

# Amaryllidaceae alkaloids of norbelladine-type as inspiration for development of highly selective butyrylcholinesterase inhibitors: synthesis, biological activity evaluation, and docking studies

Abdullah Al Mamun<sup>1</sup>, Filip Pidany<sup>1</sup>, Daniela Hulcová<sup>1,2</sup>, Jana Maříková<sup>1,3</sup>, Tomáš Kučera<sup>4</sup>, Monika Schmidt<sup>5</sup>, Maria Carmen Catapano<sup>6</sup>, Martina Hrabínová<sup>4,7</sup>, Daniel Jun<sup>4</sup>, Ľubica Múčková<sup>4,7</sup>, Jiří Kuneš<sup>3</sup>, Jiří Janoušek<sup>2</sup>, Rudolf Andrys<sup>5</sup>, Lucie Nováková<sup>6</sup>, Rozálie Peřinová<sup>1</sup>, Negar Maafi<sup>1</sup>, Ondřej Soukup<sup>4,7</sup>, Jan Korábečný<sup>4,7,\*</sup>, Lucie Cahlíková<sup>1,\*</sup>

- 1 ADINACO Research Group, Department of Pharmaceutical Botany, Faculty of Pharmacy, Charles University, Heyrovského 1203, Hradec Kralove 500 05, Czech Republic; [almamuna@faf.cuni.cz](mailto:almamuna@faf.cuni.cz) (A.A.M.); [pidany@faf.cuni.cz](mailto:pidany@faf.cuni.cz) (F.P.); [perinovr@faf.cuni.cz](mailto:perinovr@faf.cuni.cz) (R.P.); [negarm@faf.cuni.cz](mailto:negarm@faf.cuni.cz) (N.M.)
- 2 Department of Pharmacognosy, Faculty of Pharmacy, Charles University, Heyrovského 1203, 500 05 Hradec Kralove, Czech Republic; [hulcovd@faf.cuni.cz](mailto:hulcovd@faf.cuni.cz) (D.H.); [janousj2@faf.cuni.cz](mailto:janousj2@faf.cuni.cz) (J.J.)
- 3 Department of Bioorganic and Organic Chemistry, Faculty of Pharmacy, Charles University, Heyrovského 1203, 500 05 Hradec Kralove, Czech Republic; [marikoj2@faf.cuni.cz](mailto:marikoj2@faf.cuni.cz) (J.M.); [kunes@faf.cuni.cz](mailto:kunes@faf.cuni.cz) (J.K.)
- 4 Department of Toxicology and Military Pharmacy, Trebesska 1575, 500 05 Hradec Kralove, Czech Republic; [kucera-t@email.cz](mailto:kucera-t@email.cz) (T.K.); [martina.hrabinova@unob.cz](mailto:martina.hrabinova@unob.cz) (M.H.); [daniel.jun@unob.cz](mailto:daniel.jun@unob.cz) (D.J.); [lubica.muckova@unob.cz](mailto:lubica.muckova@unob.cz) (L.M)
- 5 Department of Chemistry, Faculty of Science, University of Hradec Králové, Rokitsanského 62, 50003 Hradec Králové, Czech Republic; [monika.schmidt@uhk.cz](mailto:monika.schmidt@uhk.cz) (M.S.); [rudolf.andrys@faf.cuni.cz](mailto:rudolf.andrys@faf.cuni.cz) (R.A.)
- 6 Department of Analytical Chemistry, Faculty of Pharmacy, Charles University, Heyrovského 1203, 500 05 Hradec Kralove, Czech Republic; [catapanm@faf.cuni.cz](mailto:catapanm@faf.cuni.cz) (M.C.C.); [novakoval@faf.cuni.cz](mailto:novakoval@faf.cuni.cz) (L.N.)
- 7 Biomedical Research Centre, University Hospital Hradec Kralove, Sokolska 581, 500 05 Hradec Kralove, Czech Republic; [ondrej.soukup@fnhk.cz](mailto:ondrej.soukup@fnhk.cz) (O.S.)

\*Correspondence: [cahlikova@faf.cuni.cz](mailto:cahlikova@faf.cuni.cz) (L.C.), ORCID ID: 0000-0002-1555-8870, Tel. +420 495 067 311; [jan.korabecny@fnhk.cz](mailto:jan.korabecny@fnhk.cz) (J.Ko.), ORCID ID: 0000-0001-6977-7596, Tel.: + 420 495 833 447

## Table of contents

ESI-HRMS, <sup>1</sup> H NMR and <sup>13</sup> C NMR spectra of new compounds.....	4
Figure S1. HRMS of <i>N</i> -allyl- <i>N</i> -(4-benzyloxy-3-methoxybenzyl)-2-(4-hydroxyphenyl)ethan-1-amine (3) .....	4
Figure S2. <sup>1</sup> H NMR spectrum of <i>N</i> -allyl- <i>N</i> -(4-benzyloxy-3-methoxybenzyl)-2-(4-hydroxyphenyl)ethan-1-amine (3) in CDCl <sub>3</sub> .....	4
Figure S3. <sup>13</sup> C NMR spectrum of <i>N</i> -allyl- <i>N</i> -(4-benzyloxy-3-methoxybenzyl)-2-(4-hydroxyphenyl)ethan-1-amine (3) in CDCl <sub>3</sub> .....	5
Figure S4. HRMS of <i>N</i> -allyl- <i>N</i> -(3-benzyloxy-4-methoxybenzyl)-2-(4-hydroxyphenyl)ethan-1-amine (4).....	5
Figure S5. <sup>1</sup> H NMR spectrum <i>N</i> -allyl- <i>N</i> -(3-benzyloxy-4-methoxybenzyl)-2-(4-hydroxyphenyl)ethan-1-amine (4) in CDCl <sub>3</sub> .....	6
Figure S6. <sup>13</sup> C NMR spectrum of <i>N</i> -allyl- <i>N</i> -(3-benzyloxy-4-methoxybenzyl)-2-(4-hydroxyphenyl)ethan-1-amine (4) in CDCl <sub>3</sub> .....	6
Figure S7. HRMS of <i>N</i> -allyl- <i>N</i> -(4-benzyloxy-3-methoxybenzyl)-2-(4-allyloxyphenyl)ethan-1-amine (5).....	7
Figure S8. <sup>1</sup> H NMR spectrum of <i>N</i> -allyl- <i>N</i> -(4-benzyloxy-3-methoxybenzyl)-2-(4-allyloxyphenyl)ethan-1-amine (5) in CDCl <sub>3</sub> .....	7
Figure S9. <sup>13</sup> C NMR spectrum of <i>N</i> -allyl- <i>N</i> -(4-benzyloxy-3-methoxybenzyl)-2-(4-allyloxyphenyl)ethan-1-amine (5) in CDCl <sub>3</sub> .....	8
Figure S10. HRMS of <i>N</i> -allyl- <i>N</i> -(3-benzyloxy-4-methoxybenzyl)-2-(4-allyloxyphenyl)ethan-1-amine (6).....	8
Figure S11. <sup>1</sup> H NMR spectrum of <i>N</i> -allyl- <i>N</i> -(3-benzyloxy-4-methoxybenzyl)-2-(4-allyloxyphenyl)ethan-1-amine (6) in CDCl <sub>3</sub> .....	9
Figure S12. <sup>13</sup> C NMR spectrum of <i>N</i> -allyl- <i>N</i> -(3-benzyloxy-4-methoxybenzyl)-2-(4-allyloxyphenyl)ethan-1-amine (6) in CDCl <sub>3</sub> ....	9
Figure S13. HRMS of <i>N</i> -(4-benzyloxy-3-methoxybenzyl)-2-phenylethan-1-amine (7).....	9
Figure S14. <sup>1</sup> H NMR spectrum of <i>N</i> -(4-benzyloxy-3-methoxybenzyl)-2-phenylethan-1-amine (7) in CDCl <sub>3</sub> .....	10
Figure S15. <sup>13</sup> C NMR spectrum of <i>N</i> -(4-benzyloxy-3-methoxybenzyl)-2-phenylethan-1-amine (7) in CDCl <sub>3</sub> .....	10
Figure S16. HRMS of <i>N</i> -(3-benzyloxy-4-methoxybenzyl)-2-phenylethan-1-amine (8).....	11
Figure S17. <sup>1</sup> H NMR spectrum of <i>N</i> -(3-benzyloxy-4-methoxybenzyl)-2-phenylethan-1-amine (8) in CDCl <sub>3</sub> .....	11
Figure S18. <sup>13</sup> C NMR spectrum of <i>N</i> -(3-benzyloxy-4-methoxybenzyl)-2-phenylethan-1-amine (8) in CDCl <sub>3</sub> .....	12
Figure S19. HRMS of <i>N</i> -allyl- <i>N</i> -(4-benzyloxy-3-methoxybenzyl)-2-phenylethan-1-amine (9) .....	12
Figure S20. <sup>1</sup> H NMR spectrum of <i>N</i> -allyl- <i>N</i> -(4-benzyloxy-3-methoxybenzyl)-2-phenylethan-1-amine (9) in CDCl <sub>3</sub> .....	13
Figure S21. <sup>13</sup> C NMR spectrum of <i>N</i> -allyl- <i>N</i> -(4-benzyloxy-3-methoxybenzyl)-2-phenylethan-1-amine (9) in CDCl <sub>3</sub> .....	13
Figure S22. HRMS of <i>N</i> -allyl- <i>N</i> -(3-benzyloxy-4-methoxybenzyl)-2-phenylethan-1-amine (10) .....	14
Figure S23. <sup>1</sup> H NMR spectrum of <i>N</i> -allyl- <i>N</i> -(3-benzyloxy-4-methoxybenzyl)-2-phenylethan-1-amine (10) in CDCl <sub>3</sub> .....	14
Figure S24. <sup>13</sup> C NMR spectrum of <i>N</i> -allyl- <i>N</i> -(3-benzyloxy-4-methoxybenzyl)-2-phenylethan-1-amine (10) in CDCl <sub>3</sub> .....	15
Figure S25. HRMS of <i>N</i> -(4-benzyloxy-3-methoxybenzyl)-2-(4-methoxyphenyl)ethan-1-amine (11).....	15
Figure S26. <sup>1</sup> H NMR spectrum of <i>N</i> -(4-benzyloxy-3-methoxybenzyl)-2-(4-methoxyphenyl)ethan-1-amine (11) in CDCl <sub>3</sub> .....	16
Figure S27. <sup>13</sup> C NMR spectrum of <i>N</i> -(4-benzyloxy-3-methoxybenzyl)-2-(4-methoxyphenyl)ethan-1-amine (11) in CDCl <sub>3</sub> .....	16
Figure S28. HRMS of <i>N</i> -(3-benzyloxy-4-methoxybenzyl)-2-(4-methoxyphenyl)ethan-1-amine (12).....	17
Figure S29. <sup>1</sup> H NMR spectrum of <i>N</i> -(3-benzyloxy-4-methoxybenzyl)-2-(4-methoxyphenyl)ethan-1-amine (12) in CDCl <sub>3</sub> .....	17
Figure S30. <sup>13</sup> C NMR spectrum of <i>N</i> -(3-benzyloxy-4-methoxybenzyl)-2-(4-methoxyphenyl)ethan-1-amine (12) in CDCl <sub>3</sub> .....	18
Figure S31. HRMS of <i>N</i> -allyl- <i>N</i> -(4-benzyloxy-3-methoxybenzyl)-2-(4-methoxyphenyl)ethan-1-amine (13) .....	18
Figure S32. <sup>1</sup> H NMR spectrum of <i>N</i> -allyl- <i>N</i> -(4-benzyloxy-3-methoxybenzyl)-2-(4-methoxyphenyl)ethan-1-amine (13) in CDCl <sub>3</sub> .....	19
Figure S33. <sup>13</sup> C NMR spectrum of <i>N</i> -allyl- <i>N</i> -(4-benzyloxy-3-methoxybenzyl)-2-(4-methoxyphenyl)ethan-1-amine (13) in CDCl <sub>3</sub> .....	19
Figure S34. HRMS of <i>N</i> -allyl- <i>N</i> -(3-benzyloxy-4-methoxybenzyl)-2-(4-methoxyphenyl)ethan-1-amine (14) .....	20
Figure S35. <sup>1</sup> H NMR spectrum of <i>N</i> -allyl- <i>N</i> -(3-benzyloxy-4-methoxybenzyl)-2-(4-methoxyphenyl)ethan-1-amine (14) in CDCl <sub>3</sub> .....	20
Figure S36. <sup>13</sup> C NMR spectrum of <i>N</i> -allyl- <i>N</i> -(3-benzyloxy-4-methoxybenzyl)-2-(4-methoxyphenyl)ethan-1-amine (14) in CDCl <sub>3</sub> .....	21
Figure S37. HRMS of <i>N</i> -(3-ethoxy-4-methoxybenzyl)-2-(4-hydroxyphenyl)ethan-1-amine (15) .....	21
Figure S38. <sup>1</sup> H NMR spectrum of <i>N</i> -(3-ethoxy-4-methoxybenzyl)-2-(4-hydroxyphenyl)ethan-1-amine (15) in CDCl <sub>3</sub> .....	22
Figure S39. <sup>13</sup> C NMR spectrum of <i>N</i> -(3-ethoxy-4-methoxybenzyl)-2-(4-hydroxyphenyl)ethan-1-amine (15) in CDCl <sub>3</sub> .....	22
Figure S40. HRMS of <i>N</i> -allyl- <i>N</i> -(3-ethoxy-4-methoxybenzyl)-2-(4-hydroxyphenyl)ethan-1-amine (16).....	23

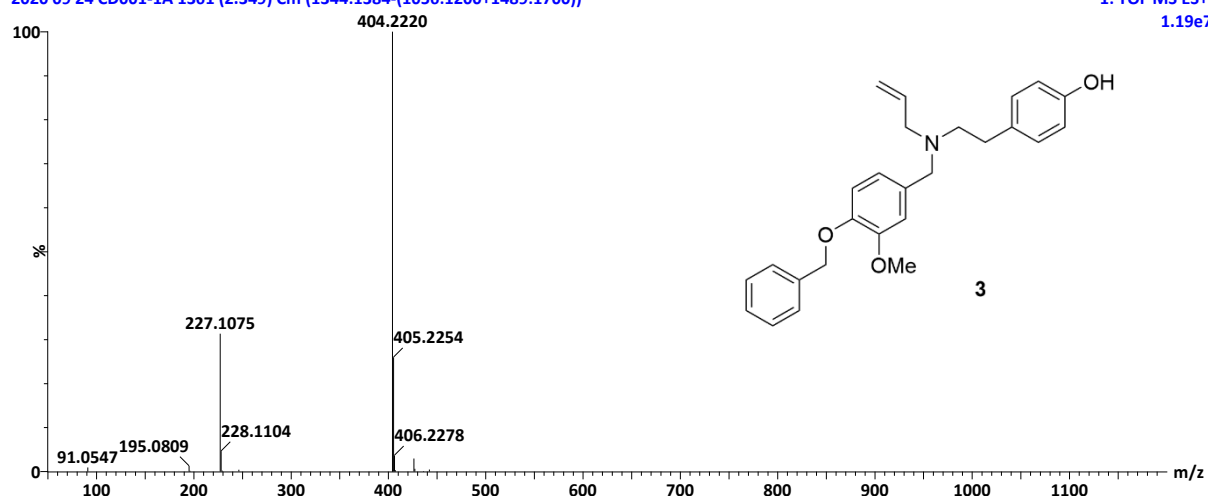
Figure S41. <sup>1</sup> H NMR spectrum of <i>N</i> -allyl- <i>N</i> -(3-ethoxy-4-methoxybenzyl)-2-(4-hydroxyphenyl)ethan-1-amine (16) in CDCl <sub>3</sub> .....	23
Figure S42. <sup>13</sup> C NMR spectrum of <i>N</i> -allyl- <i>N</i> -(3-ethoxy-4-methoxybenzyl)-2-(4-hydroxyphenyl)ethan-1-amine (16) in CDCl <sub>3</sub> .....	24
Figure S43. HRMS of <i>N</i> -allyl- <i>N</i> -(3-ethoxy-4-methoxybenzyl)-2-(4-allyloxyphenyl)ethan-1-amine (17).....	24
Figure S44. <sup>1</sup> H NMR spectrum of <i>N</i> -allyl- <i>N</i> -(3-ethoxy-4-methoxybenzyl)-2-(4-allyloxyphenyl)ethan-1-amine (17) in CDCl <sub>3</sub> .....	25
Figure S45. <sup>13</sup> C NMR spectrum of <i>N</i> -allyl- <i>N</i> -(3-ethoxy-4-methoxybenzyl)-2-(4-allyloxyphenyl)ethan-1-amine (17) in CDCl <sub>3</sub> .....	25
Figure S46. HRMS of <i>N</i> -(3-ethoxy-4-methoxybenzyl)-2-phenylethan-1-amine (18).....	26
Figure S47. <sup>1</sup> H NMR spectrum of <i>N</i> -(3-ethoxy-4-methoxybenzyl)-2-phenylethan-1-amine (18) in CDCl <sub>3</sub> .....	26
Figure S48. <sup>13</sup> C NMR spectrum of <i>N</i> -(3-ethoxy-4-methoxybenzyl)-2-phenylethan-1-amine (18) in CDCl <sub>3</sub> .....	27
Figure S49. HRMS of <i>N</i> -allyl- <i>N</i> -(3-ethoxy-4-methoxybenzyl)-2-phenylethan-1-amine (19).....	27
Figure S50. <sup>1</sup> H NMR spectrum of <i>N</i> -allyl- <i>N</i> -(3-ethoxy-4-methoxybenzyl)-2-phenylethan-1-amine (19) in CDCl <sub>3</sub> .....	28
Figure S51. <sup>13</sup> C NMR spectrum of <i>N</i> -allyl- <i>N</i> -(3-ethoxy-4-methoxybenzyl)-2-phenylethan-1-amine (19) in CDCl <sub>3</sub> .....	28
Figure S52. HRMS of <i>N</i> -benzyl- <i>N</i> -(3-ethoxy-4-methoxybenzyl)-2-phenylethan-1-amine (20).....	29
Figure S53. <sup>1</sup> H NMR spectrum of <i>N</i> -benzyl- <i>N</i> -(3-ethoxy-4-methoxybenzyl)-2-phenylethan-1-amine (20) in CDCl <sub>3</sub> .....	29
Figure S54. <sup>13</sup> C NMR spectrum of <i>N</i> -benzyl- <i>N</i> -(3-ethoxy-4-methoxybenzyl)-2-phenylethan-1-amine (20) in CDCl <sub>3</sub> .....	30
Biological assays in detail .....	31
<i>hAChE and hBuChE Inhibition Assay</i> .....	31
<i>Kinetic Study of Cholinesterase Inhibition</i> .....	31
<i>POP inhibition assay</i> .....	32
<i>MAOs inhibition assay</i> .....	32

