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SUPPORTING INFORMATION

Title: Short Synthesis of Orthogonally Protected 3α,12α-Diamino-5β-cholan-24-oic Acid, a Dipodal Steroid Scaffold for Combinatorial Chemistry

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I. Integrated analytical approach for the determination of the diastereomeric purity and identity of compounds 5a and 6a

In contrast to comparable literature,[1] in our hands ^{1}H NMR spectroscopy alone proved insufficient to accurately judge the diastereomeric purity of compounds **5a** and **6a**. Only thorough analysis by LC-MS in combination with NMR experiments and literature data resulted in the unambiguous determination of the diastereomeric purity and 3α,12α identity of these products.

The complete absence of UV-activity severely complicates the LC-MS analysis. Even at 214 nm no clear peaks could be observed in the chromatogram. We therefore had to rely on the Total Ion Chromatogram (TIC). Especially the corresponding Single Ion Chromatogram (SIC), in which only the MS signal of a particular molecular weight is shown, proved invaluable in judging the diastereomeric ratio of the compounds. The use of this technique is justified since the diastereomers can be assumed to ionize equally. The TIC on the other hand has been used as a measure for overall purity. In order to reduce tailing 0.1 % formic acid had to be added to the eluent.

Single Ion Chromatogram 1 (fig. S1 A), obtained from the crude reduction/esterification reaction mixture proved very diagnostic in identifying the formation of four diastereomers, with the 3α,3β/12α-isomers as major compounds and the 3α,3β/12β-isomers as minor compounds.

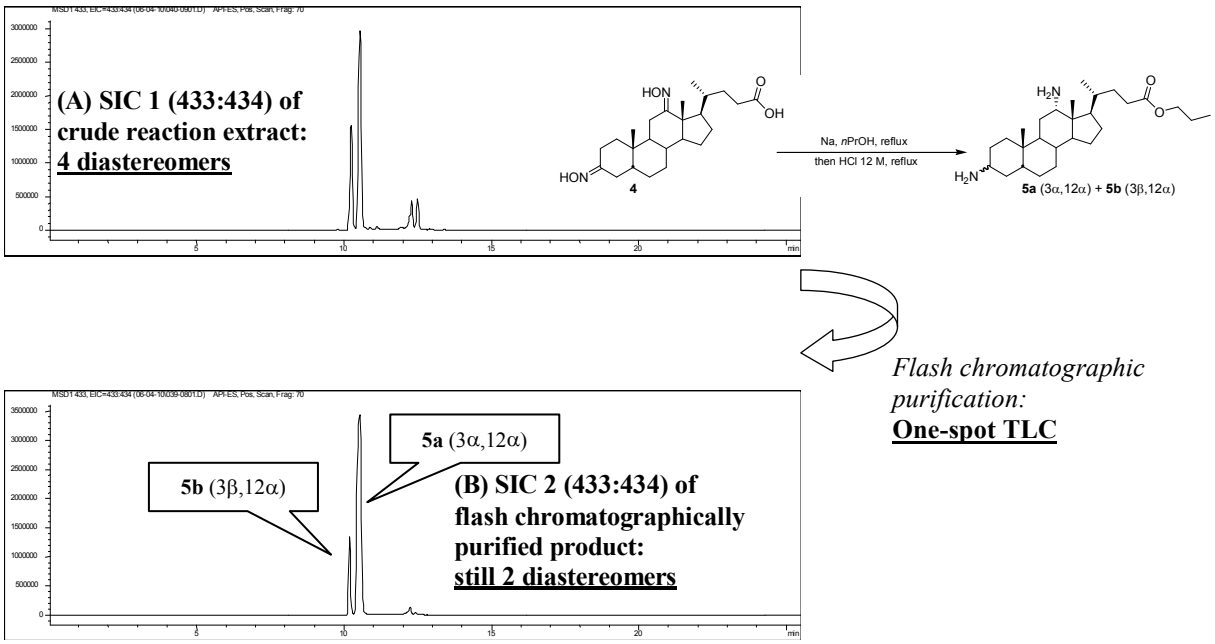

Figure S1. (A) Single Ion Chromatogram (SIC) of crude reaction extract; (B) SIC of chromatographically purified mixture **5a** + **5b** (*target molecular weight range is shown between brackets*).

The mixture of compounds having very similar Rf values on TLC could be separated by flash chromatography and one major product spot was isolated. This main spot still consisted of the two major compounds, the 3β,12α (**5b**) and 3α,12α (**5a**) isomers (diastereomeric ratio 15:85), as is clearly shown by SIC 2 (fig. S1 B). Repetitive chromatography failed to separate both compounds.

After Alloc introduction, the product mixture consequently consisted of 2 diastereomers, the C3-monoprotected products **6a** and **6b** (fig. S2 A and B). In contrast to compounds **5a** and **5b**, the monoprotected derivatives could be separated, as shown in TIC 5 (fig. S2 C) and corresponding SIC 6 (fig. S2 D).

From these chromatograms it could be concluded that compound **6a** was obtained in a satisfying overall purity (TIC 5) and an excellent diastereomeric purity (SIC 6), considering only one flash chromatographic purification is required.

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[1] a) H. Hsieh, J. G. Muller, C. J. Burrows, *Bioorg. Med. Chem.* **1995**, *3*, 823-838; b) S. Broderick, A. P. Davis, R. P. Williams, *Tetrahedron Lett.* **1998**, *39*, 6083-6086; c) A. P. Davis, M. N. Pérez-Payán, *Synlett* **1999**, 991-993; d) J. F. Barry, A. P. Davis, M. N. Pérez-Payán, M. R. J. Elsegood, R. F. W. Jackson, C. Gennari, U. Piarulli, M. Gude, *Tetrahedron Lett.* **1999**, *40*, 2849-2852; e) C. Li, A. Rehman, N. K. Dalley, P. B. Savage, *Tetrahedron Lett.* **1999**, *40*, 1861-1864; f) X. Zhou, A. Rehman, C. Li, P. B. Savage, *Org. Lett.* **2000**, *2*, 3015-3018.

