Synthesis of *tert*-Butoxycarbonyl (Boc)-Protected Purines

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Interest in the chemical synthesis of nucleic acids and their analogues as well as nucleoside antibiotics has been the driving force behind research on purines and pyrimidines. One issue involves how to mask or protect amine functionality that may be present in the nucleobases. In a typical solid-phase oligonucleotide synthesis, for example, the exocyclic amine groups of adenine, cytosine, and guanine are blocked with acyl protecting groups. At the end of synthesis, global deprotection under basic conditions gives the fully deblocked nucleic acids. With the advent of Nielsen's peptide nucleic acids (PNAs)[1] and related amide-linked oligonucleotide surrogates,[2] there is a need for protecting groups that can be removed under acidic or neutral conditions and are compatible with Fmoc-mediated solid-phase synthesis protocols. Such protecting groups would also be useful for the synthesis of DNA−peptide conjugates.[3] Unfortunately, the application of existing protecting group strategies to free nucleobases—especilly the purines—often leaves much to be desired. Although there are well-documented examples of nucleobases with acid-labile protecting groups,[4] the most common acid-labile protecting group for amines, the *tert*-butoxycarbonyl group (Boc), has, to our knowledge, not been successfully extended to the parent purine nucleobases.[5] The Boc protecting group has the additional virtue in that it can also be removed under neutral conditions.[6]

We also required an acid-labile group to protect the exocyclic amines in purine-containing α-helical peptide nucleic acids (αPNAs).[7] Boc protection was envisaged as a highly attractive strategy since this protecting group is orthogonal to our Fmoc-based SPPS protocol and, in contrast to the known acid labile monomethoxytrityl (Mmt) protecting group, can sustain mildly acidic conditions. Thus, Boc-protected αPNAs can be cleaved from the resin via mild acidolysis, and further synthetic chemistry involving conjugation, fragment condensation, etc., can be carried out. With the appropriate choice of resin/linker, on-resin deprotection and modification of a specific amine group would also be possible. One could, for example, deprotect an orthogonally protected Lys and then attach a fluorophore to the αPNA. We now report practical syntheses of Boc-protected adenine, 6-chloro-2-aminopurine (6Cl2AP), and guanine for incorporation into our αPNA monomer synthesis. Gram quantities of these Boc-protected purines can now be synthesized from inexpensive starting materials without the need for elaborate purification steps.

Our initial attempts to make the Boc-protected adenine by treating adenine with Boc_2O and a catalytic amount of DMAP were not very successful. The use of polar solvents such as DMSO and DMF (to solubilize adenine) gave mono-, bis-, and tris-Boc protected adenines, along with a major amount of free adenine. Significantly, the ratio of these products remained constant over time, and warming the reaction mixture led to a more complicated reaction mixture along with the development of highly colored species. While evaluating different reaction conditions, it was observed that use of excess (4.5 equiv) Boc_2O, a catalytic amount of DMAP, and THF as solvent gave a single product—the tris-Boc-protected adenine **3** (Scheme 1). Purification was easily effected by simple filtration through silica gel to give **3** in 90% yield. Tris-Boc adenine **3** can be converted to bis-Boc adenine **5** almost quantitatively by treatment with aq $NaHCO_3$, and the latter can be converted to the desired mono-Boc derivative **7** in very good yield by treatment with NaOH for 3 days at room temperature.

Care has to be taken during the conversion of bis-Boc adenine **5** to mono-Boc adenine **7**. The best result was obtained by stopping the reaction after 72 h by acidification and isolation of the product. Longer reaction times led to the formation of significant quantities of adenine. Once isolated, mono-Boc adenine **7** is very stable and can be stored on the benchtop for months without any degradation. Tris-Boc adenine **3** can be converted directly to **7** by treatment with NaOH without even isolating **5**. Similar chemistry was observed with 6-chloro-2-aminopurine (6Cl2AP) **2** with the exception that the mono-Boc species is quite stable to NaOH. The regiochemistry of the tris-Boc species **3** and **4** (N^7 vs N^9) was verified by UV spectroscopy since it is known that the λ_{max} for the

(1) For reviews that focus on Nielsen's contributions, see: (a) Dueholm, K. L.; Nielsen, P. E. *New J. Chem.* **1997**, *21*, 19. (b) Eriksson, M.; Nielsen, P. E. *Quart. Rev. Biophys.* **1996**, *29*, 369. (c) Uhlmann, E.; Peyman, A.; Breipohl, G.; Will, D. W. *Angew. Chem., Int. Ed.* **1998**, *37*, 2796.

(2) For a recent review that includes a section on "modified" PNAs, see: Falkiewicz, B.; *Acta Biochim. Pol.* **1999**, *46*, 503. (Also see literature cited in ref 7a.).

(3) Dreef-Tromp, C. M.; van der Maarel, J. C. M.; van den Elst, H.; van der Marel, G. A.; van Boom, J. H. *Nucleic Acids Res.* **1992**, 20, 4015.

(4) Breiphol, G.; Knolle, J.; Langner, D.; O'Malley, G.; Uhlmann, E. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 665.

(5) The Boc group has been used to protect purine-containing nucleosides, but the starting material has generally been the nucleoside and not the free bases. (a) Ubukata, M.; Isono, K. *Tetrahedron Lett.* **1986**, *27*, 3907. (b) Schirmeister, H.; Pfleiderer, W. *Helv. Chim. Acta* **1994**, *77*, 10. (c) Ubukata, M.; Osada, H.; Magae, J.; Isono, K. *Agric. Biol. Chem.* **1988**, *52*, 1117. (d) Nagatsugi, F.; Uemura, K.; Nakashima, S.; Maeda, M.; Sasaki, S. *Tetrahedron Lett.* **1995**, *36*, 421. A bis-Boc derivative of 2-amino-9-benzyloxy-6-methoxypurine has been prepared. (e) Harnden, M. R.; Wyatt, P. G. *Ibid.* **1990**, *31*, 2185. Nielsen's adenine PNA monomer has been Boc-protected starting from N^9-alkylated adenine. (f) Farèse, A.; Patino, N. Condom, R.; Dalleu, S.; Guedj, R. *Ibid.* **1996**, *37*, 1413.

(6) (a) Ceric ammonium nitrate (CAN): Hwu, J. R.; Jain, M. L.; Tsay, S.-C.; Hakimelahi, H. *Tetrahedron Lett.* **1996**, *37*, 2035. (b) Microwave irradiation: Siro, J. G.; Martín, J.; García, J. L.; Remuiñan, M. J.; Vaquero, J. J. *Synlett* **1998**, 147. (c) NaI/acetone: Ham, J.; Maeda, Choi, K.; Ko, J.; Lee, H., Jung, M. *Protein Pept. Lett.* **1998**, *5*, 257.

(7) (a) Garner, P.; Dey, S.; Huang, Y.; Zhang, X. *Org. Lett.* **1999**, *1*, 403. (b) Garner, P.; Dey, S.; Huang, Y. *J. Am. Chem. Soc.* **2000**, *122*, 2405.

(8) Ohrt, J. M.; Srikrishnan, T.; Parthasarathy, R.; Dutta, S. P.; Chheda, G. B. *J. Am. Chem. Soc.* **1978**, *100*, 5232.

(9) Coull, J. M.; Hodge, R. P. Guanine Synthons for Peptide Nucleic Acid Synthesis and Method for Production. International Patent Application No. PCT/US94/14742, December 22, 1994.

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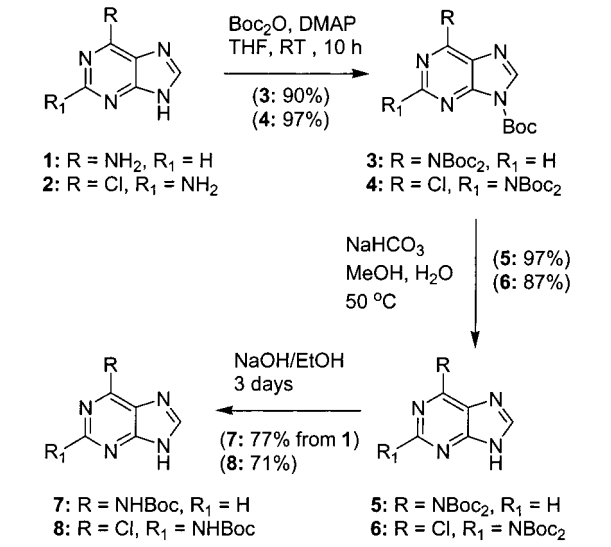
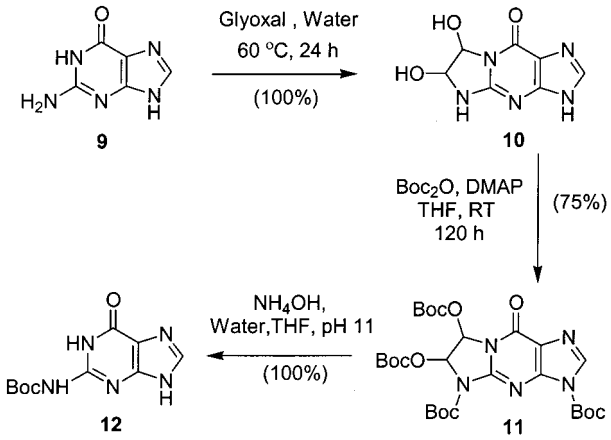
Scheme 1



Scheme 2



N^7 and N^9 regiomers of tris-acetylated adenine differ considerably (N^9: λ_{max} = 252 nm, N^7: λ_{max} = 289 nm).[8] Our data for both **3** and **4** was consistent with the N^9 regioisomers (λ_{max} = 253 nm).

Our next objective was the synthesis of Boc-protected guanine **12** (Scheme 2). Guanine **9** is well-known for its notorious insolubility in almost all solvents as well as its polyfunctional nature (imidazole, amide, and guanidine substructures), posing a challenge for any guanine synthon. Though acid-sensitive N^2 protecting groups for guanine are known,[9,4] none of them would withstand the hydrogenolysis conditions which we use to release the carboxylic acid in our αPNA monomer synthesis.[7a] To the best of our knowledge, all such derivatives are made from 6Cl2AP (**2**), which is a relatively expensive starting material.[10] Following Shapiro's procedure,[11] the glyoxal adduct **10** was synthesized from guanine (**9**) in quantitative yield. Unlike guanine itself, **10** is soluble in DMSO, DMF, and to some extent in THF. When **10** was reacted with excess Boc_2O in the presence of catalytic DMAP in THF for 5 days, a highly lipophilic tetra-Boc derivative **11** was isolated in 75% yield after filtration through silica gel. Boc-guanine **12** was obtained by exposing **11** to ammonium hydroxide in aqueous THF. Thus, starting from inexpensive starting materials, N^2-Boc-protected guanine **12** can be synthesized in high overall yield.

Experimental Section

Thin-layer chromatography (TLC) analysis was performed using either Merck silica gel 60 F-254 plates or JT-Baker Si250F plates (0.25 mm thickness). The plates were visualized first with UV illumination followed by charring with 0.3% (w/v) ninhydrin in 97:3 EtOH−AcOH. Flash chromatography was performed using silica gel (230−400 mesh). The following solvents and reagents were purified beyond commercial reagent grade: THF was distilled under Ar or N_2 from a purple solution of Na−benzophenone ketyl. Pyridine and DMSO were distilled from CaH_2 under reduced pressure. DMF was stored over activated 4 Å molecular sieves. Boc_2O was purchased from NovaBiochem. Much less expensive "grade II guanine" was obtained from Sigma. All other chemicals were obtained from Aldrich.

Tris-Boc-adenine 3. To a 100 mL Ar-flushed flask equipped with a magnetic stir bar and containing adenine (1.35 g, 10.0 mmol) and DMAP (0.122 g, 1.00 mmol) was added 50 mL of dry THF via gastight syringe. To the stirred suspension was added 9.2 mL (40 mmol) of Boc_2O under Ar atmosphere. The reaction mixture was stirred for 8 h at RT when TLC analysis indicated the presence of a single product and very little starting material. The excess THF was removed by rotary evaporation to give a yellow oil. The crude product was purified by flash chromatography on silica gel (5.0 × 10.0 cm bed, packing and elution with 7:3 hexanes−EtOAc) to give 3.87 g (90% yield) of **3** as a white foam. mp 54−55 °C (shr at 40 °C); R_f 0.45 (7:3 hexanes− EtOAc); UV (MeOH) λ_{max} 253.9, λ_{min} 232.1, ϵ 9304 M^{-1} cm^{-1};^{1}H NMR (300 MHz, $CDCl_3$) δ 9.00 (s, 1 H), 8.50 (s, 1 H), 1.70 (s, 9 H), 1.42 (s, 18 H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 154.1, 154.0, 152.5, 151.2, 150.0, 145.6, 143.3, 143.0, 87.5, 83.9, 27.9, 27.7; HRMS (EI), *m/z* calcd for $C_{21}H_{29}N_5O_6$ [M^+] 435.2117, obsd 435.2110.

Bis-Boc-adenine 5. Tris-Boc adenine **3** (10.0 mmol, unpurified) was dissolved in 400 mL of EtOAc and washed with 1 N aq HCl (30 mL) followed by brine (3 × 100 mL). The EtOAc layer was dried over Na_2SO_4 and evaporated to give a colorless oil. This oil was dissolved in 100 mL of MeOH, to which 45 mL of saturated aq $NaHCO_3$ was added. The turbid solution was stirred at 50 °C for 1 h when a clean conversion to bis-Boc protected adenine was observed by TLC. After evaporation of MeOH by rotary evaporation, 100 mL of water was added to the suspension and the aqueous layer was extracted with $CHCl_3$ (2 × 300 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated to give a white solid. The crude material was dissolved in EtOAc and filtered through silica gel (4.0 × 10.0 cm bed, packed and washed with EtOAc) to give 3.24 g (97% yield) of pure **5**. mp 148−149 °C; R_f 0.48 (EtOAc); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.97 (s, 1 H), 8.55 (s, 1 H), 1.39 (s, 18 H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 151.8, 150.2, 144.6, 84.6, 27.7; Electrospray MS, *m/z* calcd for $C_{15}H_{21}N_5O_4$ [MNa^+] 358.15, obsd 358.04.

Mono-Boc-adenine 7. Tris-Boc adenine **3** (20.0 mmol, unpurified) was dissolved in EtOH (250 mL) to which 120 mL of 1 N NaOH was added at once. The mixture was stirred for 70 h at RT when TLC analysis indicated that almost all of the starting material had been converted to mono-Boc-protected adenine. The bulk of the EtOH was removed by rotary evaporation. The aqueous suspension was cooled to 0 °C and neutralized with 1 N HCl (110 mL) and then acidified to pH 5 to 6 by addition of AcOH, with vigorous shaking and swirling. The

(10) Attempts to convert Boc-6Cl2AP **8** to Boc-guanine **12** using DABCO (Linn, J. A.; McLean, E. W.; Kelly, J. L. *J. Chem. Soc. Chem. Commun.* **1994**, *8*, 913) or sodium 2-cyanoethoxide (Hodge, R. P.; Brush, C. K.; Harris, C. M.; Harris, T. M. *J. Org. Chem.* **1991**, *56*, 1553) were unsuccessful.

(11) Shapiro, R.; Cohen, B. I.; Shiuey, S. ⁻J.; Maurer, H. *Biochemistry* **1969**, *8*, 238. The yield of this reaction became quantitative when the following modified workup was used: Upon completion of the reaction, excess water was removed by rotary evaporation at 60 °C and then 250 mL of THF was added, and the mixture was stirred. Eventually a white suspension formed, which was suction-filtered and washed with THF. After air-drying, a quantitative yield of **10** was isolated as a white solid. Compound **10** (5 mg/1 mL solvent) is completely soluble in DMSO, saturated Na_2CO_3, and dilute HCl, partially soluble in DMF and pyridine, and sparingly soluble in acetonitrile and THF.

resulting suspension was kept at 4 °C overnight and filtered to give a white solid, which was washed with water and air-dried. The filtrate was extracted with $CHCl_3$ (3 × 150 mL), dried over Na_2SO_4, and evaporated to give a white solid. Solids obtained from both sources were redissolved in 9:1 EtOAc−MeOH and, after 48 h at RT, 2.63 g of the mono-Boc-protected adenine crystallized out. The supernatant, which contained mainly tris- and bis-Boc-adenines, was concentrated by rotary evaporation to a solid and resubjected to NaOH conditions. The reaction was worked up as before to give a white solid which was purified by flash chromatography on silica gel (6.0 × 18.0 cm bed, elution with 9:1 EtOAc−MeOH) to give another 1.0 g of pure **7** (77% combined yield). Decomposed above 260 °C; R_f 0.28 (15:1 EtOAc−MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.56 (s, 1 H), 8.42 (s, 1 H), 1.50 (s, 9 H); ^{13}C NMR (75.4 MHz, DMSO-d_6) δ 160.2, 152.5, 151.1, 145.5, 145.3, 114.3, 81.0, 27.9; HRMS (FAB, CsI/NaI/glycerol matrix), *m/z* calcd for $C_{10}H_{13}N_5O_2$ [MNa^+] 258.0969, obsd 258.0966.

Tris-Boc-6Cl2AP 4. 6Cl2AP (1.42 g, 8.36 mmol) was converted to tris-Boc-6Cl2AP following the same procedure as described for adenine. After 18 h at RT, the reaction was complete as judged by TLC analysis, and the bulk of the THF was removed by rotary evaporation to give a yellow oil. The crude product was purified by flash chromatography on silica gel (3.0 × 10.0 cm bed, elution first with 9:1 hexanes−EtOAc to remove unreacted Boc_2O and then with EtOAc) to give 3.82 g (97% yield) of **4** as a white foam. mp 50−51 °C; R_f 0.63 (3:2 hexanes−EtOAc); UV (MeOH) λ_{max} 253.1, λ_{min} 232.5, ϵ 8533 M^{-1} cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.59 (s, 1 H), 1.70 (s, 9 H), 1.47 (s, 18 H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 153.7, 152.0, 151.7, 150.5, 145.4, 144.6, 130.8, 103.4, 88.0, 83.8, 27.9, 27.9; Electrospray MS, *m/z* calcd for $C_{20}H_{28}ClN_5O_6$ [MNa^+] 492.16, obsd 492.15.

Bis-Boc-6Cl2AP 6. Tris-Boc 6Cl2AP **4** (5.9 mmol, crude yellow oil obtained after extraction) was dissolved in 100 mL of MeOH, to which 45 mL of saturated $NaHCO_3$ was added. The turbid solution was stirred at 50 °C for 1 h and then at RT overnight, when clean conversion to bis-Boc-protected 6Cl2AP was observed by TLC. After evaporation of the bulk of the MeOH by rotary evaporation, 100 mL of water was added to the suspension, and aqueous layer was extracted with $CHCl_3$ (2 × 300 mL). The $CHCl_3$ layer was dried over Na_2SO_4 and evaporated to give a yellow oil, which was dissolved in EtOAc and filtered through silica gel (4.0 × 18.0 cm bed, elution with 500 mL of 95:5 EtOAc−MeOH) to give 1.89 g (87% yield) of pure bis-Boc 6Cl2AP **6**. mp 91−95 °C (foams); R_f 0.65 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (s, 1 H), 1.52 (s, 18 H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 153.0, 151.4, 151.3, 150.6, 145.1, 144.9, 129.5, 84.6, 28.0, 27.7; Electrospray MS, *m/z* calcd for $C_{15}H_{20}ClN_5O_4$ [MNa^+] 392.11, obsd 392.06.

Mono-Boc-6Cl2AP 8. Bis-Boc 6Cl2AP **6** (0.85 g, 2.3 mmol) was dissolved in 17 mL of EtOH, to which 14.5 mL of 1 N NaOH was added at once. The resulting mixture was stirred for 70 h at room temperature when TLC analysis showed complete consumption of starting material and formation of a single product. The bulk of the EtOH was removed by rotary evaporation followed by addition of 50 mL of water to the suspension and acidification to pH 4−5 with acetic acid. The aqueous suspension was extracted with $CHCl_3$ (150 mL), and the organic layer washed with brine (50 mL), dried over Na_2SO_4, and evaporated to give a white solid. Upon addition of 100 mL of Et_2O, the solid dissolved immediately and then crystallization initiated within 1 min. After 30 min at 4 °C, 0.441 g (71% yield) of colorless crystals was collected by filtration. Crystals became amorphous at 185−190 °C and began to decompose at 200 °C; R_f = 0.71 (4:1 EtOAc−MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.49 (s, 1 H), 7.94 (s, 1 H), 1.57 (s, 9 H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 153.4, 151.7, 151.4, 151.1, 145.7, 128.4, 82.4, 28.3; Electrospray MS, *m/z* calcd for $C_{10}H_{12}ClN_5O_2$ [MNa^+] 292.05, obsd 292.00.

Tetra-Boc-glyoxal-guanine Adduct 11. Glyoxal−guanine adduct **10** (1.5 g, 7.2 mmol) and DMAP were placed in flask with magnetic stir bar. After flushing the flask with Ar, 100 mL of dry THF and 9 mL (39.4 mmol) of Boc_2O were injected via gastight syringe, and the reaction was allowed to proceed for 5 days at room temperature. At this point all of the solid material had been consumed, and a dark green solution was obtained. TLC analysis showed presence of a single product. Activated carbon was added, and the reaction mixture was filtered through a pad of Celite. After evaporation of most of the THF a light brown oil was obtained which was purified by flash chromatography on silica gel (4.5 × 20.0 cm bed, 3:7 hexanes−EtOAc) to give 3.20 g (75% yield) of **11** as a light fluorescent green foam.[12] R_f 0.62 (7:3 EtOAc−hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 (s, 1 H), 6.76 (s, 1 H), 6.59 (s, 1 H), 1.68 (s, 9 H), 1.58 (s, 9 H), 1.54 (s, 9 H), 1.52 (s, 9 H).

Boc-Guanine 12. Product **11** (3.20 g, 5.36 mmol) was dissolved in 200 mL of 1:1 THF−water, to which 50 mL of NH_4-OH was added at once (pH ∼ 11). After stirring at RT for 4 h, TLC analysis showed the reaction to be complete. Excess ammonia and THF were removed by slow evaporation (RT) on a rotary evaporator (a cotton plug was used to prevent excessive foaming) when a white precipitate formed, which was suction-filtered and washed with water to give 1.29 g (97% yield) of **12** as a gray powder upon air-drying. Decomposed above 160 °C; R_f = 0.12 (8.5:0.5:1.0 EtOAc−NH_4OH−MeOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.95 (s, 1 H), 1.48 (s, 9 H); ^{13}C NMR (75.4 MHz, DMSO-d_6) δ 154.3, 154.0, 146.9, 139.3, 82.4, 27.8; Electrospray MS, *m/z* calcd for $C_{10}H_{13}N_5O_3$ [MNa^+] 274.09, obsd 274.03.

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Supporting Information Available: ^{1}H NMR spectra of compounds **3**, **4**, **5**, **6**, **7**, **8**, **11**, and **12**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) Note: It was observed by 2D TLC that the product **11** decomposes on silica gel to some extent. Instead of isolating the product **11** at this stage, the crude reaction mixture can be used for the next reaction without any problem as long as the pH is kept ∼11 during the reaction. After removal of THF and ammonia, all the impurities will remain in the aqueous supernatant layer and can be filtered off easily from the product. Since compound **11** decomposes over time, it was not possible to acquire a reliable ^{13}C spectrum and mass data.