Continuing the synthesis of (S)-α- (O-glycine)-β- (N1-thymine)-propanoic acid nucleotide

Phil Ngo Chemistry 100r – Spring 2012

Abstract

We report a synthesis of an (S)-α- (O-glycine)-β- (N1-thymine)-propanoic acid (COOH-Thy) Peptide Nucleic Acid (PNA) nucleotide and the manual solid phase peptide synthesis of an oligonucleotide chain of three COOH-Thy nucleotides. Specifically, we have oxidized (S)-α- (O-glycine)-β- (N1-thymine)-propanol (OH-Thy), an α-hydroxy acid precusor (OH-Thy), to (COOH-Thy), the desired α-hydroxy acid monomer, using a TEMPO catalyzed reaction with bis(acetoxy)iodobenzene (BAIB). We purified this compound and attempted a manual solid phase peptide synthesis procedure synthesize an oligonucleotide (Thy-Thy-Thy-Lys) using COMU as the activating agent because it is reported to reduce racemization at chiral centers alpha to the site of amide bond formation.

1. Background

Peptide -or polyamide- nucleic acids (PNA) are modified oligonucleotide analogs that entirely replace the deoxyribose backbone of DNA with, in our case, a modified (O-glycine)-propanoic acid-based polyamide backbone, though many variations exist. PNA oligonucleotides show high binding affinity[I] for natural DNA and RNA strands because there is less coulombic repulsion between the PNA backbone and natural oligonucleotides than between natural DNA strands (which carry a negative charge in physiological pH). Peptide nucleic acids could potentially be used to recruit transcription factors to DNA using PNA-peptide chimeras[II], or target drugs to cells where certain active genes cause problems because they can be used to selectively bind DNA within a cell in physiological conditions.

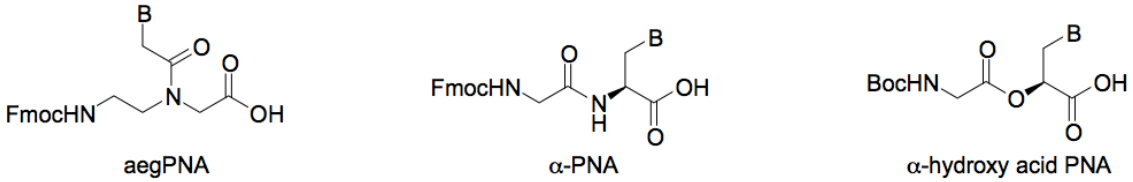


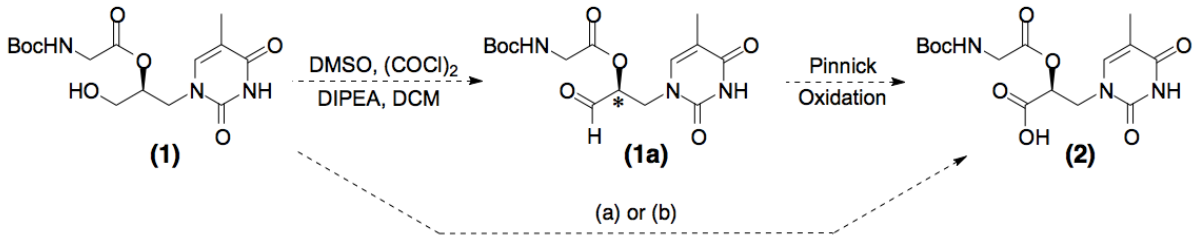
Figure 1: Various PNA types, including aegPNA, α-PNA, and α-hydroxy acid PNA, our target.

In the last several years, many types of PNA[III,IV,V,VI,VII] (see *Figure 1*) have been synthesized successfully, and here we continue the synthesis of another type, which we here call α-hydroxy acid PNA. The target is a pseudo peptide backbone with greater affinity for DNA than closely related α-PNA. α-PNA has lower affinity to DNA strands than N-(2-aminoethyl)glycine (aeg) PNA because of steric hindrance of the proton on the amide within the backbone. The α-hydroxy acid PNA that we synthesize here theoretically reduces steric hindrance by replacing the amide linkage with an ester linkage. The barrier to rotation in amide bonds is relieved in an ester, allowing increased flexibility in oligonucleotide chains and better binding affinity for DNA or RNA.

