INSTYTUT CHEMII ORGANICZNEJ POLSKIEJ AKADEMII NAUK

Synteza nowych pochodnych witaminy B_{12} i ich właściwości katalityczne

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SPIS TREŚCI

1. SPIS PUBLIKACJI WCHODZĄCYCH W SKŁAD ROZPRAWY DOKTORSKIEJ

Publikacje oryginalne:

[P1] K. ó Proinsias, **M. Giedyk**, R. Loska, M. Chromiński, D. Gryko *J. Org. Chem.* 2011, *76*, 6806, „Selective Modifications of Hydrophobic Vitamin B_{12} Derivatives at *c*- and *d*-Positions"

[P2] K. ó Proinsias, **M. Giedyk**, I. Sharina, E. Martin, D. Gryko *ACS Med. Chem. Lett.* 2012, *3*, 476, „Synthesis of New Hydrophilic and Hydrophobic Cobinamides as NO-Independent sGC Activators"

[P3] K. ó Proinsias, **M. Giedyk**, Ł. Banach, D. Rutkowska-Zbik, D. Gryko *Asian J. Org. Chem.* 2013, *2*, 504, „Selectively Modified Cobyrinic Acid Derivatives"

[P4] **M. Giedyk**, S. N. Fedosov, D. Gryko *Chem. Commun.* 2014, *50*, 4674, „An Amphiphilic, Catalytically Active, Vitamin B_{12} Derivative"

[P5] **M. Giedyk**, K. ó Proinsias, S. Kurcoń, I. Sharina, E. Martin, D. Gryko *ChemMedChem* 2014, *9*, 2344, „Small Alterations in Cobinamide Structure Considerably Influence sGC Activation"

[P6] **M. Giedyk**, K. Goliszewska, K. ó Proinsias, D. Gryko *Chem. Commun.* 2016, *52*, 1389, „Cobalt(I)-Catalysed CH-Alkylation of Terminal Olefins, and Beyond"

[P7] **M. Giedyk**, H. Shimakoshi, K. Goliszewska, D. Gryko, Y. Hisaeda *Dalton Trans.* 2016, DOI: 10.1039/C6DT00355A, „Electrochemistry and Catalytic Properties of Amphiphilic Vitamin B_{12} Derivatives in Nonaqueous Media"

Publikacje przeglądowe:

[R1] K. ó Proinsias, **M. Giedyk**, D. Gryko *Chem. Soc. Rev.* 2013, *42*, 6605, „Vitamin B_{12}: Chemical Modifications"

[R2] **M. Giedyk**, K. Goliszewska, D. Gryko *Chem. Soc. Rev.* 2015, *44*, 3391, „Vitamin B_{12}-Catalyzed Reactions"

2. SPIS WYBRANYCH WYSTĄPIEŃ KONFERENCYJNYCH

Wyniki przedstawione w niniejszej pracy zostały zaprezentowane na konferencjach:

1. International Summer School on Organic Synthesis; Gargnano, Włochy 2015:
   „New vitamin B_{12} derivatives – synthesis and their catalytic activity"
   prezentacja ustna uhonorowana nagrodą

2. Interdisciplinary FNP Conference; Warszawa 2015:
   „Vitamin B_{12} – beyond the known"
   prezentacja posterowa

3. Chemia Organiczna Wczoraj i Dziś; Warszawa 2014:
   „Synteza i Właściwości Katalityczne Nowych Pochodnych Witaminy B_{12}"
   prezentacja ustna

4. 8th International Conference of Porphyrins and Phthalocyanines; Stambuł, Turcja 2014:
   „*Novel, Amphiphilic, Catalytically Active B_{12} Derivative"*
   prezentacja posterowa

5. YoungChem2013 International Congress of Young Chemists; Poznań 2013:
   „Chemistry and Biological Activity of New Vitamin B_{12} Derivatives"
   prezentacja ustna

6. 4th European Symposium on Organic Reactivity; Praga, Czechy 2013:
   „Selective Modifications of Vitamin B_{12} Derivatives – Exploring the Reactivity of Cobyrinate Methyl Esters"
   prezentacja posterowa

7. 1st Warsaw-Cambridge Young Scientist Meeting; Warszawa 2013:
   „Synthesis of New Hydrophilic and Hydrophobic Cobinamides and Their Potential as NO-Independent sGC Activators"
   prezentacja posterowa

3. SPIS PUBLIKACJI NIEWCHODZĄCYCH W SKŁAD ROZPRAWY DOKTORSKIEJ

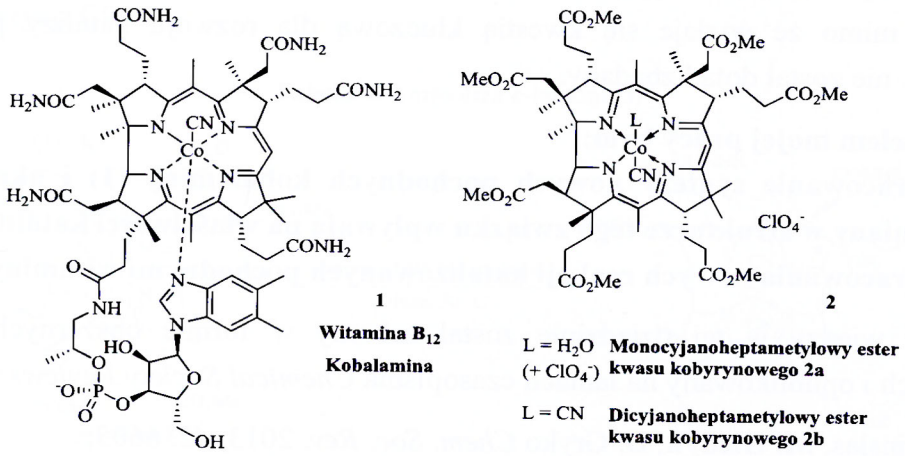
Publikacje oryginalne:

1. M. Chromiński, **M. Giedyk**, D. Gryko *ARKIVOC* 2014, *4*, 135, „Organocatalytic γ-oxidation of α,β-unsaturated aldehydes”

4. PRZEWODNIK PO ROZPRAWIE DOKTORSKIEJ

4.1 Założenia i cel pracy

Współczesna synteza organiczna nieustannie poszukuje rozwiązań, które oprócz wysokiej efektywności i szerokiego zakresu stosowalności, uwzględniają również aspekty społeczno-ekonomiczne; przede wszystkim charakteryzują się niskimi kosztami oraz niewielką szkodliwością dla środowiska naturalnego i człowieka. Trend ten jest szczególnie widoczny w katalizie, w której reakcje z użyciem kompleksów i soli metali szlachetnych m.in. palladu, rutenu, irydu etc. próbuje się zastąpić transformacjami katalizowanymi przez tanie i nietoksyczne związki żelaza, cynku, niklu czy kobaltu. To ambitne podejście jest w istocie naśladowaniem samej natury. Wymienione metale (w formie kofaktorów) wchodzą w skład holoenzymów i w sposób wydajny i selektywny katalizują reakcje chemiczne przy minimalnym nakładzie energii. Czy chemik organik może zdecydować się na odważny krok i zamiast opracowywać nowe kompleksy, skorzystać z katalizatora, który natura wytworzyła za niego? Tak, a doskonałym przykładem takiego związku jest witamina B_{12}.

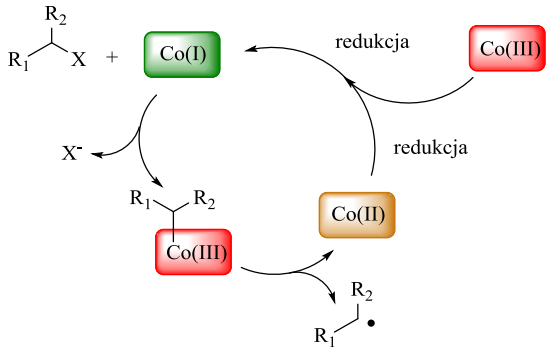


Rysunek 1. Struktura witaminy B_{12} (**1**) oraz heptametylowego estru kwasu kobyrynowego **2**.

Jest to wysoko sfunkcjonalizowany korynoid posiadający kation kobaltu na +3 stopniu utlenienia, skompleksowany wewnątrz pierścienia makrocyklicznego.[1,2] W ustroju biologicznym występuje on w formie koenzymów, których aktywność wynika z możliwości tworzenia kowalencyjnego wiązania Co-C, mogącego w odpowiednich warunkach ulegać rozerwaniu: a) heterolitycznemu lub b) homolitycznemu - generując rodniki (Schemat 1).[1,3] Ta charakterystyczna cecha witaminy B_{12} oraz jej analogów jest wykorzystywana w syntezie organicznej, gdzie wiązanie Co-C otrzymuje się na drodze redukcji stabilnego kationu kobaltu(III) do nukleofilowej formy Co(I) i następczej reakcji z elektrofilem np. halogenkiem

[1] R. Banerjee *Chemistry and Biochemistry of B_{12}*, John Wiley & Sons, 1999.
[2] B. Kräutler, B. T. Golding, D. Arigoni *Vitamin B_{12} and B_{12}-Proteins*, WILEY-VCH, 2008.
[3] K. L. Brown *Chem. Rev.* 2005, *105*, 2075.

alkilu. Dzięki temu pochodne witaminy B_{12} mogą być stosowane jako katalizatory reakcji rodnikowych, m.in. reakcji sprzęgania, addycji do wiązania podwójnego, migracji grup funkcyjnych itp.



Schemat 1. Mechanizm reakcji tworzenia rodników katalizowanej pochodnymi witaminy B_{12}.

Niestety trudnością, która ogranicza powszechne stosowanie tego związku jako katalizatora jest jego hydrofilowość i związana z nią niska rozpuszczalność w większości syntetycznie użytecznych rozpuszczalników. Próbą przezwyciężenia tej niedogodności było przekształcenie hydrofilowej kobalaminy w hydrofobowy heptaester kwasu kobyrynowego **2**, na drodze kwasowej metanolizy.[4] Jest to reakcja wydajna, jednak w jej wyniku dochodzi do istotnych zmian w strukturze katalizatora: następuje odłączenie fragmentu nukleotydowego od cząsteczki i przekształcenie pozycji *f* w ester metylowy. Ponadto, otrzymane grupy estrowe są mniej stabilne niż wyjściowe amidy. Wpływ powyższych przemian na aktywność katalityczną, mimo że wydaje się kwestią kluczową dla rozwoju katalizy pochodnymi witaminy B_{12}, nie został dotąd zbadany.

Dlatego też celem mojej pracy było:

1. **opracowanie syntezy nowych pochodnych kobalaminy (1) i określenie jak zmiany w strukturze tego związku wpływają na właściwości katalityczne,**
2. **opracowania nowych reakcji katalizowanych pochodnymi witaminy B_{12}.**

Obecny stan wiedzy w tej dziedzinie został zebrany w formie obszernych artykułów przeglądowych i opublikowany na łamach czasopisma *Chemical Society Reviews:*

[R1]K. ó Proinsias, **M. Giedyk**, D. Gryko *Chem. Soc. Rev.* 2013, *42*, 6605;
[R2]M. Giedyk, K. Goliszewska, D. Gryko *Chem. Soc. Rev.* 2015, *44*, 3391.

Podstawowym założeniem pracy była potencjalna możliwość zmiany właściwości fizykochemicznych (w tym rozpuszczalności) kobalaminy na drodze modyfikacji jej struktury w obrębie fragmentu nukleotydowego oraz łańcuchów bocznych. Założyłem również, że taka ingerencja będzie miała wpływ na otoczenie koordynacyjne centralnego kationu kobaltu, zmieniając jego właściwości redoks, a co za tym idzie, aktywność katalityczną.

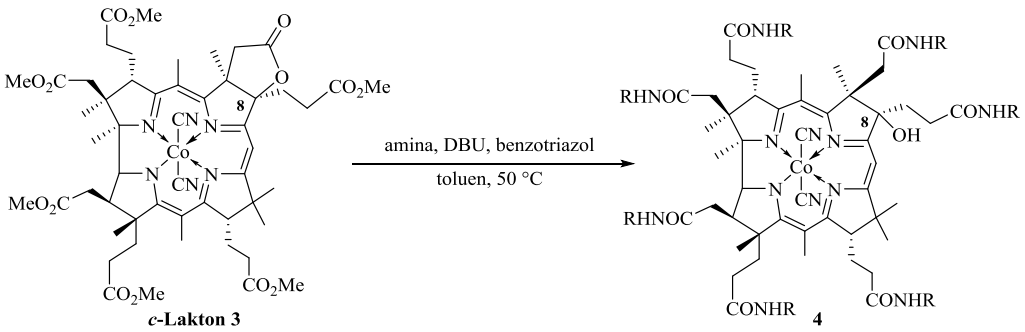
[4] L. Werthemann *Dissertation*, ETH Zürich, 1968.

4.2 Synteza i funkcjonalizacja heptaamidowych pochodnych kwasu kobyrynowego

Synteza:

W pierwszej części badań skupiłem się na syntezie nowych pochodnych kwasu kobyrynowego – analogu witaminy B_{12} pozbawionego fragmentu nukleotydowego. Moim celem była modyfikacja łańcuchów bocznych, a jako substrat wybrałem łatwy do otrzymania i stabilny heptametylowy ester kwasu kobyrynowego (**2b**). Opierając się na wstępnych wynikach otrzymanych przez Gryko i współpracowników, którzy zaobserwowali powstawanie tri- i tetraamidów pod wpływem działania nadmiaru aminy na heptaester **2b**, postanowiłem opracować procedurę aminolizy wszystkich grup estrowych, prowadzącą do heptaamidów. Trudność podjętego wyzwania polegała w dużej mierze na konieczności starannej optymalizacji warunków reakcji, tak aby transformacji ulegało wszystkich siedem grup. Każde, przecież nieuniknione, odchylenie od tej idealnej sytuacji skutkowało powstawaniem złożonej mieszaniny produktów, które mogły występować w formie regioizomerów, co czyniło proces izolacji wyjątkowo trudnym i pracochłonnym. Ponadto, w przypadku zastosowania innych amin niż etanoloamina, obserwowałem częściowe utlenianie pozycji C8.

Tabela 1. Aminoliza *c*-laktonu **3**.



Nr	Amina	Czas [h]	Produkt	Wydajność [%][a]
1	*n*-Butyloamina	48	**4a**	73 (62)
2	Etanoloamina	24	**4b**	66 (61)
3	3-Metoksypropylamina	96	**4c**	71 (57)
4	Metyloamina	72	**4d**	60[b]/85[c] (54)
5	Diglikoloamina	72	**4e**	9 (8)
6	3-Morfolinopropyloamina	96	**4f**	33 (-)

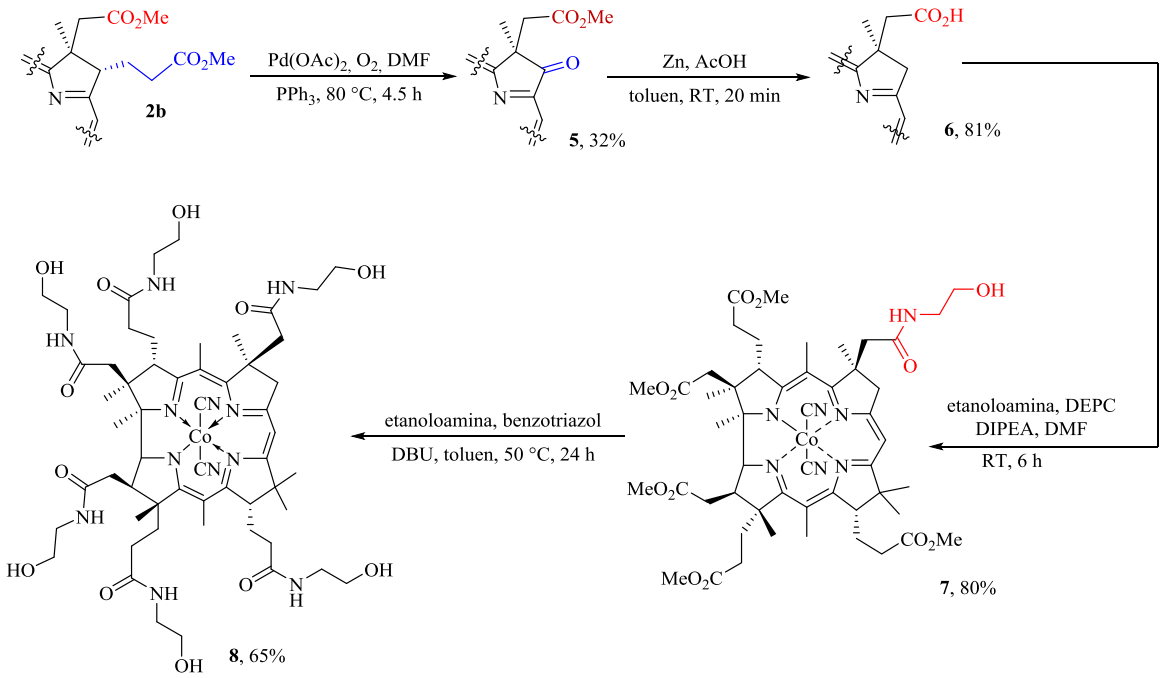
[a] Liczby w nawiasach oznaczają wydajności otrzymane z zastosowaniem $NaCN/NaN_3$ jako katalizatorów

[b] użyto roztworu $MeNH_2$/EtOH zamiast toluenu; [c] użyto roztworu $MeNH_2$/MeOH zamiast toluenu

Pomimo wymienionych trudności badania zakończyły się powodzeniem. Zastąpienie heptaestru metylowego kwasu kobyrynowego (**2b**) *c*-laktonem **3**[5] wyeliminowało problem częściowego utleniania pozycji C8, a opracowana metoda aminolizy w warunkach katalizy

[5] M. J. Pfammatter, T. Darbre, R. Keese *Helv. Chim. Acta* 1998, *81*, 1105.

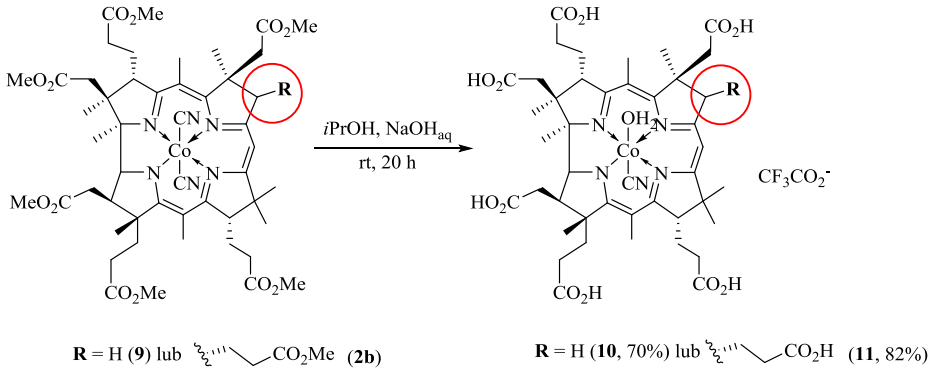
anionami cyjankowymi lub azydkowymi pozwoliła otrzymać heptaamidy zróżnicowane pod względem rozpuszczalności.**[P2]** Co więcej, prowadząc dalsze badania udoskonaliłem metodę poprzez zastosowanie benzotriazolu jako katalizatora przeniesienia grupy acylowej.[6] Pozwoliło to poszerzyć zakres stosowalności oraz zwiększyć wydajności reakcji (Tabela 1).**[P3]** Opracowaną metodę zastosowałem także do syntezy heksaetanoloamidu **8** pozbawionego łańcucha bocznego w pozycji *d*.**[P5]** Jako substratu użyłem 8-*nor*-*c*-amidu **7**, który otrzymałem zgodnie z procedurą opisaną przez Gryko i współpracowników (Schemat 2).[7]



Schemat 2. Synteza 8-*nor*-hexaetanoloamidu **8** z heptametyloestru kwasu kobyrynowego (**2b**).

Rozwinięciem i uzupełnieniem omawianych badań nad aminolizą estrowych pochodnych kwasu kobyrynowego było opracowanie warunków reakcji hydrolizy tego estru.**[P5]** Co prawda, heptakwas **11** był związkiem znanym i jego synteza została opisana w literaturze,[8] jednak moje próby zastosowania tej reakcji dla pochodnych pozbawionych łańcucha w pozycji *d* zakończyły się niepowodzeniem. Z tego powodu, opracowałem własną procedurę hydrolizy. Wykazałem, że zastosowanie roztworu wodorotlenku sodu w izopropanolu w temperaturze pokojowej oraz następcze zakwaszenie mieszaniny kwasem trifluorooctowym, w połączeniu z procedurą ekstrakcji fenolowej pozwala efektywnie przeprowadzić hydrolizę, minimalizując reakcje uboczne. Ostatecznie, otrzymałem heptakwas **11** oraz 8-*nor*-heksakwas **10** odpowiednio z wydajnościami 82% i 70% (Schemat 3).

[6] X. Yang, V. B. Birman *Org. Lett.* 2009, *11*, 1499.
[7] S. Kurcoń, K. ó Proinsias, D. Gryko *J. Org. Chem.* 2013, *78*, 4115.
[8] S. Izumi, H. Shimakoshi, M. Abe, Y. Hisaeda *Dalton Trans*. 2010, *39*, 3302.



Schemat 3. Synteza heptakwasu **11** i 8-*nor*-hexakwasu **10**.

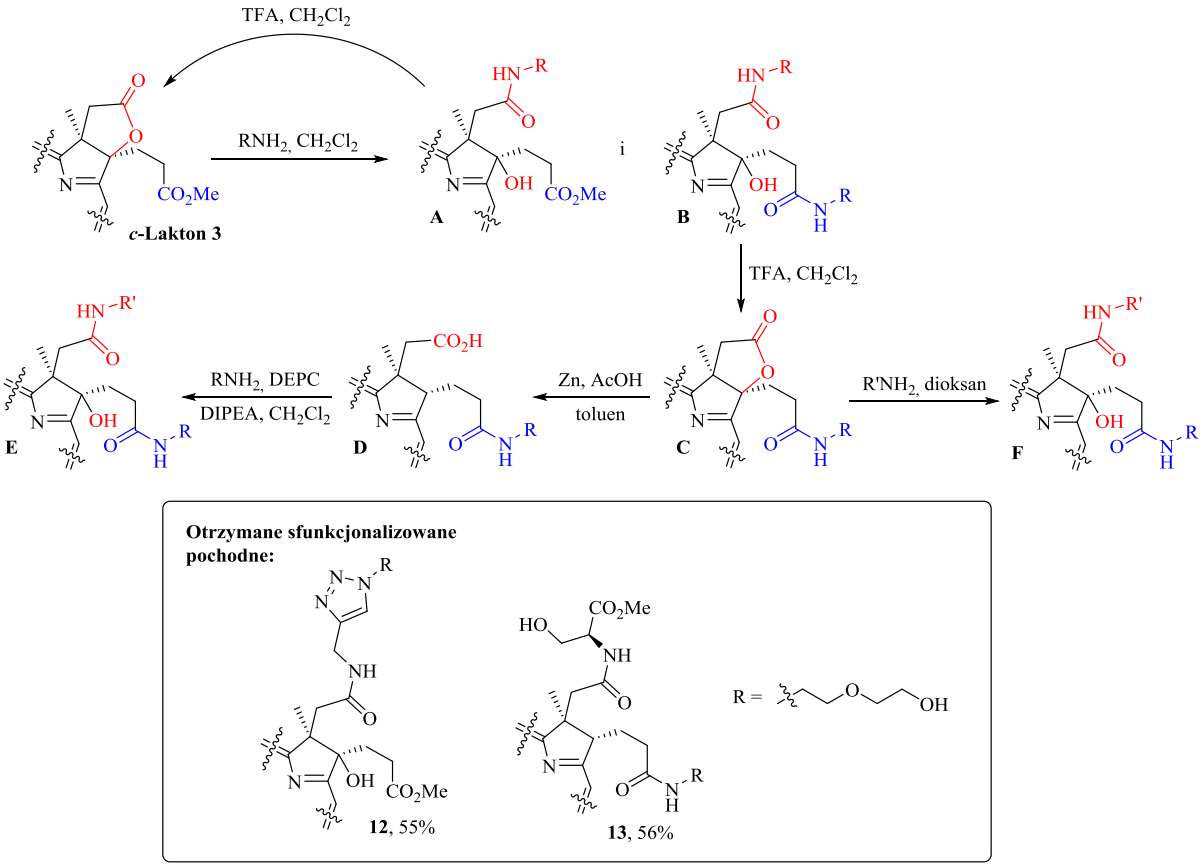
Otrzymane hepta- i heksapochodne kwasu kobyrynowego zostały poddane testom biologicznym w grupie Martina w University of Texas, Health Science Center, a niektóre z nich (hydrofilowe amidy **4b** i **8**) okazały się bardzo dobrymi aktywatorami rozpuszczalnej cyklazy guanylowej.**[P2, P5]**

Funkcjonalizacja:

Otrzymane związki są (poza jednym wspomnianym wyjątkiem) zupełnie nowymi pochodnymi witaminy B_{12}. Wobec obiecujących wyników badań biologicznych, a także biorąc pod uwagę aktualne trendy w chemii medycznej postanowiłem rozważyć możliwość zastosowania zsyntezowanych analogów jako składników budulcowych w połączeniach hybrydowych z innymi substratami o potencjalnym działaniu farmakologicznym np. peptydami, porfirynami itp. W tym celu konieczna była ich selektywna funkcjonalizacja.

Zwracając uwagę na fakt, że otrzymanie i oczyszczenie opisywanych kobinamidów jest pracochłonne i kosztowne, opracowałem procedury funkcjonalizacji, wykorzystując jako związki modelowe łatwe do zsyntetyzowania *c*-monoamidy i *c,d*-diamidy. W naszym zespole wykazano, że *c*-lakton **3** poddany reakcji z nadmiarem aminy ulega otwarciu dając *c*-amid **A**, a przy wydłużonym czasie reakcji również *c,d*-diamid **B**.[9] Oba typy związków posiadają grupę –OH w pozycji C8. Jej obecność sprawia, że amidy te są wrażliwe na działanie kwasów, pod wpływem których odtwarza się pierścień *c*-laktonu **C**. Ten z pozoru niekorzystny proces wykorzystałem do zróżnicowania podstawników w pozycjach *c* i *d*, a tym samym do funkcjonalizacji pochodnych kwasu kobyrynowego.**[P1]** Modelowe przekształcenia *c*-laktonu **3** oraz otrzymanych z niego amidów przedstawiłem na Schemacie 4. Zaznaczyłem na nim również otrzymane przeze mnie, sfunkcjonalizowane amidy **12** i **13**.

[9] K. ó Proinsias, J. L. Sessler, S. Kurcoń, D. Gryko *Org. Lett.* 2010, *12*, 4674.

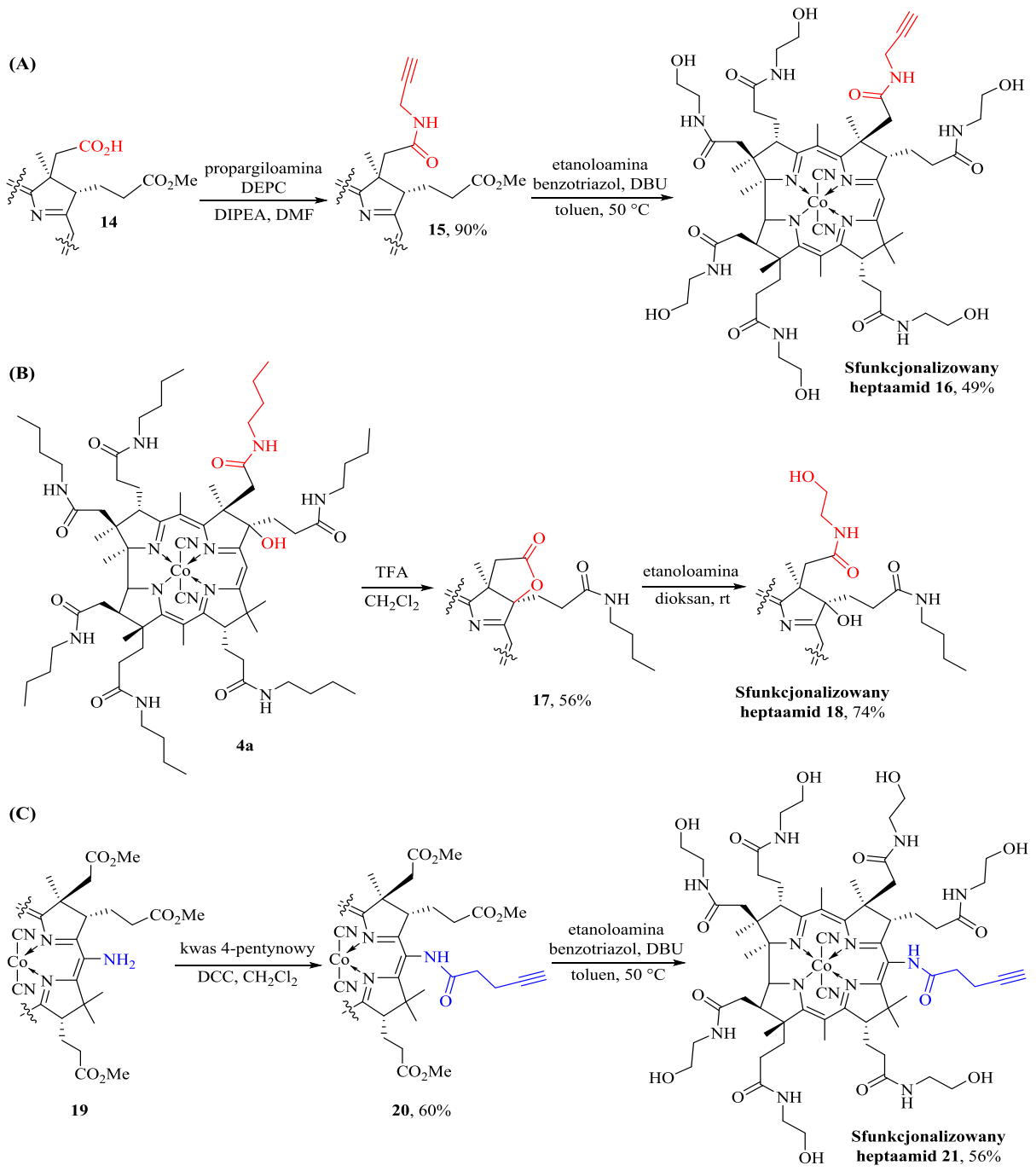


Schemat 4. Funkcjonalizacja pochodnych kwasu kobyrynowego w pozycjach *c* i *d*.

Następnie, opracowaną metodologię wykorzystałem do syntezy pochodnych heptaamidów.**[P3]** Metoda redukcji pierścienia laktonu do *c*-kwasu znalazła zastosowanie w syntezie heksaetanolo-*c*-propargiloamidu **16** (Schemat 5A). Natomiast, heksabutyloamid-*c*-lakton **17** posłużył mi jako produkt pośredni do funkcjonalizacji hydrofobowego kobinamidu **4a** (Schemat 5B). W tym celu poddałem go reakcji z nadmiarem etanoloaminy w dioksanie, co doprowadziło do selektywnego otwarcia pierścienia laktonu i otrzymania amidu **18**. Produkt ten posiada grupę –OH przy terminalnym atomie łańcucha *c*-amidowego, która może być wykorzystana w dalszych przekształceniach chemicznych np. estryfikacji, alkilowaniu itp. Ostatnim wykorzystanym przeze mnie sposobem wprowadzania grupy funkcyjnej do struktury heptaamidów była modyfikacja pozycji *mezo* heptaestru metylowego **2b** na drodze sprzęgania *mezo*-aminy **19**[10,11] z kwasem pentynowym oraz następczej reakcji aminolizy (Schemat 5C).

Z punktu widzenia przyszłych zastosowań, duża różnorodność opracowanych metod funkcjonalizacji otwiera nowe możliwości syntetyczne i umożliwia wybór najbardziej optymalnych połączeń hybrydowych omawianych związków.

[10] K. ó Proinsias, S. Kurcoń, D. Gryko *European J. Org. Chem.* 2012, *2012*, 154.
[11] A. Gossauer, K.-P. Heise, H. Götze, H. H. Inhoffen *Justus Liebigs Ann. Chem.* 1977, *1977*, 1480.



Schemat 5. Synteza sfunkcjonalizowanych hydrofilowych i hydrofobowych heptaamidów **16**, **18** i **21**.

Wyniki opisane w tym rozdziale zostały opublikowane w czterech artykułach naukowych:

[P1] K. ó Proinsias, **M. Giedyk**, R. Loska, M. Chromiński, D. Gryko *J. Org. Chem.* 2011, *76*, 6806;

[P2] K. ó Proinsias, **M. Giedyk**, I. Sharina, E. Martin, D. Gryko *ACS Med. Chem. Lett.* 2012, *3*, 476;

[P3] K. ó Proinsias, **M. Giedyk**, Ł. Banach, D. Rutkowska-Zbik, D. Gryko *Asian J. Org. Chem.* 2013, *2*, 504;

[P5] M. Giedyk, K. ó Proinsias, S. Kurcoń, I. Sharina, E. Martin, D. Gryko *ChemMedChem* 2014, *9*, 2344.

4.3 Kobalester i jego pochodne

Wszystkie opisane dotychczas pochodne nie zawierały charakterystycznego dla witaminy B_{12} fragmentu nukleotydowego związanego z pierścieniem makrocyklicznym drugorzędowym wiązaniem amidowym w pozycji *f*. Jego brak zmienia otoczenie koordynacyjne centralnego atomu kobaltu i może wpływać na jego właściwości redoks.[12,13] Dlatego też, w kolejnym etapie pracy podjąłem próby modyfikacji wyłącznie pierwszorzędowych grup amidowych. Jak dotąd, reakcje tego typu nie zostały opisane w literaturze.

Trudność, przed którą stanąłem, polegała na konieczności selektywnego przekształcenia sześciu amidów pierwszorzędowych zlokalizowanych na końcach łańcuchów bocznych kobalaminy, w obecności amidu drugorzędowego. Jak wiadomo są to stabilne grupy, których transformacje przebiegają zazwyczaj w drastycznych warunkach. Wyjątek stanowi metoda opisana przez Brocchettę i Myersa, w której przekształcenie pierworzędowych amidów w estry zachodzi w łagodnych warunkach, na drodze aktywacji grupy amidowej dimetyloacetalem dimetyloformamidu (DMF-DMA) i następczej alkoholizy wytworzonych formamidyn.[14,15] Biorąc pod uwagę, że drugorzędowe amidy powinny być w tych warunkach stabilne, uznałem tę metodę za obiecującą drogę przekształcenia kobalaminy bez naruszania pętli nukleotydowej.

Kluczowym etapem optymalizacji warunków reakcji okazał się dobór rozpuszczalnika. Użycie samego metanolu (pełniącego zarówno rolę reagenta jak i rozpuszczalnika), jak również dodatku rozpuszczalników takich jak DMF, DMSO, MeCN, *i*PrOH prowadziło do powstawania złożonej mieszaniny produktów. Dopiero zastosowanie nieklasycznego układu metanol/heksafluoroizopropanol pozwoliło mi otrzymać (z bardzo dobrą wydajnością 72%) i w pełni scharakteryzować heksaester-monoamid (kobalester, **22a**) (Schemat 6A). **Jest to jedyna znana pochodna witaminy B_{12} z zachowanym fragmentem nukleotydowym, wykazująca właściwości amfifilowe, rozpuszczająca się zarówno w rozpuszczalnikach polarnych, jak i niepolarnych.**

[12] Y. Murakami, Y. Hisaeda, T. Ozaki, Y. Matsuda *Chem. Lett.* 1988, *17*, 469.
[13] D. Lexa, J. M. Saveant *Acc. Chem. Res.* 1983, *16*, 235.
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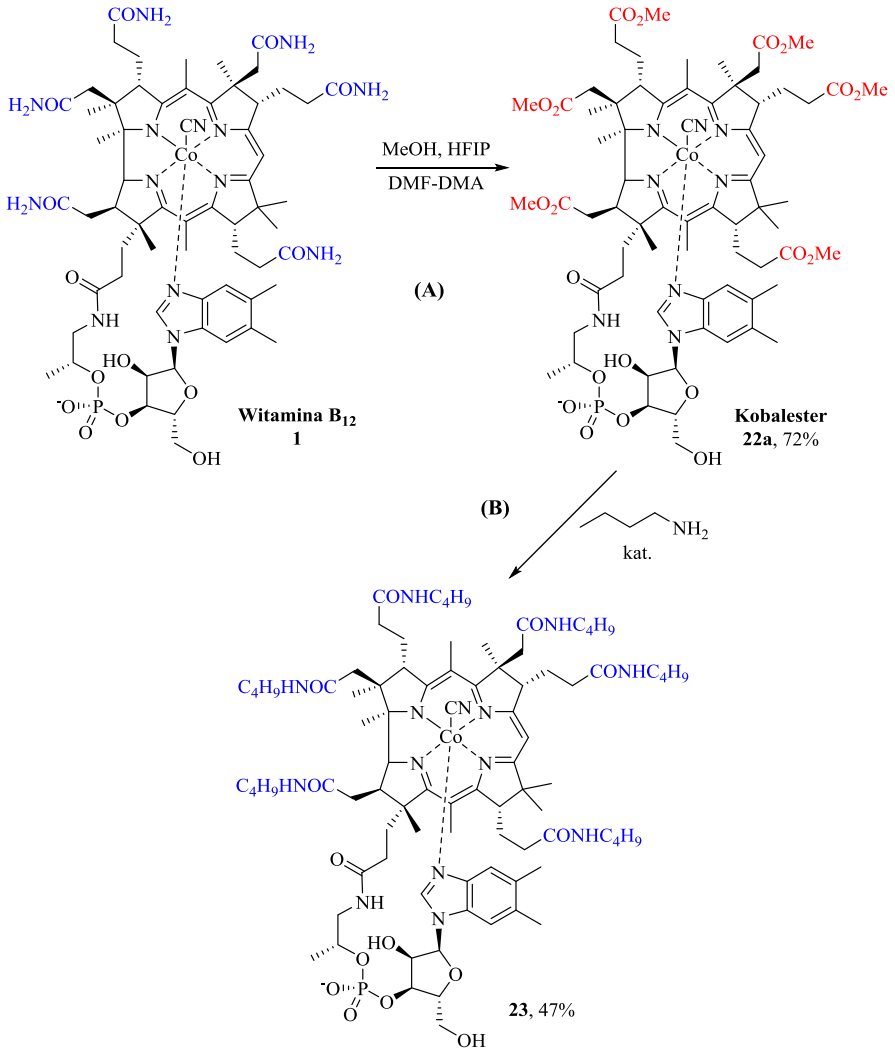
[15] T. A. Dineen, M. A. Zajac, A. G. Myers *J. Am. Chem. Soc.* 2006, *128*, 16406.



Schemat 6. Synteza kobalestru (**22a**) (A) oraz heksa(*n*-butylo)amidu **23** (B).

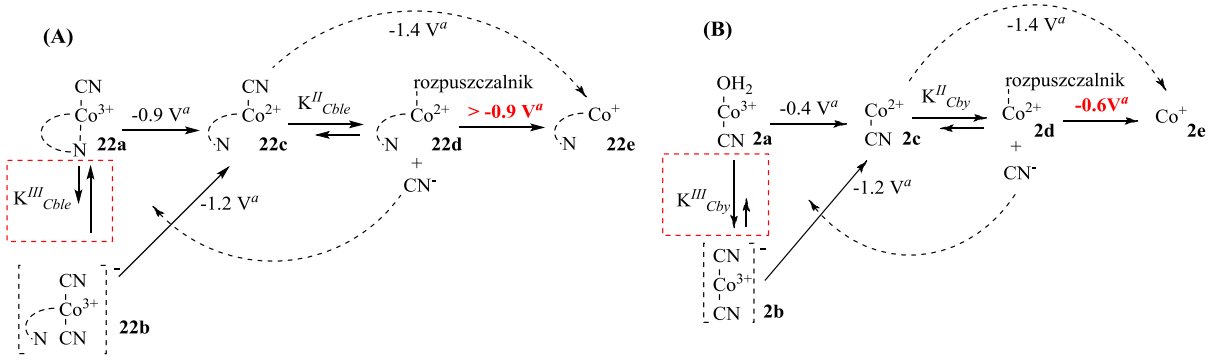
Następnie, korzystając z doświadczenia zdobytego podczas pracy nad pochodnymi kwasu kobyrynowego (rozdział 4.2) opracowałem metodę aminolizy grup estrowych prowadzącą do heksaamidowej pochodnej witaminy B_{12} **23** (Schemat 6B). Oba związki (ester **22a** i amid **23**), poza interesującymi różnicami we właściwościach fizykochemicznych, dostarczyły również ważnych danych biologicznych. **W przeciwieństwie do kobalaminy nie wiążą się one z białkami transportującymi: czynnikiem wewnętrznym, transkobalaminą oraz haptokoryną, co potwierdza kluczową rolę amidów pierwszorzędowych we właściwym dopasowaniu witaminy B_{12} do centrum wiążącego tych białek.**

Omawiane wyniki zostały opublikowane w czasopiśmie *Chemical Communications*:

[P4] M. Giedyk, S. N. Fedosov, D. Gryko *Chem. Commun.* 2014, *50*, 4674.

4.4 Właściwości elektrochemiczne pochodnych witaminy B_{12}

Podczas trzymiesięcznego stażu badawczego, który odbywałem w zespole prof. Hisaedy na Uniwersytecie Kiusiu, zbadałem właściwości elektrochemiczne nowo otrzymanego kobalesteru (**22a**) oraz porównałem go z heptaestrem metylowym kwasu kobyrynowego (**2**). Badania metodami woltamperometrii cyklicznej oraz spektroskopii UV-Vis wykazały wyraźne różnice w charakterystyce redoks obu związków. Za niewątpliwy sukces tego etapu pracy uważam określenie mechanizmów redukcji obecnego w nich kationu kobaltu na podstawie pełnego przyporządkowania sygnałów w widmach CV (Schemat 7). **Są to pierwsze badania, w których bezpośrednio określono wpływ fragmentu nukleotydowego na właściwości korynoidów w środowisku o obniżonej polarności.**



Schemat 7. Zaproponowany mechanizm redukcji kobalestru (**22a**) i heptametyloestru kwasu kobyrynowego (**2**). [a] vs. Ag-AgCl

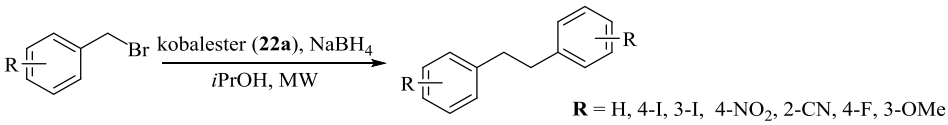
Wynika z nich, że kobalester (22a), dzięki zmniejszonemu powinowactwu do anionu cyjankowego, może być wydajniej redukowany (przy potencjale -0.9 V *vs.* Ag-AgCl) do aktywnej katalitycznie formy Co(I) (22e) niż heptaester (2). Ponadto, wykazałem istotny wpływ właściwości koordynujących rozpuszczalnika oraz otoczenia łańcuchów bocznych na charakterystykę redoks badanych pochodnych.

Wyniki te zostały opublikowane w czasopiśmie *Dalton Transactions*:

[P7] M. Giedyk, H. Shimakoshi, K. Goliszewska, D. Gryko, Y. Hisaeda *Dalton Trans.* 2016, DOI: 10.1039/C6DT00355A.

4.5 Reakcje katalityczne

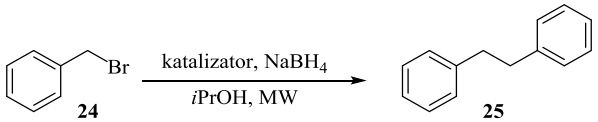
W oparciu o otrzymane wyniki potwierdzające możliwość wydajnej redukcji kobalestru (**22a**) do formy, w której kation kobaltu znajduje się na pierwszym stopniu utleniania, przeprowadziłem badania nad praktycznym wykorzystaniem mojego katalizatora w syntezie organicznej. Opracowałem metodę dimeryzacji podstawionych bromków benzylu w warunkach promieniowania mikrofalowego (Schemat 8). Do zalet metody zaliczyć należy bardzo krótki czas reakcji wynoszący 15 minut.



Schemat 8. Katalizowana kobalestrem (**22a**) dimeryzacja podstawionych bromków benzylu.

Następnie porównałem aktywność katalizatorów różniących się strukturą. Otrzymane wyniki wskazują, że zamiana estrów metylowych na grupy amidowe nie ma istotnego wpływu na wynik reakcji katalitycznej (Tabela 2, wiersz 1 i 2). Natomiast reakcja wobec związku **22a** posiadającego fragment nukleotydowy w pozycji *f* przebiegała znacznie lepiej od tej katalizowanej pochodną **2b**, w której obie pozycje aksjalne zajmowane są przez aniony cyjankowe (Tabela 2, wiersz 1 i 3).**[P4]**

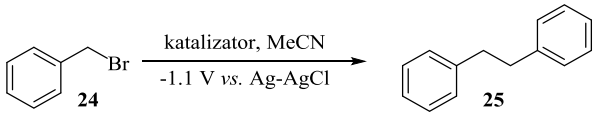
Tabela 2. Synteza bibenzylu (**25**) z zastosowaniem różnych pochodnych witaminy B_{12}.



Nr	Katalizator		Konwersja [%]	Wydajność [%]
1	Dicyjanoheptaester	**2b**	100	62
2	Heptametyloamid	**4d**	100	65
3	Kobalester	**22a**	100	84

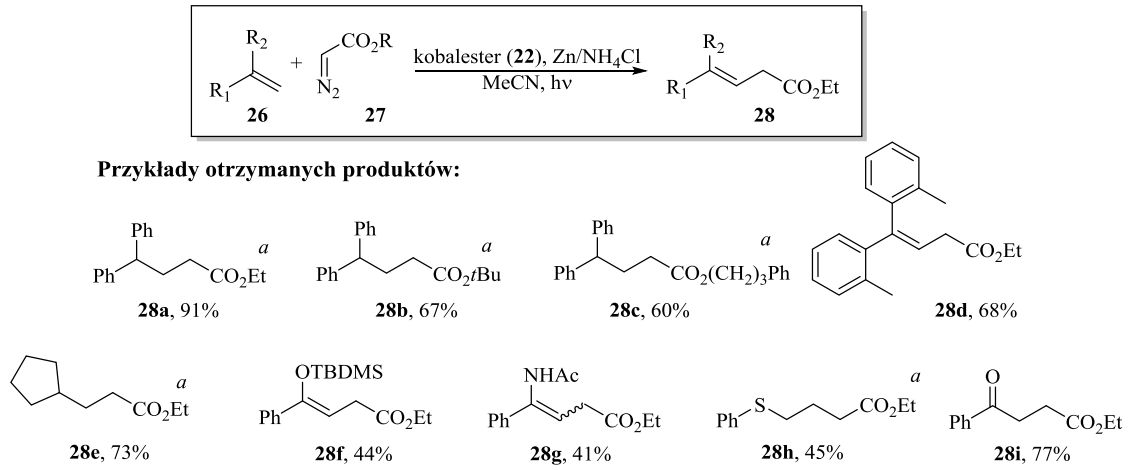
Aby zweryfikować hipotezę o pozytywnym wpływie pętli nukleotydowej na przebieg reakcji katalitycznej, przeprowadziłem elektrolizę bromku benzylu w warunkach kontrolowanego potencjału i w obecności katalizatorów: kobalestru (**22a**) oraz heptametylowego estru kwasu kobyrynowego (mono- **2a** i dicyjano **2b**) (Tabela 3).**[P7]** Potwierdziła ona różnicę między aktywnością kobalestru (**22a**) a dicyjanoheptaestru **2b**. Jednocześnie, wydajność otrzymana w obecności formy monocyjano- **2a** nie odbiegała od tej, osiągniętej z użyciem kobalestru (**22a**). Na podstawie otrzymanych wyników (w połączniu z badaniami woltamperometrii cyklicznej opisanymi w rozdziale 4.4) ustaliłem, że **obserwowane różnice wynikają z utrudnionej redukcji formy dicyjano- 2b, a w konsekwencji mniej wydajnego generowania aktywnego katalitycznie kompleksu Co(I).**

Tabela 3. Elektroliza bromku benzylu (**24**) w warunkach kontrolowanego potencjału.



Nr	Katalizator		Konwersja [%]	Wydajność [%]
1	Kobalester	**22a**	95	76
2	Monocyjanoheptaester	**2a**	88	76
3	Dicyjanoheptaester	**2b**	86	64

Ponadto, badając możliwość wykorzystania innych elektrofili jako źródeł rodników, **odkryłem nową reakcję indukowanego światłem widzialnym C-H alkilowania terminalnych olefin diazozwiązkami** (Schemat 9).**[P6]** Dotychczas ten zestaw reagentów utożsamiany był wyłącznie z cyklopropanowaniem alkenów,[16,17] a przykłady C-H funkcjonalizacji sprowadzały się jedynie do α,β-nienasyconych ketonów i oksymów.[18,19,20] Główną trudnością konieczną do przezwyciężenia było zapewnienie warunków redukujących, które umożliwiłyby wygenerowanie aktywnej formy Co(I) przy zachowaniu niezbędnej stabilności diazozwiązku. Warunek ten został spełniony dzięki użyciu aktywowanego cynku oraz dodatku NH_4Cl. Następnie, zoptymalizowałem warunki nowo odkrytej reakcji używając 1,1-difenyloetylenu (**26a**) jako modelowego substratu, ostatecznie otrzymując pożądany produkt **28a** z wydajnością 91%. Co więcej, w reakcji tej można stosować również bogate w elektrony olefiny takie jak: etery enoli, enamidy oraz tioetery winylowe. Badania mechanistyczne wykazały, że reakcja przebiega przez etap tworzenia niestabilnego kompleksu kobalt-alkil i że w reakcji tej Co(I) jest aktywną formą katalizatora.



Schemat 9. C-H alkilowanie terminalnych olefin za pomocą diazozwiązków, katalizowane kobalestrem (**22a**).
[a] przeprowadzone zostało następcze uwodornienie

Powyższe wyniki zostały opublikowane w czasopiśmie *Chemical Communications*:

[P6] M. Giedyk, K. Goliszewska, K. ó Proinsias, D. Gryko *Chem. Commun.* 2016, *52*, 1389.

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[17] Y. Chen, X. P. Zhang *J. Org. Chem.* 2004, *69*, 2431.
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4.6 Podsumowanie

Podsumowując, prowadzone przeze mnie badania doprowadziły do odkrycia nowych pochodnych witaminy B_{12} oraz określenia ich właściwości i aktywności katalitycznej w reakcjach rodnikowych. Za największe osiągnięcia badawcze uważam:

- **opracowanie metody syntezy jedynej, amfifilowej pochodnej witaminy B_{12}, którą nazwałem kobalestrem (22a)**
- **zbadanie ścieżki redukcji kationu kobaltu skompleksowanego w kobalestrze (22a) oraz porównanie jej do ścieżek innych, znanych pochodnych witaminy B_{12}**
- **opracowanie metody aminolizy estrów znajdujących się na końcu łańcuchów bocznych pochodnych witaminy B_{12}**
- **odkrycie nowej reakcji C-H alkilowania terminalnych olefin za pomocą diazozwiązków, a także innych reakcji katalizowanych pochodnymi witaminy B_{12}.**

Badania przedstawione w niniejszej rozprawie stanowią zwartą całość i jasno demonstrują użyteczność pochodnych witaminy B_{12} jako wydajnych i przyjaznych środowisku katalizatorów. Ponadto, pozwoliły one poszerzyć arsenał dostępnych metod katalitycznych w syntezie organicznej. Szczególną satysfakcję daje mi również fakt, że otrzymane wyniki prowokują do stawiania kolejnych pytań badawczych i mogą stanowić podstawę dalszych, interesujących badań w dziedzinie chemii organicznej.

5. PUBLIKACJE PRZEGLĄDOWE

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Vitamin B_{12}: chemical modifications†

Keith ó Proinsias, Maciej Giedyk and Dorota Gryko*

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www.rsc.org/csr

Vitamin B_{12} plays a key role in many metabolic processes occurring in all mammals. Over the years its biological role has been extensively studied generating a lot of interest in the chemistry of this vital molecule. This established a variety of new methodologies for the synthesis and analysis of new cobalamin derivatives as well as creative purification techniques. This tutorial review summarizes all the advancements made in this area, providing a deeper insight into vitamin B_{12} chemistry.

Key learning points
- The nomenclature of corrinoids.
- The chemistry of vitamin B_{12}.
- Selective modifications of cobalamin.
- Purification and analysis of cobalamin and its derivatives.

1 Introduction

2 Nomenclature

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Vitamin B_{12} catalysed reactions

Maciej Giedyk,[a] Katarzyna Goliszewska[ab] and Dorota Gryko*[a]

Vitamin B_{12} (cobalamin, **1**) is one of a few naturally occurring organometallic molecules. As a cofactor for adenosylcobalamin-dependent and methylcobalamin-dependent enzymes, it plays a crucial role in biological processes, including DNA synthesis and regulation, nervous system function, red blood cell formation, *etc.* Enzymatic reactions, such as isomerisation, dehalogenation, and methyl transfer, rely on the formation and cleavage of the Co–C bond. Because it is a natural, nontoxic, environmentally benign cobalt complex, cobalamin (**1**) has been successfully utilised in organic synthesis as a catalyst for Co-mediated reactions. This tutorial review concisely describes cobalamin-catalysed organic reactions that hold promise for environmentally friendly cobalt catalysis, leaving the reader with basic knowledge and the ability to harness the catalytic potential of this fascinating molecule.

Key learning points
(1) Rationale for B_{12}-catalytic activity
(2) 'Supernucleophilicity' of cobalamin(I)
(3) Radical activity of cobalamin(II)
(4) B_{12}-catalysed reactions
(5) Mechanisms of B_{12}-catalysed reactions

1 Introduction

Nature has always been the ultimate source of inspiration for scientists working across all disciplines. This statement certainly applies to catalysis, where continuous effort has been invested in mimicking and improving the function and effectiveness of enzymes. To this end, new synthetic catalysts have been developed and exploited to facilitate more efficient and selective transformations of organic compounds. However, many of these catalysts are poor replicas of naturally occurring enzymes, compromised by low stability or selectivity, high catalyst loading, or toxicity.

First isolated by Folkers and Smith in 1948, vitamin B_{12} (B_{12}, cobalamin, **1**, Fig. 1) has been recognised as a cofactor for enzymes that catalyse a range of biological processes, including isomerisation, methyltransfers, and dehalogenation.[1]

The ability of vitamin B_{12} (**1**) to catalyse these thermodynamically challenging reactions has attracted the attention of synthetic chemists, which has eventually led to the discovery of new catalytic transformations. This review highlights the application of vitamin B_{12} (**1**) as an environmentally benign catalyst for organic reactions; we leave enzymatic transformations aside, because they have been reviewed elsewhere.[1,2]

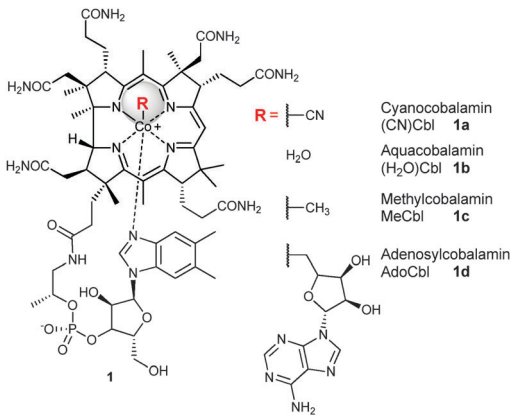

Fig. 1 Chemical structures of cobalamin and its derivatives: cyanocobalamin (**1a**), aquacobalamin (**1b**), and B_{12}-coenzymes: methylcobalamin (**1c**) and adenosylcobalamin (**1d**). Note: aquacobalamin (**1b**) bears a neutral aqua ligand, which results in a positively charged molecule; therefore, it is always accompanied by a counter-ion. For clarity, counter-ions are not shown throughout this review.

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6. PUBLIKACJE ORYGINALNE

JOC The Journal of Organic Chemistry

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Selective Modifications of Hydrophobic Vitamin B_{12} Derivatives at *c*-and *d*-Positions

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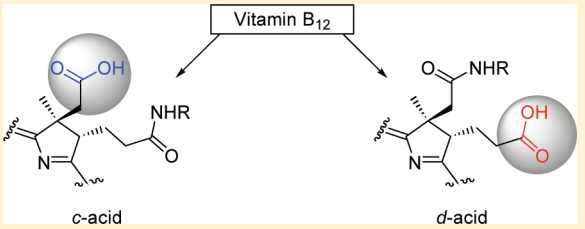
Supporting Information

ABSTRACT: The acid-sensitivity of vitamin B_{12} derived mono- and diamides was studied. It was found that the use of reductive ring-opening of the lactone moiety deactivated undesired decomposition of *c*-mono- and *c,d*-diamides under acidic conditions. As a result, reactions gave respectively *c*- or *d*-acids which were further functionalized via coupling with amino acids. Though mono- and diamides exhibited acid sensitivity, they were used for the preparation of several highly functionalized molecules showing their stability under various reaction conditions.



The importance of vitamin B_{12}, which stems from its diversified applications, is difficult to overestimate.[1] It has been investigated for many years as an oral delivery vehicle for therapeutic agents.[2−8] Most of these conjugates have been prepared via selective reactions on the cobalt center or at the 5′-hydroxy group on the ribose moiety (Figure 1).

Moreover, vitamin B_{12} derivatives have been effectively used as artificial enzymes and have been found to catalyze various organic reactions.[9] One of the most widely investigated derivatives was hydrophobic heptamethyl dicyanocobyrinate possessing terminal ester groups instead of original amides and the central cobalt atom being bound to two cyanide ligands. Hisaeda and co-workers have recently elaborated an efficient method for the dechlorination of di(4-chlorophenyl)trichloroethane (DDT) mediated by heptamethyl cobyrinate perchlorate in ionic liquids,[10] which mainly led to 1,1′-(ethylidene)bis(4-chlorobenzene) (DDO). Compounds of this type were also found to catalyze the nucleophilic addition of an alcohol to an olefin under mild conditions.[11] Moreover, to elucidate the role of potassium ion in vitamin B_{12} dependent enzymatic reactions Hisaeda's group has prepared a hydrophobic vitamin B_{12} bearing a benzo-18-crown-6 moiety.[12]

For vitamin B_{12} to realize its full potential, more efficient approaches toward selective modifications of both hydrophilic and hydrophobic derivatives have to be developed. Though various vitamin B_{12} derivatives have already been prepared and studied, there have been only a few reports describing selective preparation and use of cobalamin-derived acids. Syntheses of such compounds were usually based on vitamin B_{12} hydrolysis followed by partial esterification.[13] These approaches are non-selective, thus requiring laborious separation of regioisomers. Still, acids prepared in such a way have been successfully used in further reactions.[14,15] Selective formation of *c*-lactone and its reduction leading to either a carboxylic acid or an alcohol was reported by Keese and co-workers.[16] Therefore, further modifications of hydrophobic cobalamines have been rather limited

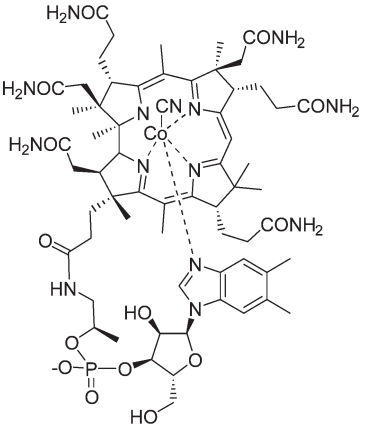


Figure 1. Vitamin B_{12}.

to reactions occurring at the *c*-position. There have been a multitude of reports detailing reactions at this site.[17,18]

Recently, we have undertaken comprehensive studies directed toward selective modifications of both hydrophilic and hydrophobic cobalamin derivatives.[19] It was shown that ring-opening of *c*-lactone **1** with various primary amines led selectively to either mono- or diamides (Scheme 1).

The goal of our research was to elaborate the vitamin B_{12} chemistry and to find superior approaches for the *regioselective* preparation of derivatives bearing complex and diversified functionalities. In particular, we were interested in strategies that could lead to bifunctional molecules under very mild conditions, thus enabling us to link a broad range of substrates to the vitamin B_{12} core.

The studies were initiated by evaluating the acid sensitivity of vitamin B_{12} derived *c*-mono- and *c,d*-diamides. When *c*-monoamides

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Selective Modifications of Hydrophobic Vitamin B_{12} Derivatives at *c*-and *d*-Positions

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Electronic Supporting Information

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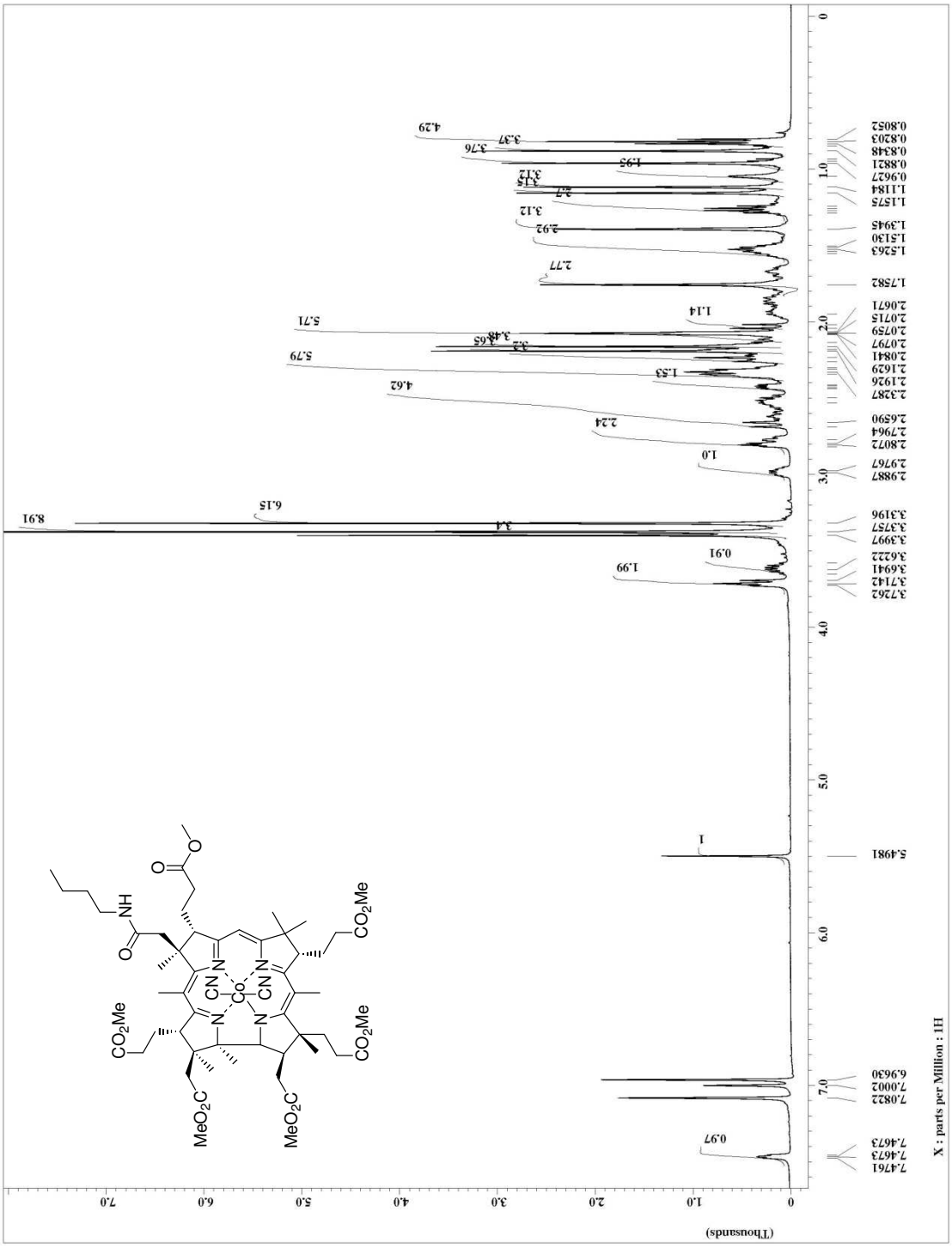
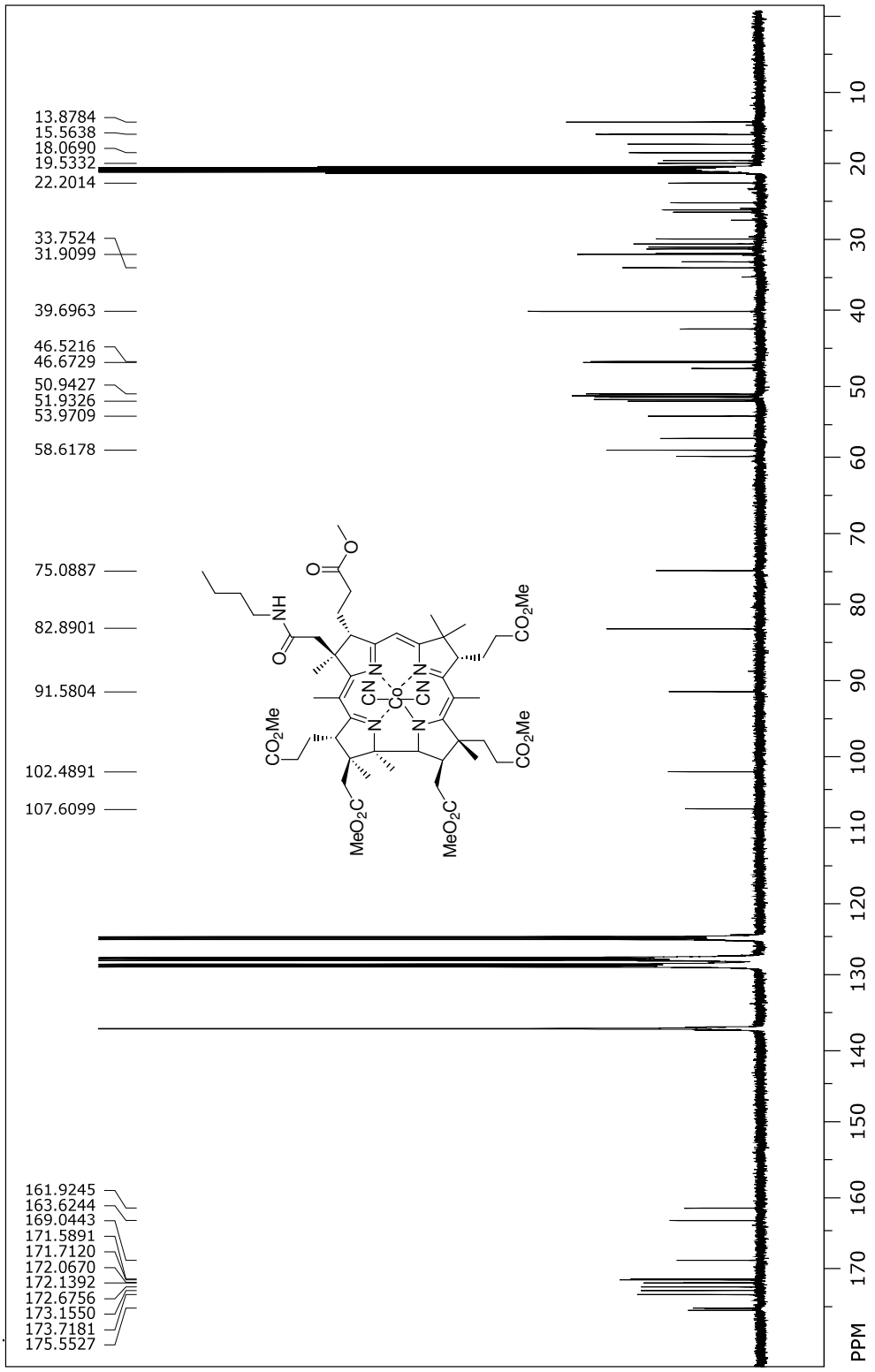
$(CN)_2$Cob(III)C1(*n*-butylamide) 3

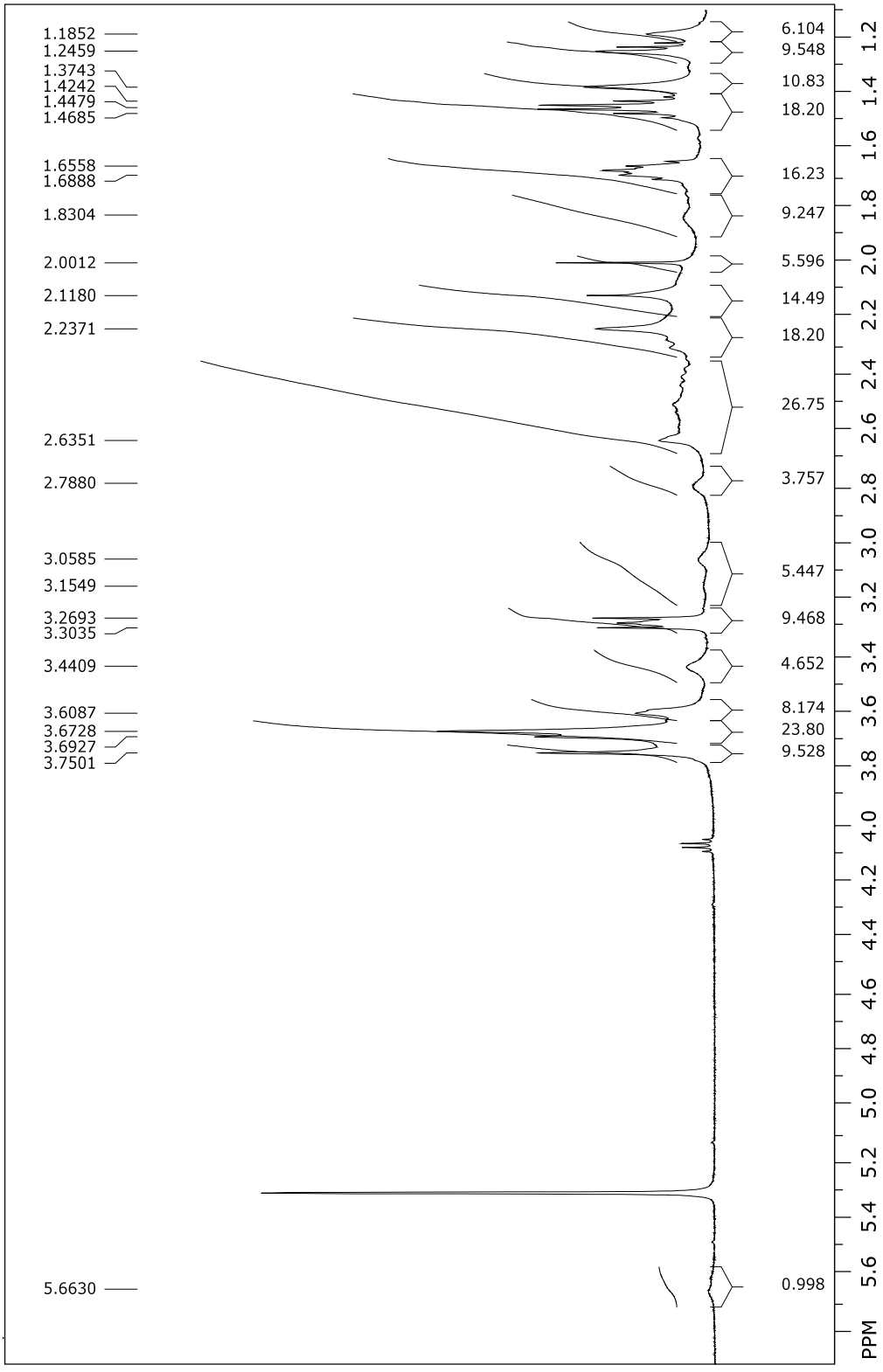


$(CN)_2$Cob(III)C1(*n*-butylamide) 3

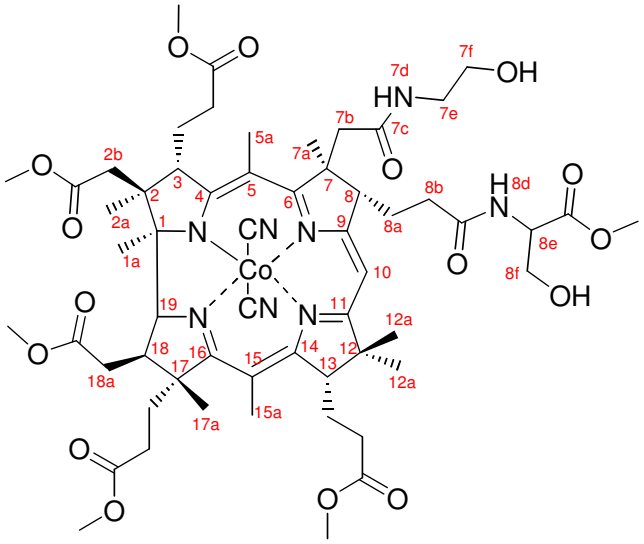


$(CN)_2$Cob(III)C1(ethanolamide)(-COOH) 5a

Note: The broadening of peaks in the ^{1}H NMR spectrum made it impossible to decipher and consequently high resolution ^{13}C spectra could not be obtained. This was caused by the presence of the acid group.



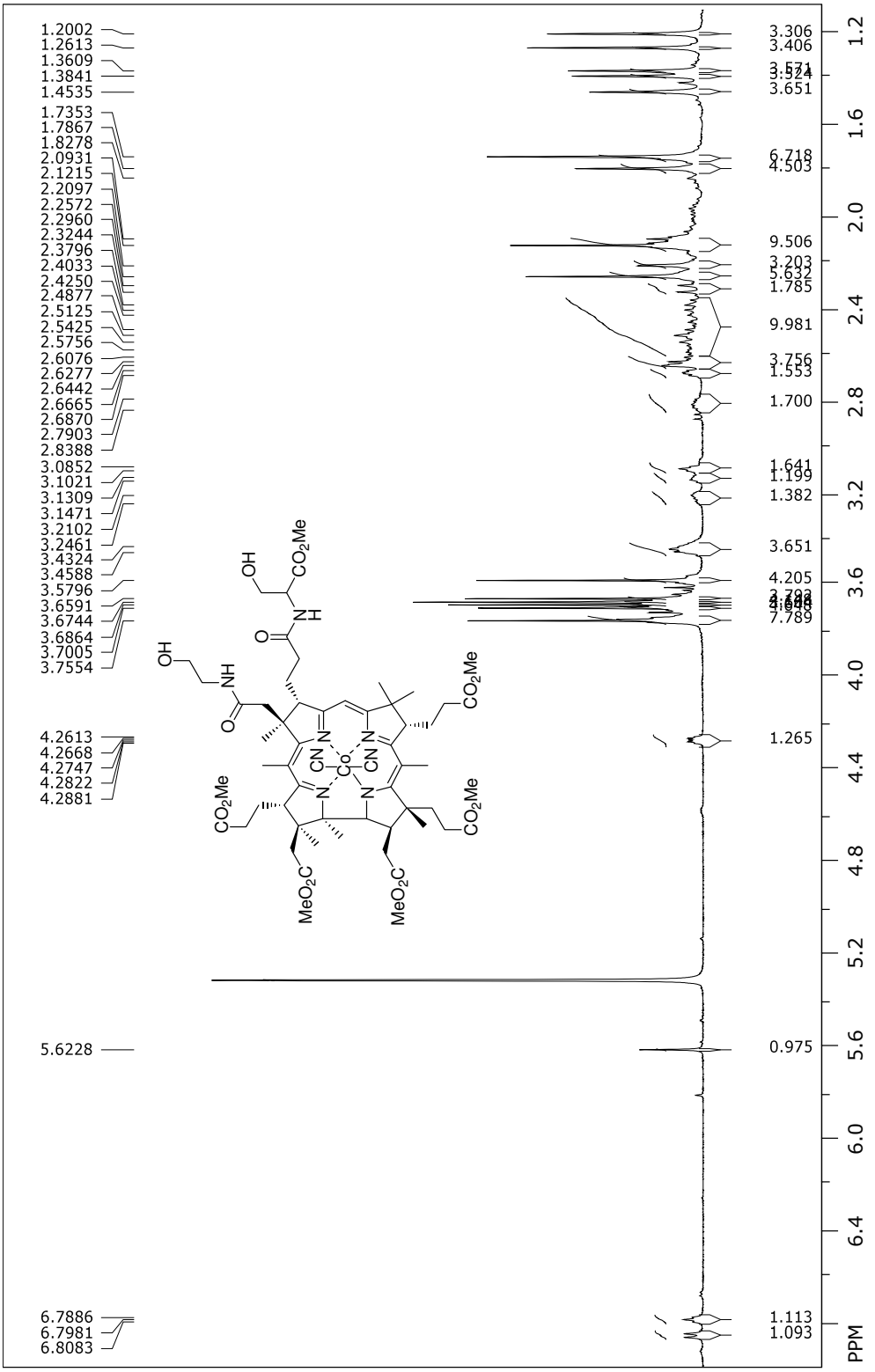
$(CN)_2$Cob(III)C1(ethanolamide)(NH-Serine-OMe) 6



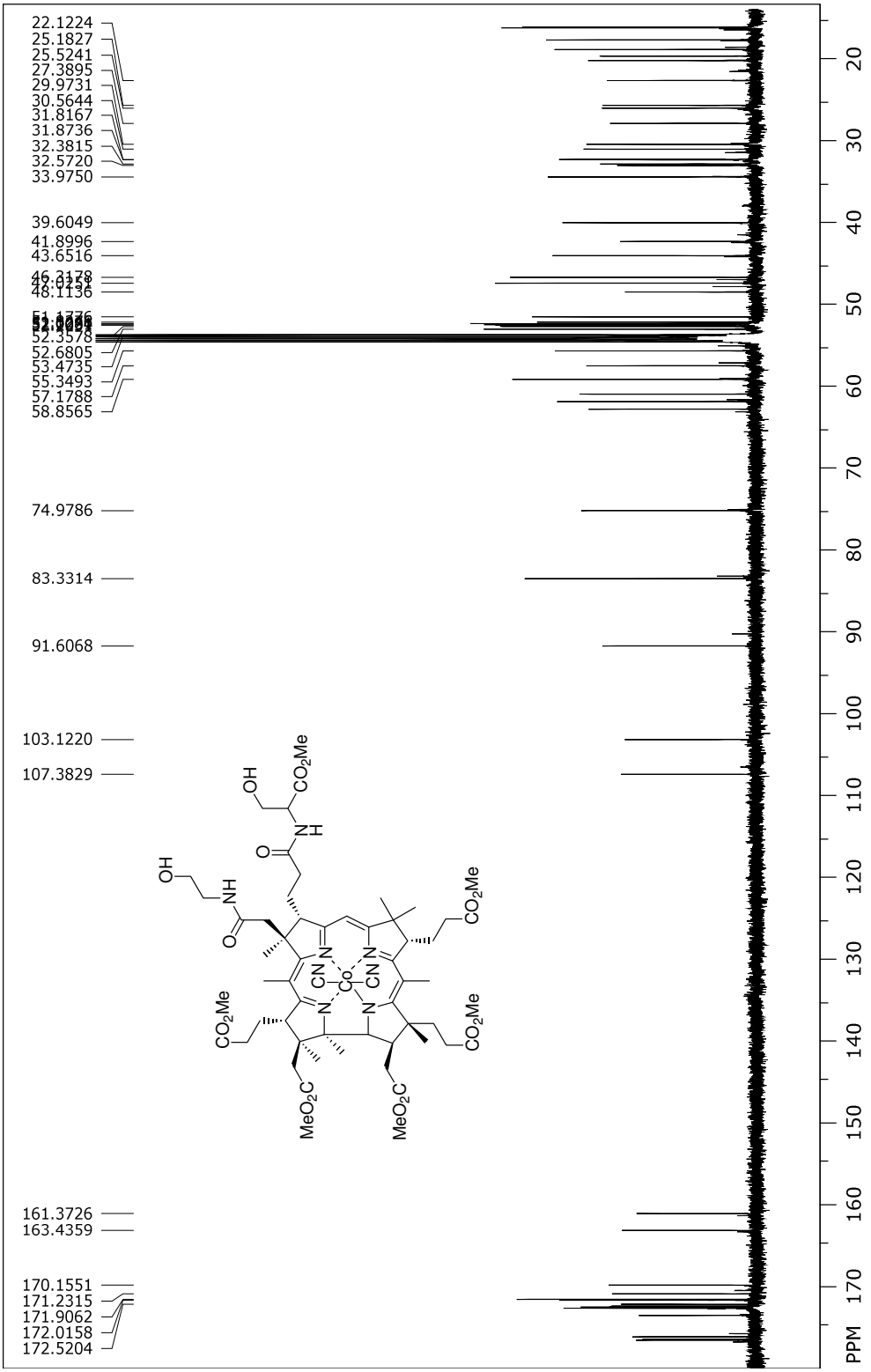
^{1}H NMR (500 MHz, CD_2Cl_2) δ (ppm); 6.86 (d, *J* = 6.8 Hz, 1H, 8d), 6.79 (t, *J* = 4.8 Hz, 1H, 7d), 5.62 (s, 1H, 10), 4.27 (dd, *J* = 2.9 and 3.9 Hz, 1H, 8e), 3.75 (s, 7H, 8f, 19 and -OC**H$_3$**), 3.70 (s, 3H, -OC**H$_3$**), 3.68 (s, 3H, -OC**H$_3$**), 3.67 (s, 3H, -OC**H$_3$**), 3.65 (s, 3H, -OC**H$_3$**), 3.57 (s, 4H, 8f and -OC**H$_3$**), 3.47-3.40 (m, 4H, 7f and 7b), 3.25-3.19 (m, 1H, 7e), 3.15-3.12 (m, 1H, 7e), 3.09 (t, *J* = 4.9 Hz, 1H, 13), 2.87-2.79 (m, 1H, 18), 2.67 (dd, *J* = 4.3 and 2.2 Hz, 1H, 3), 2.64-2.63 (m, 2H, 18a), 2.62-2.55 (m, 2H, C**H$_2$**), 2.54-2.51 (m, 1H, 2b), 2.48-2.35 (m, 4H, 8b and C**H$_2$**), 2.30 (d, *J* = 14.6 Hz, 1H, 2b), 2.25 (s, 5H, 15a, 8 and C**H$_2$**), 2.20 (s, 2H, C**H$_2$**), 2.12 (s(br), 7H, 5a, 8a and C**H$_2$**), 2.11-2.05 (m, 2H, C**H$_2$**), 1.87-1.82 (m, 1H, C**H$_2$**), 1.78 (s, 3H, 7a), 1.45 (s, 3H, 2a), 1.38 (s, 3H, 1a), 1.36 (s, 3H, 12a), 1.26 (s, 3H, 17a), 1.20 (s, 3H, 12a).

^{13}C NMR (125 MHz, CD_2Cl_2) δ (ppm); 177.1 (11), 176.9 (16), 176.4 (4), 173.8 (**C**=O), 173.0 (**C**=O), 172.8 (**C**=O), 172.7 (**C**=O), 172.5 (**C**=O), 172.0 (**C**=O), 171.9 (7c), 171.2(9), 170.8, 170.1, 163.4 (14), 161.3 (6), 107.3 (15), 103.1 (5), 91.6 (10), 83.3 (1), 74.9 (19), 62.5 (8f), 61.5 (7f), 60.6 (3), 58.8 (17), 57.1 (8), 55.3 (13), 53.4 (-O**C**H$_3$), 52.6 (-O**C**H$_3$), 52.3 (-O**C**H$_3$), 52.1 (-O**C**H$_3$), 51.9 (-O**C**H$_3$), 51.8 (-O**C**H$_3$), 51.1 (7), 48.1 (2b), 47.0 (12), 46.3 (2), 43.6 (7e), 41.9 (7c), 39.9 (18), 33.9 (8b), 32.5, 32.3, 31.9 (12a), 31.8 (19), 30.5 (8a), 29.9, 27.3, 25.7, 25.1, 22.1 (2a), 19.6 (12a), 19.1, 18.3 (17a), 17.1 (7a), 15.6 (15a), 15.5 (5a).

$(CN)_2$Cob(III)C1(ethanolamide)(NH-Serine-OMe) 6



$(CN)_2$Cob(III)C1(ethanolamide)(NH-Serine-OMe) 6



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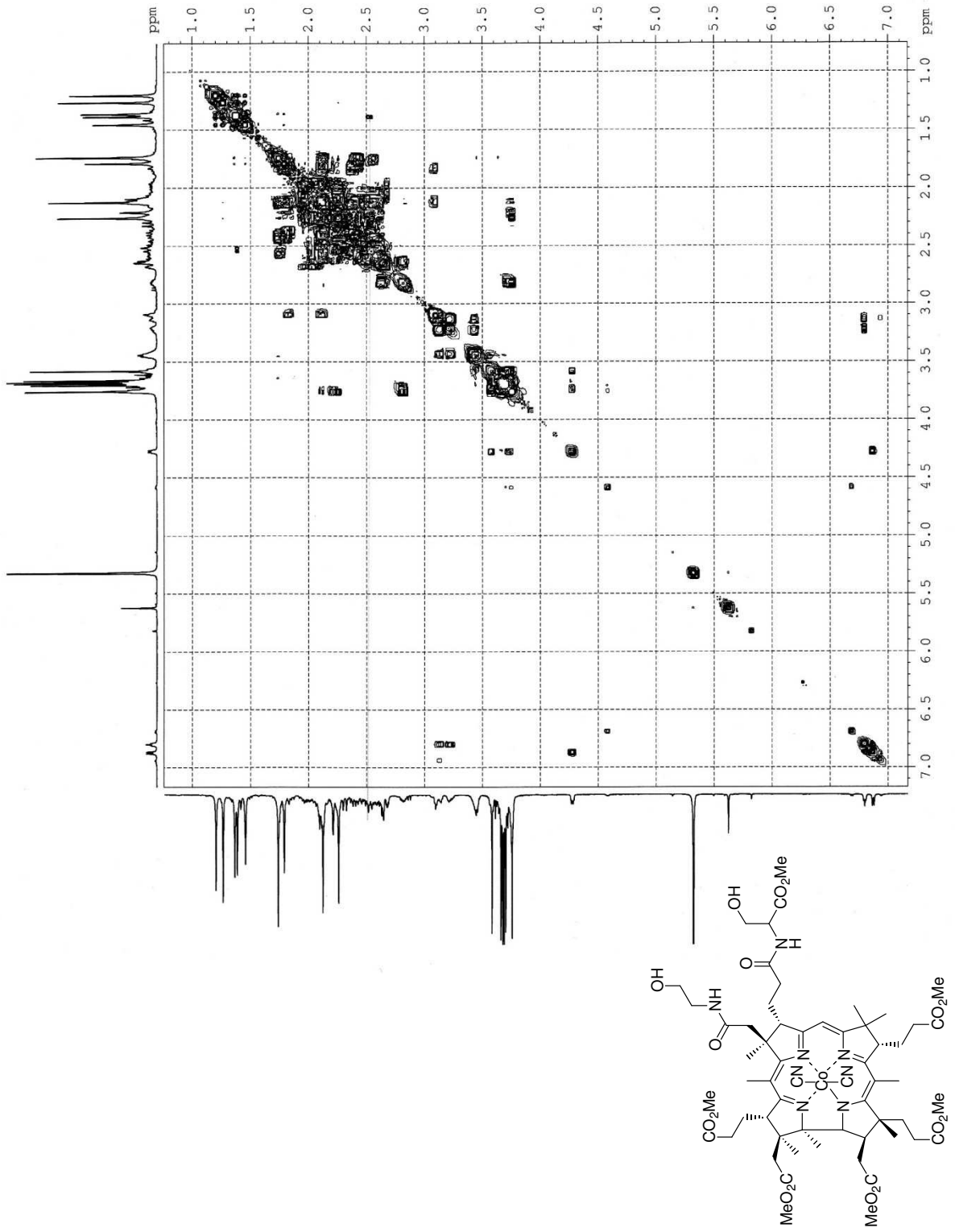
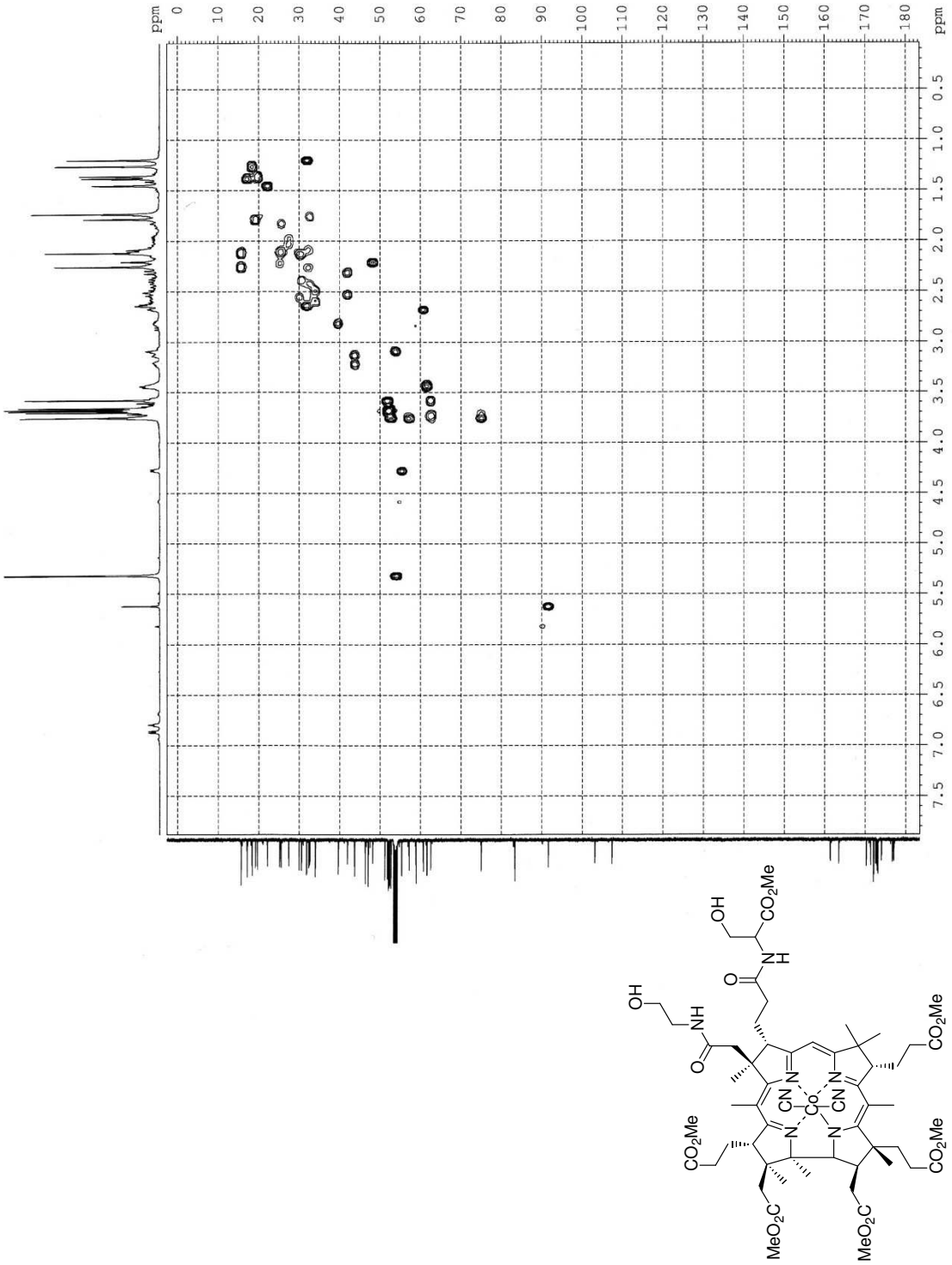
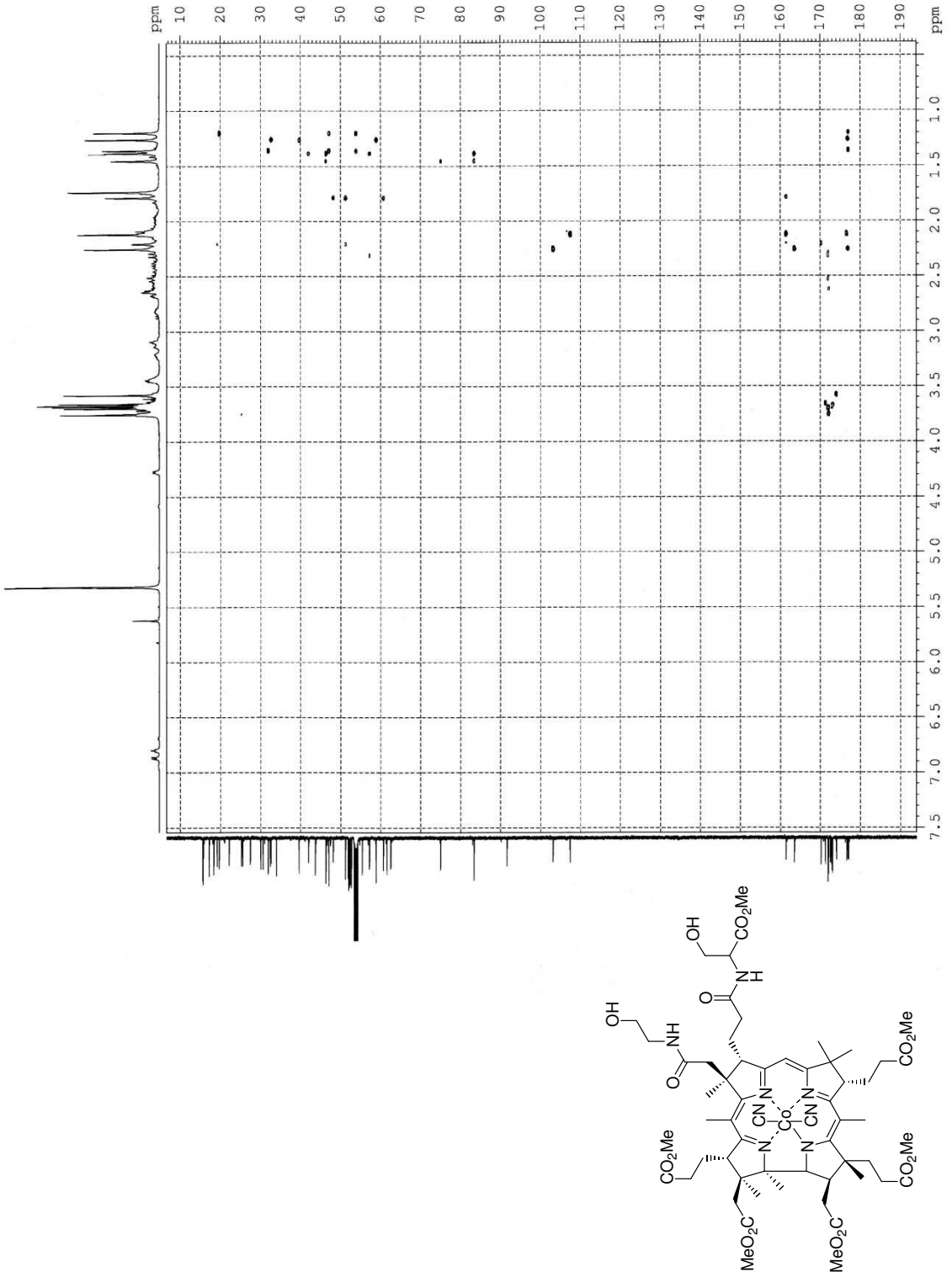


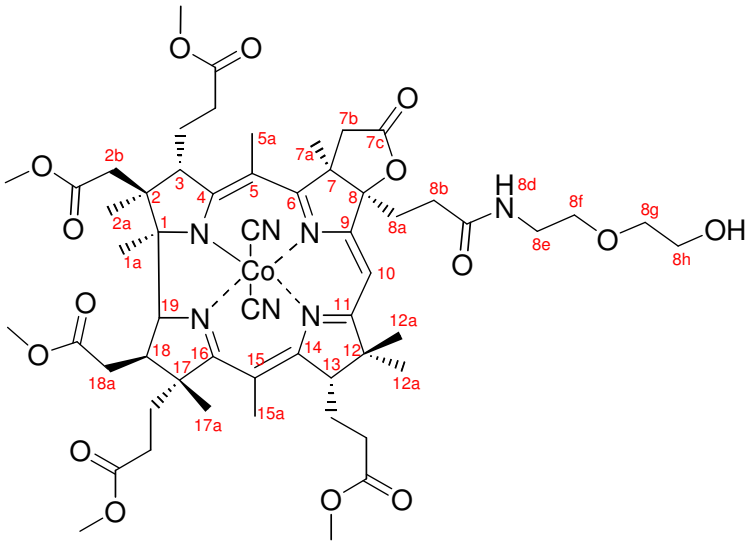
$(CN)_2$Cob(III)C1(ethanolamide)(NH-Serine-OMe) 6



$(CN)_2$Cob(III)C1(ethanolamide)(NH-Serine-OMe) 6



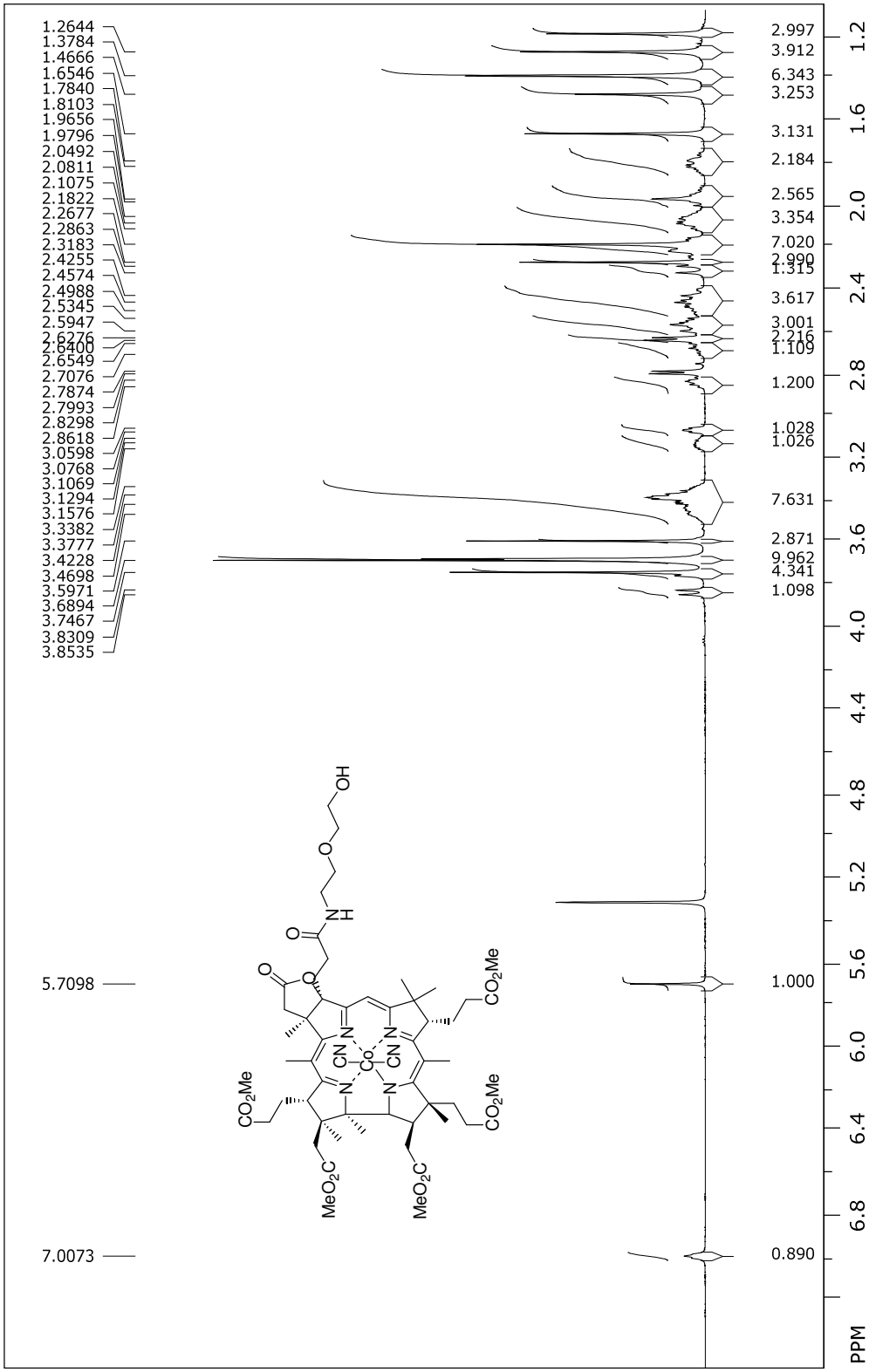
$(CN)_2$Cob(III)C1(lactone)(diglycolamide) 8



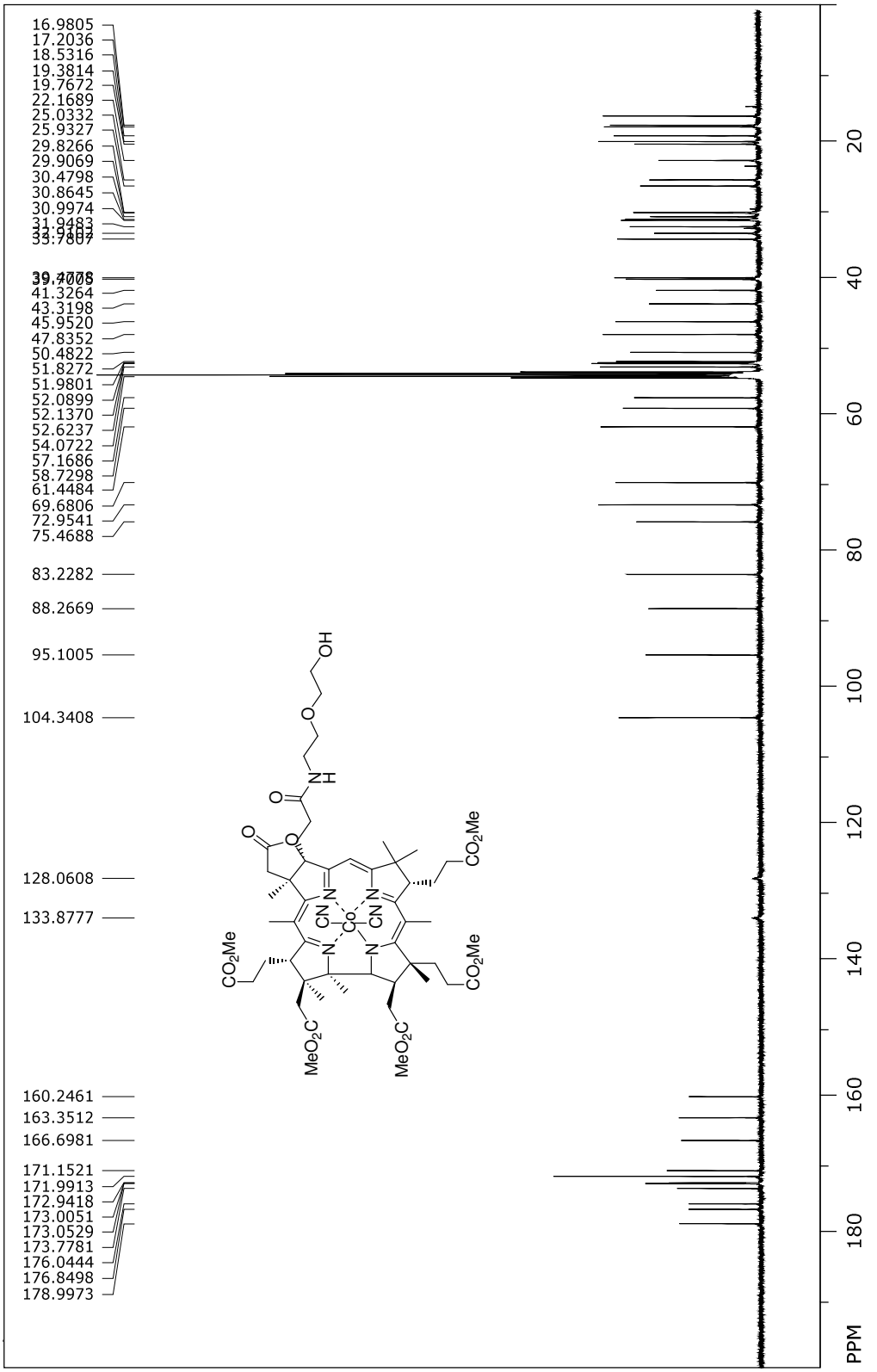
^{1}H NMR (500 MHz, CD_2Cl_2) δ (ppm); 7.00 (t, *J* = 5.0 Hz, 1H, 8d), 5.70 (s, 1H, 10), 3.84 (d, *J* = 10.8 Hz, 1H, 19), 3.76-3.74 (m, 4H, -OC**H$_3$** and 3), 3.69 (s, 7H, -OC**H$_3$** and 8f), 3.68 (s, 3H, -OC**H$_3$**), 3.59 (s, 3H, -OC**H$_3$**), 3.50-3.32 (m, 7H, 8e, 8f, 8g and 8h), 3.15-3.10 (m, 1H, 8e), 3.06 (dd, 1H, *J* = 4.5 and 1.4 Hz, 13), 2.86-2.82 (m, 1H, 18), 2.79 (d, 2H, *J* = 5.9 Hz, 7b), 2.70-2.65 (m, 1H, 8b), 2.63 (d, 2H, *J* = 6.2 Hz, 18a), 2.59-2.53 (m, 4H, 2b, 8b and C**H$_2$**), 2.50-2.39 (m, 2H, 8a and C**H$_2$**), 2.30 (d, 1H, *J* = 15.8 Hz, 2b), 2.26 (s, 3H, 15a and C**H$_2$**), 2.21-2.15 (m, 7H, C**H$_2$**, 5a and 8a), 2.11-2.02 (m, 3H, C**H$_2$**), 1.99-1.91 (m, 1H, C**H$_2$**), 1.84-1.74 (m, 2H, C**H$_2$**), 1.65 (s, 3H, 7a), 1.46 (s, 3H, 1a), 1.37 (s, 6H, 12a and 2a), 1.26 (s, 3H, 17a), 1.17 (s, 3H, 12a).