## ESI-HRMS, $^1\text{H}$ NMR and $^{13}\text{C}$ NMR spectra of new compounds

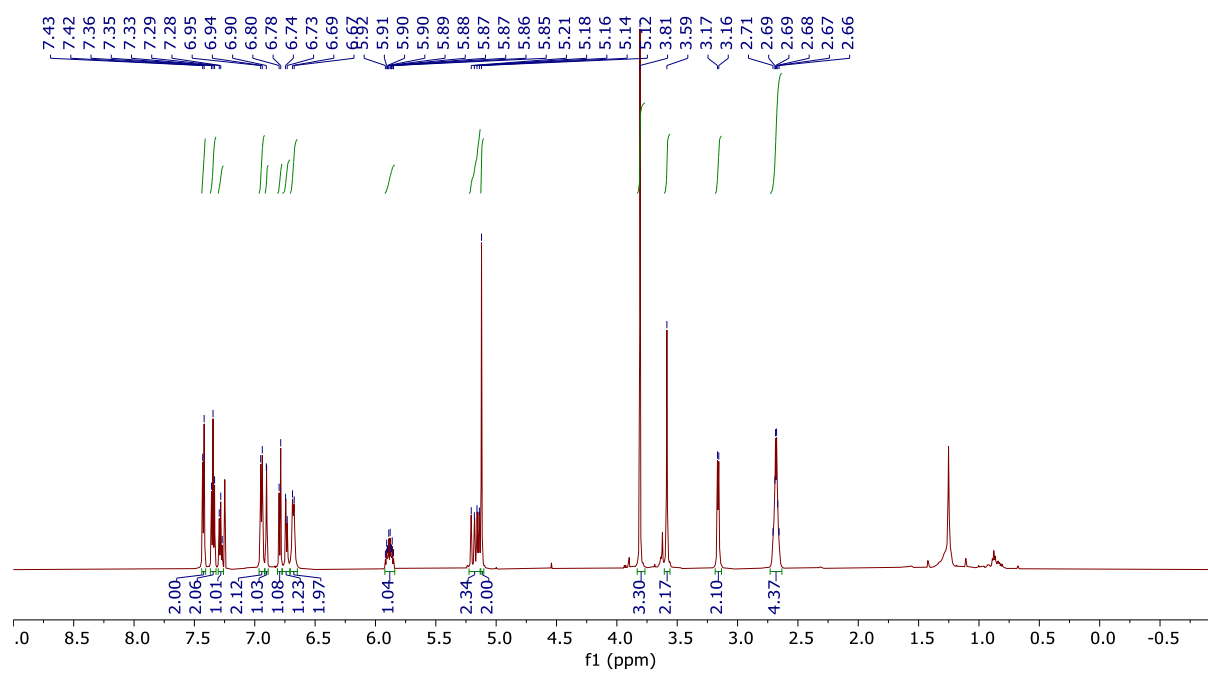
**Figure S1.** HRMS of N-allyl-N-(4-benzyloxy-3-methoxybenzyl)-2-(4-hydroxyphenyl)ethan-1-amine (**3**)  
404.2220

2020 09 24 CD001-1A 1361 (2.349) Cm (1344:1384-(1056:1200+1489:1700))

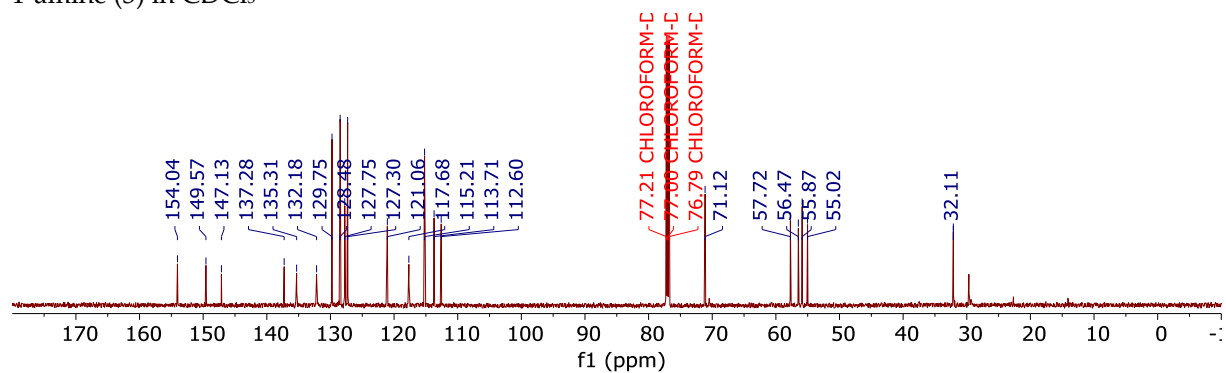
1: TOF MS ES+  
1.19e7



**Figure S2.**  $^1\text{H}$  NMR spectrum of N-allyl-N-(4-benzyloxy-3-methoxybenzyl)-2-(4-hydroxyphenyl)ethan-1-amine (**3**) in  $\text{CDCl}_3$



**Figure S3.**  $^{13}\text{C}$  NMR spectrum of *N*-allyl-*N*-(4-benzyloxy-3-methoxybenzyl)-2-(4-hydroxyphenyl)ethan-1-amine (3) in  $\text{CDCl}_3$

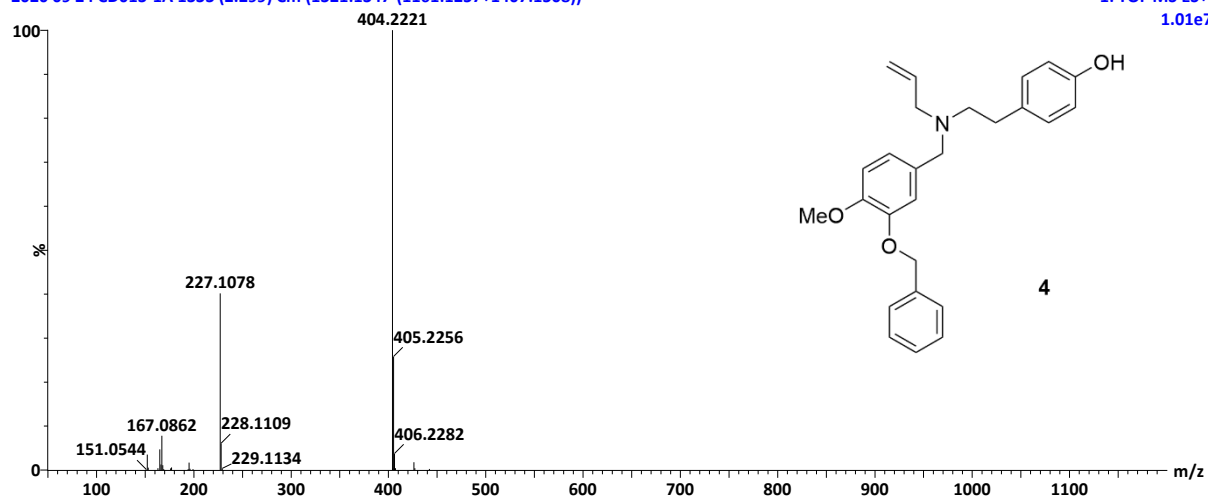


**Figure S4.** HRMS of *N*-allyl-*N*-(3-benzyloxy-4-methoxybenzyl)-2-(4-hydroxyphenyl)ethan-1-amine (4)

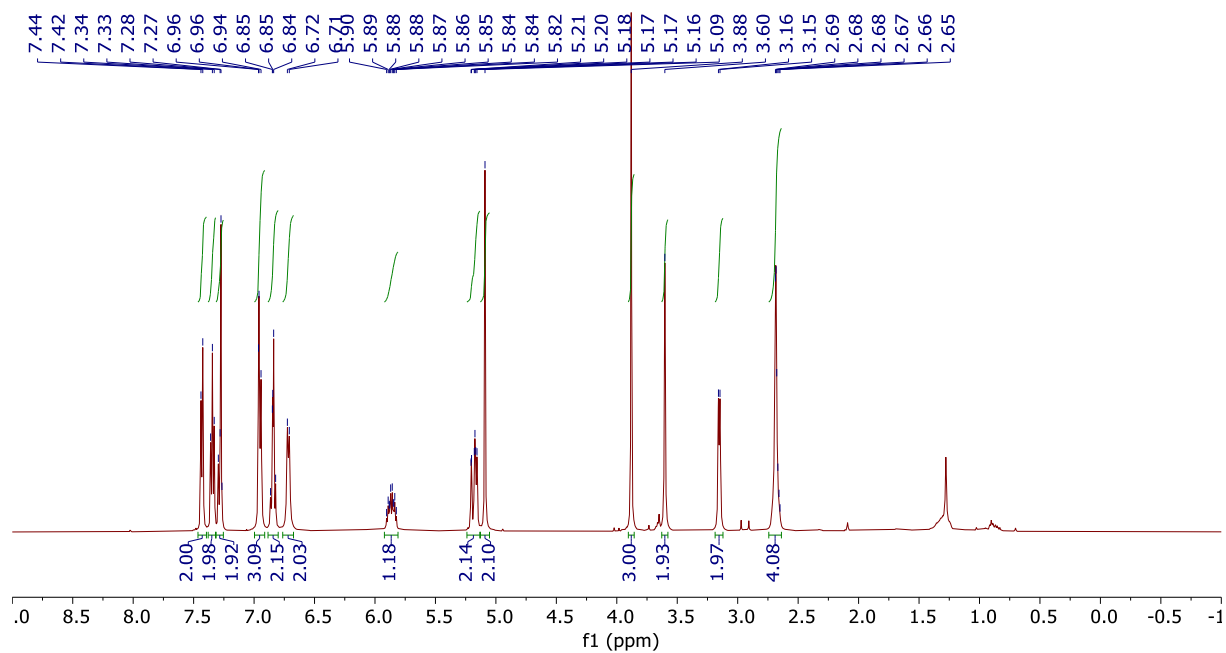
404.2220

2020 09 24 CD013-1A 1333 (2.299) Cm (1321:1347-(1161:1257+1407:1508))

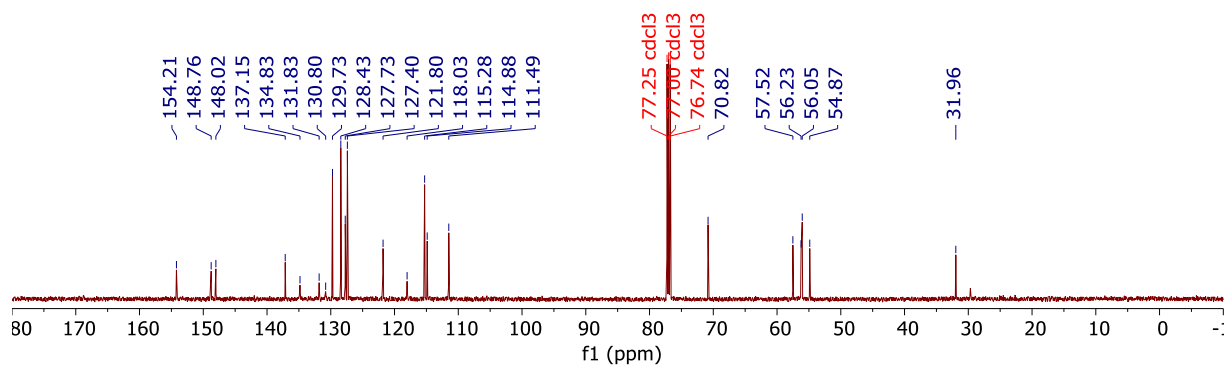
1: TOF MS ES+  
1.01e7



**Figure S5.**  $^1\text{H}$  NMR spectrum *N*-allyl-*N*-(3-benzyloxy-4-methoxybenzyl)-2-(4-hydroxyphenyl)ethan-1-amine (4) in  $\text{CDCl}_3$



**Figure S6.**  $^{13}\text{C}$  NMR spectrum of *N*-allyl-*N*-(3-benzyloxy-4-methoxybenzyl)-2-(4-hydroxyphenyl)ethan-1-amine (4) in  $\text{CDCl}_3$

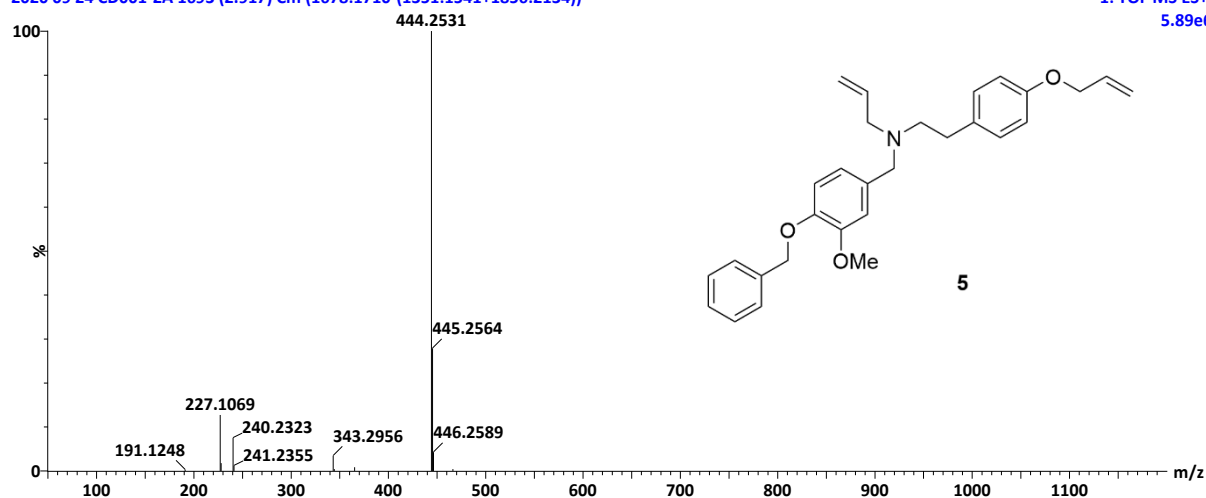


**Figure S7.** HRMS of *N*-allyl-*N*-(4-benzyloxy-3-methoxybenzyl)-2-(4-allyloxyphenyl)ethan-1-amine (**5**)

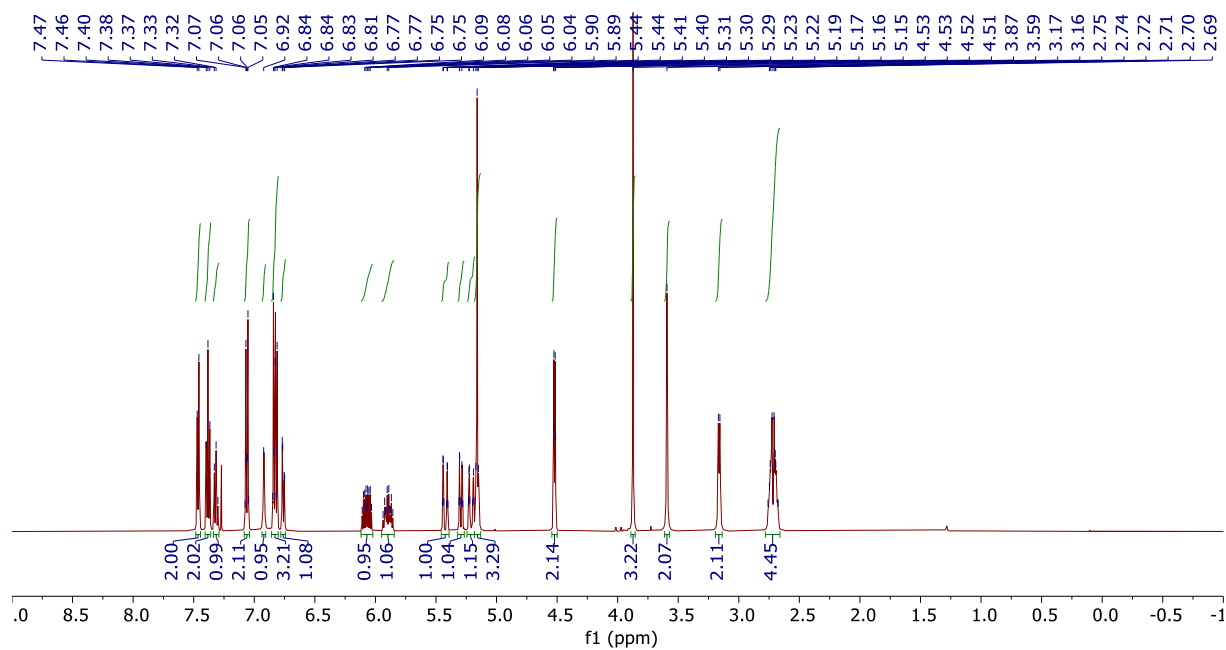
444.2533

2020 09 24 CD001-2A 1693 (2.917) Cm (1678:1710-(1351:1541+1856:2134))

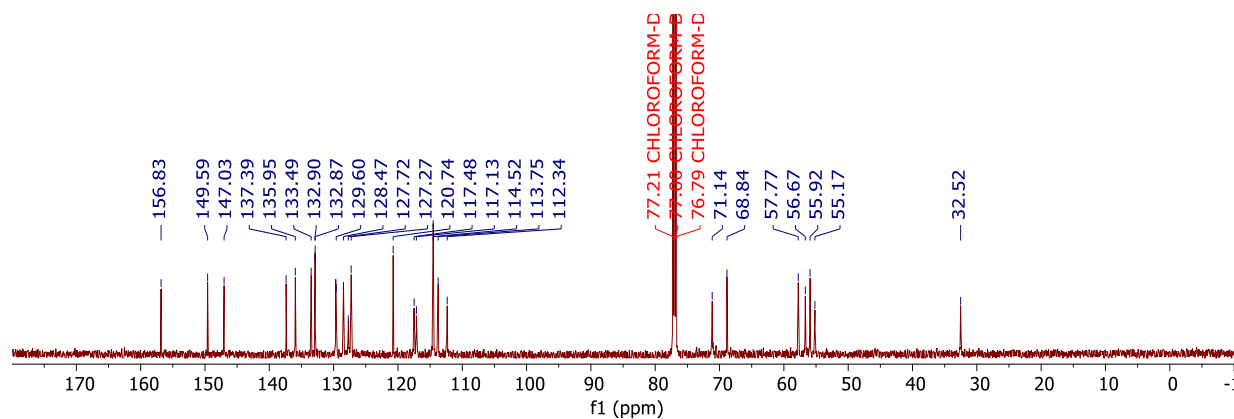
1: TOF MS ES+  
5.89e6



**Figure S8.**  $^1\text{H}$  NMR spectrum of *N*-allyl-*N*-(4-benzyloxy-3-methoxybenzyl)-2-(4-allyloxyphenyl)ethan-1-amine (**5**) in  $\text{CDCl}_3$

