

Atomic force microscopy identifying fuel pyrolysis products and directing the synthesis of analytical standards

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2 **analytical standards**
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Abstract

Here we present a new method that integrates atomic force microscopy (AFM) with analytical tools such as high-performance liquid chromatography (HPLC) with diode-array ultraviolet-visible (UV) absorbance and mass spectrometry (MS) along with synthetic chemistry. This allows the detection, identification, and quantification of novel polycyclic aromatic hydrocarbons (PAH) in complex molecular mixtures. This multi-disciplinary methodology is employed to characterize the supercritical pyrolysis products of *n*-decane, a model fuel. The pyrolysis experiments result in a complex mixture of both unsubstituted as well as highly methylated PAH. We demonstrate the AFM-driven discovery of a novel compound, benz[*l*]indeno[1,2,3-*cd*]pyrene, with the chemical structure assignment serving as input for the chemical synthesis of such molecule. The synthesis is verified by AFM and the synthesized compound is used as a reference standard in analytical measurements, establishing the first-ever unequivocal identification and quantification of this PAH as a fuel product. Moreover, the high-resolution AFM analysis detected several five- to eight-ring PAH, which represents novel fuel pyrolysis and/or combustion products. This work provides a route to develop new analytical standards by symbiotically using AFM, chemical synthesis, and modern analytical tools.

Keywords: atomic force microscopy; CO-functionalized tips; polycyclic aromatic hydrocarbons; pyrolysis; synthetic chemistry

1. Introduction

The investigation of complex molecular mixtures relies on the use of standards for experimental comparison.¹⁻⁷ Due to the limited number of molecular standards that are commercially available, thorough studies require the synthesis of new reference compounds. However, choosing the molecules to be synthesized for the characterization of a mixture with unknown components is challenging and often relies on the use of spectroscopic methods.^{1,6,7} The synthesis of the standards itself is often expensive and time consuming. As an example, the number of possible polycyclic aromatic hydrocarbons (PAH) isomers increases significantly with increasing molecular weight.⁸ Therefore, given a molecular formula, the number of unique PAH structures is large for molecules containing a combination of five- and six-membered rings. For instance, the C₂₄H₁₄ benzenoid PAH, with only six-membered rings, display 12 isomers. For the same molecular formula, but with two five-membered rings instead, there are a total of 266 isomers.⁹ In the absence of a reference standard, other analytical methods can be employed for determining the structure of unknown PAH,^{8,10} but some of these methods are limited by low concentration and need extensive purification of individual PAH in complex molecular matrices.

PAH are ubiquitous pollutants formed during the pyrolysis and/or combustion of a wide-variety of fuels and/or fuel components. The formation and subsequent growth of PAH is of practical concern for four principal reasons: (1) PAH are known precursors to soot,¹¹⁻¹³ which poses serious health hazards¹⁴⁻¹⁶ and is a major contributor to global climate change;¹⁷⁻¹⁹ (2) PAH are found in the extracts of solid deposits from catalytic hydrocracking of petroleum,¹ suggesting that PAH could be precursors to these deposits, which can clog process piping¹ and cause catalyst fouling,^{20,21} resulting in process downtime; (3) Some PAH are known to be potent carcinogens and/or mutagens,^{2,22-24} and (4) PAH are precursors to carbonaceous solids,²⁵⁻³⁰ which can clog fuel lines and injection nozzles and foul heat transfer surfaces, thereby seriously jeopardizing the safe operation of future high-speed aircraft.

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2 In the context of the pre-combustion environments^{25,31} of future high-speed aircrafts, better
3 understanding of pyrolytic reactions that lead to PAH formation and growth would facilitate the design
4 of fuel systems that are not prone to solids formation. Therefore, we conducted supercritical pyrolysis
5 experiments^{28-30,32,33} of the model fuel *n*-decane, an *n*-alkane component of jet fuels.^{34,35} An *n*-alkane
6 fuel is chosen because, under supercritical pyrolysis conditions, these jet-fuel components exhibit a high
7 propensity for solids formation.^{25,28-30} To thoroughly understand PAH formation and growth reactions,
8 particular emphasis is placed on the isomer-specific analysis of the PAH products by high-performance
9 liquid chromatography (HPLC) with diode-array ultraviolet-visible (UV) absorbance and mass
10 spectrometric (MS) detection. Previous HPLC/UV/MS analyses^{28-30,32,33} of the supercritical *n*-decane
11 pyrolysis products led to the identification of over 400 three- to nine-ring PAH, many of which contain
12 one or more methyl functionalities. The quantification of the PAH products of supercritical *n*-decane
13 pyrolysis reveals that the yields of higher-ring-number PAH are about an order of magnitude lower than
14 their lower-ring-number counterparts.²⁸⁻³⁰ In addition to the low yields of higher-ring-number PAH, *n*-
15 decane produces many alkylated PAH, which makes the identification and quantification of PAH
16 mixtures of ≥ 5 rings an enormous undertaking.

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18 Recently, high-resolution atomic force microscopy (AFM) with CO-functionalized tips showed
19 to be capable of identifying individual molecules³⁶⁻³⁹ and thus, molecular mixtures can be studied
20 molecule-by-molecule.⁴⁰⁻⁴⁴ With this approach, details about the mechanisms of on-surface organic
21 reactions^{40,42-45} and the molecular architecture and composition of complex molecular mixtures^{41,46} have
22 been obtained. Due to the single-molecule sensitivity of AFM, the detection of molecules present in
23 miniscule amounts is viable. Important for our study of fuel pyrolysis products, five- and six-membered
24 carbon rings as well as various aliphatic groups can be differentiated and identified by AFM.^{40,47,48}

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26 Here we demonstrate a method that employs atomic-resolution low-temperature AFM to identify
27 reference standards. AFM is integrated with HPLC/UV/MS and synthetic chemistry to identify

1 previously unidentified PAH products of fuel pyrolysis in two different steps. First, to identify unknown
2 PAH within the PAH-product fractions of supercritical *n*-decane pyrolysis, providing candidates for the
3 targeted synthesis of new reference standards. Second, to confirm the molecular structure of the
4 synthesized standard. With this approach, first, a novel seven-ring PAH, benz[*l*]indeno[1,2,3-*cd*]pyrene,
5 is identified by AFM. Subsequently, it is synthesized and then also confirmed by AFM. The synthesized
6 standard of benz[*l*]indeno[1,2,3-*cd*]pyrene is analyzed by HPLC/UV, providing the UV absorbance
7 spectrum, a fingerprint property of PAH, allowing the identification and quantification of this novel
8 seven-ring PAH in an isomer-specific manner as a product of supercritical *n*-decane pyrolysis.
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20 This method provides a powerful tool for directing the synthesis of target molecules and for the
21 structural confirmation of synthesized molecules, thereby providing a reference standard for subsequent
22 analysis. It is important to note that this method is not limited to a particular system but can be applied
23 to a wide range of molecular mixtures such as those found in nature, obtained from chemical synthesis,
24 and derived from fuel pyrolysis and/or combustion.
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32 **2. Experimental section**

