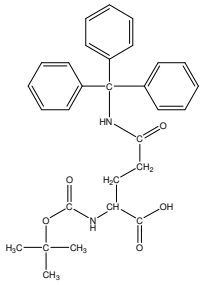
Synthesis of the inhibitor MAPI.

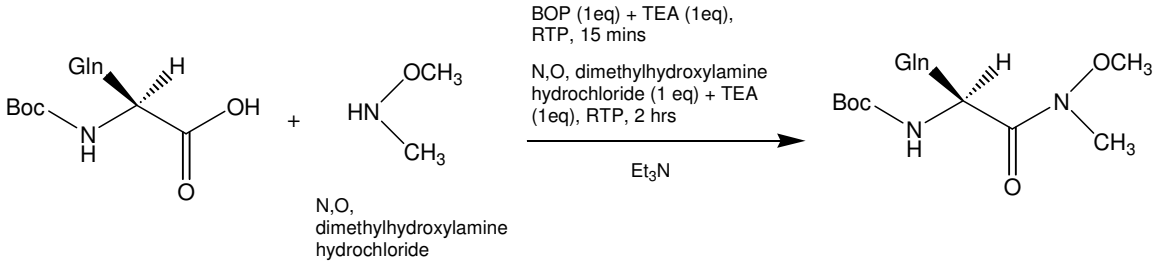
Supplementary material for 'A structural study of norovirus 3C protease specificity - binding of a designed active site-directed peptide inhibitor'. R. J. Hussey, L. Coates, R. S. Gill, P. T. Erskine, S.-F. Coker, E. Mitchell, J. B. Cooper, S. Wood, R. Broadbridge, I. N. Clarke, P. R. Lambden and P. M. Shoolingin-Jordan.

The starting material for the synthesis was N-terminal Boc-, and side-chain triphenylmethyl- (or trityl-) protected glutamine:



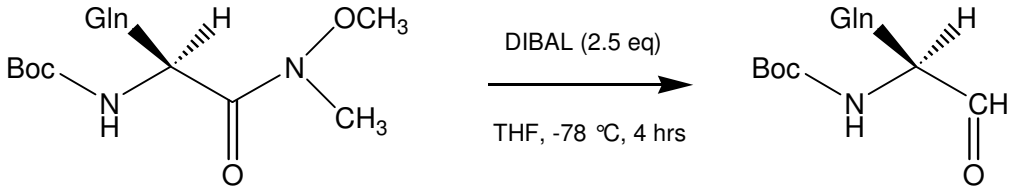
Boc-L-Tr-Gln-OH

Boc-L-Tr-Gln-OH (5 mmoles) was dissolved in 50 ml of DCM followed by addition of base TEA (1 eq) and then BOP (1 eq). The reaction mixture was then stirred at RTP for 15 minutes prior to addition of N,O,dimethylhydroxylamine hydrochloride (1 eq) and further TEA (1 eq) to neutralise the HCl produced. Following the 2 hour reaction time, production of the Weinreb amide was confirmed by TLC and mass spectrometry (MALDI-Q-TOF-MS) which was also performed for all other steps in the synthesis. Note that in the following schemes Gln indicates a trityl-protected glutamine.



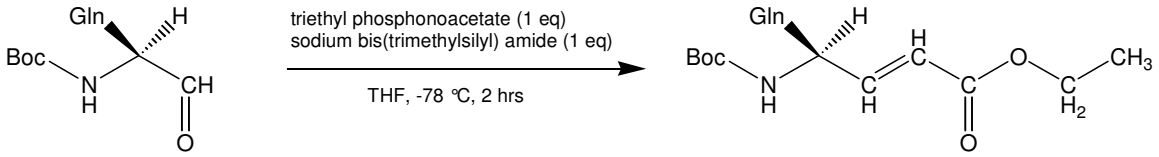
DCM was evaporated from the reaction solution using a RE with warming at 40°C. The solid product was then dissolved in 200 ml of ethyl acetate and the solution transferred to a 500 ml separating vessel. The solution was then washed with 200 ml of 1 M HCl and with 200 ml of a super-saturated $NaHCO_3$ solution in analytical grade water. The product was then dried over solid Na_2SO_4 followed by evaporation of the ethyl acetate on a RE with warming at 40°C.

The Weinreb amide glutamine derivative was then dissolved in 40 ml of THF at RTP in an RBF which was filled with inert gas and lowered into a dry ice-acetone bath to maintain a temperature of -78°C throughout. DIBAL (2.5 eq) was added dropwise by canula and the solution stirred for 4 hours. The reaction was then terminated by addition of 2 ml of methanol for 1 minute after which HCl was added by canula to a final concentration of 0.1 M to release the DIBAL and yield the aldehyde derivative glutamine. The RBF was then removed from the dry-ice-acetone bath and allowed to warm to room temperature.



The resulting suspension was dissolved in 150 ml of diethyl ether and transferred to a 500 ml separating vessel (non-equilibrating type) where it was washed three times with 100 ml of 0.1 M HCl, once with half-saturated $NaHCO_3$ and finally with water. The resulting organic phase was dried over solid anhydrous $MgSO_4$ and filtered. The diethyl ether and residual toluene (the solvent for DIBAL) were removed by evaporation at 40°C with a diaphragm pump.

An empty RBF was cooled to -78°C in a dry ice-acetone bath and triethyl phosphonoacetate (1 eq) was added followed by 20 ml of THF with stirring for 5 minutes. Sodium bis(trimethylsilyl)amide (1 eq), the triethyl phosphonoacetate activating agent, was added and the reaction mixture stirred for 20 minutes. The aldehyde glutamine derivative (Boc-L-Tr-Gln-OH) synthesised in the previous step was dissolved in 20 ml of chilled THF and then added to the RBF. The reaction was then allowed to proceed for 2 hours under inert gas at -78°C with stirring. The RBF was removed from the dry ice-acetone bath and placed in a water-ice bath to allow warming to 0°C.

