**Aqueous Phosphoric Acid as a Mild Reagent for Deprotection of
tert-Butyl Carbamates, Esters and Ethers**

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General: NMR spectra were recorded at 400 MHz unless specified otherwise. Chemical shifts are reported in part per million (ppm) with TMS as an internal standard. LCMS was recorded using API-ES ionization mode. Reagents and solvents were obtained from commercial sources and used without further purifications. Achiral HPLC analyses were carried out using a stable-bond cyano (SB-CN) column (4.6 mm×250 mm) with acetonitrile: 0.2% perchloric acid aqueous buffer (20/80 or 40/60) as mobile phase (2 mL/min) and detection at 210 nm wavelength. Chiral HPLC methods were used for the assays: AD-H column, 4.6×250 mm, hexane:ethanol:diethylamine (90/10/0.2) mobile phase, 1.5 mL/min. 35°C, 210 or 225 nm.

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One dimensional and extensive 2D NMR experiments were performed for the assignment of the proton and carbon spectra. Electrospray (ESP) mass spectrometry, with a flow injection sample introduction technique, along with tandem mass spectrometry was performed.

The preparations of products in Tables 1, 2 and 3 followed general procedures in the Experimental Section. All products were characterized by NMR and MS, in addition the products were shown to co-elute with authentic samples either from commercial sources or obtained from other established methods.

Characterization of Products in Table 1.

Entry	Product	Characterization
1	Methyl L-phenylalaninate	^1H NMR (DMSO- d_6) δ 7.28 -7.14 (m, 5H), 3.58 – 3.51 (m, 4H), 2.87 – 2.72 (m, 2H)
2	1-Benzyl L-aspartate	^1H NMR (DMSO- d_6) δ 7.36 – 7.28 (m, 5H), 5.10 (s, 2H), 3.76 – 3.72 (m, 1H), 2.57 – 2.39 (m, 2H).
3	(9-Fluorenylmethoxycarbonyl)-L-histidine	^1H NMR (DMSO- d_6) δ 8.87 – 7.22 (m, 9H), 6.82 (s, 1H), 4.25 – 4.05 (m, 5H), 3.30 – 2.88 (m, 2H)
4	N- δ -Benzyloxycarbonyl-D-ornithine	^1H NMR (DMSO- d_6) δ 7.48 – 7.25 (m, 5H), 4.97 (s, 2H), 3.07 (t, 2H, J = 6.4 Hz), 2.98 – 2.81 (m, 2H), 1.73 – 1.376 (m, 4H).
5	Methyl L-valinate	^1H NMR (DMSO- d_6) δ 3.61(s, 3H), 3.23 (d, 1H, J = 5.4 Hz), 1.97 – 1.82 (m, 1H), 0.86 – 0.80 (m, 6H).

6	Methyl (3R,4R)-4-[3-oxo-3-(6-methoxyquinolin-4-yl)propyl]piperidine-3-carboxylate	^1H NMR (DMSO- d_6) δ 9.08 (d, 1H, J = 2.8 Hz), 8.37 (d, 1H, J = 4.5 Hz), 8.21 (d, 1H, J = 2.8 Hz), 7.75 (dd, 1H, J = 4.5 and 2.8 Hz), 7.64 (d, 1H, J = 2.8 Hz), 3.91 (s, 3H), 3.62 (s, 3H), 3.39 – 2.83 (m, 7H), 2.20 – 2.04 (m, 1H), 1.94 – 1.55 (m, 5H). ^1H NMR (CDCl_3 , 400 MHz) δ 7.20-7.36 (m, 1H), 6.85-7.01 (m, 3H), 4.36-4.44 (m, 1H), 2.58-3.05 (m, 4H), 2.25-2.40 (m, 1H), 1.80-2.04 (m, 2H), 1.37-1.64 (m, 6H), 1.21 (s, 3H), 1.22 (s, 3H); ^{13}C NMR (CDCl_3 , 400 MHz) δ 179.6, 164.1, 161.8, 140.6, 130.2, 130.1, 124.9, 124.8, 116.1, 115.9, 113.7, 113.5, 80.9, 70.5, 56.4, 40.8, 39.8, 30.8, 29.5, 29.0, 26.2, 14.1. ^{13}C NMR (CDCl_3 , 400 MHz) δ 179.6, 164.1, 161.8, 140.6, 130.2, 130.1, 124.9, 124.8, 116.1, 115.9, 113.7, 113.5, 80.9, 70.5, 56.4, 40.8, 39.8, 30.8, 29.5, 29.0, 26.2, 14.1.
8	4-((tert-butyldimethylsilyloxy)methyl)piperidine	^1H NMR (DMSO- d_6) δ 6.02 (1H, br s), 3.09 (d, 2H, J = 6.8 Hz), 3.09-3.02 (2H, m), 2.75 (2H, t, J = 7.3 Hz), 1.78-1.09 (7H, m), 0.88 (s, 9H), -0.51 (s, 6H)
9	3-amino-1-benzhydrylazetidine	^1H NMR (DMSO- d_6) δ 7.39 – 7.11 (m, 10H), 4.79 (br s, 2H), 4.32 (s, 1H), 3.44 -3.38 (m, 1H), 3.31 (dd, 1H, J = 4.5 and 6.2 Hz), 2.59 (dd, 1H, J = 4.5 and 6.2 Hz)
10	7-Azabenzonorbornene	^1H NMR (CDCl_3) δ 7.23-7.18 (2H, m), 7.11-7.08 (2H, m), 4.58(2H, s), 1.95 (2H, dt, J = 7.2 and 2.5 Hz), 1.19-1.18 (2H, m).

