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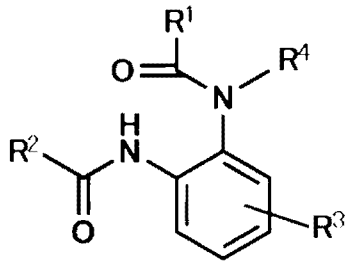
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(54)名稱

化合物、化合物之鹽、抗體修飾試劑、修飾抗體之製造方法及修飾抗體

(57)摘要

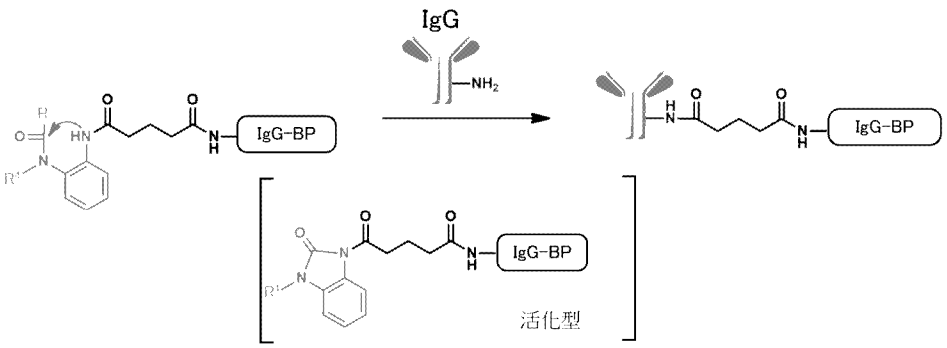
一種化合物或其鹽，該化合物由下述式 I 表示。



式I

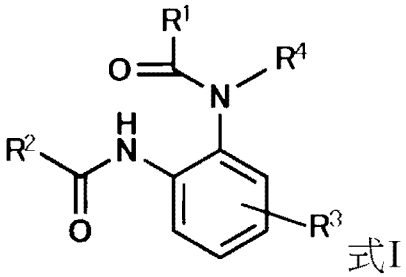
(式 I 中，R^1 係 R^1-H 之 pKa 達到 4 ～ 14 之取代基，R^2 及 R^3 之一者具有與 IgG 之 Fc 區特異性結合之 IgG 結合肽，R^4 係 H 或者經取代或未經取代之碳數 1 ～ 8 之烷基)

指定代表圖：



【圖1】

特徵化學式：



式I

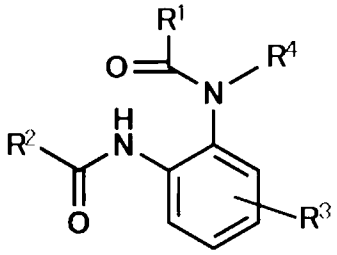
【發明摘要】

【中文發明名稱】

化合物、化合物之鹽、抗體修飾試劑、修飾抗體之製造方法及修飾抗體

【中文】

一種化合物或其鹽，該化合物由下述式I表示。



式I

(式I中，R^1係R^1-H之pKa達到4～14之取代基，R^2及R^3之一者具有與IgG之Fc區特異性結合之IgG結合肽，R^4係H或者經取代或未經取代之碳數1～8之烷基)

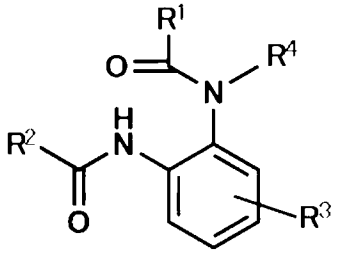
【指定代表圖】

圖1

【代表圖之符號簡單說明】

無

【特徵化學式】



式I

【發明說明書】

【中文發明名稱】

化合物、化合物之鹽、抗體修飾試劑、修飾抗體之製造方法及修飾抗體

【技術領域】

【0001】

本發明係關於一種化合物、化合物之鹽、抗體修飾試劑、修飾抗體之製造方法及修飾抗體。

【先前技術】

【0002】

業界正在開發藉由添加藥物而使功能得到增強之抗體醫藥、例如抗體藥物複合體(ADC)等。於修飾抗體時使用胺基之隨機修飾法及鉸鏈部位之硫醇修飾法等各種方法。

【0003】

專利文獻1至3中揭示有一種CCAP(Chemical Conjugation by Affinity Peptide，親和肽化學偶聯)法，其係用以製備抗體與藥物等之偶聯物(複合體)之部位特異性修飾法。

【0004】

CCAP法中，使具有與IgG之Fc區特異性結合之IgG結合肽(IgG-BP)之抗體修飾試劑與抗體進行反應。IgG-BP之規定之胺基酸殘基被N,N'-二琥珀醯亞胺戊二酸酯(disuccinimidyl glutarate；DSG)或3,3'-二硫代二丙酸二(N-琥珀醯亞胺)酯(dithiobis(succinimidyl propionate)；DSP)修飾，具有N-羥基琥珀醯亞胺(NHS)基。於抗體修飾試劑與抗體之反應中，IgG-

BP經由NHS基結合於Fc區之特定區域。

【0005】

CCAP法中，在使抗體修飾試劑與抗體反應時，IgG-BP會殘存於修飾抗體中，因此在應用於醫藥品等時，體內投予後殘存之肽之抗原性成為問題。對此，專利文獻4中揭示有一種IgG-BP不會殘留於最終產物中之CCAP法。

[先前技術文獻]

[專利文獻]

【0006】

[專利文獻1]國際公開第2016/186206號

[專利文獻2]國際公開第2017/217347號

[專利文獻3]國際公開第2018/230257號

[專利文獻4]國際公開第2018/199337號

【發明內容】

[發明所欲解決之問題]

【0007】

專利文獻1至4中所揭示之CCAP法中所使用之抗體修飾試劑之NHS基容易被水解而不穩定，不耐長期保存。為了將CCAP法應用於產業上，而需要提昇抗體修飾試劑之穩定性。

【0008】

本發明係鑒於上述實際情況而完成者，其目的在於提供一種化學上穩定且可長期保存之化合物、化合物之鹽、抗體修飾試劑、及使用該抗體修飾試劑之修飾抗體之製造方法。進而，本發明之目的在於提供一種新穎

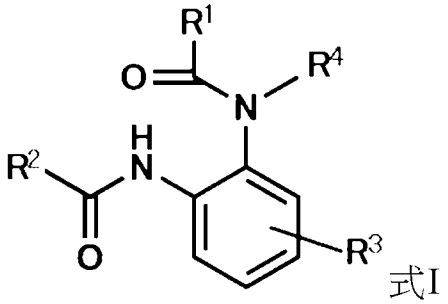
之修飾抗體。

[解決問題之技術手段]

【0009】

本發明之第1觀點之化合物由下述式I表示。

[化1]



式I

(式I中，R^1係R^1-H之pKa達到4～14之取代基，

R^2及R^3之一者具有與IgG之Fc區特異性結合之IgG結合肽，

於R^2具有上述IgG結合肽之情形時，R^3不存在，或者選自由經取代或未經取代之碳數1～8之烷基、經取代或未經取代之碳數2～8之烯基、經取代或未經取代之碳數2～8之炔基、硝基、鹵素、羧醯胺基所組成之群，

於R^3具有上述IgG結合肽之情形時，R^2為選自由經取代或未經取代之碳數1～8之烷基、經取代或未經取代之碳數2～8之烯基、經取代或未經取代之碳數2～8之炔基、胺基酸殘基數2～50之經取代或未經取代之肽鏈、聚合度2～50之經取代或未經取代之聚合物鏈及其等之組合所組成之群，

R^4為H或者經取代或未經取代之碳數1～8之烷基)。

【0010】

本發明之第2觀點之化合物之鹽係上述本發明之第1觀點之化合物之鹽。

[0011]

R^1可設為硝基苯氧基。

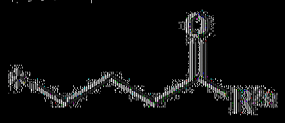
[0012]

R^2可具有上述IgG結合肽，R^3可不存在。

[0013]

R^2可以上述IgG結合肽作為R^{2a}，由下述式II表示。

[化2]



式II

(式II中，*表示式I之羰基之碳)

[0014]

於R^2具有上述IgG結合肽之情形時，IgG結合肽可經藥物、反應性官能基或標識物質修飾。

[0015]

R^3可具有上述IgG結合肽，R^2可具有藥物、反應性官能基或標識物質。

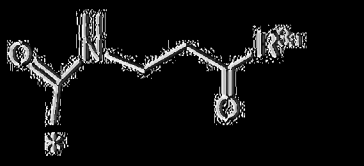
[0016]

R^3可以上述IgG結合肽作為R^{3a}，由下述式III、式IV及式V之任一者表示。

[化3]



式III



式IV



式V

(式III、式IV及式V中，*表示式I之苯環之碳)

[0017]

本說明書中記載之IgG結合肽之胺基酸序列亦可為序列編號1至115之任一者所示之胺基酸序列。

[0018]

上述IgG結合肽之胺基酸序列可為序列編號116至151之任一者所示之胺基酸序列。

[0019]

本發明之第3觀點之抗體修飾試劑包含：上述本發明之第1觀點之化合物或上述本發明之第2觀點之化合物之鹽。

[0020]

本發明之第4觀點之修飾抗體之製造方法包括如下反應步驟：使上述本發明之第3觀點之抗體修飾試劑與IgG進行反應。

[0021]

本發明之第5觀點之修飾抗體具有經由包含Lys-Gly-Gly之原子團以一價或二價結合於IgG之Fc區之Lys之藥物、反應性官能基或標識物質。

【0022】

上述原子團中所包含之Lys之側鏈之胺基被取代為上述藥物、反應性官能基或標識物質。

【0023】

上述反應性官能基可為疊氮基。

【0024】

上述IgG可為人IgG。

【0025】

上述IgG可為托珠單抗或曲妥珠單抗。

[發明之效果]

【0026】

本發明之化合物、化合物之鹽及抗體修飾試劑在化學上穩定，可長期保存。根據本發明，提供一種使用該抗體修飾試劑之修飾抗體之製造方法。根據本發明，提供一種新穎之修飾抗體。

【圖式簡單說明】

【0027】

圖1係模式性地表示藉由本發明之實施方式之化合物以IgG-BP來修飾抗體之反應的圖。

圖2係模式性地表示藉由本發明之實施方式之化合物來修飾抗體之反應的圖。

圖3係表示實施例3中將實施例1之化合物與IgG之反應溶液利用液相層析質譜儀(LC-MS)進行分析所得之結果的圖。(A)、(B)、(C)及(D)係分別表示pH值5.5、7.0、8.0及8.9之反應溶液之溶出層析圖的圖。

圖4係表示實施例3中將實施例1之化合物與IgG之反應溶液利用LC-MS進行分析所得之結果的圖。(A)、(B)、(C)及(D)係分別表示對pH值5.5、7.0、8.0及8.9之反應溶液中之IgG之波峰進行質量分析所得之結果的圖。

圖5係表示實施例3中將實施例2之化合物與IgG之反應溶液利用LC-MS進行分析所得之結果的圖。(A)、(B)、(C)及(D)係分別表示pH值5.5、7.0、8.0及8.9之反應溶液之溶出層析圖的圖。

圖6係表示實施例3中將實施例2之化合物與IgG之反應溶液利用LC-MS進行分析所得之結果的圖。(A)、(B)、(C)及(D)係分別表示對pH值5.5、7.0、8.0及8.9之反應溶液中之IgG之波峰進行質量分析所得之結果的圖。

圖7係表示實施例4中實施例2之化合物與小鼠IgG之反應溶液之利用LC-MS進行分析所得之結果的圖。(A)、(B)及(C)係分別表示添加化合物之前之pH值8.0之抗體溶液、反應2小時後之pH值8.0之溶液及反應2小時後之pH值8.9之溶液之溶出層析圖的圖。

圖8係表示實施例4中實施例2之化合物與小鼠IgG之反應溶液之利用LC-MS進行分析所得之結果的圖。(A)、(B)及(C)係分別表示對添加化合物之前之pH值8.0之抗體溶液、反應2小時後之pH值8.0之溶液及反應2小時後之pH值8.9之溶液中之IgG之波峰進行質量分析所得之結果的圖。

圖9係表示實施例5中實施例2之化合物與兔IgG之反應溶液之利用LC-MS進行分析所得之結果的圖。(A)及(B)係分別表示添加化合物之前之pH值8.0之抗體溶液及反應24小時後之pH值8.0之溶液之溶出層析圖的圖。

圖10係表示實施例5中實施例2之化合物與兔IgG之反應溶液之利用LC-MS進行分析所得之結果的圖。(A)及(B)係分別表示對添加化合物之前之pH值8.0之抗體溶液及反應24小時後之pH值8.0之溶液中之IgG之波峰進行質量分析所得之結果的圖。

圖11係表示實施例8中實施例6之化合物與人IgG之反應溶液之利用LC-MS進行分析所得之結果的圖。(A)、(B)、(C)及(D)係分別表示pH值5.5、7.0、8.0及8.9之反應溶液之溶出層析圖的圖。

圖12係表示實施例8中實施例6之化合物與人IgG之反應溶液之利用LC-MS進行分析所得之結果的圖。(A)、(B)、(C)及(D)係分別表示對pH值5.5、7.0、8.0及8.9之反應溶液中之IgG之波峰進行質量分析所得之結果的圖。

圖13係表示實施例9中實施例7之化合物與人類化IgG之反應溶液之LC-MS之結果的圖。(A)、(B)及(C)係分別表示添加化合物之前之抗體溶液、反應24小時後之pH值8.0之溶液及反應24小時後之pH值8.9之溶液之溶出層析圖的圖。

圖14係表示實施例9中實施例7之化合物與人類化IgG之反應溶液之LC-MS之結果的圖。(A)、(B)及(C)係分別表示對添加化合物之前之抗體溶液、反應24小時後之pH值8.0之溶液及反應24小時後之pH值8.9之溶液中之IgG之波峰進行質量分析所得之結果的圖。

圖15係表示試驗例2中與實施例2之化合物反應之曲妥珠單抗之肽圖之結果的圖。

圖16係表示對試驗例2中與實施例2之化合物反應之曲妥珠單抗中溶出之波峰之串聯式質譜分析(MS/MS)之結果的圖。

圖17係表示實施例10中之LC-MS之結果之圖。(A)、(B)、(C)及(D)係分別表示曲妥珠單抗(未修飾抗體)、經實施例2之化合物修飾之疊氮化曲妥珠單抗(疊氮化KGG修飾抗體)、抗癌劑與疊氮化曲妥珠單抗之反應物及疊氮化曲妥珠單抗與螢光劑之反應物之溶出層析圖的圖。

圖18係表示實施例10中之LC-MS之結果之圖。(A)、(B)、(C)及(D)係分別表示對未修飾抗體、疊氮化KGG修飾抗體、抗癌劑與疊氮化曲妥珠單抗之反應物及疊氮化曲妥珠單抗與螢光劑之反應物中溶出之抗體之波峰進行質量分析所得之結果的圖。

【實施方式】

【0028】

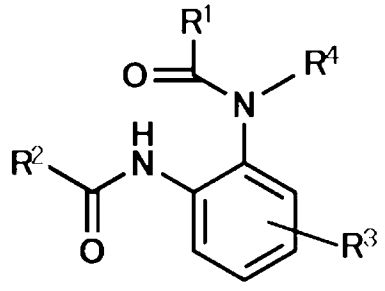
參照圖式對本發明之實施方式進行說明。再者，本發明並不受下述實施方式及圖式限定。再者，於下述實施方式中，「具有」、「包含」或「含有」這些表達亦包含「包括」或「由…構成」之含義。

【0029】

(化合物及其鹽)

本實施方式之化合物由下述式I表示。

[化4]



式I

【0030】

式I中，R^1係R^1-H之酸解離常數(pKa)達到4～14、4～12或4～10、較佳為5～9之取代基。此處之pKa係室溫(例如25℃)下之pKa。上述化合

物因R^1之脫離而成為活化中間物。R^1-H之pKa決定脫離之容易性、即轉變為活化中間物之容易性。當pKa高時，不易轉變為活化中間物，當pKa低時，容易轉變為活化中間物，但當pKa過低時，化合物有不穩定而水解之虞。較佳為R^1-H為酚類。R^1-H例如可例舉：水楊酸、苯酚、4-氟苯酚、4-硝基苯酚、2,6-二氯苯酚、N-羥基琥珀醯亞胺、2,3,5,6-四氟苯酚、五氟苯酚、胺基苯酚及二硝基苯酚等。較佳為R^1-H為4-硝基苯酚，R^1為硝基苯氧基。

【0031】

R^2及R^3之一者具有與IgG之Fc區特異性結合之IgG-BP。「IgG」可為哺乳動物、例如人及黑猩猩等靈長類；大鼠、小鼠及兔等實驗動物；豬、牛、馬、綿羊及山羊等家畜動物；以及狗及貓等寵物之IgG，較佳為人IgG(IgG1、IgG2、IgG3或IgG4)。IgG較佳為人IgG1、IgG2或IgG4、或者兔IgG，尤佳為人IgG1、IgG2或IgG4。典型地，「IgG之Fc區」意指以IgG之蛋白分解酶木瓜酶處理物之形式獲得之C末端側的片段。

【0032】

於R^2具有IgG-BP之情形時，R^3不存在，或者選自由經取代或未經取代之碳數1～8之烷基、經取代或未經取代之碳數2～8之烯基、經取代或未經取代之碳數2～8之炔基、硝基、鹵素、羧醯胺基所組成之群。於R^2具有IgG-BP之情形時，較佳為R^3不存在。

【0033】

「烷基」意指飽和烴基。烷基包含滿足碳數之條件之環式(包含縮合二環式)烷基、直鏈及支鏈烷基、以及經環式烷基取代之直鏈或支鏈烷基。作為烷基之例，可例舉：甲基、乙基、正丙基、異丙基、環丙基、正

丁基、異丁基、第二丁基、第三丁基、正戊基、1,1-二甲基丙基、1,2-二甲基丙基、2,2-二甲基丙基、1-乙基丙基、2-乙基丙基、正己基、環己基、環辛基、及1-甲基-2-乙基丙基。

【0034】

「烯基」意指具有至少1個雙鍵之烴基。烯基包含直鏈烯基及支鏈烯基這兩者。作為烯基之例，可例舉：乙烯基、正丙烯基、異丙烯基、正丁烯基、異丁烯基、第二丁烯基、第三丁烯基、正戊烯基、1,1-二甲基丙烯基、1,2-二甲基丙烯基、2,2-二甲基丙烯基、1-乙基丙烯基、2-乙基丙烯基、正己烯基及1-甲基-2-乙基丙烯基。

【0035】

「炔基」意指具有至少1個三鍵之烴基。炔基包含直鏈炔基及支鏈炔基這兩者。作為炔基之例，可例舉：乙炔基、正丙炔基、異丙炔基、正丁炔基、異丁炔基、第二丁炔基、第三丁炔基及正戊炔基等。

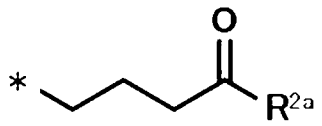
【0036】

於R^2具有IgG-BP之情形時，R^2可為IgG-BP。又，R^2還可具有連接子(連接子)，以*作為式I之羰基之碳，R^2可為*-(連接子)-(IgG-BP)。連接子並無特別限定，只要維持化合物對於Fc區之特異性結合能即可，例如選自由-NH-、-O-、-S-、-C(=O)-NH-、-NH-C(=O)-、-O-、-C(=O)-O-、-O-C(=O)-、-S-、-C(=O)-、聚氧伸烷基、胺基酸殘基、肽鏈、聚乙二醇(PEG)鏈及其等之組合所組成之群。

【0037】

適宜的是R^2由式II表示並以IgG-BP作為R^{2a}。

[化5]



式II

R^2中之IgG-BP係經由主鏈或任意之胺基酸殘基之側鏈結合於式I之羰基之碳或連接子。較佳為R^2中之IgG-BP係經由N末端之主鏈或側鏈之胺基結合於式I之羰基之碳或連接子。

【0038】

R^2中之IgG-BP可經藥物、反應性官能基或標識物質修飾。作為藥物，例如可例舉：澳瑞他汀E等澳瑞他汀、美登素、美坦辛、多柔比星、博來黴素、或其等之衍生物等抗癌劑、與血腦障壁之受體結合而促進向中樞神經轉移之藥物、或者與癌細胞等結合而使IgG能夠向細胞內轉移之藥物等靶向劑。

【0039】

作為反應性官能基，可例舉：咪唑基、羥基、胺基、硫醇基、鹵素原子、磺酸酯基、環氧基、異氰酸基、疊氮基、乙烯基、馬來醯亞胺基、硫氫基、乙炔基(-C≡CH)、二烯、炔烴、雙環[6.1.0]壬炔(BCN)、二苯并環辛炔(DBCO)基、反式環辛烯(TCO)、四吡等。較佳為反應性官能基為疊氮基，藉由與炔烴或DBCO基等之點擊反應而可利用所需分子來修飾IgG-BP。

【0040】

標識物質並無限定，例如為螢光色素、化學發光色素、放射性同位素、發光蛋白、生物素(Biotin)、綠色螢光蛋白等螢光蛋白、及過氧化酶等酵素等。較佳為標識物質為螢光色素(FAM)及FITC等螢光色素衍生物、若丹明及四甲基若丹明等若丹明衍生物、以及德克薩斯紅等螢光色素。

【0041】

利用R^2具有IgG-BP之本實施方式之化合物對IgG進行修飾之反應如圖1所示。該化合物由於具有式I所示之活性酯前驅物，故而於中性條件、較佳為弱鹼性條件下經由活化型化合物，而將IgG-BP附加於IgG之規定之胺基酸殘基、尤其是Lys殘基。

【0042】

繼而，對R^3具有IgG-BP之情形進行說明。於R^3具有IgG-BP之情形時，R^2係選自由經取代或未經取代之碳數1～8之烷基、經取代或未經取代之碳數2～8之烯基、經取代或未經取代之碳數2～8之炔基、胺基酸殘基數2～50之經取代或未經取代之肽鏈、聚合度2～50之經取代或未經取代之聚合物鏈及其等之組合所組成之群。肽鏈中之胺基酸殘基數較佳為2～30、2～20、2～10或2～5，更佳為3。作為具體之肽鏈，可例舉Lys-Gly-Gly。聚合物鏈並無特別限定，只要為公知之聚合物鏈即可，例如可例舉PEG等。再者，經取代之肽鏈包含於主鏈或側鏈上結合有取代基者。經取代之聚合物鏈包含於主鏈或側鏈上結合有取代基者。

【0043】

於R^3具有IgG-BP之情形時，R^3可為IgG-BP。又，R^3與上述R^2同樣地，可具有連接子。

【0044】

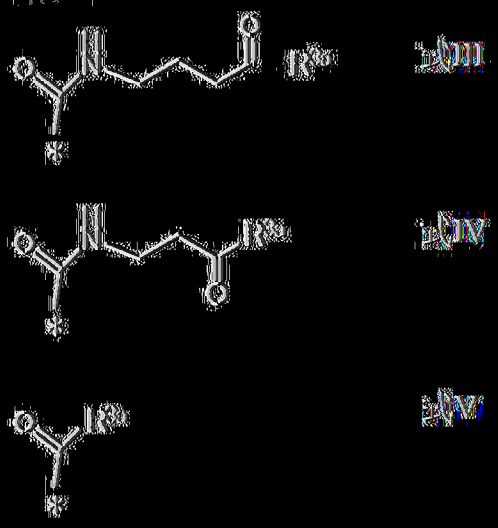
R^3中之IgG-BP係經由主鏈或任意之胺基酸殘基之側鏈結合於式I之苯環之碳或連接子。較佳為R^3中之IgG-BP經由N末端之主鏈或側鏈之胺基結合於式I之羰基之碳或連接子。

【0045】

適宜的是R^3由式III、式IV及式V之任一者表示並以IgG-BP作為R^{3a}。再者，式III、式IV及式V中，*表示式I之苯環之碳。

[0046]

[化6]



[0047]

於R^3具有IgG結合肽之情形時，R^2可具有上述之藥物、反應性官能基或標識物質。R^2中之藥物、反應性官能基或標識物質例如結合於烷基、烯基、炔基、肽鏈或聚合物鏈。於R^2例如具有Lys-Gly-Gly作為肽鏈之情形時，可將肽鏈之Lys之側鏈之胺基取代為藥物、反應性官能基或標識物質。

[0048]

利用R^3具有IgG-BP之本實施方式之化合物對IgG進行修飾之反應如圖2所示。該化合物具有式I所示之活性酯前驅物，因此經由活化型化合物與IgG結合。R^3具有IgG-BP之該化合物與R^2具有IgG-BP之情形不同，包含R^2之取代基被附加到IgG之規定之胺基酸殘基上，包含IgG-BP之R^3未被附加到IgG上。因此，於R^3具有IgG-BP之情形時，較佳為R^2具有上述之藥物、反應性官能基或標識物質。如此，可容易利用藥物、反應性官能基或

標識物質來修飾IgG。

【0049】

上述專利文獻4等中所揭示之於IgG中不會殘留IgG-BP之CCAP法在修飾IgG後需要IgG-BP之脫離反應。另一方面，若使用本實施方式之化合物，則在修飾IgG之同時，IgG-BP之脫離反應結束。

【0050】

IgG-BP較佳為與Fc中之選自依照Eu編號之Lys248、Lys246、Lys338、Lys288、Lys290、Lys360、Lys414及Lys439之部位、或其鄰近區域、較佳為Lys248或其近接區域結合，或者與蛋白A之結合區域結合之肽。例如IgG-BP亦可為具有Fc結合能之蛋白A之部分肽或其變異體。此種肽之具體例係記載於國際公開第2008/054030號、國際公開第2013/027796號、上述專利文獻1～3等中。IgG-BP可依照公知之肽之合成法、例如肽固相合成法或各文獻中記載之方法來適當製備。

【0051】

更具體而言，R^2及R^3可例舉以下之(i)～(iii)。

(i)下述式(PI)所表示之包含IgG-BP之取代基

NH_2-(連接子)-$(X^1{}_{1-3})$-C-(X^2)-(X^3)-(X^4)-(X^5)-G-(X^6)-L-(X^7)-W-C-$(X^8{}_{1-3})$-連接子)・・・式(PI)

[式(PI)中，各(連接子)獨立，為相同或不同之連接子，或者不存在，此處，連接子為RRRGS、EEGGS、$(GSGGS)_{1-3}$或者$(PEG)_{1-10}$，較佳為$(PEG)_{1-8}$或$(PEG)_{2-10}$，更佳為$(PEG)_4$，

1～3個X^1、X^2、X^3、X^4、X^5、X^6、X^7、及1～3個X^8各自彼此獨立，表示相同或不同之胺基酸殘基，

各X^1、X^2、X^3及各X^8彼此獨立，表示相同或不同之C以外之任意之胺基酸殘基，

X^4為H、R、S、或D，

X^5為選自K、C、D、E、R、V、F、L、2-胺基辛二酸、Dpr、Orn、AcOrn、AcDab、Dab、Nle、Nva、Tle、Ala(t-Bu)、及Cha中之1個胺基酸殘基，

X^6為E、N、R、或D，

X^7為I或V。

再者，Dab、Tle及Cha分別意指2,4-二胺基丁酸、第三白胺酸及β-環己基-L-丙胺酸。又，Ac及t-Bu分別意指具有乙醯基及第三丁基]