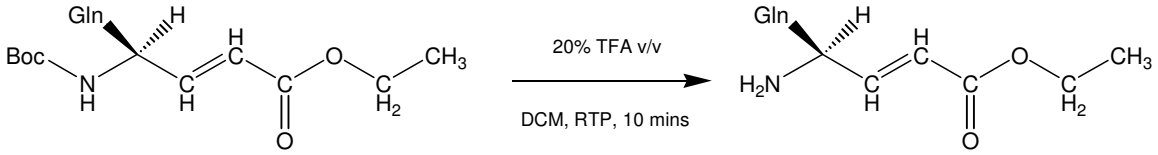


The resulting solution was decanted into a 500 ml separating vessel and 100 ml of a 0.5 M HCl was added. The mixture was washed twice with 100 ml of 50:50 ethyl

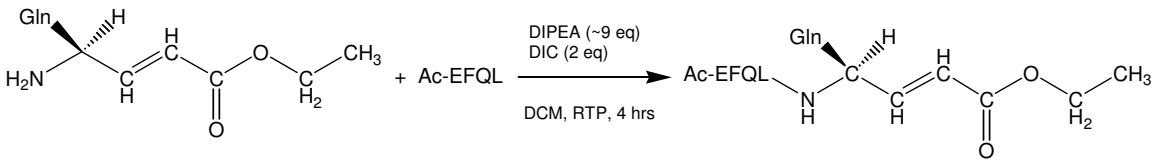
acetate:hexane and the organic phase was dried over solid Na_2SO_4 and then filtered. The solvent was evaporated from the product by use of a RE with warming at 40°C.

Coupling of the Boc-L-Tr-Gln-propenyl-ethyl-ester to the peptide Ac-EFQL was achieved in three stages: cleavage of the Boc group protecting the N-terminus of Boc-L-Tr-Gln-propenyl-ethyl ester; solution phase coupling of the deprotected L-Tr-Gln-propenyl-ethyl-ester to the peptide Ac-EFQL, and finally the cleavage of the *C*-terminal trityl side chain protecting group of Ac-EFQLQ-propenyl-ethyl-ester.

The oil containing Boc-L-Tr-Gln-propenyl-ethyl-ester was thawed and allowed to reach RT before dissolution in 50 ml of DCM. A sufficient volume of TFA to make 20% v/v was added and the solution stirred at RTP for 10 minutes. The solvent was evaporated using a RE with warming at 40°C. To remove residual TFA, 50 ml DCM was added, the RBF swirled and the solvent evaporated as before. To precipitate the L-Tr-Gln-propenyl-ethyl-ester product, 20 ml diethyl ether was added and the RBF swirled. The diethyl ether was then evaporated by use of a RE with warming at 40°C and the product re-dissolved in a further 20 ml of diethyl ether, the RBF swirled and diethyl ether evaporated as before. This was repeated using 20 ml of acetonitrile.



In a 100 ml RBF, 400 mg of L-Tr-Gln-propenyl-ethyl-ester was dissolved in 20 ml of DCM and DIPEA (~9 eq) was added to give a pH of 8. Separately, Ac-EFQL (1 eq) was dissolved in 2 ml of 50:50 DCM:DMF and added to the L-Tr-Gln-propenyl-ethyl ester followed by addition of DIC (2 eq). The reaction solution was then stirred at RT for 4 hours to allow coupling, following which the solvent was evaporated by use of a RE with warming at 40°C until the volume was reduced to approximately 5 ml. To this viscous solution, 50 ml of diethyl ether were added causing precipitation of the product: Ac-EFQLQ-propenyl-ethyl-ester with the C-terminal glutamine side chain still protected by a trityl group. To recover the product, the diethyl ether and DMF were removed by passage through a glass sinter to leave a gel of the product from which the residual ether was allowed to evaporate at RT for 1 hour.



The gel of the product Ac-EFQLQ-propenyl-ethyl-ester was transferred to a 100 ml RBF. The side chain protecting groups were then cleaved in 20 ml of 70% TFA in DCM at RTP with stirring for 1 hour. To scavenge the cleaved trityl cation, 300 µl of tri-isopropylsilane was added during this cleavage reaction. The solvent was then evaporated using a RE with warming at 40°C to leave an oil from which the product was triturated by addition of 20 ml of diethyl ether. The gel was then dissolved in 20 ml of 50:50 acetonitrile:H_2O and dried to form a white solid of the final product: Ac-EFQLQ-propenyl-ethyl-ester by freeze drying. This was dissolved in 100 µl of DMSO and loaded onto a Phenomenex C12 Jupiter reverse phase column (250 x 10 mm) with 90 Å bead size. Purity of the final product was determined by mass spectrometry with a single major peak corresponding to a M_r of 760.

Abbreviations

Ac	acetyl
Boc	butoxycarbonyl
BOP	Benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate
DCM	dichloromethane
DIBAL	diisobutyl aluminium hydride
DIC	N,N-diisopropylcarbodiimide
DIPEA	di-isopropyl ethylamine
DMF	N,N-dimethylformamide
DMSO	dimethyl sulfoxide
MALDI-Q-TOF-MS	matrix assisted laser desorption ionisation quadrapole time of flight mass spectroscopy
RBF	round bottomed flask
RE	rotary evaporator
RTP	room temperature and pressure
TEA	triethylamine

TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography