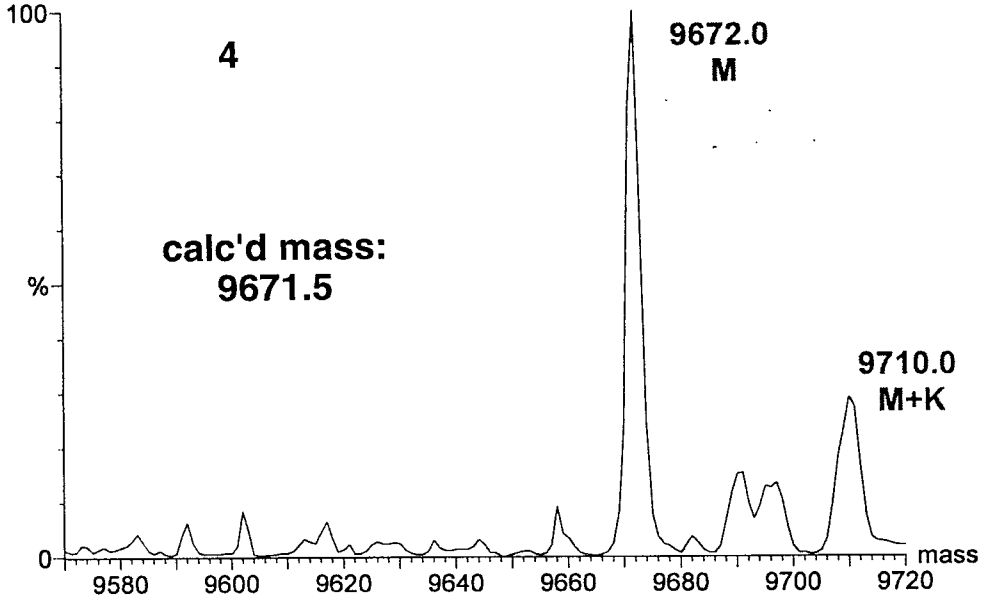
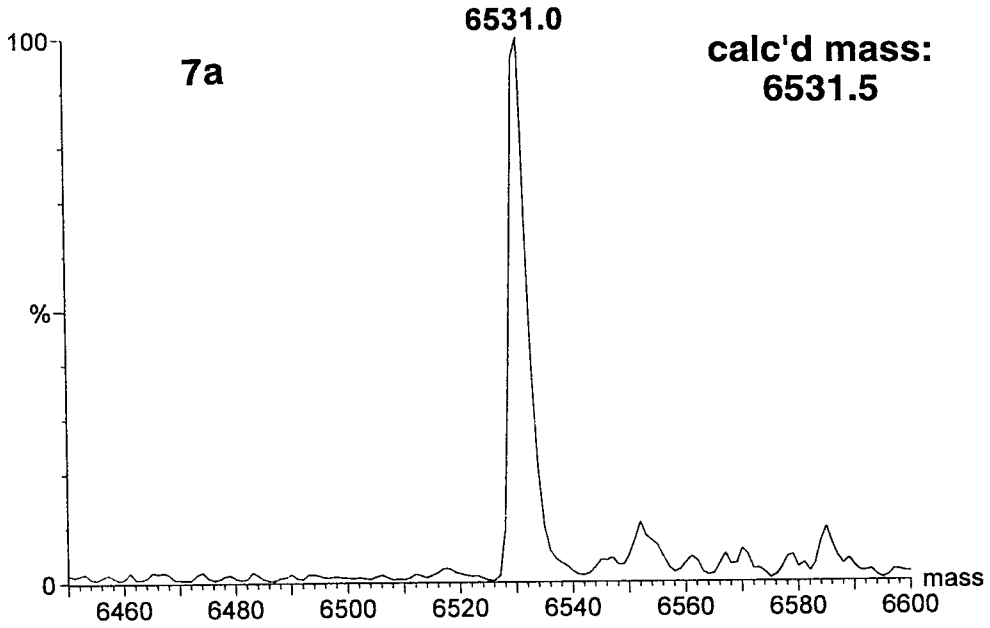
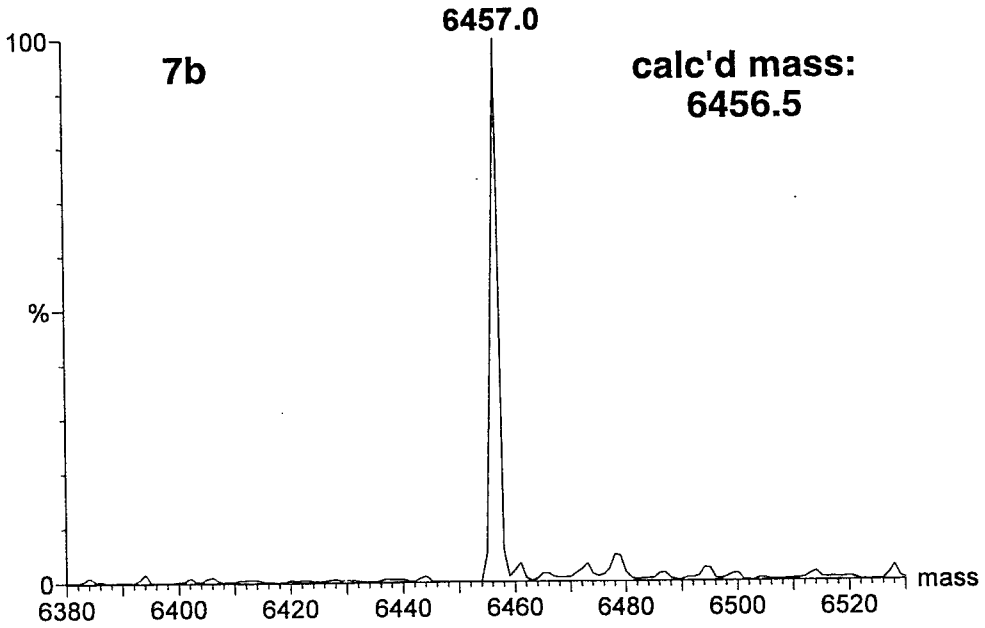
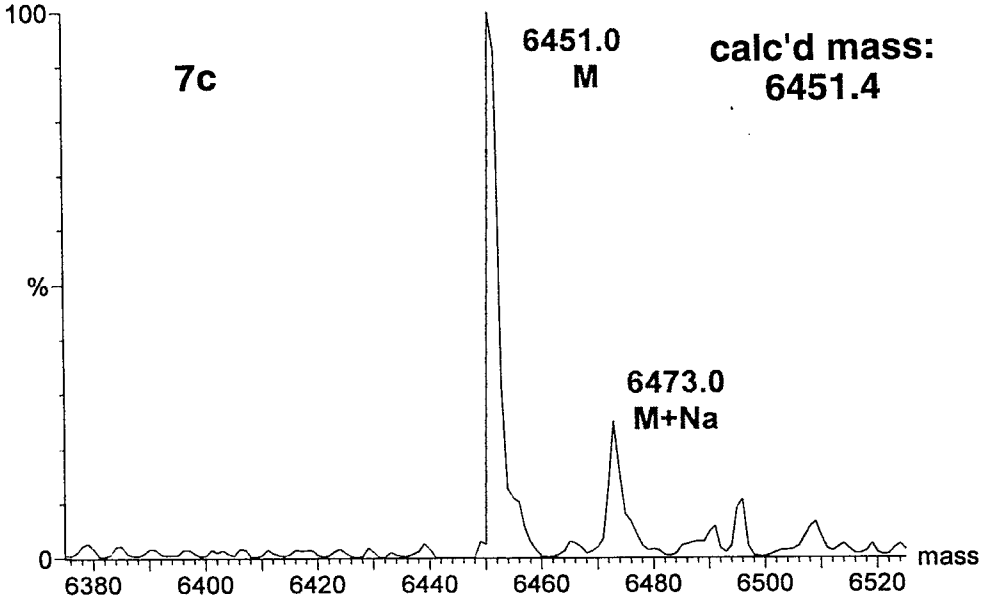
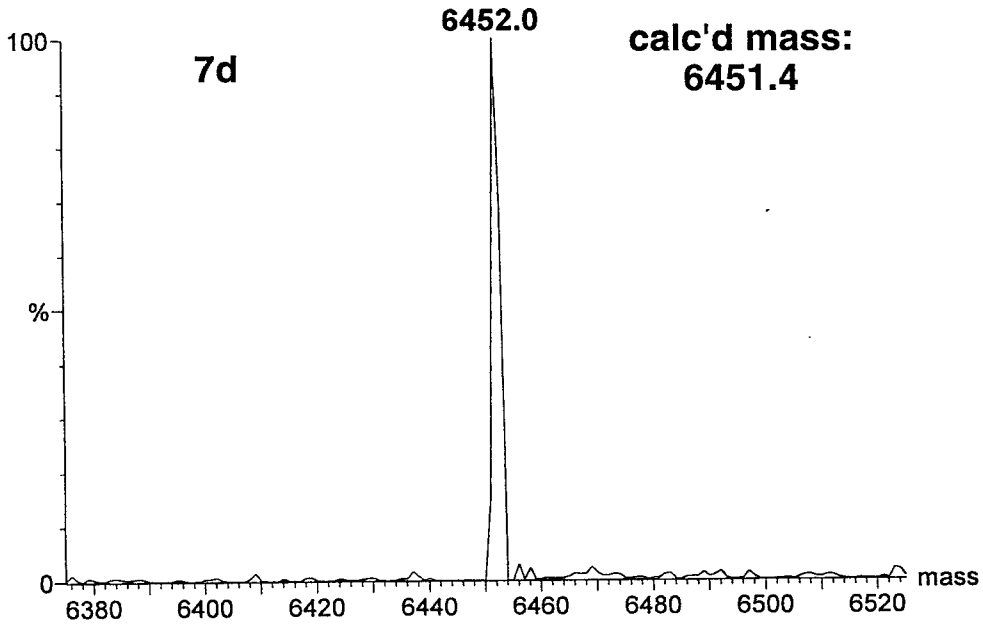
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
J. Am. Chem. Soc., 1998, 120(14), 3289-3294, DOI:10.1021/ja9740834

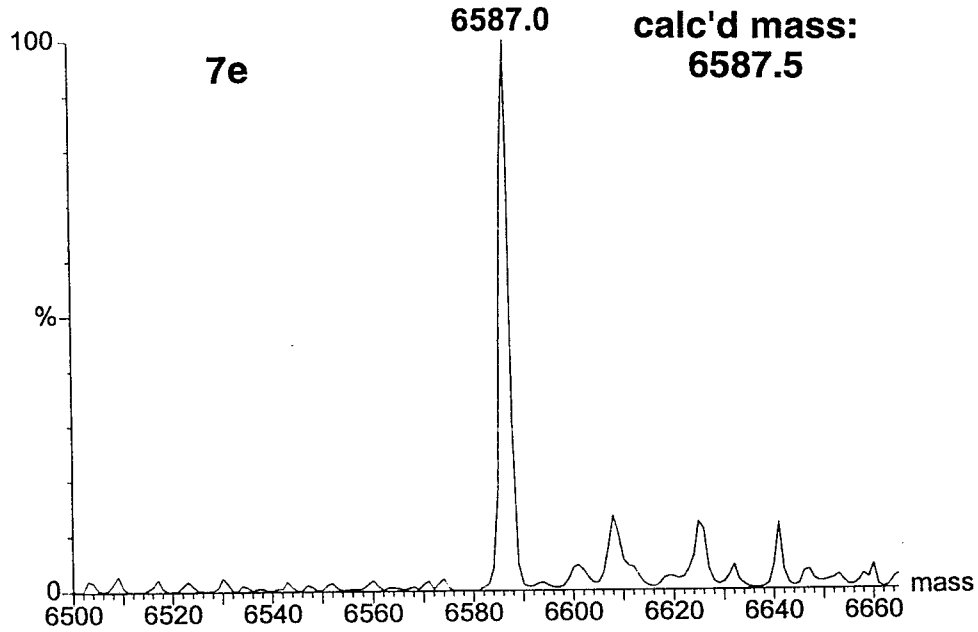


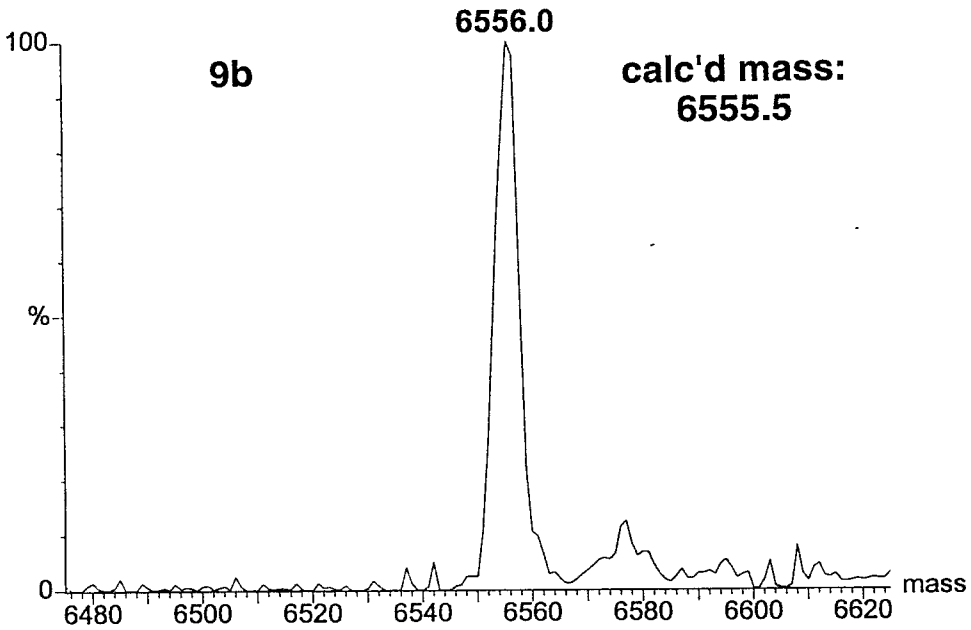












Preparation of Digestion Product of Pyrene Butyric Acid Conjugation Product. To 1-pyrenebutyric acid (25 mg, 0.1 mmol) in DMF (0.8 mL) was added (in the order listed) diisopropylethyl amine (34 mg, 0.26 mmol), PyBOP (45 mg, 0.1 mmol), 6-amino-1-hexanol (10 mg, 0.1 mmol). The