【0052】

式(PI)中，可於式(PI)之C末端胺基酸殘基之末端(-COOH)上鍵結胺基而成為(-C(=O)NH_2)基，以進行結合。又，於式(PI)中，N末端之胺基可被乙醯化。於該情形時，可於連接子中之N末端附近之適當位置處導入Lys殘基。

【0053】

作為式(PI)所表示之取代基中所包含之IgG-BP，可較佳地例舉以下之肽。

[1]$X^1{}_{1\text{-}3}$為(S、G、F、 或 無)-(D、G、A、S、P、Hcy、 或 無)-(S、D、T、N、E、或R)所表示之胺基酸序列。

[2]$X^1{}_{1\text{-}3}$為D、GPD、R、GPR、SPD、GDD、GPS、SDD、RGN、G-Hcy-D、RGP、或GPD。

[3]$X^1{}_{1\text{-}3}$為D或GPD。

[4]X^2為A、S、或T。

[5]X^2為A或T。

[6]X^2為A。

[7]X^3為Y或W。

[8]X^3為Y。

[9]X^4為H。

[10]X^5為選自A、R、K、C、D、E、L、2-胺基辛二酸、Dpr、R、F、2-胺基辛二酸、Dpr、AcOrn、AcDab、Dab、Nle、Nva、Ala(t-Bu)、及Cha中之1個胺基酸殘基。

[11]X^5為K、R、AcOrn。

[12]X^5為選自V、Dab、F、R、L、Nva、Nle、Ala(t-Bu)、及Cha中之1個胺基酸殘基。

[13]X^5為選自F、R、L、Nva、Nle、Ala(t-Bu)、及Cha中之1個胺基酸殘基。

[14]X^5為選自L、Ala(t-Bu)、及Cha中之1個胺基酸殘基。

[15]X^6為E或N。

[16]X^6為E。

[17]X^7為V。

[18]$X^8{}_{1\text{-}3}$為(S、T、或D)-(H、G、Y、T、N、D、F、Hcy、或無)-(Y、F、H、M、或無)。

[19]$X^8{}_{1\text{-}3}$為T、TFH、S、SFH、THH、TFY、TYH、或T-Hcy-H。

[20]$X^8{}_{1\text{-}3}$為T或TFH。

【0054】

作為式(PI)所表示之取代基中所包含之IgG-BP，可為以上條件之任意1個或2個以上之組合，例如可為滿足以下所記載之條件之肽：[8]與[9]、[8]與[17]、[9]與[17]、[8]與[9]與[17]、或者其等與[10]～[14]中之任一個之組合。

【0055】

更具體而言，作為IgG-BP，可例舉以下之肽(X^5與上述相同，可於N末端具有NH_2-(連接子)-基，亦可於C末端具有-NH_2基或-(連接子)-NH_2基)：

1)DCAYHX^5GELVWCT(序列編號1)

2)GPDCAYHX^5GELVWCTFH(序列編號2)

3)RCAYHX^5GELVWCS(序列編號3)

4)GPRCAYHX^5GELVWCSFH(序列編號4)

5)SPDCAYHX^5GELVWCTFH(序列編號5)

6)GDDCAYHX^5GELVWCTFH(序列編號6)

7)GPSCAYHX^5GELVWCTFH(序列編號7)

8)GPDCAYHX^5GELVWCSFH(序列編號8)

9)GPDCAYHX^5GELVWCTHH(序列編號9)

10)GPDCAYHX^5GELVWCTFY(序列編號10)

11)SPDCAYHX^5GELVWCTFY(序列編號11)

12)SDDCAYHX^5GELVWCTFY(序列編號12)

13)RGNCAYHX^5GQLVWCTYH(序列編號13)

14)G-Hcy-DCAYHX^5GELVWCT-Hcy-H(序列編號14)

15)RRGPDCAYHX^5GELVWCTFH(序列編號15)

16)DCTYHX^{5}GNLVWCT(序列編號16)

17)DCAYHX^{5}GNLVWCT(序列編號17)

18)DCTYHX^{5}GELVWCT(序列編號18)

19)DCAWHX^{5}GELVWCT(序列編號19)

20)DCTYTX^{5}GNLVWCT(序列編號20)

21)DCAYTX^{5}GNLVWCT(序列編號21)

22)DCSYTX^{5}GNLVWCT(序列編號22)

23)DCTWTX^{5}GNLVWCT(序列編號23)

24)DCTYHX^{5}GNLVWCT(序列編號24)

25)DCTYRX5GNLVWCT(序列編號25)

26)DCTYSX^{5}GNLVWCT(序列編號26)

27)DCTYTX^{5}GNLVWCT(序列編號27)

28)DCTYTX^{5}GELVWCT(序列編號28)

29)DCTYTX^{5}GRLVWCT(序列編號29)

30)DCTYTX^{5}GDLVWCT(序列編號30)

31)DCTYTX^{5}GNLIWCT(序列編號31)

32)DCAYHRGELVWCT(序列編號32)

33)GPDCAYHRGELVWCTFH(序列編號33)

34)RCAYHRGELVWCS(序列編號34)

35)GPRCAYHRGELVWCSFH(序列編號35)

36)SPDCAYHRGELVWCTFH(序列編號36)

37)GDDCAYHRGELVWCTFH(序列編號37)

38)GPSCAYHRGELVWCTFH(序列編號38)

39)GPDCAYHRGELVWCSFH(序列編號39)

40)GPDCAYHRGELVWCTHH(序列編號40)

41)GPDCAYHRGELVWCTFY(序列編號41)

42)SPDCAYHRGELVWCTFY(序列編號42)

43)SDDCAYHRGELVWCTFY(序列編號43)

44)DCTYHRGNLVWCT(序列編號44)

45)DCAYHRGNLVWCT(序列編號45)

46)DCTYHRGELVWCT(序列編號46)

47)DCAWHRGELVWCT(序列編號47)

48)DCTYTNGNLVWCT(序列編號48)

49)DCAYTNGNLVWCT(序列編號49)

50)DCSYTNGNLVWCT(序列編號50)

51)DCTWTNGNLVWCT(序列編號51)

52)DCTYHNGNLVWCT(序列編號52)

53)DCTYRNGNLVWCT(序列編號53)

54)DCTYSNGNLVWCT(序列編號54)

55)DCTYTRGNLVWCT(序列編號55)

56)DCTYTNGELVWCT(序列編號56)

57)DCTYTNGRLVWCT(序列編號57)

58)DCTYTNGDLVWCT(序列編號58)

59)DCTYTNGNLIWCT(序列編號59)

【0056】

作為R^2及R^3之例，可例舉具有以下結構之取代基。

60)GSGGS-GPDCAYHRGELVWCTFH-NH_2(序列編號60)

$(PEG)_4$-GPDCAYHRGELVWCTFH-NH_2(序列編號33)

61)GSGGS-DCAYHRGELVWCT-NH_2(序列編號61)

$(PEG)_4$-DCAYHRGELVWCT-NH_2(序列編號32)

【0057】

本說明書中肽及IgG-BP包含鍵結有官能基Z之肽。例如式(PI)所表示之取代基可於(連接子)之前鍵結官能基Z。作為此種取代基，可例舉以下之取代基。

62)乙醯基-K(Z)-RRRGS-GPDCAYHKGELVWCTFH-NH_2(序列編號62)

63)乙醯基-K(Z)-EEGGS-GPDCAYHKGELVWCTFH-NH_2(序列編號63)

64)乙醯基-K(Z)-$(PEG)_4$-GPDCAYHKGELVWCTFH-NH_2(序列編號64)

【0058】

R^2及R^3可為於以下肽之N末端或PEG上鍵結有馬來醯亞胺基、DBCO基、四吡基、TCO基之取代基，亦可為於C末端上鍵結有NH_2基之取代基。

65)RRRGS-GPDCAYHKGELVWCTFH(序列編號65)

66)EEGGS-GPDCAYHKGELVWCTFH(序列編號66)

$(PEG)_4$-GPDCAYHKGELVWCTFH(序列編號64)

【0059】

又，作為於(連接子)之前鍵結有官能基Z之取代基，可例舉以下之

肽。

67)乙醯基-K(Z)RRRGS-DCAYHKGELVWCT-NH_2(序列編號67)

68)乙醯基-K(Z)EEGGS-DCAYHKGELVWCT-NH_2(序列編號68)

69)乙醯基-K(Z)-$(PEG)_4$-DCAYHKGELVWCT-NH_2(序列編號69)

【0060】

R^2及R^3可為於以下肽之N末端或PEG上鍵結有馬來醯亞胺基、DBCO基、四唑基、TCO基之取代基，亦可於C末端上鍵結有NH_2基之取代基。

70)RRRGS-DCAYHKGELVWCT(序列編號70)

71)EEGGS-DCAYHKGELVWCT(序列編號71)

$(PEG)_4$-DCAYHKGELVWCT(序列編號69)

【0061】

(ii)下述式(PII)所表示之包含IgG-BP之取代基、或包括如下胺基酸序列之包含IgG-BP之取代基，該胺基酸序列係於(PII)之胺基酸序列中，在除X^9～X^{14}以外之位置處附加、缺失、及/或置換1個或者數個胺基酸而成

$X^9{}_{1\text{-}2}$NMQX^{10}QX^{14}RFYEALHDPNLNEEQRNAX^{11}IX^{12}SIRDDX^{13}-(連接子2)-$CONH_2$・・・(PII)

[式(PII)中，(連接子2)為$(GSGGS)_{1\text{-}3}$、$(SGSGS)_{1\text{-}3}$、或者$(PEG)_{2\text{-}10}$-Lys(較佳為$(PEG)_4$-Lys)、SGSGSK、SRRCR、SRRK(Z)R、SRRCRRCRRC、SRRK(Z)RRK(Z)RRK(Z)、或者$(PEG)_{1\text{-}8}$-Lys(較佳為$(PEG)_4$-Lys)，或者不存在，

$X^9{}_{1\text{-}2}$係選自由GF、AF、VF、LF、IF、MF、PF、FF、WF、KF、Orn-F、CF、DF、EF、β丙胺酸-F、2-胺基辛二酸-F、Dpr-F、NH_2-(PEG)n-CO(此處，n＝1-50)-F、F、K、Orn、C、D、E、2-胺基辛二

酸、Dpr、及乙醯基-K所組成之群，

X^{10}為C或Q，

X^{11}及X^{12}分別獨立地選自由R、H、D、E、S、T、N、Q、Y、及C所組成之群，

X^{13}為C或P，或不存在，

X^{14}為R、C、K、或K(Z)]。

【0062】

式(PII)之N末端胺基酸之末端(-NH_2)可乙醯化而成為(CH_3-C(=O)-NH-)基。又，連接子中所包含之Cys殘基(C)可視需要，經由馬來醯亞胺基而鍵結其他功能性分子。

【0063】

作為式(PII)所表示之取代基中所包含之IgG-BP，可較佳地例舉以下之肽。

[21]X^9為 選 自 由GF、AF、βAlaF、NH_2-$(PEG)_n$-CO(n＝2-10)-F、F、K、Orn、C、Dpr、及乙醯基-K所組成之群。

[22]X^9為選自由GF、F、K、及乙醯基-K所組成之群。

[23]X^{10}為Q。

[24]X^{11}及X^{12}分別獨立地選自由R、H、及E所組成之群。

[25]X^{11}為R。

[26]X^{12}為R或K(Z)(較佳為Z為疊氮基)。

【0064】

作為式(PII)所表示之取代基，更具體而言，可例舉以下之取代基(其中，所包含之Lys殘基亦可視需要鍵結官能基)。

72)FNMQCQRRFYEALHDPNLNEEQRNARIRSIRDDC(序列編號72)

73)GFNMQCQRRFYEALHDPNLNEEQRNARIRSIRDDC(序列編號73)

74)KNMQCQRRFYEALHDPNLNEEQRNARIRSIRDDC(序列編號74)

75)GFNMQCQKRFYEALHDPNLNEEQRNARIRSIRDDC(序列編號75)

76)KNMQCQKRFYEALHDPNLNEEQRNARIRSIRDDC(序列編號76)

77)FNMQQQRRFYEALHDPNLNEEQRNARIRSIRDD(序列編號77)

78)GKNMQCQRRFYEALHDPNLNEEQRNARIRSIRDDC(序列編號78)

79)乙醯基-FNMQQQRRFYEALHDPNLNEEQRNARIRSIRDDP-SGSGSK-NH_2(序列編號79)

80)乙醯基-FNMQCQRRFYEALHDPNLNEEQRNARIRSIRDDC-SGSGSK-NH_2(序列編號80)

81)乙醯基-FNMQQQRRFYEALHDPNLNEEQRNARIRSIRDDP-$(PEG)_4$-Lys-NH_2(序列編號81)

72-2)乙醯基-FNMQCQRRFYEALHDPNLNEEQRNARIRSIRDDC-$(PEG)_4$-Lys-NH_2(序列編號72)

82)FNMQQQCRFYEALHDPNLNEEQRNARIRSIRDD-NH_2(序列編號82)

83)FNMQQQRRFYEALHDPNLNEEQRNARIRSIRDDC-NH_2(序 列編號83)

84)FNMQQQRRFYEALHDPNLNEEQRNARIRSIRDDP-SRRK(Z)R-NH_2(序列編號84)

85)FNMQQQRRFYEALHDPNLNEEQRNARIRSIRDDP-SRRCR-NH_2(序列編號85)

86)FNMQQQCRFYEALHDPNLNEEQRNARICSIRDDP-SRRCRRCRRC-NH_2(序列編號86)

87)FNMQQQK(Z)RFYEALHDPNLNEEQRNARIK(Z)SIRDDP-SRRK(Z)RRK(Z)RRK(Z)-NH_2(序列編號87)

88)乙 醯 基-KNMQQQCRFYEALHDPNLNEEQRNARIRSIRDD-NH_2(序列編號88)

89)乙 醯 基-KNMQQQRRFYEALHDPNLNEEQRNARIRSIRDDC-NH_2(序列編號89)

90)乙 醯 基-KNMQQQRRFYEALHDPNLNEEQRNARIRSIRDDP-SRRK(Z)R-NH_2(序列編號90)

91)乙 醯 基-KNMQQQRRFYEALHDPNLNEEQRNARIRSIRDDP-SRRCR-NH_2(序列編號91)

92)乙 醯 基-KNMQQQCRFYEALHDPNLNEEQRNARICSIRDDP-SRRCRRCRRC-NH_2(序列編號92)

93)乙醯基-KNMQQQK(Z)RFYEALHDPNLNEEQRNARIK(Z)SIRDDP-SRRK(Z)RRK(Z)RRK(Z)-NH_2(序列編號93)

94)GFNMQQQCRFYEALHDPNLNEEQRNARIRSIRDD-NH_2(序 列

編號94)

95)GFNMQQQRRFYEALHDPNLNEEQRNARIRSIRDDC-NH_2(序列編號95)

96)GFNMQQQRRFYEALHDPNLNEEQRNARIRSIRDDP-SRRK(Z)R -NH_2(序列編號96)

97)GFNMQQQRRFYEALHDPNLNEEQRNARIRSIRDDP-SRRCR-NH_2(序列編號97)

98)GFNMQQQCRFYEALHDPNLNEEQRNARICSIRDDP-SRRCRRCRRC-NH_2(序列編號98)

99)GFNMQQQK(Z)RFYEALHDPNLNEEQRNARIK(Z)SIRDDP-SRRK(Z)RRK(Z)RRK(Z)-NH_2(序列編號99)

100)FNMQCQZRFYEALHDPNLNEEQRNARIRSIRDDC-NH_2序 列編號100)

101)乙 醯 基-KNMQCQZRFYEALHDPNLNEEQRNARIRSIRDDC-NH_2(序列編號101)

102)GFNMQCQK(Z)RFYEALHDPNLNEEQRNARIRSIRDDC-SRRK(Z)R-NH_2(序列編號102)

103)FNMQCQK(Z)RFYEALHDPNLNEEQRNARIRSIRDDC-NH_2(序列編號103)

104)乙 醯 基-KNMQCQK(Z)RFYEALHDPNLNEEQRNARIRSIRDDC-SRRK(Z)R-NH_2(序列編號104)

105)GFNMQCQK(Z)RFYEALHDPNLNEEQRNARIRSIRDDC-SRRK(Z)RRK(Z)RRK(Z)-NH_2(序列編號105)

106)乙 醯 基-KNMQCQK(Z)RFYEALHDPNLNEEQRNARIRSIRDDC-SRRK(Z)RRK(Z)RRK(Z)-NH_2(序列編號106)

107)GFNMQCQK(Z)RFYEALHDPNLNEEQRNARIRSIRDDC-SRRK(Z)RRK(Z)RRK(Z)-NH_2(序列編號107)

【0065】

(iii)以下之肽

108)CAWHLGELVWC(序列編號108)

109)DCAWHLGELVWCT(序列編號109)

110)DCAWHLGELVFCT(序列編號110)

111)DCAWHLGELVX^{15}CT(序列編號111)

X^{15}＝1-萘醯基、2-萘醯基、苯基、或苯并噻吩

112)CDCAWHLGELVWCTC(序列編號112)

113)CAYHLGELVWC(序列編號113)

114)DCAYHLGELVWCTF(2-Pya)(序列編號114)

115)乙 醯 基-(Lys[疊 氮])RRRGSGPDCAYHKGELVWCTFH-$CONH_2$)(序列編號115)

【0066】

考慮到IgG-BP與IgG之立體結構，於R^3具有IgG-BP之情形時，IgG-BP之胺基酸序列可為缺少序列編號72～107所示之胺基酸序列之N末端之胺基酸殘基，由序列編號116至151之任一者所示之胺基酸序列。

【0067】

R^2可鍵結於IgG-BP中所包含之Lys殘基，尤其是經由X^5之側鏈而鍵結於式I之羰基之碳或連接子，R^3可鍵結於IgG-BP中所包含之Lys殘基，

尤其是經由上述X^5之側鏈而鍵結於式I之苯環之碳或連接子。

【0068】

又，於R^2例如為上述79)、80)、81)、72-2)、88)、89)、90)、91)、92)、93)、101)、104)及106)之情形時，任意Lys殘基之側鏈可與式I之羰基之碳或連接子鍵結。同樣地，於R^3為上述79)、80)、81)、72-2)、88)、89)、90)、91)、92)、93)、101)、104)及106)之情形時，任意Lys殘基之側鏈可與式I之苯環之碳或連接子鍵結。

【0069】

再者，上述IgG-BP中，任意2個半胱胺酸殘基彼此可經由雙硫鍵而形成分子(肽)內鍵。

【0070】

R^4為H或者經取代或未經取代之碳數1～8之烷基。R^4例如為碳數1～6、1～4或1～3之烷基，較佳為乙基，更佳為甲基。

【0071】

作為本實施方式之環狀肽之鹽，並無特別限定，只要為藥理學上所容許之鹽即可，可為酸性鹽及鹼性鹽之任一種。作為鹽，例如可例舉：鋰鹽、鈉鹽及鉀鹽等鹼金屬鹽；鎂鹽及鈣鹽等鹼土金屬鹽等；鹽酸鹽、氫溴酸鹽、硫酸鹽、硝酸鹽、草酸鹽及磷酸鹽等無機酸鹽；以及乙酸鹽、丙酸鹽、己酸鹽、環戊丙酸鹽、乙醇酸鹽、丙酮酸鹽、乳酸鹽、丙二酸鹽、琥珀酸鹽、蘋果酸鹽、富馬酸鹽、酒石酸鹽、檸檬酸鹽、苯甲酸鹽、鄰(4-羥基苯甲醯基)苯甲酸鹽、桂皮酸鹽、苦杏仁酸鹽、甲磺酸鹽、乙磺酸鹽、1,2-乙二磺酸鹽、2-羥基乙磺酸鹽、苯磺酸鹽、對氯苯磺酸鹽、2-萘磺酸鹽、對甲苯磺酸鹽、樟腦磺酸鹽、葡庚糖酸鹽、3-苯基丙酸鹽、三甲

基乙酸鹽、第三丁基乙酸鹽、月桂基硫酸鹽、葡萄糖酸鹽、麩胺酸鹽、羥基萘甲酸鹽、水楊酸鹽、硬脂酸鹽、三氟乙酸(TFA)鹽、馬來酸鹽及黏康酸鹽等有機酸鹽等。

【0072】

本實施方式之化合物可基於上述式I所示之結構，利用公知之方法合成。例如於合成R^2具有IgG-BP之化合物時，可對利用肽固相合成法所合成之樹脂上之肽之胺基進行戊二醯化，使N-甲基-1,2-苯二胺縮合後，進行氯甲酸4-硝基苯酯處理。例如於合成R^3具有IgG-BP之化合物時，藉由於在樹脂上將肽伸長之途中，使用Fmoc-MeDbz(3-[(9-芴基甲氧基羰基)胺基]-4-(甲基胺基)苯甲酸)預先插入MeDbz殘基，可利用對保護肽樹脂之氯甲酸4-硝基苯酯處理而在MeDbz殘基部位上構建上述式I所示之骨架。

【0073】

本實施方式之化合物或其鹽不具有容易水解之NHS基，因此化學上穩定，可長期保存。該化合物或其鹽由於具有式I所示之活性酯前驅物，故而藉由與IgG溶混，可對Fc區之規定部位特異性地修飾。當利用反應性官能基對IgG修飾時，可經由反應性官能基使藥物或標識物質等結合於IgG。當使藥物擔載於IgG時，可獲得部位特異性地結合有藥物之抗體藥物複合體。藉此，可對IgG賦予藥物之特性，因此可應用於藥物輸送系統(DDS)等。又，藉由利用部位特異性之標識物質對IgG進行修飾，可控制標識物質之量，因此可用於IgG之追蹤、分佈及檢測等。

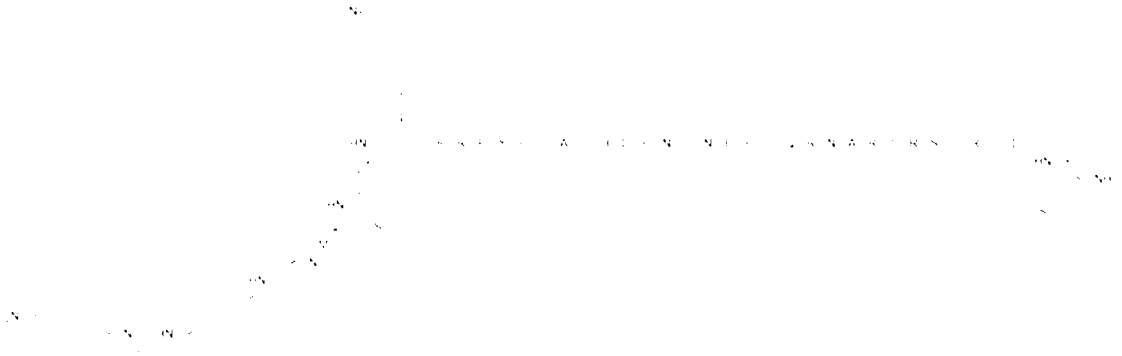
【0074】

作為本實施方式之化合物之一例之化合物A的結構如下所示。化合物A具有R^2由序列編號152表示之胺基酸序列之IgG-BP。再者，於下述化合

物A之結構中，-N-M-Q-NH-中之N、M及Q、以及RFYEALHDPNLNEEQRNARIRSIRDD表示單字母符號之胺基酸。

【0075】

[化7]

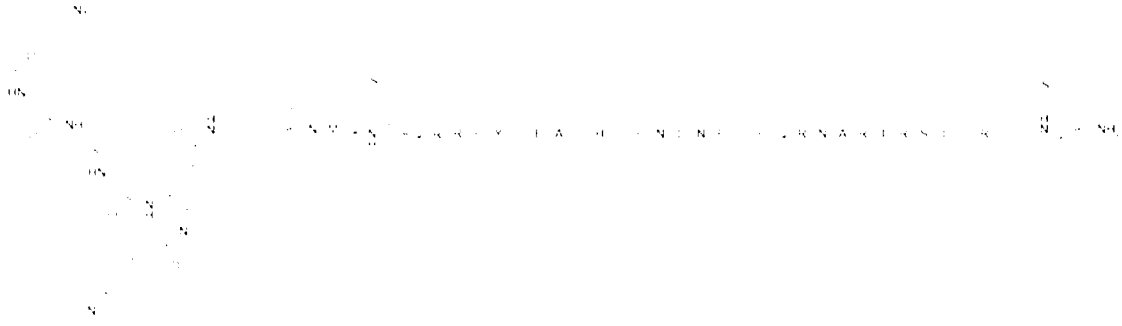


【0076】

作為本實施方式之化合物之一例之化合物B之結果如以下所示。該化合物具有R^3由序列編號153表示之胺基酸序列之IgG-BP。再者，下述化合物B之結構中，-C(=O)-N-M-Q-NH-中之N、M及Q、以及QRRFYEALHDPNLNEEQRNARIRSIRDD表示單字母符號之胺基酸。

【0077】

[化8]

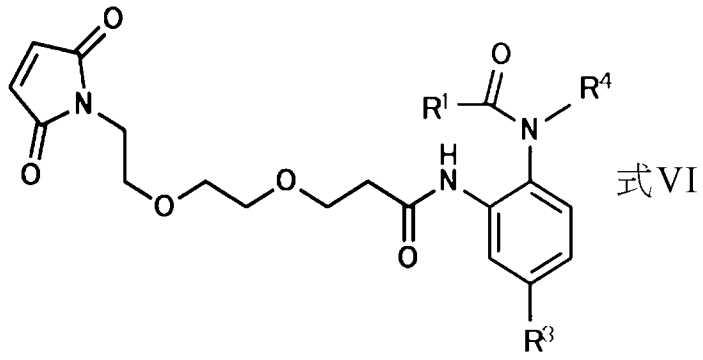


【0078】

進而，作為R^3具有IgG-BP之化合物，例示下述式VI～IX。

【0079】

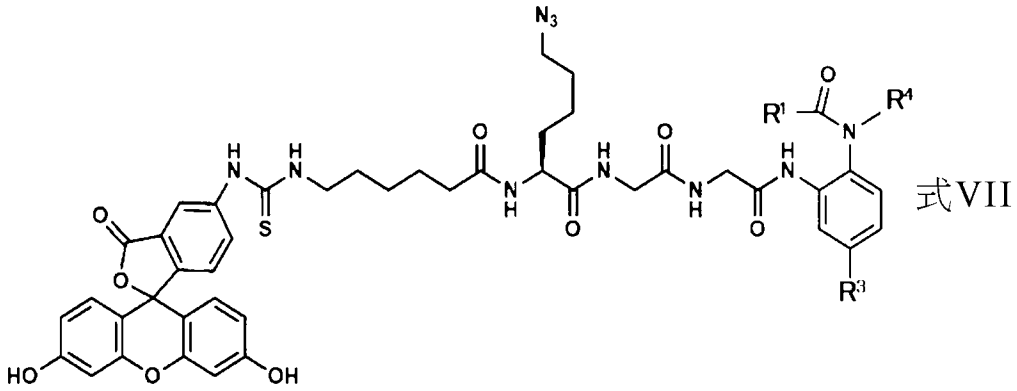
[化9]



