Amenamevir by Ugi-4CR

Xin Li[a], Tryfon Zarganes-Tzitzikas[b], Katarzyna Kurpiewska[c], and Alexander Dömling*[a]

[a]*University of Groningen, Department of Drug Design, A. Deusinglaan 1, 9713 AV,Groningen, The Netherlands.*

[b]*Alzheimer's Research UK Oxford Drug Discovery Institute, University of Oxford, Oxford OX3 7FZ, UK*

[c]*Jagiellonian University, Faculty of Chemistry, Department of Crystal Chemistry and Crystal, Physics, Biocrystallography Group, Gronostajowa 2, 30-387 Krakow, Poland.*

[d]*Palackỳ University, CATRIN, Department of Innovative Chemistry, Šlechtitelů 241/27, 779 00, Olomouc – Holice, Czech Republic*

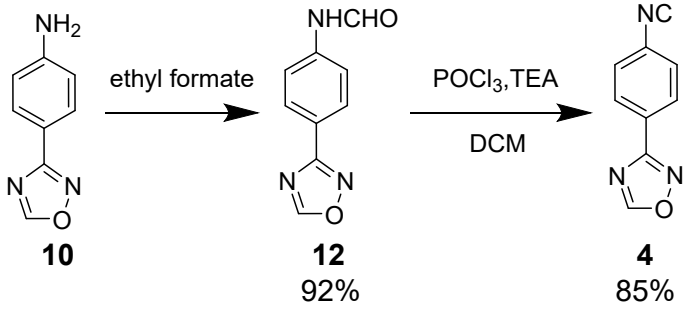
* Corresponding author E-mail: alexander.domling@upol.cz.

Contents:

1.**General Information:**

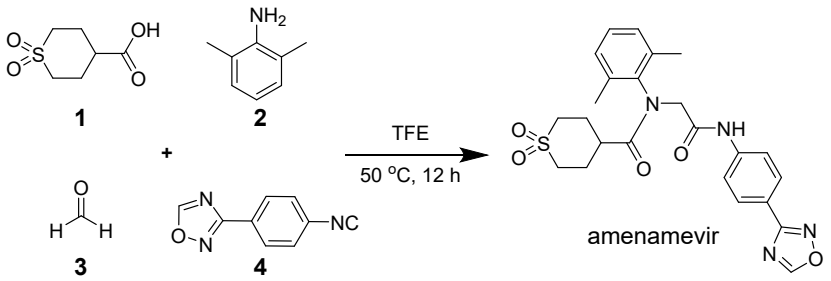
Reagents were available from commercial suppliers and used without any purification unless otherwise noted. All isocyanides were made in house by performing the Ugi procedure. The paraformaldehyde (crystalline) was purchased from Sigma Aldrich and the average length of the polymer was not specified. Other reagents were purchased from Sigma Aldrich, ABCR, Acros, Fluorochem and AK Scientific and were used without further purification. Nuclear magnetic resonance spectra were recorded on a Bruker Avance 500 spectrometer. Chemical shifts for 1H NMR were reported relative to TMS (δ 0 ppm) or internal solvent peak ($CDCl_3$ δ 7.26 ppm, CD_3OD δ 3.31 ppm or D_2O δ 4.79 ppm) and coupling con-stants were in hertz (Hz). The following abbreviations were used for spin multiplicity: s = singlet, d = doublet, t = triplet, dt = double triplet, ddd = doublet of double doublet, m = multiplet, and br = broad. Chemical shifts for 13C NMR reported in ppm relative to the solvent peak ($CDCl_3$ δ 77.23 ppm, DMSO δ 39.52 ppm, CD_3OD δ 49.00 ppm). Flash chromatography was performed on a Grace Reveleris X2 using Grace Reveleris Silica columns (12 g) and a gradient of petroleum ether/ethyl acetate (0–100%) or dichloromethane/methanol (0–20%) was applied. All microwave irradiation reactions were carried out in a Biotage Initiator™ Microwave Synthesizer. Thin layer chromatography was performed on Fluka pre-coated silica gel plates (0.20 mm thick, particle size 25 μm). Mass spectra were measured on a Waters Investigator Supercritical Fluid Chromatograph with a 3100 MS Detector (ESI) using a solvent system of methanol and CO_2 on a Viridis silica gel column (4.6 × 250 mm, 5 μm particle size) and reported as (m/z). High resolution mass spectra (HRMS) were recorded using a LTQ-Orbitrap-XL (Thermo Fisher Scientific; ESI pos. mode) at a resolution of 60000@m/z400. Melting points were obtained on a melting point apparatus and were uncorrected.

2. Synthetic Procedure I:



10 was reacted with ethyl formate at 60 °C to give *N*-[4-(1,2,4-oxadiazol-3-yl)phenyl]formamide **12**. The dehydration of **12** in CH_2Cl_2 (200 mL) can form final isocyande **4** in the presence of $POCl_3$ (1.1 equiv.) and TEA (3.0 equiv.).

Synthetic Procedure II:



The carboxylic acid **1** (1.2 equiv, 2225 mg), 2,6-dimethylaniline **2** (1 equiv, 1513 mg), paraformaldehyde **3** (1.5 equiv, 563 mg), and 4-(1,2,4-oxadiazol-3-yl)-phenyl isocyanide **4** (1.2 quiv, 2150 mg) was sequentially added into the vial with 25 mL TFE, then the reaction mixture was heated at 50 °C for 12 hours, after cooling down, the solvent was removed in vacuum and the reaction crude was purified by chromatography column to give final compound, Amenamevir.

3. Crystal sample preparation:

recrystallization in EtOH

100-200 mg sample powder (after chromatography) was added into the 25 mL flask with around 15 mL EtOH, then the mixture was reflux for a while, filtered the solution and removed undissolved material, then transfer the filtrate to a new 25 mL flask, cap the flask, and kept still for 2-4 weeks in a dark environment.

Crystal structure determination

X-ray diffraction data for single crystal of compounds **4** and **Amenamevir** was collected using Rigaku XtaLAB Synergy S Dualflex diffractometer (four circle diffractometer with a mirror monochromator) with HyPix detector and a PhotonJet CuKα radiation source (λ = 1.54184 Å) for all collected data sets. Additionally, the diffractometer was equipped with a CryoJet HT cryostat system (Oxford Instruments) allowing low temperature experiments, performed at 100 (3) K. The obtained data set was processed with CrysAlisPro software.[1] The phase problem was solved by direct methods using SUPERFLIP.[2] Parameters of obtained models were refined by full-matrix least-squares on F^2 using SHELXL-2018/3.[3] Calculations were performed using WinGX integrated system (ver. 2021.2).[4] Figure was prepared with Mercury 2020.3.0 software.[5]

All non-hydrogen atoms were refined anisotropically. All hydrogen atoms attached to carbon atoms were positioned with the idealised geometry and refined using the riding model with the isotropic displacement parameter U_{iso}[H] = 1.2 (or 1.5 (methyl groups only)) U_{eq}[C]. Crystal data and structure refinement results for presented crystal structures are shown in Table S1. The molecular geometry (asymmetric unit) observed in the crystal structures is shown in Figure S1.

Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 2213853 (**4**), CCDC 2213854 (**Amenamevir**). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

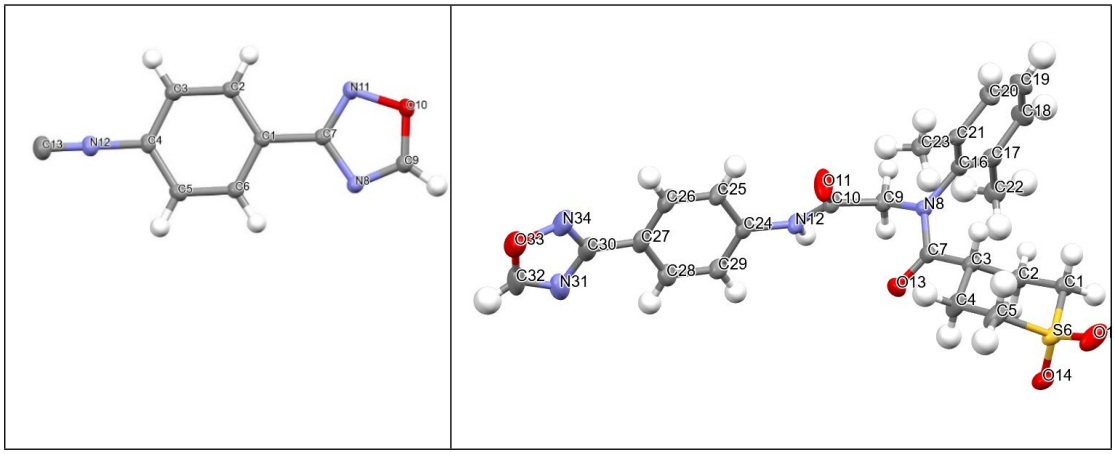


Figure S1. Molecular geometry observed in the crystal structures of compounds **4** and **Amenamevir** showing the atom labelling scheme. The positional disorder within the benzene ring is observed with equal site occupancy (50:50). Displacement ellipsoids of non-hydrogen atoms are drawn at the 30% probability level. H atoms are presented as small spheres with an arbitrary radius.

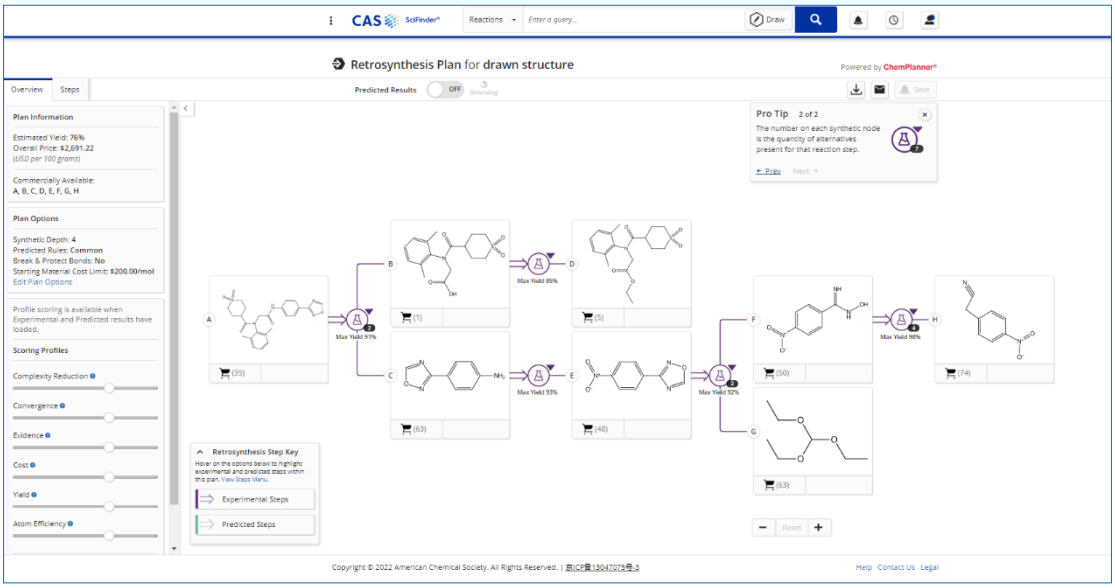
Table S1. Crystal data and structure refinement results for compounds **4** and **Amenamevir.**

	4	**Amenamevir**
Empirical moiety formula	$C_9\ H_5\ N_3\ O$	$C_{24}\ H_{26}\ N_4\ O_5\ S$
Formula weight [g/mol]	171.16	482.55
Crystal system	Monoclinic	Monoclinic
Space group	*P 2₁/n*	*I 2/a*
Unite cell dimensions	a = 3.72520(10) Å b = 11.5014(4) Å c = 18.9837(6) Å α=90° β= 91.706(3)° γ=90°	a = 19.6089(3) Å b = 13.7807(2) Å c = 17.8230(3) Å α=90° β= 101.936(2)° γ=90°
Volume [Å^3]	813.00(4)	4712.08(13)
Z	4	8
D_{calc} [Mg/m^3]	1.398	1.360