solution was stirred at room temperature for 30 min, at which time it was poured into water (10 mL), and extracted with ethyl acetate (15 mL). The organic layer was washed with 10 mM H_2SO_4 (5 mL), 1% NaOH solution (5 mL), and brine (5 mL). The organics were dried over $MgSO_4$ and concentrated. Column chromatography ($MeOH:CH_2Cl_2$; 1:19) yielded 33 mg (100%) of the amide coupling product as a white solid: mp 146-148 °C; ^1H NMR ($CDCl_3$) δ 8.28 (d, 1H, J = 9.6 Hz), 7.91-8.14 (m, 7H), 7.84 (d, 1H, J = 7.5 Hz), 3.46 (t, 2H, J = 6.6 Hz), 3.32 (t, 2H, J = 7.8 Hz), 3.12 (t, 2H, J = 6.9 Hz), 2.89 (t, 2H, J = 7.2 Hz), 2.07-2.12 (m, 2H), 1.27-1.47 (m, 8H); IR (film) 3306, 3039, 2933, 2856, 1639, 1540, 1464, 1417, 1060, 1019, 837, 715 cm^{-1}. HRMS FAB ($M + H^+$) calcd: 388.2277, found: 388.2281.

Enzymatic Digestion of 5. Snake venom phosphodiesterase (2 μL, 0.03 units/μL) and calf alkaline phosphatase (1 μL, 1 unit/μL) were added to **5** (0.5 OD) in 47 μL of Tris-acetate buffer (0.1M, pH 8.8) and $MgCl_2$ (0.015 M). The tube was vortexed, spun briefly, and immersed in 37 °C water bath for 12 h. The sample was precipitated via the addition of 3 M NaOAc (5 μL) and EtOH (180 μL) and freezing (-78 °C). The solution was centrifuged at 14,000 rpm for 10 min. The supernatants were carefully removed. The residue was resuspended in water (45 μL) and 0.3 M NaOAc (5 μL), and was reprecipitated with EtOH (180 μL). The supernatants were combined and removed *in vacuo*. The residue was resuspended in a water:acetonitrile:methanol (1:3:1) mixture (60 μL) and analyzed by reversed-phase HPLC on a Rainin C-18 Microsorb column. Gradient conditions: A, 0.01 M KH_2PO_4 (pH 6.8), 2.5% acetonitrile; B, 0.01 M KH_2PO_4 (pH 6.8), 72% acetonitrile; 0-28% B linearly over 15 min; maintained at 28% B for 20 min; 28-100% B linearly over 5 min; maintained at 100% B for 10 min.

HPLC chromatograms of: A) Enzymatic digest of deprotected oligonucleotide used to form bioconjugate **5**. B) Independently synthesized coupling product of pyrene butyric acid and 6-aminohexan-1-ol. C) Enzymatic digest of **5**.