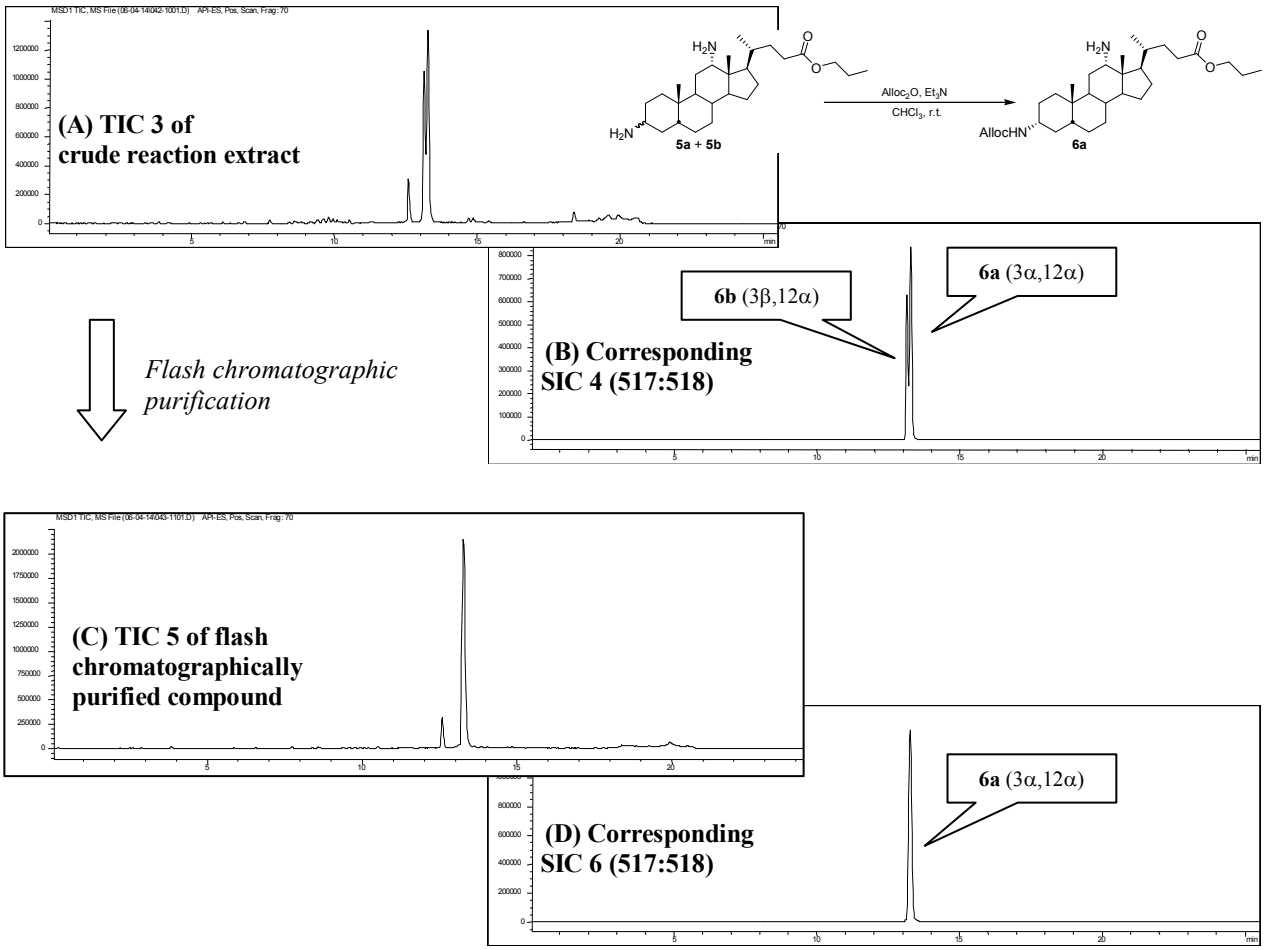
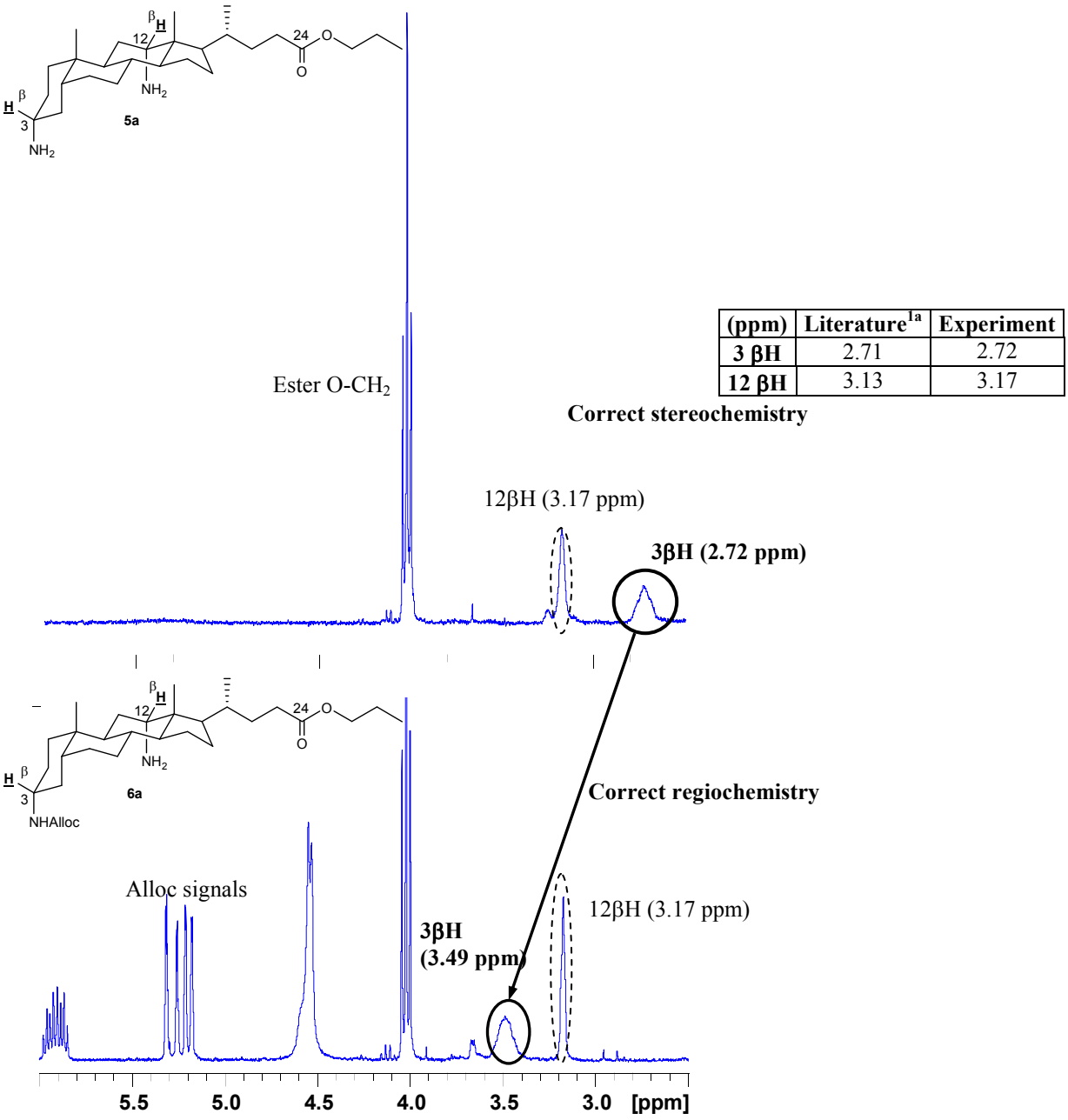


Figure S2. (A) Total Ion Chromatogram (TIC) of crude extract of the Alloc protection; (B) Corresponding Single Ion Chromatogram (SIC); (C) TIC of chromatographically purified product **6a**; (D) Corresponding SIC (*target molecular weight range is shown between brackets*).

Furthermore, after a verification step (see below, page S4) involving the removal of the C3-Alloc protecting group, LC-MS clearly confirmed the absence of the minor diamino isomer **5b** (see below, fig. S5, page S5).
In contrast to earlier published results, in our hands NMR data alone don't allow the unambiguous determination of the correct stereochemistry, mainly because of the unability to detect a reliable, clearly identifiable signal for the corresponding 3β-isomers. Not conclusive on their own, ^{1}H NMR spectra proved however valuable in determining the identity of the correct 3α,12α isomer (fig. S3 and S4). The most intense and clearly identifiable signals of the C_3-H and C_{12}-H were consistent with the 3βH,12βH geometry, thus the 3αN,12αN product being the main compound. The verification step was further supplementary to verify the stereochemistry of the scaffold, since the most reliable NMR data available in literature[1a] are those of the closely related alcohol derivatives of *n*-propylesters **5a** and **5b**. Chemical shifts proved very similar to literature data for both the 3β and 12β proton and integrations were consistent. Moreover the shape of both peaks was consistent with coupling patterns, since 3βH is more broadened (J = 39.7 Hz) due to large axial-axial couplings, in comparison with 12 βH (J = 19.1 Hz).
The isolation of the correct regioisomer after Alloc protection could also easily be derived from the downfield shift of the 3βH signal (fig. S3).

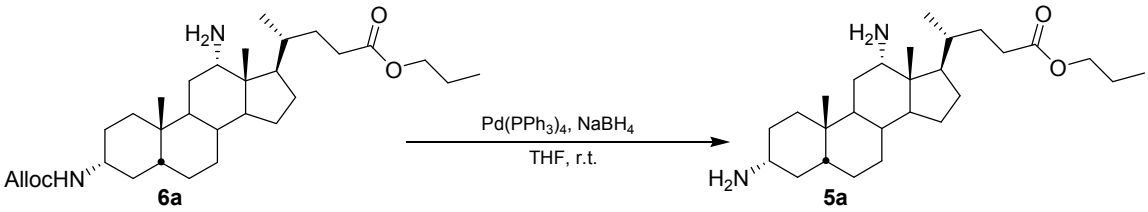


(ppm)	Literature[1a]	Experiment
3 βH	2.71	2.72
12 βH	3.13	3.17

Figure S3. ^{1}H NMR details (2.5-6.0 ppm) of **5a** + **5b** and **6a**.

In this way the correct C3α-Alloc, C12α-NH_2 diastereomer **6a** could be isolated and characterized. Whereas LC-MS experiments clearly show the satisfying overall purity (TIC 5) and excellent diastereomeric purity (SIC 6) of this compound, NMR and literature data[1a] confirm the correct stereo and regiochemistry of scaffold **6a**, prepared in only 4 steps from deoxycholic acid **3**.

Verification experiment



6a (95.8 mg; 0.1854 mmol) was dissolved in dry THF (1.9 mL) and stirred at room temperature under argon. $Pd(PPh_3)_4$ (4.5 mg; 0.0039 mmol; 0.02 eq) was added and the bright yellow solution was stirred for 5 minutes, followed by the addition of $NaBH_4$ (14.4 mg; 0.38 mmol; 2.1 eq). The mixture was stirred overnight at room temperature.[2]
After TLC check (CH_2Cl_2/MeOH 9/1 and EtOAc/MeOH/NH_4OH 28.0-30.0 % aq 9/1/1; ninhydrin and PMA) the reaction was quenched with a 1 M HCl aqueous solution (15 mL) and the mixture was transferred to a separation funnel. The mixture was made basic with a saturated $NaHCO_3$ aqueous solution (40 mL) and the product was extracted in CH_2Cl_2. The organic phase was dried on K_2CO_3, filtered and concentrated under reduced pressure.
The brownish oil was purified by flash chromatography (EtOAc/MeOH/NH_4OH 28.0-30.0 % aq 28/3/1). Although the product looked pure on TLC (EtOAc/MeOH/NH_4OH 28.0-30.0 % aq 9/1/1; chloranil or PMA), no clear NMR spectra could be obtained to determine the stereochemistry of **5a**. Therefore a solid phase extraction was performed: ~20 mg of the product was dissolved in MeOH (2 mL) and transferred into a solid phase reactor. Amberlyst 15 (wet) acidic ion-exchange resin (105.6 mg) was added and the mixture was shaken for 30 minutes. The resin was filtered and washed with CH_2Cl_2, EtOAc and MeOH. A 7 M NH_3 solution in MeOH (2 mL) was added and the reaction mixture was shaken manually, filtered and washed with MeOH. The filtrate was evaporated and dried under high vacuum to obtain diastereomerically pure **5a** (8 mg) as a colourless oil, which proved pure enough for NMR verification of the stereochemistry:

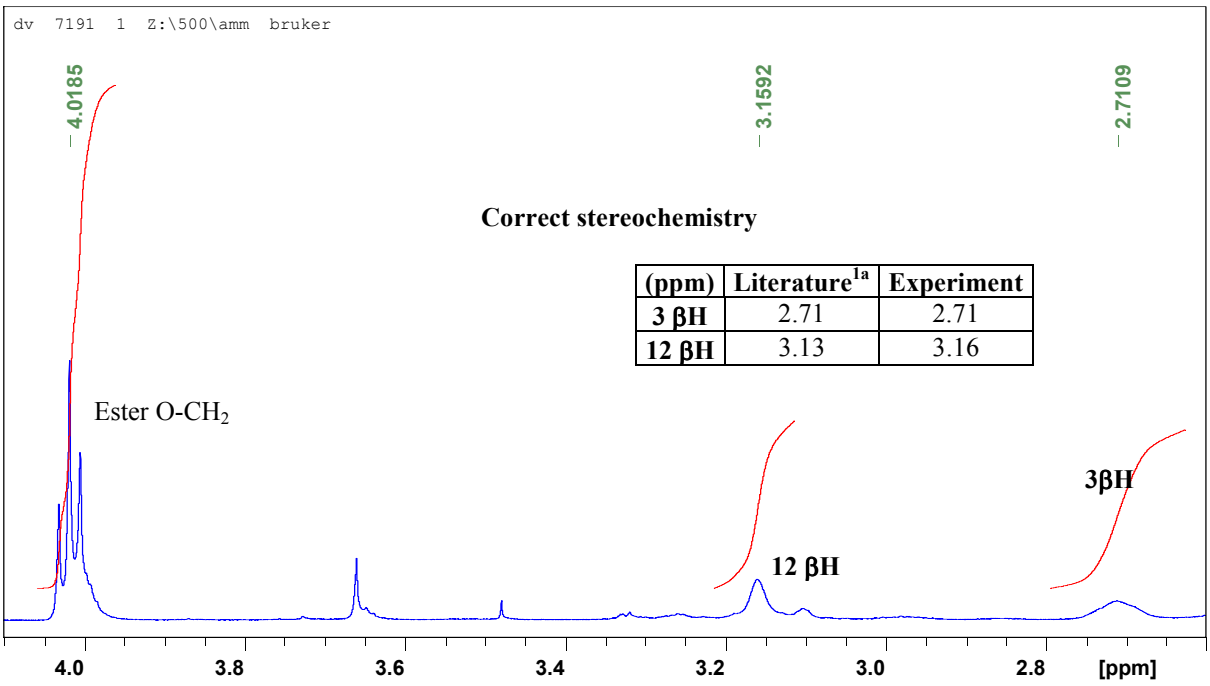


Figure S4. Detail of the ^1H spectrum of diamino ester **5a**, obtained from the verification experiment.

[2] a) R. Beugelmans, S. Bourdet, A. Bigot, J. Zhu, *Tetrahedron Lett.* **1994**, *35*, 4349-4350; b) R. Beugelmans, L. Neuville, M. Bois-Choussy, M.; J. Chastanet, J. Zhu, *Tetrahedron Lett.* **1995**, *36*, 3129-3132.

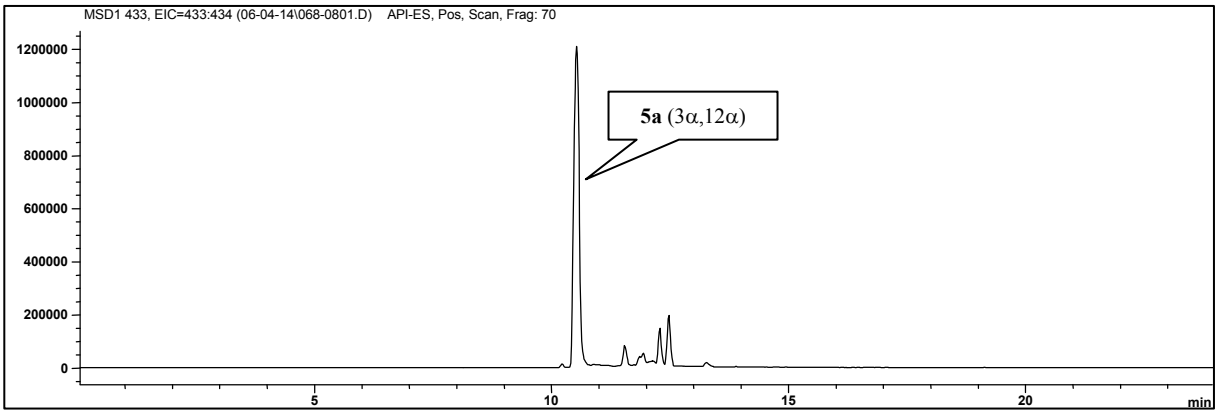


Figure S5. Single Ion Chromatogram (SIC) (*target molecular weight range: 433:434*) of diamino ester **5a**, obtained from the verification experiment.

II. ^{13}C (APT) and ^{1}H NMR spectra

All ^{1}H spectra of (deoxy)cholic acid derived products contain a region of high signal overlap between ~1.00 and ~2.00 ppm. Therefore, the detailed analysis of this region is impossible and often not mentioned[1a,b,c,3] or reported as a series of multiplets.[1e,f] Fortunately, in the preparation of scaffolds **6a** and **2** all necessary information is shifted downfield from this region. Furthermore the ^{13}C APT spectra are well-resolved and diagnostic.
Therefore, ^{13}C APT spectra (together with some spectral details) of all compounds (**4**, **5a** + **5b**, **6a**, **B** and **2**) and only the region of the ^{1}H spectra with clearly identifiable peaks of **5a** + **5b**, **6a** and **B** are included.

[3] a) V. del Amo, L. Siracusa, T. Markidis, B. Baragaña, K. M. Bhattarai, M. Galobardes, G. Naredo, M. N. Pérez-Payán, A. P. Davis, *Org. Biomol. Chem.* **2004**, *2*, 3320-3328; b) V. del Amo, K. Bhattarai, M. Nissinen, K. Rissanen, M. N. Pérez-Payán, A. P. Davis, *Synlett* **2005**, *8*, 1319-1321; c) K. M. Bhattarai, V. del Amo, G. Magro, A. L. Sisson, J. B. Joos, J. P. H. Charmant, A. Kantacha, A. P. Davis, *Chem. Comm.* **2006**, 2335-2337.

