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Amine Over-Alkylation Side Products in the Synthesis of BMS-955176

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Abstract

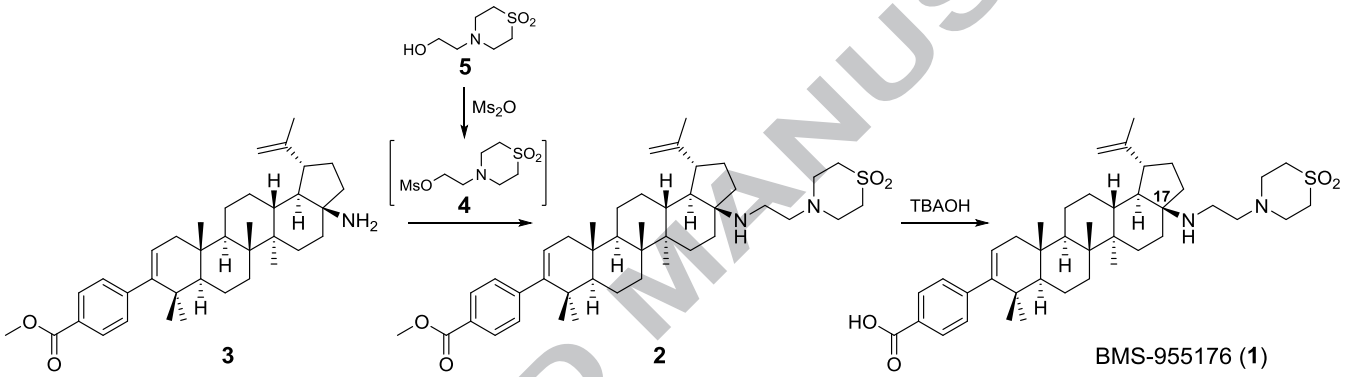
Over-alkylation side products are common in the alkylation of amines by substitution. In the synthesis of the novel HIV Maturation inhibitor BMS-955176, two over-alkylation byproducts were routinely observed at the penultimate synthetic step, in which a thiomorpholine dioxide side chain was added to the core molecule by alkylation of a primary amine. These two byproducts had drastically different HPLC relative retention times, despite both containing only one additional side chain. Adding complexity to the challenge of solving their structures was the proclivity of the two byproducts to interconvert. Positive- and negative-ion HRMS, as well as isolation and 1D and 2D-NMR were utilized to determine their structures. These byproducts were additionally problematic in that they led to daughter impurities at the API step.

Keywords

amine alkylation, BMS-955176, substitution, mass spectrometry, NMR

BMS-955176 (**1**, Scheme 1) is an orally active second generation HIV maturation inhibitor.[1,2] It was shown to overcome limitations of the first generation maturation inhibitor bevirimat by exhibiting a broader range of polymorphic coverage, and lower binding to human serum albumin.[1]

The installation of the thiomorpholine dioxide containing side chain at C17 is a key structural modification to the first generation maturation inhibitor, which improved the drug's properties.[1] The side chain is added to the amine **3** by alkylation via the *in situ* generated mesylate **4**.[3,4] Positive-ion LC-HRMS of multiple batches of the isolated product **2** consistently indicated the presence of two over-alkylation side products at RRT 0.59 and 0.92 (in neutral mobile phase)[5] with identical elemental compositions, which indicated the presence of one additional side chain. These side products did not purge completely in the crystallization of **2** (as the free base in DCM/MeOH), resulting in corresponding daughter impurities being observed in BMS-955176 (**1**).



Scheme 1. The last two steps of the synthesis of BMS-955176.

The RRT 0.59 and 0.92 impurities both showed *m/z* 866.517 by positive-ion ESI-HRMS,[6] corresponding to a neutral elemental composition of $C_{49}H_{76}N_3O_6S_2$, indicating the addition of one extra side chain. The large difference in RRT between the two was unexpected, considering that the two compounds were isomers according to positive-ion HRMS.

It was initially assumed that over-alkylation was occurring at the amine attached to C17, but it was not immediately apparent how two isomers could be generated. The possibility of alkylation of the tertiary amine of the side chain was then considered, which would create a quaternary ammonium species, and thus a second side-product (Figure 1). The observed RRTs would be consistent with these proposed structures, since the charged quaternary ammonium species would be expected to elute much earlier than a neutral species in reversed phase HPLC using a neutral mobile phase.

