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TITLE: Design and Synthesis of Bifunctional Oxime Reactivators of OP- inhibited Cholinesterases

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Organophosphorous (OP) cholinesterase inhibitors remain a significant threat to military and civilian personnel. Reactivators of OP inhibited cholinesterases can serve as OP agent antidotes but can be limited by their poor bioavailability and poor reactivation kinetics. New reactivator designs may be able to address these shortcomings. This proposal explores and evaluates the feasibly of synthesizing two new classes of oxime reactivators that use bifunctional catalysis to enhance kinetics and improve bioavailability. The synthesis of amino-functionalized aryltriflorketoxmines and 3-alkylaminopyridinium oximes are described.
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INTRODUCTION:

This objective of this project is to explore the synthetic feasibility to construct bifunctional oxime reactivators that bear pendant amine or related basic functionality. Basic functional groups have the potential to enhance the kinetic rate of oxime reactivators through participation in general acid catalysis. Three general classes of compounds have been devised in the statement of work. (I.) amine functionalized pyridinium oximes, (II) Electron deficient pyridine and benzaldoximes and (III) bifunctional analogs of MINA.

BODY:

We reduced to practice a general method for making novel bifunctional oxime AChEreactivators based on 2-PAM designed to increase the rate-limiting step of reactivation using substrate assisted catalysis. One of the key proposed strategies was to develop an approach to 2-pyridinium oximes that contains amine or similar functionality tethered to the 4-position that could act in general acid catalysis.

*General considerations for constructing 2-Pyridinium oximes:* Two general approaches were explored for the synthesis of bifunctional oximes, One strategy (I), was introduce the 2-aldehyde as a nitrile via the pyridine N-oxide. Another approach (II), was to simply perform 2-hydroxymethylation of pyridines by radical addition using ammonium persulfate (APS). Although both strategies were effective, selective reduction of the nitrile proved difficult in the presence of amide and diamine functionalities. With the availability of commercially available hydroxymethyl pyridines, the latter approach was generally more practical except for the synthesis of amidines and amidoximes (See below).

\[ \text{I.} \quad \text{FG} \quad \begin{array}{c} \text{1) MCPBA} \\ \text{2) TMSCI, ME}_2\text{NCOCl} \end{array} \rightarrow \quad \text{FG} \quad \begin{array}{c} \text{N} \end{array} \]

\[ \text{II.} \quad \text{FG} \quad \begin{array}{c} \text{1) MCPBA} \\ \text{2) TMSCI, ME}_2\text{NCOCl} \end{array} \rightarrow \quad \text{FG} \quad \begin{array}{c} \text{OH} \end{array} \]

A key compound was to develop 2-PAM analogs with amine and or diamine functionality in the 4 positions. Our original design plan called for introducing an aldehyde that could be coupled to various amines and diamines through reductive amination. Interestingly while amines readily coupled under these conditions, the diamines, called for by our design, did not couple under these conditions. Search of the literature revealed a dearth of successful examples of reductive ammination by
Our revised strategy for construction of 4 substituted bifunctional pyridinium oximes is illustrated in Scheme 1. The resulting strategy is a little longer than our initial design but provides flexibility for a generalizable scheme. A key finding from this approach has been that the oxime, which normally does not need protection in order to perform selective N-methylation of the pyridine nitrogen in the synthesis of 2-PAM, is preferentially methylated in the presence of the diamine (VI). Presumably the strongly basic diamine deprotonates the oxime under standard methylation conditions.

Protection of the oxime as the TBDMS (tButyldimethylsilyl) ether blocks O-alkylation (VII). The diamine alkylation is still competitive for N-pyridine alkylation but can be controlled by “buffering” the reaction such that the more basic amines are protonated (VIII).
Scheme 1. The synthesis of oxime 8 illustrates a general strategy for constructing bifunctional oxime reactivators with pendant general acid groups.

Compound 8 has been delivered to ICD for testing (synthesis and characterization below). This strategy is in place to make additional analogs such as the 5-substituted analog 27.

Scheme 2. Synthesis 5-substituted analogs of 2-PAM with pendant diamine.