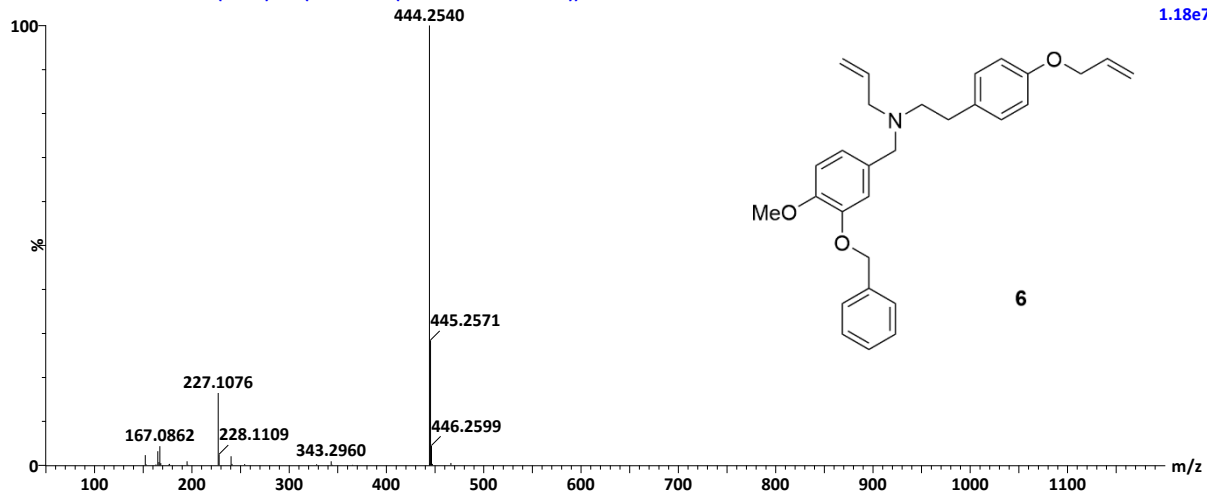


**Figure S9.**  $^{13}\text{C}$  NMR spectrum of *N*-allyl-*N*-(4-benzyloxy-3-methoxybenzyl)-2-(4-allyloxyphenyl)ethan-1-amine (5) in  $\text{CDCl}_3$



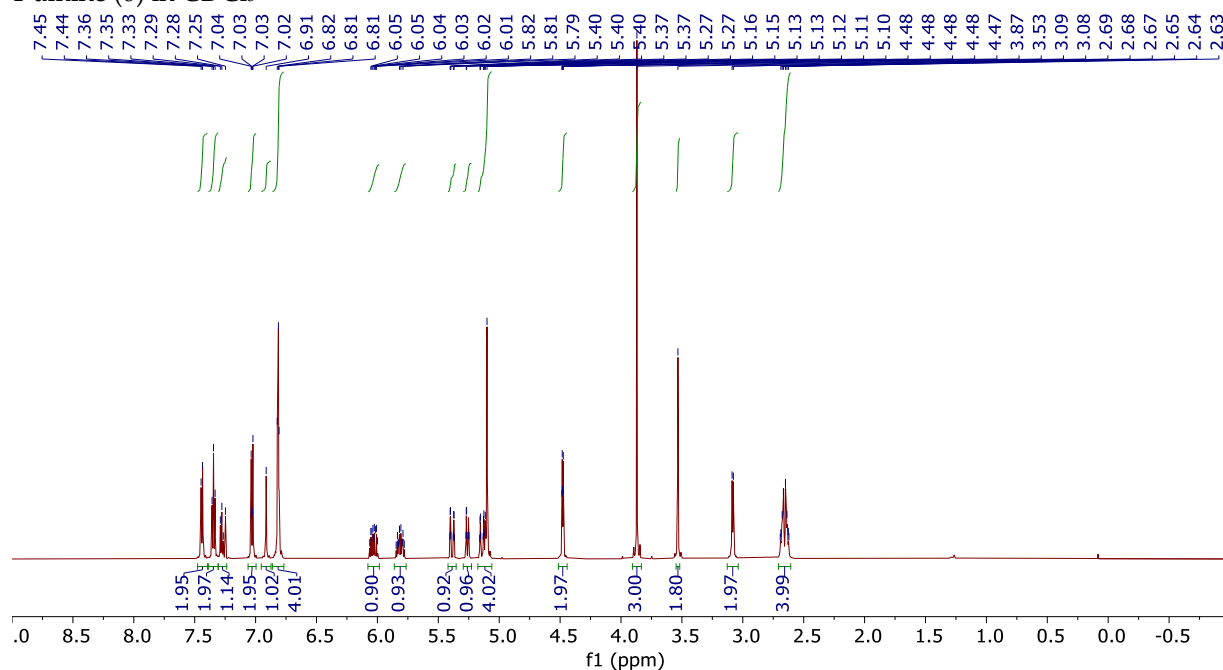
**Figure S10.** HRMS of *N*-allyl-*N*-(3-benzyloxy-4-methoxybenzyl)-2-(4-allyloxyphenyl)ethan-1-amine (6)  
444.2533

2020 09 24 CD013-2A 1673 (2.884) Cm (1661:1691-(1438:1603+1754:1929))

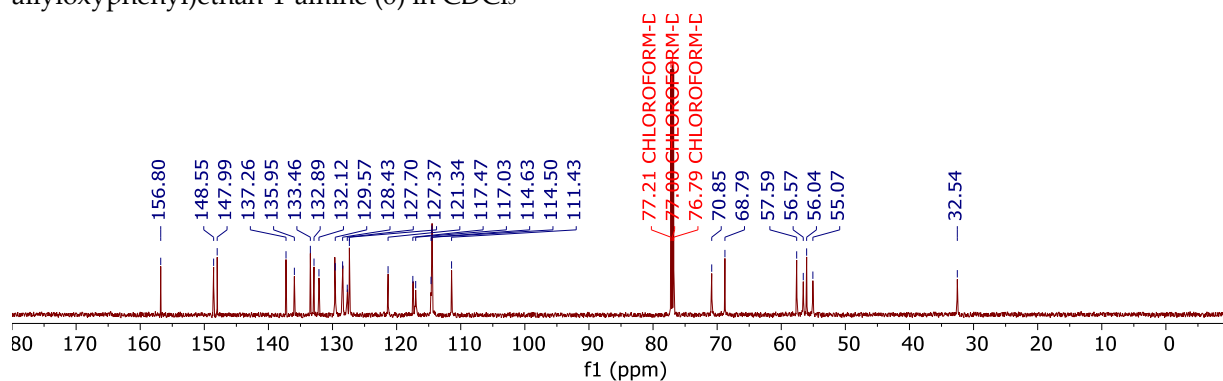




**Figure S11.**  $^1\text{H}$  NMR spectrum of *N*-allyl-*N*-(3-benzyloxy-4-methoxybenzyl)-2-(4-allyloxyphenyl)ethan-1-amine (6) in  $\text{CDCl}_3$



**Figure S12.**  $^{13}\text{C}$  NMR spectrum of *N*-allyl-*N*-(3-benzyloxy-4-methoxybenzyl)-2-(4-allyloxyphenyl)ethan-1-amine (6) in  $\text{CDCl}_3$

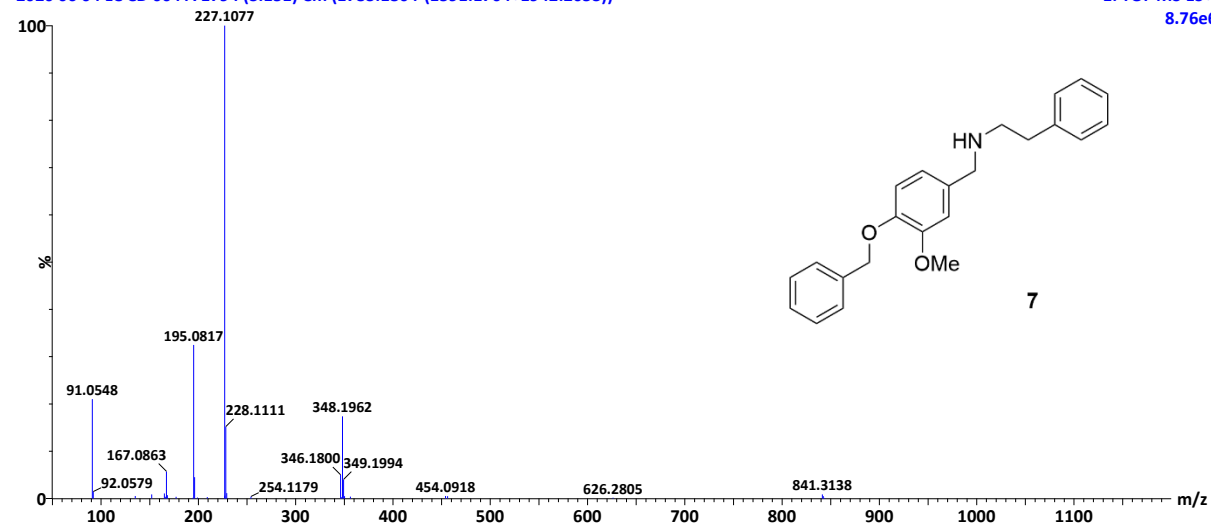


**Figure S13.** HRMS of *N*-(4-benzyloxy-3-methoxybenzyl)-2-phenylethan-1-amine (7)

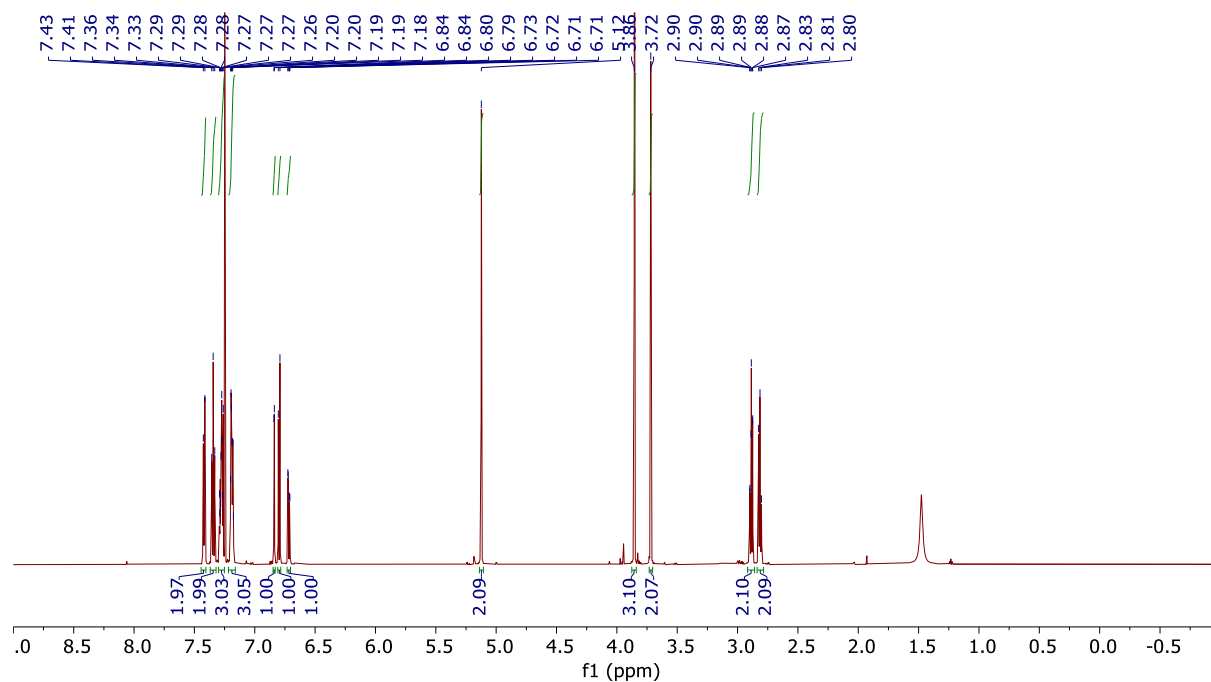
348.1958

2020 06 04 LC CD 004 A 1794 (3.151) Cm (1783:1804-(1591:1704+1942:2053))

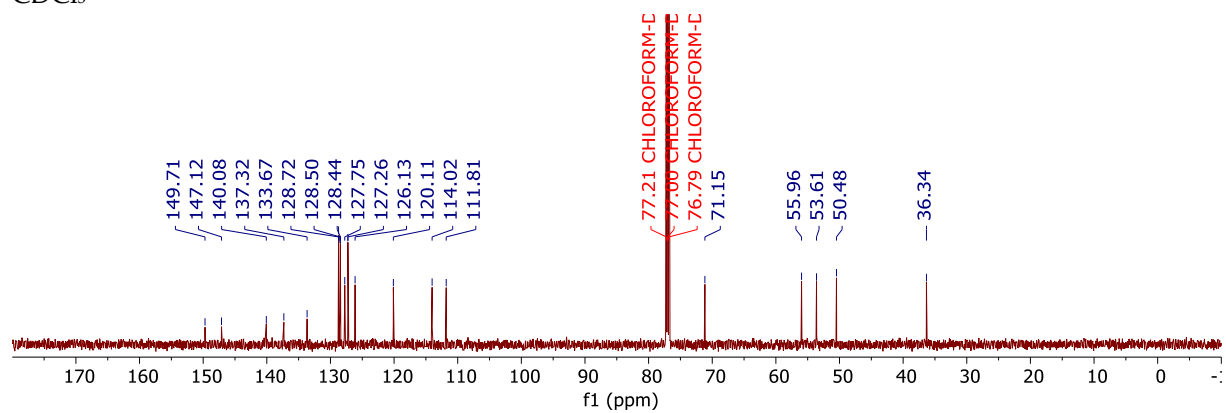
1: TOF MS ES+  
8.76e6



**Figure S14.**  $^1\text{H}$  NMR spectrum of *N*-(4-benzyloxy-3-methoxybenzyl)-2-phenylethan-1-amine (7) in  $\text{CDCl}_3$



**Figure S15.**  $^{13}\text{C}$  NMR spectrum of *N*-(4-benzyloxy-3-methoxybenzyl)-2-phenylethan-1-amine (7) in  $\text{CDCl}_3$

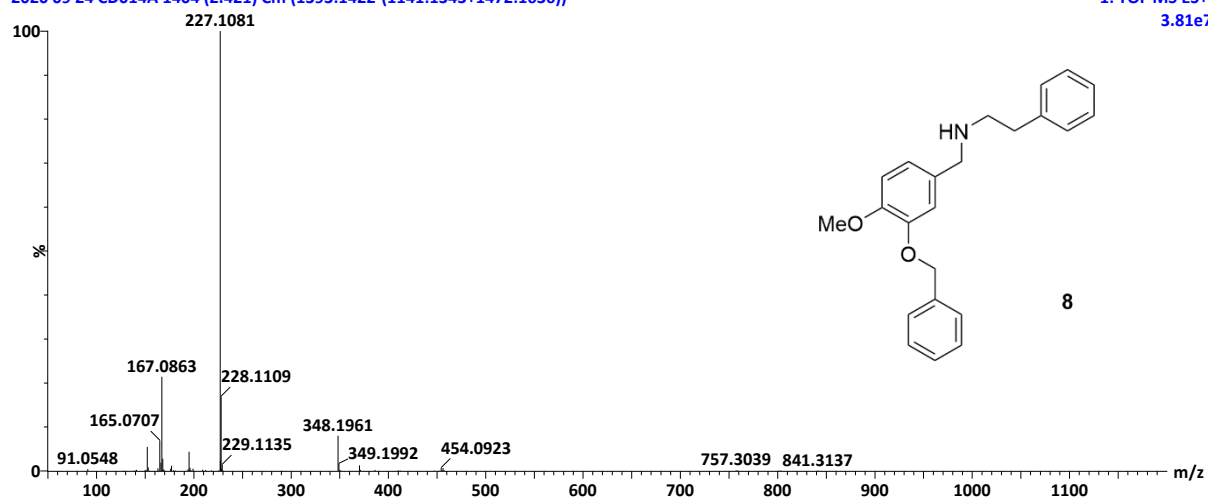


**Figure S16.** HRMS of *N*-(3-benzyloxy-4-methoxybenzyl)-2-phenylethan-1-amine (**8**)

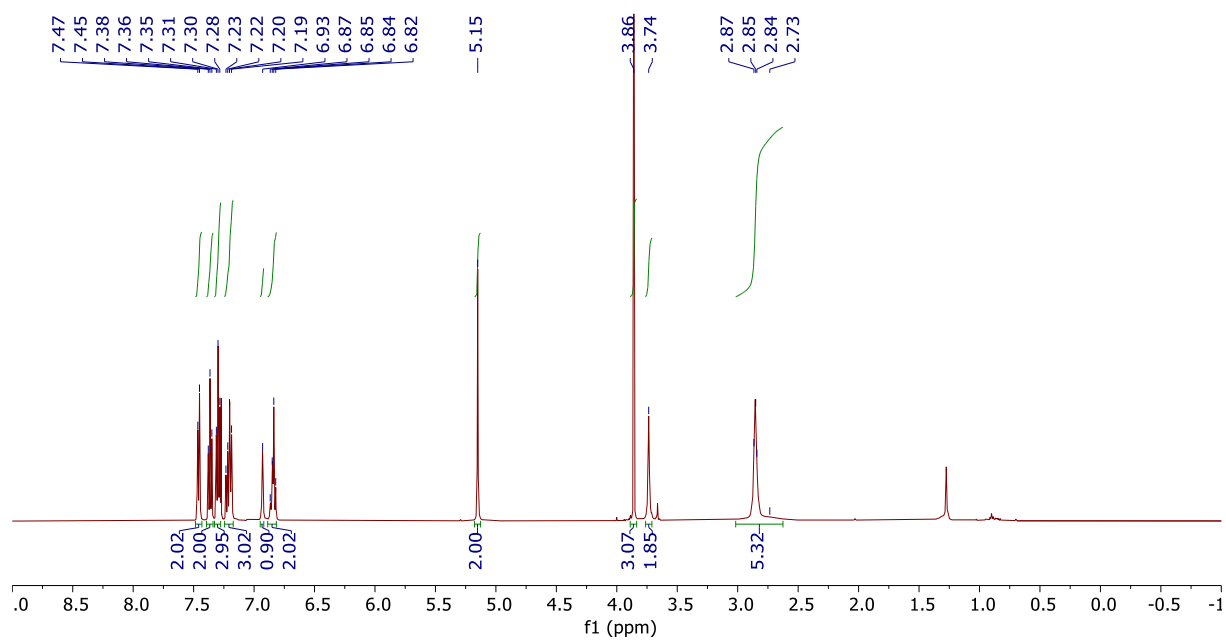
348.1958

2020 09 24 CD014A 1404 (2.421) Cm (1393:1422-(1141:1343+1472:1636))