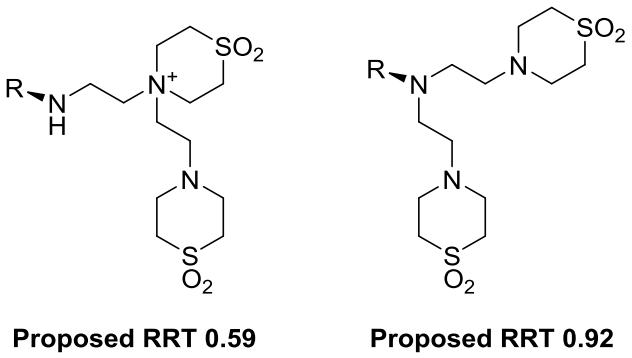


Figure 1. Initially proposed RRT 0.59 and RRT 0.92 over-alkylation side products.

This proposal was tested by negative-ion LC-ESI-HRMS, in which quaternary ammonium species can be detected via $[M^+ + 2AcO^-]^-$, and especially $[M^+ + 2TFA^-]^-$.[7] The mobile phase additive for this analysis was ammonium acetate, however, trace TFA is always observed in negative-ion mode on our system due to the frequent use of TFA-containing mobile phases. While somewhat of a nuisance in most cases, this observation was put to constructive use in this work. In negative-ion mode the RRT 0.59 peak showed *m/z* 984.542, corresponding to $[M^+ + 2AcO^-]^-$, and *m/z* 1092.487, corresponding to $[M^+ + 2TFA^-]^-$ (Figure 2a). Masses corresponding to $[M^+ + AcO^- - H]^-$ and $[M^+ + TFA^- - H]^-$ were also observed at *m/z* 924.522 and 978.490, respectively. The RRT 0.92 peak showed *m/z* 924.521, corresponding to $[M + AcO^-]^-$, and *m/z* 978.492, corresponding to $[M + TFA^-]^-$ (Figure 2b). These data confirmed that RRT 0.59 is a quaternary ammonium species and RRT 0.92 is a neutral species. While the TFA adducts resulted from the presence of trace TFA, their intensities were practically as strong as the acetate adducts, indicating that this method for quaternary ammonium detection works much better with TFA than with acetate, in agreement with the findings from Shackman et al..[7] Ions consistent with the addition of methanol to the over-alkylation products were observed co-eluting with RRT 0.92 (*m/z* 956.547 and 1010.518 in Figure 2b).

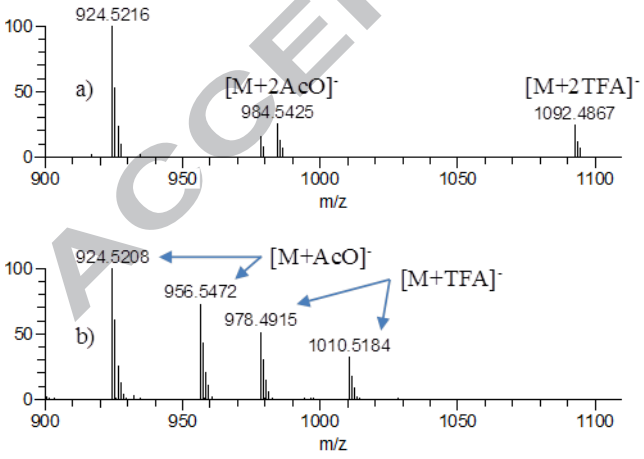


Figure 2. Negative-ion HRMS for a) RRT 0.59 and b) RRT 0.92 over-alkylation side products. Figure 2b also includes the masses for compound **8** (*m/z* 956 and 1010), which is a methanol addition product that co-eluted under these conditions. The *m/z* 978.49 peak in 2b is in 2a as well but is unlabeled in 2a.

During initial preparative HPLC isolation attempts, it was observed that the two over-alkylation side products exhibited a tendency to interconvert upon standing in the eluent. A reasonable mechanism for the conversion of the proposed neutral and quaternary ammonium species could be suggested (Figure 3), lending further plausibility to the putative structures. Additional evidence of interconversion was observed in extracted ion chromatograms of *m/z* 866.5 in positive-ion mode and *m/z* 924.5 in negative-ion mode, showing the presence of the relevant *m/z* bridging the two LC peaks (Figure 4). This is typical HPLC behavior for slowly interconverting species.[8] We consistently observed the RRT 0.92 species favored over the RRT 0.59 according to HPLC peak areas.

