4.20 (3 H, m, CH_2), 4.09-4.06 (1 H, m, CH_2), 3.71 (1 H, br, CH_2), 3.64 (1 H, br, CH_2), 3.32 (1 H, br, CH_2), 3.14 (2 H, br, CH, CH_2), 3.04 (2 H, br, CH_2), 2.19-2.17 (1 H, m, CH_2), 2.02-1.92 (5 H, m, CH_2); ^{13}C NMR (D_2O, 100 MHz) δ 158.2 (C), 157.8 (C), 153.5 (C), 151.1 (C), 136.8 (CH), 136.1 (C), 128.4 (CH), 127.9 (CH), 127.1 (CH), 115.8 (C), 87.0 (CH), 83.1 (1 C, d, $J_{C\text{-}P}$ = 8.3 Hz, CH), 73.9 (CH_2), 70.0 (CH), 67.5 (CH), 66.5 (CH_2), 65.2 (CH), 63.6 (CH), 54.4 (CH_2), 52.7 (CH_2), 37.4 (CH_2), 25.5 (CH_2), 25.2 (CH_2), 22.5 (CH_2); ^{31}P NMR (D_2O, 161 MHz) δ −10.64 (1 P, d, $J_{P\text{-}P}$ = 20.9 Hz), −11.15 (1 P, d, $J_{P\text{-}P}$ = 21.0 Hz); HRMS (ESI-TOF) calcd for $C_{26}H_{36}N_7O_{13}P_2$ $[M-H]^-$ 716.1841, found: 716.1822.



39

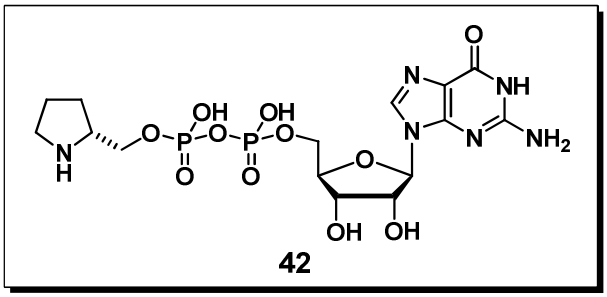
(2R)-*N*-benzyloxycarbonyl-2-hydroxymethyl pyrrolidine (39).[67] A stirred solution of D-(-)-prolinol **25** (0.60 g, 5.93 mmol) in water (15 mL) was added sodium bicarbonate (0.52 g, 5.63 mmol), followed by addition of benzyl chloroformate (0.8 mL, 5.63 mmol). The reaction mixture was stirred vigorously for 15 h, extracted with ethyl acetate for three times and dried over Mg_2SO_4. The organic layer was evaporated and the afforded oil was further purified chromatography in silica gel by using ethylacetate/hexane = 6/4

as eluent to yield a white solid. (1.40 g, 76%); R_f = 0.16 (ethyl acetate/hexane = 4/6); $[\alpha]^{20}_D$ +36.42 (*c* 5.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.37-7.31 (5 H, m, ArH), 5.14 (2 H, AB q, *J* = 2.6, 12.5 Hz, CH_2), 4.40 (1 H, br, OH), 4.00 (1 H, br, CH_2), 3.65-3.61 (2 H, m, CH,CH_2), 3.56-3.50 (1 H, m, CH_2), 3.43-3.36 (1 H, m, CH_2), 2.05-1.97 (3 H, m, CH_2), 1.67 (1 H, br, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.9 (C), 136.4 (C), 128.4 (CH), 127.9 (CH), 127.8 (CH), 67.1 (CH), 66.6 (CH_2), 60.5 (CH_2), 47.2 (CH_2), 28.4 (CH_2), 23.9 (CH_2); HRMS (ESI-TOF) calcd for $C_{13}H_{17}NO_3Na$ [M+ Na]$^+$ 258.1101, found: 258.1122.



(2R)-*N*-benzyloxycarbonyl-2-phosphoxymethyl pyrrolidine (**40**). Compound **39** (0.70 g, 2.91 mmol) and tetrabutylammonium phosphate (2.53 g, 7.28 mmol) were coevaporated in vacuo for 10 h. To the mixture of compound **34** and tetrabutylammonium phosphate in dichloromethane (10 mL) was added trichloroacetonitrile (0.9 mL, 8.71 mmol) dropwisely, which resulted an yellow solution. The reaction mixture was then stirred at room temperature for 2 days until the TLC plate showed absence of starting material. Removal of the solvent and excess trichloroacetonitrile yielded a brown oil. The mixture was then added with 4 N HCl (8

mL) and methanol (4 mL), which was kept stirring at room temperature for 12 h. The acidic solution was adjusted to pH 8 with ammonium hydroxide. The reaction mixture was evaporated and extracted with chloroform. The aqueous layer was evaporated and further purified with chromatography in silica gel by using *i*-propanol/water/ammonium hydroxide = 7/1.5/1 as eluent to give compound **40** (0.82 g, 86%); R_f = 0.38 (*i*-propanol/water/ammonium hydroxide = 7/2/1); $[\alpha]^{20}_{D}$ +34.21 (*c* 4.2, H_2O); ^{1}H NMR (D_2O, 400 MHz) δ 7.43-7.38 (5 H, m, ArH), 5.20-5.08 (2 H, m, CH_2), 4.04-3.98 (1 H, m, CH), 3.86 (1 H, br, CH_2), 3.78 (1 H, br, CH_2), 3.40 (2 H, br, CH_2), 1.98-1.93 (3 H, m, CH_2), 1.83 (1 H, br, CH_2); ^{13}C NMR (D_2O, 100 MHz) δ 156.3 (C), 136.4 (C), 128.6 (CH), 128.2 (CH), 127.6 (CH), 67.1 (1 C, d, $J_{C\text{-}P}$ = 9.1 Hz, CH_2), 64.5 (1 C, d, $J_{C\text{-}P}$ = 59.7 Hz, CH_2), 57.3 (1 C, d, $J_{C\text{-}P}$ = 34.8, CH), 46.7 (1 C, d, $J_{C\text{-}P}$ = 25.8, CH_2), 27.3 (1 C, d, $J_{C\text{-}P}$ = 58.3 Hz, CH_2), 22.6 (1 C, d, $J_{C\text{-}P}$ = 59.1 Hz, CH_2); ^{31}P NMR (D_2O, 161 MHz) δ 2.26 (1 P, d, $J_{C\text{-}P}$ = 48.2 Hz); HRMS (ESI-TOF) calcd for $C_{13}H_{17}NO_6P$ $[M-H]^-$ 314.0788, found: 314.0784.

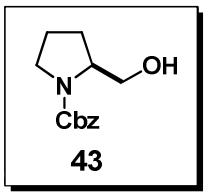


42

(2R)-guanosine diphosphate-2-hydroxymethyl pyrrolidine (**42**). To a solution of

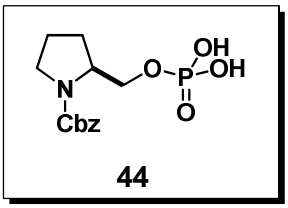
compound **40** (0.82 g, 2.51 mmol) and 10 % Pd/C (0.31 g) in water/methanol (10 mL, 1/1, v/v), hydrogen was passed through the stirred mixture for 2 h until the TLC showed no starting material remained. After filtration and evaporation, the resulting product was then coevaporated with anhydrous pyridine (3 mL for three times). GMP-morpholidate **31** (2.60 g, 5.96 mmol) was added and coevaporated with anhydrous pyridine (3 mL for three times) to a minimum volume. The coupling reaction was initiated with the addition of tatrazole (1.10 g, 14.91 mmol) and pyridine (6 mL). The reaction was stirred for 3 days under nitrogen. Pyridine was then evaporated to yield a crude product and purified by silica gel column chromatography by using *i*-propanol/water/ammonium hydroxide = 7/2/1 as eluent to give compound **42** (0.72 g, 39%); R_f = 0.25 (*i*-propanol/water/ammonium hydroxide = 7/3/1); $[\alpha]^{20}_{D}$ +9.27 (*c* 1.2, H_2O); ^{1}H NMR (D_2O, 400 MHz) δ 8.12 (1 H, s, ArH), 5.96 (1 H, d, *J* = 4.7 Hz, CH), 4.80 (1 H, s, CH, HOD), 4.51 (1 H, t, *J* = 3.2 Hz, CH), 4.36 (1 H, br, CH), 4.22-4.21 (3 H, m, CH_2), 4.04-3.99 (1 H, m, CH_2), 3.90-3.88 (1 H, m, CH), 3.32 (2 H, t, *J* = 5.7 Hz, CH_2), 2.12-1.96 (3 H, m, CH_2), 1.80-1.76 (1 H, m, CH_2); ^{13}C NMR (D_2O, 100 MHz) δ 161.1 (C), 151.5 (C), 137.4 (C), 116.0 (C), 86.6 (CH), 84.3 (1 C, d, $J_{C\text{-}P}$ = 8.7 Hz, CH), 74.1 (CH), 70.4 (CH), 63.3 (CH_2), 62.5 (1 C, d, $J_{C\text{-}P}$ = 4.0 Hz, CH_2), 60.2 (1 C, d, $J_{C\text{-}P}$ = 4.5 Hz, CH), 45.3 (CH_2), 25.6 (CH_2), 23.8 (CH_2); ^{31}P NMR (D_2O, 161 MHz) δ −11.15 (1 P, d, $J_{P\text{-}P}$ = 16.4 Hz), −11.50 (1 P, d, $J_{P\text{-}P}$ = 16.6 Hz); HRMS (ESI-TOF) calcd for

$C_{15}H_{23}N_6O_{11}P_2$ $[M-H]^-$ 525.0895, found: 525.0890.



43

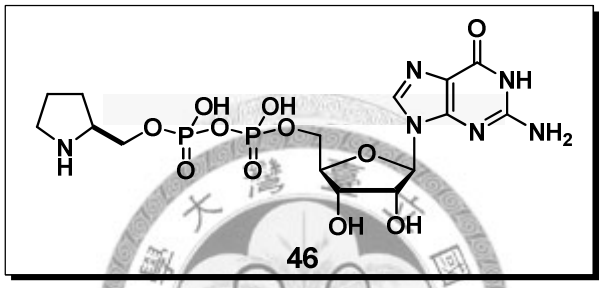
(2S)-*N*-benzyloxycarbonyl-2-hydroxymethyl pyrrolidine (43).[64] A stirred solution of L-(+)-prolinol **33** (0.60 g, 5.93 mmol) in water (15 mL) was added sodium bicarbonate (0.53 g, 5.63 mmol), followed by addition of benzyl chloroformate (0.8 mL, 5.63 mmol). The reaction mixture was stirred vigorously for 15 h, extracted with ethyl acetate for three times and dried over Mg_2SO_4. The organic layer was evaporated and the afforded oil was further purified with column chromatography by using ethyl acetate/hexane = 6/4 as eluent to yield a white solid. (1.0 g, 70%); R_f = 0.16 (ethyl acetate/hexane = 4/6); $[\alpha]^{20}_D$ −39.41 (*c* 8.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.36-7.30 (5 H, m, ArH), 5.13 (2 H, AB q, *J* = 12.5, 3.0 Hz, CH_2), 4.44 (1 H, br, OH), 3.99 (1 H, br, CH_2), 3.64 (2 H, br, CH, CH_2), 3.54-3.48 (1 H, m, CH_2), 3.42-3.35 (1 H, m, CH_2), 2.02-1.95 (1 H, m, CH_2), 1.89-1.75 (2 H, m, CH_2), 1.66-1.64 (1 H, m, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.7(C), 136.4 (C), 128.3 (CH), 127.9 (CH), 127.7 (CH), 67.0 (CH), 66.2 (CH_2), 60.4 (CH_2), 47.1 (CH_2), 28.3 (CH_2), 23.8 (CH_2); HRMS (ESI-TOF) calcd for $C_{13}H_{17}NO_3Na$ $[M+Na]^+$ 258.1101, found: 258.1116.



44

(2S)-*N*-benzyloxycarbonyl-2-phosphoxymethyl pyrrolidine (**44**). Compound **43** (0.81 g, 3.36 mmol) and tetrabutylammonium phosphate (2.90 g, 8.39 mmol) were coevaporated in vacuo for 12 h. To the mixture of compound **43** and tetrabutylammonium phosphate in dichloromethane (10 mL) was added trichloroacetonitrile (1.0 mL, 10.1 mmol) dropwisely, which resulted a yellow solution. The reaction mixture was then stirred at room temperature for 2 days until the TLC plate showed absence of starting material. Remove the solvent and excess trichloroacetonitrile carefully. The mixture was then added with 4 N HCl (8 mL) and methanol (4 mL), which was kept stirring at room temperature for 12 h. The acidic solution was adjusted to pH 8 with ammonium hydroxide. The reaction mixture was evaporated and extracted with chloroform. The aqueous layer was evaporated and further purified with chromatography in silica gel by using *i*-propanol/water/ammonium hydroxide = 7/1.5/1 as eluent to give compound **44** (0.90 g, 81%); R_f = 0.45 (*i*-propanol/water/ammonium hydroxide = 7/2/1); $[\alpha]^{20}_{D}$ −31.22 (*c* 5.0, H_2O); ^{1}H NMR (D_2O, 400 MHz) δ 7.38-7.33 (5 H, m, ArH), 5.13-4.97 (2 H, m, CH_2), 3.93 (1 H, br, CH), 3.86-3.81 (1 H, m, CH_2), 3.77-3.70 (1 H, m, CH_2), 3.33-3.27 (2 H, m, CH_2), 1.96-1.89 (3 H, m, CH_2), 1.76 (1 H, br, CH_2); ^{13}C NMR (D_2O, 100 MHz) δ 156.1 (C),

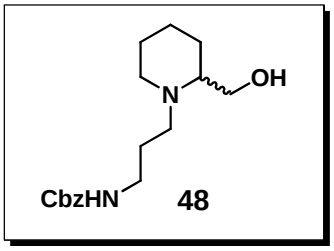
136.2 (C), 128.6 (CH), 128.1 (CH), 127.5 (CH), 67.0 (CH_2), 64.4 (1 C, d, $J_{C\text{-}P}$ = 75.0 Hz, CH_2), 57.3 (1 C, d, $J_{C\text{-}P}$ = 36.6 Hz, CH), 46.7 (1 C, d, $J_{C\text{-}P}$ = 31.8 Hz, CH_2), 27.3 (1 C, d, $J_{C\text{-}P}$ = 59.3 Hz, CH_2), 22.5 (1 C, d, $J_{C\text{-}P}$ = 49.9 Hz, CH_2); ^{31}P NMR (D_2O, 161 MHz) δ 1.72 (1 P, d, $J_{C\text{-}P}$ = 41.0 Hz); HRMS (ESI-TOF) calcd for $C_{13}H_{17}NO_6P$ $[M-H]^-$ 314.0788, found: 314.0792.



46

(2S)-guanosine diphosphate-2-hydroxymethyl pyrrolidine (**46**). To a solution of compound **44** (0.62 g, 1.91 mmol) and 10 % Pd/C (0.23 g) in water/methanol (8 mL, 1/1, v/v), hydrogen was passed through the stirred mixture for 2 h until the TLC showed no starting material remained. After filtration and evaporation, the resulting product was then coevaporated with anhydrous pyridine (3 mL for three times). GMP-morpholidate **31** (1.60 g, 3.79 mmol) was added and coevaporated with anhydrous pyridine (3 mL for three times) to a minimum volume. The coupling reaction was initiated with the addition of tatrazole (0.71 g, 9.48 mmol) and pyridine (6 mL). The reaction was stirred for 3 days under nitrogen. Pyridine was then evaporated to yield a crude product and purified by silica gel column chromatography by using *i*-propanol/water/ammonium

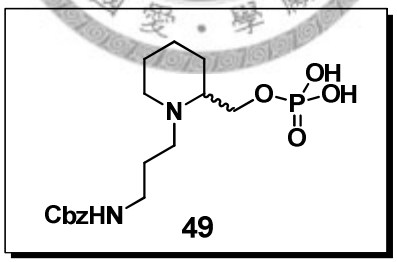
hydroxide = 7/2/1 as eluent to give compound **46** (0.52 g, 46%); R_f = 0.25 (*i*-propanol/water/ammonium hydroxide = 7/3/1); $[\alpha]^{20}_{D}$ −12.15 (*c* 1.8, H_2O); ^1H NMR (D_2O, 400 MHz) δ 8.23 (1 H, s, ArH), 5.95 (1 H, d, *J* = 5.8 Hz, CH), 4.80 (1 H, s, CH, HOD), 4.49 (1 H, t, *J* = 6.0 Hz, CH), 4.33-4.32 (1 H, br, CH), 4.09-3.99 (3 H, m, CH_2), 3.89-3.81 (2 H, m, CH, CH_2), 3.31 (2 H, t, *J* = 7.6 Hz, CH_2), 2.16-1.98 (3 H, m, CH_2), 1.96-1.88 (1 H, m, CH_2); ^{13}C NMR (D_2O, 100 MHz) δ 161.1 (C), 151.5 (C), 137.4 (CH), 116.0 (C), 86.6 (CH), 84.2 (1 C, d, J_{C-P} = 8.5 Hz, CH), 74.1 (CH), 70.4 (CH), 63.3 (CH_2), 62.5 (1 C, d, J_{C-P} = 4.0 Hz, CH_2), 60.1 (1 C, d, J_{C-P} = 4.4 Hz, CH), 45.3 (CH_2), 25.6 (CH_2), 23.8 (CH_2); ^{31}P NMR (D_2O, 161 MHz) δ −10.88 (1 P, d, J_{P-P} = 20.1 Hz), −11.26 (1 P, d, J_{P-P} = 18.9 Hz); HRMS (ESI-TOF) calcd for $C_{15}H_{23}N_6O_{11}P_2$ $[M-H]^-$ 525.0895, found: 525.0883.



48

***N*-[*N*'-(benzyloxycarbonyl)-3'-aminopropyl]-2-hydroxymethyl piperidine** (**48**). To a stirring mixture of 2-piperidinemethanol **47** (0.6 mL, 4.35 mmol) and potassium carbonate (0.62 g, 4.35 mmol) in THF (12 mL) was added compound **24** (1.10 g, 4.13 mmol) at room temperature and then stirred at 60 °C for 14 h. The reaction mixture was

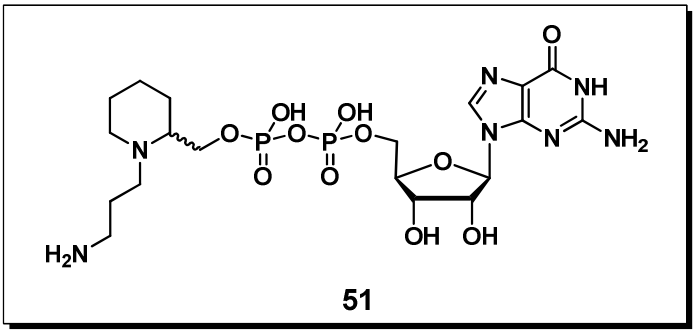
cooled to room temperature, diluted with ethyl acetate (40 mL), washed with water twice and dried over Mg_2SO_4. Removal of the solvent afforded a pure product (1.20 g, 89%). R_f = 0.23 (methanol/chloroform = 1/20); $[\alpha]^{20}_D$ −5.12 (*c* 0.3, $CDCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33-7.32 (5 H, m, ArH), 5.98 (1 H, br, NH), 5.07 (2 H, s, $PhCH_2$), 4.50 (1 H, br, OH), 3.75-3.71 (1 H, m, CH_2), 3.59-3.55 (1 H, m, CH_2), 3.22-3.21 (2 H, m, CH_2), 3.02-2.90 (2 H, m, CH_2), 2.58-2.51 (2 H, m, CH_2), 2.32-2.27 (1 H, m, CH), 1.73-1.58 (7 H, m, CH_2), 1.37-1.32 (1 H, m, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.6 (C), 136.7 (C), 128.5 (CH), 128.0 (CH × 2), 66.6 (CH_2), 62.2 (CH), 61.8 (CH_2), 50.8 (CH_2), 49.9 (CH_2), 39.2 (CH_2), 26.8 (CH_2), 26.1 (CH_2), 23.5 (CH_2), 23.0 (CH_2); HRMS (ESI-TOF) calcd for $C_{17}H_{27}N_2O_3$ $[M+H]^+$ 307.2016, found: 307.1990.



49

***N*-[*N'*-(benzyloxycarbonyl)-3'-aminopropyl]-2-phosphoxymethyl piperidine** (**49**). Compound **48** (0.10 g, 3.11 mmol) and tetrabutylammonium phosphate (2.61 g, 7.74 mmol) were coevaporated in vacuo for 10 h. To the mixture of compound **48** and tetrabutylammonium phosphate in dichloromethane (10 mL) was added trichloroacetonitrile (0.9 mL, 9.32 mmol) dropwisely, which resulted an orange solution.

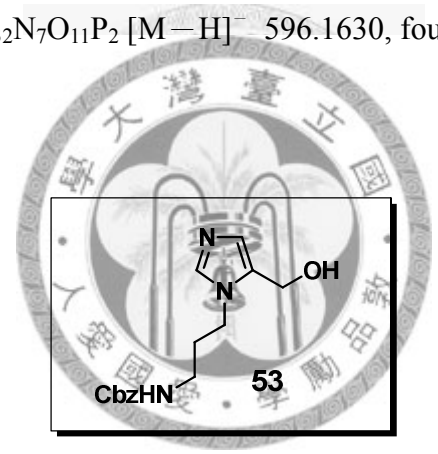
The reaction mixture was then stirred at room temperature for 2 days until the TLC plate showed absence of starting material. Removal of the solvent and excess trichloroacetonitrile carefully yielded a brown oil. The mixture was then added with 4 N HCl (8 mL) and methanol (2 mL), which was kept stirring at room temperature for 10 h. The acidic solution was adjusted to pH 8 with ammonium hydroxide. The reaction mixture was evaporated and extracted with chloroform. The aqueous layer was evaporated and further purified with chromatography in silica gel by using *i*-propanol/water/ammonium hydroxide = 7/2/1 as eluent to give compound **49** (0.93 g, 76%); R_f = 0.31 (*i*-propanol/water/ammonium hydroxide = 7/2/1); $[\alpha]^{20}_{D}$ −2.43 (*c* 1.0, H_2O); ^{1}H NMR (D_2O, 400 MHz) δ 7.36-7.35 (5 H, m, ArH), 5.03 (2 H, s, $PhCH_2$), 4.00 (1 H, br, CH_2), 3.79-3.76 (1 H, br, CH_2), 3.34-3.31 (1 H, m, CH_2), 3.17-3.14 (5 H, m, CH, CH_2), 2.92 (1 H, br, CH_2), 1.83-1.61 (7 H, m, CH_2), 1.46 (1 H, br, CH_2); ^{13}C NMR (D_2O, 100 MHz) δ 158.2 (C), 136.4 (C), 128.7 (CH), 128.3 (CH), 127.6 (CH), 66.8 (CH_2), 62.3 (CH), 61.8 (CH_2), 51.2 (CH_2), 50.1 (CH_2), 37.5 (CH_2), 25.6 (CH_2), 23.1 (CH_2), 21.8 (CH_2), 20.6 (CH_2); ^{31}P NMR (D_2O, 161 MHz) δ 3.31 (1 P, s); HRMS (ESI-TOF) calcd for $C_{17}H_{26}N_2O_6P$ $[M-H]^-$ 385.1523, found: 385.1548.



51

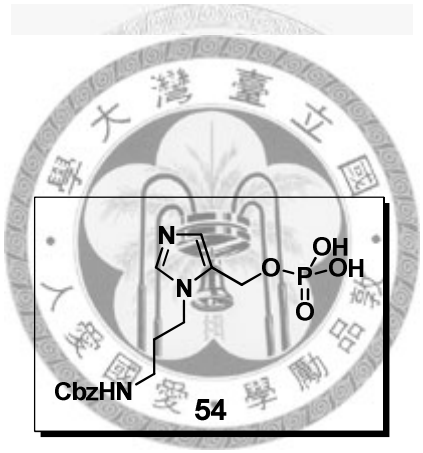
Guanosine diphosphate-*N*-(3'-aminopropyl)-2-hydroxymethyl piperidine (**51**; **YCC-5**). To a solution of compound **49** (0.19 g, 0.48 mmol) and 10 % Pd/C (0.07 g) in water/methanol (8 mL, 1/1, v/v), hydrogen was passed through the stirred mixture for 2 h until the TLC showed no starting material remained. After filtration and evaporation, the resulting product was then coevaporated with anhydrous pyridine (3 mL for three times). GMP-morpholidate **31** (0.41 g, 0.96 mmol) was added and coevaporated with anhydrous pyridine (3 mL for three times) to a minimum volume. The coupling reaction was initiated with the addition of tatrazole (0.34 g, 4.79 mmol) and pyridine (6 mL) and the reaction was stirred for two days under argon. Pyridine was then removed and the reaction mixture was washed three times with chloroform, evaporated to yield a crude product and purified by silica gel column chromatography by using *i*-propanol/water/ammonium hydroxide = 7/2/1 as eluent to give compound **51** (0.08 g, 29%); R_f = 0.2 (*i*-propanol/water/ammonium hydroxide = 7/2/1); $[\alpha]^{20}_{D}$ −4.29 (*c* 1.4, H_2O); ^{1}Hpr NMR (D_2O, 400 MHz) δ 8.05 (1 H, s, CH), 5.86 (1 H, d, *J* = 5.8 Hz, CH), 4.42 (1 H, t, *J* = 4.3 Hz, CH), 4.28 (1 H, br, CH), 4.20-4.10 (3 H, m, CH_2), 3.96-3.92 (1

H, m, CH_2), 3.38 (1 H, d, J = 12.6 Hz, CH_2), 3.27-3.25 (2 H, m, CH, CH_2), 3.17-3.08 (1 H, m, CH_2), 3.03 (2 H, t, J = 7.4 Hz, CH_2), 2.92 (1 H, t, J = 12.3 Hz, CH_2), 2.10-2.00 (2 H, m, CH_2), 1.78-1.57 (5 H, m, CH_2), 1.51-1.48 (1 H, m, CH_2); ^{13}C NMR (D_2O, 100 MHz) δ 158.8 (C), 153.9 (C), 151.6 (C), 137.3 (CH), 116.0 (C), 86.9 (CH), 83.4 (1 C, d, $J_{C\text{-}P}$ = 8.2 Hz, CH), 73.7 (CH), 70.2 (CH), 65.3 (CH_2), 63.9 (CH), 62.5 (CH_2), 52.6 (CH_2), 49.7 (CH_2), 36.6 (CH_2), 26.2 (CH_2), 22.4 (CH_2), 20.9 (CH_2 × 2); ^{31}P NMR (D_2O, 161 MHz) δ −10.76 (1 P, d, $J_{P\text{-}P}$ = 20.9 Hz), −11.25 (1 P, d, $J_{P\text{-}P}$ = 21.0 Hz); HRMS (ESI-TOF) calcd for $C_{19}H_{32}N_7O_{11}P_2$ $[M-H]^-$ 596.1630, found: 596.1628.



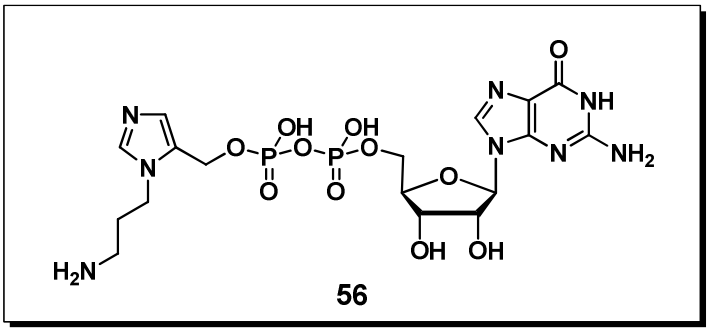
1-[*N*'-(benzyloxycarbonyl)-3'-aminopropyl]-5-hydroxymethyl imidazole (**53**). To a stirring mixture of 5-hydroxymethyl-imidazole **52** (0.4 mL, 4.07 mmol) and potassium carbonate (0.71 g, 5.32 mmol) in DMF (10 mL) was added compound **24** (1.32 g, 4.89 mmol) at room temperature and stirred for 16 h. The reaction mixture was evaporated and washed with ethyl acetate twice and dried over Mg_2SO_4. Removal of the solvent afforded a crude oil, which was purified with column chromatography by silica gel using methanol/chloroform = 1/15 as elent to give a pure product (0.70 g, 56%). R_f =

0.29 (methanol/chloroform = 1/15); $[\alpha]^{20}_{D}$ −3.21 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.36 (1 H, s, CH), 7.32-7.29 (5 H, m, CH), 6.77 (1 H, d, *J* = 4.6 Hz, CH), 5.05 (2 H, d, *J* = 8.8 Hz, CH_2), 4.49 (2 H, d, *J* = 10.1 Hz, CH_2), 3.86 (2 H, dt, *J* = 24.3, 6.8 Hz, CH_2), 3.06 (2 H, t, *J* = 5.6 Hz, CH_2), 1.88 (2 H, dt, *J* = 24.0, 6.7 Hz, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.6 (C), 137.6 (C), 136.6 (CH), 136.3 (C), 128.3 (CH), 127.9 (CH), 127.8 (CH), 127.7 (CH), 116.1 (CH), 66.4 (CH_2), 57.5 (CH_2), 44.0 (CH_2), 37.6 (CH_2), 30.9 (CH_2); HRMS (ESI-TOF) calcd for $C_{15}H_{20}N_3O_3$ $[M + H]^+$ 290.1499, found: 290.1516.



1-[*N*'-(benzyloxycarbonyl)-3'-aminopropyl]-5-phosphoxymethyl imidazole (**54**). Compound **53** (0.60 g, 1.94 mmol) and tetrabutylammonium phosphate (1.62 g, 4.84 mmol) were coevaporated in vacuo for 10 h. To the mixture of compound 18 and tetrabutylammonium phosphate in dichloromethane (12 mL) was added trichloroacetonitrile (0.6 mL, 5.81 mmol) dropwisely, which resulted an orange solution. The reaction mixture was then stirred at room temperature for 2 days until the TLC plate showed absence of starting material. Removal of the solvent and excess

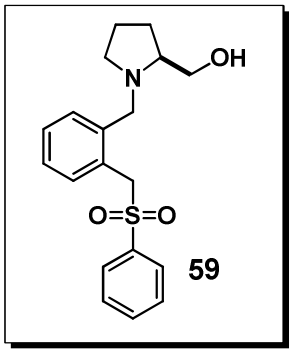
trichloroacetonitrile carefully yielded a brown oil. The mixture was then added with 4 N HCl (10 mL) and methanol (2 mL), which was kept stirring at room temperature for 10 h. The acidic solution was adjusted to pH 8 with ammonium hydroxide. The reaction mixture was evaporated and extracted with chloroform. The aqueous layer was evaporated and further purified with chromatography in silica gel by using *i*-propanol/water/ammonium hydroxide = 7/2/1 as eluent to give compound **54** (0.51 g, 66%); R_f = 0.51 (*i*-propanol/water/ammonium hydroxide = 7/3/1); $[\alpha]^{20}_{D}$ −0.56 (*c* 3.6, H_2O); ^{1}H NMR (D_2O, 400 MHz) δ 8.08 (1 H, s, CH), 7.40-7.35 (5 H, m, ArH), 7.27 (1 H, s, CH), 5.04 (2 H, s, $PhCH_2$), 4.80 (2 H, s, CH_2, HOD), 4.04 (2 H, t, *J* = 6.7 Hz, CH_2), 3.07 (2 H, t, *J* = 6.5 Hz, CH_2), 1.96 (2 H, t, *J* = 6.6 Hz, CH_2); ^{13}C NMR (D_2O, 100 MHz) δ 158.1 (C), 136.4 (C), 136.1 (CH), 134.8 (C), 128.6 (CH), 128.2 (CH), 127.5 (CH), 118.8 (CH), 66.7 (CH_2), 57.7 (CH_2), 54,3 (CH_2), 37.1 (CH_2), 29.4 (CH_2); ^{31}P NMR (D_2O, 161 MHz) δ 3.26 (1 P, s); HRMS (ESI-TOF) calcd for $C_{15}H_{19}N_3O_6P$ $[M-H]^-$ 368.1006, found: 368.0987.



56

Guanosine diphosphate-1-(3'-aminopropyl)-5-hydroxymethyl imidazole (**56**;

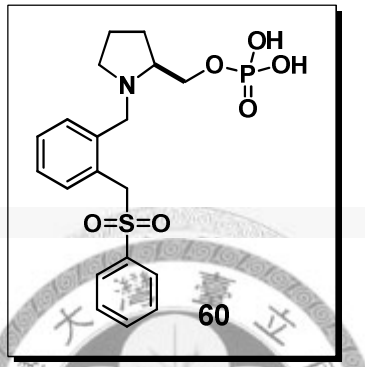
YCC-6). To a solution of compound **54** (0.23 g, 0.62 mmol) and 10 % Pd/C (0.08 g) in water/methanol (8 mL, 1/1, v/v), hydrogen was passed through the stirred mixture for 2 h until the TLC showed no starting material remained. After filtration and evaporation, the resulting product was then coevaporated with anhydrous pyridine (3 mL for three times). GMP-morpholidate **31** (0.54 g, 1.25 mmol) was added and coevaporated with anhydrous pyridine (3 mL for three times) to a minimum volume. The coupling reaction was initiated with the addition of tatrazole (0.44 g, 6.23 mmol) and pyridine (8 mL) and the reaction was stirred for two days under argon. Pyridine was then removed and the reaction mixture was washed three times with chloroform, evaporated to yield a crude product and purified by silica gel column chromatography by using *i*-propanol/water/ammonium hydroxide = 7/2/1 as eluent to give compound **56** (0.01 g, 27%); R_f = 0.35 (*i*-propanol/water/ammonium hydroxide = 7/3/1); $[\alpha]^{20}_{D}$ −25.1 (*c* 0.2, H_2O); ^{1}H NMR (D_2O, 400 MHz) δ 8.05 (1 H, s, CH), 7.57 (1 H, s, CH), 7.02 (1 H, s, CH), 5.92 (1 H, br, CH), 4.80 (3 H, s, CH, CH_2, HOD), 4.49 (1 H, br, CH), 4.33 (1 H, br, CH), 4.20 (2 H, br, CH_2), 3.98-3.93 (2 H, m, CH_2), 2.96 (1 H, br, CH_2), 2.87 (1 H, br, CH_2), 2.05 (1 H, br, CH_2), 1.84 (1 H, br, CH_2); ^{31}P NMR (D_2O, 161 MHz) δ −10.96 (1 P, d, $J_{P\text{-}P}$ = 20.4 Hz), −11.20 (1 P, d, $J_{P\text{-}P}$ = 19.7 Hz); HRMS (ESI-TOF) calcd for $C_{17}H_{25}N_8O_{11}P_2$ $[M-H]^-$ 579.1113, found: 579.1106.



59

(2S)-*N*-[2'-(phenylsulfonyl-methyl)benzyl]-2-hydroxymethyl pyrrolidine (**59**).To a stirring mixture of L-(+)-prolinol **31** (0.50 g, 4.94 mmol) and potassium carbonate (0.71 g, 4.94 mmol) in THF (15 mL) was added 2-[(phenylsulfonyl)-methyl]benzyl bromide **62** (1.52 g, 4.69 mmol) at room temperature and then stirred at 55 ^{o}C for 15 hr. The reaction mixture was cooled to room temperature, diluted with ethyl acetate (40 mL), washed with water twice and dried over Mg_2SO_4. Removal the solvent and recrystalize with chloroform and hexane to yield a white solid (1.30 g, 76%); R_f= 0.27 (ethylacetate/hexane = 1/1); $[\alpha]^{20}_{D}$ −51.88° (*c* 1.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.71 (2 H, d, *J* = 7.9 Hz, ArH), 7.62 (1 H, t, *J* = 7.5 Hz, ArH), 7.48 (2 H, t, *J* = 7.6 Hz, ArH), 7.26 (2 H, d, *J* = 3.9 Hz, ArH), 7.15 (1 H , p, *J* = 3.5 Hz, ArH), 6.99 (1 H, d, *J* = 7.6 Hz, ArH), 5.03 (1 H, d, *J* = 14.0 Hz, CH_2), 4.45 (1 H, d, *J* = 13.9 Hz, CH_2), 4.03 (1 H, d, *J* = 13.3 Hz, CH_2), 3.56 (1 H, dd, *J* = 10.9, 3.2 Hz, CH_2), 3.46 (1 H, d, *J* = 10.8 Hz, CH_2), 3.28 (1 H, d, *J* = 13.3 Hz, CH_2), 2.78 (1 H, t, *J* = 7.8 Hz, CH_2), 2.64 (1 H, d, *J* = 3.2 Hz, CH), 2.18 (1 H, q, *J* = 9.4 Hz, CH_2), 1.96-1.88 (1 H, m, CH_2), 1.80-1.73 (1 H, m, CH_2), 1.68-1.60 (2 H, m, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 139.6 (C), 138.7 (C),

133.7 (C), 132.5 (CH), 130.5 (CH), 129.0 (CH × 2), 128.9 (CH), 128.5 (CH × 2), 127.3 (CH), 127.2 (CH), 65.2 (CH), 62.9 (CH_2), 59.2 (CH_2), 57.5 (CH_2), 54.9 (CH_2), 27.6 (CH_2), 23.4 (CH_2); HRMS (ESI-TOF) calcd for $C_{19}H_{24}NO_3S$ $[M+H]^+$ 346.1471, found: 346.1472.

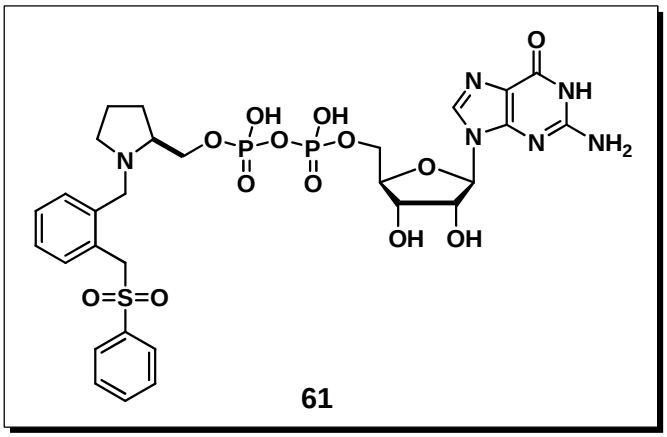


60

(2S)-*N*-[2'-(pheynlsulfonyl-methyl)benzyl]-2-phosphoxymethyl pyrrolidine (**60**).