^{13}C NMR (125 MHz, CD_2Cl_2) δ (ppm); 178.9 (11), 176.8 (16), 176.0 (4), 173.7 (**C**=O), 173.05 (**C**=O), 173.01 (7c), 172.9 (**C**=O), 171.9 (**C**=O), 171.1 (**C**=O), 166.7 (9), 163.3 (14), 160.2 (6), 133.8 (CN), 128.0 (CN), 104.3 (5 and 15), 95.1 (8), 88.2 (10), 83.2 (1), 75.4 (19), 72.9 (8f), 69.6 (8f), 61.4 (8f), 58.7 (17), 57.1 (3), 54.0 (13), 52.6 (-OC**H$_3$**), 52.1 (-OC**H$_3$**), 52.0 (-OC**H$_3$**), 51.9 (-OC**H$_3$**), 51.8 (-OC**H$_3$**), 50.4 (7), 47.8 (12), 45.9 (2), 43.3 (7b), 41.3 (2b), 39.7 (18), 39.4 (8e), 33.7 (8b), 32.9, 31.9 (18a), 31.0 (8a), 30.8 (12a), 30.4, 29.9, 29.8, 25.9, 25.0, 22.1 (1a), 19.7 (12a), 19.3 (7a), 18.5 (17a), 17.2 (5a), 16.9 (2a), 15.6 (15a).

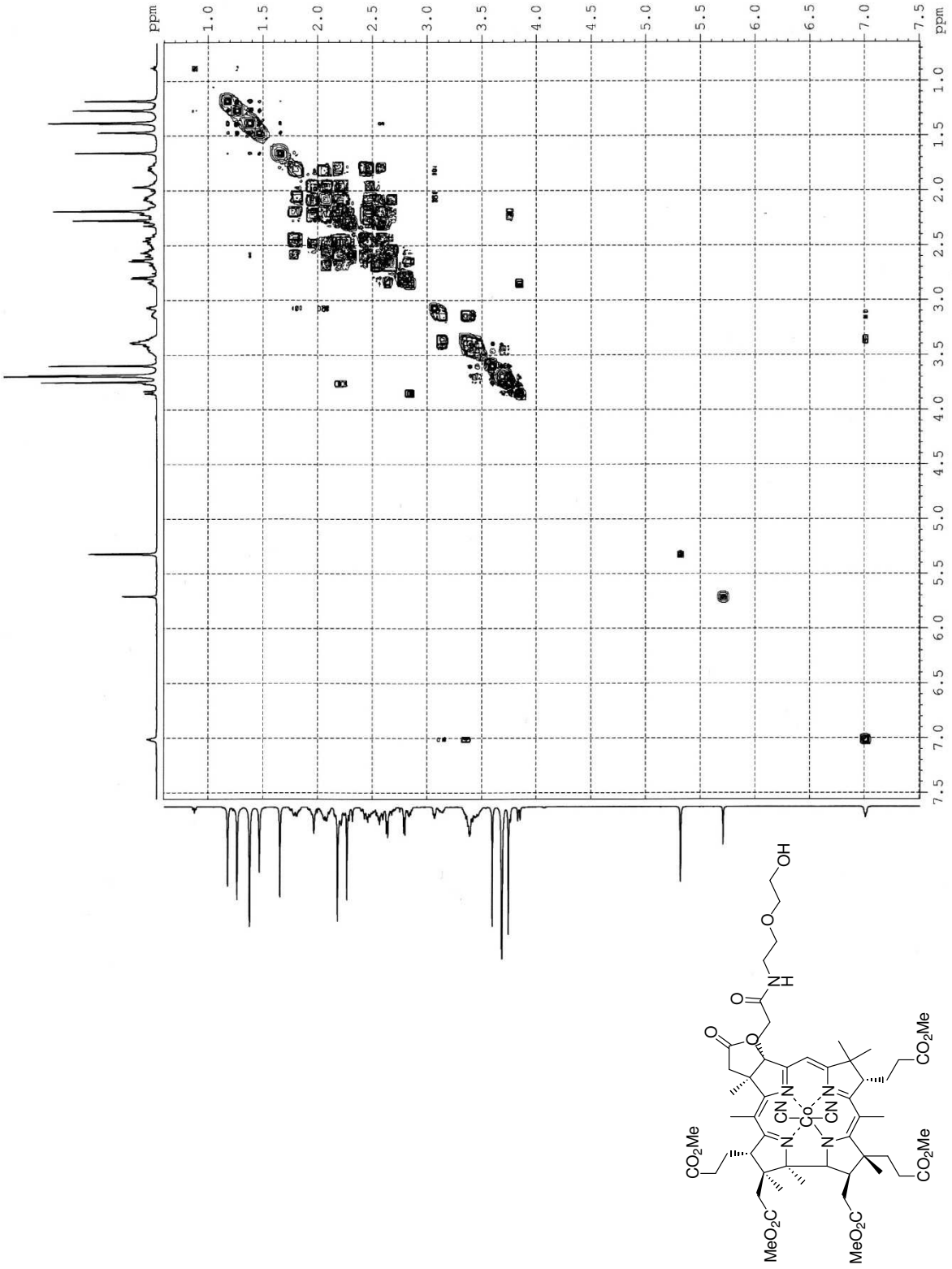
$(CN)_2$Cob(III)C1(lactone)(diglycolamide) 8



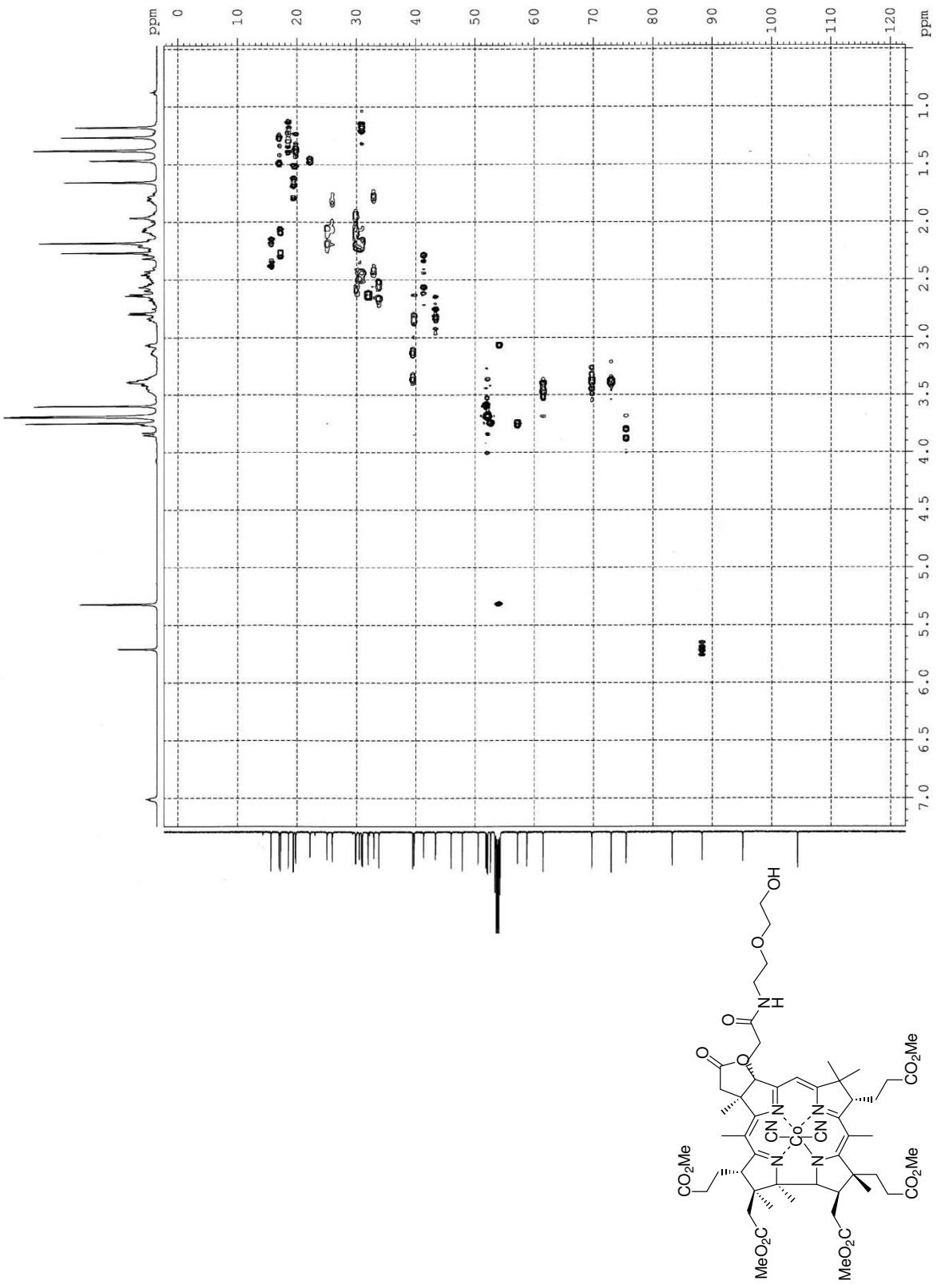
$(CN)_2$Cob(III)C1(lactone)(diglycolamide) 8



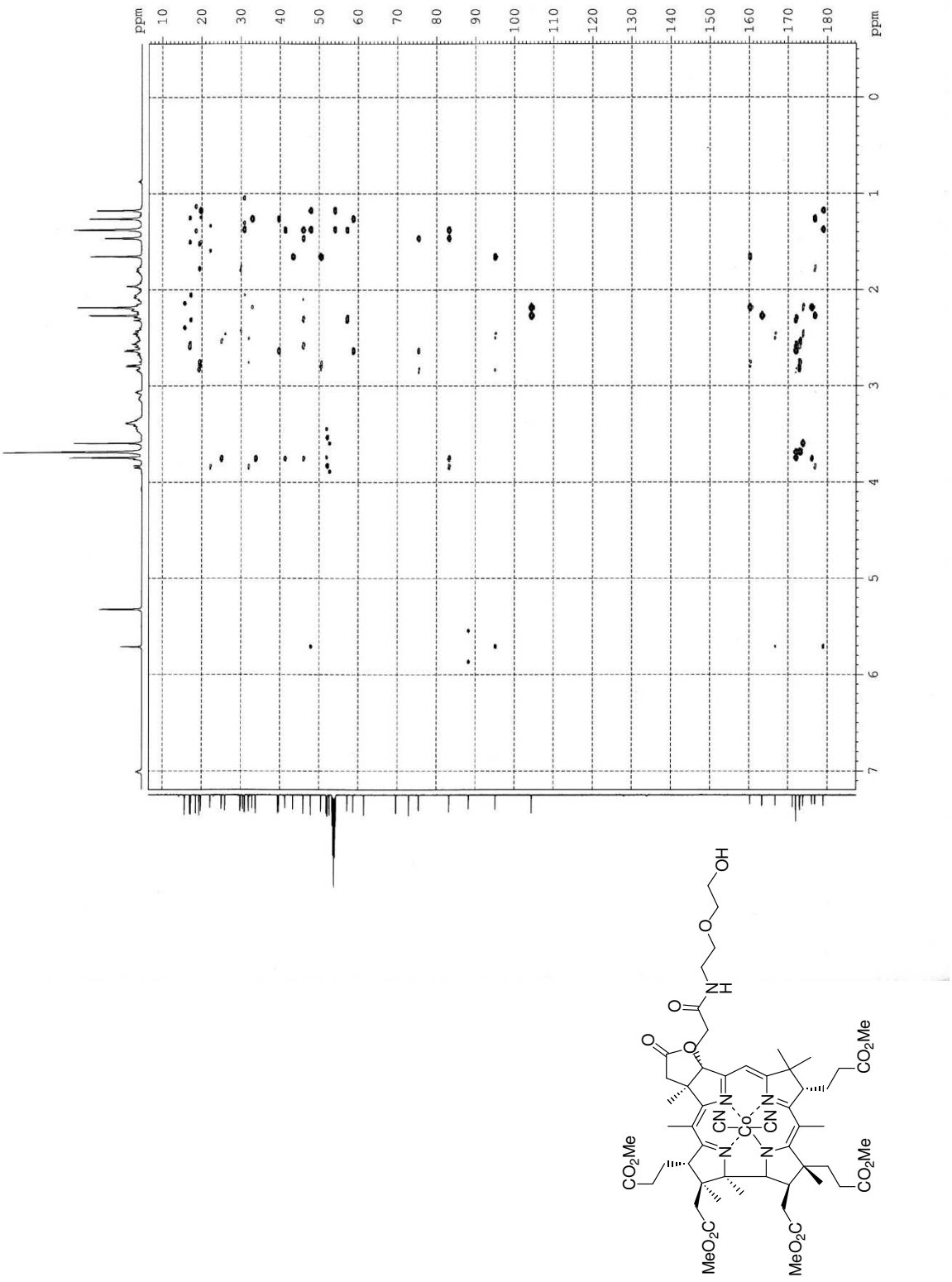
$(CN)_2$Cob(III)C1(lactone)(diglycolamide) 8



$(CN)_2$Cob(III)C1(lactone)(diglycolamide) 8

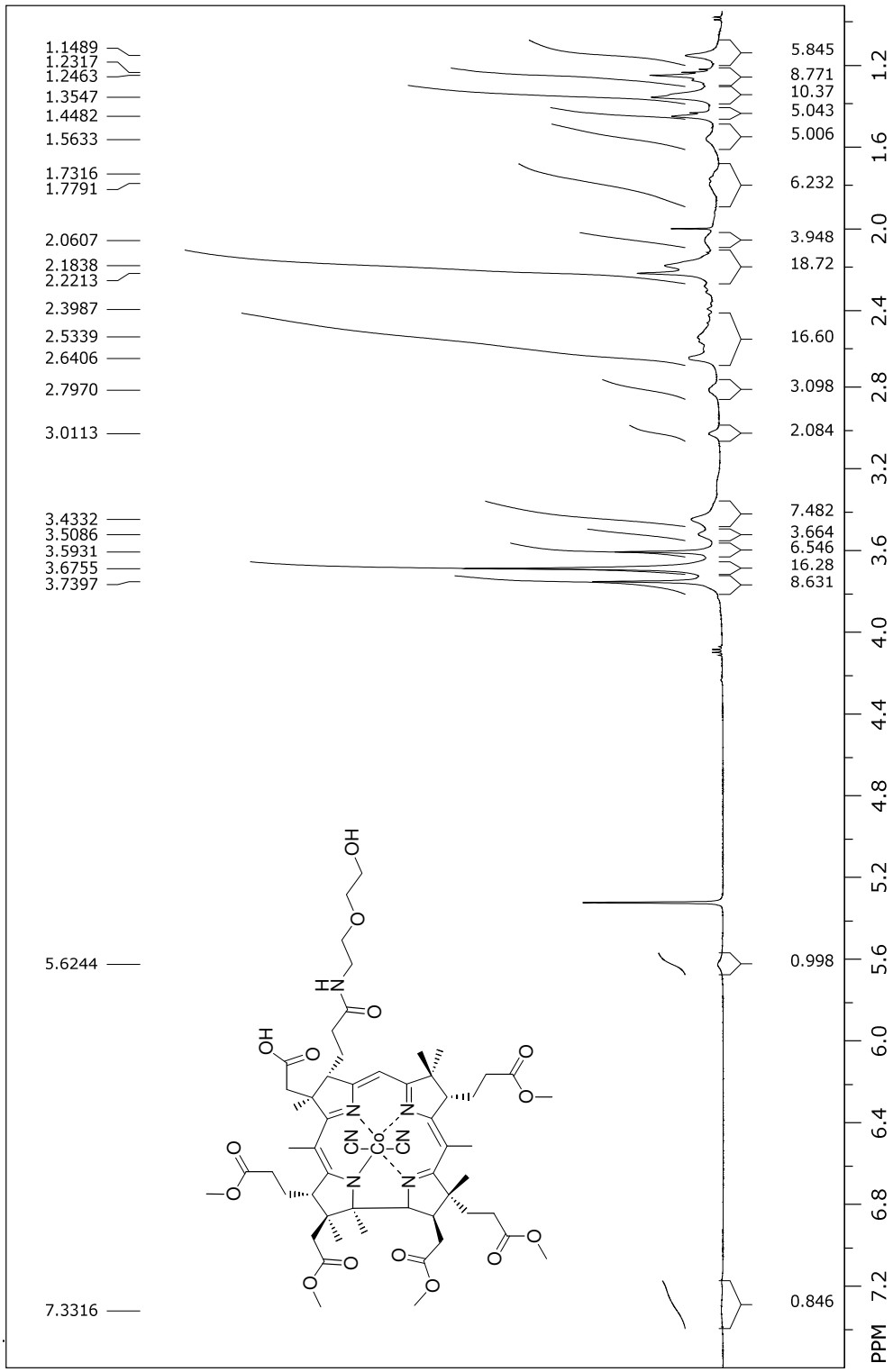


$(CN)_2$Cob(III)C1(lactone)(diglycolamide) 8

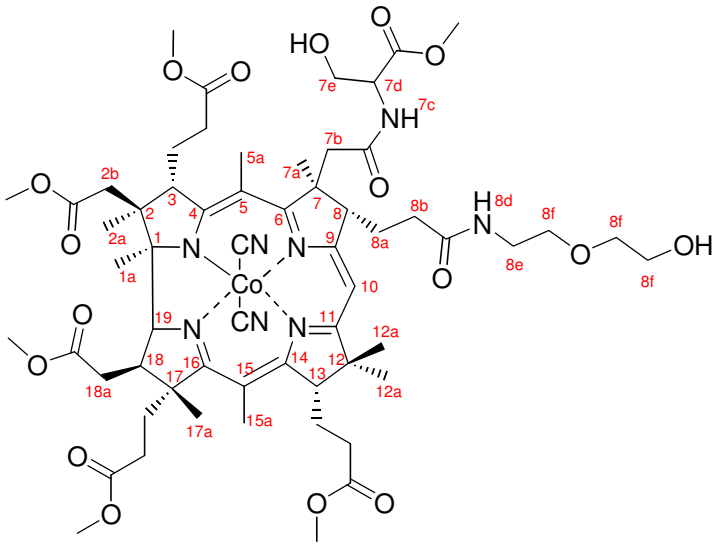


$(CN)_2$Cob(III)C1(ethionic acid)(diglycolamide) 9

Note: The broadening of peaks in the ^{1}H NMR spectrum made it impossible to decipher and consequently high resolution ^{13}C spectra could not be obtained. This was caused by the presence of the acid group.



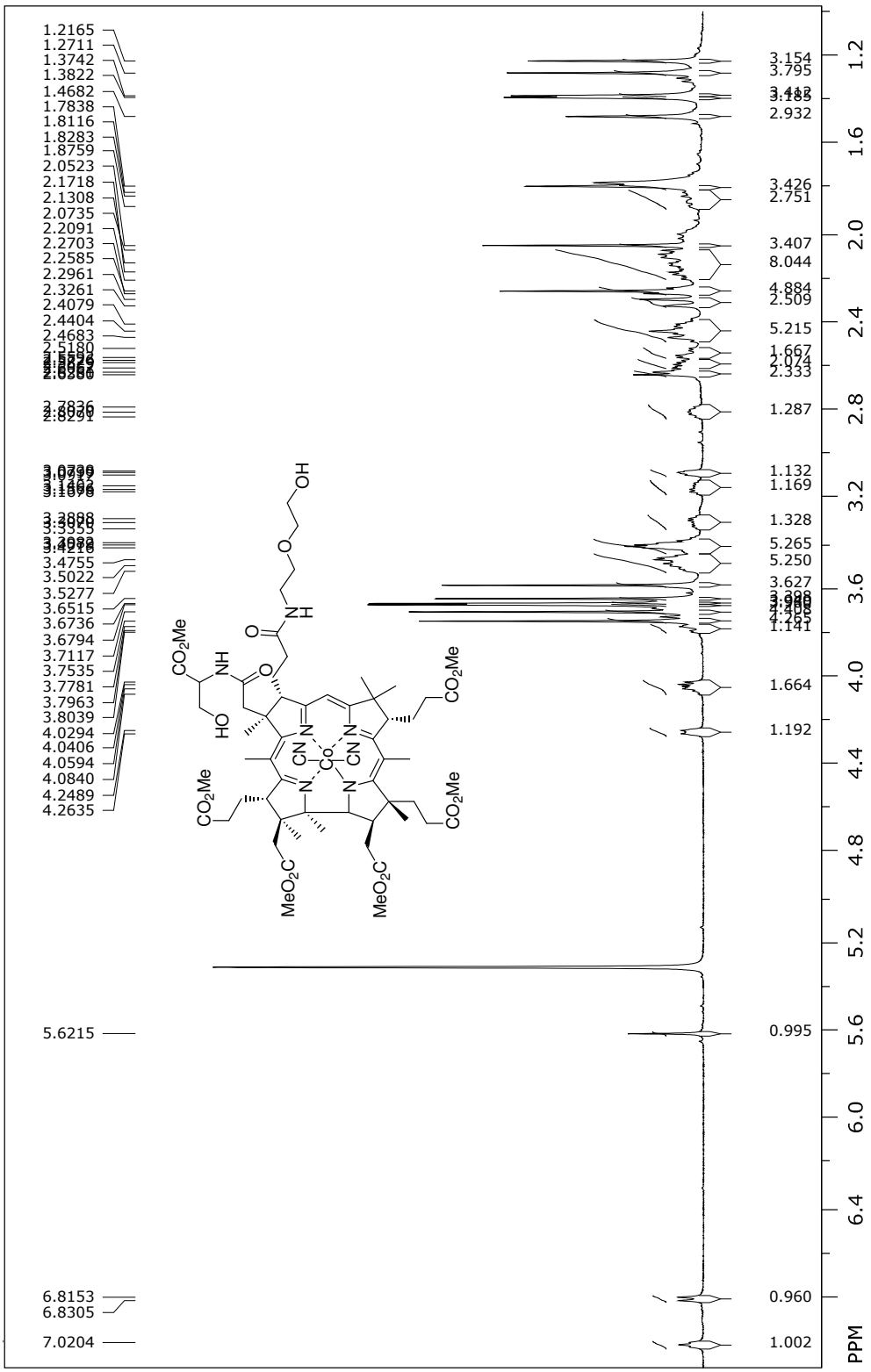
$(CN)_2$Cob(III)C1(NH-Serine-OMe)(diglycolamide) 10



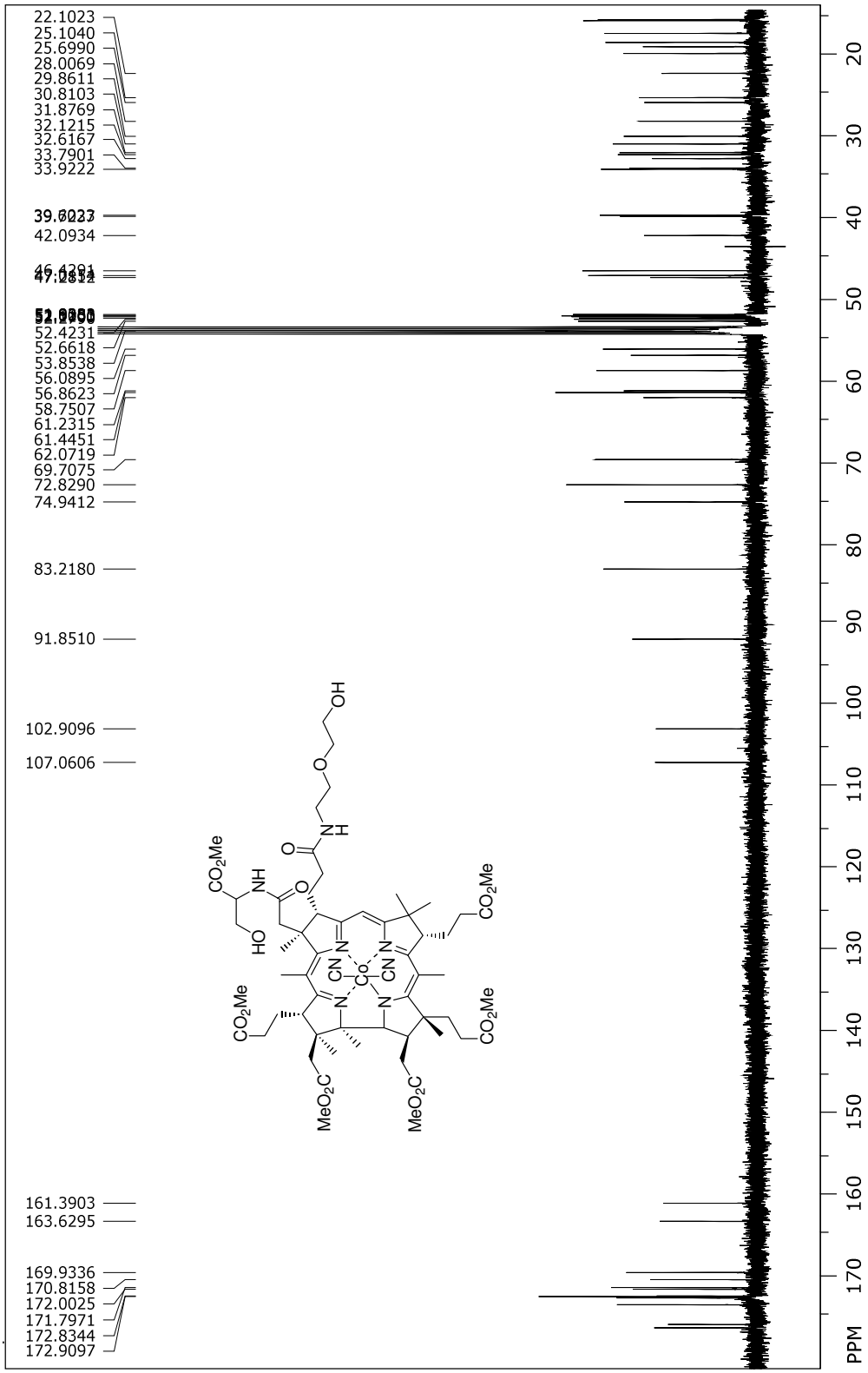
^{1}H NMR (500 MHz, CD_2Cl_2) δ (ppm); 7.02 (t, *J* = 4.9 Hz, 1H, 8d), 6.82 (d, *J* = 7.7 Hz, 1H, 7c), 5.62 (s, 1H, 10), 4.26-4.24 (m, 1H, 7d), 3.80-3.77 (m, 1H, 19), 3.75 (s, 4H, -OC**H**$_3$ and 7b), 3.71 (s, 4H, -OC**H**$_3$), 3.68 (s, 4H, -OC**H**$_3$ and 3), 3.67 (s, 3H, -OC**H**$_3$), 3.65 (s, 3H, -OC**H**$_3$), 3.59 (s, 3H, -OC**H**$_3$), 3.52-3.45 (m, 4H, 7e and 7b), 3.43-3.38 (m, 5H, 8f), 3.33-3.27 (m, 1H, 8e and 8f), 3.17-3.11 (m, 1H, 8e), 3.09-3.07 (m, 1H, 13), 2.82-2.78 (m, 1H, 18), 2.63-2.62 (m, 2H, 18a), 2.61-2.51 (m, 4H, 8a and C**H**$_2$), 2.48-2.38 (m, 5H, 2b, 8b and C**H**$_2$), 2.30 (d, *J* = 15.3 Hz, 1H, 2b), 2.25 (s, 4H, 15a, 8 and C**H**$_2$), 2.20-2.07 (m, 7H, 8a and C**H**$_2$), 2.05 (s, 3H, 5a), 1.87-1.81 (m, 2H, C**H**$_2$), 1.78 (s, 3H, 7a), 1.46 (s, 3H, 2a), 1.38 (s, 3H, 1a), 1.37 (s, 3H, 12a), 1.27 (s, 3H, 17a), 1.21 (s, 3H, 12a).

^{13}C NMR (125 MHz, CD_2Cl_2) δ (ppm); 176.77 (16), 176.73 (11), 176.3 (3), 173.9 (**C**=O), 173.0 (**C**=O), 172.9 (**C**=O), 172.0 (**C**=O), 171.8 (**C**=O), 170.8 (**C**=O), 169.9 (9), 163.6 (14), 161.3 (6), 107.0 (5), 102.9 (15), 91.8 (10), 83.2 (1), 74.9 (19), 72.9, 72.8 (8f), 69.7 (8f), 62.0 (8f), 61.4 (7e), 61.2 (7b), 58.7 (17), 56.8 (3), 56.0 (7d), 53.8 (13), 52.6 (-OC**H**$_3$), 52.4 (-OC**H**$_3$), 52.2 (-OC**H**$_3$), 52.1 (-OC**H**$_3$), 52.0 (-OC**H**$_3$), 51.9 (-OC**H**$_3$), 51.8 (7), 47.2 (8), 47.0 (12), 46.4 (2), 42.0 (2b), 39.7, 39.6 (8e), 33.9 (18), 33.7, 32.6 (12a), 32.1, 31.8, 30.0 (8a), 29.8, 28.0, 25.7, 25.1, 22.1 (2a), 19.6 (12a), 18.8 (7a), 18.2, 17.1 (19), 15.6 (15a), 15.4 (5a).

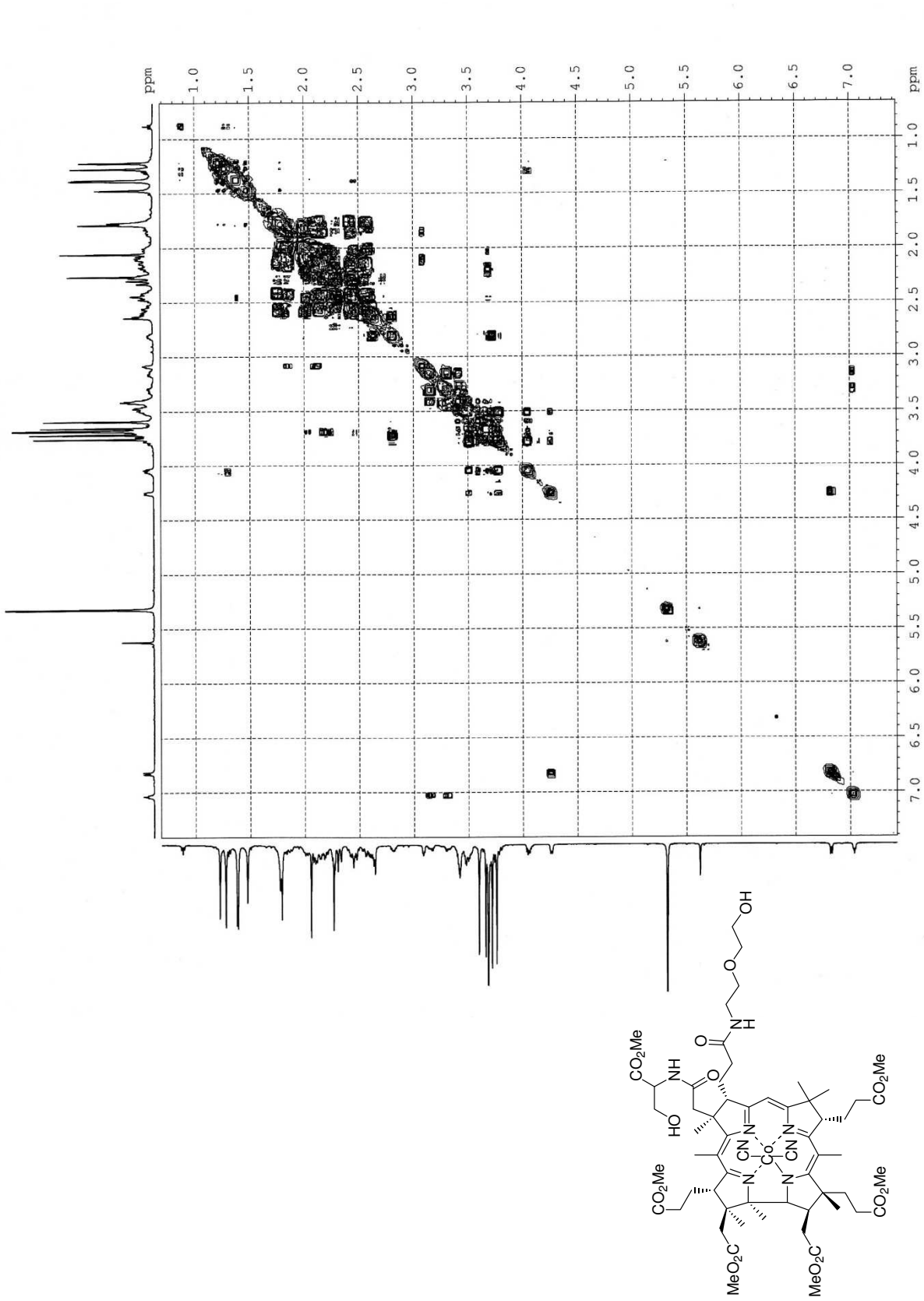
$(CN)_2$Cob(III)C1(NH-Serine-OMe)(diglycolamide) 10



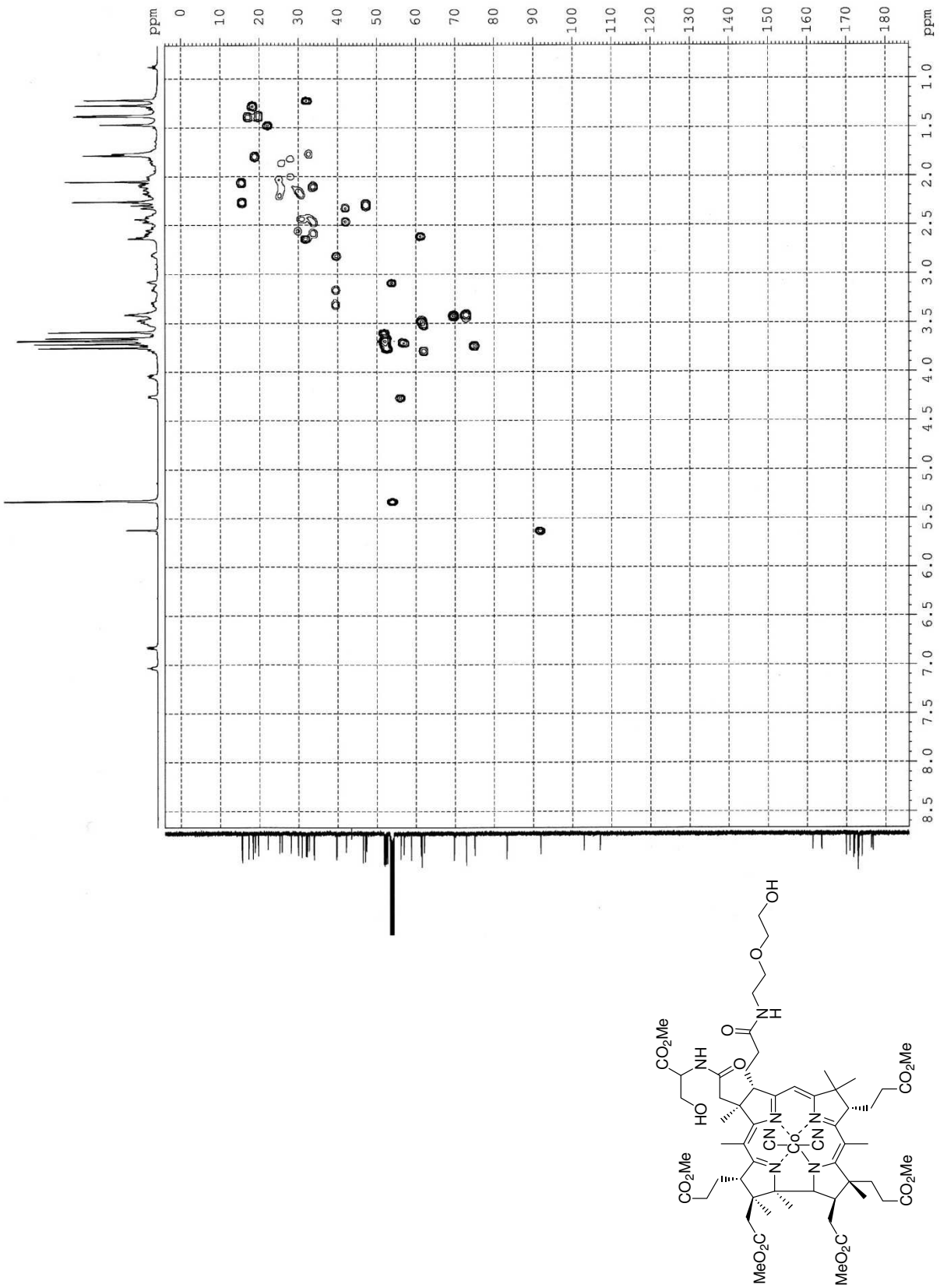
$(CN)_2$Cob(III)C1(NH-Serine-OMe)(diglycolamide) 10



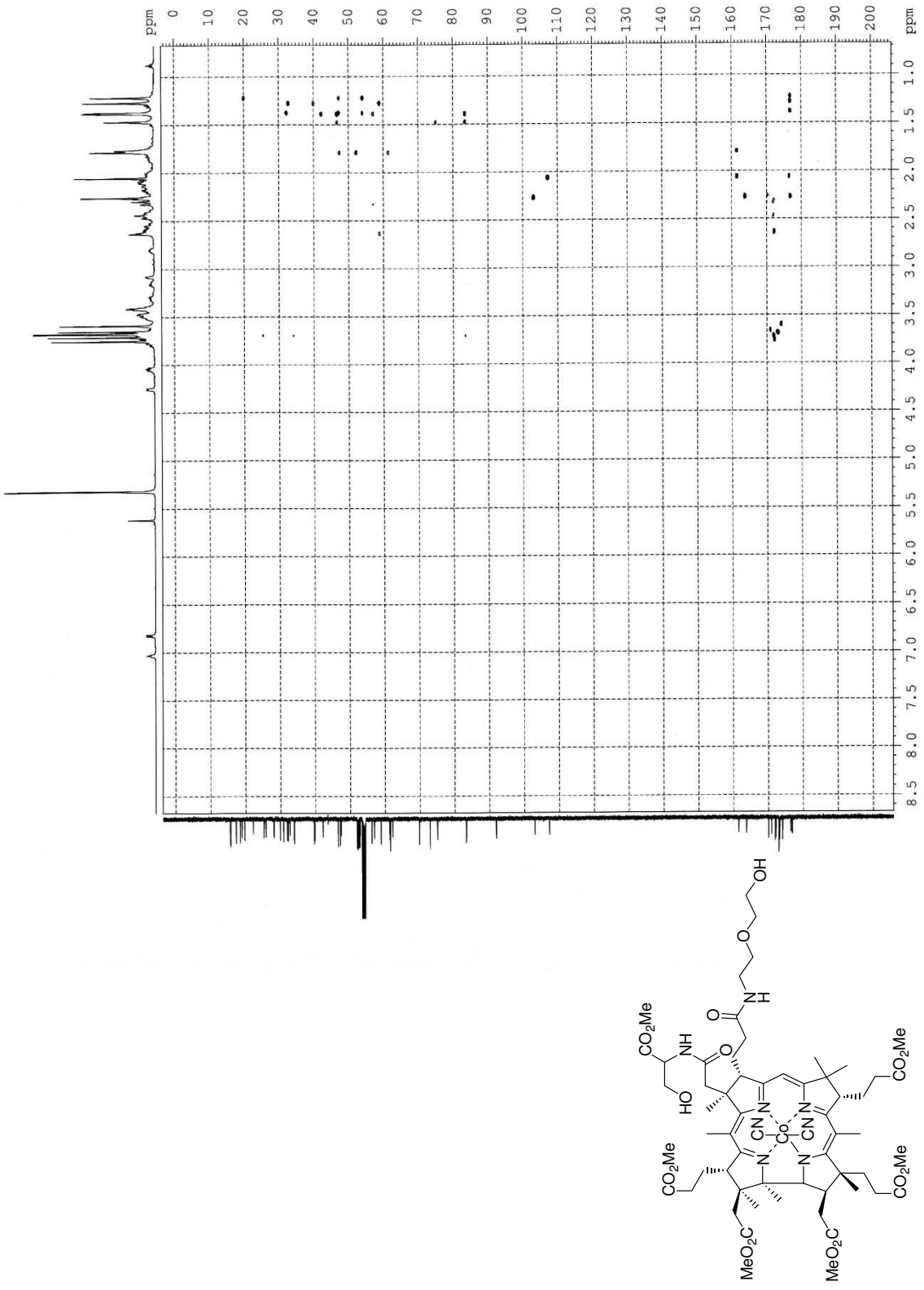
$(CN)_2$Cob(III)C1(NH-Serine-OMe)(diglycolamide) 10



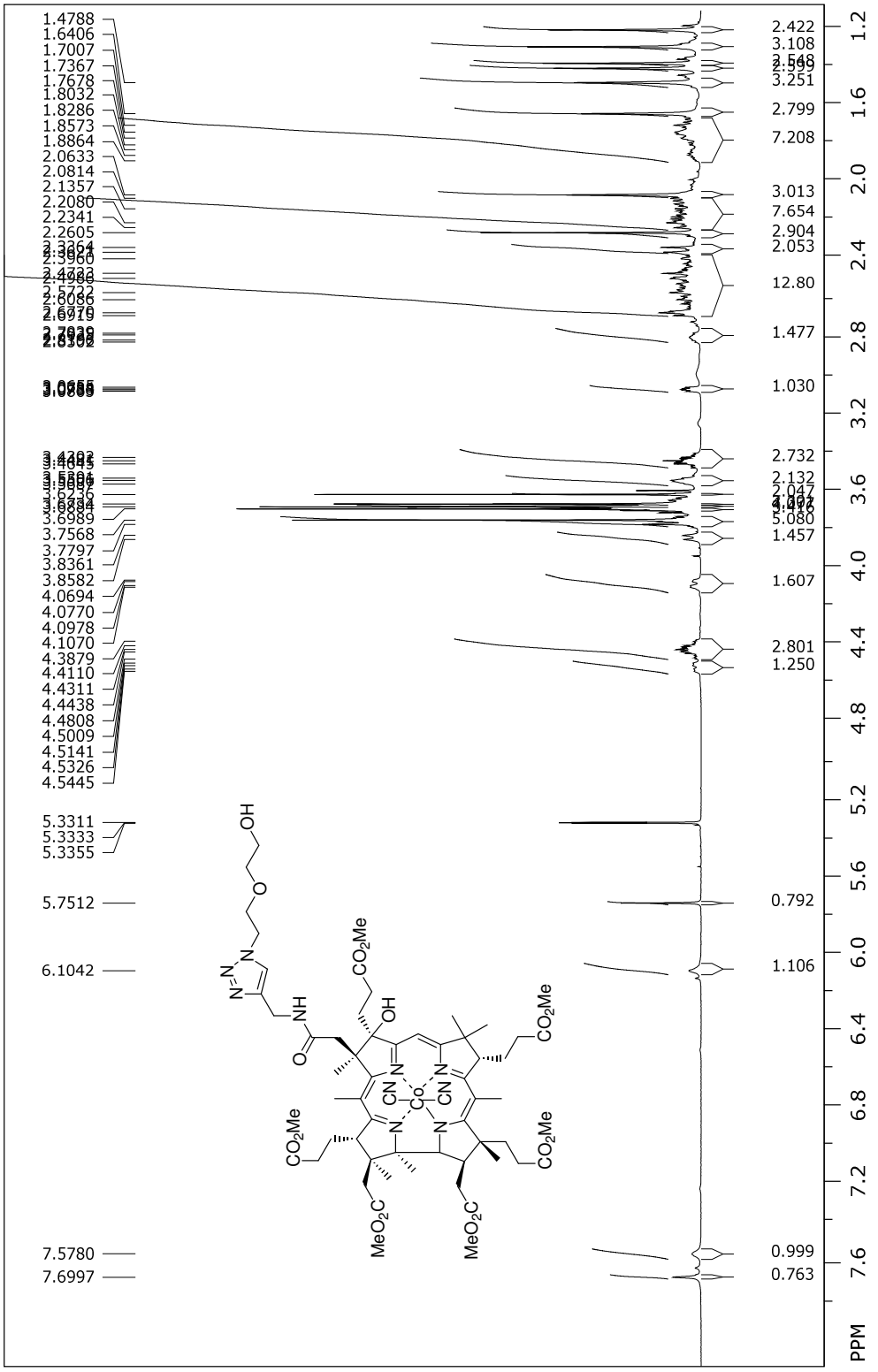
$(CN)_2$Cob(III)C1(NH-Serine-OMe)(diglycolamide) 10



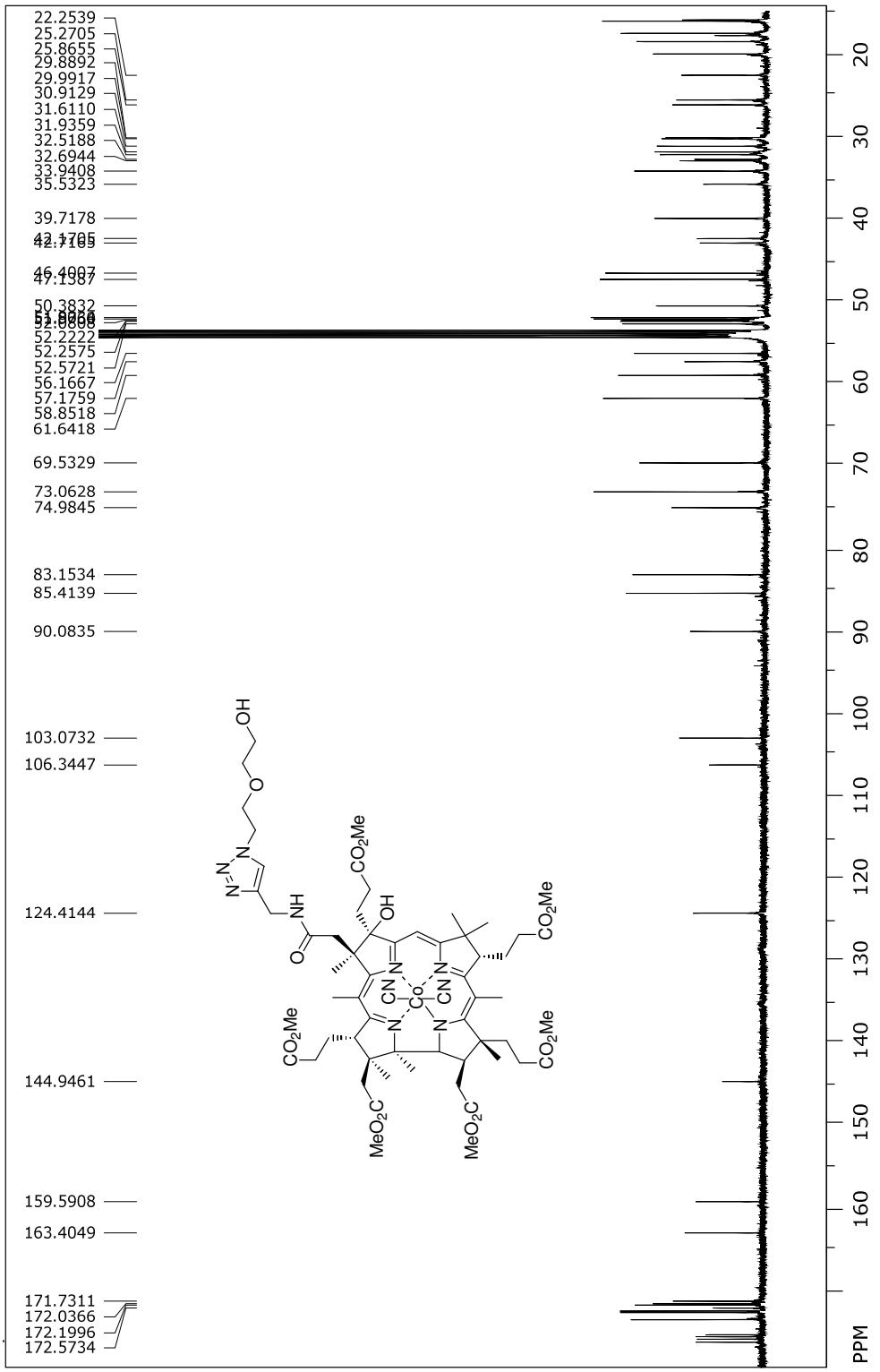
$(CN)_2$Cob(III)C1(NH-Serine-OMe)(diglycolamide) 10



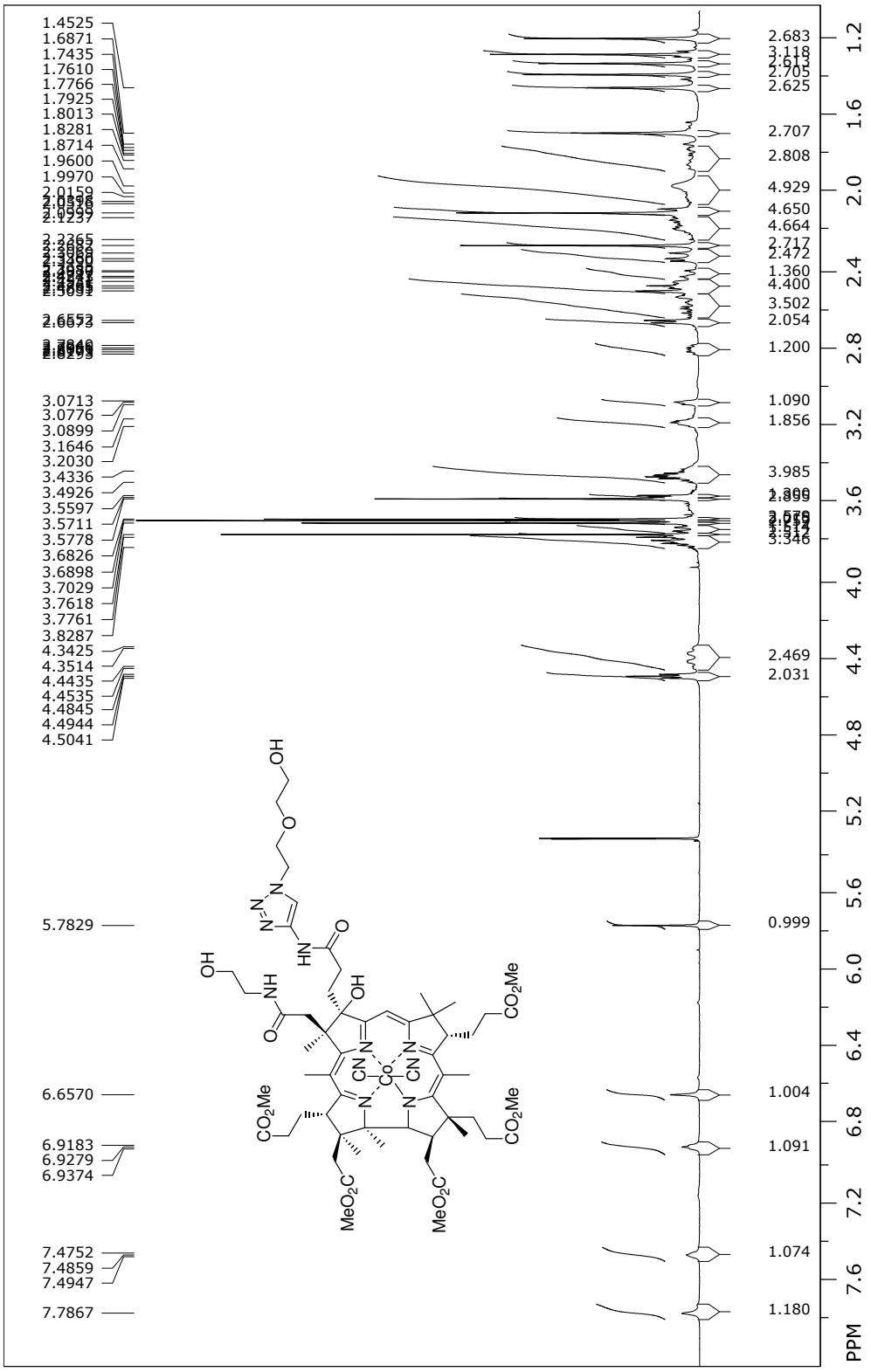
$(CN)_2$Cob(III)C1(2-{1-[2-(2-Hydroxy-ethoxy)-ethyl]-1H-[1,2,3]triazol-4-yl}-amide) 12



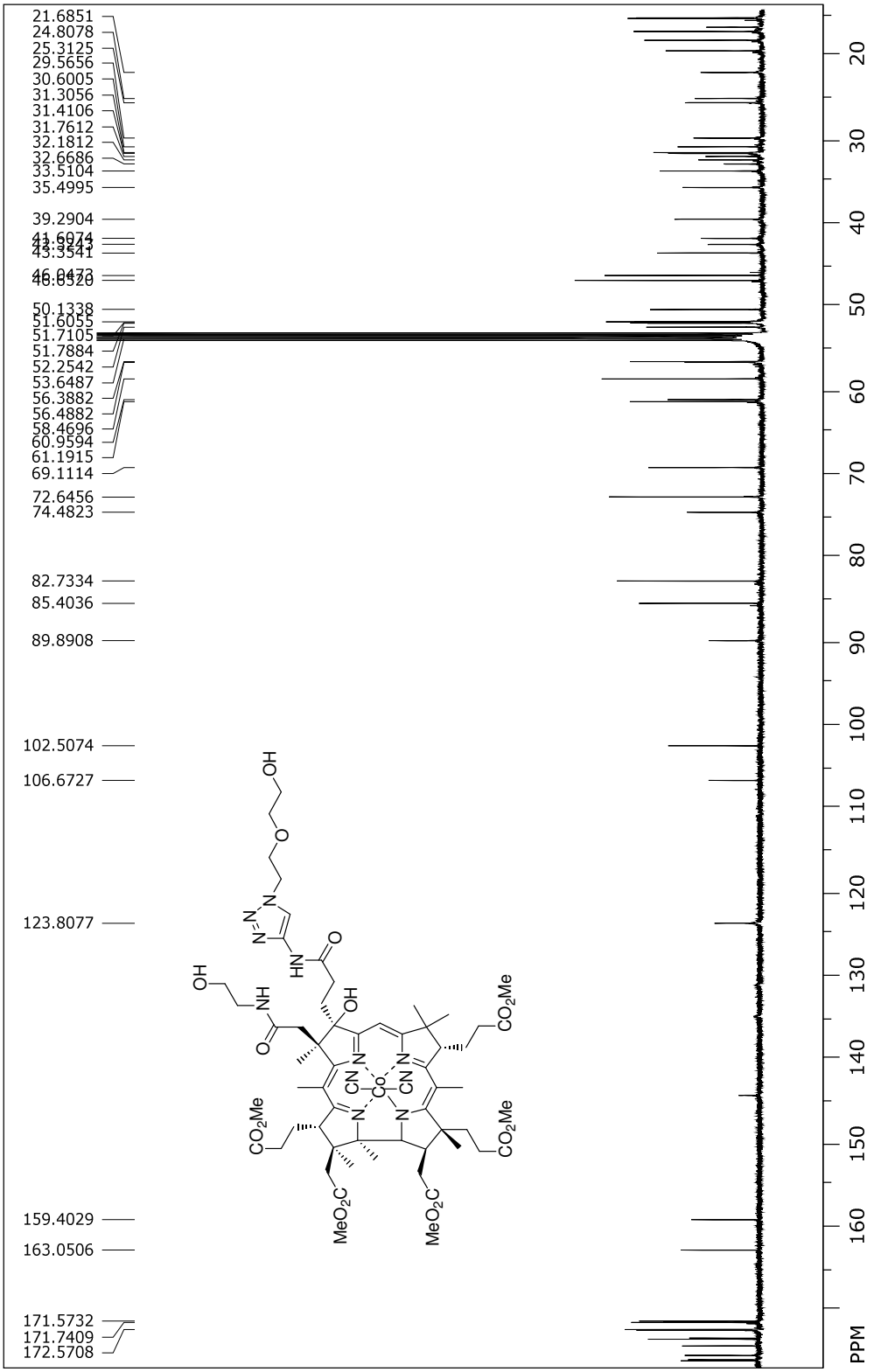
$(CN)_2$Cob(III)C1(2-{1-[2-(2-Hydroxy-ethoxy)-ethyl]-1H-[1,2,3]triazol-4-yl}-amide) 12



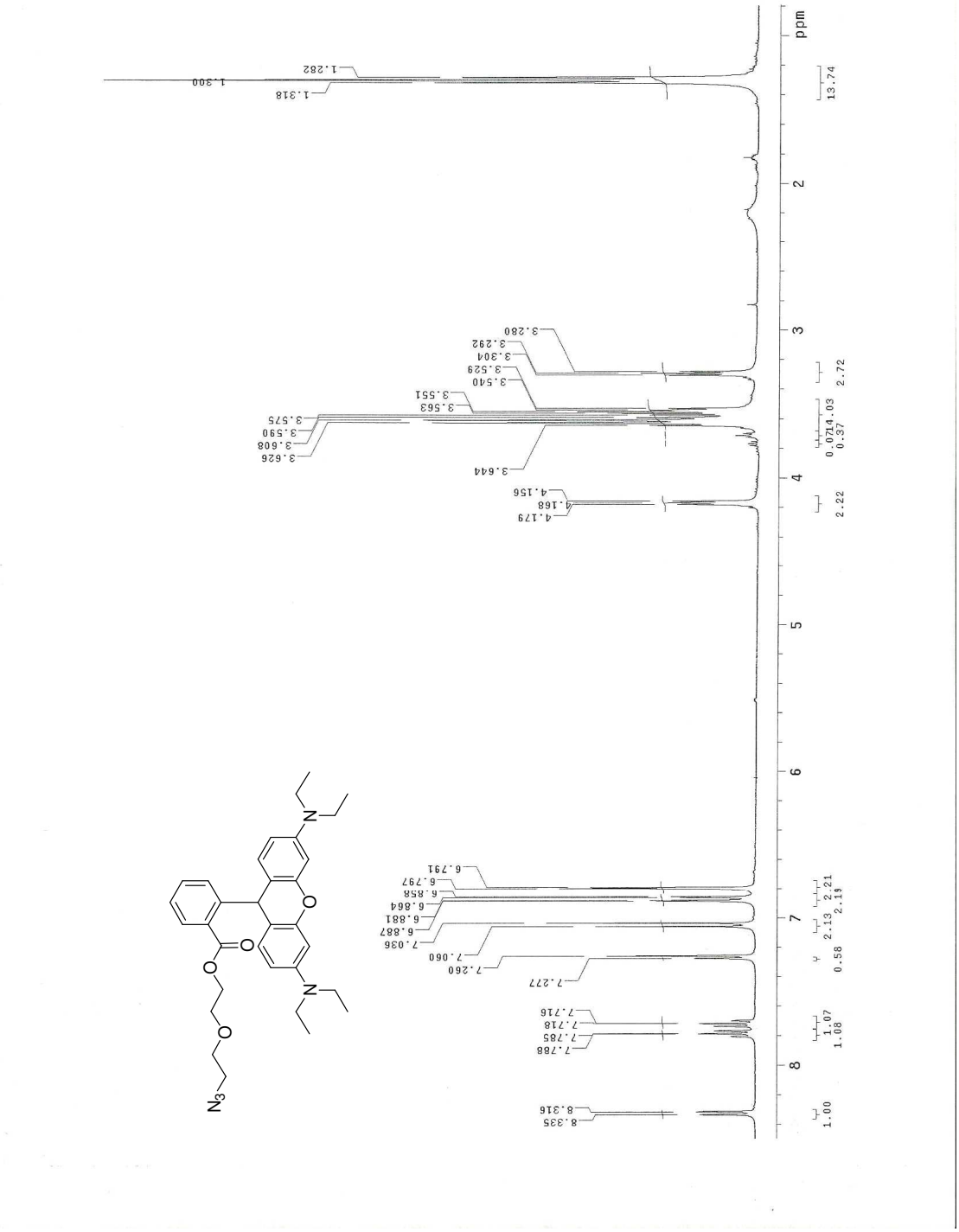
$(CN)_2$Cob(III)C1(ethanolamide)(2-{1-[2-(2-Hydroxy-ethoxy)-ethyl]-1H-[1,2,3]triazol-4-yl}-amide) 15



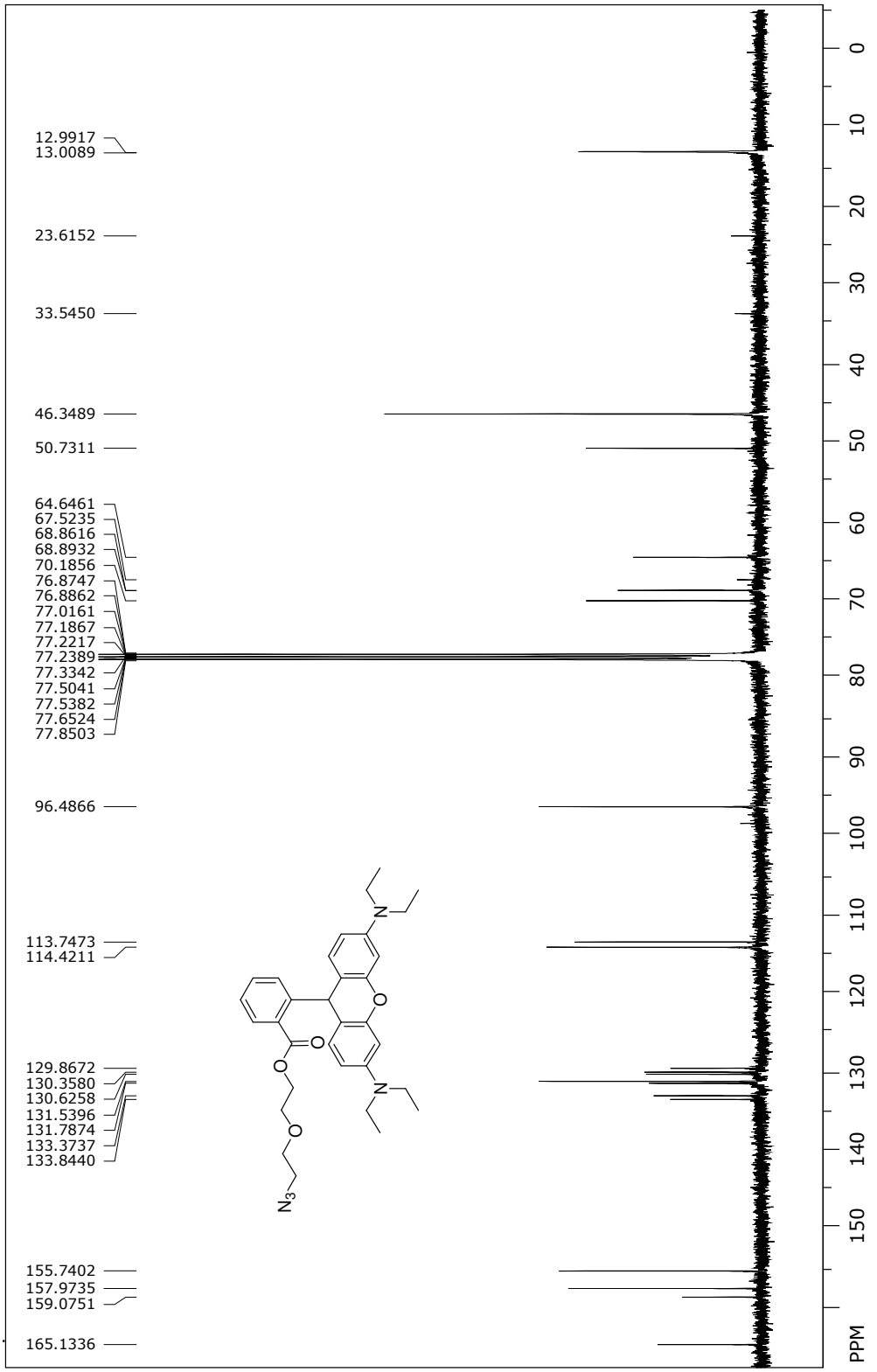
$(CN)_2$Cob(III)C1(ethanolamide)(2-{1-[2-(2-Hydroxy-ethoxy)-ethyl]-1H-[1,2,3]triazol-4-yl}-amide) 15



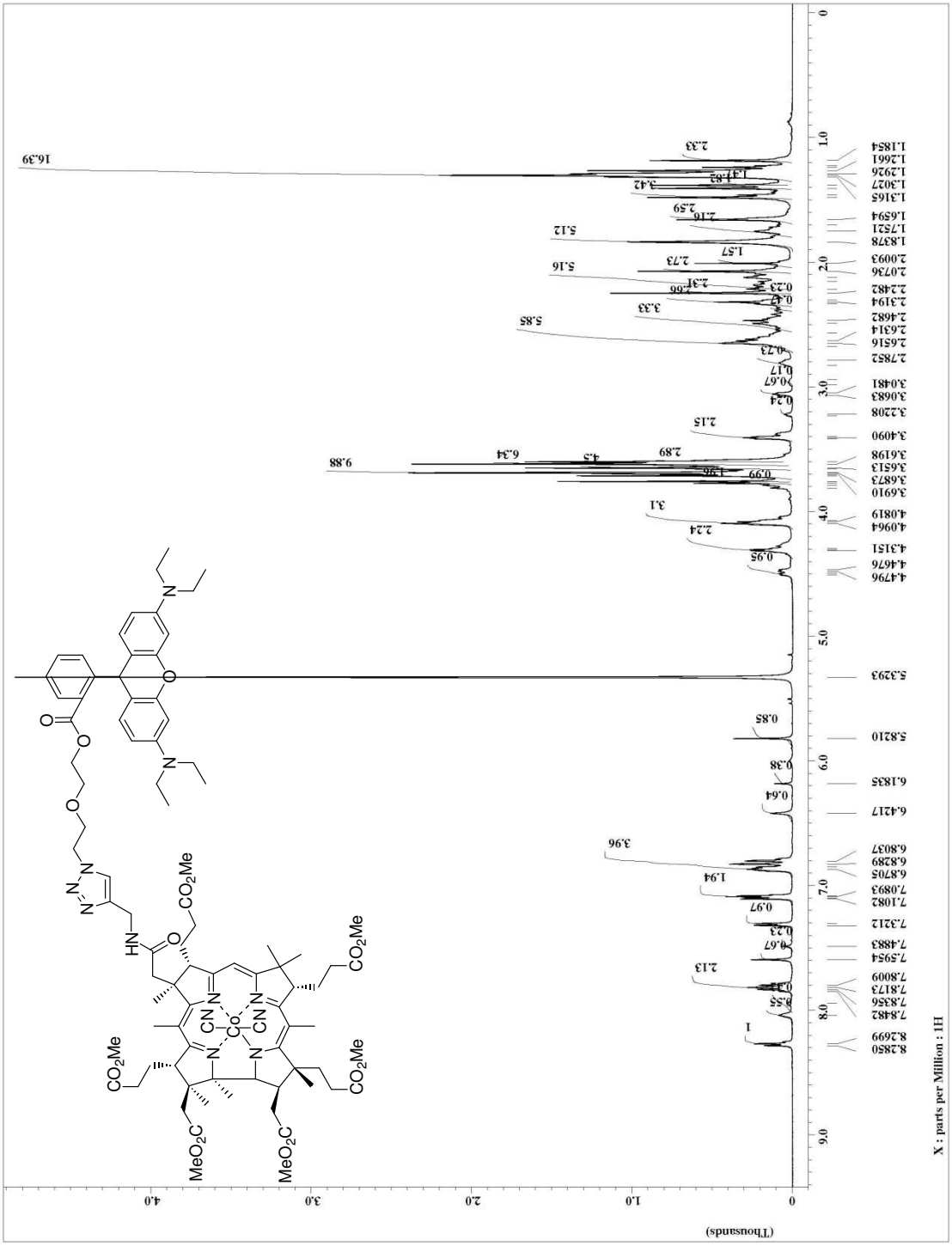
Rhodamine-2-(2-azido-ethoxy)-ethyl ester 16



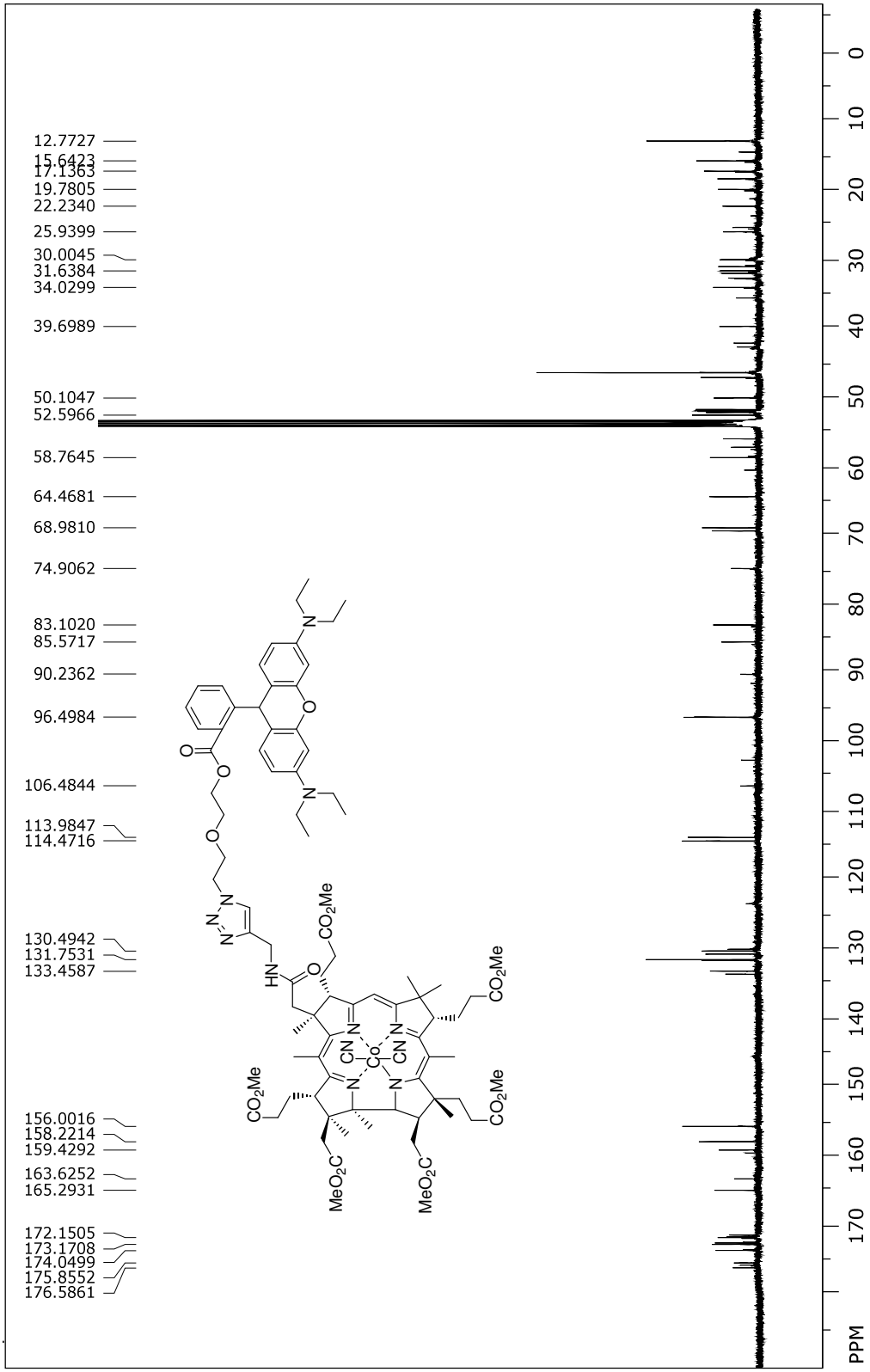
Rhodamine-2-(2-azido-ethoxy)-ethyl ester 16



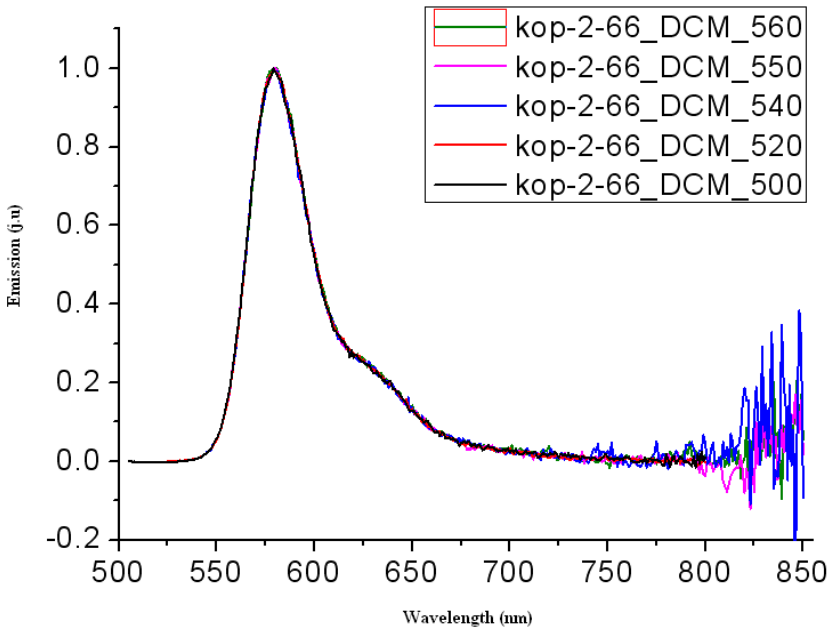
$(CN)_2$Cob(III)C1(2-{1-[rhodamine-2-(2-azido-ethoxy)-ethyl ester]-1H-[1,2,3]triazol-4-yl}-amide) 17



$(CN)_2$Cob(III)C1(2-{1-[rhodamine-2-(2-azido-ethoxy)-ethyl ester]-1H-[1,2,3]triazol-4-yl}-amide) 17

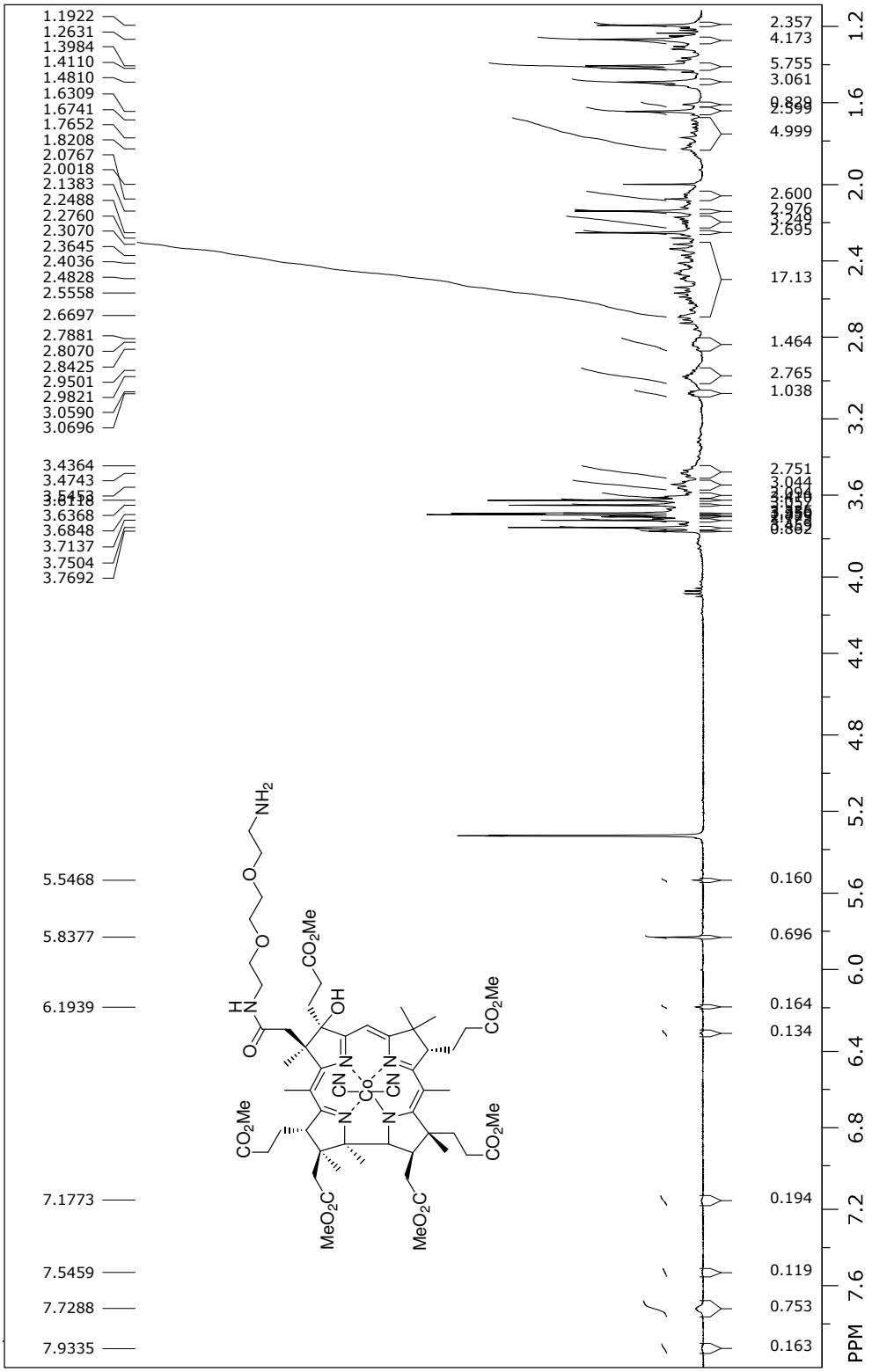


Fluorescence spectra of 17

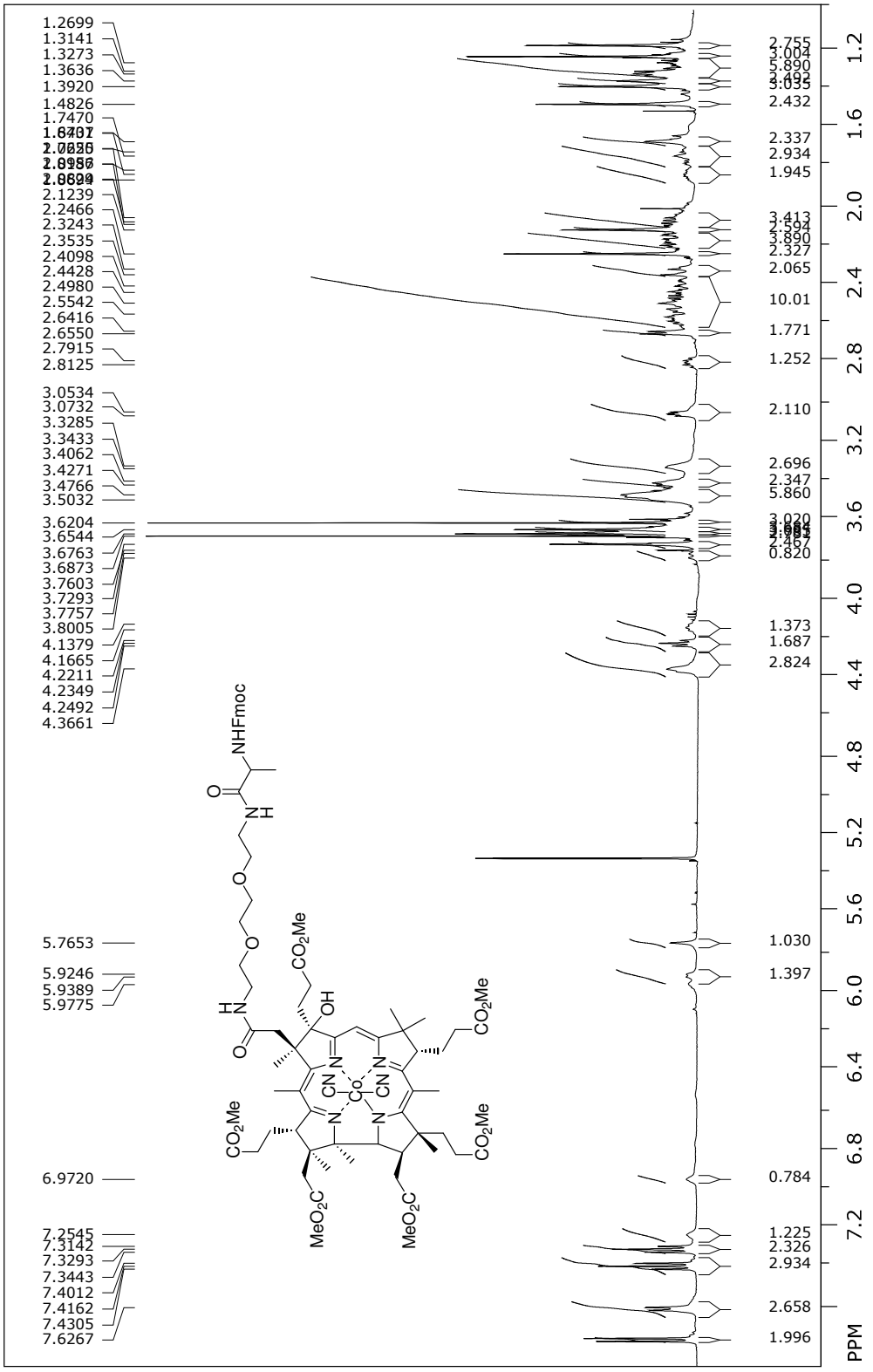


$(CN)_2$Cob(III)C1(N-[2-(2-amino-ethoxy)ethoxylethyl]-amide) 18

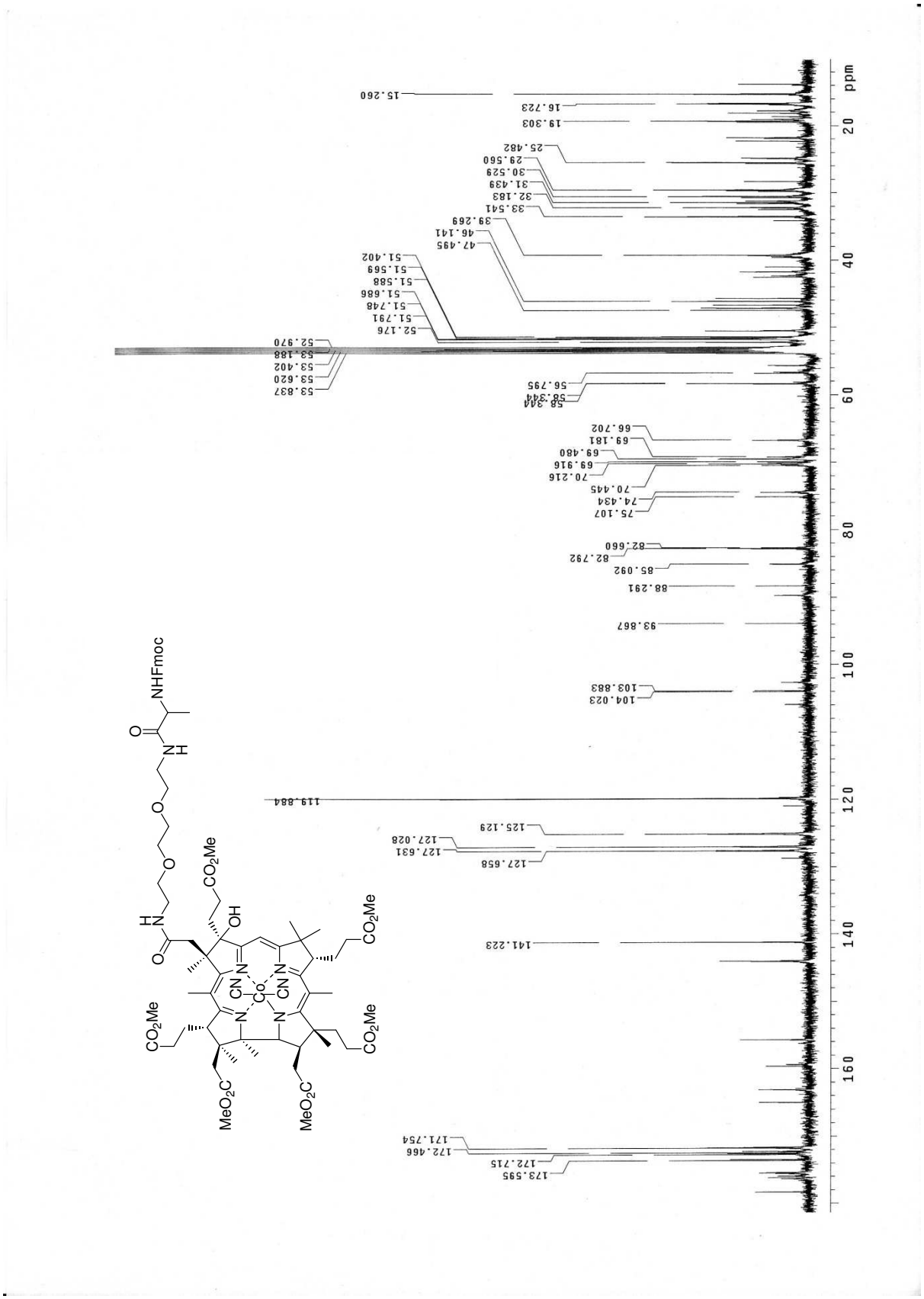
Note: Due to severe splitting of peaks in these spectra, caused by the presence of the terminal amine, the ^{1}H NMR spectra are very complicated, which made it difficult to obtain high resolution ^{13}C spectra.



