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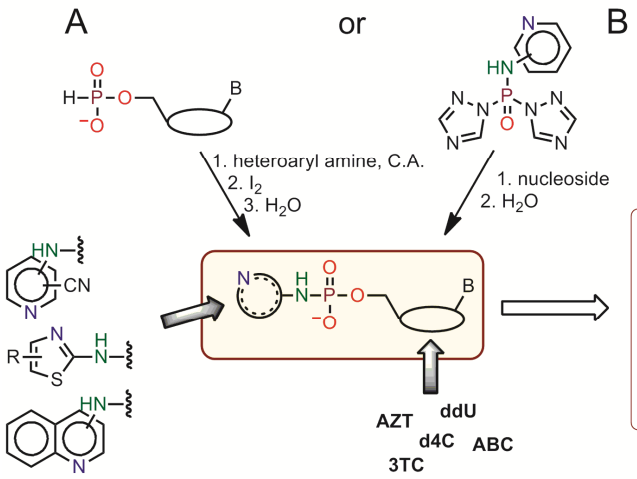

Toxicity for CEM-T4 cells:

not toxic - 100% viability at all concentrations

Anti-HIV activity:

EC_{50} = 2–20 nM for AZT derivatives

Aryl *H*-Phosphonates 18. Synthesis, properties, and biological activity of 2',3'-dideoxynucleoside (*N*-heteroaryl)phosphoramidates of increased lipophilicity

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Abstract

Recently, AZT (*N*-pyridyl)phosphoramidates were reported as a new type of potential anti-HIV therapeutics. In continuation of that work, here we present new (*N*-heteroaryl)phosphoramidate derivatives of antiviral 2',3'-dideoxynucleosides containing other types of *N*-heteroaryl moieties, particularly those with higher lipophilicity. The present studies comprise mechanistic investigations using ^{31}P NMR correlation analysis, which permitted improvements in the synthetic procedures. The obtained compounds were tested in biological systems to establish their cytotoxicity and anti-HIV activity. The results were analyzed with respect to possible correlations between biological and physico-chemical properties of the phosphoramidates studied, to get some insight into their antiviral mode of action.

Introduction

2',3'-Dideoxy derivatives of nucleosides (*e.g.* AZT, d4T, 3TC) are known to inhibit viral reverse transciriptases and some of them are used extensively in treatment of retroviral infections [1]. Nevertheless, these compounds are far from being optimal drugs and are still under thorough investigations aimed to improve their pharmacological features. One of the challenges is to overcome the AZT toxicity associated with accumulation of AZT monophosphate (AZTMP), which formed in cells rapidly from the parent nucleoside in a reaction catalyzed by thymidine kinase (TK). In contrast, phosphorylation of AZTMP to AZTDP by thymidylate kinase is slow, leading to accumulation of AZTMP and decreasing total AZT antiviral efficacy. The third phosphorylation, AZTDP → AZTTP, catalyzed by unspecific 5'-nucleoside diphosphate kinase, is rapid again [2]. Moreover, the large amounts of the accumulated AZTMP inhibit thymidylate kinase by binding to its ATP site [3], and in

consequence, affect also phosphorylation of the natural TMP, and finally, DNA replication. The simplest way to by-pass this problem would be administration of proper doses of already phosphorylated AZT; unfortunately, due to the negative charge of the phosphate residue, AZTMP it is not able to pass the cell membrane. Thus, a so-called 'pro-nucleotide approach' was developed, [4] in which the phosphate group of a therapeutic nucleotide analogue was appropriately protected and in such uncharged form, was able to surmount the lipid barrier of a cell. Subsequently, inside the cell, the phosphate protecting groups were cleaved off yielding the desired AZTMP.

Recently, we developed AZT (*N*-pyridyl)phosphoramidates (*cf*. Fig. 1) as a new type of pro-nucleotides, which exhibited high anti-HIV activity and low cytotoxicity on one hand, and very good chemical stability, resistance to enzymatic degradation, and excellent solubility in water, on the other one [5]. Additionally, preliminary tests showed low acute toxicity of these species in animal models: **7ab** (Ar = 2-pyridyl): GHS class 5 (LD_{50} = 2500 mg/kg, rats, oral); **7ab** (Ar = 4-pyridyl): GHS class 2 (LD_{50} = 50 mg/kg, rats, oral) or class 3 (LD_{50} = 200 mg/kg, rats, iv); **7ac** (Ar = 3-pyridyl): GHS class 5 (beyond classification, no lethality at 2000 mg/kg, rats, oral). In the same study, LD_{50} for AZT was found to be 2500 mg/kg (oral) and 500 mg/kg (iv) [6].

High biological activity indicated that despite their anionic character, AZT (*N*-pyridyl)phosphoramidate monoesters were able to enter the cell effectively. We rationalized this by assuming that in aqueous solutions, apart form typical ionic forms these compounds exist – to small but still significant extent – as protonated, neutral species (Fig. 1), which apparently can penetrate the cellular lipid membranes [5]. Compatibility of (*N*-pyridyl)phosphoramidates with aqueous and hydrophobic media may be also a result of the dual nature of the pyridine ring, which is both lipophilic due to its hydrocarbon fragment, and hydrophilic due to the presence of the nitrogen atom.

FIGURE 1

These speculations prompted us to study the impact of the structure, lipophilicity and basicity of the parent heterocyclic amines on anti-HIV activity of the derived nucleoside phosphoramidates.

Results and discussion

In order to elucidate the topics delineated above, three series of heteroaryl 5'-phosphoramidates were prepared: derivatives of aminoquinolines, aminocyanopyridines, and aminothiazolines. Among 14 possible isomers of aminoquinolines and aminoisoquinolines, 8

were readily commercially available and these were included in this study. Various positions of the nitrogen atoms in these isomers lead not only to different spatial arrangement of the (iso)quinoline groups, but also have a significant impact on lipophilicity and basicity of the designed nucleoside phosphoramidates (Table 1, entries 5–11). Permeability through cell membranes, which is a key feature of a compound considered as a prospective drug, usually correlates linearly with solubility of a substance in nonpolar solvents (Overton's rule), and may be conveniently expressed as an octanol-water partition coefficient, log *P* [7,8]. Most of amino(iso)quinolines have log *P* in a range of 1.2–1.9, which is roughly an order of magnitude higher than those of the unsubstituted aminopyridines (log *P* = 0.2, 0.3, and 0.5 for *m*, *p*, and *o* isomer, respectively[*]) used in the previous study [5]. Thus, phosphoramidates containing a quinoline residue may be expected to penetrate the cell membranes more effectively than their pyridyl congeners. Next, pK_a's of amines **2d–j** are spread over the range of 4.0–9.6. This should influence the acid-base equilibria of the derived phosphoramidates, and consequently, the fraction of their neutral, protonated forms under physiological pH, which are expected to surmount the cell membrane easier than the ionic forms. Finally, the aforementioned different spatial arrangement of the isomeric (iso)quinoline groups in nucleoside phosphoramidates may have additional impact on their biological activity in view of the recent findings by Herdewijn *et al.*, who observed that some nucleoside 5'-phosphoramidates can be used as substrates by HIV reverse transcriptase (without conversion into the respective triphosphates), and that structural variations in the amide part had fundamental impact on their substrate properties [9-11].

The two members in the second set of heterocyclic amines included in this study contain a nitrile group in the pyridine ring (Table 1, entries 12–13), which decreased significantly their basicity (estimated pK_a 0.5–3 vs. 6.0–6.7 for the respective aminopyridines). Thus, protonation of the amide residue in the derived phosphoramidates (*cf.* Fig. 1) should be attenuated, and this may in turn lead to an increase in population of neutral protonated forms, which are expected to be particularly effective in crossing the lipid membranes. The third group, 2-aminothiazoles (Table 1, entries 14–16), was chosen to verify whether phosphoramidates containing significantly different heteroaromatic group can be synthesized under the same conditions and if they retain the antiviral properties of (*N*-pyridyl)phosphoramidates. It is worth to note that the thiazole motif is present in many

[*] Experimental values, included in reports generated by ChemBioDraw v. 13.

molecules showing biological activity and successful syntheses of the proposed here model compounds may be a starting point for development of new bioactive derivatives.

Chemistry

The phosphoramidates of type **7** were prepared according to modified published protocols, *i.e.* by condensation of nucleoside 5'-*H*-phosphonate with an amine followed by oxidation (method A, Fig. 2) or by phosphorylation of nucleoside with bis(triazoyl)-(*N*-heteroaryl)phosphoramidates (method B, Fig. 4) [5]. All reactions were monitored by ^{31}P NMR spectroscopy.

FIGURE 2

In the method A, nucleoside *H*-phosphonates **1** [12] were condensed with heteroaryl amines of type **2** (1.1 equiv.) using diphenyl chlorophosphate (DPCP; 1.7 equiv.) as a condensing agent to yield *H*-phosphonamidates **3**, which were oxidized *in situ* to the final phosphoramidates **7** (Fig. 2). Since in same cases lower yields of the products were obtained, we inspected more carefully the course of the reactions using ^{31}P NMR spectroscopy. In the standard procedure, the addition of aqueous iodine in pyridine to the solution the *in situ* formed mixture of **3** + **4**, resulted in relatively rapid (ca. 5 min) disappearance of ^{31}P NMR signal of *H*-phosphonamidate **3** at ca 7–9 ppm with a simultaneous built-up of the signal of the final product **7** (δ_P ca. -3 ppm). Additionally, regeneration of some amounts of *H*-phosphonate **1** was observed for several amines **2**, which were more basic than pyridine. Most likely, this was a result of hydrolysis of **3**, which in these cases was rapid enough to compete with relatively sluggish iodination of *H*-phosphonamidates. Under the above conditions no intermediates could be observed in the reaction mixture.[†] However, when iodination of the mixture of **3** + **4** was carried out under anhydrous conditions, formation of two groups of signals in a 1:1 ratio was observed in the ^{31}P NMR spectra: a multiplet at ca. -7 ppm and two doublets at ca. -24 ppm. The reaction was complete within ca. 5 min, as judged from disappearance of the intermediate **3**. Also, a decrease in intensity of the signal at ca. -10 ppm, assigned to diphenyl phosphate **4**, was observed. Upon addition of an excess of water, both the new signals disappeared simultaneously within ca. 5 min, and in the final ^{31}P NMR spectrum only signals of **7** and **4** were present.

[†]Phosphoroiodidate diesters are extremely reactive, particularly in the presence of pyridine (an efficient nucleophilic catalyst for this reaction) [13,14], and the failure to detect signals of **5** (or the derived phosphopyridinium P-Py$^+$ species [14], not shown in Fig. 2) in ^{31}P NMR spectra was not surprising.

The most plausible explanation of these results is formation of a mixed pyrophosphate **6** (two diastereomers), for which the multiplet at ca. -7 ppm was assigned to the amidate ester phosphorus atom [-P(O)(OR)(NHAr)], while the two doublets at ca. -24 ppm, to the diester one [-P(O)$(OPh)_2$]. This was confirmed by spiking the reaction mixture containing the assumed mixed pyrophosphate **6** with a sample of this compound obtained in the reaction of (*N*-heteroaryl)phosphoramidate with DPCP.[‡]

These results prompted us to carry out the oxidation process in two steps: (i) oxidation of *H*-phosphonamidate **3** using anhydrous iodine in pyridine to generate pyrophosphate **6**, followed by (ii) its hydrolysis with an excess of water. Both steps proceeded with similar rate and were completed within < 5 min yielding the desired product **7** quantitatively (^{31}P NMR).

The above procedure was used successfully for the synthesis of most AZT (*N*-heteroaryl)phosphoramidates **7a*x*** (70–80% isolated yields; Table 1). A wide scope of this approach was demonstrated by preparation of several representative derivatives of ddU, d4T, ABC, and 3TC nucleosides (**7b**–**e**, Table 2). Synthesis of *H*-phosphonates **1b-e** [12] and their subsequent coupling with aromatic amines **2**, followed by oxidation (*vide supra*) proceeded nearly quantitatively (^{31}P NMR); however, due to similar R_f's of the products and other compounds present in the reaction mixtures, in several cases a repeated chromatography was required to obtain satisfactory purity of the desired nucleoside (*N*-heteroaryl)phosphoramidates. This led to 10–15% decrease in the yields (Table 2, entries 18, 20, 21, 25, 26, 28, & 29). Conversely, phosphoramidate **7cg** (d4T – 6AQ; entry 24) was prepared with an excellent isolated yield of 97%, confirming the effectiveness and high synthetic potential of the chemistry used.

FIGURE 3

Similarly as it was observed previously for 4APy (**2a**) [5], condensations of AZT *H*-phosphonate **1a** with more basic amines led to poor results (Table 1, entries 6 & 10), presumably due to coincidence of several factors. The first was an undesired superfluous activation of the initially formed *H*-phosphonamidate esters **3** (Fig. 3) with an excess of a condensing agent (diphenyl chlorophosphate, DPCP) that resulted in formation of nucleoside bis(*N*-heteroaryl)phosphordiamidites **8** (δ_P ca. 95 ppm). Since such by-products were not

[‡]Attempted isolation of pyrophosphates of type **6** failed due to their rather high reactivity. In separate experiments we found that such pyrophosphates reacted with amines and alcohols with preferential nucleophilic attack at the phosphoramidate phosphorus atom. Unfortunately, this regioselectivity was not complete (ca. 75%). Thus, potential applications of pyrophosphates **6** for synthesis of phosphoramidate diesters or phosphordiamidate monoesters require further studies.

observed for amines with pK_a < 7, it is plausible that in the presence of more basic amines the abundance of tervalent tautomers of type **3'** became significant, and their reaction with a second molecule of DPCP and the amine yielded the putative diamides **8**. Upon their oxidation, the respective bis(*N*-heteroaryl)phosphordiamidates **9** (δ_P ca. -5 ppm) were formed in amounts up to 20%. Apart from decreasing the yield of conversion of **1** to **7**, their presence hampered the chromatographic purification of the desired monoamide products.[§]

FIGURE 4

An additional problem associated with the more basic heteroaryl amines was their rapid reaction with the condensing agents, *e.g.*, DPCP or others, while such side-reaction was negligible for amines with pK_a < 7. In consequence, both the consumed amines and DPCP had to be replenished in several portions during the reaction. This in turn generated large amounts of HCl, which reduced nucleophilicity of the amines by protonation and led to their massive precipitation from the reaction mixtures in a form of hydrochlorides.

TABLE 1

TABLE 2

Thus, for incorporation of amines with pK_a > 7 into phosphoramidates **7**, an approach based on P(V) derivatives was developed (method B, Fig. 4). Thus, tris(triazoyl)phosphoramidate **10** [15] was reacted with an amine (**2**) to yield monosubstituted amides of type **11**. Similarly as the previously described di(triazoyl)[*N*-(pyridin-4-yl)]phosphoramidate **11a** [5], also derivatives of 2APy (**11b**), 4A2MQ (**11e**), 1AIQ (**11i**), and 2AQ **(11k**) precipitated from the reaction mixtures and could be readily isolated as pure products (the last were unstable during storage and should be prepared directly before use). In contrast, derivatives of other, less basic heteroarylamines remained in solution and attempts to use them directly for the next reaction steps gave poor results. Thus, the methods A and B complement each other with a borderline of pK_a ≈ 7 of the amine used.

To produce phosphoramidates **7** containing the more basic amines, the corresponding bistriazolides **11b**, **11i**, and **11k** were dissolved in pyridine (at elevated temperature, ~75 ºC, if necessary) and reacted with the added AZT to yield, after hydrolysis, products **7a*x*** (Fig. 4) in

[§]AZT bis[*N*-(pyrid-4-yl)]phosphordiamidate **9aa** appeared to be very stable under neutral and basic conditions. The half-life time in 0.1 M NaOH was ca. 10 days, and in 10% aqueous pyridine + 10% triethylamine, ca 5 days. In contrast, in 0.1 M HCl the hydrolysis was very rapid (< 3 min). In all instances, monoamidate **7aa** was formed as the final product. Initial experiments revealed high anti-HIV activity of diamidate **9aa** (EC_{50} 30 nM; EC_{90} 400 nM) and lack of cytotoxicity at concentrations up to 200 μM. Further studies on this type of compounds are in progress in this laboratory.

ca. 50–60% isolated yields. For **11e**, concomitant formation of bis(AZT) phosphate diester was observed and the yield of desired product **7ae** was low, ca. 20% after chromatographic purification.

Similarly as we noted previously for AZT (*N*-pyridyl)phosphoramidates [5], during purification of all synthesized phosphoramidates of type **7** in a form of triethylammonium salts, a significant loss of the cation took place and the products were obtained partly or completely in a form of acids. This is a serious issue since non-stoichiometric and variable amounts of the cation compromise the accuracy of sample preparation, while under-stoichiometric counter-ion contents causes solubility problems and affects the chemical stability of phosphoramidates (acid-catalyzed degradation). Thus, to obtain the products with a 1:1 anion:cation ratio, a new purification procedure was developed (see Experimental Part). The issue of the cation loss during purification is a subject of a separate publication [21].

*Stability of phosphoramidates **7***

The phosphoramidates of type **7** were examined with respect to their chemical and enzymatic stability. All of them appeared to be intact during incubation for 5 days at 37 ºC in RPMI buffer (HPLC & ^{31}P NMR analysis). A pilot experiment revealed outstandingly high stability of phosphoramidate **7aa** (AZT – 4APy) in 0.1 M HCl and 0.1 M NaOH (no hydrolysis of the phosphoramidate group within 7 days, ^{31}P NMR). For such high chemical resistance one can expect a very long shelf-life of this type of compounds. In RPMI + 10% fetal bovine serum (FBS) a rather slow decomposition was observed (Fig. 5, Tables 1 & 2), the half-life time was usually several days; however, derivatives of ddU (**7b**) were in general less stable. Most of phosphoramidates of d4T (**7c**), ABC (**7d**), and 3TC (**7e**) appeared to be completely resistant to degradation by the FBS solution. In order to compare stability of compounds with $t_{1/2} > 7$ days, the amount of phosphoramidates **7** which remained after 3 days of incubation in 10% FBS/RPMI was determined (Tables 1 & 2).

FIGURE 5

The final products of hydrolysis of the compounds **7a**–**c** in FBS solution were identified by RP HPLC as the respective nucleosides and amines of type **2**. Unfortunately, in some cases a separation of all compounds present in reaction mixtures could not be achieved, *e.g.*, for phosphoramidate **7am** the formed AZTMP and 2-aminothiazole **2m** co-migrated and the analysis of the products was hampered (Fig. 5A). For several phosphoramidates (*e.g.*, **7bo**, Fig. 5B), however, the peaks for all compounds were well resolved and their assignment was confirmed by spiking with original samples. This provided a clear-cut evidence for formation

of the anticipated nucleoside monophosphates, which, rather surprisingly, remained in a steady concentration for many days (ca. 15% of A.U. of all species absorbing at 254 nm), presumably as a result of similar rate of formation and degradation. Provided that an analogous steady-state type of kinetics is kept *in vivo*, such continuous delivery of NMTP's by gradual decomposition of phosphoramidates **7** would comply with a pro-nucleotide scenario, in which phosphoramidates in the cells are first hydrolyzed – at least partly – to monophosphate esters that serve subsequently as substrates for nucleotidyl kinases.

Peaks which could be assigned to phosphoramidates of heteroarylamines ($ArNHPO_3H_2$) that hypothetically may be formed by cleavage of the P-O bond in compounds **7**, were not detected in neither case, indicating that such pathway of decomposition is probably of minor, if any, significance.

It must be stressed that the formula of FBS-containing media is significantly different from the composition of fluids present inside cells and the above experiments should be treated just as tentative ones to get some insight into processes occurring in living cells.

Biological evaluation

The biological goal for the preparation of (*N*-heteroaryl)phosphoramidates of type **7** was their evaluation as anti-HIV agents. As a first step, we examined the parent heteroaromatic amines of type **2**, which likely are the catabolic products of the studied phosphoramidates, and found that these were of low or very low cytotoxicity (Table 1), and did not show any anti-HIV activity up to concentration of 10 μM. Thus, their biological activity was assumed to be negligible. In contrast, all AZT (*N*-heteroaryl)phosphoramidates of type **7a** were found to be potent anti-HIV agents even in nanomolar concentrations. EC_{50} values were in the range of 1–20 nM and EC_{90} values, ca. 10–200 nM (Table 1). Among them, the most promising compounds (EC_{90} < 25 nM) contained isoquinoline (**7ai** & **7aj**), cyanopyridine (**7al** & **7am**), and thiazoline amide groups (**7an**, **7ao** & **7ap**), while those with quinoline group (**7ad**–**7ah**) were in general slightly less active (EC_{90} = 30–220 nM).

All the tested phosphoramidates were practically non-toxic for CEM-T4 cells, even at the highest concentrations, which were limited only by solubility of the compounds. Thus, CC indices could not be determined and the values of selectivity indices may be only presumed. Apparently, these are very high, in many cases likely at the level of hundreds of thousands or more, making AZT (*N*-heteroaryl)phosphoramidates of type **7a** valuable candidates for further development as anti-HIV drugs.

As it was mentioned in the introduction, the working hypothesis, which governed the choice of the heteroaryl groups for phosphoramidates of type **7**, was that increased lipophilicity should facilitate cell membrane permeability and in consequence, should lead to superior pharmacological properties. To evaluate correctness of these assumptions, water-octanol partition coefficients of the compounds studied were determined (micro shake-flask partitioning approach [22]). Initial experiments revealed that octanol-water partition of phosphoramidates of type **7** was dependent on pH to a small extent only, *e.g.*, log $P_{\mathbf{7ad}}$ = -1.45 at pH 4; -1.72 at pH 7.4; -1.40 at pH 11. Thus, it was assumed that log *P* ≈ log *D* and further measurements were done at the physiological pH 7.4 only. The values of log *P* determined are included in Tables 1 and 2, and deserve some comments. (i) The influence of lipophilicity of the nucleoside residues on the total lipophilicity of phosphoramidates **7** appeared to be rather limited. Some trend of lower log *P*'s for derivatives of hydrophilic ddU [log P_{ddU} = -0.96 (lit. -1.0 [23]), log $P_{\mathbf{7bx}}$ = -1.7 – -2.4], d4T [log P_{d4T} = -0.79 (lit. -0.6 [24,25]), log $P_{\mathbf{7cx}}$ = -2.0 – -3.4], and 3TC [log P_{3TC} = -0.94 (lit. -0.93 [26]) , log $P_{\mathbf{7eo}}$ = -2.3] than those for AZT [which is equally lipo- and hydrophilic; log P_{AZT} = 0.06 (lit. -0.01 – 0.1 [27-29]), log $P_{\mathbf{7ax}}$ = -0.7 – -2.1] could be noted. However, similarly low log *P*'s were found for phosphoramidates of much more lipophilic ABC [log P_{ABC} = 1.16 (lit. 0.85 [26]), log $P_{\mathbf{7dx}}$ = -1.6 – -1.9]. (ii) There is no apparent relationship between lipophilicity of the free heteroarylamines and AZT phosphoramidates **7a*x*** containing them (Table 1). (iii) There are large discrepancies between experimental and calculated log *P* values for compounds **7**a (Table 1). These three observations indicate that the common additive methods for predicting log *P* values is not applicable for phosphoramidates of type **7**, possibly due to acid-base equilibria, electronic effects, and functional group interactions (intra- or intermolecular) etc., which apparently control the overall lipophilicity for this class of compounds.

Thus, it was not surprising to see lack of correlation between lipophilicities and antiviral potency of compounds **7** (Table 1 and 2, Fig. 6). For example, in the AZT series (**7ax**), similarly very low EC values were found for the least hydrophilic **7ai** (log *P* = -0.7, EC_{50} = 2.0 nM) and the most hydrophilic **7an** and **7ao** (log *P* = -2.1, EC_{50} = 2.1 nM), while the least antiviral effective **7ah** (EC_{50} = 20 nM) has an intermediate log *P* value of -1.5.

We also did not observe any correlation between anti-HIV activity of amides **7** and their stability in RPMI-FBS or basicity of the parent amines **2** (which should govern the isoelectric point of amides **7**, and consequently, their cell membrane permeability at given pH).

Most likely, these results reflect a complex nature of anti-HIV mode of action of the compounds studied, including involvement in acid-base equilibria, the aforementioned different cell membrane permeability, stability inside the cells, and enzyme substrate properties.

FIGURE 6

In a next set of experiments, phosphoramidates **7b**-**e** bearing other dideoxynucleoside residues (ddU, d4T, ABC, and 3TC) were tested as anti-HIV agents (Table 2). d4T and ABC nucleosides are significantly less active than AZT, and this was true for their phosphoramidates **7c** and **7d** in comparison to **7a**. Also, similarly to the AZT series, the best phosphoramidates in **7c** and **7d** series were at a similar level of anti-HIV activity as their parent nucleosides, while 3TC phosphoramidate (**7eo**) was ca. 2 orders of magnitude less active than 3TC. Nevertheless, all the studied phosphoramidates of type **7c–e** showed very low toxicity for cells, and this makes them promising compounds for further evaluation as antiviral agents.

Phosphoramidates in the **7b** series are a special case, since their parent nucleoside, dideoxyuridine (ddU), is biologically inert since is not phosphorylated by kinases to ddUMP. Moreover, even when ddU was delivered to the cells in a form of masked mono- and diphosphates, these were poorly phosphorylated to ddUTP (which is a potent HIV RT inhibitor) [30]. Previously, we reported on inhibition of HIV by ddU [*N*-(pyridin-4-yl)]phosphoramidate (EC_{50} 0.2 μM) but not by analogous 2- and 3-aminopyridine derivatives [5]. In this work, among the studied compounds of type **7b**, a moderate activity (EC_{50} 4.2 μM) was found for [*N*-(quinolin-3-yl)]phosphoramidate **7bd** only.[**] Degradation of all derivatives of type **7b** in the FBS medium proceeded in similar rates (Table 2) toward ddU *via* ddUMP (a clear-cut formation of ddUMP was observed by HPLC, *cf*. Fig. 5B). It was rather unlikely that in an anti-HIV assay the formation of ddUMP was significantly more effective for **7bd** than for **7bg**, **7bl**, or **7bo**. To explain the observed activity of some ddU phosphoramidates and differences within this group, one may invoke recent results by Herdevijn et al., who observed that some nucleoside 5'-(*N*-amino acid)phosphoramidates could mimic dNTP's in nucleic acid biosynthesis catalyzed by HIV RT and other polymerases, and that propensity of a phosphoramidate to be a substrate in such reaction depended on the structure of AA residue [9-11]. Provided that similar mode of action applies for (*N*-heteroaryl)phosphoramidates of

[**] EC_{50} values of 0.2 [5] and 4.2 μM are still much better than these of EC_{50} = 15 and 26 μM, found as the best results for classical pro-nucleotides of ddUMP [31] and ddUDP [30], respectively.

type **7**, 3-aminoquinoline derivative **7bd** itself could serve as substrate for incorporation into DNA by HIV RT compromising further chain growth, while the other amides of type **7b**, would be worse substrates.

This hypothesis may explain as well why some exceptionally stable phosphoramidates of type **7** (*e.g.* **7cd**, **7dl**, **7eo**) are able to inhibit proliferation of HIV despite their complete resistance to degradation by FBS, which may suggest a very low level of the respective nucleosides or NMP's produced inside the cells. These assumptions still require experimental verification.

Conclusions

In the chemical part of this contribution a number of new nucleoside (*N*-heteroaryl)phosphoramidates were prepared in good yields. Mechanistic investigations revealed that *in situ* oxidation of nucleoside *H*-phosphonamidates involved formation of mixed pyrophosphoramidates as intermediates. This finding led to development of an improved synthetic protocol, in which oxidation and hydrolysis were carried out as one-pot two-stage process. Additionally, side products formed in reactions in which highly basic amines were used, were identified as so far unknown nucleoside di(*N*-heteroaryl)phosphorodiamidates, which are potentially valuable as a new type of antiviral agents.

The obtained compounds were not harmful for CEM-T4 cells, while were able to suppress HIV-1 proliferation in nanomolar (AZT derivatives) or micromolar (ddU, d4T, ABC, and 3TC derivatives) concentrations.

There are no clear-cut evidences how 2',3'-dideoxynucleoside (*N*-heteroaryl)phosphoramidates of type **7** are incorporated into DNA. In a pro-nucleoside scenario, they are dephosphorylated in the cells, and can be considered as a kind of storage for gradual delivery of antiviral nucleoside analogues. However, some results (anti-HIV activity of ddU derivatives) suggest more desirable pro-nucleotide mode of action, *i.e.* cleavage of the P-N bond with formation of phosphorylated nucleosides, and by-passing the first phosphorylation stage. Alternatively, phosphoramidates **7** may be recruited as direct substrates for HIV reverse transcriptase. Anti-HIV activity of several amides that are fully resistant to degradation by FBS may point to the last mechanism.

It is intriguing that irrespective of significantly different type of the heteroaromatic residues, which resulted in variations in enzymatic stability, lipophilicity, and (presumably)

ionic equilibria, all AZT derivatives studied showed rather similar level of antiviral activity ($EC_{50} \approx 1–20$ nM; $EC_{90} \approx 10–200$ nM).

Concluding, efficient synthesis, stability, solubility, high anti-HIV activity, and very low toxicity found for the phosphoramidates studied is calling for further studies on this class of compounds as potential antiviral drugs.

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Experimental

Material and methods

^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on Varian Unity BB VT 300 MHz or Bruker Avance II 400 MHz machines. Mass spectra were recorded with the ESI technique with negative ionization with accuracy below 5 ppm. Amount of water in anhydrous solvents was controlled using Karl Fischer coulometric titration (Metrohm 684 KF coulometer). The progress of reactions was monitored by ^{31}P NMR spectroscopy. ^{31}P NMR data are collected in Table 3. HPLC analyses were performed on a Nucleosil 100-5C18 column (5.0 μm, 4.6 mm x 250 mm) using Shimadzu Prominence UFLC and Waters Breeze HPLC systems and 0 → 50%/ 30 min gradient of A+B solvent systems (A, 0.01 M aqueous triethylammonium acetate; B, A + acetonitrile, 1 : 4, v/v) at 36 ºC, flow rate 1.0 mL/min. Biological experiments were carried out as described previously [5].

2',3'-Dideoxynucleoside *H*-phosphonates 1a-e

AZT *H*-phosphonate **1a** was obtained as described previously [32]. *H*-Phosphonates of ddU (**1b**), d4T (**1c**), ABC (**1d**), and 3TC (**1e**) were prepared by a slightly modified diphenyl *H*-phosphonate procedure [12]. A nucleoside (1 mmol) was rendered anhydrous by evaporation with dry pyridine and dissolved in 5 mL of the same solvent. To this solution diphenyl *H*-phosphonate (3 equiv.) was added while stirring and left for 0.5 hour. The reaction was quenched by addition of water (1 mL) and triethylamine (TEA, 1 mL) and the solution concentrated to a viscous oil. The residue was dissolved in minimal amount of water and without work-up applied to silica-gel chromatographic column, eluted subsequently with 0 → 30% (v/v) MeOH gradient in 99:1 ethyl acetate – TEA. Fractions containing pure product were combined, evaporated and lyophilized from benzene.

3'-Azido-3'-dideoxythymidin-5'-yl *H*-phosphonate, $TEAH^+$ salt (AZT *H*-phosphonate, 1a) and **2',3'-dideoxyuridin-5'-yl *H*-phosphonate, $TEAH^+$ salt (ddU *H*-phosphonate 1b)**. Analytical data were in agreement with the literature [32].

2',3'-Didehydro-3'-deoxythymidin-5'-yl *H*-phosphonate, $TEAH^+$ salt (d4T *H*-phosphonate; 1c). Yield 72%. RP HPLC R_f 12.78 min. ^{1}H NMR (400 MHz) δ_H (D_2O) 1.21 (9H, t, J = 7.3 Hz), 1.89 (3H, d, J = 1.0 Hz), 3.21 (6H, q, J = 7.3 Hz), 4.07 (2H, m), 5.09 (1H, s), 5.98 (1H, m), 6.49 (1H, m), 6.69 (1H, d, J = 637.4 Hz), 6.95 (1H, m), 7.60 (1H, d, J = 1.2 Hz). ^{13}C NMR (100 MHz) δ_c (D_2O) 7.7, 11, 46.2, 63.4 (d, J_{PC} = 4.5 Hz), 85.4 (d, J_{PC} = 7.5 Hz), 89.5, 110.7, 124.9, 133.8, 137.7, 151.7, 166.2. HRMS *m/z* 287.1864, calcd. for $C_{10}H_{12}N_2O_6P^-$ 287.0438.

(1*S*,*cis*)-4-[2-Amino-6-(cyclopropylamino)-9*H*-purin-9-yl]-2-cyclopentene-1-methyl *H*-phosphonate, $TEAH^+$ salt (ABC *H*-phosphonate; 1d). Yield 47%. RP HPLC R_f 17.67 min. ^{1}H NMR (400 MHz) δ_H (D_2O) 0.49 (2H, m), 0.76 (2H, m), 1.17 (9H, t, J = 7.3 Hz), 1.42 (1H, m), 2.66 (2H, m), 3.06 (6H, q, J =7.3 Hz), 3.76 (2H, m), 5.22 (1H, t, J = 6.2 Hz), 5.77 (1H, m), 6.10 (1H, m), 6.61 (1H, d, J = 630.8 Hz), 7.63 (1H, s). ^{13}C NMR (100 MHz) δ_c (D_2O) 6.2, 7.6, 22.8, 33.8, 45.1 (d, J_{PC} = 7.0 Hz), 46, 58.6, 65.4 (d, J_{PC} = 4.6 Hz), 112.6, 128.9, 136.4, 137.4, 155.1, 159. HRMS *m/z* 349.1162, calcd. for $C_{14}H_{18}N_6O_3P^-$ 349.1183.

α-L-2',3'-Dideoxy-3'-thiacytadin-5'-yl *H*-phosphonate, $TEAH^+$ salt (3TC *H*-phosphonate; 1e). Yield 93%. RP HPLC R_f 12.03 min. ^{1}H NMR (400 MHz) δ_H (D_2O) 1.30 (9H, t, J = 7.3 Hz), 3.21 (6H, q, J = 7.3 Hz), 3.26 (1H, m), 3.58 (1H, dd, J = 12.3 Hz), 4.18 (1H, m), 4.31 (1H, m), 5.47 (1H, t, J = 7.2 Hz), 6.09 (1H, d, J = 7.6 Hz), 6.36 (1H, t, J = 9.9 Hz), 8.06 (1H, d, J = 7.6 Hz), 6.82 (1H, d, J = 642.4 Hz). ^{13}C NMR (100 MHz) δ_c (D_2O) 7.7, 36.2, 46.2, 63.6 (d, J_{PC} = 4.2 Hz), 83.9 (d, J_{PC} = 7.2 Hz), 86.9, 95.4, 141.5, 156, 165.2. HRMS *m/z* 292.0085, calcd. for $C_8H_{11}N_3O_5PS^-$ 292.0162.

2',3'-Dideoxynucleoside (*N*-heteroaryl)phosphoramidates 7

Method A

All phosphoramidates containing amines of pK_a < 7 were prepared from *H*-phosphonates of type **1** by a slightly modified literature methods [5,21]. Nucleoside *H*-phosphonate **1** (1 mmol) and heteroaryl amine **2d**, **2f–i**, or **2j–p** (1.1 mmol) were rendered anhydrous by the evaporation of the added pyridine (3 x 10 mL) and then dissolved in 10 mL of DCM containing 10% (v/v) of pyridine. To this, DPCP (1.7 mmol) was added, and the reaction mixture was left for 5 min yielding *H*-phosphonamidates **3** (for ^{31}P NMR data, see Table 1). Without isolation of these intermediates, a solution of iodine (2 mmol) in pyridine (1 mL) was

added, the reaction mixture was left for 5 min and then, a solution of water (50 mmol) in pyridine (1 mL) was added. After another 5 min, the excess of iodine was decomposed with the added ethanethiol, and the mixture was concentrated to an oil under reduced pressure. Crude phosphoramidate **7** was dissolved in a minimum amount of MeOH, applied to a silica-gel column, and eluted with 0–20% gradient of MeOH in ethyl acetate containing 5% TEA. Fractions containing the pure product were pooled and evaporated to dryness under reduced pressure. The residue was dissolved in a minimal amount of water containing ca. 2 equiv. (in respect to phosphoramidate) of TEA and lyophilized. Such final procedure secured a stoichiometric amount of the $TEAH^+$ cation (1 equiv.) [21], which was confirmed by ^1H NMR spectroscopy.

3'-Azido-3'-deoxythymidin-5'-yl [*N*-(quinolin-3-yl)]phosphoramidate, $TEAH^+$ salt (7ad). Yield 78%. RP HPLC R_f 20.32 min. ^1H NMR (400 MHz) δ_H (D_2O) 1.21 (9H, t, J = 7.3 Hz), 1.45 (3H, s), 2.27 (2H, m), 3.10 (6H, q, J = 7.3 Hz), 3.99 (1H, m), 4.08 (1H, m), 4.18 (2H, m), 6.01 (1H, t, J = 5.6 Hz), 6.89 (1H, m), 7.45 (2H, m), 7.56 (1H, m), 7.69 (1H, d, J = 2.6 Hz), 7.75 (1H, d, J = 8.3 Hz), 8.50 (1H, d, J = 2.6 Hz). ^{13}C NMR (100 MHz) δ_c (D_2O) 7.7, 11.1, 35.3, 46, 59.2, 64.5 (d, J_{PC} = 4.7 Hz), 81.9 (d, J_{PC} = 9.6 Hz), 84, 110.5, 119.2 (d, J_{PC} = 3.5 Hz), 125.9, 126.4, 126.6, 126.8, 127.8, 135.3, 135.9, 140.9, 143.1 (d, J_{PC} = 10.5 Hz), 151.5, 166.2. HRMS *m/z* 472.1258, calcd. for $C_{19}H_{19}N_7O_6P^-$ 472.1140.

3'-Azido-3'-deoxythymidin-5'-yl [*N*-(quinolin-5-yl)]phosphoramidate, $TEAH^+$ salt (7af). Yield 77%. RP HPLC R_f 19.26 min. ^1H NMR (400 MHz) δ_H (D_2O) 1.15 (9H, t, J = 7.3 Hz), 1.51 (3H, s), 2.18 (2H, m), 3.01 (6H, q, J = 7.3 Hz), 3.96 (2H, m), 4.03 (1H, m), 4.15 (1H, m), 5.97 (1H, t, J = 6.6 Hz), 7.22 (1H, s), 7.31 (1H, m), 7.38 (1H, m), 7.43 (2H, m), 8.44 (1H, d J = 8.4 Hz), 8.63 (1H, m). ^{13}C NMR (100 MHz) δ_c (D_2O) 7.7, 10.8, 35.3, 46.1, 59.8, 64.2 (d, J_{PC} = 4.9 Hz), 82.4 (d, J_{PC} = 9 Hz), 84.6, 110.4, 113.6 (d, J_{PC} = 2 Hz), 119.7, 119.9, 120, 129.5, 130.8, 136.6, 137.2, 146.7, 149.3, 150.9, 165.6. HRMS *m/z* 472.1172, calcd. for $C_{19}H_{19}N_7O_6P^-$ 472.1140.

3'-Azido-3'-deoxythymidin-5'-yl [*N*-(quinolin-6-yl)]phosphoramidate, $TEAH^+$ salt (7ag). Yield 73%. RP HPLC R_f 19.14 min. ^1H NMR (400 MHz) δ_H (D_2O) 1.14 (9H, t, J = 7.3 Hz), 1.51 (3H, s), 2.18 (2H, m), 3.01 (6H, q, J = 7.32), 3.94 (2H, m), 4.06 (1H, m), 4.13 (1H, m), 5.98 (1H, t, J = 6.5 Hz), 7.07 (1H, m), 7.23 (1H, dd, J = 4.4, 8.4 Hz), 7.26 (1H, d, J = 2.3 Hz), 7.40 (1H, dd, J = 2.4, 9.1 Hz), 7.70 (1H, d, J = 9.1 Hz), 7.87 (1H, d, J = 8.2 Hz), 8.43 (1H, m). ^{13}C NMR (100 MHz) δ_c (D_2O) 7.7, 10.9, 35.2, 46.2, 59.5, 64 (d, J_{PC} = 5 Hz), 82.3 (d, J_{PC} = 9.9 Hz), 84.6, 110.3, 110.5, 121.2, 123.7 (d, J_{PC} = 9.9 Hz), 126.4, 128, 128.6, 136.5, 136.7, 140.3, 145.7, 150.5, 164.9. HRMS *m/z* 472.1131, calcd. for $C_{19}H_{19}N_7O_6P^-$ 472.1140.