Despite this change in linkage, α-hydroxy acid PNA can still be coupled using standard peptide chain coupling procedures, as demonstrated here. The coupling reagent we used, an Oxyma based uronium salt, (1-Cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU)[VIII], shows better tolerance for chiral substrates and reduced levels of epimerization of chiral substrates. We have included our procedure for the peptide coupling for the reference of future researchers; however, our results are unconfirmed by mass spectrometry.

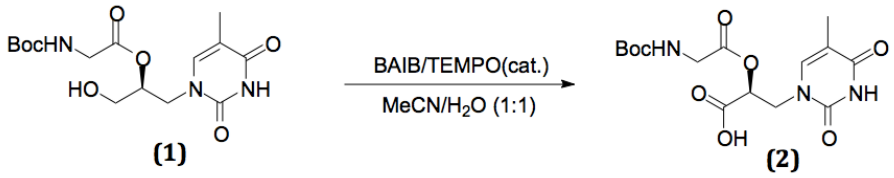
Research Results:

Scheme 1. Three initially proposed oxidations of the thymine alcohol to COOH-Thy.[a]

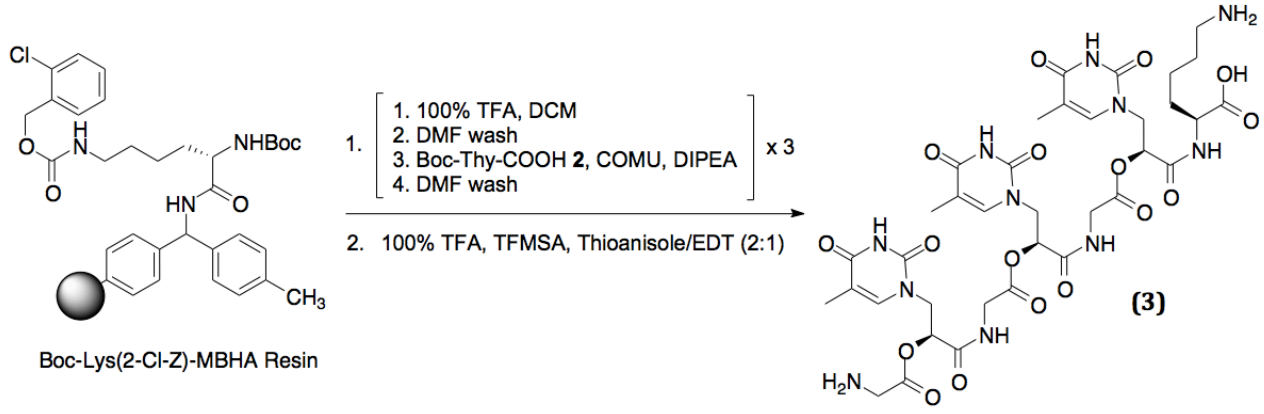


[a] Reagents and conditions: (a) $NaClO_2$/TEMPO(cat), NaClO(cat), MeCN/H_2O (1:1); (b) TPAP (10 mol%), NMO-H_2O (10 equiv.), MeCN (.25 M), rt.

Scheme 2. Revised oxidation scheme of the thymine alcohol to COOH-Thy with BAIB as co-oxidant.



Scheme 3. Peptide Coupling and Cleavage on Boc-(Cl-Lys-Z)-MBHA resin.



2. Oxidation of primary alcohol 1 to carboxylic acid 2 with catalytic TEMPO.

We attempted four oxidations of primary alcohol **1** to carboxylic acid **2,** one of which resulted in satisfactory yield. Zhao et al.[IX] reported an oxidation of primary alcohols to carboxylic acids with sodium chlorite catalyzed by (2,2,6,6-tetramethylpiperdin-1-yl)oxyl (TEMPO) and bleach. We carried out this oxidation using to the reaction conditions specified to unsatisfactory yield. We also attempted an