As a reference compound we also synthesized 2,4-diPAM.

Scheme 3. Synthesis of 2,4-diPAM
As part of objective 2 as defined in the SOW, we will assess the synthetic feasibility of making electron deficient bifunctional benzaldoximes and benketoximes and related hydroxamic acids and amidoximes as potential bioavailable AChE reactivators capable of substrate-assisted catalysis. We have explored way to synthesize non-cationic analogs of 2-PAM that can be used to lower the oxime pKa to facilitate catalysis without using a permanent charge.

Trifluoromethylketoximes: We developed a general route to the synthesis of trifluoromethylketoximes. Compound 37 was made as an isostere of the 4-substituted bifunctional oximes. Starting from 3-bromobenzylalcohol, the alcohol was protected as a THP ether and the trifluoromethylketone installed by coupling the organolithium with trifluoroacetethylpiperidine. We again used our direct acylation strategy to install the diamine side chain, which was believed to provide an amine cation for AChE binding/recognition. In vitro testing suggests that these compounds function as inhibitors. We later speculated that the trifluoro-methylketoxime may be might be forming covalent adducts through haloform chemistry.

![Chemical structures](image)

**Scheme 4.** Synthesis of bifunctional trifluoroketoxime 47.

To further modulate the trifluoromethylketoxime pKa we also synthesized analogs with 4-aminoalkoxy substituents. The alkoxy substitution should be electron donating and raise the effective pKa of the oxime. These could be made from Mitsinobu coupling of 3-bromophenol. These analogs 41 and 44 were synthesized, characterized and delivered to ICD.
Scheme 5. Synthesis of trifluoroketoxime 47.


Amideoxime: Another oxime analog we explored was the amideoxime. The amideoxime 41 was made by direct addition of hydroxylamine to the nitrile 14 which was made following the route initially described to developed to bifunctional pyridinium oximes.
Scheme 7. Synthesis of bifunctional amidoxime analog.

\textit{MINA and RS41 analogs:} We have made initial progress towards the synthesis of MINA/RS41A analogs. The remainder of the project period, expanded through no-cost extension) will be focused on making additional MINA analogs.

We have made the novel amine-functionalized nitrosoacetophenone 46 through direct nitrosylation of the acetophenone.

Scheme 8. Synthesis of $\alpha$-nitrosobenzophenone analog.

We have reduced to practice a general strategy for synthesizing a novel MINA analog that uses an amine functionalized acetophenone core.
KEY RESEARCH ACCOMPLISHMENTS:

SOW objective 1: We have successfully demonstrated the synthetic feasibility of synthesizing bifunctional pyridinium oximes.

- General strategies for synthesizing 4-aminoalkyl substituted pyridinium oximes has been achieved
- General method for 5-aminoalkyl substituted pyridine oxime has been achieved.

SOW objective 2: We have successfully demonstrated the synthetic feasibility of constructing electron deficient bifunctional benzaldoxmine, ketoximes and amidoximes.

- 4-aminoalkyl substituted pyridine amideoximes have been synthesized
- 4-aminoalkyl substituted trifluoroacetophenone oximes have been synthesized
- 4-aminoalkyl substituted trifluoroacetophenone oximes have been synthesized

SOW objective 3. Assess the synthetic feasibility of making MINA and RS41A analogs designed to support a substrate assisted bifunctional catalytic mechanism
REPORTABLE OUTCOMES:

None

CONCLUSION: We have successfully evaluated the synthetic feasibility of bifuncional pyridinium oximes and electron deficient oxime analogs as outlined in objectives on SOW objectives 1 and 2. This work has resulting in the development of a general scheme for oxime protection and for selective pyridine N-alkylation by adjusting the pH to reduce/prevent side chain alkylation. We have successfully made 7 new reactivator analogs that incorporate new functionalities that are in principle capable of supporting general acid/base catalysis. Preliminary studies suggest that trifluoroacetophenoximes are inhibitors of AChE.

In this report period work has begun on objective 3, evaluating the synthetic feasibility of constructing bifunctional MINA analogs. We hope to complete the synthesis of new MINA analogs described in objective 3 and additionally complete new pyridinium based oxime analogs.
APPENDICES:

General Procedures:

Synthesis of 2: To a solution of 1 (10g, 72.92 mmol) and H2SO4 in MeOH under refluxing was added a solution of APS (30g, 131.256 mmol) in water over 20 minutes. The solution was allowed to cool to room temperature. The MeOH was removed and solution was neutralized by Na2CO3 to pH as 8. The aqueous layer was extracted by EtOAc, combined and purified by flash column chromatography (EtOAc : Hex = 1 : 1).

Synthesis of 3: To a solution of 2 (1g, 6.5 mmol) in DCM was added p-tolunensulfonic acid (25mg, 0.1318 mmol), followed by addition of dihydropyran, stirred at RT for 2 hours, after which the reaction was poured into NaHCO3 and extracted by EtOAc. The solvent was removed by reduced pressure and the residue was purified by flash chromatography with EtOAc : DCM = 1 : 2.

Synthesis of 4: To a solution of 3 (588.3mg, 2.344mmol) in 50 ml EtOH was slowly added NaBH4 (443mg, 11.72 mmol) and than heated to reflux for 2 hours. The reaction was quenched by adding water, the EtOH was removed by reduced pressure, and aqueous layer was extracted with DCM. The residue was purified by flash chromatography with MeOH : DCM = 1 : 19.

Synthesis of 5: To a solution of 4 (152mg, 0.68 mmol) in DCM in a ice bath was added DIPEA (548 uL, 3.151 mmol) than MsCl (160 uL, 2.055 mmol), stirred for 75 minutes, after which the solution was added drop-wisely to a solution of ethylenediamine in DCM in a ice bath. The reaction was stirred for another one hour, the solvent was removed by reduced pressure and the residue was purified by flash chromatography with MeOH.NH3 : DCM = 1 : 19.

Synthesis of 6: To a solution of 5 in MeOH was added TSA in a ice bath. The reaction was stirred for 16 hours, concentrated and redissolved in CHCl3, washed with NaHCO3 and dried. The aldyhyde intermediate was applied to next step without further purification.

Synthesis of 7: To a solution of 6 (.2273 mmol) in 5 ml of EtOH was added TBDMS hydroxoyamine (147 mg, 100 mmol) and 55 uL of pyridine. The reaction was heated to 40°C for 4 hours, after which the solvent was removed by reduced pressure and the residue was purified by flash chromatography NH3.MeOH : DCM = 1 : 19.

Synthesis of 8: To a solution of 7 (11.85 mg, 0.034 mmol) in EtOH was added oxalic acid and reflux for 15 minutes, after which the solvent was removed by reduced pressure. To the residue was added THF and Dimethyl sulfate (6.42 uL, 0.068 mmol). The reaction was stirred at 50°C for over night. The precipitate was applied to reverse phase column with H2O : MeOH = 19 : 1

Synthesis of 10: To a solution of 9, 4-pyridyl carbinol (1g 9.16 mmol) and TFA (1.23 mL, 16.5 mmol) in 4 mL toluene was added a solution of acetyl chloride (1.05 mL, 14.7 mmol) in DCM drop wise. The reaction was stirred for 10 hours, after which water was added to the reaction and organic layer was dried and concentrated in vacuo. The residue was purified by flash chromatography with MeOH : DCM = 1 : 19.

Synthesis of 11: To a solution of 10 (700 mg, 4.64 mmol) in 4 mL acetone was added a solution of m-CPBA (1.37 g, 5.56 mmol) in 4 mL acetone drop wise in 4 minutes. The reaction was stirred for 4 hours, after which the solvent was removed by reduced pressure and the residue was portioned between water and ether. The organic layer was dried and concentrated in vacuo. The residue was purified by flash chromatography with MeOH : DCM = 1 : 19.
Synthesis of 12: To a solution of 11 (162.4 mg, 0.97 mmol) in DCM was added TMSCN (145 uL, 1.16 mmol), after which Me₂NCOCl (106.8 uL, 1.16 mmol) was added in three portions over thirty minutes. The reaction was stirred for over night, then the solvent was removed by reduced pressure and the residue was purified by flash chromatography with EtOAC : Hexane = 3 : 7.