product

Solvent: dmsd
Ambient temperature
UNITYplus-400
PULSE SEQUENCE
Pulse 45.0 degrees
Acq. time 2.41 sec
Width 6795.8 Hz
16 repetitions
OBSERVE H1, 399.7486325 MHz
DATA PROCESSING
F1 size 32768
Total time 1 minute

Table 1, Entry 6

Table 1 Entry 7

Solvent: dms-d₆
Acid: 4.0
pH: 7.0
Temperature: 300
Unit: ppm
Pulse sequence: zgpg30
Pulse: 45.0 degrees
Acq. time: 2.411 sec
F2: 400.146 MHz
F1: 101.253 MHz
Observations: 88.7497337 MHz
Data processing: 1
Line broadening: 0.1 Hz
F1 size: 32768
Total time: 1 minute

Table 1, Entry 7

solvent: cdcl₃
ambient temperature
ITplus-400
1SE SEQUENCE
relax. delay 0.100 sec
use 45.0 degrees
cq. time 0.685 sec
f1dh 24890.5 Hz
38 repetitions
SOLVENT 100.5163722 MHz
COUPLE H1 - 399.7487325 MHz
over 40 dB
continuously on
ALTZ-16 modulated
1A PROCESSING
the bracketing 1.0 Hz
812.33072
td1 time 3 minutes

Table 1 Entry 8

Solvent: dms
Acquired: 10/10/2019
Unit: p100-400
Pulse sequence: zgpg30
Pulse: zgpg30
Acq. time: 2.411 sec
Fid: 1000000000
F2: 100.626130 MHz
Observed: 100.626130 MHz
DATA PROCESSING
FT size: 32788
Total time: 1 minute

104316-53-02

Pulse Sequence: szpu1
Solvent: dmso
Ambient temperature
UNITYplus-400
PULSE SEQUENCE
Pulse 45.0 degrees
Acq. time 2.412 sec
Width 6794.1 Hz
16 repetitions
OBSERVE: H1, 399.6509168 MHz
DATA PROCESSING
Line broadening 0.1 Hz
F1 size 32768
Total time 1 minute