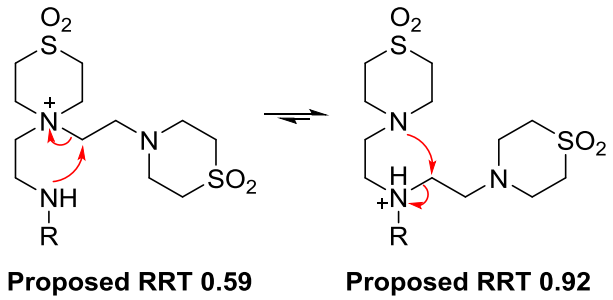


Figure 3. Possible mechanism of side chain transfer between initially proposed RRT 0.59 and RRT 0.92 over-alkylation side products.

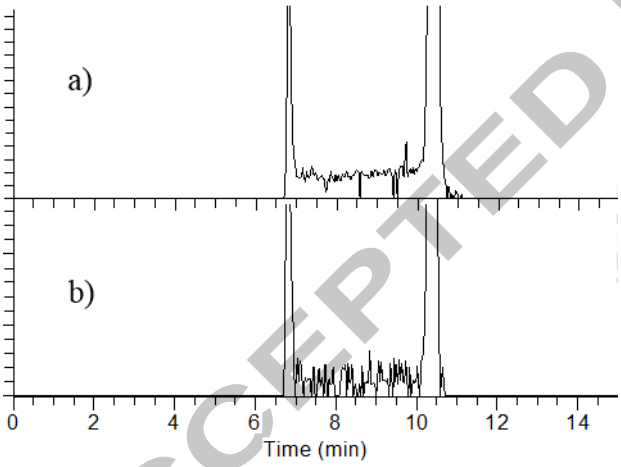


Figure 4. Extracted ion chromatograms in a) positive-ion mode (*m/z* 866.5) and b) negative-ion mode (*m/z* 924.5).

The preparative-HPLC fractions containing the two impurities were therefore combined and rotary evaporated to aqueous and subsequently extracted with chloroform. HPLC of the chloroform soluble material indicated an approximate 1:7 mixture of RRT 0.59:RRT 0.92. Despite the material being a mixture according to HPLC, NMR data were acquired at this stage without further purification.[9]

The standard set of 1D and 2D NMR data were acquired in $CDCl_3$ (^{1}H, ^{13}C, ^{1}H-^{1}H COSY, ^{1}H-^{13}C multiplicity-edited HSQC, ^{1}H-^{13}C HMBC), as well as a ^{1}H-^{15}N HMBC spectrum.[10] The ^{1}H NMR spectrum for the supposed 1:7 mixture of RRT's 0.59 and 0.92 unexpectedly showed a diagnostic set of

peaks for a vinyl group at δ_H 6.76 (*dd*, *J* = 16.6, 9.9), 6.43 (*d*, *J* = 16.6), and 6.15 (*d*, *J* = 9.9) (Figure 5 and inset). Also unexpected was that the vinyl peaks each integrated to almost 1, indicating that the isolate was a very nearly pure single species (contrary to the HPLC result). The initial proposal of over-alkylation at the C17 secondary amine of **2** was thus demonstrated to be incorrect. Over alkylation occurs only at the tertiary amine of the side chain to yield the quaternary ammonium species **6**, which ring-opens to yield the neutral vinyl species **7** (Figure 6). The C17 amine is apparently sterically hindered enough such that over-alkylation does not occur at this site, in accordance with prior observation.[1] The fact that the isolate was one species by NMR, but a mixture of **6** and **7** by HPLC was further evidence of interconversion. Full 1D and 2D NMR data for **7** are shown in Figures S1-S6 and Table S1 (Supporting Information). NMR data for compound **6** were not acquired due to its instability.