3'-Azido-3'-deoxythymidin-5'-yl [*N*-(quinolin-8-yl)]phosphoramidate, TEAH$^+$ salt (7ah). Yield 69%. RP HPLC R_f 22.35 min. ^{1}H NMR (400 MHz) δ_H (D_2O) 1.28 (6H, t, J = 7.3 Hz), 1.46 (3H, s), 2.37 (2H, m), 3.20 (6H, q, J = 7.3 Hz), 4.06 (1H, m), 4.16 (2H, m), 4.31 (1H, m), 6.03 (1H, t, J = 6.5 Hz), 7.24 (1H, s), 7.37 (1H, m), 7.41 (2H, m), 7.53 (1H, dd, J = 4.3, 8.2 Hz), 8.26 (1H, m), 8.76 (1H, m). ^{13}C NMR (100 MHz) δ_c (D_2O) 7.7, 10.7, 35.2, 46.2, 59.7, 64.5 (d, J_{PC} = 5 Hz), 82.5 (d, J_{PC} = 8.9 Hz), 84.6, 110.3, 110.5, 110.6, 121.2, 123.7 (d, J_{PC} = 9.8 Hz), 126.4, 128, 128.6, 136.5, 136.7, 140.3, 145.7, 150.5. HRMS *m/z* 472.1138, calcd. for $C_{19}H_{19}N_7O_6P^-$ 472.1140.

3'-Azido-3'-deoxythymidin-5'-yl [*N*-(isoquinolin-5-yl)]phosphoramidate, TEAH$^+$ salt (7aj). Yield 69%. RP HPLC R_f 20.07 min. ^{1}H NMR (400 MHz) δ_H (D_2O) 1.26 (6H, t, J = 7.3 Hz), 1.45 (3H, s), 2.34 (2H, m), 3.17 (6H, q, J = 7.3 Hz), 4.03 (1H, m), 4.12 (2H, m), 4.27 (1H, m), 6.00 (1H, t, J = 6.5 Hz), 7.23 (1H, s), 7.44 (2H, m), 7.73 (1H, m), 7.92 (1H, d, J = 6.2 Hz), 8.35 (1H, d, J = 6.2 Hz), 9.04 (1H, s). ^{13}C NMR (100 MHz) δ_c (D_2O) 7.7, 10.7, 35.1, 46.1, 59.8, 64.4 (d, J_{PC} = 4.7 Hz), 82.4 (d, J_{PC} = 9 Hz), 84.6, 110.4, 114.7, 117.4, 120, 127.3, 128. 128.3, 136, 136.6, 139.7, 150.6, 151.3, 165.1. HRMS *m/z* 472.1040, calcd. for $C_{19}H_{19}N_7O_6P^-$ 472.1140.

3'-Azido-3'-deoxythymidin-5'-yl [*N*-(5-cyanopyridin-2-yl)]phosphoramidate, TEAH$^+$ salt (7al). Yield 74%. RP HPLC R_f 18.84 min. ^{1}H NMR (400 MHz) δ_H (D_2O) 1.29 (9H, t, J = 7.3 Hz), 1.85 (3H, d, J = 1 Hz), 2.51 (2H, m), 3.21 (6H, q, J = 7.3 Hz), 4.07 (1H, m), 4.12 (1H, m), 4.18 (1H, m), 4.40 (1H, m), 6.13 (1H, t, J = 6.5 Hz), 7.06 (1H, d J = 8.8 Hz), 7.50 (1H, d, J = 1.2 Hz), 7.85 (1H, dd, J = 2.3, 8.9 Hz), 8.41 (1H, d, J=2.1 Hz). ^{13}C NMR (100 MHz) δ_c (D_2O) 7.7, 11.1, 35.4, 46.2, 59.6 (d, J_{PC} = 5 Hz), 64.4 (d, J_{PC} = 9.3 Hz), 82.3, 84.8, 99.2, 110.7, 117.8, 128, 137.2, 140.4, 150.9, 151.7, 157.1 (d, J_{PC} = 5.Hz), 165.7. HRMS *m/z* 447.0983, calcd. for $C_{16}H_{16}N_8O_6P^-$ 447.0935.

3'-Azido-3'-deoxythymidin-5'-yl [*N*-(6-cyanopyridin-3-yl)]phosphoramidate, TEAH$^+$ salt (7am). Yield 76%. RP HPLC R_f 18.59 min. ^{1}H NMR (400 MHz) δ_H (D_2O) 1.30 (9H, t, J = 7.3 Hz), 1.84 (3H, s), 2.51 (2H, m), 3.22 (6H, q, J = 7.3 Hz), 4.03 (1H, m), 4.14 (2H, m), 4.36 (1H, m), 6.12 (1H, t, J = 6.5 Hz), 7.39 (1H, s), 7.48 (1H, dd, J = 2.6, 8.6 Hz), 7.67 (1H, d, J = 6.5 Hz), 8.27 (1H, d, J = 2.5 Hz). ^{13}C NMR (100 MHz) δ_c (D_2O) 8.2, 11.8, 35.8, 46.6, 64.8 (d, J_{PC} = 4.8 Hz), 82.4 (d, J_{PC} = 9.3 Hz), 85.31, 111.2, 117.8, 121.3, 123.4 (d, J_{PC} = 5.6 Hz), 129.7, 137.3, 139.9 (d, J_{PC} = 8.6 Hz), 143.1, 152.9, 168.2. HRMS *m/z* 447.0845, calcd. for $C_{16}H_{16}N_8O_6P^-$ 447.0935.

3'-Azido-3'-deoxythymidin-5'-yl [*N*-(thiazol-2-yl)]phosphoramidate, TEAH$^+$ salt (7an). Yield 72%. RP HPLC R_f 17.46 min. ^{1}H NMR (400 MHz) δ_H (D_2O) 1.28 (9H, t, J = 7.3 Hz),

1.88 (3H, d, *J* = 0.8 Hz), 2.49 (2H, m), 3.20 (6H, q, *J* = 7.3 Hz), 4.06 (1H, m), 4.13 (2H, m), 4.40 (1H, q, *J* = 4.8 Hz), 6.18 (1H, t, *J* = 6.5 Hz), 6.84 (1H, d, *J* = 3.8 Hz), 7.16 (1H, d, J = 3.8 Hz), 7.63 (1H, d, *J* = 1.1 Hz). ^{13}C NMR (100 MHz) 8.2, 10.8, 11.7, 36.1, 46.6, 60.4, 64.5 (d, J_{PC} = 4.8 Hz), 82.9 (d, J_{PC} = 9.1 Hz), 84.9, 111.2, 125.4, 133.5, 137.3, 151.3, 163.9 (d, J_{PC} = 4.8 Hz), 166.1. HRMS *m/z* 428.0424, calcd. for $C_{13}H_{15}N_7O_6PS^-$ 428.0547.

3'-Azido-3'-deoxythymidin-5'-yl [*N*-(4-methylthiazol-2-yl)]phosphoramidate, TEAH$^+$ salt (7ao). Yield 75%. RP HPLC R_f 18.96 min. ^{1}H NMR (400 MHz) δ_H (D_2O) 1.27 (9H, t, *J* = 7.3 Hz), 1.86 (3H, s), 2.23 (3H, s), 2.47 (H, m), 3.18 (6H, q, *J* = 7.3 Hz), 4.04 (1H, m), 4.11 (H, m), 4.40 (1H, m), 6.14 (1H, t, *J* = 6.6 Hz), 6.77 (1H, d, *J* = 1.1 Hz), 7.60 (1H, d, *J* = 1.1 Hz). ^{13}C NMR (100 MHz) 8.2, 11.6, 36.1, 46.6, 60.3, 64.5 (d, J_{PC} = 4.9 Hz), 82.8 (d, J_{PC} = 9 Hz), 85, 111.2, 111.4, 136.9, 137.3, 151.4, 165.6 (d, *J* = 5.1 Hz), 166.3. HRMS *m/z* 442.0618, calcd. for $C_{14}H_{17}N_7O_6PS^-$ 442.0704.

3'-Azido-3'-deoxythymidin-5'-yl [*N*-(benzo[*d*]thiazol-2-yl)]phosphoramidate, TEAH$^+$ salt (7ap). Yield 69%. RP HPLC R_f 21.78 min. ^{1}H NMR (400 MHz) δ_H (D_2O) 1.27 (9H, t, *J* = 7.3 Hz), 1.75 (3H, s), 2.40 (2H, m), 3.17 (6H, q, *J* = 7.3 Hz), 4.07 (2H, m), 4.17 (1H, m), 4.36 (1H, m), 6.02 (1H, t, *J* = 6.5 Hz), 7.17 (1H, t, *J* = 7.6 Hz), 7.32 (1H, t, *J* = 7.7 Hz), 7.38 (1H, s), 7.49 (1H, d, *J* = 8.1 Hz), 7.64 (1H, d, *J* = 7.9 Hz). ^{13}C NMR (100 MHz) δ_c (D_2O) 7.7, 11.1, 35.5, 46.2, 59.3, 64.4 (d, J_{PC} = 4.9 Hz), 82.1 (d, J_{PC} = 8.4 Hz), 84.4, 110.6, 118.1, 120.7, 122.7, 125.8, 130.3, 136.7, 148.5, 150.7, 164.7, 165.4. HRMS *m/z* 478.0542, calcd. for $C_{17}H_{17}N_7O_6PS^-$ 478.0704.

2',3'-Dideoxyuridin-5'-yl [*N*-(quinolin-3-yl)]phosphoramidate, TEAH$^+$ salt (7bd). Two chromatographic purifications were required. Yield 55%. RP HPLC R_f 17.81 min. ^{1}H NMR (400 MHz) δ_H (D_2O) 1.19 (9H, t, *J* = 7.3 Hz), 1.72 (2H, m), 1.98 (1H, m), 2.24 (1H, m), 3.09 (6H, q, *J* = 7.3 Hz), 3.84 (1H, m), 4.09 (1H, m), 4.26 (1H, m), 5.06 (1H, d, J = 8.0 Hz), 5.83 (1H, dd, *J* = 3.3, 7.3 Hz), 7.07 (1H, d, *J* = 8.1 Hz), 7.42 (2H, m), 7.56 (1H, m), 7.71 (2H, m), 8.50 (1H, d, *J* = 2.6 Hz). ^{13}C NMR (100 MHz) δ_c (D_2O) 7.7, 22.7, 24.4, 30.3, 46, 66 (d, J_{PC} = 5.1 Hz), 79.4 (J_{PC} = 9.5 Hz), 85.2, 100.9, 119.2 (J_{PC} = 4.1 Hz).126, 126.2, 126.7, 126.9, 128, 135.6, 140.4 (J_{PC} = 10.4 Hz), 142.7 (J_{PC} = 10.8 Hz), 150.2, 164.6. HRMS *m/z* 417.0958, calcd. for $C_{18}H_{18}N_4O_6P^-$ 417.0969.

2',3'-Dideoxyuridin-5'-yl [*N*-(quinolin-6-yl)]phosphoramidate, TEAH$^+$ salt (7bg). Two chromatographic purifications were required. Yield 52%. RP HPLC R_f 16.70 min. ^{1}H NMR (400 MHz) δ_H (D_2O) 1.27 (9H, t, *J* = 7.3 Hz), 1.80 (1H, m), 1.95 (1H, m), 2.09 (1H, m), 2.36 (1H, m), 3.19 (6H, q, *J* = 7.3 Hz), 3.91 (1H, m), 4.14 (1H, m), 4.37 (1H, m), 5.11 (1H, m),

5.12 (1H, d, *J* = 8.0 Hz), 5.96 (1H, dd, *J* = 3.3, 7.4 Hz), 7.23 (1H, d, *J* = 8.0 Hz), 7.38 (1H, d, *J* = 2.5 Hz), 7.48 (2H, m), 7.82 (1H, d, *J* = 9.1 Hz), 8.18 (1H, d, *J* = 8.2 Hz), 8.57 (1H, d, *J* = 3.0 Hz). ^{13}C NMR (100 MHz) δ_c (D_2O) 7.7, 24.5, 30.1, 46.1, 66.1 (d, J_{PC} = 5.2 Hz), 79.6 (d, J_{PC} = 9.8 Hz), 85.6, 100.8, 110.4 (d, J_{PC} = 4.9 Hz), 121.1, 123.7 (d, J_{PC} = 9.7 Hz), 126.2, 128.8, 136.7, 139.9, 140.5, 140.9, 145.4, 150.5, 164.82. HRMS *m/z* 417.0636, calcd. for $C_{18}H_{18}N_4O_6P^-$ 417.0969.

2',3'-Dideoxyuridin-5'-yl [*N*-(5-cyanopyridin-2-yl)]phosphoramidate, TEAH$^+$ salt (7bl). Yield 67%. RP HPLC R_f 15.78 min. ^{1}H NMR (400 MHz) δ_H (D_2O) 1.30 (9H, t, *J* = 7.3 Hz), 1.92 (1H, m), 2.12 (2H, m), 2.45 (1H, m), 3.22 (6H, q, *J* = 7.33Hz), 3.96 (1H, m), 4.16 (1H, m), 4.35 (1H, m), 5.68 (1H, d, *J* = 8.1 Hz), 6.04 (1H, dd, *J* = 3.2, 7.3 Hz), 7.09 (1H, d, *J* = 8.8 Hz), 7.70 (1H, d, *J* = 8.1 Hz), 7.87 (1H, dd, *J* = 2.3, 8.9 Hz), 8.43 (1H, d, *J* = 2.0 Hz). ^{13}C NMR (100 MHz) δ_c (D_2O) 7.7, 24.4, 30.2, 46.2, 65.9 (d, J_{PC} = 5.2 Hz), 79.7 (d, J_{PC} = 9.2 Hz), 85.9, 99.2, 101.1, 110.5, 117.8, 140.6, 141.7, 150.9, 151.8, 157.1, 165.5. HRMS *m/z* 392.0699, calcd. for $C_{15}H_{15}N_5O_6P^-$ 392.0765.

2',3'-Dideoxyuridin-5'-yl [*N*-(4-methylthiazol-2-yl)]phosphoramidate, TEAH$^+$ salt (7bo). Two chromatographic purifications were required. Yield 53%. RP HPLC R_f 15.90 min. ^{1}H NMR (400 MHz) δ_H (D_2O) 1.27 (9H, t, *J* = 7.3 Hz), 1.94 (1H, m), 2.08 (2H, m), 2.25 (3H, d, *J* = 1 Hz), 2.42 (1H, m), 3.18 (6H, q, *J* = 7.3 Hz), 3.95 (1H, m), 4.13 (1H, m), 4.33 (1H, m), 5.71 (1H, d, *J* = 8.1 Hz), 6.04 (1H, dd, *J* = 3.3, 7.1 Hz), 6.80 (1H, d, *J* = 1 Hz), 7.80 (1H, d, *J* = 8.1 Hz). ^{13}C NMR (100 MHz) δ_c (D_2O) 7.7, 10.3, 24.3, 30.5, 46.1, 65.5 (d, J_{PC} = 5.3 Hz), 79.8 (d, J_{PC} = 9.1 Hz), 85.9 (d, J_{PC} = 12.9 Hz), 101.1, 125, 132, 141.6, 151, 163.9 (d, J_{PC} = 4.9 Hz), 165.7. HRMS *m/z* 387.0485, calcd. for $C_{13}H_{16}N_4O_6PS^-$ 387.0533.

2',3'-Didehydro-3'-deoxythymidin-5'-yl [*N*-(quinolin-3-yl)]phosphoramidate, TEAH$^+$ salt (7cd). Yield 67%. RP HPLC R_f 16.46 min. ^{1}H NMR (400 MHz) δ_H (D_2O) 1.25 (9H, t, *J* = 7.3 Hz), 1.39 (3H, s), 3.16 (6H, q, *J* = 7.3 Hz), 3.77 (1H, m), 4.19 (1H, d, *J* = 11.1 Hz), 5.16 (1H, d, *J* = 7.7 Hz), 5.71 (1H, d, *J* = 5.8 Hz), 6.37 (1H, d, *J* = 6.1 Hz), 6.77 (1H, m), 7.55 (2H, m), 7.66 (1H, m), 7.68 (1H, m), 7.79 (1H, d, *J* = 8.3 Hz), 8.46 (1H, d, *J* = 2.6 Hz). ^{13}C NMR (100 MHz) δ_c (D_2O) 7.7, 10.5, 46.1, 66.2 (d, J_{PC} = 5 Hz), 85 (d, J_{PC} = 11.1 Hz), 89.3, 110, 119 (J_{PC} = 3.3 Hz), 125.2, 126, 126.2, 126.9, 127, 127.7, 132.6, 135.2, 136, 140.4, 142.5 (d, J_{PC} = 11.2 Hz), 150.7, 164.3. HRMS *m/z* 429.0933, calcd. for $C_{19}H_{18}N_4O_6P^-$ 429.0969.

2',3'-Didehydro-3'-deoxythymidin-5'-yl [*N*-(quinolin-6-yl)]phosphoramidate, TEAH$^+$ salt (7cg). RP HPLC R_f 15.78 min. Yield 97%. ^{1}H NMR (400 MHz) δ_H (D_2O) 1.17 (9H, t, *J* = 7.3 Hz), 1.36 (3H, s), 3.06 (6H, t, *J* = 7.3 Hz), 3.74 (1H, m), 4.10 (1H, m), 5.05 (1H, m), 5.65 (1H, m), 6.63 (1H, m), 6.84 (1H, d, *J* = 1 Hz), 7.15 (1H, d, *J* = 2.4 Hz), 7.32 (2H, m), 7.61

(1H, d, *J* = 9.8 Hz), 7.98 (1H, d, *J*=8.1 Hz), 8.47 (1H, m). ^{13}C NMR (100 MHz) δ_c (D_2O) 7.7, 10.7, 46, 65.8 (d, J_{PC} = 4.9 Hz), 85.1 (d, J_{PC} = 10.8 Hz), 89.3, 109.7, 110 (d, J_{PC} = 4.9 Hz), 121.1, 123.2 (d, J_{PC} = 9.4 Hz), 124.8, 126.2, 128.4, 133.1, 136.1, 136.3, 139.8, 140.3, 145.5, 150.6, 164.3. HRMS *m/z* 429.0838, calcd. for $C_{19}H_{18}N_4O_6P^-$ 429.0969.

2',3'-Didehydro-3'-deoxythymidin-5'-yl [*N*-(5-cyanopyridin-2-yl)]phosphoramidate, TEAH$^+$ salt (7cl). Two chromatographic purifications were required. Yield 63%. RP HPLC R_f 15.47 min. ^{1}H NMR (400 MHz) δ_H (D_2O) 1.28 (9H, t, *J* = 7.3 Hz), 1.73 (3H, d, J = 0.8 Hz), 3.20 (6H, t, *J* = 7.3 Hz), 3.87 (1H, m), 4.15 (1H, m), 5.09 (1H, m), 5.89 (1H, m), 6.44 (1H, m), 6.81 (1H, m), 6.98 (1H, d, *J* = 8.9 Hz), 7.30 (1H, d, *J* = 1.1 Hz), 7.79 (1H, d, *J* = 8.8 Hz), 8.35 (1H, d, *J* = 2.1 Hz). ^{13}C NMR (100 MHz) δ_c (D_2O) 7.8, 10.9, 46.1, 65.9 (d, J_{PC} = 5.2 Hz), 85 (d, J_{PC} = 10.2 Hz), 89.6, 99.2, 110.2, 110.4, 117.7, 124.7, 133.5, 137.2, 140.1, 151.3, 151.6, 157 (d, J_{PC} = 5.7 Hz), 165.3. HRMS *m/z* 404.0794, calcd. for $C_{16}H_{15}N_5O_6P^-$ 404.0765.

2',3'-Didehydro-3'-deoxythymidin-5'-yl [*N*-(4-methylthiazol-2-yl)]phosphoramidate, TEAH$^+$ salt (7co). Two chromatographic purifications were required. Yield 61%. RP HPLC R_f 16.01 min. ^{1}H NMR (400 MHz) δ_H (D_2O) 1.29 (9H, t, *J* = 7.3 Hz), 1.79 (3H, d, *J* = 0.8 Hz), 2.25 (3H, d, *J* = 1.1 Hz), 3.21 (6H, q, *J* = 7.3 Hz), 3.88 (1H, m), 4.10 (1H, m), 5.11 (1H, s), 5.93 (1H, m), 6.47 (1H, m), 6.73 (1H, s), 6.88 (1H, m), 7.39 (1H, d, *J* = 1.1 Hz). ^{13}C NMR (100 MHz) δ_c (D_2O) 7.7, 10.3, 10.9, 46.2, 65 (d, J_{PC} = 4.9 Hz), 85 (d, J_{PC} = 10.2 Hz), 89.7, 110.3, 124.5, 124.8, 132, 133.7, 137.5, 151.7, 163.3, 165.9. HRMS *m/z* 399.0532, calcd. for $C_{14}H_{16}N_4O_6PS^-$ 399.0533.

(1*S*,*cis*)-4-[2-Amino-6-(cyclopropylamino)-9*H*-purin-9-yl]-2-cyclopentene-1-methyl [*N*-(5-cyanopyridin-2-yl)]phosphoramidate, TEAH$^+$ salt (7dl). Two chromatographic purifications were required. Yield 35%. RP HPLC R_f 19.39 min. ^{1}H NMR (400 MHz) δ_H (D_2O) 0.69 (2H, m), 0.99 (2H, m), 1.27 (9H, t, *J* = 7.3 Hz), 1.93 (2H, m), 2.50 (1H, m), 2.88 (1H, m), 3.10 (1H, m), 3.19 (6H, q, *J* = 7.3 Hz), 3.57 (1H, m), 3.97 (1H, m), 5.10 (1H, m), 5.88 (1H, m), 6.19 (1H, m), 6.71 (1H, d, *J* = 8.8 Hz), 7.47 (1H, dd, *J* = 2.2, 8.8 Hz), 7.50 (1H, s), 8.12 (1H, d, *J* = 1.98Hz). ^{13}C NMR (100 MHz) δ_c (D_2O) 6.3, 7.7, 22.8, 33.4, 44.8 (d, J_{PC} = 4.0 Hz), 46.1, 58.4, 67.1 (d, J_{PC} = 21.6 Hz), 98.5, 109.9, 112.6, 117.6, 128.3, 129.2, 136.5, 138, 139.9, 151.3, 154.8, 156.6, 158.7. HRMS *m/z* 466.1461, calcd. for $C_{20}H_{21}N_3O_3P^-$ 466.1510.

(1*S*,*cis*)-4-[2-Amino-6-(cyclopropylamino)-9*H*-purin-9-yl]-2-cyclopentene-1-methyl [*N*-(4-methylthiazol-2-yl)]phosphoramidate, TEAH$^+$ salt (7do). Two chromatographic purifications were required. Yield 51%. RP HPLC R_f 19.94 min. ^{1}H NMR (400 MHz) δ_H (D_2O) 0.66 (2H, m), 0.89 (2H, m), 1.26 (9H, t, *J* = 7.3 Hz), 1.42 (1H, m), 1.97 (3H, d, *J* = 1.0

Hz), 2.65 (1H, m), 2.81 (1H, s), 3.09 (1H, s), 3.18 (6H, q, *J* = 7.3 Hz), 3.66 (1H, m), 3.90 (1H, m), 5.17 (1H, m), 5.88 (1H, m), 6.17 (1H, m), 6.44 (1H, s), 7.57 (1H, s). ^{13}C NMR (100 MHz) δ_c (D_2O) 6.4, 7.7, 10.1, 22.6, 33.4, 45. (d, J_{PC} = 9.6 Hz), 46.1, 58.7, 66.8 (d, J_{PC} = 5.3 Hz), 122.6, 124.5, 128.3, 132.1, 136.6, 138, 154.8, 158.5, 163. HRMS *m/z* 461.1461, calcd. for $C_{18}H_{22}N_8O_3PS^-$ 461.1278.

α-L-2',3'-Dideoxy-3'-thiacytadin-5'-yl [*N*-(4-methylthiazol-2-yl)]phosphoramidate, TEAH$^+$ salt (7eo). Yield 69%. RP HPLC R_f 12.03 min. ^{1}H NMR (400 MHz) δ_H (D_2O) 1.27 (9H, t, *J* = 7.3 Hz), 2.22 (3H, d, *J* = 0.8 Hz), 3.12 (1H, m), 3.17 (6H, q, *J* = 7.3 Hz), 3.49 (1H, dd, *J* = 5.6, 12.2 Hz), 4.11 (1H, m), 4.24 (1H, m), 5.38 (1H, q, *J* = 7.6 Hz), 5.92 (1H, d, *J* = 7.6 Hz), 6.25 (1H, t, *J* = 5.0 Hz), 6.77 (1H, s), 7.84 (1H, d, *J* = 7.6 Hz). ^{13}C NMR (100 MHz) δ_c (D_2O) 7.7, 10.4, 36.2, 46.1, 65.3 (d, J_{PC} = 5.1 Hz), 83.4 (d, J_{PC} = 8.9 Hz), 86.7, 95.4, 125, 132, 141.1, 156.3, 163.9 (d, J_{PC} = 4.9 Hz), 165.3. HRMS *m/z* 404.0546, calcd. for $C_{12}H_{15}N_5O_5PS2^-$ 404.0257.

Method B

Phosphoramidates containing amines of pK_a > 7 were prepared by the tristriazolide approach as it was described previously [5]. The only difference was solubility of di(triazoyl)(*N*-heteroaryl)phosphoramidates of type **11** in pyridine: derivative **11k** was soluble at r.t., **11i** required heating at 75 °C for ca. 10, while **11e** dissolved only partly and was used as a suspension. ^{31}P NMR data of intermediates **11** and AZT (triazoyl)(*N*-heteroaryl)phosphoramidates **12** are listed in Table 3. Purification of the final products **7** was done as in Method A.

3'-Azido-3'-deoxythymidin-5'-yl [*N*-(quinolin-2-yl)]phosphoramidate, TEAH$^+$ salt (7ak). Yield 43%. RP HPLC R_f 22.40 min. ^{1}H NMR (400 MHz) δ_H (D_2O) 1.26 (9H, t, *J* = 7.3 Hz), 1.60 (3H, s), 2.33 (2H, t, *J* = 6.7 Hz), 3.17 (6H, k, *J* = 7.3 Hz), 4.03 (1H, m), 4.09 (1H, m), 4.20 (1H, m), 4.27 (1H, m), 5.92 (1H, t, *J* = 6.5 Hz), 7.14 (1H, s), 7.21 (1H, d, *J* = 9.1 Hz), 7.36 (1H, t, *J* = 7.4 Hz), 7.54 (1H, t, *J* = 7.4 Hz), 7.60 (1H, t, *J* = 7.7 Hz), 7.66 (1H, d, *J* = 8.0 Hz), 8.03 (1H, d, *J* = 9.1 Hz). ^{13}C NMR (100 MHz) δ_c (D_2O) 7.7, 11, 35.3, 46.2, 59.2, 64,8 (d, J_{PC} = 5.1 Hz), 82 (d, J_{PC} = 8.0 Hz), 84.2, 110.6, 112.7, 122.3, 122.8, 124.5, 127.5, 130.7, 136.4, 139.7, 150.3, 153,1, 164.9, 180.8. HRMS *m/z* 472.1062, calcd. for $C_{19}H_{19}N_7O_6P^-$ 472.1140.

3'-Azido-3'-deoxythymidin-5'-yl [*N*-(isoquinolin-1-yl)]phosphoramidate, TEAH$^+$ salt (7ai). Yield 61%. RP HPLC R_f 21.94 min. ^{1}H NMR (400 MHz) δ_H (D_2O) 1.27 (9H, t, *J* = 7.3

Hz), 1.50 (3H, s), 2.39 (2H, m), 3.18 (6H, q, *J* = 7.3 Hz), 4.10 (1H, m), 4.14 (1H, m), 4.24 (1H, m), 4.33 (1H, m), 5.94 (1H, t, *J* = 6.5 Hz), 7.17 (1H, d, *J* = 6 Hz), 7.25 (1H, s), 7.41 (1H, s), 7.56 (1H, m), 7.70 (2H, m), 7.84 (1H, d, *J* = 6.6 Hz), 8.03 (1H, d, *J* = 8.4 Hz). ^{13}C NMR (100 MHz) δ_c (D_2O) 8.1, 11.2, 35.8, 46.5, 60.1, 65 (d, *J* = 4.5 Hz), 82.8 (d, *J* = 7.5 Hz), 84.9, 110.8, 113.7, 118.6, 122.5, 126.9, 130.7, 136.8, 137, 138.6, 151.2, 152.4, 166. HRMS *m/z* 472.1144, calcd. for $C_{19}H_{19}N_7O_6P^-$ 472.1140.

3'-Azido-3'-deoxythymidin-5'-yl bis[*N*-(pyrid-4-yl)]phosphordiamidate, TEAH$^+$ salt (9aa). The compound was formed as a side product during attempted preparation of **7aa** by Method A and was isolated chromatographically from the reaction mixture. Yield ca. 10%. ^{1}H NMR (400 MHz) δ_H (D_2O) 1.71 (3H, s), 2.50 (2H, m), 4.16 (1H, m), 4.30 (1H, m), 4.39 (1H, m), 4.44 (1H, m), 6.18 (1H, t, *J* = 6.8 Hz), 6.99 (2H, dd, *J* = 1.6, 5.2 Hz), 7.01 (2H, dd, *J* = 1.6, 5.0 Hz), 7.28 (1H, d, *J* = 0.8 Hz), 8.13 (2H, d, *J* = 6.4 Hz), 8.15 (2H, d, *J* = 5.6 Hz). ^{13}C NMR (100 MHz) δ_c (D_2O) 11.6, 35.2, 59.1, 64.5, 81.3, 84.8, 110.9, 112.9, 119.7, 124.0, 129.3, 136.3, 147.1, 147.4. HRMS (positive mode) *m/z* 500.1514, calcd. for $C_{20}H_{23}N_9O_5P^+$ 500.1554.

Determination of octanol-water partition coefficient

Partition coefficients *P* were determined using micro shake-flask partitioning approach [22]. 0.01 M triethylammonium acetate buffer pH 7.4 and n-octanol stock solutions were initially saturated one with another, separated, and left for 48 h. 20 μL of 40 mM aqueous solution of a sample (ca. 2 mg/100 μL) was diluted with 480 μL of the buffer and shaken vigorously with 0.5 mL of the added octanol at 22 °C for 24 h. The mixture was centrifuged and a half of each layer was carefully pipetted out. From these, 15 μL aliquots were injected for HPLC and the ratio of concentrations in each phase was determined according to the area of appropriate peaks. For compounds of the lowest lipophilicity, a measured volume of the octanol layer was evaporated to dryness, the residue dissolved in 1/10 volume of water, and used for HPLC analysis.

TABLE 3

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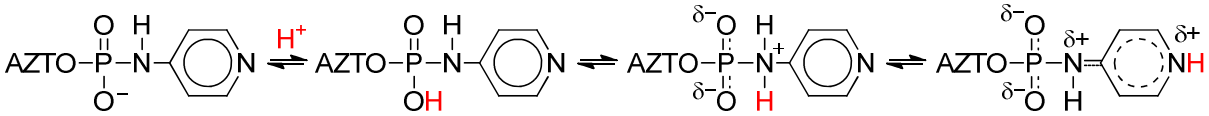
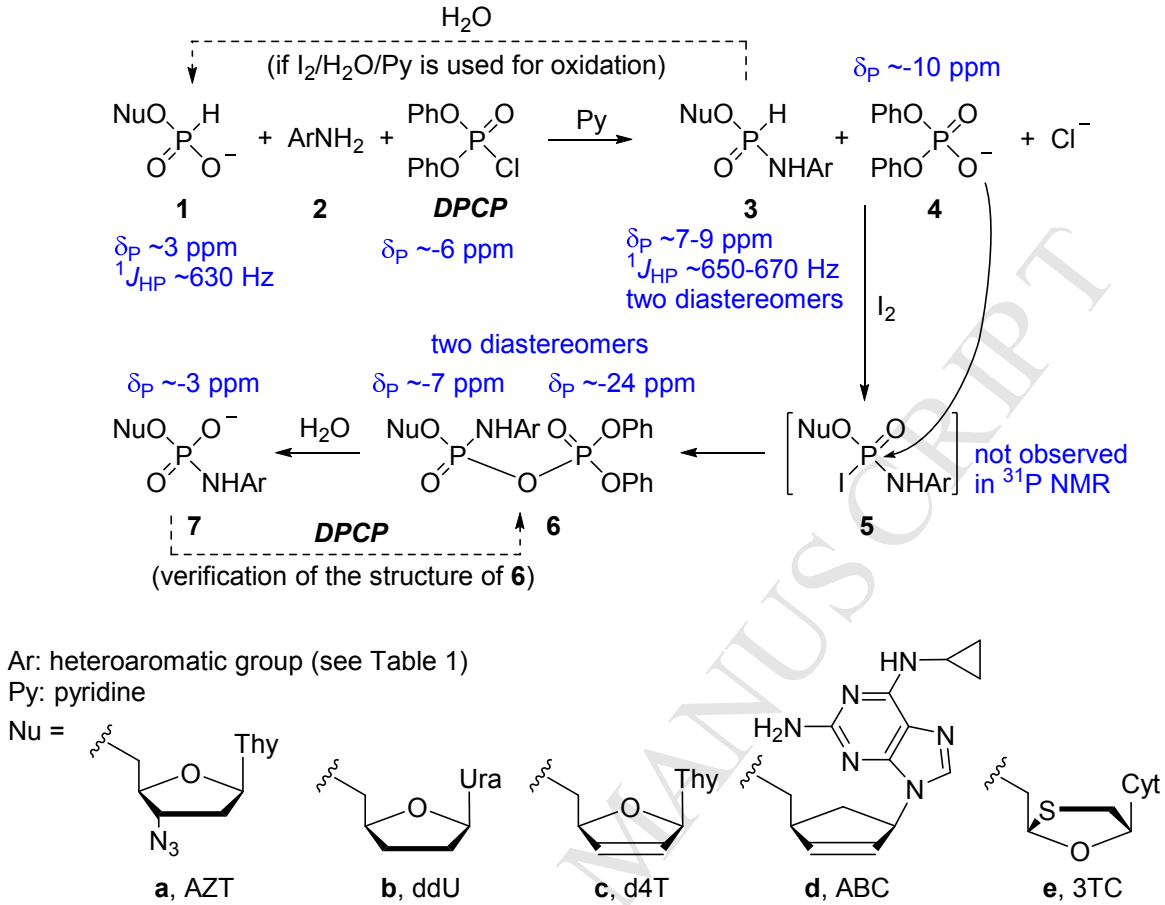


Figure 1. Possible protonation sites in AZT *N*-(pyrid-4-yl)phosphoramidic acid.



Ar: heteroaromatic group (see Table 1)
Py: pyridine
Nu =

a, AZT **b**, ddU **c**, d4T **d**, ABC **e**, 3TC

Figure 2. Synthesis of (*N*-heteroaryl)phosphoramidates **7**. Method A

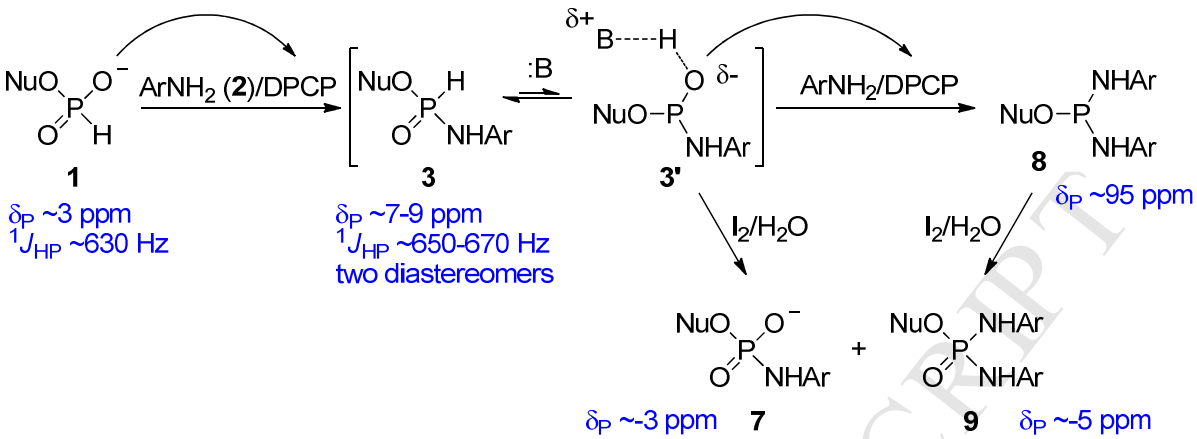


Figure 3. A putative mechanism for the formation of nucleoside di(*N*-heteroaryl)phosphordiamidates **9** for amines of pK_a > 7 (see Table 1)

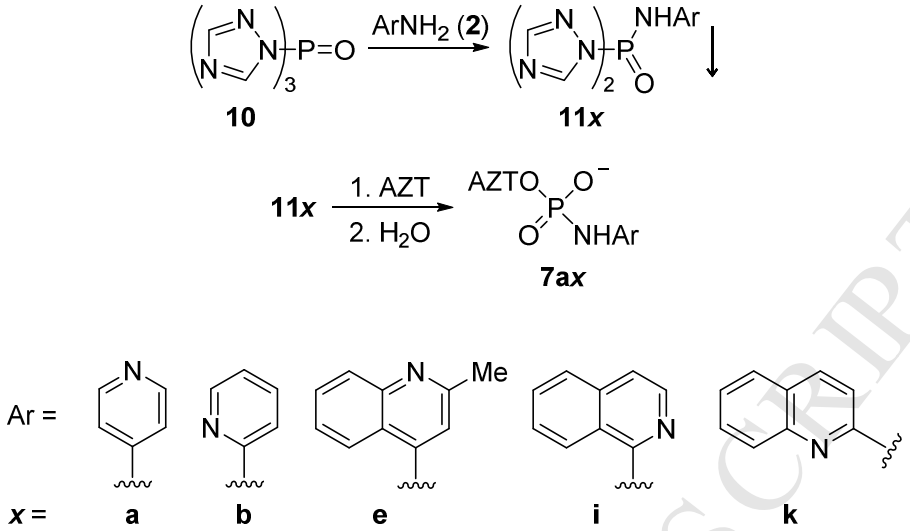


Figure 4. Synthesis of AZT (*N*-heteroaryl)phosphoramidates **7a*x***. Method B

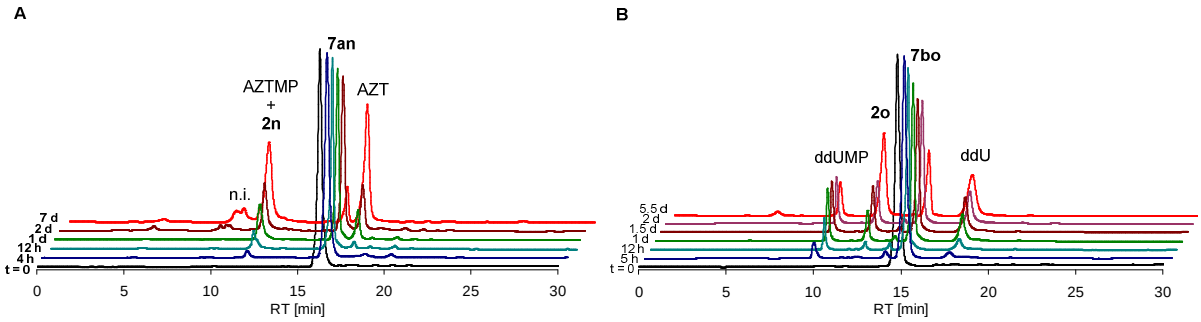


Figure 5. Exemplary HPLC traces of phosphoramidates of type **7** incubated with FBS in RPMI buffer, 37 °C. A, phosphoramidate **7an** (AZT – AT); B, phosphoramidate **7bo** (ddU – AMT); n.i. = not identified

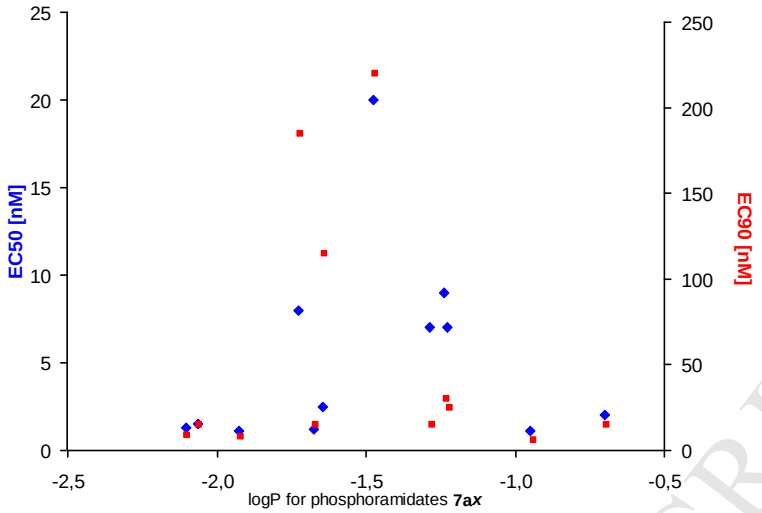
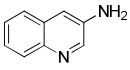
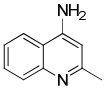
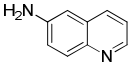
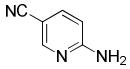
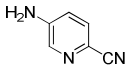
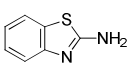