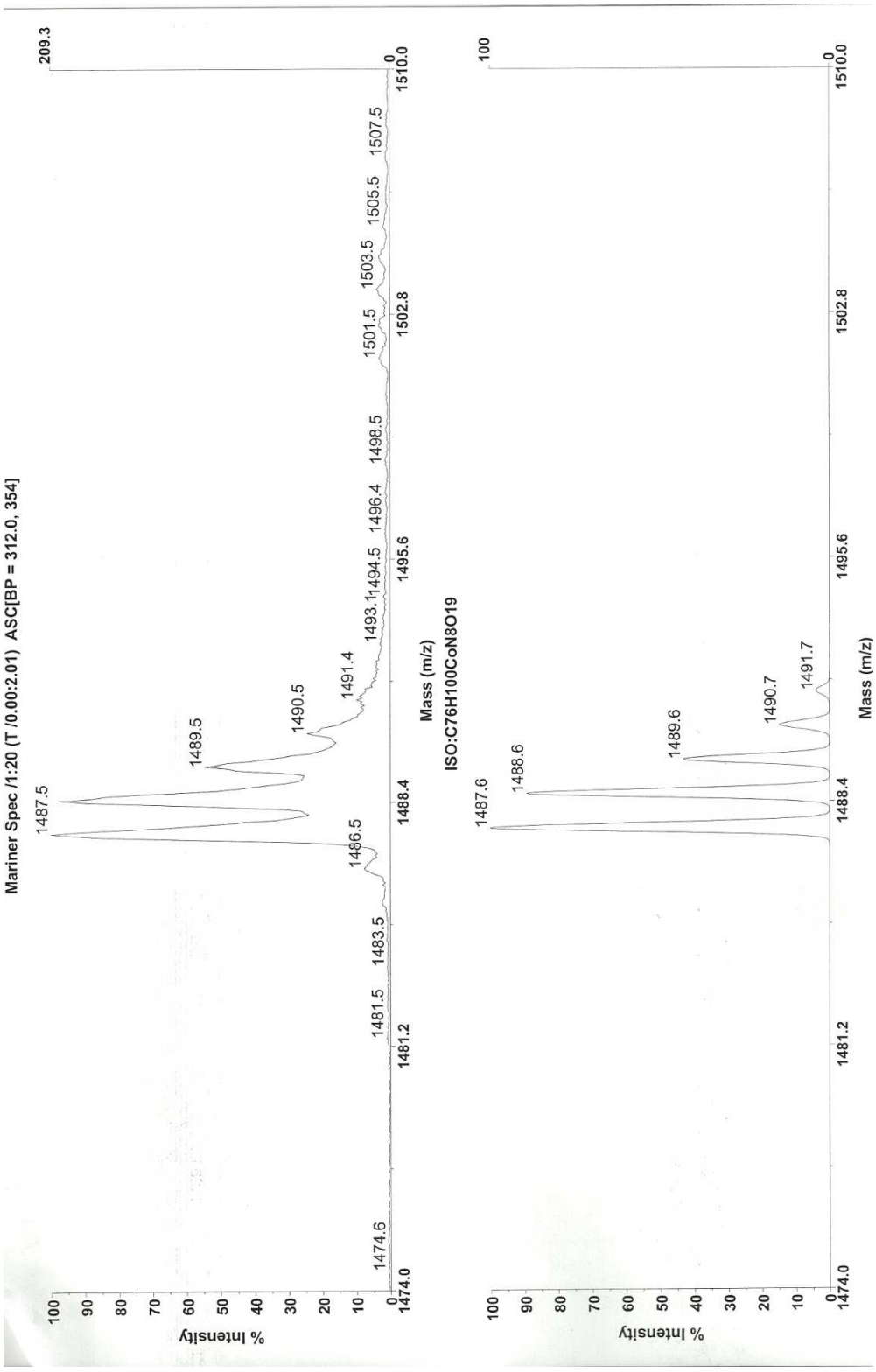
$(CN)_2$Cob(III)C1(1-{2-[2-(amino-methoxy)-ethoxy]-ethylcarbamoyl}-ethyl)-carbamic acid 9H-fluoren-9-yl methyl ester 19



$(CN)_2$Cob(III)C1(1-{2-[2-(amino-methoxy)-ethoxy]-ethylcarbamoyl}-ethyl)-carbamic acid 9H-fluoren-9-yl methyl ester 19

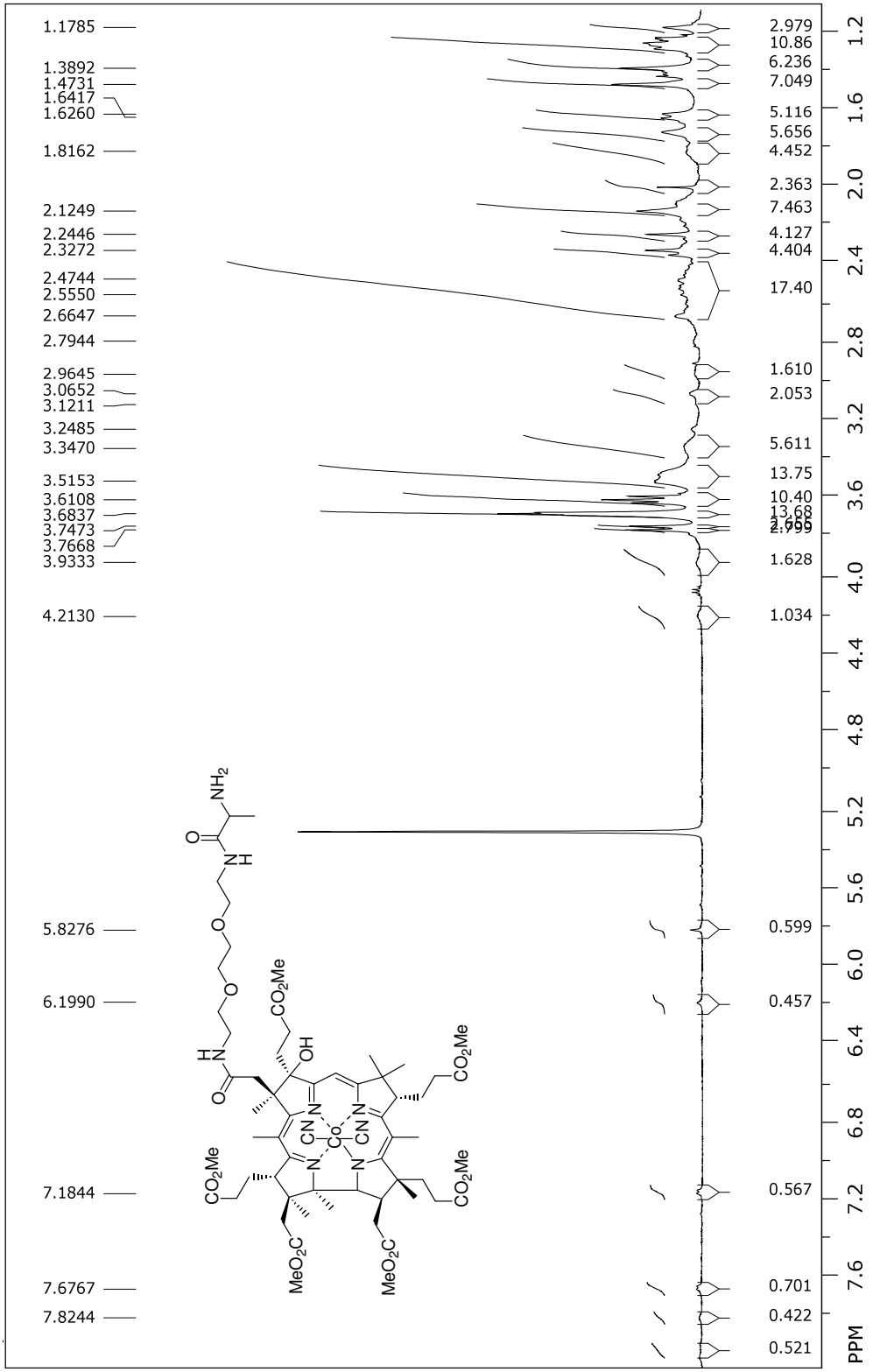


$(CN)_2$Cob(III)C1(1-{2-[2-(amino-methoxy)-ethoxy]-ethylcarbamoyl}-ethyl)-carbamic acid 9H-fluoren-9-yl methyl ester 19



$(CN)_2$Cob(III)C1(*N*-{2-[2-(amino-methoxy)-ethoxy]-ethyl}-2-amino-propionamide) 20

Note: Due to severe splitting of peaks in these spectra, caused by the presence of the terminal amine, the ^{1}H NMR spectra are very complicated, which made it difficult to obtain high resolution ^{13}C spectra.



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Synthesis of New Hydrophilic and Hydrophobic Cobinamides as NO-Independent sGC Activators

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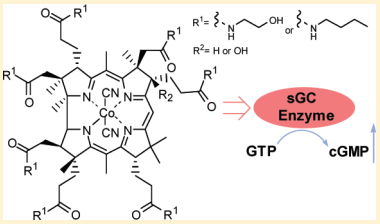
Supporting Information

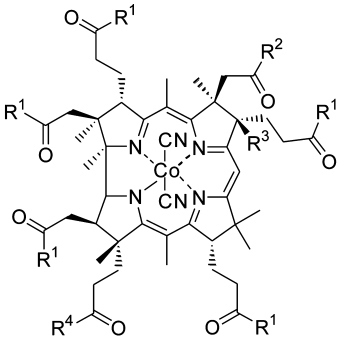
ABSTRACT: Herein, the synthesis of novel hydrophobic and hydrophilic cobinamides via aminolysis of vitamin B_{12} derivatives that activate soluble guanyl cyclase (sGC) is presented. Unlike other sGC regulators, they target the catalytic domain of sGC and show higher activity than $(CN)_2$Cbi.



KEYWORDS: *vitamin B_{12}, cobinamide, aminolysis, soluble guanyl cyclase*

Over the past decade, there has been much interest in NO-independent soluble guanyl cyclase (sGC) activators.[1,2] sGC plays an important role in cardiovascular homeostasis, platelet function, angiogenesis, and neurotransmission. It operates via activation by nitric oxide (NO), which binds to the sGC heme moiety. This induces the conversion of guanosine triphosphate (GTP) into a second messenger cyclic guanosine monophosphate (cGMP), resulting in physiological effects.[3,4] Currently, pharmacological regulation of sGC is achieved via the use of various NO releasing organic nitrates or nitrovasodilators such as nitroglycerin, isosorbide dinitrate, and isosorbide-5-mononitrate. NO-releasing activators, although effective, are known to rapidly induce tolerance and have side-effects. Therefore, the next logical step was to explore alternative methods of sGC regulation. 3-[5-Hydroxymethyl-2-furyl]-1-benzylindazole (YC-1) was the first identified NO-independent sGC regulator whose mechanism of action requires the presence of heme.[5] The discovery of YC-1 was followed by other regulators with the same mechanism of action, some with similar structural features, while others had a different structure.[6−8] Later, several heme replacing sGC activating compounds were shown to be effective regulators of sGC both in vitro and in vivo. Despite their differences, all of these regulators function by targeting the regulatory domain of sGC.

Recently, we have reported that dicyanocobinamide $(CN)_2$Cbi (Figure 1) is a novel NO-independent sGC activator with a unique mechanism of action.[9] Unlike other sGC regulators, $(CN)_2$Cbi directly targets the catalytic domain of sGC, responsible for cGMP synthesis. Moreover, it was found that $(CN)_2$Cbi exhibits a much higher activity than vitamin B_{12} itself and its cofactors adenosylcobalamine and methylcobalamine.



R^1, $R^2 = NH_2$; $R^3 = H$; $R^4 = NHCH_2CH_2(OH)CH_3$			**$(CN)_2$Cbi**
R^1, R^2, $R^4 = NHCH_2CH_2OH$		$R^3 = OH$	**1**
R^1, R^2, $R^4 = NHCH_2CH_2CH_2CH_3$		$R^3 = OH$	**2**
R^1, R^2, $R^4 = OCH_3$		$R^3 = H$	**3**
R^1, R^2, $R^4 = NHCH_2CH_2OH$		$R^3 = H$	**4**
R^1, R^2, $R^4 = NHCH_2CH_2CH_2CH_3$		$R^3 = H$	**5**
R^1, $R^4 = OCH_3$	$R^2 = OH$	$R^3 = H$	**6**

Figure 1. Hydrophobic and hydrophilic cobinamides.

The unique activation mechanism and properties of $(CN)_2$Cbi highlight the importance of developing new cobinamide derivatives to enhance sGC-targeting therapeutics. $(CN)_2$Cbi possesses seven amide groups at the periphery of the macrocycle and the central cobalt bearing two axial cyano ligands. In our previous report, we suggested that changes in sGC-activating properties of $(CN)_2$Cbi may be altered with introduction of different peripheral substitutents around the corrin macrocycle.[9] Unfortunately, there are no reports

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Synthesis of New Hydrophilic and Hydrophobic Cobinamides as NO-Independent sGC Activators

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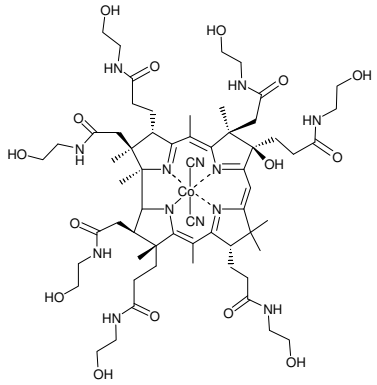
Table of Contents

General Information

Analytical grade solvents were used as received. ^{1}H and ^{13}C NMR spectra were recorded at RT on Bruker 500 MHz or Varian 500 MHz with TMS as an internal standard. DCVC (dry column vacuum chromatography) was performed using Merck Silica Gel (200-300 mesh). Thin layer chromatography (TLC) was performed using Merck Silica Gel GF254, 0.20 mm thickness. High resolution ESI mass spectra were recorded on a Mariner spectrometer. UV/Vis absorption spectra were recorded in DCM on a Perkin Elmer λ-25.

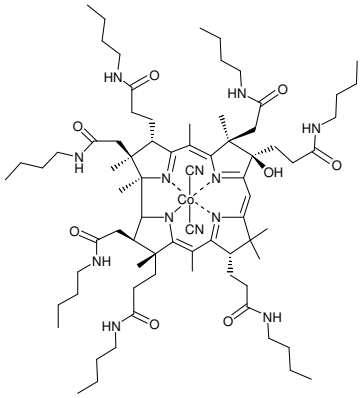
Note: In the case of compounds **1**, **2** and **4** due to overlapping of DMSO and EtOH or pentane peaks in the spectra the number of assigned protons is lower than the value stated in the molecular formula.

$(CN)_2$Cob(III)(*c*-hydroxy)heptakis(2-hydroxyethylamide) 1



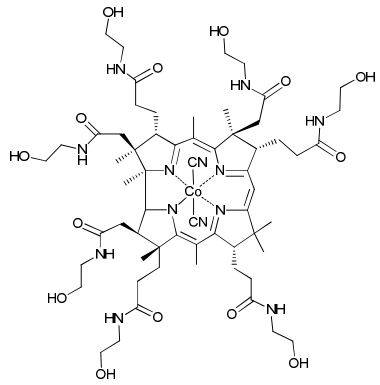
c-Lactone **7** (15 mg, 0.014 mmol) was dissolved in CCl_4 (1.0 mL) in a Schlenck tube under an argon atmosphere. Ethanolamine (0.1 mL, 1.6 mmol) and $Bu_4N^+CN^-$ (15 mg, 0.047 mmol) were then added. The mixture was stirred at 50°C for 24 h, after which it was diluted with water (10 mL) and washed with DCM (3 x 50 mL). The aqueous layer was concentrated *in vacuo* giving a purple residue, which was triturated with $Et_2O/CHCl_3$ (50 mL, 1:1) overnight. The crude product was purified using DCVC, 20-50% MeOH in DCM. Cobinamide **1** was redissolved in isopropanol, filtered through glass wool and the filtrate concentrated *in vacuo*. It was recrystalized from EtOH/Et_2O giving a purple solid (11 mg, 61%). M.p. 224-231°C. R_f 0.42, 50% MeOH in DCM. Anal. calcd. for $C_{61}H_{94}CoN_{13}O_{15}$ + EtOH + $2H_2O$: C 54.42, H 7.54, N 13.10; found: C 54.35, H 7.58, N 13.14. HRMS ESI (m/z) calcd. for $C_{60}H_{94}CoN_{12}O_{15}$ $[M\text{-}CN]^+$ 1281.6209; found 1281.6267. UV/Vis EtOH, λ_{max}, ε ($L \cdot mol^{-1} \cdot cm^{-1}$): 586 ($8.43x10^3$), 546 ($6.62x10^3$), 422 ($2.22x10^3$), 369 ($2.33x10^4$), 316 ($8.05x10^3$), 308 ($7.77.x10^3$), 280 ($8.04x10^3$). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.24 (t, *J* = 5.3 Hz, 1H), 8.20 (t, *J* = 5.4 Hz, 1H), 7.96 (m, 2H), 7.89 (t, *J* = 5.5 Hz, 1H), 7.60 (t, *J* = 5.6 Hz, 1H), 7.37 (t, *J* = 5.4 Hz, 1H), 5.71 (s, 1H), 4.79 (t, *J* = 6.2 Hz, 1H), 4.76 (t, *J* = 6.2 Hz, 1H), 4.68 (m, 3H), 4.59 (t, *J* = 5.5 Hz, 2H), 4.52 (t, *J* = 5.5 Hz, 1H), 4.34 (d, *J* = 9.1 Hz, 1H), 3.63 (d, *J* = 10.3 Hz, 1H), 3.49 (t, *J* = 5.6 Hz, 1H), 3.46-3.44 (m, 4H), 3.40-3.90 (m, 6H), 3.27-3.24 (m, 4H), 3.15-3.06 (m, 8H), 3.03-3.00 (m, 4H), 2.93-2.87 (m, 1H), 2.76 (m, 1H), 2.36-2.31 (m, 3H), 2.24-2.21 (m, 3H), 2.19 (m, 4H), 2.16-2.13 (m, 3H), 2.09 (s, 3H), 2.04-1.97 (m, 6H), 1.80-1.71 (m, 4H), 1.49 (s, 3H), 1.43 (m, 2H), 1.34 (s, 3H), 1.29 (s, 3H), 1.22 (s, 3H), 1.15 (s, 3H), 1.07 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 179.6, 178.9, 178.2, 176.1, 175.9, 174.7, 173.9, 165.4, 165.2, 105.5, 104.7, 92.3, 88.2, 85.5, 77.8, 68.2, 63.1, 63.09, 63.07, 63.04, 63.03, 62.9, 62.8, 62.7, 61.3, 58.1, 56.5, 55.9, 49.4, 49.1, 49.0, 45.0, 44.9, 44.8, 44.7, 41.9, 38.7, 36.1, 35.1, 34.9, 34.6, 34.4, 34.1, 28.9, 28.8, 24.7, 22.7, 20.8, 20.7, 19.4, 18.6, 18.4, 18.2, 18.1.

$(CN)_2$Cob(III)(*c*-hydroxy)heptakis(*n*-butylamide) 2



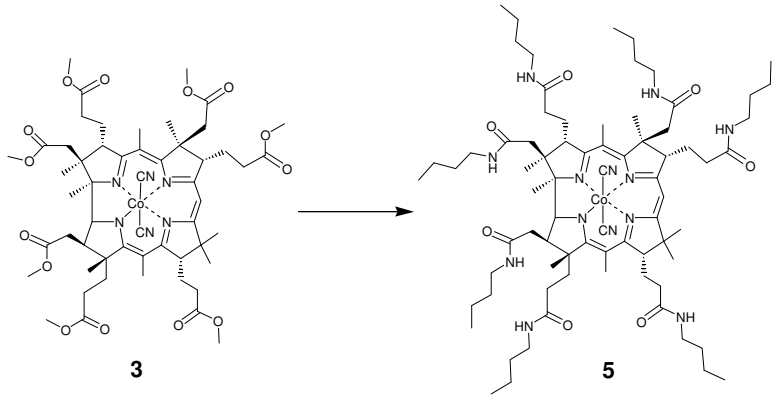
c-Lactone **7** (70 mg, 0.065 mmol) was dissoleved in CCl_4 (5.0 mL) in a Schlenck tube under an argon atmosphere. *n*-Butylamine (5.0 mL, 37 mmol) and NaN_3 (75 mg, 1.2 mmol) were then added. The mixture was stirred at 50°C for 72 h, after which it was diluted with water (20 mL) and washed with DCM (3 x 100 mL). The aqueous layer was concentrated *in vacuo* giving a purple residue, which was triturated with $Et_2O/CHCl_3$ (100 mL, 1:1) overnight. Cobinamide **2** was purified using DCVC, 2-10% EtOH in DCM. The product was recrystalized from AcOEt/pentane giving a purple solid (66 mg, 73 %). M.p. 145-148 ^oC. R_f 0.42, 5% EtOH in DCM. Anal. calcd. for $C_{75}H_{122}CoN_{13}O_8 + 3H_2O$: C 62.26, H 8.92, N 12.59; found: C 62.21, H 8.87, N 12.45. HRMS ESI (m/z) calcd. for $C_{75}H_{122}CoN_{13}O_8Na$ $[M+Na]^+$ 1414.8767; found 1414.8763. UV/Vis EtOH, λ_{max}, ε ($L \cdot mol^{-1} \cdot cm^{-1}$): 583 ($5.39x10^3$), 544 ($5.96x10^3$), 419 ($2.68x10^3$), 368 ($1.77x10^4$), 318 ($7.79x10^3$), 280 ($8.03x10^3$). ^1H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.17 (t, *J* = 4.8 Hz, 1H), 8.13 (t, *J* = 5.3 Hz, 1H), 7.94 (t, *J* = 5.3 Hz, 1H), 7.82 (t, *J* = 5.7 Hz, 1H), 7.54 (t, *J* = 5.3 Hz, 2H), 7.33 (t, *J* = 4.4 Hz, 1H), 6.65 (s, 1H), 5.64 (s, 1H), 4.49 (d, *J* = 8.8 Hz, 0.8H), 4.40 (d, *J* = 4.4 Hz, 0.2H), 3.62 (m, 1H), 3.16-2.91 (m, 16H), 2.80-2.76 (m, 2H), 2.36-2.32 (m, 2H), 2.20 (m, 2H), 2.10-1.96 (m, 9H), 1.88-1.83 (m, 3H), 1.75-1.68 (m, 4H), 1.68 (m, 4H), 1.55-1.50 (m, 5H), 1.44-1.35 (m, 10H), 1.22-1.16 (m, 12H), 1.08 (s, 4H), 0.99 (d, *J* = 6.6Hz, 1H), 0.90-0.83 (m, 16H), 0.81-0.78 (m, 5H). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 176.1, 175.7, 175.3, 172.3, 172.1, 171.6, 171.0, 170.7, 170.3, 170.2, 170.1, 162.1, 157.9, 105.8, 102.2, 88.6, 84.7, 82.4, 74.6, 67.2, 67.1, 58.3, 54.9, 54.8, 52.9, 46.0, 45.9, 38.8, 38.6, 38.3, 38.2, 38.18, 38.15, 31.3, 31.2, 31.18, 31.12, 31.0, 30.9, 30.8, 30.5, 19.9, 19.8, 19.7, 19.6, 19.56, 19.50, 19.4, 19.3, 17.4, 15.9, 15.8, 15.0, 14.6, 13.7, 13.6, 13.57, 13.56.

$(CN)_2$Cob(III)heptakis(2-hydroxyethylamide) 4



Heptamethylester **3** (15 mg, 0.014 mmol) was dissolved in toluene (1.0 mL) in a Schlenck tube under an argon atmosphere. Ethanolamine (1.0 mL, 1.6 mmol) and $Bu_4N^+CN^-$ (15 mg, 0.047 mmol) were then added. The mixture was stirred at 50°C for 24 h, after which it was diluted with water (10 mL) and washed with DCM (3 x 50 mL). The aqueous layer was concentrated *in vacuo* giving a purple residue, which was triturated with Et_2O/chloroform (50 mL, 1:1) overnight. The crude product was purified using DCVC, 20-50% MeOH in DCM. Cobinamide **4** was redissolved in isopropanol, filtered through glass wool and the filtrate concentrated *in vacuo*. Cobinamide **4** was then recrystalized from EtOH/Et_2O and dried under vacuum giving a purple solid (11 mg, 60%). M.p. 182-192°C. R_f 0.48, 50% MeOH in DCM. Anal. calcd. for $C_{61}H_{94}CoN_{13}O_{14}$ + EtOH + $2H_2O$: C 55.05, H 7.63, N 13.25; found: C 54.82, H 7.60, N 13.19. HRMS ESI (m/z) calcd. for $C_{61}H_{94}CoN_{13}O_{14}Na$ $[M+Na]^+$ 1314.6267; found 1314.6209. UV/Vis CH_3OH, λ_{max}, ε ($L \cdot mol^{-1} \cdot cm^{-1}$): 278 ($5.93x10^3$), 317 ($6.37x10^3$), 367 ($8.74x10^3$), 473 ($4.23x10^3$), 541 ($3.19x10^3$), 583 ($3.02x10^3$). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.35 (t, *J* = 5.1 Hz, 1H), 8.31 (t, *J* = 5.3 Hz, 1H), 7.99 (m, 2H), 7.91 (t, *J* = 5.3 Hz, 1H), 7.67 (t, *J* = 5.3 Hz, 1H), 7.62 (t, *J* = 5.5 Hz, 1H), 5.64 (s, 1H), 4.83 (s(br), 2H), 4.69 (s(br), 2H), 4.61 (m(br), 3H), 4.41 (d, *J* = 9.3 Hz, 1H), 3.59 (m, 2H), 3.45-3.41 (m, 4H), 3.33 (m, 11H), 3.26-3.22 (m, 1H), 3.16-3.09 (m, 8H), 3.04-3.03 (m, 6H), 2.78 (s(br), 1H), 2.63-2.56 (m, 1H), 2.46-2.37 (m, 3H), 2.30-2.25 (m, 1H), 2.18 (s, 5H), 2.14 (s, 3H), 2.09-1.99 (m, 6H), 1.93-1.72 (m, 6H), 1.51 (s, 3H), 1.33 (s, s, 6H), 1.22 (s, 3H), 1.14 (s, 3H), 1.07 (m, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 175.8, 175.7, 175.3, 172.5, 172.1, 171.6, 171.4, 171.3, 170.7, 170.5, 169.2, 162.8, 162.3, 103.5, 101.6, 90.0, 82.4, 74.7, 64.7, 59.8, 59.79, 59.77, 59.71, 59.5, 59.1, 54.8, 54.3, 52.8, 50.4, 48.6, 46.0, 45.7, 43.6, 41.7, 41.6, 41.5, 41.4, 38.5, 35.3, 32.7, 32.1, 32.0, 31.9, 31.2, 30.8, 27.4, 25.6, 25.2, 21.5, 19.4, 18.2, 17.5, 16.1, 15.2, 15.1, 14.9.

Attempted synthesis of hydrophobic cobinamide 5 from heptamethylester 3

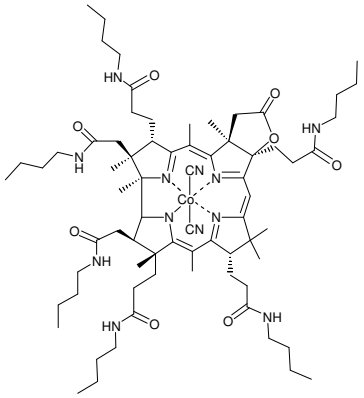


3 **5**

Heptamethylester **3** (15 mg, 0.014 mmol) was dissolved in an appropriate solvent (1.0 mL). A catalyst and *n*-butylamine (1.0 mL) were then added and the mixture was stirred at an appropriate temperature for the given time. All reactions were worked-up as described in the synthesis of cobinamide **2**.

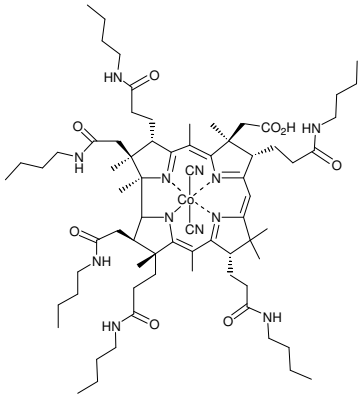
Solvent	Catalyst [eq.]	Time [h]	Temperature [^{o}C]	Yield [%] of 5
Toluene	$Bu_4N^+CN^-$; 0,5eq.	24	50	-
Toluene	$Bu_4N^+CN^-$; 0,5eq.	72	50	-
CCl_4	$Bu_4N^+CN^-$; 0,5eq.	24	RT	-
CCl_4	$Bu_4N^+CN^-$; 0,5eq.	24	50	-
CCl_4	NaN_3	72	50	-
CCl_4	NaN_3 and NaCN	72	50	-
EtOH	NaCN, excess	24	RT	-
EtOH	$Bu_4N^+CN^-$; 0,5eq.	24	RT	-
EtOH	$Bu_4N^+CN^-$; 0,5eq.	24	50	-
EtOH	$Bu_4N^+CN^-$; 0,5eq.	72	50	-
EtOH	NaN_3, excess	72	50	-
MeOH	$Bu_4N^+CN^-$; 0,5eq.	24	50	-
MeOH	$Bu_4N^+CN^-$; 0,5eq.	48	50	-
H_2O	NaCN, excess	24	50	-
Brine	$Bu_4N^+CN^-$; 0,5eq.	72	50	-

$(CN)_2$Cob(III)hexakis(*n*-butylamide)(*c*-lactone) 9



Cobinamide **2** (80 mg, 0.057 mmol) was dissolved in DCM (3.0 mL) and treated with TFA (3.0 mL, 39 mmol). The mixture was stirred at RT for 1 h, after which the acid was neutralized using $NaHCO_3$. The aqueous layer was washed with DCM and the combined organic layers were washed with aq. NaCN, dried over Na_2SO_4, filtrated and concentrated *in vacuo.* The crude product was purified using DCVC, 2-5 % EtOH in DCM. *c*-Lactone **9** was recrystalized from AcOEt/pentane and dried under vacuum giving a purple solid (55 mg, 56%). M.p. 147-150°C. R_f 0.31, 4% EtOH in DCM. Anal. calcd. for $C_{71}H_{111}CoN_{12}O_8$ + $2H_2O$: C 62.90, H 8.55, N 12.40; found: C 62.81, H 8.56, N 12.29. HRMS ESI (m/z) calcd. for $C_{70}H_{111}CoN_{11}O_8$ $[M\text{-}CN]^+$ 1292.7944; found 1292.7944. UV/Vis CH_3OH, λmax, ε ($L \cdot mol^{-1} \cdot cm^{-1}$): 280 ($9.56x10^3$), 305 ($7.64x10^3$), 317 ($8.71x10^3$), 366 ($2.82x10^4$), 419 ($2.47x10^3$), 547 ($8.11x10^3$), 856 ($9.52x10^3$). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.13 (t, *J* = 5.4 Hz, 2H), 7.92 (t, *J* = 5.4 Hz, 1H), 7.85 (t, *J* = 5.4 Hz, 1H), 7.58 (t, *J* = 5.5 Hz, 1H), 7.49 (t, *J* = 5.5 Hz, 1H), 5.60 (s, 1H), 4.40 (d, *J* = 8.7 Hz, 1H), 3.64-3.60 (m, 1H), 3.29 (d, *J* = 6.3 Hz, 1H), 3.25 (s, 1H), 3.21-3.14 (m, 1H), 3.09-2.91 (m, 12H), 2.82 (s(br), 1H), 2.61 (d, *J* = 18.7 Hz, 1H), 2.38-2.35 (m, 1H), 2.26-2.16 (m, 12H), 2.07-1.92 (m, 6H), 1.88-1.67 (m(br), 4H), 1.60 (s, 3H), 1.43-1.20 (m, 35H), 1.13 (s, 3H), 1.06 (s, 3H), 0.89-0.84 (m, 12H), 0.82-0.79 (m, 6H). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 177.7, 176.5, 175.8, 173.3, 171.8, 170.9, 170.6, 170.5, 170.3, 170.1, 164.5, 162.0, 159.5, 103.9, 103.4, 94.5, 87.0, 82.6, 75.3, 58.4, 55.2, 52.9, 49.8, 46.8, 45.8, 41.7, 38.6,38.4, 38.3, 38.2, 38.1, 35.5, 32.6, 32.2, 32.0, 31.2, 31.17, 31.5, 31.1, 30.9, 30.4, 30.0, 29.5, 26.2, 25.2, 21.6, 19.7, 19.6, 19.5, 19.3, 18.8, 17.8, 16.1, 16.0, 15.0, 13.7, 13.63, 13.60, 13.58, 13.56.

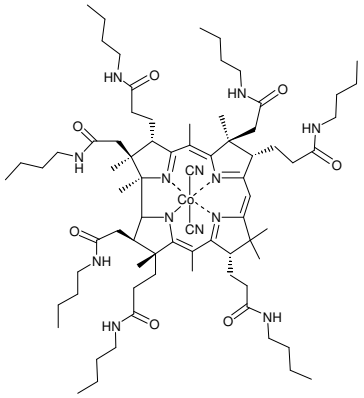
$(CN)_2$Cob(III)hexakis(*n*-butylamide)(*c*-acid) 10



c-Lactone **9** (15 mg, 0.011 mmol) was dissoleved in a degased 15 % AcOH/toluene solution (1.0 mL). Activated zinc (22 mg, 0.34 mmol) was added and the mixture was vigorously stirred at RT for 30 min. The solution was neutralized using phosphate buffer (5.0 mL) and washed with DCM. The combined organic layers were washed with aq. NaCN, dried over Na_2SO_4, filtrated and concentrated *in vacuo.* *c*-Acid **10** was crudely purified using DCVC, 5-10% MeOH in DCM, in which the most intense band was isolated. *c*-Acid **10** was recrystalized from AcOEt/pentane and dried under vacuum giving a purple solid. R_f 0.47, 10% EtOH in DCM. HRMS ESI (m/z) calcd. for $C_{70}H_{113}CoN_{11}O_8$ $[M\text{-}CN]^+$ 1294.8100; found 1294.8048.

c-Acid **10** was used without further purification. The broadening of peaks in the ^1H NMR spectrum made it impossible to decipher and consequently high resolution ^{13}C spectra could not be obtained. This was caused by the presence of the acid group.

$(CN)_2$Cob(III)heptakis(*n*-butylamide) 5



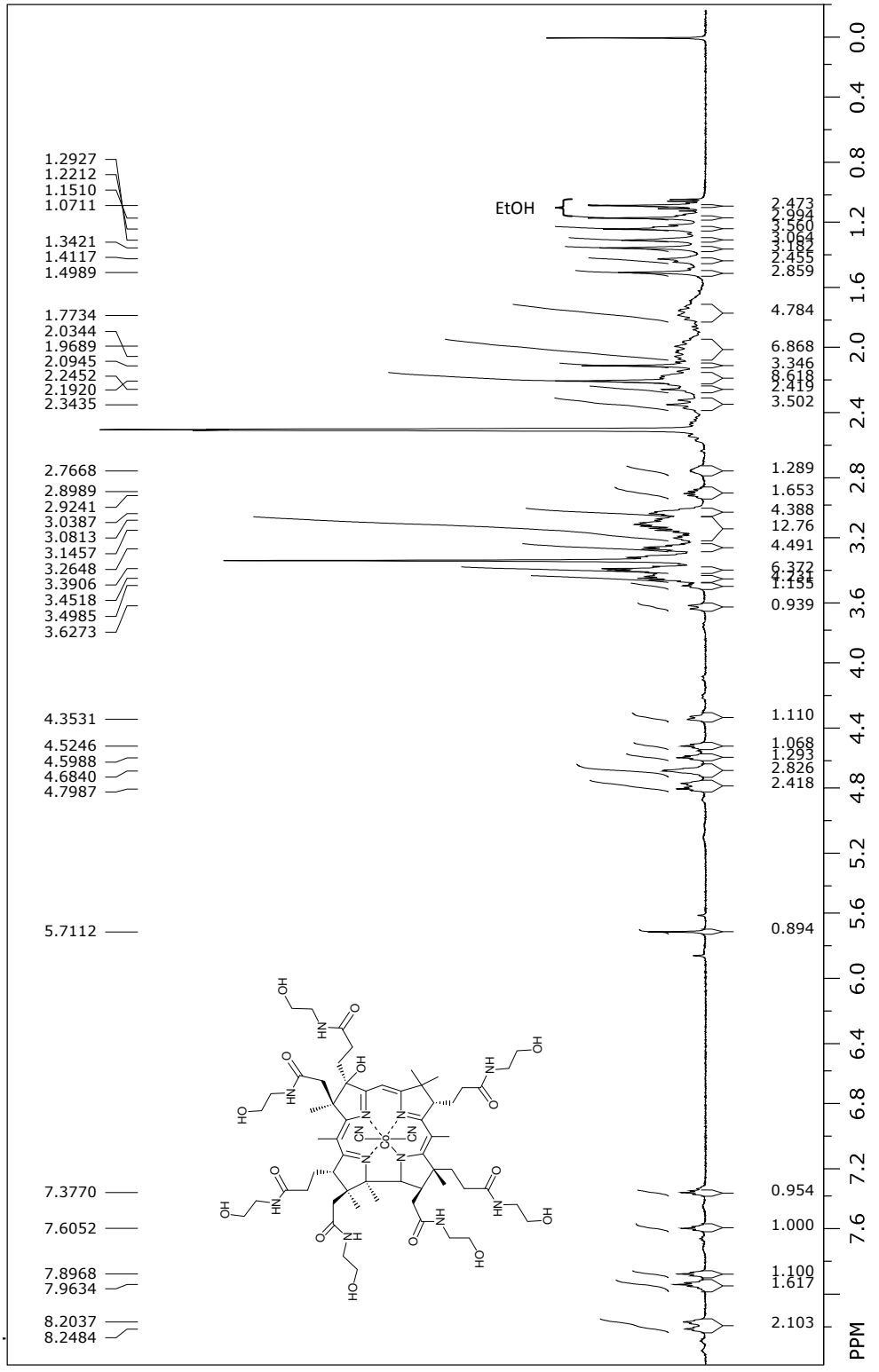
Cobinamide **10** (20 mg, 0.015 mmol) was dissolved in DMF (1.5 mL) under an argon atmosphere. *n*-Butylamine (12 µL), DIPEA (18 µL, 0.11 mmol) and DEPC (15 µL, 0.11 mmol) were then added and the mixture was stirred at RT for 24 h. The mixture was then diluted with DCM and washed with water. The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo* giving a purple residue. The crude product was purified using DCVC, 2-10% EtOH in DCM. Cobinamide **5** was recrystalized using AcOEt/pentane and dried under vacuum giving a purple solid (13 mg, 62%). M.p. 142-146 ^oC. R_f 0.44, 5% EtOH in DCM. Anal. calcd. for $C_{75}H_{122}CoN_{13}O_7 + 2H_2O$: C 63.76, H 8.99, N 12.89; found: C 63.68, H 9.07, N 12.81. HRMS ESI (m/z) calcd. for $C_{74}H_{122}CoN_{12}O_7$ $[M\text{-}CN]^+$ 1398.8814; found 1398.8815. UV/Vis CH_3OH, λ_{max}, ε ($L \cdot mol^{-1} \cdot cm^{-1}$): 279 ($1.06x10^4$), 315 ($9.48x10^3$), 368 ($2.78x10^4$), 419 ($2.75x10^4$), 544 ($8.32x10^3$), 581 ($9.43x10^3$), 940 ($7.08x10^3$). ^1H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.14-8.10 (m, 2H), 7.94 (t, *J* = 5.4 Hz, 1H), 7.84 (t, *J* = 5.4 Hz, 1H), 7.80 (t, *J* = 5.4 Hz, 1H), 7.55 (t, *J* = 5.4 Hz, 1H), 7.50 (t, *J* = 5.5 Hz, 1H), 5.65 (s, 1H), 4.45 (d, *J* = 9.0 Hz, 1H), 3.60 (d, *J* = 10.1 Hz, 1H), 3.47 (t, *J* = 5.5 Hz, 1H), 3.30 (s, 1H), 3.22-3.15 (m, 1H), 3.10-2.90 (m, 14H), 2.79 (s(br), 1H), 2.36-2.28 (m, 3H), 2.24-2.12 (m, 9H), 2.05-1.85 (m, 8H), 1.79-1.67 (m, 4H), 1.57-1.52 (m, 4H), 1.46-1.19 (m, 39H), 1.14 (s, 3H), 1.07 (s, 3H), 0.90-0.80 (m, 21H). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 175.9, 175.7, 175.4, 172.1, 171.7, 171.6, 171.0, 170.8, 170.4, 170.2, 168.8, 162.5, 162.3, 103.8, 101.7, 82.4, 74.6, 58.2, 55.0, 48.9, 46.0, 45.8, 40.0, 39.84, 39.76, 39.7, 39.6, 39.5, 39.3, 39.2, 39.0, 38.6, 38.5, 38.33, 38.27, 38.18, 38.16, 38.1, 31.9, 31.8, 31.3, 31.18, 31.17, 31.15, 31.1, 31.0, 30.9, 21.6, 19.7, 19.59, 19.56, 19.53, 19.50, 19.4, 18.2, 17.6, 16.0, 15.0, 14.9, 13.66, 13.55, 13.62, 13.56, 13.54, 13.48

Preparation and in vitro assay of recombinant human sGC enzyme.

Full-length sGC was purified from Sf9 cells as described previously.[1] Enzymatic activity was assayed using [α-^{32}P]GTP to [^{32}P] cGMP conversion assay. To evaluate the effect of (CN2)Cbi or its derivatives on sGC activity, 0.5 μg sGC was preincubated for 10 min at room temperature with indicated concentration of the tested compound in 25 mM TEA, pH 7.5, 1 mg/ml BSA, 1 mM 3-isobutyl-1-methylxanthine (IBMX), 1 mM DTT, 1 mM cGMP, 3 mM $MgCl_2$, 0.05 mg/ml creatine phosphokinase and 5 mM creatine phosphate. To assay the cGMP-forming activity of treated sGC the reaction was initiated by adding 1 mM GTP/ [α-^{32}P]GTP (~ 150000 cpm) and incubated at 37^{o}C for 10 minutes. The reaction was stopped by 400 μl of 100 mM zinc acetate followed by 500 μl of 120 mM sodium carbonate. Unreacted GTP was precipitated by centrifugation and the supernatant containing cGMP was loaded onto a 2 ml Al_2O_3 column. cGMP was eluted with 10 ml of 50 mM Tris pH 7.5 and the amount of generated [^{32}P] cGMP was calculated based on the Cherenkov counts in a beta scintillation counter.

[1]Martin, E.; Berka, V.; Tsai, A.L.; Murad, F. *Methods Enzymol., 2005,* **396,** 478-492.

$(CN)_2$Cob(III)(*c*-hydroxy)heptakis(2-hydroxyethylamide) 1



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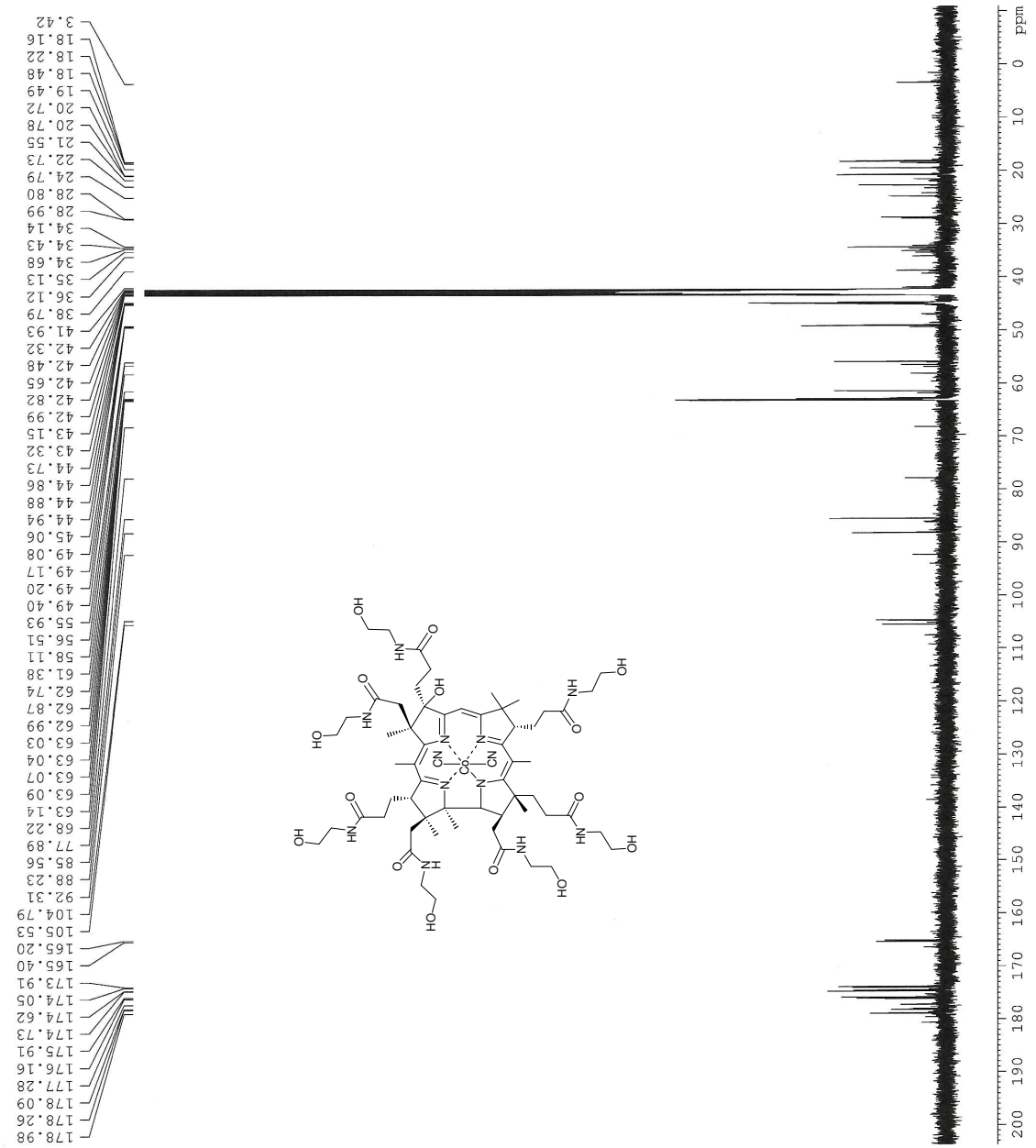

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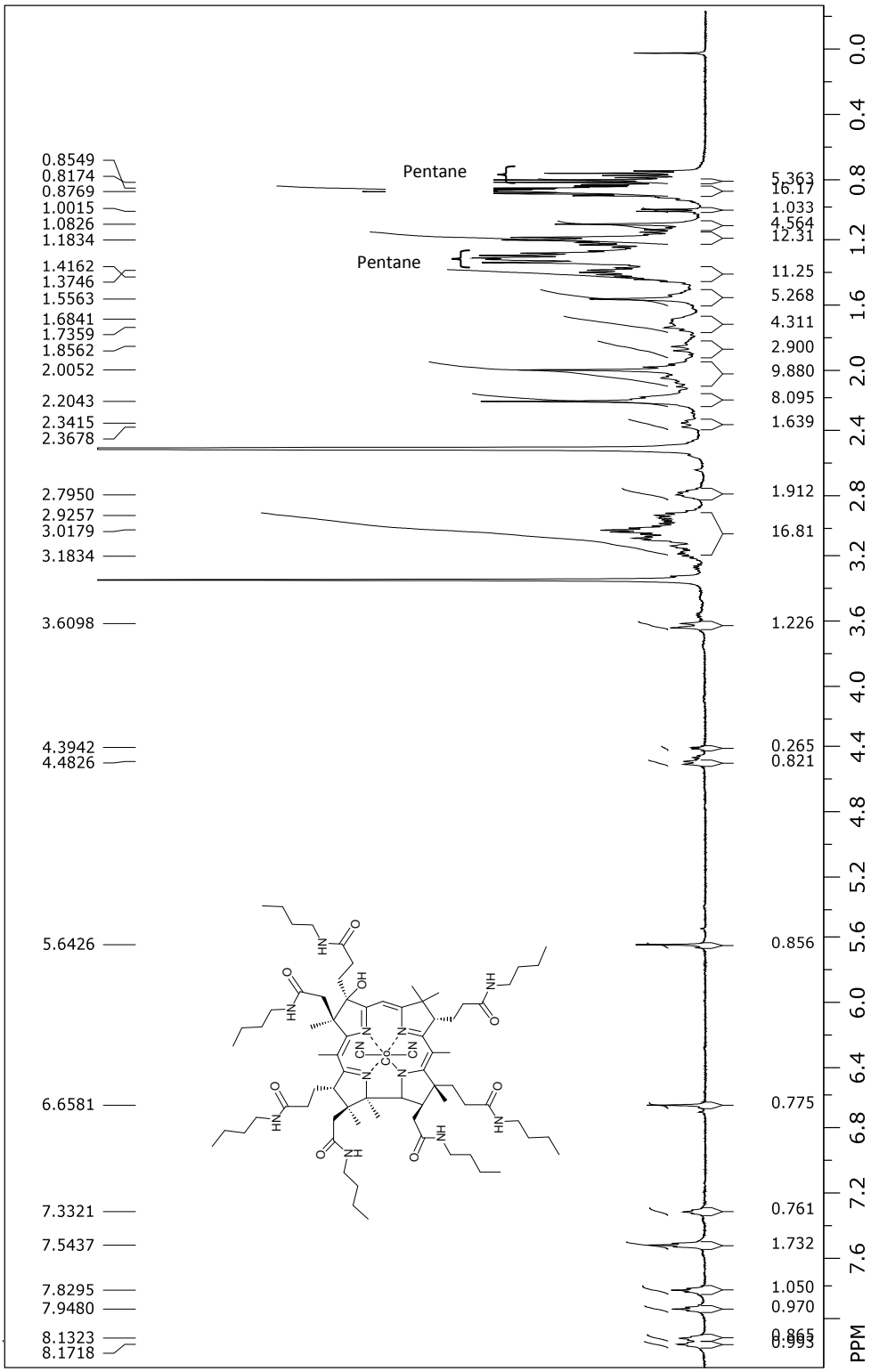

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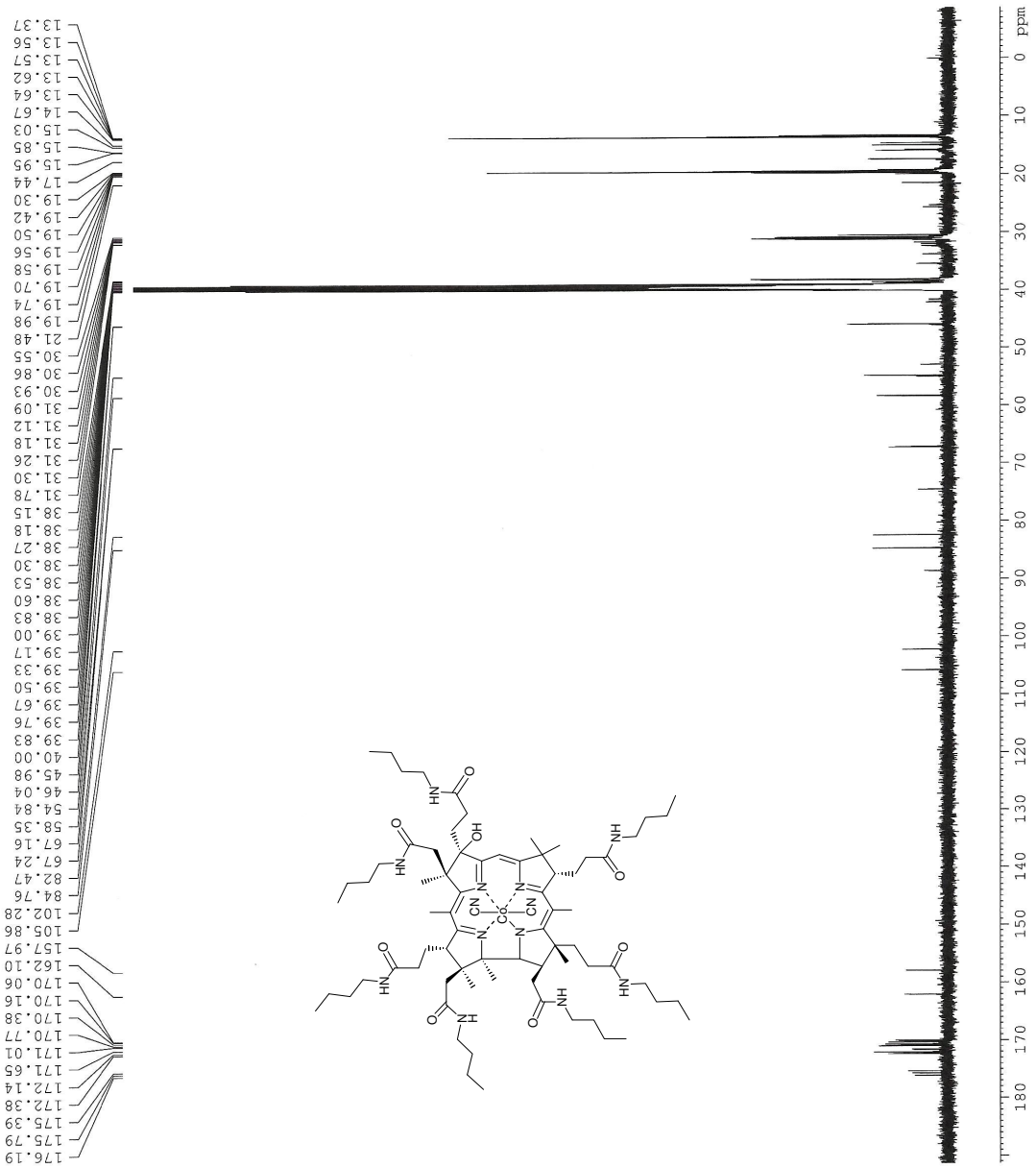

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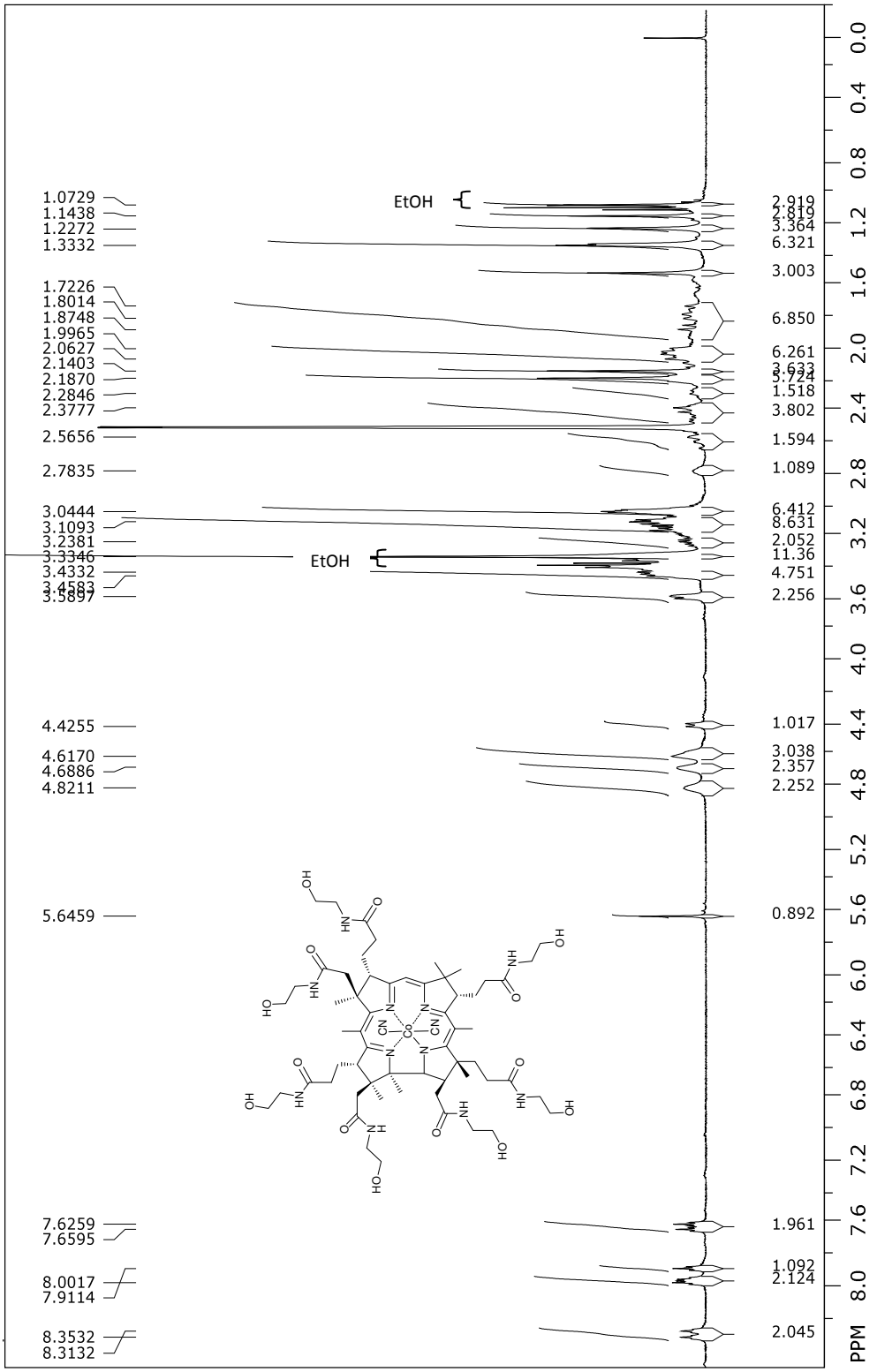

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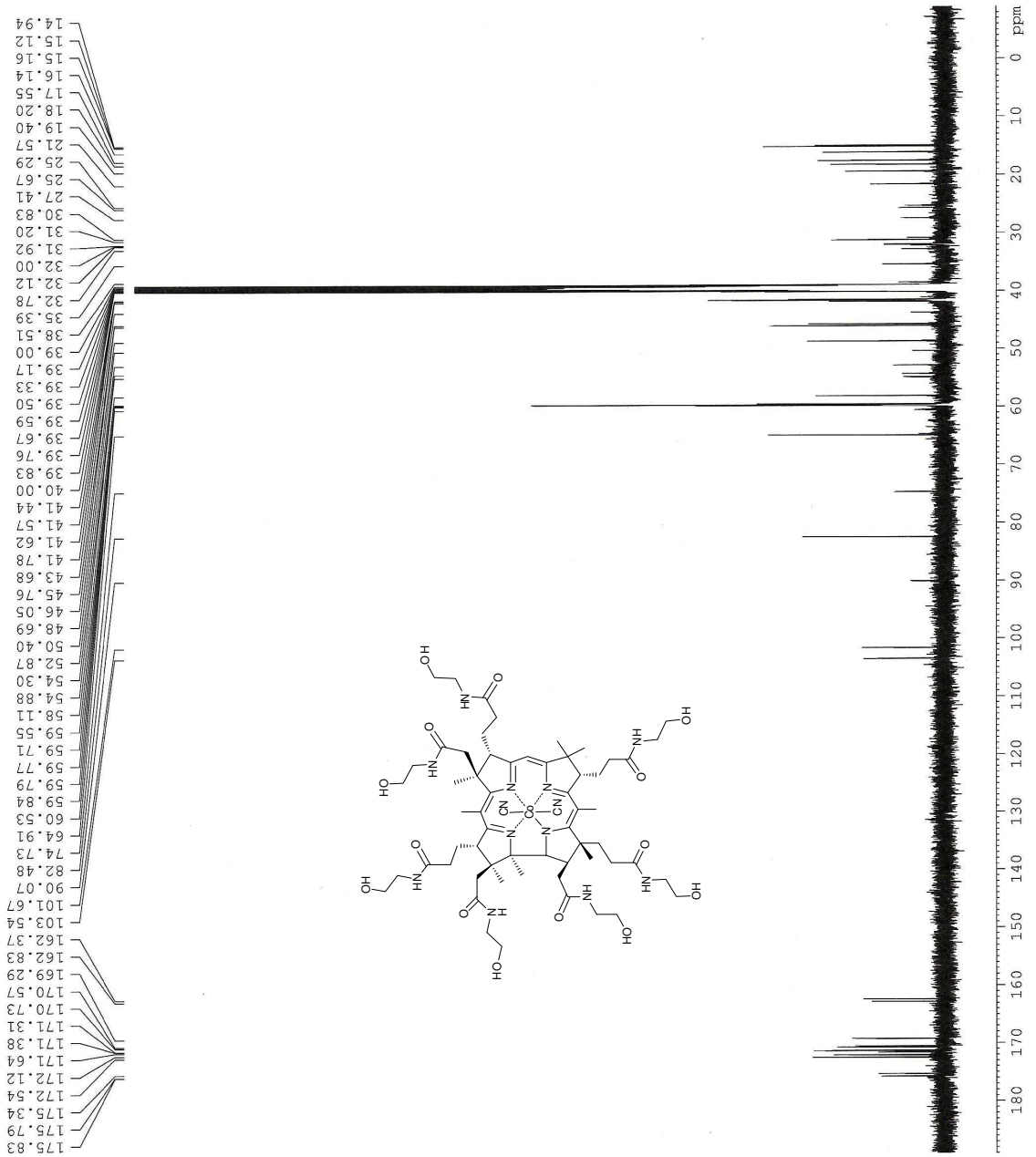
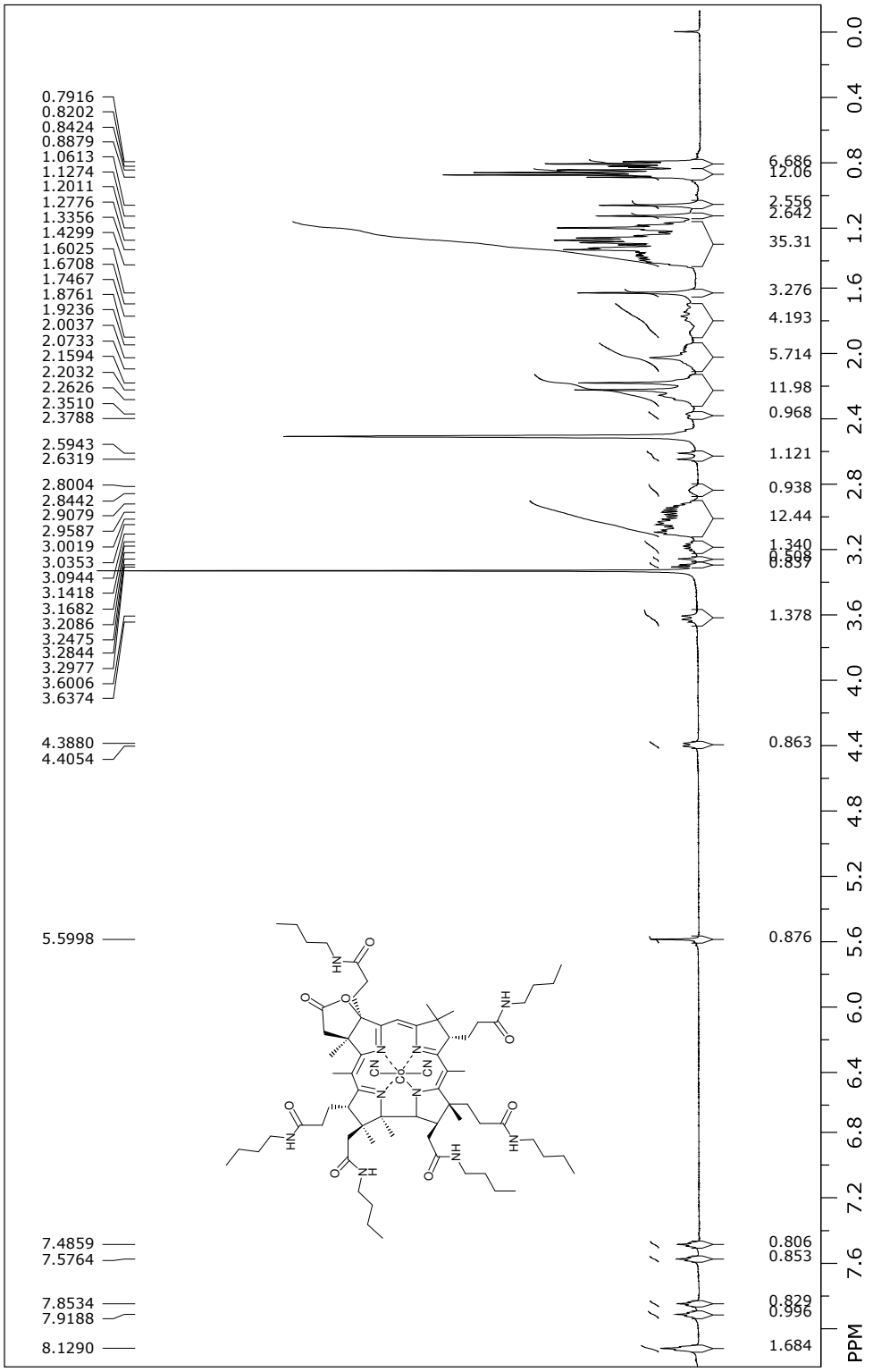


$(CN)_2$Cob(III)hexakis(*n*-butylamide)(*c*-lactone) 9



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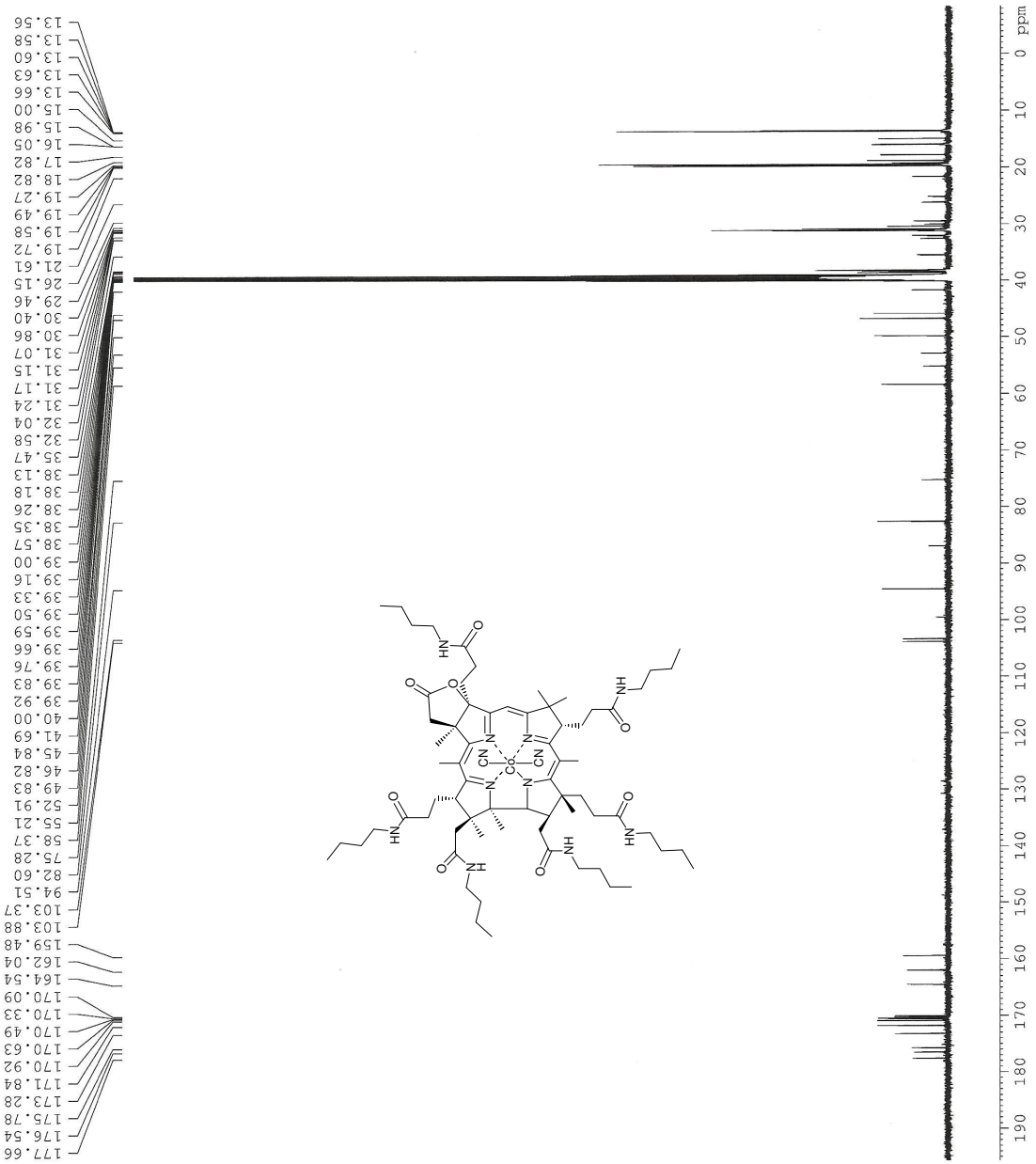
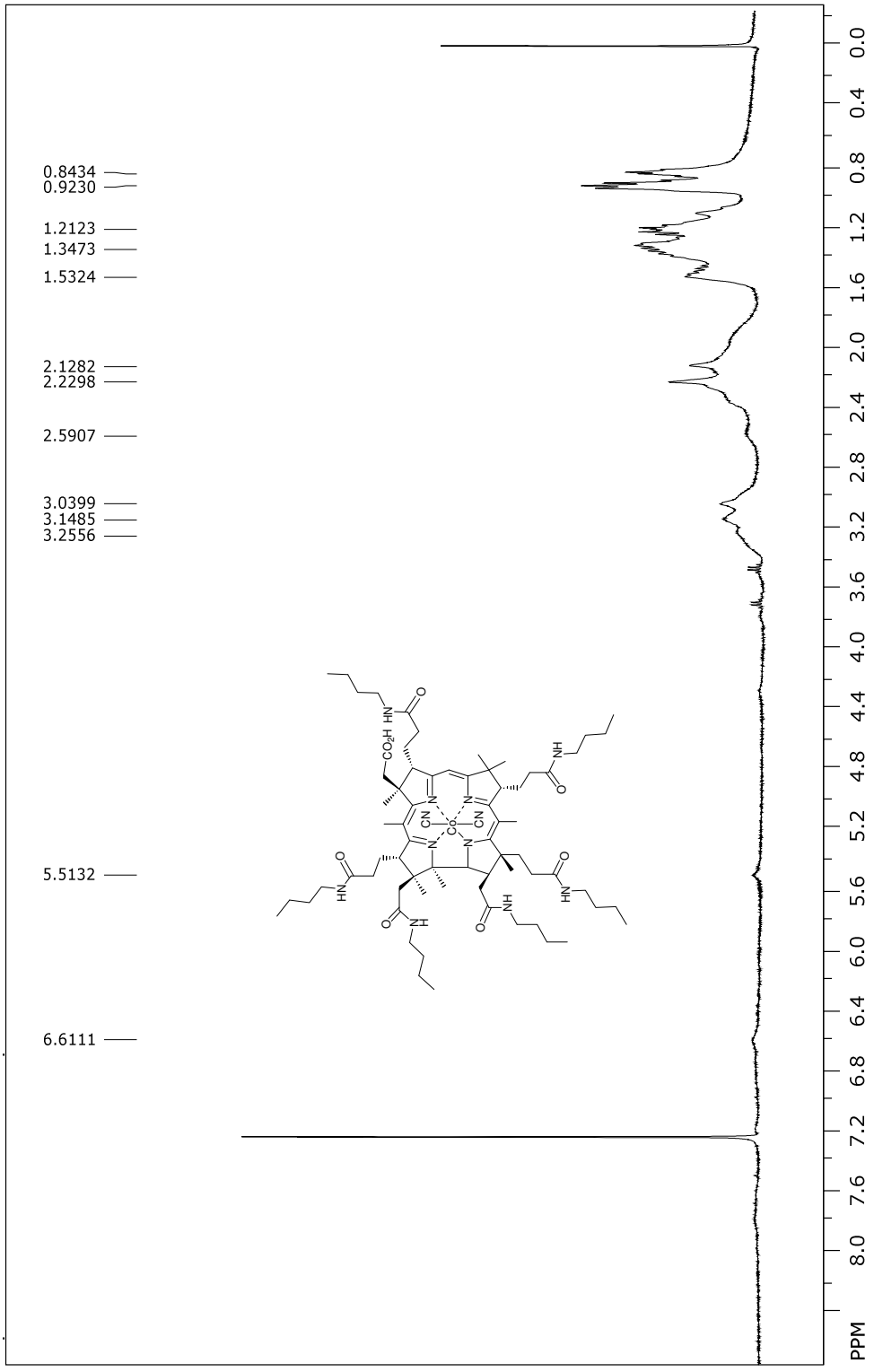


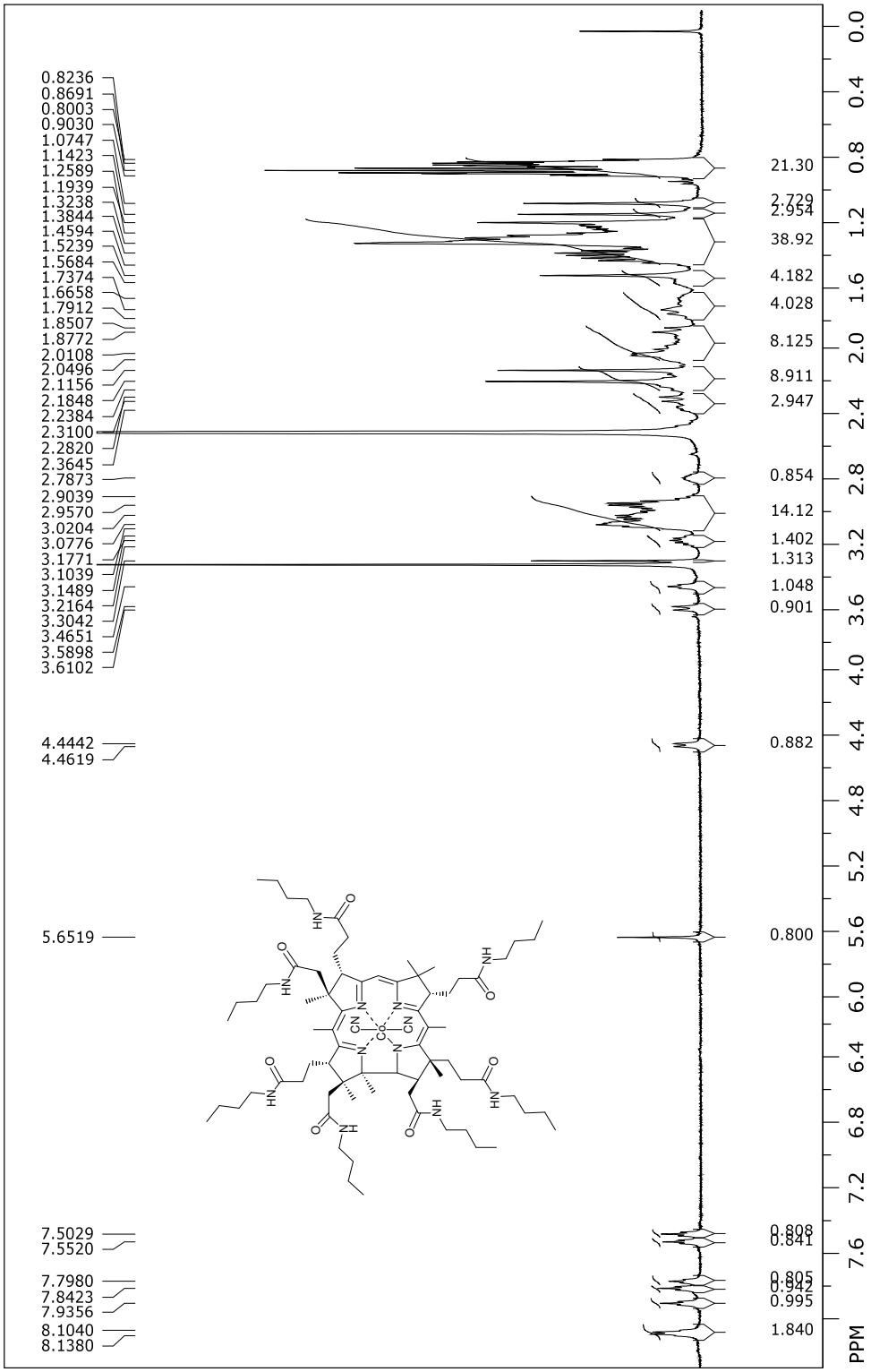
$(CN)_2$Cob(III)hexakis(*n*-butylamide)(*c*-acid) 10



Note: The broadening of peaks in the ^{1}H NMR spectrum made it impossible to decipher and consequently high resolution ^{13}C spectra could not be obtained. This was caused by the presence of the *c*-acid group.

Varian 400MHz, DSMO-d_6 at 25^{o}C

$(CN)_2$Cob(III)heptakis(*n*-butylamide) 5



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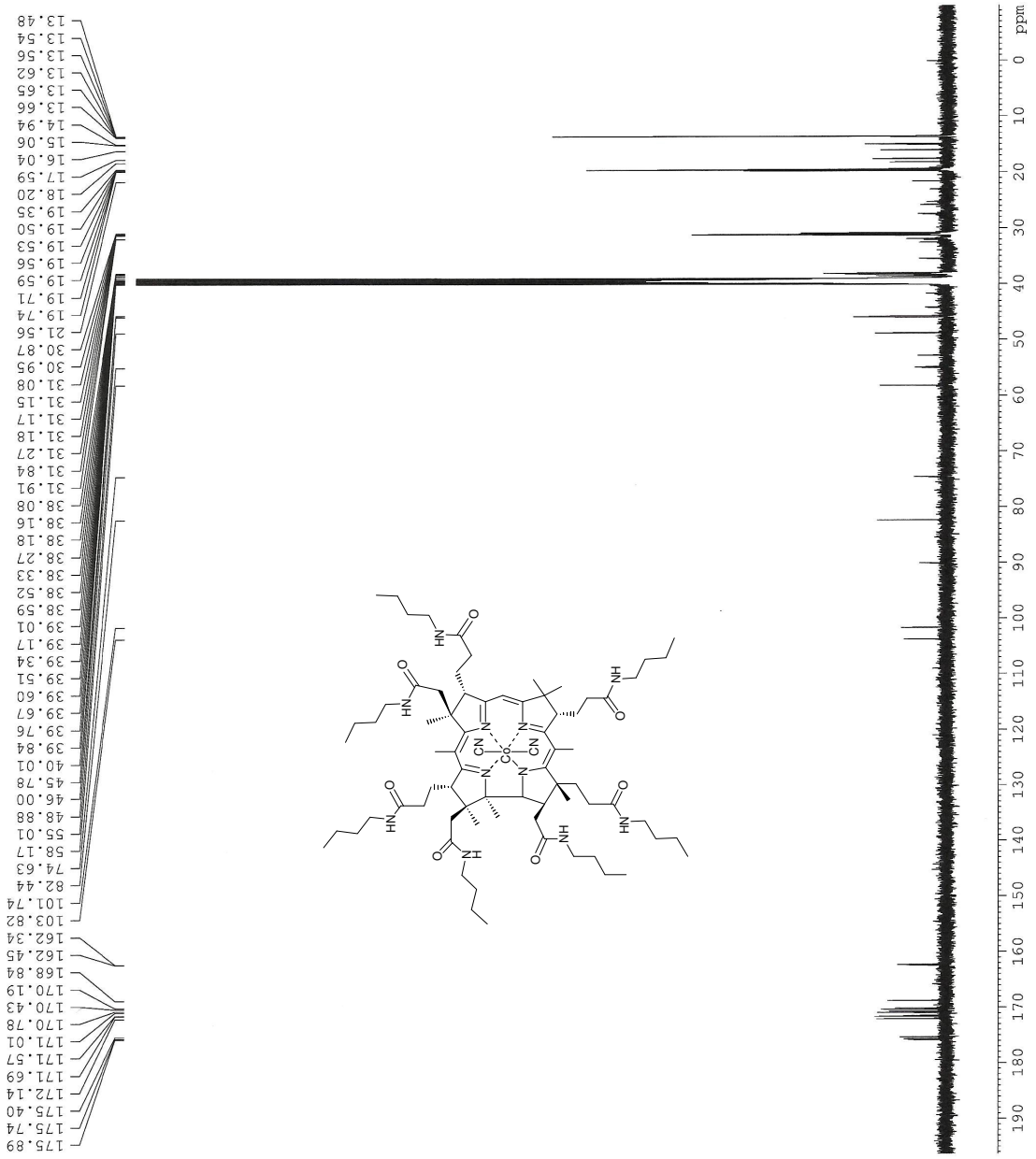

DOI: 10.1002/ajoc.201300051

Selectively Modified Cobyrinic Acid Derivatives

Keith ó Proinsias,[a] Maciej Giedyk,[a] Łukasz Banach,[a] Dorota Rutkowska-Zbik,[b] and Dorota Gryko*[a]

Abstract: A method for the synthesis of cobinamide derivatives by aminolysis of cobyrinate $(CN)_2$Cby(III)$(OMe)_6$(*c*-lactone) is described. Reactions catalyzed by a benzotriazole/1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) system afford heptaamides in good yields. During aminolysis of heptamethyl cobyrinate hydroxylation occurred at the C8 position. DFT calculations indicate that the C–H bond at this position is the weakest and, as such, is the easiest to break. The use of $(CN)_2$Cby(III)$(OMe)_6$(*c*-lactone) circumvented the problem, as the hydroxy group is already present at the 8 position and can be easily removed by *c*-lactone reformation and subsequent reduction. Moreover, selective functionalization of heptaamides at either the *c* or *meso* position was also explored.

Keywords: aminolysis · cobinamides · cobyrinic acid · lactones · vitamin B_{12}

Introduction

Dicyanocobinamide $(CN)_2$Cbi **1** (Figure 1) is a vitamin B_{12} derivative that has the 4-amidobutan-2-ol group at the *f* position and is generated by cleavage of the dimethylbenzimidazole, ribose, and phosphate moieties of vitamin B_{12}. Compound **1** has been used for the detection of cyanide in water, plants, and blood.[1] Once cyanide is bound, a significant color change from red to violet is observed and can be measured by UV/vis spectroscopy. Cobinamide has also been applied in the separation of different cobalamin-binding proteins, such as transcobalamin (TC), intrinsic factor (IF), and haptocorrin (HC), within various excreted substances from mammals and fish. For example, Greibe et al. reported that cobinamide has a high affinity for TC and IF proteins in zebrafish, whereas it binds weakly to HC.[2] A recent breakthrough by Martin and co-workers showed that $(CN)_2$Cbi acts as an NO-independent soluble guanyl cyclase (sGC) activator.[3] Unlike other NO-independent sGC activators, including 3-[5-hydroxymethyl-2-furyl]-1-benzylindazole (YC-1) and its derivatives BAY 41-2272 and BAY 41-8543 that target the regulatory domain, $(CN)_2$Cbi binds at the catalytic domain.

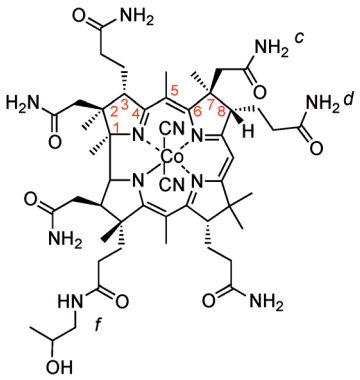

Figure 1. Dicyanocobinamide $(CN)_2$Cbi **1**.

Several chemical syntheses of $(CN)_2$Cbi **1** have been developed over the years and various protocols are currently available. The most common method relies upon treatment of vitamin B_{12} with Ce^{3+} salts in the presence of cyanides.[4] This procedure requires a long reaction time and a high reaction temperature. Brown and co-workers synthesized $(CN)_2$Cbi **1** at room temperature by using trifluoromethanesulfonic acid in 85% yield.[5] The most recent contribution to this area was by Zhou and Zelder, who discovered a simple method for cobinamide synthesis by using Zn^{2+} or Cu^{2+} salts at high temperatures.[6] Unfortunately, it is difficult to scale up such reactions.

Interestingly, particular attention has always been paid to Cbi synthesis and never to its analogues. The only attempt to synthesize heptaamide derivatives was by Munk,[7] who used cobyrinic acid as a starting material to form hetpanitrophenylesters, which, when reacted with synthetic amino acids, gave the desired products. Though effective, this procedure is an unattractive four step process. To the best of our knowledge, no attention has been paid to the possibility of creating Cbi derivatives that have various peripheral amide groups from inactivated cobyrinic acid derivatives. Recently, our group undertook this challenge and developed a method for the synthesis of heptaamides from cobyrinic acid esters.[8] As our method worked well only for

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ajoc.201300051.