式VI

【0080】

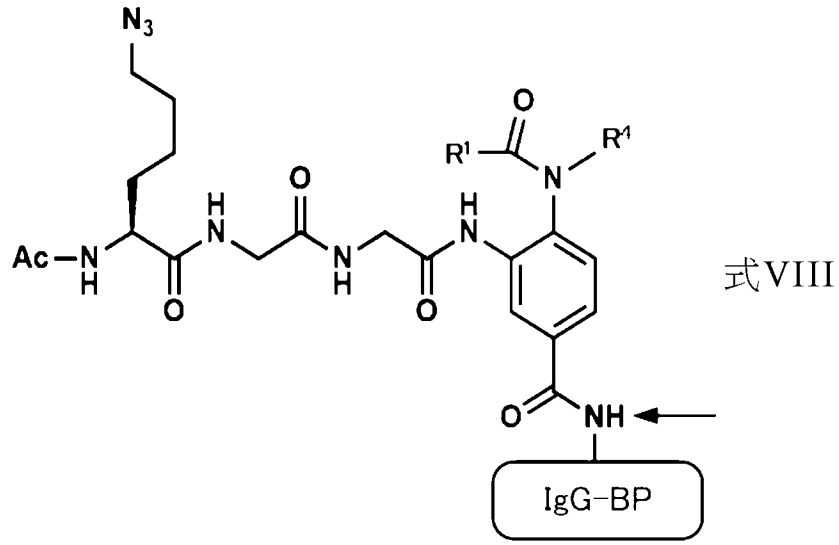
[化10]



式VII

【0081】

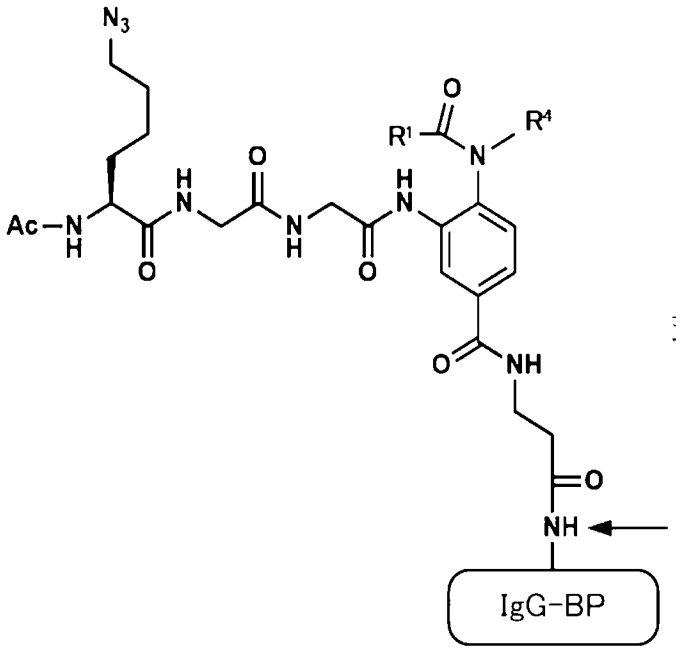
[化11]



式VIII

【0082】

[化12]



式IX

【0083】

式VIII及式IX中，IgG-BP之並非N末端之胺基酸殘基、例如Lys殘基之側鏈之胺基經由連接子與式I之苯環之碳鍵結。再者，式VIII及式IX中箭頭所表示之NH-表示Lys殘基之側鏈之胺基。

【0084】

進而，作為R^3具有IgG-BP之化合物C及化合物D，分別例示下述式X及式XI。化合物C及化合物D之IgG-BP之胺基酸序列與上述化合物B之IgG-BP相同，為序列編號153所示之胺基酸序列。

【0085】

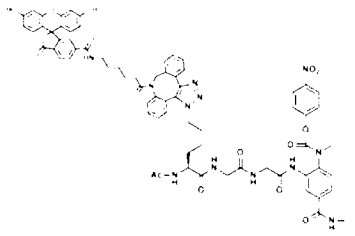
[化13]



式X

【0086】

[化14]



式XI

【0087】

於另一實施方式中，提供包含本實施方式之化合物或其鹽之抗體修飾試劑。於其他實施方式中，提供使用該抗體修飾試劑之修飾抗體之製造方法。修飾抗體之製造方法包括反應步驟。反應步驟中，使該抗體修飾試劑與IgG進行反應。

【0088】

反應步驟中之反應條件任意，只要抗體修飾試劑暴露於IgG即可，例如於pH值7.0～9.0之緩衝液中，只要將IgG與抗體修飾試劑溶混即可。緩衝液中之IgG及抗體修飾試劑之濃度係適當進行調整。緩衝液中之IgG與抗體修飾試劑之莫耳比例如為1：1～10、1：2～8或1：3～6，較佳為1：5。

【0089】

(修飾抗體)

本實施方式之修飾抗體具有原子團，該原子團係將上述式I之R^2所鍵結之羰基直至R^2包括在內。該修飾抗體較佳為藉由使上述化合物或其鹽與IgG進行反應而獲得。

【0090】

例如，該修飾抗體具有經由包含Lys-Gly-Gly之原子團以一價或二價結合於IgG之Fc區之Lys的藥物、反應性官能基或標識物質。IgG並無特別限定，尤佳為人IgG，例如為曲妥珠單抗及托珠單抗等。

【0091】

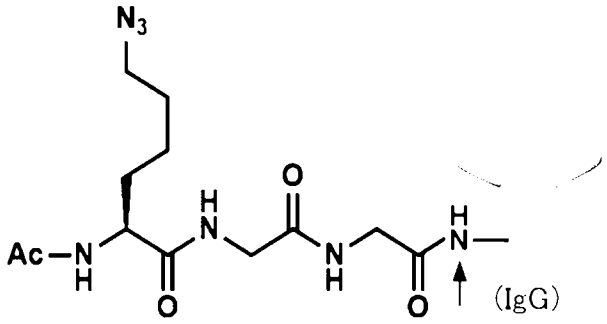
上述原子團例如可為包含Lys-Gly-Gly之肽鏈，亦可為於肽鏈上進而結合有連接子等而成之原子團。藥物、反應性官能基或標識物質可經由連接子結合於肽鏈之N末端，亦可原子團中所包含之Lys之側鏈之胺基被取代為藥物、反應性官能基或標識物質。再者，Lys-Gly-Gly之Lys之N末端可被乙醯化。

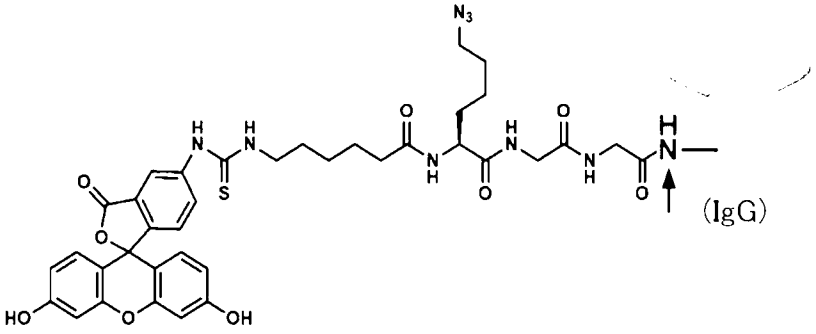
【0092】

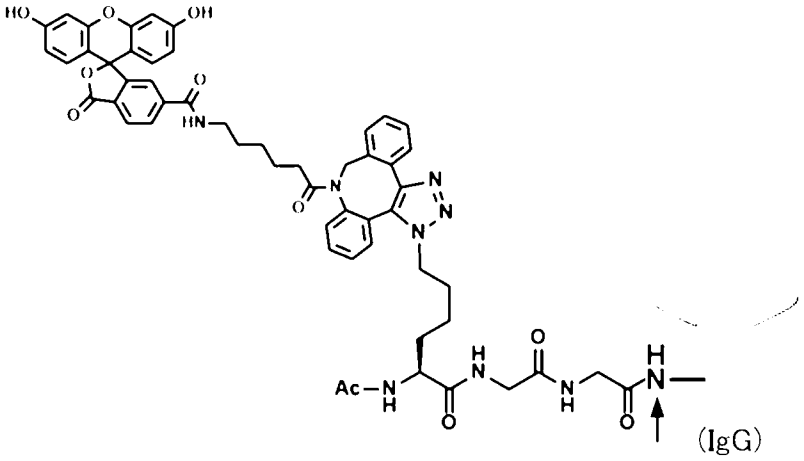
作為經由包含Lys-Gly-Gly之原子團而結合藥物、反應性官能基或標識物質而成之修飾抗體之結構，例示以下之結構。再者，箭頭所示之-NH-表示Lys殘基之側鏈之胺基。

【0093】

[化15]





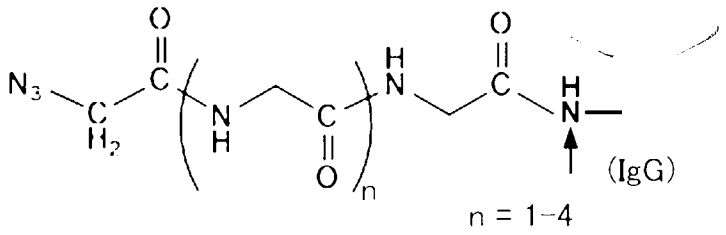


【0094】

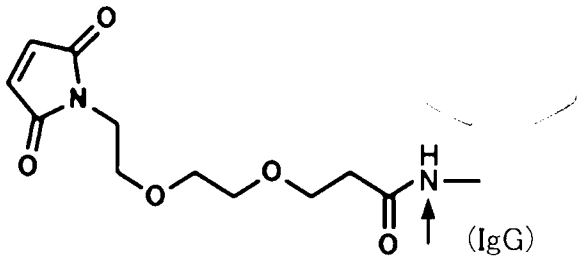
修飾抗體亦可具有經由以一價或二價結合於IgG之Fc區之Lys的包含PEG、Gly或其組合之原子團結合的上述藥物、反應性官能基或標識物質。作為該修飾抗體之結構，例示以下之結構。再者，箭頭所示之-NH-表示Lys殘基之側鏈之胺基。

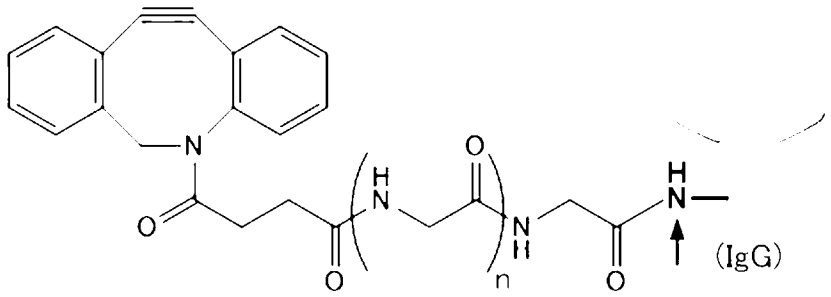
【0095】

[化16]



n = 1-4





n = 1-4

【0096】

(附記)

(附記1)一種化合物或其鹽，該化合物由上述式I所示。

(附記2)如附記1記載之化合物或其鹽，其中R^1為硝基苯氧基。

(附記3)如附記1或2記載之化合物或其鹽，其中R^2具有上述IgG結合肽，R^3不存在。

(附記4)如附記1至3中任一項記載之化合物或其鹽，其中R^2係以上述IgG結合肽作為R^{2a}，由上述式II表示。

(附記5)如附記3或4記載之化合物或其鹽，其中上述IgG結合肽經藥物、反應性官能基或標識物質修飾。

(附記6)如附記1或2記載之化合物或其鹽，其中R^3具有上述IgG結合

肽，R^2具有藥物、反應性官能基或標識物質。

(附記7)如附記1、2及6之任一項記載之化合物或其鹽，其中R^3係以上述IgG結合肽作為R^{3a}，由上述式III、式IV及式V之任一者表示。

(附記8)如附記1至7中任一項記載之化合物或其鹽，其中上述IgG結合肽之胺基酸序列係序列編號1至115之任一者所示之胺基酸序列。

(附記9)如附記6或7記載之化合物或其鹽，其中上述IgG結合肽之胺基酸序列係序列編號116至151之任一者所示之胺基酸序列。

(附記10)一種抗體修飾試劑，其包含如附記1至9中任一項記載之化合物或其鹽。

(附記11)一種修飾抗體之製造方法，其包括如下反應步驟：使附記10中記載之抗體修飾試劑與IgG進行反應。

[實施例]

【0097】

藉由以下之實施例，對本發明更具體地進行說明，但本發明並不受實施例限定。

【0098】

實施例1：化合物A之合成

以如下方式合成上述化合物A。使用0.25 mmol之Rink Amide PEG樹脂(0.55 mmol/g)，利用PurePep Chorus肽固相合成機(Gyros Protein Technologies公司製造)，反覆進行基於哌啶/NMP(1：4)之脫Fmoc、及Fmoc保護胺基酸/DIC/Oxyma(1 mmol/1 mmol/1 mmol)而構建保護肽樹脂。其中，Fmoc-Lys(N_3)之縮合係利用Fmoc-Lys(N_3)/DIC/Oxyma(0.50 mmol/0.50 mmol/0.50 mmol)實施。

【0099】

於三乙胺(35 μL)存在下，使戊二醯酸酐(0.1 g)於N-甲基-2-吡咯啶酮(NMP)中與所獲得之保護肽樹脂(0.20 mmol)之N末端胺反應1小時。其後，使用偶合試劑PyAop(0.625 g)、HOAt(0.163 g)及N,N-二異丙基乙基胺(DIEA)(0.612 mL)，使N-甲基-1,2-苯二胺二鹽酸鹽(0.234 g)與所獲得之N末端羧酸樹脂縮合。其後，使氯甲酸4-硝基苯酯(0.4 g)於二氯甲烷中反應1小時，構建PMD(苯氧基羰化N-甲基-鄰二胺基苯)(NO_2)骨架。將所獲得之樹脂利用三氟乙酸(TFA)溶液進行處理，進行脫樹脂及脫保護，利用二乙醚進行固化，獲得721 mg之粗肽(2SH體)。使所獲得之粗肽(2SH體)中之250 mg溶解於乙酸/水(1：1)中，於冰浴冷卻下緩慢地添加0.1 M之碘-甲醇溶液1當量。90秒後，利用抗壞血酸水溶液使反應淬滅，利用逆相高效液相層析法(HPLC)管柱進行純化、冷凍乾燥，藉此獲得50 mg之所需SS體肽即PMD(NO_2)-αZ34C(化合物A)。

【0100】

實施例2：化合物B之合成

以如下方式合成上述化合物B。使用0.25 mmol之Rink Amide PEG樹脂(0.55 mmol/g)，利用PurePep Chorus肽固相合成機(Gyros Protein Technologies公司製造)，反覆進行基於哌啶/NMP(1：4)之脫Fmoc、及Fmoc保護胺基酸/DIC/Oxyma(1 mmol/1 mmol/1 mmol)而構建保護肽樹脂。其中，Fmoc-MeDbz之縮合係利用Fmoc-MeDbz/HCTU/6-Cl HOBt/DIEA(1 mmol/1 mmol/1 mmol/2 mmol)實施，Fmoc-Lys(N_3)之縮合係利用Fmoc-Lys(N_3)/DIC/HOAt(0.375 mmol/0.375 mmol/0.375 mmol)實施。

【0101】

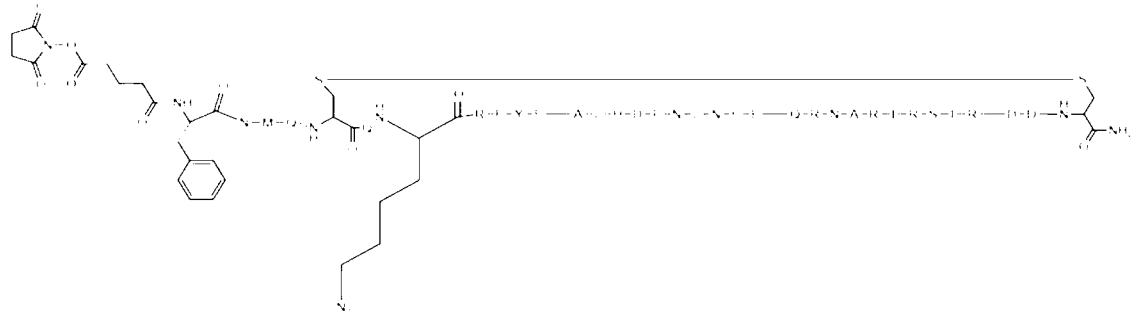
使氯甲酸4-硝基苯酯(0.5 g)於二氯甲烷中與所獲得之保護肽樹脂(0.25 mmol)反應1小時，而構建PMD(NO_2)骨架。將所獲得之樹脂利用TFA溶液進行處理，進行脫樹脂及脫保護，利用二乙醚進行固化，獲得966 mg之粗肽(2SH體)。將所獲得之粗肽利用逆相HPLC管柱進行純化、冷凍乾燥，藉此獲得147 mg之2SH體。其後，使粗肽(2SH體)溶解於乙酸/水(1：1)中，於冰浴冷卻下緩慢地添加1當量之0.1 M之碘・甲醇溶液。30秒後，利用抗壞血酸水溶液使反應淬滅，利用逆相HPLC管柱進行純化及冷凍乾燥，藉此獲得87 mg之所需之SS體肽即AzideKGG-PMD(NO_2)-αZ34C(F1Gaba，K7R)(化合物B)。

【0102】

為了比較，而合成具有DSG之下述化合物。

【0103】

[化17]



【0104】

使用0.25 mmol之Rink Amide PEG樹脂(0.55 mmol/g)，利用PurePep Chorus肽固相合成機(Gyros Protein Technologies公司製造)，反覆進行基於哌啶/NMP(1：4)之脫Fmoc、及Fmoc保護胺基酸/DIC/Oxyma(1 mmol/1 mmol/1 mmol)而構建保護肽樹脂。其中，Fmoc-Lys(N_3)之縮合係

利用Fmoc-Lys(N_3)/DIC/Oxyma(0.50 mmol/0.50 mmol/0.50 mmol)來實施。

【0105】

將所獲得之保護肽樹脂利用TFA溶液進行處理，進行脫樹脂及脫保護，將粗肽(2SH體)利用二乙醚固化並濾取。使所獲得之粗肽(2SH體)溶解於乙酸/水(1：1)中，於冰浴冷卻下緩慢地添加0.1 M之碘・甲醇溶液。60秒後，利用抗壞血酸水溶液使反應淬滅，利用逆相HPLC管柱進行純化、冷凍乾燥，藉此獲得61 mg之具有N末端游離胺成分之前驅物肽(SS體)。最後，使前驅物肽(SS體)60 mg溶解於DMSO中，緩慢地加入DSG(18 mg)及DIEA(12 μL)，將N末端之胺基轉化為丁二醯亞胺基酯並進行活化。10分鐘後，利用0.1%TFA水進行稀釋，利用逆相HPLC管柱進行純化、冷凍乾燥，藉此獲得19 mg之所需之DSG-αZ34C(以下設為比較例)。

【0106】

試驗例1：穩定性之研究

使化合物A、化合物B或比較例以達到20 mg/mL之方式溶解於DMSO中，於室溫或-20℃下靜置規定時間，利用HPLC進行分析。

【0107】

(結果)

針對化合物A、化合物B及比較例，將主峰面積相對於全部峰之面積之和之比例(HPLC純度)示於表1。於室溫及-20℃下化合物A及化合物B較比較例穩定。

【0108】

[表1]

	室溫		-20℃	
	0小時	22小時	0天	30天
比較例	85%	83%	92%	85%
化合物A	94%	93%	94%	94%
化合物B	98%	92%	98%	98%

【0109】

實施例3：化合物A及化合物B之對於人IgG之反應之評價

[與人IgG1之反應]

使化合物A或化合物B與抗體(人IgG1抗體醫藥品安挺樂(Actemra)、托珠單抗)於4種pH條件下進行反應。即，於20 mg/ml之IgG溶液20 μL中加入0.5 M 乙酸緩衝液(pH值5.5)、0.5 M 磷酸緩衝液(pH值7.0)、0.5 M Hepes緩衝液(pH值8.0)或0.5 M 碳酸氫鹽緩衝液(pH值8.9)20 μL，並添加蒸餾水138.4 μL。最後，每次加入1.56 μL之溶解於DMSO中之10 mM之化合物A或10 mM之化合物B，快速攪拌後，使總量達到200 μL(最終反應物中之IgG濃度為15.6 μM，試劑為5倍莫耳比之78 μM)。於2小時後進行取樣，利用最終濃度10%甲酸使反應終止後，利用LC-MS進行分析。

【0110】

[反應物之LC-MS分析]

將終止反應之反應溶液利用0.1%甲酸稀釋至5倍後，利用連結有蛋白質BEH C4管柱(300Å，1.7 μm, 2.1×500 mm，Waters公司製造)之BioAccord LC-MS系統(Waters公司製造)對所獲得之溶液20 μL進行分析(流速：0.4 mL/分、溶出：包含0.1%甲酸之1%CH_3CN至50%CH_3CN之線性梯度，管柱溫度：80℃)。

【0111】

(結果)

將化合物A與IgG之2小時後之反應溶液之LC-MS的結果示於圖3及圖4。圖3(A)、(B)、(C)及(D)分別為pH值5.5、7.0、8.0及8.9之反應溶液之溶出層析圖(280 nm)。2.23分鐘或2.25分鐘之波峰為與IgG之溶出對應之波峰。圖4(A)、(B)、(C)及(D)分別表示對pH值5.5、7.0、8.0及8.9之反應溶液中之IgG之波峰進行質量分析所得之結果。圖4(A)所示之pH值5.5之反應液中之質量147879 Da、148041 Da及148203 Da之分子種類分別為藉由IgG之糖鏈之多樣性檢測到之N-G0F×2(分子種類1)、N-G0F×1＋N-G1F×1(分子種類2)及N-G1F×2(分子種類3)。pH值5.5時，未觀察到藉由附加肽而得到修飾之分子種類。

【0112】

圖4(B)所示之pH值7.0之反應溶液及圖4(C)所示之pH值8.0之反應溶液中，與pH值5.5時大致同樣地，均未觀察到得到修飾之分子種類。另一方面，於圖4(D)所示之pH值8.9之反應溶液中，觀察到質量152195 Da、152357 Da及152519 Da之分子種類。其等較IgG之分子種類1、分子種類2及分子種類3之質量，分別增加了4316 Da、4316 Da及4317 Da。這大致與期待藉由化合物A而將肽附加至IgG時會增加之質量4333 Da一致，因此表明，化合物A在pH值8.9時與IgG發生反應。

【0113】

將化合物B與IgG之2小時後之反應溶液之LC-MS的結果示於圖5及圖6。圖5(A)、(B)、(C)及(D)分別為pH值5.5、7.0、8.0及8.9之反應溶液之溶出層析圖(280 nm)。1.93分或2.02分之波峰為與化合物B之溶出對應之波峰。2.25分或2.27分之波峰為與IgG之溶出對應之波峰。圖6(A)、(B)、

(C)及(D)分別表示對pH值5.5、7.0、8.0及8.9之反應溶液中之IgG之波峰進行質量分析所得之結果。如圖6(A)所示，於pH5.5時，觀察到IgG之分子種類1(147882Da)、分子種類2(148044 Da)、分子種類3(14820 8Da)，但於圖6(B)所示之pH值7.0時，分子種類1減少，148193 Da之分子種類增加。該311 Da之差係與藉由化合物B之反應所附加之疊氮化KGG之附加量(311.33 Da)一致。

【0114】

進而，於圖6(C)所示之pH值8.0及圖6(D)所示之pH值8.9時，分別增加了148505 Da及148501 Da之分子種類。該等分子種類之質量較分子種類1，增加了623 Da或619 Da，相當於疊氮化KGG之附加量(311.33)之2倍，因此認為2個疊氮化KGG附加至IgG，又，關於分子種類2，觀察到如下情況：於pH值7.0時，認為附加了1個疊氮化KGG之148353 Da及認為附加了2個疊氮化KGG之148664 Da之分子種類出現增加，於pH值8.0、及8.9時，亦在附加1個後接著附加2個。根據以上情況表明，化合物B在pH值7.0以上時與IgG發生反應。

【0115】

實施例4：化合物B對於小鼠IgG之反應之評價

[與小鼠IgG之反應]

使化合物B與小鼠IgG2抗體(抗DYKDDDDK 小鼠IgG2b單株抗體)於2個pH值條件下以如下方式發生反應。於0.98 mg/mL之IgG溶液30 μL中加入0.2 M Hepes緩衝液(pH值8.0)或0.2 M 碳酸氫鹽緩衝液(pH值8.9)22 μL。最後，每次加入8 μL之溶解於DMSO中之50 μM之化合物B，快速攪拌後，使總量達到60 μL(最終反應物中之IgG濃度為1.67 μM，試劑為4倍

莫耳比之6.67 μM)。於2小時後進行取樣，利用最終濃度5%甲酸使反應終止後，與實施例3同樣地利用LC-MS進行分析。

【0116】

(結果)

將化合物B與小鼠IgG之反應溶液之LC-MS之結果示於圖7及圖8。圖7(A)、(B)及(C)分別為添加化合物B之前之pH值8.0之抗體溶液、反應2小時後之pH值8.0之溶液及反應2小時後之pH值8.9之溶液的溶出層析圖(280 nm)。2.30分及2.53分之波峰為與小鼠IgG之溶出對應之波峰。圖8(A)、(B)及(C)分別表示對添加化合物B之前之pH值8.0之抗體溶液、反應2小時後之pH值8.0之溶液及反應2小時後之pH值8.9之溶液中的IgG之波峰進行質量分析所得之結果。圖8(A)所示之添加化合物B之前之pH值8.0之溶液中的質量150094 Da、150256 Da及150419 Da之分子種類分別為藉由IgG之糖鏈之多樣性所檢測到之N-G0F×2(分子種類1)、N-G0F×1＋N-G1F×1(分子種類2)及N-G1F×2(分子種類3)。

【0117】

如圖8(B)及(C)分別所示，於pH值8.0及pH值8.9時，分子種類1～3減少，另一方面，新增加了150569 Da、150729 Da、150884 Da及151039 Da之分子種類。質量150569 Da較分子種類2增加313 Da。質量150729 Da較分子種類3增加310 Da，較分子種類1增加635 Da。質量150884 Da較分子種類2增加628 Da。質量151039 Da較分子種類3增加620 Da。該等質量之差係相當於藉由化合物B之反應附加於抗體之疊氮化KGG之附加量(311.33 Da)或其2倍，因此觀察到在第1個之附加後接著發生第2個之附加。根據以上情況表明，化合物B在pH值8.0以上時與小鼠抗體發生反

應。

【0118】

實施例5：化合物B對於兔IgG之反應之評價

[與兔IgG之反應]

使化合物B與兔抗體(抗小鼠IgG1單株兔抗體)於pH值8.0之條件下以如下方式發生反應。於1.93 mg/mL之IgG溶液25 μL中添加0.5 M Hepes緩衝液(pH值8.0)10 μL及蒸餾水5 μL。最後，每次加入60 μL之溶解於蒸餾水中之89.3 μM之化合物B，快速攪拌後，使總量達到100 μL(最終反應物中之IgG濃度為6.7 μM，化合物B為8倍莫耳比之53.6 μM)。於2小時後進行取樣，利用最終濃度5%甲酸使反應終止後，與實施例3同樣地利用LC-MS進行分析。

【0119】

(結果)