Figure 6. Plot of log *P*'s of phosphoramidates of type **7ax** vs. EC_{50} (diamonds) & EC_{90} (squares) values

Table 1. Properties of heteroaromatic amines **2** and AZT (*N*-heteroaryl)phosphoramidates of type **7a**

Entry	#	Structure	pK_a (aq.)	log P[a]	CC_{50}[b] [μM]	CC_{90}[b] [μM]	#	Method (yield)	Calc. Clog P[c]	Exp. log P	$t_{1/2}$[d]	% of **7** after 3 d[d]	EC_{50}[e] [nM]	EC_{90}[e] [nM]	C_{max}[f] [μM]	Viability at C_{max}
1	–	–	–	–	–	–	AZT	–[g]	0.04	0.06	–	–	10	>500	300	100%
2	**2a** 4APy		9.2 [16]	0.3	>1060	>1060	**7aa**	B [5] (59%)	-0.11	-0.94	>5 d [5]	n.d.	1.1 [5]	6 [5]	200 [5]	100% [5]
3	**2b** 2APy		6.8 [16]	0.5	>1060	>1060	**7ab**	A [5] (84%)	-0.11	n.d.	5 d [5]	n.d.	2.4 [5]	29 [5]	200 [5]	100% [5]
4	**2c** 3APy		6.0 [16]	0.2	>1060	>1060	**7ac**	A [5] (92%)	-0.11	n.d.	3 d [5]	n.d.	2.3 [5]	9 [5]	200 [5]	100% [5]
5	**2d** 3AQ		5.0 [17]	1.6	1000	1400	**7ad**	A (78%)	1.27	-1.72	>7 d	78%	8	185	435	100%
6	**2e** 4A2MQ		>9.6[h]	~2[c]	n.d.	n.d.	**7ae**	B (20%)	1.77	n.d.	n.d.	n.d.	7.5	50	200	n.d.
7	**2f** 5AQ		5.5 [17]	1.2	115	160	**7af**	A (77%)	1.27	-1.23	>7 d	77%	9	30	571	100%
8	**2g** 6AQ		5.6 [17]	1.3	270	350	**7ag**	A (73%)	1.27	-1.64	>7 d	99%	2.5	115	454	100%
9	**2h** 8AQ		4.0 [17]	1.8	250	500	**7ah**	A (69%)	1.27	-1.47	7 d	73%	20	220	487	100%
10	**2i** 1AIQ		7.6 [17]	1.9	210	350	**7ai**	B (61%)	1.06	-0.69	>7 d	86%	2.0	15	218	100%
11	**2j** 5AIQ		5.6 [18]	~0.9[c]	350	1050	**7aj**	A (69%)	1.06	-1.28	>7 d	97%	7	15	356	100%
12	**2l** 6A3CNPy		~3[i]	~0.6[c]	1675	>1830	**7al**	A (74%)	-0.48	-1.92	>7 d	68%	1.2	15	418	100%
13	**2m** 5A2CNPy		~0.5[i]	~0.4[c]	>1670	>1670	**7am**	A (76%)	-0.28	-1.67	4 d	40%	1.1	8	364	100%
14	**2n** AT		5.4 [17]	0.4	1890	>1890	**7an**	A (72%)	-0.27	-2.10	2.5 d	45%	1.3	9	377	70%
15	**2o** AMT		5.6 [19]	~1.7[c]	600	>1750	**7ao**	A (75%)	0.23	-2.06	7 d	65%	1.5	15	1100	100%
16	**2p** ABT		4.5 [17]	~2.4[c]	500	1000	**7ap**	A (69%)	1.32	-1.22	24 h	23%	7	25	219	100%

[a]If not otherwise stated, the log P's are experimental values, included in reports generated by ChemBioDraw v. 13. [b]Concentration required to reduce the viability of mock-infected CEM-T4 cells by 50% or 90%, respectively, as determined by the MTT method. [c]Values calculated by ChemBioDraw v. 13. [d]In RPMI/FBS 9:1 (v/v), 37 ºC, estimated from integration of HPLC peaks. [e]Concentration required to achieve 50% and 90% (respectively) protection from virus-induced cytopathogenicity. [f]Maximal concentration used in the assay. [g]The parent nucleoside. [h]Estimated; for 4-aminoquinoline pKa = 9.6 [20]. [i]Values estimated according to the changes in basicity of pyridine (pK_a 5.2) induced separately by the cyano and amino groups in *ortho* and *meta* positions (ΔpK_a: *o*CN, -5.5; *m*CN, -3.8; *o*NH_2, +1.5; *m*NH_2, +0.8) [16]

Table 2. Properties of (*N*-heteroaryl)phosphoramidates of ddU (**7b**), d4T (**7c**), ABC (**7d**), and 3TC (**7e**)

Entry	#	Nucleoside	Amine component	Yield[a]	Exp. log *P*	$t_{1/2}$[b]	% of **7** after 3 d[b]	EC_{50} [μM]	CC_{50} [μM]	SI_{50}	EC_{90} [μM]	CC_{90} [μM]	SI_{90}
17	–[c]		–	–	-0.96	–	–	>10 [5]	–	–	–	–	–
18	**7bd**		**2d** (3AQ)	55%[d]	-2.62	1.5 d	36%	4.2	50	2.5	>10	>200	–
19	**7bg**	ddU	**2g** (6AQ)	52%[d]	-2.35	6 d	74%	>10	>>200	–	–	>>200	–
20	**7bl**		**2l** (6A3CNPy)	67%	-1.99	3.5 d	53%	>10	>>200	–	–	>>200	–
21	**7bo**		**2o** (AMT)	53%[d]	-1.71	1.5 d	40%	>10	>>200	–	–	>>200	–
22	–[c]		–	–	-0.79	–	–	0.7	170	243	3.7	360	97
23	**7cd**		**2d** (3AQ)	67%	-3.36	n.o.[e]	100%	2.5	>>200	>160	>10	>>200	–
24	**7cg**	d4T	**2g** (6AQ)	97%	-2.61	n.o.[e]	100%	>10	>>200	–	–	>>200	–
25	**7cl**		**2l** (6A3CNPy)	61%[d]	-1.99	>> 7 d	88%	6.2	>>200	>65	>10	>>200	–
26	**7co**		**2o** (AMT)	63%[d]	-2.39	>7 d	72%	1.2	>>200	>300	9.2	>>200	>>40
27	–[c]		–	–	1.16	–	–	6	>200	38	>10	>>200	–
28	**7dl**	ABC	**2l** (6A3CNPy)	35%[d]	-1.94	n.o.[e]	100%	8.5	>200	>23	>10	>>200	–
29	**7do**		**2o** (AMT)	51%[d]	-1.62	n.o.[e]	100%	>10	>200	–	–	>>200	–
30	–[c]	3TC	–	–	-0.94	–	–	0.035	>>200	>11,400	0.2	>>200	>>1,000
31	**7eo**		**2o** (AMT)	69%	-2.27	n.o.[e]	100%	6.85	>200	>29	>10	>>200	–

[a]Method A. [b]In RPMI/FBS 9:1 (v/v), 37 °C, estimated from integration of HPLC peaks. [c]The parent nucleoside. [d]Yield after two chromatographic purifications. [e]No observable decomposition within 7 days.

Table 3. ^{31}P NMR data of new intermediates and products (in DCM if not otherwise stated)

Cpd.	δ_P [ppm]	J_{HP} [Hz]	Cpd.	δ_P [ppm]	J_{HP} [Hz]
	Intermediates			Products	
1c	3.05	611.0, 7.1 (dt)			
1d	3.63	610.4, 7.3 (dt)			
1e	4.11	626.2, 8.5 (dt)			
3ad[a]	7.57; 8.09	651.1, 7.6 (dq); 652.4, 7.6 (dq)	**7ad**	-1.58	6.8 (q)
11e	-16.73	–[b]	**7ae**[d]	-4.99	6.4 (t)
3af[a]	9.00; 9.55	656.6, 8.2 (dt); 653.9, 9.2 (dt)	**7af**	-0.40	5.5 (q)
3ag[a]	7.38; 7.85	653.9, 7.9 (dq); 651.1, 8.6 (dq)	**7ag**	-1.99	5.9 (q)
3ah[a]	7.52; 8.12	667.6, 8.9 (dq); 666.7, 9.2 (dq)	**7ah**[e]	-0.39	–[b]
11i	-14.99	–[b]	**7ai**[e]	-4.20	–[b]
3aj[a]	8.62; 9.28	653.5, 8.2 (dt); 651.7, 8.7 (dt)	**7aj**	-1.49	6.4 (q)
11k	-14.7	–[b]	**7ak**	-3.09	5.5 (t)
3al[a]	6.23; 6.98	680.9, 7.3 (dt); 676.4, 8.7 (dt)	**7al**[e]	-3.49	5.5 (q)
3am[a]	6.54; 6.88	667.2, 8.7 (dq); 663.6, 8.2 (dq)	**7am**	-4.49	6.4 (q)
3an[a]	9.27; 9.62	652.7, 7.7 (dt); 649.9, 7.7 (dt)	**7an**	-4.07	6.2 (t)
3ao[a]	9.73; 9.95	646.3, 7.7 (dt); 642.5, 7.7 (dt)	**7ao**	-3.99	5.4 (t)
3ap[a]	9.65; 9.98	650.7, 7.7 (dt); 648.9, 7.7 (dt)	**7ap**[e]	-3.29	–[b]
3bd[a]	7.12; 7.57	660.7, –[b] (dm); 660.7, –[b] (dm)	**7bd**	-1.82	7.3 (q)
3bg[a]	6.75; 7.19	658.1, 8.2 (dt); 656.2, 7.8 (dt)	**7bg**	-1.87	5.5 (q)
3bl[a]	6.14; 6.60	682.8, 8.2 (dt); 680.9, 9.1 (dt)	**7bl**	-4.97	6.1 (q)
3bo[a]	9.43[c]	641.7, 7.7 (dt)[c]	**7bo**	-3.82	4.9 (q)
3cd[a]	7.12; 7.18	658.5, 6.9 (dt)[c]	**7cd**	-1.85	7.3 (q)
3cg[a]	6.84[c]	656.2, 7.3 (dt)[c]	**7cg**	-1.71	7.7 (q)
3cl[a]	9.51; 9.55	638.3, 7.0 (dt)[c]	**7cl**[e]	-2.93	–[b]
3co[a]	9.43[c]	640.1, 8.2 (dt)[c]	**7co**	-3.92	–[b]
3dl[a]	5.83; 6.05	680.0, 7.3 (dq); 681.2, –[b] (dm)	**7dl**[e]	-2.92	3.7 (t)
3do[a]	9.02; 9.09[d]	649.4, 9.8 (dq)[c]	**7do**	-3.90	3.7 (t)
3eo	10.10; 10.30[d]	628.3, –[b] (dm); 633.2, –[b] (dm)	**7eo**[f]	-2.74	6.4 (t)
12k[a,d]	-3.39; -3.54	–[b]	**8aa**	95.0	–[b]
12e[a,d]	-1.15; -1.36	–[b]	**9aa**	-5.1	–[b]
12i[a,d]	-2.75; -2.97	–[b]			

[a]Two diastereomers. [b]Unresolved. [c]Overlapped diastereomers. [d]In Py. [e]In H_2O. [f]In MeOH.

Highlights

- New 2',3'-dideoxynucleoside (*N*-heteroaryl)phosphoramidates were synthesized
- Reaction pathways were studied in detail using ^{31}P NMR correlation analysis
- New compounds are stable, water-soluble, and not toxic for CEM-T4 cells
- New AZT phosphoramidates are highly active against HIV-1 (EC_{50} = 2–20 nM)
- Some of (*N*-heteroaryl)phosphoramidates are supposed to be substrates for polymerases

Supplementary material – ^{1}H, ^{13}C, ^{31}P NMR spectra, and RP HPLC chromatograms

Aryl *H*-Phosphonates 18. Synthesis, properties, and biological activity of 2',3'-dideoxynucleoside (*N*-heteroaryl)phosphoramidates of increased lipophilicity

Krystian Kolodziej,[a] Joanna Romanowska,[a] Jacek Stawinski,[a] Jerzy Boryski,[a] Aleksandra Dabrowska,[a] Andrzej Lipniacki,[b] Andrzej Piasek,[b] Adam Kraszewski,[a*] and Michal Sobkowski[a*]

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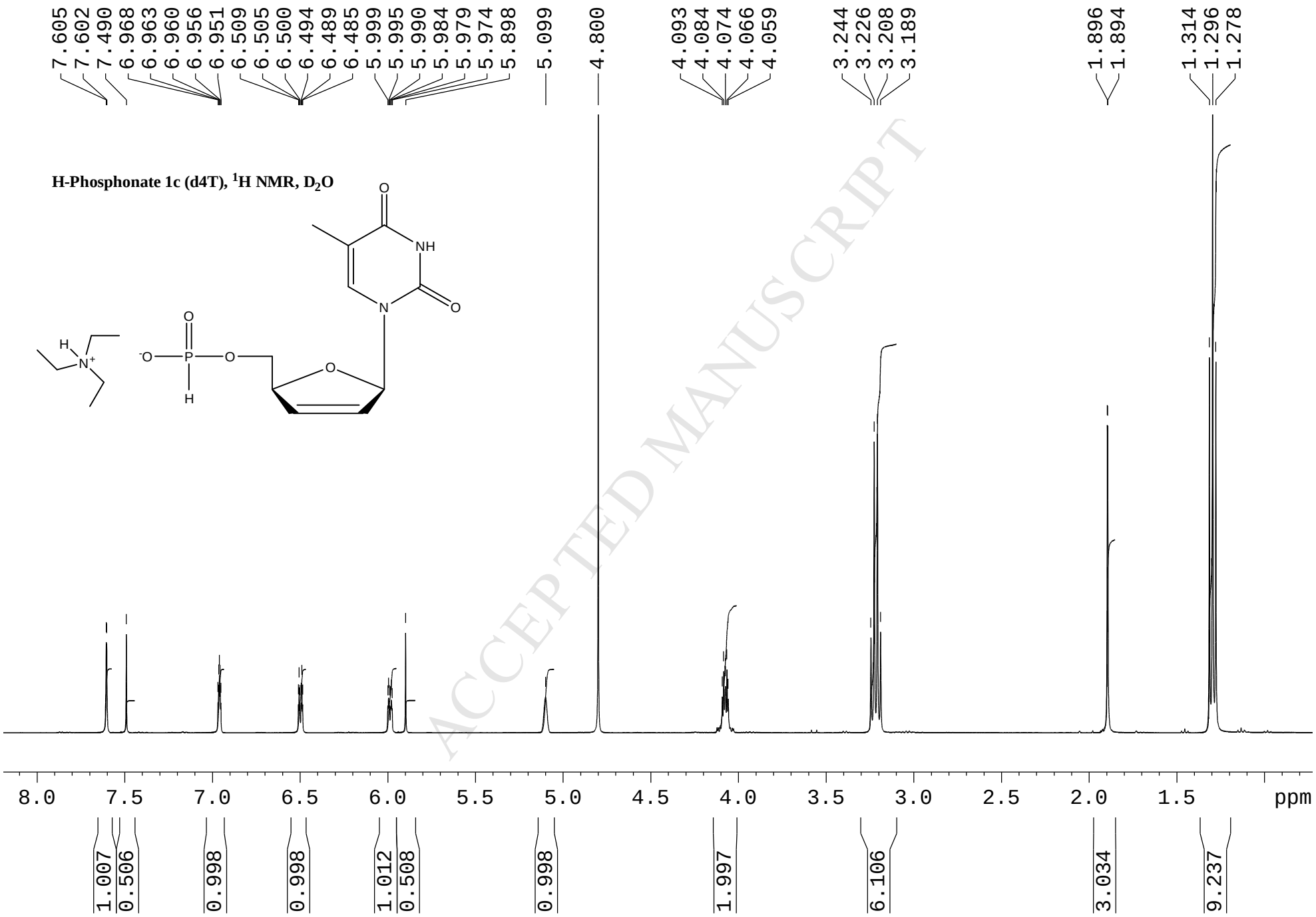
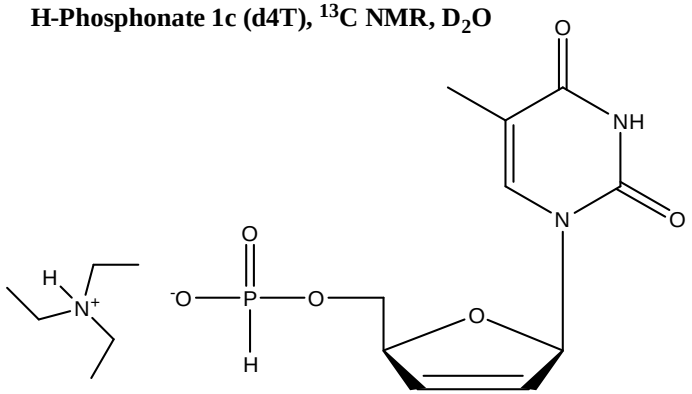
Contents



H-Phosphonate 1c (d4T), ^{13}C NMR, D_2O



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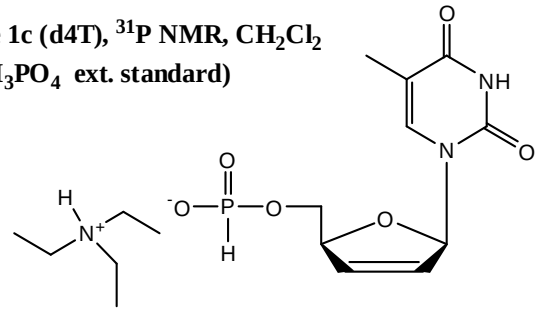

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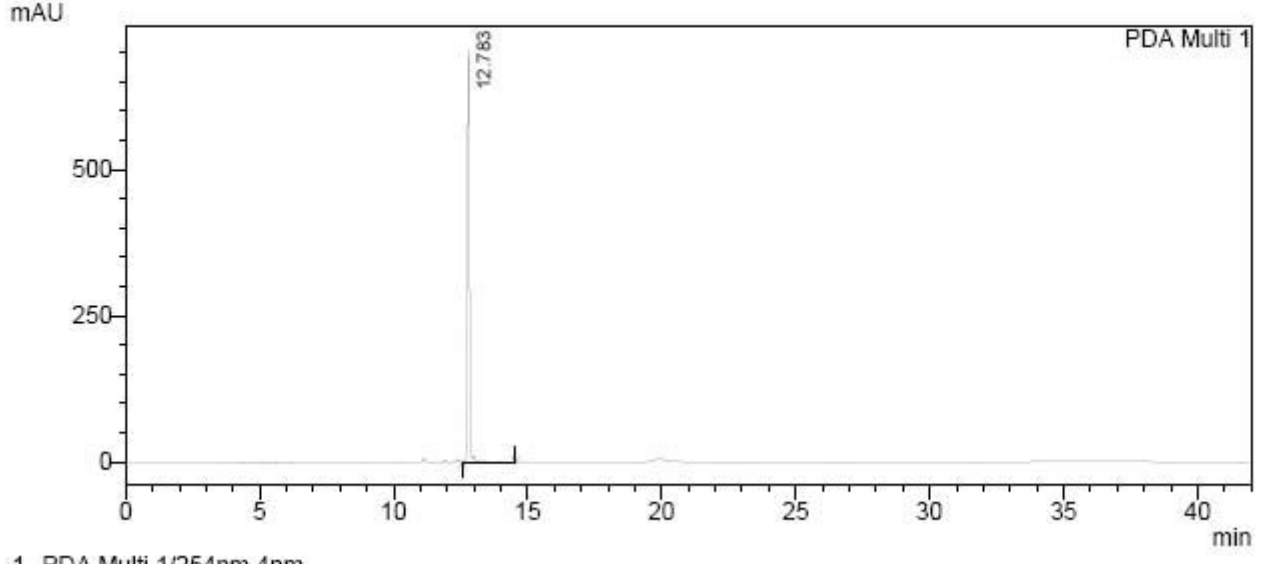

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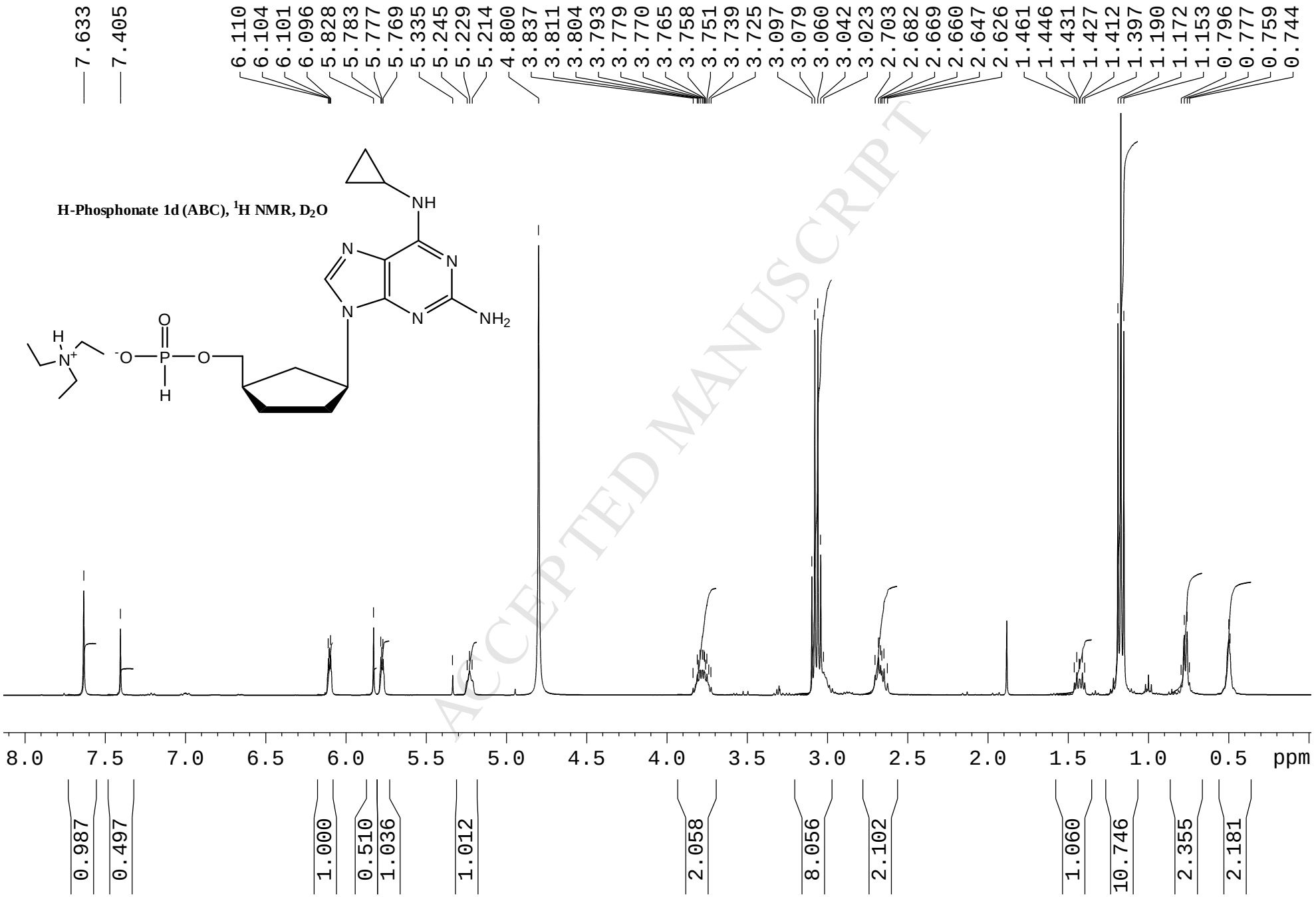

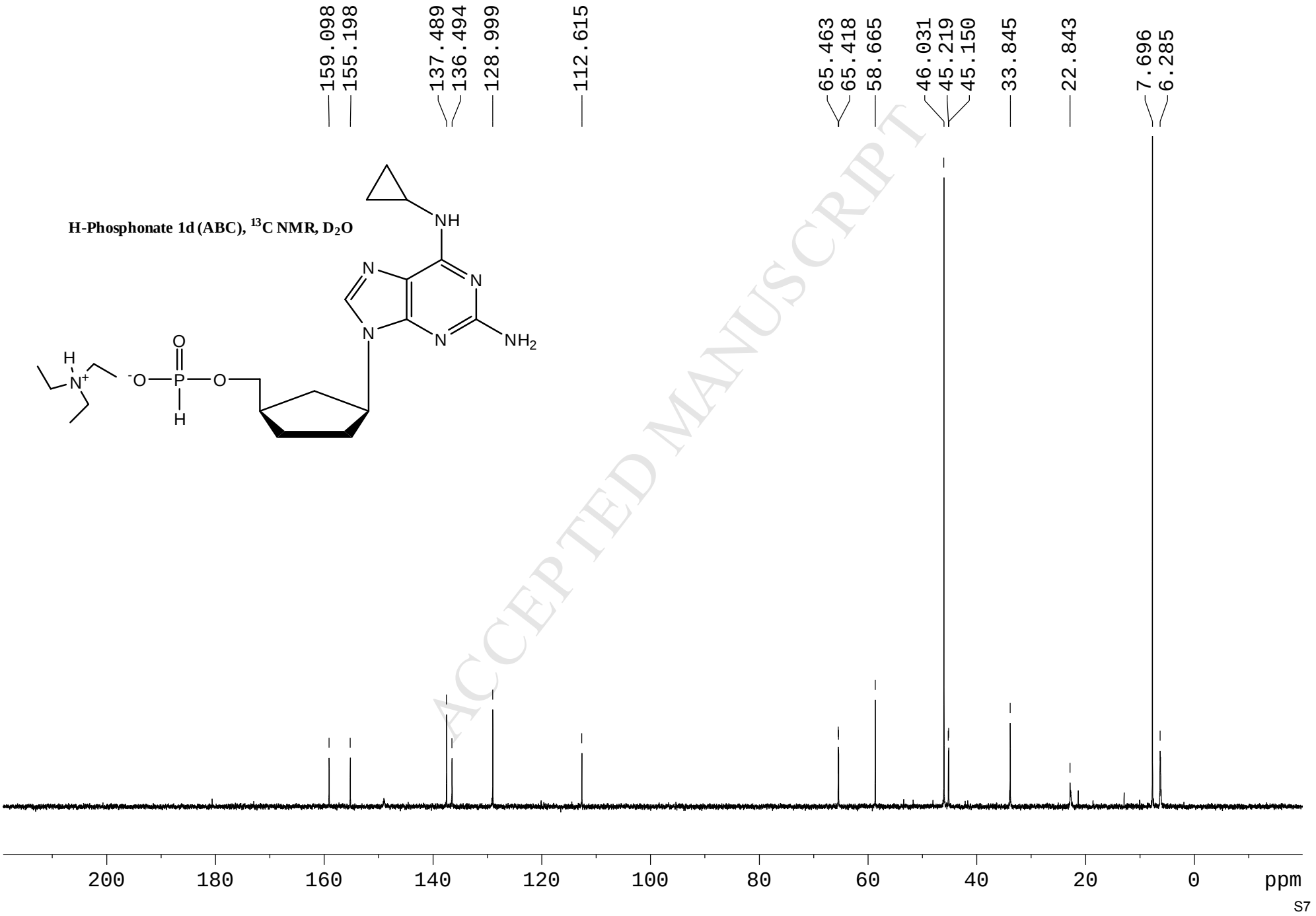

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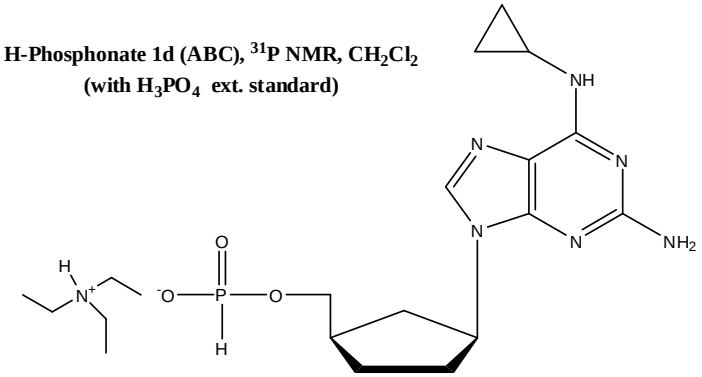

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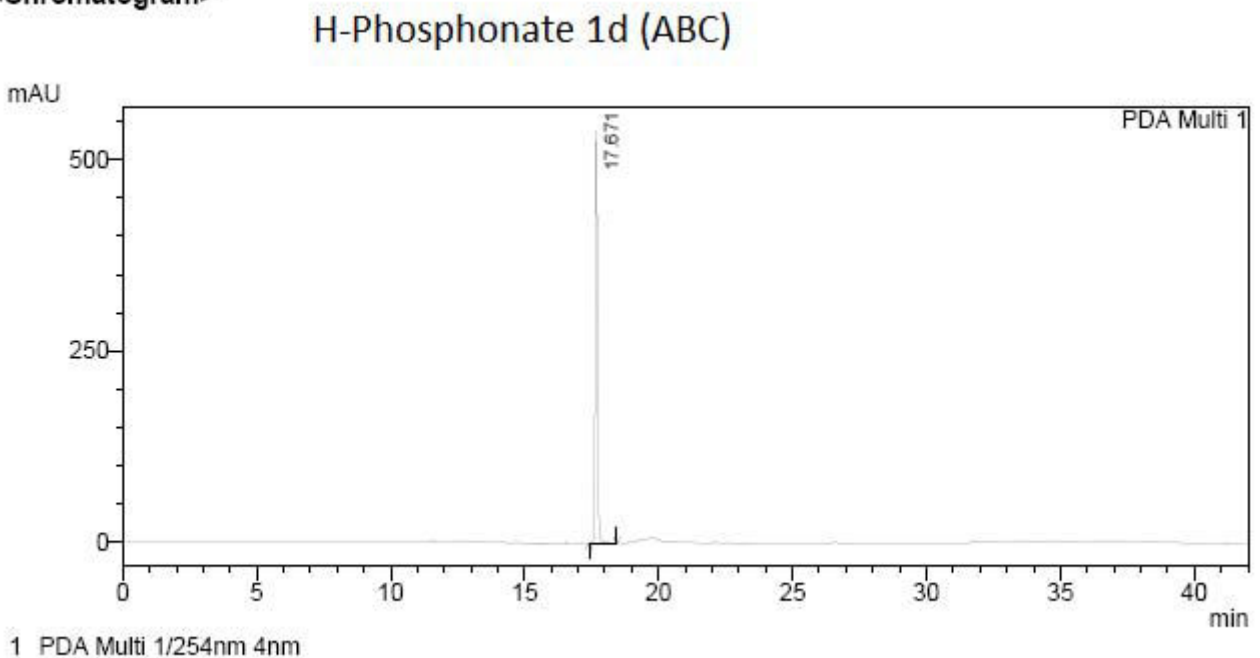
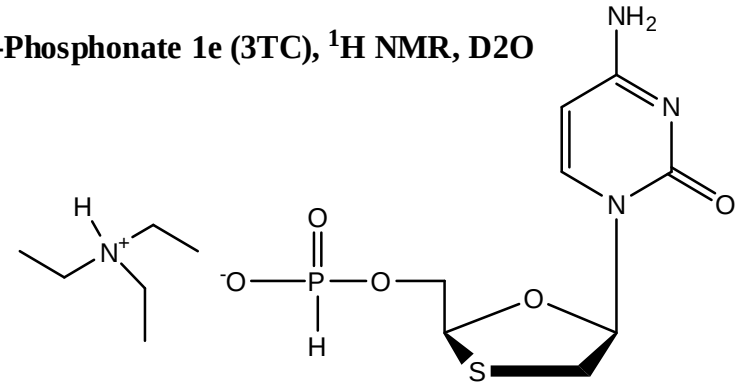


H-Phosphonate 1e (3TC), ^{1}H NMR, D2O



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8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 ppm

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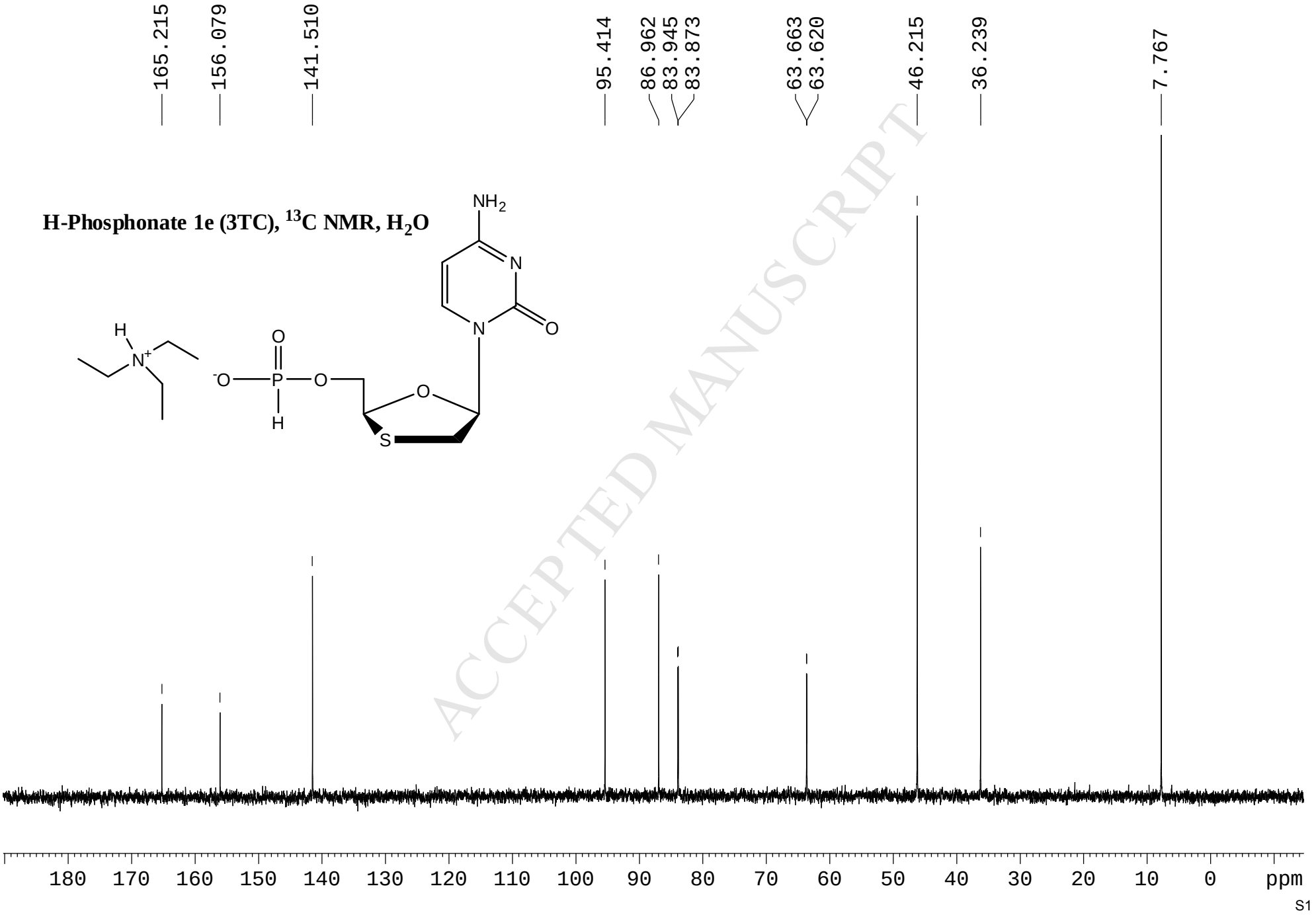
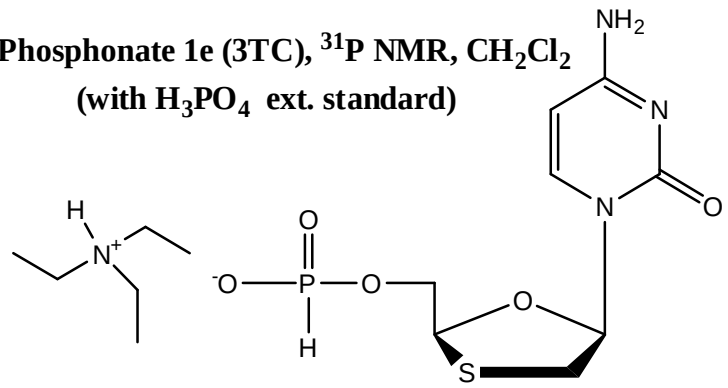


H-Phosphonate 1e (3TC), ^{31}P NMR, CH_2Cl_2
(with H_3PO_4 ext. standard)



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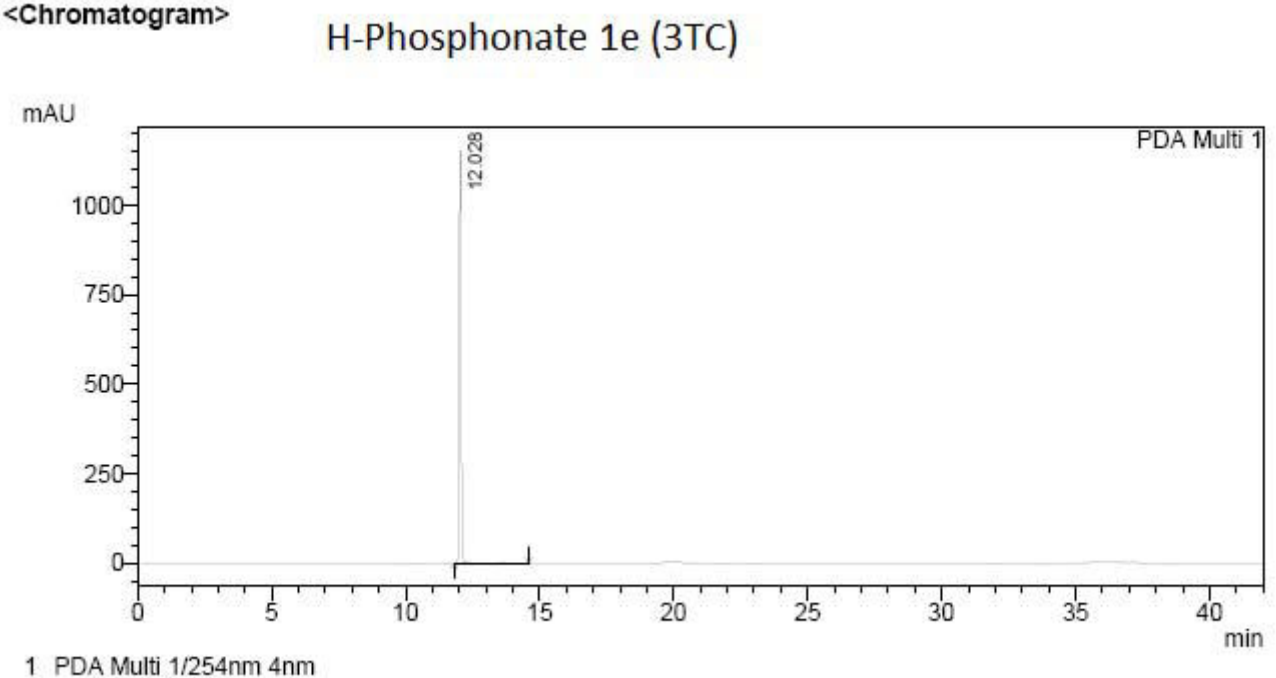
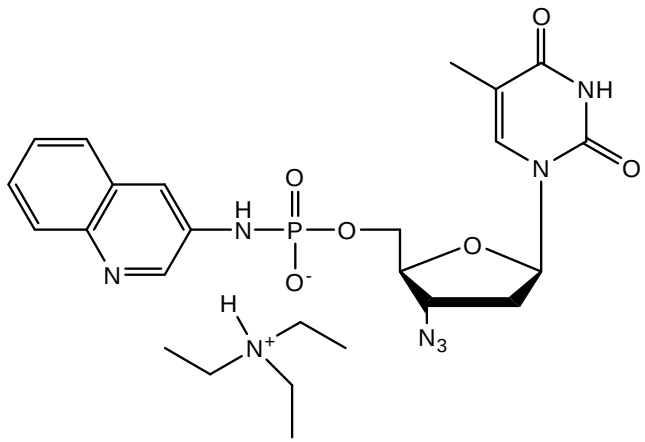
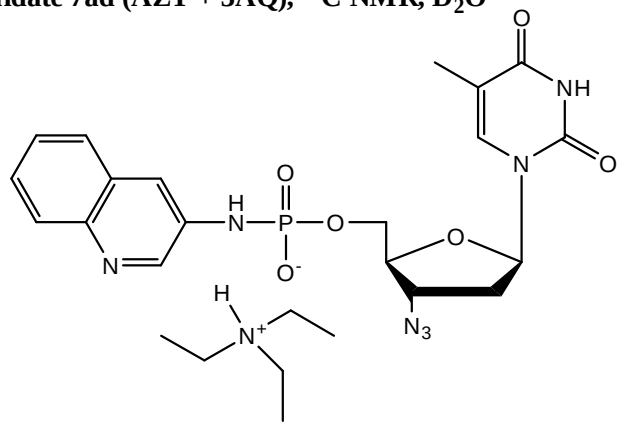