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OF ORGANIC CHEMISTRY

Supporting Information

Selectively Modified Cobyrinic Acid Derivatives

Keith ó Proinsias,[a] Maciej Giedyk,[a] Łukasz Banach,[a] Dorota Rutkowska-Zbik,[b] and Dorota Gryko*[a]

ajoc_201300051_sm_miscellaneous_information.pdf

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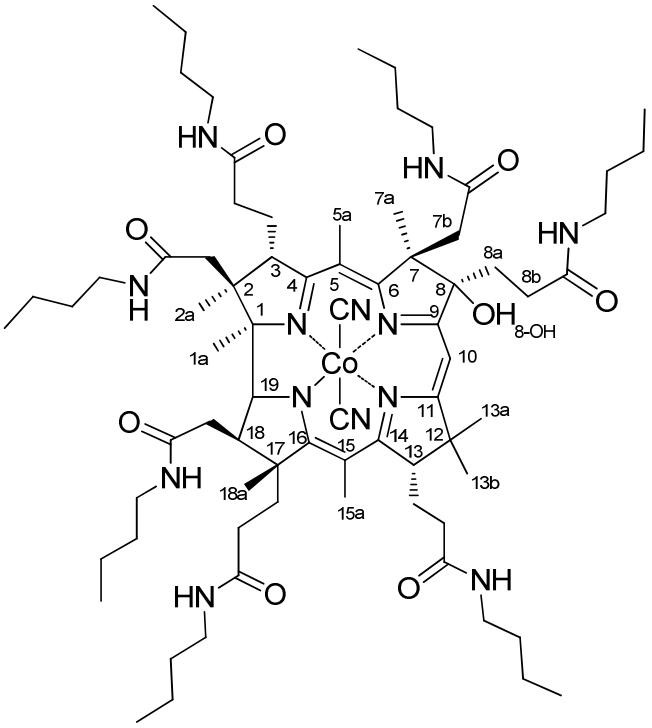
$(CN)_2$Cby(III)(*n*-butylamide)$_7$(8-OH) 5 synthesized from $(CN)_2$Cby(III)$(OMe)_7$ 3



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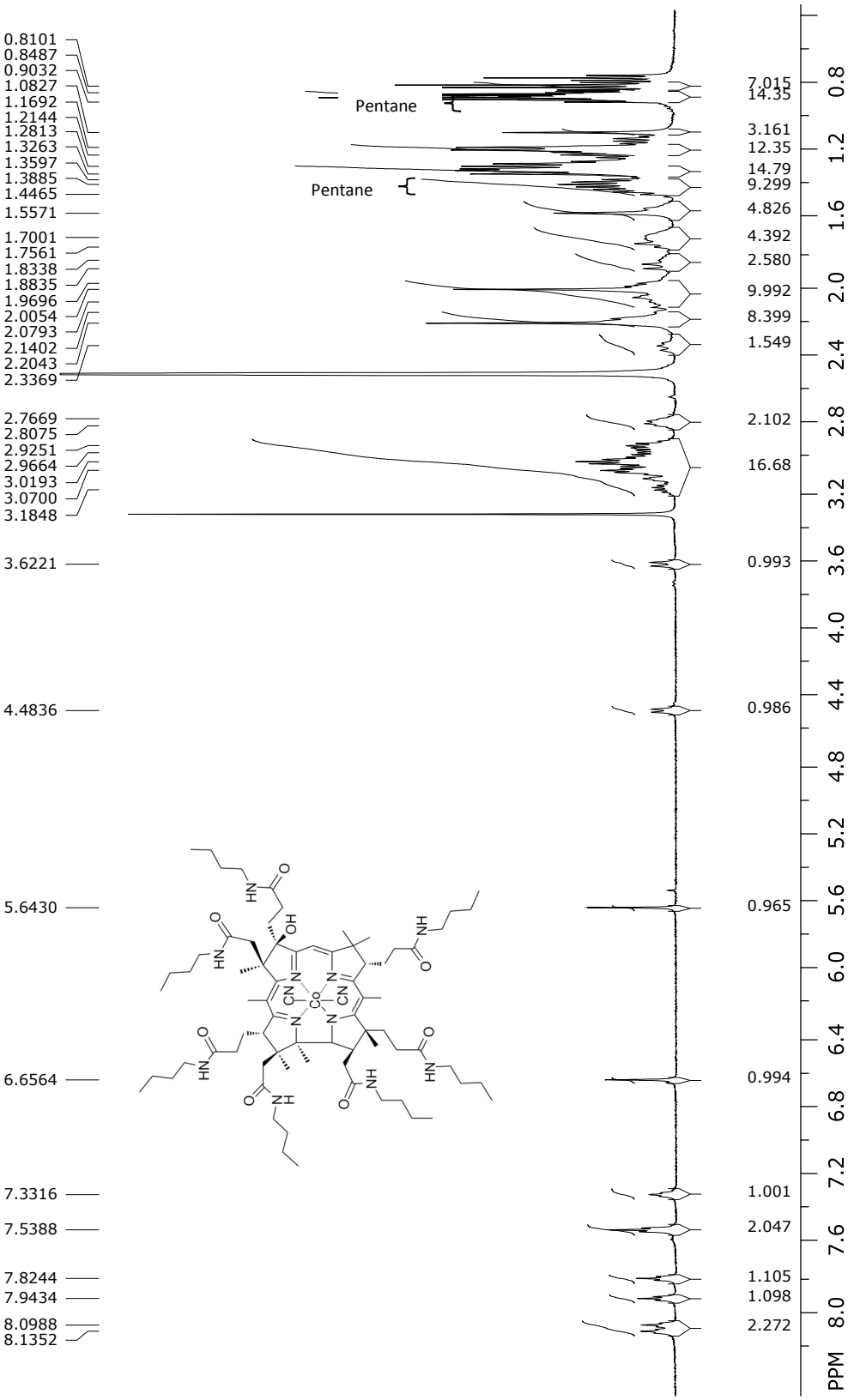

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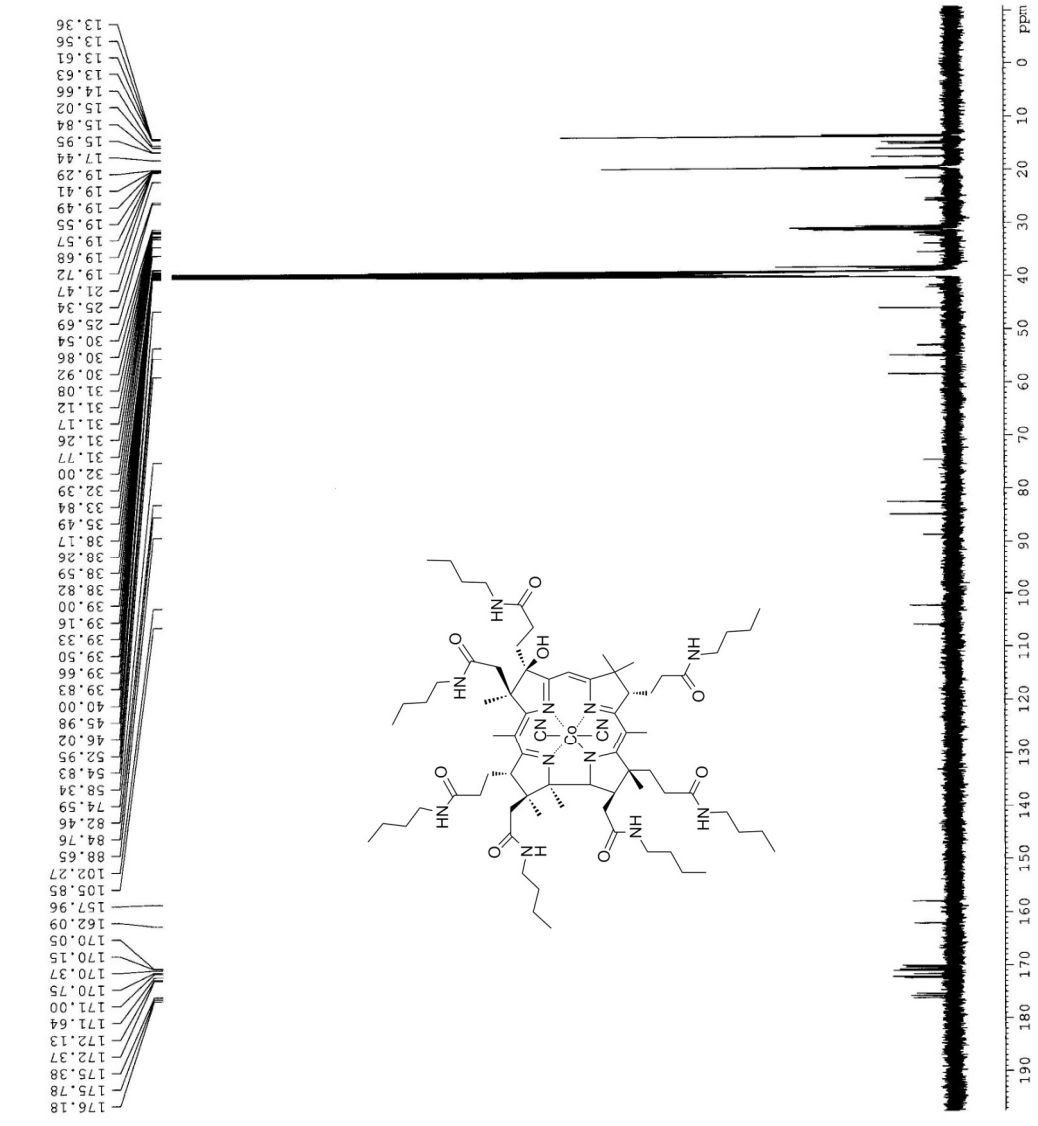

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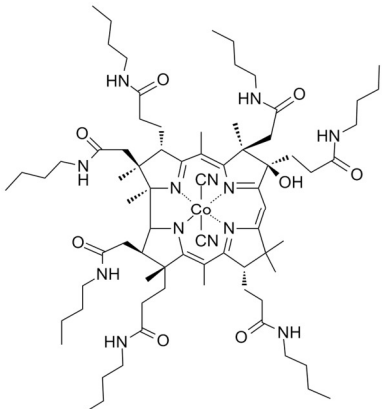
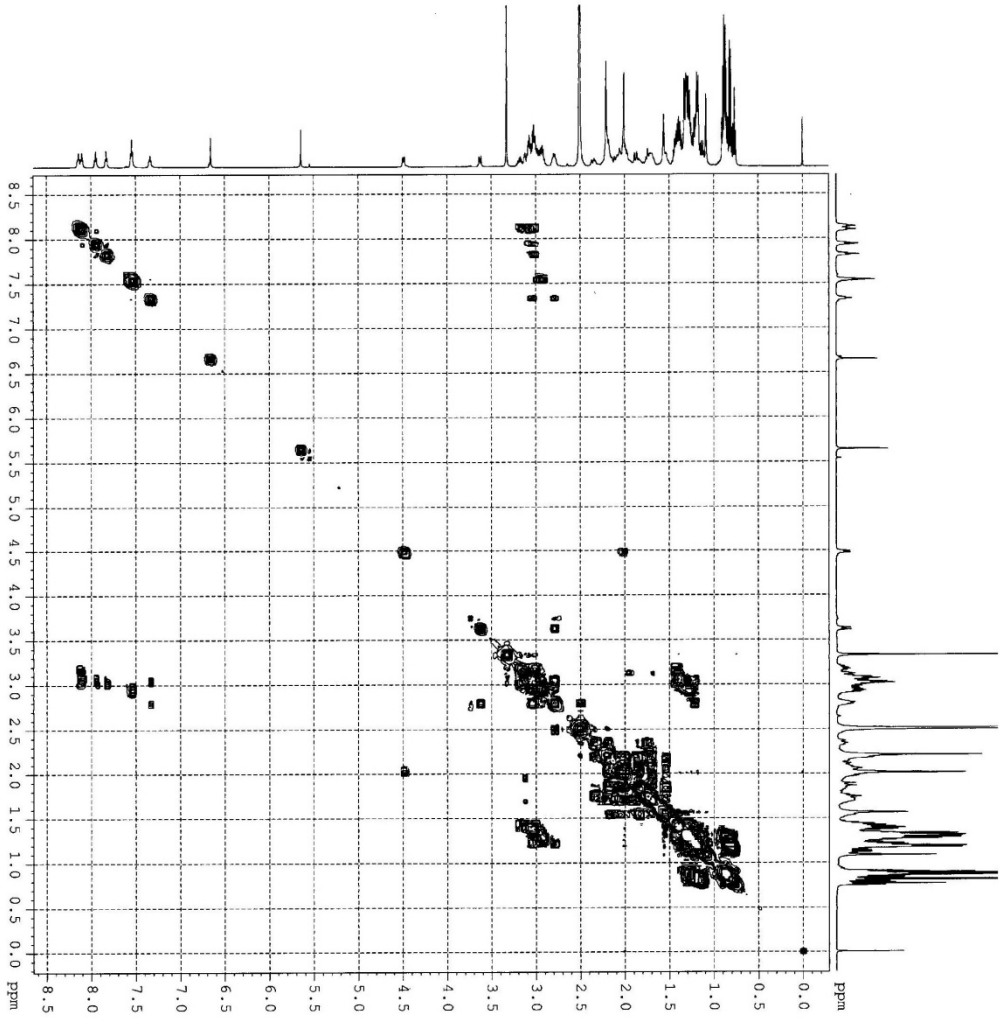
2D NMR COSY





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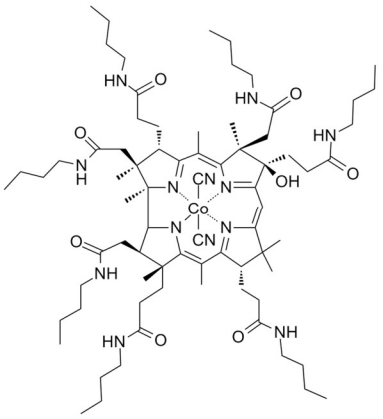
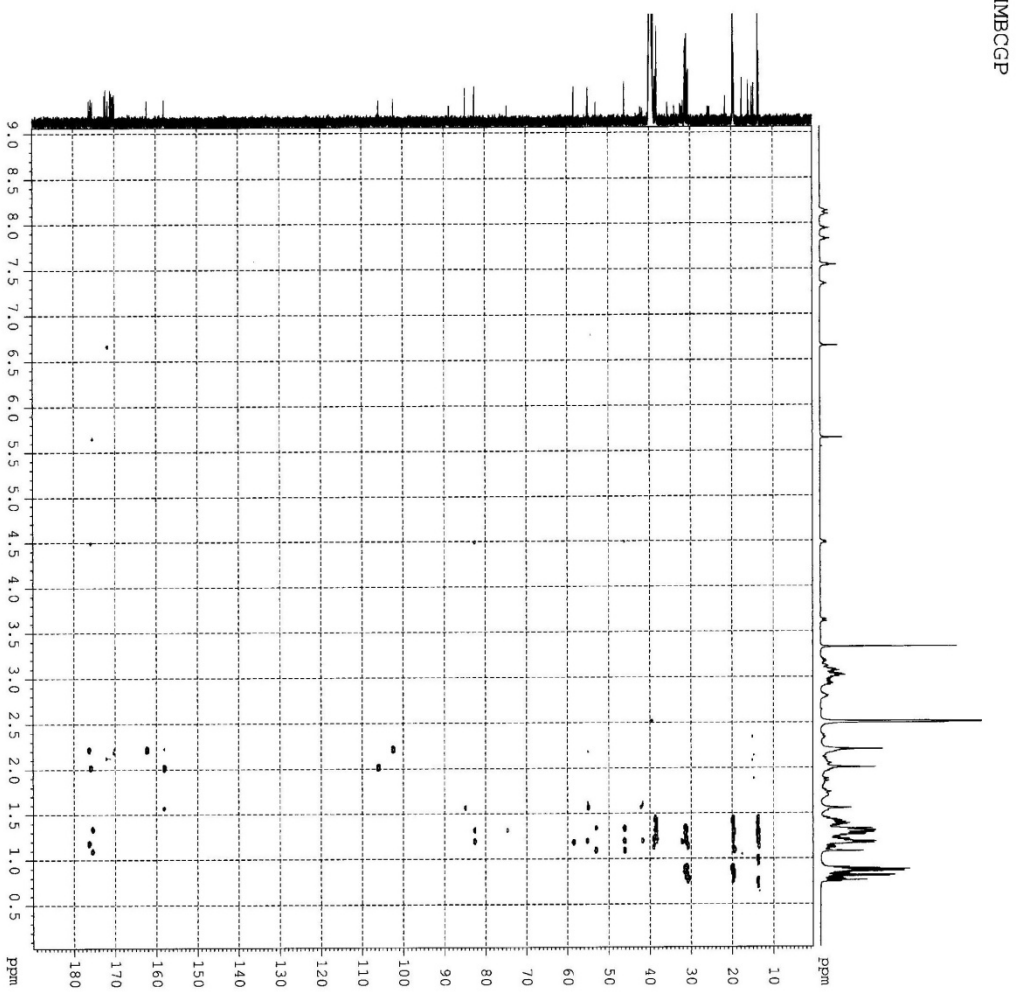
2D NMR HMBC





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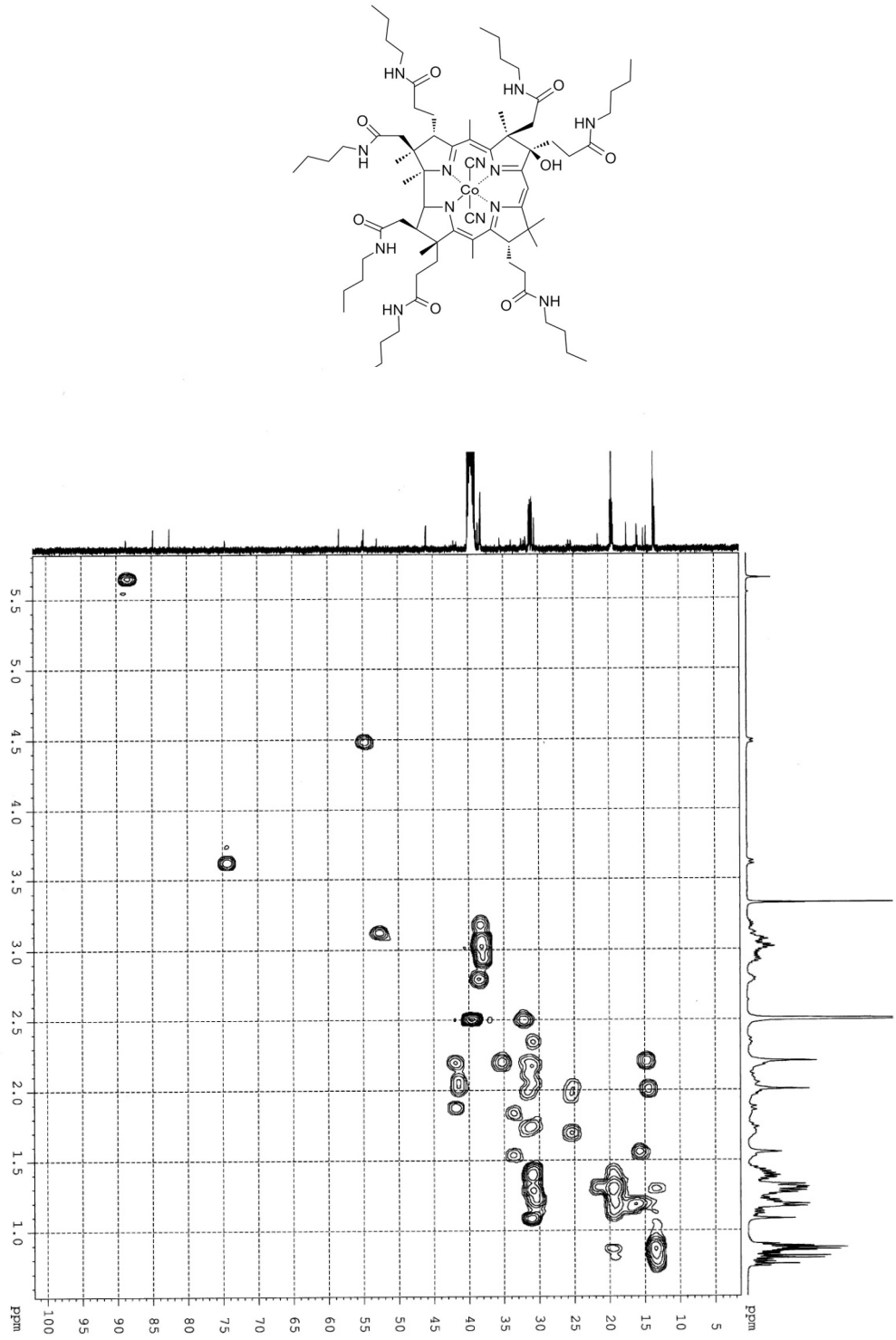
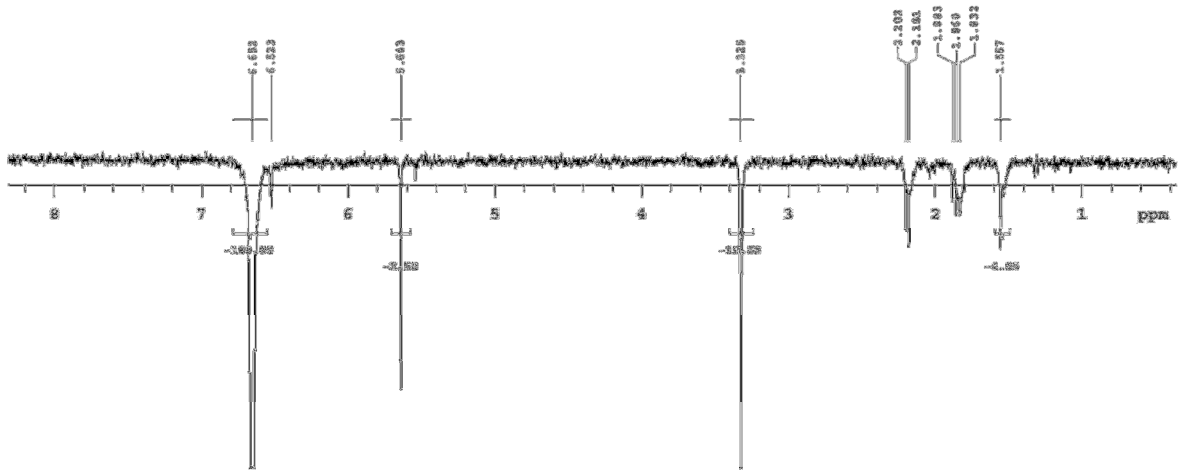
2D NMR HSQC



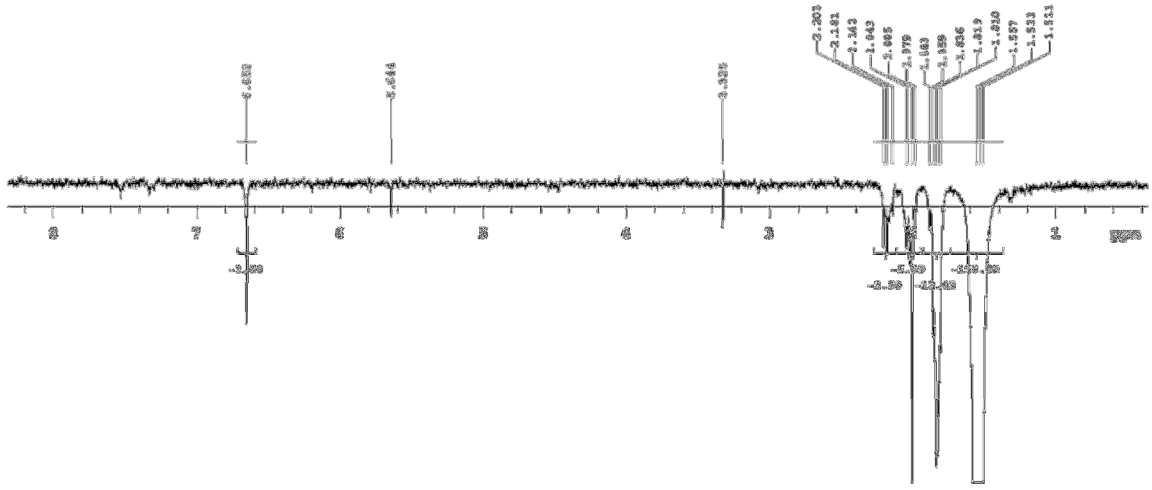
$(CN)_2$Cby(III)(*n*-butylamide)$_7$(8-OH) 5 synthesized from $(CN)_2$Cby(III)$(OMe)_7$ 3

NOESY 1D Experiments

Irradiate: **8-OH** (6.66 ppm)



Irradiate: **7a** (1.56 ppm)



$(CN)_2$Cby(III)(*n*-butylamide)$_7$(8-OH) 5 synthesized from $(CN)_2$Cby(III)$(OMe)_7$ 3

Table 1. Computed parameters of C-H bonds prone to oxidation: bond dissociation energy (BDE) and energy needed for heterolytic cleavage of the bond (ΔE) in kcal/mol, bond orders (B.O.), charges (Q), and bond lengths (R) in Å.

Position	BDE	ΔE	B.O.	Q_C	Q_H	R_{CH}	Q_CQ_H/R_{CH}
3	92.8	39.4	0.94	0.93	-0.08	1.105	-0.069
8	86.0	27.4	0.91	0.05	0.07	1.109	0.003
13	87.9	31.8	0.93	-0.22	0.11	1.108	-0.022
18	98.3	60.7	0.89	-0.07	0.16	1.111	-0.010

Atomic coordinates and total energy

BP/def2-TZVP//BP/def-SVP Total energy = -4868.048405604 a.u.

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Co	3.7967935	19.7161652	6.2030151
C	5.6165891	17.8538968	7.4554164
C	7.1717152	17.5400684	7.2814432
C	7.7938506	18.9904764	7.3437663
C	6.6578818	19.8287249	6.7664082
C	6.8532756	21.1290355	6.1595391
C	5.7680518	21.9354393	5.8360006
C	5.8177379	23.3761983	5.2621420
C	4.3565038	23.8528453	5.5630648
C	3.6195231	22.5372223	5.5321331
C	2.2726746	22.4123127	5.1683185
C	1.5202462	21.2369510	5.1973609
C	0.0692178	21.1332494	4.7323060
C	-0.3971256	19.9224059	5.6007721
C	0.9157703	19.1587350	5.7965785
C	0.9936449	17.8136605	6.1587020
C	2.2751441	17.1965431	6.4368837
C	2.5526868	15.6774193	6.5882739
C	4.0433725	15.6869730	7.1000923
C	4.5920337	17.0346186	6.5983123
C	5.1597138	17.8743935	8.9284847
N	5.5056638	19.2144830	6.8660307
N	4.4409143	21.5264654	5.8823521
N	1.9663317	20.0409499	5.6446903
N	3.3960988	17.8809932	6.4819735
C	7.7090877	16.5481598	8.3265546
C	7.4520538	16.9807709	5.8577478
C	8.9122292	16.7308179	5.5064546
O	9.8980559	17.2723208	5.9826614
C	8.2691568	19.5438804	8.7177292
C	9.6480284	19.0639422	9.1949825
C	10.8193452	19.6549928	8.4297642
O	10.8027021	20.6840842	7.7755230
C	8.2836534	21.4896315	5.8158561
C	6.8749833	24.3354783	5.8430519
C	6.0033655	23.2316771	3.7097776
C	6.0233297	24.5635210	2.9826502
O	5.0860952	25.3391202	2.8822548
C	4.1911149	24.5401631	6.9435245
C	2.7493318	24.9198690	7.2898802
C	2.6664233	25.8189731	8.5122168

O	3.5927181	26.4280141	9.0162730
C	0.0707749	20.7075380	3.2386312
C	-0.7381402	22.4306739	4.8778535
C	-0.9725574	20.2362120	7.0109227
C	-2.4222051	20.7775528	7.0889320
C	-3.3945519	19.9812343	6.2416616
O	-3.7091793	20.2441364	5.0920627
C	-0.2841512	16.9979157	6.2399483
C	2.4236011	15.0348114	5.1847378
C	1.6320403	14.9292604	7.5869809
C	1.5658108	15.5177056	9.0047504
C	0.3040876	15.1107391	9.7456889
O	-0.7879313	14.9213468	9.2342964
C	4.8942878	14.4653078	6.7216010
C	4.5678328	13.2251397	7.5362001
O	4.3463680	13.2131691	8.7351928
C	4.3712771	19.2464227	4.4140854
N	4.7937717	18.8959723	3.3683269
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H	4.0024050	24.5407363	4.7686433
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H	-1.1340499	19.3226213	5.0312895
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H	5.2911455	16.8868794	9.4097253
H	4.0956574	18.1667464	8.9942793
H	5.7239091	18.6223465	9.5134632
H	7.1445196	15.5940921	8.2980681
H	7.6322040	16.9323311	9.3600433
H	8.7726281	16.3108434	8.1306112
H	6.9276858	16.0217123	5.6866887
H	7.0500165	17.6699727	5.0789341
H	7.5211614	19.3226054	9.5033236
H	8.3035911	20.6500502	8.6452621
H	9.7918812	19.3691614	10.2556031
H	9.7542574	17.9642141	9.1867202
H	8.9529839	21.5681119	6.6960813
H	8.3606967	22.4275483	5.2483581
H	8.7246033	20.6985722	5.1727591
H	6.5646464	25.3829295	5.6545132
H	7.8667022	24.2138430	5.3717509
H	6.9962778	24.2116784	6.9356100
H	6.9428036	22.6893728	3.4967690
H	5.1670011	22.6297587	3.2982225
H	4.8015686	25.4631636	6.9688135
H	4.5899954	23.8782517	7.7412082
H	2.2712095	25.4748480	6.4508283
H	2.1103318	24.0314116	7.4659149

H	-0.9712306	20.5563403	2.8896402
H	0.6350571	19.7662520	3.0856418
H	0.5399950	21.4890072	2.6083574
H	-1.8003785	22.2443677	4.6244328
H	-0.3634363	23.2010985	4.1736763
H	-0.6797565	22.8555740	5.8990345
H	-0.2912083	20.9337719	7.5417290
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H	-2.7463768	20.7392847	8.1478697
H	-2.4841917	21.8253402	6.7476415
H	-0.5185610	16.6426589	7.2638161
H	-0.2427761	16.0971021	5.5947730
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H	1.3976940	15.1559970	4.7859535
H	0.6064087	14.8635439	7.1836069
H	1.9874048	13.8800791	7.6530241
H	2.4439061	15.2424886	9.6193280
H	1.5462476	16.6315631	8.9641335
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H	5.9568667	14.6826148	6.9627849
O	1.3908061	25.8830821	8.9775158
O	-3.8575024	18.8876547	6.9046751
O	0.5236584	15.0170173	11.0796962
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O	11.9360724	18.9079381	8.6056902
O	7.2470987	24.8152597	2.4464578
C	7.3675579	26.0585408	1.7356711
H	7.1548983	26.9170564	2.4034412
H	8.4090968	26.1007443	1.3718209
H	6.6583144	26.0954512	0.8849917
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C	13.1179406	19.3953542	7.9511482
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H	13.3668978	20.4199640	8.2925145
H	13.9258955	18.6921880	8.2195416
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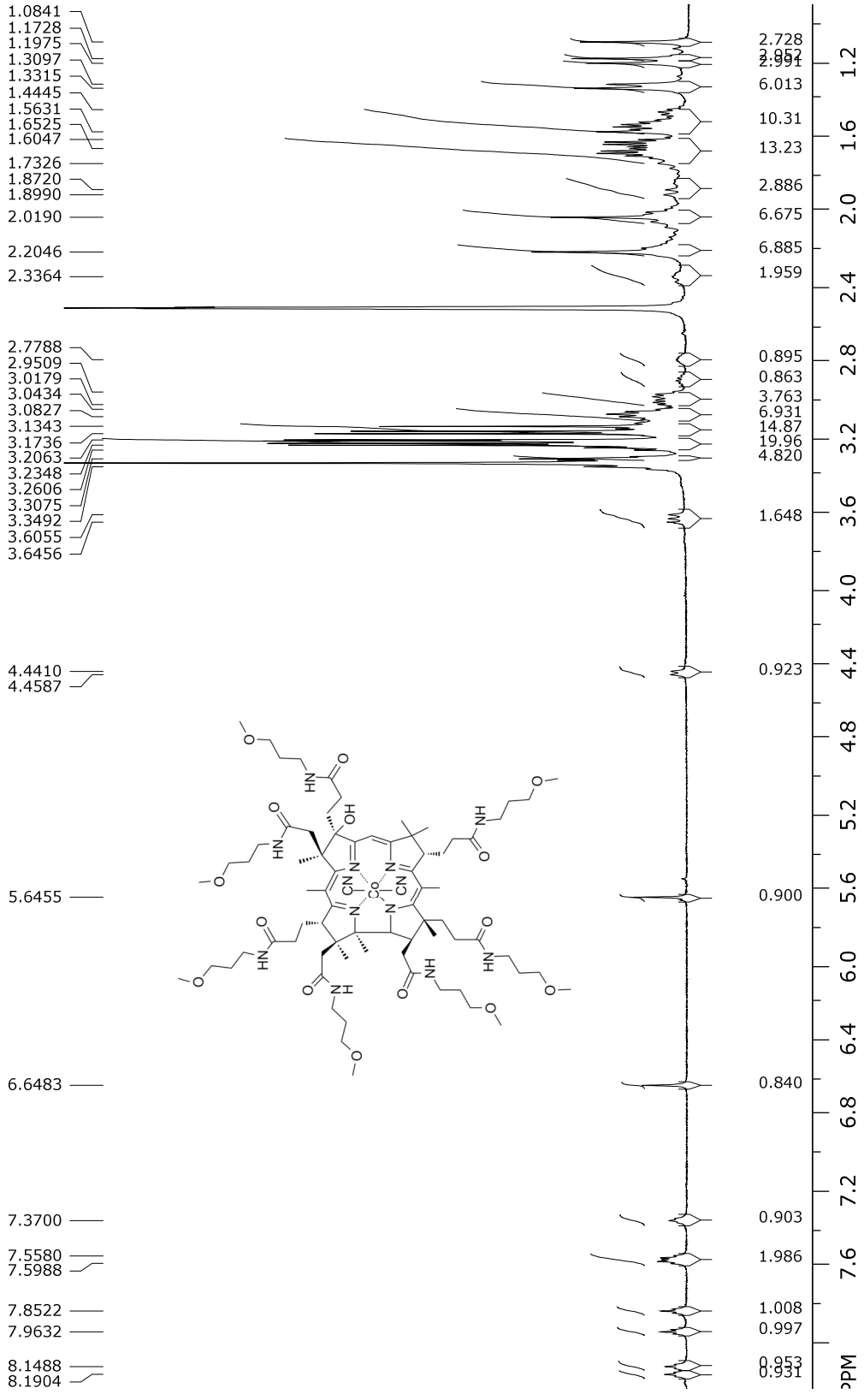
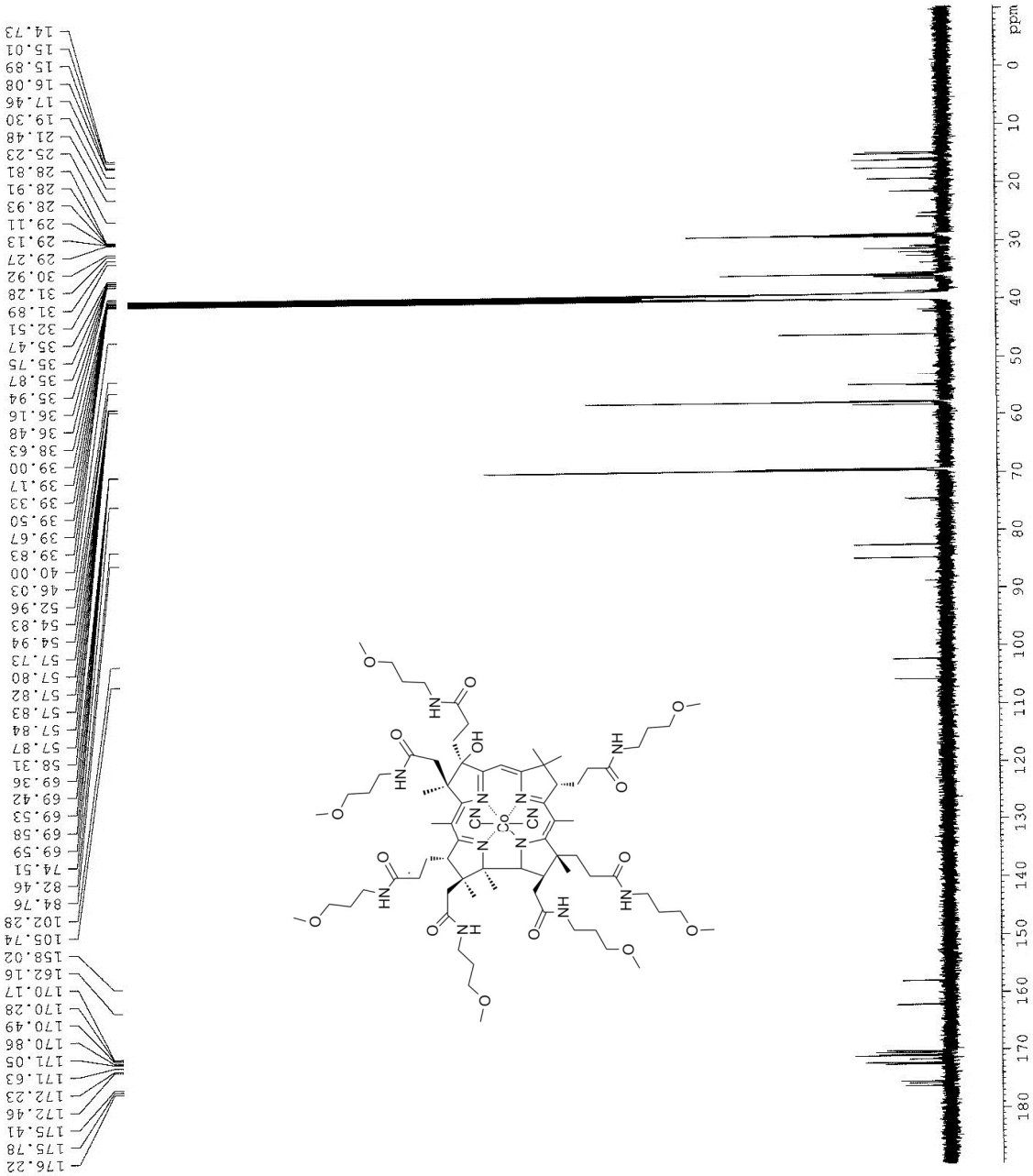
H	3.3670361	10.8618684	7.9419144
H	4.4361207	10.0741425	6.7023080
H	5.1306999	10.7150911	8.2547285
C	-0.6292296	14.7117056	11.8801123
H	-0.2756456	14.6887950	12.9258129
H	-1.4132848	15.4847151	11.7534020

H	-1.0581867	13.7302591	11.5952456
C	-4.7597932	18.0467425	6.1666887
H	-4.2818859	17.6703641	5.2405281
H	-5.0120017	17.2081327	6.8390348
H	-5.6743112	18.6054657	5.8840520
C	1.1917839	26.7318182	10.1175709
H	0.1161125	26.6699098	10.3601891
H	1.8003470	26.3870319	10.9773185
H	1.4761773	27.7786617	9.8881299
C	3.1892441	20.2505405	7.9574977
N	2.7449858	20.6275942	8.9844309

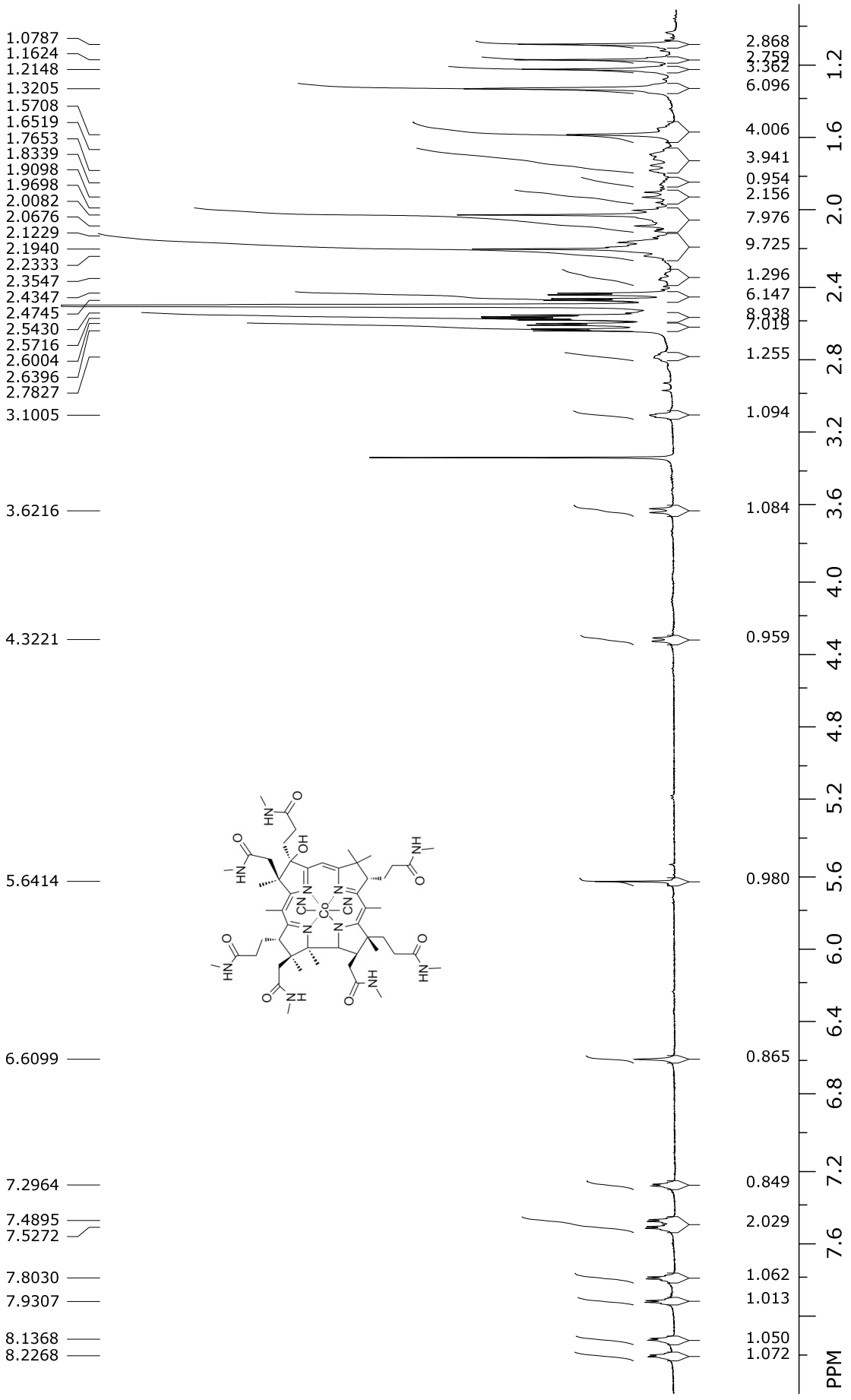
One imaginary frequency is found (-10.21 cm^{-1})



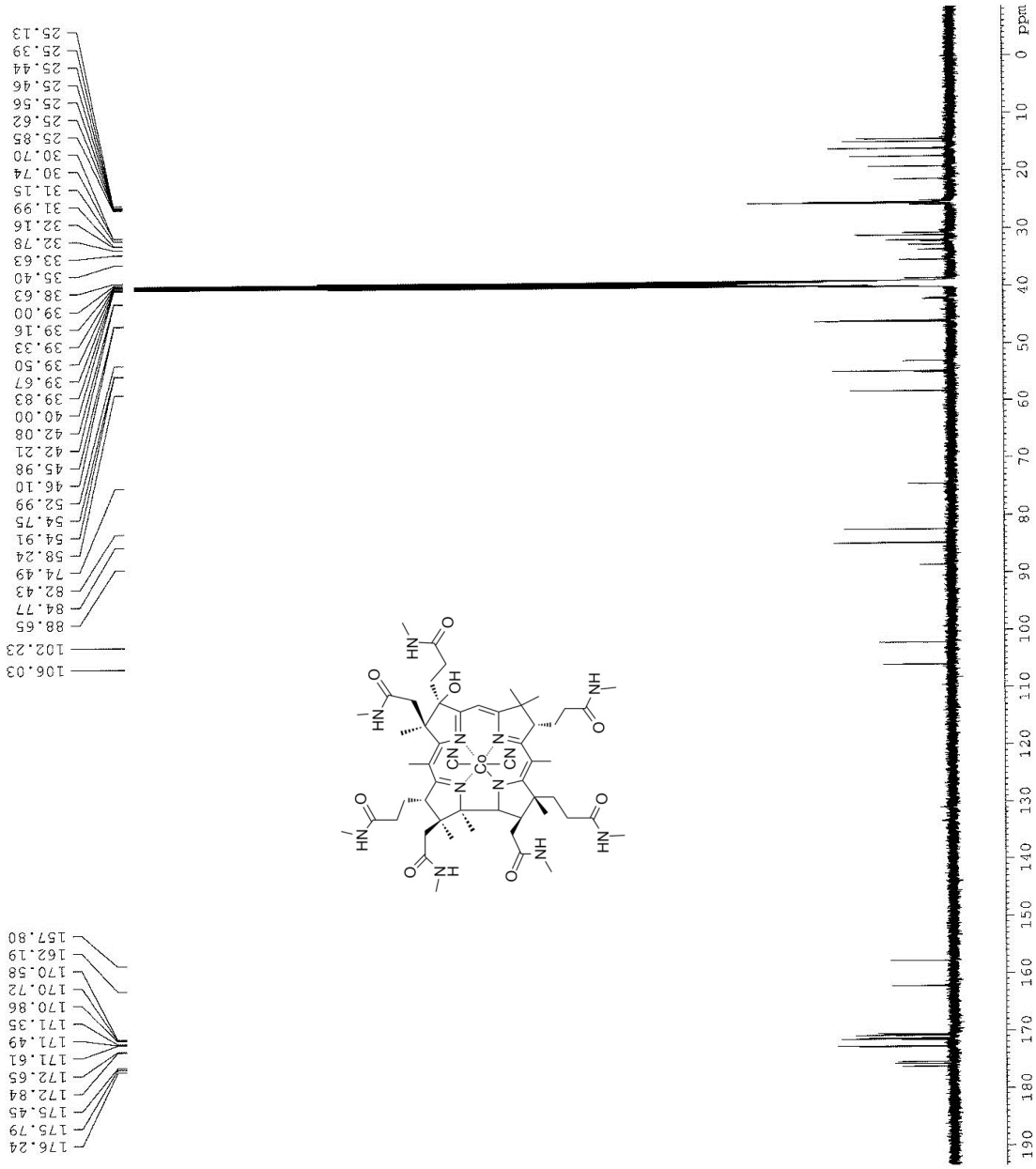
$(CN)_2$Cby(III)(3-methoxypropylamide)$_7$(8-OH) **8**

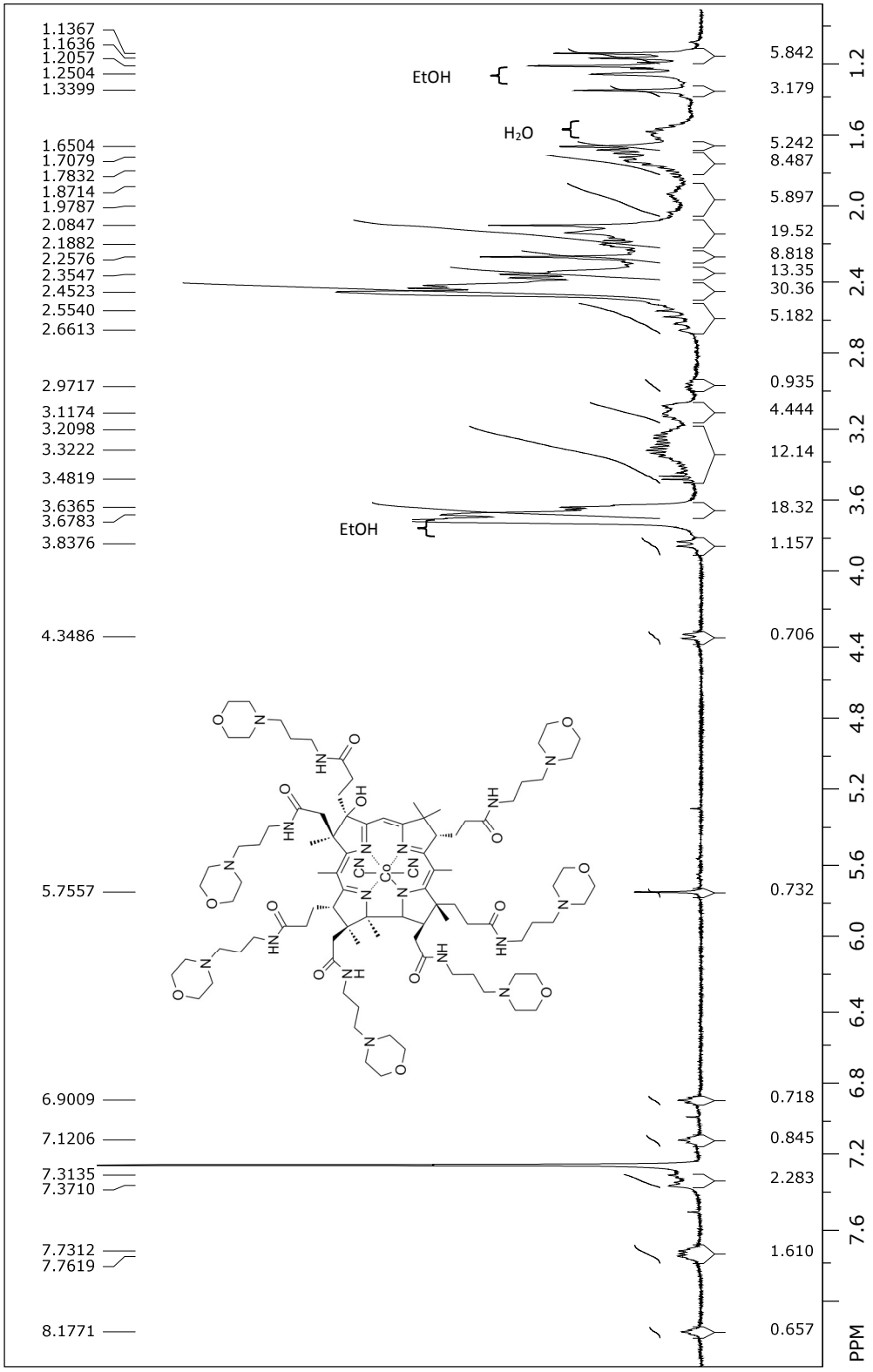


$(CN)_2$Cby(III)(methylamide)$_7$(8-OH) **9**

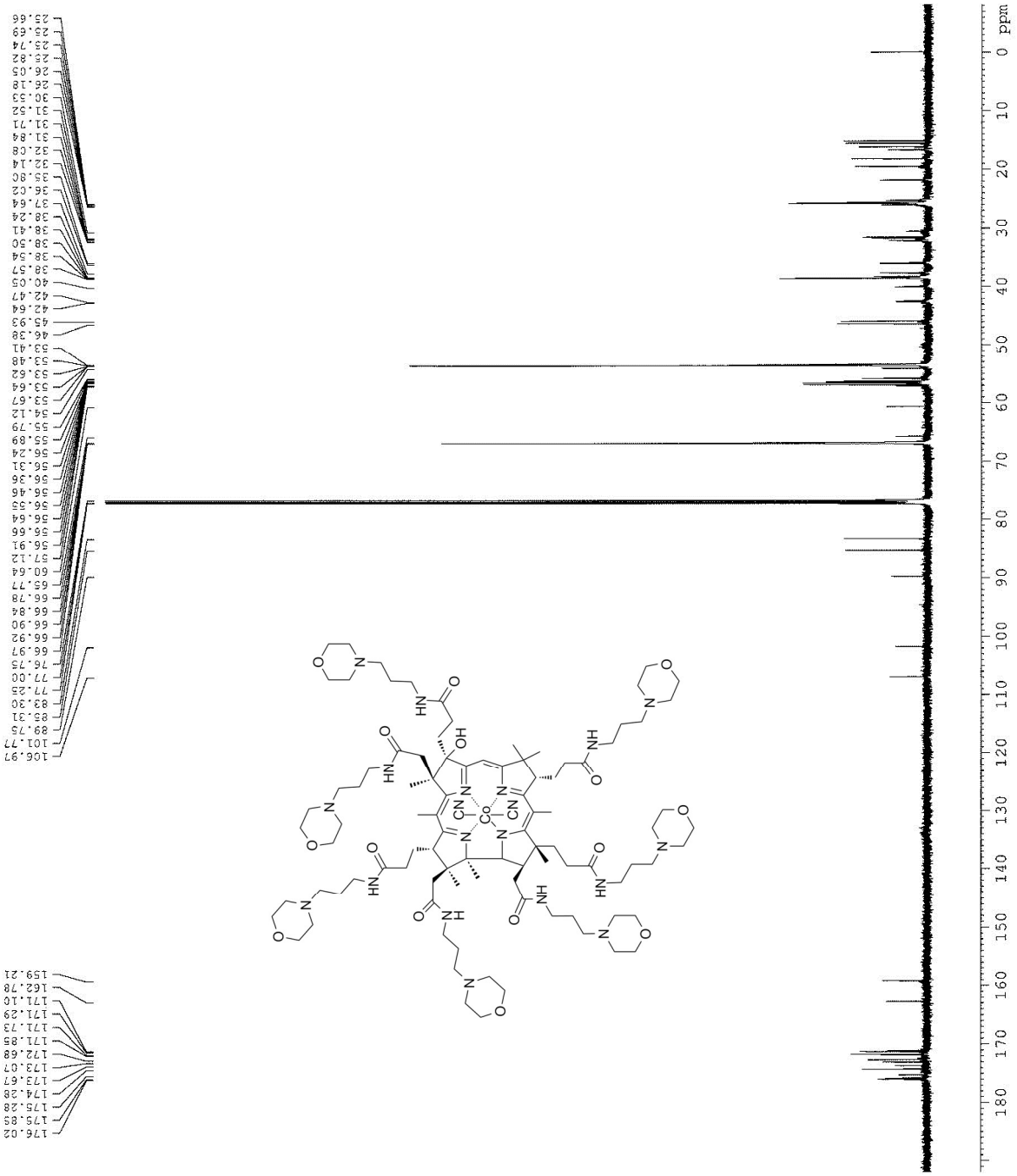


$(CN)_2$Cby(III)(methylamide)$_7$(8-OH) **9**

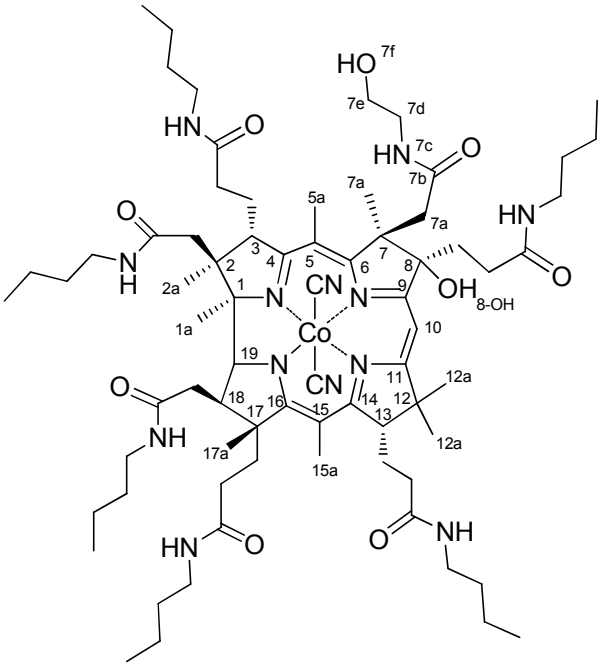




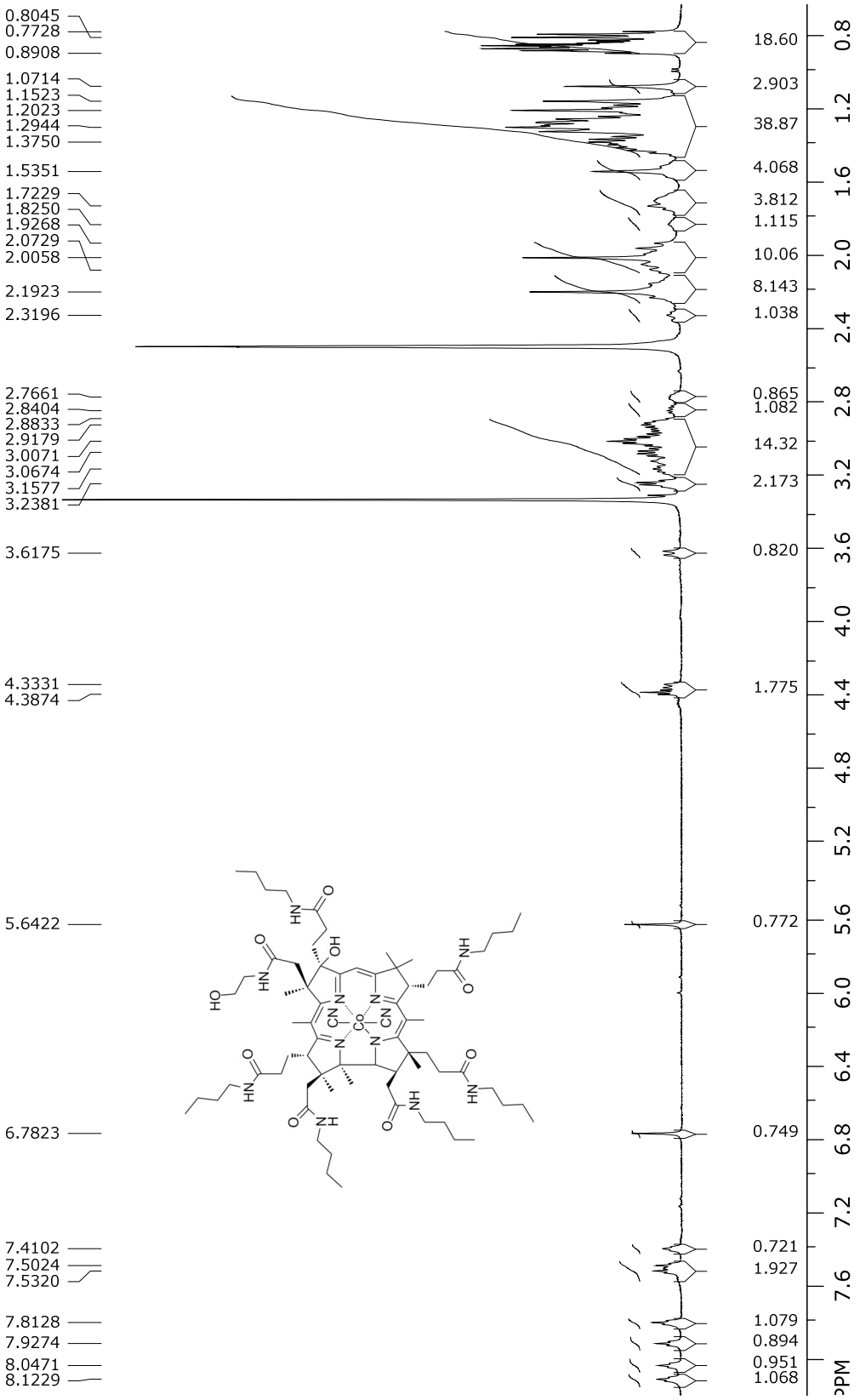
$(CN)_2$Cby(III)(3-morpholinepropylamide)$_7$(8-OH) **11**



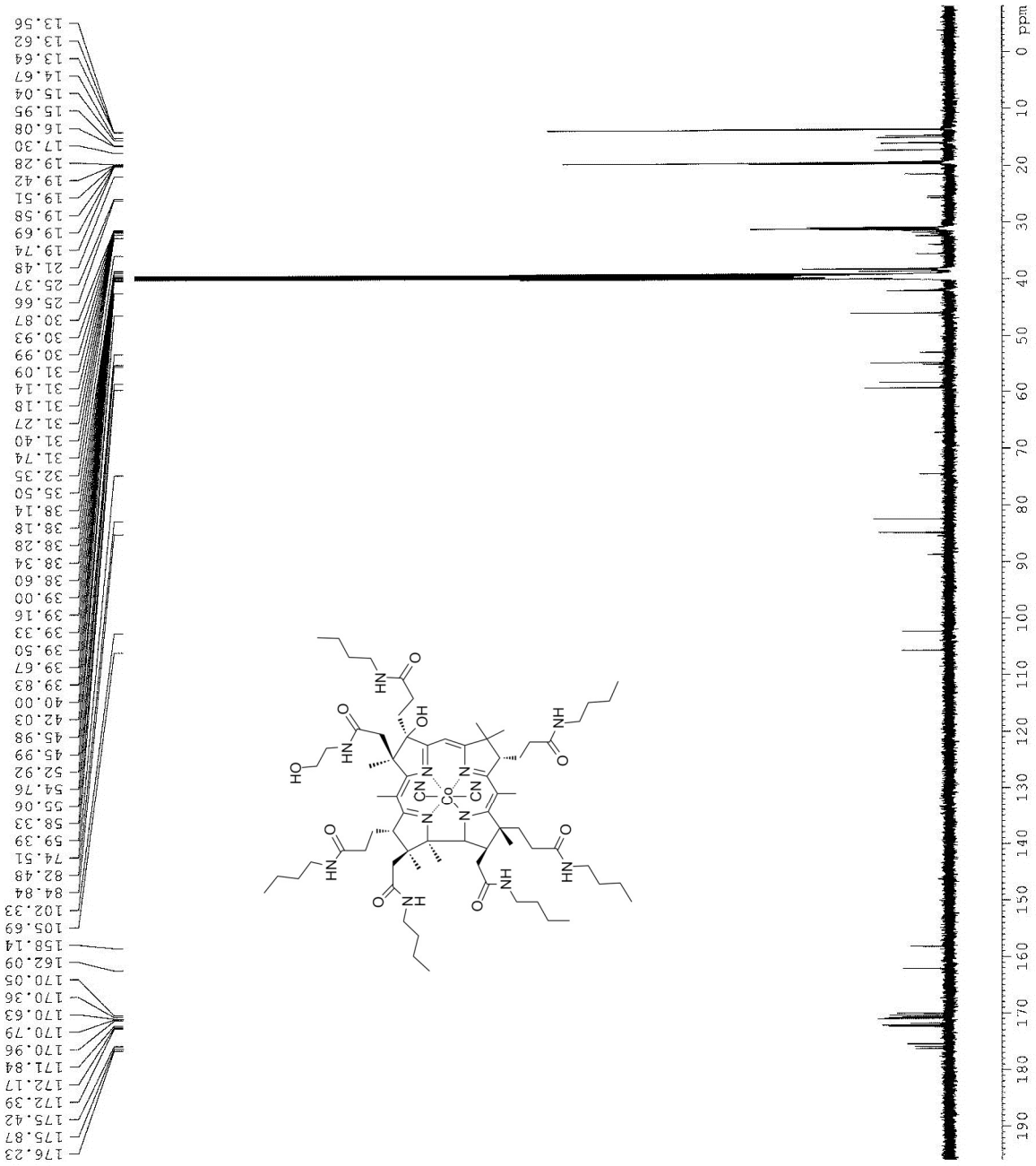
$(CN)_2$Cby(III)(*n*-butylamide)$_6$(*c*-(2-hydroxyethylamide))(8-OH) 14



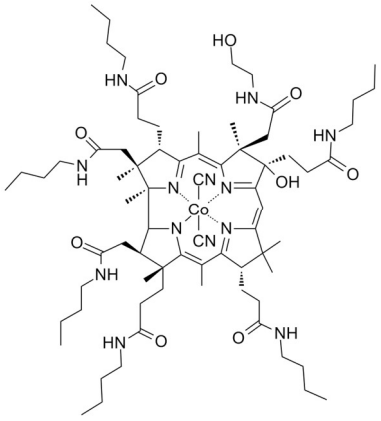
$(CN)_2$Cby(III)(*n*-butylamide)$_6$(*c*-(2-hydroxyethylamide))(8-OH) 14

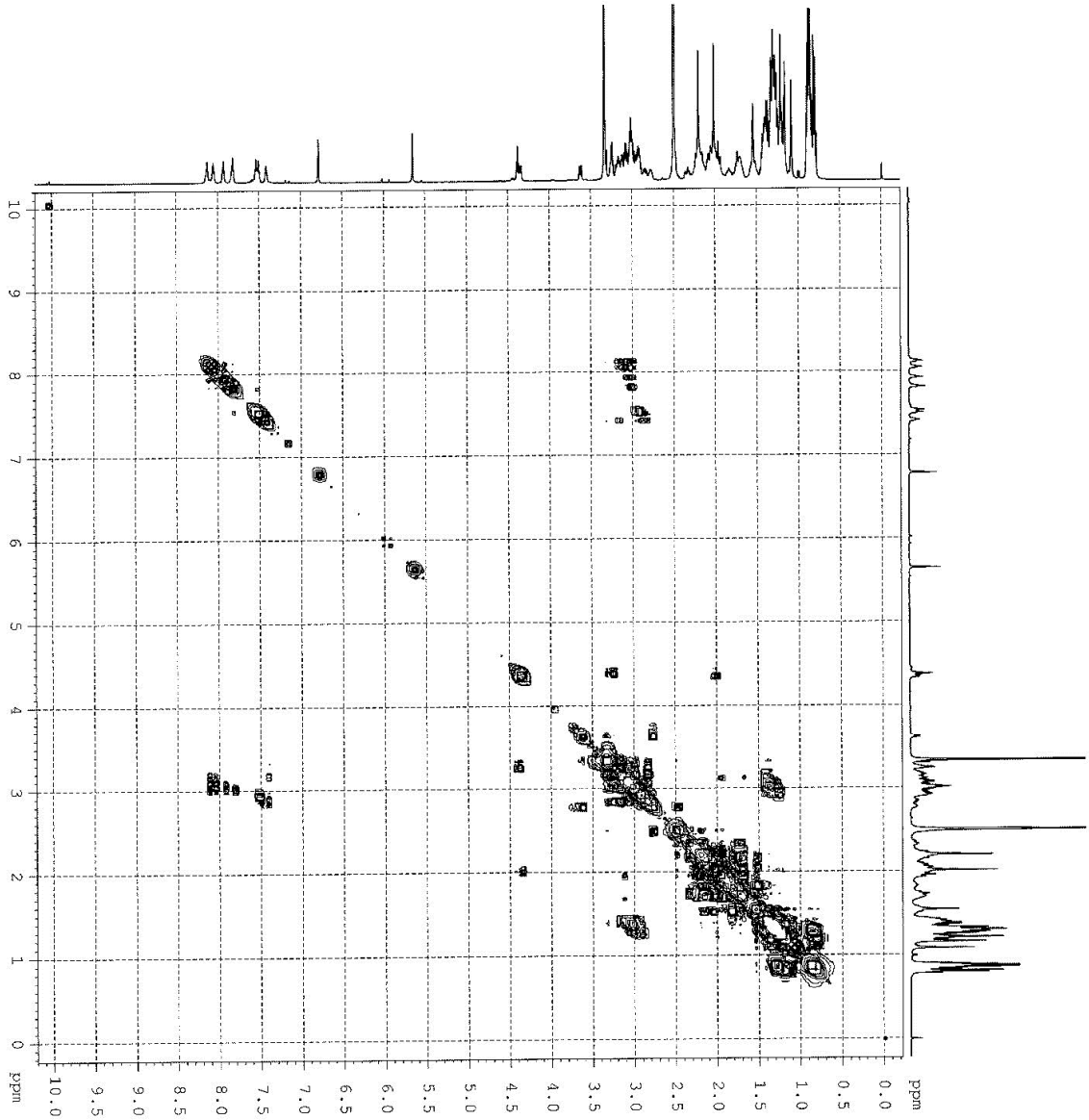


$(CN)_2$Cby(III)(*n*-butylamide)$_6$(*c*-(2-hydroxyethylamide))(8-OH) 14



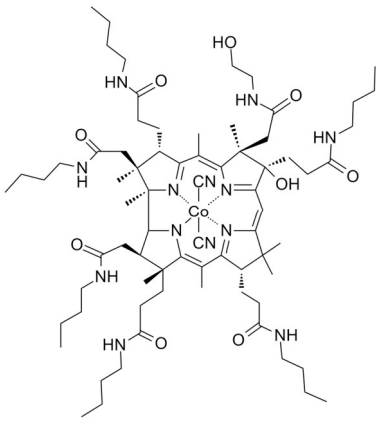
2D NMR COSY

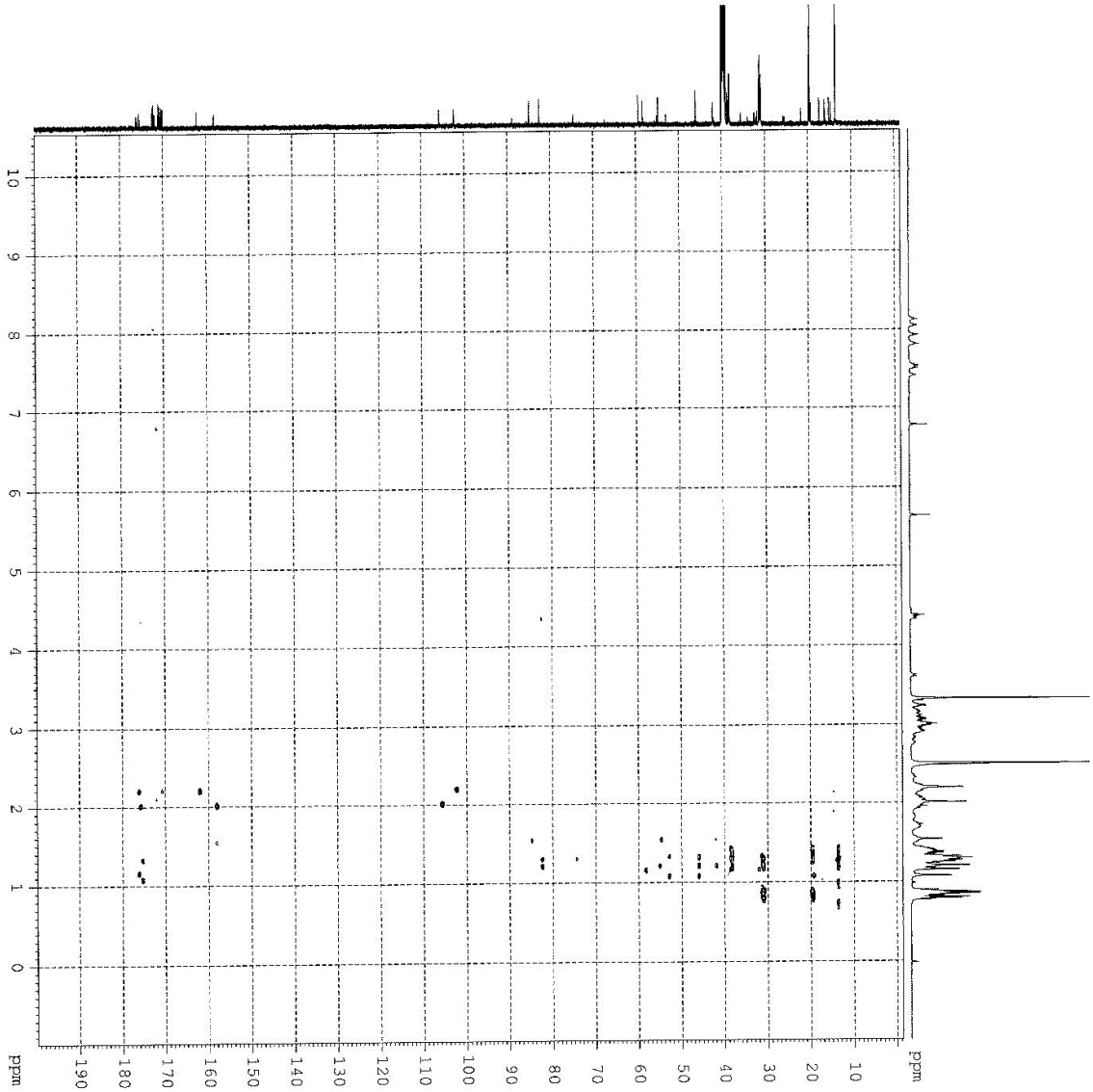




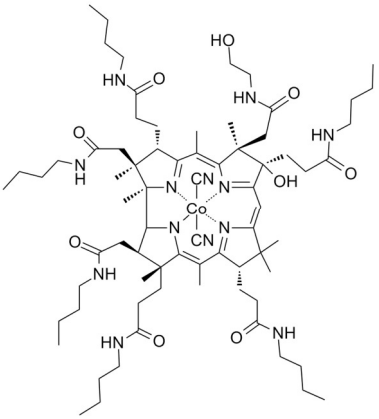
$(CN)_2$Cby(III)(*n*-butylamide)$_6$(*c*-(2-hydroxyethylamide))(8-OH) 14

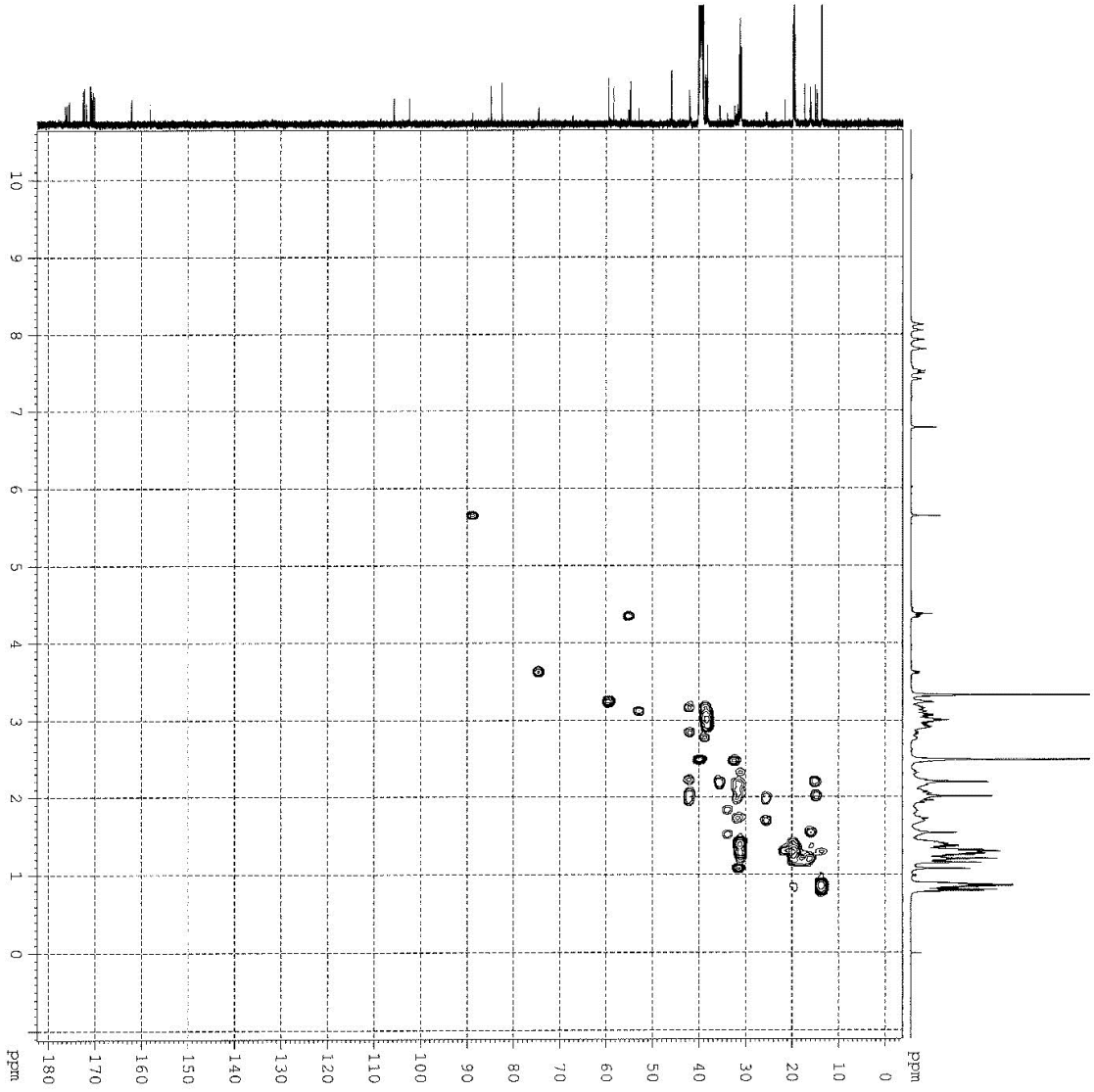
2D NMR HMBC

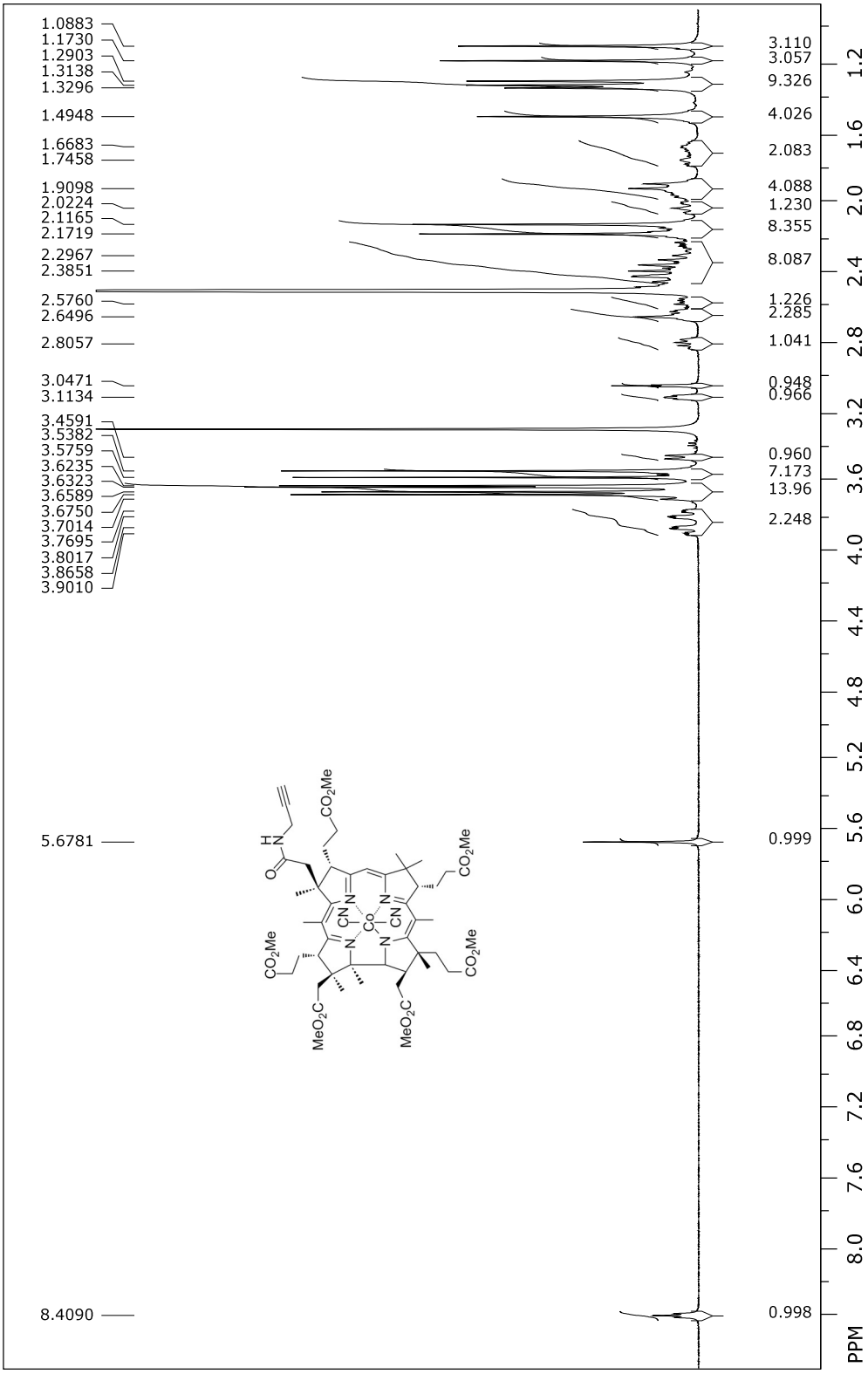




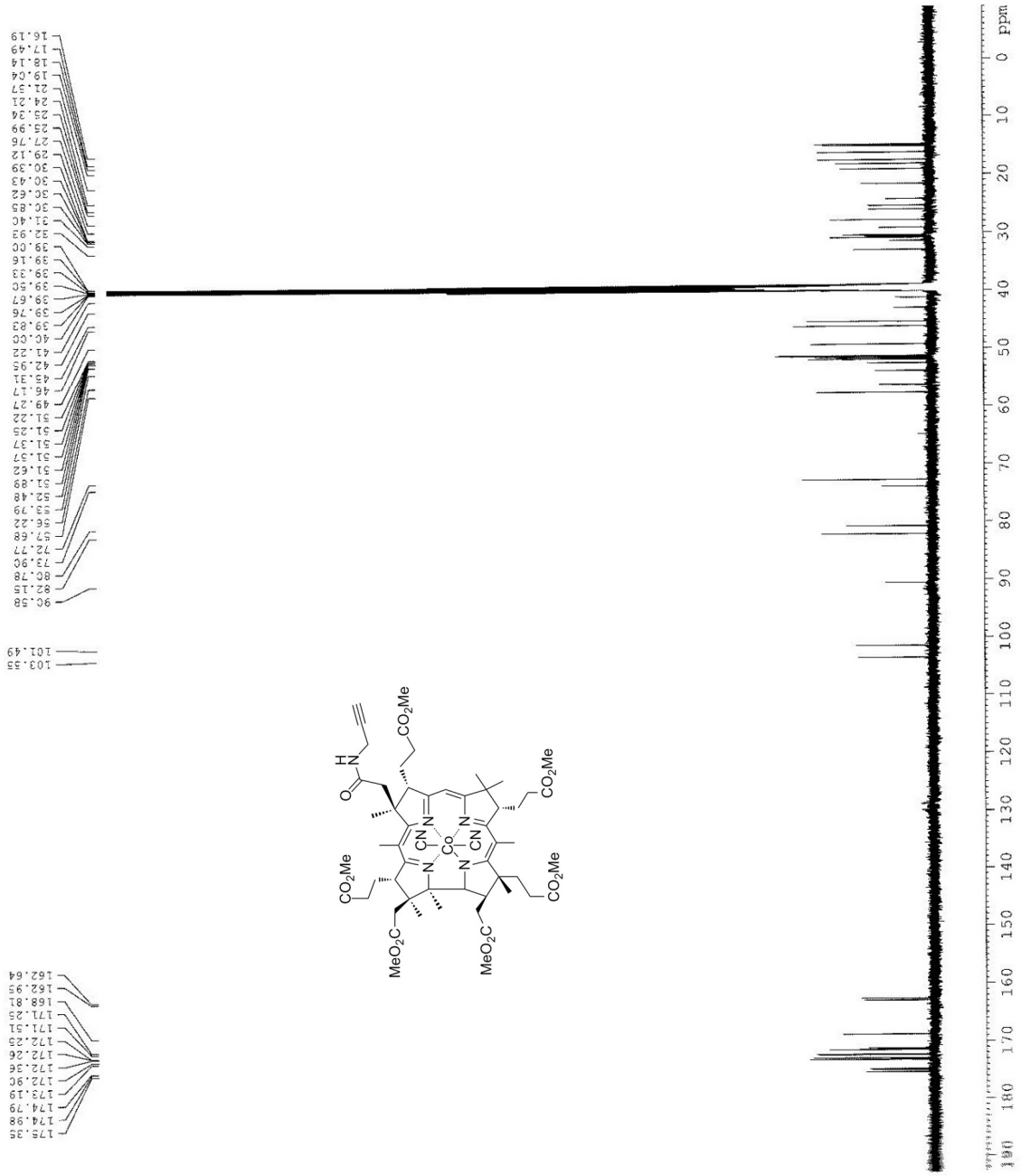
2D NMR HSQC

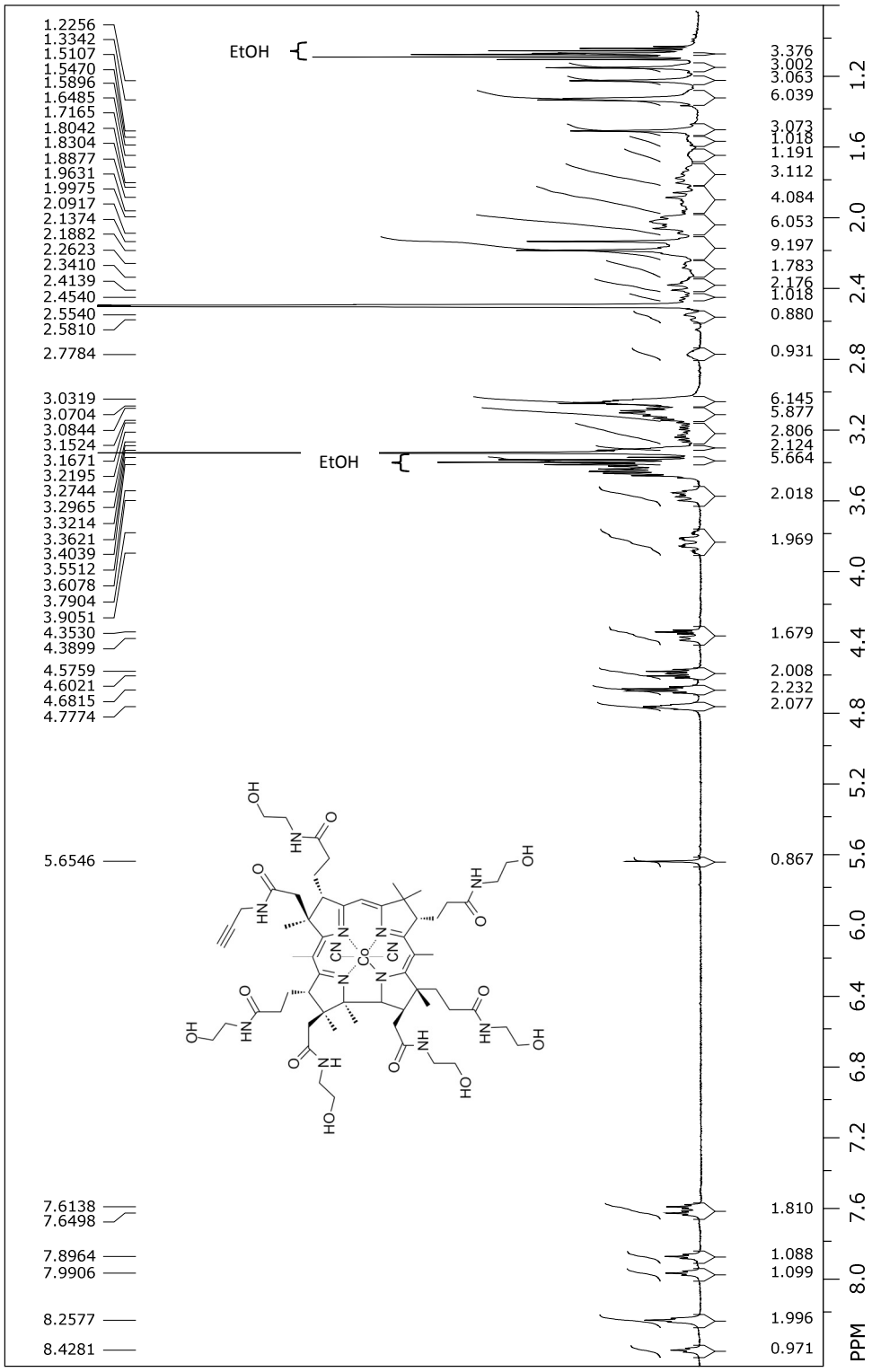




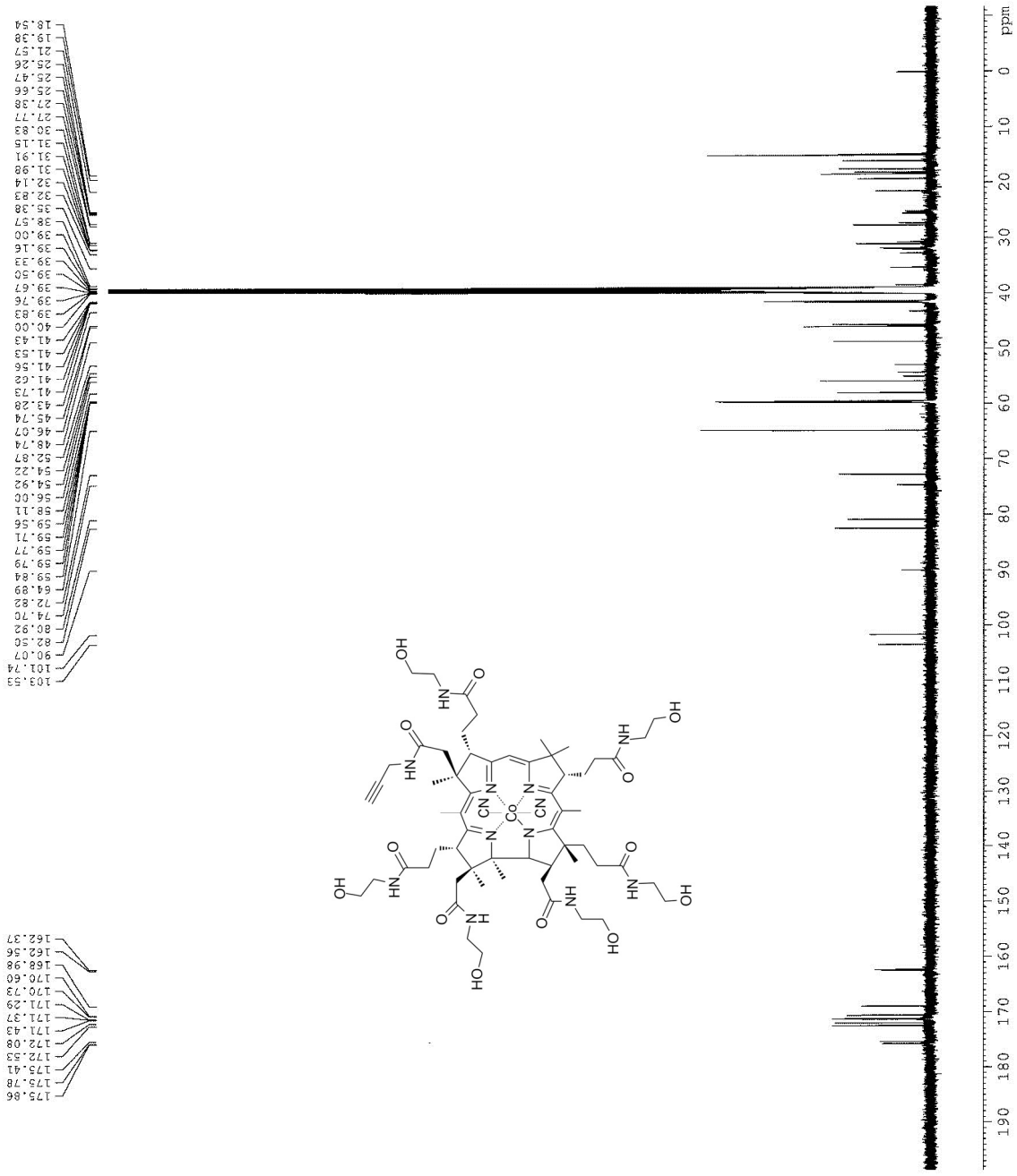


$(CN)_2$Cby(III)(*c*-propargylamide) **16**

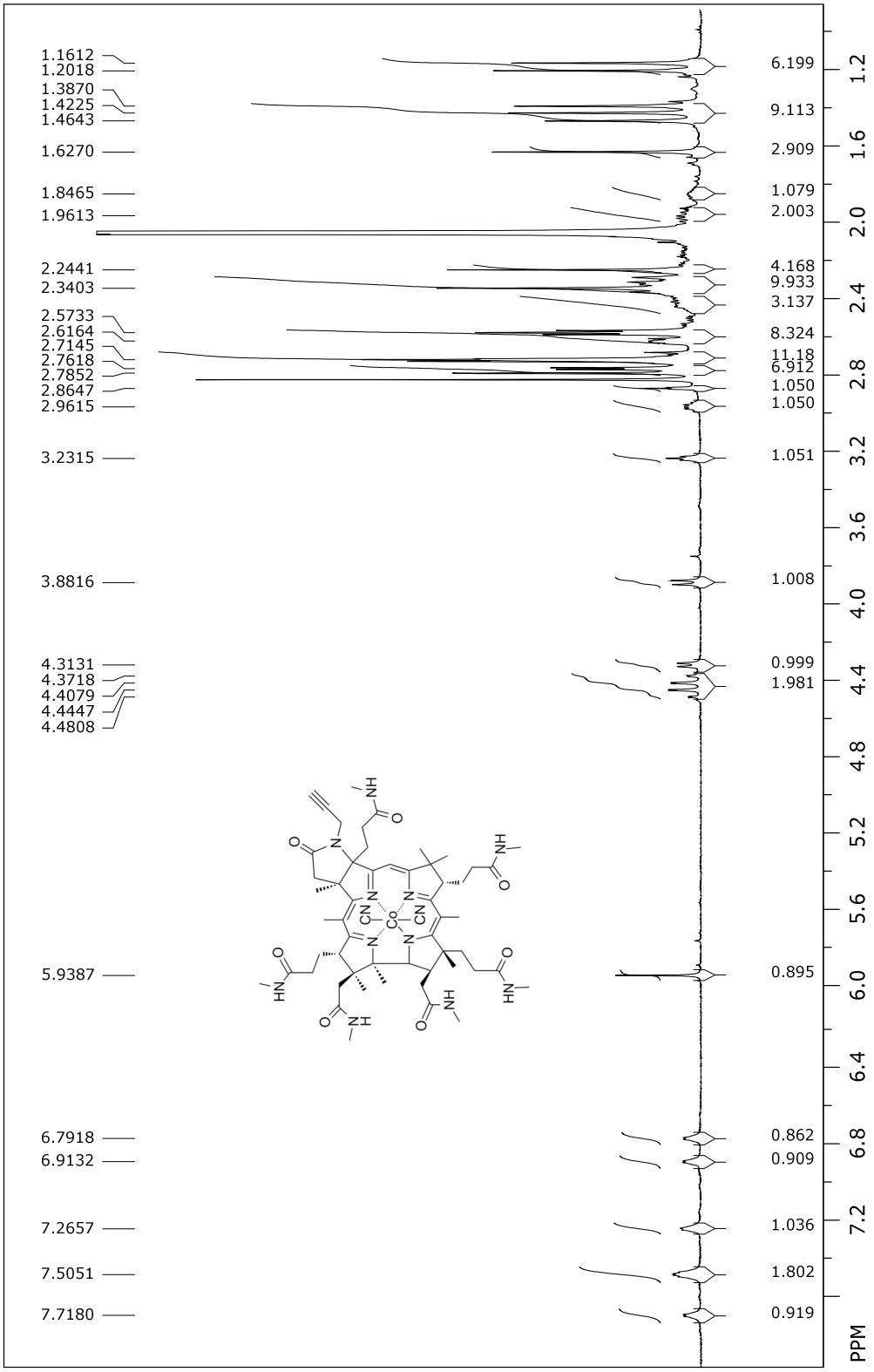




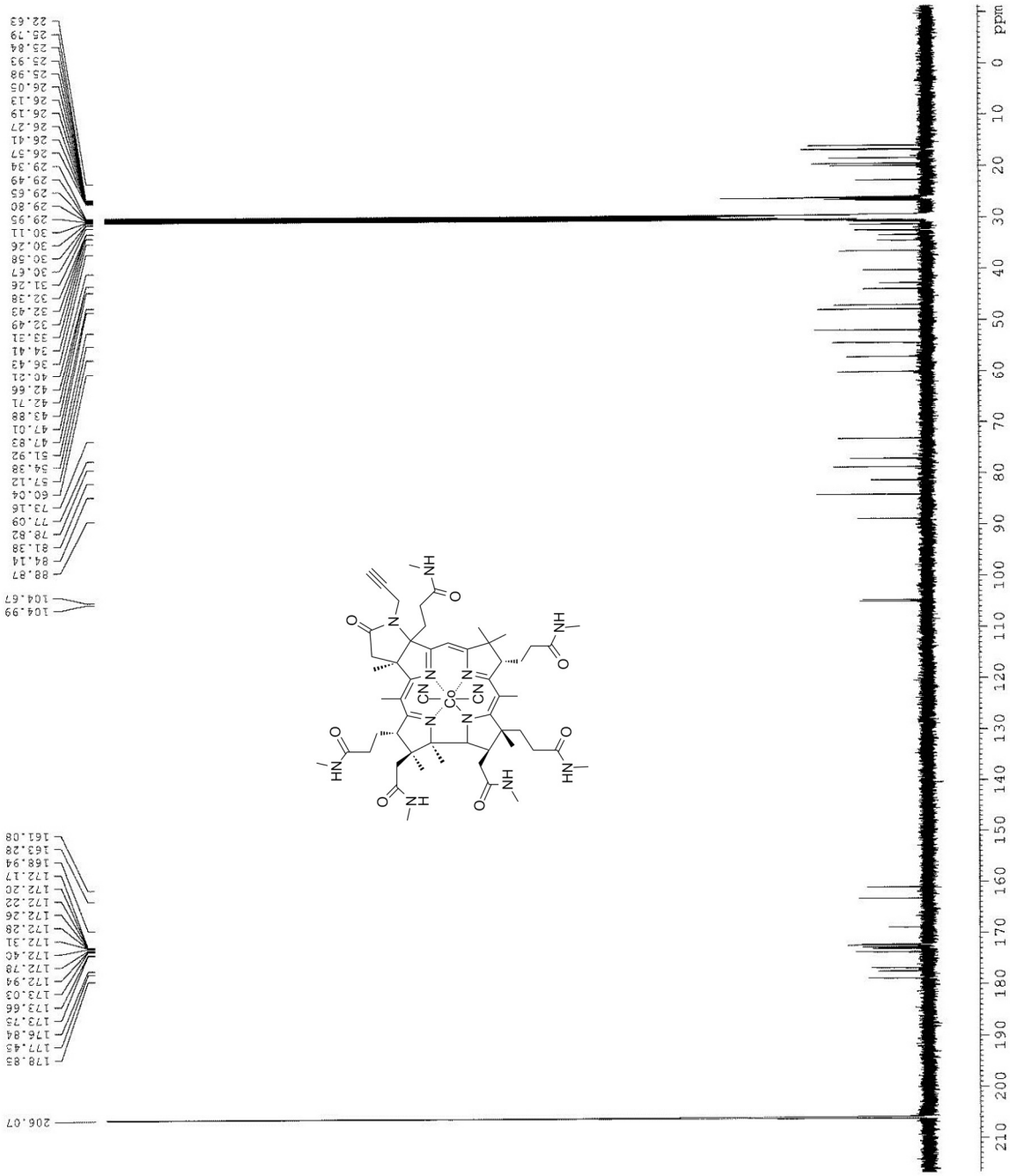
$(CN)_2$Cby(III)(2-hydroxyethylamide)$_6$(*c*-propargylamide) **17**



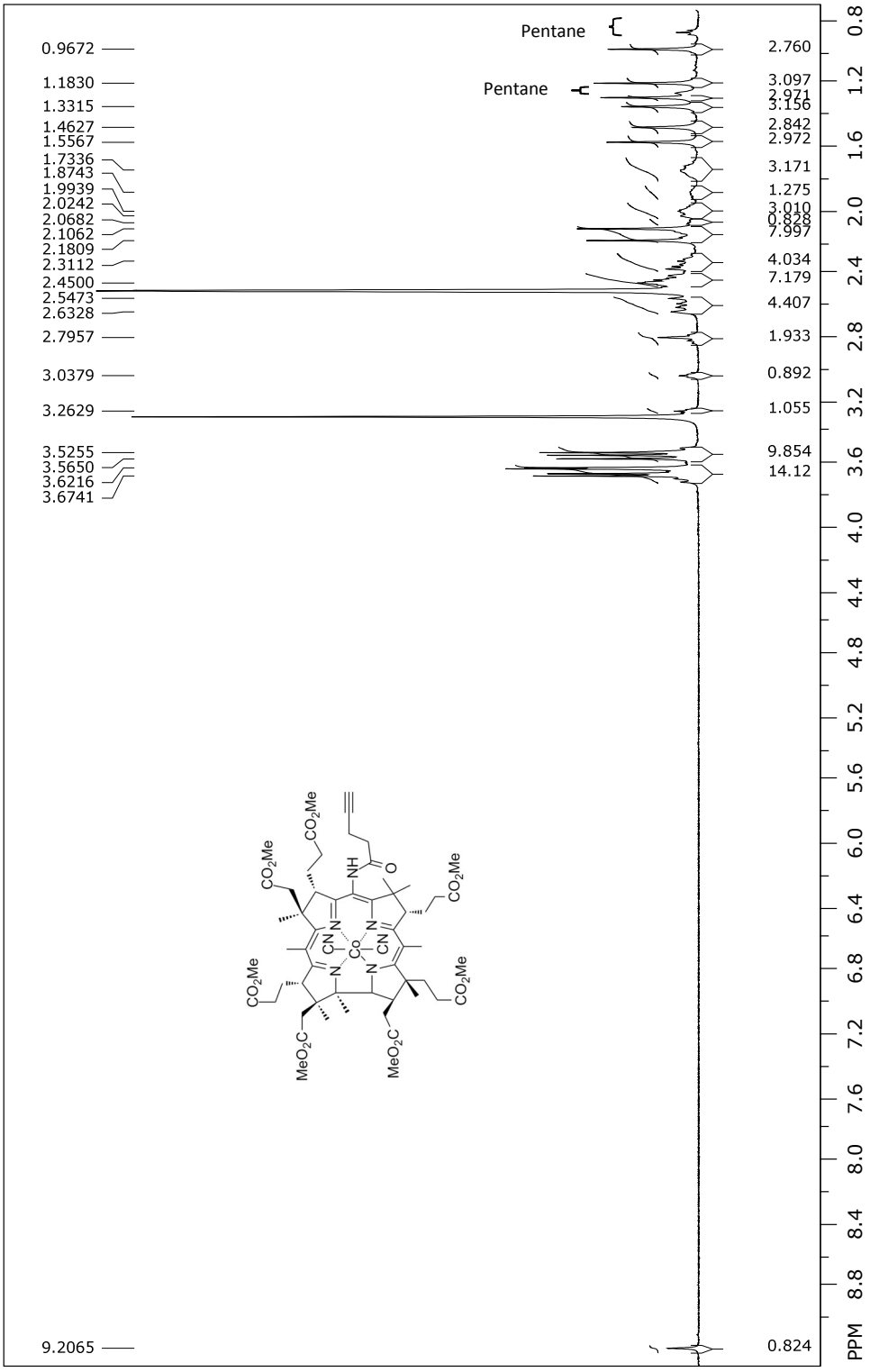
$(CN)_2$Cby(III)(methylamide)$_6$(*c*-(*N*-propargyl)lactam) **18**



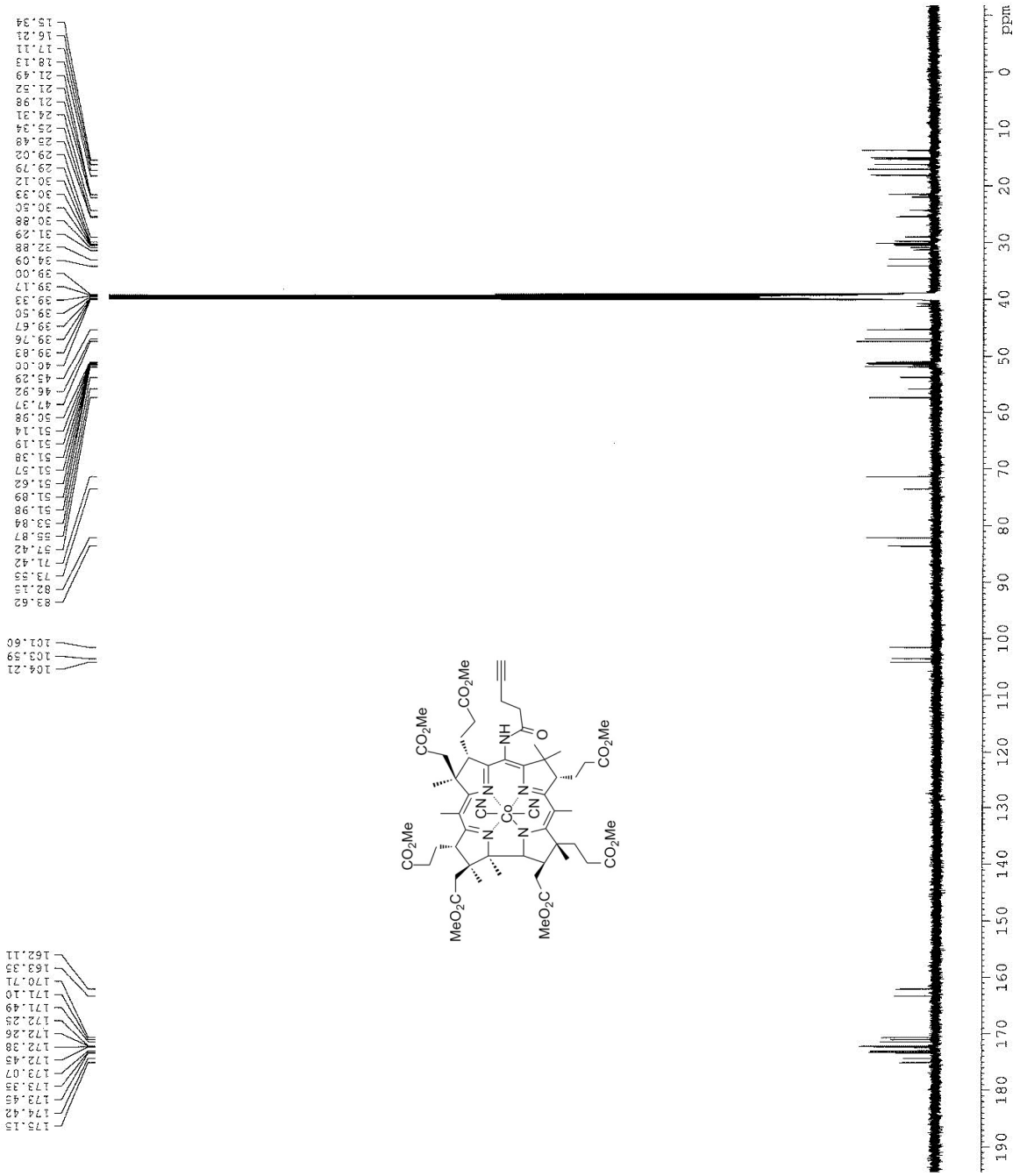
$(CN)_2$Cby(III)(methylamide)$_6$(*c*-(*N*-propargyl)lactam) **18**

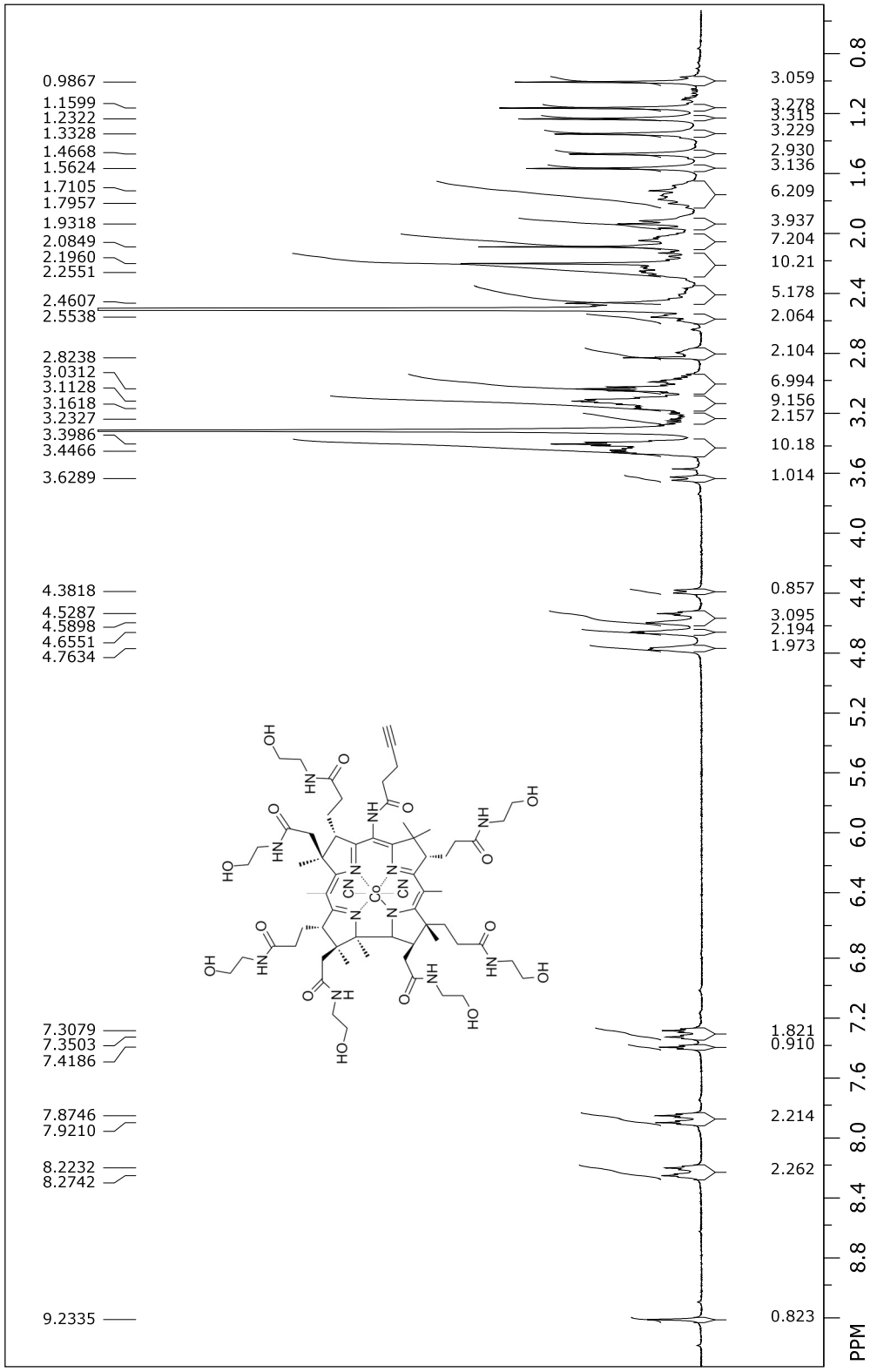


$(CN)_2$Cby(III)(10-NHCO-but-3-yn) **20**

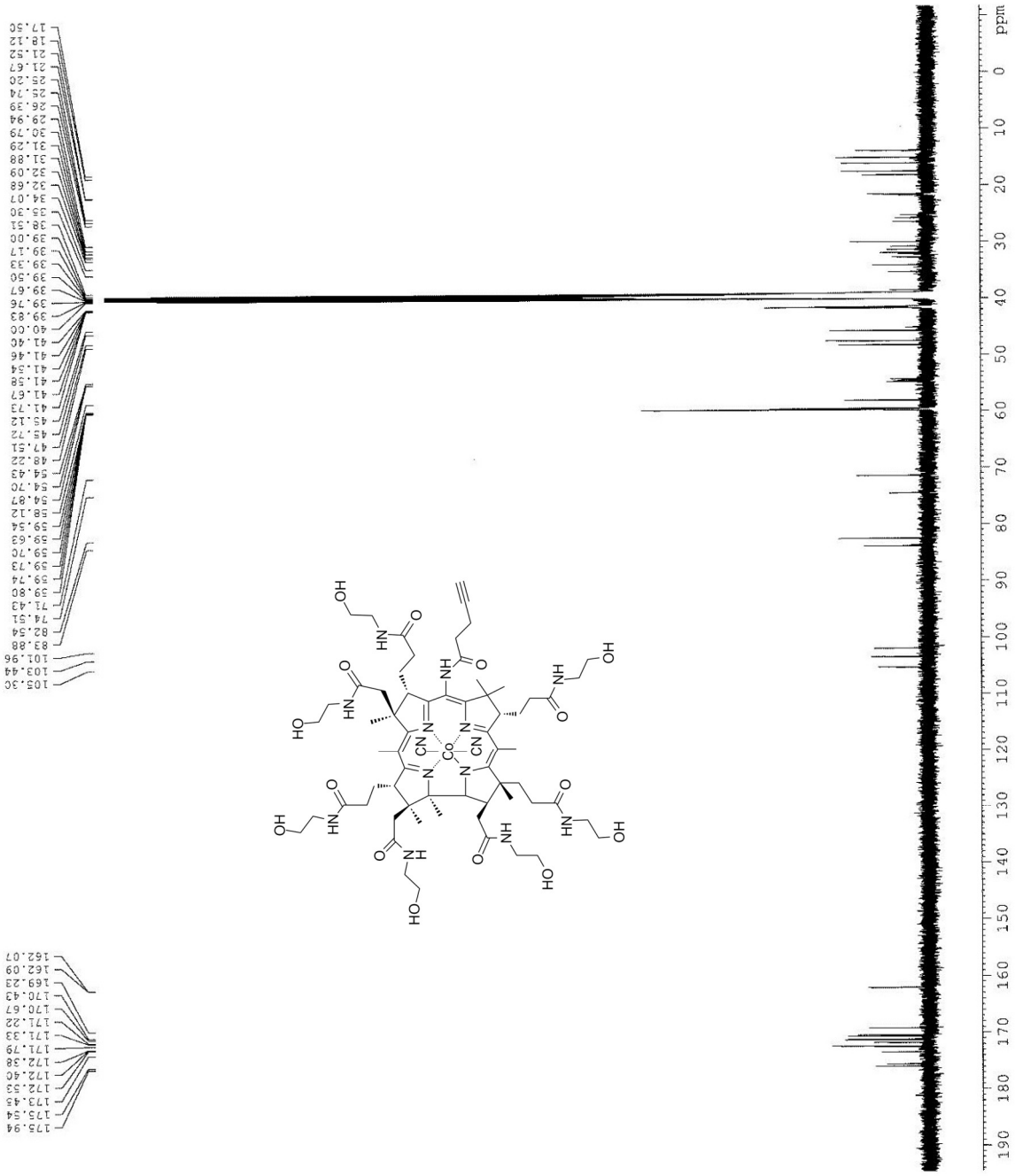


$(CN)_2$Cby(III)(10-NHCO-but-3-yn) **20**





$(CN)_2$Cby(III)(2-hydroxyethylamide)$_7$(10-NHCO-but-3-yn) 21



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An amphiphilic, catalytically active, vitamin B_{12} derivative†

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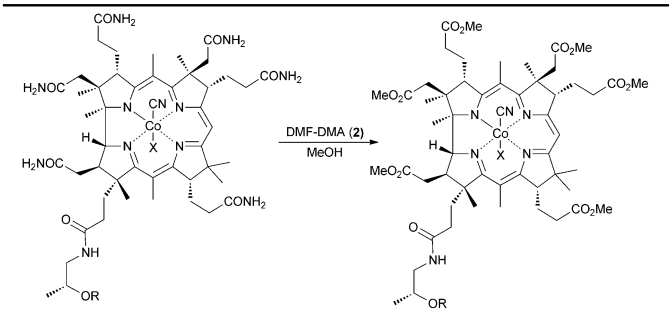
We performed the reaction of vitamin B_{12} with *N*,*N*-dimethylformamide dimethyl acetal for primary amide activation, and added MeOH as a nucleophile, to afford cobalester, the first amphiphilic cobalamin derivative. The unique combination of redox properties and solubility represents an asset for its use as a catalyst in C–C bond forming reactions.