oxidation using tetrapropylammonium perruthenate (TPAP) [X] and 4-methylmorpholine N-oxide(NMO) as oxidizing agents. These reaction conditions were found to be intolerant of the other functional groups in the reactant **1** and was abandoned when, after column chromatography, a complex mixture of products was observed in ^{1}H NMR analysis. Negative ion mode ESI high-resolution mass spectroscopy revealed the desired product **2** (M-H), however, we suspected that it was synthesized in very low yield but ionized preferentially to the starting material in negative ion mode. We also considered a Swern Oxidation[XI] of the alcohol to aldehyde, followed by an oxidation to the carboxylic acid, using reaction conditions tolerant of a wide range of functional groups, such as the Pinnick or Lindgren Oxidation. This route would have provided for high yields using time-tested oxidation methods; however, this too was abandoned because of the high risk of epimerization at the adjacent stereogenic center in **1a** (see *Figure 2*).

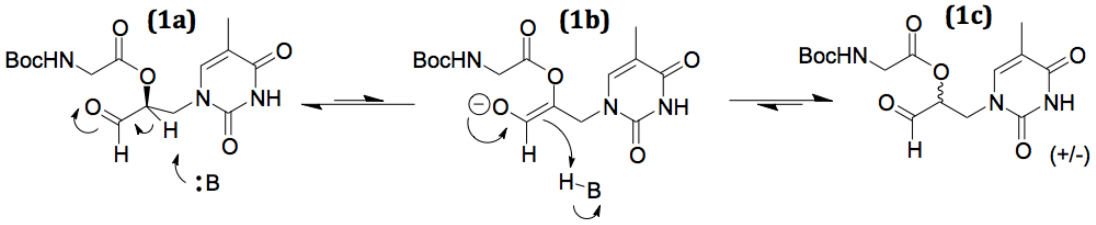


Figure 2: Stepwise oxidation of primary alcohol **1** to aldehyde **1a** to carboxylic acid **2** risks epimerization at the stereogenic center in compound **1a** due to its equilibrium with enol **1b**.

The oxidation in *Scheme 2* using bis(acetoxy)iodobenzene as a cooxidant with catalytic TEMPO[XII] gave the acid **2** in good yield after base extraction and purification on a silica gel column. Acid extraction was found not to preferentially extract the product. TLC monitoring revealed that the starting material **1** and product **2** were not separated by column chromatography. The acid, however, had a long tail and came out of the column for 20+ fractions after the UV active OH-Thy came out of the column completely, as revealed by TLC monitoring. For this reason, we switched to the use of a base extraction, which can be

done before or after column chromatography; however, if done before, addition of sodium bicarbonate solution will yield a messy white precipitate due to the presence of iodobenzene derivatives in the reaction mixture. In base extraction, the conjugate base of the COOH-Thy will remain in the aqueous layer, returning to the organic layer upon acidification. ^{1}H NMR confirms the oxidation.

3. Boc- Manual Solid Phase Peptide Synthesis of Thy-Thy-Thy-Lys 3 using COMU for activation of deprotected primary amine.

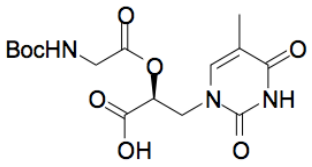
We used a Boc-Lys(2-Cl-Z)-MBHA Resin to manually synthesize a thymine oligonucleotide. The procedure[XIII] we followed allows for *in situ* neutralization of the peptide-resin TFA salt. We used a resin with a protected Lys group, which is eventually cleaved from the resin with the rest of the polypeptide chain. This extra lysine amino acid will ensure that the PNA oligonucleotide will be more soluble in physiological conditions. The activation agent COMU was chosen because of its commercial availability and decreased racemization of amino acid substrates. Because the oligonucleotide chain synthesized was short, it did not precipitate out of solution upon addition of diethyl ether. Usually, this precipitate is collected by filtration and purified. In our case, we used a solid-phase extraction column to purify the product, followed by lyophilization to dryness. The product was analyzed by mass spectrometry to reveal a number of masses close to the calculated mass of 905.32. (M+18, M+20.)