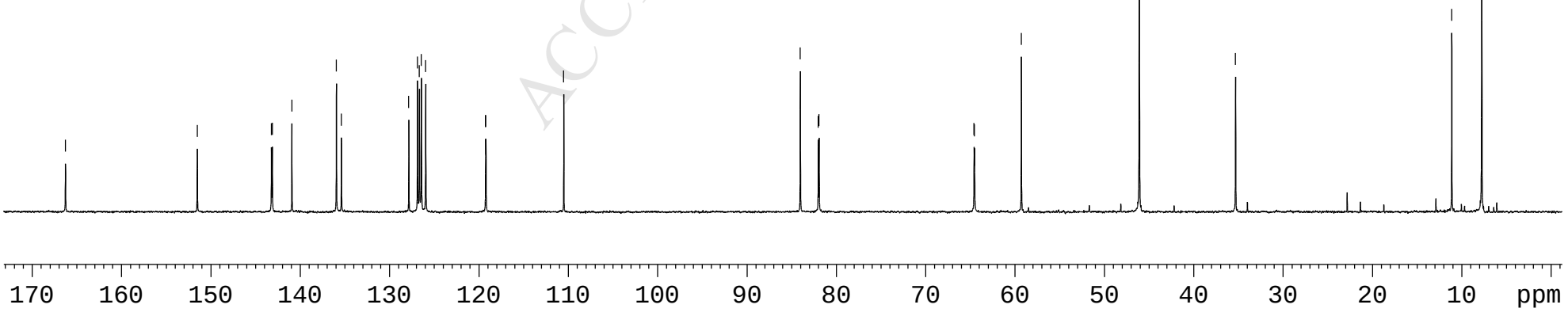
Phosphoramidate 7ad (AZT + 3AQ), ^{1}H NMR, D_2O



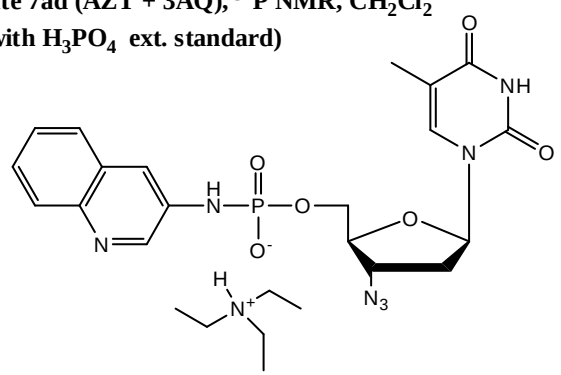
166.255
151.499
143.205
143.100
140.910
135.957
135.394
127.852
126.876
126.664
126.441
125.962
119.254
119.219
110.509
84.031
82.023
81.927
64.580
64.532
59.290
46.090
35.314
11.119
7.761

Phosphoramidate 7ad (AZT + 3AQ), ^{13}C NMR, D_2O





Phosphoramidate 7ad (AZT + 3AQ), ^{31}P NMR, CH_2Cl_2
(with H_3PO_4 ext. standard)



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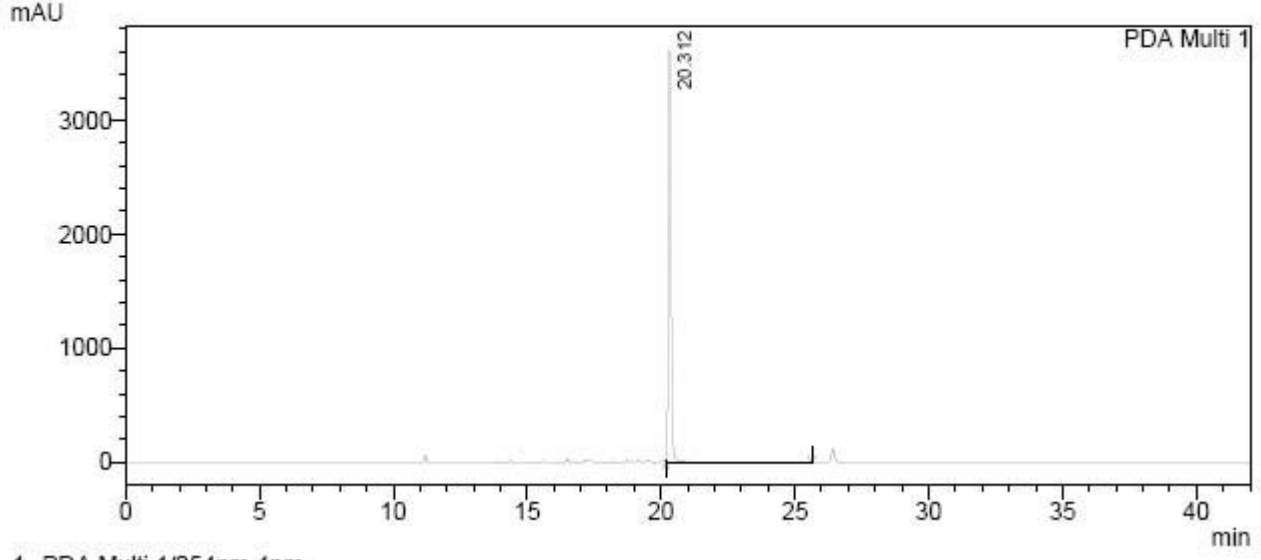
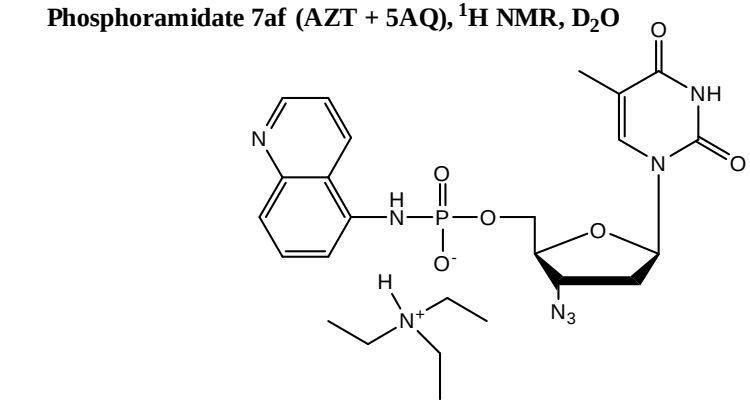
Phosphoramidate 7ad (AZT + 3AQ)



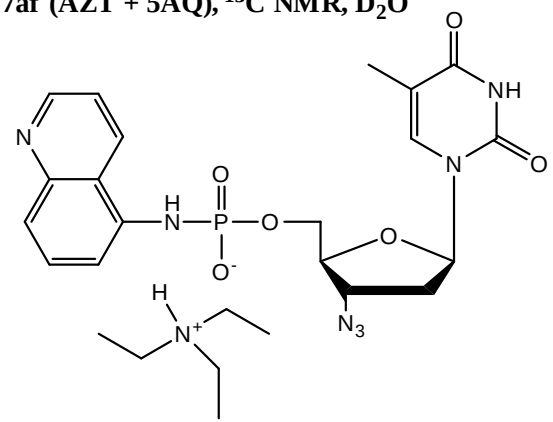
1 PDA Multi 1/254nm 4nm

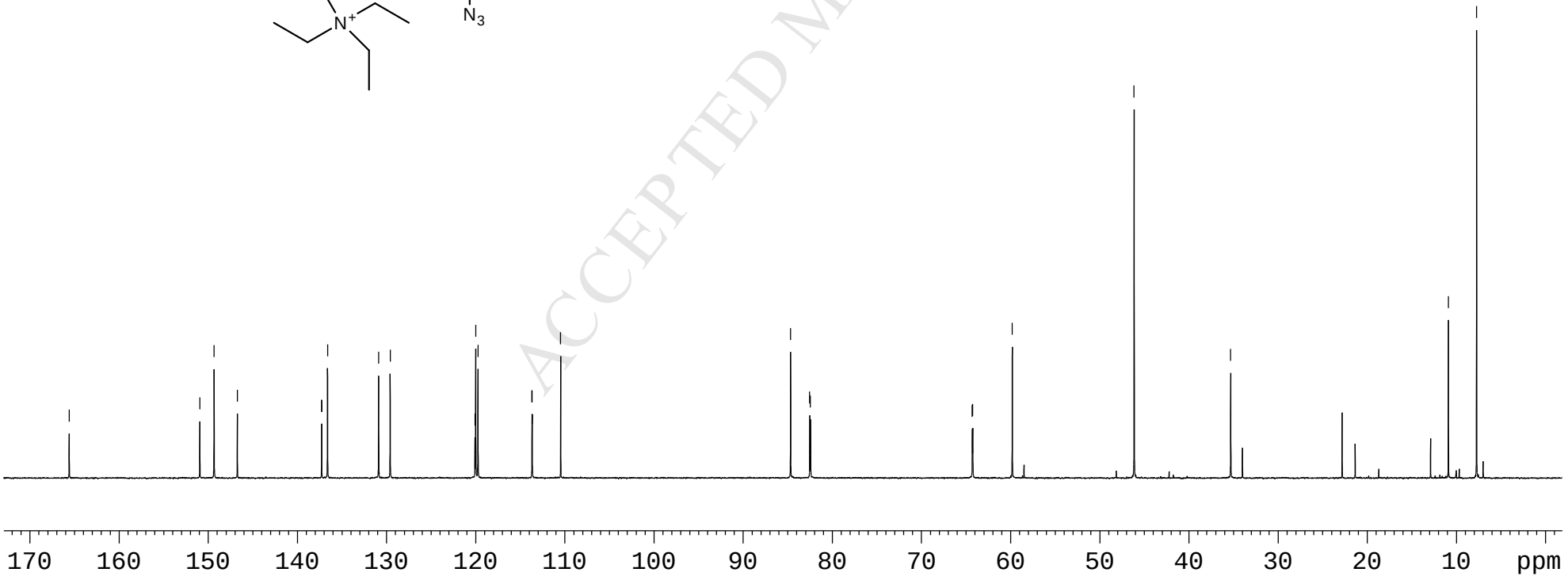
Phosphoramidate 7af (AZT + 5AQ), ^{1}H NMR, D_2O



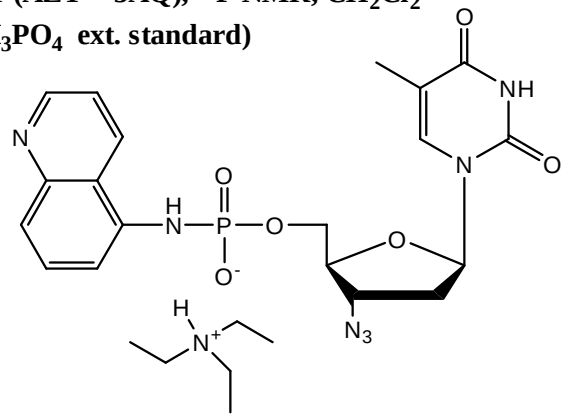
165.580
150.931
149.327
146.719
137.267
137.254
136.601
130.871
129.576
120.062
119.975
119.723
113.688
113.668
110.472
84.684
82.532
82.442
64.294
64.245
59.815
46.145
35.310
10.893
7.734

Phosphoramidate 7af (AZT + 5AQ), ^{13}C NMR, D_2O





Phosphoramidate 7af (AZT + 5AQ), ^{31}P NMR, CH_2Cl_2
(with H_3PO_4 ext. standard)



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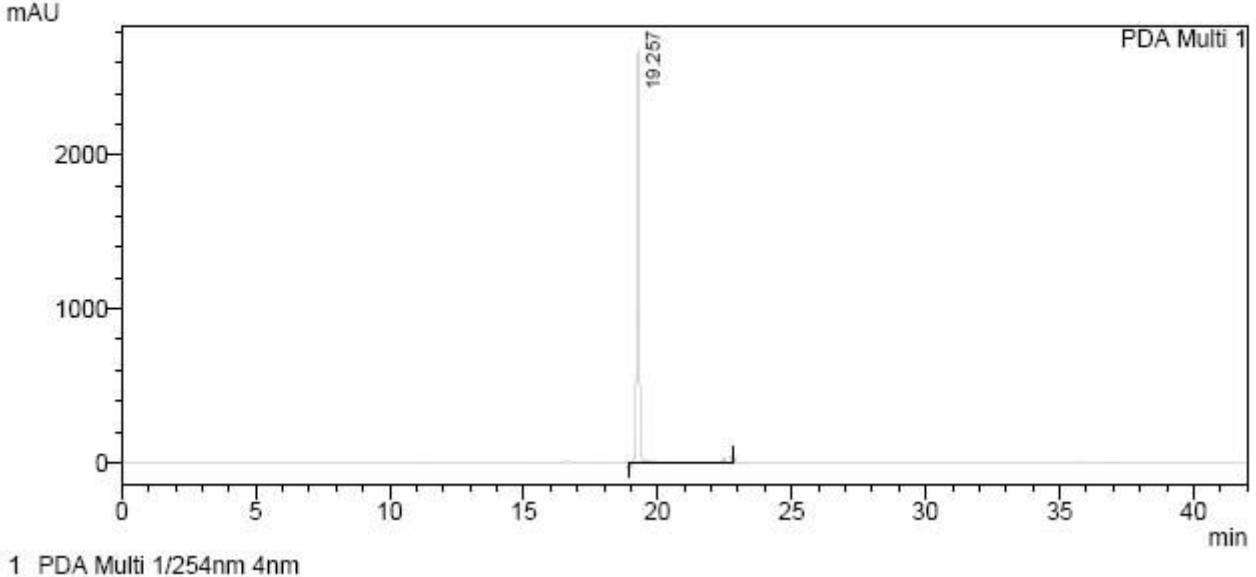
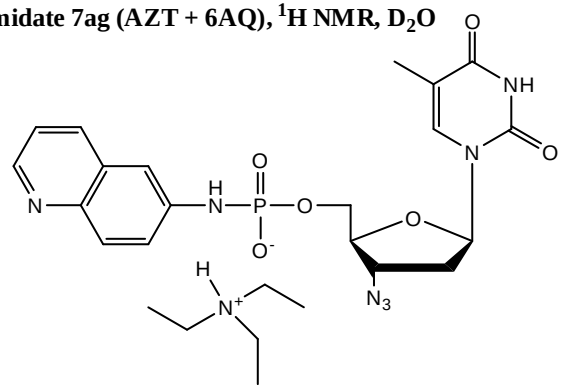
Phosphoramidate 7af (AZT + 5AQ)



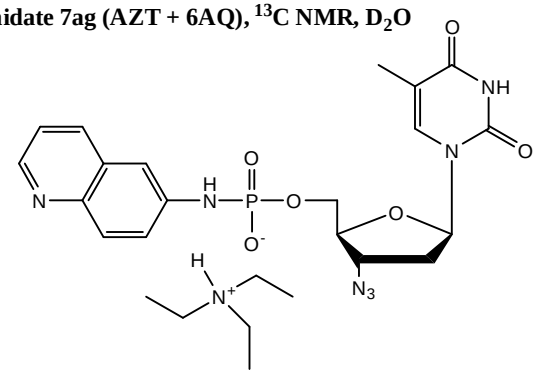
1 PDA Multi 1/254nm 4nm

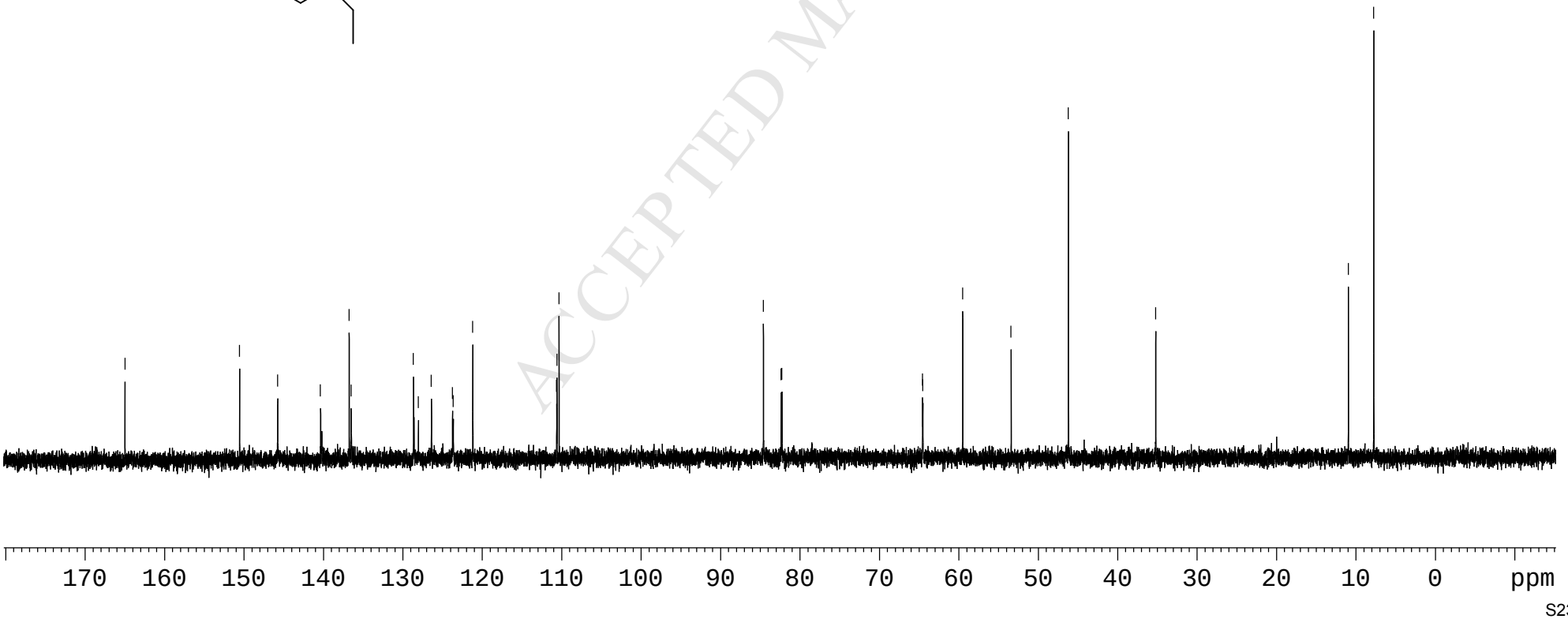
Phosphoramidate 7ag (AZT + 6AQ), ^{1}H NMR, D_2O



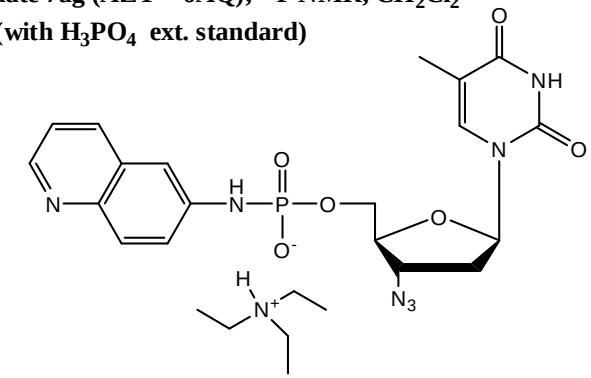
164.961
150.553
145.763
140.364
136.761
136.508
128.659
128.045
126.409
123.749
123.651
121.199
110.637
110.589
110.333
84.608
82.364
82.265
64.576
64.527
59.510
53.415
46.209
35.207
10.958
7.754

Phosphoramidate 7ag (AZT + 6AQ), ^{13}C NMR, D_2O



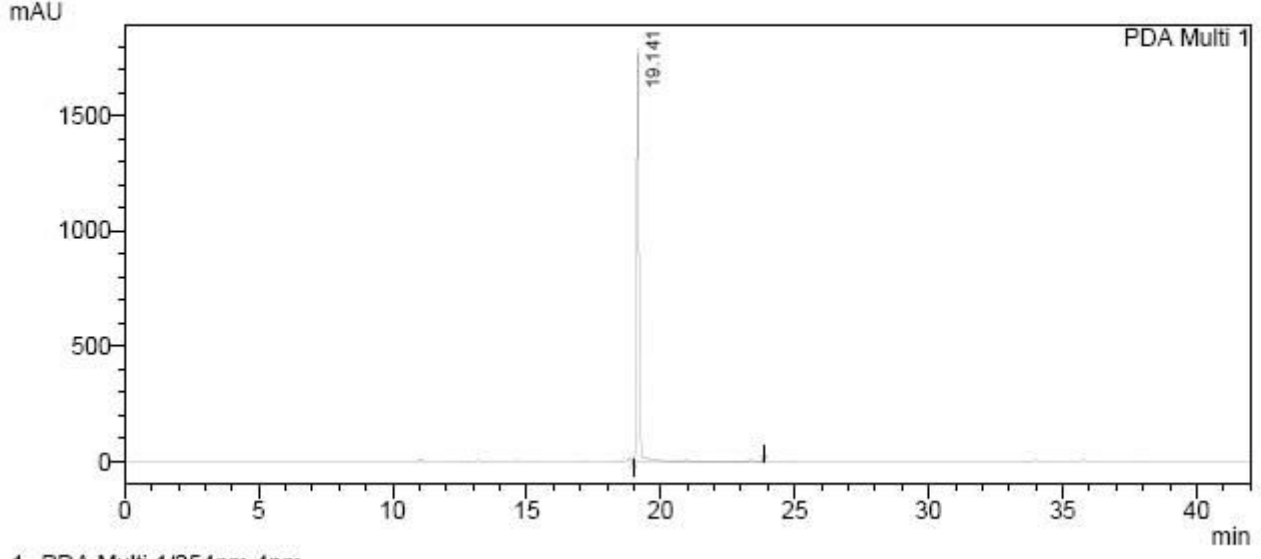


Phosphoramidate 7ag (AZT + 6AQ), ^{31}P NMR, CH_2Cl_2
(with H_3PO_4 ext. standard)



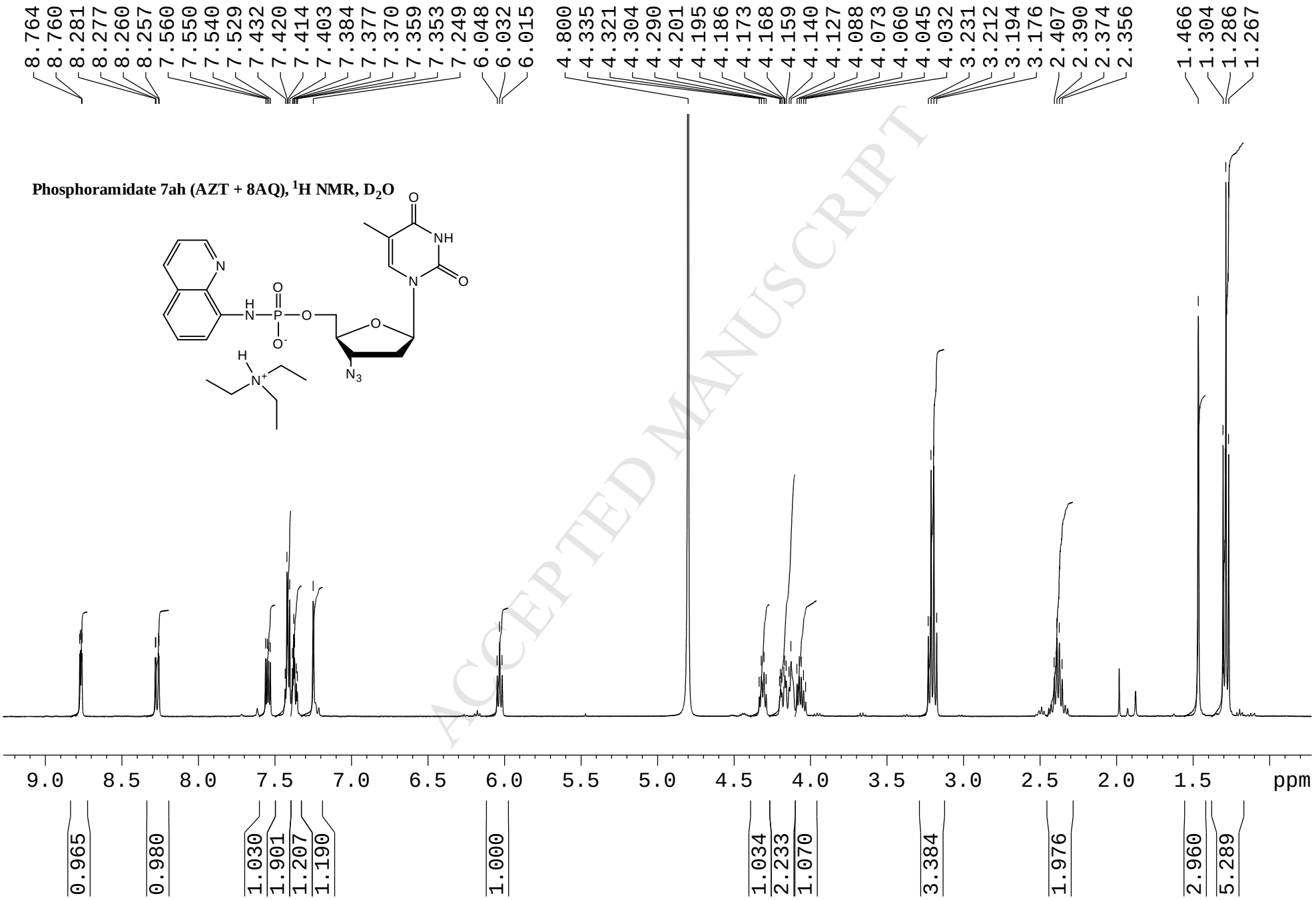
<Chromatogram>

Phosphoramidate 7ag (AZT + 6AQ)



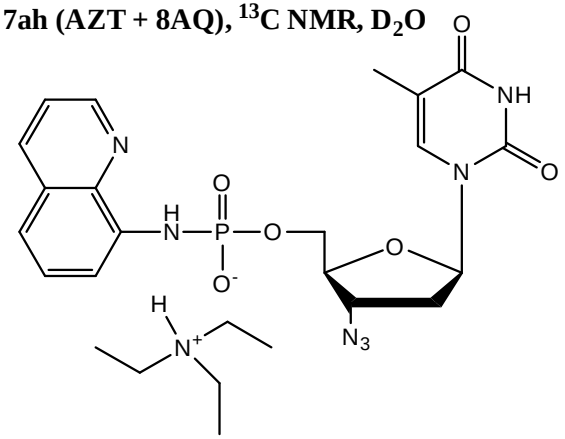
1 PDA Multi 1/254nm 4nm

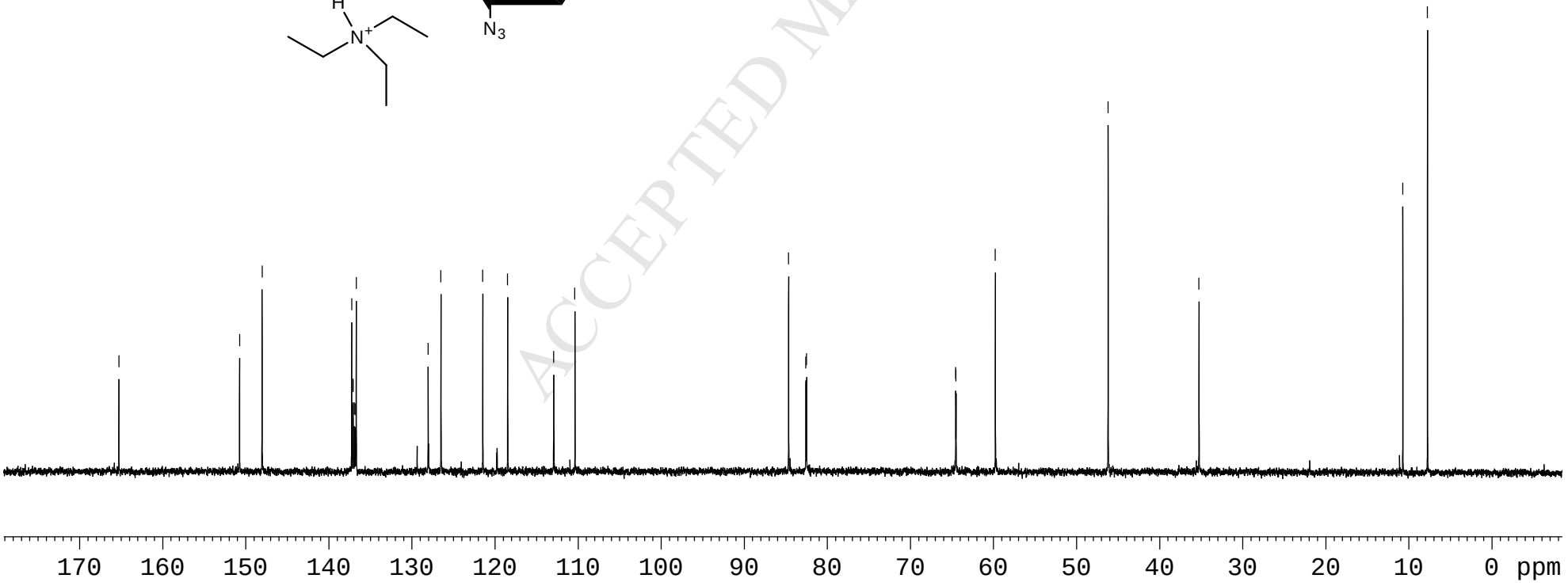
Phosphoramidate 7ah (AZT + 8AQ), ^{1}H NMR, D_2O



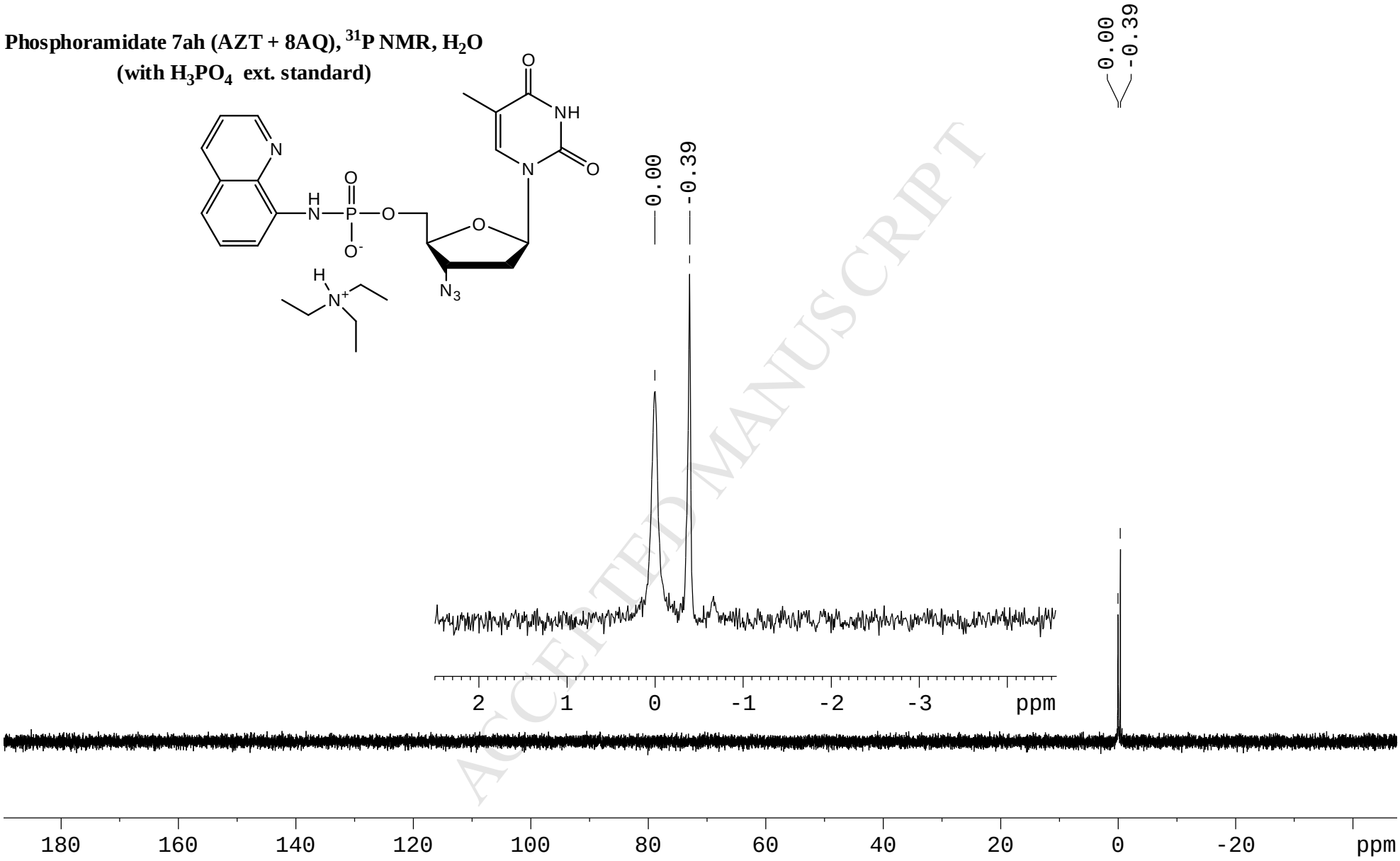
165.256
150.728
148.015
137.226
137.083
137.057
136.921
136.827
136.681
128.030
126.505
121.475
118.476
112.924
110.379
84.663
82.586
82.498
64.553
64.503
59.773
46.200
35.257
10.727
7.753

Phosphoramidate 7ah (AZT + 8AQ), ^{13}C NMR, D_2O



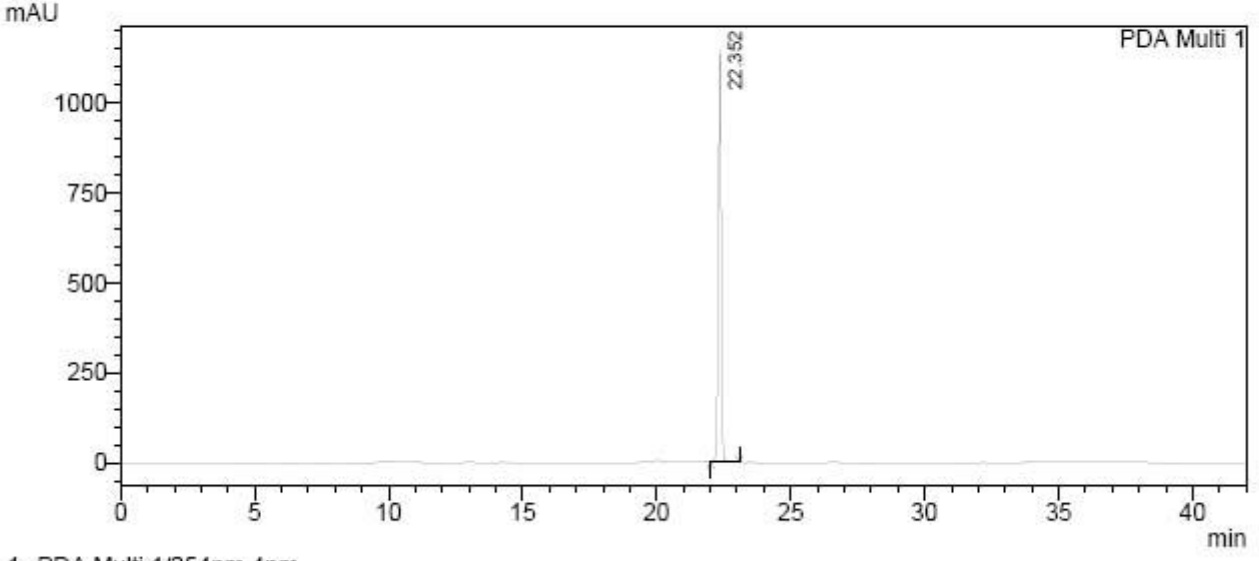


Phosphoramidate 7ah (AZT + 8AQ), ^{31}P NMR, H_2O
(with H_3PO_4 ext. standard)

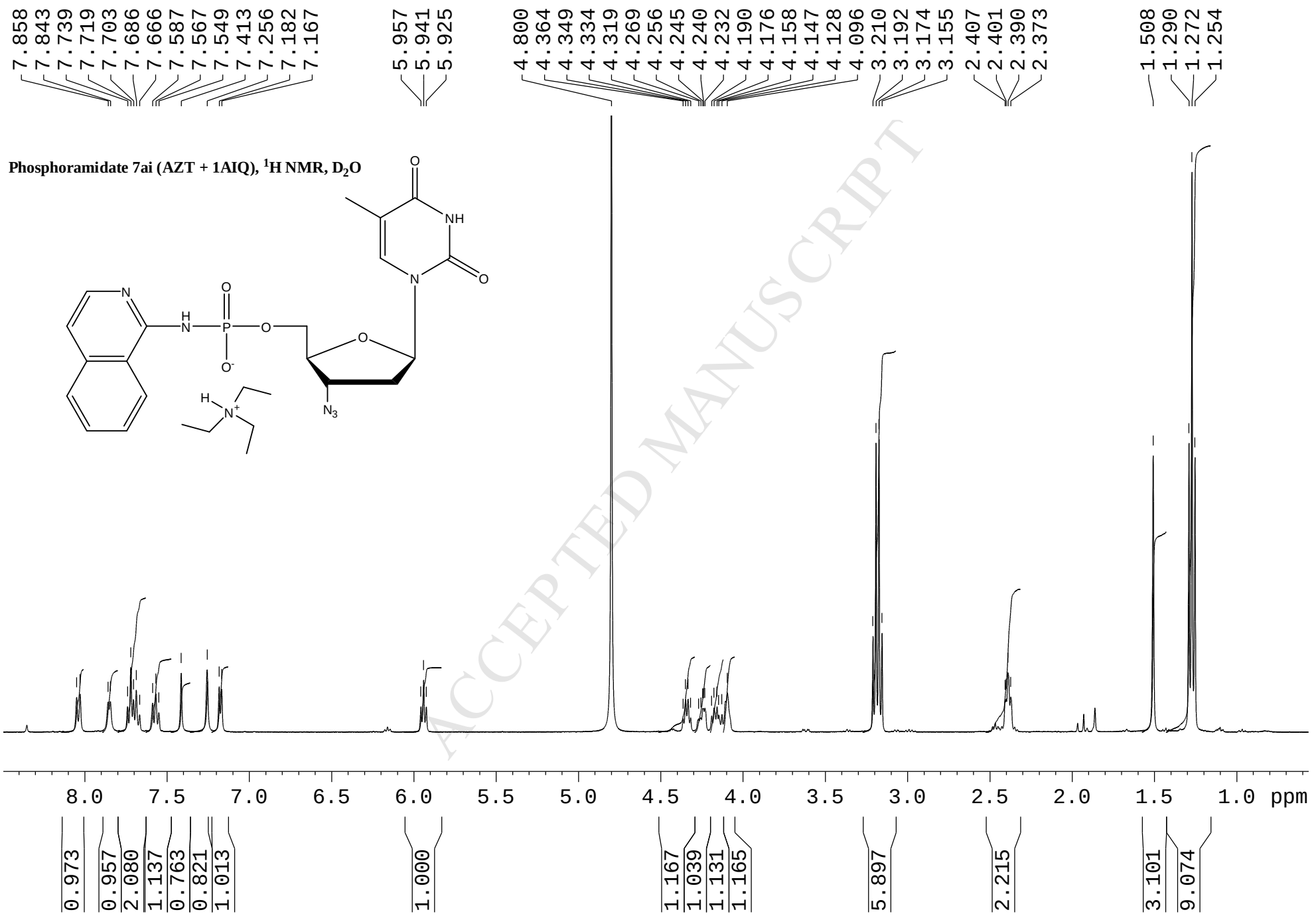


<Chromatogram>

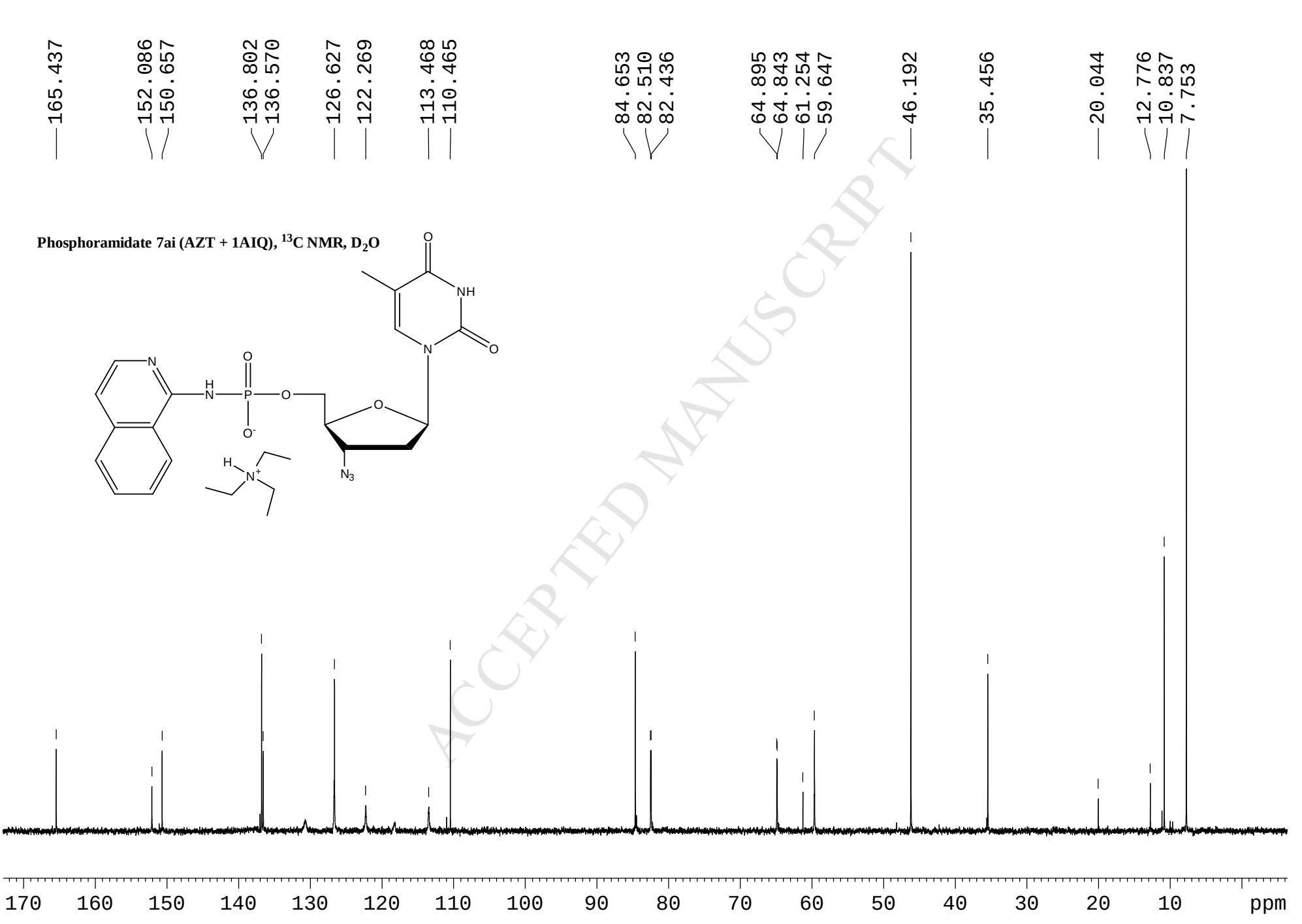
Phosphoramidate 7ah (AZT + 8AQ)