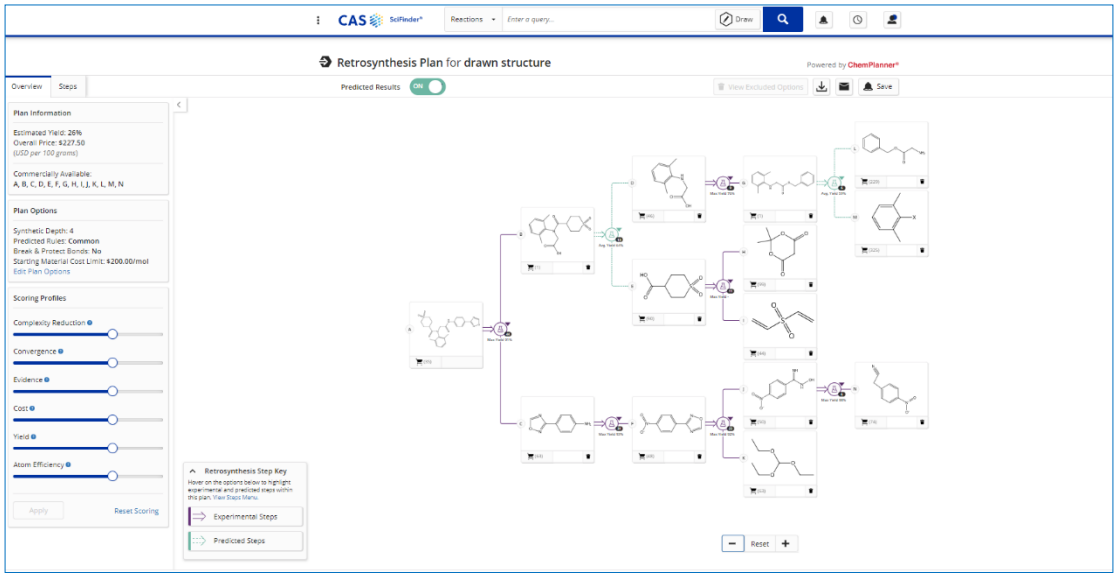
μ [mm^{-1}]	0.805	1.589
F(000)	352	2032
Crystal size [mm^3]	0.25 x 0.1 x 0.1	0.40 x 0.3 x 0.05
Θ range	4.49° to 80.61°	3.95° to 80.46°
Index ranges	-4 ≤ h ≤ 4, -14 ≤ k ≤ 14, -24 ≤ l ≤ 22	-4 ≤ h ≤ 4, -14 ≤ k ≤ 14, -24 ≤ l ≤ 22
Refl. collected	8694	30800
Independent reflections	1753 [R(int) = 0.0444]	5123 [R(int) = 0.0386]
Completeness [%] to Θ	97.9 (Θ 67.68°)	99.0 (Θ 67.68°)
Absorption correction	Multi-scan	Multi-scan
Tmin. and Tmax.	0.569 and 1.000	0.679 and 1.000
Data/ restraints/parameters	1753 / 119 / 0	5123 / 309 / 138
GooF on F2	1.083	1.085
Final R indices [I>2sigma(I)]	R1= 0.0404, wR2= 0.1040	R1= 0.0629, wR2= 0.1615
R indices (all data)	R1= 0.0362 wR2= 0.1008	R1= 0.0585 wR2= 0.1579
$\Delta\rho_{max}$, $\Delta\rho_{min}$ [$e \cdot Å^{-3}$]	0.29 and -0.21	0.54 and -0.61

4. Retrosynthesis plans from Scifinder, IBM RXN, Spaya and ASKCOS

1.1 **Scifinder**: Retrosynthesis plan **1**



1.2 **Scifinder**: Retrosynthesis plan **2**



2. IBM RXN: Retrosynthesis plan **1**

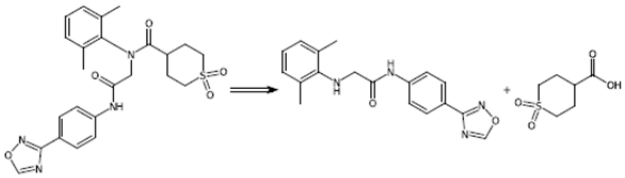
Information about the retrosynthesis

Created On: 2022-10-26T09:09:36.371000 UTC
Model: 12class-tokens-2021-05-14
Product:
O=C(N(CC(NC1C=CC(C2=NOC=N2)=CC=1)=O)C1C(C)=CC=CC=1C)C1CCS(=O)(=O)CC1
MRP: 50
FAP: 0.65
MSSR: 15
SbP: 3

Step 1

Type: Carboxylic acid + amine condensation, Confidence: 0.977

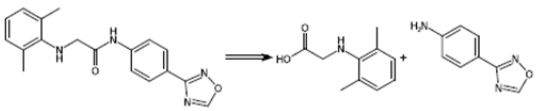
Cc1cccc(C)c1NCC(=O)Nc1ccc(-c2ncon2)cc1.O=C(O)C1CCS(=O)(=O)CC1>>O=C(N(CC(NC1C=CC(C2=NOC=N2)=CC=1)=O)C1C(C)=CC=CC=1C)C1CCS(=O)(=O)CC1



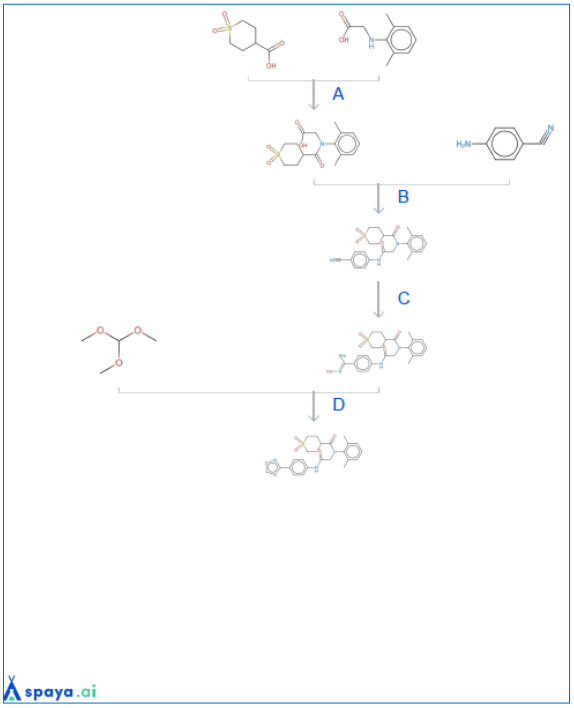
Step 2

Type: Carboxylic acid + amine condensation, Confidence: 0.968

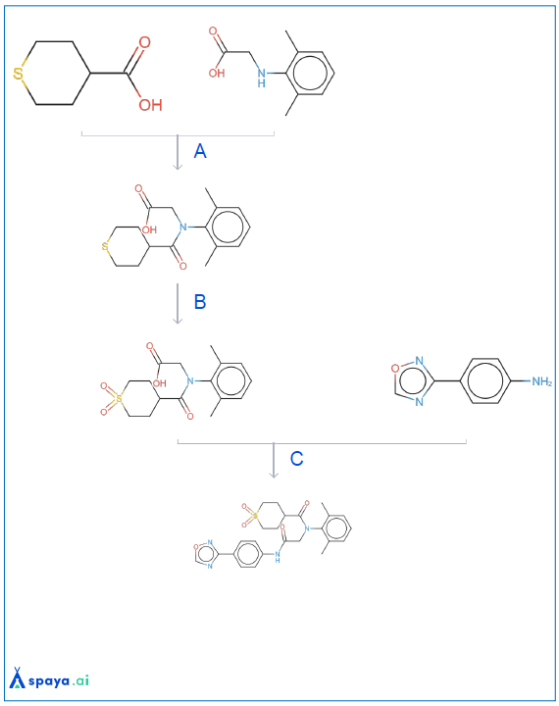
Cc1cccc(C)c1NCC(=O)O.Nc1ccc(-c2ncon2)cc1>>Cc1cccc(C)c1NCC(=O)Nc1ccc(-c2ncon2)cc1