4. Conclusion

We synthesized of a COOH-Thy **2** PNA nucleotide using a direct oxidation from the OH-Thy **1** precursor using a TEMPO catalyzed reaction with bis(acetoxy)iodobenzene as co-oxidant, minimizing the risk of enol formation that would cause epimerization at the stereogenic center alpha to the target acid. We purified this compound and used a manual solid phase peptide synthesis procedure synthesize an oligonucleotide (Thy-Thy-Thy-Lys) using COMU as the activating agent because it is reported to reduce racemization at chiral centers. Given our successful synthesis of a COOH-Thy and a short

oligonucleotide chain, other base pairs should be synthesized in order to begin testing of binding affinity for oligonucleotides. The synthesis the oligonucleotide chain is unconfirmed by mass spectrometry, but the procedure used is included for future researchers. Future researchers might also consider synthesis of other base pairs to create a library from which other PNA oligonucleotides chains can be synthesized. No other solid phase peptide synthesis conditions were attempted due to time constraints; this would be a good area for future researchers to continue synthesis.

5. Experimental Section:



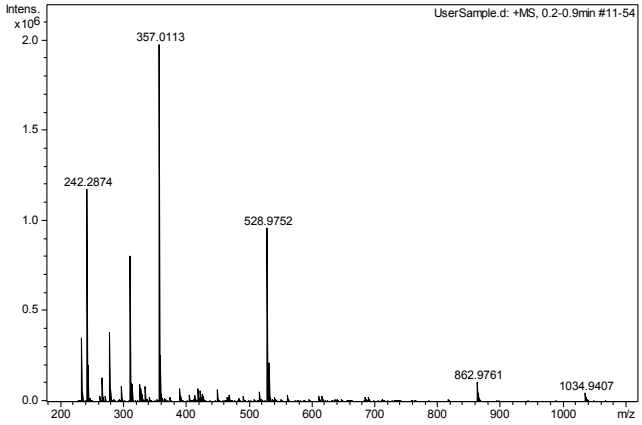
(S)-α- (O-glycine)-β- (N1-thymine)-propanoic acid (2): COOH-Thy **2**, (1.00 g, 2.80 mmol), TEMPO (87.5 mg, 0.56 mmol), and bis(acetoxy)iodobenzene (2.25 g, 7.00 mmol) were dissolved in MeCN/H_2O (1:1, 6 mL) and stirred, with the exclusion of light for 24h. Isopropanol (20 mL) was added and the mixture was extracted with 5% sodium bicarbonate (200 mL, aq), washed with ethyl acetate (3x), and the combined organic phases discarded. The aqueous layer was acidified with 6M HCl (pH <2) and extracted with ethyl acetate. The aqueous layers were combined, saturated with NaCl and extracted with ethyl acetate (3x). The organic layers were combined, dried over magnesium sulfate, concentrated, and the residue was purified with column chromatography on silica (10% MeOH, EtOAc, 1% acetic acid) to yield the acid **2** (0.849 g, 82%) a colorless oil/foam, which was used without further purification: δ_H (400 MHz, DMSO-d_6) 1.35 (9H, s), 3.32, (3H, s), 3.86 (2H, dd), 4.18 (2H, dd), 5.09 (1H, q), 7.19 (1H, t), 7.43 (1H, s), 10.80 (1H, s), 11.26 (1H, s); *m/z* HRMS (TOF ES+) $C_{15}H_{21}N_3O_8$ (M+Na)+ calcd, 394.11; found, 394.12.

Boc-Thy-Thy-Thy-Lys(2-Cl-Z)-MBHA Resin: 0.2g of Boc-Lys(2-Cl-Z)-MBHA Resin was washed with DMF (10 mL). The N^{α}-Boc group was removed by treatment with 100% TFA (1.5 mL) for 2 × 1 min followed by a 30 s flow wash with DMF (10 mL) . Boc-Thy Peptide nucleic acids (148.4 mg , 0.4 mmol) were coupled, without prior neutralization of the peptide-resin salt, as active esters preformed in

DMF (1 mL) with COMU (171.3 mg, 0.4 mmol)/DIEA (0.6 mmol) (2 min activation) as activating agents. Neutralization was performed *in situ* by adding 1.5 equiv. DIEA relative to the amount of $TFAO^{-}\square^{+}NH_3$-peptide-resin salt to the activated Boc-peptide nucleic acid/resin mixture. The resin was washed to stop the coupling reaction and remove excess reagents . All operations were performed manually in a 20 mL glass reaction vessel with a screw cap. The peptide resin was agitated by gentler inversion on a shaker during the N^{α}-deprotection and coupling steps, followed by a 30s flow wash with DMF (10mL). Coupling, washing and activation steps were repeated three times and cleaved to obtain oligonucleotide **3** using the method below.