將化合物B與兔IgG之反應溶液之LC-MS之結果示於圖9及圖10。圖9(A)及(B)分別為添加化合物B之前之pH值8.0之抗體溶液及反應24小時後之pH值8.0之溶液的溶出層析圖(280 nm)。圖10(A)及(B)分別表示對添加化合物B之前之pH值8.0之抗體溶液及反應24小時後之pH值8.0之溶液中的IgG之波峰進行質量分析所得之結果。圖10(A)所示之添加化合物B之前之pH值8.0之反應液中的質量144237 Da、144397 Da及144559 Da之分子種類分別為藉由IgG之糖鏈之多樣性所檢測到之N-G0F×2(分子種類1)、N-G0F×1＋N-G1F×1(分子種類2)及N-G1F×2(分子種類3)。

【0120】

如圖10(B)所示，分子種類1～3減少，另一方面，新增加了質量

144544 Da、144713 Da、144864 Da、145019 Da、145179 Da之分子種類。質量144544 Da較分子種類1增加307 Da。質量144713 Da較分子種類2增加316 Da。質量144864 Da較分子種類3增加305 Da，較分子種類1增加627 Da。質量145019 Da較分子種類2增加622 Da。質量1445179 Da較分子種類3增加620 Da。該等質量之差相當於藉由化合物B之反應所附加之疊氮化KGG之附加量(311.33 Da)或其2倍，因此觀察到在第1個之附加後接著發生第2個之附加。根據以上情況表明，化合物B於pH值8.0以上時與兔抗體亦發生反應。

【0121】

實施例6：化合物C之合成

以如下方式合成上述化合物C。使用0.25 mmol之Rink Amide PEG樹脂(0.48 mmol/g)，利用PurePep Chorus肽固相合成機(Gyros Protein Technologies公司製造)，反覆進行基於哌啶/NMP(1：4)之脫Fmoc、及Fmoc保護胺基酸/DIC/Oxyma(1 mmol/1 mmol/l mmol)，而構建具有N末端MeDbz之保護肽樹脂。其後，使用0.03 mmol量，依序使Fmoc-AEEA(胺基乙氧基乙氧基乙酸)及生物素縮合。其中，Fmoc-MeDbz之縮合係利用Fmoc-MeDbz/HCTU/6-Cl HOBt/DIEA(1 mmol/1 mmol/1 mmol/2 mmol)實施，Fmoc-AEEA之縮合係利用Fmoc-AEEA/DIC/Oxyma(1 mmol/1 mmol/1 mmol)實施，生物素之縮合係利用生物素/DIC/Oxyma(0.5 mmol/0.5 mmol/0.5 mmol)實施。

【0122】

使氯甲酸4-硝基苯酯(0.06 g)於二氯甲烷中與所獲得之保護肽樹脂(0.03 mmol)反應1小時，而構建PMD(NO_2)骨架。將所獲得之樹脂利用

TFA溶液進行處理，進行脫樹脂及脫保護，利用二乙醚進行固化，而獲得130 mg之粗肽(2SH體)。其後，使粗肽(2SH體)65 mg溶解於乙酸/水(1：1)中，於冰浴冷卻下，緩慢添加數當量之0.1 M之碘・甲醇溶液。60秒後，利用抗壞血酸水溶液使反應淬滅，利用逆相HPLC管柱進行純化及冷凍乾燥，藉此獲得10 mg之所需之SS體肽即Biotin-AEEA-PMD(NO_2)-aZ34C(F1Gaba，K7R)(化合物C)。

【0123】

實施例7：化合物D之合成

以如下方式合成上述化合物D。使0.62 mg之化合物B溶解於10 μL之DMSO中，將0.15 mg之2倍莫耳比之FAM-DBCO並進行靜置(最終反應物中之化合物D之濃度為10.8 mM)。1小時候，藉由高效液相層析法確認到化合物B消失，生成化合物D。不進行純化而直接用於與IgG之反應。

【0124】

實施例8：化合物C對於人IgG之反應之評價

[與人IgG之反應]

使化合物C與抗體(托珠單抗)於4種pH條件下進行反應。即，於20 mg/ml之IgG溶液20 μL中加入0.5 M 乙酸緩衝液(pH值5.5)、0.5 M 磷酸緩衝液(pH值7.0)、0.5 M Hepes緩衝液(pH值8.0)或0.5 M 碳酸氫鹽緩衝液(pH值8.9)40 μL，進而添加蒸餾水138.4 μL。最後，每次加入1.56 μL之溶解於DMSO中之10 mM之化合物C，迅速攪拌後，使總量達到200 μL(最終反應物中之IgG濃度為15.6 μM，化合物C為5倍莫耳比之78 μM)。於2小時後進行取樣，利用最終濃度10%甲酸使反應終止後，以與實施例3相同之方式利用LC-MS進行分析。

【0125】

(結果)

將化合物C與人IgG之反應2小時後之反應溶液的LC-MS之結果示於圖11及圖12。圖11(A)、(B)、(C)及(D)分別為pH值5.5、7.0、8.0及8.9之反應溶液之溶出層析圖(280 nm)。2.27分之波峰為與IgG之溶出對應之波峰。圖12(A)、(B)、(C)及(D)分別表示對pH值5.5、7.0、8.0及8.9之反應溶液中之IgG之波峰進行質量分析所得之結果。圖12(A)所示之pH值5.5之反應液中之質量147878 Da、148039 Da及148201 Da之分子種類分別為藉由IgG之糖鏈之多樣性所檢測到之N-G0F×2(分子種類1)、N-G0F×1＋N-G1F×1(分子種類2)及N-G1F×2(分子種類3)。

【0126】

如圖12(A)所示，於pH值5.5時，未觀察到經化合物C修飾之分子種類。另一方面，於圖12(B)所示之pH值7.0時，分子種類1減少，而增加了148248 Da之分子種類。該370 Da之差係與藉由化合物C之反應所附加之生物素-AEEA(以下亦簡稱為“生物素-PEG”)之附加量(372.46 Da)一致。

【0127】

進而，於圖12(C)所示之pH值8.0及圖12(D)所示之pH值8.9時，分別增加了148620 Da及148619 Da之分子種類。該等分子種類之質量較分子種類1，分別增加742 Da及743 Da。該等質量之差係相當於生物素-PEG之附加量(372.46 Da)之2倍，因此認為2個生物素-PEG附加於IgG，又，關於分子種類2，觀察到如下情況：於pH值7.0時，認為附加了1個生物素-PEG之148411 Da之分子種類出現增加，於pH值8.0及8.9時，認為附加了1

個生物素-PEG之148409 Da及認為附加了2個生物素-PEG之148782 Da之分子種類亦出現增加，於第1個之附加後接著發生了第2個之附加。根據以上情況表明，化合物C於pH值7.0以上時與人IgG發生了反應。

【0128】

實施例9：化合物D對於人類化IgG之反應之評價

[與IgG之反應]

使化合物D與抗體(抗HER2人類化單株抗體醫藥品賀癌平、曲妥珠單抗)於pH值8.0或8.9之條件下以如下方式發生。於20 mg/ml之IgG溶液20 μL中添加0.5 M Hepes緩衝液(pH值8.0)或0.5 M 碳酸氫鹽緩衝液(pH值8.9)40 μL，進而添加蒸餾水138 μL。最後，每次添加2 μL之化合物D之DMSO溶液10.8 mM，迅速攪拌後，使總量達到100 μL(最終反應物中之IgG濃度為13.5 μM，化合物D為8倍莫耳比之108 μM)。於24小時後進行取樣，利用最終濃度5%甲酸使反應終止後，與實施例3同樣地利用LC-MS進行分析。

【0129】

(結果)

將化合物D與人類化IgG之反應溶液之LC-MS之結果示於圖13及圖14。圖13(A)、(B)及(C)分別為添加化合物D之前之抗體溶液、反應24小時後之pH值8.0之溶液及反應24小時後之pH值8.9之溶液的溶出層析圖(280 nm)。2.23分之波峰為與IgG之溶出對應之波峰。圖14(A)、(B)及(C)分別為對添加化合物D之前之抗體溶液、反應24小時後之pH值8.0之溶液及反應24小時後之pH值8.9之溶液中的IgG之波峰進行質量分析所得之結果。圖14(A)中所示之添加化合物D之前之pH值8.0之反應液中的質量

148058 Da、148220 Da及148381 Da之分子種類分別為藉由IgG之糖鏈之多樣性所檢測到之N-G0F×2(分子種類1)、N-G0F×1＋N-G1F×1(分子種類2)及N-G1F×2(分子種類3)。

【0130】

如圖14(B)所示，於pH值8.0時，分子種類1～3減少，另一方面，新增加了質量149048 Da、149209 Da、149371 Da、150036 Da、150196 Da及150356 Da之分子種類。質量149048 Da較分子種類1增加990 Da。質量149209 Da較分子種類2增加989 Da。質量149371 Da較分子種類3增加990 Da。質量150036 Da較分子種類1增加1978 Da。質量150196 Da較分子種類2增加1976 Da。質量150356 Da較分子種類3增加1975 Da。該等質量之差係相當於藉由化合物D之反應所附加之FAM-DBCO-N3-KGG之附加量(988.03 Da)或其2倍，因此觀察到於第1個之附加後接著發生了第2個之附加。

【0131】

又，於pH值8.9時，亦分子種類1～3減少，另一方面，新增加了質量149077 Da、149235 Da、149399 Da、150062 Da、150224 Da及150386 Da之分子種類。質量149077 Da較分子種類1增加1019 Da。質量149235 Da較分子種類2增加1015 Da。質量149399 Da較分子種類3增加1018 Da。質量150062 Da較分子種類1增加2004 Da。質量150224 Da較分子種類2增加2004 Da。質量150386 Da較分子種類3增加2005 Da。該等質量之差大致相當於藉由化合物D之反應所附加之FAM-DBCO-N3-KGG之附加量(988.03 Da)或其2倍，因此觀察到於第1個之附加後接著發生了第2個之附加。根據以上情況表明，化合物D於pH值8.0以上時與人類化單株抗體發

生了反應。

【0132】

試驗例2：基於肽圖及質譜儀之修飾部位之特定

將曲妥珠單抗或與化合物B發生了反應之曲妥珠單抗(50 μg、25 μL之水中)與25 μL之0.2%Rapigest SF(Waters公司製造)加以混合。加入2.6 μL之100 mM二硫蘇糖醇(DTT、最終濃度5 mM)後，將溶液於60℃下保溫30分鐘。加入5.8 μL之150 mM碘乙醯胺(最終濃度15 mM)後，將該溶液於室溫、暗處中保溫30分鐘。加入12.5 μL之0.2 μg/μL胰蛋白酶，於37℃下保溫3.5小時後，加入7.85 μL之5%TFA(終濃度0.5%)以使反應終止。離心分離後，將上清液注入至與ACQUITY UPLC H-Class FTN系統(Waters公司製造)連接之ACQUITY UPLC Peptide BEH130 C18管柱(1.7 μm, 2.1×150 mm，Waters公司製造)，進行肽映射。溶出係以包含0.1%甲酸之2-90%乙腈梯度進行，將管柱烘箱之溫度保持為60℃。分離之肽之質量分析係利用與ACQUITY UPLC H-Class FTN系統(Waters公司製造)連接之Xevo G2-XS QTof質譜儀(Waters公司製造)進行，所獲得之質譜之分析係藉由MassLynx ver.4.1或UNIFI 1.9.2軟體來進行。

【0133】

(結果)

將肽圖之結果示於圖15。將與化合物B發生了反應之曲妥珠單抗(tCAP偶聯物)之結果及曲妥珠單抗之結果分別示於上段及下段。對各波峰之質量進行分析，結果在曲妥珠單抗時觀察到之在44.65分溶出之波峰(T20)顯示2844.45 Da之質量，與源自曲妥珠單抗H鏈之226-251殘基之T20肽之質量(2844.46 Da)一致。

【0134】

[表2]

片段名	胺基酸序列
T20	THTCPPCPAPELLGGPSVFLFPPKPKDTLMISR(序列編號154)

【0135】

另一方面，於tCAP偶聯物之情形時，在44.65分溶出之波峰消失，在50.77分溶出之波峰(T19-21)顯示質量4461.17 Da。該波峰如下所述，與在源自曲妥珠單抗H鏈之殘基編號222-258(Eu編號為219-255)之T19-T20-T21肽之質量上附加1個N-乙醯基Azide Lys-Gly-Gly後所得之質量4461.20一致。

【0136】

[表3]

片段名	胺基酸序列
T19-21	SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR(序列編號155)

【0137】

以上情況表示，N-乙醯基(Azide Lys)-Gly-Gly有1個附加到T19-T20-T21肽(殘基編號：222-258)上，作為其附加位置，認為Lys225、Lys249或Lys251(Eu編號為Lys222、Lys246或Lys248)為候補。此處，將tCAP偶聯物時在50.77分溶出之波峰之MS/MS之結果示於圖16。相較於作為母質量之4461.178，若追加b系列之質量資料，則檢測到與Lys-Thr-His(殘基編號：245-247)對應之質量峰之差量(b6與b3之差量)，因此表明，Lys225未引起修飾。另一方面，若追加y系列之質量資料，則檢測到與Lys-Pro(殘基編號：249-248)對應之質量峰之差量(y11與y9之差量)，因此表明，殘基編號Lys249(Eu編號為Lys246)未引起修飾。除此以外，還檢測到附加有N-乙醯基(Azide Lys)-Gly-Gly之Lys-Pro(殘基編號：251-250)之

質量峰之差量(y9與y7之差量)。以上情況明確表明，tCAP反應之主要修飾部位為殘基編號Lys251(Eu編號為Lys248)。

【0138】

實施例10：疊氮化人IgG抗體之抗癌劑修飾及螢光劑修飾

[曲妥珠單抗與抗癌劑或螢光劑之反應]

使化合物B與曲妥珠單抗反應而製備成疊氮化曲妥珠單抗，藉由點擊反應而製備上述疊氮化曲妥珠單抗與抗癌劑(DM1)或螢光劑(IRDye(商標)800CW)之偶聯物。首先，以如下方式製備用以利用抗癌劑修飾曲妥珠單抗之試劑。將溶解於DMSO中之100 mM 美登素(MedChemExpress公司製造)50 μL、溶解於DMSO中之100 mM 溴乙醯胺-dPEG(商標)4-醯胺基DBCO(QUANTA BIODESIGN公司製造)50 μL及1 M $NaHCO_3$溶液(pH值8.9)10 μL加以混合，於室溫下放置1小時。將如此製備成之DBCO-PEG4-DM1試劑溶液利用DMSO稀釋成1 mM，將1 mM DBCO-PEG4-DM1試劑溶液10 μL與製備成20 μM之疊氮化曲妥珠單抗之PBS溶液100 μL加以混合(莫耳比5：1)，並於室溫下放置0.5小時。

【0139】

於曲妥珠單抗之螢光劑修飾中，將溶解於DMSO中之1 mM IRDye(商標)800CW DBCO Infrared Dye(LI-COR Biosciences公司製造)10 μL與製備成20 μM之疊氮化曲妥珠單抗之PBS溶液100 μL加以混合(莫耳比為5：1)，並於室溫下放置1小時。對各反應液進行取樣，利用最終濃度5%甲酸使反應終止後，與實施例3同樣地利用LC-MS進行分析。

【0140】

(結果)

將疊氮化曲妥珠單抗之抗癌劑修飾後及螢光劑修飾後之反應溶液之LC-MS的結果分別示於圖17及圖18。圖17(A)、(B)、(C)及(D)分別為曲妥珠單抗(未修飾抗體)、經化合物B修飾之疊氮化曲妥珠單抗(疊氮化KGG修飾抗體)、抗癌劑與疊氮化曲妥珠單抗之反應物及疊氮化曲妥珠單抗與螢光劑之反應物之溶出層析圖(280 nm)。

【0141】

圖18(A)、(B)、(C)及(D)分別表示針對未修飾抗體、疊氮化KGG修飾抗體、抗癌劑與疊氮化曲妥珠單抗之反應物及疊氮化曲妥珠單抗與螢光劑之反應物，對溶出之各抗體進行質量分析之結果。如圖18(A)所示，於未修飾抗體時，由於糖鏈之多樣性，觀察到148058 Da、148220 Da及148381 Da這3個質量之分子種類。利用化合物B進行修飾，結果如圖18(B)所示，疊氮化KGG以一價或二價附加，作為一價，生成了148374 Da、148531 Da及148682 Da之質量之分子種類。作為二價，生成了148682 Da(與一價之波峰重疊)、148841 Da及149003 Da之質量之分子種類。根據圖18(B)之波峰比，算出0價(未修飾)：一價：二價之反應後之生成比率，為0：29：71。

【0142】

使疊氮化KGG修飾抗體與以莫耳比計為5倍量之DBCO-PEG4-DM1進行反應，結果如圖18(C)所示，生成了以一價或二價附加有DM1之分子種類。質量為149672 Da、149833 Da及149994 Da之分子種類為一價之修飾物，質量為151283 Da、151447 Da及151609 Da之分子種類為二價之修飾物。

【0143】

使疊氮化KGG修飾抗體與以莫耳比計為5倍量之IRDye(商標)800CW DBCO Infrared Dye進行反應，結果如圖18(D)所示，生成了以一價或二價附加有IRDye(商標)800CW之分子種類。質量為149630 Da、149792 Da及149954 Da之分子種類為一價之修飾物，質量為151202 Da、151365 Da及151526 Da之分子種類為二價之修飾物。

【0144】

如上所述，若使用疊氮化KGG修飾抗體，則利用點擊反應將附加有DBCO基之功能性配體附加於抗體，可容易使抗體高功能化。

【0145】

試驗例3：疊氮化KGG修飾抗體與Fc受體之親和性評價

疊氮化KGG修飾抗體對於Fc受體(FcγRI、FcγRIIa、FcγRIIb、FcγRIIIa及FcγRIIIb)之親和性分析係使用BIAcore 8K(Cytiva公司製造)於25℃下實施。將抗His標籤抗體(Cytiva公司製造)利用胺偶合法固定化至CM5感測器晶片上直至RU值約8000之量。進而將Fc受體(FcgRI-His、FcgRIIaI-His、FcgRIIbI-His、FcgRIIIaI-His或FcgRIIIbI-His、Sino Biological公司製造)以0.4 μg/mL之濃度注入至其中，藉此固定化至RU值約100之量。關於分析對象物，將以1600 nM至12.5 nM之濃度(FcγRI之情形時，1600 nM至0.05 nM之濃度)溶解於PBST緩衝液(PBS、0.005% Tween20)中之合計8種(FcγRI之情形時為16種濃度)濃度之疊氮化KGG修飾抗體或未修飾抗體以流速30 μL/分注入，獲得感測器圖譜(聚集時間：150秒、解離時間：250秒)(FcγRI之情形時，聚集時間：120秒、解離時間：480秒)。使用附帶之BIA評價軟體，藉由1：1結合模型算出解離常數(Kd)。

【0146】

另一方面，疊氮化KGG修飾抗體對於FcRn(胎兒性Fc受體)之親和性分析係使用BIAcore 8K(Cytiva公司製造)於25℃下以如下方式實施。將使用乙酸鈉pH值5.5溶液(Cytiva公司製造)製備成1.0 μg/mL濃度之FcRn(Sino Biological公司製造)注入至用於胺偶合反應之活化之CM5感測器晶片，進行固定化直至RU值約300之量。關於分析對象物，將以1600 nM至12.5 nM之濃度溶解於磷酸緩衝液(50 mM磷酸鈉、150 mM NaCl、pH值6.0)中之合計8種濃度之疊氮化KGG修飾抗體或未修飾抗體以流速30 μL/分注入，而獲得感測器圖譜(聚集時間：120秒、解離時間：150秒、再生：30秒)。使用附帶之BIA評價軟體，藉由二價結合模型算出解離常數(Kd)。再生溶液係使用100 mM 三羥甲基胺基甲烷鹽酸鹽、0.2 M NaCl、pH值8.0。

【0147】

(結果)

將評價疊氮化KGG修飾抗體及未修飾抗體與Fc受體之親和性所得之結果示於以下表中。除FcγRIIb以外，疊氮化KGG修飾抗體與未修飾抗體對於Fc受體之親和性未發現有較大差異。該情況表明，使用化合物B之修飾幾乎不會影響與對IgG抗體之效應功能重要之Fc受體的結合，而可作為一種偶聯物技術使用。

【0148】

[表4]

受體	對於未修飾抗體之Kd(nM)	對於疊氮化KGG修飾抗體之Kd(nM)
FcγRI	5.3	6.6
FcγRIIa	100	88
FcγRIIb	270	2000
FcγRIIIa	66	65
FcγRIIIb	4000	3200
FcRn	570	250

【0149】

上述實施方式係用以說明本發明者，並非限定本發明之範圍。即，本發明之範圍並非由實施方式表示，而是由申請專利範圍表示。並且，於申請專利範圍內及與其同等之發明之意義之範圍內所實施之各種變化視為本發明之範圍內。

【0150】

本發明係基於2021年9月8日提出申請之日本專利申請2021-146504號。以日本專利申請2021-146504號之說明書、申請專利範圍、圖式整體作為參照併入本說明書中。

[產業上之可利用性]

【0151】

本發明可用於修飾抗體之製造、包含抗體之複合體之製造及研究用試劑。

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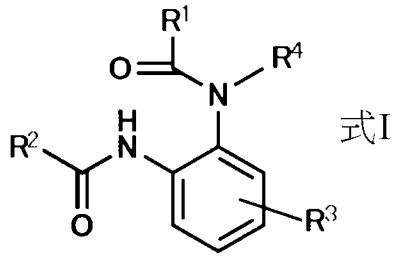
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【發明申請專利範圍】

【請求項1】

一種化合物或其鹽，該化合物由下述式I所示，

[化1]



式I

(式I中，R^1係R^1-H之pKa達到4～14之取代基，

R^2及R^3之一者具有與IgG之Fc區特異性結合之IgG結合肽，

於R^2具有上述IgG結合肽之情形時，R^3不存在，或者選自由經取代或未經取代之碳數1～8之烷基、經取代或未經取代之碳數2～8之烯基、經取代或未經取代之碳數2～8之炔基、硝基、鹵素、羧醯胺基所組成之群，

於R^3具有上述IgG結合肽之情形時，R^2係選自由經取代或未經取代之碳數1～8之烷基、經取代或未經取代之碳數2～8之烯基、經取代或未經取代之碳數2～8之炔基、胺基酸殘基數2～50之經取代或未經取代之肽鏈、聚合度2～50之經取代或未經取代之聚合物鏈及其等之組合所組成之群，

R^4係H或者經取代或未經取代之碳數1～8之烷基)。

【請求項2】

如請求項1之化合物或其鹽，其中R^1為硝基苯氧基。

【請求項3】

如請求項1或2之化合物或其鹽，其中R^2具有上述IgG結合肽，

R^3不存在。

[請求項4]

如請求項1或2之化合物或其鹽，其中R^2係以上述IgG結合肽作為R^{2a}，由下述式II表示，

[化2]



式II

(式II中，*表示式I之羰基之碳)。

[請求項5]

如請求項3之化合物或其鹽，其中上述IgG結合肽經藥物、反應性官能基或標識物質修飾。

[請求項6]

如請求項4之化合物或其鹽，其中上述IgG結合肽經藥物、反應性官能基或標識物質修飾。

[請求項7]

如請求項1之化合物或其鹽，其中R^3具有上述IgG結合肽，

R^2具有藥物、反應性官能基或標識物質。

[請求項8]

如請求項2之化合物或其鹽，其中R^3具有上述IgG結合肽，

R^2具有藥物、反應性官能基或標識物質。

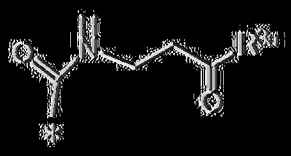
[請求項9]

如請求項1或2之化合物或其鹽，其中R^3係以上述IgG結合肽作為R^{3a}，由下述式III、式IV及式V之任一者表示，

[化3]



式III



式IV



式V

(式III、式IV及式V中，*表示式I之苯環之碳)。

[請求項10]

如請求項1或2之化合物或其鹽，其中上述IgG結合肽之胺基酸序列係序列編號1至115之任一者所示之胺基酸序列。

[請求項11]

如請求項7之化合物或其鹽，其中上述IgG結合肽之胺基酸序列係序列編號116至151之任一者所示之胺基酸序列。

[請求項12]

一種抗體修飾試劑，其包含如請求項1或2之化合物或其鹽。

[請求項13]

一種修飾抗體之製造方法，其包括使如請求項12之抗體修飾試劑與IgG進行反應之反應步驟。

[請求項14]

一種修飾抗體，其具有：經由包含Lys-Gly-Gly之原子團以一價或二價結合於IgG之Fc區之Lys的藥物、反應性官能基或標識物質。

[請求項15]

如請求項14之修飾抗體，其中上述原子團中所含有之Lys之側鏈之胺

基被取代為上述藥物、反應性官能基或標識物質。

【請求項16】

如請求項14或15之修飾抗體，其中上述反應性官能基為疊氮基。

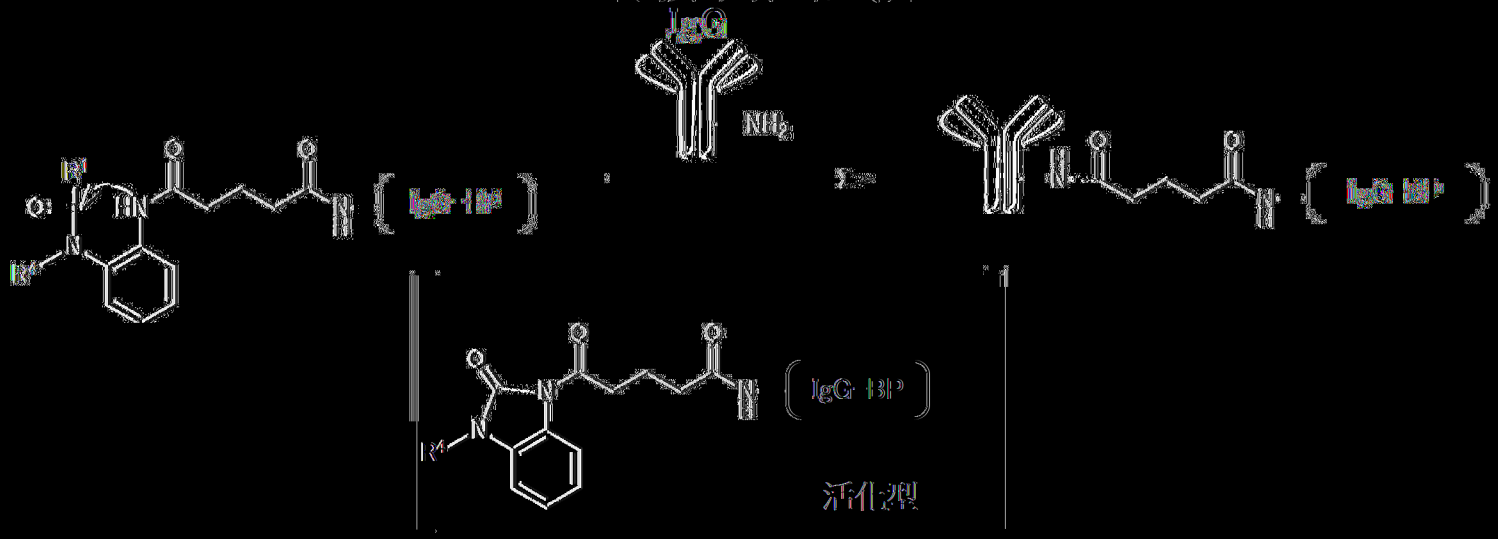
【請求項17】

如請求項14或15之修飾抗體，其中上述IgG為人IgG。

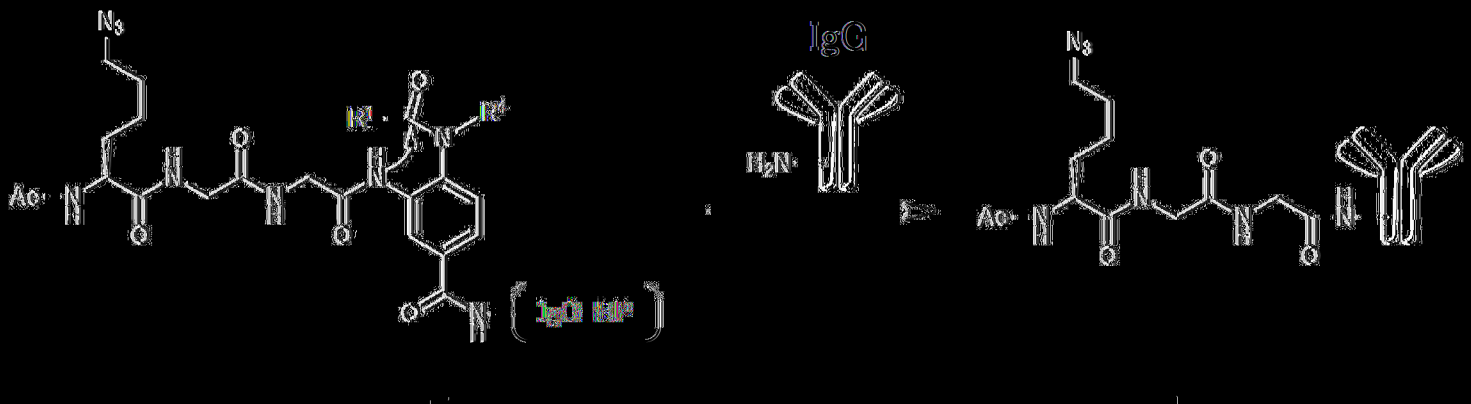
【請求項18】

如請求項14或15之修飾抗體，其中上述IgG為托珠單抗或曲妥珠單抗。

[發明圖式]