3.1 Spaya: Retrosynthesis plan **1**



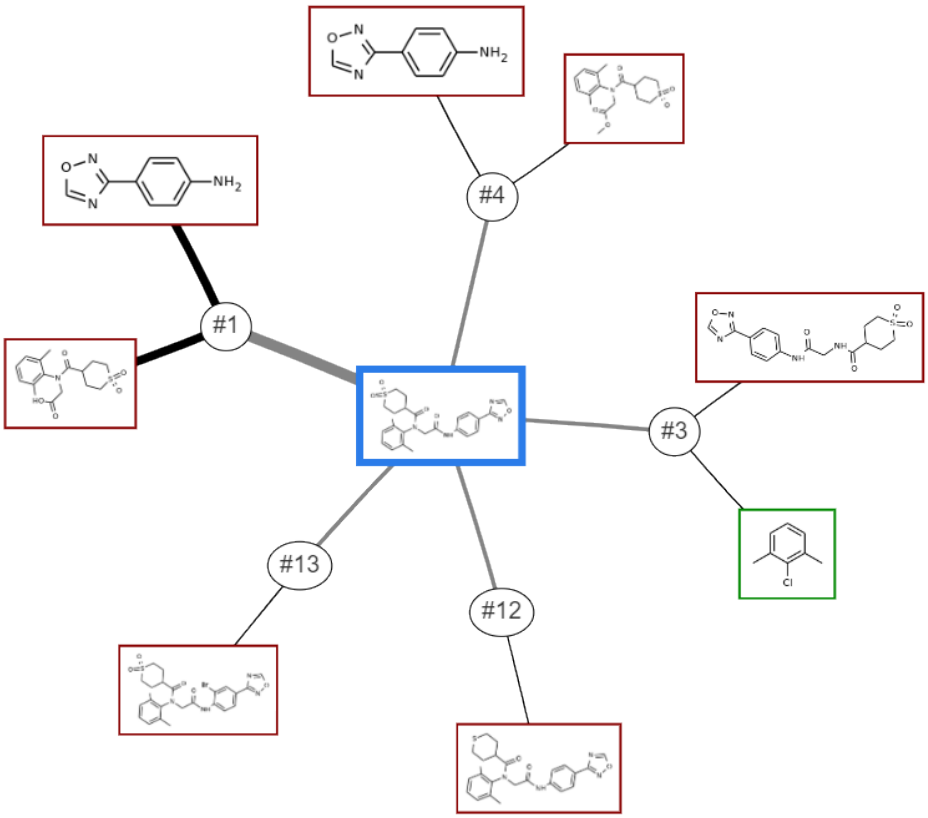
3.2



Spaya: Retrosynthesis plan **2**

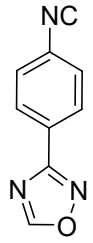
4. ASKCOS

Retrosynthesis plan **1:**



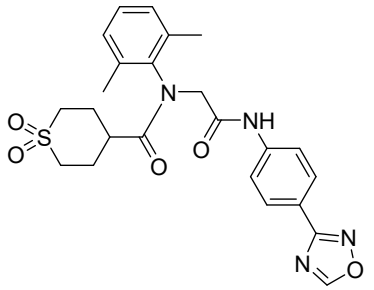
5. Characterizations of Synthesized Compounds

3-(4-Isocyanophenyl)-1,2,4-oxadiazole (**4**)



Follow general procedure **I** on 6.4 mmol scale. Yellow solid (1104 mg, 85%), m.p.: 127-128 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.80 (s, 1H), 8.16 (d, J = 8.6 Hz, 2H), 7.50 (d, J = 8.5 Hz, 2H).^{13}C NMR (126 MHz, $CDCl_3$) δ 166.6, 166.6, 165.3, 128.8, 127.4, 127.1. MS (ESI) m/z: $[M + H]^+$ 172.0433.

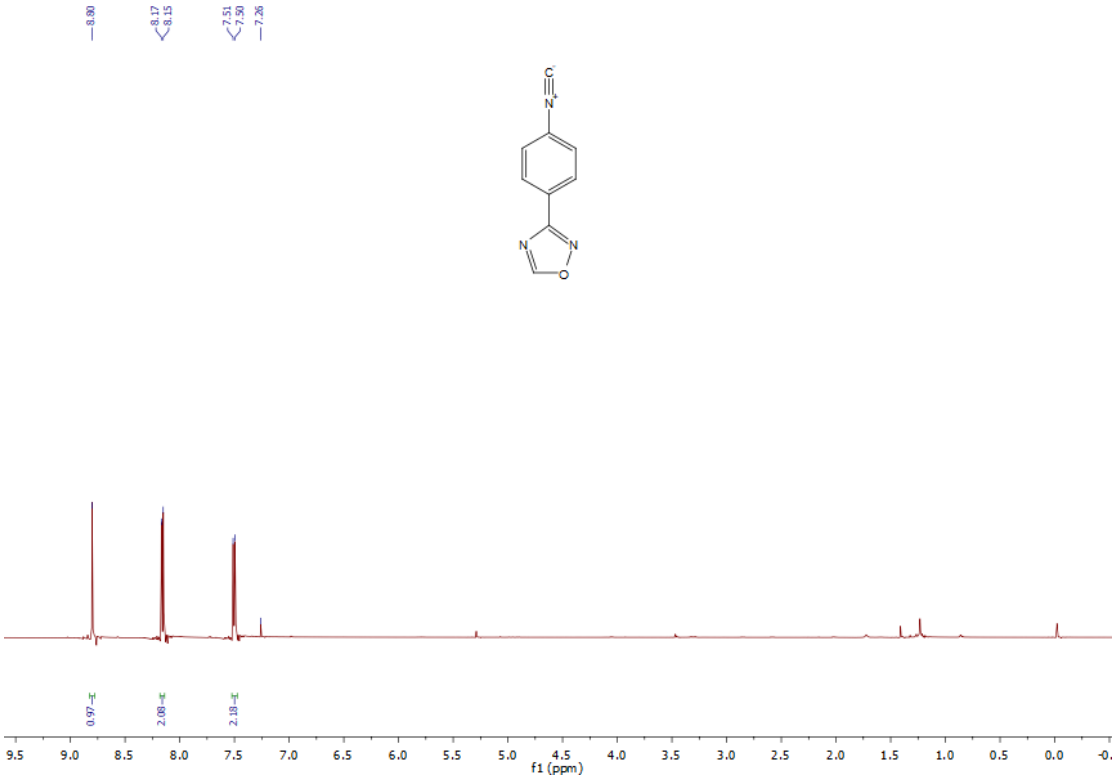
N-(2-((4-(1,2,4-oxadiazol-3-yl)phenyl)amino)-2-oxoethyl)-N-(2,6-dimethylphenyl)tetrahydro-2H-thiopyran-4-carboxamide 1,1-dioxide (**Amenamevir**)