Compound **59** (1.12 g, 2.78 mmol) and tetrabutylammonium phosphate (2.40 g, 6.94 mmol) were coevaporated in vacuo for 10 h. To the mixture of compound **59** and tetrabutylammonium phosphate in anhydrous dichloromethane (10 mL) were added trichloroacetonitrile (0.8 mL, 8.34 mmol) dropwisely, which resulted in an yellow solution. The reaction mixture was then stirred at room temperature for 2 days until the completion of the reaction was verified by TLC plate. The solvent was evaporated in vacuo. This yellow oil was then added with 4 N HCl (10 mL) ans methanol (4 mL), which was kept stirring at room temperature for 10 h. The acidic solution was adjusted to pH 8 with ammonium hydroxide. The reaction mixture was evaporated and extracted

with chloroform and water. The aqueous layer was evaporated and purified with chromatography in silica gel by using *i*-propanol/water/ammonium hydroxide = 7/2/1 as eluent to give compound **60** (0.91 g, 75%); R_f = 0.28 (*i*-propanol/water/ammonium hydroxide = 7/3/1); $[\alpha]^{20}_{D}$ +1.23 (*c* 9.8, H_2O); ^{1}Hpr NMR (D_2O, 400 MHz) δ 7.72 (2 H, d, *J* = 7.6 Hz, ArH), 7.60 (2 H, d, *J* = 7.3 Hz, ArH), 7.54 (2 H, t, *J* = 7.6 Hz, ArH), 7.46 (1 H, t, *J* = 7.6 Hz, ArH), 7.30 (1 H, t, *J* = 7.8 Hz, ArH), 7.07 (1 H, d, *J* = 7.8 Hz, ArH), 4.82 (1 H, s, CH_2), 4.27 (1 H, d, *J* = 13.9 Hz, CH_2), 4.08-3.99 (3 H, m, CH_2), 3.71 (1 H, br, CH), 3.31-3.25 (1 H, m, CH_2), 3.01-2.94 (1 H, m, CH_2), 2.20-2.11 (1 H, m, CH_2), 2.02-1.89 (3 H, m, CH_2); ^{13}C NMR (D_2O, 100 MHz) δ 135.4 (C), 135.2 (C), 133.7(C), 131.7 (CH), 130.6 (CH), 130.2 (CH), 130.1 (CH), 129.6 (CH × 2), 128.4 (CH × 2), 127.3 (CH), 68.3 (CH), 62.3 (CH_2), 58.8 (CH_2), 54.1 (CH_2 × 2), 25.6 (CH_2), 22.2 (CH_2); ^{31}P NMR (D_2O, 161 MHz) δ 3.26 (1 P, s); HRMS (ESI-TOF) calcd for $C_{19}H_{23}NO_6PS$ $[M-H]^-$ 424.0978, found: 424.0981.



61

(2S)-guanosine

diphosphate-*N*-[2'-(phenylsulfonyl-methyl)benzyl]-2-hydroxymethyl pyrrolidine (**61**; **YCC-7**). The resulting product **60** (0.82 g, 1.37 mmol) was coevaporated with pyridine (5 mL for three times). GMP-morpholidate **31** (1.20 g, 2.74 mmol) was added and coevaporated in vacuo. The coupling reaction was initiated with the addition of terazole (0.51 g, 6.85 mmol) and pyridine (6 mL). The reaction was stirred 3 days under nitrogen gas. Pyridine was then removed and the reaction mixture was washed three times with chloroform, evaporated to yield a crude product and purified with silica gel column chromatography by using *i*-propanol/water/ammonium hydroxide = 7/1/1 as eluent to give compound **61** (0.40 g, 41%); R_f = 0.22 (*i*-propanol/water/ammonium hydroxide = 7/1/1); $[\alpha]^{20}_{D}$ −3.16 (*c* 1.9, H_2O); ^{1}H NMR (D_2O, 400 MHz) δ 8.10 (1 H, s, ArH), 7.77 (1 H, t, *J* = 7.3 Hz, ArH), 7.65 (2 H, d, *J* = 8.0 Hz, ArH), 7.58 (3 H, t, *J* = 7.9 Hz, ArH), 7.42 (1 H, t, *J* = 7.5 Hz, ArH), 7.30 (1 H, t, *J* = 7.6 Hz, ArH), 7.04 (1 H, d, *J* = 7.7 Hz, ArH), 5.88 (1 H, d, *J* = 5.6 Hz, CH), 4.78-4.71 (3 H, m, CH, CH_2), 4.52 (1 H, t, *J* = 4.4 Hz, CH), 4.46 (1 H, d, *J* = 14.2 Hz, CH_2), 4.35 (1 H, br, CH), 4.26-4.23 (3 H, m, CH, CH_2), 4.20-4.15 (2 H, m, CH_2), 3.92 (1 H, br, CH), 3.42-3.38 (1 H, m, CH_2), 3.20-3.17 (1 H, m, CH_2), 2.35-2.30 (1 H, m, CH_2), 2.14-2.11 (1 H, m, CH_2), 2.04-2.00 (2 H, m, CH_2); ^{13}C NMR (D_2O, 100 MHz) δ 158.4 (C), 153.7 (C), 151.4 (C), 137.3 (CH), 135.6 (C), 135.0 (C), 133.6 (C), 131.8 (CH), 130.4 (CH), 130.0 (CH), 129.9 (CH),

129.6 (CH × 2), 128.2 (CH × 2), 127.1 (CH), 116.1 (C), 87.1 (CH), 83.5 (1 C, d, $J_{C\text{-}P}$ = 7.1 Hz, CH), 73.8 (CH), 70.2 (CH), 67.8 (CH), 65.3 (CH_2), 63.8 (CH_2), 58.7 (CH_2 × 2), 54.3 (CH_2), 25.6 (CH_2), 22.2 (CH_2); ^{31}P NMR (D_2O, 161 MHz) δ −11.14 (1 P, d, $J_{P\text{-}P}$ = 16.3 Hz), −11.61 (1 P, d, $J_{P\text{-}P}$ = 16.6 Hz); HRMS (ESI-TOF) calcd for $C_{29}H_{35}N_6O_{13}P_2S$ $[M-H]^-$ 769.1453, found: 769.1436.

第四章 參考文獻

1. Wong, C. H. *Carbohydrate-based drug discovery*, 1st Ed.; Wiley-VCH: Weinheim, 2003.
2. Dube, D. H.; Bertozzi, C. R., Glycans in cancer and inflammation- potential for therapeutics and diagnostics. *Nat. Rev. Drug Discov.* **2005**, *6*, 477-488.
3. Varki, A., Biological roles of oligosaccharides: All of the theories are correct. *Glycobiology* **1993**, *3*, 97-130.
4. Lowe, J. B.; Varki, A. *Essentials of Glycobiology*; Roberts and Company: La Jolla, 1999.
5. Compain, P.; Martin, O. R., Carbohydrate mimetics-based glycosyltransferase inhibitors. *Bioorg. Med. Chem.* **2001**, *9*, 3077-3092.
6. Kapitonov, D.; Yu, R. K., Conserved domains of glycosyltransferases. *Glycobiology* **1999**, *9*, 961-978.
7. Springer, T. A.; Lasky, L. A., Sticky sugars for selectins. *Nature* **1991**, *349*, 196-197.
8. Pang, P. C.; Tissot, B.; Drobnis, E. Z.; Sutovsky, P.; Morris, H. R.; Clark, G. F.; Dell, A., Expression of bisecting type and Lewis x/Lewis y terminated *N*-glycans on human sperm. *J. Biol. Chem*. **2007**, *282*, 36593-36602.
9. Javaud, C.; Dupuy, F.; Maftah, A.; Julien, R.; Petit, J. M., The fucosyltransferase

gene family: An amazing summary of the underlying mechanisms of gene evolution. *Genetica* **2003**, *118*, 157-170.

10. Paschinger, K.; Staudacher, E.; Stemmer, U.; Fabinil, G.; and Wilson, B. H., Fucosyltransferase substrate specificity and the order of fucosylation in invertebrates. *Glycobiology* **2005**, *15*, 463-474.
11. Lowe, J. B.; Stoolman, L. M.; Nair, R. R.; Larsen, R. D.; Berhend, T. L.; Marks, R. M., ELAM-1-dpendent cell adhesion to vascular endothelium determined by a tranfected human fucosyltransferase cDNA. *Cell* **1990**, *63*, 475-484.
12. Murray, B. W.; Wittmann, V.; Burkart, M. D.; Hung, S. C.; Wong, C. H., Mechanism of human α-1,3-fucosyltransferase V:glycosidic cleavage occurs prior to nucleophilic attack. *Biochemistry* **1997**, *36*, 823-831.
13. Kelley, L. A.; MacCallum, R. M.; Sternberg, J. E., Enhanced genome annotation using structural profiles in the program 3D-PSSM. *J. Mol. Biol.* **2000**, *299*, 499-520.
14. Izawa, M.; Kumamoto, K.; Mitsuoka, C.; Kanamori, A.; Ohmori, K.; Ishida, H.; Nakamura, S.; Kazumi, K. M.; Sasaki, K.; Nishi, T.; Kannagi, R., Expression of sialyl 6-sulfo Lewis x is inversely correlated with conventional sialyl Lewis x expression in human colorectal cancer. *Cancer Res.* **2000**, *60*, 1410-1416.
15. Taniguchi, N.; Honke, K.; Fukuda, M. *Handbook of glycosyltransferases and related genes*; Springer: Tokyo, New York, 2002.

16. Vries, T.; Knegtel, M. A.; Holmes, E. H.; Macher, B. A., Fucosyltransferase: Structure/function studies. *Glycobiology* **2001**, *11*, 119-128.

17. Lowe, J. B.; Kukowska, J. F.; Nair, R. P.; Larsen, R. D.; Marks, R. M.; Macher B. A.; Kelly, R. J.; Ernst, L. K., Molecular cloning of a human fucosyltransferase gene that determines expression of the Lewis x and VIM-2 epitopes but not ELAM-1-dependent cell Adhesion. *J. Biol. Chem*. **1991**, *266*, 17467-17477.

18. Natsuka, S.; Gersten, K. M.; Zenita, K.; Kannagi, R.; Lowe, J. B., Molecular cloning of a cDNA encoding a novel human leukocyte α-1,3-fucosyltransferase capable of synthesizing the sialyl Lewis x determinant. *J. Biol. Chem.* **1994**, *269*, 16789-16794.

19. Kaneko, M.; Kudo, T.; Iwasaki, H.; Ikehara, Y.; Nishihara, S.; Nakagawa, S.; Sasaki K.; Shiina, T.; Inoko, H.; Saitou, N.; Narimatsu, H., α1,3-Fucoslytransferase IX (FucT IX) is very highly conserved between human and mouse; molecular cloning, characterization and tissue distribution of human FucT IX. *FEBS letters* **1999**, *452*, 237-242.

20. Breton, C.; Oriol, R.; Imberty, A., Conserved structural features in eukaryotic and prokaryotic fucosyltransferases. *Glycobiology* 1998, *8*, 87-94.

21. Oriol, R.; Mollicone, R.; Cailleau, A.; Balanzino, L.; Breton, C., Divergent evolution of fucosyltranferase genes from vertebrates, invertebrates, and bacteria.

Glycobiology **1999**, *9*, 323-334.

22. Ma, B.; Wang, G.; Palcic, M. M.; Hazes, B.; Taylor, D. E., C-terminal amino acids of *Helicobacter pylori* α1,3/4-fucosyltranferases determine type I and type II transfer. *J. Biol. Chem.* **2003**, *278*, 21893-21900.

23. Ge, Z.; Chan, W. C.; Palcic, M. M.; Taylor, D. E., Cloning and heterologous expression of an α1,3-fucosyltransferase gene from the gastric pathogen *Helicobacter pylori*. *J. Biol. Chem.* **1997**, *272*, 21357-21363.

24. Wakaechuk, W. W.; Cunningham, A.; Watson, D. C.; Young N. M., Role of paired basic residues in the expression of active recombinant galactosyltransferases from the bacterial pathogen *Neisseria meningitides*. *Protein Engineering* **1998**, *11*, 295-302.

25. Martin, S. L.; Edbrooke, M. R.; Hodgman, T. C.; Eijnden, D. H.; Bird, M. I., Lewis x biosynthesis in *Helicobacter pylori*. *J. Biol. Chem.* **1997**, *272*, 21349-21356.

26. Wang, G.; Boulton, P. G.; Chan, W. C.; Palcic, M. M.; Taylor, D. E., Novel *Helicobacter pylori* α1,2-fucosyltranferase, a key enzyme in the synthesis of Lewis antigens. *Microbiology* **1999**, *145*, 3245-3253.

27. Christina, N.; Skoglund, A.; Moran, A. P.; Annuk, H.; Engstrand, L.; Normark, S., An enzymatic ruler modulates Lewis antigen glycosylation of *Helicobacter pylori* LPS during persistent infection. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 2863-2868.

28. Miyoshi, E.; Moriwaki, K.; Nakagawa, T., Biological function of fucosylation in cancer biology. *J. Biochem*. **2008**, *143*, 725–729.

29. Giannis, A., The sialyl Lewis x group and its analogus as ligands for selectins: Chemoenzymatic syntheses ans biological functions. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 178-180.

30. Lewincohn, D. M.; Bargatze, R. F.; Butcher, E. C., Leukocyte-endothelial cell recognition: Evidence of a common molecular mechanism shared by neutrophils, lymphocytes, and other leukocytes. *J. Immunol*. **1987**, *138*, 4313-4321.

31. Larsen, E.; Celi, A.; Gilbert, G. E.; Furie, B. C.; Erban, J. K.; Bonfanti, P.; Wagner, D. D.; Furie, B., PADGEM protein: A receptor that mediates the interaction of activated platelets with neutrophils and monocyter. *Cell* **1989**, *59*, 305-312.

32. Geng, J. G.; Bevilacqua, M. P.; Moore, K. L.; Mclntyre, T. M.; Prescott, S. M.; Kim, J. M.; Bliss, G. A.; Zimmerman, G. A.; MaEver, R. P., Rapid neutrophil adhesion to activated endothelium mediated by GMP-140. *Nature* **1990**, *343*, 757-760.

33. Bevilaqua, M. P.; Pober, J. S.; Mendrick, D. L.; Cotran, R. S.; Gimbrone, M. A., Identification of an inducible endothelial-leukocyte adhesion molecule. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 9238-9242.

34. Hallmann, R.; Jutila, M. A.; Smith, C. W.; Anderson, D. C.; Kishimoto, T. K.; Butcher, E. C., The peripheral lymph node homing receptor, LECAM-1, is involved

in CD18-independent adhesion of human neutrophils to endothelium. *Biochem. Biophys. Res. Commun*. **1991**, *174*, 236-243.

35. Spertini, O.; Luscinskas, F. W.; Kansas, G. S.; Munro, J. M.; Griffin, J. D.; Gimbrone, M. A.; Tedder, T. F., Leukocyte adhesion molecule-1 (LAM-1, L-selectin) interacts with an inducible endothelial cell ligand to support leukocyte adhesion. *J. Immunol.* **1991**, *147*, 2565-2573.
36. Ley, K.; Gaehtgens, P.; Fennie, C.; Singer, M. S.; Lasky, L. A.; Rosen, S. D., Lectin-like cell adhesion molecule 1 mediates leukocyte rolling in mesenteric venules in vivo. *Blood* **1991**, *77*, 2553-2555.
37. Simanek, E. E.; MaGarvey, G. J.; Jablonowski, J. A.; Wong, C. H., Selectin-carbohydrate interactions: From natural ligands to designed mimics. *Chem. Rev.* **1998**, *98*, 833-862.
38. Springer, T. A., Adhesion receptors of the immune system. *Nature* **1990**, *346*, 425-434.
39. Sun, H. Y.; Lin, S. W.; Ko, T. P.; Pan, J. F.; Liu, C. L.; Lin, C. N.; Wang, H. J.; Lin, C. H., Structure and mechanism of *Helicobacter pylori* fucosyltransferase. *J. Biol. Chem.* **2007**, *282*, 9973-9982.
40. Unligil, U. M.; Rini, J. M., Glycosyltransferase structure and mechanism. *Curr. Opi. Struc. Biol.* **2000**, *10*, 510-517.

41. Murray, B. W.; Wittmann, V.; Burkartm, M. D. ; Hung, S. C.; Wong, C. H., Mechanism of human α-1,3-fucosyltranferase V: Glycosidic cleavage occurs prior to nucleophilic attack. *Biochemistry* **1997**, *36*, 823-831.

42. Gosselin, S.; Palcic, M. M., Acceptor hydroxyl group mapping for human milk α1,3 and α1,3/4-fucosyltrnaferase. *Bioorg. Med. Chem*. **1996**, *4*, 2023-2028.

43. Hindsgaul, O.; Kaur, K. J.; Srivastava, G.; Magdalena, B. T.; Crawley, S. C.; Heerze, L. D.; Palcic, M. M., Evaluation of deoxygenated oligosaccharide acceptor analogs as specific inhibitors of glycosyltranferase. *J. Biol. Chem.* **1991**, *266*, 17858-17862.

44. Wong, C. H.; Dumas, D. P.; Ichikawa, Y.; Koseki, K.; Danishefsky, S. J.; Weston, B. W.; Lowe, J. B., Specificity, inhibition, and synthetic utility of a recombinant human α-1,3-fucosyltranferase. *J. Am. Chem. Soc.* **1992**, *114*, 7321-7322.

45. Burkart, M. D.; Vincent, S. P.; Duffels, A.; Murray, B. W.; Ley, S. V.; Wong, C. H., Chemo-enzymatic synthesis of fluorinated sugar nucleotide: Useful mechanistic probes for glycosyltransferase. *Bioorg. Med. Chem.* **2000**, *8*, 1937-1946.

46. Carchon, G.; Chretien, F.; Delannoy, P.; Verbert, A.; Chapleur, Y., Synthesis of a non-charged analogue of guanosylsiphosphofucose. *Tetrahedron Lett.* **2001**, *42*, 8821-8824.

47. Wang, Y. F.; Dumas, D. P.; Wong, C. H., Chemo-enzymatic synthesis of five-membered azasugars as inhibitors of fucosidase and fucosyltransferase: An

issue regarding the stereochemistry discrimination at transition states. *Tetrahedron Lett.* **1993**, *34*, 403-406.

48. Ichikawa, Y.; Lin, Y. C.; Dumas, D. P.; Shen, G. J.; Eduardo, G. J.; Williams, M. A.; Bayer, R.; Ketcham, C.; Walker, L. E., Chemical-enzymatic synthesis and conformational analysis of sialyl Lewis x and derivatives. *J. Am. Chem. Soc.* **1992**, *114*, 9283-9298.
49. Schuster, M.; Blechert, S., Inhibition of fucosyltransferase V by a GDP-azasugar. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1809-1811.
50. Qiao, L.; Murray, B. W.; Shimazaki, M.; Schultz, J.; Wong, C. H., Synergistic inhibition of human α-1,3-fucosyltranferase V. *J. Am. Chem. Soc.* **1996**, *118*, 7653-7662.
51. Mitchell, M. L.; Lee, L.V.; Wong, C. H., Synthesis and evaluation of six-membered GDP-iminicyclitol. *Tetrahedron Lett.* **2002**, *43*, 5691-5693.
52. Mitchell, M. L.; Tian, F.; Lee, L. V.; Wong, C. H., Synthesis and evaluation of transition-state analogue inhibitors of α-1,3-fucosyltransferase. *Angew. Chem. Int. Ed*. **2002**, *41*, 3041-3044.
53. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B., A stepwise Huisgen cycloaddition process: Copper(I)-catalyzed regioselective "ligation" of azides and terminal alkynes. *Angew. Chem. Int. Ed.* **2002**, *41*, 2596-2599.

54. Waldscheck, B.; Streiff, M.; Notz, W.; Kinzy, W.; Schmidt, R. R., α1,3-Galactosyltranferase inhibition based on a new type of disubstrate analogus. *Angew. Chem. Int. Ed. Engl.* **2001**, *40*, 4007-4011.

55. Lillelund, V. H.; Jensen, H. H.; Liang, X.; Bols, M., Recent developments of transition-state analogue glycosidase inhibitors of non-natural product origin. *Chem. Rev*. **2002**, *102*, 515-554.

56. Zhao, Y.; Thorson, J. S., A methodological comparison: The advantage of phosphorimidates in expanding the sugar nucleotide repertoire. *J. Org. Chem.* **1998**, *63*, 7568-7572.

57. Caravano, A.; Dominique, M. L.; Brondello, J. M.; Vencent, S. P.; Sinay, P., Synthesis and inhibition properties of conformational probes for the mutase-catalyzed UDP-galactopyranose/furanose interconversion. *Chem. Eur. J*. **2003**, *9*, 5888-5898.

58. Wittmann, V.; Wong, C. H., 1H-tetrazole as catalyst in phosphomorpholidate coupling reactions: Efficient synthesis of GDP-fucose, GDP-mannose, and GDP-galactose. *J. Org. Chem.* **1997**, *62,* 2144-2147.

59. Pastuszak, I.; Ketchum, C.; Hermanson, G.; Sjoberg, E. J.; Drake, R.; Elbein, A. D., GDP-L-fucose pyrophosphorylase. *J. Biol. Chem.* **1998**, *273*, 30165-30174.

60. Huey, R.; Morris, G. M.; Olson, A. J.; Goodsell, D. S., A semiempirical free energy

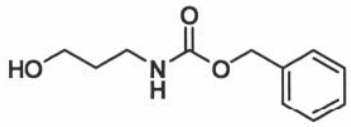
force field with charge-based desolvation. *J. Comput. Chem.* **2007**, *28*, 1145–1152.

61. Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W.; Belew, R. K.; Olson, A. J., Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *J. Comput. Chem.* **1999**, *19*, 1939–1662.
62. Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M., A program for macromolecular energy, minimization, and dynamics calculations. *J. Comput. Chem.* **1983**, *4*, 187-217.
63. http://www. accelrys.com
64. Lee, H. H.; Palmer, B. D.; Baguley, B. C.; Chin, M.; McFadyen, W. D.; Wickham, G.;Deborah, T. P.; Wakelin, P. G.; Denny, W. A., DNA-directed alkylating agents 5-acridinecarboxamide derivatives of (1,2-diaminoethane)dichloroplatinum (II). *J. Med. Chem.* **1992**, *35*, 2983-2987.
65. Wei, W. H.; Tomohiro, T.; Kodaka, M.; Okuno, H., Selective synthesis and kinetic measurement of 1:1 and 2:2 cylic compounds containing 1,4,7,10-tetraazacyclododecane and azobenzene units. *J. Org. Chem.* **2000**, *65*, 8979-8987.
66. Moffatt, J. G.; Khorana, H. G., Nucleoside polyphosphates: The synthesis and some reactions of nucleoside-5'-phosphoromorpholidates and related compounds. Improved methods for the preparation of nucleoside-5'-polyphosphates. *J. Am. Soc.*

Chem. **1961**, *83*, 649-658.

67. Dei, S.; Bellucci, C.; Buccioni, M.; Ferraroni, M.; Gualtieri, F.; Guandalini, L.; Manetti, D.; Matucci, R.; Romanelli, M. N.; Scapecchi, S.; Teodori, E., Synthesis and cholinergic affinity of diastereomeric and enantiomeric isomers of 1-methyl-2-(2-methyl-1,3-dioxolan-4-yl)-pyrrolidine, 1-methyl-2-(-methyl-1,3-oxa-thiolan-5-yl)pyrrolidine and of their iodomethylates. *Bioorg. Med. Chem.* **2003**, *11*, 3153-3164.

附錄



23

Current Data Parameters
NAME 090110-link-OH
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters

Date_	20090110
Time	17.35
INSTRUM	spect
PROBHD	5 mm BBO BB-1H
PULPROG	zg30
TD	32768
SOLVENT	CDCl3
NS	8
DS	0
SWH	6410.256 Hz
FIDRES	0.195625 Hz
AQ	2.5559540 sec
RG	71.8
DW	78.000 usec
DE	6.00 usec
TE	300.1 K
D1	2.00000000 sec
TD0	1

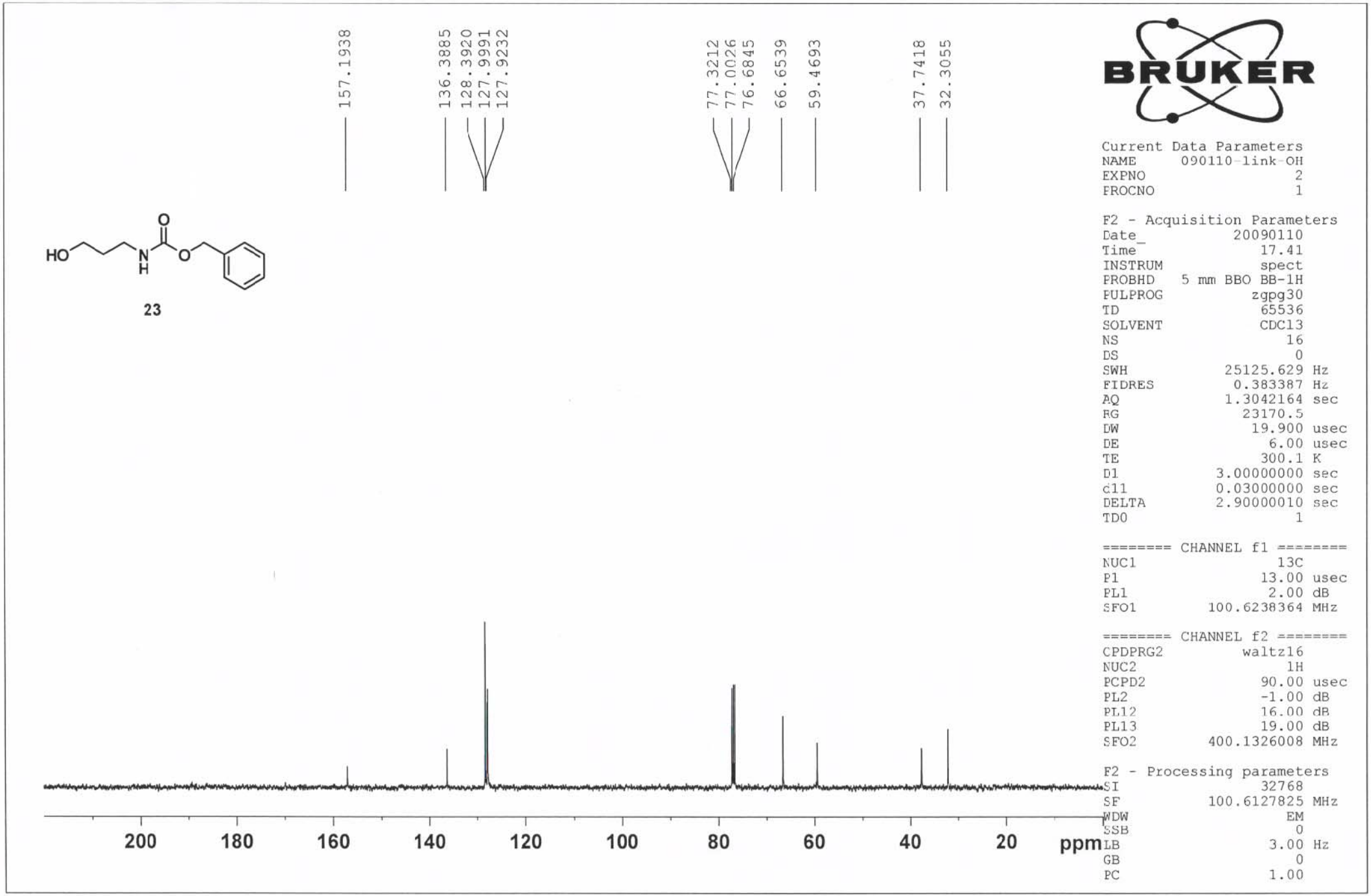
======== CHANNEL f1 ========

NUC1	1H
P1	9.70 usec
PL1	-2.00 dB
SFO1	400.1328010 MHz

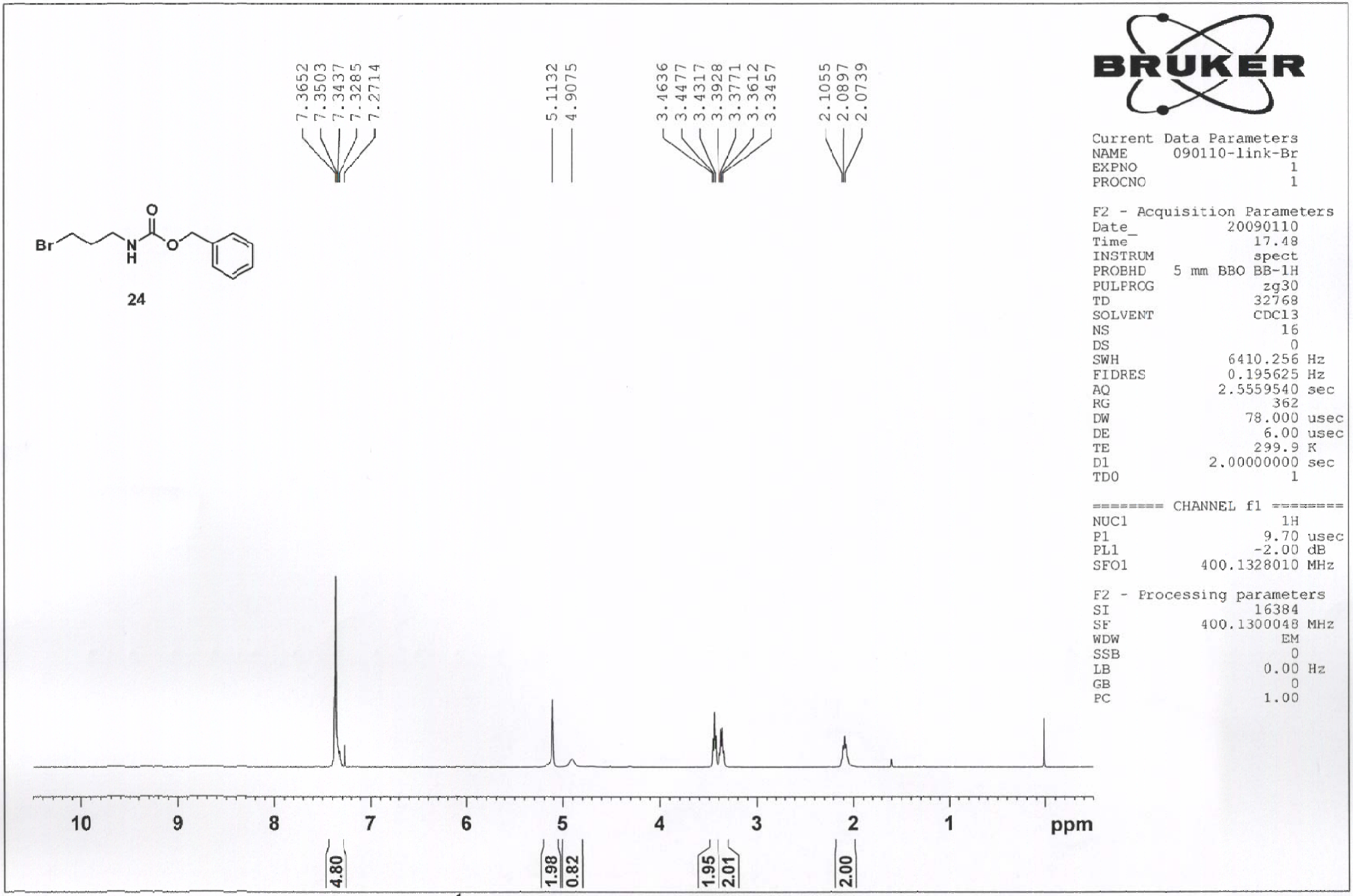
F2 - Processing parameters

SI	16384
SF	400.1300057 MHz
WDW	EM
SSB	0
LB	0.00 Hz
GB	0
PC	1.00

^{1}H spectrum of compound 23 (500 MHz, $CDCl_3$)



^{13}C spectrum of compound 23 (500 MHz, $CDCl_3$)



BRUKER

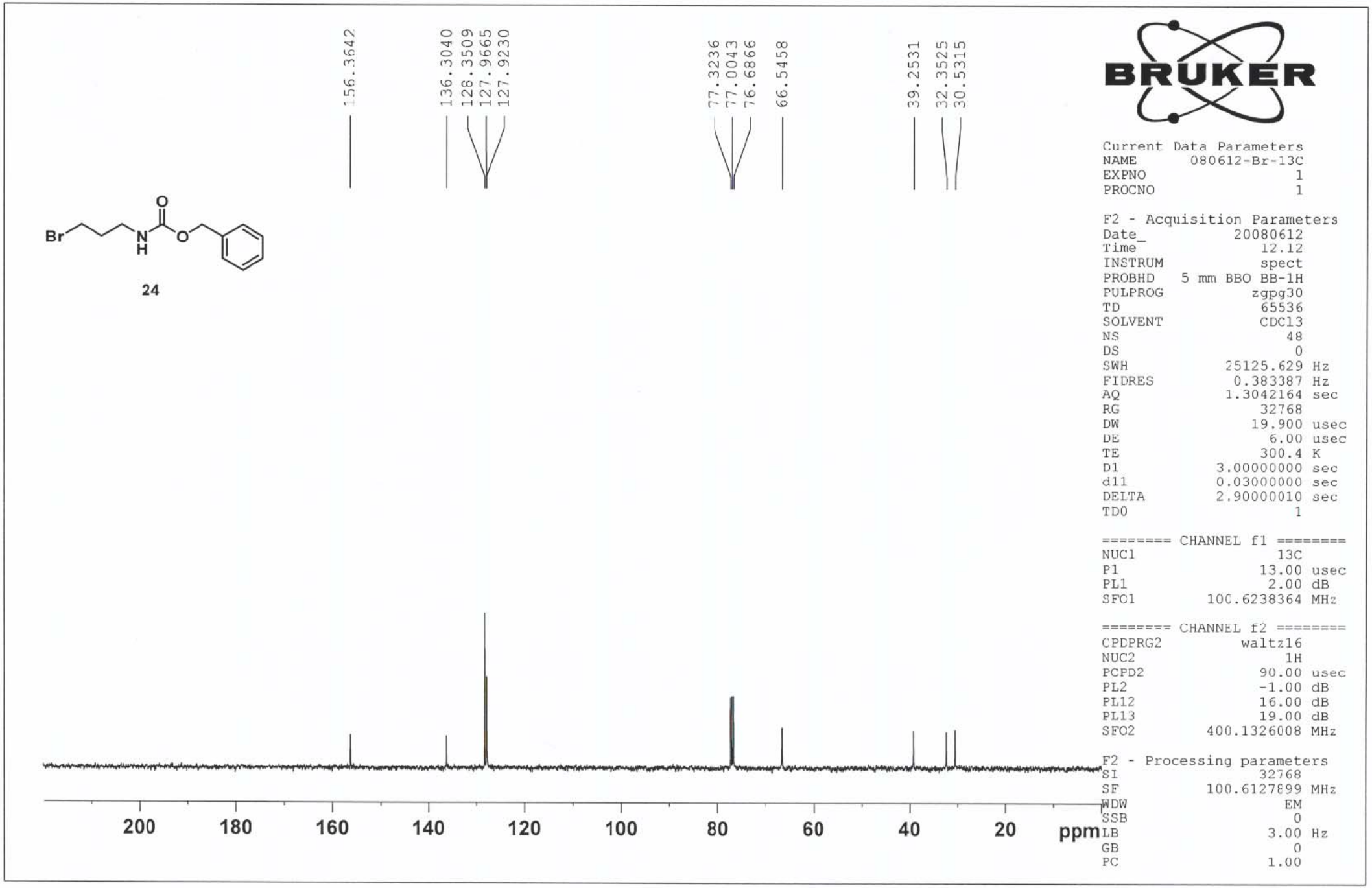
Current Data Parameters
NAME 090110-link-Br
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20090110
Time 17.48
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 16
DS 0
SWH 6410.256 Hz
FIDRES 0.195625 Hz
AQ 2.5559540 sec
RG 362
DW 78.000 usec
DE 6.00 usec
TE 299.9 K
D1 2.00000000 sec
TD0 1

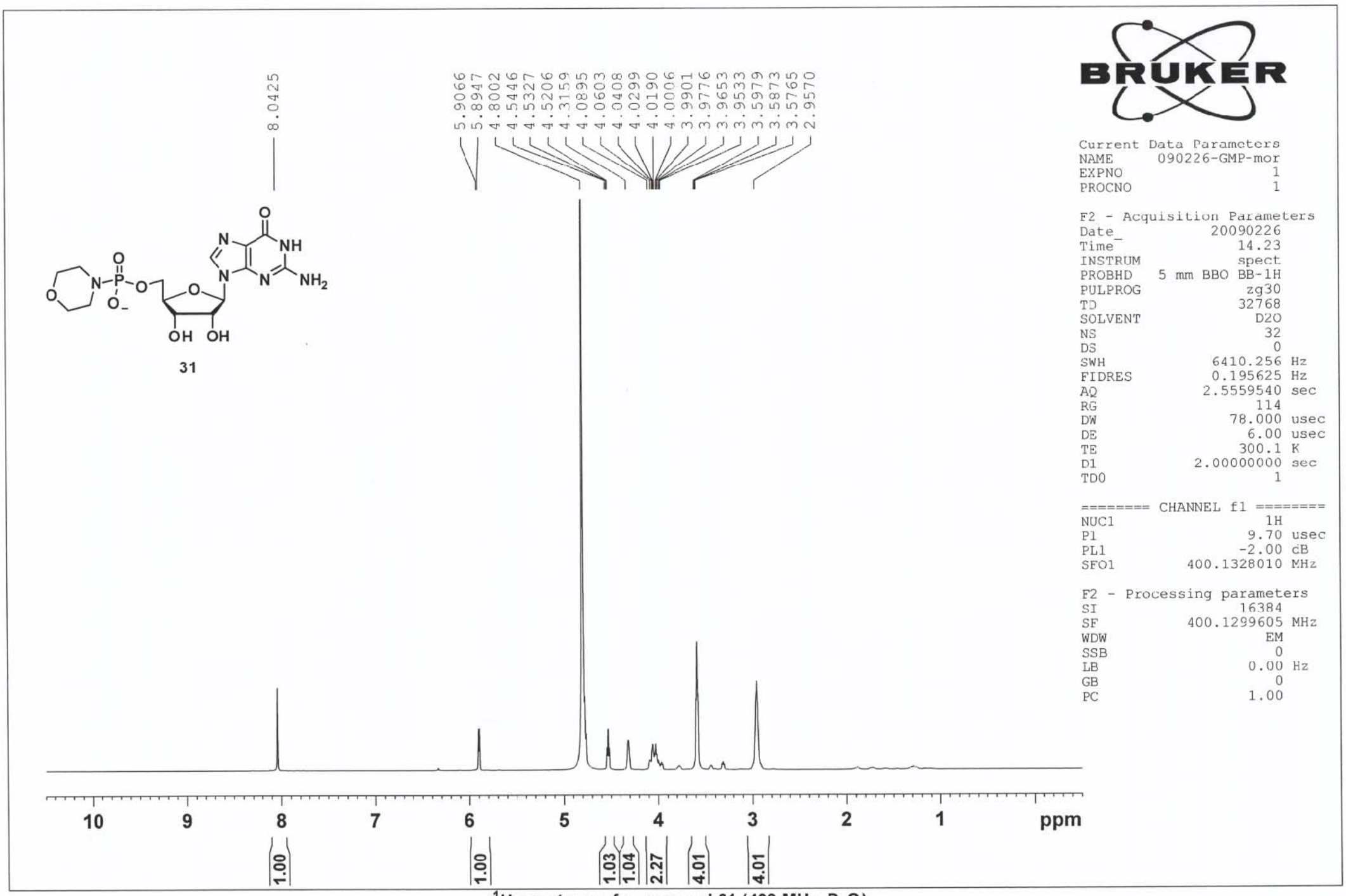
======== CHANNEL f1 ========
NUC1 1H
P1 9.70 usec
PL1 -2.00 dB
SFO1 400.1328010 MHz