Synthesis of 13: To a solution of 12 (360 mg, 2.04 mmol) in 10 ml THF was added 1N LiOH (4.1 mL). The reaction was stirred for 2 hours. THF was removed by reduced pressure and the aqueous layer was exhaustively extracted by DCM. The organic layers were combined and concentrated by vacuo. The residue was applied to next step without purification.

Synthesis of 14: To a solution of 13 (184 mg, 1.37 mmol) in 8 mL DCM was added DIPEA (550 uL, 3.151 mmol) and MsCl (122 uL, 1.58 mmol) in a ice bath. The reaction was stirred for 75 minutes and then was added to a solution of trimethylethenediamine (500 uL, 3.425 mmol) in DCM, than warmed to RT. The reaction was concentrated in vacuo and the residue was purified by flash chromatography.

Synthesis of 15: To a solution of 14 (85mg, 0.39 mmol) in 6 mL EtOH was added Hydroxylamine and pyridine. The reaction was refluxed for 4 hours, than stirred at RT for over night. The reaction was concentrated in vacuo and the residue was purified by flash chromatography with NH₃.MeOH : DCM = 1 : 9

Synthesis of 17: To a solution of 2 (347 mg, 2.065 mmol) in THF was added Dibal-H (6.2 ml, 6.2 mmol) drop wisely at -78°C for 3 hours. The reaction was quenched by addition of Sodium Sulfate Decahydrate and stirred for over night. The solvent was removed in vacuo and the residue was purified by flash chromatography Meoh : DCM = 1 : 19.

Synthesis of 18: Oxalyl Chloride (55 uL, 0.64 mmol) in DCM was cooled to -78°C, into which was added DMSO (90.1 uL, 1.28 mmol) drop wisely, in 10 minutes, solution of 17 (22.3 mg, 0.16 mmol) in DCM was added to the solution. The reaction was stirred at -78°C for 45 minutes before DIPEA (45 uL, 2.56 mmol) was added and the reaction was warmed up to 0°C. The reaction was poured into water and was extracted with DCM. The organic layers were combined and concentrated in vacuo. The product was applied to next step without further purification.

Synthesis of 19: To a solution of 18 (51.4 mg, 0.38 mmol) in 5 mL EtOH was added Hydroxylamine hydrochloride. The reaction was refluxing for 4 hours, than at RT for overnight. The solvent was removed by reduced pressure and the residue was purified by flash chromatography with MeOH : DCM = 1 : 19.

Synthesis of 20: To a solution of 19 (26.8 mg, 0.1624 mmol) in anhydrous THF was added Dimethyl Sulfate (16 uL, 0.1624 mmol) at RT, the reaction was stirred for over night. The solvent was taken out by a pippett and the precipitate was purified by a reverse column, with MeOH : H₂O = 1 : 19.

Synthesis of 22: To a solution of 21 (1.24g, 7.54 mmol) in 50 mL DCM was added p-toluenesulfonic acid , followed by addition of dihydropyran. The reaction was stirred at RT for w hours, after which the reaction was poured to Saturate NaHCO₃ and extracted by EtOAC. The organic layer was dried and concentrated in vacuo. The residue was purified by flash chromatography with EtOAC : DCM = 1 : 9.

Synthesis of 23: NaBH₄ (567.3 mg, 15 mmol) was slowly added to a solution of 22 in EtOH at RT, than the reaction was heated to reflux for 2 hours. The solution was cooled to RT and water was added. The solution was concentrated and basified by NaHCO₃ and extracted by DCM. The solution was concentrated in vacuo and the residue was purified by flash chromatography with
MeOH : DCM = 1 : 19.

Synthesis of 24: To a solution of 23 (147 mg, 0.6632 mmol) in DCM in an ice bath was added DIPEA (265 uL, 0.99 mmol) and MsCL (76 uL, 0.99 mmol), in 75 minutes, the solution was added drop wisely to a solution of trimethylehylenedimaine (194 uL, 1.3264 mmol) in an ice bath and warm up to RT. The solution was concentrated in vacuo and purified by flash chromatography with NH3.MeOH : DCM = 1 : 9.