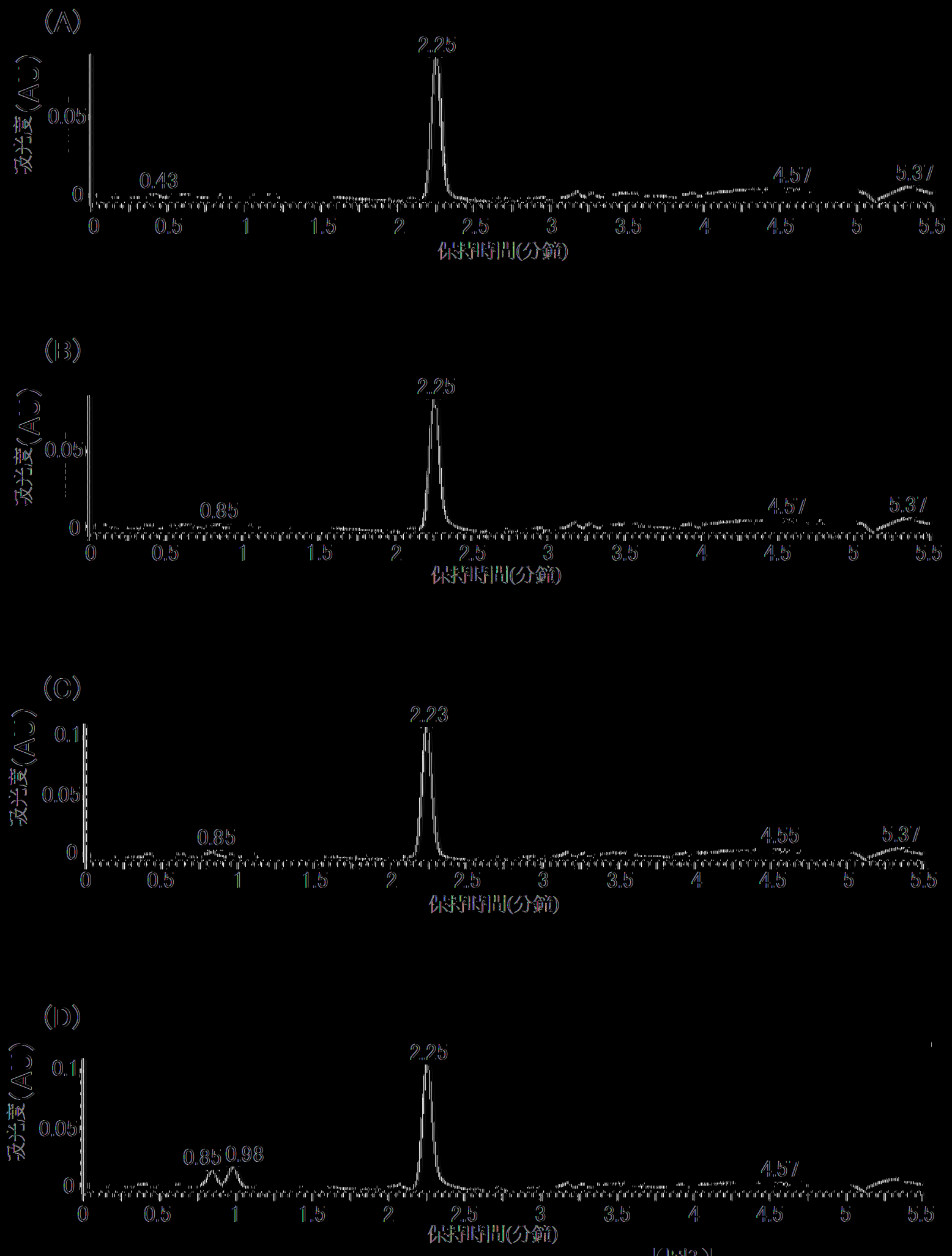

IgG
NH2
IgG-BP
IgG-BP
IgG-BP
活化型

[圖1]

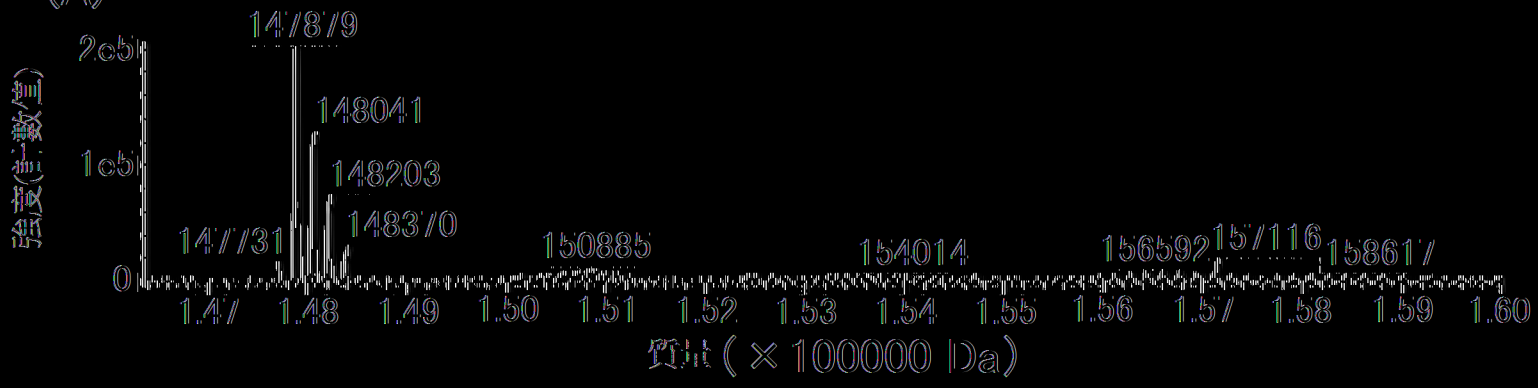

N3
Ac
IgG
H2N
IgG-BP


N3
Ac
活化型
IgG-BP

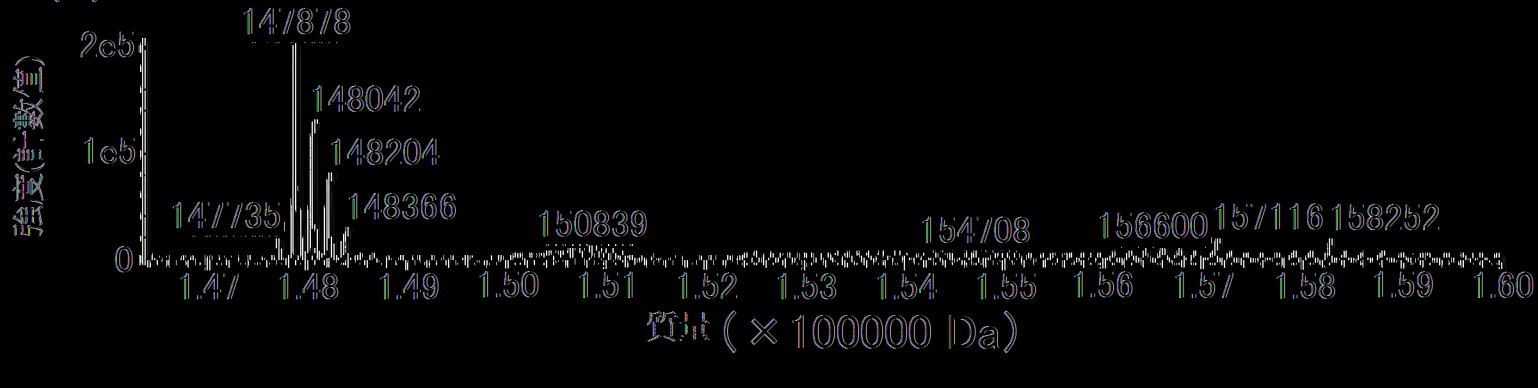
[圖2]


(A)
2.25
0.05
0
0.43
4.5左右
5.3左右
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5
吸光度(AU)
保持時間(分鐘)
(B)
2.25
0.05
0
0.85
4.5左右
5.3左右
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5
吸光度(AU)
保持時間(分鐘)
(C)
2.23
0.1
0.05
0
0.85
4.55
5.3左右
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5
吸光度(AU)
保持時間(分鐘)
(D)
2.25
0.1
0.05
0
0.85
0.98
4.5左右
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5
吸光度(AU)
保持時間(分鐘)

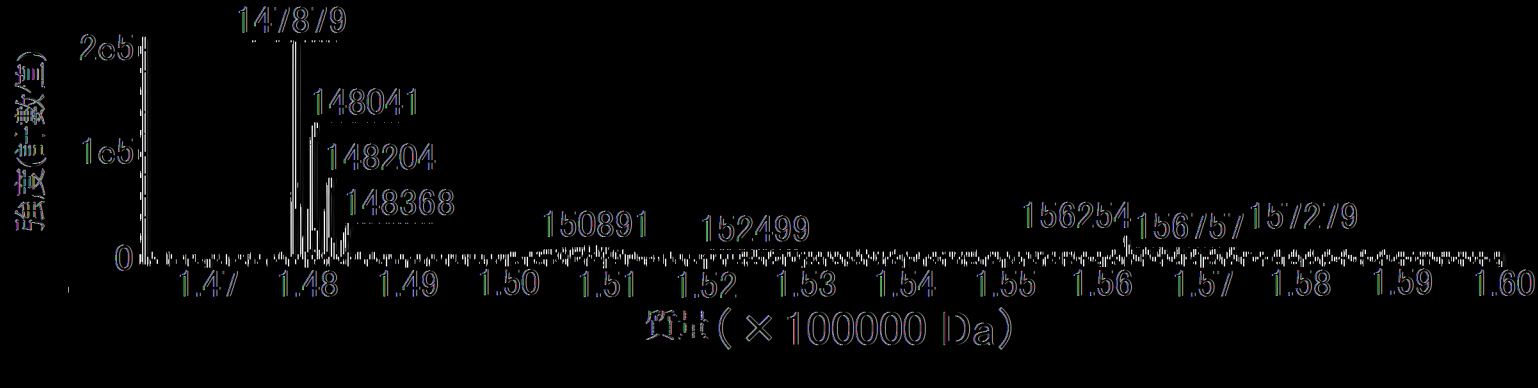
(A)


14787/9
148041
148203
148370
14777/31
150885
154014
156592
15/116
158617
2e5
1e5
0
1.47 1.48 1.49 1.50 1.51 1.52 1.53 1.54 1.55 1.56 1.57 1.58 1.59 1.60
質量（×100000 Da）

(B)


14787/8
148042
148204
148366
14777/35
150839
154708
156600
15/116
158252
2e5
1e5
0
1.47 1.48 1.49 1.50 1.51 1.52 1.53 1.54 1.55 1.56 1.57 1.58 1.59 1.60
質量（×100000 Da）

(C)


14787/9
148041
148204
148368
150891
152499
156254
156757
15727/9
2e5
1e5
0
1.47 1.48 1.49 1.50 1.51 1.52 1.53 1.54 1.55 1.56 1.57 1.58 1.59 1.60
質量（×100000 Da）

(D)

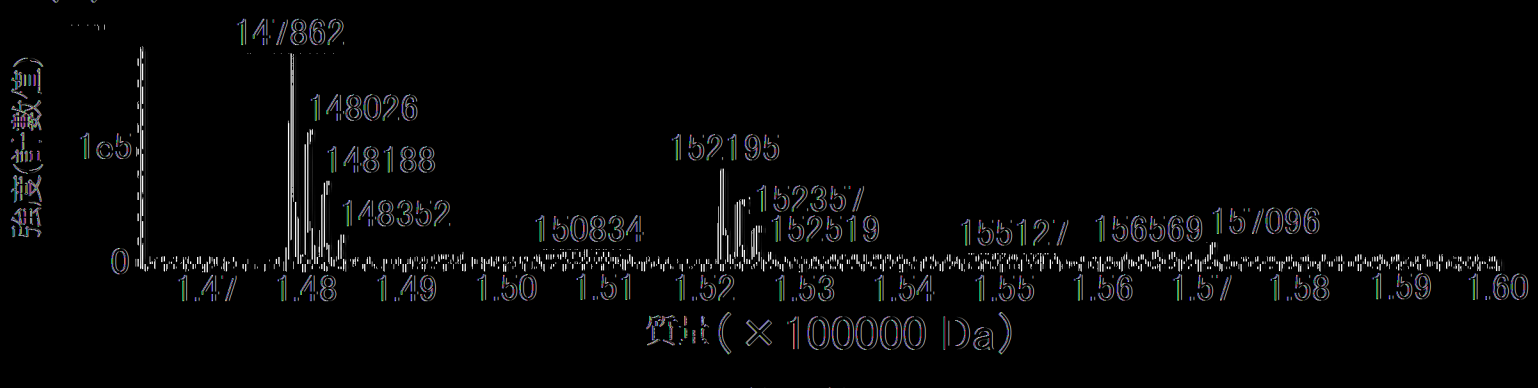
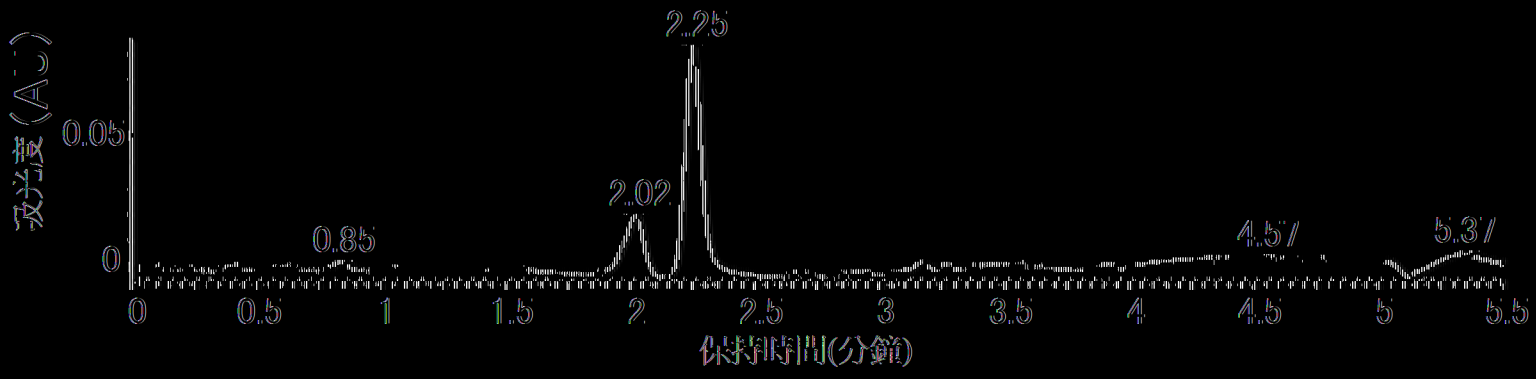
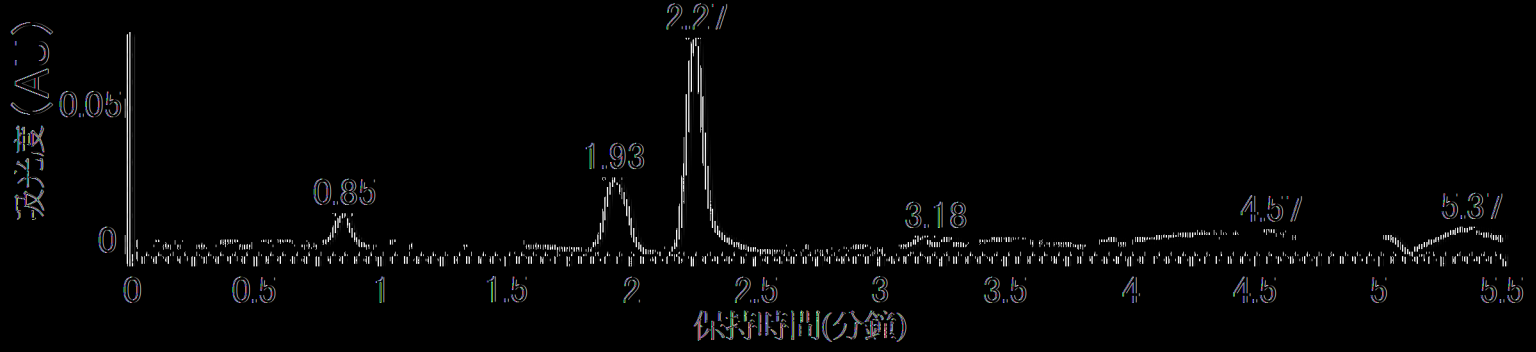

14786/2
148026
148188
148352
150834
152195
152357
152519
155127
156569
15/096
1e5
0
1.47 1.48 1.49 1.50 1.51 1.52 1.53 1.54 1.55 1.56 1.57 1.58 1.59 1.60
質量（×100000 Da）

圖4

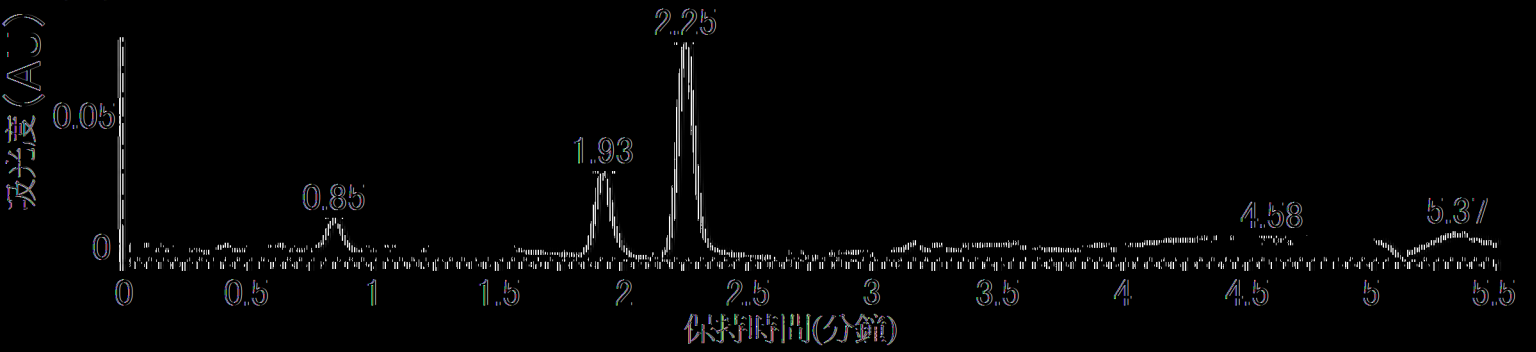
(A)


吸光度(AU)
0.05
0
0.85
2.02
2.25
4.57
5.37
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5
保持時間(分鐘)

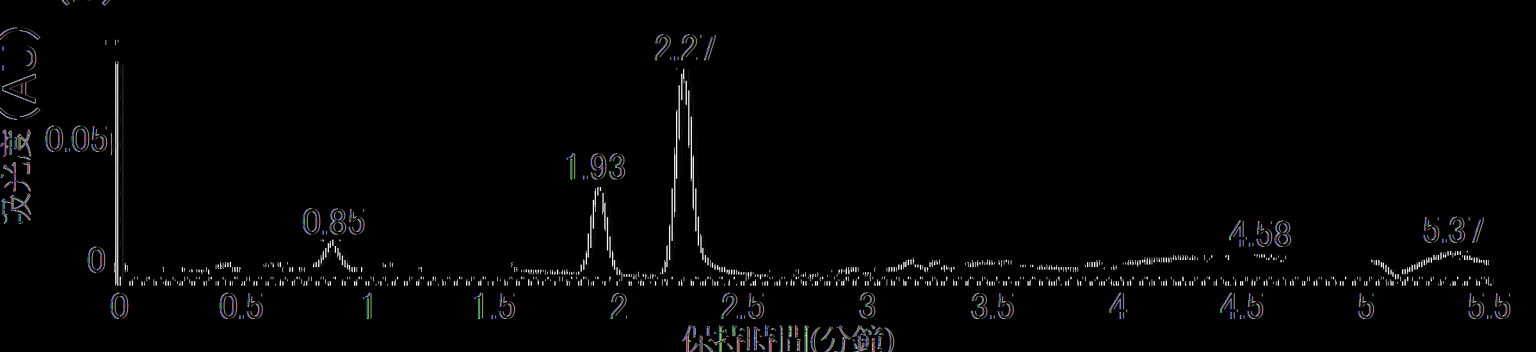
(B)


吸光度(AU)
0.05
0
0.85
1.93
2.27
3.18
4.57
5.37
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5
保持時間(分鐘)

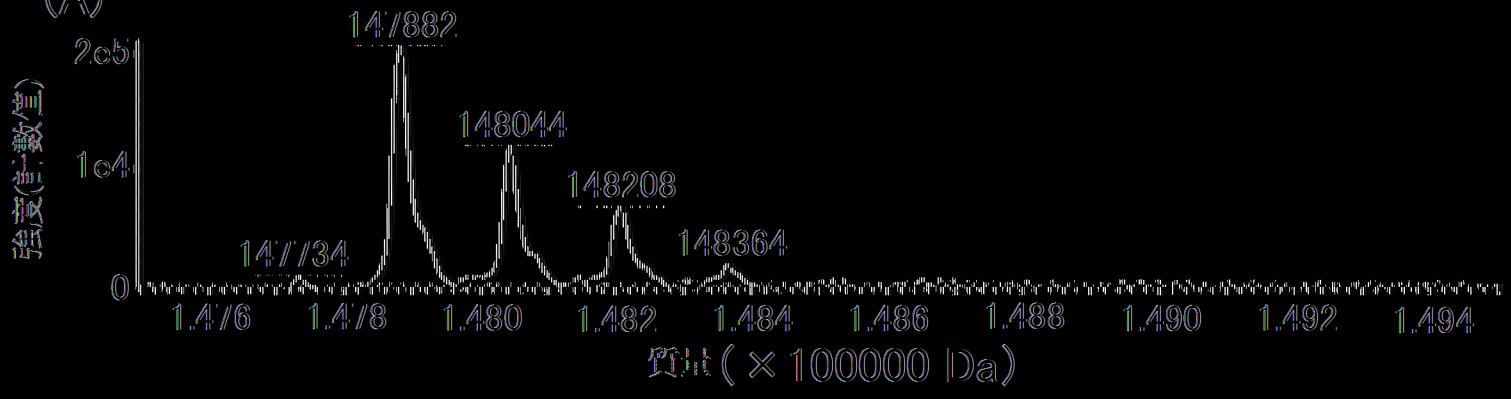
(C)


吸光度(AU)
0.05
0
0.85
1.93
2.25
4.58
5.37
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5
保持時間(分鐘)

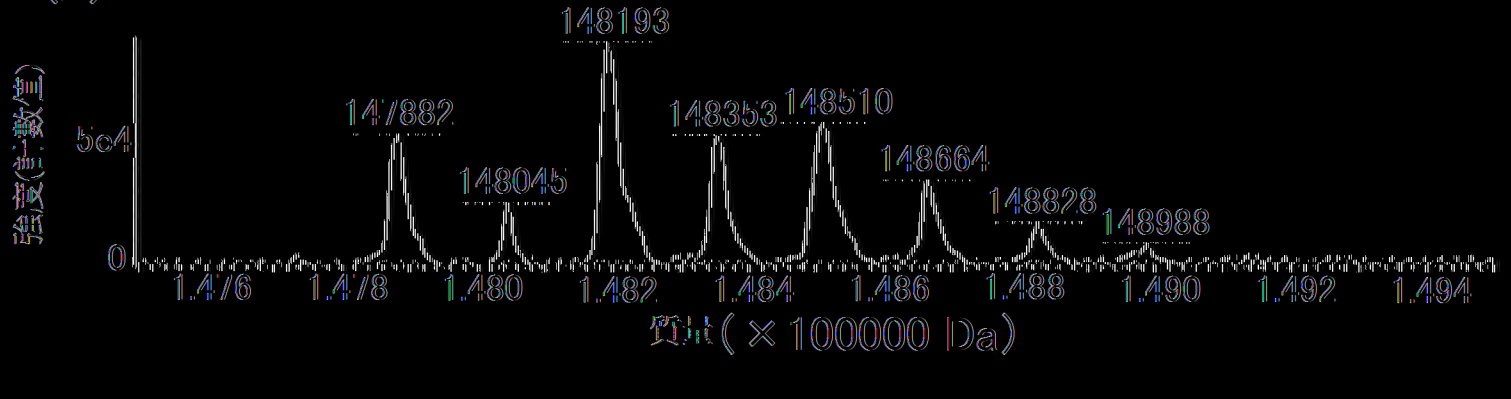
(D)


吸光度(AU)
0.05
0
0.85
1.93
2.27
4.58
5.37
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5
保持時間(分鐘)

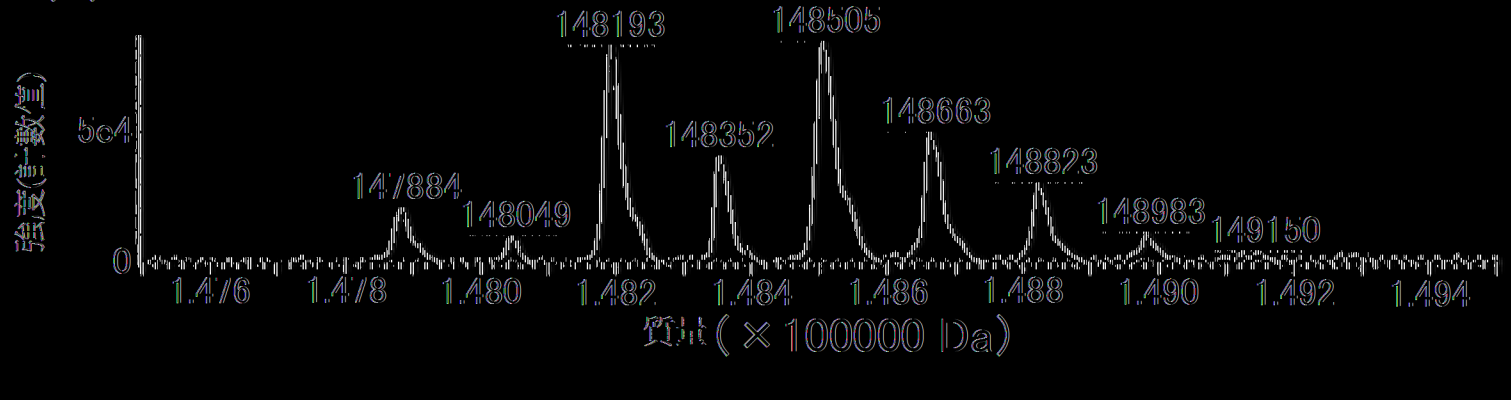
(A)


2e5
1e4
0
強度(任意単位)
147882
148044
148208
147734
148364
1.476
1.478
1.480
1.482
1.484
1.486
1.488
1.490
1.492
1.494
質量(×100000 Da)

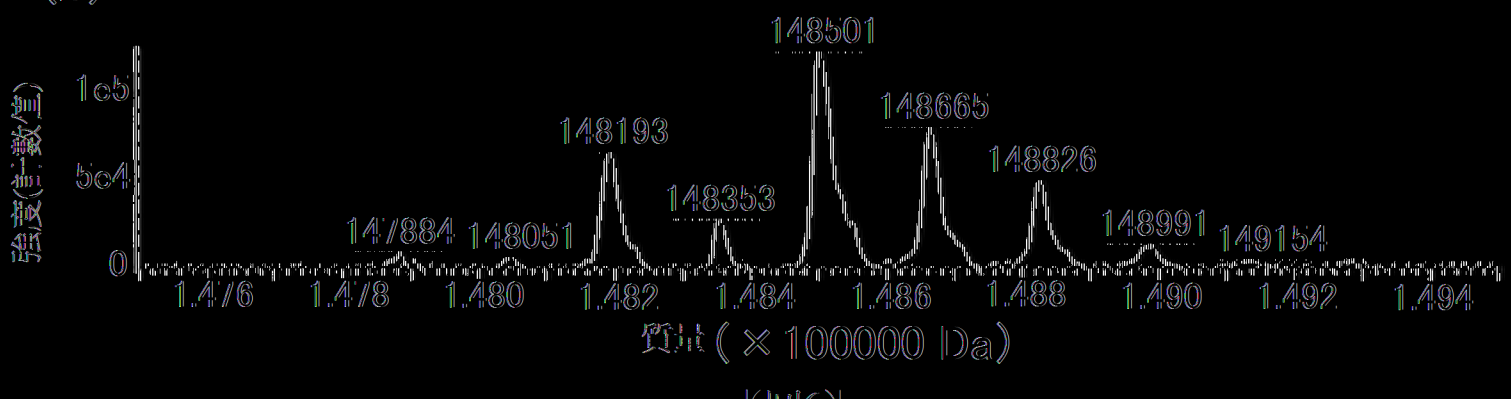
(B)


5e4
0
強度(任意単位)
148193
147882
148353
148510
148664
148045
148828
148988
1.476
1.478
1.480
1.482
1.484
1.486
1.488
1.490
1.492
1.494
質量(×100000 Da)

(C)

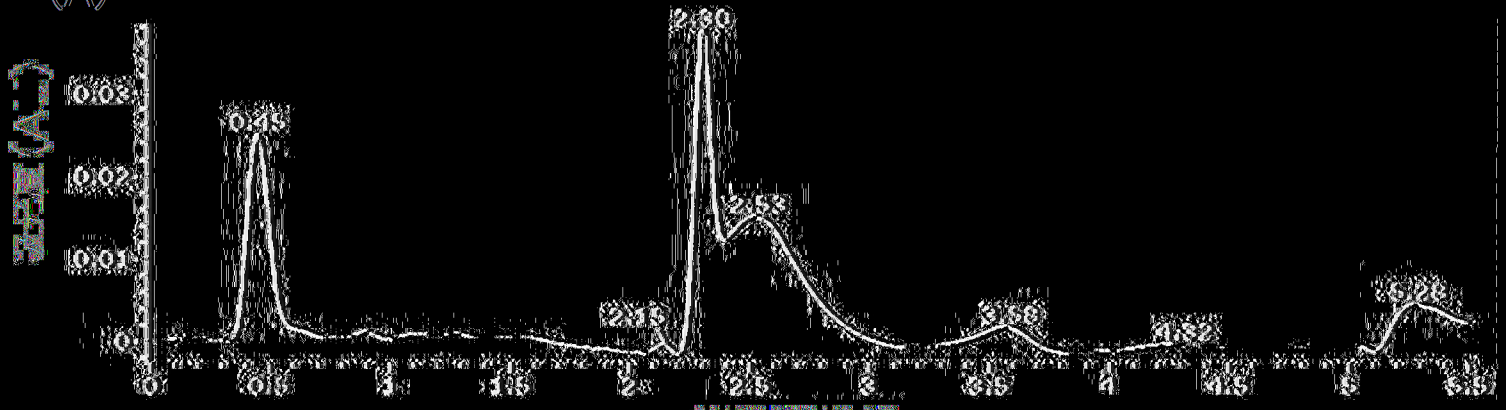

5e4
0
強度(任意単位)
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148505
148663
148352
148823
147884
148049
148983
149150
1.476
1.478
1.480
1.482
1.484
1.486
1.488
1.490
1.492
1.494
質量(×100000 Da)

(D)


1e5
5e4
0
強度(任意単位)
148501
148193
148665
148826
148353
147884
148051
148991
149154
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1.478
1.480
1.482
1.484
1.486
1.488
1.490
1.492
1.494
質量(×100000 Da)

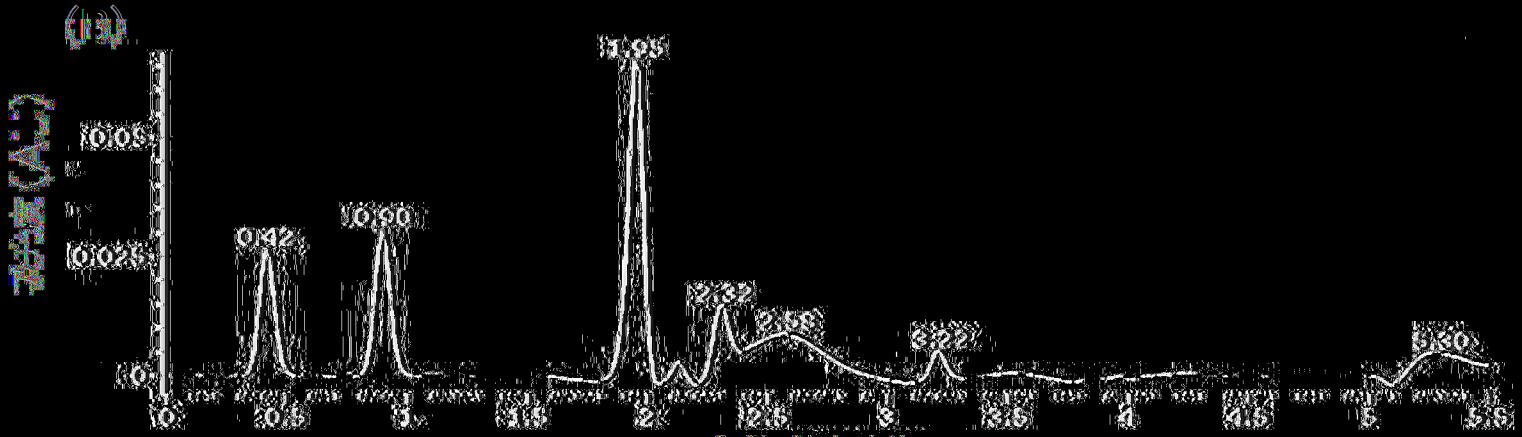
|(B|6)|

(A)



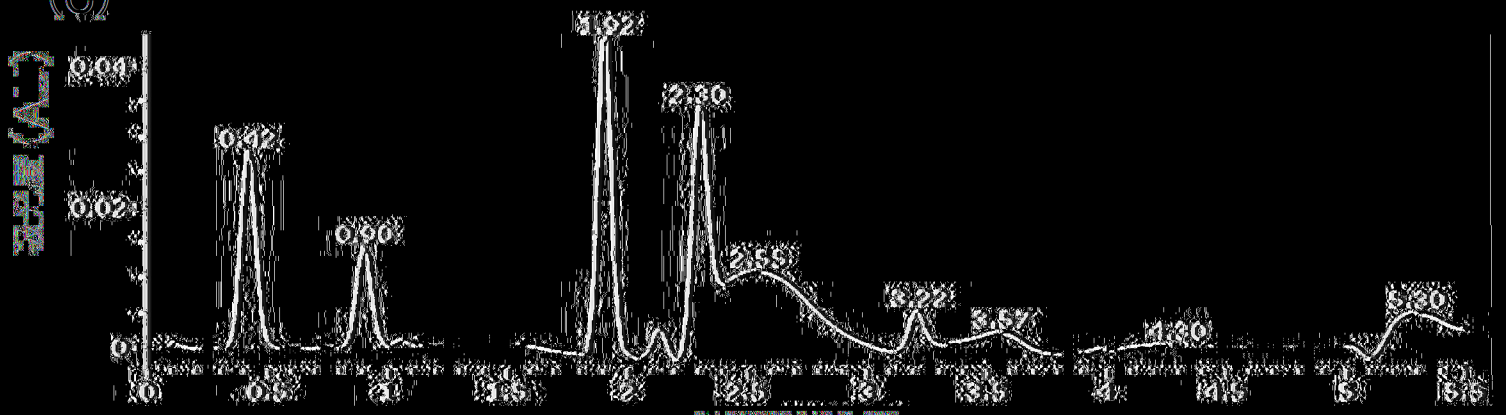
保持時間(分鐘)

(B)



保持時間(分鐘)

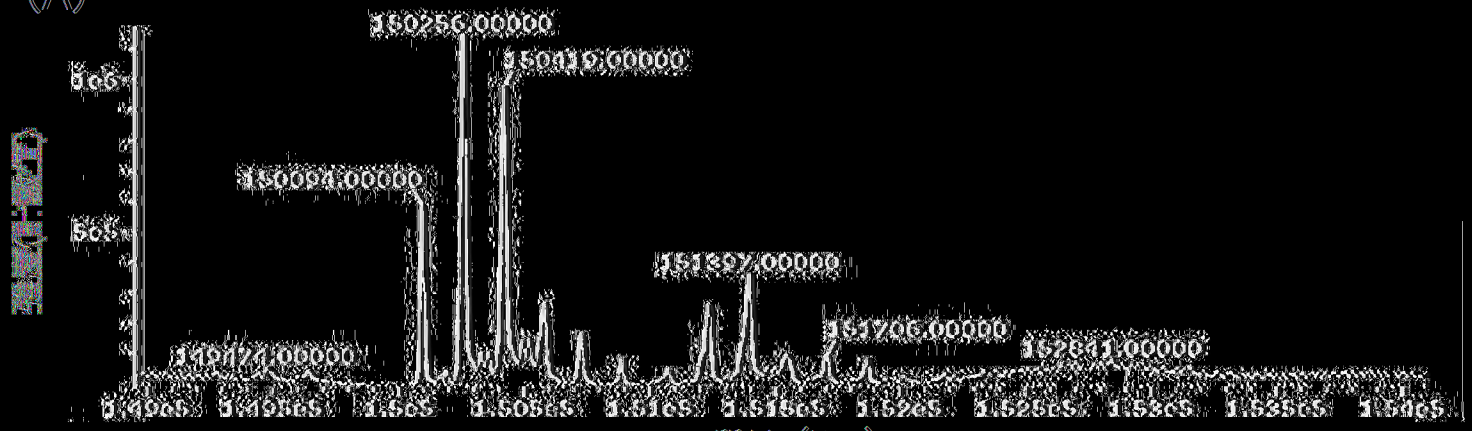
(C)



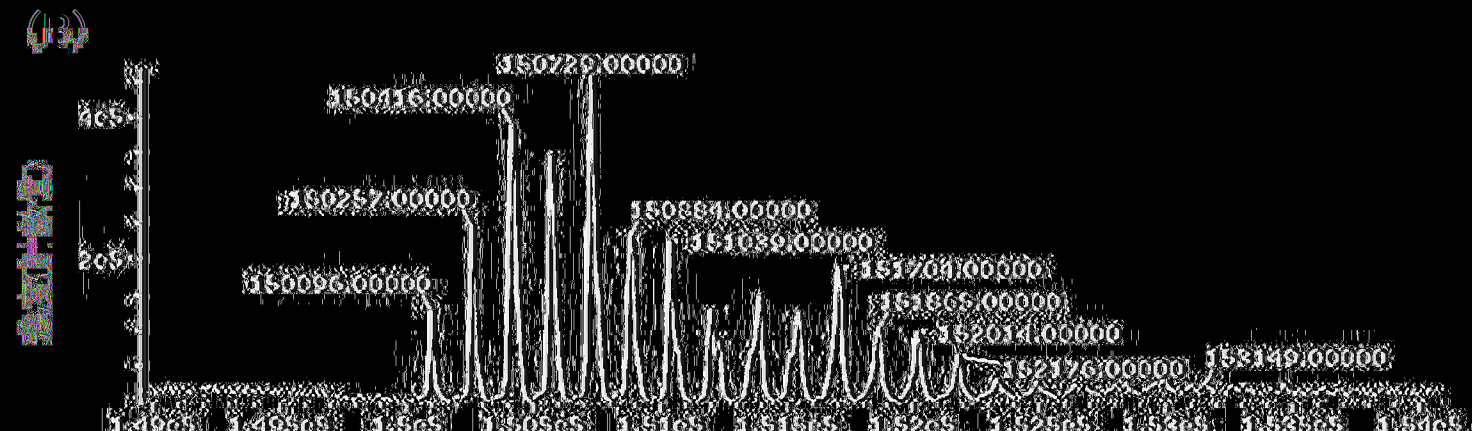
保持時間(分鐘)

|(圖7)|

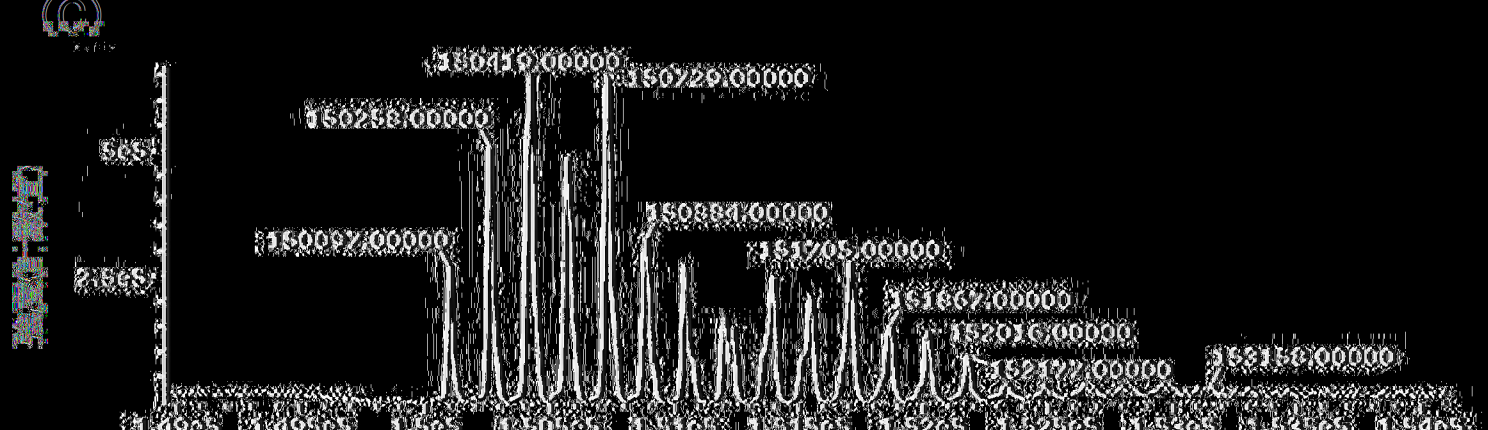
(A)


150256.00000
150119.00000
150094.00000
151397.00000
151706.00000
152841.00000
149124.00000
1.49e5
1.495e5
1.5e5
1.505e5
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1.54e5
Bit (Da)

(B)

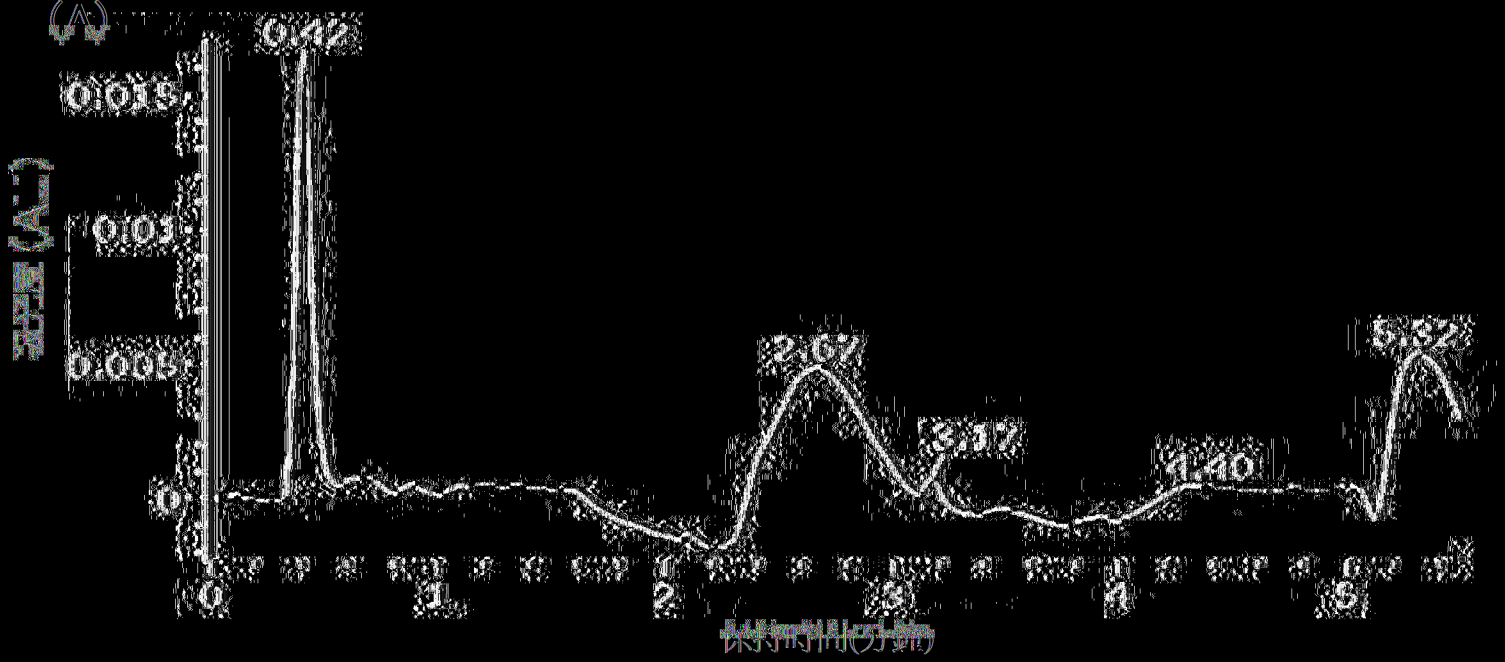

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151701.00000
150096.00000
151665.00000
152014.00000
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158149.00000
1.49e5
1.495e5
1.5e5
1.505e5
1.51e5
1.515e5
1.52e5
1.525e5
1.53e5
1.535e5
1.54e5
Bit (Da)

(C)


150119.00000
150729.00000
150258.00000
150884.00000
150092.00000
151705.00000
151662.00000
152016.00000
152127.00000
158158.00000
1.49e5
1.495e5
1.5e5
1.505e5
1.51e5
1.515e5
1.52e5
1.525e5
1.53e5
1.535e5
1.54e5
Bit (Da)

|(圖8)|

(A)


0.42
0.015
0.03
0.005
2.67
3.17
1.40
5.32
0
1
2
3
4
5

(B)

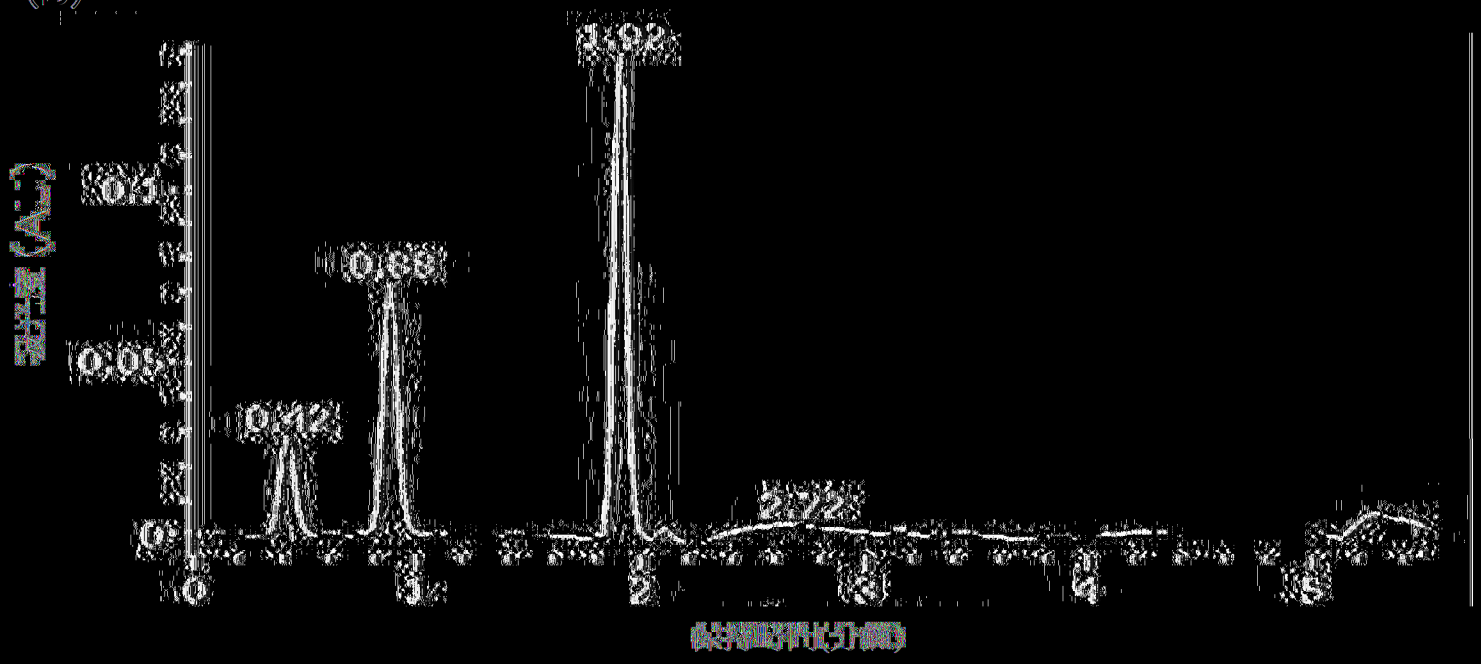

1.92
0.03
0.08
0.05
0.42
2.22
0
1
2
3
4
5

圖9

(A)

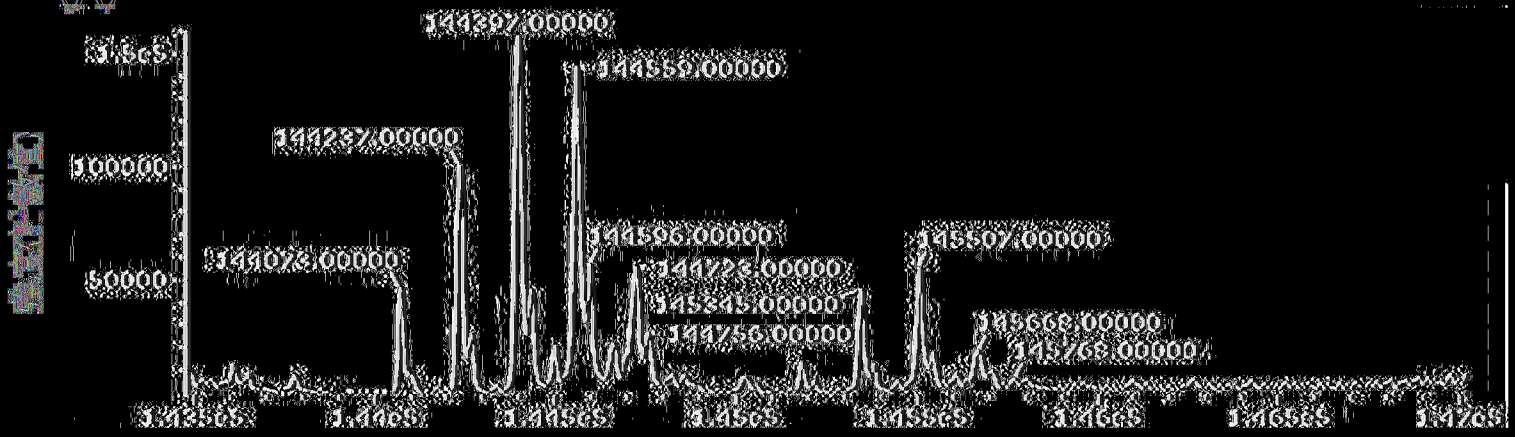


圖 (1a)

(B)

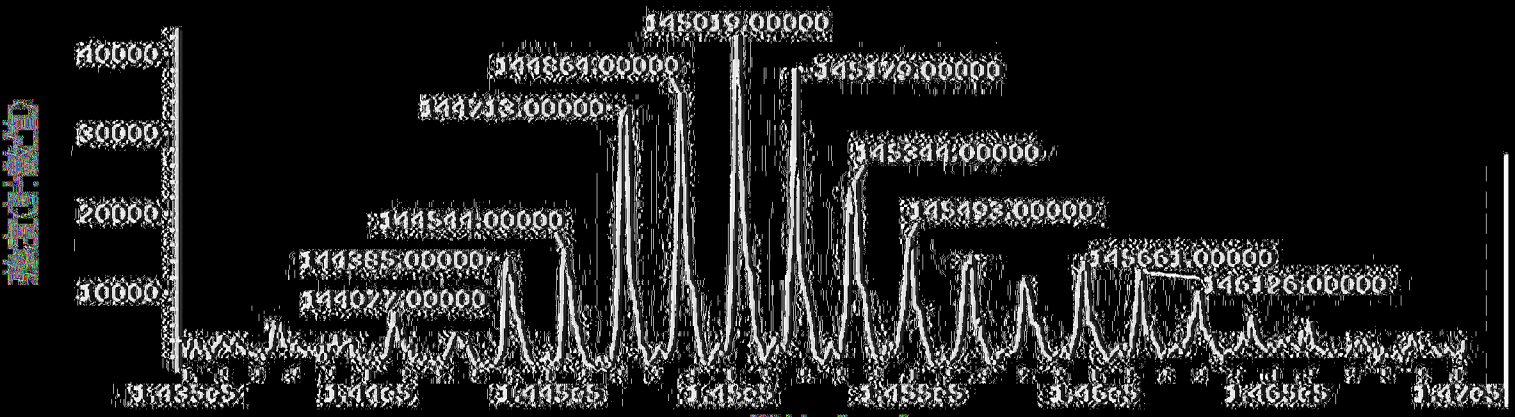
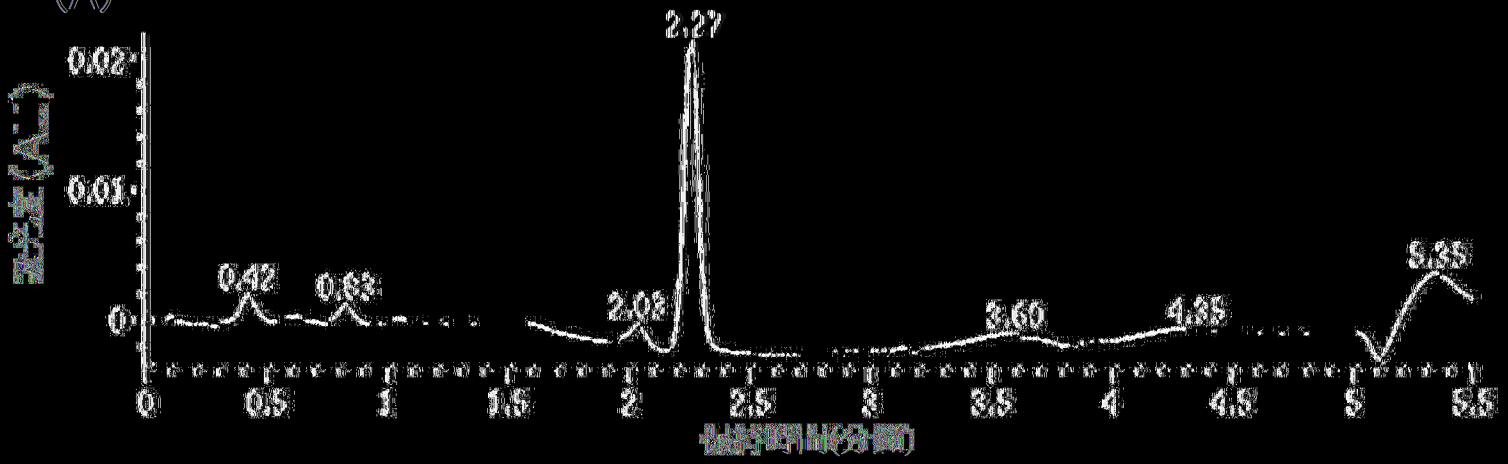


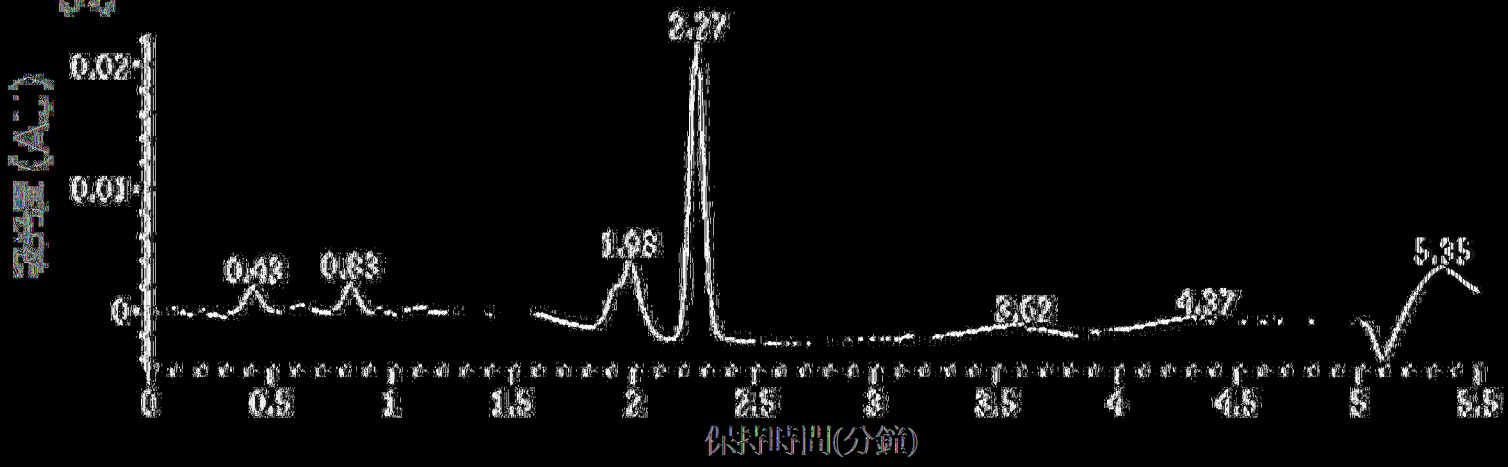
圖 (1b)

|(圖10)|

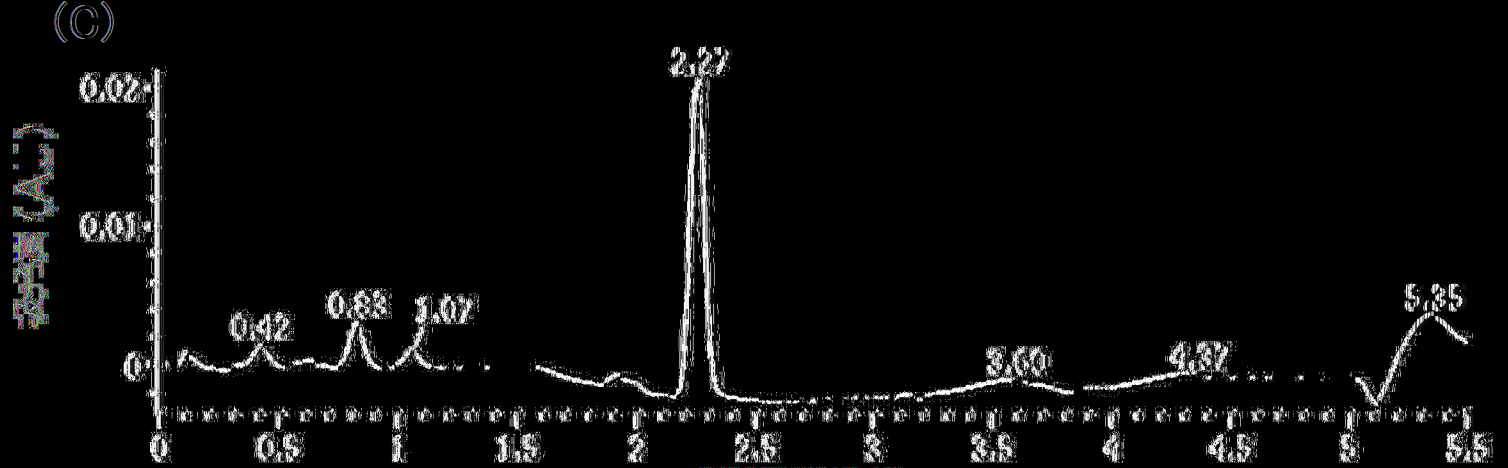
(A)


0.02
0.01
0
0.42
0.83
2.08
2.27
3.60
4.35
5.35
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
保持時間(分鐘)

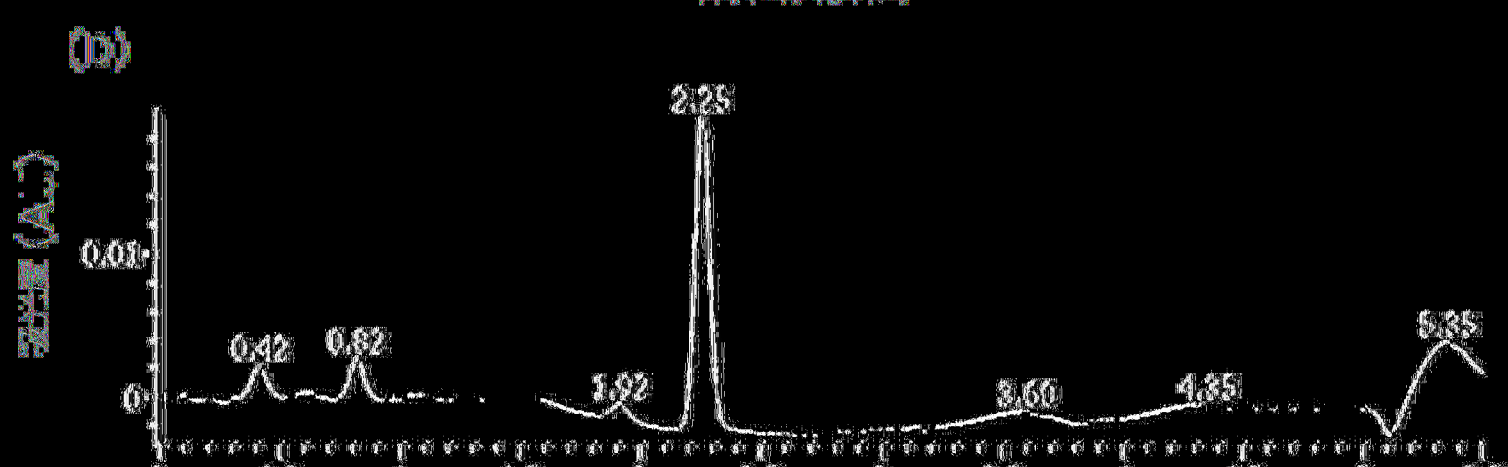
(B)


0.02
0.01
0
0.43
0.83
1.98
2.27
3.62
4.37
5.35
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
保持時間(分鐘)

(C)

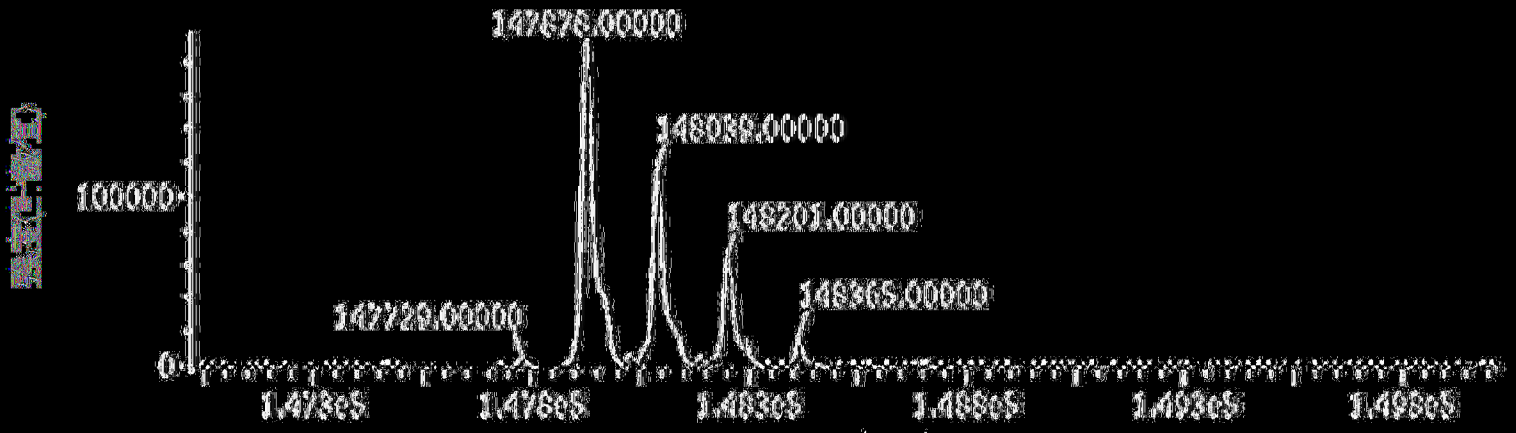

0.02
0.01
0
0.42
0.83
1.07
2.22
3.60
4.37
5.35
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
保持時間(分鐘)

(D)


0.01
0
0.42
0.82
1.92
2.25
3.60
4.35
5.35
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
保持時間(分鐘)

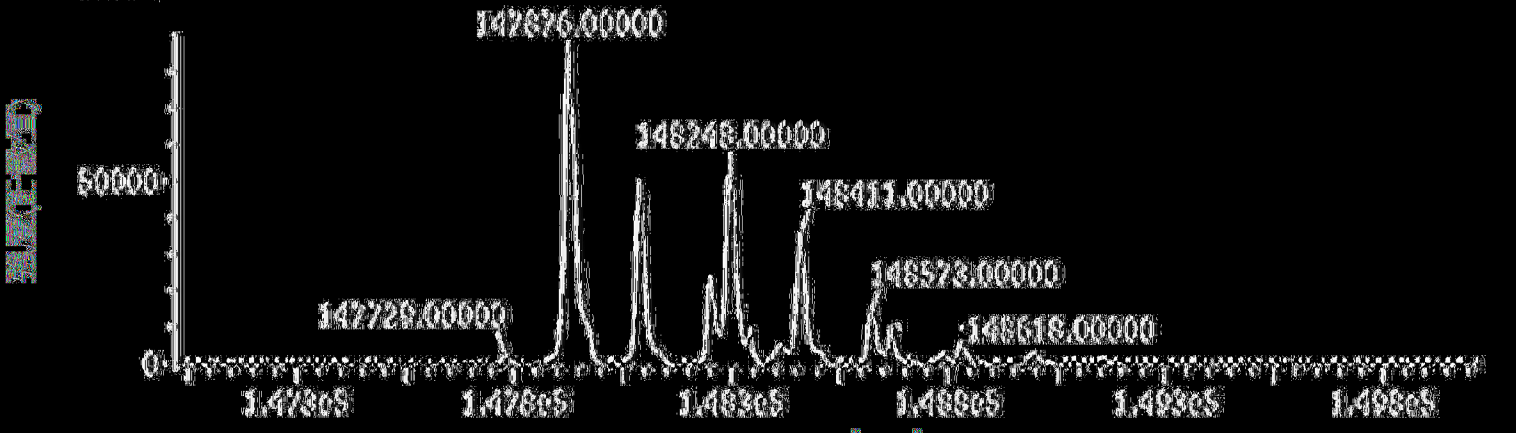
[圖1]

(A)



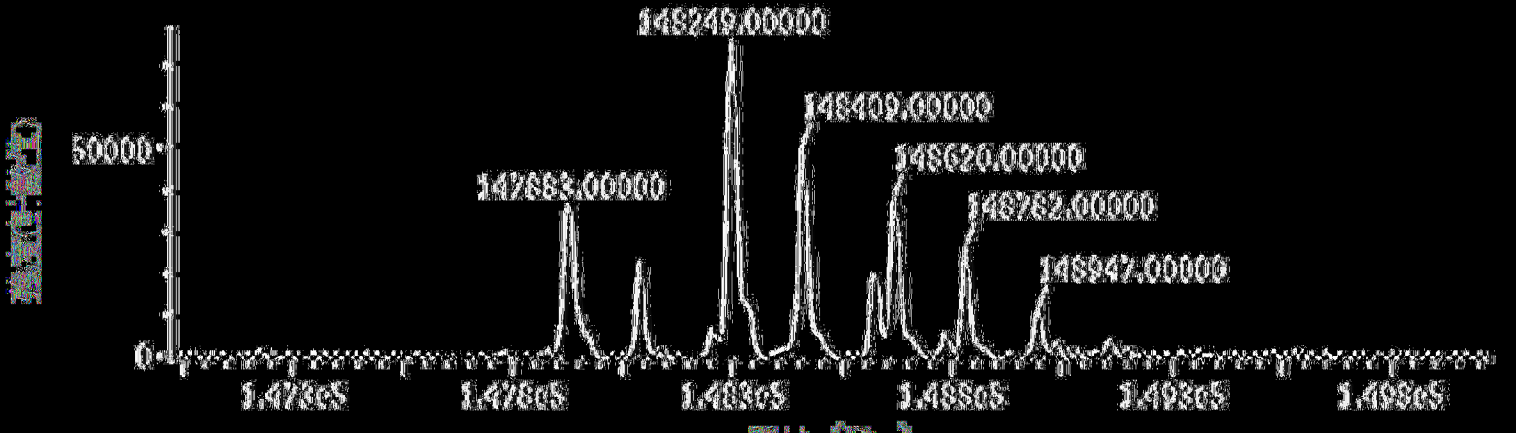
質量 (Da)

(B)



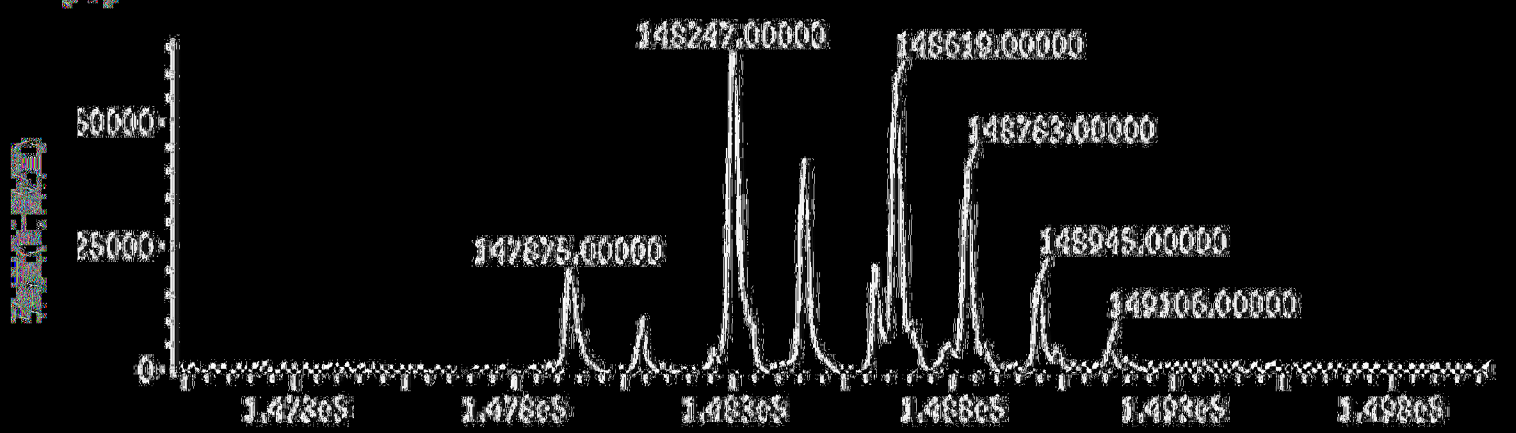
質量 (Da)

(C)



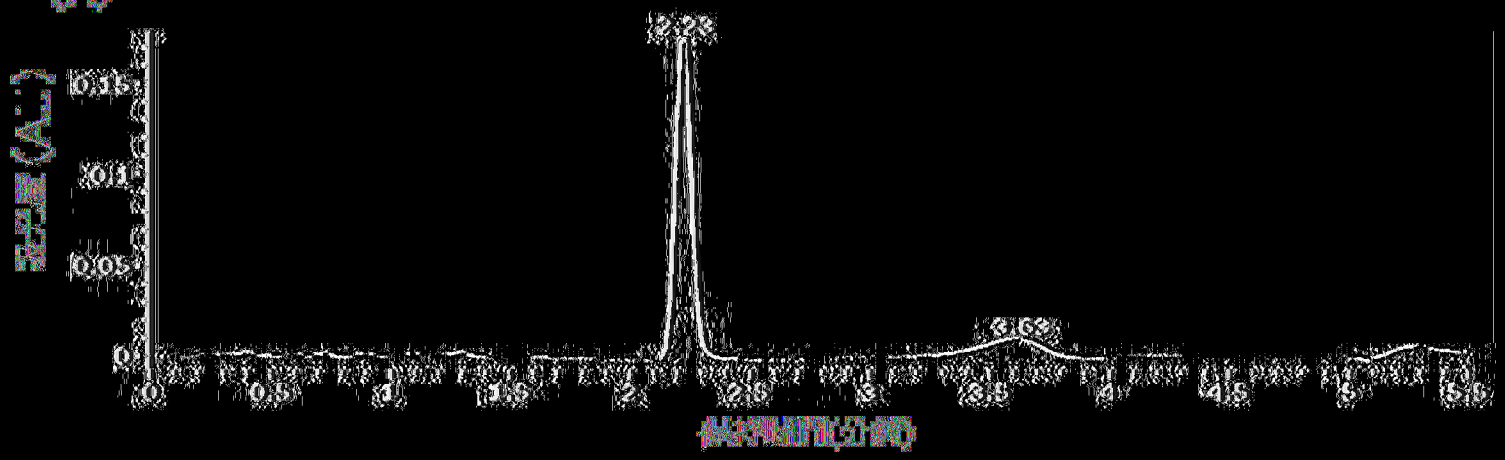
質量 (Da)

(D)



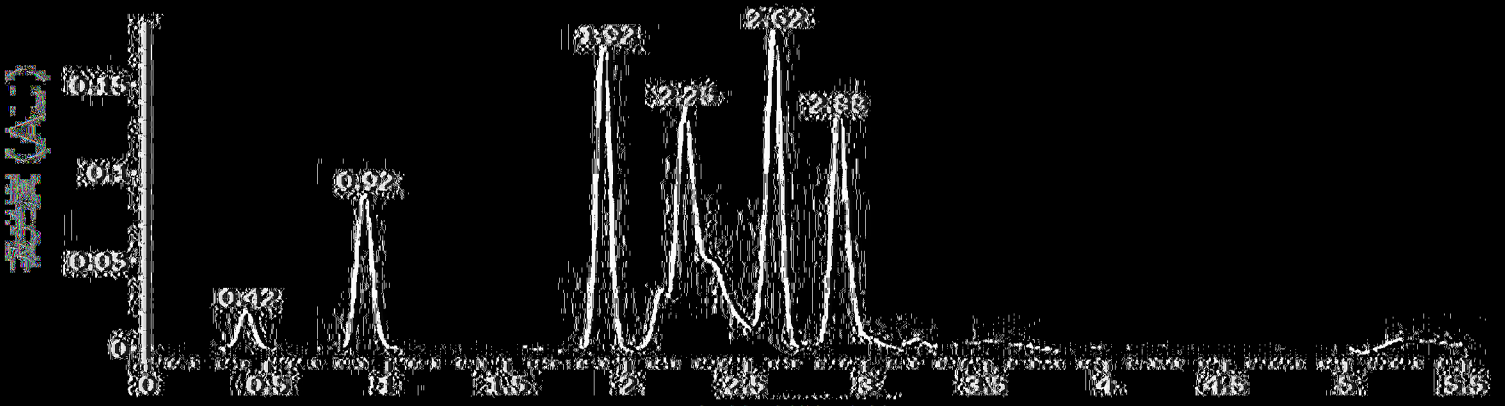
質量 (Da)

(A)



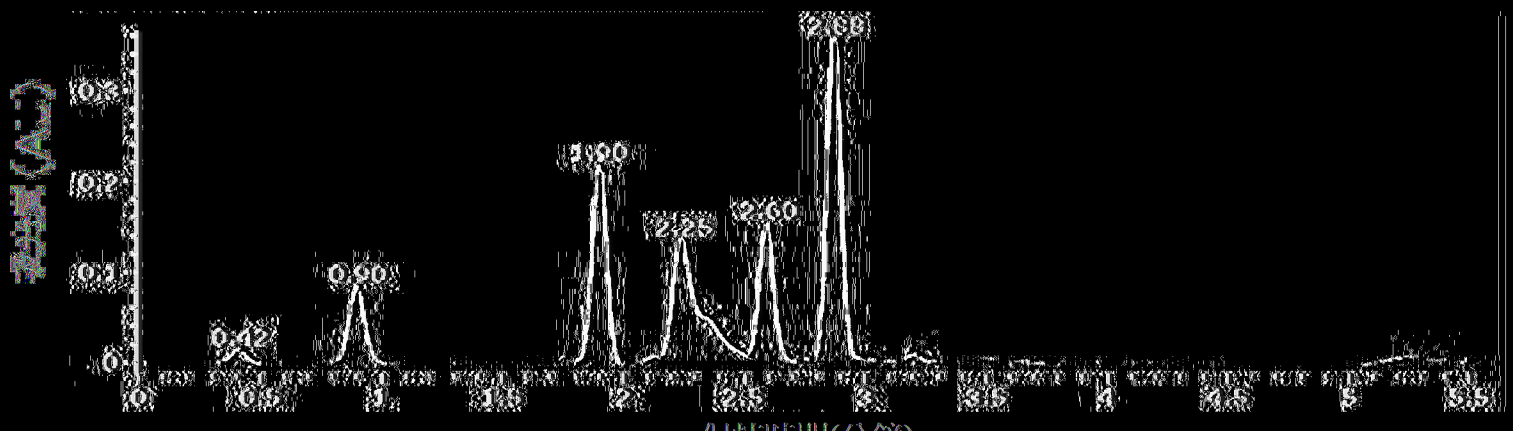
保持時間(分鐘)

(B)



保持時間(分鐘)

(C)



保持時間(分鐘)

[圖13]

(A)

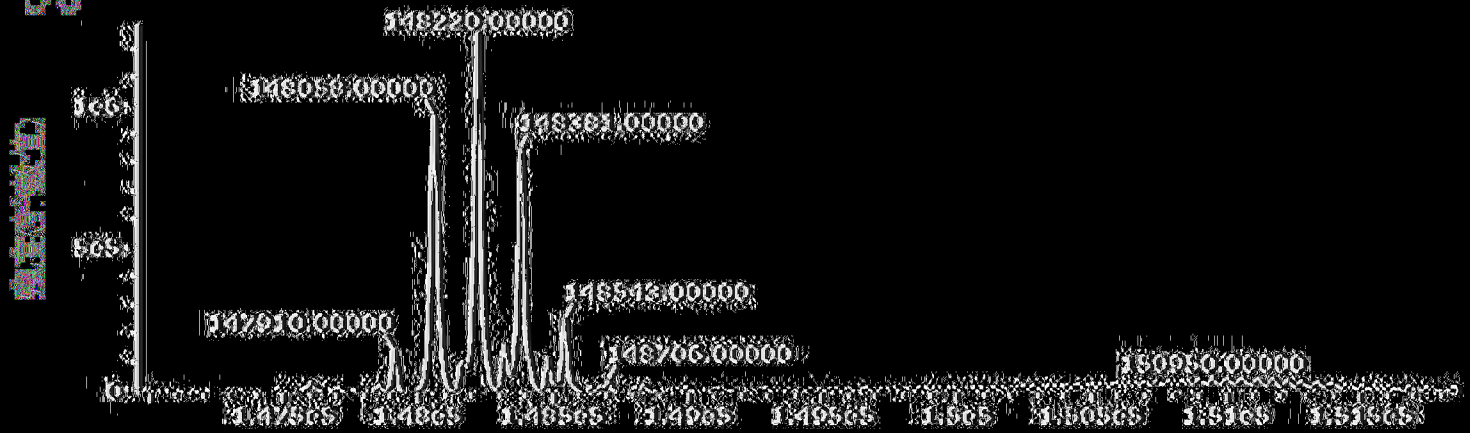


圖 (3a)