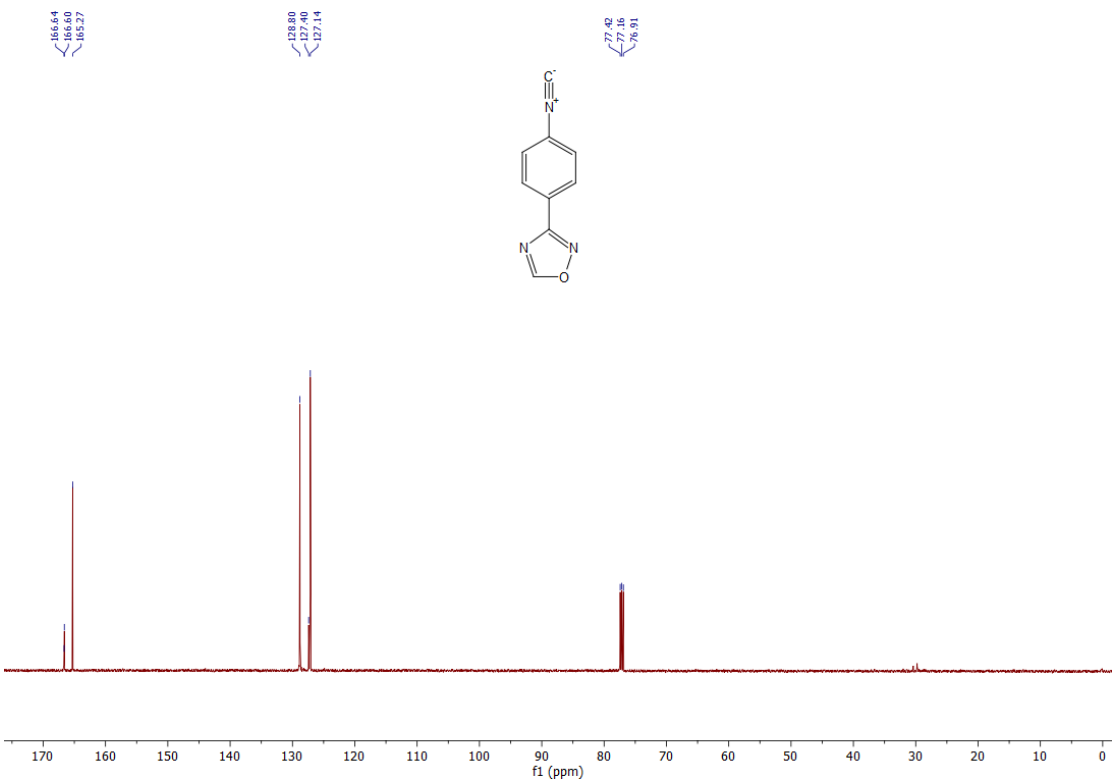
Follow general procedure **II** on 12.5 mmol scale. White solid (3.80 g, 63%), m.p.: 203-205 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.45 (s, 1H), 8.74 (s, 1H), 8.09 (d, J = 8.7 Hz, 2H), 7.68 (d, J = 8.8 Hz, 2H), 7.24(d, J = 7.9 Hz 1H), 7.17 (d, J = 7.5 Hz, 2H), 4.28 (s, 2H), 3.34 – 3.27 (m, 2H), 2.78 (ddd, J = 14.0, 10.1, 3.7 Hz, 2H), 2.43 – 2.36 (m, 2H), 2.35 – 2.33 (m, 1H), 2.22 (s, 6H), 2.11 – 2.04 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 176.2, 167.4, 167.0, 164.8, 140.7, 139.8, 135.4, 129.9, 129.5, 128.8, 122.3, 119.9, 55.9, 49.6, 36.8, 27.3, 18.3. HRMS (ESI) m/z: $[M + H]^+$ calcd for $C_{24}H_{27}O_5N_4S$, 83.1624; found, 483.1695.

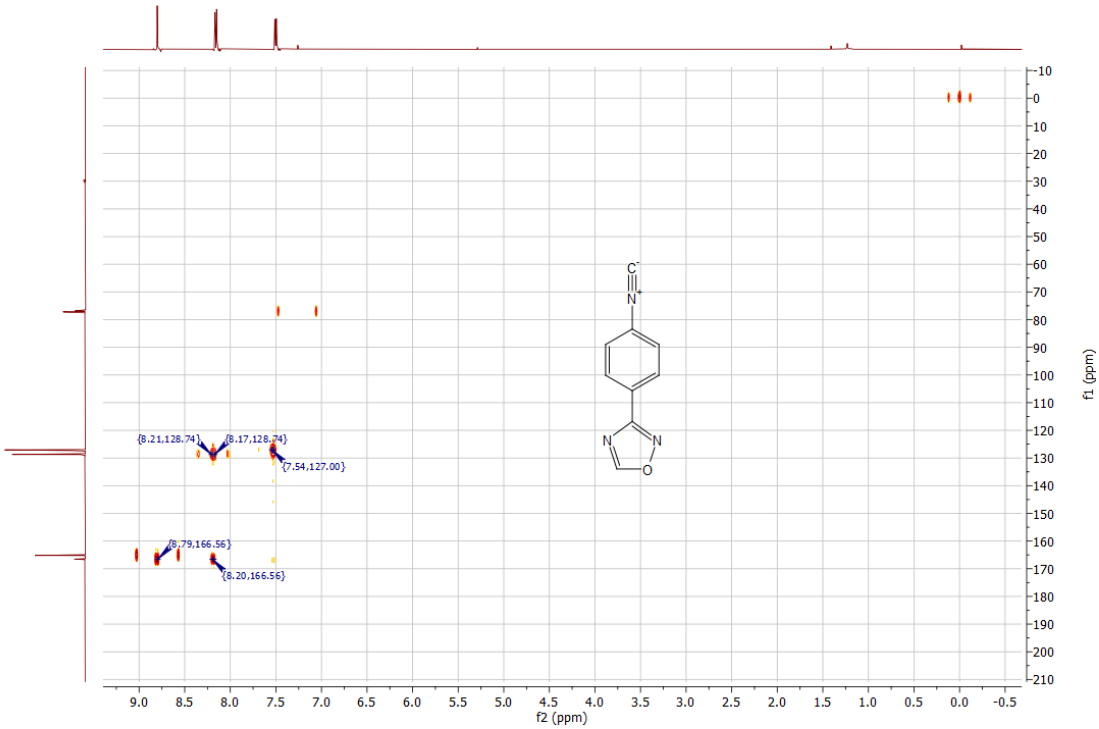
6. The ^{1}H-NMR and ^{13}C{^{1}H} NMR spectrum of final compounds