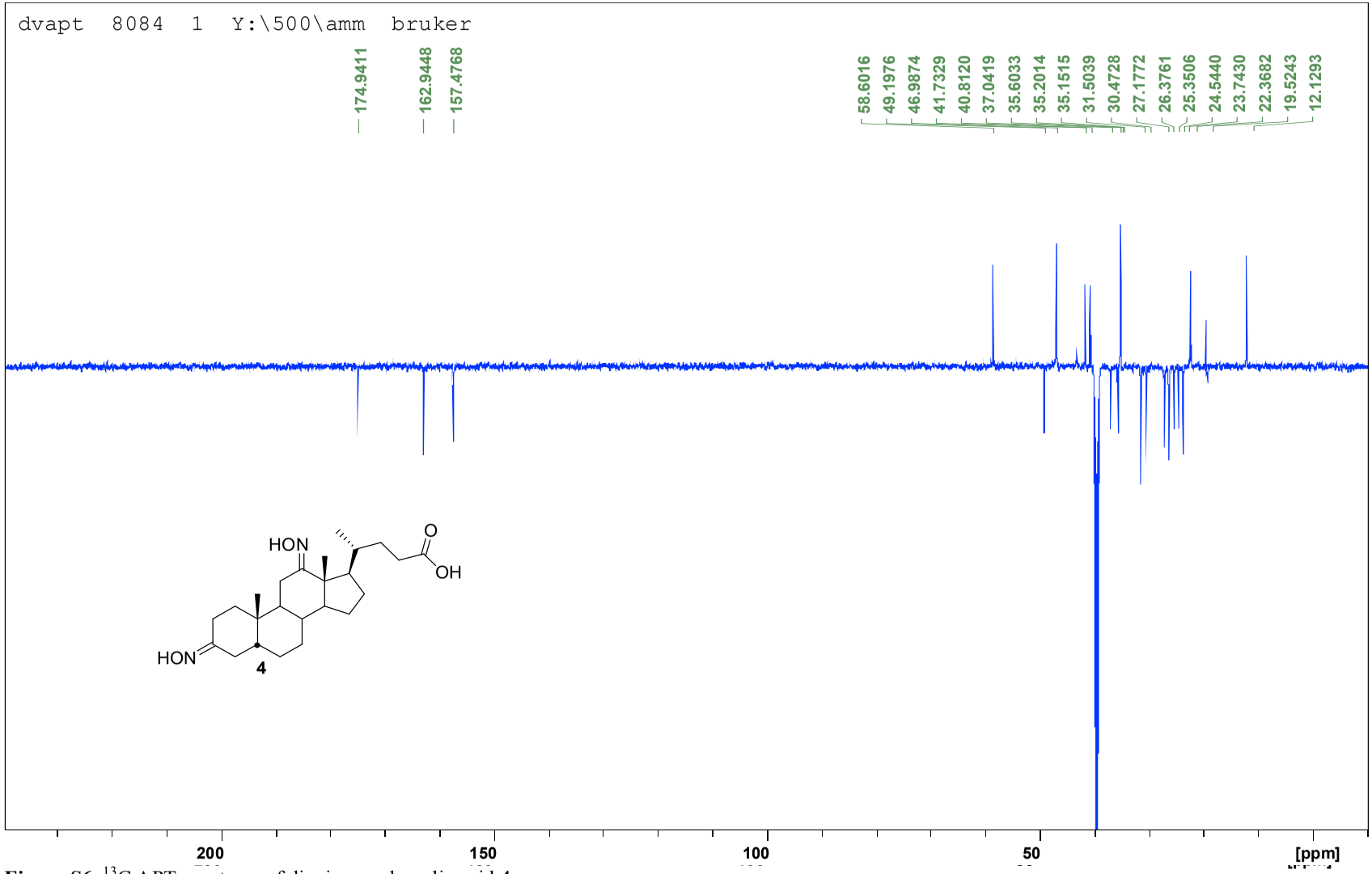


Figure S6. ^{13}C APT spectrum of dioximo carboxylic acid **4**.

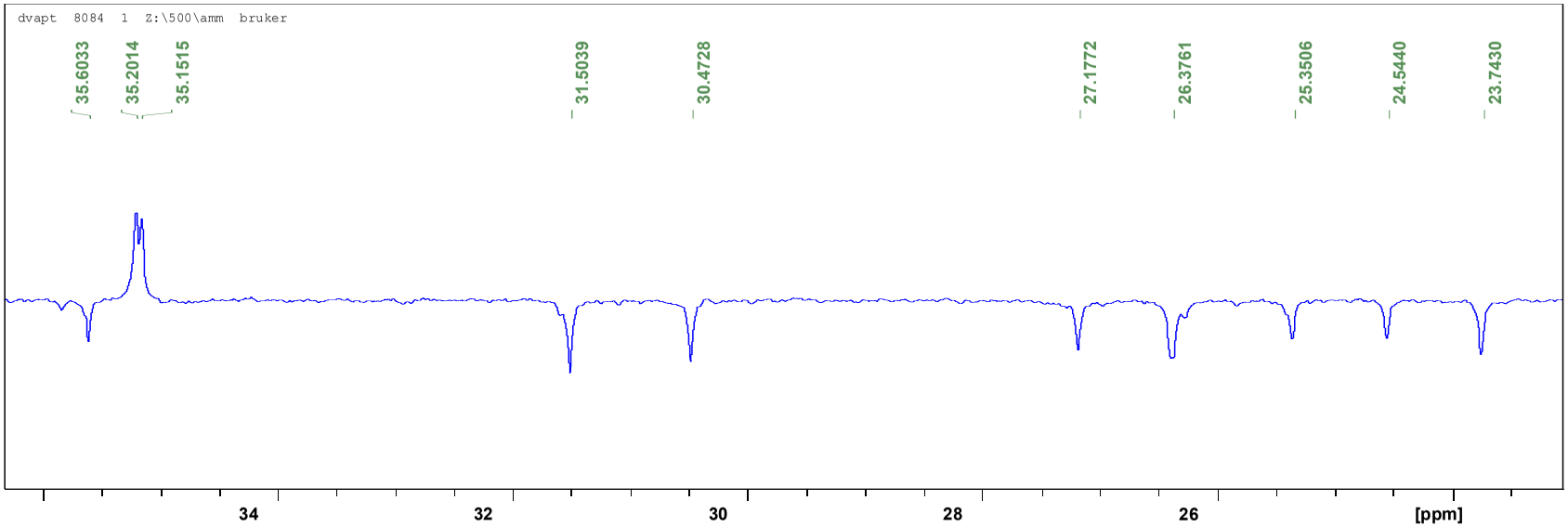


Figure S7. Detail of figure S6.

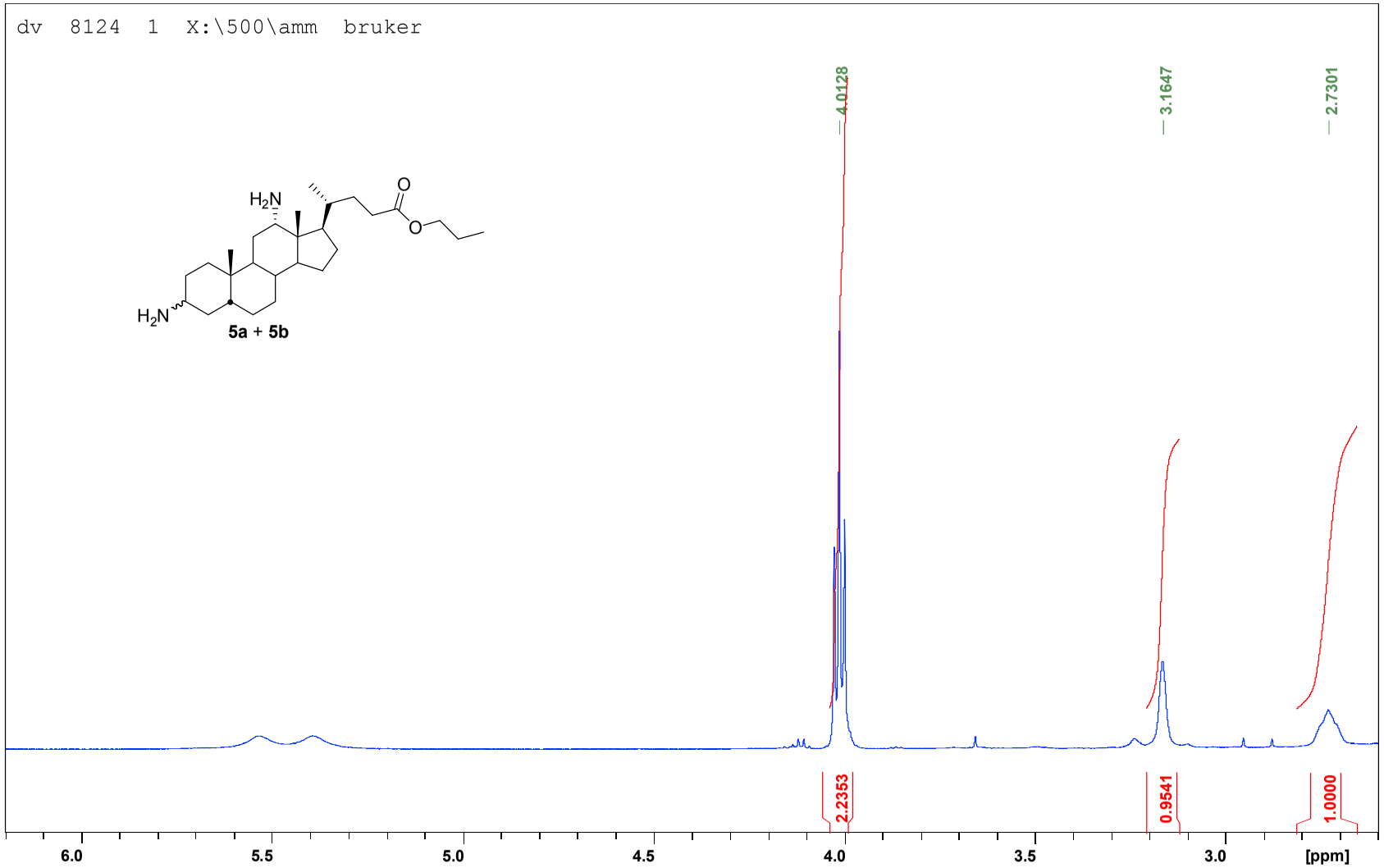


Figure S8. Detail of the ^{1}H spectrum of the diastereomeric mixture of diamino esters **5a** + **5b** (obtained after chromatography).

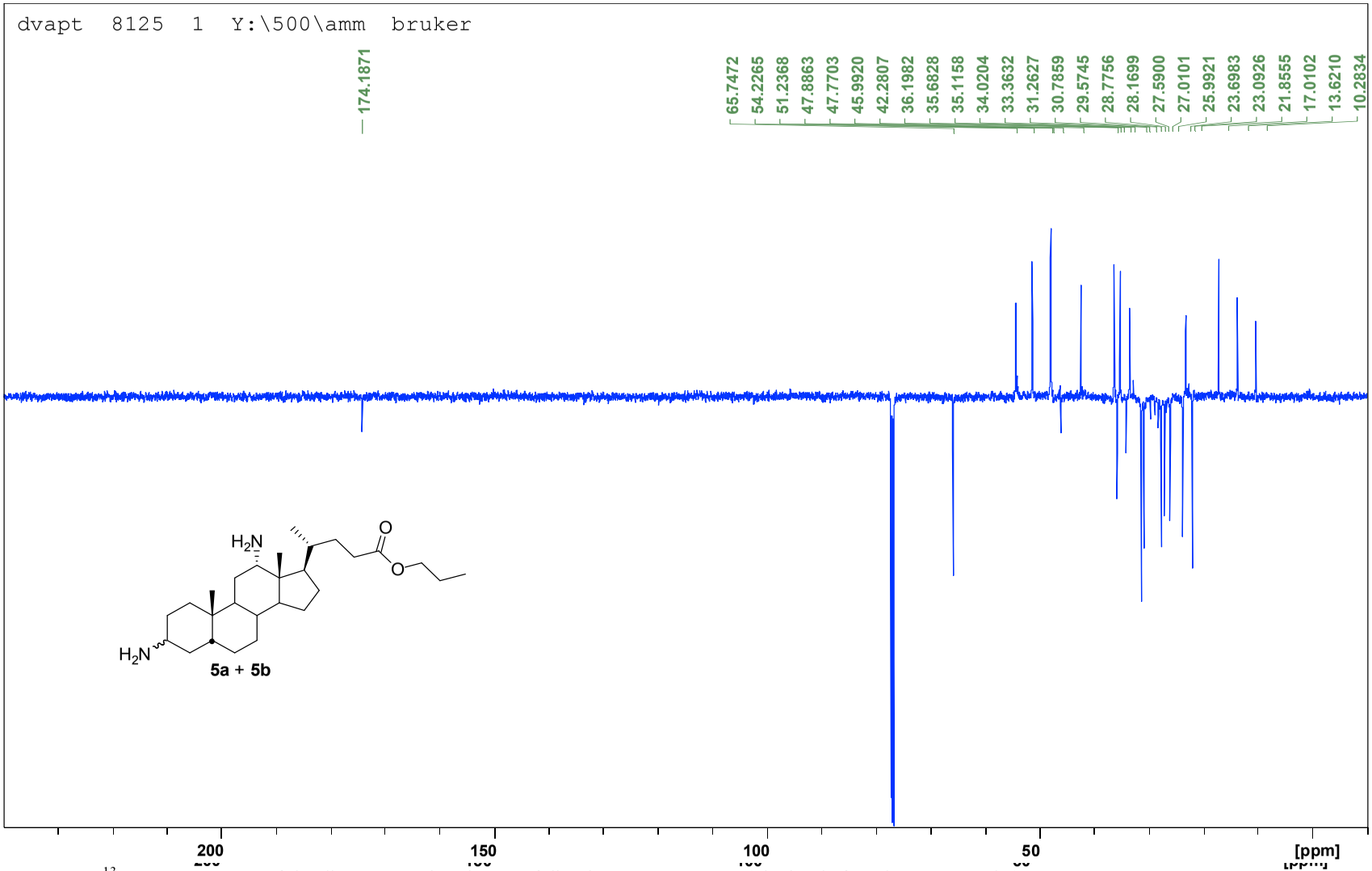


Figure S9. ^{13}C APT spectrum of the diastereomeric mixture of diamino esters **5a** + **5b** (obtained after chromatography).

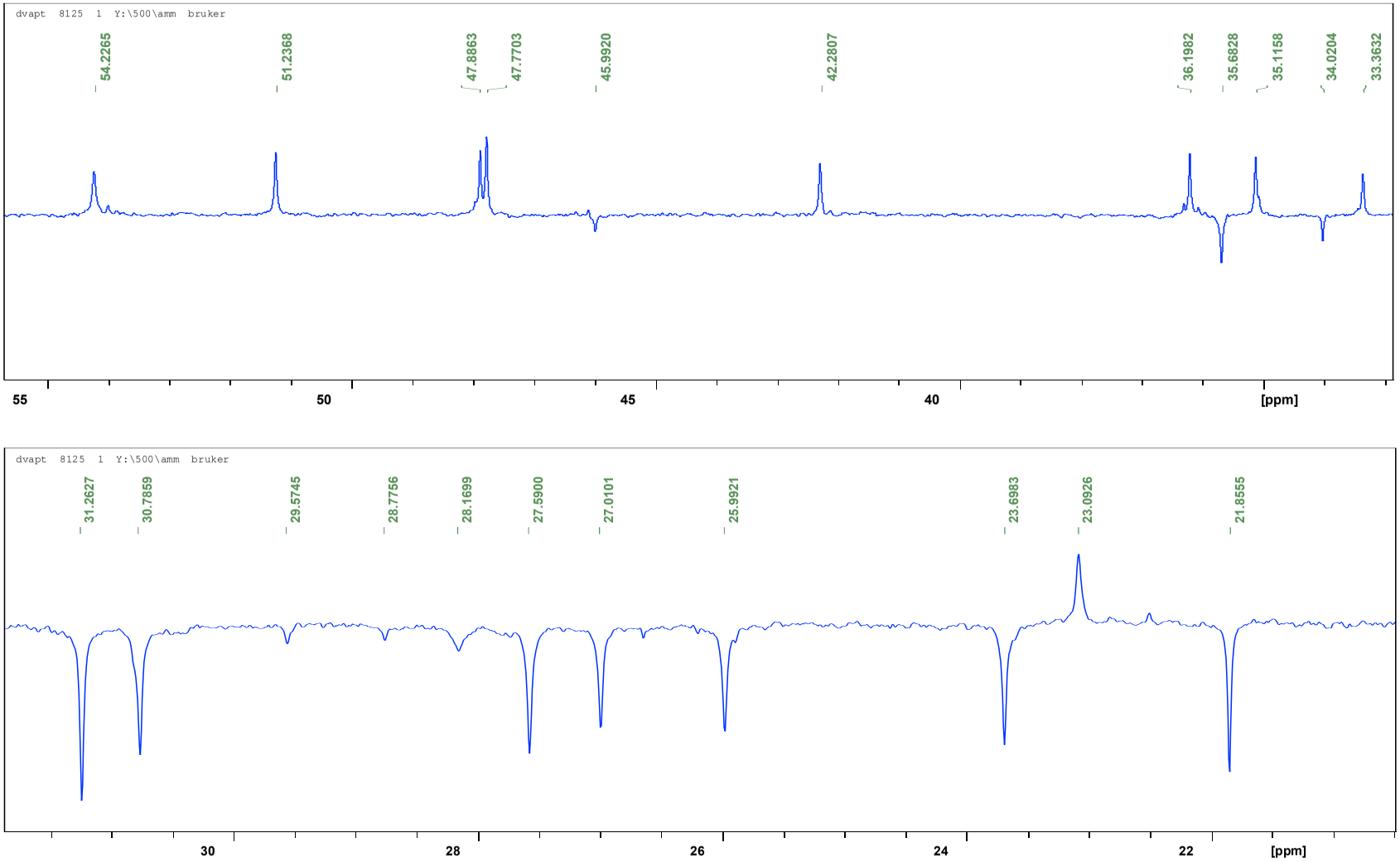


Figure S10. Details of figure S9.