Synthesis of 25: To a solution of 24 (84 mg, 0.274 mmol) in MeOH was added p-toluenesulfonic acid (208.2mg, 1.09 mmol) in an ice bath. The reaction was stirred for 16 hours, concentrated, redissolved in chloroform. The solution was washed with minimum amount of saturate NaHCO3 and dried purified by flash chromatography with NH3.MeOH : DCM = 1 : 9.

Synthesis of 26: A solution of Oxalyl chloride (55.45 uL, 0.646 mmol) in 2 mL of DCM was cooled to -78°C and was added to a solution of DMSO (91.6 uL, 100.79 mmol) drop wisely. The reaction was stirred for 10 minutes, after which a solution of 25 (72 mg, 0.323 mmol) in DCM was added over 10 minutes. The mixture was stirred at -78°C for 45 minutes before DIPEA (451 uL, 2.59 mmol) was added swiftly than the slurry was warmed up to 0°C and stirred for another 30 minutes. Than the reaction was poured to water and extracted by DCM. The solvent was removed by reduced pressure and purified by flash chromatography with MeOH : DCM = 1 : 9.

Synthesis of 27: To a solution of 26 (71 mg, 0.323 mmol) in EtOH was added H2NOH.HCl (67.35 mg, 0.969 mmol) and pyridine (78 uL, 0.969 mmol). The reaction was heated to 40°C for one hour, than lowered to RT, stirred for over night. Than the solvent was removed by reduced pressure and the residue was purified by flash chromatography with NH3.MeOH : DCM = 1 : 19.

Synthesis of 33: 3-bromobenzylalcohol(10g, 53.46 mmol) in DCM was treated with DHP(6 mL, 64.15mmol) and dry p-toluenesulfonic acid (100 mg). The reaction was stirred overnight. The reaction was extracted with H2O and DCM. The aqueous layer was extracted 3 × 100 mL DCM and the combined organic layer was washed with 3 × 50 mL brine, dried over MgSO4 and concentrated in vaccuo. The product was separated using column chromatography Hexane: Ethylacetate (90:10) as a white solid.

Synthesis of 34 (trifluoroacetylation general procedure.)
To 33(2.556 g, 9.42 mmol) in THF at -78 ºC was added n-BuLi (14.81 mL, 20.74 mmol). The reaction was stirred at -78 ºC for 30 mins. Following which 2,2,2-trifluoro-1-(pyrrolidin-1-yl)ethanone (1.23 mL, 10.362 mmol) was added dropwise. The reaction was stirred at -78 °C for 1h and allowed to warm to room temperature. Following which the reaction was worked up with saturated NH4Cl and ether. The aqueous layer was extracted with 50 mL ether. The combined organic layer was washed with brine, dried over MgSO4 and concentrated in vaccuo.

Synthesis of 35 (THP deprotection common procedure)
To 2,2,2-trifluoro-1-(3-(((tetrahydro-2H-pyran-2-yl)oxy)methyl)phenyl)ethanone (768.5 mg, 2.66 mmol) in DCM was added dry p-toluene sulfonic acid (50.71 mg, 1900.22 mmol) and the reaction was stirred at room temperature for 1 h. The reaction was stirred overnight. The reaction was extracted with H2O and DCM. The aqueous layer was extracted 3 × 100 mL DCM and the combined organic layer was washed with 3 × 50 mL brine, dried over MgSO4 and concentrated in vacuo. The product was separated using column chromatography Hexane: Ethylacetate (90:10) as a white solid.