33 **2.1 Pyrolysis experiments and HPLC/UV/MS analysis**

34 The supercritical pyrolysis experiments of the model fuel *n*-decane (99.5% pure; critical
35 temperature, 344.5 °C; critical pressure, 20.7 atm) are conducted in an isothermal, isobaric silica-lined
36 stainless-steel flow reactor described elsewhere.^{32,33} For the present study, the PAH-product mixture is
37 obtained from supercritical *n*-decane-pyrolysis experiments performed at 570 °C, 94.6 atm, and 133 sec,
38 respectively, conditions of high PAH production and onset of solids formation.^{28–30,32,33}
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50 The PAH products of supercritical *n*-decane pyrolysis are comprised of a complex mixture of
51 unsubstituted PAH and their alkylated derivatives, which requires the application of a two-dimensional
52 HPLC separation technique to achieve sufficient resolution for PAH-product identification and
53 quantification. This HPLC separation procedure was established by Wise *et al.*⁴⁹ and further developed
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1 by Bagley and Wornat^{32,33} for the analysis of the PAH products of supercritical *n*-alkane-fuel pyrolysis.
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3 The PAH products are first fractionated by normal-phase HPLC into 15 individual fractions by isomer
4 group and/or number of π -bonds. Therefore, an individual PAH-product fraction contains unsubstituted
5 PAH of a particular isomer group, along with their corresponding alkylated derivatives. In the second
6 dimension of separation, each PAH-product fraction is analyzed by reversed-phase HPLC with diode-
7 array ultraviolet-visible (UV) absorbance and mass spectrometric (MS) detection for the isomer-specific
8 identification of the PAH products. The quantification of these products is obtained from extensive
9 calibration of the UV absorbance detector with PAH reference standards.
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20 As demonstrated in our previous studies,²⁸⁻³⁰ supercritical *n*-decane pyrolysis produces PAH
21 products containing 2 to 10 aromatic rings, which are fraction collected based on the number of π -
22 bonds.^{32,33} For the scope of the present study, the two PAH-product fractions that are analyzed by AFM
23 are: (1) Fraction **P**, comprised of PAH of ≥ 5 rings (*i.e.* of aromatic carbon number ≥ 20), obtained from
24 the pre-fractionation step of our more-involved normal-phase HPLC fractionation procedure,^{32,33} and (2)
25 Fraction **F** (labelled as Fraction 11),^{32,33} primarily composed of seven-ring PAH of the isomer group
26 $C_{26}H_{14}$ (or PAH with 13 π -bonds) and their alkylated derivatives, obtained from the more-involved
27 normal-phase HPLC fractionation procedure.^{32,33}
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38 2.2 AFM measurements

39 We performed AFM experiments by using a home-built low-temperature ($T \approx 5$ K), ultrahigh
40 vacuum ($p \approx 1 \times 10^{-10}$ mbar) combined scanning tunneling microscopy (STM) and AFM setup. We
41 employed a qPlus sensor⁵⁰ operated in the frequency-modulation mode⁵¹ (resonance frequency $f_0 \approx 30$
42 kHz, spring constant $k \approx 1800$ N/m, quality factor $Q \approx 14,000$, and oscillation amplitude $A = 0.5$ Å). We
43 applied the bias voltage V to the sample. AFM measurements were acquired in constant-height mode at
44 $V = 0$ V. As substrate we used a Cu(111) single crystal partly covered by bilayer (100)-oriented NaCl
45 islands.⁵² We deposited a low coverage (approximately 0.02 nm⁻²) of CO on the surface for tip
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1 preparation by admitting CO into the UHV chamber at a sample temperature of $T \approx 10$ K. The PAH
2 fractions were separately prepared by spreading a small amount (on the order of 1 mg) of the solid, dry
3 material on a piece of Si wafer. Then, the wafer was flash-heated by resistive heating in front of the cold
4 sample.⁴¹ For the microscope tip, we used a 25 μm -thick PtIr wire, shortened and sharpened with a
5 focused ion beam. Thereafter, we prepared a clean and sharp Cu tip by repeated indentations into the Cu
6 surface. Finally, a CO-functionalized tip was created by picking-up a single CO molecule from the
7 surface.^{36,53}

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18 The assignment of the structures from the AFM images is based on previous works resolving
19 molecules atomically by AFM with CO-functionalized tips,^{36–40,42–44,48,54} taking into account the known
20 effect of distortions due to the tilting of the CO molecule at the tip.^{55,56} For example, we distinguish
21 five- and six-membered rings by the number of bond intersections and corners and by the respective
22 positions and enclosed angles of adjacent moieties. Methyl⁴¹ and other aliphatic substituents⁴⁸ are
23 assigned by their characteristic contrast that was obtained by measuring synthesized standards by AFM.
24 The presence of substitutional heteroatoms, such as nitrogen, cannot be decisively dismissed solely
25 based on the AFM results shown here.^{37,54} However, its presence amongst the PAH products can be
26 ruled out because there is no source of nitrogen, either in the fuel *n*-decane and/or added to the reaction
27 system, in the supercritical *n*-decane pyrolysis experiments.

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42 We emphasize that quantification of the abundance of compounds with the AFM is challenging,
43 because the acquisition time is on the order of 10 min for an atomically resolved image and limits the
44 number of molecules that can feasibly be imaged within a sample to a few hundred. A statistical analysis
45 of AFM data and its comparison with mass spectrometry indicated the statistical significance of the
46 sampling by AFM⁴⁶; however, the numbers of molecules to be imaged for their quantification in
47 complex mixtures by AFM would have to be increased by several orders of magnitude.

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2 Because of the preparation method employed, also the maximum size of molecules that can be
3 studied is limited. Typically, molecules of 1000 Da molecular weight or more fragment when they are
4 sublimed³⁹. The molecules investigated here are of molecular weights below 500 Da and thus
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6 fragmentation in the sublimation process is negligible. Moreover, they are mostly planar which is
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8 advantageous to obtain high resolution images with CO-tip AFM. Even considering the challenges of
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10 AFM resolving non-planar, larger and more fragile molecules, there are strategies for investigating non-
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12 planar molecules^{47,57,58,59}, it is also possible to investigate larger and more fragile molecules using
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14 electrospray ionization deposition^{60,61,62}. We envision the methodology shown here being jointly used
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16 with these strategies. For a more detailed discussion of the pitfalls and challenges of AFM for structure
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18 elucidation we refer the reader to a recent review.⁶³
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25 3. Results and Discussion

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28 By employing AFM, we first measure the supercritical *n*-decane pyrolysis product Fraction **P**.
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30 This relatively broad PAH-product fraction is analyzed by AFM to obtain an overview of the PAH
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32 produced during supercritical *n*-decane pyrolysis. AFM results showing individual PAH contained in
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34 Fraction **P** are displayed in Figure 1a. Based on the AFM measurements, a PAH structure is assigned to
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36 each AFM image. The resolved and assigned molecules in Figure 1b are: five-ring PAH **P1** and **P2**; six-
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38 ring PAH **P3**, **P4**, and **P5**; seven-ring PAH **P6** and **P7**; and eight-ring PAH **P8**. Of these eight PAH
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40 products shown in Figure 1, five are unsubstituted PAH (**P1**, **P2**, **P3**, **P6**, and **P7**) and three are
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42 methylated PAH (**P4**, **P5**, and **P8**). Additionally, these eight PAH fall under three main structural
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44 classes: benzenoid PAH (**P3**, **P4**, and **P5**), fluoranthene benzologues (**P1**, **P6**, **P7**, and **P8**), and a
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46 phenalene benzologue (**P2**). These findings are consistent with previous HPLC/UV/MS analysis of the
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48 supercritical *n*-decane pyrolysis products.^{28–30,32,33}
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54 Of the eight PAH shown in Figure 1, four (**P1**, **P3**, **P4**, and **P5**) were reported in previous
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56 HPLC/UV/MS analysis of the supercritical *n*-decane pyrolysis products.^{32,33} A reference standard of **P6**
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is available to us and the UV absorbance maxima of **P2** are published;⁶⁴ however, neither of these two PAH were previously identified as products of supercritical *n*-decane pyrolysis. It could be that **P2** and **P6** are present in amounts below the detection limit (0.3 ng of material) of our HPLC instrument or their chromatographic signal is being masked by one or more co-eluting higher-yield PAH products. Studies report **P6** as a pyrolysis product,^{65–67} but not from fuel pyrolysis experiments. Unlike **P2** and **P6**, a reference standard and/or UV spectrum of **P8** is unavailable. Presumably, the high sensitivity of AFM due to single-molecule detection enabled us to detect **P2**, **P6**, and **P8**, which were previously not reported as fuel pyrolysis and/or combustion products.