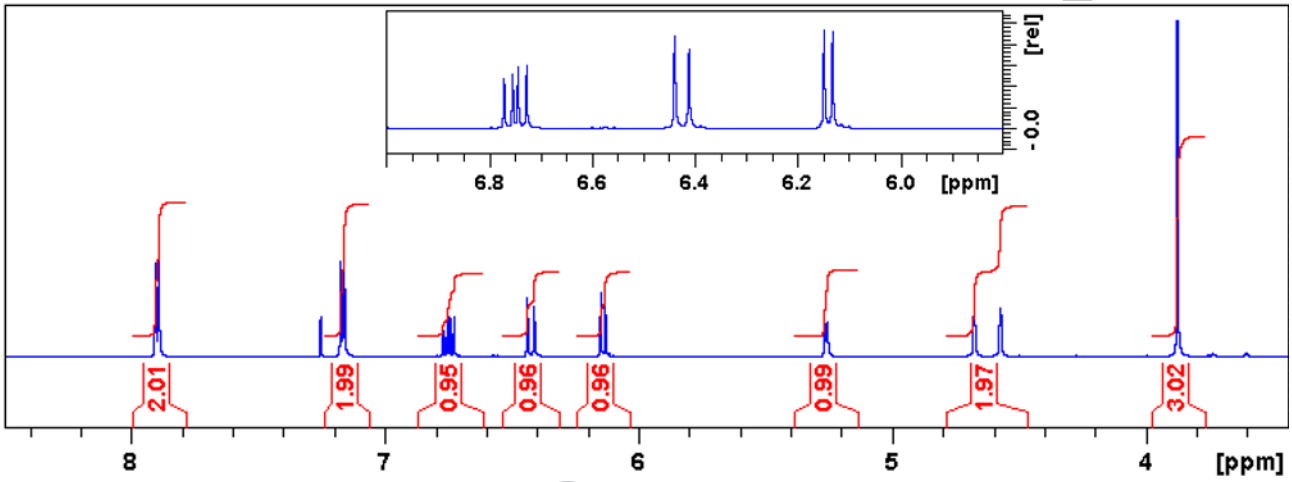


Figure 5. ^{1}H NMR ($CDCl_3$) of the downfield region for isolated RRT 0.92 over-alkylation side product (**7**) with vinyl region expanded.

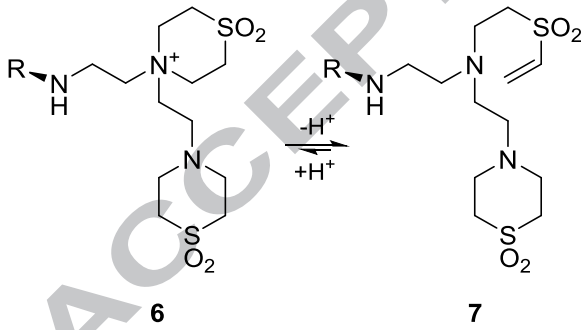


Figure 6. Actual structures of the over-alkylation side products RRT 0.59 (**6**) and RRT 0.92 (**7**).

In a prior process, the crystallization of **2** was carried out as the free base in a mixture of dichloromethane and methanol. Under these conditions, another over-alkylation impurity was observed in which an equivalent of methanol added to **7** (which had been observed in the LC-HRMS, Figure 2b).

MS/MS (Figure S7) of **8** indicated that methanol had reacted with the vinyl group via a Michael addition (Figure 7).

Side products **6** and **7** did not efficiently purge in the crystallization of **2**, thus conjugate addition was also observed in the API step which consisted of hydrolysis of the methyl ester with tetrabutylammonium hydroxide to yield the free acid (Scheme 1)[5,11]. Crystallization of **1** was carried out in isopropanol:water; therefore over-alkylation side products were observed by HRMS with a) water added across the vinyl group (RRT 0.95, **9**) and b) isopropanol added across the vinyl group (RRT 1.09, **10**) in comparable fashion to **8** (Figure 7, Figures S8-S9).

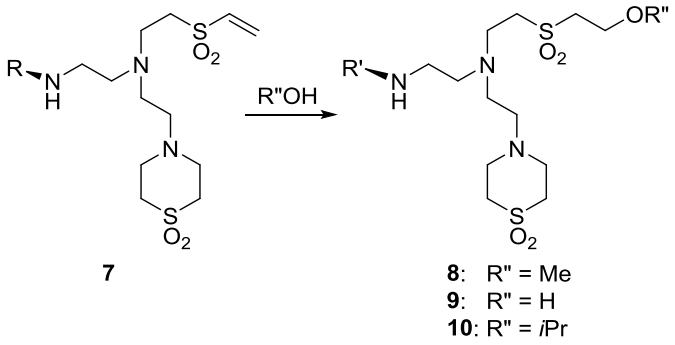


Figure 7. Conjugate addition of nucleophilic solvents to **7**. (Note: R′ vs R reflects the hydrolysis of the aromatic methyl ester, see Scheme 1)

Under the API conditions, a new impurity (RRT 0.93) was observed that would grow over time with concomitant decrease in the level of **9**.[12] HRMS of this new impurity showed *m/z* 826.483 by positive-ion ($[M + H]^+$) and 938.462 by negative-ion ($[M + TFA^-]^-$), corresponding to a neutral elemental composition of $C_{46}H_{71}N_3O_6S_2$, consistent with the net loss of C_2H_4O from **9**. This elemental composition and MS/MS indicated an unusual sulfinic acid derivative (**11**, Figure 8, Figure S11).

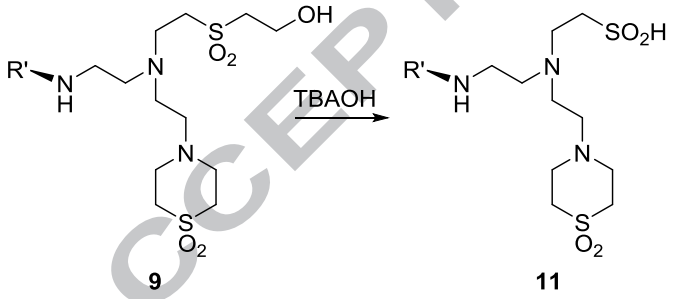
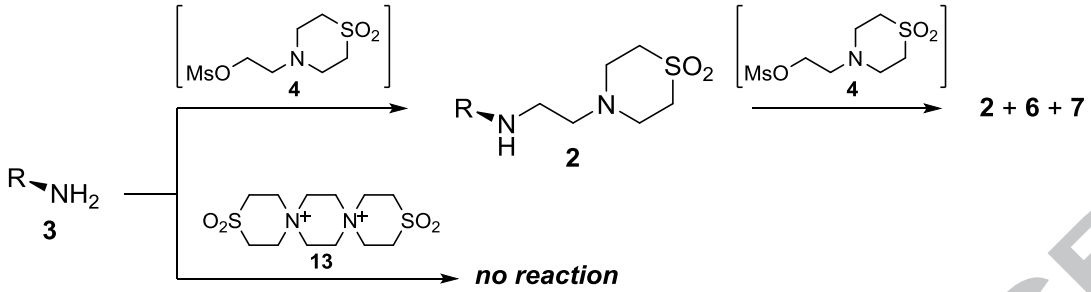


Figure 8. Sulfinic acid **11** impurity in API resulting from degradation of **9** under API conditions (with extended reaction time).

Over-alkylation could theoretically occur by two routes; a) sequential addition of each side chain, or b) dimerization of the side chain **4** followed by reaction of the dimer (**13**) with **3** (Scheme 2). The dimerization of **4** to form **13** was previously observed in the course of investigating the mechanism of the side chain addition.[13] Attempts to react **13** with **3** to produce the **6**/**7** mixture resulted in no reaction. Subjection of **2** to the alkylation conditions (DIPEA and **4** in DCM, see ref. 4) resulted in

partial conversion to **6**/**7** (Scheme 2), indicating that the over-alkylation occurs by sequential addition of two side chains.



Scheme 2. Mechanism of over-alkylation.

Over-alkylation is typically a straightforward side product of amine alkylation by substitution, however, in this case it resulted in a more complex mix of byproducts over the final two synthetic steps. The process for the API step was ultimately changed to optimize for crystal form control (THF:ACN:H_2O versus IPA:H_2O), which had the deleterious effect of poor impurity purging compared to IPA:H_2O. To compensate, the crystallization of the penultimate **2** was modified to improve purging at that step (oxalate salt in THF:H_2O versus free base in DCM:MeOH). An added benefit of these process changes was that it was no longer possible for the conjugate over-alkylation products **8** and **10** to be formed. The process change at the penultimate step led to excellent impurity purging, leading to **1** virtually free of all over-alkylation byproducts.

Acknowledgements

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[4] A typical procedure for the preparation of **2** is as follows: 1.6 equivalents of alcohol **5** were treated with 1.65 equivalents of both Ms_2O and *N*,*N*-diisopropyl ethylamine (DIPEA) in dichloromethane (DCM) to form mesylate **4**, followed by addition of compound **3** and additional DIPEA. The mixture was then stirred at 38°C until complete consumption of **3**; typically the reaction took 22 hours to complete. After base and acid wash, the product was crystallized from 1:2 DCM/methanol as the free base.

[5] Analytical HPLC conditions for analysis of **2**: Column: Waters XBridge C8, 150 × 3.0 mm, 3.5 µm; Mobile phase: A: 65:35 H_2O:MeOH with 20 mM NH_4OAc, B: MeCN; Gradient: 5-70%B from 0-3 min, 70-75%B from 3-20 min, 75-85%B from 20-23 min, 85-100%B from 23-30 min; Flow rate: 0.7 mL/min; Column temp.: 40°C; Wavelength: 235 nm.