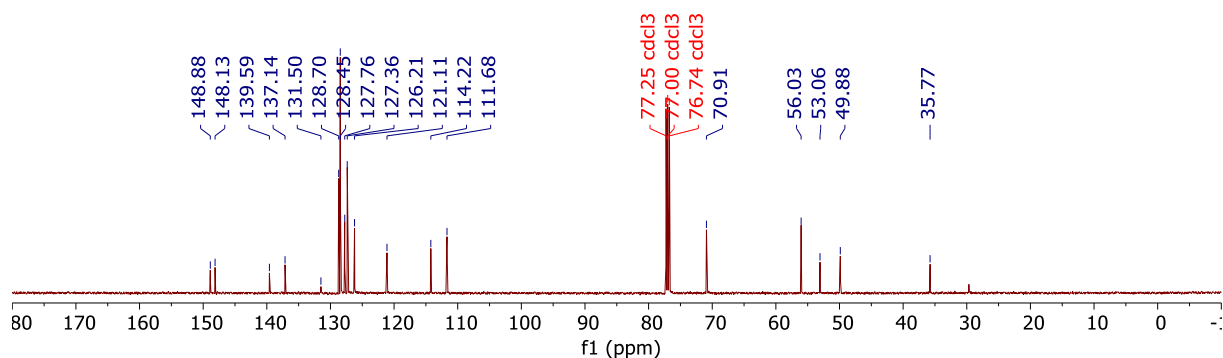
1: TOF MS ES+  
3.81e7



**Figure S17.**  $^1\text{H}$  NMR spectrum of *N*-(3-benzyloxy-4-methoxybenzyl)-2-phenylethan-1-amine (**8**) in  $\text{CDCl}_3$



**Figure S18.**  $^{13}\text{C}$  NMR spectrum of *N*-(3-benzyloxy-4-methoxybenzyl)-2-phenylethan-1-amine (8) in  $\text{CDCl}_3$

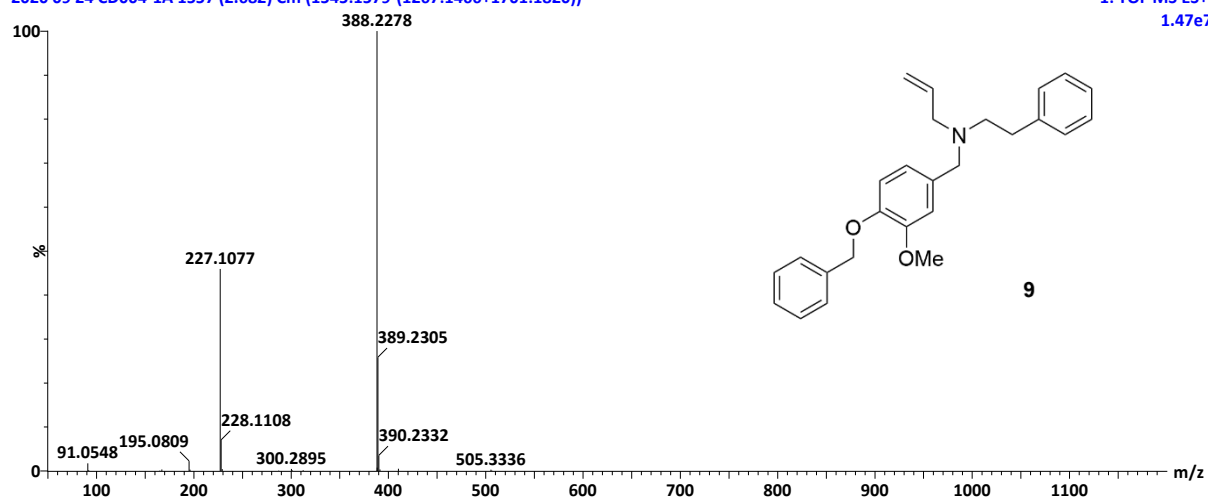


**Figure S19.** HRMS of *N*-allyl-*N*-(4-benzyloxy-3-methoxybenzyl)-2-phenylethan-1-amine (9)

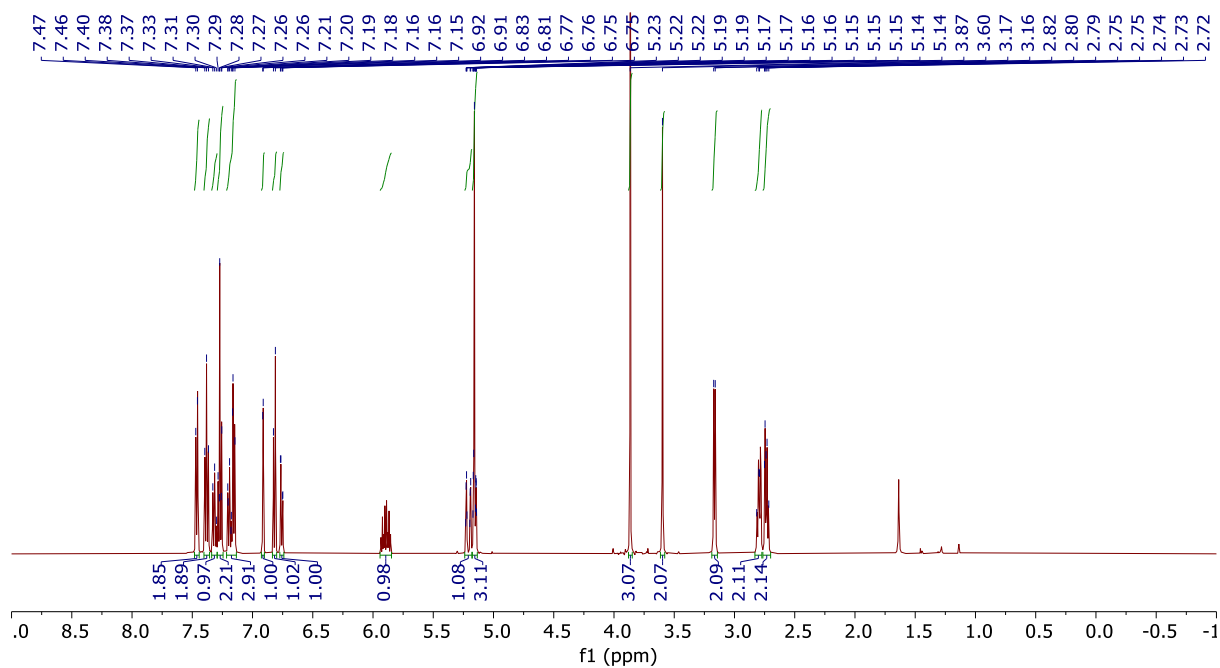
388.2271

2020 09 24 CD004-1A 1557 (2.682) Cm (1545:1579-(1267:1466+1701:1820))

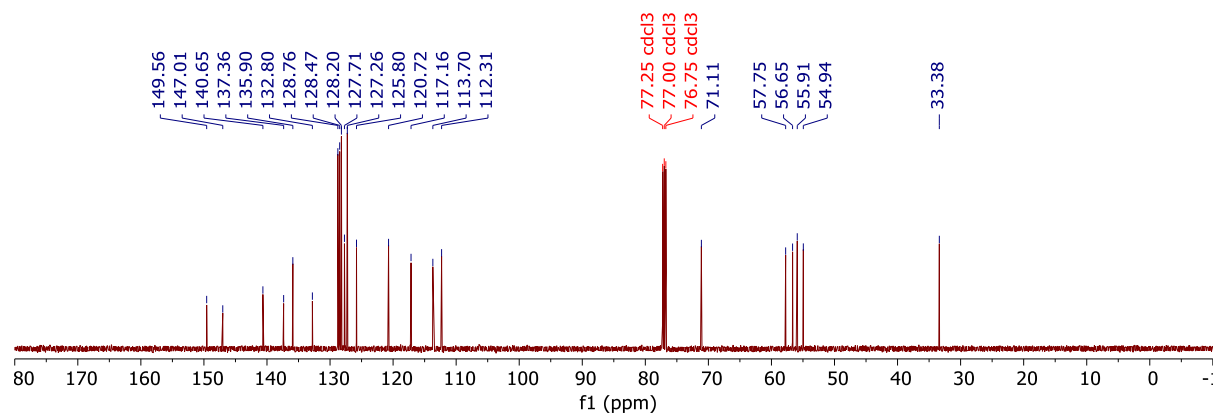
1: TOF MS ES+  
1.47e7



**Figure S20.**  $^1\text{H}$  NMR spectrum of *N*-allyl-*N*-(4-benzyloxy-3-methoxybenzyl)-2-phenylethan-1-amine (9) in  $\text{CDCl}_3$



**Figure S21.**  $^{13}\text{C}$  NMR spectrum of *N*-allyl-*N*-(4-benzyloxy-3-methoxybenzyl)-2-phenylethan-1-amine (9) in  $\text{CDCl}_3$

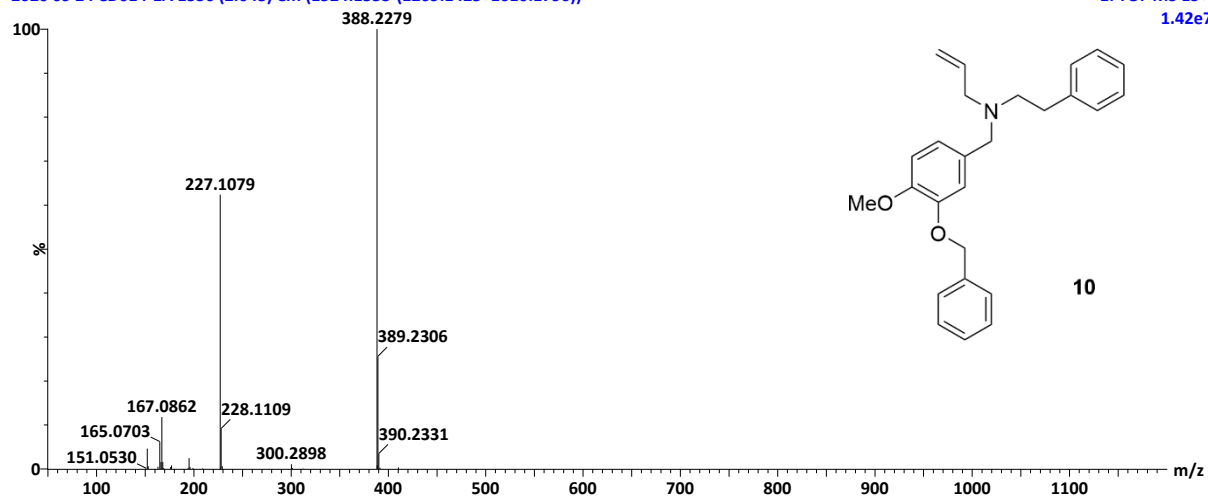


**Figure S22.** HRMS of *N*-allyl-*N*-(3-benzyloxy-4-methoxybenzyl)-2-phenylethan-1-amine (**10**)

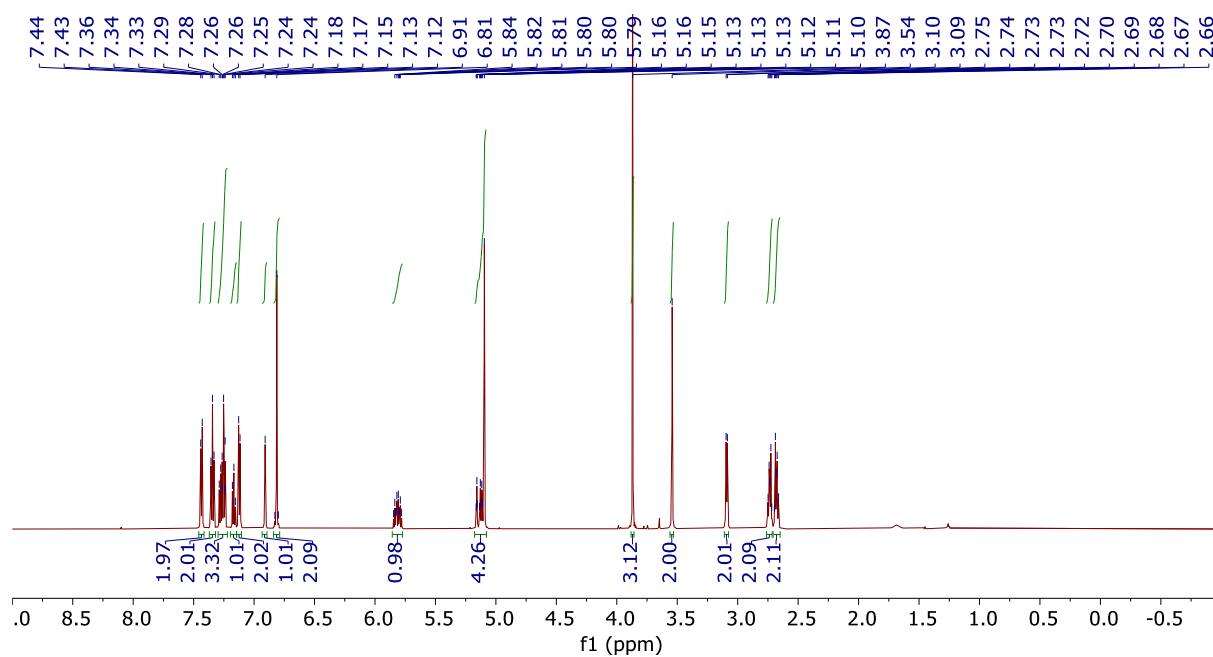
388.2271

2020 09 24 CD014-1A 1536 (2.648) Cm (1524:1553-(1269:1425+1616:1790))

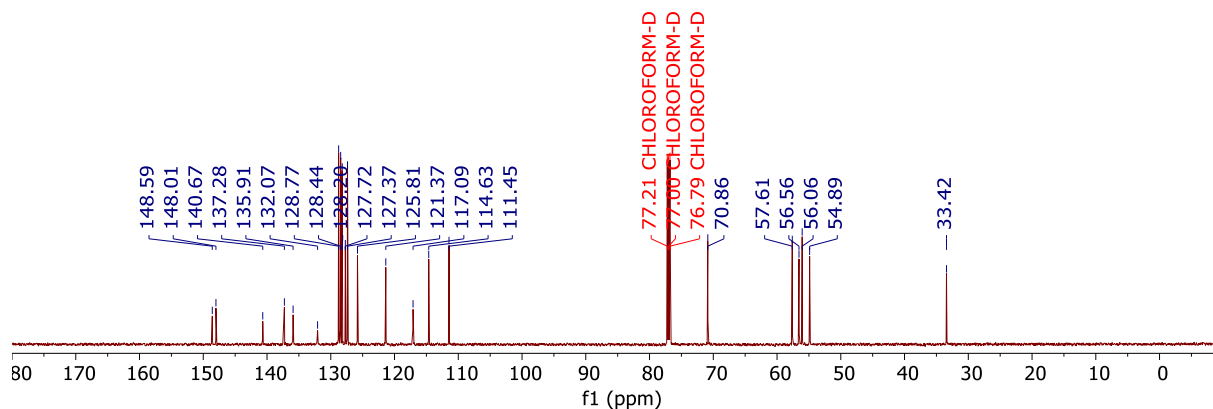
1: TOF MS ES+  
1.42e7



**Figure S23.**  $^1\text{H}$  NMR spectrum of *N*-allyl-*N*-(3-benzyloxy-4-methoxybenzyl)-2-phenylethan-1-amine (**10**) in  $\text{CDCl}_3$



**Figure S24.**  $^{13}\text{C}$  NMR spectrum of *N*-allyl-*N*-(3-benzyloxy-4-methoxybenzyl)-2-phenylethan-1-amine (10) in  $\text{CDCl}_3$