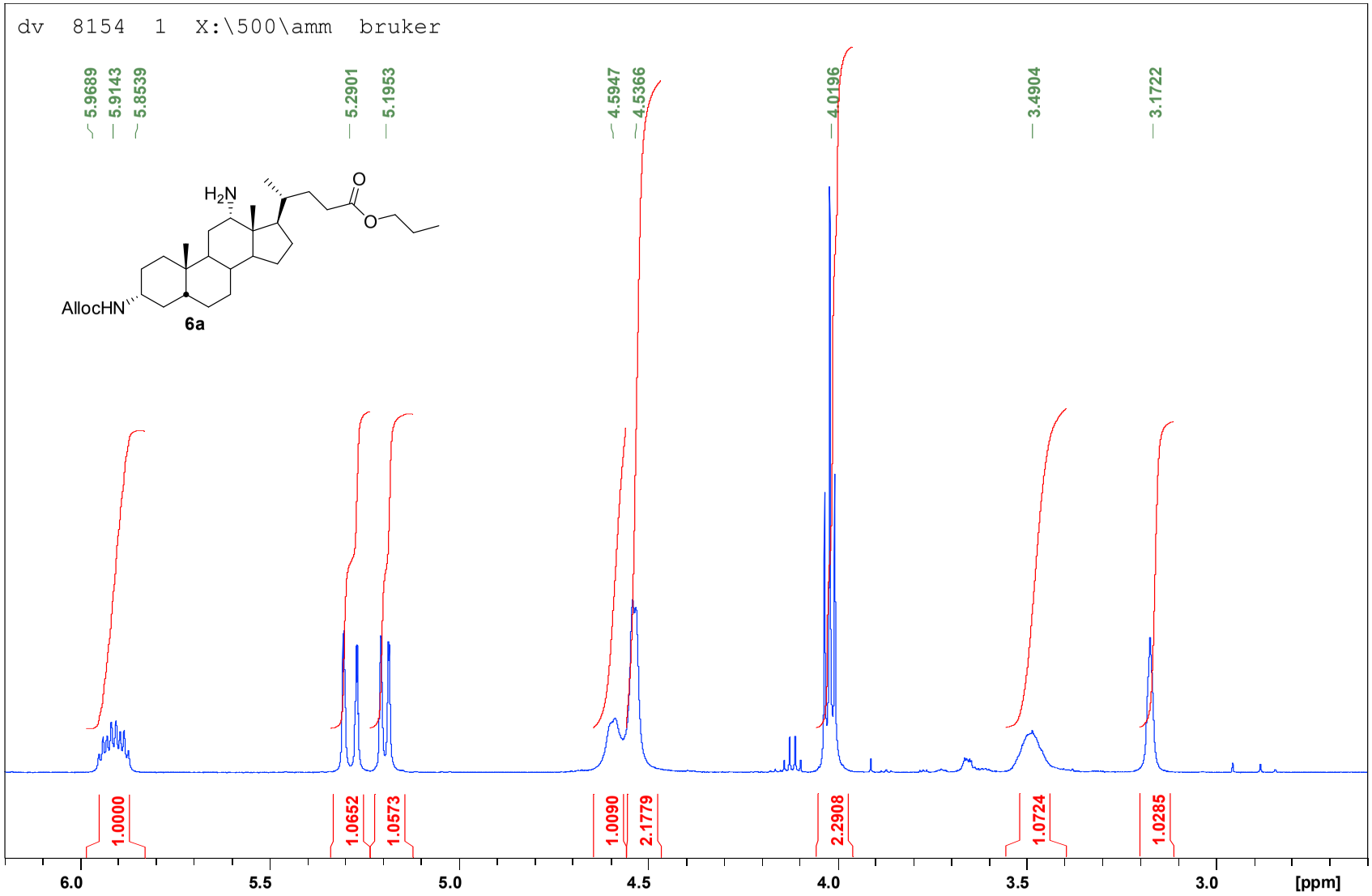


Figure S11. Detail of the ^{1}H spectrum of ester **6a**.

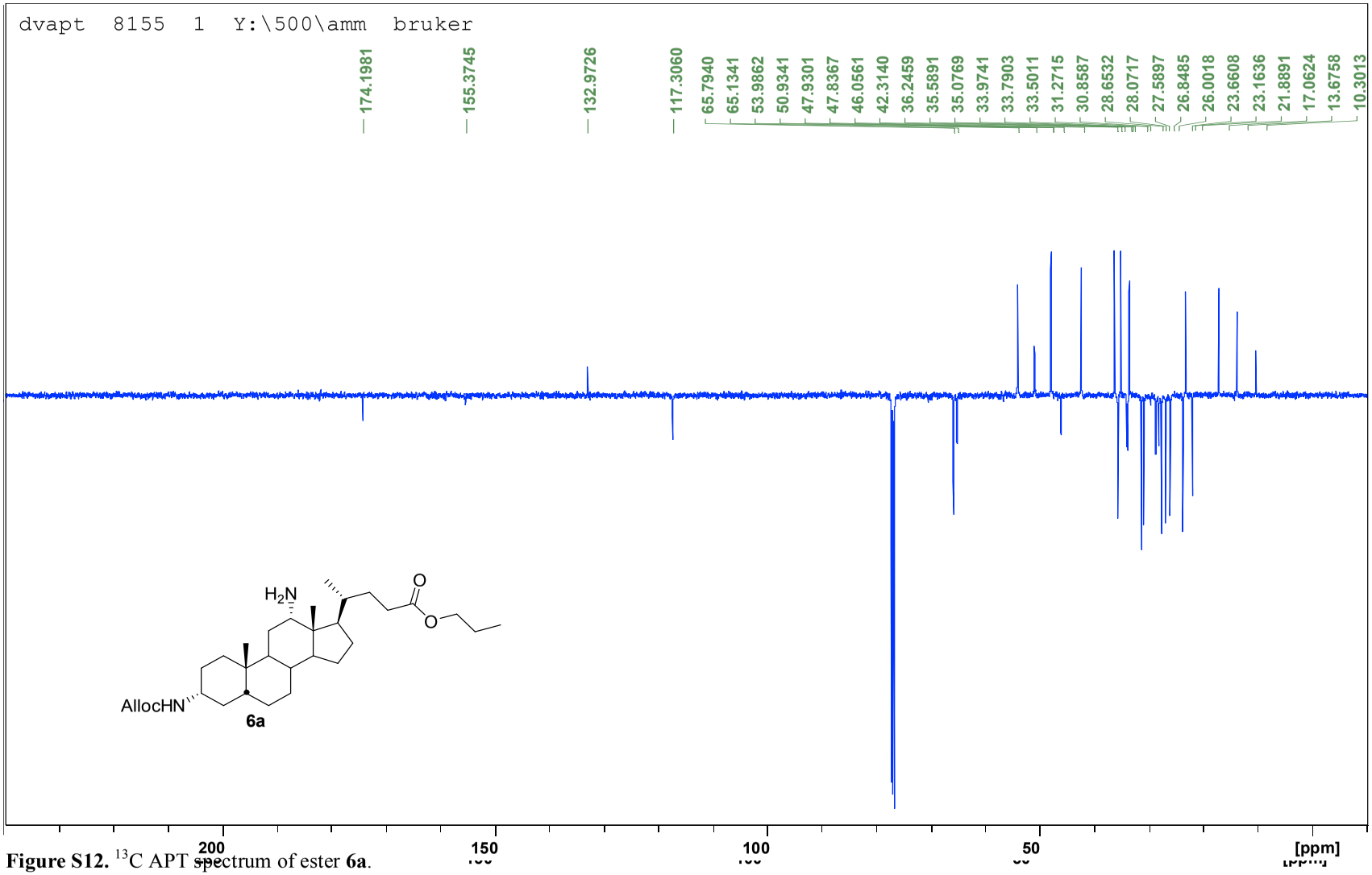


Figure S12. ^{13}C APT spectrum of ester **6a**.

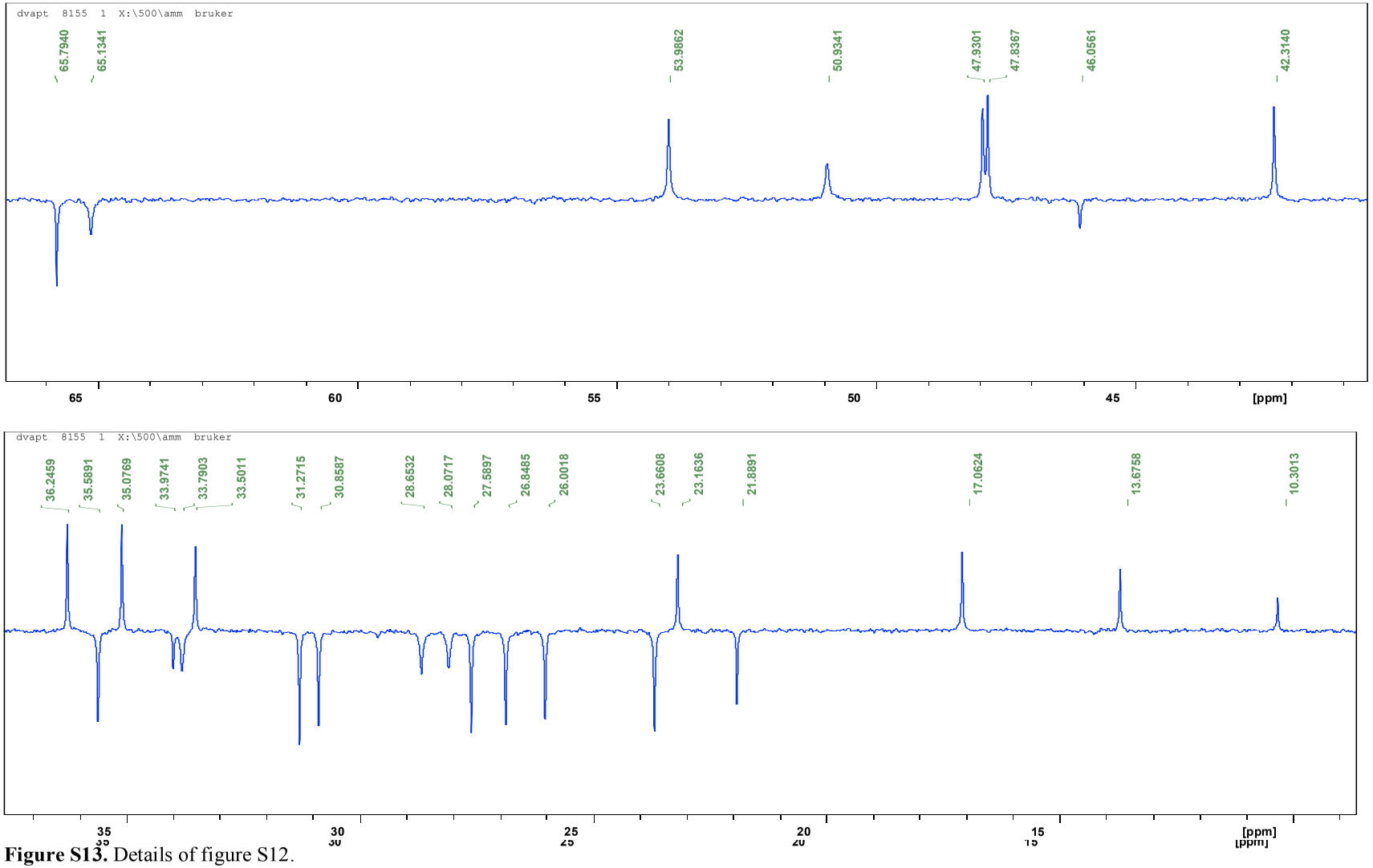


Figure S13. Details of figure S12.