F2 - Processing parameters
SI 16384
SF 400.1300048 MHz
WDW EM
SSB 0
LB 0.00 Hz
GB 0
PC 1.00

^{1}H spectrum of compound 24 (400 MHz, $CDCl_3$)



^{13}C spectrum of compound 24 (500 MHz, $CDCl_3$)



BRUKER

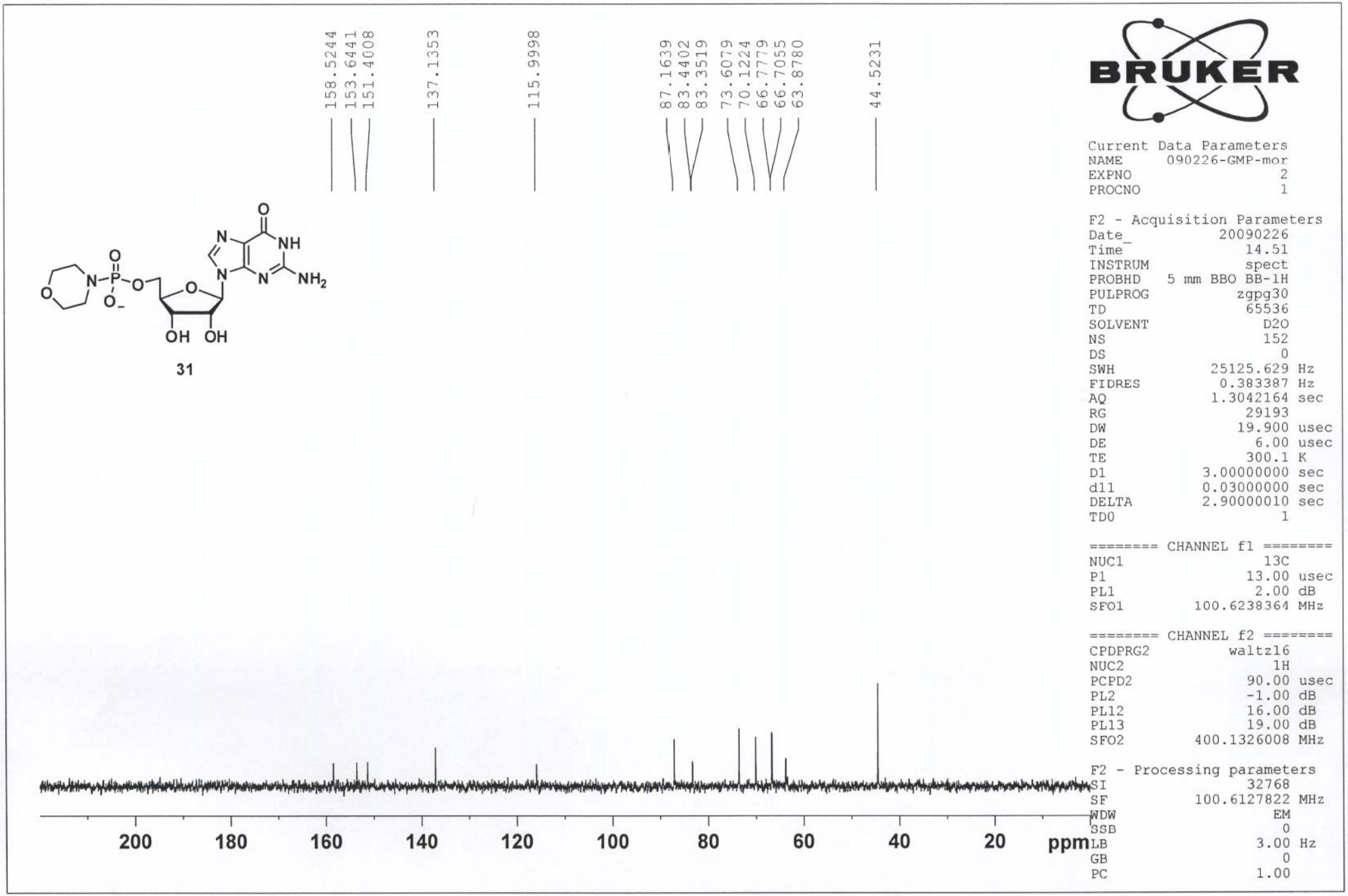
Current Data Parameters
NAME 090226-GMP-mor
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20090226
Time 14.23
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 32768
SOLVENT D2O
NS 32
DS 0
SWH 6410.256 Hz
FIDRES 0.195625 Hz
AQ 2.5559540 sec
RG 114
DW 78.000 usec
DE 6.00 usec
TE 300.1 K
D1 2.00000000 sec
TD0 1

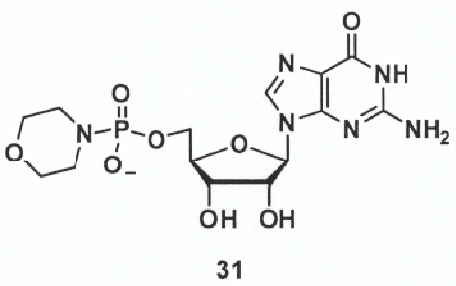
======== CHANNEL f1 ========
NUC1 1H
P1 9.70 usec
PL1 -2.00 dB
SFO1 400.1328010 MHz

F2 - Processing parameters
SI 16384
SF 400.1299605 MHz
WDW EM
SSB 0
LB 0.00 Hz
GB 0
PC 1.00

^{1}H spectrum of compound 31 (400 MHz, D_2O)



^{13}C spectrum of compound 31 (400 MHz, D_2O)



31

BRUKER

Current Data Parameters
NAME 090226-GMP-mor
EXPNO 3
PROCNO 1

F2 - Acquisition Parameters

Date_	20090226
Time	15.00
INSTRUM	spect
PROBHD	5 mm BBO BB-1H
PULPROG	zgpg30
TD	65536
SOLVENT	DMSO
NS	19
DS	0
SWH	32467.533 Hz
FIDRES	0.495415 Hz
AQ	1.0093044 sec
RG	20642.5
DW	15.400 usec
DE	6.00 usec
TE	299.9 K
D1	1.50000000 sec
d11	0.03000000 sec
DELTA	1.39999998 sec
TD0	1

======== CHANNEL f1 ========

NUC1	31P
P1	10.30 usec
PL1	1.00 dB
SFO1	161.9755930 MHz

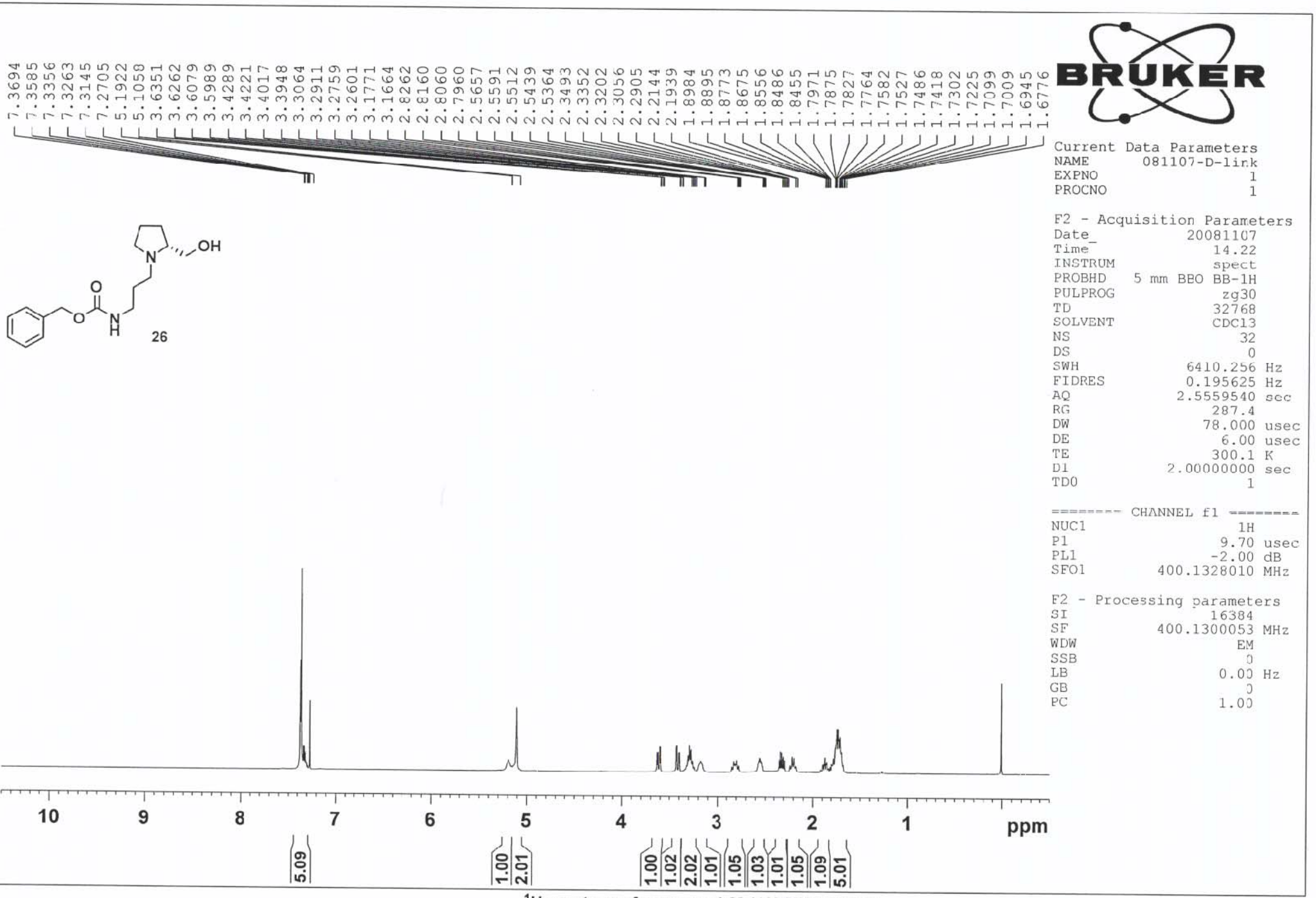
======== CHANNEL f2 ========

CPDPRG2	waltz16
NUC2	1H
PCPD2	90.00 usec
PL2	-2.00 dB
PL12	15.90 dB
PL13	18.90 dB
SFO2	400.1320007 MHz

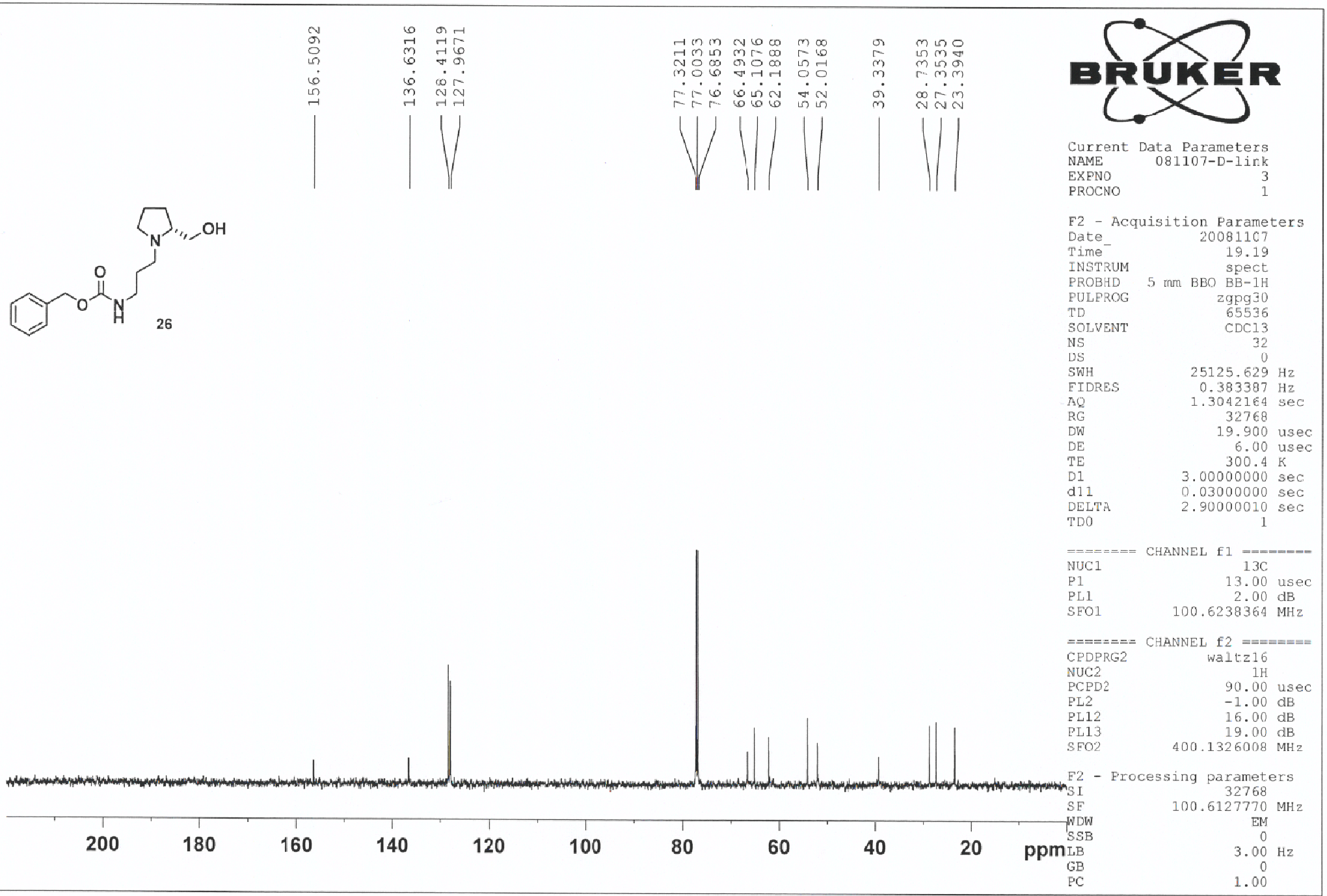
F2 - Processing parameters

SI	65536
SF	161.9755259 MHz
WDW	EM
SSB	0
LB	3.00 Hz
GB	0
PC	1.00

^{31}P spectrum of compound 31 (400 MHz, D_2O)



^{1}H spectrum of compound 26 (400 MHz, $CDCl_3$)



BRUKER

Current Data Parameters
NAME 081107-D-link
EXPNO 3
PROCNO 1

F2 - Acquisition Parameters

Date_	20081107
Time	19.19
INSTRUM	spect
PROBHD	5 mm BBO BB-1H
PULPROG	zgpg30
TD	65536
SOLVENT	CDCl3
NS	32
DS	0
SWH	25125.629 Hz
FIDRES	0.383387 Hz
AQ	1.3042164 sec
RG	32768
DW	19.900 usec
DE	6.00 usec
TE	300.4 K
D1	3.00000000 sec
d11	0.03000000 sec
DELTA	2.90000010 sec
TD0	1

======== CHANNEL f1 ========

NUC1	13C
P1	13.00 usec
PL1	2.00 dB
SFO1	100.6238364 MHz

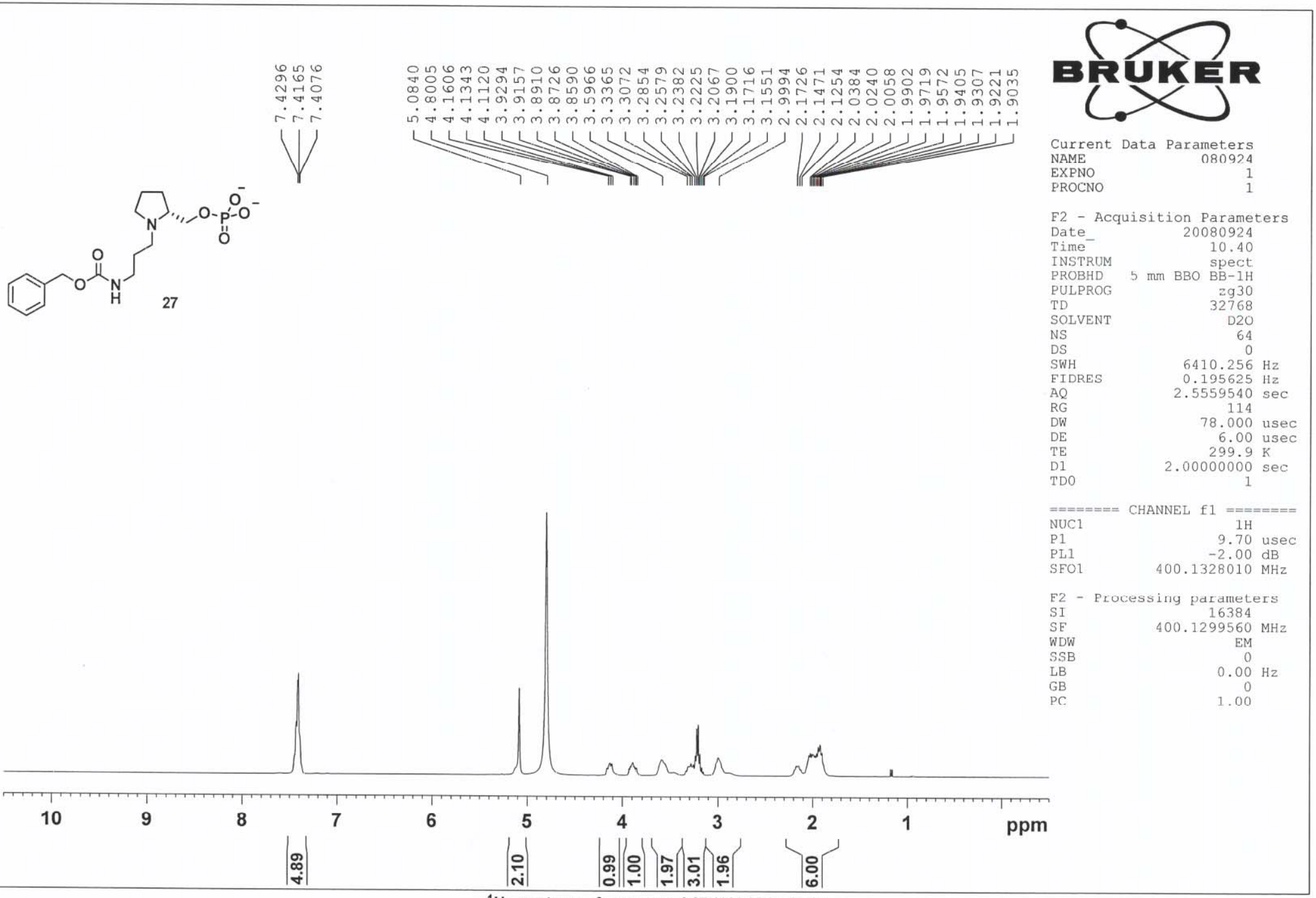
======== CHANNEL f2 ========

CPDPRG2	waltz16
NUC2	1H
PCPD2	90.00 usec
PL2	-1.00 dB
PL12	16.00 dB
PL13	19.00 dB
SFO2	400.1326008 MHz

F2 - Processing parameters

SI	32768
SF	100.6127770 MHz
WDW	EM
SSB	0
LB	3.00 Hz
GB	0
PC	1.00

^{13}C spectrum of compound 26 (400 MHz, $CDCl_3$)



BRUKER

Current Data Parameters

NAME	080924
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20080924
Time	10.40
INSTRUM	spect
PROBHD	5 mm BBO BB-1H
PULPROG	zg30
TD	32768
SOLVENT	D2O
NS	64
DS	0
SWH	6410.256 Hz
FIDRES	0.195625 Hz
AQ	2.5559540 sec
RG	114
DW	78.000 usec
DE	6.00 usec
TE	299.9 K
D1	2.00000000 sec
TD0	1

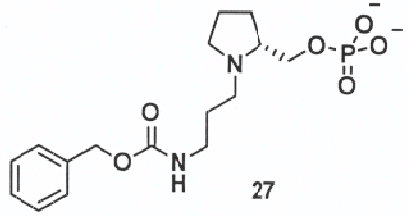
======== CHANNEL f1 ========

NUC1	1H
P1	9.70 usec
PL1	-2.00 dB
SFO1	400.1328010 MHz

F2 - Processing parameters

SI	16384
SF	400.1299560 MHz
WDW	EM
SSB	0
LB	0.00 Hz
GB	0
PC	1.00

^{1}H spectrum of compound 27 (400 MHz, D_2O)



158.1287
136.3616
128.6481
128.2383
127.5825
68.3890
66.7311
61.7975
53.8741
52.3785
37.4588
25.5334
25.2698
22.8702

200 180 160 140 120 100 80 60 40 20 ppm

BRUKER

Current Data Parameters
NAME 080924
EXPNO 2
PROCNO 1

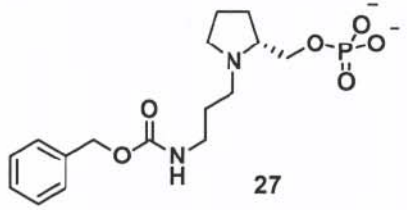
F2 - Acquisition Parameters
Date_ 20080924
Time 10.45
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zgpg30
TD 65536
SOLVENT D2O
NS 284
DS 0
SWH 25125.629 Hz
FIDRES 0.383387 Hz
AQ 1.3042164 sec
RG 20642.5
DW 19.900 usec
DE 6.00 usec
TE 300.3 K
D1 3.00000000 sec
d11 0.03000000 sec
DELTA 2.90000010 sec
TD0 1

======== CHANNEL f1 ========
NUC1 13C
P1 13.00 usec
PL1 2.00 dB
SFO1 100.6238364 MHz

======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 90.00 usec
PL2 -1.00 dB
PL12 16.00 dB
PL13 19.00 dB
SFO2 400.1326008 MHz

F2 - Processing parameters
SI 32768
SF 100.6127822 MHz
WDW EM
SSB 0
LB 3.00 Hz
GB 0
PC 1.00

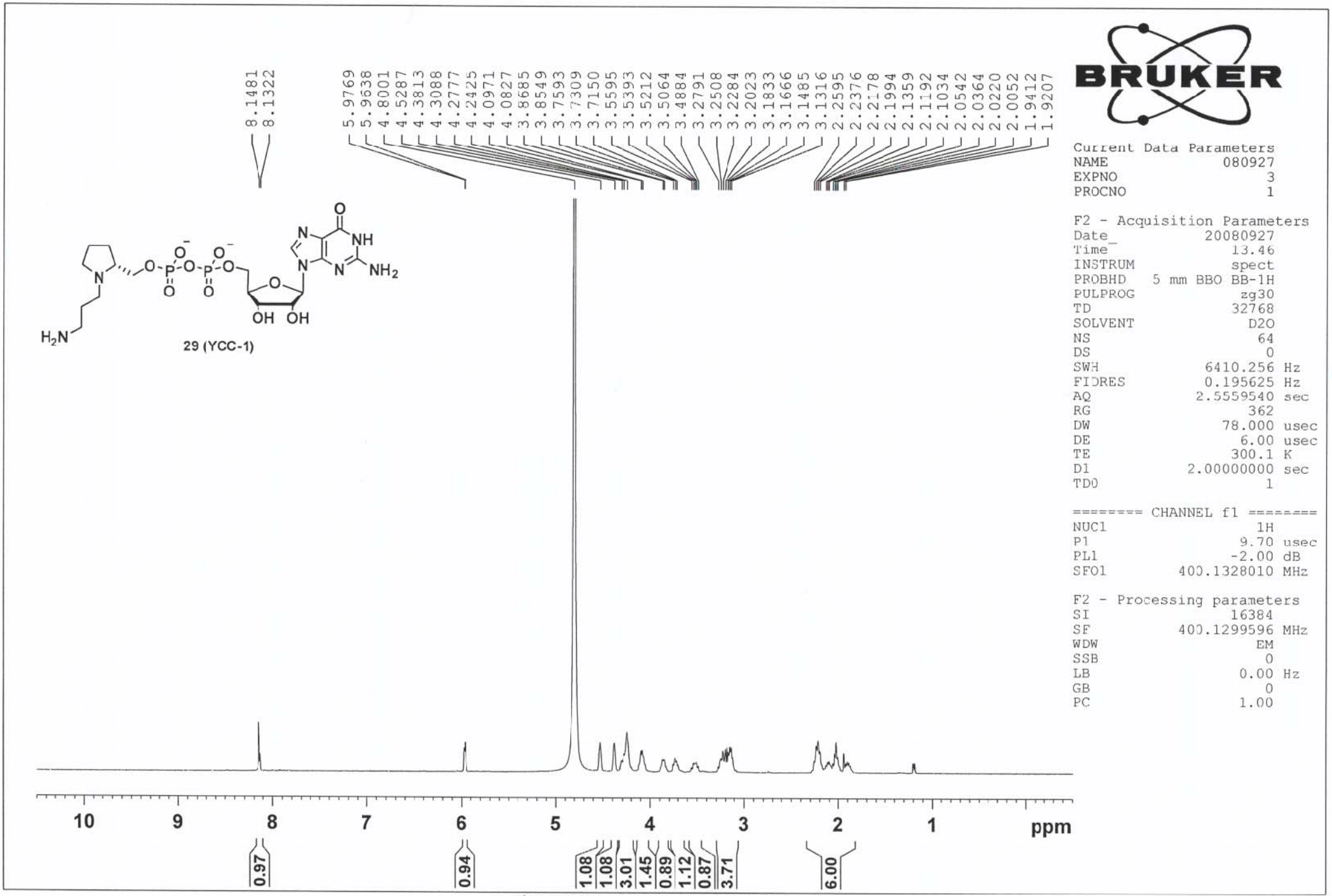
^{13}C spectrum of compound 27 (400 MHz, D_2O)



BRUKER

NAME	090511-D-Nlink-P
EXPNO	1
PROCNO	1
Date_	20090511
Time	13.49
INSTRUM	spect
PROBHD	5 mm QNP 1H/13
PULPROG	zgpg30
TD	65536
SOLVENT	D2O
NS	49
DS	0
SWH	51020.406 Hz
FIDRES	0.778510 Hz
AQ	0.6423028 sec
RG	2050
DW	9.800 usec
DE	6.50 usec
TE	300.0 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	31P
P1	9.90 usec
PL1	6.00 dB
PL1W	21.45254898 W
SFO1	202.4563350 MHz
======== CHANNEL f2 ========	
CPDPRG2	waltz16
NUC2	1H
PCPD2	90.00 usec
PL2	0.00 dB
PL12	16.60 dB
PL13	18.30 dB
PL2W	19.34152603 W
PL12W	0.42314643 W
PL13W	0.28608218 W
SFO2	500.1320005 MHz
SI	32768
SF	202.4563350 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

^{31}P spectrum of compound 27 (400 MHz, D_2O)



BRUKER

Current Data Parameters

NAME	080927
EXPNO	3
PROCNO	1

F2 - Acquisition Parameters

Date_	20080927
Time	13.46
INSTRUM	spect
PROBHD	5 mm BBO BB-1H
PULPROG	zg30
TD	32768
SOLVENT	D2O
NS	64
DS	0
SWH	6410.256 Hz
FIDRES	0.195625 Hz
AQ	2.5559540 sec
RG	362
DW	78.000 usec
DE	6.00 usec
TE	300.1 K
D1	2.00000000 sec
TD0	1

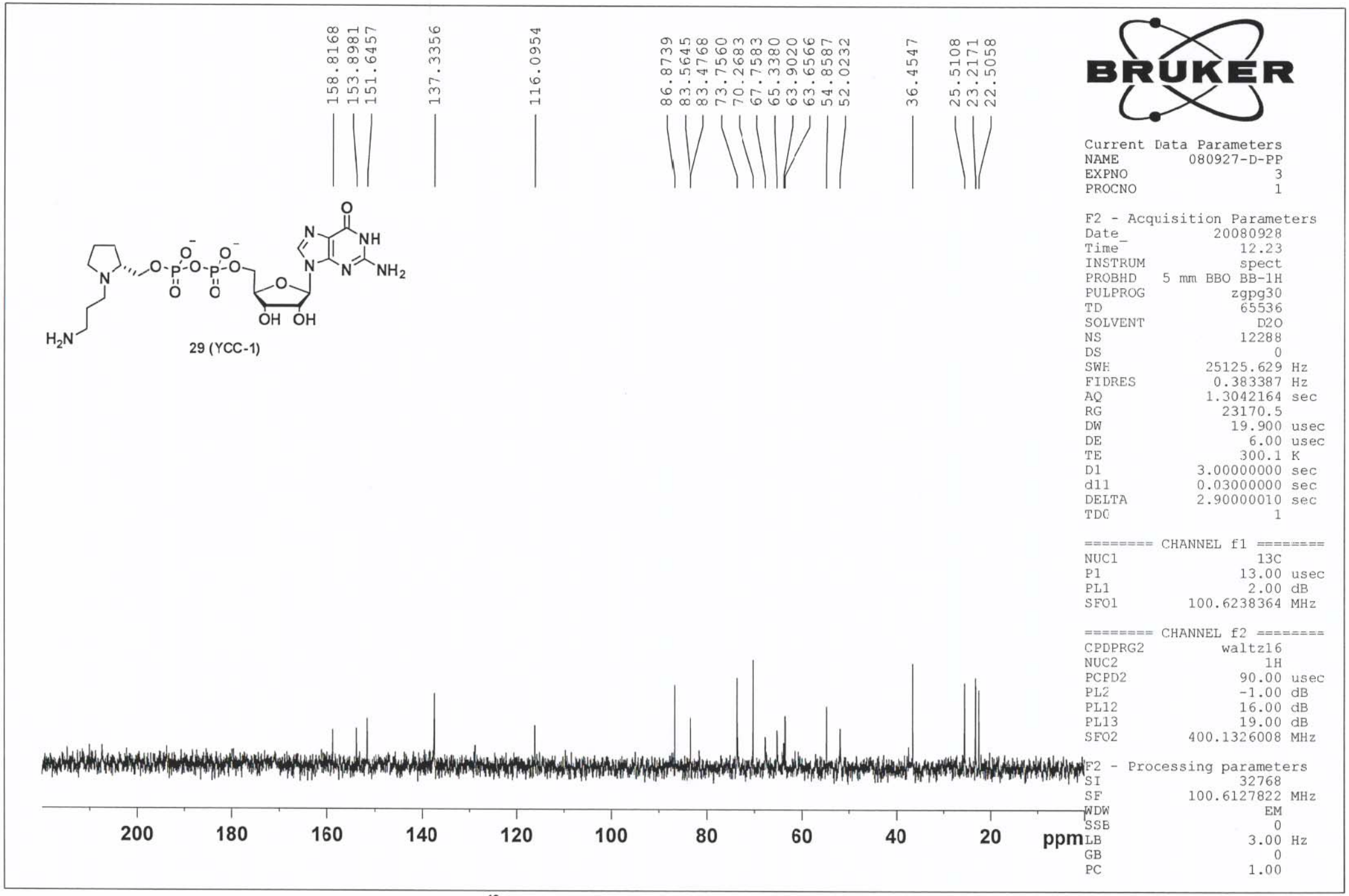
======== CHANNEL f1 ========

NUC1	1H
P1	9.70 usec
PL1	-2.00 dB
SFO1	400.1328010 MHz

F2 - Processing parameters

SI	16384
SF	400.1299596 MHz
WDW	EM
SSB	0
LB	0.00 Hz
GB	0
PC	1.00

^{1}H spectrum of compound 29 (400 MHz, D_2O)



BRUKER

Current Data Parameters

NAME	080927-D-PP
EXPNO	3
PROCNO	1

F2 - Acquisition Parameters

Date_	20080928	
Time	12.23	
INSTRUM	spect	
PROBHD	5 mm BBO BB-1H	
PULPROG	zgpg30	
TD	65536	
SOLVENT	D2O	
NS	12288	
DS	0	
SWH	25125.629	Hz
FIDRES	0.383387	Hz
AQ	1.3042164	sec
RG	23170.5	
DW	19.900	usec
DE	6.00	usec
TE	300.1	K
D1	3.00000000	sec
d11	0.03000000	sec
DELTA	2.90000010	sec
TD0	1	

======== CHANNEL f1 ========

NUC1	13C	
P1	13.00	usec
PL1	2.00	dB
SFO1	100.6238364	MHz

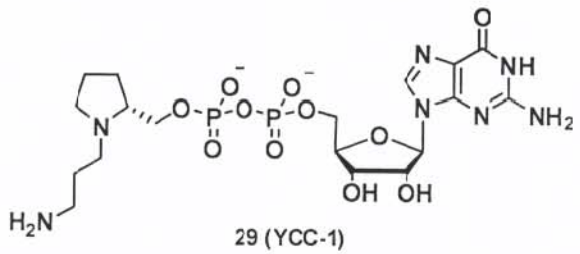
======== CHANNEL f2 ========

CPDPRG2	waltz16	
NUC2	1H	
PCPD2	90.00	usec
PL2	-1.00	dB
PL12	16.00	dB
PL13	19.00	dB
SFO2	400.1326008	MHz

F2 - Processing parameters

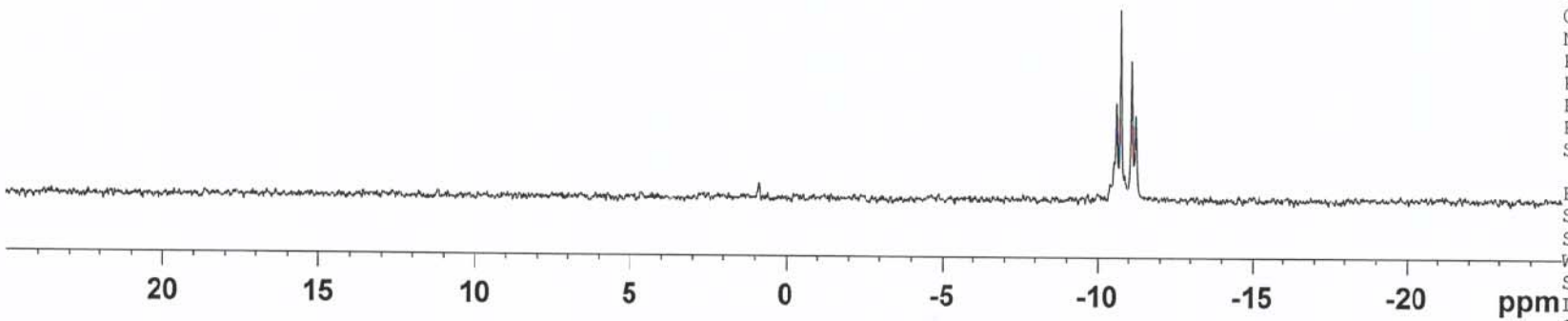
SI	32768	
SF	100.6127822	MHz
WDW	EM	
SSB	0	
LB	3.00	Hz
GB	0	
PC	1.00	

^{13}C spectrum of compound 29 (400 MHz, D_2O)



29 (YCC-1)

-10.6175
-10.7464
-11.0959
-11.2256



BRUKER

Current Data Parameters

NAME	081113-YCC-1
EXPNO	4
PROCNO	1

F2 - Acquisition Parameters

Date_	20081118
Time	19.53
INSTRUM	spect
PROBHD	5 mm BBO BB-1H
PULPROG	zgpg30
TD	65536
SOLVENT	D2O
NS	159
DS	0
SWH	32467.533 Hz
FIDRES	0.495415 Hz
AQ	1.0093044 sec
RG	29193
DW	15.400 usec
DE	6.00 usec
TE	300.1 K
D1	1.50000000 sec
d11	0.03000000 sec
DELTA	1.39999998 sec
TD0	1

======== CHANNEL f1 ========

NUC1	31P
P1	10.30 usec
PL1	1.00 dB
SFO1	161.9755930 MHz

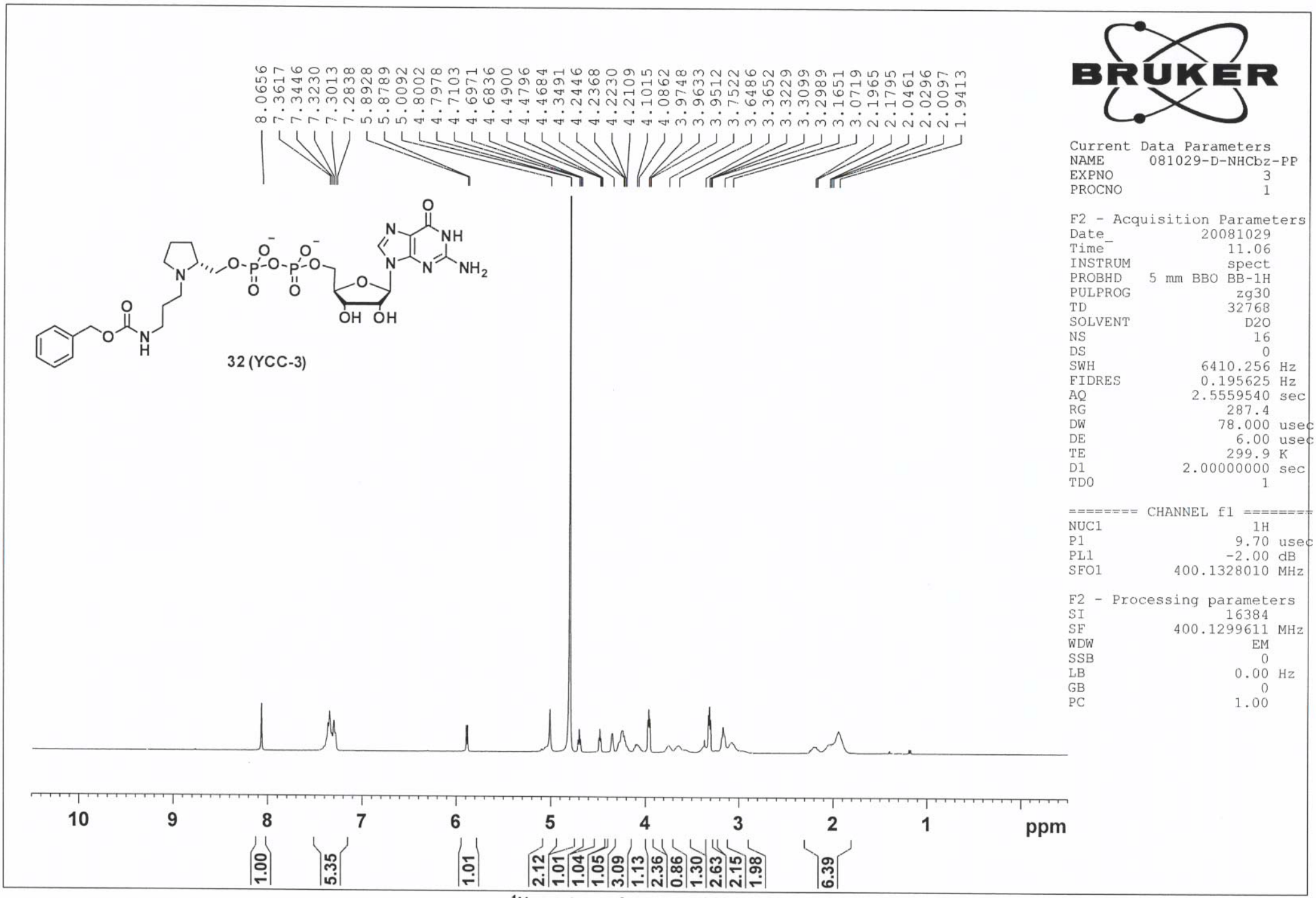
======== CHANNEL f2 ========

CPDPRG2	waltz16
NUC2	1H
PCPD2	90.00 usec
PL2	-2.00 dB
PL12	15.90 dB
PL13	18.90 dB
SFO2	400.1320007 MHz

F2 - Processing parameters

SI	65536
SF	161.9755259 MHz
WDW	EM
SSB	0
LB	3.00 Hz
GB	0
PC	1.00

^{31}P spectrum of compound 29 (400 MHz, D_2O)



BRUKER

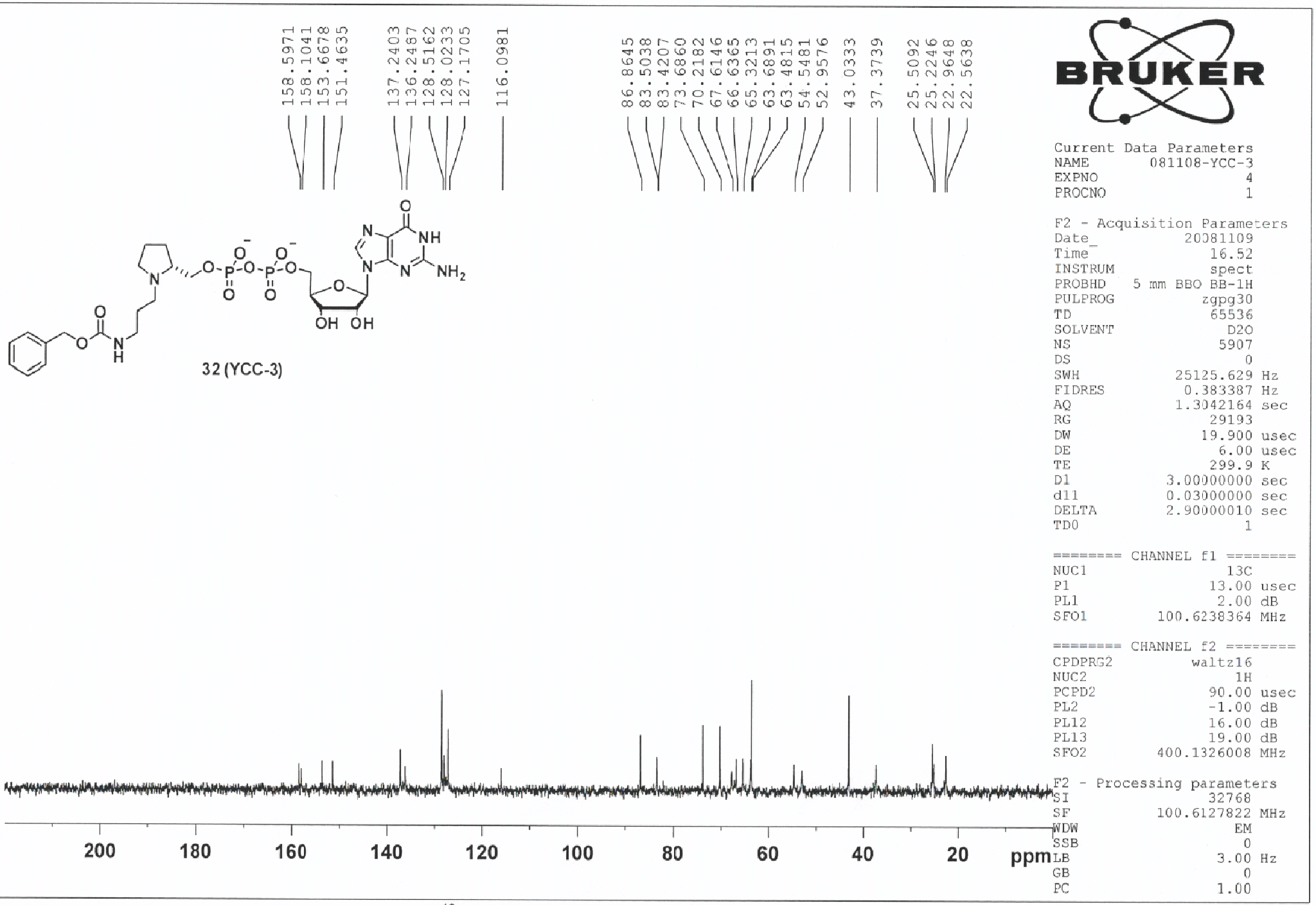
Current Data Parameters
NAME 081029-D-NHCbz-PP
EXPNO 3
PROCNO 1

F2 - Acquisition Parameters
Date_ 20081029
Time 11.06
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 32768
SOLVENT D2O
NS 16
DS 0
SWH 6410.256 Hz
FIDRES 0.195625 Hz
AQ 2.5559540 sec
RG 287.4
DW 78.000 usec
DE 6.00 usec
TE 299.9 K
D1 2.00000000 sec
TD0 1

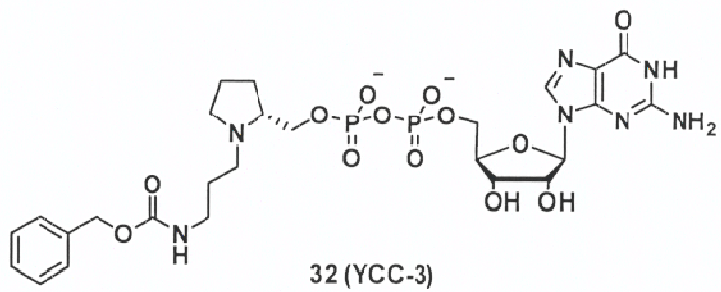
======== CHANNEL f1 ========
NUC1 1H
P1 9.70 usec
PL1 -2.00 dB
SFO1 400.1328010 MHz

F2 - Processing parameters
SI 16384
SF 400.1299611 MHz
WDW EM
SSB 0
LB 0.00 Hz
GB 0
PC 1.00

^{1}H spectrum of compound 32 (400 MHz, D_2O)



^{13}C spectrum of compound 32 (400 MHz, D_2O)



32 (YCC-3)

BRUKER

Current Data Parameters
NAME 081108-YCC-3
EXPNO 2
PROCNO 1

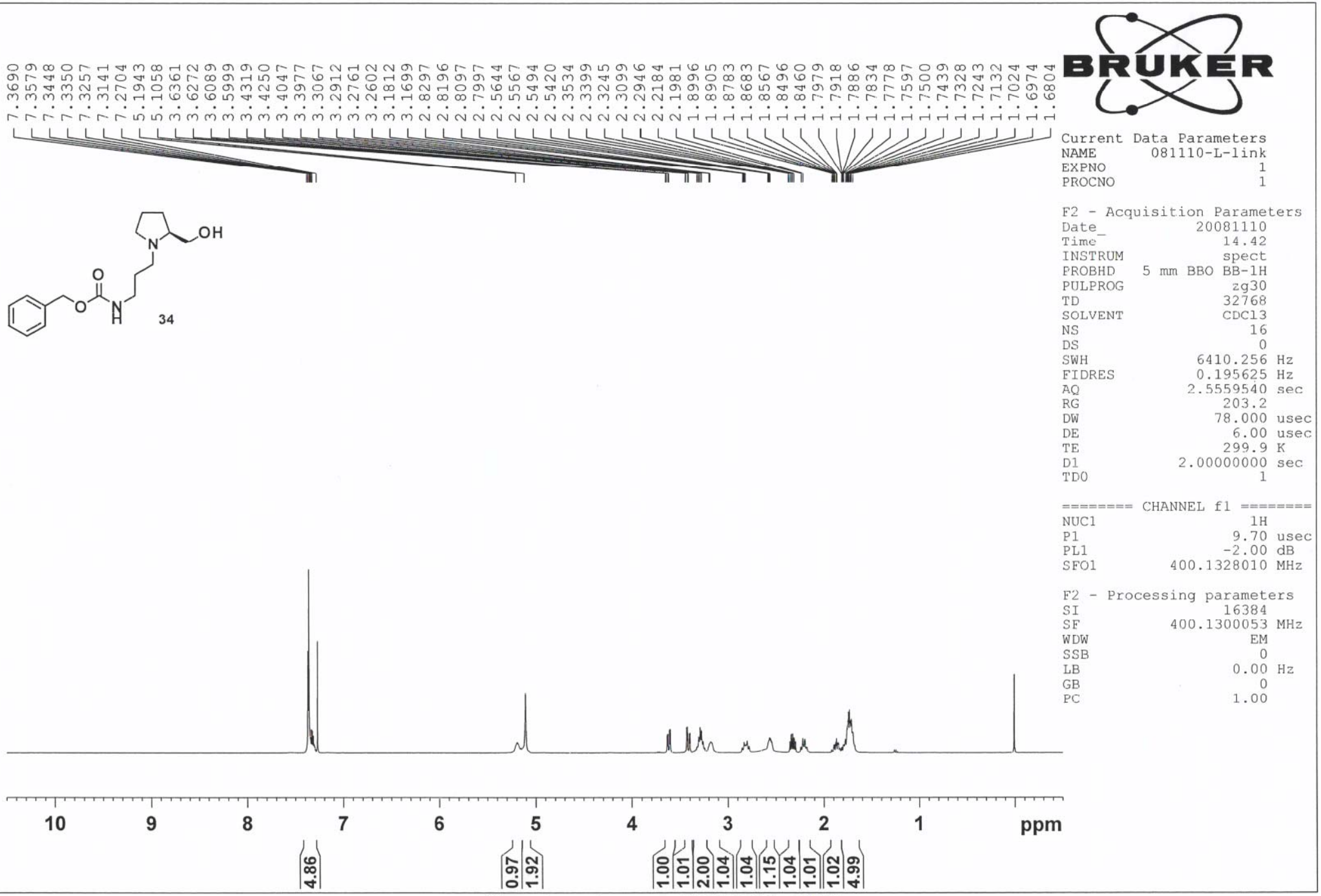
F2 - Acquisition Parameters
Date_ 20081108
Time 16.03
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zgpg30
TD 65536
SOLVENT D2O
NS 143
DS 0
SWH 32467.533 Hz
FIDRES 0.495415 Hz
AQ 1.0093044 sec
RG 29193
DW 15.400 usec
DE 6.00 usec
TE 300.3 K
D1 1.50000000 sec
d11 0.03000000 sec
DELTA 1.39999998 sec
TD0 1

======== CHANNEL f1 ========
NUC1 31P
P1 10.30 usec
PL1 1.00 dB
SFO1 161.9755930 MHz

======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 90.00 usec
PL2 -2.00 dB
PL12 15.90 dB
PL13 18.90 dB
SFO2 400.1320007 MHz

F2 - Processing parameters
SI 65536
SF 161.9755259 MHz
WDW EM
SSB 0
LB 3.00 Hz
GB 0
PC 1.00

^{31}P spectrum of compound 32 (400 MHz, D_2O)



BRUKER

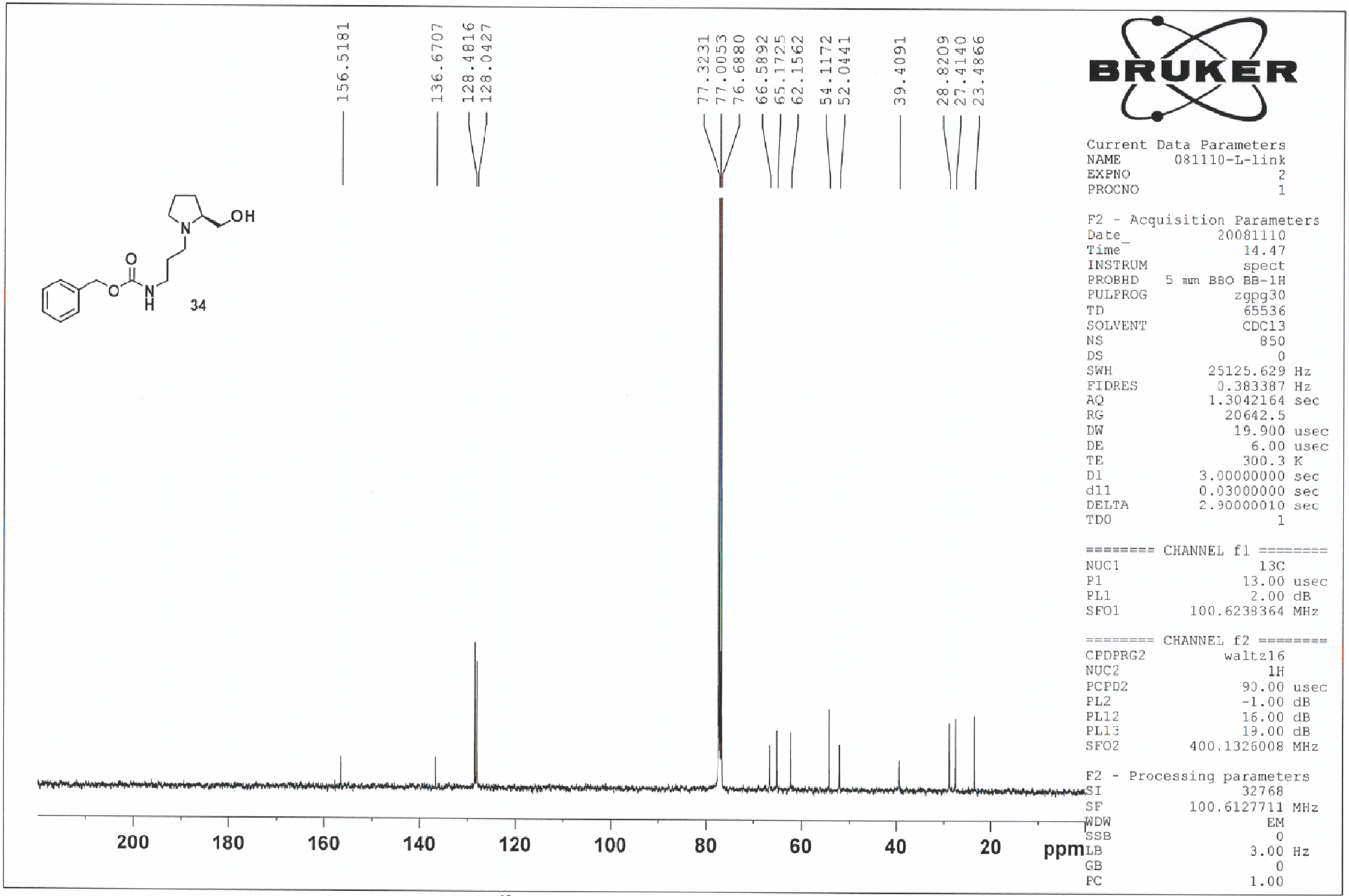
Current Data Parameters
NAME 081110-L-link
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20081110
Time 14.42
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 16
DS 0
SWH 6410.256 Hz
FIDRES 0.195625 Hz
AQ 2.5559540 sec
RG 203.2
DW 78.000 usec
DE 6.00 usec
TE 299.9 K
D1 2.00000000 sec
TD0 1

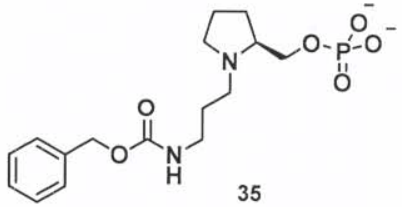
======== CHANNEL f1 ========
NUC1 1H
P1 9.70 usec
PL1 -2.00 dB
SFO1 400.1328010 MHz

F2 - Processing parameters
SI 16384
SF 400.1300053 MHz
WDW EM
SSB 0
LB 0.00 Hz
GB 0
PC 1.00

^{1}H spectrum of compound 34 (400 MHz, $CDCl_3$)



^{13}C spectrum of compound 34 (400 MHz, $CDCl_3$)



35

7.4146
7.4010
7.3917

5.0674
4.7999
4.1430
4.1161
4.0937
3.9020
3.8884
3.8629
3.8444
3.8307
3.5628
3.5343
3.3147
3.2927
3.2618
3.2436
3.2251
3.2089
3.1926
3.1758
3.1574
3.1408
2.9756
2.1518
2.1279
2.1043
2.0121
1.9948
1.9814
1.9624
1.9461
1.9276
1.9078
1.8892

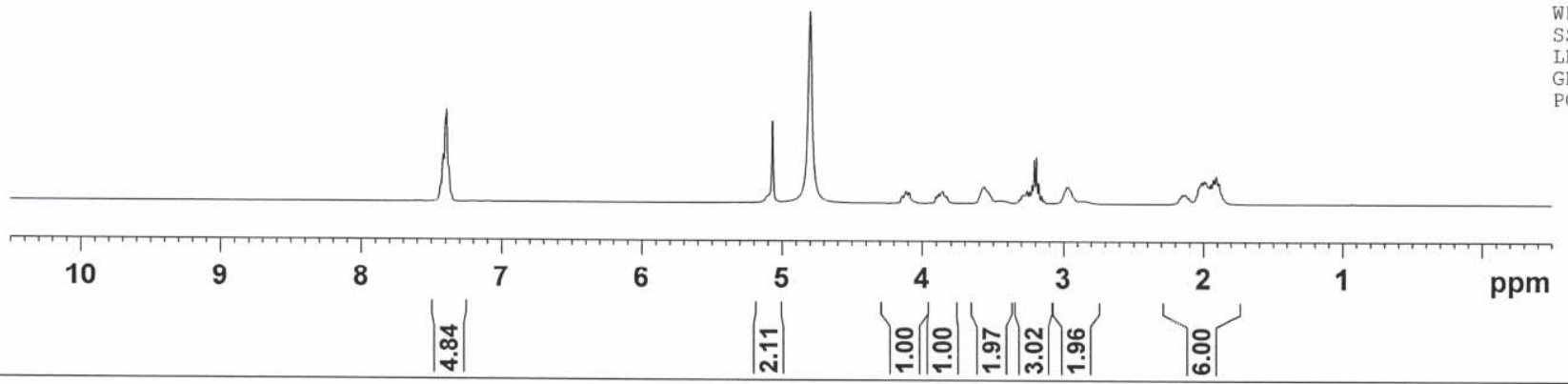
BRUKER

Current Data Parameters
NAME 081010
EXPNO 1
PROCNO 1

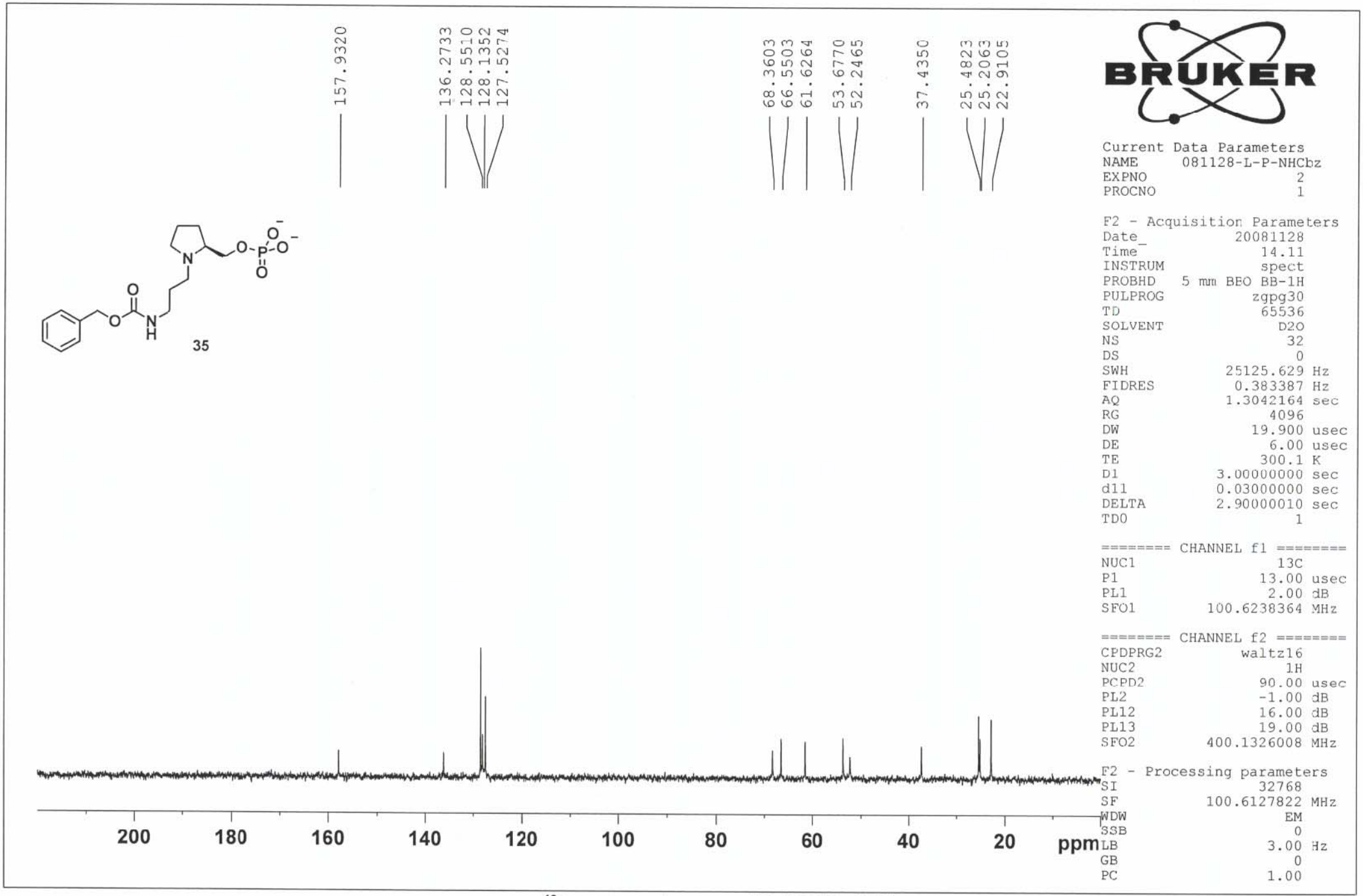
F2 - Acquisition Parameters
Date_ 20081010
Time 18.21
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 32768
SOLVENT D2O
NS 8
DS 0
SWH 6410.256 Hz
FIDRES 0.195625 Hz
AQ 2.5559540 sec
RG 57
DW 78.000 usec
DE 6.00 usec
TE 299.9 K
D1 2.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 9.70 usec
PL1 -2.00 dB
SFO1 400.1328010 MHz

F2 - Processing parameters
SI 16384
SF 400.1299552 MHz
WDW EM
SSB 0
LB 0.00 Hz
GB 0
PC 1.00



^{1}H spectrum of compound 35 (400 MHz, D_2O)



BRUKER

Current Data Parameters

NAME	081128-L-P-NHCbz
EXPNO	2
PROCNO	1

F2 - Acquisition Parameters

Date_	20081128
Time	14.11
INSTRUM	spect
PROBHD	5 mm BEO BB-1H
PULPROG	zgpg30
TD	65536
SOLVENT	D2O
NS	32
DS	0
SWH	25125.629 Hz
FIDRES	0.383387 Hz
AQ	1.3042164 sec
RG	4096
DW	19.900 usec
DE	6.00 usec
TE	300.1 K
D1	3.00000000 sec
d11	0.03000000 sec
DELTA	2.90000010 sec
TD0	1

======== CHANNEL f1 ========

NUC1	13C
P1	13.00 usec
PL1	2.00 dB
SFO1	100.6238364 MHz

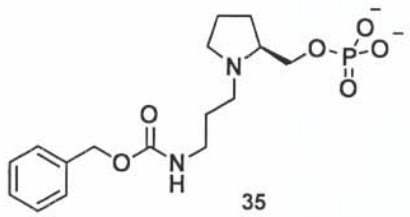
======== CHANNEL f2 ========

CPDPRG2	waltz16
NUC2	1H
PCPD2	90.00 usec
PL2	-1.00 dB
PL12	16.00 dB
PL13	19.00 dB
SFO2	400.1326008 MHz

F2 - Processing parameters

SI	32768
SF	100.6127822 MHz
WDW	EM
SSB	0
LB	3.00 Hz
GB	0
PC	1.00

^{13}C spectrum of compound 35 (400 MHz, D_2O)



35

BRUKER

Current Data Parameters
NAME 080925-L-Prollink-P
EXPNO 5
PROCNO 1

F2 - Acquisition Parameters

Date_	20080925
Time	19.00
INSTRUM	spect
PROBHD	5 mm BBO BB-1H
PULPROG	zgpg30
TD	65536
SOLVENT	DMSO
NS	320
DS	0
SWH	32467.533 Hz
FIDRES	0.495415 Hz
AQ	1.0093044 sec
RG	13004
DW	15.400 usec
DE	6.00 usec
TE	299.9 K
D1	1.50000000 sec
d11	0.03000000 sec
DELTA	1.39999998 sec
TD0	1

======== CHANNEL f1 ========

NUC1	31P
P1	10.30 usec
PL1	1.00 dB
SFO1	161.9755930 MHz

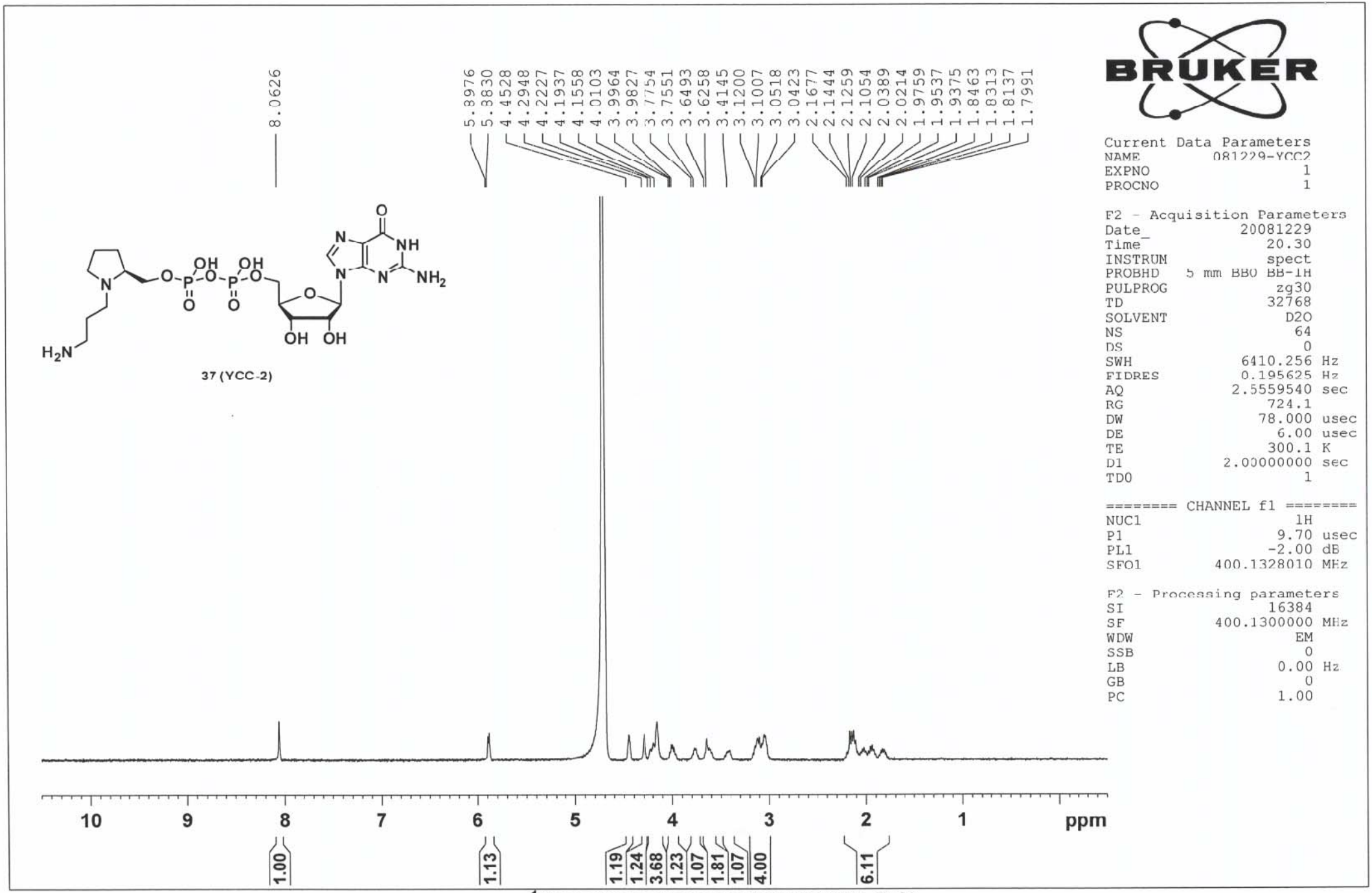
======== CHANNEL f2 ========

CPDPRG2	waltz16
NUC2	1H
PCPD2	90.00 usec
PL2	-2.00 dB
PL12	15.90 dB
PL13	18.90 dB
SFO2	400.1320007 MHz

F2 - Processing parameters

SI	65536
SF	161.9755259 MHz
WDW	EM
SSB	0
LB	3.00 Hz
GB	0
PC	1.00

^{31}P spectrum of compound 35 (400 MHz, D_2O)



BRUKER

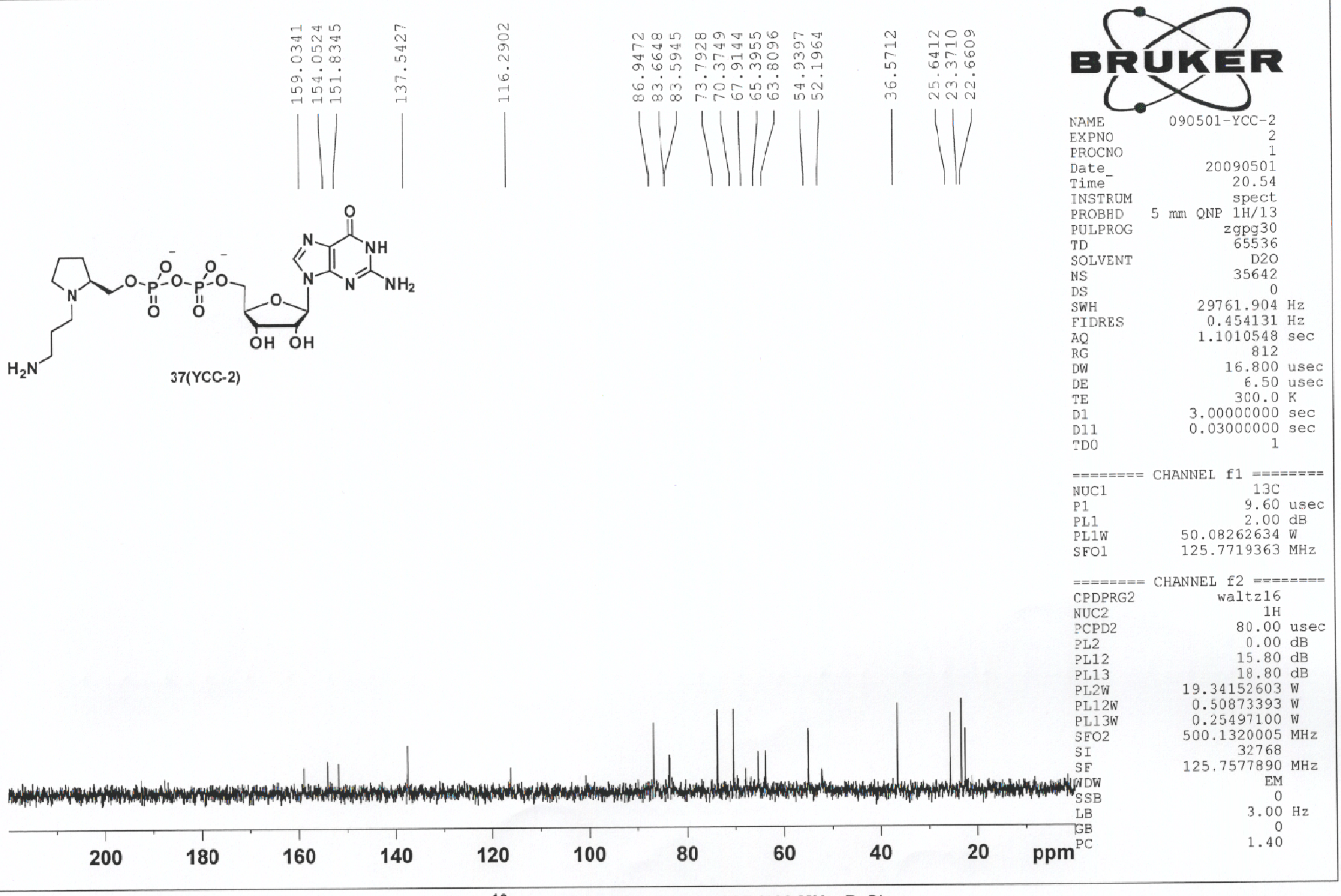
Current Data Parameters
NAME 081229-YCC2
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20081229
Time 20.30
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 32768
SOLVENT D2O
NS 64
DS 0
SWH 6410.256 Hz
FIDRES 0.195625 Hz
AQ 2.5559540 sec
RG 724.1
DW 78.000 usec
DE 6.00 usec
TE 300.1 K
D1 2.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 9.70 usec
PL1 -2.00 dB
SFO1 400.1328010 MHz

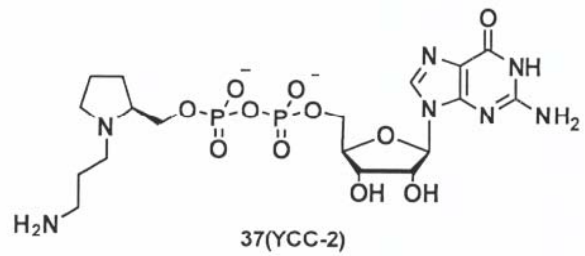
F2 - Processing parameters
SI 16384
SF 400.1300000 MHz
WDW EM
SSB 0
LB 0.00 Hz
GB 0
PC 1.00

^{1}H spectrum of compound 37 (400 MHz, D_2O)



37(YCC-2)

^{13}C spectrum of compound 37 (400 MHz, D_2O)



37(YCC-2)

-10.5960
-10.7265
-11.0753
-11.2048

BRUKER

Current Data Parameters
NAME 021108-YCC-2
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20081108
Time 15.43
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zgpg30
TD 65536
SOLVENT D2O
NS 320
DS 0
SWH 32467.533 Hz
FIDRES 0.495415 Hz
AQ 1.0093044 sec
RG 29193
DW 15.400 usec
DE 6.00 usec
TE 300.1 K
D1 1.50000000 sec
d11 0.03000000 sec
DELTA 1.39999998 sec
TD0 1

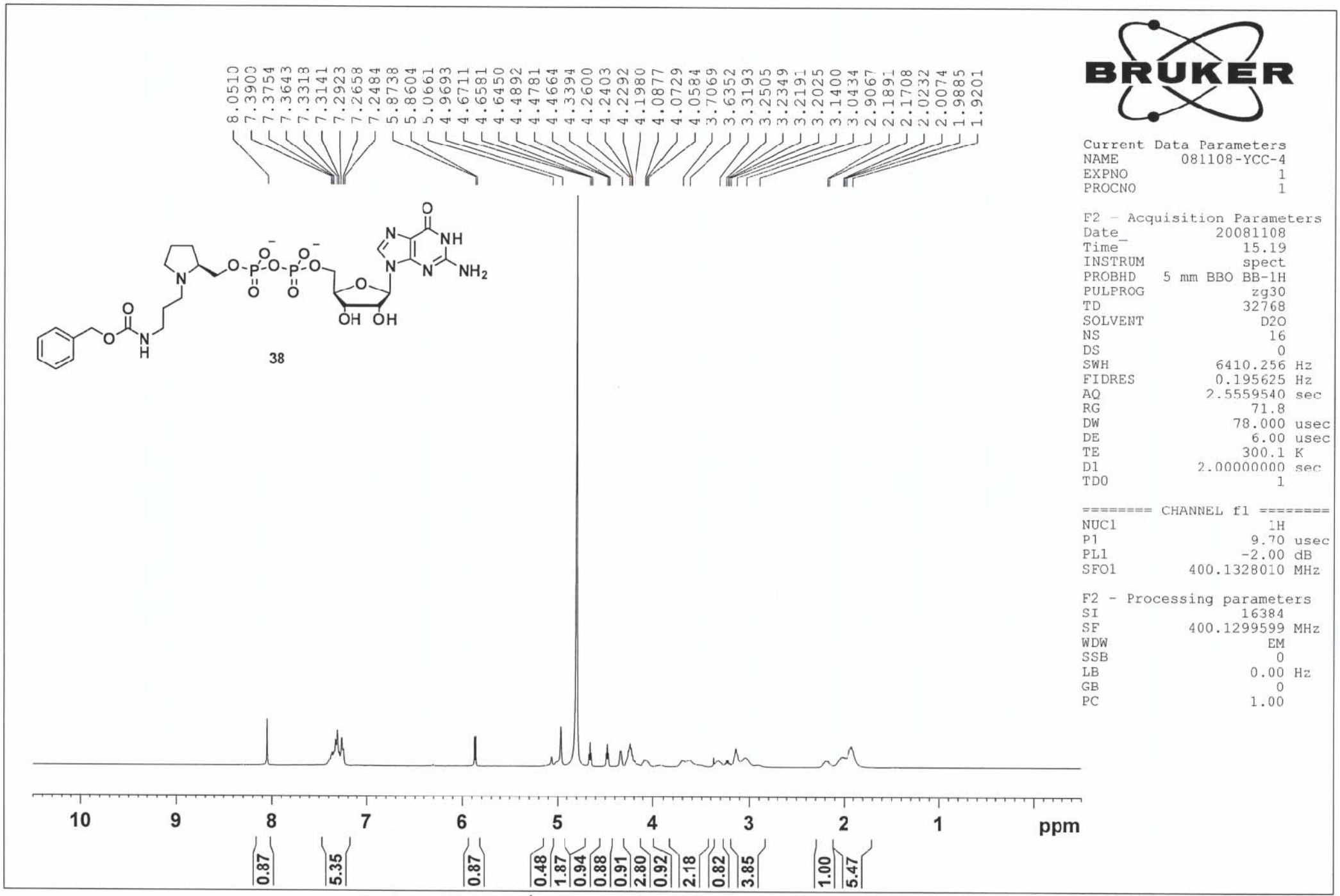
======== CHANNEL f1 ========
NUC1 31P
P1 10.30 usec
PL1 1.00 dB
SFO1 161.9755930 MHz

======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 90.00 usec
PL2 -2.00 dB
PL12 15.90 dB
PL13 18.90 dB
SFO2 400.1320007 MHz

F2 - Processing parameters
SI 65536
SF 161.9755259 MHz
WDW EM
SSB 0
LB 3.00 Hz
GB 0
PC 1.00

20 15 10 5 0 -5 -10 -15 -20 ppm

^{31}P spectrum of compound 37 (400 MHz, D_2O)



Current Data Parameters

NAME	081108-YCC-4
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20081108
Time	15.19
INSTRUM	spect
PROBHD	5 mm BBO BB-1H
PULPROG	zg30
TD	32768
SOLVENT	D2O
NS	16
DS	0
SWH	6410.256 Hz
FIDRES	0.195625 Hz
AQ	2.5559540 sec
RG	71.8
DW	78.000 usec
DE	6.00 usec
TE	300.1 K
D1	2.00000000 sec
TD0	1

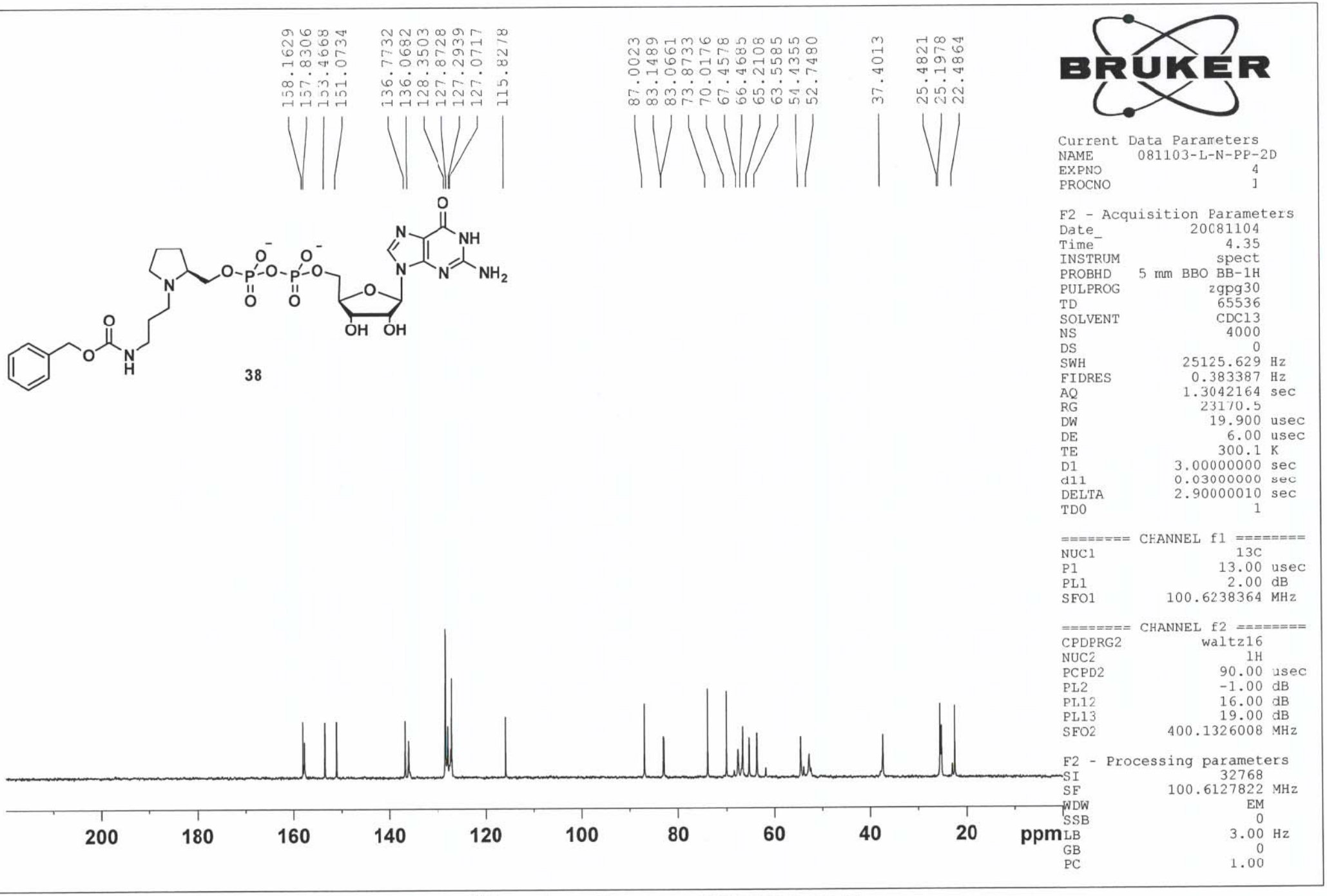
======== CHANNEL f1 ========

NUC1	1H
P1	9.70 usec
PL1	-2.00 dB
SFO1	400.1328010 MHz

F2 - Processing parameters

SI	16384
SF	400.1299599 MHz
WDW	EM
SSB	0
LB	0.00 Hz
GB	0
PC	1.00

^{1}H spectrum of compound 38 (400 MHz, D_2O)



BRUKER

Current Data Parameters
NAME 081103-L-N-PP-2D
EXPNO 4
PROCNO 1

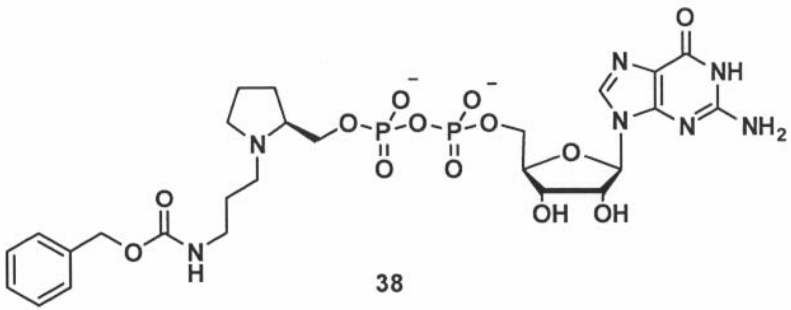
F2 - Acquisition Parameters
Date_ 20081104
Time 4.35
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 4000
DS 0
SWH 25125.629 Hz
FIDRES 0.383387 Hz
AQ 1.3042164 sec
RG 23170.5
DW 19.900 usec
DE 6.00 usec
TE 300.1 K
D1 3.00000000 sec
d11 0.03000000 sec
DELTA 2.90000010 sec
TD0 1

======== CHANNEL f1 ========
NUC1 13C
P1 13.00 usec
PL1 2.00 dB
SFO1 100.6238364 MHz

======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 90.00 usec
PL2 -1.00 dB
PL12 16.00 dB
PL13 19.00 dB
SFO2 400.1326008 MHz

F2 - Processing parameters
SI 32768
SF 100.6127822 MHz
WDW EM
SSB 0
LB 3.00 Hz
GB 0
PC 1.00

^{13}C spectrum of compound 38 (400 MHz, D_2O)



38

-10.5778
-10.7078
-11.0866
-11.2167

BRUKER

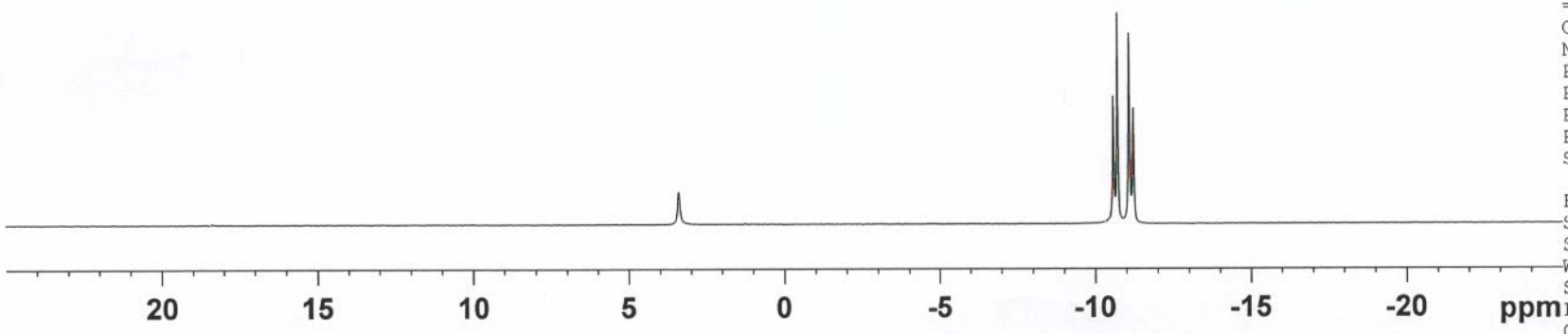
Current Data Parameters
NAME 081108-YCC-4
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20081108
Time 15.27
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 262
DS 0
SWH 32467.533 Hz
FIDRES 0.495415 Hz
AQ 1.0093044 sec
RG 13004
DW 15.400 usec
DE 6.00 usec
TE 300.1 K
D1 1.50000000 sec
d11 0.03000000 sec
DELTA 1.39999998 sec
TD0 1

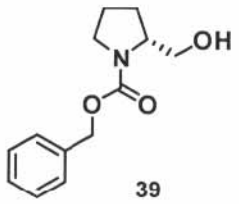
======== CHANNEL f1 ========
NUC1 31P
P1 10.30 usec
PL1 1.00 dB
SFO1 161.9755930 MHz

======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 90.00 usec
PL2 -2.00 dB
PL12 15.90 dB
PL13 18.90 dB
SFO2 400.1320007 MHz

F2 - Processing parameters
SI 65536
SF 161.9755259 MHz
WDW EM
SSB 0
LB 3.00 Hz
GB 0
PC 1.00



^{31}P spectrum of compound 38 (400 MHz, D_2O)



39

7.3668 7.3571 7.3321 7.3216 7.3102 7.2704 5.1782 5.1469 5.1404 5.1092 4.3967 4.0013 3.6514 3.6396 3.6117 3.5641 3.5464 3.5372 3.5202 3.5036 3.4251 3.4077 3.3983 3.3907 3.3810 3.3635 2.0537 2.0360 2.0187 2.0041 1.9872 1.9693 1.9213 1.9036 1.8876 1.8721 1.8560 1.8402 1.8240 1.8071 1.7909 1.7754 1.7586 1.6656

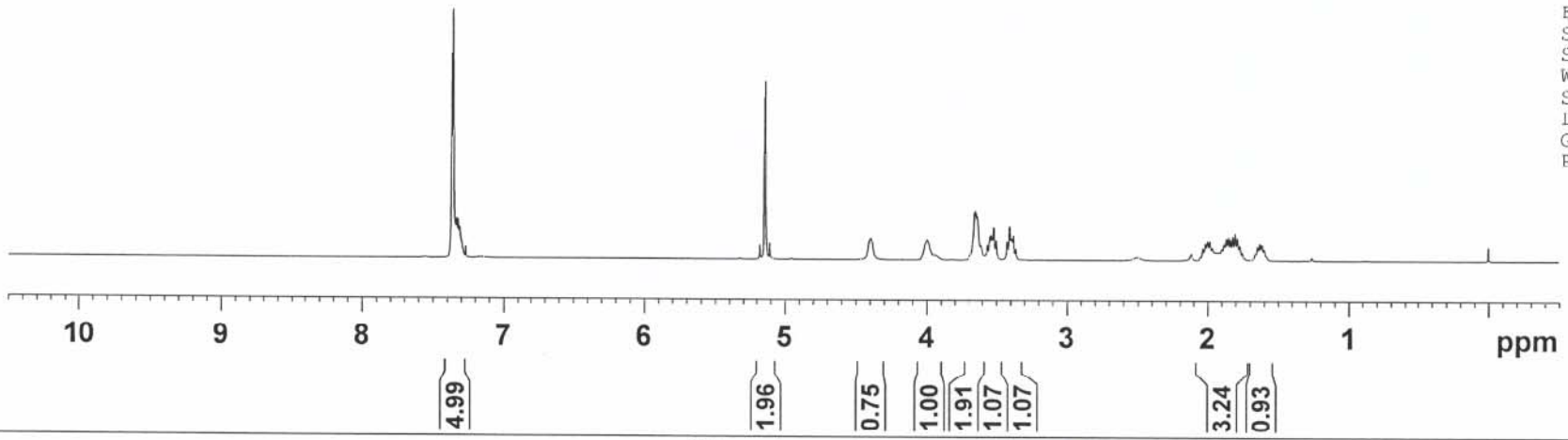
BRUKER

Current Data Parameters
NAME 081213-D-NCbz-OH
EXPNO 1
PROCNO 1

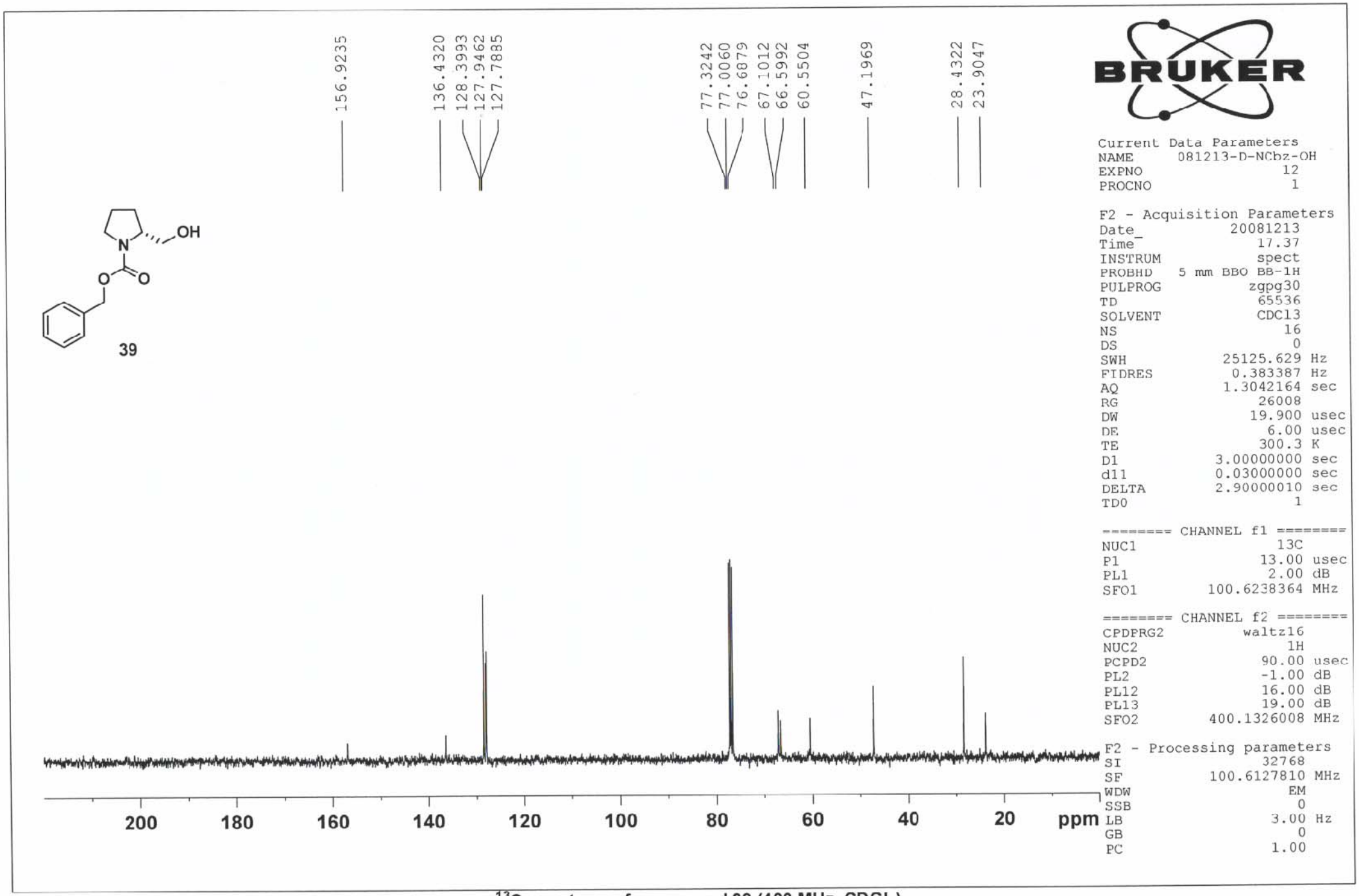
F2 - Acquisition Parameters
Date_ 20081213
Time 17.43
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 8
DS 0
SWH 6410.256 Hz
FIDRES 0.195625 Hz
AQ 2.5559540 sec
RG 64
DW 78.000 usec
DE 6.00 usec
TE 299.9 K
D1 2.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 9.70 usec
PL1 -2.00 dB
SFO1 400.1328010 MHz

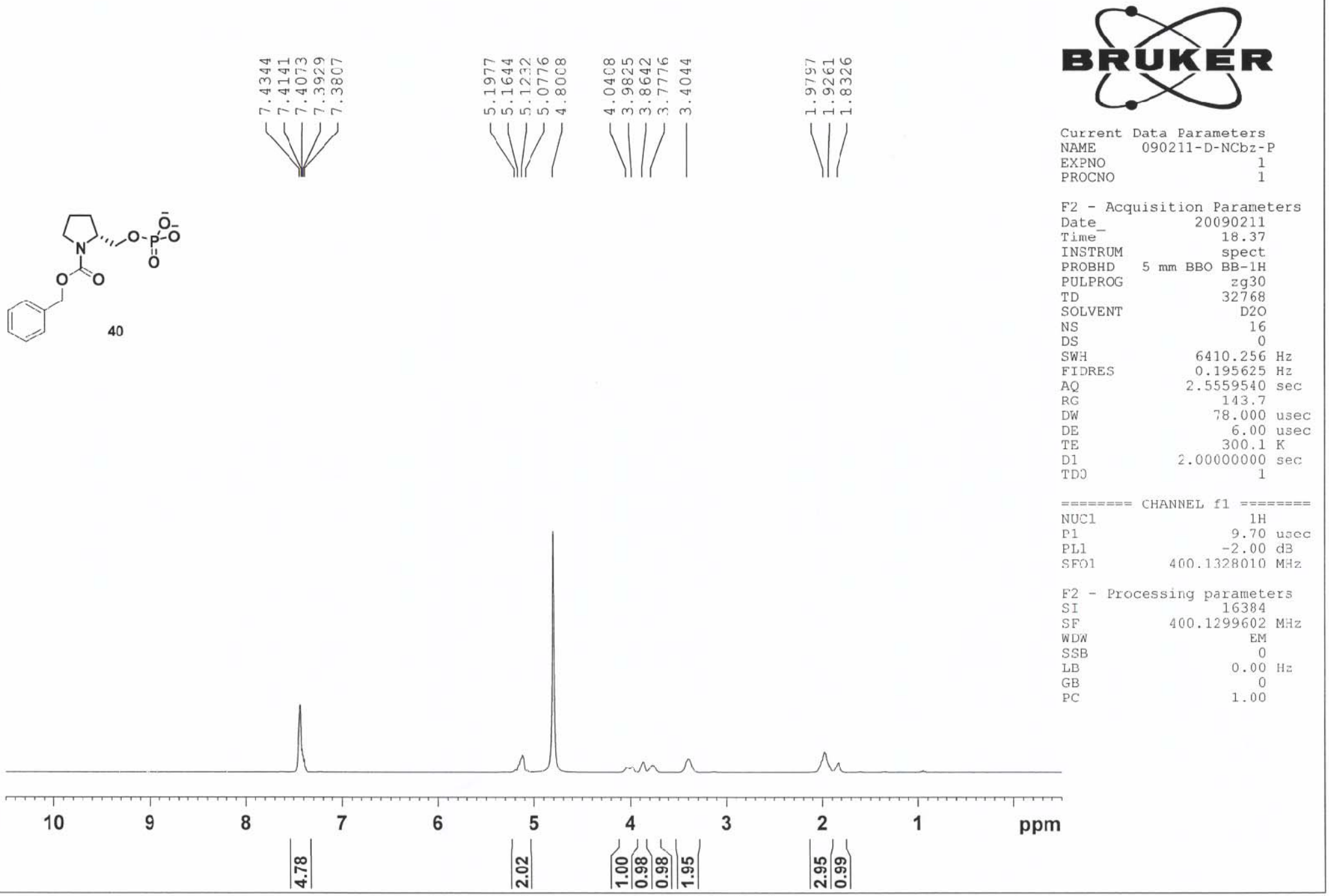
F2 - Processing parameters
SI 16384
SF 400.1300052 MHz
WDW EM
SSB 0
LB 0.00 Hz
GB 0
PC 1.00



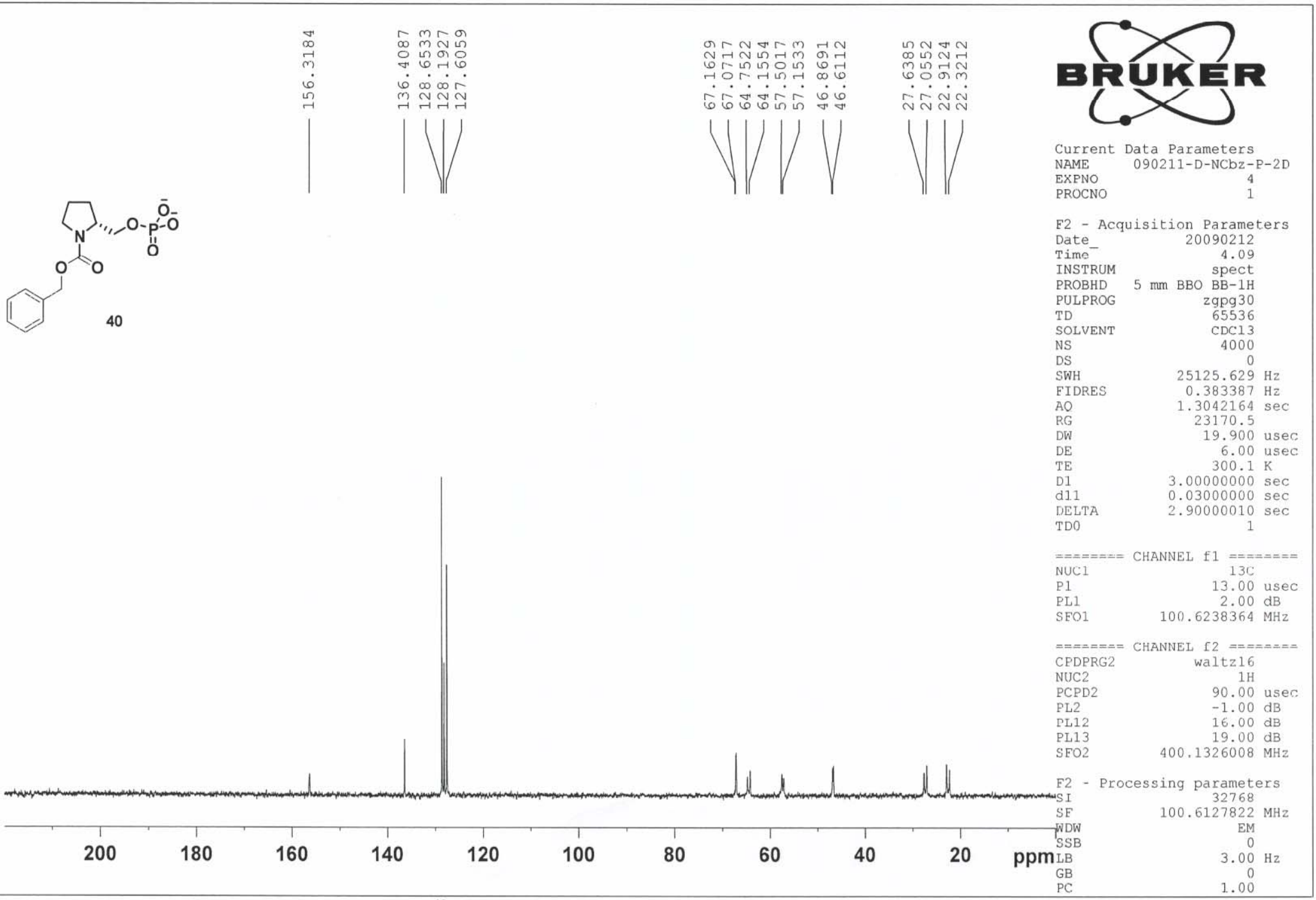
^{1}H spectrum of compound 39 (500 MHz, $CDCl_3$)



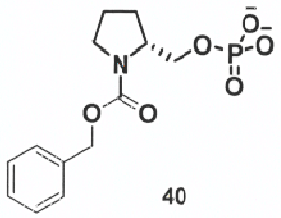
^{13}C spectrum of compound 39 (400 MHz, $CDCl_3$)



^{1}H spectrum of compound 40 (400 MHz, D_2O)

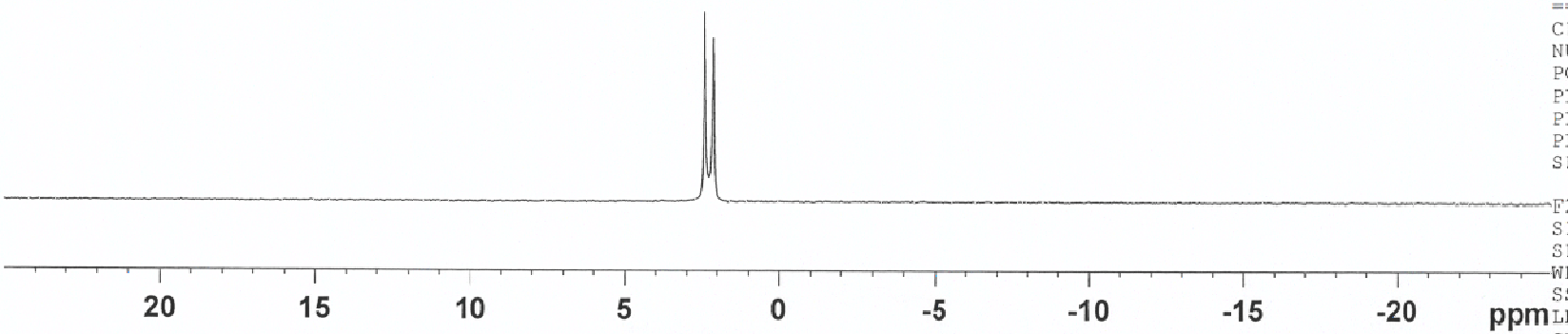


^{13}C spectrum of compound 40 (400 MHz, D_2O)



40

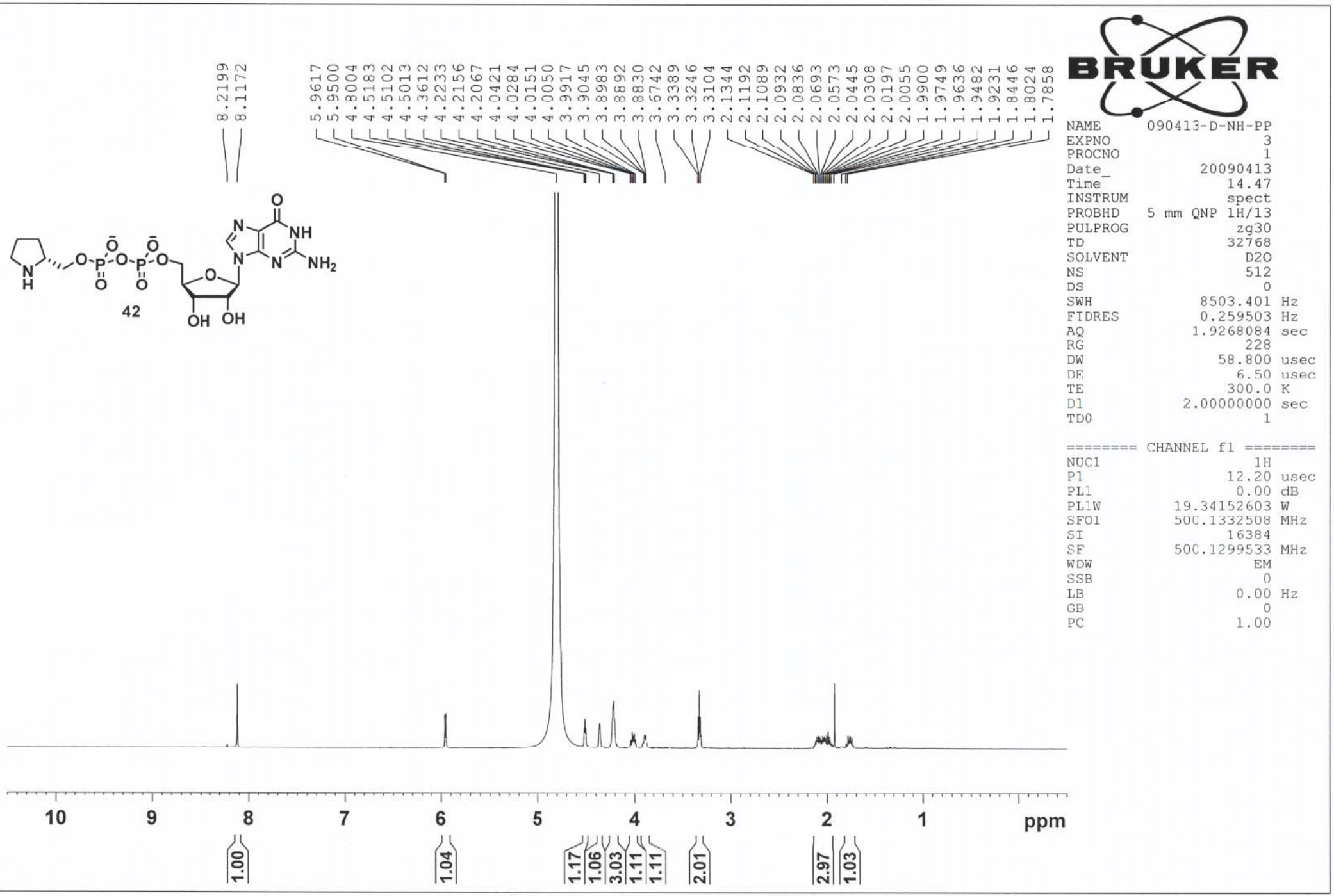
2.4129
2.1135



BRUKER

```
Current Data Parameters
NAME      090211-D-NCbz-P
EXPNO                   2
PROCNO                  1

F2 - Acquisition Parameters
Date_            20090211
Time                18.42
INSTRUM             spect
PROBHD   5 mm BBO BB-1H
PULPROG           zgpg30
TD                 65536
SOLVENT             DMSO
NS                    91
DS                     0
SWH            32467.533 Hz
FIDRES          0.495415 Hz
AQ             1.0093044 sec
RG               20642.5
DW                15.400 usec
DE                 6.00 usec
TE                300.3 K
D1             1.50000000 sec
d11            0.03000000 sec
DELTA          1.39999998 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  31P
P1                 10.30 usec
PL1                 1.00 dB
SFO1         161.9755930 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                  1H
PCPD2              90.00 usec
PL2                -2.00 dB
PL12               15.90 dB
PL13               18.90 dB
SFO2         400.1320007 MHz

F2 - Processing parameters
SI                 65536
SF          161.9755259 MHz
WDW                   EM
SSB                    0
LB                  3.00 Hz
GB                     0
PC                  1.00
```

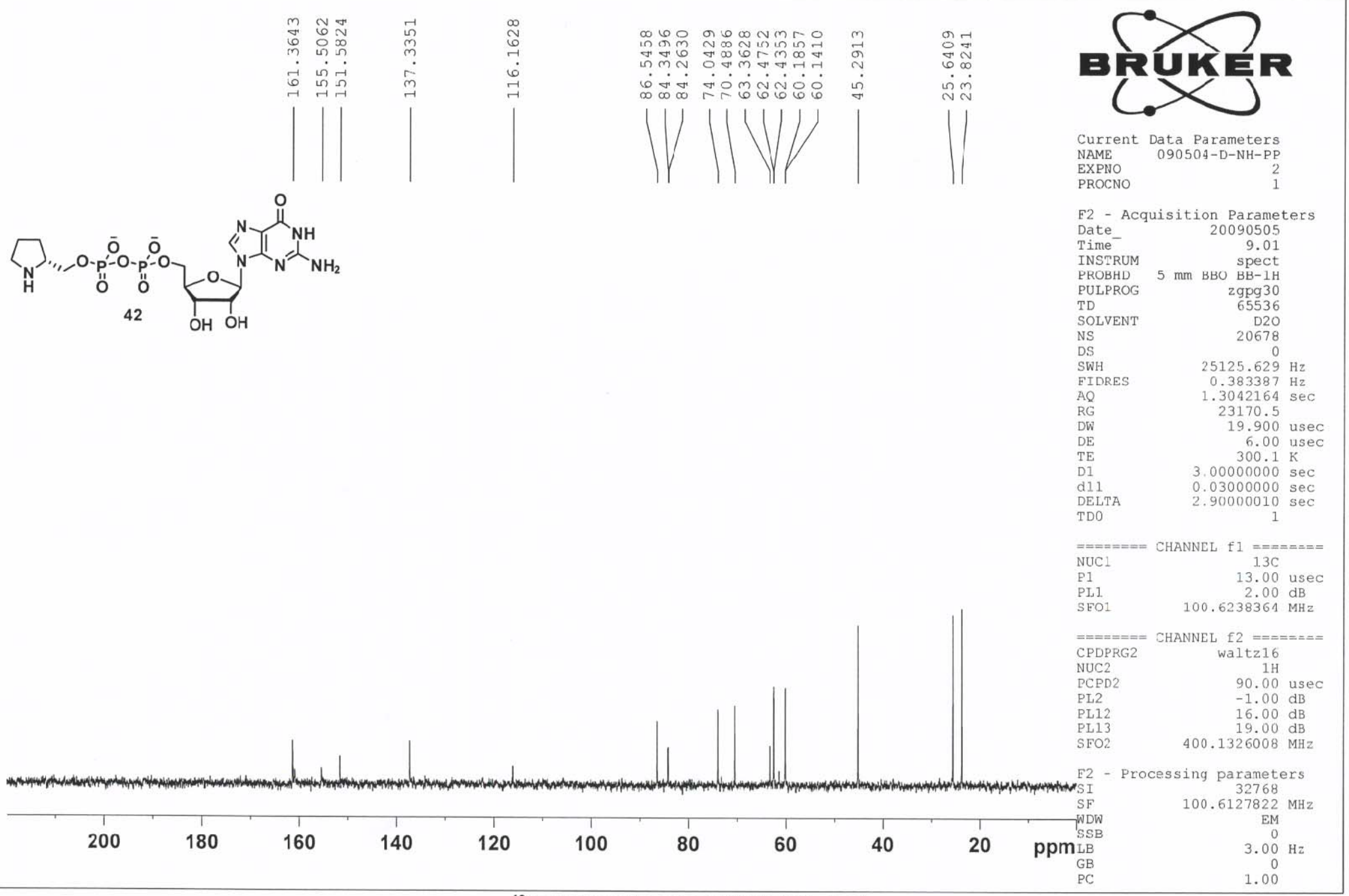
^{31}P spectrum of compound 40 (400 MHz, D_2O)



BRUKER

NAME	090413-D-NH-PP	
EXPNO	3	
PROCNO	1	
Date_	20090413	
Time	14.47	
INSTRUM	spect	
PROBHD	5 mm QNP 1H/13	
PULPROG	zg30	
TD	32768	
SOLVENT	D2O	
NS	512	
DS	0	
SWH	8503.401	Hz
FIDRES	0.259503	Hz
AQ	1.9268084	sec
RG	228	
DW	58.800	usec
DE	6.50	usec
TE	300.0	K
D1	2.00000000	sec
TD0	1	
======== CHANNEL f1 ========		
NUC1	1H	
P1	12.20	usec
PL1	0.00	dB
PL1W	19.34152603	W
SFO1	500.1332508	MHz
SI	16384	
SF	500.1299533	MHz
WDW	EM	
SSB	0	
LB	0.00	Hz
GB	0	
PC	1.00	

^{1}H spectrum of compound 42 (400 MHz, D_2O)



Current Data Parameters
NAME 090504-D-NH-PP
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters

Date_	20090505
Time	9.01
INSTRUM	spect
PROBHD	5 mm BBO BB-1H
PULPROG	zgpg30
TD	65536
SOLVENT	D2O
NS	20678
DS	0
SWH	25125.629 Hz
FIDRES	0.383387 Hz
AQ	1.3042164 sec
RG	23170.5
DW	19.900 usec
DE	6.00 usec
TE	300.1 K
D1	3.00000000 sec
d11	0.03000000 sec
DELTA	2.90000010 sec
TD0	1

======== CHANNEL f1 ========

NUC1	13C
P1	13.00 usec
PL1	2.00 dB
SFO1	100.6238364 MHz

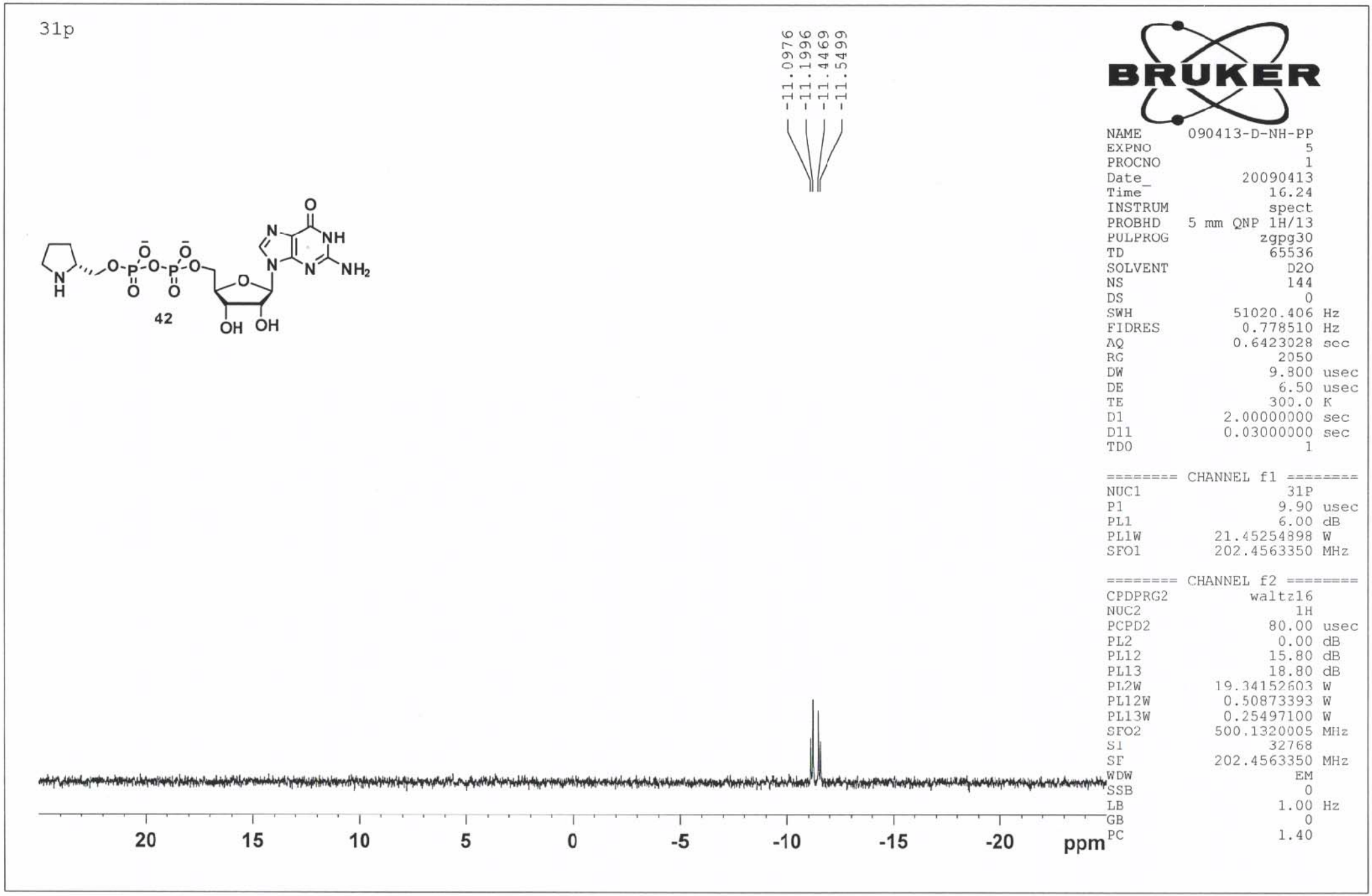
======== CHANNEL f2 ========

CPDPRG2	waltz16
NUC2	1H
PCPD2	90.00 usec
PL2	-1.00 dB
PL12	16.00 dB
PL13	19.00 dB
SFO2	400.1326008 MHz

F2 - Processing parameters

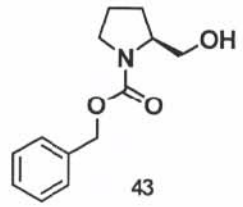
SI	32768
SF	100.6127822 MHz
WDW	EM
SSB	0
LB	3.00 Hz
GB	0
PC	1.00

^{13}C spectrum of compound 42 (400 MHz, D_2O)



NAME	090413-D-NH-PP
EXPNO	5
PROCNO	1
Date_	20090413
Time	16.24
INSTRUM	spect
PROBHD	5 mm QNP 1H/13
PULPROG	zgpg30
TD	65536
SOLVENT	D2O
NS	144
DS	0
SWH	51020.406 Hz
FIDRES	0.778510 Hz
AQ	0.6423028 sec
RG	2050
DW	9.800 usec
DE	6.50 usec
TE	300.0 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	31P
P1	9.90 usec
PL1	6.00 dB
PL1W	21.45254898 W
SFO1	202.4563350 MHz
======== CHANNEL f2 ========	
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	0.00 dB
PL12	15.80 dB
PL13	18.80 dB
PL2W	19.34152603 W
PL12W	0.50873393 W
PL13W	0.25497100 W
SFO2	500.1320005 MHz
SI	32768
SF	202.4563350 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

^{31}P spectrum of compound 42 (400 MHz, D_2O)



43

7.3555 7.3470 7.3212 7.3095 7.2993 7.2705

5.1667 5.1354 5.1280 5.0967 4.4383 3.9852 3.6364 3.5435 3.5259 3.5166 3.5001 3.4833 3.4153 3.3983 3.3814 3.3720 3.3543 2.0228 2.0145 1.9969 1.9826 1.9654 1.9474 1.8937 1.8776 1.8622 1.8461 1.8285 1.8109 1.7940 1.7780 1.7622 1.7457 1.6599 1.6447

BRUKER

Current Data Parameters
NAME 090204-L-NCbz-OH
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20090204
Time 16.59
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 16
DS 0
SWH 6410.256 Hz
FIDRES 0.195625 Hz
AQ 2.5559540 sec
RG 57
DW 78.000 usec
DE 6.00 usec
TE 300.1 K
D1 2.00000000 sec
TD0 1

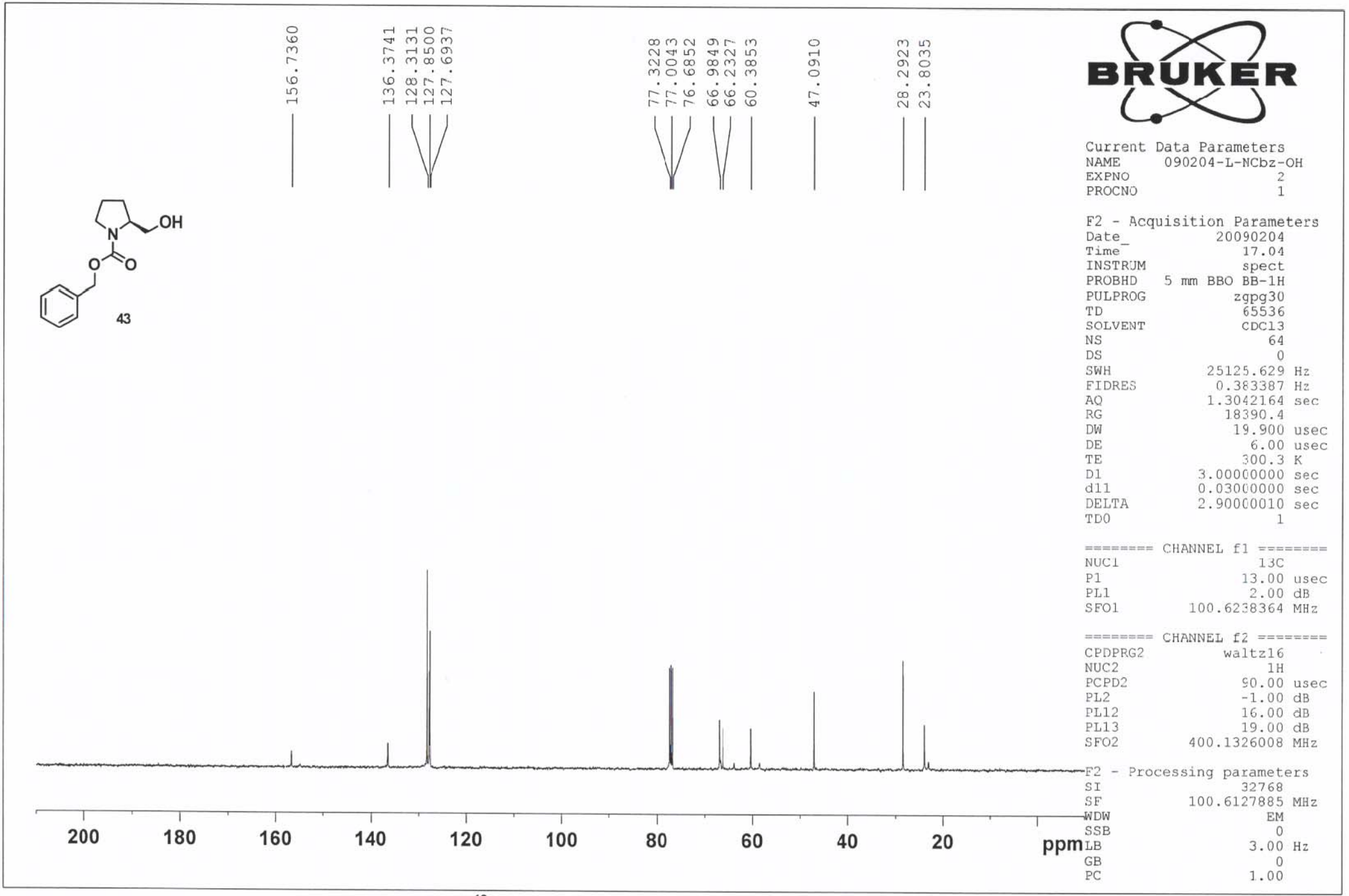
======== CHANNEL f1 ========
NUC1 1H
P1 9.70 usec
PL1 -2.00 dB
SFO1 400.1328010 MHz

F2 - Processing parameters
SI 16384
SF 400.1300048 MHz
WDW EM
SSB 0
LB 0.00 Hz
GB 0
PC 1.00

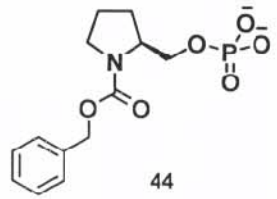
10 9 8 7 6 5 4 3 2 1 ppm

4.72 2.00 0.83 1.00 1.77 1.09 1.01 0.93 2.06 0.95

^{1}H spectrum of compound 43 (400 MHz, $CDCl_3$)



^{13}C spectrum of compound 43 (400 MHz, $CDCl_3$)



44

^{1}H spectrum of compound 44 (400 MHz, D_2O)

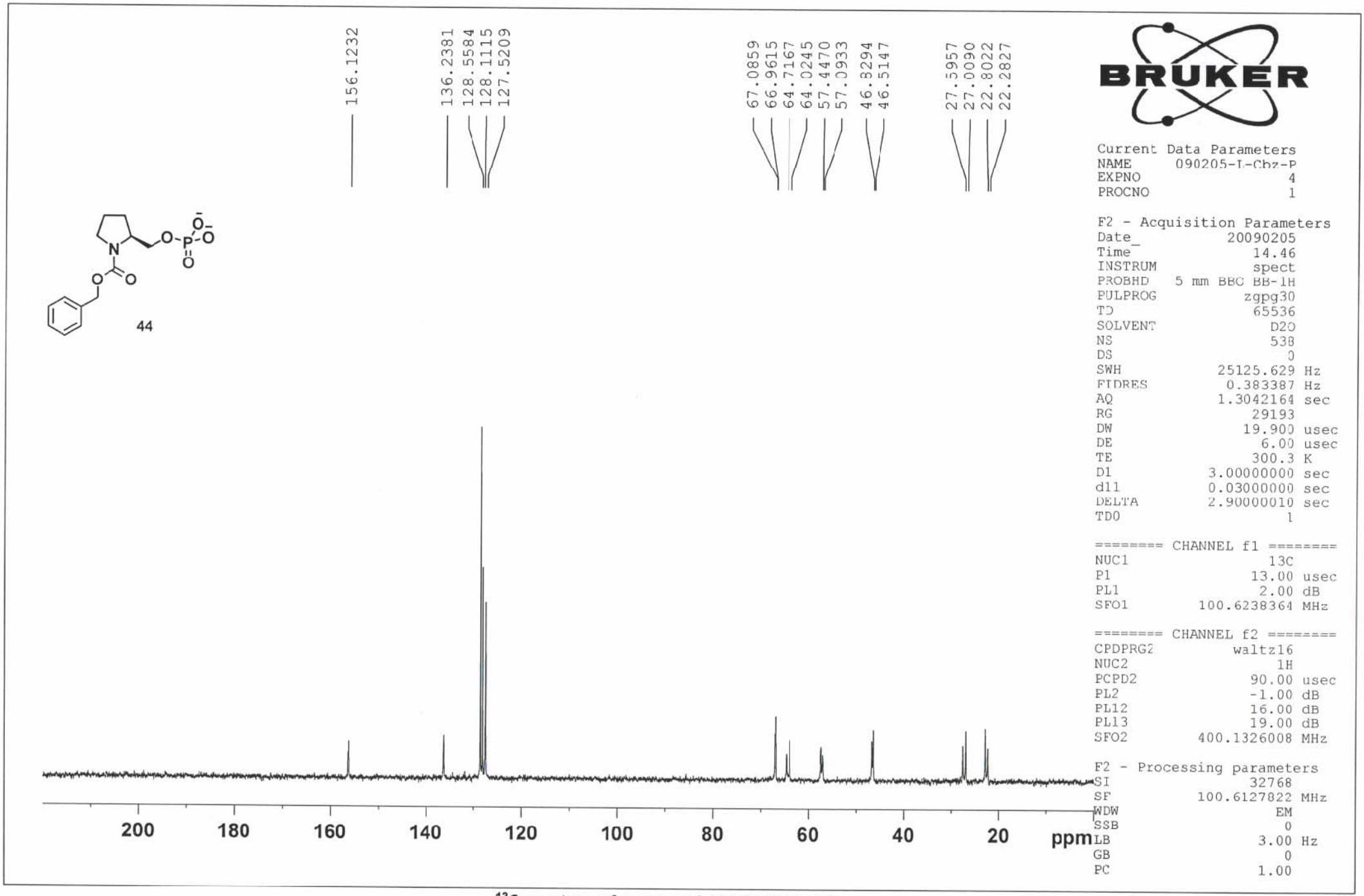
BRUKER

Current Data Parameters
NAME 090205-L-Cbz-P
EXPNO 3
PROCNO 1

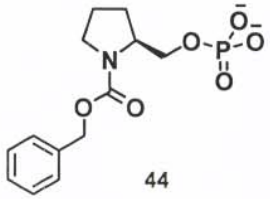
F2 - Acquisition Parameters
Date_ 20090205
Time 14.42
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 32768
SOLVENT D2O
NS 16
DS 0
SWH 6410.256 Hz
FIDRES 0.195625 Hz
AQ 2.5559540 sec
RG 90.5
DW 78.000 usec
DE 6.00 usec
TE 299.9 K
D1 2.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 9.70 usec
PL1 -2.00 dB
SFO1 400.1328010 MHz

F2 - Processing parameters
SI 16384
SF 400.1299607 MHz
WDW EM
SSB 0
LB 0.00 Hz
GB 0
PC 1.00

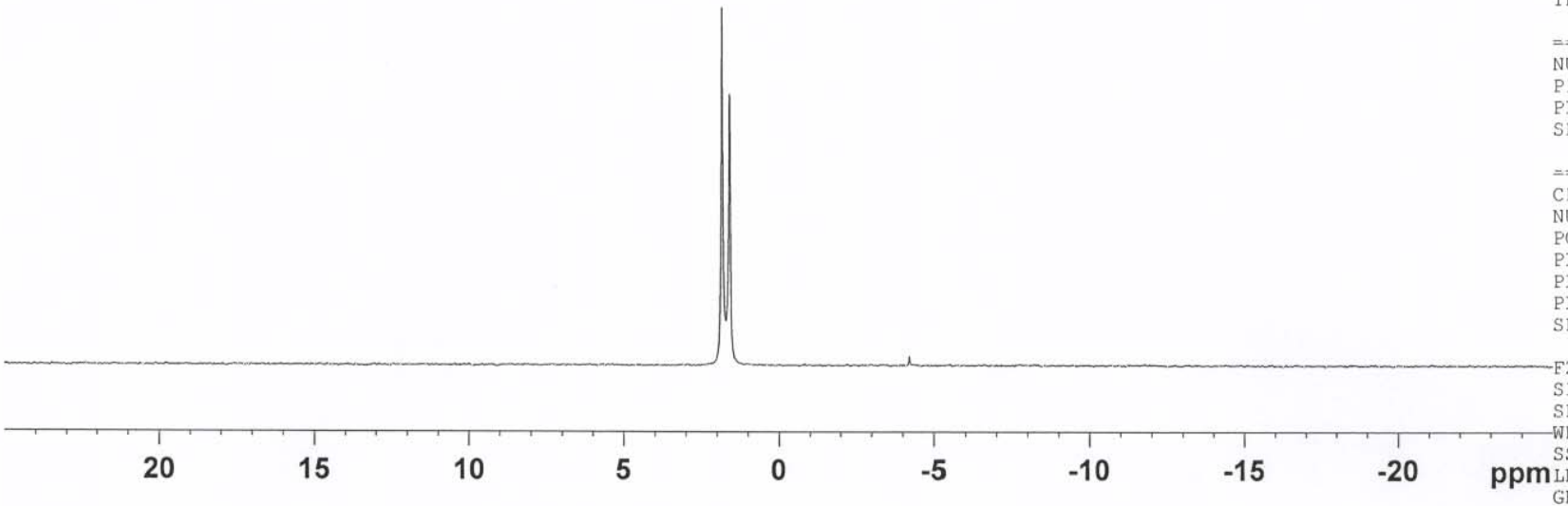


^{13}C spectrum of compound 44 (400 MHz, D_2O)



44

1.8493
1.5948



BRUKER

Current Data Parameters
NAME 090216-L-NCbz-P
EXPNO 4
PROCNO 1

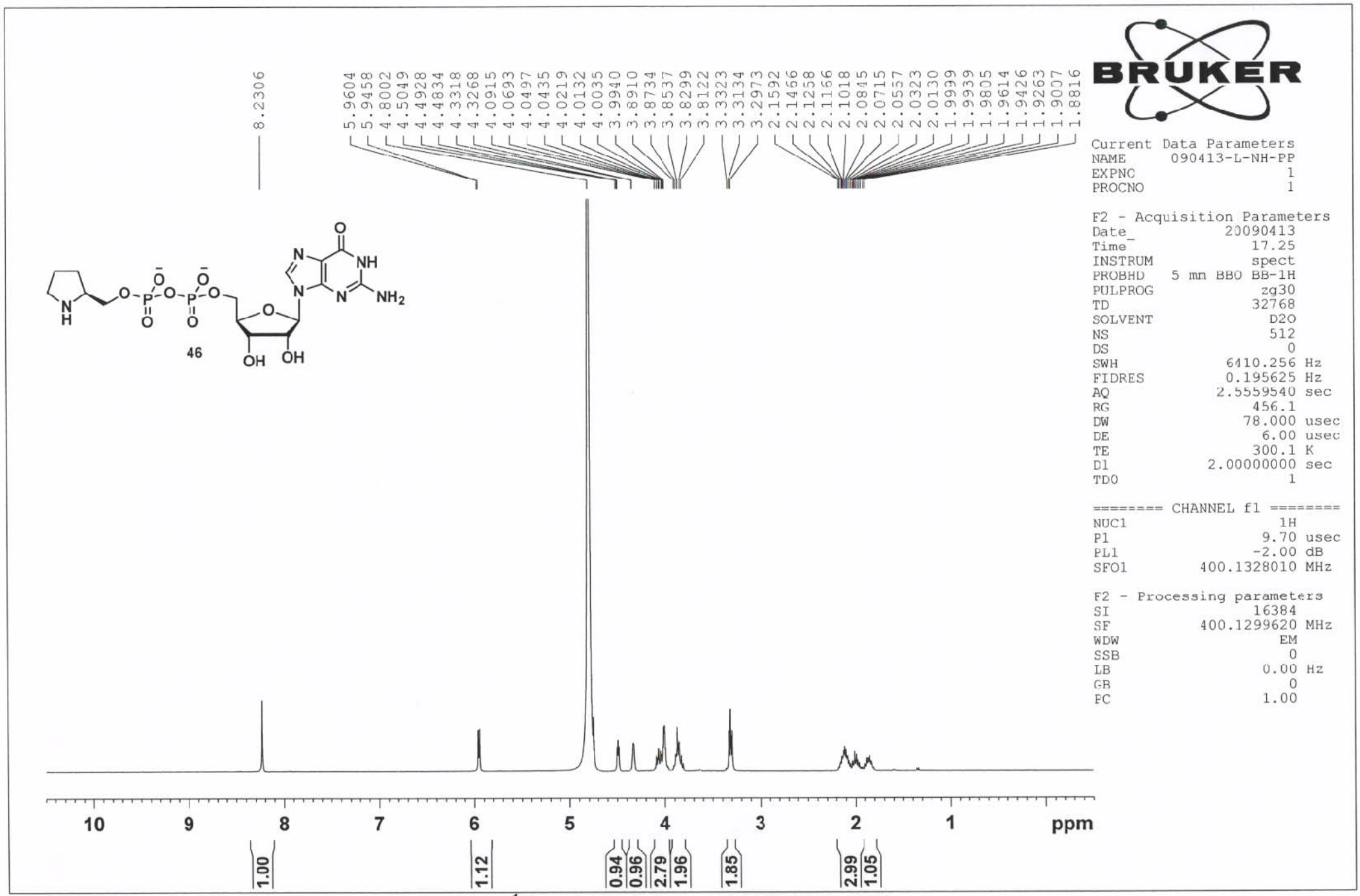
F2 - Acquisition Parameters
Date_ 20090216
Time 17.24
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 16
DS 0
SWH 32467.533 Hz
FIDRES 0.495415 Hz
AQ 1.0093044 sec
RG 14596.5
DW 15.400 usec
DE 6.00 usec
TE 300.3 K
D1 1.50000000 sec
d11 0.03000000 sec
DELTA 1.39999998 sec
TD0 1

======== CHANNEL f1 ========
NUC1 31P
P1 10.30 usec
PL1 1.00 dB
SFO1 161.9755930 MHz

======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 90.00 usec
PL2 -2.00 dB
PL12 15.90 dB
PL13 18.90 dB
SFO2 400.1320007 MHz

F2 - Processing parameters
SI 65536
SF 161.9755259 MHz
WDW EM
SSB 0
LB 3.00 Hz
GB 0
PC 1.00

^{31}P spectrum of compound 44 (400 MHz, D_2O)



BRUKER

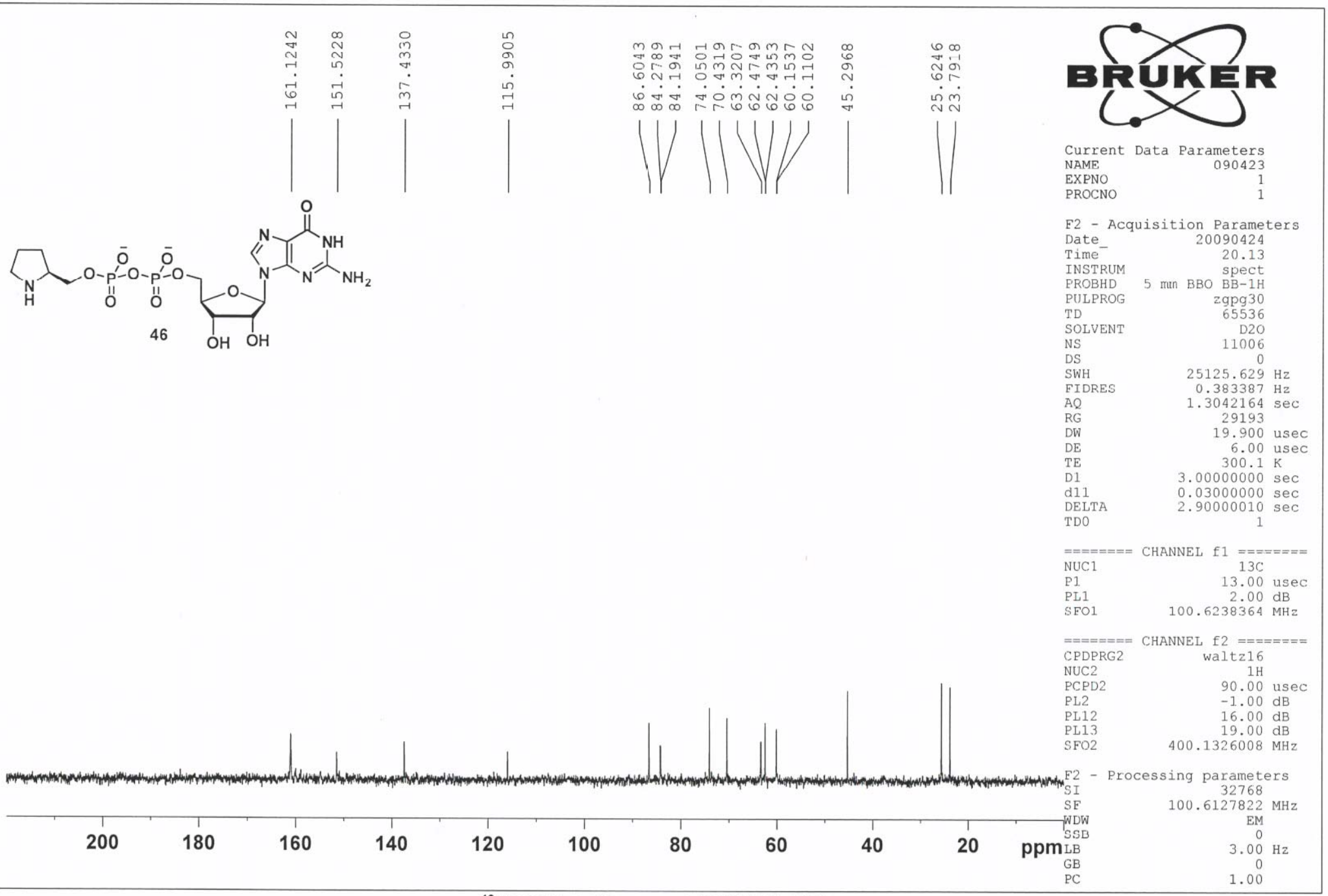
Current Data Parameters
NAME 090413-L-NH-PP
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20090413
Time 17.25
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 32768
SOLVENT D2O
NS 512
DS 0
SWH 6410.256 Hz
FIDRES 0.195625 Hz
AQ 2.5559540 sec
RG 456.1
DW 78.000 usec
DE 6.00 usec
TE 300.1 K
D1 2.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 9.70 usec
PL1 -2.00 dB
SFO1 400.1328010 MHz

F2 - Processing parameters
SI 16384
SF 400.1299620 MHz
WDW EM
SSB 0
LB 0.00 Hz
GB 0
PC 1.00

^{1}H spectrum of compound 46 (400 MHz, D_2O)



BRUKER

Current Data Parameters

NAME	090423
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	20090424
Time	20.13
INSTRUM	spect
PROBHD	5 mm BBO BB-1H
PULPROG	zgpg30
TD	65536
SOLVENT	D2O
NS	11006
DS	0
SWH	25125.629 Hz
FIDRES	0.383387 Hz
AQ	1.3042164 sec
RG	29193
DW	19.900 usec
DE	6.00 usec
TE	300.1 K
D1	3.00000000 sec
d11	0.03000000 sec
DELTA	2.90000010 sec
TD0	1

======== CHANNEL f1 ========

NUC1	13C
P1	13.00 usec
PL1	2.00 dB
SFO1	100.6238364 MHz

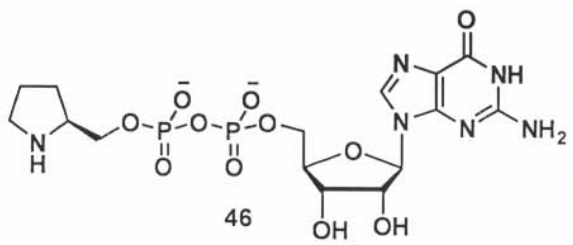
======== CHANNEL f2 ========

CPDPRG2	waltz16
NUC2	1H
PCPD2	90.00 usec
PL2	-1.00 dB
PL12	16.00 dB
PL13	19.00 dB
SFO2	400.1326008 MHz

F2 - Processing parameters

SI	32768
SF	100.6127822 MHz
WDW	EM
SSB	0
LB	3.00 Hz
GB	0
PC	1.00

^{13}C spectrum of compound 46 (400 MHz, D_2O)



46

-10.8219
-10.9467
-11.2019
-11.3193

BRUKER

Current Data Parameters
NAME 090422-L-NH-PP
EXPNO 4
PROCNO 1

F2 - Acquisition Parameters

Date_	20090422
Time	18.18
INSTRUM	spect
PROBHD	5 mm BBO BB-1H
PULPROG	zgpg30
TD	65536
SOLVENT	D2O
NS	32
DS	0
SWH	32467.533 Hz
FIDRES	0.495415 Hz
AQ	1.0093044 sec
RG	23170.5
DW	15.400 usec
DE	6.00 usec
TE	300.1 K
D1	1.50000000 sec
d11	0.03000000 sec
DELTA	1.39999998 sec
TD0	1

======== CHANNEL f1 ========

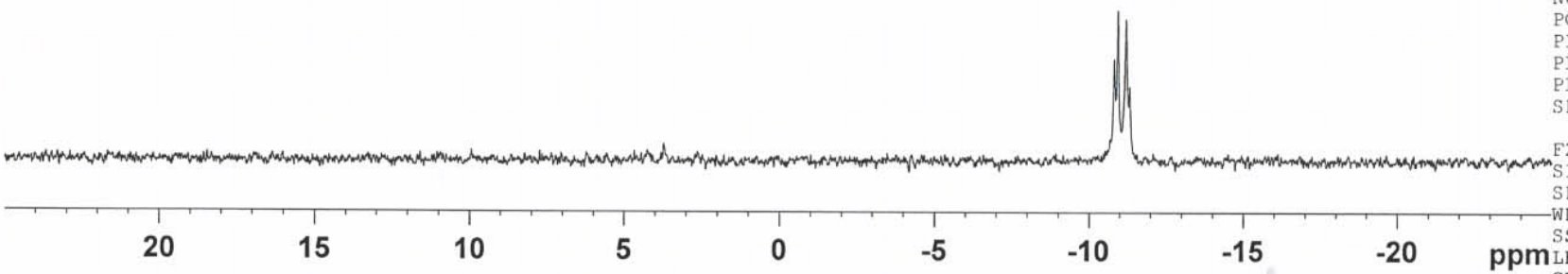
NUC1	31P
P1	10.30 usec
PL1	1.00 dB
SFO1	161.9755930 MHz

======== CHANNEL f2 ========

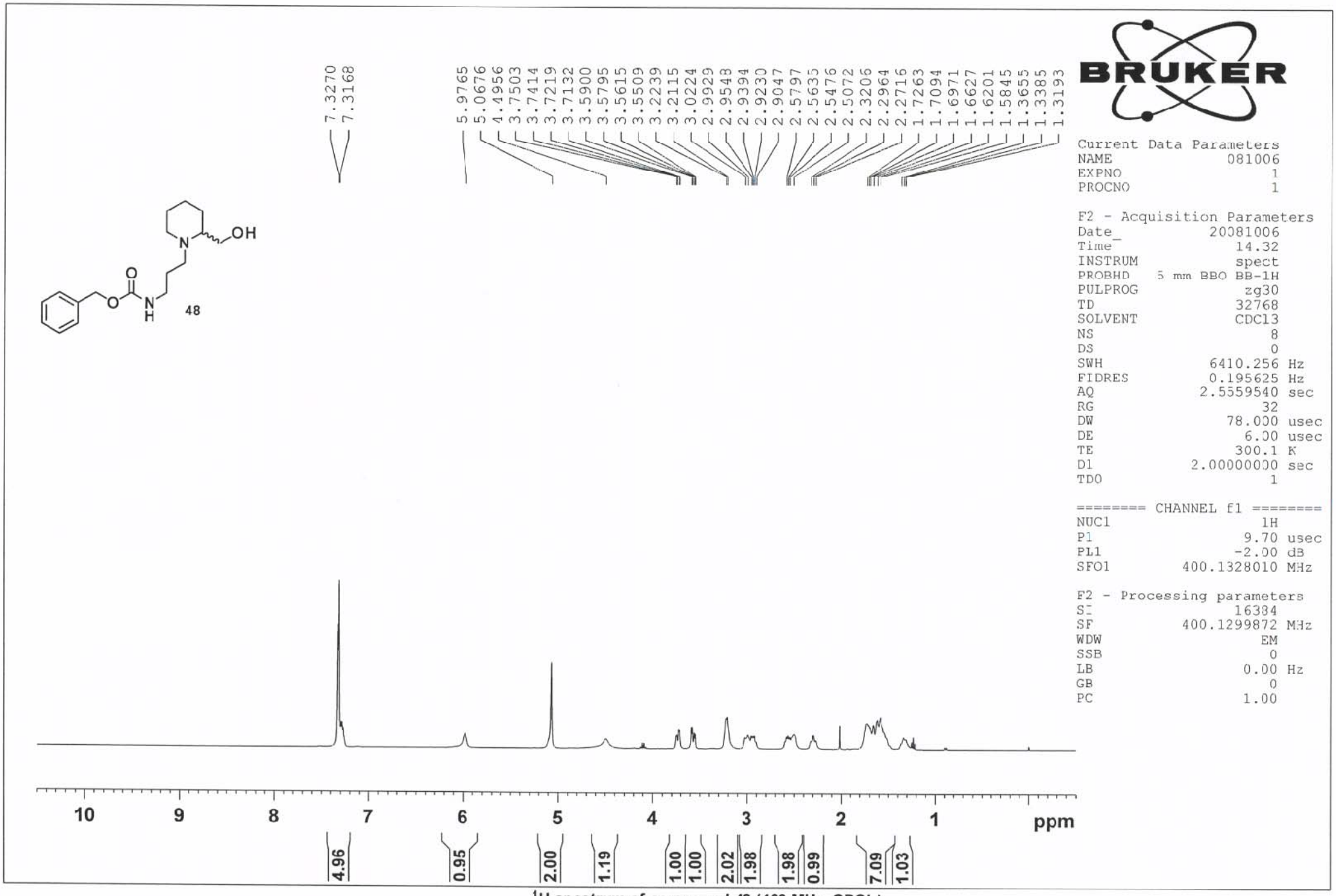
CPDPRG2	waltz16
NUC2	1H
PCPD2	90.00 usec
PL2	-2.00 dB
PL12	15.90 dB
PL13	18.90 dB
SFO2	400.1320007 MHz

F2 - Processing parameters

SI	65536
SF	161.9755259 MHz
WDW	EM
SSB	0
LB	3.00 Hz
GB	0
PC	1.00



^{31}P spectrum of compound 46 (400 MHz, D_2O)



BRUKER

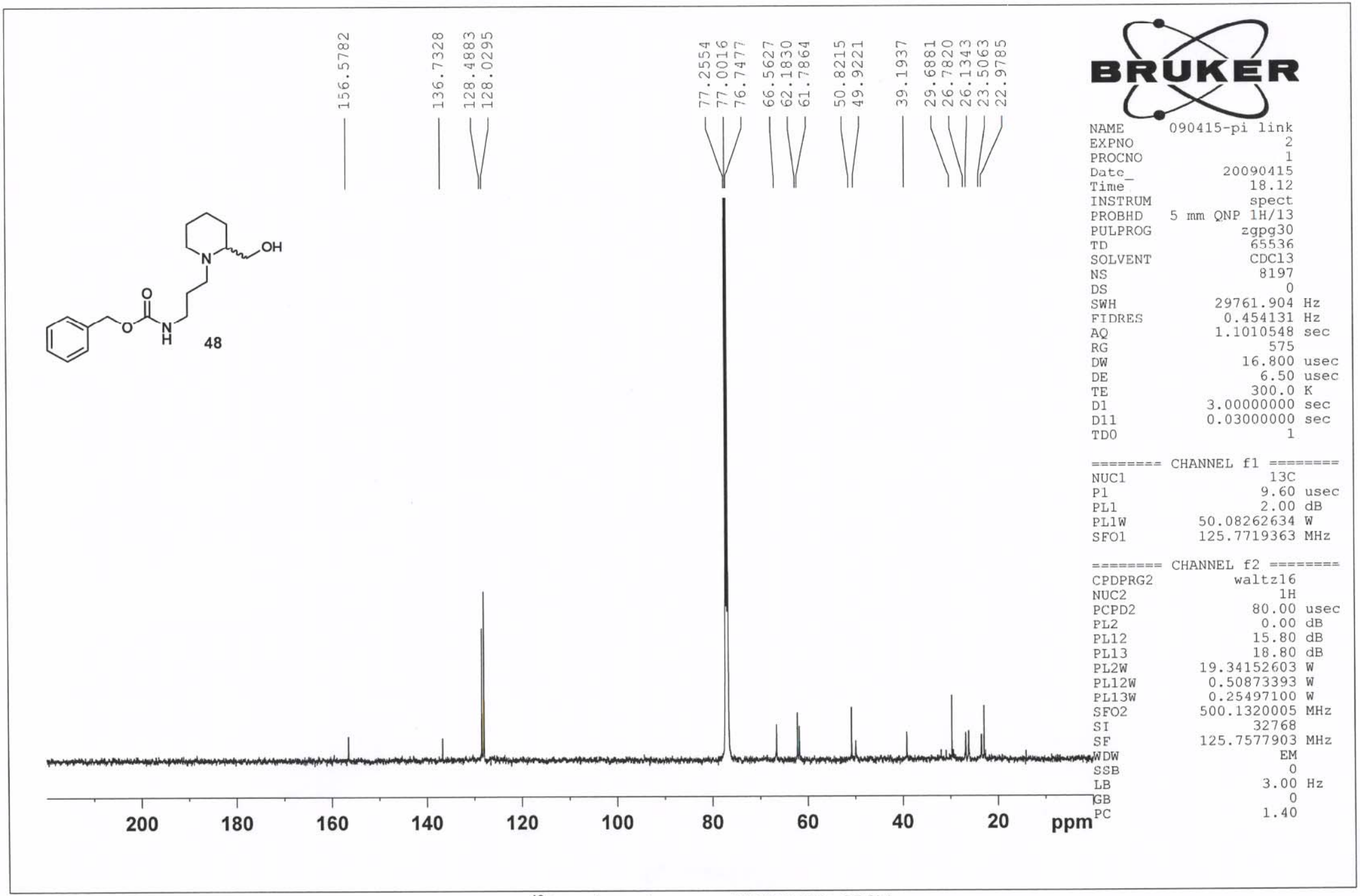
Current Data Parameters
NAME 081006
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20081006
Time 14.32
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 8
DS 0
SWH 6410.256 Hz
FIDRES 0.195625 Hz
AQ 2.5559540 sec
RG 32
DW 78.000 usec
DE 6.00 usec
TE 300.1 K
D1 2.00000000 sec
TD0 1

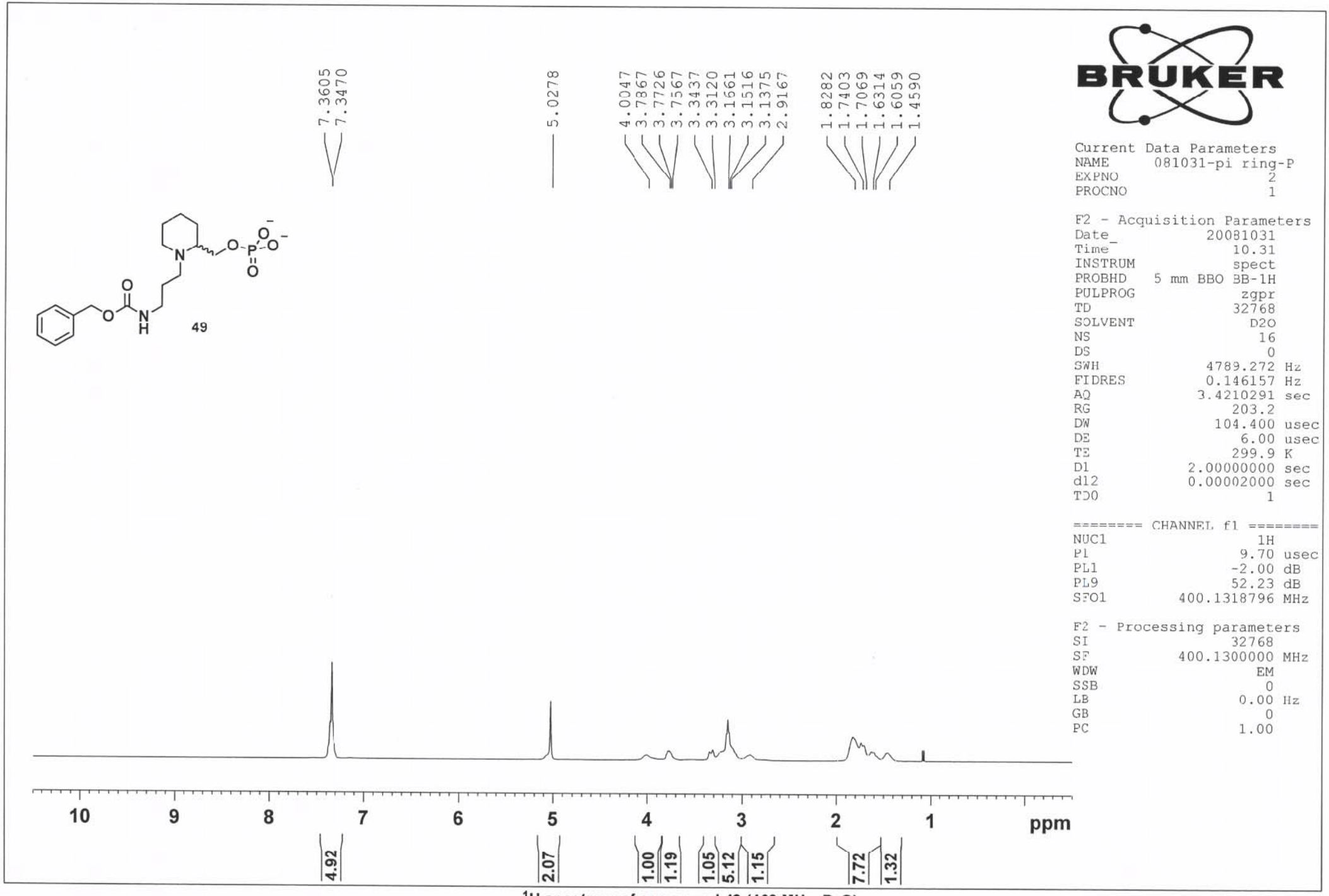
======== CHANNEL f1 ========
NUC1 1H
P1 9.70 usec
PL1 -2.00 dB
SFO1 400.1328010 MHz