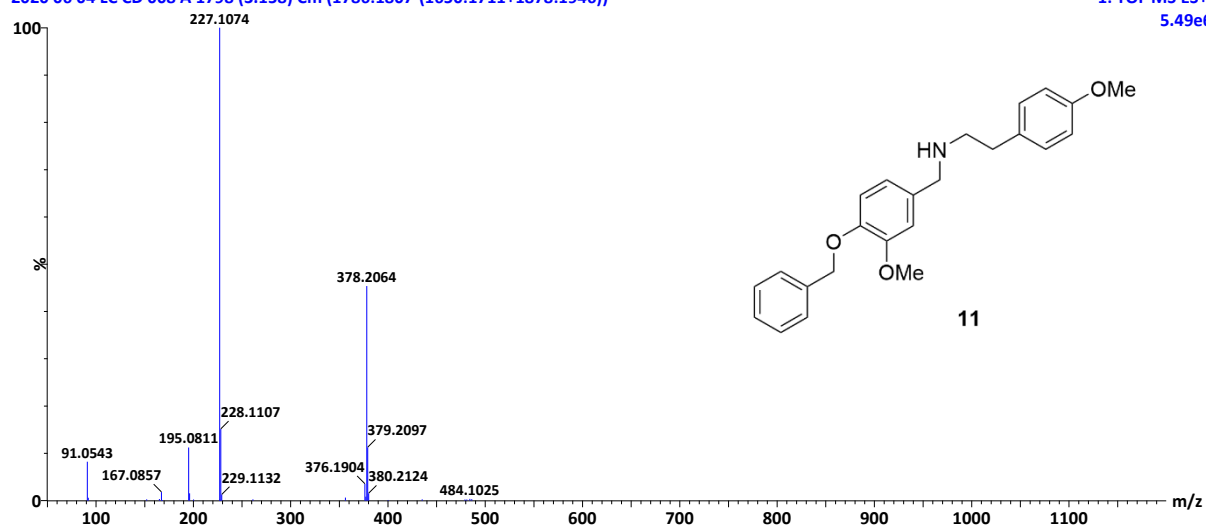


**Figure S25.** HRMS of *N*-(4-benzyloxy-3-methoxybenzyl)-2-(4-methoxyphenyl)ethan-1-amine (11)

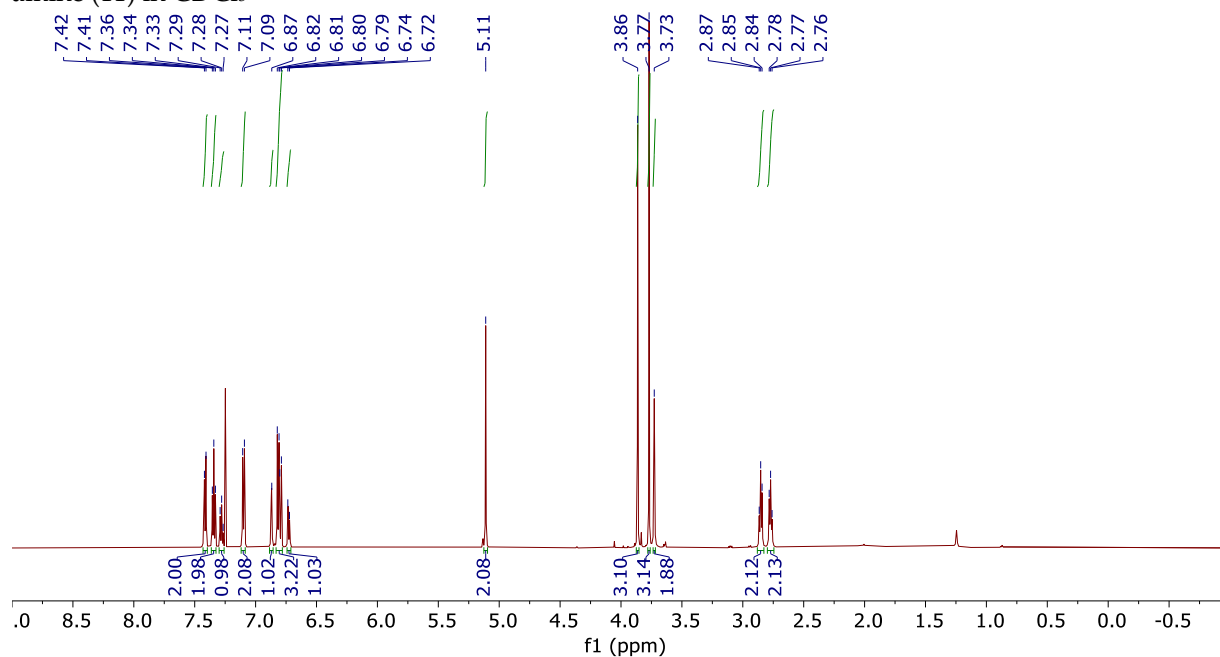
378.2064

2020 06 04 LC CD 008 A 1798 (3.158) Cm (1786:1807-(1650:1711+1878:1946))

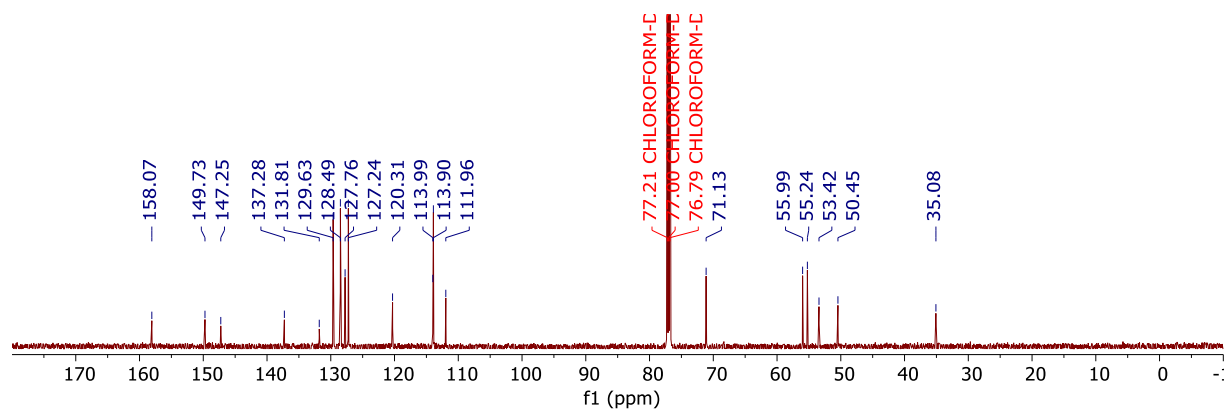
1: TOF MS ES+  
5.49e6



**Figure S26.**  $^1\text{H}$  NMR spectrum of *N*-(4-benzyloxy-3-methoxybenzyl)-2-(4-methoxyphenyl)ethan-1-amine (11) in  $\text{CDCl}_3$



**Figure S27.**  $^{13}\text{C}$  NMR spectrum of *N*-(4-benzyloxy-3-methoxybenzyl)-2-(4-methoxyphenyl)ethan-1-amine (11) in  $\text{CDCl}_3$





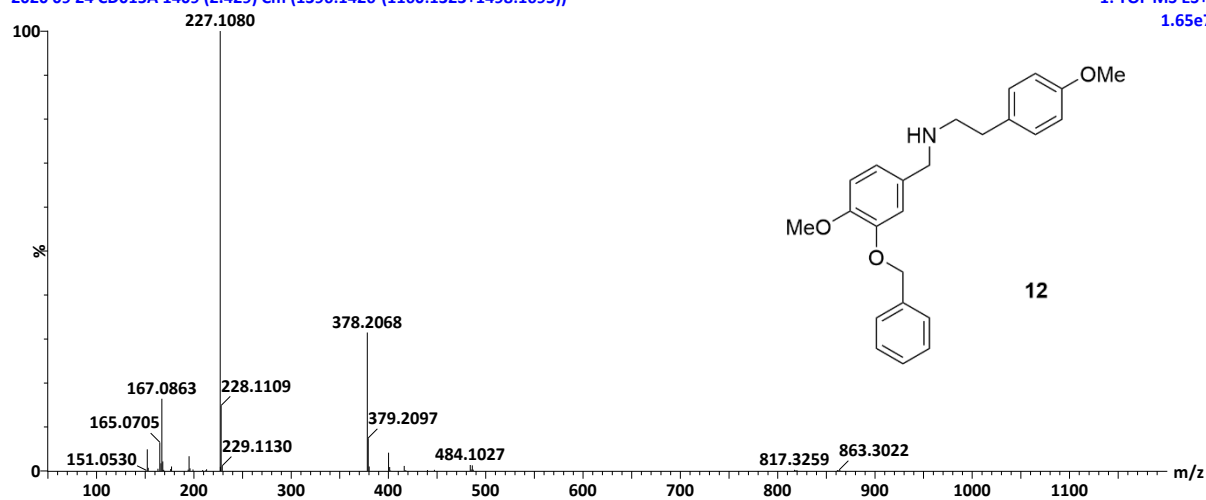
**Figure S28.** HRMS of *N*-(3-benzyloxy-4-methoxybenzyl)-2-(4-methoxyphenyl)ethan-1-amine (**12**)

378.2064

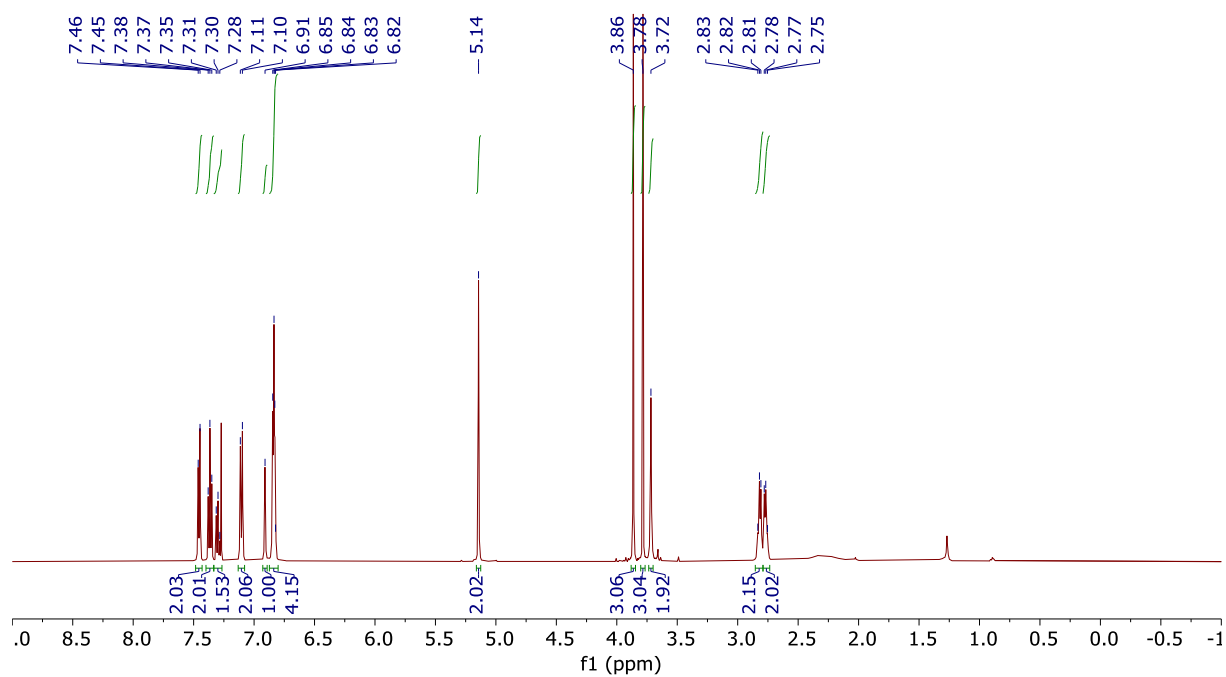
2020 09 24 CD015A 1409 (2.429) Cm (1396:1426-(1160:1323+1498:1695))

1: TOF MS ES+

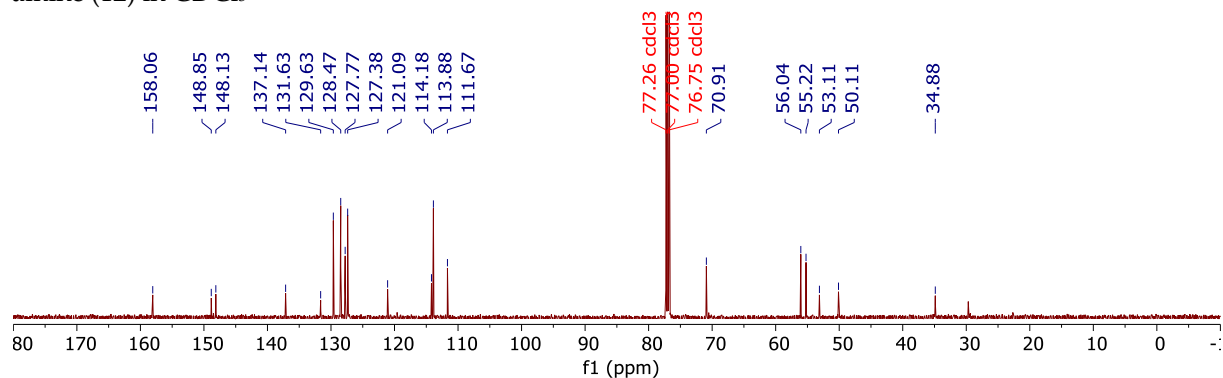
1.65e7



**Figure S29.**  $^1\text{H}$  NMR spectrum of *N*-(3-benzyloxy-4-methoxybenzyl)-2-(4-methoxyphenyl)ethan-1-amine (**12**) in  $\text{CDCl}_3$



**Figure S30.**  $^{13}\text{C}$  NMR spectrum of *N*-(3-benzyloxy-4-methoxybenzyl)-2-(4-methoxyphenyl)ethan-1-amine (12) in  $\text{CDCl}_3$

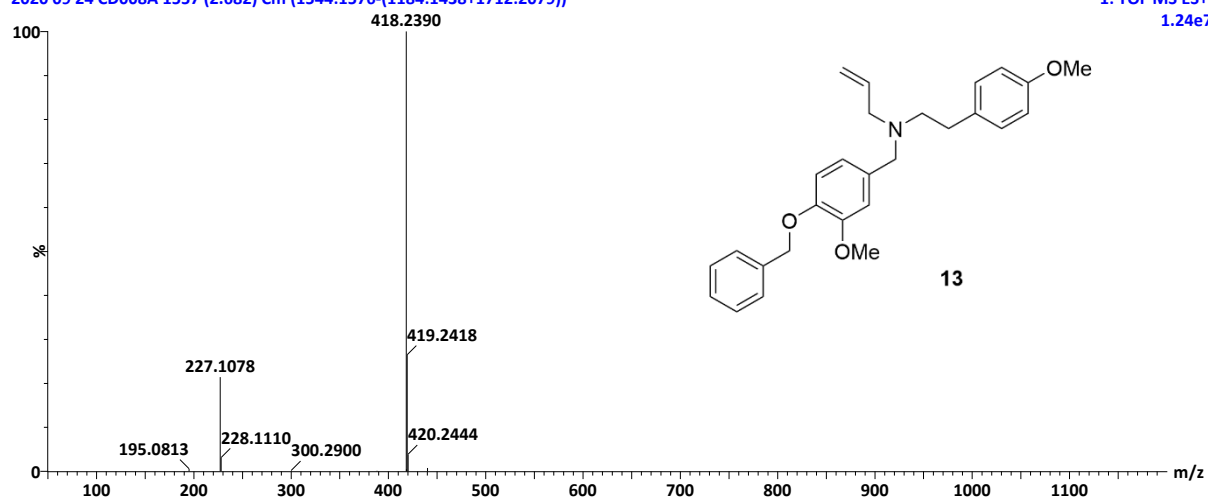


**Figure S31.** HRMS of *N*-allyl-*N*-(4-benzyloxy-3-methoxybenzyl)-2-(4-methoxyphenyl)ethan-1-amine (13)

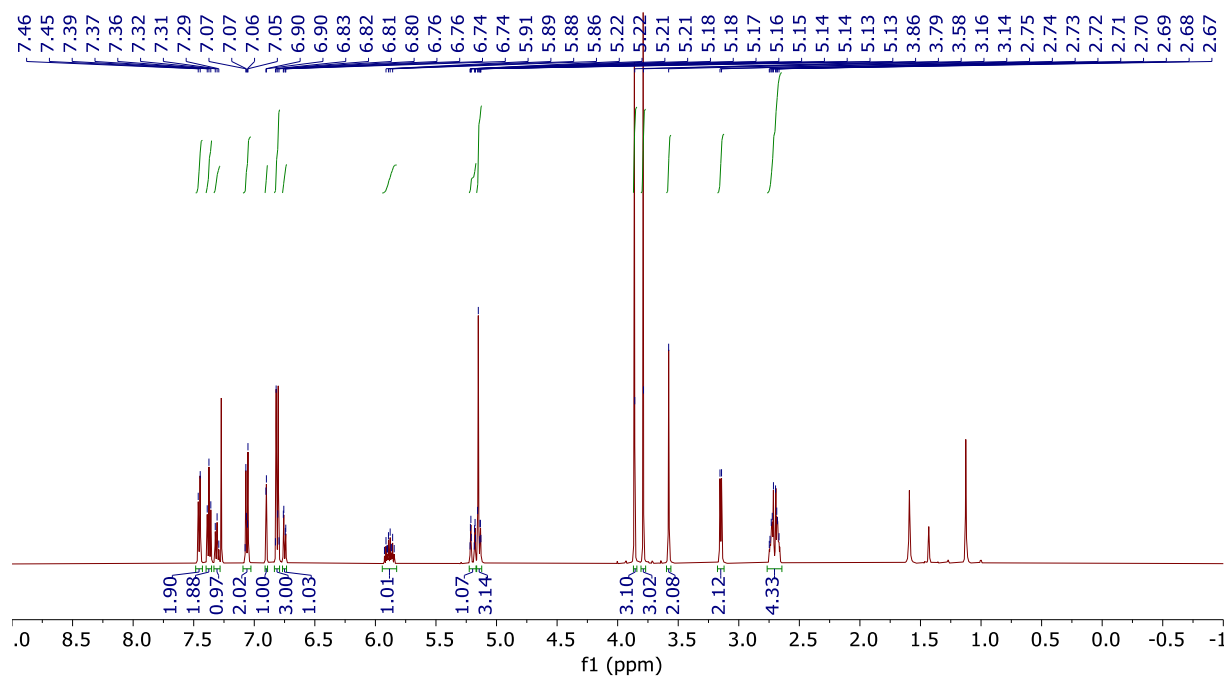
418.2377

2020 09 24 CD008A 1557 (2.682) Cm (1544:1576-(1184:1438+1712:2079))

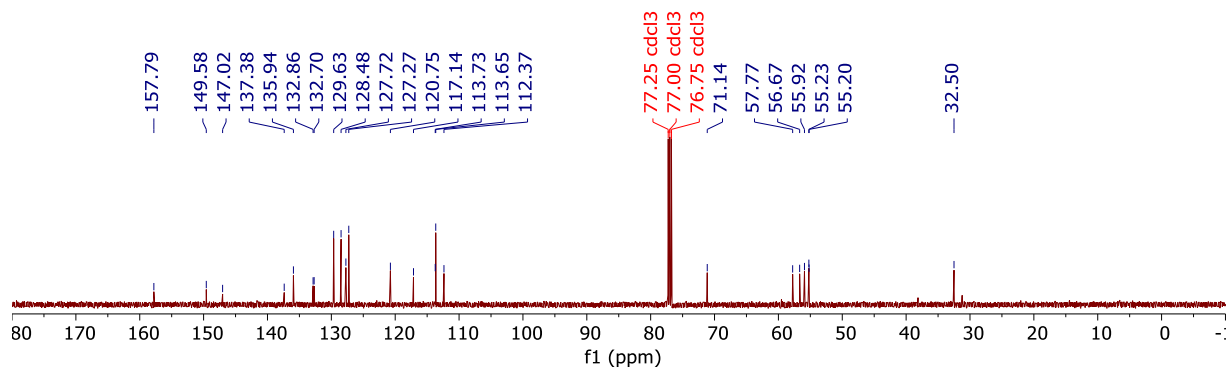
1: TOF MS ES+  
1.24e7



**Figure S32.**  $^1\text{H}$  NMR spectrum of *N*-allyl-*N*-(4-benzyloxy-3-methoxybenzyl)-2-(4-methoxyphenyl)ethan-1-amine (13) in  $\text{CDCl}_3$