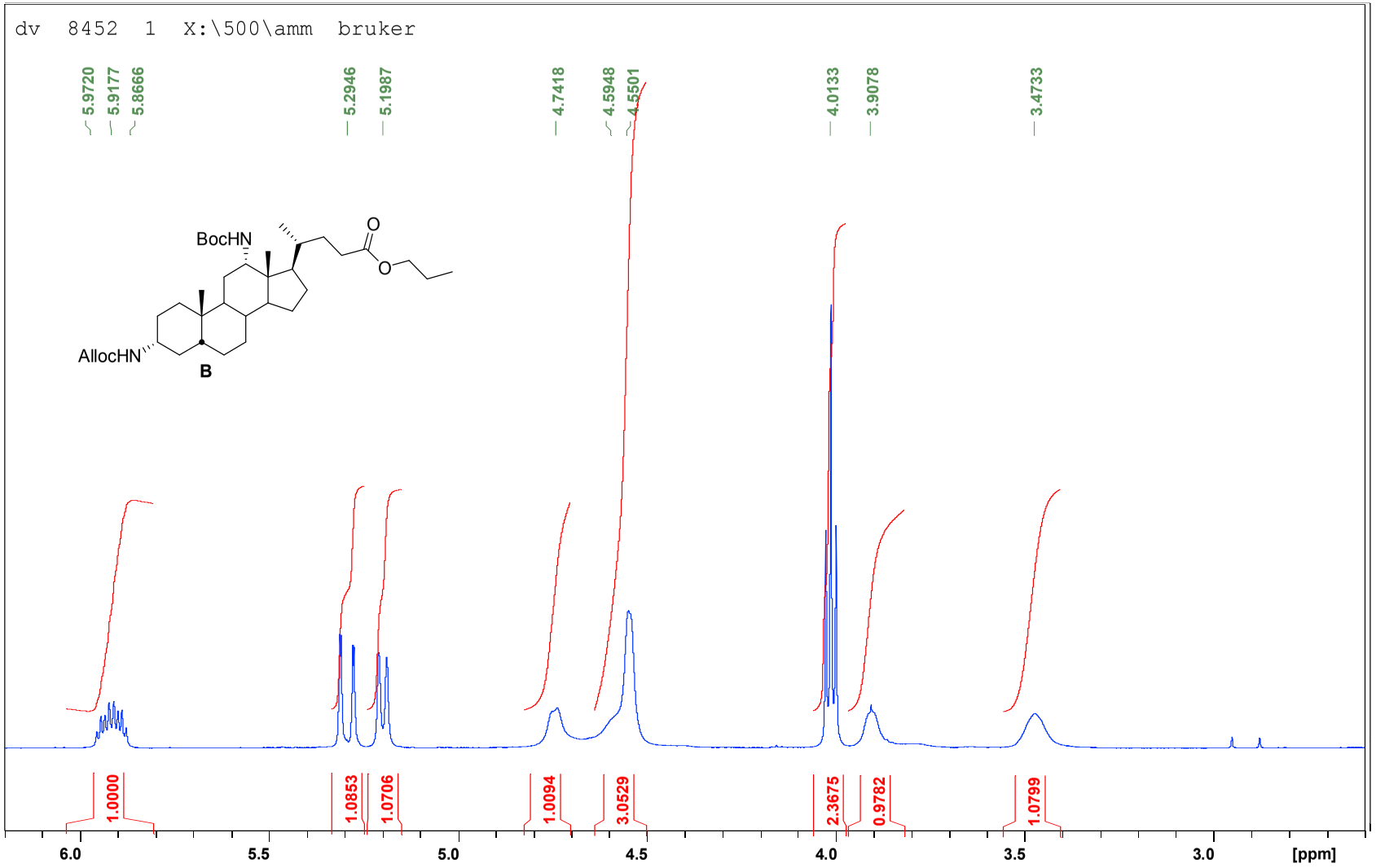


Figure S14. Detail of the ^{1}H spectrum of ester **B**.

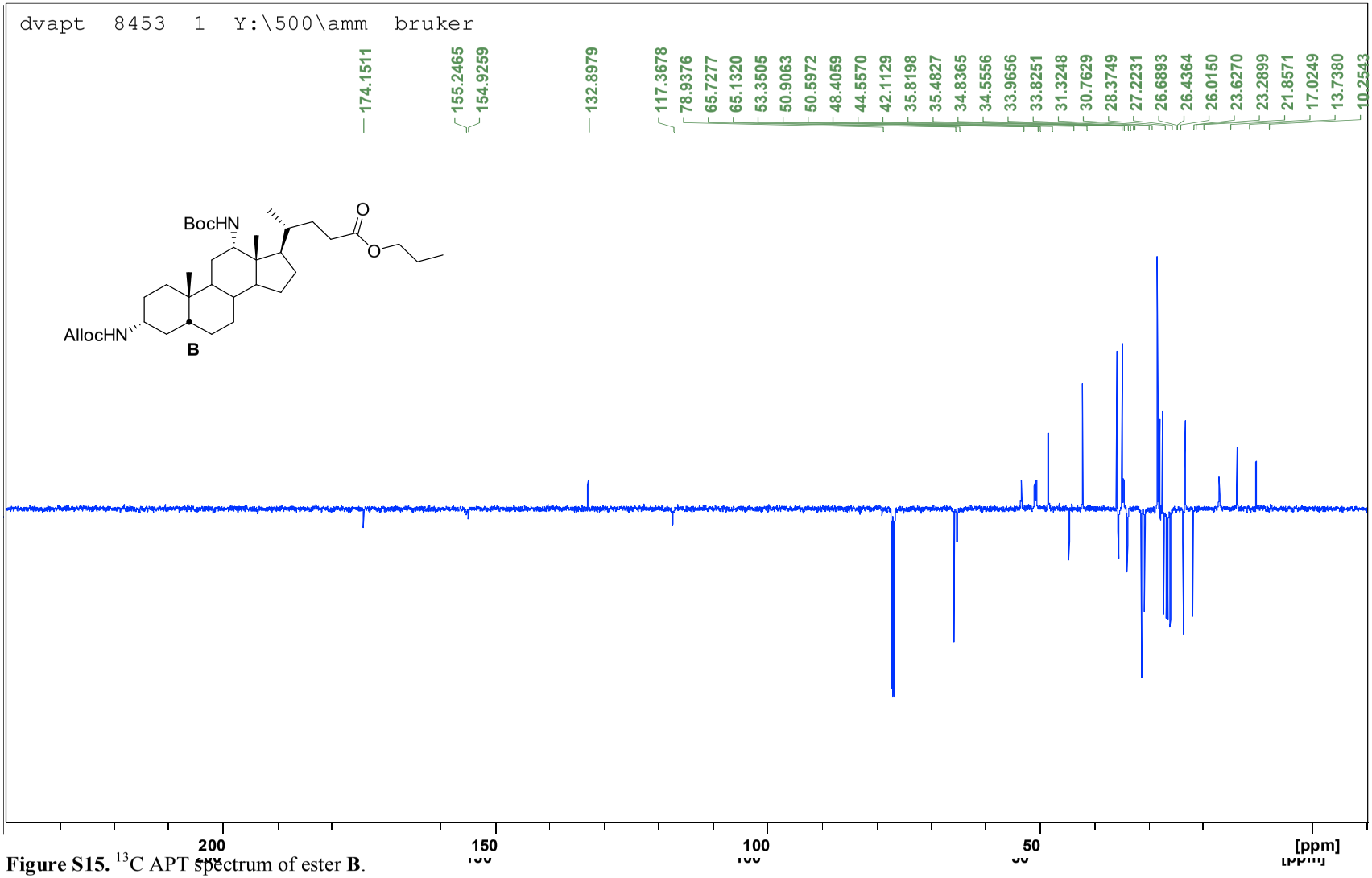


Figure S15. ^{13}C APT spectrum of ester **B**.