Thy-Thy-Thy-Lys 3: Boc-Thy-Thy-Thy-Lys(2-Cl-Z)-MBHA Resin (50 mg) and thioanisole/ethanedithiol (2:1, 75 µL) were added to a 50 mL flask and stirred for 5m. 100% TFA (1 mL) was added to the reaction flask and stirred for 10m. (Note: instruments which came into contact with EDT were rinsed in a dilute solution of bleach to neutralize the smell.) TFMSA (100 µL) was added to the reaction flask and stirred vigorously for 1.5h. Diethyl ether (5mL, 0°C) and H_20 (5mL) were added to the reaction flask, after which the resin was filtered through meduim porosity Whatman Filter paper to remove the resin beads, and reaction flask and filter paper were washed with H_20. Ethyl acetate was evaporated under reduced pressure and aqueous solution was eluted onto a pre-wetted normal phase solid-phase extraction column (10% MeOH, H_20). The column was rinsed with H_20, and the product was extracted with MeOH/EtOAc (1:1, 20mL) and the effluent was collected and solvent was removed by lyophilization to yield a salt and an oil **3**: *m/z* HRMS (TOF ES+) $C_{36}H_{47}N_{11}O_{17}$ (M+Na)+ calcd, 927.30; found, 894.6903, 1206.9062.