**Figure S33.**  $^{13}\text{C}$  NMR spectrum of *N*-allyl-*N*-(4-benzyloxy-3-methoxybenzyl)-2-(4-methoxyphenyl)ethan-1-amine (13) in  $\text{CDCl}_3$

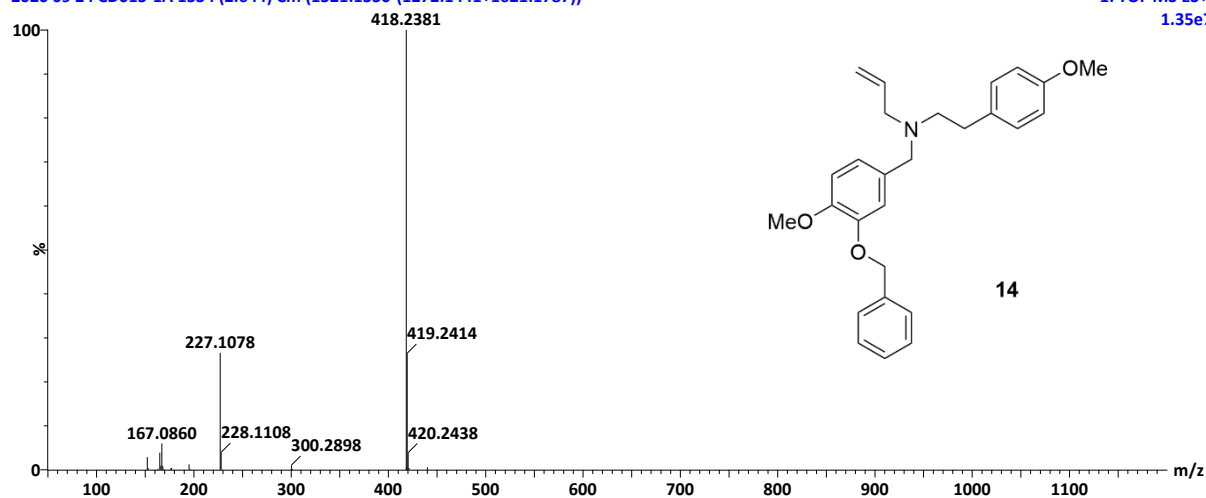


**Figure S34.** HRMS of *N*-allyl-*N*-(3-benzyloxy-4-methoxybenzyl)-2-(4-methoxyphenyl)ethan-1-amine (14)

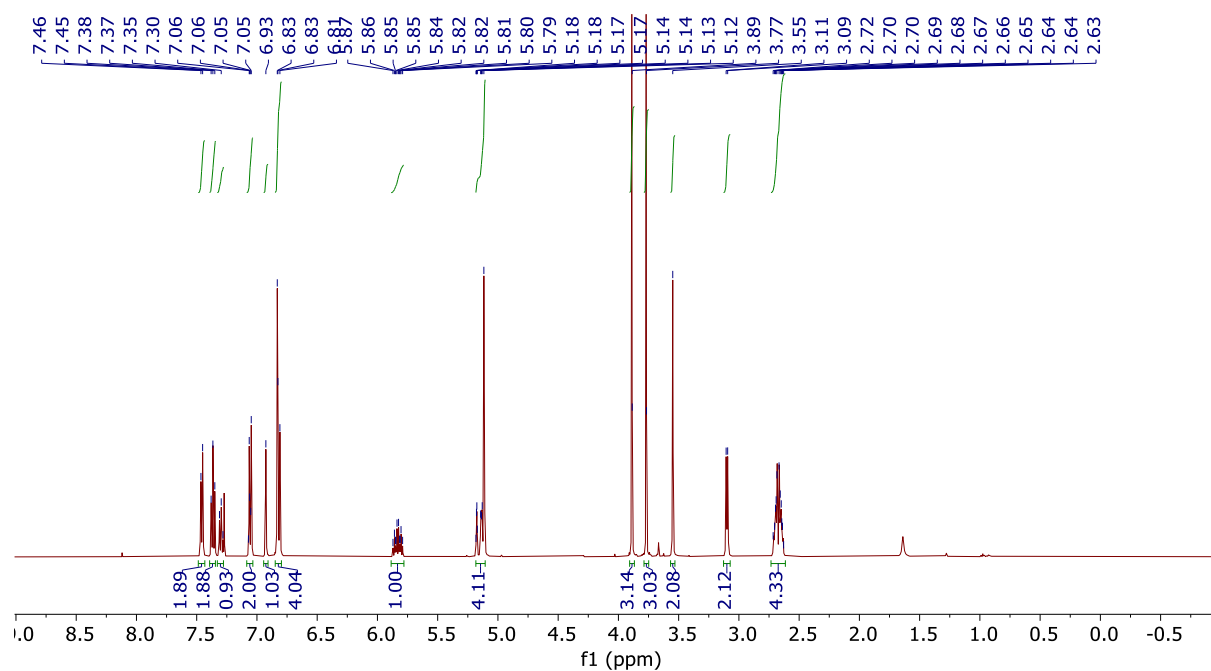
418.2377

2020 09 24 CD015-1A 1534 (2.644) Cm (1521:1550-(1272:1441+1621:1787))

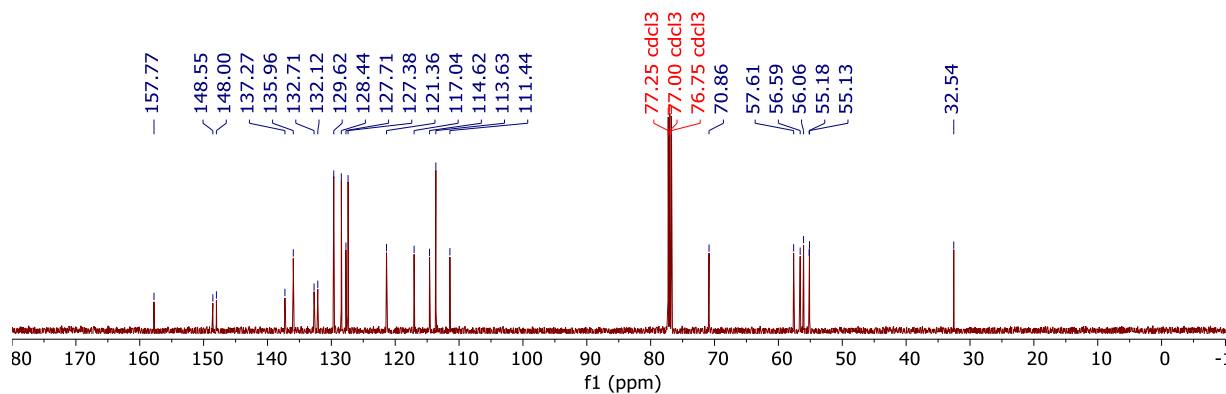
1: TOF MS ES+  
1.35e7



**Figure S35.**  $^1\text{H}$  NMR spectrum of *N*-allyl-*N*-(3-benzyloxy-4-methoxybenzyl)-2-(4-methoxyphenyl)ethan-1-amine (14) in  $\text{CDCl}_3$



**Figure S36.**  $^{13}\text{C}$  NMR spectrum of *N*-allyl-*N*-(3-benzyloxy-4-methoxybenzyl)-2-(4-methoxyphenyl)ethan-1-amine (14) in  $\text{CDCl}_3$

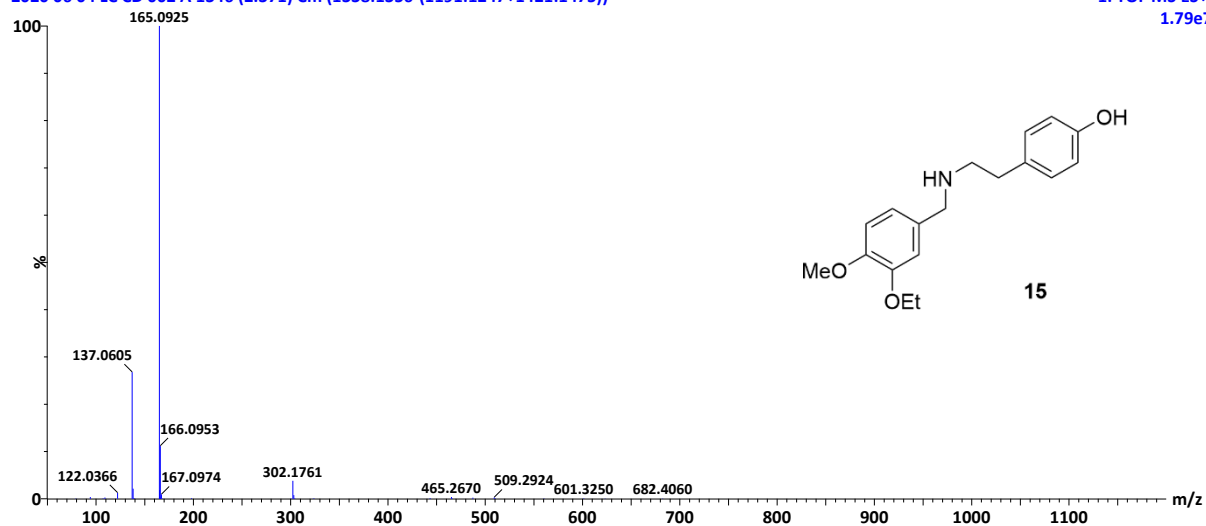


**Figure S37.** HRMS of *N*-(3-ethoxy-4-methoxybenzyl)-2-(4-hydroxyphenyl)ethan-1-amine (15)

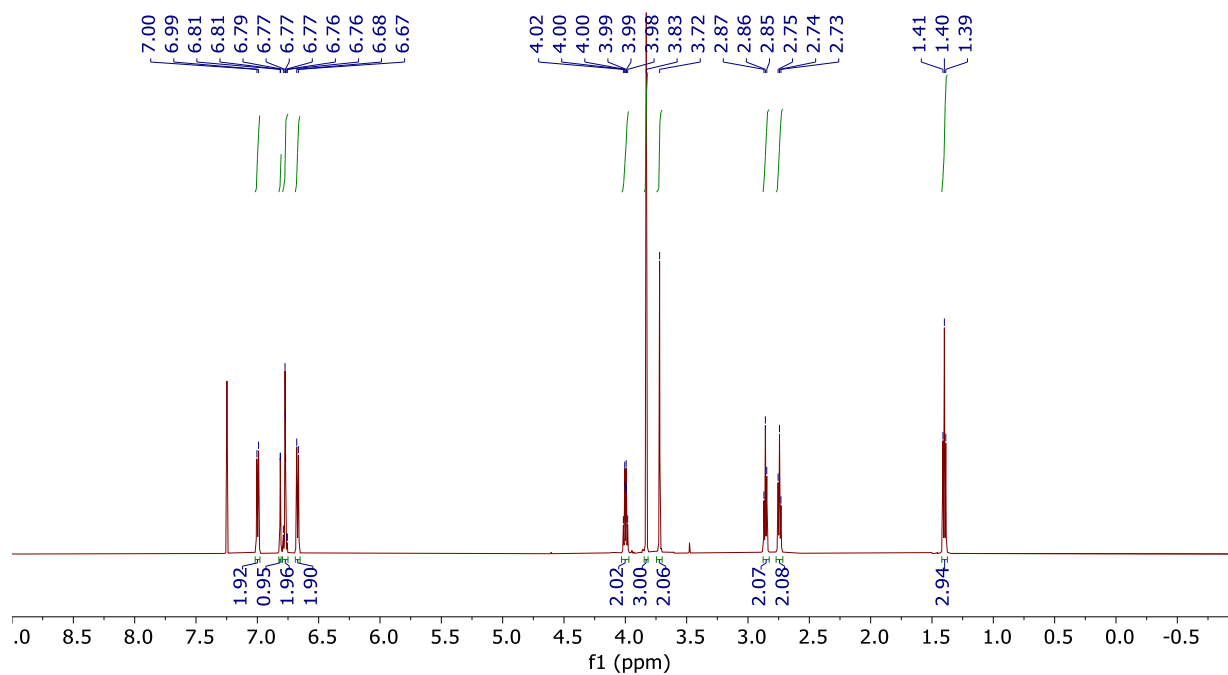
302.1751

2020 06 04 LC CD 002 A 1346 (2.371) Cm (1338:1356-(1191:1247+1421:1475))

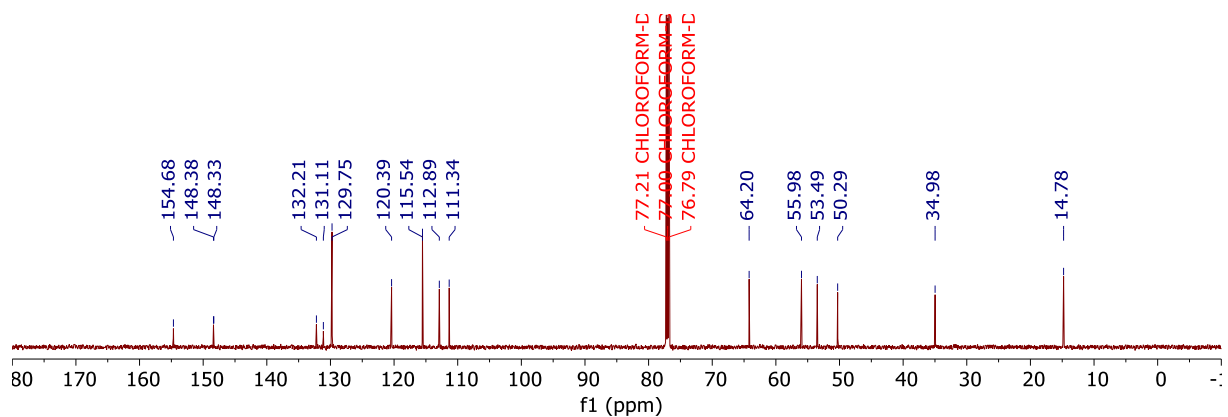
1: TOF MS ES+  
1.79e7



**Figure S38.**  $^1\text{H}$  NMR spectrum of *N*-(3-ethoxy-4-methoxybenzyl)-2-(4-hydroxyphenyl)ethan-1-amine (15) in  $\text{CDCl}_3$



**Figure S39.**  $^{13}\text{C}$  NMR spectrum of *N*-(3-ethoxy-4-methoxybenzyl)-2-(4-hydroxyphenyl)ethan-1-amine (15) in  $\text{CDCl}_3$

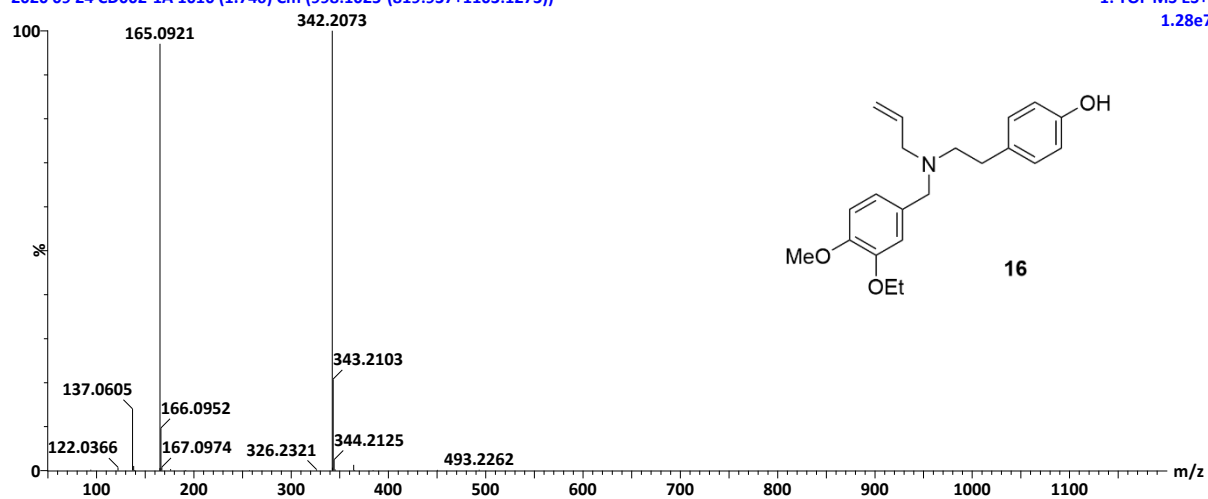


**Figure S40.** HRMS of *N*-allyl-*N*-(3-ethoxy-4-methoxybenzyl)-2-(4-hydroxyphenyl)ethan-1-amine (**16**)

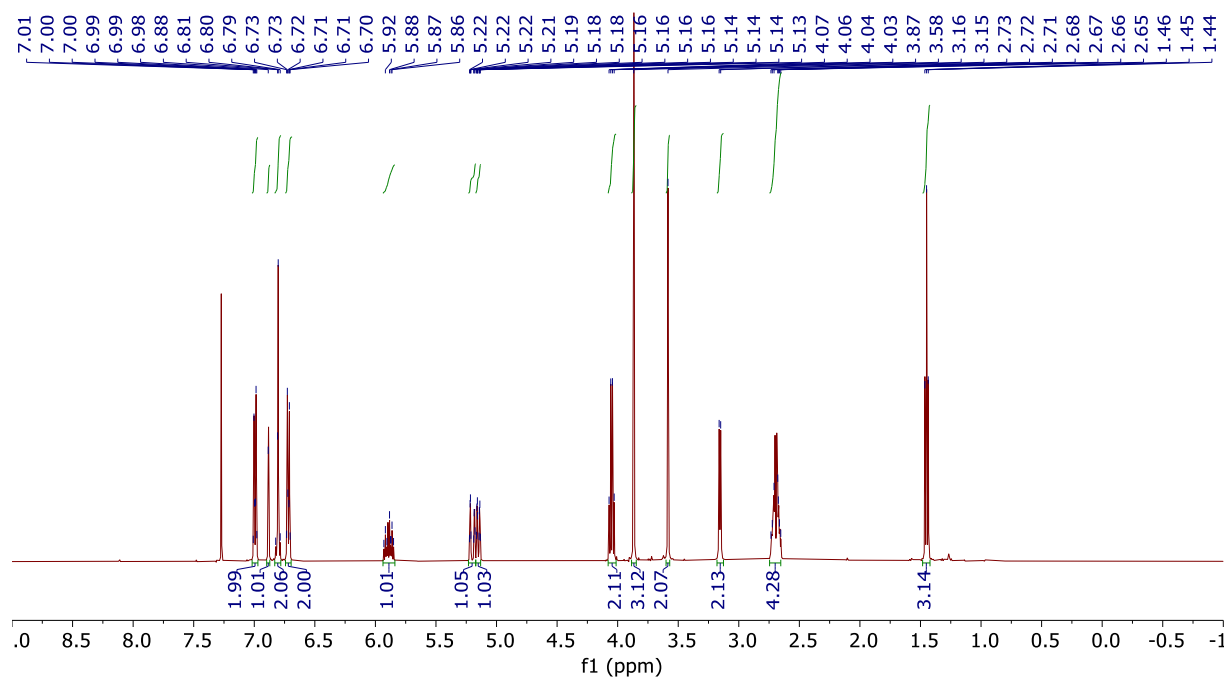
342.2064

2020 09 24 CD002-1A 1010 (1.746) Cm (998:1025-(819:937+1103:1273))