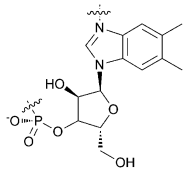
Among many biologically important metals, Co, in the form of vitamin B_{12} (cyano-cobalamin **1**), plays a unique role.[1,2] B_{12} co-enzymes are indispensable for catalyzing enzymatic rearrangements (coenzyme B_{12}, adenosylcobalamin) and methylation reactions (methylcobalamin).[1] The special ability of vitamin B_{12} (**1**) and other corrinoids to form a Co–C bond, combined with its facility in furnishing alkyl radicals *via* homolysis, has attracted the interest of many researchers, because corrinoids can be used as catalysts for C–C-bond forming reactions, which belong to the most challenging processes in organic synthesis.[3] These catalytic reactions typically involve alkyl-cobalt complexes, which are formed in reactions of Co(I) species and an electrophile or a Co(II) and a radical.[4] The most common chemical procedure utilizes the 'supernucleophilicity' of the Co(I) species (B_{12}s) toward alkylating agents, such as alkyl halides. Homolysis of the Co–C bond generates a carbon-centered radical, which can, *in situ*, disproportionate, abstract H•, or self-couple. In these reactions, B_{12} alkyl derivatives can be considered 'reversible carriers' of an alkyl radical.

To study the catalytic function of corrinoids in the hydrophobic microenvironment of enzyme active sites, Hisaeda and co-workers used cobyrinic acid derivatives as artificial enzymes. They were successfully used for dehalogenation, rearrangement, and ring expansion reactions.[4–7] These corrinoids, devoid of the nucleotide loop, possess chemical and physical properties different from those of the parent vitamin B_{12}. Importantly, in alkylcobalamins, the nucleotide enhances the ease of abstraction of the cobalt-alkyl group by an electrophile.[8]

We hypothesized that a hydrophobic derivative that retained the nucleotide loop would provide a better model for such haloenzymes and serve as better catalysts for organic reactions. This B_{12} derivative would require the conversion of the primary amide groups of cobalamin into esters. Typically, transformation of primary carboxamides into esters entails harsh reaction conditions. Brocchetta[9] and Myers,[10] however, showed that primary amides can be selectively activated and transformed into esters or secondary amides under mild conditions *via* the formation of formamidine, which subsequently reacts with nucleophiles (Table 1). Because the secondary amides remained intact, we reasoned that this methodology might be promising for selective modification of the primary amides in vitamin B_{12} leaving the nucleotide unaffected.

Table 1 Synthesis of cobalester (**3**) and cobinester (**5**)



Entry	R	X	Substrate	Product
1		—	**1**	**3**
2	H	CN	**4**	**5**

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[b] *Department of Engineering Science, Aarhus University, Denmark*

† Electronic supplementary information (ESI) available: Detailed synthetic procedures, full characterisation of new compounds, biological tests and stability tests. CCDC 981457. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c4cc01064g

Electronic Supplementary Information

for

Amphiphilic, catalytically active, vitamin B_{12} derivative

Maciej Giedyk, Sergey Fedosov, Dorota Gryko*

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General Information

All solvents and chemicals used in the syntheses were of reagent grade and were used without further purification. Tested compounds were greater than 95% chemical purity as measured by elemental analysis. A microvave-assisted synthesis was performed using microwave oven CEM Discover. UV-vis absorption spectra were measured on Jenway 7315 spectrometer and Perkin Elmer λ-25 at room temperature. High resolution ESI mass spectra were recorded on a Mariner and SYNAPT spectrometer. ^{1}H and ^{13}CNMR spectra were recorded at rt on Bruker 500 and Varian 500 MHz instruments with TMS as an internal standard. DCVC (dry column vacuum chromatography)[1] was performed using Merck Silica Gel (200-300 mesh) and Silica Gel 90 C18 (Fluka). Thin layer chromatography (TLC) was performed using Merck Silica Gel GF254, 0.20 mm thickness.

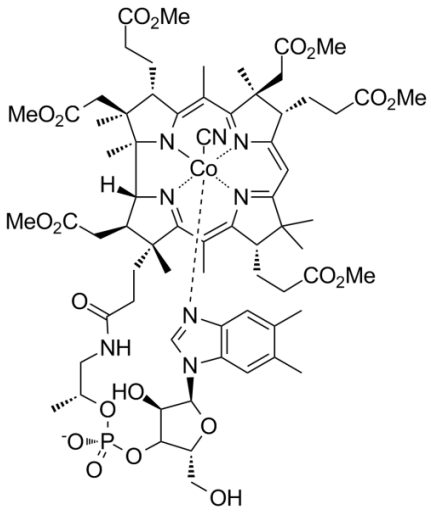
Partition *n*-octanol/water coefficient P_{OW} was determined using standard shake-flask method.[2] Concentrations were determined using UV-Vis spectroscopy.

HPLC Measurement conditions: Column: Eurospher II 100-5 C18 250 mmx4.6 mm (Knauer) with a precolumn; detection: UV-Vis, wavelength: λ=361 nm; flow rate: 1ml/min; pressure: 10 Mpa, Temperature: 30 °C. HPLC method:

Time [min]	H_2O [%]	MeCN [%]
initial	99	1
15	30	70
15	30	70

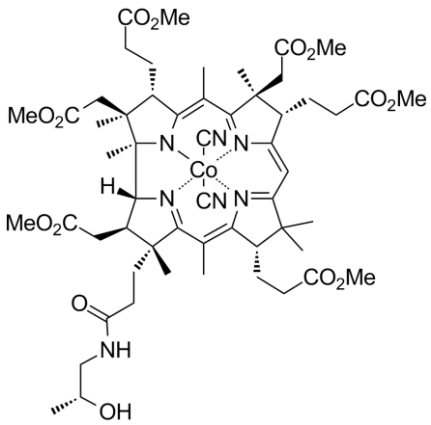
Diffractions experiments were performed on Bruker APEX-II CCD apparatus and the crystal structure was solved using SHELXL-97 software.

Cobalester (3) synthesis procedure



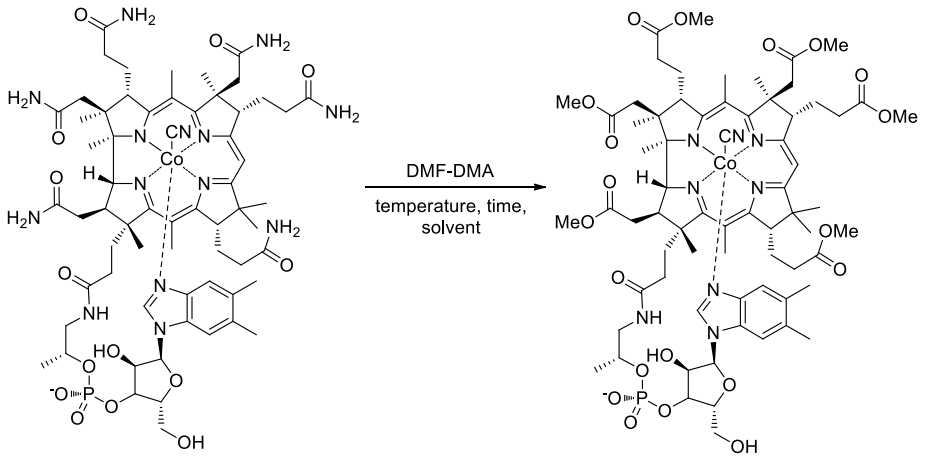
Cyanocobalamin (**1**) (15.0 mg, 0.011 mmol) was dissolved in a degased mixture of HFIP/MeOH (0.5 ml; 1:1), in a pressure tube equipped with a stirring bar. DMF-DMA (88 µl, 0.662 mmol) was added. The reaction mixture was vigorously stirred at 60 °C for 48 h in anaerobic conditions. It was then diluted with DCM (30 ml) and washed with brine (5 ml). The organic layer was concentrated in vacuo giving a red residue. It was dissolved in Et_2O (5 ml) and precipitated with hexane (20 ml. The crude product was purified using reverse phase column chromatography (gradually from 2.5 to 15% MeOH in water). Recrystalization from $CHCl_3$/hexane gave a red solid (11 mg; 72%). R_f 0.32, 20% MeOH in DCM/Toluene (1:1). logP_{OW} = 0.70. HRMS ESI (m/z) calcd for $C_{69}H_{95}CoN_8O_{20}P$ $[M+H]^+$ 1445.5732, found 1445.5707. UV/Vis (H_2O): λ_{max} (ε) = 278 (1.44x10^4), 361 (2.54x10^4), 549 (8.23x10^3). UV/Vis (DMSO): λ_{max} (ε) = 277 (1.89x10^4), 362 (2.66x10^4), 547 (9.42x10^3). UV/Vis (DCM): λ_{max} (ε) = 277 (1.51x10^4), 361 (2.52x10^4), 547 (8.65x10^3). UV/Vis (1-octanol): λ_{max} (ε) = 276 (1.48x10^4), 362 (2.58x10^4), 518 (7.52x10^3), 550 (8.63x10^3) Anal. calcd. for $C_{69}H_{94}CoN_8O_{20}P$ + $3H_2O$: C 54.54, H 6.96, N 7.17; found: C 54.17, H 7.35, N 7.17. ^{1}H NMR (500 MHz, [D4]MeOH): δ = 7.29 (s, 1H), 7.15 (s, 1H), 6.53 (s, 1H), 6.30 (d, J = 3.1 Hz, 1H), 6.01 (s, 1H), 4.77-4.71 (m, 1H), 4.37-4.30 (m, 1H), 4.18 (t, J = 3.3 Hz, 1H), 4.15-4.10 (m, 2H), 4.06-4.03 (m, 1H), 3.86-3.81 (m, 1H), 3.78-3.70 (m, 14H), 3.69 (s, 3H), 3.65 (s(br), 1H), 3.51-3.48 (m, 1H), 3.45 (s, 3H), 2.91-2.87 (m, 1H), 2.82-2.45 (m, 19H), 2.31-2.26 (m, 7H), 2.21-2.06 (m, 3H), 1.98-1.84 (m, 7H), 1.77-1.70 (m, 1H), 1.40 (s, 3H), 1.35 (s, 3H), 1.29-1.23 (m, 8H), 1.19-1.11 (m, 4H), 0.46 (s, 3H) ppm. ^{13}C NMR (125 MHz, [D4]MeOH): δ = 181.2, 180.1, 177.9, 175.1, 174.9, 174.4, 173.92, 173.90, 173.7, 173.1, 172.1, 167.12, 167.07, 143.5, 138.2, 135.6, 134.0, 131.5, 117.6, 112.6, 108.2, 105.5, 95.5, 88.1, 86.1, 83.6, 76.5, 70.7, 61.8, 60.5, 58.0, 56.1, 55.2, 52.9, 52.6, 52.4, 52.37, 52.3, 51.9, 51.7, 46.8, 43.1, 42.4, 40.6, 34.2, 33.8, 33.1, 32.7, 32.1, 31.7, 31.6, 28.7, 26.9, 26.5, 20.8, 20.6, 20.4, 20.3, 20.09, 20.06, 17.8, 17.0, 16.4, 16.1 ppm.

Cobinester (5) synthesis procedure



Cobinamide (**4**) (15.0 mg, 0.014 mmol) was dissolved in a degased mixture of HFIP/MeOH (0.5 ml; 1:1), in a pressure tube equipped with a stirring bar. DMF-DMA (88 µl, 0.662 mmol) was added. The reaction mixture was vigorously stirred at 60 °C for 48 h in anaerobic conditions. It was then diluted with DCM (30 ml) and washed with brine (5 ml). The organic layer was concentrated *in vacuo* giving a red residue. It was dissolved in Et_2O (5 ml) and precipitated with hexane (20 ml. The crude product was dissolved in DCM, washed with $NaCN_{aq}$ and purified using DCVC (gradually from 2.5 to 10% EtOH in DCM). Recrystalization from AcOEt/hexane gave a purple solid (9.1 mg; 56%). R_f 0.35, 5% EtOH in DCM. HRMS ESI (*m/z*) calcd for $C_{55}H_{78}CoN_6O_{14}$ $[M–CN]^+$ 1105.4908, found: 1105.4904; UV/Vis (DMSO): λ_{max} (ε) = 316 (1.13×10^4), 371 (3.07×10^4), 550 (1.01×10^4), 589 (1.23×10^4); Anal. calcd for $C_{56}H_{78}CoN_7O_{14}+H_2O$: C 58.48, H 7.01, N 8.52, found: C 58.13, H 7.00, N 8.43. 1H NMR (500 MHz, [D6]Acetone): δ = 7.33 (t, J = 4.0 Hz, 1H), 5.73 (s, 1H), 3.92-3.87 (m, 1H), 3.82-3.76 (m, 1H), 3.75-3.64 (m, 14H), 3.61 (s, 3H), 3.56 (s, 3H), 3.42-3.39 (m, 1H), 3.27-3.22 (m, 1H), 3.19-3.17 (m, 1H), 3.11-3.06 (m, 1H), 2.91-2.81 (m, 2H), 2.71-2.62 (m, 2H), 2.56-2.40 (m, 7H), 2.36-2.22 (m, 11H), 2.17-2.08 (m, 2H), 1.86-1.66 (m, 3H), 1.63 (s, 3H), 1.50 (s, 3H), 1.44 (s, 3H), 1.41 (s, 3H), 1.28 (s, 3H), 1.16 (s, 3H), 1.07 (d, J = 6.2 Hz, 3H) ppm. ^{13}C NMR (125 MHz, [D6]Acetone): δ = 176.84, 176.83, 176.2, 174.6, 173.9, 173.3, 173.0, 172.7, 172.5, 172.4, 172.0, 171.5, 164.3, 163.5, 104.5, 103.6, 91.4, 83.6, 75.6, 67.3, 67.2, 59.2, 57.6, 55.0, 54.2, 52.4, 52.0, 51.9, 51.8, 51.7, 51.6, 49.3, 47.94, 47.91, 47.8, 47.4, 46.7, 42.8, 42.0, 40.5, 32.1, 33.6, 32.3, 32.2, 31.9, 31.89, 31.6, 31.5, 31.4, 27.2, 26.5, 25.6, 23.3, 22.7, 21.3 ppm.

Optimization of cobalester synthesis

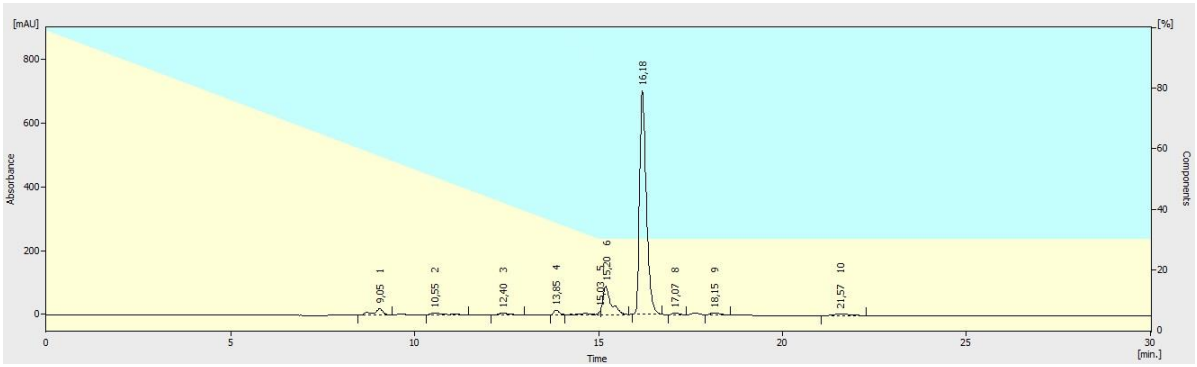


entry	T [°C]	time [h]	solvent	c [M]	DMF-DMA [eq.]**	conversion [%]	yield [%]
1	rt	1	MeOH	0,022	3	11	0
2	45	1	MeOH	0,022	3	66	0
3	45	25	MeOH	0,022	3	100	0
4	45	91	MeOH	0,022	3	100	0
5	60	1	MeOH	0,022	3	100	0
6	60	20	MeOH	0,022	3	100	0
7	60	44	MeOH	0,022	3	100	1
8	60	68	MeOH	0,022	3	100	0
9	60	44	MeOH	0,022	10	100	0
10	60	68	MeOH	0,022	10	100	0
11	60	25	MeOH/DMF*	0,022	10	100	9
12	60	25	MeOH/*i*PrOH*	0,022	10	100	traces
13	60	25	MeOH/C_6F_6*	0,022	10	100	0
14	60	25	MeOH/$C_5H_3F_8OH$*	0,022	10	100	0
15	60	25	MeOH/HFIP*	0,022	10	100	61
16	60	25	MeOH/CH_3CN*	0,022	10	100	0
17	60	25	MeOH/DMSO*	0,022	10	100	0
18	60	25	MeOH/H_2O*	0,022	10	0	0
19	60	48	MeOH/DMF*	0,022	10	100	0
20	60	48	MeOH/HFIP*	0,022	10	100	72
21	60	72	MeOH/HFIP*	0,022	10	100	65
22	60	25	MeOH/HFIP*	0,089	10	100	5
23	60	25	MeOH/HFIP*	0,011	10	100	38
24	60	48	MeOH/HFIP*	0,089	10	100	0

25	60	48	MeOH/HFIP*	0,011	10	100	54
26	60	48	MeOH/HFIP***	0,022	10	100	1
27	60	48	MeOH/HFIP****	0,022	10	100	25

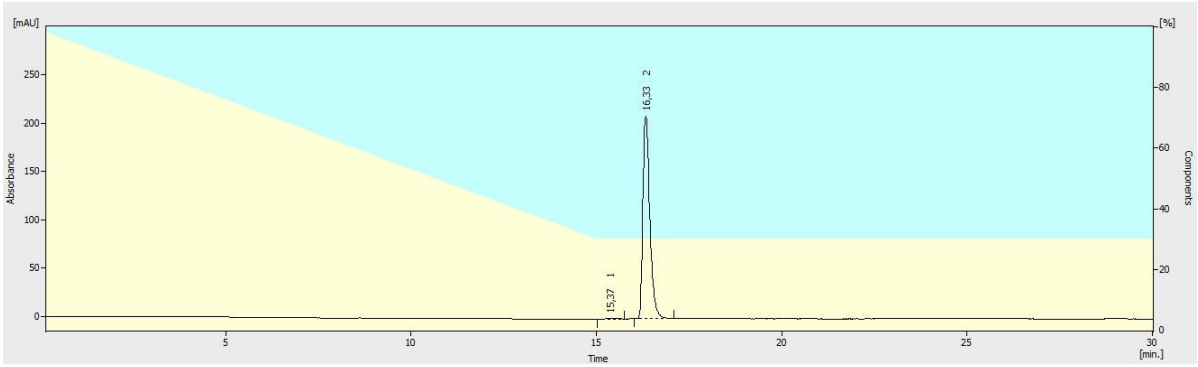
* 1:1 (MeOH:solvent) solvent ratio was used
** equivalents per one amide group
*** 5:1 (MeOH:HFIP) solvent ratio was used
**** 1:5 (MeOH:HFIP) solvent ratio was used

Copy of chromatogram of crude reaction mixture in optimal conditions (entry 20):



	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]
1	9,050	349,661	19,543	3,0	2,3	0,22	992
2	10,550	143,427	5,966	1,2	0,7	0,28	995
3	12,400	129,575	6,207	1,1	0,7	0,32	993
4	13,850	173,528	16,144	1,5	1,9	0,18	991
5	15,033	192,671	9,913	1,7	1,1	0,08	992
6	15,200	1393,044	89,108	12,0	10,3	0,22	990
7	**16,183**	**8865,269**	**702,957**	**76,3**	**81,1**	**0,22**	**960**
8	17,067	79,079	5,154	0,7	0,6	0,27	993
9	18,150	124,440	6,965	1,1	0,8	0,30	995
10	21,567	173,621	5,198	1,5	0,6	0,57	987
	Total	11624,315	867,156	100,0	100,0		

Copy of chromatogram of pure cobalester **3**:



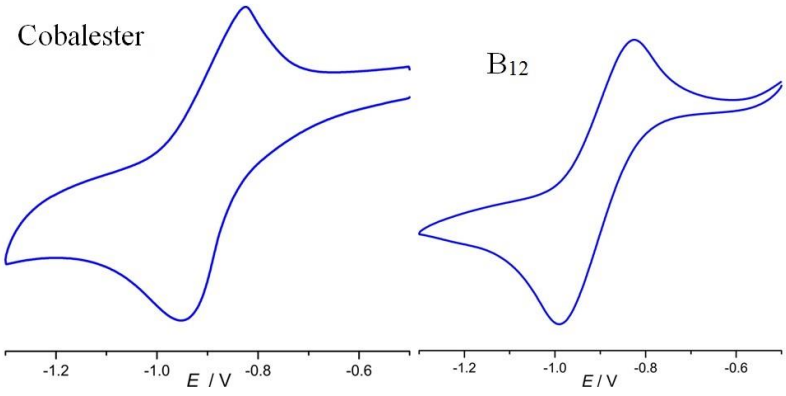
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]
1	15,367	4,064	0,240	0,2	0,1	0,28	999
2	16,333	2695,988	209,654	99,8	99,9	0,22	884
	Total	2700,052	209,894	100,0	100,0		

Cyclic voltammetry measurements

Cyclic voltammograms of vitamin B_{12} (**1**) and cobalester (**3**) were recorded in deoxygenated 0.2 M solutions of tris buffer in deionized water (C_{B12} = 1.7 mM, $C_{cobalester}$ = 0.7 mM). A three-electrode setup was used in both experiments, including glassy carbon working electrode, Ag/AgCl reference electrode and auxiliary platinum foil (scan rate v = 10 mVs^{-1}, Ar, 20 °C). For a better comparison, E values of the cobalt(III) to cobalt(II) reduction have been expressed as E^* versus $K_3Fe(CN)_6$ used as a standard in the same experimental conditions ($E_{1/2}$ = 216 mV).

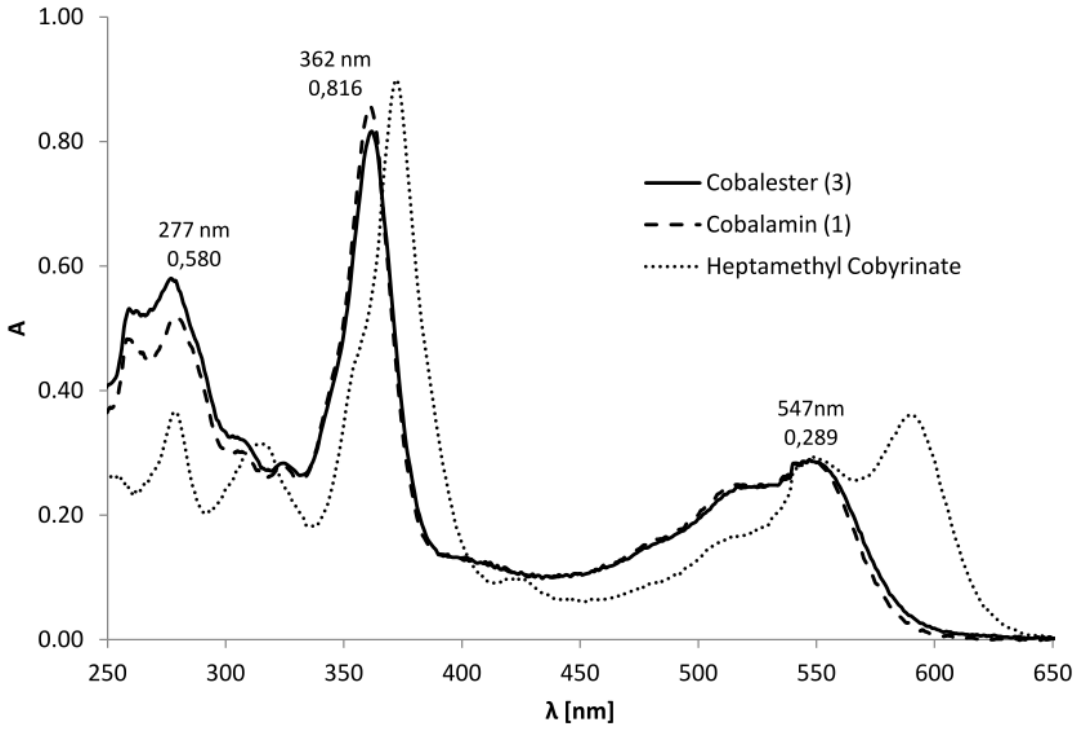
vitamin B_{12} (**1**)			cobalester (**3**)		
E_{pc}	E_{pa}	$E_{1/2}$	E_{pc}	E_{pa}	$E_{1/2}$
-0.826	-0.990	-0.908	-0.824	-0.953	-0.888
E_{pc}^*	E_{pa}^*	$E_{1/2}^*$	E_{pc}^*	E_{pa}^*	$E_{1/2}^*$
-1.042	**-1.206**	**-1.124**	**-1.040**	**-1.169**	**-1.104**

$K_3Fe(CN)_6$
$E_{1/2}$ [V]
0.216



Cyclic voltammogram of cobalester (**3**) and cobalamin (**1**).

UV/Vis spectra of cobalamin (1), cobalester (3), and heptamethyl cobyrinate in DMSO

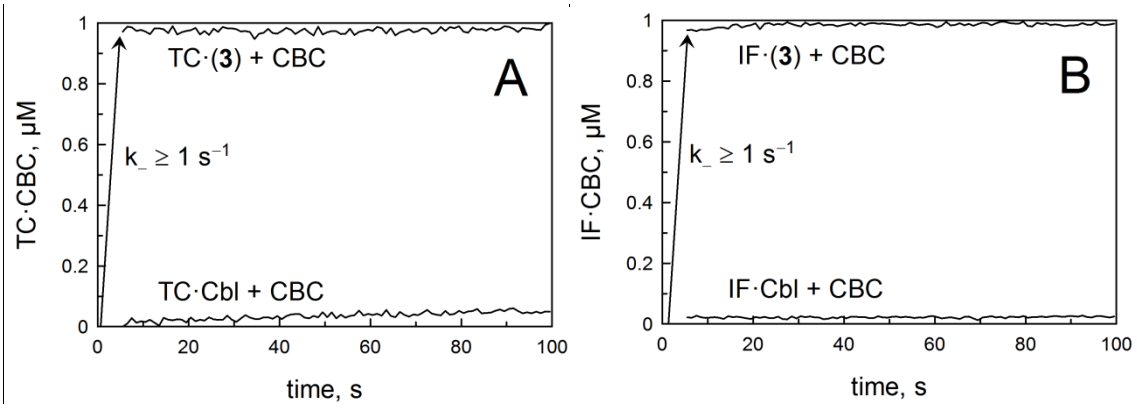


Binding of cobalester 3 to IF and TC

The potential biological properties of this new derivative **3** were examined. We tested its interactions with essential cobalamin transport proteins in an established competition assay, where the fluorescent B_{12} conjugate served as a tracer.[3] Because the peripheral primary amide groups are essential for binding to transport proteins, the biological properties of cobalester **3** were dubious. Not surprisingly, compound **3** displayed no affinity for either intrinsic factor (IF) or transcobalamin (TC). This result directly showed that the amide groups are important in the recognition process. However, these alterations do not preclude cobalester (**3**) from becoming a powerful chemical catalyst.

Dissociation experiments were conducted as follows. 1 μM binding protein (TC or IF) was incubated with 1.1 μM ligand (cobalester (**3**) or CNCbl (**1**)) for 10-20 min, whereupon 1.1 μM CBC was added. Increase in fluorescence upon formation of TC·CBC or IF·CBC was followed. Span between the fluorescence of free CBC in phosphate buffer with 0.05 mg/mL albumin and the signal of TC·CBC or IF·CBC corresponded to 100% (or 1 μM of the fluorescing complex). No trace of protein-ligand complex was found after 5 s of incubation, i.e. all protein was converted to the complex with CBC after this time. The dissociation rate constants are of above 1 s^{-1}.

The equilibrium dissociation constants can be assessed as above 10 μM.

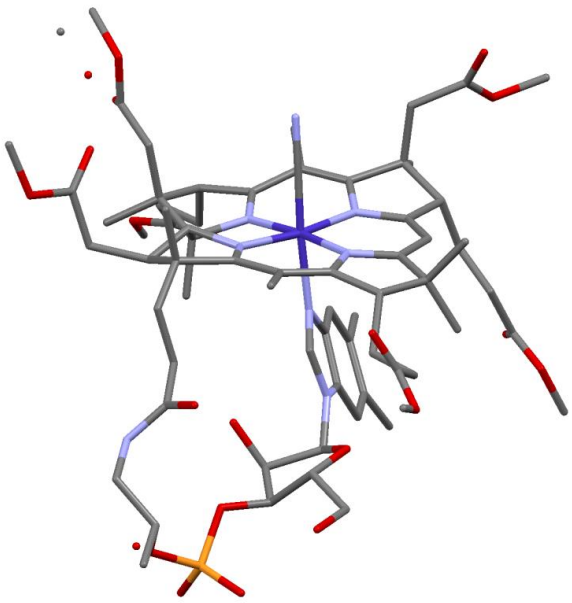


Binding cobalester (**3**) to TC (Fig. A) and IF (Fig.B).

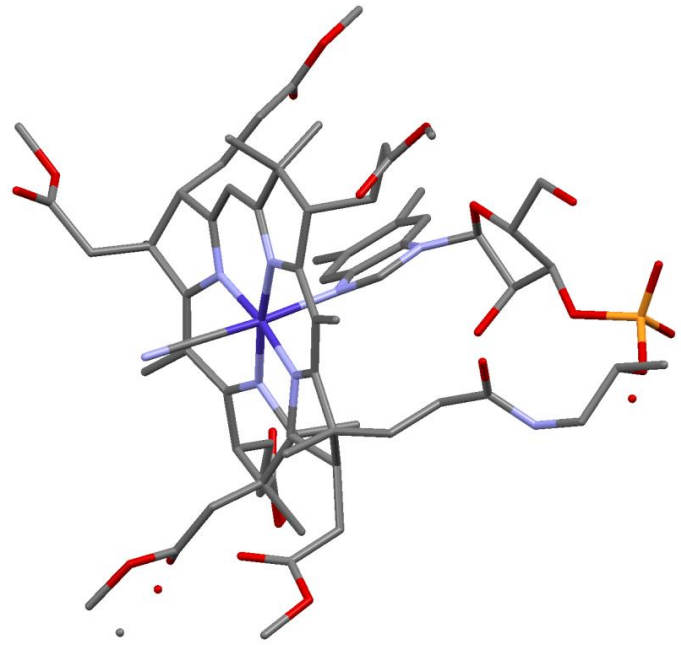
Crystallographic data and crystal structure of cobalester (3)

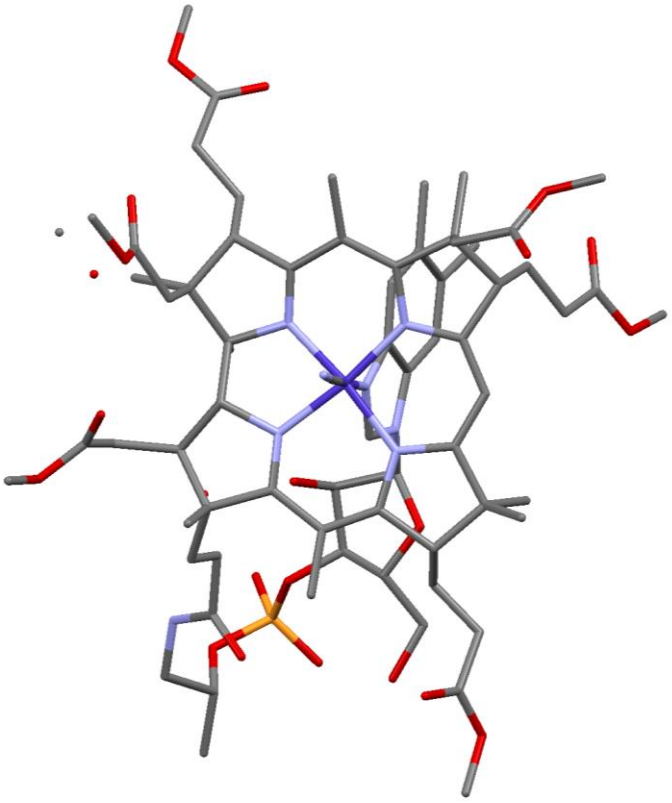
Formula	$C_{207}H_{282}Co_3N_{24}O_{61}P_3$
Space group	R3
Cell Lengths (Å)	**a** 39.7853(10) **b** 39.7853(10) **c** 14.0608(4)
Cell Angles (°)	**α** 90 **β** 90 **γ** 120
Cell Volume (Å)	19274.6
Z, Z’	Z: 3 Z': 0
R factor (%)	5.7
CCDC deposition number	981457

Cobalester (**3**) crystallized in the trigonal space group, R3. This was unusual for vitamin B_{12} analogs, which typically form orthorhombic crystals.[4–7] The axial Co-CN bond length in cobalester (**3**) (1.872 Å) is not significantly different from that in cobalamin (1) (1.858 Å).[4] In contrast, the axial Co-N bond to the purine base (2.048 Å) is almost 0.04 Å longer than that of cobalamin (1) (2.011 Å). The distances from the Co atom to the N atoms of the A and D rings are shorter than those to the N atoms of the C and B rings (Co-N_A 1.885 Å, Co-N_B 1.921 Å, Co-N_C 1.942 Å, Co-N_D 1.900 Å).



Crystal structure of cobalester (**3**) (hydrogen atoms omitted for clarity).



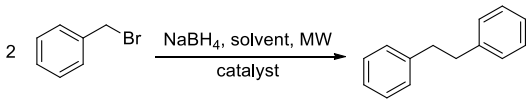


Stability test

The solubility of cobalester (**3**) exhibits features of both (CN)Cbl (**1**) and $(CN)_2Cby(OMe)_7$. It dissolves well in both polar (water, MeOH, DMSO) and nonpolar (DCM, $CHCl_3$) solvents. In buffered solutions, the compound was stable for 3 h between pH 7 and 2, but at pH = 10, it rapidly decomposed to carboxylic salts. pH Stability was performed by incubating solutions of cobalester (**3**) in buffers of pH ranging from 2 to 12 ($7x10^{-4}$ M) and conversion was controlled using HPLC.

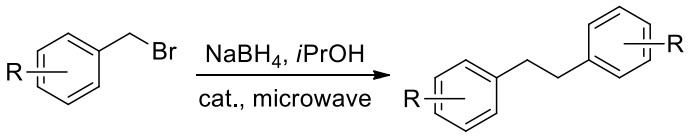
entry	**pH**	**temperature [°C]**	**time [h]**	**conversion [%]**
1	2	rt	1	0
2	2	rt	5	4
3	4	rt	1	0
4	4	rt	5	0
5	7	rt	1	0
6	7	rt	5	0
7	7	rt – UV lamp irradiation	1	0
8	7	rt – UV lamp irradiation	5	0
11	7	90	1	11
12	7	90	5	87
13	10	rt	1	80
14	10	rt	5	100
15	12	rt	1	99
16	12	rt	5	100

Optimization of benzylbromides homocoupling



entry	benzyl bromide [mmol]	solvent	solvent volume [ml]	catalyst loading [%]	$NaBH_4$ [eq.]	temperature [°C]	time [min]	yield [%]
1	0.25	iPrOH	1	1.5	2.0	120	10	19
2	0.25	iPrOH	1	1.5	2.0	120	15	84
3	0.25	Dioxane	1	1.5	2.0	120	15	traces
4	0.25	THF	1	1.5	2.0	120	15	-
5	0.25	n-BuOH	1	1.5	2.0	120	15	80
6	0.25	iPrOH	1	1	2.0	120	15	84
7	0.25	iPrOH	1	0.5	2.0	120	15	86
8	0.25	iPrOH	1	0.25	2.0	120	15	42
9	0.25	iPrOH	0.25	0.5	2.0	120	15	37
10	0.25	iPrOH	1	0.5	1.0	120	15	54
11	0.25	iPrOH	0.25	0.5	0.5	120	15	traces
12	0.25	iPrOH	0.25	0.5	1.0	120	15	traces
13	0.25	iPrOH	0.5	0.5	1.0	120	15	traces
14	0.25	iPrOH	1	0.5	2.0	90	15	84
15	0.25	iPrOH	1	0.5	2.0	60	15	10
16	0.25	iPrOH	1	-	2.0	90	15	-
17	0.25	iPrOH	1	0.5	2.0	reflux (oil bath)	15	traces
18	0.25	iPrOH	1	0.5	2.0	reflux (oil bath)	60	58

General bibenzyl synthesis procedure



A MW reaction vessel equipped with a stirring bar was charged with cobalester (**3**) (1.8 mg, 0.5 mol%) and $NaBH_4$ (19 mg, 0.5 mmol). Degased isopropanol (1.0 ml) and subsequently appropriate benzyl bromide (0.25 mmol) were added under anaerobic atmosphere. The reaction mixture was heated to 90 °C in a microwave reactor for 15 minutes (maximum power 300 W; ramping time not included). After cooling down to rt it was filtered through a celite pad and the filtrate concentrated in vacuo. The crude product was purified using column chromatography.

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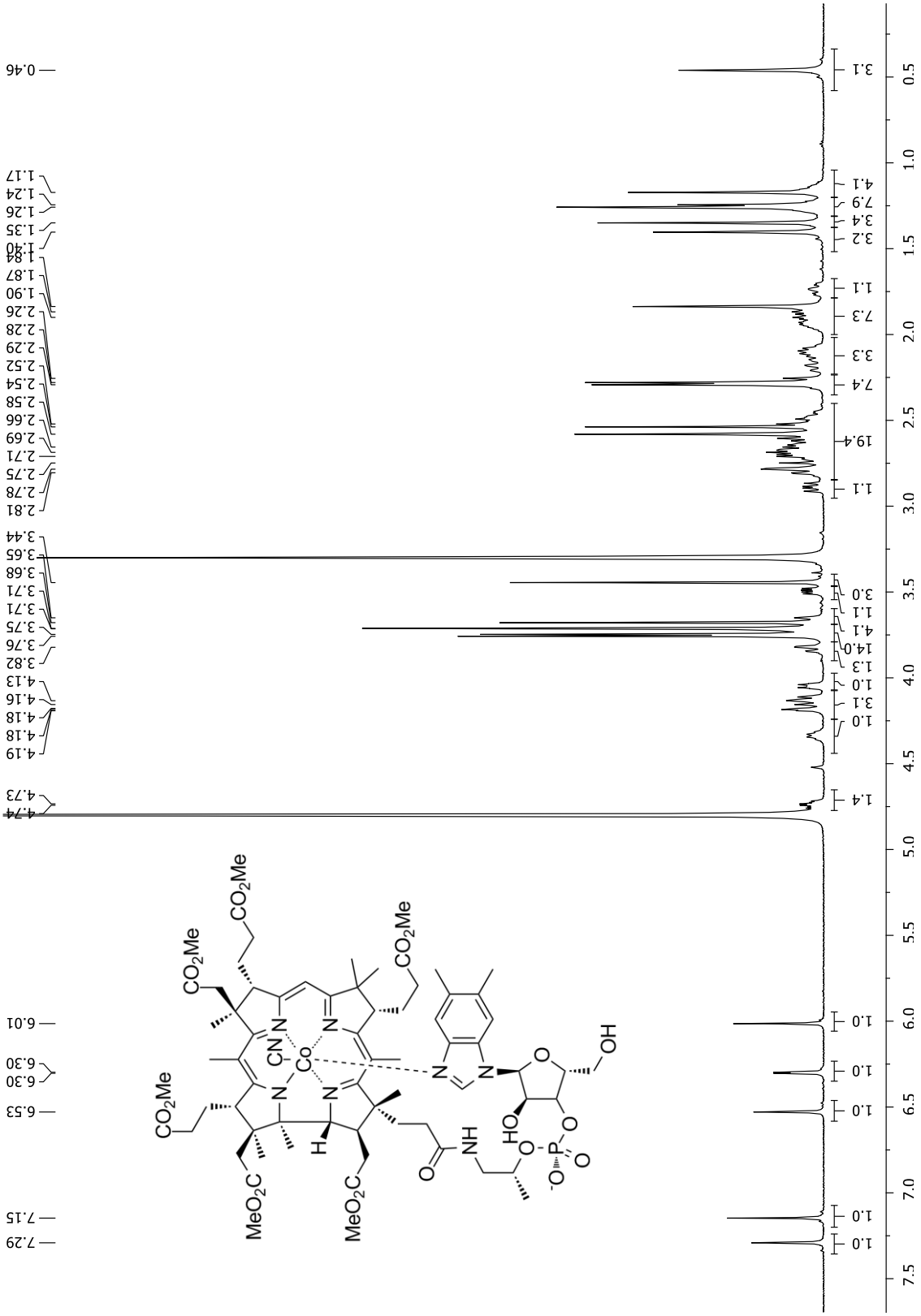
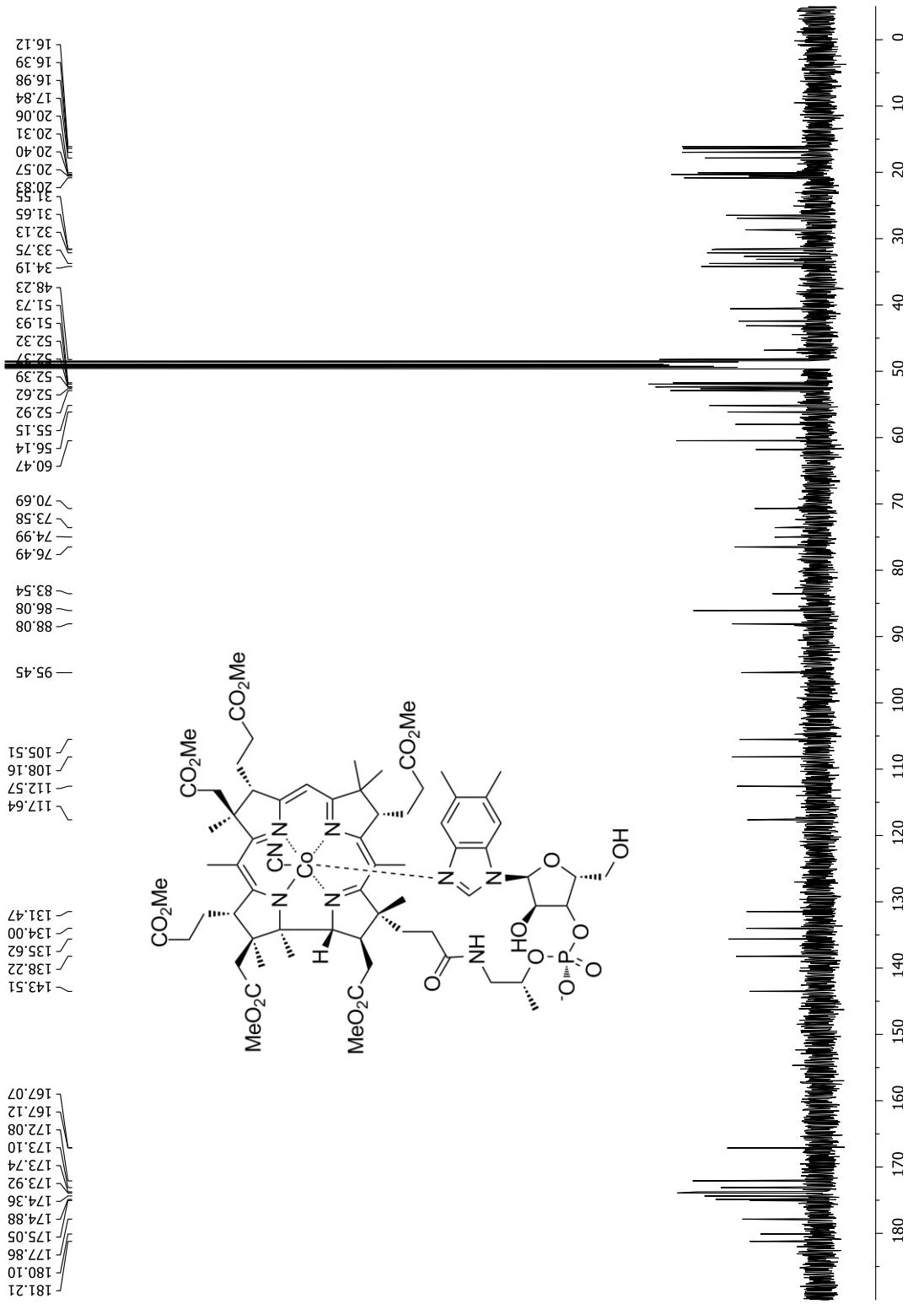
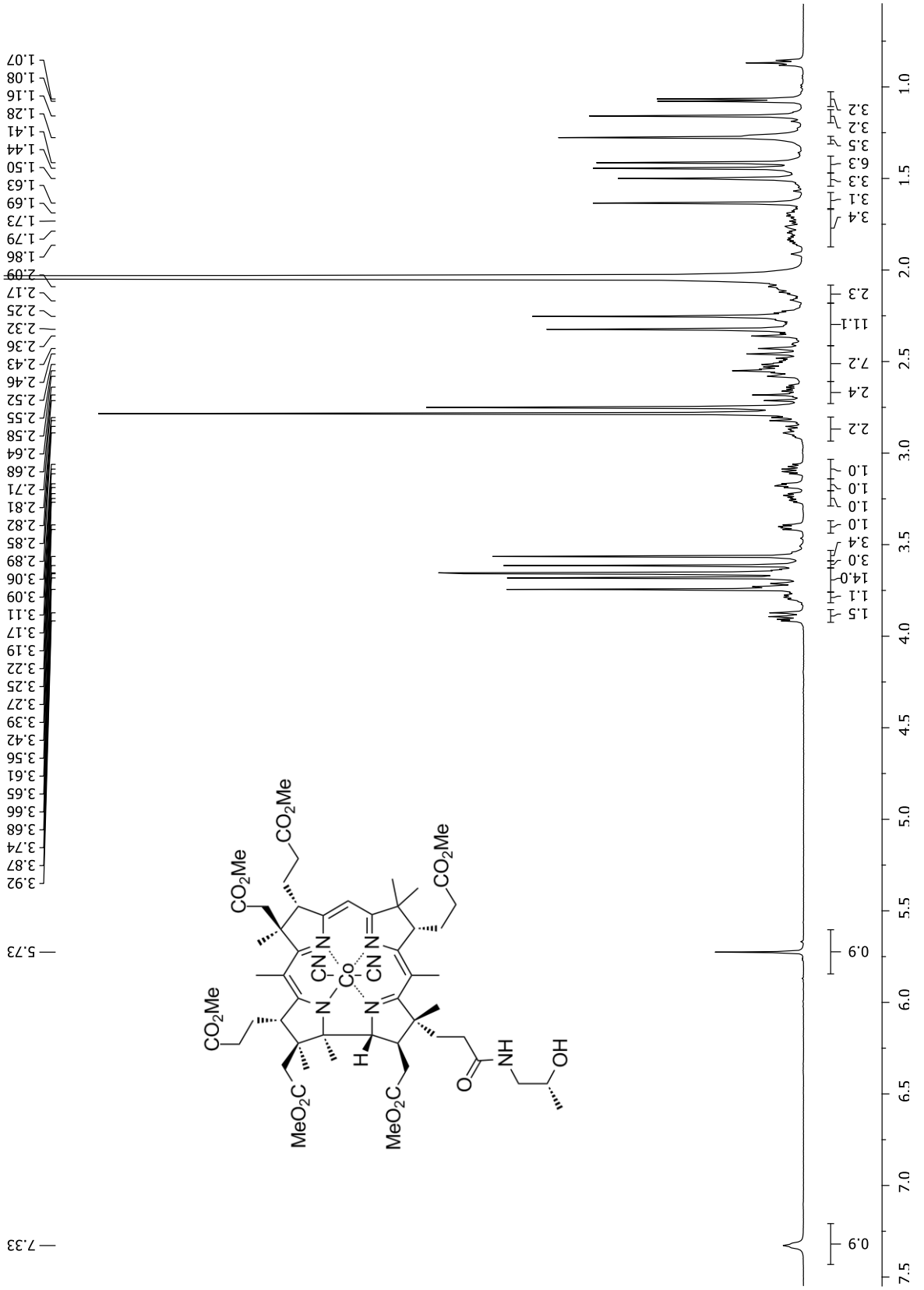
Cobalester (3) - ^{1}H NMR



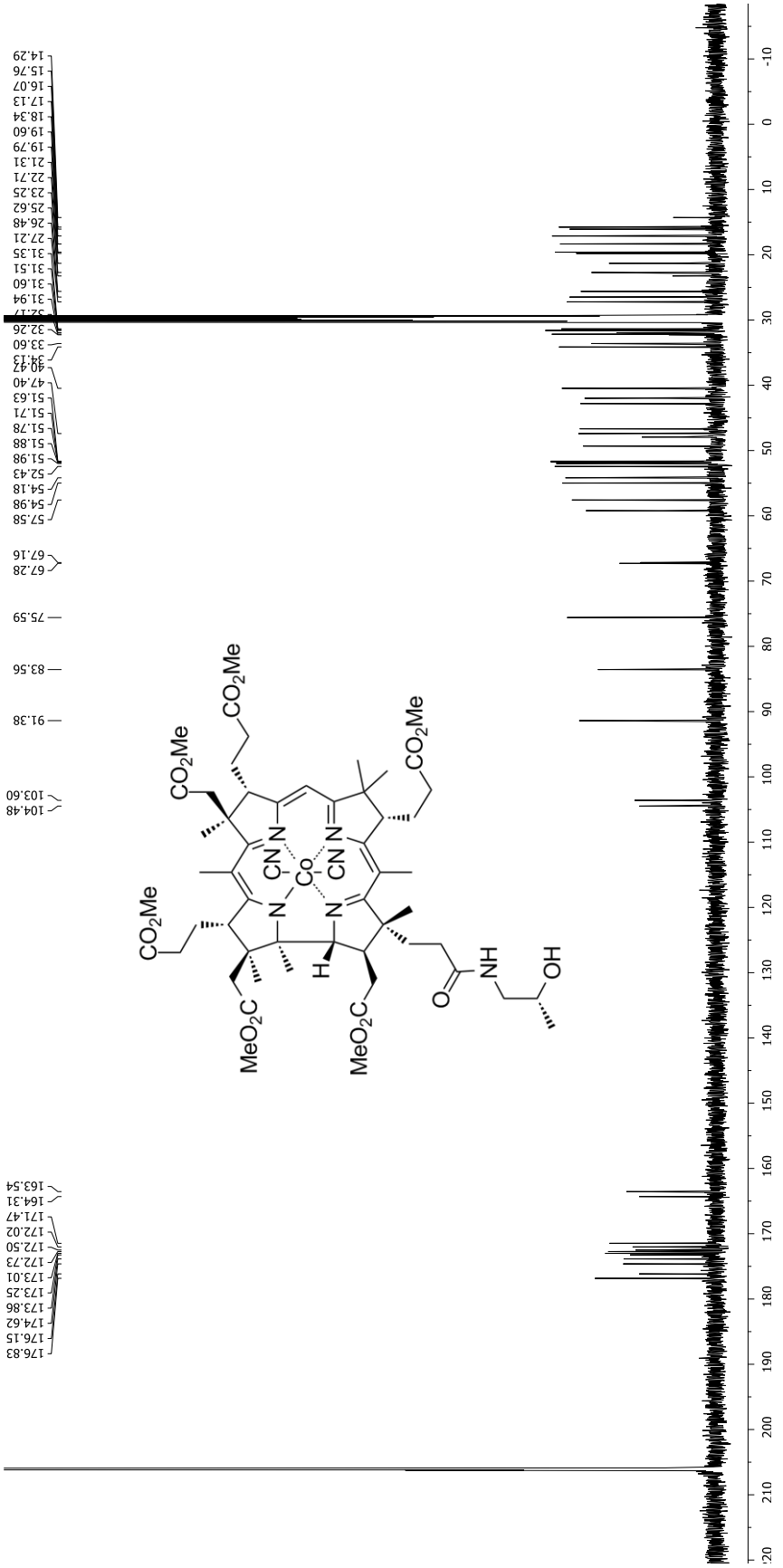
Cobalester (3) – ^{13}C NMR



Cobinester (5) – ^{1}H NMR



Cobinester (5) – ^{13}C NMR



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Small Alterations in Cobinamide Structure Considerably Influence sGC Activation

Maciej Giedyk,[a] Keith ó Proinsias,[a] Sylwester Kurcoń,[a] Iraida Sharina,[b] Emil Martin,*[b] and Dorota Gryko*[a]

Specially designed B-ring-modified cobalamin derivatives were synthesized and tested as potential activators of soluble guanylyl cyclase (sGC). Herein, we disclose the influence of substituents at the *c*- and *d*-positions in hydrophilic and hydrophobic cobyrinic acid derivatives on their capacities to activate sGC. The presence of the amide group at *c*-/*d*-position in cobyrinic acid derivatives strongly influence the level of sGC activation. Removal of the *d*-position altogether has a profound effect for hydrophobic compounds. In contrast, little differences were observed in hydrophilic ones.

Introduction

Soluble guanylyl cyclase (sGC) is a heme protein that in response to NO stimulation catalyzes the conversion of guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP), an important cellular secondary messenger. From a structural point of view, sGC is a heterodimer composed of two subunits, α and β, each having a multidomain organization with three distinct functional domains: an N-terminal heme-binding/regulatory domain, a central signal transducing/dimerization domain, and a C-terminal catalytic domain.[1–4] Activation of sGC by endogenous NO, a ligand for the enzyme's prosthetic heme group, elevates cellular cGMP levels, affecting a wide range of physiological processes such as vascular homeostasis, vasorelaxation, angiogenesis, neurotransmission, and platelet function.[5,6] Therefore, sGC is an important drug target, especially for the treatment of such cardiovascular diseases as angina pectoris, heart failure, and pulmonary hypertension.[7] While various NO-dependent and -independent pharmacological sGC activators are being developed,[8–10] they all target the same heme-binding domain. Hence, new strategies for sGC regulation that are not focused on the heme domain must be explored.

It was recently reported that dicyanocobinamide ($(CN)_2$Cbi) and its derivatives can directly activate sGC by targeting sites outside the heme-binding region.[11–13] Introduction of other hydrophilic amides to the peripheral side chains dramatically improves sGC activation. Hydrophobic cobinamide analogues display a more modest maximal activation, but in contrast show an improved EC_{50} value, providing insight into the difference between hydrophilic and hydrophobic cobalamins.

The exact binding site for cobinamide and its derivatives remains unknown. In-depth structure–activity relationship studies will generate approaches and tools suitable for identification of new sGC binding sites. It is therefore an inspiring task to carefully investigate this novel mode of sGC activation. The main goal of this report is to disclose the influence of substituents at the *c*- and *d*-positions of cobyrinic acid derivatives on their capacities to activate sGC, highlighting the role that substituents at these specific locations play in sGC binding or activation.

Results and Discussion

Chemistry

We recently reported the selective synthesis of novel cobinamide derivatives starting from heptamethyl cobyrinate (**1**).[13,14] A small library of various heptaamides was obtained, among which hydrophilic product **2**, bearing seven ethanolamide residues, displayed the highest activity toward sGC (Scheme 1).

The replacement of ethanolamide groups with other aliphatic amides led to decreased biological activity, suggesting the importance of terminal hydroxy groups. It was also shown that the presence of an OH group at the *d*-position influences the activation of sGC. It was therefore apparent that the B-ring of the corrinoid, within which substituents *c* and *d* are located, plays an important role in binding the enzyme active site. We decided to scrupulously study this relationship by selective modifications at certain positions of the parent molecule.

Substituents at *c*- and *d*-positions are perhaps the easiest to modify, and their alteration represents a useful means for functionalization of cobalamin derivatives.[15–17] Our research group recently developed a method for removal of the *d* side chain from heptamethyl cobyrinate (**1**).[18] Palladium-catalyzed cleav-

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CHEMMEDCHEM
Chemistry & Drug Discovery

Supporting Information

Small Alterations in Cobinamide Structure Considerably Influence sGC Activation

Maciej Giedyk,[a] Keith ó Proinsias,[a] Sylwester Kurcoń,[a] Iraida Sharina,[b] Emil Martin,*[b] and Dorota Gryko*[a]

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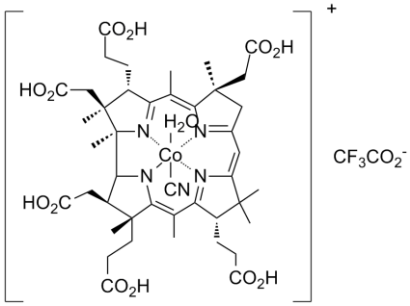
General Information

All solvents and chemicals used in the syntheses were of reagent grade and were used without further purification. Tested compounds were greater than 95% chemical purity as measured by elemental analysis. A microvave-assisted synthesis was performed using microwave oven CEM Discover. UV-vis absorption spectra were measured on Jenway 7315 spectrometer and Perkin Elmer λ-25 at room temperature. High resolution ESI mass spectra were recorded on a Mariner and SYNAPT spectrometer. ^{1}H and ^{13}CNMR spectra were recorded at rt on Bruker 500 and Varian 500 MHz instruments with TMS as an internal standard. Elemental analyses were obtained from the Institute of Organic Chemistry PAS. DCVC (dry column vacuum chromatography) was performed using Merck Silica Gel (200-300 mesh). Thin layer chromatography (TLC) was performed using Merck Silica Gel GF254, 0.20 mm thickness.
c-Lactone **11** and *c*-acid **12** were synthesized according to literature procedures.[1] Heptamethyl cobyrinate **1** was obtained from vitamin B_{12}.[2]

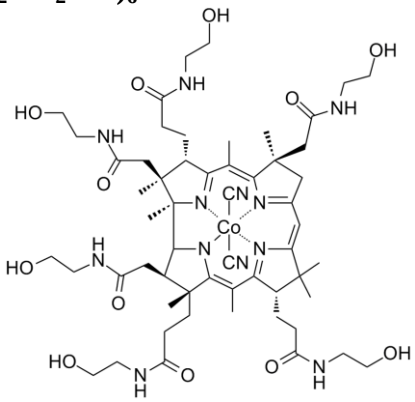
$(CN)_2$-8-*nor*-Cby(III)$(OH)_6$ 6



Hexaamethyl ester **5** (30 mg, 0.028 mmol) was dissolved in *i*PrOH (2 mL). $NaOH_{aq}$ (2 mL, 4 mol/L) was then added and the mixture was stirred at rt for 20 h after which it was acidified with TFA (2 mL). PhOH/DCM solution was added (1:1, 10 mL) and the mixture was washed with water. Organic layer was diluted 10 times with Et_2O and extracted with freshly added water (10 ml). Aqueous layer was then washed with DCM and concentrated *in vacuo*. Phenol extraction procedure was repeated twice. Hexaacid **6** was recrystalized from *i*PrOH/acetone giving a red solid (20 mg, 70%): mp: 183 °C; UV/Vis (EtOH): λ_{max} (ε)=522 ($7.40x10^3$), 492 ($7.81x10^3$), 402 ($4.13x10^3$), 354 ($2.29x10^4$), 272 nm ($1.07x10^4$); HRMS-ESI (m/z) $[M-H_2O]^+$ calcd for $C_{43}H_{55}CoN_5O_{12}$: 892.3179, found: 892.3165; Anal. calcd. for $C_{45}H_{57}CoF_3N_5O_{15}$: C 52.79, H 5.61, N 6.84, found: C 52.83, H 5.69, N 6.68.

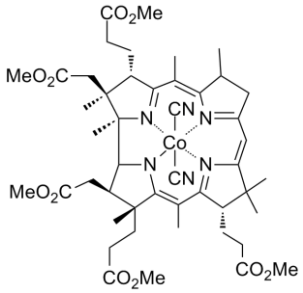
Note: Broadening of peaks in the ^1H NMR spectrum, caused by the presence of the $-CO_2H$ groups, made it impossible to decipher and consequently high resolution ^1H or ^{13}C spectra could not be obtained.

$(CN)_2$-8-*nor*-Cby(III)$(NHCH_2CH_2OH)_6$ 8



Following the reported procedure,[3] product **8** was isolated as a purple solid (11 mg, 65%): R_f=0.24 (MeOH/DCM 40%); ^{1}H NMR (500 MHz, [D6]DMSO): δ=8.42 (t, *J*=5.9 Hz, 1H), 8.38 (t, *J*=5.5 Hz, 1H), 8.10 (t, *J*=5.5 Hz, 1H), 7.96 (t, *J*=5.5 Hz, 1H), 7.89 (t, *J*=5.5 Hz, 1H), 7.60 (t, *J*=5.5 Hz, 1H), 5.56 (s, 1H), 4.90-4.82 (m, 2H), 4.67 (bs, 3H), 4.55 (bs, 1H), 4.37 (d, *J*=8.7 Hz, 1H), 3.69 (d, *J*=17.8 Hz, 1H), 3.60 (d, *J*=10.5 Hz, 1H), 3.48-3.35 (m, 13H including residual Et_2O), 3.26-3.00 (m, 14H), 3.83-3.73 (m, 2H), 2.66-2.61 (m, 1H), 2.45-2.34 (m, 3H), 2.31-2.24 (m, 1H), 2.23-2.13 (m, 9H), 2.10-1.97 (m, 5H), 1.91 (bs, 1H), 1.82-1.71 (m, 3H), 1.66-1.58 (m, 2H), 1.49 (s, 3H), 1.33 (s, 3H), 1.30 (s, 3H), 1.22 (s, 3H), 1.13 (s, 3H), 1.11-1.03 ppm (m, 9H including residual Et_2O); ^{13}C NMR (125 MHz, [D6]DMSO): δ=175.7, 175.5, 175.3, 172.4, 171.29, 171.25, 170.7, 170.6, 169.3, 167.8, 164.1, 162.5, 102.9, 101.5, 82.4, 74.9, 64.8, 62.0, 59.81, 59.76, 59.7, 59.5, 58.0, 56.0, 55.0, 52.8, 48.2, 46.0, 45.9, 45.0, 42.0, 41.8, 41.6, 41.54, 41.49, 40.0, 39.83, 39.76, 39.7, 39.6, 39.5, 39.3, 39.2, 39.0, 38.5, 35.5, 32.2, 32.0, 31.0, 30.8, 25.4, 23.7, 21.7, 19.3, 18.5, 17.7, 16.0 ppm; UV/Vis (MeOH): λ_{max} (ε)=578 ($8.83x10^3$), 538 ($7.75x10^3$), 367 ($2.53x10^4$), 308 ($9.43x10^3$), 276 nm ($1.10x10^4$); HRMS-ESI (m/z) $[M+Na]^+$ calcd for $C_{56}H_{85}CoN_{12}O_{12}Na$: 1199.6540, found: 1199.5634; Anal. calcd. for $C_{56}H_{85}CoN_{12}O_{12}$ + $5H_2O$: C 53.07, H 7.56, N 13.26, found: C 53.05, H 7.46, N 13.22.

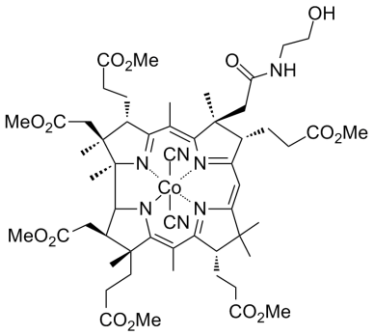
$(CN)_2$-*7,8-nor*-Cby(III)$(OMe)_5$ 10



(15 mg, 0.015 mmol) was dissolved in decalin (1 mL) in a MW test tube equipped with a stirring bar. It was degassed using vacuum-argon procedure and placed in microwave reactor. All manipulations were carried out in anaerobic conditions, in the darkness. The reaction mixture was heated to 210 °C (150W) for 5 minutes (ramping time not included). After cooling down to r.t. it was treated with degassed glacial acetic acid (3 mL) and zinc dust (600 mg, 9.2 mmol). The reaction mixture was vigorously stirred for 2 minutes at r.t., filtered through a celite pad and washed with MeOH/DCM (5%) solution. It was neutralized with $NaHCO_{3aq}$ and organic layer was washed with $NaCN_{aq}$, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Compound **10** was then purified using DCVC, 0-5% MeOH in DCM. Recrystalization from benzene/pentane gave a red solid as a mixture of two epimers (4.9 mg, 35%): R_f=0.31 (MeOH/DCM 6%); UV/Vis (DCM): λ_{max} (ε)=276 (7.23×10^3), 313 (6.55×10^3), 370 (1.83×10^4), 546 (6.04×10^3), 586 nm (7.29×10^3); HRMS-ESI (*m/z*) $[M–CN]^+$ calcd for $C_{46}H_{61}CoN_5O_{10}$: 904.3907, found: 904.3911; Anal. calcd for $C_{47}H_{63}CoN_6O_{10}+2H_2O$: C 58.38, H 6.98, N 8.69, found: C 58.72, H 6.87, N 8.31.

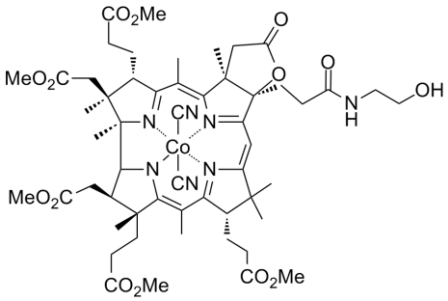
It was impossible to obtain a clear and precise ^1H NMR spectra and a ^{13}C spectrum was not recorded because the compound exists as a mixture of epimers and additionally splitting of peaks caused by the labile cyano ligand was observed.

$(CN)_2$Cby(III)(*c*-$NHCH_2CH_2OH$)$(OMe)_6$ 13



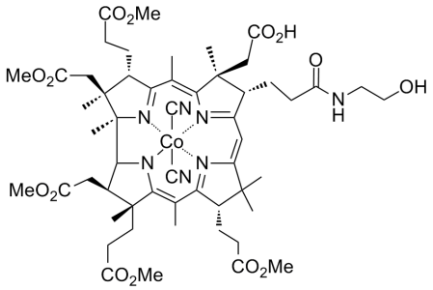
Following the reported procedure,[3] product **13** was isolated as a purple solid (37 mg, 80%): R_f=0.55 (EtOH/DCM 10%); mp: 113 °C; ^{1}H NMR (500 MHz, [D6]Acetone): δ=7.18 (t, *J*=5.2 Hz, 1H), 5.77 (s, 1H), 3.86 (d, *J*=10.2 Hz, 1H), 3.74-3.70 (m, 8H), 3.65 (s, 3H), 3.64 (s, 3H), 3.60 (s, 3H), 3.56 (s, 3H), 3.44-3.40 (m, 2H), 3.24-3.19 (m, 2H), 3.10-3.05 (m, 1H), 2.93-2.80 (m, 4H), 2.66-2.42 (m, 9H), 2.36-2.27 (m, 5H), 2.26-2.18 (m, 3H), 2.17-2.09 (m, 6H), 1.87-1.79 (m, 5H), 1.66-1.58 (m, 1H), 1.54 (s, 3H), 1.45 (s, 3H), 1.42 (s, 3H), 1.33 (s, 3H), 1.19 ppm (s, 3H); ^{13}C NMR (125 MHz, [D6]Acetone): δ=176.7, 176.5, 174.2, 173.9, 173.24, 173.16, 173.0, 172.61, 172.58, 170.4, 164.1, 162.2, 107.5, 103.2, 92.0, 83.7, 75.5, 61.5, 61.3, 59.5, 59.2, 57.6, 54.1, 52.5, 52.07, 52.06, 51.9, 51.8, 51.63, 51.58, 47.5, 47.4, 47.0, 43.73, 43.71, 43.6, 42.4, 40.4, 34.1, 32.9, 31.9, 31.8, 31.5, 31.4, 30.3, 30.1, 30.0, 29.8, 29.7, 29.5, 29.3, 26.6, 26.5, 25.6, 22.7, 19.7, 19.2 ppm; UV/Vis (DCM): λ_{max} (ε)=589 ($9.44x10^3$), 549 ($7.68x10^3$), 372 ($2.38x10^4$), 315 ($8.42x10^3$), 229 nm ($2.95x10^4$); HRMS-ESI (m/z) $[M+Na]^+$ calcd for $C_{55}H_{76}CoN_7O_{14}Na$: 1140.4688, found: 1140.4691; Anal. calcd. for $C_{55}H_{76}CoN_7O_{14}$: C 59.08, H 6.85, N 8.77, found: C 59.30, H 7.03, N 8.53.

$(CN)_2$Cby(III)(*c*-lactone)(*d*-$NHCH_2CH_2OH$)$(OMe)_5$ 15



Following the reported procedure,[3] product **15** was isolated as a purple solid (56 mg, 54%): R_f=0.55 (EtOH/DCM 10%); mp: 130 °C; ^{1}H NMR (500 MHz, [D6]Acetone): δ=6.88 (t, *J*=5.2 Hz, 1H), 5.77 (s, 1H), 3.95 (d, *J*=9.4 Hz, 1H), 3.95 (d, *J*=9.2 Hz, 1H), 3.74 (s, 3H), 3.71-3.64 (m, 10H), 3.58 (s, 3H), 3.40-3.37 (m, 2H), 3.253 (t, *J* =5.5 Hz, 1H), 3.12-3.09 (m, 2H), 3.07 (s, 1H), 2.94-2.89 (m, 2H), 2.84-2.80 (m, 1H), 2.72-2.65 (m, 2H), 2.63-2.44 (m, 6H), 2.42-2.22 (m, 11H), 2.20-1.15 (m, 1H), 2.12-2.07(m, 2H), 1.91-1.79 (m, 3H), 1.76 (s, 3H), 1.53 (s, 3H), 1.47 (s, 3H), 1.45 (s, 3H), 1.32 (s, 3H), 1.17 ppm (s, 3H); ^{13}C NMR (125 MHz, [D6]Acetone): δ=179.3, 177.2, 176.6, 174.1, 173.28, 173.27, 173.1, 173.0, 172.6, 172.0, 167.2, 163.9, 161.2, 105.0, 104.9, 95.8, 88.6, 83.8, 76.1, 61.9, 61.7, 59.3, 57.7, 54.3, 52.5, 52.1, 52.0, 51.8, 51.7, 51.0, 48.2, 46.7, 43.3, 43.1, 43.0, 42.93, 42.90, 41.9, 40.4, 34.0, 33.0, 32.6, 32.0, 31.4, 30.9, 30.6, 30.3, 30.1, 30.0, 29.8, 29.7, 29.5, 29.3, 26.5, 25.5, 23.3, 22.6, 19.8 ppm; UV/Vis (DCM): λ_{max} (ε)=592 ($1.20x10^4$), 553 ($1.02x10^4$), 369 ($3.15x10^4$), 318 ($9.97x10^3$), 230 nm ($3.74.x10^4$); HRMS-ESI (m/z) $[M+Na]^+$ calcd for $C_{54}H_{72}CoN_7O_{14}Na$: 1124.4367, found: 1124.4398; Anal. calcd. for $C_{54}H_{72}CoN_7O_{14}$: C 58.85, H 6.58, N 8.90, found: C 58.77, H 6.81, N 8.67.

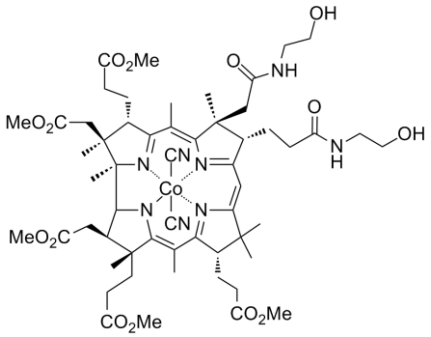
$(CN)_2$Cby(III)(*c*-acid)(*d*-$NHCH_2CH_2OH$)$(OMe)_5$ 16



Following the reported procedure,[3] product **16** was crudely purified and used as a purple solid without further purification. HRMS-ESI (m/z) $[M\text{-}CN]^+$ calcd for $C_{53}H_{74}CoN_6O_{14}$: 1077.4595, found: 1077.4596.

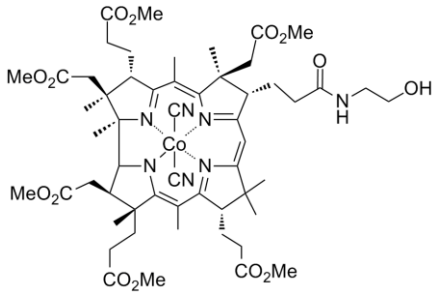
Note: Broadening of peaks in the ^1H NMR spectrum, caused by the presence of the $-CO_2H$ groups, made it impossible to decipher and consequently high resolution ^1H or ^{13}C spectra could not be obtained.

$(CN)_2$Cby(III)(*c*,*d*-$NHCH_2CH_2OH)_2(OMe)_5$ 17



Following the reported procedure,[3] product **17** was isolated as a purple solid (9 mg, 60%): R_f=0.43 (EtOH/DCM 10%); mp: 127 °C; ^{1}H NMR (500 MHz, [D6]Acetone): δ=7.06 (t, *J*=5.1 Hz, 1H), 7.86 (t, *J*=5.1 Hz, 1H), 5.86 (s, 1H), 3.92 (d, *J*=9.9 Hz, 1H), 3.77-3.68 (m, 9H), 3.65 (s, 3H), 3.64 (s, 3H), 3.56 (s, 3H), 3.44-3.37 (m, 4H), 3.27-3.20 (m, 2H), 3.12-3.07 (m, 3H), 2.97-2.87 (m, 3H), 2.84-2.81 (m, 1H), 2.66-2.60 (m, 2H), 2.58-2.43 (m, 6H), 2.35 (s, 3H), 2.27-2.12 (m, 12H), 1.89-1.82 (m, 2H), 1.80-1.77 (m, 4H), 1.51 (s, 3H), 1.46 (s, 3H), 1.42 (s, 3H), 1.34 (s, 3H), 1.19 ppm (s, 3H); ^{13}C NMR (125 MHz, [D6]Acetone): δ=177.1, 177.0, 176.7, 174.2, 173.6, 173.5, 173.20, 173.17, 173.0, 172.6, 170.4, 164.0, 162.5, 107.2, 103.3, 92.2, 83.8, 75.6, 62.0, 61.8, 61.5, 61.4, 60.1, 59.3, 57.6, 54.1, 52.5, 52.1, 52.0, 51.8, 51.69, 51.67, 47.4, 47.3, 46.9, 43.7, 43.11, 43.08, 42.3, 40.4, 34.1, 33.3, 32.9, 32.3, 31.9, 31.8, 31.1, 30.3, 30.1, 30.0, 29.6, 29.5, 29.3, 28.5, 26.2, 25.4, 23.3, 22.6, 19.6 ppm; UV/Vis (DCM): λ_{max} (ε)=590 ($1.03x10^4$), 550 ($8.59x10^3$), 371 ($2.72x10^4$), 316 ($9.30x10^3$), 229 nm ($3.42x10^4$); HRMS-ESI (m/z) $[M+Na]^+$ calcd for $C_{56}H_{79}CoN_8O_{14}Na$: 1169.4945, found: 1169.4946; Anal. calcd. for $C_{56}H_{79}CoN_8O_{14}$ + H_2O: C 57.72, H 7.01, N 9.62, found: C 57.55, H 7.10, N 9.56.

$(CN)_2$Cby(III)(*d*-$NHCH_2CH_2OH$)$(OMe)_6$ 18



Following the reported procedure,[4] product **18** was isolated as a purple solid (19 mg, 63%): R_f=0.55 (EtOH/DCM 10%); mp: 125 °C; ^{1}H NMR (500 MHz, [D6]Acetone): δ=6.79 (t, *J*=5.1 Hz, 1H), 5.82 (s, 1H), 3.94 (d, *J*=9.0 Hz, 1H), 3.75-3.73 (m, 4H), 3.69-3.65 (m, 13H), 3.56 (s, 3H), 3.45 (t, *J*=5.1, 1H), 3.37 (t, *J*=5.7, 2H), 3.21 (t, *J*=5.0 Hz, 1H), 3.12-3.04 (m, 2H), 2.91-2.79 (m, 3H), 2.71-2.64 (m, 2H), 2.62-2.42 (m, 6H), 2.35-2.29 (m, 4H), 2.28-2.07 (m, 10H), 1.97-1.81 (m, 4H), 1.66 (s, 3H), 1.50 (s, 3H), 1.46 (s, 3H), 1.41 (s, 3H), 1.31 (s, 3H), 1.16 ppm (s, 3H); ^{13}C NMR (125 MHz, [D6]Acetone): δ=177.3, 176.7, 176.5, 174.2, 173.5, 173.2, 173.1, 173.0, 172.7, 171.4, 164.5, 163.9, 104.6, 103.3, 91.7, 83.7, 75.6, 62.0, 61.9, 59.1, 57.7, 55.9, 54.2, 52.5, 52.0, 51.94, 51.91, 51.8, 51.7, 48.9, 47.4, 46.6, 43.09, 43.06, 42.9, 41.9, 40.3, 34.2, 33.0, 32.8, 32.3, 32.0, 31.4, 31.2, 30.3, 30.1, 30.0, 29.8, 29.7, 29.5, 29.3, 28.8, 26.3, 25.5, 23.3, 22.6, 19.7, 19.4 ppm; UV/Vis (DCM): λ_{max} (ε)=589 ($1.00x10^4$), 547 ($8.48x10^3$), 371 ($2.60x10^4$), 315 ($9.13x10^3$), 229 nm ($3.35x10^4$); HRMS-ESI (m/z) $[M+Na]^+$ calcd for $C_{55}H_{76}CoN_7O_{14}Na$: 1140.4680, found: 1140.4703; Anal. calcd. for $C_{55}H_{76}CoN_7O_{14}$: C 59.08, H 6.85, N 8.77, found: C 58.79, H 6.81, N 8.63.