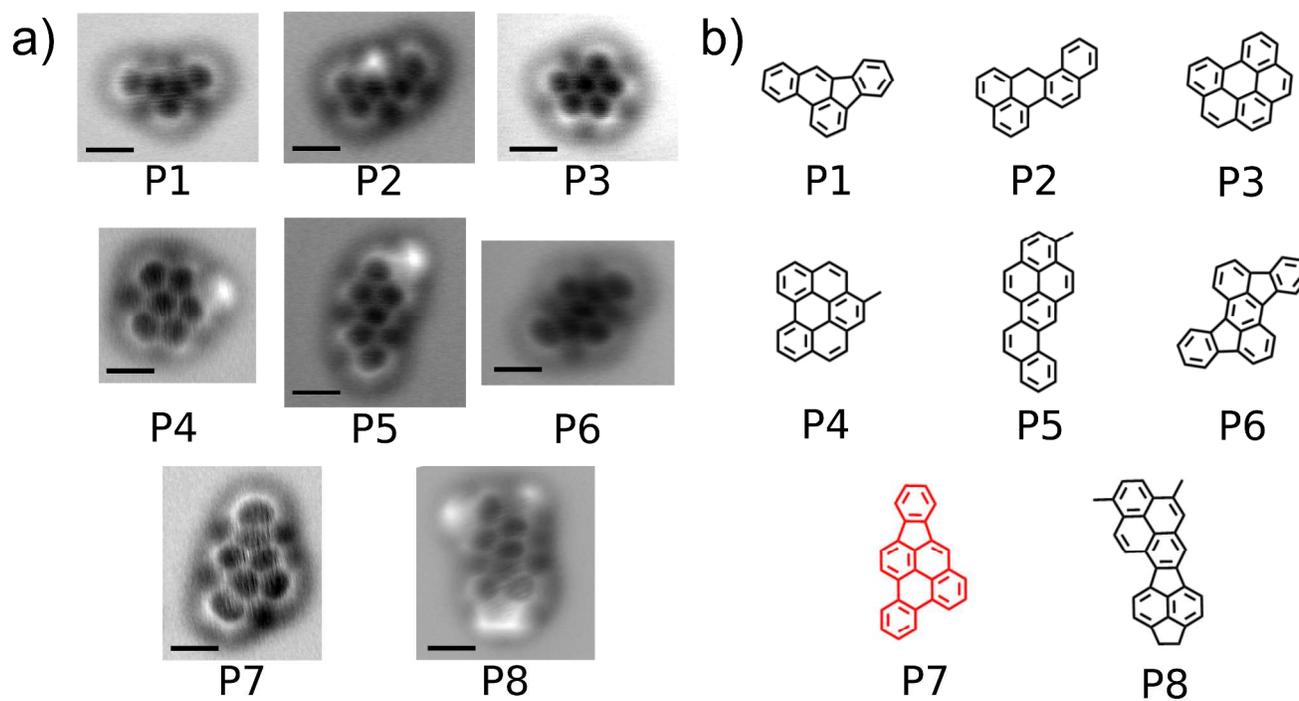


Figure 1. Fraction **P** of the products of *n*-decane pyrolysis at 570 °C, 94.6 atm, and 133 sec measured by AFM: (a) Constant-height AFM measurements with a CO-functionalized tip. Scale bars are 5 Å. **P1**, **P2**, **P3** and **P5** were measured on Cu(111); **P4**, **P6**, **P7**, and **P8** were measured on bilayer NaCl on Cu(111). (b) Proposed chemical structures: benzo[*b*]fluoranthene (**P1**), 7*H*-benzo[*hi*]chrysene (**P2**), benzo[*ghi*]perylene (**P3**), 1-methylbenzo[*ghi*]perylene (**P4**), 1-methylnaphtho[2,1-*a*]pyrene (**P5**), rubicene (**P6**) benz[*l*]indeno[1,2,3-*cd*]pyrene (**P7**), and 7,10-dimethyl-1,2-dihydrocyclopenta[5,6]acenaphtho[1,2-*a*]pyrene (**P8**).

Notably, we also detected with AFM a seven-ring $C_{26}H_{14}$ PAH benz[*l*]indeno[1,2,3-*cd*]pyrene (**P7**, in Figure 1), whose synthesis and/or identification as a fuel product has previously not been reported in the literature. Therefore, based on the AFM detection of **P7**, we undertook the synthesis of this novel molecule to facilitate its unequivocal identification and quantification in *n*-decane's products by HPLC/UV/MS.

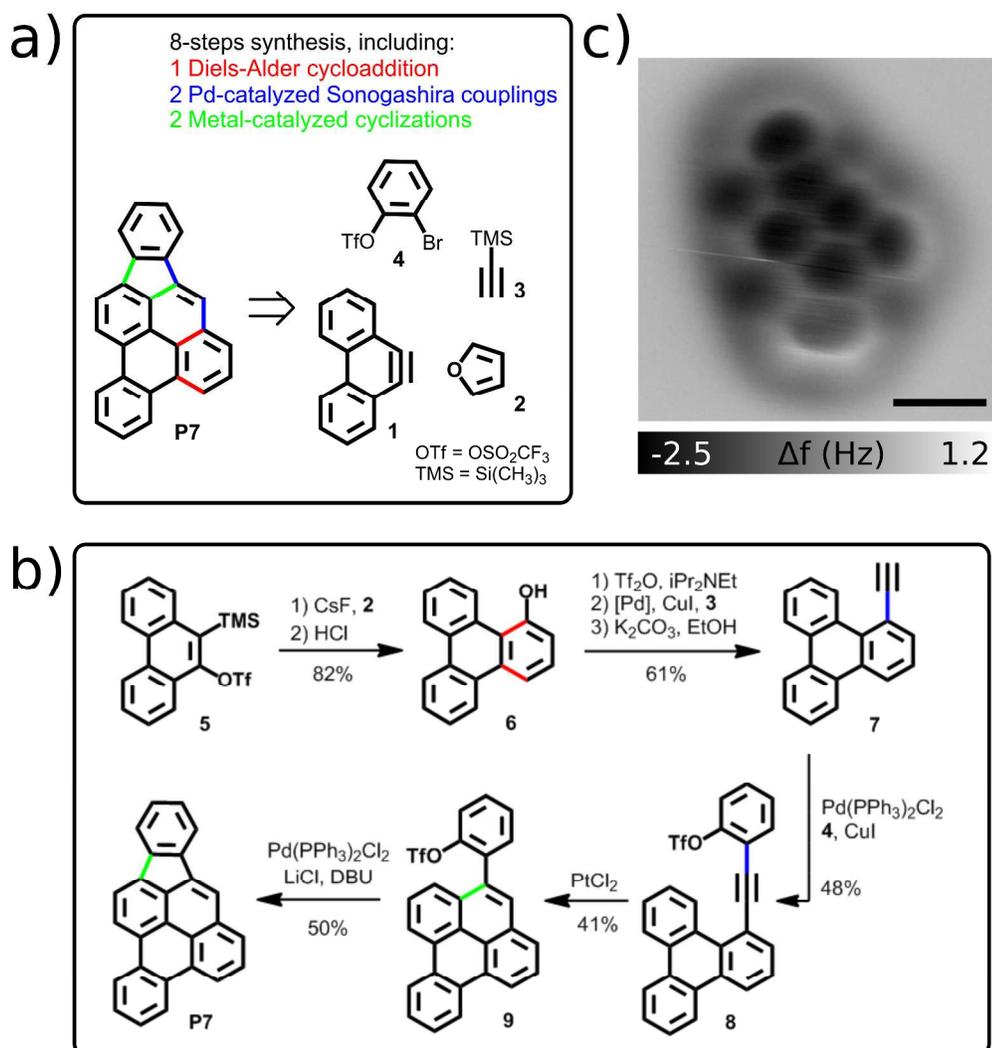


Figure 2. Synthesis of benz[*l*]indeno[1,2,3-*cd*]pyrene (**P7**): (a) Retrosynthetic analysis, (b) Synthetic route to **P7**, and (c) AFM image of the synthesized compound on Cu(111), confirming the successful preparation of **P7**. Scale bar is 5 Å.