Synthesis of 36 (trimethyldiaminne displacement common procedure): To 2,2,2-trifluoro-1-(3-(hydroxymethyl)phenyl)ethanone (225.8 mg, 1.106 mmol) in DCM was added
Methansulfonylchloride (100 uL, 139.42 mmol) and DIPEA (423 uL, 314.44 mmol). The reaction was stirred at room temperature for 1 h. The reaction mixture was worked up with H2O and DCM. The reaction was extracted with H2O and DCM. The aqueous layer was extracted 3 × 50 mL DCM and the combined organic layer was washed with 3 × 30 mL brine, dried over MgSO4 and concentrated in vacuo. The crude product in 10 mL DCM was treated with N,N,N'-trimethylene-1,2-diamine and reaction stirred overnight. The reaction was extracted with H2O and DCM. The aqueous layer was extracted 3 × 100 mL DCM and the combined organic layer was washed with 3 × 50 mL brine, dried over MgSO4 and concentrated in vacuo. The product was separated using column chromatography DCM: MeOH (90:10) as a yellow oil.

Synthesis of 37 (Oxime formation general procedure)
To the ketone 36 (340 mg, 1,123 mmol) in 1 ml of pyridine was added Hydroxylamine hydrochloride (312.15 mg, 4.492 mmol). The reaction was stirred overnight at 50 ºC. After which pyridine was evaporated and the residue was applied on the silica column and product was separated using DCM:MeOH (NH3).

Synthesis of 39 (Mitsunobo reaction general procedure): To 3-bromophenol and triphenylphosphine in THF at 0 ºC was added 2-dimethylaminoethanol. To this solution was added diisopropylazidodicarboxylate. The mixture was allowed to stir at room temperature overnight. The reaction was worked up with H2O and the aqueous layer was extracted with ether. The organic layer was washed with 3 brine, dried over MgSO4 and concentrated in vaccuo. The residue was dissolved in minimum amount of hexane and cooled at -20 ºC to crystallize triphenyl phosphineoxide. The supernatant was applied on to silica column to separate the product using DCM:MeOH.

Synthesis of 40 (Trifluoroacetylation general procedure): To 2-(3-bromophenoxy)-N,N-dimethylethanamine (683.8 mg, 2.8 mmol) in THF at -78 ºC was added n-BuLi (5 mL, 7 mmol). The reaction was stirred at -78 ºC for 30 mins. Following which 2,2,2-trifluoro-1-(pyrrolidin-1-yl)ethanone (668 uL, 795.31 mmol) was added dropwise. The reaction was stirred at -78 ºC for 1h and allowed to warm to room temperature. Following which the reaction was worked up with saturated NH4Cl and ether. The aqueous layer was extracted with 50 mL ether. The combined organic layer was washed with brine, dried over MgSO4 and concentrated in vaccuo.

Synthesis of 41: Oxime 41 was made from ketone 40 following the same proceed common procedure used to make compound 37.

Synthesis of 42: Ether 42 was made from 3-bromophenol by Mitsunobo reaction following the same general procedure was applied to compound 38.

Synthesis of 43: Compound 43 was made by trifluoroacetylation of compound 42 following the same general procedure used to make compound 40.

Synthesis of 44: Oxime 44 was made from ketone 43 using the same general procedure used to make compound 37.

Synthesis of 45
To 5ml of Ethanol was added NaH (75 mg, 3.12 mmol) in small portions to generate sodiumethoxide. To a solution of 1-(3-(2-(dimethylamino)ethoxy)phenyl)ethan-1-one (534.3 mg, 2.6 mmol) in 500 uL EtOH was added the freshly made Sodium ethoxide dropwise. To this mixture was added dropwise isoamylnitrite (335.04 mg, 2.86 mmol). The mixture was stirred overnight. The solvent was evaporated and the product separated on reverse phase c-18 column using H2O:MeOH (90:10). The solvent was evaporated and residual water was removed by
lyophylization. to yield a yellow solid.
**PROTON_16**

**PROTON_16**

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DL: 1.00020060 sec
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D2: 1.00000000 sec
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t: 298.2 K
DL: 1.00020060 sec
TOO: 1
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**CHANNEL f1**

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F1: 4.90 db
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SF: 400.1324710 MHz
SI: 37.68
SR: 400.1324020 MHz
NORM: 50
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FB: 1.00
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AQ: 0.363819 Hz
BG: 18.654576 sec
BG: 512
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**CHANNEL f2**

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P2: 18.00 usec
T5: 288.2 K
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D1: 1.00000000 sec
D2: 1.00000000 sec
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**NUC2**

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