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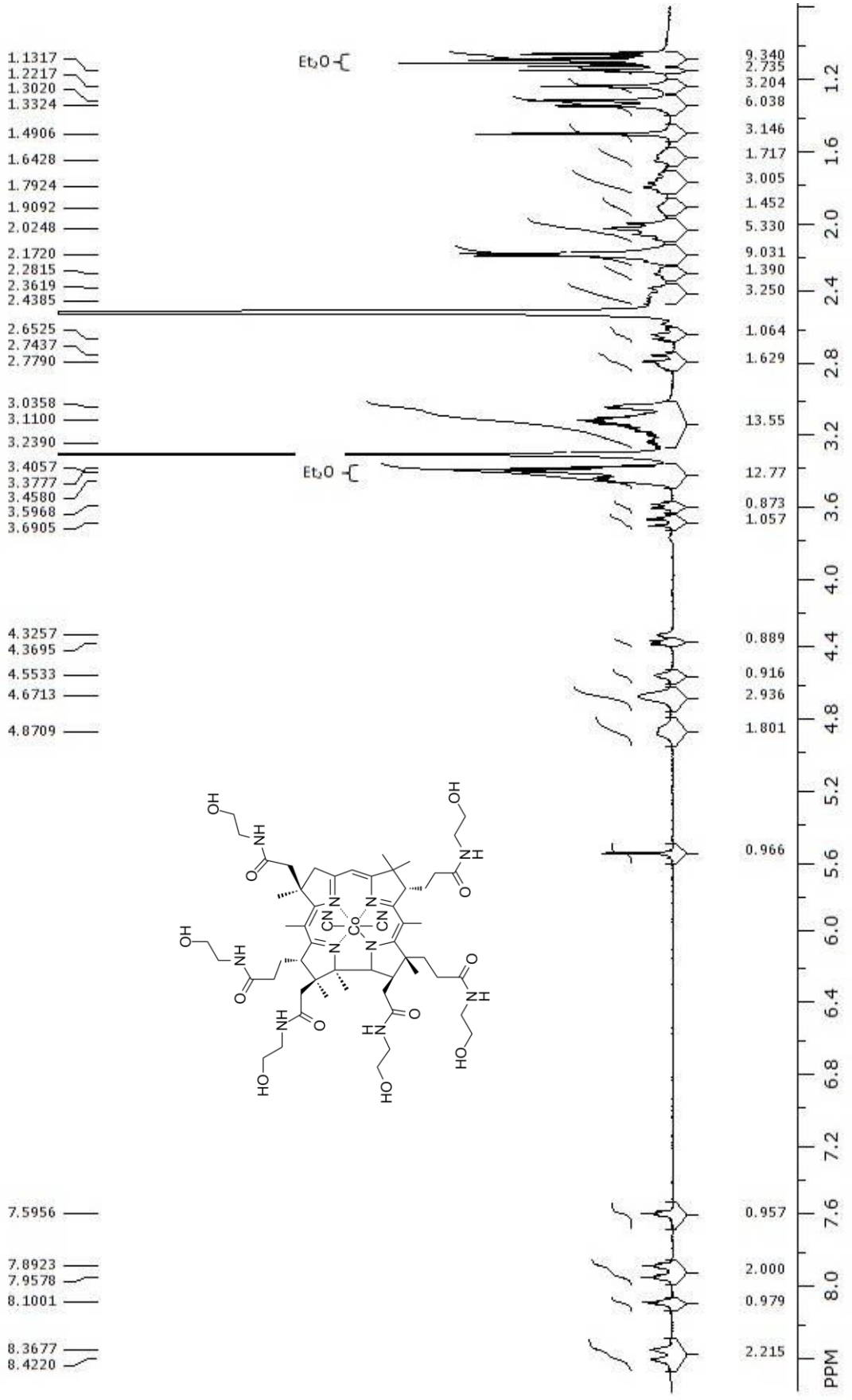
$(CN)_2$-8-*nor*-Cby(III)$(NHCH_2CH_2OH)_6$ 8



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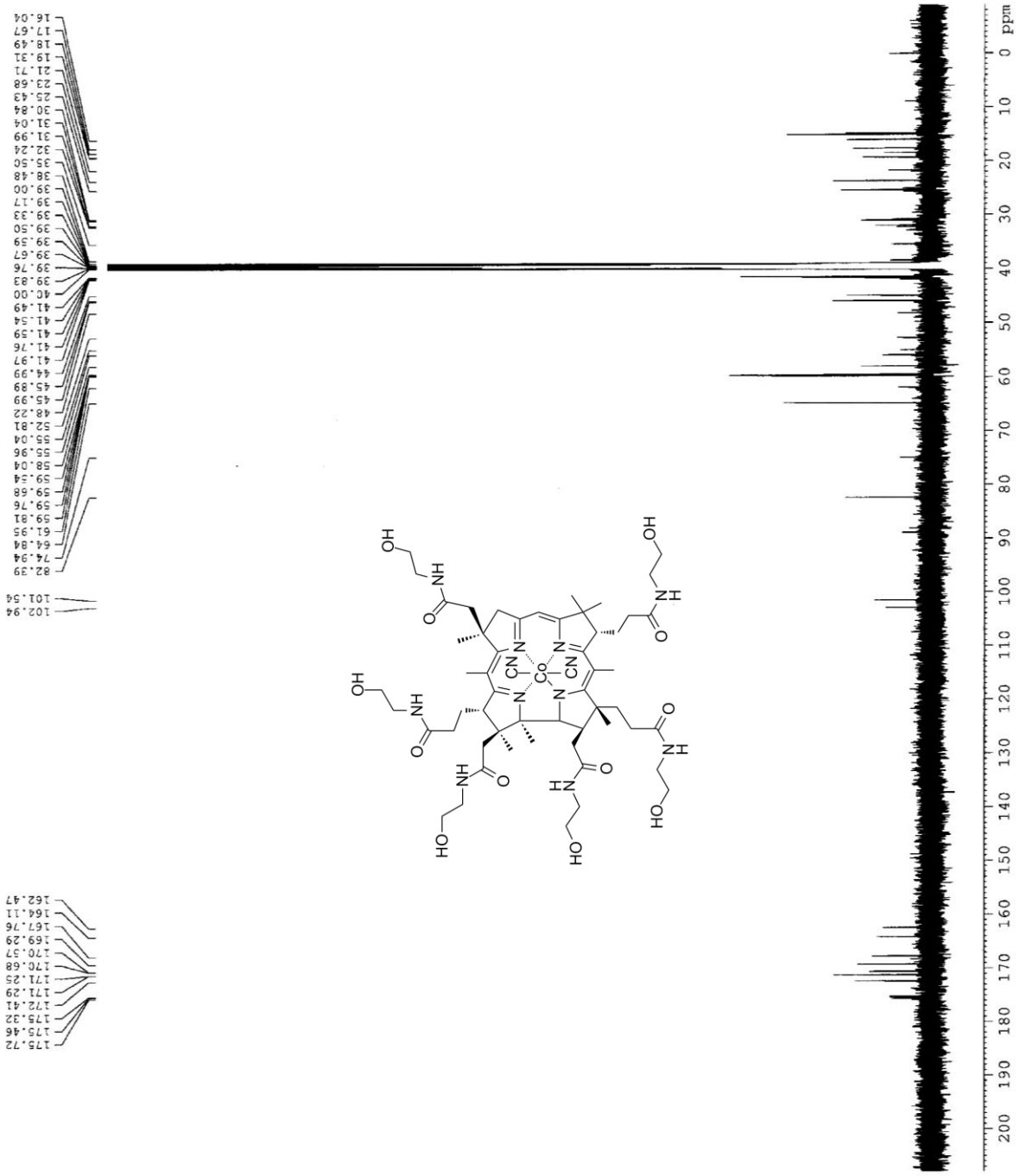
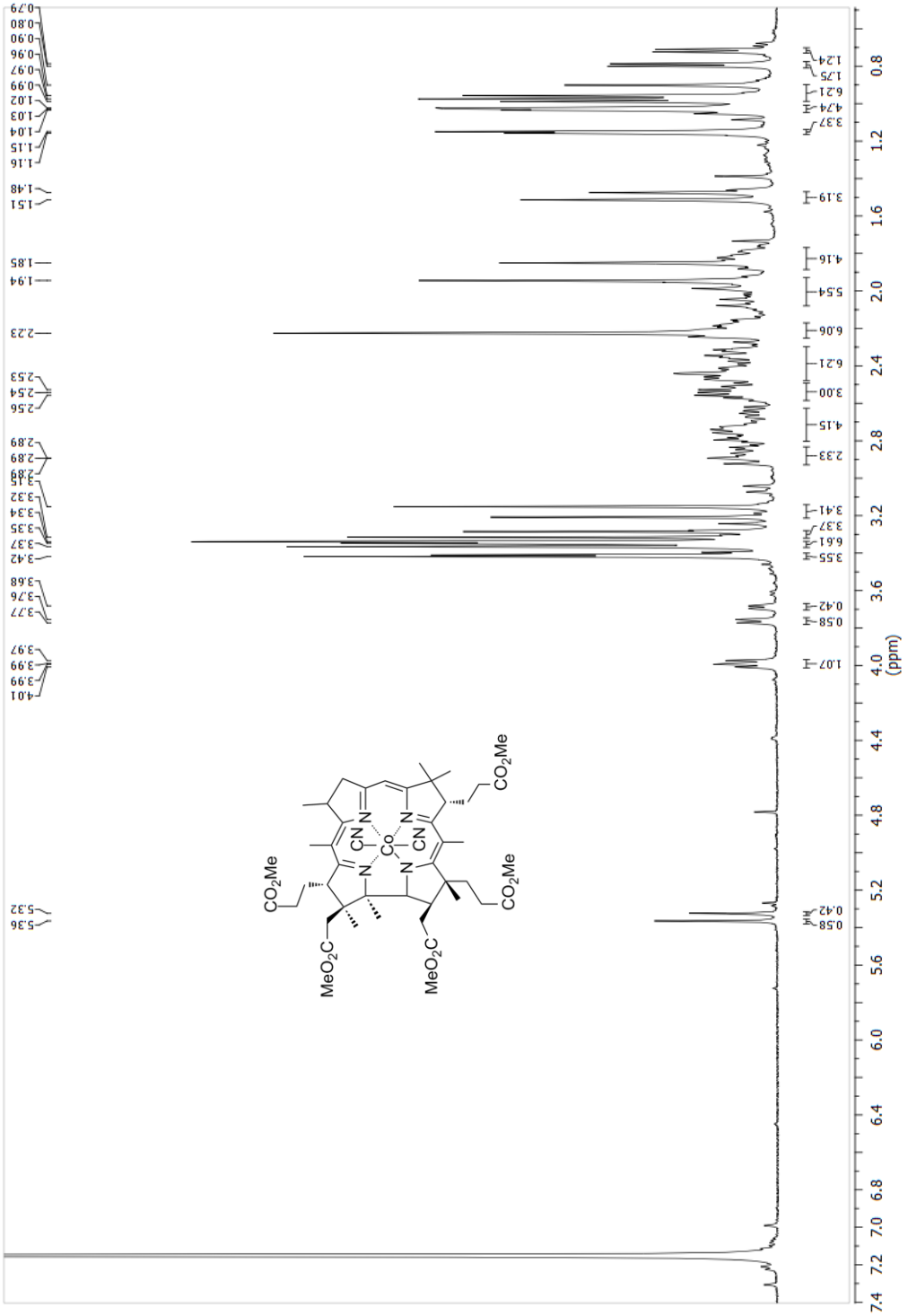
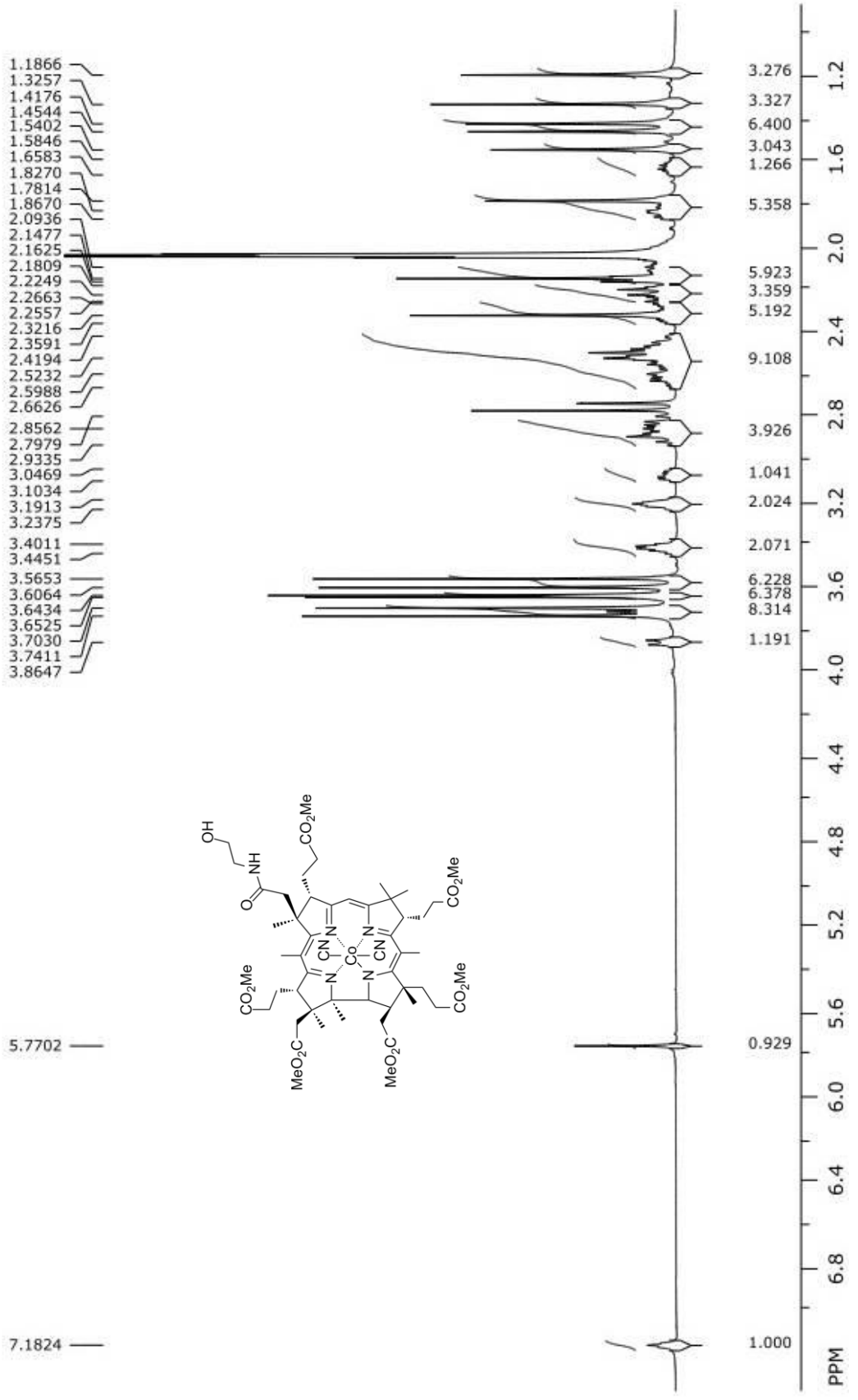


$(CN)_2$-7,8-*nor*-Cby(III)$(OMe)_5$ 10



$(CN)_2$Cby(III)(*c*-$NHCH_2CH_2OH$)$(OMe)_6$ 13



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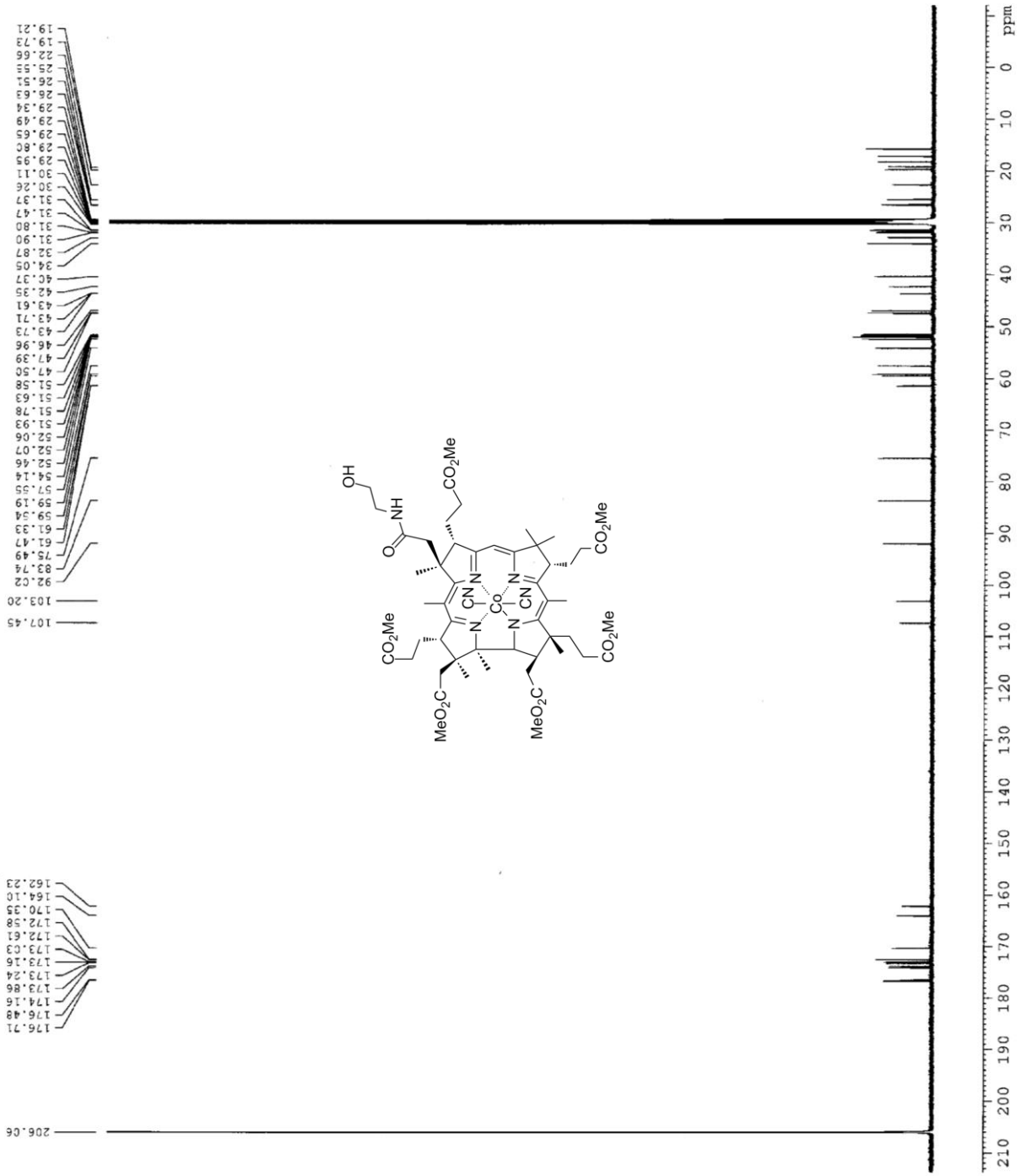

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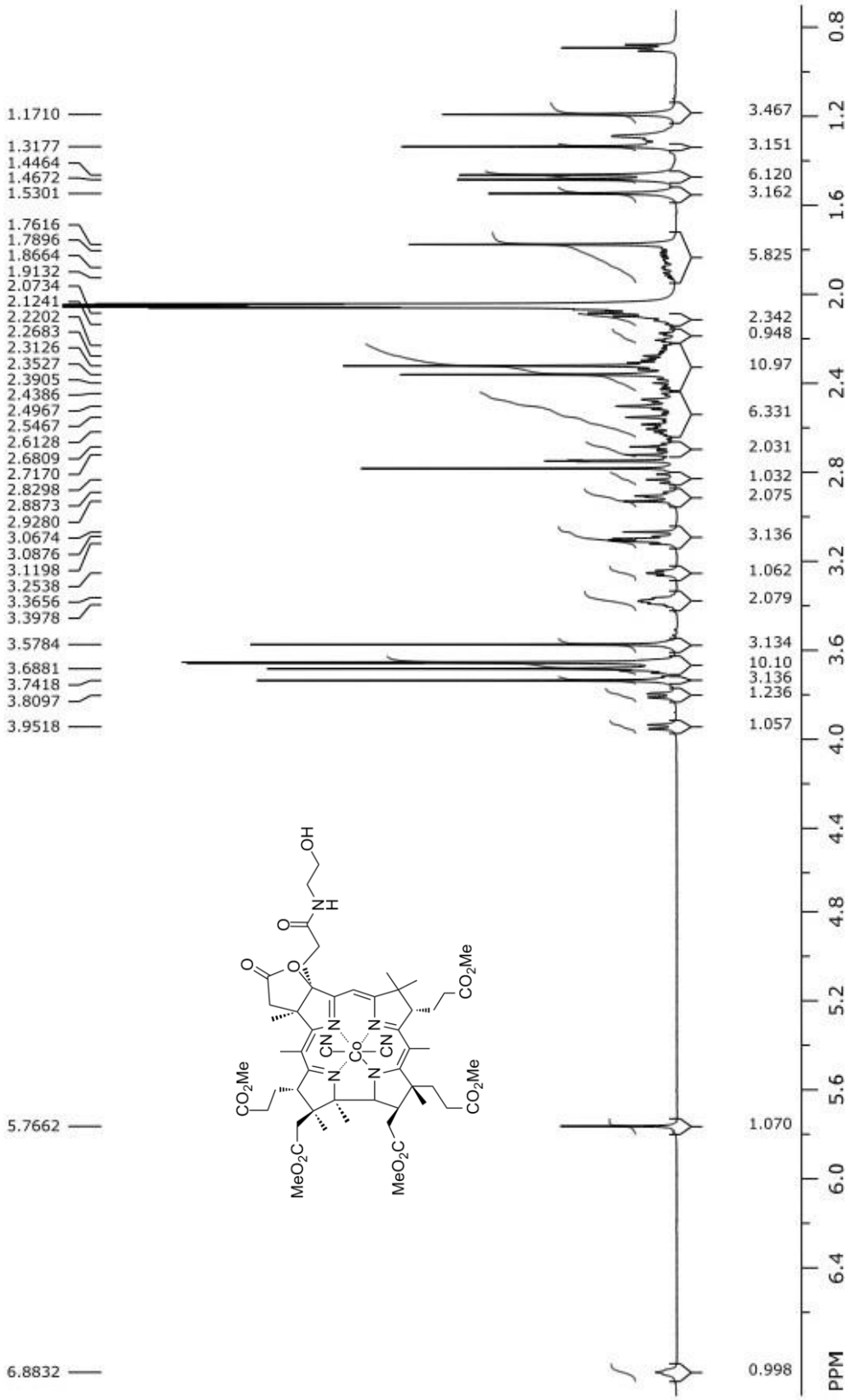

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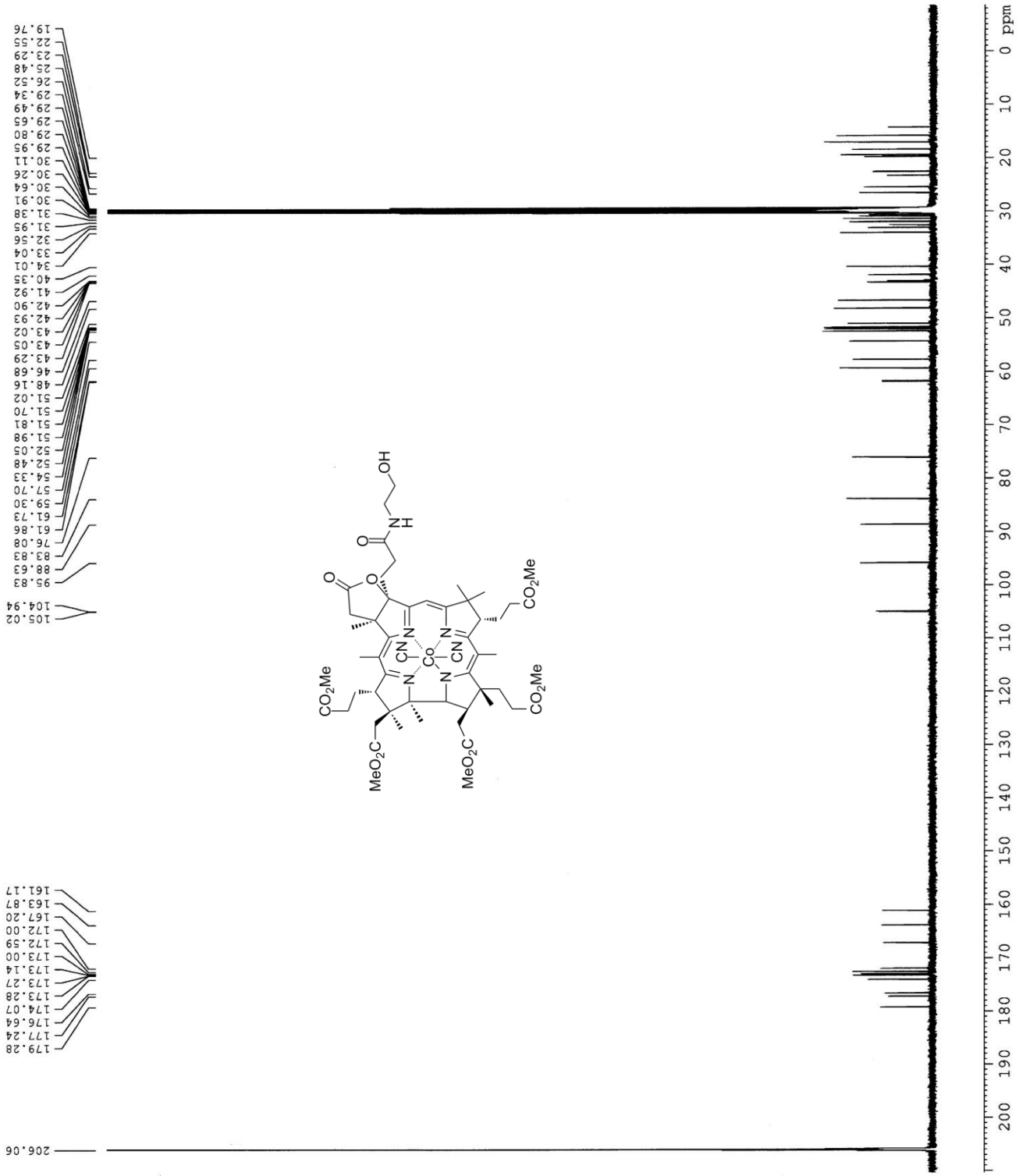

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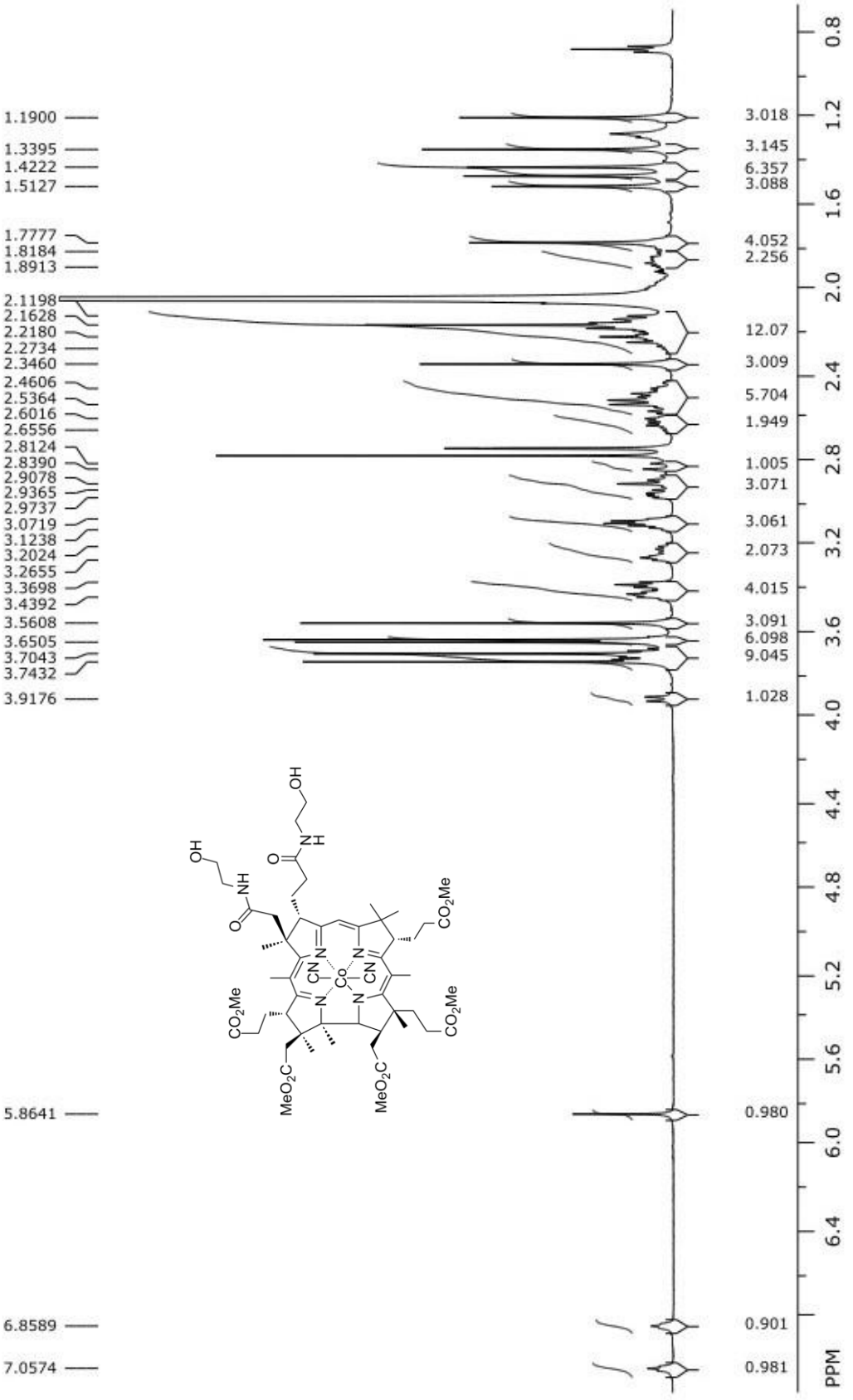

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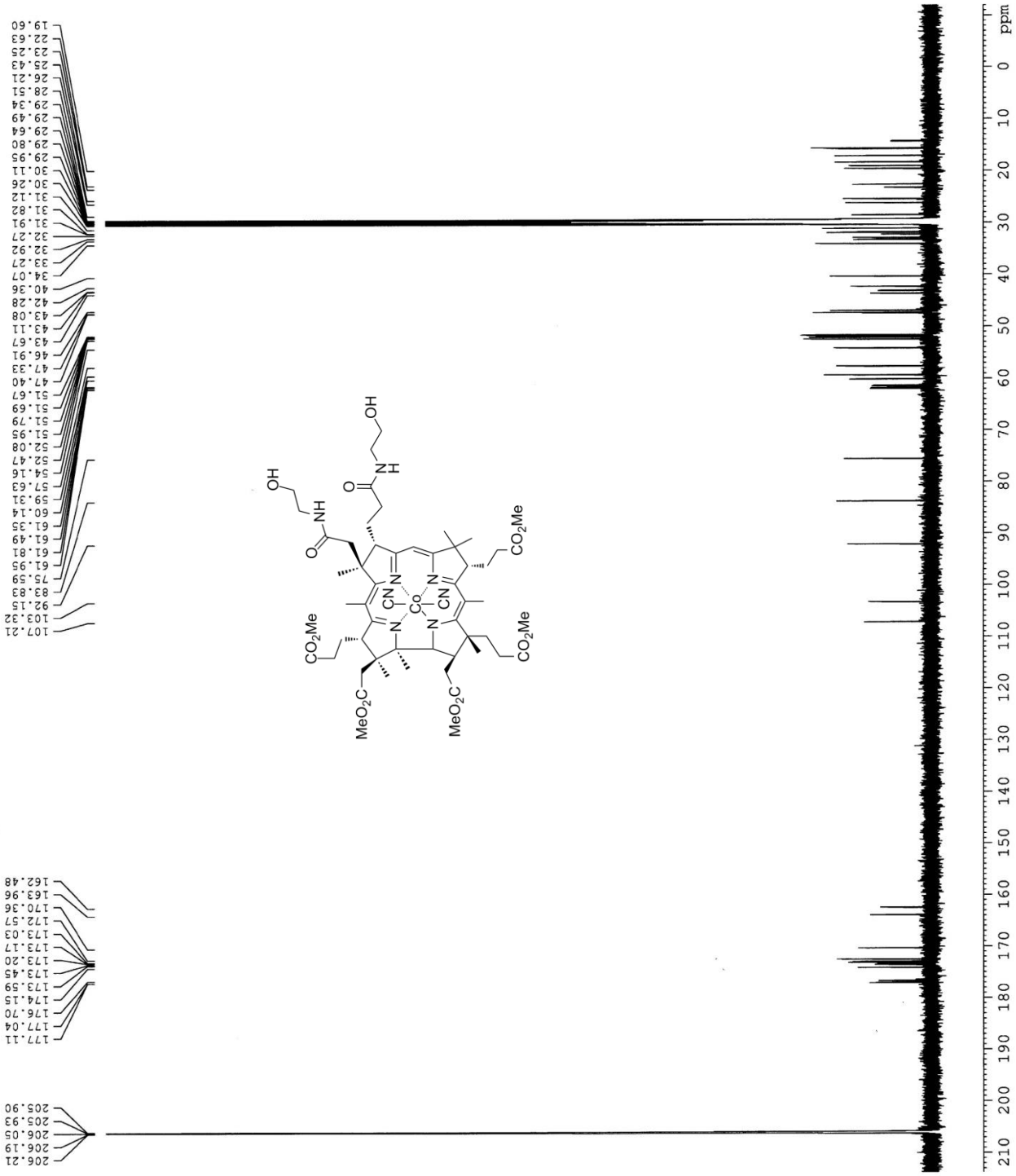

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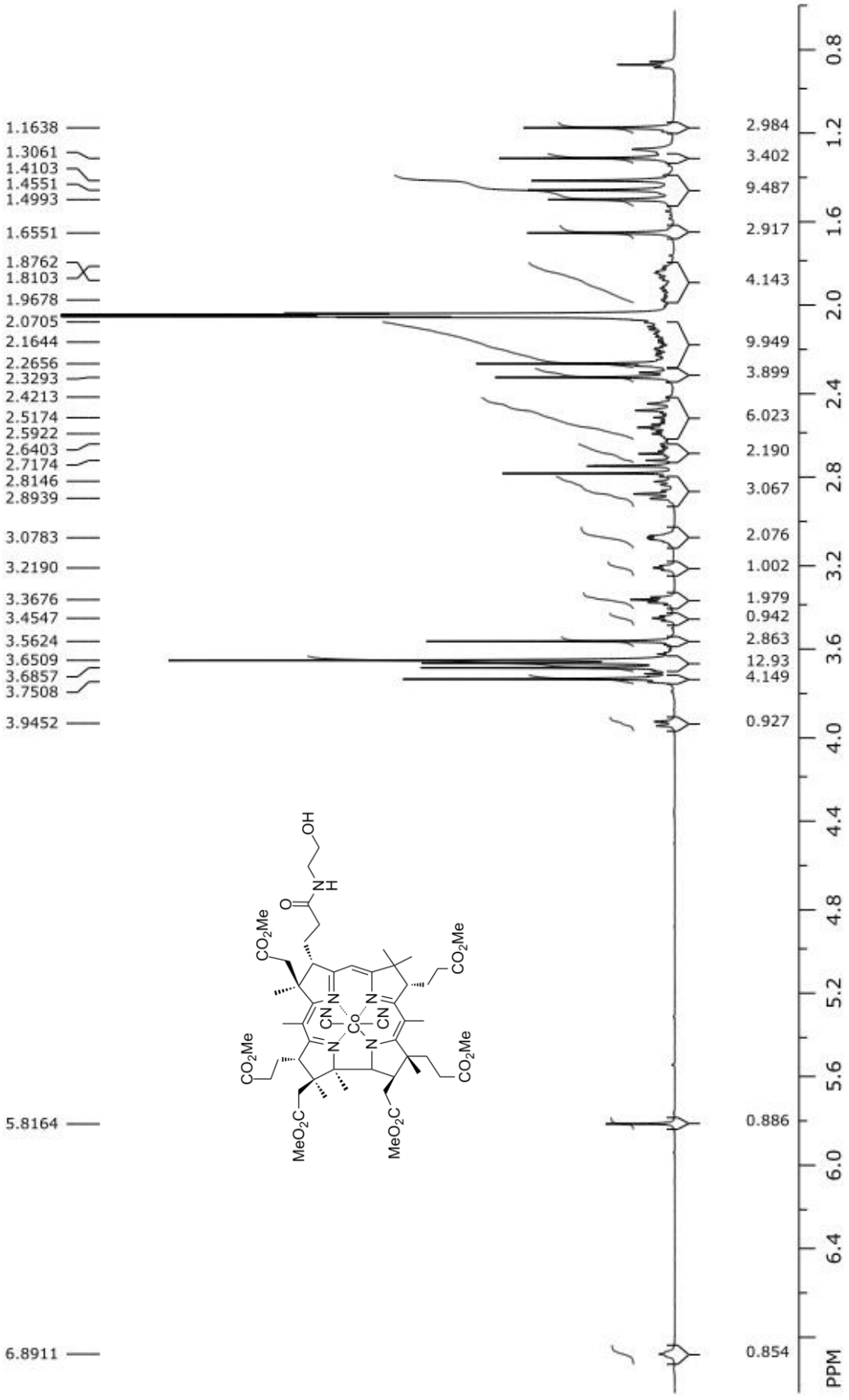
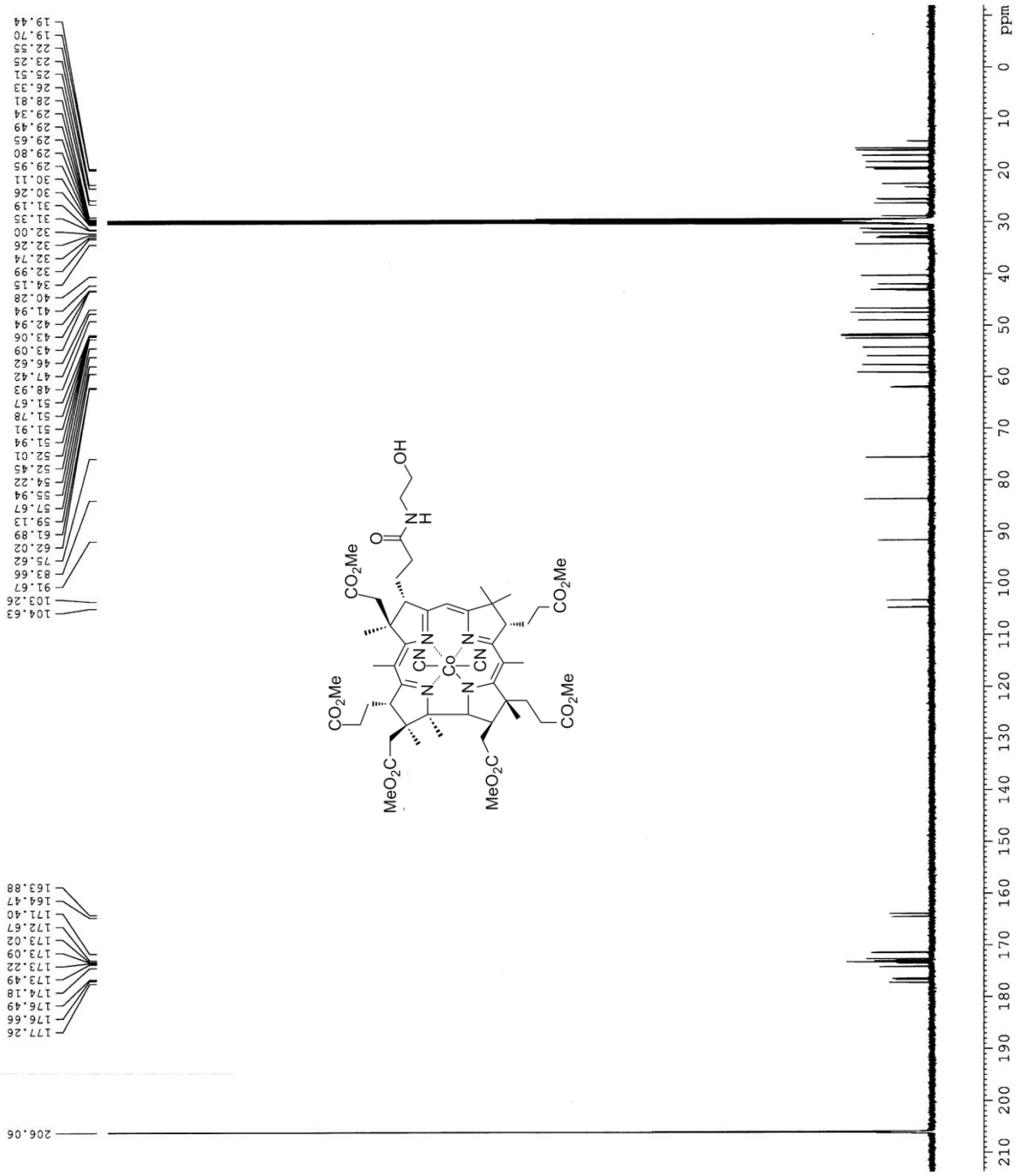


$(CN)_2$Cby(III)(*d*-$NHCH_2CH_2OH$)$(OMe)_6$ 18



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Cobalt(I)-catalysed CH-alkylation of terminal olefins, and beyond†

Maciej Giedyk, Katarzyna Goliszewska, Keith ó Proinsias and Dorota Gryko*

Cobalester, a natural nontoxic vitamin B_{12} derivative, was found to catalyse unusual olefinic sp^2 C–H alkylation with diazo reagents as a carbene source instead of the expected cyclopropanation.

Diazocarbonyl compounds are important and useful reagents in organic chemistry due to their ease of integration into numerous chemical transformations under mild conditions.[1] Particularly, they are utilised in cyclopropanation, direct insertion into sp^3 C–H, aromatic sp^2 C–H, and sp C–H bonds as well as heteroatom–H bonds.[2–7] Until recently, C–H functionalisation was mainly achieved with Rh and Cu catalysts,[1d] along with other group 11 metals.[8] Nowadays, methodologies avoiding the use of harmful and toxic reagents have attracted a lot of attention with Fe,[9] Co,[10] and Cr[11] being recognised as valuable catalysts.

In this regard, vitamin B_{12} (**1**, Cbl, cobalamin, Fig. 1), being a natural, environmentally benign Co-complex, bears advantages over other transition metal compounds. Its catalytic properties are inherently connected with the ability to form alkyl derivatives *via* Co(II) and Co(I) reduced forms.[12] To date, 'supernucleophilicity' of the Co(I) species (B_{12}s) and radical character of the Co(II) form (B_{12}r) have been investigated in numerous chemical reactions, such as dehalogenation, additions to activated alkenes, functional group migration, dimerisation, cyclopropanation and cyclopropane ring opening.[13] In 2004 Chen and Zhang reported that the reaction of styrenes with ethyl diazoacetate (EDA) in the presence of (HO)Cbl led to cyclopropane derivatives (Scheme 1).[14]

Their proposed mechanism suggests that the reaction involves the formation of Co(II) species that when reacted with EDA generates a C-centred alkylcobalamin radical. On the other hand, carbene complexes, formed *via* reaction of diazo compounds with metal salts, including cobalt, are known to trap nucleophiles and insert into C–H bonds.[1a] On this basis, we questioned whether and how diazo reagents, those of electrophilic character, would react with 'supernucleophilic' B_{12}s. We presumed that the reaction should provide alkylcobalamins that upon heating or light irradiation would generate radicals that could either disproportionate, abstract $H^{\bullet}$, or self-couple.

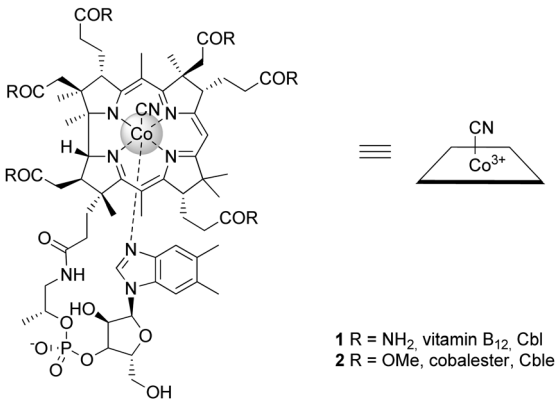
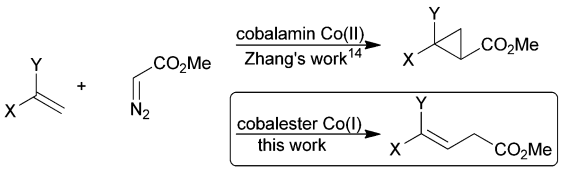


Fig. 1 Structure of vitamin B_{12} and cobalester.

Our recent investigations into vitamin B_{12}-catalysed dimerisation of benzyl bromides led us to use newly developed cobalester (**2**, Cble(III)) as a catalyst for the designed reaction.[15] Firstly, EDA was reacted with cobalester (**2**) in the presence of a reducing system (Zn/NH_4Cl) to prove the formation of hypothesised alkylcobalamin. Indeed, mass spectrometry analysis (ESI MS) of the reaction mixture showed a peak at 1506.8 corresponding to the desired alkylcobalester (see the ESI†). Following this observation, EDA was reacted with 1,1-diphenylethene (**3**) in the presence of Cble (**2**) under light irradiation (Scheme 2, Table 1, entry 3).



Scheme 1 Functionalisation of olefins with EDA.

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† Electronic supplementary information (ESI) available: Details of optimization and mechanistic studies, and spectroscopic data for all new compounds. See DOI: 10.1039/c5cc07363d

Electronic Supplementary Information

for

Cobalt(I)-catalyzed CH-alkylation of terminal olefins, and beyond

Maciej Giedyk, Katarzyna Goliszewska, Keith ó Proinsias and Dorota Gryko*

Table of Contents

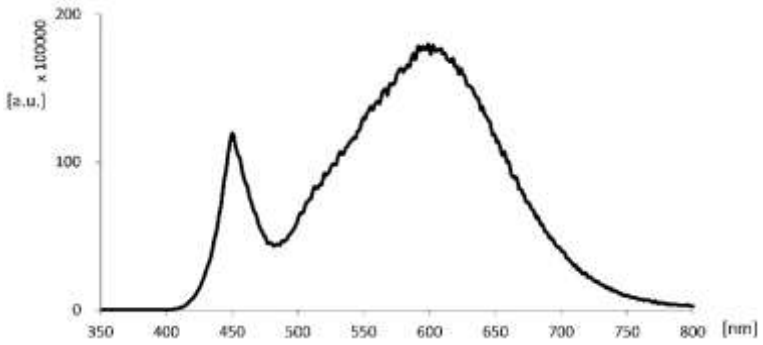
1. General Information

✓ All solvents and chemicals used in the syntheses were of reagent grade and were used without further purification. High resolution ESI mass spectra were recorded on a Mariner and SYNAPT spectrometer. ^{1}H and $^{13}CNMR$ spectra were recorded at rt on Bruker 400 and Varian 600 MHz instruments with TMS as an internal standard. EPR spectrum was recorded on Magnettech MS200 spectrometer. Thin layer chromatography (TLC) was performed using Merck Silica Gel GF254, 0.20 mm thickness.

✓ ND_4Cl used in deuterium experiment contained 98 atom% of deuterium.

✓ Photo-induced reactions were performed using a homemade photoreactor equipped with two LED light bulbs (300 Lm; warm light).

Emission spectrum of the LED light bulbs used:



✓ Cobalester (Cble **2**) was synthesized according to the reported procedure on 1 g scale.[1]

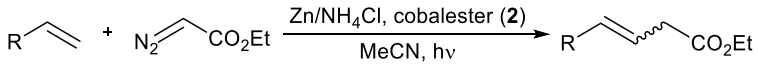
✓ -*O*TBDMS-Protected ketone **11** was synthesized according to the reported procedure.[2]

✓ Enamide **13** was synthesized according to the reported procedure.[3]

✓ In all reactions activated Zn powder was used. Activation process comprised: 1) washing with 10% HCl, 2) grinding, 3) washing with water, MeOH and Et_2O, 4) drying *in vacuo*.

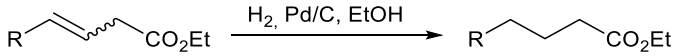
2. Description of general synthetic procedures

Representative procedure for C-H functionalization of olefins:



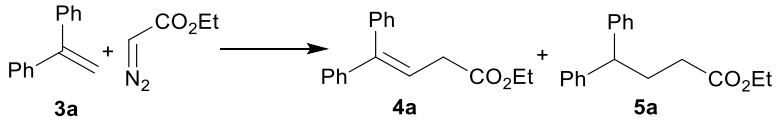
A reaction vessel equipped with a stirring bar was charged with cobalester (**2**) (14 mg, 2 mol%), Zn (196 mg, 3.0 mmol) and NH_4Cl (90 mg, 1.7 mmol). Degassed MeCN (5.0 mL) was added and then the reaction mixture was vigorously stirred in anaerobic conditions until cobalester (**2**) was fully reduced (color change was observed from red to green). Subsequently, an olefin (0.5 mmol) and ethyl diazoacetate (156 µl, 1.5 mmol) were added and the reaction was irradiated with visible light from LEDs (300 Lm; warm light). Progress of the reaction was monitored using thin layer chromatography (TLC). When the reaction was completed, it was diluted with Et_2O (20 mL), filtered through the cotton wool and concentrated in vacuo. The crude product was purified by flash chromatography (SiO_2).

Representative procedure for hydrogenation step:



Crude olefin was dissolved in EtOH (5.0 mL) and hydrogenated in a flow of hydrogen in the presence of Pd/C (30 mg) at room temperature for 2 h. The reaction mixture was filtered, washed with EtOH and concentrated in vacuo. The crude product was purified by flash chromatography (SiO_2).

3. Optimization studies



1. Background reactions

Entry	Light	Temperature [°C]	Conversion [%]	Yield [%]	4a:5a ratio
1	-	RT	63	34	1 : 1.4
2	LED	15	78	28	1 : 2.6
3	**LED**	**-**	**94**	**74**	**1 : 2.6**
4	-	42	94	65	1 : 1.3
5	-	60	86	68	1 : 1.5

Reaction conditions: Ph_2CCH_2 (0.5 mmol), EDA (3 equiv.), Zn (3 equiv.), NH_4Cl (3.4 equiv.), catalyst **2** (1 mol%), MeCN (2.5 mL), 18 h

2. Optimization of the solvent

Entry	Solvent	Additive	Conversion [%]	Yield [%]
1	**MeCN**	**-**	**n.d.**	**71**
2	Dry MeCN	-	85	63
3	Dry DMF	-	56	15
4	Dry MeOH	-	16	9
5	MeCN	H_2O (1 equiv.)	82	46
6	THF	H_2O (1 equiv.)	80	58
7	Acetone	H_2O (1 equiv.)	74	49

Reaction conditions: Ph_2CCH_2 (0.5 mmol), EDA (3 equiv.), Zn (3 equiv.), NH_4Cl (3.4 equiv.), catalyst **2** (1 mol%), 18 h, light: 2x300 Lm LED warm light

3. The influence of an amount of NH_4Cl

Entry	NH_4Cl [equiv.]	Conversion [%]	Yield [%]	4a:5a ratio
1	1.1	12	10	n.d.
2	2.2	90	48	n.d.
3	**3.4**	**94**	**74**	**2.7 : 1**
4	4.9	95	70	n.d.
5	5.6	85	63	n.d.

Reaction conditions: Ph_2CCH_2 (0.5 mmol), EDA (3 equiv.), Zn (3 equiv.), NH_4Cl, catalyst **2** (1 mol%), MeCN (2.5 mL), 18 h, light: 2x300 Lm LED warm light

4. Optimization of reaction time

Entry	Time [h]	Conversion [%]	Yield [%]
1	3	34	12
2	6	53	30
3	**18**	**94**	**74**

Reaction conditions: Ph_2CCH_2 (0.5 mmol), EDA (3 equiv.), Zn (3 equiv.), NH_4Cl (3.4 equiv.), catalyst **2** (1 mol%), MeCN (2.5 mL), light: 2x300 Lm LED warm light

5. Optimization of EDA equivalents

Entry	EDA [equiv.]	Conversion [%]	Yield [%]
1	2.0	95	34
2	**3**	**94**	**74**
3	4.0	93	48

Reaction conditions: Ph_2CCH_2 (0.5 mmol), EDA, Zn (3 equiv.), NH_4Cl (3.4 equiv.), catalyst **2** (1 mol%), MeCN (2.5 mL), 18 h light: 2x300 Lm LED warm light

6. Determination of the optimal concentration

Entry	Solvent [mL]	Conversion [%]	Yield [%]
1	1	98	56
2	**2.5**	**94**	**74**
3	5	82	42

Reaction conditions: Ph_2CCH_2 (0.5 mmol), EDA (3 equiv.), Zn (3 equiv.), NH_4Cl (3.4 equiv.), catalyst **2** (1 mol%), MeCN, 18 h light: 2x300 Lm LED warm light

7. The influence of a catalyst loading on the alkylation reaction

Entry	Catalyst 2 [% mol]	Conversion [%]	Yield [%]	4a:5a ratio
1[a]	0	33	3	n.d.
2	0.5	61	7	n.d.
3	1	94	74	2.7 : 1
4	2	97	80	3 : 1
5 [b]	**2**	**91**	**86**	**3.7 : 1**
6	3	99	76	4 : 1
7	5	100	91	6 : 1

Reaction conditions: Ph_2CCH_2 (0.5 mmol), EDA (3 equiv.), Zn (3 equiv.), NH_4Cl (3.4 equiv.), catalyst **2**, MeCN (2.5 mL), 18 h light: 2x300 Lm LED warm light; [a] 5.6 equiv of NH_4Cl was used; [b] 5 mL of MeCN was used

8. Determination of the optimal amount of Zn

Entry	Zn [equiv.]	NH_4Cl [equiv.]	Conversion [%]	Yield [%]	4a:5a ratio
1	0.9	3.4	50	38	2 : 1
2	1.8	3.4	93	85	2 : 1
3	3	3.4	91	86	3.7 : 1
4	**6**	**3.4**	**99**	**91**	**5 : 1**
5	6	2.2	91	80	8 : 1
6	6	1.1	90	79	13 : 1

Reaction conditions: Ph_2CCH_2 (0.5 mmol), EDA (3 equiv.), Zn, NH_4Cl, catalyst **2** (2 mol%), MeCN (5 mL), 18 h light: 2x300 Lm LED warm light

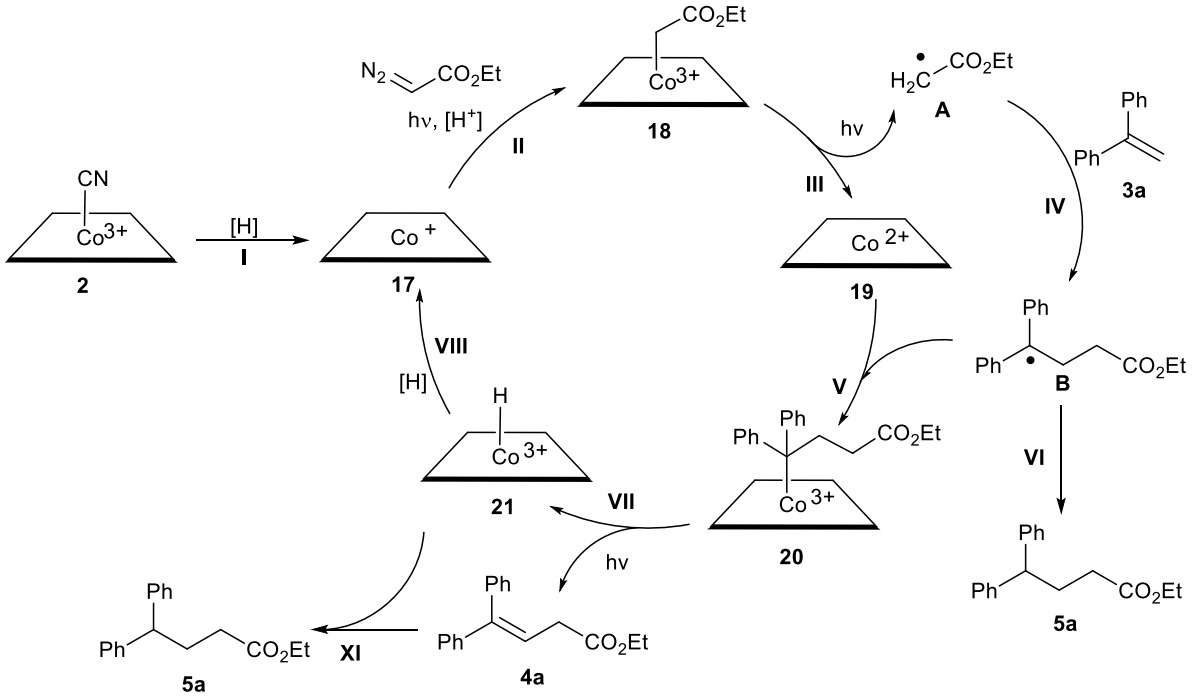
9. Thermally induced reactions

Entry	Light	Temperature [°C]	Conversion [%]	Yield [%]	4a:5a ratio
1	**LED**	**-**	**99**	**91**	**1 : 5.0**
2	-	42	80	76	1 : 1.8
3	-	60	90	62	1 : 2.5

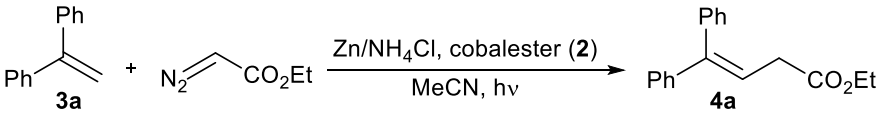
Reaction conditions: Ph_2CCH_2 (0.5 mmol), EDA (3 equiv.), Zn (6 equiv.), NH_4Cl (3.6 equiv.), catalyst **2** (2 mol%), MeCN (5 mL), 18 h light: 2x300 Lm LED warm light

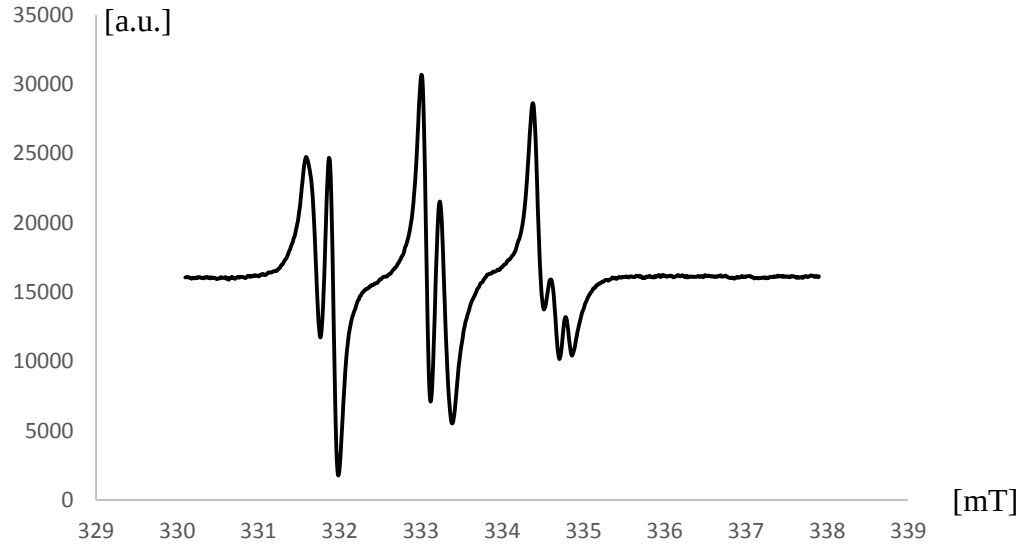
4. Mechanistic considerations

4a. Proposed Mechanism



4b. EPR spectroscopy



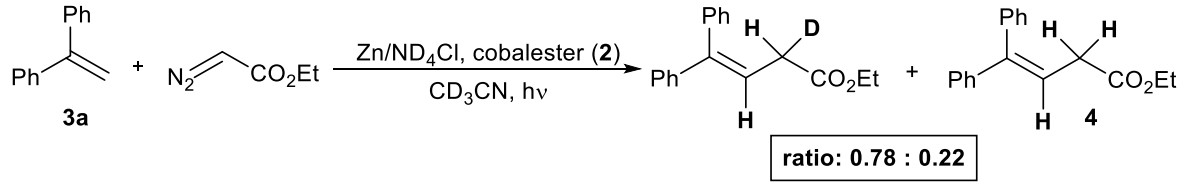


EPR spectra of the reaction mixture was recorded at 9,3 GHz, after 15 min of stirring;

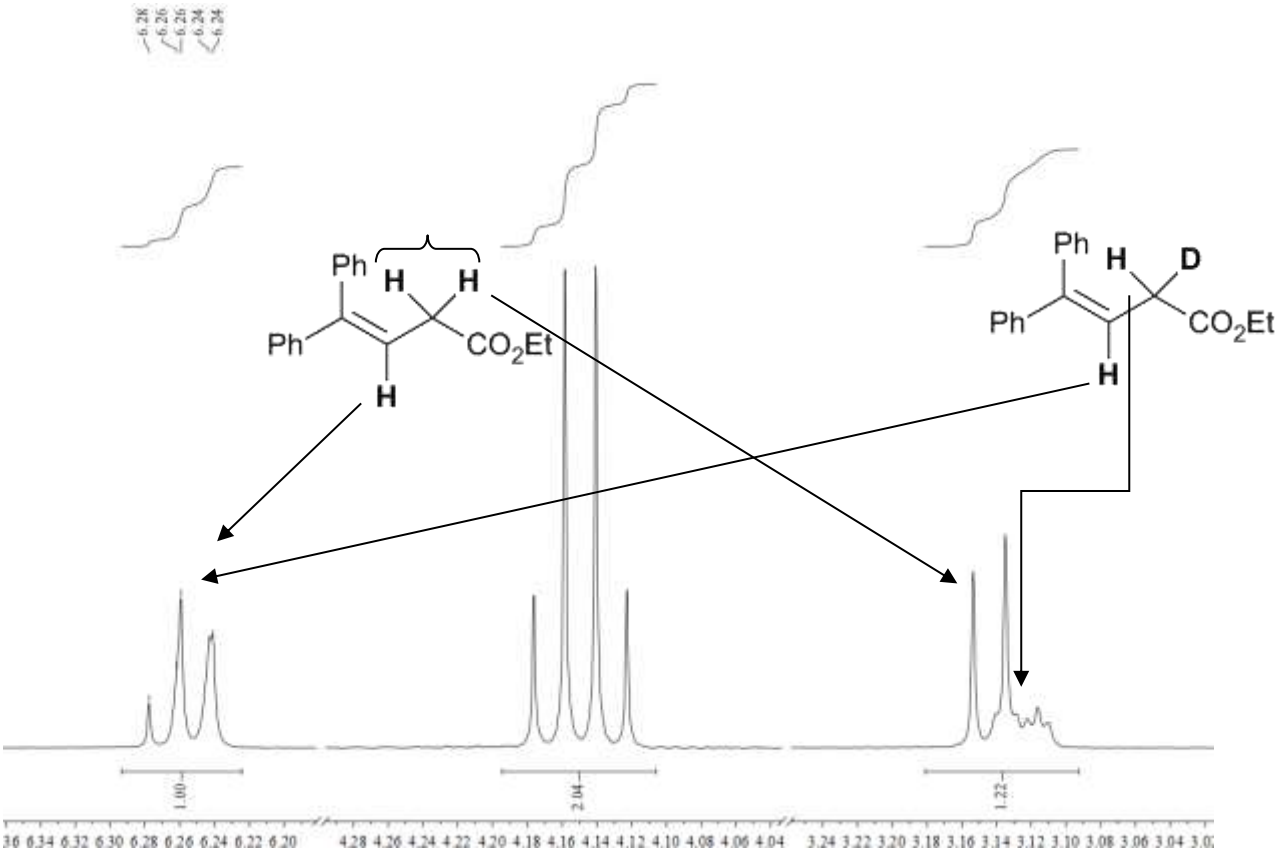
spin trap:	*N-tert*-Butyl-α-phenylnitrone;
central magnetic field:	333 mT;
sweep width:	7,9 mT;
modulation amplitude:	0,06 mT;
microwave strength:	6,3 mW;
sweep time:	30 s;
number of scans:	16

The experiment revealed the presence of radicals in the reaction mixture, suggesting radical mechanism of the reaction.

4c. Experiment with deuterated reagents

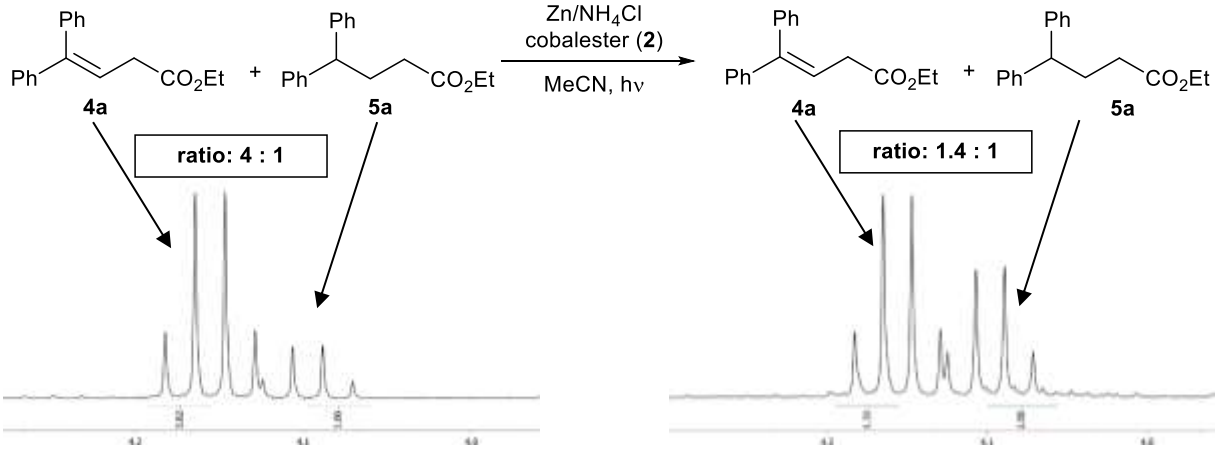


Conditions: substrate (0.5 mmol, 1.0 equiv.), EDA (1.5 mmol, 3 equiv.), cobalester **2** (2 mol%), Zn (6 equiv.), ND_4Cl (3.4 equiv.), CD_3CN (5 mL), light, 18 h



To prove the hypothesis of external proton incorporation at the α-position to the ester group, the experiment with ND_4Cl in CD_3CN was performed. The integration 1.22 for the signal at 3.14 ppm demonstrates that deuterated product is present, though in the mixture with protonated species (which can be explained by the presence of moisture), in 0.78 : 0.22 ratio. This result supports the proposed mechanism, in which alkylated cobalester **18** forms from the reaction of nucleophilic Co(I) species **17** with EDA.

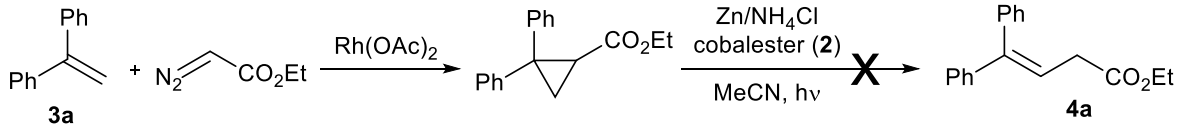
4d. Cobalester (**2**)-catalyzed saturation of double bonds



Conditions: cobalester **2** (2 mol%), Zn (6 equiv.), ND_4Cl (3.4 equiv.), MeCN (5 mL), light, 18 h

The mixture of unsaturated product **4a** and byproduct **5a** (ratio 4:1) was treated with cobalester (**2**) in reductive conditions and irradiated with light. After 18 h composition of the mixture changed to 1.4:1 showing that hydrogenation of olefin **4a** can occur after the desired C-H functionalization, during the reaction.

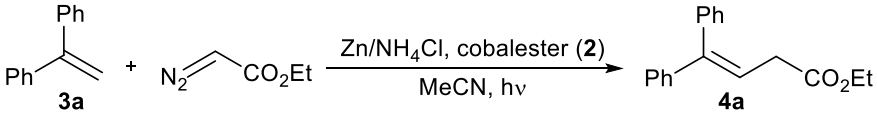
4e. Verification of cyclopropane-intermediate mechanism

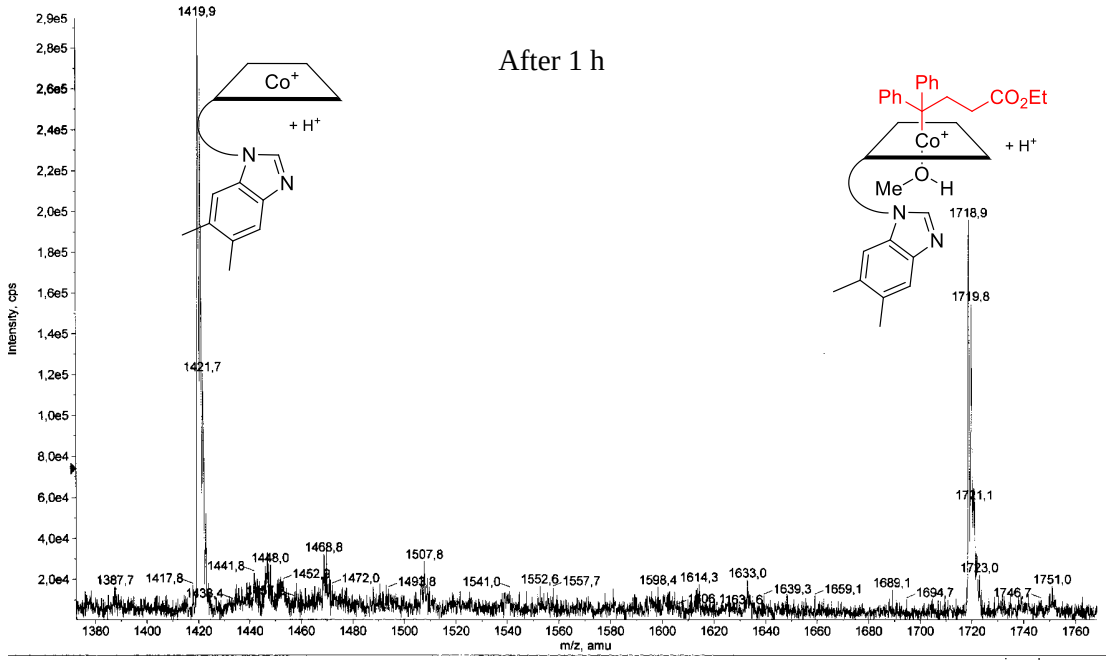


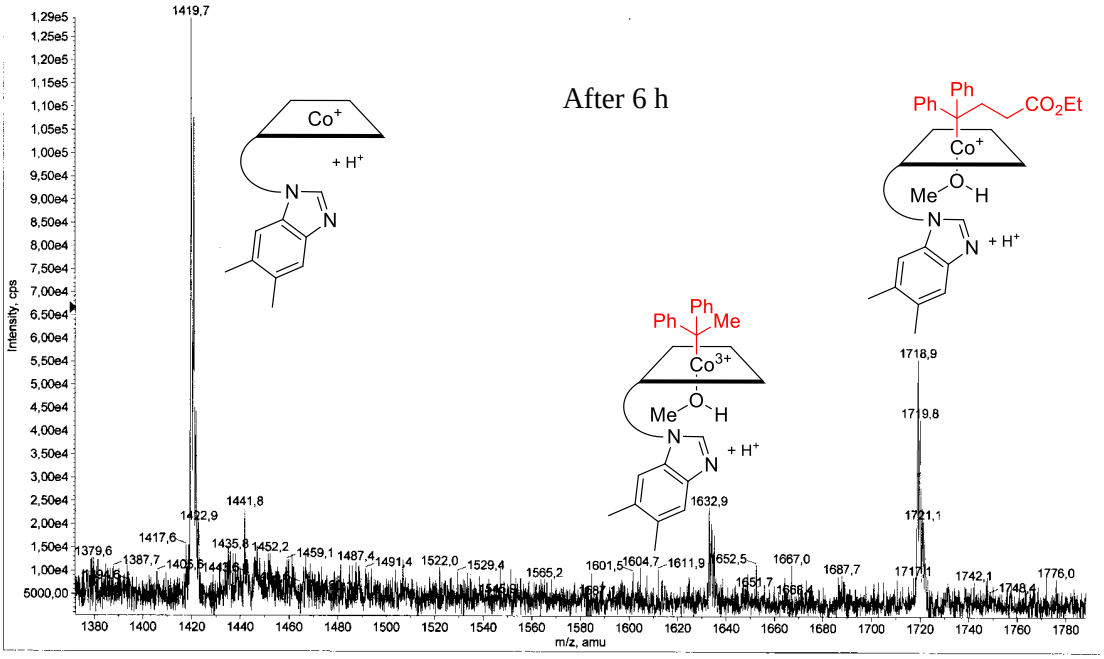
Substituted cyclopropane was synthesized using standard rhodium-catalyzed approach.[4] It was subsequently subjected to our model, cobalt-catalyzed reaction conditions. No conversion was observed (cyclopropane species was recovered), thus excluding cyclopropane-intermediate pathway from the considered reaction mechanism.

4f. Mass spectrometry studies

1) Composition of reaction mixtures were studied using LR mass spectrometry

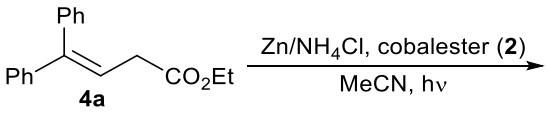


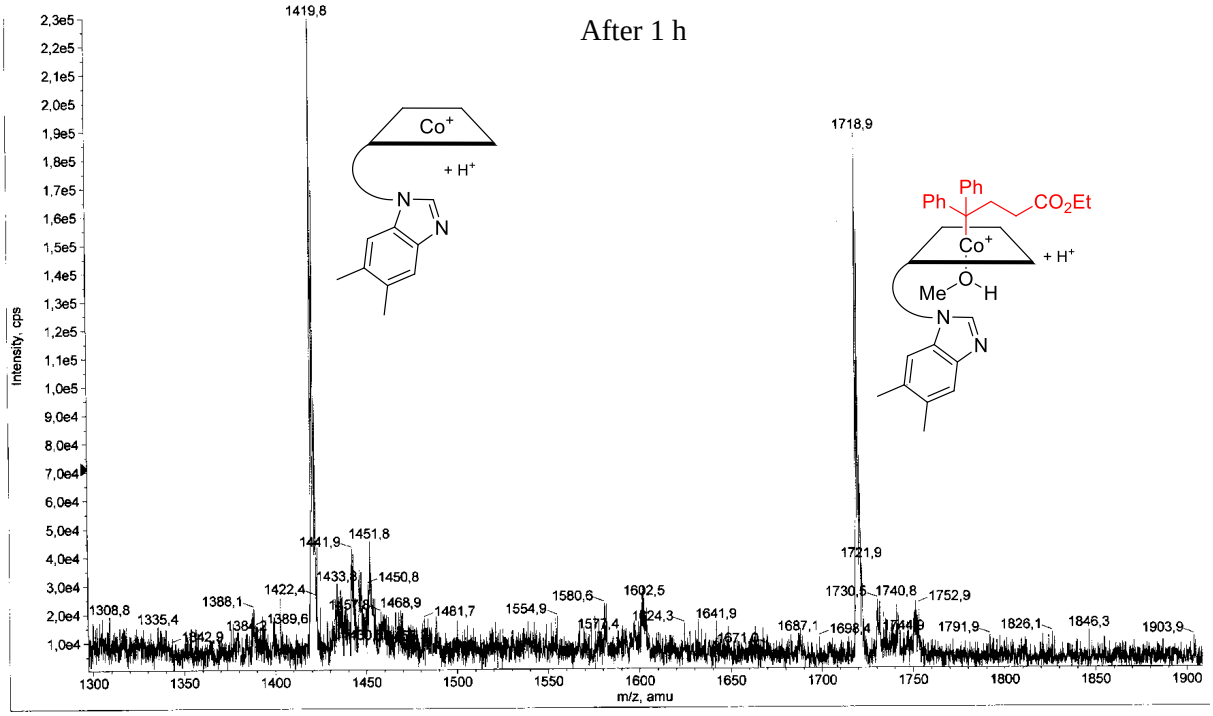




Initially, only the catalyst and the catalyst-product complex was observed suggesting high reaction rate for the alkylation of the catalyst with EDA and subsequent reaction with olefin. Presumably hydrocobaltation is the slowest step and after 6 h , when the concentration of EDA decreases, the interaction between the catalyst and olefin **5** becomes competitive.

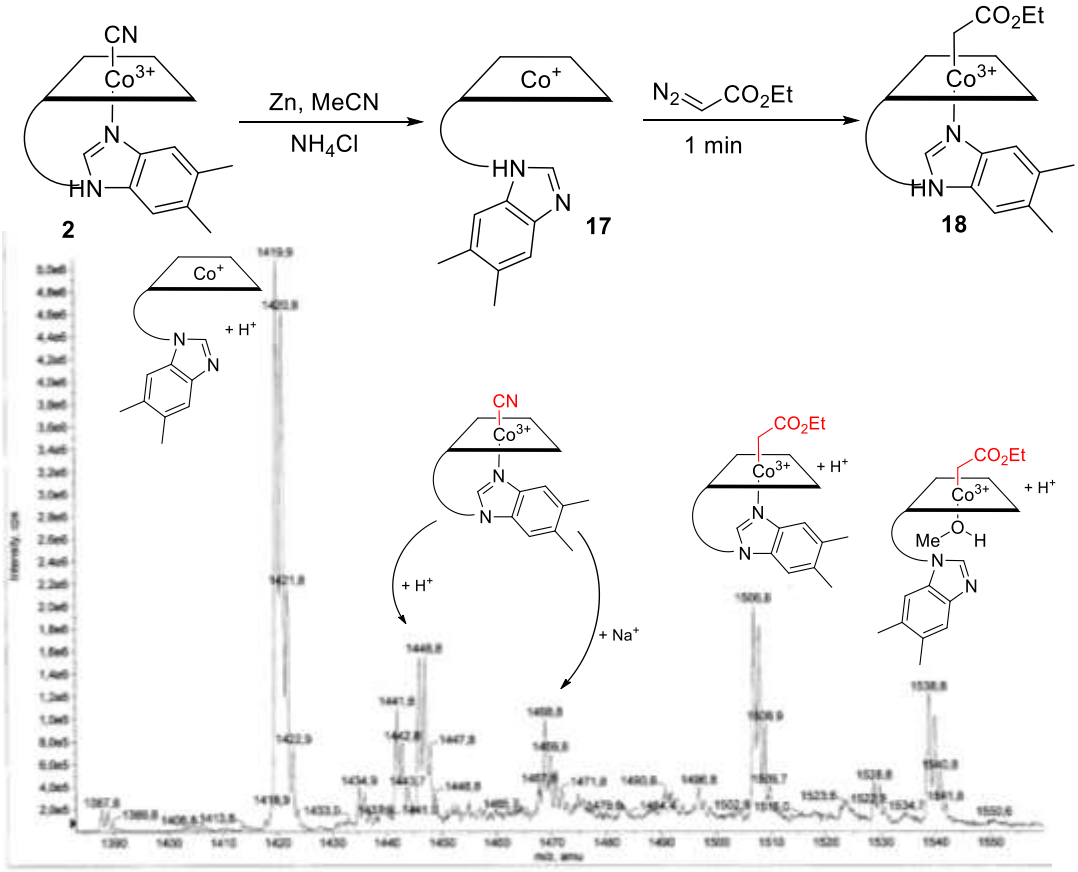
2)

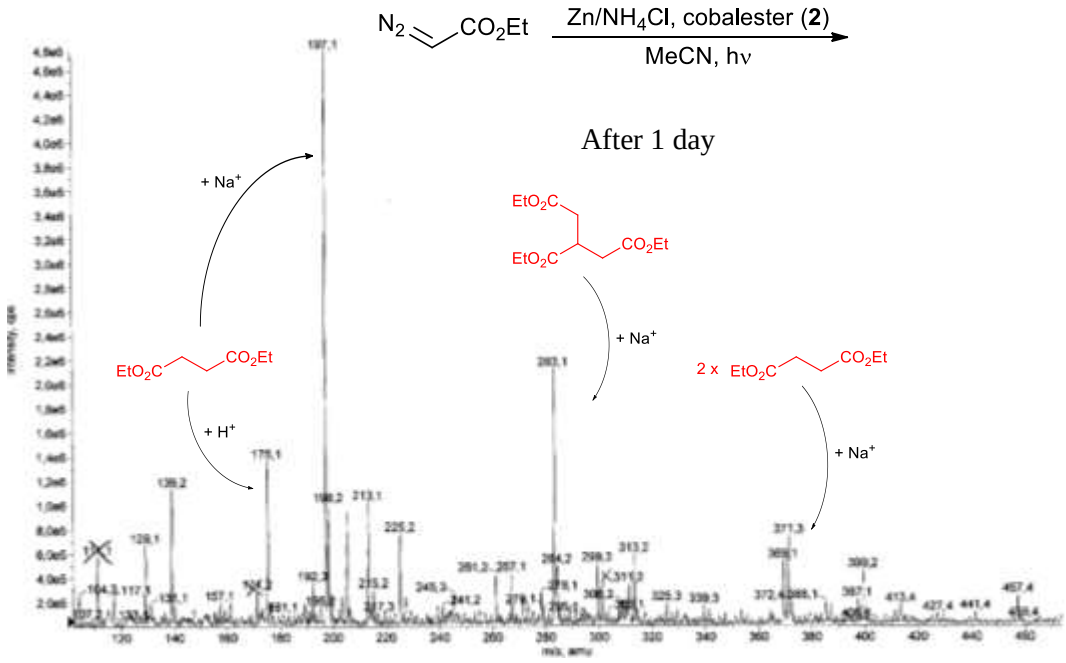




LR ESI spectrum shows peak at 1718.9 corresponding to the catalyst alkylated with product **4a**. We assume that initially formed unsaturated product can undergo hydrogenation catalyzed by cobalester (**2**).

3)

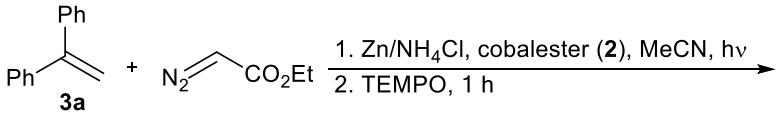


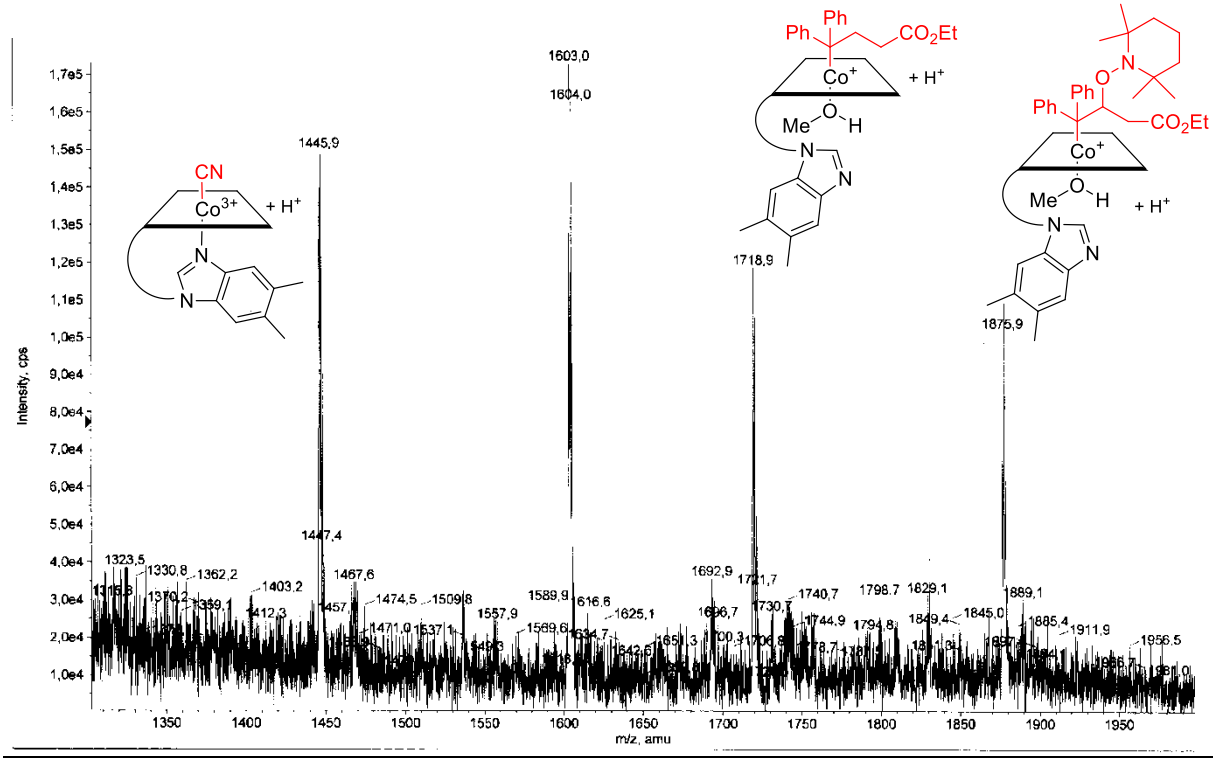


LR ESI experiment revealed that upon treatment of the reduced catalyst **17** with only EDA, alkylated cobalester **18** is formed (peaks at 1506.8 and 1538.8). Hence, vitamin B_{12}

derivatives can be alkylated with diazocompounds. Subjecting EDA to standard reaction conditions results in dimers, trimers etc. formation.

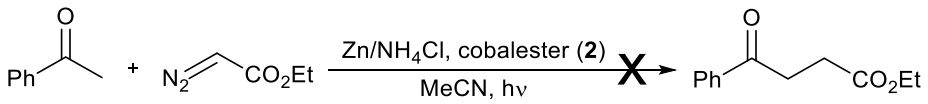
4)





When TEMPO was used as a radical scavenger, a cobalester-product-TEMPO adduct was observed, which further supports the radical mechanism.

4g. Background experiment for reactions of OTMS- and OTBDMS-protected ketones **9** and **11**

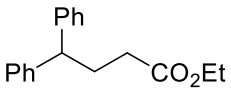


Standard conditions: substrate (0.5 mmol, 1.0 equiv.), EDA (1.5 mmol, 3 equiv.), cobalester **2** (2 mol%), Zn (6 equiv.), NH_4Cl (3.4 equiv.), MeCN (5 mL), light, 18 h

In order to prove that the presence of double bond is necessary for C-H functionalization at the α-position, the reaction of acetophenone with EDA was performed. The reaction led only to the recovery of ketone, thus excluding the mechanism proposed by Gutsche and Hillman.[5]

5. Scope and analytical data for newly synthesized compounds

Ethyl 4,4-diphenylbutanoate (5a)[6]



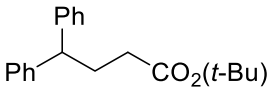
Anal. calcd for $C_{18}H_{20}O_2$: C 80.56, H. 7.51, found: C 80.66, H 7.59

HRMS ESI calcd for $C_{18}H_{20}O_2$ $[M+H]^+$ 268.1463, found: 268.1461

^{1}H (400 MHz, $CDCl_3$): δ = 7.31-7.23 (m, 8H), 7.20-6.97 (m, 2H), 4.10 (q, *J* = 7.1 Hz, 2H), 3.94 (t, *J* = 8.2 Hz, 1H), 2.43-2.35 (m, 2H), 2.30-2.24 (m, 2H), 1.23 (t, *J* = 7.2 Hz, 3H) ppm.[6]

^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.4, 144.2, 128.5, 127.9, 126.3, 60.3, 50.6, 32.8, 30.6, 14.2 ppm.

t-Butyl 4,4-diphenylbutanoate (5b)



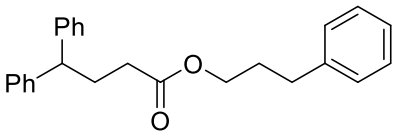
Anal. calcd for $C_{20}H_{24}O_2$: C 81.04, H. 8.16, found: C 80.91, H 8.15

HRMS ESI calcd for $C_{20}H_{24}O_2Na$ $[M+Na]^+$ 319.1667, found: 319.1674

^{1}H (400 MHz, $CDCl_3$): δ = 7.29-7.22 (m, 8H), 7.19-7.15 (m, 2H), 3.92 (t, *J* = 7.9 Hz, 1H), 2.36-2.31 (m, 2H), 2.19-2.15 (m, 2H), 1.43 (s, 9H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.8, 144.3, 128.5, 127.9, 126.3, 80.15, 50.5, 34.0, 30.8, 28.1 ppm.

3-Phenyl-*n*-propyl 4,4-diphenylbutanoate (5c)



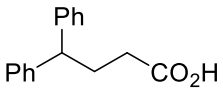
Anal. calcd for $C_{25}H_{26}O_2$: C 83.76, H. 7.31, found: C 83.85, H 7.26

HRMS ESI calcd for $C_{25}H_{26}O_2Na$ $[M+Na]^+$ 381.1830, found: 381.1832

^{1}H (400 MHz, $CDCl_3$): δ = 7.28-7.22 (m, 10H), 7.18-7.13 (m, 5H), 4.05 (t, *J* = 6.5 Hz, 2H), 3.93 (t, *J* = 7.8 Hz, 1H), 2.64 (t, *J*=7.7 Hz, 2H), 2.38 (q, *J*=7.6 Hz, 2H), 2.26 (t, *J* = 7.5 Hz, 2H), 1.92 (m, 2H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.4, 144.2, 141.2, 128.6, 128.5, 128.4, 127.9, 126.4, 126.1, 63.8, 50.6, 32.8, 32.3, 30.7, 30.3 ppm.

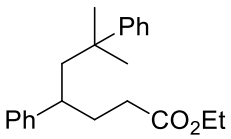
4,4-Diphenylbutanoic acid (5d)[6]



^{1}H (400 MHz, $CDCl_3$): δ = 11.25 (s(br), 1H), 7.29-7.22 (m, 8H), 7.19-7.15 (m, 2H), 3.94 (t, *J* = 7.6 Hz, 1H), 2.42-2.36 (m, 2H), 2.33-2.29 (m, 2H) ppm.[6]

^{13}C NMR (100 MHz, $CDCl_3$): δ = 179.7, 143.9, 128.6, 127.8, 126.4, 50.4, 32.5, 30.3 ppm.

Ethyl 4,6-diphenyl-6-methylheptanate (5e)



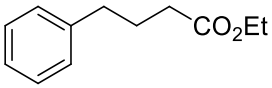
Anal. calcd for $C_{22}H_{28}O_2$: C 81.44, H. 8.70, found: C 81.34, H 8.82

HRMS ESI calcd for $C_{22}H_{28}O_2$ $[M+H]^+$ 324.2089, found: 324.2095

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.26-7.09 (m, 8.9H, overlapping with $CHCl_3$), 6.97-6.94 (m, 2H), 4.04-3.96 (m, 2H), 2.38-2.30 (m, 1H), 2.12-1.97 (m, 2H), 1.95-1.82 (m, 2H), 1.91-1.65 (m, 2H), 1.24 (s, 3H), 1.16 (t, *J* = 7.1 Hz, 3H), 1.11 (s, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.5, 149.1, 145.9, 128.2, 127.9, 127.8, 125.9, 125.8, 125.4, 60.1, 51.3, 42.2, 38.4, 34.0, 32.4, 30.7, 28.3, 14.2 ppm.

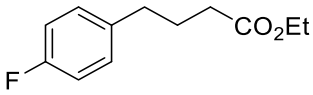
Ethyl 4-phenylbutanoate (5f)[7]



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.31-7.25 (m, 2H), 7.21-7.16 (m, 3H), 4.13 (q, *J* = 7.2 Hz, 2H), 2.65 (t, *J* = 7.8 Hz, 2H), 2.32 (t, *J* = 7.6 Hz, 2H), 1.96 (quin, *J* = 7.6 Hz, *J* = 7.5 Hz, 2H), 1.25 (t, *J* = 7.1 Hz, 3H) ppm.[7]

^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.5, 141.4, 128.5, 128.4, 125.9, 60.2, 35.1, 33.7, 26.5, 14.2 ppm.

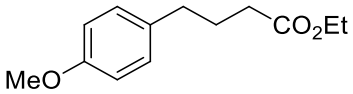
Ethyl 4-(4-fluorophenyl)butanoate (5g)[8]



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.13 (dd, *J* = 8.5, 5.5 Hz, 2H), 7.01 – 6.92 (m, 2H), 4.13 (q, *J* = 7.1 Hz, 2H), 2.62 (t, *J* = 7.6 Hz, 2H), 2.30 (t, *J* = 7.4 Hz, 2H), 1.94 (m, 2H), 1.25 (t, *J* = 7.1 Hz, 3H). ppm.[8]

^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.3, 162.5, 160.1, 137.03, 137.00 129.8, 129.7, 115.2, 115.0, 60.3, 34.3, 33.5, 26.6, 14.2 ppm.

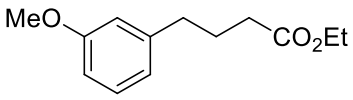
Ethyl 4-(4-methoxyphenyl)butanoate (5j)[8]



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.11-7.07 (m, 2H), 6.85-6.80 (m, 2H), 4.12 (q, *J* = 7.1 Hz, 2H), 3.78 (s, 3H), 2.59 (t, *J* = 7.6 Hz, 2H), 2.30 (t, *J* = 7.7 Hz, 2H), 1.92 (quin, *J* = 7.6 Hz, *J* = 7.5 Hz, 2H), 1.25 (t, *J* = 7.2 Hz, 3H) ppm.[8]

^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.5, 157.9, 133.5, 129.4, 113.8, 60.2, 55.2, 34.2, 33.6, 26.8, 14.2 ppm.

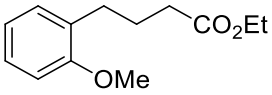
Ethyl 4-(3-methoxyphenyl)butanoate (5k)[8]



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.21-7.16 (m, 1H), 6.79-6.72 (m, 3H), 4.12 (q, *J* = 7.2 Hz, 2H), 3.79 (s, 3H), 2.63 (t, *J* = 7.8 Hz, 2H), 2.32 (t, *J* = 7.3 Hz, 2H), 1.95 (quin, *J* = 7.6 Hz, *J* = 7.4 Hz, 2H), 1.25 (t, *J* = 7.2 Hz, 3H) ppm.[8]

^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.4, 159.7, 143.1, 129.3, 120.9, 114.2, 111.3, 60.2, 55.1, 35.2, 33.7, 26.4, 14.2 ppm.

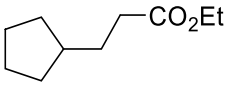
Ethyl 4-(2-methoxyphenyl)butanoate (5l)[8]



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.20-7.09 (m, 2H), 6.90-6.81 (m, 2H), 4.12 (q, *J* = 7.1 Hz, 2H), 3.81 (s, 3H), 2.66 (t, *J* = 7.7 Hz, 2H), 2.32 (t, *J* = 7.8 Hz, 2H), 1.92 (quin, *J* = 7.6 Hz, *J* = 7.5 Hz, 2H), 1.25 (t, *J* = 7.2 Hz, 3H) ppm.[8]

^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.7, 157.5, 130.0, 127.2, 120.4, 110.2, 60.1, 55.2, 34.0, 29.5, 25.1, 14.2 ppm.

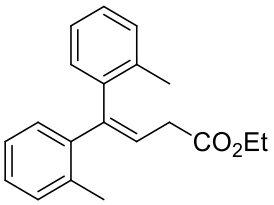
Ethyl 3-(cyclopentyl)propionate (5n)[9]



^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.11 (q, *J* = 7.2 Hz, 2H), 2.30 (t, *J* = 7.8 Hz, 2H), 1.81-1.44 (m, 9H), 1.24 (t, *J* = 7.2 Hz, 3H), 1.15-1.02 (m, 2H) ppm.[9]

^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.0, 60.1, 39.7, 33.7, 32.4, 31.2, 25.1, 14.2 ppm.

Ethyl 4,4-di(2-methylphenyl)but-3-enoate (5o)



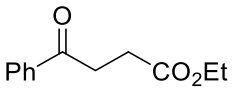
Anal. calcd for $C_{20}H_{22}O_2$: C 81.60, H 7.53, found: C 81.53, H 7.43

HRMS ESI calcd for $C_{20}H_{22}O_2Na$ $[M+Na]^+$ 317.1517, found: 317.1513

^{1}H NMR (400 MHz, $CDCl_3$): δ = δ 7.22-7.06 (m, 8H), 5.96 (t, 7.2 Hz, 1H), 4.13 (q, *J* = 7.2 Hz, 2H), 3.08 (t, *J* = 7.4 Hz, 2H), 2.32 (s, 3H), 2.09 (s, 3H), 1.25 (t, *J* = 7.1 Hz, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.8, 143.6, 141.7, 139.2, 136.3, 135.6, 130.9, 130.5, 130.4, 129.8, 127.4, 127.0, 125.5, 124.7, 60.6, 35.3, 21.0, 19.9, 14.2 ppm.

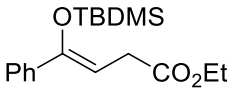
Ethyl 4-oxo-4-phenylbutanoate (10)[10]



^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.00-7.94 (m, 2H), 7.58-7.52 (m, 3H), 4.15 (q, *J* = 7.2 Hz, 2H), 3.30 (t, *J* = 7.1 Hz, 2H), 2.74 (t, *J* = 7.2 Hz, 2H), 1.25 (t, *J* = 7.2 Hz, 3H) ppm.[10]

^{13}C NMR (100 MHz, $CDCl_3$): δ = 198.1, 172.8, 136.6, 133.1, 128.6, 128.0, 60.6, 33.4, 28.3, 14.2 ppm.

Ethyl 4-phenyl-4-(*tert*-butyldimethylsiloxyl)but-3-enoate (12)



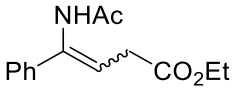
Anal. calcd for $C_{18}H_{28}O_3Si$: C 67.46, H. 8.81, found: C 67.48, H 8.73

HRMS ESI calcd for $C_{18}H_{28}O_3NaSi$ $[M+Na]^+$ 343.1705, found: 343.1707

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.47-7.43 (m, 2H), 7.31-7.25 (m, 3H, overlapping with $CHCl_3$), 5.29 (t, *J* = 7.0 Hz, 1H), 4.16 (q, *J* = 7.2 Hz, 2H), 3.25 (d, *J* = 7.1 Hz, 2H), 1.27 (t, *J* = 7.1 Hz, 3H), 0.98 (s, 9H), -0.06 (s, 6H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.1, 151.6, 139.0, 127.92, 127.90, 126.2, 103.0, 60.5, 31.9, 25.8, 18.3, 14.3, -4.1 ppm

Ethyl 4-phenyl-4-(*N*-acetylamine)but-3-enoate (14a)



as a mixture of E/Z stereoisomers with restricted rotation of the C-N amide bond

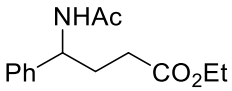
Anal. calcd for $C_{14}H_{17}NO_3$: C 68.00, H 6.93, N 5.66, found: C 68.14, H 6.98, N 5.43

HRMS ESI calcd for $C_{14}H_{17}NO_3Na$ $[M+Na]^+$ 270.1106, found: 270.1111

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.49-7.22 (m, 5H), 7.24 (br s, 0.4H), 6.93 (br s, 0.1H), 6.64 (br s, 0.5H), 6.52 (t, *J* = 8.0 Hz, 0.5H), 6.02 (t, *J* = 7.4 Hz, 0.1H), 5.94 (t, *J* = 7.2 Hz, 0.4H), 4.21-4.08 (m, 2H), 3.28 (d, *J* = 7.4 Hz, 0.2H), 3.20 (d, *J* = 7.1 Hz, 0.8H), 3.04 (d, *J* = 7.8 Hz, 1H), 2.16 (s, 1.2H), 2.04 (s, 1.5H) 1.73 (s, 0.3H), 1.30-1,23 (m, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.0, 171.9, 168.2, 137.3, 136.7, 136.5, 128.9, 128.7, 128.5, 128.4, 125.9, 116.0, 110.0, 61.0, 60.7, 34.2, 34.0, 24.5, 23.5, 14.2 ppm

Ethyl 4-phenyl-4-(*N*-acetylamine)butanoate (14b)



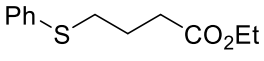
Anal. calcd for $C_{14}H_{19}NO_3$: C 67.45, H 7.68, N 5.62, found: C 67.33, H 7.48, N 5.50

HRMS ESI calcd for $C_{14}H_{19}NO_3Na$ $[M+Na]^+$ 272.1263, found: 272.1265

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37-7.23 (m, 5H, overlapping with $CHCl_3$), 6.01 (d, *J* = 4.2 Hz, 1H), 4.98 (q, *J* = 8.3 Hz, 1H), 4.11 (q, *J* = 7.2 Hz, 2H), 2.41-2.26 (m, 2H), 2.21-2.03 (m, 2H), 1.97 (s, 3H), 1.24 (t, *J* = 7.2 Hz, 3H) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.6, 169.3, 141.5, 128.8, 127.6, 126.5, 60.6, 53.2, 31.3, 30.8, 23.4, 14.2 ppm

Ethyl 4-(phenylthio)butanoate (16)[11]



^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.36-7.25 (m, 4H), 7.20-7.15 (m, 1H), 4.12 (q, *J* = 7.2 Hz, 2H), 2.96 (t, *J* = 7.0 Hz, 2H), 2.45 (t, *J* = 7.2 Hz, 2H), 1.96 (quin, *J* = 7.4 Hz, *J* = 7.3 Hz, 2H), 1.25 (t, *J* = 7.2 Hz, 3H) ppm.[11]

^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.9, 136.1, 129.4, 128.9, 126.0, 60.4, 33.0, 32.9, 24.4, 14.2 ppm.