Table 1, entry 10

Filename: 104316-53-02_h1.fid
unitym: 050719

Entry	Product	Characterization
1	Benzoic acid	^1H NMR (CD_3OD) δ 8.02 (t, 2H, $J = 7.5$), 7.58 (d, 1H, $J = 7.5$), 7.42 (t, 2H, $J = 7.5$), 4.93 (1H, br s)
2	N-Phthaloylglycine	^1H NMR (CD_3OD) δ 7.88-7.78 (m, 4H), 4.40 (s, 2H)
3	N-(Carbobenzyloxy)-L-Glutamic Acid	^1H NMR (CD_3OD) δ 7.38 – 7.23 (m, 5H), 5.03 (s, 2H), 4.96 (br s, 1H), 4.22-4.18 (m, 1H), 2.40 (t, 2H, $J = 6.7$ Hz), 2.22-2.04 (m, 1H), 1.98-1.84 (m, 1H).
4	Benzyl Malonate	^1H NMR (CD_3OD) δ 7.38-7.24 (m, 5H), 5.17 (s, 2H), 3.40 (s, 2H).
5	N-(9-Fluorenylmethoxycarbonyl)-L-Aspartic Acid	^1H NMR (CD_3OD) δ 7.80 – 7.24 (m, 8H), 4.96 (br s, 2H), 4.60 – 4.19 (m, 3H), 2.91-2.60 (m, 2H)
6	N-(Benzyloxycarbonyl)-D-Aspartic Acid	^1H NMR (CD_3OD) δ 7.38-7.21 (m, 5H), 5.20 – 4.92 (m, 3H), 4.68 -4.65 (m, 1H), 2.88 – 2.72 (m, 2H)
7	2'-O-Acetyl-11-N-carboxymethy-11,12-cyclocarbamate ketolide (4)	See page S28

Table 2, Entry 1

Solvent: cd_3OD
Acidulant temperature
UNIT: plus 900
PULSE SEQUENCE
Pulse 45.0 degrees
Acq. time 2.431 sec
Width 6785.6 Hz
10 repetitions
DSERVE H1: 388.743087 MHz
DATA PROCESSING
FT size 32768
Total time 1 minute

Table 2, Entry 2

Table 2, Entry 3

Solvent: cd3od
Ambient temperature
UNITplus-400
PULSE SEQUENCE
Pulse 45.0 degrees
Acq. time 2.411 sec
Width 6795.8 Hz
16 repetitions
OBSERVE H1, 399.7483087 MHz
DATA PROCESSING
FT size 32768
Total time 1 minute

Table 2 Entry 4

Table 2 Entry 5

Table 2 Entry 6

Solvent: CDCl_3
Ambient temperature
Unit: ppm-400
Pulse sequence
Pulse: 45.0 degrees
Acq. time: 2.41 sec
Vid: 6795.0 Hz
15 acquisitions
OBSERVE: M1, 389.742807 MHz
DATA PROCESSING
F1: 8122.82750
Total time: 1 minute

Characterization of Products in Table 3

Entry	Product	Characterization
1	(2S,4R)-1-(benzyloxycarbonyl)-4-hydroxypyrrolidine-2-carboxylic acid	^1H NMR (DMSO- d_6) δ 12.62 (s, 1H), 7.38 – 7.23 (m, 5H), 5.19 – 4.98 (m, 3H), 4.38 – 4.18 (m, 2H), 3.47 – 3.31 (m, 2H), 2.20-2.04 (m, 1H), 1.97 - 1.91(m, 1H)
2	N-benzyloxycarbonyl-L-Serine	^1H NMR (DMSO- d_6) δ 7.39 – 7.22 (m, 5H), 5.01 (s, 2H), 4.05 – 4.01 (m, 1H), 3.62- 3.59 (m, 1H), 3.36 (s, 1H).
3	N-benzyloxycarbonyl-L-Threonine-Benzyl Ester	^1H NMR (DMSO- d_6) δ 7.39 – 7.22 (m, 10H), 5.18-4.98 (m, 5H), 4.80 (d, 1H, J = 6.6 Hz), 4.12-4.03 (m, 2H), 1.07 (d, 3H, J = 6.6 Hz)
4	4-bromophenol	^1H NMR (DMSO- d_6) δ 9.68 (s, 1H), 7.29 (d, 2H, J = 8.7 Hz), 6.71 (d, 2H, J = 8.7 Hz).
5	N-benzyloxycarbonyl-4-(R)-Hydroxy-L-Proline	^1H NMR (DMSO- d_6) δ 10.24 (s, 1H), 9.74 (s, 1H), 7.39 (dd, 1H, J = 8.3 and 2.1 Hz), 7.35 (d, 1H, J = 2.1 Hz), 6.9 (d, 1H, J = 8.3 Hz)

Characterization of Compound 4.

Both NMR and MS data are consistent with **4** below.