F2 - Processing parameters
SI 16384
SF 400.1299872 MHz
WDW EM
SSB 0
LB 0.00 Hz
GB 0
PC 1.00

^{1}H spectrum of compound 48 (400 MHz, $CDCl_3$)



^{13}C spectrum of compound 48 (400 MHz, $CDCl_3$)



BRUKER

Current Data Parameters
NAME 081031-pi ring-P
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters

Date_	20081031
Time	10.31
INSTRUM	spect
PROBHD	5 mm BBO BB-1H
PULPROG	zgpr
TD	32768
SOLVENT	D2O
NS	16
DS	0
SWH	4789.272 Hz
FIDRES	0.146157 Hz
AQ	3.4210291 sec
RG	203.2
DW	104.400 usec
DE	6.00 usec
TE	299.9 K
D1	2.00000000 sec
d12	0.00002000 sec
TD0	1

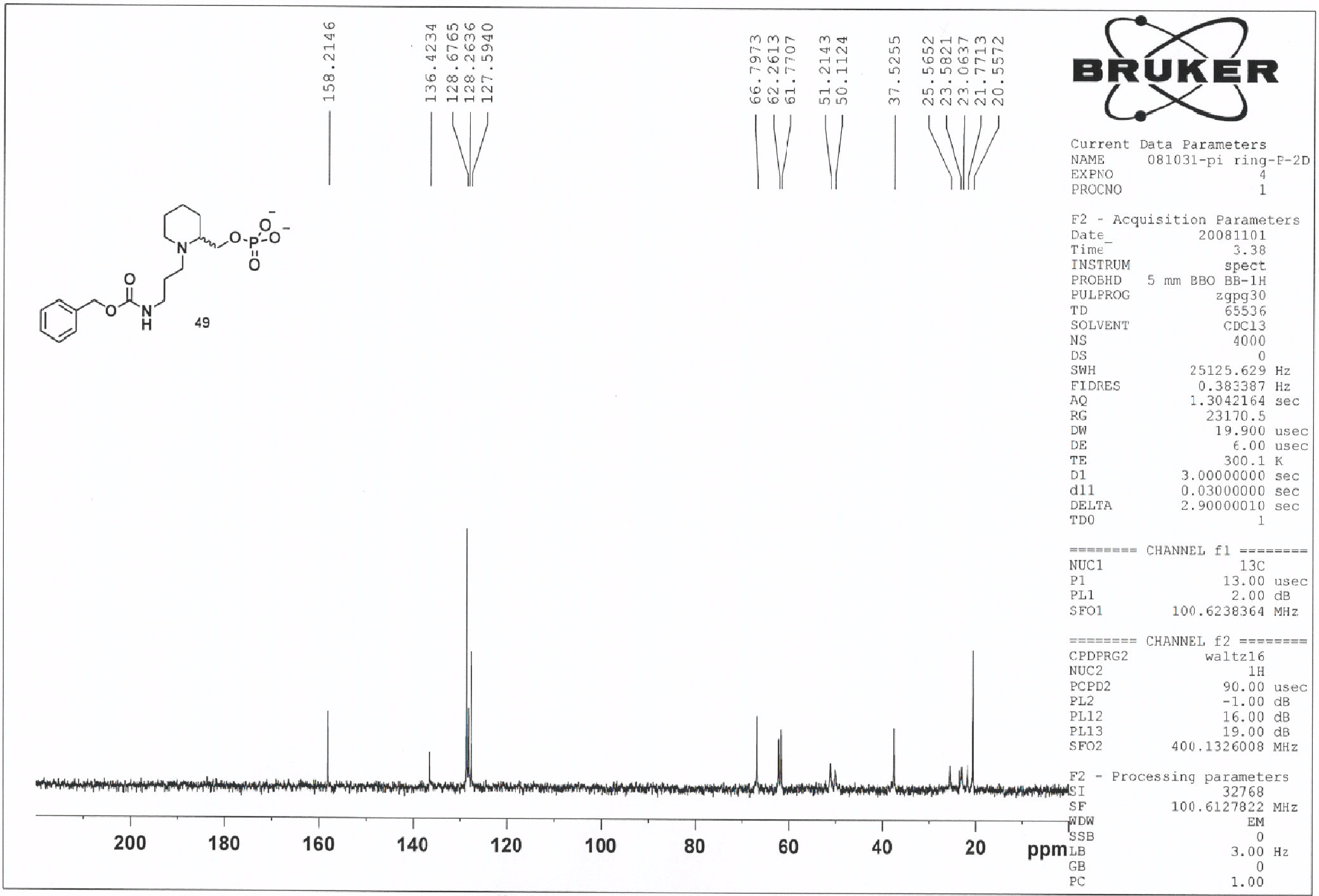
======== CHANNEL f1 ========

NUC1	1H
P1	9.70 usec
PL1	-2.00 dB
PL9	52.23 dB
SFO1	400.1318796 MHz

F2 - Processing parameters

SI	32768
SF	400.1300000 MHz
WDW	EM
SSB	0
LB	0.00 Hz
GB	0
PC	1.00

^{1}H spectrum of compound 49 (400 MHz, D_2O)



BRUKER

Current Data Parameters
NAME 081031-pi ring-P-2D
EXPNO 4
PROCNO 1

F2 - Acquisition Parameters

Date_	20081101
Time	3.38
INSTRUM	spect
PROBHD	5 mm BBO BB-1H
PULPROG	zgpg30
TD	65536
SOLVENT	CDCl3
NS	4000
DS	0
SWH	25125.629 Hz
FIDRES	0.383387 Hz
AQ	1.3042164 sec
RG	23170.5
DW	19.900 usec
DE	6.00 usec
TE	300.1 K
D1	3.00000000 sec
d11	0.03000000 sec
DELTA	2.90000010 sec
TD0	1

======== CHANNEL f1 ========

NUC1	13C
P1	13.00 usec
PL1	2.00 dB
SFO1	100.6238364 MHz

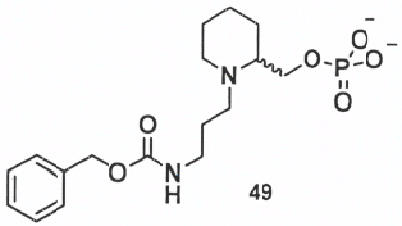
======== CHANNEL f2 ========

CPDPRG2	waltz16
NUC2	1H
PCPD2	90.00 usec
PL2	-1.00 dB
PL12	16.00 dB
PL13	19.00 dB
SFO2	400.1326008 MHz

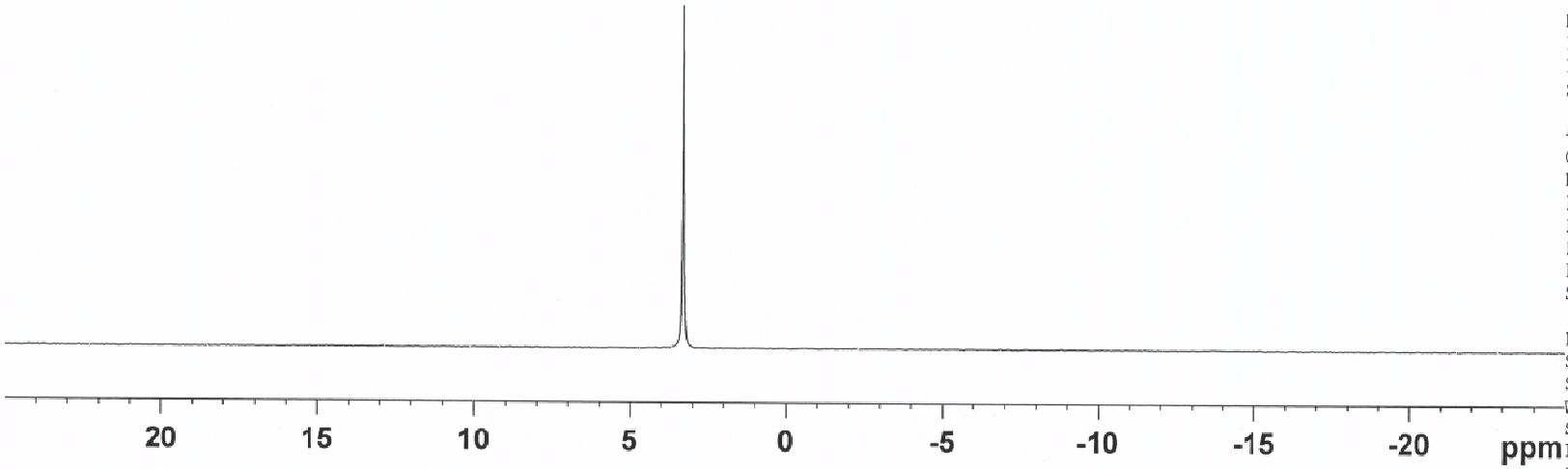
F2 - Processing parameters

SI	32768
SF	100.6127822 MHz
WDW	EM
SSB	0
LB	3.00 Hz
GB	0
PC	1.00

^{13}C spectrum of compound 49 (400 MHz, D_2O)



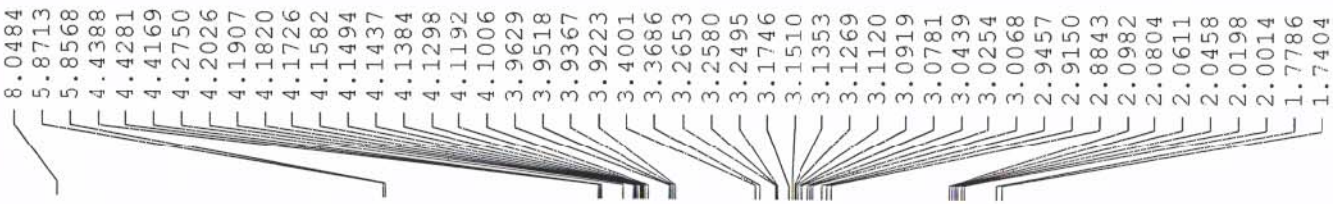
3.3057

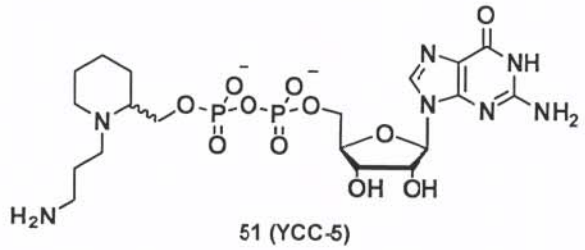


BRUKER

```
Current Data Parameters
NAME      081031-pi ring-P
EXPNO                    4
PROCNO                   1

F2 - Acquisition Parameters
Date_             20081031
Time                 10.44
INSTRUM              spect
PROBHD    5 mm BBO BB-1H
PULPROG           zgpg30
TD                 65536
SOLVENT              DMSO
NS                     140
DS                       0
SWH            32467.533 Hz
FIDRES          0.495415 Hz
AQ              1.0093044 sec
RG                   29193
DW                15.400 usec
DE                 6.00 usec
TE                300.1 K
D1             1.50000000 sec
d11            0.03000000 sec
DELTA          1.39999998 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                   31P
P1                10.30 usec
PL1                1.00 dB
SFO1         161.9755930 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                  1H
PCPD2             90.00 usec
PL2               -2.00 dB
PL12              15.90 dB
PL13              18.90 dB
SFO2         400.1320007 MHz

F2 - Processing parameters
SI                 65536
SF          161.9755259 MHz
WDW                   EM
SSB                    0
LB                 3.00 Hz
GB                     0
PC                  1.00
```

^{31}P spectrum of compound 49 (400 MHz, D_2O)





51 (YCC-5)

Current Data Parameters

NAME	081204-pi ring-PP
EXPNO	2
PROCNO	1

F2 - Acquisition Parameters

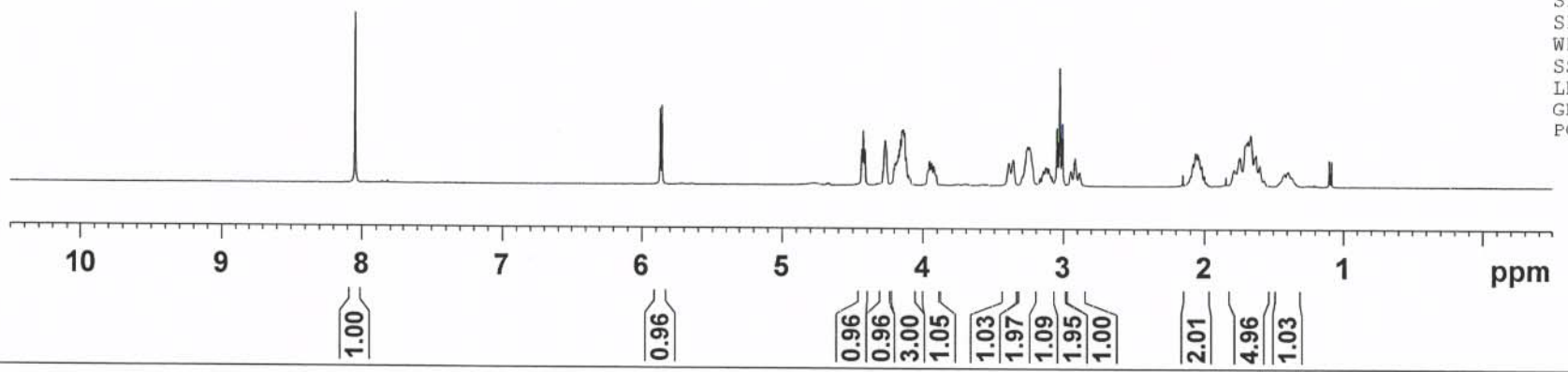
Date_	20081204
Time	10.25
INSTRUM	spect
PROBHD	5 mm BBO BB-1H
PULPROG	zgpr
TD	32768
SOLVENT	D2O
NS	64
DS	0
SWH	4789.272 Hz
FIDRES	0.146157 Hz
AQ	3.4210291 sec
RG	574.7
DW	104.400 usec
DE	6.00 usec
TE	299.9 K
D1	2.00000000 sec
d12	0.00002000 sec
TD0	1

======== CHANNEL f1 ========

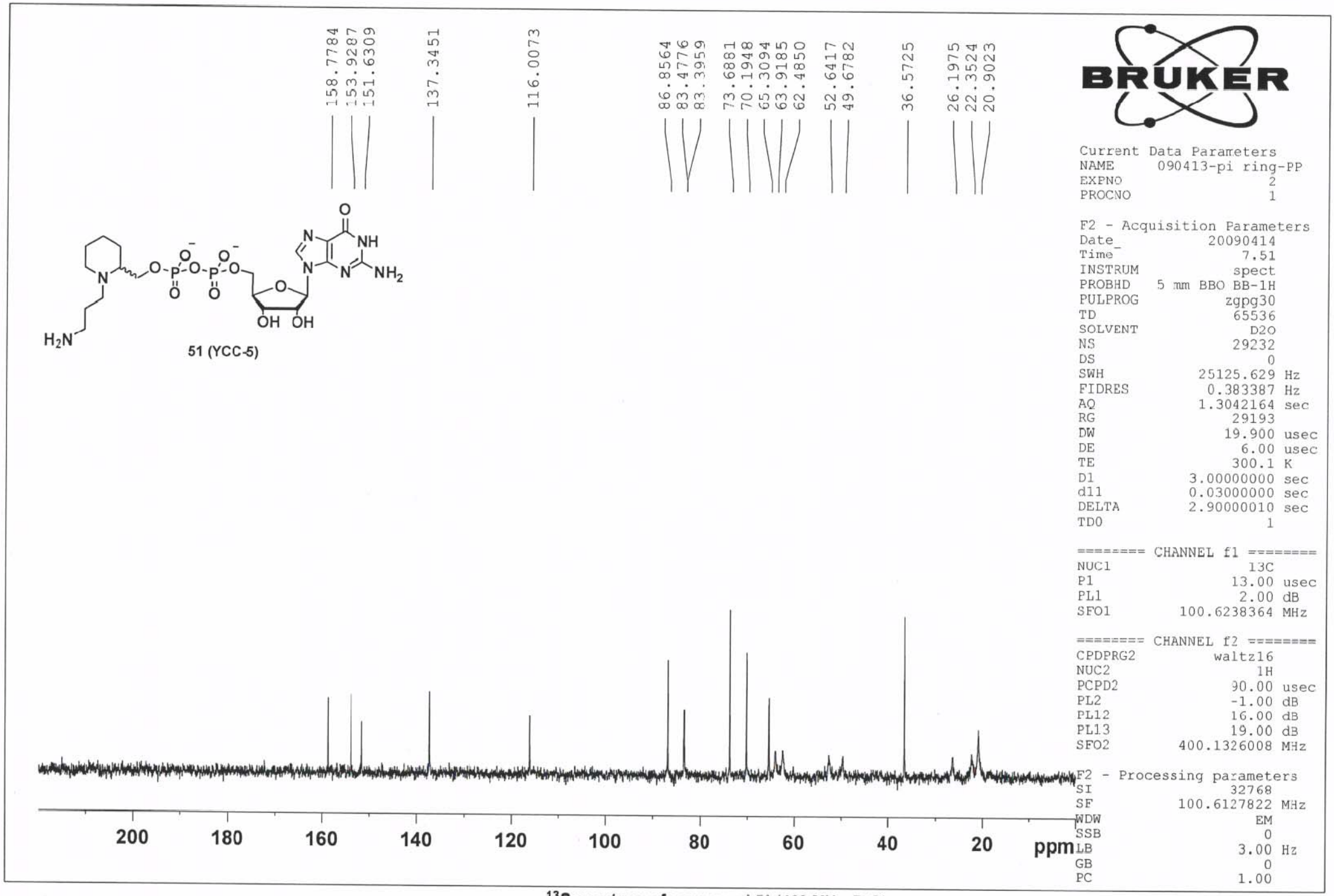
NUC1	1H
P1	9.70 usec
PL1	-2.00 dB
PL9	52.23 dB
SFO1	400.1318808 MHz

F2 - Processing parameters

SI	32768
SF	400.1300000 MHz
WDW	EM
SSB	0
LB	0.00 Hz
GB	0
PC	1.00

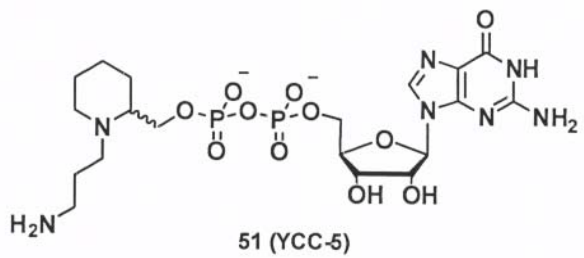


^{1}H spectrum of compound 51 (400 MHz, D_2O)



51 (YCC-5)

^{13}C spectrum of compound 51 (400 MHz, D_2O)



51 (YCC-5)

-10.6918
-10.8214
-11.1802
-11.3105

BRUKER

Current Data Parameters
NAME 081204-pi ring-PP
EXPNO 3
PROCNO 1

F2 - Acquisition Parameters

Date_	20081204
Time	10.37
INSTRUM	spect
PROBHD	5 mm BBO BB-1H
PULPROG	zgpg30
TD	65536
SOLVENT	DMSO
NS	163
DS	0
SWH	32467.533 Hz
FIDRES	0.495415 Hz
AQ	1.0093044 sec
RG	29193
DW	15.400 usec
DE	6.00 usec
TE	300.3 K
D1	1.50000000 sec
d11	0.03000000 sec
DELTA	1.39999998 sec
TD0	1

======== CHANNEL f1 ========

NUC1	31P
P1	10.30 usec
PL1	1.00 dB
SFO1	161.9755930 MHz

======== CHANNEL f2 ========

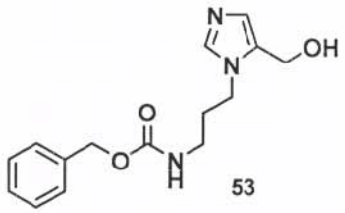
CPDPRG2	waltz16
NUC2	1H
PCPD2	90.00 usec
PL2	-2.00 dB
PL12	15.90 dB
PL13	18.90 dB
SFO2	400.1320007 MHz

F2 - Processing parameters

SI	65536
SF	161.9755259 MHz
WDW	EM
SSB	0
LB	3.00 Hz
GB	0
PC	1.00

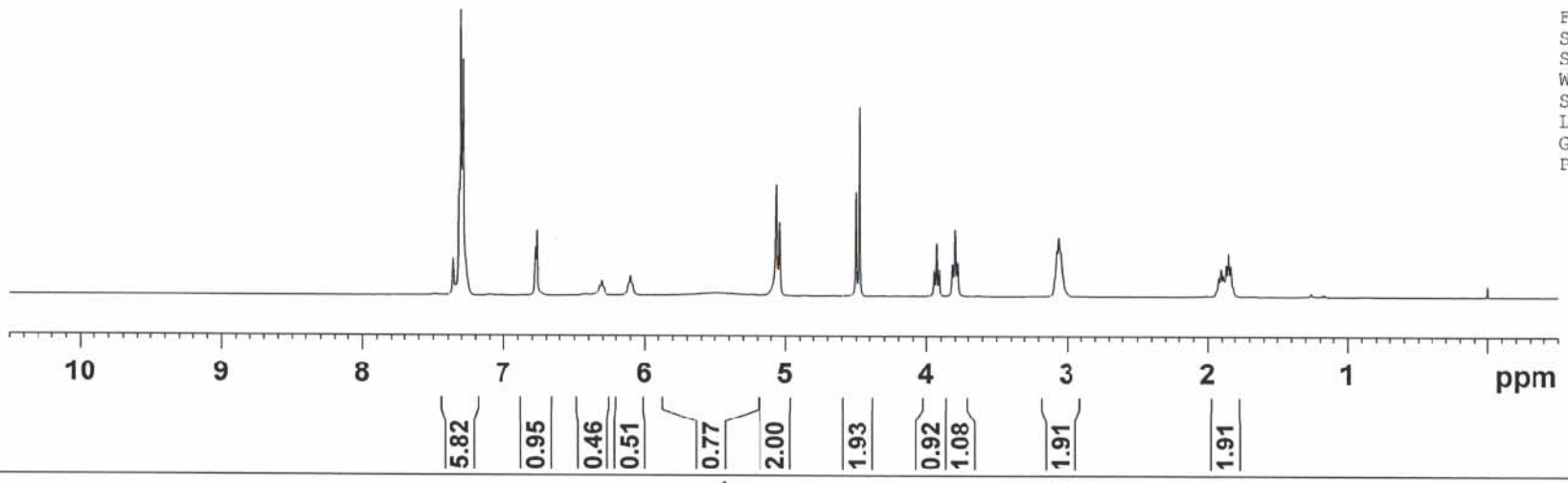
20 15 10 5 0 -5 -10 -15 -20 ppm

^{31}P spectrum of compound 51 (400 MHz, D_2O)



53

7.3555
7.3158
7.3057
7.2885
6.7770
6.7654
6.3070
6.0980
5.0628
5.0409
4.5021
4.4770
3.9451
3.9280
3.9108
3.8169
3.7998
3.7827
3.0731
3.0593
1.9235
1.9074
1.8908
1.8634
1.8467
1.8302



^{1}H spectrum of compound 53 (400 MHz, $CDCl_3$)

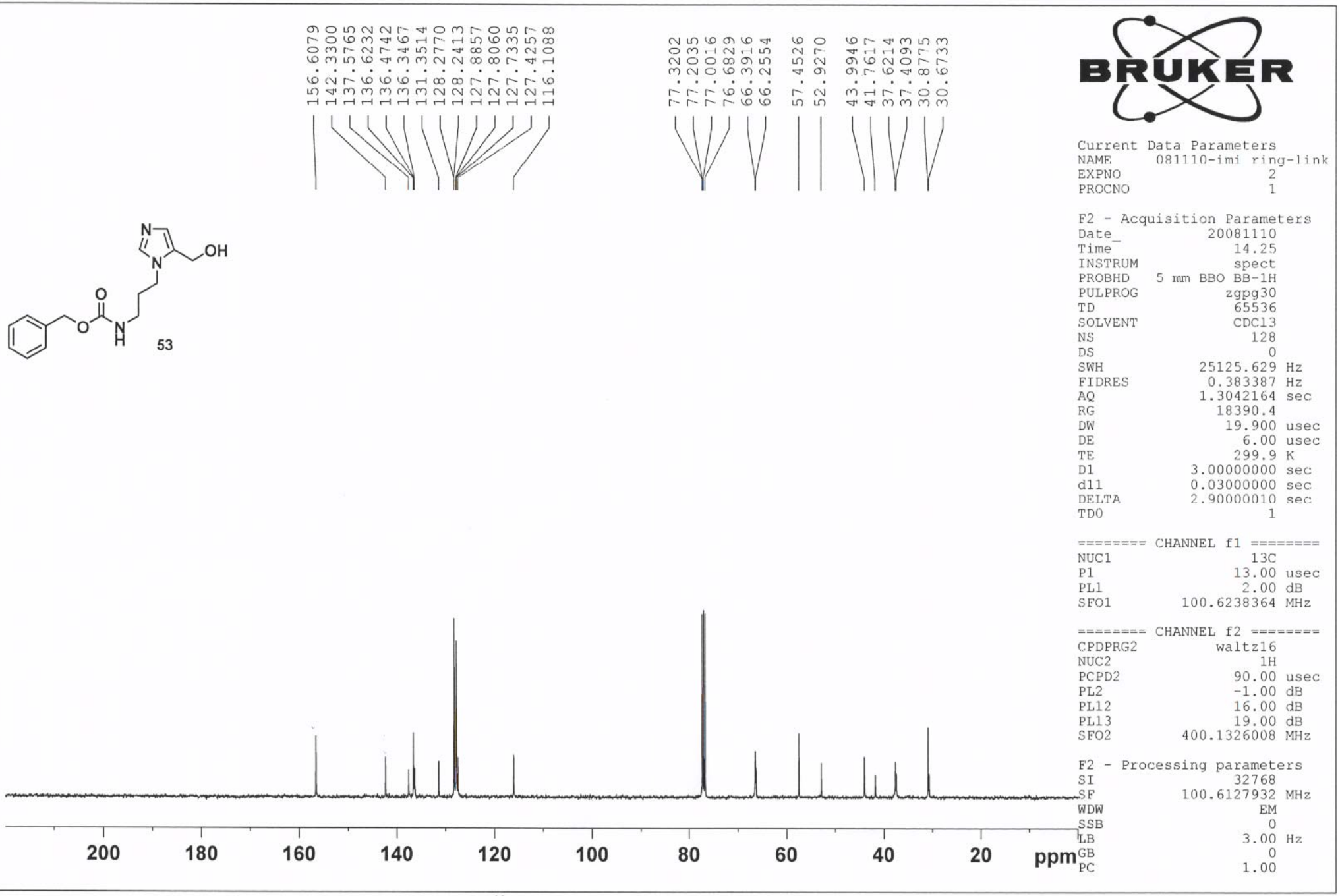
BRUKER

Current Data Parameters
NAME 081110-imi ring-link
EXPNO 1
PROCNO 1

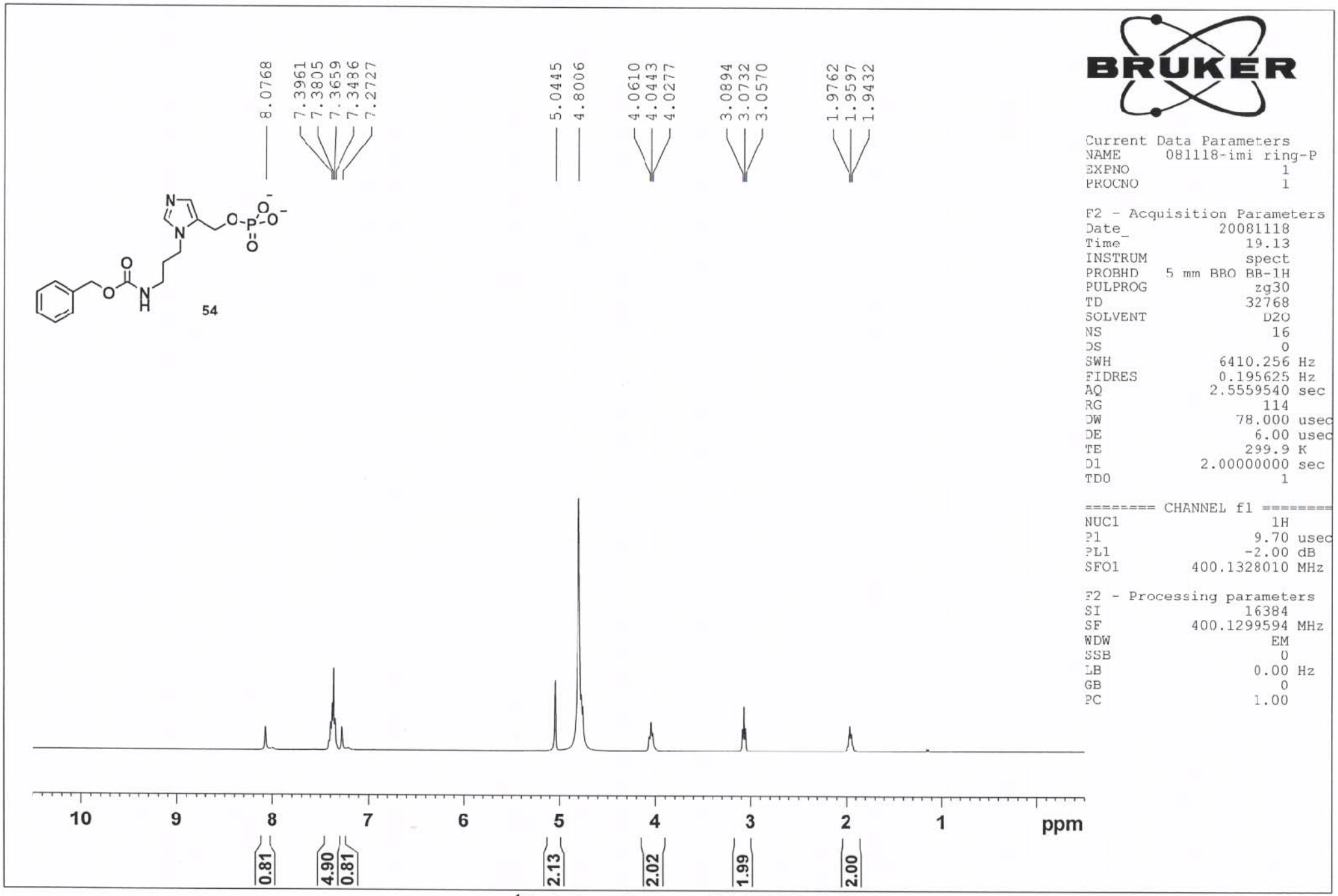
F2 - Acquisition Parameters
Date_ 20081110
Time 14.10
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 8
DS 0
SWH 6410.256 Hz
FIDRES 0.195625 Hz
AQ 2.5559540 sec
RG 32
DW 78.000 usec
DE 6.00 usec
TE 299.9 K
D1 2.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 9.70 usec
PL1 -2.00 dB
SFO1 400.1328010 MHz

F2 - Processing parameters
SI 16384
SF 400.1299991 MHz
WDW EM
SSB 0
LB 0.00 Hz
GB 0
PC 1.00



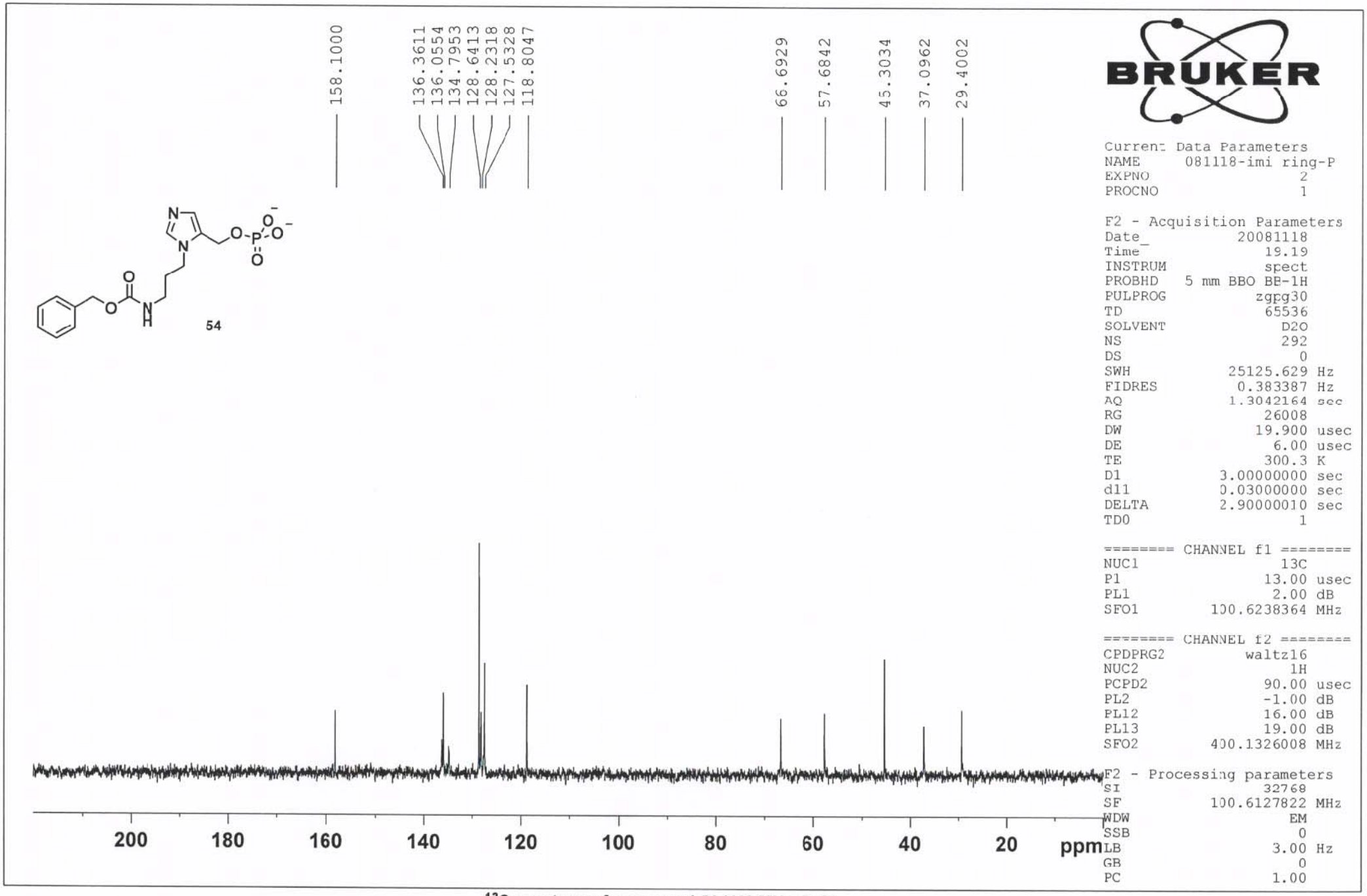
^{13}C spectrum of compound 53 (400 MHz, $CDCl_3$)



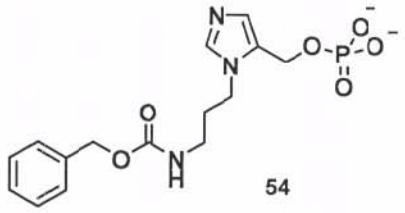
BRUKER

```
Current Data Parameters
NAME      081118-imi ring-P
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_              20081118
Time                 19.13
INSTRUM              spect
PROBHD   5 mm BBO BB-1H
PULPROG               zg30
TD                   32768
SOLVENT                D2O
NS                      16
DS                       0
SWH             6410.256 Hz
FIDRES          0.195625 Hz
AQ              2.5559540 sec
RG                     114
DW                78.000 usec
DE                 6.00 usec
TE                299.9 K
D1             2.00000000 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                    1H
P1                  9.70 usec
PL1                -2.00 dB
SFO1         400.1328010 MHz

F2 - Processing parameters
SI                   16384
SF          400.1299594 MHz
WDW                     EM
SSB                      0
LB                0.00 Hz
GB                       0
PC                1.00
```

^{1}H spectrum of compound 54 (400 MHz, D_2O)



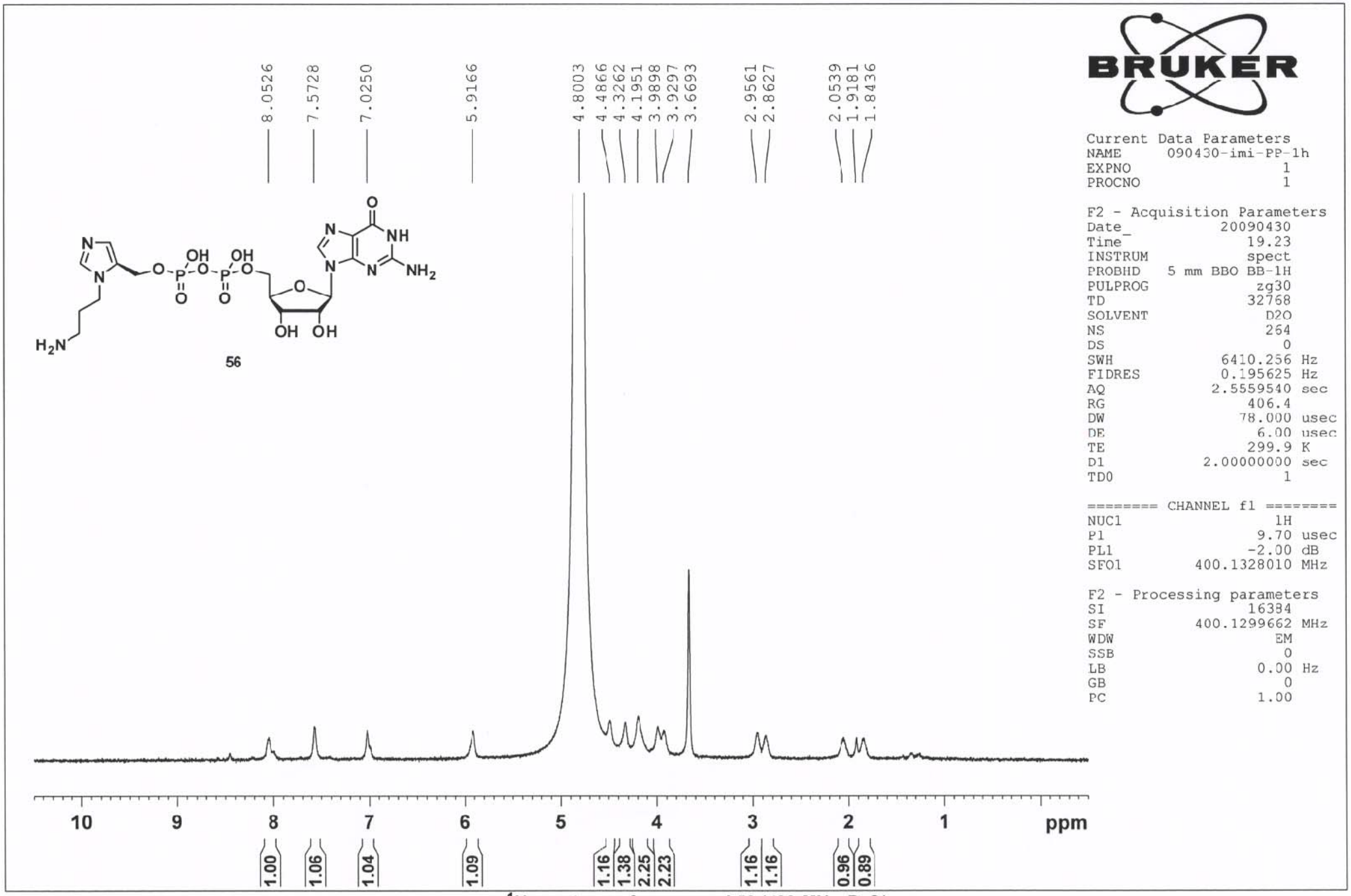
^{13}C spectrum of compound 54 (400 MHz, D_2O)