6. References

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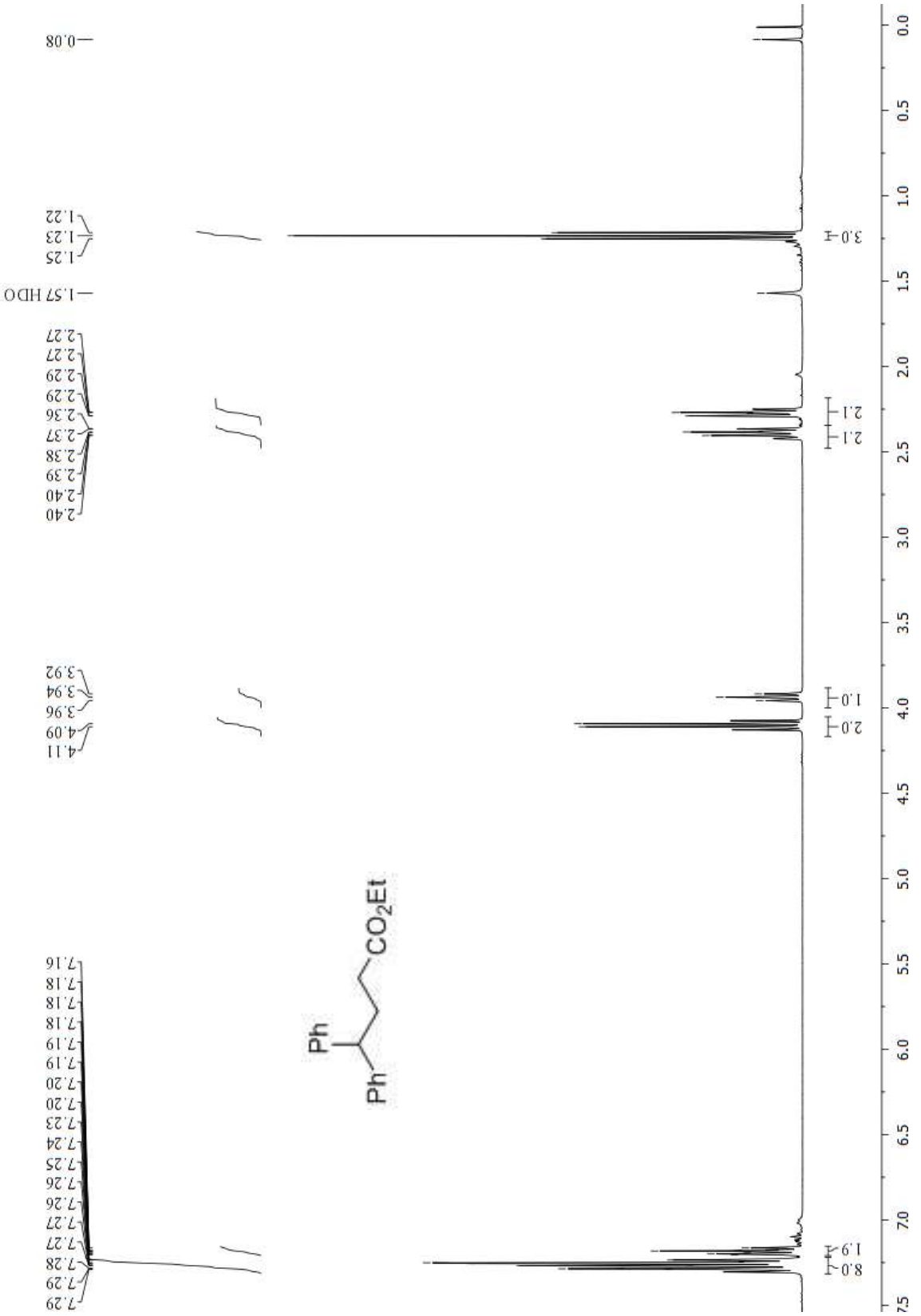
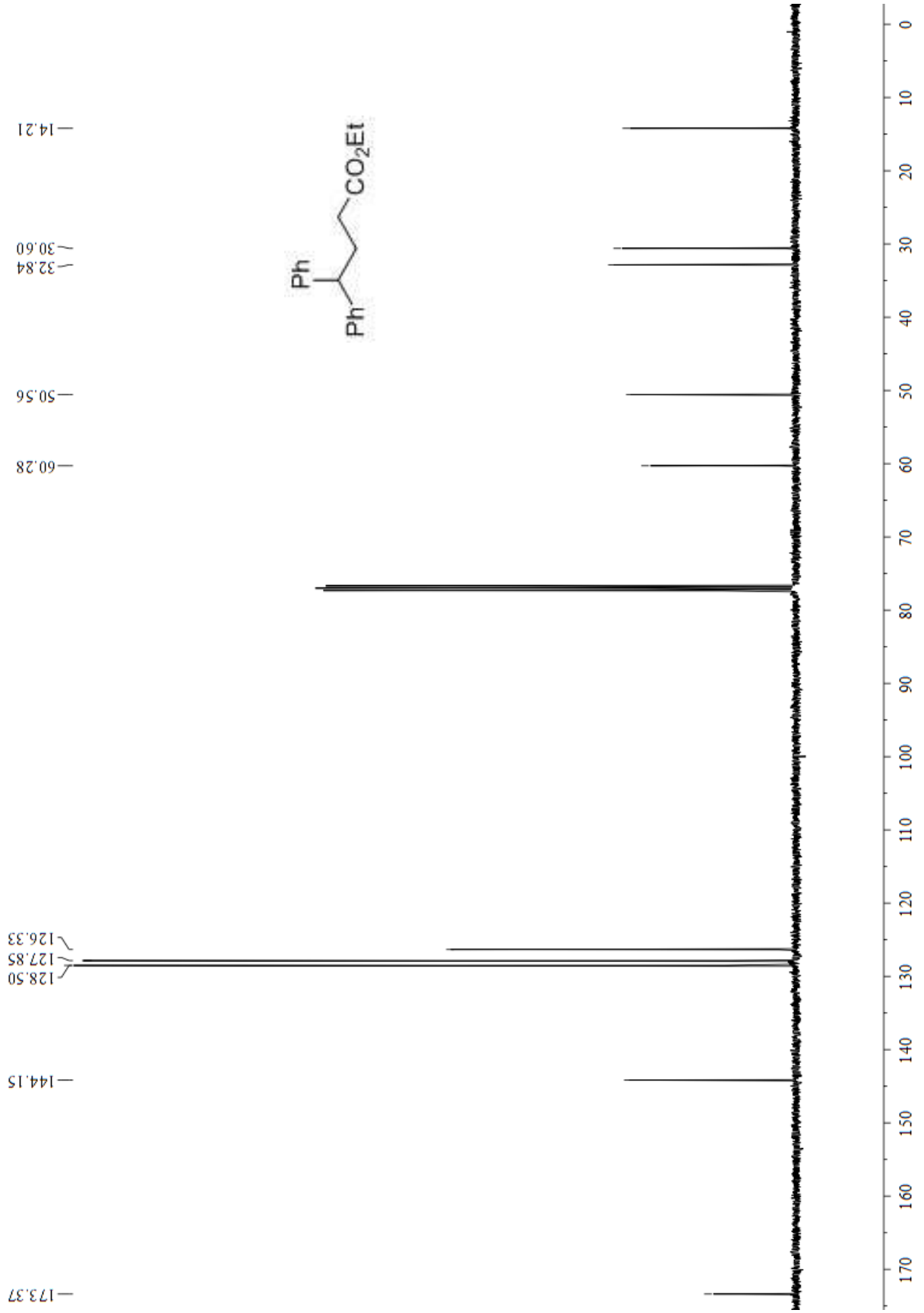
7. ^{1}H and ^{13}C NMR spectra

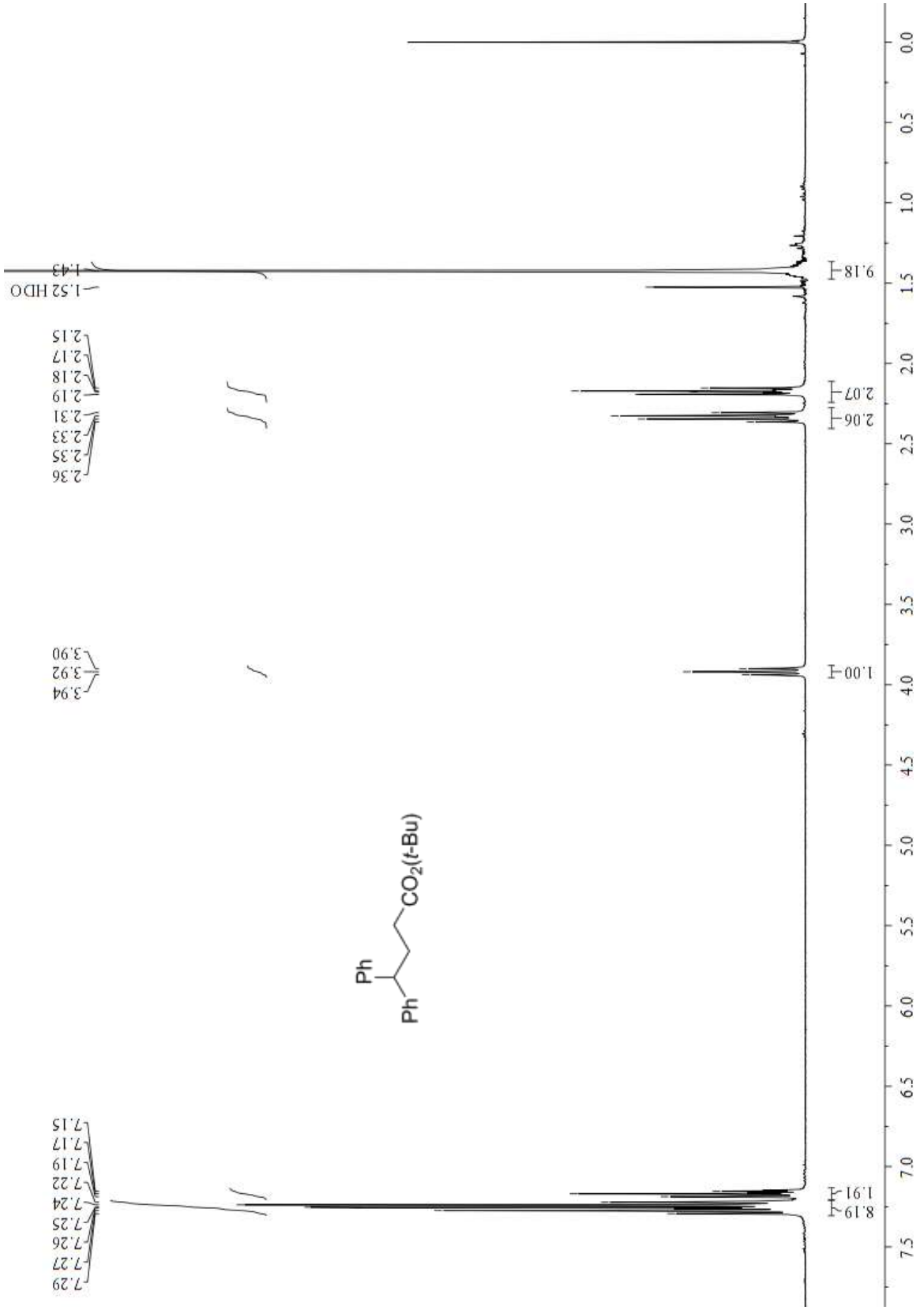
Ethyl 4,4-diphenylbutanoate (5a) - ^{1}H NMR



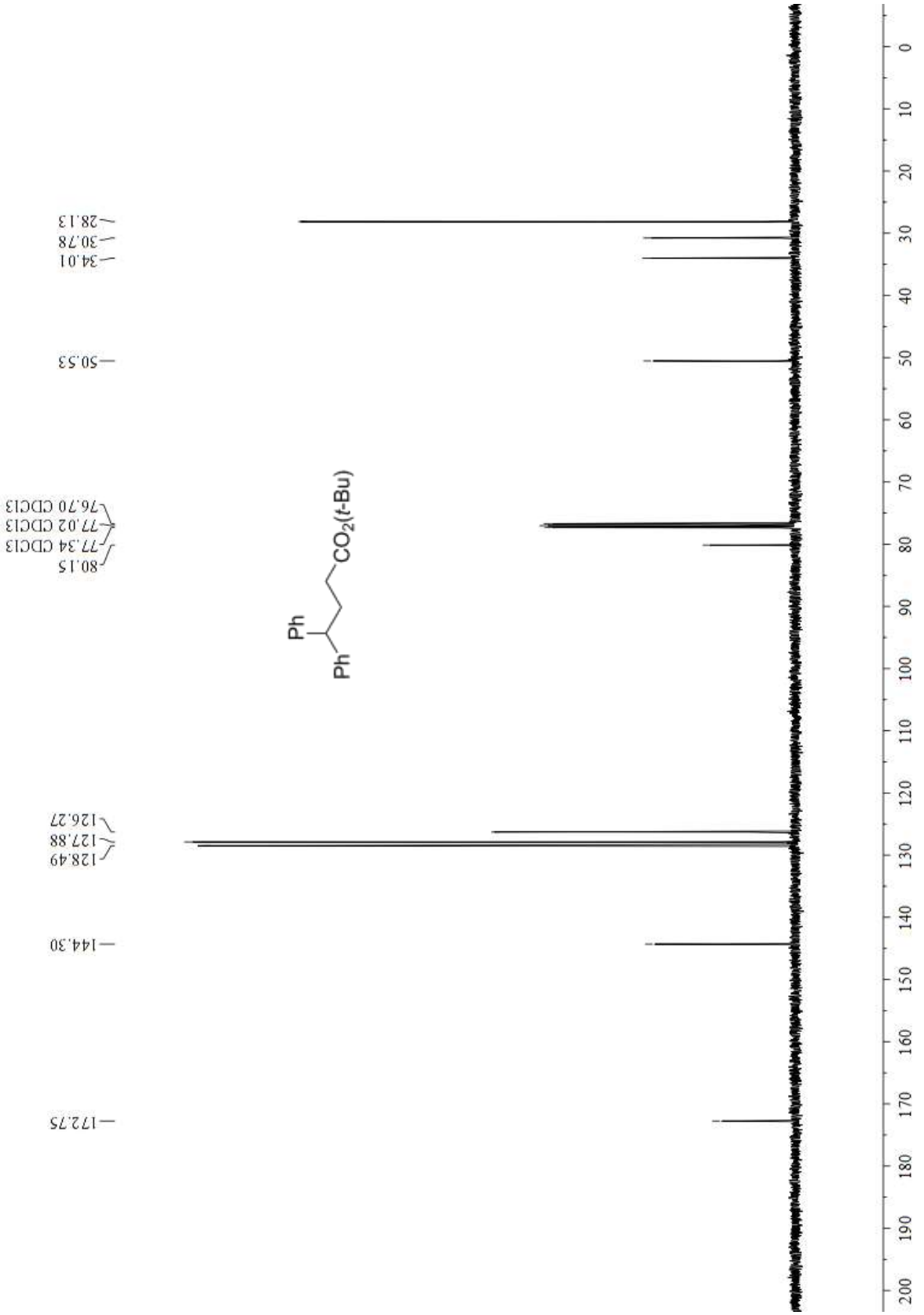
Ethyl 4,4-diphenylbutanoate (5a) – ^{13}C NMR



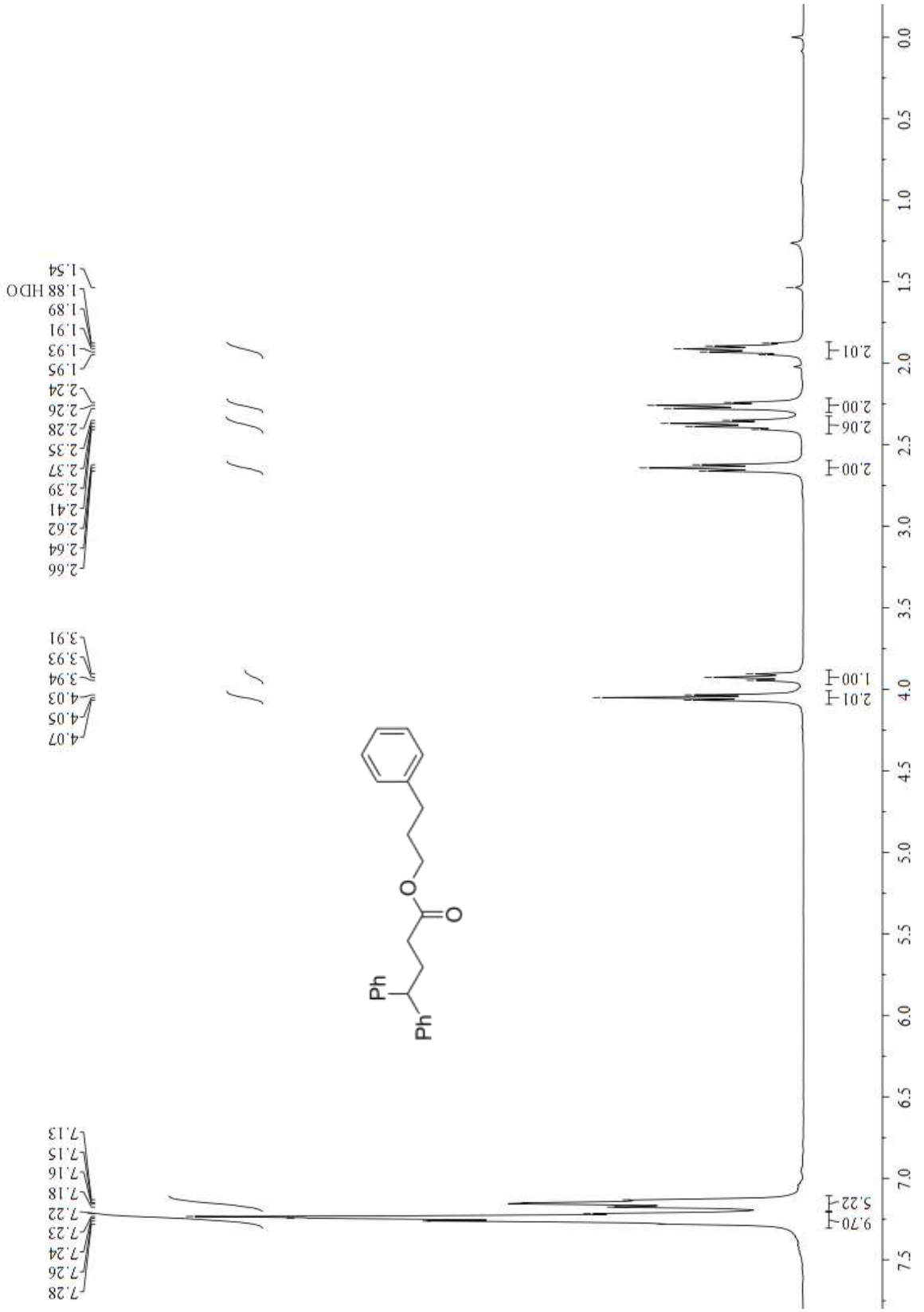
_t_-Butyl 4,4-diphenylbutanoate (5b) - ^{1}H NMR



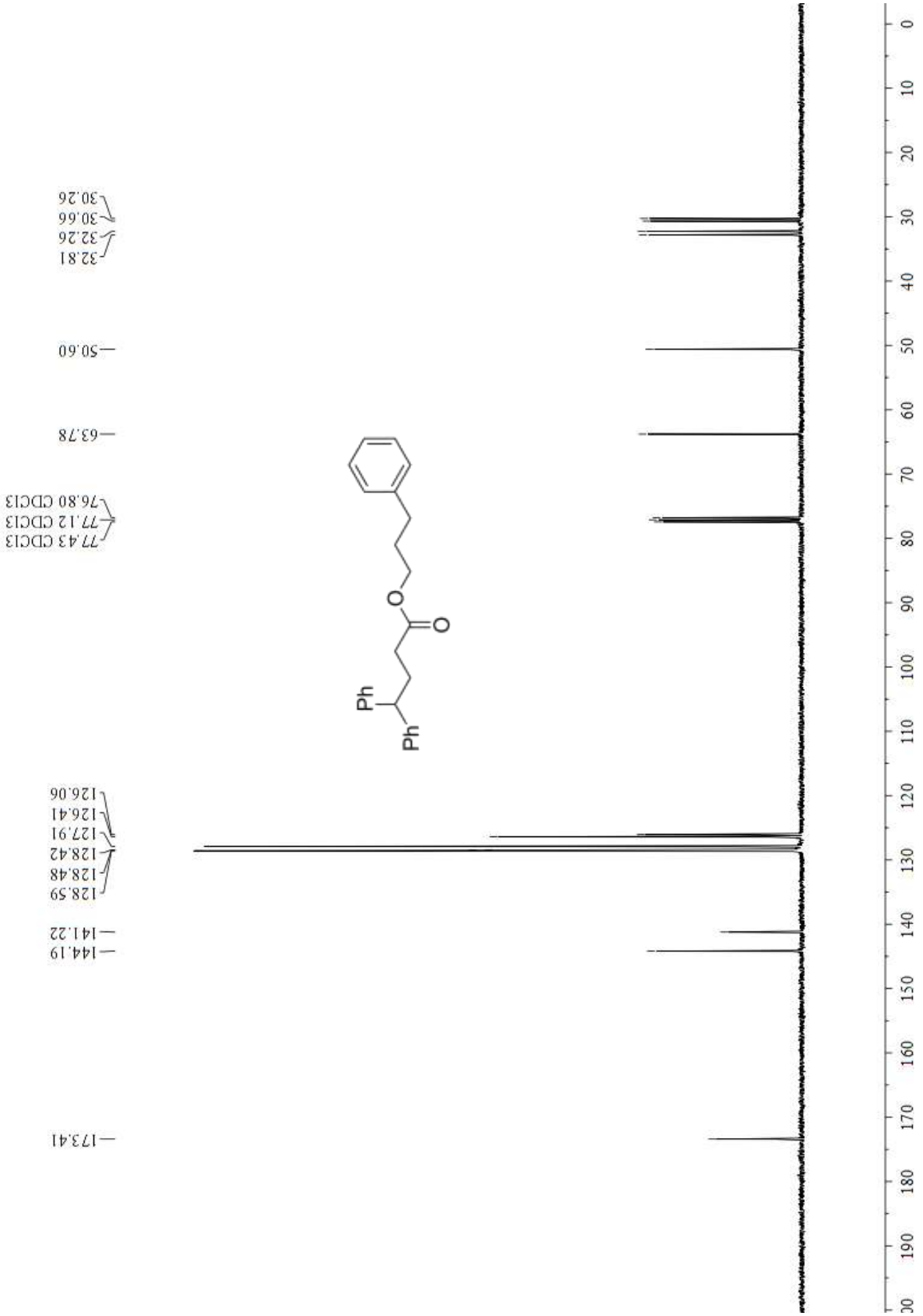
_t_-Butyl 4,4-diphenylbutanoate (5b) – ^{13}C NMR



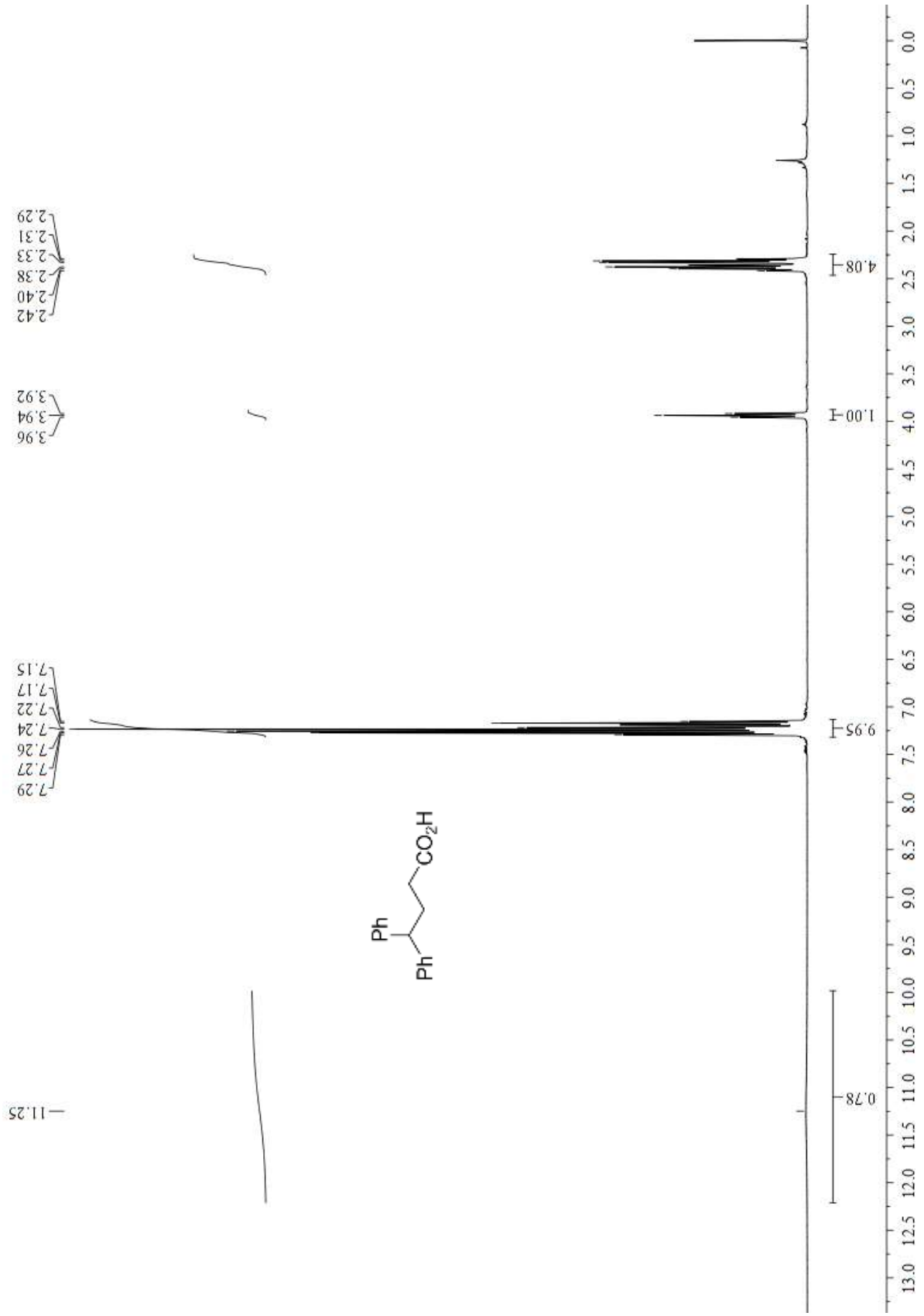
3-Phenyl-*n*-propyl 4,4-diphenylbutanoate (5c) - ^{1}H NMR



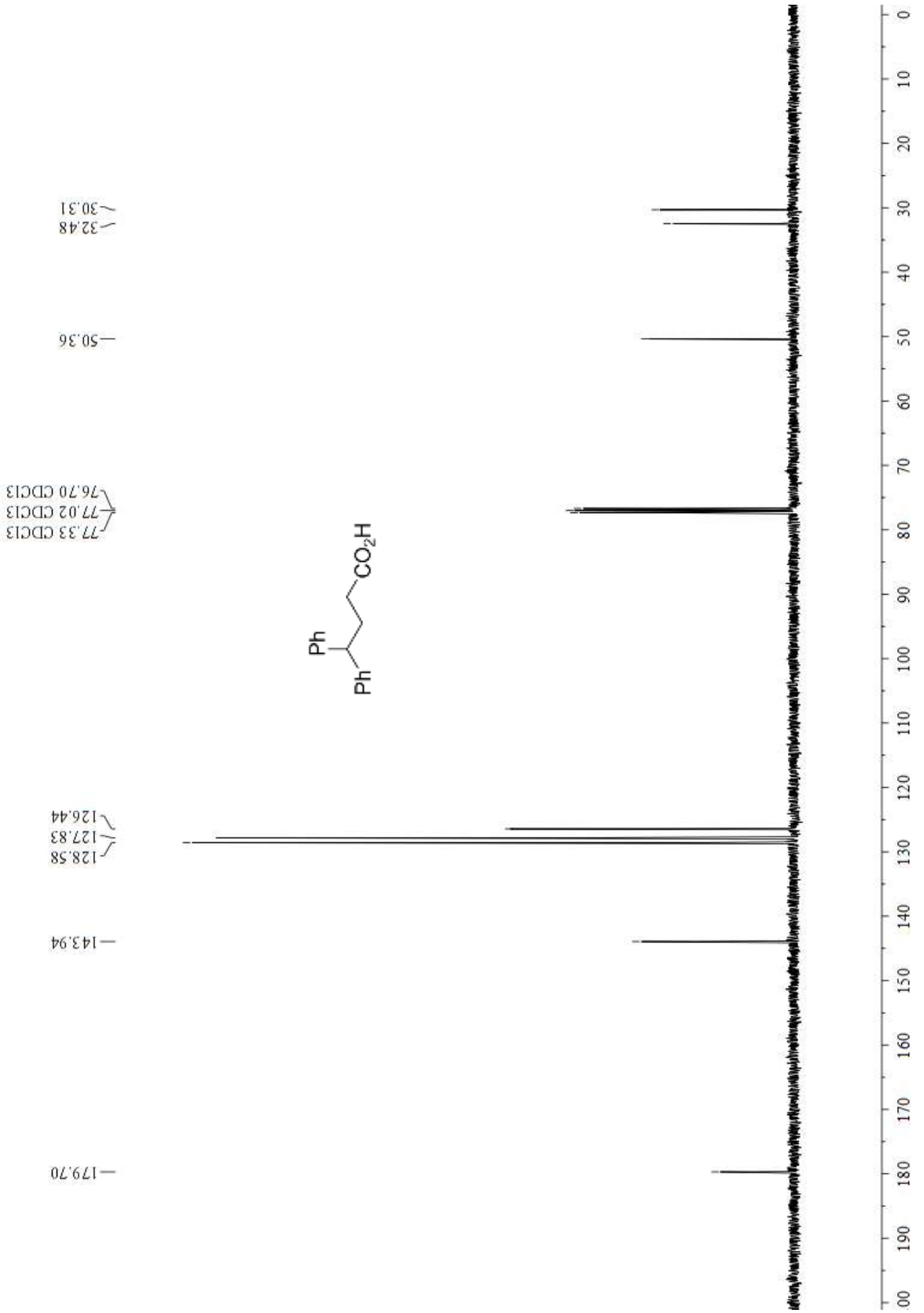
3-Phenyl-*n*-propyl 4,4-diphenylbutanoate (5c) – ^{13}C NMR



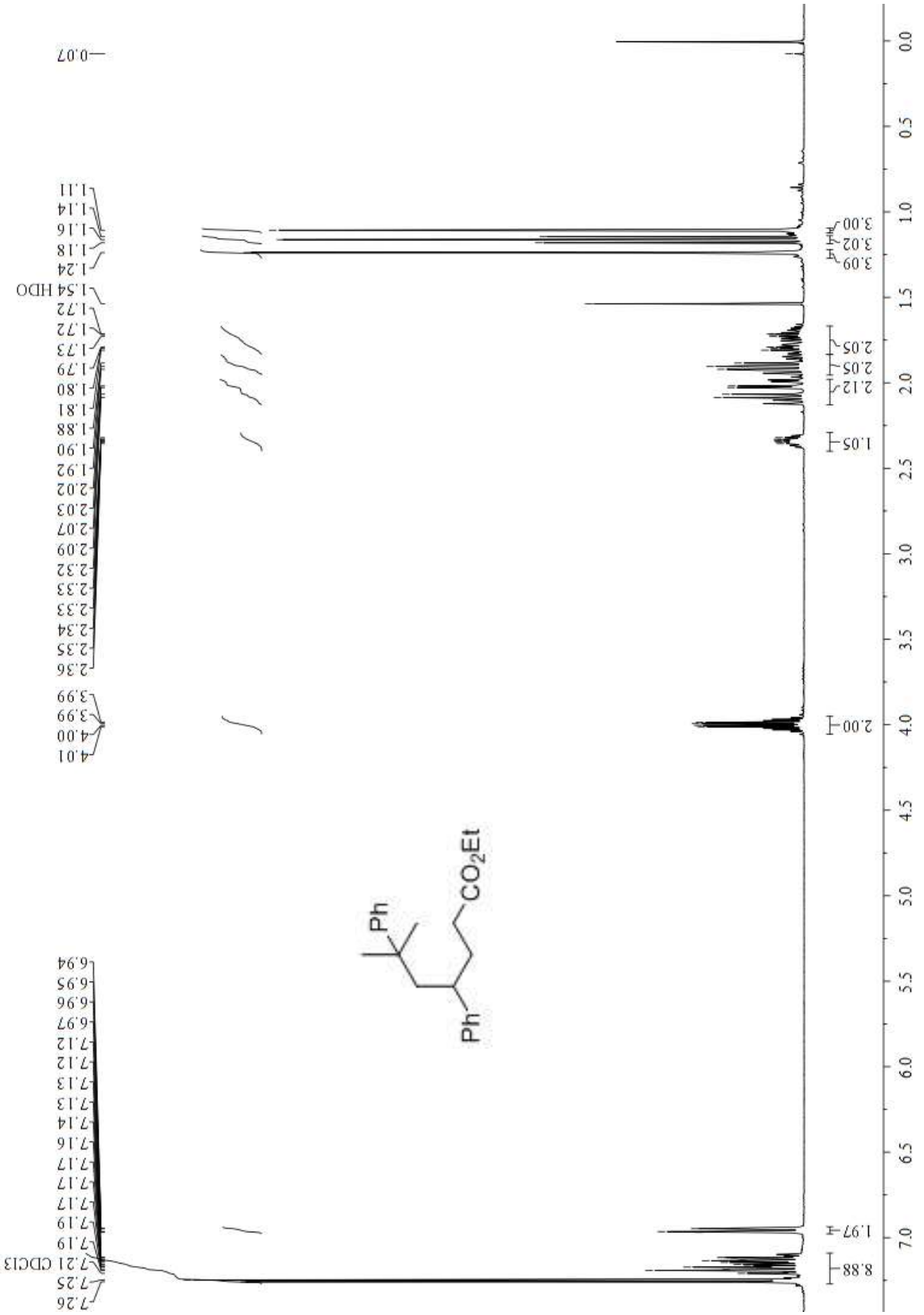
4,4-Diphenylbutanoic acid (5d) - ^{1}H NMR



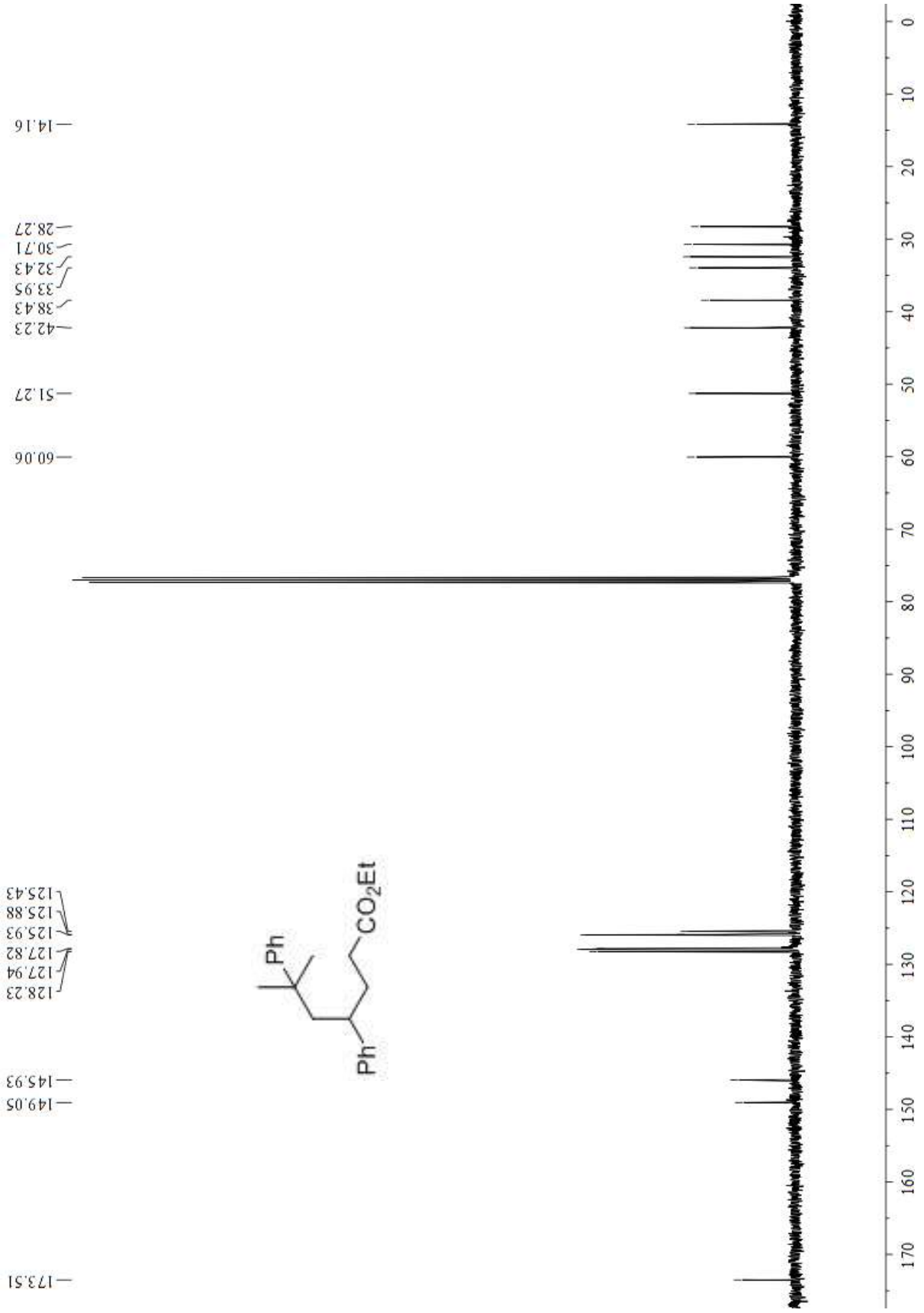
4,4-Diphenylbutanoic acid (5d) – ^{13}C NMR



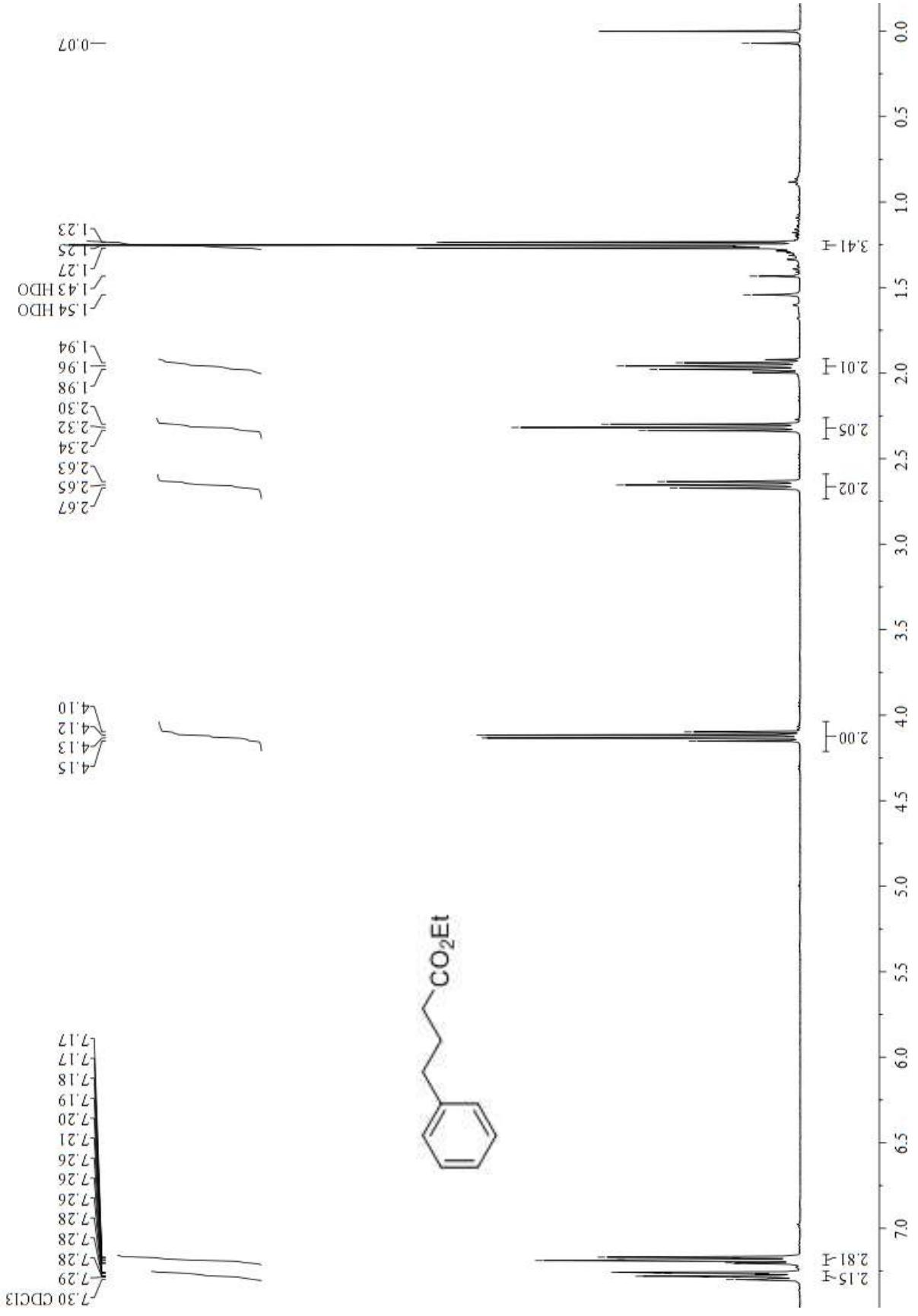
Ethyl 4,6-diphenyl-6-methylheptanate (5e) – ^{1}H NMR



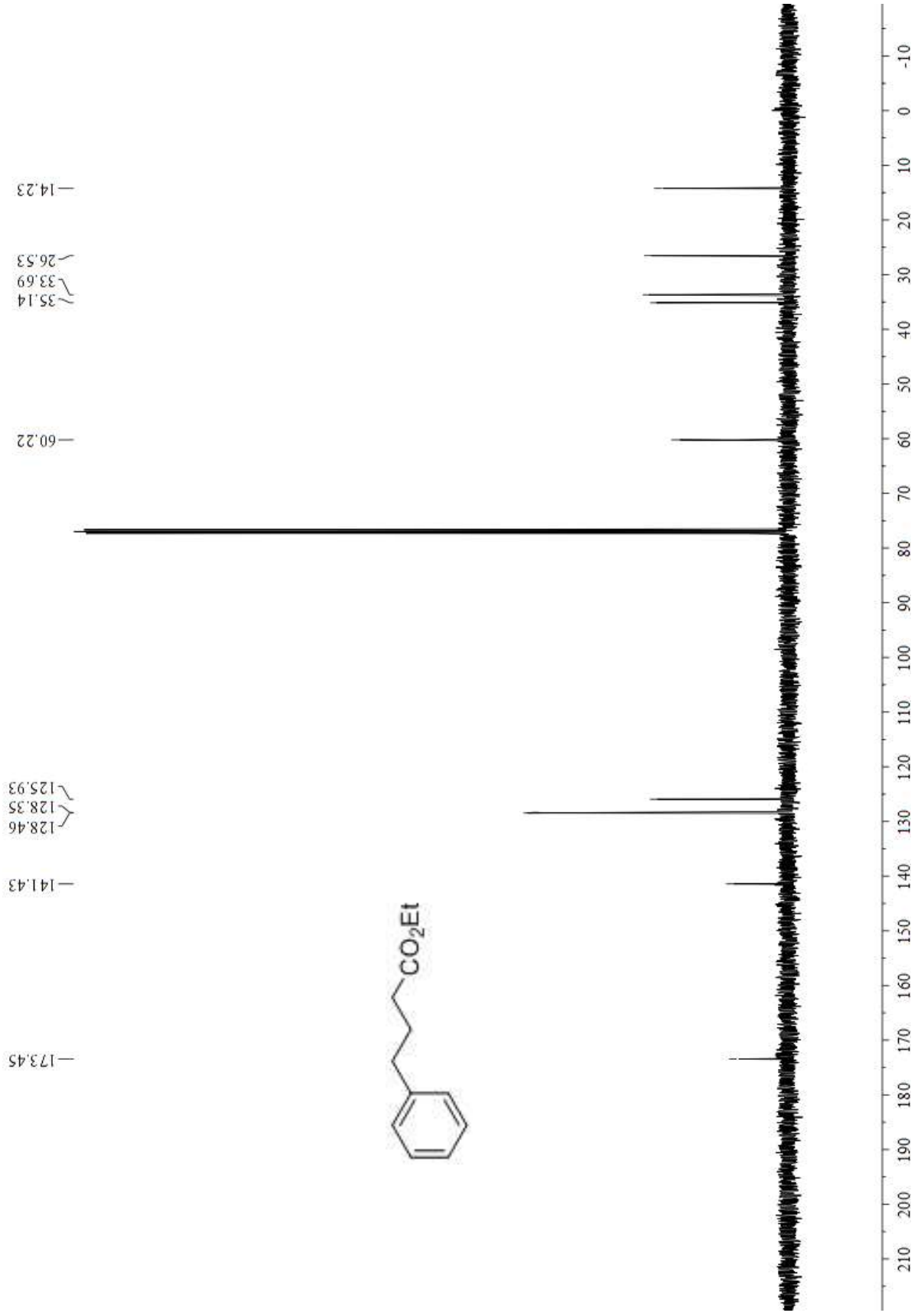
Ethyl 4,6-diphenyl-6-methylheptanate (5e) – ^{13}C NMR



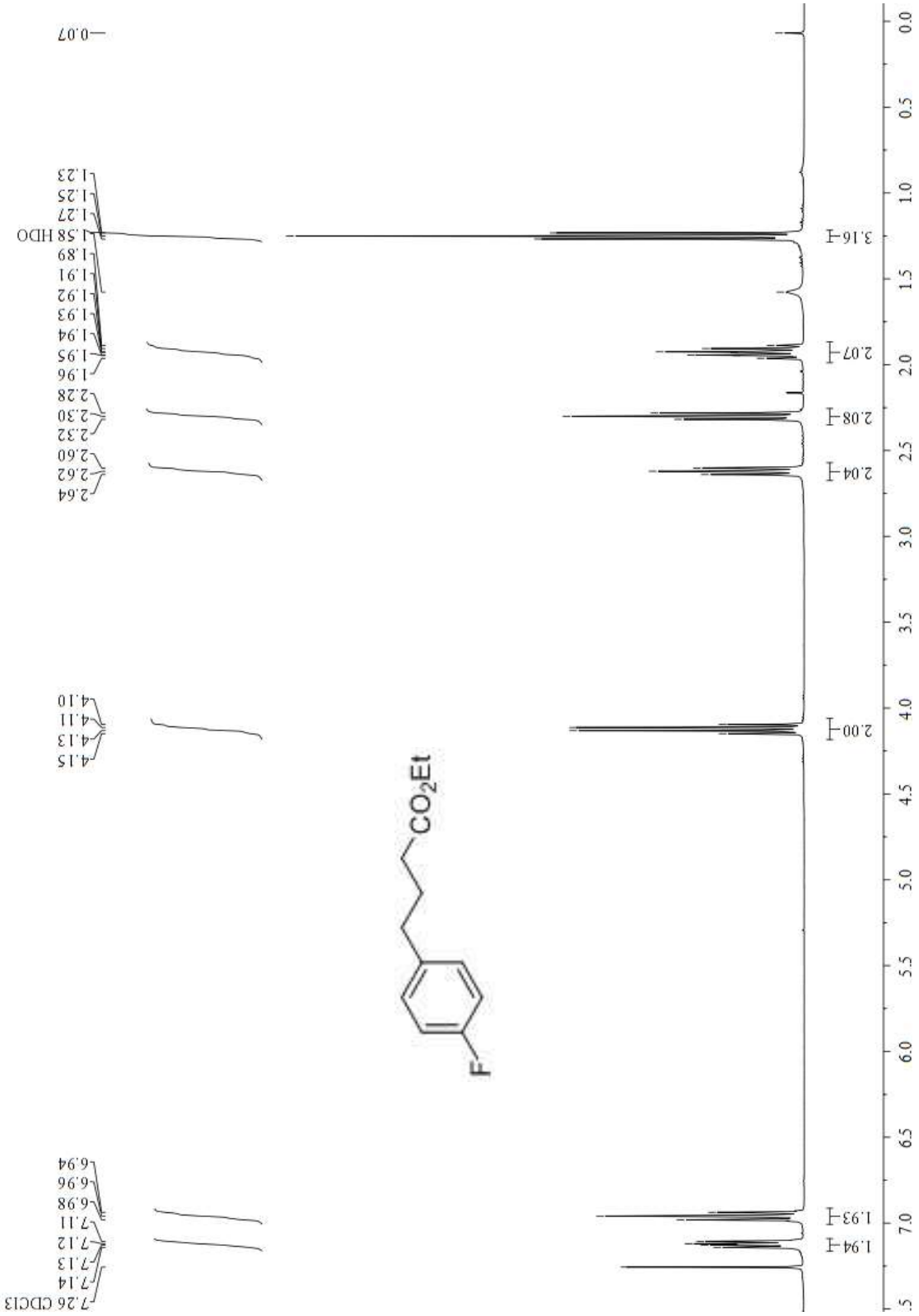
Ethyl 4-phenylbutanoate (5f) – ^{1}H NMR



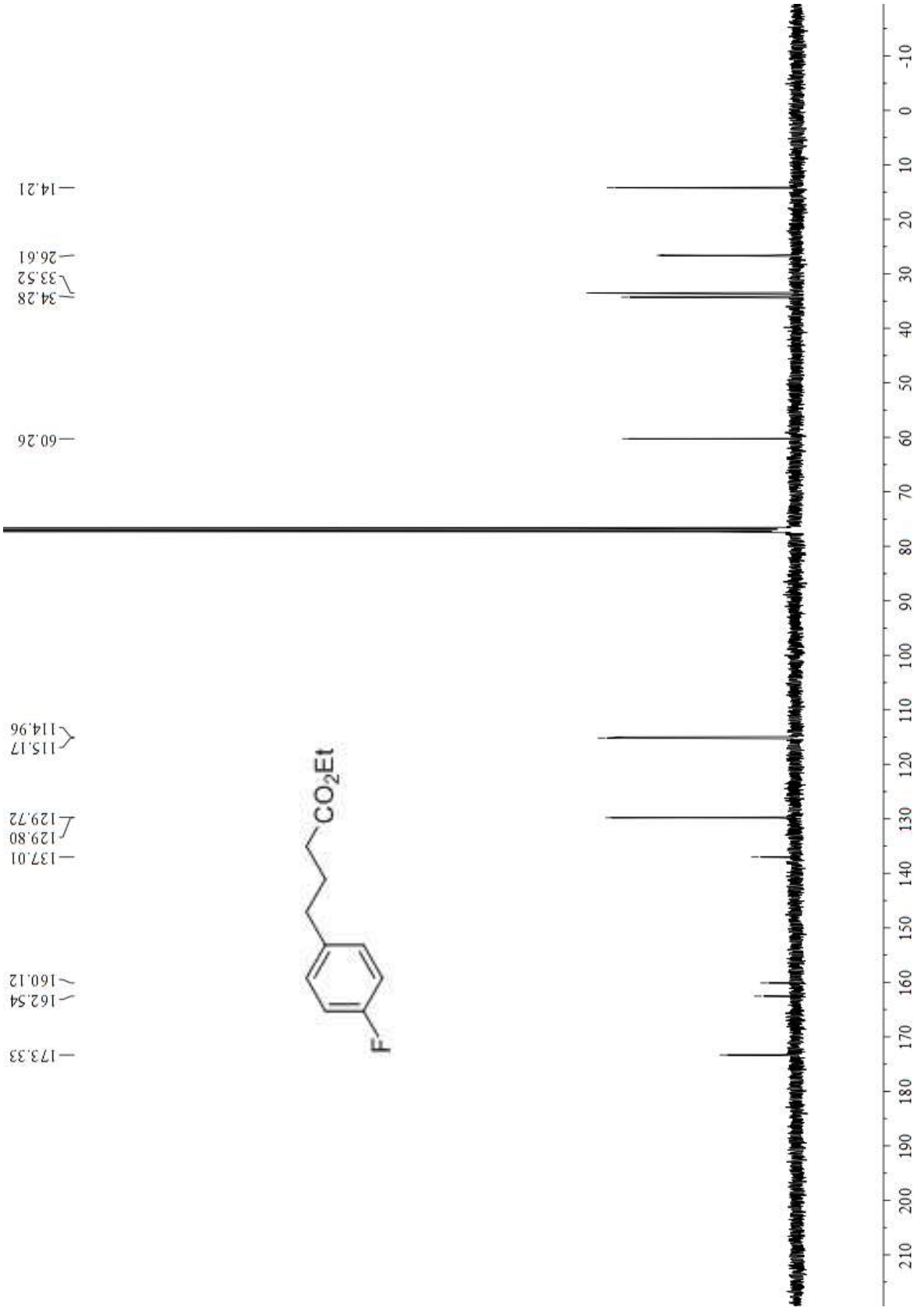
Ethyl 4-phenylbutanoate (5f) – ^{13}C NMR



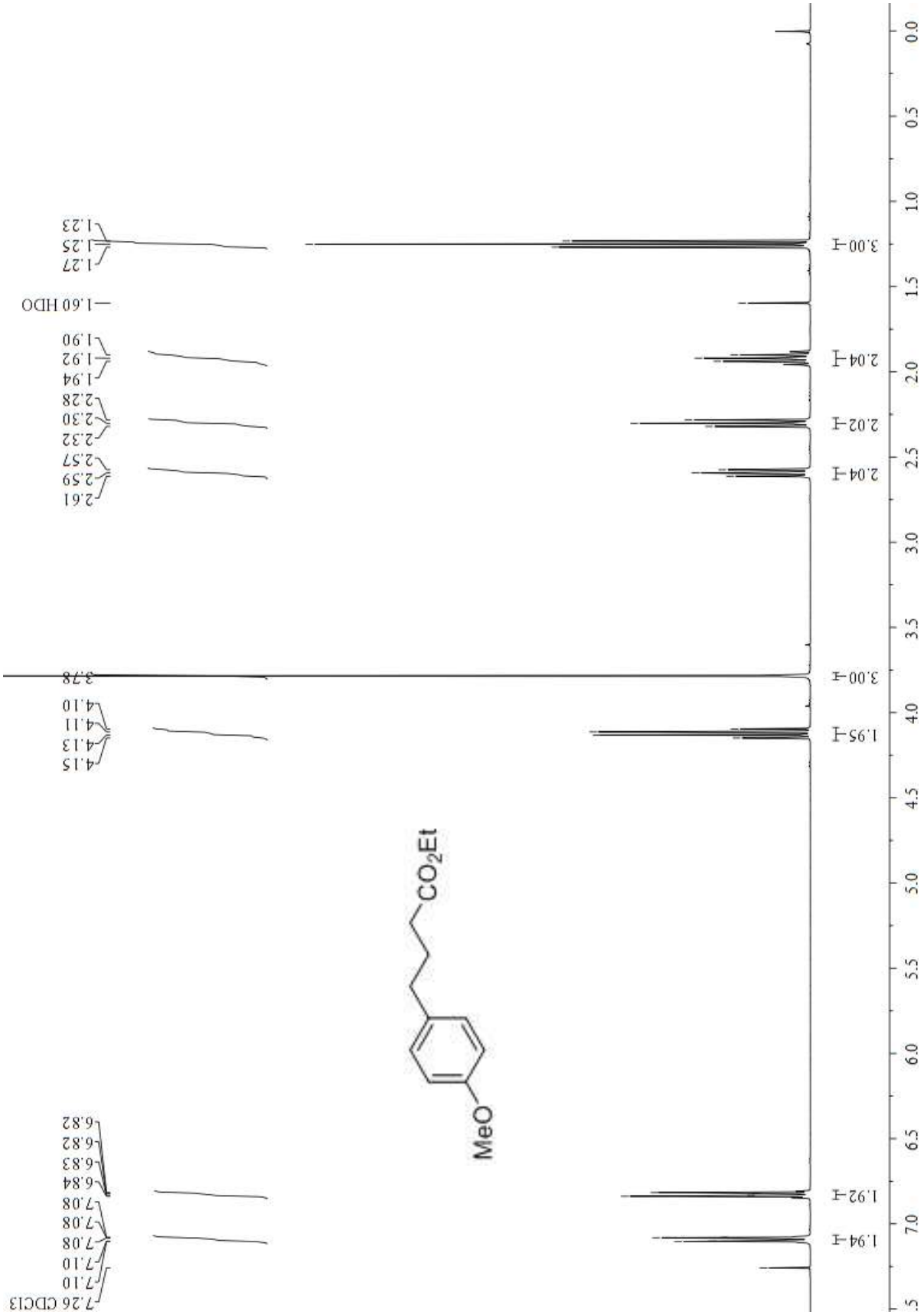
Ethyl 4-(4-fluorophenyl)butanoate (5g) – ^{1}H NMR



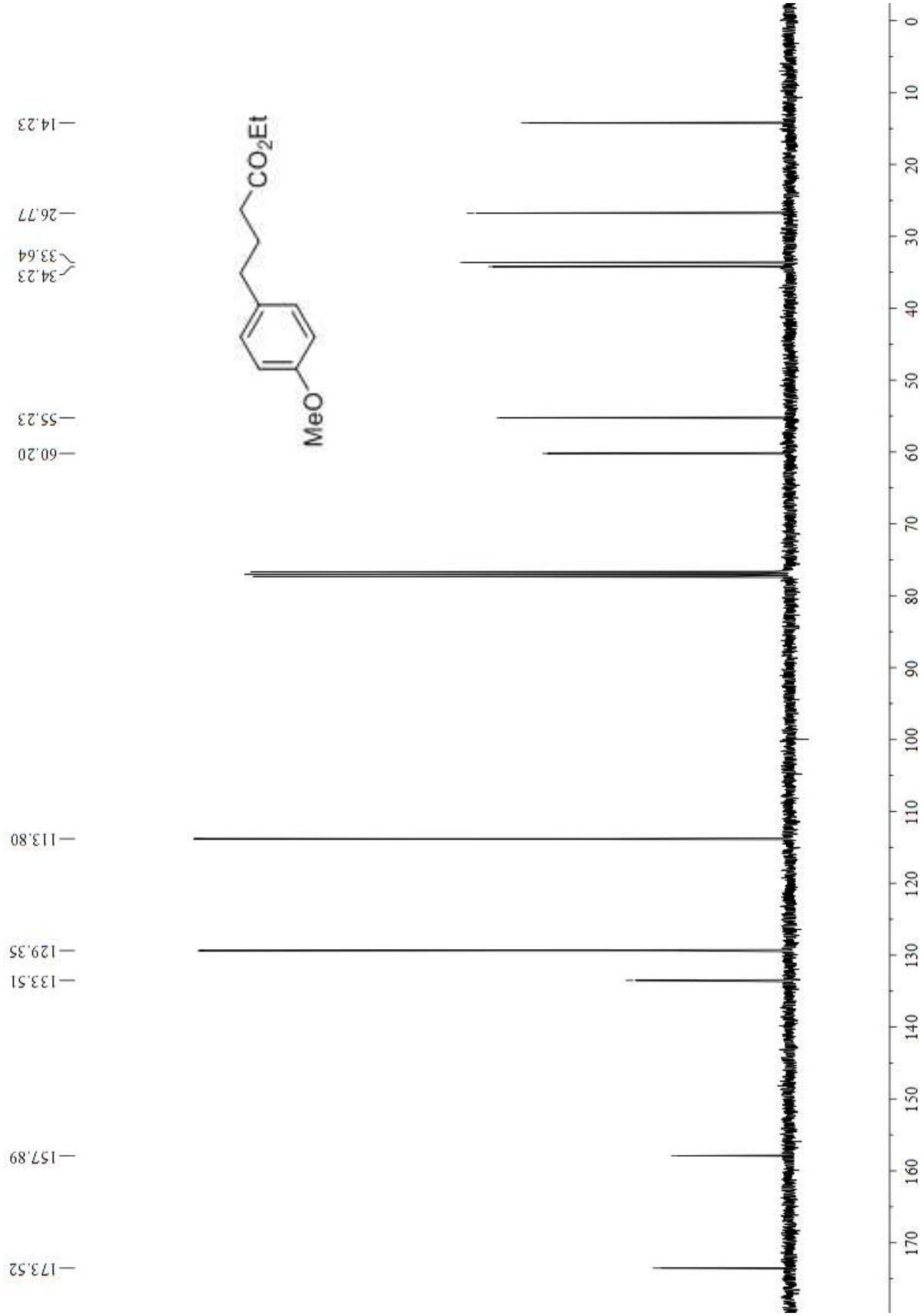
Ethyl 4-(4-fluorophenyl)butanoate (5g) – ^{13}C NMR



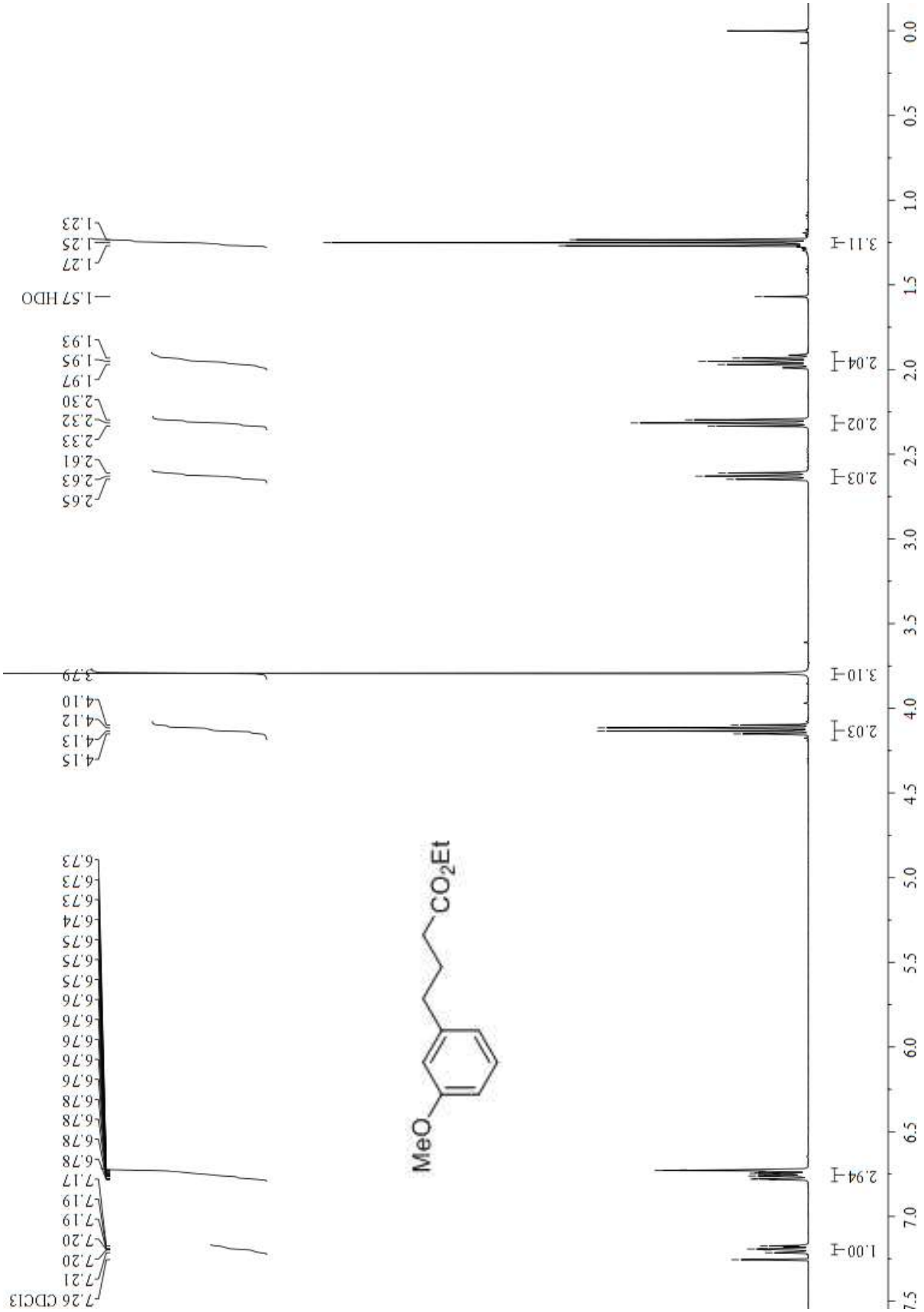
Ethyl 4-(4-methoxyphenyl)butanoate (5j) – ^{1}H NMR



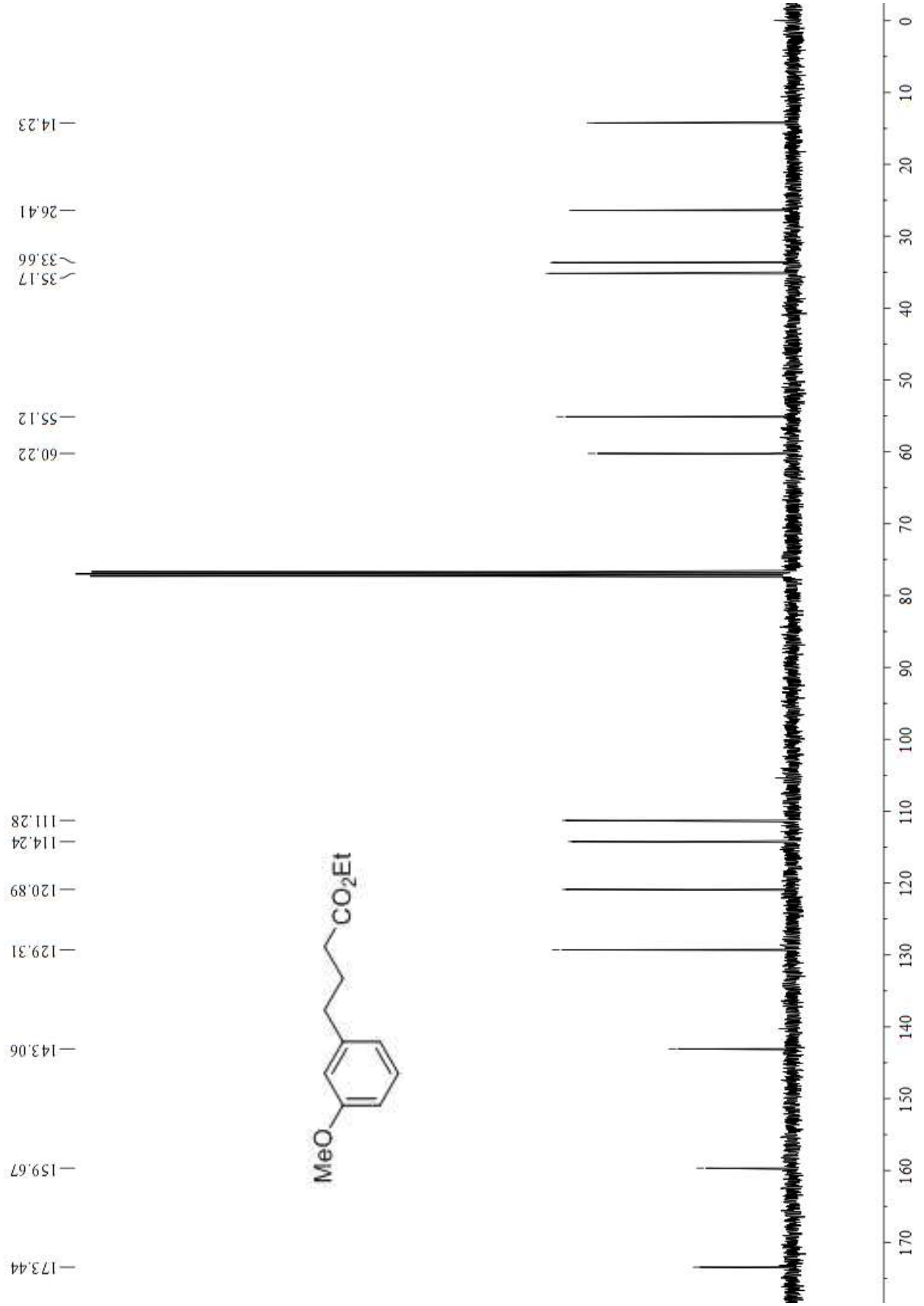
Ethyl 4-(4-methoxyphenyl)butanoate (5j) – ^{13}C NMR



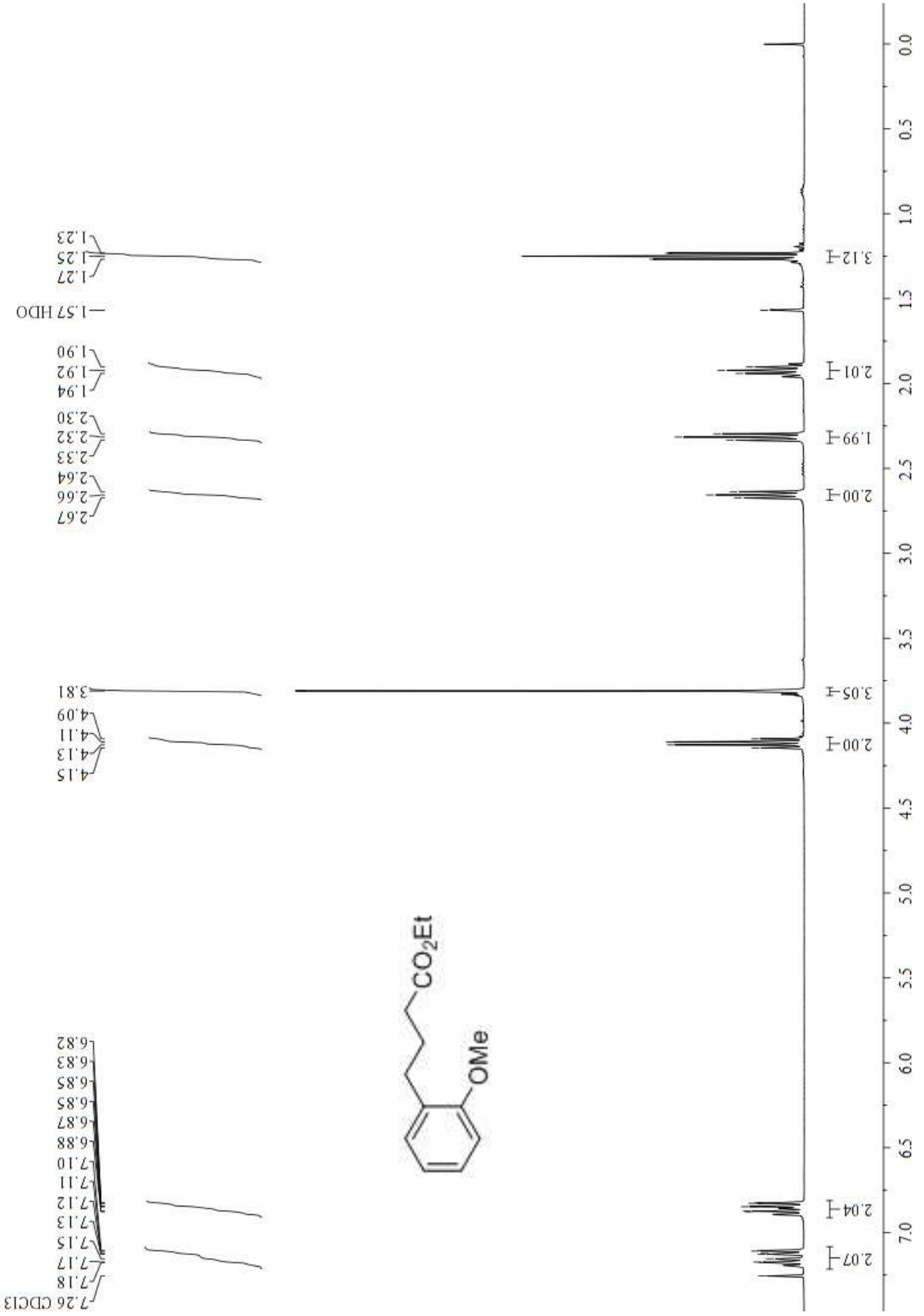
Ethyl 4-(3-methoxyphenyl)butanoate (5k) – ^{1}H NMR



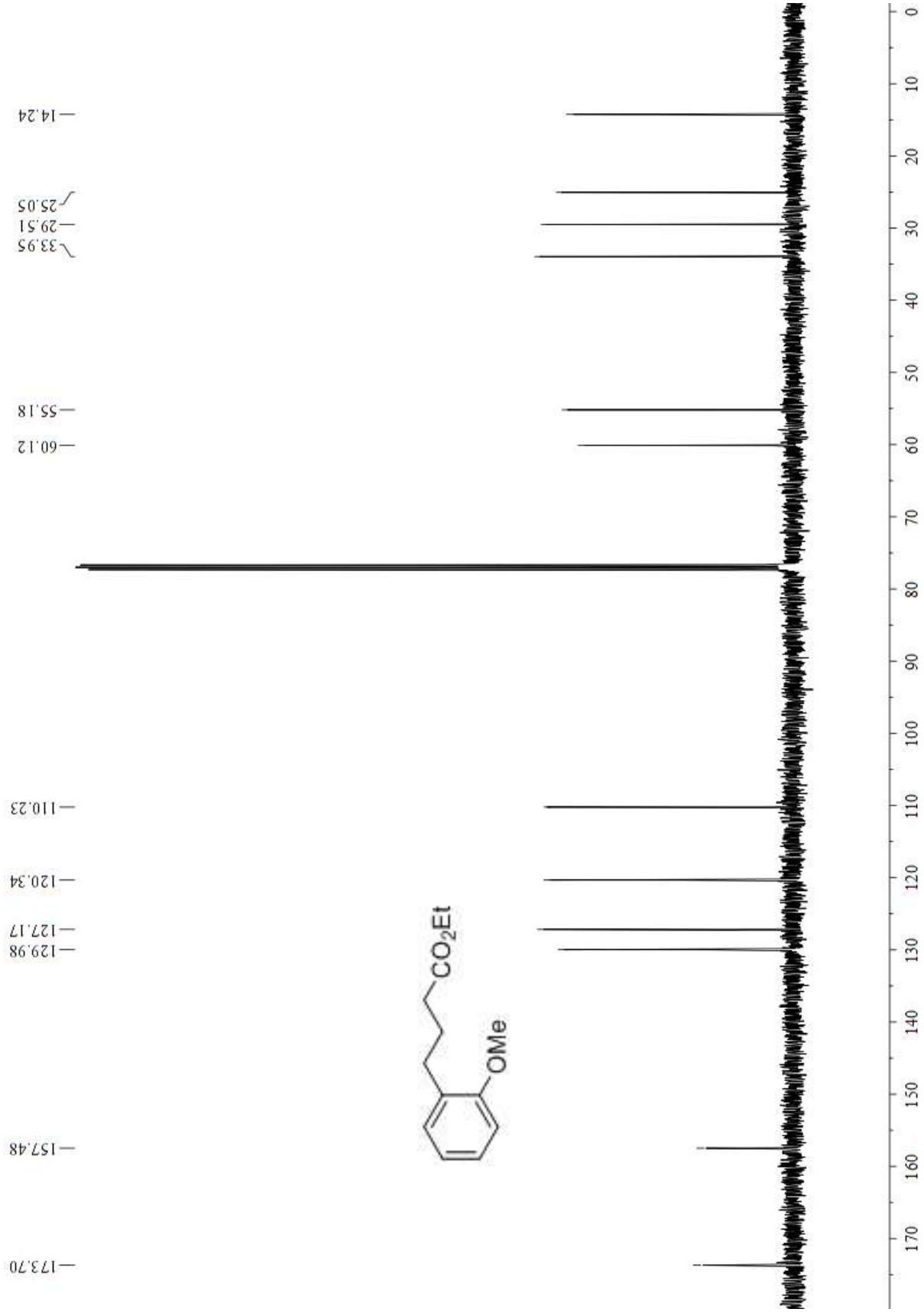
Ethyl 4-(3-methoxyphenyl)butanoate (5k) – ^{13}C NMR



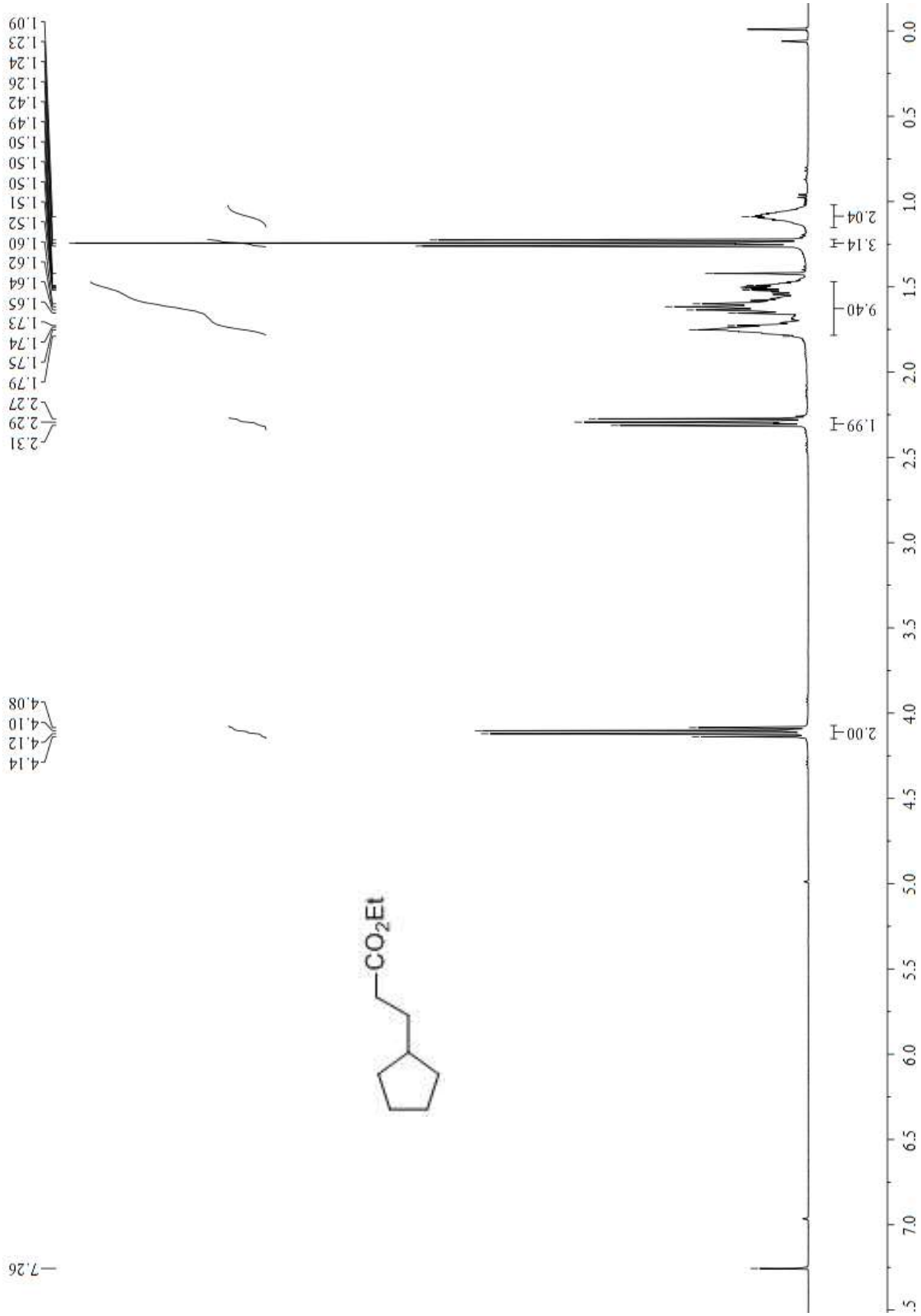
Ethyl 4-(2-methoxyphenyl)butanoate (5l) – ^{1}H NMR



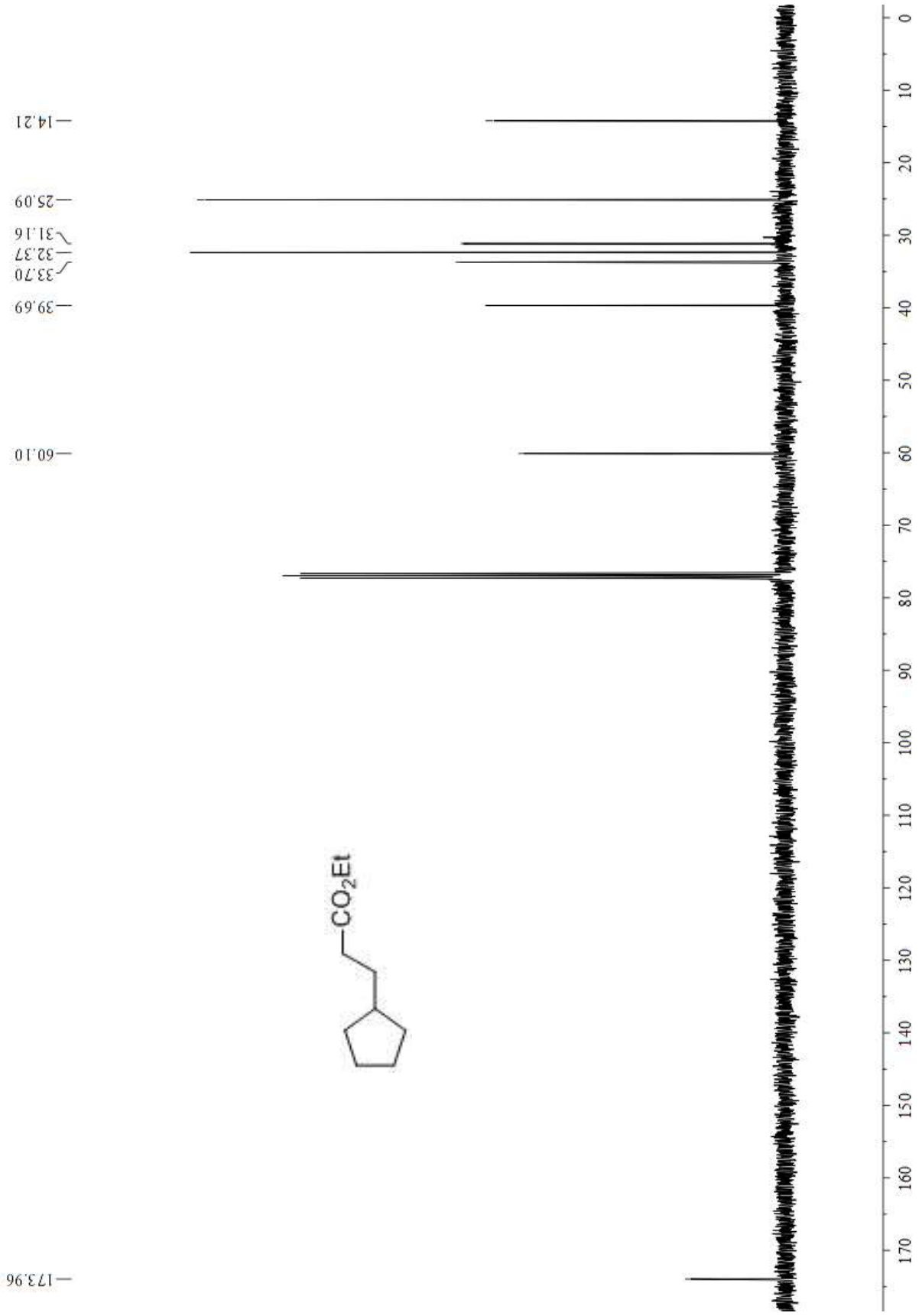
Ethyl 4-(2-methoxyphenyl)butanoate (5l) – ^{13}C NMR



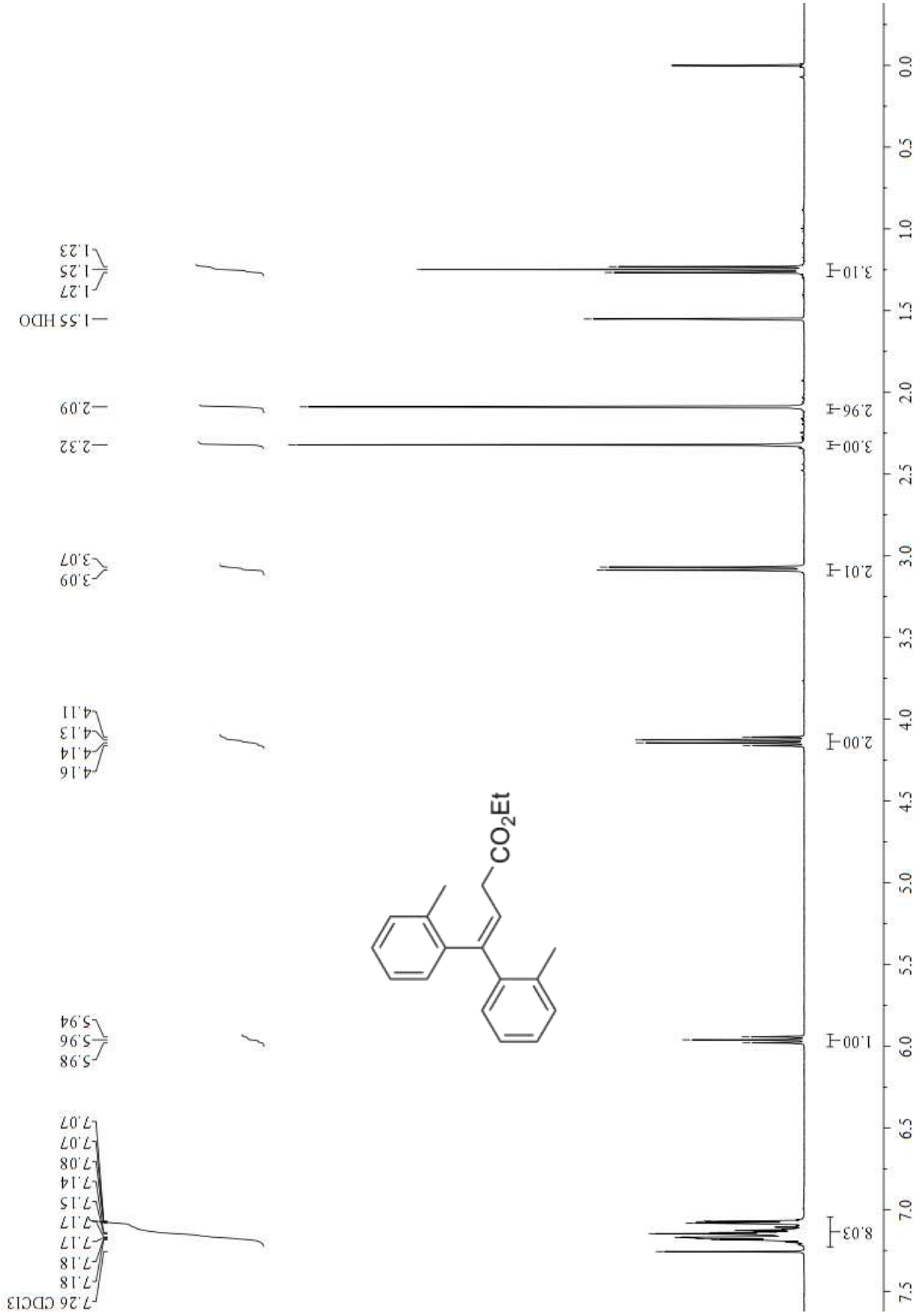
Ethyl 3-(cyclopentyl)propionate (5n) – ^{1}H NMR



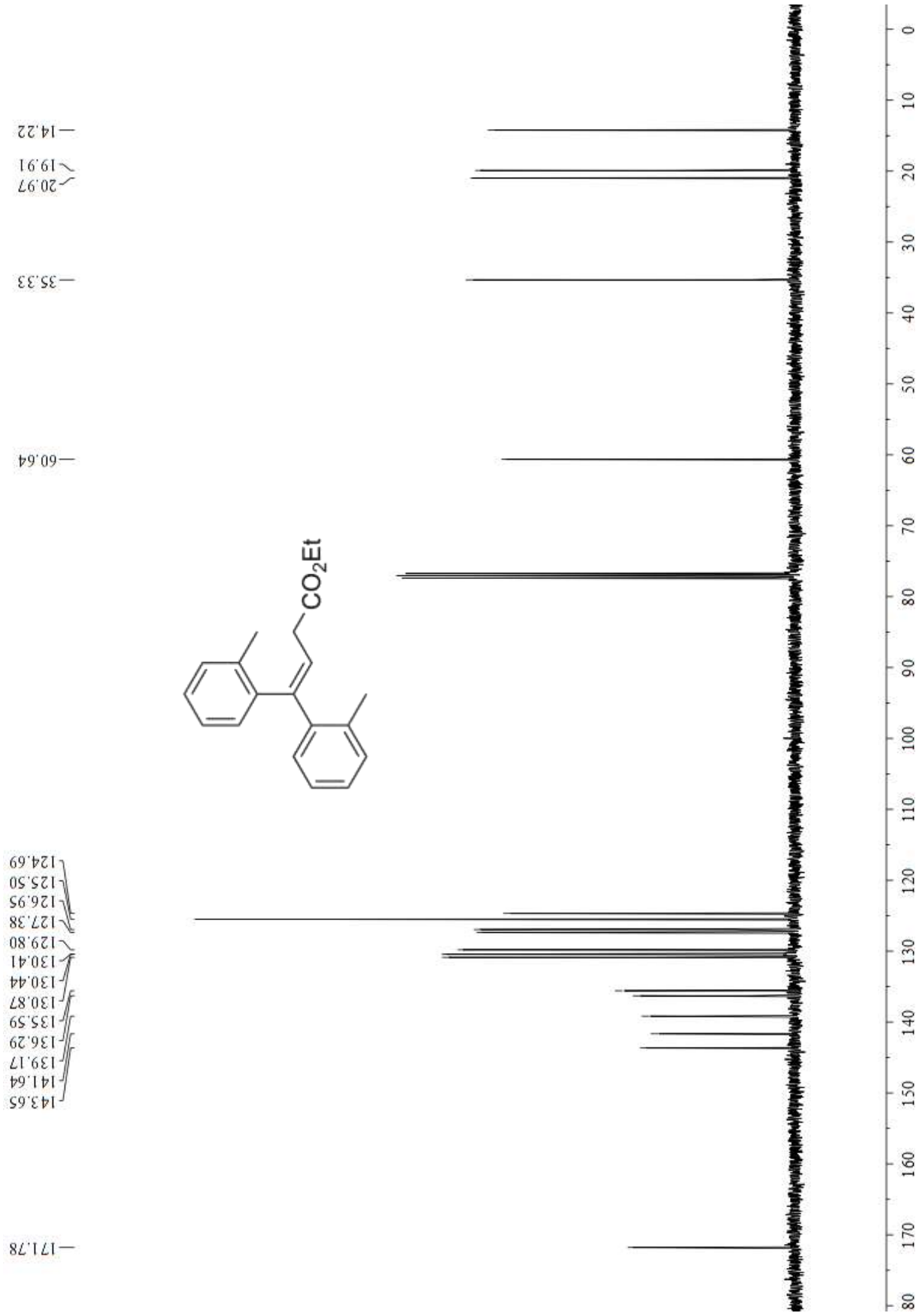
Ethyl 3-(cyclopentyl)propionate (5n) – ^{13}C NMR



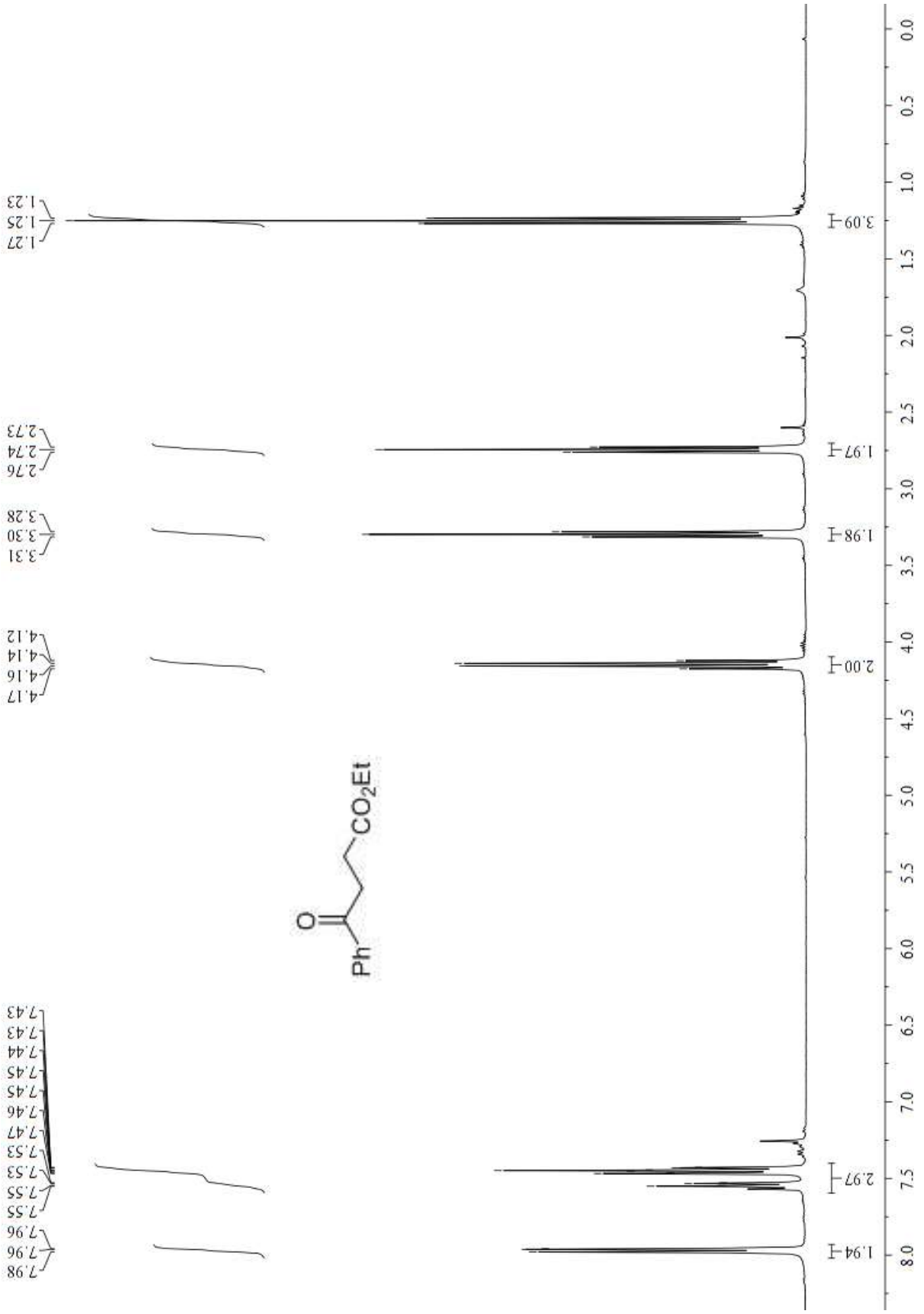
Ethyl 4,4-di(2-methylphenyl)but-3-enoate (5o) – ^{1}H NMR