Compound **P7** was prepared in eight steps based on the retrosynthetic analysis shown in Figure 2a. The synthesis was initiated by a Diels-Alder cycloaddition of aryne **1**, generated by fluoride-induced decomposition of triflate **5**, with furan **2** followed by acid treatment to obtain 1-triphenylenol **6** in 82% yield (Figure 2b).⁶⁸ Transformation of compound **6** into the corresponding triflate, followed by Pd-catalyzed Sonogashira coupling and base-induced desilylation afforded terminal alkyne **7** in 61% yield. Again, a Pd-catalyzed Sonogashira coupling between alkyne **7** and bromide **4** led to compound **8**, which was treated with PtCl_2 to induce a cycloisomerization to obtain triflate **9**.⁶⁹ Finally, Pd-catalyzed cyclization of compound **9** afforded **P7** in 50% yield (see supporting information for details). AFM measurements unequivocally confirmed the structure of the synthesized standard as benz[*l*]indeno[1,2,3-*cd*]pyrene (**P7**, Figure 2c), which is then used as reference standard for HPLC/UV/MS analysis.

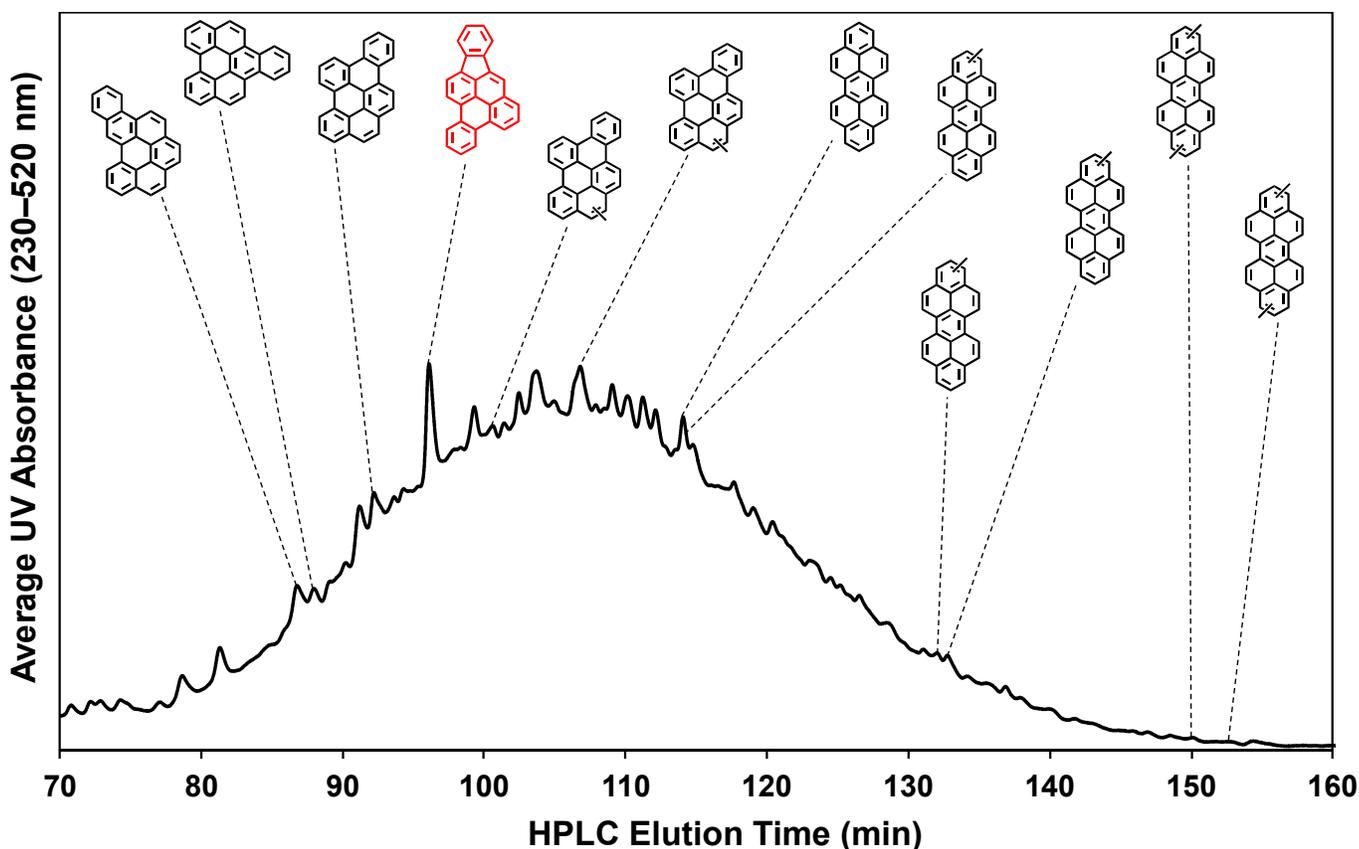


Figure 3. Reversed-phase HPLC chromatogram of Fraction **F** of the products of *n*-decane pyrolysis at 570 °C, 94.6 atm, and 133 sec. The 11 black structures, which include four unsubstituted C₂₆H₁₄

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2 benzenoid PAH, five singly-methylated PAH, and two doubly-methylated (or ethylated) PAH are
3 reported in literature.³³ The red structure corresponds to the newly identified PAH **P7**.
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9 Normal-phase HPLC analysis of the standard of this newly synthesized C₂₆H₁₄ PAH **P7** reveals
10 that it elutes in PAH-product Fraction **F** of *n*-decane pyrolysis, which mainly contains PAH with 13 π -
11 bonds, *i.e.* C₂₆H₁₄ PAH and their alkylated derivatives.^{32,33} By employing the reversed-phase HPLC
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13 method, which is used to analyze the Fraction **F**,³³ we determined that **P7** has a HPLC elution time of
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15 96.1 min. This elution time corresponds to the single largest peak in the reversed-phase HPLC
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17 chromatogram of Fraction **F**, displayed in Figure 3. Previous HPLC analysis of Fraction **F** led to
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19 identification of 11 PAH (black structures in Figure 3).³³ However, due to the previous lack of a
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21 reference standard and/or UV spectrum that matched with the chromatographic peak eluting at 96.1 min
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23 in Fraction **F**, this HPLC peak was left unassigned. The mass spectrum of this unidentified PAH, shown
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25 in Figure 4a, eluting at the same time as **P7**, portrays the primary ion at 326, which corresponds to the
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27 molecular weight of a C₂₆H₁₄ PAH (same as **P7**). In Figure 4b, we present the UV spectrum of the
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29 unknown C₂₆H₁₄ PAH product of supercritical *n*-decane pyrolysis (in black), along with that of the
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31 synthesized standard of benz[*l*]indeno[1,2,3-*cd*]pyrene (**P7**, in red). Figure 4b displays the excellent
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33 agreement between the UV spectra of the product/standard pair, establishing the unequivocal
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35 identification of benz[*l*]indeno[1,2,3-*cd*]pyrene (**P7**) as a product of supercritical *n*-decane pyrolysis.
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37 The quantification of **P7** reveals that for *n*-decane pyrolysis experiments conducted at 570 °C, 94.6 atm,
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39 and 133 sec, this newly identified molecule accounts for 5.9% of the mass of Fraction **F**, which is
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41 equivalent to 0.267 μ g of **P7** per g of *n*-decane fed to the reactor. Therefore, by employing AFM in
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43 conjunction with HPLC/UV/MS and synthetic chemistry, we established the exact structural identity of
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45 the novel seven-ring C₂₆H₁₄ PAH benz[*l*]indeno[1,2,3-*cd*]pyrene.
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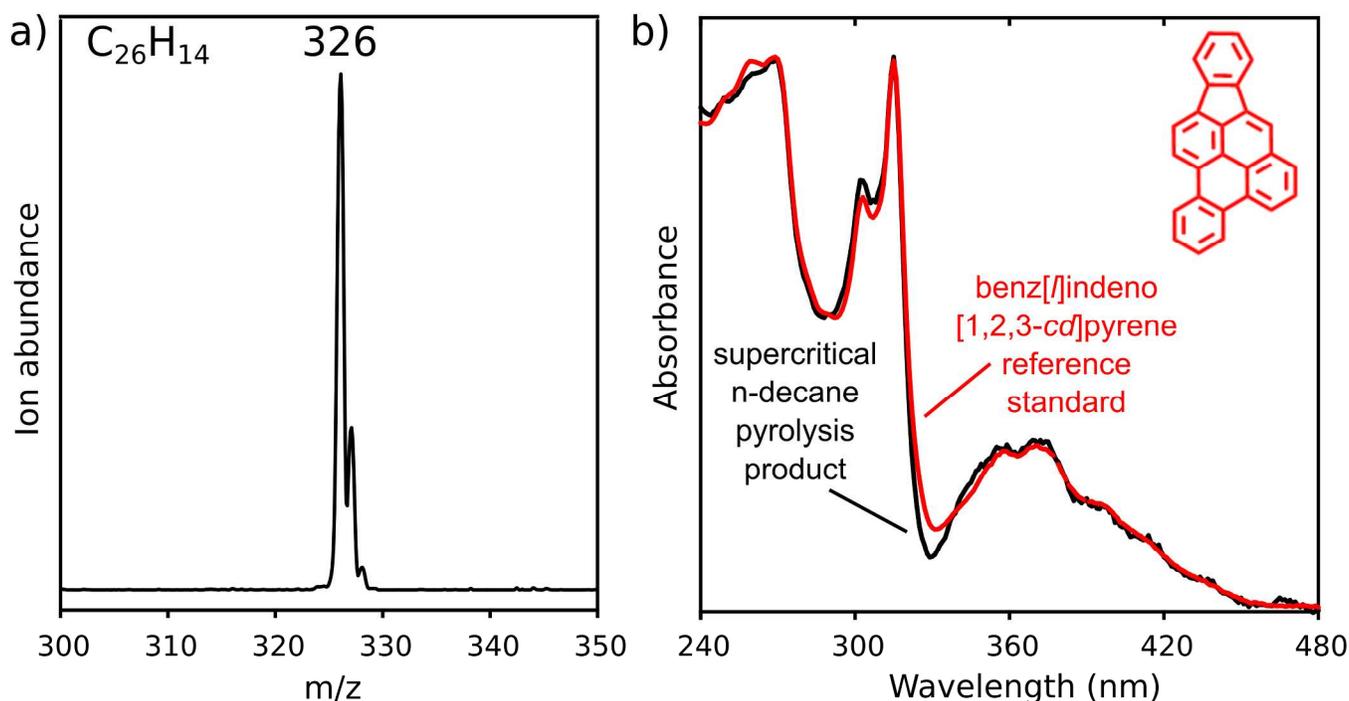


Figure 4. (a) Mass spectrum of the supercritical *n*-decane pyrolysis product, eluting at 96.1 min in the HPLC chromatogram in Figure 3; and (b) UV absorbance spectrum (in black) of the supercritical *n*-decane pyrolysis product, eluting at 96.1 min in the HPLC chromatogram in Figure 3, along with the UV absorbance spectrum (in red) of the reference standard of **P7**.

Because our HPLC analysis revealed that benz[1]indeno[1,2,3-*cd*]pyrene (**P7**) elutes in Fraction **F** of the products of *n*-decane pyrolysis, we further analyzed this PAH-product fraction by AFM to search for the presence of additional unknown PAH. The AFM images of six molecules measured in Fraction **F**, labelled **F1** to **F6**, along with tentatively assigned chemical structures are shown in Figure 5. The AFM measurements of Fraction **F** indicate that all of the imaged molecules have side groups attached to the PAH core and, expectedly, the most common side groups are methyl. Most of the molecules are seven-ring PAH, except for **F5** and **F6**, which contain eight and six rings, respectively. Each of these six PAH detected by AFM have an aromatic core that contains 13 π -bonds, which is consistent with our previous findings^{32,33} that showed that Fraction **F** primarily contains PAH with 13 π -bonds. Three of these six molecules (**F1**, **F4**, and **F5**) have an aromatic core that corresponds to a C₂₆H₁₄ PAH. The aromatic cores of **F2** and **F3** correspond to C₂₇H₁₆ PAH and that of **F6** corresponds to a

$C_{26}H_{16}$ PAH. Although the aromatic cores of **F2**, **F3**, and **F6** constitute a slightly larger molecular formula than $C_{26}H_{14}$ they still contain 13 π -bonds, consistent with them eluting in Fraction **F**.³³

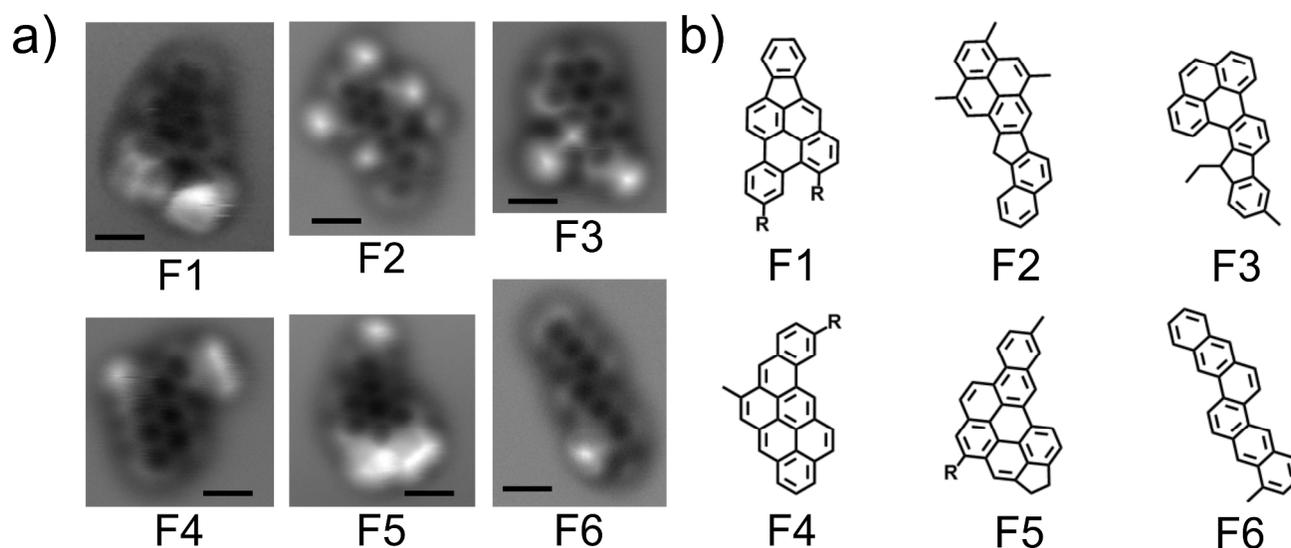


Figure 5. Fraction **F** of the products of *n*-decane pyrolysis at 570 °C, 94.6 atm, and 133 sec measured by AFM: (a) AFM measurements. Scale bars are 5 Å. **F4** and **F5** were measured on bilayer NaCl, all the other molecules were measured on Cu(111). (b) The PAH cores of the proposed chemical structures are benz[*l*]indeno[1,2,3-*cd*]pyrene (**F1**), benz[4,5]indeno[2,1-*a*]pyrene (**F2**), fluoreno[2,3-*e*]pyrene (**F3**), benz[*a*]anthanthrene (**F4**), 4,5-dihydrodibenzo[*b,ghi*]cyclopenta[*lm*]perylene (**F5**), and dibenzo[*b,k*]chrysene (**F6**).

F1 is a substituted derivative of benz[*l*]indeno[1,2,3-*cd*]pyrene (**P7**), whose two side groups could not be identified. The bright appearance on the bottom right-hand side of the AFM image of **F1**, in Figure 5a, indicates a non-planar, bulky side-group at this position. Assignment of these groups might become possible in the future when more AFM characteristics for fingerprinting of molecular moieties will have been measured.^{46,70} Of these six PAH shown in Figure 5, UV spectra and/or reference standards are available for benz[*l*]indeno[1,2,3-*cd*]pyrene, dibenzo[*b,ghi*]perylene, and dibenzo[*b,k*]chrysene, which correspond to the aromatic cores of **F1**, **F5**, and **F6**, respectively, but for none of their substituted counterparts. The reference standards or UV spectra of **F2**, **F3**, and **F4** and/or

1 their unsubstituted counterparts are unavailable to us. Therefore, it is certainly plausible that some of the
2 unidentified peaks in the HPLC chromatogram of Fraction F, in Figure 3, could correspond to one or
3 more of these novel PAH detected by AFM. The joint usage of STM orbital imaging with AFM can
4 further aid in structure determination of unknown PAH in fuel-product mixtures, an example being F4
5 (more details in the supporting information). In this case, the benz[*a*]anthanthrene motif is properly
6 identified by comparing the experimentally obtained lowest unoccupied molecular orbital (LUMO)
7 image with the calculated LUMO via density functional theory. This is the first time any of these six
8 PAH have been reported as fuel pyrolysis and/or combustion products.

19 20 21 **4. Conclusion**

22 This study shows that an AFM-based strategy, in conjunction with synthetic chemistry and analytical
23 tools, is advantageous for investigating molecular ensembles. We showcase the detection, identification,
24 synthesis, and quantification of a novel seven-ring PAH product, benz[*I*]indeno[1,2,3-*cd*]pyrene, of
25 supercritical *n*-decane pyrolysis. Aided by the AFM capability of probing single molecules, even at
26 lower concentrations than 1 ppm, novel molecules within fuel pyrolysis products were reported. The
27 demonstrated multi-disciplinary approach directs the production of chemical standards in a cost-
28 effective and time-saving manner and should generally be applicable in fields where a diverse range of
29 PAH are being formed.

30 31 32 33 34 35 36 37 38 39 40 41 42 **5. Acknowledgments**

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Supplementary Material: Additional AFM measurements, chemical structures, and synthetic procedures.

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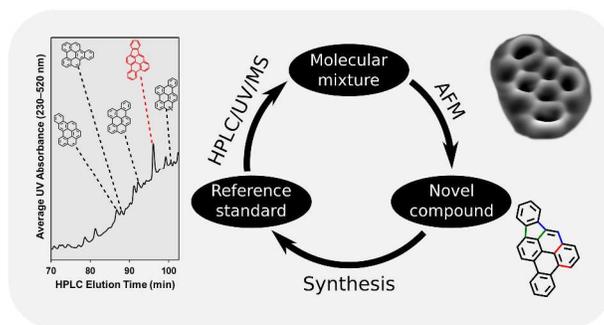
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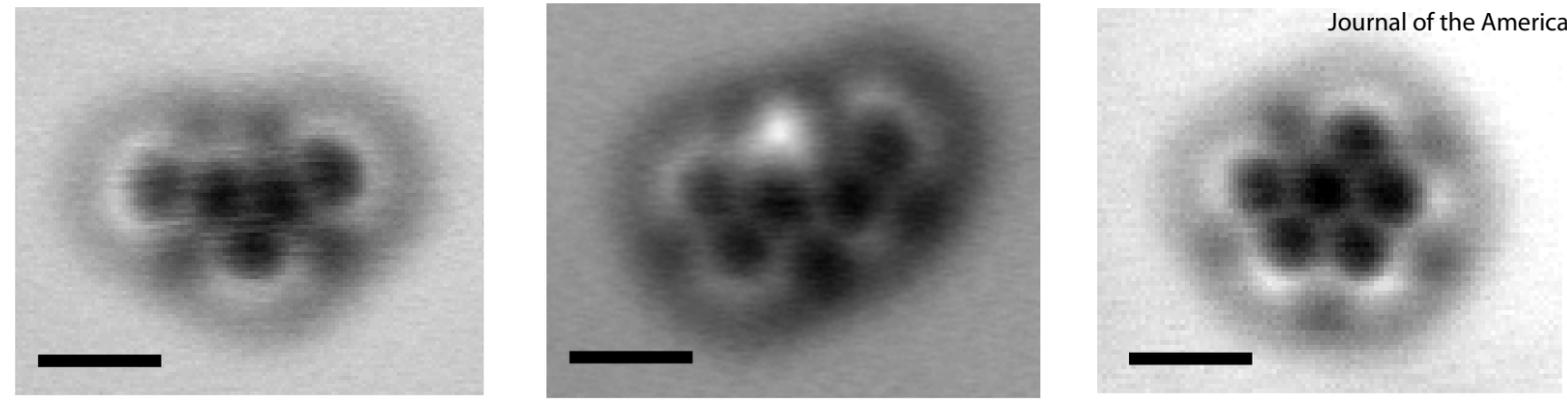
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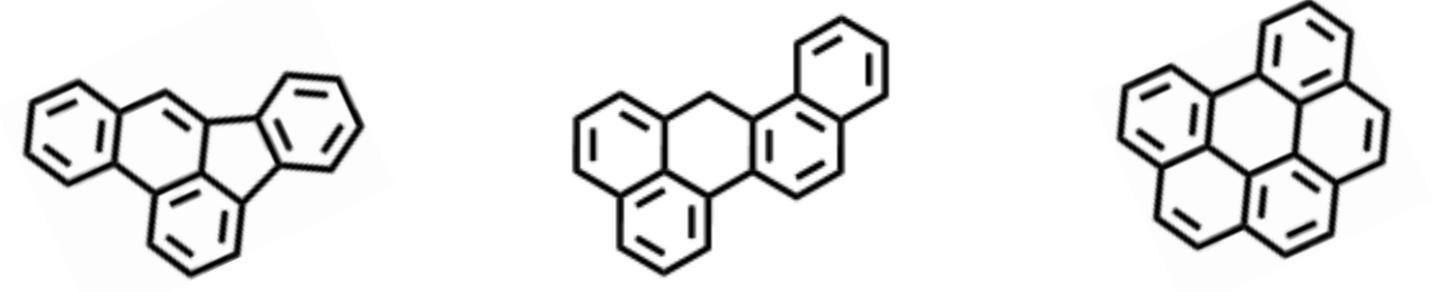


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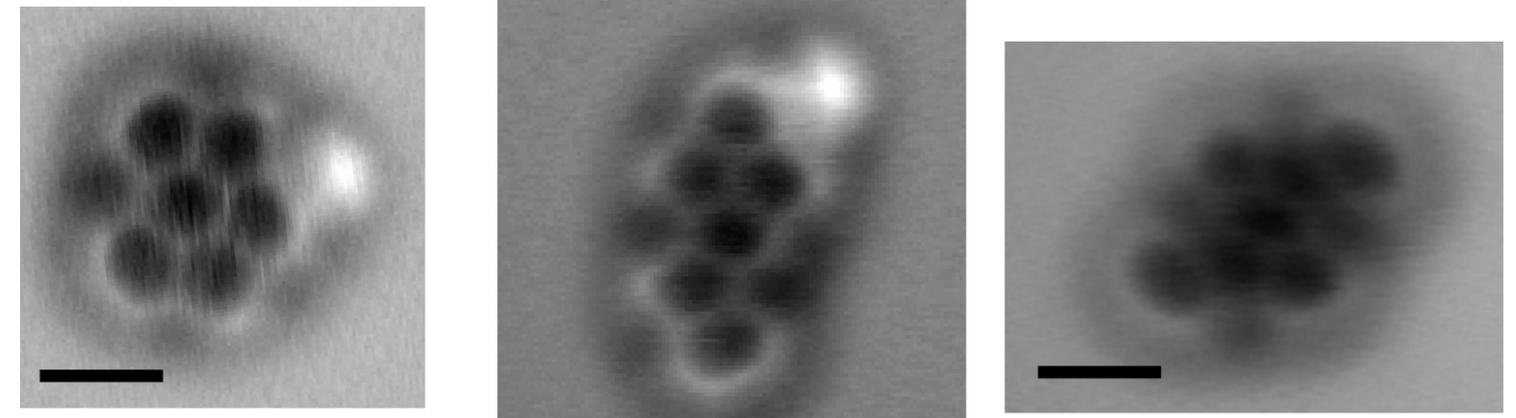
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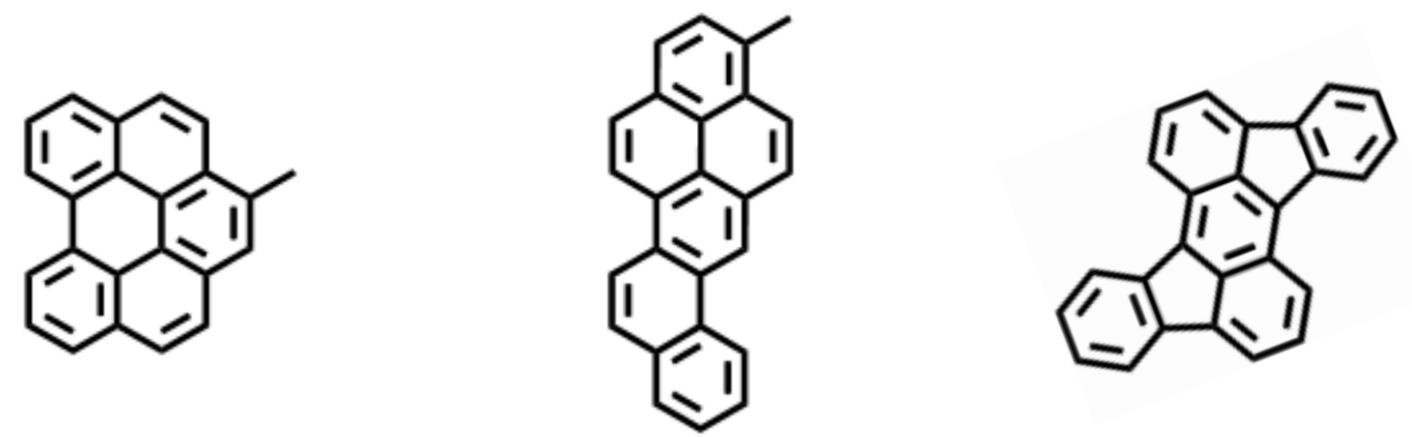
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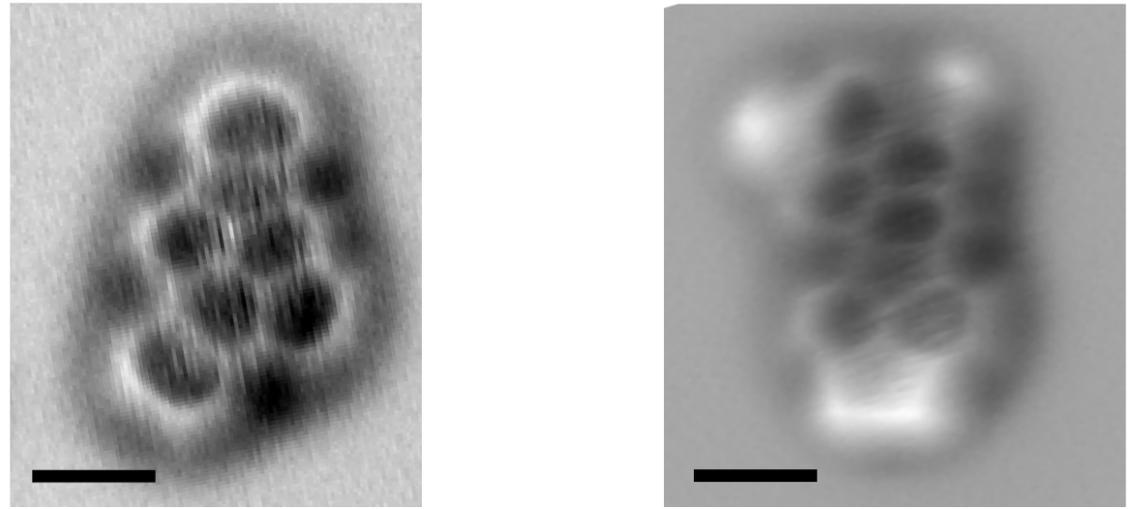
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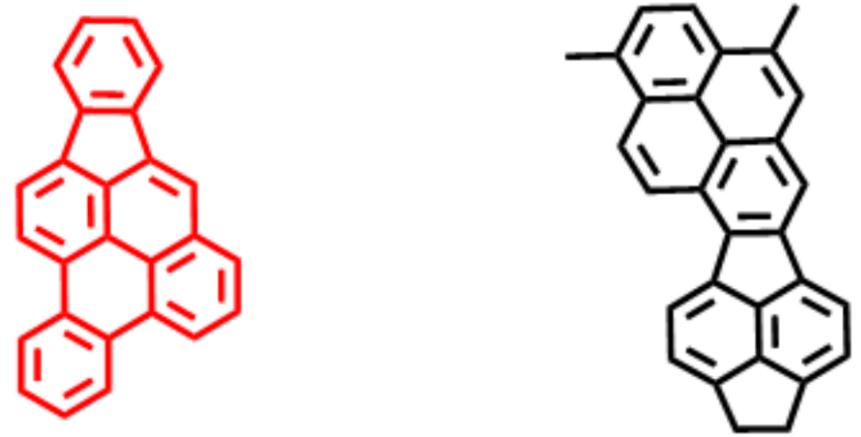
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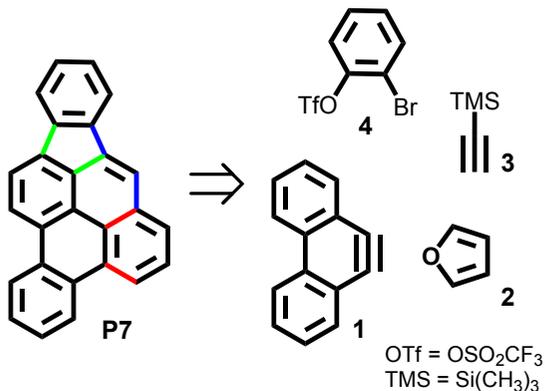


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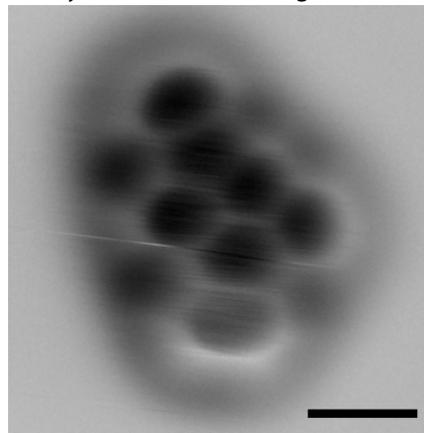
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8-steps synthesis of P7
 1 Diels-Alder cycloaddition
 2 Pd-catalyzed Sonogashira couplings
 2 Metal-catalyzed cyclizations



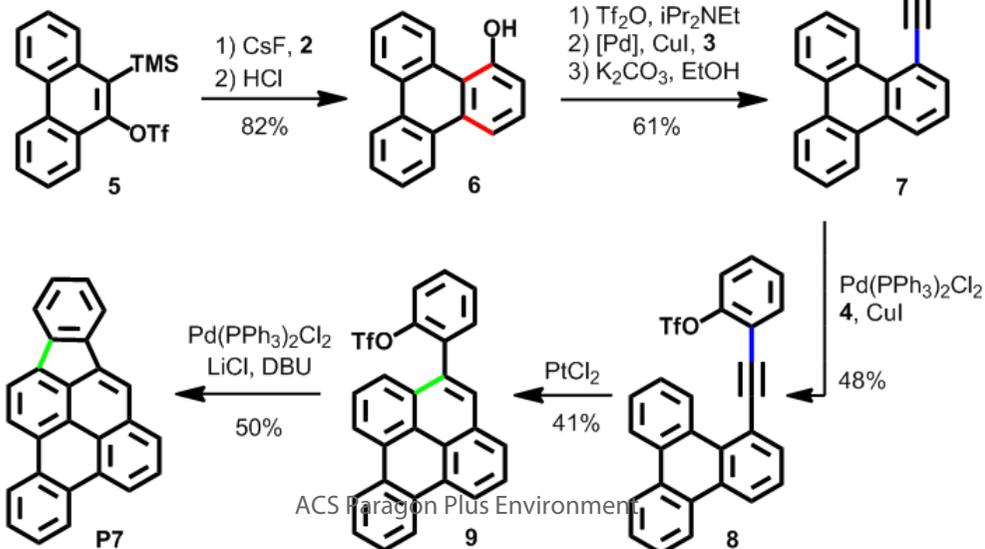
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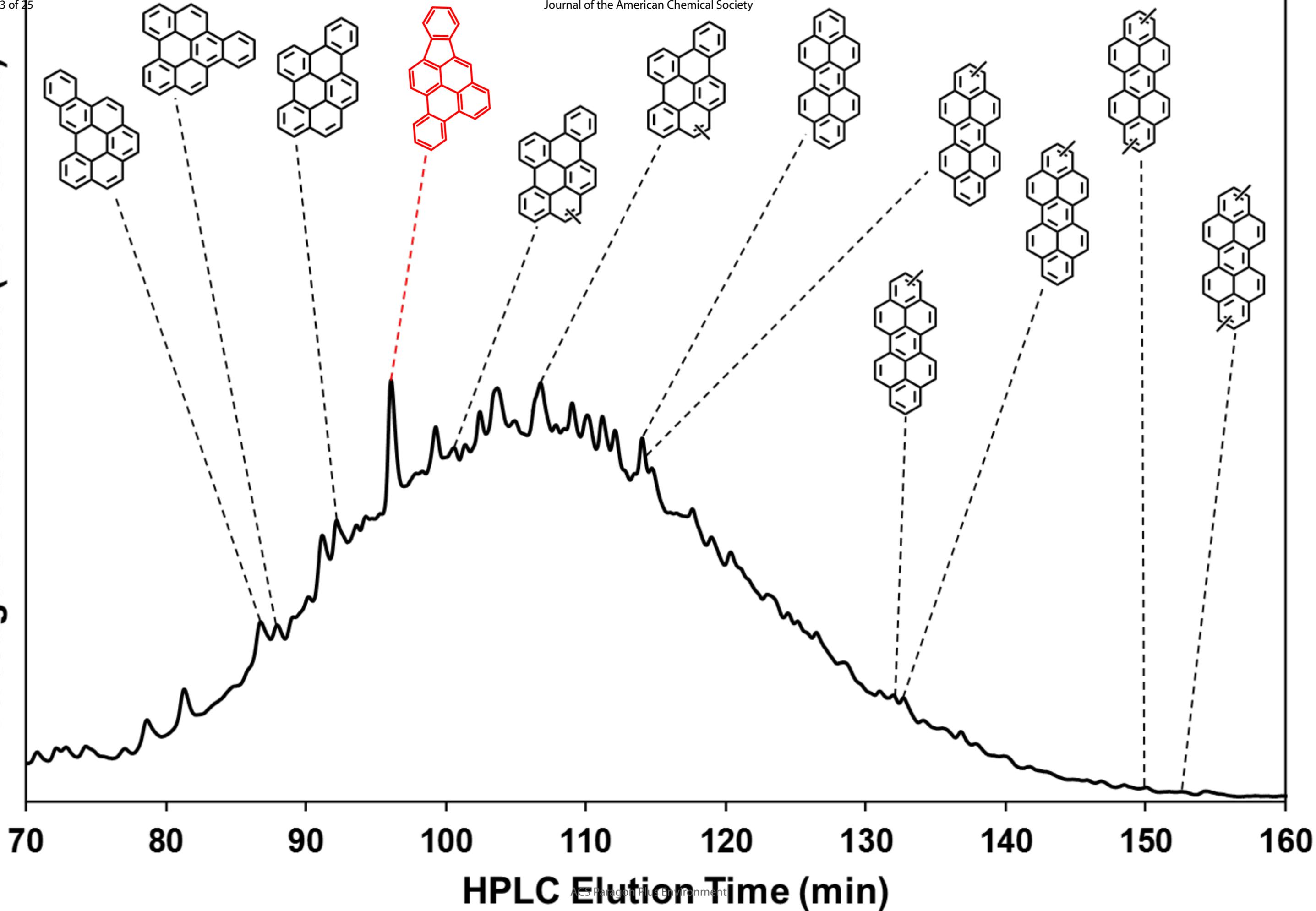


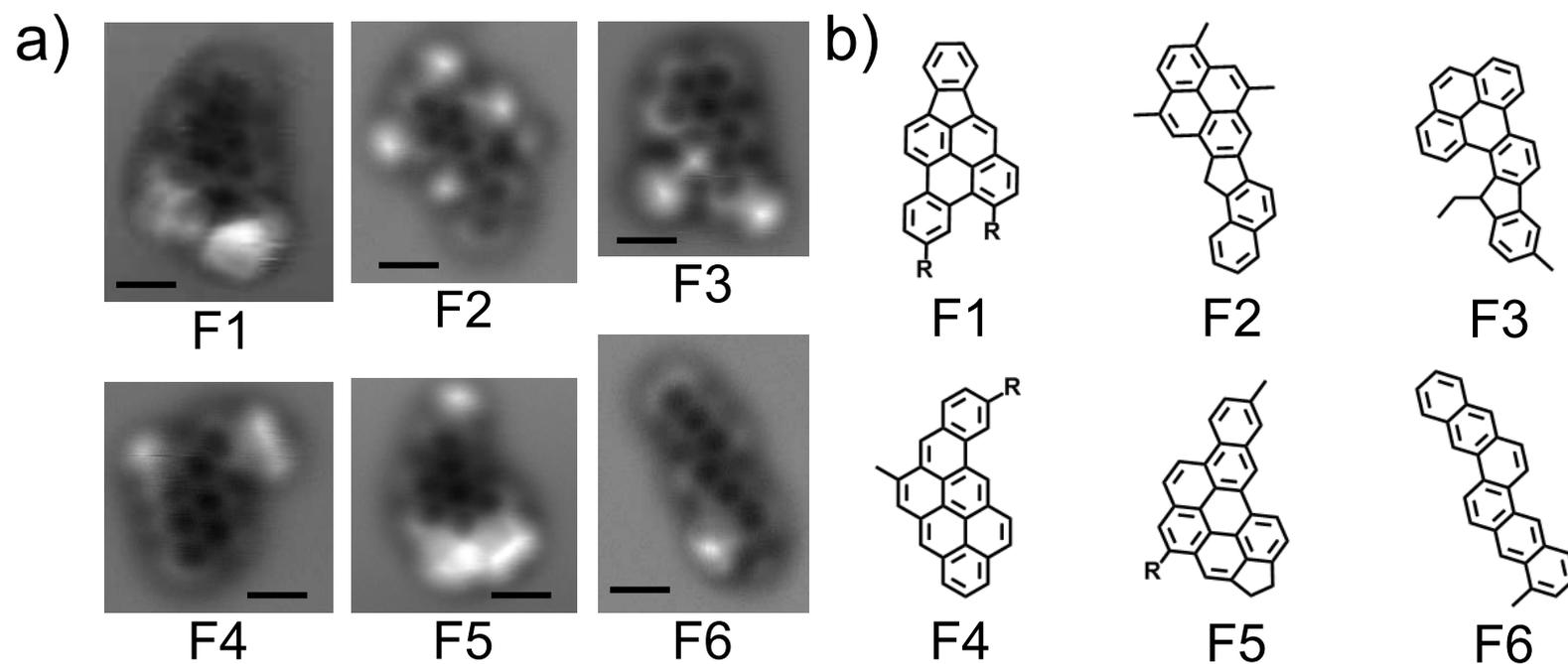
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