BRUKER

```
Current Data Parameters
NAME      081118-imi ring-P
EXPNO                    4
PROCNO                   1

F2 - Acquisition Parameters
Date_             20081118
Time                 19.41
INSTRUM              spect
PROBHD   5 mm BBO BB-1H
PULPROG           zgpg30
TD                65536
SOLVENT             DMSO
NS                    41
DS                     0
SWH           32467.533 Hz
FIDRES         0.495415 Hz
AQ            1.0093044 sec
RG              20642.5
DW               15.400 usec
DE                6.00 usec
TE               299.9 K
D1           1.50000000 sec
d11          0.03000000 sec
DELTA        1.39999998 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  31P
P1                 10.30 usec
PL1                 1.00 dB
SFO1         161.9755930 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                  1H
PCPD2              90.00 usec
PL2                -2.00 dB
PL12               15.90 dB
PL13               18.90 dB
SFO2         400.1320007 MHz

F2 - Processing parameters
SI                 65536
SF          161.9755259 MHz
WDW                   EM
SSB                    0
LB                  3.00 Hz
GB                     0
PC                  1.00
```

^{31}P spectrum of compound 54 (400 MHz, D_2O)



BRUKER

Current Data Parameters
NAME 090430-imi-PP-1h
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters

Date_	20090430
Time	19.23
INSTRUM	spect
PROBHD	5 mm BBO BB-1H
PULPROG	zg30
TD	32768
SOLVENT	D2O
NS	264
DS	0
SWH	6410.256 Hz
FIDRES	0.195625 Hz
AQ	2.5559540 sec
RG	406.4
DW	78.000 usec
DE	6.00 usec
TE	299.9 K
D1	2.00000000 sec
TD0	1

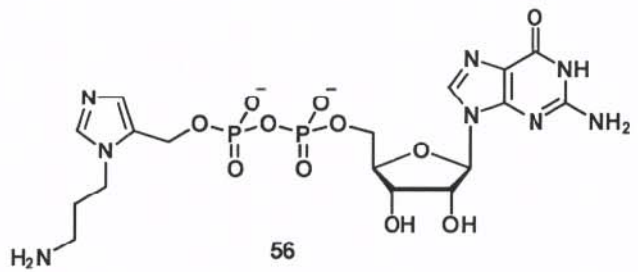
======== CHANNEL f1 ========

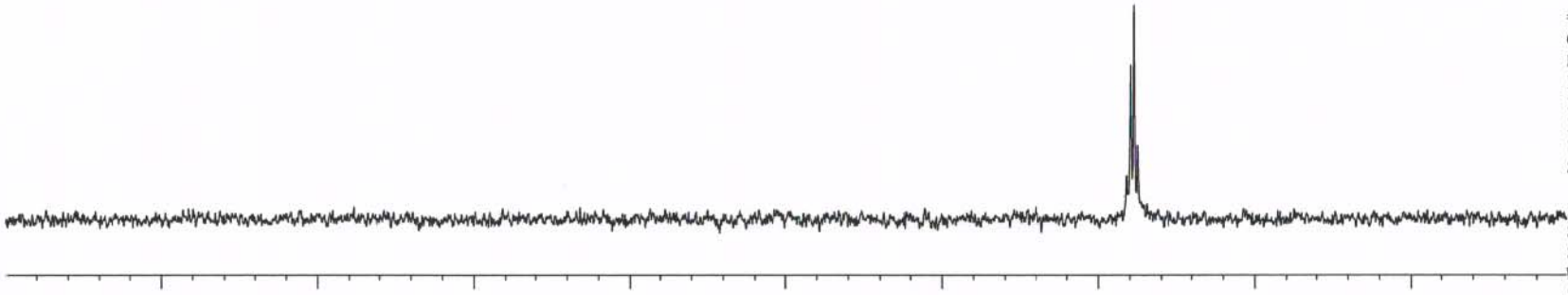
NUC1	1H
P1	9.70 usec
PL1	-2.00 dB
SFO1	400.1328010 MHz

F2 - Processing parameters

SI	16384
SF	400.1299662 MHz
WDW	EM
SSB	0
LB	0.00 Hz
GB	0
PC	1.00

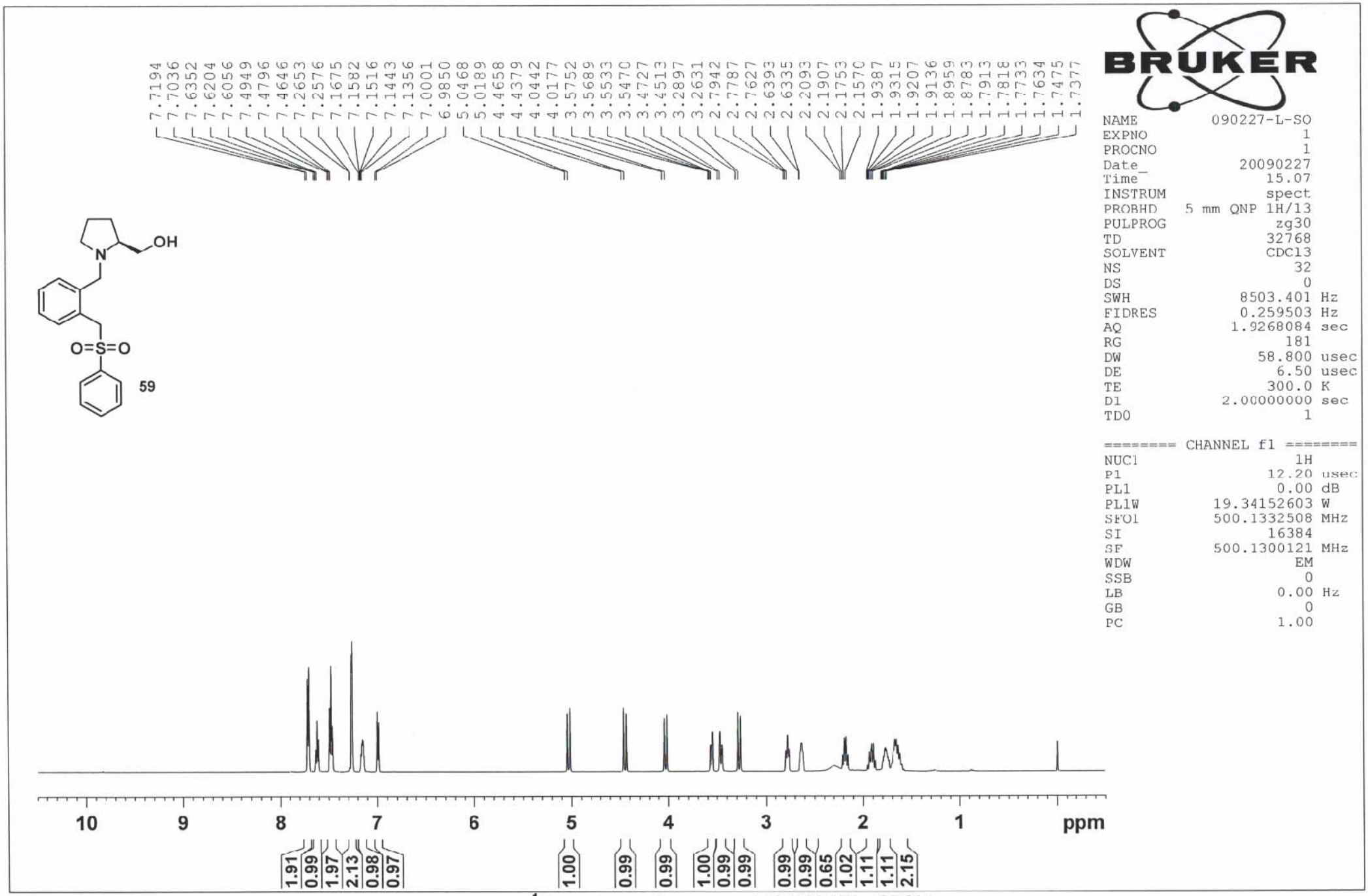
^{1}H spectrum of compound 56 (400 MHz, D_2O)



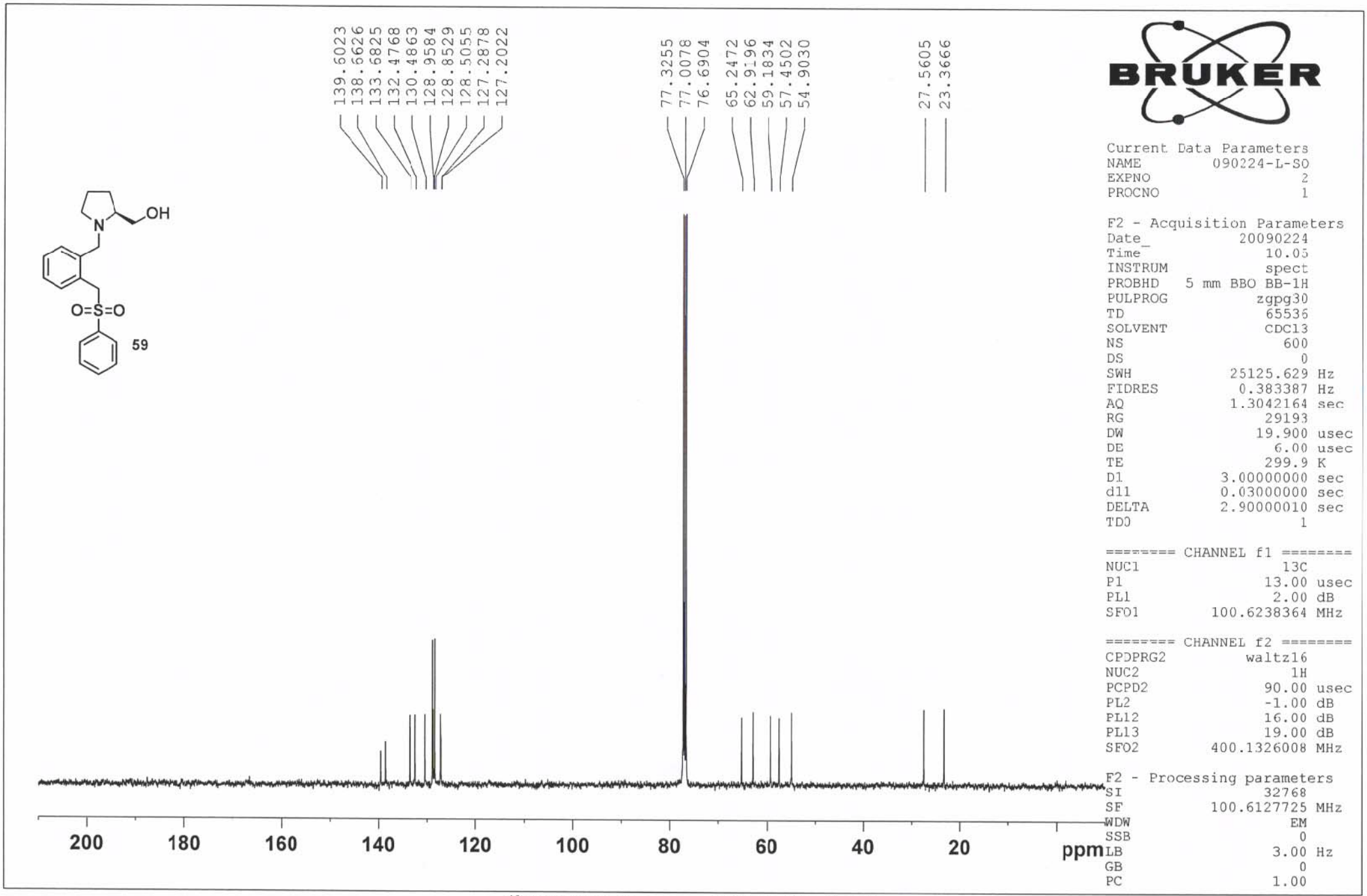



```
Current Data Parameters
NAME      090430-imi-PP-1h
EXPNO                    3
PROCNO                   1

F2 - Acquisition Parameters
Date_             20090406
Time                 12.11
INSTRUM              spect
PROBHD   5 mm BBO BB-1H
PULPROG           zgpg30
TD                65536
SOLVENT             D2O
NS                     32
DS                      0
SWH           32467.533 Hz
FIDRES         0.495415 Hz
AQ            1.0093044 sec
RG                 29193
DW                15.400 usec
DE                 6.00 usec
TE                300.4 K
D1           1.50000000 sec
d11          0.03000000 sec
DELTA        1.39999998 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  31P
P1                10.30 usec
PL1                1.00 dB
SFO1        161.9755930 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                  1H
PCPD2             90.00 usec
PL2               -2.00 dB
PL12              15.90 dB
PL13              18.90 dB
SFO2        400.1320007 MHz

F2 - Processing parameters
SI                 65536
SF          161.9755259 MHz
WDW                   EM
SSB                    0
LB                 3.00 Hz
GB                     0
PC                  1.00
```

^{31}P spectrum of compound 56 (400 MHz, D_2O)



^{1}H spectrum of compound 59 (400 MHz, $CDCl_3$)



BRUKER

Current Data Parameters
NAME 090224-L-SO
EXPNO 2
PROCNO 1

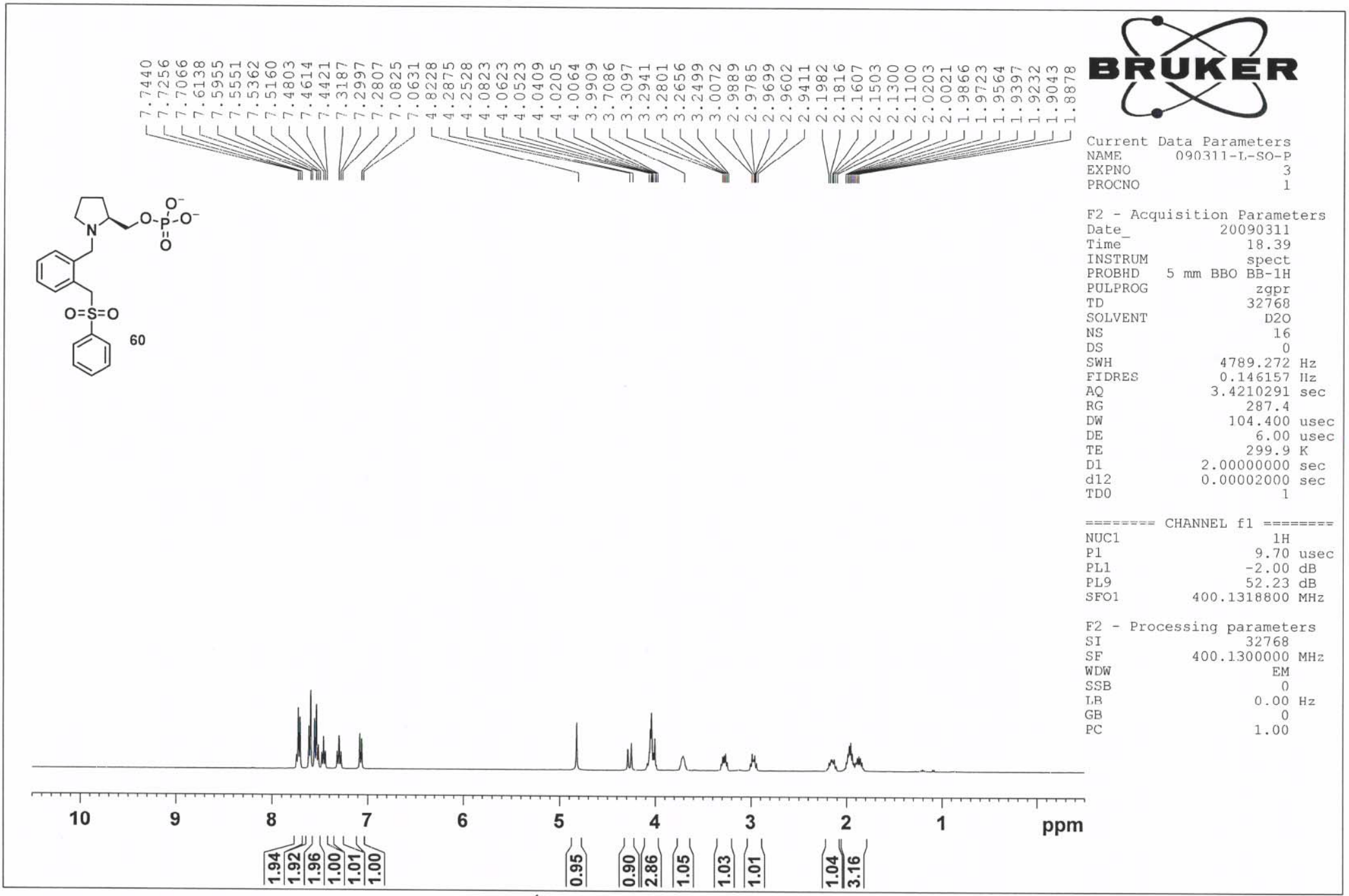
F2 - Acquisition Parameters
Date_ 20090224
Time 10.05
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 600
DS 0
SWH 25125.629 Hz
FIDRES 0.383387 Hz
AQ 1.3042164 sec
RG 29193
DW 19.900 usec
DE 6.00 usec
TE 299.9 K
D1 3.00000000 sec
d11 0.03000000 sec
DELTA 2.90000010 sec
TD0 1

======== CHANNEL f1 ========
NUC1 13C
P1 13.00 usec
PL1 2.00 dB
SFO1 100.6238364 MHz

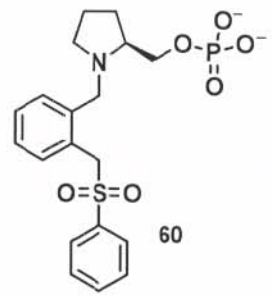
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 90.00 usec
PL2 -1.00 dB
PL12 16.00 dB
PL13 19.00 dB
SFO2 400.1326008 MHz

F2 - Processing parameters
SI 32768
SF 100.6127725 MHz
WDW EM
SSB 0
LB 3.00 Hz
GB 0
PC 1.00

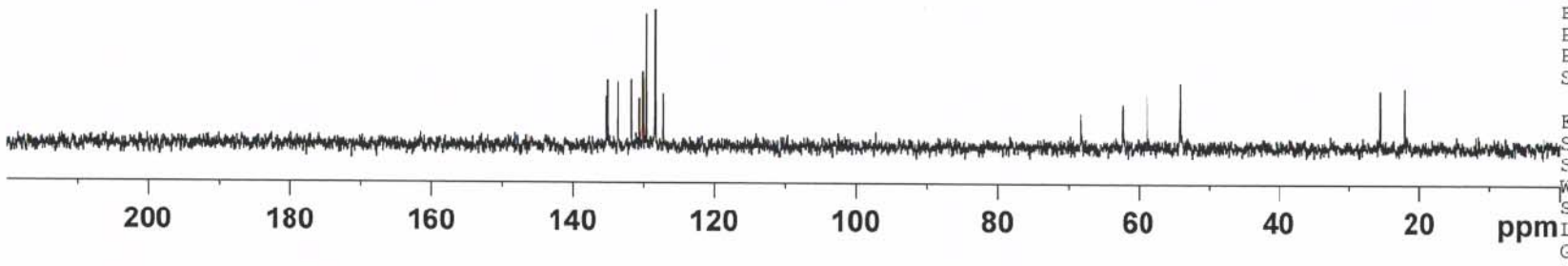
^{13}C spectrum of compound 59 (400 MHz, $CDCl_3$)



^{1}H spectrum of compound 60 (400 MHz, D_2O)



60



135.3707
135.1917
133.6895
131.6738
130.6210
130.1573
130.0819
129.6350
128.3780
127.2916

68.2527
62.2713
58.7953
54.0626

25.5972
22.2163

BRUKER

Current Data Parameters
NAME 090413-L-SO-P
EXPNO 1
PROCNO 1

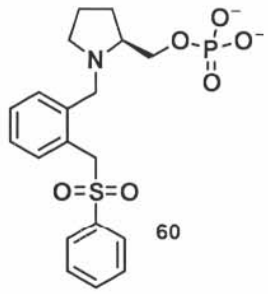
F2 - Acquisition Parameters
Date_ 20090413
Time 21.48
INSTRUM spect
PROBHD 5 mm BBO BB-1H
PULPROG zgpg30
TD 65536
SOLVENT D2O
NS 954
DS 0
SWH 25125.629 Hz
FIDRES 0.383387 Hz
AQ 1.3042164 sec
RG 29193
DW 19.900 usec
DE 6.00 usec
TE 299.9 K
D1 3.00000000 sec
d11 0.03000000 sec
DELTA 2.90000010 sec
TD0 1

======== CHANNEL f1 ========
NUC1 13C
P1 13.00 usec
PL1 2.00 dB
SFO1 100.6238364 MHz

======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 90.00 usec
PL2 -1.00 dB
PL12 16.00 dB
PL13 19.00 dB
SFO2 400.1326008 MHz

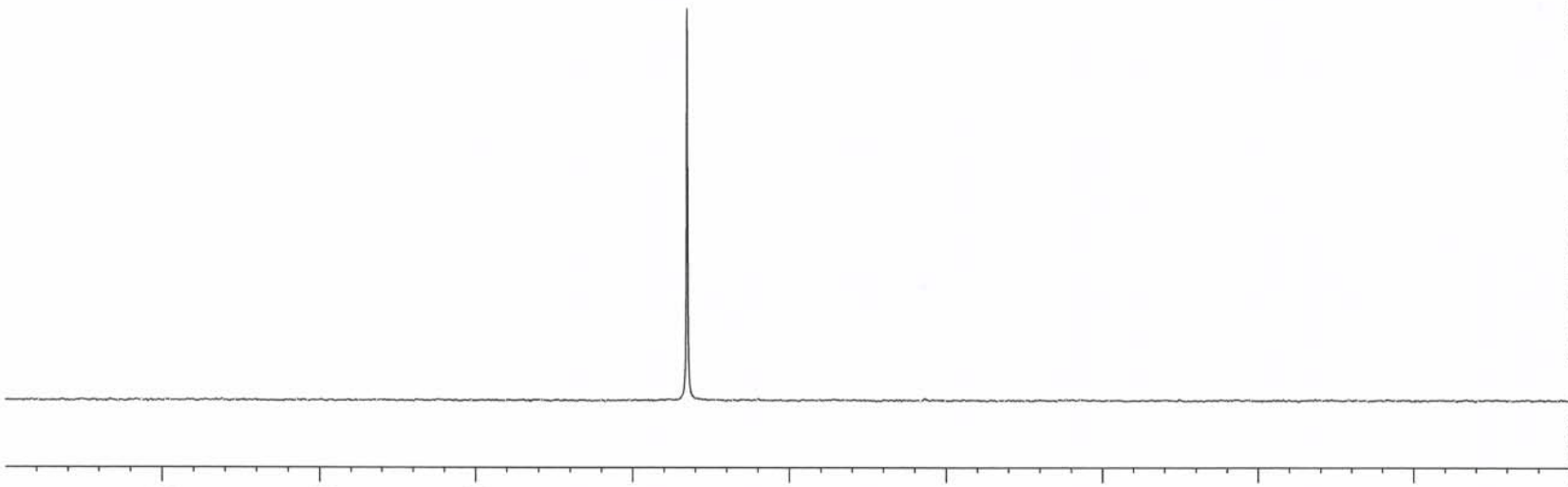
F2 - Processing parameters
SI 32768
SF 100.6127822 MHz
WDW EM
SSB 0
LB 3.00 Hz
GB 0
PC 1.00

^{13}C spectrum of compound 60 (400 MHz, D_2O)



60

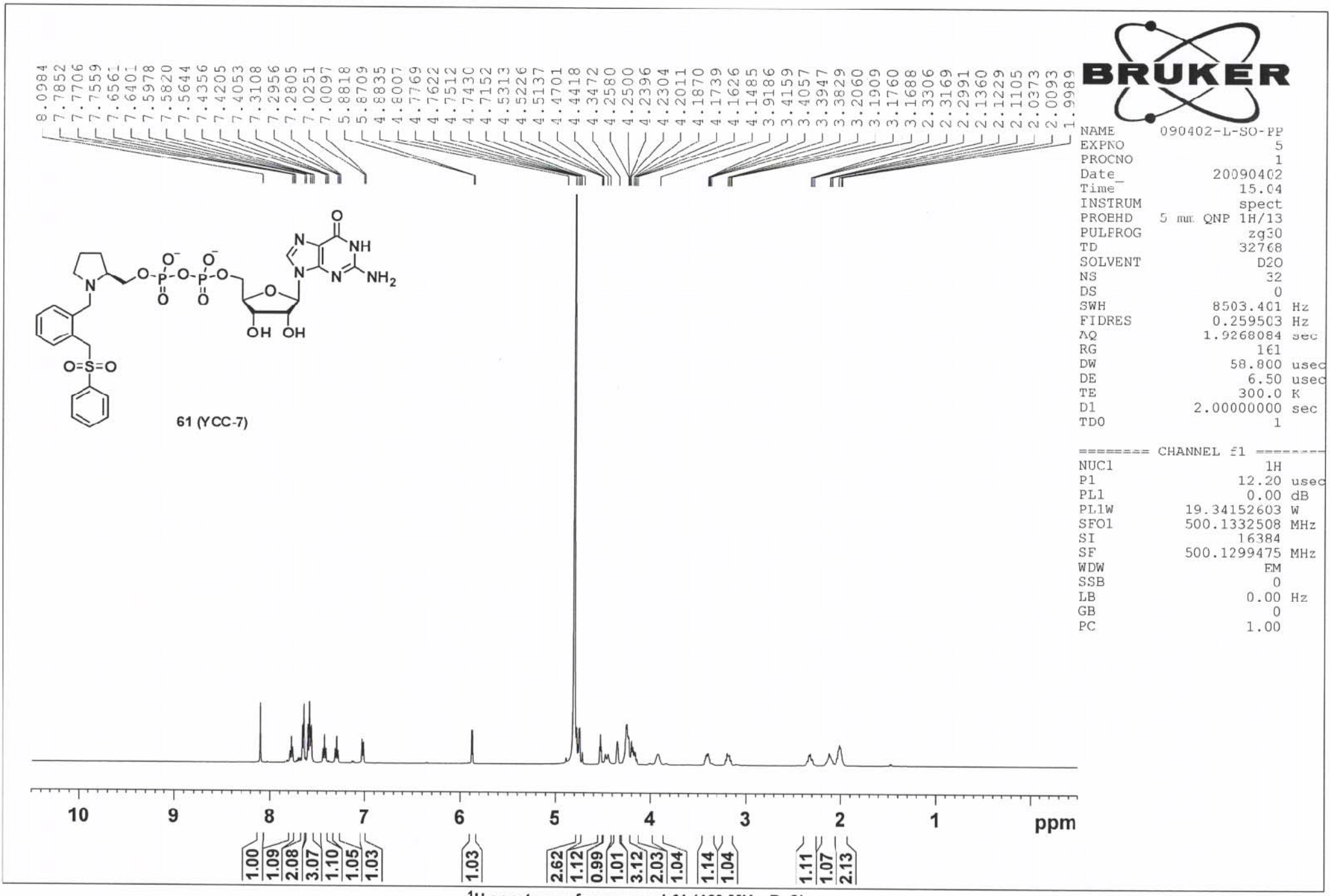
3.2550



BRUKER

```
Current Data Parameters
NAME       090312-L-SO-P
EXPNO                 3
PROCNO                1

F2 - Acquisition Parameters
Date_          20090312
Time              14.20
INSTRUM           spect
PROBHD   5 mm BBO BB-1H
PULPROG         zgpg30
TD               65536
SOLVENT            D2O
NS                  16
DS                   0
SWH          32467.533 Hz
FIDRES        0.495415 Hz
AQ            1.0093044 sec
RG              23170.5
DW             15.400 usec
DE              6.00 usec
TE              300.1 K
D1          1.50000000 sec
d11         0.03000000 sec
DELTA       1.39999998 sec
TD0                  1

======== CHANNEL f1 ========
NUC1                31P
P1             10.30 usec
PL1             1.00 dB
SFO1       161.9755930 MHz

======== CHANNEL f2 ========
CPDPRG2        waltz16
NUC2                1H
PCPD2          90.00 usec
PL2            -2.00 dB
PL12           15.90 dB
PL13           18.90 dB
SFO2       400.1320007 MHz

F2 - Processing parameters
SI                65536
SF         161.9755259 MHz
WDW                  EM
SSB                  0
LB             3.00 Hz
GB                   0
PC                1.00
```

^{31}P spectrum of compound 60 (400 MHz, D_2O)



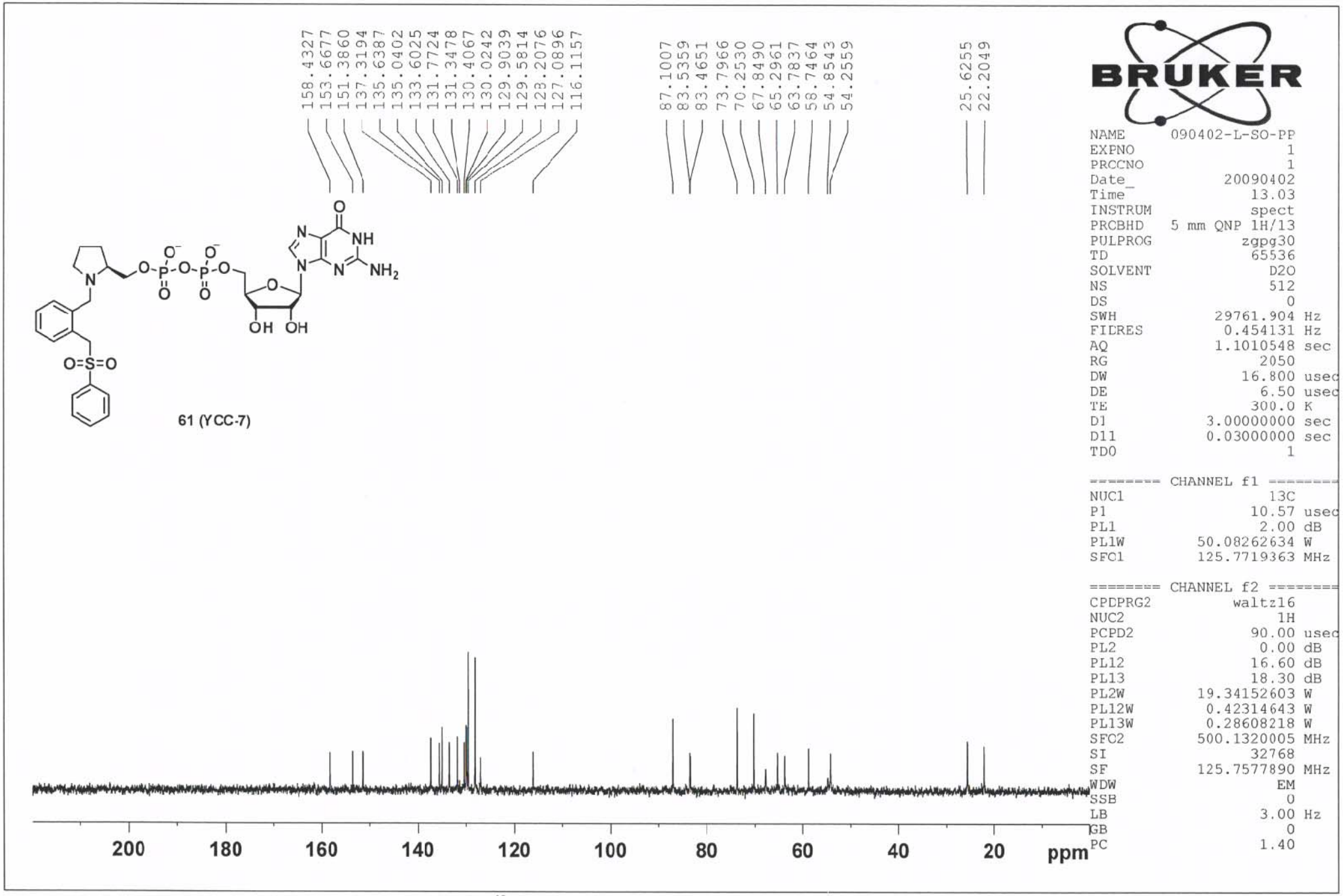
BRUKER

NAME	090402-L-SO-PP
EXPNO	5
PROCNO	1
Date_	20090402
Time_	15.04
INSTRUM	spect
PROBHD	5 mm QNP 1H/13
PULPROG	zg30
TD	32768
SOLVENT	D2O
NS	32
DS	0
SWH	8503.401 Hz
FIDRES	0.259503 Hz
AQ	1.9268084 sec
RG	161
DW	58.800 usec
DE	6.50 usec
TE	300.0 K
D1	2.00000000 sec
TD0	1

======== CHANNEL f1 ========

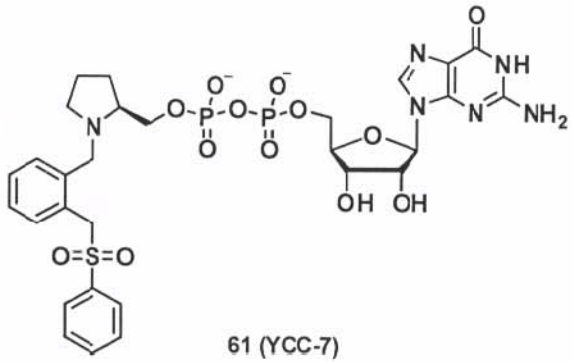
NUC1	1H
P1	12.20 usec
PL1	0.00 dB
PL1W	19.34152603 W
SFO1	500.1332508 MHz
SI	16384
SF	500.1299475 MHz
WDW	EM
SSB	0
LB	0.00 Hz
GB	0
PC	1.00

^{1}H spectrum of compound 61 (400 MHz, D_2O)



NAME	090402-L-SO-PP
EXPNO	1
PROCNO	1
Date_	20090402
Time	13.03
INSTRUM	spect
PROBHD	5 mm QNP 1H/13
PULPROG	zgpg30
TD	65536
SOLVENT	D2O
NS	512
DS	0
SWH	29761.904 Hz
FIDRES	0.454131 Hz
AQ	1.1010548 sec
RG	2050
DW	16.800 usec
DE	6.50 usec
TE	300.0 K
D1	3.00000000 sec
D11	0.03000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	13C
P1	10.57 usec
PL1	2.00 dB
PL1W	50.08262634 W
SFO1	125.7719363 MHz
======== CHANNEL f2 ========	
CPDPRG2	waltz16
NUC2	1H
PCPD2	90.00 usec
PL2	0.00 dB
PL12	16.60 dB
PL13	18.30 dB
PL2W	19.34152603 W
PL12W	0.42314643 W
PL13W	0.28608218 W
SFO2	500.1320005 MHz
SI	32768
SF	125.7577890 MHz
WDW	EM
SSB	0
LB	3.00 Hz
GB	0
PC	1.40

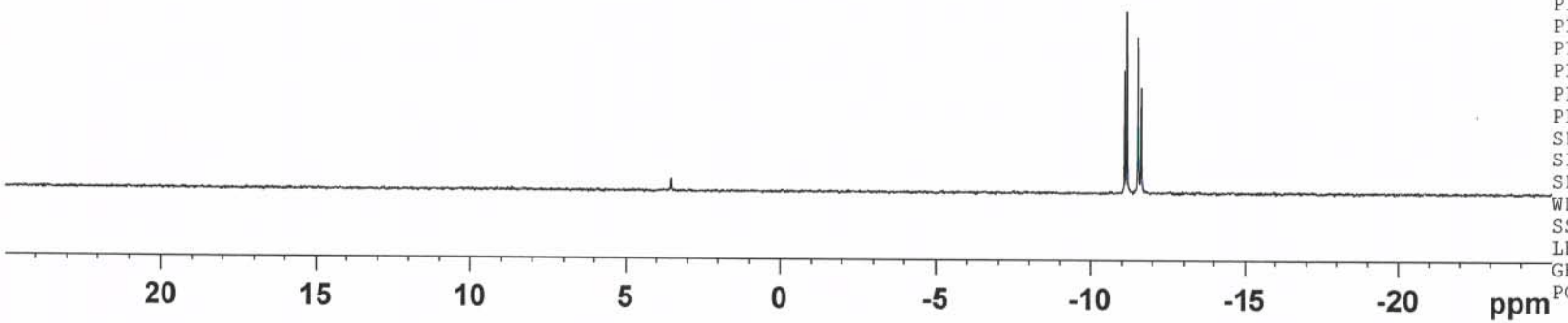
^{13}C spectrum of compound 61 (400 MHz, D_2O)



61 (YCC-7)

3.5305

-11.0825
-11.1839
-11.5545
-11.6574



NAME	090402-L-SO-PP
EXPNO	7
PROCNO	1
Date_	20090402
Time	15.16
INSTRUM	spect
PROBHD	5 mm QNP 1H/13
PULPROG	zgpg30
TD	65536
SOLVENT	D2O
NS	10
DS	0
SWH	51020.406 Hz
FIDRES	0.778510 Hz
AQ	0.6423028 sec
RG	2050
DW	9.800 usec
DE	6.50 usec
TE	300.0 K
D1	2.00000000 sec
D11	0.03000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	31P
P1	9.90 usec
PL1	6.00 dB
PL1W	21.45254898 W
SFO1	202.4563350 MHz
======== CHANNEL f2 ========	
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	0.00 dB
PL12	15.80 dB
PL13	18.80 dB
PL2W	19.34152603 W
PL12W	0.50873393 W
PL13W	0.25497100 W
SFO2	500.1320005 MHz
SI	32768
SF	202.4563350 MHz
WDW	EM
SSB	0
LB	1.00 Hz
GB	0
PC	1.40

^{31}P spectrum of compound 61 (400 MHz, D_2O)