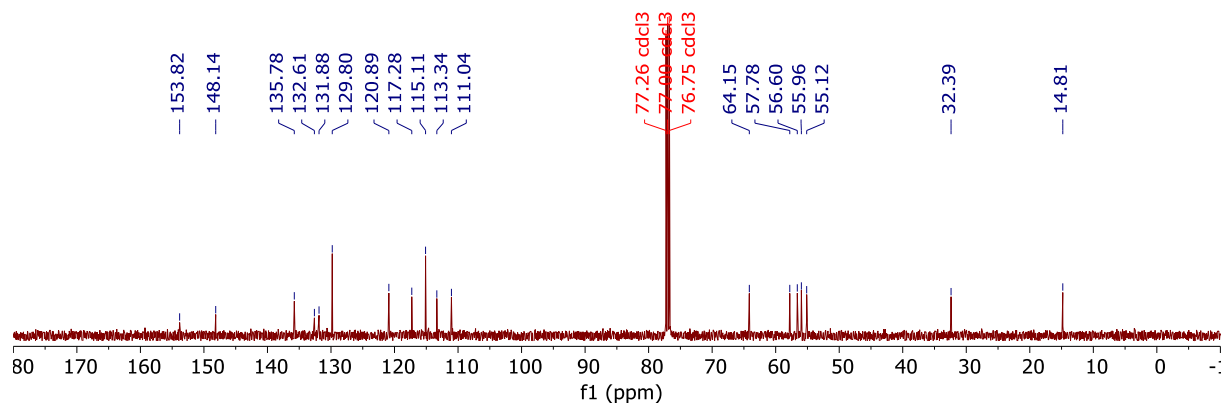
1: TOF MS ES+  
1.28e7



**Figure S41.**  $^1\text{H}$  NMR spectrum of *N*-allyl-*N*-(3-ethoxy-4-methoxybenzyl)-2-(4-hydroxyphenyl)ethan-1-amine (**16**) in  $\text{CDCl}_3$



**Figure S42.**  $^{13}\text{C}$  NMR spectrum of *N*-allyl-*N*-(3-ethoxy-4-methoxybenzyl)-2-(4-hydroxyphenyl)ethan-1-amine (16) in  $\text{CDCl}_3$

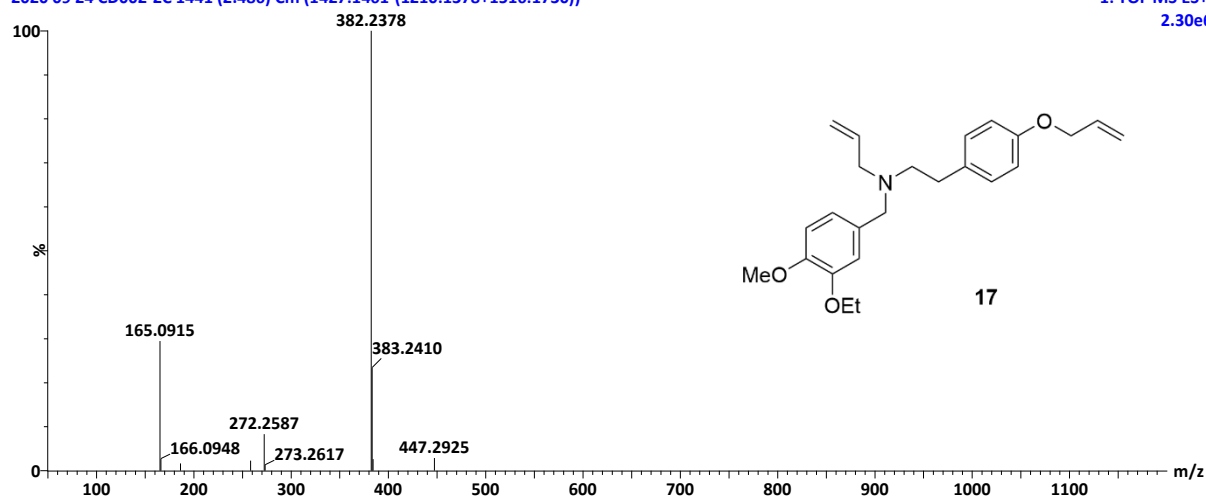


**Figure S43.** HRMS of *N*-allyl-*N*-(3-ethoxy-4-methoxybenzyl)-2-(4-allyloxyphenyl)ethan-1-amine (17)

382.2377

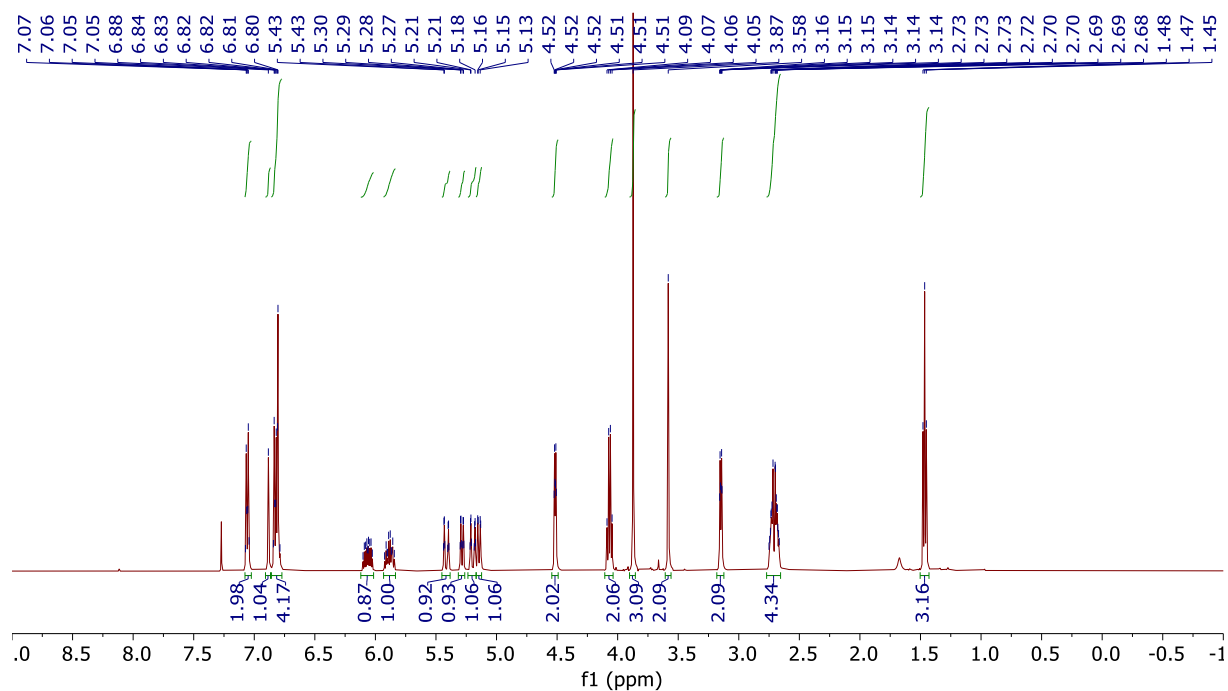
2020 09 24 CD002-2C 1441 (2.486) Cm (1427:1461-(1210:1378+1516:1730))

1: TOF MS ES+  
2.30e6

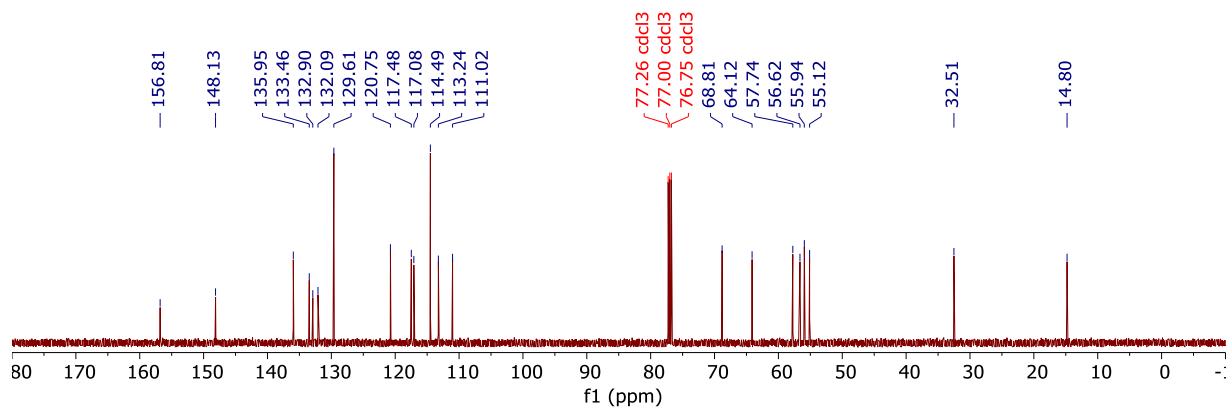




**Figure S44.**  $^1\text{H}$  NMR spectrum of *N*-allyl-*N*-(3-ethoxy-4-methoxybenzyl)-2-(4-allyloxyphenyl)ethan-1-amine (17) in  $\text{CDCl}_3$



**Figure S45.**  $^{13}\text{C}$  NMR spectrum of *N*-allyl-*N*-(3-ethoxy-4-methoxybenzyl)-2-(4-allyloxyphenyl)ethan-1-amine (17) in  $\text{CDCl}_3$

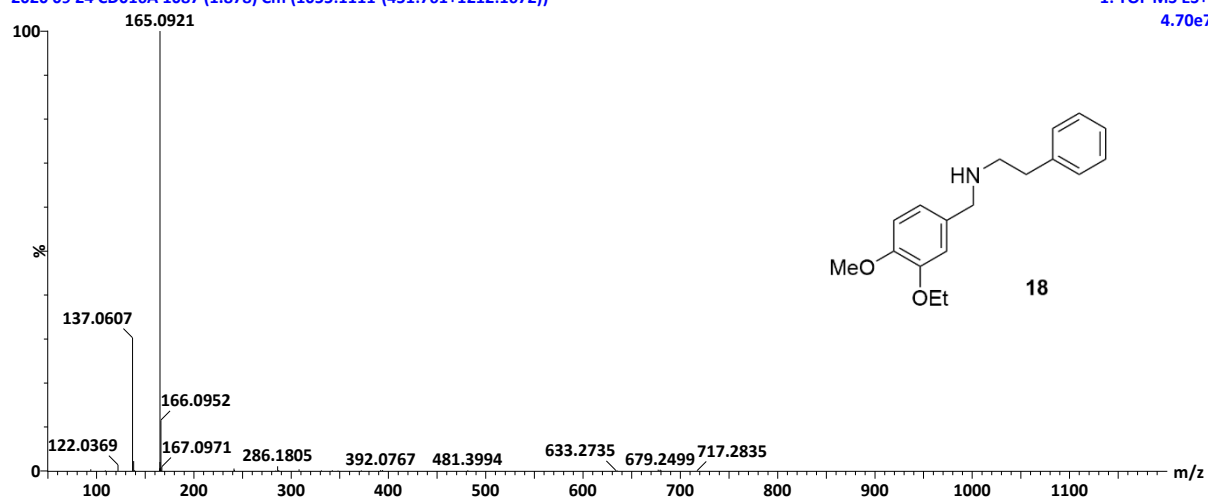


**Figure S46.** HRMS of *N*-(3-ethoxy-4-methoxybenzyl)-2-phenylethan-1-amine (**18**)

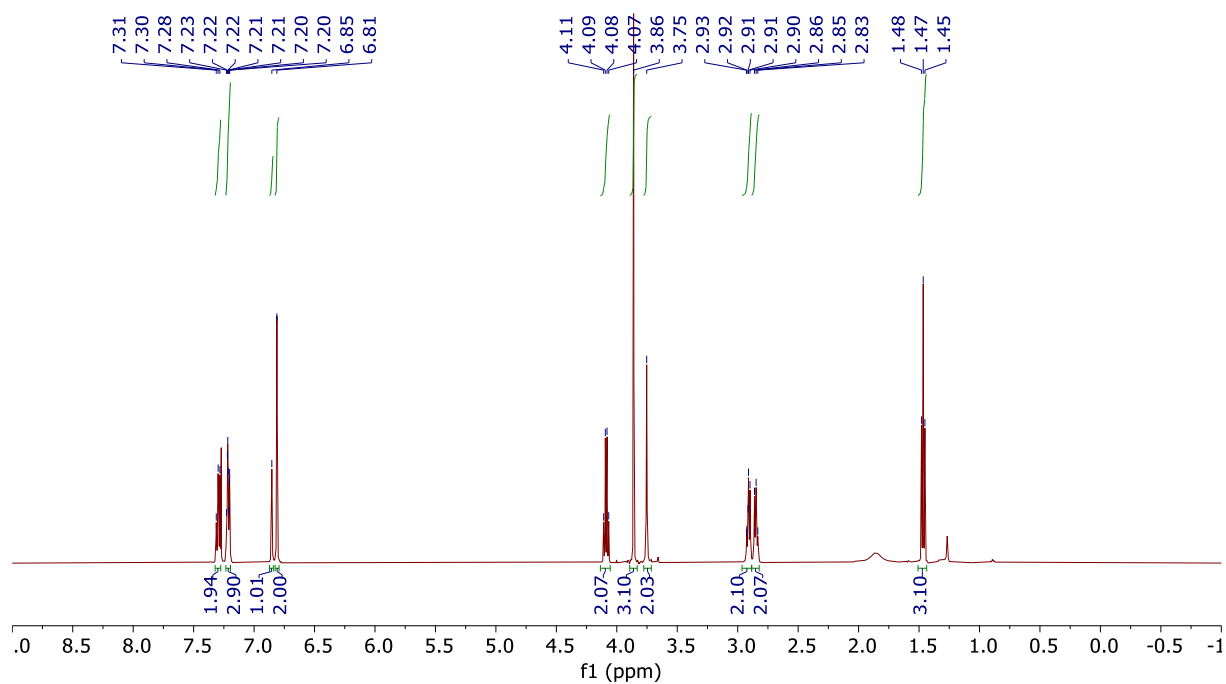
286.1802

2020 09 24 CD016A 1087 (1.878) Cm (1055:1111-(451:761+1212:1672))

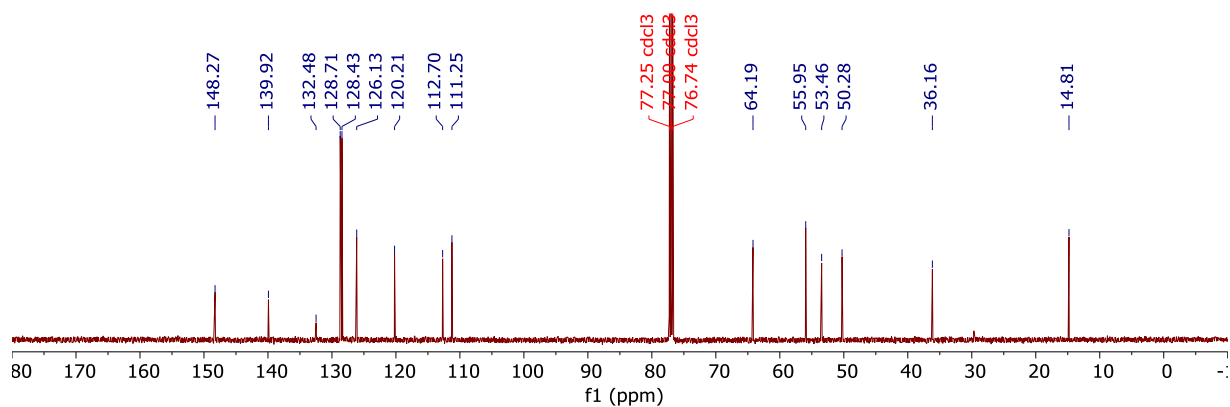
1: TOF MS ES+  
4.70e7



**Figure S47.**  $^1\text{H}$  NMR spectrum of *N*-(3-ethoxy-4-methoxybenzyl)-2-phenylethan-1-amine (**18**) in  $\text{CDCl}_3$



**Figure S48.**  $^{13}\text{C}$  NMR spectrum of *N*-(3-ethoxy-4-methoxybenzyl)-2-phenylethan-1-amine (18) in  $\text{CDCl}_3$