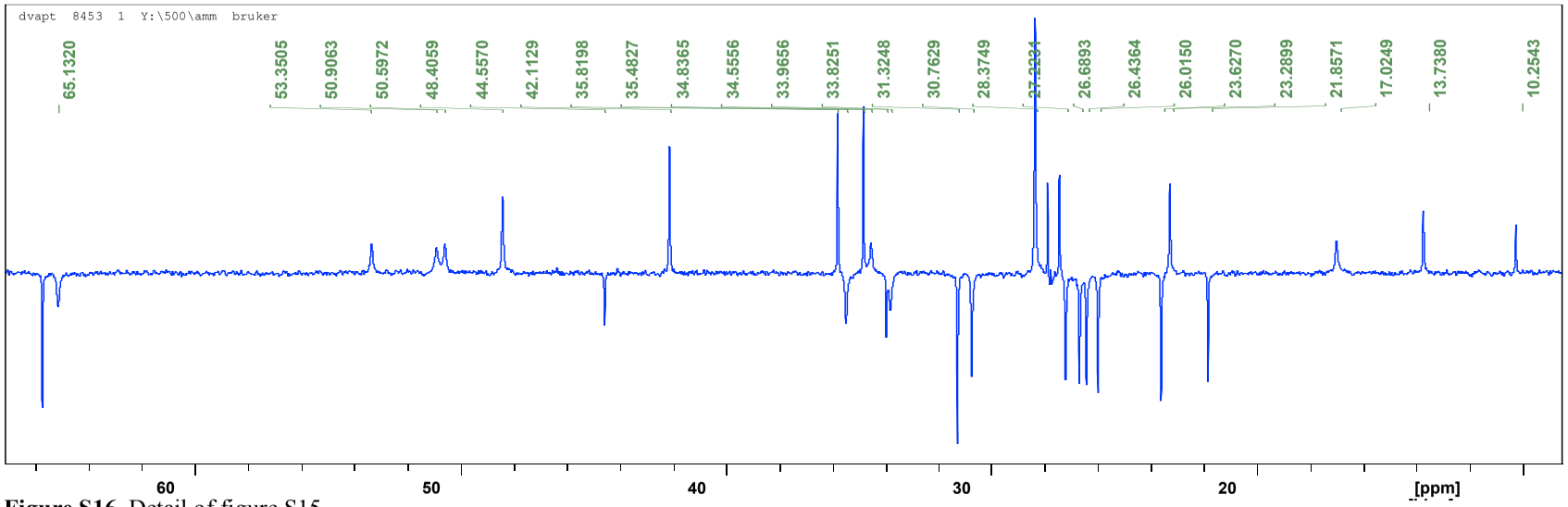


Figure S16. Detail of figure S15.

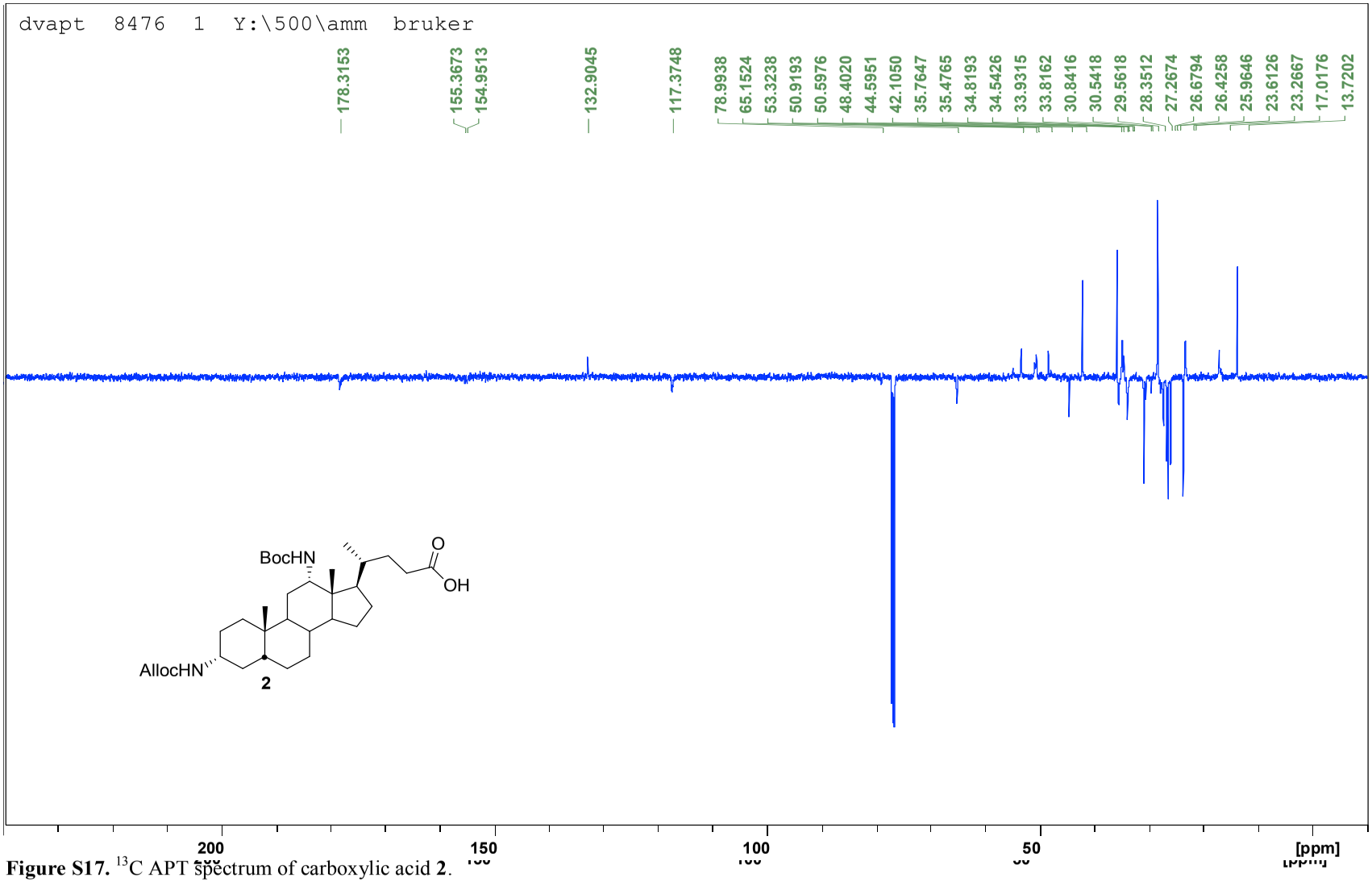


Figure S17. ^{13}C APT spectrum of carboxylic acid **2**.

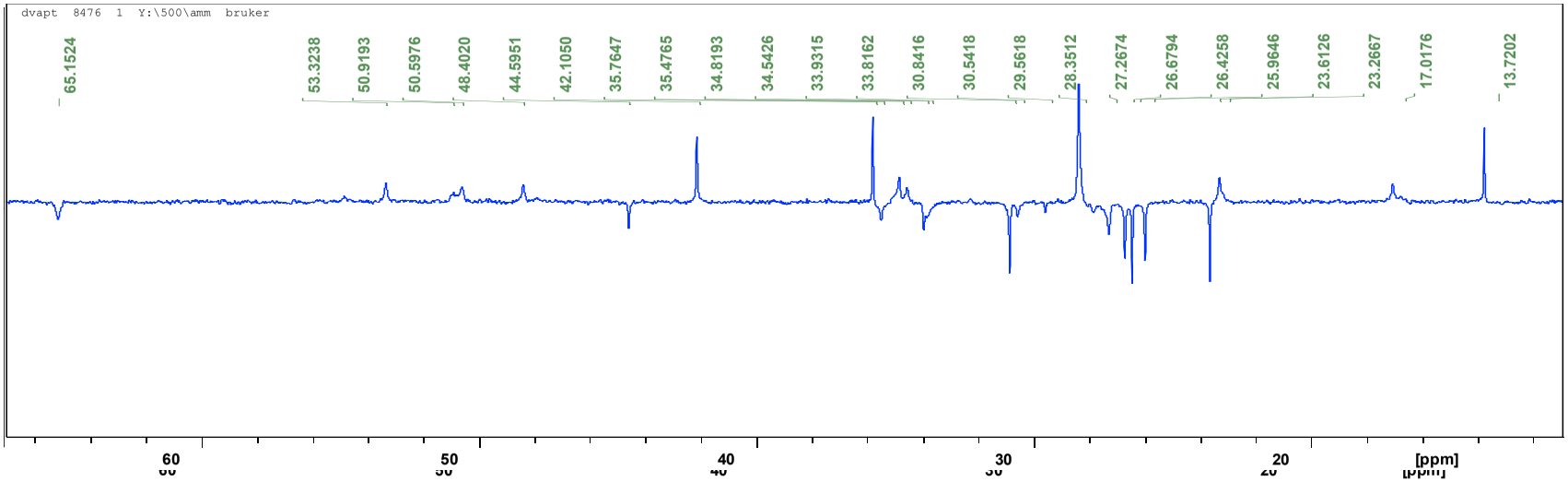


Figure S18. Detail of figure S17.