[6] A Thermo Q-Exactive Orbitrap with HESI ionization source was used for HRMS coupled with a Waters Acquity UPLC. HRMS conditions: Spray voltage: 3.5 kV (+), 3.0 kV (-); Capillary temp.: 250°C; Sheath Gas: 45; Aux. Gas: 10; Spare Gas: 2; S-Lens RF Level: 50.

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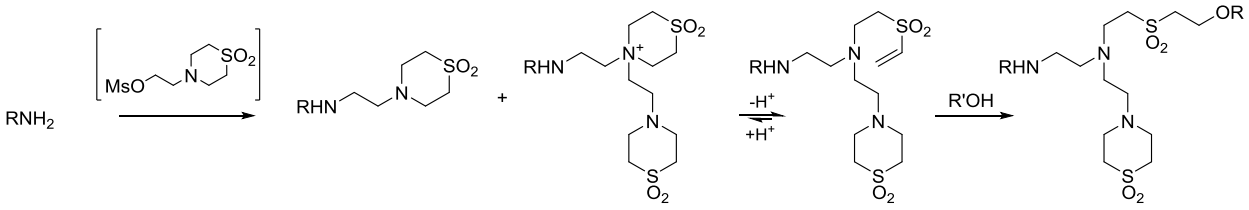
[9] A batch of **2** was enriched in the over-alkylation impurities, prepared by following the same procedure as in ref. 4 except using 3.2 equivalents of mesylate **4**. A portion of this material (1 g) was brought up in 25 mL of 4:1 THF:MeCN for isolation. A Waters FractionLynx system with a Micromass ZQ mass spectrometer was used for preparative HPLC isolation of **7**. Column: Waters XBridge C8, 150 × 19 mm, 5 µm; Mobile phase: A: 65:35 H_2O:MeOH with 20 mM NH_4OAc, B: MeCN; Gradient: 5-75%B from 0-3 min, 75%B from 3-15 min, 75-100%B from 15-18 min, 100%B from 18-21 min; Flow rate: 22 mL/min; Column temp.: ambient; Wavelength: 235 nm; Number of injections: 50; Injection volume: 0.5 mL. Fractions were collected from 7.9 to 8.4 min and 13.1 to 13.9 min. These fractions were combined. The resulting solution was rotary evaporated to aqueous, then extracted once with an equal volume of chloroform. The chloroform layer was passed through anhydrous sodium sulfate and rotary-evaporated to dryness (26 mg).

[10] A Bruker Avance III 600 NMR spectrometer (operating at 600.67 MHz for ^{1}H, 151.04 MHz for ^{13}C, 60.87 MHz for ^{15}N) was used with a TCI CryoProbe and TopSpin v3.5 software. The isolate was dissolved in $CDCl_3$ (Cambridge Isotope Laboratories).

[11] Methyl ester **2** was treated with 1.5 eq. of tetrabutylammonium hydroxide in a mixture of isopropanol (IPA) and water. The mixture was heated to 60°C and maintained overnight. Once the reaction was complete, 3 eq of aqueous hydrochloric acid was added followed by additional isopropanol. Crystallization was effected by heating to 80°C and controlled cooling to 0°C. The solids were isolated as an IPA solvate which converted to the neat HCl salt upon drying.

[12] Analytical HPLC conditions for analysis of **1**: Column: Waters XBridge C8, 150 × 3.0 mm, 3.5 µm; Mobile phase: A: H_2O with 0.1% TFA, B: 70:30 MeCN:MeOH with 0.1% TFA; Gradient: 30-95%B from 0-22 min, 95%B from 22-26 min; Flow rate: 0.7 mL/min; Column temp.: 40°C; Wavelength: 235 nm.

[13] When isolated compound **4** was solubilized in acetonitrile at room temp., after 1h a white solid had precipitated out of solution and was shown by x-ray crystallography, HRMS, and NMR to be the dimer **13** (Figures S11-S13).



Highlights:

- Use of negative-ion mass spectrometry to differentiate between interconverting byproduct species; a positively charged quaternary ammonium species and a neutral species

- Isolation and NMR to determine neutral byproduct species structure

- Detection and elucidation of daugher synthetic byproducts derived from the initial byproduct

- Determination of mechanism of over-alkylation