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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.

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**).

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 (Fig. 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.

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 $[M^+ + 2TFA^-]^-$ (Fig. 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 $[M + TFA^-]^-$ (Fig. 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]

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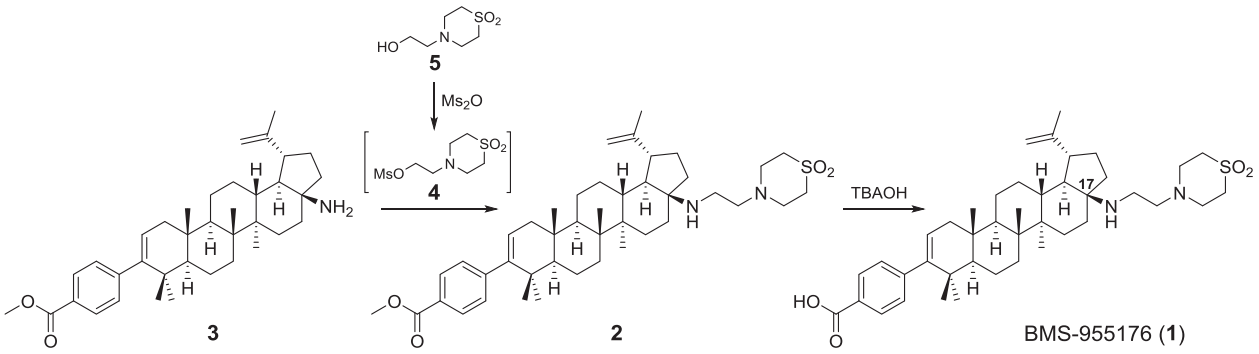

Scheme 1. The last two steps of the synthesis of BMS-955176.

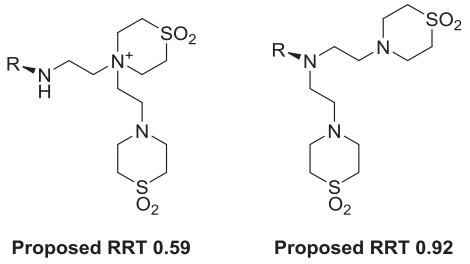

Fig. 1. Initially proposed RRT 0.59 and RRT 0.92 over-alkylation side products.

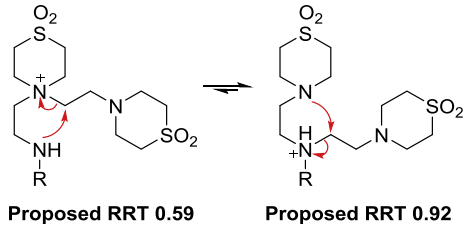

Fig. 3. Possible mechanism of side chain transfer between initially proposed RRT 0.59 and RRT 0.92 over-alkylation side products.

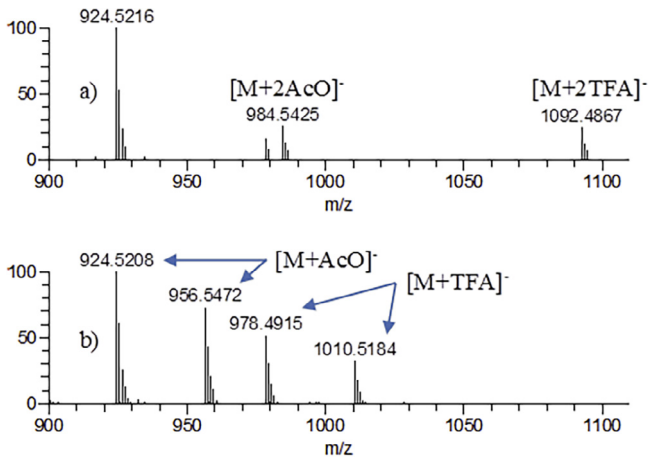

Fig. 2. Negative-ion HRMS for a) RRT 0.59 and b) RRT 0.92 over-alkylation side products. b) also includes the masses for compound **8** (*m*/*z* 956 and 1010), which is a methanol addition product that co-eluted under these conditions. The *m*/*z* 978.49 peak in 2b is in 2a as well but is unlabeled in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

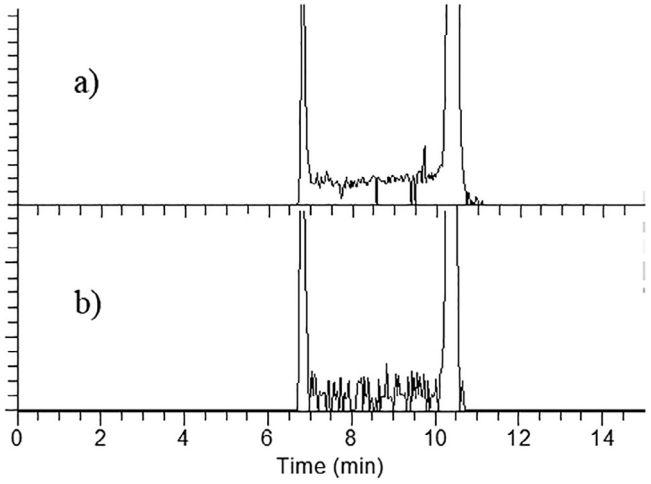

Fig. 4. Extracted ion chromatograms in a) positive-ion mode (*m*/*z* 866.5) and b) negative-ion mode (*m*/*z* 924.5).

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 Fig. 2b).

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 (Fig. 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 (Fig. 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.

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 $CDCl_3$ (^1H, ^{13}C, ^1H–^1H COSY, ^1H–^{13}C multiplicity-edited HSQC, ^1H-^{13}C HMBC), as well as a ^1H-^{15}N HMBC spectrum.[10] The ^1H 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) (Fig. 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