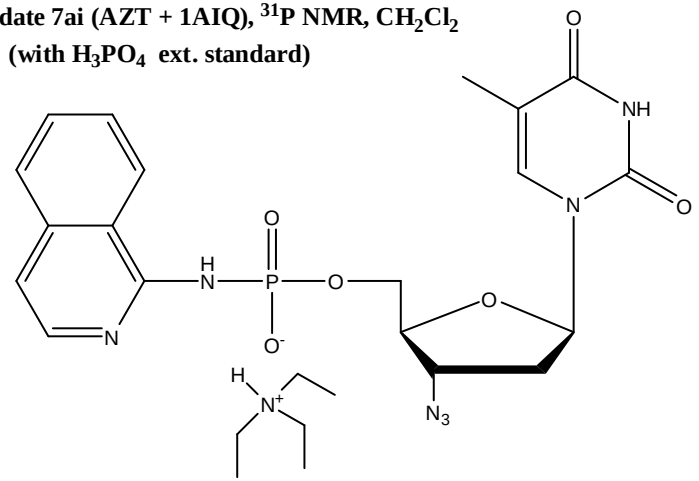
1 PDA Multi 1/254nm 4nm



Phosphoramidate 7ai (AZT + 1AIQ), ^{13}C NMR, D_2O



Phosphoramidate 7ai (AZT + 1AIQ), ^{31}P NMR, CH_2Cl_2
(with H_3PO_4 ext. standard)



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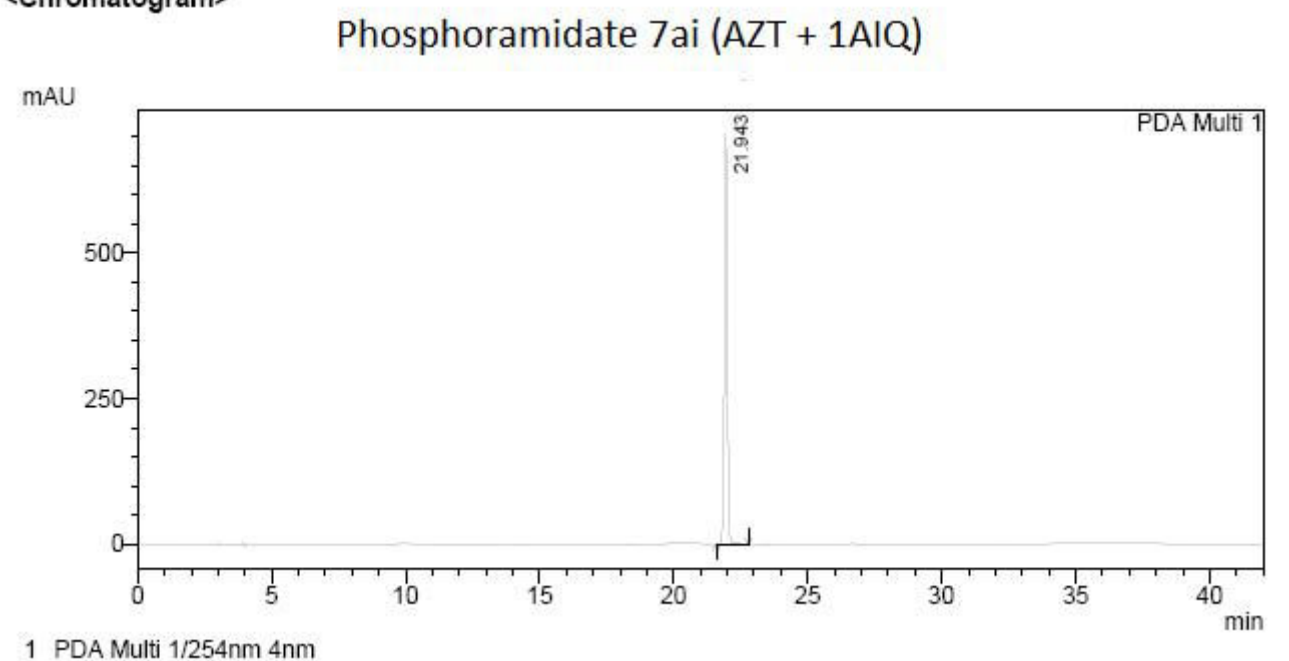
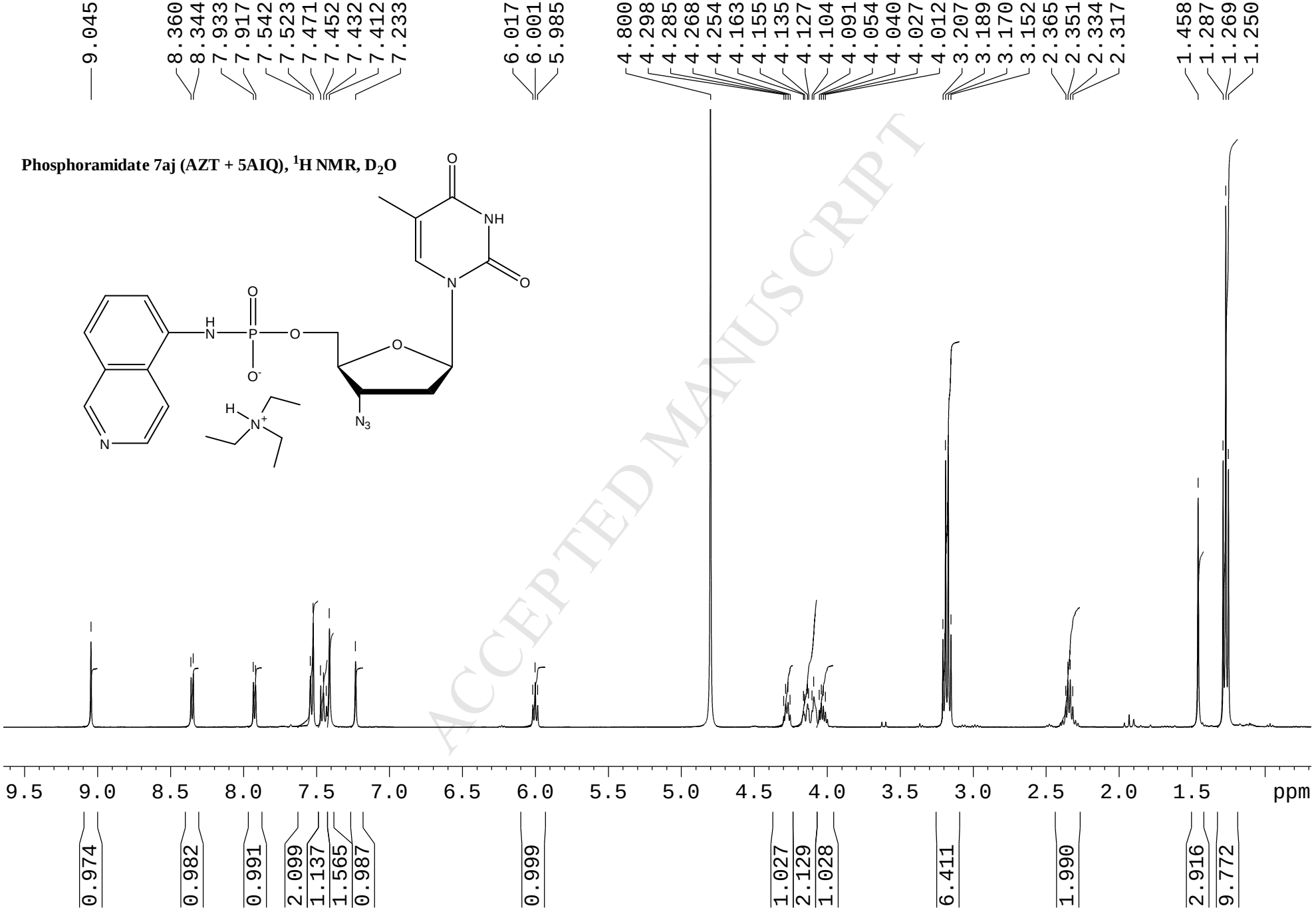
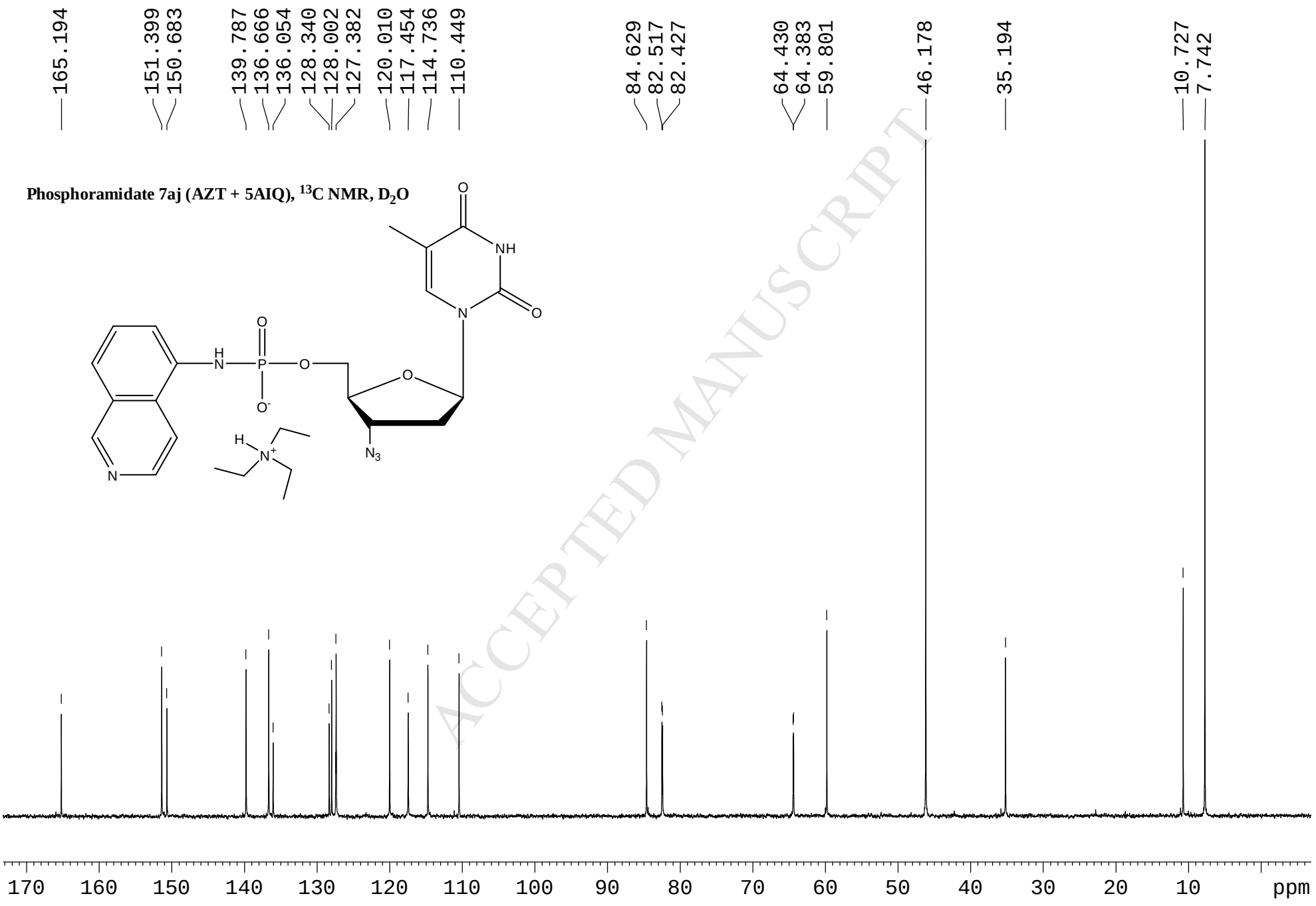


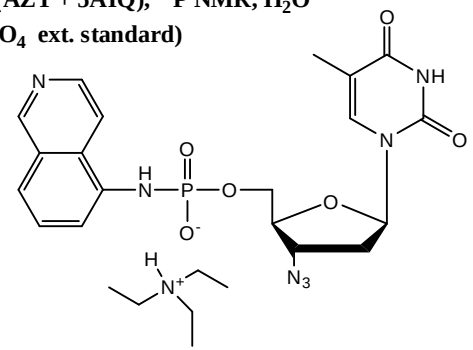
Phosphoramidate 7aj (AZT + 5AIQ), ^{1}H NMR, D_2O



Phosphoramidate 7aj (AZT + 5AIQ), ^{13}C NMR, D_2O



Phosphoramidate 7aj (AZT + 5AIQ), ^{31}P NMR, H_2O
(with H_3PO_4 ext. standard)



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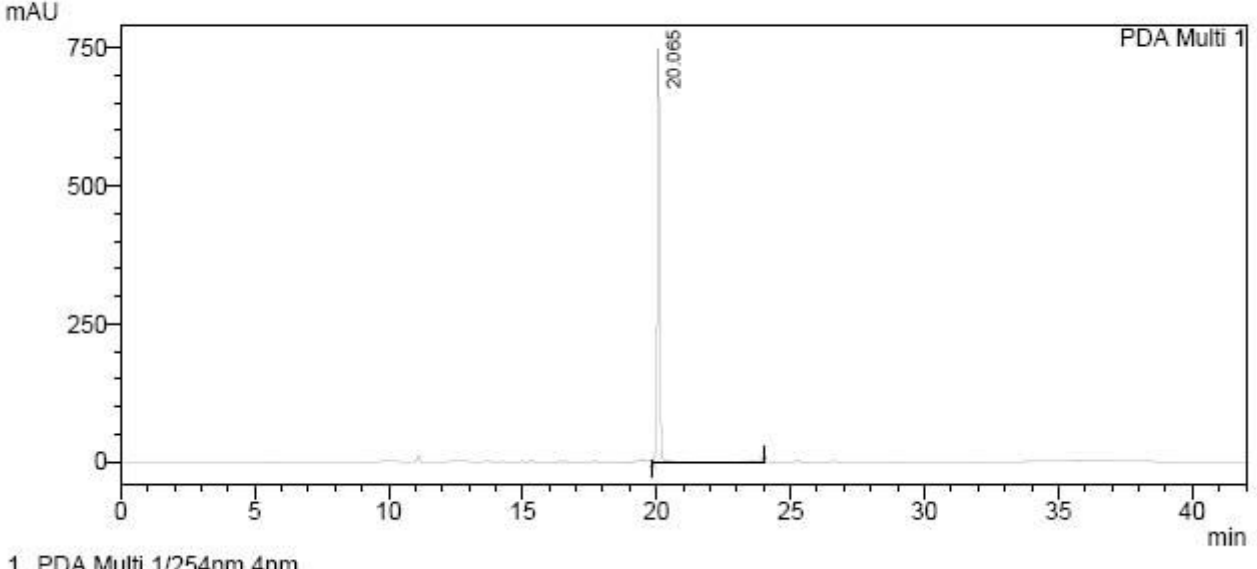
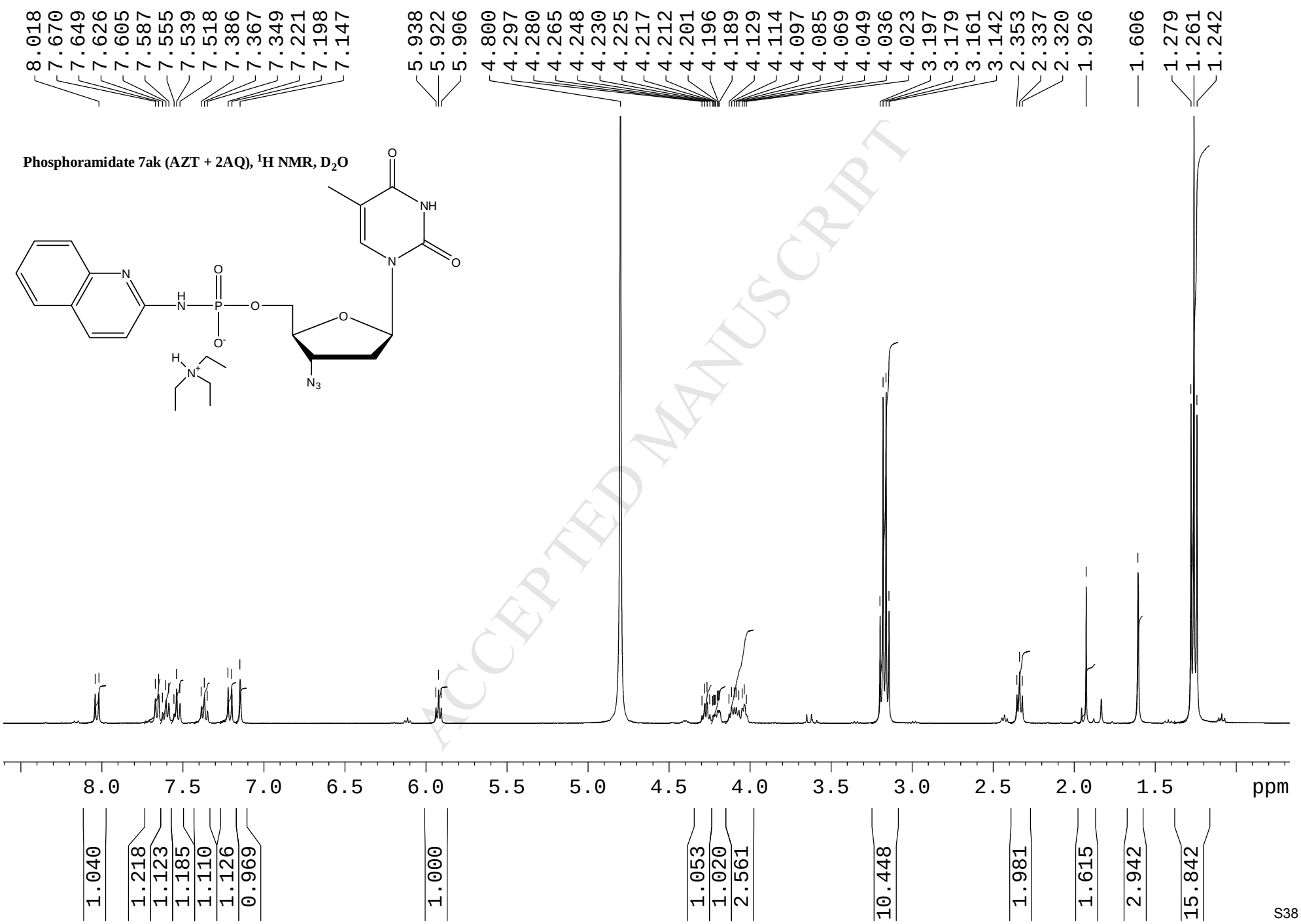
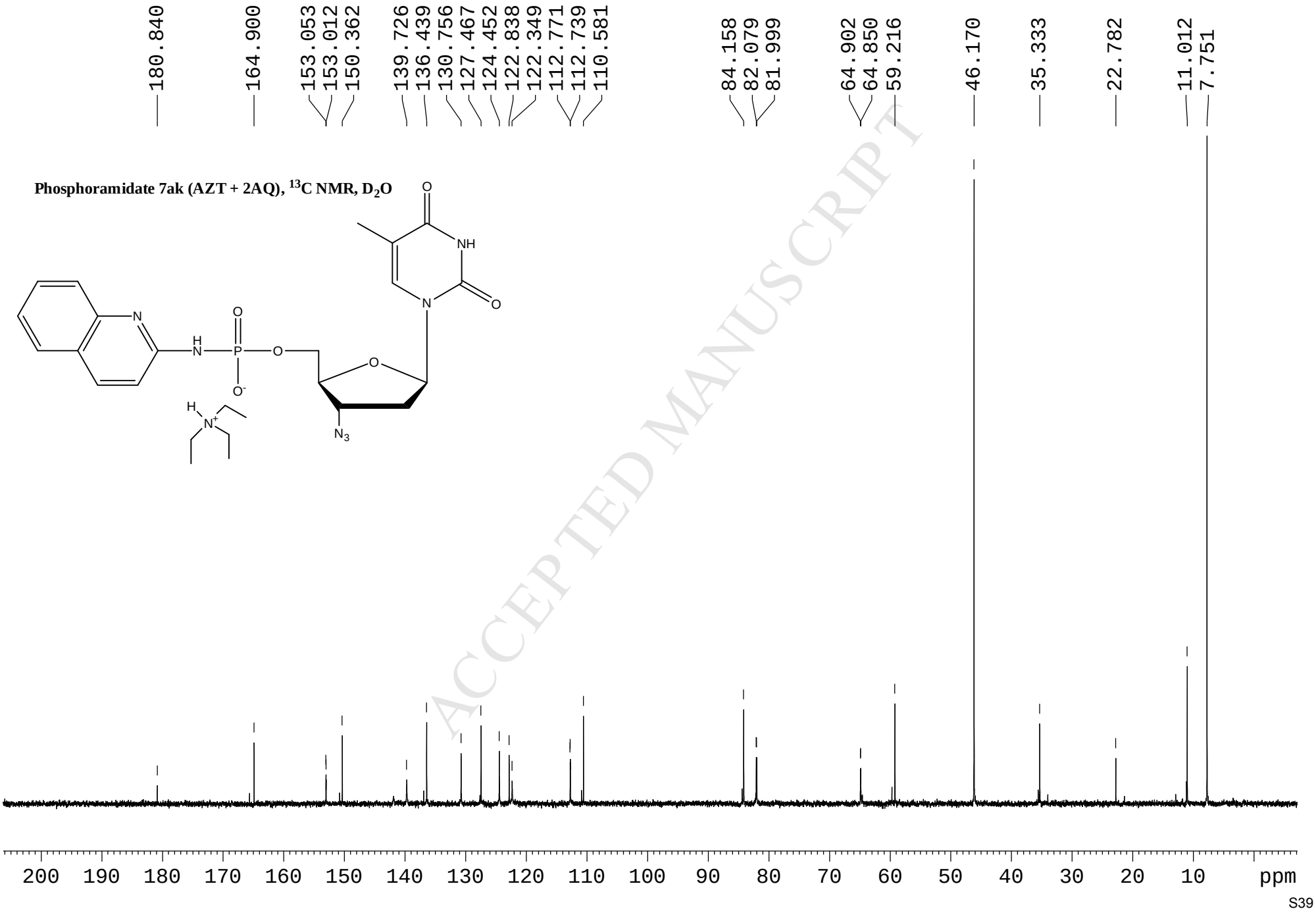
Phosphoramidate 7aj (AZT + 5AIQ)

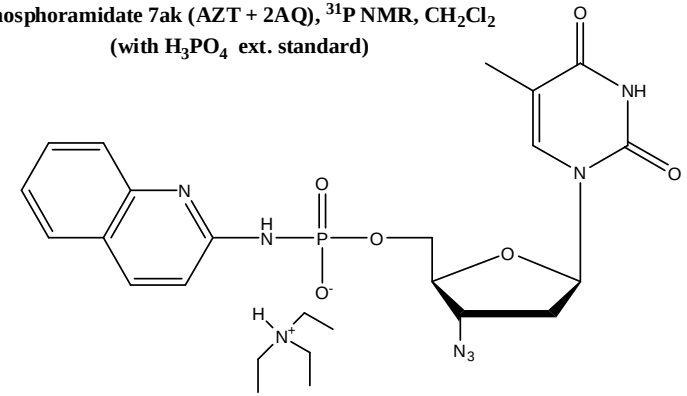


1 PDA Multi 1/254nm 4nm





Phosphoramidate 7ak (AZT + 2AQ), ^{31}P NMR, CH_2Cl_2
(with H_3PO_4 ext. standard)



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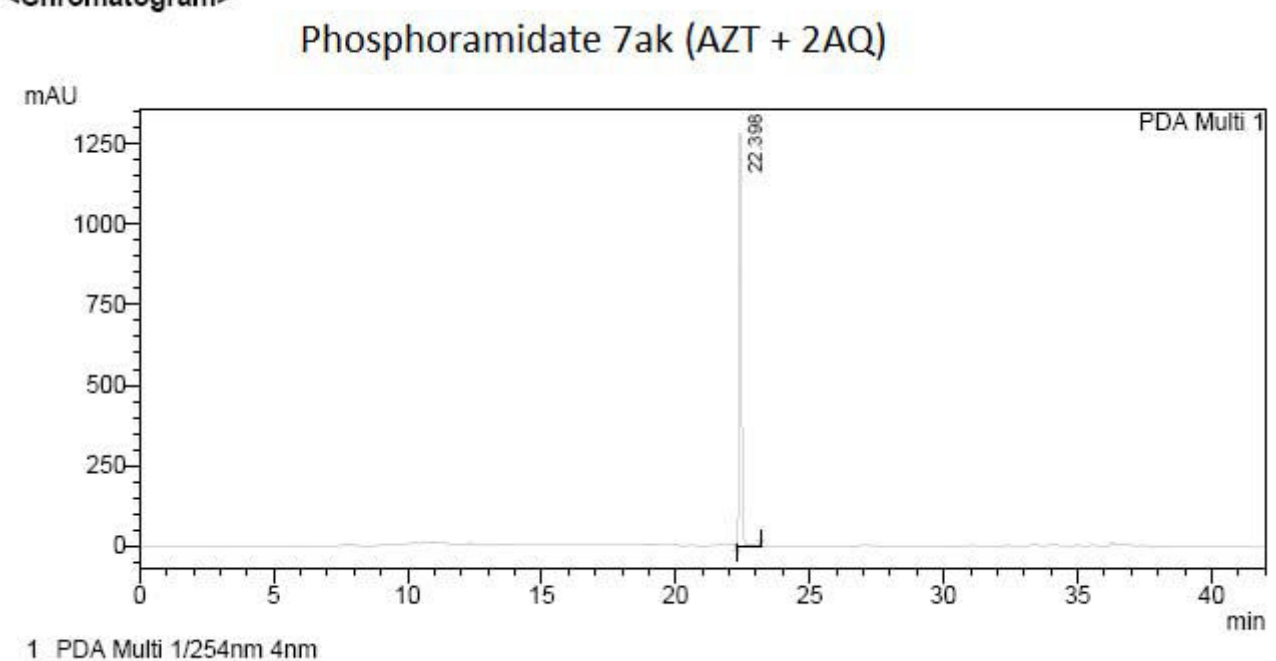
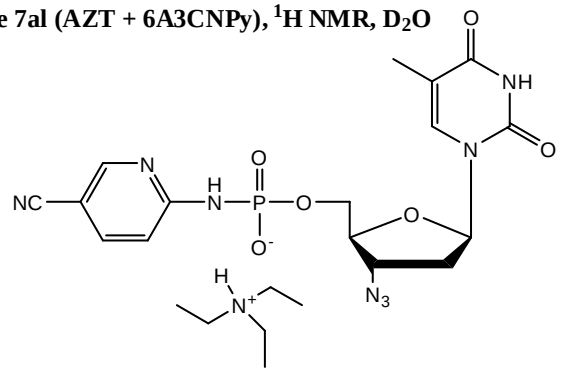


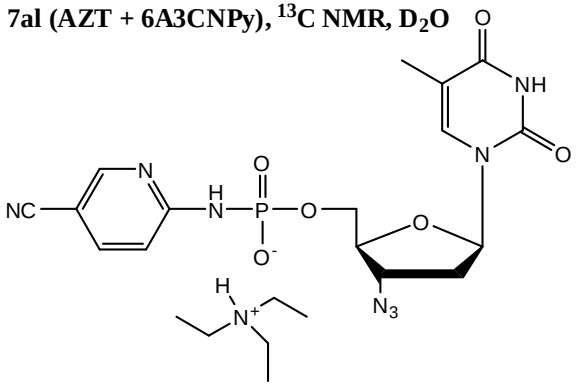
1 PDA Multi 1/254nm 4nm

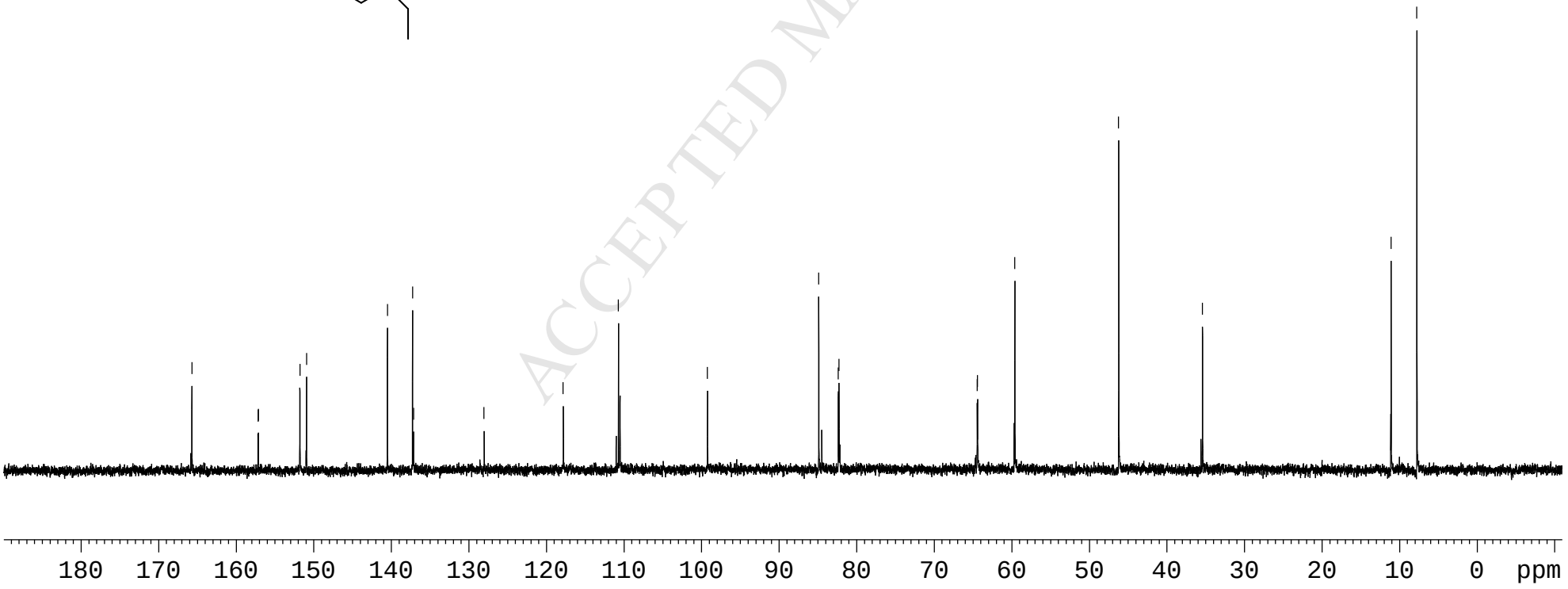
Phosphoramidate 7al (AZT + 6A3CNPy), ^{1}H NMR, D_2O



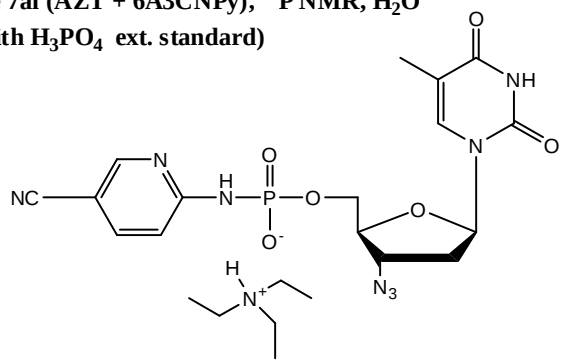
165.698
157.165
157.115
151.770
150.909
140.484
137.244
137.104
128.042
117.834
110.712
99.239
84.894
82.374
82.284
64.463
64.413
59.608
46.218
35.413
11.098
7.769

Phosphoramidate 7al (AZT + 6A3CNPy), ^{13}C NMR, D_2O



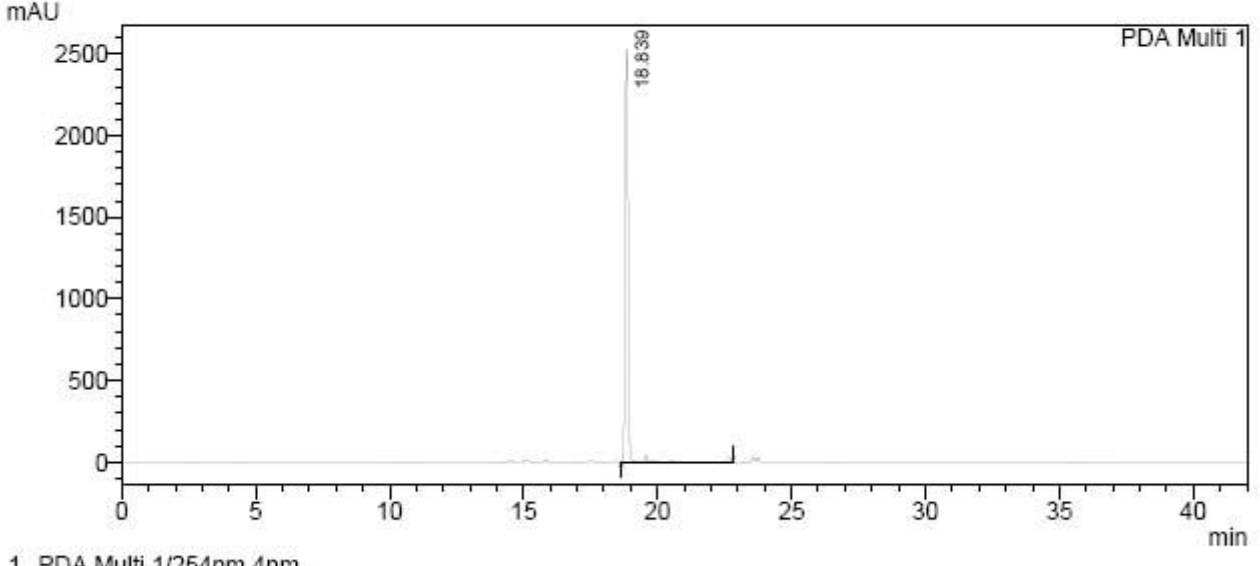


Phosphoramidate 7al (AZT + 6A3CNPy), ^{31}P NMR, H_2O
(with H_3PO_4 ext. standard)



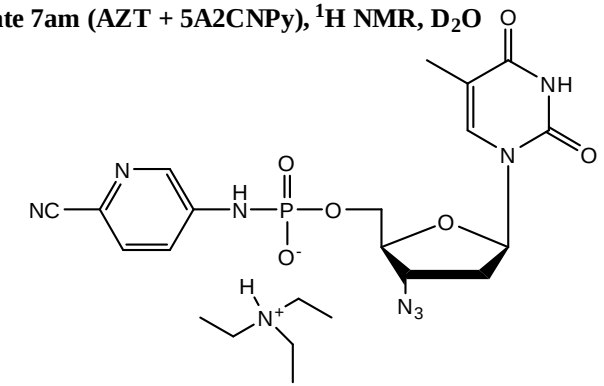
<Chromatogram>

Phosphoramidate 7al (AZT + 6A3CNPy)



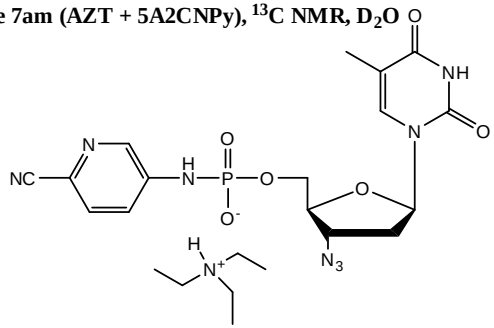
1 PDA Multi 1/254nm 4nm

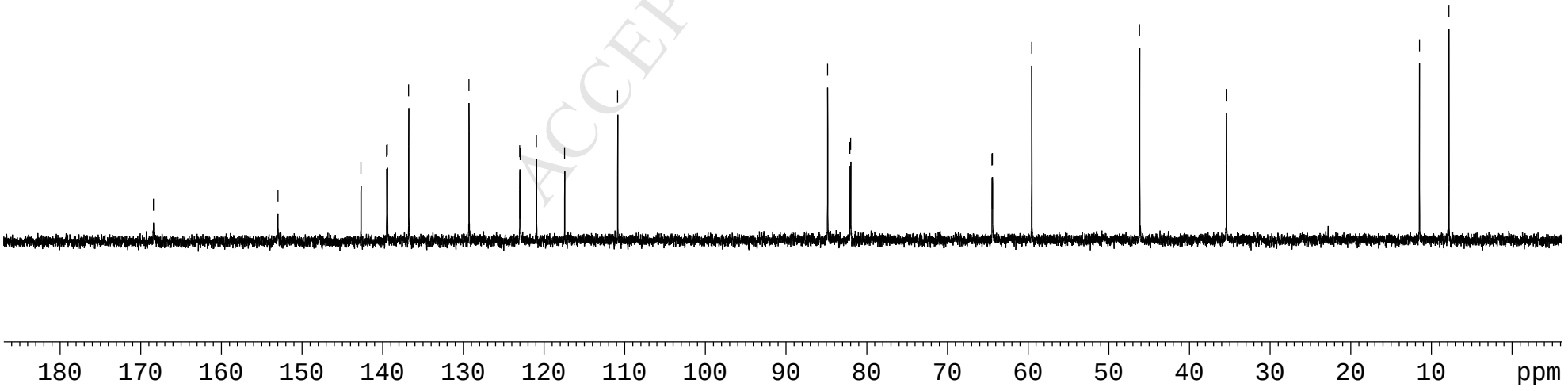
Phosphoramidate 7am (AZT + 5A2CNPy), ^{1}H NMR, D_2O



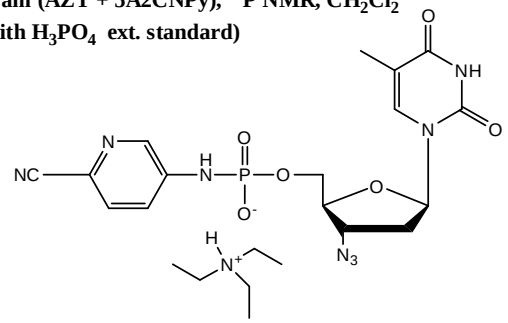
168.389
152.975
142.687
139.498
139.411
136.779
129.298
123.006
122.948
120.923
117.443
110.873
84.843
82.063
81.971
64.453
64.405
59.542
46.157
35.404
11.471
7.825

Phosphoramidate 7am (AZT + 5A2CNPy), ^{13}C NMR, D_2O





Phosphoramidate 7am (AZT + 5A2CNPy), ^{31}P NMR, CH_2Cl_2 (with H_3PO_4 ext. standard)



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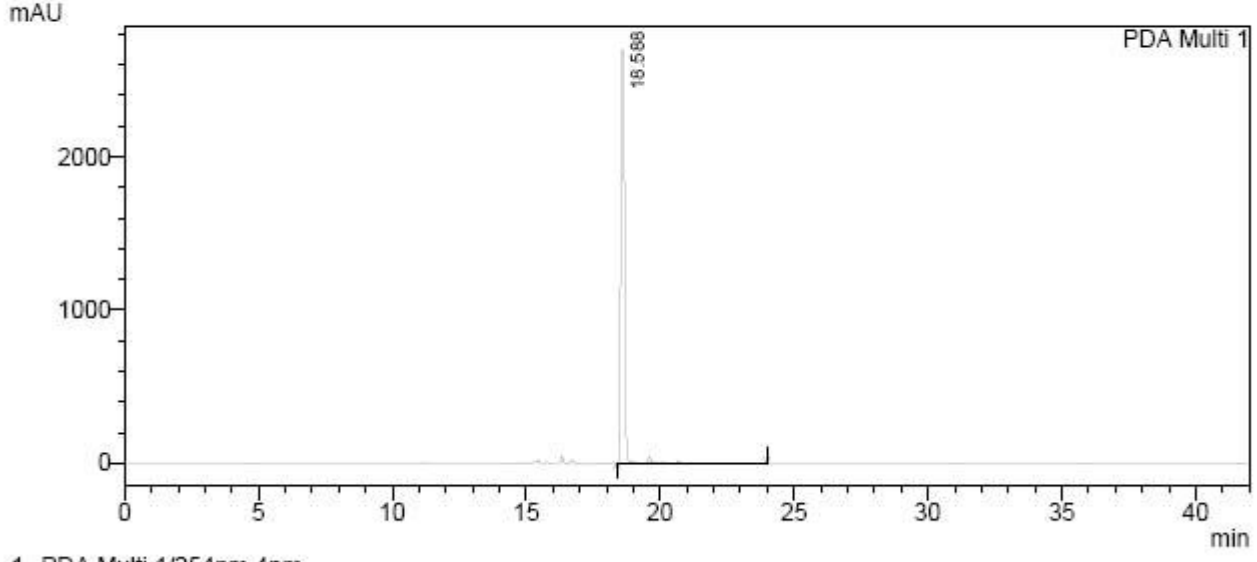
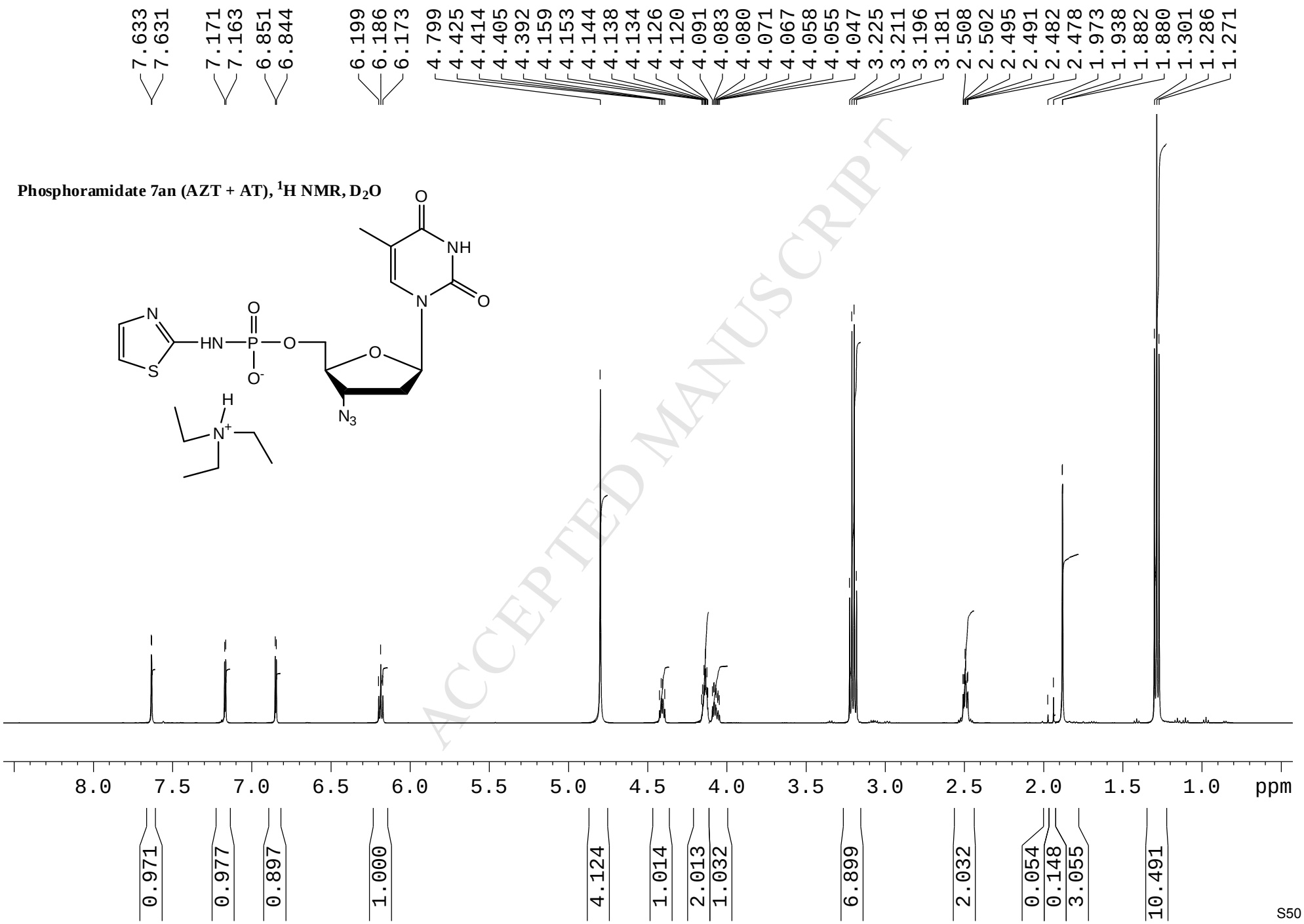
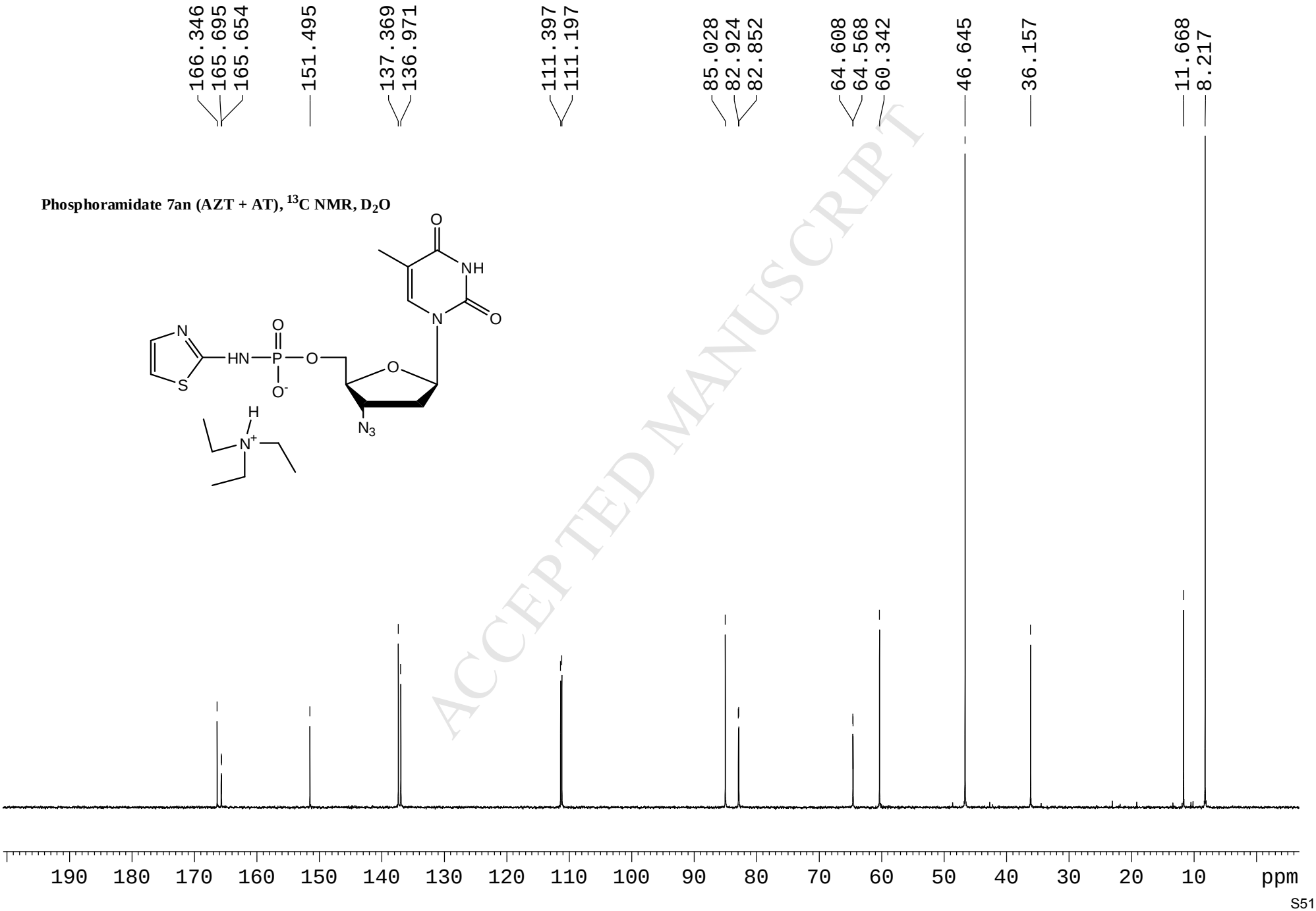
Phosphoramidate 7am (AZT + 5A2CNPy)



1 PDA Multi 1/254nm 4nm

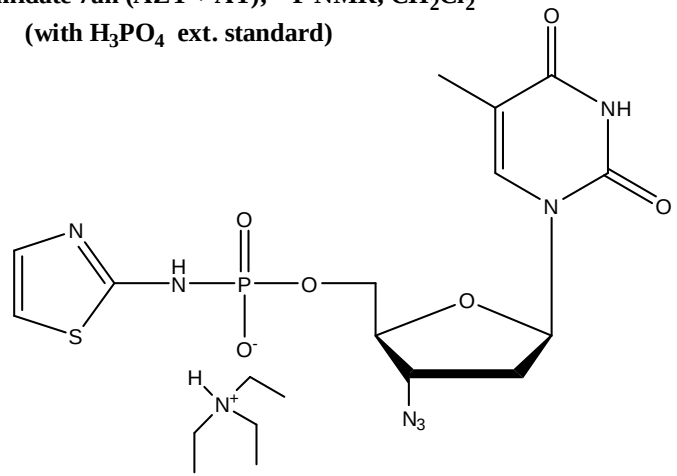
Phosphoramidate 7an (AZT + AT), ^{1}H NMR, D_2O





Phosphoramidate 7an (AZT + AT), ^{13}C NMR, D_2O

Phosphoramidate 7an (AZT + AT), ^{31}P NMR, CH_2Cl_2
(with H_3PO_4 ext. standard)



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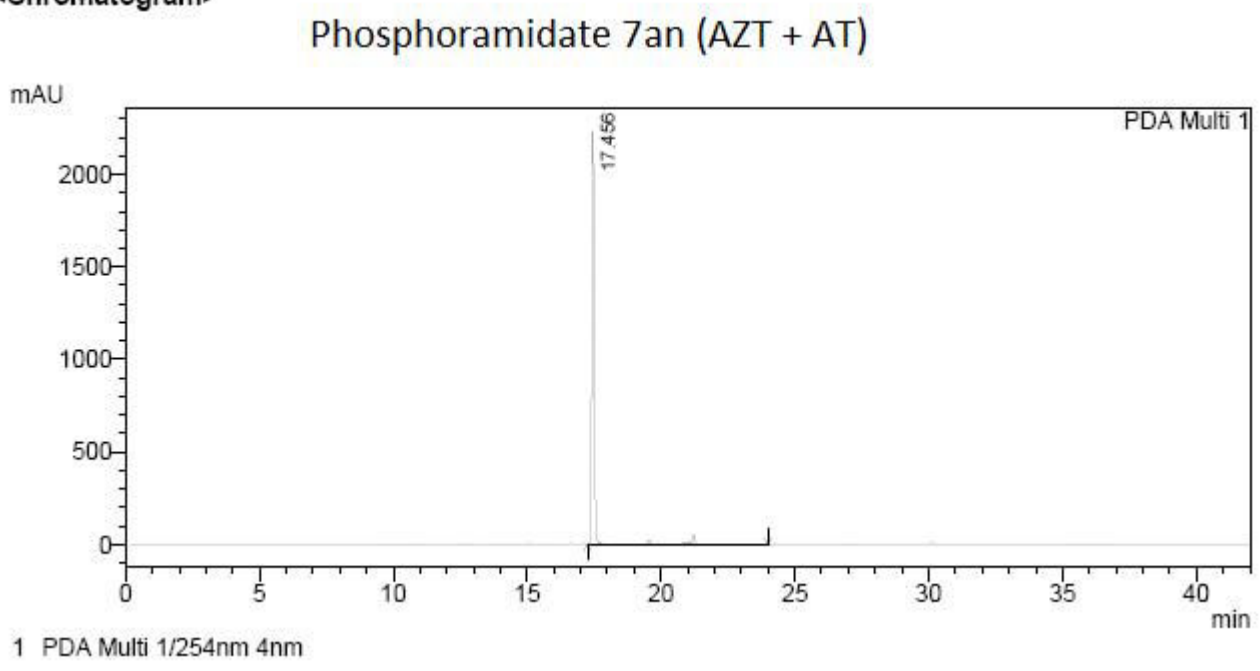
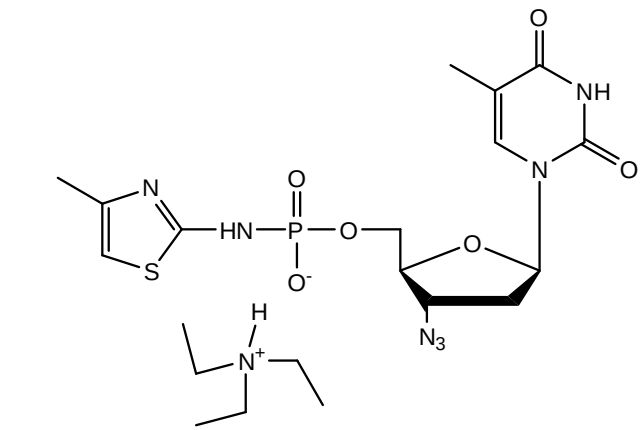
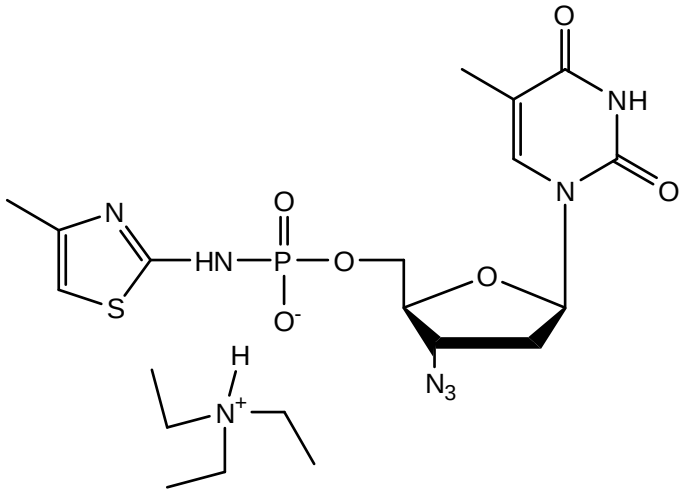


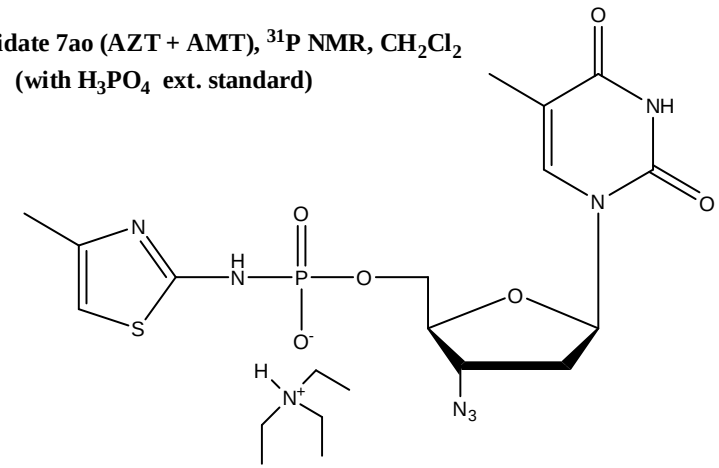
Phosphoramidate 7ao (AZT + AMT), ^{1}H NMR, D_2O



Phosphoramidate 7ao (AZT + AMT), ^{13}C NMR, D_2O



Phosphoramidate 7ao (AZT + AMT), ^{31}P NMR, CH_2Cl_2 (with H_3PO_4 ext. standard)



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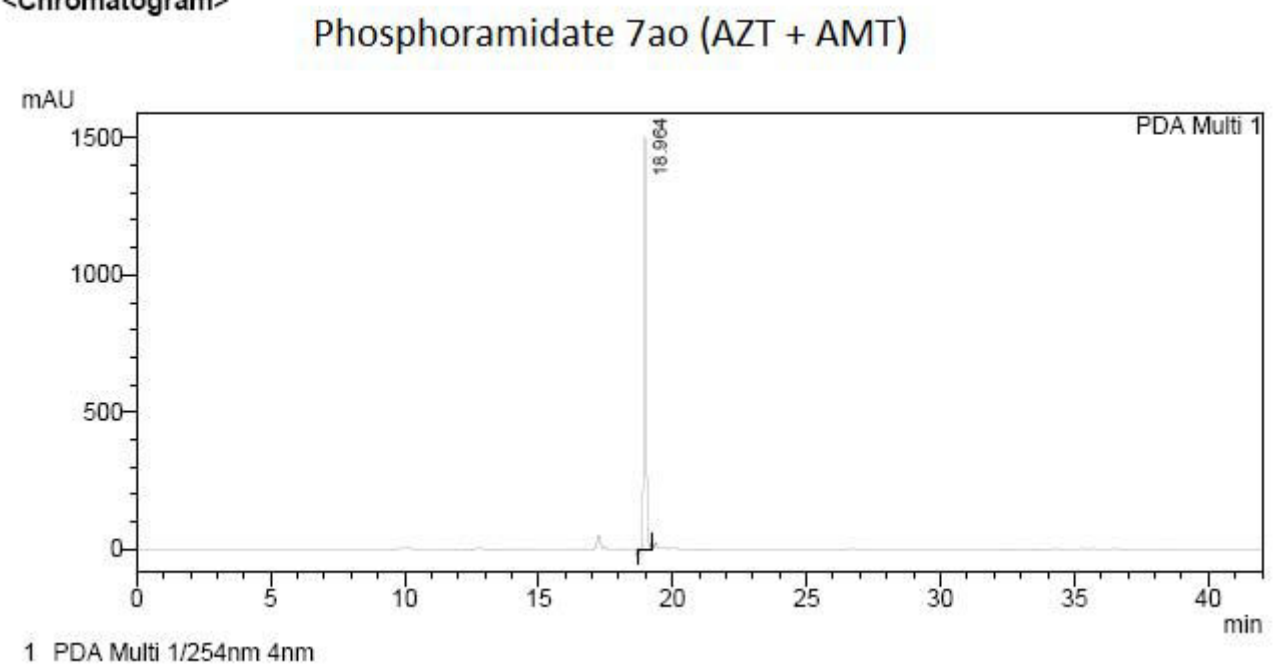
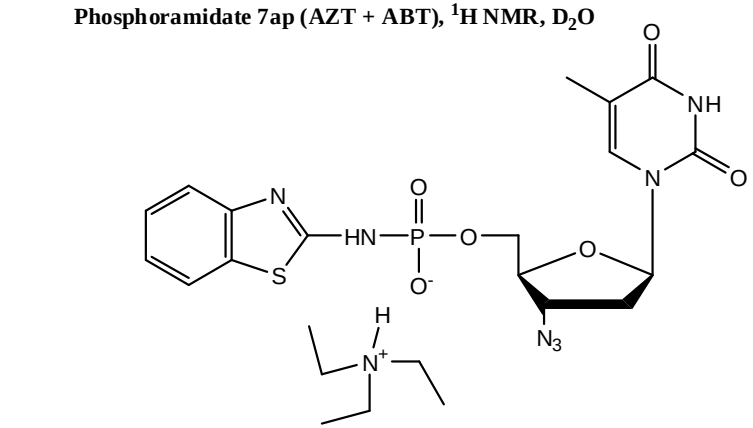
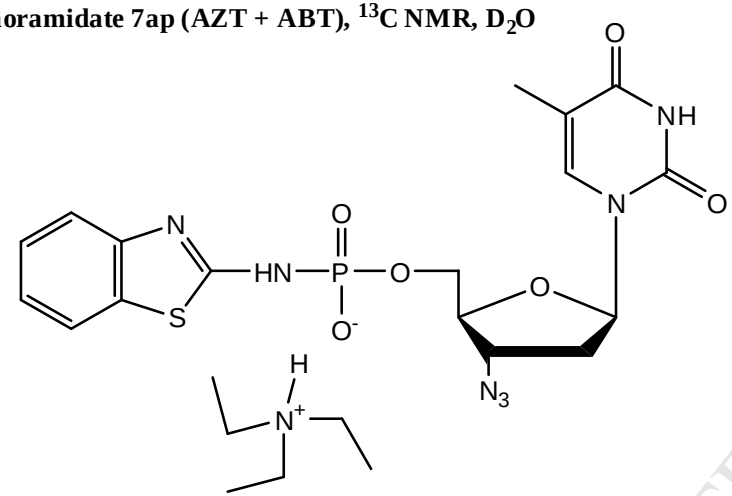


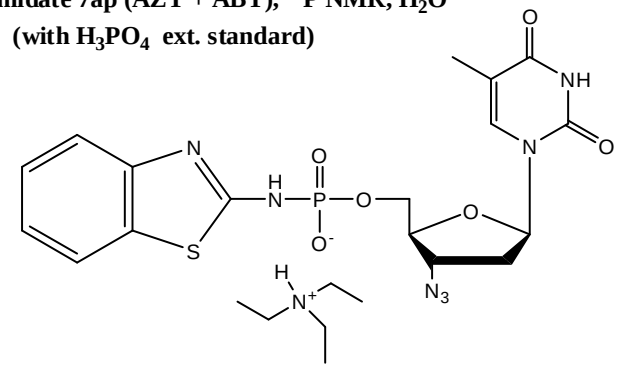
Phosphoramidate 7ap (AZT + ABT), ^{1}H NMR, D_2O



Phosphoramidate 7ap (AZT + ABT), ^{13}C NMR, D_2O



Phosphoramidate 7ap (AZT + ABT), ^{31}P NMR, H_2O
(with H_3PO_4 ext. standard)



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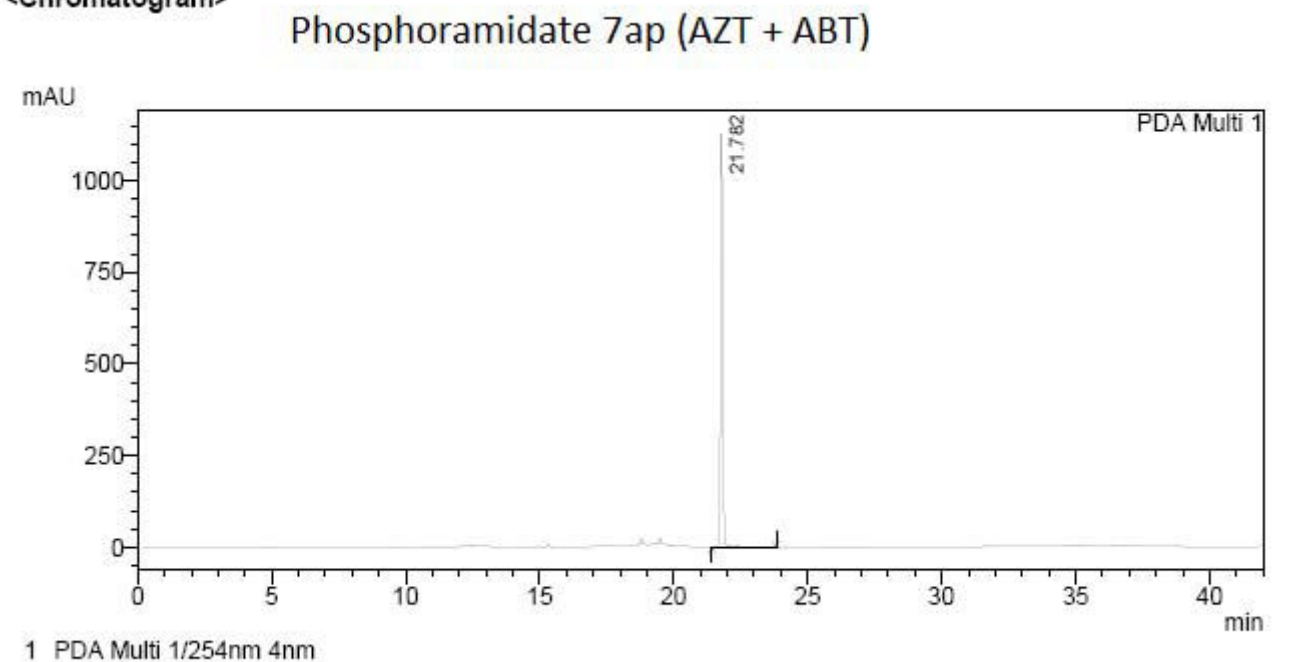
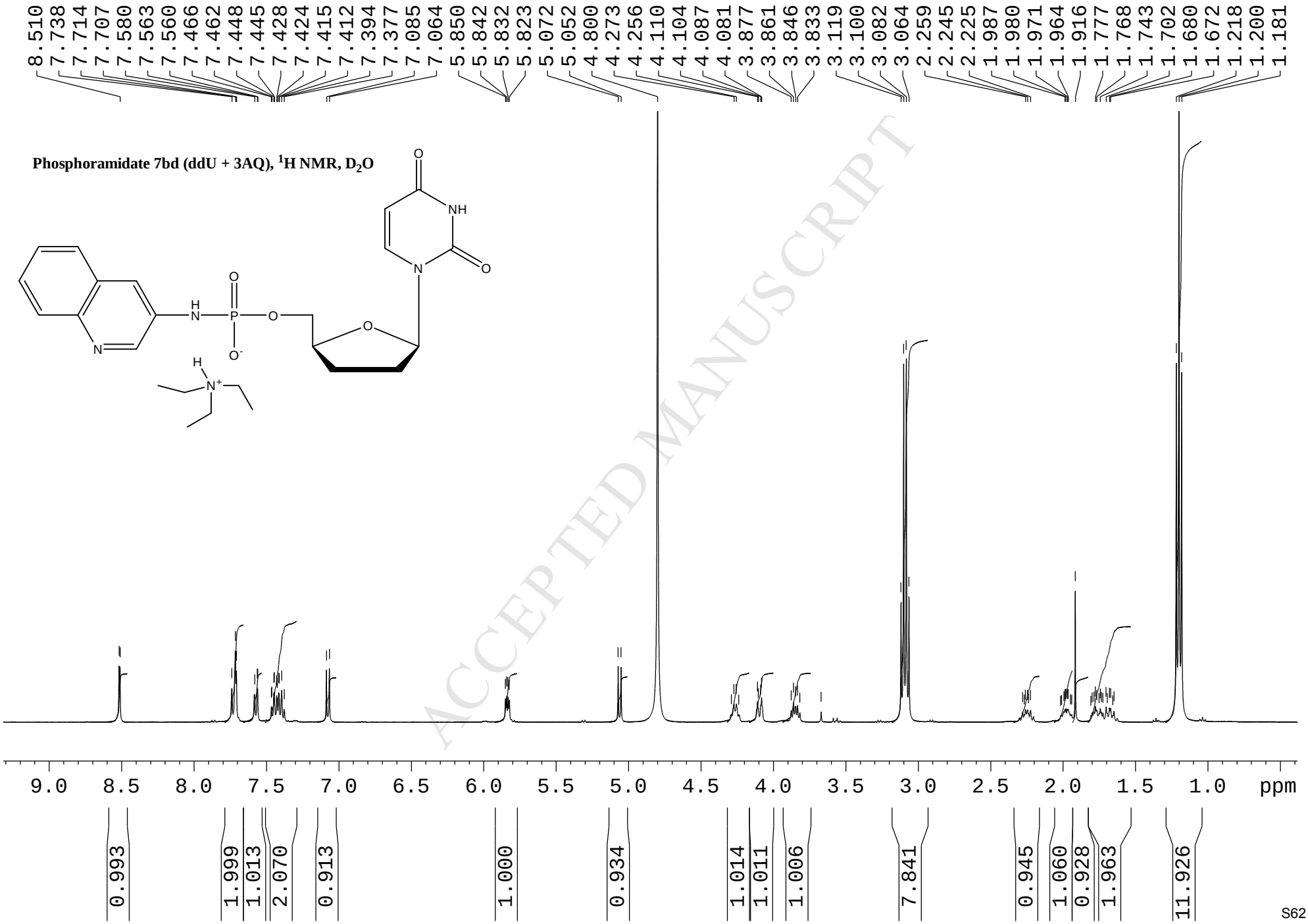
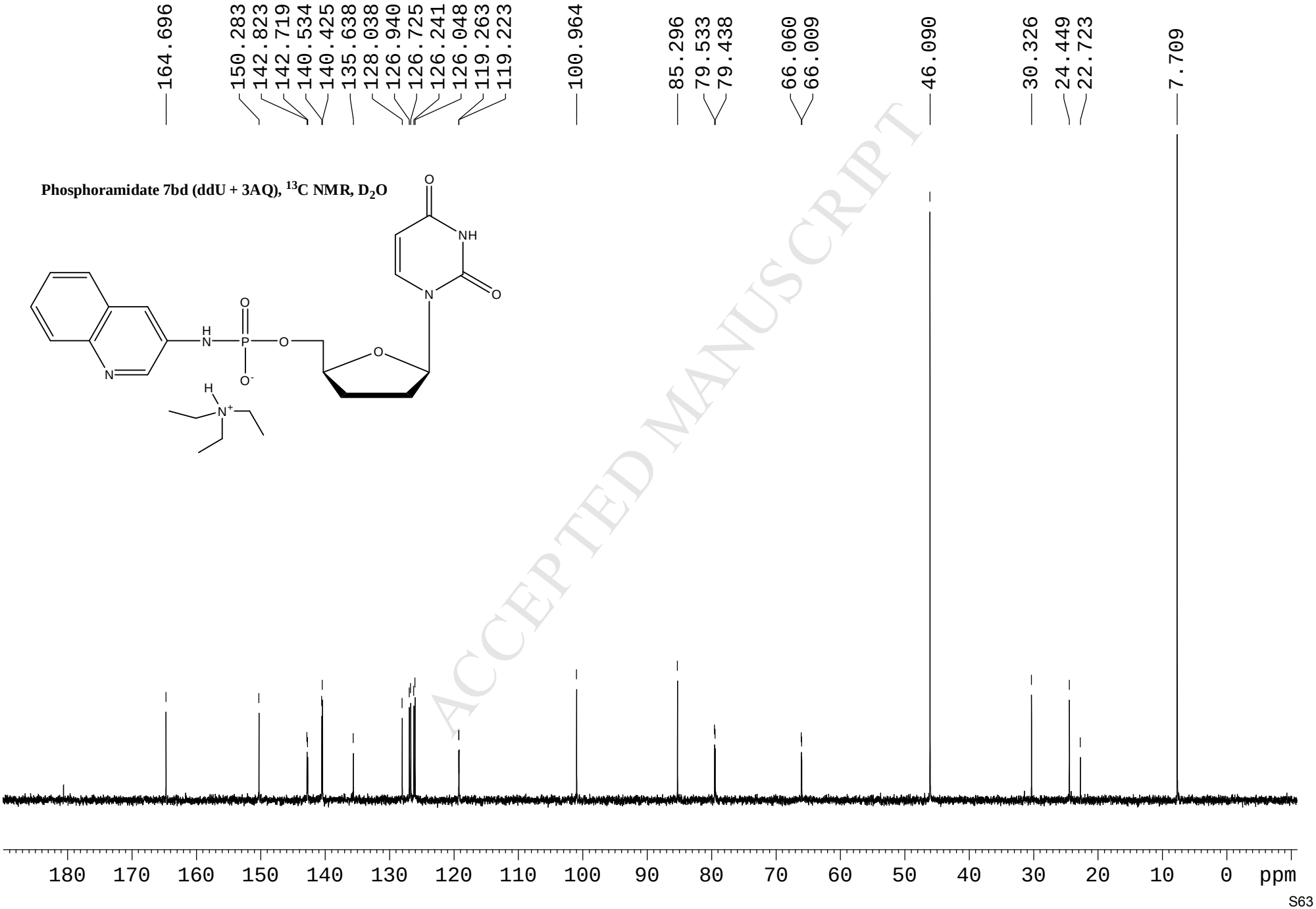
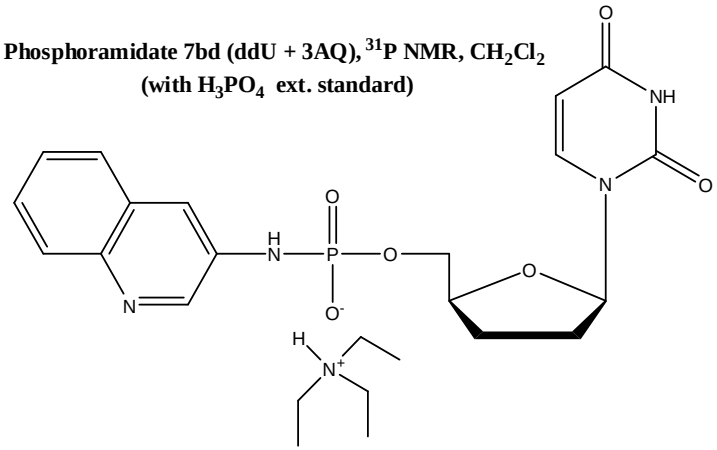






Phosphoramidate 7bd (ddU + 3AQ), ^{31}P NMR, CH_2Cl_2 (with H_3PO_4 ext. standard)



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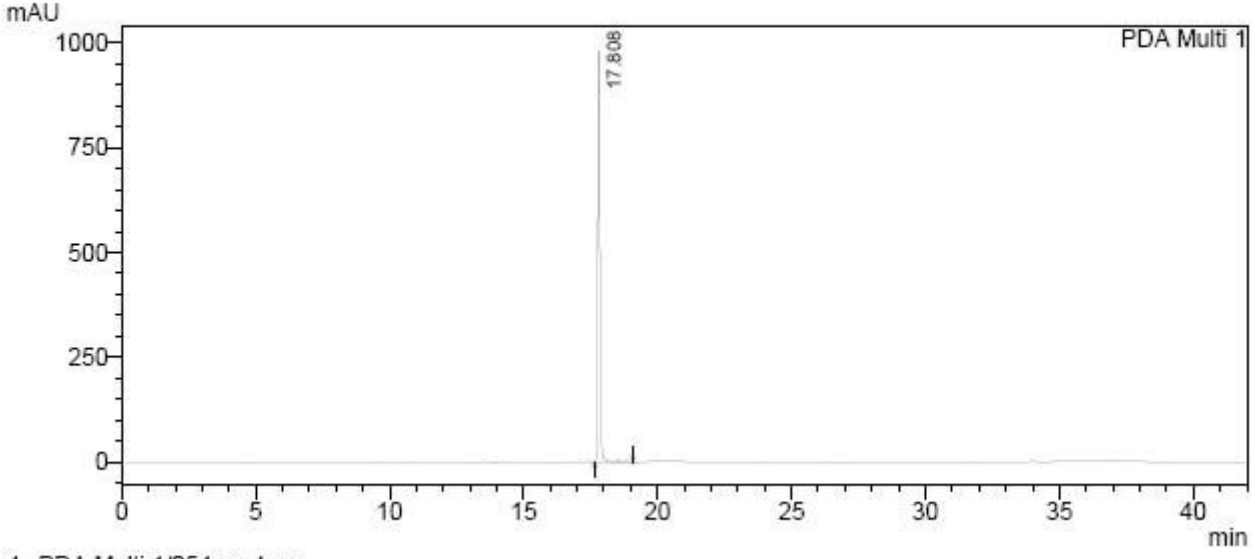
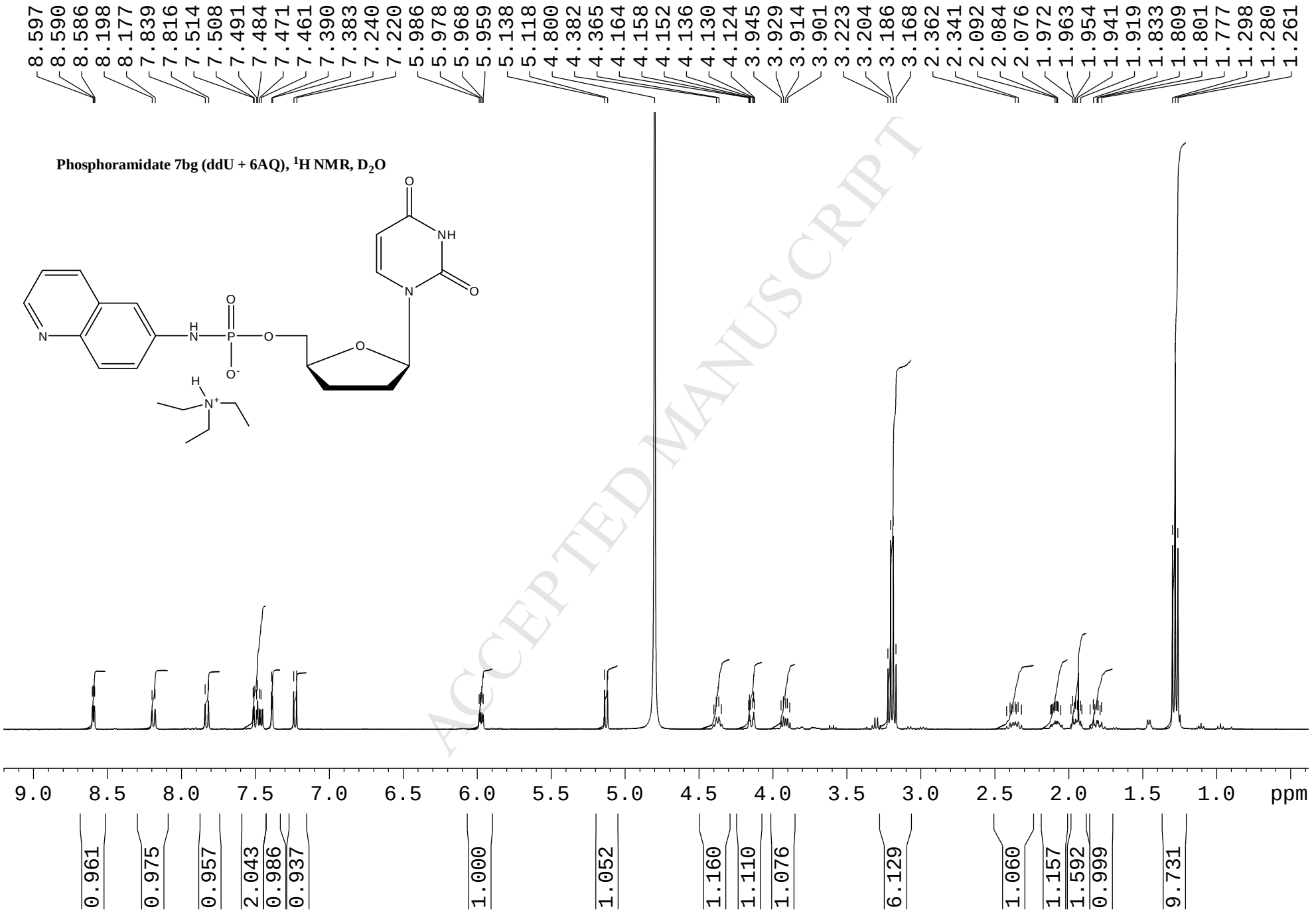
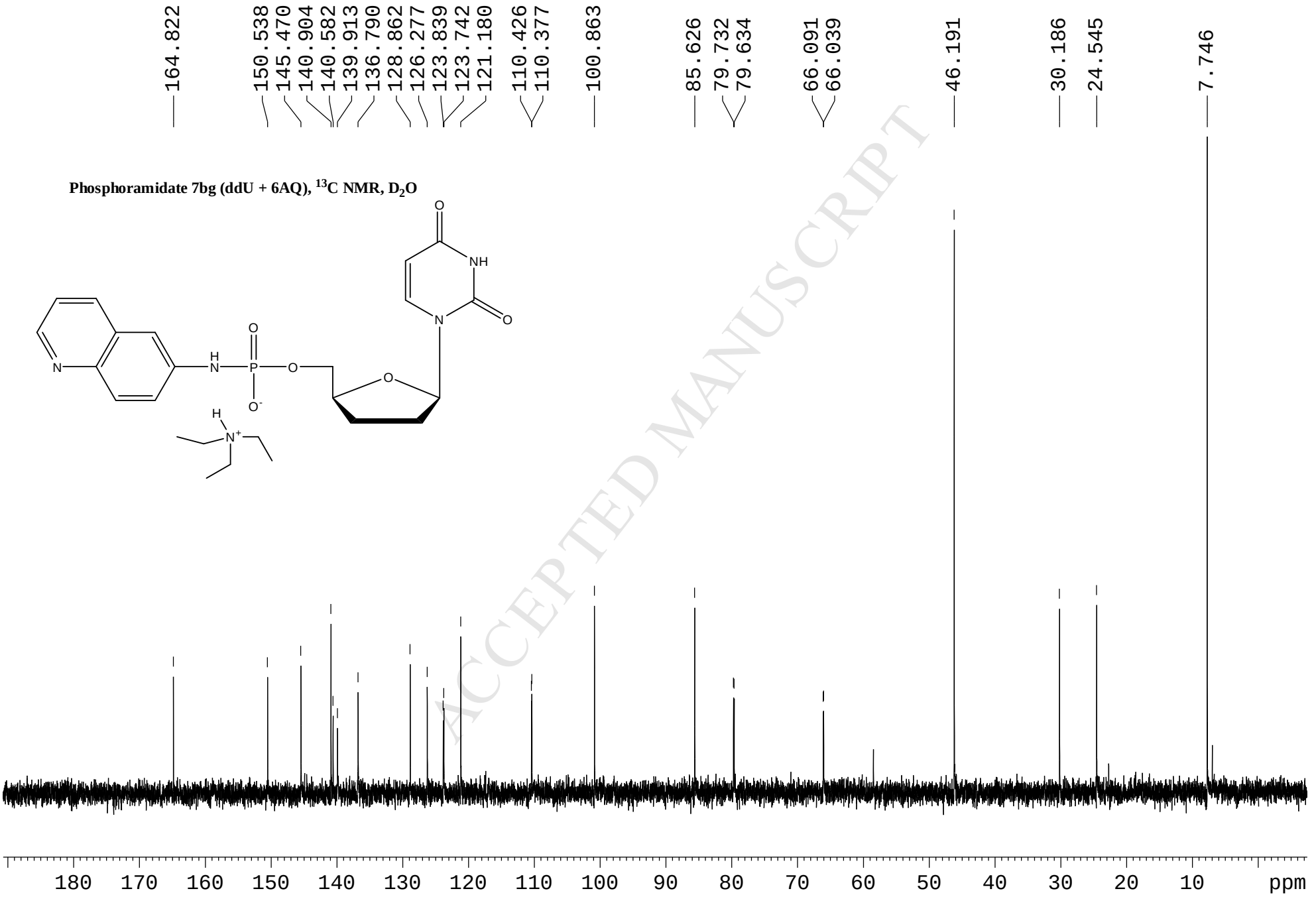
Phosphoramidate 7bd (ddU + 3AQ)

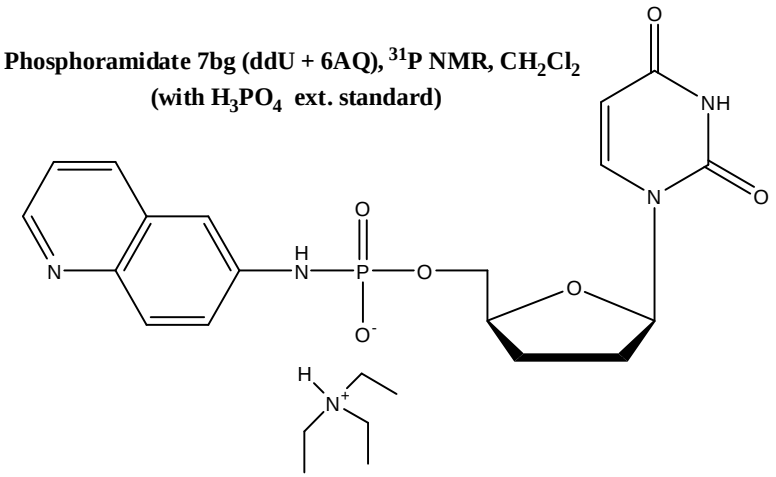


1 PDA Multi 1/254nm 4nm





Phosphoramidate 7bg (ddU + 6AQ), ^{31}P NMR, CH_2Cl_2
(with H_3PO_4 ext. standard)



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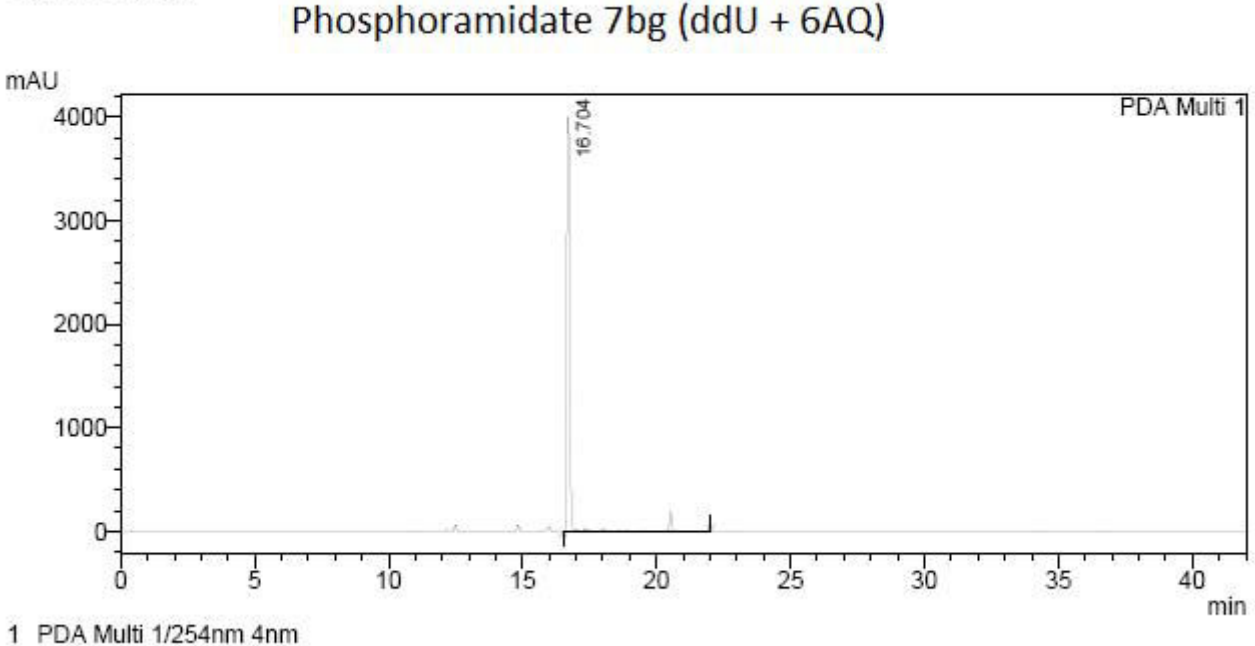
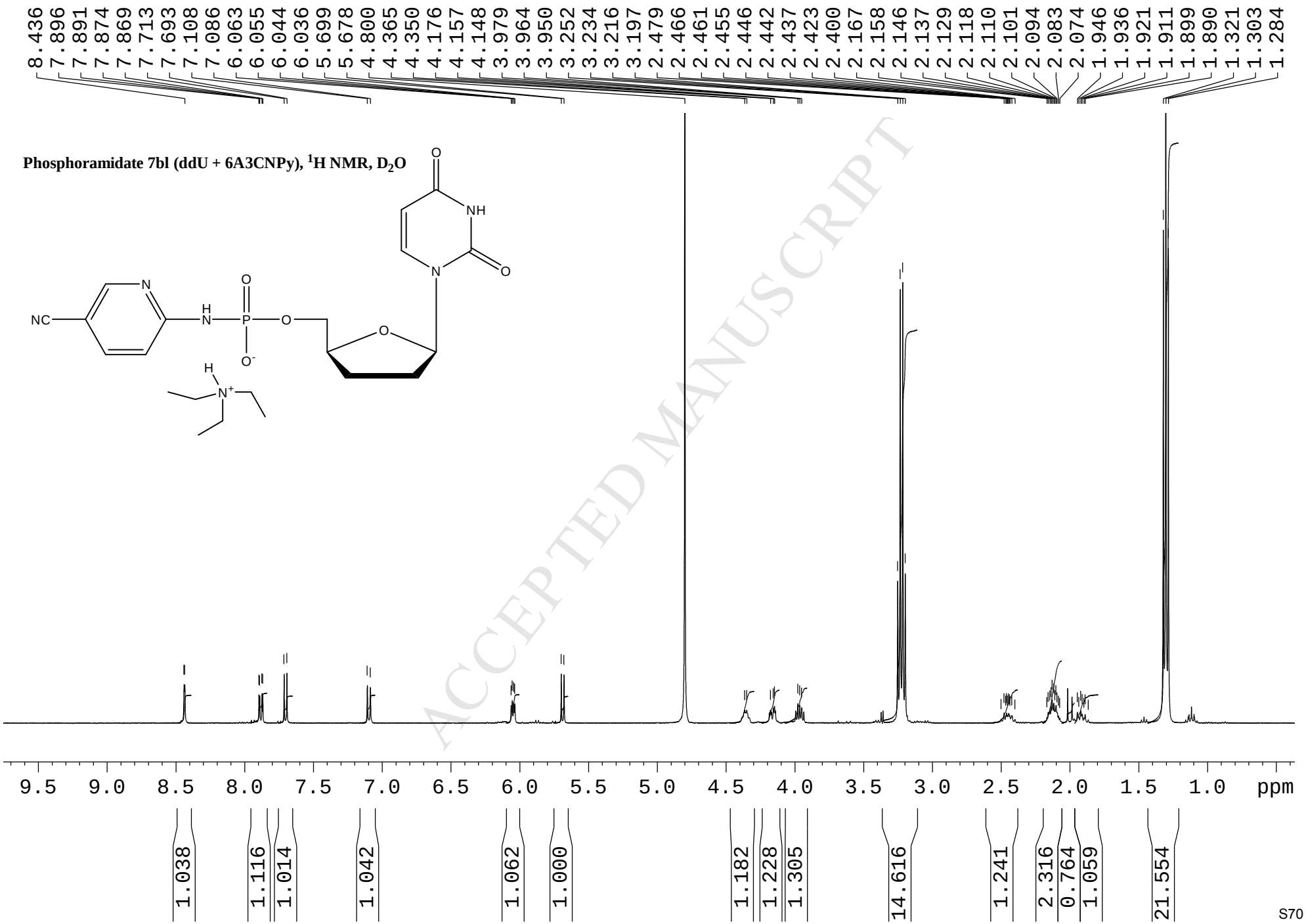
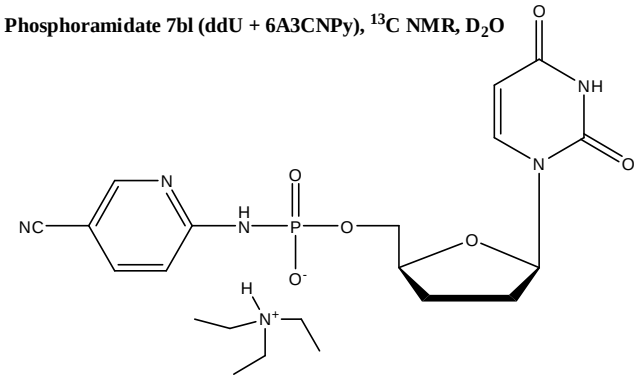
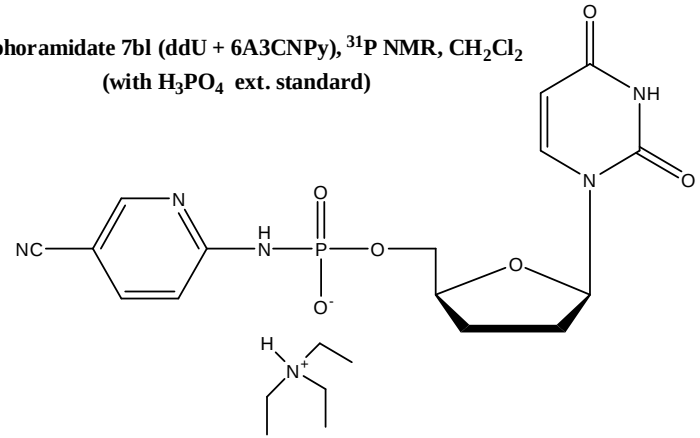




Phosphoramidate 7bl (ddU + 6A3CNPy), ^{13}C NMR, D_2O



Phosphoramidate 7bl (ddU + 6A3CNPy), ^{31}P NMR, CH_2Cl_2 (with H_3PO_4 ext. standard)



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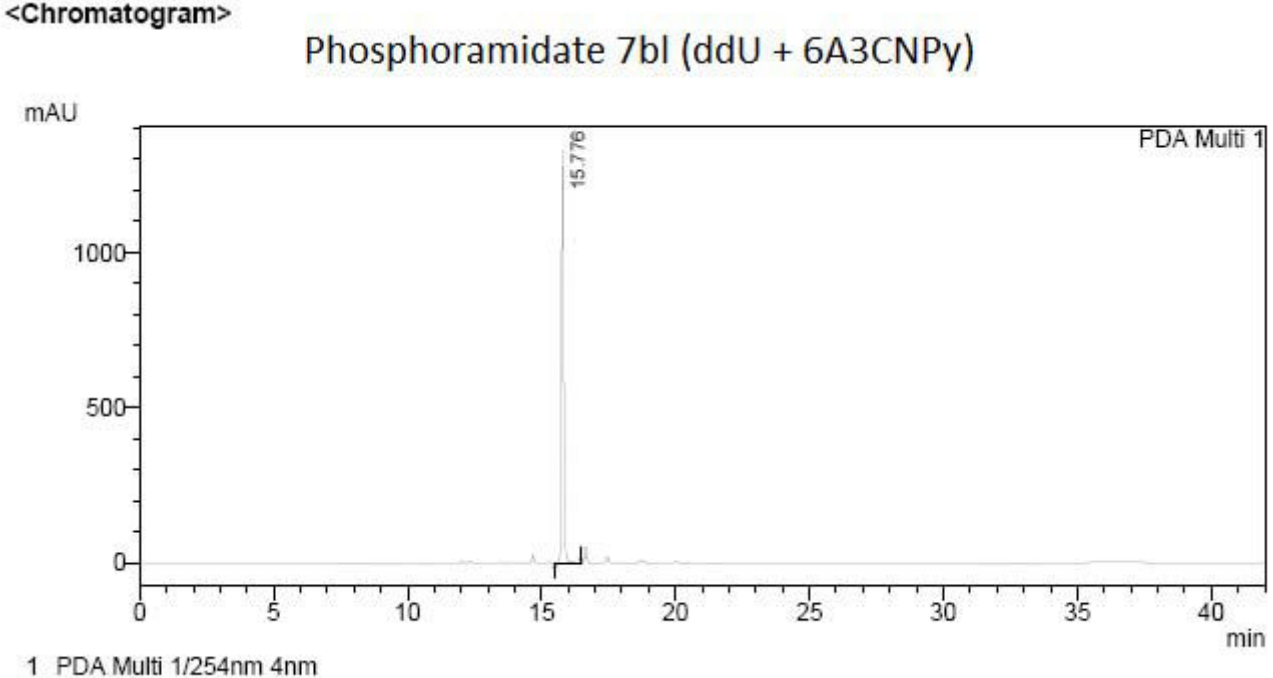
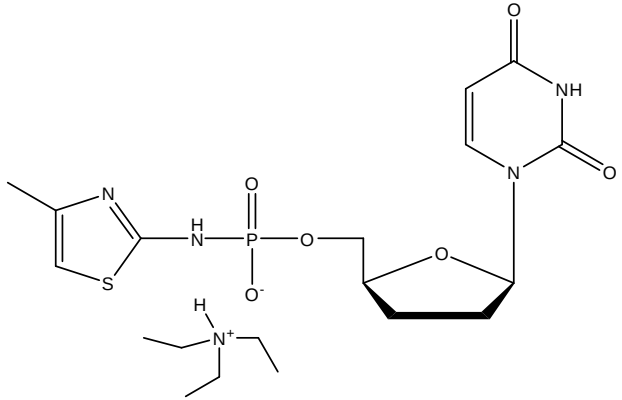
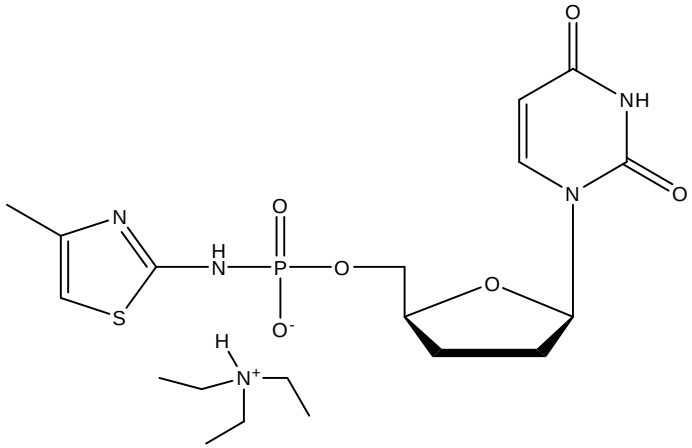


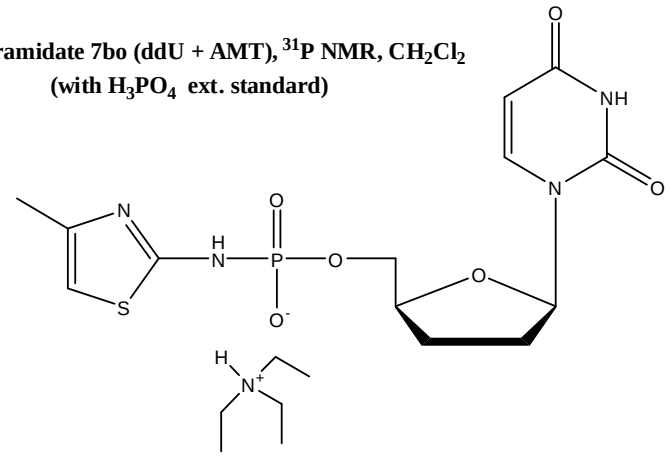
Phosphoramidate 7bo (ddU + AMT), ^{1}H NMR, D_2O



Phosphoramidate 7bo (ddU + AMT), ^{13}C NMR, D_2O



Phosphoramidate 7bo (ddU + AMT), ^{31}P NMR, CH_2Cl_2 (with H_3PO_4 ext. standard)



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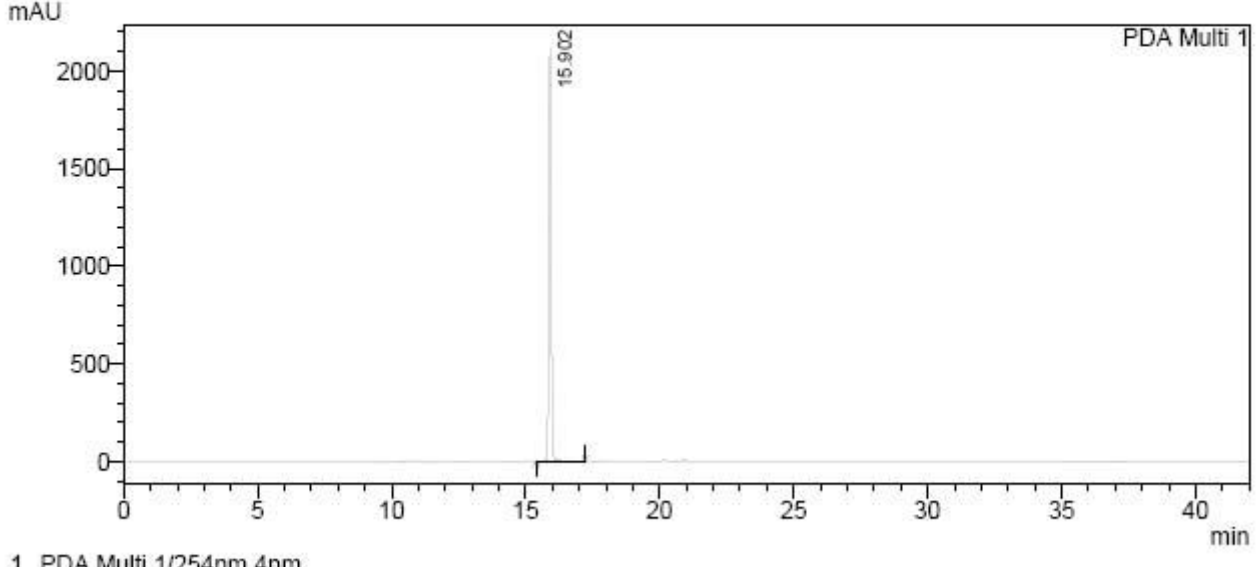
Phosphoramidate 7bo (ddU + AMT)



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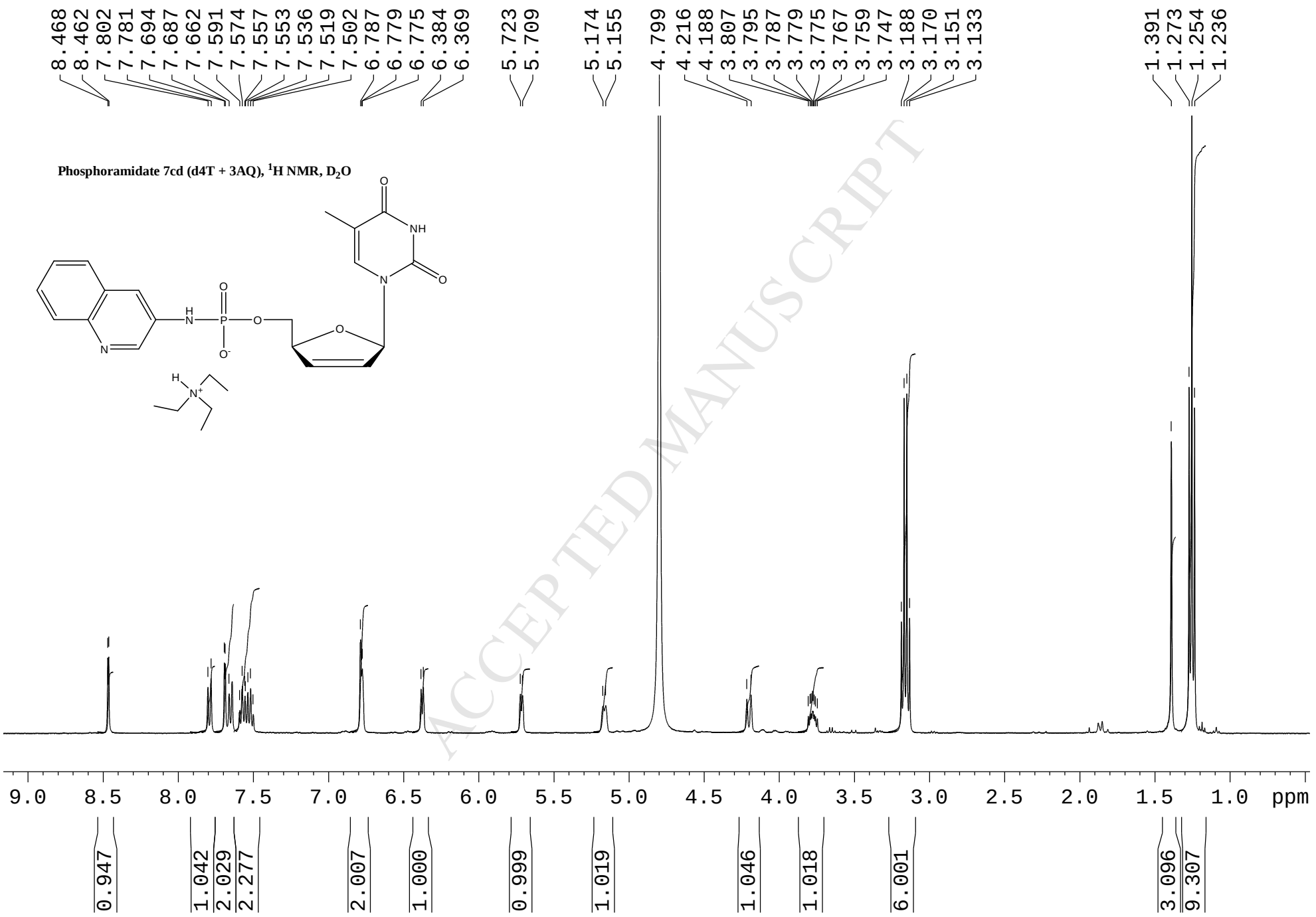
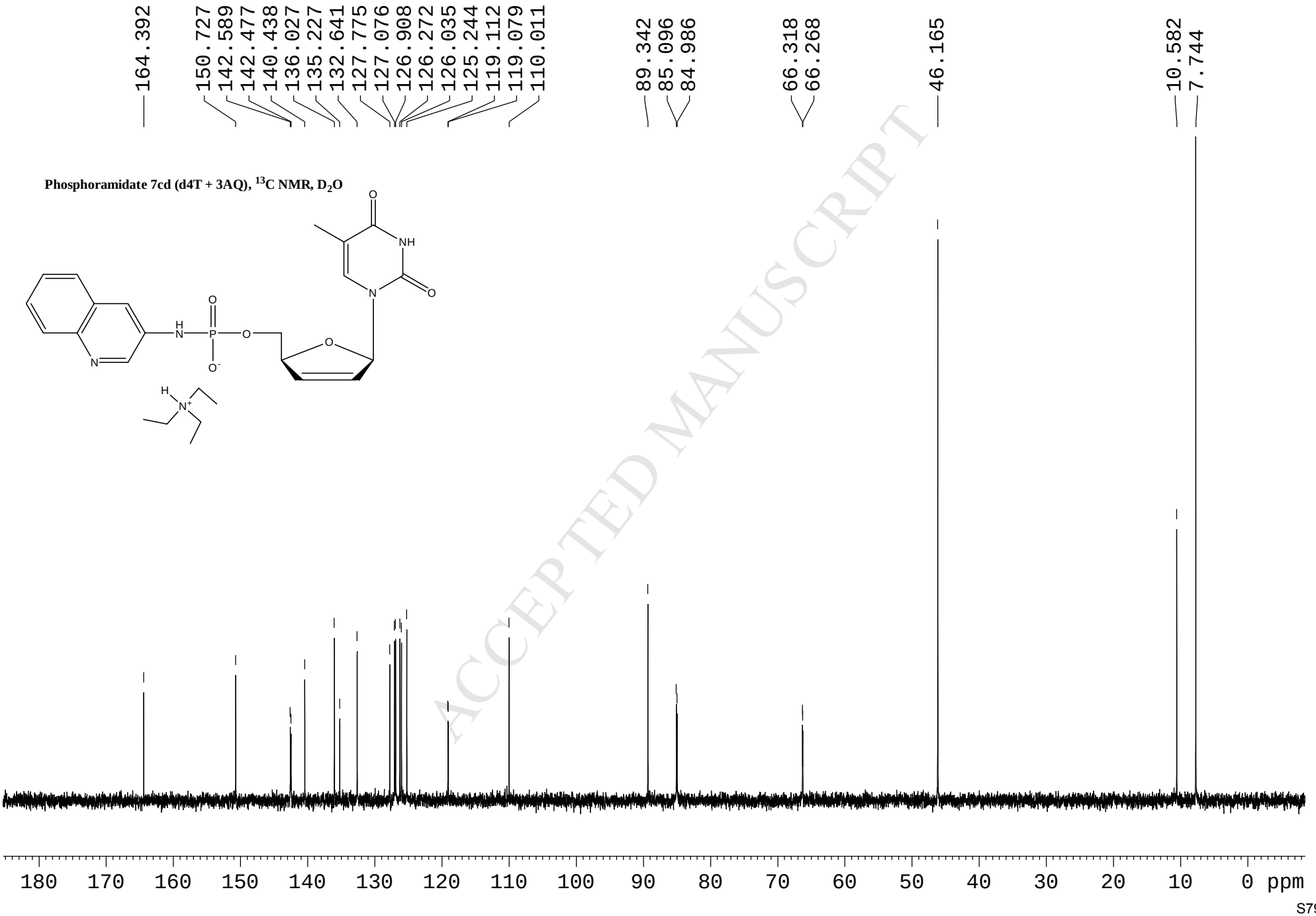
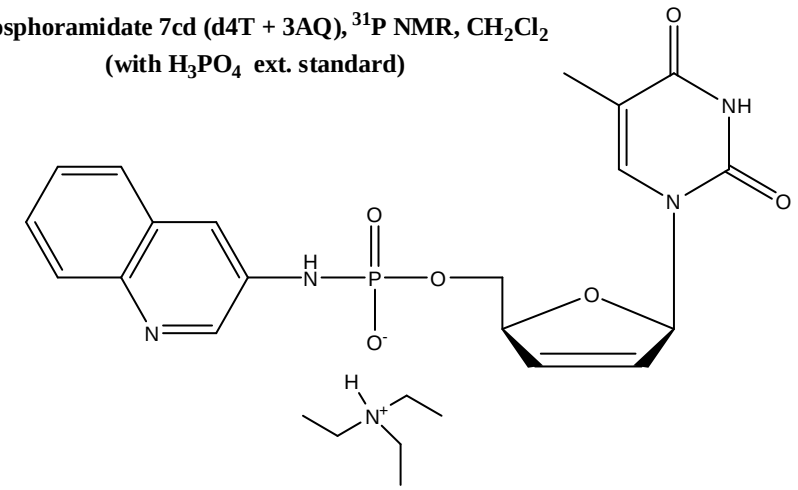




Phosphoramidate 7cd (d4T + 3AQ), ^{31}P NMR, CH_2Cl_2
(with H_3PO_4 ext. standard)



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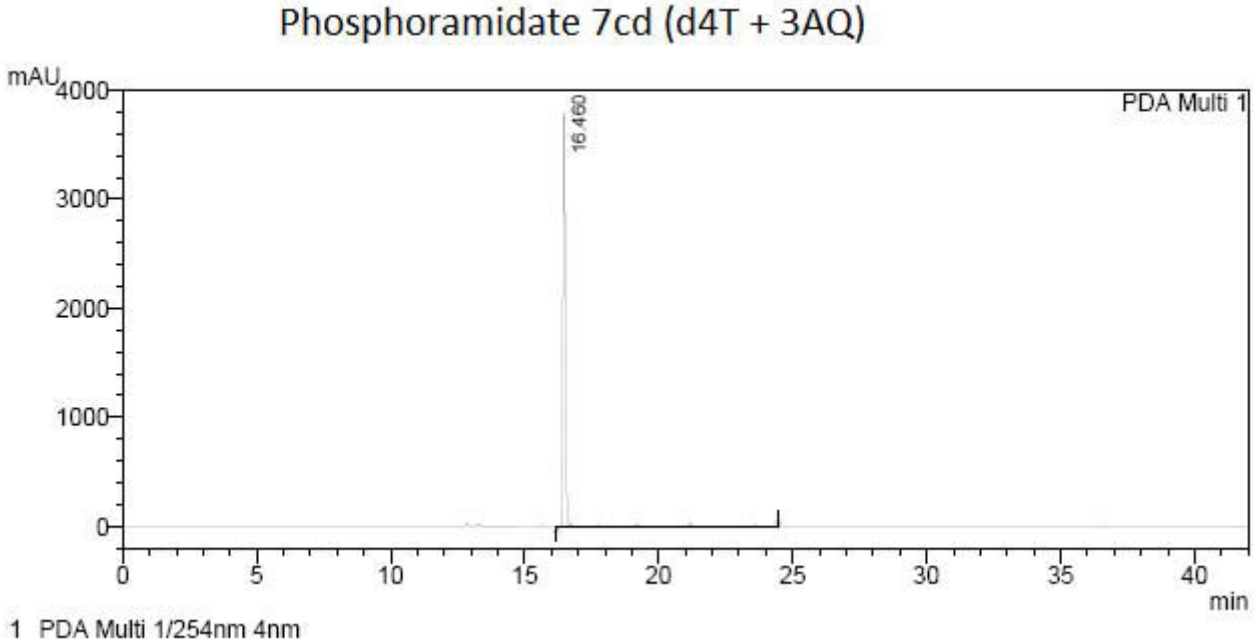

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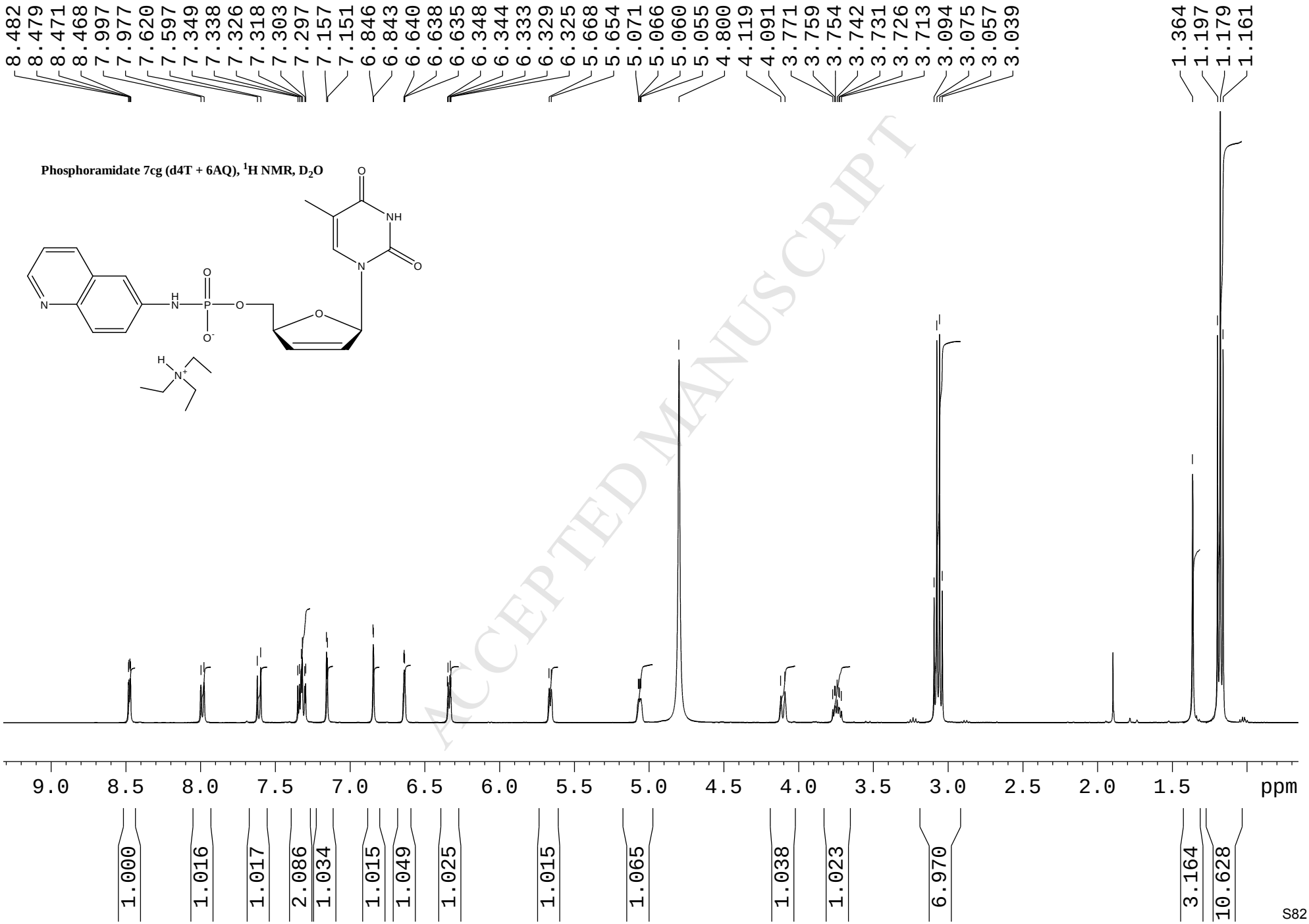
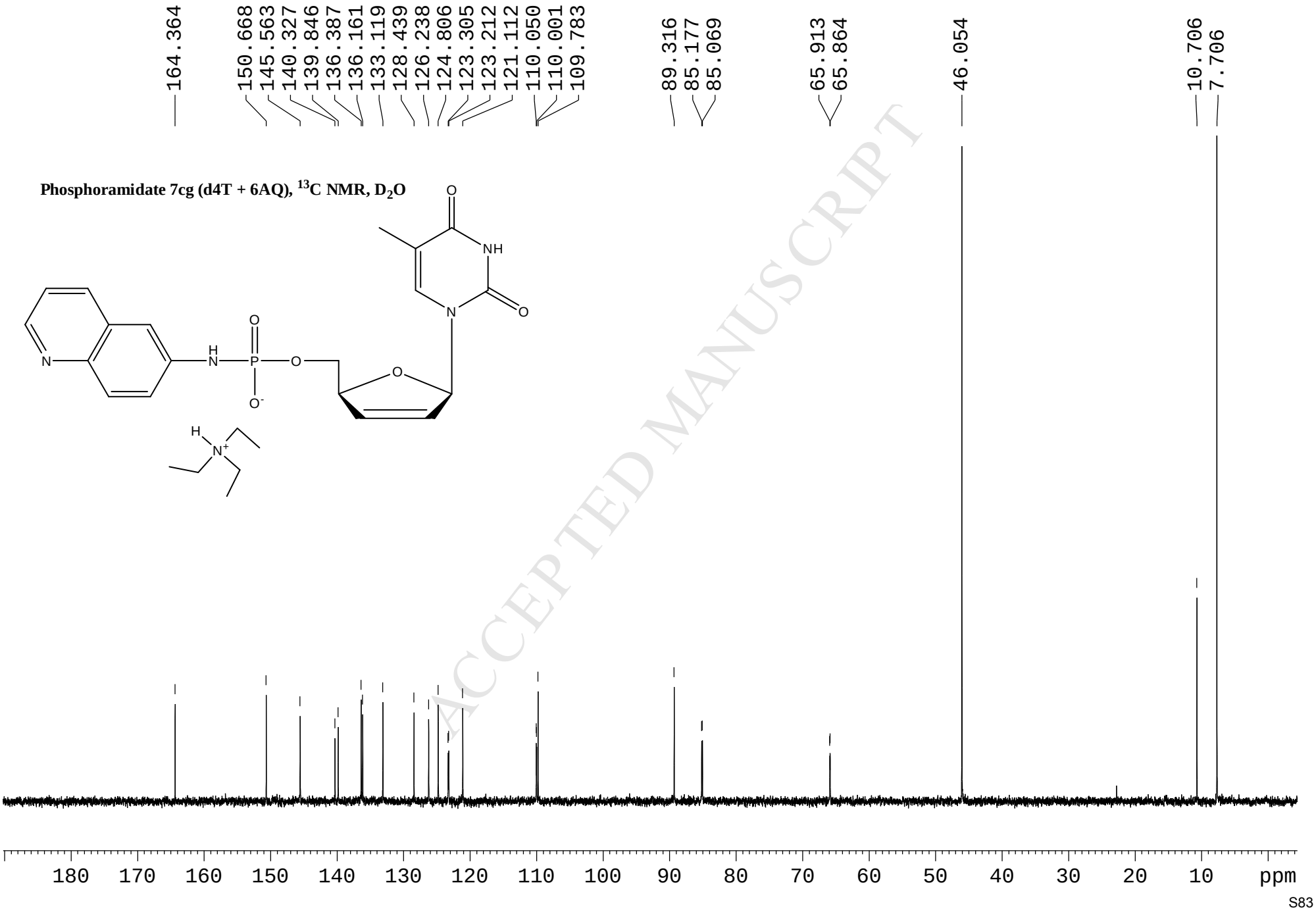
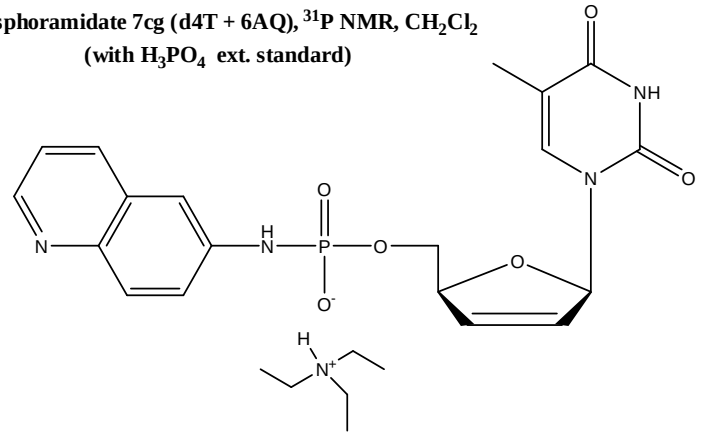