(B)

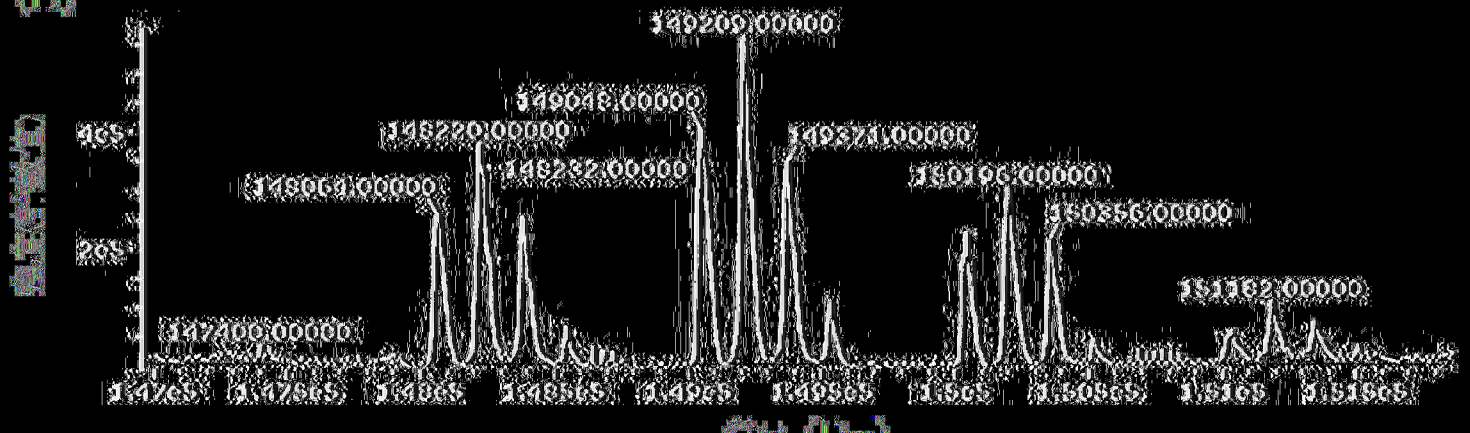


圖 (3b)

(C)

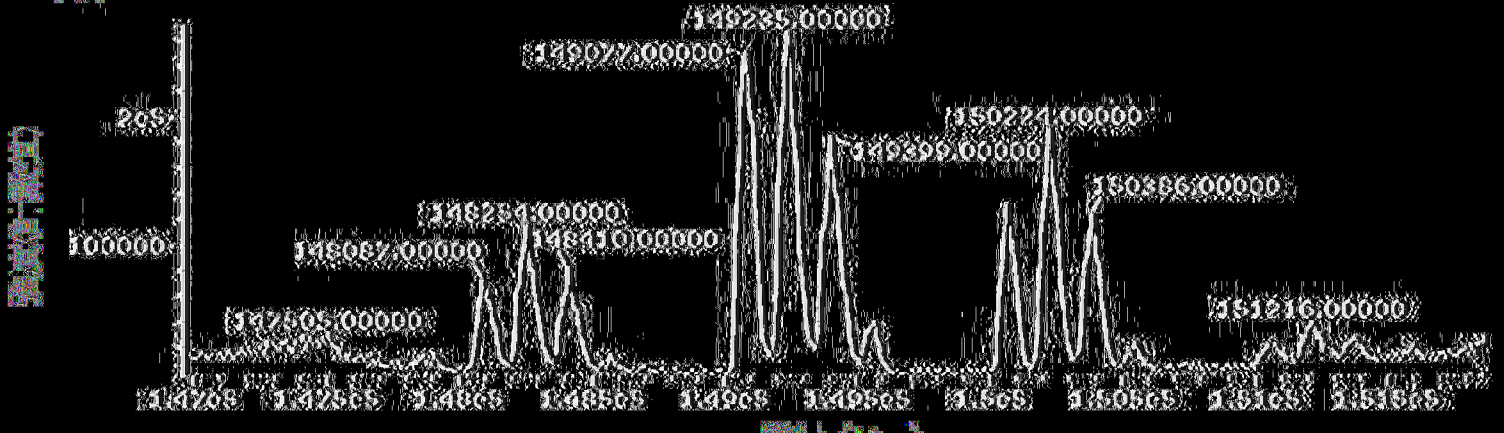
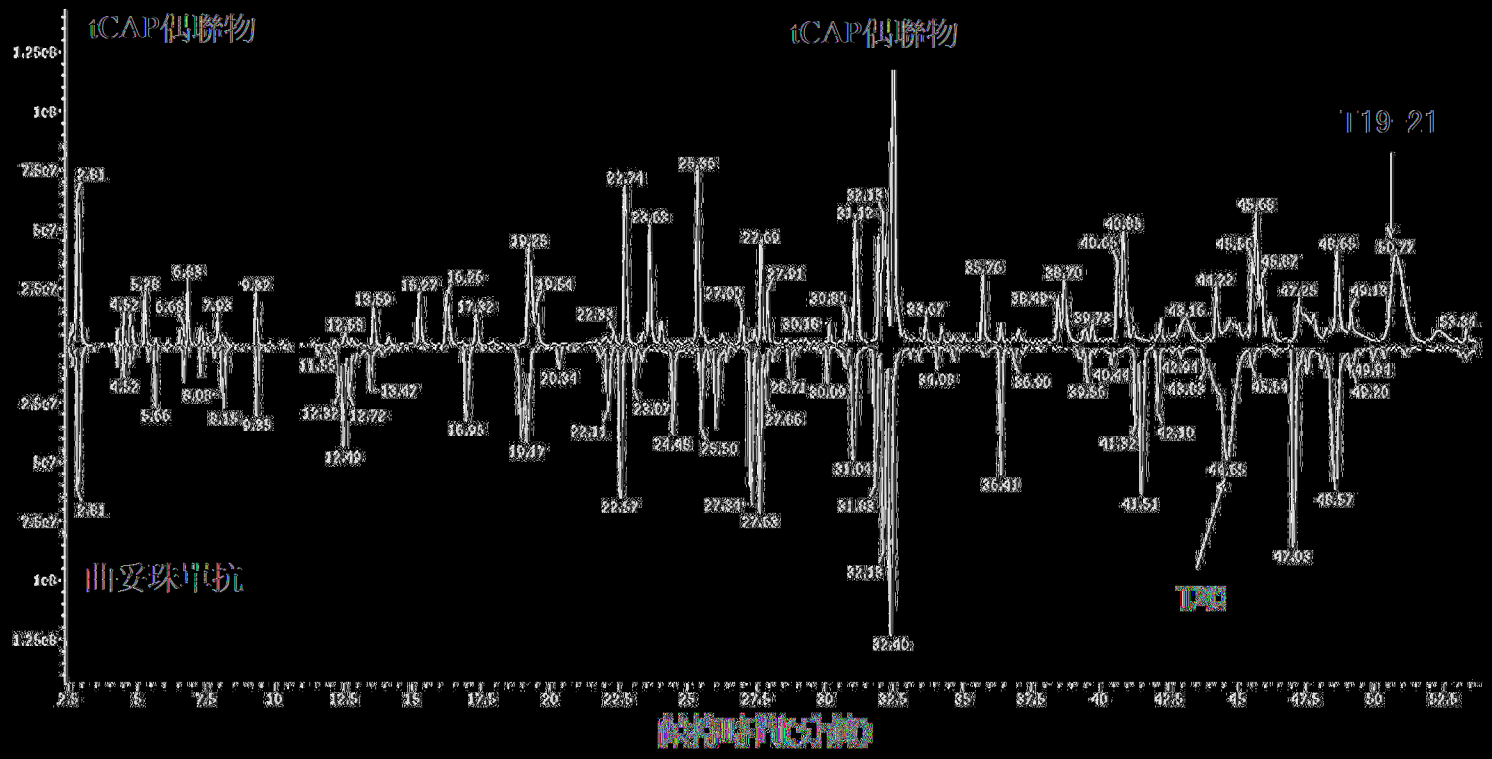
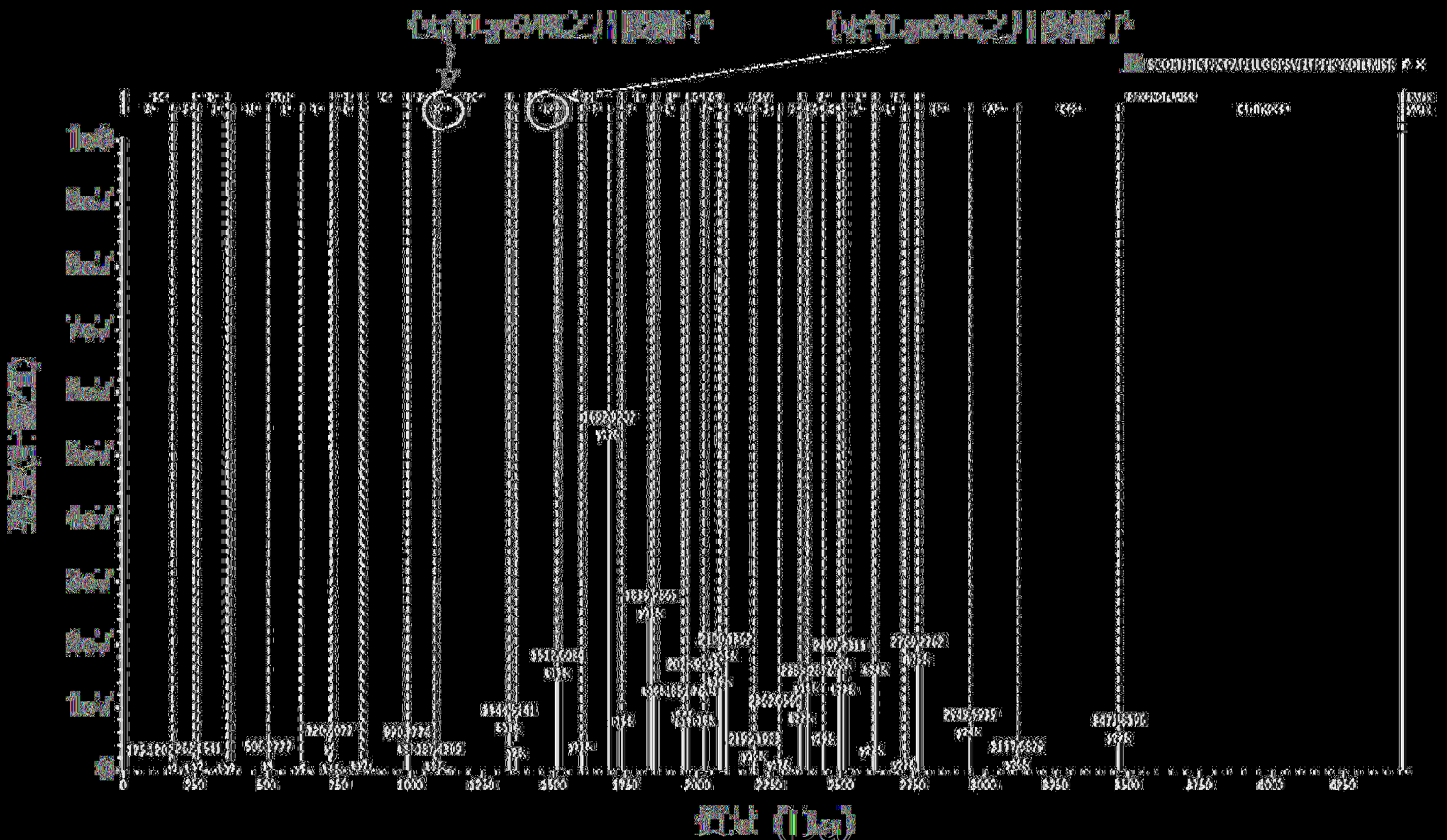


圖 (3c)

[圖 4]

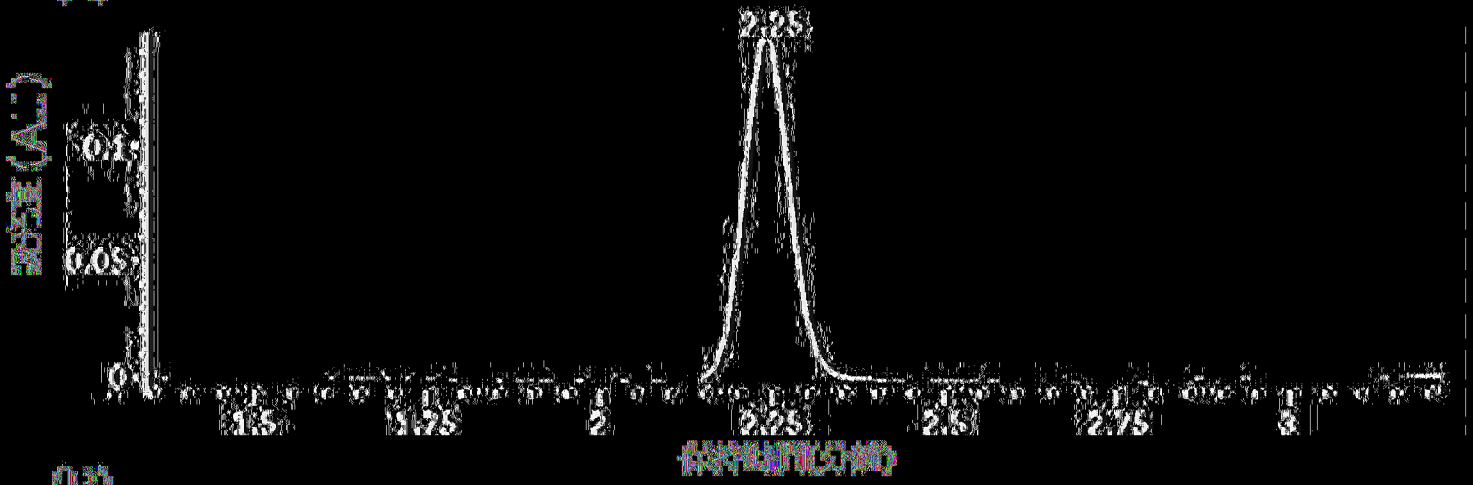

tCAP偶聯物
tCAP偶聯物
119 21
曲妥珠單抗
滯留時間(分鐘)

[圖15]

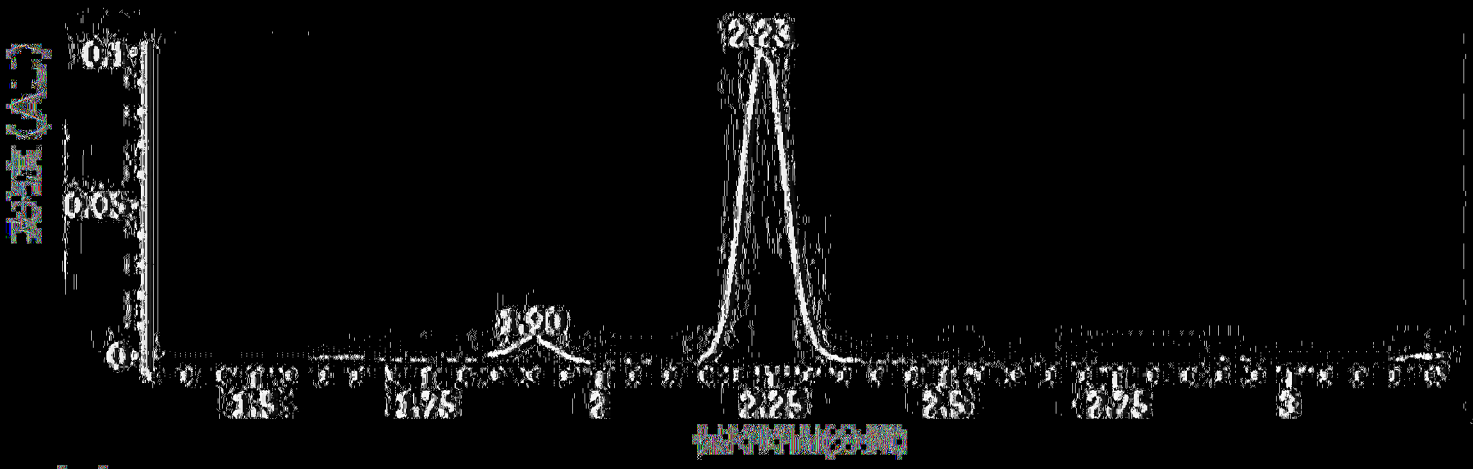

tCAP偶聯物之抗體鏈
tCAP偶聯物之抗體鏈
強度 (Da)

[圖16]

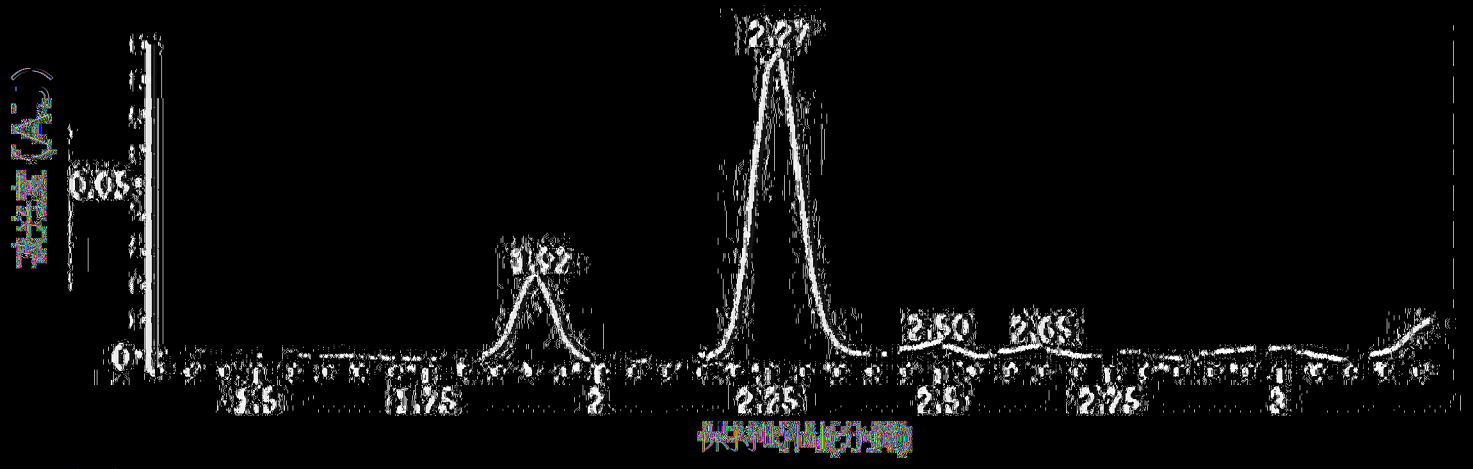
(A)


2.25
0.1
0.05
0
1.5
1.75
2
2.25
2.5
2.75
3

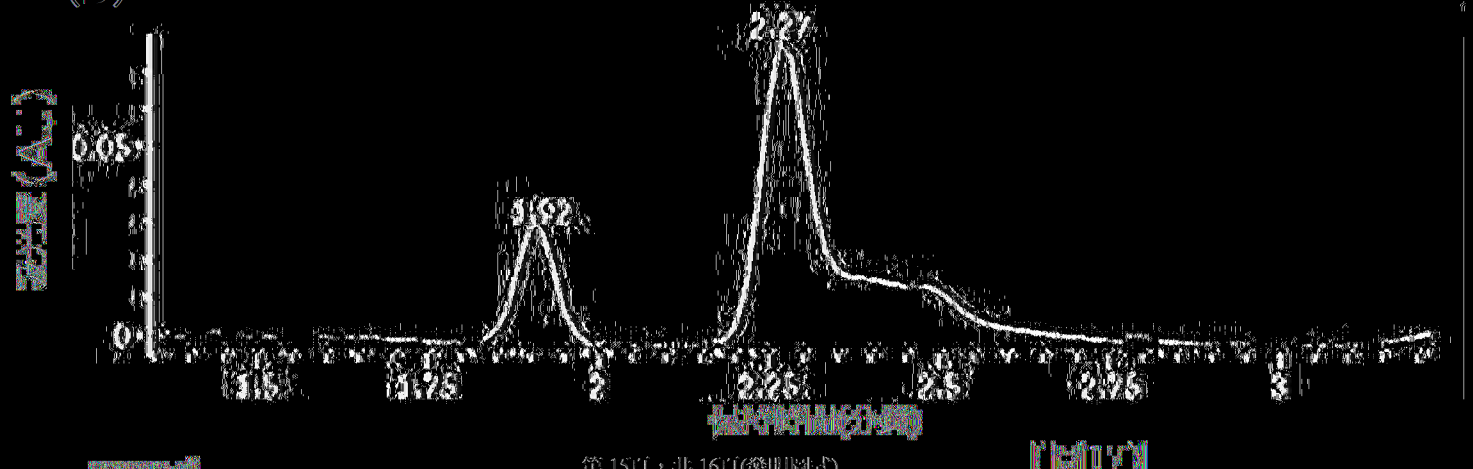
(B)


2.23
1.90
0.1
0.05
0
1.5
1.75
2
2.25
2.5
2.75
3

(C)


2.27
1.92
2.50
2.65
0.05
0
1.5
1.75
2
2.25
2.5
2.75
3

(D)


2.27
1.92
0.05
0
1.5
1.75
2
2.25
2.5
2.75
3

(A)

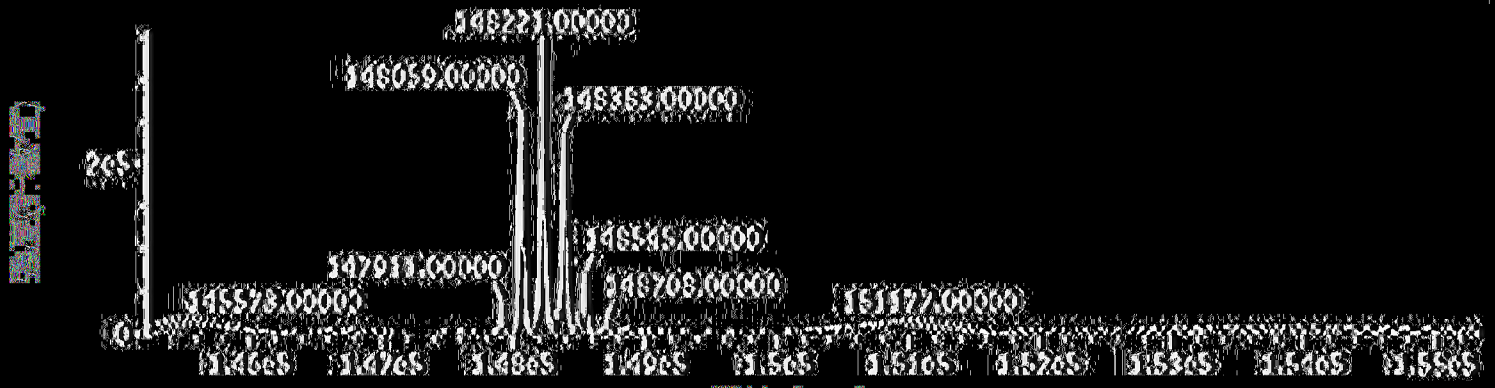


圖 1 (Da)

(B)

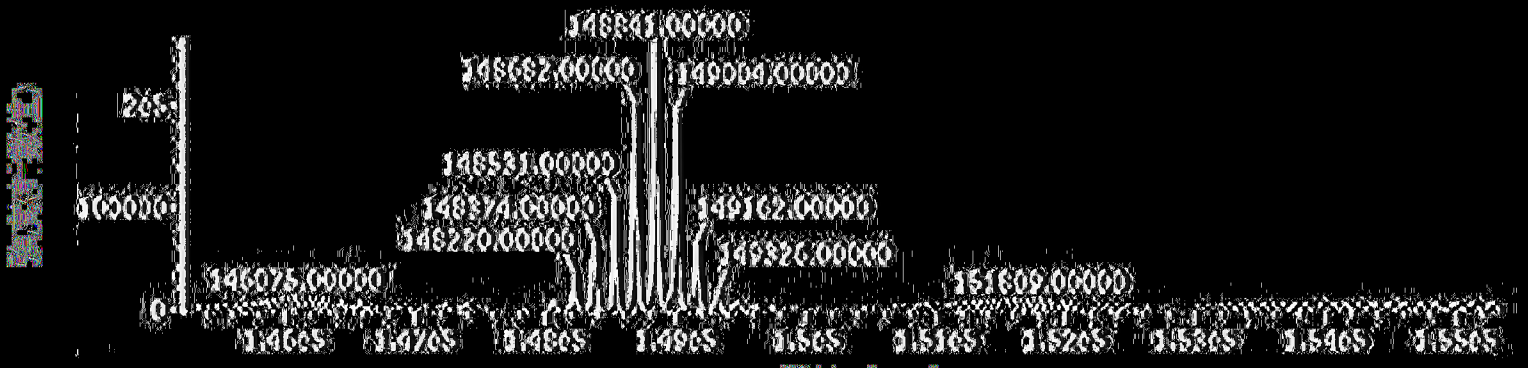


圖 1 (Da)

(C)

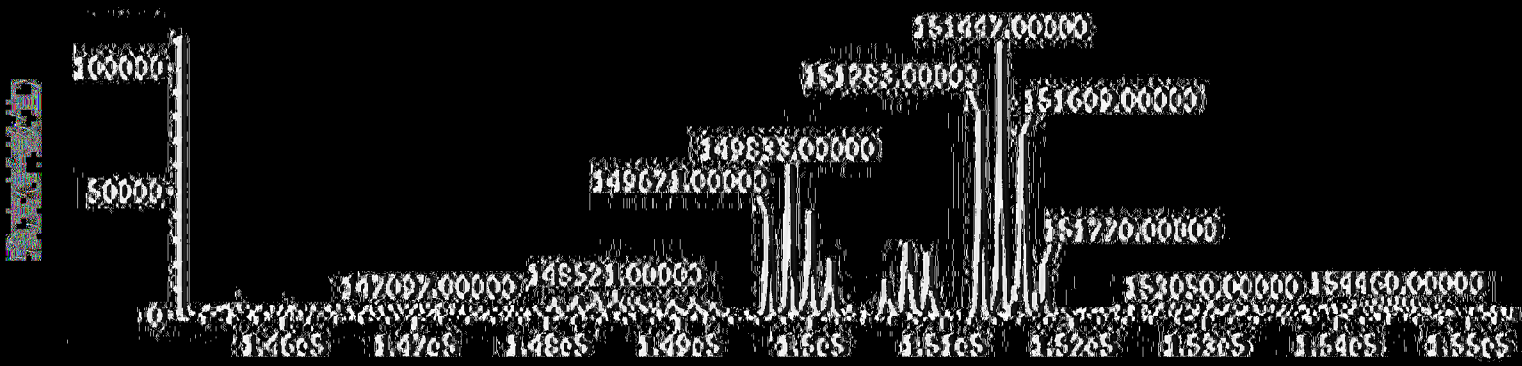


圖 1 (Da)

(D)



圖 1 (Da)

|(圖 18)|