Compound 4

MW = 712.8

$\text{C}_{35}\text{H}_{56}\text{N}_2\text{O}_{13}$

LC-MS**Sample Preparation**

A sample of compound **4** was prepared at a concentration of ~ 1 mg/ml in 50% acetonitrile/ 50% 20mM ammonium acetate pH:3.1.

Instrumentation

Nominal mass MS and MS-MS collision induced decomposition experiments were performed using the API quadrupole time of flight tandem mass spectrometer. For MS-MS studies, argon was used in the collision cell. Static and dynamic calibrations were performed in electrospray ionization mode with a solution of sodium iodide/cesium iodide. Interface and mass spectrometer tuning parameters were optimized on the drug substance by repeated flow injections.

The HPLC instrument used for these experiments was an modular system consisting of quaternary solvent pump, autosampler, UV/VIS detector, column oven and membrane solvent degasser. Mobile phase was mixed from individual components by the quaternary solvent pump and was composed of 50% 20mM ammonium acetate and 50% acetonitrile. Column temperature was ambient and the flow rate was 1 ml/min.

NMR

Sample Preparation

Approximately 12 mg of sample were dissolved in 0.8 mL of 99.9% chloroform-*d* (CDCl_3) containing 0.05% w/w tetramethylsilane (TMS). The 1D proton and 1D carbon spectra were referenced using the TMS signal and set equal to 0.00 ppm.

Instrumentation

All 1D and 2D data were collected at 298K using a 5mm gradient broadband observe probe on a NMR spectrometer operating at 600 MHz.

Experiments

The following data were collected: 1D proton, 1D carbon, ^1H - ^1H gradient DQFCOSY (Double Quantum-Filtered Correlation SpectroscopY), ^1H - ^1H NOESY (Nuclear Overhauser Enhancement SpectroscopY),

^1H - ^{13}C multiplicity edited HSQC (Heteronuclear Single Quantum Coherence), ^1H - ^{13}C BIRDHMBC (Bilinear Rotation Decoupling-filtered Heteronuclear Multiple Bond Correlation), ^1H - ^1H ROESY (Rotating frame Overhauser Enhancement SpectroscopY), and LED with bipolar gradient pulse pair DOSY (Diffusion Orded SpectroscopY).

RESULTS AND DISCUSSION

LC/MS

Figure 1 presents the mass spectrum for compound **4**

Figure 1: Mass spectrum for **Compound 4**

The spectrum is dominated by a species at m/z 713, consistent with a relative molecular mass (r.m.m.) of 712 Daltons. This is consistent with the expected structure for compound **4**. Figure 2 presents the MS/MS collision induced dissociation (CID) spectrum of the m/z 713 species acquired at collision cell voltages of 20 and 40 V.

The spectra contain a main fragment at m/z 200 likely due to cleavage between the oxygen and the sugar ring system. A small fragment at m/z 158 is likely due to cleavage between the sugar ring oxygen and the macrolide ring followed by loss of the acetyl group.

NMR

The 1D proton spectrum shows the expected chemical shifts, multiplicities and integrations that are consistent with structure **1** and the main component in the sample. The 1D carbon spectrum is consistent with structure **1** based upon chemical shift. The 2D spectra show the expected vicinal and geminal ^1H - ^{13}C and ^1H - ^1H correlations. Key homonuclear and heteronuclear correlations are indicated in figure 3. Proton and carbon assignments are tabulated in table 1.

The stereochemistry of this compound was not explicitly evaluated.

Additional components at approximately equimolar amounts as the main component were detected in this sample. In the 1D proton spectrum three multiplets at 8.63, 7.71 and 7.31 ppm do not show any correlations to resonances any other components as determined by multiple NMR data. In the HMBC spectrum the proton resonance at 8.63 ppm shows correlations to carbons at

123.9 and 136.4 ppm, which correspond to the proton resonances at 7.31 and 7.71 ppm, respectively. The protons at 7.31 and 7.71 ppm are further coupled to a carbon at 149.3 ppm which correspond to the proton multiplet at 8.63 ppm. Heteronuclear H, C-correlations were determined through analysis of the multiplicity edited HSQC spectrum. In addition, these protons are homonuclear coupled in the COSY data.

Additional resonances detected in the 1D proton spectrum at 1.20, 2.76 and 3.01 ppm also do not show any correlations to resonances any component as determined by the analysis of multiple NMR data.

Figure 3. Structure 1 with arrows indicating key 2D long-range ($^nJ_{CH}$, $n=2,3$) heteronuclear couplings (shown in red) and geminal homonuclear couplings (shown in blue).

CARBON #	CARBON (PPM)	H's ATTACHED	PROTON (PPM)	PROTON MULTIPLICITY
1	100.9	1	4.39	d
3	68.8	1	3.56	m
4	30.8	2	1.37 1.84	m m
5	62.5	1	3.01	m
6	70.9	1	4.80	t
8	39.7	3	2.33	s
9	39.7	3	2.33	s
10	20.9	3	1.26	d
13	45.7	2	4.25 4.53	d d
14	173.1	0		
16	39.1	1	3.01	m
17	44.9	1	2.57	m
18	216.4	0		
19	61.8	1	3.69	s
20	82.6	0		
21	78.2	1	5.71	d
22	39.4	2	1.53 1.64	m

23	77.6	1	4.16	d
24	77.9	0		
26	168.7	0		
27	51.4	1	3.81	q
28	204.6	0		
29	47.0	1	3.01	m
31	14.5	3	1.05	d
34	18.2	3	1.16	d
35	14.1	3	1.13	d
36	19.3	3	1.26	s
37	22.8	2	1.53 2.00	m m
38	14.8	3	1.49	s
39	10.8	3	0.89	t
41	15.8	3	1.35	d
42	49.1	3	2.53	s
44	157.6	0		
46	170.0	0		
47	21.4	3	2.09	s

s=singlet, d=doublet, dd=doublet of doublets, dt=doublet of triplets, dq=doublet of quartets,

t=triplet, tt=triplet of triplets, q=quartet, m=multiplet

Table 1. Table of carbon assignments, number of protons on each carbon, proton assignments and multiplicities.

CONCLUSIONS

The 1D proton spectrum shows the expected chemical shifts, multiplicities and integrations that are consistent with structure 1. The 1D ^{13}C spectrum is consistent with structure 1 based upon chemical shift. The 2D spectra show the expected vicinal and geminal ^1H - ^{13}C and ^1H - ^1H correlations. Therefore, the spectra are consistent with compound **4**.

The MS and MS/MS spectra are consistent with compound **4**.

Compound 4

Chemical Shift (ppm)	Integration
~8.6	1.16
~7.2	0.61
~7.1	1.16
~5.8	0.93
4.5 - 2.0	0.92, 0.86, 0.91, 0.99, 0.92, 2.32, 1.15, 1.01, 4.24, 0.82, 1.14, 2.93, 5.66, 2.81, 0.39, 0.80, 0.79, 0.77, 0.64, 3.30, 3.08, 5.92, 1.77, 2.93, 2.89, 3.12, 3.00

~12 mg in 0.8 mL CDCl₃ by SSW
600 MHz, BB0 probe @ 25 °C

Compound 4

~12 mg in 0.8 mL CDCl₃ by SSW
600 MHz, BB0 probe @ 25 °C

Compound 4

~12 mg in 0.8 mL CDCl₃ by SSW
600 MHz, BB0 probe @ 25 °C

Compound 4

Compound 4

Chemical shift (ppm): 220.0, 204.6, 177.2, 174.6, 173.1, 170.0, 168.7, 157.6, 149.3, 136.4, 123.9, 100.9, 82.6, 78.2, 77.9, 77.6, 77.2, 77.0, 76.8, 70.9, 68.8, 62.5, 61.8, 51.4, 49.1, 47.0, 45.7, 44.9, 44.6, 39.7, 39.4, 39.1, 30.8, 29.6, 29.9, 22.8, 21.4, 21.4, 21.0, 20.9, 19.4, 19.3, 18.2, 18.2, 15.8, 15.8, 14.8, 14.8, 14.5, 14.5, 14.4, 14.4, 10.9, 10.8, 8.4, 0.0.

[illegible]

ROESY
 ~12 mg in 0.8 mL CDCl₃ by SSW
 600 MHz, BB0 probe @ 25 °C

Compound 4