^{1}H NMR spectrum of **4** (500 MHz, $CDCl_3$)



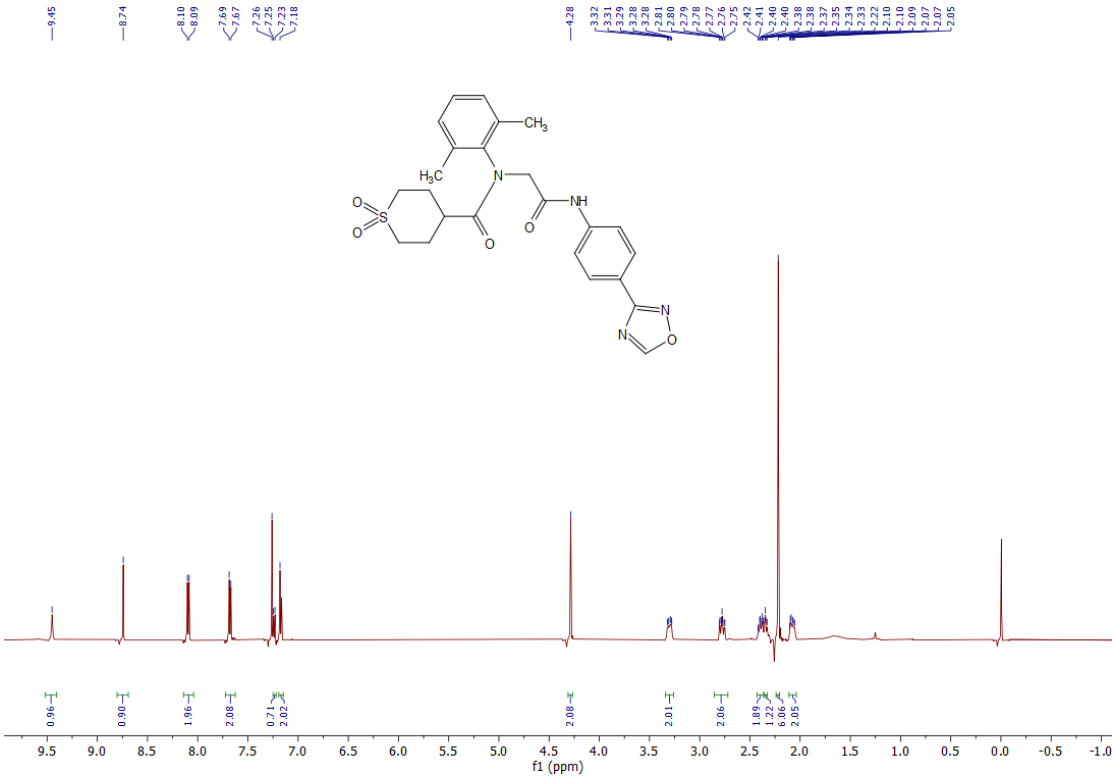
^{13}C NMR spectrum of **4** (500 MHz, $CDCl_3$)



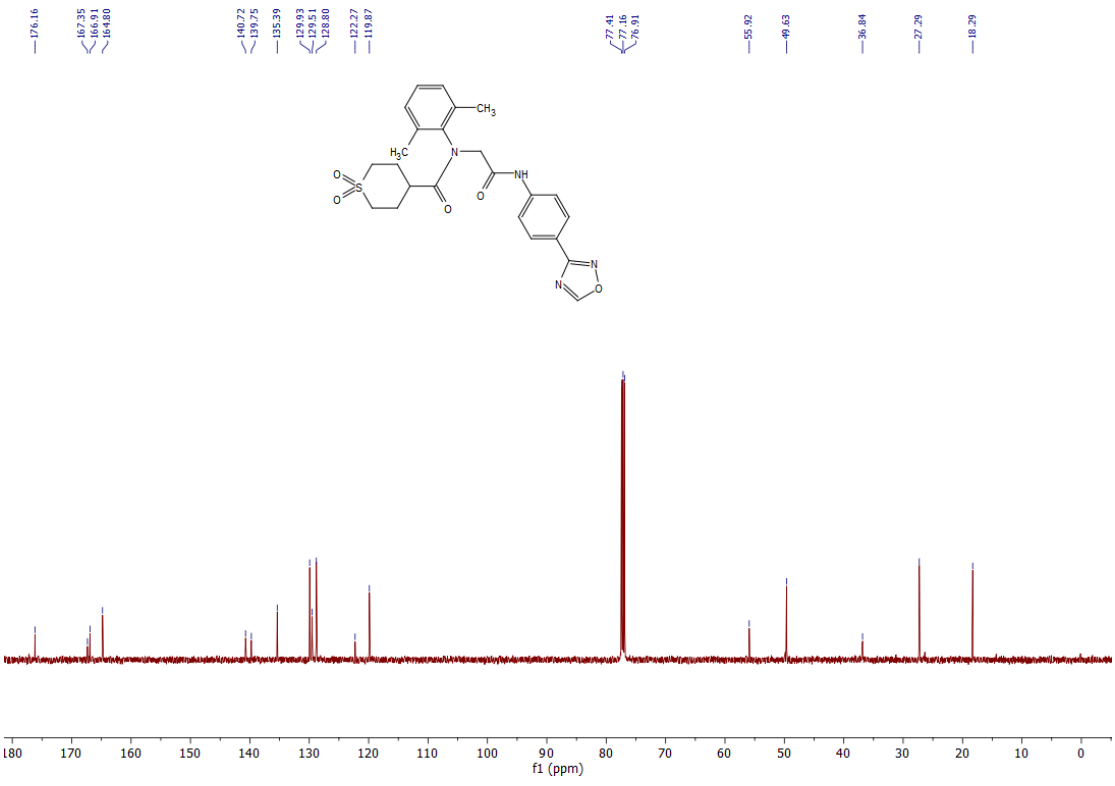


^{1}H-^{13}C HMBC NMR spectrum of **4** (500 MHz, $CDCl_3$)