HRMS: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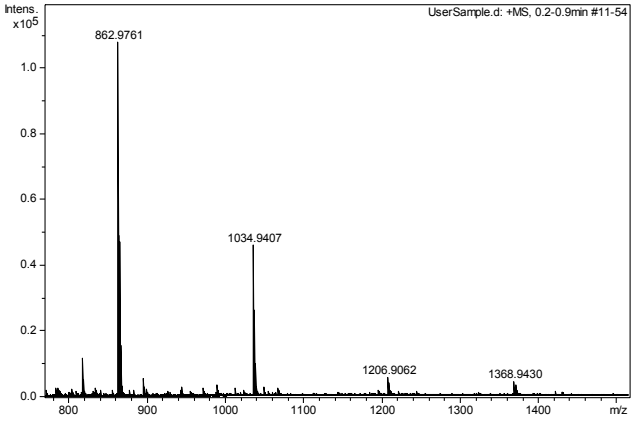
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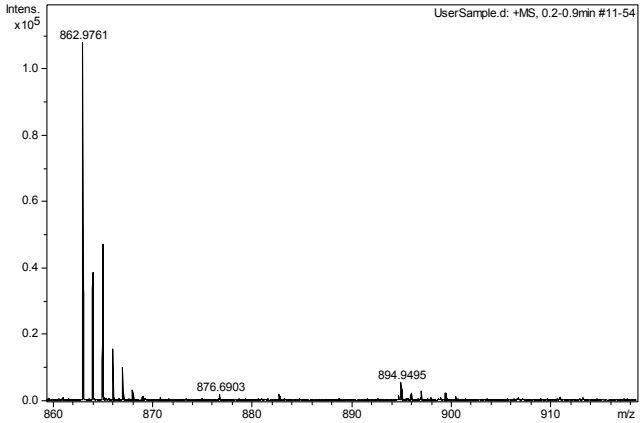
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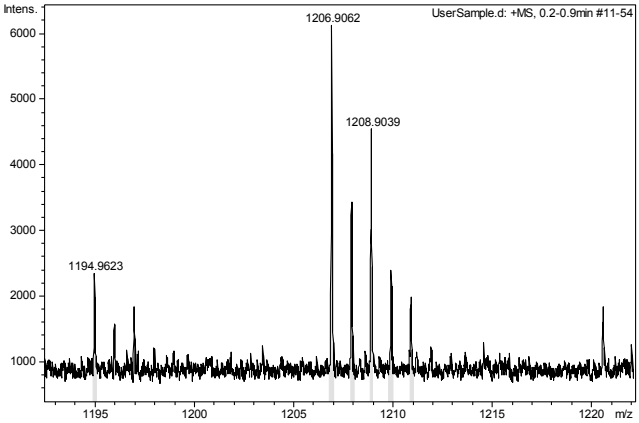
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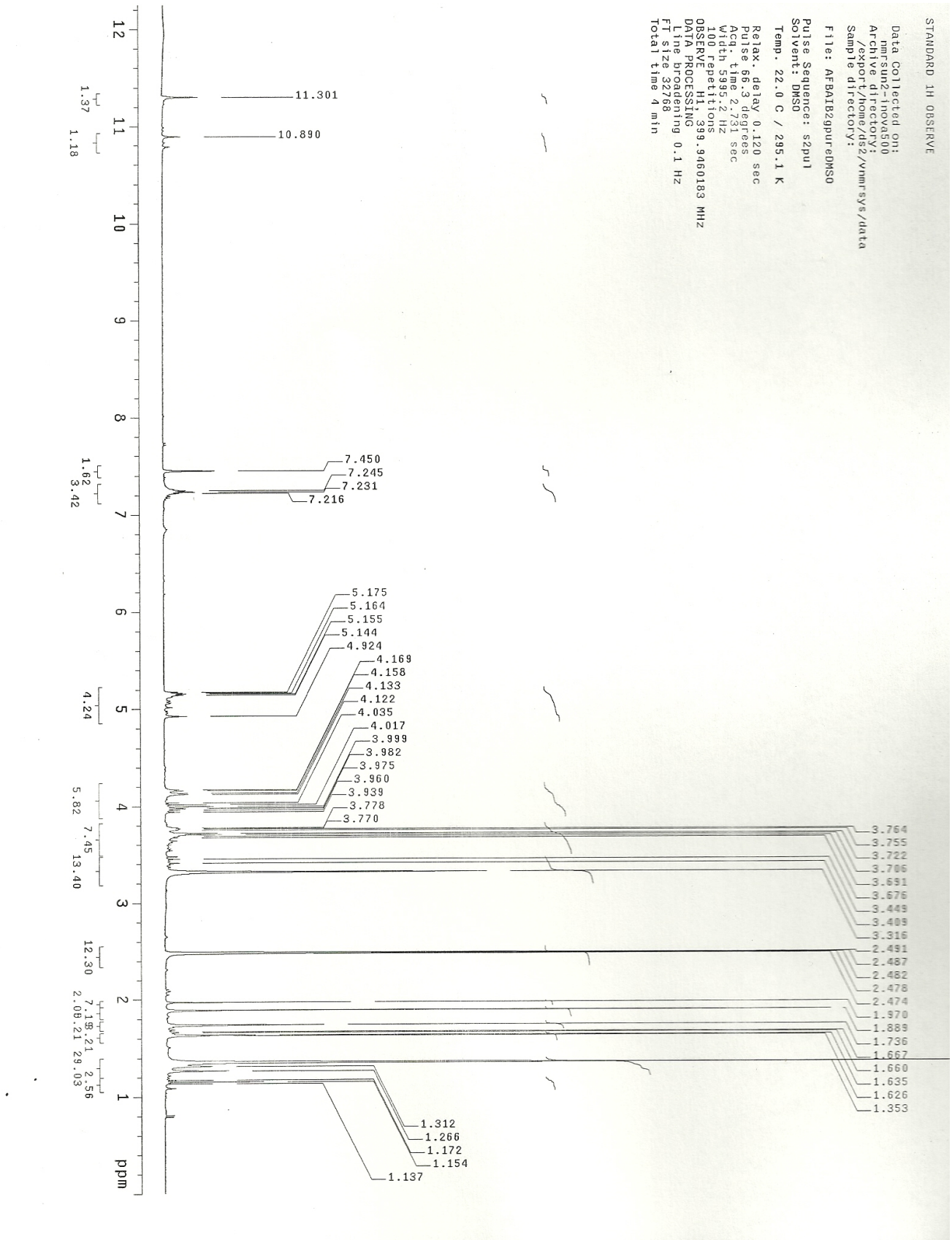

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