Phosphoramidate 7cg (d4T + 6AQ), ^{31}P NMR, CH_2Cl_2
(with H_3PO_4 ext. standard)



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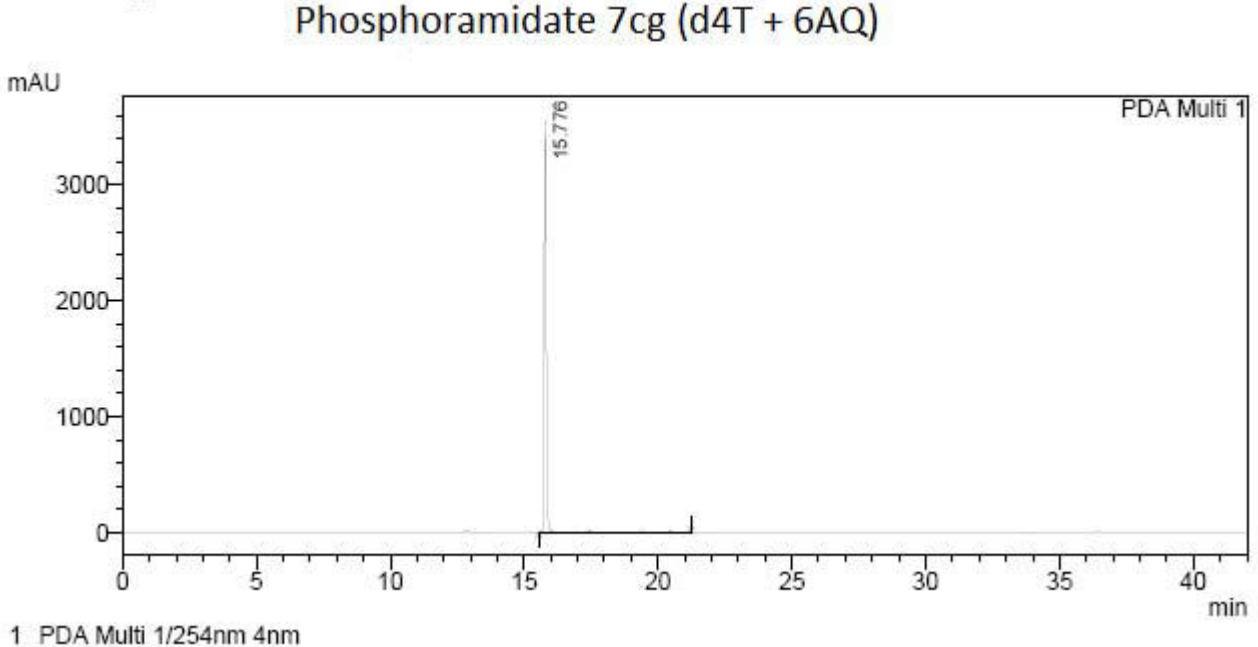

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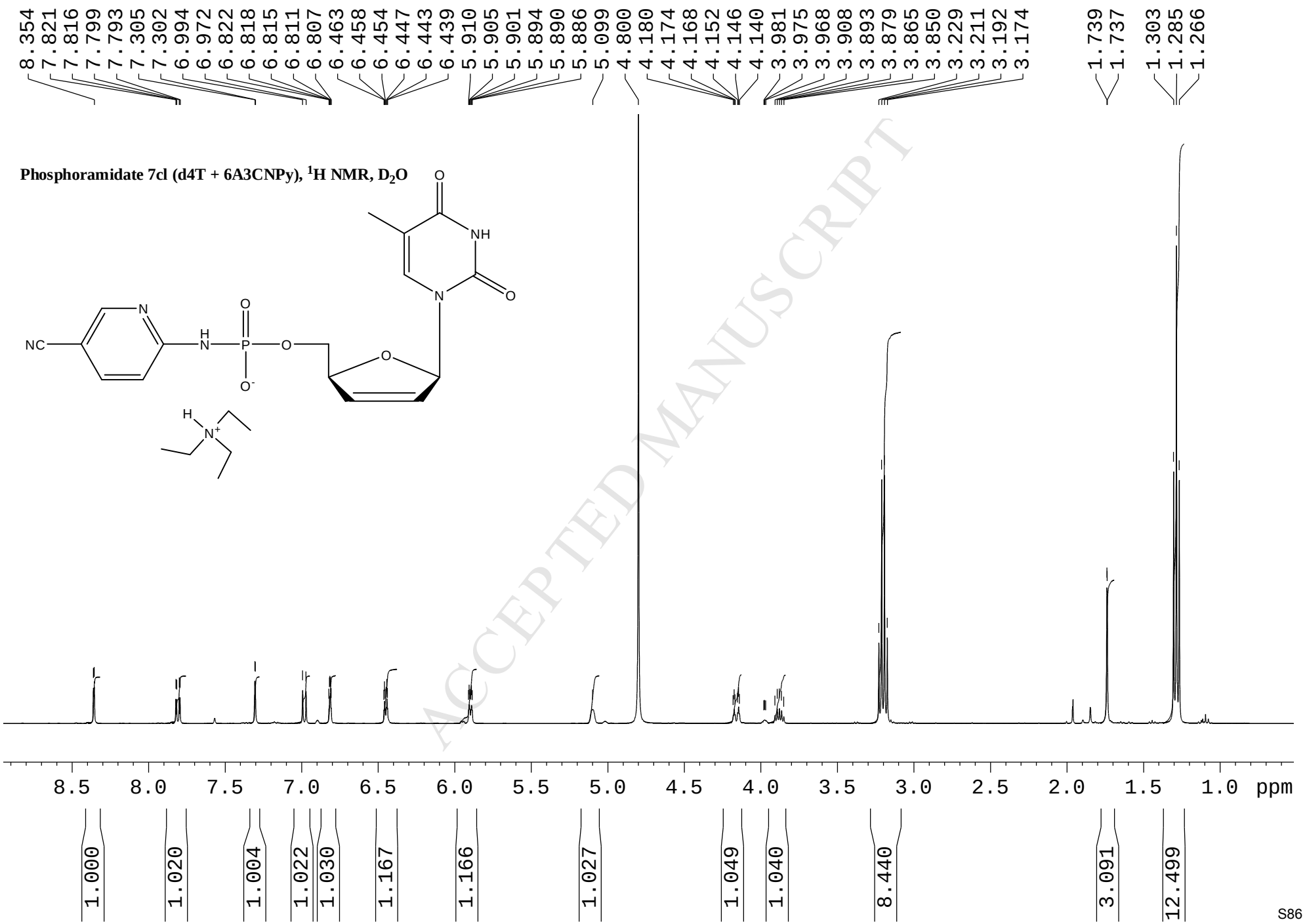
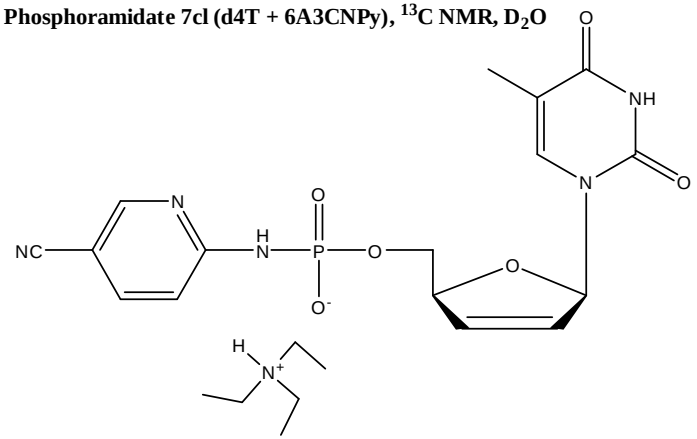
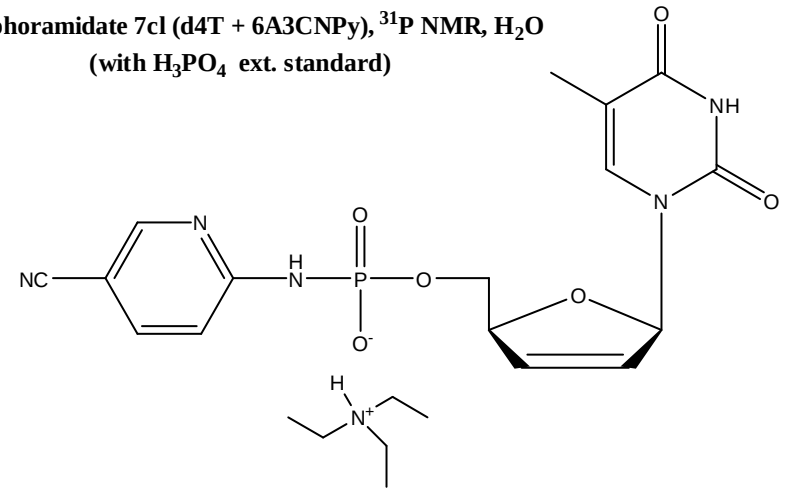


Phosphoramidate 7cl (d4T + 6A3CNPy), ^{13}C NMR, D_2O



Phosphoramidate 7cl (d4T + 6A3CNPy), ^{31}P NMR, H_2O
(with H_3PO_4 ext. standard)



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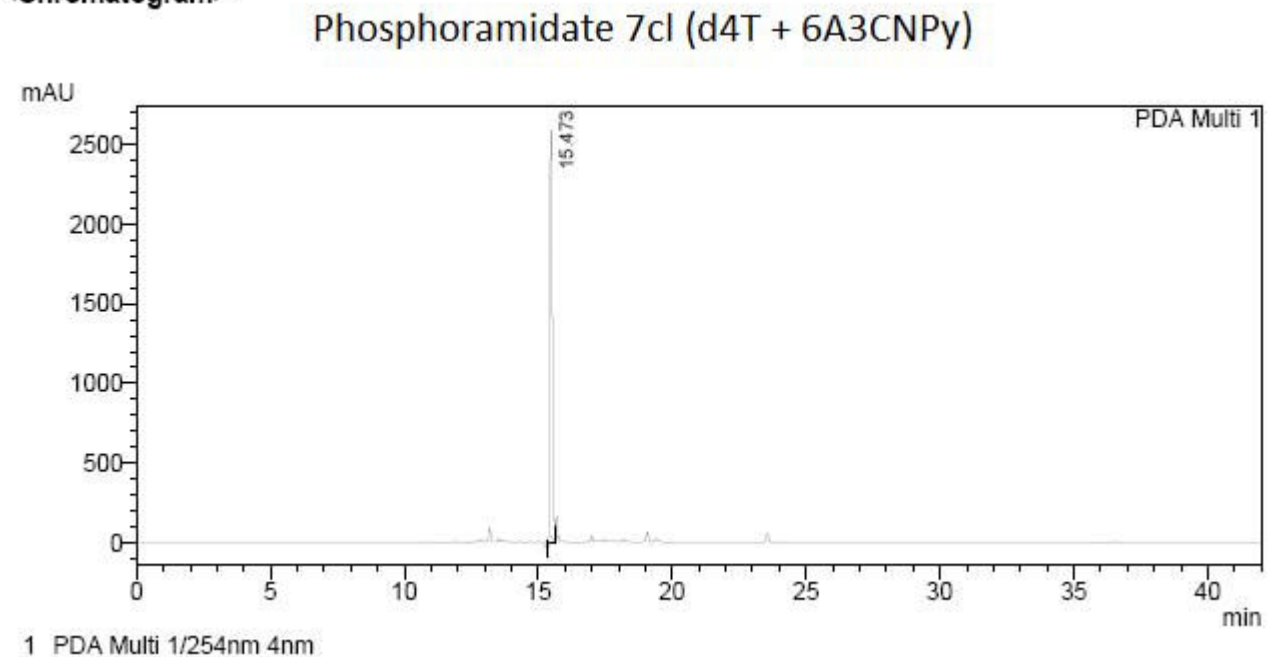
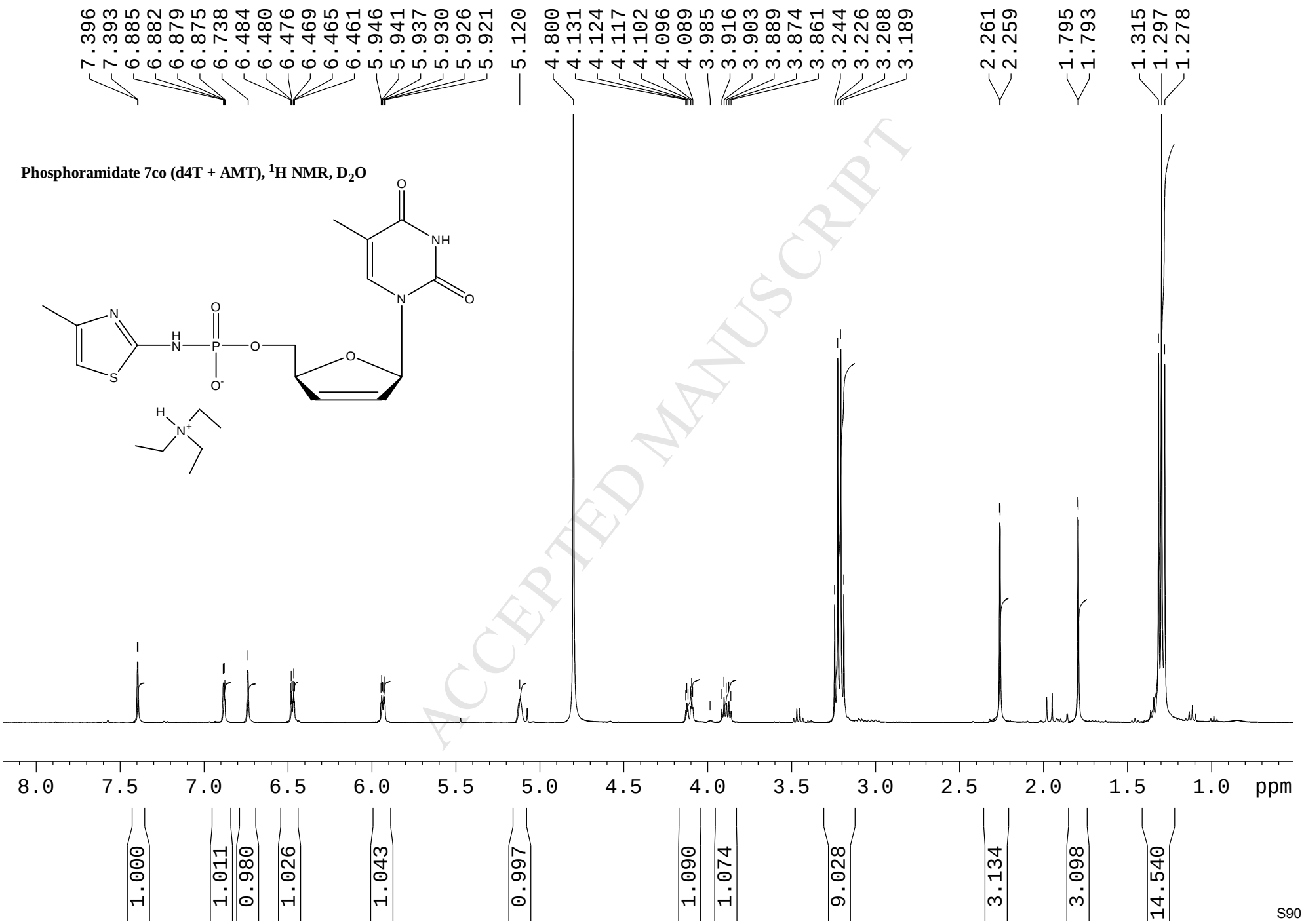
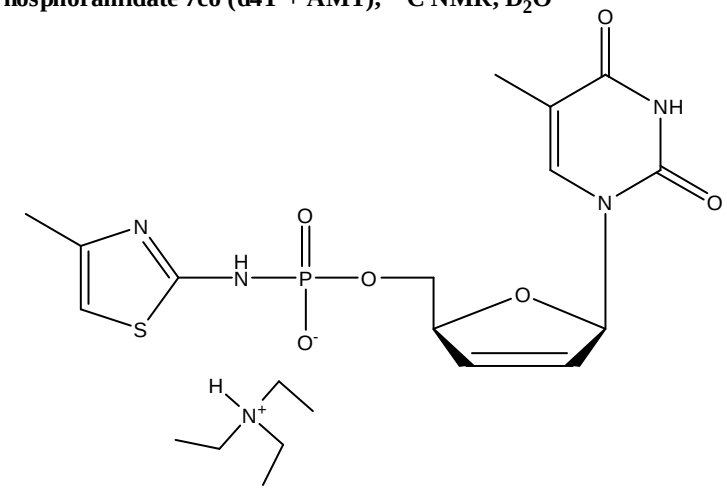
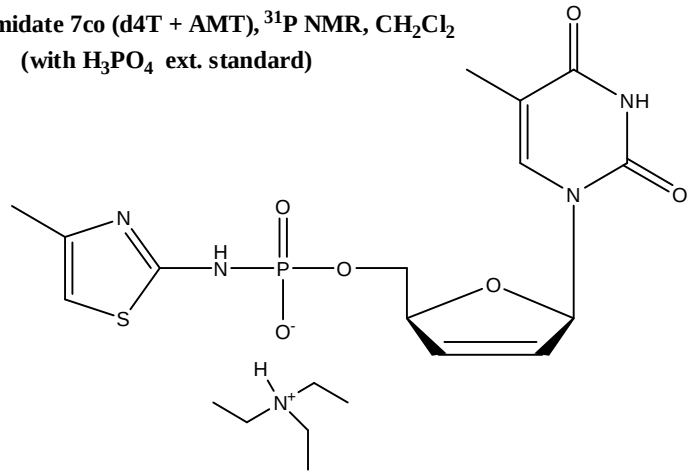




Phosphoramidate 7co (d4T + AMT), ^{13}C NMR, D_2O



Phosphoramidate 7co (d4T + AMT), ^{31}P NMR, CH_2Cl_2
(with H_3PO_4 ext. standard)



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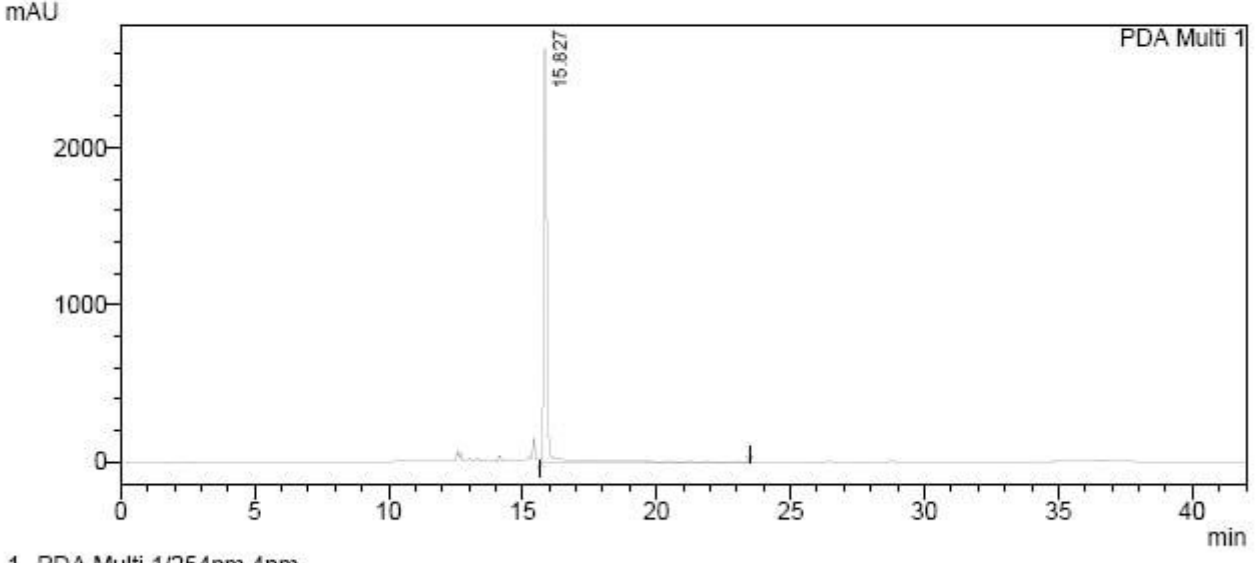
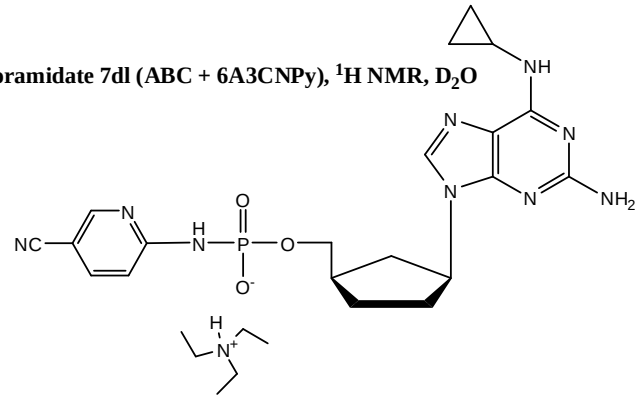
Phosphoramidate 7co (d4T + AMT)

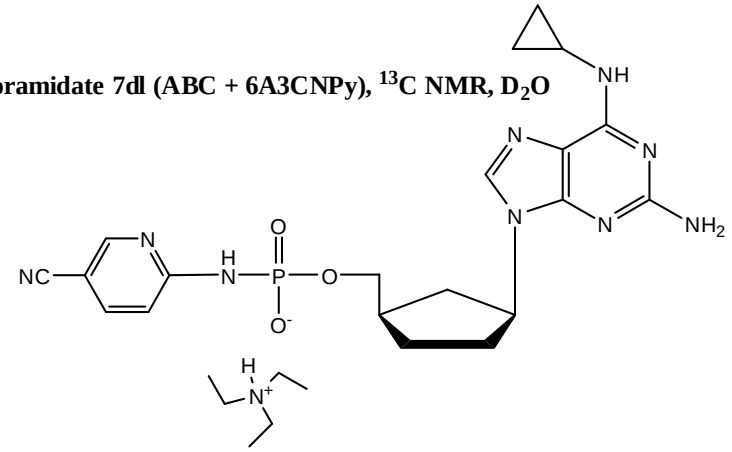


1 PDA Multi 1/254nm 4nm

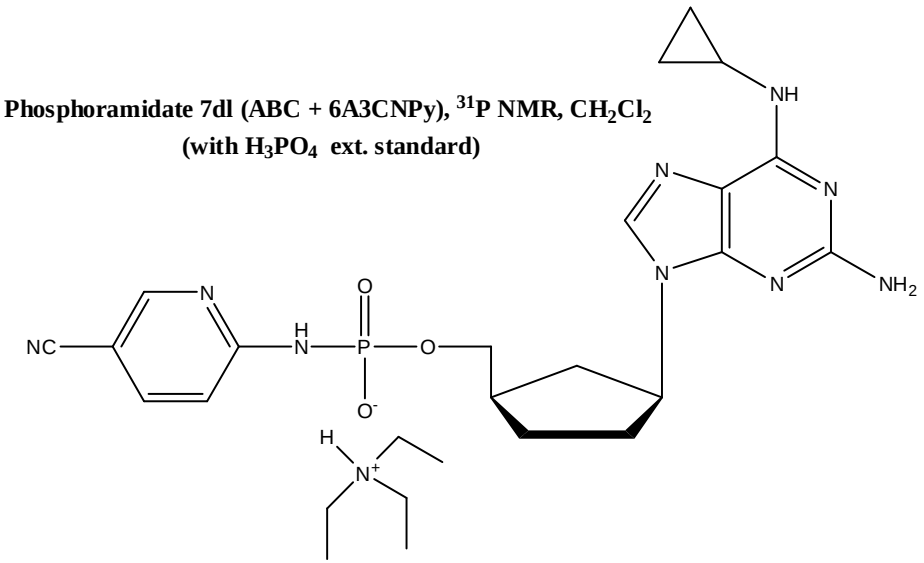
Phosphoramidate 7dl (ABC + 6A3CNPy), ^{1}H NMR, D_2O



Phosphoramidate 7dl (ABC + 6A3CNPy), ^{13}C NMR, D_2O



Phosphoramidate 7dl (ABC + 6A3CNPy), ^{31}P NMR, CH_2Cl_2
(with H_3PO_4 ext. standard)



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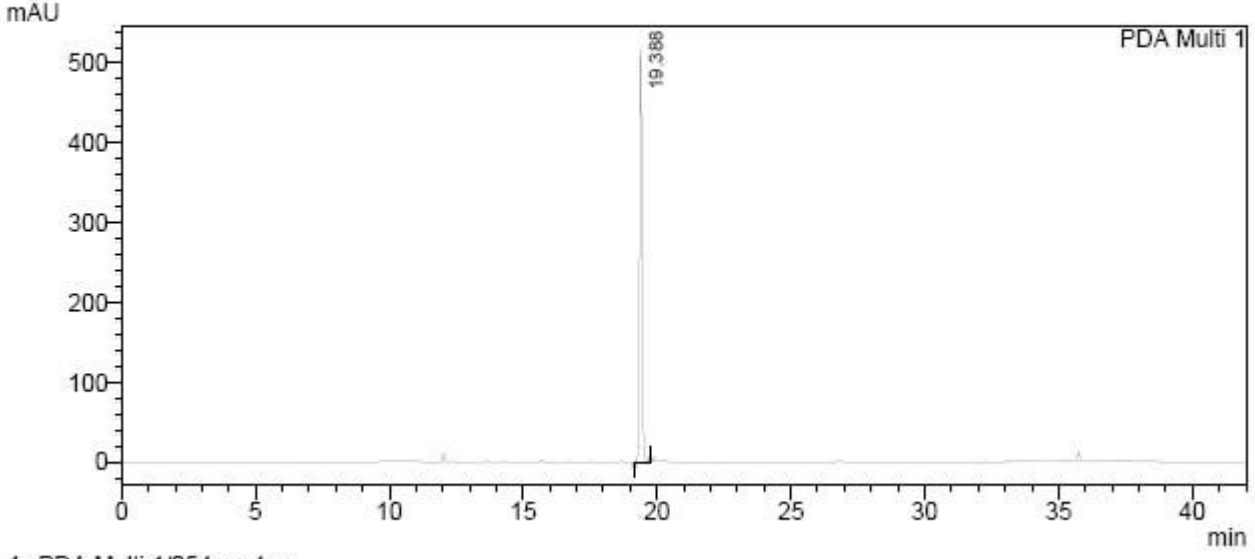
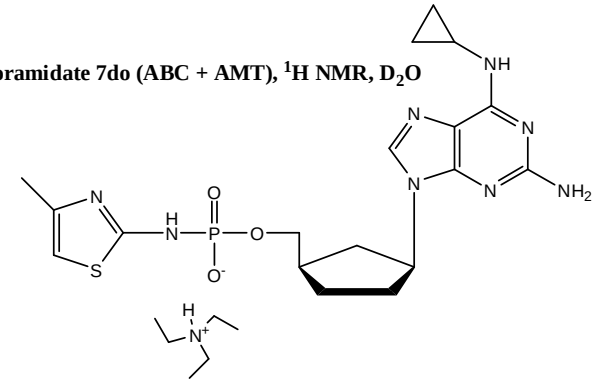
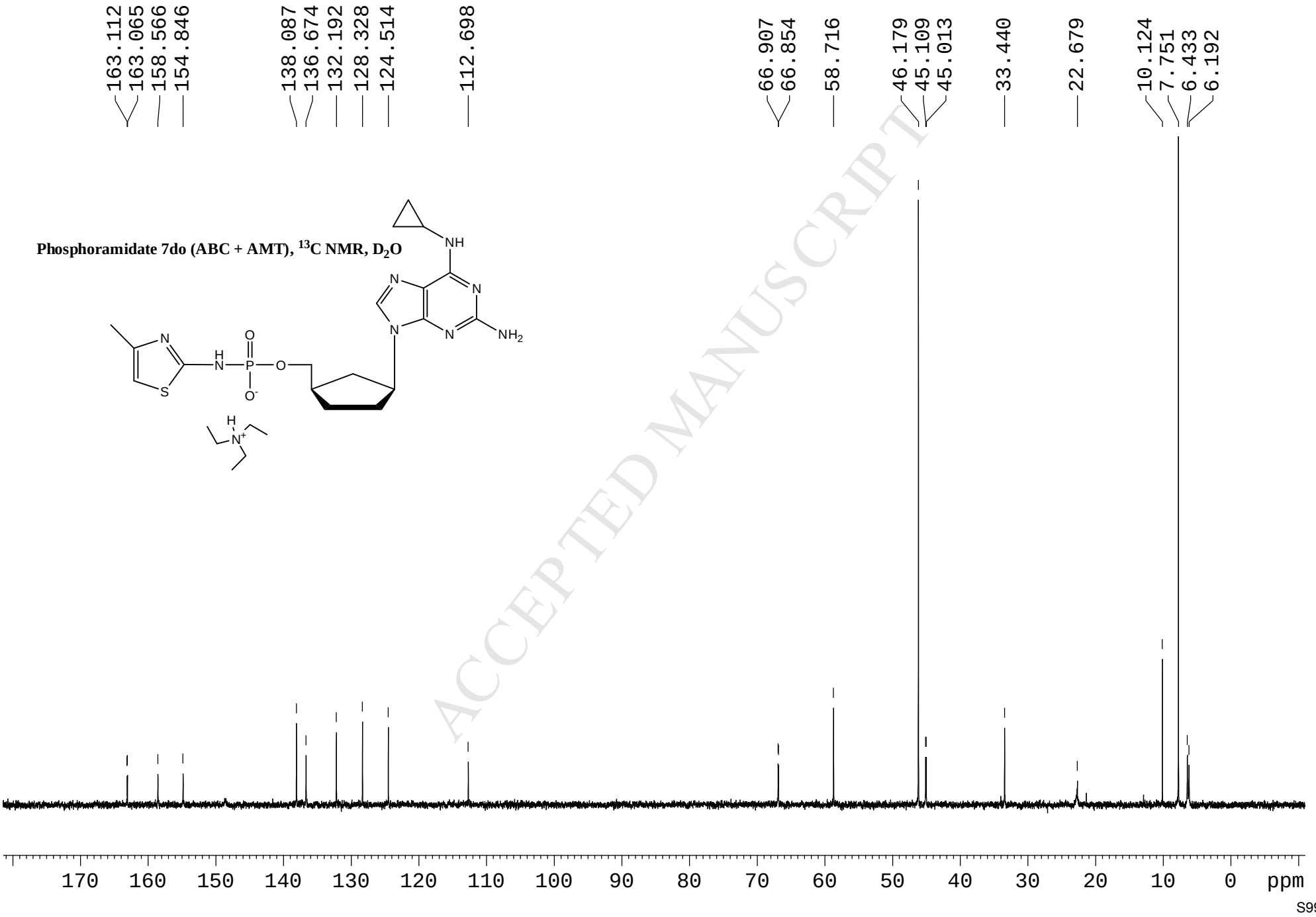
Phosphoramidate 7dl (ABC + 6A3CNPy)



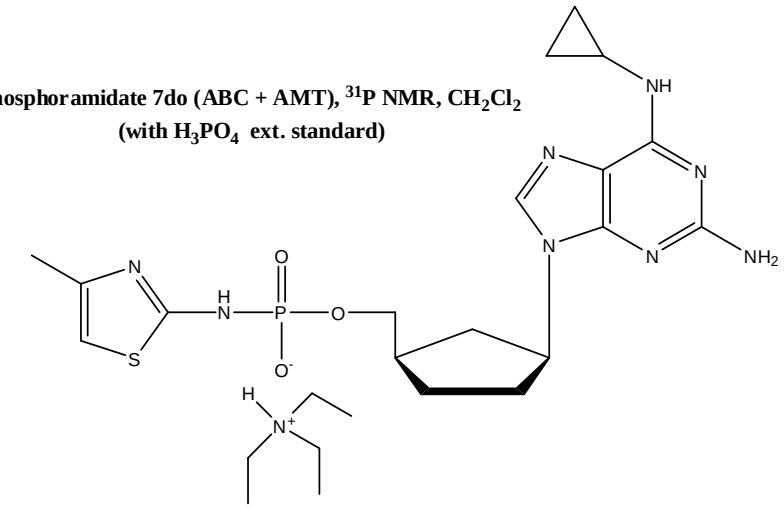
1 PDA Multi 1/254nm 4nm

Phosphoramidate 7do (ABC + AMT), ^{1}H NMR, D_2O





Phosphoramidate 7do (ABC + AMT), ^{31}P NMR, CH_2Cl_2 (with H_3PO_4 ext. standard)



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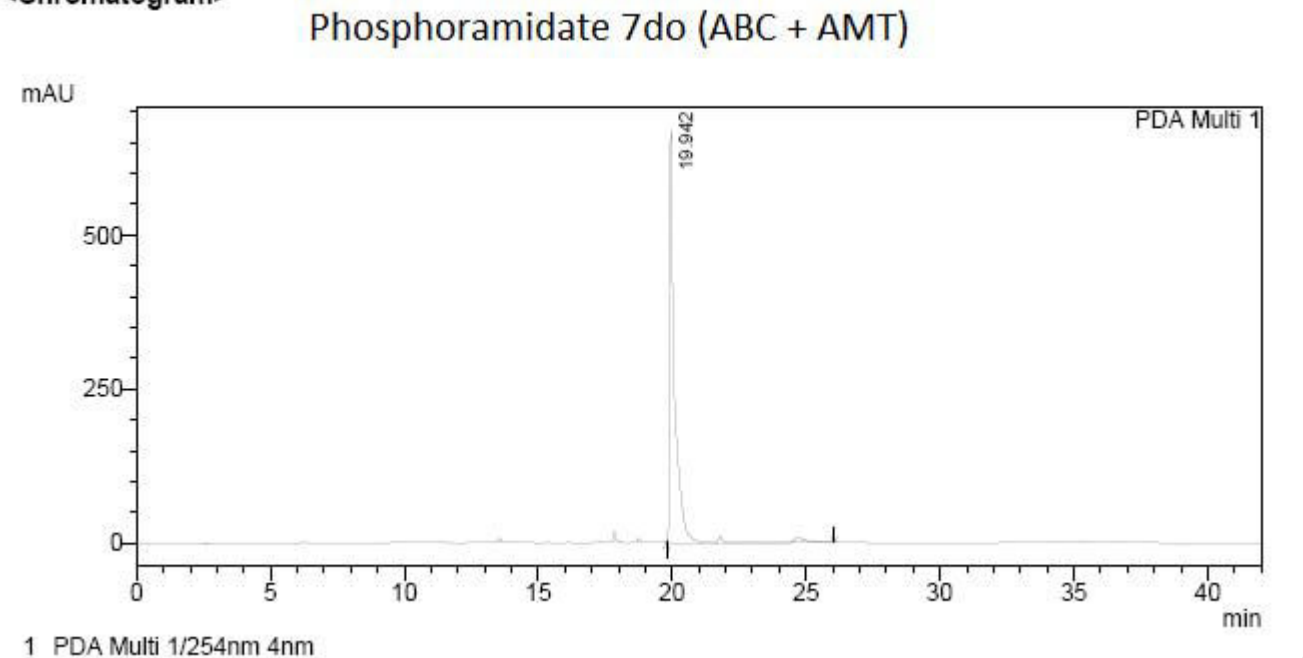
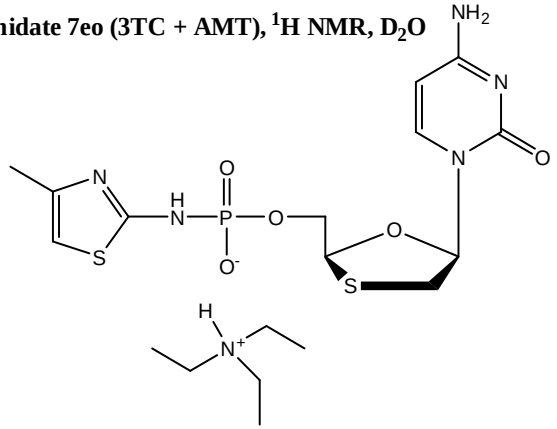
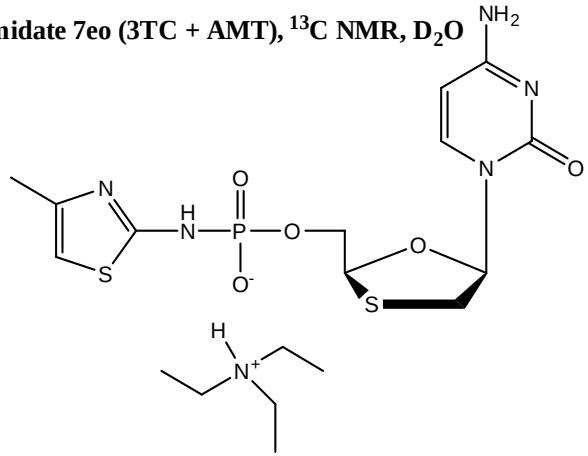


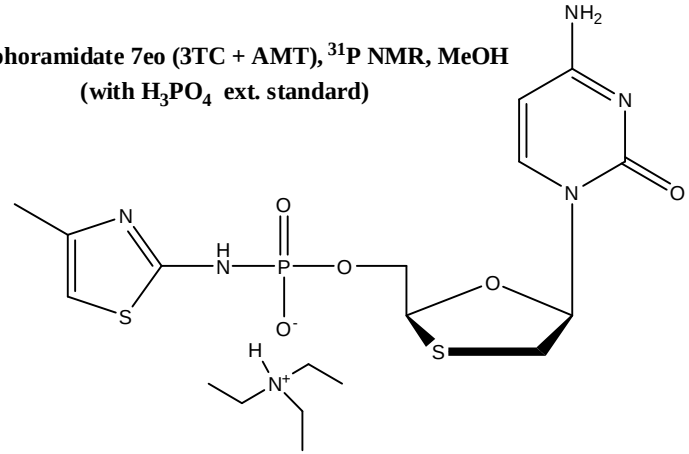
Phosphoramidate 7eo (3TC + AMT), ^{1}H NMR, D_2O



Phosphoramidate 7eo (3TC + AMT), ^{13}C NMR, D_2O



Phosphoramidate 7eo (3TC + AMT), ^{31}P NMR, MeOH
(with H_3PO_4 ext. standard)



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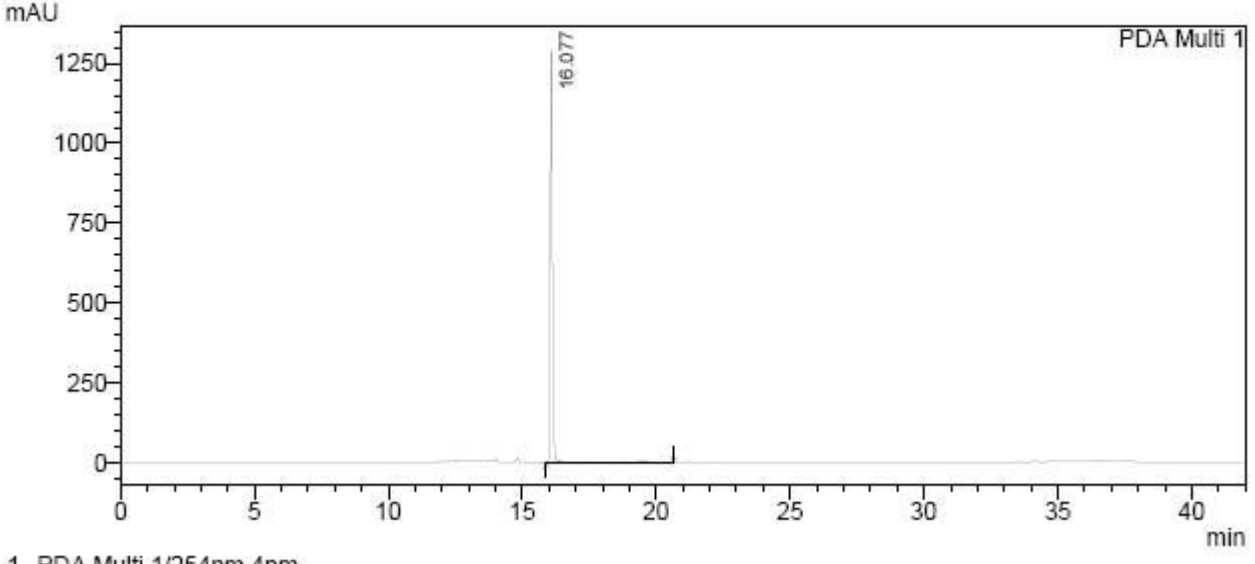
Phosphoramidate 7eo (3TC + AMT)



1 PDA Multi 1/254nm 4nm