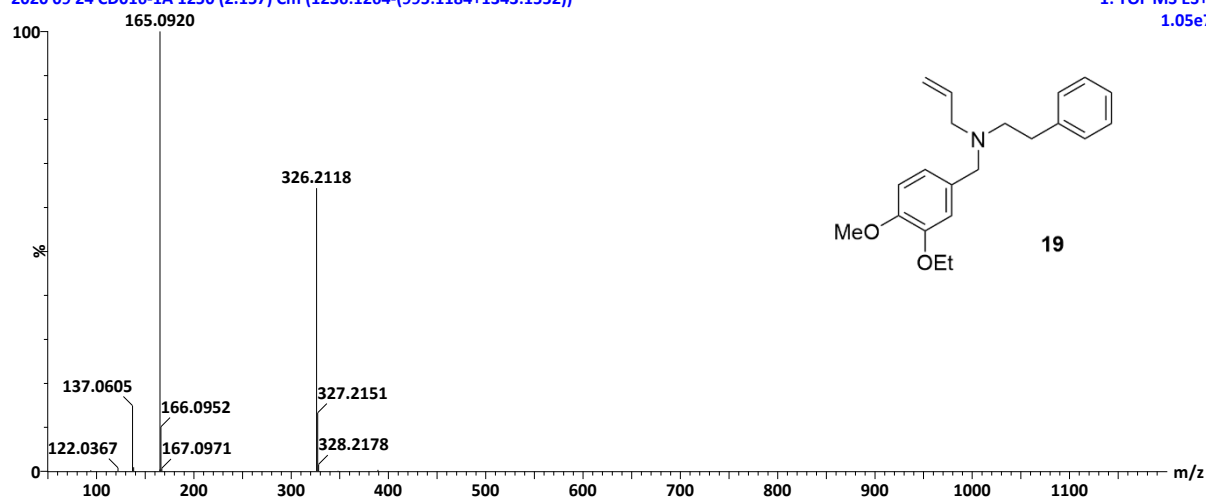


**Figure S49.** HRMS of *N*-allyl-*N*-(3-ethoxy-4-methoxybenzyl)-2-phenylethan-1-amine (19)

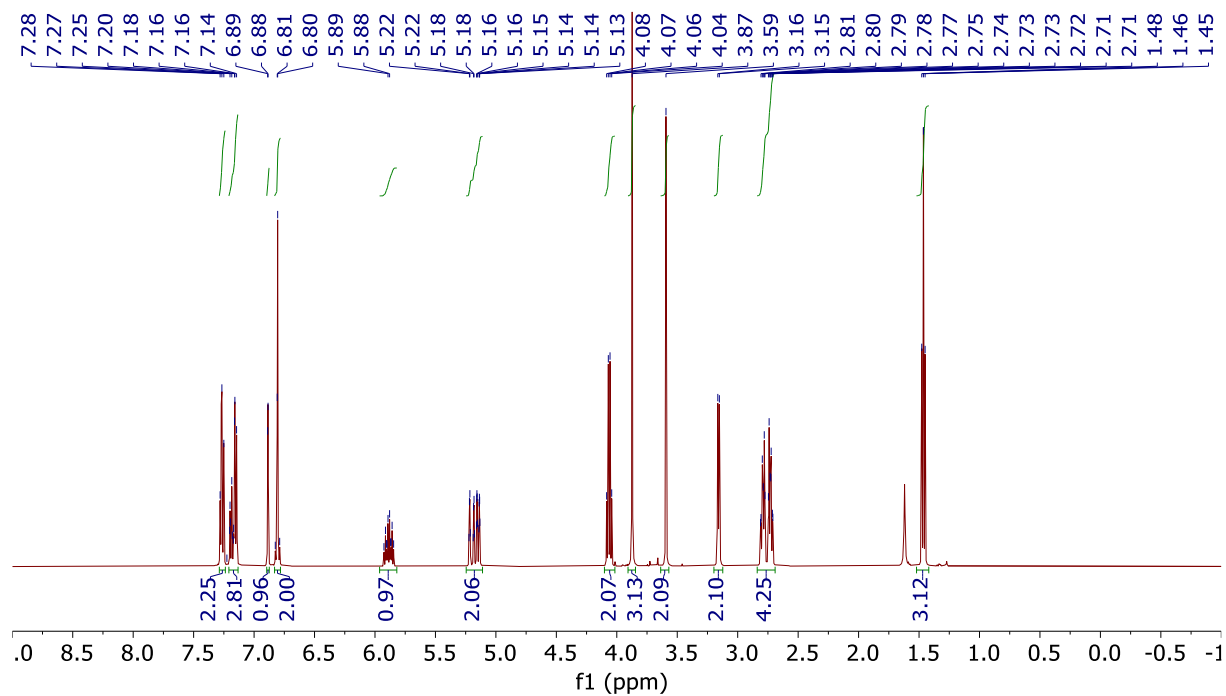
326.2115

2020 09 24 CD016-1A 1250 (2.157) Cm (1236:1264-(995:1184+1343:1552))

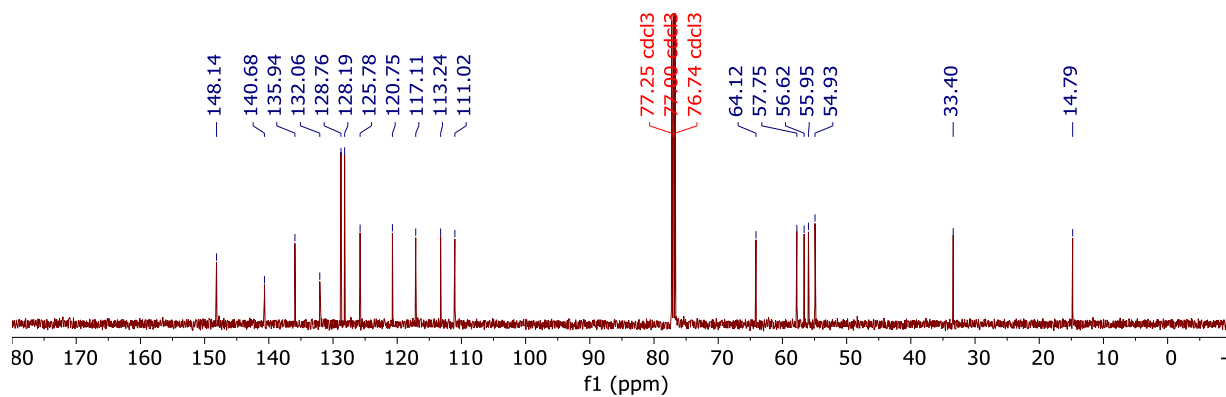
1: TOF MS ES+  
1.05e7



**Figure S50.**  $^1\text{H}$  NMR spectrum of *N*-allyl-*N*-(3-ethoxy-4-methoxybenzyl)-2-phenylethan-1-amine (19) in  $\text{CDCl}_3$



**Figure S51.**  $^{13}\text{C}$  NMR spectrum of *N*-allyl-*N*-(3-ethoxy-4-methoxybenzyl)-2-phenylethan-1-amine (19) in  $\text{CDCl}_3$

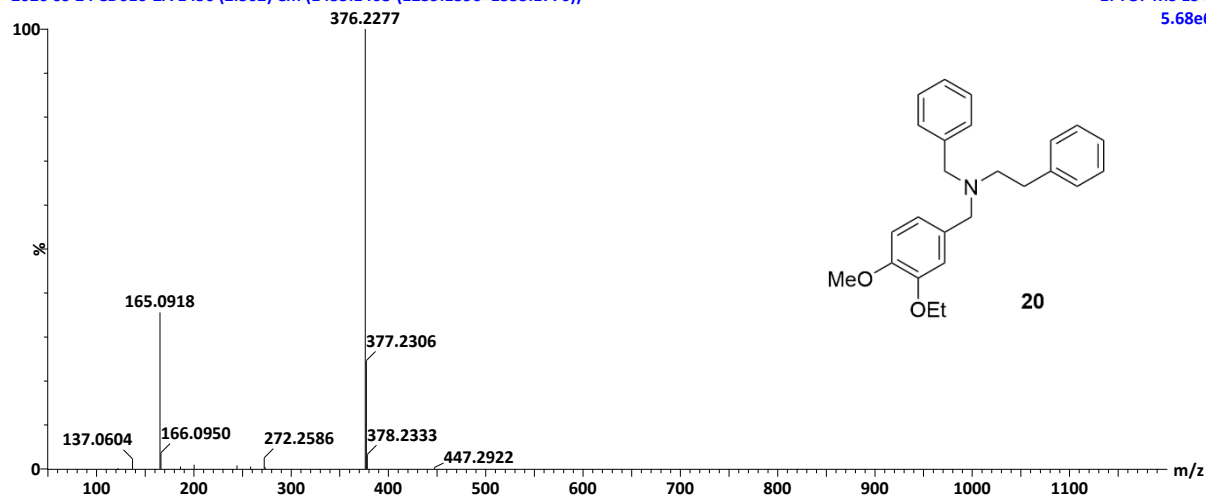


**Figure S52.** HRMS of *N*-benzyl-*N*-(3-ethoxy-4-methoxybenzyl)-2-phenylethan-1-amine (**20**)

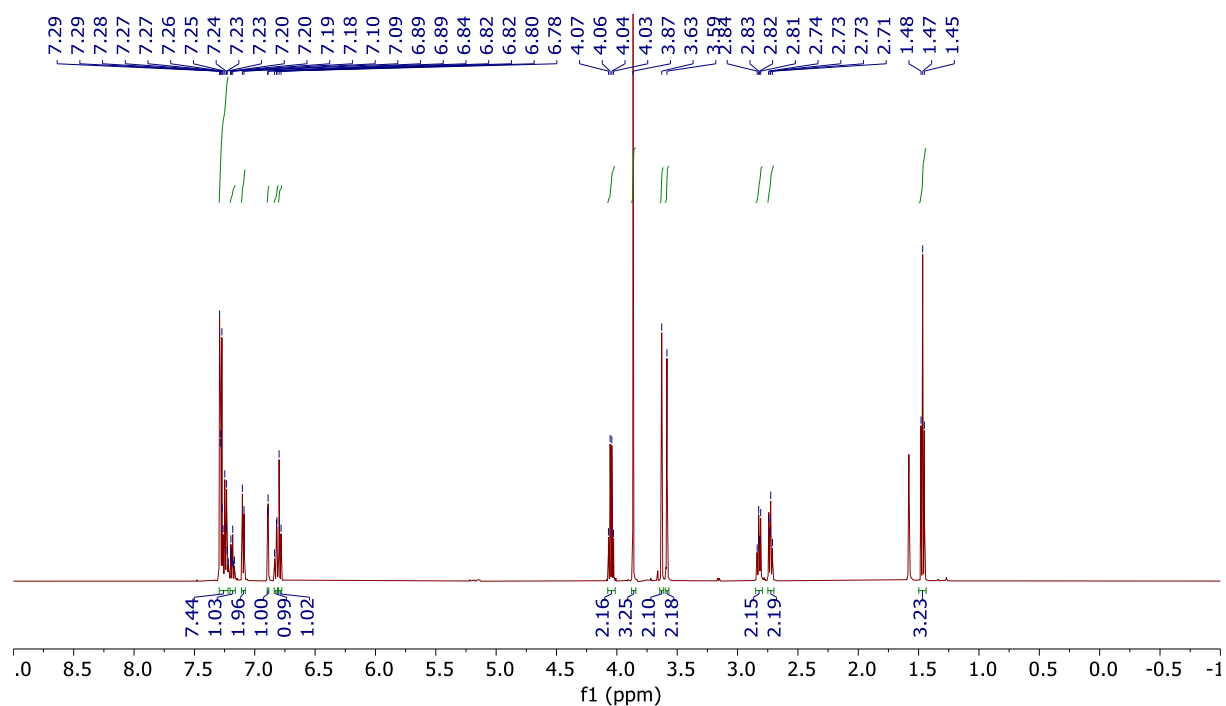
376.2271

2020 09 24 CD016-2A 1450 (2.502) Cm (1435:1468-(1239:1396+1553:1776))

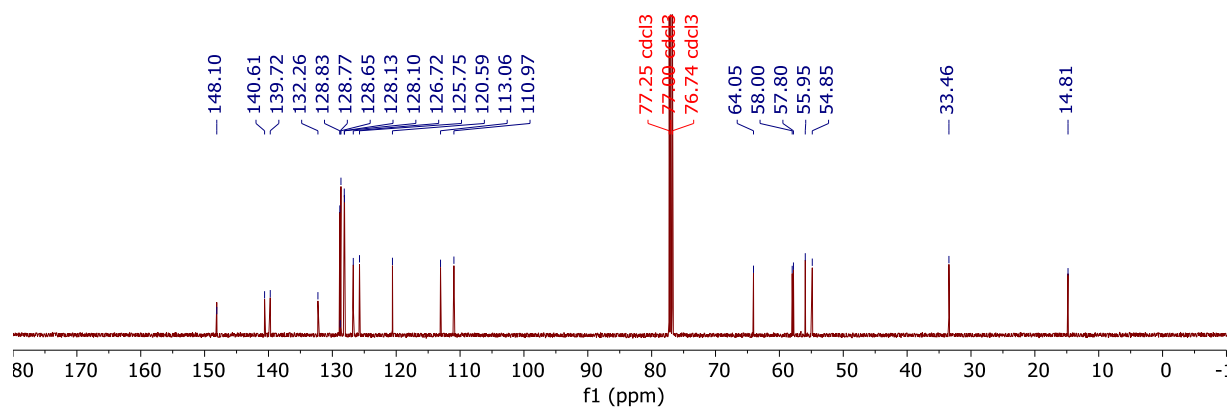
1: TOF MS ES+  
5.68e6



**Figure S53.**  $^1\text{H}$  NMR spectrum of *N*-benzyl-*N*-(3-ethoxy-4-methoxybenzyl)-2-phenylethan-1-amine (**20**) in  $\text{CDCl}_3$



**Figure S54.**  $^{13}\text{C}$  NMR spectrum of *N*-benzyl-*N*-(3-ethoxy-4-methoxybenzyl)-2-phenylethan-1-amine (20) in  $\text{CDCl}_3$



## Biological assays in detail

### *hAChE and hBuChE Inhibition Assay*

The inhibitory activities of prepared compounds and standards against human recombinant AChE (E.C. 3.1.1.7) and human plasma BChE (E.C. 3.1.1.8) were determined using modified Ellman's method [1-3] and expressed as IC<sub>50</sub> (the concentration of the compound that is required to reduce 50% of cholinesterase activity). Human recombinant AChE, phosphate buffer (PB, pH = 7.4), 5,5'-dithio-bis(2-nitrobenzoic) acid (Ellman's reagent, DTNB), acetylthiocholine (ATCh), butyrylthiocholine (BTCh), and other used compounds were purchased from Sigma-Aldrich (Prague, Czech Republic). Human plasma was used as a source of BChE and was prepared from heparinized human blood. Blood was centrifuged for 20 minutes (4 °C, 2300 × g) by Hettich Universal 320R centrifuge. The plasma was separated and stored at -80 °C. During the measurement, 96-well microplates from polystyrene (ThermoFisher Scientific, Waltham, MA, USA) were used.

The solutions of the corresponding cholinesterase in PB were prepared up to the final activity 0.002 U/μL. The assay medium (100 μL) consisted of cholinesterase (10 μL), DTNB (20 μL of 0.01 M solution), and PB (40 μL of 0.1 M solution). The solutions of the tested compounds (10 μL of different concentrations) were pre-incubated for 5 minutes in the assay medium and then a solution of the substrate (20 μL of 0.01 M ATCh or BTCh iodide solution) was added to initiate the reaction. The increase of absorbance was measured at 412 nm using Multimode microplate reader Synergy 2 (BioTek Inc., Winooski, VT, USA). For the calculation of the resulting measured activity (the percentage of inhibition I) following formula was used:

$$I = \left(1 - \frac{\Delta A_i}{\Delta A_0}\right) \times 100$$

where  $\Delta A_i$  indicates absorbance change provided by adequate enzyme exposed to corresponding inhibitor and  $\Delta A_0$  indicates absorbance change when a solution of PB was added instead of a solution of inhibitor. Software Microsoft Excel (Redmont, WA, USA) and GraphPad Prism version 6.07 for Windows (GraphPad Software, San Diego, CA, USA) were used for the statistical data evaluation.

### *Kinetic Study of Cholinesterase Inhibition*

The kinetic study *hBuChE* was performed by using above mentioned modified Ellman's method. The values of  $V_{\max}$  and  $K_m$  of the Michaelis-Menten kinetics as well as the values of  $K_i$  and  $K_{i'}$  were calculated by nonlinear regression from the substrate velocity curves. Linear regression was used for the calculation of Lineweaver-Burk plots. All calculations were performed using GraphPad Prism software version 6.07 for Windows (San Diego, CA, USA).

### ***POP inhibition assay***

POP (EC 3.4.21.26) was dissolved in phosphate-buffered saline (PBS; 0.01 M Na/K phosphate buffer, pH 7.4, containing 137 mM NaCl and 2.7 mM KCl); the specific activity of the enzyme was 0.2 U/mL. The assay was performed in standard polystyrene 96-well microplates with a flat and clear bottom. Stock solutions of tested compounds were prepared in DMSO (10 mM). Dilutions ( $10^{-3}$  to  $10^{-7}$  M) were prepared from the stock solution with deionized H<sub>2</sub>O; the control was performed with the same DMSO concentration. POP substrate, (Z)-Gly-Pro-p-nitroanilide, was dissolved in 50% 1,4-dioxane (5 mM). For each reaction, PBS (170  $\mu$ L), tested compound (5  $\mu$ L), and POP (5  $\mu$ L) were incubated for 5 min at 37 °C. Then, substrate (20  $\mu$ L) was added, and the microplate was incubated for 30 min at 37 °C. The formation of p-nitroanilide, directly proportional to the POP activity, was measured spectrophotometrically at 405 nm using a microplate ELISA reader (Multimode microplate reader Synergy 2, BioTek Instruments, Winooski, VT). The inhibition potency of tested compounds was calculated by nonlinear regression analysis and was expressed as an IC<sub>50</sub> value (concentration of inhibitor which causes 50% POP inhibition). All calculations were performed using GraphPad Prism software version 6.07 for Windows (GraphPad Software).

### ***MAOs inhibition assay***

The reaction mixture contains 2.5mg/mL MAO-A or 6.25mg/mL MAO-B enzyme (Merck, Germany) and inhibitor in final concentrations of 1, 5, 8, 10, 15, 30, 50, and 80mM in 50 mM potassiumphosphate buffer with 20% (v/v) glycerol (pH 7.5). The mixture was pre-incubated at 37 °C for 5 min and subsequently, substrate kynuramine was added to the final concentration of 60mM in the case of MAO-A and 30mM in the case of MAO-B. The final volume of the reaction mixture was 0.1 mL. The whole reaction mixture was incubated at 37 °C for 30 min. The reaction was stopped by the addition of 200mL acetonitrile:methanol mixture (1:1) and cooling down to 0 °C. The sample was then centrifuged (16.500 g) for 10 min. The deamination product of kynuramine formed during the enzymatic reaction 4-hydroxyquinoline (4-HQ) was determined by UHPLC-MS on a Zorbax RRHD Eclipse plus C18 column (2.1 mm x 50 mm, 1.8mm) (Agilent Technologies, USA), by using a 6470 Series Triple Quadrupole mass spectrometer (Agilent Technologies, USA) as detector with electrospray ionisation – positive ion mode. Three m/zMRM transitions were followed for kynuramine (165.1→30.2, 165.1→118.0, 165.1→136.0) and 4-HQ (146.1→51.1, 146.1→77.0, 146.1→91.0). Eluents: (A) 0.1% formic acid in water; (B) 0.1% formic acid in acetonitrile. IC<sub>50</sub> of individual compounds were determined by nonlinear regression using GraphPad Prism 8.0 (GraphPad Software, USA).