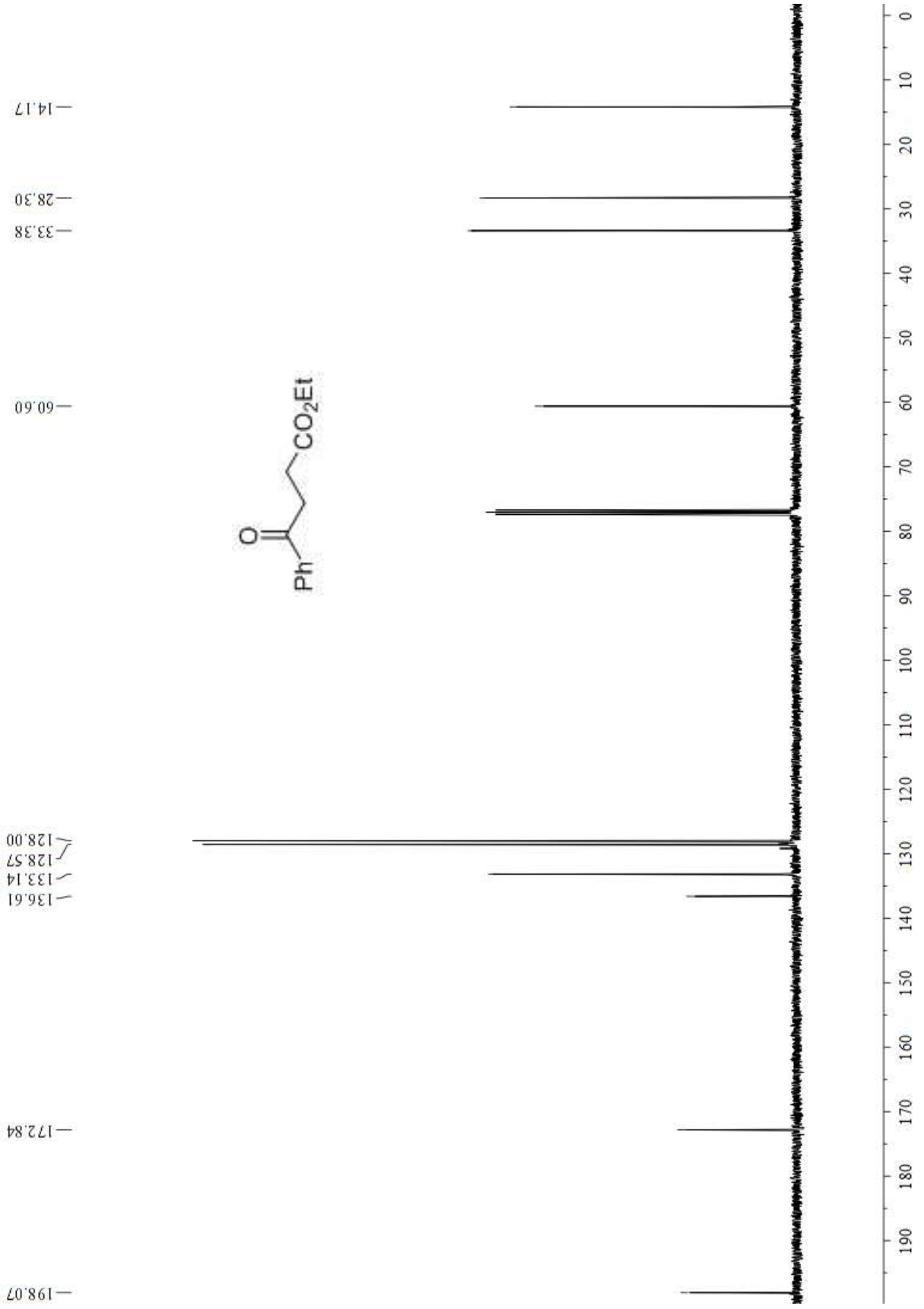
Ethyl 4,4-di(2-methylphenyl)but-3-enoate (5o) – ^{13}C NMR



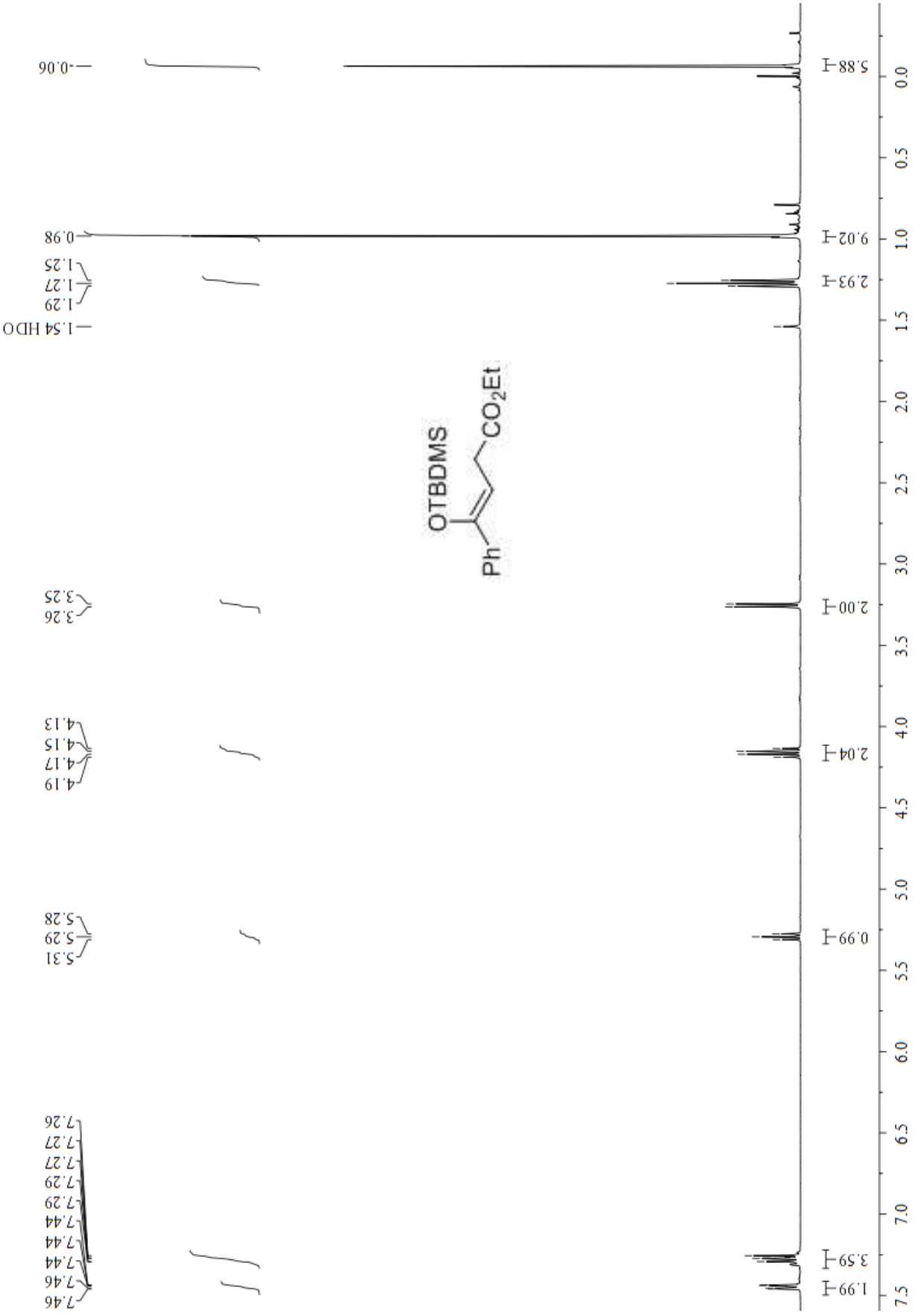
Ethyl 4-oxo-4-phenylbutanoate (10) – ^{1}H NMR



Ethyl 4-oxo-4-phenylbutanoate (10) – ^{13}C NMR

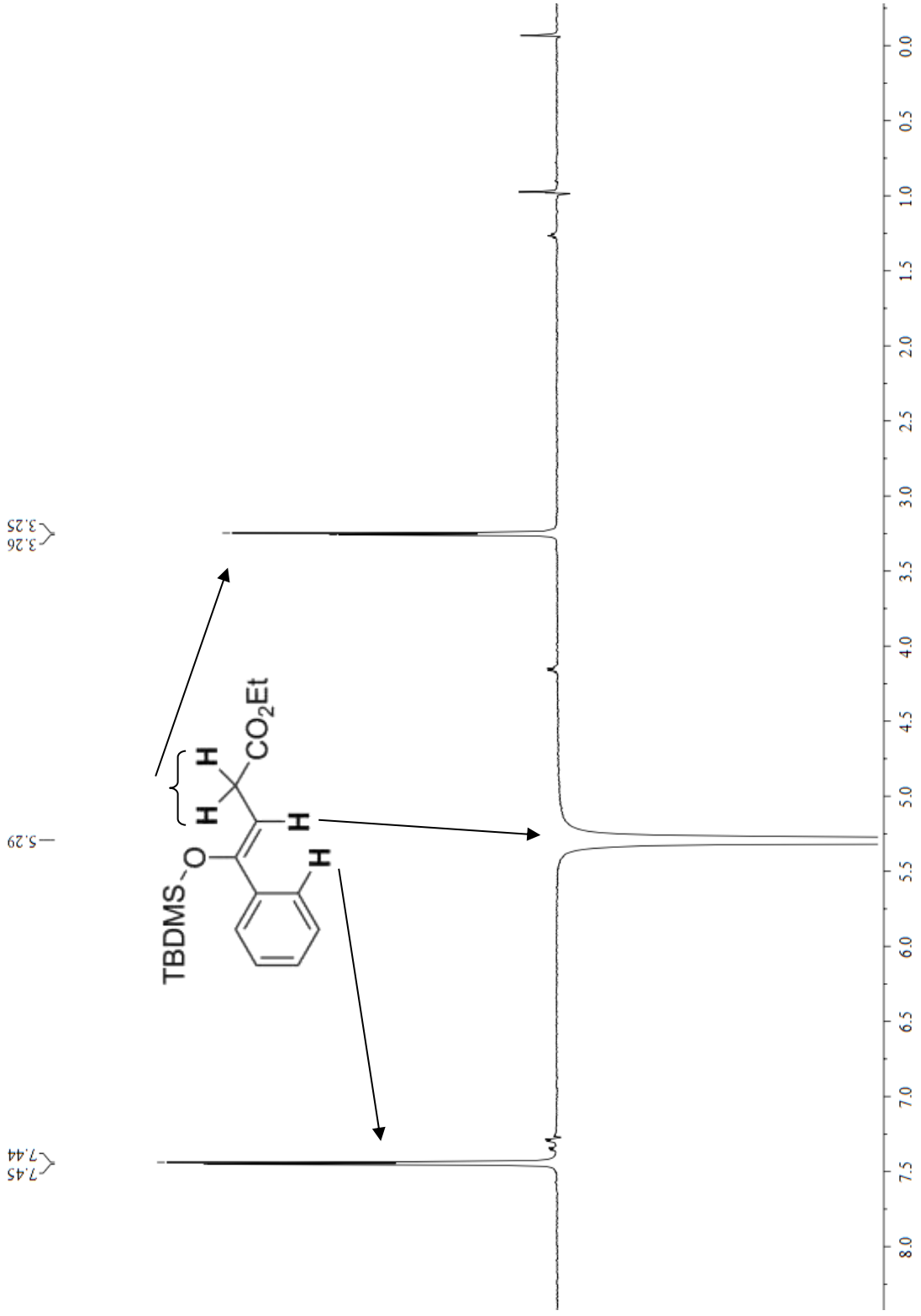


Ethyl 4-phenyl-4-(tert-butyldimethylsiloxyl)but-3-enoate (12) – ^{1}H NMR

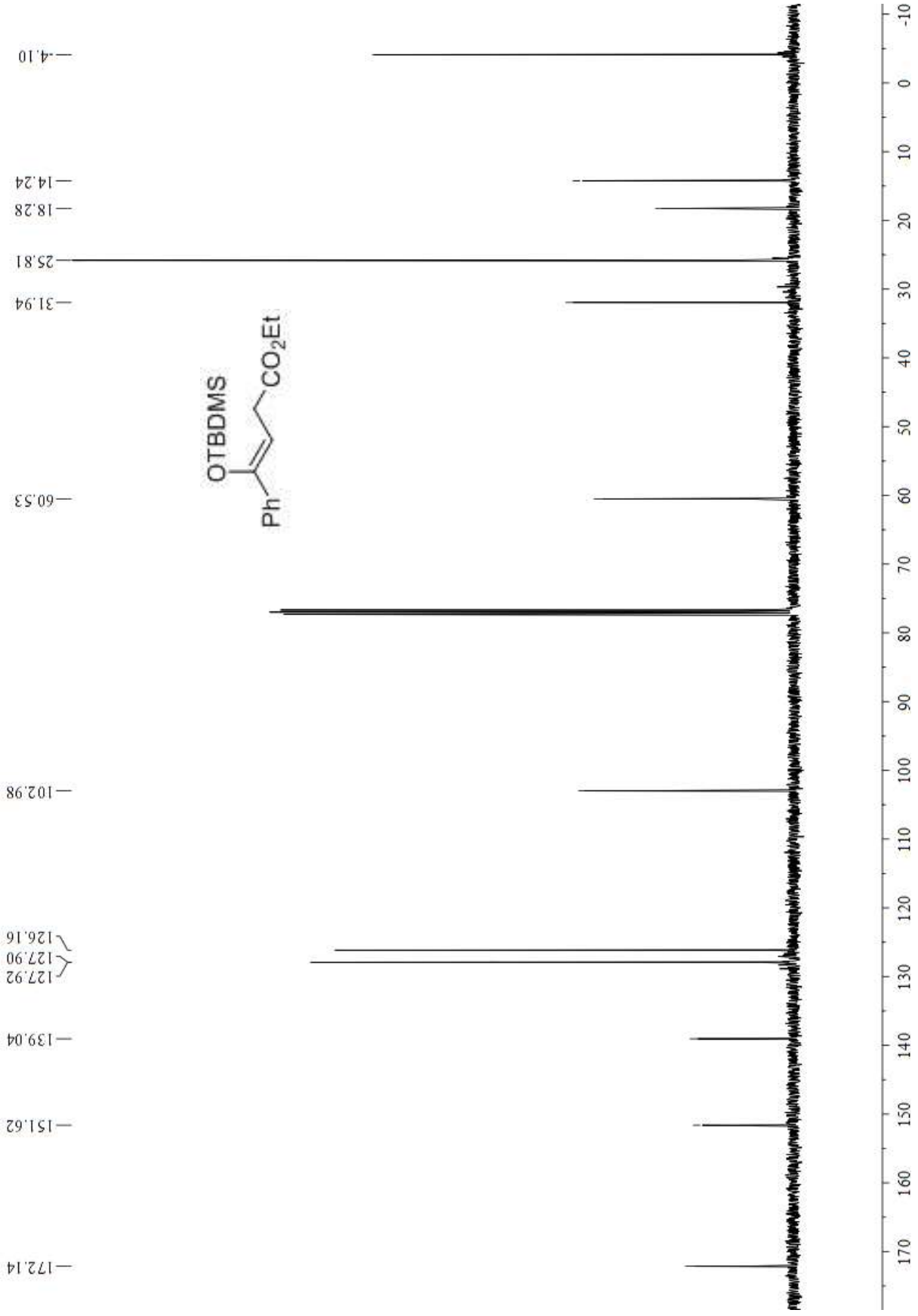


Ethyl 4-phenyl-4-(tert-butyldimethylsiloxyl)but-3-enoate (12) – 1D NOESY

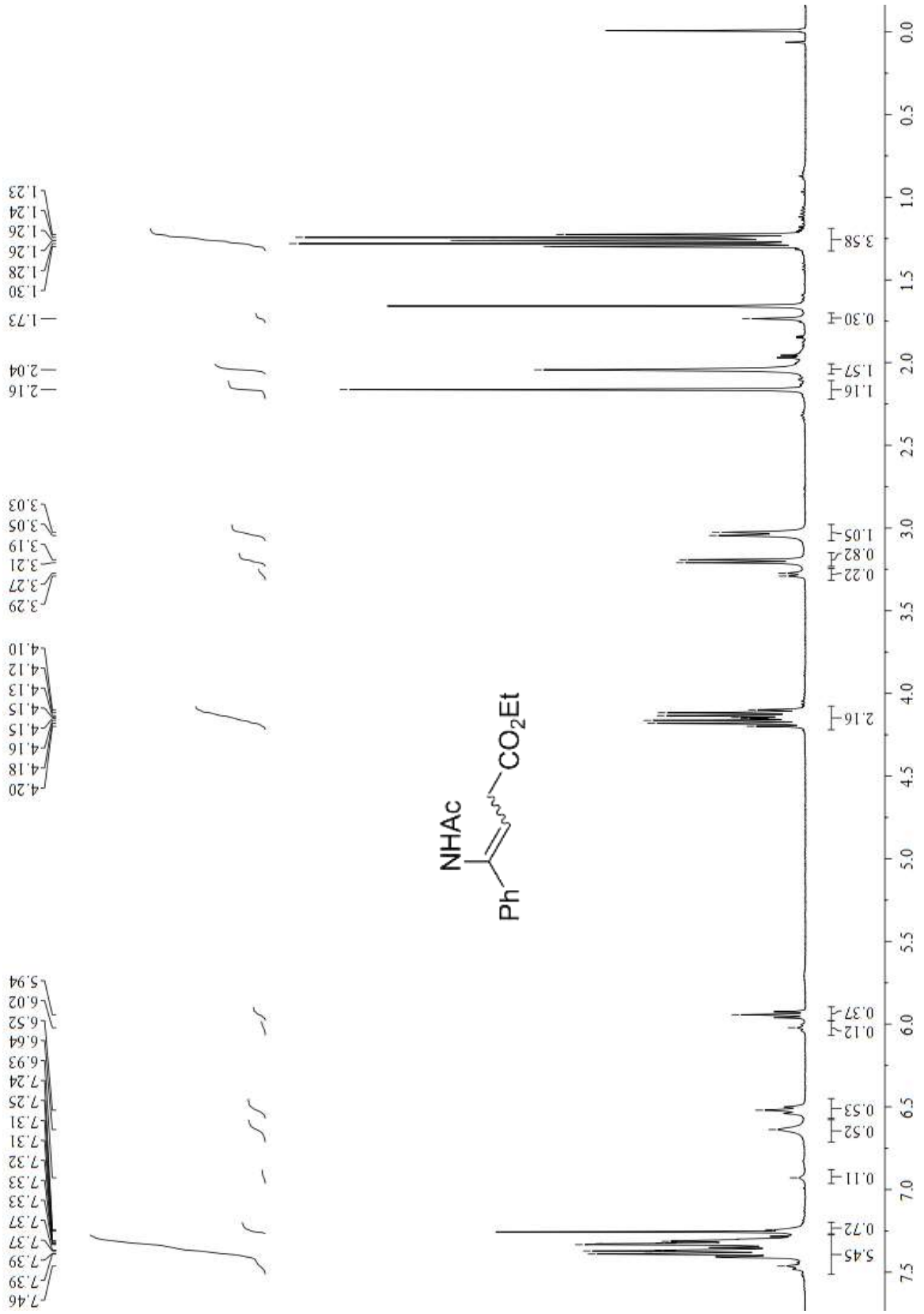
Upon irradiation of the vinyl proton, the interaction with aromatic proton in *-orto* position was observed, thus proving the *Z* configuration of the compound.



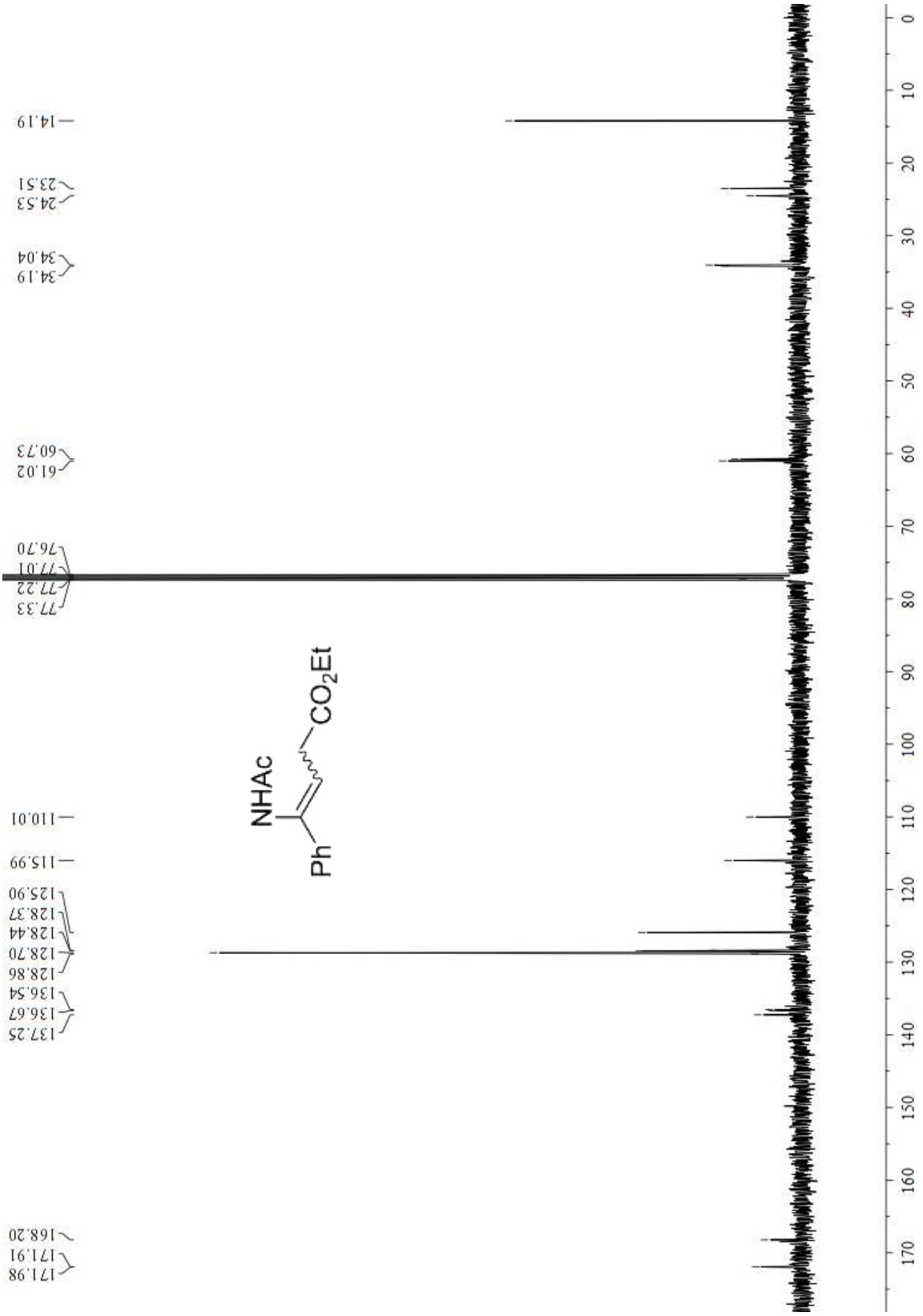
Ethyl 4-phenyl-4-(tert-butyldimethylsiloxyl)but-3-enoate (12) – ^{13}C NMR



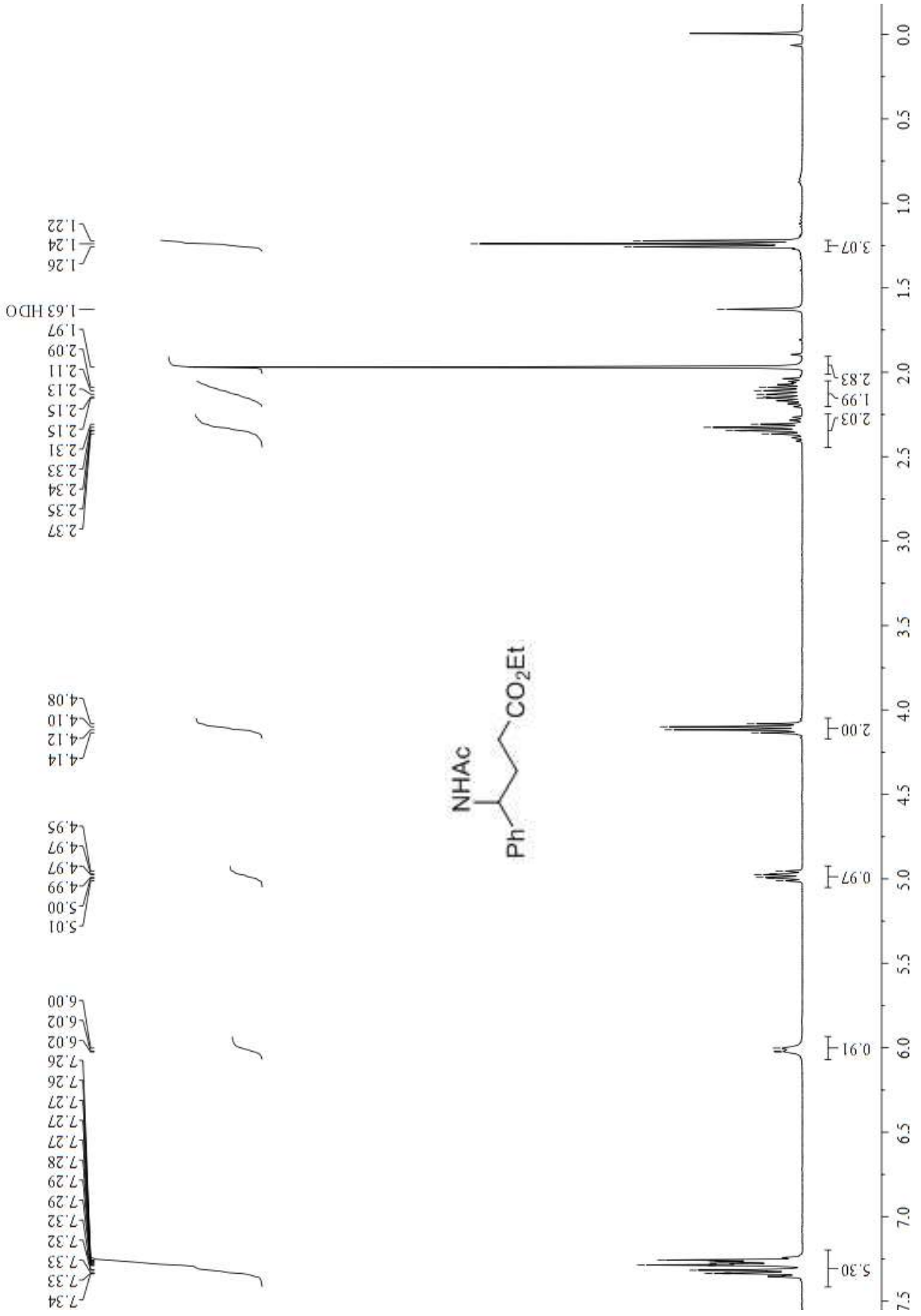
***E*- and *Z*- Ethyl 4-phenyl-4-(*N*-acetylamine)but-3-enoate (14a) – ^{1}H NMR**



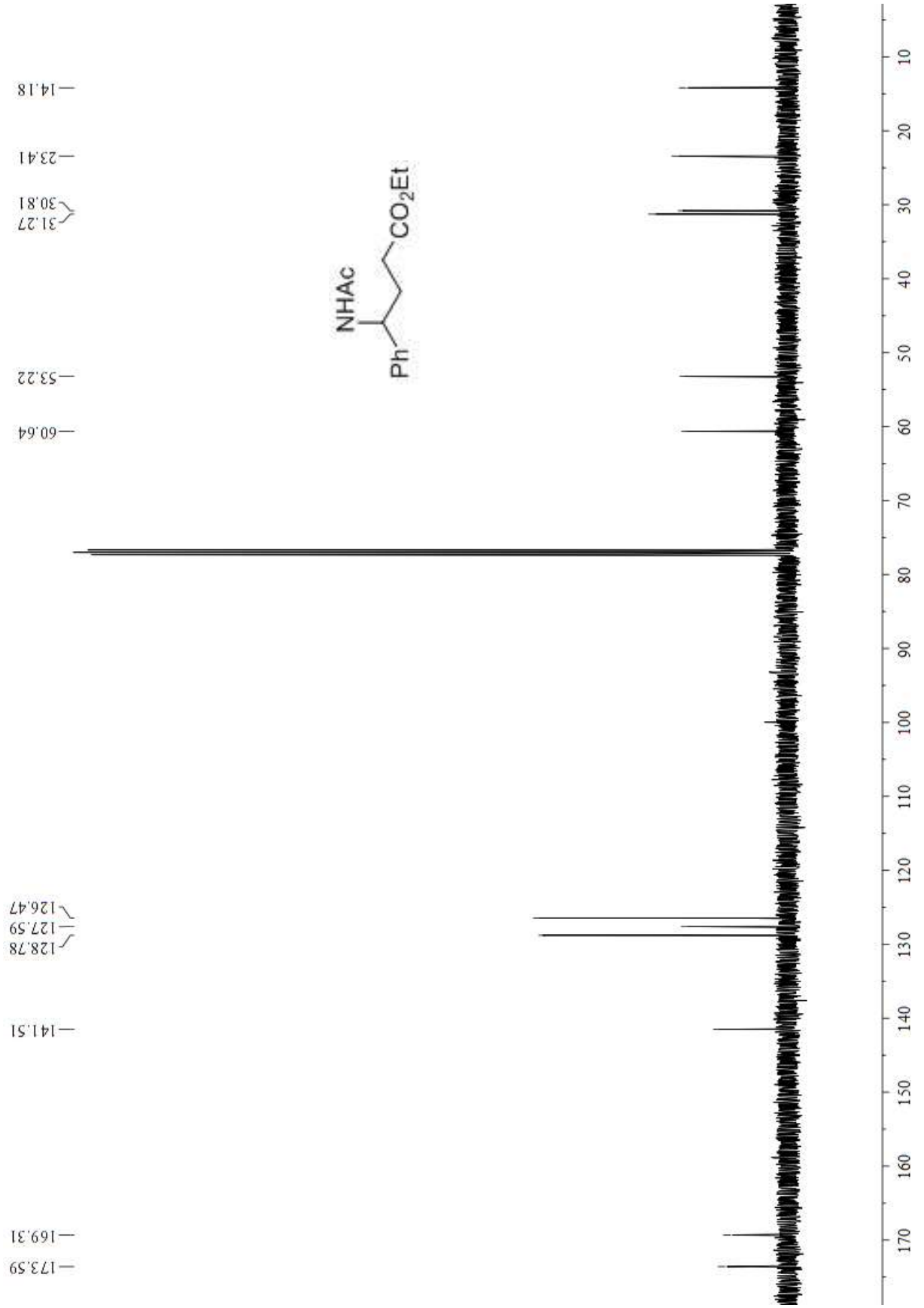
***E*- and *Z*- Ethyl 4-phenyl-4-(*N*-acetylamine)but-3-enoate (14a) – ^{13}C NMR**



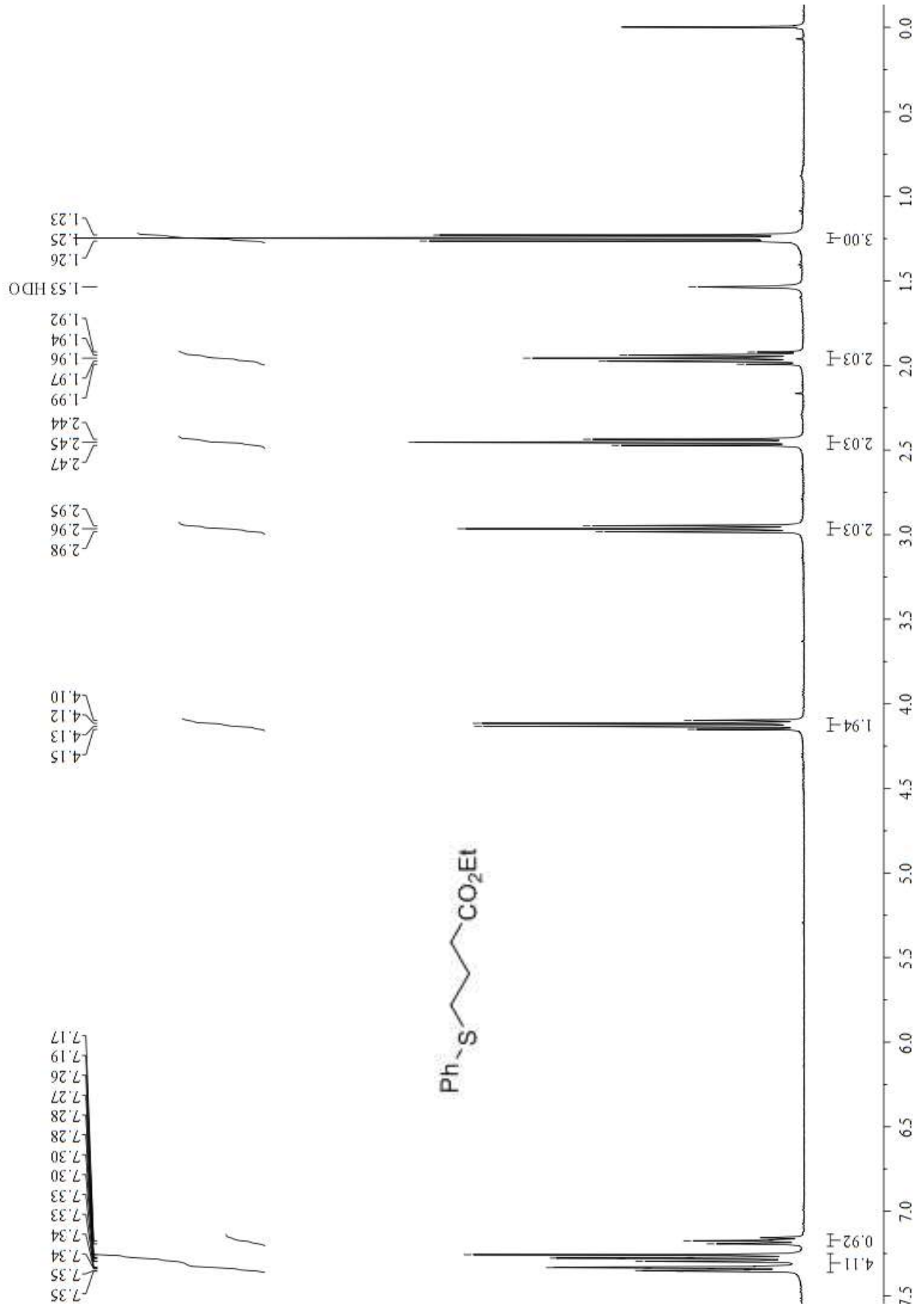
Ethyl 4-phenyl-4-(*N*-acetylamine)butanoate (14b) – ^{1}H NMR



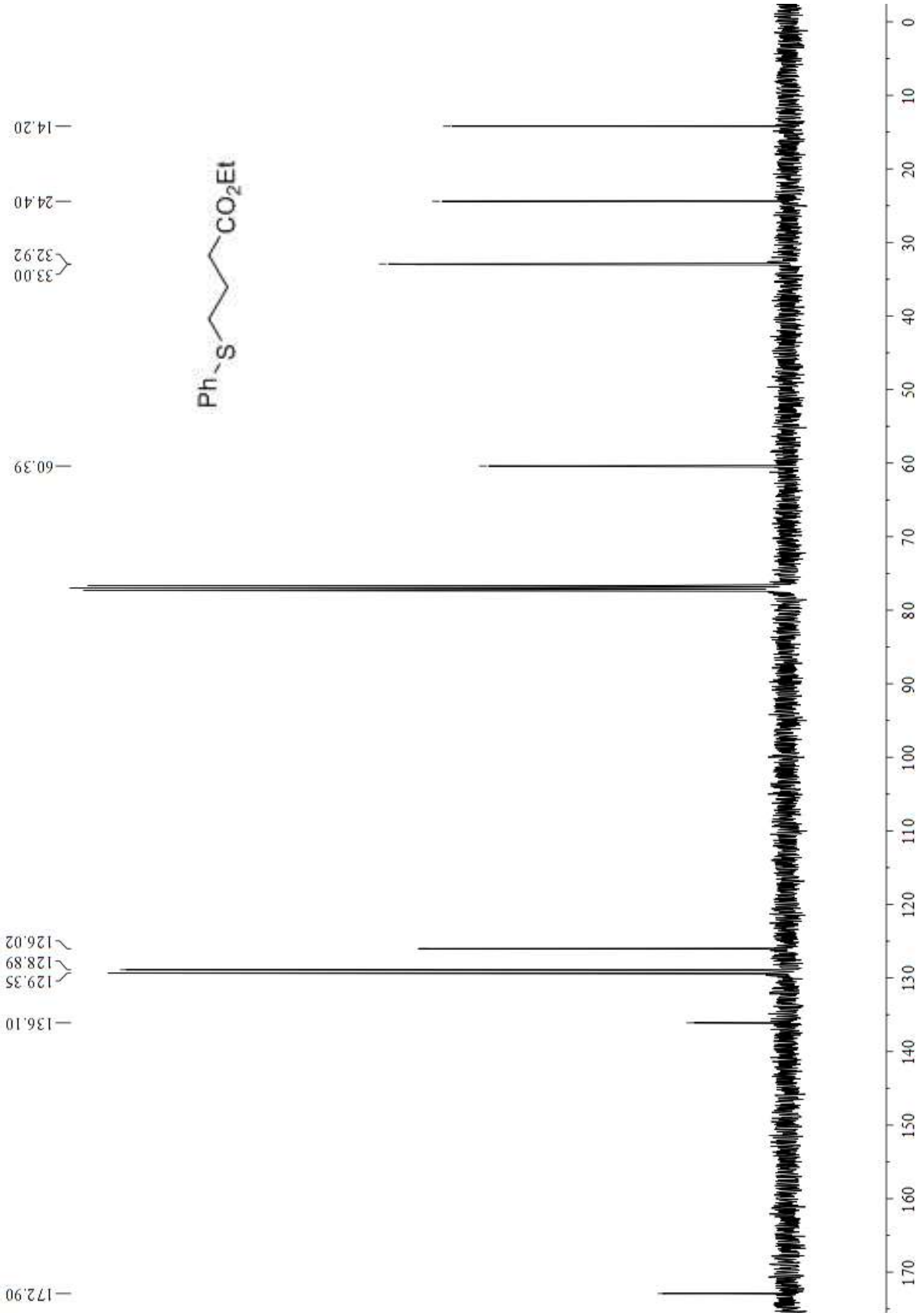
Ethyl 4-phenyl-4-(*N*-acetylamine)butanoate (14b) – ^{13}C NMR



Ethyl 4-(phenylthio)butanoate (16) – ^{1}H NMR



Ethyl 4-(phenylthio)butanoate (16) – ^{13}C NMR



Dalton Transactions

PAPER

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Electrochemistry and catalytic properties of amphiphilic vitamin B_{12} derivatives in nonaqueous media†

M. Giedyk,[a] H. Shimakoshi,*[b] K. Goliszewska,[a] D. Gryko*[a] and Y. Hisaeda*[b]

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www.rsc.org/dalton

The reduction pathway of cobalester (CN)Cble, an amphiphilic vitamin B_{12} derivative, was investigated in organic solvents under electrochemical conditions and compared with mono- and dicyanocobyrinates. The redox characteristics were determined using cyclic voltammetry and spectroelectrochemical methods. The presence of a nucleotide moiety in B_{12}-derivative impedes the *in situ* formation of dicyano-species thus facilitating the (CN)Co(III) to Co(I) reduction. The (CN)Cble shows stepwise reduction to Co(I) *via* (CN)Co(II). The reduction of (CN)Co(II)/Co(I) was found to depend on cyanide-solvent exchange equilibrium with weakly coordinating solvents and bulky peripheral chains promoting intact (CN)Co(II) species existence. The studied complexes were also utilized as catalysts in bulk electrolysis of benzyl bromide affording bibenzyl in very good yield.

Introduction

Vitamin B_{12} (cyanocobalamin **1**) is a highly functionalised organometalic complex comprising a corrin ring with the central cobalt cation and peripheral amide chains as shown in Fig. 1.[1,2] As a cofactor for B_{12}-dependent enzymes it plays an important role in the normal functioning of mammalian organisms.[3–5] Inspired by nature, scientists successfully employed cyanocobalamin (**1**) and its derivatives as catalysts in organic synthesis, due to their ability to form and selectively cleave Co–C bonds.[6–9] The scope of B_{12}-catalyzed reactions is continuously expanding, now including such processes as dehalogenation, addition to double bonds, ring expansion, C–H functionalization *etc*.

The catalytic properties of vitamin B_{12} derivatives rely on redox chemistry of the central cobalt cation with the reduction of Co(III) to active Co(II) or Co(I) forms being critical.[10] Therefore, the understanding of their electrochemistry becomes an essential element for the rational design and successful application of B_{12}-catalyzed reactions.

Even though the natural, nontoxic, and stable cyanocobalamin (**1**) may appear as an ideal catalyst, it suffers from low solubility in synthetically useful organic solvents such as DCM, MeCN, THF *etc*. Hence, modification of its structure is commonly performed giving access to more lipophilic derivatives.[11] For example, the synthesis and utilization of heptamethyl cobyrinates (*e.g.* monocyanocobyrinate **3**) have been well established.[12–17] An alternative approach was recently reported by Gryko *et al.* who synthesized cobalester ((CN)Cble, **2**) – a derivative possessing six ester groups on the periphery of the corrin ring with the nucleotide fragment intact that displays good solubility both in aqueous and in organic media.[18] A direct comparison of derivatives with and without the nucleotide loop became thereby possible.

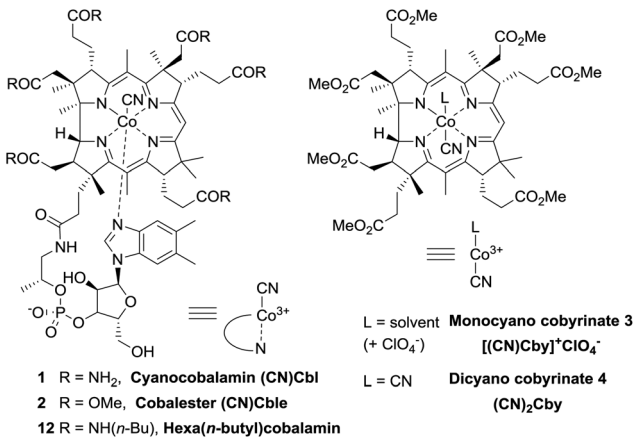

Fig. 1 Structures and abbreviations of vitamin B_{12} derivatives.

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[b]Department of Chemistry and Biochemistry, Graduate School of Engineering, Kyushu University, Fukuoka, 819-0395, Japan. E-mail: shimakoshi@mail.cstm.kyushu-u.ac.jp, yhisatcm@mail.cstm.kyushu-u.ac.jp

†Electronic supplementary information (ESI) available: Details of mechanistic studies, spectroscopic data of (CN)Cble (**2**), $[(CN)Cby]^+ClO_4^-$ (**3**), and hexa(*n*-butyl)cobalamin (**12**). See DOI: 10.1039/c6dt00355a

Electronic Supplementary Information

for

Electrochemistry and Catalytic Properties of Amphiphilic Vitamin B_{12} Derivatives in Nonaqueous Media

M. Giedyk, H. Shimakoshi,* K. Goliszewska, D. Gryko,* and Y. Hisaeda*

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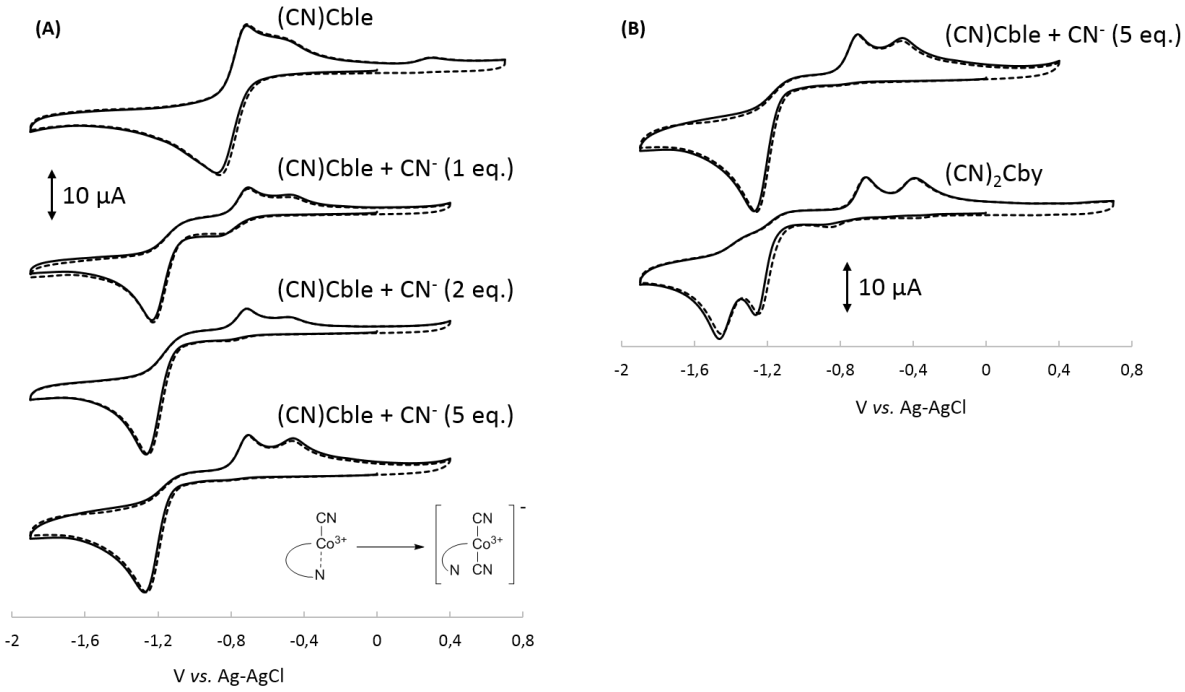
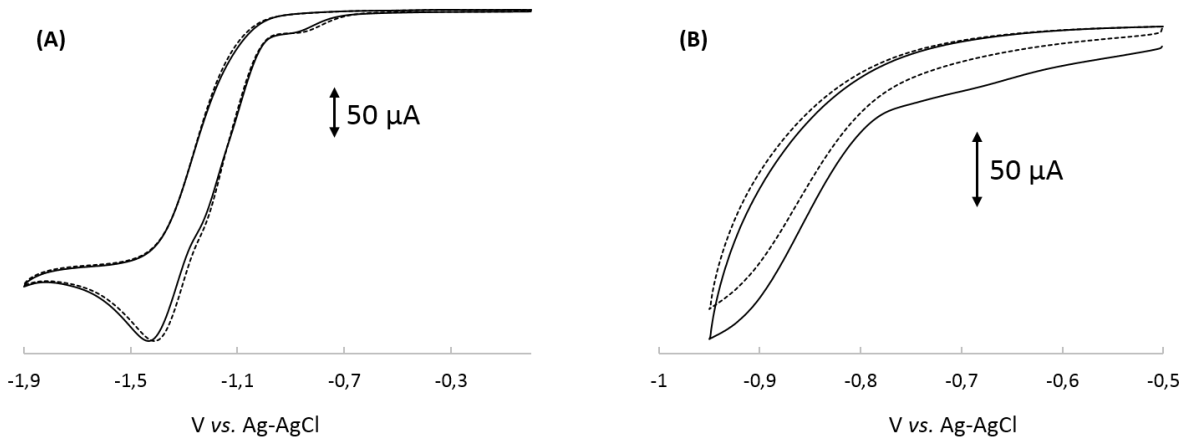
Cyclic voltammetry of (CN)Cble (2) upon addition of cyanide



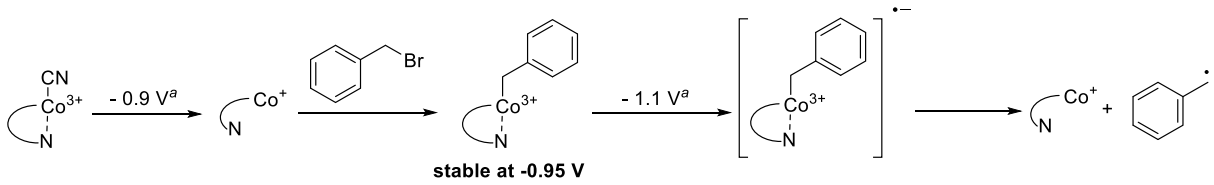
Cyclic voltamperometry spectra of (CN)Cble (**2**) upon addition of external cyanide ($Bu_4N^+CN^-$) (A) and comparison between $[(CN)_2Cble]^-$ and $(CN)_2Cby$ (B).

Conditions: 1.0 mM MeCN solutions of: cyanocobalester (**2**) and $(CN)_2Cby$ (**4**); All solutions contained 0.1 M $Bu_4N^+ClO_4^-$ and measurements were carried out at room temperature under a nitrogen atmosphere using GC working electrode and Pt counter electrode; sweep rate: 0.1 V/s.

Cyclic v voltammetry of (CN)Cble (2) upon addition of benzyl bromide



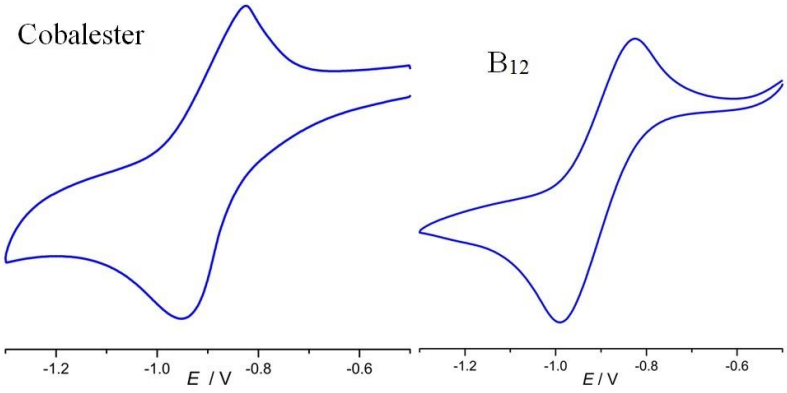
Cyclic voltamperometry spectra of (CN)Cble (**2**) upon addition of benzyl bromide (10 eq.) When the scan was reversed at -0.95 V *vs.* Ag-AgCl (B), no reversible wave was observed. This implies that once Co(I) species is generated, it rapidly reacts with the electrophile forming cobalt-carbon bond:



Cyclic voltammetry measurements of (CN)Cble (2) and cyanocobalamin (1) in aqueous solution[1]

Results presented belowe were published in supplementary information of reference 1:

Cyclic voltammograms of vitamin B_{12} (**1**) and cobalester (**2**) were recorded in deoxygenated 0.2 M solutions of tris buffer in deionized water (C_{B12} = 1.7 mM, $C_{cobalester}$ = 0.7 mM). A three-electrode setup was used in both experiments, including glassy carbon working electrode, Ag/AgCl reference electrode and auxiliary platinum foil (scan rate v = 10 mVs^{-1}, Ar, 20 °C).



Cyclic voltammogram of cobalester (**2**) and cobalamin (**1**).

[1] M. Giedyk S. N. Fedosov and D. Gryko, *Chem. Commun.*, 2014, **50**, 4674.

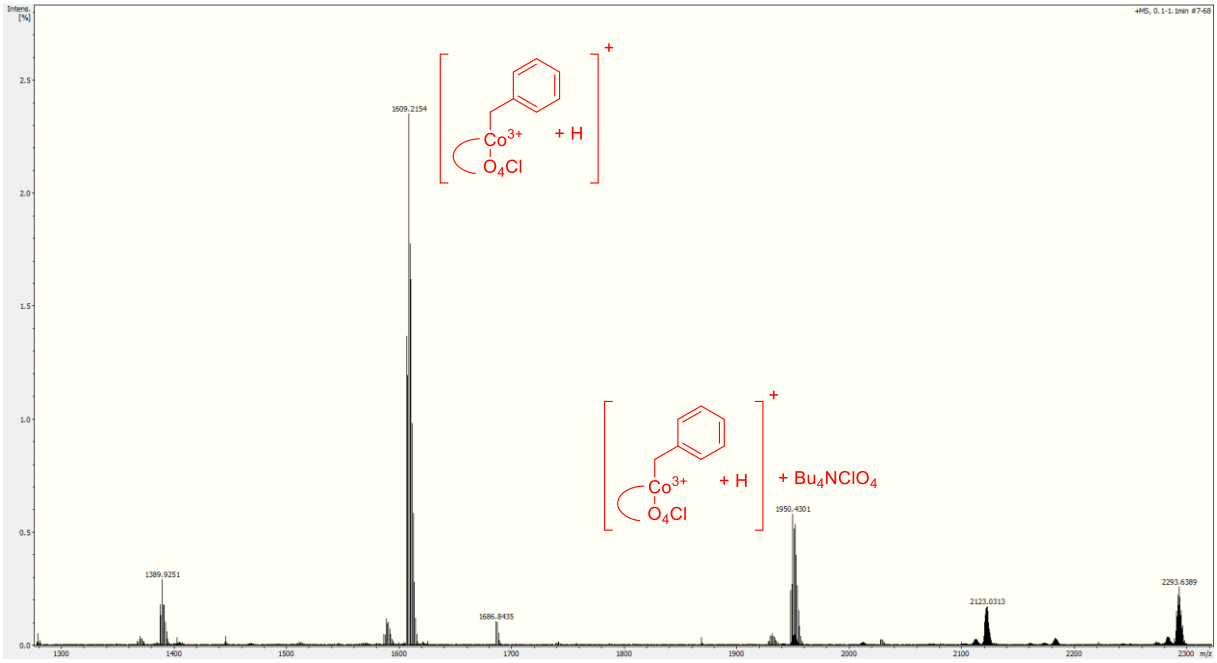
Comparison of redox potentials

for (CN)Cble (2), cobyrinate 3 and 4 and cyanocobalamin (1)

	E [V *vs.* Ag-AgCl]							
	3 in MeCN	**4** in MeCN	**10** in MeCN	**12** in MeCN	**2** in MeCN	**2** in THF	**2** in H_2O	**1** in H_2O
$(CN)Co^{3+}/(CN)Co^{2+}$	-0.4	-	-	-0.9	-0.9	-0.9	-1.0	-1.0
$(solvent)Co^{2+}/Co^{+}$	-0.6	-	-0.6	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
$(CN)Co^{2+}/Co^{+}$	-1.4	-1.4	-	-1.4	-	-1.4	-	-
$[(CN)_2Co^{3+}]/(CN)Co^{2+}$	-1.2	-1.2	-	-	- / -1.2[a]	-	*n.d.*	*n.d.*
$Co^{+}/(solvent)Co^{2+}$	-0.6	-0.7	-0.6	-0.6	-0.7	-0.5	-0.8	-0.8
$(CN)Co^{2+}/(CN)Co^{3+}$	-0.3	-0.4	-	-0.3	-0.5	-0.3	*n.d.*	*n.d.*
$(solvent)Co^{2+}/(solvent)Co^{3+}$	0.6	-	0.6	0.4	0.3	0.5	*n.d.*	*n.d.*

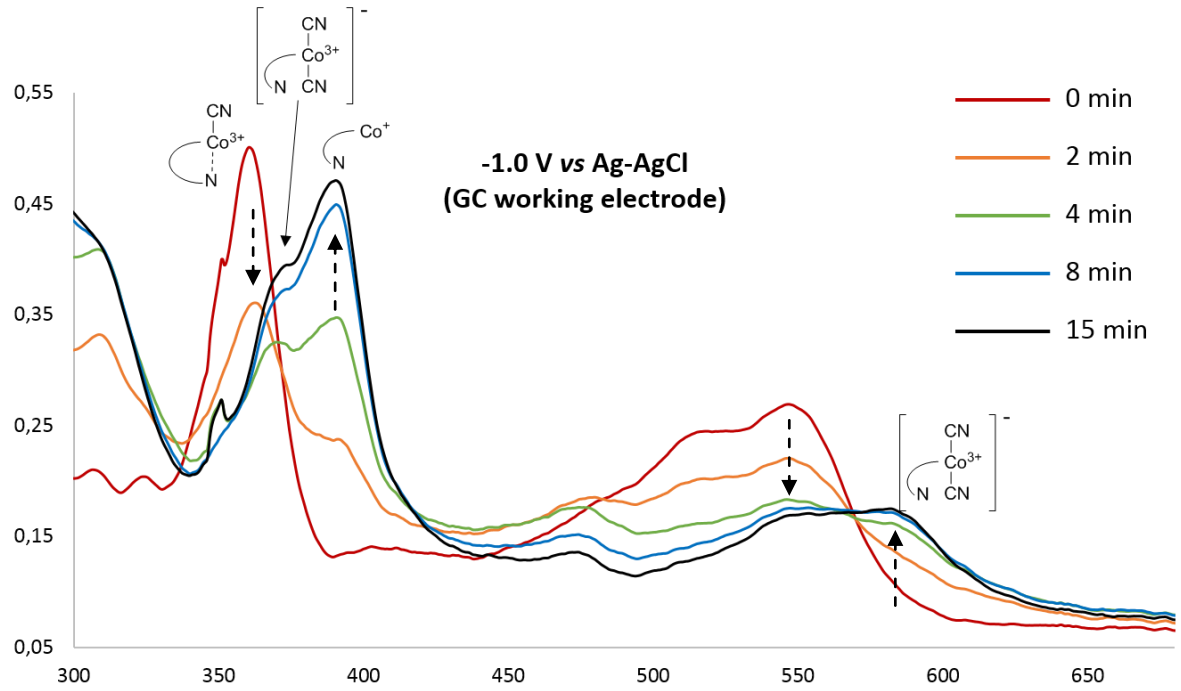
[a] a reduction at this potential was observed when 5 eq. of cyanide was added

ESI MS analysis of benzyl bromide dimerization reaction mixture

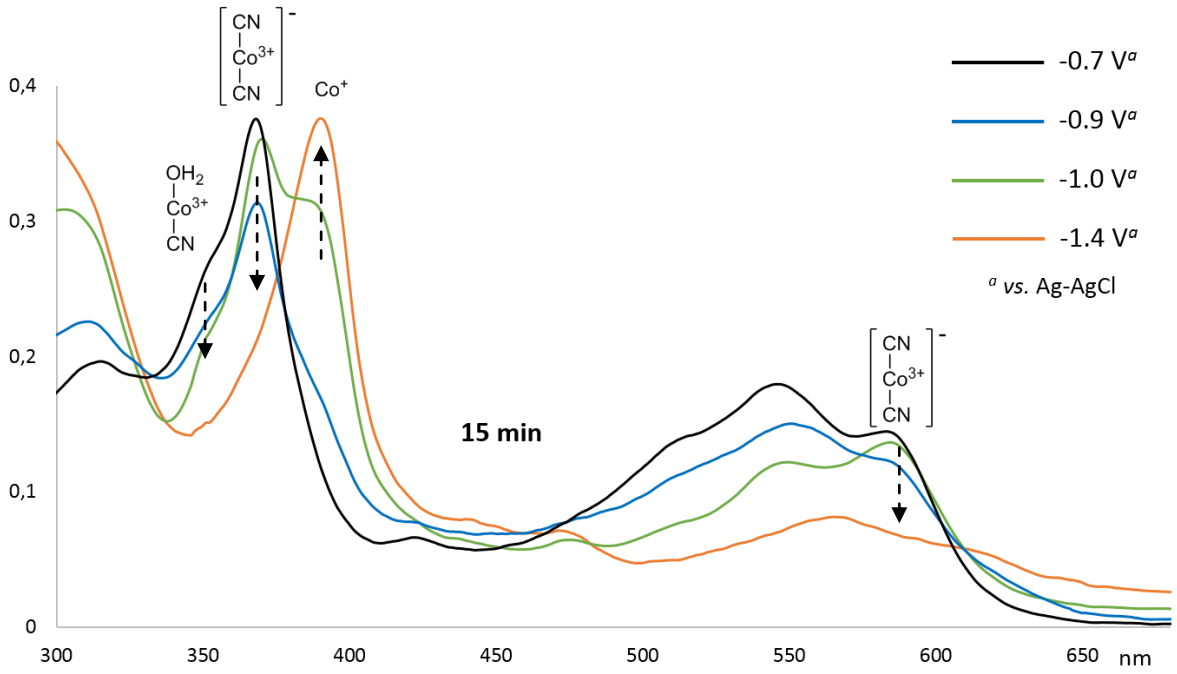


Reaction mixture was analyzed (after 15 min) using ESI MS in the dark. It indicated facile formation of cobalt-benzyl intermediate with m/z = 1609.2.

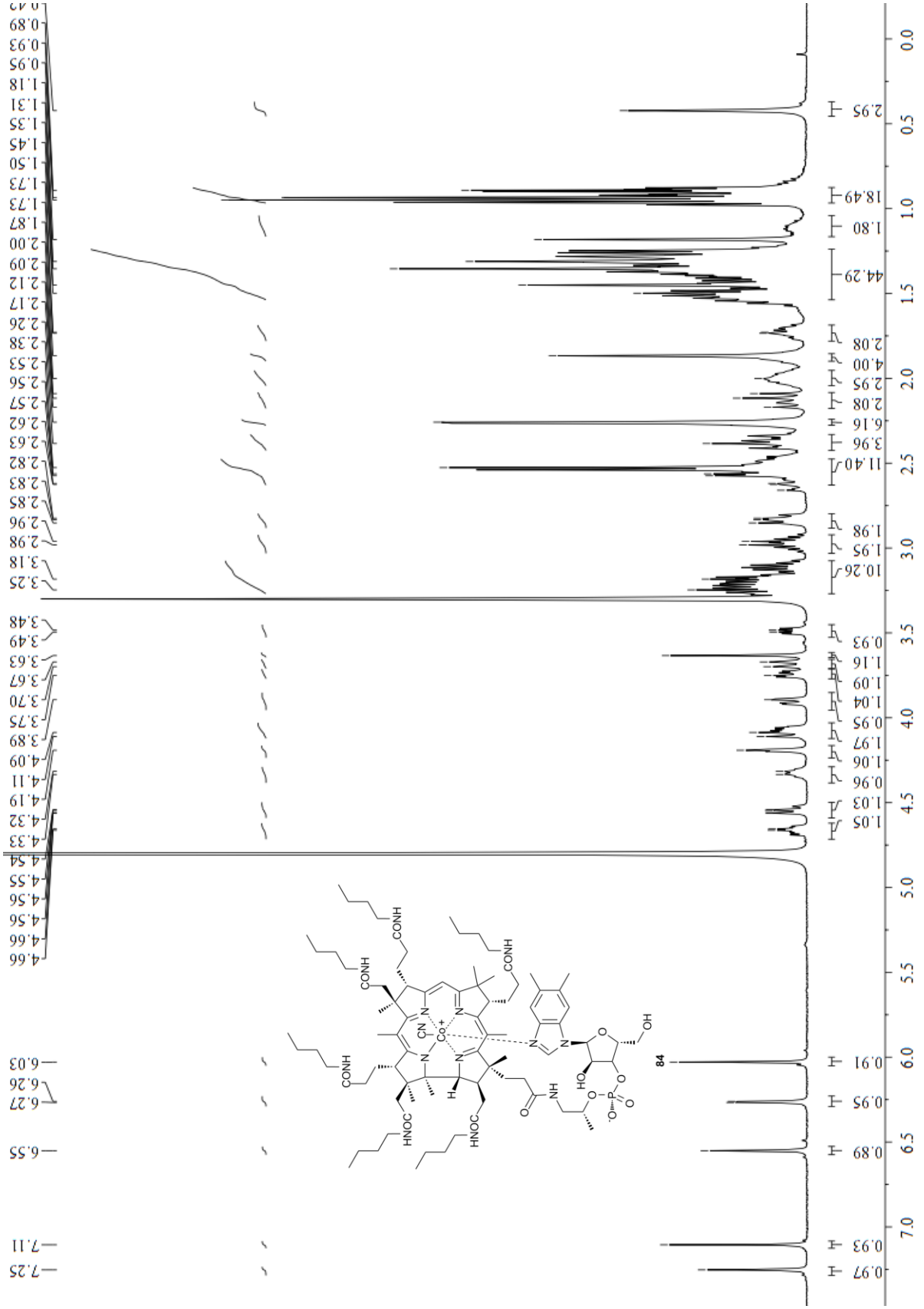
UV-Vis controlled electrolysis of (CN)Cble (2) in MeCN



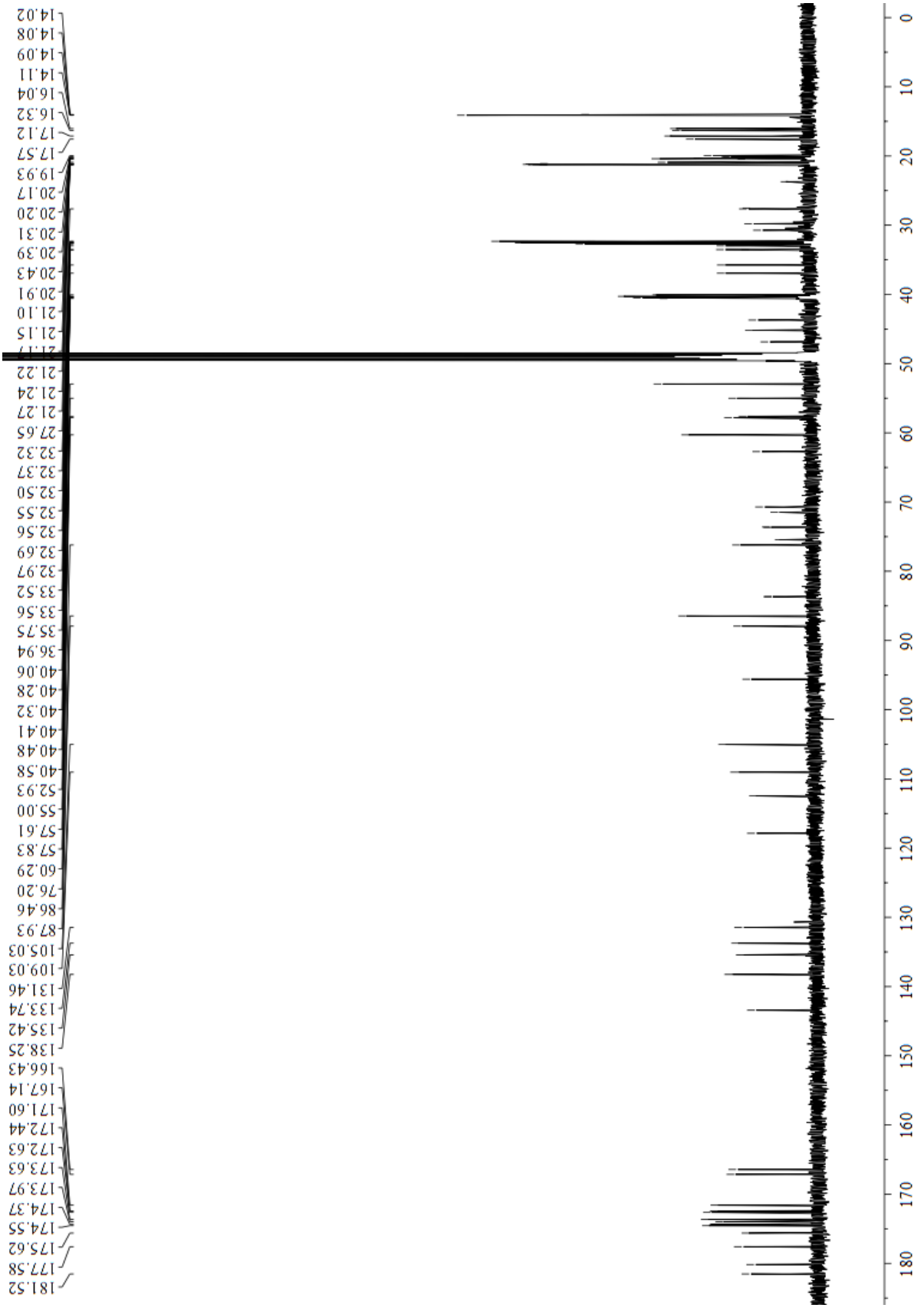
UV-Vis controlled electrolysis of monocyano cobyrinate 3 in MeCN



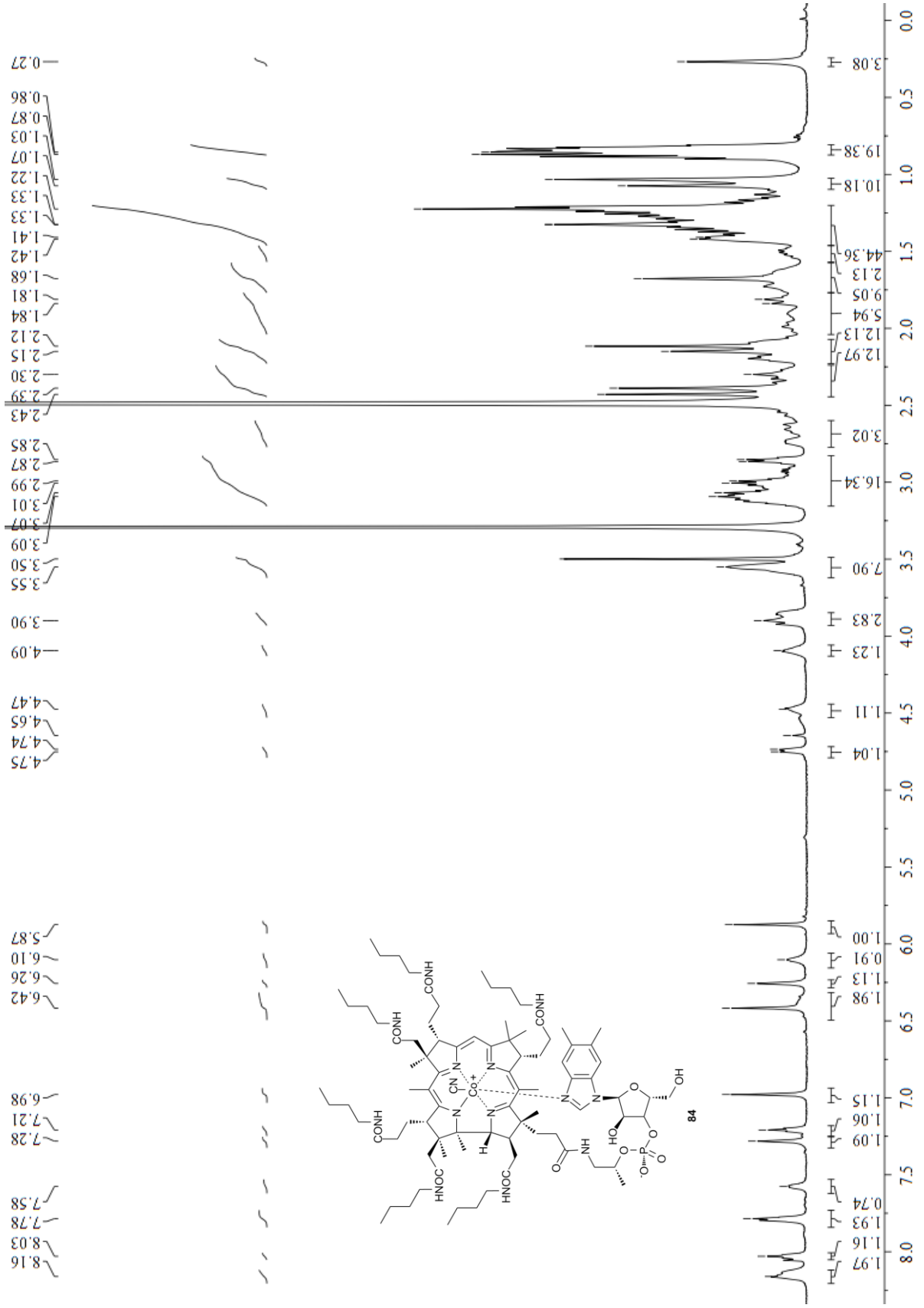
^{1}H NMR spectra of hexa(*n*-butyl)cobalamin (12) in CD_3OD



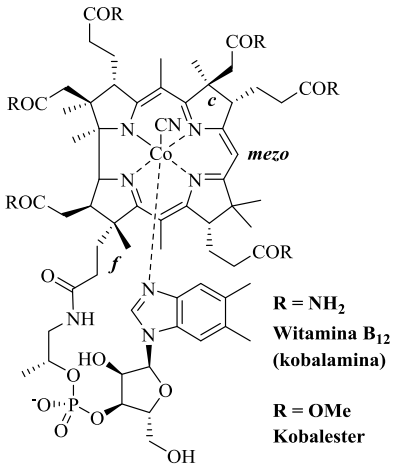
^{13}C NMR spectra of hexa(*n*-butyl)cobalamin (12) in CD_3OD



^{1}H NMR spectra of hexa(*n*-butyl)cobalamin (12) in d_6-DMSO



7. STRESZCZENIE W JĘZYKU POLSKIM



Witamina B_{12} należy do grupy składników odżywczych, niezbędnych do prawidłowego funkcjonowania organizmów wszystkich ssaków. Występuje ona w ustroju biologicznym w formie koenzymów, a ich aktywność wynika z obecności kowalencyjnego wiązania Co-C, które w odpowiednich warunkach ulega rozerwaniu stając się źródłem rodników. Ta charakterystyczna cecha witaminy B_{12}, w połączeniu z jej nietoksycznością, sprawia, że może być ona wykorzystana jako nowoczesny katalizator reakcji rodnikowych.

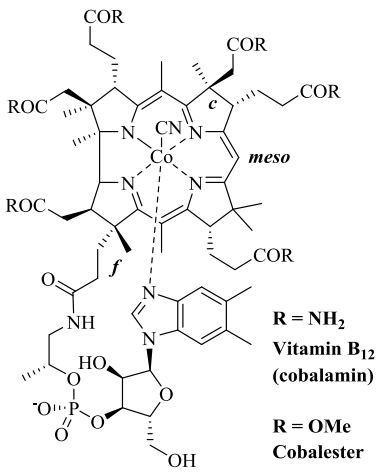
Celem moich badań była synteza nowych pochodnych kobalaminy, określenie jak zmiany w strukturze tego związku wpływają na jego właściwości katalityczne oraz opracowanie nowych reakcji katalizowanych pochodnymi witaminy B_{12}.

W pierwszej części badań opracowałem metodę aminolizy grup estrowych znajdujących się na zewnątrz pierścienia heptaestru metylowego kwasu kobyrynowego, otrzymując pochodne heptaamidowe, zróżnicowane pod względem struktury i właściwości fizykochemicznych. Następnie, związki te poddałem selektywnej funkcjonalizacji w pozycjach *c* lub *mezo* w sposób umożliwiający projektowanie dalszych połączeń hybrydowych. Wykorzystując opracowaną metodologię otrzymałem również 8-*nor*-heksaamid oraz analogiczny heksakwas.

Drugi obszar badań stanowiły modyfikacje pierwszorzędowych grup amidowych obecnych w kobalaminie, bez naruszania fragmentu nukleotydowego znajdującego się w pozycji *f*. W ich wyniku udało mi się opracować wydajną metodę przekształcenia amidów w estry metylowe, na drodze aktywacji grupy amidowej dimetyloacetalem dimetyloformamidu i następczej metanolizy wytworzonych formamidyn. **Otrzymany związek, który nazwałem kobalestrem, jest jedyną znaną pochodną witaminy B_{12} z zachowanym fragmentem nukleotydowym, która wykazuje właściwości amfifilowe i rozpuszcza się zarówno w rozpuszczalnikach polarnych, jak i niepolarnych**. Kobalester, jak i otrzymaną z niego heksbutylokobalaminę scharakteryzowałem metodami elektrochemicznymi oraz określiłem mechanizm redukcji centralnego kationu kobaltu w warunkach aprotycznych.

Ukoronowaniem prowadzonych przeze mnie badań było odkrycie nowej, indukowanej światłem reakcji C-H funkcjonalizacji olefin diazozwiązkami. Proces ten, zamiast typowego cyklopropanowania, prowadzi z bardzo dobrymi wydajnościami do produktów C-H alkilowania, a zakres jego stosowalności obejmuje również alkeny podstawione grupami elektronodonorowymi. Ponadto, opracowałem procedury dimeryzacji bromków benzylu w warunkach elektrolizy i promieniowania mikrofalowego.

8. STRESZCZENIE W JĘZYKU ANGIELSKIM / ABSTRACT IN ENGLISH



Vitamin B_{12} is a complex biomolecule of vast importance for normal functioning of all mammalian organisms. Its various forms play the role of coenzymes and posess covalent Co-C bond that can be selectively cleaved, becoming a source of radicals. This characteristic feature, together with its nontoxicity, renders vitamin B_{12} a useful catalyst for radical reactions.

The goal of my studies was to synthesize new cobalamin derivatives and determine how do alterations in the molecular structure influence their catalytic properties. I also aimed to develop new organic reactions catalyzed by vitamin B_{12} derivatives.

At the first stage of my work I have established a method for the aminolysis of ester groups which are present at the periphery of the corrin ring in heptamethyl cobyrinate. A small library of heptaamides was obtained with diversified structure and physicochemical properties. They were subsequently functionalized at *c* and *meso* positions allowing for further conjugation. Aminolysis and hydrolysis reactions were also used for the synthesis of 8-*nor*-hexaamide and 8-*nor*-hexaacid derivatives.

Another part of my research focused on the modification of the primary amide groups present in cobalamin, leaving the nucleotide fragment at *f* position intact. As a result, I developed an efficient method for the transformation of these amides into ester groups by the activation of amide group with dimethylformamide dimethylacetal and subsequent methanolysis. The desired compound (cobalester) is the only amphiphilic vitamin B_{12} derivative possessing a nucleotide loop intact, being soluble in both polar and nonpolar solvents. Along with its hexaamide derivative, it was fully characterized by electrochemical means and the mechanism of the reduction of the central cobalt atom was determined.

Finally, I have discovered a new, light-induced, cobalester-catalyzed reaction of olefins with diazo compounds, leading to unexpected sp^2 C-H alkylation instead of the usual cyclopropanation. Moreover, procedures for benzyl bromide homocoupling in electrolytic or microwave conditions were developed.

In summary, my studies demonstrate the usefulness of vitamin B_{12} derivatives as efficient, environmentally benign catalysts in organic synthesis. They also provide tools for modifying their structure, allowing to adjust catalyst properties for desired application.

9. OŚWIADCZENIA AUTORÓW PUBLIKACJI[21]

mgr inż. Maciej Giedyk

Oświadczam, że mój wkład w powstanie poniższych publikacji polegał na:

1. M. Giedyk, H. Shimakoshi, K. Goliszewska, D. Gryko, Y. Hisaeda *Dalton Trans.* 2016, DOI: 10.1039/C6DT00355A „Electrochemistry and Catalytic Properties of Amphiphilic Vitamin B_{12} Derivatives in Nonaqueous Media"

Współopracowałem koncepcję badań, zsyntezowałem część substratów oraz wykonałem wszystkie eksperymenty. Dane eksperymentalne poddałem analizie oraz wyciągnąłem wnioski dotyczące zakresu i mechanizmu obserwowanych przemian. Następnie przygotowałem manuskrypt wraz z opisem części eksperymentalnej.

2. M. Giedyk, K. Goliszewska, K. ó Proinsias, D. Gryko *Chem. Commun.* 2016, 52, 1389-1392 „Cobalt(I)-Catalysed CH-Alkylation of Terminal Olefins, and Beyond"

*Współopracowałem koncepcję i metodykę badań, wykonałem wszystkie eksperymenty mające na celu wyjaśnienie mechanizmu reakcji oraz przedstawiłem ich interpretację. Ponadto przeprowadziłem syntezę substratów **11** i **13**, a także wykonałem część eksperymentów mających na celu zbadanie zakresu stosowalności i ograniczeń metody, otrzymując związki **5a,b,c,d,n,o**, **10**, **12**, **14a**, **14b**, **16**. Uczestniczyłem także w przygotowaniu manuskryptu.*

3. M. Giedyk, K. ó Proinsias, S. Kurcoń, I. Sharina, E. Martin, D. Gryko *ChemMedChem* 2014, 9, 2344-2350 „Small Alterations in Cobinamide Structure Considerably Influence sGC Activation"

*Zaplanowałem i wykonałem większość syntez chemicznych wydzielając związki **6**, **7**, **8**, **13**, **15**, **16**, **17** i **18** oraz przeprowadziłem interpretację wyników chemicznych i biologicznych. Opracowałem metodę pełnej hydrolizy heptaestru **1** i heksaestru **5** do odpowiednich kwasów. Brałem także czynny udział w pisaniu manuskryptu.*

4. M. Giedyk, S. N. Fedosov, D. Gryko *Chem. Commun.* 2014, 50, 4674-4676 „An Amphiphilic, Catalytically Active, Vitamin B_{12} Derivative"

Współopracowałem koncepcję i metodykę badań. Zaplanowałem i wykonałem syntezę wszystkich związków przedstawionych w manuskrypcie, w tym opracowałem metodę syntezy kobalestru (3) i kobinestru (5). Następnie otrzymane związki poddałem pełnej analizie oraz

[21] Numeracja związków wymienionych w tym rozdziale odnosi się do numerów znajdujących się w oryginalnych publikacjach.

przeprowadziłem interpretację wyników chemicznych i biologicznych. Otrzymane wyniki przygotowałem w formie manuskryptu.

5. K. ó Proinsias, M. Giedyk, Ł. Banach, D. Rutkowska-Zbik, D. Gryko *Asian J. Org. Chem.* 2013, 2, 504-513 „Selectively Modified Cobyrinic Acid Derivatives"

*Zaplanowałem i wykonałem większość syntez chemicznych, otrzymując związki **4**, **5**, **8**, **9**, **10**, **11**, **14**, **16**, **17**, **20** i **21**, które następnie poddałem pełnej analizie oraz przeprowadziłem interpretację wyników (w tym pełne przyporządkowanie sygnałów NMR korynoidu na podstawie widm 2D NMR). Brałem także udział w przygotowywaniu części eksperymentalnej do manuskryptu.*

6. K. ó Proinsias, M. Giedyk, I. Sharina, E. Martin, D. Gryko *ACS Med. Chem. Lett.* 2012, 3, 476-479 „Synthesis of New Hydrophilic and Hydrophobic Cobinamides as NO-Independent sGC Activators"

*Zaplanowałem i wykonałem wszystkie syntezy chemiczne, a otrzymane związki **1**, **2**, **4**, **5**, **9** i **10** poddałem pełnej analizie. Brałem również udział w interpretacji wyników badań biologicznych oraz przygotowaniu części eksperymentalnej do manuskryptu.*

7. K. ó Proinsias, M. Giedyk, R. Loska, M. Chromiński, D. Gryko *J. Org. Chem.* 2011, 76, 6806-6812 „Selective Modifications of Hydrophobic Vitamin B_{12} Derivatives at *c*- and *d*-Positions"

*Przeprowadziłem wieloetapową syntezę chemiczną prowadzącą do amidowych pochodnych **10** i **12** wraz z oczyszczeniem i poddaniem związków pełnej analizie.*

8. M. Giedyk, K. Goliszewska, D. Gryko *Chem. Soc. Rev.* 2015, 44, 3391-3404 „Vitamin B_{12}-Catalyzed Reactions"

Zgromadziłem i dokładnie zapoznałem się z aktualną literaturą naukową dotyczącą opisywanego zagadnienia, a następnie zaplanowałem kształtu manuskryptu. Brałem także czynny udział w jego pisaniu.

9. K. ó Proinsias, M. Giedyk, D. Gryko *Chem. Soc. Rev.* 2013, 42, 6605-6619 „Vitamin B_{12}: Chemical Modifications"

Zgromadziłem i dokładnie zapoznałem się z aktualną literaturą naukową dotyczącą opisywanego zagadnienia. Brałem także czynny udział w pisaniu manuskryptu.

15/03/2016 Maciej Giedyk

14.03.2016 Dryko

prof. dr hab. Dorota Gryko

Oświadczam, że mój wkład w powstanie poniższych publikacji polegał na:

1. M. Giedyk, H. Shimakoshi, K. Goliszewska, D. Gryko, Y. Hisaeda *Dalton Trans.* 2016, DOI: 10.1039/C6DT00355A „Electrochemistry and Catalytic Properties of Amphiphilic Vitamin B_{12} Derivatives in Nonaqueous Media"

Współopracowałam koncepcję badań i przeprowadziłam korektę manuskryptu.

2. M. Giedyk, K. Goliszewska, K. ó Proinsias, D. Gryko *Chem. Commun.* 2016, 52, 1389-1392 „Cobalt(I)-Catalysed CH-Alkylation of Terminal Olefins, and Beyond"

Współopracowałam koncepcję badań, uczestniczyłam w planowaniu eksperymentów i interpretacji wyników, a także pisaniu manuskryptu.

3. M. Giedyk, K. ó Proinsias, S. Kurcoń, I. Sharina, E. Martin, D. Gryko *ChemMedChem* 2014, 9, 2344-2350 „Small Alterations in Cobinamide Structure Considerably Influence sGC Activation"

Współopracowałam koncepcję badań i zaplanowałam część eksperymentów, a także interpretowałam ich wyniki. Przeprowadziłam korektę manuskryptu.

4. M. Giedyk, S. N. Fedosov, D. Gryko *Chem. Commun.* 2014, 50, 4674-4676 „An Amphiphilic, Catalytically Active, Vitamin B_{12} Derivative"

Współopracowałam koncepcję badań, interpretowałam ich wyniki, a także pisałam manuskrypt.

5. K. ó Proinsias, M. Giedyk, Ł. Banach, D. Rutkowska-Zbik, D. Gryko *Asian J. Org. Chem.* 2013, 2, 504-513 „Selectively Modified Cobyrinic Acid Derivatives"

Współopracowałam koncepcję badań, interpretowałam ich wyniki, a także pisałam manuskrypt.

6. K. ó Proinsias, M. Giedyk, I. Sharina, E. Martin, D. Gryko *ACS Med. Chem. Lett.* 2012, 3, 476-479 „Synthesis of New Hydrophilic and Hydrophobic Cobinamides as NO-Independent sGC Activators"

Współopracowałam koncepcję badań, interpretowałam ich wyniki, a także pisałam manuskrypt.

7. K. ó Proinsias, M. Giedyk, R. Loska, M. Chromiński, D. Gryko *J. Org. Chem.* 2011, 76, 6806-6812 „Selective Modifications of Hydrophobic Vitamin B_{12} Derivatives at *c*- and *d*-Positions"

Współopracowałam koncepcję badań, interpretowałam ich wyniki, a także pisałam manuskrypt.

8. M. Giedyk, K. Goliszewska, D. Gryko *Chem. Soc. Rev.* 2015, 44, 3391-3404 „Vitamin B_{12}-Catalyzed Reactions"

Opracowałam koncepcję przeglądu i uczestniczyłam w jego pisaniu.

9. K. ó Proinsias, M. Giedyk, D. Gryko *Chem. Soc. Rev.* 2013, 42, 6605-6619 „Vitamin B_{12}: Chemical Modifications"

Opracowałam koncepcję przeglądu i uczestniczyłam w jego pisaniu.

11.03.2016 Dgryko

Keith ó Proinsias, PhD

I hereby declare that my contribution to below mentioned publications is as follows:

1. M. Giedyk, K. Goliszewska, K. ó Proinsias, D. Gryko *Chem. Commun.* 2016, 52, 1389-1392 „Cobalt(I)-Catalysed CH-Alkylation of Terminal Olefins, and Beyond"

*I performed the synthesis and purification of compounds **5g,h,i** and I was involved in the optimization studies. I also participated in correcting of the manuscript.*

2. M. Giedyk, K. ó Proinsias, S. Kurcoń, I. Sharina, E. Martin, D. Gryko *ChemMedChem* 2014, 9, 2344-2350 „Small Alterations in Cobinamide Structure Considerably Influence sGC Activation"

I participated in writing the manuscript and interpreting biological data.

3. K. ó Proinsias, M. Giedyk, Ł. Banach, D. Rutkowska-Zbik, D. Gryko *Asian J. Org. Chem.* 2013, 2, 504-513 „Selectively Modified Cobyrinic Acid Derivatives"

I participated in establishing the methodology of presented studies and writing the manuscript. I was also involved in performing optimization studies.

4. K. ó Proinsias, M. Giedyk, I. Sharina, E. Martin, D. Gryko *ACS Med. Chem. Lett.* 2012, 3, 476-479 „Synthesis of New Hydrophilic and Hydrophobic Cobinamides as NO-Independent sGC Activators"

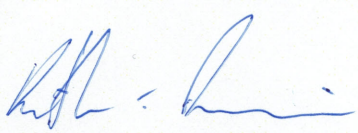
I participated in establishing the methodology of presented studies and in writing the manuscript.

5. K. ó Proinsias, M. Giedyk, R. Loska, M. Chromiński, D. Gryko *J. Org. Chem.* 2011, 76, 6806-6812

I participated in establishing the methodology of presented studies and in writing the manuscript. I also performed modifications in position d.

6. K. ó Proinsias, M. Giedyk, D. Gryko *Chem. Soc. Rev.* 2013, 42, 6605-6619 „Vitamin B_{12}: Chemical Modifications"

I was involved in planning and writing the manuscript.



mgr inż. Katarzyna Goliszewska

Oświadczam, że mój wkład w powstanie poniższych publikacji polegał na:

1. M. Giedyk, H. Shimakoshi, K. Goliszewska, D. Gryko, Y. Hisaeda *Dalton Trans.* 2016, DOI: 10.1039/C6DT00355A „Electrochemistry and Catalytic Properties of Amphiphilic Vitamin B_{12} Derivatives in Nonaqueous Media"

*Otrzymałam i oczyściłam heksa(n-butylo)kobalaminę (**12**).*

2. M. Giedyk, K. Goliszewska, K. ó Proinsias, D. Gryko *Chem. Commun.* 2016, 52, 1389-1392 „Cobalt(I)-Catalysed CH-Alkylation of Terminal Olefins, and Beyond"

*Przeprowadziłam część reakcji mających na celu zbadanie zakresu stosowalności i ograniczeń metody; konkretnie wydzieliłam i oczyściłam związki **5e,f,j,k,l,m**. Uczestniczyłam także w przygotowaniu opisu części eksperymentalnej.*

3. M. Giedyk, K. Goliszewska, D. Gryko *Chem. Soc. Rev.* 2015, 44, 3391-3404 „Vitamin B_{12}-Catalyzed Reactions"

Zgromadziłam aktualną literaturę dotyczącą opisywanego zagadnienia oraz uczestniczyłam w edycji manuskryptu.

08.03.2016 r.

Katarzyna Goliszewska

prof. Yoshio Hisaeda

I hereby declare that my contribution to below mentioned publications is as follows:

1. M. Giedyk, H. Shimakoshi, K. Goliszewska, D. Gryko, Y. Hisaeda *Dalton Trans.* 2016, DOI: 10.1039/C6DT00355A „Electrochemistry and Catalytic Properties of Amphiphilic Vitamin B_{12} Derivatives in Nonaqueous Media"

I supervised the electrochemical studies. I was also involved in correcting the manuscript.

Yoshio Hisaeda

March 9, 2016

Hisashi Shimakoshi, assoc. prof.

I hereby declare that my contribution to below mentioned publications is as follows:

1. M. Giedyk, H. Shimakoshi, K. Goliszewska, D. Gryko, Y. Hisaeda *Dalton Trans.* 2016, DOI: 10.1039/C6DT00355A „Electrochemistry and Catalytic Properties of Amphiphilic Vitamin B_{12} Derivatives in Nonaqueous Media"

I planned initial electrochemical experiments and supervised their execution. I was also involved in writing the manuscript.

Hisashi Shimakoshi

Iraida Sharina, PhD

I hereby declare that my contribution to below mentioned publications is as follows:

1. M. Giedyk, K. ó Proinsias, S. Kurcoń, I. Sharina, E. Martin, D. Gryko *ChemMedChem.* 2014, 9, 2344-2350 „Small Alterations in Cobinamide Structure Considerably Influence sGC Activation"

I performed biological studies.

2. K. ó Proinsias, M. Giedyk, I. Sharina, E. Martin, D. Gryko *ACS Med. Chem. Lett.* 2012, 3, 476-479 „Synthesis of New Hydrophilic and Hydrophobic Cobinamides as NO-Independent sGC Activators"

I performed biological studies.

Iraida Sharina, PhD
Assistant Professor,
University of Texas Health Science Center in Houston
McGovern Medical School

Emil Martin, PhD

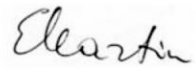
I hereby declare that my contribution to below mentioned publications is as follows:

1. M. Giedyk, K. ó Proinsias, S. Kurcoń, I. Sharina, E. Martin, D. Gryko *ChemMedChem.* 2014, 9, 2344-2350 „Small Alterations in Cobinamide Structure Considerably Influence sGC Activation"

I performed biological studies and participated in their interpretation. I was also involved in writing the biological part of the manuscript.

2. K. ó Proinsias, M. Giedyk, I. Sharina, E. Martin, D. Gryko *ACS Med. Chem. Lett.* 2012, 3, 476-479 „Synthesis of New Hydrophilic and Hydrophobic Cobinamides as NO-Independent sGC Activators"

I co-developed the concept of the studies, performed biological studies and participated in their interpretation. I was also involved in writing the biological part of the manuscript.



Emil Martin, PhD
Associate Professor,
University of Texas Health Science Center in Houston
McGovern Medical School

Sergey N. Fedosov, PhD

I hereby declare that my contribution to below mentioned publications is as follows:

1. M. Giedyk, S. N. Fedosov, D. Gryko *Chem. Commun.* 2014, 50, 4674-4676 „An Amphiphilic, Catalytically Active, Vitamin B_{12} Derivative"

I performed the biological studies on protein – ligand interactions and provided their interpretation. I was also involved in writing the biological part of the manuscript.

Dr. Sergey Fedosov
Dept. Molecular Biology and Genetics
Aarhus University,
Science Park, Gustav Wieds Vej 10C
8000 Aarhus C
Denmark
E-mail: snf@mb.au.dk
Tel: (45) 871 555 21
Mobile: (45) 40 12 30 01
Fax: (45) 86 13 65 97

dr hab. Dorota Rutkowska-Żbik

Oświadczam, że mój wkład w powstanie poniższej publikacji:

1. K. ó Proinsias, M. Giedyk, Ł. Banach, D. Rutkowska-Zbik, D. Gryko *Asian J. Org. Chem.* 2013, 2, 504-513 „Selectively Modified Cobyrinic Acid Derivatives"

polegał na:

Przeprowadziłam obliczenia kwantowo-mechaniczne dla heptaestru ***3*** *oraz dokonałam analizy i interpretacji otrzymanych danych. Przygotowałam także część manuskryptu dotyczącą badań obliczeniowych.*

Kraków, 7.03.2016

dr inż. Sylwester Kurcoń

Oświadczam, że mój wkład w powstanie poniższych publikacji polegał na:

1. M. Giedyk, K. ó Proinsias, S. Kurcoń, I. Sharina, E. Martin, D. Gryko *ChemMedChem.* 2014, 9, 2344-2350 „Small Alterations in Cobinamide Structure Considerably Influence sGC Activation"

*Przeprowadziłem syntezę opisanych wcześniej związków **3**, **4** i **5** oraz związku **10**, które M. Giedyk wykorzystał jako substraty do dalszych badań.*[1]

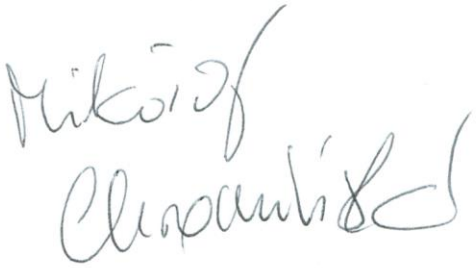
[1] S. Kurcoń, K. ó Proinsias, D. Gryko *J. Org. Chem.* 2013, 78, 4115-4122.

dr inż. Mikołaj Chromiński

Oświadczam, że mój wkład w powstanie poniższych publikacji polegał na:

1. K. ó Proinsias, M. Giedyk, R. Loska, M. Chromiński, D. Gryko *J. Org. Chem.* 2011, 76, 6806-6812 „Selective Modifications of Hydrophobic Vitamin B_{12} Derivatives at *c*- and *d*-Positions"

*Przeprowadziłem syntezę pochodnej rodaminy B (**16**), a następnie połączyłem ją z pochodną **11** na drodze CuAAC (synteza związku **17**). Ponadto wykonałem analizę otrzymanych związków.*



dr Rafał Loska

Oświadczam, że mój wkład w powstanie poniższych publikacji polegał na:

1. K. ó Proinsias, M. Giedyk, R. Loska, M. Chromiński, D. Gryko *J. Org. Chem.* 2011, 76, 6806-6812 „Selective Modifications of Hydrophobic Vitamin B_{12} Derivatives at *c*- and *d*-Positions"

Otrzymałem i oczyściłem linkery, które zostały wykorzystane do otrzymania pochodnych ***12***, ***15***, ***16*** *oraz* ***19***.

R Loska
04.03.2016

mgr inż. Łukasz Banach

Oświadczam, że mój wkład w powstanie poniższych publikacji polegał na:

1. **K. ó Proinsias, M. Giedyk, Ł. Banach, D. Rutkowska-Zbik, D. Gryko *Asian J. Org. Chem.* 2013, 2, 504-513 „Selectively Modified Cobyrinic Acid Derivatives"**

Przeprowadziłem syntezę związku 18, a także brałem udział badaniach optymalizacyjnych.

B. Org. 386/16