^{1}H NMR spectrum of **Amenamevir** (500 MHz, $CDCl_3$)



^{13}C NMR spectrum of **Amenamevir** (500 MHz, $CDCl_3$)



7. Environmental factor (E-factor), Process Mass Intensity (PMI) and Atom Economy (AE) calculations (1g of Amenamevir)

The reaction conditions of **Amenamevir** synthesis were taken from literature.[6-8] While calculating the E-factors, we didn't consider the amount of silica gel used for flash column chromatography as it is generally not reported. In our case, for a 1 mmol scale reaction we used 15 g of 100 - 200 mesh size silica for the purification in only one step (one-pot synthesis of Amenamevir). However, the amount of silica gel that is employed on the other three methods is considerably higher due to the purification of at least two steps and the increased scale of the reactions described.

Therefore, the inclusion of silica gel waste would considerably increase the difference in E-factor between our reported synthesis and the previous two patents.

The three equations that we used to calculate **E-factor**, **PMI** and **AE*** in **1g** of **Amenamevir** syntheses were listed as follow:

(1) **E-factor** = Mass (**waste)**/Mass (**product**)

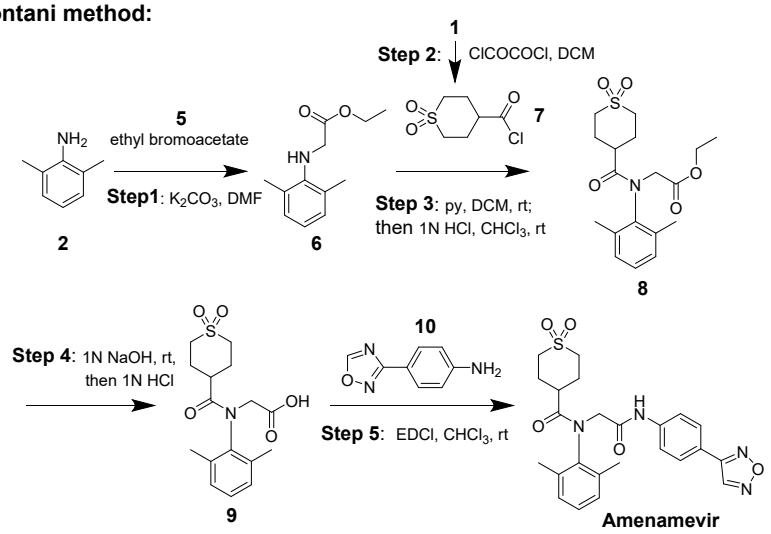
(2) **PMI** = Mass (**total**)/Mass (**product**) = E-factor + 1

(3) **AE** = (Molecular mass of **desired product**/Sum of molecular masses of **all reactants**) X 100%

**For the AE calculation any material that is incorporated into an intermediate or product during the synthesis is taken into account. This includes protecting groups, catalysts used in stoichiometric quantities and acids or bases used for hydrolysis. Solvents, reagents or materials used in catalytic quantities are omitted from the analysis, as they do not contribute atoms to an intermediate and/or product.*[#]

7.1 Kontani, T.; Miyata, J.; Hamaguchi, W.; Kawano, T.; Kamikawa, A.; Suzuki, H.; Sudo, K. US 20050032855A1, **2005**.

Kontani method:



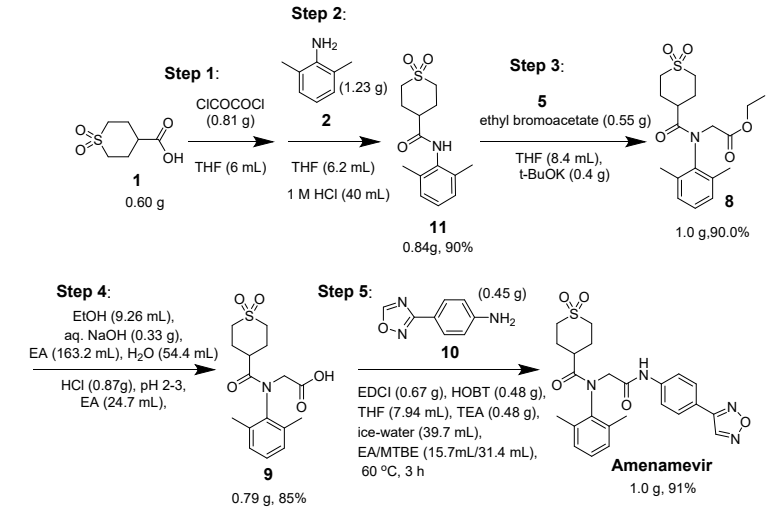
Unfortunately, this first-issue patent does not specify the amounts of reactants used, reaction times, yields, and spectral characteristics, so the **E-factor**, **PMI** and **AE** cannot be calculated. Nevertheless, it is clear that

NO.	Reagents	Amount (g)
1	tetrahydro-2H-thiopyran-4-carboxylic acid 1,1-dioxide (**1**)	0.6 g
2	oxalyl dichloride	0.8 g
3	THF	28.5 mL*0.89 g/mL = 32.0 g
4	2,6-dimethylaniline (**2**)	1.2 g
5	1 M HCl	40 mL*1.06 g/mL = 42.4 g
6	ethyl bromoacetate (**5**)	0.55 g
7	*t*-BuOK	0.4 g
8	EtOH	9.3 mL*0.79 g/mL = 7.3 g
9	aq. NaOH	0.3 g
10	EA	203.6 mL*0.90 g/mL = 183.2 g
11	H_2O	94.1 mL*1.0 g/mL = 94.1 g
12	HCl	0.87 g
13	4-(1,2,4-oxadiazol-3-yl)aniline (**10**)	0.45 g
14	EDCI	0.67 g
15	HOBT	0.48 g
16	TEA	0.48 g
17	MTBE	31.4 mL*0.74 g/mL = 23.2 g
18	Total	389.0 g

five steps are employed in the reaction, and a strong base (NaOH) and acid (HCl) are used in the synthetic route.

7.2 Zhang, X, M.; Zhao, X.; Gao C.; Zhang Z.; Chen, J.; Zhen, Y. CN 108623577A, **2018**.

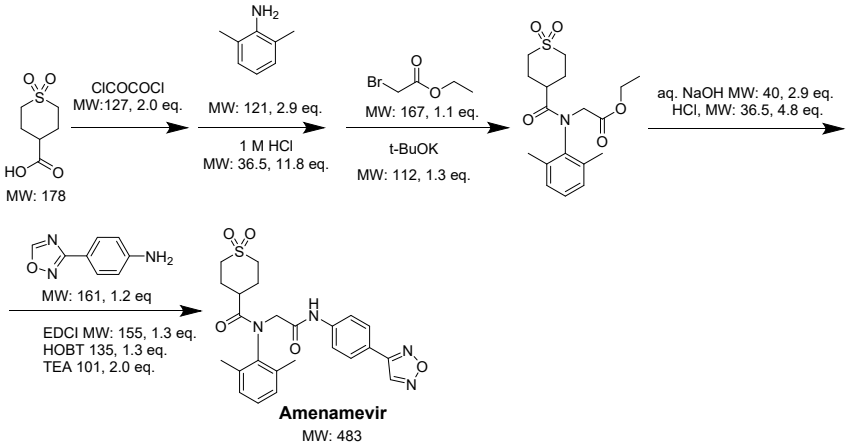
Xumeng method:



Amount of product = 1.0 g

E-Factor = Amount of waste/Amount of product = (389.0-1) g/1g = 388.0

PMI = E-factor + 1 = **389.0**



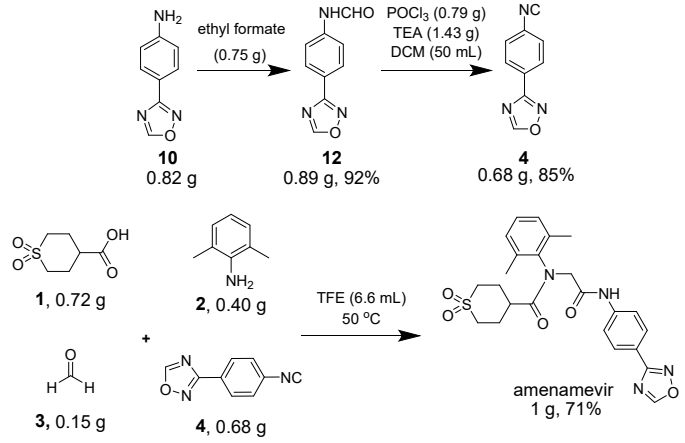
AE = (483/(178+127*2+121*2.9+36.5*11.8+167*1.1+112*1.3+40*2.9+36.5*4.8+161*1.2+155*1.3+135*1.3+101*2.0)*100% = (483/2428.3)*100% = 19.9%

Extraction solvent volume: In order to do the calculation and comparison, we settle the same solvent volume value for the extraction process: for every 1 mmol reactant, 20 mL organic solvent and 20 mL H_2O were used.

Drying agents: the amounts of drying agents (Na_2SO_4, $MgSO_4$, $CaSO_4$, etc.) were not take into account in our calculations.

7.3 In this work:

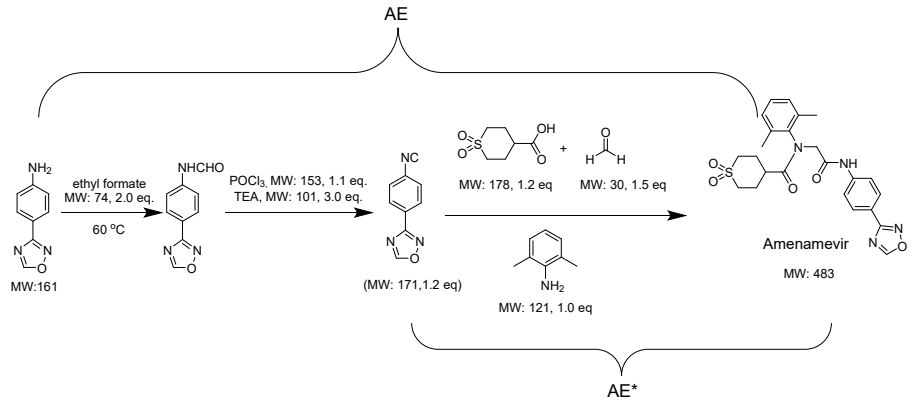
Our Method:



Amount of product = **1.0 g**

E-Factor = Amount of waste/Amount of product = (86.5-1) g/1g = 85.5

PMI = E-factor + 1 = **86.5**



AE = (483/(161+74*2.0+153*1.1+101*3.0+178*1.2+30*1.5+121*1.0)*100% = (483/1160)*100% = 41.6%

Including isocyanide synthesis step.

NO.	Reagents	Amount (g)
1	4-(1,2,4-oxadiazol-3-yl)aniline (**10**)	0.82 g
4	ethyl formate	0.75 g
5	$POCl_3$	0.79 g
6	TEA	1.43 g
7	DCM	50 mL*1.33 g/mL = 66.5 g
8	tetrahydro-2H-thiopyran-4-carboxylic acid 1,1-dioxide (**1**)	0.72 g
9	2,6-dimethylaniline (**2**)	0.40 g
10	formaldehyde (**3**)	0.15 g
11	3-(4-isocyanophenyl)-1,2,4-oxadiazole (**4**)	0.68 g
12	TFE	6.6 mL*1.52 g/mL = 10.03 g
18	Total	86.5 g

AE* = (483/(171*1.2+178*1.2+30*1.5+121*1.0)*100% = (483/584.8)*100% = 82.6%

*Without isocyanide synthesis step.

References:

1. Rigaku-Oxford Diffraction; CrysAlisPro Oxford Diffraction Ltd, Abingdon, England V 1. 171. 36. 2.(release 27-06-2012 CN (2006)

2. Palatinus, L. Chapuis, G., J. Appl. Cryst. 2007, 40, 786.

3. Sheldrick, G. M. ActaCryst. 2008, A64, 112-122.

4. Farrugia, L.J. Journal of Applied Crystallography 2012, 45, 849-854.

5. Macrae C. F., Edgington P.R., McCabe P., Pidcock E., Shields G.P., Taylor R., Towler M., & van de Streek J., J. Appl. Cryst. 2006, 39, 453-457.

6. A. Dömling and T. Zarganes-Tzitzikas, WO 2020038812A1, 2020.

7. T. Kontani, J. Miyata, W. Hamaguchi, T. Kawano, A. Kamikawa, H. Suzuki and K. Sudo, US 20050032855A1, 2005.

8. X, M. Zhang, X. Zhao, C. Gao, Z. Zhang, J. Chen and Y. Zhen, CN 108623577A, 2018.